Montefiore

DEPARTMENT OF BIOCHEMISTRY

Departmental Guide for First Year Students

Departmental Graduate Student Policies, Guidelines & Procedures, and Faculty Research Interests

2019 – 2020

(Updated as of 8/2/19)

PREFACE

To Prospective Graduate Students in the Department of Biochemistry

Welcome to the Graduate Program in the Department of Biochemistry. This booklet introduces you to departmental policies and procedures that help you earn a Ph.D. degree in Biochemistry. The topics range from acceptance into the Department for thesis study to your defense of a Ph.D. thesis. Included are discussions of our graduate course requirements, faculty advisement, a list of courses led by members of the department and summaries of the research of our faculty.

This information will help to organize your graduate studies and plan your development as a research investigator. Once you have selected a thesis mentor, your advisory committee and thesis research mentor will become your principal guides along the road to your Ph.D. The Biochemistry office staff, current Biochemistry graduate students, and Biochemistry faculty are all available to help you and to make your tenure in the Department as productive and enjoyable as possible.

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I. *GENERAL INFORMATION*

A. *Einstein Graduate Program in the Biomedical Sciences:*

Officials and Office Personnel
Einstein Graduate Program Office: Room 201 Belfer Building

Dr. Victoria Freedman
Associate Dean for Graduate Studies in the Biomedical Sciences
Assistant Professor of Microbiology and Immunology
(718) 430-2872

Ms. Sheila Cleeton
Executive Director and Registrar
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Director, Medical Scientist Training Program
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Dr. Joan W. Berman
Senior Academic Advisor, Graduate Division
Professor of Pathology and Microbiology & Immunology
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B. *Department of Biochemistry*

Officials and Office Personnel: Room 308 Forchheimer Building

Dr. Steven Almo
Professor and Chairman of the Department of Biochemistry
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Ms. Leslie Goodwin
Administrator, Department of Biochemistry
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Ms. Patricia Corr
Secretary to the Chairman
(718) 430-2814

Departmental Office Staff, (718) 430-2814
Ms. Cheryl Bates, Ms. Celia James, Ms. Joanna Vega

II. *DEPARTMENTAL REQUIREMENTS*

Students who are granted a Ph.D. degree from the Department of Biochemistry have demonstrated their ability to (*i*) design, conduct and evaluate high quality, independent research and (*ii*) possess broad-based knowledge of the field of Biochemistry. Students are expected to demonstrate their acquisition of these skills principally in their thesis research and through graduate course performance, student journal club participation, seminar attendance and performance on the qualifying examination.

A. Research Requirement

The Ph.D. degree in Biochemistry is a research degree. Research training begins with laboratory research rotations during the 1st year of study and continues with the selection of a thesis research advisor and development of an original research project.

Students' typically rotate in three laboratories during their 1st year. Students who matriculate directly to the laboratory of a member of the department are required to rotate in at least two other laboratories in order to gain breadth in their research training. Students who matriculate with a M.S. or equivalent research experience and have selected a thesis laboratory may request of the departmental oversight committee exemption from laboratory rotations if the request is endorsed by their mentor and advisory committee.

Students should plan to complete the majority of their course work including required departmental courses during the first year. (The department recognizes that special circumstances may delay completion of the required courses.) Advanced courses appropriate to their thesis research may be taken in the second and subsequent years.

Once students join a thesis laboratory in the department, their advisory committee and the departmental oversight committee monitor their progress closely. Ph.D. thesis research involves meaningful, critical thinking and the execution of ideas in the laboratory through the use of the scientific method. Students are expected to embrace full-time research upon declaration of their thesis mentor following a twelve-month calendar. The qualifying exam is scheduled to take place during the latter half of the second year, allowing timely advancement to candidacy. Upon joining a thesis laboratory, it is expected that students' research continue in concert with their course work and examinations.

The research conducted by a Biochemistry Ph.D. candidate should be an original contribution to scientific knowledge. The quality of the candidate's research is expected to be equivalent to that found in reputable, refereed scientific journals. Research progress is documented by the reports of the student's advisory committee meetings and by the student's progress reports submitted before these meetings. Filing these reports with the Chairman of Biochemistry and the Chairperson of the department graduate training oversight committee is a departmental requirement. Reports of student advisory committees should discuss research progress and research.

B. Course Requirements for the Ph.D. Degree in Biochemistry

First year students must fulfill the general course requirements of the graduate division. While thesis research is necessarily highly focused, a broad foundation in chemistry and modern biology is required to carry out scholarly studies in biochemistry. A student's curriculum should provide breadth and fundamental information as well as specialized education in the chosen area of research. A complete list of the courses offered by the Graduate Division is maintained at <http://www.einstein.yu.edu/education/phd/graduate-curriculum/fall-course-block.aspx>. The Biochemistry faculty has identified three courses that all of our students should endeavor to complete during their 1st year of study. These courses are:

Required Courses for Ph.D. and M.D.-Ph.D. Students (taught annually):

(Block 1) Biochemistry – This course continues building the foundation in understanding of biological macromolecules, energetics, biochemical methods and enzymatic activity presented in undergraduate biochemistry courses.

(Block 2) Gene Expression: Beyond the Double Helix - This course covers the molecular mechanisms of biological information content of cellular processes such as transcription, translation, splicing and replication.

(Block 3) Human Metabolism: Regulation and Disease - This course explores the metabolic pathways relevant to human health and disease.

Students may request exemption from these courses if they have completed substantial graduate level courses on these topics during either their undergraduate or masters training. Students should contact the leader(s) of each course upon matriculation for a review of their placement request. The decision of a course leader on an exemption request is final.

First year students are encouraged to seek out faculty for advice on crafting an effective curriculum that will support their future studies in the disciplines of biochemistry, cell biology and biophysics studied by members of our department. Since a goal of the new graduate curriculum is to complete the majority of a student's didactic training during the first year, serious thought should be given about charting the optimal pathway through the available options remembering that educational breath as well as depth is important to future success. Students who have joined a thesis laboratory should discuss additional course work with their mentor and advisory committee members.

Students must pass the required courses to be in good academic standing with the department. Students who fail a course may take it one additional time; passage will restore a student's good academic standing. (Students should be aware that a grade of 'fail' is a permanent mark on their academic transcripts.) Lastly, students may not graduate with an incomplete on their academic record. Departmental students not in good standing are placed on academic probation. A written plan by the student's advisory committee for remediation will be reviewed by the departmental oversight committee who will convey to the student and his/her mentor what will be required for a return to good academic standing.

C. Biochemistry Student Journal Club and Works in Progress Seminars

Upon declaring the Department of Biochemistry for their thesis research, graduate students participate in the Journal Club (JC) and Works in Progress (WIP) held at noon on Fridays in the departmental seminar room. Attendance at the weekly meetings begins immediately. Presentations by the student begin at the start of the semester following declaration. This is typically the fall semester of the second year for students entering the department from the rotational pathway. Each student presents one journal club and one works in progress each calendar year. Students completing their thesis studies *may* be excused from the journal club presentation by the faculty advisor to the program. However, all students will present yearly works in progress until they successfully defend their thesis.

For both journal club and works-in-progress presentations, students are randomly assigned a faculty advisor for their presentation at the beginning of the semester. Journal club and works in progress presentation scheduling is at the discretion of the faculty advisor to the program as discussed below. A written record for each Journal Club and Works in Progress is filed with the departmental office. Forms for evaluation of the Journal Club and Works in Progress performance is in the Appendix. The criteria for the evaluations are discussed below. A detailed description of Journal Club and Works in Progress guidelines can be found in the Appendix.

Journal Club Guidelines and Procedures

A journal club presentation is an analysis of a recent scientific article chosen for its general interest and importance. The Journal Club requirement is met by (i) presentation of literature seminars in consultation with the assigned faculty member; and (ii) participation in the discussion during and following seminar presentations. Students are required to attend the Biochemistry Journal Club and Works in Progress during each semester they are enrolled in the Biochemistry Graduate Program. Rotational students are also encouraged to attend.

The student and faculty advisor will jointly select an article that is outside the scope of the student's thesis research. The assigned faculty advisor will mentor the student through a critical reading of the journal article and preparation of the student's presentation. The faculty advisor will meet with the student prior to the JC to provide guidance for the presentation. The faculty advisor along with a second faculty reader will be present during the presentation and provide the student with critical feedback on the effectiveness of the presentation. Each faculty member can serve only once per semester as a journal club advisor, but can be a reader for more than one presentation. A student's thesis advisor can neither advise nor be a reader for their journal club presentation. However, thesis advisors are expected to be present during their student's journal club presentations and provide critical, if informal, feedback. In addition, journal clubs will be evaluated by fellow students. The purpose of these evaluations is to help students develop the skill of public presentation.

At least three weeks before the presentation date, in consultation with the faculty advisor, the student must choose the article to be presented. The articles must be primary research papers, not review articles or short research communications. The selected article must be from an area

not directly related to the student's thesis research; students can take best advantage of the expertise of their faculty advisor by selecting a paper that the advisor can comment on with some authority. A pdf file of the selected article should be provided to the faculty advisor to the program or the current student organizer at least one week before the presentation date. If the student does not provide a copy of the article in time, he/she will be responsible for the article's distribution.

The faculty advisor will be available to guide the Journal club preparation process during the three weeks following article selection and prior to the presentation. In the event that the faculty advisor cannot fulfill his/her responsibility, the faculty reader can substitute as the advisor. The reader position will then be assumed by another faculty member in attendance at the presentation. The student should make every effort to give a practice talk before the presentation date. After the presentation, both the faculty advisor and reader will meet with the student to discuss the presentation. The journal club presentation should contain Introduction and background, Experimental methods, Results, Conclusions and Critique sections as described below. Students should aim for a 20 minute presentation in order to allow time for discussion during and at the end of the presentation. Careful consideration should be given to the time allocated to each of these sections in order to craft an effective, enjoyable and informative presentation.

- Introduction and Background: Informs the audience on both basic concepts and latest developments impacting the article that is being presented. The background should point out the questions answered by the article to be presented. In crafting their presentation, students should remember that many in the audience will be unfamiliar with the subject of the presented paper. It is very important to put the paper in context. Why is the work important?
- Experimental methods: Describes the experimental techniques used in the article. Common techniques can be described in a few phrases or sketches. Specialized or novel techniques should be explained in greater detail. It is often helpful to an audience to know why a particular technique and not another was used.
- Results: All data acquired in the experiments should be presented and described in great detail. The student should assume that the audience does not know the significance of the results. Do not simply recapitulate the results. Explain them!
- Conclusions: Describes the inferences drawn by the authors from the data obtained. The student should refer back to result slides as needed.
- Critique: This is the most important part of the journal club. The student will present his/her opinion regarding strengths and weaknesses of the article. The critique should be treated as a peer-reviewed publication process. Do you believe that this paper will have an impact on its field? If so, why?

Students may use Microsoft PowerPoint or other graphics display aids. However, fancy formatting and animation should only be used if warranted by the nature of the article. In preparing the presentation, the emphasis should be in reviewing the science, not on demonstrating computer graphics skill. Students should keep their slides simple, only presenting the information that they wish to convey to their audience.

Works in Progress

The works in progress presentation is prepared with the thesis mentor as advisor. Additionally, the faculty advisor for the WIP presentation will provide outside critique and guidance on the presentation. The student should arrange a meeting with the faculty advisor prior to the WIP. This presentation should contain Introduction and background, Experimental methods, Results and Conclusion sections.

- Introduction and Background: Informs the audience on “the big picture” and where your thesis project fits into this big picture. The background should point out what questions you hope to answer with your research.
- Experimental methods: Describes the experimental techniques that will be used in your research. Common techniques can be described in a few phrases or sketches. Specialized or novel techniques should be explained in greater detail.
- Results: Results are not required for a works in progress. Second year students often do not have results to present. In this instance the student should focus his/her presentation on the background and experimental methods to be used.

Each section can be as long as necessary, taking into account that the presentation should allow plenty of time for questions. Each student should aim for a 20 minute presentation to allow time for questions and discussion during and at the end of the presentation.

D. Student Advisory Committees

Upon declaration of a thesis laboratory within the department, an advisory committee must be formed by the student and mentor consisting of three additional faculty (two of whom must be members of the department). The role of the advisory committee is to advise the student during their Ph.D. research, nurture and evaluate their scholarly development and ultimately grant permission to defend the Ph.D. thesis. The faculty who comprise a student’s advisory committee can play a central role in graduate student training by providing additional areas of expertise and perspective to the conduct of a student’s thesis study.

The Department of Biochemistry requires that advisory committee meetings be held twice each year. Initially, a committee can provide guidance in crafting the thesis project, selection of courses and preparation for the qualifying examination. Subsequently, they can evaluate the laboratory work that constitutes the research component of the Ph.D. degree. The regular meetings of these committees and the filing of written reports of their content with the departmental are required for a student to remain in good departmental standing.

The departmental oversight committee will review the composition of a student’s advisory committee and must approve changes made in subsequent years. Both the mentor and the advisory committee must endorse a request to the departmental Chairman for a leave of absence. Any other changes in a student’s status will be reviewed by the same procedure. The

Chairman of the Department and Chairperson of the departmental student oversight committee are *ex officio* members of all advisory committees and may attend a meeting at their discretion.

Every student is responsible to schedule the two required advisory meetings. Additional meetings may be scheduled as needed as requested by the student, mentor or committee. Prior to a meeting, the student must distribute to the committee a short (1 - 2) page summary of academic and research progress since the last meeting. A synopsis of the meeting and committee recommendations will be summarized by the committee chairperson and communicated to the student and his/her mentor.

The student progress reports and the Committee reports on all meetings are included in the student's academic file. Copies of these reports are also distributed to the Chairman of the Department of Biochemistry and the Chairperson of the departmental oversight committee. The written reports of the required advisory committee meetings must be on file with the departmental office before the Ph.D. degree is granted. An advisory committee report form is included in the Appendix.

E. Research Seminars in Biochemistry

Students enrolled in the Graduate Program are required to attend the research seminars presented in the Seminar Programs of the Department of Biochemistry. Notices are posted on the Departmental bulletin board near the Departmental Office.

F. Qualifying Examination

The Graduate Division administers the qualifying examination for advancement to candidacy for the Ph.D. degree. Passage of the qualifying examination is required for continued study in the department.

G. Thesis Examination

The preparation and defense of the Ph.D. thesis in Biochemistry is a culmination of a student's independent laboratory research. During the course of thesis research, the advisory committee will assist the student and the thesis advisor in defining the nature and scope of the research project that will form the basis of the doctoral dissertation. The Graduate Division has established procedures for preparation of the thesis and its defense that can be found on the program's website. As per the Department of Biochemistry requirements, one member of the thesis committee should be an outside examiner.

Students must remain in residence until the thesis research has been completed to the satisfaction of the Ph.D. mentor and the advisory committee. A student must request a waiver of this requirement from the departmental oversight committee who will then make a recommendation to the Chairman of the Department for a final decision.

H. Time to Degree

The faculty of the department shares the national concern with the increasing time to achieve the Ph.D. degree. Our goal is to reverse this trend. Students typically complete their graduate

training 4 to 6 years from their entry into the graduate school. Written permission of the mentor, the student advisory committee, the departmental oversight committee and the Chairman of the Department of Biochemistry is required for study to continue beyond 6 calendar years. The Graduate School requires that students admitted to doctoral programs register each semester, unless a leave of absence has been applied for and granted. This requirement applies to students engaged in thesis research who are not enrolled in any courses.

III. ***Faculty and Research Interests***

Members of the Graduate Faculty in Biochemistry serve as thesis research advisors for Ph.D. research. The Graduate Faculty of Biochemistry are tenure-track faculty with a primary appointment in the Department of Biochemistry or with a secondary appointment in Biochemistry and a primary appointment in a clinical department that does not grant a Ph.D. degree (*e.g.*, Medicine). Permission of the Chairman of the Department of Biochemistry is required of faculty with a secondary appointment in the department to serve as a thesis research mentor. Emeritus Professors are not eligible as Ph.D. mentors of Biochemistry Graduate Students.

IV. ***Contents of On-Line Appendices for School wide policies.***

- Admissions Procedures for the PhD: http://einstein.yu.edu/education/phd/prospective-students/admissions.aspx and MD-PhD Programs: http://einstein.yu.edu/education/mstp/admissions/
- Journal Club and Works-In-Progress Seminar Guidelines and Procedures: http://einstein.yu.edu/docs/departments/biochemistry/guide-for-1st-year-students.pdf
- Thesis and Final Examination Guidelines and Procedures: http://einstein.yu.edu/education/phd/current-students/thesis.aspx
- Graduate Courses Taught by Biochemistry Faculty http://einstein.yu.edu/departments/biochemistry/graduate-studies/phd-courses.aspx

- Acknowledgement of Research Support – All publications, theses, reports, etc. resulting from research conducted during the candidate's degree program must include an appropriate acknowledgement of the sources of funding. Examples include research grants to the faculty sponsor, training grants, equipment grants for equipment essential to the research, core facilities, scholarship support provided by the school, and fellowships from external sources.

Graduate Faculty in Biochemistry
Statements of Research Interest

Name	Research Interest
Almo, Steven C.	*Structural Immunology & Functional Annotation*
Blanchard, John S.	*Chemical Mechanisms of Enzymatic Catalysis*
Brenowitz, Michael	*Cellular Regulation by Reversible Macromolecular Assembly and Association*
Bresnick, Anne	*Mechanisms of Cell Motility and Tumor Metastasis*
Callender, Robert	*Protein Structure & Dynamics: Enzymatic Catalysis & Protein Folding*
Charron, Maureen J.	*Molecular Basis of Metabolic Disease (Obesity & Diabetes)*
Cowburn, David	*Physical Biochemistry*
Gavathiotis, Evripidis	*Chemical and Structural Biology of Cell Death and Survival Signaling*
Lai, Jonathan	*Peptide and Protein Engineering*
Schramm, Vern L.	*Enzymatic Transition States and Logical Inhibitor Design*
Sidoli, Simone	Technology for chromatin biology
Shechter, David	*Chromatin and the Biochemistry of Epigenetic Information*
Steinman, Howard M.	*Acquisition of Virulence Phenotypes by Bacterial Pathogens*
Willis, Ian M.	*Gene Transcription, Regulation of Cell Growth and Metabolism*

Faculty with Secondary Appointments in Biochemistry

(For Research Interests, please see their respective departments.)

Backer, Jonathan M. Molecular Pharmacology	*Signaling by Phosphoinositide Kinases*
Fiser, Andras Systems & Computational Biology	*Bioinformatics and Computational Biology*
Hatcher, Victor B. Associate Dean for CME and Research Administration	*Nuclear Import & Export in Human Endothelial Cells*

DR. STEVEN ALMO

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Structural Immunology & Functional Annotation

For the past two decades, the major scientific focus of my laboratory has been high-throughput structure discovery and functional annotation. My laboratory has been directly involved in a number of large-scale programs involved in technology development and high-throughput applications. I am the Director of the Einstein Macromolecular Therapeutics Development Facility, which provides a wide array of proteins services to the Einstein community, and served as PI of the New York Structural Genomics Research Consortium (NYSGRC), one of the four large-scale high-throughput structure discovery centers supported by the NIGMS Protein Structure Initiative. I served as Director of the Protein Expression Core for the Northeast Biodefense Center (one of the NIAID-funded Regional Centers of Excellence) and was a major participant in the Immune Function Network, an NIGMS-funded program on the mechanistic dissection of innate and adaptive immunity. I also served as co-PI of the Enzyme Function Initiative, an NIGMS-supported Glue Grant focused on the development, implementation and dissemination of strategies for the large scale annotation of enzyme function. My laboratory has made extensive contributions to the structural, functional and mechanistic analysis of the cell surface and secreted proteins that modulate adaptive and innate immunity, and we have developed a series of platforms for the high-throughput evaluation of protein interactions and their functional implications. Finally, we have a number of programs that depend on the design of new selectivities and affinities for the realization of novel biologics that will greatly benefit the proposed work. Based on my experience with protein expression/purification and biochemical, biophysical, structural, functional characterization and mechanistic analysis of immune cell surface receptors, as well as my training record, I am well qualified to serve as mentor on this Supplement.

I. Strategies for Functional Annotation and Metabolism Discovery. The number of newly reported protein sequences inferred from genome sequencing continues to grow at a rate that severely outpaces the assignment of function through comparative genomics or direct biochemical analysis. This situation results in a large proportion of unannotated and misannotated protein sequences precluding the discovery of novel enzymes, activities, and metabolic pathways important to (1) understanding the contributions of the gut microbiome to human health, (2) the realization of new chemical processes for industry, and (3) our understanding of critical environmental issues, including global nutrient cycles and the evolution of complex microbial communities. To address these challenges our laboratory is devising experimental strategies based on the solute binding protein (SBP) components of small molecule transport systems, since the first step in a catabolic pathway is frequently the passage of a metabolite across the cellular membrane by SBP-dependent transport machinery. The ability to identify the initial reactant (or a closely related molecule) for a catabolic pathway provides an immediate toe-hold by placing significant constraints on the regions of chemical space that need to be considered and, in conjunction with knowledge of colocalized and coregulated genes, begins to define details of the *in vivo* biochemical transformations operating within the metabolic pathway. Using our high-throughput infrastructure we produced and screened 158 TRAP SBPs against a small molecule library by differential scanning fluorimetry (DSF). These efforts led to the

identification of 40 new TRAP SBP ligands, the generation of experiment-based annotations for 2084 individual SBPs in 71 isofunctional clusters, and the definition of numerous metabolic pathways, including novel catabolic pathways for the utilization of ethanolamine as sole nitrogen source and the use of D-Ala-D-Ala as sole carbon source[1]. Other comparable large scale functional annotation studies were performed for the Isoprenoid Synthase[2] and Haloacid Dehalogenase[3] Superfamilies.

1. Vetting MW, Al-Obaidi N, Zhao S, San Francisco B, Kim J, Wichelecki DJ, Bouvier JT, Solbiati JO, Vu H, Zhang X, Rodionov DA, Love JD, Hillerich BS, Seidel RD, Quinn RJ, Osterman AL, Cronan JE, Jacobson MP, Gerlt JA, **Almo SC** (2015) "Experimental strategies for functional annotation and metabolism discovery: targeted screening of solute binding proteins and unbiased panning of metabolomes." *Biochemistry* **54**, 909-931.
2. Wallrapp FH, Pan JJ, Ramamoorthy G, Almonacid DE, Hillerich BS, Seidel R, Patskovsky Y, Babbitt PC, **Almo SC**, Jacobson MP, Poulter CD (2013) "Prediction of function for the polyprenyl transferase subgroup in the isoprenoid synthase superfamily." *Proc Natl Acad Sci U S A* **110**, 196-202.
3. Huang H, Pandya C, Liu C, Al-Obaidi NF, Wang M, Zheng L, Toews Keating S, Aono M, Love JD, Evans B, Seidel RD, Hillerich BS, Garforth SJ, **Almo SC**, Mariano PS, Dunaway-Mariano D, Allen KN, Farelli JD. (2015) "Panoramic view of a superfamily of phosphatases through substrate profiling." *Proc Natl Acad Sci U S A* **112**, 74-83.
4. Gizzi AS, Grove TL, Arnold JJ, Jose J, Jangra RK, Garforth SJ, Du Q, Cahill SM, Dulyaninova NG, Love JD, Chandran K, Bresnick AR, Cameron CE, **Almo SC** (2018) "A naturally occurring antiviral ribonucleotide encoded by the human genome." *Nature* **558**, 610-614.

II. High-throughput Protein Production Infrastructure. Despite a multitude of recent technical breakthroughs speeding high-resolution structural and functional analysis of biological macromolecules, production of sufficient quantities of well-behaved, active protein continues to represent the rate-limiting step in many structure discovery and functional annotation efforts. These challenges are amplified when considered in the context of ongoing large scale efforts to systematically define structure, function and mechanism of a wide range of macromolecules including multi-domain eukaryotic proteins, secreted proteins, and ever larger macromolecular assemblies. As part of our programs at Einstein, we have established robust bacterial expression platforms for the high-throughput discovery of new metabolism. Unique to the Almo group is the world's first integrated system for high-throughput functional and structural biology of oxygen sensitive proteins. This resource has allowed for the recapitulation of the entire high-throughput protein production and crystallization pipeline within an oxygen-free environment (see http://www.nysgrc.org/psi3/anaerobic.html). We have also established high-throughput eukaryotic expression platforms, including insect and mammalian-based systems, which represents a unique resource in academics. We have extensively described the capabilities of our protein production platforms in the literature[4]. These capabilities are being leveraged to realize a wide range of cutting-edge platform technologies, including receptor-ligand deorphaning, epitope discovery, the generation of novel biologics and the development of new clonal-specific T cell strategies for the treatment of malignancies and autoimmunity[5].

5. **Almo SC**, Garforth SJ, Hillerich BS, Love JD, Seidel RD, Burley SK (2013) "Protein production from the structural genomics perspective: achievements and future needs." *Curr Opin Struct Biol* **23**, 335-44.
6. Samanta D, Mukherjee G, Ramagopal UA, Chaparro RJ, Nathenson SG, DiLorenzo TP, **Almo SC** (2011) "Structural and functional characterization of a single-chain peptide-MHC molecule that modulates both naive and activated CD8+ T cells." *Proc Natl Acad Sci U S A* **108**, 13682-13687.

III. Structural, functional and mechanistic analysis of the cell surface and secreted proteins that modulate adaptive and innate immunity. Cell surface receptors and adhesion molecules are the gatekeepers of cellular function, and are responsible for the detection of signals arising from developmental, morphogenetic and environmental cues central to normal physiology and pathology. Notably, these receptors and ligands are not only therapeutic targets, but soluble versions of these molecules are themselves widely exploited therapeutics for the treatment of autoimmune diseases, infectious diseases and malignancies. High resolution structural characterization and biochemical analyses of these complexes are mechanistically invaluable as they define the chemical and physical determinants underlying receptor:ligand specificity, affinity, oligomeric state, and valency. We have made significant contributions in these areas, including the structures of complexes of CTLA-4:B7-2[6], PD-1:PD-L2[7], DcR3:TL1A[8], DcR3:LIGHT, DcR3:FasL and HVEM:LIGHT, as well as B7-H3, B7-H4[9], TIM-3, NTB-A, CD84, GITRL, TIGIT, CRTAM, nectins and CD160, all of which are potential/proven targets for immunotherapy. These structures defined the determinants responsible for receptor:ligand recognition, which are being leveraged to generate a wide range of variants with altered biochemical properties (e.g., affinities, selectivities) to probe mechanism and provide new functional/therapeutic insights. A major challenge in these efforts is the fact that many, if not most, receptor:ligand pairs remain undefined and thus cannot be structurally characterized or exploited for immunotherapy. To address this bottleneck, we are developing experimental platform technologies for the rapid, systematic and affordable identification of cell surface protein-protein interacting partners and the mapping of protein interaction interfaces. This same platform provides powerful approaches to generate costimulatory receptors and ligands with a wide range of affinities and selectivities, which can leveraged for the design of "tunable" immune modulators.

7. Schwartz J-C, Zhang X, Fedorov AA, Nathenson SG, **Almo SC** (2001) "Structural Basis for Costimulation by the Human CTLA-4/B7-2 Complex." *Nature* **410**, 604-608.

8. Lázár-Molnár E, Yan Q, Cao E, Ramagopal U, Nathenson SG, **Almo SC** (2008) "Crystal structure of the complex between programmed death-1 (PD-1) and its ligand PD-L2." *Proc Natl Acad Sci* U S A. **105**, 10483-10488.

9. Zhan C, Patskovsky Y, Yan Q, Li Z, Ramagopal U, Cheng H, Brenowitz M, Hui X , Nathenson SG, **Almo SC** (2011) "Decoy Strategies: The Structure of TL1A-DcR3 Complex." *Structure* **19**, 162-171.

10. Jeon H, Vigdorovich V, Garrett-Thomson SC, Janakiram M, Ramagopal UA, Abadi YM, Lee JS, Scandiuzzi L, Ohaegbulam KC, Chinai JM, Zhao R, Yao Y, Mao Y, Sparano JA, **Almo SC,** Zang X (2014) "Structure and cancer immunotherapy of the B7 family member B7x." *Cell Rep* **9**, 1089-1098.

11. Liu W, Garrett SC, Fedorov EV, Ramagopal UA, Garforth SJ, Bonanno JB, **Almo SC** (2019) "Structural Basis of CD160:HVEM Regognition." *Structure* **S0969-2126 (19)** 30172-30178 [Epub ahead of print].

12. Ramagopal UA, Liu W, Garrett-Thomson SC, Bonanno JB, Yan Q, Srinivasan M, Wong SC, Bell A, Mankikar S, Rangan VS, Deshpande S, Korman AJ, **Almo SC** (2017) "Structural basis for cancer immunotherapy by the first-in-class checkpoint inhibitor ipilimumab." *Proc Natl Acad Sci USA* **114,** E4223-E4232.

DR. JOHN BLANCHARD

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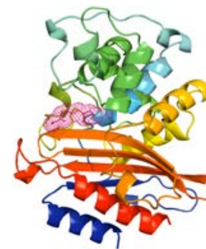

M. tuberculosis β-lactamase

Chemical Mechanisms of Enzymatic Catalysis

One focus of this laboratory is the mechanistic and structural description of enzymes that are essential for the viability of bacterial and parasitic pathogens. Through a combination of recombinant DNA methods, protein purification, kinetic and chemical mechanistic analysis and three-dimensional structural determination, we hope to develop these enzymes into targets for subsequent inhibitor evaluation, and eventual drug design. A major effort is underway to clone, sequence, express, enzymatically characterize and crystallize the enzymes involved in amino acid and vitamin biosynthesis in *Mycobacterium tuberculosis*, the causative pathogen in tuberculosis. We have mechanistically characterized seven of the eight enzymes in the L-lysine biosynthetic pathway, and many of those involved in pantothenate (Vitamin B5) biosynthesis. Future studies are focused on the completion of our functional and structural characterization of the remaining enzymes in these important pathways, as well as enzymes central to the biosynthesis of *L*-arginine and *L*-leucine.

We also have an interest in the mechanisms of by which bacteria become resistant to extant antibacterial compounds. The aminoglycoside class of antibiotics acts by inhibiting prokaryotic protein synthesis. Clinical resistance to aminoglycosides is due to the expression of enzymes that modify the drug, especially those that catalyze the N-acetylation of the drug. We have mechanistically and structurally characterized two such enzymes from *Salmonella enterica* and *M. tuberculosis* that differ in the regioselectivity of acetylation, and have defined the molecular basis for the differing regioselectivity. We have also identified a structurally unique protein from *M. tuberculosis* that causes resistance to fluoroquinolones, a second important class of antibacterials that inhibit DNA gyrase. Finally, we have identified a chromosomally encoded β-lactamase in *M. tuberculosis* whose expression is responsible for the resistance of this organism to the important β-lactam class of antibiotics. We have kinetically and structurally characterized the inhibition of this enzyme by clavulanate, a β-lactamase inhibitor. We have shown recently that the combination of this inhibitor with a very slowly hydrolyzed substrate, meropenem, is effective in killing TB, including those exhibiting an extensively drug resistant phenotype. A recent case report has appeared in which the combination of meropenem and clavulanate cured a 14-year-old girl infected with extensively drug-resistant (XDR) tuberculosis.

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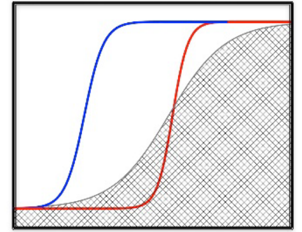

Cellular Regulation by Reversible Macromolecular Assembly and Association

Biology is a dynamic process. Among the myriad array of reversible association reactions that constitute life, small molecules bind to proteins, proteins self-associate and bind to other proteins and nucleic acids and nucleic acids fold and bind to each other in elaborate processing, signaling and regulatory cascades. What is common to these processes is the physical chemistry that underlies these interactions. For example, electrostatic interactions mediate both the binding of proteins to DNA and the folding of RNA. Proteins that mimic the electrostatic character of DNA may competitively regulate DNA binding by other proteins. Our laboratory seeks answers to questions related to the structure – function relationships that govern macromolecular function by combining quantitative analysis with innovative approaches.

- The longest running programmatic theme of our laboratory is the study of the mechanisms by which proteins recognize and bind specific sequences of DNA. We have turned our attention to proteins involved in epigenetic regulation exploring the biophysics of an epigenetic regulatory methyl-CpG binding protein MeCP2 whose disruption is a cause of the neurological disorder Rett Syndrome.
- Our interest in RNA structure and folding has led us to explore the packaging and delivery of RNA therapeutics. We are using a biophysical method that quantitates the size and density of RNA delivery vehicles in support of their use as novel therapeutics.
- We have developed and utilize a high-throughput method to map protein-protein interactions using amino acid side chain oxidation by the hydroxyl radical to measure solvent accessibility as a tool for mapping the molecular interfaces of regulatory complexes and protein therapeutics.

Representative Publications

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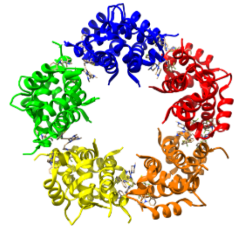

Mechanisms of Cell Motility and Tumor Metastasis

Cell migration, cytokinesis and the establishment and maintenance of cell morphology are fundamental force-requiring processes of all eukaryotic cells. Actin and myosin-II are essential cytoskeletal components of contractile processes in nonmuscle cells. Although it is recognized that myosin-II filament dynamics are under strict temporal and spatial control, the mechanisms controlling filament assembly in higher eukaryotes are not known. We are specifically addressing how covalent modification and noncovalent interactions with novel regulatory proteins mediate the subcellular localization, organization and assembly of myosin-II during chemotactic motility.

Phosphorylation of nonmuscle myosin-II on the heavy chain regulates filament assembly and is attributed to several kinases. Most recently, we showed that heavy chain phosphorylation regulates the chemotactic motility of tumor cells. Moreover, genes coding for proteins that modulate the myosin-II regulatory pathway are up-regulated in invasive tumor cells. Given these findings, we are examining the intermediary signaling pathways in tumor cells that regulate heavy chain phosphorylation and the subsequent effects on motility and invasion. We are using an interdisciplinary approach that combines biochemistry and structural biology to define the physical and chemical features underlying the regulation of myosin-II assembly by phosphorylation, and molecular and cellular techniques coupled with fluorescence microscopy to investigate how phosphorylation regulates myosin-II dynamics in vivo.

We are also studying S100A4, a member of the S100 family of Ca^{2+}-binding proteins that is directly involved in tumor metastasis and regulates tumor cell motility by promoting the monomeric, unassembled state of myosin-II. Thus S100A4 is an excellent target for investigating the mechanisms controlling the localized assembly/disassembly of myosin-II that are relevant to motility, development and metastasis. We are taking a global approach to dissecting S100A4 function; biochemical and structural approaches are being used to identify the mechanisms by which S100A4 regulates myosin-II assembly, intravital imaging studies will evaluate the impact of S100A4 expression on metastasis in live animal models; and a S100A4 knockout mouse has been developed to examine S100A4 function in normal physiology.

Numerous studies indicate that S100A4 is not simply a marker for metastatic disease, but rather has a direct role in metastatic progression. These observations suggest that S100A4 is an excellent target for therapeutic intervention. We developed several assays to identify small molecules that disrupt the interaction of S100A4 with myosin-IIA. Our efforts are now focused on obtaining high-resolution x-ray structures of S100A4 bound to small molecule inhibitors to identify the chemical and structural determinants involved in S100A4 inhibition, and biochemical and cell-based analyses to evaluate the selectivity and potency of lead compounds. These studies will provide the biochemical and structural foundation for the design of second generation S100A4 inhibitors.

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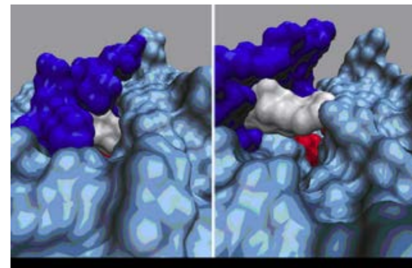

Protein Structure & Dynamics: Enzymatic Catalysis & Protein Folding

THE STRUCTURES AND DYNAMICS OF PROTEINS

Our work is centered on studying the structural and dynamical properties of proteins in order to understand the molecular mechanisms of protein function. New and powerful spectroscopic methods have been developed to obtain the vibrational spectra of specific protein groups and/or bound ligands, even within large proteins, as well as monitor the kinetics of protein structural changes that take place in protein folding and in enzymatic catalysis down to the picosecond time scale. Sub-millisecond motions in proteins, which are clearly important for function, have been largely unstudied because of here-to-fore technical limitations.

Structure and Dynamics of Enzymes

It is well known that enzymes are able to carry out the catalysis of specific chemical reactions because of the specific positioning of atoms and atomic groups and also because of specific motions that these or other atomic groups take. For example, bond polarization and/or proton transfer between a bound substrate and the surrounding protein is a common motif of enzymatic catalysis. Atomic motion often shows up as the movement of a loop that closes over the bound substrate acting to exclude solvent, recruit key residues into the active site, and properly position the bound substrate. It has been recently conjectured that certain protein vibrational motions are organized to be directed along the reaction coordinate in order to 'promote' catalysis. We measure the structure of enzymes complexed with inhibitors and substrates using vibrational spectroscopic tools, determining the Raman and IR spectra of specific bonds within the complex. Vibrational spectroscopy yields a very high resolution of structure (better than 0.01 Å), and changes on this order in the bond length of certain bonds are key to understanding enzymatic catalysis. We have recently developed kinetic approaches that can measure molecular motions in proteins from 10 picoseconds to minutes based on initiating chemical and structural changes employing laser induced temperature jump spectroscopy using optical and vibrational probes to follow structural changes.

The Dynamics of Protein Folding

We also wish to understand how proteins arrive at their three dimensional structure (the protein folding problem). A number of studies are underway to understand the thermodynamics of folding. In addition, the crucial kinetic events of protein folding occur faster than the conventional millisecond time scale of stopped-flow mixing techniques. The early kinetic events (down to nanoseconds) in the folding process are being studied using advanced techniques that initiation chemistry on fast time scales.

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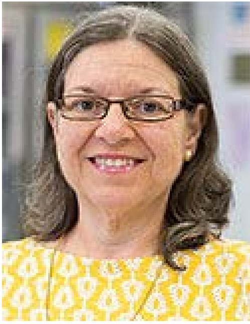

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Molecular Basis of Metabolic Disease (Obesity & Diabetes)

The global epidemics of Type 2 Diabetes Mellitus (T2DM), obesity and the Metabolic Syndrome cannot be explained simply by genetics and/or current lifestyles. Data suggests these adult diseases have their origin in the intrauterine and early postnatal environment. Epidemiological and animal studies have demonstrated that incidence of T2DM and obesity is increased in offspring whose mothers were themselves diabetic or obese pre-pregnancy, perpetuating disease prevalence. Using mouse models we have shown that exposure to a maternal high fat diet predisposes offspring to future development of these metabolic diseases. Combined these data strongly suggest epigenetic alterations of the fetal genome are the cause of increased incidence of disease in adults. At present we have identified significant changes in DNA methylation, as well as changes in histone modifications, associated with altered gene expression in offspring exposed to a maternal high fat diet. Additionally, we are interested in identifying the components of the maternal diet that are linked to the poor metabolic phenotype of offspring. By varying dietary fat content and adding antioxidants to the maternal diet, we hope to alter the disease susceptibility of offspring. The results of these studies may alter the guidelines recommended for a healthy pregnancy/postpartum diet.

We have expanded our mouse work in the field of the "Developmental Origin of Health and Disease" to study the epigenetic basis of early childhood obesity in children. We are conducting a major prospective study at Einstein/Montefiore using $CD3^+$ T-cells purified from the umbilical cords of healthy term infants (intrauterine growth restricted and appropriate for gestational age) born to mothers at the Weiler Hospital to understand the molecular mechanism underlying early childhood obesity during the first 24 months of life. Recent studies have identified a key role for $CD3^+$ T-cells in the initiation and regulation of adipose tissue inflammation and insulin resistance. We are characterizing the DNA methylation profiles of $CD3^+$ T-cells from infants at birth and 24 months old and we are measuring T-cell function, metabolism and gene expression. We will use this information to determine whether T-cell DNA methylation and functional profiles are associated with infant growth velocity and adiposity in the first 24 months of life. Our ultimate goal is to characterize the epigenetic mechanisms underlying programmed T-cell dysfunction in healthy-term newborns as a critical early step in understanding obesity-associated inflammation preceding the onset of childhood obesity.

Additional studies are focused on two members of the glucose transporter gene family (GLUT4 and GLUT8). GLUT4 is insulin and exercise responsive and is the major glucose transporter expressed in cardiac and skeletal muscle and adipose tissue. GLUT8 is a member of the glucose transporter gene family that is expressed in many tissues (including brain, liver, placenta and various tumor cells). By using transgenic and gene knockout mouse models we study the role of GLUT4 and GLUT8 in whole body and organ specific glucose utilization in normal and disease states.

In contrast to our studies on insulin action and glucose uptake we also study the glucagon receptor. Glucagon elevates serum glucose levels. We cloned the glucagon receptor gene and studied its regulation by hormones and nutrients. We are studying the role of glucagon action in the pathophysiology of T2DM using gene knockout and transgenic mouse models.

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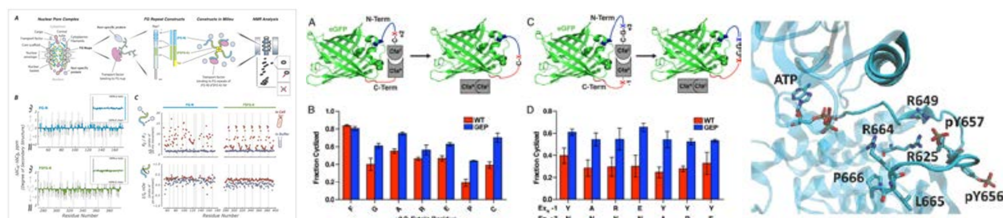

PHYSICAL BIOCHEMISTRY

Much of the machinery of the cell -- enzymes, transport factors, signaling complex, transcriptional and translational devices -- involve proteins' interaction with other proteins, with other bio-macromolecules and with low molecular weight ligands. The large scale systems analysis of these interactions is highly complex. Using and developing the tools of structural biology, molecular dynamics simulation, molecular biology and protein engineering, we are attempting to provide such analysis in leading edge systems of practical biological interest.

In many proteins the role of 'unstructured' regions can be assessed using improved NMR and scattering measurements probing how multiple weak interactions can give rise to specificity and selectivity of biological activity. These interactions can also be probed *in cell* using direct observation of multiple expressed protein systems by NMR, for a direct study of protein-protein interactions. A new area of application is using these methods to understand the dynamic structures of the FG-rich Nuclear Pore proteins, and their interactions with carriers and cargo. Novel functional roles of 'unfoldable' protein regions are being discovered. The nuclear pore transport mechanism contains potential significant targets for next generation therapeutics in cancer and infectious disease, which are being targeted for validation.

The mechanism of the intein reaction, internal splicing of proteins, is of general interest for protein engineering and as a model for several posttranslational modification mechanisms involving thioesterification. NMR is being used as an essential tool for probing this unusual reaction involving breaking and making peptide bonds, with substantial flexibility of the coordinating entities. All these studies also involve developing new analytical applied mathematical methods.

Protein kinases are critical mediators in development, differentiation, and homeostasis. Genetic and phenotypic modifications of their activities and of related phosphatases are commonly associated with many diseases states including infections, cancers, autoimmunity and developmental disorders. Recent advances have introduced selective inhibitors to these enzymes, and the potential for understanding the chemical biology of their interactions and for therapeutics are significant. A substantial part of their regulation and substrate interactions involve major molecular movements ("dynamics") which are probed using NMR and other methods adding to static structural information from NMR or crystallography.

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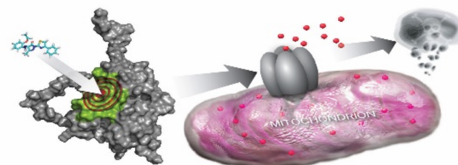

Chemical and Structural Biology of Cell Death and Survival Signaling

The Gavathiotis laboratory investigates mechanisms of protein-protein interactions in cell death and cell survival signaling such as apoptosis, mitochondrial dynamics and autophagy, which are deregulated in cancer and other diseases. We use mechanistic insights to develop chemical probes that can be used for target identification and validation and serve as the basis for novel therapeutics. We aim to develop prototype therapeutics and therapeutic strategies that we evaluate in preclinical disease models together with our collaborators in academia and industry. We are an interdisciplinary group that has expertise in structural and chemical biology, medicinal chemistry, drug design, computational and experimental screening, biochemical and cell biology approaches and in vivo pharmacology.

Molecular Mechanisms of Cell Death and Cell Survival Signaling

1. Programmed cell death is a genetically controlled physiological process that rids the body of unwanted or malfunctioning cells to maintain the normal development and homeostasis of multicellular organisms. Deregulation of **cell death and cell survival programs** leads to variety of disease conditions and understanding the molecular mechanisms that govern these signaling pathways is both fundamentally important and medically relevant. Our focus is the protein interaction network of the **BCL-2 family of proteins** and its role in regulating **apoptosis**. We currently expland our work in mechanisms of **selective autophagy**, **mitochondrial fusion and fission** and **mitochondrial permeability transition-driven necrosis.** Using structural biology, biochemical, biophysical and cell biology studies, we aim to elucidate the mechanisms of protein-protein interactions and define the very determinants that **modulate life and death decisions in healthy and malignant cells.**
2. Aberrant regulation of survival signaling pathways can lead to uncontrolled cell growth and proliferation leading to malignant transformation and tumorigenesis. Constitutive activation of the mitogen activated protein kinase **(MAPK) signaling pathway**, resulting from mutations in key components of the pathway or by mutations in upstream activators of the pathway, is a highly frequent event in **human cancer**. Abnormal activity of the MAPK singaling is also present in developmental syndromes termed **RASopathies.** We are using chemical and structural approaches to **elucidate and target novel mechanisms** that regulate critical components of the MAPK signaling pathway e.g. RAS, RAF, MEK and ERK proteins. Our goals are to advance our understanding of the structure-function relationships regulating important components of the MAPK signaling pathway and provide new avenues for drug development overcoming resistance mechanisms to current treatments.

Chemical Biology and Drug Discovery of Pathological Protein-Protein Interactions

- We apply **high-throughput screening**, **structure-based drug design** and **medicinal chemistry** to discover and develop **small molecules and peptide-based probes** that modulate the function of protein-protein interactions. We use these probes to interrogate the signaling pathways and **understand the biological mechanisms**. Probes are also used as templates for the **development of novel therapeutics**. Our **targets** include but are not limited to proteins of the mitochondrial cell death pathway, chaperone-mediated autophagy and mitochondrial dynamics that are validated in genetic models and are considered challenging or **"undruggable"**. For example, we have identified the **first-in-class**: 1) **small molecule activators of pro-apoptotic BAX** and demonstrated a new paradigm for pharmacologic induction of apoptosis in cancer, 2) **small molecule activators of chaperone-mediated autophagy** that protect cells from oxidative stress and proteotoxicity 3) **small molecule mitofusin activators** that promote mitochondrial fusion 4) **allosteric BAX inhibitors** that inhibit apoptosis and necrosis 5) **small molecule allosteric BRAF inhibitors** that overcome resistance to FDA-approved inhibitors. We work towards a **"chemical toolbox"** of activators and inhibitors of **major cell death and cell survival pathways** to enable us to manipulate cell signaling and fate decision in physiological and disease conditions and provide **new research tools and future therapeutics.**

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Peptide and Protein Engineering

Our group is broadly interested in the application of peptide, protein and antibody engineering methods for the discovery and development of novel immunotherapies and vaccines. Projects are highly interdisciplinary and involve aspects such as phage display, structure-based protein design, bispecific antibody engineering, structural biology, virology, and cancer biology. Current direction include:

1. Engineered antibodies as virus immunotherapies. The use of monoclonal antibodies (mAbs) as therapeutics (immunotherapy) has been highly successful for oncology and other indications, but application of mAbs to viral immunotherapy is only now emerging. mAbs offer an advantage of being highly specific, with little adverse effects. Furthermore, mAbs can engage in Fc-related functions that may serve to promote clearance of infections. We have been using state-of-the-art protein engineering methods to identify, characterize, and evaluate novel virus immunotherapies against Ebola virus, Dengue virus, and Chikungunya virus. We focus on developing new therapeutics by protein engineering that may have features that are not possible with conventional (natural) antibodies.

2. Immunogen design for flavivirus vaccine discovery. Dengue virus is a mosquito-transmitted flavivirus that causes hundreds of millions of human infections world-wide each year. There are four serotypes of Dengue (DENV1-4) that co-circulate in hyperendemic regions. Dengue virus vaccine design has been complicated by the recent emergence of Zika virus (ZIKV), another flavivirus. We have been using structure-guided protein engineering to develop novel immunogens that elicit DENV and ZIKV protective antibody responses. Our strategy focuses on common susceptible epitopes that may be structurally engineered.

3. Dissection and engineering of protein-protein and protein-antibody interactions by phage display. Phage display is a combinatorial technique that permits the selection of binding clones from highly diverse protein libraries. We have used phage display to dissect critical determinants underlying specific protein-protein and protein-antibody interactions. Ultimately, this information can be utilized to design new proteins or antibodies with enhanced function; and contributes generally to our knowledge of protein recognition. Projects focus on applications in T-cell immunology, chronic lymphocytic leukemia, and viral vaccine design.

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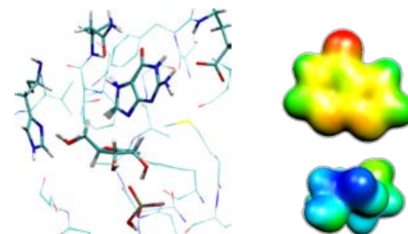

Enzymatic Transition States and Logical Inhibitor Design

Enzymes catalyze virtually all of the chemical transformations necessary for biological life. Knowledge of the transition-state structure of enzymatic reactions permits the design of powerful inhibitors. Methods have been developed in this laboratory for the experimental determination of the geometry and charge features that characterize enzymatic transition states. This information is used for the logical design of transition-state analogues. Chemical synthesis is accomplished by our chemistry collaborators at the Victoria University of Wellington, New Zealand. Our novel transition state analogues have the potential to be new drugs Specific projects include:

Human genetic deficiency of purine nucleoside phosphorylase causes a specific T-cell insufficiency. Our inhibitors of this enzyme are powerful anti T-cell agents and anti-gout candidates. Immucillin-H (Mundesine®) was approved for use in Japan for peripheral T-cell lymphoma (PTCL). DADMe-Immucillin-H has completed phase 2 clinical trials for gout. We plan to characterize the enzyme-drug interactions at the kinetic and atomic levels to define their exceptional drug characteristics.

Purine salvage is essential for growth of parasitic protozoa. A family of powerful inhibitors has been prepared against two target enzymes from the malaria parasite. Promising results have been obtained in cell culture and in infected primates. Drug discovery efforts continue for a critical enzyme of purine salvage in malaria parasites.

Human cancers are genetically unstable. The epigenetic changes make cancer cells more susceptible to agents that disrupt epigenetic control. Regulatory methylation of proteins and DNA are our epigenetic anticancer targets. S-Adenosylmethionine is the source for methyl transfer reactions, essential in cancer cells. We are targeting three enzymes in the epigenetic pathways. Our goal is to developed powerful transition state analogue inhibitors with anticancer activity.

Enzymatic transition states have lifetimes of a few femtoseconds (10^{-15} sec) but catalyze reactions on the millisecond time scale (10^{-3} sec). We make isotopically heavy enzymes to understand what happens at the fsec timescale. These studies are textbook-changing studies into the fundamentals of catalysis. We collaborate with computational quantum chemists at the University of Arizona, nice to visit in January and February.

Students in this laboratory can receive training in enzymology, catalysis, protein expression, inhibitor design, computer modeling, inhibitor synthesis, and in drug metabolism studies in cells and animals. Active collaborations occur with laboratories specializing in NMR, X-ray crystallography, mass spectroscopy, synthetic organic chemistry, cancer and medicine. Projects can be designed to include several of these

research approaches through active collaborative research programs. Graduates from this laboratory are well trained for future careers as university researchers, the pharmaceutical industry or biomedical research.

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A more complete list from this laboratory is available at:

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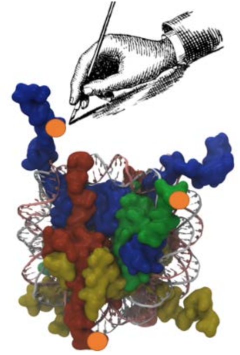

How to Write a Histone Code:
Chromatin and the Biochemistry of Epigenetic Information

We focus on understanding chromatin, the complex of DNA and histones that form the eukaryotic genome. Post-translational modifications of histones, and deposition of histone variants, establish a "histone code" of transcriptional regulation and other chromatin-mediated transactions, and constitute much of the epigenome. Epigenetic information is the landscape on which the dynamic usage of genetic information is encoded. We are particularly interested in mechanisms and biological function of these processes in embryonic development, how this process is misregulated in cancer cells, and how to drug components of the machinery.

We utilize a unique and interdisciplinary approach to address these questions, including: protein biochemistry and quantitative enzymology; structural biology (NMR, crystallography, SAXS); cancer cell culture and genome wide studies (RNA-Seq and ChIP-Seq); and cell free extracts of the frog *Xenopus laevis.* These complementary tools allow us to probe evolutionarily conserved mechanisms specifying critical chromatin and epigenetic events. Our combined use of rigorous *in vitro* studies–along with *in vivo* studies in the frog and in cancer cells–provides an uncompromised approach to fully understanding epigenetic phenomena and how to apply this knowledge towards improving human health. We are currently pursuing two major aims:

Histone chaperone molecular mechanisms - Chaperone proteins bind to histones and escort them for chromatin deposition; they also remove and deposit histones during transcription, replication, and repair of the DNA. However, little is understood about how histone chaperones mechanistically accomplish these complex tasks. Specifically, it is not clear how chaperones bind to histones to both stabilize them and prevent aggregation with DNA, nor is it understood how they cooperate to deposit histones into nucleosomes. Chaperones are diverse proteins with little to no sequence similarity. Strikingly, they all have distinct structural and functional properties. We propose that the major clue to chaperone molecular mechanisms lies in their only conserved feature: their acidic-stretches embedded in intrinsically disordered regions (IDRs). Our studies use novel approaches to target these chaperone IDRs and their post-translational modifications, particularly the unusual glutamylation of the acidic IDRs.

Protein arginine methyltransferases (PRMTs): structure, enzymology, and biological function- PRMTs1-9 catalyze the post-translational methylation of protein arginines, including in histones, RNA-binding proteins, and splicing factors. They are critical for early development; they are also outstanding candidates for cancer chemotherapy because misregulation of their activity contributes to proliferative and invasive cellular phenotypes. It is unclear how PRMTs select their substrates, how their activity is regulated, and what

arginine methylation does in the cell to regulate biological function. We are currently focused on the major enzymes PRMT1 and PRMT5.

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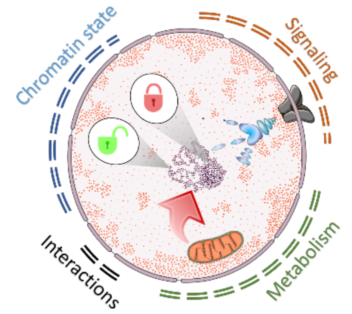

Technology for chromatin biology

Our lab develops and applies methods to study proteins associated to chromatin. We optimize technology mostly adopting mass spectrometry to investigate histone post-translational modifications and their role in regulating chromatin accessibility in health and disease. The lab moves in two major aspects of science:

(1) Basic and fundamental biology

We develop and exploit methods for the analysis of proteomes to identify mechanisms of disease etiology, and target those mechanisms to discover new potential therapeutic perspectives. Specifically, we want to determine "context-dependent" roles of histone marks depending on (i) how accessible they are on chromatin, (ii) what other PTMs they co-exist with and (iii) what is their effect on protein recruitment and DNA readout. This will allow us to identify chromatin markers that play a fundamental role in phenotypic changes (development or diseases), and eventually target their readers for therapy. The lab is equipped with technology to perform state-of-the-art proteomics, including identification and quantification of hundreds of histone PTMs in a single analysis. We will use mass spectrometry to define new quantitative dimensions in systems biology, including protein synthesis rate, nucleosome deposition rate, PTM catalysis rate and accessibility of PTMs on chromatin. We are also proficient with genomics strategies to map genome-wide localization of chromatin elements and quantify transcripts. Our model system is tumor cells grown as three-dimensional spheroids, as they model more accurately the growth and the cell-cell interconnectivity of solid tissues compared to flat cultures.

(2) Translational biomedicine

We investigate the potential of histone marks in diagnostics. Histone PTMs are already targeted in selected pathology assays, but very few of them are considered as biomarkers. Histones are the most abundant and heterogeneous (i.e. modified) protein family in the cell, so they are a highly suitable biomarker candidate. We exploit the high accuracy, robustness and speed of our new detection methods to identify unique features correlating with selected genomics states. This line of research will develop automated sample preparation and clustering tools for live spectra matching with reference databases.

Selected publications

One minute analysis of histone post-translational modifications by direct injection mass spectrometry. *Sidoli et al. Genome Research, 2019*

Metabolic labeling in middle-down proteomics allows for investigation of the dynamics of the histone code. *Sidoli et al. Epigenetics & Chromatin, 2017*

Low resolution data-independent acquisition (DIA) in an LTQ-Orbitrap allows for simplified and fully untargeted analysis of histone samples. *Sidoli et al. Analytical Chemistry, 2015*

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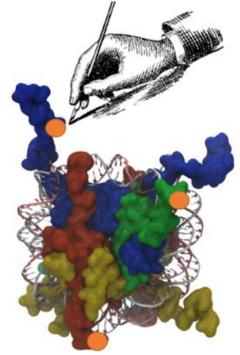

How to Write a Histone Code:
Chromatin and the Biochemistry of Epigenetic Information

We focus on understanding chromatin, the complex of DNA and histones that form the eukaryotic genome. Post-translational modifications of histones, and deposition of histone variants, establish a "histone code" of transcriptional regulation and other chromatin-mediated transactions, and constitute much of the epigenome. Epigenetic information is the landscape on which the dynamic usage of genetic information is encoded. We are particularly interested in mechanisms and biological function of these processes in embryonic development, how this process is misregulated in cancer cells, and how to drug components of the machinery.

We utilize a unique and interdisciplinary approach to address these questions, including: protein biochemistry and quantitative enzymology; structural biology (NMR, crystallography, SAXS); cancer cell culture and genome wide studies (RNA-Seq and ChIP-Seq); and cell free extracts of the frog *Xenopus laevis.* These complementary tools allow us to probe evolutionarily conserved mechanisms specifying critical chromatin and epigenetic events. Our combined use of rigorous *in vitro* studies–along with *in vivo* studies in the frog and in cancer cells–provides an uncompromised approach to fully understanding epigenetic phenomena and how to apply this knowledge towards improving human health. We are currently pursuing two major aims:

Histone chaperone molecular mechanisms - Chaperone proteins bind to histones and escort them for chromatin deposition; they also remove and deposit histones during transcription, replication, and repair of the DNA. However, little is understood about how histone chaperones mechanistically accomplish these complex tasks. Specifically, it is not clear how chaperones bind to histones to both stabilize them and prevent aggregation with DNA, nor is it understood how they cooperate to deposit histones into nucleosomes. Chaperones are diverse proteins with little to no sequence similarity. Strikingly, they all have distinct structural and functional properties. We propose that the major clue to chaperone molecular mechanisms lies in their only conserved feature: their acidic-stretches embedded in intrinsically disordered regions (IDRs). Our studies use novel approaches to target these chaperone IDRs and their post-translational modifications, particularly the unusual glutamylation of the acidic IDRs.

Protein arginine methyltransferases (PRMTs): structure, enzymology, and biological function- PRMTs1-9 catalyze the post-translational methylation of protein arginines, including in histones, RNA-binding proteins, and splicing factors. They are critical for early development; they are also outstanding candidates for cancer chemotherapy because misregulation of their activity contributes to proliferative and invasive cellular phenotypes. It is unclear how PRMTs select their substrates, how their activity is regulated, and what

arginine methylation does in the cell to regulate biological function. We are currently focused on the major enzymes PRMT1 and PRMT5.

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Emmanuel S. Burgos, Ryan O. Walters, Derek M. Huffman and **David Shechter.** A simplified characterization of S-adenosyl-L-methionine-consuming enzymes with 1-Step EZ-MTase: a universal and straightforward coupled-assay for in vitro and in vivo setting. *Chem. Sci.*, **2017**

Christopher Warren and **David Shechter.** Fly Fishing for Histones: Catch and Release by Histone Chaperone Intrinsically Disordered Regions and Acidic Stretches. *J Mol Biol*. **2017**

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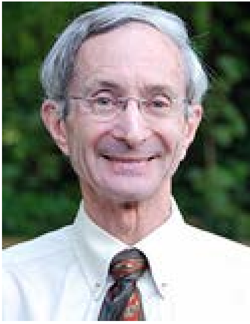

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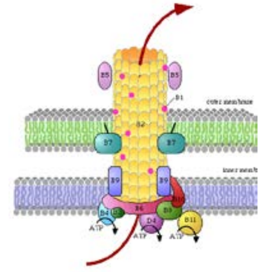
Depiction of a Type 4 Secretion Machine



Acquisition of Virulence Phenotypes by Bacterial Pathogens

Pathogenic Mechanisms of the Legionnaires' Disease Bacterium

The fresh water bacterium, *Legionella pneumophila*, is the causative agent of Legionnaires' disease, a potentially fatal pneumonia spread by aerosolization of the bacterium from air conditioning systems, shower heads and humidifying devices. This highly-publicized outbreak in Manhattan emphasized the importance of Legionella in community public health education.

> New York Times JUNE 16, 2017
> *Legionnaires' Outbreak on Upper East Side Kills One and Sickens Six*
> By SARAH MASLIN NIR
> One person is dead and six other people have been sickened in an outbreak of Legionnaires' disease on the Upper East Side of Manhattan, the city health department announced on Friday.
> … "We know that this is an organism that exists in our environment, and we don't expect to be able to eradicate it," said Dr. Mary T. Bassett, the health commissioner. "From a public health point of view, we want to be able to get a handle on clusters that may have a common source, but we hardly ever are able to identify them."

The ability of *L. pneumophila* to replicate within and evade killing by pulmonary macrophages requires a Type Four Secretion System (TFSS) which secretes *Legionella* proteins into host cells. These bacterial proteins then influence the fate of internalized *Legionella*.

Research in my laboratory demonstrated that following exposure to conditions that mimic the fresh water environment *Legionella*, the Dot/Icm T4SS—previously associated with all virulence phenotypes—is no longer required. We implicated the Lvh T4SS as a functional alternative to the Dot/Icm T4SS. The Lvh T4SS is of particular interest because its locus is on a mobile genetic element, pLP45, that can exist either integrated in the bacterial chromosome or excised as an episomal element. Excision of pLP45 interrupts a non coding RNA, lpr0035, that is essential for efficient replication of *L. pneumophila* in macrophage and amoebae hosts.

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- **My laboratory is now closed. As the Assistant Dean for Biomedical Science Education, I am involved in medical school education, curricular change and educational administration. I also teach in Grad School Molecular Genetics and Human Metabolism courses.**
- **I mentor MD students in research on medical education and am a consultant for organizations that integrate clinical and basic science in medical education and prepare MD students for their Step 1 exam.**

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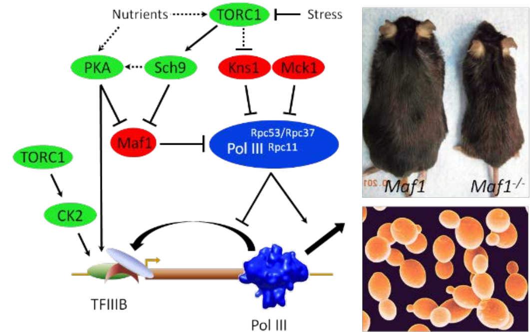

Gene Transcription, Regulation of Cell Growth and Metabolism

Signaling Pathways and Transcriptional Regulation in Growth Control, Cancer and Obesity
Our laboratory is conducting basic research on the mechanisms of eukaryotic transcriptional regulation in response to nutrients and environmental and cellular stress. We are interested in defining the signaling pathways and the mechanisms that regulate transcription of ribosomal components and transfer RNAs since these processes are critically important for controlling cell growth. Deregulation of cell growth control is a key event in cell transformation and tumorigenesis and is relevant to a broad range of human diseases. In addition, as the synthesis of new protein synthetic capacity constitutes ~85% of nuclear gene transcription, the tight coordinate control of this process is critical for metabolic economy. Our research programs span genetics, molecular biology, biochemistry and structural biology and utilize budding yeast and mice as model experimental systems. Much of our current focus is on Maf1, a conserved master regulator of gene transcription that confers resistance to diet-induced obesity when knocked out in the mouse.

Obesity Resistance in *Maf1* Knockout Mice
Mice with a whole body knockout of *Maf1* are resistant to diet-induced obesity and non-alcoholic fatty liver disease. These phenotypes are associated with reduced food intake, increased energy expenditure and metabolic inefficiency. The lean phenotype of *Maf1* KO mice is supported by their wasteful use of metabolic energy through the enhanced synthesis and turnover (futile cycling) of tRNAs and hepatic lipids. Our findings suggest that MAF1 may be a desirable drug target for treating obesity in humans. Current research on this unique model is focused on understanding the molecular and metabolic basis of obesity resistance. We are also pursuing structure-function studies on MAF1 and we are developing approaches to obtain small molecule inhibitors of MAF1.

Genetic Arrays, Gene Networks and Functional Genomics
Synthetic genetic array analysis and other systematic genome-wide genetic approaches such as synthetic dosage lethality and suppression are being conducted by robotic pinning of high density arrays of yeast strains. This technology enables the mapping of genetic interaction networks, defines the function of genes and establishes functional relationships between biochemical pathways. These genetic array-based approaches are being used to interrogate a range of biological processes including transcriptional regulation as described above. The robot also serves as a resource to other researchers at Einstein and elsewhere who are working in yeast or in mammalian systems on genes that have homologs in yeast. The integration of genetic interaction data with other large scale datasets such as DNA microarray, RNA and ChIP-sequencing and protein-protein interaction data is used to inform testable hypotheses of the systems level behavior of genes and their products.

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STUDENT JOURNAL CLUB EVALUATION

Date:___________________________

Graduate Student:_______________________

Title of Journal Article: ____

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Citation:____

Critique (Taking in account the organization and clarity of the presentation):

Strong points of the presentation:

Weak points of the presentation (areas needing improvement):

Journal Club Faculty/Student Consultant

Name:___________________________ Signature:_____________________________ Biochemistry Journal Club procedures 4

DEPARTMENT OF BIOCHEMISTRY

WORKS IN PROGRESS EVALUATION

***Date:*___________________________**

***Graduate Student:*_______________________**

***Title of Presentation:* ____**

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Critique (Taking in account the organization and clarity of the presentation):

Strong points of the presentation:

Weak points of the presentation (areas needing improvement):

Journal Club Faculty/Student Consultant

***Name:*___________________________ *Signature:*_____________________________**