Department of Biochemistry

Graduate Student Handbook 2017-18

Stanford University
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FACULTY

Emeritus Professors:	Robert L. Baldwin, Paul Berg, Patrick O. Brown, Douglas Brutlag, David S. Hogness, A. Dale Kaiser, I. Robert Lehman
Chairman:	Suzanne R. Pfeffer
Professors:	Steven Artandi, Philip Beachy, Gilbert Chu, Ronald W. Davis, James E. Ferrell, Jr., Daniel Herschlag, Peter Kim, Mark A. Krasnow, Suzanne R. Pfeffer, James A. Spudich, Julie Theriot
Associate Professors:	Rhiju Das, Pehr Harbury, Rajat Rohatgi, Aaron Straight
Assistant Professors:	Onn Brandman, Lingyin Li, Julia Salzman, Ellen Yeh
Courtesy Professors:	Chaitan Khosla, Sharon Long
Graduate Advisor:	Aaron Straight

The Department of Biochemistry is located in the Beckman Center for Molecular and Genetic Medicine and is part of Stanford's School of Medicine. Advanced courses in more specialized areas emphasize the most recent developments in biochemistry, biophysics, cell biology, and molecular biology. These courses include the physical chemistry of proteins and nucleic acids, membrane biology and biochemistry, the cytoskeleton, mechanisms and regulation of nucleic acid replication and recombination, the biochemistry of bacterial and animal viruses, the molecular basis of morphogenesis, and the structure and function of both eukaryotic and prokaryotic chromosomes.

COURSES

199. Undergraduate Research—Investigations sponsored by individual faculty members. Prerequisite: consent of instructor.
1-18 units, Aut, Win, Spr, Sum (Staff)

200. Applied Biochemistry–(Enrollment limited to MD candidates) Fundamental concepts of biochemistry as applied to clinical medicine. Topics include thermodynamics, enzyme kinetics, vitamins and cofactors, metabolism of carbohydrates, lipids, amino acids and nucleotides, and the integration of metabolic pathways. Clinical case studies discussed in small-group problem-based learning sessions.
2 units, Aut (Harbury, Cowan)

202. Biochemistry Bootcamp—(Open to first year Biochemistry students or consent of instructor) Hands-on, five-day immersion in biochemical methods and practice, theory and application of light microscopy, and computational approaches to modern biological problems.
1 unit, Aut (Brandman, Harbury, Pfeffer, Straight, Theriot)

205. Molecular Foundations of Medicine—(For medical students) Topics include DNA structure, replication, repair, and recombination; gene expression, including mechanisms for regulating transcription and translation; chromosome structure and function; gene cloning, protein engineering, and genomics. Patient presentations and journal clubs illustrate how molecular biology affects the practice of medicine.
3 units, Aut (Chu, Krasnow)

209A. The Human Genome and Disease–(Same as BIO 109A, BIO 209A, BIOC 109A, HUMBIO 158) The variability of the human genome and the role of genomic information in research, drug discovery, and human health. Concepts and interpretations of genomic markers in medical research and real life applications. Human genomes in diverse populations. Original contributions from thought leaders in academia and industry and interaction between students and guest lecturers. Students with a major, minor or coterm in Biology: 109A/209A or 109B/209B may count toward degree program but not both.
3 units, Win (Davis, Heller)

209B. The Human Genome and Disease: Genetic Diversity and Personalized Medicine–(Same as BIO 109B, BIO 209B, BIOC 109B) Continuation of 109A/209A. Genetic drift: the path of human predecessors out of Africa to Europe and then either through Asia to Australia or through northern Russia to Alaska down to the W. Coast of the Americas. Support for this idea through the histocompatibility genes and genetic sequences that predispose people to diseases. Guest lectures from academia and pharmaceutical companies. Prerequisite: Biology or Human Biology core. Students with a major, minor or coterm in Biology: 109A/209A or 109B/209B may count toward degree program but not both.
3 units, Spr (Davis, Heller, Ghanta)

215. Frontiers in Biological Research—(Same as DBIO 215, GENE 215) Literature discussion in conjunction with the Frontiers in Biological Research seminar series hosted by Biochemistry, Developmental Biology, and Genetics in which distinguished investigators present current work. Students and faculty meet beforehand to discuss papers from the speaker's primary research literature. Students meet with the speaker after the seminar to discuss their research and future direction, commonly used techniques to study problems in biology, and comparison between the genetic and biochemical approaches in biological research.
1 unit, Aut, Win (Harbury, Villeneuve, Pringle)

220. Chemistry of Biological Processes—(Same as CSB 220) The principles of organic and physical chemistry as applied to biomolecules. Goal is a working knowledge of chemical principles that underlie biological processes, and chemical tools used to study and manipulate biological systems. Prerequisites: organic chemistry and biochemistry, or consent of instructor.
3 units, Alternate Years, Given Next Year (Wandless)

221. The Teaching of Biochemistry—Required for teaching assistants in Biochemistry. Practical experience in teaching on a one-to-one basis, and problem set design and analysis. Familiarization with current lecture and text materials; evaluations of class papers and examinations. Prerequisite: enrollment in the Biochemistry Ph.D. program or consent of instructor.
3 units, Aut, Win, Spr, Sum (Staff)

224. Advanced Cell Biology—(Same as BIO 214, MCP 221) For PhD students. Current research on cell structure, function, and dynamics. Topics include complex cell phenomena such as cell division, apoptosis, compartmentalization, transport and trafficking, motility and adhesion, differentiation, and multicellularity. Current papers from the primary literature. Prerequisite for undergraduates: BIO 129A,B, and consent of instructor.
4 *units, Win (Pfeffer, Kopito, Theriot, Jonikas, Rohatgi)*

236. Biology by the Numbers–(Same as APPPHYS 236) Topics in biology from a quantitative perspective. Subjects vary. 2012-13 focus: evolution, from basic principles of evolutionary dynamics to fundamental quantitative questions that are far from being answered; from early life, metabolic processes, and molding of earth by microbes to spread of human epidemics; from analysis of genomes and molecular phylogenies to aspects of multi-cellular development. Prerequisites: Familiarity with ordinary differential equations and probability. Biology background not required.
3 units, Alternate Years, Given Next Year (Fisher)

241. Biological Macromolecules—(Same as BIOPHYS 241, SBIO 241) The physical and chemical basis of macromolecular function. Forces that stabilize biopolymers with three-dimensional structures and their functional implications. Thermodynamics, molecular forces, structure and kinetics of enzymatic and diffusional processes, and relationship to their practical application in experimental design and interpretation. Biological function and the level of individual molecular interactions and at the level of complex processes. Case studies in lecture and discussion of classic and current literature. Enrollment limited to 30. Prerequisites: None; background in biochemistry and physical chemistry preferred but material available for those with deficiency; undergraduates with consent of instructor only.
3-5 units, Win (Das, Ferrell, Harbury, Huang, Bryant)

257. Currents in Biochemistry—Seminars by Biochemistry faculty on their ongoing research. Background, current advances and retreats, general significance, and tactical and strategic research directions.
1 unit, Aut (Spudich)

299. Directed Reading—Prerequisite: consent of instructor.
1-18 units, Aut, Win, Spr, Sum (Staff)

350. Development of Thesis Research–Biochemistry 2nd year PhD students with permission of instructor only. Students place their thesis research into a broader scientific perspective, identify important questions to ask, and learn to communicate these clearly. Series of roundtable discussions with students and faculty about the proposed research topics. Initial focus on developing the equivalent of specific aims for research grants.
2 units, Aut, (Das, Harbury, Rohatgi)

360. Developing an Original Research Proposal–Biochemistry 3rd year PhD students with permission of instructor only. Students develop new research directions. Topics well outside of student's research topic must be chosen. Series of discussion groups with faculty and students. Students present possible outside research topics followed by presentation of important questions and approaches to answer these questions. Focus is on developing the equivalent of specific aims for research grants.
1 unit, Win (Straight, Theriot, Brandman)

370. Medical Scholars Research–Provides an opportunity for student and faculty interaction, as well as academic credit and financial support, to medical students who undertake original research. Enrollment is limited to students with approved projects.
4-18 units Aut, Win, Spr, Sum (Staff)

399. Graduate Research and Special Advanced Work—Investigations sponsored by individual faculty members. Prerequisite: consent of instructor.
1-18 units, Aut, Win, Spr, Sum (Staff)

459. Frontiers in Interdisciplinary Biosciences—(Same as BIOE 459, BIO 459, CHEMENG 459, CHEM 459, PSYCH 459. Students register through their affiliated department.) For specialists and non-specialists. Sponsored by the Stanford BioX Program. Three seminars per quarter address scientific and technical themes related to interdisciplinary approaches in bioengineering, medicine, and the chemical, physical, and biological sciences. Leading investigators from Stanford and the world present breakthroughs and endeavors that cut across core disciplines. Pre-seminars introduce basic concepts and background for non-experts. Registered students attend all pre-seminars; others welcome. See http://biox.stanford.edu/courses/459.html. Recommended: basic mathematics, biology, chemistry, and physics.
1 unit, Aut, Win, Spr (Robertson)

802. TGR Dissertation – Terminal Graduate Registration course for doctoral programs. Work on the thesis disseration must be evaluated.
0 units, Aut, Win, Spr, Sum (Staff)

This information, together with the latest Stanford University time schedule, is also available through Axess at: http://axess.stanford.edu/

There are excellent graduate level courses taught by faculty in other departments in the Medical School as well as by faculty in Biology and Chemistry. These courses enhance the breadth and depth of graduate education, providing students with an understanding of the multidisciplinary nature of modern biochemistry. Students are also encouraged to come up with areas for courses, which can then be organized in conjunction with one or more members of the faculty.

DEPARTMENT REQUIREMENTS FOR THE Ph.D.

1. Timetable and requirements:

a. The first two or three quarters of a student's first year involve research rotations, which are typically one per quarter, but students sometimes opt for two shorter rotations per quarter. Rotations are set up by direct arrangement between the student and the appropriate faculty member. A thesis advisor can be selected as early as the end of the second quarter, which we promote in order to allow students to get started on their thesis project as soon as possible. A thesis advisor should be selected no later than the end of the third quarter.

b. A committee to review graduate student progress is formed as soon as the student chooses an advisor. In consultation with the advisor, the student chooses the committee, which consists of the advisor and two other Biochemistry Department faculty members. A faculty member from another department may serve as a member of the committee in addition to the advisor and the two Biochemistry faculty.

c. Students present two research proposals (the first on his/her proposed thesis research, the second on an outside area) and one Journal Club presentation. These are described in detail below.

d. Each year the student will also meet with his/her committee to review degree progress and goals. Beyond year 5, students will meet with their committee every quarter.

e. It is expected that the Ph.D. thesis and oral examination will be completed within five years; students may finish in three years. The student, the student's advisor, and the student's committee should work together to meet this goal. This policy is designed to encourage timely progress toward the degree and to protect the long-term interests of our students, as an extended graduate tenure impinges on the exploration and experience of new scientific areas and endeavors; the exposure to alternate scientific environments, approaches to science, and mentoring styles; and the opportunity for rapid advancement to an independent career in science and related fields. NIH also feels strongly about the importance of timely progress to the degree, and expects training programs to have effective policies in place to ensure this. Continued funding beyond the summer quarter of the student's fifth year (and beyond) is contingent upon documented timely progress toward the degree and successful petitioning by the student to a departmental committee. In the petition form, the student must: (i) describe the proposed plan and timeline to completion of the degree, and (ii) explain and justify the need for additional research and/or writing time. Extensions beyond G6 are expected to be granted only under exceptional, well-justified circumstances. If a student has completed all the requirements for the degree but needs to finish papers for journal submission, they should consider doing so following completion of the degree by continuing for a short period in the lab as a postdoctoctoral fellow.

f. The above requirements are set by the Biochemistry Department. There are also three University requirements: (i) a student must be admitted to candidacy for the Ph.D. degree no later than the end of the second year (see page 24), (ii) the Ph.D. degree must be completed within 6 years, and (iii) a student must be registered continuously to the end of the Ph.D. degree, unless he or she obtains an approved leave of absence.

2. Course requirements

a. Students graduating with a Ph.D. in Biochemistry from Stanford are expected to be generally proficient in four core scientific areas relevant to biochemical research as well as in the specific scientific areas most relevant to their particular thesis projects. The four core proficiency areas are: Quantitative Biochemistry and Biophysics, Genetics, Molecular Biology, and Cell Biology. Proficiency can be demonstrated by successfully completing graduate-level courses in each of these four areas, or by other means with permission of the graduate advisor.

b. Additionally, four universal G1 courses are required of all students and are normally taken in the first year:

BIOS 200 – Foundations in Experimental Biology (Autumn Quarter)
BIOC 215 – Frontiers in Biological Research (Two Quarters – Autumn & Winter)
BIOC 257 – Currents in Biochemistry (Autumn Quarter)
MED 255 – Responsible Conduct of Research (intensive one-day class, Any Quarter)

c. Ph.D. students must complete a total of at least six graduate-level courses, including core requirements and electives (but not including the above mini-courses):

Core Proficiency Requirement 1: Quantitative Biochemistry and Biophysics
Recommended course: BIOC 241 – Biological Macromolecules
Some additional courses in this area:
BIOC 220 – Chemistry of Biological Processes
SBIO 242 – Methods in Molecular Biophysics

Core Proficiency Requirement 2: Genetics
Recommended course: GENE 205 – Advanced Genetics
Some additional courses in this area:
DBIO 210 – Developmental Biology
GENE 211 – Genomics

Core Proficiency Requirement 3: Molecular Biology
Recommended course: CSB 250 – Biology of Chromatin-Templated Processes
Some additional courses in this area:
BIO 256 – Epigenetics
BIOC 236 – Biology By the Numbers
Several intensive mini-courses may also suffice for this requirement depending approval by the graduate advisor

Core Proficiency Requirement 4: Cell Biology
Recommended course: BIOC 224 – Advanced Cell Biology
Some additional courses in this area:
MCP 256 – How Cells Work: Energetics, Compartments and Coupling in Cell Biology
CSB 210 – Signal Transduction Pathways and Networks

d. Elective courses:

The elective component of the Ph.D. curriculum empowers each student to design his or her own graduate coursework experience. Choice of elective courses will depend on each student's scientific interests. Many students end up choosing to take more than the minimal requirement of six graduate-level courses in order to satisfy a desire for both breadth and depth in their graduate coursework. Graduate-level courses from any science, engineering or mathematics department at Stanford may be used toward the elective course requirement, not merely those in the biosciences. Undergraduate courses may be counted toward the elective course requirement with the permission of the graduate advisor. Courses listed above within the four core proficiency areas may also be chosen as electives.

Students may wish to consider choosing a few elective courses that will expose them to areas of biological research that lie outside the core proficiency areas most relevant to biochemistry but are nevertheless extremely important in 21st century biology. Examples of such areas include: organismal-level biology and physiology, human health and disease, ecology and evolution, systems-level analysis of biological systems, bioinformatics, physical biology and biological chemistry. Various Stanford departments offer graduate-level courses in all these areas, and new courses are constantly being developed. The graduate advisor and other departmental faculty, as well as other graduate students, can provide valuable input and advice on elective course opportunities.

21st century research will continue to rely heavily on computer power in all areas. Incoming Ph.D. students who do not already have some experience in computer programming and computer algorithms are strongly encouraged to acquire familiarity with basic programming approaches during their time here. Several classes focused on biological topics use basic programming within the course for problem sets and projects. Examples that are accessible to all students, including those lacking any programming experience, include: GENE 211 Genomics (PERL), PATH 218 Computational Analysis of Biological Images (Java), and BIOC 225 Interdisciplinary Approaches to the Cytoskeleton (MatLab). For students who already have some programming skills, several more advanced computation-based classes have been highly recommended by Biochemistry Ph.D. students in recent years, including CS 248 Computer Graphics, CS 221 Artificial Intelligence, and BIOMEDIN 214 Representations and Algorithms for Computational Molecular Biology.

Many students find that small, in-depth, literature-based courses that focus closely on a narrowly-defined topic are among their most rewarding intellectual experiences in graduate school, providing an important opportunity to think very deeply about the literature and discuss it at a sophisticated level. Literature-based courses extend the experience of BIOC 215 Frontiers into areas of special interest. Examples of literature-based courses include BIOC 210 Advanced Topics in Membrane Trafficking and BIOC 230 Molecular Interventions in Human Disease.

Students are invited and encouraged to design their own elective courses! Department faculty are happy to work together with students interested in learning more about a specific area to develop directed reading or special topics courses.

e. Sample schedules:

Students arrive with a diverse range of experience and training. We have assembled examples of potential course schedules that reflect how students with varying expertise and interests might structure their coursework. Note: all students will take BIOC 215 Frontiers during Q2 and Q3, and BIOC 257 Currents during Q1, as well as MED 255 Responsible Conduct of Research at some point during the first year, so these courses have been omitted from the sample schedules for clarity.

Student A has a very strong background in physical chemistry and biophysics and has already learned most of the material covered in BIOC 241 Macromolecules. He/She is interested in studying force generation by molecular motors.

Q1: BIOS 200 - Foundations in Experimental Biology

Q2: BIOC 224 - Advanced Cell Biology
CSB 210 - Signal Transduction Pathways and Networks

Q3: CSB 250 – Biology of Chromatin Templated Processes
MCP 232 - Advanced Imaging Lab in Biophysics
APPPHYS 294 - Cellular Biophysics

Summer - Begin thesis research. Prepare for 1st proposal.

Q4: 1st proposal

Q5: BIOC 236 - Biology by the Numbers

Q6: BIOE 335 - Molecular Motors

Student B has a strong background in cell biology and developmental biology. He/She is interested in studying the development of the lung and understanding how pathogens attack the lung.

Q1: BIOS 200 - Foundations in Experimental Biology

Q2: BIOC 224 - Advanced Cell Biology
MI 210 - Advanced Pathogenesis of Bacteria, Viruses and Eukaryotic Parasites

Q3: CSB 250 – Biology of Chromatin Templated Processes
DBIO 210 - Developmental Biology

Summer - Begin thesis research. Prepare for 1st proposal.

Q4: 1st proposal
BIOC 230 - Molecular Interventions in Human Disease

Q5: BIOC 241 - Biological Macromolecules

Q6: BIOC 210 - Advanced Topics in Membrane Trafficking
PATH 218 - Computational Analysis of Biological Images

Student C has a strong background in molecular biology and genetics and has already learned most of the material covered in Gen203 Advanced Genetics. He/She is interested in studying catalytic mechanisms of enzymes.

Q1: BIOS 200 - Foundations in Experimental Biology

Q2: BIOC 224 - Advanced Cell Biology
SBIO 242 - Methods in Molecular Biophysics

Q3: CSB 250 – Biology of Chromatin Templated Processes
BIOC 220 - Chemistry of Biological Processes

Summer - Begin thesis research. Prepare for 1st proposal.

Q4: 1st proposal

Q5: GENE 211 – Genomics
BIOC 241 - Biological Macromolecules

Q6: Focus on thesis research

f. Students are expected to attend the departmental seminar series.

3. Teaching

Students are required to gain experience in mentoring, instruction or teaching for one quarter. Possibilities for formal teaching assistantships include: BIOC 205 (Aut), BIOC 241 (Spr), BIO 42 (Win), BIO109a/209a (Win), BIO 109b/209b (Spr), CHEM 181 (Aut), CHEM 183 (Win), HUMBIO 200 (Aut, Win, Spr) and others as approved by your advisor. Students receive academic credit as teaching assistants in these by enrolling in BIOC 221 (The Teaching of Biochemistry) and notifying the Student Services Coordinator of the TA course selected. Teaching involves attending all lectures, holding office hours to answer questions from students in the courses, compiling problem sets and answers, helping compile, supervise and grade exams, and assisting with organizational matters including distribution of handouts. More detailed descriptions of TA responsibilities will be provided by the course instructor. Other mentoring and instruction opportunities in local community programs are also encouraged as routes to fulfill this requirement. In addition, direct supervision and mentoring of undergraduate or junior students in the laboratory can also suffice to fulfill this requirement.

4. Fellowship Applications

All first year students are expected to submit predoctoral fellowship applications to the National Science Foundation. Additional information regarding deadlines can be found on-line at: https://www.fastlane.nsf.gov/grfp/.

Students should schedule a meeting with the Graduate Advisor by October 14 to discuss their areas of research for these applications, and each student will choose a faculty member to develop, read and critique their proposals.

All third year students are required to adapt their 1st Proposals into fellowship applications. For US Citizens and Permanent Residents, we expect that you will submit an application for the NIH F31 NRSA Individual Predoctoral Fellowship (due in early December). For international students on visas, we expect that you will submit an application to the American Heart Association to Western States Affiliates Predoctoral Fellowship (due in January) and/or to the internal Bio-X Predoctoral Fellowship (due in January). All students are encouraged to seek out other fellowship opportunities for which they might be eligible.

5. Rotations

Research rotations are critical for students in choosing their thesis lab. In addition, rotations broaden a student's research experience and familiarize students with ongoing research projects. Rotation possibilities and experiences are discussed quarterly with the Graduate Advisor, or more frequently if desired. Rotations are set up by discussion of the student directly with the faculty member of interest. The first rotation must be carried out in the Biochemistry Home Program; subsequent rotations can be carried out with faculty in any Home Program throughout the Biosciences. Rotations are typically one quarter in length, but the student can arrange more, shorter rotations. Rotations longer than one quarter are strongly discouraged, as the primary purpose of the rotation is to find a suitable thesis lab, not to obtain publication quality results.

Students can choose their thesis lab any time after the end of the second quarter. Some students carry out an additional rotation in the third quarter and then choose their thesis lab. Although students have on occasion carried out a rotation over the summer quarter, this is highly discouraged, as it is typically in the student's best interests to initiate his/her thesis research to maximize the period that can be devoted to this.

6. Research Proposals and Committee Meetings

General Philosophy

The Biochemistry faculty recognizes that students admitted to our program are among the best-prepared and most motivated scientists in training throughout the world. We feel that students will best explore their creative potential and develop their intellectual and analytical skills through frequent collegial interactions with faculty. In this spirit, committee and proposal meetings are designed to allow an open and exciting exchange of scientific ideas and results. Through this, the student learns to develop, organize, and present his/her ideas and results while benefiting from the experience and insights of committee members. These meetings also provide an opportunity to identify areas for students to focus on as they develop as independent scientists.

Choosing a Thesis Committee

The thesis committee is chosen by the student, in consultation with his/her advisor. It is made up of at least three faculty members: the student's advisor and two other faculty members from the Biochemistry department. Students are encouraged to include an additional faculty member from outside of the Biochemistry department whose expertise and perspective the student believes will be valuable. Additional members are possible, but it should be recognized that scheduling complications may arise with a larger committee.

The Biochemistry faculty are committed to direct and frequent interactions with, and mentoring of, all students within the department; such close interactions are often critical for obtaining the strongest reference letters possible at the close of one's graduate training. Nevertheless, there may be instances in which students would like to have a larger number of committee members from outside of Biochemistry and fewer from within the department. In such cases, the student should petition the Graduate Advisor, briefly explaining the underlying reasons.
Finally, students can invite to any committee or proposal meeting any Stanford faculty member whom they believe would enhance discussion and provide valuable feedback.

Scheduling Committee and Proposal Meetings

All meetings will be scheduled by your advisor's Administrative Assistant (AA), after receiving notice from the Student Services Coordinator. While this removes the substantial burden of arranging meetings from the student, the student maintains the responsibility to rapidly communicate information about his/her course schedule and other commitments to the AA. The committee meeting should be 90 minutes in length. For students in their fourth year and beyond, an additional 15 minutes should be used to discuss future plans.

Handy Proposal and Committee Meeting Timeline

	Autumn	Winter	Spring	Summer
1st year	**Rotations**	**Rotations**	**Rotations**	
2nd year	**1st Proposal Course Meetings (BIOC 350)**	**1st Proposal Defense/ Committee Meeting (Jan)**		
3rd year	**Submit Fellowship Applications**	**2nd Proposal Course Meetings (BIOC 360)** **Committee Meeting**	**2nd Proposal Defense (May)**	
4th year	**< Committee** **<Journal**	**Meeting >** **Club**	**Presentation>**	
5th year	**<Journal**	**Committee Meeting** **Club**	**Presentation>**	**Committee Meeting**
6th year	**Committee Meeting**	**Committee Meeting**	**Committee Meeting**	**Committee Meeting**

Research Proposals

Proposal Timeline and Topics

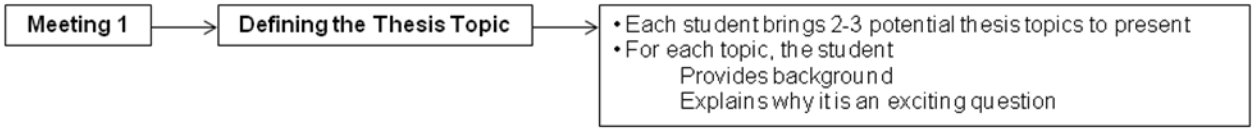
Proposal	Due Date	Topic
1st proposal	2nd quarter (Jan) of 2nd year	Thesis research
2nd proposal	3rd quarter of 3rd year	Outside research area

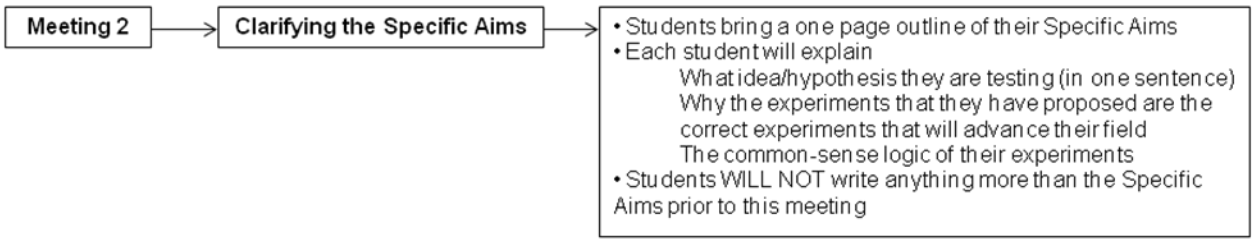
Notes:

- Any proposal can be completed at an earlier date. The 1st proposal must be completed within 8 months of joining a lab.
- Any exceptions to the above timeline must be discussed with the graduate advisor.
- See below for description of the format and expectations for each proposal.

Research Proposal Format

- All Proposal Defense meetings will be scheduled for 90 minutes and will not extend longer. This includes the time for faculty consultation and post-meeting discussion.
- At the end of each Proposal Defense meeting, the student and faculty committee members will together decide whether feedback will be given as a group or individually. Students are encouraged to take advantage of their committee members for feedback directly following proposal and committee meetings as well as at any other time. It may be useful in these discussions to articulate clear goals for the upcoming year.
- Meetings leading up to the Proposal Defense (as detailed below) are based on the Yamamoto Model for writing a qualifier proposal. This procedure emphasizes the importance of working out research ideas through discussion before any writing begins:





Both meetings will include other students and 2-3 faculty members with differing areas of expertise.

1st Proposal:

Enroll in BIOC 350 Autumn Quarter of your 2nd Year

In the first proposal the student describes and discusses his/her planned thesis research.

The goal of the first proposal, and the process leading up to it, is for the student to take ownership of her/his project. Not unlike the Velveteen rabbit, who became a real rabbit, this is the process by which the student becomes a real scientist. The distinguishing characteristic of the PhD is the intellectual component. This component consists of the following:

i. Knowledge of the background and intellectual history of a field;
ii. Critical appraisal of the experiments, models, and directions of that field;
iii. An ability to pose important questions in the field;
iv. An ability to derive an experimental plan to address one or more important questions in the field.

Experiments can be carried out by technicians without the requirement of a doctoral degree. In contrast, the intellectual aspect of one's project is here emphasized, recognizing of course that experimental planning and experiments are also critical. Further, a corollary of the above is that knowledge of multiple fields will render one a more powerful scientist. While the preparation time before the first proposal is limited, such broadening will greatly enhance one's ability to identify and solve scientific questions and is thus expected to develop throughout one's graduate experience.

There is no expectation at this meeting for a given amount of initial data to be presented. Rather, the following is expected:

- A thorough (deep and broad) understanding of the literature that provides the background directly leading to the student's thesis project and the literature pertinent to framing the thesis question and carrying out the proposed research.
- A clear and compelling description of why the proposed research question is interesting and important.
- A clear experimental plan, with contingencies, for carrying out the research. A crude estimated timeline should be presented.
- Most generally, the student should have developed the intellectual skills required to articulate the question being addressed, its importance, and how it will be addressed in a thoughtful, well-directed, and logical manner.

How to prepare:

Preparation for the 1st proposal starts as soon as one joins a lab.

- You should have regular meetings with your advisor and discuss your ideas and possible thesis projects, with project ideas coming from both you and your advisor.
- You should read the literature in your area, broadly and deeply. Your advisor may give you references to read, but you should not rely on nor limit yourself to those.

- You should become familiar with other projects in the lab and how they relate to your project. This typically is accomplished by talking with lab mates (typically best when specific times are set aside to talk) and by reading the prior literature from your lab.
- Read multiple papers *each* week. Over time you will then accumulate a comprehensive background. On the other hand, if you read only when you are forced to, you will have to rely on others for insights and directions in your research.
- It is not possible to prepare for the first proposal simply by taking one week or even one month off from research prior to the 1st proposal and reading all pertinent literature in this compressed period. Such a strategy is counter to the development of a thesis project and the necessary depth of understanding.
- Students should take approximately 1-2 weeks prior to the proposal meeting to prepare the written and oral presentations and to reflect on preliminary data, if any relevant data has been obtained.

Class Leading Up to the 1st Proposal:

Second year graduate students will enroll in BIOC 350, Development of Thesis Research, during the Autumn quarter. The class will meet once a week to prepare you for writing and presenting your proposal. Three instructors (the Proposal Steering Committee) will teach the class. A syllabus for the class will be available on its coursework site.

Second year graduate students will defend their proposals before their Thesis Committee, during a single week in January of the Winter quarter. See below for guidelines for the written and oral presentation.

The student's advisor is asked to be present at the 1st Proposal Committee Meeting to meet with the committee before the student presentation and for the discussion of the committee after the proposal, but may not be present for the presentation and question period. At least one member of the Proposal Steering Committee will be part of the committee at this meeting.

Students will adapt their 1st Proposal into fellowship applications for submission in Autumn quarter of their 3rd year. It is strongly recommended that all rising 3rd year students take BIOS 242, Writing Successful NIH Fellowships and K Awards, in Autumn quarter of their 3rd year in order to help with this process.

Specific Guidelines for Written Proposals:

(Also view the example proposal in the Appendix by Sara Ocon.)

Proposal summary

This should be handed out to your committee at least one day prior to the committee meeting. The first and second proposals are no more than 5 pages in length (single spaced, including figures, excluding references). Suggested lengths for the sections are: **Title**, maximum 80 characters -- the title is important and defines the point of your project; **Specific Aims**, 1/2 page preferred, 1 page maximum; **Background and Significance**, 1 page preferred, 2 pages maximum; **Research Design and Methods**, 2 pages; and **Progress Report**, 1/2 page, but remember that the point of the proposal is not to emphasize preliminary results.

Some may approach the outline above for the first and second proposals. Another example is as follows:

i. *Specific aims:* Present a small number of experiments (less than five) that you view as most important to the model or to discriminate between models. What specific experimental goals do you actually plan to accomplish over the period of the proposal (3-4 years)?

OR

i*. *Specific aims for novel experimental approach:* Describe a sequence of intermediate controls that will validate the full experimental approach in a stepwise fashion. In addition, describe one biochemical experiment that you view as the most important application of the novel experimental approach. What would be learned that cannot be learned with existing methods?

ii. *Background*: This should be limited to one page, if at all possible. The background should not be covered encyclopedically. Rather, the background relevant to points ii and iii below should be concisely covered.

iii. *Model:* Present one or more models of the biochemical phenomenon that account for all or most of the facts presented in the Background. Specifically, what is hypothesized to be happening at the molecular level? Detail inconsistencies between models and experimental data. The model can be taken directly from the literature, but the student should have thought through the model thoroughly. The proposal should include at least one central figure that describes the model pictorially.

OR

iii*. *Novel experimental approach*: Describe the conceptual basis of the experimental approach and the means to implement it. Justify each technically challenging step by a direct precedent from the experimental literature. The proposal should include at least one central figure that describes the approach pictorially.

iv. *Long term goals:* What are the broad, important questions, and why are they important? Often the hardest part of a project is figuring out what question, if answered, would lead to significant insights.

The Oral Proposal:

- A critical feature of the oral presentation is planning and time management. There is a tendency to present too much background information. This should be kept to a minimum, with presentation only of those prior results that provide the intellectual underpinning and are directly relevant to the proposed thesis research.
- The major question to be asked and/or hypothesis to be addressed should be clearly and simply stated, right in the beginning. This should take only a minute or two. Then the background and the proposed experiments should center around this statement.
- The general background should take <5 minutes. With a comprehensive understanding of the area, you should be able to pick out the critical background and field questions on directly and distally related points that your committee may raise.
- The student should be prepared to describe the importance of the proposed research and its relevance beyond the stated project.

- Presenting an estimated timeline for the proposed research can be very helpful in prioritizing experiments.
- It is critical to have considered details of the experimental plan. It is not necessary to present all of this, but very often it is the details of an experiment that provide the greatest challenges or prevent an experiment from working.
- When a proposal is satisfactorily completed, the student should have the written proposal (including the title and date of completion) approved and signed by the faculty advisor and members of the committee and placed in the student's file (see Student Services Coordinator).

2nd Proposal

Students enroll in BIOC 360 for Winter Quarter of the 3rd Year. This course will meet using an agreed-upon schedule with the 3rd year students and the faculty on the Proposal Steering Committee.

The aim of the 2nd proposal is for the student to formulate and address incisively an outstanding scientific question outside of his/her research area. This is meant to be a fun opportunity for you to explore independently an unfamiliar field about which you have long been curious, and to exercise your skills in framing scientific questions precisely and in designing experiments to address those questions in a definitive way. This proposal entails attending BIOC 360 class meetings in Winter quarter, preparation of 1-page written proposal summarizing the question and appropriate specific aims, and a final oral presentation presented to members of the Biochemistry Department. The public presentations should be completed by the end of the Winter Quarter.

IMPORTANT INFORMATION ABOUT THE PROPOSAL:

Suppose you are starting your own lab, say as an independent fellow. What ideas and experiments will you present as your first grant application, given that you are free to work in any research area related to biochemistry (broadly defined)? For purposes of the 2nd proposal, the scope should be such that one or two people could complete your experiments within about three years (you and a technician, if needed). In other words, we are looking for you to identify the most important question in a field and come up with experiments to address it, rather than simply describing an entire field and all of the experiments that would keep a lab or the field going. Do not propose an endless sequence of experiments that would take up the time of multiple labs for a decade. There are always many experiments one can dream up; the trick is to narrow the list down to the best experiments that get to the heart of the problem you have identified.

The 1-page written proposal should include:

- A brief introductory paragraph including a single sentence statement of the question to be addressed, or the hypothesis to be tested. (An alternative is to present an idea for the development of new technology.)
- A brief summary of experimental specific aims, with an explanation of why the proposed experiments are the killer experiments that will advance the chosen field.

The oral presentation should be aimed for the general audience of the members of the Biochemistry Department, who are not likely to be experts in your chosen field. The presentation should include sufficient background information to convince your audience that your question is interesting and vital, and you should clearly demonstrate the incisiveness of your experimental logic in designing the experiments within the specific aims. The entire presentation should take no more than 30 minutes, of which at least 10 minutes should be reserved for questions and discussion with the audience. Members of the steering committee as well as other faculty members, postdoctoral fellows, graduate students, and research staff will be invited to give you written feedback on your proposal. The goal is for everyone to learn about interesting and diverse fields, and to enjoy critical thinking about how best to make progress at the leading edge of science.

Committee Meetings

One thesis committee meeting is required per year in years 2-4, two in year 5, and one per quarter in year 6 and beyond. The first committee meeting is covered by the 1st proposal, as this is a presentation of the student's proposed thesis research. However, in the years in which the student has his/her 2nd Proposal or Journal Club Presentation, additional thesis committee meetings are required. Individual Development Plan (IDP) meetings are a requirement for all Biosciences PhD students and are in addition to the thesis committee meeting requirements:

Student Year	IDP Meeting Due Date	Committee Meeting Due Date	Notes
Year 1	Within 30 days of joining thesis lab		
Year 2	August 1	2nd quarter	Committee Mtg same as 1st Proposal
Year 3	August 1	2nd quarter	
Year 4	August 1	1st or 2nd quarter	Committee Mtg requires a 1-page written progress report
Year 5	August 1	2nd and 4th quarters	Committee Mtg requires a "5th Year and beyond degree-progress petition"
Years 6+	August 1	Each quarter	Committee Mtg requires a "5th Year and beyond degree-progress petition"

Notes:

- Students can request scheduling of an additional committee meeting at any time.
- Any exceptions to the above timeline must be discussed with the graduate advisor.
- To receive a committee meeting waiver, written approval must be sent to the Student Services Coordinator by both the student's advisor and the graduate advisor.
- It is the expectation of the Biochemistry faculty that the Ph.D. project should be carried out and defended in five years or less (see policy 1.e. on page 7). Students enrolling for a sixth year must petition to the Graduate Advisor with a timeline for graduation and a statement of post-graduation plans. This petition will be required for registration.

Thesis Committee Meeting Format

- Committee meetings will be scheduled for 90 minutes and will not extend longer. This includes the time for faculty consultation and post-meeting discussion.
- Students are encouraged to take advantage of their committee members for feedback during the committee meetings as well as any other time. It may be useful in these discussions to articulate clear goals for the upcoming year.

Individual Development Plan (IDP) and Annual Planning Meetings

Your Individual Development Plan (IDP) and annual planning meeting with your advisor are intended to help you:

- **Take ownership** of your training and professional development.
- **Pause and reflect!** Amidst daily research activities, it is easy to lose sight of longer-term goals.
- **Think intentionally** about your short-, mid- and long-term training and development goals.
- **Identify and use resources** to help you achieve your goals.
- **Have open and direct dialogue** with your mentor(s).
- **Establish clear expectations/steps.**

As of March 31, 2014, the Committee on Graduate Admissions and Policy (CGAP) has adopted a new policy requiring all Biosciences PhD candidates and their mentors in the Schools of Medicine and H&S to create and discuss their Individual Development Plans (IDPs) on an annual basis. This annual IDP meeting is in addition to any required Committee Meetings (as noted above).

Students and their advisors share responsibility for completing the IDP, as well as the consequences of not completing the IDP by the deadlines below. Failure to comply with IDP requirements will

- negatively impact Stanford's ability to receive NIH funding; and
- incur a hold on student registration that prevents stipends from being funded.

Key Deadlines

Action	First Year Students	All Other Students
Schedule a planning and mentoring meeting with your advisor	Within 30 days of joining your thesis lab	Before June 1
Download and complete the appropriate IDP form. (Ideally, share the completed form with your advisor in advance.)	Before your meeting	Before your meeting
Hold your annual planning/ mentoring meeting with advisor	Within 30 days of joining your thesis lab	By August 1
Verify that you and your advisor met to discuss your IDP	Within 30 days of joining your thesis lab	By August 1

See http://biosciences.stanford.edu/current/idp/ for more information and IDP forms, including extensive FAQs and resources for both faculty and students.

Questions? Please email somcareers@stanford.edu

7. Ph.D. thesis and oral examination

a. Students must complete a draft of the Ph.D. thesis that is acceptable to the reading committee, which is typically, but not necessarily, the same as the proposal committee. Students must also have completed their two departmental research proposals and their Journal Club presentation before the Ph.D. oral examination can be scheduled.

b. Reprints of a student's published work may be included in the thesis. However, if a publication is jointly authored, the student must describe in the thesis his/her role in that work. In addition, the thesis should contain a general introduction and a general conclusion.

c. At the oral examination, a student will first present to an open audience a seminar on the thesis, after which there is an open question period. Then, the examining committee meets in private with the candidate for further discussion of the general area of the research work and to test the candidate's command of biochemistry and fitness for scholarly pursuits.

d. Please see the Student Services Coordinator for the appropriate instructions and forms before establishing a Dissertation Reading Committee and Oral Examination. Your committee must consist of at least five members: four examiners (your thesis committee plus one) and one University chair (chair cannot have appointment in same department as you or your advisor). Approval to deviate from this assemblage requires approval by the graduate advisor and your thesis advisor.

e. Graduate student funding will end with the thesis defense and only on special conditions, on a case by case basis, will a postdoc position be offered.

f. Deadlines for submitting your thesis to the Registrar's Office and applying to graduate can be found on the Registrar's website at: https://registrar.stanford.edu/students/dissertation-and-thesis-submission. You must be registered in the quarter in which you will turn in your thesis. Tuition can be reduced in your final quarter by completing a "Petition for Graduation Quarter." Please see the Student Services Coordinator for this form. Other graduation information can be found on the Registrar's website under the Academics tab at: https://registrar.stanford.edu/students.

Notes on thesis preparation

University regulations specify the composition of the examination committee and the format of the dissertation defense. Students should refer to the booklet *Directions for Preparing Doctoral Dissertations*, available from the Registrar's Office, for specific information. You can also obtain this information at: https://registrar.stanford.edu/students/dissertation-and-thesis-submission. These guidelines should be read carefully before final preparation of the manuscript to avoid costly and time-consuming revisions. Previously published dissertations may not be a good guide to preparation of the manuscript, as the directions have changed. Published papers may be included in dissertations; however, they must meet the University's format guidelines. Manuscripts and figures submitted for publication during the doctoral program should be retained for later reformatting and inclusion in the dissertation.

SEMINAR PROGRAMS

The Department of Biochemistry hosts a biweekly seminar series (autumn-spring quarters) entitled Frontiers in Biology. Seminars are at 4 PM on Wednesdays in the Clark Center Auditorium, alternating with the Developmental Biology and Genetics seminar programs. Graduate students and postdoctoral fellows select and sponsor several of the seminar speakers.

Graduate students are expected to attend all of the Biochemistry Department-sponsored seminars. This ensures broad training within our graduate program.

Lectures by Students and Postdoctoral Trainees

An important aspect of the training of every graduate student is the development of speaking skills. Opportunities are offered for trainees to speak about their ongoing research in a seminar setting at group meetings, conferences and the annual departmental research conference each fall.

Group Meetings

Each faculty member in the department has weekly group meetings in which the students and postdoctoral fellows take turns presenting their experiments and/or discussing papers from the literature.

Journal Clubs (partial list):

Biochemistry Journal Club

Our Biochemistry Department Journal Club is designed to bring together students, post-docs and faculty to discuss current topics in the literature. Every 4-6 weeks, one 4th or 5th year student and one Post-doc or Faculty member will present a research discussion based on recent papers or developing scientific topics of general interest. This provides a unique opportunity for students to hone their presentation skills and to bring the department up to date with regard to recent scientific developments. Each student will prepare by selecting potential papers for journal club and then discussing the papers with a faculty member before making a final selection. Once a final paper has been selected, the student will prepare and present a talk to the department. It is recommended that each student review their presentation with a faculty member before presenting Journal Club in order to obtain useful feedback on how to organize and convey scientific ideas. Faculty will provide feedback after the presentation to help students learn the art of communicating science. This forum is designed to help advanced students as they begin to present at scientific conferences and apply for post-doctoral and professional positions. Journal club presentations fulfill the third presentation requirement for the PhD degree, if the topic selected is entirely distinct from that presented as part of either the first or second proposals.

Molecular Biophysics

Stanford research groups with molecular interests gather on the second Wednesday each month and listen to two talks of approximately 30 minutes about ongoing research. Talks begin at 12:00noon. All interested researchers are encouraged to attend regularly. For a current schedule or further information contact Kathleen Guan, kguan@stanford.edu, 3-7576. You can also view seminar announcements on the Medical School On-Line Seminar Calendar at http://www.med.stanford.edu/seminars/.

Bay Area RNA Club

The Bay Area RNA Club meets semi-annually on the UCSF campus. Detailed information at: http://barc.ucsf.edu/ - click on Bay Area RNA Club.

STUDENT SERVICES

Registration

Instructions on registration procedures and payment of fees can be found on the Registrar's website at https://registrar.stanford.edu/students. Graduate students are required by the University to register for Autumn, Winter, Spring and Summer quarters either for 10 units, or TGR until the degree is received. Students receiving stipend checks must register for 10 units or TGR in order to receive a check. Leaves of absence require department approval before departure.

Registration Process

Access to Stanford student privileges (housing, financial aid, access to courses and facilities, etc.) is contingent upon timely and accurate completion of the following primary activities each term:

1. Confirm, through Axess, that the University has your correct address and phone number.
2. Ensure that your University bill is paid. If it is not or if you believe there is an error, please see the Student Services Coordinator (Joella Mesa) immediately.
3. Clear all holds that may block your ability to enroll in classes.
4. File your study list (the list of courses in which you wish to enroll) and maintain that study list throughout the term, via Axess.
5. If all of your holds have been cleared and Axess does not allow you to enroll, contact the Student Services Center (650-723-7772, 2nd floor in Tresidder Union) for assistance.

Deadlines are set for each of these activities. For example, there are dates set each quarter for submission of the study list, for dropping or adding courses or units, for changing the grading basis for an enrolled course, for withdrawing from a course, etc. Deadlines are published in the Academic Calendar and found on the Registrar's website:
https://registrar.stanford.edu/resources-and-help/stanford-academic-calendar.

Graduate Student Tracking (GST) System

The Biosciences Graduate Student Tracking System (GST) is a secure online resource for Ph.D. students, faculty, and student services administrators (SSAs); its ultimate goal is to provide support in the related areas of student academic progress, alumni tracking, admissions and training grant application/renewal. It can be accessed via this link: https://med.stanford.edu/gst/ with SUNet ID authentication. Information on the system is provided at https://biosciences.stanford.edu/current/gst/index.html. Students are asked to enter their Lab Rotations as well as IDP and Thesis Committee Meetings into this system.

Terminal Graduate Registration (TGR)

Students who have: 1) been admitted to candidacy, 2) completed all required course work, and 3) have satisfied the residency requirement of 135 units are required to register for TGR status. Under TGR, tuition fees are substantially reduced.

Students registered in TGR status must enroll each quarter in a TGR course (course #802 for doctoral programs) in the Biochemistry department, with their advisor as the instructor, and for 0 units. Work on the thesis dissertation must be evaluated each quarter for academic progress and graded as follows: "N" indicating satisfactory progress, "P" for a final grade when all requirements have been completed.

Study List

Preliminary Study Lists are due on the first day of classes. Final Study Lists are due approximately three weeks after the start of the quarter. Students should complete this carefully and submit it by the listed deadlines (otherwise late fees may be assessed and the course grades possibly delayed). Students submit their study lists through AXESS.

Student Record in the AXESS System

Each student is responsible for ensuring that the University has his or her correct mailing address and telephone number. Addresses and phone numbers should be updated through AXESS, the on-line system for student information. Students can also examine records of their courses and the grades they have received; making it easier to change incorrect information or spot incomplete grades. Incoming students should receive information about AXESS from the Registrar's Office through email; continuing students should consult their AXESS account for more information regarding procedures and University policy.

Candidacy

Admission to candidacy is a judgment by the faculty in the department of the student's potential to successfully complete the requirements of the degree program. Students are expected to complete department qualifying procedures and apply for candidacy by the summer quarter of their second year in the Ph.D. program. This form is forwarded to the Registrar's Office and indicates that the student is formally qualified for the Ph.D. degree and is in good academic standing. The form requires listing completed Stanford course work with at least 3 units of course work taken with each of four Stanford faculty members.

Once a student is admitted to candidacy, the status is valid for five years; subject to termination by the department if progress is unsatisfactory. In special circumstances, it may be renewed by the submission and approval of a new application, or extended upon the chairman's recommendation.

Units and Residency

The University's minimum unit requirement for the Ph.D. degree is satisfactory completion of 135 units of course work, reading, and/or research at Stanford. At least three units must be taken with each of four different Stanford faculty members.

Post-graduation Planning

After receiving the Ph.D., many graduates become postdoctoral fellows and research associates in other laboratories before entering research or tenure-track academic positions. At least one and a half years before the expected Ph.D. date, the student should consult their advisor concerning career plans and the strategies and conventions of obtaining fellowships, postdoctoral sponsors or employment in industry. Although applications for postdoctoral fellowships are normally made in the last year of graduate study, decisions regarding sponsors must be completed before the application process begins.

Vaden Student Health Center

The Vaden Student Health Center provides medical care, including a range of counseling and mental health services, to regularly enrolled Stanford students. The center is located at 866 Campus Drive and has a full-time staff of physicians, mental health professionals and nurses. It provides, for free or for modest fees, a program of medical and psychological services to students holding current student I.D. cards.

For hours of operation see https://vaden.stanford.edu/about/hours. Call 650-498-2336 for information and appointments.

Stanford University requires all new students to have completed an Entrance Medical Record. Information on entrance requirements and forms is provided at https://vaden.stanford.edu/about/entrance-health-requirements.

Health Insurance

Stanford students are required to enroll in the Stanford health insurance plan, Cardinal Care, paid along with registration or tuition fees, or provide evidence of satisfactory coverage with an external carrier. Cardinal Care is the comprehensive student health insurance plan sponsored by Stanford University featuring access to Stanford Medical Center. Coverage information for 2017-18 can be found at https://vaden.stanford.edu/insurance/cardinal-care-overview-and-benefits.

Students are automatically enrolled in Cardinal Care unless they waive coverage and have other health insurance. Students must waive coverage before the first quarter in which they are enrolled each academic year (normally this is Autumn quarter). Cardinal Care is waived in Axess by the deadlines listed at https://vaden.stanford.edu/insurance/choosing-your-insurance/important-deadlines. Stanford health insurance charges appear on quarterly University bills (autumn, winter, spring quarters). The phone number for the Insurance Desk at Vaden is 723-2135.

Dental benefits are now available through the Cardinal Care insurance plan. Benefits are administered by Delta Dental of California. Coverage includes diagnostic and preventive services at 100% with no deductible when an in network Delta Dental PPO dentist is used. Consult your Cardinal Care insurance plan for more information. Information is available on the Vaden website at https://vaden.stanford.edu/insurance/dental-and-vision-insurance-options.

Campus Health Service Fee

This is a mandatory fee that applies to all undergraduate and graduate students enrolled on the Stanford campus ($210/qtr, 2017-18). It covers many services provided by Vaden Health Center, including primary care medical visits, psychological evaluation and short-term therapy at Counseling and Psychological Services (CAPS), and access to health and wellness programs.

Specific details regarding this fee and its implementation may be found at https://vaden.stanford.edu/insurance/health-insurance-overview/insurance-vs-campus-health-service-fee.

Information about the services provided by Vaden Health Center may be found at http://vaden.stanford.edu.

Stipends

Entering students are offered a stipend and tuition. Students are required to apply for predoctoral fellowships from the National Science Foundation during their first year in residence. Applications are available on the Web and are due in November. Students are also encouraged to apply for other outside fellowships. Departmental funds are used to supplement support from all sources to an annual minimum level of $38,736 (2017-18). ASSU fees, late fees, etc. are the responsibility of each student. Additionally a one-time document fee of $250 must be paid by the student. Health insurance will be paid by the department if not covered by fellowship institutional allowances. Students may receive stipends quarterly or semi-monthly (based on funding source). A U.S. Social Security number is required to receive any funds disbursed by Stanford.

For those students on fellowships who are paid quarterly, the stipend checks are issued approximately two weeks before the quarter begins (provided you are enrolled in classes for the quarter) and are mailed by the Student Financial Services Office to the student's address in Axess. Fellowship stipends are taxable but are not subject to withholding or reporting by Stanford. Students receiving stipends are responsible for making any necessary estimated tax payments. Federal Form 1040-EZ and California Form 540-ES are available at https://sfs.stanford.edu/taxes/resources. These forms are also available on the IRS' website.

Students who are paid semi-monthly will be paid on the 7th and the 22nd of the month (or on the preceding work day if these dates fall on a weekend or holiday). Salary assistantships are taxable and subject to withholding, and are reported by Stanford on a W-2 form. International students may qualify for federal "tax treaty exemption" - if one exists between the US and their country. Direct-deposit is also available, apply through AXESS.

Students in the Department of Biochemistry including MSTP students who declare biochemistry as their home program will be provided $250 per year for the first four years of their thesis program for books and other incidentals relevant to their training. (In the case of the MSTP students, if they declare biochemistry in their 2nd year then they can use funding in years 2 through 5.) Students in their first year can choose to take advance payment of their future

years' allotments to apply to a more expensive item such as a computer. If a student does not take advantage of the full $250 supplement within that year, the student is not eligible to "roll over" the money to use in the future. Requests to use department funding should be submitted in writing to the Graduate Student Advisor. See the Student Services Coordinator for information.

Tuition

Tuition (10 units) is fully covered by research assistantships or traineeships. Tuition paid by the department is paid directly to the University. Students will receive tuition credit on their University bill.

Tax

Stipends are subject to income tax, but not withholding, so the student must pay estimated taxes (form 1040ES). Please view information found at https://sfs.stanford.edu/taxes should you have a question regarding tax status or payments.

I-9 Requirement

Any individual receiving salaried compensation must have on file a correctly completed I-9 form (Employment Eligibility) prior to commencement of work. International students who are not U.S. permanent residents must have a valid passport and visa with either an I-94 card or an I-20 ID card carrying an employment authorization stamp in order to file an I-9. See the Student Services Coordinator for completion of this form.

Academic Standing Policy

Enthusiasm, intellectual growth, and the ability for bench research are essential elements for success in the Biochemistry Graduate Training Program. In cases where a Ph.D. candidate may find these elements to be lacking, the faculty urge the candidate to consider alternate – and likely more rewarding – career development paths at an early stage, with time to explore and excel in another field during their prime years. A graduate committee may also judge that a candidate is not making sufficient progress to complete the Ph.D. degree in a timely fashion, in which case the committee will increase its level of mentoring through quarterly meetings with the candidate and the establishment of concrete, short-term research goals. Continued lack of progress can lead to dismissal from the program. For more information, see the University's policy at: (http://exploredegrees.stanford.edu/graduatedegrees/#degreeprogresstext).

Vacation Policy

Graduate student quarter breaks are not like that of undergraduates because of the continuous nature of research progress. Students have a finite time in graduate school to complete their thesis work. Their success/failure will depend on the choices they make and their dedication to their research.

Graduate students are allowed to be away 15 days per year (not including Christmas Day and New Years Day). Generally only a portion is used at winter break. All vacation time is to be scheduled as to minimize disruption to their research.

Patent Policy

Stanford's patent and copyright policies apply to any student working on a research project, regardless of the source of aid. You must agree to this policy by completing the form "SU Patent Agreement (SU-18)" located in the AXESS system. The policies allow inventors/creators to retain all rights to inventions and copyrightable materials unless certain exceptions apply. The most important exception is that Stanford claims title to inventions and copyrightable materials (including computer software) made under sponsored research in order to grant sponsors the licensing or other rights required under the agreement. Stanford also claims title to copyrighted material under the following circumstances:

- The work is created for University purposes in the course of employment;
- The work is commissioned by the University;
- The work is supported by a direct allocation of funds through the University pursuit of a specific project;
- Other arrangements are required as agreed in writing

Housing

On Campus

Graduate Housing at Stanford accommodates single students as well as those coming to Stanford with a spouse, same-gender or opposite-gender domestic partner, and/or children. Graduate residences include studios and apartments with up to four bedrooms. All Stanford student housing is smoke-free, and pets are not allowed. Campus rents range between $795/mo (4 bedroom, quad occupancy) and $2,010/mo (studio, single occupancy) for the academic year 2017-18. All housing assignments are made through a lottery system. If you are new to Stanford and enrolled in a graduate degree program, you are guaranteed housing for your first year of study if you apply by the Lottery deadline and indicate as the final choice on your application that you are willing to live in any residence for which you are eligible. Housing information can be found at: http://studenthousing.stanford.edu.

Off Campus

Many students live off-campus. Community Housing Services provides helpful information on their website at http://offcampus.stanford.edu, including listings of rentals available in the local area.

The following web sites are also good sources for off-campus housing.

Short term housing:
https://rde.stanford.edu/studenthousing/short-term-visitors

Stanford News housing ads:
http://news.stanford.edu/classified-ads/

Palo Alto Weekly:
http://www.paloaltoonline.com/index.php

San Francisco Chronicle:
http://www.sfgate.com/

Local newspapers are also a good source of off-campus housing. The Peninsula has several newspapers including the *Palo Alto Weekly*, published on Tuesdays and Fridays, and *The San Jose Mercury News* and the *San Francisco Chronicle,* published daily.

Transportation

Cars

Permits are required for parking on campus. Rates for 2017-18 are: $396/year for "Resident" (allow you to park at your campus dorm or apartment) and "C" permits (allow parking in C lots). $1,116/year for "A" permits (allow parking in any lot). Both A & C permits are available to commuters (students not living on campus). Carpool and vanpool permits are also available to eligible persons. For more information call the Parking and Transportation Office at 723-9362 or visit their web site, http://transportation.stanford.edu/.

Additional automobile resources include:

- The Dept. of Motor Vehicles in Santa Clara: 3665 Flora Vista Ave, 800-777-0133; and in Redwood City: 300 Brewster, 800-777-0133. It is recommended that you call in advance to set up an appointment.
- California State Automobile Association: 430 Forest Ave., Palo Alto, 650-321-0470.

Bicycles

The California Vehicle Code requires registration of bicycles to aid in identification and recovery if stolen. The Campus Bike Shop at Tresidder Union registers bicycles. Call 650-723-9300 or visit their website at http://campusbikeshop.com/ for information. You can also register your bicycle at the Parking & Transportation Office at 340 Bonair Siding. Engravers are available at the Police Station to engrave a license number or Stanford student identification number on bicycle frames. Stolen bicycles should be reported to the Police Station (650-723-9633).

Bicyclists must follow the same rules of the road as automobile drivers, not pedestrians. Palo Alto and other nearby cities have established a network of bike lanes and paths marked with signs and painted lines to make biking safer. Helmets are recommended but not required. Please note that bicycles are not permitted within the Beckman Center.

Marguerite Shuttle

The Marguerite is the main campus public transport and is free. It operates Monday through Friday all year except on University holidays. Shuttles run to various locations around campus, Palo Alto, SLAC, Menlo Park, and Mountain View. Maps and time schedules are available at https://transportation.stanford.edu/marguerite.

Airport Transportation

The Super Shuttle (415-558-8500, www.supershuttle.com) and Bayporter Express (415-467-1800, www.bayporter.com) are van services that will bring you and your baggage directly to the Stanford campus from SFO, SJC, or OAK. Super Shuttle discounts of $6 can be obtained using Stanford's Student Discount code of T9X58 (see link to information on student discount at https://transportation.stanford.edu/transit/community-shuttles). Both shuttles operate on a regular basis during the day and early evening. Call for more information and reservations. You also may dial 7-0901 on a white airport courtesy phone for pick-up locations. The trip from San Francisco to Stanford takes approximately 40 minutes and from San Jose to Stanford about 30 minutes.

SamTrans bus number KX leaves SFO every half hour to hour, between 6:00 AM and 11:00 PM. Only one piece of small carry-on luggage is allowed on the bus. The ride takes about one hour and the closest bus stop is the Stanford Shopping Center. Schedule details can be found at: http://www.samtrans.com/schedules.html and http://transit.511.org/schedules/index.aspx#m1=S&m2=bus&routeid=7916&cid=SM.
From the Shopping Center you may take a taxi a short distance to campus, the Marguerite during operating hours, or take the Santa Clara County bus number 35 to campus. Call 1-800-660-4287 for Santa Clara County bus times and route lines.

There are also other taxi, limousine and van services to and from the airports but they are more expensive.

Health and Safety

Stanford University's health and safety mission is to provide a safe and healthy environment for faculty, students, and staff, protect the University resources against losses arising from various types of occurrences like fires and explosions, and to assure compliance with federal, state and local health, safety and environmental regulations. The University Environmental Health and Safety Office (https://ehs.stanford.edu/) manages health and safety programs for the Medical School such as:

- Health Physics (Radiation Safety)
- Biosafety
- Industrial Hygiene & Fire Safety
- Chemical Safety

Each person working in a lab is required to be trained in the specific hazards of his or her job. Laboratory safety is a component of the orientation to a new lab. It is the Principal Investigator, the Research Associate/Assistant and the departmental Lab Manager's responsibility to provide information and training about lab equipment, procedures and chemicals. To assist, Environmental Health and Safety conducts a course in health physics. New students need to complete the following before they can handle radioactivity:

- Statement of Training Experience
- Take a class and a test (or just a test depending on experience)
- Film badge request

The Medical School has its own Health and Safety Program Office (see http://med.stanford.edu/medfacilities.html). The School's program provides the Lab Manager with safety information and regulatory compliance strategies. The office assists individuals and groups in resolving safety problems. Safety resources include:

- Environmental Health & Safety Office (24 hrs), 3-0448
- Health Physics, 3-3201
- Jessica Metzger, Biochemistry Lab Manager, 3-6303
- Jodee Jenkerson, Biochemistry Lab Services Coordinator, 3-6301

The School's Health and Safety Program and University EH&S also have many references and video tapes you can borrow. Each lab should have a copy of the Radiation Safety Manual, Stanford Biohazardous Materials Guidelines, the Beckman Laboratory Safety Manual, Stanford Safety Manual, and the Department of Biochemistry Guidebook.

Facilities

The Department of Biochemistry, housed on the fourth floor of the Beckman Center, is part of the Medical Center complex. Most laboratory space and equipment is shared and members of different laboratory groups are intermingled. This is a popular and efficient way to promote collaboration and intellectual interaction.

Facilities include numerous state-of-the art microscope imaging units, darkrooms, computer stations, glassware and media preparation rooms, a conference room and a library. The Beckman Center houses a Protein and Nucleic Acid (PAN) core facility equipped for the synthesis and characterization of macromolecules. The Fluorescence Activated Cell Sorter Facility is located on the ground floor along with Munzer Auditorium, PAN Facility, Cell Sciences Imagining Facility and the cafeteria.

Stockroom

A stockroom with common lab supplies is available in Room B432. All ordering of supplies and small equipment is handled through the stockroom.

Glassware Facilities

The glassware facilities are located in room B431. The staff in this facility are responsible for picking up, washing, wrapping and sterilizing the department's glassware.

Computer Resources

Stanford University (http://www.stanford.edu) enjoys one of the most extensive and varied computing environments of any campus in the country. The Stanford University Network, SUNet connects over 20,000 mainframes, microcomputers and advanced workstations with the Internet.

The BioInformatics Resource in the Beckman Center provides both SUN SparcServers for analysis of biological data and sequences and Silicon Graphics Servers for molecular modeling (http://cmgm.stanford.edu/facilities/br/). The resource also provides connection to the Internet and World-Wide-Web, e-mail, file and printing services. Every desk in the Beckman Center is wired for high-speed ethernet connection to SUNet. To set up your own computer, call 650-725-8000 and self register. The network allows each computer to access University and Medical School card catalogs, Medline, bookstore and a wide variety of other information resources.

Administrative/Social

Daily Department Refreshments
In order to encourage and facilitate interaction among the various labs, the department provides cookies and fruit at 4 PM in the 4th floor lobby. Everyone is encouraged to attend. Department socials are sponsored by labs and held about every three weeks, Friday's at 5 PM, in the lobby or on the LKSC lawn.

Supply Room

There's a supply room on the fourth floor available for everyone's use. It includes microwave ovens, a refrigerator, water cooler and photocopier. The room is regularly stocked with office supplies.

Department Mailboxes

Department mailboxes are arranged in the lobby across from the business office (room B400). They are arranged by lab group. Please check your mailbox regularly.

Mail moves between departments and offices at Stanford by interdepartmental (ID) mail. All ID mail should include the four digit Stanford mail code. Biochemistry is mail code 5307. There is a complete list of mail codes in the Stanford Directory. Stanford mail codes are the same as ZIP+4 codes used by the U.S. Postal Service.

Beckman Bistro

The Bistro is located on the ground level of the Beckman Building and is open from 7:30 AM to 3:00 PM Monday through Friday. Breakfast and lunch selections are served as well as a large variety of snack items. Seating is available both indoors and outdoors. The adjacent CCSR building has a café that serves breakfast and lunch from 7:30 AM to 5 PM, Monday-Friday, and the LKSC building has the Med Café open 7:00 AM to 7:00 PM.

Card Key Security System

A card key security system has been installed in the Beckman Center and other external buildings within the Medical Center. The Beckman Center has six ground floor doors plus the RAF tunnel door keyed. These doors are also equipped with closed circuit cameras. There is a telephone outside the main front doors to accommodate visitors without card keys. No access card is needed between 7 AM - 7 PM, Monday through Friday (not including holidays). See Jodee Jenkerson in Room B432 to obtain a card key as well as keys to the lab and shared rooms.

Department Library

The department library is located in B402 and is used for study, seminars, and group meetings. Audiovisual equipment is available for use in the library. To reserve the room, contact your advisor's AA or the Biochemistry Office Assistant.

Conference Room

The department's conference room is located in B475. It is used for group meetings and study. Audiovisual equipment is available for use in the room. To reserve the room, contact your advisor's AA or the Biochemistry Office Assistant.

Bulletin Boards

Bulletin boards located throughout the department display departmental and University information and announcements as well as job opportunities. Upcoming seminars are also displayed on a weekly basis outside B400 and can also be checked via the computer network.

Post Office

The post office at Stanford is a branch of the Palo Alto U.S. Postal Service and is located at 531 Lasuen Mall. The hours are 9-5, Monday-Friday. Post Office boxes are available for annual or semi-annual rental, in a variety of sizes. The zip code for post office boxes at the Stanford University branch is 94309. The ZIP code for all other addresses on campus is 94305. The ZIP code for the Biochemistry Department is 94305-5307.

Banking

The Wells Fargo Bank in Tresidder Memorial Union and the Stanford Federal Credit Union at Tresidder and on Pampas Lane are conveniently located on campus. You can use your student identification card in tandem with Wells Fargo Bank for ATM services. Automatic Teller Machines for Bank of America, Stanford Federal Credit Union, and Wells Fargo Bank are on the second floor of Tresidder and near the Hospital Emergency entrance.

The Li Ka Shing Center for Learning and Knowledge (LKSC)

The LKSC is located next door to Beckman. The fourth floor houses student only facilities, including a fitness center, entertainment area, kitchenette, lounge, variety of study areas including open and banquette seating, computer cluster, soft seating, small group study rooms, project rehearsal area, and traditional quiet reading room. Key card access is required and is limited to students in School of Medicine programs. For information on access and the fitness center waiver form, please see http://biosciences.stanford.edu/current/incoming/id-cards.html.

Tresidder Memorial Union

Tresidder Memorial Union is a center of community activity on the Stanford campus. It is located at White Plaza and houses food services; meeting rooms; two pleasant patios; a campus information center; the American Express Travel service; a ticket office for campus and Bay Area events (including BASS); banking services including automatic tellers for Stanford Federal Credit Union and Bank of America, a Wells Fargo branch office with express stops and walk-up windows, an office for account handling and loan applications; Pulse, the University Copy Center; a recreation center offering Stairmasters, stationary bikes, nautilus equipment, free weights; and a hairstyling shop. Tresidder Express carries groceries, magazines and sundries. TMU is also the home of the Associated Students of Stanford University, and Student Organization Services.

Bechtel International Center

Bechtel International Center is located at 584 Capistrano Way (https://bechtel.stanford.edu/). Staff at the Bechtel International Center provide support not just to international students but also to their spouses and to American students. Informal English classes, English conversation practice and language exchanges are among the many programs and services offered to students and their spouses. Counseling on immigration concerns, intercultural adjustment and administrative support for visa processing (in liaison with departments and other campus offices) are also part of the I-Center's service to international students. The I-Center is also the campus administrative office for awards enabling American students to study and conduct research overseas.

Stanford Bookstore

The Stanford Bookstore was incorporated as a nonprofit cooperative in 1987. The main branch is located at White Plaza. New and used textbooks are shelved by courses under the school or department. MICRODISC handles computer hardware and software needs. Also sold are medical, technical, and general books, paperbacks, clothing, souvenirs, stationery, supplies, art prints, and gifts; and there is a photocopying service. Other branches around campus and at the Shopping Center are listed at http://visit.stanford.edu/activities/shopping.html.

Lane Medical Library

Lane Medical Library is in the Medical Center and online at http://lane.stanford.edu/index.html. Services include general reference, in-depth consulting in all aspects of literature research, journal article file management, or any other information access/management needs (e.g., database design); training programs in bibliographic database searching (e.g. Medline), microcomputer/telecommunication based information access support, and training in general library skills.

Lane Medical Library's research collections cover clinical medicine and its specialties, basic sciences, public health, nursing and related fields. With over 3,000 journal titles and approximately 300,000 volumes, the collections rank among the best in the West. Access to bibliographic information was greatly improved with the introduction of Lane's Online Information System (LOIS). Since it is an integrated system, patrons can see if a title is on the shelf, if it is checked out, and when it is due back. LOIS can be accessed 24 hours a day from labs, wards, offices and homes. Access to journal article information is available through online databases of ovid, mdconsult, pubmed, lane catalog, shine, e-journals (http://lane.stanford.edu/biomed-resources/ej.html) as well as at SearchWorks (https://searchworks.stanford.edu/), Stanford's online library database. A list of Stanford libraries can be accessed at: http://library.stanford.edu/.

ANNUAL EVENTS

Departmental Research Conference

The Department holds its annual scientific research conference in October. All laboratory groups present talks or posters on current research, and attendance is mandatory. This year's conference is scheduled for October 4-6, 2017 at Chaminade Conference Center in Santa Cruz, CA.

RESOURCES

- Biochemistry Department - http://biochemistry.stanford.edu/
- Biochemistry Department Calendar of Events – http://biochemistry.stanford.edu/events/
- Stanford University Bulletin: Courses and Degrees - http://exploredegrees.stanford.edu/
- Stanford Directory - http://stanfordwho.stanford.edu/
- Graduate Academic Policies and Procedures - http://web.stanford.edu/group/gap/index.html
- Vice Provost for Graduate Education - https://vpge.stanford.edu/
- Presentation Resources (requires SUNet ID entry) - http://med.stanford.edu/biochemistry/education/resources/
- Oral Communications Program – https://undergrad.stanford.edu/programs/oral-communication-program
- Stanford Report - http://news.stanford.edu/stanford-report/

AFFIRMATIVE ACTION

The department is committed to increasing representation of women and members of minority groups in its graduate and postdoctoral training programs and particularly encourages applications from such candidates.

CLUBS & ORGANIZATIONS

Stanford Biosciences Student Association (SBSA)
http://sbsa.stanford.edu/

Biomedical Association for the Interest of Minority Students (BioAIMS)
http://bioaims.stanford.edu/

Graduate Student Council
http://gsc.stanford.edu/

Associated Students of Stanford University
http://assu.stanford.edu/

Student Activities and Leadership
https://sal.stanford.edu/

Academic Resources
http://www.stanford.edu/gateways/students.html

Administrative and Lab Services Telephone and E-mail Directory

(Unless noted, staff members are in B400)

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Appendix

Control of Spatial and Temporal Organization of Myosin during Cytokinesis

Sara Ocon, First Proposal, Dept of Biochemistry

1. SPECIFIC AIMS

The long-term goal of this project is to understand how myosin II is able to be spatially organized in a cell-cycle and condition-specific manner. In particular, this project seeks to identify extragenic factors responsible for localization of myosin at the cleavage furrow during cytokinesis. The identification of these factors and the characterization of their roles will help distinguish between current models of myosin II localization control or establish how elements of these models may work together.

A. I will use a genetic screen to find factors that help to localize and/or assemble myosin II at the cleavage furrow in *Dictyostelium discoideum*.

1. I will use cDNA complementation to define suppressors of R1880P myosin, a mutant defective in thick filament assembly.

B. I will characterize the genetic interactions between the suppressors and myosin II.

1. I will test for bypass suppression by expressing the suppressors in myosin-II-null *Dictyostelium* cells.
2. I will express the suppressors in wild-type *Dictyostelium* cells to determine the effect of their overexpression on wild-type myosin II.
3. I will express the suppressors in a strain of *Dictyostelium* that carries 3xAsp myosin II, a different myosin II defective in assembly and localization.
4. I will knockout the suppressor genes by homologous recombination.

C. I will characterize the cell biology of these factors to better understand the role they play in myosin II assembly or localization.

1. I will use fluorescence microscopy to determine the localization pattern of CFP-tagged suppressors.
2. I will determine the localization pattern of YFP-tagged myosin II in cells expressing the suppressors.
3. I will use multicolor fluorescence microscopy to determine the spatial and temporal organization of the CFP-suppressors and YFP-myosin co-expressed in *Dictyostelium*.

D. I will begin biochemical characterization of the suppressors.

1. I will determine if the suppressors are found in the triton-insoluble cytoskeleton of *Dictyostelium* cells.
2. I will determine if expression of the suppressors affects the amount of myosin II found in the triton-insoluble cytoskeleton of *Dictyostelium*.
3. I will purify suppressors that appear to localize with myosin II and test them for direct binding to purified myosin II.
4. I will determine the effect of purified suppressors on *in vitro* myosin II filament assembly.

2. BACKGROUND AND SIGNIFICANCE

Nonmuscle myosin II is used by cells to aid in locomotion, cytokinesis, and in determination of cell shape[1-3]. The ability of nonmuscle myosin II (hereafter referred to as myosin) to perform these functions depends critically on its ability to localize to different places in both a cell cycle and condition-specific manner[4, 5]. For instance, myosin is known to accumulate in the cleavage furrow of cells undergoing cytokinesis where it is believed to interact with actin filaments to help generate the contractile force necessary to cleave the cell (Figure 1A)[6, 7]. In addition, in *Dictyostelium discoideum* myosin translocates to the posterior cortex of cAMP-stimulated chemotaxing cells where it is believed to be

involved in locomotion either by generating contractile force at the back of the cell or by aiding in detachment of the cell (Figure 1B)[7, 8]. The importance of myosin in these processes has been clearly demonstrated in *Dictyostelium* myosin heavy chain null cells (*mhcA*-). These cells exhibit impaired chemotaxis and cannot undergo cytokinesis in suspension culture, although they can divide on a substrate[1]. *D. discoideum* is a cellular slime mold that aggregates to form fruiting bodies upon starvation. However, *mhcA*- cells cannot develop past the mound stage (Figure 2)[2, 9].

Both the function and proper localization of myosin are dependent on its ability to form bipolar thick filaments[4, 5]. *Dictyostelium* myosin is a hexameric protein consisting of two heavy chains and two pairs of light chains. The heavy chains contain N-terminal globular actin-binding motor domains and C-terminal α-helical domains that entwine to form long coiled-coil tails (Figure 3). Two myosin tails associate to form parallel dimmers, and dimers associate to form antiparallel tetramers. Finally, the rapid addition of parallel dimers to the tetramers completes the formation of mature bipolar thick filaments[10]. A cold-sensitive myosin mutant with an arginine to proline mutation at amino acid 1880 in the coiled–coil tail was shown to be assembly incompetent at low temperature[11]. *Dictyostelium* cells only expressing R1880P myosin are unable to divide in suspension culture or develop past the finger-like stage at the non-permissive temperature of 13 °C (Figure 2)[11]. Thus, thick filament formation is vital for myosin function in the cell. Thick filament formation is phosphor-ylation state dependent. Phosphorylation of three key threonine residues, T1823, T1833, and T2029, on the heavy chain tail disrupts filament formation *in vitro* and *in vivo*[12-14]. Four myosin heavy chain kinases, MHCK-A, B, C and PKC, have been characterized[15]. Myosin phosphatases have been purified from *Dictyostelium* cell extracts, but, to date, no molecular characterization is available of a phosphatase known to be biologically significant for myosin dephosphorylation[16-18]. The question of how thick filament formation and localization are related has been addressed by introducing mutant myosins into *mhcA- Dictyostelium* cells. First, myosin in which threonines 1823, 1833, and 2029 were replaced with alanines (3xAla myosin) was introduced into cells. This myosin cannot be phosphorylated, and thus is always assembled into thick filaments[4]. Myosin in these cells is correctly localized to the cleavage furrow during cytokinesis and to the posterior cortex of chemotaxing cells[14]. However, 3xAla myosin shows greater cortical localization and cells expressing this mutant myosin both move and divide more slowly than wild type cells[4, 14]. Thus, phosphorylation seems to be important for correct recycling of myosin. Myosin in which threonines 1823, 1833, and 2029 were mutated to aspartic acids (3xAsp) to mimic the phosphorylated state was also introduced into *mhcA- Dictyostelium* cells. This myosin acts like constitutively phosphorylated myosin and is almost completely unable to assemble into filaments. Cells containing 3xAsp myosin have a similar phenotype to *mhcA*- cells in that they cannot divide in suspension culture nor develop correctly. Fluorescence localization studies have shown that 3xAsp myosin remains soluble in the cytoplasm and is not localized to the cortex nor the cleavage furrow of

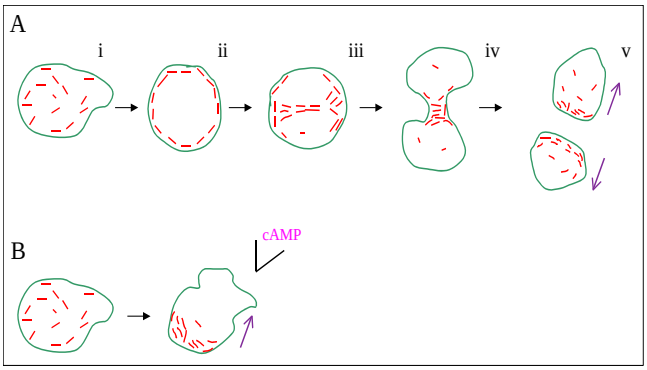

Figure 1. Schematic of Myosin II localization in *Dictyostelium discoideum*. Myosin filaments are represented as red rods. The direction of movement of cells is represented by purple arrows. **A**. Localization of myosin during cytokinesis. **i**, In interphase cells myosin is diffuse throughout the cell. **ii**, During metaphase myosin becomes localized to the general cortex. **iii**, At anaphase myosin is clearly localized to the cell equator. **iv**, At telophase myosin is enriched at the cleavage furrow. **v**, In the resulting daughter cells myosin is localized at the posterior of the cells as they migrate away from one another. **B**. Localization of myosin in chemotactic cells towards cAMP. Myosin is clearly localized at the posterior of the moving cells. (Figure adapted from Yumura, 1997).

dividing cells[4, 14]. Thus, correct localization and activity of myosin depends critically on its ability to form filaments. Therefore, a complete understanding of myosin localization will necessarily involve understanding the mechanisms and perhaps location-specific nature of myosin phosphorylation control.

The molecular mechanisms of how myosin is localized to the exact midpoint of the cell during cytokinesis in *Dictyostelium* are still very much a mystery. It is known that the location of the mitotic spindle is crucial to determining where the cleavage furrow will occur,[19] however, how the spindle encodes this information is unknown. One model of myosin translocation involves cortical flow of all equatorial proteins to the furrow as a gradient of tension is produced from pole to equator. The collection of cell surface receptors at the furrow provided some of the earliest evidence for this theory[20]. In addition, microinjected actin filaments and fluorescent myosin filaments have been visualized moving toward the furrow[21, 22]. More recently, atomic force microscopy has revealed that indeed there is a stiffness gradient from pole to equator beginning in late anaphase in potorous triactylis kidney cells[23]. In the classic cortical flow model myosin helps create this tension by generating contractile force as it tugs on actin filaments. However, myosin null *Dictyostelium* cells still accumulate other proteins at the furrow suggesting there may be another mechanism. This tension gradient could be generated by

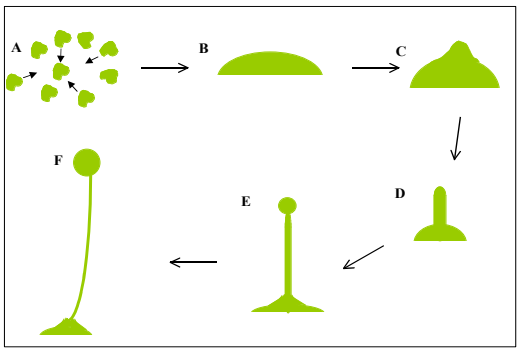

Figure 2. Schematic of the developmental process in *Dictyostelium discoideum*. **A.** Starving cells begin to aggregate by chemotaxis towards secreted cAMP. **B**. The cells first form a mound. *MhcA*- cells cannot progress past this point. **C.** Cells have begun to migrate up to form a stalk. **D**. In this finger-like stage the stalk is clearly visible. Cells expressing only R1880P myosin do not proceed beyond this point at low temperature. **E**. In this immature fruiting body the spore-filled head has formed. **F**. In the mature fruiting body the stalk is much longer and the spore-filled head is larger.

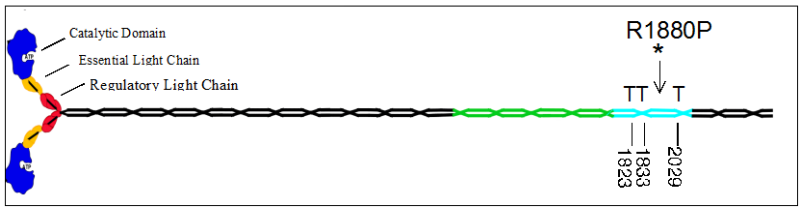

Figure 3. Schematic Drawing of *Dictyostelium discoideum* Myosin-II. The catalytic actin-binding domain, essential light chains, and regulatory light chains are shown in blue, yellow, and red respectively. The green section of the coiled-coil tail represents the amino acids necessary and sufficient for thick filament assembly. The three Ts mark the positions of threonines whose phophorylation inhibits filament formation. The asterisk marks the position of arginine 1880. Mutation of R1880 to proline causes a cold-sensitive assembly defect.

other myosin isoforms or a completely unrelated mechanism such as actin network rearrangements[24]. It has been suggested that myosin is simply translocated passively to the furrow bound to flowing actin filaments in the cortex[25]. However, Zang and Spudich (1998) showed that GFP-myosin tail chimeras completely lacking the actin-binding domain are localized normally to the cleavage furrow[26]. Thus, only the tail and its ability to form filaments are necessary for the localization of myosin to the cleavage furrow.

These data have led to a missing ligand model. In this model some currently unknown protein capable of binding only thick filaments is responsible for the correct translocation of myosin to the furrow[24, 27, 28]. The ligand is predicted to be filament specific because 3xAsp myosin does not localize to the furrow. The ligand could be an actin binding protein that links myosin to the cortical actin as it flows toward the cell equator or simply a protein that itself is localized to the furrow and has a high affinity for filamentous myosin. Indeed, Schroeder and Otto (1988) showed that myosin remained at the cleavage furrow of sea urchin eggs even when the actin was depolymerized with gelsolin[29]. However, data from

Yumura (2001) showing that the on and off rates of myosin turnover at the furrow are nearly identical argues against the existence of a high affinity myosin binding protein continuously located at the furrow[27].

Studies with 3xAla and 3xAsp myosin have clearly shown that localization to the cortex requires myosin to be assembled into thick filaments. Thus, it has been suggested that phosphorylation control by MHCKs and myosin phosphatases is responsible for the general translocation of myosin into and out of the cortex, but that cortical flow is responsible for determining the specific localization within the cortex[24]. However, recent evidence suggests that the picture is more complicated. Fluorescence recovery after photobleaching studies have shown that individual myosin monomers within cortical filaments in the cleavage furrow exchange rapidly with soluble monomers in the cytoplasm, suggesting myosin reaches the furrow directly through the cytoplasm. However, in cells containing only 3xAla myosin, which cannot disassemble, equatorial fluorescence bleached in early telophase before formation of the furrow was replaced by the unbleached flanking cortical myosin[27]. Thus, cortical flow does seem to play a role in the initial localization of myosin to the furrow. In addition, the various MHCKs have been shown to have specific and unique localization patterns, and thus phosphorylation control may not only determine general cytoplasmic/coritcal translocation, but may also help determine where in the cortex myosin is localized[30, 31]. In summary, the various models of cortical flow, phosphorylation control, and perhaps even ligand specific binding most likely work together to affect the complex changes in myosin localization over the lifetime of the cell.

3. RESEARCH DESIGN AND METHODS

A. I will use a genetic screen to find factors that help to localize and/or assemble myosin II at the cleavage furrow in *Dictyostelium discoideum*.

I will find suppressors of the R1880P myosin mutation using a *Dictyostelium* complementation/mulitcopy suppression library developed in our lab. Until recently no such library existed, and such genetics could not be performed in *Dictyostelium*. However, Robinson and Spudich (2000) were able to build a library by cloning cDNAs created from both substrate-attached and suspension-grown cultures. Its potential was demonstrated when the library was used to find four suppressors of a *cortexillin I-* phenotype[32]. However, library complementation/suppression still remains a relatively unexplored technology in *Dictyostelium*. I will introduce this library into *Dictyostelium* cells that have had the *myosin R1880P* gene integrated into an *mhcA-* background. These cells have a strong cytokinesis defect when grown at 1ow temperature presumably due to R1880P myosin's reduced ability to assemble into thick filaments. However, at the permissive temperature of 24 ^{o}C myosin R1880P is able to assemble at about 50% wild type levels, and cells expressing it can grow at wild-type rates and develop full fruiting bodies[11]. Thus, it is clear that the ability of myosin R1880P to assemble into filaments is easily affected, and therefore it is a good candidate for suppression.

Cells transformed with the library will be selected for growth in suspension at 13 ^{o}C. In addition, before selection in suspension and periodically during selection, samples of the cells will be removed and screened for the ability to develop into full fruiting bodies. Plasmids will be isolated from those strains that can grow in suspension at 13 ^{o}C and/or develop fully. The recovered plasmids will then be retransformed into the R1880P myosin expressing cell lines to ensure that the suppression can be recapitulated and is truly due to the genes present on the plasmids. Sequencing the plasmids from the selected cell lines will determine which genes can suppress the myosin R1880P phenotype.

B. I will characterize the genetic interactions between the suppressors and myosin II.

1. I will test for bypass suppression by expressing the suppressors in myosin-II-null *Dictyostelium* cells.

Myosin null *Dictyostelium* cells expressing the suppressors will be assayed for growth in suspension and development on bacterial lawns. Because *Dictyostelium* myosin null cells can undergo cytokinesis on an attached substrate, there certainly must be other proteins capable of maintaining a stiffness gradient from

the poles to the equator significant enough to result in contraction at the midzone during cytokinesis. However, to divide in suspension without myosin may be a formidable task, and finding strains that can do so may be difficult.

2. I will express the suppressors in wild-type *Dictyostelium* cells to determine the effect of their overexpression on wild-type myosin II.

The cytokinetic and developmental phenotypes of these cells may provide clues to the biological role of the suppressors. For instance, a 3xAla phenotype could suggest that the suppressor is indeed involved in promoting filament assembly.

3. I will express the suppressors in a strain of *Dictyostelium* that carries 3xAsp myosin, a different myosin II defective in assembly and localization.

By testing for rescue of myosin function, it can be determined if suppression is specific for the R1880P mutation. In addition, the 3xAsp cytokinetic and developmental defects are much more severe. Thus, rescue of this mutation would suggest that the suppressor radically affects the equilibrium between monomers and filaments.

4. I will knockout the suppressor genes by homologous recombination.

For suppressors that give interesting phenotypes I will create knockout strains in *Dictyostelium* to test the effect of loss of function of the suppressors. Later cell biological and biochemical experiments can then be performed in these strains.

C. I will characterize the cell biology of these factors to better understand the role they play in myosin II assembly or localization.

1. I will use fluorescence microscopy to determine the localization pattern of CFP-tagged suppressors.

The library vector was designed to allow *gfp* to be subcloned into either the 5' or 3' end of the inserted genes[32]. I will modify the *gfp* insert to *cfp*. If, for instance, one of the suppressors is the "missing ligand" that binds myosin filaments, a CFP fusion should be localized to the cleavage furrow during cytokinesis. The localization will be performed in R1880P myosin expressing cells as well as in myosin null cells in order to reveal if the localization of the suppressors is myosin dependent.

2. I will determine the localization pattern of YFP-tagged myosin II in cells expressing the suppressors.

The localization pattern of myosin R1880P will be tested in the presence and absence of the suppressors. To do this, the mutant myosin will be subcloned into a YFP-fusion vector, and fluorescence microscopy will be used to determine the localization pattern of the mutant myosin in *Dictyostelium mhcA*- cells. If the suppressors do indeed help to assemble or localize myosin, then the YFP-myosin R1880P should be translocated more readily to the cortex and to the cleavage furrow in cell lines also expressing the suppressors. I will also test for myosin localization in the suppressor knockout strains to determine if the localization of myosin depends on the suppressors.

3. I will use multicolor fluorescence microscopy to determine the spatial and temporal organization of the CFP-suppressors and YFP-myosin co-expressed in the same cell.

The use of YFP-myosin R1880P will allow for co-localization studies of myosin and the CFP-suppressors. This can be done in a time-resolved fashion to determine if suppressors that arrive at the cleavage furrow do so before, after, or simultaneously with myosin.

D. I will begin biochemical characterization of the suppressors.

1. I will determine if the suppressors are found in the triton-insoluble cytoskeleton of *Dictyostelium* cells.

To assay for the presence of the suppressors in the cell cortex, an HA tag can be fused to the suppressor genes and expressed in *Dictyostelium*. The cells will then be lysed in the presence of triton X-100 which solublizes most proteins except those bound to the cortex. The cell extracts will then be centrifuged and western blots will be performed with anti-HA antibodies to determine if the suppressor proteins are pulled down into the pellet.

2. I will determine if expression of the suppressors affects the amount of myosin II found in the triton insoluble cytoskeleton of *Dictyostelium.*
If a suppressor does indeed promote filament assembly and cortical localization of R1880P myosin, then expression of the suppressor should result in an increase in the percentage of R1880P myosin found in the triton-insoluble cytoskeleton. In addition, a suppressor that promotes filament assembly and localization might be expected to result in over-assembly of wild type myosin as the 3xAla mutation does. However the effect of over-assembly on the phenotype of cells is very subtle and can be hard to determine by just looking at growth rates and development. However, 80% of 3xAla myosin is cortically associated as compared to only 17% of wild type myosin[4]. Thus, measuring the amount of cortical myosin in wild type cells in the presence and absence of the suppressors may reveal over-assembly.

3. I will purify suppressors that appear to localize with myosin II and test them for direct binding to purified myosin II.
Presence in the cortex and/or co-localization with myosin by fluorescence may be good indicators of suppressors that could potentially interact directly with myosin. Therefore, I will choose the suppressors that appear to be good candidates and purify these proteins from *Dictyostelium*. Co-sedimentation with filamentous myosin can be used as an easy assay for direct myosin binding.

4. I will determine the effect of purified suppressors on *in vitro* myosin II filament assembly.
In addition, the effect of the suppressor proteins on the ability of myosin to assemble can be determined by assaying for sedimentation of myosin filaments in the presence of varying amounts of the suppressor proteins.

In conclusion, the above are the immediate experiments necessary to begin the characterization of genes found in the suppression screen. However, the sequence of the genes found may reveal clues to their possible functions, and this would necessarily determine the kinds of experiments performed. For instance, if a suppressor gene is found that has high homology to a phosphatase, then clearly its ability to remove phosphates from myosin will immediately be tested. An international effort to sequence the *Dictyostelium* genome is currently underway and should be finished within a couple of years. In addition, the *Dictyostelium* cDNA Project has created, sequenced, and characterized the transcriptional profile of over 6,000 cDNAs from cells at various stages of development[33, 34]. Thus, there is a high probability that information will be available for the suppressor genes found in this study.

4. PROGRESS REPORT

We have created *Dictyostelium* cell lines with the *myosin II r1880p* gene under control of the *actin 15* promoter integrated into an *mhcA*- background. These cell lines are only able to develop short fingers upon starvation even at room temperature. Preliminary data suggest they cannot grow in suspension at 13 ^{o}C and grow with doubling times about 1.5 times longer than wild type cells at 22 ^{o}C. The *myosin II* gene has been amplified and sequenced from these lines and does contain the R1880P mutation. Two of these cell lines express myosin at wild-type levels as determined by western blot analysis. Currently, I am optimizing conditions for transformation of these two lines with the library vector.
In addition, I have performed further work verifying the validity of suppressors found in a genetic screen using the cDNA library developed in our lab. Robinson and Spudich (2000) genetically and cell biologically characterized a *cortexillin I* loss of function suppressor named *dynacortin*. Their genetic and fluorescence localization work suggested the suppressor might interact with actin to increase the global stiffness of the cell. I was able to follow up on this work and characterize dynacortin biochemically. I was able to show that the protein binds and bundles actin filaments into complex arrays by sedimentation experiments and electron microscopy with purified dynacortin[35]. Certainly, this activity is also consistent with the genetic and cell biological data. It suggests that the experimental design of the present proposal, namely the isolation of suppressors using the cDNA library, followed by a genetic and cell biological characterization, and finally a biochemical characterization, is likely to yield significant results.

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