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 of 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
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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MEMBRANE TRANSPORTERS and MALARIA

MALARIA PURINE TRANSPORTERS & ANTIMALARIAL DRUG DEVELOPMENT

Malaria is a major public health problem affecting large areas of the world. About 600,000 people, mostly children and pregnant woman, die each year due to malaria. Malaria is caused by unicellular parasites from the *Plasmodium* species that grow inside erythrocytes. *Plasmodium falciparum* causes the most lethal form of malaria. *Plasmodium* species parasites are purine auxotrophs and require an exogenous source of purines to survive. They import purine precursors from the host erythrocyte via equilibrative nucleoside transporters (ENTs). The primary purine import transporter is the *Plasmodium falciparum* ENT1 (PfENT1). Knockout of PfENT1 is lethal at purine concentrations found in human plasma (<10 μM). This suggests that PfENT1 inhibitors might kill parasites and 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\textsubscript{50} of 5-50 nM. The compounds kill *P. falciparum* parasites in culture with micromolar IC\textsubscript{50} values. We have also received PfENT1 inhibitors from GlaxoSmithKline (GSK) based on their use of our assays to screen their 1.8 million compound library. We are characterizing the hits and exploring the structure-activity relationships (SAR) for the compounds to identify more potent derivatives. We are currently working with medicinal chemists to identify more potent derivatives of the hits that may serve 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.

Selected References:


Using the information derived from high resolution X-ray crystallography, we exploit the structure of individual proteins to understand the formation and regulation of macromolecular assemblies. In particular, our lab is focused on the regulation and assembly of the actin cytoskeleton, and the general structural features of trans-membrane signal transduction pathways. Our structural results are used to design mutant proteins with defined in vitro activities and then use genetic systems in yeast, Drosophila and mice to determine the in vivo consequences of a particular activity. This multidisciplinary approach places us in the unique position to obtain in vivo structure-function relationships for proteins regulating major aspects of cell biology.

We are studying a number of proteins involved in the formation and localization of filamentous actin networks in the cell. We have solved the structures of proteins that are responsible for the assembly, dynamics and crosslinking of actin filaments. Most recently, we have focused our efforts on the structural analysis of the scaffolding proteins that participate in the formation of multicomponent assemblies that direct the site specific, de novo nucleation of actin filaments, and link these dynamic events with phosphoinositide and small G-protein signaling pathways. Elucidating the mechanisms underlying the dynamic behavior of the actin cytoskeleton is essential to understanding cell locomotion, cytokinesis, and complex morphogenetic and developmental programs in multicellular organisms. These same mechanisms underlie pathological states such as neoplastic transformation and tumor metastasis and the invasive and motile mechanism used by Listeria and Shigella and a range of enteropathogenic bacteria.

We have solved the first structures of allergens, which are responsible for the clinical symptoms of allergy, including rhinitis, conjunctivitis and asthma. The structures of these proteins provide novel targets for the design of therapeutic agents which block the primary events in the allergic response. The spatial information derived from these allergens is allowing us to obtain novel structural information about the receptor-mediated events which trigger histamine release, and will provide general principles about signaling pathways dependent on receptor clustering events. As an extension of this work, we have recently begun to examine the structures of proteins that modulate the cell surface events involved in T-cell activation.

Our laboratory also has active collaborations on structure-based drug design for a number of proteins that are prime candidates as targets for anti-malarials, autoimmunity and graft rejection. Our laboratory has also initiated a very large scale structural genomics program that aims to develop and apply high throughput methodologies with the goal of producing a structural model for every member of the human proteome. More information about the proteomics program can be obtained at http://proteome.bnl.gov.

A number of projects are available to students with interest in specific areas of cell biology or who desire training in structural analysis.

Selected References


Dr. Branch Directs Einstein’s Gruss Magnetic Resonance Research Center, which supports a wide variety of imaging studies in both animals and man including brain injury and disease, liver disease, cancer, hemoglobinopathies and other disorders using MRI, PET and CT and MR-Guided High Frequency Focused Ultrasound (MRg-HIFU). Dr. Branch personal research focuses on the application of MR-based measures of function and microstructure to understand pathophysiological changes associated with disease. Diffusion Tensor Imaging (DTI) provides detailed information regarding tissue water movements, which elucidates alterations in tissue integrity, while perfusion, permeability and function are measured using dynamic MR based methods, which can be coupled with metabolic spectroscopy to comprehensively describe tissue state. Combined with MRI based measures of tissue perfusion and physiology, DTI is applied to characterize evolving tissue pathology in mild head trauma, sickle cell anemia, liver cancer and other diseases. Translational application of these approaches is a key feature of our research.

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)

Select Bibliography:

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 is 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 these methods to understand the dynamic structures of the FGFR. Several kinases and phosphatase systems including Crk, Src, Abl, PTPN22 and receptor kinase FGFR are under study.

Selected References

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.

Selected Publications:
PROTEIN DYNAMICS AND REACTIVITY/NANOPARTICLE DRUG DELIVERY/NITRIC OXIDE

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 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; 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 N₂O₃.

Selected Recent References

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 B_{12} dependent 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 kinesins using spin-labeling techniques; HF-EPR/ENDOR of a variety of protein and substrate-derived radical species.

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 the regulatory mechanisms of the enzyme to determine the various factors that control its activity. 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


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, carcinogenesis, zebrafish development, and now drug discovery. Our laboratory has been the leader in understanding PGT action and regulation, from whole-mouse knockouts down to the molecular level, including site-directed and cysteine-scanning mutagenesis. 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 while eating more than controls. By screening a small molecular library and carrying out subsequent lead compound development, we have developed a series of high-affinity PGT inhibitors that, in pre-clinical studies, mitigate diet-induced obesity.

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 govern 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 negatively 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 activities of microtubule regulatory proteins in a clinical context.
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.

Selected bibliography:
Nucleotide binding and hydrolysis induces a disorder-order transition in the kinesin neck-linker region.
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.
 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 (TDO) 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.

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