DEPARTMENT OF BIOCHEMISTRY

Departmental guide for first year students

Departmental graduate student policies, guidelines and procedures, and faculty research interests

2016 – 2017

(Updated as of 8/26/16)
Welcome to the Graduate Program in the Department of Biochemistry. This booklet introduces you to departmental policies and procedures that help you earn a Ph.D. degree in Biochemistry. The topics range from acceptance into the Department for thesis study to your defense of a Ph.D. thesis. Included are discussions of our graduate course requirements, faculty advisement, a list of courses led by members of the department and summaries of the research of our faculty.

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

A. Einstein Graduate Program in the Biomedical Sciences:

Officials and Office Personnel

Einstein Graduate Program Office: Room 201 Belfer Building

**Dr. Victoria Freedman**
Associate Dean for Graduate Programs in the Biomedical Sciences
Assistant Professor of Microbiology and Immunology
430-2872

**Ms. Sheila Cleeton**
Assistant Director and Registrar, Graduate Division
430-2345

**Dr. Myles Akabas**
Professor of Physiology and Biophysics, Neuroscience and Medicine
Director, Medical Scientist Training Program
430-3360

**Dr. Joan W. Berman**
Senior Academic Advisor, Graduate Division
Professor of Pathology and Microbiology & Immunology
430-2587

B. Department of Biochemistry

Officials and Office Personnel: Room 308 Forchheimer Building

**Dr. Steven Almo**
Professor and Chairman of the Department of Biochemistry
430-2813

**Ms. Leslie Jefferson**
Administrator, Department of Biochemistry
430-2270

**Ms. Patricia Corr**
Secretary to the Chairman
430-2814

Departmental Office Staff, 430-2814

Ms. Shaunice Dawkins, Ms. Celia James, Ms. Joanna Vega
II. DEPARTMENTAL REQUIREMENTS

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

A. Research Requirement

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

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

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

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

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

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

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

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

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

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

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

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

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

C. Biochemistry Student Journal Club and Works in Progress Seminars

Upon declaring the Department of Biochemistry for their thesis research, graduate students participate in the Journal Club and Works in Progress held at noon on Fridays in the departmental seminar room. Attendance at the weekly meetings begins immediately. Presentations by the student begin at the start of the semester following declaration. This is
typically the fall semester of the second year for students entering the department from the rotational pathway. Each student presents one journal club and one works in progress each calendar year. Fifth year or senior 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 journal club presentations, students are randomly assigned a faculty advisor for their presentation at the beginning of the semester. Journal club and works in progress presentation scheduling is at the discretion of the faculty advisor to the program as discussed below. A written record for each Journal Club and Works in Progress is filed with the departmental office. Forms for evaluation of the Journal Club and Works in Progress performance is in the Appendix. The criteria for the evaluations are discussed below. A detailed description of Journal Club and Works in Progress guidelines can be found in the Appendix.

D. Student Advisory Committees

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

*The Department of Biochemistry requires that advisory committee meetings be held twice each year.* Initially, a committee can provide guidance in crafting the thesis project, selection of 2nd 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.

The departmental oversight committee will review the composition of a student’s advisory committee and must approve changes made in subsequent years. Both the mentor and the advisory committee must endorse a request to the departmental Chairman for a leave of absence. Any other changes in a student’s status will be reviewed by the same procedure. The Chairman of the Department and Chairperson of the departmental student oversight committee are *ex officio* members of all advisory committees and may attend a meeting at their discretion.

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

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

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

F. Qualifying Examination

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

G. Thesis Examination

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

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

H. Time to Degree

The faculty of the department shares the national concern with the increasing time to achieve the Ph.D. degree. Our goal is to reverse this trend. Students typically complete their graduate training 4 to 6 years from their entry into the graduate school. Written permission of the mentor, the student advisory committee, the departmental oversight committee and the Chairman of the Department of Biochemistry is required for study to continue beyond 6 calendar years. The Graduate School requires that students admitted to doctoral programs register each semester, unless a leave of absence has been applied for and granted. This requirement applies to students engaged in thesis research who are not enrolled in any courses.

III. Faculty and Research Interests

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

IV. Contents of On-Line Appendices

The appendices previously included in this document are being transferred to the departmental website. In the interim, copies of all documents can be obtained from the offices of the Biochemistry Department or the Graduate Division.
The following subjects are discussed in the Appendices:

- Responsibilities of the Biochemistry Graduate Committee and the Academic Standards Committee
- Admissions Procedures for the PhD and MD-PhD Programs
- Journal Club and Works-In-Progress Seminar Guidelines and Procedures
- Thesis and Final Examination Guidelines and Procedures
- Acknowledgement of Research Support
- Graduate Courses Taught by Biochemistry Faculty
# Graduate Faculty in Biochemistry
## Statements of Research Interest

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<td>Structural Immunology &amp; Functional Annotation</td>
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<td>Blanchard, John S.</td>
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<td>Brenowitz, Michael</td>
<td>Structure &amp; Function of Proteins and Nucleic Acids</td>
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<td>Schramm, Vern L.</td>
<td>Enzymatic Transition States and Logical Inhibitor Design</td>
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<td>Steinman, Howard M.</td>
<td>Acquisition of Virulence Phenotypes by Bacterial Pathogens</td>
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<td>Willis, Ian M.</td>
<td>Gene Transcription, Regulation of Cell Growth and Metabolism</td>
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Faculty with Secondary Appointments in Biochemistry
(For Research Interests, please see their respective departments.)

Angeletti, Ruth H.
Developmental & Molecular Biology

Proteomics and Mass Spectrometry

Backer, Jonathan M.
Molecular Pharmacology

Signaling by Phosphoinositide Kinases

Briehl, Robin W.
Physiology & Biophysics

Physical Chemistry, Structure & Pathogenesis of HbS

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
The major focus of our lab is the structural, functional and mechanistic analysis of the cell surface molecules responsible for controlling the human immune response. These studies support the development of new immunotherapies to treat a wide range of malignancies, autoimmune diseases and infectious diseases. A second area of interest is the development of new strategies for enzyme functional annotation and the discovery of new metabolism, with the long-term goal of defining the entire metabolic repertoire present in nature. These programs leverage extensive automation and robotics for high-throughput cloning, protein production, X-ray structure determination, receptor-ligand de-orphaning and function discovery.

**Structural, functional and mechanistic analysis of the cell surface and secreted proteins that modulate human 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 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\(^1\), PD-1:PD-L2\(^2\), DcR3:TL1A\(^3\), DcR3:LIGHT, DcR3:FasL and HVEM:LIGHT, as well as B7-H3, B7-H4\(^4\), TIM-3, NTB-A, CD84, GITRL, TIGIT, CRTAM, nectins and CD160, all of which are potential/proven targets for immunotherapy. These efforts have resulted in a new paradigm for immunotherapy, which allows for “clonal-specific” modulation of human T cells. In contrast to all existing immunotherapies, which result in global modulation (i.e., all T cell are effected), our strategy selectively and uniquely modulates only disease relevant T cells, leaving the vast majority of the repertoire untouched, thus eliminating considerable undesirable and life-threatening side effects. Directly related to these efforts is the development of new approaches for receptor-ligand deorphaning. While a large number of cell surface molecules are implicated in immune modulation, the vast majority are uncharacterized in terms of their extracellular binding partners. Using our highly automated platforms, we are seeking to define the entire set of extracellular interactions (e.g., the ecto-interactome).


**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. For example, using our high-throughput infrastructure we expressed, purified and screened 158 SBPs against a small molecule library by differential scanning fluorimetry (DSF). These efforts led to the identification of 40 new SBP ligands, the generation of experiment-based annotations for 2084 individual SBPs, and the definition of numerous novel metabolic pathways\textsuperscript{5}. Other comparable large scale functional annotation studies were performed for the Isoprenoid Synthase\textsuperscript{6} and Haloacid Dehalogenase\textsuperscript{7} Superfamilies.

One of the greatest challenges in this area of functional annotation is the ability to identify new metabolites and new protein:metabolite interactions. The achievement of these goals is hindered by limitations associated with the physical and virtual libraries used in experimental and computational approaches. The great expanse of chemical space and the unknown array of biologically relevant transformations represent continuing and significant obstacles. Indeed, all current approaches for metabolite discovery suffer from our incomplete understanding of metabolomes and the difficulties associated with the synthesis of many important metabolites; i.e., we can only study those metabolites which are both known to exist and can be obtained in sufficient quantities for experimentation. To overcome these bottlenecks, we are pursuing “metabolome panning”, in which entire metabolomes of culturable organisms are used as screening libraries, with recombinant target proteins acting as affinity reagents to select cognate (or near-cognate) small molecule binding partners from the expression host or lysates generated from the organism of origin\textsuperscript{8}. The strategy of “metabolite panning” affords unique opportunities, as the complete metabolomes 1) contain previously unknown metabolites\textsuperscript{8}, 2) contain metabolites which present significant synthetic challenges, and 3) are composed of components which, by definition, are physiologically relevant. These approaches, in combination with informatics and additional experimental confirmation, represent novel strategies for the discovery of new metabolites, new metabolism and new biology.


High-throughput Protein Production. Despite a multitude of recent technical breakthroughs advancing 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. We have established high-throughput bacterial expression platforms, and unique to the Almo group is the world’s first integrated system for high-throughput functional and structural biology of oxygen-sensitive proteins. This resource has allowed for the recapitulation of the entire high-throughput protein production and crystallization pipeline within an oxygen-free environment (see http://www.nysgrc.org/psi3/an aerobic.html). We have also established robust high-throughput eukaryotic expression platforms, including insect and mammalian-based systems. We have extensively described the capabilities of our protein production platforms in the literature\textsuperscript{9}. 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.

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

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

\textbf{Selected References}

The Reversible Acetylation and Inactivation of \textit{Mycobacterium tuberculosis} Acetyl-CoA Synthetase is Dependent on cAMP. Xu, H., Hegde, SS and \textbf{Blanchard, JS} (2011) \textit{Biochemistry} \textbf{50}, 5883-5892.


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 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 causes the neurological disorder Rett Syndrome.

- Our interest in RNA structure and folding embraces RNA aptamers, small RNA molecules selected to bind to proteins and cells with high affinity as potential diagnostic tools or therapeutics. We are studying the structure and thermodynamics of aptamer – protein complexes in order to illuminate the principals of aptamer binding and perhaps build biologically efficacious aptamers.

- We are developing and utilizing 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.

Selected Recent Publications


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.

**Selected References**


THE STRUCTURES AND DYNAMICS OF PROTEINS

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

Structure and Dynamics of Enzymes

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

The Dynamics of Protein Folding

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

Selected References


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 (IU) 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. A systems biology approach is currently underway to address this hypothesis. Specifically, genome wide changes in gene expression, DNA methylation and histone modifications are being characterized in livers at late gestation (e18.5), post-weaning (5 wks) and at 6 mos of age. 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. Future studies that incorporate ChIP-Seq and genome wide HELP assays will be used to define the epigenetic basis underlying IU exposure to a maternal high fat diet. Additionally, we are interested in identifying the components of the maternal diet that are linked to a poor metabolic phenotype later in life of offspring. By varying dietary fat content (amount and type of fat) 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.

Additional studies are focused on two members of the glucose transporter gene family (GLUT4 and GLUTx1/GLUT8). GLUT4 is insulin and exercise responsive and is the major glucose transporter expressed in cardiac and skeletal muscle and adipose tissue. GLUTx1/GLUT8 is a newly identified member of the glucose transporter gene family that is expressed in many tissues (including testis, brain, liver, placenta and various tumor cells) in addition to GLUT4-expressing tissues (e.g. heart, adipose, muscle). Glucose homeostasis depends mainly on controlled changes in glucose transport in insulin-responsive tissues such as muscle and adipose 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 (e.g. diabetes mellitus and obesity). GLUT8 is upregulated in several tissues in response to GLUT4 ablation and may represent a novel anti-diabetic target. We are also studying the potential role of GLUT8 in tumor cell growth and metabolism that may lead to novel chemotherapeutic modalities. We have shown that GLUT8 is significantly upregulated in human endometrial adenocarcinoma and in rodent mammary tumors. GLUT8 expression was highest in the most aggressive human tumors that may provide a growth advantage. Present studies are directed toward understanding the regulation of GLUT8 in normal and disease states (e.g. T2DM and cancer).

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. Novel insight gained from these studies demonstrate a role for glucagon in normal development of pancreatic islets and in hepatic substrate utilization, energetics and survival. Additionally, gene knockout studies provided proof of concept that pharmacologic inhibition of hepatic glucagon action can lower fasting glycemia and may be useful in treating hepatic insulin resistance in diabetics.
Selected References


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 mechanism of the intein reaction, internal splicing of proteins, is of general interest for protein engineering and as a model for several post translational 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. Improved procedures are being developed for sample preparation, by segmental isotopic labeling of proteins, so that only selected domains are studied, and by other protein engineering approaches to assess the roles of post translational modifications. Several kinases and phosphatase systems including Csk, Abl, PTPN22 and receptor kinase FGFR are under study.

Selected References

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Areas of Research: chemical biology, structural biology, medicinal chemistry, drug discovery, protein-protein interactions, structure-function, cell death, mitochondria, autophagy, oncogenes, cancer, cardiovascular disease

The Gavathiotis laboratory's research aims to elucidate and target molecular signaling mechanisms of cell death and cell survival that are deregulated in cancer and other diseases. Our goal is to translate our mechanistic and structural insights of protein interactions into novel pharmacological strategies and chemical probes that can be used for target validation and serve as the basis for novel therapeutics. To achieve our goal, we take an interdisciplinary approach using chemical synthesis, structure-based design, structural biology (NMR and X-ray crystallography), biochemistry, cellular and in vivo pharmacology.

Molecular Mechanisms of BCL-2 Family Proteins in Cell Death Regulation
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 programs leads to variety of disease conditions and understanding the molecular mechanisms that govern cell death signaling pathways is both fundamentally important and medically relevant. Our focus is the protein interaction network of the BCL-2 family of proteins and its role in regulating apoptosis, necrosis and mitochondrial dynamics. Our current work, using structural biology, biochemical, biophysical and cell biology studies, aims to elucidate the mechanisms of protein-protein interactions and post-translational modifications to define the very determinants that modulate life and death decisions in healthy and malignant cells.

Chemical Biology of Cell Death and Chaperone-Mediated Autophagy
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 proteins. We use these probes to interrogate the signaling pathways and understand the biological mechanisms. Probes are also used as templates for the development of novel therapeutics. Our targets include but are not limited to proteins of the mitochondrial cell death pathway and chaperone-mediated autophagy that are highly validated in in vivo models and are considered challenging or "undruggable". For example, using structure-based drug design we identified: 1) the first small-molecule activator of pro-apoptotic BAX and demonstrated a new paradigm for pharmacologic induction of apoptosis and ii) the first class of small molecules that activate chaperone-mediated autophagy and protect cells from oxidative stress and proteotoxicity.

Molecular Mechanisms and Targeting of the MAPK/ERK Signaling Pathway
Aberrant regulation of cellular signaling pathways can lead to uncontrolled cell growth and proliferation leading to malignant transformation and tumorigenesis. Constitutive activation of the mitogen activated protein kinase (MAPK) signaling pathway is a highly frequent event in human cancer, which results from mutations in key components of the pathway or by mutations in upstream activators of the pathway. We are using chemical and structural approaches to elucidate and target novel mechanisms that regulate critical components of the MAPK signaling pathway e.g. RAS, RAF, MEK and ERK proteins. Our goals are to advance our understanding of the structure-function relationships regulating important components of the MAPK signaling pathway and provide new avenues for drug development overcoming resistance mechanisms to current therapies.

Selected References


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Transporter Structure, Function, and Inhibition using NMR Spectroscopy

Membrane proteins are responsible for transmembrane signaling, energy transduction, and ion and metabolite transport – making them important players in infectious disease, genetic disorders, and cancer. Despite their importance, their numbers (20 to 35% of the protein sequences in all genomes), and the need for structure to understand their function, they only make up a tiny fraction of the structures in the Protein Data Bank. Obviously these proteins have resisted routine structural analysis. Our overall research goals are to develop and apply new solution conditions and NMR methods to determine structures of membrane transporters and pumps, alone and as drug-protein complexes, in order to understand how they function and guide the development process of new or improved antibiotics.

The F₀ portion of the F₁F₀ ATP synthase is one focus of present efforts in the lab, both as a model system and because of its importance. This complex is responsible for synthesizing the vast majority of cellular ATP - over 80 pounds of ATP per day in the average human. In the ATP synthase, H⁺ translocation across the membrane through F₀ provides the driving force for ATP synthesis on F₁. A ring of ten copies of subunit-c make up the bulk of the F₀ complex. This subunit is responsible both for translocating H⁺ through F₀, and generating long range conformational changes transmitted over 100 Å to the ATP binding sites on F₁. We have determined the complete structures of subunit-c in both its protonation states, providing a detailed picture of the conformational changes linked to energy transduction. The c-subunit of the ATP synthase was recently shown to be a useful, genus-specific target for antibiotics (against M. tuberculosis and Strep. pneumonia, for example). Hence we are investigating the structural basis for the specificity of inhibitors with the human, M. tuberculosis, Strep. pneumonia, and E. coli homologs of the protein.

A simpler, but still biomedically important transporter is the small multidrug resistance pump from Staphylococcus aureus. Bacteria have developed several methods to resist the lethal effects of antibiotics. The broadest spectrum resistance results from the action of Multidrug Resistant Pumps (MDRs), which extrude a range of compounds of quite diverse chemical structure. The Small Multidrug Resistance pumps (SMRs) are 100-110 residue dimeric proton-drug antiporters that contain the full multidrug transport machinery, stripped to its barest essentials. Hence they are ideal transporters for a comprehensive structural and functional understanding of drug transport and inhibition in a medically important MDR. We are determining the structures of the conformations that make up the functional cycle of an SMR, and identifying the binding determinants for multiple drugs and inhibitors using solution NMR.

And finally, newer inter-group collaborative efforts include the fusion machinery of HIV, Ebola, and Marburg viruses, a structural analysis of Alzheimer's precursor protein processing inhibition, and RNA aptamer/protein complexes.
Selected References


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Peptide and protein chemistry

Our lab combines biochemistry, biophysics, organic chemistry, virology, and cell biology to study and engineer proteins and peptides for biomedical applications. We are currently engaged in several active lines of research:

1. Engineering of Novel Virus Immunotherapeutics

There has been much recent interest in the use of monoclonal antibodies (mAbs), or cocktails thereof, for treatment of viral diseases. We are using state-of-the-art protein engineering technologies to develop novel immunotherapeutics against several viruses. An example is the use of "synthetic antibody technology" to develop the first humanized protective mAbs against the Sudan ebolavirus species (see below and ACS Chem. Biol., 2014, 9, 2263), the result of a large multi-institution collaborative effort. Synthetic antibody technology is a phage-based approach that allows the production and screening of very large ($>10^{10}$) protein libraries. There are five species of ebolavirus and, while many potential mAbs are available for the Zaire ebolavirus species, few are available for Sudan ebolavirus despite its high pathogenicity and increasing prevalence. Subsequent efforts are focused on using nascent protein engineering technologies to address unmet immunotherapeutic needs for this and other viruses.

![Figure - Protein engineering of novel, human, protective Sudan ebolavirus antibodies.](image)

2. Mechanisms of Viral Membrane Fusion

Membrane-bound viruses require fusion between the host and viral membranes for infection. Although the details of the mechanism for membrane fusion differ among viruses, a common conformational state is the "extended" or "prehairpin" intermediate, in which the viral fusion subunit spans both the host and viral membranes. Collapse of this extended intermediate into a stable hairpin-like structure provides the energetic driving force for membrane fusion. We are using biophysical and structural methods to dissect fine details of the fusion process (below and: Biochemistry, 2015, 54, 1589; J. Mol. Biol., 2014, 426, 1452; Structure, 2013, 21, 1085 as examples).
3. Protein Structure and Molecular Recognition

We use combinatorial biochemistry, and traditional biophysical approaches to decipher determinants for protein structure and molecular recognition. These studies illuminate the requirements for specificity and affinity at intermolecular interfaces and provide new tools and potential therapeutics.

Selected References


The Levy Lab draws from biological, chemical and combinatorial approaches to both understand fundamental biological interactions, as well as design novel diagnostics, therapeutics and biotools.

Research in the Levy Lab focuses in two main areas. In the first area, we are focused on developing new methods for the identification of cell and tissue specific targeting agents. Of particular interest is the development of cell specific and cell surface receptor specific aptamers. These nucleic acid based affinity agents hold great promise for the design of novel diagnostics as well as therapeutic agents and offer many advantages over other affinity reagents used for targeting such as antibodies. Importantly, because aptamers can be readily generated in vitro, their properties and specificity can be finely tuned.

Recently, we and others have demonstrated that aptamers are capable of directing delivery of cargoes to cells. Of particular interest is the targeted delivery of siRNA. The ability to specifically target and deliver cargoes to cells using nucleic acids opens a new niche in the field of targeting and delivery.

Current research in my lab aims to build on these initial results to identify novel nucleic acids capable of targeting or delivering cargoes to cells. We are currently performing a variety of different in vitro selection experiments against multiple cell types and cell surface receptors, including receptors expressed on the surface of the blood brain barrier, pancreatic beta cells, cancer cells and dendritic cells. In addition, we are developing strategies to perform selections in whole organisms to identify aptamers which can home to specific tissues and organs. In parallel with these efforts, we are further developing the use of aptamers as components in compound delivery reagents. To this end, we are exploring their use for targeted delivery of liposomes as well as nanoparticles such as quantum dots, colloidal gold, and dendrimers.

In the second area, we are utilizing a new technique, in vitro compartmentalization (IVC), to engineer novel protein-ligand interactions and functions. IVC provides the potential to screen very large (10⁹-10¹¹) combinatorial libraries for function. The method uses water-in-oil emulsions to form femtoliter-sized reaction vessels and thus avoids many complications and ‘bottlenecks’ incurred using more traditional in vivo protein evolution techniques. Evolved proteins with altered properties are potentially very useful tools for a variety of biotechnology and nanotechnology applications. In addition, they can provide important insight into the nature of high affinity protein-ligand interactions and substrate specificity. Current work focuses on developing orthologs of the streptavidin-biotin couple as well as on further developing, adapting and optimizing a class of bacterial proteins called sortases for in vivo labeling and imaging applications.

**Selected References**


Chromatin and the Biochemistry of Epigenetic Information

Our research interests are focused on understanding chromatin, the complex of DNA, histones, and other proteins that constitute the physiological form of the genome. In particular, we are interested in the role of histone post-translational modifications and histone chaperones in “writing” an epigenetic state, how this process is misregulated in cancers, and how to drug components of the machinery.

Epigenetics is a phenomenon important for an overall increase in the complexity of the genome without changes in gene sequence. Post-translational modifications of histones, and deposition of histone variants, establish a “histone code” of activation or repression of transcription and other chromatin-mediated transactions, and constitute a major part of the epigenome. Epigenetic information is information content “on top of” the DNA-encoded genetic material. Epigenetic information is the landscape on which the dynamic usage of genetic information is encoded.

We primarily utilize protein biochemistry and enzymology, structural biology, embryos of the frog *Xenopus laevis*, and cultured cancer cells in our studies. These tools allow us to probe evolutionarily conserved mechanisms specifying critical events in chromatin biology and in maternal and zygotic control of development that are misregulated in cancer. Our combined use of rigorous *in vitro* studies along with *in vivo* studies in the frog and in cultured cells provides an uncompromised approach to fully understanding epigenetic phenomena.

We are currently pursuing a number of specific research avenues, including:

- determination of the biochemical mechanisms and biological function of the essential PRMT5-MEP50 histone arginine methyltransferase complex and identification of new methylarginine “reader” proteins
- applying cutting-edge rational approaches to design small molecule chemical probes and lead molecules for drug screening for PRMT5
- analyzing the histone code specified by PRMT5-catalyzed histone methylation in embryos and breast/lung cancer cells to promote EMT and metastasis
- Determining how phosphorylation, methylation, and glutamylation of histone chaperones Nucleoplasmin, Nucleophosmin (the most frequently mutated gene in AML) and Nap1 occur and how these post-translational modifications regulate histone deposition activity
- Using quantitative techniques (hydrogen-deuterium exchange, NMR, binding studies, activity assays) to understand histone chaperone binding and release of histones

These studies are designed to probe the molecular role of chromatin components in the establishment of the embryonic state and have direct bearings on understanding basic events in development and cancer. Our approach provides a unique “bottom-up” molecular understanding of the role of egg components, such as pre-deposition histones, histone modifications, and histone chaperones, in writing the embryonic chromatin state.
Selected References


Histone H2A and H4 N-Terminal Tails are Positioned by the MEP50 WD-Repeat Protein for Efficient Methylation by the PRMT5 Arginine Methyltransferase. Emmanuel S. Burgos, Carola Wilczek, Takashi Onikubo, Jeffrey B. Bonanno, Janina Jansong, Ulf Reimer and David Shechter. *Journal of Biological Chemistry*, 2015.


Enzymes catalyze virtually all of the chemical transformations necessary for biological life. Knowledge of the transition-state structure of enzymatic reactions permits the design of powerful inhibitors. Methods have been developed in this laboratory for the experimental determination of the geometry and charge features that characterize enzymatic transition states. This information is used for the logical design of transition-state analogues. These have the potential to be powerful biologically active agents. 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. Two of these inhibitors are now in human clinical trials against human T-cell cancers and gout. Three T-cell cancer indications for one drug have received orphan drug status from the FDA. One of these purine nucleoside phosphorylase inhibitors is pending approval as an anticancer drug.

Purine salvage is essential for growth of parasitic protozoa. A family of powerful inhibitors has been prepared against these enzymes from the malaria parasite. Promising results have been obtained in cell culture studies. One of these inhibitors stops the growth of malaria parasites in primate malaria. New inhibitors are being synthesized as more powerful drugs.

S-adenosylmethionine recycling and methyl transfer reactions are essential in cancer cells. We targeted an enzyme in this pathway and developed powerful transition state analogue inhibitors. They show strong anticancer effects in mouse xenografts and are in evaluation for human clinical trials.

Epigenetic control of gene expression is a central feature of cancer. We are targeting DNA and protein methyltransferases to develop transition state analogue inhibitors as anticancer agents.

Protein dynamics in catalysis studies the fast (femtosecond) motions in enzyme catalytic sites that leads to formation of the transition state. Quantum chemical calculations are coupled to experimental work with isotopically heavy enzyme atoms to shift bond vibrational frequency.

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

Selected References


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. The recent, highly-publicized outbreak in the Bronx emphasized the importance of community public health education in a Town Hall meeting held Monday August 17, 2015.

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 maturation and fate of internalized *Legionella*.

Research in our 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. The focus of our research is Lvh T4SS that we implicated 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 amoeba hosts.

Future directions of our research are
- Defining the role of non-coding lpr0035 in modulating expression of known Legionella virulence regulators
- Determining if Lvh T4SS proteins can functionally substitute for Dot/Icm components
- Characterizing the number and location of the pLP45 by FISH technology

Selected References


Signaling Pathways and Transcriptional Regulation in Growth Control, Cancer and Obesity

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

Obesity Resistance in Maf1 Knockout Mice

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

Genetic Arrays, Gene Networks and Functional Genomics

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


