Graduate Fellowship FAQs

1. How will I be funded if I don't have a fellowship?
    a. Federal (NIH/NSF) training grant
    b. Stanford endowment
    c. Research assistantship (RA) from research grants
    d. Teaching assistantship (TA)
2. Why should I apply for fellowships?
    a. Prestige
    b. Slight increase in stipend
    c. Fringe benefits – supercomputing time, travel support, networking events
    d. Practice writing grants
    e. Gives you more freedom to pick advisors/labs
    f. Relieves financial pressure on your advisor
    g. Relieves teaching requirements
3. Why shouldn't I apply for fellowships?
    a. You're not eligible. For NSF this means you're not a US citizen or you've no longer in the first two years of graduate school
4. What fellowships am I eligible for?
    a. Nationally competitive
        i. NSF GRFP – 11/18, http://www.nsfgrfp.org/
        ii. DoD NDSEG – 12/16, http://ndseg.asee.org/
        iii. DOE CSGF – 1/10, http://www.krellinst.org/csgf/
        iv. DOE CGSF – may not be offered this year, http://scgf.orau.gov/
        v. DHS DHSED – may not be offered this year, http://www.orau.gov/dhsed/
        vi. NPSC – 11/30, http://www.npsc.org/
        vii. DoD SMART – 12/1, http://smart.asee.org/
        viii. Hertz – 10/31, http://www.hertzfoundation.org/
        ix. Soros – 11/1, http://www.pdsoros.org/
        x. NIH F31 – 12/13, 4/13, 8/13, http://grants.nih.gov/training/nrsa.htm
        xi. Ford Foundation – 11/14, http://sites.nationalacademies.org/pga/fordfellowships/
    b. Stanford internal
        i. SGF – nominated in spring, http://sgf.stanford.edu/
        ii. SIGF / Bio-X – Late March, http://biox.stanford.edu/grant/grad2010.html
        iii. Training Grant in Biotechnology - Late July, http://cheme.stanford.edu/faculty/nih/
5. How long are the tenure of fellowships?
    a. Generally 3 years
6. How many biophysics students have won various fellowships over the past 5 years (out of 24 students total although not all 25 were eligible for each fellowship)
    a. NSF: 10

Graduate Fellowship FAQs

   b. NSDEG: 1
   c. DHS: 1
   d. Hertz: 1
   e. SGF: several
   f. Others: 0
7. What are the requirements for the NSF and NDSEG applications?
   a. NSF
      i. Application: 3 essays, 3 recommendations, Transcript, NO GRE
      ii. Choosing a subfield
      iii. the three essays
      iv. intellectual merit and broader impacts criteria
   b. NDSEG
      i. 3000 characters summary of goals
      ii. 400 character essays on leadership experience, teamwork experience, community & volunteer work
      iii. CV (publications, presentations, awards, honors, research/work experience, memberships),
      iv. GRE, Transcript
      v. 3 recommendations
   c. Others if there's time
8. What to do once you get a fellowship / FAQs
   a. Terms and conditions of fellowships
      i. Very minimal
      ii. Usually you are free to pursue whatever is interesting to you, you're not obligated to pursue what you propose in the applications
   b. What happens if you get more than one fellowship
      i. Often you can't accept multiple fellowships
      ii. But you can still write on your CV that you won them
   c. Requirements for keeping fellowships
      i. Maintain good academic standing
      ii. Yearly progress reports
9. Resources
   a. NSF GRFP Presentation
      http://www.drexel.edu/fellowships/search/fellowships/NSF%20GRFP/
   b. Advice for applying for NSF, NDSEG
      http://www.stanford.edu/~pgbovine/fellowship-tips.htm
   c. More advice for NSF
      http://gradschool.missouri.edu/financial/assistantships-fellowships/fellowships/external/nsf-research-fellowship/

Graduate Fellowship FAQs

d. NSF, NDSEG sample essays
   https://www.stanford.edu/group/stbl/cgi-bin/fellowships/
e. NSF sample essays, advice
   https://jshare.johnshopkins.edu/deyler1/public_html/NSFappguide.htm
f. NSF sample essays
   http://rachelcsmith.com/academics/nsf.htm
   http://www.anat.stonybrook.edu/IDPAS/student_grants/pastapplications.html
   http://grads.astro.cornell.edu/sample_essays/
   http://www.alexhunterlang.com/nsf-fellowship
   http://web.mit.edu/campsoup/www/fellowship.html

NSF Graduate Research Fellowship

Intellectual Merit Criterion

NSF Official Statement

Panelists will consider factors including: the strength of the academic record, the proposed plan of research and whether it is potentially transformative, the description of previous research experience, references, Graduate Record Examinations (GRE) General and Subject Tests scores, and the appropriateness of the choice of institution relative to the proposed plan for graduate education and research.

NSF Bullet points

- How important is the proposed activity to advancing knowledge and understanding within its own field or across different fields?
- How well qualified is the proposer (individual or team) to conduct the project? (If appropriate, the reviewer will comment on the quality of prior work.)
- To what extent does the proposed activity suggest and explore creative, original, or potentially transformative concepts?
- How well conceived and organized is the proposed activity?
- Is there sufficient access to resources?

Broader Impacts Criterion

NSF Official Statement

The broader impacts criterion includes contributions that infuse learning with the excitement of discovery, and assure that the findings and methods of research are communicated in a broad context and to a large audience.

A strong application will encourage diversity, broaden opportunities, and enable the participation of all citizens-women and men, underrepresented minorities, and persons with disabilities-in science and research.

In addition to reaching a broad audience, a strong application must demonstrate how it will enhance scientific and technical understanding, while benefiting society.
Applicants may provide characteristics of their background, including personal, professional, and educational experiences, to indicate their potential to fulfill the broader impacts criterion.

NSF Bullet points

- How well does the activity advance discovery and understanding while promoting teaching, training, and learning? Eg.
    - Include students (e.g., K-12, undergraduate science majors, non-science majors, and /or graduate students) as participants in the proposed activities as appropriate.
    - Establish special mentoring programs for high school students, undergraduates, graduate students, and technicians conducting research.
- How well does the proposed activity broaden the participation of underrepresented groups (e.g., gender, ethnicity, disability, geographic, etc.)?
    - Include students from underrepresented groups as participants in the proposed research and education activities.
    - Mentor early-career scientists and engineers from underrepresented groups who are submitting NSF proposals.

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- To what extent will it enhance the infrastructure for research and education, such as facilities, instrumentation, networks, and partnerships?
    - Identify and establish collaborations between disciplines and institutions, among the U.S. academic institutions, industry and government and with international partners.
    - Stimulate and support the development and dissemination of next-generation instrumentation, multi-user facilities, and other shared research and education platforms.
- Will the results be disseminated broadly to enhance scientific and technological understanding?
    - Partner with museums, nature centers, science centers, and similar institutions to develop exhibits in science, math, and engineering.
    - Give science and engineering presentations to the broader community (e.g., at museums and libraries, on radio shows, and in other such venues.).
    - Make data available in a timely manner by means of databases, digital libraries, or other venues such as CD-ROMs.
    - Integrate research with education activities in order to communicate in a broader context.
- What may be the benefits of the proposed activity to society?
    - Demonstrate the linkage between discovery and societal benefit by providing specific examples and explanations regarding the potential application of research and education results.

Phillip Guo Comments

*Broader Impacts (mostly expressed within your Personal Statements Essay) makes or breaks your application***.** Alone, it cannot secure you a victory, but coupled with great technical qualifications (the other criterion, *Intellectual Merit*), it can vastly improve your chances of winning. Every single person whom I've asked about the NSF fellowship tells me to spend lots of time thinking about and addressing Broader Impacts. They've told me stories about how their friends barely missed winning because their Broader Impacts weren't, well, broad enough. You must infuse Broader Impacts throughout all of your essays, but the *Personal Statements Essay* gives you the most opportunities to do so. Let's go through the 4 parts of the criterion one-by-one:

1. *effectively integrate research and education at all levels, infuse learning with the excitement of discovery, and assure that the findings and methods of research are communicated in a broad context and to a large audience*
    - This means not only that you should be enthusiastic about learning for your own hot self, but more importantly, that you should be enthusiastic about sharing knowledge with others. TAing, tutoring, and mentoring are good examples. Even better, if you have done anything with science education, especially for children in underprivileged areas, that would be golden. This should go in your *Personal Statements Essay* and in your short answers about your teaching experiences.
2. *encourage diversity, broaden opportunities, and enable the participation of all citizens-women and men, underrepresented minorities, and persons with disabilities-in science and research*
    - I think that this is the most influential point in Broader Impacts. The diversity point. This should definitely go in your *Personal Statements Essay*. Find SOME way to incorporate this point. If you are a woman, part of an underrepresented minority, or have some disability, now is the time and place to mention it somehow (always tastefully and sparingly, of course; it should not be your main selling point). Have no fear or shame. This is a game, and your strongest competitors who are in these demographics will mention their minority status. I know NSF winners in these demographics who've told me how they mentioned their status in their essays, even though they felt that it sounded a bit cheesy. Whatever. It's better to be a bit cheesy and win over $100k than to stubbornly stick to your principles and lose out. After all, the primary criteria for evaluation is your technical merit, so if you are really well-qualified anyways, nobody should think that you got this fellowship 'just because you're part of X or Y group'. What if you aren't part of any underrepresented minority? Well, think about anything you have done to promote diversity in science and education, and write about those experiences (if any).
3. *enhance scientific and technical understanding*
    - This one is like doing science for the sake of science. Don't worry about it too much because the boring technical aspects of your application, such as your *Previous Research* and *Proposed Research* essays should cover it pretty well.
4. *benefit society*
    - This is really about the impact of your work, both previous and proposed. Remember: impact, real world impact. You're not doing science in a bubble.

NSF Graduate Research Fellowship

Out of all 3 fellowship applications, I spent by far the most time on the NSF. It was definitely time well spent, though, because it made doing the other 2 applications, especially the NDSEG, much easier.

NSF Graduate Research Fellowship

Previous Research Experience

NSF Prompt

Describe any scientific research activities in which you have participated, such as experience in undergraduate research programs, or research experience gained through summer or part-time employment or in work-study programs, or other research activities, either academic or job-related. Explain the purpose of the research and your specific role in the research, including the extent to which you worked independently and/or as part of a team, and what you learned from your research experience. In your statement, distinguish between undergraduate and graduate research experience. At the end of your statement, list any publications and/or presentations made at national and/or regional professional meetings.

If you have no direct research experience, describe any activities that you believe have prepared you to undertake research.

NSF Fellows are expected to become globally-engaged knowledge experts and leaders who can contribute significantly to research, education, and innovations in science and engineering.

You MUST provide specific details in this essay that address BOTH the NSF Merit Review Criteria of Intellectual Merit and Broader Impacts in order for your application to be competitive. Please refer to the Program Announcement for further information on the NSF Merit Review Criteria (examples of Broader Impacts activities).

NSF Bullet points

- What are all of your applicable experiences?
- For each experience, what were the key questions, methodology, findings, and conclusions?
- Did you work in a team and/or independently?
- How did you assist in the analysis of results?
- How did your activities address the Intellectual Merit and Broader Impacts criteria?

Phillip Guo Comments

This essay is pretty plain vanilla. Just follow the prompt and write about all the research projects in which you have been involved, making sure to hit all the points the prompt requires. Briefly describe each project, your particular role in it, how much you worked per week, what you learned from the experience, what impact it had on the world (however small), how it motivated you to want to pursue a Ph.D. (if it did at all), etc.

The flow of my essay was one of build-up and climax. I wrote about 4 projects in chronological order, each one requiring more sophistication and experience than the previous one. The climax came as I described my most significant (which was my most recent) project. I spent the first page describing the first three projects, and the second page solely dedicated to describing the final and most significant one. I suggest devoting more space to your most significant project, because it emphasizes your greatest strength; it also provides good context for your next essay, Proposed Research, as well as ample space for making cross-references between your essays. Don't devote an equal amount of space to each of your projects unless you truly feel that they are all of equal significance.

If you have published (or submitted) papers, in addition to listing them at the end of this essay in a mini-bibliography like the prompt requires, make references to them in the essay itself in order to put those papers in their proper context.

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Proposed Plan of Research

NSF Prompt

In a clear, concise, and original statement, present a complete plan for a research project that you plan to pursue while on fellowship tenure and how you became interested in the topic. Your statement should demonstrate your understanding of research design and methodology and explain the relationship to your previous research, if any.

NSF Fellows are expected to become globally-engaged knowledge experts and leaders who can contribute significantly to research, education, and innovations in science and engineering.

You MUST provide specific details in this essay that address BOTH the NSF Merit Review Criteria of Intellectual Merit and Broader Impacts in order for your application to be competitive. Please refer to the Program Announcement for further information on the NSF Merit Review Criteria (examples of Broader Impacts activities).

Format: Include the title, key words, hypothesis, research plan (strategy, methodology, and controls), anticipated results or findings, and literature citations. If you have not formulated a research plan, your statement should include a description of a topic that interests you and how you propose to conduct research on that topic.

In addition to review of the Intellectual Merit and Broader Impacts of your proposal, research topics discussed in your proposed plan will be used to determine eligibility. Refer to the Field of Study eligibility criterion in the program announcement.

NSF Bullet points

- What issues in the scientific community are you most passionate about?
- Do you possess the technical knowledge and skills necessary for conducting this work, or will you have sufficient mentoring and training to complete the study?
- Is this plan feasible for the allotted time and institutional resources?
- How will my research contribute to the "big picture" outside the academic context?
- How can I draft a plan using the specified research proposal format?
- How does your proposed research address the Intellectual Merit and Broader Impacts criteria?

Phillip Guo Comments

This essay is a real doozie. It will take a lot of thinking, planning, and discussion with your advisor and colleagues to come up with a research proposal that makes for a strong essay. What the reviewers are looking for is whether you can write a technical proposal for a project that is feasible, impactful, and realistic given your particular expertise. However, nobody is going to follow-up with you and force you to actually work on the project you proposed.

I would strongly suggest writing a proposal that's somehow based on work that you have already done, so that you can tie it in with your Previous Research essay (cross-references!!!). In other words, don't just make up some cool topic out of thin air. If you're not that excited about your current work and desperately want to switch to something completely different once you start your Ph.D., now is not the time to express your discontent. It's dangerous to propose a research project that's not related to what you've already done because it dampens your credibility. Show, don't tell. If you make a proposal that's based on your current work, you have more opportunities to show rather than tell. However, don't fall into the trap of simply proposing a small step forward for your current research (a slight tweak here and there), because that just makes you sound lazy and unoriginal. The reviewers want to see something innovative. Don't be afraid to make a

bigger, more ambitious conceptual leap, as long as it's grounded in your current work, even if you have no clue whether it's fully tractable to solve the problem you've proposed (just don't leap as far out as cold fusion or perpetual motion).

Oh yeah, you know how the prompt says: If you have not formulated a research plan, your statement should include a description of a topic that interests you and how you would propose to conduct research on that topic. Well, don't ever take this cop-out route! Formulate an actual research plan! Nothing is stopping you, other than lack of effort. Taking this cop-out route is a fast path to the land of loser.

A good research proposal will contain solid anchoring to your previous work, a well-motivated research problem, citations from 4 or 5 references to show that you are familiar with the body of existing work on this problem, and a proposed solution to the problem that sounds somewhat tractable. I know, 2 pages isn't much room at all, but everyone else has the same space constraints too.

The prompt wants you to satisfy the requirement of Intellectual Merit, but that is pretty straightforward. That simply means, write something that's technically competent and shows that you know what you're doing.

In order to satisfy the requirement of Broader Impacts in this essay, you will need to argue for why this research benefits society and the world (feel free to stretch a bit, but don't be too corny). And no, simply advancing the 'state of the art' in your field isn't sufficiently broad an impact, because that doesn't impact the people in the rest of the world who know nothing about your field. Think broader.

NSF Graduate Research Fellowship

Personal Statement

NSF Prompt

Describe any personal, professional, or educational experiences or situations that have prepared you or contributed to your desire to pursue advanced study in science, technology, engineering, or mathematics. Describe your competencies and evidence of leadership potential. Discuss your career aspirations and how the NSF fellowship will enable you to achieve your goals.

NSF Fellows are expected to become globally-engaged knowledge experts and leaders who can contribute significantly to research, education, and innovations in science and engineering. The purpose of this essay is to demonstrate your potential to satisfy this requirement. Your ideas and examples do not have to be confined necessarily to the discipline that you have chosen to pursue. You MUST provide specific details in this essay that address BOTH the NSF Merit Review Criteria of Intellectual Merit and Broader Impacts in order for your application to be competitive. Please refer to the Program Announcement for further information on the NSF Merit Review Criteria (examples *of Broader Impacts activities).*

NSF Bullet points

- Why are you fascinated by your research area?
- What examples of leadership skills and unique characteristics do you bring to your chosen field?
- What personal and individual strengths do you have that make you a qualified applicant?
- How will receiving the fellowship contribute to your career goals?
- How do these activities address the Intellectual Merit and Broader Impacts criteria?

Phillip Guo Comments

Ok, pay real close attention. This is the essay that will make or break your application. Stop laughing. I'm not kidding. It sounds like a foofy fluffy feel-good personal statement essay, but trust me, the people I knew who didn't take this essay seriously barely missed out on winning an award, getting an honorable mention instead and receiving written reviews along the lines of "you're very strong technically, but you just don't have enough broader impacts." Pretty much all top contenders are technically marvelous, so what makes the difference between a win and a near-win is Broader Impacts, which are best expressed in this essay.

See the next section for more details about Broader Impacts, but the name of the game in this essay is to incorporate as much Broader Impacts as humanly possible while still following the prompt. In previous years, there was a separate essay specifically asking you to address Broader Impacts, but in my year, they eliminated that essay, so this is your primary opportunity to discuss Broader Impacts.

Don't be too selfish in this essay; don't toot your own horn too much because the reviewers don't care about you as a person. Don't just make it all me, me, me, like about how deeply passionate you are about your field ever since you played with your first chemistry or electronics set when you were 6 years old. Instead, try to talk about your own work and interests in relation to other people, such as leadership, mentoring, TAing, learning from research colleagues, working on a team, etc. Show your passion for your field by cross-referencing your internship and research experiences (to weave together different parts of your app) instead of just recounting wonderful childhood nerd stories that nobody except your mother cares about.

DoD National Defense Science & Engineering (NDSEG) Fellowship

NDSEG Summary of Goals Prompt

In your own words, provide a summary of your educational program objectives and your long-range professional goals.

- As part of this statement, we are interested in your ideas about:
  - *the kinds of research in which you would like to be engaged* during your graduate study or in the longer term or
  - *specific research questions that interest you* and how you became interested in them.
- *Please discuss these research interests in sufficient detail for an expert who is technically competent in your field to judge your understanding of the questions to be addressed.* This includes relevant hypotheses and approaches one might take to answering the questions and other research principles required to investigate the research area you identify.
- *We are interested in not only the science, but also your longer-term goals and how the science fits into your life as an individual.* We do not want this to look like a grant submission.

The statement you present in this part of the application should be reflective of your ability to think independently and creatively, as well as your ability to write about your research or study plans accurately, thoughtfully, and concisely. The panelists evaluating your responses will be highly qualified professionals and faculty members, generally with doctoral degrees in the discipline you have selected. Be sure to include in your written response all relevant information pertaining to your goals.

Phillip Guo Comments

General Philosophy and Format
Despite its military-sounding name, I've heard that the NDSEG actually has fairly civilian intentions: The fellowships are given out to ensure that America has a steady supply of capable future scientists, because presumably America will be a stronger and safer place if our best and brightest don't escape to other naughty countries to make doomsday weapons for them. So don't worry. If you win this fellowship, you won't have to enlist in the military for mandatory service or anything. This application isn't due until January (after all your grad school apps), so if you've done a solid job on the NSF and Hertz applications, it should be trivial to fill out this one (there are no long essays, only a few short essays and fill-in-the-blanks). You can easily finish it in a few days over winter break.

You don't need to tailor your application specifically for the military
When working on this application, don't treat it like the military brass or super-duper-hardcore patriots will be scrutinizing you, waiting to pick out any glimmer of sedition. Don't try to purposely impress them by casting your accomplishments in military terms (unless, of course, your research really does have direct military applications). Just relax and don't let the name of the fellowship intimidate you. As far as I know, respectable scientific researchers will be reading your application, just like for the NSF.

Study what the funding agencies are looking for
The area of specialty you choose to write in really matters a lot. Here is why ... taken from a passage from the *Proposed discipline & area of specialization* section of the NDSEG application instructions:

Walter Reed Army Institute of Research (2001-2002). In high school I worked with Dr. Richard Bauman to evaluate the neuroprotective efficacy of cyclosporine, a T cell immunosuppressant. We simulated injury in rats by simultaneous blunt head trauma and hypoxia. Cyclosporine was administered immediately post-injury and evaluated by comparing neurological and physiological function pre- and post-injury. Specifically, I designed three vestibulomotor assessment tasks and then evaluated treated and control rat performance on each of these tasks pre- and post-injury. Currently Dr. Bauman is collaborating with industry to develop a formulation appropriate for first-response treatment. My internship at Walter Reed was invaluable. It provided me my first research experience and instilled in me a commitment to translational medicine.

Biotechnology Division, National Institute of Standards and Technology (2003). To gain experience in rigorous, quantitative biology, I next worked with Dr. Adolfas Gaigalas, a physicist, who was trying to improve fluorescence quantification technology to facilitate comparison of biological assays across different instruments. I worked with Dr. Gaigalas to reduce fluorophore photodegradation by temporally modulating the intensity of the excitation laser. I incorporated excitation intensity modulation hardware and software into an existing flow cytometry system and showed that this reduced the photodegradation of fluorescein. Working with Dr. Gaigalas was a great experience. He taught me how to think quantitatively about biological systems, design rigorous experiments, and, most importantly, how to be a patient, persevering scientist.

McGovern Institute for Brain Research, MIT (2004-2006). Combining my interests in neuroscience and quantitative biology from working at Walter Reed and NIST, I chose to spend the later half of college working with Professor Michale Fee to improve the accuracy of electrode implantation in songbirds to facilitate the study of specific brain areas that generate complex behaviors. First, I worked with Professor Fee to develop a device that predicts the location of brain structures by 1) constructing a 2-dimensional model of the cranial midline using a laser, cylindrical lens, CCD camera and image processing software, and 2) comparing the midline model to a library of models to which we planned to associate the locations of various brain structures by burn lesioning and sectioning. Initial data, however, suggested there was little correlation between midline topology and brain architecture, leading us to switch to an x-ray based approach. Second, I developed software to trigger and acquire data from an x-ray camera and worked with two other students to build hardware controls for the x-ray source and develop cranial bone segmentation software. In contrast to my experience as NIST, working with Professor Fee taught me to be an impatient scientist – to quickly take charge, make decisions, and solve problems.

Graduate Program in Translational Medicine, Stanford University (2006-2007). To better prepare myself to address medically relevant problems, I decided to enroll in the translational medicine masters program at Stanford and complete the pre-clinical medical curriculum. The goal of my first two projects under this program was to learn how to think quantitatively about problems in translational medicine while addressing two specific medical problems: 1) infection diagnosis, and 2) antibiotic resistance. I initiated both projects to fulfill course requirements and consequently conducted both projects independently with only published literature and publicly available data. The goal of my first project was to develop a broadly applicable scheme for rapid infection diagnosis. To achieve this I developed software that BLASTs expressed sequence tags (ESTs), which in the future could be sequenced from biopsies of infections, against a database of sequences of viral, bacterial, and parasitic pathogens. In lieu of testing the software with real data, I constructed and used simulated infection EST data to characterize the software's performance

and tolerance for contamination by commensal organisms and human tissue. I concluded that at reasonable contamination levels (eg. 75%) the software identifies the correct pathogen in 80% of cases. When EST infection sequencing becomes feasible, I hope to further develop this technology.

My second project in graduate school was inspired by an article by Drs. Nathan Alder and Steven Theg that enumerated several factors that affect the energetic cost of molecular transport across membranes, and a review by Dr. Christopher Higgins, which discussed the energetic requirements of multi-drug transporters. Combining these ideas, I reasoned that one solution to the problem of antibiotic resistance due to multi-drug transporters is to derivatize existing antibacterials to increase their energetic cost of efflux by bacterial multi-drug transporters, thereby decreasing their rate of efflux. For my project I wrote a review summarizing this idea. Working on these projects with limited resources was a very instructive exercise. It forced me to explore the breadth of publicly available data and become a more resourceful scientist.

Department of Chemical & Systems Biology, Stanford University (2007). To gain experience in model-driven experimental biology more strongly grounded in first physics and chemistry principles, I chose to work on a systems biology project with Professor Jim Ferrell for my first formal graduate research project. Specifically, I worked to test the predictions of two opposing models of mitosis regulation: 1) a single master regulator model where a single protein initiates each mitotic event that predicts that the time intervals between mitotic events are correlated, and 2) a regulatory chain model, which predicts that these time intervals are uncorrelated because their initiations are controlled by a series of proteins. With another graduate student, I discriminated between these models by measuring the timing intervals between the G2/M transition, centrosome separation, nuclear accumulation of cyclin B1, and nuclear envelope breakdown by double thymidine block and microscopy of HeLa cells transfected with fluorescently labeled cyclin B1 and a nuclear marker, MBS. After learning how to culture cells, transfect DNA, and optimize experimental conditions, we concluded that the time intervals between mitotic events are uncorrelated, supporting the regulatory chain model. Perhaps most importantly, I learned the value of model-driven biology – using a quantitative model to plan new experiments and using those experiments to refine the model.

Department of Bioengineering, Stanford University (2007-present). Currently I am working with Professor Markus Covert to gain additional experience in systems biology. I am developing mathematical tools for analyzing systems-level flow cytometry cancer data collected by our collaborators in the Department of Medicine to infer how B cell signaling networks in follicular lymphoma patients differ from those of healthy individuals. To accomplish this I am developing web-based software that enables scientists and clinicians to perform supervised Bayesian structural inference of signaling networks, build consensus models of signaling networks across patients by network alignment, and visualize systems level data as animated network diagrams. I recently reported this work at two conferences on computational biology and interdisciplinary cancer biology [1,2]. With the support of an NSF fellowship, in the future I hope to leverage my neuroscience and systems biology expertise to elucidate the basic mechanisms of synaptic plasticity, which in the future I will hopefully use to inform the pathophysiology and treatment of memory disorders.

Posters & Presentations

[1]. National Cancer Institute Integrated Cancer Biology Program Meeting. 2007.

[2]. Biological Computation at Stanford Symposium. 2007.

[3]. United States Army/George Washington University Joint Symposium. 2001, 2002.

[4]. Walter Reed Research Center/Naval Medical Research Center Joint Symposium. 2001, 2002.

Title. A Systems Biology Approach for Understanding Memory

Keywords. synaptic plasticity, systems biology, computational neuroscience

Introduction. One of the most intriguing questions in neurobiology is how short term neural activity at synapses produces long-term memory. This process called synaptic plasticity is presently believed to result from the simple switch-like behavior of the following linear pathway: 1) presynaptically released glutamate binds post-synaptic AMPA receptors, 2) Ca^{2+} enters and increases the resting potential, 3) NMDA channel Mg^{2+} plug is expelled, 4) upon subsequent stimulation glutamate activates both AMPA and NMDA Ca^{2+} channels, 5) intracellular Ca^{2+} increases further, 6) a Ca^{2+}-dependent protein kinase cascade upregulates immediate early genes, 7) immediate early genes increase AMPA transcription resulting in increased basal calcium conductance, resting potential, and responsiveness to pre-synaptic signals [1]. However, a recent review of AMPA trafficking by Bredt and Nicoll indicates that AMPA expression is not switch-like, but rather is highly dynamic [2]. Furthermore, a recent theoretical study by Fusi showed that synaptic switching is an inefficient mechanism for memory storage and does not produce permanent memory [3].

Hypothesis. I propose to use a systems biology approach to test an alternative hypothesis that synaptic plasticity is not caused by a linear pathway, but instead arises from the dynamics of a complex biochemical network. A recent theoretical study by Fusi, Drew, and Abbot showed that unlike linear pathways, biochemical networks can plausibly account for memory storage and long-term retention [4]. I propose to use the newly emerging field of systems biology to investigate this theory. I propose to 1) verify my hypothesis by using electrophysiology to confirm two predictions of this study, and 2) use experimental and computational systems biology to build, test, and refine Bayesian and partial differential equation (PDE) models of the biochemistry of synaptic plasticity. The key feature of this approach is the use of systems biology to systematically refine models of synaptic plasticity. Specific features of this approach include: 1) use of high-throughput data and machine learning algorithms to objectively consider the space of all possible biochemical networks, 2) use of probabilistic Bayesian models to tolerate noise and encapsulate non-linear interactions, and 3) integration of an expected information gain metric to inform experimental design.

Research Plan and Anticipated Results. In the first months of the study, I plan to confirm two predictions of the Fusi-Drew-Abbott model: 1) repeated long-term potentiation decreases plasticity, and 2) depotentiation increases plasticity. To validate the former I plan to collaborate with Professor Mark Schnitzer to compare synaptic plasticity between sensory deprived and non-deprived mouse visual neurons. Specifically I will electrically stimulate pairs of pre- and post-synaptic cells in visual cortex slices to induce potentiation and measure resultant changes in post-synaptic resting potentials. Per my hypothesis, I expect to observe diminished potentiation in sensory non-deprived neurons. Similarly, I will evaluate the later prediction by comparing the post-synaptic resting potential in sensory non-deprived neurons stimulated with low frequency current with that of unstimulated sensory non-deprived neurons. According to my hypothesis, I expect to observe greater potentiation in the low-frequency stimulated cells.

I plan to spend the following six months using publicly available data to construct and refine an initial Bayesian model of the biochemical network responsible for synaptic plasticity. First I will build a consensus list of the genes and proteins involved in synaptic plasticity from those indicated in pathway and ontology databases. Second, I will construct my initial Bayesian model by connecting these biochemicals with interactions documented in biochemical pathway and protein-protein

interaction databases. Where applicable I will employ linear stability analysis to disambiguate the direction of each interaction [5]. Third, in collaboration with Dr. Karen Sachs, I will refine my model by applying a supervised Metropolis Bayesian structural inference algorithm to publicly available interventional microarray data. Specifically I will use my initial model to seed and constrain a search for the models with the highest posterior probability. This search will likely highlight edges for which only weak evidence existed in the literature as well as suggest several previously undocumented edges. During the following six months I will experimentally verify each of these predictions using yeast two-hybrid and co-immunoprecipitation assays. This will give me the opportunity to learn how to clone, transfect, and engineer fusion DNA, analyze protein complexes by gel electrophoresis and mass spectrometry, and culture mammalian and yeast cell lines. I expect these experiments to positively confirm approximately 50% of the previously unknown interactions and negatively confirm nearly all of the predicted extraneous edges.

I will spend the remainder of my second year developing a theoretical framework for modeling the complex spatiotemporal dynamics of synaptic plasticity with my research advisor Professor Markus Covert. Specifically I will develop a PDE model which relaxes the standard systems biology assumption of spatial isotropy. This is necessary because neurons have significant subcellular organization. I will employ the following assumptions: 1) separation of time scales – because electrical activity, biochemical signal propagation, and changes in gene expression occur on distinct time scales, each will be modeling assuming the other processes are time-independent, and 2) discrete anisotropy – neurons will be modeled as consisting of a small number of compartments. These simplifying assumptions will enable me to make quantitative, experimentally verifiable predictions of the dynamics of synaptic plasticity at minimal computational cost.

In parallel I will construct three experimental tools for observing the complex dynamics of synaptic plasticity: 1) fluorescent signaling protein fusion constructs, 2) FRET donor-acceptor constructs, and 3) siRNAs for each gene and protein-protein interaction in the network. The former two tools will permit me to use fluorescence microscopy during my third year to quantitate the expression, localization, and interactions of the multiple proteins involved synaptic plasticity with high spatiotemporal resolution. The later will enable me to iteratively improve my PDE model by 1) fitting the model's kinetic parameters to new interventional data, and 2) at each iteration using the refined model to select the next experiment as that with the maximum expected information gain.

Using the systematic approach outlined above, I expect to construct a quantitative, physiologically accurate model of synaptic plasticity within three years. In the future I plan to use my model to investigate how memory is perturbed in Alzheimer's disease by 1) testing amyloid β-protein theory, and 2) identifying drug targets for reversing its pathologic synaptic plasticity.

Personal Statement. With the support of my collaborators, I believe mapping the biochemical network of synaptic plasticity is feasible within the time frame outlined here. Furthermore, I believe this study will be very instructive for learning how to build, test, and refine models of cell physiology, and will prepare me for a career as an independent systems neurobiologist.

Statement of Originality. I hereby declare that this submission is my own work.

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Florence Tabrys is an 84-year old grandmother from Redwood City, CA. When I met Florence at Stanford's student run Arbor Free Clinic last year, we had an engaging conversation about her grandchildren and my experiences at Stanford. Unfortunately, Florence is not the healthy 84-year old woman she appears to be. Despite being able to cook dinner for her and her husband each evening, she has difficulty with more complex tasks such as driving and paying bills. Florence, along with 15 million other elderly persons worldwide, has Alzheimer's disease, a slowly progressive and incurable disorder.

My scientific goals are two-fold: 1) to use systems biology in conjunction with traditional neurobiology to quantitatively understand and model the biochemistry of memory, and 2) later in my career to use this model to inform our understanding of memory disorders. I believe that systems biology is ideally suited to attaining these goals because systems biology provides a rigorous framework for building and systematically refining quantitative physiological models.

I first learned about systems biology in 2004 at MIT's annual Systems Biology Symposium. As luck had it, the symposium included a panel discussion with David Botstein, Leroy Hood, and Marc Kirschner entitled "Research and Education in New Biology", which enumerated the skills necessary to practice quantitative, model-driven experimental biology: solid background in physics, chemistry, mathematics, and biology; sharp experimental intuition; and, perhaps most importantly, knowledge of how to use physics and mathematics to gain unique insights into biology. Throughout college I directed my studies toward acquiring these skills and becoming a systems biology researcher. In my first three years of college I took foundational courses in physics, chemistry, mathematics, biology and neuroscience. This prepared me for advanced courses in biophysics, systems biology, and computational biology in my senior year. In parallel I spent my summers working with scientists such as Professor Michale Fee who had trained as physicists or engineers and were quantitatively addressing neurobiological problems.

After college I decided to step back from systems biology and neuroscience and enroll in the new Masters of Science in Medicine program at Stanford. My goal in participating in this program is to prepare myself for the second phase of my career during which, in addition to continuing to elucidate the biochemical basis of memory, I will collaborate with physicians to translate advances in the basic science of memory into novel treatments for memory disorders. In this way I hope to be as prepared as possible to help design new treatments for Alzheimer's disease and other memory disorders so that my generation and my childrens generation are never robbed of their memory and independence the way Florence will likely be in her last years. The Masters of Medicine program has vastly expanded my knowledge of biomedicine, and more importantly it has given me the opportunity to think about how quantitative science can be used to advance human health. The program has also enabled me to interact with clinicians, including the post-doctoral fellow in the Department of Medicine with whom I am currently collaborating to use flow cytometry and bioinformatics to map signaling networks in tumors of individual patients and predict patients' responses to treatment and prognosis based on the signaling network active in their tumor. In total, I believe that with my combined training in quantitative neurobiology and translational medicine I am uniquely prepared and eager to pursue an academic career first focused on the systems biology of memory, and later expanded to the translational medicine of memory disorders.

Beyond the research lab, I am actively involved in scientific outreach, education, and leadership development. As a freshman and sophomore, I volunteered 3 hours each Saturday to refurbish

computers for low-income families, and I tutored students in electricity and magnetism 2 hours each Thursday. Sophomore year I also served as secretary and regional representative of MIT Hillel. Junior year, I spent 5 hours each week of the spring semester designing graphics for a web-based physics tutoring program for high school students and early undergraduates. That same year I also gained invaluable leadership and management experience as president of my fraternity. This position required me to manage all aspects of the fraternity including membership recruitment and retention, external relations, building maintenance, and budgeting. During the summer between my junior and senior years, I read and recorded over 100 hours of calculus textbooks for blind and dyslexic students. Senior year of college I served as secretary of the Order of Omega Honor Society. In this capacity I created and maintained a website to disseminate information to our members. This past year I've become involved with three mentoring programs – one for low-income Bay area high school students interested in careers in science and medicine (SMYSP), another for underrepresented Bay area pre-medical students (SUMMA), and a third for pre-graduate or pre-medical Stanford undergraduates (BioBridge). Each of these programs has paired me with one high school or college student with whom I meet monthly to discuss and work toward their biomedical career goals. Currently I also organize a biophysics student journal club.

Of these experiences, by far the most meaningful and gratifying has been mentoring a high school student, Fernando Rios. Born in Jalisco, Mexico into a working class family and having immigrated to the United States only five years ago, Fernando does not have the benefit of friends and family who can serve as role models for higher education, help him apply to college, or explore biomedical career options. I met Fernando at SMYSP. Over the summer we met each Thursday evening to get to know each other and talk about Fernando's dream of becoming the first in his family to graduate from college. Since then we've worked together to achieve his goal. In particular, I've helped Fernando succeed in his AP classes and write college and scholarship application essays. Knowing that Fernando will be the first in his family to attend college in part because of my support strengthens my commitment to science outreach.

Throughout graduate school I plan to continue to mentor low-income and underrepresented high school and college students. In addition, I hope to strengthen the systems biology community at Stanford and nationally with three projects: 1) with Stanford faculty I plan to develop a project-based systems biology course in which graduate students will build quantitative models of biological systems using publicly available data, 2) I will help my research advisor Professor Markus Covert develop self-guided systems biology workbook that will teach undergraduates how to build mathematical models of biological systems, and 3) I hope to organize a biological network modeling competition to help centralize modeling efforts in the systems biology community. This competition will follow the style of the Critical Assessment of Techniques for Protein Structure Prediction (CASP); teams will compete to infer the structures of synthetic biological networks.

In conclusion, I believe that an NSF graduate fellowship will help me pursue my professional goal of easing Florence and her family's suffering by enabling me to continue to learn how to practice rigorous quantitative systems neurobiology toward becoming an academic systems neurobiologist. Specifically, an NSF graduate fellowship will enable me to continue to work with my advisor Markus Covert to build quantitative models of synaptic plasticity. Furthermore, an NSF graduate fellowship will allow me to continue my efforts to help young students such as Fernando explore careers in science and promote quantitative biology education.

NDSEG Summary of Goals

My professional goals are: 1) develop high-throughput experimental technologies to explore complex biological systems, 2) use systems biology to study host-pathogen interactions, 3) translate basic science discoveries into antimicrobial and bacterioprotective pharmaceuticals, and 4) promote systems biology. These goals stem from my long-standing commitment to use systems biology to advance human health.

In graduate school I plan to investigate intestinal epithelial cell-*E. coli* interactions using a systems biology approach. The long-term goal of this work is to identify targets for novel antimicrobial and bacterioprotective pharmaceuticals to combat the rising antimicrobial resistance problem.

First, I will identify specific intercellular epithelial-bacterial interactions using the split ubiquitin yeast two-hybrid system. Second, I will construct Bayesian models of the bacterial and epithelial intracellular protein networks using gene expression profiles of *E. coli* knockouts and epithelial siRNA knockdowns. Third, I will develop a high-throughput method for monitoring interacting cells which uses flow cytometry to simultaneously quantitate the expression of fluorescently labeled proteins in conjugated pairs of bacterial and epithelial cells. Pairs of cells will be distinguished computationally by the temporal spacing of flow cytometry events and surface marker expression. Data from this conjugate flow cytometry technique applied to wild type bacterial and epithelial cells, in addition to the intercellular interactions identified by the split ubiquitin yeast two-hybrid screen, will be used to combine the two individual Bayesian models into a single model of bacterial-epithelial interactions. Fourth, I will simulate pharmaceutical perturbations to the combined model to identify potential bacterial targets for antimicrobials and epithelial targets for bacterioprotective drugs. Finally, I will experimentally verify each predicted target using *E. coli* knockouts, epithelial siRNA knockdowns, and two bacterial growth competition assays of 1) knockout versus wild type bacteria on wild type epithelial cells, and 2) wild type bacteria on wild type versus siRNA knockdown epithelial cells.

In graduate school I also hope to promote systems biology in two ways: 1) develop a project-based course where students learn to model biological systems using publicly available data, and 2) organize a network modeling competition similar to CASP to unite the systems biology community.

In the future I hope to translate validated drug targets into novel pharmaceuticals. To prepare for this phase of my career, I am pursuing a Masters degree in Medicine where I am learning about human pathophysiology side-by-side with the same medical students who will be my future clinical collaborators. An NDSEG fellowship will allow me to investigate host-pathogen interactions and continue to prepare for an academic career in biomedical research and translational medicine.

Previous Research Statement

Conducting research has allowed me to gain a depth of knowledge and perspective in biochemistry and structural biology far beyond that usually obtainable in an undergraduate curriculum. The laboratory setting has served as an excellent preview of graduate school and the research environment, confirming my desire to dedicate my work to academic research.

Following my sophomore year in 2005, I participated in a summer undergraduate research internship at Rice University through the NSF Integrative Graduate Education and Research Traineeship (IGERT) program. I worked in a developmental biology lab, studying cell cycle regulation in zebrafish. My particular project was an investigation of Wee kinase function and gene expression. Wee kinases play a significant role in the developmental regulation of cell cycle progression, delaying entry into mitosis if, for example, errors occur in the DNA synthesis phase of a maturing cell. Working with a team of graduate students, I used RT-PCR to determine that zebrafish *wee* genes are expressed maternally and zygotically, and I assisted in developing a useful PCR-based assay to screen for carriers of a lethal recessive mutation in one of two known zebrafish *wee* genes. That summer, for the first time, I played a part in the process of generating and expanding knowledge. My contribution will enable other scientists to make even more progress in the field. This revelation had an enduring impact.

The IGERT program was my initiation into the world of scientific research. Working in a research lab was vastly different from prior experiences in any lab course I had ever taken. That summer I learned to appreciate the entire research process—asking a question, generating a hypothesis, designing experimental procedures and controls and, of course, troubleshooting when obstacles inevitably arose. My experience at Rice motivated me to seek additional research opportunities at my undergraduate institution, Colorado State University (CSU). It also instilled in me a desire to encourage others to participate in research, in hopes that they might benefit from the experience as I have (see Personal Statement for details).

At CSU in the fall of 2005, I began research in a structural biology lab headed by Dr. Karolin Luger, a pioneer and leader in the field of chromatin structure and function. Studies in the Luger Lab revolve around the fundamental repeating unit of chromatin—the nucleosome, which comprises 146 bp of DNA tightly wrapped around eight core histone proteins[1]. Initially, I worked with a graduate student to study the molecular mechanism of two anticancer drugs, FK317 and Psoralen, in the context of chromatin. While these drugs have been shown to covalently crosslink two strands of DNA, we found that organization of DNA into nucleosomes effectively protects the DNA against drug-mediated crosslinking. These findings were recently published[2], and serve as a major step in understanding and ultimately improving the mechanism by which these DNA crosslinking agents act to disrupt DNA replication in rapidly dividing cancer cells.

In 2006, my advisor encouraged me to apply for a Beckman Scholarship, which would fund my research in the Luger Lab through the summer of 2007. The application required an extensive project proposal, which I presented to a committee of professors from various departments. Preparing the proposal allowed me to think independently about potential research directions for a project of my own, and to consider possible obstacles and alternative outcomes. In addition, I developed valuable skills in communicating my ideas in a scientific context.

Winning the Beckman Scholarship was a rite of passage. The new project I implemented was in collaboration with a yeast geneticist at another university, which enabled me to extend my aims into another field of research. While I received informative training from graduate students and was in frequent contact with my advisor and collaborator, for the most part I worked independently on my project. The research involved histone-mediated gene silencing, which is

achieved via histone modification and/or through interactions with suppressor proteins. Key amino acid mutations within histone H3 (a nucleosome core histone) have been shown to inhibit normal gene silencing *in vivo* in yeast[3]. I showed that these amino acid mutations do not affect nucleosome integrity, but may instead disrupt suppressor protein binding to histone H3. I used X-ray crystallography to obtain high-resolution atomic structures of nucleosomes containing the amino acid mutations, and of a nucleosome in complex with a portion of a suppressor protein (Sir3) known to interact with nucleosomes in yeast[4]. Knowledge of these structures will be useful in understanding an interaction intimately involved in the process of histone-mediated gene silencing, thus building on existing findings in the field.

I presented the findings and interpretations of my most recent project in an undergraduate research symposium at CSU, where my poster received Highest Honors distinction. I also attended two Beckman Conferences in California, where I learned about the research of fellow scholars in diverse areas of expertise. In turn, I was able to present my work to many students and professors in fields ranging from organic chemistry to biomedical engineering. It was highly beneficial to use each other as sounding boards, bouncing around theoretical, experimental, and technical ideas and approaches in an effort to enhance the impact of our work.

As a first-year graduate student at Stanford University, I have found a home in the Structural Biology Department. I am making significant progress in a lab that uses biophysical and structural approaches to understand in molecular detail neuronal communication through neurotransmitter (nt) release into the synapse. Under the guidance of Dr. William Weis, I am studying the protein machinery required for the docking and fusion of nt-containing vesicles at the pre-synaptic membrane. I collected diffraction data at the synchrotron on protein crystals that I obtained in less than a month, and am becoming more familiar with the software programs used to process the data. I am currently building my model into electron density maps. The entire process has increased my understanding of crystallography immensely.

My solid research background has prepared me exceptionally well for graduate school and beyond. I have become enamored with the technique of X-ray crystallography, and look forward to contributing to many fields, solving problems from a structural and mechanistic perspective. Financial support from the NSF Fellowship would greatly facilitate my involvement in harnessing the powerful tool of X-ray crystallography to resolve the classic duality of 'structure and function' into a unified understanding of the molecular basis of life.

Selected Presentations

- 9th Annual Beckman Scholars Symposium (07/2007). **Effect of Histone H3 E73D Mutation on *in vitro* Chromatin Silencing.**
- CSU Celebrate Undergraduate Research & Creativity Showcase (04/2007). **Effect of Histone H3 E73D Mutation on *in vitro* Chromatin Silencing.**
    - Award: Highest Honor for Poster Presentation
- Beckman Scholars Program Project Proposal (03/2006). **Effects of DNA Cross-linking Agents, FK317 and Psoralen, on Linear and Nucleosomal DNA.**
- NSF IGERT Summer Research Internship Program at Rice University (08/2005). **Wee Kinase Function and Gene Expression in Zebrafish.**

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Munc18a Regulation of SNARE Complex Assembly

Keywords: neuron, exocytosis, membrane fusion, Munc18a, SNAREs, Syntaxin1a, PKC

In the brain, neurons communicate across specialized intercellular junctions called synapses. Arrival of an action potential in a pre-synaptic neuron triggers a calcium (Ca^{2+}) influx, which leads to exocytosis of pre-synaptic vesicles and release of neurotransmitters for uptake via receptors in the post-synaptic neuron. Fusion of synaptic vesicles to the pre-synaptic membrane is a critical step in Ca^{2+}-triggered exocytosis, and is mediated by Soluble NSF Attachment protein Receptors (SNAREs). Three neuronal SNARE proteins, Syntaxin1a, SNAP-25 and VAMP combine to form a four-helix bundle, a stable complex essential for membrane fusion[1] (Figure 1). A neuronal protein, Munc18a, is required for synaptic vesicle fusion and regulates SNARE complex assembly through its interaction with Syntaxin1a. Syntaxin1a contains a conserved N-terminal peptide (aa 1-9) followed by the Habc domain, a linker, and the H3 domain involved in SNARE protein binding (Figure 2). Munc18a binds the N-peptide, Habc domain and H3 domain of Syntaxin1a, rendering the latter inaccessible to its SNARE binding partners, effectively preventing SNARE complex assembly[2,3].

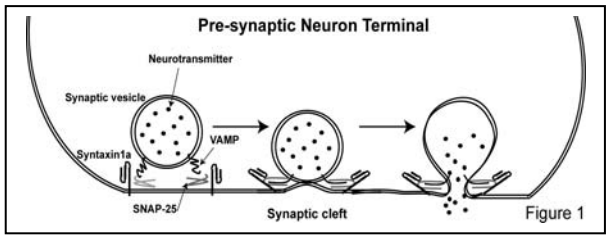

Figure 1

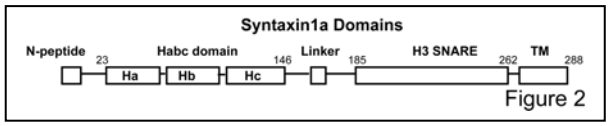

Figure 2

A recent finding shows that Munc18a in complex with Syntaxin1a lacking its N-peptide allows SNARE complex assembly *in vitro* upon addition of SNAP-25 and VAMP[3]. Hence the interaction of the N-peptide of Syntaxin1a with Munc18a plays a key role in the regulation of SNARE complex assembly. This result prompted us to solve the crystal structure (unpublished) of Munc18a bound to Syntaxin1a lacking its N-peptide (Syx^{25-262}) for comparison with the known crystal structure[2] of Munc18a in complex with full-length Sytnaxin1a (Syx^{1-262}). We found only slight conformational differences between the two complexes. Importantly, one such difference is seen in the vicinity of two serines (S306 and S313) on Munc18a that are known phosphorylation sites for protein kinase C (PKC)[4]. PKC phosphorylation of Munc18a enhances pre-synaptic vesicle fusion and neurotransmitter release[5], but the molecular mechanism of this pathway is unclear. I hypothesize that the synergistic actions of Syntaxin1a N-peptide removal and PKC-catalyzed phosphorylation of Munc18a expose the SNARE-binding domain of Syntaxin1a, thus facilitating SNARE complex assembly (Figure 3). The following aims will elucidate the roles of Munc18a phosphorylation and Syntaxin1a N-peptide removal in SNARE complex assembly.

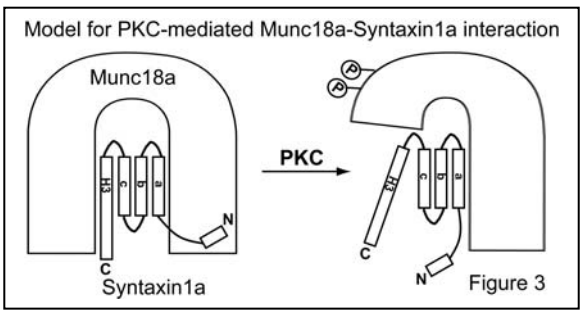

Figure 3

Aim 1*: Determine quantitatively the thermodynamics of the Munc18a-Syntaxin1a interaction.* To test the combined effects of Munc18a phosphorylation and Syntaxin1a N-peptide removal, I will use Isothermal Titration Calorimetry (ITC) to measure the *in vitro* thermodynamics of interactions between PKC-phosphorylated Munc18a and full-length Syntaxin1a (Syx^{1-262}) or Syntaxin1a lacking its N-peptide (Syx^{25-262}). Munc18a will be phosphorylated *in vitro* using PKC, ATP, and Ca^{2+} (to activate PKC). Phosphorylation efficiency will be determined in a separate experiment using γ-^{32}P labeled ATP and subjecting the reaction sample to SDS-PAGE,

followed by protein staining and autoradiography. Combining Syx^{1-262} or Syx^{25-262} with non-phosphorylated Munc18a will serve as an experimental control. If phosphorylation is inefficient, three different Munc18a phosphomimetic mutants will be used for ITC: $Munc18a^{S306E}$, $Munc18a^{S313E}$ and the double mutant $Munc18a^{S306E/S313E}$. Munc18a mutants will be generated using site-directed mutagenesis. The single phosphomimetic Munc18a mutants binding either form of Syntaxin1a will indicate the relative contributions of the individual phosphorylation sites to complex stability. Based on my hypothesis, the combination of Syx^{25-262} and the PKC-phosphorylated Munc18a (or $Munc18a^{S306E/S313E}$) should have the weakest affinity, as this interaction is predicted to confer the SNARE-binding conformation of Syntaxin1a, with fewer residues in contact with Munc18a (Figure 3).

Aim 2: ***Determine whether phosphorylation of Munc18a and removal of Syntaxin1a N-peptide facilitate SNARE complex assembly.*** Syntaxin1a, SNAP-25 and VAMP assemble into a highly stable SNARE complex *in vitro*, which is SDS-resistant and dissociates only upon boiling[6]. I will take advantage of this property to test the effect of Munc18a phosphorylation on *in vitro* SNARE complex assembly. Each Syntaxin1a (Syx^{1-262} or Syx^{25-262}) will be pre-incubated with PKC-phosphorylated Munc18a or with the different phosphomimetic Munc18a mutants described above. Munc18a will be used in excess to eliminate any free Syntaxin1a. SNAP-25 and VAMP will then be added and the samples will be subjected to SDS-PAGE, with and without boiling. The components of the boiled samples should run as separate bands on an SDS gel. Non-boiled samples in which SNARE complex formation occurs will run as two bands corresponding to Munc18a and the intact SNARE complex. According to my hypothesis, loss of Syntaxin1a N-peptide or phosphorylation of Munc18a should facilitate SNARE complex assembly. A more quantitative fluorescence-based assay will be used to measure the rate of SNARE complex formation. Upon addition of SNAP-25 and fluorescently-labeled VAMP, the phosphorylated Munc18a-Syx^{25-262} combination is expected to yield the fastest SNARE complex assembly rate.

Aim 3: ***Determine whether phosphorylated Munc18a exposes the SNARE binding domain in Syntaxin1a lacking its N-peptide.*** Given my hypothesis, I expect phosphorylation of Munc18a to induce a conformational change such that the H3 domain of Syx^{25-262} no longer interacts with Munc18a, but is instead exposed for SNARE protein binding (Figure 3). I will obtain high-resolution atomic structures of the PKC-phosphorylated Munc18a-Syx^{25-262} complex, and/or of any Munc18a phosphomimetic mutants in complex with Syx^{25-262} to confirm or refute the hypothesis. Initial crystal growth conditions will be similar to those that yielded the crystals used to solve the wt Munc18a-Syx^{1-262} structure[2]. If necessary, a more general crystallization screen will be carried out. Crystal diffraction will be screened at the Stanford Synchrotron Radiation Laboratory (SSRL). The structures will be computed via molecular replacement using the structure of the Munc18a-Syx^{1-262} complex.

Studying the molecular machinery responsible for the exquisite regulation of synaptic neurotransmission is essential to our understanding of memory and learning. My experience with X-ray crystallography as an undergraduate has cultivated a deep interest and enthusiasm to study protein structure and function in graduate school. Ultimately, I hope to solve relevant problems investigating the structural basis of biology at the molecular and cellular levels.

Statement of Originality: I certify this proposal represents my own original work.

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Personal Statement

In the words of William Yeats, “Education is not the filling of a pail, but the lighting of a fire.” My fire was ignited at The Denver Waldorf School, where—much like in Waldorf schools all around the world—my teachers brought creative and unconventional methods to the classroom in an effort to cultivate well-rounded people with the capacity for using their hearts, hands and bodies, not just their heads. My Waldorf experience included building a boat, riding a unicycle, dancing through the multiplication tables, and learning multiple foreign languages. My early education left deep imprints, and imbued me with the notion that education is a journey that must be experienced, embraced, and shared in creative ways, rather than passively absorbed.

It was as a Waldorf student that I first picked up a violin and learned to play. More than a decade of experience performing in symphonies and string quartets has taught me a lot about being a leader and a team player. As a section leader in various orchestras, conductors have depended on me to bring a sea of violinists together to perform as a single entity. Playing in smaller ensembles, I learned when to take initiative and when to step back and appreciate the contributions of other players. There are similar dynamics among individuals and institutions in science. Interactions with superiors, subordinates, and peers influence the work and creative contributions of an individual scientist. The leadership and teamwork skills I developed as I musician have been a tremendous asset in my maturation as a scientist.

As an undergraduate at Colorado State University (CSU) I was active in Students as Leaders in Science (SLS). SLS members serve as judges in local high school science fairs and expose even younger students to science through demonstrations and hands-on experiments. I fondly remember working with fellow undergrads to put on a workshop for elementary school students in which we reviewed the major parts of the cell in a fun, interactive presentation, and helped the students observe cells and organelles under a microscope. It was highly rewarding to see the same unbridled joy of discovery in the kids that I experienced as a young Waldorf student. As a member of the Luger Lab at CSU, I assisted my research advisor and lab mates with a guided lab tour for some underprivileged high school students participating in a summer research program. We showed them state of the art equipment in the lab, and related our research goals to various experimental techniques utilized in the lab. What struck me about this experience was my impression that many of the students seemed to have walked away with an appreciation, if not enthusiasm, for what goes on in a real laboratory. We were able to make “research” more tangible to them—perhaps they could see themselves some day wearing white lab coats and conducting experiments of their own.

Another opportunity in the Luger Lab enabled me to spend a summer mentoring Jon, an undergrad with no previous research experience. Being a mentor was more of a challenge than I thought it would be. Like a new parent, my responsibilities increased drastically. Suddenly, I was accountable for the work of two people, not just my own. I had to plan ahead, assign tasks, and conduct experiments around two schedules. I explained the underlying rationale of each experiment, interpreted data, and made executive decisions on how to proceed. In the end, it was truly gratifying not only to pass on various technical skills, but to fan the flames of excitement in another aspirant researcher (Jon has recently joined a lab where he intends to conduct long-term research). Mentoring embodies several important values I learned early on at Waldorf, including the effectiveness of hands-on, interactive training, and the desire to share my knowledge and passions.

My passion for structural biology was largely inspired by Dr. David Fahrney, a scientist and teacher who dedicated more than thirty-five years to the Biochemistry & Molecular Biology Department at CSU. Dr. Fahrney transformed the way biochemistry is taught to students at CSU,

creating and incorporating interactive molecular modeling and internet-based instruction into his lectures. Visualization of molecular structures in three dimensions dramatically enhanced my ability to conceptualize the molecular world. It is one thing, for example, to read about hemoglobin in a textbook. Observing this highly dynamic protein in animation, changing conformations through the binding and release of oxygen molecules, makes a much greater impression. In line with Dr. Fahrney's efforts, I think it is important to integrate innovative molecular models and corresponding tutorials into science classes at every level of education. I hope to initiate this endeavor as a TA in graduate school. Beyond the classroom, I have a vision to incorporate didactic tutorials, much like those Dr. Fahrney invented, alongside atomic structures currently available in the online protein database. This would facilitate the dissemination of knowledge useful to experts, novices, and everybody in between.

For me the main appeal of structural biology is its breadth of application. Structural biology today is a gateway to new, interdisciplinary areas of research. In less than six months of working in crystallography, I have moved from the study of chromatin to membrane fusion proteins and neurobiology. I cannot think of a more exciting and promising field to be involved in. The financial support and prestige associated with the NSF fellowship would afford opportunities during and beyond my graduate studies, as I work to master the art of X-ray crystallography. I am looking forward to a career that will accord some measure of freedom to think independently, to choose research directions and priorities, and to motivate and inspire others as a research team leader, mentor, and teacher. As a university professor, I aspire to be the Dr. Fahrney of my generation.

I became a believer in the importance and power of diversity in science through my involvement with the Society for the Advancement of Chicanos and Native Americans in Science (SACNAS) chapter at CSU. At the 2006 National Conference, a vision of a new generation of scientists united conferees with hope and optimism. It was not a naïve hope that science and technology will purge society of all its problems, but a hope that a broader base of scientists will be able to lead science and technology in directions thus far unexplored, to weave a bigger net, so to speak, to cast over the problems of society. It is not simply science itself that limits us, but the lack of a vision of what to do with it. The emergence of new visions for the field depends critically on expanding the pool of participants to include individuals from varied cultures and backgrounds. My commitment to science is thus inseparable from my commitment to finding ways to broaden participation in it.

As a first-year graduate student at Stanford University, I plan to enhance the accessibility of scientific research to as many people as possible. To that end, I continue to be involved with SACNAS, increasing awareness of its mission and attending future SACNAS conferences to encourage and recruit SACNAS members to participate in summer research programs and graduate studies at Stanford and elsewhere. I have an idea for a seminar series I would like to implement through Stanford's Center for Teaching and Learning. Targeting research-oriented high school, undergraduate, and graduate students, the seminars will focus on developing and honing skills in public speaking, professional networking, résumé writing, designing scientific posters, and finding and applying for internships. I am currently involved in a Pre-Grad Mentoring program through a Stanford organization called BioBridge, mentoring and encouraging undergraduates to pursue advanced degrees in the sciences.

Wherever my journey takes me, I will continue to cherish the values instilled in me early on in my Waldorf education. Through my experience as a leader, mentor, and role model to future scientists, I am well on my way in making a positive difference with my head, hands, and heart.

Intellectual Merit

Overall Assessment: Excellent
Your research experience and academic record place you suggest the very highest potential for success in independent biochemistry/structural biology research. Your research project was written at a level approaching that of a seasoned researcher. However, it would have enhanced your proposal even further had you spent a little more time contextualizing the importance of 3-D structures to understanding the mechanisms of membrane fusion, and how you would use the information you would obtain in your studies.

Overall Assessment: Excellent
The applicant is exceptional in a number of ways, from her perfect grade point average in college at Colorado State University, to her significant experience in performing and communicating high-quality research, to her well-written and original proposal that builds on a rotation project at Stanford.

Overall Assessment: Excellent
This candidate has excellent academic credentials, but curiously did not take advanced mathematics (mult variabl calc and diff eq). She has chosen to perform structural biology studies at an outstanding institution. She writes an engaging and imaginative personal statement. She has received numerous academic and scientific awards. Her research project, probing the mechanism of vesicle fusion is timely and interesting. She has a clear, testable hypothesis. The only weakness is the absence of a discussion of the significance of the problem.

Broader Impacts

Overall Assessment: Very Good
Your statements and references indicate that you very effectively convey your excitement for science and learning. You suggest innovations for using structural data in teaching. Through your involvement with SACNAS you propose to enable the participation of underrepresented groups directly in your graduate research. You could have been somewhat more specific as to how you would specifically carry this out.

Overall Assessment: Excellent
Every indication is that this applicant will become a role model in science. Her involvement with the Society for the Advancement of Chicanos and Native Americans in Science (SACNAS) was unexpected and interesting. Her current involvement with the BioBridge mentoring program at Stanford suggests that her interest in encouraging promising young minority scientists was not simply a passing fad.

Overall Assessment: Good
This applicant is a proven leader. She was active in a Student leadership group that served as judges in local science fairs. She mentored high school and undergraduate students in the laboratory where she worked. She has a well defined history and plan to continue working with the community. She has worked with SACNAS as an undergrad and is currently involved in a mentoring program.

Previous Research Experience

Background

At the University of Illinois, I joined Professor Laura H. Greene's group in the spring of 2007 as an undergraduate research student. Her research focuses on strongly correlated electron systems, some of which include unconventional superconductors. I contributed to her tunneling spectroscopy project, which aims to characterize the order parameter in these materials. This parameter is a wave function that describes how energy gaps are distributed across the Fermi surface of a superconductor with respect to momentum direction. These gaps result from a phase change in the superconductor when electron pairs form. While this pair formation phenomenon has been described in conventional superconductors by the Bardeen, Cooper and Schrieffer (BCS) theory, a successful theory explaining the phenomenon in unconventional superconductors has eluded condensed-matter physicists for many years. This theory is important because many unconventional superconductors have high critical temperatures, that is, electric currents can flow through these materials with no resistance at more reasonable temperatures. High-temperature superconductors have been put into use in particle accelerators and magnetic levitation trains, so the technology of unconventional superconductivity has benefited human activities while the theoretical background has yet to be established.

One of the difficulties in doing so results from the anisotropy of the order parameters in unconventional superconductors. That is, the size of the energy gap varies with respect to momentum direction. Thus, it is important to achieve high momentum resolution in experiments. To do this, Prof. Greene's group uses a technique called was point contact tunneling spectroscopy (PCTS). The technique is called so because the electrical conductance from a normal metal into a superconductor in a micrometer-wide junction is measured at various voltages. Momentum resolution is provided by tunneling effects between the two electrodes in such junction, because the probability of particle transmission across a tunneling barrier decreases exponentially as the particle path deviates from the direction normal to the barrier. The voltage across the junction is proportional to the the energy in the superconductor, so plotting junction current versus voltage reveals the essential features of the superconductor's order parameter. An important phenomenon that can be measured using PCTS is Andreev reflection. In certain crystal orientations where there is a finite energy gap, an electron coming from the normal metal can interact with the unconventional order parameter, giving rise to a hole reflected back into the normal metal and to the transmission of an electron Cooper pair, a process that is detectable in PCTS measurements as a conductance peak at zero voltage. Again, since voltage is proportional to energy in these experiments, conductance data can reveal the strength of the electron interactions caused by Andreev reflection.

Intellectual Merit

As a member of Prof. Greene's research group, I was responsible for developing a process to create a tunneling barrier for junctions designed to study unconventional superconductors such as $CeCoIn_5$. We chose aluminum as the normal metal in these junctions because it is known to oxidize readily in air. This oxide layer, if thin enough, would provide the desired tunneling effects.

The group had not yet develop a method to establish micrometer-wide junctions using aluminum, so I learned a lot about electrochemistry to come up with an etching technique that produced electrodes of the desired dimensions. To check the quality of my products, I learned how to operate scanning and transmission electron microscopes to characterize the size, and surface smoothness of my electrodes.

When the group was satisfied with the sharpness of my electrodes, I researched the current literature to develop an anodizing method to create an oxide layer on these electrodes. To survey the quality of these layers, I used transmission electron microscopy to estimate layer thickness and X-ray crystallography to determine the chemical composition of the oxide layers. Once I could produce electrodes of similar quality reliably, I learned how to use the group's PCTS probing system under the supervision of Professor Wan Kyu Park. In my measurements, I investigated the conductance behavior across the aluminum oxide layer into a niobium electrode, a conventional superconductor. The data I collected produced typical conductance curves that could be used to calculate the size of the energy gap in superconducting niobium. However, I could not reproduce my results reliably. I

hypothesized that the layers produced were either too thick or too brittle to provide good tunneling effects. Therefore, I spent the last few months of my research appointment optimizing the anodizing process.

Broader Impacts

As a research student, I also participated in the group's weekly meetings, giving frequent reports on my progress. I participated in the 2007 Research Experience for Undergraduates (REU) program hosted by my home university, where I communicated the importance and results of my research to undergraduate students from many universities across the country. In the fall of 2007, I took a course to write a senior thesis based on one of my REU papers (http://physics.illinois.edu/undergrad/reu/2007/Tan_Jiongyi.pdf). In January of 2008, I presented my research findings at the Undergraduate Research Symposium to students and faculty. As a recognition of my research accomplishments, I was awarded the Robert Hetrick Prize for outstanding undergraduate research projects and senior theses.

Characterizing the mechano-chemistry of ClpXP using optical tweezers

Background

Proteins are one of the most important building blocks of life: they catalyze chemical reactions, help cells communicate with each other and provide them with structure. The central dogma of molecular biology explains the various processes that lead to protein synthesis; a gene is transcribed and delivered to ribosomes, which then assemble amino acid sequences as dictated by the transcript. These processes are not infallible, however, and synthesized proteins may be dysfunctional. To prevent the accumulation of these proteins, cells have developed a variety of coping mechanisms, one of which involves the unfolding and degradation of proteins by proteases. Biophysicists are interested in their mechano-chemical behavior, which describes how they convert the chemical energy stored in adenosine triphosphate (ATP) molecules into useful mechanical work.[1] The aim of my research is to use single-molecule biophysics to characterize the mechano-chemical behavior of ClpXP, an *E. Coli* molecular motor made up of a six-member ring of AAA+ ATPases (ClpX) and a tetradecameric ring of serine proteases (ClpP). Single-molecule methods will reveal real-time details about the unfolding and degradation performed by ClpXP that are not accessible by bulk experiments due to the difficulty of synchronizing a population of molecules except for very short times.

To build a model describing the mechano-chemistry of ClpXP, scientists must understand the kinetics of unfolding and degradation by ClpXP. The kinetics are described mainly by how quickly the motor works, and how this work is divided into steps such as substrate recognition, binding to ATP, unfolding, translocation and finally degradation. ClpX is the unfoldase that exerts force on the substrate to unfold it and then translocate it into ClpP, the protease. I plan to study each unit separately, and I will first focus on studying ClpX. To do so, I will use an experimental technique developed in the laboratory of Professor Carlos J. Bustamante that will allow us to play "tug-of-war" with a single ClpX hexamer as it unfolds and translocates a single green fluorescent protein (GFP), our substrate of choice (Figure 1). As ClpX unfolds and translocates GFP, its extension will change, leading to measurable changes in the external forces applied to the system. We can calculate the kinetic rates of unfolding and translocation by analyzing extension vs. time plots.[2] These plots will also provide information about any intermediate conformational states of the substrate during unfolding. Once the behavior of ClpX is understood, I will study how ClpP modulates the mechano-chemistry of ClpX.

Method

In the proposed experiments, I will use optical tweezers to apply external forces to a single GFP as it is being unfolded and translocated by ClpX. Optical tweezers rely on laser beams that provide a balance of scattering and gradient forces that traps dielectric objects stably near the focal point of the beams, permitting the adjustment and measurement of object position and forces.[3] To study ClpX, a laser will be used to produce two beams to trap two dielectric beads. One bead will be coated with anti-digoxigenin (anti-DIG), which will be bound to a molecule of GFP using DIG and a DNA "handle." The GFP will be tagged with an ssrA-titin tag that ClpX can recognize.[5] The second bead will be covered with streptavidin, which is attached to ClpX using biotin. Then, the laser beams will be moved to form a tether between ClpX and GFP, as shown in Fig. 1. Upon formation of this tether, the instrument can both apply and measure a tensional force between the beads, and activity of ClpX results in real-time changes in force and extension.

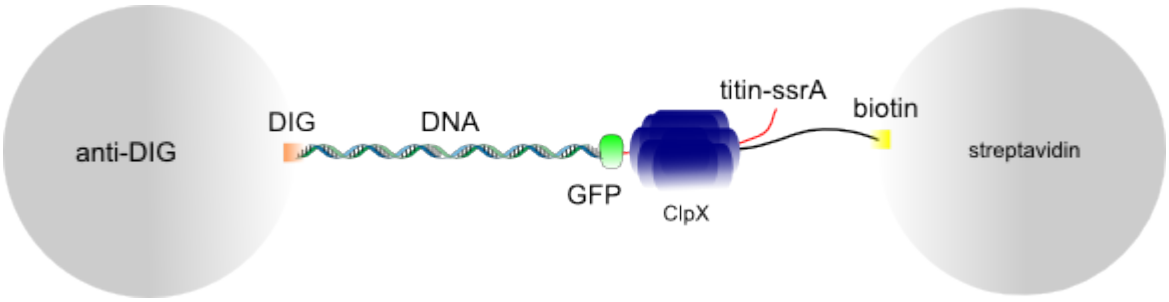

Figure 1. Schematic diagram of the setup to study GFP unfolding and degradation by ClpXP

Hypothesis

In a recent experiment, ClpXP was found to be rather resilient; it can apparently bind to ATP to power unfolding and translocation even if most of its six subunits are impaired or disabled by mutations.[4] While there is a finite probability of binding, unfolding, and translocation as long as ClpXP has at least one fully-working subunit, the kinetic rates of these processes diminish as the number of mutant subunits raises. In addition, the ClpX variants sustaining the slower rates turn out to be less energy efficient. I hypothesize that a ClpX variant with mutant subunits must take many more mechano-chemical steps than the fully-functioning wild type ClpX. The mutations are designed to affect ClpX's ability to bind to ATP, to hydrolyze it for energy, to recognize substrate tags, to unfold substrate, to translocate it, or any combination of these processes. To work optimally, then, a mutant unit would have to cycle through these processes for smaller substrate sections, which would explain the higher energy costs. If this hypothesis is true, then I anticipate that these cycles will result in many more intermediate conformational states in the extension plots obtained from the unfolding and translocating activity of mutants.

Merits

These experiments will provide details that cannot revealed by bulk methods. For example: the average step size of ClpX, its kinetic rates at saturating and limiting ATP concentrations, its response to external mechanical forces, how rate changes with the substrate sequence, the dynamic coordination among subunits, the processivity of the motor, the origin and duration of any pauses in the motor's activity, or the size, frequency and sequence dependance of slips, etc.

Knowing how proteases act on substrate proteins will help scientists understand how proteins are recycled in a cell. What's more, there are diseases that are caused by the accumulation of dysfunctional proteins. The research proposed here will provide some of the molecular basis underlying these pathological conditions.

Broadly speaking, the success of this research project and others in the biophysics research field will promote interdisciplinary learning. As a student with a physics background, I hope to show how physics can be used to explain biological phenomena. Working in Prof. Bustamante's laboratory has reaffirmed my belief that individuals with diverse academic and cultural backgrounds can collaborate to produce results of the highest calibre.

I have two years of work experience serving Hispanic students--all qualify for free or reduced-lunch--in Oakland, CA, and I hope to be a role model to them. The high school where I worked took pride in preparing their students for college, and I will take advantage of my relationships with teaching colleagues and of my current position at Berkeley to get my ex-students excited about the brave new world of single-molecule biophysics, a field that is not easily accessible to minority student populations. Based on my teaching experience, I know that many students in Oakland do not know about the educational opportunities are available in the vicinity. Guest lectures on current science, or a field trip to the research facilities where I work would make my ex-students aware of the possibilities. Finally, I hope that my contributions to the community will naturally broaden as I become a more accomplished researcher.

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[2] Keller, D. and Carlos Bustamante. *Biophysical Journal*. 2000; 78:541-56
[3] Ashkin, et al. *Optics Letters*. 1986; 11(5):288-90.
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[5] Jabri, Evelyn. *Nature Structural Biology*. 2003; 10:676

Personal Statement

Pursuit

I am reading Ayn Rand's *Atlas Shrugged*, and the quote that has terrified me since I took up that book is: "The most despicable kind of man, is the man without purpose." I do not want Rand to look down on me, and to tackle the problem of purpose, I have tried to follow Richard Feynman's problem-solving algorithm: 1) write down the problem, 2) think very hard, 3) and write down the answer. For the past few months, my problem has been the following: I cannot stop thinking about biophysics after hearing one of Dr. Mina Bissell's lectures on physics and cancer research. After thinking very hard, I have concluded that the best solution is to beat that thought into submission and either turn it into a reality, or let it help me realize I have made the wrong choice. Testing this answer has become my purpose, and advanced study is the most appropriate way of fulfilling it. There is risk involved, but the most rewarding investments in life come with great risk.

Competence

I have the fortune of learning well. Such fortune comes from the luxury of taking on one project at a time. Before taking college calculus, I taught myself how to differentiate and integrate. I was awarded Honors in Calculus at Pasadena City College. In college, I would take at least two weeks to compose a paper that would reflect my thoughts most candidly. My efforts paid off, and in 2008 I was the sole recipient of the Robert Hetrick Prize, which recognizes outstanding undergraduate research projects and senior theses (see previous research experience). During my senior year, I took on cooking and now prepare Chinese, Peruvian, Indian, Thai, Korean and other kinds of dishes from memory with much gusto. I even roast a delicious Thanksgiving turkey, and I am vegetarian. At age five, I almost drowned in a public pool; a lifeguard had to take me home to my parents. A year ago, I bought a copy of Terry Laughlin's *Total Immersion* and have taught myself how to swim freestyle with rigorous form. Last year, I taught Algebra I, English and language arts, U.S. history and physical science to eight-graders without any formal preparation. At least 88% of them scored in the proficient or advanced levels in standardized state tests. Most recently, my conversations with Professor Carlos J. Bustamante at the University of California at Berkeley have landed me a research position in his laboratory, where I will use optical tweezers to understand how ClpX unfolds proteins for further degradation. You see, I have been rewarded for taking initiative and risks--at worst, I would have been wrong, but then I am good at learning, even from mistakes.

Broader Impact

Finishing a project successfully requires gumption. As Robert Pirsig puts it in *Zen and the Art of Motorcycle Maintenance: An Inquiry into Values*, "If you are going to repair a motorcycle, an adequate supply of gumption is the first and most important tool. If you haven't got that you might as well gather up all the other tools and put them away, because they won't do you any good." I wrote about the gumption that was my motive power through undergraduate education in my admissions essay. It was an account of how I had become an eminent condensed-matter physicist who discovered a high-temperature superconductor that would solve the world's problems of electrical inefficiency. Driven by such an image, I worked for Laura H. Greene, Professor of Physics at the University of Illinois at Urbana-Champaign. And while I was rewarded for the quality of my research, the more important reward was learning that experiments rarely work as expected, and that simple hypotheses are hard to formulate and even harder to test. I had realized that the gumption expressed in my admissions essay was not going to be enough to motivate another six years of research. Just as you step away from a paper to overcome writer's block, I stepped away from the idea of graduate school.

After graduation, my senior thesis award and success in academia motivated me to find out if I was any good at teaching. After teaching in inner-city classrooms in Oakland, CA, I determined that I can be an effective teacher, and I am as idealistic as ever. More importantly, though, I found that solving simple problems could provide enough motivation to refocus on greater goals. If I had not chosen to find out how to tell a student not to eat in class, or how to teach them to write the correct assignment headings automatically, I would have never achieved as much as I did in two years or have enjoyed it as much. Four of my students would have never qualified to attend Johns Hopkins University's Center for Talented Youth, a summer program where they would take courses to enrich their academic experience. In this sense, the gumption behind my research efforts will

come in two ways: the sheer intellectual excitement in investigating how certain molecular motors recycle proteins, and the pleasure of solving “little” problems such as figuring out the right buffer solution, sorting out noise problems, and even configuring software properly. My teaching experience has taught me to appreciate smaller victories, which will make research--with all its obstacles--something to look forward to doing.

Aspiration

I do not know enough biophysics to pick a single problem that I will be able to solve in six years or so. But my interest in this field is enough to make me take a fifty percent pay cut to work in Prof. Bustamante laboratory and teach myself biochemistry. I plan to use this knowledge and my work experience in his lab to pick a good research project when the time is appropriate. I already have successful teaching experience, so I plan to teach well and focus on research quality. After honing in research skills, I will better positioned to know if my intellectual curiosity can be realized in a career in academic research, industry, education, or a combination of these.

The graduate research fellowship provided by the National Science Foundation will help me afford the preparation necessary to perform biophysical research of the highest calibre and make contributions to my field of study in both the classroom and the laboratory environment. The merits of my undergraduate research project and the contributions I have made to public education in the last two years make me a prime candidate to receive a fellowship. Thank you for your consideration.

Intellectual Merit

Overall Assessment: Excellent
Your letters, academic record and research accomplishments speak to the highest potential for success in research. Your writing is unusually thoughtful and enjoyable to read. Your proposal using optical tweezers to dissect the motor properties of ClpXA protease interesting and creative. One improvement might have been to develop a model based on the inactive subunit experiments you referenced.

Overall Assessment: Very Good
The applicant has very good academic credentials and has demonstrated the ability to conduct research as an undergraduate. The proposal is very well written and indicates a strong likelihood of success. The applicant will receive excellent multidisciplinary training in single molecule biophysics.

Overall Assessment: Excellent
The applicant has excellent academic record and received one merit award. The proposed research project on the application of optical tweezers is good but not exceptional. However, the lack of evidence in the participation in scientific conferences and no record of research publications limit the interest in the applicant.

Broader Impacts

Overall Assessment: Very Good
Your dedication to teaching and communication are well-documented. Your diverse background provides a unique perspective and the potential to provide a role model for other multi-racial scientist. The broader impact criteria could have been greatly strengthened by providing more detailed plans about how you would carry out such activities during the NSF period.

Overall Assessment: Excellent
The applicant has demonstrated a strong commitment to science education and the outreach plans described in the proposal are very realistic and likely to have a positive impact. The candidate has a good appreciation of the problems involved in broadening participation by underrepresented minorities and has worked in this direction. The description of the broader impact of the research could have been improved.

Overall Assessment: Very Good
The general application and broader impact of the proposed research is of very limited nature. The applicant participated in working with under-represented student group as a teach. This shows the tenacity of the applicant to reach the community. However, more active participation in conferences and involvement in scientific publications enhance the potential of the application.

The path to my thesis project, which incorporates cell biology, biophysics and neuroscience, is somewhat unconventional. During the summer of 2005, I began working in Ka Yee Lee's laboratory in the Department of Chemistry. Under the guidance of Eva Chi, a post-doctoral fellow in the lab, I carried out experiments to elucidate the effects of model cell membranes on the aggregation of the Alzheimer's protein amyloid-beta (Aβ) protein into amyloid fibrils. In light of research suggesting that Aβ interacting with cell membranes "template" Aβ into the fibrillar form seen in the brains of Alzheimer's patients, the effects of cell membrane components, such as charged phospholipids and gangliosides, on Aβ fibril formation were examined. We used a number of analytical techniques, including thioflavin-T fluorescence, a classic amyloid dye used to monitor fibril formation, size exclusion chromatography and dynamic light scattering to determine whether the lipid templated β-sheet structures could lead to fibril formation. I found that in water, anionic PG vesicles uniquely induced the formation of Aβ fibrils whereas cationic and neutral vesicles had no effect on fibril formation [1]. These results yielded a hypothetical mechanism for *in vivo* Aβ fibrillogenesis. Since negatively charged phospholipids reside only in the inner leaflet of the cell membrane, it is possible that as oxidative damage to the membrane accumulates, negatively charged phospholipids are flipped outward and exposed to the extracellular environment. These negatively charged lipids are then able to interact with Aβ in a potentially deadly capacity. Later experiments included testing the ability of the air-water interface to seed Aβ into fibrils [2].

Following my work at the University of Chicago, I sought out an additional research experience that focused on more biological models. Darrell Irvine's work at MIT captivated me because of its unique blend of immunology and material science. I applied to the Irvine Group for a research position. Fortuitously, Professor Irvine was looking for a technical assistant to aid his graduate student, Yuki Hori, on her project to develop a novel cancer vaccine. I began work on Ms. Hori's project to generate injectable polysaccharide alginate hydrogels that can attract immune cells to the injection site *in vivo* (mouse models) for immunotherapy. Our system uses a novel technique for gelation: alginate microspheres containing excess Ca^{2+} were co-injected with soluble alginate to form a gel via divalent cross-linking. Our gels, when co-injected with dendritic cells and other immunostimulatory factors, are designed to circumvent the natural defenses a tumor has against the body's immune system by locally boosting immune response at the tumor site. For roughly two-thirds of my time I aided Ms. Hori with mammalian cell harvesting and culture, alginate particle synthesis, *in vivo* work and assays such as ELISA, flow cytometry and rheometry [3]. For my independent project, I quantified the amount of Ca^{2+} bound in our microspheres and characterized the redistribution of calcium from the microspheres to the soluble alginate *in vitro* using a calcium-sensing dye and fluorescence microscopy [4].

After a year at MIT, I switched coasts and began my graduate studies at Stanford. My first rotation was with Sarah Heilshorn, a professor in the department of Material Science and Engineering. I had heard Dr. Heilshorn give an amazing talk at MIT on tunable biomaterials for regenerative medicine and was excited to explore this aspect of bioengineering. For my rotation, I expressed and purified synthetic polypeptides, which were then cross-linked to form hydrogels for cell encapsulation. I then assessed the efficiency of cross-linking reaction using a free amine sensor. The overall goal of the project was to identify cross-linking conditions that would allow cells and growth factors to be safely encapsulated in hydrogels. My next rotation took place in Bianxio Cui's laboratory in the Department of Chemistry. I was intrigued by the Cui Group's use of advanced biophysical techniques to image dynamic processes in live cells. For my rotation project, I used total internal reflection fluorescence (TIRF) to image the transport of growth

factors tagged with quantum dots in primary neurons. I learned to culture primary rat neurons and work with a laser-based imaging system. I also learned how to make the microfluidic chips used in the lab to control the patterning of neurons. My third and final rotation led me to the Meyer Lab in the Chemical and Systems Biology department. There, I was immersed in the world of cell signaling. I worked on the characterization of a G1 phase sensor developed by the lab. I began to use the lab's automated epifluorescence microscope and learned MATLAB in order to write a program to detect the translocation of the sensor from the cytosol to the nucleus of neuronal-like PC12 cells.

The Meyer Lab specializes in taking multiple approaches to study dynamic processes and signaling pathways in cells, which I found very exciting. The lab often performs high-throughput screens combined with quantitative imaging to discover proteins involved in a variety of cellular processes. Confocal, TIRF and epifluorescence imaging are used to examine cells transfected with an ever expanding library of biosensors. The lab also uses computation biology to inform and describe experiments and data. The resources available to me, combined with the interdisciplinary environment provided by the Bio-X program at Stanford, made the Meyer lab an easy and enthusiastic choice for my thesis project. Although I took an unconventional path to my thesis project, my prior experiences have enriched and refined my approach to the dynamic process of axon specification. My research at the University of Chicago taught me the power of using biophysical studies to distill biological processes to controllable parameters. However, my studies at MIT have shown me the necessity of verifying the same principles in a biological context. Thus, when addressing question of neuronal polarization, I included both biophysical and live cell experiments in my experimental approach. My proposal also includes techniques acquired over my rotations such as primary neuronal culture, TIRF and protein purification. Taken together, my previous projects have provided me a valuable platform to continue my development as a scientist.

Publications

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[4] Y Hori, **AM Winans** and DJ Irvine. Modular injectable matrices based on alginate solution/microsphere mixtures that gel in situ and co-deliver immunomodulatory factors. *Acta Biomaterialia* (2009) **5**(4), 969-982.

Selected Presentations

- "β-sheet templating of amyloid- β by anionic phosphatidylglycerol lipid membrane". **Amy M. Winans,** Eva Y. Chi and Ka Yee C. Lee. Chicago Area Undergraduate Research Symposium, Chicago, IL, April 2007, *invited* (presenter)
- "β-sheet templating of amyloid- β protein by anionic phosphatidylglycerol membranes". **Amy M. Winans**, Eva Y. Chi, Canay Ege, Jaroslaw Majewski, Kristian Kjaer and Ka Yee C Lee. 233rd American Chemical Society National Meeting, Chicago, IL, March 2007 (presenter)

Summary: Hippocampal neurons polarize to form one axon and several dendrites during central nervous system development. Previous studies have shown that kinesin 1 accumulates in axon post specification [1,2], however the mechanism by which kinesin 1 partitions and contributes to axon formation is unknown. Here, I propose to investigate using live cell dynamics and *in vitro* single molecule imaging experiments the hypothesis that microtubule modification (e.g., acetylation and detyrosination) are responsible for kinesin 1 partitioning and contribute to axon specification.

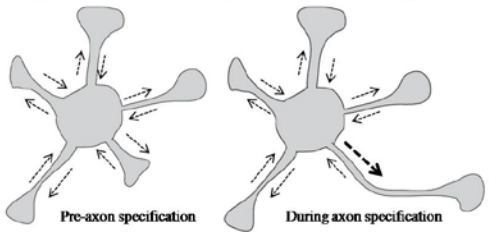

Figure 1: dashed arrows symbolize direction of growth..

Hypothesis: *Neuronal polarization is initiated by the stabilization and modification of microtubules, which leads to partitioning of active transport into the future axon.*

Aim 1: Establish the effect of MT modification, specifically tubulin detyrosination and acetylation, on kinesin binding and processivity using single molecule imaging using reconstituted component in an *in vitro* system.

Aim 2: With time-lapse live cell imaging, correlate kinesin transport and MT quantity and growth with neurite extension and retraction using primary hippocampal neurons transfected with YFP-kinesin and CFP-tubulin. The addition of drugs and siRNA will be used to test the role of microtubule modification in axon specification.

Introduction: Hippocampal neurons (HNs), which are responsible for long-term memory and spatial awareness, serve as a popular model for *in vitro* neuron polarization studies. *In vitro*, HNs sprout four to five neurites that stochastically retract and elongate (Figure 1). Within several days, one neurite "wins", growing faster than its companions, leading to its eventual specification as the axon [3]. Growing evidence supports the hypothesis that MT modification may be responsible for the partitioning of certain kinesins into a single neurite. Separate studies have shown the selective affinity of kinesin 1 for both acetylated (Acet-tub) [4] and detyrosinated (Glu-tub) [5] tubulin, although the effect of the modifications together has yet to be reconciled. The motor domain of kinesin 1, which lacks the inhibitory tail region, is found to localize in a single neurite then switch between neurites during differentiation, finally residing in the axon post-specification, while kinesin 3 distributes uniformly in all neurites [2]. Furthermore, overexpression and knock-down of many microtubule associated proteins (MAPs) lead to multi-axon or un-differentiated phenotypes [6]. The following experiments are designed to uniquely examine the role of modified MT's in kinesin partitioning at both the biophysical, *in vitro* level and on the level of cell dynamics.

Single molecule measurement of the effect of microtubule modification on kinesin motility

The following assays will be used to probe the claim in my hypothesis that MT modifications can cause the partitioning of kinesins. Motor domains of kinesin 1 and kinesin 3 tagged with YFP or quantum dots will be used in a single molecule imaging assay to measure motor processivity on MT that underwent different modification regimes. Truncation is necessary since kinesins lacking cargo will fold into inert structures. Acet-tub will be harvested from HNs treated with the histone deacetylase (HDAC) inhibitor trichostatin A. Purified HDAC will be added after isolation to produce un-modified MTs, followed by addition of carboxy-terminal peptidase A to produce Glu-tub. After each step, MTs will be re-polymerized if necessary, conjugated to streptavidin, and attached to a biotin-coated glass coverslip. A small amount of rhodamine labeled tubulin will be added to enable imaging of the polymerized microtubules. I will verify the presence of tubulin modifications using *in vitro* immunostaining using Acet-tub and Glu-tub specific

antibodies. Total internal reflection fluorescence (TIRF) will be used to track the signal from labeled kinesins. By fitting a single Gaussian to the imaged point-spread function, I can then extract velocity and processivity parameters. Based on my hypothesis, I expect kinesin 1 to have higher processivity along both Glu-tub and Acet-tub over unmodified MT's, whereas kinesin 3 will display no favoritism.

Correlation of neurite growth rates with microtubule behavior using live cell imaging

To test my global hypothesis in live cell studies, a robust time-lapse microscopy protocol will be established that allows transfection of multiple fluorescent biosensors in primary hippocampal neurons followed by 12-24 hours of imaging. A new electroporation method developed in the lab will be used to electroporate adherent cells with small volumes of concentrated mRNA to increase cell viability and induce even protein expression of multiple biosensors [7]. An automated epifluorescent microscope will be used to image 100+ cells during a single time-lapse experiment. Neurite growth rates will be obtained through software I wrote to extract neurite length through a RFP-CAAX membrane marker (Figure 2). Kinesin partitioning will be assessed using the previously-described constitutively active YFP tagged kinesin 1 and 3 constructs. CFP-tubulin and CFP-EB1, a MT plus-end binding protein, will be used to measure MT quantity and MT polymerization and collapse rates. This live cell imaging will establish the temporal correlation between kinesin partitioning and axon specification as well as establish the basal level of partitioning in an unperturbed system.

Perturbation of microtubule modification in live cell imaging

Trichostatin A will be added during an imaging run to increase Acet-tub levels. Through the quantitative imaging analysis described above, the effect of acetylation on MT and neurite dynamics will be definitively established. Co-transfection with truncated kinesins will also reveal partitioning caused by acetylation and partitioning rates can be determined. Next, Ttl, the enzyme responsible for re-tyrosinating Glu-tub, will be knocked-down to increase the amount of Glu-tub. Although the time-scale of RNAi is several days, information on MT, kinesin and neurite dynamics in the presence of increased Glu-tub can still be extracted. Immunostaining will be used to verify the presence of MT modifications. It is expected that these experiments show the increased transport and selective partitioning of kinesin 1 into the fastest growing neurite upon upregulation of MT modification. Furthermore, the dependence of kinesin transport on total number of MT's versus MT modifications will be determined.

Conclusion: These experiments will establish a molecular mechanism underlying kinesin 1 partitioning and its role in axon specification. My study highlights the importance of MTs and MAPs as drug targets for promoting neuron growth or regeneration, especially in the context of neurodegenerative disorders such as Parksinson's and Alzheimer's Disease. The study will be aided by the exceptional resources provided by the Biophysics program at Stanford. Labs that lead the field in molecular motor research, such as the Spudich and Block Groups, will be valuable for the successful implementation of my *in vitro* work. Furthermore, the microscopy systems in Meyer lab, including automatic epifluorescence imaging and TIRF, have allowed my experimental design to better probe my hypothesis.

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As my second grueling year at the University of Chicago came to a close, my friends and I started to talk about our lives after college. One night, the question rolled around: if you had the talent to do anything in the world, what would you do? I answered "a pianist". My answer startled me. I was a third of my way to acquiring a B.S. in Biological Chemistry and I struggled to maintain a practice regime for the piano. Nonetheless, I realized that I had no clear conception of what a future in science would bring to my life. The classes I took gave me facts and formulas, but no excitement or anticipation. Fortunately, over the next few years, a series of opportunities and experiences shaped my views on science and research, and guided me to my current standing as a second year graduate student in the Biophysics program at Stanford University.

The summer after my second year, I sought out an undergraduate research position to supplement my coursework. Professor Ka Yee Lee kindly offered me project exploring the biophysical mechanism of Alzheimer's Disease under the guidance of a post-doctoral fellow. The project was rewarding: it yielded two publications over the two and a half years I worked in the lab. In the summer of 2006, after a year in Lee Lab, I was accepted into the Beckman Scholars Program. My research as an undergraduate, coupled with this outstanding program, breathed life into my conception of science. Rather than poring over problem sets and exams, I helped design and implement experiments aimed towards illuminating the mechanism of a devastating neurodegenerative disease. As a Beckman Scholar, I met weekly, four quarters of the year, with U of C faculty members and fifteen other undergraduates involved in similar integrative research programs. Two twenty-minute research presentations stretched to ninety minutes as the undergraduate physicists, chemists and biologists in the room, led by Professor Stephen Kron, discussed and critiqued methods, motivation, results and general theory. These meetings introduced me to the joys of scientific discussion and problem solving sessions and cultivated in me a deep excitement for the broad array of interdisciplinary studies at the forefront of research.

Since college, I have been in four different research environments tackling a wide array of projects. Every new laboratory brought a host of new techniques and concepts in disparate fields such as material science, immunology and neuroscience. One of the most valuable pieces of advice came from a young Materials Science and Engineering professor at Stanford, and the only female faculty member in that department. She told me that research was fueled by two separate passions. First, the scientist needs to have a real love of problem solving and the tactile manipulations driving the project. Secondly, even when frustrated by failed protocols and constant repetition, the scientist is driven by the belief that her work has value in the larger tapestry of her field, and may play some small, important role in furthering scientific progress. I took that advice to heart. I joined the Meyer lab, which features a unique and powerful combination of cell biology, biophysics, imaging and modeling. My thesis project's end goal, to establish a predictive model of neuron polarization, has powerful ramifications for regenerative medicine. As each month passes, I grow more excited about my thesis project and career in academia, where I would continue to promote interdisciplinary training, from the classroom to the laboratory, helping to shape future generations of young scientists. And although I still play the piano, I have been able to discover the joy I used to find solely in music in my research as well.

Since high school, I have taken time to teach and mentor younger students who had not received the same academic advantages I experienced. In high school, I volunteered for Project READ, a program that paired high school students with struggling second and third grade readers (often ESL). I also channeled my love of science into Science Explorers, where I helped design and then teach science enrichment curricula to middle and high school students during a three-week summer camp. These curricula included energy production and use in the US and

alternative solutions for the imminent energy crisis. In a society that constantly struggles to get elementary students interested in school, it was inspiring to see our students brainstorm innovative ways to conserve energy.

In college, my life became dominated by classes, research, and my involvement in our dormitory's council. As President of the Shoreland Council, I found myself trying to better the lives of more than 650 individuals in a 100-year-old building with a council of twenty people. I developed my organization, communication and leadership skills through weekly meetings and numerous large-scale events run during the year. Ultimately, I found the experience rewarding in a way completely unrelated to academics. Furthermore, the skills that I acquired over my years of leadership serve me now as a teaching assistant and will continue to be valuable in the future.

Though my work in Shoreland Council enriched my college experience, I resolved to return to outreach activities in graduate school. During the 2008-2009 school year, I volunteered for the East Palo Alto Tennis and Tutoring program, which serves underprivileged and minority youngsters of East Palo Alto (the less privileged area of Palo Alto). I acted as a tutor and mentor two young ninth grade women for four hours a week. I often emphasized the importance of a solid foundation in mathematics, regardless of one's gender or future career plans, through additional problem sets and the telling of my own experiences. To further feed my affinity for teaching, I sought out a teaching assistantship for Biological Macromolecules, an intensive graduate course that tackles the physical and chemical principles underlying biology. Since teaching is not a requirement for my program, I have worked to maintain my thesis research in addition to prepping small group paper discussions, office hours, and class time. Nonetheless, the experience has enhanced my desire to pursue a career in academia.

As a whole, my teaching experiences have taught me the necessity of cultivating the love of science and mathematics from an early age. SPLASH, a new volunteer teaching opportunity at Stanford, provides an opportunity for Stanford students to design and teach enrichment classes to teenage kids from all over the bay area. Since participation in the program is free, it has the ability to draw students from all ethnic and socio-economic backgrounds. I, along with two other women in the Biophysics program, recently co-taught classes on molecular motors to over 30 students. For future SPLASH sessions, I proposed a set of classes designed to convey the richness and significance of science. Through the telling of the great discoveries of science, such as the discovery of insulin and unraveling the double-helix, we can explore the scientific method, basic biology and chemistry, and, most importantly, the human faces and real implications behind scientific research. Just as my perception of science grew from a sterile set of figures and pictures to a dynamic processes intimately connected to my colleagues and my community, I hope this class series can convey a small portion of this to our students. Finally, SPLASH can also be used as a platform to reach young woman interested in math and science. My co-teachers and I benefited from strong female role models in science. Here, we have the opportunity to leverage our success as graduate students in the biophysical sciences against the reticence of young women to enter these traditional "male dominated" fields.

I am fascinated by the process of evolution—that natural selection can act on mutations in a linear sequence of DNA and alter the structure and thus the function of proteins. Though this fascination has held for many years, my research experiences thus far have been diverse, and reflect my flexibility and readiness to approach this topic from different angles. Through the process of discovering and refining my interests, I have gained a deep respect for several modes of scientific inquiry—evolutionary analysis, genetics, structural biology, and biochemistry—a breadth of experience that has equipped me to ask truly interdisciplinary questions.

My first experience in a lab was in the summer of 2007, when I was an NSF REU fellow in Howard Ochman's lab at the University of Arizona. There I explored the hypothesis that linear chromosomes have evolved because they promote recombination. To this end, I worked with postdoctoral fellow Pradeep Reddy Marri, investigating recombination rates between twenty strains of *A. tumefaciens*, the only species of bacteria known to have both a linear and a circular chromosome. We employed a multilocus sequence typing approach to look for a correlation between chromosome geometry and genetic stability. Our analysis showed that there is not significantly more recombination on the linear chromosome, suggesting that increased recombination is not the reason bacteria have evolved linear chromosomes. I not only learned to amplify divergent genes and manually check sequencing data, but also tested computer programs to detect recombination within and between genes on the linear and circular chromosomes. After the summer, I was thrilled to keep up correspondence with Professor Ochman, watching my results develop into a manuscript that would be published in *Genetics* the following year.

When I returned to Amherst in the fall, I began work in Caroline Goutte's lab, using genetic approaches to examine vulval development in *C. elegans*. The projects I worked on were focused on Sao-1, a new mutation identified by the Goutte lab as a suppressor of Aph-1, a component of the Notch pathway that interacts with genes homologous to human presenilins—proteins linked to early-onset Alzheimer's disease. I used fluorescence microscopy and performed classical genetic crosses and temperature shift experiments to observe the effects of Sao-1 both alone and in conjunction with other well-studied mutations in an effort to further elucidate its function. I enjoyed working in the Goutte lab, and learned much about designing experiments and thinking analytically. However, this experience with classical genetics led me to the realization that I desire a more molecular understanding of biology, and I consequently chose to spend the summer of 2008 working in an X-ray crystallography lab.

As a SURF fellow at the Rockefeller University, I worked in Seth Darst's lab with graduate student Joe Osmundson. Over the summer, I subcloned, expressed, purified, and set up crystallization trials for the RNA polymerase α-subunit that is encoded in the plastid of *P. falciparum*, the causative agent of malaria. Over one million people die of malaria each year, the majority of them women and children in sub-Saharan Africa, and parasite resistance to current anti-malarial drugs is a growing problem. The RNA polymerase from the *P. falciparum* plastid closely resembles bacterial RNA polymerase, probably because the plastid itself is the relic of an ancient event of endosymbiosis, and is a promising drug target because there already exist clinically approved antibiotics known to bind bacterial RNA polymerase. These drugs, however, are only mildly effective at combating malaria. We wanted to determine the structure of this protein in order to explore how it has diverged from bacterial RNA polymerase, perhaps gaining insights that could aid in anti-malarial drug design. By the end of the summer, I had two crystal hits and was able to collect preliminary diffraction data on them. Unfortunately, both hits were salt crystals. Being at Rockefeller was empowering and eye-opening, allowing me to participate in research at the forefront of biology. Yet even more significantly, I realized that while

exploring the basic biological question I find fascinating—how the structures of proteins change over time—I could also focus my energies on a project that had the potential to benefit millions.

For my senior thesis, I worked with Amherst chemistry professor Anthony Bishop on developing a yeast-based assay to test inhibitors of protein tyrosine phosphatases (PTPs), signaling proteins that are involved in many essential cellular processes, including growth and differentiation, and linked to diseases such as diabetes and cancer. The assay monitors the ability of yeast cells to grow when v-Src, a tyrosine kinase known to inhibit yeast growth by over-phosphorylation, is co-expressed with a phosphatase domain in the presence of different inhibitors. If the phosphatase is active in yeast cells, it rescues the cells from v-Src-induced growth arrest, but if the phosphatase is inhibited, it is unable to rescue growth, allowing a high-throughput readout of phosphatase inhibition. The motivation for developing such an assay was to test the *in vivo* inhibition of several human PTPs the Bishop lab has specifically sensitized to inhibition by the small molecule FlAsH. The ultimate goal is to use this chemical tool to obtain otherwise inaccessible information about signaling pathways and drug target validation. Starting in the summer of 2009 and continuing through the summer of 2010, I refined the yeast assay, using western blots to demonstrate that every PTP that is expressed in yeast is able to rescue growth, and that this rescue is correlated to a global reduction in the amount of phosphotyrosine. However, the growth curves did not prove sensitive enough to respond to changes in the dosage of different known PTP inhibitors or to FlAsH. I then tried using anti-phosphotyrosine western blots to detect inhibition, but the variation between lanes in the westerns was too high to be conclusive. My work illuminated the limitations of this system, and suggested future approaches that could prove more successful at detecting the *in vivo* inhibition of phosphatases. The culmination of my research was a 114-page thesis for which I received top honors from my department. As the only person working with yeast in the Bishop lab, I learned independence—researching techniques and troubleshooting protocols on my own—but also the importance of seeking help from others, both within and outside my department.

For graduate school, I chose to apply to biophysics programs. A biophysical accounting of how protein structure and function evolve is a powerful approach for understanding how modern-day proteins came to be and is potentially critical in the field of enzyme design—helping to improve current methods of evolving existing enzymes to catalyze new chemical reactions—with the potential to impact the areas of alternative energy, environmental remediation, health, and others. I chose to come to Stanford to work with Daniel Herschlag, whose interest in protein evolution and widely recognized expertise in the field of mechanistic enzymology represent an ideal match for my research interests. In his lab, I have begun to characterize the physical adaptations that underlie the ability of proteins from the same superfamily to utilize similar catalytic mechanisms while specializing in different overall reactions. I have long wanted to directly examine the ways in which evolution acts on protein structure and function; with the expertise and resources in the Herschlag lab, I am now in the perfect position to do so.

Publications & Posters

- Marri, P. R., **L. K. Harris**, K. Houmiel, S. C. Slater, and H. Ochman (2008). **The effect of chromosome geometry on genetic diversity**. *Genetics*. 179: 511-516.
- Harris, L. K. **A Cell-Based Assay for Inhibition of Divergent Protein Tyrosine Phosphatases**. BA thesis. Amherst College, 2010.
- Rockefeller University Summer Undergraduate Research Fellowship Program (08/2008). Poster: **Determining the structure of the C-terminal domain of the plastidic RNA polymerase α-subunit in *Plasmodium falciparum***.
- NSF REU Summer Research Program at the University of Arizona (08/2007). Poster: **The Effect of Chromosome Geometry on Bacterial Genetic Diversity**.

From One Function to Another: The Physical Strategies of Protein Evolution

Key Words: protein evolution, enzyme design, phosphoryl transfer, superfamily, AP, iPGM

Introduction: Cellular chemistry is carried out by enzymes that have evolved to specifically and efficiently catalyze the reactions that are necessary for life. A physical and energetic accounting of the mechanisms through which natural selection acts on enzymes to achieve this specificity and catalytic ability would advance our basic understanding of how current biological systems came to be, and could further aid in efforts to design proteins for use in the areas of alternative energy, environmental remediation, health, and others. Rather than designing enzymes *de novo*, equipped with a physical understanding of the strategies used by evolution to convert an enzyme with one function into another, extant enzymes could be altered to catalyze different reactions. Yet it is difficult to reconstruct the past. Much evolutionary research has focused on sequence data, applying phylogenetic approaches. Structural data has also been used, both in comparing many related structures and in the reconstruction of ancestral enzymes.[1] Directed evolution studies have tried to recreate and characterize molecular evolution experimentally. I propose to take a distinct and complementary approach, systematically and thoroughly characterizing the physical differences between two enzymes from the same superfamily that utilize similar catalytic mechanisms and apparati, but that have evolved to catalyze different reactions.

The two proteins I will compare are alkaline phosphatase (AP) and cofactor-independent phosphoglycerate mutase (iPGM). AP is arguably one of the best-studied enzymes, and its mechanism of phosphate hydrolysis using two Zn^{2+} ions and proceeding through a phosphoserine intermediate has been thoroughly characterized.[2] iPGM catalyzes the interconversion of 2- and 3-phosphoglycerates (2PGA, 3PGA) and is structurally homologous to AP, containing a nearly identical active site—the main difference being the incorporation of two Mn^{2+} ions rather than two Zn^{2+} ions. In the mutase reaction, phosphate is removed from glycerate and transferred to the active site serine. Glycerate then repositions in the active site, and phosphate is transferred back to a different glycerate hydroxyl group. Crystal structures show that iPGM is composed of two domains that are closely associated when substrate is bound, but swing over 10 Å apart when no substrate is present. One domain contains the conserved active site, while the other contains at least one catalytic residue and other residues thought to be involved in glycerate positioning.[3]

Hypothesis: <u>The following adaptations that distinguish iPGM from AP are proposed to allow iPGM to specifically catalyze the mutase reaction, instead of phosphate hydrolysis: 1) binding interactions with glycerate, 2) the substitution of Zn^{2+} for Mn^{2+}, and 3) the relegation of catalytic residues from the active site to a second domain that must open and close on the same timescale as catalysis</u>. To investigate this hypothesis, I will monitor the fate of the phosphoenzyme intermediate (**Fig. 1**), and thus iPGM's ability to promote glycerate phosphorylation and the mutase reaction over phosphate hydrolysis and an AP-like phosphatase reaction. I will then both mutate iPGM and exchange Zn^{2+} for Mn^{2+} and observe how these changes affect the ratio of hydrolysis to PGA formation, as well as their influence on the rate of enzyme opening and closing to determine what role, if any, this mechanism plays in promoting the mutase reaction.

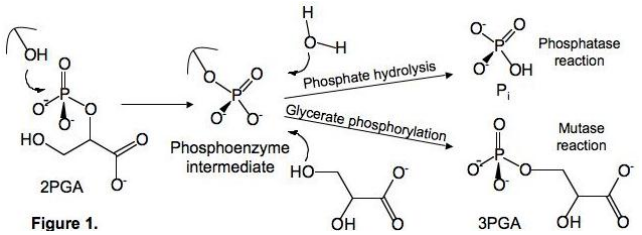

Figure 1.

Aim 1: ***Compare rates of phosphate hydrolysis and glycerate phosphorylation***

I will first establish a kinetic assay to monitor the fate of the phosphorylated iPGM intermediate. (A) The below outlines a strategy for generating a stable form of this intermediate. iPGM will be

exposed to phosphorylation agents such as *para*-nitrophenylphosphate that contain radioactive phosphate (^{32}P) and the fraction of enzyme that is radioactively labeled will be determined. By varying the temperature, buffer, and pH of the reaction and comparing the fraction of protein that is phosphorylated in every condition, I will define reaction conditions that optimally stabilize the phosphoenzyme intermediate. (B) The ratio of phosphate hydrolysis to glycerate phosphorylation will be measured using a pulse-chase experiment. iPGM will be pre-incubated with ^{32}P labeled phosphorylation agent, and then excess glycerate and non-radioactive phosphorylation agent will be added. The ratio of hydrolysis to PGA formation will be determined by monitoring the fate of ^{32}P—either as inorganic phosphate or phosphoglycerate—using thin layer chromatography.

Aim 2: ***Measure the rates of conversion and the equilibrium between open and closed iPGM***

I will use two different biophysical techniques to measure the equilibrium between open and closed iPGM. (A) Donor and acceptor fluorophores will be attached to both domains of iPGM, and FRET traces from individual proteins will be monitored using fluorescence microscopy. The FRET signal is correlated to the proximity of the two fluorophores, allowing the determination of opening and closing rates for individual molecules, as well as the equilibrium between the two states and thus the ΔG for closing. These same measurements will be repeated in the presence of phosphorylation agent and glycerate to determine the effects these molecules have on closure. (B) Small-angle X-ray scattering (SAXS) can provide information about the conformational behavior of a protein. I will collect SAXS diffraction data for iPGM in solution in the absence and presence of phosphorylation agent and glycerate. This data will be used to reconstruct low-resolution shapes of the different conformations of iPGM, which, through further analysis, can provide information about the relative numbers of molecules that adopt each conformation.[4]

Aim 3: ***Characterize functional implications of amino acid and metal substitutions***

Once the above schemes are developed, I will be able to probe the contributions individual residues and metal ligands make to the mutase reaction. I will make mutations in iPGM as well as exchange Zn^{2+} for Mn^{2+}, and measure the functional effects. The results will be interpreted in comparison to AP and in the context of evolution. Residues involved in catalysis and glycerate binding will be identified, as well as more complicated situations, such as the following two examples. (A) Mutation of a residue reduces PGA formation drastically and phosphate hydrolysis slightly, and shifts the equilibrium toward the open state. One model is that this mutation causes the enzyme to close less frequently, and that closure is not only essential for glycerate binding, but also involved in the chemical step since the rate of hydrolysis is also reduced. (B) Substitution of Zn^{2+} for Mn^{2+} increases hydrolysis, decreases PGA formation, and does not change the $\Delta G_{closing}$. These results would suggest a model in which Zn^{2+} enhances the hydrolysis reaction to the point where the phosphoenzyme intermediate is so short-lived that glycerate is simply not phosphorylated. In this model, the incorporation of Mn^{2+} in iPGM would be an important adaptation for slowing down the hydrolysis reaction and promoting PGA formation.

Conclusion: The scheme outlined above presents a novel approach to studying the physical mechanisms of protein evolution. In comparing related enzymes that catalyze distinct reactions, I hope to elucidate the specific adaptations in iPGM that have allowed it to promote the mutase reaction. This information will not only deepen our basic understanding of how proteins evolve, but it also has the potential to impact the field of enzyme design.

Statement of Originality: I assert that this proposal is my own work.

References: 1. Ortlund, E.A. et al. *Science,* 317: 1544-1548 (2007) **2.** Coleman, J.E. *Annu. Rev. Biophys. Biomol. Struct.*, 21: 441-483 (1992) **3.** Nukui, M. et al. *Biophys J.*, 92: 977-988 (2007) **4.** Svergun, D.I. *Biophys J.*, 76: 2879-2886 (1999)

Communication is the real work of leadership. –Dr. Nitin Nohria, Harvard University

The final performance of our tour was drawing to a close. After a year of preparation and ten days of traveling, performing, and sightseeing, we were about to sing our final set. As the president of the Amherst College Concert Choir, I had organized our group during the school year and helped prepare for this, our tour of Argentina. Our last pieces were traditional Argentine folk songs, and as we started, a thrill passed through the auditorium. I suddenly felt more connected to an audience than I ever had before. Our forty American voices were singing words that many of us did not understand, yet we were able to sir deep emotion in our audience. The music had enabled us to communicate, despite the language barrier. Similarly, there are barriers to be bridged in science—between disciplines, between the scientific community and the public, and for students who doubt their own scientific abilities. Effective communication is the antidote to these problems. It is my hope that by communicating scientific ideas both within and outside the scientific community, I will broaden the reach of science and deepen understanding and collaboration.

My own passion for science began in AP biology my sophomore year in high school, and is due to a teacher—Mrs. Weiland. I attended an all-girls Catholic high school in downtown Phoenix, and up until that point had harbored dreams of being an author or film director. From the very beginning of the class, Mrs. Weiland progressed at breakneck speed through the topics of biochemistry, cell biology, genetics, evolution, and ecology. She communicated each topic with absolute clarity, and I was amazed at how everything could fit together so beautifully. Her emphasis was on molecular biology, but she constantly reminded us to think within the context of evolution—a perspective that continues to influence my research interests. In addition to her ability to communicate scientific ideas effectively, Mrs. Weiland's sheer excitement about science was infectious, and after taking her class I began to reassess my goals and consider a career in basic science. She was one of the smartest women I'd ever met, and my tremendous respect for her coupled with her obvious passion for science made all the difference.

Knowing first-hand the impact a teacher can make, I have in turn spent a lot of time working as a tutor and teaching assistant. In high school, I tutored several girls at a school in downtown Phoenix where most of the students were Hispanic and came from low-income backgrounds. I struggled to balance simply being their friend and making them do their work, and learned that the more excitement I showed about a topic, the more attentive they were. I was thrilled whenever my way of communicating an idea suddenly made a concept clear in their minds. In college I also worked as a tutor. Many students come to Amherst with less preparation in the sciences than others and are overwhelmed by the introductory courses. As a tutor, I was able to review topics with them, paraphrasing and giving examples, to convince them that the material was indeed accessible, and that they shouldn't give up on science. In addition to tutoring, I worked as a lab TA for intro molecular biology and quantum chemistry, and a course TA for intro chemistry. In the lab, I was able to teach concepts and techniques while the students witnessed phenomena that they had previously only heard about. As a course TA, I attended class and held help sessions, working problems and reviewing concepts in front of groups of up to twenty. At first I was terrified to have so many students listening to my every word, copying down what I wrote on the chalkboard, but after several sessions I was able to relax, and simply speak conversationally. I learned to make eye contact with as many students as possible, and to read their faces to determine whether or not my approach was coming across effectively.

In addition to teaching students, I believe it is also essential to bring science to the general public. At Amherst, a small liberal arts college, the majority of students are not

scientists. I enjoyed writing an article published in Amherst's science magazine, *The Element*, targeting this audience. It was a challenge to use language that was accessible to everyone while not oversimplifying the subject matter. I was especially pleased that the final product spurred discussion among humanities and science majors alike. This summer I attended a workshop on using protein visualization software, and learned about the website www.proteopedia.org, where registered users can create pages that correspond to entries of the PDB. Users add text and create 3D scenes depicting different aspects of the structures that can change interactively in response to clicks from the viewer. The result is a free platform for communicating both functional and structural information about proteins. Later in the summer I held a workshop of my own, and taught students from my lab and another in the department how to use the website, and several students went on to write articles that they planned to submit for incorporation into Proteopedia.

Not coming from a scientific family and attending a small liberal arts college, I did not know anything about getting involved in research when I began at Amherst. Consequently, during my first year I turned to my biology professor, Caroline Goutte, for help. Caroline helped me secure summer research fellowships at the University of Arizona and Rockefeller University, hired me to work in her lab during my sophomore year and part of my junior year, and has been an amazing source of career advice. She is an incredible mentor, and has played an integral part in my journey toward becoming a scientist. Realizing the importance of such a mentor, I wanted to do the same for other Amherst students, so this summer after graduation I chose to remain on campus as an HHMI post-baccalaureate fellow and supervise the HHMI program, which allows undergraduates from diverse backgrounds to conduct scientific research. I lived in the same dorm as my students, ate in the same dining hall, and was a resource for them as they tried their hand at research for the first time. I held dorm events, arranged outings, and fostered a community where students could talk to me and to each other about the joys and struggles of "actual" science. I also arranged tours of research labs at UMass Worcester, where many of the students saw for the first time what a large research center is like, and realized that there are more options than medical school for the scientifically inclined. On top of these activities, I also organized a lunchtime lecture series for my students and the rest of the Amherst community. I asked professors from different science departments to present their research, and every week over lunch we heard experts from different fields discussing topics they were passionate about. I felt strongly that my students should be exposed to the different types of research going on at Amherst, and many professors also chose to attend to finally hear what their long-time colleagues in different departments actually study. No forum of this sort had been held at Amherst, spurring scientific discussion between scientists from different disciplines, and after one lecture I even overheard a geologist and a physical chemist discussing the possibility of a collaboration.

In the history of science class I took at Amherst, the most striking theme that emerged was that the greatest advances come at the boundaries of disciplines. As a professor at a university, I plan to conduct research that brings together the fields of evolution and biophysics, a powerful combination that will deepen our understanding of life as well as potentially aid in enzyme design. As a graduate student at Stanford, I will continue my work as a TA and mentor for younger students, especially women, who are considering careers in research. I also plan to start an online blog, where I will discuss current scientific discoveries in terms that will be accessible to the public, as well as demystify life as a scientific researcher. Receiving an NSF fellowship would be an honor, and give me the freedom to continue communicating information and excitement about science within the scientific community and to the general public, in an effort to deepen understanding and advance discovery.

Intellectual Merit

Overall Assessment: Excellent
The applicant has a very strong undergraduate background both in coursework and research experiences. The applicant clearly has considerable intellectually maturity and depth. The long-term aim to use biochemical techniques to study protein evolution is a strength. The proposal is well-written, and is demanding but reasonable for a graduate student. However, it is not clear how innovative the proposal truly is. The inclusion of alternatives for the event that the hypotheses are not born out would also improve the proposal.

Overall Assessment: Excellent
The applicant's potential for successful career in science has been recognized through numerous scholarship awards. This applicant has extensive research experience in several areas of biophysics experiment and modeling and is a co-author of one publication. The research plan and personal statement are clearly formulated and well-written.

Overall Assessment: Excellent
The applicant has a great deal of undergraduate research experiences at Amherst, U. Arizona, Rockefeller under REU and HHMI, and proposes an experimental plan to understand how two proteins from the same superfamily with similar catalytic machinary have evolved different catalysis reactions. Her academic record is stellar.

Broader Impacts

Overall Assessment: Good
The applicant has a strong track-record in tutoring, in public dissemination of scientific knowledge and in promoting cross-disciplinary engagement. However, these considerable strengths are weakened by a lack of plans to continue in such broadening activities.

Overall Assessment: Very Good
This applicant has demonstrated commitment to broader impact activities and is likely to continue making important contribution in this area. A research plan that links into outreach activities would make the application even stronger.

Overall Assessment: Very Good
The applicant has been involved in a spectrum of outreach activities including teaching, scientific writing, and mentoring students in which she exhibited great leadership as a HHMI fellow that fostered undergraduate research at Amherst.

My path toward applying physics to biological topics in graduate research began in the winter of my freshman college year. Eager to begin hands-on experiments and looking to explore physics research possibilities, I enrolled in a one-month exploratory program for undergraduates which I extended into an eight-month research project. Working with Prof. Min-Chang Lee of the Plasma Science and Fusion Center of MIT, I studied plasma physics and related fields, including waveguide theory, transmission line theory and circuit analysis. Along with Prof. Lee's other students, I participated in weekly group discussions and conducted several lab experiments pertaining to antenna radiation theory and diode circuit construction. A few months later, we prepared for field experiments at a two-week summer program, the Polar Aeronomy and Radio Science summer school at the University of Alaska. My work with this group solidified my decision to pursue a bachelor's degree in physics and motivated me to continue with experimental physics research.

From my junior spring until my college graduation I worked with Prof. Bernd Surrow of the Department of Physics at MIT. In his lab, I assembled and characterized several triple-GEM (Gas-Electron Multiplier) prototype detectors. Triple-GEM detectors are micro-pattern, gaseous, particle detectors first developed by Dr. Fabio Sauli at CERN in 1996 [1]. GEM foils are thin sheets of polymer material, metal-clad on both sides and chemically perforated with a high-density foil pattern. When voltages are applied to the upper and lower metal layers of a foil, the resulting electric fields within the holes draw in electrons from above the foil, causing charge multiplication within the holes. The transferred ionization pattern below the foil is a close mirror of the pattern which existed above the foil; multiple foils may be stacked to repeatedly amplify the charge distribution. A 2D readout board below the foils collects the charge distribution. After assembling the detectors, I recorded the detector gain distribution of two prototypes containing different sets of GEM foils using a Fe-55 radioactive test source. Since my graduation, the group has presented the detector at a conference [P1], and a publication is currently in review [P2]. Working with Prof. Surrow taught me important laboratory electronics techniques, and I also learned how to prepare and organize an 80-page bachelor's thesis.

During my senior year of college, I attended several lectures by biophysicists and became interested in applying my skills in experimental physics to biological research topics. These lectures strongly influenced my decision to pursue doctoral research in the Applied Physics department at Stanford University, where students frequently engage in interdisciplinary biophysics research typically involving cutting edge optical technologies. As I entered Stanford, I decided to first focus on the field of fiber optics in an effort to develop a base for future work integrating optics and photonics into biophysics research.

During my first quarter of graduate school at Stanford, I worked with Prof. Shanhui Fan and Dr. Michel Digonnet to study the effect of magnetic fields on fiber-optic gyroscopes, which are currently under development for use in aircraft navigation. I set up and began measurements to determine the Verdet constant of air-core fiber by looking at the Faraday effect. The Faraday effect is described as follows: for a given length L of fiber exposed to a constant magnetic field B parallel to the direction of travel of the light in the fiber, the angle of linearly polarized light will shift by an amount $F=VBL$, where F is the angular shift and V is the Verdet constant for the fiber.

For my experimental setup, linearly polarized IR light was coupled into a fiber that passed through the center of a 10 cm long solenoid. The magnetic field within the solenoid was modulated at 10 Hz. After exiting the solenoid, the light was incident upon a polarizing beam splitter cube; the two resulting beam powers A and B were transmitted to a lock-in amplifier, along with the 10 Hz reference signal, to obtain (A-B). The quantity T = (A-B)/(A+B) is related to F, the

polarization angle shift caused by the Faraday effect. We studied this quantity to understand how stray magnetic fields experienced by air-core fiber-optic gyros in aircraft navigation systems will affect the reliability of the device. The strong foundation in fiber optics that I gained from this research rotation has proved invaluable to my current research efforts to apply fiber-optic techniques to *in vivo* fluorescence imaging.

My graduate thesis research in the laboratory of Prof. Mark Schnitzer, who has joint appointments in Applied Physics and Biological Sciences, spans the exciting intersection of optics and neuroscience. A longstanding goal in the field of brain imaging has been to develop imaging techniques for observing brain activity at the cellular scale in behaving mammalian subjects. Physicists interested in biology are well-suited for combining quantitative analysis with optics theory to do innovative biophysics research. With the goal of correlating mammalian behavior and learning with underlying cellular mechanisms, the lab has developed a miniature fiber-optic epifluorescence microendoscope for brain imaging in freely moving mice [2]. Light is delivered via a flexible fiber-optic bundle to the microendoscope, which is mounted directly to the mouse's head. Micro-lenses within the microendoscope guide the light from the fiber bundle to the imaging plane within the brain. The optical design utilizes a 1 mm diameter gradient refractive index (GRIN) objective lens for minimally invasive imaging of deep brain structures, such as the hippocampus. Fluorescence generated in the imaging plane is collected by the microendoscope, passed through the fiber bundle, and imaged onto a high speed CCD camera. The fiber bundle is mounted in a commutator to eliminate torsional forces due to head rotation during awake behavior.

I am currently using the microendoscope to investigate blood flow dynamics in microvasculature of the CA1 hippocampal area. Before imaging, I inject a small amount of dextran-conjugated fluorescein into the mouse's tail vein. Fluorescein is excited by blue light and emits green fluorescence, so red blood cells, which do not absorb the fluorescein, show up as moving dark patches within the microvasculature. Last September, I gave a research talk at a Stanford symposium about the lab's research using this microendoscope [T1]. My next project will involve imaging cellular activity using neuronal calcium-sensitive fluorescent probes, such as Oregon Green Bapta-1 488. I plan to study neuronal activity in the hippocampus and cerebellar cortex. In my proposed plan of research essay, I present the novel approach to develop a new microendoscope for *in vivo* chronic imaging studies of the expression of Arc, a protein associated with learning and memory.

Drawing upon my undergraduate and graduate training in physics, my laboratory experience, and my first year's work in fiber optics, I am well prepared for my current work in optics and neuroscience. I am excited to work in this stimulating field combining physics and biology and believe that my graduate years will be excellent preparation for my career as an innovative biophysicist.

Publications & **Talks.** [P1] F Simon *et al. Development of Tracking Detectors with industrially produced GEM Foils.* Nuclear Science Symposium Conference Record, IEEE. **2**:660, 2006. [P2] F Simon *et al. Development of Tracking Detectors with industrially produced GEM Foils.* Submitted Sept 2007, IEEE TNS. [T1] LD Burns. *Imaging cellular level brain activity in freely moving mice using fiber-optic microscopy.* Stanford Photonics Research Symposium, 2007.
Citations. [1] F Sauli. *GEM: A new concept for electron amplification in gas detectors.* Nuclear Instrum Meth. **A386**:531, 1997. [2] BA Flusberg *et al. High-speed cellular level brain imaging in freely moving mice using fluorescence microendoscopy.* Manuscript in preparation.

In vivo time-lapse imaging of Arc expression in freely behaving mice

Keywords: Arc, learning, memory, fluorescence microendoscopy

Introduction: Understanding neural network dynamics is key to identifying the biological basis of memory. This requires methods for simultaneously recording cellular activity of multiple neurons in freely behaving animals; current methods which observe neurons singly are insufficient. To meet the need for such experimental technologies, I propose to use my expertise in optics to develop a portable microendoscope (PME) for epifluorescence microscopy. I will then use the PME to study hippocampal expression of Arc (Activity Regulated Cytoskeleton-associated protein) [1], an immediate early gene (IEG) associated with learning and memory.

Arc is the most promising IEG to study in memory research due to its low basal expression, its regulation of synaptic plasticity, and its necessity for memory formation [2]. Most importantly, Arc expression has been shown to be upregulated in 40% of CA1 hippocampal neuronal dendrites immediately following patterned neuronal activity in response to a novel stimulus, peaking approximately 45 minutes post-stimulation, and slowly relaxing to basal levels over several hours [3]. Additionally, recent *in vitro* studies indicate that repeated exposures subsequently downregulate Arc expression in as much as 60% of those neurons [4]. The details of the complex regulation and function of Arc remain unknown. I believe that animal age and time of initial exploration contribute to the regulation of Arc expression. Consequently, I believe Arc expression must be studied in freely behaving individual animals. *In vitro* studies which explore Arc expression outside the context of behavior and head-fixed *in vivo* studies such as [5] which do not allow free exploratory behavior I believe are insufficient.

Traditionally neuroscience has attracted a wide array of researchers including physicists, psychologists, computer scientists, and electrical engineers, who have each advanced the field by contributing unique perspective. Physicists most notably advanced whole-brain functional imaging with the advent of nuclear magnetic resonance-based technologies such as fMRI. Here I propose to continue this tradition of collaboration between neuroscience and physics, by employing recent advances in optics to characterize the dynamics of Arc expression in individual animals, revealing new insights into the role of Arc in memory formation.

Hypothesis: I hypothesize: 1) the strength of Arc upregulation increases with initial exploration time; 2) younger mice have stronger and more persistent Arc expression compared to aged animals.

Research plan: I propose to study the temporal dynamics of Arc expression in two phases: 1) development (1 year) of the PME, and 2) experimental studies (2 years) which use the PME to quantitate Arc expression over time in individual freely behaving mice. With the guidance of my advisor, Prof. Mark Schnitzer, I believe the project is feasible within the presented timeline.

The PME will have two important improvements compared to Prof. Schnitzer's first generation of head-mounted microendoscopes [6] described in my previous research experience essay. First, the PME will have a light source and camera integrated into the microscope headmount to minimize motion artifacts by fixing the orientation of the camera with respect to the mouse brain. This improvement will eliminate troublesome de-rotation analysis of the data. Second, the PME will allow greater motion by, and chronic imaging of, the mouse. To realize the integrated camera, I will collaborate with Prof. Abbas El Gamal of the Electrical Engineering Department to incorporate a tiny CMOS camera in the PME design. Additionally I will collaborate with a mechanical engineering doctoral student in my lab to design and fabricate housing which is lightweight (less than 4g) and permits chronic implantation in freely behaving mice. Utilizing my expertise in optics theory, I will design the optics for the PME. The design will incorporate commercial and custom

micro-lenses, including gradient refractive index (GRIN) lenses, miniature achromatic lenses, and micro-ball lenses. Data acquisition from the imaging camera and power to the imaging camera and LED will be accomplished using a flexible, lightweight bundle of wires. Development of the PME is already in progress. The development phase has and will continue to allow me to work collaboratively in a group spanning multiple disciplines.

In the second phase of the study, I plan to investigate the temporal dynamics of Arc expression through behavioral experiments with heterozygous Arc-GFP knock-in mice. In these mice, the Arc promoter drives GFP as well as Arc expression, thus Arc expression is quantitated by the strength of GFP fluorescence. Broadly, I will allow a mouse to explore a novel environment several times over 4 days, using the chronic PME for time-lapse imaging over 4 hours following each exploration. I will image once every 20 minutes. Experiments will be structured by combining several variants of these 4-day/single environment trainings.

Specifically, to test my two-part hypothesis, I will begin by varying the time of initial exploration (TIE), setting subsequent exploration sessions to 10 minutes. On day 1, after mounting the PME on the mouse's head, I will visualize all of the neurons in the chosen CA1 hippocampal field of view by rapidly placing the mouse in several different novel environments. On day 2 I will begin several 4-day trials described above, varying the length of the TIE between 1 and 30 minutes. I will analyze the GFP fluorescence data for each 4-day trial to quantitate both the number of neurons expressing Arc and the strength of expression, using the initial neuronal map and a basal fluorescence signal for references. I will analyze the fluorescence data along with memory formation, as measured by behavioral analysis over the 4-day trials. I will compare my results to previously published *in vitro* and *in vivo* head-fixed studies to compare statistical trends. The entire procedure will be repeated for N=10 young mice. Secondly, I will test the effect of animal age on Arc expression. I will again use 4-day trials, repeating trials every two weeks for 6 months per mouse and allowing 10 minute exploration sessions. I will analyze the time-lapse fluorescence and behavior data following a similar protocol as noted above, repeating for N=10 young mice.

For experimental controls, I will repeat these experiments with homozygous knock-in mice, where the Arc promoter only drives GFP expression. I will repeat the experiments using wild-type mice to collect baseline behavioral data. Finally, I will do time-lapse imaging in heterozygous knock-in caged mice to obtain the basal Arc expression dynamics.

Anticipated results: I expect to see that initial Arc expression as well as memory formation will increase with TIE. I believe that Arc reactivation, shown by analysis of the percent of neurons that express Arc during later exposures, and memory formation will increase with TIE but decrease with age for a constant TIE. In the homozygous knock-in control mice, I expect that Arc expression will be regulated by session time and animal age, and memory formation will suppressed. I expect that basal Arc levels in caged animals will remain stable. Upon completion of the study, results will be reported in the neuroscience literature.

Personal motivation: With my training in physics and neuroscience I believe I am thoroughly prepared to conduct the proposed research. In particular, the optical design portion of the project will draw on my physics training. I am excited to propose this project. I believe it will provide excellent preparation for my career as a biophysics researcher and is worthy of support by the NSF.

Statement: I attest that this research proposal is my own work.

Literature citations: [1] GL Lyford *et al*. Neuron **14**:433, 1995. [2] N Plath *et al*. Neuron **52**:437, 2006. [3] JF Guzowski *et al*. Nat Neurosci **2**:1120, 1999. [4] JF Guzowski *et al*. PNAS **103**:1077, 2005. [5] KH Wang *et al*. Cell **126**:389, 2006. [6] BA Flusberg *et al*. Manuscript in preparation.

With formal training in physics and a longstanding interest in discovering how the world works, I am a scientist who works at the exciting interface between physics and biology. My graduate research passion is for *in vivo* fluorescence microendoscopy. Headed by Professor Mark Schnitzer, my lab has developed novel techniques to image brain activity in deep structures of the brain during free behavior, and I am eager to lead the development of more techniques to track cellular network activity in freely behaving animals. Last September I gave a research talk at the Stanford Photonics Research Symposium. I was very excited to share my lab's research with the scientists and professionals attending the symposium. I look forward to giving talks and writing papers about my research endeavors of the coming years. In my proposed plan of research essay, I describe a project to study the temporal dynamics in individual animals of Arc, a neuronal activity reporter protein which regulates memory formation. Broader impacts of this project include advancing understanding of memory-related diseases, such as Alzheimer's. In my previous research experience essay, I present my background in experimental research and detail some of my current work. In this essay, I describe my journey to develop myself as a responsible scientist and my goals for the future, which will be all the more attainable with the support of the National Science Foundation.

My cultivation to become a scientist began in my early years. Since my nursery school days I have been fascinated by figuring out *how* things work and *why* they function as they do. In elementary school, I excelled in math and science while continually observing and commenting on my surroundings. In middle school, I skipped a year of math and took honors courses in chemistry and biology, where my curiosity about natural phenomena continued to mature. In high school, I realized my enthusiasm and passion for physics when I took two years of physics classes with Mrs. Kristy Beauvais. Her perpetual energy for teaching, love for physics, and devotion to her students came through every day in class, and I eagerly absorbed everything she taught. To her, no question was insignificant and each student deserved personal attention; for me, she is an unforgettable teacher and a role model who showed me that having passion for what I do will light up my life. I strive to emulate her when I work with young students, and my goal is to bring an equal enthusiasm for teaching to my academic career.

I believe that responsible scientists should be proficient at communicating with and educating the larger community about their work and should have solid leadership skills. I am actively involved in giving back to the broader community, and I have been for many years. In high school, my curiosity for biology and medical science led me to volunteer for 380 hours at the Emerson Hospital in Concord, MA, and I worked with my Girl Scout troop on a year-long Silver Award project collecting donations for a battered women's shelter in Boston. In college, one of my first activities was an annual CityDays volunteer program, where I helped ready a public school kindergarten room for the new school year. I participated in CityDays again the next fall, this time with additional responsibility as a project leader. In my junior year, I developed leadership skills as a sorority executive board member. As Vice-President of Alumnae Relations, I improved alumnae networking for recent graduates' employment searches, facilitated communication between local alumnae and current undergraduates, and published a bi-annual newsletter describing chapter events and alumnae updates. Additionally, I volunteered at annual Pan-Hellenic Children's Carnivals for the Cambridge community, helped to raise campus awareness of domestic violence, and participated in city-wide Charles River clean-up days.

An especially valuable teaching and leadership experience for me was as a tutor for Shania, a Cambridge public school fifth-grade student, during my sophomore year of college. I was paired with Shania through the Outreach Tutor program coordinated by the Public Service Center at MIT.

I worked with Shania twice weekly on her English and writing skills at the Cambridge YMCA after-school program. I prepared engaging language and writing activities and also aided her with school assignments. Being a tutor reinforced to me that teaching is not as easy as simply presenting information; the teacher is responsible for ensuring that the information is processed and that the student can successfully internalize the lessons.

Since starting graduate school, I've pursued community involvement through new avenues. I am actively involved in mentorship programs to support women in sciences and to support younger students. Last year I joined a local mentoring program for women in sciences which pairs graduate students with older women scientists as mentors. I participate in monthly discussion sessions on how women can continue to succeed in science-related careers and have a very enriching mentoring relationship. At one meeting my mentor, a post-doctoral student, shared notable tips and strategies for interview techniques which I successfully applied during my interview for my thesis research lab. I look forward to becoming a mentor for female graduate students to help instill confidence at persevering in competitive scientific research. Separately, this fall I will mentor a biological sciences undergraduate student through a mentoring program for pre-graduate students, and next summer I will participate in a mentoring program for under-represented local high school students interested in biomedical sciences, who would often be first-generation college students. I look forward to helping to increase the diversity of college students through these programs and find mentoring to be a very meaningful experience.

I also enjoy working with young students to build their enthusiasm for science. In January 2007, I volunteered as a Science Fair Judge for local middle school students. I judged two different projects and had great discussions about the methodology and strong points of the projects. I will continue volunteering for this event over the coming years and will encourage my colleagues to volunteer as well. Last spring, I participated in Stanford's annual Community Day for the surrounding towns' families by volunteering to run a booth about the wonders of fluorescence. I displayed various fluorescent materials and fielded questions about the interactive display from young inquisitive children as well as from their equally inquisitive parents. In 2008, I would like to become a science mentor for a local fifth-grade classroom, visiting several times over the year to have discussions with the class about current science in the news and to share my excitement about how science allows us to understand the workings of the world.

My academic and intellectual abilities support my potential to become a scientific innovator. I have excelled in rigorous physics coursework at MIT, earning induction into Phi Beta Kappa and Sigma Pi Sigma, a national physics honor society. My undergraduate thesis on the development of prototype triple-GEM (Gas-Electron Multiplier) particle detectors, described in my previous research experience essay, contributed to the implementation of a GEM detector into the Solenoidal Tracker at the Relativistic Heavy Ion Collider. At Stanford, I have taken graduate classes in physics, advanced microscopy imaging techniques, and signal processing in neural circuits while doing biophysics graduate research. Being involved in both collaborative and independent projects, I utilize leadership and research planning skills daily.

In conclusion, I believe I embody the sprit of the NSF graduate fellowship program. I have already taken many steps towards developing myself as an experimental scientist, and the future lies ahead of me. Being supported by the National Science Foundation will allow me to freely pursue my current passion for *in vivo* fluorescence microendoscopy, to apply quantitative methods to research on biological problems, and to achieve my career goal of becoming a responsible academic researcher who is actively involved with the greater community.