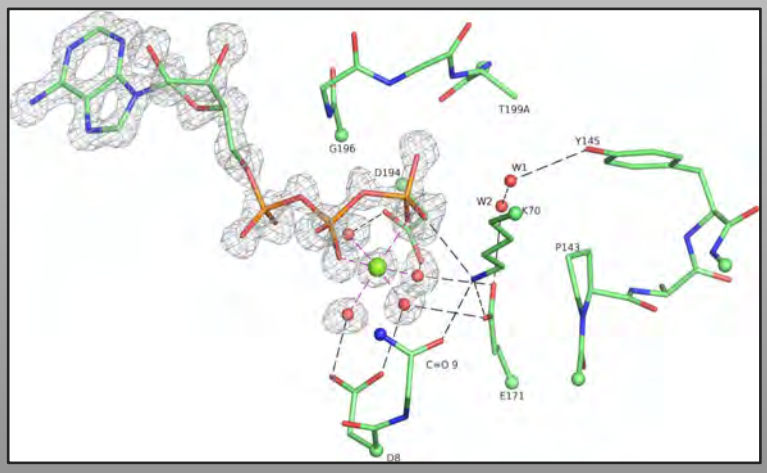
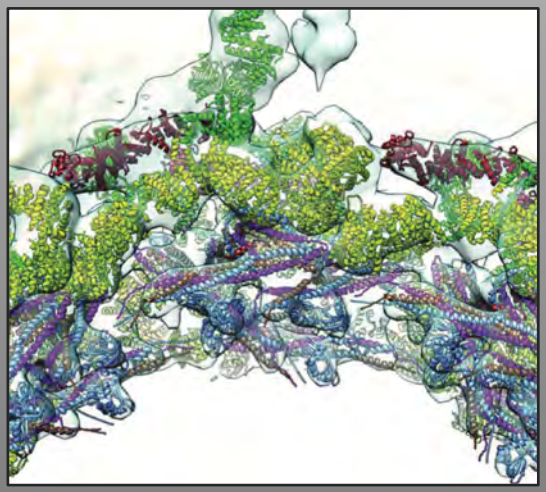
Gulf Coast Consortia
QUANTITATIVE BIOMEDICAL SCIENCES

Emerging Trends in Cellular and Molecular Biophysics Symposium

December 3, 2019

BioScience Research Collaborative
6500 Main St., Event Hall
Houston, Texas





Gulf Coast Consortia

QUANTITATIVE BIOMEDICAL SCIENCES

The Gulf Coast Consortia (GCC), located in Houston, Texas, is a dynamic, multi-institution collaboration of basic and translational scientists, researchers, clinicians and students in the quantitative biomedical sciences, who benefit from joint training programs, topic-focused research consortia, shared facilities and equipment, and exchange of scientific knowledge. Working together, GCC member institutions provide a cutting-edge collaborative training environment and research infrastructure beyond the capability of any single institution. GCC training programs currently focus on Biomedical Informatics, Computational Cancer Biology, Molecular Biophysics, Neuroengineering, Pharmacological Sciences, Precision Environmental Health Sciences and Antimicrobial Resistance. GCC research consortia gather interested faculty around research foci within the quantitative biomedical sciences, and currently include Antimicrobial Resistance, Nanox, Mental Health, Innovative Drug Discovery and Development, Translational Pain Research, Theoretical and Computational Neuroscience, Single Cell Omics, Regenerative Medicine, Translational Imaging and Cellular and Molecular Biophysics. Current members include Baylor College of Medicine, Rice University, University of Houston, The University of Texas Health Science Center at Houston, The University of Texas Medical Branch at Galveston, The University of Texas M. D. Anderson Cancer Center, and the Institute of Biosciences and Technology of Texas A&M Health Science Center.

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BRUKER

1st Annual GCC Cellular and Molecular Biophysics Symposium

Dec. 3, 2019

Agenda

8:00 Breakfast and Poster Setup

9:00 Welcome

Session 1: Cellular Biophysics
Convener: Irina Serysheva, Univ. of Texas Health Science Center Houston

9:05 *Progress and Potential of Cryo-electron Tomography*
Grant Jensen, Cal Tech University

9:45 *Electron Cryo-Tomography Reveal Axons Present a Barrier to Mitochondrial Transport*
Neal Waxham, Univ. of Texas Health Science Center Houston

10:05 *Structural Study of Bacterial Multidrug Efflux Pumps*
Zhao Wang, Baylor College of Medicine

10:25 *Channelrhodopsin Structure/Function and Optogenetics*
John Spudich, Univ. of Texas Health Science Center Houston

10:45 Break

Session 2: Trainees
Convener: George Phillips, Rice Univ.

11:00 Selected Abstracts

11:40 Flash Poster Data Blitz

12:00 Lunch and Poster Session (Posters 12:45-1:45)

Session 3: Enzyme and Computational Biophysics
Convener: Ming Zhou, Baylor College of Medicine

1:45 *Pressure-sensing WNK Kinases*
Elizabeth Goldsmith - Univ. of Texas Southwestern

2:25 *Replication Mechanisms of a Small dsRNA Virus*
Jane Tao, Rice Univ.

2:45 *Physics of Memory and Learning – From the Perspective of Interacting Biomolecules*
Margaret Cheung, Univ. of Houston

Agenda

3:05 *Hydrophobicity and Solubility*
Monte Pettitt, Univ. of Texas Medical Branch at Galveston

3:25 Break

Session 4: Molecular Biophysics
Convener: Xiaodong Cheng, MD Anderson Cancer Center

3:40 *Allosteric Control of Hsp70 Chaperone Activity*
Wayne Hendrickson, Columbia Univ.

4:20 *Defining Dynamic DNA Repair Responses*
John Tainer, MD Anderson Cancer Center

4:40 *Cryo-EM Structures of Transcriptional Mediator Complexes*
Kuang-Lei Tsai, Univ. of Texas Health Science Center Houston

5:00 *What is THE Structure of a Protein When it has More than One? Time to Move Beyond Static Snapshots*
George Philips, Rice Univ.

5:20 Reception

Grant Jensen, PhD
Investigator, Howard Hughes Medical Institute
Professor, Biology and Biophysics

Electron Cryotomography: Present Capabilities and Future Potential

Grant Jensen is an Investigator of the Howard Hughes Medical Institute and a Professor of Biology and Biophysics at the California Institute of Technology in Pasadena, California. He earned his doctorate working on electron microscopy of RNA polymerase and other protein complexes with Dr. Roger Kornberg (who later won the Nobel prize for structural studies of transcription). Next Grant continued his work in protein electron microscopy as a Damon Runyon-Walter Winchell post-doctoral fellow under the supervision of Dr. Kenneth Downing at the Lawrence Berkeley National Lab. Here his interests expanded to include electron tomography of whole cells. Grant launched his own lab at Caltech starting in 2002. At Caltech his research has focused on three main areas: the ultrastructure of small cells, the structural biology of HIV, and the further development of cryo-EM technology. Together with his colleagues he has now published ~150 papers in these areas (see http://www.jensenlab.caltech.edu/publications.html). His lab has developed a searchable tomography database and populated it with ~40 thousand cryotomograms of over 100 different viral and microbial samples. Among his most prominent discoveries has been the structure and function of the bacterial type VI secretion system, a "poison-tipped spring-loaded molecular dagger," and the architecture of the type IV pilus responsible for cell motility. Meanwhile his teaching has centered on biophysical methods, including most recently the creation of a popular 14-hour online course "Getting started in Cryo-EM."

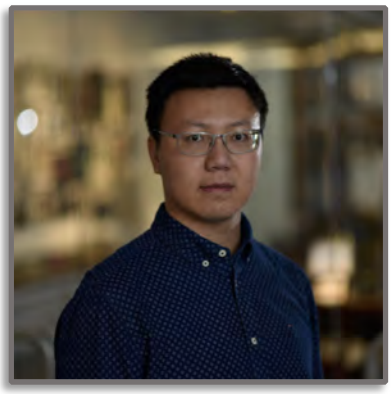
Abstract: In the last ten years electron cryotomography (cryo-ET) has made it possible to visualize large macromolecular assemblies inside intact cells in a near-native, "frozen-hydrated" state in 3-D to a few nanometers resolution. Increasingly, atomic models of individual proteins and smaller complexes obtained by X-ray crystallography, NMR spectroscopy, or other methods can be fit into cryotomograms to reveal how the various pieces work together inside cells. A few good pictures is therefore sometimes all that is really needed to distinguish between competing models. To illustrate these points, I will present examples of current results from our recent work on bacterial secretion systems and eukaryotic stress responses. The range of cellular samples that cryo-ET can reveal is dramatically expanding with FIB-milling, and will likely soon become dramatically more useful with correlated light and electron microscopy (CLEM) targeting. Two major developments in cryo-ET technology further suggest that cryo-ET will become an important new method for determining the structures of single particles and small proteins as well. First, highly eucentric stages are now allowing tilt-series to be recorded in seconds rather than minutes, opening the possibility of high resolution single particle tomography. Second, cryo-ET of very small crystals (“nanocrystals”) offers compelling potential advantages over X-ray crystallography. I will explain these advances and their projected implications with examples from our work.

M. Neal Waxham, PhD
William M. Wheless III, Professor in Biomedical Sciences
Professor, Neurobiology & Anatomy

Electron Cryo-Tomography Reveal Axons Present a Barrier to Mitochondrial Transport

Dr. Waxham is currently the Wheless Professor of Biomedical Sciences and Professor of Neurobiology and Anatomy at McGovern Medical School-Houston. He received his PhD in Immunology and Infectious Disease from Johns Hopkins University in 1984 and his BS in Microbiology from Penn State University in 1978. His laboratory studies the structure and function of synapses using computational, biochemical and biophysical approaches and more recently, electron cryomicroscopy and cryotomography.

Abstract: This talk will emphasize our recent efforts to characterize the high resolution structural features of "near native" neuronal processes and synapses using cryoelectron tomography. Hippocampal neurons were grown directly on EM grids, cryopreserved and imaged in an electron microscope under cyrogenic conditions. Well preserved axonal processes and presynaptic varicosities were abundant and of an ideal thickness to produce high quality tomographic reconstructions. The question posed in this study was what structural barriers might be presented to the transport of mitochondria within the densely packed environment of the thin axonal processes. In a typical mammalian neuron, the presynaptic varicosities are sites of vesicular release of neurotransmitter and can number in the tens of thousands all of which must be supported through a single, immensely long, branching axon. The distance of synapses from the soma present unique cell biological challenges, including how to support the immense energy demands necessary to maintain synaptic transmission. Using electron cryotomography, we quantified the magnitude of 3D spatial restrictions within axons and varicosities to assess how they may influence the subcellular distribution and morphology of mitochondria. Segmented tomographic reconstructions revealed major morphological adaptations of mitochondria due to restricted physical dimensions between presynaptic varicosities and axons. Furthermore, analysis of mitochondrial membranes revealed distinct structural changes between the inner and outer mitochondrial membranes, suggesting independent regulation of their properties to adapt to the spatial constraints within axons. These findings offer a new perspective on the remarkable structural plasticity of mitochondrial membranes but raise the question of whether the restricted dimensions and crowded space within axons lead to underappreciated barriers to mitochondrial transport specifically and perhaps other organelle transport more broadly.



Zhao Wang, PhD
Assistant Professor
Verna and Marrs McLean Department of Biochemistry and Molecular Biology

Structural Study of Bacterial Multidrug Efflux Pumps

The ultimate goal of my research is to gain a deeper understanding of biological nano-machines by CryoEM/CryoET techniques and computer reconstruction, and the use of structures to reveal their structure-based functional mechanisms. Electron microscopy has been one of the primary techniques used for my studies. Structural studies of macromolecular complex structures have been a keystone of my scientific career when I joined National Center for Macromolecular Imaging (Baylor College of Medicine, Houston TX) as a Research Associate. I published numerous important structures, including the first structures of nuclear receptor coactivator complexes, multidrug efflux pumps, and several ion channels. I pioneered using the first direct electron detection device (DDD), a newly designed equipment that enables a 'resolution revolution' in single-particle CryoEM and I am developing a novel protocol of data collation and processing using this camera. Our work first develops the 'damage compensation' analysis strategy, which is now commonly used by the EM community. Having a broad background in biochemistry and structural biology, with specific training and expertise in CryoEM of membrane proteins, I started as an independent investigator at the Department of Biochemistry and Molecular Biology (Baylor College of Medicine, Houston TX) in 2018. Be trained in experimental & computational biophysics, my laboratory research involving in the development of experimental and computational methodologies for CryoEM/CryoET, and application on research of following biological systems: First, the structure, assembly, and mechanism of the resistance-nodulation-cell division (RND)-superfamily of efflux pumps; Second, Structures of nuclear receptors and co-activators, which have great relevance to genetic and reproductive diseases, disorders of metabolism and diabetes, and cancers; Last, A major area of interest in cation ion channels involving many critical functions such as IP3R and TRPV.

Abstract: Multidrug efflux pumps actively expel a wide range of toxic substrates from the cell and play a major role in intrinsic and acquired drug resistance. In Gram-negative bacteria, these pumps form tripartite assemblies that span the cell envelope. However, the in situ structure and assembly mechanism of multidrug efflux pumps remain unknown. Here we report, for the first time, the in situ structure of the Escherichia coli AcrAB-TolC multidrug efflux pump obtained by electron cryo-tomography and subtomogram averaging. Our data point to a sequential assembly process beginning with AcrAB subcomplex in living bacteria, which provides the structural basis for the design of efflux pump inhibitors. The cellular efflux pump observation is in marked contrast to the behavior of the purified complex, indicating that both the structure and function of the pump depend critically on interactions with other molecules and cellular components in living cells.

John Spudich, PhD

Robert A. Welch Distinguished Chair in Chemistry
Director, Center for Membrane Biology
Professor, Department of Biochemistry & Molecular Biology

Channelrhodopsin Structure/Function and Optogenetics

John Spudich, professor of Biochemistry and Molecular Biology at UTHealth McGovern Medical School, holds the Robert A. Welch Distinguished Chair in Chemistry and is Director of the Center for Membrane Biology. A pioneer in the field of microbial rhodopsins, he discovered the first with a sensory function (phototaxis signaling), demonstrated worldwide presence of sensory rhodopsins with diverse signaling mechanisms, as well as new light-driven ion pumps (e.g. proteorhodopsins), and elucidated relationships between ion transport and sensory signaling mechanisms in the rhodopsin superfamily. Phototaxis receptors that function as light-gated channels, cation-conducting channelrhodopsins (CCRs) and, more recently, anion-conducting channelrhodopsins (ACRs), identified by Spudich and coworkers, have become highly noteworthy because of their use as genetically-targeted tools for photocontrol of neuron firing (optogenetics). Optogenetics has transformed research on neural circuitry, and is showing promise for gene therapy for neurological diseases, for example for vision restoration for which clinical trials are in progress. Dr. Spudich has published over 200 research articles and commentaries and two books on photosensory biology. He is an elected fellow of the American Academy of Arts and Sciences.

Abstract: Microbial rhodopsins are a family of photoactive retinylidene proteins widespread throughout the microbial world. They are notable for their diversity of function, using variations of a shared seven-transmembrane helix design and similar photochemical reactions to carry out distinctly different light-driven energy and sensory transduction processes. Their study continues to advance our understanding of how evolution modifies protein scaffolds to create new protein chemistry.

Use of microbial rhodopsins as genetically targeted tools to control membrane potential with light has given rise to the new technology of optogenetics, which has had transformative impact on research in neurophysiology. Cation-conducting channelrhodopsins (CCRs) enabling targeted photoinduced neuron firing and light-driven transporters and more recently anion-conducting channelrhodopsins (ACRs) enabling neuron photosuppression have become established as effective tools for analysis of brain circuitry. Channelrhodopsins have also begun to be tested for optogenetic gene therapy in animal models of neurological diseases, and clinical trials are in progress for channelrhodopsin-based vision restoration to the blind.

Recent progress includes our X-ray crystal structure of the best studied and most used ACR, GtACR1 from Guillardia theta. The structure obtained in collaboration with the laboratories of Lei Zheng in the Center for Membrane Biology and Meitian Wang at the Swiss Light Source, Paul Scherrer Institute, in Villigen, Switzerland, revealed a preexisting channel in the dark state closed by three well-defined constrictions, one of which is formed by the photoactive site itself. The GtACR1 tunnel is the only ion conductance pathway so far imaged in atomic structures of channelrhodopsins. These findings have led to new insights into the conductance mechanism.

Elizabeth J. Goldsmith, PhD
Patti Bell Brown Professorship
Biochemistry

WNK Kinases are Cellular Homeostats that Sense Pressure and Ions

Elizabeth Goldsmith did her Ph.D. work in the laboratory of David Eisenberg at UCLA, and then studied with Max Perutz at the MRC laboratories in Cambridge and Robert Fletterick at the University of California, San Francisco. She has made several contributions to our understanding of proteins, mainly through crystallographic studies. In her own lab, she determined the structure of a serpin protease inhibitor and showed through kinetic assays that the active conformer is not the most stable configuration of the serpin polypeptide. She was involved at the design level in a project directed by Joseph Sambrook to make a second generation, mutant, tissue plasminogen activator that is used in the treatment of heart attack and stroke. Over the past 25 years, she has made several discoveries concerning protein kinases including structures of an inactive and active kinase in the exact same sequence, the structural basis of MAP kinase docking interactions, and elucidation of mechanisms of regulation of the environmental sensing WNK kinases.

Abstract: WNK [With No lysine (K)] kinases are cytoplasmic serine/threonine protein kinase that sense and adjust the intracellular milieu. They were discovered in a strategy to find stress activated kinases and shortly thereafter found to be associated with familial forms of hypertension and hypotension. The familial disease is treated by diuretics that target cation- chloride co-transporters (NCC's and NKCC's). This association led us to inquire whether WNKs are regulated by chloride or osmotic stress, known effectors of NCC's and NKCC's. Data will be presented that the kinase domains of WNK kinase 1 and 3 are inhibited by chloride and potassium and activated by osmotic stressors Given the association with blood pressure regulation, we further inquired whether these proteins are activated by hydrostatic pressure applied at 190 kPa, and found that they are so activated. The activation and inhibition work through an autophosphorylation mechanism. Autophosphorylation and substrate phosphorylation was tracked by mass spectrometry, ATP depletion assays, and by in gel staining for phospho-proteins. Applied osmotic pressure or hydrostatic pressure induces dissociation and autophosphorylation. Crystallography of the pressure sensor shows that it is an asymmetric dimer housing excess bound water. The crystallographically observed asymmetric dimer was observed by SAXS. Application of hydrostatic pressure in a SEC-MALs instrument (700 kPa) induces monomer formation. Implications and future directions will be discussed.

UT Southwestern

Yizhi Jane Tao, PhD
Professor
BioSciences

Replication Mechanisms of a Small dsRNA Virus

Born in China, Yizhi Jane Tao received a B.Sc. degree in biology from Peking University in Beijing, China, in 1992. She later moved to West Lafayette, Indiana, where she received her Ph.D. in biological science while studying under Michael Rossmann at Purdue University. She completed a postdoctoral fellowship under Stephen C. Harrison at Harvard University in 2002. Upon completing her postdoctoral studies, she joined the faculty of Rice University, where she has made important contributions to the study of influenza, hepatitis, and birnaviruses.

Abstract: Picobirnaviruses (PBV) are small, dsRNA viruses with a bisegmented genome that have been repeatedly isolated from fecal samples of diseased and healthy humans and broad range of animal species. In humans, picobirnavirus has been isolated from patients with acute gastroenteritis. Several infection mechanisms have been proposed for picobirnavirus: that it infects mammals in a manner similar to rotavirus or norovirus, that it infects bacteria that colonize mammalian guts, or that it infects mitochondrial endosymbionts. In the three decades since its discovery, a culture system or an animal model for picobirnavirus has not been described, posing a barrier for mechanistic understanding of this virus and its lifecycle. Using various biophysical methods as well as enzymatic and biochemical assays, our laboratory aims to tease apart the picobiranvirus infection mechanisms including viral gene expression, assembly, genome replication and cell attachment.

Margaret S. Cheung, PhD
Moores Professor of Physics, Chemistry, and Computer Science

Physics of Memory and Learning – From the Perspective of Interacting Biomolecules

Dr. Cheung is Moores Professor of Physics at the University of Houston. She graduated from the National Taiwan University with a bachelor's degree in chemistry and received her Ph.D. in physics from the University of California, San Diego. She carried out theoretical biological physics and bioinformatics research as a Sloan Postdoctoral Fellow at the University of Maryland and started her lab at the University of Houston in 2006. Dr. Cheung's research focuses on protein folding inside a cell, calmodulin dependent calcium signaling, protein motors, actomyosin dynamics, and quantum efficiency in organic photovoltaics. She is particularly interested in developing multi-physics models that bridge the dynamics across wide temporal and spatial scales in subcellular biology and materials, and designing computational algorithms that integrate high-performance computing resources across heterogeneous systems. She is a fellow of the American Physical Society, a Senior Scientist at the Center for Theoretical Biological Physics and an Adjunct Professor of Bioengineering at Rice University.

Abstract: The calcium (Ca2+) signaling pathway is integral to learning and memory formation in neurons. Short transient Ca2+ around the entry sites activate Ca2+-binding proteins such as calmodulin (CaM). The prototypical pathway describes CaM as encoding a Ca2+ signal by selectively activating downstream CaM-dependent proteins through molecular binding. However, CaM's intrinsic Ca2+-binding properties alone appear insufficient to decode rapidly fluctuating Ca2+ signals. It has been proposed that the temporally varying mechanism for producing target selectivity requires CaM-target interactions that directly tune the Ca2+-binding properties of CaM through reciprocal interactions, thus making a distinctive signaling decision in a cell. In this presentation, I will first focus on the binding mechanism of CaM and its target, which requires mutually and conformationally-induced changes in both participants Then, I will reveal how a target mechanistically tunes CaM's affinity for Ca2+ by examining its binding with neurogranin (Ng) and CaM-dependent kinase II (CaMKII). These two targets are biochemically known to tune CaM's affinity for Ca2+ in opposite directions in postsynaptic neuronal cells. I will further discuss about the active role of CaM/CaMKII in organizing the structure and dynamics of actin networks in a dendritic spine, underlying how a chemical reaction at a molecular scale transmits to a designated mechanical response at a micron scale. My group has employed an integrative approach of quantum mechanical calculations, all-atomistic molecular dynamics, and coarse-grained molecular simulations to investigate these problems across a wide scale in both space and time.

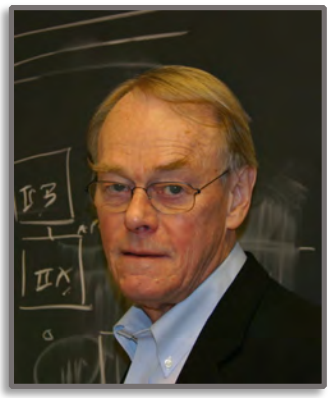
University of Houston

B. Montgomery Pettitt, PhD
Professor, Biochemistry & Molecular Biology
Director, Sealy Center for Structural Biology and Molecular Biophysics

Hydrophobicity and Solubility

B. Montgomery Pettitt is Robert A. Welch Distinguished University Professor of Chemistry at the University of Texas Medical Branch where he is in the Departments of Biochemistry and Molecular Biology as well as Pharmacology and Toxicology. He was a postdoctoral fellow at the University of Texas at Austin and an NIH Fellow at Harvard University. He spent 27 years at the University of Houston before moving to UTMB. He directs the Sealy Center for Structural Biology and Molecular Biophysics, a research center serving UTMB faculty and students with facilities for structural biology (x-ray, NMR and cryoEM) and scientific computing for use in the areas of simulation and modeling. His research focuses on understanding proteins and DNA in solution to understand signaling in cells. Signaling that becomes unregulated leading to cancer and other diseases is central to the medical motivation in his laboratory. His theoretical work relies on techniques in statistical mechanics and high performance scientific computing.

Abstract: Hydrophobicity is a phenomenological concept that has been related to protein folding, aggregation and association. Authors use a variety of different definitions from surface science to biochemistry. We consider the use of the solubility limit to characterize the forces responsible for aggregation. We calculate the solubility limit of penta-peptides in water by simulating the phase separation in an over saturated solution. The thermodynamic driving forces are decomposed in terms of enthalpy and entropy. We demonstrate that fluctuations in conformation and hydration number of peptide in monomeric form are correlated with the solubility of the peptide.



Wayne Hendrickson, PhD
Professor
Biochemistry and Molecular Biophysics

Molecular and Structural Basis of HSP70 Activity

Wayne A. Hendrickson is a University Professor at Columbia University in the Department of Biochemistry and Molecular Biophysics and Violin Family Professor in the Department of Physiology and Cellular Biophysics. He is also Scientific Director of the New York Structural Biology Center. Hendrickson has a B.A. from the University of Wisconsin at River Falls, a Ph.D. in biophysics at Johns Hopkins University with Warner Love, and postdoctoral research experience with Jerome Karle at the Naval Research Laboratory. He and his colleagues use x-ray crystallography and electron microscopy as well as biochemical and cellular analyses to study biomolecular properties in atomic detail. The current emphasis is on membrane receptors and cellular signaling, molecular chaperones and protein folding, viral proteins and HIV infection, and technology for membrane protein production and analysis. Hendrickson's advances in diffraction methodology have contributed significantly to the emergence of structural biology as a major force in modern biology and molecular medicine.

Abstract: Heat-shock proteins of 70 kDa (Hsp70s) are vital in nearly all forms of life, notably important for protein folding. Hsp70s use ATP binding and hydrolysis at a nucleotide-binding domain (NBD) to control the binding and release of client polypeptides at a substrate-binding domain (SBD); however, the mechanistic basis for these allosteric actions has been elusive. We have developed a theoretical model of allosteric equilibria among Hsp70 conformational states to explain functional characteristics of structure-inspired mutants. A restraining state (R) restricts ATP hydrolysis and binds peptides poorly; whereas a stimulating state (S) hydrolyzes ATP rapidly and has high intrinsic affinity for substrates but with rapid binding kinetics. This model for allosteric regulation is supported by new Hsp70 DnaK structures in the postulated S state and improved R-state structures; additional biochemical tests further corroborate the theory. The basis for R-state blockage of ATP hydrolysis is revealed from a series of high-resolution (1.3 - 1.8 Å) structures of NBD-ATP. These structures define a catalytic mechanism that entails steering of the Pγ phosphate into a scissile orientation, positioning of a water molecule for nucleophilic attack, deprotonation of the attacking water by a catalytic base, and formation of a pentacovalent phosphorane transition state.

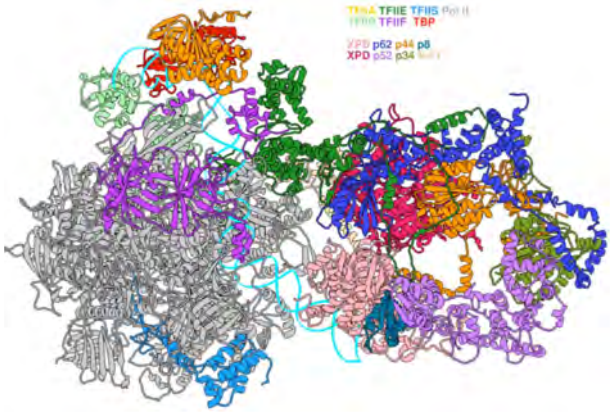
John A. Tainer, PhD
Robert A. Welch Chair in Chemistry
Director SIBYLS, Advanced Light Source,
Director Structural Biology

Defining Dynamic DNA Repair Responses

John Tainer received his undergraduate training in Zoology and Anthropology at Duke's Trinity College and his Ph.D. in Biochemistry and Structural Biology also from Duke University. He was on the faculty at Scripps as a tenured Professor for 20 years. He joined Lawrence Berkeley Lab in 2000 as a Senior Scientist where he designed, built, and continues to run the synchrotron beamline Structurally Integrated Biology for Life Sciences (SIBYLS). From his work at MD Anderson, he received a National Cancer Institute Outstanding Investigator award for Mesocale And Nanoscale Technologies Integrated by Structures for DNA Repair Complexes (MANTIS-DRC) along with a CPRIT Multi-Investigator Award for BRCA Answers from Cancer Interactome Structures (BACIS). He has solved >350 macromolecular structures with coordinates and X-ray diffraction data deposited in the Protein Data Bank for researchers worldwide. He has published 26 papers recommended by the Faculty of 1000, and his papers have been cited over 30,000 times (Web of Science H-index 106). He works on developing and applying methods for integrating biophysical data with cell biology to address biological questions. His analyses provide metrics and parameters to quantitatively assess flexibility, measure intermolecular distances, define data to model agreement, and reduce false positives. His funded research aims to build foundational knowledge on DNA damage responses and their connections for cancer biology impacting patient care.

Abstract: Pol II depends on key general transcription factors (GTFs: TFIIA, TFIIB, TFIID, TFIIF, TFIIS, TFIIE and TFIIH) to recognize promoter DNA and assemble with the polymerase into a pre-initiation complex (PIC) to begin transcription. Within this PIC complex, transcription Factor IIH (TFIIH) plays critical and distinctly different helicase roles in transcription initiation and in Nucleotide Excision Repair. The helicase mechanisms for TFIIH activities in these two pathways are not understood, and published models of TFIIH are missing key parts of TFIIH subunits p62, p52, p44, p34, and XPB. We determined a largely complete TFIIH model based on advanced computational modeling plus integration of published cryoEM maps and crystal structures [1-5]. Strikingly, newly modeled regions interlace TFIIH subunits together like "molecular rigging" and are positioned for previously unexpected regulatory functions. The density maps identify key TFIIE/p62 interactions linking the PIC core to TFIIH. Furthermore, p62 caps the ATP binding site in XPD helicase subunit and both XPB and p62 are positioned against the ssDNA binding interface of XPD, indicating that the XPD repair functions are blocked in the apo TFIIH. The mostly complete TFIIH structure enabled molecular dynamics revealing which elements of TFIIH, independent of protein chain, move together as a community or act as "the gears of the TFIIH machine". We find that XPD is a dynamic anchor point and that promoter opening is linked to the global motions and dynamic networks within TFIIH. Notably, 31 of 34 single site patient mutations map to XPD and point to XPD as a central pivot point for TFIIH motions. Thus, the perplexing association of TFIIH with three different diseases is clarified by the finding that disease mutations cluster into distinct classes based on subunit assembly, XPD function, and networked communities. We are moreover finding analogous results with the XPG nuclease and a replication restart complex.

Fig. 1. PIC defined by Cryo-EM, crystallography,



University of Texas MD Anderson Cancer Center

Kuang-Lei Tsai, PhD
Assistant Professor
Biochemistry and Molecular Biology

Cryo-EM Structures of Transcriptional Mediator Complexes

Dr. Tsai's research interests focus on the use of single-particle cryo-electron microscopy (cryo-EM) and computational image analysis to determine the structures of macromolecular complexes that carry out key biological functions, such as transcription and gene regulation. In particular, he is interested in the functional relevance of large-scale conformational changes in macromolecular complexes. His research group uses advanced cryo-EM, X-ray crystallography and biochemical approaches to understand how dysfunction of specific macromolecular complexes drives cancer cell development.

Abstract: The eukaryotic Mediator complex, including a core and a Cdk8 kinase module, plays an essential role in the regulation of RNA polymerase II transcription. A detailed understanding of Mediator's structure and interactions is critical for determining how the complex influences transcription initiation and conveys regulatory information to the basal transcription machinery. My talk will focus on the structure of the Cdk8 kinase module and describe how the Cdk8 is non-canonically activated through conserved Med12 residues recurrently mutated in human tumors.

University of Texas Health Science Center Houston

George Phillips, PhD
Ralph and Dorothy Looney Professor, Biochemistry & Cell Biology
Professor, Chemistry

What is THE Structure of a Protein When it has More than One? Time to Move Beyond Static Snapshots

About Dr. Phillips research: The overall goal of the research in our laboratory is to relate the three-dimensional structure and dynamics of proteins to their biological functions. We use techniques of X-ray crystallography and other biophysical methods to elucidate the molecular structures, dynamics, and functions of proteins. Extensive use is made of modern computational methods to analyze the structures and their dynamics.

One project we have underway is directed towards obtaining an atomic description of the basis for binding of oxygen and other ligands to heme proteins. Detailed three-dimensional structures are being determined for modified myoglobins and hemoglobins and other novel heme proteins, such as nitrobindin, a protein we discovered that reversibly binds nitric oxide and is found throughout the animal kingdom. We are also working to develop new techniques for observing the dynamics of proteins and nucleic acids using diffuse X-ray scattering analysis and molecular dynamics simulations. The result will be a transition from "snapshots" of macromolecules to the generation of "movies" of molecules in action.

Organisms have proteins that are highly adapted to the growing conditions in the environment. We have determined structures of enzymes from hyperthermophilic bacteria to reveal aspects of the connections of protein structure to dynamics, which is an integral part of proteins' designs. We have also developed new methods of improving the thermostability of proteins for potential commercial improvements.

Members of the laboratory are also involved in the field of structural genomics, the solving of structures whose function may not yet be known. The structures often give clues about the functions. We are currently working on structures of enzymes involved in natural product biosynthesis in order to help produce lead compounds for cancer pharmaceutical discovery. More recently, members of my laboratory have become involved in the Great Lakes Bioenergy Research Center, whose mission is to contribute basic science results to the development of biofuel, particularly cellulosic ethanol.

A longstanding interest in the laboratory is also computational biology. This activity entails the development and application of modern algorithms from computer science and applied mathematics to solve interesting biological problems.

Poster Presenters

First	Last	Institution	Title	Poster #
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Poster #1

Directed Evolution of a Potent Inhibitor of the Methicillin-Resistant *Staphylococcus aureus* (MRSA) Resistance Enzyme, PBP2a

Boragine D_1, Adamski C_1, Mehta S_2, Palzkill $T_{1,2}$

1. Department of Biochemistry and Molecular Biology, Baylor College of Medicine
2. Department of Pharmacology and Chemical Biology, Baylor College of Medicine

Corresponding Author: Timothy Palzkill, Department of Pharmacology and Chemical Biology, Baylor College of Medicine, One Baylor Plaza, Houston, Tx, timothyp@bcm.edu

Abstract:

Antibiotic resistance has manifested into a global health epidemic. One of the most widespread human pathogens, Methicillin-resistant *Staphylococcus aureus* (MRSA) encodes a novel penicillin-binding-protein, PBP2a. Production of PBP2a by MRSA confers resistance to nearly all β-lactam antibiotics by continued peptidoglycan cell wall synthesis, even at high concentrations of antibiotic. The transpeptidase domain of PBP2a shares structural homology with class A β-lactamases, bacterial enzymes that inactivate β-lactam antibiotics. Class A β-lactamases, such as TEM-1, are inhibited by protein-based inhibitors named β-lactamase inhibitory proteins (BLIPs) and BLIP-II potently inhibits TEM-1 with a K_D 0.48pM. It was previously found that BLIP-II also weakly binds to PBP2a in the low micromolar range (K_D 1.5μM), in contrast to BLIP-II's potent inhibition of TEM-1. We hypothesize that through directed evolution, we can re-engineer BLIP-II for altered specificity to potently bind PBP2a. A directed evolution approach using phage display affinity selection was used to identify a BLIP-II double mutant, N50A:Y113H, that enhanced the binding affinity to PBP2a 30-fold to a K_D of 50nM. An additional directed evolution cycle, starting with the tighter binding BLIP-$II_{N50A:Y113H}$ template, was then performed to select for PBP2a binding while driving the binding affinity down to low nanomolar range. To date, we have identified several BLIP-$II_{N50A:Y113H}$ mutants that exhibit an additional ~3-5-fold enhancement in binding affinity to PBP2a and have also identified a G205W mutant that improved binding an additional 50-fold with a K_D ~1nM. This is a >1,500-fold enhancement in binding affinity to PBP2a compared to wild-type BLIP-II. However, the BLIP-$II_{N50A:Y113H}$ mutants resulted in >5,000-fold loss in binding to TEM-1, K_i 0.09nM, thereby indicating a change in selectivity compared to wild-type BLIP-II. Each BLIP-II mutant resulted in enhanced binding affinity to PBP2a, while simultaneously weakening the binding affinity to TEM-1. Thus, the evolved BLIP-II variants have shifted from high affinity for □-lactamases and low affinity towards PBPs, towards high affinity for PBPs with reduced affinity for □-lactamases. These results suggest that BLIP-II can be further optimized and serve as a scaffold for developing potential PBP2a inhibitors.

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Poster #2

Investigating Cholesterol Orientation in Lipid Bilayer by Raman Spectroscopy

Steven Demers,[1] Mathieu Simeral,[1] Aobo Zhang,[1] Hannah Hughes,[1] and Jason Hafner[1,2]

[1]Department of Physics, Rice University, Houston, TX
[2]Department of Chemistry, Rice University, Houston, TX

Corresponding author: Jason Hafner[1,2], 6100 Main MS-550, Houston, TX 77005, hafner@rice.edu

Gold nanostructures focus light to a molecular length scale at their surface, creating the possibility to determine molecular structures. The high optical intensity leads to surface enhanced Raman scattering (SERS) from nearby molecules. SERS spectra contain information on molecular position and orientation relative to the surface but are difficult to interpret quantitatively. Here we describe a ratiometric analysis that combines SERS and unenhanced Raman spectra with theoretical calculations of the optical field and molecular polarizability. This method can determine specific interfacial structure under ambient conditions, with microscopic quantities of material, and without molecular labels. Previous investigations found that phospholipid bilayers are normal to gold nanorods' surface when they encapsulate the nanorods. While the exact quantity varies by cell type, cholesterol is the next most abundant molecule in cellular membranes behind phospholipids. From molecular dynamics studies, not only do cholesterol molecules interact with the lipid headgroup but also forms pairs and interdigitate within the tail region of the phospholipid bilayer. The results of our structural analysis applied to cholesterol in phospholipid membranes will be presented. Of particular interest is the conformational changes exhibited by bilayer to the presence of cholesterol. Upcoming investigations include examining different anti-inflammatory drugs and different extracellular vesicles.

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Poster #3

Use of a Cell-free Protein Synthesis System and Coupled Enzyme Reactions to Synthesize Natural Products

Alex Ditzel[1], George N. Phillips Jr.[1,2]
1. Department of BioSciences, Rice University
2. Department of Chemistry, Rice University

Corresponding author: Alex Ditzel, Department of BioSciences, Rice University, 6100 Main Street, Houston, TX, Email: AlexDitzel@rice.edu

Natural products are a great source of pharmaceuticals, providing a majority of all small molecule drugs that exist today. However, due to the difficulty in producing them, pharmaceutical companies shifted away from natural products and towards synthetic chemistry because of the ease of screening large libraries of synthetic molecules. Unfortunately, very few of these synthetic molecules were biologically active in a useful way, so many companies want to shift focus back towards natural products, but it can be difficult to produce a range of products and screen them. Creating natural products through organic synthesis can take years of effort and synthesis *in vivo* in heterologous hosts can also be difficult and time consuming. Therefore, in order to allow for easier screening and production of natural products, I will be demonstrating the use of a novel cell-free system to assemble natural products *in vitro* using coupled enzyme reactions. This allows for higher throughput screening with the help of robotic automation while also allowing for complete control of the constituents of the system so that metabolic engineering becomes greatly simplified or eliminated. This may allow for the production of products that are very difficult to assemble within cells and may allow for more molecules to be screened for biological activity than traditional cell-based expression methods.

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Poster #4

Structures of DNA Replisome and Catalytic Mechanisms of DNA Synthesis

Yang Gao[1], Wei Yang[2]
1. Department of Biosciences, Rice University
2. Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health

Corresponding author: Yang Gao, Department of Biosciences, Rice University, 6100 Main St., Houston, TX, 77030. E-mail: yg60@rice.edu

Abstract. DNA polymerase, helicase, primase and their accessory factors form an integral complex named replisome to fulfill the complicated task of parental DNA unwinding and continuous leading-strand and discontinuous lagging-strand DNA synthesis. Using a model replisome system from Bacteriophage T7, we have obtained a series of high resolution cryo-electron microscopic structures of replisomes engaging in DNA unwinding and leading and lagging strand DNA synthesis. The structures revealed that hexameric DNA helicase unwinds DNA in a sequential hand-over-hand mechanism, akin to that of AAA+ peptide translocase unfolding proteins. A T-shaped replication fork is sandwiched between DNA polymerase on the leading strand and DNA helicase on the lagging strand. The two enzymes pull two daughter strands DNA to the opposite directions to cooperatively unwind parental DNA. One or more lagging strand DNA polymerases can be attached to the other side of ring-shaped helicase and a zinc binding domain from primase shuffles between active sites of primase and lagging strand polymerase to mediate discontinuous lagging strand DNA synthesis. Moreover, to explore the catalytic mechanism of DNA synthesis, we employed the time-resolved crystallography to record the DNA synthesis reaction. We proved that DNA polymerase with well aligned DNA, dNTP and two metal ions are not adequate for the chemical reaction. Only upon the arrival of a transiently bound third metal ion the reaction proceeds. The third metal ion is present transiently and not directly coordinated by polymerase, explaining why it escaped from detection for decades.

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Poster #5

Measuring the Mechanical Forces During Protein Biosynthesis via EF-G Crosslinking

Gavriliuc M[1], Wang Y[1]
1. Department of Biology and Biochemistry, University of Houston, 3455 Cullen Blvd, Houston, TX 77204

Corresponding author: Dr. Yuhong Wang, Department of Biology and Biochemistry, University of Houston, 4800 Calhoun Road, Houston, Texas, E-mail: ywang60@uh.edu

The ribosome is the complex molecular machine found in all living cells that is responsible for the synthesis of all proteins. The ribosome is associated with various protein factors, including the GTPase Elongation Factor G (EF-G). EF-G is responsible for catalyzing tRNA and mRNA translocation on the ribosome, however, the mechanism of this translocation remains elusive. A recent crystallographic study has implied large conformational changes of EF-G during translocation. Previous studies observed only the elongated, post-conformational state; however, a compact, pre-translocation state has recently been seen. The question regarding the biological relevance of these conformational changes remains. To answer this, we have generated double-cysteine EF-G that is internally crosslinked, to itself, with various lengths of crosslinkers. If the large conformational change does occur in solution, then translocation will be affected by the crosslinking. To observe the effects of the crosslinking on translocation, we are measuring the force EF-G generates when crosslinked with different crosslinker lengths. Restricting the movement of EF-G will also restrict the force that can be generated by EF-G. This study will help to gain a better understanding of the mechanism of translocation and how the ribosome can efficiently translate mRNA into protein.

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Poster #6

Effects of Inhibition of the Catalytic Domain of Histone Lysine Demethylase KDM5

John R. Horton[1], Qin Chen[1], Xu Liu[2], Clayton B. Woodcock[1], Paula Vertino[3], Haian Fu[4], Xing Zhang[1], and Xiaodong Cheng[1]

[1]Department of Epigenetics and Molecular Carcinogenesis, University of Texas MD Anderson Cancer Center, Houston, TX 77030
[2]Department of Biochemistry, Emory University School of Medicine, Atlanta, GA, 30322
[3]Winship Cancer Institute and Department of Radiation Oncology, Emory University, Atlanta, GA 30322
[4]Department of Pharmacology and Emory Chemical Biology Discovery Center, Emory University, Atlanta, GA 30322

Accumulating evidence indicates a crucial role for KDM5 family members of histone demethylases (A, B, C, and D) either as oncogenic drivers or tumor repressors (1–4). For instance, in ER+ breast cancer cells, KDM5B is overexpressed and knockdown of KDM5B in MCF7 (ER+) cells induces growth arrest through increased activity of the TGF-β signaling pathway (3). KDM5 enzymatic activities are specific for removing methyl groups from trimethylated and dimethylated histone 3 lysine 4 (H3K4me3/me2) – a chromatin mark that is associated with regions of accessible chromatin, including gene promoters and enhancers. Our laboratory's recent studies, as well as that of others, on the development of KDM5 inhibitors have shown that inactivation of KDM5 enzymatic activity by small molecule inhibitors suppresses the growth of subtypes of human cancer cells, suggesting that KDM5 inhibition could be exploited for cancer treatment.

Our laboratory has made some interesting observations with the examination of crystal structures of the catalytic domain of KDM5A with over twenty small molecule inhibitors (5,6) in combination with *in vivo* experiments. The KDM demethylases belong to a larger family of dioxygenases that contain Fe(II) and α-ketoglutarate as cofactors in their active site. Thus far, we have looked at inhibitors that displace α-ketoglutarate and partially utilize the metal for their binding to the enzyme. The lessons learned give potential strategies which hopefully can be utilized in the successful design of selective and potent epigenetic inhibitors of KDM5. We hope that in the long term such an inhibitor could be developed into a new cancer therapeutic.

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Poster #7

Viral RNA Polymerase Forms Amyloids

Kaundal S[1], Jain N[1], Pollet J[2], Prasad BVV[1]

1. Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, Texas
2. Pediatrics-Tropical Medicine, Baylor College of Medicine, Houston, Texas

Corresponding author: BVV Prasad, Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, Texas, E-mail: vprasad@bcm.edu

Noroviruses responsible for severe gastroenteritis, are members of the Caliciviridae family of positive sense RNA viruses. Currently human norovirus infection is responsible for 2 million deaths annually, yet there are currently no effective vaccines/antivirals available. RNA-dependent RNA polymerase (RdRp) is considered a promising drug target because of its important role in genome replication and in the synthesis/amplification of subgenomic RNA. Here, by using combination of size exclusion chromatography and analytical ultracentrifugation we show that RdRp of GII.4 norovirus has a tendency of forming higher order oligomer at 22 and 37 °C. The existence of higher order oligomers leads to investigate the of amyloidogenic property of RdRp. Bioinformatics-based sequence analysis carried out using three independent web-based servers suggests that RdRp has multiple hot-spots spread across the sequence that may help in the formation of amyloid-like fibrils. Using a combination of transmission electron microscopy (TEM) analysis along with dye based assays including Thioflavin-T based fluorescence and Congo red binding assays, we discovered that RdRp forms amyloid-like fibrils at physiologically relevant conditions *in vitro*. We further show that RdRp forms amyloid inclusions in *Escherichia coli* by using confocal imaging.

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Poster #8

Structure Determination of Epigenetic Complexes using Electron Microscopy

Gundeep Kaur[1], Ren Ren[1], Celine Shuet Lin Kong[1,2], Janani Kumar[1], Xing Zhang[1] and Xiaodong Cheng[1]
[1]Department of Epigenetics and Molecular Carcinogenesis, The University of Texas MD Anderson Cancer Center, Houston, Texas
[2]Program in Cancer Biology and Program in Genetics and Epigenetics, The University of Texas MD Anderson Cancer Center, UTHealth Graduate School of Biomedical Sciences, Houston, Texas

Corresponding author: Xiaodong Cheng, Department of Epigenetics and Molecular Carcinogenesis, The University of Texas MD Anderson Cancer Center, Houston, Texas. Email: xcheng5@mdanderson.org

TRIM28 is the transcriptional co-repressor of the Kruppel-associated box-containing zinc finger proteins (KRAB-ZFPs), which is the largest and most rapidly diverging family of the transcription factors in mammals. During early embryogenesis, TRIM28 interacts with KRAB and results in silencing of retroviral elements. TRIM28 is a master regulator which recruit several players involved in epigenetic modification such as DNA methyltransferases (Dnmt). Dnmt cause DNA methylation and convert the nucleobase cytosine (C) occurring predominantly in cytosine-phosphate-guanine (CpG) islands to 5-Methylcytosine (5mC). Dnmt have three isoforms: 3A, 3B and 3L. Of the three members of de novo Dnmt3 families, catalytically inactive DNMT3L acts as an essential cofactor for Dnmt3A and Dnmt3B in embryonic and germ cells. In somatic cells where Dnmt3L is not expressed, inactive isoforms of Dnmt3B, such as Dnmt3B3, have been shown to act as cofactors Dnmt3A and Dnmt3B. In *Arabidopsis thaliana (At)*, bifunctional DNA glycosylases within DEMETER family, *At*DML3, are known to directly excise modified cytosines (5mC and 5-hydroxymC). However**,** no mammalian homolog has been identified for *At*DML3. **At present, no structural information is available for any of the epigenetic complexes (Dnmt3A/3B/DNA, DML3 and TRIM28/KRAB), so we aim to determine their structures using electron microscopy (EM)**. We used negative staining EM to determine the low-resolution structures of all the epigenetic complexes. Determining their structure(s) will help us in delineating the molecular function and mechanisms involved in DNA methylation, demethylation and transcription repression carried out by these epigenetic complexes.

The study is funded by CPRIT (RR160029)

Poster #9

Structural Interrogation of Proteins Involved in the Biosynthesis of 10-Membered Enediyne Anticancer Natural Products

Abigael Kosgei*[1], Mitch Miller[1], David Xu[1], George N. Phillips[1]
1. Department of Biosciences, Rice University
   6100 Main Street Houston, Texas 77005

Enediynes natural products bind to the minor groove of DNA in such a way that they can abstract two hydrogen atoms from the sugars of the opposite strands causing double-stranded DNA cleavage. To ascertain the biochemical functionalities of proteins encoded by genes involved in the biosynthetic pathway of 10-membered ring enediynes, our lab utilizes X-ray crystallography to determine the atomic structures of these proteins, which shed a light to their functionality.

My project is geared towards determining the atomic structure of CalU17, a protein encoded by a gene involved in the biosynthesis of a 10-membered ring enediyne natural product, calicheamicin (CAL). I cloned CalU17 with His Tag and expressed it in *E. coli* expression cells. It was soluble. I purified the CalU17 protein using immobilized metal affinity chromatography with Ni-NTA resin then crystallized using sitting drop method with and without His Tag.

From the X-ray diffraction data, CalU17 crystallized in the tetragonal space group $P4_32_12$. I solved the phase problem using molecular replacement. The final structure of CalU17 had a resolution of ~2 Å. We hope to elucidate the role CalU17 plays in the biosynthetic pathway of CAL from the structure. The results will inform how 10-membered ring enediyne natural products are synthesized and will expand our ability to modify and generate a library of novel small molecules that will be used for drug screening.

Poster #10

Structural Studies of Hepatitis B Virus Surface Antigen

Lin Z

1. Department of Biosciences, Rice University

Corresponding author: Yizhi Jane Tao, Rice University, 6100 Main St, Houston, TX, E-mail: ytao@rice.edu

The Hepatitis B Virus (HBV), a member of *Hepadnaviridae* family, is a serious pathogen that causes chronic Hepatitis B, which affects over 240 million people in the world. HBV vaccines first became available in 1982, but they cannot cure established infections and their effectiveness in preventing blood-borne transmission from an infected mother to her infant is about 90%. In order to develop more effective drugs for HBV infection, it is essential to elucidate the structure of HBV viral protein. Such data would not only inform us about the enzymatic mechanism of viral protein, but also provide insights into the potential protein-drug interaction, and mutation-mediated drug resistance.

We preferentially target on viral polymerases and surface antigen based on the result of previous research. Viral surface antigen on human HBV is chosen on after an *E.coli* expression screening on viral polymerases and surface antigen, even including homogeneous viral protein from the HBV infecting different organisms. Three types of antigens are known to be on the human HBV envelope, but currently no drug is designed for HBV antigen. Crystal structure of the HBV surface antigen will indicate the potential drug binding pocket.

Two type of the protein has been to screen for protein crystal: cytosol truncation of the HBV surface antigen named LM, and the protein complex composed of single chain variable fragment (scFv) and LM, called 2H5-LM. The LM protein crystal is either big with “aggregated” form, or small with acceptable shape which has a clear and sharp edge on single crystal. Both forms of the crystals tend not to diffract under LS-CAT beamline. The scFv could act as a crystallization chaperone to LM protein, forming a more stable complex for crystallization. Nevertheless, 2H5-LM is crystallized in similar condition and the protein crystal displayed identical morphology.

We’ve recently tried on analytical ultracentrifugation (AUC) on 2H5, LM, and their complex. According to the interpretation of our collaborator, 2H5 with stable monomeric form binds to LM protein in 1 to 1 ratio. LM individual is heterogeneous with slightly difference between high and low protein concentration. The heterogeneity is most likely to be multiple protein conformation, which can be the folding issue during the expression. LM is a monomer by itself. The result illuminates me to try trypsin digestion preferentially, and further screen the expression other method, such as insect cell, mammalian cell and cell free etc, because heterogeneous protein is not able to pack into the well-diffracted protein crystal.

We acknowledge Welch Foundation to support the HBV research.

Poster #11

Structure and Biological Function Analysis of the Influenza D Virus M Protein

Xiaotong Lu[1]

1. Department of Biochemistry and cell biology, Rice University

Corresponding author: Yizhi Jane Tao, Department of Biochemistry and cell biology, Rice University, 6100 Main St, Houston, TX 77005, E-mail: ytao@rice.edu

Influenza D virus is a novel virus discovered in 2012 that can infect living stocks like bovine and swine. Until now, no human infection from this virus has been observed. However, due to its ability to replicate under 37 ℃ which is the normal human lung temperature, influenza D virus may acquire the potential to cause pandemic influenza in human. Influenza D virus M gene encodes two proteins: the DM1 protein and also the precursor DM protein which is further processed into the DM2 protein. DM1 and DM2 are involved in virus budding and viral genome release, respectively. However, the detailed mechanisms of these two processes remain unclear. For my PhD project, I will perform structural and biochemical studies of both DM and DM1 in order to find potential targets for the design of drug candidates. For DM, my preliminary finding that DM can be expressed as a soluble protein in E. coli with a well-defined oligomeric structure suggests that DM may indeed have additional functions. Since the structure of a protein can often shed light on its function, I will first solve the structure of DM using recombinant proteins and then use the structure to design functional assays to determine if DM has special functions other than simply serving as a protein precursor. For DM1, my preliminary finding that the N-terminal domain of DM1 can generate good crystals which might give information about its structure. For full length DM1, the gel filtration data shows that its oligomerization status is related to the buffer pH which may support the hypothesis that DM1 is involved in the vRNP release process.

Funding source: The Welch Foundation

Poster #12

Targeting the *Leishmania* Hsp100 N Domain to Prevent Infective Parasite Stage Differentiation

Jonathan M. Mercado[1,2], Jungsoon Lee[2], Nuri Sung[2], Christian Florian Teh-Poot[3], Liliana Estefania Villanueva-Lizama[3,4,5] Maria Elena Bottazzi[4,5,6], Kathryn Jones[4,6], Sukyeong Lee[2], Julio Vladimir Cruz-Chan[3,4,5], Francis T.F. Tsai[2,6*]

1. Department of Molecular and Cellular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA.
2. Department of Biochemistry and Molecular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA.
3. Dr. Hideyo Noguchi Regional Research Center, Autonomous University of the Yucatan, Mérida, Yuc 97000, MX.
4. Texas Children's Hospital Center for Vaccine Development, Houston, TX 77030, USA
5. Department of Pediatrics, National School of Tropical Medicine, Baylor College of Medicine, Houston, TX 77030, USA
6. Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX 77030, USA.

*Corresponding author: Francis T.F. Tsai, Department of Biochemistry, Baylor College of Medicine, 1 Baylor Plz, Houston, Texas 77030, ftsai@bcm.edu.

Leishmaniasis is a vector-borne lesion forming disease endemic in many developing countries caused by the *Leishmania* parasite. *Leishmania spp.* are dimorphic, protozoan parasites that spread to mammalian hosts during the blood meals of infected female sandflies. *Leishmania* exist as either non-infective promastigotes in the sandfly gut or infectious amastigotes that are responsible for the clinical manifestation of Leishmaniasis. Promastigote-to-amastigote differentiation is triggered during mammalian infection and is accompanied by *Leishmania* Hsp100 chaperone expression. *Leishmania* Hsp100 is a member of the Hsp100 family of protein foldases and is particularly similar to the ClpB/Hsp104 subfamily of disaggregases. Hsp104 chaperones are absent in animal cells making *Leishmania* Hsp100 a potential target for the treatment of Leishmaniasis. *Leishmania* Hsp100 is a multi-domain protein consisting of an N domain, an M domain, and two AAA+ domains. We recently showed the N domain of yeast Hsp100 contributes towards the recovery of proteins from an aggregated state where mutations in the N domain abolish chaperone activity. Therefore, I hypothesize that *Leishmania* Hsp100 activity can be depleted by small molecule inhibitors targeting the N domain that block *Leishmania* stage differentiation. To exploit *Leishmania* Hsp100 for drug design, I determined the crystal structure of the *L. mexicana* Hsp100 N domain at 1.4 Å resolution and developed an assay to assess for N domain function. The outcome of my work may be used to combat Leishmaniasis and related human infections caused by trypanosome parasites.

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Poster #13

Integration of Results from Time-Resolved Serial Crystallography and Spectroscopy in the Catalysis of Ceftriaxone by Beta-Lactamase

Olmos, Jr., J. L.[1], Chaires, H.A.[1], Phillips, Jr., G. N. [1,2]

1. Department of BioSciences, Rice University, Houston, TX, 77005, USA
2. Department of Chemistry, Rice University, Houston, TX, 77005, USA

The visualization of protein motions is one of the many exciting, cutting-edge uses of an X-ray free electron laser. Time-resolved mix-and-inject-serial (MISC) crystallography studies have directly visualized the reaction catalyzed by the enzyme, β-lactamase (Olmos *et al.*). This class of enzymes confers antibiotic resistance by hydrolyzing a broad spectrum of antibiotics in many bacteria, including *Mycobacterium tuberculosis*. The previous MISC results using the third-generation antibiotic, ceftriaxone, showed the development of an intermediate and the approach to a steady state involving product inhibition. While this approach has directly shown the course of the reaction in near-atomic detail, the technique has some practical limitations stemming from the packing of molecules in the crystal, the need for high concentrations of substrate, and the lack of synchrony throughout the crystal. Nevertheless, kinetic behavior can be determined from the structural information, including the postulation of product inhibition, which was subsequently confirmed by Szyperski, *et al.* (unpublished).[2]

To further connect the crystallography result to enzymology, the enzymatic reaction was studied in solution under conditions that more closely mimic the reaction in the microcrystals. In particular, UV-vis spectroscopy was employed to monitor the activity under various conditions. The solution reaction rates are indeed variable and dependent upon the components of the solution used for crystallization. Compared to the standard conditions for assays, pH and other buffer dependent effects were observed on the enzymatic activity.

Taken together, the integrated results of MISC and UV-vis spectroscopy show new insights for the basic enzymological activity of a medically important enzyme.

Corresponding author: Jose Luis Olmos, Jr., Biosciences, Rice University, 6500 Main St. BRC 703, Houston, Texas, e-mail: olmos@rice.edu

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Poster #14

Generation and Characterization of Recombinant Human Picobirnavirus (hPBV)

Yu Ouyang[1] and YizhiJane Tao[1]
[1]Department of BioSciences, Rice University, Houston, TX,77030

Human picobirnavirus (hPBV) is the type species in the *Picobirnaviridae* family. As its name indicates, hPBV is a small (35 nm in diameter, i.e. "pico"), non-enveloped, bi-segmented double-stranded RNA (i.e. "bi-RNA") virus. The bi-segmented RNA genome of hPBV has only at most four predicted open reading frames (functionally unknown ORF1 and ORF2, a capsid protein, and an RNA-dependent RNA polymerase). HPBV is proposed to be a possible gastroenteritis cause or to play a synergistic role in the infection associated with the primary enteric cause. Diarrhea is a common complication in immune-compromised patients and the second most common cause of death in infants. Interestingly, though the correlation between hPBV and diarrhea is still controversial, a recent study suggested some associations between hPBV abundance and enteric graft-versus-host disease (GHVD). Up to date, picobirnaviruses (PBVs) have been isolated from a wide variety of animals, but no report has claimed to successfully propagate PBV in any cell culture or animal model in the laboratory. An analysis of 81 picobirnavirial sequences in the NCBI database revealed that bacterial ribosomal binding site (RBS) is abundantly enriched in picobirnaviral genome near the start of predicted open reading frames, which is rarely observed in eukaryotic viruses. Therefore, we hypothesize that hPBV is a prokaryotic virus propagating in human enteric tract, but not excluding the possibility of hPBV as a eukaryotic virus infecting mitochondrion.

In this project, we expressed viral RNA segments in *E. coli* Rosetta cells. We were successfully detected each viral protein expression. The results indicate the predicted bacterial RBS on viral RNA gene segments is functional to initiate protein translation. The recombinant hPBV virus particles were then purified by CsCl gradient ultracentrifuge and followed by iodixanol gradient ultracentrifuge. The purifiled VLPs were characterized for its protein and RNA composition, as well as its morphology under TEM. RT-qPCR results reveal that the purified virus particles prefer to package viral RNA segments. We are still optimizing our protocol to generate a reproducible good quality of recombinant hPBV by *E. coli.* The recombinant hPBV can later be used as a platform for future hPBV studies.

Funding Source: Welch Foundation, NIH

Poster #15

Mechanism of Neutralization of Human GII Noroviruses by Cross-reactive Human IgA

Salmen W[1], Hu L[2], Alvarado G[3], Atmar R[1], Estes M[1], Crowe J[3], Prasad B[1,2]

1. Department of Molecular Virology and Microbiology, Baylor College of Medicine
2. Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine
3. Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center

Corresponding Author: B.V.V. Prasad, Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, 1 Baylor Plaza, Houston, Tx Email: vprasad@bcm.edu

Human noroviruses cause approximately 685 million cases of acute gastroenteritis and are responsible for an estimated 50,000 deaths worldwide in children under the age of five. Since 2002, the predominating circulating strain of norovirus is the rapidly evolving GII.4 strain. Until recently, there was little understanding of the mechanism by which the host mounts antibody-mediated neutralization against norovirus. Recent studies have identified a panel of human monoclonal antibodies (mAbs) from patients previously infected with a GII.4 strain. Within this panel, the neutralizing IgA antibody, NORO-320 was identified, which cross-reacts with viruses from several GII genotypes including GII.3, GII.4, GII.6, GII.12, and GII.17. To understand the mechanism by which NORO-320 mediates neutralization, we determined the crystal structure of the protruding domain (P-domain) of the GII.4 capsid protein, VP1, in complex with NORO-320 antigen-binding fragment (Fab). This crystal structure revealed that the Fab binds nearly perpendicular to the P-domain dimer near a region that is close the shell domain, which is responsible for capsid assembly and integrity. Sequence analysis indicates that the NORO-320 binding site in the P-domain is conserved in viruses of the GII.3, GII.4, GII.6, GII.12, and GII.17 genotypes, thereby providing a structural basis for the observed cross-reactivity of NORO-320. As this epitope is distant from the characterized histo-blood group antigen (HBGA) binding site, the mechanism of neutralization is not by directly blocking the receptor interaction. Our crystallography data suggests that the close proximity of the antibody binding site to the shell domain may facilitate neutralization by disruption of capsid integrity. To test this hypothesis, we are performing negative-stain of NORO-320 Fab in complex with virus-like particles of GII.4 to observe particle integrity. This study will provide insight into the mechanism by which the human adaptive immune system can elicit broadly neutralizing antibodies against a rapidly evolving virus.

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Poster #16

Expression and Characterization of the Human Astrovirus (HAstV) VP90 Capsid Protein for Investigating the Structural Features of the Proteolysis-Mediated Viral Maturation Process

Ykema MR[1], Xun M[1], Tao YJ[1]

1. Department of Biosciences, Rice University

Corresponding author: Matthew Ykema, Department of Biosciences, Rice University, 6100 Main Street, Houston, TX, Email: mry3@rice.edu

The human astrovirus (HAstV) is a non-enveloped virus with a positive-sense RNA genome and causes gastroenteritis in infants, the elderly and immunocompromised individuals. The virus has no vaccine, a high mutation rate, and emerging clinical symptoms like lethal encephalitis. Further structural and biophysical analysis must be done on the capsid protein to determine how it allows the virus to enter the host cell and transport its genome. The HAstV capsid is coded by the viral genome as a 90 kD polyprotein labeled as VP90, which forms a non-infectious particle. The capsid undergoes posttranslational modification through sequential proteolytic cleavages to form the mature infectious state. Little is understood of the infectious domains, cleavage sites, or capsid structural dynamics. We plan to express the immature virus-like particle using an E. coli expression system, simulate its maturation in vitro, then investigate these isolated intermediate particles with structural studies and cell culture testing.

Multiple VP90 capsid protein constructs have been expressed in E. coli and purified using HisTrap and size exclusion chromatography. Constructs with short N-terminal truncations had better expression and solubility when compared to full length constructs. Size-exclusion chromatography and transmission electron microscopy have indicated the capsid protein is forming a number of oligomeric states. A dimeric form of VP90 was screened for the formation of protein crystals for use in X-ray crystallography, but no crystals have been observed. Larger oligomeric states are being investigated through electron microscopy to determining the assembly pathways and the antigenic properties of these particles. Once icosahedral virus-like particle formation has been confirmed, the particles will be used for structural reconstruction using cryo-electron microscopy, in vitro maturation assays, and liposome infiltration assays. This research is funded by the Welch Foundation (C-1565 to YJT) and (HAMBP T32GM008280 to MY)

Poster #17

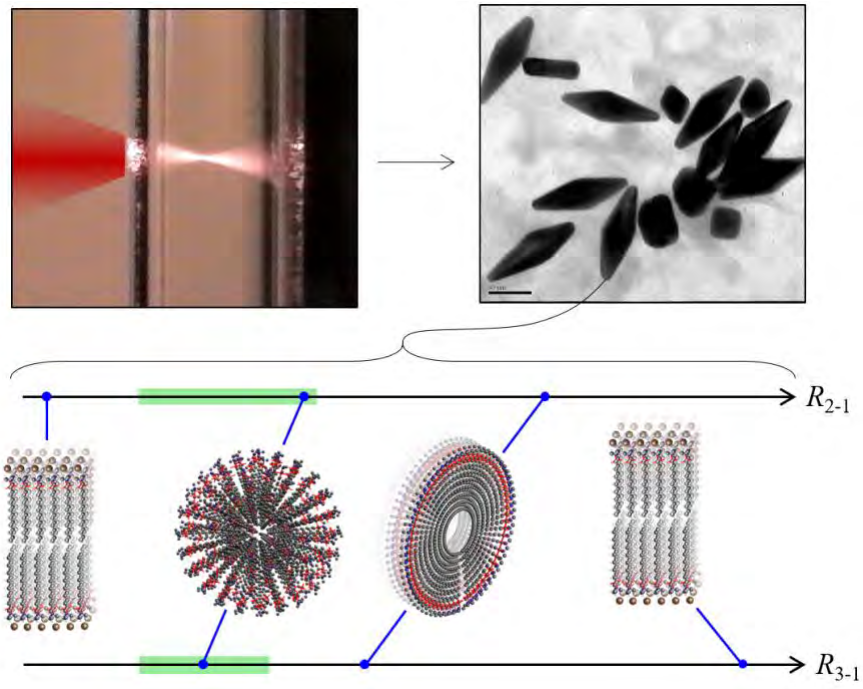
Surfactant Structure on Gold Nanoparticles from Structural Analysis by Enhanced Raman Scattering

Aobo Zhang[1], Steven M.E. Demers[1], Hannah J. Hughes[1], Mathieu L. Simeral[1], Mohammad Abdul-Moqueet[3], Kathryn M. Mayer[3], and Jason H. Hafner[1,2]

1. Department of Physics and Astronomy, Rice University
2. Department of Chemistry, Rice University
3. Department of Physics and Astronomy, The University of Texas at San Antonio

Corresponding author: Jason H. Hafner, Department of Physics and Astronomy, Department of Chemistry, Rice University, 6100 Main Street, Houston, Texas 77005, Email: hafner@rice.edu

The cetyltrimethylammonium (CTAB) surfactant layer that binds to the surface of gold nanoparticles has a significant influence on nanoparticle synthesis, surface chemistry, and applications in plasmonics. Recent molecular dynamics simulations suggest that CTAB may adsorb to the nanoparticle surface as a micelle. Structural analysis by enhanced Raman scattering (SABERS) was applied to three vibrational modes of CTAB on gold bipyramids in solution. This method combines unenhanced and enhanced Raman spectra, finite element calculations of the electromagnetic near-field of the nanoparticle, and time-dependent density functional theory calculations of the Raman tensors of the adsorbed molecule. Of the CTAB bilayer and micelle structures considered, the results were only consistent with an adsorbed spherical micelle at the bipyramid tip.



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Poster #18

A Purification Scheme Enabling Native-Condition Structural Studies of the Nematode Virus Orsay

Jim L. Zhang[1], Junhua Pan[2], Matthew Ykema[1], Miao Jin[1], Ying Zhou[1], Yizhi Jane Tao[1]
[1]*Department of Biosciences, Rice University, Houston, Texas*
[2]*Department of Biological Chemistry and Molecular Pharmacology, Harvard University, Cambridge, MA*

Corresponding author: Jim L. Zhang, Department of Biosciences, Rice University, Houston, Texas 77251, E-mail: jlz4@rice.edu

Orsay is the first known natural virus capable of infecting *C. elegans*, a valuable laboratory model. The non-enveloped virus presents a promising opportunity to develop a host-pathogen system capable of modeling eukaryotic viral infection. Orsay's (+)-ssRNA genome encodes for a capsid protein (CP), an associated protein δ, and an RNA-dependent viral polymerase. δ, which has been demonstrated to facilitate viral entry and nonlytic egress, can be expressed either as a free or fusion (CP-δ) protein. Prior research efforts have successfully structured Orsay CP and δ proteins individually through crystallography. However, a composite structure of the CP-δ fusion protein remains unverified.

Here, we report an iodixanol-based purification protocol capable of isolating Orsay virions (CP-δ) with substantial yield and purity. Iodixanol, when subjected to high-speed ultracentrifugation, is able to form a continuous density gradient. In the process, any proteins within the solution are also separated and isolated by density. When this technique was applied to a concentrated solution of supernatant derived from Orsay-infected *C. elegans*, fractions of pure and concentrated CP-δ were isolated from surrounding host proteins. The resulting samples of CP-δ serve as viable candidates for high resolution structuring through cryogenic electron microscopy. Preliminary Cryo-EM screening and data collection has already provided a preliminary model confirming the presence of CP-δ asymmetrically distributed across five-fold vertices within the viral capsid.

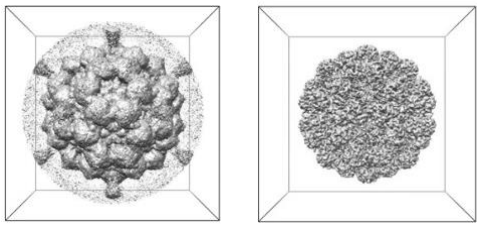

Figure 1. Preliminary Cryo-EM Structure of the Composite Orsay Virion

With the structure of CP-δ, we hope to further characterize the still-unknown mechanistic interactions that occur between Orsay and *C. elegans* host proteins – particularly during the process of viral host entry.

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Poster #19

Three Dimensional Structures Associated with Photoreceptor Cilia by Cryo-electron Tomography

Zhang Z, He F, Wensel TG

Department of Biochemistry and Molecular Biology, Baylor College of Medicine

Corresponding author: Theodore G. Wensel, Department of Biochemistry and Molecular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX, Email: twensel@bcm.edu

The light-sensing portion of rod and cone photoreceptor cells, known as the outer segment (ROS), is a modified primary cilium. The ROS is attached to the visual cell body by a connecting cilium. Axonemal components of this cilium extend into the ROS, an organelle which undergoes continuous renewal throughout life. The formation of new disks is believed to result from plasma membrane evaginations at the distal end of the connecting cilium, but the mechanism responsible for disk morphogenesis is not yet understood. At the heart of the connecting cilium is a nine + zero microtubule-based axoneme that emerges from a centriolar structure termed the basal body complex, which plays fundamental roles in cell division, motility and signaling. The relationship between the microtubules of the centriole and the axoneme is poorly understood.

We have used cryo-electron tomography (cryoET) of isolated rods combined with sub-volume averaging by *EMAN2* to determine the three-dimensional structure of centrioles in the basal bodies. The 3D density map reveals a tapered cylindrical structure composed of nine MT bundle from the plus (triplet) end to the minus (doublet) end of centriole. These centrioles were on average 400 nm long. Three structural domains were identified along the proximal (triplet)-distal (doublet) axis.

The proximal core region ~170 nm consists of a triplet microtubule having a 13 protofilament A-tubule and 10 protofilament B- and C-tubules, and are decorated with a pinhead structure. In the ~170 nm to 340 nm region, the pinhead disappears and the C-tubule is partially missing. In the distal ~60 nm, the centrioles are dominated by doublets, with an abrupt transition from triplets with partial C-tubules into doublets, and a narrower diameter and geometry that is more reminiscent of an axoneme. The structural features of the pinhead and incomplete C-tubule resemble other mammalian centrioles, with a ~200 nm proximal core region. The commonality of core-region length, and the abrupt transition to centrioles, suggests a conserved length-setting mechanism.

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Poster #20

Developing Small-Molecule Inhibitors for Human Norovirus Protease
-Discovery of A Novel Allosteric Inhibitor

Zhao B[1], Viskovska MA[4], Hu L[4], Nie S[2], Yu Z[3], Matzuk MM[3], Song Y[2], Estes MK[1 5], Prasad BVV[1 4]
1. Department of Molecular Virology and Microbiology, Baylor College of Medicine
2. Department of Pharmacology and Chemical Biology, Baylor College of Medicine
3. Department of Pathology and Immunology, Baylor College of Medicine
4. Department of Biochemistry and Molecular Biology, Baylor College of Medicine
5. Department of Medicine, Baylor College of Medicine

Corresponding author: B. V. Venkataram Prasad, Department of Biochemistry and Molecular Biology, Baylor College of Medicine, 1 Baylor Plaza, Houston, Texas, E-mail: vprasad@bcm.edu

Norovirus, a leading cause of acute gastroenteritis around the world, accounts for approximately 685 million cases and 50,000 child deaths annually. Unfortunately, no licensed vaccines or antiviral drugs are available for norovirus infection. Potent inhibitors against protease of GI.1 norovirus have been identified by our lab previously. However, these substrate-based peptidic inhibitors are less effective against GII.4 norovirus protease. As GII.4 variants have become the predominant genotype that causes 80% of global pandemics, it is necessary to develop potent inhibitors not only against GII.4 genotype but also human norovirus in general. The objectives of this study are to identify potent small molecule inhibitors toward protease of human norovirus and to investigate the mechanism of inhibition. We have developed a high throughput screening assay to identify lead chemical compounds from a novel small molecule library. Biochemical analyses and in vitro culture assays are used to investigate cytotoxicity and to confirm the inhibition activity toward GII.4 norovirus protease. X-ray crystallography studies are used to characterize the binding of compounds in complex with GII.4 norovirus protease. By screening of a library containing 800 compounds, we have discovered a novel set of non-toxic and non-peptidic protease inhibitors. Structure of one inhibitor in complex with GII.4 norovirus protease has been determined. Previous work has shown that inhibitors of GI.1 norovirus protease occupy the substrate binding pockets and interact with the catalytic triad to abolish protease activity. Our structural studies have indicated substantial changes in substrate binding pockets and active site of GII.4 norovirus protease, which contribute to the differential inhibition activities of the inhibitors. In this study, we have identified non-toxic inhibitors that were not designed based on the substrate binding pockets of norovirus protease. Discerning the structural basis of how GII.4 protease interact with novel inhibitors in comparison to GI.1 protease will provide a rational basis for designing and optimizing inhibitors that are effective for both genotypes.

This study is supported by National Institutes of Health Grant NIH PO1 AI057788 and Robert Welch Foundation Grant Q1279

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