Department of Biochemistry
School of Medicine
University of Washington
Seattle Campus

Program Review
May 2 – 3, 2022

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Part A: Required Background Information

Section I: Overview of Organization

Mission and Organizational Structure

<u>Overall mission</u>

Our mission is to contribute to the betterment of society and improve the health of the public by

- Discovering and disseminating new knowledge in the molecular mechanisms of biology and disease,
- Educating a diverse student body of undergraduate, graduate, and medical students in the underlying principles of biochemistry, and
- Participating in and contributing to local, national, and global scientific endeavors.

<u>Values</u>

- **Curiosity.** We are driven to create new knowledge through scholarship, research, and innovation.
- **Integrity.** We strive to act honestly and ethically in all situations.
- **Excellence.** We strive to produce scholarly work of the highest standard.
- **Respect**. We believe that diversity promotes excellence. We celebrate the uniqueness of every member of our department. We strive to foster an inclusive environment through mutual respect and value the perspective of everyone.
- **Teamwork**. We encourage collaborations, which increase our collective impact.
- **Service**. We are public servants supported largely by taxpayer dollars. We take this responsibility seriously and are dedicated to the betterment of society in our community, state, country, and world.
- **Communication**. We are experts in our fields, and we enthusiastically share our knowledge and ideas through our scholarly work, teaching, and outreach.

<u>Degrees offered</u>

- Ph.D. in Biochemistry
- M.A. in Biochemistry
- M.S. in Biochemistry
- Bachelor's degrees (B.A. and B.S.) in Biochemistry are conferred by the Department of Chemistry in the College of Arts and Sciences through collaboration with the Department of Biochemistry.

This review is focused on our graduate program. During the Biochemistry review, the agreed plan is to consider only the aspects of the undergraduate program that relate to the graduate program, such as the graduate teaching clerkship.

<u>Enrollment and graduation patterns</u>

The enrollment patterns and the colleges of entrants to our graduate program in the last twelve years is given in Appendix A. (Please use the bookmark feature in Adobe Acrobat to navigate through our Appendices.) Several conclusions can be made from these data.

1. Our outreach program in 2012 was successful because we significantly increased our pool of applicants (a jump from 51 to 169 applicants). Our pool has averaged 172 applicants since then.
2. We attract about one fifth of our students from Washington state and one fourth from California.
3. All our students arrive with research experience but the amount of experience has varied from 5 to 96 months in recent years.
4. Between 3 and 10 students have entered our program each year for the last twelve years, with an average of 6.5 per year. We try to match the number of entrants with the number of labs accepting students. To do so, we identify what labs will be accepting students and adjust the number of students accepted accordingly. If we accept all qualified students (27-28), the number of entrants tends to be large (9-10 students). When we are more selective and accept only 18-23 students, the number of entrants tends to be small (4-5 students).
5. We recruit fewer than one foreign student per year.
6. We recruit on average one URM per year.
7. The mean GPA of the applicant pool is 3.5 and of our entrants is 3.7.

These facts suggest that our graduate program is healthy and attracting talented students.

Our time to graduation in the last five years was 5.3 years. We have graduated 67 students with a Ph.D. and 4 with a master's degree since 2010. After graduation, 65% of our students pursued a postdoctoral position. Eighteen percent moved directly into a staff scientist position in industry or academia. Of the 34 doctoral students who graduated between 2010 and 2016, eight are in faculty positions, ten are scientists in academia or industry, two are managers in industry, six have other positions in industry, one is a lawmaker, one is a medical technologist, one is a forensic toxicologist, one started his own brewing company, one is a homemaker, one is retired and we have lost contact with two. Ninety percent of our students are using their degrees in their careers. A list of the current positions of our 71 students who graduated with a master's degree or a Ph.D. in the last twelve years is given in Appendix A.

<u>Organizational structure</u>

The Department of Biochemistry is one of 14 basic science departments in the School of Medicine at the University of Washington (Appendix B) (out of 31 total departments in the School). The Institute for Protein Design (IPD) is an institute in our department. At the UW, institutes are not independent of departments, and directors of institutes report to their chair. Chairs report directly to the Dean of Medicine. Organizational charts for the Department of Biochemistry and the IPD are given in Appendix C. Also included in Appendix C is a pie chart showing the composition of the department.

<u>Unit support of academic services</u>

We support staff directly involved in education (Erin Kirschner - Associate Director of Graduate Programs, Teri Alvarado - Undergraduate Coordinator, and Cameron Rosaeur - Undergraduate Lab Coordinator) with funds provided by the State of Washington to the Department of Biochemistry. Erin Kirschner provides all academic advising for graduate students. Ms. Kirschner supports both our departmental graduate

program and the Biological Physics, Structure, and Design interdisciplinary graduate program, which provides half her support. She is an exceptional staff member.

Many IPD staff are supported by funding provided by the state specifically for IPD administrative support. The department supports all other departmental and IPD staff with funds derived from external sources, for example, indirect costs returned to the department, gift funds, or grant funds designated for administration. (Foundation grants often include administrative staff salaries and benefits as direct costs.) These staff include the head administrator, the chair's assistant, and staff providing grant support, fiscal services, purchasing, and human resources. IPD pays for departmental administrative staff in proportion to their annual expenses.

Each lab provides its own technology support, with Tonya Hirtzel, the chair's assistant, providing additional support as needed. The department and IPD pay the UW technology fee, which pays for internet and telephone access.

<u>Shared governance</u>

As recommended by the prior review, we have developed an extensive committee system that runs the department. (See Department of Biochemistry Standing Committees 2021-2022 in Appendix D.) Faculty and students volunteer to serve on these committees each year. Note that to ensure student input in key aspects of student life, students can serve on the Graduate Admissions Committee, the fellowship committees, the Diversity, Equity and Inclusion Committee and the Retreat Committee. Students also have their own committees to organize features of student life including student seminars and student lunches with seminar speakers. (See 2021-2022 Biochemistry Student Jobs, Appendix D.) The students also organize the Milton Gordon lecture whose speaker is invited and hosted by the graduate students.

Faculty meetings are held regularly on the second Tuesday of each month from October to June. The chair prepares an agenda each month and sends it to the faculty before each meeting, and minutes are taken at each meeting. Copies of all agenda and minutes are kept on the departmental server and are available to all faculty. Anyone can suggest an agenda item by contacting the chair, the chair's assistant, or the departmental administrator. A graduate student representative attends all faculty meetings and shares the minutes with the other students.

Students raise concerns with Erin Kirschner, the Associate Director of Graduate Programs. The chair, Professor Jim Hurley (the Graduate Program Coordinator) and Assistant Professor Andrea Wills (Assistant Graduate Program Coordinator) meet regularly with Ms. Kirschner (at least once a month, usually more often) to discuss any issues that have arisen. The Chair, the Graduate Program Coordinator and the Assistant Graduate Program Coordinator are also available to meet with students, but students usually share their concerns with Erin Kirschner, the Associate Director, or their advisor first.

The chairs of the 14 School of Medicine basic science departments meet formally every month in the academic year to share and discuss concerns with the Vice Dean for Research and Graduate Education. The Basic Science Chairs also meet informally every Friday to plan, problem solve and share information. All 31 chairs of the School of

Medicine meet once each month to share input with each other and with the Dean of the School of Medicine.

The Dean of the School of Medicine is readily available for individual meetings with the chair. The chair also consults regularly with the Vice Dean for Administration and Finance, the Director of Personnel Policy and the Senior Director of Business and Regulatory Affairs in the School of Medicine.

Budget and Resources

Overview

The Department of Biochemistry is in a stable financial state. A budget that recaps fiscal year 2021 (July 1, 2020 – June 30, 2021) is given in Appendix E with total expenses of $40M per year. (As requested, budgets provided to the department by the School of Medicine from four biennia are also in Appendix E but are harder to interpret as they combine faculty startup funds with other departmental funds, restricted funds with unrestricted funds, and IPD funds with other departmental funds.) The Department of Biochemistry (excluding IPD) ended FY21 with a carryforward of $1.6M in unrestricted funds, $1.6M committed to faculty startup funds, $2.4M in other restricted funds (Appendix E), and $17M in committed grant funding (not shown).

The Department of Biochemistry (excluding IPD labs) has four main sources of revenue: funds from the State of Washington (17%), indirect costs from grants (7%), direct costs from grants (61%), and funds from endowments, gifts, and royalties (15%). 99% of the state funding is for tenure lines for the faculty in the department. Tenured and tenure-track faculty, have three portions of their salary. The state tenure line (the “A” portion) represents on average 75% their salary (80% for junior faculty and 70% for senior faculty). On average the “B” portion is 25% of the salary and is paid by grants or gifts. To fund our graduate program, we charge faculty 20% of the A line as “recapture,” so an average tenured or tenure track faculty receives 60% of their salary from the State. (Recapture is forgiven for the first three years as an Assistant Professor.) Regular faculty who are “WOT” (without tenure for reasons of funding) receive an average of 48% of their salary from departmental funds in recognition of their critical contributions to service and teaching.

Administrative staff are paid by several mechanisms. Our education staff are paid from scarce state funds because we receive few funds for teaching. Indirect costs, external funds, and funds from IPD pay for other departmental staff. Our administration costs are ~4% of our total expenses.

Grants and gifts (including training grants and fellowships to students, and grants and gifts to advisors) pay for graduate students, postdocs, and lab staff. Whether or not they are taking graduate students, faculty contribute from their grants to pay first-year graduate students through RA-ships (research assistantships) while the students are doing their rotations (through faculty salary recapture as mentioned above). Unlike the College of Arts and Sciences, we do not receive teaching assistantship funds. (Note that our graduate students participate in a teaching clerkship for two quarters, see below.)

The IPD is in excellent financial shape with extensive reserves (Appendix E). The IPD is funded by funds from the State of Washington (4%), grants (45%), and funds from endowments and gifts (51%). The IPD ended FY21 with a carryforward of $28M in gifts and royalties and $34M in committed grant funding. The IPD's constant focus is raising funds (gifts, grants, and royalties) to support their $25M per year expenses.

Training grants and fellowships

Faculty in our department participate in 12 training grants. One faculty member, Justin Kollman, is co-PI of the Molecular Biophysics Training Grant, and Biochemistry administrative staff manage two training grants marked with *. (Number of biochemistry faculty currently involved; number of Biochemistry student years funded by a training grant in last 7 years)

Training in Molecular and Cellular Biology (10, 8)
*Training in Molecular Biophysics (10, 18)
Diabetes, Metabolism and Endocrinology Training (3, 0)
*Interdisciplinary Training Grant in Cancer Research (5, 3)
Vision Research Training Grant (3, 2)
Genetic Approaches to Aging Research (2, 5) (Ended April 2021)
Graduate Training in Neuroscience (2, 0)
Interdisciplinary Training in Genomic Science (2, 0)
Medical Scientist Training Program (2, 0)
Comprehensive Training in Inter-Disciplinary Oral Health Research (1, 0)
Drug Action, Metabolism and Kinetics (1, 0)
Training in Molecular Pharmacology of Abused Drugs (1, 0)

In addition, in the last seven years, 11 student years have been funded by an NSF fellowship, eight by an F31, and eight by other fellowships. In the last seven years, our students have been funded by training grants or fellowships for 65/229 student years. In other words, with a time to graduation of 5.3 years, our students are funded on average by the department for 0.8 years, by a fellowship or training grant for 1.5 years, and by the grants or other funds of their thesis advisor for 3.0 years. As training grant funding has declined, we would like to obtain more fellowship funding for our students. An alternative would be to receive teaching assistant funds for our students as do students in the departments in the College of Arts and Sciences ($100,000's per year).

Evaluation of current funding, human capital, and other resources

Discussions about number, types and salaries of administrative staff are frequent and continuing between the head administrator of Biochemistry (Zulfiya Lafi), the Chief Strategy and Operations Officer of the IPD (Lance Stewart) and the chair. Ms. Lafi and Dr. Stewart in turn consult with their staff. We aim to strike a balance between having enough staff for the ever increasing responsibilities for the IPD and Biochemistry without being overstaffed. Historically, we have tended to be slightly understaffed, especially after having to lay off one staff member due to the School of Medicine temporarily borrowing $165,000 of our annual state funds in FY17-FY21. Those funds have now been returned and we are in the process of hiring someone to staff the front desk.

Several years ago, an analysis of our efficiency in grant submission was found to be at the median for all the basic science departments.

The School of Medicine began encouraging cost savings in 2017. This included efforts to centralize all HR, grant submissions and visas. We have not participated in this centralization, and recently the School has realized that the centralized system has not served everyone well. In the Department, we are continually considering the most efficient ways to administer the department and IPD, while maintaining excellent customer service for our faculty, postdoctoral fellows, and students.

Future financial plans

For the future, one departmental goal is to continue to increase grant funding. We have been on an upward trajectory since 2014 (Figure 1), and this has occurred across the department and for several reasons. Our junior faculty obtained more grant funding as they established their labs, and our senior faculty have maintained their funding. The IPD grant funding has also increased although with a greater percentage of foundation grants that pay lower indirect costs. Continuing to grow grant funding depends on junior faculty obtaining second grants, hiring more junior faculty, senior faculty maintaining funding and continued success of the IPD. To achieve this goal, we provide feedback on grant proposals as requested, strategize on where to send grants, and encourage collaboration. We provide matching funds for equipment requests. We also have a dedicated team to submit grants and shepherd them through the university bureaucracy.

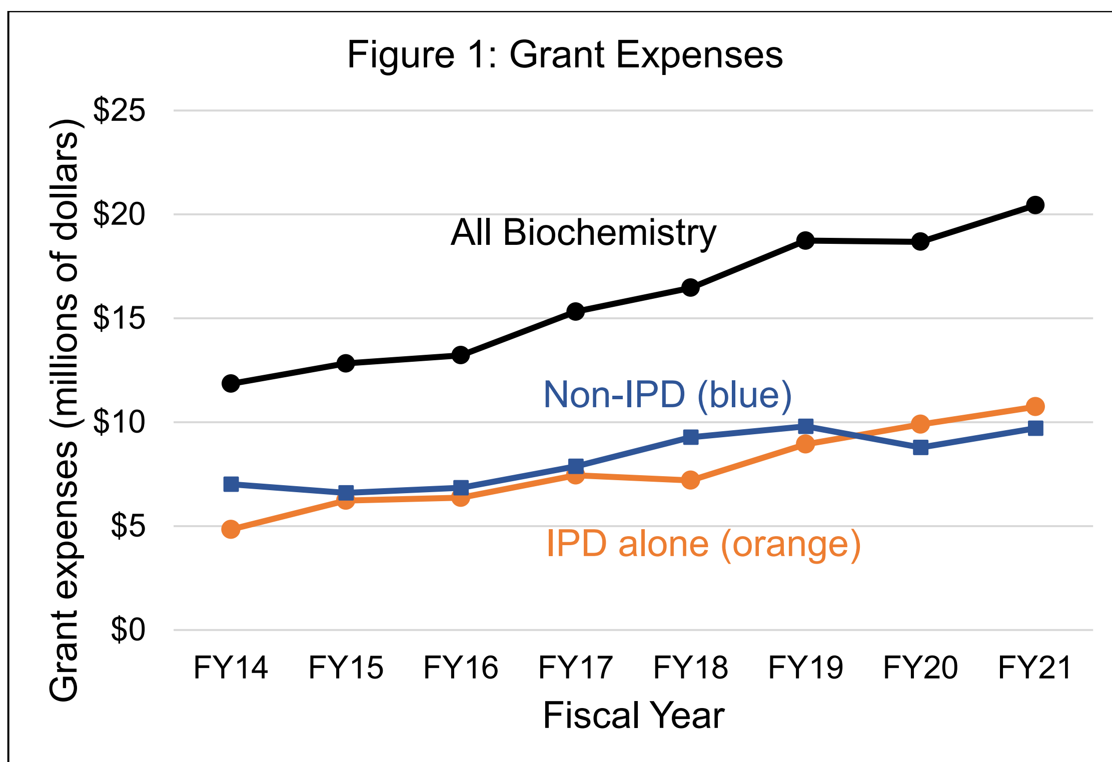


Figure 1: Grant expenditures per fiscal year not including $6 M in grant funding for cryo-electron microscopy equipment in 2016. Does not reflect indirect cost recovery, which depends on indirect cost rate of grants funded. The average indirect cost rate of grants in the department

fluctuates as the ratio of grants from Federal sources (indirect cost rate 55.5%) to grants from foundations (indirect cost rate 8-15%) fluctuates. In FY21, 5.6% of grant expenses were returned to the department as indirect costs.

A second departmental goal is to increase gift funding for general departmental use. Ten years ago, we had one endowed professorship. Since then, we have been fortunate to raise funds for two endowed chairs and one endowed professorship. Even so, we have more distinguished faculty than we have endowed positions. We also raised funds for an endowed lectureship and an endowed graduate research fund, which is used to buy new laptops for incoming graduate students and to pay for scientific training outside of the university. Twenty-five years ago, an endowed fund was set up to pay for graduate students to travel to two scientific meetings during their time as a graduate student. These are all due to generous gifts from donors. See Appendix F for a list of endowed funds.

We have increased our outreach with a newsletter and by direct contact by the chair. The chair meets monthly with an advancement officer from the School of Medicine to strategize on funding opportunities. We are working to raise additional funds for research, our graduate program and our "Friends of Biochemistry" account.

A third departmental goal is to increase graduate student funding by fellowships. We already have a course to teach grant writing to first year graduate students. Every student writes an NSF proposal and all those eligible submit their proposal. This course is taught by Dana Miller, Associate Professor. Since its inception, we have increased our success rate from very few over many years to seven in seven years (including BPSD students who also take this course). We have also had twelve honorable mentions in 8 years. Encouraged by the success of the NSF grant-writing course, Andrea Wills, Assistant Professor, began an F31 grant writing course for third year graduate students.

Finally, we have actively developed a translational investigator program. (This was largely the work of Lance Stewart (CSOO of IPD), IPD faculty, and the chair.) The Translational Investigator Program gives talented postdoctoral fellows extra time, funding, and business help to develop their discoveries into products for licensing to their own or other companies. As a result of this program, IPD has spun out seven companies and licensed extensive technologies to three others. The financial benefits of this program are becoming obvious as equities from sale of three of the companies have already been realized and funds from the sale of two additional companies are due to arrive in November 2022. Royalties might provide a long-term revenue stream to the IPD and to Biochemistry.

Academic Unit Diversity

<u>Diversity Plan</u>

The Biochemistry department is committed to creating an environment that is welcoming and supportive of researchers from all backgrounds, particularly those from historically excluded groups. We have worked to recruit more diverse graduate students and faculty to join our department and work with UW and SOM to support these students and faculty. To enhance the diversity of our graduate student population, each year the department sends the Associate Director of Graduate Programs, Erin

Kirschner, and a student representative to the annual conferences of SACNAS (Society for the Advancement of Chicanos and Native Americans in Science) and ABRCMS (Annual Biomedical Research Conference for Minority Students), as well as the annual NIH Graduate & Professional School Fair. In addition, in coordination with other basic science biomedical departments, the Biochemistry department sends a faculty member to either SACNAS or ABRCMS every other year to recruit URM graduate students for all the basic science biomedical departments and programs at the UW. We ask graduate student applicants to explain how they will contribute to a diverse learning community at UW as part of the application, and this is a key factor in choosing candidates to admit into our program. Sixty-five percent of our graduate students are women and 12% nonbinary, and 29% of our students are from historically excluded populations.

Diversifying the faculty is slower, but we are also making progress in this area. Of the regular faculty, 8/23 are women and at least 2 are LGBTQIA+. In the past 10 years, we have hired two Asian-American junior faculty, and our most recent hire is Latina. We are currently finishing another junior faculty search. In this search, we implemented some best practices for hiring diverse faculty, including training of the search committee, focusing on diversity statements provided by applicants, and using high-quality rubrics to evaluate the candidates. We have offered tenure-track positions to two highly qualified candidates, a Latina and an Asian-American woman. We are currently negotiating with both candidates.

Diversity committee representation

Our department diversity committee has six members, two white females are co-chair, one is LGBTQIA+. The ex officio member is Erin Kirschner.

Diversity of Department

	Gender	White	Asian	Hispanic	African American	Pacific Islander	LGBTQIA+
Faculty	8 F/15 M	18	3	1	0	1	2
Admin Staff	13 F/5 M	10	8	0	0	0	unk
Graduate students	22F/8M/4NB	22	6*	4	1	1	unk

* 3 US Citizens, 1 US Resident, 2 International students.

Institutional Resources

The Graduate Opportunities and Minority Achievement Program (GO-MAP) seeks to create an inclusive environment for all students. Several Biochemistry students have participated in GO-MAP programming, which includes seminars and workshops held throughout the year. GO-MAP also host regular social events that have allowed our students to connect with students in other graduate programs. This has helped to build community for some of our students.

The Associate Director intentionally promotes the GO-MAP program to prospective candidates at recruitment events such as the SACNAS and ABRCMS conferences.

Additionally, GO-MAP flyers are included in our orientation packets for incoming students. Programming and event information is distributed throughout the year to current students.

Outreach strategies to underrepresented minority students

Over the last nine years we have made a concerted effort to increase our recruitment of underrepresented minorities. Part of our strategy has been to regularly attend the annual SACNAS and ABRCMS conferences, where we connect with talented students from a variety of ethnicities and backgrounds. Our Associate Director has attended both of these national events every year since 2013 and was joined by many UW affiliates, including Biochemistry students. With experience, and by cooperating with numerous other UW programs that also attend, we have developed a strategy for making meaningful connections with prospective students. Attendees often return several times throughout their undergraduate careers, so there are opportunities for sustained relationships with those who have connected with us in past years. There are also opportunities to coach students about the rewards and challenges of a research-focused graduate program like ours. We consider it an honor to educate prospective candidates about possible scientific careers, and to help them find the career path that is best for them, even if it does not involve our program.

The pandemic made it more challenging to make strong connections as all the conferences have been held remotely. However, we believe that return to in-person conferences will solve this problem.

For the last five years, our Associate Director has also attended the annual NIH Graduate & Professional School Fair, on the Bethesda campus of the NIH (and virtually during the pandemic). This event is not specifically tailored to minorities but attracts a high number of talented post baccalaureate students, many of whom are from underrepresented groups. In addition, the Associate Director attended the Equity & Excellence Access in Higher Education conference in 2019, organized by the Association on Higher Education and Disability (AHEAD). This annual meeting provides training for program administrators about how to enhance program support for students with disabilities. As an example, one of our 1st year students for Autumn 2020 enrollment met our Associate Director at an on-campus outreach event three years before and that factored into the candidate's decision to accept our offer.

Initiatives to create a supportive environment for students

Beyond just increasing the recruitment of diverse students and faculty, we believe it is imperative to create an environment that enables the success for our students and faculty from historically excluded populations despite the systemic barriers they face. As part of this, we connect our students to UW campus resources staffed by experts including Hall Health counseling center, Q Center, GSSE, SOM affinity groups, and various student groups including UW's award-winning student SACNAS chapter. David Kimelman, Biochemistry Professor, was the chapter's faculty advisor until he retired in August 2021. We also require that first year students in our graduate program take Bioc533, which starts with an introduction to graduate school to help introduce students to some of the "unwritten rules" of academia to help lower barriers for historically excluded and first-generation students.

Additionally, in 2020, the current students in the Biochemistry and BPSD Ph.D. programs initiated the formation of our student buddy program. This program pairs current graduate students with incoming graduate students so that each one has a designated "buddy" prior to orientation. Buddies usually meet at least once a month. In addition, the leaders organize group buddy activities on a monthly basis to connect more people. Our new students have emphasized how much they enjoy this program and how it has helped them to transition to Seattle and graduate school. This program is completely student initiated and led. That investment in the new students from their peers has created an even stronger sense of community in our department.

We include graduate student representatives on nearly all departmental committees so that students can contribute to departmental governance. Our department has instituted a parental leave policy for graduate students, providing at least 8 weeks of paid leave for new parents.

Our DEI committee (co-chaired by Associate Professors Dana Miller and Suzanne Hoppins) has recently begun regular DEI "learning groups" - we discussed the 1619 podcast episode on the origins of health care disparities in our first meeting.

The seminar committee (chaired by Associate Professor Dana Miller) ensures that the series includes underrepresented minority scientists and women so that our students and trainees have first-hand examples of successful academics from different groups. Finally, there are several Biochemistry faculty who are Faculty Allies. The Faculty Allies program aims to ensure a healthy learning environment for all students and trainees (see more below).

Institutional resources to recruit and retain faculty from underrepresented identities

In the last two years, substantial resources have been developed.
The Provost has a DEI STEM Faculty recruitment initiative.

> *"The UW will provide startup funds to assist in hiring faculty at the UW Seattle campus. These funds are reserved for recruitment of faculty in the STEM disciplines (broadly defined) with a proven history of supporting and mentoring BIPOC students, post-docs, and/or early-career colleagues; conducting research that benefits underrepresented or underserved communities, and/or significantly participating in programs and activities that are aimed at increasing diversity and inclusion in their field."*

We are requesting these funds to support our recent offer to a faculty candidate.
The School of Medicine has an active office of Faculty Affairs, with the following mission statement:

> *"The mission of the UW School of Medicine Office of Faculty Affairs is to foster a thriving community of faculty by helping individuals develop as clinicians, educators, scientists, and leaders over the course of their career; promoting a climate of inclusion, support, and collaboration; and advocating for equity and courageous innovation."*

Resources from the Office of Faculty Affairs include a Bias Reporting Tool to formally report incidents of racism, bullying, and other negative biases ranging from one-time micro-aggressions to more severe and sustained behavior. The office also sponsors

many workshops and activities to support well-being. Resources from the Office of HealthCare Equity includes a Healthcare Equity Toolkit.

Strategies to support career success of faculty

The Department of Biochemistry recognizes that we have opportunities to improve our support of underrepresented members of our faculty. Of the twelve junior faculty we have hired since 2010, four were women. This brings the total number of women on our faculty to eight (of 23 total faculty members). Two of the faculty who have joined our department are Asian and one is a Latina.

All of our junior faculty have been successful. All four of the women added to our faculty since 2010 secured federal funding, and three have already been promoted to Associate Professor with tenure. Formal support for junior faculty includes mentoring committees, which meet annually. Each junior faculty member chooses their own mentoring committee. In addition to annual meetings, junior faculty can meet informally with members of their committee as necessary. All the women junior faculty have benefitted from generous mentoring from the senior women in our faculty; men and women faculty have specifically mentioned that Dr. Rachel Klevit has been particularly generous with her time and advice.

Section II: Teaching & Learning

Student Learning Goals and Outcomes

Ph.D. in Biochemistry

Learning Goals

1. Display mastery in a specific area in the broad field of Biochemistry
2. Display mastery of research methodology including:
    a. Define a specific problem
    b. Test hypotheses
    c. Design and perform well-controlled experiments
    d. Critically analyze data
3. Present results effectively in several formats, including poster, short talk, long talk and at least one manuscript.

Curriculum

First year students take 4-5 courses each quarter, which includes a laboratory rotation each quarter. Some of these are core courses, others cover special topics. The choice of elective courses depends on their interests, past experience, and previous coursework. A sample syllabus for the Advanced Biochemistry course is given in Appendix G.

Sample Class Schedule for 1st year studies

Autumn	Winter	Spring	Summer
BIOC 520 Seminar (1 credit)	BIOC 520 Seminar (1 credit)	BIOC 520 Seminar (1 credit)	BIOC 600 Laboratory Research (2 credits)
BIOC 540 Literature Review (2 credits)	BIOC 541 Literature Review (2 credits)	BIOC 542 Literature Review (2 credits)	Biomedical Research Integrity Lectures – 3 required
BIOC 581 Laboratory Rotation (4 credits)	BIOC 581 Laboratory Rotation (4 credits)	BIOC 581 Laboratory Rotation (4 credits)	
BIOC 530 Advanced Biochemistry (3 credits)	Elective Class	Elective Class	
BIOC 533 Grant Writing (1 credit)	Elective Class	Elective Class	
Student Seminar – Weekly	Student Seminar – Weekly	Student Seminar – Weekly	
	BIOC 533 Ethics 101 (1 credit)		

Second year students participate for two quarters in BIOC650, the teaching clerkship course for 6 credits. BIOC650 instructs students on how to teach effectively. Discussions in BIOC650 are moderated by faculty who teach the undergraduate courses and therefore are familiar with the challenges posed by, for example, teaching large classes or teaching lab classes. This course also provides students with experience teaching and evaluating undergraduates. This takes place either in small discussion groups associated with a large undergraduate lecture course (BIOC 440, 441 or 442) or the honors biochemistry course (BIOC 450 and 451), or in an undergraduate biochemistry laboratory class (BIOC 426). A sample syllabus for the teaching clerkship is given in Appendix G.

In subsequent years, students rarely take courses and instead perform their thesis research under the guidance of their advisor and thesis committee.

Evaluation

Our graduate students are evaluated in several ways at several stages of their careers.

These are the minimal requirements to continue to remain in good academic standing

- Maintain a cumulative 3.0 GPA
- Hold the first committee meeting by the Winter Quarter of the second year
- Secure a lab placement for thesis research by the end of Spring Quarter in the first year
- Complete the General Exam during the Autumn Quarter of the third year
- Conduct annual committee meetings

- Miss no more than 3 Graduate Student Seminars per quarter

During their first year, our students are evaluated in their Biochemistry and Literature Review courses by the quality of their short, written assignments, and/or oral presentations. They also complete three research rotations and are evaluated by their ability to think through a research problem, their willingness to learn, their effort, and to a small degree, the results produced.

In their second year, our graduate students are evaluated in their teaching clerkship by their completion of the course activities. (See sample syllabus in Appendix G).

In their third year, our graduate students are evaluated by a general exam where they present and defend their thesis proposal and answer questions previously provided by the thesis committee. The following steps are required:

1. Two weeks before the exam, the Thesis Committee should receive a detailed thesis proposal that has been reviewed and approved by their thesis advisor (8 to 10 single-spaced pages excluding figures and tables). The Thesis Proposal normally includes an abstract, background, objectives, key experiments and techniques, anticipated problems, alternative approaches, significance, and future directions.
2. One week before the General Exam, the Thesis Committee should either accept the initial proposal as a basis for the oral examination, request modest modification, or require major modification followed by resubmission. This should be done by email from each member of the committee to the student and cc'd to the GPC.

The format for the General Examinations is as follows:

1. 20-30 minute presentation of the research proposal to the thesis committee (with minimal interruptions).
2. 30-45 minutes of questions from the committee about the thesis proposal.
3. 30-45 minutes of questions from the committee about the specific biochemistry topics proposed by the committee members. These questions should be discussed in the committee meeting that occurs in the second year and proposed shortly thereafter.

Finally, our students are evaluated by their dissertation, which demonstrates their ability to conduct original research that advances a field of biochemistry and to have met the learning goals. The written thesis is evaluated by a reading committee of three faculty members. The graduate student then defends the thesis in a public hour-long presentation and answers questions from the audience followed by questions from the thesis committee.

<u>M.S. in Biochemistry</u>

Learning Goals

1. Present a thorough understanding of a specific area in the broad field of Biochemistry

2. Present a thorough understanding of research methodology including:
    a. Define a specific problem
    b. Test hypotheses
    c. Design and perform well controlled experiments
    d. Critically analyze data
3. Present results effectively to their committee and in a written Master's thesis.

Evaluation

Evaluation is the same as for Ph.D. student during the first year. At that point a student who has completed at least 36 credits and has performed significant rotation or thesis research, but chooses not to continue toward the Ph.D., may be eligible for the M.S. degree. Research required for the M.S. degree need not be conclusive, or publishable; however, a formal M.S. thesis must be written in consultation with the thesis advisor and Thesis Committee and a formal thesis presentation must be made to the Thesis Committee as required by the Graduate School's Summary of Requirements. M.S. candidates may also request to present their thesis work in a seminar open to the entire department.

<u>M.A. in Biochemistry</u>

Learning Goals

1. Present an understanding of a specific area in the broad field of Biochemistry
2. Present an understanding of research methodology including:
    a. Test hypotheses
    b. Design a well controlled experiment
    c. Critically analyze data.

Evaluation

Evaluation is the same as for Ph.D. student during the first year. At that point a student who has completed at least 36 credits and has performed significant rotation, but chooses not to continue toward the Ph.D., may be eligible for the M.A. degree.

Methods to Assess Student Satisfaction

We gather formal student evaluations for our biochemistry course and our literature review course. Ms. Kirschner (Associate Director of Graduate Programs) meets regularly with students during their first two years to check in and assess their satisfaction and try to proactively discover problems. Jim Hurley, the Graduate Program Coordinator and Andrea Wills, the Assistant Graduate Program Coordinator is also available for student consultation, as is the chair. After the general exam in the third year, feedback is student directed. Students have a meeting of their thesis committee each year and can provide feedback there or meet with Ms. Kirschner, Dr. Hurley, Dr. Wills, or the chair upon request.

<u>CORGE survey</u>

In 2019, the Council on Research and Graduate Education performed a climate survey. The anonymous survey was conducted throughout the School of Medicine, with responses from 122 (21%) graduate students and 49 (14%) postdoctoral fellows responded. We have no specific results for Biochemistry, nor do we know how many

Biochemistry students or postdocs responded. However, 60% of the women reported experiencing some type of harassment.

Graduate student survey

In February and March 2020, we gathered two surveys from graduate students in our department. One was not anonymous, and we received immediate feedback that a survey should be anonymous. We asked the graduate student president, Sam Witus, to gather an anonymous survey (since the common online survey methods reveal the participant) and provide us with the information.

Seventeen of our 31 Biochemistry students replied to the survey that was not anonymous. Three of the 6 URMs replied.

Eight of the 87 students in Biochemistry labs (including students from Biochemistry, BPSD, Molecular and Cellular Biology, and Neuroscience) replied to the anonymous survey.

Response to feedback

Response to CORGE survey

In response to the results of the CORGE survey in 2019, the Office of Research and Graduate Education began a Faculty Allies program. This program trains faculty from each department to act as allies for graduate students and postdoctoral fellows. A description of the program:

> "The UW Allies program serves graduate students and postdoctoral scholars in basic science labs at UW. Allies are faculty volunteers who provide trainees with support during conflict with mentors and lab mates, assistance in navigating institutional barriers to success, and connection with institutional and community services. Allies stand in solidarity with trainees, so they don't have to face challenges alone."

Four of our faculty members serve as Allies.

Specific Responses to Graduate Student Feedback Through the Years

In 2017, we received feedback that the paid parental leave for graduate students of seven days provided by the union contract was insufficient. A committee of faculty worked with the Associate Director to gather information about leave policies at the UW and those provided by training grants. Based on this research, the Parental Leave Committee recommended that the Department of Biochemistry provide 8 weeks of paid parental leave. The faculty approved this policy and it is now in place and has been used.

We have also received feedback that we did not properly prepare students for their teaching experience. We received this feedback several times over the last 10 years including in the latest anonymous survey mentioned above. As our first response to this feedback (7 years ago), the Associate Director organized a forum where graduate students who had success in their teaching experience could present a workshop for students who were about to begin their teaching experience. This workshop has always been well received but was not enough. In 2019, we developed a new teaching clerkship course. (See sample syllabus in Appendix G.)

Our grant writing course was revamped based on feedback from students to include a more extensive peer review process. We also added another grant writing course based on student feedback. Our recent survey revealed that students value the literature review course. One student gave rave reviews to the Biostatistics course. Most students and most faculty did not want additional courses in the 2020 survey. However, in 2022, students suggested that we expand the list of accepted electives to include additional courses in data science. We await a formal proposal from the students. Other students stated that they would like additional courses in their area of developmental biology. These courses have historically been provided by other programs in the School of Medicine including the Molecular and Cellular Biology Program.

In 2020, the students strongly encouraged that the Diversity, Equity and Inclusion Committee be revamped and include students, and we did so. In 2020, the students proposed to develop a student buddy system. We approved and the entire system is run by students (described above).

Instructional Effectiveness

Evaluation

We obtain formal student evaluations of the faculty who teach our core curriculum graduate classes.

Junior faculty have three faculty members to provide mentorship on teaching and other matters. A formal one-day course provided by the Molecular Cell Biology program in 2020 on mentoring and training graduate students was well received.

Note that, although not part of this review, all junior faculty teaching undergraduate courses are evaluated and receive suggestions on their undergraduate teaching by our Peer Evaluation Teaching Committee on the following schedule: Assistant Professors, every year and Associate Professors, every other year. Faculty teaching undergraduate courses also receive mentorship on teaching from the chair and from senior faculty teaching in the same courses. Several faculty members have received valuable guidance from the Center for Teaching and Learning.

Teaching and Mentoring Outside the Classroom

A major component of our day-in and day-out teaching is done in our labs as mentors and thesis advisors to our graduate students and as members of their thesis committees. The majority of regular faculty (12/20) are a thesis advisor for at least one graduate student (Appendix H).

Adjunct faculty have participated in our graduate program since 2015. They bring diverse scientific interests, some more clinical and others more chemical than our faculty. In the first two years, 3 students joined Adjunct faculty labs, two have graduated and one is nearing graduation. When we expanded our graduate class in 2020, many students rotated in Adjunct faculty labs and four chose to join one in 2020 (Appendix H).

Ensuring steady progress

The time to graduation in our department had been a steady 6.25 years for decades. In 2012, the department hired Ms. Kirschner and she instituted organizational principles to

be sure the students met each program requirement on time (e.g. general exam in Fall Quarter of the 3rd year, committee meeting every year). Ms. Kirschner actively advises students and helps in problem solving. She checks in with the junior students regularly. Even these brief meetings help students maintain steady academic progress early in their career by helping students overcome hurdles before they become roadblocks. Due to these efforts, in the last five years, our time to graduation has dropped to 5.3 years, nearly a year shorter than previously.

Preparing for the next phase

Thesis advisors are a critical piece in helping students plan their next steps. Training grants require preparation of an Individual Development Plan to be discussed with the advisor and are a good way to start the conversation early in the career of our students. The department has recommended individual development plans for all students for several years. On several occasions, the department has brought in alumni to speak to students about their careers outside of academia. Since 2000, the Bioscience Careers Committee (made up of students) has organized a seminar series where Ph.D.'s who have not gone into academia give a seminar and interact with students to talk about their careers. This series is funded by the Graduate School, interdisciplinary graduate programs and the Basic Science departments in the School of Medicine. Example of talks given:

> "Always carry a notebook: My career in science writing and media relations" by James Urton, Ph.D. from UW News.
>
> "Storyteller, Writer and Scientist" by Adam Ruben, a writer, comedian and molecular biologist.
>
> "A mission-driven career: Solving national security challenges in biodefense" by Rachel Bartholomew, a Senior Research Scientist and Technical Team Lead in Global Security, Technology and policy Group at Pacific Northwest National Laboratory.

We encourage students to take advantage of the resources provided by the UW including The Career and Internship Center and CoMotion, the technology transfer office that also provides internships and trainings on innovation and commercialization. Three of our students have done internships in Industry in the last 6 years.

The Pandemic

Although we were asked not to focus on the pandemic, this self-study would not be complete without at least one section about our pandemic experience and its impact on our department and graduate program. The State of Washington was somewhat unusual in that Governor Inslee declared all biomedical researchers as essential workers very early in the pandemic, early April 2020. The Basic Science Chairs developed safety protocols and let students, staff scientists, postdocs and faculty come back into the labs, although at first with only one person per 600 square feet (essentially one person per lab room), By summer 2020 almost everyone could be back in the lab if they chose to do so. Research was not back to normal by any means, but labs stayed open, mice colonies were kept alive, and people learned to analyze data and write at home and only come to lab to do experiments. Group meetings and journal clubs were

done on zoom. Because we had to stay 6 feet apart with masks on, human interactions were certainly not normal. And, of course, if you had children or elderly parents to care for, it was very tough, childcares were closed or rarely open, nursing homes not a place you wanted your elders to be.

I don't want to diminish the suffering, but work did go on, people did research, wrote grants, and wrote papers. Even excluding the 40 papers published about SARS-CoV-2 in 2021, 111 papers were published from our department that year. Fourteen students graduated during the pandemic, which is the same number that graduated the 2 years before the pandemic.

In addition to the negative impacts of the childcare crisis and homeschooling on parents in our department, the other group that has suffered is the cohort of students that entered in September 2020. Although labs were open, people spent as little time in lab as needed to do an experiment and then worked from home. There was little socializing or just sitting around chatting. Many of the rotation projects had a large remote component. All classes were remote. The 2019 cohort was settled in a lab when the pandemic began and seemed to have weathered the pandemic well. The 2021 cohort who benefitted from more normal lab operations and in person classes also seem to be progressing well. However, the students in the 2020 cohort have suffered. Three out nine were unhappy in their first lab. All three had chosen an adjunct faculty member as their thesis advisor. It seems that the combination of remote learning and doing research in a lab that was not tightly linked to our department was an unfortunate combination. The Department has helped two of those students transition to new labs for a fourth rotation, and the third student has chosen to receive a Master's degree. We have provided support both financially and otherwise through this process.

Section III: Scholarly Impact

Overview

In the last twelve years, our departmental faculty has undergone a major renewal. In 2009, among our faculty, there were 16 professors, 4 associate professors and 1 assistant professor for a total of 21 faculty members. Since then, eight faculty have retired, one tenured faculty member and three other faculty members have joined other institutions or departments. Since 2009, we have hired 11 junior faculty and now have 8 professors, 7 associate professors and 5 assistant professors among the regular faculty for a total of 20 faculty members. One of the assistant professors will be promoted to Associate Professor with tenure on July 1, 2022. We also have two senior joint professors (one will retire June 30th, 2022) and one research associate professor. In twelve years, we have moved from a median of 21 years of service at the UW in 2010 to a median of 12 years in 2022, which is evidence of a rejuvenation of the department. We have 13 adjunct faculty. Our current faculty are listed in Appendix I, and their biosketches are given in the last appendix Appendix K.

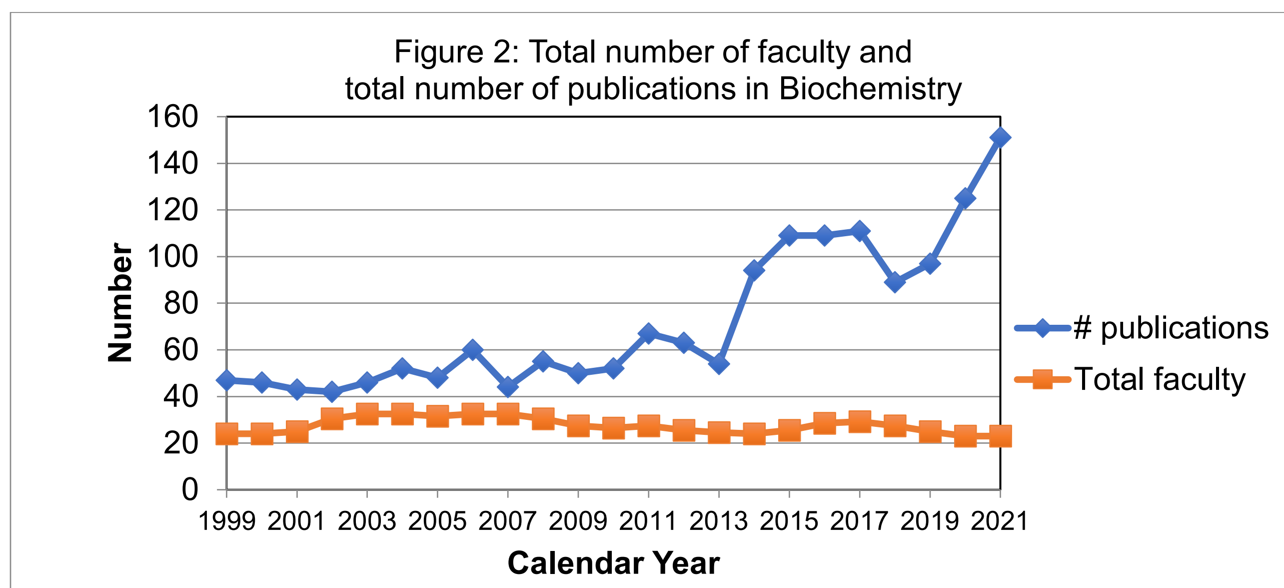
There are many positive implications of this shift towards a more balanced faculty. The new faculty bring new ideas, new science, new methods, and energy. Since many of the faculty who retired were minimally funded, the new faculty bring new funding as shown by our increase in total funding mentioned above. The challenge has been the burden on the current senior faculty, who have been carrying a heavy administrative and

mentoring load along with their normal teaching and research responsibilities. They deserve a huge thanks from all of us.

For over 30 years, our department has had diverse scientific interests from structural biology to molecular mechanisms of development, from femtosecond reactions in photosynthesis to signaling in the retina. In the last twelve years, we have hired faculty in three broad areas: protein design (3 new faculty), structural biology (2 new faculty), and molecular mechanism (6 new faculty). The last faculty member we hired was Thelma Escobar, who filled a joint position with the Institute for Stem Cell and Regenerative Medicine. Brief descriptions of the current scientific interests of the regular and adjunct faculty are given in Appendix I. We currently have 4 faculty working in aspects of protein design, 4 faculty in structural biology with interests ranging from metabolic polymers to ubiquitination to coronaviruses to drug design, and 12 faculty in molecular mechanism with the following interests represented: neuronal signaling, stress response, photoreceptor signaling, degeneration and energy metabolism, mitochondrial diseases, development, regeneration, mitosis, membrane trafficking, and cachexia. This diversity of scientific interests is seen as a strength of our program by our graduate students as judged from our 2020 survey where 11/17 of the students mentioned it as an advantage.

Scholarly Impact

Scholarly impact can be measured in multiple ways, none of them perfect. The first is by number of publications. The number of publications from the department is increasing (Figure 2). The large increase in 2014 from an average of 70 publications per year to 100 per year was fueled by increased productivity from a large group of our faculty. The most recent increase was fueled by research on coronavirus.



A second measure of scholarly impact is the honors and awards received by our faculty. These are listed on pages 5-9 of Appendix I. We have a number of distinguished faculty among our ranks including three members of the National Academy and 3 HHMI Investigators.

A third measure of scholarly impact is the **h**-index. Suggested in 2005 by Jorge Hirsch, the **h**-index is defined as the maximum value of **h** such that the given author has published **h** papers that have each been cited at least **h** times. Richard Palmiter, who is also the longest standing HHMI Investigator in the world, has the highest **h**-index of 154 with the second highest number of publications (510). David Baker, our second HHMI Investigator, is a close second with an **h**-index of 140 and 597 publications. Among our senior faculty, the median **h**-index is 51 with a median number of publications of 151.

Graduate student awards and impact

Lexi Walls was awarded the highly competitive Hal Weintraub award in 2019. Jennifer Bocanegra, Andrew Muenks and Rachel Flores were awarded NSF fellowships. Celia Bisbach, Anika Burrell, and Hannah Arbach were awarded F31 fellowships from NIH, Arianne Caudal was awarded an American Heart Association Fellowship, and Asis Hussein was awarded an ISCRM fellowship. Other recent awards include Best Poster at the International Xenopus Conference to Anneke Kakebeen and first place at the Minority Affairs Poster Competition at the American Society for Cell Biologists to Rachel Flores. Anika Burrell and Ha Dang both received student scholar awards from the Microscopy Society of America for outstanding papers. Unfortunately, we do not have a record of every award received over the last 10 years.

Our students impact their fields by performing cutting edge research and disseminating their results through publications. Our 34 doctoral students who graduated in the last 5 years have published over 100 papers with a median of 2.5 papers per student and 1 first author paper per student. We strongly encourage students to have at least one submitted first-author manuscript before graduation. Thirty-two of the 34 graduate students published a first-author paper and all were authors on at least one publication.

Our students also disseminate their research findings by poster and oral presentations at scientific meetings. Travel to meetings is funded by the Schultz Fellowship in our department (two meetings per graduate career), by training grants or by their advisor’s grants.

Our students are a community of active and engaged citizens. In the survey completed in 2020, 11 of the 17 respondents mentioned outreach activities such as: Pacific Science Center Science Communication Fellow, participating in Science at the Market (present science to shoppers at local farmers’ markets), volunteer at “Girls in Science” at Burke Museum, science judge at Bio-Expo science fairs, public lecturer at Town Hall Seattle, volunteer at ROOTS (Rising Out Of The Shadows Young Adult Shelter), volunteer at GEMS (Girls in Engineering, Math, and Science), member of ENGAGE board of directors (to promote science communication in the community), volunteer at SACNAS and ABRCAMS, and tutors at CLUE (UW’s free multidisciplinary study center).

Postdoctoral fellows

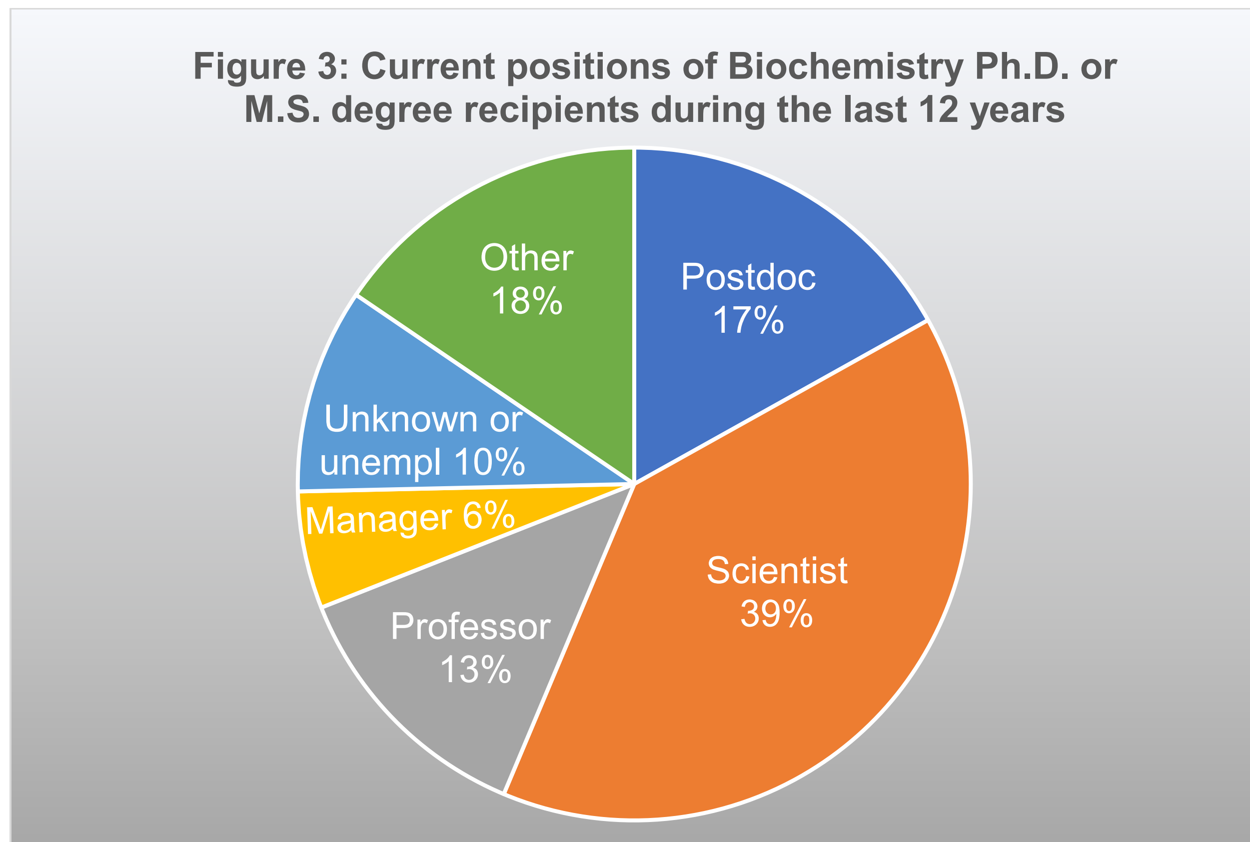
Postdoctoral fellows make up a quarter of our research staff (see Appendix C) and play an important role in performing research and in training graduate students, undergraduates, and junior postdoctoral scholars. A few postdoctoral fellows participate in active-learning exercises in our didactic graduate courses. This review has not been

focused on training of postdoctoral fellows, even though that is an important topic. Briefly, our postdoctoral fellows receive training from their mentors, senior scientists in the lab and senior postdoctoral fellows. They can also receive valuable training at the School of Medicine Future Faculty Fellows two-day workshop and then participate in apprenticeship teaching.

Postdoctoral fellows in the IPD can receive training to become Translational Investigators to develop their discoveries into products to be licensed and commercialized in their own startup companies.

Impact of program graduates

Of the 71 graduate students who graduated in the last 12 years with a Ph.D. or M.S. degree, 90% are postdoctoral fellows or have full time jobs as shown in Figure 3. The current positions of students are listed in Appendix A; all who are working are either postdoctoral fellows or working in careers that utilize their degree.



Influences of advances and changes in the field

In the rapidly moving field of science, change is a constant. Our senior faculty have responded to their swiftly moving fields by changing their lines of research as fields have saturated and new approaches become possible. The existing diversity of interests and the intellectual caliber of the faculty provide an environment that makes these kinds of shifts not only possible but rewarding. Examples include moves from metallothionein to neuronal signaling, from signaling in photoreceptors to energy metabolism, from calcium signaling to ubiquitination and intrinsically disordered

proteins, from calcium signaling to the mitotic cytoskeleton, from Wnt signaling in the mesoderm in Xenopus to neuromesodermal progenitors in Zebrafish, from protein structure prediction to protein design, from Drosophila development to stem cell metabolism, and from membrane trafficking to pathogenic bacteria. We have adopted new technologies and contributed to their development including transgenic animals, optogenetics, cross-linking mass spectrometry, NMR, hydrogen exchange, metabolic mass spectrometry, optical trapping, vastly improved computational algorithms and induced pluripotent stem cells. Probably the best examples of how fast our faculty can shift research focus are the 68 papers published by our department on SARS-CoV-2 in the last two years.

No chair or leader knows where the next great breakthrough will be, what knowledge will be needed to help humanity. Twenty years ago, few could have imagined that a small group in China studying viruses in bats would lay the foundation for understanding the current pandemic. Instead, we need a curious faculty striving to do the best science possible with the most modern tools, a faculty who is nimble enough to change fields or technologies, and passionate enough to follow their instincts to the next great discovery. By assembling and supporting a curious, nimble, passionate faculty, we ensure that our students and postdoctoral fellows will receive the training they need to make the best contributions to science possible.

Challenges of funding

Challenges in funding both educational activities and research activities impact scholarly impact, the topic of this section.

<u>Funding educational activities</u>

A main challenge for the department is funding our educational activities. Our tenured and tenure-track faculty members' educational activities are funded by their tenure lines, but we have substantial other educational costs including funding graduate and undergraduate advisors, lecturers, faculty without tenure ("WOT"), and undergraduate lab equipment and supplies. In FY22, we will spend $395K on these other educational expenses and will receive $117K in funding for that purpose. That is a mismatch, and we fund the excess from scarce departmental funds which negatively impacts our ability to hire tenure-track faculty. To make matters worse, the funding provided for teaching will be reduced to $75 K over the next two years as the Provost reclaims our teaching funds, while expenses increase. If the department were provided funds specifically for teaching based on our teaching load of 800 undergraduate students per quarter, then we could increase our scholarly impact in research and in training graduate students by hiring additional faculty with funds currently being used to fund these other educational activities.

<u>Funding research activities</u>

Most departmental research funding (except for the IPD) is from the NIH and all but one of our faculty are so funded. The NIH has been going through funding boom and bust cycles for as long as I have been a faculty member. A study done a few years ago showed that the lowest retention rate for first time renewals was the cohort who received their first grant in 1989 with more junior faculty in that cohort losing their grants than the 1997 or 2003 cohort (also bad times)

(https://nexus.od.nih.gov/all/2014/10/28/retention-of-first-time-r01-awardees/). Nearly all the senior faculty members in my department have experienced at least a temporary loss of a major NIH grant once in their career. On the flip side, several faculty members have kept R01's funded for decades (one faculty member for 37 years). Fortunately, we have bridge funding available from the Provost, which requires matching funds from the Department. About $100,000 (Provost plus department) is available, which is enough to keep one key staff member. This bridge funding has proven invaluable and allowed the labs to restore their funding and get back to doing great science.

However, the bridge funding is not enough to continue paying the faculty member's full salary. Many faculty have had to give up a portion of their salary during the loss of one of their grants. (As stated above, on average, faculty receive 60% of their salary from their tenure line.) The few faculty on soft money are even more vulnerable. This is obviously unacceptable, but with tight departmental budgets, we have had few options. It should be noted that we guarantee funding to all Biochemistry graduate students in good academic standing, and if necessary, the department pays the students, so no student goes without funding even if a lab has lost its funding.

All except one of the regular faculty are now funded, most by NIH R01 or R35 funding mechanisms. One challenge is that the modular budgets have not increased in years and at least NIGMS has been cutting first time R01 grant recipients by 24% (so that no specific aims can be changed) to $197,000/year. The exception is early Investigator R35 awards from NIGMS, which provide $250,000/year. These have provided valuable funding to two junior faculty members. The challenge is that a faculty member with an R35 from NIGMS cannot receive a second grant from NIGMS. Funding a lab long term on one grant is stress-inducing.

While the amount of dollars/grant is not increasing, cost of a graduate student (RA-ship plus tuition) has been increasing by ~5% each year. The annual salary of a postdoctoral fellow is scheduled to increase 6% per year over the next 6 years because of the Washington State Minimum Wage Act. To make matters worse, the number of training grant slots have been cut, and we have no funds to pay teaching assistantships. This has put many of the junior faculty in a bind of how to afford to pay the graduate students, who are so important to their research and whom they are deeply committed to training. One solution is that they write additional grants. Alternatively, the students must seek their own funding. As mentioned above, we are beginning to train students to write F31 individual fellowship grants and have had some success from institutes except NIGMS. The second priority as we work to increase our departmental revenue is to be able to provide support to graduate students who are participating in the teaching clerkship.

Collaboration

The faculty at the University of Washington are remarkably collaborative. Every member of the Biochemistry faculty is collaborating with other faculty, most with two or more groups both locally and globally. In most cases, the collaboration is interdisciplinary and brings together expertise from different fields. Of the 98 publications from our departmental faculty in 2019, 75% were collaborations with at least one other faculty member, half outside of the Seattle area.

The positive impacts are manifold. Bringing together different types of expertise allows us to consider and answer biological questions with a wider view and a bigger toolkit. The pandemic has shown us how important it is not only for our science but also for well-being to be actively engaged with the broader scientific community. It is in our Mission Statement.

The Basic Science graduate programs, the Office for Graduate and Research Education in the School of Medicine, and the Interdisciplinary programs in the Graduate School also work together on issues of graduate education, including diversity and inclusion, compensation, training, curriculum, and well-being of our students.

Promotion and Tenure

The departmental promotion and tenure policies are given in Appendix J. These are kept current and were revised in the last five years to add criteria for professionalism, diversity and equity. The departmental Advancements and Promotions Committee developed the language for the changes, which was then adopted by a vote of the faculty.

Expectations for junior faculty are shared in several ways. All documents are available on the departmental shared drive with links from the departmental website. The timeline for promotion to tenure is presented to each faculty member every year at their annual meeting with the chair. The expectations for tenure are provided as is an evaluation of how each faculty member is progressing on the timeline. Areas of good progress and areas for improvement are noted. All of this is put in writing in the letter summarizing the annual meeting. The promotion process is also reviewed by the chair with greater and greater detail as the faculty member approaches the tenure decision.

The tenure decision is an “up or out” decision, begins in the 5th year of appointment, and takes 1.5 years for the full process. Tenure clock extensions are given for increased family care responsibilities, serious illness or because of loss productivity during the pandemic (up to two years).

Mentoring

The role of the mentoring committee is to mentor a beginning faculty member in all aspects of his/her professional development. In consultation with the chair, a junior faculty member selects at least 3 senior faculty members to serve formally as a mentoring committee. The Chair will ask the members to serve. A junior faculty member should meet at least annually with all 3 committee members, preferably as a group; additional informal meetings are encouraged whenever useful or necessary. The mentoring committees are encouraged to provide mentees with concise written summaries of the annual meetings. These meetings and any written summaries are confidential; however, the mentoring committee may advise the Chair on how the department may be helping or hindering a faculty member’s progress.

In addition to formal mentoring, the School of Medicine is setting up peer-to-peer support groups. Currently these are intended for faculty in the clinical departments but will be expanded to the Basic Science departments in the future.

Section IV: Future Directions

As I sit here on the tail end of a pandemic of COVID-19, I believe that the field of biochemistry is more important than ever. Right now, in my department, three labs are working directly on SARS-CoV-2, vaccines, diagnostics or therapeutics. We know as much as we do about this virus and this disease because of years of research from many fronts, but certainly from biochemical research exploring the structure and function of related viral proteins. In turn that research depended on years of prior research, much of it not focused on viruses, but focused on technology, protein function, protein structure, computation, and molecular cell biology. Development of the mRNA vaccines occurred quickly only because of 60 years of prior research starting with the discovery of mRNA.

We don't know what discoveries now will enable our future responses to crises in human health. Instead, we need to continue our work to build and support a cadre of biochemists doing their best work and following their best instincts with all their energy.

Nine years ago, when I applied to be Chair of Biochemistry, I presented the following vision to the Dean:

"Imagine a world where you walk into your doctor's office with a genetic illness, your genome is sequenced, causative mutations are found, and a new drug is designed to treat you personally. Fanciful? Perhaps, yet on a limited scale this is already happening.

Genome sequences are becoming cheaper and easier to assemble and interpret, with many advances being made here in our Department of Genome Sciences. But what holds us back is a broader understanding of the variation we find in each individual's genome. There are a few thousand mutations currently identified that predict the probability of an illness. Yet there are 2×10^7 base pairs that encode proteins. Even when one can predict the probability of disease, a molecular understanding of the illness may well be lacking entirely. I propose to build a biochemistry department that will enable us to understand the molecular consequences of genetic variation. As we approach the era of the $100 genome, biochemistry is more important than ever to provide molecular descriptions of cellular processes. Through these descriptions will come a deeper understanding of the molecular basis of disease and new avenues for therapies."

If anything, that vision was too narrow, for now we know we must understand not only the products of the human genome but the products of the genome of each new pathogen, of each new organism, even those we do not believe will be pathogens. We must understand molecular mechanism not only of human disease but of biology, so we know how to respond to the next crisis, because we don't know where or how that will originate.

My plan presented to Dean Ramsey nine years ago included six goals for the department:

1. Build the IPD. We have a truly unique opportunity to build an unparalleled, one-of-a-kind institute. The IPD could become a "company factory" and attract the best and the brightest to the UW.

2. Develop a world-class group of faculty in structural biology with expertise in structural analysis working at resolutions from the atomic level to the cellular level.
3. Build a world-class faculty in molecular mechanism of biology and disease.
4. Modernize our teaching approach.
5. Adjust faculty salaries to be appropriate to their level of experience.
6. Increase the diversity in our department at all levels.

Looking at where we are with these goals provides an opportunity to plan for the future. We have achieved the first goal beyond our wildest dreams due to the hard work of many members of the School of Medicine but especially David Baker, with his clear vision and focused determination. We are always working to continue funding this world-class endeavor. Along those lines, a new building for the IPD was just approved by the Regents, a building that will be shared with the Brotman-Baty Institute (a genomics institute in the Department of Genome Sciences).

For the second goal, we hired Justin Kollman and David Veesler, who then raised $8 M to build a state-of-the-art cryo-EM facility, which was used to determine the structure of SARS-CoV-2 spike protein in April 2020. This center also has kept Seattle at the front of the cryo-EM wave and enabled the Fred Hutchinson Cancer Research Center to hire a new structural biology faculty member. Our microscopes include a Krios and a Glacios to provide atomic-level resolution. HHMI has just provided funding for an Aquilos cryo-FIB-SEM to provide cellular-level resolution.

For the third goal, we hired Suzanne Hoppins, Andrea Wills, Young Kwon and Thelma Escobar with research in mitochondrial fusion and fission, regeneration, cachexia and cell dissemination, and epigenetic mechanisms underlying development, all top areas in human disease research.

However, we cannot stop there. I believe we should continue to hire faculty every three years, to avoid a situation like we had 11 years ago when the department sat on the precipice of a faculty decreasing by half in a few years just by retirements. Since it is by joining together all our expertise that we succeed, we should continue with a department of diverse scientific interests and hire in each area, protein design, structural biology, and molecular mechanism. I propose hiring on a cycle so that all three areas maintain a critical mass and momentum.

Our major challenge to execute this plan is to find funds for startup packages for new faculty members. These packages now cost between $1.7 M and $2 M. While I have been chair, funds for hiring faculty came from the School of Medicine, the Provost, IPD funds, and the departmental royalty funds (from Earl Davie's discovery and licensing of Factor IX in blood clotting). Thelma Escobar was a joint hire with ISCRM with nearly half her startup provided by ISCRM. The two new offers being made currently will be funded by the departmental share of equity from the recent sales of IPD startup companies, and the Earl W. Davie/Zymogenetics endowment. The IPD itself has a startup package set aside for an additional faculty member. After that it will be challenging to stay on schedule to hire an additional faculty member every three years without an additional influx of funds. This is the third reason to pursue philanthropy and additional grant funding, and to support additional translational activities. (The other two reasons

mentioned above are the ability to support faculty through brief losses of funding and to fund our teaching clerkships.)

We have had moderate success on the fourth goal, modernizing our teaching methods. We were able to modernize our lab course with the significant funding from the School of Medicine to buy modern equipment. We have hired a lecturer, Michael Holt, to develop and implement additional strategies for improvement. There have been remarkably few resources for modernizing our lecture courses. We have no institutional support for teaching assistants and little to help faculty adopt new methods. Nevertheless, we have made improvements in our lectures and made progress in flipping the classroom. The pandemic and the move to remote teaching accelerated these changes. We have enhanced the training of our graduate students in their teaching clerkship. We plan to continue to develop more active-based learning strategies.

As to the fifth goal to adjust faculty salaries, we retained tenure lines upon retirement and used a portion of those lines to move our faculty to at least the 50th percentile according of AAMC standards for biochemistry faculty. When I started, some faculty were at the 5th percentile. However, this is not a sustainable strategy and because recent raises have only been 2%, we are at a serious risk of falling behind again. Our goal is to keep our faculty salaries at or above the 50th percentile.

The sixth goal is to increase the diversity of department at all levels. In 2020, we completely revamped our Diversity Equity and Inclusion Committee and included students and postdocs on the committee. Since then, the committee has instituted a training for the whole department, revamped our website by adding a new Diversity section and added content to highlight the diverse backgrounds of our trainees and faculty. They are currently developing a strategic plan. As described above, we are finishing another junior faculty search. In this search, we implemented some best practices for hiring diverse faculty, including training of the search committee, focusing on diversity statements provided by applicants, and using high-quality rubrics to evaluate the candidates. We have offered tenure-track positions to two highly qualified candidates, a Latina and an Asian-American woman. We are currently negotiating with both candidates.

In summary, we have made progress on each of the goals I set nine years ago, and they provide good guidance for our continued progress towards ensuring a diverse, vibrant and active faculty engaged in their research and teaching, and a diverse successful student body working together to contribute to the betterment of society and the health of the public.

Acknowledgments

I would like to thank Erin Kirschner for working with me on this report (starting summer 2019), in organizing, gathering data and information, writing and editing, Christina Larmore for additional help with the data and for gathering the data about the current positions of our students, Tonya Hirtzel for gathering and assembling information from the faculty, Zulfiya Lafi for information and editing, Dana Miller, Suzanne Hoppins and Young Kwon for help writing the Diversity section, Eric Muller, Justin Kollman, Rachel

Klevit and Jim Hurley for comments prior to submission, all the faculty for help and prompt replies to my requests and the students for providing feedback.

Part B: Unit-Defined Questions

How might we better prepare graduate students for broader biomedical career paths within the current structure of (NIH)-grant-funded graduate support?

Sixty-five percent of our graduates pursue postdoctoral positions in Academia. Of the twenty-six graduates who started a postdoc more than five years ago:

- seven are assistant professors,
- nine are staff scientists in industry or academia
- six have other jobs in industry
- one is a forensic toxicologist,
- one is homemaker
- one is retired
- one is unknown.

I conclude that graduates are finding jobs in science-related areas. I think the paths from postdoctoral fellow to industry scientist or professor are relatively well understood.

The question above pertains to the other 35% of our students, who are headed towards other types of careers. I do not believe we are educating too many students. The world will not be a better place if we educate fewer people in the rigors of critical thought and problem solving. Our job then is to help our students find the career path that they can follow with passion and energy. Our constraint is that, of the 5.3 years it requires for a Ph.D. in our program, 3.0 years are funded by their thesis advisor's grant, usually an NIH grant. Giving students time for teaching opportunities at area schools or internships in industry can stall the progress of a grant. Note that we do have an internship program in industry, but only three students have taken advantage of that in the last five years. The good news is that the PIs on two training grants at UW believe that NIH is beginning to value non-academic careers in our graduates.

What can we do to help our students? In our survey of graduate students, many felt they had good career support. This is reassuring, but as I watch my own students find their way, I believe we can do more. As mentioned above, the Bioscience Careers Committee (made up of students) has organized a seminar series where Ph.D.'s who have not gone into academia give a seminar and interact with students to talk about their careers. One simple idea is to take advantage of the Bioscience Careers program already in place and connect our graduates with this committee as possible speakers. (For example, our student who became a lawyer would be an interesting speaker.)

Another idea is to invite graduates back to our department to spend a day presenting a talk and interacting with our students. We have done this twice but will do it more often. Other suggestions from the committee are welcomed.

We strive to recruit students with a broader range of life experiences than traditionally found in STEM and not only recruit students with advantaged backgrounds. As a small program, how do we meet the varied educational needs of our entering students?

This question is about how we train all our students whether they had a privileged background or not, whether they have had many advantages or few.

The first area of required expertise is the ability to write scientifically. At present, writing is taught in the first-year grant writing class and by the professors teaching the literature review course. More training for the latter in how to provide useful feedback could improve the effectiveness of these activities. A dedicated writing course that is shared among relevant departments would be a way to leverage faculty resources and experience more effectively.

The second required expertise is how to navigate a lab. All our students arrive with research experience, but it can be as little as 3 months and often quite different from being a graduate student in a lab. The first training in laboratory navigation is implicitly carried out by the first rotation advisor and the senior students and postdocs in the first rotation lab. But, some of these people might not have had formal training in how to train students in the research enterprise and might employ the “sink-or-swim” method. Several students in our survey commented on how they had a rocky start in their rotations because of the lack of experience of their mentor.

One model for training is the Postbaccalaureate Research Education Program (PREP) program, which is an excellent NIH program to provide “support to work as apprentice scientists in a mentor’s laboratory.” The PREP program provides training for students from diverse backgrounds before they start a Ph.D. program. Approximately 40 institutions have PREP grants including the UW. Our graduate program has taken several PREP students and found them well prepared. Ideally such a program could be expanded to help an even a larger group of students. Judging from our experience, it is an effective way to give students that have not had every advantage a head start.

Short of an expansion of the PREP program, our School or University needs a way to train students in general workings of a lab when they arrive. Anecdotally, I have heard of at least one university that does a new graduate student “bootcamp.” For example, a workshop that is organized and led by senior graduate students and, possibly, postdoctoral fellows, for incoming first-year students could be held during the first week of Fall quarter when things in the first rotation are only ramping up. Alternatively, we should provide more mentorship training for our faculty, students and postdocs, but it is not clear what would be most effective. Suggestions from the committee based on their experiences would be appreciated.

Appendix A

Graduate Enrollment Data Biochemistry Graduate Program Academic Years 2010-2021

	Applicants			Entrants				
Academic Year	Total	Domestic	Average GPA	Total	Domestic	Average GPA	Percent URM	Months prior research
21-22	179	138	3.5 (2.5-4)	9	9	3.5 (3.1 - 4.0)	11%	23.3 (9.4 - 60)
20-21	209	154	3.5 (2.6-4)	9	9	3.8 (3.5 - 4.0)	11%	24.5 (5 - 96)
19-20	157	111	3.4 (2.8-4)	4	4	3.5 (3.1 - 4.0)	50%	18.8 (9.4 - 36)
18-19	182	134	3.5 (2.6 – 4.0)	6	5	3.8 (3.6 – 4.0)	17%	13.9 (6-18)
17-18	185	140	3.6 (2.5 – 4.0)	4	3	3.9 (3.7 – 4.0)	0%	16.4 (9.5 – 26)
16-17	170	126	3.6 (2.5 – 4.0)	6	5	3.7 (3.1 – 4.0)	33%	26.4 (7.5 – 38)
15-16	131	94	3.6 (2.5 – 4.0)	10	9	3.7 (3.1 – 3.9)	10%	20.0 (9.5 - 37.5)
14-15	170	119	3.5 (2.3 – 4.0)	5	4	3.7 (3.2 – 4.0)	0%	20.4 (3.0 – 59.0)
13-14	169	120	3.48 (2.7 – 4.0)	9	8	3.59 (3.12 – 3.98)	11%	25.8 (5.6 – 84.0)
12-13	169	120	3.50 (2.6 – 4.0)	9	8	3.42 (3.1 – 3.86)	45%	14.4 (10.0 – 18.0)
11-12	51	45	3.57 (2.98 – 4.0)	3	2	3.55 (3.28 – 3.78)	0%	21.2 (9.7 – 36.0)
10-11	52	47	3.55 (2.9 – 3.99)	4	4	3.70 (3.45 – 3.86)	25%	9.3 (2.3 – 24.0)

Appendix A

Colleges Attended by Entrants to Biochemistry Graduate Program

21-22	20-21	19-20	18-19	17-18	16-17	15-16	14-15	13-14	12-13	11-12	10-11
Harvard U	Cal State U San Bernardino	Inter-Amer U Puerto Rico Hato Rey	Colorado Mesa U	Arizona State U	U Wisconsin-Madison	Mount Holyoke College	U Massachusetts-Amherst	Universidad de los Andes	ESCPE Lyon (France)	UCSD	U Idaho
Rensselaer Polytechnic Inst.	New Mexico State U	Texas A & M Univ	U of Arizona	Cornell U	Pomona College	UCSB	Istanbul Technical U	Boston U	Western WA U	U of WA	UCSD
Union College	Salem College	Texas State U	UCLA	Regis College	UC Berkeley	U of Iowa	Western WA U	U Wisconsin Madison	Bastyr U	U of BC	U Colorado Boulder
UC Berkeley	Scripps College	U of WA	U of Kansas	U of Oregon	Mount Holyoke College	Bates College	Truman State U	Portland St U	U of WA		U of WA
UC Berkeley	Suny at Binghamton		U of Missouri Columbia	U of WA	Western WA U	UCLA	Georgia Tech	U Nevada Reno			
U Mass-Amherst	UCLA		U of WA		California State U – Long Beach	UCSD		Colorado St U			
U of Saint Thomas	Utah State U					California Institute of Technology		UCSB			
U Wisconsin La Crosse	Western WA U					U of New Hampshire		U Penn			
Western WA U	Western WA U					U of WA		U of WA			
						Arizona State U					

Appendix A

	Year Graduated	Degree	Current Position	Current Employer	Lab or Department
1	2010	Ph.D.	Director of Medical Writing Communication	Loxo Oncology	N/A
2	2010	Ph.D.	Assistant Professor of Cell Biology	Roger Williams University (Boston area)	
3	2010	Ph.D.	Basic Life Research Associate Scientist	Stanford University Department of Biology	Judith Frydman Lab
4	2010	Ph.D.	Director of Regulatory Affairs	Loxo Oncology	N/A
5	2010	Ph.D.	Scientist	Pacific Northwest National Laboratory	Chemical and Biological Signature Sciences Group
6	2010	Ph.D.	Retired		
7	2010	Ph.D.	Associate Patent	Global Prior Art	N/A
8	2010	Ph.D.	Research Scientist	University of Utah/HHMI	Erik Jorgensen Lab
9	2010	Ph.D.	Senior Developer / Head of Customer Success	CompilerWorks	N/A
10	2010	Ph.D.	Assistant Professor	U Mass Medical School	Department of Microbiology and Physiological Systems
11	2010	Ph.D.	Senior Computational Scientist	Cyclica (Toronto)	N/A
12	2010	Ph.D.	Senior Counsel, Office of Program Research	Washington State House of Representatives	N/A
13	2010	Ph.D.	Assistant Professor of Biochemistry	Medical College of Wisconsin	Biochemistry
14	2011	Ph.D.	Assistant Professor of Medicine	Duke University	Human Vaccine Institute
15	2011	Ph.D.	Associate Director of Regulatory Affairs	Loxo Oncology	N/A
16	2011	Ph.D.	Assistant Professor of Neurosciences	Northwestern University	Feinberg School of Medicine
17	2011	Ph.D.	Founder	Postdoc Brewing Company	N/A
18	2012	Ph.D.	Staff Scientist	Fred Hutchinson Cancer Research Center	Jim Olson Lab
19	2012	Ph.D.	Assistant Investigator	Allen Institute for Immunology	N/A
20	2012	Ph.D.	Assistant Professor of Molecular Micro and Immunology	OHSU	School of Medicine
21	2013	Ph.D.	Assistant Professor of Chemical Physiology and Biochemistry	OHSU	
22	2013	Ph.D.	No current position found		
23	2013	Ph.D.	Senior Principal Scientific Researcher	Genentech	N/A
24	2014	Ph.D.	Computation Scientist	Science and Technology Facilities Council	
25	2014	Ph.D.	Forensic Toxicologist	Armed Forces Medical Examiner System	N/A
26	2014	Ph.D.	Senior Scientist	STEMCELL Technologies	N/A
27	2014	Ph.D.	Senior Scientist in Oncology Biocscience	AstraZeneca	
28	2015	Ph.D.	No current position found		
29	2015	Ph.D.	Senior Manager of Corporate Development	Seattle Genetics	N/A
30	2016	Ph.D.	Homemaker		
31	2016	Ph.D.	Scientist I	Adaptive Biotechnologies Corp	
32	2016	Ph.D.	Business Development Manager	Roche	N/A
33	2016	Ph.D.	Tenure Track Faculty	South Seattle Community College	Biology Department
34	2017	Ph.D.	Principal Scientist	Draper (Cambridge, MA)	N/A
35	2017	Ph.D.	Postdoctoral Researcher	University of Washington	Smita Yadav Lab (Pharmacology)
36	2017	Ph.D.	Scientific Software Developer	Cyrus Biotechnology	N/A
37	2017	Ph.D.	No current position found		
38	2017	Ph.D.	Scientist	Neoleukin Therapeutics	Leo Stamatatos Lab
39	2018	Ph.D.	Scientist II	Tectonic Therapeutic	
40	2018	Ph.D.	Senior Scientist in Advanced Drug Discovery	AstraZeneca	
41	2018	Ph.D.	Medical Laboratory Technologist	Atlanta Piedmont Hospital	
42	2018	Ph.D.	Senior Machine Learning Scientist	EchoNous (Seattle area)	N/A
43	2018	Ph.D.	Research Scientist	University of Washington	Jay Neitz Lab
44	2018	Ph.D.	Scientist	LegoChem Biosciences	N/A
45	2018	Ph.D.	Translational Investigator	University of Washington	Biochemistry
46	2018	Ph.D.	No current position found		
47	2019	Ph.D.	Head of Electron Microscopy	University of Washington	Institute for Protein Design
48	2019	Ph.D.	Senior Scientist	A Alpha Bio	N/A
49	2019	Ph.D.	Associate Scientist	WuXi App Tech	
50	2019	Ph.D.	Computational Chemist	Encodia	N/A
51	2019	Ph.D.	Postdoctoral Researcher	University of Pennsylvania	Benjamin Prosser Lab
52	2019	Ph.D.	Research Scientist	University of Washington	David Veesler Lab
53	2020	Ph.D.	Postdoctoral Fellow	University of North Carolina	Amy Gladfelter Lab
54	2020	Ph.D.	Postdoctoral Scholar-Fellow	University of Washington	Susan Brockerhoff Lab
55	2020	Ph.D.	Postdoctoral Researcher	University of Colorado Boulder	Lee Niswander Lab
56	2020	Ph.D.	Lecturer	University of Washington	N/A
57	2021	Ph.D.	Postdoctoral Fellow	Fred Hutchinson Cancer Research Center	Daphne Avgousti Lab

58	2021	Ph.D.	Research Scientist	Promega Corporation	N/A
59	2021	Ph.D.	Postdoctoral Fellow	Stanford University	Joseph Wu Lab
60	2021	Ph.D.	Scientist I, Protein Biophysics	Vir Biotechnology, Inc.	N/A
61	2021	Ph.D.	Protein Scientist	Impossible Foods	N/A
62	2021	Ph.D.	Protein Engineer Scientist	Cyrus Biotechnology	N/A
63	2021	Ph.D.	Postdoctoral Scholar	UCSF	Daniel Southworth Lab
64	2021	Ph.D.	Postdoctoral Scholar	University of Washington	Hannele Ruohola-Baker Lab
65	2021	Ph.D.	Postdoctoral Scholar	University of California, San Francisco	Natalia Jura and Kliment Verba Labs
66	2021	Ph.D.	Postdoctoral equivalent	University of Washington	Suzanne Hoppins Lab
67	2021	Ph.D.	Postdoctoral Scholar	University of Washington	Michael MacCoss Lab
68	2010	M.S.	No current position found		
69	2011	M.S.	Manager, Project Engineering	Alvotech	N/A
70	2012	M.S.	Senior Site Reliability Engineer	Cargo Signal	N/A
71	2012	M.S.	Engineering Manager	Google	N/A

Basic Science Departments in the School of Medicine

*Department of Biochemistry

*Department of Bioengineering[1]

*Department of Bioethics and Humanities

Department of Biological Structure

*Department of Biomedical Informatics and Medical Education

Department of Comparative Medicine

*Department of Genome Sciences

*Department of Global Health[2]

*Department of Health Metrics Sciences

*Department of Immunology

*Department of Microbiology

Department of Laboratory Medicine and Pathology

*Department of Pharmacology

Department of Physiology and Biophysics

*Have departmental graduate programs that are actively recruiting

[1]Joint with College of Engineering

[2]Joint with School of Public Health

Interdisciplinary Graduate Programs

*Biological Physics, Structure and Design

Molecular and Cellular Biology

Molecular Medicine and Mechanisms of Disease

Neuroscience

Pathobiology

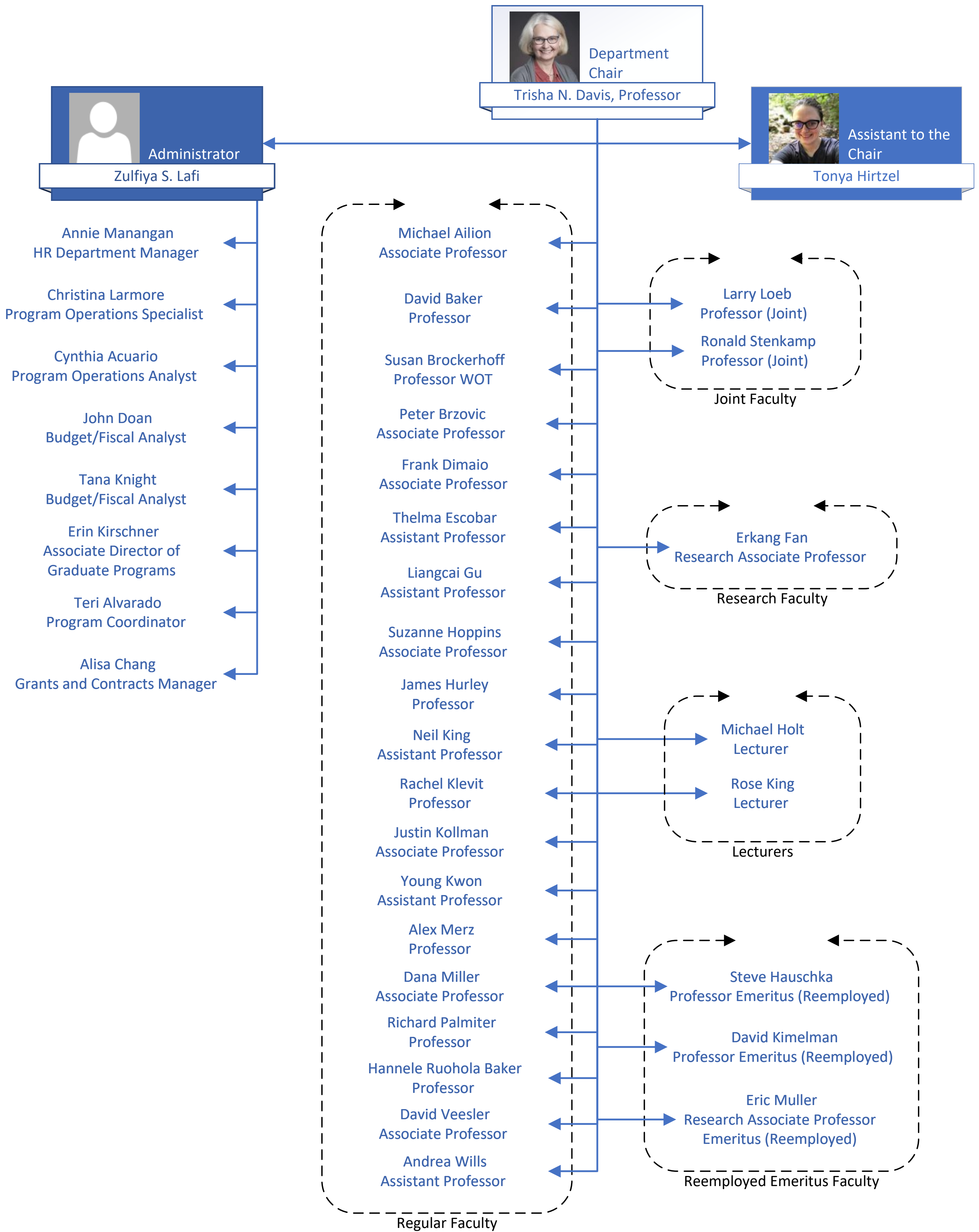
*not degree-granting, students receive degree from the department of their chosen mentor.

Science Departments in College of Arts and Sciences with Relationships with Biochemistry

Department of Biology (Collaborators and joint equipment efforts)

Department of Chemistry (Collaborators, joint equipment efforts, adjunct faculty and runs undergraduate Biochemistry Program)

Appendix C



IPD in The School of Medicine of the University of Washington

Paul Ramsey, M.D.
Dean of the School of Medicine

IPD ADVISORY BOARD

Bryan White
Acting Chair

Clara Wu Tsai MBA
George Church, Ph.D.
Rob Hershberg, M.D. Ph.D.

BIOCHEMISTRY DEPARTMENT

Trisha Davis, Ph.D.
Chair of Department of Biochemistry

David Baker, Ph.D.
Director of IPD
Professor of Biochemistry
HHMI Investigator

Lance Stewart, Ph.D. MBA [3]
Chief Strategy & Operations Officer

IPD ADMIN [2]

IPD CORE LABS [2]

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Development Council
Bruce and Jeannie Nordstrom
Honorary Chairs

SCIENTIFIC COUNCIL

Scientific Council
David Urdal, Ph.D., Chair

Judy Wasserheit, M.D., MPH
Trisha Davis, Ph.D.
Stanley Fields, Ph.D.
William Catterall, Ph.D.
Stanley Riddell, M.D.
Wes Van Voorhis, M.D. Ph.D.
Bob Waterson, M.D. Ph.D.
Mark Wener, M.D.
Julie Overbaugh, Ph.D.

MEMBER LABS [1]

Frank DiMaio, Ph.D.
Neil King, Ph.D.
Gaurav Bhardwaj, Ph.D.
Liangcai Gu, Ph.D.

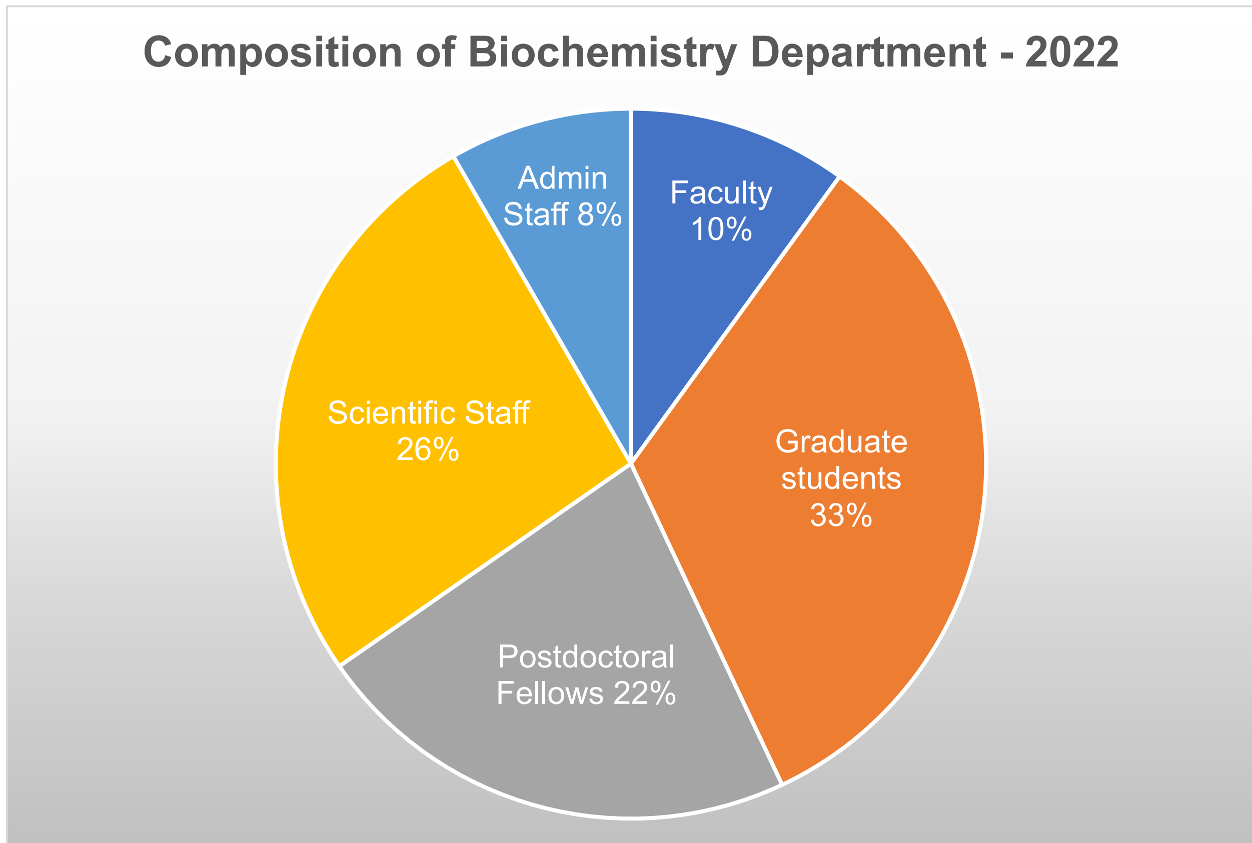
TRANSLATIONAL INVESTIGATORS [1]

Stephanie Berger, Ph.D.
George Ueda, Ph.D.
James Lazarovits, Ph.D.
Tim Craven, Ph.D.
Anindya Roy, Ph.D.

AFFILIATE LABS [1]

Phil Bradley, Ph.D.

[1]All faculty, whether in the Member Labs, the Translational Investigators or in Affiliate Labs have supervisory reports to their Chairs.
Postdoctoral fellows and students have supervisory reports to the faculty member at the head of the lab.
Dashed lines indicate scientific advisory roles of David Baker.
[2] Dual report to David Baker and Lance Stewart.
[3] Dual report to David Baker and Trisha Davis



Biochemistry has 300 total members not including 46 undergraduates working part time in our labs or as reader/graders. Faculty includes all ranks including Acting Instructors and Lecturers.

Appendix D

Department of Biochemistry
Standing Committees 2021-2022

Appointments and Promotions
Klevit, Chair
Merz, Palmiter,
Ruohola-Baker

Undergraduate Education
Hoppins, Chair
Kollman, Ruohola-Baker

Graduate Education
Hurley, Chair
Wills, Assistant Chair
Kwon, DiMaio
Student: no volunteers

Graduate Admissions
Hurley, Chair
(Ailion on sabbatical), Kwon, Veesler, King, DiMaio
Students: Adams and Rutter
Ex officio: Erin Kirschner

NIH Training Grants
CMBTG: Steering Committee: Davis
Vision TG: Steering Committee: Klevit
MBTG: PI, Zheng
Co-PI: Kollman
Selection Committee: Brzovic
Steering Committee: Davis

BPSD Graduate Program
Lee, Co-director
Maly, Co-director

BPSD Graduate Admissions
Gu (DiMaio when Ailion not on sabbatical)

Interdisciplinary Graduate Training
MCB Steering Committee: Hoppins
MSTP Advisory Board: Baker, King

Retreat
Hoppins
Students: Criswell, Pranoto
Ex officio: Erin Kirschner

Teaching Evaluation Committee
Brockerhoff, Chair
Palmiter, (Ailion on sabbatical)

Seminar Committee
Miller, Chair
Kwon, DiMaio, King

Neurath Seminar Selection Committee
Palmiter, Chair
Miller, (Ailion on sabbatical), Wills

Schultz Fellowship Committee
Kollman, Chair
Gu, King, Student: no volunteers

Urdal Fellowship Committee
Brockerhoff, Chair
Hoppins, Student: no volunteers

Equipment Committee
Klevit, Natalie Stone (staff)

Cryo EM Committee
Kollman, Co-Director
Veesler, Co-Director

Diversity
Miller, Hoppins, Co-chairs, Kwon
Students: Muenks, Cardenas
Postdocs: Hass, Levy
Ex officio: Erin Kirschner

Parental Leave Committee
Wills, Chair, Hoppins, Kwon

Faculty Search Committee
Ruohola-Baker, Chair
DiMaio, Hoppins, Kollman

Faculty Senate Representative
Kollman

CORGE
Empty

IGEM coordinator
Gu

Faculty Allies
Hoppins, Merz, Miller, Wills

Medical School A&P
Merz
Brockerhoff

Appendix D

Sign up: First come first serve		
Student Job	Quarter Active	Student Sign Up
StuSem Email Contact	Fall	Grace Hendricks
(1 student/quarter)	Winter	Justin Applegate
	Spring	Morgan Jones
StuSem Lunch setup	Fall	Stephanie Sloat
(2 students/quarter)		Yuxin Pan
	Winter	Jenny Bocanegra
		Chau Vuong
	Spring	Amy Bounds
		Samantha Zepeda
Invited Speaker Lunches	Fall	Dane Zambrano
(1 student/quarter)	Winter	Zhaoqian Wang
	Spring	Megi Rexhepaj
Recruitment Organizers	Fall/Winter	Anika Burrell
(5 students total)		Marisa Brandys
		Alejandra Cabrera
		Audrey O'Neill
		Adam Nguyen
Rotation talk setup	Fall	Sam Witus
(1 student/quarter)	Winter	Annabel Vernon
	Spring	Ilya Veil
Party Czars	Fall	Ilya Veil
(2 students/quarter)		Megi Rexhepaj
	Winter	Carson Adams
		Halli Benasutti
	Spring	Megi Rexhepaj
		Cullen Demakis
Department Retreat Organizers	Summer/Fall	Cameron Criswell
(2 students total)		Inez Pranoto
GPSS Student Senators	N/A	
(2 students total)		
Schultz Fellowship Committee	All	
Urdal Fellowship Committee	All	
Graduate Education Committee	All	

Elected Jobs 2021-22	
Student President	Dane Zambrano
DEI Committee	Andrew Muenks
	Jorge Cardenas
Admissions Committee	Chloe Adams
	Kaitlyn Rutter
Faculty Chalk Talk	Jeet Patel
Faculty Meeting Rep	Meerit Said

Appendix E

	IPD				Department (Non-IPD)				Total Department	
Revenue Source	Carryforward from FY20 ($1000's)	Revenue FY21 ($1000's)	Expenses FY21 ($1000's)	Carryforward at end of FY21 ($1000's)	Carryforward from FY20 ($1000's)	Revenue FY21 ($1000's)	Expenses FY21 ($1000's)	Carryforward end of FY21 ($1000's)	Revenue ($1000's)	Expenses ($1000's)
State funding	$2	$1,056	$1,058	$0	$7	$2,679	$2,687	$0	$3,735	$3,745
WWAMI funds	$0	$0	$0	$0	$19	$64	$83	$0	$64	$83
Indirect costs, departmental share	$0	$0	$0	$0	$140	$1,156	$761	$535	$1,156	$761
Grants and Contracts*		$10,732	$10,732	$0		$9,700	$9,700		$20,432	$20,432
Start up funds reserved for faculty	$1,228	$0	$32	$1,196	$792	$1,088	$294	$1,586	$1,088	$326
Gifts/Endowments/Royalties Restricted	$30,058	$11,956	$13,508	$28,505	$2,214	$808	$668	$2,354	$12,764	$14,176
Gifts/Endowments/Royalties Unrestricted	$0	$56	$4	$52	$604	$485	$4	$1,084	$540	$8
Totals	$31,287	$23,799	$25,333	$29,753	$3,775	$15,980	$14,196	$5,559	$39,779	$39,529

*Grant revenues are recognized to the extent of realized expenses, so there is no carryforward.

Expenses	IPD	Non-IPD Dept	Total Department
Faculty Salary/Benefits	$4,740	$4,379	$8,892
Staff Salary/Benefits	$7,084	$5,933	$9,406
Operations	$4,397	$5,098	$16,484
Totals	$16,220	$15,410	$34,782

Appendix E

Biennium 2013: Biochemistry Non-IPD and IPD funds								
Fund Source	FY14 beginning balance	FY14 Revenue	FY14 Expense	FY14 Total	FY15 Revenue	FY15 Expense	FY15 Total	Grand Total Biennium 2013
ENDOPGIFT	(1,626,689)	(3,738,136)	826,281	(4,538,544)	(4,541,817)	1,241,240	(3,300,577)	(7,839,121)
GRANT/CONT	(7,682,773)	(19,501,083)	11,848,498	(15,335,358)	(27,117,855)	12,833,153	(14,284,702)	(29,620,060)
GOF	0	(2,426,882)	2,316,227	(110,655)	(3,479,609)	3,590,264	110,655	0
WWAMI	0	(62,315)	61,098	(1,217)	(62,809)	64,026	1,217	0
R&T	(58,301)	(10,000)	9,140	(59,161)	(14,378)	14,640	262	(58,899)
ROYALTIES	(4,173,339)	(801,152)	1,075,032	(3,899,459)	(1,334,344)	1,720,949	386,605	(3,512,854)
DOF	0	(433,384)	90,972	(342,412)	0	389,771	389,771	47,359
RCR	0	(2,155,828)	2,010,952	(144,876)	(1,397,438)	1,383,880	(13,558)	(158,434)
Total	(13,541,102)	(29,128,779)	18,238,199	(24,431,683)	(37,948,250)	21,237,923	(16,710,328)	(41,142,010)

Biennium 2015: Biochemistry Non-IPD and IPD funds (includes most of Biennium 2015: IPD on next page)*								
Fund Source	FY16 beginning balance	FY16 Revenue	FY16 Expense	FY16 Total	FY17 Revenue	FY17 Expense	FY17 Total	Grand Total Biennium 2015
ENDOPGIFT	(7,946,541)	(2,687,094)	3,172,935	(7,460,701)	(8,970,226)	4,742,126	(4,228,100)	(11,688,800)
GRANT/CONT	(19,958,883)	(14,864,903)	13,214,340	(21,609,446)	(28,233,976)	15,319,761	(12,914,215)	(34,523,662)
GOF	0	(3,587,598)	3,874,603	287,005	(3,718,459)	3,431,454	(287,005)	0
WWAMI	0	(57,422)	52,235	(5,187)	(59,623)	64,810	5,187	0
R&T	(58,899)	4,378	2,442	(52,079)	(10,000)	498	(9,502)	(61,581)
ROYALTIES	(3,512,854)	(1,285,628)	1,640,608	(3,157,874)	(2,058,338)	1,912,707	(145,632)	(3,303,505)
DOF	0	(242,641)	231,746	(10,895)	0	(1,110)	(1,110)	(12,005)
RCR	0	(1,878,197)	1,799,762	(78,435)	(1,507,057)	1,125,477	(381,580)	(460,015)
Totals	(31,477,177)	(24,599,106)	23,988,669	(32,087,614)	(44,557,679)	26,595,724	(17,961,955)	(50,049,569)

Biennium 2017: Biochemistry Non-IPD funds and and some IPD Grant funds.								
Fund Source	FY18 beginning balance	FY18 Revenue	FY18 Expense	FY18 Total	FY19 Revenue	FY19 Expense	FY19 Total	Grand Total Biennium 2017
ENDOPGIFT	(1,178,720)	(1,648,235)	1,507,585	(1,319,371)	(946,639)	772,277	(174,362)	(1,493,733)
GRANT/CONT	(21,237,105)	(17,035,204)	18,088,432	(20,183,877)	(44,301,946)	17,642,623	(26,659,323)	(46,843,200)
SELFSUST	(9,264)	0	2,524	(6,740)	0	36	36	(6,704)
GOF	0	(2,602,754)	2,572,907	(29,847)	(2,756,646)	2,786,493	29,847	0
WWAMI	0	(60,163)	49,671	(10,492)	(62,164)	72,656	10,492	0
R&T	(61,581)	0	326	(61,256)	0	1,245	1,245	(60,011)
ROYALTIES	(1,929,642)	(866,689)	887,822	(1,908,508)	(29,664)	343,183	313,519	(1,594,989)
DOF	0	(12,005)	8,543	(3,462)	(536,447)	79,460	(456,987)	(460,449)
RCR	0	(1,752,458)	1,454,830	(297,628)	(966,547)	1,071,012	104,465	(193,163)
Totals	(24,416,313)	(23,977,508)	24,572,640	(23,821,181)	(49,600,054)	22,768,985	(26,831,069)	(50,652,249)

Biennium 2019: Biochemistry Non-IPD funds and most IPD grant funds								
Fund Source	FY20 beginning blance	FY20 Revenue	FY20 Expense	FY20 Total	FY21 Revenue	FY21 Expense	FY21 Total	Grand Total Biennium 2019
ENDOPGIFT	(1,651,484)	(758,450)	510,365	(1,899,569)	(777,411)	426,733	(350,678)	(2,250,246)
GRANT/CONT	(46,983,157)	(16,669,744)	18,189,688	(45,463,212)	(21,187,495)	19,075,510	(2,111,985)	(47,575,197)
SELFSUST	(6,704)	0	1,079	(5,626)	0	934	934	(4,692)
GOF	0	(2,737,939)	2,730,492	(7,447)	(2,679,295)	2,686,742	7,447	0
WWAMI	0	(61,695)	42,643	(19,052)	(63,525)	82,577	19,052	(0)
R&T	(60,011)	0	(9,782)	(69,793)	0	4,966	4,966	(64,827)
ROYALTIES	(1,594,989)	(491,855)	203,345	(1,883,499)	(1,607,715)	341,464	(1,266,250)	(3,149,750)
DOF	0	(476,037)	89,056	(386,981)	(31,972)	195,264	163,292	(223,689)
RCR	0	(1,206,746)	941,969	(264,777)	(1,159,312)	800,917	(358,395)	(623,172)
Totals	(50,296,345)	(22,402,466)	22,698,855	(49,999,956)	(27,506,725)	23,615,108	(3,891,617)	(53,891,572)

* In 2016, IPD was given its own org code (sub to Biochemistry) and slowly GOF, gifts and royalties for the IPD were switched to the IPD Org code

Appendix E

Biennium 2015: IPD (Overlaps with Biennium 2015 on previous page)								
Fund source	FY16 beginning balance	FY16 Revenue	FY16 Expense	FY16 total	FY17 Revenue	FY17 Expense	FY17 total	Grand total Biennium 2015
ENDOPGIFT	(6,950,484)	(2,365,309)	2,805,598	(6,510,195)	(6,694,430)	4,212,280	(2,482,150)	(8,992,345)
GRANT/CONT	(13,252,665)	(4,784,126)	6,028,615	(12,008,176)	(448,388)	5,926,876	5,478,488	(6,529,688)
GOF	0	(1,004,431)	1,157,139	152,708	(1,013,774)	861,066	(152,708)	0
ROYALTIES	(850,065)	(758,733)	575,148	(1,033,650)	(955,372)	615,158	(340,214)	(1,373,864)
Total	(21,053,214)	(8,912,599)	10,566,500	(19,399,313)	(9,111,964)	11,615,380	2,503,416	(16,895,897)

Biennium 2017: IPD GOF, Gifts, Royalties and some Grants								
Fund source	FY18 beginning balance	FY18 Revenue	FY18 Expense	FY18 total	FY19 Revenue	FY19 Expense	FY19 total	Grand total Biennium 2017
ENDOPGIFT	(10,518,186)	(16,298,217)	6,610,025	(20,206,378)	(17,460,540)	9,045,894	(8,414,646)	(28,621,024)
GRANT/CONT	(3,520,644)	(903,832)	2,755,762	(1,668,714)	(10,831,231)	1,064,764	(9,766,467)	(11,435,181)
GOF	0	(1,025,621)	922,197	(103,424)	(1,057,385)	1,160,809	103,424	0
ROYALTIES	(1,373,864)	(1,413,787)	663,812	(2,123,840)	(1,549,753)	740,546	(809,207)	(2,933,047)
Total	(15,412,694)	(19,641,457)	10,951,796	(24,102,356)	(30,898,909)	12,012,013	(18,886,896)	(42,989,252)

Biennium 2019: IPD GOF, Gifts and Royalties and a few Grants								
Fund Source	FY20 beginning blance	FY20 Revenue	FY20 Expense	FY20 Total	FY21 Revenue	FY21 Expense	FY21 Total	Grand Total Biennium 2019
ENDOPGIFT	(28,463,273)	(11,851,358)	13,995,062	(26,319,569)	(9,584,102)	12,312,591	2,728,489	(23,591,080)
GRANT/CONT	(133,901)	(798,146)	503,428	(428,619)	(4,839,836)	1,356,937	(3,482,899)	(3,911,518)
GOF	0	(1,059,677)	1,057,810	(1,867)	(1,055,981)	1,057,848	1,867	0
ROYALTIES	(2,933,047)	(1,719,363)	658,404	(3,994,006)	(2,371,592)	1,259,118	(1,112,474)	(5,106,480)
Total	(31,530,221)	(15,428,544)	16,214,703	(30,744,062)	(17,851,511)	15,986,495	(1,865,016)	(32,609,078)

Key:	
COSTCENTER	Costcenters were added to report in FY18. We have two costcenters: the Biochemistry Stock Room and the Electron Microscopy center
ENDOPGIFT	Endowment operating funds and gift funds and also includes funds for faculty startup packages.
GRANT/CONT	Grants and Contracts
SELFSUST	Self Sustaining
GOF	General Operating Funds: State funds, our tenure lines
WWAMI	Funds from WWAMI for teaching. WWAMI stands for Washington, Wyoming, Alaska, Montana and Idaho and teaches medical students across the five states.
R&T	Research and Training
ROYALTIES	Royalties for department and also includes funds for faculty startup packages.
DOF	Designated Operating Funds: State funds for department and also includes funds for faculty startup packages.
RCR	Research Cost Recovery: Departmental Share of Indirect Costs also includes funds for faculty startup packages.

Endowed Funds in the Department of Biochemistry

Endowed Chairs and Professorships

Earl W. Davie and Zymogenetics Endowed Chair – held by Chair of Biochemistry

Edmond H. Fischer and Washington Research Fund Endowed Chair – held by Rachel Klevit (current rules required to be held by new hires in the future)

Henrietta and Aubrey Davis Endowed Professorship – held by David Baker

Hans Neurath Endowed Chair newly created, January 2022.

Other Endowed Funds

Benjamin Schultz Endowed Research Fund in Biochemistry (Travel for graduate students)

David L. Urdal Endowed Research Fund in Biochemistry (Laptops for first year graduate students)

Paul M. Bornstein Endowed Fund (Paul M Bornstein endowed lecture).

Petersen, Donald and Jo Anne Endowment for Accelerating Advancements in Alzheimer's Disease Research at the Institute for Protein Design

Appendix G

BIOC 530 Autumn 2019 Class Schedule

Monday	Wednesday	Friday
	September 25 Ethan Merritt X-ray Crystallography 1	September 27 Ethan Merritt X-ray Crystallography 2
September 30 Ethan Merritt X-ray Crystallography 3	October 2 David Baker Forces 1	October 4 David Baker Forces 2
October 7 David Baker Forces 3	October 9 Justin Kollman Macromolecular Assemblies 1	October 11 Justin Kollman Macromolecular Assemblies 2
October 14 Valerie Daggett Protein Dynamics 1	October 16 Valerie Daggett Protein Dynamics 2	October 18 David Baker Protein Folding 1
October 21 David Baker Protein Folding 2	October 23 Ethan Merritt X-ray Crystallography 4	October 25 David Baker Protein Folding in vivo
October 28 David Baker Sequence Comparison	October 30 David Baker Structure Prediction	November 1 David Baker Protein Design
November 4 Alan Weiner RNA 1	November 6 Alan Weiner RNA 2	November 8 Alan Weiner RNA 3
November 11 NO CLASS	November 13 Rachel Klevit NMR 1	November 15 Rachel Klevit NMR 2
November 18 David Veesler Electron Microscopy 1	November 20 David Veesler Electron Microscopy 2	November 22 Liangcai Gu DNA Synthesis for Protein Characterization
November 25 Liangcai Gu DNA Sequencing for Protein Characterization	November 27 Ning Zheng Signaling & Protein Modification 1	November 29 NO CLASS
December 2 Ning Zheng Signaling & Protein Modification 2	December 4 Dustin Maly Chemical Biology 1	December 6 Dustin Maly Chemical Biology 2

BIOC 650 Biochemistry Clerkship in Scientific Teaching
Sample Syllabus

Course Description:
The pedagogical requirement addressed by this course is direct experience in teaching undergraduate Biochemistry classes (Introduction to Biochemistry or Biochemistry lab). This course features a strong emphasis on the pedagogy of the educational experience that includes weekly meetings with instructors for training in teaching techniques and classroom management, attending class lectures, holding office hours, leading study section(s), developing homework/quizzes, grading short answer exams and management of course website all under the direct oversight and mentorship of regular course instructors. By the end of this course, the graduate student will have developed skills, abilities and insights as a science educator and communicator through the sustained support and guidance of the instructor of record.

Student Learning Objectives:
- Organize and prepare a lesson or lab
- Write questions to assess knowledge
- Prepare learning activities

Grading Guidelines: Course is graded Credit/No Credit. To receive credit for the class, students must complete a minimum of 80% of course activities.

Student Evaluations:
- Weekly presentations to undergraduate students (50%)
- Prepared materials to assess performance of undergraduate students (30%)
- Prepared learning activities for undergraduate students (20%)

Class Meetings:
Students will participate in a hands on teaching experience in the classroom for either the BIOC 440 series or the BIOC 426 lab classes. This requires students to attend class meeting times. Additionally, students will meet once a week with their professors to cover the following discussion assignments.

Appendix G

Reading List: "Scientific Teaching" by Jo Handelsman, Sarah Miller, Christine Pfund
Chapters 1-5

Week	Reading	Discussion assignment
1	“Scientific Teaching” Chapter 1: *Scientific Teaching*	What is constructivism?
2	“Scientific Teaching” Chapter 2: *Active Learning*	How to enhance active participation
3	“Scientific Teaching” Chapter 3: *Assessment*	Preparation and analysis of a midterm exam.
4	“Scientific Teaching” Chapter 4: *Diversity*	Implicit bias
5	“Scientific Teaching” Chapter 5: *A framework for constructing a teachable unit.*	How to prepare meaningful problem sets and activities
6		Preparation and analysis of a midterm exam.
7		Each student presents an example of a successful problem set or activity.
8		Additional presentations
9		Prepare questions for Final Exam
10		Analysis of the Final Exam

Appendix H

Students and their Thesis Advisor				
Name	**YR**	**Enrollment Year**	**Department**	**Lab**
Clarke, Charlie	1	2021	Biochemistry	Rotation
Eisenach, Helen	1	2021	Biochemistry	Rotation
Gonske, Sara	1	2021	Biochemistry	Rotation
Green, Akimi	1	2021	Biochemistry	Rotation
Hurwitz, Sophie	1	2021	Biochemistry	Rotation
Kinfu, Elias	1	2021	Biochemistry	Rotation
Krishna, Rohith	1	2021	Biochemistry	Rotation
Mackey, Emma	1	2021	Biochemistry	Rotation
Tipps, Samuel	1	2021	Biochemistry	Rotation
Applegate, Justin	2	2020	Biochemistry	Merz
Bounds, Amy	2	2020	Biochemistry	Hoppins
Cardenas, Jorge	2	2020	Biochemistry	Maly
Hendricks, Grace	2	2020	Biochemistry	King
Jones, Morgan	2	2020	Biochemistry	Rotation
Rexhepaj, Megi	2	2020	Biochemistry	Rotation
Veil, Ilya	2	2020	Biochemistry	Zheng
Vernon, Annabel	2	2020	Biochemistry	Kwon
Vuong, Chau	2	2020	Biochemistry	Ailion
Bocanegra, Jennifer	3	2019	Biochemistry	Hoppins
Brandys, Marisa	3	2019	Biochemistry	King
Criswell, Cameron	3	2019	Biochemistry	King
Zepeda, Samantha	3	2019	Biochemistry	Veesler
Adams, Chloe	4	2018	Biochemistry	King
Muenks, Andrew	4	2018	Biochemistry	DiMaio
Pan, Yuxin	4	2018	Biochemistry	Gu
Rutter, Kaitlyn	4	2018	Biochemistry	Brockerhoff
Said, Meerit	4	2018	Biochemistry	Baker
Zambrano, Dane	4	2018	Biochemistry	King
Adams, Carson	5	2017	Biochemistry	DiMaio
Benasutti, Halli	5	2017	Biochemistry	Chamberlain
Pranoto, Inez	5	2017	Biochemistry	Kwon
Burrell, Anika	6	2016	Biochemistry	Kollman
Cabrera, Alejandra	6	2016	Biochemistry	Kwon
Witus, Samuel	6	2016	Biochemistry	Klevit

Appendix H

Adjunct Thesis Advisors*					
Name		**Enrollment Year**	**Department**	**Lab**	**Graduation**
Caudal, Arianne	6	2015	Biochemistry	Tian	**SUM 21**
Maker, Allison	7	2015	Biochemistry	Gumbiner	**AUT 21**
Benasutti, Halli	5	2017	Biochemistry	Chamberlain	
Cardenas, Jorge	2	2020	Biochemistry	Maly	**MS in WIN 22 Planned**
Jones, Morgan	2	2020	Biochemistry	~~Mougous~~	**Moved to a fourth rotation in Feb., 22**
Rexhepaj, Megi	2	2020	Biochemistry	~~Chamberlain~~	**Moved to a fourth rotation in Mar., 22**
Veil, Ilya	2	2020	Biochemistry	Zheng	
*Before 2015, students were not allowed to do their thesis research in Adjunct Professor labs. This list shows all students who chose an Adjunct Professor as a thesis advisor and the outcome.					

Appendix I

Name	Title	Appointments in these Units
Escobar, Thelma	Assistant Professor	Biochemistry
Gu, Liangcai	Assistant Professor	Biochemistry Genome Sciences - Adjunct
King, Neil	Assistant Professor	Biochemistry
Kwon, Young	Assistant Professor	Biochemistry
Ailion, Michael	Associate Professor	Biochemistry
Brzovic, Peter	Associate Professor*	Biochemistry
Dimaio, Frank	Associate Professor	Biochemistry
Hoppins, Suzanne	Associate Professor	Biochemistry
Kollman, Justin M	Associate Professor	Biochemistry
Miller, Dana	Associate Professor	Biochemistry
Veesler, David J	Associate Professor HHMI Investigator	Biochemistry
Wills, Andrea	Associate Professor (effective July 1, 2022)	Biochemistry
Baker, David	Professor HHMI Investigator	Biochemistry Bioengineering (Medicine) - Adjunct Chemical Engineering - Adjunct Genome Sciences - Adjunct Paul G. Allen School of Computer Science & Engineering - Adjunct Physics - Adjunct
Brockerhoff, Susan E	Professor*	Biochemistry Ophthalmology - Adjunct
Davis, Trisha N	Professor	Biochemistry
Hurley, James B	Professor	Biochemistry Ophthalmology - Adjunct
Klevit, Rachel	Professor	Biochemistry Chemistry - Adjunct Pharmacology - Adjunct
Merz, Alex	Professor	Biochemistry Physiology and Biophysics - Adjunct
Palmiter, Richard	Professor HHMI Investigator	Biochemistry Genome Sciences - Adjunct
Ruohola-Baker, Hannele	Professor	Biochemistry Bioengineering (Medicine) - Adjunct Biology - Adjunct Genome Sciences - Adjunct Oral Health Sciences - Adjunct
Loeb, Lawrence A	Professor (Retiring June 30, 2022)	Pathology Biochemistry - Joint
Stenkamp, Ronald E	Professor	Biological Structure Biochemistry - Joint

Appendix I

Fan, Erkang	Research Associate Professor	Biochemistry Biological Structure - Joint
Hauschka, Stephen D (Retired - Active)	Professor Emeritus	Biochemistry
Kimelman, David (Retired - Active)	Professor Emeritus	Biochemistry
Muller, Eric D (Retired - Active)	Research Associate Professor Emeritus	Biochemistry
*Without tenure for reasons of funding		

Appendix I

Name	Title	Current Scientific Interest	h-index
Escobar, Thelma	Assistant Professor	Epigenetic mechanisms that shape developmental systems	12
Gu, Liangcai	Assistant Professor	Protein barcoding, in situ sequencing, de novo engineering of chemically induced dimerization	18
King, Neil	Assistant Professor	Custom-designed protein nanomaterials as platform technologies for medical applications	25
Kwon, Young	Assistant Professor	Drosophila model of cell dissemination and systemic organ wasting	6
Ailion, Michael	Associate Professor	Biological function of neuromodulators from their cellular mechanisms of action to their roles in behavior.	21
Brzovic, Peter	Associate Professor	Structure and function of the breast and ovarian cancer tumor suppressor protein BRCA1 and other protein complexes critical to ubiquitin signaling pathways	32
Dimaio, Frank	Associate Professor	Computational approaches to problems of biomolecular structure determination, typically using sparse experimental data	50
Hoppins, Suzanne	Associate Professor	Mechanism and regulation of mitochondrial fusion	15
Kollman, Justin M	Associate Professor	Structure and function of metabolic enzyme polymers and bacterial cytoskeleton	22
Miller, Dana	Associate Professor	Molecular mechanisms that animals use to maintain homeostasis in changing conditions (low oxygen and exposure to hydrogen sulfide)	10
Veesler, David J	Associate Professor HHMI Investigator	Structure and function of macromolecular complexes involved in viral/bacterial pathogenesis and immunity	42
Wills, Andrea	Associate Professor (effective July 1, 2022)	Molecular and cellular basis of differentiation in vertebrate development and regeneration	10
Baker, David	Professor HHMI Investigator	Design of macromolecular structures and functions	140
Brockerhoff, Susan E	Professor	Mechanisms associated with photoreceptor degeneration	26
Davis, Trisha N	Professor	Formation and regulation of the mitotic cytoskeleton	44
Hurley, James B	Professor	Develop a new conceptual framework for how energy production and distribution influence function and viability of photoreceptor neurons	60
Klevit, Rachel	Professor	Applying cutting edge technologies to challenging systems including protein ubiquitination and small heat shock proteins.	58
Loeb, Lawrence A.	Professor (Retiring June 30, 2022)	Relationship between mutations and human cancer.	91
Merz, Alex	Professor	Understand the molecular signals and effectors that control organelle biogenesis	29
Palmiter, Richard	Professor HHMI Investigator	Developing and using transgenic technology to study neuronal circuits that mediate appetite and body weight regulation	154
Ruohola-Baker, Hannele	Professor	Molecular biology of stem cells and on the use of Drosophila as model organisms for human diseases	41
Stenkamp, Ronald	Professor	Understanding biomolecular functions in terms of their structures including G-protein coupled receptors and cell adhesion proteins.	49
Fan, Erkang	Research Associate Professor	Structure based drug discovery for tropical diseases	37
Kimelman, David	Professor - Emeritus (Retired - Active)	Molecular mechanisms underlying early vertebrate embryonic development from signaling and transcription to morphogenesis using zebrafish as model system.	33
Hauschka, Stephen D	Professor Emeritus (Retired - Active)	Development of skeletal and cardiac muscle regulator gene cassettes for expression of genes especially for gene therapy	59

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Muller, Eric D	Research Associate Professor Emeritus (Retired - Active)	Organization and function of microtubule organizing centers	25

Appendix I

Name	Title	Primary Department	Current Scientific Interest
Aitchison, John	Adjunct Professor	Pediatrics	Yeast cell biology, nucleocytoplasmic transport, peroxisomal assembly and function
Bedalov, Antonio	Adjunct Professor	Medicine: Oncology	Cancer Biology; Gene Expression; Cell Cycle and Chromosome Biology; Genetics; Genomics and Evolution
Bush, Matthew	Adjunct Assoc Prof	Chemistry	Development and application of mass spectrometry and ion mobility spectrometry techniques
Chamberlain, Jeffrey	Adjunct Professor	Neurology	Muscular dystrophy; dystrophin structure and function; gene therapy
Daggett, Valerie	Adjunct Professor	Bioengineering	Molecular dynamics; protein unfolding/folding
Gelb, Michael	Adjunct Professor	Chemistry	Lipid mediator production and inflammation; design of anti-parasite agents; proteomic methodolog
Gumbiner, Barry	Adjunct Professor	Pediatrics	Cell adhesion in morphogenesis and disease
Jung, Barbara	Adjunct Professor	Medicine	Colorectal cancer, tumor genetics, and activin signaling and tumor progression
Lopez, Jose	Adjunct Professor	Medicine: Hematology	Platelet adhesion; von Willebrand factor; vessel inflammation and occlusion
Maly, Dustin	Adjunct Professor	Chemistry	Chemical biology of signal transduction
Mougous, Joseph	Adjunct Professor	Microbiology	Mechanisms of interbacterial interactions
Tian, Rong	Adjunct Professor	Anesthesiology & Pain Medicine	Mitochondrial biology, immunometabolism, stem cell and cardiovascular diseases
Zheng, Ning	Adjunct Professor	Pharmacology	Ubiquitin ligase machinery in eukaryotic biology

Faculty Honors and Awards

Assistant Professors

Liangcai Gu

2008 Robert Scarborough Award for Graduate Excellence in Medicinal Chemistry, American Chemical Society
2009 Young Investigator Award, Society for Industrial Microbiology
2009 – 2012 Jane Coffin Childs Postdoctoral Fellowship Award
2017 University of Washington Innovation Award
2018 Safeway Early Career Award in Cancer Research
2020 Cutting-Edge Basic Research Awards (CEBRA), National Institute on Drug Abuse

Neil King

2008 Audree Fowler Fellowship in Protein Science, University of California, Los Angeles, CA
2009 – 2010 Dissertation Year Fellowship, University of California, Los Angeles, CA
2013 PDB Molecule of the Month (September 2013: Designed Protein Cages)
2016 *Science* Breakthrough of the Year runner–up (citing Bale et al. and Hsia et al.)
2018 Amgen Young Investigator Award

Young Kwon

2007 Paul Ehrlich Research Award, Johns Hopkins University
2009 Tom Elkins Memorial Lecture Award
2009 – 2011 Damon Runyon Cancer Research Fellowship
2016 Regular Grant Award, Edward Mallinckrodt, Jr. Foundation
2021 – 2024 Kuni Foundation Cancer Research Grant

Andrea Wills

2007 Ruth Sager Memorial Fellowship
2010* Stanford Dean's Fellowship
2010* Stanford Pediatric Research Fund Fellowship
2010 – 2013 NIH NRSA fellowship 1F32 DK089643–01. Investigating the transcriptional regulation of liver specification in *Xenopus tropicalis.*
2013 Talk award, West Coast Society for Developmental Biology meeting
2014 John Gurdon Prize, Xenopus Meeting
2014 Hide Mangold Postdoctoral Symposium award, SDB meeting
2014 Travel award, Society for Developmental Biology meeting
2014 – 2015 Katherine McCormick Advanced Postdoctoral Fellowship
2021 International Xenopus Board Young Investigator Award
2021 Science in Medicine New Investigator Lecture, University of Washington School of Medicine
*These awards both had overlap with my NRSA fellowship, and therefore were declined

Associate Professors

Michael Ailion

1989 – 1993 National Merit Scholarship, University of Utah
1989 – 1993 President's Scholarship, University of Utah
1994 NSF Predoctoral Fellowship awarded (declined)
1994 – 1999 Howard Hughes Medical Institute Predoctoral Fellowship, University of Washington
2004 Life Science Research Foundation Postdoctoral Fellowship awarded (declined)
2004 – 2007 Helen Hay Whitney Postdoctoral Fellowship, University of Oregon/University of Utah
2008 – 2010 NIH K99 Pathway to Independence Award, University of Utah
2010 – 2011 NIH K99 ARRA Supplement, University of Utah
2013 – 2017 Ellison Medical Foundation New Scholar Award, University of Washington
2015 – 2020 NSF CAREER Award, University of Washington

Frank Dimaio

2001 – 2002 University of Wisconsin Fellowship
2002 – 2006 Computation and Informatics in Biology and Medicine Graduate Fellowship
2017 UW Innovation Award

Suzanne Hoppins

2000 Jaedon Bland Memorial Graduate Scholarship
2000 – 2001 Faculty of Science Graduate Teaching Assistantship Scholarship
2001 – 2002 Graduate Intern Tuition Supplement
2001 – 2005 Alberta Heritage Foundation for Science and Engineering Research Graduate Scholarship
2002 Province of Alberta Graduate Student Scholarship
2003 J Gordin Kaplan Graduate Student Award
2003 – 2005 Walter H. Johns Graduate Fellowship
2003 – 2005 National Science and Engineering Research Council (NSERC) Post Graduate Scholarship B
2009 2nd place in poster competition at FASEB Mitochondrial assembly & dynamics in health and disease, Tucson, AZ.
2011 – 2014 NIH Pathway to Independence Award (K99/R00)

Justin Kollman

1998 Graduate Assistance in Areas of National Need (GAANN) Award, US. Dept. of Education
2006 Ruth L. Kirschstein National Research Service Award, National Institutes of Health
2014 Research Scholar Junior 1 Career Award, Fonds de Recherche Santé Québec
2014 New Investigator Salary Award, Canadian Institutes of Health Research
2016 Science in Medicine New Investigator Lecture, University of Washington School of Medicine

Dana Miller

1994 National Merit Scholarship Finalist
1997 Golden Key National Honor Society Scholarship Winner
1997 Phi Beta Kappa, Gamma Chapter of Colorado
1998 *Summa cum laude*, University of Denver
2005 NIH Loan Repayment Program Award
2005 NIH NRSA Fellowship
2009 NIH K99/R00 Pathway to Independence Award
2010 Nathan Shock Center for Excellence in the Biology of Aging Junior Faculty Award
2011 – 2014 Ellison New Scholar in Aging
2015 – 2020 NIEHS ONES (Outstanding New Environmental Scientist) Award

David Veesler

2005 Masters Fellowship, French Ministry of Higher Education and Research.
2006 Ph.D. Fellowship, French Ministry of Higher Education and Research.
2008 ProteomeBinders Fellowship
2009 Application Note Award for outstanding achievement in light scattering, Wyatt Technology.
2011 Thesis Award, French Crystallographic Association.
2011 Marie–Curie International Outgoing postdoctoral Fellowship.
2012 Fall Research Symposium Award, The Scripps Research Institute.
2013 Presidential Scholar Award, Microscopy Society of America.
2017 Young Investigator Outstanding Recognition – FEI
2017 Pew Scholar in Biomedical Sciences
2018 – Burroughs Wellcome Fund Investigator in the Pathogenesis of Infectious Diseases
2020 – National Institutes of Health Director's Pioneer Award
2021 – HHMI Investigator

Professors

David Baker

1983, 1984 Harvard College Scholarship
1985 University of California Regents' Fellowship
1985 – 1988 National Science Foundation Predoctoral Fellowship

1990 – 1993 Life Sciences Research Foundation Postdoctoral Fellowship
1994 National Science Foundation Young Investigator Award
1994 Packard Fellowship in Science and Engineering
1995 Beckman Young Investigator Award
2000 – 2005 HHMI Associate Investigator
2000 Protein Society Young Investigator Award
2002 International Society for Computational Biology Overton Prize
2003 – 2004 Director, Biomolecular Structure and Design Graduate Program (BMSD)
2004 AAAS Newcomb–Cleveland Prize
2004 Foresight Institute Feynman Prize
2005 – HHMI Investigator
2006 – National Academy of Sciences
2008 Sackler Prize in Biophysics
2009 – American Academy of Sciences
2011 University of Washington Inventor of the Year Award
2012 Biochemical Society Centenary Award
2014 American Chemical Society David Perlman Memorial Award
2017 – Henrietta and Aubrey Davis Endowed Professorship in Biochemistry
2018 Solvay Public Lecture
2018 Protein Society Hans Neurath Award
2019 The Audacious Project Recipient
2020 – Fellow, American Institute for Medical and Biological Engineering
2021 The Breakthrough Prize, Life Sciences

Susan Brockerhoff

1987 Graduated CCNY summa cum laude
1994 – 1996 NIH Postdoctoral Fellowship F32 NS009611
1996 – 1997 NIH Postdoctoral Fellowship F32 EY006762
2018 Invited speaker for Distinguished Lecture Series Program at the Cole Eye Institute

Trisha N. Davis

1975 – 1976 University of California Regents' Scholar
1977 – 1980 Predoctoral Fellow, National Science Foundation
1983 – 1985 Postdoctoral Fellow, Anna Fuller Fund
1988 – 1991 Searle Scholar, Searle Foundation
2005 UW School of Medicine/Center of Excellence Mentoring Award, UW School of Medicine
2008 – 2009 Humboldt Research Award, Alexander von Humboldt Foundation
2012 – Fellow, American Association for the Advancement of Science
2013 – Earl W. Davie/Zymogenetics Endowed Chair in Biochemistry
2015 – Member, Washington State Academy of Sciences
2018 – Fellow, American Society for Cell Biology
2020 – Fellow, American Academy of Arts and Sciences
2022 – Winge-Lindegren Address at Yeast Genetics Meeting

James Hurley

1975 American Institute of Chemists Award for Scholastic Achievement
1977 – 1979 NIH Cell Biology Trainee
1980 – 1982 Helen Hay Whitney postdoctoral fellow with Dr. Lubert Stryer in the Department of Structural Biology at Stanford University, investigating phototransduction;
1982 – 1983 Helen Hay Whitney postdoc with Dr. Melvin I. Simon at UCSD, isolating cDNA for transducin;
1985 – 1989 HHMI Assistant Investigator
1989 – 1995 HHMI Associate Investigator
1996 – 2001 HHMI Investigator
2014 Alcon Research Institute Award

Rachel E. Klevit

1978 Outstanding Senior Woman, American Association of University Women

1978	Phi Beta Kappa, Phi Beta Kappa
1978	Rhodes Scholar, The Rhodes Trust
1983	Postdoctoral Fellowship, American Cancer Society
1988	Margaret Oakley Dayhoff Award, Biophysical Society
1989	Established Investigatorship, American Heart Association
1990	DuPont Young Investigator Award, The Protein Society
1990	Award for Excellence in Chemistry, ICI Pharmaceuticals
1991	Van't Hoff Award, Royal Netherlands Academy of Arts and Sciences
1993	Brittingham Visiting Scholar, Dept. of Chemistry, Univ. Wisc./Madison
1998 –	AAAS Fellow, American Association for the Advancement of Science
2009	Scholar's Week Keynote Speaker, Western Washington University
2012	Excellence in Mentoring Award, University of Washington School of Medicine
2015	Fritz Lipmann Award, American Society for Biochemistry and Molecular Biology
2016	Dorothy Crowfoot Hodgkins Award, The Protein Society
2016	Distinguished Scientist in Medicine, University of Washington
2016	Scottish Institute of Cell Signalling Ub Lectureship
2016 –	Elected to Washington Academy of Sciences
2017 –	Edmond H. Fischer – Washington Research Foundation Endowed Chair in Biochemistry
2021 –	Member, National Academy of Sciences

Larry Loeb (Joint with Pathology)

1957 – 1961	Salk Scholarship in Medicine, City of New York
1957	Brittain Award in Moral Philosophy, City College of New York
1957	Baskerville Award in Chemistry, City College of New York
1966	Fellow, American College of Physicians
1966	Seventh Annual Misra-Baptia Medal, Lucknow Med. School, India
1985 – 1999	Outstanding Investigator Award, National Cancer Institute
1986	Fellow, American Association for the Advancement of Science
1987 – 1988	Vice President, American Association for Cancer Research
1988 – 1989	President, American Association for Cancer Research
1988	Abbot Laboratory Distinguished Scientist Lectureship
1988	Lorenzini Foundation Lecturer, Urbino Italy
1988	Visiting Lecturer for the Foundation for Promotion of Cancer Research Distinguished Scientist, Tokyo, Japan
1990	Sixth Founder's Day Lecture, Tata Memorial Cancer Centre Bombay, India
1991	Ignoble Award, Department of Biochemistry, University of Washington
1992	Distinguished Professor in Cancer Research, Eppley Cancer Center
1993	Fessinger-Springer Memorial Lecture, MARC Research Symposium
1995	Nakahara Memorial Lecture, "Mutator Phenotype and Spontaneous Mutagenesis"
1998	Environmental Mutagen Society Annual Award for Excellence in Basic Science
2001	President-Elect and Program Chairman, Environmental Mutagen Society
2002	President, Environmental Mutagen Society
2002	Burroughs Wellcome Visiting Professorship in the Basic Medical Sciences
2003	Lubomir Hnlilica Memorial Lecture, "A mutator phenotype in cancer"
2005	Princess Takamatsu Annual Lectureship (Japan)
2006	Distinguished Scientist Lecturer, University of Washington on Mammalian DNA Repair
2008	AACR Princess Takamatsu Award
2008	Gulbenkian lectureship, Lisbon, Portugal
2008	J.B. Little Award, Harvard

Alexey Merz

1992	HHMI Undergraduate Research Fellow
1996 – 1999	NIH Predoctoral Trainee (T32 AI07472)
1996 – 1997	N.L. Tartar Research Award, OHS Foundation
1997	Sears Award for Excellence in Graduate Medical Education
2000	Paper of the Year (Nature 407:98), OHSU Graduate Council
2000 – 2003	Damon Runyon Cancer Research Foundation Postdoctoral Fellow (DRG–1598)

2003 – 2004 NIH Postdoctoral Trainee (T32 AR07576)
2010 – 2013 American Cancer Society Research Scholar
WWAMI Student Award for Excellence in Medical Education.

Richard D. Palmiter

1976 – HHMI Investigator
1988 Elected to National Academy of Sciences
1988 Elected to American Academy of Arts and Sciences
1994 Charles–Leopold Mayer Award, French Academy of Sciences
2004 Recipient of the Julius Axelrod Medal

Hannele Ruohola-Baker

1986 – 1989 Nordic Yeast Research Program Predoctoral Fellowship
1989 Predoctoral award from the Oskar Oflund Foundation
1989 Academy of Sciences Award (Finland)
1989 – 1991 EMBO Postdoctoral Fellowship
1992 – 1994 ACS Senior Postdoctoral Fellowship
1995 – 1997 Basil O'Connor Starter Scholar Research Award
1995 – 2000 American Heart Association Established Investigator Award
1996 – 2000 Pew Fellow in Biomedical Sciences, Pew Memorial Trust
2005 American Heart Association Award (best grant)
2007 Tietze Award
2008 – 2010 Elected Drosophila Board Representative

Ronald Stenkamp (Joint with Biological Structure)

1969 Phi Beta Kappa.
1972 Phi Lambda Upsilon.
2014 Fellow, American Crystallographic Association.

Peter S. Brzovic

1985 Graduate Fellowship, University of California, Riverside
1989 Graduate Student Association Council President, Dept. of Biochemistry, Univ. of Calif., Riverside
1990 Scholarship for Young Participants from Abroad, 8th International Symposium on Vitamin B6 and Carbonyl Catalysis
1991 Fellowship from UCR Training Program in Cellular and Molecular Endocrinology, University of California, Riverside
1995 American Cancer Society Fellowship, American Cancer Society

Erkang Fan

1983 – 1987 Scholarship for Outstanding Performance in Undergraduate Study, Peking University.
1992 – 1993 Andrew Mellon Predoctoral Fellowship, University of Pittsburgh.
1994 – 1996 NIH Postdoctoral Fellowship.

Department of Biochemistry

Guidelines for Faculty Appointments and Promotions

This document is a set of "Guidelines" rather than a set of "Rules". This is in recognition of the fact that every appointment is unique and requires flexibility in our expectations and requirements of potential appointees. Departure from these guidelines in individual cases is possible by a vote of approval by the Biochemistry Faculty. These guidelines are specific to the Department of Biochemistry. For general UW policies with regard to promotions and appointments, please see the Faculty Code (http://www.washington.edu/admin/rules/policies/FCG/FCGTOC.html).

Appointments and promotions are contingent upon demonstrated excellence in teaching, research and service appropriate to rank as described below. Excellence must be demonstrated in each area. Assessment of excellence is weighted proportionately to the amount of time spent in each activity.

OVERVIEW: PROFESSIONALISM AND DIVERSITY

Professionalism (All Ranks):
The members of the Biochemistry Faculty are committed to maintaining and modeling the highest professional and ethical standards in matters relating to our academic and scientific endeavors. The Department of Biochemistry (BIOC) is committed to upholding the Statement on Faculty Professional Conduct approved by the faculty of Biochemistry (available at: Faculty Professionalism Guidelines) and modelled on the UW Graduate School guidelines on professionalism (http://www.grad.washington.edu/mentoring/good-practice/professionalism.shtml). Non-compliance with the standards will be considered in all appointment and promotion reviews.

Diversity and Equity (All Ranks):
Per UW Faculty Code: "In accord with the University's expressed commitment to excellence and equity, contributions in scholarship and research, teaching, and service that address diversity and equal opportunity ***may be included*** among the professional and scholarly qualifications for appointment and promotion outlined below." From the Faculty Code, Volume II, Part II, Chapter 24, Section 24-32.
URL: http://www.washington.edu/admin/rules/policies/FCG/FCCH24.html#2432

Examples of commitment to excellence and equity though contributions in scholarship and research, teaching, and service that address diversity and equal opportunity that may be found in the CV and other materials (from the University of Washington, Director of the Office for Faculty Advancement: source UC Berkeley):

For example, faculty who have

- engaged in service to increase participation in science, education, humanities, fine arts, or social sciences by groups historically under-represented in higher education.

- contributed to pedagogies addressing different learning styles.

- significant experience in teaching students who are under-represented in higher education.

- research interests in subjects that will contribute to diversity and equal opportunity in higher education.

Assistant Professor

The criteria for appointment to Assistant Professor include:

- Excellent record of research accomplishment during graduate and postdoctoral work in any area of modern biochemistry including, but not limited to, molecular biology, cell biology, structural biology, biophysics, or developmental biology.
- The candidate should have made important contributions to the field as demonstrated by first-author papers in critically reviewed journals.
- The candidate should demonstrate extraordinary promise of continued independent scholarly achievement.

Terms of appointment:

- The initial appointment is normally for a term of three academic years, with the possibility of a second three-year term.
- The appointee may become a member of the graduate faculty and may supervise graduate students.

The appointee is reviewed according to the following guidelines:

- Before the end of the second year, a thorough review will be conducted by the Appointments and Promotion Committee. Based on this review, a recommendation will be made to the department faculty.
- Mandatory review resulting in either promotion or non-renewal must be made before the end of the sixth year of appointment.

Associate Professor

The criteria for promotion to Associate Professor include

- Continued scholarly achievement as evidenced by an excellent record of scientific publications, student mentoring, and effectiveness in classroom teaching

- Continued success in achieving an appropriate level of independence and in securing a level of external support sufficient to maintain a vigorous research program.
- Candidates for promotion are expected to have served on departmental committees or to have contributed to other important departmental functions; service on School of Medicine or University committees will also be taken into account.
- The candidate must have achieved a national reputation as indicated by letters of support from outside the University.
- Must continue to demonstrate high standards of professional integrity and conduct.

Terms of appointment:

- Appointments as Associate Professor to state-funded positions are for indefinite terms.
- Associate Professors may be appointed without tenure (WOT) by virtue of the source of salary funding.
- The appointee may be a member of the graduate faculty and may supervise graduate students.

Professor

The criteria for promotion to Professor include those listed above for promotion to Associate Professor. In addition, the candidate is expected to:

- Have achieved a strong international reputation
- Have made a significant impact on the field, as evidenced both by publications and letters of support from outside the University.

Terms of the appointment:

- Appointments as Professor to state-funded positions are for indefinite terms.
- Professors may be appointed without tenure (WOT) by virtue of the source of salary funding.
- The appointee may be a member of the graduate faculty and may supervise graduate students.

Research Assistant Professor

The criteria for appointment to Research Assistant Professor are similar to those for appointment to Assistant Professor, except that research faculty:

- Are not required to teach in the regular instructional program, except insofar as required by funding sources. Although teaching by research faculty is encouraged, it is not required and only compensated if greater than *de minimis* in amount (please see UW guidelines for definition, http://www.washington.edu/research/maa/fec/faqs.html).
- Usually occupy space within the Biochemistry Department assigned to members of the regular faculty ranks.
- Can be appointed to the graduate faculty, but do not normally serve as thesis advisor or chair of a graduate student supervisory committee.

Terms of the appointment:

- The initial appointment is normally for a term of three academic years.
- These appointments are limited by the duration of salary funding.

The appointee is reviewed according to the following guidelines:

- After a thorough review conducted before the end of the second year, a second three-year appointment may be made.
- Mandatory review resulting in either promotion or non-renewal must be made by the end of the sixth year of appointment.

Research Associate Professor

The criteria for promotion to this position are similar to those for promotion to Associate Professor. In addition, there should be an emphasis on the following:

- Research productivity after appointment to Research Assistant Professor as evidenced by first--and corresponding--author publications
- Grant and contract support as Principal (or co-Principal) Investigator
- Mentoring effectiveness within the home laboratory.
- The candidate should have achieved a national reputation as evidenced by letters of support from outside the University.

Terms of the appointment:

- Appointments at this rank may be for terms of up to five years and are renewable.
- These appointments are limited by the duration of salary funding.
- A Research Associate Professor usually occupies space within the Biochemistry Department assigned to a member of the regular faculty ranks.

The appointee is reviewed according to the following guidelines:
- Renewal decisions must be made in the fall of the last year of the appointment term.

Research Professor

The criteria for appointment to Research Professor include those for appointment to Professor. In addition, there should be an emphasis on the following:
- Research productivity and grant and contract support, not on teaching effectiveness or departmental service.

Terms of the appointment:
- A Research Professor usually occupies space within the Biochemistry Department assigned to a member of the regular faculty ranks.
- Appointments to this rank may be made for up to five years and are renewable.
- These appointments are limited by the duration of salary funding.

The appointee is reviewed according to the following guidelines:
- Renewal decisions must be made in the fall of the last year of the appointment term.

Adjunct Faculty Appointees

Adjunct faculty have a significant interest in the research and scholarly activities of our department. Adjunct faculty:
- Are welcome at department activities, such as faculty meetings, faculty seminars and departmental retreats.
- Generally do not have formal departmental responsibilities, although they may occasionally serve on departmental committees, or give occasional lectures or classes, which would benefit from their expertise.

Terms of the appointment:
- Adjunct Faculty do not vote on appointments and promotions.
- Adjunct Faculty are not eligible to serve as thesis advisor for graduate students enrolled in the department graduate program.
- The adjunct faculty rank is determined by rank in the primary department.

Joint Faculty Appointees

Joint faculty, like adjunct appointees, have a significant interest in the research and scholarly activities of our department. Joint Faculty have the following privileges and responsibilities:

- They are welcome at departmental activities, such as faculty meetings, faculty seminars and departmental retreats.
- In contrast to adjunct, joint appointees assume significant departmental responsibilities, most commonly a substantial teaching load (for example, 5 weeks of a 10-week lecture course for undergraduates or first year medical students).
- Joint faculty vote on appointments and promotions
- Joint faculty are eligible to take rotating graduate students, and can serve as thesis advisor for graduate students enrolled in the department graduate program.

Terms of the appointment:

- The joint faculty rank is determined by rank in the primary department.

Acting Instructor

The criteria for appointment to Acting Instructor include:

- The appointment must be supported by a faculty vote, a letter from the chair, and four letters of recommendation.

Terms of the appointment:

- The appointment carries no explicitly defined duties, instructional or otherwise, and no national search is required. Further information about this appointment may be found at: (http://www.washington.edu/admin/acadpers/job_descriptions/Acting_Instructor.html).
- The position is not intended as a transition in our department to an appointment of greater rank such as Assistant Professor or Research Assistant Professor; however appointment to Acting Instructor does not preclude such a transition.
- The Acting Instructor title describes an annual appointment that may be reviewed for renewal for up to three academic years (a total of four years).
- An Acting Instructor can serve as Principal (or co-Principal) Investigator for a grant or contract.
- An Acting Instructor occupies space assigned to a regular faculty member and performs research related to the faculty member's interests.

Part-time Lecturer

Individuals are occasionally appointed to the part-time position of Lecturer for the purpose of enhancing the educational missions of the Department. The following are the principal characteristics of this position:

- Individuals appointed to the position of Lecturer must have demonstrable expertise in the relevant subject area(s), commensurate with the level of the course(s) that will be taught.
- Lecturers are hired to teach courses and not to carry out research and/or supervise graduate students.
- These individuals will generally have attained a doctorate degree.
- The normal appointment period of a part----time Lecturer shall be for one quarter. The terms of employment will be set out in a letter of employment from the Chair.

*The position of **full-time Lecturer** has been used only rarely by the Department of Biochemistry. The criteria for this appointment are the same as above, except that a formal competitive national search is required.*

Affiliate Appointments and Promotions

The criteria for appointment to the affiliate faculty include:

- An affiliate appointment recognizes the professional contribution to the department and/or institution by an individual whose principal employment responsibilities lie outside the colleges or schools of the University of Washington.
- Level of appointment and promotion will not automatically be linked to the individual's title or promotion at the parent institution, but will be determined by the nature and intensity of the contribution by the individual to the academic and/or scientific programs of the department and the University of Washington.

OMB No. 0925-0001 and 0925-0002 (Rev. 10/2021 Approved Through 09/30/2024)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Michael Ailion

eRA COMMONS USER NAME (credential, e.g., agency login): MICHAELAILION

POSITION TITLE: Associate Professor of Biochemistry

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
University of Utah, Salt Lake City, UT	B.S.	09/1989	08/1993	Biology
University of Utah, Salt Lake City, UT	B.A.	09/1989	08/1993	English
University of Washington, Seattle, WA	Ph.D.	09/1994	06/2000	Molecular and Celllular Biology
University of Oregon, Eugene, OR	Postdoc	06/2003	04/2005	Neurobiology
University of Utah, Salt Lake City, UT	Postdoc	05/2005	11/2011	Neurobiology and Genetics

A. Personal Statement

The main project in my research group is to understand the biological function of neuromodulators (neuropeptides and biogenic amines), from their cellular mechanisms of action to their roles in behavior. Our current focus is to understand neuromodulatory signaling at the molecular and cellular level. We study the biogenesis and regulated release of dense-core vesicles, the organelles that release neuromodulators, as well as the G protein signal transduction pathways that regulate and respond to neuromodulatory signals. Our work has primarily used the nematode *C. elegans* as a model system, but in the last few years we have extended our work into mammalian neuroendocrine cell lines and mice, with a focus on insulin secretion.

In a second distinct project in the lab, we investigate the genetics of hybrid incompatibility both within *C. elegans* and in other *Caenorhabditis* species. We identify genes responsible for hybrid incompatibility and study their molecular mechanisms of action at the cellular level, with the long-term goal of determining the evolutionary forces and molecular pathways that underlie speciation events.

We have received funding for all of our projects. Work on the dense-core vesicle project has been supported by an R01 award from NIGMS, now beginning year 5 of a five-year grant ending in February 2023 (R01 GM121481, *Proteins important for dense-core vesicle function*). We also share an R01 award from NINDS (with Jihong Bai at the Fred Hutchinson Cancer Research Center) that supports our work on G protein signal transduction pathways that modulate neuronal activity, now in year 3 of a four-year grant ending in December 2023 (R01 NS109476, *Signaling pathways that modulate neuronal activity*, Ailion and Bai Co-PIs). Work on the hybrid incompatibility project has been supported for the past 5+ years by an NSF CAREER award, which is now in a no-cost extension (*CAREER: Hybrid incompatibility genes in Caenorhabditis species*).

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2021-2022 Visiting professor, École Normale Supérieure de Lyon, Lyon, France
2019- Affiliated faculty, UW Medicine Diabetes Institute
2018- Associate Professor, Department of Biochemistry, University of Washington, Seattle, WA
2017- Affiliate Investigator, Nutrition Obesity Research Center, University of Washington
2016- Affiliate Investigator, Diabetes Research Center, University of Washington
2012- Faculty, Neuroscience Graduate Program, University of Washington

2012-	Faculty, Molecular and Cellular Biology Graduate Program, University of Washington
2011-2018	Assistant Professor, Department of Biochemistry, University of Washington, Seattle, WA
2005-2011	Postdoctoral Fellow, Department of Biology, University of Utah, Salt Lake City, UT
2003-2005	Postdoctoral Fellow, Institute of Neuroscience, University of Oregon, Eugene, OR
2000-2001	Postdoctoral Research Associate, Dept. of Genetics, University of Washington, Seattle, WA
1993-1994	Research Assistant, Department of Biology, University of Utah, Salt Lake City, UT

Honors and Awards

2015-2020	NSF CAREER Award, University of Washington
2013-2017	Ellison Medical Foundation New Scholar Award, University of Washington
2010-2011	NIH K99 ARRA Supplement, University of Utah
2008-2010	NIH K99 Pathway to Independence Award, University of Utah
2004-2007	Helen Hay Whitney Postdoctoral Fellowship, University of Oregon/University of Utah
2004	Life Science Research Foundation Postdoctoral Fellowship awarded (declined)
1994-1999	Howard Hughes Medical Institute Predoctoral Fellowship, University of Washington
1994	NSF Predoctoral Fellowship awarded (declined)
1989-1993	National Merit Scholarship, University of Utah
1989-1993	President's Scholarship, University of Utah

Memberships and Other Professional Activities

2022	Member, NSF review panel, MCB/Genetic Mechanisms
2022	Ad hoc member, NIDA study section ZDA1 SXC-G (01)
2019-2023	Regular member, NIH SYN/NC study section
2015-2019	Ad hoc member, NIH Synapses, Cytoskeleton, and Trafficking study section (SYN)
2013-	American Society for Cell Biology
2008-	Genetics Society of America
1993-	Phi Beta Kappa

Reviewer: *bioRxiv*, *Cell Reports*, *Curr. Biol.*, *Development*, *Dev. Biol.*, *Dev. Cell*, *eLife*, *EMBO*, *Genetics*, *G3*, *J. Cell Biol.*, *J. Neurosci.*, *Micropubl. Biol., Mol. Biol. Cell*, *Nature*, *Neuron*, *PLOS Genetics*, *PLOS One, PNAS*, *Review Commons*, *Science Advances*
Guest editor: *PLOS Genetics*, *eLife*
Ad hoc grant reviewer: NSF, Israel Science Foundation, Graduate Women in Science Fellowship Program, Murdock College Research Program for Life Sciences

C. Contributions to Science

1. **Regulation of vitamin B12 synthesis in the bacterium *Salmonella typhimurium***

As an undergraduate, I worked on the regulation of vitamin B12 synthesis. Vitamin B12 is one of the largest nonpolymeric molecules found in nature and is synthesized only in bacteria. To understand the physiological relevance of B12 synthesis and use in *Salmonella*, I studied the regulation of two large operons: the *cob* operon consisting of genes for B12 synthesis and the adjacent *pdu* operon consisting of genes for the B12-dependent degradation of propanediol, a potential carbon and energy source. I discovered that both operons are induced under identical conditions and identified the proximal transcription factor *pocR* as well as the Crp and Arc signaling proteins that mediated global control. I also showed that the active coenzyme form of B12 represses the *cob* operon and that synthesis of this form is controlled by enzymes in the *pdu* operon. I isolated mutants defective in B12 repression, but rather than identifying a traditional repressor protein, I found that these mutations mapped to a regulatory region at the 5’ end of the *cob* mRNA. B12 directly regulates folding of this mRNA leader sequence into a secondary structure that controls translation of the *cob* operon.

- Bobik, T.A., **M. Ailion** and J.R. Roth. (1992) A single regulatory gene integrates control of vitamin B12 synthesis and propanediol degradation. J. Bacteriol. 174: 2253-66. PMCID: PMC205846
- **Ailion, M.**, T.A. Bobik and J.R. Roth. (1993) Two global regulatory systems (Crp and Arc) control the cobalamin/propanediol regulon of *Salmonella typhimurium*. J. Bacteriol. 175: 7200-8. PMCID: PMC206861

- Chen, P., **M. Ailion**, T. Bobik, G. Stormo and J. Roth. (1995) Five promoters integrate control of the *cob/pdu* regulon in *Salmonella typhimurium*. J. Bacteriol. 177: 5401-10. PMCID: PMC177344
- **Ailion, M.** and J.R. Roth. (1997) Repression of the *cob* operon of *Salmonella typhimurium* by adenosylcobalamin is influenced by mutations in the *pdu* operon. J. Bacteriol. 179: 6084-91. PMCID: PMC179512

2. **Molecular genetics of dauer formation in *C. elegans***

For my Ph.D. dissertation, I studied the regulation of dauer formation in the nematode *Caenorhabditis elegans*. In response to unfavorable environmental conditions, *C. elegans* arrests development and forms a dauer larva. Dauer formation is regulated by several environmental stimuli, including a pheromone and temperature, and serves as an excellent model system to dissect how a simple nervous system integrates sensory and internal signals to mediate a binary developmental decision. To understand this process at a molecular and cellular level, I performed genetic screens for mutants that affect the dauer decision. I discovered a new environmental stimulus (high temperature) that specifically induces dauer formation and identified a number of new genes involved in this response, including components of a TGF-β pathway and two protein kinases (PDK and AKT that act downstream of insulin signaling. My work helped identify the components of this insulin receptor signal transduction pathway that serves a central role in regulating metabolism and aging.

- **Ailion, M.**, T. Inoue, C.I. Weaver, R.W. Holdcraft and J.H. Thomas. (1999) Neurosecretory control of aging in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. 96: 7394-7397. (Correction: Proc. Natl. Acad. Sci. 96: 10944). PMCID: PMC22096
- Paradis, S., **M. Ailion**, A. Toker, J.H. Thomas and G. Ruvkun. (1999) A PDK1 homolog is necessary and sufficient to transduce AGE-1 PI3 kinase signals that regulate diapause in *Caenorhabditis elegans*. Genes Dev. 13: 1438-1452. PMCID: PMC316759
- **Ailion, M.** and J.H. Thomas. (2000) Dauer formation induced by high temperatures in *Caenorhabditis elegans*. Genetics 156: 1047-1067. PMCID: PMC1461313
- **Ailion, M.** and J.H. Thomas. (2003) Isolation and characterization of high-temperature-induced dauer formation mutants in *Caenorhabditis elegans*. Genetics 165: 127-144. PMCID: PMC1462745

3. Molecular genetics of natural variation and speciation in *Caenorhabditis*

Though *C. elegans* had been extensively used as a model organism in the lab, there was little work studying evolution or natural variation in this species. I have helped establish *C. elegans* as a model for studying the genetic basis of evolutionary processes such as natural variation and speciation. I first undertook a systematic analysis of natural variation in the dauer-related traits of temperature and pheromone sensitivity. I surprisingly found little variation in the thermal limit among different isolates of the species regardless of their geographic origin, but ample variation in pheromone response. My colleagues and I identified the mutations that cause pheromone insensitivity in several lab-evolved strains and determined their cellular mechanisms. We showed that specific selection pressures may lead to reproducible parallel evolutionary changes within and between species, indicating that evolution may not be as random as generally thought.

More recently, I analyzed genetic incompatibility within *C. elegans* in an effort to identify genes involved in incipient speciation events. Although post-zygotic reproductive isolation occurs by the accumulation of genetic incompatibilities between populations, few incompatibility genes have been identified molecularly in any species. As a postdoc, I discovered a fascinating genetic incompatibility between two strains of *C. elegans* mediated by a novel selfish genetic element consisting of a sperm-expressed paternal effect toxin and its zygotically-expressed antidote. In my lab, we continue to work on the cellular basis of this incompatibility and have begun studying hybrid incompatibility in other *Caenorhabditis* species. We have recently discovered a cytoplasmic-nuclear incompatibility in the male-female species *C. nouraguensis* and are characterizing its molecular mechanism. We also discovered that hybridization between *C. nouraguensis* and its sister species *C. becei* promotes asexual reproduction and have characterized the genetic mechanism by which diploid eggs are produced. These projects have been aided greatly by our finding that rotten fruit is the natural habitat of *Caenorhabditis* nematodes in the wild, which spurred an international effort that has led to the discovery and isolation of dozens of new species in this genus.

- McGrath, P.T., Y. Xu, **M. Ailion**, J. Garrison, R.A. Butcher and C.I. Bargmann. (2011) Parallel evolution of domesticated Caenorhabditis species targets pheromone receptor genes. Nature 477: 321-325. PMCID: PMC3257054

- Seidel, H.S., **M. Ailion**, J. Li, A. van Oudenaarden, M.V. Rockman and L. Kruglyak. (2011) A novel sperm-delivered toxin causes late-stage embryo lethality and transmission ratio distortion in *C. elegans*. PLOS Biol. 9: e1001115. PMCID: PMC3144186 **Cover article*
- Lamelza, P. and **M. Ailion**. (2017) Cytoplasmic-nuclear incompatibility between wild-isolates of *Caenorhabditis nouraguensis*. G3 7: 823-834. PMCID: PMC5345712
- Lamelza, P., J.M. Young, L.M. Noble, L. Caro, A. Isakharov, M. Palanisamy, M.V. Rockman, H.S. Malik and **M. Ailion**. (2019) Hybridization promotes asexual reproduction in *Caenorhabditis* nematodes. PLOS Genet. 15: e1008520. PMCID: PMC6946170

4. Molecular and cellular mechanisms of dense-core vesicle biogenesis

A current major project in my laboratory is to identify and explain cellular and molecular mechanisms of neuromodulator function. Despite their broad biological and medical importance, surprisingly little is known about the mechanisms of neuromodulator release and the signaling pathways that mediate the response to these compounds. Neuromodulators are released from a unique organelle, the dense-core vesicle (DCV), and typically activate G protein-coupled receptors and second messenger pathways. However, the cell biology and biochemistry of these critical pathways remain poorly defined. We have used forward genetic screens in the nematode *C. elegans* to identify proteins involved in DCV biogenesis and release. We identified two new effectors of the Rab2 small GTPase (RUND-1 and CCCP-1), as well as the endosomal trafficking complex EARP and its interactor EIPR-1. We are currently working out the molecular and cellular mechanisms by which these proteins act using *C. elegans* and mammalian cells, with a focus on insulin secretion.

- **Ailion, M.***, M. Hannemann, S. Dalton, A. Pappas, S. Watanabe, J. Hegermann, Q. Liu, H-F. Han, M. Gu, M.Q. Goulding, N. Sasidharan, K. Schuske, P. Hullett, S. Eimer and E.M. Jorgensen*. (2014) Two Rab2 interactors regulate dense-core vesicle maturation. Neuron 82: 167-180. PMCID: PMC3997996 **Corresponding authors.*
- Topalidou, I., J. Cattin-Ortolá, A.L. Pappas, K. Cooper, G.E. Merrihew, M.J. MacCoss and **M. Ailion**. (2016) The EARP complex and its interactor EIPR-1 are required for cargo sorting to dense-core vesicles. PLOS Genet. 12: e1006074. PMCID: PMC4871572
- Cattin-Ortolá, J., I. Topalidou, A. Dosey, A.J. Merz and **M. Ailion**. (2017) The dense-core vesicle maturation protein CCCP-1 binds RAB-2 and membranes through its C-terminal domain. Traffic 18: 720-732. PMCID: PMC5650531
- Topalidou, I, J. Cattin-Ortolá, B. Hummer, C.S. Asensio and **M. Ailion**. (2020) EIPR1 controls dense-core vesicle cargo retention and EARP complex localization in insulin-secreting cells. Mol. Biol. Cell 31:59-79. PMCID: PMC6938272

5. A new Gq signal transduction pathway

From a screen for suppressors of an activated Gq mutant in *C. elegans*, we identified a new signal transduction pathway downstream of Gq that acts in parallel to the canonical phospholipase Cβ pathway. This new pathway is conserved and consists of the RhoGEF Trio, the small GTPase Rho, and culminates in activation of the NCA/NALCN ion channel, a novel channel of the voltage-gated cation channel family that may serve as a sodium leak channel to help set the resting membrane potential. We have additionally shown that this pathway is regulated by dopamine and that the output of this pathway is modulated by an ERK MAP kinase signaling pathway that surprisingly operates independently of its canonical trigger, the small GTPase Ras. We are currently identifying other components of these pathways and determining how they regulate the NCA channel.

- Williams, S.L., S. Lutz, N.K. Charlie, C. Vettel, **M. Ailion**, C. Coco, J.J. Tesmer, E.M. Jorgensen, T. Wieland and K.G. Miller. (2007) Trio's Rho-specific GEF domain is the missing $G\alpha_q$ effector in *C. elegans*. Genes Dev. **21:** 2731-2746. PMCID: PMC2045128
- Topalidou, I., P.-A. Chen, K. Cooper, S. Watanabe, E.M. Jorgensen and **M. Ailion**. (2017) The NCA-1 and NCA-2 ion channels function downstream of Gq and Rho to regulate locomotion in *Caenorhabditis elegan*s. Genetics 206: 265-282. PMCID: PMC541947
- Topalidou, I., K. Cooper, L. Pereira and **M. Ailion** (2017) Dopamine negatively modulates the NCA ion channels in *C. elegans*. PLOS Genet. 13: e1007032. PMCID: PMC5638609
- Coleman, B, I. Topalidou and **M. Ailion** (2018) Modulation of Gq-Rho signaling by the ERK MAPK pathway controls locomotion in *Caenorhabditis elegans*. Genetics 209: 523-535. PMCID: PMC5972424

Complete List of Published Work:

https://www.ncbi.nlm.nih.gov/myncbi/1DoCiiV3f2DAV/bibliography/public/?sortby=pubDate&sdirection=descending

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: BAKER, DAVID

eRA COMMONS USER NAME (credential, e.g., agency login): DABAKER

POSITION TITLE: Professor of Biochemistry, University of Washington; Investigator, Howard Hughes Medical Institute (HHMI); Adjunct Professor of Computer Science, Physics, Genome Sciences, Bioengineering, Chemical Engineering

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
Harvard University, Cambridge, MA	BA	1984	Biology
Univ of California, Berkeley, CA	PHD	12/1989	Biochemistry
Univ of California, San Francisco, CA	Postdoctoral Fellow	1993	Biophysics

A. Personal Statement

My colleagues and I have developed the Rosetta computational methodology for predicting and designing macromolecular structures, interactions, and functions. We have used this methodology to predict the structures and interactions of naturally occurring biomolecules, and to design new proteins with new structures, interactions and functions. We have made substantial advances in ab initio protein structure prediction, and more recently the generation of high-resolution structure models from limited co-evolution experimental data.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2012 -	Director, Institute for Protein Design, University of Washington, Seattle, WA
2005 -	Investigator, Howard Hughes Medical Institute
2004 -	Professor, University of Washington, Department of Biochemistry, Seattle, WA
2004 -	Adjunct Professor of Bioengineering, Computer Science, Genome Sciences, Chemical Engineering, and Physics, University of Washington, Seattle, WA
2000 - 2005	Associate Investigator, Howard Hughes Medical Institute
2000 - 2004	Associate Professor, University of Washington, Department of Biochemistry, Seattle, WA
1993 - 2000	Assistant Professor, University of Washington, Department of Biochemistry, Seattle, WA

Honors

2021	Prize in Life Sciences, Breakthrough
2020	Fellow, American Institute for Medical and Biological Engineering
2018	Public Lecture, International Solvay Institutes
2018	Hans Neurath Award, Protein Society
2017	Henrietta and Aubrey Davis Endowed Professorship in Biochemistry, University of Washington
2014	Perlman Memorial Award, American Chemical Society
2012	Centenary Award, Biochemical Society
2011	Inventor of the Year Award, University of Washington
2009	Member, American Academy of Sciences
2008	Sackler Prize in Biophysics, Tel Aviv University
2007	Editorial Board, Proceedings of the National Academy of Sciences
2006	Member, National Academy of Sciences
2004	Feynman Prize, Foresight Institute
2004	Newcomb Cleveland Prize, American Academy of Sciences

2002	Overton Prize, International Society for Computational Biology
2000	Young Investigator Award, Beckman
1994	Fellowship in Science and Engineering, Packard
1994	Young Investigator Award, National Science Foundation
1993	Fellowship, Boyer Foundation

C. Contribution to Science

1. **How proteins fold.** We investigated the extent to which amino acid sequence determines protein folding rates by using selection methods to identify very different sequences that folded to the same structure. While the stabilities of these proteins were generally less than the native protein, the folding rates were as often faster as slower, suggesting that evolution has not optimized sequences for rapid folding. Since introducing substantial sequence variation did not significantly affect protein folding rates, we sought other factors that determined the rate of folding. A series of experimental and computational analyses established that protein folding rates and mechanism are largely determined by the topology of the native structure. A particularly notable observation was that protein folding rates are strongly correlated with the contact order of the native structure (the sequence separation between contacting residues in the protein structure), with low contact order proteins folding orders of magnitude faster than high contact order proteins.
   a. Rocklin GJ, Chidyausiku TM, Goreshnik I, Ford A, Houliston S, Lemak A, Carter L, Ravichandran R, Mulligan VK, Chevalier A, Arrowsmith CH, Baker D. Global analysis of protein folding using massively parallel design, synthesis, and testing. Science. 2017 Jul 14;357(6347):168-175. PubMed Central PMCID: PMC5568797.
   b. Riddle DS, Grantcharova VP, Santiago JV, Alm E, Ruczinski I, Baker D. Experiment and theory highlight role of native state topology in SH3 folding. Nat Struct Biol. 1999 Nov;6(11):1016-24. PubMed PMID: 10542092.
   c. Alm E, Baker D. Prediction of protein-folding mechanisms from free-energy landscapes derived from native structures. Proc Natl Acad Sci U S A. 1999 Sep 28;96(20):11305-10. PubMed Central PMCID: PMC18029.
   d. Plaxco KW, Simons KT, Baker D. Contact order, transition state placement and the refolding rates of single domain proteins. J Mol Biol. 1998 Apr 10;277(4):985-94. PubMed PMID: 9545386.

2. **Protein structure prediction and determination of macromolecular structure from sparse data sets.** Guided by the insights gained in our studies of protein folding mechanism, we developed the Rosetta ab initio structure prediction methodology which builds protein structures by fragment assembly. The CASP blind structure prediction experiments showed that the Rosetta protein structure prediction methodology was a significant improvement over previous approaches. We developed methods for efficiently refining protein models in a physically realistic all atom potential, coupled this with lower resolution conformational search methods, and showed that not only monomeric protein structures but also protein-protein complexes (the docking problem), higher order symmetric protein assemblies, membrane proteins, and RNA structures could be modeled accurately by searching for the lowest energy state provided the space to be searched was not too large. We then showed that the Rosetta approach could generate quite accurate models of more complex systems when provided with limited experimental data to guide conformational sampling. Rosetta supplemented with experimental data has become a powerful and widely used approach to solve macromolecular structures using sparse NMR data (CS-Rosetta), low-resolution x-ray diffraction data (MR-Rosetta), cryo-electron microscopy data, and co-evolution sequence information (Gremlin-Rosetta).
   a. Ovchinnikov S, Park H, Varghese N, Huang PS, Pavlopoulos GA, Kim DE, Kamisetty H, Kyrpides NC, Baker D. Protein structure determination using metagenome sequence data. Science. 2017 Jan 20;355(6322):294-298. PubMed Central PMCID: PMC5493203.
   b. DiMaio F, Terwilliger TC, Read RJ, Wlodawer A, Oberdorfer G, Wagner U, Valkov E, Alon A, Fass D, Axelrod HL, Das D, Vorobiev SM, Iwaï H, Pokkuluri PR, Baker D. Improved molecular replacement by density- and energy-guided protein structure optimization. Nature. 2011 May 26;473(7348):540-3. PubMed Central PMCID: PMC3365536.
   c. Raman S, Lange OF, Rossi P, Tyka M, Wang X, Aramini J, Liu G, Ramelot TA, Eletsky A, Szyperski T, Kennedy MA, Prestegard J, Montelione GT, Baker D. NMR structure determination for larger proteins

using backbone-only data. Science. 2010 Feb 19;327(5968):1014-8. PubMed Central PMCID: PMC2909653.
   d. Gray JJ, Moughon SE, Kortemme T, Schueler-Furman O, Misura KM, Morozov AV, Baker D. Protein-protein docking predictions for the CAPRI experiment. Proteins. 2003 Jul 1;52(1):118-22. PubMed PMID: 12784377; NIHMSID: NIHMS308621.

3. **Design of protein structure and immunogens**. The Rosetta structure prediction methodology described above searches for the lowest energy structure for a given sequence, and we realized that we could invert the process to search for the lowest energy sequence for a desired structure—the protein design problem. We demonstrated proof-of-concept for de novo protein design with the design of TOP7, a novel protein with a fold not found in nature, and later developed general principles for designing hyperstable idealized alpha-beta proteins and helical bundles. With the capability of designing stable protein structures in hand, we developed methods for stabilizing both linear and complex epitopes from pathogen proteins, and showed that these could elicit neutralizing antibodies in animals, opening up computational design approaches to developing improved vaccines.
   a. Hosseinzadeh P, Bhardwaj G, Mulligan VK, Shortridge MD, Craven TW, Pardo-Avila F, Rettie SA, Kim DE, Silva DA, Ibrahim YM, Webb IK, Cort JR, Adkins JN, Varani G, Baker D. Comprehensive computational design of ordered peptide macrocycles. Science. 2017 Dec 15;358(6369):1461-1466. PubMed Central PMCID: PMC5860875.
   b. Correia BE, Bates JT, Loomis RJ, Baneyx G, Carrico C, Jardine JG, Rupert P, Correnti C, Kalyuzhniy O, Vittal V, Connell MJ, Stevens E, Schroeter A, Chen M, Macpherson S, Serra AM, Adachi Y, Holmes MA, Li Y, Klevit RE, Graham BS, Wyatt RT, Baker D, Strong RK, Crowe JE Jr, Johnson PR, Schief WR. Proof of principle for epitope-focused vaccine design. Nature. 2014 Mar 13;507(7491):201-6. PubMed Central PMCID: PMC4260937.
   c. Koga N, Tatsumi-Koga R, Liu G, Xiao R, Acton TB, Montelione GT, Baker D. Principles for designing ideal protein structures. Nature. 2012 Nov 8;491(7423):222-7. PubMed Central PMCID: PMC3705962.
   d. Kuhlman B, Dantas G, Ireton GC, Varani G, Stoddard BL, Baker D. Design of a novel globular protein fold with atomic-level accuracy. Science. 2003 Nov 21;302(5649):1364-8. PubMed PMID: 14631033.

4. **Design of small molecule binding, catalysis, protein interactions, and self-assembly**. A grand challenge in computational protein design is creating new binding proteins de novo for use in therapeutics and diagnostics. We have developed general methods for designing proteins which bind with high affinity/specificity to sites of interest of therapeutic importance on protein targets, both human and pathogen (influenza, bacterial toxins, oncogenic proteins). We developed general methods for designing catalysts for arbitrary chemical reactions starting from a description of the reaction transition state geometry, and used the approach to design catalysts for a number of reactions not catalyzed by naturally occurring enzymes. We have developed an approach to computationally designing self-assembling nanomaterials and used it to design new proteins that self assemble into regular tetrahedral, octahedral and icosahedral structures as well as two dimensional layers with near atomic-level accuracy. We are now developing these self-assembling materials for targeted delivery and vaccine applications.
   a. Chevalier A, Silva DA, Rocklin GJ, Hicks DR, Vergara R, Murapa P, Bernard SM, Zhang L, Lam KH, Yao G, Bahl CD, Miyashita SI, Goreshnik I, Fuller JT, Koday MT, Jenkins CM, Colvin T, Carter L, Bohn A, Bryan CM, Fernández-Velasco DA, Stewart L, Dong M, Huang X, Jin R, Wilson IA, Fuller DH, Baker D. Massively parallel de novo protein design for targeted therapeutics. Nature. 2017 Oct 5;550(7674):74-79. PubMed Central PMCID: PMC5802399.
   b. King NP, Bale JB, Sheffler W, McNamara DE, Gonen S, Gonen T, Yeates TO, Baker D. Accurate design of co-assembling multi-component protein nanomaterials. Nature. 2014 Jun 5;510(7503):103-8. PubMed Central PMCID: PMC4137318.
   c. Tinberg CE, Khare SD, Dou J, Doyle L, Nelson JW, Schena A, Jankowski W, Kalodimos CG, Johnsson K, Stoddard BL, Baker D. Computational design of ligand-binding proteins with high affinity and selectivity. Nature. 2013 Sep 12;501(7466):212-216. PubMed Central PMCID: PMC3898436.
   d. Siegel JB, Zanghellini A, Lovick HM, Kiss G, Lambert AR, St Clair JL, Gallaher JL, Hilvert D, Gelb MH, Stoddard BL, Houk KN, Michael FE, Baker D. Computational design of an enzyme catalyst for a stereoselective bimolecular Diels-Alder reaction. Science. 2010 Jul 16;329(5989):309-13. PubMed Central PMCID: PMC3241958.

5. **Involving the general public in Science.** We created a distributed computing project called Rosetta@home in which volunteers donate spare cycles on their computers to carry out protein folding trajectories. We extended Rosetta@home to the interactive multiplayer online game Foldit which allows players to guide the course of the protein structure prediction and design calculations. By relying on human intuition and 3-D problem solving skills, Foldit players have made a number of important contributions: solved the structure a retroviral protease, developed new algorithms for finding low-energy protein conformations, and designed a novel synthetic enzyme by large-scale redesign of the active site.
    a. Cooper S, Khatib F, Baker D. Increasing public involvement in structural biology. Structure. 2013 Sep 3;21(9):1482-4. PubMed PMID: 24010706.
    b. Eiben CB, Siegel JB, Bale JB, Cooper S, Khatib F, Shen BW, Players F, Stoddard BL, Popovic Z, Baker D. Increased Diels-Alderase activity through backbone remodeling guided by Foldit players. Nat Biotechnol. 2012 Jan 22;30(2):190-2. PubMed Central PMCID: PMC3566767.
    c. Khatib F, Cooper S, Tyka MD, Xu K, Makedon I, Popovic Z, Baker D, Players F. Algorithm discovery by protein folding game players. Proc Natl Acad Sci U S A. 2011 Nov 22;108(47):18949-53. PubMed Central PMCID: PMC3223433.
    d. Khatib F, DiMaio F, Cooper S, Kazmierczyk M, Gilski M, Krzywda S, Zabranska H, Pichova I, Thompson J, Popović Z, Jaskolski M, Baker D. Crystal structure of a monomeric retroviral protease solved by protein folding game players. Nat Struct Mol Biol. 2011 Sep 18;18(10):1175-7. PubMed Central PMCID: PMC3705907.

Complete list of published work in MyBibliography:
https://www.ncbi.nlm.nih.gov/sites/myncbi/david.baker.2/bibliography/40635205/public/

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Brockerhoff, Susan Elizabeth

eRA COMMONS USER NAME (credential, e.g., agency login): SBROCKERHOFF

POSITION TITLE: Professor of Biochemistry

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
City College of NY, NYC	B.S.	01/1987	Biochemistry
University of Washington, Seattle	Ph.D.	03/1993	Biochemistry
Harvard University, MA	Postdoctoral	12/1996	Molecular Cell Biology Photoreceptor Function

A. Personal Statement

My lab uses biochemical, genetic, cell biological and physiological approaches to address important unanswered questions in photoreceptor biology. I have a long-standing interest in dissecting mechanisms associated with photoreceptor degeneration. We exploit the experimental advantages of the zebrafish model organism by using our expertise with live imaging to analyze molecular events at subcellular resolution in single cells. I was trained in genetics and biochemistry working with yeast as a graduate student with Trisha Davis. As a postdoctoral fellow I worked with John Dowling and conducted the first behavioral screen in zebrafish that successfully identified mutations that affect cone photoreceptor function. I have broad experimental training and many years experience as a primary PI on research projects. I have an active research program and I have trained many students and postdoctoral fellows since joining the Biochemistry Department as a faculty member in 1997.

B. Positions and Honors

1997- 2007 Research Assistant Professor of Biochemistry, University of Washington, Seattle, WA
2007- 2013 Associate Professor of Biochemistry, University of Washington, Seattle, WA
2013- current Professor of Biochemistry, University of Washington, Seattle, WA

Other Experience and Professional Memberships

1995-current	Member, ARVO
1995-current	Member, Society for Neuroscience
1998	NSF Peer Review Committee adhoc reviewer
2005	NIH Peer Review Committee adhoc reviewer: Zebrafish Genetic Tools
2008-2011	NIH Peer Review Committee adhoc reviewer: F05, Cell Biology and Development
2010	Reviewer for Research Corporation for Science Advancement
2011	Grant Reviewer for Fight for Sight, UK
2011	NIH Peer Review Committee adhoc reviewer: BDPE (NEI) and F03A, Neurodevelopment, Synaptic Plasticity and Neurodegeneration
2011-2014	Faculty representative on the IACUC at UW
2012	NIH Peer Review Committee adhoc reviewer: ZRG1 CB-Z(55 and 56) and SEP, ZRG1 CB-G(02), Zebrafish Genetic Tools and Screens.
2013, 2014	NIH Peer Review Committee adhoc reviewer: Cell Biology, Developmental Biology, and Bioengineering, F05-D(21)
2013, 2015	NIH Peer Review Committee adhoc reviewer; BVS (NEI)
2016-2019	NIH Peer Review Committee regular member; BVS (NEI)
2019-2020	Chair, NIH Peer Review Committee, BVS (NEI)
2021	NIH Peer Review, ZGR1 CB-H (55) MIRA for ESIs (R35) SEP (NIGMS)

Honors

1987	Graduated CCNY summa cum laude
1994-1996	awarded F32 NS009611
1996-1997	awarded F32 EY006762
2018	Invited speaker for Distinguished Lecture Series Program at the Cole Eye Institute

C. Contributions to Science

1. My career in vision science began when I was a postdoctoral fellow. I joined John Dowling's lab to initiate a genetic screen for zebrafish vision mutants. I was inspired by the pioneering work done in *Drosophila* by William Pak and Seymour Benzer. I tested several visual behavioral assays and chose the measurement of the optokinetic kinetic response in larvae as a primary screen mainly because this behavior is sensitive and reproducible. The screen was targeted to identify cone mutations since only cone photoreceptors are functional in young zebrafish larvae. I initially screened mutagenized larvae generated in Wolfgang Driever's lab (then at MGH) and then conducted a genetic screen in John Dowling's lab. I analyzed thousands of mutagenized larvae and identified several mutants that had defective optokinetic responses. Together with colleagues in the Dowling lab, I developed a method for recording electroretinogram responses from larval zebrafish to identify the subset of mutations that altered the function of the outer retina. This was the first behavioral screen done in zebrafish that successfully identified vision mutations. The screen and zebrafish mutants I identified are described in the following peer-reviewed publications.

   Brockerhoff SE, Hurley JB, Janssen-Bienhold U, Neuhauss CF, Driever W, Dowling JE (1995) A behavioral screen for isolating zebrafish mutants with visual system defects. Proc Natl Acad Sci USA 92:10545-10549.

   Brockerhoff SE, Hurley JB, Niemi GA, Dowling JE (1997) A new form of inherited red-blindness identified in zebrafish. J Neurosci 20:1-8.

   Fadool JM, Brockerhoff SE, Hyatt GA, Dowling JE (1997) Mutations affecting eye morphology in the developing zebrafish *(Danio rerio)*. Developmental Genetics 20:1-8.

   Allwardt BA, Lall AB, Brockerhoff SE, Dowling JE (2001) Synapse formation is arrested in retinal photoreceptors of the zebrafish nrc mutant. J Neurosci 21:2330-2342.

2. At the University of Washington, we continued identifying vision mutants, determined the molecular identity of mutations, and conducted detailed characterizations of mutants. We successfully identifed the causal genetic lesions in 7 different mutants. To do this I set up and implemented all of the gene mapping and identification methods in my own lab. Our findings revealed novel functions for these genes. We found mutations in genes that altered a variety of key functions of cone photoreceptors, including synaptic transmission, phototransduction, endocytic trafficking, metabolism and signal processing. Our mutants have become a widely used resource for the vision community. The following are some of the key publications that describe those studies.

    Van Epps HA, Hayashi M, Lucast L, Stearns GW, Hurley JB, De Camilli P, Brockerhoff SE (2004) The zebrafish *nrc* mutant reveals a role for the polyphosphoinositide phosphatase synaptojanin 1 in cone photoreceptor ribbon anchoring. J Neurosci 24:8641-8650.

    Taylor MR, Hurley JB, Van Epps HA, Brockerhoff SE (2004) A zebrafish model for pyruvate dehydrogenase deficiency: rescue of neurological dysfunction and embryonic lethality using a ketogenic diet. Proc Natl Acad Sci U S A 101:4584-4589.

    Stearns G, Evangelista M, Fadool JM, Brockerhoff SE (2007) A mutation in the cone-specific pde6 gene causes rapid cone photoreceptor degeneration in zebrafish. J Neurosci 27:13866-13874.

    Lewis AA, Wilson N, Stearns G, Johnson N, Nelson R, Brockerhoff SE (2011) Celsr3 is required for normal development of GABA circuits in the inner retina. PLoS Genetics Aug;7(8):e1002239 PMC3154962

3. Key scientific findings from our mutant characterizations combined with technological advances motivated several studies of cone photoreceptor function and signalling. These projects have exploited the experimental strategies available in zebrafish such as the ability to easily generate transgenic strains and conduct live imaging experiments. For example, in our analysis of mechanisms of cone cell degeneration, we used blastula stage transplants and generated mosaic retinas to determine whether degenerating cones could influence the health and viability of neighboring healthy cones. This study revealed that cone to cone mediated death is fundamentally different than rod to cone mediated death. We also expressed genetically-encoded Ca^{2+} sensors in cones and tested the hypothesis that excessive intracellular Ca^{2+} levels within the cell body cause photoreceptor degeneration induced by Pde6 mutations. For this analysis, we conducted extended time-lapse imaging experiments and correlated the morphological changes occuring in real-time in individual dying cone cells with changes in cell body [Ca^{2+}]. Our results challenge the prevailing view that *rd1* degeneration is driven simply by global [Ca^{2+}] elevations that overload the endogenous Ca^{2+} buffering and clearance mechanisms within the cell body. Our analyses of mechanisms of cell death have important implications for the development of neuroprotection strategies in retinitis pigmentosa models of retinal degeneration.

    Holzhausen LC, Lewis AA, Cheong KK, Brockerhoff SE (2009) Differential role for synaptojanin 1 in rod and cone photoreceptors. J Comp Neurol 517:633-644. PMC3071606

    Lewis A, Williams P, Lawrence O, Wong RO, Brockerhoff SE (2010) Wild-type cone photoreceptors persist despite neighboring mutant cone degeneration. J Neurosci 30:382-389. PMC280545

    Ma EY, Lewis A, Barabas P, Stearns G, Suzuki S, Krizaj D, Brockerhoff SE (2013) Loss of Pde6 reduces cell body Ca^{2+} transients within photoreceptors. Cell Death Dis. Sep 12;4:e797. PMC3789190

    George AA, Hayden S, Stanton G, Brockerhoff SE (2016) Arf6 and the 5'phosphatase of Synaptojanin 1 regulate autophagy in cone photoreceptors. Inside Cell. Apr;1(2):117-133. PMC4844074

4. A recent and ongoing scientific study is to examine how photoreceptor mitochondria integrate changes in $[Ca^{2+}]_i$. These investigations were stimulated in part by our work using genetically-encoded Ca^{2+} sensors. We are defining the key role of mitochondria in regulating photoreceptor Ca^{2+} homeostasis and we are discovering unique functions for the mitochondrial calcium uniporter in retina and in rod verses cone photoreceptors.

   Giarmarco MM, Cleghorn WM, Sloat SR, Hurley JB, Brockerhoff SE (2017) Mitochondria maintain distinct Ca^{2+} pools in cone photoreceptors. J Neurosci 23: 2689-16. PMC5338755

   Giarmarco MM, Brock DC, Robbings BM, Cleghorn WM, Tsantilas KA, Kuch KC, Ge W, Rutter KM, Parker ED, Hurley JB, Brockerhoff SE (2020) Daily mitochondrial dynamics in cone photoreceptors. Proc Natl Acad Sci U S A. (2020) Nov 17;117(46):28816-28827. PMC7682359.

   Hutto RA, Bisbach CM, Abbas F, Brock DC, Cleghorn WM, Parker ED, Bauer BH, Ge W, Vinberg F, Hurley JB, & Brockerhoff SE (2020) Increasing Ca^{2+} in Photoreceptor Mitochondria Alters Metabolites, Accelerates Photoresponse Recovery, and Reveals Adaptations to Mitochondrial Stress Cell Death and Differentiation. Cell Death Differ. Mar;27(3):1067-1085. PMC7206026

   Bisbach CM, Hutto RA, Poria D, Cleghorn WM, Abbas F, Vinberg F, Kefalov VJ, Hurley JB, Brockerhoff SE (2020) Mitochondrial Calcium Uniporter (MCU) deficiency reveals an alternate path for Ca^{2+} uptake in photoreceptor mitochondria. Sci Rep. Sep 29;10(1):16041. doi: 10.1038/s41598-020-72708-x. PMC7525533.

For a more complete bibliography, copy and paste the following URL into your web browser.
http://www.ncbi.nlm.nih.gov/sites/myncbi/susan.brockerhoff.1/bibliography/40787546/public/?sort=date&direction=ascending

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing

R01EY026020 Brockerhoff (MPI with Hurley) 01/01/2016 – 12/31/2024
Photoreceptor mitochondria and Ca^{2+} Dynamics
Specific Aim 1: Define daily and circadian changes in photoreceptor mitochondria function and structure.
Specific Aim 2: Evaluate mitochondrial clearance and biogenesis in wild type zebrafish cones.
Specific Aim 3: Examine the fate of damaged mitochondria due to elevated matrix Ca2+ or mitochondrial dysfunction.

R21EY031546 Brockerhoff (MPI with Kollman) 04/01/2020 – 03/31/2022
IMPDH structure and function in retinal degeneration
Specific Aim 1: Conduct biochemical and structural studies for IMPDH1 mutations.
Specific Aim 2: Characterize cellular, vision, and metabolic phenotypes of Y12A, R105W, R224P and IMPDH1a KO zebrafish compared to WT.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: BRZOVIC, PETER S

eRA COMMONS USER NAME (credential, e.g., agency login): PBRZOVIC

POSITION TITLE: ASSOCIATE PROFESSOR

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of California, San Diego	BA	06/1984	Chemistry
University of California, Riverside	PHD	10/1991	Biochemistry

A. Personal Statement

As a faculty member, graduate student education and training is one of our most important duties. In return, the contributions that graduate students make toward our ongoing research efforts are immeasurable, and so I've worked to support the Department of Biochemistry graduate program in several ways. I've mentored or co-mentored at least 12 graduate students, helping to develop research projects and guide them through graduate school toward their degree. I have been a member of the graduate faculty since 2008 and served on the thesis committees for five students. From 2010-2017 I served on the Graduate Education Committee and am served on the Graduate Student Admissions Committee until 2020. I've been an active participant in Bioc540, a literature review course that covers current topics in Biochemistry and aims to develop students critical thinking and writing skills. Finally, I founded the Seattle Ubiquitin Research Group which brings researchers throughout the greater Seattle area together to meet bi-monthly to share and discuss current research in Ubiquitin signaling. A primary goal of this group is to give graduate students and post-docs a forum to highlight their ongoing work and interact with the broader scientific community.

My research at the University of Washington builds on my long-standing interests in understanding protein structure and function, the allosteric regulation of protein activity, and deciphering networks of protein interactions involved in the assembly of macromolecular complexes. Much of my present research focuses on 1) the structure and function of the breast and ovarian cancer tumor suppressor protein BRCA1 and 2) mechanisms of assembly that govern the function of macromolecular complexes involved in ubiquitin transfer and signaling. Using a variety of structural, biophysical, and biochemical approaches we have begun to understand key interactions important in the assembly of various protein complexes critical to Ubiquitin signaling pathways. Recently, this effort has expanded to include the structures and biological activities of effector proteins from pathogenic bacteria that integrate into or hijack eukaryotic ubiquitin signaling systems. These bacterial systems provide a great deal of insight not only into mechanisms of bacterial pathogenesis, but also about the eukaryotic pathways that they target.

1. Brzovic PS, Rajagopal P, Hoyt DW, King MC, Klevit RE. Structure of a BRCA1-BARD1 heterodimeric RING-RING complex. Nat Struct Biol. 2001 Oct;8(10):833-7. PubMed PMID: 11573085.
2. Pruneda JN, Littlefield PJ, Soss SE, Nordquist KA, Chazin WJ, Brzovic PS, Klevit RE. Structure of an E3:E2~Ub complex reveals an allosteric mechanism shared among RING/U-box ligases. Mol Cell. 2012 Sep 28;47(6):933-42. doi: 10.1016/j.molcel.2012.07.001. Epub 2012 Aug 9. PubMed PMID: 22885007; PubMed Central PMCID: PMC3462262.

3. DaRosa PA, Harrison JS, Zelter A, Davis TN, Brzovic P, Kuhlman B, Klevit RE. (2018) A Bifunctional Role for the UHRF1 UBL Domain in the Control of Hemi-methylated DNA-Dependent Histone Ubiquitylation. Mol Cell. 72(4):753-765. Epub 2018 Nov 1. PMID: 30392931 PMCID: PMC6239910
4. Witus SR, Burrell AL, Farrell DP, Kang J, Wang M, Hansen JM, Pravat A, Tuttle LM, Stewart MD, Brzovic PS, Chatterjee C, Zhao W, DiMaio F, Kollman JM, Klevit RE. BRCA1/BARD1 site-specific ubiquitylation of nucleosomal H2A is directed by BARD1. Nat Struct Mol Biol. 2021 Mar;28(3):268-277. doi: 10.1038/s41594-020-00556-4. Epub 2021 Feb 15. PubMed PMID: 33589814; PubMed Central PMCID: PMC8007219.

For a full publication list, see https://www.ncbi.nlm.nih.gov/myncbi/peter.brzovic.1/bibliography/public/

B. Positions and Honors

Positions and Employment

1991 - 1993	Post-Graduate Trainee, Training Program in Cellular and Molecular Endocrinology, Dept. of Biochemistry, University of California Riverside
1993 - 1998	Postdoctoral Fellow, Department of Biochemistry, University of Washington
1998 - 2006	Research Scientist, Department of Biochemistry, University of Washington
2007 - 2013	Research Assistant Professor, Department of Biochemistry, University of Washington
2013 – 2020	Research Associate Professor, Department of Biochemistry, University of Washington
2020 - current	Associate Professor, Without Tenure, Dept. of Biochemistry, University of Washington

Other Experience and Professional Memberships

2012 - current	Member, American Association for the Advancement of Science (AAAS)

Honors

1985	Graduate Fellowship, University of California, Riverside
1989	Graduate Student Association Council President, Dept. of Biochemistry, Univ. of Calif., Riverside
1990	Scholarship for Young Participants from Abroad, 8th International Symposium on Vitamin B6 and Carbonyl Catalysis
1991	Fellowship from UCR Training Program in Cellular and Molecular Endocrinology, University of California, Riverside
1995	American Cancer Society Fellowship, American Cancer Society

C. Contributions to Science

1. Understanding the structure and function of BRCA1, the breast and ovarian cancer tumor suppressor protein. Solving the NMR structure of the BRCA1/BARD1 heterodimeric RING E3 ligase provided the first structural insights into a region of the breast tumor suppressor protein that harbors the most frequent and penetrant cancer-associated mutations. This was also the first structure of a heterodimeric RING-RING E3 Ubiquitin ligase complex. Multiple strategies were derived from this work to interrogate the function of BRCA1 *in vivo*, including the development of general structure-based mutations that selectively disrupt specific BRCA1 E3 interactions. The solution structure of the BRCA1/BARD1 RING heterodimer was the catalyst for our further studies on Ubiquitin transfer reactions and subsequent contributions to the field advancing our understanding of the function of RING E3 ligases and their E2 ubiquitin conjugating enzyme partners. This effort is rapidly expanding to understanding the interaction of BRCA1/BARD1 with nucleosomes and the role of BRCA1's E3 ligase activity in epigenetic regulation.

   a. Witus SR, Burrell AL, Farrell DP, Kang J, Wang M, Hansen JM, Pravat A, Tuttle LM, Stewart MD, Brzovic PS, Chatterjee C, Zhao W, DiMaio F, Kollman JM, Klevit RE. BRCA1/BARD1 site-specific ubiquitylation of nucleosomal H2A is directed by BARD1. Nat Struct Mol Biol. 2021 Mar;28(3):268-277.

doi: 10.1038/s41594-020-00556-4. Epub 2021 Feb 15. PubMed PMID: 33589814; PubMed Central PMCID: PMC8007219.

b. Christensen DE, Brzovic PS, Klevit RE. E2-BRCA1 RING interactions dictate synthesis of mono- or specific polyubiquitin chain linkages. Nat Struct Mol Biol. 2007 Oct;14(10):941-8. PubMed PMID: 17873885.

c. Brzovic PS, Keeffe JR, Nishikawa H, Miyamoto K, Fox D 3rd, Fukuda M, Ohta T, Klevit R. Binding and recognition in the assembly of an active BRCA1/BARD1 ubiquitin-ligase complex. Proc Natl Acad Sci U S A. 2003 May 13;100(10):5646-51. PubMed PMID: 12732733; PubMed Central PMCID: PMC156255.

d. Brzovic PS, Rajagopal P, Hoyt DW, King MC, Klevit RE. Structure of a BRCA1-BARD1 heterodimeric RING-RING complex. Nat Struct Biol. 2001 Oct;8(10):833-7. PubMed PMID: 11573085.

2. Investigation of Ubiquitin transfer complexes and key protein interactions in Ubiquitin signaling pathways. Our work on interactions between RING E3s and activated E2~Ub conjugates helped define the allosteric mechanism that many RING/U-Box Ub E3 ligases utilize to activate E2~Ub conjugates to facilitate Ub transfer. This effort led to the identification of a widely conserved E3 “linchpin” residue and helped solve the long-standing enigma in the Ub field as to how the largest class of ligases activate Ub transfer. This work allowed researchers to introduce mutations that selectively affect Ub-transfer activity without disrupting the assembly of Ub-transfer complexes.

   a. Pruneda JN, Littlefield PJ, Soss SE, Nordquist KA, Chazin WJ, Brzovic PS, Klevit RE. Structure of an E3:E2~Ub complex reveals an allosteric mechanism shared among RING/U-box ligases. Mol Cell. 2012 Sep 28;47(6):933-42. PubMed PMID: 22885007; PubMed Central PMCID: PMC3462262.

3. Characterization of the functional diversity of Ubiquitin-conjugating enzymes. Humans employ ~40 different E2 Ubiquitin conjugating enzymes to manage the transfer of Ub and various Ub-like proteins. Our ongoing studies continue to provide insights into the important functional differences that differentiate these E2s and the various strategies used by E2s and E3s to catalyze Ub transfer and selectively modify substrates. Recent work on the epigenetic regulator UHRF1 uncovered an unexpected strategy for the recruitment of a cognate E2~Ub and described how binding affects domain interactions regulate the assembly of an active E3 ligase complex. We also found that E2s can function outside canonical Ub transfer pathways and perform regulatory roles in mediating protein activity. This is true in human cells, such as the interactions of E2~Ub conjugates with the de-ubiquitinase OTUB1 but is also exploited by pathogenic bacteria during invasion. We found that binding of an E2~Ub conjugate to the bacterial effector protein OspG stabilizes an active conformation of the bacterial kinase that is required as part of its role in mediating pathogenesis.

   a. DaRosa PA, Harrison JS, Zelter A, Davis TN, Brzovic P, Kuhlman B, Klevit RE. (2018) A Bifunctional Role for the UHRF1 UBL Domain in the Control of Hemi-methylated DNA-Dependent Histone Ubiquitylation. Mol Cell. 72(4):753-765. Epub 2018 Nov 1. PMID: 30392931 PMCID: PMC6239910

   b. Stewart MD, Ritterhoff T, Klevit RE, Brzovic PS. E2 enzymes: more than just middle men. Cell Res. 2016 Apr;26(4):423-40. PubMed PMID: 27002219; PubMed Central PMCID: PMC4822130.

   c. Vittal V, Shi L, Wenzel DM, Scaglione KM, Duncan ED, Basrur V, Elenitoba-Johnson KS, Baker D, Paulson HL, Brzovic PS, Klevit RE. Intrinsic disorder drives N-terminal ubiquitination by Ube2w. Nat Chem Biol. 2015 Jan;11(1):83-9. PubMed PMID: 25436519; PubMed Central PMCID: PMC4270946.

   d. Pruneda JN, Smith FD, Daurie A, Swaney DL, Villén J, Scott JD, Stadnyk AW, Le Trong I, Stenkamp RE, Klevit RE, Rohde JR, Brzovic PS. E2~Ub conjugates regulate the kinase activity of Shigella effector OspG during pathogenesis. EMBO J. 2014 Mar 3;33(5):437-49. PubMed PMID: 24446487; PubMed Central PMCID: PMC3989626.

   e. Wenzel DM, Lissounov A, Brzovic PS, Klevit RE. UBCH7 reactivity profile reveals parkin and HHARI to be RING/HECT hybrids. Nature. 2011 Jun 2;474(7349):105-8. PubMed PMID: 21532592; PubMed Central PMCID: PMC3444301.

4. Though bacteria do not employ ubiquitin signaling as a means of intracellular communication, bacterial effector proteins often target or exploit eukaryotic ubiquitin signaling pathways to promote pathogenesis. Understanding the functions and mechanisms of effector proteins provides insight not only into strategies of pathogenesis but also informs our understanding of eukaryotic signaling pathways. Detailed studies of

SspH/IpaH E3 Ubiquitin ligase effectors from *Salmonella* and *Shigella* and various effectors such as SdeA from *Legionella* greatly expands our understanding of mechanisms of Ubiquitin transfer that are helpful to understanding eukaryotic systems and offer potential strategies for intervention in infectious diseases.

   a. Puvar K, Iyer S, Fu J, Kenny S, Negrón Terón KI, Luo ZQ, Brzovic PS, Klevit RE, Das C. Legionella effector MavC targets the Ube2N~Ub conjugate for noncanonical ubiquitination. Nat Commun. 2020 May 12;11(1):2365. PubMed PMID: 32398758; PubMed Central PMCID: PMC7217864.
   b. Matt J. Cook, Scott P. Delbecq, Thomas P. Schweppe, Miklos Guttman, Rachel E. Klevit, and Peter S. Brzovic (2019) The ubiquitin ligase SspH1 from Salmonella uses a modular and dynamic E3 domain to catalyze substrate ubiquitylation *J. Biol. Chem.* 294:783-793 Epub 2018 Nov 20. PMID 30459234 PMID:30459234 PMCID: PMC6341402
   c. Akturk A, Wasilko DJ, Wu X, Liu Y, Zhang Y, Qiu J, Luo ZQ, Reiter KH, Brzovic PS, Klevit RE, Mao Y. (2018) Mechanism of phosphoribosyl-ubiquitination mediated by a single Legionella effector. Nature. 557(7707):729-733. Epub 2018 May 23. PMID: 29795346.
   d. Levin I, Eakin C, Blanc MP, Klevit RE, Miller SI, Brzovic PS. (2010) Identification of an unconventional E3 binding surface on the UbcH5~Ub conjugate recognized by a pathogenic bacterial E3 ligase. Proc Natl Acad Sci U S A. 107(7):2848-53. PMID: 20133640

5. Understanding the role of disordered regions in transcriptional activation and regulation. Structural characterization of the complex formed by an acidic transcription activator (Gcn4) and a subunit of the Mediator Complex (Gal11) led to the discovery that intrinsically disordered regions of Gcn4 interact with Gal11 domains via the formation of "fuzzy" complexes. This structural insight helped address long-standing questions in the field and advanced our understanding as to how disordered regions of transcription factors can work to help assemble the transcriptional machinery. This work also describes how a single region of Gal11 can recognize multiple diverse transcription activation domains.

   a. Brzovic PS, Heikaus CC, Kisselev L, Vernon R, Herbig E, Pacheco D, Warfield L, Littlefield P, Baker D, Klevit RE, Hahn S. The acidic transcription activator Gcn4 binds the mediator subunit Gal11/Med15 using a simple protein interface forming a fuzzy complex. Mol Cell. 2011 Dec 23;44(6):942-53. PubMed PMID: 22195967; PubMed Central PMCID: PMC3246216.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/peter.brzovic.1/bibliography/public/

D. Additional Information: Research Support and/or Scholastic Performance

1R01CA260834-02 (Klevit RE [PI], Brzovic, P [MPI]

April 2021 to March 2026

Defining the role of BARD1 in nucleosomal ubiquitylation

This project aims to define how BARD1 functions with BRCA1 to contribute to DNA damage repair and transcriptional repression with a focus on its interactions with nucleosomal substrates.

Completed Research Support

2R01GM088055-18 (Klevit RE [PI], Brzovic, P & Chaterjee S [Co-Investigators])

September 2017 to August 2021

Expanding Roles of E2 and E3 Enzymes in Ubiquitin Transfer

Work conducted under this proposal will expand our knowledge as to how E2s and E3s recognize substrates and function in regulating selective Ubiquitin Transfer to substrate proteins.

R01 GM098503-05 (Brzovic, Peter S. [PI], Klevit, RE [Co-Investigator])

August 2012 to May 2018

Unconventional Interactions of Human Ubiquitin Conjugating Enzymes

This proposal is a NIH R01 in which we propose to investigate the non-canonical interactions involving E2 ubiquitin-conjugating enzymes.

Northwest Regional Center of Excellence Career Development Project (Brzovic, P.I.) November 2009 to October 31, 2012

Pathogenic virulence factors and host protein ubiquitination machinery

The major goals of this Northwest Regional Center of Excellence Career Development Project are to investigate effector proteins produced by pathogenic bacteria that interact with eukaryotic proteins involved in ubiquitin transfer.

Role PI

Royalty Research Fund, University of Washington (Brzovic, P.I.) July 2009 to June 2010

Structural and functional characterization of bacterial effector proteins that hijack eukaryotic ubiquitin signaling pathways.

Role: PI

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Davis, Trisha

eRA COMMONS USER NAME (credential, e.g., agency login): tdavis

POSITION TITLE: Professor and Chair

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of California, Santa Cruz, CA	B.A.	06/1976	Biology and Computer Science
Yale University, New Haven, CT	Ph.D.	12/1983	Molecular Biophysics and Biochemistry
University of California, Berkeley, CA	Postdoctoral Fellow	11/1987	Biochemistry

A. Personal Statement

I am a biochemist, who is also trained in computer science, genetics and biophysics. My diverse background enables me to combine multiple approaches to answer biological questions and to communicate easily with collaborators with expertise in computational biology, structural biology, biophysics and engineering. We have two longstanding (more than 10 years) highly successful collaborations with Dr. David Agard and Dr. Charles Asbury that enable an interdisciplinary approach focused on answering biological questions and not limited by the technologies required. My lab has made major advances in understanding centrosome assembly, microtubule nucleation and kinetochore function as described in the “Contributions to Science” with over 70 publications in these areas (4 recent examples are listed below). I have trained 33 students and postdocs (19 women and 2 URMs), and they have pursued diverse careers, which include faculty in tier 1 research institutions, science librarian at UC Berkeley, lawyers in biotechnology companies and the legislature and senior scientists in biotechnology companies. As director of a P41 technology development center for 15 years, (the Yeast Resource Center), I promoted technology development and dissemination. Over 100 labs profited each year from quality mass spectrometry, FRET and other technologies provided by the center. My lab has also focused on large-scale dissemination of data and published 12 papers describing new public databases and web tools. I have served on NIH study sections and multiple special emphasis panels to review other technology centers. More recently, as chair of my department, I have supported my senior faculty to serve on study section.

1. Hamilton GE, Helgeson LA, Noland CL, Asbury CL, Dimitrova YN, Davis TN. Reconstitution reveals two paths of force transmission through the kinetochore. Elife. 2020 May 14;9. doi: 10.7554/eLife.56582. PubMed PMID: 32406818; PubMed Central PMCID: PMC736768
2. Brilot A, Lyon A, Zelter A, Viswanath S, Maxwell A, MacCoss MJ, Muller EG, Sali A, Davis TN, Agard DA. CM1-driven assembly and activation of yeast γ-tubulin small complex underlies microtubule nucleation BioRxiv. 2020 doi: https://doi.org/10.1101/2020.11.21.392803
3. King BR, Meehl JB, Vojnar T, Winey M, Muller EG, Davis TN. Microtubule associated proteins and motors required for microtubule array formation in *S. cerevisiae.* Genetics. 2021. In press.
4. Kim JO, Zelter A, Umbreit NT, Bollozos A, Riffle M, Johnson R, MacCoss MJ, Asbury CL, Davis TN. The Ndc80 complex bridges two Dam1 complex rings. Elife. 2017 Feb 13;6PubMed PMID: 28191870; PubMed Central PMCID: PMC5354518.

B. Positions and Honors

Positions and Employment

1987 - 1994	Assistant Professor, University of Washington, Biochemistry, Seattle, WA
1994 - 2001	Associate Professor, University of Washington, Biochemistry, Seattle, WA
2001 -	Professor, University of Washington, Biochemistry, Seattle, WA
2001 - 2016	Director, Yeast Resource Center, an NIGMS Biomedical Technology Research Center, Seattle, WA
2008 - 2009	Alexander von Humboldt Fellow, Max Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany
2011 - 2013	Acting Chair, University of Washington, Biochemistry, Seattle, WA
2013 -	Chair, University of Washington, Biochemistry, Seattle, WA
2013 -	Earl W. Davie/ZymoGenetics Endowed Chair in Biochemistry, University of Washington

Other Experience and Professional Memberships

1982 -	Member, American Society for Microbiology
1987 -	Member, American Association for the Advancement of Science
1988 -	Member, Genetics Society of America
1989 -	Member, American Society for Biochemistry and Molecular Biology
1994 -	Member, American Society of Cell Biology
1994 - 1998	Member, Microbial Physiology Study Section, National Institutes of Health
1995 - 2002	Editorial Board Member, Molecular Biology of the Cell
2001 - 2004	Editorial Board Member, Molecular and Cellular Proteomics
2002 - 2002	Member, NIH Special Emphasis Panel for CDF-2 Site Visit
2003 - 2003	Ad hoc Member, NIH Study Section: CDF-4
2003 - 2006	Monitoring Editor, Molecular Biology of the Cell
2004 - 2004	Member, NIH Study Section for SBIB Site Visit
2006 - 2008	Member, Scientific Working Group for NIAID Biodefense Proteomics Research Program
2007 - 2008	Member, ORNL Center for Molecular and Cellular Sciences Scientific Advisory Committee
2008 - 2014	Member, NIH Special Emphasis Panels
2012 - 2012	Ad hoc Member, NIGMS Council
2016 - 2021	Member, University of Washington Research Advisory Board

Honors

1977	Predoctoral Fellow, National Science Foundation
1983	Postdoctoral Fellow, Anna Fuller Fund
1988	Searle Scholar, Searle Foundation
2005	UW School of Medicine/Center of Excellence Mentoring Award, UW School of Medicine
2008	Humboldt Research Award, Alexander von Humboldt Foundation
2012	Fellow, American Association for the Advancement of Science
2015	Member, Washington State Academy of Sciences
2018	Fellow, American Society of Cell Biologists
2020	Member, American Academy of Arts and Sciences

C. Contributions to Science

1. How kinetochores couple to dynamic microtubules under tension.

Kinetochores attach to microtubules with a striking combination of strength and plasticity. The attachments are mobile and robust under tension but destabilized in response to regulatory signals that selectively cue the release of incorrect attachments. My first contribution focuses on how kinetochores form load-bearing attachments to dynamic microtubules. When we began this work, there were two prevailing models: biased diffusion and the conformational wave model. In collaboration with Dr. Charles Asbury, we showed that the Dam1 complex and the Ndc80 complex can couple cargo to both assembling and disassembling microtubules under tension (d). The Ndc80-based coupling likely occurs through biased diffusion and is conserved from yeast to humans (d). We found that the Ndc80 complex bridges two Dam1 complex rings

rather than the one ring presumed in all current models (c). This dual connection has significant implications for efforts at reconstituting load-bearing attachments. The Ska complex in human cells is thought to either be a functional analog of the Dam1 complex or to act solely as a scaffold for protein phosphatase. We have shown that *in vitro* the Ska complex behaves as a functional analog to the Dam1 complex in that it strengthens microtubule attachments made by the human Ndc80 complex (b). Finally, we have mapped the path of force transmission from the microtubule through to the nucleosome by reconstituting the entire path from recombinant components (a).

a. Hamilton GE, Helgeson LA, Noland CL, Asbury CL, Dimitrova YN, Davis TN. Reconstitution reveals two paths of force transmission through the kinetochore. Elife. 2020 May 14;9. doi: 10.7554/eLife.56582. PubMed PMID: 32406818; PubMed Central PMCID: PMC7367685.
b. Helgeson LA, Zelter A, Riffle M, MacCoss MJ, Asbury CL, Davis TN. The human Ska complex and Ndc80 complex interact to form a load-bearing assembly that strengthens kinetochore microtubule attachments Proc. Natl. Acad. Sci. USA, 115:2740-2745. PMCID: PMC5856539
c. Kim JO, Zelter A, Umbreit NT, Bollozos A, Riffle M, Johnson R, MacCoss MJ, Asbury CL, Davis TN. The Ndc80 complex bridges two Dam1 complex rings. Elife. 2017 Feb 13;6 PubMed PMID: 28191870; PubMed Central PMCID: PMC5354518.
d. Powers AF, Franck AD, Gestaut DR, Cooper J, Gracyzk B, Wei RR, Wordeman L, Davis TN, Asbury CL. The Ndc80 kinetochore complex forms load-bearing attachments to dynamic microtubule tips via biased diffusion. Cell. 2009 Mar 6;136(5):865-75. PubMed PMID: 19269365; PubMed Central PMCID: PMC2749323.

2. Error correction mechanisms of kinetochore-microtubule attachments

Kinetochore–microtubule attachments that are too stable can be detrimental. Improper connections made during initial kinetochore capture by the mitotic spindle could result in chromosome misalignment if allowed to remain attached. The accuracy of chromosome segregation during each cell division depends on a mechanism for the targeted release of aberrant attachments. The prevailing model was that phosphorylation by the Aurora B kinase detached incorrect attachments. In collaboration with the Asbury lab, we provided support for this model by showing that phosphoregulation of kinetochore attachments occurs at two interfaces: the microtubule kinetochore interface and the interface between kinetochore complexes. Specifically, phosphorylation of the Dam1 complex decreases the cooperativity of microtubule binding and its affinity for Ndc80 complex (a, d (also see b in contribution 1)). In mammalian cells, the story is more complicated. We showed that in addition to regulating attachment stability, Aurora B controls microtubule dynamics through phosphorylation of the Ndc80 complex (c). Our results explained a longstanding but surprising observation that Aurora B activity did not always result in kinetochore detachment, but instead kinetochores tracked depolymerizing microtubules to the spindle pole where they are corrected. We also discovered an alternative method for regulating the association of the Ndc80 complex with the kinetochore. Microtubule binding by the Ndc80 complex is auto-inhibited by tight bending of the Ndc80 complex around a flexible region in the middle of the long coiled coil (a). Autoinhibition is relieved by binding to the MIND complex. This mechanism ensures that the Ndc80 complex binds tightly to microtubules only when it is associated with the kinetochore and explains why the Ndc80 complex localizes at the kinetochore and is not distributed all along the mitotic spindle. In conclusion, we have shown that the error correction kinase not only destabilizes the interface with the microtubule as was generally believed, but also destabilizes the interfaces between complexes and changes microtubule dynamics. We discovered a novel regulatory mechanism to target Ndc80 complex to the microtubule.

a. Scarborough EA, Davis TN, Asbury CL. Tight bending of the Ndc80 complex provides intrinsic regulation of its binding to microtubules. Elife. 2019 May 2;8. doi: 10.7554/eLife.44489. PubMed PMID: 31045495; PubMed Central PMCID: PMC6516834.
b. Kudalkar EM, Scarborough EA, Umbreit NT, Zelter A, Gestaut DR, Riffle M, Johnson RS, MacCoss MJ, Asbury CL, Davis TN. Regulation of outer kinetochore Ndc80 complex-based microtubule attachments by the central kinetochore Mis12/MIND complex. Proc Natl Acad Sci U S A. 2015 Oct 13;112(41):E5583-9. PubMed PMID: 26430240; PubMed Central PMCID: PMC4611631.
c. Umbreit NT, Gestaut DR, Tien JF, Vollmar BS, Gonen T, Asbury CL, Davis TN. The Ndc80 kinetochore complex directly modulates microtubule dynamics. Proc Natl Acad Sci U S A. 2012 Oct 2;109(40):16113-8. PubMed PMID: 22908300; PubMed Central PMCID: PMC3479545.
d. Tien JF, Umbreit NT, Gestaut DR, Franck AD, Cooper J, Wordeman L, Gonen T, Asbury CL, Davis TN. Cooperation of the Dam1 and Ndc80 kinetochore complexes enhances microtubule coupling and is

regulated by aurora B. J Cell Biol. 2010 May 17;189(4):713-23. PubMed PMID: 20479468; PubMed Central PMCID: PMC2872917.

3. *Mechanism of microtubule nucleation*

Spindle morphogenesis requires spatially controlled microtubule nucleation, but a key question is how microtubule nucleation is regulated. At the center of this question is γ-tubulin, which is required for nucleation. We were the first to reconstitute the small γ-tubulin complex required for nucleation. Our work in collaboration with the Agard lab established that the γ-tubulin complex acts as a template for nucleation and suggested the hypothesis that the γ-tubulin small complex is activated by a conformational transformation that orients the γ-tubulins to match the geometry of a microtubule (d, c). We have found two additional factors are required for nucleation by the γ -tubulin complex. First assembly of the γ -tubulin ring is dependent on oligomerization of the protein that targets the ring to the centrosome (Spc110) (b). Second, the polymerase activity of XMAP215 and its homologs promote nucleation (a). In conclusion, we have established the hypothesis that the γ-tubulin complex acts in concert with other factors to spatially regulate microtubule nucleation.

a. King BR, Moritz M, Kim H, Agard DA, Asbury CL, Davis TN. XMAP215 and γ-tubulin additively promote microtubule nucleation in purified solutions. Mol Biol Cell. 2020 15:2187-2194. PubMed PMID: 32726183. PubMed Central PMCID: PMC7550701
b. Lyon AS, Morin G, Moritz M, Yabut KC, Vojnar T, Zelter A, Muller E, Davis TN, Agard DA. Higher-order oligomerization of Spc110p drives γ-tubulin ring complex assembly. Mol Biol Cell. 2016 Jul 15;27(14):2245-2258. PubMed PMID: 27226487; PubMed Central PMCID: PMC4945142.
c. Kollman JM, Greenberg CH, Li S, Moritz M, Zelter A, Fong KK, Fernandez JJ, Sali A, Kilmartin J, Davis TN, Agard DA. Ring closure activates yeast γTuRC for species-specific microtubule nucleation. Nat Struct Mol Biol. 2015 22:132-137. PubMed PMID: 25599398; PubMed Central PMCID: PMC4318760.
d. Kollman JM, Polka JK, Zelter A, Davis TN, Agard DA. Microtubule nucleating gamma-TuSC assembles structures with 13-fold microtubule-like symmetry. Nature. 2010 466:879-882. PubMed PMID: 20631709; PubMed Central PMCID: PMC2921000.

4. *Higher order protein structure*

As Director of the P41 Yeast Resource Center and now as a co-investigator, I have focused on developing methods to obtain structural information of protein complexes and then using that information for integrative structural modeling. We developed fluorescence resonance energy transfer as a method to analyze the structure of protein complexes in living cells. Our intuitive metric can be applied even if using a simple wide-field fluorescent microscope. Our method has been adopted to describe the organization of 5 protein complexes including the yeast centrosome (see bibliography). In a second complementary approach, we developed methods to perform cross-linking of protein complexes *in vitro* and then identify the cross-linked peptides by mass spectrometry. This project has been a collaboration with the MacCoss and Moritz labs. We developed the mass spectrometry methods and a new algorithm (Kojak) that can accurately identify protein cross-links and performs as well as or better than the current algorithms (a). We developed a web application (now widely used) to visualize and publicly disseminate cross-linking data and the underlying mass spectrometric data (b). Using these methods, we have applied cross-linking technology and revealed new structural information about 7 protein complexes (c, d and see bibliography and other contributions).

a. Hoopmann MR, Zelter A, Johnson RS, Riffle M, MacCoss MJ, Davis TN, Moritz RL. Kojak: efficient analysis of chemically cross-linked protein complexes. J Proteome Res. 2015 May 1;14(5):2190-8. PubMed PMID: 25812159; PubMed Central PMCID: PMC4428575.
b. Riffle M, Jaschob D, Zelter A, Davis TN. ProXL (Protein Cross-Linking Database): A Platform for Analysis, Visualization, and Sharing of Protein Cross-Linking Mass Spectrometry Data. J Proteome Res. 2016 Aug 5;15(8):2863-70. doi: 10.1021/acs.jproteome.6b00274. Epub 2016 Jun 24. PubMed PMID: 27302480; PubMed Central PMCID: PMC4977572.
c. Greenberg CH, Kollman J, Zelter A, Johnson R, MacCoss MJ, Davis TN, Agard DA, Sali A. Structure of γ-tubulin small complex based on a cryo-EM map, chemical cross-links, and a remotely related structure. J Struct Biol. 2016 Jun;194(3):303-10. doi: 10.1016/j.jsb.2016.03.006. PubMed PMID: 26968363; PubMed Central PMCID: PMC4866596.
d. Zelter A, Bonomi M, Kim JO, Umbreit NT, Hoopmann MR, Johnson R, Riffle M, Jaschob D, MacCoss MJ, Moritz RL, Davis TN. The molecular architecture of the Dam1 kinetochore complex is defined by

cross-linking based structural modelling. Nat Commun. 2015 Nov 12;6:8673. doi: 10.1038/ncomms9673. PubMed PMID: 26560693; PubMed Central PMCID: PMC4660060.

5. *Calmodulin has essential functions in addition to signal transduction.*

When I began my career, calmodulin had been extensively studied as the mediator of calcium ion signaling. Shortly after beginning as an Assistant Professor at the University of Washington, my lab discovered that calmodulin has essential functions outside of calcium ion signaling (d). In fact, in yeast, the only essential functions are performed independently of calcium ions. We discovered calmodulin is a structural component of the centrosome and is required to attach microtubules to the core of the centrosome in both yeast and human cells (b, c). Coming full circle, we recently discovered that calmodulin is directly required to strengthen attachments of microtubules to the centrosome (a).

a. Fong KK, Sarangapani KK, Yusko EC, Riffle M, Llauró A, Graczyk B, Davis TN, Asbury CL. Direct measurement of the strength of microtubule attachment to yeast centrosomes. Mol Biol Cell. 2017 Jul 7;28(14):1853-1861. PubMed PMID: 28331072; PubMed Central PMCID: PMC5541836.
b. Flory MR, Moser MJ, Monnat RJ Jr, Davis TN. Identification of a human centrosomal calmodulin-binding protein that shares homology with pericentrin. Proc Natl Acad Sci U S A. 2000 May 23;97(11):5919-23. PubMed PMID: 10823944; PubMed Central PMCID: PMC18534.
c. Sundberg HA, Goetsch L, Byers B, Davis TN. Role of calmodulin and Spc110p interaction in the proper assembly of spindle pole body components. J Cell Biol. 1996 Apr;133(1):111-24. PubMed PMID: 8601600; PubMed Central PMCID: PMC2120774.
d. Geiser JR, van Tuinen D, Brockerhoff SE, Neff MM, Davis TN. Can calmodulin function without binding calcium? Cell. 1991 Jun 14;65(6):949-59. PubMed PMID: 2044154.

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/sites/myncbi/trisha.davis.1/bibliography/40752211/public/?sort=date&direction=descending

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

R35 GM130293-03
Davis, Trisha N. (PI)
03/01/19-01/31/24
Molecular Analysis of Chromosome Segregation
Role: PI

P41 GM103533-25
MacCoss, Michael (PI)
09/30/97-03/31/22
Comprehensive Biology: Exploiting the Yeast Genome
Role: Project leader on "Higher order protein structure" subproject

Completed Research Support

R01 GM040506
Davis, Trisha N. (PI)
07/01/88-01/31/19
Molecular Analysis of the Kinetochore
Role: PI

P01 GM105537
Winey, Mark (PI)
09/01/14-08/31/19
Microtubule Nucleation and its Regulation
Role: Co-investigator

CURRICULUM VITAE **MARCH 2022**
FRANK DIMAIO, PHD

1. PERSONAL DATA

Place of Birth: Pennsylvania, US
Citizenship: US

2. EDUCATION

1996-2001	B.S., Computer Science, Pennsylvania State University
2001-2002	M.S., Computer Sciences, University of Wisconsin – Madison
2002-2007	Ph.D., Computer Sciences, University of Wisconsin – Madison

3. POSTGRADUATE TRAINING

2007-2012	Postdoctoral Fellowship, Biochemistry Department, University of Washington Postdoctoral Advisor: Dr. David Baker

4. FACULTY POSITIONS HELD

2012-2014	Research Assistant Professor Biochemistry Department, University of Washington
2014-2018	Assistant Professor Biochemistry Department, University of Washington
2018-	Associate Professor Biochemistry Department, University of Washington

5. HOSPITAL POSITIONS HELD

None

6. HONORS

2001-2002	University of Wisconsin Fellowship
2002-2006	Computation and Informatics in Biology and Medicine Graduate Fellowship

7. BOARD CERTIFICATION

None

8. LICENSURE

None

9. PROFESSIONAL ORGANIZATIONS

None

10. TEACHING RESPONSIBILITIES

2015-	Instructor, GENOME 541, *Introduction to Computational Molecular Biology*
2016-	Instructor, BIOC 405, *Introduction to Biochemistry*

2017	Organizer, BPSD 541*, Literature Review*

A. Postdoctoral Fellows in Laboratory

2017-	Guangfeng Zhou, Ph.D.
2014-2017	Ryan Pavlovicz, Ph.D.

B. Graduate Students

2021-	Marisa Brandys, Biochemistry Student
2018-	Carson Adams, Biochemistry Student
2018-	Gabriella Reggiano, BPSD Student
2016-2021	Dan Farrell, Biochemistry Student (graduated)
2014-2018	Zibo Chen, BPSD Student *(co-advised*) (graduated)
2014-2017	Brandon Frenz, Biochemistry Student (graduated)
2012-2015	Ray Wang, BPSD Student *(co-advised*) (graduated)

11. EDITORIAL RESPONSIBILITIES

Reviewer of over 25 journal articles from journals including: *Nature, Cell, Nature Methods, Nature Structure and Molecular Biology, PNAS, Structure, Journal of Structural Biology, Scientific Reports*

12. SPECIAL NATIONAL RESPONSIBILITIES

2017-2020	Review panel, LCLS X-Ray FEL facility
2019, 2020	Ad hoc reviewer NIH study section, Molecular Structure and Function D
2012	Ad hoc reviewer NIH study section, Bioengineering Sciences and Technology

Program committee member

2008	International Conference On Machine Learning (ICML)
2008	Assn. for the Advancement of Artificial Intelligence (AAAI)
2008	Brazilian Symposium on Bioinformatics (BSB)
2009	International Joint Conference on Artificial Intelligence (IJCAI)
2010	Intelligent Systems for Molecular Biology (ISMB)
2013	Assn. for the Advancement of Artificial Intelligence (AAAI)

Tutorials on software I've developed for density-guided model building

2015	Keystone Symposium on Hybrid Methods. Lake Tahoe, NV
2016	New York Structural Biology Center, NY
2016	CECAM workshop on Cryo-EM Modeling, Jülich, Germany
2017	Vanderbilt University, TN
2018	University of Michigan, MI
2018	Stockholm, Sweden
2019	Cold Spring Harbor, New York, NY
2020	*CryoEM Winter* School, Vienna, Austria

13. SPECIAL LOCAL RESPONSIBILITIES

2014-2015	Biochemistry Graduate Admissions Committee
2014-2017	Review panel WRF-IPD Innovation Postdoctoral Fellowship Program
2016-2019	Biological Physics, Structure and Design (BPSD) Admissions Committee
2016-2019	Biochemistry Seminar Committee

14. RESEARCH FUNDING

NIH/NIGMS R01 GM123089, 2017-2022
Protein structure determination from low-resolution experimental data
$196,500 direct/yr.
PI: DiMaio

Defense Threat Reduction Agency - GRANT13030942, 2021-2023
Machine Learning for De Novo Design of Macrocyclic Peptides as Strain-Specific Proteome Affinity Reagents
PI: Baker

Defense Advanced Research Projects Agency - HR0011-21-2-0012, 2021-2025
Host augmentation for targeted bacterial eradication using designed chimeric macrocycles and miniproteins
PI: Mougous

Defense Threat Reduction Agency - GRANT13030960, 2021-2024
Forcefield-guided Machine Learning Methods for Flexible Ligand Docking
PI: DiMaio

NIH/NIGMS R01 GM125351-01, 2018-2021
Protein structure determination from low-resolution experimental data
$270,000 direct/yr.
PI: Gordon

UW Innovation Award 2017-2019
A general approach for label-free, ELISA-like detection of small molecules
$500,000 over two years
PIs: Gu, DiMaio

15. BIBLIOGRAPHY

A. Refereed Publications

1. Z. Wang, M. Amaya, A. Addetia, H.V. Dang, G. Reggiano, L. Yan, A.C. Hickey, **F. DiMaio**, C.C. Broder, D. Veesler (2022). Architecture and antigenicity of the Nipah virus attachment glycoprotein. *Science*. doi: 10.1126/science.abm5561.

2. H. Arlt, X. Sui, B. Folger, C. Adams, X. Chen, R. Remme, F. A. Hamprecht, **F. DiMaio**, M. Liao, J.M. Goodman, R.V. Farese Jr, T.C. Walther (2022). *Nat Struct Mol Biol*. doi: 10.1038/s41594-021-00718-y
3. L.S. Ferro, Q. Fang, L. Eshun-Wilson, J. Fernandes, A. Jack, D.P. Farrell, M. Golcuk, T. Huijben, K. Costa, M. Gur, **F. DiMaio**, E. Nogales, A. Yildiz (2022). *Science* 375(6578):326-331.
4. N.A.P. Lieberman, M.J. Lin, H. Xie, L. Shrestha, T. Nguyen, M.L. Huang, A.M. Haynes, E. Romeis, Q.Q. Wang, R.L. Zhang, C.X. Kou, G. Ciccarese, I. Dal Conte, M. Cusini, F. Drago, S.I. Nakayama, K. Lee, M. Ohnishi, K.A. Konda, S.K. Vargas, M. Eguiluz, C.F. Caceres, J.D. Klausner, O. Mitjà, A. Rompalo, F. Mulcahy, E.W. Hook, S.A. Lukehart, A.M. Casto, P. Roychoudhury, **F. DiMaio**, L. Giacani, A.L. Greninger (2021). *PLoS Negl Trop Dis*15(12):e0010063.
5. I. Anishchenko, S.J. Pellock, T.M. Chidyausiku, T.A. Ramelot, S. Ovchinnikov, J. Hao, K. Bafna, C. Norn, A. Kang, A.K. Bera, **F. DiMaio**, L. Carter, C.M. Chow, G.T. Montelione, D. Baker (2021). De novo protein design by deep network hallucination. *Nature*. 600(7889):547-552.
6. J. Koehler Leman, S. Lyskov, S.M. Lewis, J. Adolf-Bryfogle, R.F. Alford, K. Barlow, Z. Ben-Aharon, D. Farrell, J. Fell, W.A. Hansen, A. Harmalkar, J. Jeliazkov, G. Kuenze, J.D. Krys, A. Ljubetič, A.L. Loshbaugh, J. Maguire, R. Moretti, V.K. Mulligan, M.L. Nance, P.T. Nguyen, S. Ó Conchúir, S.S. Roy Burman, R. Samanta, S.T. Smith, F. Teets, J.K.S. Tiemann, A. Watkins, H. Woods, B.J. Yachnin, C.D. Bahl, C. Bailey-Kellogg, D. Baker, R. Das, **F. DiMaio**, S.D. Khare, T. Kortemme, J.W. Labonte, K. Lindorff-Larsen, J. Meiler, W. Schief, O. Schueler-Furman, J.B. Siegel, A. Stein, V. Yarov-Yarovoy, B. Kuhlman, A. Leaver-Fay, D. Gront, J.J. Gray, R. Bonneau (2021). Ensuring scientific reproducibility in bio-macromolecular modeling via extensive, automated benchmarks. *Nat Commun.* 12(1):6947.
7. J.M. Hansen, A. Horowitz, E.M. Lynch, D.P. Farrell, J. Quispe, **F. DiMaio**, J.M. Kollman (2021). Cryo-EM structures of CTP synthase filaments reveal mechanism of pH-sensitive assembly during budding yeast starvation. *Elife*. 10:e73368.
8. M.K. Rathinaswamy, U. Dalwadi, K.D. Fleming, C. Adams, J.T.B. Stariha, E. Pardon, M. Baek, O. Vadas, **F. DiMaio**, J. Steyaert, S.D. Hansen, C.K. Yip, J.E. Burke (2021). Structure of the phosphoinositide 3-kinase (PI3K) p110γ-p101 complex reveals molecular mechanism of GPCR activation. *Sci Adv*. 7(35):eabj4282.
9. N. Kacherovsky, L.F. Yang, H.V. Dang, E.L. Cheng, I.I. Cardle, A.C. Walls, M. McCallum, D.L. Sellers, **F. DiMaio**, S.J. Salipante, D. Corti, D. Veesler, S.H. Pun (2021). Discovery and Characterization of Spike N-Terminal Domain-Binding Aptamers for Rapid SARS-CoV-2 Detection. *Angew Chem*. 60(39):21211-21215.
10. M. Baek, **F. DiMaio**, I. Anishchenko, J. Dauparas, S. Ovchinnikov, G.R. Lee, J. Wang, Q. Cong, L.N. Kinch, R.D. Schaeffer, C. Millán, H. Park, C. Adams, C.R. Glassman, A. DeGiovanni, J.H. Pereira, A.V. Rodrigues, A.A. van Dijk, A.C. Ebrecht, D.J. Opperman, T. Sagmeister, C. Buhlheller, T. Pavkov-Keller, M.K. Rathinaswamy, U. Dalwadi, C.K. Yip, J.E. Burke, K.C. Garcia, N.V. Grishin, P.D. Adams, R.J. Read, D. Baker (2021). Accurate prediction of protein structures and interactions using a three-track neural network. *Science*. 2021 373(6557):871-876.
11. C.M. Bryan, G.J. Rocklin, M.J. Bick, A. Ford, S. Majri-Morrison, A.V. Kroll, C.J. Miller, L. Carter, I. Goreshnik, A. Kang, **F. DiMaio**, K.V. Tarbell, D. Baker (2021).

Computational design of a synthetic PD-1 agonist. *Proc Natl Acad Sci U S A*. 20;118(29):e2102164118.

12. D. Mann, J. Fan, K. Somboon, D.P. Farrell, A. Muenks, S.B. Tzokov, **F. DiMaio**, S. Khalid, S.I. Miller, J.R.C. Bergeron (2021). Structure and lipid dynamics in the maintenance of lipid asymmetry inner membrane complex of A. baumannii. *Commun Biol*. 29;4(1):817.
13. J. Wu, T.B. Blum, D.P. Farrell, **F. DiMaio**, J.P. Abrahams, J. Luo (2021). Cryo-electron Microscopy Imaging of Alzheimer's Amyloid-beta 42 Oligomer Displayed on a Functionally and Structurally Relevant Scaffold. *Angew Chem* 60(34):18680-18687.
14. G. Chi, R. Ebenhoch, H. Man, H. Tang, L.E. Tremblay, G. Reggiano, X. Qiu, T. Bohstedt, I. Liko, F.G. Almeida, A.P. Garneau, D. Wang, G. McKinley, C.P. Moreau, K.D. Bountra, P. Abrusci, S.M.M. Mukhopadhyay, A. Fernandez-Cid, S. Slimani, J.L. Lavoie, N.A. Burgess-Brown, B. Tehan, **F. DiMaio**, A. Jazayeri, P. Isenring, C.V. Robinson, K.L. Dürr (2021). Phospho-regulation, nucleotide binding and ion access control in potassium-chloride cotransporters. *EMBO J*. 40(14):e107294.
15. G. Dai, T.K. Aman, **F. DiMaio**, W.N. Zagotta. Electromechanical coupling mechanism for activation and inactivation of an HCN channel. *Nat Commun*. 14;12(1):2802.
16. S.R. Witus, A.L. Burrell, D.P. Farrell, J. Kang, M. Wang, J.M. Hansen, A. Pravat, L.M. Tuttle, M.D. Stewart, P.S. Brzovic, C. Chatterjee, W. Zhao, **F. DiMaio**, J.M. Kollman, R.E. Klevit. BRCA1/BARD1 site-specific ubiquitylation of nucleosomal H2A is directed by BARD1. *Nat Struct Mol Biol*. 28(3):268-277.
17. H. Park, G. Zhou, M. Baek, D. Baker, **F. DiMaio** (2021). Force Field Optimization Guided by Small Molecule Crystal Lattice Data Enables Consistent Sub-Angstrom Protein-Ligand Docking. *J Chem Theory Comput*. 17(3):2000-2010.
18. C.L. Lawson, A. Kryshtafovych, P.D. Adams, P.V. Afonine, M.L. Baker, B.A. Barad, P. Bond, T. Burnley, R. Cao, J. Cheng, G. Chojnowski, K. Cowtan, K.A. Dill, **F. DiMaio**, D.P. Farrell, J.S. Fraser, M.A. Herzik Jr, S.W. Hoh, J. Hou, L.W. Hung, M. Igaev, A.P. Joseph, D. Kihara, D. Kumar, S. Mittal, B. Monastyrskyy, M. Olek, C.M. Palmer, A. Patwardhan, A. Perez, J. Pfab, G.D. Pintilie, J.S. Richardson, P.B. Rosenthal, D. Sarkar, L.U. Schäfer, M.F. Schmid, G.F. Schröder, M. Shekhar, D. Si, A. Singharoy, G. Terashi, T.C. Terwilliger, A. Vaiana, L. Wang, Z. Wang, S.A. Wankowicz, C.J. Williams, M. Winn, T. Wu, X. Yu, K. Zhang K, H.M. Berman, W. Chiu (2021). Cryo-EM model validation recommendations based on outcomes of the 2019 EMDataResource challenge. *Nat Methods*. 18(2):156-164.
19. C.S. Rule, Y.J. Park, J.R. Delarosa, S. Turley, W.G.J Hol, S. McColm, C. Gura, **F. DiMaio**, K.V. Korotkov, M. Sandkvist (2020). Suppressor Mutations in Type II Secretion Mutants of Vibrio cholerae: Inactivation of the VesC Protease. *mSphere*. 16;5(6):e01125-20.
20. J.B. Maguire, H.K. Haddox, D. Strickland, S.F. Halabiya, B. Coventry, J.R.Griffin, S.V.S.R.K. Pulavarti, M. Cummins, D.F. Thieker, E. Klavins, T. Szyperski, **F. DiMaio**, D. Baker, B. Kuhlman (2021). Perturbing the energy landscape for improved packing during computational protein design. *Proteins*. 89(4):436-449.
21. Y. Zhang, L.M. Prach, T.E. O'Brien, **F. DiMaio**, D.M. Prigozhin, J.E. Corn, T. Alber, J.B. Siegel, D.J. Tantillo (2020). Crystal Structure and Mechanistic Molecular

Modeling Studies of Mycobacterium tuberculosis Diterpene Cyclase Rv3377c. *Biochemistry* 59(47):4507-4515.

22. B. Frenz, S.M. Lewis, I. King, **F. DiMaio**, H. Park, Y. Song (2020). Prediction of Protein Mutational Free Energy: Benchmark and Sampling Improvements Increase Classification Accuracy. *Front Bioeng Biotechnol*. 8:558247.
23. V.K. Mulligan, C.S. Kang, M.R. Sawaya, S. Rettie, X. Li, I. Antselovich, T.W. Craven, A.M. Watkins, J.W. Labonte, **F. DiMaio**, T.O. Yeates, D. Baker (2020). Computational design of mixed chirality peptide macrocycles with internal symmetry. *Protein Sci*. 29(12):2433-2445.
24. N. Mashtalir, H. Suzuki, D.P. Farrell, A. Sankar, J. Luo, M. Filipovski, A.R. D'Avino, R. St Pierre, A.M. Valencia, T. Onikubo, R.G. Roeder, Y. Han, Y. He, J.A. Ranish, **F. DiMaio**†, T. Walz†, C. Kadoch† (2020). A Structural Model of the Endogenous Human BAF Complex Informs Disease Mechanisms. *Cell*. 183(3):802-817.e24. *†co-corresponding*
25. R.E. Pavlovicz, H. Park, **F. DiMaio** (2020). Efficient consideration of coordinated water molecules improves computational protein-protein and protein-ligand docking discrimination. *PLoS Comput Biol*. 16(9):e1008103.
26. D.P. Farrell, I. Anishchenko, S. Shakeel, A. Lauko, L.A. Passmore, D. Baker, **F. DiMaio** (2020). Deep learning enables the atomic structure determination of the Fanconi Anemia core complex from cryoEM. *IUCrJ*. 7(Pt 5):881-892.
27. C. Xu, P. Lu, T.M. Gamal El-Din, X.Y. Pei, M.C. Johnson, A. Uyeda, M.J. Bick, Q. Xu, D. Jiang, H. Bai, G. Reggiano, Y. Hsia, T.J. Brunette, J. Dou, D. Ma, E.M. Lynch, S.E. Boyken, P.S. Huang, L. Stewart, **F. DiMaio**, J.M. Kollman, B.F. Luisi, T. Matsuura, W.A. Catterall, D. Baker D (2020). Computational design of transmembrane pores. *Nature*. 585(7823):129-134.
28. B. Basanta, M.J. Bick, A.K. Bera, C. Norn, C.M. Chow, L.P. Carter, I. Goreshnik, **F. DiMaio**, D. Baker (2020). An enumerative algorithm for de novo design of proteins with diverse pocket structures. *Proc Natl Acad Sci U S A*. 117(36):22135-22145.
29. H.K. Bhargava, K. Tabata, J.M. Byck, M. Hamasaki, D.P. Farrell, I. Anishchenko, **F. DiMaio**, Y.J. Im, T. Yoshimori, J.H. Hurley (2020). Structural basis for autophagy inhibition by the human Rubicon-Rab7 complex. *Proc Natl Acad Sci U S A*. 117(29):17003-17010.
30. N.S. Al-Otaibi, A.J. Taylor, D.P. Farrell, S.B. Tzokov, **F. DiMaio**, D.J. Kelly, J.R.C. Bergeron (2020). The cryo-EM structure of the bacterial flagellum cap complex suggests a molecular mechanism for filament elongation. *Nat Commun*. 11(1):3210.
31. J.K. Leman, B.D. Weitzner, S.M. Lewis, J. Adolf-Bryfogle, N. Alam, R.F. Alford, M. Aprahamian, D. Baker, K.A. Barlow, P. Barth, B. Basanta, B.J. Bender, K. Blacklock, J. Bonet, S.E. Boyken, P. Bradley, C. Bystroff, P. Conway, S. Cooper, B.E. Correia, B. Coventry, R. Das, R.M. De Jong, **F. DiMaio**, L. Dsilva, R. Dunbrack, A.S. Ford, B. Frenz, D.Y. Fu, C. Geniesse, L. Goldschmidt, R. Gowthaman, J.J. Gray, D. Gront, S. Guffy, S. Horowitz, P.S. Huang, T. Huber, T.M. Jacobs, J.R. Jeliazkov, D.K. Johnson, K. Kappel, J. Karanicolas, H. Khakzad, K.R. Khar, S.D. Khare, F. Khatib, A. Khramushin, I.C. King, R. Kleffner, B. Koepnick, T. Kortemme, G. Kuenze, B. Kuhlman, D. Kuroda, J.W. Labonte, J.K. Lai, G. Lapidoth, A. Leaver-Fay, S. Lindert, T. Linsky, N. London, J.H. Lubin, S. Lyskov, J. Maguire, L. Malmström, E. Marcos, O. Marcu, N.A. Marze, J. Meiler, R. Moretti, V.K. Mulligan, S. Nerli, C. Norn, S. Ó'Conchúir, N. Ollikainen, S. Ovchinnikov, M.S.

Pacella, X. Pan, H. Park, R.E. Pavlovicz, M. Pethe, B.G. Pierce, K.B. Pilla, B. Raveh, P.D. Renfrew, S.S.R. Burman, A. Rubenstein, M.F. Sauer, A. Scheck, W. Schief, O. Schueler-Furman, Y. Sedan, A.M. Sevy, N.G. Sgourakis, L. Shi, J.B. Siegel, D.A. Silva, S. Smith, Y. Song, A. Stein, M. Szegedy, F.D. Teets, S.B. Thyme, R.Y. Wang, A. Watkins, L. Zimmerman, R. Bonneau (2021). Macromolecular modeling and design in Rosetta: recent methods and frameworks. *Nat Methods*. 17(7):665-680.

32. E.G.B. Evans, J.L.W. Morgan, **F. DiMaio**, W.N. Zagotta, S. Stoll (2020). Allosteric conformational change of a cyclic nucleotide-gated ion channel revealed by DEER spectroscopy. *Proc Natl Acad Sci U S A*. 117(20):10839-10847.
33. P.R. Stoddard, E.M. Lynch, D.P. Farrell, A.M. Dosey, **F. DiMaio**, T.A. Williams, J.M. Kollman, A.W. Murray, E.C. Garner (2020). Polymerization in the actin ATPase clan regulates hexokinase activity in yeast. *Science*. 367(6481):1039-1042.
34. Y.J. Park, A.C. Walls, Z. Wang, M.M. Sauer, W. Li, M.A. M.A. Tortorici, B.J. Bosch, **F. DiMaio**, D. Veesler (2019). Structures of MERS-CoV spike glycoprotein in complex with sialoside attachment receptors. *Nat Struct Mol Biol*. doi: 10.1038/s41594-019-0334-7.
35. H.M. Berman, P.D. Adams, A.A. Bonvin, S.K. Burley, B. Carragher, W. Chiu, **F. DiMaio**, T.E. Ferrin, M.J. Gabanyi, T.D. Goddard, P.R. Griffin, J. Haas, C.A. Hanke, J.C. Hoch, G. Hummer, G. Kurisu, C.L. Lawson, A. Leitner, J.L. Markley, J. Meiler, G.T. Montelione, G.N. Phillips Jr, T. Prisner, J. Rappsilber, D.C. Schriemer, T. Schwede, C.A.M. Seidel, T.S. Strutzenberg, D.I. Svergun, E. Tajkhorshid, J. Trewhella, B. Vallat, S. Velankar, G.W. Vuister, B. Webb, J.D. Westbrook, K.L. White, A. Sali (2019). Federating Structural Models and Data: Outcomes from A Workshop on Archiving Integrative Structures. *Structure*. 227(12):1745-1759.
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37. H.T. Chou, L. Apelt, D.P. Farrell, S.R. White, J. Woodsmith, V. Svetlov, J.S. Goldstein, A.R. Nager, Z. Li, J. Muller, H. Dollfus, E. Nudler, U. Stelzl, **F. DiMaio***†*, M.V. Nachury*†*, T. Walz*†* (2019). The Molecular Architecture of Native BBSome Obtained by an Integrated Structural Approach. *Structure*. 27(9):1384-1394. *†co-corresponding*
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131. **F. DiMaio**, J. Shavlik and G. Phillips (2005). Pictorial structures for molecular modeling: Interpreting density maps. *Advances in Neural Information Processing Systems (NIPS)* 17, Vancouver, Canada.
132. D. Gopan, **F. DiMaio**, N. Dor, T. Reps and M. Sagiv (2004). Numeric domains with summarized dimensions. *Proceedings of Tools and Algorithms for the Construction and Analysis of Systems (TACAS)*, Barcelona, Spain.
133. **F. DiMaio** and J. Shavlik (2004). Learning an approximation to inductive Logic programming clause evaluation. *Proceedings of the Fourteenth International Conference on Inductive Logic Programming*, Porto, Portugal.

B. Book Chapters

1. **F. DiMaio** (2017). *Rosetta Structure Prediction as a Tool for Solving Difficult Molecular Replacement Problems*. In: A. Wlodawer, M. Jaskolski and Z. Dauter, editors, Protein Crystallography: Methods and Protocols. Springer.
2. **F. DiMaio** (2012). *Protein structure modelling with Rosetta*. In: R. Read, A. Urzhumtsev & V. Lunin, editors, Present and Future Methods for Biomolecular Crystallography, pp. 353- 362. Springer.

3. **F. DiMaio**, A. Soni and J. Shavlik (2008). *Machine Learning in Structural Biology: Interpreting 3D Protein Images*. In S. Mitra, S. Datta, T. Perkins & G. Michailidis, editors, Introduction to Machine Learning and Bioinformatics, pp. 237-276. Chapman & Hall/CRC Press.

16. OTHER

Select Presentations and Invited Talks

F. DiMaio (2022). *Keystone Symposium on Hybrid Methods*. Lake Tahoe, NV (*invited talk*)
F. DiMaio (2021). *Cryo-EM Winter School*, Vienna, Austria, (*virtual invited talk*)
F. DiMaio (2020). *Invited virtual seminar*, NIH
F. DiMaio (2020). *Invited seminar*, Purdue University, Indiana
F. DiMaio (2020). *Cryo-EM Winter School*, Vienna, Austria, (*invited talk + seminar)*
F. DiMaio (2019). *Biophysics Society Meeting.* Baltimore, MD. (*invited talk + Satellite Meeting talk*).
F. DiMaio (2018). *Protein Engineering Canada.* University of British Colombia, Vancouver, BC, Canada. (*invited talk*).
F. DiMaio (2018). *Invited seminar*, University of Pittsburgh.
F. DiMaio (2017 *Invited seminar*, University of California – San Diego.
F. DiMaio (2017). *Symposium on Modeling Immunity.* Vanderbilt University, TN. (*invited talk*).
F. DiMaio (2017). *Invited seminar*, Columbia University, NY. (*invited talk*).
F. DiMaio (2017). *Invited seminar*, University of Oxford. (*invited talk*).
F. DiMaio (2017). *Invited seminar*, Oregon Health & Science University (*invited talk*).
F. DiMaio (2016). *Invited seminar*, Florida State University. (*invited talk*).
F. DiMaio (2015). *Gordon Research Conference on 3D electron microscopy*. New London, New Hampshire. (*invited talk*).
F. DiMaio (2015). *Keystone Symposium on Hybrid Methods*. Lake Tahoe, NV (*invited talk*)
F. DiMaio (2015). *Biophysics Society Meeting*. Baltimore, MD (*speaker, New and Notable session*).
F. DiMaio (2014). *NRAMM CryoEM Workshop.* La Jolla, CA. (*invited talk*)
F. DiMaio (2014). High accuracy structure determination from near-atomic-resolution cryoEM maps. *Gordon Research Conference on 3D electron microscopy*. Barcelona, Spain (*poster presentation selected for talk*).
F. DiMaio (2014). *wwPDBx Hybrid Methods Task Force Meeting*. Hinxton, UK.
F. DiMaio (2013). Model validation and *de novo* models. *Workshop on Theoretical Model Validation and PDBx/mmCIF Data Exchange Format.* Rutgers, NJ. (*invited talk*)
F. DiMaio (2013). *Phenix Workshop.* Berkeley, California. (*invited talk*)
F. DiMaio (2013). *The CCP4 Study Weekend: Molecular Replacement.* Nottingham, UK. (*invited talk*)
F. DiMaio (2012). *Present and Future Methods for Biomolecular Crystallography*, Erice, Italy. (*invited talks*)
F. DiMaio (2012). *Gordon Research Conference on Diffraction Methods in Structural Biology*, Lewiston, Maine. (*invited talk*)

F. DiMaio (2011). *NE-CAT Workshop on Advances in Moderate to Low Resolution Phasing and Refinement*, New York, NY. (*invited talk*)

F. DiMaio (2011). The Pacific Symposium on Biocomputing (PSB) Workshop on Validation and Modeling of Cryo-EM Structures, Kona, Hawaii. (*invited talk*)

F. DiMaio (2007). Guiding particle filtering with marginal approximations: An application in protein image interpretation. *The Learning Workshop*, San Juan, Puerto Rico.

F. DiMaio (2006). Modeling protein backbones with pairwise Markov fields. *ISMB Satellite Meeting on Structural Bioinformatics and Computational Biophysics (3Dsig)*, Fortaleza, Brazil.

F. DiMaio (2006). Tracing protein backbones in electron density maps using a Markov random field model. *The Learning Workshop*, Snowbird, Utah.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Escobar, Thelma

eRA COMMONS USER NAME (credential, e.g., agency login): escobt01

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
University of California, Los Angeles (UCLA), Los Angeles, CA	BS	06/2007	Molecular Biology
Jonhs Hopkins University, Baltimore, MD	PhD	08/2014	Biology and Immunology
New York University School of Medicine, New York, New York	Postdoctoral Fellow	04/2021	Biochemistry

A. Personal Statement

A long-term goal of my research is to understand the mechanisms and functional consequences of chromatin dynamics in maintaining cellular identity. My research experiences thus far have enabled me the opportunity to explore different model organisms, RNA regulation, chromatin dynamics, as well as particular areas of stem cell and immunology. During my doctoral training as part of the Johns Hopkins University - NIH Graduate Partnership Program, I focused on mechanisms regulating gene expression and cellular differentiation by investigating how microRNAs and chromatin changes influence immune cell differentiation and function (1-3). Through these studies, I learned various mouse models and next generation sequencing techniques that are well suited for my role in this project. Some examples include, generating a conditional knockout mouse, loss-of-function studies in primary cells, and various mouse models of disease states such as experimental autoimmune uveitis (EAU) and experimental autoimmune encephalomyelitis (EAE). Furthermore, as a postdoctoral fellow in the laboratory of Dr. Danny Reinberg, I became proficient in biochemistry and the epigenetic mechanisms determinant to cellular identity. Using an innovative system to specifically access how pre-existing, parental nucleosomes and their associated PTMs segregate during DNA replication, I uncovered the local nucleosome recycling of repressed chromatin domains (4), a key epigenetic mechanism for sustaining cellular identity. Follow-up experiments to identify the molecular mechanism pertaining to inheritance of repressed chromatin domains in mouse embryonic stem cells have led me to this proposal in which for my own research program, I propose to merge my expertise in chromatin dynamics, stem cells, and immune biology to study hematopoietic development in health and disease.

1. Escobar TM, Oksuz O, Saldaña-Meyer R, Descostes N, Bonasio R, Reinberg D. Active and Repressed Chromatin Domains Exhibit Distinct Nucleosome Segregation during DNA Replication. Cell. 2019 Oct 31;179(4):953-963.e11. PubMed Central PMCID: PMC6917041.
2. Escobar TM, Kanellopoulou C, Kugler DG, Kilaru G, Nguyen CK, Nagarajan V, Bhairavabhotla RK, Northrup D, Zahr R, Burr P, Liu X, Zhao K, Sher A, Jankovic D, Zhu J, Muljo SA. miR-155 activates cytokine gene expression in Th17 cells by regulating the DNA-binding protein Jarid2 to relieve polycomb-mediated repression. Immunity. 2014 Jun 19;40(6):865-79. PubMed Central PMCID: PMC4092165.
3. Hu G, Tang Q, Sharma S, Yu F, Escobar TM, Muljo SA, Zhu J, Zhao K. Expression and regulation of intergenic long noncoding RNAs during T cell development and differentiation. Nat Immunol. 2013 Nov;14(11):1190-8. PubMed Central PMCID: PMC3805781.

4. Escobar T, Yu CR, Muljo SA, Egwuagu CE. STAT3 activates miR-155 in Th17 cells and acts in concert to promote experimental autoimmune uveitis. Invest Ophthalmol Vis Sci. 2013 Jun 10;54(6):4017-25. PubMed Central PMCID: PMC3680004.

B. Positions and Honors

Positions and Employment

2021 -	Assistant Professor, University of Washington Department of Biochemistry and Institute for Stem Cell & Regenerative Medicine (ISCRM), Seattle, WA
2014 - 2021	Postdoctoral fellow with Dr. Danny Reinberg, New York University School of Medicine, Department of Biochemistry, New York, NY
2007 - 2008	Postbaccalaureate research with Dr. Ira Pastan, National Cancer Institute, Laboratory of Molecular Biology, Bethesda , MD
2004 - 2007	Undergraduate Research with Dr. Alexander van der Bliek, UCLA, Department of Biological Chemistry, Los Angeles, CA

Other Experience and Professional Memberships

2005 - 2007	Trainee, NIH sponsored Maximizing Access to Research Careers (MARC) Program
2007 - 2008	Trainee, NIH Academy Postbaccalaureate Program

Honors

2007	Women for Change 2007 Student Leadership Award, UCLA
2007	Chancellor's Service Award, UCLA
2006	Science Poster Day Dean's Prize Award Recipient, UCLA

C. Contribution to Science

1. The stable and heritable information of gene expression that is distinct from DNA sequences, known as epigenetics, must be sustained through mitotic cell division. To gain insights into how parental nucleosome segregate across DNA replication and contributes to epigenetic inheritance, I devised a method that permanently labels H3.1- and H3.2-containing nucleosomes at desired actively transcribed or repressed genes in mouse embryonic stem cells (mESCs). This system allows following the fate of the “marked” parental nucleosome upon DNA replication when mESCs are in a pluripotent state or enter into a specific differentiation program. I determined that repressed chromatin comprises epigenetic features by uncovering local parental nucleosome recycling of facultative heterochromatin and I showed the dispersed consequence of active chromatin domains. These findings suggest mechanisms for inheriting a transcriptional repressed state and I am now investigating possible histone chaperones that function to sustain epigenetic memory across cellular division. I recently co-authored a Review that will be published in Nature Reviews Genetics highlighting the importance of parental nucleosome segregation for the inheritance of cellular identity.

    a. Escobar TM, Loyola A, Reinberg D. Parental nucleosome segregation and the inheritance of cellular identity. Nat Rev Genet. 2021 Jun;22(6):379-392. PubMed PMID: 33500558.
    b. Escobar TM, Oksuz O, Saldaña-Meyer R, Descostes N, Bonasio R, Reinberg D. Active and Repressed Chromatin Domains Exhibit Distinct Nucleosome Segregation during DNA Replication. Cell. 2019 Oct 31;179(4):953-963.e11. PubMed Central PMCID: PMC6917041.

2. Unraveling the mechanisms that control gene expression via changes in chromatin accessibility is of fundamental importance to understanding normal cellular identity as well as disease states. Nucleosomal histones are key for chromatin accessibility in which an extraordinary array of chemical modifications, have profound effects of chromatin structure and gene expression. A number of histone deposition pathways are relatively well understood, but we had yet to fully understand the means by which nuclear histones are chaperoned through DNA replicated and repair. Histones are categorized as replication-dependent and -independent. Replication-dependent histones are synthesized in S-phase to counter the dilution of nucleosomal histones due to the duplication of DNA content. In this study, we performed a comprehensive

biochemical purification scheme resulting in a detailed description surveying the stable interactions between replication coupled histone H3.1 and histone chaperone protein complexes. We identified 11 histone chaperones that interact with H3.1 and showcase the diverse roles of chaperons in the forefront of replication. These H3.1 purifications provided a reference to the soluble H3.1 protein complexes that exist in vivo and insights into the diverse functions undertaken by histone chaperones in mammalian cells.

   a. Campos EI, Smits AH, Kang YH, Landry S, Escobar TM, Nayak S, Ueberheide BM, Durocher D, Vermeulen M, Hurwitz J, Reinberg D. Analysis of the Histone H3.1 Interactome: A Suitable Chaperone for the Right Event. Mol Cell. 2015 Nov 19;60(4):697-709. PubMed Central PMCID: PMC4656108.

3. Transcriptional and epigenetic programs initiated within CD4+ T helper (Th) cells must work together to promote changes in gene expression, ultimately resulting in production of CD4+ Th subset signature cytokines. microRNA (miRNA) provide a layer of post-transcriptional gene expression in addition to the more classical transcriptional networks. MiRNA-155 has been shown to regulate vast immune developmental programs and has a critical role in promoting the differentiation of CD4+ Th cells towards the Interleukin 17 producing lineage (Th17). The central question of my thesis research was to determine the mechanism for how miRNA-155 promotes Th17 development. I identified a molecular network for in which miR-155 can regulate the chromatin landscape of Th17 cells by targeting Jarid2, a protein that recruits the Polycomb repressive complex 2 (PRC2) to mediate gene silencing. This mechanistic study of how miR-155 regulates gene expression was one of the first findings that supported a regulatory circuit between microRNAs and chromatin modifiers in the control of immune cell function.

   a. Escobar TM, Kanellopoulou C, Kugler DG, Kilaru G, Nguyen CK, Nagarajan V, Bhairavabhotla RK, Northrup D, Zahr R, Burr P, Liu X, Zhao K, Sher A, Jankovic D, Zhu J, Muljo SA. miR-155 activates cytokine gene expression in Th17 cells by regulating the DNA-binding protein Jarid2 to relieve polycomb-mediated repression. Immunity. 2014 Jun 19;40(6):865-79. PubMed Central PMCID: PMC4092165.

4. Uveitis is driven by the inflammation and tissue destruction of the uvea by the CD4+ T cell sub-population, Th17 cells. The transcription factor Signal Transducers and Activators of Transcription (STAT) 3 plays a critical role in Th17 development and loss of STAT3 prevents experimental autoimmune uveitis. As my thesis research focused on how the microRNA (miRNA) 155 promotes Th17 differentiation, I began a collaboration with the laboratory of Dr. Charles Egwuagu to assess whether STAT3 and miRNA-155 act in concert to promote Th17 cell development and autoimmunity. We found that STAT3 targets miRNA-155 for high expression in Th17 cells and in turn, miRNA-155 functions to promote an inflammatory environment in experimental autoimmune uveitis. Thus, STAT3 and/or miRNA-155 targeting may have the therapeutic potential for modulating uveitis and other Th17-driven diseases.

   a. Escobar T, Yu CR, Muljo SA, Egwuagu CE. STAT3 activates miR-155 in Th17 cells and acts in concert to promote experimental autoimmune uveitis. Invest Ophthalmol Vis Sci. 2013 Jun 10;54(6):4017-25. PubMed Central PMCID: PMC3680004.

5. Reorganization of the nuclear chromatin environment is a fundamental component of gene regulation. To identify and characterize how distal regulatory elements influence the tissue specific regulation of nuclear hormone receptor, we assessed how chromatin modifications changes, specifically DNA methylation, affects glucocorticoid receptor (GR) binding. We found that tissue-specific chromatin accessibility; DNA methylation and transcription factor binding have an integral role in the gene expression regulation of nuclear receptors.

   a. Wiench M, John S, Baek S, Johnson TA, Sung MH, Escobar T, Simmons CA, Pearce KH, Biddie SC, Sabo PJ, Thurman RE, Stamatoyannopoulos JA, Hager GL. DNA methylation status predicts cell type-specific enhancer activity. EMBO J. 2011 Jun 24;30(15):3028-39. PubMed Central PMCID: PMC3160184.
   b. Wiench M, Hager GL. Expanding horizons for nuclear receptors. EMBO Rep. 2010 Aug;11(8):569-71. PubMed Central PMCID: PMC2920441.

Complete List of Published Work in My Bibliography:
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BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Fan, Erkang

eRA COMMONS USER NAME (credential, e.g., agency login): erkang

POSITION TITLE: Research Associate Professor of Biochemistry and Biological Structure

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Peking University, Beijing, P. R. China	B.Sc.	07/1987	Chemistry
University of Pittsburgh, Pittsburgh	Ph.D.	08/1993	Organic Chemistry
University of California, Berkeley	Postdoctoral	1993 – 1996	Bioorganic Chemistry

A. Personal Statement

The main research area of my laboratory is structure-based drug discovery for tropical diseases. We have active projects that span from screening of compound library to medicinal chemistry for lead optimization. All our projects are collaborative in nature, and involve multiple research groups with expertise in biochemistry, structural biology, organic/medicinal chemistry, pharmacology, and parasitology. These offer unique opportunities for students who study in my group to gain multidisciplinary research experiences.

B. Positions and Honors

Positions and Employment

1988 – 1993: Graduate Research Associate, University of Pittsburgh, Pittsburgh.
1993 – 1996: Postdoctoral Fellow, University of California, Berkeley.
1996 – 2002: Research Assistant Professor of Biological Structure, University of Washington, Seattle.
2002 – 2020: Research Associate Professor of Biochemistry and Biological Structure, University of Washington, Seattle.
2020 – Research Associate Professor of Biochemistry, University of Washington, Seattle.

Other Experience and Professional Memberships

Member, American Chemical Society (1991 – present)
Editorial Board Member of BMC Chemical Biology (2008 – 2013)
Editorial Board Member of Scientific Reports (2011 – present)
Ad hoc reviewer for NIH Study Sections (NIAID-BARDA, IMST-G, DDR, IDM-T, IDM-Y, IMM-N, IDM-X, IDM-A, AIDC-C, AIDC-Y, and RCB-3), National Science Foundation, and various private funding agencies

Honors

1994 - 1996: NIH Postdoctoral Fellowship.

C. Contributions to Science

1. My early independent research career focused on the development of multivalent protein inhibitors using a structure-based design, where I am the leading PI on the project. Our modular approach toward such inhibitors allowed for stepwise optimization of each relevant module and the subsequent combination of

those optimal modules to achieve excellent potency. This also allowed for the design and synthesis of multivalent protein inhibitors that match the target protein not only in dimension, but also in surface features. We pioneered the use of dynamic light scattering techniques to study the solution behavior of multivalent ligand-protein complexes. We also extended the “molecular dimerizer” concept into a multivalent approach that can realize high affinity using relatively weak-affinity hetero-bivalent ligands. This holds the potential of extending the application of multivalent inhibitors for intracellular targets.

a. **E. Fan**, Z. Zhang, W. E. Minke, Z. Hou, C. L. M. J. Verlinde and W. G. J. Hol, "High affinity pentavalent ligands of E. coli heat-labile enterotoxin by modular structure-based design", *J. Am. Chem. Soc.*, **2000**, *122*, 2663-2664.
b. Z. Zhang, E. A. Merritt, M. Ahn, C. Roach, Z. Hou, C. L. M. J. Verlinde, W. G. J. Hol, and **E. Fan**, "Solution and Crystallographic Studies of Branched Multivalent Ligands that Inhibit the Receptor-Binding Process of Cholera Toxin", *J. Am. Chem. Soc.*, **2002**, *124*, 12991-12998.
c. Z. Zhang, J. C. Pickens, W. G. J. Hol, and **E. Fan**, "Solution and solid-phase syntheses of guanidine-bridged, water-soluble linkers for multivalent ligand design", *Org. Lett.*, **2004**, *6*, 1377-1389.
d. J. Liu, Z. Zhang, X. Tan, W. G. J. Hol, C. L. M. J. Verlinde and **E. Fan**, "Protein Heterodimerization through Ligand-Bridged Multivalent Pre-organization: Enhancing Ligand Binding toward Both Protein Targets", *J. Am. Chem. Soc.*, **2005**, *127*, 2044-2045.

2. Along the research line of combating infectious diseases caused by protozoan parasites, I participated in structural genomics projects as co-investigator and project manager at different stages. The main research activities from my research group were to provide chemical materials that may enhance protein crystallization, design fragment-based libraries for ligand discovery of proteins under study in the genomics project, develop compound library screening techniques suitable for handling the demand of a structural genomics project, and screen for protein ligand hits that maybe optimized for therapeutic development. Several of our currently active medicinal chemistry projects (see #3 below and Section D of Research Support) are the successful outcome of our structural genomics studies.
    a. Y. Ogata, T. Carter, L. Scampavia, **E. Fan**, and F. Turecek, "Automated Affinity Chromatography Measurements of Compound Mixtures Using a Lab-on-Valve Apparatus Coupled to Electrospray Ionization Mass Spectrometry", *Anal. Biochem.*, **2004**, *331*, 161-168.
    b. J. Bosch, M. A. Robien, C. Mehlin, E. Boni, A. Riechers, F. S. Buckner, W. C. Van Voorhis, P. J. Myler, E. A. Worthey, G. DeTitta, J. R. Luft, A. Lauricella, S. Gulde, L. A. Anderson, O. Kalyuzhniy, H. M. Neely, J. Ross, T. N. Earnest, M. Soltis, L. Schoenfeld, F. Zucker, E. A. Merritt, **E. Fan**, C. L. M. J. Verlinde, and W. G. J. Hol, "Identifying Novel Lead Compounds For Inhibiting Nucleoside 2-deoxyribosyltransferase In Trypanosoma brucei by Fragment Cocktail Crystallography", *J. Med. Chem.*, **2006**, *49*, 5939-5946.
    c. **E. Fan**, D. Baker, S. Fields, M. Gelb, F. Buckner, W. Van Voorhis, E. Phizicky, M. Dumont, C. Mehlin, E. Grayhack, M. Sullivan, C. Verlinde, G. DeTitta, D. Meldrum, E. Merritt, T. Earnest, M. Soltis, F. Zucker, P. Myler, L. Schoenfeld, D. Kim, L. Worthey, D. LaCount, M. Vignali, J. Li, S. Mondal, A. Massey, B. Carroll, S. Gulde, J. Luft, L. DeSoto, M. Holl, J. Caruthers, J. Bosch, M. Robien, T. Arakaki, M. Holmes, I. Le Trong, and W. Hol, "Structural Genomics of Pathogenic Protozoa: An Overview", in *Methods in Molecular Biology, Vol. 426: Structural Proteomics: High-throughput Methods*, Eds. Kobe, B., Guss, M. and Huber, T., Humana Press Inc., Totowa, NJ, **2008**, pp 497-513.
    d. C. L. M. J. Verlinde, **E. Fan**, S. Shibata, Z. Zhang, Z. Sun, W. Deng, J. Ross, J. Kim, L. Xiao, T. L. Arakaki, J. Bosch, J. M. Caruthers, E. T. Larson, I. LeTrong, A. Napuli, A. Kelly, N. Mueller, F. Zucker, W. C. Van Voorhis, E. A. Merritt, and W. G. J. Hol, "Fragment-based cocktail crystallography by the Medical Structural Genomics of Pathogenic Protozoa Consortium", *Curr. Topics Med. Chem.*, **2009**, *9*, 1678-1687. PMCID: PMC2897734.

3. Currently, the majority of my research activities are related to medicinal chemistry. As PI or co-investigator, I have been actively involved in several drug discovery projects using traditional medicinal chemistry as well as structure-based design approaches. The highlights of my research contribution in this area include the delivery of a preclinical candidate plus a backup for treating malaria in a project funded by the Medicines for Malaria Venture. Other projects are in hit-to-lead, advanced lead

optimization, or candidate selection stages. Positive outcomes from these projects will have significant impacts on several neglected infectious diseases.

a. A. B. Vaidya, J. M. Morrisey, Z. Zhang, S. Das, T. M. Daly, T. D. Otto, N. J. Spillman, M. Wyvratt, P. Siegl, J. Marfurt, G. Wirjanata, B. F. Sebayang, R. N. Price, A. Chatterjee, A. Nagle, M. Stasiak, S. A. Charman, I. Angulo-Barturen, S. Ferrer, M. B. Jimenez-Diaz, M. S. Martinez, F. Javier Gamo, V. M. Avery, A. Ruecker, M. Delves, K. Kirk, M. Berriman, S. Kortagere, J. Burrows, **E. Fan**, L. W. Bergman, "Pyrazoleamide compounds are potent antimalarials that target Na^+ homeostasis in intraerythrocytic Plasmodium falciparum", *Nature Communications*, **2014**, *5*:5521, doi: 10.1038/ncomms6521. PMCID: PMC4263321.
b. Z. Zhang, C. Y. Koh, R. M. Ranade, S. Shibata, J. R. Gillespie, M. A. Hulverson, W. Huang, J. Nguyen, N. Pendem, M. H. Gelb, C. L. M. J. Verlinde, W. G. J. Hol, F. S. Buckner, **E. Fan**, "5-Fluoroimidazo[4,5-b]pyridine Is a Privileged Fragment That Conveys Bioavailability to Potent Trypanosomal Methionyl-tRNA Synthetase Inhibitors", *ACS Infectious Diseases* **2016**, *2*, 399-404, PMCID:PMC5108244.
c. O. Faghih, Z. Zhang, R. M. Ranade, J. R. Gillespie, S. A. Creason, W. Huang, S. Shibata, X. Barros-Alvarez, C. Verlinde, W. G. J. Hol, **E. Fan**, F. S. Buckner, "Development of Methionyl-tRNA Synthetase Inhibitors as Antibiotics for Gram-Positive Bacterial Infections", *Antimicrob. Agents Chemother.* **2017**, *61*, e00999-17, PMCID: PMC5655057.
d. W. Huang, M. A. Hulverson, R. Choi, S. L. M. Arnold, Z. Zhang, M. C. McCloskey, G. R. Whitman, R. C. Hackman, K. L. Rivas, L. K. Barrett, K. K. Ojo, W. C. Van Voorhis, **E. Fan**, "Development of 5-Aminopyrazole-4-carboxamide-based Bumped-Kinase Inhibitors for Cryptosporidiosis Therapy", *Journal of Medicinal Chemistry* **2019**, *62*, 3135-3146. PMCID: PMC6559944.

Complete list of published Work:
https://pubmed.ncbi.nlm.nih.gov/collections/61425447/?sort=pubdate

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

R01 AI152358 Fan/Buckner (PIs) 04/01/2020 – 03/31/2024
NIH/NIAID
"Developing methionyl tRNA synthetase inhibitors as therapeutics for Chagas disease"
The goal of this project is to develop novel compounds for treatment of Chagas disease.
Role: Contact-PI responsible for medicinal chemistry

R01 AI155536 Staker (PI) 11/15/2020 – 10/31/2025
NIH/NIAID
"Structural Analysis and Inhibitor Optimization of Cryptosporidium N-myristoyltransferase for Drug Discovery"
The goal of this project is to optimize N-myristoyltransferase inhibitors for cryptosporidiosis treatment.
Role: Co-Investigator responsible for medicinal chemistry

R01 AI155412 Van Voorhis (PI) 01/01/2021 – 12/31/2025
NIH/NIAID
"Optimization of Lead BKIs for Cryptosporidiosis Therapy"
The goal of this project is to deliver a pre-clinical lead for cryptosporidiosis therapy, targeting Calcium-dependent Protein Kinase 1, with bumped-kinase inhibitors (BKIs).
Role: Co-Investigator responsible for medicinal chemistry

R01 HD102487 Van Voorhis (PI) 03/15/2021 – 02/28/2026
NIH/NICHD
"Bumped-Kinase Inhibitor Drug Development for Toxoplasmosis"
The overall goal of this project is to optimize Bumped-Kinase Inhibitors (BKIs) for central nervous system penetration and pregnancy safety to be suitable for the treatment of toxoplasmosis.
Role: Co-Investigator responsible for medicinal chemistry

R01 AI158524 Ojo (PI) 04/14/2021 – 03/31/2025
NIH/NIAID

“Methionyl-tRNA Synthetase inhibitors can be developed as novel Giardiasis therapeutics”
This is the continuation/replacement of R56 AI146067. This project will develop inhibitors of Giardia lamblia methionyl-tRNA synthetase as potential drugs for effective shorter-course treatment of symptomatic and asymptomatic giardiasis. It will use innovative techniques to profile and optimize the pharmacokinetic/pharmacodynamic relationships and chemical properties of functional groups to deliver safe preclinical compounds for efficient treatment of clinical giardiasis.
Role: Co-Investigator responsible for medicinal chemistry

Pending
R44 AIxxxxxx Lipka (PI) 07/01/2022 – 06/30/2025
NIH/NIAID
“Development of MRS-2541, a Methionyl-tRNA Synthetase Inhibitor, for Gram Positive Bacterial Infections”
The goal of this project is to perform scale up and subsequent preclinical pharmacology and toxicology assessments of a candidate compound MRS-2541 for development as an oral antibiotic treatment.
Role: Co-Investigator responsible for chemistry

Completed Research Support (in the past three years)
R56 AI146067 Ojo (PI) 09/03/2020 – 08/31/2021
NIH/NIAID
“Development of Cell-active Giardia lamblia Methionyl-tRNA Synthetase Inhibitors as a New Therapeutic for Giardiasis”
The goal of this project is to develop cell-active inhibitors of Giardia lamblia Methionyl-tRNA Synthetase as novel, preclinical lead for treatment of giardiasis.
Role: Co-Investigator responsible for medicinal chemistry

R01 AI097177 Fan/Buckner (PIs) 05/01/2012 – 04/30/2021
NIH/NIAID
“Structure-based Optimization of *T. brucei* methionyl tRNA Synthetase Inhibitors.
The goals of this project are to develop inhibitors of protein synthesis in *Trypanosoma brucei* for treating African sleeping sickness.
Role: Contact-PI responsible for medicinal chemistry

R21 AI140881 Ojo (PI) 02/01/2019 – 01/31/2021
NIH/NIAID
“Development of Dual Efficacy Therapeutic Against Cryptosporidiosis and Giardiasis”
The goal is to screen and identify hits from kinase inhibitor libraries for drug discovery against cryptosporidiosis and giardiasis.
Role: Co-Investigator responsible for medicinal chemistry

R44 AI134190 Lipka (PI) 08/03/2017 – 01/31/2021
NIH/NIAID
“Development of methionyl-tRNA synthetase inhibitors for Gram positive bacterial infections”
The goal of this project is to optimize a novel series of antibiotic compounds in preclinical development.
Role: Co-Investigator responsible for medicinal chemistry

R21 AI137815 Staker (PI) 12/23/2017 – 11/30/2020
NIH/NIAID
“Structural analysis and inhibitor optimization of Cryptosporidium N-myristoylation”
The major goal is to use structure-based design for inhibitor optimization of Cryptosporidium n-myristoyltransferase (NMT) for drug discovery.
Role: Co-Investigator responsible for medicinal chemistry

1R01 HD080670 Van Voorhis (PI) 10/01/2014 – 06/30/2020
USDA # 2014-06183 Van Voorhis (PI) 09/01/2014 – 08/30/2019
NICHHD and USDA
“Dual Use Therapeutics for Cryptosporidiosis, Toxoplasmosis, and Neosporosis”

The main goal of this study is to test the use of bumped kinase inhibitors for the therapy of animal diseases and in human health, particularly as it relates to children ages 0-18 months in Africa and Asia. The focus is on dual use therapeutics and to bring the drugs to the point where an Investigational New Drug Application for children and a New Animal Drug Application can be filed. This is a grant co-funded by USDA and NICHHD.
Role: Co-Investigator responsible for medicinal chemistry

R01 AI111341 Van Voorhis (PI) 03/15/2014 – 2/28/2020
NIH/NIAID
"Bumped Kinase Inhibitors: Novel Therapeutics for cryptosporidiosis and toxoplasmosis"
The major goal is to develop clinical candidates based on CDPK1 inhibitors for cryptosporidiosis and toxoplasmosis.
Role: Co-investigator responsible for medicinal chemistry

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Gu, Liangcai

eRA COMMONS USER NAME (credential, e.g., agency login): guliangcai

POSITION TITLE: Assistant Professor of Biochemistry

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Lanzhou University, China	B.S.	06/1998	Chemistry
Tsinghua University, China	M.S.	06/2002	Biochemistry
University of Michigan, Ann Arbor	Ph.D.	12/2008	Medicinal Chemistry
Harvard Medical School, Boston	Postdoctoral	07/2015	Genetics

A. Personal Statement

My research focuses on two distinct technology innovations: i) de novo engineering of ligand- or light-inducible protein interaction and ii) spatial (or in situ) DNA sequencing. Although they appear to belong to distinct fields and are hard to simultaneously tackle for a junior PI, I was motivated by novelty and significance of these approaches and encouraged by my previous training and experience in tackling similar challenges. In the past six years, I built a multidisciplinary research team, obtained five NIH and four other local grants, and successfully made the technologies working in my lab. Our new platform technologies enable (i) the design of highly specific protein dimerization systems controlled by arbitrary small-molecular ligands or light as in vivo sensors and actuators, and (ii) the single-cell, spatial transcriptome mapping of tissues without dissociating cells. They have led to exciting applications and collaborations, such as drug and light-controlled cell and gene therapies, optical biosensors for drug addiction, and spatial transcriptomics of neuronal plasticity and cellular senescence. Interestingly, our efforts on in vivo drug sensors and the spatial RNA sequencing technology have been intertwined and bound under an exciting new direction, precision pharmacology, the next major pursue of my lab.

B. Positions and Honors

2008-2009 Research Fellow, University of Michigan, Ann Arbor, MI
2009-2015 Research Fellow, Harvard Medical School, Boston, MA
2015- Assistant Professor, Department of Biochemistry, University of Washington, Seattle, WA

Honors

2008 Robert Scarborough Award for Graduate Excellence in Medicinal Chemistry, American Chemical Society
2009 Young Investigator Award, Society for Industrial Microbiology
2009 - 2012 Jane Coffin Childs Postdoctoral Fellowship Award
2017 University of Washington Innovation Award
2018 Safeway Early Career Award in Cancer Research
2020 Cutting-Edge Basic Research Award (CEBRA), National Institute on Drug Abuse

C. Contributions to Science

1. **Structural and Mechanistic Enzymology of Curacin Biosynthetic Pathway.** My PhD research with Professor David Sherman at the University of Michigan focused on structural and mechanistic enzymology of natural product biosynthesis. I *in vitro* reconstituted a 65-enzyme biosynthetic pathway of curacin A, an

anticancer bacterial metabolite, and studied structural and mechanistic details of several unusual biochemical reactions, providing a basis for engineering of the enzymes into other pathways to produce valuable chemicals. My work led to the discoveries of a widely used polyketide β-branching mechanism (Gu, et al., JACS, 2006), an unexpected polyketide chain initiation mechanism (Gu, et al., Science, 2007), two highly homologous but mechanistically diverged pathways that lead to halogenation and cyclopropanation (Gu, et al., Nature, 2009), a decarboxylative polyketide chain termination mechanism useful for biofuel biosynthesis (Gu, et al., JACS, 2009), and an intriguing strategy that nature uses to improve the catalytic efficiency of multienzyme reactions (Gu, et al., ACIE, 2011).

a. Gu L., Geders T.W., Wang B., Gerwick W.H., Håkansson K., Smith J.L., Sherman D.H. (2007). GNAT-like strategy for polyketide chain initiation. Science. 318(5852), 970-4.
b. Gu L., Wang B., Kulkarni A., Geders T.W., Grindberg R.V., Gerwick L., Håkansson K., Wipf P., Smith J.L., Gerwick W.H., Sherman D.H. (2009). Metamorphic enzyme assembly in polyketide diversification. Nature, 459(7247), 731-5.
c. Gu L., Wang B., Kulkarni A., Gehret J.J., Lloyd K.R., Gerwick L., Gerwick W.H., Wipf P., Håkansson K., Smith J.L., Sherman D.H. (2009). Polyketide decarboxylative chain termination preceded by *O*-sulfonation in curacin a biosynthesis. J. Am. Chem. Soc., 131(44), 16033-5.
d. Gu L., Eisman E.B., Dutta S., Franzmann T.M., Walter S., Gerwick W.H., Skiniotis G., Sherman D.H. (2011). Tandem acyl carrier proteins in the curacin biosynthetic pathway promote consecutive multienzyme reactions with a synergistic effect. Angew. Chem. Int. Ed. Engl., 14;50(12), 2795-8.

2. **Protein Barcoding and In Situ Sequencing Technology Development**. My postdoctoral research with Professor George Church at Harvard Medical School was to develop a single-molecular-interaction sequencing (SMI-seq) technology that can fundamentally improve the quality and cost-effectiveness of high-throughput protein-protein interaction analysis. SMI-seq involves attaching a barcoding DNA to proteins and analyzing single-molecule proteins and complexes in parallel by *in situ* sequencing their barcodes (Gu, et al., Nature, 2014). Because all proteins are assayed *en masse* and *in situ* detected by polony sequencing, SMI-seq allows highly quantitative comparison of binding affinities under standard conditions. By coupling the barcoding method to a nanodisc technology, we demonstrated its application in the functional screening of cell membrane receptors, for example, G protein-coupled receptors. Moreover, highly multiplex protein interaction profiling was demonstrated by a 'one-pot' antibody library vs. antigen library binding screening, which is impossible to perform with other techniques. In addition to natural and recombinant proteins, SMI-seq is applicable to de novo proteins (for example, those with unnatural amino acids or modifications), nucleic acids and barcoded small molecules.
    a. Gu, L., Li, C., Aach, J., Hill, D.E., Vidal, M., & Church, G.M. (2014). Multiplex single-molecule interaction profiling of DNA-barcoded proteins. Nature, 515(7528), 554-7.

3. **Creating Chemically Induced Dimerization for Sensing and Actuation**. Chemically induced dimerization (CID) systems, in which two proteins dimerize only in the presence of a small molecule ligand, offer versatile tools for small molecule sensing and actuation. Since the concept of using CID systems as a molecular switch was introduced by Schreiber, Crabtree, and coworkers in 1993 (Spencer, et al., Science, 1993), a few naturally occurring CID systems, such as rapamycin-inducible FKBP/FRB, gibberellin-inducible GAI/GID1 complexes, and their derivatives, have been engineered as genetically encoded biosensors to dissect or manipulate important biological processes. Although CID is applicable to biosensing, the applications had been hampered by the inability to create CID for a given ligand. My lab developed a combinatorial binders-enabled selection of CID (COMBINES-CID) method to select two CID proteins from a vastly diverse synthetic nanobody library (>10^9) for a given ligand—an 'anchor binder' that first binds to a ligand, and a 'dimerization binder' that only binds to the anchor binder–ligand complex not the unbound anchor binder. As a proof-of-principle (Kang, et al., JACS, 2019), we successfully created and characterized CIDs for cannabidiol (CBD). Selected CIDs showed high sensitivity and specificity and fast kinetics, and were used to construct biosensors for *in vitro* detection of CBD. This method provides a general solution to ligand biosensing because unlike other methods, it does not rely on finding or modifying naturally occurring proteins that bind to ligands of interest.
    a. Kang, S., Davidsen, K., Gomez-Castillo, L., Jiang, H., Fu, X., Li, Z., Liang, Y., Jahn, M., Moussa, M., DiMaio, F. & Gu, L. (2019) COMBINES-CID: An efficient method for de novo engineering of highly specific chemically induced protein dimerization systems, J. Am. Chem. Soc., 141(28), 10948-52.

    b. Spencer D.M., Wandless T.J., Schreiber S.L. & Crabtree G.R. (1993) Controlling signal transduction with synthetic ligands. Science, 262(5136), 1019-1024.

4. **Creating Light Induced Dimerization for Deep-Tissue Optogenetics**. Protein dimerization systems that can be controlled by red light with increased tissue penetration depth are a highly needed tool for clinical applications such as cell and gene therapies. However, existing red light-induced dimerization systems are all based on phytochrome photoreceptors and naturally occurring binding partners with complex structures and suboptimal in vivo performance (Kaberniuk, et al., Nat. Methods, 2016; Redchuk, et al., Nat. Chem. Biol., 2017), limiting mammalian applications. My lab developed an efficient, generalizable method (COMBINES-LID) for creating highly specific light-induced dimerization systems (Huang, et al., 2020, under review). It involves a two-step binder screen (column chromatography-based phage display and yeast two-hybrid) of a combinatorial nanobody library to obtain binders that selectively engage a light-activated form of a photoswitchable protein or domain not the dark form. Proof-of-principle was provided by creating nanobody-based, red light-induced dimerization (nanoReD) systems comprising a truncated bacterial phytochrome sensory module using a mammalian endogenous chromophore, biliverdin, and a light-form specific nanobody. Selected nanoReD systems were biochemically characterized and exhibited significant lower dark activity and higher induction specificity for in vivo activation of gene expression than the existing red light LIDs. Our method opens new opportunities for creating genetically encoded actuators for the optical manipulation of biological processes.
    a. Kaberniuk, AA.; Shemetov, A.A. & Verkhusha, VV. (2016) A bacterial phytochrome-based optogenetic system controllable with near-infrared light. Nat. Methods, 13, 591-597.
    b. Redchuk, TA; Omelina, ES.; Chernov, K. G. & Verkhusha, V. V. (2017) Near-infrared optogenetic pair for protein regulation and spectral multiplexing. Nat. Chem. Biol. 13, 633-639.
    c. Huang,Z., Li,Z., Zhang X., Dong,R., Kang, S., Sun, L., Fu,Z., Vaisar D., Watanabe,K. & Gu, L. (2020) Creating red light-switchable protein dimerization systems as genetically encoded actuators with high specificity. ACS Synth. Biol., 9, 3322-33.

5. **Spatial transcriptomics with high resolution and sensitivity**. Current technologies for acquiring spatial, transcript information from tissue sections rely on either RNA probes or spatial barcodes. The former methods require a priori knowledge for probeset formulation; the latter have yet to achieve single cell resolution and/or transcript capture efficiencies approaching dissociative, single-cell methods. My laboratory recently developed a novel, spatial transcriptome assay called polony (or DNA cluster)-indexed library-sequencing (PIXEL-seq). It improves upon other spatial barcode methods by employing "continuous" polony oligos arrayed across a customized gel surface. In terms of assay performance, PIXEL-seq attains ≤ 1 μm resolution and captures >1,000 UMIs/10×10 μm^2. In other words, this global, naive platform achieves subcellular spatial transcriptome mapping while maintaining high transcript capture efficiencies.
    a. Fu, X.; Sun, L.; Dong, R.; Chen J.Y.; Lin, Y.; Palmiter, R.D.; Lin, S. & Gu, L. (2021) Continuous polony gels for tissue mapping with high resolution and RNA capture efficiency. bioRxiv, doi: https://doi.org/10.1101/2020.03.17.435795.

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/liangcai.gu.2/bibliography/public/

D. Research Support

Ongoing Research Support

UG3CA268096 Gu (PI) 09/24/21 - 08/31/23
National Institute of Health / OD&NCI
PIXEL-seq-Based Spatial, Multi-Omic Profiling for Senescent Cell Mapping with Single-Cell Resolution
The major goals of this project are to develop PIXEL-seq-based multi-omics assays to map senescent cells in human tissues.

R21DA051555 Gu (PI) 04/01/21 - 03/31/23
National Institute of Health / NIDA

Genetically Encoded Optical Biosensors for Dissecting Brain Distribution and Metabolism of Cannabinoids
The major goals of this project are to develop and in vitro validate THC and 11-Hydroxy-THC biosensors.

R61DA051489 Bruchas/Gu (MPI) 06/01/20 – 05/31/23
National Institute of Health / NIDA
Optopharmacology and Sensors for Dissecting Opioid Action In Vivo
The major goals of this project are to develop and use optopharmacological and biosensor (morphine and methadone) tools for understanding how mu-opioids mediate effects in the brain to alter brain function, inhibit pain, and alter motivation.

R21DA051194 Gu (PI) 04/01/20 - 03/31/22
National Institute of Health / NIDA
Genetically Encoded Fluorescent Biosensors for Single-Cell and Subcellular Opioid Pharmacokinetics
The major goal of this project is to develop and in vitro validate fentanyl biosensors.

R35GM128918 Gu (PI) 8/01/18 - 07/31/23
National Institute of Health / NIGMS Total Project Cost: $1,974,002
De Novo Engineering of Small Molecule-Actuatable Biosensors for Cell Therapy
The major goal of this project is to develop and use drug-controlled protein biosensors to control in vivo behaviors of therapeutic cells.

UW/NSTG collaboration Gu (PI) 10/01/21 - 6/31/22
NanoString Technologies
Pilot Experiments to Characterize Unique Classes of Spatial Genomic Technologies on Fresh Frozen and FFPE Human Cancer Tissues
The major goal of this project is to characterize the potential of three spatial genomic technologies for analyzing transcriptome and proteome in morphologically intact human cancer tissues.

R41MH130299 Gu (subaward PI) 04/01/22 - 03/31/23
National Institute of Health / NIMH
Scalable, non-dissociative single-cell RNA sequencing for mapping the brain in health and disease
The major goals of this STTR Phase I project to optimize, scale, and commercialize PIXEL-seq for the brain mapping.

Completed:
65-5339 Gu/DiMiao (MPI) 05/01/17-04/30/20
U of Washington Innovation Award
A General Approach for Label-Free, ELISA-Like Detection of Small Molecules
Development of computational design and high-throughput screening approaches to engineer chemically induced binders to detect small-molecule drugs for point-of-care testing.

New Investigator Support Grant, CCSG Gu (PI) 08/01/17-07/31/18
Fred Hutch/UW Cancer Consortium
Developing a ‘Library vs. Library’ Screening of TCR Mimic Antibodies Targeting Neoantigens
The goal of this project is to develop a cost-effective approach to screen TCR mimic antibodies against neoantigens by SMI-seq.

CCSG Pilot grant Gu (PI) 4/16/18-4/15/19
Fred Hutch/UW Cancer Consortium
Design of Near-Infrared Light-Actuatable Biosensors for Precise Spatiotemporal Control of CAR T-Cell Therapy
The major goal of this project is to engineer near-infrared light-controlled protein dimerization systems to improve safety and efficacy of CAR-T cell therapy.

OMB No. 0925-0001 and 0925-0002 (Rev. 12/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Stephen D. Hauschka

eRA COMMONS USER NAME (credential, e.g., agency login): hauschka

POSITION TITLE: Professor of Biochemistry

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Amherst College	Biology	06/1962	Biology
Johns Hopkins University & Carnegie Inst. Embryol.	Biology	06/1966	Biology
University of Washington	Post-Doc	07/1967	Biochemistry

A. Personal Statement: I've been working on mechanisms of striated muscle development & gene regulation since 1963. Specific expertise related to this proposal comes from many decades of at-the-bench muscle cell culture and muscle gene regulation experience. Our lab was the first to develop methods for clonal analysis of human skeletal muscle cells, and we also discovered several of the growth factors and mechanisms related to myoblast growth & differentiation *(see refs in sections 1 & 3 of "Contributions to Science").* I continue to do such studies on a daily basis in conjunction with current research focused on identifying skeletal & cardiac muscle gene enhancer & promoter regions, the control elements within these, and their relevant transcription factors **(Ref-1)**; and this basic information has been used for the design of several hundred skeletal- & cardiac Muscle-Specific Expression Cassettes (MSECs) that transcribe virtually any product in these cell types over a 3-log range **(Ref-2)** *(also see Salva et al Ref in Section-5 of "Contributions to Science")*. These MSECs are now being use by us, Eric Olson, Charlie Gershbach and others for muscle-specific CRISPR-mediated gene corrections **(Refs-3 & 4)**. Of particular relevance to this proposal, we merged our research group with Jeff Chamberlain's group 2 years ago, and thus collaborate seamlessly in the same Lab and AAV Vector Core space on our muscle gene therapy studies.

Ref-1: Tai, PW, et al. (2011). Differentiation and fiber type-specific activity of a muscle creatine kinase intronic enhancer. Skeletal Muscle. 2011Jul 7;1:25. doi: 10.1186/2044-5040-1-25. PMID: 21797989

Ref-2: Himeda, CL, Chen X, and Hauschka S (2011). Design and testing of regulatory cassettes for optimal activity in skeletal and cardiac muscles. Methods Mol Biol. 709: 3-19. PMID: 21194018

Ref-3: Bengtsson, NE, et al. (2017). Muscle-specific CRISPR/Cas9 dystrophin gene editing ameliorates pathophysiology in a mouse model for Duchenne muscular dystrophy. Nat Commun 14;8: 14454. PMID: 28195574.

Ref-4: Bengtsson, NE, et al. (2022). Comparison of dystrophin expression following gene editing and gene replacement in an aging preclinical DMD animal model.

B. Positions and Honors:

1967-Present Professor, Dept. of Biochemistry, University of Washington

Grant Review Panels:

1975-1976: NIH Cell Biology Study Section.
1977-1978: NSF Developmental Biology Study Section.
1982-1988: NIH Molecular Cytology Ad Hoc Member (every other panel mtg.).
1989-2000: MDA Scientific Advisory Committee Study Section & MDA Gene Therapy Steering Com.
2005-2008: Am. Society of Gene Therapy Ethics Committee.

Editorial Boards: *Growth Factors; Molecular Therapy; J. Neuromuscular Diseases*

Honors:

1966 - 1967 Howard Hughes Medical Institute Post-doctoral Fellowship
1967 - 1977 Howard Hughes Medical Institute Faculty Fellowship
1981: University of Washington Outstanding Undergraduate Professor Award.
1992-2002: NIH Merit Award.
2011: University of Washington Undergraduate Research Mentor-of-the-Year Award

C. Contribution to Science: *The "Contribution Topics" below are listed in the general chronological order in which the studies were carried out. Current research is focused primarily on topics 4 & 5 that apply basic knowledge from our muscle gene regulation studies (topic-4) to the applied research goal of designing improved Muscle-Specific Expression Cassettes (topic-5).*

1. Clonal Analysis of Myogenesis: We were the first lab to develop clonal assays for myoblasts derived from chick & human limb buds of different developmental ages **(Ref-1)**. We detected differences between muscle colony-forming (MCF) cells based on their differential requirements for conditioned media & serum components **(Ref-2)**. Our identification of stage-specific MCF cells also led to the discovery of Neural Tube and Ectodermal Ridge influences on different MCF cell types, as well as to the identification of collagen-fibronectin & FGF as critical components for survival & differentiation of distinct MCF types. In related studies we used quantitative MCF cell assays to delineate the spatial domains of different myogenic lineages in developing limb buds **(Ref-3)**, and to determine when different MCF cells migrate from somites into limb buds **(Ref-4)**. Our studies served as the conceptual foundation for discrete myogenic cell types within the skeletal muscle lineage, and led to the first reproducible protocol for making permanent clonal myoblast cell lines (MM14 cells) **(Topic-3, Ref-1)**, and diploid satellite cell-derive lines from many normal, mutant & NMD mouse models. **This experience & the FGF myoblast proliferation studies described in #3, are directly applicable to the studies described in this proposal.**

Ref-1: Hauschka, SD. (1974). Clonal analysis of vertebrate myogenesis. 3. Developmental changes in the muscle colony-forming cells of the human fetal limb. Dev Biol. 37: 345-368. PMID: 4826281.
Ref-2: White NK, et al. (1975). Clonal analysis of vertebrate myogenesis. IV. Medium-dependent classification of colony-forming cells. Dev Biol. 44:346-361. PMID: 1132597.
Ref-3: Rutz R, Haney C, and S. Hauschka (1982). Spatial analysis of limb bud myogenesis: a proximo-distal gradient of muscle colony-forming cells in chick embryo leg buds. Dev Biol. 90: 399-411. PMID: 7075868.
Ref-4: Seed J. & S Hauschka (1984). Temporal separation of the migration of distinct myogenic precursor populations into the developing chick wing bud. Dev Biol. 106: 389-393. PMID: 6500180.

2. Analysis of Myogenic Induction Mechanisms: Classic embryologic tissue deletion/transplantation and cell marking studies had shown that most vertebrate skeletal muscle cells are derived from somitic mesoderm and that their formation requires inductive signals from neural tube and/or notocord. To determine the bio-chemical basis of induction we developed an *in vitro* single somite assay that delineated the developmental & somite stage-specificity of myogenically committed and uncommitted somites. We then showed that cells in uncommitted somites became myogenic when co-cultured with either neural tube or notocord, and that Wnt-1 is capable of replacing the neural tube inductive signals **(Ref-1)**. Subsequent studies showed that FGF & TGF-1 as well as IGF-1 & -2, Insulin and Sonic Hedgehog, can synergize to induce somite myogenesis **(Refs 2 &**

3); and myogenic steps were correlated with temporal changes in myogenic transcription factors **(Ref-4)**. These studies then contributed to strategies for manipulating hES cell and iPSC differentiation.

Ref-1: Stern HM, et al. (1995). Myogenesis in paraxial mesoderm: preferential induction by dorsal neural tube and by cells expressing Wnt-1. Development 121: 3675-3686. PMID: 8582280.
Ref-2: Stern HM, et al. (1997). Synergistic interactions between bFGF and a TGF-beta family member may mediate myogenic signals from the neural tube. Development. 124:3511-3523. PMID: 9342044
Ref-3: Pirskanen A, et al. (2000). IGFs, insulin, Shh, bFGF, and TGF- 1 interact synergistically to promote somite myogenesis in vitro. Dev Biol 224: 189-203. PMID: 10926759.
Ref-4: Kiefer JC and SD Hauschka (2001). Myf-5 is transiently expressed in nonmuscle mesoderm and exhibits dynamic regional changes within the presegmented mesoderm and somites I-IV. Dev Biol 232: 77-99. PMID: 11254349.

3. Commitment to Terminal Differentiation & the roles of Fibroblast & Epidermal Growth Factors (FGF & EGF) & their Receptors in Myogenesis: By the 1980s it was well known that in vitro muscle differentiation could be triggered by serum removal and the mechanism was presumed to be due to a growth-related repression of myogenesis by unknown serum factors. Coincident to the discovery of Fibroblast Growth Factor (FGF)by Gospodarowicz we had shown that mouse myoblast differentiation was repressed by an embryo extract component. Hypothesizing that EE contained FGF, we purified it from bovine brains, showed that FGF1 & 2 repress myoblast differentiation. **(Ref-1)**, and that replicating myoblasts deprived of FGF for as little as 4 hoursundergo an "irreversible" commitment to terminal differentiation only in the G1 cell cycle phase **(Ref-1)**. This discovery played a major role in refuting the concept of *"quantal cell cycles"* that were thought to initiate the onset of terminal differentiation many cycles prior to differentiation. Coincident with these studies we were thefirst lab to isolate & characterize the FGF receptor (FGFR) **(Ref-2)**. Knowledge from these studies facilitated the temporal analysis of FGFR as well as EGFR, following FGF removal, and led to a mechanistic explanationof the irreversible post-mitotic phenotype of committed myocytes via the repression of FGFR & EGFR transcription & the disappearance of these cell surface receptors; thus rendering committed myocytes unable to detect & respond to subsequent FGF or EGF mitogenic signals **(Refs-3 & 4)**. This has become a general mechanistic paradigm for muscle differentiation.

Ref-1: Clegg CH, et al. (1987). Growth factor control of skeletal muscle differentiation: commitment to terminal differentiation occurs in G1 phase and is repressed by fibroblast growth factor. J Cell Biol. 105: 949-956. PMID: 3624313.
Ref-2: Olwin BB and SD Hauschka (1986). Identification of the fibroblast growth factor receptor of Swiss 3T3 cells and mouse skeletal myoblasts. Biochemistry. 25: 3487-3492. PMID: 3013291.
Ref-3: Olwin BB and SD Hauschka (1988). Cell surface fibroblast and epidermal growth factor receptors are permanently lost during skeletal muscle terminal differentiation in culture. J. Cell Biol.197:761-769. PMID: 2843547
Ref-4: Templeton TJ and SD Hauschka (1992). FGF-mediated aspects of skeletal muscle growth and differentiation are controlled by a high affinity receptor, FGFR1. Dev Biol. 154: 169-181. PMID: 1426624.

4. Mechanisms of Muscle Gene Regulation: Our muscle gene studies began at a time when no muscle enhancers or control elements had been identified, and when regulatory factors such as MyoD had not been envisioned. We focused on the M-creatine kinase (MCK) gene after showing it was rapidly activated during terminal differentiation, and that its mRNA levels exceeded 1000 per myonucleus. After cloning, manually sequencing & testing regions within MCK's 5'-flanking DNA for transcriptional activity we were the first group to identify a muscle gene enhancer. We then used deletion & mutation strategies to identify the enhancer's most active control elements and found 2 tandem so-called E-box motifs that were specifically bound by a nuclear factor (MEF1) whose activity was present in differentiated skeletal muscle but not in replicating myoblasts or any other cell types **(Ref-1)**. Coincident with our studies the Weintraub lab identified MyoD as a muscle factor that – when over-expressed in non-muscle cells – converted them to myoblasts. Collaborations between our groups then identified our unknown MEF1 factor as MyoD and the enhancer E-Box motifs as the sites of MyoD binding in MCK and many other muscle genes **(Ref-2)**. These studies provided mechanistic foundations for understanding how the transcriptional activation of hundreds of different muscle genes is coordinated during differentiation. While we and others then identified many other muscle gene control elements based on their conserved sequences and correlations with known transcription factor (TF) binding site data, we also found

conserved DNA motifs that exhibited transcriptional activity but lacked clear matches to the TF data bases. We thus developed a differential TF-DNA motif binding assay and used it in conjunction with quantitative proteomics to identify candidate TFs. This led to identifying TFs with novel binding sites (e.g., Six4), TFs with no previously known roles in muscle gene regulation; e.g., MAZ **(Ref-3),** and identified synergisms between known and previously unknown muscle TFs (e.g., SRF & KLF3) that play novel roles in muscle gene regulation **(Ref-4)**. Identifying these myogenic control elements and their cognate TFs provided the field with basic information for understanding the intracellular signaling mechanisms responsible for expressing muscle genes at different levels in response to environmental & physiological signals.

Ref-1: Buskin JN and SD Hauschka (1989). Identification of a myocyte nuclear factor that binds to the muscle-specific enhancer of the mouse muscle creatine kinase gene. Mol Cell Biol. 9: 2627-2640. PMID: 2761542
Ref-2: Lassar AB, et al. (1989). MyoD is a sequence-specific DNA binding protein requiring a region of myc homology to bind to the muscle creatine kinase enhancer. Cell. 58: 823-831. PMID: 2550138
Ref-3: Himeda Cl, et al. (2008). Quantitative proteomic identification of MAZ as a transcriptional regulator of muscle-specific genes in skeletal and cardiac myocytes. Mol Cell Biol. 28:H6521-6535. PMID: 18710939.
Ref-4: Himeda, C.L., et al. (2010). KLF3 regulates muscle-specific gene expression and recruits SRF to KLF binding sites. *Mol. Cell. Biol*. 30: 3430-3443. PMID: 20404088.

5. Design of optimal Muscle-Specific Expression Cassettes (MSECs): Because current viral vectors for gene therapy exhibit little cell type specificity, they can – when used in conjunction with ubiquitously expressed promoters such as CMV -- cause safety issues by producing therapeutic products in off-target cells. CMV can also activate oncogenes via rare integration events. These pitfalls can be lessened by designing MSECs. However, since native muscle genes have lower transcriptional activities than CMV they require modifications to enhance their activities. Also, since AAV has an ~4.8 kb packaging size limit, MSECs must be miniaturized in order to package therapeutic cDNAs in the 3.5 - 4.2 kb range. Our initial studies created active MCK-based MSECs of ~ 0.6kb, then a 3X more active ~ 0.8kb RC containing MCK enhancer/promoter regions plus an α-Myosin Heavy Chain enhancer **(Ref-1)**. Later improvements created MSECs <0.5kb with activities ~ 2X> CMV in pure muscle cultures. "CK8e," whose *in vivo* expression occurs only in skeletal & cardiac muscle has now been used in diverse muscle disease gene therapy studies, as well as for diseases in which non-muscle therapeutic products (growth & parathyroid hormones, and liver arginase) are secreted by muscle fibers **(Ref-2)**. Additionally, we've designed cardiac-specific cassettes containing components from the human cardiac troponin T enhancer/promoter, and used these to simultaneously express elevated levels of the two protein sub-units of ribonucleotide reductase in heart muscle via systemic AAV delivery (PMID: 26388461). Improved MSECs have provided increased expression of novel micro-dystrophins **(Ref-3)**, and have been used to correct dystrophin mutations tissue specifically in skeletal & cardiac muscle via CRISPR/Cas9 **(Refs-4).** Importantly, several of our MSECs are being used in on-going human Clinical Trials and appear to be functioning well. **Our continually improving MSEC designs are directly relevant to all Specific Aims in this proposal.**

Ref-1: Salva, M.Z., et al. (2007) Design of novel tissue-specific regulatory cassettes for high-level rAAV-mediated expression is skeletal and cardiac muscle. *Molecular Therapy* 15: 320-329. PMID: 17235310.
Ref-2: Hu C, et al. (2014). Myocyte-mediated arginase expression controls hyperarginemia but not hyperammonemia in arginase-deficient mice. Mol Ther 22: 1792-1802. PMID: 24888478.
Ref-3: Ramos, J.N. et al. (2019). Development of novel micro-dystrophins with enhanced functionality. Mol Ther. 27(3):623-635. PMID: 30130494.
Ref-4: Bengtsson, NE, et al. (2021). Dystrophin gene-editing stability is dependent on dystrophin levels in skeletal but not cardiac muscles. Mol Ther. 5: S1525-0016PMID: 33160075.

Url for PubMed Papers: **http://www.ncbi.nlm.nih.gov/pubmed/?term=Hauschka+S**

Numerous Book Chapters & papers in non-PubMed journals are not included in this list.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Suzanne Hoppins

eRA COMMONS USER NAME (credential, e.g., agency login): shoppins

POSITION TITLE: Associate professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Alberta, Edmonton, Alberta, Canada	BSc	06/2000	Mol. Biol. & Genetics
University of Alberta, Edmonton, Alberta, Canada	PhD	11/2005	Mol. Biol. & Genetics
University of Davis, California, Davis, CA, USA	n/a	04/2013	Mol. & Cell Biology

A. PERSONAL STATEMENT

The long-term goal of my lab is to understand how mitochondrial dynamics are integrated with cellular physiology. Mitochondria are remarkable organelles and the dynamic processes of fusion, division and transport are central to their function. Both mitochondrial fusion and mitochondrial division are mediated by dynamin superfamily proteins (DSPs), large GTPases that self-assemble into structures that harness mechanochemical work to remodel membranes. This proposal is focused on mechanistic understanding of the mitochondrial outer membrane fusion machine, composed of two mitofusin paralogs, Mfn1 and Mfn2. Using mouse embryonic fibroblasts to probe the unique functions of Mfn1 and Mfn2, I reconstituted mitochondrial fusion *in vitro* as a postdoctoral fellow. In my own lab, this powerful assay has revealed fusion defects in disease-associated mitofusin variants, which were masked in cells, allowing us to gain critical insight into the mechanism and regulation of mitofusin activity. We have established additional biochemical assays to examine GTP-dependent assembly of mitofusins, including co-immunoprecipitation of the tethering complex and BN-PAGE of oligomers in the same mitochondrial membrane. Significantly, these assays demonstrate for the first time that GTP-dependent assembly *in cis* is required for mitofusin-mediated fusion. *With this biochemical analysis pipeline, we are poised to develop a mechanistic framework for how DSPs mediate membrane tethering and fusion.* I have the technical expertise required to execute the experiments proposed in this grant. I have extensive experience in assay development, microscopy, cell culture models, and biochemical techniques. Since establishing my lab at the University of Washington, I have recruited and trained productive, creative and technically sound graduate and undergraduate students. We are poised to execute the experiments outlined in this research proposal and make significant contributions to our understanding of the mechanism of mitochondrial fusion and the pathophysiology of CMT2A. The Covid-19 pandemic has somewhat slowed our progress. UW School of Medicine closed all labs in April 2020 and opening has been slowly staged but will be complete by September 2021. In addition, my two elementary-aged children attend public schools, which were fully remote from April 2020 – April 2021 and hybrid from April 2021 – June 2021.

B. RESEARCH AND/OR PROFESSIONAL EXPERIENCE

Employment:

2000-2005 Graduate Student with Frank Nargang Ph.D., Department of Genetics, University of Alberta, Edmonton, Alberta, Canada

2005-2013 Postdoctoral Fellow with Jodi Nunnari Ph.D., Department of Molecular and Cellular Biology, University of California, Davis, USA

2013-2019 Assistant Professor, Department of Biochemistry, University of Washington, Seattle, WA, USA
2019-present Associate Professor, Department of Biochemistry, University of Washington, Seattle, WA, USA

Honors:

2000	Jaedon Bland Memorial Graduate Scholarship
2000-2001	Faculty of Science Graduate Teaching Assistantship Scholarship
2001-2002	Graduate Intern Tuition Supplement
2001-2005	Alberta Heritage Foundation for Science and Engineering Research Graduate Scholarship
2002	Province of Alberta Graduate Student Scholarship
2003	J Gordin Kaplan Graduate Student Award
2003-2005	Walter H. Johns Graduate Fellowship
2003-2005	National Science and Engineering Research Council (NSERC) Post Graduate Scholarship B
2009	2nd place in poster competition at FASEB Mitochondrial assembly & dynamics in health and disease, Tucson, AZ.
2011-present	NIH Pathway to Independence Award (K99/R00)

Professional societies and public advisory committees:
American society of cell biology

C.CONTRIBUTIONS TO SCIENCE

As I have established my own lab, we have built on my postdoctoral work and have continued to focus on the mechanism of mitochondrial outer membrane fusion. We characterized unique functional variants of the Mitofusins that are associated with the neurodegenerative disorder CMT2A. Our data indicate that Mitofusins interact across two membranes to form a physical tether in a nucleotide-dependent manner. We have also adapted native gels to monitor nucleotide-dependent assembly of Mitofusin and show that Mfn1 and Mfn2 assemble independently of the opposite paralog. We have characterized a novel class of Mitofusin mutants that efficiently tether mitochondria but have defects in nucleotide-dependent assembly and mitochondrial fusion *in vitro*. From this, we suggest that higher order assembly is required for efficient Mitofusin-mediated fusion following an initial tethering event. To gain insight into the functional differences that define Mfn1 and Mfn2, we have characterized chimeric proteins in cells and in vitro. These data indicate that nucleotide dependent assembly is a unique feature of each protein and that a discrete domain (MISR) influences this activity.

Engelhart EA, **Hoppins S*** (2019). *A* catalytic domain mutant of Mitofusin that requires a wild-type paralog for function uncouples mitochondrial outer membrane tethering and fusion. ***J. Biol. Chem.*** 294:8001-8014. PMC6527157

Sloat SR, Whitley BN, Engelhart EA, **Hoppins S*** (2019). Identification of a Mitofusin specificity region that confers unique activities to Mfn1 and Mfn2. ***Mol. Biol. Cell*** 30(17):2309-2319. PMC6743458

Samanas NB, Engelhart EA, **Hoppins S*** (2020) Mfn2 requires Hinge 1 integrity for efficient nucleotide-dependent assembly and membrane fusion. **Life Sci Alliance** Apr 3;3(5):e201900527. PMC7136618

We had the exciting opportunity to collaborate with clinicians to characterize novel mutant variants of the mitochondrial division machine, Drp1. The variants were all identified by whole exome sequencing of patients diagnosed with a mitochondrial disorder. The clinical report of five individuals built upon and expanded the clinical spectrum associated with Drp1 variants in patients. Our functional analysis of the Drp1 variants, some of which were novel, determined the impact of each variant on mitochondrial division activity and its dominant negative activity in wild type cells. We observed varying degrees of loss of function and dominant negative activity in the group and some discordance with the severity of the clinical symptoms. This highlights the complexity of the disease and suggests that additional factors, such as tissue specific functions, may be at play.

Whitley BN, Lam C, Cui H, Haude K, Bai R, Escobar L, Hamilton A, Tarnopolsky MA, Brady L, Dengle L, Snyder M, Picker J, Lincoln S, Lackner LL, Glass IA, **Hoppins S** (2018). Aberrant Drp1-mediated

mitochondrial division presents in humans with variable outcomes. **Hum Mol Genet** 27(21):3710-3719. PMC6196655

The mitochondrial-focused high-density genetic interaction map presented here represents an exciting and unprecedented tool for mitochondrial biologists. The MITO-MAP provides valuable insight into mitochondrial functions and the relationship between the ER and mitochondria. This work also characterized a conserved complex identified in the MITO-MAP termed MitOS, which includes Mitofilin/Fcj1. I discovered that the complex forms a unique scaffold-like structure required for normal mitochondrial inner membrane architecture.

Hoppins, S.*, Collins, S.C. *****, Cassidy-Stone, A., Hummel, E., DeVay, R.M., Lackner, L.L., Westermann, B., Schuldiner, M., Weissman, J.S., Nunnari, J.M. (2011) A mitochondrial-focused genetic interaction map reveals a scaffold-like complex required for inner membrane organization in mitochondria. *****equal contribution **Journal of Cell Biology** 195(2):323-40. PMC3971754.

The main focus of my postdoctoral work was to study the mechanism of mammalian fusion. The Molecular Cell paper describes the first biochemical reconstitution of mammalian mitochondrial fusion in vitro. The assay facilitated the discovery that Mfn1 and Mfn2 work synergistically as we show that the heterotypic Mfn1-Mfn2 complex is more effective for mitochondrial fusion than either homotypic complex. We also investigated the role of Bax in mitochondrial fusion in both healthy and apoptotic conditions. We propose a model where apoptotic-inactive Bax stimulates fusion via MFN2 homotypic complexes. In contrast, apoptotic active Bax appears to negatively regulate mitochondrial fusion in vitro, concomitant with outer membrane permeabilization.

Hoppins, S. (2014) The regulation of mitochondrial dynamics. **Curr Opin Cell Biol** 29:46-53.
Hoppins, S., Edlich, F., Cleland, M., Banerjee, S., McCaffery, J.M., Youle, R., Nunnari, J.M. (2011) The soluble form of Bax regulates mitochondrial fusion via MFN2 homotypic complexes. **Molecular Cell** 41(2):150-160. PMC3072068
Hoppins, S., Nunnari, J.M. (2009) The molecular mechanism of mitochondrial fusion. **Biochim Biophys Acta** 1793(1):20-6.
Hoppins, S.*, Lackner, L.L.*, Nunnari, J.M. (2007) The Machines that Divide and Fuse Mitochondria. **Annual Review of Biochemistry** 76: 751-80. *equal contribution

My first project in the Nunnari lab was characterization of mitochondrial fusion in the model system *S. cerevisiae*. My work defined the unique topology of Ugo1 in the mitochondrial outer membrane and demonstrated that Ugo1 forms functionally significant dimers. Furthermore, this work provides insight into the mechanism of mitochondrial fusion and suggests that Ugo1 is required for both outer and inner membrane fusion events, following membrane tethering, at the lipid mixing step of membrane fusion. I also mentored and collaborated with a graduate student, Rachel DeVay, in the characterization of the role of the conserved mitochondrial inner membrane dynamin related protein, Mgm1. We found that the isoform that is tethered to the mitochondrial inner membrane has negligible GTPase activity and it is the short, soluble isoform that couples the catalytic cycle to membrane fusion.

Hoppins, S., Horner J.S., Song C., McCaffery, J.M. Nunnari, J.M. (2009) Mitochondrial outer and inner membrane fusion required a modified carrier protein. **Journal of Cell Biology** 184(4):569-81. PMC2654124
DeVay R.M., Dominguez-Ramirez, L., Lackner, L.L., **Hoppins, S.**, Stahlberg, H., Nunnari J.M. (2009) Coassembly of Mgm1 isoforms requires cardiolipin and mediates mitochondrial inner membrane fusion. **Journal of Cell Biology** 186: 793-803. PMC2753158

Complete list of published works in MyBibliography
https://www.ncbi.nlm.nih.gov/myncbi/suzanne.hoppins.1/bibliography/public/

D. Research Support

Ongoing research support

5R01 GM 118509 Hoppins (PI) 02/01/2017 – 01/31/2022
Determining the mechanism of mitochondrial outer membrane fusion.
The goal of this grant is to gain insight into the mechanism of mitofusin-mediated membrane fusion.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: James B. Hurley

eRA COMMONS USER NAME (credential, e.g., agency login): jhurley

POSITION TITLE: Professor of Biochemistry

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
SUNY College of Environmental Science & Forestry	B.S.	05/1975	Chemistry
University of Illinois, Champaign, IL	Ph.D.	12/1979	Physiol/Biophys
Stanford University, Stanford, CA	Postdoc	06/1981	Biochemistry
UCSD/California Institute of Technology	Postdoc	12/1984	Biochemistry

A. Personal Statement

The primary focus of my current research is to develop a new conceptual framework for how energy production and distribution influence function and viability of photoreceptor neurons. I am qualified to do this because of my successful experience on this current project and my previous success in helping establish a conceptual framework for phototransduction. To understand energy metabolism I am using a strategic plan similar to what I used for phototransduction: develop or adapt whatever methods are necessary, and focus on the most fundamental questions. My colleagues and I develop methods to answer key questions. These include isolating and purifying proteins, developing enzymatic assays, cloning, expression, ERG analysis, mass spectrometry of proteins and metabolites. I've trained 18 graduate students and 17 postdoctoral fellows. 14 of them established independent academic appointments and competed successfully for independent funding. I have helped to mentor them in their new positions and I also have played a significant role in mentoring several of the new faculty in our department at the University of Washington.

Ongoing and recently completed projects that I would like to highlight include:

RO1 EY06641 Hurley, James B. (PI)
12/15/19 - 11/30/23
Determinants of Rod and Cone Response Characteristics

RO1 EY017863 Hurley, James B. (PI)
4/1/18 – 3/31/23
Control of Photoreceptor Metabolism

R21 EY032597 Hurley, James B. (PI)
5/1/21 – 4/30/23
Respiration in vivo in the retina and RPE

Citations:

- Bisbach, C.M., Hass, D.T., Robbings, B.M., Rountree, A.M., Sadilek, M., Sweet, I.R. and Hurley, J.B. (2020). Succinate can shuttle reducing power from the hypoxic retina to the O_2-rich pigment epithelium. Cell Rep 31:107606 PMC7273505.

- Kanow, M.A., Giarmarco, M.M., Jankowski, C.S.…**Hurley, J.B**. (2017). Biochemical adaptations of the retina and retinal pigment epithelium support a metabolic ecosystem in the vertebrate eye. Elife e28899. PMC5617631.
- Du, J., Rountree, A., Cleghorn, W.M., Contreras, L., Lindsay, K.J., Sadilek, M., Gu, H., Djukovic, D., Raftery, D., Sartrústegui, J., Kanow, M., Chan, L., Tsang, S.J., Sweet, I.R., **Hurley, J.B.** (2016). Phototransduction influences metabolic flux and nucleotide metabolism in mouse retina. J. Biol. Chem. 291:4698-710. PMC4813492.
- **Hurley, J.B.**, Lindsay, K.J., Du, J. (2015). Glucose, lactate and shuttling of metabolites in vertebrate retinas. J. Neurosci. Res. 93: 1079-92. PMC4720126

B. Positions, Scientific Positions and Honors

1996- present Professor of Biochemistry, University of Washington
1996-2001, HHMI Investigator and Professor of Biochemistry, Department of Biochemistry;
1989-1995, HHMI Associate Investigator and Associate Professor of Biochemistry;
1985-1989, HHMI Assistant Investigator and Assistant Professor of Biochemistry, Univ. of Washington;
1983-1984, Research Fellow with Dr. Melvin I. Simon at Caltech, sequencing G-proteins;
1982-1983, Helen Hay Whitney postdoc with Dr. Melvin I. Simon at UCSD, isolating cDNA for transducin;
1980-1982, Helen Hay Whitney postdoctoral fellow with Dr. Lubert Stryer in the Department of Structural Biology at Stanford University, investigating phototransduction;
1976-1979, graduate student with Dr. T. G. Ebrey at the University of Illinois, thesis on primary photochemistry of visual pigments and biochemical processes associated with vision.

Honors and Awards: American Institute of Chemists Award for Scholastic Achievement, 1975; NIH Cell Biology Trainee,1977-1979; Helen Hay Whitney Postdoctoral Fellowship, 1980-1983; 2014 Alcon Research Institute Award.

C. Contributions to Science

Research:

1. **A G-protein (transducin) a plays a central role in phototransduction**. As a graduate student with Tom Ebrey I was interested in how photoreceptors transduce light into electrical and chemical signals. At the time, the prevailing model was that stimulation of rhodopsin released Ca^{2+} into the photoreceptor cytoplasm and this this altered plasma membrane conductance. I focused my research on an alternative model based on reports by Bitensky and Liebman that revealed effects of light on cyclic nucleotide metabolism. After completing my Ph.D. I continued this research as a postdoc with Lubert Stryer. I helped identify and reconstitute three key components of this signaling cascade, a phosphodiesterase, a G-protein and an inhibitor subunit of the phosphodiesterase.
   - **Hurley, J. B.** (1980) Isolation and recombination of bovine rod outer segment cGMP phosphodiesterase and its regulators. *Biochem. Biophys. Res. Comm.* **92**:505. *Cited 48 times*.
   - Fung, B. K.-K., **Hurley, J. B.** and Stryer, L. (1981) Flow of information in the light triggered cyclic nucleotide cascade of vision. *Proc. Natl. Acad. Sci. USA* **78**:152. *Cited 719 times*.
   - **Hurley, J. B.** and Stryer, L. (1982) Purification and characterization of the regulatory subunit of the cyclic GMP phosphodiesterase from retinal rod outer segments. *J. Biol. Chem*. **257**:11094. *Cited 279 times*.
   - **Hurley, J. B.**, Simon, M.I., Teplow, D. B., Robeshaw, J. D. and Gilman, A. G. (1984) Homolgies between signal transducing G proteins and *ras* gene products. *Science* **226**:860. *Cited 477 times.*
2. **Rods and cones use distinct proteins for phototransduction**. While isolating cDNA clones for the transducin and phosphodiesterase subunits I found that there were distinct forms of transducin alpha subunits and phosphodiesterase inhibitor subunits in rods vs. in cones. In subsequent experiments my colleagues and I used this finding colleagues and I to characterize cone-transducin-deficient mice and zebrafish and to establish the visual sensitivities of scotopic and photopic vision in mice. The finding also has been used widely by other investigators in attempts to explain the differences in response sensitivity and kinetics between rods and cone.
   - Lerea, C. L., Somers, D. E., **Hurley, J. B**., Kloch, I. B. and Bunt-Milam, A. H. (1986) Identification of specific transducin α subunits in retinal rod and cone photoreceptors. *Science* **234**:77. *Cited 229 times*.
   - Hamilton, S. and **Hurley, J. B**. (1990) Characterization of a retinal cone phosphodiesterase inhibitor. *J. Biol. Chem*. **265**:11259-11264. *Cited 65 times*.
   - Lerea, C. L., Bunt-Milam, A. H., Kloch, I. and **Hurley, J. B**. (1989) Transducin is present in red, green

and blue sensitive photoreceptors in the human retina. *Neuron* **3**:367-376. *Cited 106 times*.

- Nathan, J., Reh, R., Ankoudinova, I., Andoudinova, G., Chang, B., Heckenlively, J. and **Hurley, J.B**. (2006). Scotopic and photopic visual thresholds and spatial and temporal discrimination evaluated by behavior analysis of mice in a water maze. *Photochem. Photobiol*. 82:1489-94. *Cited 14 times*.

3. **Membrane guanylyl cyclases and Ca^{2+}-sensitive regulatory proteins control cyclic GMP synthesis**. My colleagues and I investigated biochemical mechanisms responsible for synthesizing cGMP in photoreceptors. We identified the guanylyl cyclases and one of the regulatory proteins that imparts Ca^{2+}-sensitivity to the cyclase. We contributed much of the fundamental information about structures of these proteins, how they function and the consequences of mutations in these proteins that cause retinal disease.
   - Dizhoor, A. M., Olshevskaya, E. V., Laura, R., Lowe, D. G., and **Hurley, J. B.** (1994) The human photoreceptor membrane guanylyl cyclase, RetGC, is regulated by calcium and a soluble activator. *Neuron.* **12**:1345-1352. *Cited 207 times*.
   - Dizhoor, A.M., Olshevskaya, E.V., Henzel, W.J., Wong, S.C., Stults, J.T., Ankoudinova, I. and **Hurley, J.B.** (1995) Cloning, sequencing and expression of a 24 kDa Ca^{2+}-binding protein activating photoreceptor membrane guanylyl cyclase. *J. Biol. Chem.* **270**:25200-25206. *Cited 222 times*.
   - Tucker, C. L. Woodcock, S.C., Kelsell, R.E., Ramammurthy, V., Hunt, D.M. and **Hurley, J.B.** (1999) Biochemical analysis of a dimerization domain mutation in RetGC-1 associated with dominant cone-rod dystrophy*. Proc Natl Acad Sci U S A.* **96**:9039-44. *Cited 65 times*.
   - Ramamurthy, V., Tucker, C.L., Wilkie, S.E., Daggett, V., Hunt, D.M. and **Hurley, J.B.** (2001). Interactions within the coiled-coil domain of RetGC-1 guanylyl cyclase are optimized for regulation rather than for high affinity. *J. Biol. Chem*. **276**:26218-26219. *Cited 49 times*.

4. **Rhodopsin is phosphorylated differentially at specific sites *in vivo***. My colleagues and I developed a rapid quench and mass spectrometry-based method to analyze phosphorylation of rhodopsin that occurs in live retinas. We linked our measurements of phosphorylation of rhodopsin directly with measurements of dark-adaptation by performing biochemical and electrophysiological analyses under identical *in vivo* conditions. Our mass spectrometry method also allowed us to identify the sequence with which sites on rhodopsin become phosphorylated and dephosphorylated in vivo following photo-stimulation.
   - Kennedy, M.J., Lee, K.A., Niemi, G.A., Craven, K.B., Garwin, G.G., Saari, J.C. and **Hurley, J.B.** (2001) Multiple phosphorylation of rhodopsin and the *in vivo* chemistry underlying rod photoreceptor dark adaptation. *Neuron*. **31***:*87-101. *Cited 93 times*.
   - Kennedy, M.J., Dunn, F.A. and **Hurley, J.B.** (2004). Visual pigment phosphorylation but not transducin translocation can contribute to light adaptation in zebrafish cones. *Neuron* 41:915-28. *Cited 39 times*.
   - Nair, K.S., Hanson, S.M., Mendez, A., Gurevich, E.V., Kennedy, M.J., Shestopalov, V.I., Visnhivestskiy, S.A., **Hurley, J.B.**, Gurevich, V.V. and Slepak, V.Z. (2005). Light-dependent redistribution of arrestin in vertebrate rods is an energy-independent process governed by protein-protein interactions. *Neuron* 46:555-567. *Cited 108 times*.
   - Lee, K.A., Nawrot, M., Garwin, G.G., Saari, J.C., **Hurley, J.B.** (2010). Relationships among visual cycle retinoids, rhodopsin phosphorylation, and phototransduction in mouse eyes during light and dark adaptation. Biochemistry 49:2454-63. *Cited 4 times*.

5. **Zebrafish is a powerful genetic tool for vision research**. As a biochemist I became concerned that most of the genes that we had identified that were linked to phototransduction were found by in vitro biochemical assays. I sought out a more unbiased approach based on in vivo function. I collaborated with Sue Brockerhoff and John Dowling to develop a screen for blind zebrafish. Sue Brockerhoff and her colleagues then identified the mutant genes and I collaborated with her to develop biochemical and physiological methods to help characterize several of the mutatnts.
   - Brockerhoff, S.E., **Hurley, J.B.**, Janssen-Bienhold, U., Neuhauss, S.C.F., Driever, W. and Dowling, J.E. (1995) A behavioral screen for isolating zebrafish mutants with visual system defects. *Proc. Natl. Acad. Sci. USA* **92**:10545-10549. *Cited 233 times*.
   - Brockerhoff, S.E., **Hurley, J.B.**, Niemi, G.A. and Dowling, J.E. (1997) A new form of inherited red-blindness identified in zebrafish. *J. Neurosci.* **17**:4236-4242. *Cited 60 times*.
   - Brockerhoff, S.E., Rieke, F., Matthews, H.R., Taylor, M.R., Kennedy, B., Ankoudinova, I., Niemi, G.A., Tucker, C.L., Xiao, M., Cilluffo, M.C., Fain, G.L. and **Hurley, J.B.** (2003). Light stimulates a transducin-independent increase of cytoplasmic Ca^{2+} and suppression of current in cones from the zebrafish mutant nof. *J. Neurosci*. **23**:470-80. *Cited 56 times*.

- Taylor , M.R., **Hurley, J.B.** Van Epps, H.A. and Brockerhoff, S.E. (2004). A zebrafish model for pyruvate dehydrogenase deficiency: rescue of neurological dysfunction and embryonic lethality using a ketogenic diet. *Proc. Natl. Acad. Sci. USA* 101:4584-9. *Cited 34 times*.

Complete list of published work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/41158377/?sort=date&direction=ascending

BIOGRAPHICAL SKETCH

NAME: David Kimelman

eRA COMMONS USER NAME: dkimelman

POSITION TITLE: Professor emeritus

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Stanford University, Stanford, CA	B.S.	1979	Chemistry
Harvard, University (Mentor: Bryan Roberts)	Ph.D	1985	Mol. Biol.
UC San Francisco (Mentor: Marc Kirschner)	Postdoc	1989	Dev. Biol.

A. Personal Statement

I have been working on the study of early vertebrate development for over 35 years, starting first in frogs (*Xenopus*) and more recently studying zebrafish. After having run a quite successful lab for over 30 years (my work has >17,000 citations), I felt that though the science was going well I wanted to move on to other challenges and so I officially retired Sept. 1, 2021. However, during the last year running my lab I started a collaboration with a postdoc in Cole Trapnell's lab in Genome Sciences at the UW, working intensively with her both intellectually and experimentally, and I became very impressed with Cole, the outstanding people in his lab, and the huge potential impact of the technology he has developed for understanding embryonic development. Thus, when Cole asked me to continue working with him post-retirement I readily agreed, since I felt this gave me the new challenge I was looking for, and I felt that I could really help the people in Cole's lab with their research, both experimentally and intellectually. Cole and my research interests are very synergistic, combining my long history in studying vertebrate embryos with Cole's amazing technological prowess, and I am certain this is going to lead to many exciting new developments. We already have two collaborative papers submitted, and more work in the pipeline. Finally I would note that while I am listed as 40% time since this is the limit emeritus faculty are officially allowed to work at the UW, I am working full time in the lab since I am very excited about this work.

a) Talbot, W.S., Trevarrow, B., Halpern, M.E., Melby, A.E., Farr, G., Postlethwait, J.H., Jowett, T., Kimmel, C.B., and Kimelman, D. (1995). A homeobox gene essential for zebrafish notochord development. Nature *378*, 150-157.

b) Martin, B.L. & Kimelman, D. (2008) Regulation of canonical Wnt signaling by Brachyury is essential for posterior mesoderm formation. *Dev Cell* **15**, 121-133.

c) Martin, B.L. & Kimelman, D. (2012) Canonical Wnt signaling dynamically controls multiple stem cell fate decisions during vertebrate body formation. *Dev Cell* **22**, 223-232.

d) Ye, Z., Braden, C. R., Wills, A. & Kimelman, D. 2021. Identification of in vivo Hox13 binding sites reveals an essential locus controlling zebrafish brachyury expression. *Development,* dev185298.

B. Positions and Honors.
Professional Experience

1985 - 1989	Postdoctoral Fellow with Marc Kirschner Department of Biochemistry and Biophysics, U.C.S.F., San Francisco Early patterning events in *Xenopus laevis*.
1990 -1995	Assistant Professor, Dept. of Biochemistry, University of Washington, Seattle Molecular regulation of early *Xenopus* development.
1995 - 1996	Associate Professor, Dept. of Biochemistry, University of Washington, Seattle
1996 – 1999	Associate Professor, Dept. of Biochemistry, University of Washington, Seattle Adjunct Associate Professor, Department of Zoology Molecular regulation of early *Xenopus* and zebrafish development.
1999 – 2021	Professor, Dept. of Biochemistry, University of Washington, Seattle Adjunct Professor, Department of Biology (was Zoology) Molecular regulation of early vertebrate development.
2022-present	Professor emeritus, Dept. of Biochemistry, University of Washington, Seattle

Honors and Awards
B.S. with Honors with Distinction
Phi Beta Kappa
NSF Predoctoral Fellow
NIH Postdoctoral Fellow
Searle Scholar
Basil O' Connor Award from the March of Dimes

Professional Duties
Cell Editorial Board, 1997-2008
Developmental Biology Editorial Board, 2000-2021
Developmental Cell Editorial Board, 2003-2021
Genes & Development Editorial Board, 2007-2021
Zebrafish TechnoFish Section Editor 2014-2021
Faculty of 1000, 2001-2008
Director, Cellular and Molecular Biology Training Grant (33 positions), U.W., 1996-2013
Member, NSF Study section, Developmental Mechanisms, 1999-2004
Faculty advisor, student SACNAS chapter, 2015-2021
Member, NIH Study section, DEV2, 2016-2021

C. Contributions to Science (17,495 citations as of January 1, 2022)

1. **Identification of mesoderm inducing factors**

   Working as a postdoc in Marc Kirschner's lab, I identified FGF and TGF-β as the factors that regulate mesoderm induction in *Xenopus*. This work solved one of the long-standing problems in developmental biology and spurred work in many systems.

   a) Kimelman, D., and Kirschner, M. (1987). Synergistic induction of mesoderm by FGF and TGF- β and the identification of an mRNA coding for FGF in the early *Xenopus* embryo. Cell *51*, 869-877.

   b) Kimelman, D., Abraham, J.A., Haaparanta, T., Palisi, T.M., and Kirschner, M.W. (1988). The presence of fibroblast growth factor in the frog egg: its role as a natural mesoderm inducer. Science *242*, 1053-1056.

c) Kimelman, D., and Kirschner, M.W. (1989). An antisense mRNA directs the covalent modification of the transcript encoding fibroblast growth factor in *Xenopus* oocytes. Cell *59*, 687-696.

2. Regulation of the embryonic body axis by the Wnt signaling pathway

In my own lab, we demonstrated that the kinase GSK-3 is an essential component of the embryonic Wnt signaling pathway, identified a novel inhibitor of GSK-3 required to form the dorsal-ventral axis, revealed how Wnt signaling works to regulate a key target gene, and provided the first evidence that GSK-3 directly regulates β-catenin stability by phosphorylating it. These studies revealed key aspects of the Wnt signaling pathway and showed how the embryo uses this pathway to regulate early axis formation.

a) Pierce, S.B., and Kimelman, D. (1995). Regulation of Spemann organizer formation by the intracellular kinase Xgsk-3. Development *121*, 755-765.

b) Yost, C., Torres, M., Miller, J.R., Huang, E., Kimelman, D., and Moon, R.T. (1996). The axis-inducing activity, stability, and subcellular distribution of β-catenin is regulated in *Xenopus* embryos by glycogen synthase kinase 3. Genes Dev *10*, 1443-1454.

c) Brannon, M., Gomperts, M., Sumoy, L., Moon, R.T., and Kimelman, D. (1997). A β-catenin/XTcf-3 complex binds to the *siamois* promoter to regulate dorsal axis specification in *Xenopus*. Genes Dev *11*, 2359-2370.

d) Yost, C., Farr, G.H., 3rd, Pierce, S.B., Ferkey, D.M., Chen, M.M., and Kimelman, D. (1998). GBP, an inhibitor of GSK-3, is implicated in *Xenopus* development and oncogenesis. Cell *93*, 1031-1041.

3. Regulation of early embryonic development by homeobox and T-box transcription factors

Using first *Xenopus* and later zebrafish, we have revealed how non-Hox homeobox factors and T-box factors regulate patterning in the early vertebrate embryo, from germ layer patterning to tissue specific identity to morphogenesis.

b) Talbot, W.S., Trevarrow, B., Halpern, M.E., Melby, A.E., Farr, G., Postlethwait, J.H., Jowett, T., Kimmel, C.B., and Kimelman, D. (1995). A homeobox gene essential for zebrafish notochord development. Nature *378*, 150-157.

c) Griffin, K.J., Amacher, S.L., Kimmel, C.B., and Kimelman, D. (1998). Molecular identification of *spadetail*: regulation of zebrafish trunk and tail mesoderm formation by T-box genes. Development *125*, 3379-3388.

d) Bjornson, C.R., Griffin, K.J., Farr, G.H., 3rd, Terashima, A., Himeda, C., Kikuchi, Y., and Kimelman, D. (2005). *Eomesodermin* is a localized maternal determinant required for endoderm induction in zebrafish. Dev Cell *9*, 523-533.

e) Manning, A.J., and Kimelman, D. (2015). Tbx16 and Msgn1 are required to establish directional cell migration of zebrafish mesodermal progenitors. Dev Biol *406*, 172-185.

4. Regulation of neuromesodermal progenitors in zebrafish.

We showed that the key vertebrate embryonic transcription factor Brachyury acts by maintaining Wnt signaling and inhibiting Retinoic Acid signaling in the progenitor population that forms the anterior-posterior axis, and is essential because it creates the niche for the progenitors. We showed that the progenitors are bipotential neuromesodermal cells whose fate is regulated by Wnt signaling, which activates Tbx16 at high levels, pushing cells into a mesodermal fate and suppressing the progenitor fate. Finally, we have discovered that the posterior *hox* genes play an essential role in the regulation of these cells, including through the regulation of *brachyury/tbxta*. These findings are critical for understanding how the early embryonic body is established.

a) Martin, B.L., and Kimelman, D. (2008). Regulation of canonical Wnt signaling by Brachyury is essential for posterior mesoderm formation. Dev Cell *15*, 121-133.

b) Martin, B.L., and Kimelman, D. (2012). Canonical Wnt signaling dynamically controls multiple stem cell fate decisions during vertebrate body formation. Dev Cell *22*, 223-232.

c) Ye, Z. and Kimelman, D. (2020). Hox13 genes are required for mesoderm formation and axis elongation during early zebrafish dev. Development *147*, 1-12.

d) Ye, Z., Braden, C. R., Wills, A. & Kimelman, D. 2021. Identification of in vivo Hox13 binding sites reveals an essential locus controlling zebrafish brachyury expression. *Development,* dev185298.

5. <u>Structural analysis of the Wnt signaling pathway</u>

With my collaborator Wenqing Xu (U.W.) we used a combination of structural biology and biochemistry to provide key insights into the mechanism of the Wnt signaling pathway, including the first structure of β-catenin bound to one of its partners.

a) Graham, T.A., Weaver, C., Mao, F., Kimelman, D., and Xu, W. (2000). Crystal structure of a β-catenin/Tcf complex. Cell *103*, 885-896.

b) Graham, T.A., Clements, W.K., Kimelman, D., and Xu, W. (2002). The crystal structure of the β-catenin/ICAT complex reveals the inhibitory mechanism of ICAT. Mol Cell *10*, 563-571.

c) Xing, Y., Clements, W.K., Kimelman, D., and Xu, W. (2003). Crystal structure of a β-catenin/axin complex suggests a mechanism for the β-catenin destruction complex. Genes Dev *17*, 2753-2764.

d) Xing, Y., Clements, W.K., Le Trong, I., Hinds, T.R., Stenkamp, R., Kimelman, D., and Xu, W. (2004). Crystal structure of a β-catenin/APC complex reveals a critical role for APC phosphorylation in APC function. Mol Cell *15*, 523-533.

Complete List of Published Work in MyBibliography
http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/40672752/?sort=date&direction=ascending

D. Research Support

<u>Completed Research Support</u>

1R01GM079203 (Kimelman) 7/1/17-6/30/21
NIH/NIGMS

Morphogenetic mechanisms regulating directed cell migration required to form the vertebrate posterior body
This project involves the study of the mesodermal progenitors that form the trunk and tail in zebrafish using transgenic zebrafish lines to study the mechanisms that regulate their movement to form the musculature of the body.
Role: P.I.

R21 EB 020898-01A1 5/15/2016 to 3/31/2018
NIH/NIBIB
An inducible protein knockout strategy based on an orthogonal, ligand-activated E3 ubiquitin ligase
This project involves the development of a new system for inducible protein degradation. My lab has a small portion of this award for testing this system in zebrafish as an in vivo animal model
Role: co-P.I.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: King, Neil

eRA COMMONS USER NAME: neilpking

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	END DATE	FIELD OF STUDY
Northwestern University, Evanston, IL	BS	2004	Biomedical Engineering
University of California, Los Angeles, Los Angeles, CA	PhD	2010	Biochemistry
University of Washington, Seattle, WA	Postdoctoral Fellow	2014	Biochemistry

A. Personal Statement

The long-term goal of my group is to develop custom-designed protein nanomaterials as platform technologies for medical applications. I recently established a general computational method for designing novel self-assembling protein nanomaterials with atomic-level accuracy. Using this as a foundation, my group designs novel protein-based systems for i) next-generation nanoparticle vaccines and ii) targeted delivery of drugs and biologics. A major and unique focus of our work is tailoring the supramolecular structure of these materials to specific applications. We use cutting-edge computational protein design as well as traditional protein engineering to construct functional, application-oriented materials, and we determine their biochemical and biophysical properties using a variety of techniques. My earlier training in X-ray crystallography and biophysical analysis of protein stability and folding enables thorough characterization of the materials in my group. We work with a network of world-class collaborators to test and refine the functional performance of the materials (e.g., as vaccines). By constructing design-build-test cycles with minimal iteration time, we aim to develop our novel materials into mature technologies that can be translated and commercialized to realize their ultimate purpose: public health impact.

Publications that highlight my experience and qualifications for this project include:

1. Arunachalam PS, Walls AC, Golden N, Atyeo C, Fischinger S, Li C, Aye P, Navarro MJ, Lai L, Edara VV, Röltgen K, Rogers K, Shirreff L, Ferrell DE, Wrenn S, Pettie D, Kraft JC, Miranda MC, Kepl E, Sydeman C, Brunette N, Murphy M, Fiala B, Carter L, White AG, Trisal M, Hsieh CL, Russell-Lodrigue K, Monjure C, Dufour J, Spencer S, Doyle-Meyer L, Bohm RP, Maness NJ, Roy C, Plante JA, Plante KS, Zhu A, Gorman MJ, Shin S, Shen X, Fontenot J, Gupta S, O'Hagan DT, Van Der Most R, Rappuoli R, Coffman RL, Novack D, McLellan JS, Subramaniam S, Montefiori D, Boyd SD, Flynn JL, Alter G, Villinger F, Kleanthous H, Rappaport J, Suthar MS, **King NP**, Veesler D, Pulendran B. Adjuvanting a subunit COVID-19 vaccine to induce protective immunity. *Nature*. 2021 Apr 19; PubMed PMID: 33873199.
2. Boyoglu-Barnum S, Ellis D, Gillespie RA, Hutchinson GB, Park YJ, Moin SM, Acton OJ, Ravichandran R, Murphy M, Pettie D, Matheson N, Carter L, Creanga A, Watson MJ, Kephart S, Ataca S, Vaile JR, Ueda G, Crank MC, Stewart L, Lee KK, Guttman M, Baker D, Mascola JR, Veesler D, Graham BS*, **King NP***, Kanekiyo M*. Quadrivalent influenza nanoparticle vaccines induce broad protection. *Nature*. 2021 Mar 24; PubMed PMID: 33762730. (*equal contributions)
3. Walls AC, Fiala B, Schäfer A, Wrenn S, Pham MN, Murphy M, Tse LV, Shehata L, O'Connor MA, Chen C, Navarro MJ, Miranda MC, Pettie D, Ravichandran R, Kraft JC, Ogohara C, Palser A, Chalk S, Lee EC, Guerriero K, Kepl E, Chow CM, Sydeman C, Hodge EA, Brown B, Fuller JT, Dinnon KH 3rd, Gralinski LE, Leist SR, Gully KL, Lewis TB, Guttman M, Chu HY, Lee KK, Fuller DH, Baric RS, Kellam P, Carter L, Pepper M, Sheahan TP, Veesler D*, **King NP***. Elicitation of Potent Neutralizing Antibody Responses by Designed Protein Nanoparticle Vaccines for SARS-CoV-2. *Cell*. 2020 Nov 25;183(5):1367-1382.e17. PubMed Central PMCID: PMC7604136. (*equal contributions)
4. Marcandalli J, Fiala B, Ols S, Perotti M, de van der Schueren W, Snijder J, Hodge E, Benhaim M, Ravichandran R, Carter L, Sheffler W, Brunner L, Lawrenz M, Dubois P, Lanzavecchia A, Sallusto F, Lee

KK, Veesler D, Correnti CE, Stewart LJ, Baker D, Loré K, Perez L*, **King NP***. Induction of Potent Neutralizing Antibody Responses by a Designed Protein Nanoparticle Vaccine for Respiratory Syncytial Virus. *Cell*. 2019 Mar 7;176(6):1420-1431.e17. PubMed Central PMCID: PMC6424820. (*equal contributions)

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2017–	Assistant Professor, Department of Biochemistry, University of Washington, Seattle, WA
2014–2017	Acting Assistant Professor, Department of Biochemistry, University of Washington, Seattle, WA

Other Experience and Professional Memberships

2017–	Co-founder and Chair of Scientific Advisory Board, Icosavax, Inc., Seattle, WA

Honors

2020	*Nature Biotechnology*'s academic spinouts of 2019 (Icosavax)
2018	Amgen Young Investigator Award
2016	*Science* magazine Breakthrough of the Year runner-up (citing Bale et al. and Hsia et al.)
2013	PDB Molecule of the Month (September 2013: Designed Protein Cages)
2009	UCLA Dissertation Year Fellowship; University of California, Los Angeles
2008	Audree Fowler Fellowship in Protein Science; University of California, Los Angeles

C. Contributions to Science

1. Computational design of self-assembling protein nanomaterials

I conceived and developed general computational methods for designing novel self-assembling protein nanomaterials with atomic-level accuracy, a long-standing goal in protein engineering. These methods have opened the door to accurately designing ordered protein materials spanning several length scales and protein-based molecular machines that are tailored to particular applications. The methods have been made freely available to non-profit users as part of the Rosetta molecular modeling software and are widely regarded as the state of the art in the field.

a. Bale JB, Gonen S, Liu Y, Sheffler W, Ellis D, Thomas C, Cascio D, Yeates TO, Gonen T, **King NP***, Baker D*. Accurate design of megadalton-scale two-component icosahedral protein complexes. *Science*. 2016 Jul 22;353(6297):389-94. PubMed Central PMCID: PMC5485857. (*equal contributions)

b. Hsia Y, Bale JB, Gonen S, Shi D, Sheffler W, Fong KK, Nattermann U, Xu C, Huang PS, Ravichandran R, Yi S, Davis TN, Gonen T, **King NP**, Baker D. Design of a hyperstable 60-subunit protein dodecahedron. [corrected]. *Nature*. 2016 Jul 7;535(7610):136-9. PubMed Central PMCID: PMC4945409.

c. **King NP***, Bale JB*, Sheffler W*, McNamara DE, Gonen S, Gonen T, Yeates TO, Baker D. Accurate design of co-assembling multi-component protein nanomaterials. *Nature*. 2014 Jun 5;510(7503):103-8. PubMed Central PMCID: PMC4137318. (*equal contributions)

d. **King NP**, Sheffler W, Sawaya MR, Vollmar BS, Sumida JP, André I, Gonen T, Yeates TO, Baker D. Computational design of self-assembling protein nanomaterials with atomic level accuracy. *Science*. 2012 Jun 1;336(6085):1171-4. PubMed Central PMCID: PMC4138882.

2. Structure-based design of next-generation nanoparticle vaccines

Vaccines are among the most impactful medical interventions yet discovered, but progress in developing safe and effective vaccines for several important pathogens such as HIV has been slow. Scaffolding engineered forms of pathogen-derived proteins on protein nanoparticles is a promising approach to increase the immunogenicity of subunit vaccines and elicit humoral responses against neutralizing epitopes. Over the past four years, my group has devoted substantial effort to developing next-generation nanoparticle vaccines

for a variety of indications using our designed protein nanomaterials. The nanoparticles have yielded promising immunogenicity and protection data in mice, cotton rats, ferrets, rabbits, and non-human primates and result in high levels of antigen-specific B cell activation *in vitro*. Our work in influenza vaccine design has highlighted how nanoparticle immunogens can be used to elicit antibody responses that provide broadly protective immunity against a wide variety of viruses. These are highly collaborative projects involving multiple PIs; my group has led the nanoparticle design, engineering, production, and biochemical/biophysical/antigenic characterization efforts, and in 2020 we extended our capabilities into running immunogenicity studies and analyzing serological responses in-house. Our nanoparticle vaccine platform has been rapidly adopted in the field, and three vaccine candidates from our group have either entered human clinical trials in early 2021 (SARS-CoV-2 and influenza) or will enter in the latter half of the year (RSV).

a. Boyoglu-Barnum S, Ellis D, Gillespie RA, Hutchinson GB, Park YJ, Moin SM, Acton OJ, Ravichandran R, Murphy M, Pettie D, Matheson N, Carter L, Creanga A, Watson MJ, Kephart S, Ataca S, Vaile JR, Ueda G, Crank MC, Stewart L, Lee KK, Guttman M, Baker D, Mascola JR, Veesler D, Graham BS*, **King NP***, Kanekiyo M*. Quadrivalent influenza nanoparticle vaccines induce broad protection. *Nature*. 2021 Mar 24; PubMed PMID: 33762730. (*equal contributions)
b. Walls AC, Fiala B, Schäfer A, Wrenn S, Pham MN, Murphy M, Tse LV, Shehata L, O'Connor MA, Chen C, Navarro MJ, Miranda MC, Pettie D, Ravichandran R, Kraft JC, Ogohara C, Palser A, Chalk S, Lee EC, Guerriero K, Kepl E, Chow CM, Sydeman C, Hodge EA, Brown B, Fuller JT, Dinnon KH 3rd, Gralinski LE, Leist SR, Gully KL, Lewis TB, Guttman M, Chu HY, Lee KK, Fuller DH, Baric RS, Kellam P, Carter L, Pepper M, Sheahan TP, Veesler D*, **King NP***. Elicitation of Potent Neutralizing Antibody Responses by Designed Protein Nanoparticle Vaccines for SARS-CoV-2. *Cell*. 2020 Nov 25;183(5):1367-1382.e17. PubMed Central PMCID: PMC7604136. (*equal contributions)
c. Brouwer PJM, Antanasijevic A, Berndsen Z, Yasmeen A, Fiala B, Bijl TPL, Bontjer I, Bale JB, Sheffler W, Allen JD, Schorcht A, Burger JA, Camacho M, Ellis D, Cottrell CA, Behrens AJ, Catalano M, Del Moral-Sánchez I, Ketas TJ, LaBranche C, van Gils MJ, Sliepen K, Stewart LJ, Crispin M, Montefiori DC, Baker D, Moore JP, Klasse PJ, Ward AB*, **King NP***, Sanders RW*. Enhancing and shaping the immunogenicity of native-like HIV-1 envelope trimers with a two-component protein nanoparticle. *Nat Commun.* 2019 Sep 19;10(1):4272. PubMed Central PMCID: PMC6753213. (*equal contributions)
d. Marcandalli J, Fiala B, Ols S, Perotti M, de van der Schueren W, Snijder J, Hodge E, Benhaim M, Ravichandran R, Carter L, Sheffler W, Brunner L, Lawrenz M, Dubois P, Lanzavecchia A, Sallusto F, Lee KK, Veesler D, Correnti CE, Stewart LJ, Baker D, Loré K, Perez L*, **King NP***. Induction of Potent Neutralizing Antibody Responses by a Designed Protein Nanoparticle Vaccine for Respiratory Syncytial Virus. *Cell*. 2019 Mar 7;176(6):1420-1431.e17. PubMed Central PMCID: PMC6424820. (*equal contributions)

3. Design of protein-directed hybrid biomaterials

Complex biological processes are often performed by self-organizing nanostructures comprising multiple classes of macromolecules, such as ribosomes (proteins and RNA) or enveloped viruses (proteins, nucleic acids, and lipids). Although approaches have been developed for designing self-assembling structures consisting of either nucleic acids or proteins, strategies for engineering hybrid biological materials are only beginning to emerge. We have recently begun to extend our efforts to include the design of hybrid biological materials comprising multiple classes of biomolecules. In one example, in collaboration with the Sundquist lab at the University of Utah, we designed self-assembling protein nanocages that direct their own release from cells inside small vesicles in a manner that resembles some viruses. These hybrid biomaterials can fuse their membranes with target cells and deliver their contents, thereby transferring cargoes from one cell to another. In another example, in collaboration with the Baker and Pun labs at UW, we engineered our designed protein nanomaterials to encapsulate their own genomes in the form of a bicistronic mRNA, thereby linking genotype to phenotype. This enabled improvement of several functional properties of the designed materials through evolution, including long circulation half-life in mice. We have several additional projects ongoing in this area.

a. Butterfield GL, Lajoie MJ, Gustafson HH, Sellers DL, Nattermann U, Ellis D, Bale JB, Ke S, Lenz GH, Yehdego A, Ravichandran R, Pun SH, **King NP***, Baker D*. Evolution of a designed protein assembly

encapsulating its own RNA genome. *Nature*. 2017 Dec 21;552(7685):415-420. PubMed Central PMCID: PMC5927965. (*equal contributions)

b. Votteler J, Ogohara C, Yi S, Hsia Y, Nattermann U, Belnap DM, **King NP***, Sundquist WI*. Designed proteins induce the formation of nanocage-containing extracellular vesicles. *Nature*. 2016 Dec 8;540(7632):292-295. PubMed Central PMCID: PMC5729044. (*equal contributions)

4. Structure, stability, and folding of knotted proteins and proteins from hyperthermophiles

My early research used X-ray crystallography and a variety of biochemical, biophysical, and computational techniques to study the structure, stability, and folding of two classes of proteins: proteins with complex topologies such as knots and slipknots, and disulfide-containing proteins from hyperthermophilic archaea that use intracellular disulfide bonding as a stabilizing structural adaptation. These studies suggested answers to questions regarding the puzzling existence of knots and other complex topologies in proteins and how they might form, and extended the evidence of widespread intracellular disulfide bonding in certain thermophilic organisms to include stabilizing disulfides at protein-protein interfaces.

a. Sayre TC, Lee TM, **King NP**, Yeates TO. Protein stabilization in a highly knotted protein polymer. *Protein Eng Des Sel.* 2011 Aug;24(8):627-30. PubMed Central PMCID: PMC3165941.
b. **King NP**, Jacobitz AW, Sawaya MR, Goldschmidt L, Yeates TO. Structure and folding of a designed knotted protein. *Proc Natl Acad Sci USA*. 2010 Nov 30;107(48):20732-7. PubMed Central PMCID: PMC2996448.
c. **King NP***, Lee TM*, Sawaya MR, Cascio D, Yeates TO. Structures and functional implications of an AMP-binding cystathionine beta-synthase domain protein from a hyperthermophilic archaeon. *J Mol Biol.* 2008 Jun 27;380(1):181-92. PubMed Central PMCID: PMC2577872. (*equal contributions)
d. **King NP**, Yeates EO, Yeates TO. Identification of rare slipknots in proteins and their implications for stability and folding. J Mol Biol. 2007 Oct 12;373(1):153-66. PubMed PMID: 17764691.

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Klevit, Rachel E.

eRA COMMONS USER NAME (credential, e.g., agency login): KLEVIT

POSITION TITLE: Professor of Biochemistry

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Reed College	B.A.	10/1978	Chemistry
Oxford University	D. Phil.	10/1981	Chemistry
Duke University	Postdoctoral Fellow	09/1983	Dept. of Microbiology & Immunology
University of Washington	Postdoctoral	06/1984	Dept. of Chemistry

A. Personal Statement

Throughout my 30+-year career I have applied cutting edge techniques to challenging systems of high biological importance. My lab solved the earliest structures of a number of high impact protein classes, including (Cys_2,His_2) DNA-binding zinc fingers and the BRCA1/BARD1 RING ubiquitin ligase by solution-state NMR, the human small heat shock protein, αB-crystallin (HSPB5), by a hybrid approach to apply solid-state NMR methods, SAXS, and EM, and a "fuzzy complex" of a transcription activator/mediator. Ever since we first solved the structure of the BRCA1/BARD1 RING domain and it was shown to be a Ubiquitin E3 ligase, I have been fascinated by fundamental questions in how protein ubiquitylation is carried out. In the early 2000's, little was known about how an E3 specifies what E2 Ub-conjugating enzyme to use or how an E2 selects its protein targets. A majority of E3s, with BRCA1/BARD1 as a prime example, do not have recognizable substrate-binding domains, making the identification of *bona fide* cellular targets a major challenge and barrier in the field. We felt that we couldn't reasonably look for substrates until we knew which was the correct E2 of the three dozen in humans that works with BRCA1/BARD1. At the time the prevailing wisdom was that an E3 would work with a single E2, so our discovery that BRCA1/BARD1 works with nine E2s was groundbreaking; it has since become a paradigm that E3s can work with numerous E2s and the choice of E2 dictates the modification (mono-ubiquitin, poly-Ub, and which type of chain) deposited on the target. The ability to work with so many E2s posed a new challenge to identification of BRCA1/BARD1 substrates, namely the difficulty of designing a truly ligase-dead mutant of BRCA1 that is dead for *all* its E2s. This has led to an underlying conflict in the BRCA1/BARD1 field: use of a BRCA1 mutant that was presumed to be ligase-dead but is *not* led to the provocative conclusion that BRCA1/BARD1 ligase activity is not relevant to its tumor suppression function. This conclusion flies in the face of some of the most penetrant missense mutations in BRCA1 breast cancer families that specifically abrogate its E3 ligase activity but caused the field to move away from further investigations of BRCA1 as an E3 ligase. Interest was rekindled when a novel ubiquitin mark placed on nucleosomal histone H2A by BRCA1/BARD1 was shown to be required for its role in DNA damage repair and, more recently by us, in transcriptional repression of certain genes. Importantly, our new cryo-EM structure of BRCA1/BARD1 RINGs atop a nucleosome reveal that it is the under-studied subunit, BARD1, that determines placement of the Ub marks. My lab's years of experience in BRCA1/BARD1, protein ubiquitylation, and structural biology place us in a strong position to finally uncover the role(s) of enigmatic BARD1 and its more famous partner, BRCA1.

Ongoing and recently completed projects of relevance to this application that I would like to highlight:

1998/12/21-2021/06/30 R01 GM088055, NIGMS KLEVIT, RACHEL E. (PI)
"Expanding Roles of E2 and E3 Enzymes in Ubiquitin Transfer"

This grant has supported the studies in protein ubiquitylation in my lab since 1998. Our work on E2s and E3s will continue with support of a new R35 grant that has been approved for funding. Start date pending.

2021/04-2026/03 1R01CA260834, NCI KLEVIT, RACHEL & BRZOVIC, PETER (co-PIs)
"Defining the role of BARD1 in nucleosomal ubiquitylation"
This grant supports structural, biochemical, and cell biological studies on the BRCA1/BARD1 E3 ligase complex function in modifying chromatin, with a focus on the BARD1 subunit.

Citations:

1. Brzovic, P., Rajagopal, P., Hoyt, D.W., King, M. C., and Klevit R. E. (2001). Structure of a BRCA1-BARD1 heterodimeric RING-RING complex. Nature Structural Biology. 8(10), 833-7.
2. Christensen, D. E., Brzovic, P. S., and Klevit, R. E. (2007). E2:BRCA1 RING interactions dictate the synthesis of either mono- or specific poly-ubiquitin chain linkages. Nature Structural & Molecular Biology, Oct 14(10): 941-948.
3. Stewart MD, Zelin E, Dhall A, Walsh T, Upadhyay E, Corn JE, Chatterjee C, #King MC, #Klevit RE. BARD1 is necessary for ubiquitylation of nucleosomal histone H2A and for transcriptional regulation of estrogen metabolism genes. Proc Natl Acad Sci U S A. 2018 Jan 24.
4. Witus SR, Burrell AL, Farrell DP, Kang J, Wang M, Hansen JM, Pravat A, Tuttle LM, Stewart MD, Brzovic PS, Chatterjee C, Zhao W, DiMaio F, Kollman JK, Klevit RE. (2021). BRCA1/BARD1 site-specific ubiquitylation of nucleosomal H2A is directed by BARD1. Nat Struct Mol Biol. 2021 Mar;28(3):268-277. doi: 10.1038/s41594-020-00556-4. Epub 2021 Feb 15. PMID: 33589814.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2017 – present	Edmond H. Fischer - Washington Research Foundation Endowed Chair in Biochemistry, University of Washington
2008 – present	Adjunct Professor of Pharmacology, Univ. of Washington, Dept. of Pharmacology, Seattle,WA
2005 - 2015	Director, Molecular Biophysics Training Program at UW, Univ. of Washington, Seattle, WA
1996 - present	Professor of Biochemistry, Univ. of Washington, Dept. of Biochemistry, Seattle, WA
1984 - 1996 -	Adjunct Professor of Chemistry, University of Washington, Dept. of Chemistry, Seattle, WA
1995 - 2000	Founding Director, Graduate Program in Biomolecular Structure & Design, Univ. of WA 1986 Research Associate (Faculty), Univ. of Washington, Dept. of Chemistry, Seattle, WA
1991 - 1996	Associate Professor of Biochemistry, Univ. of Washington, Dept. of Biochemistry, Seattle, WA
1991 - 1993	Associate Editor, Protein Science, The Protein Society
1986 - 1991	Assistant Professor of Biochemistry, Univ. of Washington, Dept. of Biochemistry, Seattle, WA

Other Experience and Professional Memberships

2014 - 2016	Co-organizer, FASEB Summer Conference on Ubiquitin & Cellular Regulation
2011	Chair, NIH ZRG1 BCMB-R Study Section
2005 - 2006	Chair, NIH ZRG1 BCMB-B Conflicts in Biophys. & Macromol. Biophysics Study Section 2011 -
2004 - 2004	Chair, NIH ZRG1 BPC-B Conflicts in Biophysics and Chemistry Study Section 2004 - 2004 Chair, NIH ZRG1 BST-C(90) Special Emphasis Panel
2003 - 2003	Co-Chair, Proteins Gordon Conference, Gordon Research Conference 2003 - 2013 Advisory Board, Seattle Girls School
2002 - 2008	Review Committee, California Breast Cancer Research Program
2002 - 2007	Editorial Board, Journal of Biological Chemistry
1996 - 1996	Program Committee, American Society for Biochemistry and Molecular Biology 2002 - 2002 External Review Committee, University of Arizona Dept. of Biochemistry
1993 - 1997	Regular Study Section Member (BBCB), NIH
1994 - 1994	External Review Committee, Simon Fraser University Dept. of Chemistry 1994 - 1994 External Review Committee, University of British Columbia Dept. of Chemistry

1994 - 1994	Nominating Committee, American Society for Biochemistry and Molecular Biology 1995 - 1998 Council Member, The Protein Society
1991 - 1992	Chair, Nominating Committee, American Chemical Society 1992 - 1992 Nominating Committee, Biophysical Society
1991 - 1991	Nominating Committee, The Peptide Society
1990 - 1995	Comm. for Equal Opportunities for Women, Amer. Soc. for Biochemistry & Molecular Biology
1990 - 1993	Program Committee, American Peptide Symposium
1989 - 1994	Scientific Advisory Board, Damon Runyon-Walter Winchell Cancer Fund 1990 - 1993 Council member, Biophysical Society
1989 - 1991	Nominating Committee, The Protein Society

Honors

2021	Elected to National Academy of Sciences
2017	Endowed Chair in Biochemistry, University of Washington Edmond H. Fischer – Washington Research Foundation
2016	Elected to Washington Academy of Sciences
2016	Scottish Institute of Cell Signalling Ub Lectureship
2016	Distinguished Scientist in Medicine, Univ. of Washington
2016	Dorothy Crowfoot Hodgkins Award, The Protein Society
2015	Fritz Lipmann Award, American Society for Biochemistry and Molecular Biology
2012	Excellence in Mentoring Award, University of Washington School of Medicine
2009	Scholar's Week Keynote Speaker, Western Washington University
1998	AAAS Fellow, American Association for the Advancement of Science
1993	Brittingham Visiting Scholar, Dept. of Chemistry, Univ. Wisc./Madison
1991	Van't Hoff Award, Royal Netherlands Academy of Arts and Sciences
1990	Award for Excellence in Chemistry, ICI Pharmaceuticals
1990	DuPont Young Investigator Award, The Protein Society
1989	Established Investigatorship, American Heart Association
1988	Margaret Oakley Dayhoff Award, Biophysical Society
1983	Postdoctoral Fellowship, American Cancer Society
1978	Rhodes Scholar, The Rhodes Trust
1978	Phi Beta Kappa, Phi Beta Kappa
1978	Outstanding Senior Woman, American Association of University Women

C. Contribution to Science

1. Studies on protein ubiquitylation. I was drawn into the field of protein ubiquitylation in the late 1990's, when I and colleague Peter Brzovic solved the NMR structure of the heterodimeric RING complex, BRCA1/BARD1 and it was shown to be a ubiquitin E3 ligase. Since then, my lab has made numerous ground-breaking discoveries, of which four are presented here.

- **NMR structure of BRCA1/BARD1 was the largest protein complex solved *de novo* by NMR.** The structure was the first RING heterodimer and serves as the paradigm example in the field. (Brzovic, PS, Rajagopal, P., Hoyt, DW, King, MC, and **Klevit RE.** (2001). Structure of a BRCA1-BARD1 heterodimeric RING-RING complex. NATURE STRUCTURAL BIOLOGY. 8(10), 833-7.)
- **BRCA1 works with ten human E2s that dictate the ubiquitin product.** Two concepts generated by our efforts to identify the relevant E2s for BRCA1 are now general paradigms in the Ub field: 1) an E3 can work with multiple E2s to generate different modification types and 2) poly-Ub chains are built by the combined action of a priming and a chain-extending E2. (Christensen, DE, Brzovic, PS, and **Klevit, RE** (2007)

E2:BRCA1 RING interactions dictate the synthesis of either mono- or specific poly-ubiquitin chain linkages. Nature Structural & Molecular Biology, Oct 14(10): 941-948. *Featured in Editorial "New Partners for BRCA1." Faculty of 1000 evaluated article.*)

- **Mechanism of allosteric activation by RING-type E3 ligases.** These studies solved the long- standing enigma as to how the largest class of ligases activates Ub transfer. Our "linchpin" residue mutation is widely used to selectively disrupt Ub-transfer activity without affecting the assembly of Ub- transfer complexes (Pruneda JN, Littlefield PJ, Soss SE, Nordquist KA, Chazin WJ, Brzovic PS, **Klevit RE**. Structure of an E3:E2~Ub complex reveals an allosteric mechanism shared among RING/U-box ligases. Mol Cell. 2012 Sep 28;47(6):933-42. PMCID: PMC3462262)
- **RING-Between-RINGs are a unique mechanistic class of E3s that contain an active-site Cys.** This discovery was paradigm shifting and has catalyzed parkin research by providing a mechanistic understanding that was completely lacking. Our identification of the active-site Cys has provided a means to inactivate parkin and other RBR E3s for cellular investigations. Virtually all the structural, molecular, and cellular biology studies on parkin since 2011 have made use of our discovery. (Wenzel DM, Lissounov, A, Brzovic, PS, **Klevit, RE** (2011) UbcH7 reactivity profile reveals Parkin and HHARI to be RING/HECT hybrids. Nature. 2011 Jun 2;474(7349):105-8. PMCID: PMC3444301. *Faculty of 1000 evaluated article.*)

2. Studies on small heat shock proteins. We are fascinated by human sHSPs because they exhibit unique and mysterious properties that are essential to their function. Studying this family of proteins expands the way in which we think about protein structure and how it leads to function.

- **First pseudo-atomic model for mammalian sHSP.** We combined data from solid-state NMR, SAXS, and EM to generate the first model for a human sHSP oligomer. (Jehle S, Vollmar B, Bardiaux B, Dove KK, Rajagopal P, #Gonen T, #Oschkinat H, and #Klevit RE. 2011. The N-terminal domain of αB-crystallin provides a conformational switch for multimerization and structural heterogeneity. Proc Natl Acad Sci U S A. 2011 Apr 19;108(16):6409-14.)
- **Defined a step-wise model by which subunits are recruited to HSPB5 oligomers.** We developed a simple approach with which to dissect the mechanism of oligomer assembly based on our growing understanding of 1) IxI interactions and 2) dimer interface interactions. (Delbecq SP, Rosenbaum JC, **Klevit RE**. A Mechanism of Subunit Recruitment in Human Small Heat Shock Protein Oligomers. Biochemistry. 2015 Jun 22. PMCID: PMC4512712.)
- **Discovered that the disordered N-terminal region (NTR) that is essential for HSPB1 function is quasi-ordered.** We performed the first residue-level solution-state NMR and HDX/MS on a full-length sHSP and discovered that the "disordered" NTR participates in specific and well-defined interactions. We proposed a novel quasi- ordered model for HSPB1. (Clouser AF, Baughman HE, Basanta B, Guttman M, #Nath A, #Klevit RE. Interplay of disordered and ordered regions of a human small heat shock protein yields an ensemble of 'quasi-ordered' states. Elife. 2019 Oct 1;8).
- **Discovered a novel mechanism by which HSPB1 is activated to chaperone Tau against aggregation.** We leveraged our new quasi-ordered model for HSPB1 and discovered that release of a tethered sub-region of the NTR activates the chaperone activity of HSPB1 towards Tau. Our mechanistic model is contrary to a previously published model and provides an explanation for observations that were contrary to that model. (Baughman HER, Pham T-HT, Adams CS, #Nath A, and #Klevit RE. Release of a disordered domain enhances HspB1 chaperone activity toward tau. Proc Natl Acad Sci U S A., 2020 Feb 11;117(6):2923-2929.)

3. Contributions to the field of intrinsically-disordered proteins. I became intrigued with the concept of functionally active intrinsically disordered proteins and have had several opportunities to contribute to a field whose impact continues to grow at rapid pace.

- **Transcriptional activator/co-activator form a fuzzy complex**. Brzovic PS, Heikaus CC, Kisselev L, Vernon R, Herbig E, Pacheco D, Warfield L, Littlefield P, Baker D, **Klevit RE***, and Hahn S*. The acidic transcription activator Gcn4 binds the Mediator subunit Gal11/Med15 using a simple protein interface forming a fuzzy complex. Mol Cell. 2011 Dec 23;44(6):942-53. PMCID: PMC3246216 Featured as "Editor's Choice" in Science, 335 (6067) 27, January 2012. (*Two corresponding authors)
- **Discovered the mechanism for a ubiquitin-conjugating enzyme (E2) that uses an intrinsically disordered region to recognize intrinsically disordered substrate.** (Vittal V, Wenzel DM, Scaglione

KM, Shi L, Duncan ED, Basur V, Elenitoba-Johnson KSJ, Baker D, Paulson HL, Brzovic PS, **Klevit RE**. Intrinsic Disorder drives N-terminal ubiquitination by Ube2w. Nat Chem Biol. 2015 11:83-9.)

4. Early & general contributions (pre-1990). With my doctoral training in (1D) protein NMR, I was well positioned to be among the earliest investigators to apply multi-dimensional NMR to proteins.

- **Interactions between calmodulin (CaM) and the first identified calmodulin-binding domain, "M13."** This was the first example of a CaM-binding domain from a *bona fide* CaM target. Co-author Blumenthal identified the sequence in myosin light chain kinase and I demonstrated that M13 undergoes a disorder-to-order transition upon CaM binding. These findings were foundational for widely used technologies of tandem-affinity-purification "TAP"-tag and designed calcium sensors used in cell biology. (**Klevit, R.E.,** Blumenthal, D.K. Wemmer, D.E., and Krebs, E.G. (1985). Interaction of Calmodulin and a Calmodulin-Binding Peptide from Myosin Light Chain Kinase: Major Spectral Changes in Both Occur as a Result of Complex Formation. BIOCHEMISTRY 24, 8152-8157.)
- **First unknown protein structure solved completely by 2DNMR approaches.** 90-residue HPr was first to be solved *without prior knowledge of a crystal structure*. I performed these studies single- handedly using protein supplied by co-author Waygood. Published as a three-part series, the studies helped propel the use of NMR in structural biology. (**Klevit, R.E.** and Waygood, E.B. (1986). Two- dimensional ^{1}H NMR Studies of Histidine-Containing Protein from Escherichia coli. 3. Secondary and Tertiary Structure As Determined by NMR. BIOCHEMISTRY 25, 7774-7781.)
- **Demonstration that Asp is a good structural as well as functional mimic for phosphorylated serine.** We established the proof-of-principle for this now ubiquitous approach in one of the earliest studies that combined the then new techniques of site-directed mutagenesis AND protein NMR. (Wittekind, M., Reizer, J., Deutscher, J., Saier, M., and **Klevit, R.E.** (1989). Common Structural Changes Accompany the Functional Inactivation of HPr by Seryl Phosphorylation or by Serine to Aspartate Substitution. BIOCHEMISTRY, 28, 9908-12.)
- **First experimentally-determined structure of a Cys_2-His_2 zinc finger**. In addition to the structure itself, this paper catalyzed many subsequent NMR and crystallographic studies on other zinc finger proteins by describing a general method for the proper re-folding of the domains, which had been a hurdle. The structural insights and predictions regarding its sequence-specific DNA binding serve as a cornerstone for the design of artificial restriction enzymes called novel zinc finger nucleases that facilitate genome editing. (Parraga, G.E., Horvath, S., Eisen, A., Taylor, W.E., Hood, L., Young, E.T., and **Klevit, R.E.** (1988). Zinc-Dependent Structure of a Single Finger Domain of Yeast ADR1. SCIENCE, 241, 1489-1492.)

For full publication list, see http://www.ncbi.nlm.nih.gov/myncbi/collections/bibliography/40652089/ .

OMB No. 0925-0001 and 0925-0002 (Rev. 12/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Kollman, Justin M.

eRA COMMONS USER NAME (credential, e.g., agency login): JKOLLMAN

POSITION TITLE: Associate Professor of Biochemistry

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Menlo College, Atherton, CA	BS	05/1998	Biology
University of California, San Diego, CA	PhD	05/2005	Biology
University of California, San Francisco, CA	Postdoc	02/2012	Biochemistry & Biophysics

A. Personal Statement

My graduate training was primarily in X-ray crystallography, where I made significant contributions to our understanding of the assembly and structure of blood clots. I had a brief introduction to cryo-EM during my graduate work and, impressed by the possibilities it offered for studying large macromolecular complexes, I chose to focus on this approach as my primary structural tool during my postdoc in David Agard's lab at UCSF and in my independent positions. I have had the opportunity to participate in the recent rapid technological developments in cryo-EM that are revolutionizing the field, and I am now using these tools to make a significant impact in studies of the macromolecular complexes that drive intracellular organization. Importantly, I have always coupled my structural work with functional studies in vitro and in vivo, often in collaboration with other experts in the field. With this combined approach, I uncovered the mechanism of microtubule nucleation by γ-tubulin complexes, and am now focusing my efforts on newly discovered metabolic filaments and a number of bacterial contractile and motile systems. In 2014 I relocated my laboratory from McGill University to the University of Washington. Here, I have played an instrumental role in establishing the Arnold and Mabel Beckman Cryo-EM Center, leading the successful effort to upgrade our facilities to include cutting edge microscopes, detector technologies, and computational infrastructure. My lab currently focuses on the structural biology of complex self-assembling protein structures, including metabolic enzyme polymers and force-generating bacterial nanomachines.

Ongoing projects that I would like to highlight include:

R01 AI153048-01A1
Kollman (PI)
4/15/21-3/31/25
Structural basis for differential regulation and selective inhibition of human CTP synthases 1

R01 GM118396-06
Kollman (PI)
04/01/16-02/28/25
The structure and function of metabolic filaments

R21 EY031546-02
Brockerhoff & Kollman (MPI)
04/01/20-03/31/2022
IMPDH structure and function in retinal degeneration

R01 GM127648-04
Kollman (PI)
6/01/18-5/31/22
Structure and function of the R-body, a piston-like nanomachine

Citations:

a. Lynch EM and **Kollman JM*** (2020) Coupled structural transitions enable highly cooperative regulation of human CTPS2 filaments *Nat. Struct. Mol. Biol.* 27:42-48 PMC6954954

b. Lynch EM, Dimattia MA, Albanese S, van Zundert GCP, Hansen JM, Quispe J, Kennedy M, Verras A, Borrelli K, Toms AV, Kaila N, Kreutter KD, McElwee JJ, **Kollman JM*** (2021) Structural basis for isoform-specific inhibition of human CPTS1. Proc. Natl. Acad. Sci. USA 118:e2107968118 PMC8641951

c. Burrell AL, Nie C, Said M, Simonet JC, Fernández-Justel D, Johnson MC, Quispe J, Buey RM, Peterson JR, **Kollman JM***. (2021) IMPDH1 retinal variants control filament architecture to tune allosteric regulation. Nat. Struct. Mol. Biol. 29:47-58

d. Hansen JM, Horowitz A, Lynch EM, Farrell DP, Quispe J, DiMaio F, **Kollman JM*** (2021) Cryo-EM structures of yeast CTPS filaments and the functional consequences of regulated assembly. eLife 2021;10:e73368 PMC8641951

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2012-2014	Assistant Professor, Department of Anatomy & Cell Biology, McGill University, Montréal, QC
2014	Assistant Professor (Associate Member), Department of Biochemistry, McGill University, Montreal, QC
2014-present	Adjunct Assistant Professor, Department of Anatomy & Cell Biology, McGill University, Montreal, QC
2014-2019	Assistant Professor, Department of Biochemistry, University of Washington, Seattle, WA
2014-present	Director, Arnold and Mabel Beckman Cryo-EM Center, University of Washington, Seattle, WA
2019-present	Associate Professor, Department of Biochemistry, University of Washington, Seattle, WA

Other Experience and Professional Membership

2005-present	Member, American Society for Cell Biology
2012-2016	Member, Groupe de Recherche Axé sur la Structure des Protéines
2015, 2018	Organizer, Special Interest Subgroup on Metabolic Enzyme Polymers, American Society for Cell Biology Annual Meeting
2018-present	Instructor, Cryo-EM Course, Cold Spring Harbor Laboratories
2019, 2021	Ad Hoc Member, Molecular Structure and Function C Study Section, NIH
2019-present	Member, User Review Committee, National Center for CryoEM Access and Training
2019-present	Member, User Review Committee, Pacific Northwest Center for Cryo-EM
2019-present	Member, Advisory Board, Centre for Cellular Evolution, Adaptation and Diversity Studies, McGill University
2020-present	co-Directory, Molecular Biophysics Training Program, University of Washington

Honors

1998	Graduate Assistance in Areas of National Need (GAANN) Award, US. Dept. of Education
2006	Ruth L. Kirschstein National Research Service Award, National Institutes of Health
2014	Research Scholar Junior 1 Career Award, Fonds de Recherche Santé Québec
2014	New Investigator Salary Award, Canadian Institutes of Health Research
2016	Science in Medicine New Investigator Lecture, University of Washington School of Medicine

C. Contributions to Science

1. **Metabolic enzyme polymers.** My lab has been at the forefront of studying regulation of metabolic enzymes through formation of large-scale polymers, a relatively newly discovered but widespread phenomenon. My group has provided the first structural and mechanistic insight into enzyme polymerization and its regulatory role. Our initial focus has been on two critical regulatory enzymes in nucleotide biosynthesis, CTP synthetase and IMP dehydrogenase, which both form filamentous polymers. Because of the critical importance of *de novo* nucleotide biosynthesis, both enzymes are targets for immunosuppressant, chemotherapeutic, and antiparasitic therapies. We have shown that polymerization directly affects enzyme activity, reflecting a novel mechanism of regulation. We have recently expanded these efforts to include collaborative studies of polymerizing glycolytic enzymes. These efforts are providing a framework for understanding the function of the dozens of polymerizing enzymes that have been identified to date, and are positioning my group as a leader in this new and quickly developing field.
    a. Lynch EM, Hicks DR, Shepherd M, Endrizzi JA, Maker A, Hansen JM, Barry RM, Gitai Z, Baldwin EP, **Kollman JM*** (2017) Human CTP synthase filament structure reveals the active enzyme conformation. *Nat. Struct. Mol. Biol.* 24,507-514 PMC 5472220
    b. Webb BA, Dosey AM, Wittman T, **Kollman JM**, Barber DL. (2017) The glycolytic enzyme Phosphofructokinase-1 assembles into filaments. *J. Cell. Biol.* 216,2305-2313 PMC 5551713
    c. Stoddard PR, Lynch EC, Farrell DP, Justman QA, Dosey AM, William TA, **Kollman JM**, Murray AW, Garner EC (2020) Independent evolution of polymerization in the Actin ATPase clan regulates hexokinase activity *Science* 367:1039-1042 PMC7846450
    d. Johnson MC, **Kollman JM*** (2020) Cryo-EM structures demonstrate human IMPDH2 filament assembly tunes allosteric regulation *eLife* Jan 30;9. pii: e53243 PMC7018514
2. **Bacterial cell biology.** Subcellular organization in bacteria has been traditionally overlooked, but in recent years it has become clear that despite their relatively small size bacteria employ complex mechanisms to organize their internal spaces. Our work in this field focuses on bacterial cytoskeletal systems, primarily bacterial actin homologs. An important class of bacterial actins drives plasmid segregation, which is important for ensuring stable inheritance of very large plasmids, many of which encode toxins or virulence factors. In collaboration with Dyche Mullins at UCSF I determined the structure of the plasmid segregating filament AlfA, demonstrating a unique filament architecture that is tied to unusual filament dynamics. Another unique bacterial actin, MamK, is required to position and organize membrane-bound bacterial organelles. In collaboration with Arash Komeili at UC Berkeley I have determined the structure of MamK, which provided insight into cytoskeletal-organelle interactions in bacteria. Together, these novel actin structures demonstrate how protomers with strongly conserved tertiary structure evolve new functional and dynamic properties through changes to assembly contacts that give rise to dramatic changes in quaternary structure.
    a. Polka JK, **Kollman JM**, Mullins RD (2013) Accessory factors promote AlfA-dependent plasmid segregation by regulating filament nucleation, disassembly, and bundling. *Proc. Natl. Acad. Sci. USA* 111, 2176-2181 PMC3926056
    b. Bergeron JR, Hutto R, Ozyamak E, Hom N, Hansen J, Draper O, Byrne ME, Keyhani S, Komeili A, **Kollman JM*** (2016) Structure of the magnetosome-associated actin-like MamK filament at subnanometer resolution. *Protein Sci.* 26, 93-102 PMC5192964
    c. Usluer G, Dimaio F, Yang SK, Hansen JM, Polka JP, Mullins RD**, Kollman JM*** (2018) Cryo-EM structure of the bacterial actin AlfA reveals unique assembly and ATP-binding interactions and the absence of a conserved subdomain. *Proc. Natl. Acad. Sci. USA* 115,3356-3361 PMC5879662
    d. Morris RM, Cain KR, Hvorecny KL**, Kollman JM** (2020) Lysogenic symbiosis in SAR11. Nat. Microbiol. 5:1011-1015 PMC7387148
3. **Microtubule Cytoskeleton.** My postdoctoral work focused on elucidating the molecular mechanisms of microtubule nucleation. Microtubule nucleation is controlled *in vivo* by γ-tubulin complexes, which define the timing and location of new microtubule growth. The core γ-tubulin complex, γ-Tubulin Small Complex

(γTuSC) had been shown to self-assemble into a large ring-like structure called the γ-Tubulin Ring Complex (γTuRC), but the mechanism by which γTuRC initiates microtubule growth was unknown. I determined the structure of the free γTuSC, demonstrating a high degree of flexibility. I discovered that an adaptor protein, important for γTuSC localization, has the unexpected ability to promote γTuSC oligomerization in to rings. I exploited this to form stable γTuSC oligomers for structure determination by cryo-EM. The orientation and arrangement of γ-tubulin in the structure strongly supports a template mechanism for nucleation, in which γTuSC oligomers provide the first layer of tubulin in the microtubule and define its helical geometry. We subsequently demonstrated that the nucleating activity of γTuRC is conformationally regulated by inter-conversion between an open and a closed state, mediated by the flexibility I observed in the γTuSC subunit. The high-resolution structure of the more active closed state also allowed us to build a pseudo-atomic model of the entire γTuRC that provides significant insight into its assembly and function. Taken together, my work on γ-tubulin complexes has defined the mechanism of microtubule nucleation, created a new understanding of the link between localization and assembly of γTuRC, and provided a structural framework for interpreting existing biochemical and cell biological data and for generating new hypotheses about γTuRC function.

a. **Kollman JM**, Polka JK, Zelter A, Davis TN, Agard DA. (2010) Microtubule nucleating γ-TuSC assembles structures with 13-fold microtubule-like symmetry. *Nature* 466, 879-882. PMC2921000

b. Guillet V, Knibiehler M, Gregory-Pauron L, Remy M-H, Cemin C, Raynaud-Messina B, Bon C, **Kollman JM**, Agard DA, Mourey L, Merdes A (2011) Insight into γ-tubulin complex assembly from the crystal structure of GCP4. *Nat. Struct. Mol. Biol.* 18, 915-919. PMC3703858

c. **Kollman JM**, Greenberg CH, Li S, Moritz M, Zelter A, Fong KK, Fernandez J-J, Sali A, Kilmartin J, Davis TN, Agard DA (2014) Ring closure activates yeast γTuRC for species-specific microtubule nucleation. *Nat. Struct. Mol. Biol.* 22,132-137. PMC4318760

d. Chaban S, Jariwala S, Hsu C-T, Redemann S, **Kollman JM**, Müller-Reicher T, Sept D, Bui KH, Brouhard GJ (2018) The Structure adn Dynamics of *C. elegans* Tubulin Reveals the Mechanistic Basis of Microtubule Growth. Dev. Cell 47,191-204

4. **Design of large-scale protein assemblies.** In recent years, working in collaboration with investigators at the Institute for Protein Design at the University of Washington, including Frank DiMaio and David Baker, my group has been contributing to design of self-assembling protein structures, including unbounded filamentous and two-dimensional arrays and defined nanomachines. Our efforts focus on validation of designed structures using cryo-EM, and the iterative refinement of designs. *De novo* designed proteins hold enormous potential as platforms for novel therapeutic and nanotechnology development.

a. Shen H[#], Fallas JA[#], Lynch E[#], Sheffler W, Parry B, Jannetty N, Decarreau J, Wagenbach M, Chen J, Wang L, Dowling Q, Oberdorfer G, De Yoreo J, Wordeman L, Jacobs-Wagner C, **Kollman JM**, Baker D. (2018) *De novo* design of self-assembling helical protein filaments. Science 362:705-709 PMC6637945

b. Brunette TJ, Bick MJ, Hansen JM, **Kollman JM**, Baker D (2020) Modular repeat protein sculpting using rigid helical junctions. Proc. Natl. Acad. Sci 117:8870-8875 PMC7183188

c. Xu C, Lu P, Gamal TM, Pei X-Y, Johnson MC, Uyeda A, Bick MJ, Jiang D, Ma D, Lynch E, Boyken SE, Huang PS, Stewart L, **Kollman JM**, Luisi BF, Matsuura T, Catterall WA, Baker D (2020) Computational design of transmembrane pores. Nature 585:129-134 PMC7483984

d. Ben-Sasson AJ, Watson J, Sheffler W, Johnson MC, Bittleston A, Logeshwaran S, Decarreau J, Jiao F, Chen J, Mela I, Drabek AA, Jarrett SM, Blacklow SC, Kaminski CF, Hura GL, De Yoreo JJ, Ruohola-Baker H, **Kollman JM**, Delivery E, Baker D (2020) Design of biologically active binary protein 2D materials. Nature 589:648-473 PMC7483984

Complete list of published works:
https://pubmed.ncbi.nlm.nih.gov/?term=kollman%20JM%20OR%20kollman%20justin

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Young Kwon

eRA COMMONS USER NAME (credential, e.g., agency login): YOUNG_KWON

POSITION TITLE: Assistant Professor of Biochemistry

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Yonsei University	B.S.	02/1997	Biology
Yonsei University	M.S.	08/1999	Biology
Johns Hopkins University	Ph.D.	04/2008	Genetics
Harvard Medical School	Postdoctoral training	08/2015	Genetics

A. Personal Statement

My research goal is to elucidate the molecular mechanisms underlying the maintenance of the adult tissue pattern and physiology. Currently, my lab is focusing on understanding 1) how oncogenic transformation of an epithelial tissue affects the hemostasis of the distant tissues and 2) how transformed cells disseminate from an intact organ using *Drosophila melanogaster* as a model. During my postdoctoral training, I developed a novel *Drosophila* model of systemic organ wasting. This model allows us to dissect the molecular mechanisms underlying the tissue wasting process using the advanced genetic and genomic tools available in *Drosophila*. Recently, my lab established a *Drosophila* model of cell dissemination, whereby expression of Ras^{V12} oncogene in intestinal epithelial cells induced them to move out from the intestine and enter the hemocoel – the fly's body cavity. Using this model, we described actin- and cortactin-rich invasive protrusions resembling invadopodia observed in cancer cells and the course of cell dissemination in a native context. Furthermore, this model provides a unique opportunity for screening genes involved in the dissemination process. We have identified several candidate genes through a genetic screening and plan to characterized them in *Drosophila* and mouse models of metastasis.

Lee J, Ng K, Dombek K, and **Kwon YV.** Tumors overcomes the action of the wasting factor ImpL2 by elevating Wnt/Wingless. ***PNAS*** (2021)

Lee J, Cabrera A, Nguyen C, and **Kwon YV**. Dissemination of Ras^{V12}-transformed cells requires the mechanosensitive channel Piezo. ***Nature Communications*** (2020)

Kwon Y, Song W, Droujinine IA, Hu Y, Asara JM and Perrimon N. Systemic organ wasting induced by localized expression of the secreted insulin/IGF antagonist *ImpL2.* ***Developmental Cell.*** (2015) April 6;33:36-46

Kwon Y, Vinayagam A, Sun X, Dephoure N, Gygi SP, Hong P and Perrimon N. The Hippo signaling pathway interactome. ***Science*** (2013) Nov 8;343(6159):737-40

B. Positions and Honors

Professional Positions

2008-2015 Postdoctoral Research Fellow , lab of Dr. Norbert Perrimon at Harvard Medical School

2015-present Assistant Professor, Department of Biochemistry, University of Washington

Awards

2007 Paul Ehrlich Research Award, Johns Hopkins University

2009 Tom Elkins Memorial Lecture Award

2009-2011 Damon Runyon Cancer Research Fellowship

2016 Regular Grant Award, Edward Mallinckrodt, Jr. Foundation

Distinguished Lectures

2009 Tom Elkins memorial lecture, The neurobiology of *Drosophila* conference at Cold Spring Harbor

2015 Future of Cancer Science Symposium, The University of Texas MD Anderson Cancer Center

C. Contributions to Science

Complete List of Published Work in MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/14GzcvVz3ElQw/bibliography/public/

1. Establishment of a *Drosophila* Model of Cell Dissemination

Dissemination of transformed cells is a key process in metastasis. Despite its importance, how transformed cells disseminate from an intact tissue and enter the circulation is poorly understood. Using a fully developed tissue, *Drosophila* midgut as a model, my lab described the morphologically distinct steps and the cellular events occurring over the course of Ras^{V12}-transformed cell dissemination. Notably, we found that Ras^{V12}-transformed cells formed the Actin- and Cortactin-rich invasive protrusions that were important for breaching the extracellular matrix (ECM) and the visceral muscle layer. Furthermore, we uncovered the essential roles of the mechanosensory channel Piezo in orchestrating the cell dissemination process by impinging on multiple steps. Recently, my lab elucidated the roles of E-cadherin and intracellular calcium signaling in cell dissemination. Collectively, our study establishes an *in vivo* model for studying how transformed cells migrate out from a complex tissue and identifies new molecular players in the cell dissemination process.

Lee J, Cabrera A, Nguyen C, and **Kwon Y**. Dissemination of Ras^{V12}-transformed cells requires the mechanosensitive channel Piezo. ***Nature Communications*** (2020)

Cabrera AJH, Barry BM, and **Kwon YV**. The roles of distinct Ca^{2+} signaling mediated by Piezo and inositol triphosphate receptor (IP3R) in the remodeling of E-cadherin during cell dissemination. ***bioRxiv*** (2021); ***Review Commons*** (under review) https://www.biorxiv.org/content/10.1101/2021.11.10.467957v1

2. Establishment of a Non-mammalian Model of Cachexia-like Wasting

Wasting, characterized by an involuntary loss of body mass, is observed under conditions of extreme starvation and in the context of diseases, including cancers. Although multiple systemic factors and signaling pathways involved in wasting are identified, the underlying mechanisms are not clearly understood. Moreover, effective treatment options for pathological wasting conditions such as cancer cachexia are not currently available. During my postdoctoral study, I developed a model for systemic organ wasting in adult *Drosophila*, whereby overproliferation induced by activation of Yorkie, the Yap1 oncogene ortholog, in intestinal stem cells led to wasting of the ovary, fat body, and muscle. We found that these organ-wasting phenotypes are associated with a reduction in systemic insulin/IGF signaling due to increased expression of the secreted insulin/IGF antagonist ImpL2 from the overproliferating gut. Altogether, my study provides the first in-depth characterization

of systemic wasting phenotypes in *Drosophila*, highlighting striking similarities in the wasting phenotypes between *Drosophila* and mammals. Importantly, my study uncovered a novel tumor-derived systemic wasting factor, ImpL2, which is the ortholog of insulin-like growth factor binding proteins (IGFBPs) in mammals. Recently, my lab addressed why tumors were not wasted away even though they expressed high levels of the wasting factor ImpL2. We found that tumors could evade the action of ImpL2 by locally elevating Wg/Wnt expression, implying the inhibition of Wg/Wnt could be an efficient way to treat the tumors associated with cachexia. Altogether, this unique model will allow us to dissect the genetic basis of cachexia-like wasting.

Kwon Y, Song W, Droujinine IA, Hu Y, Asara JM and Perrimon N. Systemic organ wasting induced by localized expression of the secreted insulin/IGF antagonist *ImpL2*. ***Developmental Cell.*** (2015) April 6;33:36-46

Lee J, Ng KGL, Dombek KM, and **Kwon**. Tumors overcomes the action of the wasting factor ImpL2 by elevating Wnt/Wingless. ***PNAS*** (2021)

3. Construction of Hippo signaling pathway protein-protein interaction network

The Hippo pathway is a critical regulator of metazoan organ growth and tumor formation, but our knowledge of the composition and the structure of this signaling network is incomplete. To identify additional players in the signaling pathway, I generated a functional Hippo signaling pathway protein-protein interaction network using proteomics and functional genomics. I performed an in-depth analysis of the network of interactions, which revealed a snapshot of the overall organization of the Hippo signaling network, and enabled me to identify many new components of the Hippo pathway. In particular, I showed that a new member of the α-arrestin family (Leash) is a novel negative regulator of growth. Moreover, the network has been also used by other researchers to identify new mechanisms that regulate Hippo signaling. Further characterization of the Hippo signaling pathway protein-protein interaction network will help to uncover additional mechanisms that regulate this signaling pathway. Given the importance of Hippo signaling in oncogenesis, findings from these studies will be useful for developing novel intervening strategies for cancers.

Kwon Y, Vinayagam A, Sun X, Dephoure N, Gygi SP, Hong P and Perrimon N. The Hippo signaling pathway interactome. ***Science*** (2013) Nov 8;343(6159):737-40

Sun X, Hong P, Kulkarni M, **Kwon Y**, Perrimon N. PPIRank - an advanced method for ranking protein-protein interactions in TAP/MS data. ***Proteome Sci.*** (2013) Nov 7;11(Suppl 1):S16.

Neumuller RA*, Wirtz-Peitz F*, Lee S, Kwon Y, Buckner M, Hoskins RA, Venken KJT, Bellen HJ, Mohr SE and Perrimon N. Stringent analysis of gene function and protein-protein interactions using fluorescently tagged genes. ***Genetics*** (2012) Mar;190(3):931-40.

4. Identification of the role of TRPA1 in avoidance of a natural insect repellent Citronellal

Insects avoid plants-produced insect repellents, such as citronellal, which is the main component of citronellal oil. However, the molecular pathways through which insects sense botanical repellents was not unknown. Using *Drosophila* as a model, we showed that the Ca(2+)-permeable cation channel TRPA1 plays essential roles in sensing citronellal in *Drosophila* and *Anopheles gambiae*. Overall, our study provides insights into the roles of insect TRPA1 channels in sensing citronellal, and raises the possibility that mosquito TRPA1 may be a target for developing improved repellents to reduce insect-borne diseases such as malaria.

Kwon Y*, Kim SH*, Ronderos DS*, Lee Y, Akitake B, Woodward OM, Guggino WB, Smith DP, Montell C. *Drosophila* TRPA1 channel is required to avoid the naturally occurring insect repellent citronellal. ***Curr Biol***. (2010)

5. Identification of the molecular mechanism through which phosphoinositides regulate TRP channels

Although multiple TRP channels were regulated by phosphoinositides (PIs), the mechanisms through which PIs regulate TRP channels were obscure. Using biochemical approaches, we showed that TRPC6 bound directly to PIs, and the PIs binding disrupted the association of calmodulin (CaM) with TRPC6. Strikingly, our study also suggests that PI-mediated disruption of CaM binding appears to be a theme that applies to other TRP channels,

as well as to the voltage-gated channels KCNQ1 and Ca(v)1.2. Altogether, our findings elucidated the molecular mechanism underlying the regulation of TRP channels by PIs.

Kwon Y, Hofmann T, Montell C. Integration of phosphoinositide and calmodulin mediated regulation of TRPC6. ***Mol Cell***. (2007) 25:491-503

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

R35GM128752, NIH, NIGMS (2018~2023)

The Molecular Basis of Basal Cell Extrusion in Drosophila Intestinal Epithelium

The project is to elucidate the molecular mechanisms underlying dissemination of Ras^{V12}-transformed cells from the *Drosophila* intestine.

Discovery Grants for Cancer Research, Kuni Foundation (2022~2024)

Targeting the metabolic vulnerability of tumor-derived extracellular vesicles for cancer therapy

The project is to elucidate the mechanisms by which tumor-derived extracellular vesicles maintain their integrity for develop strategies to eliminate them.

Completed Research Support

Mallinckrodt Grant, Edward Mallinckrodt, Jr. Foundation (2016-2020)

Discovering the Genetic Basis of Cachexia-like Wasting

The goal of the proposed study is to elucidate the mechanisms underlying tissue wasting caused by localized tumor using Drosophila as a model.

New Investigator Support from Cancer Center Support Grant (CCSG), Fred Hutch/University of Washington Cancer Consortium (2016)

Discovering a novel role of Yap1/Yorkie in alternative polyadenylation

The goal of the project is to study the role of Yorkie and Yap1 in regulation of 3’UTR shortening.

OMB No. 0925-0001 and 0925-0002 (Rev. 10/2021 Approved Through 09/30/2024)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Merz, Alexey Jarrell

eRA COMMONS USER NAME: ajmerz

POSITION TITLE: Professor of Biochemistry

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Reed College	BA	06/1992	Cell Biology
Oregon Health & Science University	PhD	12/1999	Microbiology & Immunology
Duke University School of Medicine	Visiting Scientist	04/2000	Biophysics
Dartmouth Geisel School of Medicine	Postdoctoral	08/2004	Biochemistry

A. Personal Statement

As a graduate student I published landmark papers on *Neisseria*-host interactions and reported the first direct observations of pilus retraction in any bacterial system. I joined W. Wickner's group as a postdoc due to the group's foundational contributions in both bacterial and eukaryotic membrane biochemistry. Major efforts in my laboratory at the University of Washington focus on fundamental molecular mechanisms that underlie biogenesis and dynamics of endolysosomal organelles and the Golgi. My group's approach is biology-driven, cross-disciplinary, and collaborative, emphasizing high-leverage tools and technologies. These include quantitative light microscopy, classical forward genetics, deep scanning mutagenesis, protein biophysical chemistry, reconstitution of complex organelle dynamics using cell-free systems and chemically defined components, cryo EM, and mass spectrometric methods to reveal the architecture of proteins and protein complexes. In addition to our work on fundamental aspects of eukaryotic cell biology, we work on bacterial pathogens including *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pseudomonas*, and *Francisella*. I follow the examples of graduate and postdoctoral mentors who emphasized that if you take care of your trainees and reports, strong science will emerge.

Active research support

2021 *Advanced light microscopy equipment.*
NIH R01 GM077349-14S1. PI: Merz
$206,000 direct.

2006–'25 *Dynamics of endomembrane docking and fusion*.
NIH R01 GM077349-14. PI: Merz
$1.5M direct + indirect over 4 years

2021-'23 *Molecular basis of pilus-mediated gonococcal adhesion.*
NIH R21 AI155991-01. PI: Merz
$420k direct + indirect over 2 years.

2019-'23 *Mechanisms of AP-3 function in vesicle formation and Golgi maturation.*
NIH R01 GM130644-02. PI: Merz; collaboration w/ Co-PI G. Odorizzi (CU-Boulder).
$2M direct+indirect over 4 years, shared between the two laboratories.

Representative papers

i. **Merz AJ**, So M, Sheetz MP. Pilus retraction powers bacterial twitching motility. *Nature*. 2000. doi: 10.1038/35024105. PubMed PMID: 10993081.

ii. Schwartz ML, **Merz AJ**. 2009. Capture and release of partially zipped trans-SNARE complexes on intact organelles. *J Cell Biol* 185:535–549. PMCID: PMC2700395.

iii. Schwartz ML, Nickerson DP, Lobingier BT, Plemel RL, Duan M, Angers CG, Zick M, **Merz AJ**. 2017. Sec17 (α-SNAP) and an SM-tethering complex regulate the outcome of SNARE Zippering in vitro and in vivo. *eLife*, doi: 10.7554/eLife.27396 PMCID: PMC3196633

iv. Leveille CL, Cornell CE, **Merz AJ***, Keller SL*. 2022. Yeast cells actively tune their membranes to phase separate at temperatures that scale with growth temperatures. *PNAS*, 10.1073/pnas.2116007119 **Corresponding authors.*

B. Positions, Scientific Appointments, and Honors

Positions and scientific appointments

2019— Professor of Biochemistry; Professor of Physiology & Biophysics, University of Washington School of Medicine, Seattle, WA.

2012-19 Associate Professor of Physiology & Biophysics, University of Washington School of Medicine, Seattle, WA.

2010-19 Associate Professor of Biochemistry, University of Washington School of Medicine, Seattle, WA.

2004–10 Assistant Professor of Biochemistry, University of Washington School of Medicine, Seattle, WA.

2000–04 Postdoctoral Fellow, Dartmouth Geisel School of Medicine, Hanover, NH.

1989 Statistical Software Test & Validation, Red Brick Systems (acquired by IBM).

Professional activities

NIH Cell Structure and Function (CSF2), temporary member, 2022–.

UW System Chemical and Physical Safety Committee (CAPS), 2019–.

Co-Chair, Symposia on Protein and Organelle Quality Control, 2013 ASCB Meeting.

ASBMB Membership Committee, 2011–2014.

Ad hoc NIH grant review, 2012.

Ad hoc grant review, UK Medical Research Council, 2012.

Co-Chair, Minisymposium on Membrane Fusion and Fission, 2011 ASCB Annual Meeting.

Referee: *Biophys J · Curr Biol · Dev Cell · eLife · Euk Cell · F1000 Biol · Genetics · J Bac · JBC · JCB · JCS · J Neurosci · MBoC · Mol Microbiol · Nanotechnology · Nature · NCB · NSMB · Nat Protocols · PNAS · Science · Traffic · etc.*

Member: ASCB, ASBMB.

Honors and awards

WWAMI Student-initiated Award for Excellence in Medical Education, 2017.

American Cancer Society Research Scholar, 2010–2013.

NIH Postdoctoral Trainee (T32 AR07576), 2003–2004.

Damon Runyon Cancer Research Foundation Postdoctoral Fellow (DRG-1598), 2000–2003.

Paper of the Year (*Nature* 407:98), OHSU Graduate Council, 2000.

Sears Award for Excellence in Graduate Medical Education, 1997.

N.L. Tartar Research Award, OHS Foundation, 1996–1997.

NIH Predoctoral Trainee (T32 AI07472), 1996–1999.

HHMI Undergraduate Research Fellow, 1992.

C. Contributions to Science

1. How bacteria crawl

As a visiting scientist in M. Sheetz's group I established how the second major mechanism of bacterial motility works. Using laser tweezers force spectroscopy, and engineered *N. gonorrhoeae* cells, I made the first direct observations of bacterial pilus retraction, measured the force and velocity of the retraction event, and demonstrated that retraction requires the PilT ATPase (*i*). Retraction entails depolymerization of 2,000 pilin subunits per second from the pilus base, and type IV pili are arguably the strongest processive motors in biology. My experiments were soon followed by complementary work from Skerker and Berg (*ii*). Together, our two papers resolved a twenty-year argument over the mechanism by which pili allow bacteria to crawl over surfaces (*iii*), and opened new avenues of investigation into mechanisms of bacterial motility, biofilm assembly, DNA uptake, and type 2 secretion.

- *i.* **Merz AJ**, So M, Sheetz MP. Pilus retraction powers bacterial twitching motility. 2000. *Nature*. doi: 10.1038/35024105. PMID: 10993081
- *ii.* Skerker JM, Berg HC. Direct observation of extension and retraction of type IV pili. 2001. *Proc Natl Acad Sci*. doi: 10.1073/pnas.121171698 PMID: 11381130
- *iii.* **Merz AJ**, Forest KT. Bacterial surface motility: slime trails, grappling hooks and nozzles. 2002. *Curr Biol*. doi: 10.1016/s0960-9822(02)00806-0 PMID: 11967173

2. Bacterial interactions with target cells

As a grad student with M. So, I set up the first infection model for pathogenic *Neisseria* that employed polarized human epithelial cells cultured on a permeable support (*i*). Using that system, I discovered that *N. gonorrhoeae* (GC) can traverse an epithelial monolayer without disrupting its barrier function. Moreover, adhesion, entry, and traversal were strongly dependent on the presence of type IV pili. In subsequent work (*i-iii*), I discovered that epithelial cells exhibit rapid responses to GC attachment including rearrangements of microvilli and the underlying cytoskeleton, and accumulation of tyrosine-phosphorylated proteins. I called the resulting structures cortical plaques, after the focal adhesion plaques that anchor the eukaryotic cytoskeleton to the extracellular matrix. Further analyses revealed that cortical plaque formation requires type IV pili and is strongly dependent on pilus retraction. These are probably the first experiments showing that mechanical force is a signal passed from bacteria to host cells (*iii*). In collaboration with J. Mougous and colleagues, we contributed the pivotal discovery that type VI bacterial secretion systems deliver antibacterial toxins (*iv*).

- *i.* **Merz AJ**, Rifenbery DB, Arvidson CG, So M. Traversal of a polarized epithelium by pathogenic *Neisseriae*: facilitation by type IV pili and maintenance of epithelial barrier function. 1996. *Mol Med*. PMCID: PMC2230138.
- *ii.* **Merz AJ**, Enns CA, So M. Type IV pili of pathogenic *Neisseriae* elicit cortical plaque formation in epithelial cells. 1999. *Mol Microbiol*. doi: 10.1046/j.1365-2958.1999.01459.x PMID: 10383771.
- *iii.* **Merz AJ**, So M. Interactions of pathogenic *Neisseriae* with epithelial cell membranes. 2000. *Annu Rev Cell Dev Biol*. doi: 10.1146/annurev.cellbio.16.1.423 PMID: 11031243.
- *iv.* Hood RD, Singh P, Hsu F, Güvener T, Carl MA, Trinidad RR, Silverman JM, Ohlson BB, Hicks KG, Plemel RL, Li M, Schwarz S, Wang WY, **Merz AJ**, Goodlett DR, Mougous JD. A type VI secretion system of Pseudomonas aeruginosa targets a toxin to bacteria. *Cell Host Microbe*. 2010. doi: 10.1016/j.chom.2009.12.007 PMID: 20114026.

3. Organelle biogenesis

My group at the University of Washington has sought to understand how Rab small GTP-binding proteins and their effectors organize traffic and regulate fusion. We discovered two Rab7 binding sites within the HOPS effector complex and three more Rab5 binding sites within CORVET, the HOPS sister complex. We also mapped subunit contact architecture within these ~700 kDa complexes (*i*). These results, along with parallel work from competing groups, laid the groundwork for recent X-ray and EM structures. We were first to demonstrate that Rab5 is indispensable for biogenesis of the endosomal multivesicular body, and first to show

that Rab5 has an important role in autophagy (*ii*). Further, we discovered that a single yeast Rab GAP, Gyp3, delineates the boundary between the early Rab5 compartment and the late Rab7 compartment (*ii*). A major focus has been the development of chemically-defined reconstitutions of vesicle docking and fusion. This led to our unprecedented discovery (*iii*) that yeast Rab5, and a subset of other Rabs, can operate autonomously as a minimal tethering machinery — a layer of function that functions in parallel with classical Rab effectors. To our knowledge this is the *only* chemically defined system to date that fully recapitulates reversible, GEF- and GAP-regulated, Rab-dependent membrane tethering. More recently we developed a chemically defined system that recapitulates core aspects of COPII vesicle docking and fusion; this led to our discovery of a hidden tethering activity within the essential SNARE cofactor Sly1 (*iv*).

i. Plemel RL, Lobingier BT, Brett CL, Angers CG, Nickerson DP, Paulsel A, Sprague D, **Merz AJ**. 2011. Subunit organization and Rab interactions of Vps-C protein complexes that control endolysosomal membrane traffic. *Mol Biol Cell* 22:1353–1363. PMCID: PMC3078060

ii. Nickerson DP, *Russell MRG, *Lo SY Milnes J and **Merz AJ**. 2012. Termination of isoform-specific Vps21/Rab5 signaling at endolysosomal organelles by Msb3/Gyp3. *Traffic*, doi: 10.1111/j.1600-0854.2012.01390.x PMCID: PMC393931 *Equal contributors.

iii. Lo SY, Brett CL, Plemel RL, Vignali M, Fields S, Gonen T, **Merz AJ**. 2011. Intrinsic tethering activity of endosomal Rab proteins. *Nat Struct Mol Biol* 19:40–47. PMCID: PMC3252480

iv. Duan M, Plemel RL, Takenaka T, Lin A, Delgado BM, Nattermann U, Nickerson DP, Mima J, Miller EA, **Merz AJ**. Gatekeeper helix activates Golgi SM protein Sly1 and directly mediates vesicle tethering. *bioRχiv* [preprint], doi: 10.1101/2020.01.16.906719

4. SNARE cofactors in membrane fusion

Most intracellular membrane fusion events are executed by SNARE proteins. After fusion, SNARE complexes are pulled apart by the ATPase Sec18 (NSF) and its essential adapter, Sec17 (α-SNAP). We've discovered that Sec17 has additional roles not only after fusion, but before — during membrane docking. These novel functions help to explain the essential functions *in vivo* of a third family of essential SNARE cofactors, the SM (Sec1/Munc-18) proteins. In 2009 we reported for the first time that Sec17 executes a positive role *upstream* of fusion (*i*): Sec17 can bind to stalled pre-fusion *trans*-SNARE complexes and trigger their fusogenic activity. In parallel work we discovered that — again, entirely unexpectedly — Sec17 can promote the loading of SM (Sec1/Munc18) family proteins onto SNARE complexes (*iii*). Finally, Sec17 has not only positive effects before fusion, but negative effects. Remarkably, the SM selects whether Sec17 stimulates fusion, or inhibits (*v*). We have proposed a kinetic partitioning model (*iii and iv*), in which the SM functions as a discriminator that promotes accurate SNARE-mediated fusion both directly, and by actively repressing inhibitory side-reactions.

i. Schwartz ML, **Merz AJ**. 2009. Capture and release of partially zipped trans-SNARE complexes on intact organelles. *J Cell Biol* 185:535–549. PMCID: PMC2700395.

ii. Lobingier BT, **Merz AJ**. 2012. Sec1/Munc18 protein Vps33 binds to SNARE domains and the quaternary SNARE complex. *Mol Biol Cell* 23:4611–4622. PMCID: PMC3510022.

iii. Lobingier BT, Nickerson DP, Lo SY, **Merz AJ**. 2014. SM proteins Sly1 and Vps33 co-assemble with Sec17 and SNARE complexes to oppose SNARE disassembly by Sec18. *eLife* 16;3:e02272. PMCID: PMC4060006.

iv. Schwartz ML, Nickerson DP, Lobingier BT, Plemel RL, Duan M, Angers CG, Zick M, **Merz AJ**. 2017. Sec17 (α-SNAP) and an SM-tethering complex regulate the outcome of SNARE Zippering in vitro and in vivo. *eLife*, doi: 10.7554/eLife.27396 PMCID: PMC3196633

5. Microdomains and phase separation in biological membranes

Using ultrasensitive fluorescence measurements on intact organelles, we demonstrated that membrane docking involves a complex and ordered assembly of over a dozen proteins (*i*) and numerous specific lipids (*ii*). This on-the-fly choreography assembles the machinery that marks the incipient membrane fusion site and catalyzes the fusion event. This year, in collaboration with the Keller lab, we discovered that "polka dots" first observed on the yeast vacuole fifty years ago are in fact formed through the separation of two co-existing liquid phases: a liquid

disordered phase and a “raft like” liquid ordered phase (*iii*). The lipid raft concept has been immensely controversial in part because the term is not rigorously defined. Our experiments, combined with prior work from Prinz and many others, establish these yeast vacuole domains as perhaps the first definitive example of liquid-liquid membrane phase segregation in unperturbed living cells. Because many of the same proteins and lipids are segregated into distinct domains during spontaneous domain formation, and during vacuole tethering and docking, we hypothesize that Rab-mediated docking may trigger phase separation to organize lipids and proteins at incipient fusion sites.

v. Wang L, Seeley ES, Wickner W, **Merz AJ**. 2002. Vacuole fusion at a ring of vertex docking sites leaves membrane fragments within the organelle. *Cell* 108:357–369. PMID: 11853670.

vi. Fratti RA, Jun Y, **Merz AJ**, Margolis N and Wickner WT. 2004. Interdependent assembly of specific regulatory lipids and membrane proteins into the vertex ring domain of docked vacuoles. *Journal of Cell Biology* DOI: 10.1083/jcb.200409068. PMCID: PMC2172599

vii. Rayermann SP, Rayermann GE, Cornell CE, **Merz AJ***, Keller SL. 2017*. Hallmarks of reversible phase separation in living, unperturbed cell membranes. *Biophys J*, doi: 10.1016/j.bpj.2017.09.029 PMCID: 5768407. **Corresponding authors.*

viii. Leveille CL, Cornell CE, **Merz AJ***, Keller SL. 2017*. 2022. Yeast cells actively tune their membranes to phase separate at temperatures that scale with growth temperatures. *PNAS*, doi: 10.1073/pnas.2116007119 **Corresponding authors.*

Complete Lists of Published Work:

NCBI: *http://www.ncbi.nlm.nih.gov/sites/myncbi/alexey.merz.1/bibliography/41140445/public/*

Google Scholar: *https://scholar.google.com/citations?user=HkExtJsAAAAJ&hl=en&oi=ao*

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Dana Miller

eRA COMMONS USER NAME (credential, e.g., agency login): dlmiller

POSITION TITLE: Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Denver, Denver CO	BS	06/1998	Biology and Biochemistry
The Johns Hopkins University, Baltimore MD	PhD	10/2003	Biochemistry
The Fred Hutchinson Cancer Research Center, Seattle WA	postdoctoral	07/2010	Molecular Genetics

A. Personal Statement

My research group that uses *C. elegans* to explore how animals respond to H_2S and hypoxia (decreased O_2). We are one of the only groups in the world that has leveraged the *C. elegans* system to understand the molecular and genetic mechanisms that mediate the responses to defined changes in the gaseous environment, and we have discovered many novel phenotypes and genes and pathways that reveal fundamental aspects of cellular physiology and homeostasis. Our work on hypoxia in particular has focused on analysis of how proteostasis pathways are coordinated during stress. I have established multi-disciplinary collaborations with colleagues who are primarily structural biologists (Dove *et al.* 2017 PNAS), focused on revealing the physiological effects of mutations in E2/E3 ubiquitin ligases that are well-characterized structurally. I also have a nascent collaboration with the Gardner lab focused on how ubiquitin and SUMO (the small ubiquitin-like modifier) coordinate cellular proteostasis during stress. As such, my lab has expertise in molecular and genetic approaches to studying enzymes such as UBC-18, an E2 that works with RBR E3 ligases (similar to Parkin), *in vivo*. My lab uses genetic, genomic, cell biological, developmental, and biochemical approaches.

Since starting my lab in 2010, I have trained 36 undergraduate and high school students, 4 graduate students, and 6 postdoctoral fellows. Graduate students trained in my lab have gone on to work in nonprofits, small pharma start-up companies, and in high school curriculum development. One student transitioned from my lab to a PhD program in clinical psychology. Many of the undergraduates who have trained in my lab have gone on to MD and/or PhD programs. The postdoctoral fellows I have trained are working in teaching and science policy, as a staff scientist, and one is applying to medical school. I have served (or am serving on) 32 graduate thesis committees. My service work is largely focused on promoting access and inclusion in the basic biomedical sciences. For example, I served as one of the first faculty members of the MCB DEI committee, I am currently co-chair of the Biochemistry DEI committee, I helped initiation the Basic Sciences DEI task force, and I am an active member of the SOM Allies leadership council.

B. Positions and Honors

Positions and employment

1999-2003	Pre-doctoral research associate (Joel F. Shildbach, advisor), Department of Biology, The Johns Hopkins University, Baltimore, MD
2003-2010	Post-doctoral research fellow (Mark B. Roth, mentor), Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA

2010-2017	Assistant Professor, Department of Biochemistry, University of Washington School of Medicine, Seattle, WA.
2017-present	Associate Professor, Department of Biochemistry, University of Washington School of Medicine, Seattle, WA.

Faculty Affiliations at UW

Fred Hutchinson/University of Washington Cancer Consortium
Molecular and Cellular Biology Graduate Program
Neuroscience Graduate Program

Other Experience and Professional Membership

Faculty Advisor, Genetics Society Early Career Scientist Subcommittee on Diversity and Inclusion
Editorial Board, *Journal of Gerontology: Biological Sciences*
Member, National Scientific Advisory Council for the American Federation for Aging Research (AFAR)
Ad hoc member, NIH Cellular Mechanisms of Aging and Disease (CMAD) study section.
Scientific Organizer (with Esther Verheyen) 2019 Northwest Developmental Biology Meeting
Scientific Organizer (with Sean Curran) 2014 *C. elegans* Topic Meeting: Aging, Metabolism, Pathogenesis, Stress, and Small RNAs.
Ad-hoc member NIH ONES Review study section
Member, NIH/NIEHS special emphasis panel for K99/R00 awards.

Selected Honors

1994	National Merit Scholarship Finalist
1997	Golden Key National Honor Society Scholarship Winner
1997	Phi Beta Kappa, Gamma Chapter of Colorado
1998	*Summa cum laude*, University of Denver
2005	NIH Loan Repayment Program Award
2005	NIH NRSA Fellowship
2009	NIH K99/R00 Pathway to Independence Award
2010	Nathan Shock Center for Excellence in the Biology of Aging Junior Faculty Award
2011	Ellison New Scholar in Aging
2015	NIEHS ONES (Outstanding New Environmental Scientist) Award

C. Contribution to Science

Link to full bibliography: DLMiller PubMed bibliography

Physiological responses to hydrogen sulfide

Hydrogen sulfide (H_2S) is an endogenous signaling molecule that influences a wide array of cellular processes. My lab has pioneered the use of *C. elegans* to understand molecular and genetic mechanisms that mediate the biological effects of H_2S. As a postdoctoral fellow, I discovered that *C. elegans* grown in H_2S are thermotolerant and live 70% longer than controls without H_2S and showed that increased lifespan in H_2S requires the conserved sirtuin orthologue, *sir-2.1*. I also showed that HIF-1 is required for all of the early transcriptional changes in H_2S and revealed a role for *skn-1*/Nrf2 in mediating the transcriptional response to H_2S. In my own lab, we have discovered that H_2S can prevent hypoxia-induced defects in proteostasis and found that the sulfide-quinone oxidoreductase, *sqrd-1,* also has a role to prevent activation of the mitochondrial and ER UPR in H_2S. In recent work, we have discovered that increased expression of a single protein, RHY-1, can rescue the lethality of *hif-1* mutants in H_2S. This recent work suggests that there is a novel cellular mechanism that can modulate H_2S toxicity, and is the focus of the current application.

Relevant publications:

Horsman, J. W., Heinis, F. I., and **Miller, D. L.** 2019 “A novel mechanism to prevent H_2S toxicity in *C. elegans*”. *Genetics* (213): 481-490. PMID: 31371406.

Topalidou, I., and **Miller, D. L.** 2017 “*Caenorhabditis elegans* HIF-1 is broadly required for survival in hydrogen sulfide”. G3 7(11): 3699-3704. PMID: 28889102, PMCID: PMC5677155.

Horsman, J. W., and **Miller, D. L.** 2016 "Mitochondrial SQRD-1 is essential to maintain translation in hydrogen sulfide". *JBC* 291(10): 5320-5325. PMID: 26677221, PMCID: PMC4777863.

Miller, D. L., Budde, M. W. and Roth, M. B. 2011 "HIF-1 and SKN-1 Coordinate the Transcriptional Response to Hydrogen Sulfide in *C. elegans*." *PLoS ONE* 6(9): e25476. PMID: 21980473, PMCID: PMC3183046.

Epigenetic bookmarking of hydrogen sulfide

Changes in environmental conditions can have long-lasting effects of the epigenetic landscape. These effects can influence disease susceptibility, sensitivity to drugs or toxins, and even aging. We have found that *C. elegans* transiently exposed to low H_2S can survive subsequent exposure to otherwise lethal high H_2S, even after 72 hours in normal room air (without H_2S). We have found that after a short adaptation to low H_2S animals develop a bookmark to H_2S that facilitates a more robust transcriptional response upon subsequent exposure to high H_2S. We have identified the conserved SWI/SNF chromatin-remodeling complex as well as histone methyltransferase and demethylase enzymes that are required to maintain the H_2S bookmark. We are continuing to define the mechanism by which exposure to H_2S is translated into epigenetic changes.

Relevant publications:

Fawcett, E. M., Johnson, J. K., Braden, C. R., Garcia, E. M., and **Miller, D. L.** "Epigenetic bookmarking of H_2S exposure in *Caenorhabditis elegans*" *In review*. Pre-print available on BioRXiv doi**:** https://doi.org/10.1101/619734.

Integrated responses to hypoxia

Oxygen is essential for animal development, metabolism, and physiology. However, there is great diversity in how well animals can successfully respond to hypoxia. Even different cells within the same organism are differently sensitive to hypoxia. In order to understand the fundamental cellular responses to hypoxia, we have characterized cellular and organism responses to specific hypoxic environments. Our studies show that the response to hypoxia depends greatly on the specific amount of oxygen that is available. Moreover, we show that different cell types have different responses to hypoxia. Autophagy is upregulated almost exclusively in the intestine, whereas reproduction and development are regulated by neuronal cues. We have also discovered specific hypoxic conditions disrupt proteostasis, and that short periods of fasting can prevent hypoxia-induced protein aggregation and proteotoxicity. We have identified a counterintuitive role for the insulin/IGF-like receptor, DAF-2, in mediating the protective effects of fasting in hypoxia. DAF-2 works independently of the downstream FOXO transcription factor DAF-16 in this situation.

Relevant publications:

Iranon N. N., Jochim B. E., and **Miller, D. L.** 2019 "Fasting prevents hypoxia-induced defects of proteostasis in *C. elegans*". *PLoS Genetics* 15(6): e1008242. https://doi.org/10.1371/journal. pgen.1008242.

Chapin, H. C., Okada, M., Merz, A. J., and **Miller, D. L.** 2015. "Tissue-specific autophagy responses to aging and stress in *C. elegans*." *Aging* (7): 419-434 PMID: 26142908, PMCID: PMC4505168.

Fawcett, E. M., Hoyt, J. M., Johnson, J. K., and **Miller, D. L.** 2015. "Hypoxia disrupts proteostasis in *Caenorhabditis elegans*." *Aging Cell* (14): 92-101 PMID: 25510338, PMCID: PMC4326909.

Leiser, S. F., Miller, H., Rossner, R., Fletcher, M., Leonard, A., Primitivo, M., Rintala, N., Ramos, F. J., **Miller, D. L**., and Kaeberlein, M. 2015. "Cell nonautonomous activation of flavin-containing monooxygenase promotes longevity and health span." *Science* 350: 1375-8. PMID: 26586189, PMCID: PMC4801033.

Oligomerization of F-plasmid TraM

Conjugation is a common mechanism for bacteria to exchange genetic material, and may contribute to the spread of virulence factors and antibiotic resistance. In my dissertation research I characterized the oligomerization of the protein TraM, which is required for conjugation of F-plasmid episomes. Efficient transfer of the F-plasmid requires that the donor cell extend a pilus, which contacts a recipient cell. The pilus the retracts, bringing the two cells into close apposition. When stable mating pairs have formed, the episome is nicked at a specific DNA sequence, unwound, and transferred through the pilus into the recipient cell. TraM is essential for conjugation because it transmits the signal that a stable mating pair has formed that initiates DNA unwinding and transfer. However, the mechanism by which TraM transmits this signal is unknown. In my

dissertation research, I characterized the biophysical properties of TraM using protein denaturation and analytical ultracentrifugation. TraM is a tetramer in solution, but my research revealed a monomeric folding intermediate, and suggested that oligomerization is important for TraM to transmit the mating signal that initiations conjugative DNA transfer.

Relevant publications:

Miller, D. L. and Schildbach, J. F. 2013. "Evidence for a monomeric intermediate in the reversible unfolding of F factor TraM". JBC 278: 10400-10407. PMID 12529360.

Street, L. M., Harley, M. J., Stern, J. C., Larkin, C, Williams, S. L., **Miller, D. L.**, Dohm, J. A., Rodgers, M. E., and Schildbach, J. F. 2003. "Subdomain organization and catalytic residues of the F Factor TraI relaxase domain." Biochim Biophys Acta: 1646: 86-99. PMID: 12637015.

D. Research Support

Completed

2015-2020
R01 AG044378 (PI)
NIH/NIA
Mechanisms integrating hypoxia response with proteostasis

2015-2020
R01 ES024958 (PI)
NIH/NIEHS ONES (Outstanding New Environmental Scientist) Award.
Mechanisms of hydrogen-sulfide effects on the epigenetic landscape.

2017-2018
EDGE pilot project (co-PI with Rich Gardner)
NIEHS/UW
Conserved SUMOylation pathways in heavy metal exposure

2015-2016
Glenn Award for Research in Biological Mechanisms of Aging
Glenn Foundation for Medical Research

2011-2015
Ellison Medical Foundation New Scholars in Aging (PI)
"Understanding the Effects of Hydrogen Sulfide on Protein Homeostasis and Aging"

NIGMS R01GM088055 (Klevit, PI)
Collaborative structure/function investigation of novel ubiquitin enzymes using C. elegans.
Role: collaborator

2010-2013
NIA R00 AG033050
"Mechanism of the response to hydrogen sulfide"

2010-2012
Nathan Shock Center Junior Faculty Award
Mechanisms of environmental influence on protein homeostasis

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Richard D. Palmiter

POSITION TITLE: Investigator of Howard Hughes Medical Institute

and Professor of Biochemistry and Genome Sciences, University of Washington

eRA COMMONS USER NAME (credential, e.g., agency login):RPalmiter

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Duke University, Durham NC	A.B.	1964	Zoology
Stanford University, Palo Alto CA	Ph.D.	1968	Biology

A. Personal Statement

I started my career studying hormone action and transcriptional regulation during development of the chicken oviduct. My laboratory also cloned the first metallothionein genes and studied their regulation of hormones, metals and DNA methylation. Subsequently, we identified the first mammalian zinc efflux transporters (Slc30a1, a2 and a3). In the early 1980's I helped develop transgenic technology in collaboration with Dr. Ralph Brinster. During this 15-year collaboration, we identified promoter/enhancer elements for cell-specific gene expression for growth hormone, albumin, hemoglobin, protamine, and elastase genes and used them to study development, physiology and malignancy. In the mid-1980's we began to apply these transgenic techniques to neurobiology and in the mid-1990's we began using gene targeting technology to inactivate genes involved in catecholamine biosynthesis. I have been supported by HHMI since 1976. HHMI support has allowed me freedom to work in the lab myself and to pursue diverse research directions. Being in the lab most of the time allows me to set realistic goals and to solve problems as they arise. My reputation and experience have allowed me to recruit the best students and with their help to develop exciting new areas of research. I strive for every student to have their own phenomenon to study, but to share methodology and collaborate with each other when it is most efficient. We have a long tradition of collaborating effectively with other laboratories when it is mutually beneficial. Our laboratory typically has 4 technicians, 4 graduate students, 4 senior fellows, and several undergraduate students. We have weekly lab meetings where students discuss their research progress and the relevant recent literature. Over 85% of the 60 graduate students and senior fellows who have trained in my lab now have medically relevant positions. I have an excellent track record of developing new technologies and applying them to new areas of investigation. Our research is described in over 500 publications, with more than 100 in premier (impact factor >10) journals. These publications can be found at PubMed under 'Palmiter, R.D.' Our main ongoing projects involve using genetically engineered mice and viruses to decipher neural circuits that control innate behaviors, especially those involving body weight regulation and responses to threats, including painful stimuli.

B. Positions and Honors

Research and Professional Experience

1968-1971 **Postdoctoral Fellow** with Robert Schimke, Dept Pharmacology, Stanford University, Stanford CA

1971-1973 **Research Associate** with Norman Carey, Dept. Biochemistry, GD Searle Research Labs, High Wycombe, England

1973-1974 **Research Associate** with Fotis Kafatos, The Biological Laboratories, Harvard University, Cambridge MA

1974-1978 **Assistant Professor**, Dept. Biochemistry, University of Washington, Seattle WA

1976-present **Investigator** of Howard Hughes Medical Institute

1978-1981 **Associate Professor**, Dept Biochemistry, University of Washington, Seattle

1981-present **Professor**, Dept. Biochemistry, University of Washington, Seattle, WA

2000 (6 mo) **Acting Chairman**, Dept. Biochemistry, University of Washington, Seattle

2001-present **Adjunct Professor,** Dept. Genome Sciences, University of Washington, Seattle WA

Honors and Awards

1988 Elected to National Academy of Sciences

1988 Elected to American Academy of Arts and Sciences

1994 Charles-Leopold Mayer Award, French Academy of Sciences

2004 Recipient of the Julius Axelrod Medal

C. Contributions to Science

1. During my post-doctoral years and early career as an assistant professor, I pursued the mechanisms by which steroid hormones (estrogen, progesterone, testosterone and glucocorticoids) controlled the development of the chicken oviduct with particular emphasis on the transcription regulation of the genes encoding egg white proteins (ovalbumin, conalbumin, ovomucoid and lysozyme). These projects resulted in about 40 publications. The research culminated with the first demonstration that steroid hormones act the transcriptional level.

McKnight, G.S., and **Palmiter, R.D.** (1979) Transcriptional Regulation of the Ovalbumin and Conalbumin Genes by Steroid Hormones in Chick Oviduct. J. Biol. Chem. 254, 9050-9058.

Palmiter, R.D., Mulvihill, E.R., Shepherd, J.H. and McKnight, G.S. (1981) Steroid Hormone Regulation of Ovalbumin and Conalbumin Gene Transcription: A Model Based Upon Multiple Regulatory Sites and Intermediary Proteins. J. Biol. Chem. 256, 7910-7916.

During this time, we also developed methods for sequencing proteins translated in reticulocyte lysates. This technology was used to determine the sequence of signal peptides from several secreted proteins prior to advent of DNA cloning. This effort is represented in about 10 publications. We discovered that ovalbumin, a major secreted protein, is secreted without a removable signal sequence.

Palmiter, R.D., Gagnon, J., Ericsson, L.H. and Walsh, K.A. (1977) Precursor of Lysozyme: Amino Acid Sequence of an NH_2-terminal Extension. J. Biol. Chem. 252, 6386-6393.

Palmiter, R.D., Gagnon, J., and Walsh, K.A. (1978) Ovalbumin: A Secreted Protein without a Transient Hydrophobic Leader Sequence. Proc. Natl. Acad. Sci. USA. 75, 94-98.

2. In 1979, I decided to switch focus from regulation of chicken genes to the mouse metallothionein genes because of the available cell lines. We were the first to clone a metallothionein gene, sequence the gene, and demonstrate that it was transcriptionally regulated by hormones and metals (zinc and cadmium). We also the first to demonstrate that the genes are amplified in response to selection for cadmium resistance, that they can be inactivated by DNA methylation and reactivated by UV irradiation. We ultimately cloned the entire locus which encompasses 4 metallothionein genes. We ultimately made mice lacking metallothionein genes and

mice that over-expressed these genes to study the functions of these proteins in vivo. My efforts to elucidate the mechanism of transcriptional regulation of metallothionein genes lead to the discovery of zinc transporters that transport zinc out of the cytoplasm – either across the plasma membrane (ZnT1, or into vesicles (Znt2 and ZnT3). We discovered that ZnT3 is responsible for transport of zinc into synaptic vesicles where it functions as a neuromodulator.

Durnam, D.M., Perrin, F., Gannon, F. and **Palmiter, R.D.** (1980) Isolation and Characterization of the Mouse Metallothionein-I Gene. Proc. Natl. Acad. Sci. U.S.A. 77, 6511-6515.

Beach, L.R. and **Palmiter, R.D**. (1981) Amplification of the Metallothionein-I Gene in Cadmium-Resistant Mouse Cells. Proc. Natl. Acad. Sci. U.S.A. 78, 2110-2114.

Compere, S.J. and **Palmiter, R.D.** (1981) DNA Methylation Controls the Inducibility of the Mouse Metallothionein-I Gene in Lymphoid Cells. Cell 25, 233-240.

Cole, T.B., Wenzel, H.J., Kafer, K.E., Schwartzkroin, P.A., and **Palmiter, R.D.** (1999) Elimination of Zinc for Synaptic Vesicles in the Intact Mouse Brain by Disruption of the ZnT3 Gene. *Proc. Natl. Acad. Sci. USA* **96**, 1716-1721.

3. Our research on metallothionein led to development of transgenic technology in collaboration with Ralph Brinster (Univ. Pennsylvania). We were the first to achieve expression of transgenes in mice. We used this technology to study genes that (a) control growth and (b) promote cancer. We also used this technology to study spermatogenesis and replace diseased liver. We also identified the first enhancer elements for cell-specific gene expression including those involved in expression of elastase, protamine, albumin and beta globin. This collaboration extended over 15 years in resulted in over 140 publications.

Brinster, R.L., Chen, H.Y., Trumbauer, M., Senear, A.W., Warren, R., **Palmiter, R.D.** (1981) Somatic Expression of Herpes Thymidine Kinase in Mice following Injection of a Fusion Gene into Eggs. Cell 27, 223-231

Palmiter, R.D., Brinster, R.L., Hammer, R.E., Trumbauer, M.E., Rosenfeld, M.G., Birnberg, N.C. and Evans, R.M. (1982) Dramatic Growth of Mice that Develop from Eggs Microinjected with Metallothionein-Growth Hormone Fusion Genes. Nature 300, 611-616.

Brinster, R.L., Chen, H.Y., Messing, A., van Dyke, T., Levine, A.J., and **Palmiter, R.D.** (1984) Transgenic Mice Harboring SV40 T-Antigen Genes Develop Characteristic Brain Tumors. Cell 37, 367-369.

Ryan, T.M., Townes, T.M., Reilly, M.P., Asakura, T., **Palmiter, R.D**., Brinster, R.L., and Behringer, R.R. (1990) Human Sickle Hemoglobin in Transgenic Mice. Science 247, 566-568.

4. In the mid-1980's we began to focus on using transgenic techniques to study neurobiology with an emphasis on catecholamines and by mid-1990's we began to use gene targeting technology to inactivate genes involved in catecholamine biosynthesis. We developed dopamine-deficient mice and used them to study the role of dopamine in a variety of behaviors including cognition. We also studied the role of NMDA receptors in dopamine neurons and their targets. This research includes over 50 publications.

Thomas, S. A., Matsumoto, A.M. and **Palmiter, R.D.** (1995) Norepinephrine is Essential for Mouse Development. *Nature* 374, 643-646.

Zhou, Q-Y and Palmiter R.D. (1995) Dopamine-deficient Mice Are Severely Hypoactive, Adipsic and Aphagic. *Cell* 83, 1197-1209.

Thomas, T.A and **Palmiter, R.D.** (1997) Impaired Maternal Behavior in Mice Lacking Norepinephrine and Epinephrine. *Cell* **91**, 583-592.

Zweifel, L.S., Argilli, E., Bonci, A., and **Palmiter, R.D.** (2008) Role of NMDA Receptors in Dopamine Neurons for Plasticity and Addictive Behaviors. *Neuron* **59,** 486-496. PMC2556153

5. There has been an ongoing interest during the last 20 years in genes and neuronal circuits that mediate appetite and body weight regulation. This work started with you genetic inactivation of the neuropeptide Y (*Npy*) gene, which at the time was thought to be critical for feeding behavior; however, inactivation of the NPY gene had no effect on body weight regulation. Inactivation of agouti-related protein and GABA signaling by the hypothalamic NPY neurons was also without effect. However, ablation of the NPY/AgRP neurons in adult mice led to starvation indicating that the neurons are important. We discovered that the starvation phenotype was

due to loss of inhibition of neurons in the parabrachial nucleus (PBN) and that mice could survive loss of NPY/AgRP neurons if the function of the PBN neurons was suppressed for at least a week, suggesting that some form of adaptation occurs. We identified the neurons in the PBN that mediate the anorexia phenotype as those that express calcitonin gene-related protein (CGRP). These CGRP neurons project to the lateral capsule of the amygdala. Optogenetic or chemogenetic activation of CGRP neurons (or their processes in the amygdala) is sufficient to inhibit feeding by hungry mice. We have shown that the CGRP neurons mediate anorexia caused by visceral malaise (eating too much, nausea, or illness) and they are critical for establishing a conditioned taste aversion. Most recently, we have discovered that these neurons also mediate the unconditioned pain stimuli (e.g. foot shock) and are a critical input for fear conditioning.

Carter, M.E., Soden, M.E., Zweifel, L.S., **Palmiter, R.D.** (2013) Genetic identification of a neural circuit that suppresses appetite. *Nature*, **503**,111-114. PMC3878302

Han, S., Soleiman, M.T., Soden, M.E., Zweifel, L.S., **Palmiter, R.D.** (2015) Elucidating an Affective Pain Circuit that Creates a Threat Memory. *Cell*, 162, 363-374. PMC4512641

Campos CA, Bowen AJ, Schwartz MW, **Palmiter RD.** (2016) Parabrachial CGRP Neurons Control Meal Termination. *Cell Metab*. 23:811-820. PMC4867080

Campos CA, Bowen AJ, Roman CW, and **Palmiter RD**. (2018) Encoding danger by parabrachial CGRP neurons. *Nature* 555, 617-622. PMC 6129987

Chen JY, Campos CA, Jarvie BC, **Palmiter RD.** (2018) Parabrachial CGRP Neurons Establish and Sustain Aversive Taste Memories. *Neuron* 100, 891-899

D. Research Support

Active:

PI: Richard Palmiter

Agency: Howard Hughes Medical Institute (Appointment approved until 6/30/25). This is not a grant. HHMI supports my salary, that of several technicians, a senior scientist, equipment, mouse costs, and most of the supplies

Goals: **"***Mouse molecular genetics".* HHMI has supported our research since 1976. During recent years we have applied genetic and viral technologies to study the role of specific neurons in mediating mouse behavior and physiology.

PI: Richard Palmiter

Agency: National Institutes of Health. R01-HD24908 (07/01/2017-06/30/2022) "*Anorexia neural-circuit delineation***".** This research is focused on the role of neurons expressing calcitonin gene related protein (CGRP) in suppressing feeding. Direct costs: $196,000/year

OMB No. 0925-0001 and 0925-0002 (Rev. 10/2021 Approved Through 93/30/2024)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Hannele Ruohola-Baker

eRA COMMONS USER NAME (credential, e.g., agency login):

hannele POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Helsinki, Helsinki, Finland	BA, MS	1978-1984	Biochemistry
University of Helsinki/Yale University	PhD	1985-1989	Cell Biology
University of California, San Francisco, HHMI	Postdoctoral	1989-1993	Developmental Biology

A. Personal Statement

My laboratory studies the molecules and cellular properties that are required for stem cell states and their differentiation capacity, both in normal and pathological situations. During the recent years, my laboratory has shown that microRNAs and HIF pathways, and metabolism in general play key roles in regulating adult and embryonic stem cell self-renewal in model organisms as well as in hESC and iPSC. The laboratory presently works on three questions: 1) metabolic determinants of stem cells, 2) the molecular mechanism of reversible quiescence/diapause and 3) decoding regeneration using computer designed proteins and organoids. The mechanisms that govern the stem cell regenerative competence still remain unclear. My laboratory, in collaboration with the Institute for Protein Design (IPD) has developed novel computer designed protein-based tools to dissect and govern regeneration by signaling pathways and precise epigenetic regulation. We have recently generated computer designed Ang/Tie2 super-agonists for vascular protection in regeneration. This unprecedented, valency based exactness to manipulate Tie2 signaling pathway allows us to analyze, and plausibly in the future treat inflammation induced traumatic brain injuries as well as cytokine storm, sepsis (***Zhao et al. EMBO Rep. 2021; Divine et al. Science 2021; Ben-Sasson et al. Nature 2021***). We have worked to combine designed Tie2 agonists and COVID-19 Spike protein-binders to combat the deadly virus, SARS-CoV-2 utilizing human organoids (***Hunt et al. in review; Helms et al. JCI Ins. 2021***). We work on utilizing the artificial intelligence guided, designed de novo proteins to govern epigenomic landscape of precise genes in development and aging (***Levy et al. Cell Rep. 2022*****).**

1. Levy S, Somasundaram L, Raj IX, Ic-Mex D, Phal A, Schmidt S, Ng WI, Mar D, Decarreau J, Moss N, Alghadeer A, Honkanen H, Sarthy J, Vitanza N, Hawkins RD, Mathieu J, Wang Y, Baker D, Bomsztyk K, Ruohola-Baker H (2022) dCas9 Fusion to Computer Designed PRC2 Inhibitor Reveals Functional TATA Box in Distal Promoter Region. Cell Rep. 38(9):110457.

2. Zhao YT, Fallas JA, Saini S, Ueda G, Somasundaram L, Zhou Z, Xavier I, Ehnes D, Xu C, Carter L, Wrenn S, Mathieu J, Sellers DL, Baker D, Ruohola-Baker H.(2021) F-domain valency determines outcome of signaling through the angiopoietin pathway. EMBO Rep. 6;22(12):e53471

3. Divine R, Dang HV, Ueda G, Fallas JA, Vulovic I, Sheffler W, Saini S, Zhao YT, Raj IX, Morawski PA, Jennewein MF, Homad LJ, Wan YH, Tooley MR, Seeger F, Etemadi A, Fahning ML, Lazarovits J, Roederer A, Walls AC, Stewart L, Mazloomi M, King NP, Campbell DJ, McGuire AT, Stamatatos L, Ruohola-Baker H, Mathieu J, Veesler D, Baker D. (2021) Designed proteins assemble antibodies into modular nanocages. Science. 372(6537):eabd9994.

4. Ben-Sasson AJ, Watson JL, Sheffler W, Johnson MC, Bittleston A, Somasundaram L, Decarreau J, Jiao F, Chen J, Mela I, Drabek AA, Jarrett SM, Blacklow SC, Kaminski CF, Hura GL, De Yoreo JJ, Kollman JM, Ruohola-Baker H, Derivery E, Baker D. (2021) Design of Biologically active binary protein 2D materials. Nature. 589(7842):468-473.

B. Positions and Honors

1986-1989 NYRP predoctoral fellow, Department of Cell Biology, Yale University, School of Medicine
1989-1992 EMBO Postdoctoral fellow, laboratory of Dr. Lily and Y-N Jan, UCSF
1992-1993 ACS Postdoctoral Fellow, laboratory of Dr. Lily and J-N Jan, UCSF
1993-2000 Assistant Professor of Biochemistry and Genetics, University of Washington, Seattle, WA
2000-2004 Associate Professor of Biochemistry and Genome Sciences, University of Washington
2002-present Member of Graduate Program in Neurobiology and & Behavior
2004-present Professor of Biochemistry, University of Washington, Seattle
2004-present Adjunct Professor of Genome Sciences, University of Washington, Seattle
2005-present Member of The Center of Human Development and Disability, UW, Seattle
2006-present Member of The Institute of Stem Cell and Regenerative Medicine, UW, Seattle
2007-present Member of The Cancer Consortium, Fred Hutchinson Cancer Research Center, Seattle
2009-present Adjunct Professor of Biology, University of Washington,
2011-present Associate Director, ISCRM, UW, Seattle
2014-present Adjunct Professor of Bioengineering, UW, Seattle
2015-present Adjunct Professor of Oral Health Sciences, School of Dentistry, UW, Seattle

Other Experience and Awards

1986-1989 Nordic Yeast Research Program Predoctoral Fellowship
1989 Predoctoral award from the Oskar Oflund Foundation
1989 Academy of Sciences Award (Finland)
1989-1991 EMBO Postdoctoral Fellowship
1992-1994 ACS Senior Postdoctoral Fellowship
1995-1997 Basil O'Connor Starter Scholar Research Award
1995-2000 American Heart Association Established Investigator Award
1996-2000 Pew Fellow in Biomedical Sciences, Pew Memorial Trust
2005 American Heart Association Award (best grant)
2008-2010 Elected Drosophila Board Representative
2007 Tietze Award
2011 Co-founder of Sphingo Pharmaceuticals, Inc.
2011 Inventor on the UW patent "Compositions and Methods for Treating Degenerative Muscle Conditions" (nationalized in the US) 45399.03WO2
2012 Undergraduate Research Mentor Award, UW
2013 Inventor of the filed UW patent "Methods and compositions to modulate RNA processing" 46335.02WO2
2013 Science In Medicine Lecture, University of Washington
2013 Organizer of the 54th Drosophila Research Conference
2014 Inventor of the filed UW patent "New composition and method on cardiac maturation" 46541.02WO2 2016 Inventor of the UW patent No. UW 46541.04US2 Methods for maturing cardiomyocytes and uses thereof.
2016-2019 Inventor of the UW patent No. US 9,416,369 B2, Methods and compositions to modulate RNA processing; Inventor of the UW patent No. U.S. Provisional Application No. 62/546,438, compositions and methods for enhancing maturation states of healthy and diseased cardiomyocytes; Inventor of the UW Provisional Patent 48477.01US1 Titled Precise gene activation via novel designed proteins mediating epigenetic remodeling, and UW Provisionla Patent 62/925113 Titled Computational Design of Tie2 Superagonists and Superantagonists
2018 Keynote Speaker, Metabolomics 2018
2019 Roger E. Koeppe Endowed Lecture, Oklahoma State University
2019 Cascadian Meeting
2019 III International Course on Theoretical and Applied Systems Biology. Rio de Janeiro, Brazil
2020 ISCCR COVID Symposium
2021 Kroc Symposium
2021 XI Brazilian Association of Cell and Gene Therapy Keynote Speaker

Study sections: 2020-2021 European Research Council (ERC), 2010-present NIH Study sections (Ad hoc), 2019 FDA/NIH Site Visit, 2014 NCI Site visit, 2008 NIH hESC PPG Study Section, 2007 NIH Study Section (DEV1, Ad hoc); 2005-2009 American Heart Association Review Panel; 2004-2008 (Ad hoc) National Institute of Health, Study Section for Skeletal Muscle Biology and Exercise Physiology; 2004 American Cancer Society, Review Panel; 2001 National Institute of Health, Study Section for Reproductive Sciences (RO1); 2001-2003 National Institute of Health, Study Section for RO3 Grants; 2000 National Cancer Institute Scientific Review Group for Program Projects (study section and parent committee).

C. Contributions to Science

1) My laboratory has studied the function of cellular metabolic remodeling in early development. We have shown the role of hypoxia inducible transcription factors (HIF1a and HIF2a) in the acquisition of stemness. Hypoxia can induce the reversal of human ESC early differentiation and HIFs are important for the metabolism of primed stem cell state. Previous studies showed that aggressive cancers display gene expression signatures characteristic of ESCs and are commonly exposed to hypoxic environments. HIFs may connect these two facts: hypoxia, through HIFs can induce a human embryonic stem cell-like transcriptional program in cancer cells. We used the reprogramming assay to analyze HIF1a and HIF2a function in stem cell acquisition and found that constitutive activation of HIF1a and HIF2a result in opposite outcomes during reprogramming; while constitutive activation of HIF1 is beneficial, constitutive activation of HIF2 is inhibitory for iPSC induction. One of the goals of the laboratory now is to identify and manipulate the metabolic determinants of stem cells by dissecting the metabolic control of epigenetic dynamics between the earliest embryonic stem cell stages. The working hypothesis is that differential metabolites between pluripotent stages may control epigenetic dynamics and signaling and the goal is to identify the epigenetic modifiers that are controlled by metabolic differences.

   a. Sperber H, Mathieu J, Wang Y, Ferreccio A, Hesson J, Xu Z, Fischer KA, Devi A, Detraux D, Gu H, Battle SL, Showalter M, Valensis C, Bielas JH, Ericson NG, MargarethaL, Robitaille AM, Margineantu D, Fiehn O, Hockenbery D, Blayu CA, Raftery D, Margolin A, Hawkins RD, Moon RT, Ware CB and **Ruohola-Baker H**. (2015) The metabolome regulates the epigenetic landscape during naïve to primed human embryonic stem cell transition, Nature Cell Biology, 17:1523-35.
   b. Mathieu J, Zhou W, Xing Y, Sperber H, Ferreccio A, Agoston Z, Kuppusamy K, Moon R and **Ruohola-Baker H**. (2014) Hypoxia Inducible Factors have distinct and stage-specific roles during reprogramming of human cells to pluripotency. Cell Stem Cell, 14:592-605. PMID: 24656769
   c. Mathieu J, Zhang Z, Nelson A, Lamba D, Reh TA, Ware C and **Ruohola-Baker H**. (2013) Hypoxia induces reentry of committed cells into pluripotency. Stem Cells, 31:1737-48. PMID:23765801
   d. Zhou W, Choi M, Margineantu D, Margaretha L, Cavanaugh C, Hesson J, Blau CA, Horwitz MS, Hockenbery D, Ware C, and **Ruohola-Baker H**. (2012) HIF1α induced switch from bivalent to exclusively glycolytic metabolism during ESC to EpiSC/hESC transition, EMBO J, 31:2103-16. .PMID: 22446391.

2) We have shown miRNA function in stem cells and regeneration. In particular, we dissected the function of key microRNAs regulating cell division.

   a. Hatfield SD, Shcherbata HR, Fischer KA, Nakahara K, Carthew RW, **Ruohola-Baker H**. (2005) Stem cell division is regulated by the microRNA pathway. Nature. 435:974-8.
   b. Qi J, Yu JY, Shcherbata HR, Mathieu J, Wang J, Seal S, Zhou W, Stadler B, Bougin D, Wang L, Nelson A, Ware C, Raymond C, Lim LP, Magnus J, Ivanovska I, Diaz R,Ball A, Cleary M and **Ruohola-Baker H**. (2009) microRNAs regulate human embryonic stem cell division. Cell Cycle, 8 (22). PMID: 19823043
   c. Yu J-Y, Reynolds SH, Hatfield S, Shcherbata HR, Fischer KA, Ward EJ, Long D, Ding Y, and **Ruohola-Baker H**. (2009) Dicer-1 dependent p21/p27/Dacapo suppression acts downstream of Insulin Receptor in regulating cell division of Drosophila germline stem cells. Development, 136:1497-507.
   d. Miklas JW, Clark E, Levy L, Detraux D, Leonard A, Beussman K, Showalter MR, Smith AT, Hofsteen P,Yang X, Macadangdang J, Manninenk T, Raftery R, Madan A, Suomalainen A, Kim DH, Murry CE, Fiehn O, Sniadecki NJ, Wang Y, **Ruohola-Baker H** (2019) The human disease gene TFPa/HADHA is required for fatty acid beta- oxidation and cardiolipin re-modeling in human cardiomyocytes. Nature Comm.10(1):4671

3) My laboratory revealed a new mechanism for stem cell protection against apoptosis; the dying transiently amplifying daughter cells send a protective signal that makes the stem cell resistant to programmed cell death. Since aggressive cancer cells, cancer stem cells are also highly apoptosis resistant, they may utilize similar mechanisms to escape chemo- and radiotherapies. Furthermore, we have studied the process during aging and shown that FOXO plays a critical function in stem cell aging.

   a. Artoni F, Kreipke RE, Palmiera O, Dixon C, Goldberg Z, **Ruohola-Baker H** (2017) Loss of FOXO rescues stem cell aging in Drosophila germ line, eLIFE, Sep 19;6. pii: e27842. doi: 10.7554/eLife.27842
   b. Xing Y, Su TT, **Ruohola-Baker H**. (2015) Tie mediated signal from apoptotic cells protects stem cells in Drosophila melanogaster. Nature Commun, 6:7058. PMID: 25959206.
   c. Shcherbata HR, Ward EJ, Fischer KA, Yu J-Y, Reynolds SH, Chen C-H, Xu P, Hay BA and **Ruohola-Baker H**. (2007) Stage-Specific Differences in the Requirements for Germline Stem Cell Maintenance in the Drosophila Ovary. Cell Stem Cell, 1:685-698**.**
   d. Ward EJ, Shcherbata HR, Reynolds S, Fischer KA, Hatfield S, and **Ruohola-Baker H**. (2006) Stem Cells Signal to the Niche through the Notch pathway in the Drosophila ovary. Curr. Biol. 16:2352-2358.

4) My laboratory showed that miRNAs are critical for human post-natal maturation process. In metazoans a switch in energy metabolism from glycolysis to fatty acid (FA) oxidation is observed during fetal to adult heart transition. Many heart diseases, for example most muscular dystrophies are manifested as late onset heart defects, during cardiomyocyte postnatal maturation. However, human cardiomyocyte maturation is poorly understood process at the moment. Using large scale profiling methods, my laboratory showed that the molecular signatures in *in-vitro* matured hESC derived cardiomyocytes are similar to those seen in the *in-vivo* derived mature cardiac tissues, thus enabling them to be used as excellent model to identify the metabolites and microRNAs that accelerate human cardiac maturation. The laboratory has already found key microRNAs in the process and shown that microRNA let-7 controls growth, force and the fundamental metabolic switch to fatty acid utilization in cardiomyocyte maturation. Hence let-7 and its targets now reveal themselves as key tools for disease interventions. We have also shown that sphingosine 1-phosphate (S1P) pathway suppresses dystrophic muscle phenotypes and have dissected the molecular mechanism for S1P action in this process.

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   b. Nguyen-Tran D-H, Hait N, Sperber H, Qi J, Fischer K, Ieronimakis N, Pantoja M, Hays A, Allegood J, Reyes M, Spiegel S and **Ruohola-Baker H**. (2014) Molecular Mechanism of Sphingosine-1-Phosphate Action in Duchenne Muscular Dystrophy, Disease Models & Mechanisms, 7:41-54. PMID:24077965
   c. Pantoja M, Fischer KA, Ieronimakis N, Reyes M, and **Ruohola-Baker H**. (2013) Genetic elevation of Sphingosine 1-Phosphate suppresses dystrophic muscle phenotypes in Drosophila. Development, 140:136-46. PMID: 23154413
   d. Shcherbata HR, Yatsenko AS, Patterson LB, Sood VD, Nudel U, Yaffe D, Baker D, and **Ruohola-Baker H**. (2007) Dissecting Muscle and Neuronal Disorders in Drosophila model for Muscular Dystrophy. EMBO J. 26:481-93.

5) My laboratory established the function of Notch pathway in stem cells and mitotic to endocycle transition in Drosophila melanogaster.

   a. Schaeffer V, Althauser C, Shcherbata HR, Deng W-M, and **Ruohola-Baker H**. (2004) Notch-dependent expression of Fizzy-related/Hec1/Cdh1 is required for the mitotic-to-endocycle transition in Drosophila follicle cells, Curr. Biol, 14:630-636.
   b. Deng W-M, Althauser C, and **Ruohola-Baker H**. (2001) Notch-Delta signaling induces a transition from mitotic cell cycle to endocycle in Drosophila follicle cells, Development, 128:4737-4746
   c. Jordan K, Clegg N, Blasi J, Morimoto A, Sen J, Stein D, McNeill H, Deng W-M, Tworoger M, and **Ruohola-Baker H**. (2000) The homeobox gene mirror links EGF signaling to embryonic dorso-ventral axis formation through Notch activation. Nature Genetics, 24, 429-433.
   d. Bryant Z, Subrahnmanyan L, LaTray L, Tworoger M, Liu C-R, Li M-J, van den Engh G, and **Ruohola-Baker H**. (1999) Characterization of differentially expressed genes in purified Drosophila follicle cells: towards a general strategy for cell type-specific developmental analysis. Proc Natl Acad Sci U S A. 96, 5559-5564.

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OMB No. 0925-0001 and 0925-0002 (Rev. 12/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Ronald Eugene Stenkamp

eRA COMMONS USER NAME (credential, e.g., agency login): RSTENKAMP

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Univ. of Oregon, Honors College, Eugene	B.A.	06/70	Chemistry
Univ. of Washington, Seattle	M.Sc.	06/71	Chemistry
Univ. of Washington, Seattle	Ph.D.	06/75	Chemistry

A. Personal Statement

I'm a structural biologist with many years of experience in dealing with a variety of crystallographic problems. I have collaborated with several Biochemistry faculty and students here at the Univ. of Washington and this has led to my obtaining and supporting a joint appointment in the Department. My interactions with the Department have been extensive over the past 40 years, predating my official joint appointment.

What I'm particularly interested in and proud of is my continuing interactions with UW students on crystallographic topics and projects. I do this via collaborations, literature review courses, and a fundamentals of crystallography course (BStr515). The latter has been taught here for over 50 years, with me as the principal instructor for the past 35. The course covers X-ray diffraction, symmetry, and the solution and refinement of crystal structures. Most of the biophysics students doing crystallography here at the UW have taken the course, and we've all learned from it.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

1970-1973 Teaching Assistant, Dept. of Chemistry, Univ. of Washington.
1974-1975 Research Assistant, Dept. of Chemistry, Univ. of Washington.
1975 Senior Fellow, Dept. of Biological Structure, Univ. of Washington.
1975-1977 Amer. Canc. Soc. Postdoctoral Fellow, Molec. Biophys. and Biochem., Yale Univ.
1977-1986 Postdoctoral Fellow, Research Associate, Research Asst. Prof., Dept. of Biological Structure, Univ. of Washington.
1986-1987 Res. Assoc. Prof., Dept. of Biol. Structure, Univ. of Washington.
1987-2002 Associate Professor, Dept. of Biol. Structure. (Adjunct Assoc. Prof., Dept. of Chemistry, Dept. of Biochemistry), Univ. of Washington.
2002- Professor, Dept. of Biol. Structure (Joint - Biochemistry), Univ. of Washington.

Honors

1969 Phi Beta Kappa.
1972 Phi Lambda Upsilon.
2014 Fellow, American Crystallographic Association

C. Contributions to Science

My research contributions are aimed at understanding biomolecular functions in terms of their structures. Knowing the three-dimensional structures of molecules, i.e., the locations of the atoms forming them, is extremely useful in understanding how they work and carry out their biological and biochemical functions. I'm fortunate that I've found projects where my expertise in X-ray crystallography has augmented the skills of collaborators in characterizing significant types of scientific problems. Listed here are some of the projects I've worked on.

1. G protein-coupled receptors (GPCRs). GPCRs are membrane proteins important for transmitting environmental signals into cells. There are a large number of members of this protein class, and they are important for all sorts of signal transmission. They respond to external stimuli (light or ligands) with a conformational change that results in setting off G protein signaling pathways inside cells. There is consider pharmaceutical interest in these molecules, and they form the targets for many drugs and therapeutic agents.

The first crystal structure of a GPCR was that of rhodopsin, one of the photopigments found in vertebrate retinas that forms the molecular basis for vision. Our first paper, published in 2000, has been cited nearly 4500 times, partly because the first GPCR structure was useful for modeling other GPCRs. I was a member of the team that solved and refined that structure and showed the locations of the various structural motifs that respond to the conformational shift of retinal, the protein's chromophore, when it absorbs a photon. Our later work on rhodopsin was in search of the light activated conformation and its complex with transducin, its G protein.

a. Palczewski, K., T. Kumasaka, T. Hori, C.A. Behnke, H. Motoshima, B.A. Fox, I. Le Trong, D.C. Teller, T. Okada, **R.E. Stenkamp**, M. Yamamoto and M. Miyano 2000 Crystal Structure of Rhodopsin: A G Protein-Coupled Receptor. Science, 289, 739-745.
b. Teller, D.C., T. Okada, C.A. Behnke, K. Palczewski and **R.E. Stenkamp** 2001 Advances in Determination of a High-Resolution Three-Dimensional Structure of Rhodopsin, A Model of G-Protein-Coupled Receptors (GPCRs). Biochemistry, 40, 7761-7772. PMCID: PMC1698954.
c. Salom, D., D.T. Lodowski, **R.E. Stenkamp**, I. Le Trong, M. Golczak, B. Jastrzebska, T. Harris, J.A. Ballesteros and K. Palczewski 2006 Crystal structure of a photoactivated deprotonated intermediate of rhodopsin. Proc. Natl. Acad. Sci., USA, 103, 16123-16128. PMCID: PMC1637547.
d. **Stenkamp, R.E**. 2018 Identifying G protein-coupled receptor dimers from crystal packings. Acta Crystallographica, D74, 655–670.

2. Non-heme iron proteins. Metalloproteins containing a binuclear iron complex bridged by carboxylates and a mu-oxygen atom carry out various enzymatic reactions and serve as oxygen transport and storage proteins in a few marine invertebrates. Hemerythrin is the oxygen binding protein in sipunculids, where it serves as a hemoglobin analog, just as hemocyanin, a copper containing protein, serves in some other marine invertebrates. As a graduate student, post-doc, and young faculty member, I worked to determine the structure of hemerythrin and its iron complex. The protein subunit consists of four roughly parallel alpha helices, and the iron atoms are bound to the protein via histidine and carboxylate-containing side-chains. Our structure determinations provided hints for inorganic chemists as they tried to model this (at the time) novel complex. Over the past 30 years, a number of important enzymes have been found to contain similar complexes.

a. **Stenkamp, R. E**., L. C. Sieker, L. H. Jensen and J. Sanders-Loehr 1981 Structure of the binuclear iron complex in metazidohemerythrin at 2.2 Å resolution. Nature, 291, 263-264.
b. **Stenkamp, R. E**., L. C. Sieker, L. H. Jensen, J. D. McCallum, and J. Sanders-Loehr. 1985 Active site structures of deoxyhemerythrin and oxyhemerythrin. Proc. Natl. Acad. Sci., USA., 82, 713-716. PMCID: PMC397116.
c. Holmes, M.A., I. Le Trong, S. Turley, L.C. Sieker and **R.E. Stenkamp** 1991 Structures of deoxy and oxy hemerythrin at 2.0 Å resolution. J. Mol. Biol., 218, 583-593.
d. Sieker, L.C., M. Holmes, I. Le Trong, S. Turley, B.D. Santarsiero, M.-Y. Liu, J. LeGall and **R.E. Stenkamp** 1999 Alternative metal binding sites in rubrerythrin: Which is the functional binuclear center? Nature Structural Biology, 8,308-309.

3. Blood clotting proteins. Clotting of blood is a well-regulated process involving a series of enzymes in an enzymatic cascade so a clot can form quickly when needed. Most of the enzymes are serine proteases that convert zymogens to active enzymes in a particular order so the process can be amplified and controlled.

After thrombin cleaves fibrinogen to make fibrin and the initial clot, factor XIII, a transglutaminase, is activated and crosslinks the fibrin subunits to stabilize the overall structure. Dave Teller and I led a group of researchers in solving the structure of factor XIII which led to the idea that the transglutaminase crosslinking reaction was similar in mechanism to that of the cysteine proteases, but run in reverse. We also determined the structure of other proteins involved in clotting. Our structures formed the basis for work by other groups that has yielded structures of most of the proteins involved in this very important biomedical process.

a. Yee, V.C., L.C. Pedersen, I. Le Trong, P.D. Bishop, **R.E. Stenkamp** and D.C. Teller 1994 Three-dimensional structure of a transglutaminase: Human blood coagulation factor XIII. *Proc. Natl. Acad. Sci., U.S.A.,* 91, 7296-7300. PMCID: PMC44386.
b. Pedersen, L.C., V.C. Yee, P.D. Bishop, I. Le Trong, D.C. Teller and **R.E. Stenkamp** 1994 Transglutaminase factor XIII uses proteinase-like catalytic triad to crosslink macromolecules. *Protein Science,* 3, 1131-1135. PMCID: PMC2142901.
c. Fox, B.A., V.C. Yee, L.C. Pedersen, I. Le Trong, P.D. Bishop, **R.E. Stenkamp** and D.C. Teller 1999 Identification of the calcium binding site and a novel ytterbium site in blood coagulation factor XIII by X-ray crystallography. J. Biol. Chem., 274, 4917-4923.

4. Protein-ligand interactions. Interactions between proteins and between proteins and other types of ligands are ubiquitous in biological systems. Understanding the thermodynamics and kinetics of these interactions is important for drug design, signal-transduction, metabolism, etc. To understand these in terms of molecular structures, I've collaborated with Pat Stayton and Terry Lybrand to apply mutagenesis techniques to the streptavidin-biotin system to correlate biophysical, computational and structural information about the tight binding of these two molecules. Binding constants for biotin with various streptavidin mutants are around 10^{13}-10^{14} M, and such large binding constants and binding energies make them more tractable for experimental measurements. We've looked at a number of mutants with substantially reduced binding affinities, but in only a few cases have we seen changes in the equilibrium structures in the crystalline state that might correlate with those different binding constants. These results however are consistent with molecular dynamics simulations indicating that the kinetics of biotin binding, i.e., on-rates and off-rates, are different for some mutations. Our crystallographic results, because they show only small structural changes, reinforce the idea that binding kinetics might be very important to consider in processes such as drug design.

a. Freitag, S., V. Chu, J.E. Penzotti, L.A. Klumb, R. To, I. Le Trong, T.P. Lybrand, **R.E. Stenkamp** and P.S. Stayton 1999 A Structural Snapshot of an Intermediate on the Streptavidin-Biotin Dissociation Pathway, Proc. Natl. Acad. Sci., USA, 96, 8384-8389. (S. Freitag, V. Chu, and J. Penzotti should be considered co-first authors). PMCID: PMC17525.
b. Hyre, D.E., L.M. Amon, J.E. Penzotti, I. Le Trong, **R.E. Stenkamp**, T.P. Lybrand and P.S. Stayton 2002 Early mechanistic events in biotin dissociation from streptavidin, Nature Structural Biology, 9, 582-585.
c. Baugh, L., I. Le Trong, D.S. Cerutti, N. Mehta, S. Gülich, P.S. Stayton, **R.E. Stenkamp** and T.P. Lybrand. 2012 Second Contact Shell Mutation Diminishes Streptavidin-Biotin Binding Affinity Through Transmitted Effects on Equilibrium Dynamics, Biochemistry, 51, 597-607. PMCID:PMC3320214.
d. Baugh, L., I. Le Trong, P.S. Stayton, **R.E. Stenkamp**, and T.P. Lybrand. 2016 A Streptavidin Binding Site Mutation Yields an Unexpected Result: An Ionized Asp128 Residue is Not Essential for Strong Biotin Binding. Biochemistry, 55, 5201-5203. PMID:27603565; PMCID:PMC5030189

5. Cell-adhesion proteins. For several years, I've collaborated with Wendy Thomas (Bioengineering), Evgeni Sokurenko (Microbiology) and Rachel Klevit (Biochemistry) and their students on studies of the cell adhesion protein, FimH. *E. coli* bacteria have cellular appendages called fimbria on their surfaces. These rod-like structures are made up of several proteins with FimH located at the tip of the fimbria. FimH has two domains, one to connect to the rest of the fimbria and one to bind to mannose residues expressed on other surfaces. FimH binds to these mannose receptors and causes the bacteria to adhere to those surfaces. FimH binds to mannose in what's called a "catch bond". These interactions tighten when the binding molecules are subjected to lateral forces or flow. This is of considerable interest due to *E. coli's* participation in urogenital infections. Through crystallographic studies and structure analyses, my group has provided structural information useful for understanding the biophysical results generated by our collaborators.

a.. Le Trong, I., P. Aprikian, B.A. Kidd, M. Forero-Shelton, V. Tchesnokova, P. Rajagopal, V. Rodriguez, G. Interlandi, R. Klevit, V. Vogel, **R.E. Stenkamp**, E.V. Sokurenko and W.E. Thomas. 2010 Structural Basis for Mechanical Force Regulation of the Adhesin FimH via Finger Trap-like β Sheet Twisting. *Cell,* **141**, 645–655. DOI 10.1016/j.cell.2010.03.038, PMCID: PMC2905812.

b. Le Trong, I., P. Aprikian, B.A. Kidd, W.E. Thomas, E.V. Sokurenko and **R.E. Stenkamp** 2010 Donor Strand Exchange and Conformational Changes During *E. coli* Fimbrial Formation, Journal of Structural Biology, **172**, 380-388. PMCID: PMC2964381.

c. Magala, P., R.E. Klevit, W.E. Thomas, E.V. Sokurenko and **R.E. Stenkamp**. 2020. DOI 10.1002/prot.25840, PMID:31622514, PMCID:PMC7058522.

d. Kisiela, D.I., Magala, P., Interlandi, G., Carlucci, L.A., Ramos A., Tchesnokova, V., Basanta, B., Yarov-Yarovoy, V., Avagyan, H., Hovhannisyan, A., Thomas, W.E., **Stenkamp, R.E**., Klevit, R.E., and Sokurenko, E.V.. 2021 Toggle switch residues control allosteric transitions in bacterial adhesins by participating in a concerted repacking of the protein core, PLOS pathology, 17(4), DOI 10.1371/journal.ppat.1009440.

Complete List of Published Work in MyBibliography:
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BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Veesler, David

eRA COMMONS USER NAME (credential, e.g., agency login): dveesler

POSITION TITLE: Associate Professor of Biochemistry

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Aix-Marseille University (France)	M.Sc.	06/2006	X-ray crystallography
Aix-Marseille University (France)	Ph.D	06/2011	X-ray crystallography
The Scripps Research Institute (CA, USA)	Postdoctoral	2011-2014	Cryo-electron microscopy

A. Personal Statement

The long-term goal of my research program is to contribute to eradicating infectious diseases using a multi-disciplinary approach involving structural biology, immunology and protein engineering to obtain multi-scale data ranging from atom to whole cell. My lab is focusing on understanding the structure and function of macromolecular complexes involved in viral/bacterial pathogenesis and immunity to provide avenues for creating next-generation vaccines and therapeutics.

One of the main lines of research in my lab is to elucidate how pathogens gain access to host cells with a special emphasis on the roles of glycoproteins decorating the surface of enveloped viruses. I have been at the forefront of this field by developing innovative and integrative methods to study viral glycoproteins (in particular coronavirus spike glycoproteins). More specifically, I am studying (i) the mechanisms of membrane fusion between enveloped viruses and target cells, and (ii) the humoral immune response elicited by viruses upon infection or vaccination of humans and animals. We use this information to guide the design of vaccines and inhibitors to combat emerging, re-emerging and endemic viruses.

Ongoing and recently completed projects that I would like to highlight include:

R01GM120553
Veesler (PI)
9/1/16-8/31/21
Structural Studies of Coronavirus Fusion Proteins

DP1AI158186
Veesler (PI)
9/30/20-7/31/25
Unraveling the bat humoral immune response against zoonotic viruses to guide the design of next-generation therapeutics

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2021– Present Investigator, Howard Hughes Medical Institute
2020– Present Associate Professor, Department of Biochemistry, University of Washington, Seattle, WA
2020– Present Consultant, Vir Biotechnology, San Francisco, CA
2015 – 2020 Assistant Professor, Department of Biochemistry, University of Washington, Seattle, WA

Honors
2021: Investigator, Howard Hughes Medical Institute
2020: NIH Director's Pioneer Award
2020: Amgen Young Investigator Award
2018: Burroughs Wellcome Fund Investigator in the Pathogenesis of Infectious Diseases
2017: Pew Scholar in Biomedical Sciences
2017: Young Investigator Outstanding Recognition – FEI (now Thermo-Fisher Scientific)
2013: Microscopy & Microanalysis Presidential Scholar Award - Microscopy Society of America
2012: Fall Research Symposium Award - The Scripps Research Institute
2011: Marie-Curie International Outgoing Postdoctoral Fellowship (IOF-FP7)
2011: Thesis Award - French Crystallographic Association
2009: Application Note Award for Outstanding Achievement in Light Scattering - Wyatt Technology Corporation
2008: ProteomeBinders Fellowship
2006: Ph.D. Fellowship - French Ministry of Higher Education and Research

C. Contributions to Science

1. Coronaviruses are zoonotic viruses responsible for mild respiratory tract infections and fatal pneumonia in humans worldwide. The spike glycoprotein is responsible for coronavirus entry into target cells and is the main target of neutralizing antibodies upon infection. My lab obtained the first atomic-level description of a coronavirus spike glycoprotein in the prefusion and postfusion conformations using cryoEM providing snapshots of the start and end points of the membrane fusion reaction. We pioneered the integration of cryoEM and cutting-edge glycoproteomics to characterize coronavirus spike glycoproteins and revealed the presence of a composite glycan shield, comprising up to one hundred N-linked oligosaccharides, obstructing the surface of these glycoproteins. Our work suggested coronaviruses use molecular trickery, based on epitope masking with glycans and activating conformational changes, to evade the immune system of infected hosts, in a manner similar to that described for HIV-1.
   a. Walls AC, Tortorici MA, Bosch BJ, Frenz B, Rottier PJM, DiMaio F, Rey FA* and **Veesler D***. Cryo-electron microscopy structure of a coronavirus spike glycoprotein trimer. *Nature.* (2016). 531:114-117. [PMC5018210] *Co-correspondence
   a. Walls AC, Tortorici MA, Frenz B, Snijder J, Rey FA, DiMaio F, Bosch BJ and **Veesler D**. Glycan shield and epitope masking of a coronavirus spike protein observed by cryo-electron microscopy. *Nat Struct Mol Biol.* (2016) 23(10):899-905. PMID: 27617430 [PMC5515730]
   b. Walls AC, Tortorici MA, Snijder J, Xiong X, Bosch BJ, Rey FA and **Veesler D**. Tectonic conformational changes of a coronavirus spike glycoprotein promote membrane fusion. *Proc Natl Acad Sci. (2017) 114(42):11157-62.* [PMC5651768]
   c. Xiong X, Tortorici MA, Snijder J, Yoshioka C, Walls AC, Li W, McGuire AT, Rey FA, Bosch BJ and **Veesler D**. Glycan shield and fusion activation of a deltacoronavirus spike glycoprotein fine-tuned for enteric infections. J Virol (2018) 92(4):e01628-17. [PMCID 5790929]

2. Coronavirus attachment to host receptors is a key determinant of tropism and participate in modulating zoonotic transmissions. To understand attachment of coronaviruses to sialic acids at the surface of host cells, we determined high-resolution structures of the MERS-CoV and HCoV-OC43 spike glycoproteins in complex with sialoside receptors. This work identified the (sialoside) receptor-binding sites, which we functionally validated using site-directed mutagenesis and pseudovirus entry assays, thereby providing a structural framework for blocking viral entry through targeting these sites of vulnerability. A few weeks after the release of the SARS-CoV-2 genome sequence, my lab identified angiotensin-converting enzyme 2 (ACE2) as a *bona fide* receptor for SARS-CoV-2 entry into target cells, and demonstrated that SARS-CoV-2

interacts at least as well as SARS-CoV with human ACE2, in line with the high-transmissibility of the newly emerged coronavirus. We determined cryoEM structures of the SARS-CoV-2 spikes in two distinct, functionally relevant conformations, providing a blueprint that has been used by thousands of research groups worldwide for mapping emerging mutations as well as designing vaccines and inhibitors of viral entry. We most recently showed that membrane lectins (DC-SIGN, L-SIGN and SIGLEC1) participate in ACE2-mediated SARS-CoV-2 entry into host cells by acting as attachment receptors modulating viral neutralization of several classes of antibodies. Finally, we discovered an unprecedented example of antibody-mediated receptor functional mimicry for SARS-CoV, which has since been observed many times for SARS-CoV-2 and elucidated the unique nature of coronavirus membrane fusion activation and host cell invasion.

a. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT and **Veesler D**. Structure, function and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell (2020) 181(2):281-292. [PMC7102599]
b. Walls AC, Xiong X, Park YJ, Tortorici MA, Snijder J, Quispe J, Cameroni E, Gopal R, Dai M, Lanzavecchia A, Zambon M, Rey FA, Corti D and **Veesler D**. Unexpected receptor-functional mimicry elucidates activation of coronavirus fusion. Cell (2019) 176(5):1026-1039. [PMCID: 6751136]
c. C, Li Z, Koerhuis D, Boons GJ, Bosch BJ, Rey FA, DeGroot RJ and **Veesler D**. Structural basis for human coronavirus attachment to sialic acid receptors. Nat Struct Mol Biol. (2019) 26(6):481-489. [PMC6554059]
d. Lempp FA, Soriaga L, Montiel-Ruiz M, Benigni Fabio, Noack J, Park YJ, Bianchi S, Walls AC, Bowen JE, Zhou J, Kaiser H, Agostini M, Meury M, Dellota Jr E, Jaconi S, Cameroni E, Virgin HW, Lanzavecchia A, **Veesler D**, Purcell L, Telenti A, Corti D. Membrane lectins enhance SARS-CoV-2 infection and influence the neutralizing activity of different classes of antibodies. (2021) Nature.

3. To understand the immune response in COVID-19 patients, we worked collaboratively with Davide Corti's group at Vir Biotech to identify a site of vulnerability targeted by all SARS-CoV-2 N-terminal domain (NTD) neutralizing antibodies and showed they are potent neutralizers *in vitro* and protect hamsters against SARS-CoV-2 challenge *in vivo*. We demonstrated that NTD antibodies represent a key part of the immune response against SARS-CoV-2 and exert a selective pressure participating in the emergence of so-called variants of concern harboring NTD mutations/deletions enabling neutralization escape. We carried out high-throughput structural and serological analysis to delineate an RBD antigenic map and reveal that the viral spike receptor-binding domain (RBD) is immunodominant and accounts for 90% of plasma neutralizing activity. We isolated and characterized two RBD-specific, broadly neutralizing sarbecovirus monoclonal antibodies, designated S309 and S2X259, isolated from SARS-CoV and SARS-CoV-2-infected individuals and showed that they protect hamsters against SARS-CoV-2 challenge. A derivative of S309 (VIR-7831) received an emergency use authorization from the FDA and is used in the clinic worldwide.
   a. McCallum M, Marco A, Lempp F, Tortorici MA, Pinto D, Walls AC, Beltramello M, Chen A, Liu Z, Zatta F, Zepeda S, di Iulio J, Bowen JE, Montiel-Ruiz M, Zhou J, Rosen LE, Bianchi S, Guarino B, Fregni CS, Abdelnabi R, Caroline Foo SY, Rothlauf PW, Bloyet LM, Benigni F, Cameroni E, Neyts J, Riva A, Snell G, Telenti A, Whelan SPJ, Virgin HW, Corti D*, Pizzuto MS*, **Veesler D*.** N-terminal domain antigenic mapping reveals a site of vulnerability for SARS-CoV-2. Cell (2021) 184(9):2332-2347. [PMC7962585] *Co-correspondence
   b. Pinto D, Park YJ, Beltramello M, Walls AC, Tortorici MA, Bianchi S, Jaconi S, Culap K, Zatta F, De Marco A, Peter A, Guarino B, Spreafico R, Cameroni E, Case JB, Chen RE, Havenar-Daughton C, Snell G, Telenti A, Virgin HW, Lanzavecchia A, Diamond MS, Fink K, **Veesler D***, Corti D*. Cross-neutralization of SARS-CoV-2 by a Human Monoclonal SARS-CoV Antibody. Nature (2020) 583(7815)290-295. *Co-correspondence
   c. Piccoli L, Park YJ, Tortorici MA, Czudnochowski N, Walls AC, Beltramello M, Silacci-Fregni C, Pinto D, Rosen LE, Bowen JE, Acton OJ, Jaconi S, Guarino B, Minola A, Zatta F, Sprugasci N, Bassi J, Peter A, De Marco A, Nix JC, Mele F, Jovic S, Rodriguez BF, Gupta SV, Jin F, Piumatti G, Lo Presti G, Pellanda AF, Biggiogero M, Tarkowski M, Pizzuto MS, Cameroni E, Havenar-Daughton C, Smithey M, Hong D, Lepori V, Albanese E, Ceschi A, Bernasconi E, Elzi L, Ferrari P, Garzoni C, Riva A, Snell G, Sallusto F, Fink K, Virgin HW, Lanzavecchia A, Corti D*, **Veesler D***. Mapping Neutralizing and Immunodominant Sites on the SARS-CoV-2 Spike Receptor-Binding Domain by Structure-Guided High-Resolution Serology. Cell (2020) 183(4):1024-1042. *Co-correspondence
   d. Tortorici MA, Czudnochowski N, Starr TN, Marzi R, Walls AC, Zatta F, Bowen JE, Jaconi S, Iulio JD, Wang Z, De Marco A, Zepeda SK, Pinto D, Liu Z, Beltramello M, Bartha I, Housley MP, Lempp FA,

Rosen LE, Dellota E, Kaiser H, Montiel-Ruiz M, Zhou J, Addetia A, Guarino B, Culap K, Sprugasci N, Saliba C, Vetti E, Giacchetto-Sasselli I, Fregni CS, Abdelnabi R, Foo SC, Havenar-Daughton C, Schmid MA, Benigni F, Cameroni E, Neyts J, Telenti A, Snell G, Virgin HW, Whelan SPJ, Bloom JD, Corti D*, **Veesler D***, Pizzuto MS*. Structural basis for broad sarbecovirus neutralization by a human monoclonal antibody. (2021) Nature. *Co-correspondence

4. Based on our antibody discovery effort, we designed a subunit vaccine multivalently displaying the SARS-CoV-2 RBD in a highly immunogenic array using a self-assembling protein nanoparticle (developed by Neil King's group) to focus antibody responses on this key domain of vulnerability. The vaccine elicits 10-fold higher neutralizing antibody titers than the '2P' prefusion-stabilized spike despite a 5-fold lower dose in mice and efficiently activates germinal center B cells and T follicular helper cells in in mice and NHPs. Antibodies elicited by the RBD nanoparticle vaccine target multiple distinct epitopes, are resilient to a broad spectrum of variants, and protect mice and non-human primates from SARS-CoV-2 challenge using four different clinical adjuvants. This vaccine is currently being evaluated in phase III clinical trials. We designed mosaic and cocktail sarbecovirus multivalent RBD-nanoparticle vaccine candidates eliciting broad sarbecovirus neutralizing activity and protecting mice against SARS-CoV MA15 challenge even in the absence of the SARS-CoV RBD in the vaccine. This study provided proof-of-principle that sarbecovirus RBD-nanoparticle induce protective immunity against heterotypic challenge and will enable advancement of broadly protective sarbecovirus vaccines to the clinic.
    a. Walls AC, Fiala B, Schäfer A, Wrenn S, Pham MN, Murphy M, Tse LV, Shehata L, O'Connor MA, Chen C, Navarro MJ, Miranda MC, Pettie D, Ravichandran R, Kraft JC, Ogohara C, Palser A, Chalk S, Lee EC, Kepl E, Chow CM, Sydeman C, Hodge EA, Brown B, Fuller JT, Dinnon KH, Gralinski LE, Leist SR, Gully KL, Lewis TB, Guttman M, Chu HY, Lee KK, Fuller DH, Baric RS, Kellam P, Carter L, Pepper M, Sheahan TP, **Veesler D***, King NP*. Elicitation of potent neutralizing antibody responses by designed protein nanoparticle vaccines for SARS-CoV-2. Cell (2020) 183(5):1367-1382. [PMC7604136] *Co-correspondence
    b. S Arunachalam P, Walls AC, Golden N, Atyeo C, Fischinger S, Li C, Aye P, Navarro MJ, Lai L, Edara VV, Roltgen K, Rogers K, Shirreff L, Ferrell DE, Wrenn S, Pettie D, Kraft JC, Miranda MC, Kepl E, Sydeman C, Brunette N, Murphy M, Fiala B, Carter L, White AG, Trisal M, Hsieh CL, Russell-Lodrigue K, Monjure C, Dufour J, Doyle-Meyer L, Bohm RB, Maness NJ, Roy C, Plante JA, Plante KS, Zhu A, Gorman MJ, Shin S, Shen X, Fontenot J, Gupta S, O Hagan DT, Most RV, Rappuoli R, Coffman RL, Novack D, McLellan JS, Subramaniam S, Montefiori D, Boyd SD, Flynn JL, Alter G, Villinger F, Kleanthous H, Rappaport J, Suthar M, King NP, **Veesler D**, Pulendran B. Adjuvanting a subunit SARS-CoV-2 nanoparticle vaccine to induce protective immunity in non-human primates. Nature (2021)
    c. Walls AC, Miranda MC, Pham MN, Schäfer A, Greaney A, Arunachalam PS, Navarro MJ, Tortorici MA, Rogers K, O'Connor MA, Shireff S, Ferrell DE, Brunette N, Kepl E, Bowen JE, Zepeda SK, Starr T, Hsieh CL, Fiala B, Wrenn S, Pettie D, Sydeman C, Johnson M, Blackstone A, Ravichandran R, Ogohara C, Carter L, Tilles SW, Rappuoli R, O'Hagan DT, Van Der Most R, Van Voorhis WC, McLellan JS, Kleanthous H, Sheahan TP, Fuller DH, Villinger F, Bloom J, Pulendran B, Baric R, King NP* and **Veesler D*.** Elicitation of broadly protective sarbecovirus immunity by receptor-binding domain nanoparticle vaccines. (2021) Cell. *Co-correspondence

5. Nipah virus (NiV) and Hendra virus (HeV) are zoonotic henipaviruses responsible for recurrent outbreaks of encephalitis and respiratory illness with fatality rates of 50-100%. No vaccines or licensed therapeutics currently exist to protect humans against NiV or HeV. Henipaviruses enter host cells by fusing the viral and cellular membranes via the concerted action of the attachment (G) and fusion (F) glycoproteins, the main targets of the humoral immune response upon infection. In collaboration with Chris Broder's lab at Uniform Services University, we isolated, cloned and humanized a panel of F-specific henipavirus monoclonal antibody and showed they cross-neutralized NiV and HeV under BSL-4 containment. CryoEM analysis along with triggering and fusion assays demonstrated the antibodies bind to distinct, prefusion-specific quaternary antigenic sites, conserved in NiV F and HeV F glycoproteins, and prevents membrane fusion and viral entry. We further showed that therapeutic administration (up to several days after infection) of F-specific antibodies protect ferrets challenged with lethal doses of NiV or HeV. These data provided proof-of-concept for using

antiviral monoclonal antibodies targeting the henipavirus F glycoprotein as a potential treatment of human NiV and HeV infections.

a. Dang HV, Cross RW, Borisevich V, Bornholdt ZA, West BR, Chan YP, Mire CE, Da Silva SC, Dimitrov AS, Yan L, Amaya M, Navaratnarajah CK, Zeitlin L, Geisbert TW, Broder CC, Veesler D. Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. Nat Struct Mol Biol. (in press).
b. Dang HV, Chan YP, Park YJ, Snijder J, Da Silva SC, Vu B, Yan L, Feng YR, Rockx B, Geisbert TW, Mire CE, Broder CC* and **Veesler D***. An antibody against the F glycoprotein inhibits Nipah and Hendra virus infections. Nat Struct Mol Biol. (2019) 26(10):980-87. [PMCID6858553].
c. Mire CE, Chan YP, Borisevich V, Cross RW, Yan L, Agans KN, Dang HV, **Veesler D**, Fenton KA, Geisbert TW and Broder CC. A cross-reactive humanized monoclonal antibody targeting fusion glycoprotein function protects ferrets against lethal Nipah virus and Hendra virus infection. J Infect Dis. (2020) 221:S471-S479 [PMC7199785]
d. Cheliout Da Silva S, Yan L, Dang HV, Xu K, Epstein JH, **Veesler D**, Broder CC. Functional Analysis of the Fusion and Attachment Glycoproteins of Mojiang Henipavirus. Viruses (2021) 13(3):517.

Complete List of Published Work

http://www.ncbi.nlm.nih.gov/pubmed/?term=veesler+d

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Andrea Elizabeth Wills

eRA COMMONS USER NAME (credential, e.g., agency login): WILLS.ANDREA

POSITION TITLE: Assistant Professor of Biochemistry, University of Washington, Seattle

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Pomona College	BA	05/2002	Biology
University of California, Berkeley	PhD	06/2009	Molecular and Cell Biology
University of California, Berkeley	Postdoctoral		Molecular and Cell Biology
Stanford University	Postdoctoral		Genetics

A. Personal Statement

The research focus of my lab is to understand the molecular mechanisms that enable or limit regenerative healing using vertebrate models. To this end, we employ high-throughput genomics technologies to generate testable hypotheses about the cell biological mechanisms that are being prioritized as cells undergo regeneration. We then leverage emerging cell biological methods, biochemical assays, live and fixed imaging strategies, and classical embryological tools to test these hypotheses. For example, our ATAC-Seq and RNA-Seq analyses (Chang et al., 2017, *Genesis*; Kakebeen et al., 2020, *eLife*; Patel el al., 2022, *Dev Biol.*), integrated with our new data on nutrient mobilization in regeneration (Williams et al., 2021, *Dev Biol*) have steered us to a novel hypothesis and a metabolic model for regeneration. This model represents a new direction for my lab that has grown out of my own long history studying transcriptional regulation of cell fate decisions, now blended with our emerging expertise in metabolism. **Scientific Expertise:** My scientific expertise in the molecular basis of cell fate specification was developed during my graduate work in the lab of Richard Harland, where I articulated new functions for BMP antagonism, Wnt and FGF signaling in gastrulation and neural plate specification (Wills et al., 2006; Wills et al., 2008;, Wills et al., 2010), and contributed to the *Xenopus tropicalis* genome project (Ullsten et al., 2009). As a postdoctoral fellow, I developed genomic strategies and computational pipelines for interrogating transcription and transcription factor occupancy in the early embryo (Yoon, Wills et al., 2010; Wills et al., 2014; Wills et al, 2015). In my own lab, we have turned this expertise in transcriptional regulation and genomics to understanding the molecular and cell biological basis of regeneration, with a particular emphasis on chromatin organization and cell biology of the tadpole tail (Chang et al., 2016; Arbach et al., 2018; Kakebeen et al., 2019; Kakebeen et al., 2020; Kakebeen and Wills 2020; Williams et al., 2021; Patel et al., 2022). **Selected Support:** Work in our lab has been supported by my R01 grant from NINDS focusing on spinal cord regeneration (2017-2022) and R03 from NICHD focusing on chromatin remodeling events in regeneration (2017-2019). Work forming the foundation and preliminary data for much of this proposal was supported by pilot grants from the UW Research Royalties Foundation and the John H. Tietze foundation.

B. Positions and Honors

Post-baccalaureate positions

- Research Assistant 06/02-08/02 Monterey Bay Aquarium Research Institute Supervisor: Steven Haddock
- Post-graduate researcher 10/02-08/03 University of California, Davis (Plant Bio.) Supervisor: Julin Maloof
- Postdoc 06/09-08/09 University of California, Berkeley (Mol. and Cell Bio.) Supervisor: Richard Harland
- Postdoc 09/09-08/15 Stanford School of Medicine (Genetics) Supervisor: Julie Baker
- Assistant Professor 09/15-present University of Washington (Biochemistry)

Academic and Professional Honors

Honors:

- Invited Teaching assistant, MBL Embryology course, Woods Hole, MA (2009-2012)
- Invited lecturer, Cold Spring Harbor *Xenopus* course, 2016 to present
- Invited faculty, MBL Embryology Course, 2020-to present

Awards:

- Ruth Sager Memorial Fellowship, 2007
- NIH NRSA fellowship 1F32 DK089643-01. July 2010-July 2013. Investigating the transcriptional regulation of liver specification in *Xenopus tropicalis.*
- Stanford Pediatric Research Fund Fellowship, 2010*
- Stanford Dean's Fellowship, 2010*
- John Gurdon Prize, Xenopus Meeting, 2014
- Hilde Mangold Award, 2014
- Katherine McCormick Advanced Postdoctoral Fellowship, 2014-2015
- John H. Tietze award (University of Washington), 2021
- International Xenopus Society early career award, 2021

**These awards both had overlap with my NRSA fellowship, and therefore were declined*

Membership in Scholarly Societies:

- AAAS member: 2006-2007
- Society for Developmental Biology member: 2007-present
- Genetics Society of America: 2014-present
- ASCB member, 2016-present

C. Contributions to Science

1. Discovery of transcriptional mechanisms contributing to neural specification and patterning in *Xenopus*. During my graduate work I discovered new mechanisms contributing to the initial specification of the neural plate. I found that in *Xenopus*, Twisted gastrulation (Tsg) acts as a cooperative factor for the BMP antagonist Chordin, serving to enhance Chordin's opposition of BMP signaling. This activity is critical both for the full specification of the neural plate, and for the specification of the anteriormost neural plate tissue. In the absence of Tsg the neural plate is reduced, and the forebrain is lost. A longstanding controversy in neural plate specification is the differential contribution of FGF signaling and BMP antagonism to neural plate specification. I helped to clarify the roles these pathways play by identifying that each pathway contributes to a unique spatial domain within the early *Xenopus* embryo, and that both pathways are required for the full specification of the neural plate.

Selected References:

- Wills A, Harland RM, Khokha MK. 2006. Twisted gastrulation is required for forebrain specification and cooperates with Chordin to inhibit BMP signaling during X. tropicalis gastrulation. *Dev. Biol.* 289(1):166-78. PMID:16321373
- Wills AE1, Choi VM, Bennett MJ, Khokha MK, Harland RM. 2010. BMP antagonists and FGF signaling contribute to different domains of the neural plate in *Xenopus*. 337(2):335-50. *Dev Biol.* PMC2812634

2. Development of genomic analysis methods for *Xenopus*. I have helped to establish multiple genomic resources for the *Xenopus* community, and generated published protocols for multiple types of genomic analysis in this species that are now standard use in the field. I was a contributing author to the *Xenopus tropicalis* genome project, in which my role was to provide photographs of corresponding stages in *X. tropicalis* and X. laevis, and validate predicted EST sequences. During my postdoctoral work, I contributed to the development of RNA-Seq methods in *X. tropicalis*, culminating in the publication of the developmental transcriptome for X. tropicalis. Continuing in this vein, I developed ChIP-Seq methods for *X. tropicalis* and *X. laevis*, detailing both the wetlab work and the computational analysis pipeline. I contributed to new transgenic techniques in this system. I have continued to work to bring new protocols to the community through my development of ATAC-Seq for this system.

Selected References:

- Hellsten U, Harland RM, Gilchrist MJ, Hendrix D, Jurka J, Kapitonov V, Ovcharenko I, Putnam NH, Shu S, Taher L, Blitz IL, Blumberg B, Dichmann DS, Dubchak I, Amaya E, Detter JC, Fletcher R, Gerhard DS, Goodstein D, Graves T, Grigoriev IV, Grimwood J, Kawashima T, Lindquist E, Lucas SM, Mead PE, Mitros T, Ogino H, Ohta Y, Poliakov AV, Pollet N, Robert J, Salamov A, Sater AK, Schmutz J, Terry A, Vize PD, Warren WC, Wells D, Wills A, Wilson RK, Zimmerman LB, Zorn AM, Grainger R, Grammer T, Khokha MK, Richardson PM, Rokhsar DS. 2010. The genome of the Western clawed frog *Xenopus tropicalis. Science.* 2010. 328(5978):633-6. PMC2994648
- Tan MH, Au KF, Yablonovitch AL, Wills AE, Chuang J, Baker JC, Wong WH, Li JB. 2013. RNA sequencing reveals a diverse and dynamic repertoire of the *Xenopus tropicalis* transcriptome over development. *Genome Res*. 23(1):201-16. PMC3530680
- Loots GG1, Bergmann A, Hum NR,Oldenburg CE, Wills AE, Hu N, Ovcharenko I, Harland RM. 2013. Interrogating transcriptional regulatory sequences in Tol2-mediated *Xenopus* transgenics. *PLoS One*. 8(7):e68548. PMC3713029
- Wills AE, Gupta R, Chuong E, Baker JC. 2014. Chromatin immunoprecipitation and deep sequencing in Xenopus tropicalis and *Xenopus laevis*. Methods. 2014. 66(3):410-21. PMC4084882

3. Discovery of transcription factor/chromatin interactions driving cell fate specification. I have helped to define how transcription factors interact with chromatin modifications to allow cell fate specification, a difficult problem to tackle in mammalian development, but tractable in externally-developing *Xenopus*. Using a combination of ChIP-Seq and functional analyses, my colleagues and I interrogated interactions between Smad2/3, FoxH1, and chromatin domains during embryonic cell fate specification in embryos and human ES cells. We found a unique bivalent chromatin signature associated with endoderm specification. Continuing this work, I interrogated Smad2/3 interactions with chromatin during the initial specification of germ layers in *X. tropicalis*. I used ChIP-Seq to show that enhancer marks are established at chromatin prior to stable Smad2/3 association, and that Smad2/3 binds chromatin at regions that are already marked as enhancers. This established a hierarchy of gene regulatory events in early embryogenesis. Using a combination of genomic and functional analyses, I demonstrated that the E protein class of bHLH factors act as critical cofactors for Nodal signaling in embryos, co-occupying their genomic targets. I went on to show that this activity is critical for early embryonic development, and that in the absence of E2a, Smad2/3 is unable to activate transcription of critical early developmental genes.

Selected References:

- Kim SW, Yoon SJ, Chuong E, Oyolu C, Wills AE, Gupta R, Baker J. 2011. Chromatin and transcriptional signatures for Nodal signaling during endoderm formation in hESCs. Dev Biol. 357(2):492-504. PMID: 21741376
- Gupta R, Wills A, Ucar D, Baker J. 2014. Developmental enhancers are marked independently of zygotic Nodal signals in Xenopus.395 (1):38-49. PMC4517478
- Yoon SJ*, Wills AE*, Chuong E, Gupta R, Baker JC. 2011. HEB and E2A function as SMAD/FOXH1 cofactors. *Genes Dev.* 25(15):1654-61. PMC3182016. *co-first author
- Wills AE, Baker JC. 2015. E2a is necessary for Smad2/3-dependent transcription and the direct repression of lefty during gastrulation. 32(3):345-57. PMC4510980

4. Developing tools and resources for studying cell fate specification, growth and metabolism in *Xenopus* regeneration. In my lab at UW, we have focused on elucidating the cell biological and molecular mechanisms that enable complex tissue regeneration, using *Xenopus* as our model. To facilitate these studies we have developed transcriptomic, cell biological, genetic and biochemical tools for this system. These have

included flow cytometric strategies for isolating cells from an auto fluorescent or background, RNA-Seq and ATAC-Seq assays of bulk and cell type-specific samples, inducible loss of function strategies, lineage-restricted CRISPR, and imaging approaches. Among other observations, several of these strategies were applied to discover that the epidermal cells of the *Xenopus* tail fin have elaborately branched nuclei.

Selected References:

- Arbach HE, Harland-Dunaway M, Chang JK, Wills AE. 2018. Extreme nuclear branching in healthy epidermal cells of the *Xenopus* tail fin. *J. Cell Sci* 131 (18). PMC6176923
- Kakebeen AD, Chitsazan AD, Wills AE. Tissue disaggregation and isolation of specific cell types from transgenic Xenopus appendages for transcriptional analysis by FACS. *Dev. Dyn* 2021 PMC8088453.

5. Defining the mechanisms of cell fate specification, growth and patterning in *Xenopus* tail regeneration. We have applied the strategies listed above to define the transcriptional dynamics of regeneration. Initially we used bulk sequencing of whole tail tissue, and more recent work currently in revision examines single-cell transcriptomics in sorted cell populations. We have found that regenerating progenitor cells segregate their functions, placing an early emphasis on differentiation. We have also used ATAC-Seq to examine chromatin accessibility dynamics as regeneration progresses in bulk tissue and in isolated neural progenitors, some of which is currently under review. We identified identified several dozen candidate transcription factors including HIF1a and FoxO1 whose binding sites have dynamic accessibility that predict changes in gene expression, leading to the discovery that HIF1a is required for Wnt target gene expression in development and regeneration. An additional study identified a new role for nutritional stores and metabolic constraints underlying the regeneration refractory period.

Selected References:

- Chang, J, Baker, J, Wills A. 2017. Transcriptional dynamics of tail regeneration in *Xenopus tropicalis*. *Genesis* 55 (1-2). PMID 28095651
- Kakebeen AD, Chitsazan AD, Williams MC, Saunders LM, Wills AE. 2020. Chromatin accessibility dynamics and single cell RNA-Seq reveal new regulators of regeneration in neural progenitors. *Elife.* Apr 27;9:e52648. PMC7250574
- Williams MC, Patel JH, Kakebeen AD, Wills AE. 2021. Nutrient availability contributes to a graded refractory period for regeneration in *Xenopus tropicalis*. *Dev. Biol.* 473 59-70 PMC8061425
- Patel JH, Schattinger P, Takayoshi E, Wills AE. Hif1α and Wnt are required for posterior gene expression during *Xenopus tropicalis* tail regeneration. In press, *Dev. Biol.*

Complete Bibliography in MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/andrea.wills.1/bibliography/public/

OMB No. 0925-0001 and 0925-0002 (Rev. 10/2021 Approved Through 09/30/2024)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: AITCHISON, JOHN

eRA COMMONS USER NAME (credential, e.g., agency login): JOHN_AITCHISON

POSITION TITLE: Co-Director and Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
McMaster University, Hamilton, Ontario	BS	04/1986	Biochemistry
McMaster University, Hamilton, Ontario	PHD	04/1992	Biochemistry
Rockefeller University, New York, New York	Post-Doctoral	05/1997	Cell Biology

A. Personal Statement

My laboratory is focused on developing and applying systems approaches to reveal systems levels insights into cell biology, host-pathogen interactions, and infectious disease. We initially began our research in model systems and now apply the resulting advances to infectious disease. This has allowed us to develop and build on high throughput technologies and computational biology (including proteomics, transcriptomics, genomics, phenomics, and integrative modeling of structures and networks) to establish a systems cell biology foundation for application to infectious disease research. Large scale systems-based data are generated and integrated using network-based and machine learning approaches in the context of host-pathogen interactions, and immune responses associated with infections from viruses such as SARS-CoV-2 and dengue, protozoans causing malaria, and bacteria such as M. tuberculosis.

a. Mast FD, Navare AT, van der Sloot AM, Coulombe-Huntington J, Rout MP, Baliga NS, Kaushansky A, Chait BT, Aderem A, Rice CM, Sali A, Tyers M, Aitchison JD*. Crippling life support for SARS-CoV-2 and other viruses through synthetic lethality. J Cell Biol. 2020 Oct 5;219. PubMed Central PMCID: PMC7659715
b. Neal ML, Wei L, Peterson E, Arrieta-Ortiz ML, Danziger SA, Baliga NS, Kaushansky A, Aitchison JD*. A systems-level gene regulatory network model for Plasmodium falciparum. Nucleic Acids Res. 2021 May 21;49(9):4891-4906. PubMed PMID: 33450011
c. Mast FD, Fridy PC, Ketaren NE, Wang J, Jacobs EY, Olivier JP, Sanyal T, Molloy KR, Schmidt F, Rutkowska M, Weisblum Y, Rich LM, Vanderwall ER, Dambrauskas N, Vigdorovich V, Keegan S, Jiler JB, Stein ME, Olinares PDB, Hatziioannou T, Sather DN, Debley JS, Fenyö D, Sali A, Bieniasz PD, Aitchison JD*, Chait BT*, Rout MP*. Highly synergistic combinations of nanobodies that target SARS-CoV-2 and are resistant to escape. Elife. 2021 Dec 7;10:e73027. doi: 10.7554/eLife.73027. PubMed Central PMCID: PMC8651292
d. Neal ML, Duffy FJ, Du Y, Aitchison JD*, Stuart KD*. Preimmunization correlates of protection shared across malaria vaccine trials in adults. NPJ Vaccines. 2022 Jan 14;7(1):5. doi: 10.1038/s41541-021-00425-1. PubMed Central PMCID: PMC8760258

*corresponding authors

B. Positions, Scientific Appointments, and Honors

<u>Positions and Employment</u>

2020 – present Professor, Dept. of Pediatrics, School of Medicine, University of Washington
2018 – present Co-Director and Principal Investigator, Seattle Children's Research Institute, Seattle, WA

2018 – present President Emeritus, Seattle Biomedical Research Institute, dba Center for Infectious Disease Research, Seattle, WA
2017 – present Affiliate Professor, Institute for Systems Biology, Seattle, WA
2003 – present Affiliate/Adjunct Professor, Dept of Biochemistry, Faculty of Medicine, University of Washington, Seattle, WA
2002 – present Adjunct Professor, Dept of Biochemistry and Molecular Biology, Faculty of Medicine, University of British Columbia, Vancouver, BC
2002 – present Adjunct Professor, Dept of Cell Biology, Faculty of Medicine, University of Alberta, Canada
2017 - 2018 President and Director, Seattle Biomedical Research Institute, dba Center for Infectious Disease Research, Seattle, WA
2011 - 2018 Professor, Seattle Biomedical Research Institute, dba Center for Infectious Disease Research, Seattle, WA
2015 - 2017 Chief Science Officer, Seattle Biomedical Research Institute, dba Center for Infectious Disease Research, Seattle, WA
2005 - 2017 Professor, Institute for Systems Biology, Seattle, WA
2013 - 2015 Scientific Director, Seattle Biomedical Research Institute, dba Center for Infectious Disease Research, Seattle, WA
2011 - 2013 Director of Integrative Biology, Seattle Biomedical Research Institute, dba Center for Infectious Disease Research, Seattle, WA
2011 - 2013 Senior Vice President, Executive Director of Integrative Biology, Institute for Systems Biology, Seattle, WA
2006 - 2011 Associate Director, Institute for Systems Biology, Seattle, WA
2000 - 2005 Associate Professor, Institute for Systems Biology, Seattle, WA
1997 - 2000 Assistant Professor, Dept of Cell Biology, Faculty of Medicine, University of Alberta, Canada
1995 - 1997 Associate, Howard Hughes Medical Institute, Lab of Cell Biology, The Rockefeller University, New York

Other Experience and Professional Memberships

2021 – present Scientific Advisory Board, Smart Health Initiative (SHI) at King Abdullah University of Science and Technology (KAUST)
2019 – present Board Member, Washington Global Health Association
2018 – present Member, Scientific Advisory Board, P01, Notre Dame, South Bend, IN
2014 – present Scientific Advisor and Founder, PreCyte Inc., Seattle, WA
2012 – present Scientific Advisory Board Chair, Institut de Recherches Cliniques de Montreal (IRCM)
2012 – present Editorial Board Member, In Silico Biology, Institute for Systems Biology
2010 – present Editorial Board Member, BMC Biology
2009 – present Editorial Board Member, Journal of Cell Biology (JCB)
2003 – present Ad hoc service on several NIH panels: Special Emphasis Panel for the Centers of Excellence in Complex Biomedical Systems Research; NIGMS Program Projects; NIGMS Special Emphasis Panel, Modeling and Biological Systems; Center for Scientific Review (CSR) Cell Biology Integrated Review Group; NIGMS EUREKA Scientific Review; New Innovator Award (DP2); Center for Scientific Review's pilot study to evaluate the relative quality of grant applications; Genomics, Computational Biology and Technology (GCAT); Research Centers for Cancer Systems Biology Consortium Research Centers (U54); Function and Dynamics

	Study Section (NCSD); Special Emphasis Panel, Bioengineering Sciences and Technologies IRG (BST).
2012 - 2019	Gairdner Medical Review Panel Member, Gairdner Foundation
2009 - 2019	College of CSR Reviewers, NIH
2016	Panel Member, US Army Medical Research and Material Command State of the Science Meeting: Systems Biology of Drug-Resistant Infectious Diseases
2016	Reviewer, FWF Der Wissenschaftsfonds, Germany
2012 - 2015	Collaborative Research & Innovation Opportunities (CRIO), Review Panel Member, Alberta Innovates Health Solutions (AIHS)
2011	Review Panel Member, Swiss National Science Foundation
2009	Systems Biology Working Group Member, The Global HIV Vaccine Enterprise, Global Vaccine Initiative

Honors

1998	Canadian Foundation for Innovation Researcher, Canadian Foundation for Innovation
1997	Scholarship (declined September 2000), Alberta Heritage Foundation for Medical Research
1997	Scholarship (declined September 2000), Medical Research Council of Canada
1995	Fellowship, Howard Hughes Medical Institute Research Associate
1992	Post-Doctoral Fellowship, Medical Research Council of Canada
1992	Post-Doctoral Fellowship (declined), Natural Sciences Engineering Research Council of Canada
1991	Department of Biochemistry Scholarship, McMasters University
1988	Postgraduate Studentship, Natural Sciences and Engineering Research Council of Canada

C. Contributions to Science

1. Systems Cell Biology - Organelle Biogenesis

As a founding faculty member of the Institute for Systems Biology we sought to develop systems cell biology approaches as part of the institute's larger goal of pioneering systems biology in medical research through multidisciplinary research, technology development, 'omics, and computational biology. We chose yeast peroxisomes as an important and amenable model system because they are ubiquitous, poorly understood and are highly controllable by shifts in carbon sources. We approached our studies on biogenesis of peroxisomes as an integrated program from signaling through transcription, translation, and organelle biogenesis per se as a systems level understanding of biogenesis is important for rational intervention and control of peroxisomes in synthetic biology and medicine. This integrated approach continues and remains unique to the field. We developed numerous technologies, analysis tools and systems approach, novel at the time, including quantitative (phospho) proteomics, high-throughput imaging, global transcriptional network inference, dynamic deterministic, stochastic modeling of transcriptional networks and machine learning. These approaches enabled us to discover and characterize numerous mechanisms controlling peroxisome biogenesis. Discoveries we made are fundamental to the field and to potential treatment of peroxisome biogenesis disorders.

a. Smith JJ, Marelli M, Christmas RH, Vizeacoumar FJ, Dilworth DJ, Ideker T, Galitski T, Dimitrov K, Rachubinski RA, Aitchison JD. Transcriptome profiling to identify genes involved in peroxisome assembly and function. J Cell Biol. 2002 Jul 22;158(2):259-71. PubMed Central PMCID: PMC2173120.
b. Marelli M, Smith JJ, Jung S, Yi E, Nesvizhskii AI, Christmas RH, Saleem RA, Tam YY, Fagarasanu A, Goodlett DR, Aebersold R, Rachubinski RA, Aitchison JD. Quantitative mass spectrometry reveals a role for the GTPase Rho1p in actin organization on the peroxisome membrane. J Cell Biol. 2004 Dec 20;167(6):1099-112. PubMed Central PMCID: PMC2172632.
c. Saleem RA, Rogers RS, Ratushny AV, Dilworth DJ, Shannon PT, Shteynberg D, Wan Y, Moritz RL, Nesvizhskii AI, Rachubinski RA, Aitchison JD. Integrated phosphoproteomics analysis of a signaling network governing nutrient response and peroxisome induction. Mol Cell Proteomics. 2010 Sep;9(9):2076-88. PubMed Central PMCID: PMC2938103.
d. Mast FD, Herricks T, Strehler KM, Miller LR, Saleem RA, Rachubinski RA, Aitchison JD. ESCRT-III is required for scissioning new peroxisomes from the endoplasmic reticulum. J Cell Biol. 2018 Jun 4;217(6):2087-2102. doi: 10.1083/jcb.201706044. Epub 2018 Mar 27. PubMed Central PMCID: PMC5987711.

2. Nuclear organization and transport

I began research on nuclear transport and the nuclear pore complex as a post-doc in the lab of Nobel Laureate Guenter Blobel where, through early proteomics and reverse genetics approaches we discovered and characterized novel nuclear pore complex (NPC) proteins (nucleoporins) and the family of transport factors (called karyopherins, aka importins) responsible for transporting proteins into the nucleus. Our original discovery of two novel karyopherins, along with others at time, catalyzed the characterization of this family and their roles in transport into and out of the nucleus. We used comprehensive quantitative proteomics, reverse genetics and yeast cell biology approaches with Brian Chait and Mike Rout to complete the inventory and the NPC, develop an architectural map of the relative position of each nucleoporin, and based on these data, proposed a novel virtual gating model for transport through the NPC. Although the precise mechanisms remain controversial and under intensive investigation, the fundamental concepts underlying the virtual gating model remain generally accepted by the field. This structure/function map of the NPC continues to evolve; larger collaborations, data integration and integrated modeling have led to a sub-nanometer precision structure of a yeast NPC. We continue studies on the NPC structure and function.

a. Aitchison JD, Blobel G, Rout MP. Kap104p: a karyopherin involved in the nuclear transport of messenger RNA binding proteins. Science. 1996 Oct 25;274(5287):624-7. PubMed PMID: 8849456.
b. Rout MP, Aitchison JD, Suprapto A, Hjertaas K, Zhao Y, Chait BT. The yeast nuclear pore complex: composition, architecture, and transport mechanism. J Cell Biol. 2000 Feb 21;148(4):635-51. PubMed Central PMCID: PMC2169373.
c. Aitchison JD, Rout MP. The yeast nuclear pore complex and transport through it. Genetics. 2012 Mar;190(3):855-83. PubMed Central PMCID: PMC3296253
d. Kim SJ, Fernandez-Martinez J, Nudelman I, Shi Y, Zhang W, Raveh B, Herricks T, Slaughter BD, Hogan JA, Upla P, Chemmama IE, Pellarin R, Echeverria I, Shivaraju M, Chaudhury AS, Wang J, Williams R, Unruh JR, Greenberg CH, Jacobs EY, Yu Z, de la Cruz MJ, Mironska R, Stokes DL, Aitchison JD, Jarrold MF, Gerton JL, Ludtke SJ, Akey CW, Chait BT, Sali A, Rout MP. Integrative structure and functional anatomy of a nuclear pore complex. Nature. 2018 Mar 22;555(7697):475-482. doi: 10.1038/nature26003. Epub 2018 Mar 14. PubMed Central PMCID: PMC6022767.

3. NPC and control of gene expression

Our studies on the nuclear pore complex and individual nucleoporins revealed the first yeast nucleoporin, Nup2, that dynamically associates with the NPC. We further showed that Nup2 is involved in gene expression, specifically gene silencing, epigenetic gene expression and chromatin boundaries by interaction with chromatin-bound proteins and recruitment. These papers contributed to a new way of thinking about the NPC; not as a stable structure, but rather as a dynamic assembly with a multitude of activities and serving as a beacon, or scaffold in the nuclear envelope to localize activities, and confer localized epigenetic control of specialized chromatin regions such as sub telomeric domains. We later showed that Nup170, which is much more stable in the NPC, also that also plays a critical role in localized chromatin structure and gene silencing. The role of the NPC in control of gene expression is now considered fundamental to our understanding of how chromatin organization and the NPC coordinate activities within the nucleus in numerous contexts, development, differentiation and response to external stimuli and immune evasion by protozoans. This remains a very active field of investigation.

a. Dilworth DJ, Suprapto A, Padovan JC, Chait BT, Wozniak RW, Rout MP, Aitchison JD. Nup2p dynamically associates with the distal regions of the yeast nuclear pore complex. J Cell Biol. 2001 Jun 25;153(7):1465-78. PubMed Central PMCID: PMC2150724.
b. Dilworth DJ, Tackett AJ, Rogers RS, Yi EC, Christmas RH, Smith JJ, Siegel AF, Chait BT, Wozniak RW, Aitchison JD. The mobile nucleoporin Nup2p and chromatin-bound Prp20p function in endogenous NPC-mediated transcriptional control. J Cell Biol. 2005 Dec 19;171(6):955-65. PubMed Central PMCID: PMC2171315.
c. DuBois KN, Alsford S, Holden JM, Buisson J, Swiderski M, Bart JM, Ratushny AV, Wan Y, Bastin P, Barry JD, Navarro M, Horn D, Aitchison JD, Rout MP, Field MC. NUP-1 Is a large coiled-coil nucleoskeletal protein in trypanosomes with lamin-like functions. PLoS Biol. 2012;10(3):e1001287. PubMed Central PMCID: PMC3313915
d. Van de Vosse DW, Wan Y, Lapetina DL, Chen WM, Chiang JH, Aitchison JD*, Wozniak RW*. A role for the nucleoporin Nup170p in chromatin structure and gene silencing. Cell. 2013 Feb 28;152(5):969-83. PubMed Central PMCID: PMC3690833.

4. **Systems approaches and infectious disease**

Building on our systems cell biology foundation we applied advances to infectious disease research. This led to fundamental insights into numerous infectious diseases, including viral host-pathogen interactions and the development the novel concept of viral-induced vulnerability for host-based therapeutics. Extending earlier work in yeast technology development, we developed a high throughput imaging approaches to reveal heterogeneity and antibiotic heteroresistance in clinical isolates of *Mycobacterium tuberculosis*, enabling detection of resistance in ~24-30h. We have also used machine learning approaches to identify determinants of severe malaria in children and adults and correlates predictive of active disease and containment in tuberculosis. These same computational approaches are also being used to identify correlates of vaccine efficacy for malaria and HIV.

a. Carpp LN, Rogers RS, Moritz RL, Aitchison JD. Quantitative proteomic analysis of host-virus interactions reveals a role for Golgi brefeldin A resistance factor 1 (GBF1) in dengue infection. Mol Cell Proteomics. 2014 Nov;13(11):2836-54. PubMed Central PMCID: PMC4223476.
b. Herricks T, Donczew M, Mast FD, Rustad T, Morrison R, Sterling TR, Sherman DR, Aitchison JD. ODELAM, rapid sequence-independent detection of drug resistance in isolates of Mycobacterium tuberculosis. Elife. 2020 May 13;9:e56613 PubMed PMID: 32401195; PubMed Central PMCID: PMC7263823.
c. Plumlee CR, Duffy FJ, Gern BH, Delahaye JL, Cohen SB, Stoltzfus CR, Rustad TR, Hansen SG, Axthelm MK, Picker LJ, Aitchison JD, Sherman DR, Ganusov VV, Gerner MY, Zak DE, Urdahl KB Ultra-low Dose Aerosol Infection of Mice with Mycobacterium tuberculosis More Closely Models Human Tuberculosis. Cell Host Microbe. 2021 Jan 13;29(1):68-82.e5. PubMed PMID: 33142108
d. Navare AT, Mast FD, Olivier JP, Bertomeu T, Neal M, Carpp LN, Kaushansky A, Coulombe-Huntington J, Tyers M, Aitchison JD. Viral protein engagement of GBF1 induces host cell vulnerability through synthetic lethality. bioRxiv [Preprint]. 2020 Nov 6:2020.10.12.336487. doi: 10.1101/2020.10.12.336487. PMCID: PMC7654857.

5. **Modeling complex regulatory networks in yeast and infectious disease**

Transcriptional responses being central to cells responding to environmental cues and infection, we have placed efforts in understanding these responses through transcriptional profiling, analysis of chromatin, data integration, global network inference and dynamic network modeling. These approaches have led to significant insights into how network architecture endows cells with complex, reliable and predictable control of transcriptional responses while simultaneously controlling cellular heterogeneity. The predictable dynamics of transcriptional responses are relevant to antibiotic resistance mechanisms, correlates of immunity and infection, pharmacologic intervention and synthetic biology applications.

a. Ramsey SA, Smith JJ, Orrell D, Marelli M, Petersen TW, de Atauri P, Bolouri H, Aitchison JD. Dual feedback loops in the GAL regulon suppress cellular heterogeneity in yeast. Nat Genet. 2006 Sep;38(9):1082-7 PubMed PMID: 16936734
b. Smith JJ, Ramsey SA, Marelli M, Marzolf B, Hwang D, Saleem RA, Rachubinski RA, Aitchison JD. Transcriptional responses to fatty acid are coordinated by combinatorial control. Mol Syst Biol. 2007;3:115. PubMed Central PMCID: PMC1911199
c. Litvak V, Ratushny AV, Lampano AE, Schmitz F, Huang AC, Raman A, Rust AG, Bergthaler A, Aitchison JD, Aderem A. A FOXO3-IRF7 gene regulatory circuit limits inflammatory sequelae of antiviral responses. Nature. 2012 Oct 18;490(7420):421-5. PubMed Central PMCID: PMC3556990.
d. Schoggins JW, MacDuff DA, Imanaka N, Gainey MD, Shrestha B, Eitson JL, Mar KB, Richardson RB, Ratushny AV, Litvak V, Dabelic R, Manicassamy B, Aitchison JD, Aderem A, Elliott RM, García-Sastre A, Racaniello V, Snijder EJ, Yokoyama WM, Diamond MS, Virgin HW, Rice CM. Pan-viral specificity of IFN-induced genes reveals new roles for cGAS in innate immunity. Nature. 2014 Jan 30;505(7485):691-5. doi: 10.1038/nature12862. PubMed Central PMCID: PMC4077721.

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/john.aitchison.1/bibliography/43975020/public/?sort=date&direction=descending

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Bedalov, Antonio MD, PhD

eRA COMMONS USER NAME (credential, e.g., agency login): abedalov

POSITION TITLE: Professor, FHCRC; Professor, University of Washington

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Univ. of Zagreb School of Medicine, Zagreb, Croatia	MD	1989	Medicine
Univ. of Zagreb School of Medicine, Zagreb, Croatia	PhD	1998	Medical Sciences
University of Connecticut Health Center, Farmington, CT	Post-Doctoral Fellow	1993	Medicine
Baylor College of Medicine, Internal Medicine	Resident	1996	Internal Medicine
University of Washington, Seattle, WA	Hematology /Oncology Fellow	1997	Hematology/Oncology
Fred Hutchinson Cancer Research Center, Seattle, WA	Post-Doctoral Fellow	1999	Post-Doctoral Fellow

A. Personal Statement

~~My position at the Fred Hutchinson Cancer Research Center allows me to combine clinical oncology and basic research. In my role as a physician, I am constantly confronted by genomic instability and aging as manifested in cancer. As a scientist, I am driven to understand the genetic and biological bases of these complex phenomena.~~

My laboratory is focused on the biology of sirtuins including their role in genome replication and aging as well as therapeutic targets in cancer. To this end, we use yeast as a model system both to dissect the role of sirtuins in genome maintenance and for identification of small molecule inhibitors of this class of enzymes[1]. My laboratory has made important contributions to the sirtuin field including identification of *HST1* (homologue of *SIR2*) as an NAD sensor and a regulator of cellular NAD homeostasis and establishing the role of *HST3* and *HST4* in DNA damage response during S-phase. As an expert in chromatin biology and aging, I am a standing member in the Cellular Mechanisms of Aging and Development (CMAD) study section.

In addition to traditional yeast genetic approaches, we have expanded our studies to exploration of natural variation in aging-related traits such as telomere maintenance and replicative senescence. This proposal is a direct result of leveraging naturally occurring genetic diversity to gain insights into genetics of replicative aging.

Our discovery of a polymorphism in the ribosomal DNA (rDNA) origin of replication that extends replicative lifespan led us to a model in which incomplete genome replication is the life-limiting event during replicative senescence[2]. This model that was bolstered by demonstrating that Sir2 promotes replication in the gene-rich euchromatic portion of the genome by suppressing origin activity in the repetitive heterochromatic rDNA[3]. One intriguing possibility for how Sir2 inhibits activation of rDNA origins is suggested by our recent discovery that, by suppressing transcription, Sir2 prevents repositioning of pre-replicative helicase from its loading site, where this helicase is constrained by high nucleosome occupancy, to an adjacent region that is largely nucleosome-free[4]. These findings have broad implications, because late replication and transcriptional silencing are two of

the hallmark of repetitive genomic regions from yeast to humans, and both transcription and replication are accelerated in these regions during aging and carcinogenesis.

In our current proposal, we explore the mechanism that underlies the association between transcription and replication timing, making use of high-resolution approaches that we have developed to assess nucleosome and replication factor dynamics. I have assembled a research team with expertise in chromatin structure and replication. Dr. David MacAlpine is an expert in yeast chromatin and replication, and we have recently published a paper together. Dr. Steve Henikoff is a colleague at my institution who has pioneered the techniques for chromatin profiling we are exploiting in this proposal. My own expertise in yeast genetics, chromatin biology, genome stability and aging will enable me to direct the overall research program.

1. **Bedalov A**, Gatbonton T, Irvine WP, Gottschling DE, Simon JA. (2001) Identification of a small molecule inhibitor of Sir2. *Proc. Natl. Acad. Sci. U S A.* 98, 15113-15118.2.

2. Kwan EX, Foss EJ, Tsuchiyama S, Alvino GM, Kruglyak L, Kaeberlein M, Raghuraman MK, Brewer BJ, Kennedy BK, **Bedalov A.** (2013) A natural polymorphism in rDNA replication origins links origin activation with calorie restriction and lifespan. *PloS Genet.* 9(3):e1003329.

3. Foss E.J., Lao U, Dalrymple E, Adrianse RL, Loe T, and **Bedalov A.** (2017) *SIR2* Suppresses Replication Gaps and Genome Instability by Balancing Replication Between Repetitive and Unique Sequences. *Proc. Natl. Acad. Sci. U S A.*, 114:552-557.

4. Foss EJ, Gatbonton-Schwager T, Thiesen A, Taylor E, Soriano R, Lao U, **MacAlpine D** and Bedalov A. (2019) *SIR2* suppresses transcription-mediated displacement of Mcm2-7 replicative helicases at the ribosomal DNA repeats. *PloS Genet.* 10.1371/journal.pgen.1008138. PMID: 31083663 PMCID: PMC6532929.

B. Positions and Honors

<u>Employment</u>

2021-present	Professor, Department of Medicine, University of Washington, Seattle, WA Adjunct Professor, Department of Biochemistry, University of Washington
2019-present	Professor, Fred Hutchinson Cancer Research Center, Seattle, WA
2010-2019	Associate Member, Fred Hutchinson Cancer Research Center, Seattle, WA
2010-present	Associate Professor, Department of Medicine, University of Washington, Seattle, WA Adjunct Associate Professor, Department of Biochemistry, University of Washington
2005-2010	Adjunct Assistant Professor, Department of Biochemistry, University of Washington
2004 – 2010	Assistant Member, Fred Hutchinson Cancer Research Center, Seattle, WA Assistant Professor, Department of Medicine, University of Washington, Seattle, WA
1999 – 2004	Associate in Clinical Research, Fred Hutchinson Cancer Research Center, Seattle, WA Instructor in Medicine, University of Washington, Seattle, WA

<u>Awards</u>

2002 – 2006	Ellison Medical Foundation New Scholar Award in the Biology of Aging

<u>NIH Study Section Membership</u>

Cellular Mechanisms in Aging and Development (CMAD), Permanent Member, 2018-2024
Special Emphasis Panel, Cancer Research Workforce Diversity, ZRG1 OBT-D ad hoc (2018)
Cellular Mechanisms in Again and Development (CMAD) ad hoc (2016)
Drug Discovery and Molecular Pharmacology (DMP) ad hoc (2013, 2015, 2016, 2017)
Special Emphasis Panel, Oncology Fellowship, ZRG1 F09A-D, ad hoc (2014, 2015, 2017)
Molecular Neurogenetics (MG) ad hoc 2012

<u>Boards</u>

1996 American Board of Internal Medicine
2011 American Board of Medical Oncology

C. Contributions to Science

1) Identification of the first inhibitor of Sir2.

When I began my postdoctoral studies, the field of epigenetic regulation was still in its infancy. Discovered through phenotypic screens in model organisms, most of today's well-understood epigenetic regulators, including Sir2, had no known biochemical or enzymatic activities. Using phenotypic screens in yeast, I identified the first sirtuin inhibitor, splitomicin, as a small molecule that phenocopies deletion of *SIR2*, and later discovered a selective inhibitor of the Sir2 homolog, Hst2. These small molecule probes allowed conditional inactivation of siruins for mechanistic studies and for dissecting the cellular roles of sirtuins across species and specifically in cancer (see below). Furthermore, our success in identification of Sir2 inhibitors underscores the power of phenotypic screens in identifying small molecule probes for modulating epigenetic states.

Bedalov A, Gatbonton T, Irvine WP, Gottschling DE, Simon JA. (2001) Identification of a Small Molecule Inhibitor of Sir2p. *Proc. Natl. Acad. Sci. U S A*. 98, 15113-15118. PMCID: PMC64992.

Hirao M, Posakony J, Nelson M, Hruby H, Jung M, Simon JA, **Bedalov A**. (2003) Identification of selective inhibitors of NAD+-dependent deacetylases using phenotypic screens in yeast. *J Biol Chem.*;278(52):52773-82.

Posakony J, Hirao M, Stevens S, Simon JA, **Bedalov A**. (2004) Inhibitors of Sir2: evaluation of splitomicin analogues. *J Med Chem*. 2004 May 6;47(10):2635-44.

Posakony J, Hirao M, **Bedalov A** Identification and characterization of Sir2 inhibitors through phenotypic assays in yeast. (2004) *Comb Chem High Throughput Screen.* 2004 (7):661-8. Review.

Newcomb B, **Bedalov A**. (2009) Identification of inhibitors of chromatin modifying enzymes using the yeast phenotypic screens. *Methods Mol Biol.* 548:145-60. PMCID: PMC2925398.

2) Defining the cellular roles of yeast Sir2 homologues: Hst1 as a key regulator of NAD biosynthesis and Hst3, in genome replication and DNA damage response.

Besides Sir2, yeast contains four additional sirtuins, Hst1-4. The function of sirtuins as well as oxydoruductive enzymes that require NAD as a cofactor, depend critically on the availability of NAD. We found that cellular NAD levels in yeast are regulated by a negative feedback loop featuring Hst1 as an NAD sensor. When NAD levels are adequate, Hst1 represses NAD biosynthesis genes; however, when NAD levels are reduced, repression of NAD biosynthesis genes is alleviated, leading to increased NAD biosynthesis and restoration of NAD levels. Our results described for the first time the regulatory circuitry that monitors and maintains cellular NAD levels. Similar feedback mechanisms involving sirtuins have been recently show to regulate circadian NAD fluctuations in mammals.

My laboratory identified Hst3 as the critical enzyme that deactylates histone H3 at lysine K56 during S-phase and the mechanism that controls Hst3 protein level during genotoxic stress. Acetylation of histone H3K56 is an important and conserved histone modification, carried out by SIRT6 in mammals and critical for genome maintenance across species.

Bedalov A, Hirao M, Posakony J, Nelson M, Simon JA. (2003) NAD-dependent deacetylase Hst1p controls biosynthesis and cellular NAD+ levels in Saccharomyces cerevisiae. *Mol Cell Biol.* (19):7044-54. PMCID: PMC193940.

Thaminy S, Newcomb B, Kim J, Gatbonton T, Foss E, Simon JA, and **Bedalov A**. (2007). HST3 is regulated by MEC1-dependent proteolysis and controls the S phase checkpoint and sister chromatid cohesion by deacetylating histone H3 at lysine 56. *J Biol Chem*. 282:37805-14.

3) Aging-related phenotypes as polygenic traits and discovery of rDNA origin activation as modulator or genome-wide replication and replicative lifespan.

Most of what we have learned the genetics of aging comes from model organisms where the effects of the single gene changes can be examined independently from other genetic alterations. However, because natural

populations are genetically diverse, differences in aging among individuals are more likely to result from the integration of effects of polymorphisms at multiple loci. In order to gain insight into the genetics of aging-related traits in outbred systems, we have employed a yeast model consisting of haploid progeny derived from a cross between wild and laboratory yeast. We found that telomere length, chronologic aging and replicative aging are all highly heritable traits that exhibit transgressive segregation and are controlled by multiple QTL. Identification of the wild rDNA variant that extends replicative lifespan through a single nucleotide change that reduces rDNA origin activity, serves as a basis for this proposal. In addition to aging traits, this experimental system enabled us to identify non-transcriptional mechanisms as a driving force in shaping proteome diversity in the outbred populations.

Gatbonton T, Imbesi M, Nelson M, Akey J, Ruderfer D, Kruglyak L, Simon J and **A Bedalov**. (2006) Telomere length as a quantitative trait: Genome-wide survey of the deletion mutants and genetic mapping of the telomere length control genes in S. cerevisiae. *PloS Genet*. Mar 17;2(3):e35

Kwan EX, Foss E, Kruglyak L, **Bedalov A**. (2011) Natural polymorphism in *BUL2* links cellular amino acid availability with chronological aging and telomere maintenance in yeast. *PLoS Genet*. (8):e1002250. PMCID: PMC316192

Foss EJ, Radulovic D, Shaffer SA, Goodlett DR, Kruglyak L, **Bedalov A**. (2011) Genetic variation shapes protein networks mainly through non-transcriptional mechanisms. *PLoS Biol*. 2011 (9):e1001144. PMCID: PMC3167781.

Kwan EX, Foss EJ, Tsuchiyama S, Alvino GM, Kruglyak L, Kaeberlein M, Raghuraman MK, Brewer BJ, Kennedy BK, **Bedalov A**. (2013) A natural polymorphism in rDNA replication origins links origin activation with calorie restriction and lifespan. *PLoS Genet*. 9(3):e1003329. PMCID: PMC3591295

Foss E.J., Lao U, Dalrymple E, Adrianse RL, Loe T, and Bedalov A. (2017) *SIR2* Suppresses Replication Gaps and Genome Instability by Balancing Replication Between Repetitive and Unique Sequences. *Proc. Natl. Acad. Sci. U S A.*, 114:552-557.

Foss EJ, Gatbonton-Schwager T, Thiesen A, Taylor E, Soriano R, Lao U, MacAlpine D and **Bedalov A**. (2019) *SIR2* suppresses transcription-mediated displacement of Mcm2-7 replicative helicases at the ribosomal DNA repeats. *PloS Genet.* 10.1371/journal.pgen.1008138.

5) Sirtuins as therapeutic targets in cancer.

NAD-dependent deacetylases have been implicated in control of cellular responses to stress and in tumorigenesis through deacetylation of important regulatory proteins, including p53 and the BCL6 oncoprotein. An analogue of splitomicin, which we named cambinol and that inhibits human Sirt1 and Sirt2 led us to the discovery that germinal center B-cell lymphomas are highly sensitive to sirtuin inhibition and that increased activation of P53 and inactivation of BCL6 constitutes a likely basis for the anti-lymphoma activity. In a separate study, employing APC^{min} mice, we demonstrated that genetic inactivation of SIRT1 reduces growth of intestinal polyps in this mouse model. Our results demonstrate that sirtuin inhibition presents an attractive therapeutic strategy in specific tumor contexts.

Heltweg B, Gatbonton T, Schuler AD, Posakony J, Goehle S, Kollipara R, DePinho R, Simon J and **A Bedalov**. (2006) Antitumor activity of a small molecule inhibitor of human Sir2 enzymes. *Cancer Res*. 15;66(8):4368-77.

Leko V, Varnum-Finney B, Li H, Gu Y, Flowers D, Nourigat C, Bernstein ID, **Bedalov A.** (2012) SIRT1 is dispensable for function of hematopoietic stem cells in adult mice. *Blood*. 119(8):1856-60. PMCID: PMC3293640

Leko V, Park GJ, Lao U, Simon JA, **Bedalov A**. (2013) Enterocyte-Specific Inactivation of SIRT1 Reduces Tumor Load in the APC $^{+/min}$ Mouse Model. *PLoS One.* 8:e66283. PMCID: PMC3682947

Mahajan SS, Scian M, Sripathy S, Posakony J, Lao U, Loe TK, Leko V, Thalhofer A, Schuler AD, **Bedalov A,** Simon JA**.** (2014) Development of Pyrazolone and Isoxazol-5-one Cambinol Analogues as Sirtuin Inhibitors *J. Med. Chem.*, *57*, 3283–3294 PMCID: PMC4002067

Complete List of Published Work in MyBibliography:

https://www.ncbi.nlm.nih.gov/sites/myncbi/1XYVakMkPmLQQ/bibliography/40431981/public/?sort=date&direction=descending

D. Research Support

Active

R01 GM117446 (Bedalov, A.) 02/01/16 – 2/30/2024
NIH / NIGMS
Origin Firing at Repetitive Sequences and Genome Replication
The goal of this proposal is to deterimine whether activation of replication origins at repetetive ribosomal DNA origins shapes replication of the rest of the genome.

RETT SYNDROME RESEARCH TRUST (Bedalov, A.) 11/01/2014 – 5/30/2024
Genetic and Pharmacologic Reactivation of Mecp2 on the Silent X-chromosome as a Therapeutic Approach to Rett Syndrome
The goal of this proposal is to determine the reactivation level of Mecp2 and other genes on the inactive X-chromosome upon pharmacological and genetic inhibition of targets identified in the reporter based Mecp2 reactivation screen.

R01 CA206462-01A1 (Simon, J.A., Bedalov, A.) 07/07/16 - 06/30/22 NCE
NIH/NCI
SIRT2 Inhibitors for the Treatment of B-cell Lymphoma
We hypothesize that selective sirtuin SIRT2 inhibition exerts anti-lymphoma activity by restoring the cellular acetylation balance in GC-like DLBCL. We will test this hypothesis in the following aims. Aim 1: Optimize SIRT2 inhibitors. Aim 2: Determine mechanisms underlying SIRT2 inhibitor anti-GCBCL activity. Aim 3: Define SIRT2 function in vivo in normal germinal center and in lymphomagenesis. Aim 4: Assess efficacy of SIRT2 analogues in vivo.

Completed

No completed projects to list.

OMB No. 0925-0001 and 0925-0002 (Rev. 10/2021 Approved Through 09/30/2024)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Bush, Matthew Francis

eRA COMMONS USER NAME (credential, e.g., agency login): MFBUSH

POSITION TITLE: Assistant Professor of Chemistry

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
Carleton College, Northfield, MN	BA	06/2003	Chemistry
University of California, Berkeley, CA	PHD	05/2008	Chemistry
University of Cambridge	Postdoctoral Fellow	12/2009	Chemistry
University of Oxford	Fellow	06/2011	Chemistry

A. Personal Statement

My experience in state-of-the-art biological mass spectrometry, biophysics, and lasers make me ideally suited to carry out the proposed studies. My laboratory focuses on understanding the structure and assembly of protein complexes, particularly those that are heterogeneous, dynamic, and interact with complex chemical matrixes. Towards that end, we develop and apply mass spectrometry based technologies for interrogating the shapes (1), stoichiometry (2,3), and interaction partners (4) of intact protein complexes. Since beginning my independent career at the University of Washington, my laboratory has used native mass spectrometry to probe the structures of a complexes from the Type II Secretion System of Vibrio cholerae (3) and the ubiquitin proteasome system (4).

1. Lu C, Turley S, Marionni ST, Park YJ, Lee KK, Patrick M, Shah R, Sandkvist M, Bush MF, Hol WG. Hexamers of the type II secretion ATPase GspE from Vibrio cholerae with increased ATPase activity. Structure. 2013 Sep 3;21(9):1707-17. PubMed Central PMCID: PMC3775503.
2. Xing W, Busino L, Hinds TR, Marionni ST, Saifee NH, Bush MF, Pagano M, Zheng N. SCF(FBXL3) ubiquitin ligase targets cryptochromes at their cofactor pocket. Nature. 2013 Apr 4;496(7443):64-8. PubMed Central PMCID: PMC3618506.
3. Bush MF, Hall Z, Giles K, Hoyes J, Robinson CV, Ruotolo BT. Collision cross sections of proteins and their complexes: a calibration framework and database for gas-phase structural biology. Anal Chem. 2010 Nov 15;82(22):9557-65. PubMed PMID: 20979392.
4. Ekeowa UI, Freeke J, Miranda E, Gooptu B, Bush MF, Pérez J, Teckman J, Robinson CV, Lomas DA. Defining the mechanism of polymerization in the serpinopathies. Proc Natl Acad Sci U S A. 2010 Oct 5;107(40):17146-51. PubMed Central PMCID: PMC2951428.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2019 -	Affiliate Faculty, eScience Institute, University of Washington, Seattle, WA
2019 -	Associate Professor, Department of Chemistry, University of Washington, Seattle, WA
2019 -	Adjunct Associate Professor, Department of Biochemistry, School of Medicine, University of Washington, Seattle, WA
2014 - 2019	Adjunct Assistant Professor of Biochemistry, School of Medicine, University of Washington, Seattle, WA

2012 - Faculty, Molecular Engineering & Sciences Institute, University of Washington, Seattle, WA
2011 - 2019 Assistant Professor of Chemistry, University of Washington, Seattle, WA
2011 - Faculty, Biological Physics, Structure & Design Program, University of Washington, Seattle, WA

Honors

2017 Arthur F. Findeis Award for Achievements by a Young Analytical Scientist, American Chemical Society, Division of Analytical Chemistry
2014 Research Fellow, Alfred P. Sloan Foundation
2014 Young Investigator Award in Analytical Chemistry, Eli Lilly and Company
2013 Research Award, American Society for Mass Spectrometry
2010 Waters Research Fellow, Waters Co.
2010 Junior Research Fellow, Jesus College, University of Oxford

C. Contribution to Science

1. Native Mass Spectrometry (2009–present) is a technique that uses ions of intact noncovalent complexes to probe the stoichiometries, shapes, and distributions of those complexes in solutions. My laboratory determined that the complex between a mammalian cryptochrome (mCRY2) and the E3 ubiquitin ligase that regulates its degradation (FBXL3-Skp1) can assemble even in the absence of phosphorylation (in a). My laboratory used native mass spectrometry to characterize factors that control the oligomeric state of the ATPase of the Type II Secretion System of Vibrio cholerae, which led to high-resolution characterization of the biologically relevant form of that complex (in b). Most recently, my laboratory developed an integrative mass spectrometry strategy to quantify and sequence the bound substrates of a C-end ubiquitin ligase (in c and d).

   a. Canzani D, Rusnac DV, Zheng N, Bush MF. Degronomics: Mapping the Interacting Peptidome of a Ubiquitin Ligase Using an Integrative Mass Spectrometry Strategy. Anal Chem. 2019 Oct 15;91(20):12775-12783. PubMed Central PMCID: PMC6959985.
   b. Rusnac DV, Lin HC, Canzani D, Tien KX, Hinds TR, Tsue AF, Bush MF, Yen HS, Zheng N. Recognition of the Diglycine C-End Degron by CRL2^{KLHDC2} Ubiquitin Ligase. Mol Cell. 2018 Dec 6;72(5):813-822.e4. PubMed Central PMCID: PMC6294321.
   c. Lu C, Turley S, Marionni ST, Park YJ, Lee KK, Patrick M, Shah R, Sandkvist M, Bush MF, Hol WG. Hexamers of the type II secretion ATPase GspE from Vibrio cholerae with increased ATPase activity. Structure. 2013 Sep 3;21(9):1707-17. PubMed Central PMCID: PMC3775503.
   d. Xing W, Busino L, Hinds TR, Marionni ST, Saifee NH, Bush MF, Pagano M, Zheng N. SCF(FBXL3) ubiquitin ligase targets cryptochromes at their cofactor pocket. Nature. 2013 Apr 4;496(7443):64-8. PubMed Central PMCID: PMC3618506.

2. Ion Mobility Mass Spectrometry (2009–present) is an emerging technology for characterizing the shapes of ions and is highly complementary to mass spectrometry. I developed new ion mobility spectrometry devices that enable the direct determination of collision cross sections with high sensitivity. Measurements made using these devices are now used to standardize and validate a large fraction of ion mobility spectrometry experiments performed world-wide for the analysis protein complexes, peptides, and small molecules (in a&b). My laboratory is now developing the next generation of ion mobility spectrometry instrumentation that will enable the multidimensional ion mobility analysis of biomolecules ranging from metabolites to protein complexes (in c&d).

   a. Allen SJ, Eaton RM, Bush MF. Structural Dynamics of Native-Like Ions in the Gas Phase: Results from Tandem Ion Mobility of Cytochrome c. Anal Chem. 2017 Jul 18;89(14):7527-7534. PubMed PMID: 28636328.

b. Allen SJ, Giles K, Gilbert T, Bush MF. Ion mobility mass spectrometry of peptide, protein, and protein complex ions using a radio-frequency confining drift cell. Analyst. 2016 Feb 7;141(3):884-91. PubMed PMID: 26739109.
c. Bush MF, Campuzano ID, Robinson CV. Ion mobility mass spectrometry of peptide ions: effects of drift gas and calibration strategies. Anal Chem. 2012 Aug 21;84(16):7124-30. PubMed PMID: 22845859.
d. Bush MF, Hall Z, Giles K, Hoyes J, Robinson CV, Ruotolo BT. Collision cross sections of proteins and their complexes: a calibration framework and database for gas-phase structural biology. Anal Chem. 2010 Nov 15;82(22):9557-65. PubMed PMID: 20979392.

3. Computational Ion Mobility Mass Spectrometry (2012–present) is focused on the methods, algorithms, and methodologies used to process, analyze, and interpret the data produced in ion mobility mass spectrometry experiments. Although my lab has long focused on developing methods to standardize ion mobility mass spectrometry, workflows to extract critical information from ion mobility mass spectrometry data, and methods to visualize results from ion mobility mass spectrometry experiments, more recently we have focused on developing theory to support experiment design (in a) and computational methods to predict collision cross sections for atomistic models (in b-d). My laboratory is now developing methods to compare experimental ion mobility arrival-time distributions and atomistic models that include representations of experimental uncertainty and broadening during separations.

   a. Canzani D, Laszlo KJ, Bush MF. Ion Mobility of Proteins in Nitrogen Gas: Effects of Charge State, Charge Distribution, and Structure. J Phys Chem A. 2018 Jun 28;122(25):5625-5634. PubMed PMID: 29864282.
   b. Lee JW, Davidson KL, Bush MF, Kim HI. Collision cross sections and ion structures: development of a general calculation method via high-quality ion mobility measurements and theoretical modeling. Analyst. 2017 Nov 6;142(22):4289-4298. PubMed PMID: 29034911.
   c. Laszlo KJ, Bush MF. Effects of Charge State, Charge Distribution, and Structure on the Ion Mobility of Protein Ions in Helium Gas: Results from Trajectory Method Calculations. J Phys Chem A. 2017 Oct 12;121(40):7768-7777. PubMed PMID: 28910102.
   d. Davidson KL, Bush MF. Effects of Drift Gas Selection on the Ambient-Temperature, Ion Mobility Mass Spectrometry Analysis of Amino Acids. Anal Chem. 2017 Feb 7;89(3):2017-2023. PubMed PMID: 28208256.

4. Ion/Ion Reactions of Biomolecular Ions (2012–present). Reacting multiply charged biomolecules with monoanions results in proton transfer, which reduces the charge state of the ion, or electron transfer, which also results in covalent bond fragmentation. We also use ion/ion proton transfer reactions to increase the accuracy of charge state assignment and the resolution of interfering protein complexes in native mass spectrometry (in a) and to probe the effects of charge state on the structures of protein ions in the gas phase (in b-d).

   a. Gadzuk-Shea MM, Bush MF. Effects of Charge State on the Structures of Serum Albumin Ions in the Gas Phase: Insights from Cation-to-Anion Proton-Transfer Reactions, Ion Mobility, and Mass Spectrometry. J Phys Chem B. 2018 Nov 1;122(43):9947-9955. PubMed PMID: 30351115.
   b. Laszlo KJ, Bush MF. Interpreting the Collision Cross Sections of Native-like Protein Ions: Insights from Cation-to-Anion Proton-Transfer Reactions. Anal Chem. 2017 Jul 18;89(14):7607-7614. PubMed Central PMCID: PMC5589449.
   c. Laszlo KJ, Munger EB, Bush MF. Folding of Protein Ions in the Gas Phase after Cation-to-Anion Proton-Transfer Reactions. J Am Chem Soc. 2016 Aug 3;138(30):9581-8. PubMed Central PMCID: PMC4999245.
   d. Laszlo KJ, Bush MF. Analysis of Native-Like Proteins and Protein Complexes Using Cation to

Anion Proton Transfer Reactions (CAPTR). J Am Soc Mass Spectrom. 2015 Dec;26(12):2152-61. PubMed Central PMCID: PMC4655144.

5. Zwitterion Formation in Amino Acids (2003–2008). All naturally occurring amino acids are nonzwitterionic when isolated in the gas phase, despite existing as zwitterions in solution over a wide pH range. I used mass spectrometry, infrared spectroscopy, and computational chemistry to probe the formation of zwitterions in amino acids. During these studies, I determined that the diameter of the metal ion (in a and d), the extent of microhydration (in b), the proton affinity of the proton accepting group (in d), and the charge of the metal ion (in c) can all make the zwitterionic form of an amino acid lowest in energy. These studies provide fundamental insights into the contributions of noncovalent interactions to the structure of biological molecules.

    a. Bush MF, Oomens J, Williams ER. Proton affinity and zwitterion stability: new results from infrared spectroscopy and theory of cationized lysine and analogues in the gas phase. J Phys Chem A. 2009 Jan 15;113(2):431-8. PubMed PMID: 19128186.
    b. Bush MF, Oomens J, Saykally RJ, Williams ER. Effects of alkaline earth metal ion complexation on amino acid zwitterion stability: results from infrared action spectroscopy. J Am Chem Soc. 2008 May 21;130(20):6463-71. PubMed PMID: 18444620.
    c. Bush MF, Prell JS, Saykally RJ, Williams ER. One water molecule stabilizes the cationized arginine zwitterion. J Am Chem Soc. 2007 Nov 7;129(44):13544-53. PubMed PMID: 17929811.
    d. Bush MF, O'Brien JT, Prell JS, Saykally RJ, Williams ER. Infrared spectroscopy of cationized arginine in the gas phase: direct evidence for the transition from nonzwitterionic to zwitterionic structure. J Am Chem Soc. 2007 Feb 14;129(6):1612-22. PubMed Central PMCID: PMC2675882.

OMB No. 0925-0001 and 0925-0002 (Rev. 12/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Jeffrey S. Chamberlain, Ph.D.

eRA COMMONS USER NAME (credential, e.g., agency login): Chamberl

POSITION TITLE: : Professor and McCaw Endowed Chair

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Rice University, Houston, TX	B.A.	05/1978	Biochemistry
University of Washington, Seattle, WA	Ph.D.	02/1985	Biochemistry
Baylor College of Medicine, Houston, TX	Post-doc	02/1990	Molecular Genetics

A. Personal Statement

My group has a long-standing interest in Duchenne muscular dystrophy, with a focus on gene therapy. This therapeutic focus has been pursued in parallel with studies of the mechanisms leading to the muscular dystrophies, the organization of dystrophin and the dystrophin-glycoprotein complex (DGC), and approaches to muscle gene delivery. We performed extensive structure/function studies on dystrophin and on how the DGC is assembled in striated muscles, and have pioneered methods for gene delivery to skeletal and cardiac muscles. These studies enabled us to develop and test dozens of potentially therapeutic dystrophin constructs, including mini- & micro-dystrophins. *We were the first group to develop micro-dystrophins (starting in 1996), and also the first to show that AAV could be used for systemic gene delivery to muscle (2004)*. Our vectors are being tested by Sarepta, Genethon and Solid Biosciences in clinical trials for gene therapy of DMD, and will be used in upcoming clinical trials by Ultragenyx & Roche. Together with the Hauschka lab we isolated the first muscle-specific enhancer element (from the MCK gene) and this has been engineered to make hundreds of muscle-specific expression cassettes (MSECs) for use in AAV and transgenic animals. Current efforts involve developing improved mini- & micro-dystrophins, testing them in multiple muscle types and models, & improving the safety & efficacy of gene delivery with AAV. Our group is also focused on advancing vectors for disorders such as LGMD2i and LGMD2a. We have also refined an extensive set of assays on muscle contractile properties, morphology and physiology and work with collaborators on applying these assays to their models. As Director of the *Seattle Wellstone Muscular Dystrophy Specialized Research Center* I have the privilege of interacting with an outstanding group of scientists and clinicians to advance research into treatments for the muscular dystrophies, and our Center has an important role in supplying muscular dystrophy and vector reagents and methods to labs worldwide. In this regard, our Center works closely with many collaborators to design, produce and provide advice on the use of viral vectors, which we produce in high titer and high purity in our Viral Vector lab. Our Core is able to produce numerous serotypes of AAV, including AAVs 1, 2, 6, 8, 9 and AAVMyo. We have also generated a number of adenoviral vectors, some of which are being used in human clinical trials for COVID-19 & cancer by ImmunityBio.

Ongoing and recently completed projects that I would like to highlight include:
P50 AR065139
Chamberlain JS (PI)
09/01/2014-08/31/2023
Senator Paul D. Wellstone Muscular Dystrophy Specialized Research Center

R01 AR40864-31A1
Chamberlain JS (PI)
04/01/1991 – 2/28/2026
Dystrophin Replacement

Citations (from >280): See also 'MyBibliography':
https://www.ncbi.nlm.nih.gov/myncbi/jeffrey.chamberlain.1/bibliography/public/

Google Scholar h-index, 1/2022: 92; https://scholar.google.com/citations?user=gaTbyBAAAAAJ

1. Cox GA, Cole N, Matsamura K, Phelps SF, **Hauschka SD**, Campbell KP, Faulkner JA, **Chamberlain JS**. (1993). Overexpression of dystrophin in transgenic *mdx* mice eliminates dystrophic symptoms without toxicity. Nature; 364:725-729.
2. Gregorevic P, Blankinship MJ, Allen J, Crawford RW, Meuse L, Miller D, Russell DW and **Chamberlain JS** (2004): Systemic delivery of genes to striated muscles using adeno-associated viral vectors. Nature Medicine; 10:828-834. PMC1365046.
3. Gregorevic P, Blankinship MJ, Minami E, Allen JA, Haraguchi M, Meuse L, Finn E, Adams M, Froehner SC, Murry CE and **Chamberlain JS** (2006): Systemic delivery of rAAV6-microdystrophin preserves muscle function and extends lifespan in a murine model of severe muscular dystrophy. Nature Medicine; 12:787-789. PMC4244883.
4. **Bengtsson NE**, Hall JK, Odom GL, Phelps MP, Andrus CR, Hawkins RD, **Hauschka SD**, Chamberlain JR and **Chamberlain JS** (2017): Muscle-specific CRISPR/Cas9 editing of the dystrophin gene ameliorates pathophysiology in a mouse model of DMD. Nat Comm; 8:14454. DOI: 10.1038/ncomms14454

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2021-Present	Skygene Bio, Co-founder/Scientific Advisory Board
2021-Present	Myogene, Scientific Advisory Board
2020-Present	International Fibrodysplasia Ossificans Progressiva Association (IFOPA), Scientific Advisory Board
2019-Present	Gilbert Family Foundation, Scientific Advisory Board
2018-Present	KineaBio, Co-founder/Scientific Advisory Board
2016-Present	Jesse's Journey, Scientific Review Board
2015-Present	Solid Biosciences, Scientific Advisory Board, *chair*
2010-Present	Cellular, Tissue and Gene Therapies Advisory Committee, Center for Biologics Evaluation and Research, Food & Drug Administration: Special Govt. Employee:
2003-2010	Etubics Inc., co-founder, SAB (now part of *ImmunityBio*)
2008-Present	McCaw Endowed Chair in Muscular Dystrophy
2003-Present	Adjunct Professor, Dept. of Biochemistry, University of Washington
2002-Present	Professor, Dept. of Medicine, Division of Medical Genetics, University of Washington
2001-Present	Professor, Dept. of Neurology, University of Washington School of Medicine, Seattle, WA
1999-2000	Professor, Dept. of Human Genetics, U. Michigan Medical School, Ann Arbor, MI
1999-2000	Interim Director, Center for Gene Therapy, U. Michigan Medical School, Ann Arbor, MI
1994-1999	Associate Professor, Dept. of Human Genetics, U. Michigan Medical School, Ann Arbor, MI
1990-1994	Assistant Professor, Dept. of Human Genetics, U. Michigan Medical School, Ann Arbor, MI.
2018, 2021	NIH, SMEP study section, ad hoc reviewer
2017-Present	Fondazione Telethon, ad hoc grant reviewer
2018-2019	Japan Agency for Medical Research and Development, grant reviewer
1995-2018	Muscular Dystrophy Association, Scientific Advisory Committee
2012-2015	Fondazione Telethon, Milan, Italy, Scientific Advisory Committee
2005-2009	Cellular, Tissue and Gene Therapies Advisory Committee, Center for Biologics Evaluation and Research, Food & Drug Administration, Member

1989-1995 Muscular Dystrophy Association, Fellowship Review subcommittee
Editorial Boards: Mol Ther, Skel Mus, J Neuromusc Disord, J Gene Med

Honors

2021-2024	VP, President-elect, and President (sequential) American Society for Gene and Cell Therapy
2021-2024	Program Committee, American Society for Gene and Cell Therapy
2015-	Fellow, American Association for the Advancement of Science
2015-2017	Council Member, American Association for the Advancement of Science
2012-2015	Board of Directors, American Society for Gene and Cell Therapy
2006-2015	NIH MERIT Award
1999-2002	Board of Directors, American Society for Gene Therapy
1995	Gift of Hope Award, Jones Intercable Inc.
1995	Faculty Recognition Award, University of Michigan
1994	Leadership Award, Service Merchandise Inc.
1991	Basil O'Conner Starter Scholar Research Award, March of Dimes Birth Defects Foundation
1987-1990	Post-doctoral Fellowship, Muscular Dystrophy Association

C. Contributions to Science

1. While a post-doc with Dr. C. Thomas Caskey I worked with another post-doc (Richard Gibbs) and a Research Scientist (Joel Ranier Chamberlain) to study the *mdx* mouse and the human dystrophin gene. We were the first group to study dystrophin mRNA expression in mice, revealing that *mdx* mice had reduced dystrophin mRNA expression and that the gene was expressed in both muscle and the CNS. A second focus was applying the recently discovered method of PCR to diagnose genetic disorders. Here we invented and patented the technique of *Multiplex PCR*. At the time, PCR had only been used to diagnose 2 or 3 genetic disorders, and no one had amplified more than one sequence in a single reaction. Our first application of the multiplex approach was to develop a technique where nine different genomic regions from the human dystrophin gene could be PCR amplified in a single reaction, enabling pre- and post-natal diagnosis and carrier detection for more than half of all DMD deletion cases, or approximately 35% of all cases. At the time, DNA diagnosis for DMD involved southern analysis using cDNA and genomic fragments. For many years our multiplex PCR method was the most widely used method worldwide for DMD DNA diagnostics, and it has been used in thousands of studies of basic and clinical issues. Our multiplex manuscript has been cited over 1,900 times (Google Scholar, 2/2022).
   a. **Chamberlain JS**, Pearlman JA, Muzny DM, Gibbs RA, Ranier JE, Reeves AA and Caskey CT: Expression of the murine Duchenne muscular dystrophy gene in muscle and brain. Science 1988; 239:1416-18.
   b. **Chamberlain JS**, Gibbs RA, Ranier JE, Nguyen PN and Caskey CT: Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification. Nucleic Acids Res 1988; 516:11141-11156.
   c. **Chamberlain JS**, Chamberlain JR, Fenwick RG, *et al*.: Diagnosis of Duchenne and Becker muscular dystrophies by polymerase chain reaction: A multicenter study. JAMA 1992; 267:2609-2615.

2. Upon establishing my own lab at the University of Michigan I began studies to characterize various *mdx* mouse models of DMD and explore their use for therapeutic development. At the time many groups felt the *mdx* mouse was NOT a model for DMD, but linkage and expression analyses by our group and others suggested otherwise. My group generated the first intact full-length cDNA clones for human dystrophin (using small cDNAs provided by Lou Kunkel) and for mouse dystrophin (our own cloning). We found the genetic mutations in the *mdx*^{2cv}, 3cv, 4cv and 5cv mice, and published the first study to show that DMD could be cured by genetic therapy (in transgenic *mdx* mice; see Nature 1993 from section A). We've long used the *mdx* mouse to study pathophysiology and dystrophin function in transgenic *mdx* models, and now focus on the *mdx*^{4cv} line and the CXMD dog.

a. Cox GA, Phelps SF, Chapman VM and **Chamberlain JS**: New *mdx* mutation disrupts expression of muscle and nonmuscle isoforms of dystrophin. Nature Genet 1993; 4:87-93.
b. Phelps SF, Hauser MA, Cole NM, Rafael JA, Hinkle RT, Faulkner JA, **Chamberlain JS**: Expression of full-length and truncated dystrophin mini-genes in transgenic *mdx* mice. Hum Mol Genet 1995; 4:1251-1258.
c. Judge L, Banks G, Arnett A and **Chamberlain JS**: Expression of the dystrophin isoform Dp116 preserves functional muscle mass and extends lifespan without preventing dystrophy in severely dystrophic mice. Hum Molec Genet 2011; 20:4978-90. PMC3221536.
d. Swiderski K, Shaffer SA, Gallis B, Odom GL, Arnett AL, Edgar JS, Baum DM, Chee A, Naim T, Gregorevic P, Murphy KT, Goodlett DR, Lynch GS and **Chamberlain JS**: Phosphorylation within the cysteine-rich region of dystrophin enhances its association with β-dystroglycan and identifies a potential novel therapeutic target for skeletal muscle wasting. Hum Molec Genet 2014; 23:6697-6711. PMC4245040.

3. A major area of focus has been on vector development, including the use of muscle-specific regulatory cassettes for gene delivery to muscle. These efforts pursued a dual strategy of developing vectors that could carry large genes while also testing smaller dystrophin expression cassettes. Various studies looked at plasmid vectors, retroviral vectors and adenoviral vectors, and led to the development of E2b-deleted and 'gutted" adenoviral vector systems able to deliver full-length dystrophin cDNAs into skeletal muscles. These studies were also aided immensely from collaborations with the lab of Stephen Hauschka (U. Washington). While a graduate student with Hauschka I cloned the mouse muscle creatine kinase (MCK) gene, from which the first muscle-specific enhancer element was identified. Hauschka's lab went on to develop a large number of muscle-specific gene regulatory cassettes that we have used in our gene therapy vectors and by other labs worldwide. These regulatory elements have proven extremely useful for AAV delivery of dystrophin and Cas9, and several are in the clinic. We also explored retroviral and lentiviral vectors to permanently target muscle stem cells and their progenitors. Our adenoviral vectors have been licensed to ImmunityBio and are being tested as a genetic vaccine in human clinical trials for cancer and COVID-19.

a. Hartigan-O'Connor D, Kirk C, Crawford R, Mule J and **Chamberlain JS**: Immune evasion by muscle specific gene expression in dystrophic muscle. Mol Ther 2001; 4:525-533
b. DelloRusso C, Scott J, Hartigan-O'Connor D, Salvatori G, Barjot C, Robinson AS, Crawford RW, Brooks SV and **Chamberlain JS**: Functional correction of adult *mdx* mouse muscle using gutted adenoviral vectors expressing full-length dystrophin. Proc Nat'l Acad Sci 2002; 99:12979-12984.
c. Kimura E, Li S, Gregorevic P, Fall BM and **Chamberlain JS**: Dystrophin delivery to muscles of *mdx* mice using lentiviral vectors leads to myogenic progenitor targeting and stable gene expression. Mol Ther 2010; 18:206-213. PMC2839215.
d. Odom GL, Gregorevic P, Allen JA, Finn E and **Chamberlain JS**: Gene therapy of *mdx* mice with large truncated dystrophins generated by recombination using rAAV6. Mol Ther 2011; 19:36-45. PMC3017440.

4. We have focused extensively on structure/function studies of dystrophin. These studies evolved from our first efforts to generate transgenic mice carrying dystrophin expression cassettes. In this work we collaborated extensively with the lab of John Faulkner (Michigan). Faulkner's lab was among the world leaders in studying muscle mechanical properties, and his group and mine collaborated extensively to apply physiological studies to our transgenic and vector treated *mdx* mice. Some highlights of our research were the discovery that mini-dystrophins with enhanced function could be generated by merging discreet functional domains (rather than by deletion of individual exons, as in exon skipping), identifying that the dystrophin C-terminal domain was not needed for function (due to redundant binding sites for members of the dystrophin-glycoprotein complex), and the development of a variety of mini- and micro-dystrophin (and utrophin) cDNAs.

a. Rafael JA, Cox GA, Jung D, Campbell KP, and **Chamberlain JS**: Forced expression of dystrophin deletion constructs reveals structure-function correlations. J Cell Biol 1996; 134:93-102.
b. Crawford GE, Faulkner JA, Crosbie RH, Campbell KP, Froehner SC, and **Chamberlain JS**: Assembly of the dystrophin-associated protein complex does not require the dystrophin COOH-terminal domain. J Cell Biol 2000; 150:1399-1410.

c. Harper S, Hauser M, DelloRusso C, Duan D, Crawford RW, Phelps SF, Harper HA, Robinson AS, Engelhardt JF, Brooks SV and **Chamberlain JS**: Modular flexibility of dystrophin: Implications for gene therapy of Duchenne muscular dystrophy. Nature Med 2002; 8:253-261.
d. Banks G, Judge L, Doremus C, Finn E, Allen J and **Chamberlain JS**: The polyproline site in hinge 2 influences the functional capacity of truncated dystrophins. PLoS Genetics 2010; 6:e1000958. PMC2873924

5. A major focus of current research is on developing methods for muscle gene therapy. We were the first group to publish a method for systemic gene delivery to striated muscles throughout an adult animal, using vascular delivery of AAV (see Gregorevic 2004, 2006 in section A). At the time, AAV had only been used for intramuscular injection or for delivery to the liver. This discovery has transformed my research program, allowing us to focus heavily on methods for gene therapy of DMD using vascular delivery of AAV/micro-dystrophin vectors. Our third generation vector (AAV-CK8-μDys5) has been licensed by my instituiton to Solid Biosciences, which began a human clinical trial in early 2018. Our first generation vector (AAV-MHCK7-ΔR4-R23/ΔCT μDys) is being used by Sarepta/Genethon/Roche in their clinical trials. We have also adapted muscle-specific AAV vectors for gene editing of dystrophin and other genes.

a. Arnett LHA, Konieczny P, Ramos JN, Hall J, Odom GL, Yablonka-Reuveni Z, Chamberlain JR and **Chamberlain JS**: Adeno-associated viral (AAV) vectors do not efficiently target muscle satellite cells. Mol Ther: Meth Clin Develop. 2014; 1:14038. doi: 10.1038/mtm.2014.38. Open Access Journal. PMC4288464.
b. **Bengtsson NE**, Hall JK, Odom GL, Phelps MP, Andrus CR, Hawkins RD, **Hauschka SD,** Chamberlain JR and **Chamberlain JS**: Muscle-specific CRISPR/Cas9 editing of the dystrophin gene ameliorates pathophysiology in a mouse model of DMD. Nat Comm 2017; 8:14454. PMC5316861.
c. Ramos JN, Hollinger K, **Bengtsson NE**, Allen JM, **Hauschka SD** and **Chamberlain JS**: Development of novel micro-dystrophins with enhanced functionality. Mol Ther 2019; 27:623-635. doi: 10.1016/J.ymthe.2019.01.002. PMC6403485. *Open Access*
d. **Bengtsson NE**, Tasfaout H, **Hauschka SD** and **Chamberlain JS**: Dystrophin gene editing stability is dependent on dystrophin levels in skeletal but not cardiac muscles. Mol Ther 2022; 29:1070-1085. Published on-line: https://doi.org/10.1016/j.ymthe.2020.11.003. PMC in process; NIHMS 1653991.
e. **Bengtsson NE**, Crudele JM, Klaiman JM, Halbert CL, **Hauschka SD** and **Chamberlain JS**: Comparison of dystrophin expression following gene editing and gene replacement in an aged preclinical DMD animal model. Mol Ther 2022; *in press*.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Valerie Daggett

eRA COMMONS USER NAME (credential, e.g., agency login): vdaggett

POSITION TITLE: Professor, University of Washington

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Reed College, Portland OR	BA	06/1983	Chemistry
University of California, San Francisco, CA (Advisors: Irwin Kuntz and Peter Kollman)	PhD	06/1990	Pharmaceutical Chemistry
Stanford University, Stanford, CA (Advisor: Michael Levitt)	Postdoc	01/1993	Structural Biology

A. Personal Statement

I am well suited to act as a consultant for the computational studies proposed. I have been involved in developing methods and software for the simulation of proteins for over 30 years. My lab was the first to perform thermal unfolding simulations of proteins. We were the first to characterize denatured, intermediate and transition states during the process in MD simulations. This required much work to ensure that the high temperatures required to see something in a reasonable period of time corresponded to what is probed experimentally (often in the direction of folding, not unfolding). Validation was critical and we worked in parallel, with experimentalists. There is great synergy between our experimental and computational approaches. Along the way we were the first to simulate proteins in mixed solvents and to simulate urea denaturation of a protein and compare it with thermal denaturation. Also, through multiple, simulations we have demonstrated that the unfolding process is essentially an activated process such that increasing the temperature increases the rate without changing the pathway, and we directly demonstrated microscopic reversibility in simulations at the Tm of proteins, for the first time. Along the way we have had to develop novel analysis techniques and much software, as none of these things had been done before. Our approach is to use standard MD methods with our force field parameters and procedures that have been tested over a 30-year period and then ask interesting scientific questions. The work proposed here is very much in line with what my lab has been doing for years and what Dr. Childers did in my lab as a PhD student, in the sense that he will be using simulations and modeling to provide insight into CMT via close collaboration with experimentalists to validate the simulations and check their predictions, and flesh out interactions that may be contributing to disease.

Ongoing projects:

NIH/NIAMS P30 Award AR074900-01
Role: Co-PI 4/1/2019 – 3/31/2024
UW Center for Translational Muscle Research. *Goal*: To provide a unifying resource and state of the art approaches to enhance muscle research and facilitate novel insights to muscle pathologies.

NIH/NIA Award R01AG067476
Daggett (PI) 4/14/2020-3/31/2025
Conformational heterogeneity and alpha-sheet: Determinants of toxicity in Abeta variants *Goal*: Characterize Abeta toxic oligomers in vitro and human samples, effect of alpha-sheet designs.

NIH/NIAID Award R11AI156017
Role: Co-PI 12/1/2020-11/30/2022

Engineering a denaturant-resistant polymerase for direct nucleic acid diagnostics *Goal*: Use MD simulations to determine vulnerabilities in structure to stabilize it for use in point-of-care diagnostic test,

Citations:

1. **Daggett, V**. α-sheet: The toxic conformer in amyloid diseases? *Acc. Chem. Res*., **39**, 594-602, 2006.
2. Hopping, G., Kellock, J., Barnwal, R.P., Law, P., Bryers, J.D., Varani, G., Caughey, B., **Daggett, V**. Designed alpha-Sheet Peptides Inhibit Amyloid Formation by Targeting Toxic Oligomers. *eLIFE* **3**: e01681, 2014. PMC4091096
3. Shea, D., Hsu, C.-C., Bi, T., Paranjapye, N., Childers, M., Cochran, J., Tomberlin, C.P., Wang, L., Paris, D., Zonderman, J., Varani, G., Link, C., Mullan, M., **Daggett, V**., α-sheet secondary structure in amyloid β-peptide drives aggregation and toxicity in Alzheimer's Disease, *Proc. Natl. Acad. Sci. USA*, **116**, 8895-8900, 2019. PMC6500163

B. Positions and Honors

Professional Experience at the University of Washington, City, State:

2018-present	Member of Molecular and Cellular Biology Program
2016-present	Adjunct Professor of Chemical Engineering
2014-present	Member of Molecular Engineering Program
2007-present	Professor of Bioengineering, College of Engineering and School of Medicine
2007-2010	Director, Biological Physics Structure and Design Program
2005-2007	Adjunct Professor of Bioengineering
2005--present	Member of Computational Biology Program
2003-2007	Professor of Medicinal Chemistry
2002--present	Member of Neuroscience Program
2002--present	Professor of Biomedical and Health Informatics, Adjunct
1998--present	Professor of Biochemistry, Adjunct
1998- -present	Member of Center for Nanotechnology
1996--present	Founding Member of Biomolecular Structure and Design Program
1993--present	Member of Molecular Biophysics Program
1993-2003	Assistant then Associate Professor, Department of Medicinal Chemistry

Honors and Awards:

2015 Elected	College of Fellows, American Institute of Medical and Biological Engineering, for outstanding contributions to protein engineering and design via the characterization of protein unfolding and structural changes linked to disease.
2011 Elected	Fellow of the Biophysical Society, for technical innovations in molecular dynamics
1995-1998	Young Investigator Award, Office of Naval Research
1994-1995	RPG Award, NSF
1990-1993	Katherine McCormick Fund for Women
1990-1993	Jane Coffin Childs Foundation Postdoctoral Fellowship for Medical Research
1990-1993	University of California Regents Graduate Opportunity Fellowship, U.C.S.F.

Invited participant National Academy of Sciences' symposium on the Frontiers of Science (11/96, 8/98).
Special Programs Grant Reviews: National Academy of Sciences/NRC, DOE, NIH, Wellcome Trust, Biotechnology & Biological Sciences Research Council, U.K., ERC, and private foundations.
Charter Member of NIH Macromolecular Structure and Function B Study Section, July 2005-08. (BBCA prior)
Chair of 2004 Biopolymers Gordon Conference, vice chair 2002
Chair of 2017 Computational Aspects of NMR Gordon Conference, Vice Chair 2015
Editor of first volume on Protein Simulations for *Advances in Protein Chemistry,* 2003
Elected to the Nominating Committee for the Protein Society, 2000-2003
Elected Biophysical Society Council Member, 2007-2010.
Co-organizer of the Annual Protein Society Meeting (2002)
US Department of Energy (DOE) *Innovative and Novel Computational Impact on Theory and Experiment* (INCITE) award for research in "Molecular Dynameomics." 2005, One of 3 given in US.
Nobel Foundation Symposium on Protein Folding: From Mechanisms to Impact on Cells, Stockholm, 2017

Professional Memberships and Activities:

Senior Editor of *Protein Engineering Design and Selection (PEDS)* with Alan Fersht (January 2004-2020)
Editorial Boards (current): *Biochemistry* (2003-present), *Structure* (1995-present), *Current Opinion in Structural Biology* (2012-present)
Editorial Boards (former): *Folding and Design* (1995-2000), *Protein Science* (1996-2003), Principal Editor for *TheScientificWorld* (2000-2001) and Structural Biology section of Biomed Central, (2001-2003), *Biomedical Computation Review (BCR)* (2005-present), Board of Reviewing Editors *eLife* (2012 – 2015)
Participant/lecturer for Royal Society Discussion Meeting on New Science from High Performance Computing I represented the field of Biomolecular Modeling (10/2001)
Contributing faculty member to 'Faculty of 1000 Biology', February 2005 – 2007.
Editor, *Current Opinion in Structural Biology*, Folding and Binding Issue, 2007 and 2009.
Visiting Professor, Chemistry Department Cambridge University, Member of the Room, Gonville and Caius, and Center for Protein Engineering, Medical Research Council, 2004.
Lise Meitner Visiting Professorship, Lund University, 2010.

C. Contributions to Science

1. **Developed approach of studying protein unfolding by molecular dynamics simulations**. I have been involved in protein folding studies, the subject of my undergraduate thesis, since 1982. At that time I was performing experiments aimed at mapping the folding of bovine pancreatic trypsin inhibitor (BPTI), both wild type and a chemically modified form. I was frustrated by the lack of mechanistic detail one could obtain and I became interested in computational approaches to the problem. So, I went to UCSF for graduate school and worked with Professors Peter Kollman and Tack Kuntz. I became proficient in modeling and was a contributor to AMBER, but it was not yet possible to study protein folding at that time for a variety of reasons, so I focused on small helical peptides. Even so, it was becoming clear that peptides and proteins don't sample all conformations and that there are pathways to folding, but simulations of such pathways were beyond the abilities of AMBER and available computing power. Around that time Professor Michael Levitt (2013 Chemistry Nobel Laureate) published an impressive and quite realistic simulation of the native state of BPTI, and I wanted to try to apply his approaches to the problem and I joined his lab at Stanford as a Jane Coffin Childs postdoctoral fellow. His methods turned out to be much more robust and I was able to generate more reasonable thermal unfolding trajectories of BPTI. This led to the first publication of protein unfolding simulations in solution. Over the years we spent many years validating the methods, the water model, the applicability of unfolding to folding, mapping pathways in great detail, and making predictions of rates, structures, phi-values, interactions, effects of mutations on the process, demonstration of microscopic reversibility, and showing that unfolding is an activated process, allowing us to use temperature to accelerate the process without altering the pathway. I collaborated with many experimentalists to test our predictions, but my most productive and long-lasting collaboration (since 1993) has been with Sir Alan Fersht (Cambridge University and MRC). I also was always more interested in unfolding than folding because of the many advantages of studying unfolding computationally, but also because of its relevance to disease, including amyloid diseases and other disorders due to mutation that lead to instability and unfolding.
    a. **Daggett, V.** and M. Levitt, A Model of the Molten Globule State from Molecular Dynamics Simulations, *Proc. Natl. Acad. Sci. USA,* **89**: 5142-5146, 1992. (1st simulations of protein unfolding)
    b. Li, A. and **V. Daggett**. Characterization of the Transition State of Protein Unfolding Using Molecular Dynamics: Chymotrypsin Inhibitor 2, *Proc. Natl. Acad. Sci. USA*, **91**: 10430-10434, 1994. (1st TS model)
    c. Bond, K C.J., Wong, K., Clarke, J., Fersht, A.R. and **V. Daggett**, Characterization of Residual Structure in the Thermally Denatured State of Barnase by Simulation and Experiment: Description of the Folding Pathway, *Proc. Natl. Acad. Sci. USA*., **94**: 13409-13413, 1997. (1st detailed model of denatured state)
    d. Mayor, U., Johnson, C.M., Grossmann, J.G., Sato, S., Jas, G.S., Freund, S.M.V., Guydosh, N.R., Alonso, D.O.V., **Daggett, V.** and A.R. Fersht, The Complete Folding Pathway of a Protein from Nanoseconds to Microseconds, *Nature*, **421**, 863-867, 2003. (combined MD and experiment mapping EnHD folding)

2. **Performed the first simulations related to amyloidogenesis and protein misfolding.** As mentioned above, I have long had an interest in unfolding for biological reasons, which began when the first experimental studies of the Amyloid-beta peptide were coming out and it was becoming clear that most so-called misfolding diseases are not due to misfolding as a competition between correct folding or misfolding, they are due to unfolding or partial unfolding, followed by aggregation. My lab published the first simulations in the amyloid area on Abeta and prion peptides (as part of a Program Project Grant of Dr Stan Prusiner (1997 Medicine Nobel Laureate) in 1995. We published the first simulation of the conversion of the hamster prion protein in

2001 and we followed that up with studies of other species and human mutants and we get a consistent consensus mechanism of conversion, which is in very good agreement with experiment. In fact, one of the key regions of the protein we predicted to form nonnative sheet structure, a hallmark of the conversion, was confirmed through a crystal structure over 10 years after publication.

a. DeMarco, M.L. and **V. Daggett**, From Conversion to Aggregation: Protofibril Formation of the Prion Protein, *Proc. Natl. Acad. Sci. USA,* **101**, 2293-2298*,* 2004***.*** PMCID: PMC356944 (1st simulation of prion protein conversion by us in 2001, but this follow-up study presents both conversion and a detailed model of the infectious oligomer)
b. Armen, R.S., Bernard, B., Day, R., Alonso, D.O.V. and **V. Daggett**, Characterization of a possible amyloidogenic precursor in glutamine-repeat neurodegenerative diseases**,** *Proc. Natl. Acad. Sci. USA,* **102**, 13433-13438, 2005*.*
c. Kirshenbaum, K. and **V. Daggett,** pH Dependent Conformations of the Amyloid β(1-28) Peptide Fragment Explored Using Molecular Dynamics, *Biochemistry*, **34**: 7629-7639, 1995. (1st simulation in field)
d. Kazmirski, S., D.O.V. Alonso, F.E. Cohen, S. Prusiner and **V. Daggett**. Theoretical Studies of Sequence Effects on the Conformational Properties of a Fragment of the Prion Protein: Implications for Scrapie Formation. *Chemistry & Biology,* **2**: 305-315, 1995. (1st simulation in field)

3. **Performed the first simulations showing the importance of dynamics in explaining the effects of sequence variation in disease-associated proteins due to single-nucleotide polymorphisms**. In addition to looking at the effects of solvent and temperature on protein stability and unfolding, we began to introduce known disease-causing mutations and evaluated their effects on structure, dynamics and function, leading to the first simulations of the products of single-nucleotide polymorphisms. While there are deleterious effects to the mutations (and often advantageous effects as well), the mutations are generally tolerated, and are not directly lethal. In fact, studies of the static structures of these variants rarely shed light on the pathology and we showed that dynamic characterizations are necessary to explain the phenotypes. This work began with predictions from simulations and then we performed various experimental studies to test the predictions.

a. Rutherford, K., Bennion, B.J., Parson, W.W. and **V. Daggett**, The 108M polymorph of human catechol *O*-methyltransferase is prone to deformation at physiological temperatures, *Biochem*., **45**, 2178-2188, 2006.
b. Rutherford, K., Parson, W.W. and **V. Daggett**, The Histamine *N*-Methyltransferase T105I Polymorphism Affects Active Site Structure and Dynamics, *Biochemistry*, **47**, 893-901, 2008.
c. Rutherford, K. and **V. Daggett**. Polymorphisms and Disease: Hotspots of Inactivation in Methyltransferases, *Trends in Biochem. Sci*., **35**, 531-538, 2010.
d. Bromley, D., Anderson, P.C., **Daggett, V.** Structural consequences of mutations to the alpha-tocopherol transfer protein associated with the neurodegenerative disease ataxia with vitamin E deficiency. *Biochemistry*, **52**: 4264-4273, 2013. PMC3727652

4. **Developed new MD methods, big data information management system and visual analytics framework**. I began working on methods and code development in 1985 and continued when I moved to the Levitt lab. There I worked on new methods for robust MD, and a new fully flexible water model and validating its use as a function of temperature. This work continued when I started my own lab in 1993. These improvements allowed for realistic and experimentally validated unfolding trajectories of proteins. I was putting these changes into AMBER for distribution, but unfortunately Peter Kollman died and we then proceeded to write our own molecular modeling package, *in lucem* molecular mechanics, that incorporated modern software engineering approaches and accounted for modern hardware etc. In terms of the protein conformational studies, we wanted to not only explore the effects of temperature on proteins, but also mixed solvents. This led to the first simulation of a protein in an aqueous organic solvent. Also, the control in pure water became the first simulation of hydrophobic collapse of a protein. We followed up with the first simulations of chemical denaturation using 8 M urea and the effect of counteracting osmolytes. Approximately 15 years ago we had simulated and validated the unfolding properties of a variety of proteins and we loaded these trajectories into a database that could be queried to address more general questions about protein folding/unfolding. It became evident that our sampling was inadequate so we began our Dynameomics effort, which contains native state and multiple unfolding simulations of representatives of all known autonomous protein folds. This required much development in the area of databases, informatics, as well as visual analytics for big data. Our Dynameomics project produced the largest collection of protein

simulations and structures in the world, 10^5 more structures than the PDB.

a. Levitt, M. Hirshberg, R. Sharon, and **V. Daggett**, Potential Energy Function and Parameters for Simulations of the Molecular Dynamics of Proteins and Nucleic Acids in Solution, *Computer Physics Commun.* **91**: 215-231, 1995. (Force field and methods used for our simulations)

b. Alonso, D.O.V. and **V. Daggett**, Molecular Dynamics Simulations of Protein Unfolding and Limited Refolding: Characterization of Partially Unfolded States of Ubiquitin in Methanol and in Pure Water, *J. Mol. Biol.*, **247**: 501-520, 1995. (1st mixed solvent protein simulation, co-solvent parameters and procedures also developed)

c. Van der Kamp, M.W., Anderson, P.C., Beck, D.A.C., Benson, N.C., Jonsson, A.L., Merkley, E.D., Schaeffer, R.D., Scouras, A.D., Simms, A., Toofanny, R.D., and **V. Daggett**. Dynameomics: A comprehensive database of protein dynamics. *Structure*, **18**, 423-435, 2010. (Description of Dynameomics Project) PMC2892689

d. Bromley, D., Rysavy, S.J., Su, R., Toofanny, R.D., Schmidlin, T., **Daggett, V**. DIVE: A data intensive visualization engine. *Bioinformatics*, **30**, 593-595, 2014. (Development of visual analytics software platform for mining large complex databases) (Also associated patent application) PMC3928528

5. **Discovered alpha-sheet structure in MD simulations, proposed it is linked to toxicity in amyloidogenesis, and performed experiments validating the hypothesis.** During our studies of a variety of amyloidogenic proteins we 'discovered' a new secondary structure, which we dubbed alpha-sheet and we proposed that it is a general and toxic amyloid intermediate adopted by such proteins under amyloidogenic conditions independent of sequence and starting structure. We later found that the structure in our simulations (which we originally called α-sheet had been predicted 50 years earlier, and dismissed, by Pauling and Corey). We have published numerous simulations in this area and in 2007 I moved to the Bioengineering Department so that I could set up a wet lab and pursue experimental studies to test this hypothesis. We have now shown that compounds designed to be complementary to the 'toxic' conformation do indeed bind the toxic oligomers, thereby blocking toxicity and aggregation in different mammalian disease systems, including Abeta, Alzheimer's Disease and transthyretin, systemic amyloidosis and heart disease, as well as amyloid-stabilized biofilms associated with both gram-negative and gram-positive bacteria. These anti-alpha-sheet compounds represent a new class of molecules that selectively target the toxic oligomers.

a. Armen, R.S., DeMarco, M.L., Alonso, D.O.V. and **V. Daggett**, Pauling and Corey's alpha-pleated sheet structure may define the prefibrillar amyloidogenic intermediate in amyloid disease *Proc. Natl. Acad. Sci. USA*, **101**, 11622-11627, 2004.

   Articles written about our discovery:
   Nature, "New Role for Pauling's Ribbons" by Christopher Surridge, **430**, 739, 2004.
   Science, "A Neatly Pleated Alpha Sheet" by Orla Smith, **305**, 1534, 2004.

b. Hopping, G., Kellock, J., Barnwal, R.P., Law, P., Bryers, J.D., Varani, G., Caughey, B., **Daggett, V**. Designed alpha-Sheet Peptides Inhibit Amyloid Formation by Targeting Toxic Oligomers. *eLIFE* **3**: e01681, 2014. PMC4091096

c. Kellock, J., Hopping, G., Caughey, B., **Daggett, V**. Peptides composed of alternating L- and D-amino acids inhibit amyloidogenesis in three distinct amyloid systems independent of sequence. *Journal of Molecular Biology* **428**: 2317-2328, 2016. PMC4884539

d. Maris, N.L, Shea, D., Bleem, A., Bryers, J.D., **Daggett, V**. Chemical and physical variability in structural isomers of an L/D alpha-sheet peptide designed to inhibit amyloidogenesis. *Biochemistry*, **57**: 507-510, 2018. PMC5801053

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Michael H. Gelb

eRA COMMONS USER NAME (credential, e.g., agency login): mhgelb

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of California, Davis, CA	B.S.	06/1979	Chemistry/Biochemistry
Yale University, New Haven, CT	Ph.D.	11/1982	Biochemistry

A. Personal Statement

The Gelb lab has worked on clinical enzymology for the past 22 years. He was the first to develop tandem mass spectrometry for the analysis of lysosomal enzymes in dried blood spots, and this work led to the first newborn screening of a lysosomal storage disease in the USA. Over the past several years, the Gelb lab has developed tandem mass spectrometry newborn screening assays for several lysosomal storage diseases. This work provided a key component leading to the addition of Pompe disease and MPS-I to the federal Recommended Newborn Screening Panel (RUSP). Newborn screening for lysosomal storage diseases is now live in several states in the USA and in a few countries worldwide (Taiwan, Italy and soon in Holland and Japan). The Gelb lab reagents have been commercialized by PerkinElmer for worldwide distribution. Quality control standards are provided by the Centers for Disease Control and Prevention. Additional work has led to newborn screening methods for other inborn errors of metabolism

a. Hong X, Daiker J, Sadilek M, Ruiz-Schultz N, Kumar AB, Norcross S, Dansithong W, Suhr T, Escolar ML, Ronald Scott C, Rohrwasser A, **Gelb MH.** “Toward newborn screening of metachromatic leukodystrophy: results from analysis of over 27,000 newborn dried blood spots.”*Genet Med.* **2021** Mar;23(3):555-561.PMID: 33214709
b. Hong X, Daiker J, Sadilek M, DeBarber AE, Chiang J, Duan J, Bootsma AH, Huidekoper HH, Vaz FM, **Gelb MH.** Toward newborn screening of cerebrotendinous xanthomatosis: results of a biomarker research study using 32,000 newborn dried blood spots. *Genet Med.* **2020** Oct;22(10):1606-1612. PMID: 32523054
c. Elliott, S.; Buroker, N.; Cournoyer, J.J.; Potier, A.M.; Trometer, J.D.; Elbin, C.; Schermer, M.J.; Kantola, J.; Boyce, A.; Tureček, F.; **Gelb,** M.H.; **Scott,** C.R. “Pilot study of newborn screening for six lysosomal storage diseases using Tandem Mass Spectrometry.” *Mol Genet Metab.* **2016,** *118*, 304-309. PMCID: PMC5318163.
d. **Gelb,** M.H.; **Scott,** C.R.; Tureček, F. “Newborn screening for lysosomal storage diseases.” *Clin Chem.* **2015**, *61*, 335-46. PMCID: PMC4345406.

B. Positions and Honors

Positions

University of California, Davis	B.S., Chemistry and Biochemistry	06/1979
Yale University	Ph.D. in Biochemistry	11/1982
Brandeis University	Postdoc with R. H. Abeles	06/1983-08/1985
University of Washington	Assistant Professor of Chemistry	09/1985-08/1991
University of Washington	Adjunct Assistant Professor of Biochemistry	09/1986-08/1991

University of Washington	Associate Professor of Chemistry	09/1991-08/1995
University of Washington	Adjunct Associate Professor of Biochemistry	09/1991-08/1995
University of Washington	Professor of Chemistry	09/1995-
University of Washington	Adjunct Professor of Biochemistry	09/1995-

Awards

American Cancer Society Postdoctoral Fellowship (1983-1985).
Merck New Faculty Career Development Award (1986).
NIH Research Career Development Award (1990-1995).
Alfred P. Sloan Research Fellow (1991-1993).
ICI Pharmaceuticals Group 1991 Award for Excellence in Chemistry.
Pfizer Award in Enzyme Chemistry (Division of Biological Chemistry, American Chemical Society (1993)).
Winner of the Medicines for Malaria Venture Project of the Year (2003).
NIH Merit Award (2007).
Paul Hopkins Faculty Award of the Chemistry Department, University of Washington (2006-2007).
Harry and Catherine Jaynne Boand Endowed Professorship in Chemistry, University of Washington (2008-20014).
Fellow of the American Association for the Advancement of Science (2009-).
Esselen Award in Chemistry in the Public Interest (Northeastern Section American Chemical Society) (2014).
Professor Boris and Barbara L. Weinstein Endowed Chair in Chemistry, University of Washington (2014-).
University of Washington Annual Faculty Lecture Award (2018).
Repligen Award in Biological Chemistry, American Chemical Society (2018)

Honors

NIH Pharmacology Study Section Ad Hoc Member (June, 1989).
Editorial Board Member, Archives of Biochemistry and Biophysics (July, 1993-2003).
NIH Pharmacology Clinical Services Special Emphasis Panel, Ad Hoc Member (May, 1996).
NIH Biochemistry Study Section, Ad Hoc Member (October, 1996).
NIH Biochemistry Study Section, Regular Member (October, 1997-2000).
Co-Chairman, Gordon Conference in Enzymes, Co-Enzymes, and Metabolic Pathways, Kimball Union Academy, Meriden, NH (July, 1997).
NIH Physical Biochemistry Study Section, Ad Hoc Member (June, 1997).
Editorial Advisory Board, "Current Option in Chemical Biology" (January, 1998).
American Chemical Society, Division of Biological Chemistry, Chair of Nominating Committee (1999).
American Chemical Society, Division of Biological Chemistry, Chair of Nominating Committee (2000).
Member of the Scientific Advisory Board, Cell Therapeutics Inc., Seattle, WA (January, 2000-2005).
NIH Bioorganic and Natural Product Study Section, Ad Hoc Member (October, 2000).
NIH Physical Biochemistry Study Section, Regular Member (March, 2001).
Editorial Advisory Board, "Journal of Biological Chemistry" (2002-2007).
NIH Physical Biochemistry Study Section (October, 2002-2006).
NIH Therapeutic Approaches to Genetic Diseases Study Section (June, 2015 and July, 2021 onward).

C. Contributions to Science

1. Phospholipases A_2. The Gelb lab has been a world leader in the study of phospholipases A_2 over the past 30 years. Many of the human secreted phospholipases A_2 were first identified, cloned and studied by the Gelb lab. Together with Profs. M. K. Jain and O. G. Berg, we developed the only reliable methods for analysis of the kinetics of these enzymes including substrate specificity and inhibition. We also showed for the first time that human group X secreted phospholipase A2 plays a critical role in generation of eicosanoids in the airways in asthmatic models in and in human asthma.

a. Jain, M.K.; Yuan, W.; Gelb, M.H. "Competitive Inhibition of Phospholipase A_2 in Vesicles." *Biochemistry,* **1989**, *28*, 4135-4139. PMCID: PMC3442112.
b. Valentin, E.; Ghomashchi, F.; Gelb, M.H. "Novel human secreted phospholipase A_2 with homology to the group III bee venom enzyme." *J. Biol. Chem.*, **2000**, *275,* 7492-7496. PMID: 10713052. doi: 10.1074/jbc.275.11.7492

c. Bartoli, F.; Lin, H-K.; Ghomashchi, F.; Gelb, M.H.; Jain, M.K.; Apitz-Castro, R. "Tight-Binding Inhibitors of the 85-kDa Phospholipase A_2 but not the 14-kDa Phospholipase A_2 Inhibit the Release of Free Arachidonate in Thrombin-Stimulated Human Platelets." *J. Biol. Chem.*, **1994**, *22*, 15625-15630. PMID: 8195211.
d. Henderson, W.R., Jr.; Chi, E.Y.; Bollinger, J.G.; Tien, Y.T.; Ye, X.; Castelli, L.; Rubtsov, Y.P.; Singer, A.G.; Chiang, G.K.; Nevalainen, T.; Rudensky, A.Y.; Gelb, M.H. "Importance of group X-secreted phospholipase A_2 in allergen-induced airway inflammation and remodeling in a mouse asthma model." *J. Exp. Med.* **2007**, *204,* 865-877, PMCID: PMC2118555.

2. Protein Prenylation. The Gelb lab discovered protein prenylation, the attachment of farnesyl and geranylgeranyl groups to the C-termini of proteins in eukaryotic cells. We also showed that heterotrimeric G proteins and GTP binding proteins including Ras, Rab, etc., are prenylated. Prenylation of Ras is required for its oncogenic potential, and our discovery led to a massive campaign in the pharmaceutical industry for inhibitors of protein farnesyltransferase as anti-cancer agents. We also developed inhibitors of protein farnesyltransferase as anti-parasite agents.

a. Farnsworth, C.C.; Wolda, S.L.; Gelb, M.H.; Glomset, J.A. "Human Lamin B Contains a Farnesylated Cysteine Residue." *J. Biol. Chem.,* **1989,** *264*, 20422-20429. PMCID: PMC3443689.
b. Farnsworth, C.C.; Gelb, M.H.; Glomset, J.A. "Identification of Geranylgeranyl-Modified Proteins in HeLa Cells." *Science,* **1990,** *247*, 320-322. PMCID: PMC3442113.
c. Yamane, H.K.; Farnsworth, C.C.; Xie, H.; Howald, W.; Fung, B.K.-K.; Clarke, S.; Gelb, M.H.; Glomset, J.A. "Brain G-proteins Contain an All-trans-Geranylgeranyl-Cysteine Methyl Ester on Their Carboxyl Termini." *Proc. Nat. Acad. Sci. USA,* **1990**, *87*, 5868-5872. PMCID: PMC54430.
d. Farnsworth, C.C.; Seabra, M.C.; Ericsson, L.H.; Gelb, M.H.; Glomset, J.A. "Rab Geranylgeranyl Transferase Catalyzes the Geranylgeranylation of Adjacent Cysteines in the Small GTPases, Rab1A, Rab3A, and Rab5A." *Proc. Natl. Acad. Sci. USA*, **1994,** *91*, 11963-11967. PMCID: PMC45356.

3. Anti-parasite therapeutics. The team at University of Washington led by Gelb, Buckner and Van Voorhis is one of the most successful academic-based centers for drug discovery for parasitic diseases (malaria, African sleeping sickness, Chagas disease and Leishmaniasis). Our program on protein farnesyltransferase inhibitors was awarded the Medicines for Malaria Venture Project of the Year in 2003. Currently we are pursuing several drug candidates that are curative in animal and primate models of African sleeping sickness, Chagas disease and Leishmaniasis with the goal of advancing 1-2 compounds into clinical trials.

a. Gelb, M.H. and Hol, W.G.J. "Parasitology. Drugs to Combat Tropical Protozoan Parasites." *Science*, **2002,** *297,* 343-344, PMID: 12130767. DOI: 10.1126/science.1073126.
b. Bendale, P.; Olepu, S.; Suryadevara, P.K.; Bulbule, V.; Rivas, K.; Nallan, L.; Smart, B.; Yokoyama, K.; Ankala, S.; Pendyala, P.R.; Floyd, D.; Lombardo, L.J.; Williams, D.K.; Buckner, F.S.; Chakrabarti, D.; Verlinde, C.L.; Van Voorhis, W.C.; Gelb, M.H. "Second generation tetrahydroquinoline-based protein farnesyltransferase inhibitors as antimalarials." *J. Med. Chem.*, **2007,** *50,* 4585-4605, PMCID: PMC2894570.
c. Kraus, J.M.; Verlinde, C.L.M.J.; Karimi, M.; Lepesheva, G.I.; Gelb, M.H.; Bucker, F.S. "Rational Modification of a candidate cancer drug for use against chagas disease." *J. Med. Chem.*, **2009,** *52,* 1639-1647; Erratum *J. Med. Chem.*, **52,** 4549; *J. Med. Chem.*, **52,** 4979, PMCID: PMC2715367.
d. Tatipaka, H.B.; Gillespie, J.R.; Chatterjee, A.K.; Norcross, N.R.; Halverson, M.A.; Ranade, R.M.; Nagendar, P.; Creason, S.A.; McQueen, J.; Duster, N.A.; Nagle, A.; Supek, F.; Molteni, V.; Wenzler, T.; Brun, R.; Glynne, R.; Buckner, F.S.; Gelb, M.H. "Substituted 2-Phenylimidazopyridines: A new class of drug leads for human African Trypanosomiasis." *J. Med. Chem.*, **2014,** *57,* 828-835, PMCID: PMC3962778.

4. Applications of Mass Spectrometry in Medicine. Together with Prof. R. Aebersold, the Gelb lab developed Isotope-Coded Affinity Tags (ICAT), which was the first wide-spread reagent for quantitative proteomics. The reagents were commercialized by Applied Biosystems and used by thousands of researchers worldwide.

The Gelb laboratory developed tandem mass spectrometry for newborn screening of inborn errors of metabolism with special emphasis on lysosomal storage diseases. Our lab developed the first-ever newborn

screening program for Pompe disease (Taiwan); this contributed to the successful treatment of dozens of newborns (the infantile form of the disease is usually fatal in the first 1-2 years of life), and lead to the Secretary of Health and Human Services recommendation that Pompe disease be added to nationwide recommended uniform screening panel (RUSP). We also developed preeminent technology for newborn screening of mucopolysaccharidosis-I (Hurler syndrome), leading to the recent recommendation by the Discretionary Committee on Hereditary Diseases and Newborn Screening (DACHDNC) to add Hurler disease to the newborn screening panel. Our laboratory developed the technology for newborn screening of Krabbe disease, as well as mass spectrometry methods for newborn screening of other lysosomal storage diseases: Fabry, Gaucher, Niemann-Pick-A/B, MPS-II, MPS-IIIA, MPS-IIIB, MPS-IVA, and MPS-VI.

Our methods have been commercialized recently by Perkin Elmer Corp. Worldwide launching of the first-ever kit for newborn screening of a panel of lysosomal storage diseases occurred in early 2016.

a. Li, Y.; Scott, C.R.; Chamoles, N.A.; Ghavami, A.; Pinto, B.M.; Tureček, F.; Gelb, M.H. "Direct Multiplex Assay of Lysosomal Enzymes in Dried Blood Spots for Newborn Screening." *Clin. Chem.*, **2004,** *50,* 1785-1796, PMCID: PMC3428798.
b. Hong, X. Y.; Sadilek, M.; Gelb, M. H., "A highly multiplexed biochemical assay for analytes in dried blood spots: application to newborn screening and diagnosis of lysosomal storage disorders and other inborn errors of metabolism." *Genet Med.* **2020,** *22* (7), 1262-1268. PMID: 32307446
c. Hong, X. Y.; Daiker, J.; Sadilek, M.; Ruiz-Schultz, N.; Kumar, A. B.; Norcross, S.; Dansithong, W.; Suhr, T.; Escolar, M. L.; Scott, C. R.; Rohrwasser, A.; Gelb, M. H., "Toward newborn screening of metachromatic leukodystrophy: results from analysis of over 27,000 newborn dried blood spots." *Genet Med.* **2020**. PMID: 33214709
d. Hong, X. Y.; Daiker, J.; Sadilek, M.; DeBarber, A. E.; Chiang, J.; Duan, J.; Bootsma, A. H.; Huidekoper, H. H.; Vaz, F. M.; Gelb, M. H., "Toward newborn screening of cerebrotendinous xanthomatosis: results of a biomarker research study using 32,000 newborn dried blood spots." *Genet Med.* **2020,** *22* (10), 1606-1612. PMID: 32523054 PMCID: PMC7529987

PubMed link to my publications: https://www.ncbi.nlm.nih.gov/pubmed/?term=gelb%2C+michael+h.

OMB No. 0925-0001 and 0925-0002 (Rev. 12/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

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NAME: Gumbiner, Barry M.

eRA COMMONS USER NAME (credential, e.g., agency login): GUMBINERNIH

POSITION TITLE: Professor of Pediatrics

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Cincinnati, Cincinnati, Ohio	B.S.	1976	Biochemistry
University of California San Francisco, San Francisco, CA	Ph.D.	1982	Neurosciences
Massachusetts Institute of Technology, Cambridge, MA	Postdoc	1982-1983	Cell biology
European Molecular Biology Laboratory, Heidelberg, Germany	Postdoc	1983-1985	Cell biology

A. Personal Statement

I have a broad and diverse base of research experience in cell biology, biochemistry, developmental biology, and physiology. I obtained my Ph.D. in Neurosciences at the University of California San Francisco (UCSF) where I discovered that proteins are sorted into distinct pathways for constitutive and regulated secretion. During my postdoctoral research with Kai Simons at the EMBL I studied the role of cell-cell junctions in the development of epithelial polarity, beginning my career-long interest in cadherins, tissue morphogenesis, and epithelial barrier function. I became an Assistant Professor at the University of California San Francisco (UCSF), where my laboratory investigated the functions of cadherins and catenins in tissue development. We set up the early *Xenopus* embryo as a model for cell junction formation and tissue morphogenesis. The combination of our biochemical work identifying β-catenin as a cadherin-associated protein and the use of the *Xenopus* embryos, led to our discovery of β-catenin as the mediator of the Wnt signaling pathway. This initiated my long-term interest in signal transduction and developmental biology. In 1992 I moved to the Memorial Sloan-Kettering Cancer Center where we studied the mechanisms controlling β-catenin signaling and the role of Wnt-β-catenin signaling in embryonic patterning. We also continued to study cadherins at many levels: the role of cadherin regulation in tissue morphogenesis, the cellular mechanisms regulating cadherins, and the biophysical analysis of the structure-function of the cadherin ectodomain. I moved my laboratory to the University of Virginia in 2002 to become Chair of Cell Biology. In addition to our studies on cadherin regulation and structure-function, my laboratory began studies on the role of cadherins in the contact inhibition of proliferation and tumor growth and metastasis. I moved my laboratory in 2015 to the Seattle Children's Research Institute as a Professor at the University of Washington to return to full time research and teaching in order to enhance these research programs and develop new efforts and collaborations in structural biology, cancer biology, and inflammation. My appointment as Adjunct Professor of Biochemistry and association with the Fred Hutchinson Cancer Research Center provide excellent interactions in basic molecular-cellular science and cancer biology, including structural biology/biophysics and signaling.

Throughout my career I have ventured into new areas beyond my immediate experience to make significant new discoveries, learning from colleagues and/or collaborating in order to acquire the necessary expertise. As a result, I have developed a very wide range of interests and expertise, ranging from biophysical/structural through cell biological to developmental and physiological, which will serve well for this project. Furthermore, I have a very good record of training graduate students and postdoctoral fellows who have gone on to successful independent careers and populate the cell adhesion and Wnt signaling fields. This provides evidence that I am able to inspire and lead trainees in my laboratory to achieve significant accomplishments.

B. Positions, Scientific Appointments, and Honors

<u>Positions</u>

10/15- present	Adjunct Professor, Dept. of Biochemistry, University of Washington
07/15 – present	Professor of Pediatrics, University of Washington and Center for Developmental Biology and Regenerative Medicine, Seattle Children's Research Institute
2002 – 2015	Professor and Chairperson, Dept. of Cell Biology, University of Virginia
1996 - 2002	Member with tenure, Program in Cellular Biochemistry and Biophysics, Memorial Sloan-Kettering Cancer Center, New York, NY and Professor, Department of Cell and Developmental Biology, Weill Cornell Medical College, New York, NY
1992 - 1996	Associate Member, Program in Cellular Biochemistry and Biophysics, Memorial Sloan-Kettering Cancer Center, New York, NY and Associate Professor, Department of Cell and Developmental Biology, Weill Cornell Medical College, New York, NY
1992	Associate Professor with tenure, Departments of Pharmacology and Physiology, University of California, San Francisco
1986 - 1992	Assistant Professor, Departments of Pharmacology and Physiology, University of California, San Francisco

<u>Scientific Appointments</u>

2018 – 2022	Associate Program Leader, Cancer Basic Biology Program, Fred Hutchinson and University of Washington Cancer Consortium, Seattle, WA
2011 - present	Editorial Board of Current Opinion in Cell Biology
2001 - present	Section Head – Cell Adhesion, Faculty of 1000
2003 - 2020	Ad Hoc Member of the NIGMS Advisory Council (9/2002) and several NIH study sections (PBC- 6/2003, ICI-6/2007, NIGMS MIRA-6/2018, SEP ZRG1 CB-J(02)-6/2019, NIGMS young invest MIRA-2/2020.
2009 - 2014	Board of Scientific Counselors, National Institute of Child Health and Human Development, NIH
2003 - 2014	Scientific Advisory Board, Max-Planck-Institute of Molecular Biomedicine, Münster, Germany
1999	Chair of the Cell Contact and Adhesion Gordon Conference
1995 - 2012	Editorial Board, Current Biology
7/95 - 7/04	Editorial Board, Journal of Cell Biology (including monitoring editor)
6/94, 10/95-7/00	Cell Biology and Physiology-1 (CBY-1) Study Section, Chairperson final 2 yrs
2003 - 2008	Participant in NIH workshops (CSR review of Cell Biology study sections-12/03, NIGMS Future of Structural Genomics-10/08, NICHD Scientific Vision Development-2/2011)
1993 – 2005	Member of several academic review committees (Graduate Program in Cell Biology, U. Connecticut Farmington-11/93, State of Washington review of Fred Hutchinson Cancer Center proposal for new graduate program - 4/93, Review of Developmental Biology Center at UC Irvine - 05/2003, Review of Molecular Cell Developmental Biology Dept. at Mt. Sinai School of Medicine-11/2005)
1992 - 1995	Section Editor for Current Opinions in Cell Biology

<u>Honors and Awards</u>

2018-2022	NIH MIRA (Maximizing Investigators Research Award) R35GM122467
2013	Elected Fellow of the American Association for the Advancement of Science (AAAS)
2002-2015	Harrison Distinguished Professor of Cell Biology, University of Virginia
2000-2010	NIH MERIT Award, R37 GM37432, "Catenin and Cadherin Signaling in Development and Cancer"
1996–1998	Editorial Board, Molecular Biology of the Cell
1994–1999	Irma T. Hirschl Career Scientist Award
1988-1992	Established Investigatorship Award. American Heart Association
1982–1985	Helen Hay Whitney Postdoctoral Fellowship
1981-1982	Mary Morrow Prize for Excellence in Scientific Writing, First Prize, Univ. of Calif., San Francisco
1981-1982	University of California Chancellor's Fellowship for graduate study
1976-1979	National Science Foundation Graduate Fellowship
1976	Summa Cum Laude with Honors for Independent Study, University of Cincinnati

C. Contributions to Science

I. E-cadherin control of junctional complex and epithelial barrier function

When I began my postdoctoral work, the epithelial junctional complex had been described morphologically, but nothing was known about molecular constituents or functional regulation. I identified E-cadherin (uvomorulin, L-CAM) as functionally important for the formation of the epithelial permeability barrier [1]. My own independent group at UCSF went on to show that uvomorulin/E-cadherin controls the state of the epithelial junctional complex [2]. The same protein had been described as important for overall cell-cell recognition and adhesion events, but these were the first findings demonstrating direct control of cell junction and barrier formation. They provided initial insights into a major concept in cell and cancer biology, the role of E-cadherin in regulating the epithelial-mesenchymal transition (EMT), by virtue of its control of epithelial junction assembly.

We also participated in the identification of specific protein components of the tight junction and found that occludin proteins of the tight junction can regulate paracellular permeability without affecting epithelial cell morphology or the overall organization of the epithelial junctional complex [3], in contrast to E-cadherin. Since then, other groups have found that the claudins constitute the main sealing elements of the tight junction and function in association with occludin.

1. Gumbiner, B. and Simons, K. (1986) A functional assay for proteins involved in establishing an epithelial occluding barrier: Identification of a uvomorulin-like polypeptide. J. Cell Biol. 102, 457-468. PMCID: PMC2114088.

2. Gumbiner, B.* Stevenson, B. and Grimaldi, A. (1988) The role of the cell adhesion molecule uvomorulin in the formation and maintenance of the epithelial junctional complex. J. Cell Biol. 107, 1575-1587. PMCID: PMC2115263. * - senior author/PI

3. Wong, V. and **Gumbiner, B.M.** (1997) A synthetic peptide corresponding to the extracellular domain of occludin perturbs the tight junction permeability barrier. J. Cell Biol., 136, 399-409. PMCID: PMC2134825.

II. Basic molecular mechanisms of cadherin-mediated adhesion

At the early stage of this field, we wanted to identify the molecular linkages between E-cadherin and the cytoskeleton. Ozawa and Kemler identified catenin polypeptides on gels, but we were the first to identify β-catenin as a homolog of the *Drosophila* Armadillo protein; by sequencing and cloning the cDNA encoding it [4].

We learned a lot about the basic molecular and cellular mechanisms controlling cadherin function. Early on we provided evidence that oligomerization and clustering of cadherins in the plasma membrane were important for formation of functional adhesive interactions between cells [5,6]; now widely accepted concepts. We discovered that cadherin juxtamembrane domain and p120-catenin played an important role in cadherin clustering and adhesive strengthening in addition to the known cytoskeletal anchoring via β- and α- catenins [6]. In an important collaboration with Larry Shapiro's group, we solved the first 3D crystal structure of an entire cadherin ectodomain (domains EC1-EC5), that of Xenopus C-cadherin [7]. Our contributions were to express the full ectodomain in mammalian cells and produce it in large quantities, to purify it to homogeneity sufficient for crystallization, and to encourage Shapiro to attempt to crystallize such an elongated glycoprotein. The solution of the full ectodomain revealed several insights which had not been realized from the previous structure of the EC1-2 fragment; including the idea that the W2 residue-mediated strand exchange forms the adhesive bond rather than cis dimer, and the curved structure of the ectodomain facilitated by the inter-EC domain calcium binding sites. This structure has become the paradigm for understanding classical cadherins.

4. McCrea, P., Turck, C.W., and **Gumbiner, B.** (1991) A homolog of the armadillo protein in Drosophila (plakoglobin) associated with E cadherin. Science, 254, 1359-1361. PMID:1962194.

5. Brieher, W.M., Yap, A.S., and **Gumbiner, B.M.** (1996) Lateral dimerization is required for the homophilic binding activity of C-cadherin. J. Cell Biol., 135:487-496. PMCID:PMC2121050.

6. Yap, A.S., Niessen, C.M., and **Gumbiner, B.M.** (1998) The juxtamembrane region of the cadherin cytoplasmic tail supports lateral clustering, adhesive strengthening and interaction with p120ctn. J. Cell Biol., 141:779-790. PMCID: PMC2132752.

7. Boggon, T. J. , Murray, J., Chappuis-Flament, S., Wong, E., **Gumbiner, B.M.**, and Shapiro, L. (2002) C-Cadherin ectodomain structure and implications for cell adhesion mechanisms. Science, 296:1308-1313. PMID: 11964443.

III. Role of β-catenin in Wnt signaling.

We identified β-catenin as a homolog of the *Drosophila* Armadillo protein [4], which was known to mediate wingless (Wnt) signaling in *Drosophila*, and we also showed that β-catenin mediates Wnt signaling in vertebrates in studies of embryonic axis induction in *Xenopus* [8,9,10]. We discovered that β-catenin is present in the nucleus as well as at cell-cell contacts and that its signaling activity is associated with its translocation into the nucleus [8,9]. Other groups demonstrated shortly after that β-catenin associates with TCF transcription factors. In a collaboration with Frank Costantini's group we discovered that a cytoplasmic protein called Axin functioned along with glycogen synthase kinase 3 (GSK3) to antagonizes β-catenin signaling activity and nuclear localization [10]; additional work by many groups showed that this occurred through regulated degradation of β-catenin by the so-called "destruction complex". We showed that the association of β-catenin with cadherins also antagonizes Wnt-β-catenin signaling in *Xenopus* embryos and it does so by preventing β-catenin nuclear translocation [9]. We found that this antagonism of Wnt-β-catenin signaling by cadherins is general and also acts in tumor cells to inhibit Wnt-β-catenin-dependent cell proliferation [11]. This concept of cadherin inhibition of Wnt-β-catenin signaling is now widely recognized and commonly invoked as important in many contexts (including the EMT) as a major mechanism of controlling β-catenin signaling.

8. Funayama, N., Fagotto, F., McCrea, P., and **Gumbiner, B.M.** (1995) Embryonic axis induction by the armadillo repeat domain of β-catenin: evidence for intracellular signaling. J. Cell Biol., 128, 959-968. PMCID: PMC2120405.

9. Fagotto, F., Funayama, N., Glück, U. and **Barry M. Gumbiner** (1996) Binding to cadherins antagonizes the signaling activity of β-catenin during axis formation in Xenopus. J. Cell Biol., 132, 1105-1114. PMCID: PMC2120760

10. Zeng, L., Fagotto, F., Zhang, T., Hsu, W., Vasicek, T.J., Perry, W.L. III, Lee, J.J., Tilghman, S.M., **Gumbiner, B.M.**, and Costantini, F. (1997) The mouse Fused locus encodes Axin, an inhibitor or the Wnt signaling pathway that regulates embryonic axis formation. Cell, 90:181-192. PMID: 9230313.

11. Gottardi, Cara J., Wong Ellen, and **Gumbiner, B.M.** (2001) E-cadherin suppresses cellular transformation by inhibiting β-catenin signaling in an adhesion-independent manner. J. Cell Biol., 153: 1049–1059. PMCID: PMC2174337

IV. Roles and mechanisms of cadherin allosteric regulation at the cell surface

My group was the first to demonstrate that dynamic regulation of cadherin adhesive activity at the cell surface mediates cell rearrangements during tissue morphogenesis. We wondered how cells in the *Xenopus* gastrula could rearrange and exchange neighbors during convergent extension to mediate tissue elongation. We discovered that the adhesive activity of *Xenopus* C-cadherin was downregulated in response to morphogenesis inducing growth factors, and we developed a C-cadherin activating antibody to demonstrate that this downregulation was required for tissue morphogenesis [12]. We've also identified activating mAbs for E-cadherin in epithelial cells [13] and activating mAbs for vascular endothelial cadherin (VE-cadherin) that inhibit endothelial leakiness induced by physiological agents [14]. The concept of dynamic cadherin regulation is now widely recognized as important for different cadherins in a wide variety of morphogenetic and physiological processes, including tissue morphogenesis in embryos, angiogenesis and vasculogenesis, regulation of vascular permeability, and collective cell migration. Many mechanisms have been proposed to explain cadherin regulation, but our findings showed that cadherins are regulated directly at the cell surface independent of any changes in levels of the protein [12,13,14,15].

We've begun to understand how changes in cadherin structure mediate dynamic regulation of adhesion at the cell surface. Activity-associated conformations appear to be propagated from the cytoplasmic domain all through the extracellular domains. Monoclonal antibodies to E-cadherin that either rapidly activate its adhesive activity or distinguish active and inactive states of E-cadherin on the surface of cells recognize conformational epitopes at various sites in different EC domains [13], and activation of adhesion is also triggered by changes in p120-catenin serine phosphorylation on the cytoplasmic side [13]. In a collaboration with Deborah Leckband's group, we provided direct biophysical evidence for allosteric regulation of the adhesive binding activity of E-cadherin, and ongoing work with cryo-EM, X-ray crystallography, and molecular dynamic simulations of cadherins in complex with activation Fabs reveals structural changes with activating antibody binding (submitted). Importantly, we have found that the activity state of E-cadherin at the cell surface has critical roles *in vivo* because it controls mouse mammary tumor metastasis [15] and epithelial barrier function and inflammation in mouse models of inflammatory bowel disease [16].

12. Zhong, Y, Brieher, W.M., and **Gumbiner, B.M.** (1999) Analysis of C-cadherin regulation during tissue morphogenesis with an activating antibody. J. Cell Biol., 144:351-359. PMCID:PMC2132887.
13. Petrova, Y.I., M.M. Spano, and **B.M. Gumbine**r, (2012) Conformational epitopes at cadherin calcium-binding sites and p120-catenin phosphorylation regulate cell adhesion. Molec. Biol. Cell. 23:2092-2108. PMCID: PMC3364174
14. Park, K-S, Schecterson, L, and **Gumbiner, BM** (2021) Enhanced endothelial barrier function by monoclonal antibody activation of vascular endothelial cadherin. Am. J. Physiol. Heart Circ. Physiol. 320: H1403–H1410. PMID: 33577432, PMCID: PMC8260392
15. Na, TY, Schecterson, Mendonsa, A.M., and Gumbiner, B.M. (2020) The functional activity of E-cadherin controls tumor cell metastasis at multiple steps. Proc. Natl. Acad. Sci. 117 (11) 5931-5937. PMCID: PMC7084067.
16. Bandyopadhyay, C, Schecterson, L, and **Gumbiner, B.M.** (2021) E-cadherin activating antibodies limit barrier dysfunction and inflammation in mouse inflammatory bowel disease, Tissue Barriers, 9:4, 1940741, DOI: 10.1080/21688370.2021.1940741 PMID: 34402758, PMCID: PMC8794503

V. Cadherins, contact Inhibition of proliferation, and the Hippo signaling pathway.

Contact inhibition has long been recognized to be a mechanism controlling proliferation in continuous cell layers and tissues, and the lack of contact inhibition is a well-known property of cancer cells. E-cadherin had been implicated in contact inhibition, but it was not known whether this was due to a direct signaling function or simply due to its adhesive function, which could indirectly facilitate the formation of other types of cell interactions. Therefore, we first demonstrated that the formation of the E-cadherin homophilic bond directly mediates a growth inhibitory signal in epithelial cells, independent of other types of cell contacts [17]. Although we previously found that E-cadherin could inhibit β-catenin-Wnt signaling [12], this occurred independent of its role in mediating cell-cell adhesion. Through investigating the mechanism of the adhesion-dependent growth inhibitory signaling, we discovered that it is mediated to a large extent through stimulation of the Hippo signaling pathway through regulation of the nuclear localization of the transcriptional activator YAP [18]. We also found that cell-cell contact controls the levels of the YAP transcriptional effectors, the TEAD proteins, by a distinct pathway involving the regulation of its palmitoylation and stability [19]. These findings are important because the Hippo pathway, YAP, and TEADs have been found to regulate growth of a wide variety of tissues during development, regeneration, and tumorigenesis, and it provides a new explanation for the role of E-cadherin in the control of tissue and cancer growth.

Contact inhibition acts antagonistically to mitogenic growth factor signaling to control cell proliferation [e.g. 17]. We discovered an important mechanism for this antagonism; finding that several different mitogenic factors inhibit the Hippo pathway, resulting in the nuclear localization of YAP to activate growth promoting target genes [20]. This effect was mediated by growth factor stimulation of PI3Kinase-PDK-1 signaling, but independent of its main downstream effectors Akt [20], providing evidence for a new pathway of growth factor signaling.

17. Perrais, M., Chen, X., Perez-Moreno, M., and **Gumbiner, B.M.** (2007) Homophilic ligation of E-cadherin directly inhibits cell growth and EGF receptor signaling independent of other cell interactions. Mol. Biol. Cell., 18:2013-2025. PMCID: PMC1877107. 96
18. Kim, N.G., Koh, E.J., and **Gumbiner, B.M.** (2011) E-cadherin mediates contact inhibition of cell proliferation through Hippo signaling pathway components. Proc. Natl. Acad. Sci.., 108: 11930-11935. PMCID: PMC3141988 109
19. Kim NG, Gumbiner BM. Cell contact and Nf2/Merlin-dependent regulation of TEAD palmitoylation and activity. Proc Natl Acad Sci U S A. 2019 May 14;116(20):9877-9882. PMCID: PMC6525549.
20. Fan, R., Kim, NG, and **Gumbiner, B.M.** (2013) Regulation of Hippo Pathway by Mitogenic Growth Factors via PI3-Kinase and Phosphoinositide-Dependent Kinase-1. Proc. Natl. Acad. Sci, 110(7): 2569-2574. PMCID: PMC3574943

Complete List of Published Works:

https://pubmed.ncbi.nlm.nih.gov/?term=%28%28%28%28%28%28%28%28%28adhesion%29+OR+Adrenocorticotropic+Hormone%29+OR+polarity%29+OR+epithelial%29+OR+cadherin%29+OR+catenin%29+OR+wnt%29+OR+xenopus%29+AND+Gumbiner+B%29+OR+Gumbiner+BM&sort=date

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

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NAME: Jung, Barbara H.

eRA COMMONS USER NAME (credential, e.g., agency login): bhjung

POSITION TITLE: Professor of Medicine

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Ludwig-Maximilians-Univ, Munich, Germany	M.D.	06/1996	Internal Medicine
Sidney Kimmel Cancer Center, San Diego, CA	Post-Doc Fellow	06/1996-05/1999	Colon Cancer
Univ. of Calif., San Diego, La Jolla, CA	Residency	06/1999-06/2001	Internal Medicine
Univ. of Calif., San Diego, La Jolla, CA	Fellowship	07/2001-06/2004	Gastroenterology

A. Personal Statement

I am a practicing Gastroenterologist with a clinical focus in hereditary GI diseases. My research centers on the mechanisms of GI epithelial cell growth and motility and specifically the modulation of growth suppressive and pro-migratory signaling by TGFβ family members. Previous work from my lab has demonstrated the importance of the primary receptor of the growth suppressive TGFβ family member activin in GI epithelia. Currently, my group investigates canonical and non-canonical activin signaling including activation of AKT and downstream targets enhancing epithelial to mesenchymal transition and cell migration. In addition, we have explored their respective effects on colon epithelia and the surrounding stroma. We have also brought our expertise in activin signaling to the problem of acute pancreatitis and observed that worse disease is associated with elevated serum activin. In mouse models of acute pancreatitis, blocking activin with a neutralizing antibody decreased disease severity without reducing pancreas regeneration. As the Chair of the Department of Medicine at the University of Washington, I am committed to promoting translational research. I also directly interact with the GI fellows while being on clinical service and through educational activities. I have developed a research team leveraging the collection of clinical data from acute pancreatitis patients (Papachristou and Yazici), manipulating murine models of acute pancreatitis to understand the potential for translation of activin inhibition to the clinical setting (Jung, Bialkowska, and Husain laboratories) and delving into the molecular mechanisms of activin in acute pancreatitis to develop new clinical targets (Jung and Husain laboratories). Under my leadership at UIC, we have competed for and been awarded National Pancreas Foundation Designation. My clinical practice allows us to formulate clinically relevant questions with near future impact as in the proposed studies. My role as Department Chair while involved, permits me to leverage resources to support translational studies in my Department as well as protect time for my own studies.

B. Positions and Employment

2004-2007 Assistant Adjunct Professor of Medicine
University of California, San Diego, Division of Gastroenterology, La Jolla, CA
2007-2009 Assistant Professor of Medicine
University of California, San Diego, Division of Gastroenterology, La Jolla, CA
2009-2011 Assistant Professor of Medicine

Northwestern Feinberg School of Medicine, Division of Gastroenterology, Chicago, IL
2011-2013 Associate Professor of Medicine
Northwestern Feinberg School of Medicine, Division of Gastroenterology, Chicago, IL
2013- 2017 Associate Professor of Medicine
University of Illinois at Chicago and Chief, Division of Gastroenterology & Hepatology
Chicago, IL
2017-2019 Professor of Medicine
University of Illinois at Chicago and Chief, Division of Gastroenterology & Hepatology
Chicago, IL
Sept. 2019- Professor of Medicine
University of Washington, and Chair, Department of Medicine, Seattle, WA

Other Experience and Professional Memberships
2001- Member, Gastroenterology Research Group (GRG)
President 2014-2016
2002- Member, Women in Cancer Research (WICR)
2005- Member, AACR
2005- Member, AGA
2012- Fellow, AGA (AGAF)

Honors
93-94 Deutsche Akademischer Austausch Dienst" (DAAD) Award
96-99 Deutsche Forschungsgemeinschaft" (DFG) Fellowship
2003 Foundation for Digestive Health and Nutrition (FDHN) Fellow to Faculty Transition Award
2003 Giannini Family Foundation Research Fellowship Award
2006 San Diego Padres "Medical All Star"
2007 UCSD Division of Gastroenterology Excellence in Clinical Teaching Award
2011 Northwestern Division of Gastroenterology Excellence in Clinical Teaching Award
2015 College of Medicine Team Member Servant Leadership Award, UIC
2016 Selected as UIC Healthy City Collaborative Researcher of the Month
2016 Hugh R. Butt Family Named Visiting Professor, Mayo Clinic, Rochester, MN
2016 Thomas J. Layden Endowed Professorship, University of Illinois at Chicago, IL
2019 Robert G. Petersdorf Endowed Chair in Medicine, University of Washington, Seattle, WA

C. Contributions to Science

1. *Activin signaling in colon cancer and pancreatitis*. Understanding the changes in prometastatic cell signaling pathways in colon cancer is critical to the development of new therapeutic approaches and for the development of individualized treatment regimens. We were the first to show that disruption of activin signaling through mutation, loss of heterozygosity or promoter hypermethylation of *ACVR2,* activin's primary receptor, leads to increased local colon tumor growth, but a decrease in migration in colon cancer cells in an *ACVR2*-dependent manner. Then, we extended those studies to include other TGFβ super family (TGFβ, activin and BMP) members regulating colon cancer cell growth. We observed the bone morphogenetic proteins (BMPs) signaling and growth suppression of colon cancer cells is mediated by SMAD4-dependent signaling facilitated by p21 through improved protein stability. In addition, activin and TGFβ driven growth inhibition of CRC cells utilizes the canonical SMAD4-dependent pathway. In contrast, our studies demonstrate that activin signaling is instrumental in promoting a metastatic phenotype in CRC cells is independent of SMAD4 activation and utilizes the Akt/PI3K pathway while TGFβ induced cell migration utilizes MAP/ERK activation underscoring the importance of both activin and TGFβ in advanced colon cancer. Recently, we have expanded our studies to other GI disease including pancreatitis. We were able to show that increased activin levels in acute pancreatitis is associated with worse patient outcome and show evidence that blocking activin *in vivo* may be a novel therapeutic approach.

a) **Jung B**, Beck SE, Fiorino A, Doctolero RT, Smith EJ, Bocanegra M, Cabrera BL, and Carethers JM. (2007) Functional correlation of growth suppression with activin treatment in microsatellite unstable, activin type 2 receptor (ACVR2)-restored colorectal cancer cells. **Gastroenterology** 132(2):633-44.PMCID: PMC4139066

b) Bauer J, Sporn JC, Cabral J, Gomez J, and **Jung B**. (2012) Effects of activin and TGFβ on p21 in colon cancer. **PLoS One** 7(6):e39381., PMCID: PMC3383701
c) Bauer J, Akagi N, Principe D, Bishehsari F, Spehlmann M, Eckmann L, Grippo P and **Jung B**. (2015) Activin and TGFβ use diverging mitogenic signaling in advanced colon cancer. **Molecular Cancer** 14:182,: PMCID: PMC4619565
d) Staudacher JJ, Yazici C, Carroll T, Bauer J, Pang J, Krett N, Xia Y, Wilson A, Papchristou G, Witcomb DC, Fantuzzi G and **Jung B**. (2017) Activin in acute pancreatitis: Potential risk-stratifying marker and novel therapeutic target. **Sci Rep** 7(1):12786 PMCID PMC5630611
e) Thomas, AT, Castellanos K, Mancinelli G, Xia Y, Yazici C, Fantuzzi G, Hwang S, Krett N, Papachristou G, Whitcomb D, and **Jung B**. (2020) Activin A modulates inflammation in acute pancreatitis and strongly predicts severe disease in patients with high BMI. **Clinical and Translational Gastroenterology** 2020;11:e00152. PMCID PMC7263641

2. *Targeting DNA repair in colon cancer.* Homologous recombination (HR) repair to DNA requires functional BRCA1, and loss of HR is associated with increased risk of cancer. While colon cancer expresses BRCA1, mutations to BARD1, a dimerization partner required for BRCA1 function, are detected in colon cancer. We have observed that expression of splice variants of *BARD1* in colon cancer patients are associated with a poor outcome indicating that BARD1 splice variants may be useful as biomarkers to improve risk stratification for colon cancer patients. We have explored the mechanistic impact of a specific BARD1 variant, namely BARD1β and observed that its expression leads to increased cellular migration indicative of a more metastatic phenotype. In addition, expression of the BARD1β variant imparts increased sensitivity to poly (ADP-ribose) polymerase (PARP) inhibitors which is further enhanced by DNA replication inhibitors such as irinotecan. These data indicate that BARD1 status of CRC tumors may select for a population of patients that could have a favorable response to therapies which include PARP-1 inhibition combined with DNA replication inhibitors. Currently, we are proposing a clinic trial based on BARD1 status with PARP-1 inhibitors in refractory CRC.

a) Sporn JC, Hothorn T, and **Jung B**. (2011) BARD1 expression predicts outcome in colon cancer. **Clin Cancer Res**. 17(16):5451-62. PMCID: PMC3372413
b) Ozden, O, Bishehsari, F, Bauer, J, Park, Jana, A, S-H Baik, SH, Sporn, JC, Staudacher, JJ, Yazici, C, Krett, N and **Jung B.** (2016) Expression of an Oncogenic BARD1 Splice Variant Impairs Homologous Recombination and Predicts Response to PARP-1 Inhibitor Therapy in Colon Cancer. **Science Reports** 6:26273; PMCID:4873788

3. *Activin and TGFβ regulation and the TME*. After dissecting activin and TGFβ signaling in colon cancer epithelial cells (see 1.), we next turned our attention to the assessment of the role of the tumor stroma in advanced CRC and pancreatic cancer signaling. For this, we have determined contributions of stromal TGFβ and immune infiltration in patient tumors as well as models of colon and pancreatic cancer. Loss of TGFβ signaling in human colon tumors was associated with increased inflammatory burden. Given the importance of epithelial-stromal interaction in tumorigenesis and recent acknowledgement of not only TGFβ's but also activin's role in stromal effects in CRC, we measured activin secretion following TGFβ treatment of colonic stromal cells as well as stromal cells in co-culture with epithelial colon cancer cells. We found that GI stromal cells amplify TGFβ pro-oncogenic function through thus far unrecognized induction and utilization of activin signaling. To explore the impact of attenuated TGFβ signaling on tumor biology, we have generated mouse models of both colon and pancreatic cancers with either systemic or epithelial cells specific deficiency of TGFβ receptor (TGFBR). In colon cancer model mice with a systemic decrease in TGFBR, we observed increased inflammation and accelerated tumor formation which was exacerbated in the model with epithelial specific decrease in TGFBR. In our mouse models of pancreatic cancer, we found that epithelial suppression of TGFβ signals facilitated pancreatic tumorigenesis whereas global loss of TGFβ signaling protected against tumor development vial inhibition of tumor associated fibrosis, stromal TGFβ1 production and the resultant restoration of anti-tumor immune function. Human pancreatic ductal adenocarcinoma (PDAC) has extensive stromal involvement and we elucidated activin A expression which was significantly higher in cancer patients, with expression in stroma correlated with shorter survival. Since pancreatic cancer has a high stiffness and stromal cells play a critical biophysical role in cancer progression, we found in CRC that increased tumor microenvironment stiffness leads to stromal cell mediated activin A signaling. An acknowledgement of our prominence in the field is evidenced by the integrated model of TGFβ superfamily signaling we were asked to present in our recent review published in *Gastroenterology*.

a) Principe DR, DeCant B, Vitello D, Mangan RJ, Wayne EA, Mascarinas E, Diaz A, Bauer J, McKinney RD, Khazaie K, Pasche B, Dawson, DW, Munshi HG, Grippo PJ and **Jung BH.** (2016) Loss of TGFβ Signaling Promotes Colon Cancer Progression and Tumor-Associated Inflammation. **Oncotarget,** published online June 4, 2016. PMID 27270652
b) Principe DR, DeCant B, Mascarinas E, Wayne EA, Akagi N, Bauer J, Hwang R, Pasche B, Dawson, DW, Fang D, Bentram DJ, Munshi HG, **Jung BH** and Grippo PJ. (2016) Stromal-Derived TGFβ Facilitates Pancreatic Cancer Development via Repression of the Cytotoxic Response. **Cancer Research** 79(9): 2525-2536. PMCID: PMD4873388.
c) Staudacher JJ, Bauer J, Jana A, Tian J, Carroll T, Mancinelli G, Ozden O. Krett N, Gusman G, Kerr D, Grippo P, and **Jung B.** (2017) Activin signaling is an essential component of the TGF-β induced pro-metastatic phenotype in colorectal cancer. **Scientific Reports** 7:5569 PMCID: PMC4619565**.**
d) Mancinelli G, Torres C, Krett N, Bauer J, Castellanos K, McKinney R, Dawson D, Guzman G, Hwang R, Grimaldo S, Grippo P, Jung B. (2021) Role of stromal activin A in human pancreatic cancer and metastasis in mice. Sci Rep. 2021;11(1):7986. PMCID: PMC8042028.
e) Bauer J, Emon MAB, Staudacher JJ, Thomas AL, Zessner-Spitzenberg J, Mancinelli G, Krett N, Saif MT, Jung B. (2020) Increased stiffness of the tumor microenvironment in colon cancer stimulates cancer associated fibroblast-mediated prometastatic activin A signaling. Sci Rep. 2020;10(1):50. PMCID: PMC6952350.
f) **Jung B,** Staudacher JJ, and Beauchamp D. (2017) Transforming growth factor β super family signaling in development of colorectal cancer. **Gastroenterology**, 152(1):36-52 PMCID: PMC5550896

My Bibliography URL:
http://www.ncbi.nlm.nih.gov/sites/myncbi/barbara.jung.1/bibliography/40834917/public/?sort=date&direction=ascending

D. Additional Information: Research Support and/or Scholastic Performance
ACTIVE

2R01CA141057-06A1 Jung (PI) 09/01/10-06/30/2022
NIH NCI
Mechanisms of colon cancer metastasis: combined role of activin and TGFbeta
The main goal of this study is to understand the mechanisms and cross-regulation of the activin and TGFβ signaling pathways that are important is colon cancer metastasis to allow functional assessment *in toto* and to better select patients for treatments based on individual tumor biology.

1U54MD012523-01 Winn, Ramierez-Valles, Daviguls (mPIs) 09/2301/17-06/30/2022
NIH NIMHD
"Chicago Health Equity Research Center (CHER)"
A program project grant that explores the impact of environmental stress on the development of cancer.
Role: Co-I

1R01 R01DK114126-01 Sun (PI) 04/01/18-03/31/22
NIH NIDDK
How Vitamin D Receptor Influences Intestinal Barrier
This study will investigate the role of intestinal VDR in regulating tight junctions.
Role: Co-I

RECENTLY COMPLETED

R01CA204808-02 Gaskins, Mutlu, Ridlon, Tussing-Humphreys (mPIs) 08/24/2016-07/31/2021

NIH NCI
Diet modulation of bacterial sulfur and bile acid metabolism and colon cancer risk
A human diet intervention study that tests the central hypothesis that the bile salt taurocholic acid is a key diet-controlled metabolite whose metabolism by specific colonic bacteria yields a carcinogen and a tumor-promoter.
Role: Co-I

Pilot Study #2018-01 Jung (PI) 11/16/2017-11/15/2019
UIC Center for Clinical and Translational Sciences
"Delineating mechanisms of health disparity in acute pancreatitis"
A pilot grant to investigate if clinical parameters in African Americans can contribute to increased acute pancreatitis and to investigate the role of activin in the development of acute pancreatitis and whether inhibition of activin can provide therapeutic benefit.

Pilot Project Jung/Fantuzzi (PI) 07/01/2016-06/30/2017
American Gastroenterological Association (AGA)
Activin as a therapeutic target and prognostic marker in pancreatitis
The aim of this project is to examine if activin is increased with severity of pancreatitis in mouse models and in the serum of acute pancreatitis patient and determine if that is correlated with disease severity.

UIC-CCTS Jung (PI) 03/10/2017-02/28/2018
"*Development of a high throughput screen (HTS) for identification of a novel activin pathway inhibitor"*
A pilot study to develop an indicator cell line for a high throughput screen of small molecule inhibitors to identify candidates which inhibit activin actions as a potential therapeutic.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: José A. López

eRA COMMONS USER NAME (credential, e.g., agency login): joselz

POSITION TITLE: Member, Bloodworks Research Institute

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
New Mexico Institute of Mining & Technology	B.S.	1977	Chemistry
University of New Mexico, Albuquerque, NM	M.D.	1981	Medicine
University of Washington, Seattle, WA	Intern/Res.	1981-1984	Internal Medicine
University of Washington, Seattle, WA	Clinical Fellow	1984–1985	Hematology-Oncology
University of Washington, Seattle, WA	Sr. Fellow	1986–1989	Biochemistry

A. Personal Statement

For almost 3 decades, I have led an active laboratory investigating the hemostatic system and its role in disease. The areas studied have been very broad, although primary focus has been on the two proteins that mediate the initial stage of platelet adhesion, the platelet membrane glycoprotein (GP) Ib-IX-V complex and its vessel wall ligand, von Willebrand factor (VWF). This work has involved studies that range from basic biochemical or genetic studies to patient studies of interventions for diseases such as thrombotic thrombocytopenic purpura and sickle cell disease. We continue to look at the involvement of this adhesive system in numerous diseases, including trauma hemostasis, bleeding and clotting disorders, atherosclerosis, microangiopathies, sickle cell disease, sepsis, HIV, and malaria.

Ongoing and recently completed projects

R01 HL137991
Chung (PI), Role: Co-investigator
9/01/17 – 6/30/21
The Biology of VWF Self-Association

R35 HL145262
López (PI)
3/15/19 – 2/28/26
Molecular and Translational Studies in Hematologic Disorders

R01 HL078610
Lindner (PI), Role: Co-investigator
4/01/19 – 2/28/23
Molecular Imaging of Platelets and Oxidative Stress in Atherosclerosis

Andy Hill CARE Fund
Chung/López (PI)
7/01/20 – 6/30/21
Development of Single-Chain Variable Fragment Antibodies to Block SARS-CoV-2 infection

R01 HL153072
Stolla (PI), Role: Co-investigator
7/01/20 – 6/30/25
Role of TRPM8 in Cold-stored Platelet Transfusions

R61 HL154250
Zheng/Dong (PI), Role: Co-investigator
9/01/20 – 8/31/25
3D Models of the Blood-Brain Barrier for Studying Trauma-induced Cerebral and Systemic Injuries

R01HL158606
Lood (PI), Role: Co-Investigator
8/25/21 – 6/30/26
Role of Mitochondria in SLE and its Cardiovascular Complications

Relevant Citations

1. Sullam, P.M., Hyun, W.C., Szöllösi, J, Dong, J.-f., Foss, W.M., and **López, J.A.** (1998) Physical proximity and functional interplay of the glycoprotein Ib-IX-V complex and the Fc receptor FcγRIIA on the platelet plasma membrane. J. Biol. Chem. 273(9):5331–36.
2. Zheng, Y., Chen, J., and **López, J.A.** (2015) Flow-driven assembly of VWF fibers and webs in *in vitro* microvessels. Nat Commun. 2015; 6:7858-68. PMCID: PMC4522708.
3. Feghhi, S., Munday, A.D., Tooley, W.W., Rajsekar, S., Fura, A.M., Kulman, J.D., **López, J.A.**, Sniadecki, N.J. (2016) Glycoprotein Ib-IX-V complex transmits cytoskeletal forces that enhance platelet adhesion. Biophys J. 111:601-608. PMCID: PMC4982925.
4. Interlandi G., Yakovenko O., Tu A.Y., Harris J., Le J., Chen J., **López, J.A.**, Thomas W.E. (2017) Specific electrostatic interactions between charged amino acid residues regulate binding of von Willebrand factor to blood platelets. J Biol Chem. 292:18608-18617. PMCID: PMC5682969.
5. Nagao R.J., Marcu R., Wang Y., Wang L., Arakawa C., DeForest C., Chen J., **López, J.A.**, Zheng Y. (2019) Transforming endothelium with platelet-rich plasma in engineered microvessels. Adv Sci (Weinh). 6:1901725. PMCID: PMC6918119.

B. Positions and Honors

Professional Experience

2020 –	Chief Scientific Officer, Bloodworks Northwest, Seattle, WA
2016 –	Full Member, Bloodworks Research Institute, Seattle, WA
2015 – 2016	Chief Scientific Officer, Bloodworks Northwest, Seattle, WA (corporate name change)
2012 – 2015	Chief Scientific Officer, Puget Sound Blood Center, Seattle, WA
2006 –	Professor, Departments of Medicine and Biochemistry, University of Washington, Seattle, WA
2006 – 2012	Executive Vice-President for Research, Puget Sound Blood Center, Seattle, WA
2004 – 2006	Vice Chair for Research, Department of Medicine, Baylor College of Medicine, Houston, TX
2001 – 2006	Professor of Medicine, Baylor College of Medicine, Houston, TX
1999 –	Program in Cardiovascular Sciences, Baylor College of Medicine, Houston, TX
1999 –	Adjunct Associate Professor, Department of Bioengineering, Rice University, Houston, TX
1996 – 2001	Associate Professor, Department of Molecular and Human Genetics, Baylor College of Medicine
1996 – 2001	Associate Professor of Medicine, Baylor College of Medicine, Houston, TX
1994 – 1996	Assistant Professor of Medicine, Baylor College of Medicine, Houston, TX
1989 – 1994	Assistant Professor of Medicine in Residence, University of California, San Francisco, CA
1989 – 1994	Scientist I, Gladstone Institute of Cardiovascular Disease, San Francisco, CA

Honors and Awards

2014–	Associate Editor, Blood Journal
2014	Distinguished Scientist Award, Society for Advancement of Chicanos and Native Americans in Science (SACNAS)

2014	Distinguished Alumni Achievement Award, New Mexico Institute of Mining and Technology Alumni Association
2010	Elected Member, Association of American Physicians
2009 – 2015	Member, NIH Hemostasis Thrombosis Study Section
2005	Investigator Recognition Award as part of the 12th Biennial Awards for Contributions to Hemostasis at the XXth Congress of the International Society on Thrombosis and Hemostasis in Sydney, Australia
2003	Appointed Co-chairman, Scientific Subcommittee on Platelet Physiology, Scientific and Standardization Committee, International Society of Thrombosis and Haemostasis
2003	Appointed Co-chairman, The American Society of Hematology Minority Recruitment Initiative
2003	American Society of Hematology, ASH Scholars Study Section
2003	American Society of Hematology, Committee on Government Affairs
2002	Elected Vice-Chairman, Gordon Conference on Hemostasis, Chairman for meeting in 2006
1999 – 2004	Associate Editor, *Circulation*
1997 & 1998	Commendation for Excellence in Research, Baylor College of Medicine
1997	NIH Hematology I Study Section
1996	Elected Member, American Society for Clinical Investigation
1996 – 2001	Established Investigator, American Heart Association
1987	Young Investigator Award, XIth International Congress on Thrombosis and Hemostasis, Brussels
1981	Alpha Omega Alpha Medical Honor Society
1981	Henry J. Kaiser Foundation Merit Award, given to 50 outstanding minority medical students in the U.S.
1977	*Summa Cum Laude*, NMIMT
1977	Morris F. Stubbs Award, given to top Chemistry Graduate, NMIMT

C. Contribution to Science

1.) My earliest project as a trainee was to determine the primary structure of platelet glycoprotein (GP) Ib. With the help of colleagues, I was successful in determining the structures of its two polypeptide chains GPIbα and GPIbβ, through cloning of the cDNAs for the two polypeptides. This work not only revealed for the first time the amino acid sequences of these important polypeptides, it was also the first time that it was suggested that there was a family of proteins with a shared motif containing leucine-rich repeats. The cDNAs were made available to investigators throughout the world and were used in numerous studies including those to develop drugs to block platelet adhesion. These reagents were also useful in studies of the biosynthesis of the complex in which we revealed that each of three polypeptides, GPIbα, GPIbβ, and GPIX were necessary for the synthesis of a functional complex on the platelet surface. This proposal was later borne out by studies of patients with the GPIb-IX-V deficiency disorder, the Bernard-Soulier syndrome, which showed that the disorder could be produced by mutations to the genes encoding any one of the three polypeptides. The cell lines also allowed us to determine how the individual polypeptides interact with each other, and the cellular locations and timing of the complex's synthesis, and important post-translational modifications of the complex, including the functionally important tyrosine sulfation of GPIbα. During this time we also determined the biochemical and genetic nature of a GPIbα size polymorphism, which we found was due to variable numbers of tandem repeats within the stalk-like macroglycopeptide region. The cell lines produced in the studies were also made widely available to investigators throughout the world and are still used to explore the functions of the complex.

1. **López, J.A.**, Chung, D.W., Fujikawa, K., Hagen, F.S., Papayannopoulou, T., and Roth, G.J. (1987) Cloning of the α-chain of human platelet glycoprotein Ib: A transmembrane protein with homology to leucine-rich α2-glycoprotein. Proc. Natl. Acad. Sci. USA. 84:5615-5619. PMCID: PMC298913.
2. **López, J.A**., Chung, D.W., Fujikawa, K., Hagen, F.S., Davie, E.W., and Roth, G.J. (1988) The α and β-chains of human platelet glycoprotein Ib are both transmembrane proteins containing a leucine-rich amino acid sequence. Proc. Natl. Acad. Sci. USA. 85:2135-2139. PMCID: PMC279943.
3. **López, J.A.,** Ludwig, E., and McCarthy, B.J. (1992) Polymorphism of human glycoprotein Ibα results from a variable number of tandem repeats of a thirteen amino acid sequence in the mucin-like macroglycopeptide region: Structure/function implications. J. Biol. Chem. 267:10055-10061.

4. **López, J.A.**, Leung, B., Reynolds, C.C., Li, C.Q., and Fox, J.E.B. (1992) Efficient plasma membrane expression of a functional platelet glycoprotein Ib-IX complex requires the presence of its three subunits. J. Biol. Chem. 267:12851-59.

2.) New ligands and functions of the GPIb-IX-V complex. We continued our work on the functions of the GPIb complex and found that this complex was importantly associated with the Fc receptor, FcγRIIa, on the platelet surface, an association that accounted for some of the signaling properties of the receptor and for the fact that antibodies against the complex could sometimes block Fc receptor functions, such as activation of platelets by immune complexes. We also discovered two new counter receptors for GPIbα, P-selectin and the leukocyte integrin, Mac-1. Both of these interactions have been shown to be important in the roles of platelets in inflammation, and the Mac-1 interaction has been the target of drug development to treat diseases such as multiple sclerosis.

1. Sullam, P.M., Hyun, W.C., Szöllösi, J, Dong, J.-f., Foss, W.M., and **López, J.A.** (1998) Physical proximity and functional interplay of the glycoprotein Ib-IX-V complex and the Fc receptor FcγRIIA on the platelet plasma membrane. J. Biol. Chem. 273(9):5331–36.
2. Romo, G.M., Dong, J.-F., Schade, A., Kansas, G.S., McIntire, L.V., Berndt, M.C., and **López, J.A.** (1999) The glycoprotein Ib-IX-V complex is a platelet counter-receptor for P-selectin. J. Exp. Med. 190:803–13.
3. Simon, D.I., Xu, H., Chen, Z., Ballantyne, C.M., Zhang, L., Berndt, M.C., **López, J.A.** (2000) Platelet glycoprotein Ibα is a counter-receptor for the leukocyte integrin Mac-1 (CD11b/CD18). J. Exp. Med. 192(2):193–204. PMCID:PMC2193258
4. Shrimpton, C.N., Borthakur, G., Larrucea, S., Cruz. M.A., Dong, J.F. and **López, J.A.** (2002) Localization of the adhesion receptor glycoprotein Ib-IX-V complex to lipid rafts is required for platelet adhesion and activation. J. Exp. Med. 196(8):1057–66. PMCID:PMC2194038

3.) Discovery of ULVWF strings on the endothelial surface and exploration of VWF functions. Our findings in this area include: that newly secreted VWF multimers assemble under flow into long string-like structures, demonstration that the ultralarge form of VWF (ULVWF) can bind platelets in the absence of shear stress or modulators, that VWF is prone to oxidation by leukocyte oxidants and that this oxidation renders it hyperadhesive and uncleavable by ADAMTS13, that N-acetylcysteine can reduce VWF multimers to smaller forms and attenuate thrombosis in a mouse model of TTP (we are now studying the effects of NAC in human patients with TTP), that VWF is abundant and hyperadhesive in sickle cell disease and its quantity correlates with hemolysis in patients with this disorder, and use of an *in vitro* system of endothelialized microvessels to study the effects of hydrodynamic force and vessel size and geometry on VWF self-association and the ability of self-associated VWF to bind platelets.

1. Dong, J.F., Moake, J.L., Nolasco, L., Bernardo, A., Arceneaux, W., Shrimpton, C.N., Schade, A.J., McIntire, L.V., Fujikawa, K., and **López, J.A.** (2002) ADAMTS-13 rapidly cleaves newly secreted ultra-large von Willebrand factor multimers on the endothelial surface under flowing conditions. Blood. 100(12):4033–39.
2. Chen, J., Reheman, A., Gushiken, F.C., Nolasco, L., Moake, J.F., Ni, H., and **López, J.A.** (2011) N-acetylcysteine reduces the size and activity of von Willebrand factor in human plasma and mice. J. Clin. Invest. 121(2):593-603. PMCID: PMC3026714.
3. Zheng, Y., Chen, J., and **López, J.A**. (2015) Flow-driven assembly of VWF fibers and webs in *in vitro* microvessels. Nat Commun. 2015; 6:7858-68. PMCID: PMC4522708.
4. Chung, D.W., Chen, J., Ling, M., Fu, X., Blevins, T., Parsons, S., Le, J., Harris, J., Martin, T.R., Konkle, B.A., Zheng, Y., and **López, J.A**. (2016) High-density lipoprotein modulates thrombosis by preventing von Willebrand factor self-association and subsequent platelet adhesion. Blood. 127:637-45. PMCID: PMC4742551.

4.) Service to the scientific community. Throughout my scientific career, I have been very active in the service of the scientific community. These activities include service on numerous grant review study sections for organizations such as the American Heart Association, The American Society of Hematology, the National Blood Foundation, and numerous other foundations, in addition to twice serving as a regular member of NIH study sections,

first the Hematology I Study Section then the Hemostasis and Thrombosis Study Section. I served as the chair of the latter study section. I have chaired the Gordon Conference on Hemostasis and have been elected to chair the FASEB Conference on Proteases for 2017. In 2013, I was Co-Chair of the Scientific Program for the annual meeting of the American Society of Hematology, being responsible for all of benign hematology at the meeting. I have served as Associate Editor for two of the most prominent journals in hematology and cardiovascular disease, *Circulation* and *Blood*. I have also been very active in activities aimed at increasing the participation of individuals from minority groups in science. I was one of the founders of the ASH Minority Recruitment Initiative, and served as a co-chair of the Committee on Diversity for seven years.

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/myncbi/jose.lopez.1/bibliography/40738934/public/?sort=date&direction=ascending

OMB No. 0925-0001 and 0925-0002 (Rev. 12/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

NAME: **Maly, Dustin James**

eRA COMMONS USER NAME (credential, e.g., agency login): MALYDU

POSITION TITLE: Professor of Chemistry, Professor of Biochemistry

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Wisconsin, Madison WI	B.S.	05/1997	Chemistry
University of California, Berkeley	Ph.D.	05/2002	Chemistry
University of California, San Francisco	Postdoctoral	09/2006	Chemical Biology

A. Personal Statement

The long-term goal of my research program is to develop and apply new biochemical and chemical genetic techniques for studying the function of protein families involved in mammalian signal transduction. Through the application of these techniques we hope to identify and validate new protein kinase drug targets. My research program combines techniques in chemistry, biochemistry, protein engineering, chemoproteomics, and cell biology to interrogate intracellular signaling processes. We have established a number of productive collaborations at UW. For example, we have established highly productive collaborations with David Baker's (Biochemistry), Doug Fowler's (Genome Sciences) Mike Guttman's (Medicinal Chemistry), and Shao-En Ong's (Pharmacology) labs. Several publications that have resulted from our collaborative efforts are listed below:

Ahler, E.; Register, A. C.; Chakraborty, S.; Fang, L.; Dieter, E. M.; Sitko, K. A.; Vidadala, R. S. R.; Trevillian, B. M.; Golkowski, M.; Gelman, H.; Stephany, J. J.; Rubin, A. F.; Merritt, E. A.; ^Fowler, D. M.; ^Maly, D. J. "A combined approach reveals a regulatory mechanism coupling Src's kinase activity, localization, and phosphotransferase-independent functions" *Mol. Cell* **2019**, *74*, 393-408. (**PMCID**: PMC6474823) (^=co-corresponding authors)

Potter, Z. E.; Lau, H-T; Chakraborty, S.; Fang, L.; Guttman, M.; Ong, S-E.; Fowler, D. M.; Maly, D. J. "Parallel Chemoselective Profiling for Mapping Protein Structure" *Cell Chem. Biol.* **2020**, *27*, 1084-96. (**PMCID**: PMC7484201)

Foight, G. W.; Wang, Z.; Wei, C. T.; Greisen, P. J.; Warner, K.; Cunningham-Bryant, D.; Park, K.; Brunette, T. J.; Sheffler, W.; Baker, D.; Maly, D. J. "Multi-input chemical control of protein dimerization for programming graded cellular responses" *Nat. Biotech.* **2019**, *37*, 1209-16. (**PMCID**: PMC6776690).

Fang, L.; Chakraborty, S.; Dieter, E. M.; Potter, Z. E.; Lombard, C. K.; Maly, D. J. "A Chemoproteomic Method for Profiling Inhibitor-Bound Kinase Complexes" *J. Am Chem. Soc.* **2019**, *141*, 11912-22. (**PMCID**: PMC6688853)

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments:

2014- Professor, Department of Chemistry, University of Washington, Seattle
2014- Professor, Department of Biochemistry, University of Washington, Seattle
2006-2014 Assistant/Associate Professor, Department of Chemistry, University of Washington, Seattle
2002-2006 Postdoctoral Fellow, Department of Cellular and Molecular Pharmacology, University of California, San Francisco (Advisor: Prof. Kevan M. Shokat)

1997-2002 Graduate Research Assistant, Department of Chemistry, University of California, Berkeley (Advisor: Prof. Jonathan A. Ellman)
Member, NIH SBCB Study Section (2018-2024)
Co-Director Biological Physics, Structure and Design Ph.D. Program (2019-present)
Editorial Board Member, *Current Research in Chemical Biology* (CRCHBI) (2019-present)
Editorial Board Member, *Journal of Biological Chemistry* (2014-2019)
Co-Founder and Scientific Advisory Board Member of OptiKira (2014-present)
Member of NCI Chemical Biology Laboratory Intramural Assessment Team (2017)
Ad Hoc Member, NIH SBCB Study Section (2013, 2016, 2017)
Ad Hoc Member, NIH MSFA Study Section (2013, 2014, 2016)
Ad Hoc Member, NIH BCMB-10 Study Section (2017)
Ad Hoc Member, NIH ZRG1-EMNR-R-56 (2015)
Ad Hoc Member, NIH ZRG1-F04A-W-20 (2015)
Ad Hoc Member, NIH ZRG1-IDM-S-02 (2014)
Ad Hoc Member, NIH MSFE Study Section (2012)
Ad Hoc Member, NIH SBCA Study Section (2010)

Honors:

Raymon E. and Rosellen M. Lawton Distinguished Scholar in Chemistry (2014)
Camille Dreyfus Teacher-Scholar Award (2012)
Sloan Research Fellowship (2011)
NSF Career Award (2010)
Life Science Research Foundation Postdoctoral Fellowship (Pfizer Fellow) (2003-2006)
Susan G. Komen Breast Cancer Foundation Postdoctoral Fellowship (2003)
UC-Berkeley Outstanding Graduate Student Instructor Award (2001)
Hilldale Undergraduate Research Fellowship (1996)

C. Contributions to Science

1. Over the last ten years my lab has studied the conformational flexibility of protein kinase active sites. We have discovered that small molecule inhibitors that stabilize distinct ATP-binding site conformation are able to modulate long distance allosteric effects on domains that are distal to the site of inhibitor binding. Using conformation-selective inhibitors, we have been able to modulate the ability of non-receptor tyrosine kinases (Src- and Abl-family) and MAPKs (p38 and Erk) to engage in inter-molecular binding interactions. We have also shown that conformation-selective inhibitors can control the post-translational phosphorylation and dephosphorylation of these kinases, which affects their sub-cellular localization.

a. Chakraborty, S.; Inukai, T.; Fang, L.; Golkowski, M.; Maly, D. J. “Targeting dynamic ATP-binding site features allows discrimination between highly homologous protein kinases” *ACS Chem. Biol.* **2019**, *14,* 1249-59. (**PMCID**: PMC6642640)

b. Fang, L.; Vilas-Boas, J.; Chakraborty, S.; Potter, Z. E.; Register, A. C.; Seeliger, M. A.; Maly, D. J. “How ATP-competitive inhibitors allosterically modulate tyrosine kinases that contain a Src-like regulatory architecture” *ACS Chem. Biol.* **2020**, *15*, 2005-16. (**PMCID**: PMC7700871)

c. Lombard, C. K.; Davis, A. L.; Inukai, T.; Maly, D. J. “Allosteric modulation of JNK docking-site interactions with ATP-competitive inhibitors” *Biochemistry* **2018**, *57*, 5897-5909. (**PMCID**: PMC6338552)

d. Krishnamurty, R.; Brigham, J. L.; Leonard, S. E.; Ranjitkar, P.; Dale, E. J.; Larson, E. T.; Merritt, E. A.; Maly, D. J. “Active site profiling reveals coupling between domains in SRC-family kinases” *Nat. Chem. Biol.* **2013**, 43-50. (**PMCID**: PMC3522794)

2. It has long been believed that the high selectivity that the clinically approved drug Gleevec has for its targets, c-Abl and BCR-Abl, is due to the ability of the Abl kinase domain to adopt a unique inactive conformation. However, in collaboration with John Kuriyan (UC, Berkeley), we have shown that this is not the case and that many other tyrosine kinases are able to adopt a similar inactive ATP-binding site conformation. Furthermore, we have shown that Gleevec’s unique interaction with a flexible region of Abl’s kinase active site, called the P-loop, underlies the selectivity of this drug.

These observations provide insight into the underlying mechanism of the most frequently observed Gleevec-resistant mutations in the clinic and suggest strategies for overcoming resistance.

a. Seeliger, M. A.; Ranjitkar, P.; Kasap. C.; Shan, Y.; Shaw, D. E.; Shah, N. E.; Kuriyan, J.; Maly, D. J. "Equally Potent Inhibition of c-Src and Abl by Compounds that Recognize Inactive Kinase Conformations." *Cancer Res.* **2009**, *69*, 2384-2392. (**PMCID**: PMC2678021)

b. Ranjitkar, P.; Brock, A. M.; Maly, D. J. "Affinity Probes that Target a Specific Inactive Conformation of Protein Kinases." *Chem. Biol.* **2010**, *17*, 195-206. (**PMCID**: PMC2871157)

c. Hari, S. B.; Merritt, E. A.; Maly, D. J. "Sequence Determinants of a Specific Inactive Protein Kinase Conformation" *Chem. Biol.* **2013**, *20*, 806-815. (**PMCID**: PMC3742363)

d. Hari, S. B; Perera, B. G. K.; Ranjitkar, P.; Seeliger, M. A.; Maly, D. J. "Conformation-selective inhibitors reveal differences in the activation and phosphate-binding loops of the tyrosine kinases Abl and Src" *ACS Chem. Biol.* **2013**, *8*, 2734-2743. (**PMCID**: PMC3880807)

3. **We have developed a new chemical genetic method for controlling the activity of intracellular enzymes with small molecules. This method involves the use of protein engineering to introduce intramolecular autoinhibitory domains into a protein of interest. We have used this concept to confer temporal and graded control over the activities of a number of important proteins–including Rho GTPases, Ras GTPase, Cre Recombinase, and Cas9.**

a. Rose, J. C.; Stephany, J. J.; Valente, W. J.; Trevillian, B. M.; Dang, H. V.; Dickinson, M. S.; Bielas, J. H.; ^Maly, D. J.; ^Fowler D. M. "DSB-ddPCR and a rapidly inducible Cas9 variant enable exploration of editing kinetics." *Nat. Methods* **2017**, *14*, 981-986. (**PMCID**: PMC5730411)

b. Foight, G. W.; Wang, Z.; Wei, C. T.; Greisen, P. J.; Warner, K.; Cunningham-Bryant, D.; Park, K.; Brunette, T. J.; Sheffler, W.; Baker, D.; Maly, D. J. "Multi-input chemical control of protein dimerization for programming graded cellular responses" *Nat. Biotech.* **2019**, *37*, 1209-16. (**PMCID**: PMC6776690).

c. Cunningham-Bryant, D.; Dieter, E. M.; Foight, G. W.; Rose, J. C.; Maly, D. J. "A Chemically-Disrupted Proximity System for Controlling Dynamic Cellular Processes" *J. Am Chem. Soc.* **2019**, *141*, 3352-5. (**PMCID**: PMC6492022)

d. Rose, J. C.; Huang, P-S.; Camp, N. D.; Ye, J.; Leidal, A. M.; Goreshnik, I.; Trevillian, B. M.; Dickinson, M. S.; Cunningham-Bryant, D.; Debnath, J.; Baker, D.; Wolf-Yadlin, A.; Maly, D. J. "A computationally engineered RAS rheostat reveals RAS/ERK signaling dynamics" *Nat. Chem. Biol.* **2017**, *13*, 119-26. (**PMCID:** PMC5161653)

4. **In collaboration with the Papa and Oakes Labs at UCSF, we have shown that it is possible to develop small molecules that target the ATP-binding site of IRE1α but allosterically affect the activity of its RNase domain from a distance. Furthermore, we have shown that these small molecule IRE1α inhibitors are cytoprotective under a number of ER stress conditions. These small molecule inhibitors have allowed us to test the efficacy of targeting the terminal UPR (tUPR) in a number of disease models.**

a. Feldman, H. C.; Ghosh, R.; Auyeung, V. C.; Mueller, J. L.; Kim, J.; Potter, Z. E.; Perera, B. G. K.; Vidadala, V. N.; Olivier, A.; Backes, B. J.; Zikherman, J.; ^Papa, F. R.; ^Maly, D. J. "ATP-Competitive Partial Antagonists of the IRE1α RNase Segregate Outputs of the UPR" *Nat. Chem. Biol.* **2021**, *17, 1148–56*. (**PMCID:** *in process*)

b. Ghosh, R.; Wang, L.; Wang, E. S.; Perera, B. G. K.; Alavi, M. V.; Igbaria, A.; Morita, S.; Prado, K.; Thamsen, M.; Caswell, D.; Weiberth, K. F.; Hari, S. B.; Mitra, A. K.; Bhhatarai, B.; Macias, H.; Snapp, E. L.; German, M. S.; Backes, B. J.; Schürer, S. C.; Gould, D. B.; Maly, D. J.; Oakes, S. A.; Papa, F. R. "Allosteric inhibition of the IRE1α RNase preserves cell viability and function during endoplasmic reticulum stress." *Cell* **2014**, *158*, 534–548. (**PMCID:** PMC4244221)

c. Wang, L.; Perera, B. G. K.; Hari, S. B.; Bhhatarai, B.; Backes, B. J.; Seeliger, M. A.; Schürer, S. C.; Oakes, S. A.; Papa, F. R.; Maly, D. J. "Divergent allosteric control of the IRE1α endoribonuclease using kinase inhibitors." *Nat. Chem. Biol.* **2012**, 982-989. (**PMCID:** PMC3508346)

d. Morita, S.; Villalta, S. A.; Feldman, H. C.; Register, A. C.; Rosenthal, W.; Hoffmann-Petersen, I. T.; Mehdizadeh, M.; Ghosh, R.; Wang, L.; Colon-Negron, K.; Meza-Acevedo, R.; Backes, B. J.; ^Maly, D. J.; ^Bluestone, J. A.; ^Papa, F. R. "Targeting ABL-IRE1α signaling spares ER-stressed pancreatic β-cells to reverse autoimmune diabetes" *Cell Metab.* **2017**, *25*, 883-97. (**PMCID:** PMC5497784)

Complete List of Published Work in MyBibliography:

http://www.ncbi.nlm.nih.gov/sites/myncbi/dustin.maly.1/bibliography/40430581/public/?sort=date&direction=descending

BIOGRAPHICAL SKETCH

NAME: Mougous, Joseph David

eRA COMMONS USER NAME : mougous

POSITION TITLE: Associate Professor of Microbiology

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Western Washington University	B.S.	06/1999	Biochemistry
University of California, Berkeley	Ph.D.	12/2004	Molecular and Cell Biology
Harvard Medical School	Postdoctoral	07/2007	Microbiology

A. PERSONAL STATEMENT

Research in my lab focuses on dissecting molecular mechanisms of interbacterial antagonism, through structural, biochemical, genetic and bioinformatic approaches. One specific area of interest has been the study of the effectors and mechanism of action of the bacterial type VI secretion system (T6SS). We were the first to show that in many species, the T6SS targets bacterial cells; we published the first biochemical characterization of its effector proteins and we have identified several widespread effector superfamilies. We also demonstrated that T6SSs can be classified as three distinct types; the largely bacterial-targeting $T6SS^{i}$ of Proteobacteria and $T6SS^{iii}$ of Bacteroidetes, and the Francisella pathogenicity island (FPI) encoded $T6SS^{ii}$. We went on to demonstrate that the $T6SS^{iii}$ of *Bacteroides* spp. mediates interbacterial antagonism in the human gut. Our structural and biochemical studies of T6SS effector proteins have led to the identification of diverse toxin mechanisms. We have also shown that bacteria benefit from the simultaneous production of multiple secreted effectors with different biochemical activities as a result of synergistic toxicity and variability in toxin efficacy across differing environmental conditions. Additionally, we have demonstrated that the Esx secretion system of Gram positive bacteria functions analogously to the T6SS in that it can be used to delivery toxic effectors between bacteria, and genes encoding these effectors proteins are widespread throughout the Firmicutes phylum. Finally, a recent focus of research in the lab is the development of biotechnological applications for antibacterial toxins. This includes the repurposing of a deaminase toxin from *Burkholderia* to engineer the first mitochondrial genome editor. Besides producing high quality science, a second key theme of the laboratory is training. Trainees gain independence by driving projects from conception through publishing. I have successfully mentored trainees at all levels, including undergraduates, graduate students, postdoctoral fellows, and senior scientists. My former trainees have gone on to an array of desirable positions, including becoming postdoctoral researchers in top-notch laboratories and assistant professors at high-caliber academic institutions. Trainees in the Mougous laboratory have published well and been awarded prestigious awards and fellowships for their work under my mentorship. Representative recent publications from my group include:

a. Ting S.Y., LaCourse K.D., Ledvina H.E., Zhang R., Radey M.C., Kulasekara H.D., Somavanshi R., Bertolli S.K., Gallagher L.A., Kim J., Penewit K.M., Salipante S.J., Xu L., Peterson S.B., Mougous J.D. Discovery of coordinately regulated pathways that provide innate protection against interbacterial antagonism. *Elife.* 2022 Feb 17;11:e74658. (PMC Pending)

b. de Moraes M.H., Hsu F., Huang D., Bosch D.E., Zeng J., Radey M.C., Simon N., Ledvina H.E., Frick J.P., Wiggins P.A., Peterson S.B., Mougous J.D. An interbacterial DNA deaminase toxin directly mutagenizes surviving target populations. *Elife*. 2021 Jan 15;10:e62967. (PMC7901873)

c. Mok B.Y., de Moraes M.H., Zeng J., Bosch D.E., Kotrys A.V., Raguram A., Hsu F., Radey M.C., Peterson S.B., Mootha V.K., Mougous J.D., Liu D.R. A bacterial cytidine deaminase toxin enables CRISPR-free mitochondrial base editing. *Nature*. 2020 Jul;583(7817):631-637. (PMC7381381)

d. Ross B.D., Verster A.J., Radey M.C., Schmidtke D.T., Pope C.E., Hoffman L.R., Hajjar A.M., Peterson

S.B., Borenstein E., Mougous J.D. Human gut bacteria contain acquired interbacterial defence systems. Nature. 2019 Nov;575(7781):224-228. (PMC6938237)

e. Ting S., Bosch D.E., Mangiameli S.M., Radey M.C., Huang S., Park Y., Kelly K.A., Filip S.K., Goo Y.A., Eng J.K., Allaire M., Veesler D., Wiggins P.A., Peterson S.B., Mougous J.D. Bifunctional immunity proteins protect bacteria against FtsZ-targeting ADP-ribosylating toxins. *Cell* 2018 Nov 15; 175:1-13. (PMC6239978)

B. POSITIONS AND HONORS

Positions and Employment

2005-2007	Postdoctoral Fellow, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA
2007-2008	Acting Assistant Professor, Dept. of Microbiology, University of Washington, Seattle, WA
2008-2013	Assistant Professor, Dept. of Microbiology, University of Washington, Seattle, WA
2013-2018	Associate Professor, Dept. of Microbiology, University of Washington, Seattle, WA
2014-2018	Adjunct Associate Professor, Dept. of Biochemistry, University of Washington, Seattle, WA
2018-	Professor, Dept. of Microbiology, University of Washington, Seattle, WA
2018-	Adjunct Professor, Dept. of Biochemistry, University of Washington, Seattle, WA

Other Experience and Professional Memberships

2004-	Member, American Society of Microbiology
2007-2009	User, Molecular Foundry
2013-	Ad hoc member, NIH Study Section

Honors

1999	Outstanding Chemistry Graduate, Western Washington University, Bellingham, WA
2000	Ford Foundation Predoctoral Fellow, University of California, Berkeley, CA
2005	Damon Runyon Postdoctoral Fellow, Harvard Medical School, Boston, MA
2011	Burroughs Wellcome Investigator in the Pathogenesis of Infectious Disease, University of Washington, Seattle, WA
2011	Irving S. Sigal Memorial Award, American Society for Microbiology
2015	Investigator, Howard Hughes Medical Institute
2016	Editorial Board Member, Current Opinion in Microbiology
2018	Finalist, Blavatnik National Award for Young Scientists
2019	Finalist, Blavatnik National Award for Young Scientists
2021	National Academy of Sciences Award in Molecular Biology

C. CONTRIBUTION TO SCIENCE

1. Role of the type VI secretion system in interbacterial interactions
The primary focus of my laboratory is dissecting the molecular mechanisms that underlie interbacterial interactions within microbial communities. We found that the type VI secretion system (T6SS), which is a complex secretion apparatus widely distributed in Gram-negative bacteria, mediates interbacterial antagonism through the contact-dependent delivery of toxic protein effectors. My group investigates the phenotypic significance of this system in both pathogens and environmental bacteria, including *Pseudomonas aeruginosa*, *Burkholderia thailandensis*, *B. pseudomallei*, *Vibrio cholerae*, *Escherichia coli* and *Bacteroides* spp. To better understand the role of T6S in polymicrobial communities, my laboratory also studies regulation of the T6S apparatus. We have identified both phosphorylation-dependent and -independent pathways that control activation of the system. These pursuits have necessitated our development of technologies for analysing interbacterial competition using quantitative single-cell methods. Moreover, our work has led to the observation that T6S is one arm of a broader cellular program activated in response to attack by competitor cells. We have also investigated the physiological benefits conferred by maintaining diverse effector arsenals. Finally, our studies of the T6SS in *Bacteroides* spp. have led to the discovery that the pathway is important for mediating interbacterial interactions in the human gut.

   a. LaCourse K.D., Peterson S.B., Kulasekara H.D., Radey M.Cc, Kim J., Mougous J.D. Conditional toxicity and synergy drive diversity among antibacterial effectors. *Nat Microbiol*. 2018 Apr;3(4):440-446. (PMC5876133)

b. Verster A.J., Ross B.D., Radey M.C., Bao Y., Goodman A.L., Mougous J.D., Borenstein E. The Landscape of Type VI Secretion across Human Gut Microbiomes Reveals Its Role in Community Composition. *Cell Host Microbe.* 2017 Sep 13;22(3):411-419.e4. (PMC5679258)

c. Russell A.B., Wexler A.G., Harding B.N., Whitney J.C., Bohn A.J., Goo Y.A., Tran B.Q., Barry N.A., Zheng H., Peterson S.B., Chou S., Gonen T., Goodlett D.R., Goodman A.L., Mougous J.D. A type VI secretion-related pathway in Bacteroidetes mediates interbacterial interactions. Cell Host Microbe. 2014 Aug 13;16(2):227-36 (PMC4136423)

d. Hood R.D., Singh P., Hsu F., Güvener T., Carl M.A., Trinidad R.R., Silverman J.M., Ohlson B.B., Hicks K.G., Plemel R.L., Li M., Schwarz S., Wang W.Y., Merz A.J., Goodlett D.R., and Mougous J.D. A type VI secretion system of *Pseudomonas aeruginosa* targets a toxin to bacteria. *Cell Host Microbe.* 2010 Jan 21;7(1):25-37. (PMC2831478)

2. Identification of exported interbacterial toxins, their mechanism of action and export mechanism
Another area of research in my group is the identification and characterization of secreted antibacterial toxins. We identified the first three effectors of the T6SS through our work in *P. aeruginosa*, and these studies revealed that many T6S effectors function as toxin–immunity modules. Using an interdisciplinary approach, we characterized the activities for several of these toxins and determined the first X-ray crystal structure of a T6S effector. Since these early findings, we have used a diverse array of informatic and proteomic approaches to discover and characterize superfamilies of T6S effectors. Our investigations in this area are yielding insights into essential processes in bacteria, as these universally conserved pathways are the targets of interbacterial toxins. Furthermore, my group has defined two distinct modes of effector secretion. For one of these mechanisms, we have dissected the molecular interactions that mediate substrate recognition by the apparatus. More recently, we discovered that the Esx secretion system of Gram positive bacteria also exports antibacterial proteins, and we structurally and biochemically characterized two such secreted toxins.

a. Ting S., Bosch D.E., Mangiameli S.M., Radey M.C., Huang S., Park Y., Kelly K.A., Filip S.K., Goo Y.A., Eng J.K., Allaire M., Veesler D., Wiggins P.A., Peterson S.B., Mougous J.D. Bifunctional immunity proteins protect bacteria against FtsZ-targeting ADP-ribosylating toxins. *Cell* 2018 Nov 15; 175:1-13. (PMC6239978)

b. Whitney JC, Quentin D., Sawai S., LeRoux M., Harding B.N., Ledvina H.E., Tran B.Q., Robinson H., Goo Y.A., Goodlett D.R., Raunser S., and Mougous J.D. An Interbacterial NAD(P)+ Glycohydrolase Toxin Requires Elongation Factor Tu for Delivery to Target Cells. *Cell*. 2015 Oct 22;163(3):607-19. (PMC4624332).

c. Silverman J.M., Agnello D.M., Zheng H., Andrews B.T., Li M., Catalano C.E., Gonen T., Mougous J.D. Haemolysin coregulated protein is an exported receptor and chaperone of type VI secretion substrates. *Mol Cell.* 2013 Sep 12;51(5):584-93. (PMC3844553)

d. Russell A.B., Leroux M., Hathazi K., Agnello D.M., Ishikawa T., Wiggins P.A., Wai S.N., Mougous J.D. Diverse type VI secretion phospholipases are functionally plastic antibacterial effectors. *Nature*. 2013 Apr 25;496:343-347 (PMC3652678)

3. Biotechnological applications of interbacterial toxins
The work of our group and others has led to the recognition that toxins employed by interbacterial antagonism represent a diverse set of proteins with a number of remarkable features. These include their incredible potency, their wide range of target molecules, their modularity (many of these toxins are polymorphic, containing c-terminal structural domains) and the fact that each can be inactivated by a dedicated immunity protein that prevents self-intoxication. We have recently begun capitalizing on these features toward a number of biotechnological applications. In the first instance, we developed programmed inhibitor cells (PICs) that employ toxins secreted by the T6SS system to specifically eliminate chosen target organisms. Targeting by PICs is mediated by display of single-chain antibodies that recognize target-specific surface antigens. We demonstrated that PICs can achieve highly efficient removal of a target species from a complex, mammalian gut-derived community without detectable collateral damage. Our

second application of an interbacterial toxin involved repurposing a double-stranded DNA targeting deaminase and T6SS substrate from *Burkholderia cenocepacia* for genome editing of mitochondria. The biochemical activity of this toxin is unprecedented, and it enabled editing of the mitochondrial genome through fusion to TALE proteins, which, unlike Cas9-based genome editors, do not require nucleic acid guides unable to penetrate the mitochondrion. Additional applications of interbacterial toxin are currently being investigated.

a. Mok B.Y., de Moraes M.H., Zeng J., Bosch D.E., Kotrys A.V., Raguram A., Hsu F., Radey M.C., Peterson S.B., Mootha V.K., Mougous J.D., Liu D.R. A bacterial cytidine deaminase toxin enables CRISPR-free mitochondrial base editing. *Nature.* 2020 Jul;583(7817):631-637. (PMC7381381)
b. Ting S.Y., Martínez-García E., Huang S., Bertolli S.K., Kelly K.A., Cutler K.J., Su E.D., Zhi H., Tang Q., Radey M.C., Raffatellu M., Peterson S.B., de Lorenzo V., Mougous J.D. Targeted depletion of bacteria from mixed populations by programmable adhesion with antagonistic competitor cells. *Cell Host Microbe* 2020 Aug 12;28(2):313-321. (PMC7725374)

4. Domestication of interbacterial toxins by eukaryotes
One discovery that resulted from our study of a superfamily of T6S cell wall-degrading toxins is the observation that several genes encoding these toxins were domesticated by a diverse range of eukaryotes, including the Lyme disease tick vector. Notably, we found that these genes were acquired through multiple, independent horizontal gene transfer events and that the biochemical and *in vivo* antibacterial functions of the domesticated enzymes were conserved. This work provided a rare example of functional inter-kingdom gene transfer. My group went on to show that contrary to existing dogma, the microbial community associated with the environment in which these horizontally acquired toxins act, the tick midgut, is of limited diversity. These findings provide a potential explanation for the lack of interbacterial antagonism mechanisms in an organism specifically adapted for this environment, the Lyme disease pathogen *Borrelia burgdorferi*.

a. Ross B.D., Hayes B., Radey M.C., Lee X., Josek T., Bjork J., Neitzel D., Paskewitz S., Chou S., Mougous J.D. *Ixodes scapularis* does not harbor a stable midgut microbiome. *ISME J.* 2018 Nov;12(11):2596-2607. PMC pending.

b. Chou S., Daugherty M.D., Peterson S.B., Biboy J., Yang Y., Jutras B.L., Fritz-Laylin L.K., Ferrin M.A., Harding B.N., Jacobs-Wagner C., Yang X.F., Vollmer W., Malik H.S., Mougous J.D. Transferred interbacterial antagonism genes augment eukaryotic innate immune function. *Nature* 2015 Feb 5; 518: 98-101. (PMC6194123)

5. Role of T6SS effectors in bacterial pathogenesis
While our group and others have shown that the primary function of the T6SS is the delivery of toxic effectors between bacteria, it has become apparent that some bacterial pathogens employ this protein targeting mechanism to deliver effectors to host cells. One such organism is the environmental saprophyte and causative agent of melioidosis, *Burkholderia pseudomallei. B. pseudomallei* encodes six distinct T6SSs. My group demonstrated that only one of these is important for mediating pathogenesis, T6SS-5. We also identified the first secreted substrate of this system and demonstrated that this effector is key to mediating the role of T6SS-5 in virulence. We have since gone on to define secreted substrates for a second host targeting T6SS, that of *Francisella tularensis.* The T6SS of this organism had long been known to be crucial for pathogenesis, but the secreted substrates had eluded identification. Using quantitative mass spectrometry, we identified four secreted effectors of this system. Surprisingly, two of the substrates we identified were encoded outside of the *Francisella* pathogenicity island that harbors the T6SS. We have gone on to demonstrate that one of these substrates, OpiA, is the founding member of a superfamily of bacterial phosphatidylinositol 3-kinases, that acts on the Francisella-containing phagosome and promotes bacterial escape into the cytoplasm. Current efforts in the laboratory are directed toward further defining the mechanisms by which OpiA and other secreted effectors facilitate *F. tularensis* pathogenicity.

a. Ledvina, H.E., Kelly, K.A., Eshraghi, A., Plemel, R.L., Peterson, S.B., Lee, B., Steele, S., Adler, M., Kawula, T.H., Merz, A.J., Skerrett, S.J., Celli, J., Mougous, J.D. A phosphatidylinositol 3-kinase effector alters phagosomal maturation to promote intracellular growth of Francisella. *Cell Host Microbe*. 2018 Aug 8;24(2):285-295. (PMC6394229)

b. Eshraghi A., Kim J., Walls A.C., Ledvina H.E., Miller C.N., Ramsey K.M., Whitney J.C., Radey M.C., Peterson S.B., Ruhland B.R., Tran B.Q., Goo Y.A., Goodlett D.R., Dove S.L., Celli J., Veesler D., Mougous J.D. Secreted effectors encoded within and outside of the Francisella Pathogenecity Island promote intramacrophage growth. *Cell Host Microb.* 2016 Mar 8;21(3):286-289. (PMC5384264)

c. Schwarz S., Singh P., Robertson J.D., LeRoux M., Skerrett S.J., Goodlett D.R., West T.E., Mougous J.D. VgrG-5 is a Burkholderia type VI secretion system-exported protein required for multinucleated giant cell formation and virulence. *Infect Immun.* 2014 Apr;82(4):1445-52. (PMC3993412)

d. Schwarz S., West T.E., Boyer F., Chiang W.C., Carl M.A., Hood R.D., Rohmer L., Tolker-Nielsen T., Skerrett S.J., and Mougous J.D. *Burkholderia* type VI secretion systems have distinct roles in eukaryotic and bacterial cell interactions. *PLoS Pathog.* 2010 Aug 26;6(8). (PMC2928800)

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/pubmed/?term=Mougous

D. RESEARCH SUPPORT

Ongoing Research Support

Investigator Award (Mougous) 09/1/15-08/31/21
Howard Hughes Medical Institute
“Mechanisms of contact-mediated interbacterial antagonism”
This award provides support for ongoing research efforts and is not restricted to a specific project.

R01 AI080609 (Mougous), NIH/NIAID 06/17/09 – 03/31/25
“Mechanisms for sensing and responding to interbacterial antagonism”
Role: Principal investigator
The goals of this study are to characterize mechanisms by which the Gac/Rsm global regulatory system of *Pseudomonas* species provides protection from bacterial antagonists.

HR0011-21-2-0012 UW (MPI Mougous/Baker/Bhardwaj/DiMaio/Woodward) DARPA 8/2/2021 – 8/1/2023
“Host augmentation for targeted bacterial eradication using designed chimeric macrocycles and miniproteins”
Role: Principal Investigator
The work put forth in this proposal seeks to develop a new therapeutic paradigm in the arena of infectious disease. Because our protein-targeting chimeric (PROTAC) and new chimeric strategies (NewTACs) are orthogonal to the traditional antimicrobial drug approach, they overcome currently circulating and widespread resistance mechanisms. We have selected three pathogen targets that provide a representative cross section of pathologies and diseases to develop our system against: Pseudomonas aeruginosa, Acinetobacter baumannii, and Francisella tularensis. Our team has in-depth knowledge and hands-on experience with these organisms, which will both maximize successful targeting and facilitate conclusive studies to validate the mechanistic basis of eradication.

R01AI145954 (MPI Dove/Mougous) 7/1/19 – 6/30/24
“Identity, function and control of Francisella effectors encoded outside its pathogenicity island”
Role: Principal investigator
The *Francisella* genus constitutes a diverse group of host-associated bacteria, including the highly virulent human pathogen that causes tularemia, *F. tularensis*. In this study, we seek to identify secreted proteins that enable *F. tularensis* subspecies to grow within host cells, and to define the mechanisms by which these proteins act. This work will provide insight into what differentiates pathogenic *Francisella* and it will contribute to our basic understanding of the means by which pathogens subvert human cellular defenses.

A163579 (Mougous) Washington Research Foundation
1/1/2021 – 12/31/2021
Role: Principal Investigator
This award is to support the commercial development of programmed inhibitor cells for the targeted eradication of individual bacterial species from complex communities.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Tian, Rong

eRA COMMONS USER NAME (credential, e.g., agency login): rongtian

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
West China University of Medical Sciences, China	MD	07/1986	Medicine
University of Aarhus, Denmark	PHD	12/1992	Pharmacology
Brigham & Women's Hosp, Harvard Medical School	Postdoctoral	12/1996	Biophysics and Biochemistry

A. Personal Statement

My research focuses on the molecular mechanisms regulating cell metabolism and energetics. A long-term goal of my laboratory is to understand the role of mitochondria and metabolism in the pathogenesis of human diseases, in particular, cardiovascular diseases. We have utilized molecular and genetic approaches to identify and perturb specific regulators in the key pathways of energy metabolism in mice and subsequently interrogated the physiological and biochemical responses in vitro and in vivo. Our work in the past two decades focused on the oxidative metabolism and bioenergetics in ischemic heart disease, obesity and diabetes, and heart failure using mouse models of altered glucose, fatty acid and amino acid metabolism. We also seek to decipher the mechanistic link between impaired mitochondrial function and cellular stress response. Results of these studies identified an important role of cell metabolism and redox state in diseases caused by mitochondrial dysfunction including cardiovascular, inflammatory and neurological pathologies. To translate these observations, we are conducting mechanistic studies in heart failure patients as well as clinical trials of mitochondria-targeted therapy. In addition, we have developed partnerships to take a systems approach, such as quantitative mitochondrial protein interactome and metabolomics, to investigate metabolic mechanisms of human diseases.

Ongoing and recently completed projects that I would like to highlight include:

1. **R01HL149695** (PI: Tian)
07/01/2020-06/30/2024
Mitochondrial metabolism and macrophage function post MI

2. **R01HL144937** (MPI: O'Brien/Tian)
09/01/2019-08/31/2024
Mechanistic Studies of NAD+/NADH in Human Heart Failure

3. **R01 HL142628** (PI: Tian)
07/01/2018-06/30/2022
Mitochondrial function and glycolytic switch in pathological cardiac hypertrophy.

4. **R01 HL144778** (PI: Bruce; Co-I: Tian)
07/01/2019-06/30/2023

The heart failure interactome

5. **R01 HL110349** (PI: Tian, R)
04/01/2017 – 03/31/2022 (NCE)
NAD+/NADH ratio, protein acetylation and mitochondrial function

Citations:

1. Karamanlidis G, Lee CF, Garcia-Menendez L, Kolwicz Jr. SC, Suthammarak W, Gong G, Sedensky MM, Morgan PG, Wang W, **Tian R**. Mitochondrial Complex I Deficiency increases protein acetylation and accelerates heart failure. *Cell Metab*. 2013, 2013 Aug 6;18(2):239-50. PMCID: PMC3779647.
2. Li T, Zhang Z, Kolwicz Jr. SC, Abell L, Roe ND, Kim M, Zhou B, Cao Y, Ritterhoff J, Gu H, Raftery D, Sun H, **Tian R.** Defective branched-chain amino acid (bcaa) catabolism disrupts glucose metabolism and sensitizes the heart to ischemia-reperfusion injury. *Cell Metab*. 2017 Feb 7;25(2):374-385. doi: 10.1016/j.cmet.2016.11.005. PMID: 28178567
3. Zhou B, Wang DD, Qiu Y, Airhart S, Liu Y, Stempien-Otero A, O'Brien KD, Tian R. Boosting NAD Level Suppresses Inflammatory Activation of PBMC in Heart Failure. *J Clin Invest*. 2020 Nov 2;130(11):6054-6063. PMID: 32790648
4. Ritterhoff J, Young S, Villet O, Shao D, Neto FC, Bettcher LF, Hsu YA, Kolwicz Jr. SC, Raftery D, **Tian R**. Metabolic Remodeling Promotes Cardiac Hypertrophy by Directing Glucose to Aspartate Biosynthesis. *Circ Res* 2020 Jan 17; 126(2): 182-196. PMID: 31709908; PMC9162292

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2020- Editor-in-Chief, Journal of Molecular and Cellular Cardiology
2020- Adjunct Professor of Department of Medicine - Cardiology, University of Washington, Seattle, WA
2014- Academic Editor, PLOS Biology
2013- Adjunct Professor of Pathology, University of Washington, School of Medicine, Seattle, WA, U.S.A.
2009- Professor of Anesthesiology & Pain Medicine and Bioengineering, Adjunct Professor of Biochemistry, University of Washington, Seattle, WA
2009- Director, Mitochondria and Metabolism Center, University of Washington, Seattle, WA
2007- Circulation Research editorial board, Consulting Editor (2016-2019)
2007- Journal of Molecular and Cellular Cardiology, associate editor (2019) editor-in-chief (2020-)
2007-2011 Board of directors, Society of Heart and Vascular Metabolism
2006- Circulation editorial board; section editor (2006-09)
2006- International Society for Heart Research: Councilor: 2006-12, Treasurer: 2018-2019 of the North American Section; Executive Council of the International (2020-present)
2005-2009 Associate Professor of Medicine, Harvard Medical School, Boston, MA
2005-2009 Marketing and Communication Committee of the American Heart Association
2005-2006 CV section Award Committee of the American Physiological Society
2004-2005 Steering Committee of the CV section of American Physiological Society
2002-06, 2016-20 Leadership committee of the American Heart Association Basic CV Science Council
2002-2006 Chair, Committee on Communication and newsletter editor for the American Heart Association Basic Cardiovascular Science Council
2002-2016 NIH chartered study section (CVA: 02-03; MIM: 03-06; MIST:14-16)
2000-present Special emphasis panels for the CSR, NHLBI, NIDDK, NIEHS
2000-present *Ad Hoc* reviewer for the Program Project Grant Review Committee of NHLBI
1999-2005 Assistant Professor of Medicine, Harvard Medical School, Boston, MA.
1997-1999 Instructor in Medicine, Harvard Medical School, Boston, MA.

Honors

2021 George E. Brown Memorial Lecture, American Heart Association

2019 Berne Lecturer, American Physiological Society
2019 Fischer Lecturer, Tulane University
2017 College of Fellows, American Institute for Medical and Biological Engineering (AIMBE)
2017 Bernard and Joan Marshall Distinguished Investigator, British Society for CV Research
2017 Research Achievement Award of the International Society for Heart Research
2010 Distinguished Achievement Award, American Heart Association Council on Basic Science
2008 Election to the American Society of Clinical Investigation
2004 Young Investigator Award of the American Physiological Society
2003-2007 Established Investigator Award of the American Heart Association
1999 Scholars in Medicine at Harvard Medical School
1998 First Award by NIH
1998 Scientist Development Grant Award of the American Heart Association

C. Contributions to Science

1. Investigating mitochondrial mechanisms in the pathogenesis of heart failure. We have identified a number of mechanisms linking mitochondrial function to cardiac sensitivity to stress including substrate metabolism, NAD(H) redox and mitochondrial turnover. We have also developed novel technology to investigate mitochondrial protein interactome in the heart during normal and stress conditions.

   a. Karamanlidis G, Nascimben L, Couper GS, Shekar PS, Tian R. Defective DNA replication impairs mitochondrial biogenesis in human failing hearts. *Circ Res.* 2010, 109:1541—1549. PMID: 20339121 PMCID: PMC2880225.

   b. Schweppe DK, Chavez JD, Lee CF, Caudal A, Kruse SE, Stuppard R, Marcinek DJ, Shadel GS, Tian R, Bruce JE. Mitochondrial protein interactome elucidated by chemical cross--linking mass spectrometry. *Proc Natl Acad Sci USA.* 2017 Feb 14; 114(7):1732-1737. doi: 10.1073/pnas. 1617220114. PMID: 28130547

   c. Zhou B, Tian R. Mitochondrial dysfunction in pathophysiology of heart failure. *J Clin Invest*. 2018 Aug 31; 128(9):3716-3726. doi: 10.1172/JCI120849. Epub 2018 Aug 20. PMID: 30124471

   d. Shao D, Kolwicz SC, Wang P, Roe ND, Villet O, Nishi K, Hsu YA, Flint GV, Caudal A, Wang W, Regnier M, Tian R. Increasing Fatty Acid Oxidation Prevents High Fat Diet Induced Cardiomyopathy through Regulating Parkin Mediated Mitophagy *Circulation* 2020 Sep 8;142(10):983-997. doi: 10.1161/CIRCULATIONAHA.119.043319. Epub 2020 Jun 29. PMID: 32597196

2. Targeting NAD metabolism for therapy. To translate novel mechanisms identified in the lab into therapy we have focused on the role NAD metabolism in mitochondrial signaling and bioenergetics. Our results suggest that NAD sensitive mechanisms are promising therapeutic targets for a variety of chronic diseases associated with mitochondrial dysfunction including heart failure.

   a. Lee CF, Chavez J, Garcia-Menendez L, Choi YS, Roe N, Chiao YA, Edgar J, Goo YA, Goodlett, Bruce J, Tian R. Normalization of NAD+ Redox Balance as a Therapy for Heart Failure. *Circulation* 2016 Sep 20;134(12):883-94.

   b. Wang W, Karamanlidis G, Tian R. Novel Targets for Mitochondria Medicine. *Sci Transl Med*. 2016 Feb 17;8(326):326rv3. doi: 10.1126/scitranslmed.aac7410. PMID: 26888432

   c. Lee CF, Caudal A, Nagana Abell L, Gowda GA, Tian R. Targeting NAD+ metabolism as interventions for mitochondrial disease. *Sci Rep*. 2019 Feb 28;9(1):3073. doi: 10.1038/s41598-019-39419-4.

   d. Zhou B, Wang DD, Qiu Y, Airhart S, Liu Y, Stempien-Otero A, O'Brien KD, Tian R. Boosting NAD Level Suppresses Inflammatory Activation of PBMC in Heart Failure. *J Clin Invest*. 2020 Aug 13:138538. doi: 10.1172/JCI138538. Online ahead of print. PMID: 32790648

3. The role of metabolic reprogramming in cardiac injury and pathological remodeling. We have investigated the functional significance of altered substrate metabolism using a variety of bioengineered mouse models and multi-nuclear NMR spectroscopy. Our studies identified mechanistic links between alterations in

glucose, fatty acids and amino acids metabolism and cardiac stress response through both energy provision (catabolic metabolism) and non-energy provision (anabolic and signaling functions) pathways.

a. Luptak I, Balschi JA, Xing Y, Leone TC, Kelly DP, Tian R. Decreased contractile reserve in PPARα null hearts can be rescued by increasing glucose transport and utilization. *Circulation* 2005; 112:2339-2346.

b. Yan J, Young ME, Cui L, Lopaschuk GD, Liao R, Tian R. Increased glucose uptake and oxidation in mouse hearts prevents high fatty acid oxidation but causes cardiac dysfunction in diet—induced obesity. *Circulation.* 2009; 119:2818—2828. Epub 2009 May 18. PMID:19451348 PMCID: PMC2765220.

c. Kolwicz Jr., SC, Olson DP, Marney LC, Garcia-Menendez L, Synovec RE, Tian R. Cardiac-specific deletion of acetyl CoA carboxylase 2 (ACC2) prevents metabolic remodeling during pressure-overload hypertrophy. *Circ Res* 2012, Aug 31; 111(6): 728-38. PMID: 22730442; PMCID: PMC3434870.

d. Shao D, Villet O, Zhang Z, Choi SW, Yan J, Ritterhoff J, Gu H, Djukovic D, Christodoulou D, Kolwicz SC Jr, Raftery D, Tian R. Glucose promotes cell growth by suppressing branched-chain amino acid degradation. *Nat Commun*. 2018 Jul 26; 9(1):2935. doi: 10.1038/s 41467-018-05362-7. PMID: 30050148

4. Stem cell Metabolism. We characterized the relationship between mitochondrial metabolism and maturation of human pluripotent stem cell derived cardiomyocytes. Furthermore, we sought to enhance the maturation of hiPSC-CM by targeting oxidative metabolism.

a. Dai DF, Danoviz ME, Wiczer B, Laflamme MA, Tian R. Mitochondrial Maturation in Human Pluripotent Stem Cell Derived Cardiomyocytes. *Stem Cells Int*. 2017, 2017:5153625. doi: 10.1155/2017/5153625. Epub 2017 Feb 27. PMID: 28421116

b. Lee CF, Cao Y, Tian R. Failed power plant turns into mass murder: New insight on mitochondrial cardiomyopathy. *Circ Res*. 2018 Jan 5;122(1):11—13. doi: 10.1161/CIRCRESAHA.117.312288. PMID:29301837

c. Yang X, Rodriguez ML, Leonard A, Sun L, Fischer KA, Wang Y, Ritterhoff J, Zhao L, Kolwicz SC Jr, Pabon L, Reinecke H, Sniadecki NJ, Tian R, Ruohola-Baker H, Xu H, Murry CE. Fatty Acids Enhance the Maturation of Cardiomyocytes Derived from Human Pluripotent Stem Cells. *Stem Cell Rep*. 2019 Sep 12. pii: S2213-6711(19)30307-8. doi: 10.1016/j.stemcr.2019.08.013. [Epub ahead of print] PMID: 31564645

5. Elucidating the role of AMP-activated protein kinase (AMPK) in cardiac biology and diseases. We are the first to report that AMPK cascade in the heart acts as a signaling intermediary connecting the cardiac energy status to the regulation of metabolism. Mutations of the γ2-subunit of AMPK lead to aberrant kinase activity and the development of cardiomyopathy in humans.

a. Xing Y, Musi N, Fujii N, Zou L, Luptak I, Hirshman MF, Goodyear LJ, Tian R. Glucose metabolism and energy homeostasis in mouse hearts overexpressing dominant negative α2 subunit of AMP-activated protein kinase. *J Biol Chem* 2003, 278:28372-28377. PMID: 12766162

b. Luptak I, Shen M, He H, Hirshman MF, Musi N, Goodyear LJ, Yan J, Wakimoto H, Morita H, Arad M , Seidman CE, Seidman JG, Ingwall JS ,Balschi JA, and Tian R. Aberrant activation of AMP-activated protein kinase remodels metabolic network in favor of cardiac glycogen storage. *J Clin Invest.* 2007;117(5):1432-9. PMCID: PMC1847536.

c. Kim M, Hunter RW, Garcia-Menendez L, Gong G, Yang YY, Kolwicz SC Jr, Xu J, Wang W, Sakamoto K, Tian R. Mutation in the γ2-subunit of AMPK Stimulates Cardiomyocyte Proliferation and Hypertrophy Independent of Glycogen Storage. *Circ Res.* 2014 Mar 14;114(6):966-75. Epub 2014 Feb 6. PMCID: PMC3971100.

d. Cao Y, Bojjireddy N, Kim M, Li T, Zhai P, Nagarajan N, Sadoshima J, Palmiter RD, Tian R. Activation of γ2-AMPK suppresses ribosome biogenesis and protects against myocardial ischemia/reperfusion

injury. *Circ Res.* 2017 Aug23. pii: CIRCRESAHA.117.311159. doi: 10.1161/CIRCRESAHA.117.311159. [Epub ahead of print] PMID: 28835357

Complete List of Published Work in MyBibliography (a total of 125):
http://www.ncbi.nlm.nih.gov/sites/myncbi/1BUIslaiMWcAd/bibliography/42777656/public/?sort=date&direction=asc ending

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Ning Zheng

eRA COMMONS USER NAME (credential, e.g., agency login): nzheng

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Fudan University, Shanghai, PR of China	B.S.	08/1991	Scientific Conservation of Historical Relics
University of Texas Southwestern Medical Center at Dallas, Dallas, TX	Ph.D	08/1997	Molecular Biophysics
Memorial Sloan-Kettering Cancer Center, New York, NY	Postdoctoral	07/2002	Structural Cancer Biology

A. Personal Statement

Research in my laboratory is aimed at dissecting the molecular and structural mechanisms by which protein-protein, protein-nucleic acids, and protein-small molecule interactions control eukaryotic biology and human diseases. We emphasize on challenging yet fundamental questions that will have cross-disciplinary significances and impacts. We have a long-standing interest in the E3 enzymes regulating protein ubiquitination, their roles in signaling, and their therapeutic potentials. We have also developed a second research program focusing on membrane protein structure biology and structural analyses of selected ion channels, transporters, and membrane pores that play an important role in pharmacology and plant biology. We have a strong track record in purifying, characterizing, and determining the crystal structures of multi-component protein assemblies. Besides research, I serve as the chair of a graduate level course entitled “Drug Discovery and Emerging Therapeutics”, give lectures on PK/PDs in our medical curriculum, and contribute to several courses on protein structure and function. I have mentored and co-mentored more than fifteen graduate students and more than twenty postdoctoral fellows in my lab.

B. Positions, Scientific Appointments, and Honors

Positions

2012-now	Professor, Department of Pharmacology, University of Washington, Seattle, WA
2008-now	Investigator, Howard Hughes Medical Institute
2007-2012	Associate Professor, Department of Pharmacology, University of Washington, Seattle, WA
2002-2007	Assistant Professor, Department of Pharmacology, University of Washington, Seattle, WA
1997-2002	Post-Doctoral Fellow with Dr. Nikola Pavletich, Memorial Sloan-Kettering Cancer Center, New York, NY
1991-1997	Graduate Research Assistant with Dr. Lila M. Gierasch, University of Texas Southwestern Medical Center at Dallas, Dallas, TX/University of Massachusetts at Amherst, Amherst, MA

Scientific Appointments

2004-2011	Ad hoc reviewer for NIH study sections, BBCB, MSFC, BCMC, CB_J.
2007-2011	Ad hoc reviewer for NSF
2011	Member of HHMI-GBMF Plant Science Investigator Selection Committee
2011-2017	Regular member of the NIH study section MSFC

2012 Ad hoc reviewer for NIH Director's Pioneer Awards Program.

Honors

2004 Pew Scholar in Biomedical Sciences
2004 Searle Scholar (declined)
2007 Burroughs Wellcome Investigator in Pathogenesis of Infectious Diseases
2008 Howard Hughes Medical Institute Investigator
2015 AAAS Fellow
2020 Washington State Academy of Sciences Member

Ongoing and recently completed projects

HHMI Zheng (PI) 10/01/08 – 8/31/2025
Supports from HHMI are not project-orientated. A Visiting Scholar from Henan Univ. in China participates in the studies.

NIH – R01 HD097408 (Zheng, PI, Chatterjee, Co-PI) 07/01/2020 – 06/30/2025
Structure and Mechanism of the SET1/COMPASS H3K4 Methyltransferase Complex

NSF – IOS 1901566 (Xiao, PI, Zheng, Consultant) 08/01/2020 – 07/31/2023
Probing the Molecular Interactions at the Extrahaustorial Membrane Interface

BeyondSpring Inc. Zheng (PI) 05/01/2016 – 04/30/2020
Discovery of Ubiquitin Ligase Reprogramming Agonists.

NIH - 2R01HL112808-06 Catterall (PI, Zheng as Co-PI) 01/01/2018 – 12/31/2019
Structural basis for Antiarrhythmic Drug Action. Zheng currently serves as a Collaborator.

Citations:

1. Hsu PL, Shi H, Leonen C, Kang J, Chatterjee C*, & **Zheng N***. 2019. Structural Basis of H2B Ubiquitination-Dependent H2K4 Methylation by COMPASS. **MOL. CELL** 76:712-723. (*Co-corresponding).
2. Jiang D, Shi H, Tonggu L, Gamal El-Din TM, Lenaeus MJ, Zhao Y, Yoshioka C, **Zheng N***, & Catterall WA*. 2020. Structure of the Cardiac Sodium Channel. **CELL** 180:122-134. (*Co-corresponding).
3. Wang H, Shi H, Rajan H, Canarie ER, Hong S, Simoneschi D, Pagano M, Bush MF, Stoll S, Leibold EA, & **Zheng N**. 2020. FBXL5 Regulates IRP2 Stability in Iron Homeostasis via an Oxygen-Responsive [2Fe2S] Cluster. **MOL. CELL**. 78:31-45.

C. Contributions to Science

Contribution 1. Cullin-RING ubiquitin ligase complexes. As a postdoc fellow in Dr. Nikola Pavletich's lab, I determined the first crystal structure of the SCF E3 complex, which is the prototype of the cullin-RING ubiquitin ligase superfamily. After setting up my own lab, we have delineated for the first time how a 120kDa protein, CAND1, binds and regulates the assembly of SCF. We have further defined a new class of cullin-RING E3s, known as the DDB1-CUL4A-RBX1 E3 complex by not only determining the crystal structure of this entire complex, but also identifying a family of WD40-repeat proteins, DCAFs, as the missing substrate receptors. Our studies of DDB1-CUL4A-RBX1 complex also established for the first time the mechanism by which a viral protein hijacks and reprograms a cellular ubiquitin ligase machinery for degrading host's anti-viral factor.

1. **N Zheng**, B Schulman, JJ Miller, P Wang, PD Jeffrey, C Chu, DM Koepp, SJ Elledge, M Pagano, RC Conaway, JW Conaway, JW Harper, and NP Pavletich 2002. Structure of the Cul1-Rbx1-Skp1-Fbox^{Skp2} Ubiquitin-Protein Ligase Complex. **NATURE** 416: 703-709 (*Cover story*).

2. Goldenberg SJ, Cascio TC, Shumway SD, Garbutt KC, Liu J, Xiong Y, & **Zheng N.** 2004. Structure of the Cand1-Cul1-Roc1 Complex Reveals Regulatory Mechanisms for the Assembly of the Multisubunit Cullin-Dependent Ubiquitin Ligases. **CELL** 119: 5170528.

3. Li T, Chen X, Garbutt KC, Zhou P, & **Zheng N** 2006. Crystal structure of DDB1 in complex with simian virus 5 V protein: viral hijack of A propeller cluster in ubiquitin ligase. **CELL** 124:105-117.

4. Angers S, Li T, Yi X, MacCoss MJ, Moon RT*, & **Zheng N***. 2006. Molecular Architecture and Assembly of the DDB1-Cul4A Ubiquitin Ligase Machinery. **NATURE** 443:590-593. (*Co-correspondence) ♦

Contribution 2. Ubiquitin Ligase-based Plant Hormone Receptors. My lab has made seminal contributions to the plant biology field by reporting the structural mechanism by which the F-box protein TIR1 functions as the *bona fide* receptor for auxin, a plant hormone that controls almost every aspect of plant physiology. Our results directly answer a central question in plant biology that was raised in Darwin's time and had puzzled scientists over a century. In doing so, our study establishes the first structural model of a plant hormone receptor, which has since triggered a cascade of studies of other key plant hormone receptors. Our group have been closely involved in the identification and characterization of the receptors for jasmonates, salicylic acid, and strigolactones. Beyond the plant biology field, our research on these F-box-based plant hormone receptor have made profound impact to drug discovery targeting human ubiquitin ligases.

1. Tan X, Calderon-Villalobos LIA, Sharon M, Zheng C, Robinson CV, Estelle M, & **Zheng N**. 2007. Mechanism of Auxin Perception by the TIR1 Ubiquitin Ligase. **NATURE** 446:640-645 (*Cover story*).

2. Sheard LB, Tan X, Mao H, Withers J, Ben-Nissan G, Hinds TR, Kobayashi Y, Hsu F, Sharon M, Browse J, He SY, Rizo J, Howe GA & **Zheng N**. 2010. Jasmonate perception by inositol-phosphate-potentiated COI1–JAZ co-receptor. **NATURE** 468:400-5.

3. Fu Z, Yan S, Saleh A, Wang W, Ruble J, Oka N, Mohan R, Spoel SH, Tada Y, Zheng N, & Dong X. 2012. NPR1 paralogues, NPR3 and NPR4, are receptors of the immune signal salicylic acid in plants. **NATURE** 486:228-32.

4. Shabek N, Ticchiarelli F, Mao H, Hinds TR, Leyser O, **Zheng N**. 2018. Structural plasticity of D3-D14 Ubiquitin Ligase in Strigolactone Signaling. **NATURE** 563:652-656.

Contribution 3. Deubiquitinase Complex and Regulation. Structural biology of deubiquitinases (DUBs) represents a new direction of our research. DUBs are intracellular iso-peptidases, which are able to cleave ubiquitin off target proteins. It is expected that their activities are tightly regulated. Most available DUB structures are of individual catalytic domains and cannot address the question as to how they can be allosterically regulated by interacting partners. We have made the first breakthrough by determining the crystal structure of the entire four-subunit histone H2B DUB module found in the transcription coactivator complex, SAGA. Besides delineating the functional role of each subunit, we discovered for the first time how a small zinc-finger domain of one subunit is "strategically" anchored next to the catalytic triad of the DUB catalytic domain, playing a crucial role in enzyme activation. Part of our current research is focused on two other prototypical DUB regulatory proteins, which likely activates the target deubiquitinase via long-range allosteric mechanisms.

1. Köhler A*, Zimmerman E, Schneider M, Hurt E, & **Zheng N*.** 2010. Structural Basis for Assembly and Activation of the Hetero-tetrameric SGA Histone H2B Deubiquitinase Module. **CELL** 141:606-617. (*Co-correspondence)

2. Li H, Lim KS, Kim H, Hinds TR, Jo U, Mao H, Weller CE, Sun J, Chatterjee C, D'Andrea AD, & **Zheng N**. 2016. Allosteric Activation of Ubiquitin-Specific Proteases by β-Propeller Proteins UAF1 and WDR20. **MOL CELL**. 63:249-260.

Contribution 4. Structural Biology of Circadian Clock. Our research in protein ubiquitination led us to the mammalian circadian clock field, which has been pioneered by geneticists but lacks in-depth biochemical and mechanistic understanding of the molecular clockwork. Through structure-function studies of the key components of the clock pathway, we have made several breakthroughs in revealing the structural basis and regulatory mechanisms of the transcriptional and translational negative feedback loop that drives the circadian

cycle in mammalian cells. We reported the first crystal structures of the human cryptochrome-2 protein in a total of five different functional states, including apo, FAD-bound, drug-bound, ubiquitin ligase-bound, and in complex with its obligate partner, Period). These studies have established the missing structural framework for understanding the molecular underpinnings of the mammalian circadian clock.

1. Xing W, Busino L, Hinds TR, Marionni ST, Saifee NH, Bush MF, Pagano M, **Zheng N.** 2012. SCF-Fbxl3 Ubiquitin Ligase Targets Cryptochromes at Their Cofactor Pocket. **NATURE** 496: 64-67.

2. Nangle S, Xing W, **Zheng N.** 2013. Crystal Structure of Mammalian Cryptochrome in Complex with A Small Molecule Competitor of Its Ubiquitin Ligase. **CELL RES.** 23:1417-9.

3. Nangle S, Rosensweig C, Koike N, Tei H, Takahashi J*, Green CB, & Zheng N*. 2014. Molecular Assembly of the Mammalian Period-Cryptochrom Circadian Clock Core Complex. **eLIFE** 3:e03674 (*Co-corresponding).

Contribution 5. Structural Biology of Voltage-gated Ion Channels and Transporters. Voltage-gated sodium channels (Na_V) play a key role in generating action potential in most excitable cells and are the targets of local anaesthetics, antiarrhythmics and antiepileptic drugs. Despite the long history of Na_V channel research, the sheer size of the vertebrate Na_V channels made their structural determination highly challenging. In close collaboration with Bill Catterall's group within our own department, we made our first attempt of membrane protein crystallography and succeeded in solving the long-waited first structure of a voltage-gated sodium (Na_V) channel. This has been recognized as a “milestone” achievement by the ion channel field. We have subsequently determined the structure of the channel in its inactivated state and converted its ion selectivity toward calcium. In parallel to these studies, we have begun investigating structure-function relationship of eukaryotic transporters. We reported the first plant nitrate transporter structure in 2014.

1. Payandeh J, Scheuer T, **Zheng N***, & Catterall WA* 2011. The crystal structure of a voltage-gated sodium channel. **NATURE** 475:353-358. (*Co-correspondence)

2. Payandeh J, Gamal El-Din, TM, Scheuer T, **Zheng N***, & Catterall WA*. 2012. Crystal structure of a voltage-gated sodium channel in two potentially inactivated states. **NATURE** 486:135-9. (*Co-correspondence).

3. Sun J, Bankston JR, Payandeh J, Hinds TR, Zagotta WN, & Zheng N. 2014 Crystal structure of the Plant Dual-affinity Nitrate Transportor NRT1.1. **NATURE** 701:73-7..

4. Tang L, El-Din TM, Swanson TM, Pryde DC, Scheuer T, **Zheng N***, & Catterall WA*. 2016. Structural basis for inhibition of a voltage-gated Ca_{2+} channel by Ca_{2+} antagonist drugs. **NATURE** 537:117-21. (*Co-correspondence)

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/pubmed/?term=Zheng+N+AND+(University+of+Washington+%5BAD%5D+OR+Sloan+%5BAD%5D)+NOT+Exome