GRADUATE STUDIES IN

PHYSIOLOGY & BIOPHYSICS

FROM MOLECULE TO MAN

ALBERT EINSTEIN COLLEGE OF MEDICINE OF YESHIVA UNIVERSITY
Prospective Students

*Physiology* is one of the oldest disciplines in medical science. It is the study of how living organisms function ranging from basic molecular interactions to the mechanism of organ performance in the whole animal. On the other hand, *Biophysics* is one the newest disciplines in medical science. It is the determination of the chemical, physical, and mathematical basis for biological activity and often utilizes the latest sophisticated technology. Recent advances in molecular biology, spectroscopy, microscopy, and theoretical modeling allow for a bridging of these two disciplines. The integration of physiology and biophysics with these advanced techniques provides a multidisciplinary approach to biomedical research that leads to understanding of the latest biological problems at the molecular, cellular, organ, and whole body level.

The diverse research programs in our Department are enhanced by five state-of-the-art facilities and centers: The Biomolecular Laser Research Center, The Electron Paramagnetic Resonance Facility, and The Rapid Kinetics Facility. These biophysical facilities and centers act as conduits through which the sophisticated physical and chemical techniques are introduced into the biological arena. They provide powerful research tools to probe structure, function and dynamics of biological molecules with unprecedented detail and poise the Department to make significant advances in genomics and proteomics as we move ahead in the new millennium.

An emphasis in our program is to take advantage of the variety of tools that are available within the department rather than relying on only a single technique to address a problem. Providing accessibility to a broad range of expertise and technologies is a major part of the departmental strategy. As a result, students are exposed to many different techniques, concepts and investigators. A highly interactive and collegial environment fosters appropriate interactions among students and faculty to promote the cross-disciplinary investigations.

As Chairman of the Department of Physiology and Biophysics, I am committed to creating a research environment based on the idea that our research should be creative, high impact, forward looking and fun. I invite you to join us on a voyage of discovery, innovation and challenge.

Sincerely,

Denis L. Rousseau Professor and Chairman
The Department of Physiology & Biophysics plays a unique role in biological research. It is in effect a conduit through which the powerful techniques and tools of the physical sciences are brought to bear on significant problems of biological importance. The range of problems being addressed in the Department runs the gamut from understanding functionally important atomic scale motions of proteins to characterizing complex behavior on the cellular through organelle level. The tools being used to pursue these cutting edge problems include state of the art instrumentation for magnetic resonance, laser and synchrotron radiation spectroscopies as well as extensive computer modeling.

The strength of the Department stems not only from the significant problems that are being aggressively addressed by the departmental faculty, but also from the resources and the collaborative spirit with the department.

The Department houses several world class spectroscopy facilities:

**Biomolecular Laser Research Center (BLRC)**

The BLRC is composed of three interrelated laser oriented facilities. The laser spectroscopy facility (LSF) contains an extensive array of state-of-the-art laser spectroscopic tools devoted to studying structure, function and dynamics in isolated biomolecules. The laser imaging and microscopy facility (L1MF) focuses on interfacing laser spectroscopy with microscopy to study complex systems at the molecular level. The third facility, devoted to laser based diagnostic tools for clinical applications, is still in the development stage.

**Pulsed EPR Facility**

The EPR facility consists of a number of state-of-the-art spectrometers that have been constructed at Einstein. Both theoretical work and experiments are being carried out to define the structure of metal binding sites in metalloproteins and to determine the orientation and distance of substrates to metal centers at active sites of metalloenzymes.

**Rapid Kinetics Facility**

The Rapid Kinetics Facility provides instrumentation for the study of rapid biological reactions. It consists of an integrated rapid mixing system that allows stopped flow, continuous flow, freeze quench and chemical quench experiments to be carried out. In the stopped or continuous flow modes reactions can by studied by absorption, fluorescence, Raman scattering and circular dichroism. In the chemical and freeze quench modes reactive intermediates can be trapped and then characterized by electron paramagnetic resonance, nuclear magnetic resonance, or other spectroscopies. The mixing time limitation is one millisecond. Rapid mixers are also available in which two solutions can be completely mixed within 100 microseconds.
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ION CHANNELS and MEMBRANE TRANSPORTERS: Structure, Function and Pharmacology

ION CHANNEL STRUCTURE, FUNCTION AND DYNAMICS
Neurotransmitter-gated ion channels are essential components in synaptic transmission. Our work focuses on the GABA\textsubscript{A} receptor, the major post-synaptic inhibitory neurotransmitter receptor in brain. It is the target for drugs used clinically in the treatment of anxiety and epilepsy, and for general anesthesia and insomnia. GABA\textsubscript{A} receptors are members of a gene superfamily that includes receptors for glycine, acetylcholine, and serotonin. Our goals are to understand the structural bases for the functional properties of the channel and to understand the molecular interactions by which drug binding modulates structure and channel activity. We use a combination of techniques including site-directed mutagenesis, heterologous expression, covalent chemical modification and electrophysiology. These studies have identified the residues lining the channel, the location of channel blocker binding sites and identified conformational changes occurring during channel gating and modulation by drugs including valium and general anesthetics such as propofol.

MALARIA DRUG RESISTANCE TRANSPORTERS AND PARASITE PHYSIOLOGY
Malaria is a major public health problem in the developing world causing millions of deaths per year. Using heterologous expression systems we have characterized the expression and transport properties of nucleoside transporters (PfENT1 & PfENT4), sodium-hydrogen exchangers (PfNHE) and the Plasmodium falciparum chloroquine resistance transporter (PfCRT). These proteins are involved in metabolite and drug transport and in drug resistance. We are currently exploring the properties of a family of nucleoside transporters to identify the transporters involved in AMP and Immucillin transport and developing a high throughput assay for PfENT1 inhibitors to identify novel anti-malarial drugs.

Selected References:


**Key Words:** Sickle cell disease, sickle cell hemoglobin, red cells, polymerization, translational approach to therapy

Sickle cell hemoglobin (HbS) forms long, rod-like, polymers when deoxygenated. These rigidify and damage red cells, producing microvascular obstruction and pathogenesis in sickle cell disease. The HbS system has structural, thermodynamic and kinetic properties in common with other long protein polymers (e.g. tubulin, flagellin, actin, tobacco mosaic virus, beta-amyloid) including helical structure, nucleation controlled kinetics, entropy and excluded volume driven reactions, and phase separation. Thus, it is interesting both for its pathogenic significance and its mechanisms.

HbS polymerization and gelation (i.e. polymer cross-linking) have been studied by physical chemical methods (e.g. light scattering, ultracentrifugation, viscometry, NMR), but these methods reflect average properties of vast numbers of fibers without observation of individual fibers as they nucleate, grow and cross-link. Electron microscopy damages gel structure and fails to reveal events in real time. Conventional microscopy does not resolve the thin (200A diameter) fibers. Using differential interference contrast (DIC) microscopy we overcome these limitations and observe individual HbS fibers in real time as they nucleate, grow and cross-link to form the final solid-like gel. These studies demonstrate two mechanisms of nucleation of new fibers, mechanisms of fiber growth and depolymerization, diverse cross-linked and branching structures and their mechanisms of formation.

Our work now has four facets: (1) We are undertaking a pilot clinical study in which we hypothesize that failure of complete polymer dissolution in the lungs exacerbates pathology and also provides a rationale for new, pulmonary assist-based, therapy. (2) We continue to examine mechanisms of the depolymerization of hemoglobin S fibers in laboratory, physical-chemical and imaging studies. (3) Having previously shown that sickle HbS fibers are very stiff, we now characterize gel viscoelasticity and the underlying gel structures and fiber interactions, the basis of red cell rigidity and a major factor in vaso-occlusion. (4) We study changes in red cell membrane viscoelasticity resulting from polymerization-induced cellular damage. Disciplines and methodologies for these studies include physical chemistry, polymer physics, imaging and structural biology, and a pilot clinical trial. (5) We also plan work on the mechanisms by which sickle trait blood inhibits pathogenesis in falciparum malaria.

**Selected References:**


My laboratory is interested in understanding urogenital pathology at the physiological, biochemical and molecular level. The ultimate goal of these investigations is to develop new treatments for, or methods to diagnose, urogenital disease. Our recent research has focused on the role of two very different proteins, the MaxiK channel and Opiorphins in urogenital smooth muscle tissue function.

The MaxiK potassium channel, encoded by the Slo gene, plays an important role in regulating smooth muscle tone. We have shown the Slo gene is differentially spliced in smooth muscle tissue when animals age or are diabetic—two conditions that result in urogenital pathology. We have found that animals with erectile or bladder dysfunction can be treated using naked gene therapy of vectors expressing the MaxiK channel. We were the first group in the World to investigate the potential of vectors expressing MaxiK for the treatment of human erectile or bladder dysfunction in Phase I in clinical trials.

The other proteins that we are investigating for a role in urogenital smooth muscle function are a recently identified family of pentapeptides called Opiorphins. They act as potent neutral endopeptidase inhibitors. The rat homologue of Opiorphin (Sialorphin) can cause relaxation of corporal smooth muscle tissue through an affect involving changes in the activity of G-protein coupled receptors. We have expanded these studies to show that overexpression of Opiorphins can result in priapism, and that in Sickle cell disease (which causes a high incidence of priapism in patients) there is up regulation of Opiorphins. We are investigating the translational potential to use the observations to prevent priapism in patients with Sickle cell disease.

We recently demonstrated that Opiorphins produced in the corporal smooth muscle tissue can have systemic effects, causing changes in the blood pressure of diabetic rats. These findings potentially link the association between erectile dysfunction and cardiovascular disease.

Selected Publications:
Cutaneous drug delivery offers many advantages over alternative routes of administration. It provides safe passage, especially hydrophilic ones, from impenetrable skin barriers. A complicating factor is that the skin can impede the delivery of molecules, especially hydrophilic ones, from safe passage. Novel delivery systems have been generated here at AECOM through nanotechnology, which are raising the exciting prospect for controlled and sustained drug delivery across the impenetrable skin barrier.

A major focus of this work has been the utilization of a novel nanoplatform to deliver nitric oxide (NO-np) and nitric oxide intermediates, such as Nitrosoglutathione. Interest in the therapeutic potential of nitric oxide (NO) has been growing exponentially over the past few decades. This interest is a direct result of findings demonstrating an ever-expanding range of functionalities associated with NO under physiological conditions. These established properties not only have direct therapeutic implications for the treatment of infections, modulation of vasoactivity, angiogenesis, and wound healing, but also provide a basis for our understanding of many diseases ranging from asthma to psoriasis. Harnessing this potential has proven difficult as reflected by the intense but relatively unsuccessful efforts to develop therapeutically useful NO delivery devices/vehicles. Clinical use of these materials has been limited due to cost, cytotoxicity, instability of the chemical compounds, potential carcinogenicity, and development of tolerance to the NO releasing substances. The NO-np overcomes many of the existing limitations associated with the current NO releasing strategies as it does not rely on complex chemistry to generate or donate NO nor does it rely on external enzymatic conversion. In vitro and in vivo pre-clinical studies are on-going to further enhance this platform and elucidate its potential as a therapeutic in multiple clinical arenas.

**Selected Recent References**


ION-CONDUCTING CHANNELS INCORPORATED INTO PLANAR LIPID BILAYER

The objectives of the research in this laboratory are two-fold: (1) to obtain a detailed understanding both of the mechanism(s) by which channels are opened and closed (i.e., gated) and of the physico-chemical factors that govern transport through open channels; (2) to determine the relationship of the translocation of proteins and polypeptide chains across membranes to the formation and existence of wide-lumen channels. The methodology employed is the study at both the macroscopic and single-channel level of the size, ion-selectivity, voltage-dependent properties, and stochastic behavior of channels incorporated into planar phospholipid bilayer membranes. These channels include those inserted into membranes by bacterial proteins such as diphtheria toxin, tetanus toxin, botulinum toxin, anthrax toxin, and colicins of the E1 class (E1, Ia, Ib and A). With respect to the first objective: the genes for the channel-forming proteins mentioned above have all been cloned and sequenced, so that detailed models of channel structure and gating can be developed. Moreover, models can be stringently tested by comparing properties of specifically modified channels (formed by site-mutated proteins) with their predicted behavior. With respect to the second objective: all of the above-mentioned bacterial toxins consist of at least three domains, only one of which is necessary for channel formation; one of the other domains can be an enzyme that must cross a vesicular membrane to enter the cytosol and thereby cause cell intoxication. Whether and how this enzymatic domain of the toxin crosses planar bilayers in conjunction with the opening and closing of the channel-forming domain is being investigated.

References
Jakes KS, Finkelstein A. The colicin Ia receptor, Cir, is also the translocator for colicin Ia. Mol Microbiol. 2010 Feb;75(3):567-78.
Nanodermatology: Utilizing Nanotechnology to Target Skin and Soft Tissue Disease

Dr. Friedman's research program focuses on the translational and pre-clinical applications of nanomaterial-based therapeutics for the treatment of skin and soft tissue diseases, specifically multidrug-resistant infections and acceleration of wound healing. Cutaneous drug delivery offers many advantages over alternative routes of administration with regards to target specific impact, decreased systemic toxicity, avoidance of first-pass metabolism, variable dosing schedules, and broadened utility to diverse patient populations. A complicating factor is that the skin has evolved mechanisms to impede exogenous molecules, especially hydrophilic ones, from safe passage. Novel delivery vehicles being generated here at AECOM through nanotechnology, which are raising the exciting prospect for controlled and sustained drug delivery across the impenetrable skin barrier.

A major focus of this work has been the utilization of a novel nanoplatform to deliver nitric oxide (NO-np) and nitric oxide intermediates, such as Nitrosoglutathione. Interest in the therapeutic potential of nitric oxide (NO) has been growing exponentially over the past few decades. This interest is a direct result of findings demonstrating an ever-expanding range of functionalities associated with NO under physiological conditions. These established properties not only have direct therapeutic implications for the treatment of infections, modulation of vasoactivity, angiogenesis, and wound healing, but also provide a basis for our understanding of many diseases ranging from asthma to psoriasis. Harnessing this potential has proven difficult as reflected by the intense but relatively unsuccessful efforts to develop therapeutically useful NO delivery devices/vehicles. Clinical use of these materials has been limited due to cost, cytotoxicity, instability of the chemical compounds, potential carcinogenicity, and development of tolerance to the NO releasing substances. The NO-np overcomes many of the existing limitations associated with the current NO releasing strategies as it does not rely on complex chemistry to generate or donate NO nor does it rely on external enzymatic conversion. In vitro and in vivo pre-clinical studies are ongoing to further enhance this platform and elucidate its potential as a therapeutic in multiple clinical arenas.

Selected Recent References

PROTEIN DYNAMICS AND REACTIVITY/NANOPARTICLE DRUG DELIVERY/BLOOD SUBSTITUTES

Dr. Friedman's research program has several components. I). Integrated investigations of structure, structural dynamics and function on protein samples both in solution and in unusual solid state matrices (sol-gels and glassy matrices) at both ambient and cryogenic temperatures. Laser probe techniques with time resolution capabilities starting at less than a trillionth of a second are used to provide atomic level resolution for both structural and functional properties. Sol-gel encapsulation is used to trap functional intermediates. ii) A recent new translational research focus is the development of nano and micro particles including paramagnetic nanoparticles for sustained slow targeted release of therapeutic agents such as NO, curcumin and chemotherapy drugs. iii) Development of hemoglobin-based blood substitutes and suitable solid-state matrices for long term storage of the product. iv) Probing the mechanism through which hemoglobin generates nitric oxide from nitrite.

Selected Recent References


Roche, C.J. and J.M. Friedman NO reactions with sol-gel and solution phase samples of the ferric nitrite derivative of HbA, Nitric Oxide, 2010, 22, 180.


Roche, C.J. and J.M. Friedman NO reactions with sol-gel and solution phase samples of the ferric nitrite derivative of HbA, Nitric Oxide, 2010, 22, 180.


INVESTIGATION OF PROTEIN/SUBSTRATE INTERMEDIATES USING ADVANCED EPR SPECTROSCOPY

The goal of our research is to determine protein function through the investigation of intermediate state structures generated along a given reaction pathway. These intermediate states involve transient forms of the protein, cofactor and substrate. In a variety of enzyme systems, intermediates include paramagnetic species in the form of metals and/or organic radicals. In addition, for systems which lack endogenous paramagnetic species, it is often advantageous to introduce a stable radical "spin label" to serve as a reporter of protein structure and dynamics. Electron paramagnetic resonance (EPR) spectroscopy is well suited for the characterization of all of these classes of paramagnetic species. Thus our primary experimental tools for structural characterization involve advanced EPR techniques, including electron spin echo envelope modulation (ESEEM), electron nuclear double resonance (ENDOR), and Hyperfine Correlation Spectroscopy (HYSCORE). Pulsed electron double resonance (PELDOR) techniques are used to measure distances between mutagenically introduced spin labels out to 50 Å. Quantum mechanical simulations of experimental spectra are developed from first principles for the accurate determination of spectral parameters. For proteins which are not amenable to NMR or crystallographic techniques, homology modeling and molecular dynamics calculations are used to generate structures which can be tested using EPR techniques. Instrumentation and spectroscopic techniques are implemented as required by the systems under investigation. A primary focus in this regard is the development and application of high frequency cw and pulsed EPR/ENDOR spectroscopy. HF-EPR/ENDOR extends EPR spectroscopy to high magnetic field strengths and enhances the capabilities of the technique to determine molecular and electronic structure.

Examples of projects currently under study include: structure of protein active sites and substrate intermediates in coenzyme B12-dependent enzymes such as glutamate mutase and ribonucleoside triphosphate reductase; identity and structure of radicals generated during catalysis by prostaglandin H synthase (COX or PGHS) and cytochrome c oxidase; molecular structure determination of the regulatory proteins phosphoinositide 3-kinases (p85/p110) using spin-labeling techniques; structural determination of copper binding prion proteins and corresponding model compounds; HF-EPR/ENDOR of a variety of protein and substrate-derived radical species.

Selected References:
MAGNETIC RESONANCE IMAGING STUDIES OF MICE

Our lab focuses on developing and applying MRI based methods to study mouse models of human disease. Through synergistic collaborations with other labs we study mouse models of cardiac hypertrophy, heart failure, diabetic cardiomyopathy, chagasic cardiomyopathy, obesity, cancer and atherosclerosis. We also incorporate other technologies (microPET, non-invasive blood pressure monitoring and ECG) in multimodality studies of mice and are involved in studies aimed at developing targeted MRI and microPET agents.

Serial in vivo MRI studies permit the evaluation of the same mouse over a chronic time period. An understanding of the changes in morphology and function that occur in the organs of the living animal during the progression of disease is useful for designing strategies to prevent or limit development of debilitating disease in humans.

Selected Recent References:


3: de Souza AP, Sieberg R, Li H, Cahill HR, Zhao D, Araújo-Jorge TC, Tanowitz HB, and Jelicks LA, The role of selenium in intestinal motility and morphology in a murine model of Trypanosoma cruzi infection. Parasitol Res. 2010 May;106(6):1293-8


THEORETICAL STUDIES IN BIOPHYSICS AND CELL BIOLOGY

The research in my group is focused on development and application of novel computational and theoretical methods for studying dynamical processes in biological systems that span over a multitude of spatial and temporal scales. Currently, we are pursuing two major directions.

1. An integrated computational approach for understanding microtubule-associated proteins (MAPs) from the level of the structure and dynamics of individual molecules to the level of their function, regulation and interactions in vivo. MAPs control the assembly dynamics of microtubules and play essential roles in a broad range of important cellular processes such as mitosis, motility, polarization and morphogenesis. We strive to address this problem from both top-down and bottom-up perspectives and established close collaborations with experimental colleagues in cell biology, structural biology and biophysics. The research in this direction consists of four synergistic components: (1) automated image analyses for extracting quantitative and detailed information from experimental data, (2) coarse-grained systems-level simulations of spindle dynamics, (3) simple kinetic model of single molecule dynamics, and (4) atomistic simulations for understanding the molecular mechanism underlying the structure-function relationships of mitotic proteins.

2. Understanding the detailed dynamics of individual biomolecules. Protein dynamics are essential for protein function, but the molecular mechanism of how dynamics impact function remains elusive. Moreover, protein dynamics are strongly influenced by environmental factors such as solvent, confinement and osmolytes. The research along this direction focuses on inter-relationship between solvent dynamics, protein dynamics and protein function. In collaboration with experimental colleagues, we are using atomistic simulations to elucidate the molecular mechanism of the “solvent-slaving” phenomenon (protein dynamics are "slaved" to solvent dynamics), and how to use environmental factors to modulate protein dynamics and function.

Selected References:


In our laboratory the mechanisms and properties of two enzymes, cytochrome c oxidase and nitric oxide synthase, are being investigated.

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, electron paramagnetic resonance 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 overproduction of NO.

References


THEORETICAL STUDIES IN BIOPHYSICS AND BIOCHEMISTRY

The research in my group has four broad aims. First we wish to develop approximate methods that allow the practical prediction of the dynamics of chemical reactions in biomolecular systems. Because even in large biological systems, many events are governed by quantum mechanics, we seek to develop methods that allow the study quantum processes in complex systems. Some enzymatic reactions seem to proceed almost entirely by quantum mechanical tunneling. Using methods we have developed we have uncovered the extraordinary possibility that in certain enzyme systems, evolution has developed a catalyst that maximizes quantum tunneling by making a barrier to reaction thinner, rather than the conventional view that enzymes always work to make barriers to reaction smaller. This concept of directed protein motions, which we have termed promoting vibrations, is now an area of international research focus.

The second aim of my work is to understand the atomic level processes by which enzymes accomplish their extraordinary specific catalytic effect. We employ such methods as Transition Path Sampling to develop detailed pictures of enzymatic mechanism.

The third aim of my research is to develop and apply new methods to the study of protein complexes that make up the machinery of cells. In particular, with Jil Tardiff's group, we are interested in understanding the function of and how mutations effect the cardiac sarcomere at the molecular level.

The fourth aim of research in my group is to develop methods that will allow us to understand the basic dynamics of condensed phases. In particular, we seek to understand how liquids behave, how they influence chemical reaction, and finally how these effects are manifest in biological systems.

References:
MECHANISMS OF MITOSIS

During mitosis, microtubules and an array of associated proteins are assembled into the spindle. This highly dynamic machine is used to capture, move and separate chromosomes into the daughter cell products of cell division. A primary goal of my laboratory’s research is to elucidate the molecules and mechanisms that build the spindle and move chromosomes on it. Relevant ongoing projects include:

Elucidate the molecular pathway underlying poleward microtubule flux (see Rogers, Rogers, and Sharp, Journal of Cell Science, 2005)
Subunits within spindle microtubules flux from the spindle equator toward mitotic spindle poles. This phenomenon, termed poleward microtubule flux, reels chromosomes into spindle poles and also regulates spindle length. We recently showed that the microtubule destabilizing enzyme, KLP10A, is required for poleward flux in Drosophila embryos and S2 cells (Rogers et al, Nature, 2004) and are currently working to uncover additional components required for this fundamental and conserved process.

Determine the functions and interactions of chromosome-associated motor proteins (see Sharp, Rogers, and Scholey, Nature, 2000)
Distinct classes of microtubule motors are associated with chromosome arms and kinetochores. We are working to understand how these motors are utilized, individually and in concert, to drive spindle assembly and chromosome segregation. Our recent work suggests that the kinetochore-motors, cytoplasmic dynein and KLP59C, work cooperatively to generate poleward forces on chromosomes (Sharp and Rogers, Cell Cycle, 2004).

Discovery of Novel Mitotic Proteins.
To generate a complete inventory of proteins involved in chromosome segregation, we have collaborated with the Genomics Institute of the Novartis Research Foundation to screen the entire human genome for essential mitotic proteins. We are now elucidating the functions and mechanisms of action of novel proteins identified in this screen.

Understand the etiology of aneuploidy.
Defects in chromosome segregation can result in cells with too many or too few chromosomes (aneuploidy), which in turn may proliferate into cancerous tumors. We are now determining which of the novel proteins identified in our genome screen influence chromosome number. This will lead to the identification of molecular hotspots and weak links in the maintenance of ploidy and may help guide the development of anti-tumor agents.

Selected recent publications.
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 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. Cryo-electron microscopy is an ideal technique to obtain medium to high-resolution information of big macromolecular complexes such as the one formed by the motors proteins and their tracks. To trap different structural intermediates we use non-hydrolysable ATP analogues and rapid mixing techniques. To detect conformational changes in aqueous solutions as the proteins work, we developed a fluorescence polarization microscope that allows determining the orientation and mobility of a single fluorophore.

References:


The Structural and Functional Basis of Thin-Filament Cardiomyopathies

The studies in my lab utilize an integrative physiologic and biophysical approach to delineate the underlying pathophysiologic links between structural alterations in thin filament proteins and the resultant complex cardiovascular phenotypes. The basic paradigm for this work is focused on a unique subset of Familial Hypertrophic Cardiomyopathy. We have developed a unique set of independent transgenic mouse models that express five independent mutant cardiac Troponin T (cTnT) proteins exclusively in the murine heart and our initial characterization of these models suggested that the range of clinical phenotypes observed in patients with cTnT-related cardiomyopathies are a direct reflection of the multi-functional role of the thin filament in regulating contractile function. Our current research approaches include:

1) The role of the cardiac sarcomere as a “scaffolding” for myocellular signaling is a recent and exciting area of research that focuses on the mechanisms that determine how external stimuli are transduced to the nucleus and result in specific changes in cardiac remodeling. The pathways that link the sarcomere to the nucleus are poorly understood. We have previously shown that independent cTnT mutations lead to specific downstream alterations in transcriptional activation. We are in the process of classifying patterns for each of the five independent cTnT mutations via a three-timepoint set of long-oligo microarray studies. The results will allow us to eventually correlate the temporal expression patterns to the specific alteration in thin filament function at the level of the sarcomere. The long-term goal is to identify potential pathways for intervention in disease progression.

2) We have recently shown that independent cTnT mutations also differentially alter intracellular Ca2+ homeostasis and myocardial energetics -- changes that may partially underlie the observed clinical phenotype of early sudden cardiac death. We are currently expanding these studies via measurements of intracellular Ca2+ kinetics in isolated adult ventricular myocytes from each of our mutant cTnT lines. Correlating these studies to myocellular mechanics will provide a robust framework for understanding how alterations in contractility at the molecular level modulate Ca2+ handling mechanisms and subsequently lead to pathogenic remodeling.

3) We have recently shown that independent cTnT mutations also differentially alter intracellular Ca2+ homeostasis and myocardial energetics -- changes that may partially underlie the observed clinical phenotype of early sudden cardiac death. We are currently expanding these studies via measurements of intracellular Ca2+ kinetics in isolated adult ventricular myocytes from each of our mutant cTnT lines. Correlating these studies to myocellular mechanics will provide a robust framework for understanding how alterations in contractility at the molecular level modulate Ca2+ handling mechanisms and subsequently lead to pathogenic remodeling.

References:


My primary research involves the use of imaging as a tool to study the pathophysiology of hydrocephalus. Hydrocephalus, a disease which is most common in newborns and young children, although it is also one of the causes of dementia in the elderly, is characterized by an increased accumulation of fluid in the brain. It is associated with brain development, cognitive and motor function delays and deficits. In collaboration with researchers at the University of Utah, we are working on characterizing the role of brain pulsations in the development and progression of ventricular dilation in hydrocephalus. We use MRI and multi-photon confocal microscopy to image blood and cerebrospinal fluid flow in an animal, and are now working with the Einstein Behavioral Core to evaluate potential biomarkers of disease severity and of recovery following shunting of the fluid from the brain. The work obviously has important clinical application, and we are working on improved imaging techniques for quantifying pulsatile fluid flow in the brain and its relationship to hydrocephalus and recovery following shunt surgery.

I am also interested in a technique called MR-spectroscopy (MRS), in which MRI can be used to quantify concentrations of common metabolites in the brain. We use MRS to understand white and grey matter degradation in multiple sclerosis. Using techniques based on principle component analysis and metabolomic analysis, we are able detect changes in MS lesions as well as in "normal-appearing" white matter in the MS brain. We also use diffusion tensor imaging, a technique which can detect changes in myelin microstructure, as well as volumetric analyses to understand the effect of hippocampal atrophy, for example, on cognitive function. This work is ongoing in collaboration with investigators at Stony Brook University.

Finally, I am involved in MRI pulse sequence development, i.e. manipulating the MRI machine to extract new types of information from MRI images.
STRUCTURE, FUNCTION AND FOLDING of PROTEINS

Proteins are the building blocks for all life forms. They are produced in ribosomes as nonstructured nascent polypeptides, which subsequently fold into functional proteins. The first goal of my research program is to understand the general principle underlies protein-folding reactions. The current interest is to dissect the folding pathways of hemoglobins, fatty acid binding proteins and \( \alpha \)-synuclein. The second goal of my research focuses on the studies of the structure-function relationships in heme proteins, including bacterial hemoglobins, as well as mammalian Indoleamine 2,3-dioxygenase (IDO), Tryptophan 2,3-dioxygenase and Nitric oxide synthases (NOS). In this research program, a wide array of spectroscopic tools, including optical absorption, fluorescence, circular dichroism and UV/VIS resonance Raman scattering, are utilized to study various biological processes. With the state-of-the-art rapid solution mixing technique developed in my laboratory, along with conventional stopped-flow and nanosecond laser flash photolysis systems, we are able to follow biological reactions from nanosecond to hours.

Reference