Albert Einstein College of Medicine

Montefiore

DEPARTMENT OF BIOCHEMISTRY

Departmental Guide for First-Year Students

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

2024 – 2025

(Updated as of 8/12/2024)

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 will 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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A. GENERAL INFORMATION

A. Einstein Graduate Program in the Biomedical Sciences:

- Officials and Graduate Office Personnel – Click Here
- Admissions Procedures for the PhD – Click Here
- Admissions Procedures for the MD-PhD Program – Click Here
- Thesis and Final Examination Guidelines and Procedures – Click Here

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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Administrator, Department of Biochemistry
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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 the 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 one other laboratory to gain breadth in their research training. Students who matriculate with an M.S. or equivalent research experience and have selected a thesis laboratory may obtain an exemption from laboratory rotations if the request is endorsed by their mentor, their advisory committee, and the Chair of Biochemistry.

Students should plan to complete much of their course work including required departmental courses during the first year. (The department recognizes that special circumstances may delay the 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, an advisory committee will be assembled; this advisory committee is responsible for monitoring the student's progress. Ph.D. thesis research involves meaningful, critical thinking and the execution of ideas in the laboratory using the scientific method. Students are expected to embrace full-time research upon the declaration of their thesis mentor. 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 continues in concert with their coursework 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 progress reports with both the Chair of Biochemistry and the Graduate Division office is a departmental requirement. Reports of student advisory committees should discuss research progress and future plans.

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. Click Here – for a list of the courses offered by the Graduate Division. The Biochemistry department has specific course requirements that should be completed during the 1st year of study.

Courses Requirements for Ph.D. and M.D.-Ph.D. Students in the Department of Biochemistry:

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

In addition to the Block 1 course, students are required to take one of the following two 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.

OR

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

To complete the required coursework, the following courses are highly recommended for many department students:

- Molecular Genetics
- Stem Cells
- Molecular Cell Biology
- Development and Disease

Students may request an exemption from the required courses if they have completed substantial graduate-level course work in Biochemistry during their undergraduate or masters training. Students should contact the leader(s) 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 biochemistry and related disciplines studied by members of our department. Since a goal of the graduate curriculum is for students to complete much of their didactic training during the first year, serious thought should be given about charting the optimal

pathway through the available options remembering that educational breadth as well as depth is important to future success. Students who have joined a thesis laboratory should discuss additional coursework 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 who are not in good standing or who fail to progress in Thesis Research are referred to the Academic Affairs Committee (AAC) of the Graduate Division for review. As part of this review, the student's advisory committee will prepare a written plan for the student, the mentor and the AAC, outlining the requirements 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 and is mandatory for students in the Biochemistry Department. Presentations by the student begin at the start of the semester following the 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 are 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 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. A student's thesis advisor cannot serve as an advisor 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 the 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. If the faculty advisor cannot fulfill his/her responsibility, another faculty member should be sought to substitute as the advisor. The student should make every effort to give a practice talk before the presentation date. After the presentation, the faculty advisor will meet with the student to discuss the presentation. The journal club presentation should contain the Introduction and background, Experimental methods, Results, Conclusions and Critique sections as described below. Students should aim for a 20-minute presentation 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 to craft an effective, enjoyable, and informative presentation.

- Introduction and Background: Informs the audience on both basic concepts and the 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? It is also important to give proper attribution to the lead and communicating authors.
- 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 the result slides as needed.
- Critique: This is the most important part of the journal club. The student will present his/her opinion regarding the 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 on reviewing the science, not on demonstrating computer graphics skills. 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 an 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. At least one and preferably two members of this committee should 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 which comprises 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 department are required for a student to remain in good departmental standing.

As the direction of a student's thesis research may change as the project progresses, the student and their advisor may wish to make changes to the composition of a student's advisory committee. Such changes must be approved by the Chair of the advisory committee. The Chair of the Department is an *ex officio* member of all advisory committees and may attend a meeting at his/her discretion. Other policies governing student academics and responsibilities can be found in the Academic Policies and Guidelines of the Sue Golding Graduate Division, which is available on the program's website – Click Here.

Students are responsible for scheduling the two required advisory meetings each year. 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.

Student progress reports and Advisory Committee reports on all meetings are included in the student's academic file. Copies of these reports are also distributed to the Chair of the Department of Biochemistry. The written reports of the required advisory committee meetings must be on file with the departmental office before the Ph.D. degree is granted. Advisory committee guidelines and other relevant forms can be found on the Graduate Division's website – Click Here.

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, on the Einstein homepage Calendar of Events and in e-blast announcements.

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. 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 Chair of the Advisory Committee who will then make a recommendation to the Chair of the Department for a final decision. The Graduate Division has established procedures for the preparation of the thesis and its defense that can be found on the program's website. As per these procedures, the thesis committee is composed of a minimum of 5 tenure track faculty. The chair must be a senior member of the faculty (professor or associate professor), with experience serving on a defense committee; the chair does not have to be a member of the student's home department but usually is. As per the Department of Biochemistry requirements, one member of the thesis committee should be an outside examiner.

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. The written permission of the mentor, the student advisory committee, and the Chair 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 Chair 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. Citations and Acknowledgements

Acknowledgment of Research Support – All publications, theses, reports, etc. resulting from research conducted during the candidate's degree program must include an appropriate acknowledgment 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

Almo, Steven C.	*Structural Immunology, Biologics & Enzyme Functional Annotation*
Brenowitz, Michael	*Cellular Regulation by Reversible Macromolecular Assembly and Association*
Bresnick, Anne	*Mechanisms of Cell Motility and Tumor Metastasis*
Callender, Robert	*Professor Emeritus, Department of Biochemistry*
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*
Gennerich, Arne	*Molecular Mechanisms of Microtubule-based Motor Proteins in Health and Disease*
Grove, Tyler	*Leveraging Anaerobic Biochemistry for Novel Therapeutics*
Kitamura, Seiya	*Chemical Biology, Medicinal Chemistry, and Drug Discovery*
Lai, Jonathan	*Peptide and Protein Engineering*
Maianti, J.P.	*Reprogramming, Inhibiting and Sensing Enzymes with DNA-Encoded Library Discovery Platforms*
Rousseau, Denis	*Structure, Function, Dynamics and Time-Resolved X-ray Crystallography of Cytochrome c oxidase*

Satir, Birgit H.	*Professor Emerita, Department of Biochemistry*
Schramm, Vern L.	*Enzymatic Transition States and Logical Inhibitor Design*
Shechter, David	*Chromatin and the Biochemistry of Epigenetic Information*
Sidoli, Simone	*Technology for Chromatin Biology*
Sosa, Hernando	*Structure and Function of Biological Macromolecular Machines*
Steinman, Howard M.	*Assistant Dean for Biomedical Science Education*
Willis, Ian M.	*RNA Polymerase III Transcription in Health and Disease*
Yeh, Syun-Ru	*Molecular Mechanisms and Structural Biology of Hemeproteins*

Faculty with Secondary Appointments in Biochemistry

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

Backer, Jonathan M. Molecular Pharmacology	*Signaling by Phosphoinositide Kinases*
Chang, Roger Systems & Computational Biology	*Structural Systems Biology*
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*
Schuster, Victor Medicine (Nephrology) Senior Vice-Dean	*Prostanoid Signaling in Metabolism and Metabolic Disease*

DR. STEVEN ALMO

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Structural Immunology & Enzyme Functional Annotation

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. We have contributed to efforts focused on the large-scale annotation of enzyme function, including the discovery of novel antivirals and new targets for controlling inflammatory processes.

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 to treat cancer, autoimmune diseases and infectious diseases.

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(3):909-31. PMCID: PMC4310620

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(13):196-202. PMCID: PMC3612614
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(16):74-83. PMCID: PMC4413258

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. 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].

4. 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(3):335-44. PMCID: PMC4163025
5. 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(33):13682-7. PMCID: PMC3158197

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 be leveraged for the design of "tunable" immune modulators.

6. 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. PMID: 11279501; DOI: 10.1038/35069112

7. 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**:10483-8. PMCID: PMC2492495

8. 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**:162-71. PMCID: PMC3065972

9. 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**, 1089-98. PMCID: PMC4250833

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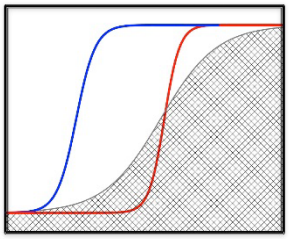

Cellular Regulation by Reversible Macromolecular Assembly and Association

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

Wang, Q., Aleshintsev, A., Bolton, D., Zhuang, J., Brenowitz, M., Gupta R., Ca(II) and Zn(II) Cooperate To Modulate the Structure and Self-Assembly of S100A12 (2019) Biochemistry 58(17), 2269-2281

Warren, C., Matsui, T., Karp., J.M., Onikubo, T., Cahill, S., Brenowitz, M., Cowburn, D., Girvin, M., & Shechter, D. (2017) Histone Binding and Release by the Chaperone Nucleoplasmin is Controlled by Intramolecular Dynamics, *Nature Communications*, 8(1), 2215

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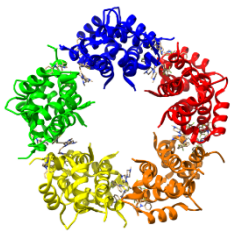

Mechanisms of Cell Motility and Tumor Metastasis

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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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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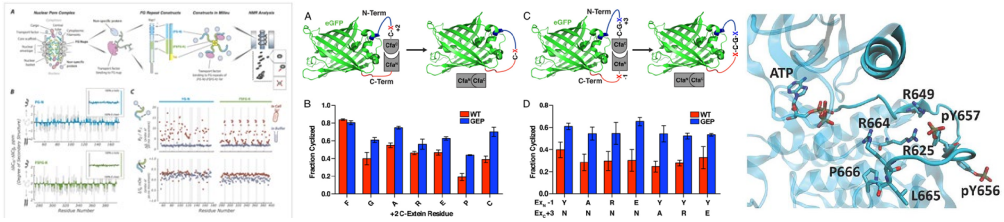

PHYSICAL BIOCHEMISTRY

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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Molecular mechanisms of microtubule-based motor proteins in health and disease

The research of our laboratory is focused on the development of advanced high-resolution and single-molecule microscopy techniques and their application to study how biomolecular motors work and generate biological motion. In particular, the Gennerich Lab combines single-molecule biophysics with molecular biology and biochemistry to study the molecular mechanisms underlying cell division, intracellular organelle and mRNA transport, as well as the molecular mechanisms underlying the regulation of transcription elongation by RNA polymerases. Current research is focused on the molecular functions of the microtubule motors kinesins and cytoplasmic dynein (molecular machines that harness the chemical energy of ATP hydrolysis to perform mechanical work in eukaryotic cells), the regulation of RNA polymerase II-based transcription elongation through nucleosomes, and the molecular mechanism of transcription elongation by RNA polymerase III. We use a multidisciplinary approach integrating ultrasensitive single-molecule assays (high-resolution optical trapping and single-molecule fluorescence microscopy) and genetic approaches such as homologous recombination to dissect the mechanisms of microtubule- and DNA-based motor proteins. Our long-term goal is to understand the fundamental design principles of biomolecular motors and the molecular basis of human diseases with underlying defects in motor function.

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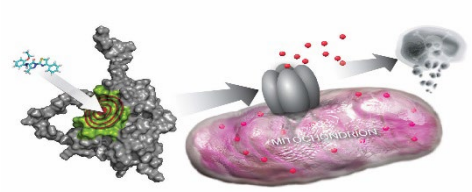

Chemical and Structural Biology of Cell Death and Survival Signaling

The Gavathiotis laboratory investigates mechanisms of BCL-2 family proteins and other key proteins in cell death and cell survival pathways such as apoptosis, mitochondrial dynamics, selective autophagy and oncogenic signaling. We harness mechanistic and structural insights to develop first-in-class small molecules that can be used for target identification and validation and serve as the basis for novel therapeutics. Our work in these pathways has pioneered novel mechanisms, pharmacological strategies and first-in-class small molecules targeting challenging and "undruggable" targets. In this process, we have developed innovative approaches based on computational and biophysical methods as well as chemical strategies to enable the discovery and design of small molecules. Our studies have led to several prototype therapeutics that are undergoing development towards Investigational New Drug (IND) application for oncology and aging-associated diseases. 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

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. While our primary focus is the protein interaction network of the **BCL-2 family of proteins** and its role in regulating **apoptosis,** we have expanded our research in understanding related pathways and mechanisms of proteins involved in **mitochondrial fusion and fission, selective autophagy, MAPK signaling pathway** and **senescence**. Using chemical and structural biology, biochemical, biophysical and cell biology studies, we aim to elucidate the mechanisms that define the very determinants that **modulate life and death decisions in healthy and malignant cells.** These studies advance our understanding of fundamental biological mechanisms and provide novel targets for drug development.

Here is list of key contributions of **molecular mechanisms and insights** from our laboratory:
1) mechanism of pro-apoptotic BAX activation pathway and the role of the BAX trigger site in regulation of BAX conformational activation and mitochondrial translocation, oligomerization and outer membrane permeabilization in apoptosis (*Nature 2008, Molecular Cell 2010*, *Nature Chemical Biology 2012, J. Biological Chemistry 2015, Molecular Cell 2016*). **2)** Structure of autoinhibited BAX dimer and mechanism of BAX autoinhibition regulating apoptosis (*Molecular Cell 2016*) **3)** Allosteric mechanism of BRAF activation by inhibitors and structural basis of RAF inhibitors activity against oncogenic BRAF (*Cancer Cell 2016*) **4)** Structural basis of Mitofusins conformational activation and inhibition regulating mitochondrial fusion and fission *(Nature 2016).* **5)** Allosteric mechanism of BAX inhibition and discovery of BAX small molecule allosteric site regulating BAX conformational activation *(Nature Chemical Biology 2019).* **6)** Allosteric mechanism of BRAF dimerization control and discovery of BRAF allosteric site *(Nature Communications 2020).* **7)** Allosteric mechanisms of BAX inhibition through engagement of the BAX trigger site and α1-α2 loop (Molecular Cell 2016, *Nature Communications 2021).* **8)** Mechanisms of small molecule BAX activation in cancer cells and induction of apoptosis and its regulation by anti-apoptotic proteins BCL-XL and BCL-2 (Cancer Cell 2017, *Nature Communications 2022)* **9)** Connecting inhibition of mitochondrial fusion to fission, mitochondrial outer membrane permeabilization, cytochrome *c* release, caspase-3/7 activation and DNA damage (*Nature*

Communications 2022). **10)** Mechanism of selective Chaperone-mediated Autophagy (CMA) activation through stabilization of RARα/NCoR1 interaction and small-molecule allosteric site *(Nature Communications 2022).* **11)** Mechanism of resistance to BH3 mimetics in AML through increased levels of mitofusin-2, mitochondria-ER contacts and mitophagy *(Cancer Discovery 2023).*

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 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** to understand biological mechanisms and prototypes for the development of **novel therapeutics**. Our **protein targets** include but are not limited to proteins of the mitochondrial cell death pathway, chaperone-mediated autophagy, mitochondrial dynamics, oncogenic kinase signaling and are typically validated in genetic models but they are considered challenging or **"undruggable"**.

Our laboratory has identified the following **first-in-class small-molecules**:
1) **activators of pro-apoptotic BAX** that demonstrated a new paradigm for pharmacologic induction of apoptosis in cancer (*Nature Chemical Biology 2012, Cancer Cell 2017, Nature Communications 2022*). 2) **activators of chaperone-mediated autophagy** through targeting RARa that protect cells from oxidative stress and proteotoxicity (*Nature Chemical Biology 2013*). 3) **activators** of **mitofusins** that promote mitochondrial fusion, increase mitochondrial respiration and restore mitochondrial motility in CMT2A neuropathy (*Science 2018, Nature Communications 2022*). 4) **allosteric BAX inhibitors** that inhibit apoptosis and necrosis and protect from chemotherapy-induced cardiotoxicity (*Nature Chemical Biology 2019, Nature Cancer 2020*). 5) **allosteric BRAF inhibitors** that overcome resistance to FDA-approved inhibitors in melanoma and colorectal tumors (*Nature Communications 2020*). 6) **competitive BAX inhibitors** that inhibit cell death and protect from chemotherapy-induced cytotoxicity (*Nature Communications 2021*). 7) **inhibitors** of **mitofusins** that inhibit mitochondrial fusion, promote mitochondrial fission, induce mitochondrial-outer membrane permeabilization,caspase-3/7 activation and sensitize pro-apoptotic drugs (*Nature Communications 2022*). 8) **stabilizers of NCoR1/RARa** that activate chaperone-mediated autophagy and protect from neurodegeneration in Alzheimer's models, protect from retinal degeneration and induce rejuvenation of old hematopoietic stem cells (*Nature 2021, Cell 2021, Nature Communications 2022, Cancer Discovery 2023*). 9) **kinase inhibitors** with rationally designed kinetic selectivity and favorable pharmacological properties as lead cancer therapeutics. Our integrative methodologies have identified novel targets and mechanism of action for **two FDA-approved inhibitors** presenting novel pharmacological and **clinical opportunities** towards novel therapies (*Nature Communications 2020, Nature Communications 2021*).

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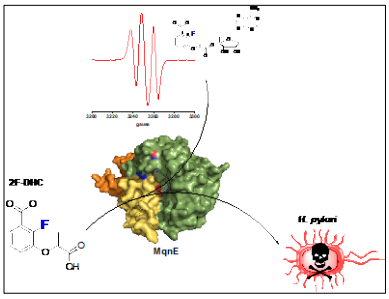

Leveraging Anaerobic Biochemistry for Novel Therapeutics

Metalloenzymes catalyze some of the most complex chemical reactions found in Nature. The Grove lab studies and exploits a wide range of oxygen-sensitive metalloenzymes for fundamental mechanistic analysis, functional annotation and inhibitor/lead discovery. The primary focus of our lab is on catalytic iron-sulfur cluster (Fe/S) enzymes involved in human health and disease. To exploit these enzymes, the Grove lab utilizes a highly multi-disciplinary approach that includes a range of classical enzymology analyses, high-resolution crystallography, mass spectrometric methods, spectroscopic approaches (e.g., Mössbauer and electron paramagnetic resonance spectroscopies), and high-throughput inhibitor screening, that allow us to define the chemical and physical determinants responsible for substrate selectivity and reactivity. In addition, we take advantage of a variety of cell biological approaches, so as to move beyond the accrual of *in vitro* activities and to begin defining the complex *in vivo* biological functions. As a new lab, we have many projects in nascent stages:

1) High-throughput drug discovery targeting anaerobic enzymes
2) Metabolic profiling and optimization of novel antivirals
3) Mechanistic understanding of Fe/S enzymes as a strategy for drug development
4) Discovery of novel chemistry of radical SAM enzymes
5) Anaerobic biochemistry involved in epigenetic reprogramming

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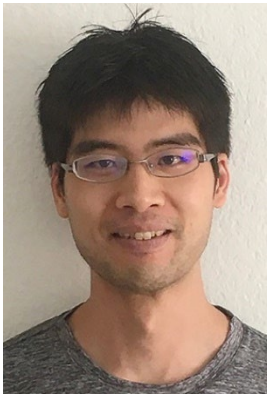

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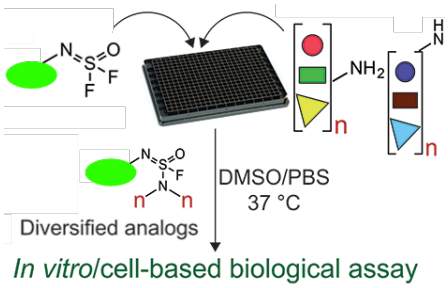

Chemical Biology, medicinal chemistry, and drug discovery

Our laboratory aims to fundamentally change the paradigms of chemical biology and medicinal chemistry, with an ultimate goal to improve human health.

The current COVID pandemic demonstrates how infectious diseases collectively represent tremendous and unpredictable challenges for humankind and how novel antivirals are urgently needed to continually improve and replenish our arsenal of therapeutics. The rate at which lead molecules derived from high-throughput screens are optimized for eventual human use (e.g. bench-to-bedside) is significantly hampered, as the modern drug discovery process is entirely reliant on the manual iteration of small molecule syntheses with biological assays. This dependence on skilled medicinal chemists makes medicinal chemistry incredibly time consuming and costly.

Our research program focusses on the development and expansion of each component of the structure-activity relationship paradigm with an emphasis on targeting host-microbe interactions both infectious microbes and commensal microbiome, and more recently cancer, with an ultimate goal to fundamentally change the medicinal chemistry and chemical biology, and eventually to improve human health.

1. **Development of fundamental methodologies in medicinal chemistry** Our laboratory aims to develop and apply generally applicable methodologies in medicinal chemistry that accelerate the drug development using the state-of-art click chemistry and other technologies.

2. **Chemical biology of host-microbe interactions** We aim to advance our understanding of host-microbe interactions, in particular microbiome, with cutting-edge chemical biology technologies, including chemical probes, HT assays, and chemical proteomics to characterize the biological roles of target proteins in the host-microbe interactions.

3. **Expanding the modalities and function of small molecules** Traditionally, inhibition of biological pathways was the major biological functions of small molecules while recently other functions of small molecules are explored such as degradation or activation of target proteins. We aim to explore new modalities and functions of small molecules. These efforts enable us to modulate biological targets that are currently considered undruggable.

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Antibody Isolation, Protein and Peptide 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. In addition, we are using B cell sorting to identify then characterize naturally-occurring antibodies for a variety of purposes. Projects are highly interdisciplinary and involve aspects such as phage display, structure-based protein design, bispecific antibody engineering, structural biology, virology, immunology and cancer biology. Current direction include:

4. 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.

5. 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.

6. 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.

7. Isolation of monoclonal antibodies by single B cell sorting. We have recently identified a novel set of human antibodies targeting alphaviruses such as Chikungunya virus and are utilizing this technique in other cases.

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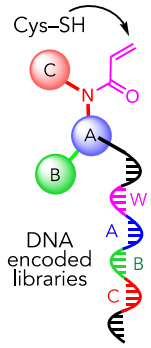

Reprogramming, Inhibiting and Sensing Enzymes with DNA-Encoded Covalent Ligands

Program 1. Covalent Ligand Discovery Using an Electrophilic DNA-Encoded Library (eDEL) Platform. Covalent mechanisms have the potential to massively expand the fraction of the proteome targeted by small-molecules, and by consequence expand the scope of biomedical problems that can be intervened. However, only a small fraction of all known and unknown pockets having reactive residues has ever been explored with covalent ligands. Across academia and the pharmaceutical sector there is a scarcity of structurally diverse screening collections having appropriately validated warheads, and a lack of technologies to identify covalently targetable pockets in allosteric and non-catalytic domains. The eDEL platform is enabled by the distinctive innovation of masked-warhead installation reagents (**Fig 1, top**), which altogether will solve a number of incompatibilities that have hindered both the assembly and screening of covalent libraries in the past. This previously untapped approach will support the synthesis and late-stage purification of the largest reported small-molecule collections of >100 million per warhead, including the acrylamides that are well validated in human therapeutics. The eDEL screens will feature improved dual-enrichment and dual-barcoding using interaction-dependent PCR (ID-PCR) and stringent biotin-affinity selection that capture a multiplexed snapshot of all combinations of target-ligand pairs in one pot (**Fig. 1, bottom**). Multiplexing intra-experiment controls will improve hit-calling and addresses the problem of stochastic noise from the warhead reactivity. In larger scale, multiplexing will enable unbiased discovery campaigns using hundreds of targets at the same time. The approach will be exemplified by a screen of all 350 human WD40 beta-propeller domains, which were selected for their strong potential to discover covalent probes to block protein-protein interactions in oncology and epigenetics targets, and to develop a new types of covalent E3-ligase binding Protacs.

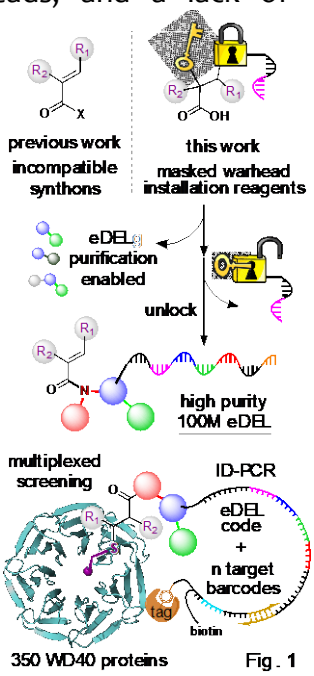

Fig. 1

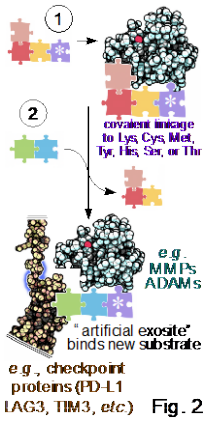

Fig. 2

Program 2. Commandeering Enzymes using Modular Reprogramming and Retargeting of Non-Cognate Substrate Interactions to Treat Human Diseases. Bioorthogonal chemistry has achieved unprecedented milestones over the past decade, which sets the stage for the next frontier of *in vivo* chemistry: manipulating components of the native proteome without pre-installed genetic modifications. Reprogramming enzymes represents a new therapeutic modality to convey spatiotemporal, multi-turnover, and tissue/cell-type targeting selectivity features that are not achievable with conventional small molecules or biologics. However, we have not yet discovered the methods and the parameters to systematically reprogram enzyme-substrate interactions in native biological contexts. This program will create the first generalizable toolkit of modular reactants to control and retarget pleiotropic enzymes towards processing non-cognate substrates for

therapeutically relevant purposes. Several methods will be devised to exploit fast and chemoselective proximity-directed bioorthogonal reactions (**Fig. 2, top**), and subsequently connect the enzyme to an "artificial exosite" comprising a modular small-molecule ligand or nanobody that will recruit the unnatural substrate (**Fig. 2, bottom**). The challenge of navigating the vast combinatorial space of the various tunable components, substitution sites, and tether lengths, can be addressed by a DNA-barcoding library that is scalable to explore a large collection of proteins. The ligands and bioreactants proposed herein are suitably biocompatible for research in animal and xenograft models, which will enable the exemplified proof-of-concept case studies of enzyme reprogrammers that modulate the coagulation cascade and that inactivate checkpoint proteins for tumor-localized T-cell activation as a new modality for immuno-oncology therapeutics (**Fig. 2, bottom**).

Program 3. DNA-Encoded Discovery of High-Specificity Substrates for Bioorthogonal Quantitative Enzymology Ex Vivo and In Vivo. Our ability to interrogate the complexity of biological pathways depends on our toolkit of probes and reporters, particularly on their potency, specificity, sensitivity, and compatibility with living systems. However, investigators are often forced to make qualitative and indirect inferences about biochemical events due to the lack of substrate reporters to monitor the activity of many enzymes directly and selectively. Current approaches such as substrate profiling are limited to proteases using natural peptide sequences, which typically lack the aforementioned qualities to be used as enzyme probes within a biological context. To address this technological gap in the field of enzymology, we will establish the first DNA-encoded substrate library (DESL) platform to discover reporter substrates that disassemble through a variety of enzyme-triggered mechanisms (**Fig. 3, top**). The DESL platform is uniquely tailored for small molecule-like substrate scaffolds that can be engineered as activity-based reporters for a broad range of enzymes in signaling cascades, post-translational modifications and epigenetics, which are challenging to study with other methods. The substrates identified by PCR amplification and DNA sequencing, can be re-configured for a host of highly sensitive and quantitative readouts such as fluorescence, luminescence, and isotopic mass-tags, upon replacement of the DNA barcodes (**Fig. 3, bottom**). More broadly, the DESL small-molecule structures are poised to be systematically optimized beyond basic research applications in biomedical fields such as non-invasive activity-based diagnostics, pro-drugs and antibody-drug conjugates, high-throughput phenotypic screens, and cleavage-dependent biosensor applications.

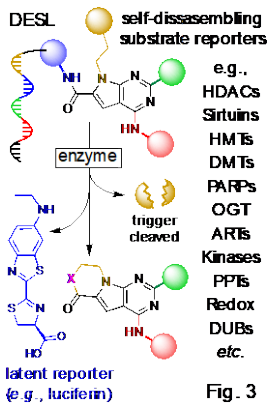

Fig. 3

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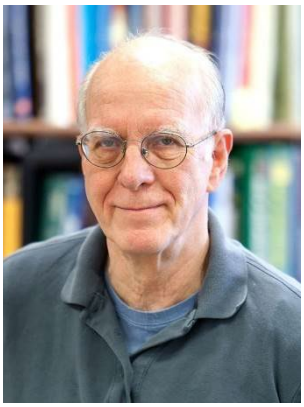

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Structure, function, dynamics and time resolved X-ray crystallography of Cytochrome c oxidase

In our laboratory the mechanisms and properties of two enzymes, cytochrome c oxidase and nitric oxide synthase, are being investigated as well as the molecular basis of protein folding. Cytochrome c oxidase is the terminal enzyme in the electron transfer chain. Physiologically, it reduces oxygen to water and utilizes the excess energy to translocate protons across the mitochondrial membrane. The enzyme is responsible for over 90% of the oxygen consumption by living organisms in the biosphere; yet the mechanism of its basic function, the coupling between the redox processes and proton translocation is undetermined. Our objective is to obtain a quantitative description of the manner by which oxygen is reduced to water by exploiting laser spectroscopic methods and rapid mixing techniques developed in our laboratory. These studies will allow us to identify all of the intermediates in the catalytic reaction and thereby establish the molecular basis for one of the most important processes in bioenergetics. Nitric oxide has been found to play many diverse physiological roles ranging from a neurotransmitter, a vasodilator and a cytotoxic agent. The enzyme that catalyzes the formation of NO from oxygen and arginine is nitric oxide synthase, a very complex enzyme containing several cofactors and a heme group which is part of the catalytic site. We have discovered that NO the enzymatic product, inhibits the enzyme and are now studying the mechanism of the inhibition process. In addition, we are studying a variety of inhibitors of the enzyme to sort between the many mechanisms of inhibition that are possible in nitric oxide synthase. These studies will serve as a foundation for the development of drugs that can be used to treat many different syndromes associated with the under- or over-production of NO. How a protein folds into its three dimensional structure is one of the central questions in molecular biology and in the biotechnology industry. To advance the understanding of protein folding, it is necessary to determine the structures and the kinetics of the intermediates in the folding pathway. For this, we developed submillisecond mixers in which folding can be initiated in less than 100 microseconds, a time scale that is over an order of magnitude faster than previously possible. This already has allowed us to discover a new model that accounts for the folding of cytochrome c from 100 microseconds to the formation of the native state. Many new experiments with several different techniques will be done to test the generality of this model and its possible role in other proteins.

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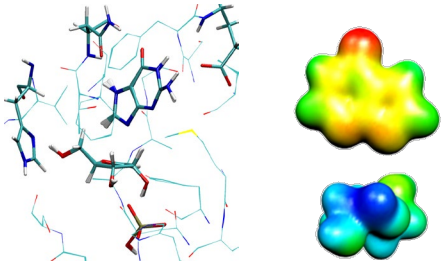

Enzymes catalyze virtually all of the chemical transformations necessary for biological life. Knowledge of the transition-state structure of enzymatic reactions permits the design of powerful inhibitors. Methods have been developed in this laboratory for the determination of geometry and charge features 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 (Ulodesine®) has completed phase 2 clinical trials for gout. We are characterizing the enzyme-drug interactions at the kinetic, atomic and drug-resistant 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. Galidesivir®, first characterized here, is in clinical trials for Covid-19 and Yellow Fever in Brazil.

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 develop 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.

Clostridium difficile is a growing health problem. We use transition state theory to design and synthesize antitoxins to fight this growing infectious disease threat.

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. We collaborate in NMR, X-ray crystallography, mass spectroscopy, synthetic organic chemistry, cancer and

medicine projects. 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:

https://www.ncbi.nlm.nih.gov/myncbi/1F_tvj88Sd_kO/bibliography/public/

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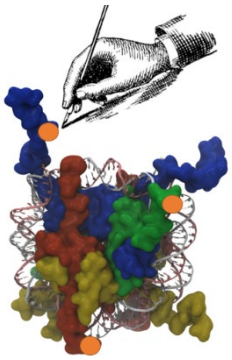

Recipes for Regulation of the Genome:
Charge, Grease, and Intrinsic Disorder

Our goal to understand how the eukaryotic genome is regulated. In the nucleus, DNA is packaged with histones into chromatin, forming a complex substrate for all genetic transactions. Post-translational modifications of histones, RNA-binding proteins, and other nuclear factors regulate how the genome is assembled, transcribed, and processed. This "epigenetic" information is the landscape on which the dynamic usage of genetic information is encoded. Many of these epigenetic regulatory proteins are enriched in intrinsically disordered regions that are subject to hydrophobic or charge-shifting modifications like methylation, acetylation, and glutamylation.

To improve our basic understanding of biology, human disease, and to develop new therapeutic approaches, we aim to understand how these epigenetic modifications work. Our studies are critically important for understanding diseases such as lung cancer and leukemia and have wide-ranging implications for developmental biology and even neuroscience. In this context, our primary research interests are in understanding the basic biochemistry, drug discovery, cell biology, and cancer biology of:

- Protein Arginine Methyltransferases (PRMTs 1-9), their protein substrates, and the "readers" of methylarginine
- Histone chaperone proteins (NPM1, NPM2, and NAP1) and structural and molecular mechanisms of their intrinsically disordered regions
- Chaperone post-translational glutamylation and deglutamylation by the TTLL and CCP enzymes

We utilize a unique and interdisciplinary approach to address these topics, including: protein biochemistry and quantitative enzymology; structural biology (NMR, crystallography, CryoEM); cancer cell culture and genome wide studies (RNA-Seq, ChIP-Seq, and RNA-processing); quantitative microscopy, and occasionally 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 cellular studies–provides an uncompromised approach to fully understanding epigenetic phenomena and how to apply this knowledge towards improving human health.

Students in the lab will obtain strong training in critical thinking, experimental design, data analysis, literature review, writing, and all aspects of the modern scientific career paths. Students are encouraged to think independently and drive the research forward.

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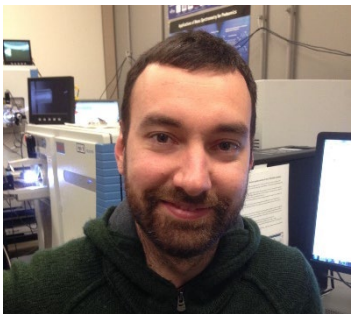

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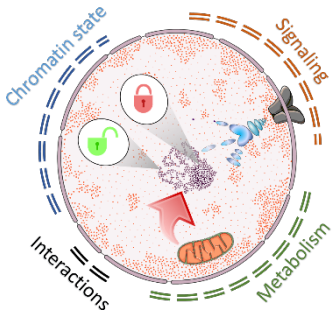

Technology for chromatin biology

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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Structure and Function of Biological Macromolecular Machines

In my laboratory, we use combination of biophysical approaches such as **cryo-electron microscopy** and **single molecule fluorescence microscopy** to elucidate the mechanism of action of macromolecular complexes involved in key cellular functions. Currently our main focus is on proteins associated with the **cytoskeleton** and in particular the **kinesin** superfamily of motor proteins. Kinesins play essential roles in intracellular motile processes such as organelle transport and cell division. Understanding how kinesins and other cytoskeletal proteins work will help the development of treatment for several human diseases. Absence or malfunction of kinesins has been associated with neuro-developmental defects, motor neuron disease, Alzheimer's disease, retinitis pigmentosa and liver and kidney diseases. Kinesins are also becoming an important target for anti-cancer drugs.

There are more than 100 different proteins that belong to the kinesin superfamily (41 in humans) which is defined by the presence of a catalytic or motor domain (~340 amino acids) where the chemical energy from ATP hydrolysis is coupled to mechanical work production. The motor domain is highly conserved among all kinesins, yet there are kinesins with very different functionalities. Most kinesins are molecular motors that walk or generate forces along microtubules but there are several kinesins that depolymerize microtubules and are important regulators of microtubule dynamics in-vivo.

It is still not fully clear what conformational changes do kinesins go through during movement or how very similar motor domains can perform seemingly very different functions, such as walking or depolymerizing microtubules. Using cryo-electron microscopy and other approaches we are elucidating the mechanism of action of cytoskeletal motors and other biological machines at the atomic level. Using these approaches we are also investigating how small molecules with therapeutic potential bind and modulate the activity of cytoskeletal proteins.

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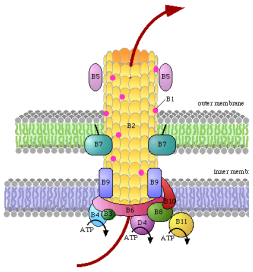
Depiction of a Type 4 Secretion Machine



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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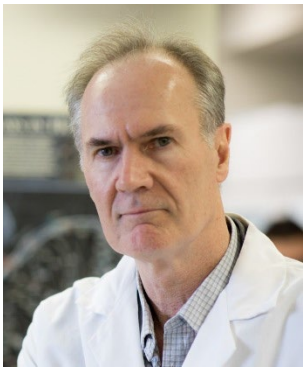
- Although my laboratory is now closed, I continue to advise Biochemistry students on Journal Club and WIP presentations. I also teach in the Graduate School Human Metabolism and Disease course.

- As Einstein's Assistant Dean for Biomedical Science Education, I teach MD students and play an active role in the Office of Medical Education in curricular change, educational administration and integration of foundation science with clinical curriculum.



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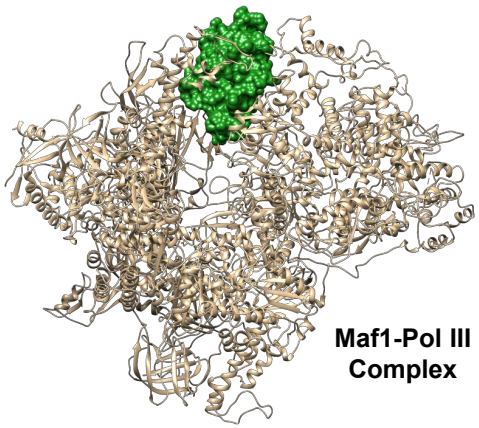

Maf1-Pol III Complex

RNA Polymerase III Transcription in Health and Disease

Our laboratory studies gene transcription by RNA polymerase (Pol) III and the functional impact of this system on normal and disease processes. The products of Pol III transcription are small non-coding RNAs that have diverse and expanding functions in eukaryotic cells. These include RNAs that are central players in protein synthesis and cell growth, molecules that function in RNA processing, protein secretion and autophagy, and other regulatory RNAs. Proper regulation of Pol III transcription is critical for balanced growth and its deregulation is a key event in cell transformation and tumorigenesis. Conversely, loss of function mutations in Pol III subunits are known to cause neurodegeneration and to compromise the innate immune response following viral infection. Our research seeks to understand the molecular bases of these pathologies and to discover novel biological functions and mechanisms that are impacted by perturbing Pol III transcription. Our programs span genetics, molecular biology, biochemistry, metabolism and structural biology and utilize budding yeast and mice as model experimental systems. Two important areas of concentration are highlighted below.

Mechanisms of Obesity Resistance in *Maf1* Knockout Mice

Maf1 is a master negative regulator of Pol III transcription that controls ribosome and tRNA synthesis at the downstream end of critical nutrient- and stress-signaling pathways. Remarkably, mice with a whole body knockout of *Maf1* are resistant to diet-induced obesity and have increased health span. Obesity resistance in these mice is associated with increased energy expenditure and metabolic inefficiency. Our current mechanistic understanding of these phenotypes is based on a novel futile RNA cycle hypothesis wherein deregulated Pol III transcription serves as an energy sink, consuming energetically costly nucleotides in the wasteful synthesis of RNA that does not accumulate and is mostly degraded. Current research on this unique model is focused on understanding global (whole body) and cell-specific molecular and metabolic changes that enhance energy expenditure and contribute to the lean phenotype. MAF1 targeted therapeutics may be effective in treating obesity and improving health span.

Mechanisms of Neurodegeneration in Pol III-related Leukodystrophy

Pol III-related leukodystrophy was recently identified as a genetically inherited neurodegenerative disease. The disease typically manifests in children as a progressive decline in motor function and cognitive regression with additional variable neurological and non-neurological features. We have developed several mouse models of this disease and have characterized numerous behavioral and neuropathological phenotypes. Our research seeks to understand the mechanisms of pathogenesis in different neural cell populations and their molecular basis. We are also conducting pre-clinical testing of genetic and pharmacologic strategies as potential therapeutic treatments.

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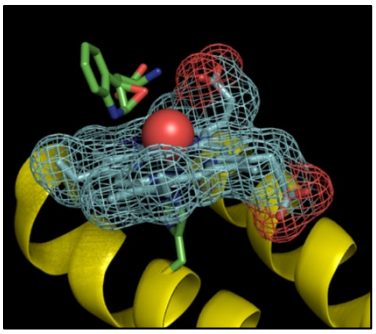

Molecular Mechanisms and Structural Biology of Hemeproteins

We are interested in understanding how proteins, in particular hemeproteins, work within their cellular environment in health and disease. The three-dimensional structures of hemeproteins are designed by nature to perform a variety of biological functions raging from delivering electrons, transporting oxygen, sensing small gas molecules, to oxidizing inert organic substances. These biological activities are typically finely tuned by translational / post-translational regulations and/or by intermolecular interactions with small cellular metabolites or macromolecules in response to environmental stimuli. Our current research is focused on the comprehension of structure-and-function relationships of a group of human hemeproteins called dioxygenases, including tryptophan dioxygenase (TDO) and two isoforms of indoleamine 2,3-dioxygenase (IDO1 and IDO2), at the molecular level.

The three dioxygenases catalyze the first and rate-limiting step of the Kynurenine pathway, the major metabolic pathway of tryptophan (Trp), by degrading it to N-formyl Kynurenine. Through controlling the Trp metabolism, these enzymes downregulate autoimmunity by depleting Trp, the least abundant essential amino acid required for immune cell growth, and by producing immunosuppressive Trp metabolites. The malfunction and/or dysregulation of the dioxygenases leads to a wide variety of disorders, such as inflammatory diseases, cardiovascular diseases, diabetes, mental disorders, and cancer. Comprehending the functional and regulatory mechanisms of these enzymes in healthy and disease states can thus identify novel opportunities for targeted therapies.

To achieve our research goals, we employ diverse and complementary approaches spanning from spectroscopy, crystallography, high throughput screening, medicinal chemistry, protein engineering to computational biology. We develop novel techniques, such as spectroscopy-guided X-ray crystallography and serial crystallography, to understand the evolution of protein allostery, the choreography of biochemical pathways and enzyme catalysis.

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DEPARTMENT OF BIOCHEMISTRY
STUDENT JOURNAL CLUB EVALUATION

Date:_____ ***Graduate Student:_____***

Title of Journal Article, Journal, Year:

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Critique & Thoughts on:

Introduction: Was the significance of the paper made clear? Was sufficient background information provided to understand the presentation?	
Explanation of methods: Were the usefulness and necessity of the methods elaborated upon? Detailed description given for unique work?	
Presentation of Figures: Were the figures understandable as described and were they sufficient to convey the **main** results?	
Summary of Conclusions: Were the overall findings summarized and r**elated back to the background/original intent** of the paper?	
Student's Critiques of the paper: Were strengths and weaknesses of the paper discussed by the student prior to the Q&A session?	

Additional feedback (positive highlights, any weak points, thoughts on Q&A, presentation skills, etc.):

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Journal Club Faculty/Student Consultant

Name:_____ ***Signature:_____***

DEPARTMENT OF BIOCHEMISTRY
WORKS IN PROGRESS EVALUATION

Date:_____ ***Graduate Student:_____***

Title of Presentation: _____

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Critique & Thoughts on:

Introduction: Is the **current state** of the scientific field addressed? How does the student's thesis work fit into it?	
Experimental Methods: Are the methods described clearly enough so that you understand why they are appropriate for the intended results?	
Results: If results are present, **are they related back to the introduction** and critically assessed?	
Q&A: Does the student convey the boundaries of their understanding and perform impromptu critical thinking toward questions?	

Additional feedback (positive highlights, any weak points, presentation skills, etc.):

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Works-in-Progress Faculty/Student Consultant

Name:_____ ***Signature:_____***