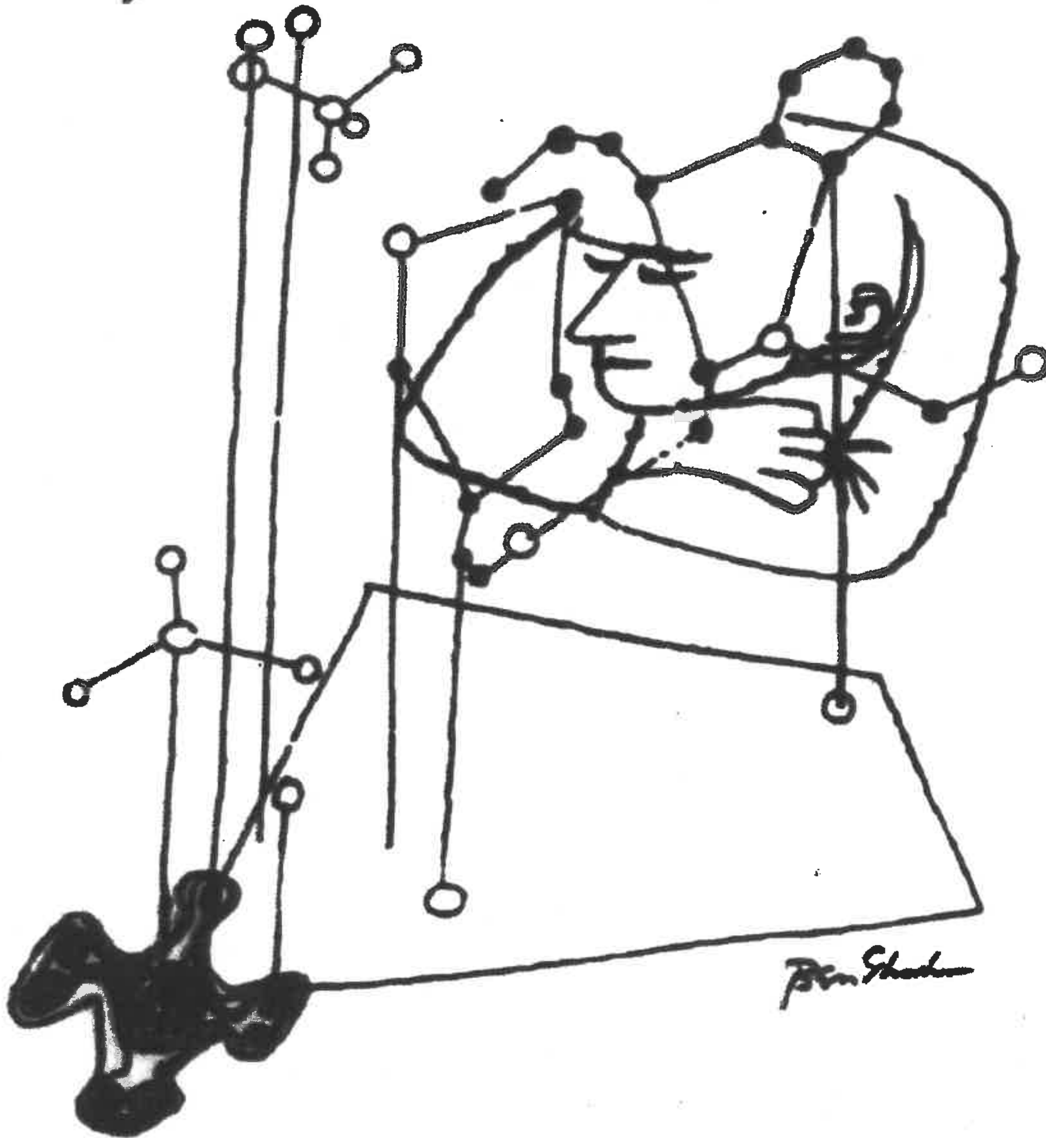
GRADUATE STUDIES IN

PHYSIOLOGY & BIOPHYSICS



FROM MOLECULE TO MAN

ALBERT EINSTEIN COLLEGE OF MEDICINE

Albert Einstein College of Medicine

Montefiore

Science at the heart of medicine

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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 Nuclear Magnetic Resonance Facility, The Biomolecular Laser Research Center, The Electron Paramagnetic Resonance Facility, The Rapid Kinetics Facility, and The Center for Synchrotron Biosciences. 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, Ph.D.
Professor and Chairman

DLR:ps

DEPARTMENTAL RESOURCES

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.

DEPARTMENT OF PHYSIOLOGY & BIOPHYSICS

ALBERT EINSTEIN COLLEGE OF MEDICINE

FACULTY RESEARCH INTERESTS

Faculty	Research Interest
AKABAS, MYLES	*MEMBRANE TRANSPORTERS AND MALARIA*
ALMO, STEVE	*STRUCTURAL IMMUNOLOGY & FUNCTIONAL ANNOTATION*
BRANCH, CRAIG	*FUNCTIONAL AND MICRO-STRUCTURAL IMAGING OF CNS DISEASES: DIFFUSION TENSOR IMAGING OF MILD HEAD TRAUMA*
COWBURN, DAVID	*PHYSICAL BIOCHEMISTRY*
DAVIES, KELVIN	*THE ROLE OF OPIORPHINS IN SMOOTH MUSCLE PHYSIOLOGY*
FRIEDMAN, JOEL M.	*PROTEIN DYNAMICS AND REACTIVITY/NANOPARTICLE DRUG DELIVERY/BLOOD SUBSTITUTES*
GERFEN, GARY	*INVESTIGATION OF PROTEIN/SUBSTRATE INTERMEDIATES USING ADVANCED EPR SPECTROSCOPY*
ROUSSEAU, DENIS	*BIOENERGETICS, ENZYMATIC FUNCTION AND PROTEIN DYNAMICS*
SCHUSTER, VICTOR	*MOLECULAR BIOLOGY OF PROSTAGLANDIN TRANSPORT*
SHARP, DAVID	*MECHANISMS OF MITOSIS*
SOSA, HERNANDO	*STRUCTURE AND FUNCTION OF MACROMOLECULAR MACHINES*
WAGSHUL, MARK	*MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY*
YEH, SYUN-RU	*STRUCTURE, FUNCTION AND FOLDING OF PROTEINS*

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MEMBRANE TRANSPORTERS and MALARIA

MALARIA PURINE TRANSPORTERS & ANTIMALARIAL DRUG DEVELOPMENT

Malaria is a major public health problem affecting large areas of the world. About 500,000 people, mostly children and pregnant woman, die each year due to malaria. Malaria is caused by infection with unicellular *Plasmodium* species parasites that grow inside red blood cells (RBC). *Plasmodium falciparum* causes the most lethal form of malaria. *Plasmodium* species parasites are purine auxotrophic. They require an exogenous source of purines to proliferate. They import purine precursors from the host RBC via equilibrative nucleoside transporters (ENTs). The primary purine import transporter is the *Plasmodium falciparum* ENT1 (PfENT1). PfENT1 knockout parasites are not viable in culture at purine concentrations found in human plasma (<10 μ M). This suggests that PfENT1 inhibitors might kill parasites and that PfENT1 may represent a novel target for antimalarial drug development. We developed a robust yeast-based high throughput screen to identify PfENT1 inhibitors. We have screened a 65,000 compound library and identified 171 hits. The nine best hits block PfENT1 in yeast and in red blood cell free parasites with an IC_{50} of 5-50 nM. The compounds kill *P. falciparum* parasites in culture with micromolar IC_{50} values. GlaxoSmithKline (GSK) used our assays to screen their 1.8 million compound library. They gave us six of the best hits. Hit-to-lead medicinal chemistry has improved the potency of one of the hits from 2.9 μM. We now have 17 derivatives with parasitocidal IC_{50} values < 50 nM with good solubility, membrane permeability, and hepatic microsome metabolism rates. Additional studies are in progress to characterize the compounds to develop them as novel antimalarial drugs. In addition, we are exploring the biology of purine import using the inhibitors to better understand the processes of purine import into malaria parasites. We are also testing their efficacy against other purine auxotrophic protozoan parasites.

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Structural Immunology & Functional Annotation

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

I. Strategies for Functional Annotation and Metabolism Discovery. The number of newly reported protein sequences inferred from genome sequencing continues to grow at a rate that severely outpaces the assignment of function through comparative genomics or direct biochemical analysis. This situation results in a large proportion of unannotated and misannotated protein sequences precluding the discovery of novel enzymes, activities, and metabolic pathways important to (1) understanding the contributions of the gut microbiome to human health, (2) the realization of new chemical processes for industry, and (3) our understanding of critical environmental issues, including global nutrient cycles and the evolution of complex microbial communities. To address these challenges our laboratory is devising experimental strategies based on the solute binding protein (SBP) components of small molecule transport systems, since the first step in a catabolic pathway is frequently the passage of a metabolite across the cellular membrane by SBP-dependent transport machinery. The ability to identify the initial reactant (or a closely related molecule) for a catabolic pathway provides an immediate toe-hold by placing significant constraints on the regions of chemical space that need to be considered and, in conjunction with knowledge of colocalized and coregulated genes, begins to define details of the *in vivo* biochemical transformations operating within the metabolic pathway. Using our high-throughput infrastructure we produced and screened 158 TRAP SBPs against a small molecule library by differential scanning fluorimetry (DSF). These efforts led to the identification of 40 new TRAP SBP ligands, the generation of experiment-based annotations for 2084 individual SBPs in 71 isofunctional clusters, and the definition of numerous metabolic pathways, including novel catabolic pathways for the utilization of ethanolamine as sole nitrogen source and the use of D-Ala-D-Ala as sole carbon source[1]. Other comparable large scale functional annotation studies were performed for the Isoprenoid Synthase[2] and Haloacid Dehalogenase[3] Superfamilies.

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II. High-throughput Protein Production Infrastructure. Despite a multitude of recent technical breakthroughs speeding high-resolution structural and functional analysis of biological macromolecules, production of sufficient quantities of well-behaved,

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

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

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FUNCTION & MICRO-STRUCTURAL IMAGING OF CNS DISEASES: DIFFUSION TENSOR IMAGING OF MILD HEAD TRAUMA

Dr. Branch is Director of Einstein's Gruss Magnetic Resonance Research Center, the Blaufox MicroPET/SPECT/CT Center and is Co-Director of the EGL Integrated Imaging Program at Einstein. These centers support a wide variety of translational imaging studies in both animals and man. Dr. Branch's personal research interests include brain injury and disease, cancer metastases, hemoglobinopathies and other disorders using MRI, PET and CT and MR-Guided High Frequency Focused Ultrasound (MRg-HIFU), in which he employs MR-based measures of function and microstructure to understand pathophysiological changes associated with cerebral and metastatic disease. A principal interest is the study of cerebral metabolic disorders involving anemia, particularly that associated with Sickle Cell Disease and its associated increase in the risk for stroke. Combining functional MRI and metabolic magnetic resonance spectroscopy with Diffusion Tensor Imaging (DTI), his research attempts to elucidates how alterations in tissue structure are related to tissue function. Translational application of MRI and photonic approaches are key features of his research.

Dr. Branch oversees or facilitates research involving a wide variety disease and imaging technology.

Particular interests include:

- Magnetic resonance imaging (MRI)
- Diffusion tensor imaging (DTI) of brain
- MRI/PET/CT of Animal Models
- MRI of brain disorders associated with hemoglobinopathies
- Imaging of macrophage / microglial induced inflammation
- High Frequency Focused Ultrasound (MRI-guided HIFU)

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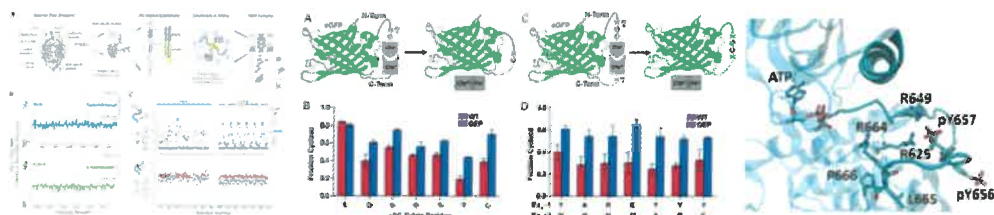

PHYSICAL BIOCHEMISTRY

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

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

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

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

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THE ROLE OF OPIORPHINS IN SMOOTH MUSCLE PHYSIOLOGY

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.

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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. Sol-gel encapsulation is used to trap functional intermediates. ii) Basic and translational translational focusing on the development of nano and micro particles including paramagnetic nanoparticles for sustained slow targeted release of therapeutic agents such as NO, curcumin, plasmids, siRNA and chemotherapy drugs; iii) treatment of vascular inflammation and hemoglobinopathies with nanoparticles. iv) Use of nanoparticles for drug delivery across the blood brain barrier for treatment of glioblastoma; v) Development of hemoglobin-based blood substitutes and suitable solid-state matrices for long terms storage of the product; iv) Probing the mechanisms and functional consequences of reactions through which hemoglobin generates nitric oxide and N_2O_3.

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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 (HF) 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 B_{12}-dependent ribonucleoside triphosphate reductase and the iron-sulfur cluster containing enzyme *Mtb* dihydroxyacid dehydratase; identity and structure of radicals generated during catalysis by prostaglandin H synthase (COX or PGHS), cytochrome c oxidase and 2-oxoglutarate dehydrogenase, molecular structure determination of kinesins and phosphoinositide 3-kinase using spin-labeling techniques; HF-EPR/ENDOR of a variety of protein and substrate-derived radical species.

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BIOENERGETIC, ENZYMATIC FUNCTION AND PROTEIN DYNAMICS

In our laboratory the mechanisms and properties of cytochrome *c* oxidase, the terminal enzyme in the electron transfer chain, are being investigated.

Physiologically, cytochrome *c* oxidase 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, rapid mixing techniques and crystallography. 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.

A new approach we are using to determine the structures of cytochrome *c* oxidase intermediates is Serial Femtosecond X-Ray Crystallography (SFX). This technique uses an X-Ray free electron laser (XFEL) which generates femtosecond X-Ray pulses to determine the crystal structure of nanocrystals of the enzyme in a flowing solution. The only XFEL available in this country is at SLAC, the Stanford Linear Accelerator Laboratory, where we go several times a year to make our measurements. With this techniques we are able to obtain crystal structures without radiation damage and we can determine the structures of kinetic and photochemical intermediates for the first time. This technique promises to unlock the secrets of how the oxygen reduction process brings about conformational changes that regulate proton translocation.

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Molecular Biology of Prostaglandin Transport

Prostaglandins (PGs) are context-dependent signaling molecules that signal diverse and important biological functions. In 1995, our laboratory discovered the prostaglandin (PG) transporter "PGT", a finding that we have extended to human and mouse genetics, fat metabolism, renal protection, and drug discovery. Our laboratory has been the leader in understanding PGT action and regulation, from whole-mouse knockouts down to the molecular level. We have advanced the hypothesis that PG signaling is akin to neuronal signaling, i.e. the signaling molecule is released and then taken up again by the same cell. Our global PGT knockout mice have low body fat and increased muscle mass while eating more food than controls, and are thus a model for prevention of sarcopenic obesity. We have developed a series of high-affinity PGT inhibitors that, in pre-clinical studies, mitigate diet-induced obesity and prevent renal toxic injury.

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MECHANISMS OF MITOSIS

The life of a cell in multicellular organisms is complex and proceeds through multiple stages, beginning with its "birth" from the division of preexisting cells, movement from its "birth" site to a distal target, differentiation into a form designed for a specialized task and then, finally, its death. All of these events are in one way or another influenced by microtubules, intrinsically dynamic and structurally polar polymers of alpha/beta-tubulin further organized into higher order arrays that vary according to the immediate needs of the cell. While probably best known as directional railways for the motor driven transport of intracellular cargos, microtubules also form the spindle apparatus that separates chromosomes and defines the site of cell cleavage during mitosis/meiosis, provide structural support for the formation of elongate cell shapes and regulate the behaviors of other cytoskeletal networks, such as actin, through mechanisms that remain poorly understood. The broad objective of my research program is to identify the fundamental molecular mechanisms that goven the formation and function of the microtubule cytoskeleton and determine how these contribute to human health and disease.

Specific ongoing research projects include:

I) Determining the mechanisms of chromosome segregation. The mitotic spindle is a self-organizing microtubule-based machine that segregates chromosomes into identical daughter nuclei during cell division. Defects in spindle assembly and the movement of chromosomes on it give rise to cells with too many or two few chromosomes (aneuploidy) which is a hallmark of tumorigenesis. Previous work from my laboratory has shown that the mitotic spindle moves chromosomes by a Pacman-Flux mechanism involving the coordinated activities of microtubule depolymerizing and severing enzymes (e.g. Rogers et al, Nature, 2004; Zhang et al, The Journal of Cell Biology, 2007, Rath and Sharp, Chromosome Research, 2011)
II) Determining the roles of microtubules in cell motility. The ability of cells to migrate from their sites of origin to distal targets is fundamental to the development and maintenance of multicellular organisms. Defects in cell migration have also been linked to numerous human pathologies ranging from mental retardation to cancer metastasis. Decades of work have established that somatic cell motility is driven by a polarized actomyosin network that, among other things, promotes protrusion of the membrane at the cell front (leading edge) and contractility at the rear. Much less is understood about the contributions of microtubules to these processes. However, we recently showed that the microtubule severing enzyme, Katanin, localizes to the cell cortex and netatively regulates cell motility by suppressing actin-based protrusions (Zhang et al, Nature Cell Biology, 2011) We have since identified a number of additional microtubule regulatory proteins (some of which are entirely uncharacterized in the literature) that control distinct parameters of cell movement. Elucidation of the specific functions and mechanisms of action of these is a major current thrust of my research program.
III) Development of novel therapeutics. We have found that specific microtubule regulatory proteins can be targeted to alter various aspects of human cell motility both in vitro and in vivo. We are currently building on these findings to develop novel therapies to enhance wound healing, treat spinal cord injury and cardiovascular disease, and prevent cancer metastasis. We are working closely with the Friedman, Nosanchuk and Zhou labs to develop and test nanoparticle-based approaches to manipulate the activiites of microtubule regulatory proteins in a clinical context.

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Structure and Function of Biological Macromolecular Machines

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

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

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

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MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY

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 metabolomics 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 are 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.

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STRUCTURE, FUNCTION AND INHIBITION of PROTEINS

Human indoleamine 2,3-dioxygenase (IDO) and tryptophan dioxygenase (TDO) are two key cancer immunotherapeutic targets. They catalyze the first and rate-limiting step of the kynurenine pathway by inserting dioxygen into the substrate L-Tryptophan. Although IDO is a monomer, while TDO is a tetramer, their active sites share high structural similarity, posing serious challenges for rational design of selective inhibitors. The goal of our current research focuses on understanding structure and function relationships of IDO and TDO and designing selective inhibitors targeting them. In this research program, a wide array of approaches, including molecular biology, enzymology, optical spectroscopies, X-ray crystallography, calorimetry and computational modeling methods, are employed to interrogate these two physiologically important enzymes.

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