2nd Annual Cellular & Molecular Biophysics Conference

December 9-11, 2020

Gulf Coast Consortia

QUANTITATIVE BIOMEDICAL SCIENCES

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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, 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 AI in Healthcare, Antimicrobial Resistance, Cellular and Molecular Biophysics, Innovative Drug Discovery and Development, Immunology, Mental Health, Regenerative Medicine, Single Cell Omics, Theoretical and Computational Neuroscience, Translational Imaging and Translational Pain Research. 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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Ming Zhou, Baylor College of Medicine
Michael Zhu, UT Health Houston

Agenda

Wednesday, December 9: Single Molecule Techniques

9:00 Welcome - John Tainer, MD Anderson Cancer Center and Jane Tao, Rice University

Convener: **Michael Zhu**, University of Texas Health Science Center Houston

9:05 Keynote Speaker
Unraveling the Specificity and Inhibition of CRISPR-Cas Nucleases
Ilya Finkelstein, University of Texas Austin

9:45 *Glutamate Receptors are the Single Molecule Level*
Vasanthi Jayaraman, University of Texas Health Science Center Houston

10:15 Trainee Talk
Structural Interrogation of Enzymes Involved in the Biosynthesis of Enediyne Natural Products
Abigael Kosgei, Rice University

10:25 Break

Convener: **Ching-Hwa Kiang,** Rice University

10:40 Keynote Speaker
Cohesin SA1 and SA2 are RNA Binding Proteins that Localize to RNA Containing Regions on DNA
Hong Wang, North Carolina State University

11:20 *3D Single-molecule Super-resolution Light Sheet Imaging Throughout Mammalian Cells*
Anna-Karin Gustavsson, Rice University

11:50 Trainee Talk
Long-range Coupled Motions Underlie Ligand Recognition by a Chemokine Receptor
Vinay Nair, University of Texas Health Science Center Houston

12:00-12:40 pm Poster Session 1

Thursday, December 10: Computational Modeling

Convener: **Jim Briggs**, University of Houston

9:00 *Drugging KRAS with Allosteric Non-covalent Inhibitors*
Alex Gorfe, University of Texas Health Science Center Houston

Agenda

9:30 Keynote Speaker
Harnessing the Power of Whole Genome Sequencing in Analysis of Mutagenesis in Human Cells
Serena Nik-Zainal, University of Cambridge

10:10 Break

Convener: **Jianpeng Ma**, Univeristy of Houston

10:20 *De Novo Modeling Building in CryoEM Density Maps*
Matt Baker, University of Texas Health Science Center Houston

11:00 Keynote Speaker
The Coming of Age of De Novo Protein Design
David Baker, University of Washington

Convener: **Jim Briggs**, University of Houston

11:40 Trainee Talk
Exploring the Energy Landscape of Chromosomes: Transitions Between Interphase and Mitotic Phase
Vinícius Contessoto, Rice University and São Paulo State University

11:50 Trainee Talk
Cryo-EM Reconstruction of a Covalently Linked Viral Fiber
Jim Zhang, Rice University

12:00-12:40 pm Poster Session 2

Friday, December 11: CryoEM, CryoTM and MicroED

Convener: **Yimo Han**, Rice University

9:00 Keynote Speaker
MicroED: Conception, Practice and Future Opportunities
Tamir Gonen, University of California Los Angeles

9:40 *Cryo-EM Analysis of IP3R Channel in a Lipid Bilayer*
Mariah Baker, University of Texas Health Science Center Houston

10:20 Break

Convener: **Ming Zhou**, Baylor College of Medicine

10:30 Keynote Speaker
Structure, Dynamics and Interactions in the Regulation of Macromolecular Function Studied by Cryo-EM

Agenda

Eva Nogales, Lawrence Berkeley National Lab

11:10 *Deep learning Gaussian Mixture Models of Molecular Variability in CryoEM*
Steve Ludtke, Baylor College of Medicine

11:40 *A Viral Genome Packaging Motor Transitions Between Cyclic and Helical Symmetry to Translocate dsDNA*
Marc Morais, University of Texas Medical Branch at Galveston

12:00 Closing Remarks
Jane Tao, Rice University

Keynote Speakers

David Baker, PhD
Head of the Institute for Protein Design
Professor of Biochemistry

The Coming of Age of De Novo Protein Design

David Baker is the director of the Institute for Protein Design, a Howard Hughes Medical Institute Investigator, a professor of biochemistry and an adjunct professor of genome sciences, bioengineering, chemical engineering, computer science, and physics at the University of Washington. His research group is a world leader in protein design and protein structure prediction. He received his Ph.D. in biochemistry with Randy Schekman at the University of California, Berkeley, and did postdoctoral work in biophysics with David Agard at UCSF. Dr. Baker is a member of the National Academy of Sciences and the American Academy of Arts and Sciences. Dr. Baker is a recipient of the Breakthrough Prize in Life Sciences, Irving Sigal and Hans Neurath awards from the Protein Society, the Overton Prize from the ISCB, the Feynman Prize from the Foresight Institute, the AAAS Newcomb Cleveland Prize, the Sackler prize in biophysics, and the Centenary Award from the Biochemical society. He has also received awards from the National Science Foundation, the Beckman Foundation, and the Packard Foundation. Dr. Baker has published over 500 research papers, been granted over 100 patents, and co-founded 11 companies. Seventy-five of his mentees have gone on to independent faculty positions.

Abstract: Proteins mediate the critical processes of life and beautifully solve the challenges faced during the evolution of modern organisms. Our goal is to design a new generation of proteins that address current day problems not faced during evolution. In contrast to traditional protein engineering efforts, which have focused on modifying naturally occurring proteins, we design new proteins from scratch based on Anfinsen's principle that proteins fold to their global free energy minimum. We compute amino acid sequences predicted to fold into proteins with new structures and functions, produce synthetic genes encoding these sequences, and characterize them experimentally. I will describe the de novo design of fluorescent proteins, membrane penetrating macrocycles, transmembrane protein channels, allosteric proteins that carry out logic operations, and self-assembling nanomaterials and polyhedra. I will also discuss the application of these methods to COVID-19 challenges.

Ilya Finkelstein, PhD
Associate Professor
Molecular Biosciences

Unraveling the Specificity and Inhibition of CRISPR-Cas Nucleases

Dr. Ilya Finkelstein is an Associate Professor of Molecular Biosciences at the University of Texas at Austin. Prior to his position at UT-Austin, Dr. Finkelstein received a B.S. from the University of California, Berkeley, a Ph.D. in Chemistry from Stanford University, and completed postdoctoral training at Columbia University Medical Center. Dr. Finkelstein's lab is investigating the molecular mechanisms of genome maintenance, CRISPR biology, and epigenetic inheritance. His group addresses these questions by combining biophysics, advanced microscopy and micro-/nano-scale engineering approaches. He ultimately hopes to apply these insights to understand the molecular underpinnings of and potential therapeutic avenues for malignancies and other diseases.

Abstract: CRISPR-Cas nucleases cleave genomic sites that are complementary to their CRISPR RNA (crRNA). However, all Cas nucleases bind and cleave near-cognate "off-target" sites, leading to adverse gene editing outcomes. I will describe several biophysical platforms for benchmarking diverse Cas nucleases. NucleaSeq, the nuclease sequencing pipeline, exhaustively measures cleavage kinetics and captures the time-resolved identities of cleaved products for a large library of partially crRNA-matched DNAs. The same DNA library is used to measure the binding specificity of each enzyme on repurposed next-generation DNA sequencing chips. This integrated workflow allowed us to benchmark the cleavage and binding specificities of Cas12a and five Cas9 variants for >105 DNAs containing mismatches, insertions, and deletions. I will also describe how Cas12a is inhibited by nucleosomes. Nucleosome unwrapping determines the extent to which both steps of Cas12a binding–PAM recognition and R-loop formation–are inhibited by the nucleosome. Nucleosomes inhibit Cas12a binding to DNA targets that extend beyond the canonical ~146 basepair core particle. Relaxing DNA wrapping within the nucleosome by reducing DNA bendability, adding histone modifications, or introducing a target-proximal nuclease-inactive Cas9 enhances DNA cleavage rates over 10-fold. Surprisingly, Cas12a readily cleaves DNA within chromatin-like phase separated nucleosome arrays. Taken together, these results highlight the importance of biophysical studies for understanding off-target and chromatin cleavage activities of Cas nucleases.

University of Texas Austin

Tamir Gonen, PhD
Professor
Biological Chemistry and Physiology

MicroED: Conception, Practice and Future Opportunities

Tamir Gonen is a membrane biophysicist and an expert in electron crystallography and cryo EM. Gonen is a professor of Biological Chemistry and Physiology at the David Geffen School of Medicine of the University of California, Los Angeles and an Investigator of the Howard Hughes Medical Institute and a Member of the Royal Society of New Zealand. In 2011 while leading a lab at the HHMI Janelia Research Campus he began developing Microcrystal Electron Diffraction (MicroED) as a new method for structural biology. With this method Dr Gonen has pushed the boundaries of cryoEM and determined several previously unknown structures at resolutions better than 1Å. Gonen authored more than 100 publications and several of his past trainees are now faculty around the world at top universities.

Abstract: My laboratory studies the structures of membrane proteins that are important in maintaining homeostasis in the brain. Understanding structure (and hence function) requires scientists to build an atomic resolution map of every atom in the protein of interest, that is, an atomic structural model of the protein of interest captured in various functional states. In 2013 we unveiled the method Microcrystal Electron Diffraction (MicroED) and demonstrated that it is feasible to determine high-resolution protein structures by electron crystallography of three-dimensional crystals in an electron cryo-microscope (CryoEM). The CryoEM is used in diffraction mode for structural analysis of proteins of interest using vanishingly small crystals. The crystals are often a billion times smaller in volume than what is normally used for other structural biology methods like x-ray crystallography. In this seminar I will describe the basics of this method, from concept to data collection, analysis and structure determination, and illustrate how samples that were previously unattainable can now be studied by MicroED. I will conclude by highlighting how this new method is helping us understand major brain diseases like Parkinson's disease; helping us discover and design new drugs; shedding new light on chemical synthesis and small molecule chemistry; and showing us unprecedented level of details with sub atomic resolutions.

University of California Los Angeles

Serena Nik-Zainal, MD, PhD
CRUK Advanced Clinician Scientist

Harnessing the Power of Whole Genome Sequencing in Analysis of Mutagenesis in Human Cells

Dr. Nik-Zainal is a CRUK Advanced Clinician Scientist and Honorary Consultant in Clinical Genetics in Cambridge, UK. Serena went to the UK as a PETRONAS scholar from Malaysia in 1993, obtaining a First in Physiology at University of Cambridge before completing her medical degree in 2000. She trained as a physician and specialized in Clinical Genetics. She undertook a PhD at the Wellcome Sanger Institute in 2009 pioneering exploration of breast cancers through whole genome sequencing (WGS).

Dr. Nik-Zainal demonstrated how detailed downstream analyses of all mutations present in WGS breast cancers could reveal mutational signatures, imprints left by mutagenic processes that have occurred through cancer development. She also identified a novel phenomenon of localised hypermutation termed 'kataegis'. Dr. Nik-Zainal continues to explore large cancer datasets using computational approaches while investigating biological underpinnings of mutational signatures through cell-based model systems. She led a clinical project, Insignia recruiting patients with DNA repair/replication defects, aging syndromes and neurodegeneration, and is also focused on advancing the field of mutational signatures into the clinical domain.

Abstract: Mutational signatures are the imprints of DNA damage and DNA repair processes that have been operative during tumorigenesis. They are biologically informative, reporting on the processes that have contributed to the developmental history of each patient's cancer. In this lecture, on behalf of my team and my collaborators, I shall provide an update on the field, focusing on validation of these abstract mathematical concepts, untangling the mechanisms underpinning mutation patterns in human somatic cells, and describing the new insights that we have gained through combinations of computational analysis and experiments in cell-based systems. We showcase how mutational-signature-based clinical algorithms have been developed, describe the path taken in translating these towards medical utility and highlight some of the hurdles that need to be navigated in this type of translational research.

University of Cambridge

Eva Nogales, PhD
Professor
Biochemistry, Biophysics and Structural Biology

Structure, Dynamics and Interactions in the Regulation of Macromolecular Function Studied by Cryo-EM

Eva received her bachelor's degree in physics from the Universidad Autónoma de Madrid (Spain) and her doctorate in biophysics from the University of Keele (UK). During her postdoctoral work at LBNL with Ken Downing she determined the structure of tubulin by electron crystallography. She joined UC Berkeley in 1998 and HHMI in 2000. She is also a Senior Faculty Scientist at LBNL. Eva's research is dedicated to gaining mechanistic insight into the dynamics of the microtubule cytoskeleton and the regulation of gene expression at the transcriptional level using cryo-EM as a major tool. Eva has been the recipient of the Dorothy Crowfoot Hodgkin Award from the Protein Society, the Mildred Cohn Award from ASBMB, the Keith R Porter Lecture Award from ASCB and the LBNL Director's Award for Exceptional Science Achievement. She is a fellow of ASCB and of the Biophysical Society, an elected member of the National Academy of Sciences and the American Academy of Arts & Sciences, and an elected associate member of EMBO. In 2020 she serves as president of ASCB.

Abstract: Cryo-electron microscopy (cryo-EM) has emerged as a powerful structural biology technique that overcomes some of the bottlenecks of other methods used for structure determination. In particular, the structural analysis of large and flexible macromolecular assemblies that cannot be obtained in large amounts is now possible by cryo-EM. Among such samples are large protein complexes that are required for the regulation of gene expression at the transcriptional level. In my presentation I will cover some of the progress my lab has made concerning such human molecular machinery.

University of California Berkeley

Hong Wang, PhD
Associate Professor
Physics

Cohesin SA1 and SA2 are RNA Binding Proteins that Localize to RNA Containing Regions on DNA

Dr. Hong Wang obtained her Ph.D from the University of North Carolina at Chapel Hill in 2003 under the supervision of Dr. Dorothy Erie. She received postdoctoral training at NIEHS from 2204 to 2008, and at the Hillman Cancer Center (University of Pittsburgh) from 2008 to 2011 under the mentorship of Drs. Bennett Van Houten and Patricia Opresko. Her graduate and postdoctoral work using AFM imaging and tracking of quantum dot- labeled proteins on DNA revealed how DNA mismatch repair and nucleotide excision repair proteins search and recognize DNA damage at the single- molecule level. In 2009, she received the NIH Pathway to Independent Award (K99/R00) to study protein-DNA interactions at telomeres. She joined the Physics Department at North Carolina State University in 2012. Since then, single-molecule AFM and fluorescence imaging studies from her lab have revealed structures and dynamics of various biological pathways, including telomere maintenance, mitochondrial DNA replication, chromatin structures, epigenetic regulations, and sister telomere cohesion. Currently, her lab's main focus is to understand how the shelterin protein complex modulates higher-order DNA structures at telomeres and how cohesin binds to specific DNA sequences and R-loops.

Abstract: The cohesin complex plays important roles in diverse biological processes including sister chromatid cohesion, DNA double-strand break (DSB) repair, re-start of stalled replication forks, and maintenance of 3D chromatin organization. Since the discovery of the cohesin complex, the long-held view is that cohesin binds DNA through the entrapment of DNA inside the ring subunits (SMC1, SMC3, and RAD21 in humans), and the fourth subunit (SA1/STAG1 or SA2/STAG2) serves supporting roles. Recently, SA2 was identified as 1 of only 12 genes that are significantly mutated in four or more cancer types. Despite the importance of cohesin SA1 and SA2, their biophysical properties are largely unknown. Using single-molecule AFM imaging and tracking of quantum dot-labeled protein on DNA tightropes, we discovered that cohesin SA1 and SA2 are single-stranded (ss) and double-stranded (ds) DNA and RNA binding proteins. SA1 displays similar DNA binding affinities for ds and ssDNA, and binds specifically to double-stranded telomeric sequences mediated through its N-terminal AT-hook domain. Due to SA2's higher binding affinities for ssDNA than for dsDNA,

it recognizes intermediate DNA structures during DNA replication and double-strand break (DSB) repair, such as a dsDNA end, single-stranded overhang, flap, fork, and ssDNA gap. Furthermore, cohesin SA1 and SA2 bind to various RNA containing nucleic acid substrates, including ssRNA, dsRNA, dsRNA with an overhang, RNA:DNA hybrids, a model R-loop substrate, and long ssRNA transcripts. Strikingly, cohesin SA1 and SA2 preferentially localize to regions on dsDNA that contain RNA. These discoveries of previously unknown DNA and RNA binding activities of cohesin SA1 and SA2 open up new directions of research to unravel the mechanisms underlying their diverse cellular functions.

Plenary Speakers

Mariah Baker, PhD
Research Assistant Professor
Biochemistry and Molecular Biology

Cryo-EM Analysis of IP3R channel in a Lipid Bilayer

Dr. Baker received her Ph.D. from Baylor College of Medicine in the Structural and Computational Biology and Molecular Biophysics graduate program. Her early research work on voltage- and ligand-gated ion channels and their molecular regulation shaped her perspective on the importance of elucidating protein structures to inform and interpret biochemical studies. Since then, she has focused on the structural analysis of membrane proteins using single-particle electron cryo-microscopy and computational modeling. During her postdoctoral work at the National Center for Macromolecular Imaging she was a co-developer of a computational approach for de novo atomic modeling from EM density maps using a novel implementation of the traveling salesman algorithm. In 2012, she joined Irina Serysheva's lab as a senior postdoc in the Department of Biochemistry and Molecular Biology at UTHealth McGovern Medical School and played a key role in analyzing the first near-atomic resolution structure of the full-length, tetrameric Ca2+ release channel, IP3R, by single-particle cryo-EM. Dr. Baker is currently an Assistant Professor at UTHealth and a Cryo-EM specialist for the UTHealth Cryo-EM Core. Her current research is committed to answering key questions regarding how ion channels process complex regulatory signals, and how these signals affect channel structure and function.

Abstract: Inositol 1,4,5-trisphosphate receptors (IP3Rs) are tetrameric intracellular cation channels ubiquitously expressed in mammalian cells and located predominantly in the endoplasmic reticulum (ER) membranes. IP3Rs mediate Ca2+ release from the ER into the cytosol and thereby involved in many physiological processes. IP3R structures in closed and ligand-bound states have been captured by single-particle cryo-EM under conditions in which channel protein was solubilized with detergent. However, ion channels reside in biological membranes, where lipids have important structural and regulatory roles. Here, we present the structure of full-length neuronal type IP3R reconstituted in lipid nanodisc determined by single-particle electron cryo-microscopy.

The lipid-bound structure shows improved features that enabled us to build an atomistic model of IP3R1 including regions that were not previously resolved. Our study suggest conserved locations of protein-integrated lipids among homotetrameric ion channels that is critical for their structural and functional integrity despite the diversity of structural mechanisms for their gating.

Matthew Baker, PhD
Assistant Professor
Biochemistry and Molecular Biology

De Novo Modeling Building in CryoEM Density Maps

Matthew Baker, PhD, recently joined The University of Texas Health Science Center at Houston as an Assistant Professor in the Department of Biochemistry and Molecular Biology. Dr. Baker's laboratory focuses on the development and application of computational modeling tools for the analysis of macromolecular assemblies. With over 20 years of experience in electron cryomicroscopy (cryoEM) and computational modeling, his pioneering efforts produced the first C-alpha backbone and all-atom models directly from near-atomic resolution cryoEM density maps without the aid of a structural template. Today, Dr. Baker's class-leading computational tools are used to analyze and model a wide range of challenging structures, including cancer immunotherapeutic, viruses and membrane proteins. In addition to his research, Dr. Baker is the faculty liaison and mentor for BRASS, a scholarship program for incoming graduate students at BCM, and the director of Brain Labs, a STEM outreach program in neuroanatomy.

Abstract: Over the course of the last two decades, single particle electron cryomicroscopy (cryoEM) has emerged from "blob-ology" and can now routinely produce structures at resolutions that readily allow for atomistic interpretations directly from a density map without the aid of structural templates. This rapid improvement in obtainable resolutions is in part due to the technological advancements in electron microscopes, imaging hardware and tools for data processing. Concurrently, new model building tools have been developed, enabling atomistic model generation in complex density maps at resolutions better than 5Å. Our computational modeling software, Pathwalking, is a robust suite of tools that can rapidly and reliably generate accurate models directly from near-atomic resolution density maps without any structural template or a priori knowledge. In fact, the core algorithm in Pathwalking is agnostic to the protein sequence, building a model based purely on the optimization of protein geometry in the density map. As Pathwalking is extremely quick (on the order of a few seconds for even the largest proteins), the software can generate a gallery of potential models, as well as a probabilistic model, in minutes. These tools have been used on a number of challenging cryoEM density maps, including membrane proteins and viruses, to enable the discovery of macromolecular structure and function.

Alemayehu Gorfe, PhD
Associate Professor
Integrative Biology and Pharmacology

Drugging KRAS with Allosteric Non-covalent Inhibitors

Dr Gorfe joined the Department of Integrative Biology and Pharmacology (IBP) of the McGovern Medical School of the University of Texas Health Science Center in Houston (UTHealth) in 2009 as a tenure-track Assistant Professor, and promoted to Associate Professor with tenure in 2014. Prior to that, he attended Graduate School at the University of Tromoe, Norway (MSc in Physical Chemistry) and the University of Zurich, Switzerland (PhD in Biochemistry), and received excellent postdoctoral training at the University of Zurich with Prof. Amedeo Caflisch and at the University of California San Diego with Prof J. Andrew McCammon. His laboratory strives to discover therapies for unmet health challenges through a detailed study of bio-molecular dynamics and interactions. Supported by grants from the NIH and CPRIT, Dr Gorfe's laboratory develops and applies cutting-age techniques in the areas of molecular simulation, computational structural biology, and experimental biophysical and cell-based assays.

McGovern Medical School
University of Texas Health Science Center Houston

Anna-Karin Gustavsson, PhD
Norman Hackerman-Welch Young Investigator
Assistant Professor
Chemistry

3D Single-molecule Super-resolution Light Sheet Imaging Throughout Mammalian Cells

Dr. Gustavsson joined the faculty at Rice University in the summer of 2020 as a CPRIT Scholar and the Norman Hackerman-Welch Young Investigator Chair. Dr. Gustavsson received her PhD in Physics from the University of Gothenburg, Sweden, in 2015. Her work focused on studying dynamic responses in single cells by combining and optimizing techniques such as fluorescence microscopy, optical tweezers, and microfluidics. Upon completion of her graduate work, Dr. Gustavsson joined the group of Nobel Laureate W. E. Moerner at Stanford University as a Postdoctoral Fellow in 2015. Her research focused on the development and application of 3D single-molecule super-resolution microscopy for cellular imaging, and included the implementation of light sheet illumination for optical sectioning of mammalian cells. Dr. Gustavsson's work has been recognized with multiple honors, awards, and fellowships, most notably the *FEBS Journal Richard Perham Prize for Young Scientists* in 2012, the 3-year *Swedish Research Council International Postdoc Fellowship* in 2016, the *PicoQuant Young Investigator Award* in 2018, the *NIH K99/R00 Pathway to Independence Award* in 2019, and the *CPRIT Recruitment of First-Time Tenure-Track Faculty Members Award* in 2020.

Vasanthi Jayaraman, PhD
Professor
Biochemistry and Molecular Biology

Glutamate Receptors are the Single Molecule Level

Dr. Jayaraman's graduate training is in physical chemistry, and she worked on spectroscopic investigations of hemoglobin under the guidance of Dr. Thomas Spiro at Princeton University, and as a postdoctoral associate she used rapid kinetic electrophysiological measurements to study ligand gated ion channels under the guidance of Dr. George Hess at Cornell University. When she started as an independent investigator she was hence in a unique position to be able to combine these two areas of expertise and developed a research program focusing on understanding the role of structure-dynamics in dictating ion channel function. Over the last 23 years she has developed and used cutting edge spectroscopic tools to gain an understanding of conformational changes in membrane proteins with a focus on ionotropic glutamate receptors. These studies have provided insight into changes starting at the level of specific ligand-protein non-covalent interactions, which control conformational changes in the protein, and ultimately dictate function. During this time she has trained 13 doctoral students, 15 postdoctoral fellows and several undergraduate and high school students. She has mentored several women and minority students, and have played an active role in the Committee of Professional Opportunities for Women in the Biophysical Society, organizing career development events. Most recently she started the Women Faculty Forum at the McGovern Medical School at The University of Texas Health Science Center, and made significant strides; for instance they were able to initialize day care for sick faculty children and support travel for women faculty to career development opportunities. She has also been actively involved in the Biophysical Society where she was elected to serve on the Executive Board, she was the co-chair for the 2016 Annual Biophysical Society Meeting, and she is an associate editor for the Channels and Transporters section of the Biophysical Journal.

Abstract: Glutamate receptors mediate excitatory responses in the mammalian central nervous system, and ultimately control motor and cognitive functions. AMPA mediate fast synaptic transmission by opening cation ion permeable channels upon binding glutamate. We use a combination of mutagenesis, single molecule FRET and

McGovern Medical School
University of Texas Health Science Center Houston

luminescence resonance energy transfer methods, along with electrophysiological measurements to map the conformational landscape that the underlies this gating process of channel opening and desensitization in this protein. We also investigate how the gating properties are modulated by the presence auxiliary subunits such as gamma2 and gamma 8, as well as small molecule inhibitors, and determine the changes in the conformational landscape that underlie these modulatory functional changes.

Steven J. Ludtke, PhD
Professor
Biochemistry and Molecular Biology

Deep learning Gaussian Mixture Models of Molecular Variability in CryoEM

For the last 24 years, Ludtke's group has focused on CryoEM/CryoET and structural biology, applying these techniques to a wide range of biological systems, including: nuclear receptor complexes, nuclear pore complexes, ion channels, RNA/DNA, chaperonins, and a variety of other systems. Much of this work has been focused around development of the EMAN software suite. This software has been used in solving a significant fraction of the structures in the EMDatabank, and remains widely used in the field. In addition to developing new methods within EMAN, his group engages in a wide range of biological collaborations, through the CryoEM Core at BCM which he directs, and previously, through the National Center for Macromolecular Imaging, the CryoEM P41 center he codirected for 15 years. The core has recently expanded under a CPRIT core facility award, allowing us to update our equipment and begin moving more aggressively into cellular CryoET. Recent developments include deep-learning techniques to improve CryoET capabilities (now achieving 0.5 nm resolution within cells) new mathematical techniques for studying biomolecule flexibility in solution and many others.

Baylor College of Medicine

Marc Morais, PhD
Associate Professor
Biochemistry & Molecular Biology

A Viral Genome Packaging Motor Transitions Between Cyclic and Helical Symmetry to Translocate dsDNA

The Morais laboratory uses a combination of structural, biophysical, biochemical, and computational approaches to study the structures and functions of complex macromolecular assemblies and machines. By integrating results from X-ray crystallography, cryo-electron microscopy, small angle X-ray scattering, and traditional biochemical/biophysical methods with molecular simulations, we are able to probe how changes in molecular structure give rise to biological function. In particular, we interested in understanding the rules that govern self-assembly of viral capsids, as well as how various components in molecular motors coordinate their activities to efficiently generate force. By understanding the structure-function relationship at the atomic scale, we hope to apply this knowledge towards the development of therapeutics and the rational design of novel nano-motors and machines.

Abstract: Molecular segregation and biopolymer manipulation require the action of molecular motors to do work by applying directional forces to macromolecules. The additional strand conserved E (ASCE) ring motors are an ancient family of molecular motors responsible for diverse tasks involving biological polymer manipulation (e.g. protein degradation and chromosome segregation). Viruses also utilize ASCE segregation motors to package their genomes into their protein capsids against considerable energetic barriers. Indeed, they are the most powerful molecular motors in nature, and thus provide a unique window into the mechanochemistry of force-generation found in this broad class of molecular motors. We show by CryoEM focused image reconstruction that ASCE ATPases in viral dsDNA packaging motors adopt helical symmetry complementary to their dsDNA substrates. In contrast results from X-ray crystallography showing these ATPases can also adopt planar ring conformations. Taken together with complementary long time-scale MD simulations, these results suggest that viral dsDNA packaging motors translocate dsDNA via stepwise helical-to-planar ring transitions that are tightly coordinated by ATP binding, hydrolysis, and release.

University of Texas Medical Branch at Galveston

Trainee Talk Speakers

Vinícius Contessoto, PhD
Postdoctoral Fellow

Exploring the Energy Landscape of Chromosomes: Transitions Between Interphase and Mitotic Phase

Dr. Contessoto has a Bs.c in Biological Physics and have Ph.D. in Molecular Biophysics from São Paulo State University studying protein folding and engineering. He is currently working with prof. José Onuchic as a Postdoc in the Center for Theoretical Biological Physics, CTBP, at Rice University. In his postdoc, they are studying chromatin structure and dynamics.

Abstract: Understanding the genome architecture and how chromosomes are organized during different phases of the cell is a significant challenge which involves scientists from diverse areas of knowledge. Recent studies of synchronized HIC maps of DT40 chicken cell line in different phases provide information of the genome organization during this transition. Here, using maximum entropy approach in coarse-grained chromosome model, we investigate the folding-unfolding dynamics of chromosomal structures. In other words, we studied the transition of chromosomes between the interphase to mitotic phase. Some preliminary results indicate that the genome structural organization during the mitotic phase might be related to the modulation of non-specific long-range interactions. This modulation leads the chromosome structure to lose some compartments associated with the long genomic distance contacts. These preliminary findings suggest that the local (short genomic distances) interactions play an essential role in the condensation process which might be related to an extrusion process by condensin.

Abigael Kosgei
Graduate Student

Structural Interrogation of Enzymes Involved in the Biosynthesis of Enediyne Natural Products

Ms. Kosgei is a fourth year Graduate student in the Bioscience Department at Rice University. She received her Bachelor's Degree in Biochemistry from Claflin University, South Carolina. She is interested in structural biology in the field of crystallography and cryogenic electron microscopy. Her research focus is on investigating enzymes involved in biosynthesis of enediyne natural products and those that modify collagen.

Abstract: The need for novel drugs to treat diseases such as cancer and to curb antibiotic-resistance has prompted scientists to use natural products that are a great resource for discovery of drugs, especially small molecule drugs. Most natural products owe their existence to the enzymatic machinery that runs inside microbes and plants. The biosynthetic machinery is made of enzymes that work in a specific fashion to render a complex natural product framework. The structural and functional diversity generated by these natural architects has stimulated interest in elucidating their underlying enzymology. Through understanding the natural products biosynthetic pathway, we can exploit the catalytic ability of enzymes and use them as biocatalysts for structural diversification, activity optimization, and generation of previously unobserved natural products with novel antitumor functions. The enediynes natural products show great potential as anticancer antibiotics. They 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. The study of the genes in the biosynthetic gene cluster of the 10-membered ring enediynes, calicheamicin (CAL) and dynemicin (DYN), will help us understand the role each enzyme plays in their respective biosynthetic pathways. X-ray crystallography was used to perform structural analysis of proteins CalU17 and DynF. Dimeric structure of DynF was solved to a 1.6 angstroms resolution. Palmitic acid was bound within the dimeric beta barrel suggesting that DynF may be involved in binding the precursor polyene, Heptaene, required for the biosynthesis of dynemicin core. Further biochemical characterization will be done to ascertain the role of DynF in the biosynthetic gene cluster of dynemicin. The His-tag on and tag-off structures of CalU17 were solved to ~2.5 angstroms. CalU17 may have a conserved Ca2+ binding site and hypothesized to catalyze the oxidation of peptidyl cysteine to formylglycine (fGly).

Institution

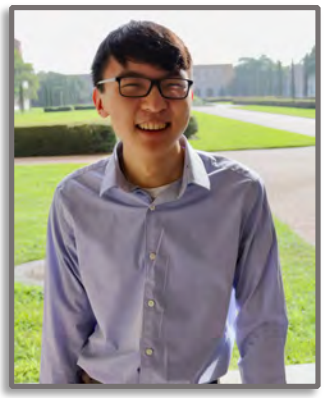
Further characterization of CalU17 and DynF, and determination of their functions will expand our abilities to modify and generate a library of novel small molecules that will be used for drug screening.

Vinay Nair
Graduate Student

Long-range Coupled Motions Underlie Ligand Recognition by a Chemokine Receptor

Vinay Nair is a PhD student graduate student at the MD Anderson UTHealth Graduate School of Biomedical Sciences. His research in the lab of Alemayehu Gorfe focuses on understanding the biology of the K-Ras protein using a mix of computational modeling and biochemical & cell-based experiments. Vinay graduated from the University of Pune, India with a 5 Yrs. Integrated MS in Biotechnology. He is a fellow of the CPRIT Innovation in Cancer Prevention Research Training Program.

Abstract: Chemokines are unusual class-A GPCR agonists because of their large size (~10 kDa) and binding at two distinct receptor sites: N-terminal domain (Site-I, unique to chemokines) and a groove defined by extracellular loop/transmembrane helices (Site-II, shared with all small molecule class-A ligands). Structures and sequence analysis reveal that the receptor N-terminal domains (N-domains) are flexible and contain intrinsic disorder. Using a hybrid NMR-MD approach, we characterized the role of Site-I interactions for the CXCL8-CXCR1 pair. NMR data indicate that the CXCR1 N-domain becomes structured on binding and that the binding interface is extensive with 30% of CXCL8 residues participating in this initial interaction. MD simulations indicate that CXCL8 bound at Site-I undergoes extensive reorganization on engaging Site-II with several residues initially engaged at Site-I also engaging Site-II. We conclude that structural plasticity of Site-I interactions plays an active role in driving ligand recognition by a chemokine receptor.



Jim Zhang
Undergraduate-graduate Student

Cryo-EM Reconstruction of a Covalently Linked Viral Fiber

Jim Zhang is a junior undergraduate-graduate student working in the Yizhi Jane Tao Laboratory at Rice University in Houston, Texas, which investigates the structural and molecular virology of RNA viruses. His current work focuses on (1) the native structure of Orsay, the first and only known virus capable of naturally infecting the laboratory model Caenorhabditis elegans; and (2) the potential role of C. elegans FSHR-1, a G-protein coupled receptor, in facilitating Orsay viral entry.

Orsay remains the only known virus capable of naturally infecting Caenorhabditis elegans, a valuable laboratory model. The non-enveloped virus thus presents a promising opportunity to develop a host-pathogen system capable of modeling gastrointestinal eukaryotic viral infection. Orsay itself contains a (+)-ssRNA genome encoding for capsid protein (CP), viral fiber δ, and an RNA-dependent RNA polymerase. δ, which has been implicated in facilitating viral entry and nonlytic egress, can be expressed as either a free or covalently fused CP-δ protein for incorporation into the viral capsid. Previous studies have successfully structured recombinant forms of both Orsay CP and δ; however, a composite structure of the native, infectious virion containing CP-δ remains unsolved.

Here, we report an iodixanol-based purification scheme capable of isolating CP-δ containing Orsay virions. The resulting samples of CP-δ served as viable candidates for high-resolution structural analysis through single-particle cryogenic electron microscopy (Fig. 1). Preliminary screening and data collection confirmed the presence of CP-δ asymmetrically distributed about five-fold vertices within the viral capsid. Following alignment to a unique vertex, the resulting electron density clearly displayed the full-length viral fiber, which was subsequently mapped using prior-solved recombinant structures.

Rice University

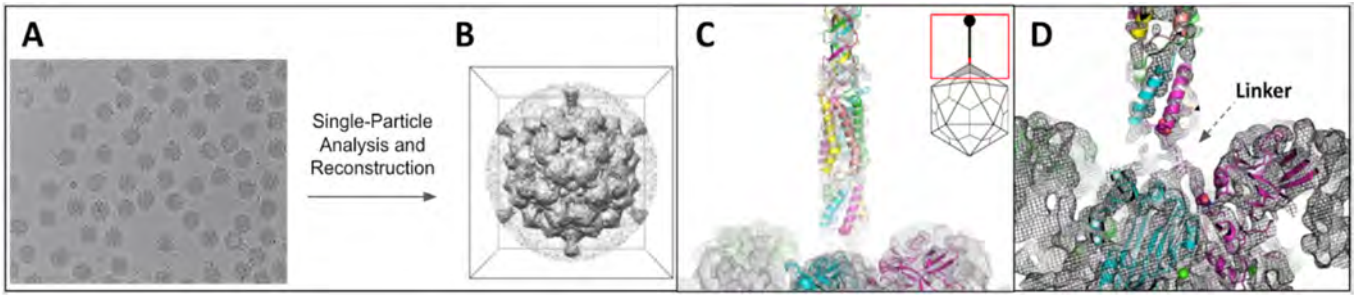


Figure 1. Preliminary Cryo-EM Structure of the Composite Orsay Virion. (A) A representative cryo-electron micrograph and (B) initial electron density map of the Orsay virion following single-particle analysis. (C) Following refinement and additional data collection, CP-δ can be distinguished and mapped using prior solved, recombinant Orsay structures (D) in addition to the elusive linker region connecting the spike and capsid.

Additional data collection is required to further refine resolution, particularly about the 29-amino acid linker region and fiber-containing capsid pentamer. With these structures, 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 entry.

Poster Presenters

First Name	Last Name	Institution	Poster Title
Nayara	Alcântara-Contessoto	RU and São Paulo State University	*Investigating the Effect of Anti-cancer Compound (Piperlongumine) on the Physical Properties of Cancer Cells Using Atomic Force Microscopy*
Cecilia	Bores	UTMB	*Genome Packaging in Bacteriophages: The Effect of DNA – Capsid Interactions*
Ahmad	Borzoi	Syracuse University	*A Data-Driven Statistical Field Theory of Active Matter*
Caleb	Chang	RU	*Observing a Polymerase Incorporate a Wrong Nucleotide*
Miriam	Gavriliuc	University of Houston	*Measuring the Mechanical Forces of Elongation Factor G Mediated Translocation*
Zihan	Lin	RU	*Structual and Functional Studies of Colletotrichum Camelliae Filamentous Virus 1 Methyltransferase and RNA-dependent RNA polymerase*
Xiaotong	Lu	RU	*Structure and Biological Function Analysis of the Influenza D Virus M Protein*
Guolin	Ma	TAMU	*Optogenetic Engineering to Probe the Molecular Choreography of Cell Signaling*
Yu	Ouyang	RU	*Characterization of the ORF2 Protein in Recombinant Human Picobirnavirus (hPBV)*
Chi-Lin	Tsai	MDACC	*EXO5-DNA Structure and BLM Interactions Control DNA Resection for Replication Restart*
Matthew	Ykema	RU	*Expression and Characterization of the Human Astrovirus (HAstV) VP90 Capsid Protein for Investigating the Features of the Proteolysis-Mediated Viral Maturation Process*

Investigating the Effect of Anti-cancer Compound (Piperlongumine) on the Physical Properties of Cancer Cells Using Atomic Force Microscopy

Alcântara-Contessoto NS[1,2], Bezerra DP[3], Barbosa-Filho JM[4], Lian I[5], Cornélio ML[1], Kiang CH[2]

[1]Physics Department, São Paulo State University, São José do Rio Preto, SP, Brazil.
[2] Department of Physics & Astronomy, Rice University, Houston, TX, USA.
[3]Centro de Pesquisas Gonçalo Moniz, FIOCRUZ, Salvador, BA, Brazil.
[4]Lab. de Tec. Farmacêutica, Federal University of Paraíba, João Pessoa, PB, Brazil.
[5]Department of Biology, Lamar University, Beaumont, TX, USA.

Natural compounds have shown a great potential for cancer treatment. Piperlongumine (PL), a natural anti-tumor compound derived from pepper species, has been demonstrated anti-cancer effect on Hela cells, and toxicity of PL against Hela cells has been investigated. Here we focus on understanding the mechanical properties of Hela cells under PL treatment, using Atomic Force Microscopy (AFM) based single-cell manipulation technique. We used AFM to pull single Hela cells and acquired the force-distance curve. The characteristic force curves showed stepwise patterns, and we analyzed the step force and found that, under treatment of PL, the most probable step force shifted to higher values compared to the non-treated cells. This change was observed in cells on substrates of different stiffness. These effects may be due to the change in the cytoskeleton structure of Hela cells after PL treatment, possibly due to microtubule destabilization by PL. Therefore, understanding the PL effects on the cellular physical properties beyond the biochemical mechanisms of PL's anti-cancer effects, may help in the development of effective therapeutic drugs against cancers.

This work was supported by Conselho Nac. Des. Cient. Tecnológico (CNPq # 141714/2017-4), and the Welch Foundation (C-1632).

Genome Packaging in Bacteriophages: The Effect of DNA – Capsid Interactions

Bores C[1], Pettitt BM[1]

1. Department of Biochemistry and Molecular Biology, UTMB

Corresponding author: Cecilia Bores, Biochemistry and Molecular Biology, UTMB, 301 University Blvd, Galveston, TX, Email: cbquijan@utmb.edu.

DNA packaging and ejection are two critical moments in dsDNA bacteriophages lifecycle. The understanding of these two processes is decisive for the effective application of phages as an alternative to antibiotics or in gene therapy. The forces needed to pack the DNA molecule to near crystalline density combined with the geometrical constrains and the chemical structure of the phage major capsid protein determine the conformation of the confined DNA. In particular, features such as DNA orientation, the layered structure near to the wall and especially the detailed structure of the outer layer are strongly linked to the correlation between the DNA and the inner wall of the phage proteinaceous capsid, rather than the polymeric structure of the DNA itself. We have performed Molecular Dynamics simulations, using oxDNA model for the dsDNA molecule, to mimic the packing process in phage ϕ29. We have analyzed the DNA conformation by means of density profiles and correlation functions finding results that depend on the injection forces or on the geometry, size and flexibility of the capsid model. DNA structure predicted by these simulations show features that agree with cryoEM and X-ray diffraction experiments. However, the atomic roughness and charge distribution of the inner surface of the capsid shown by cryoEM results may be important for the existence of DNA patterns next to the capsid wall of certain viruses. In an effort to include the detailed atomic structure of the capsid in our simulations, we have developed a coarse-grained model coupling an atomistic approach for the capsid with the oxDNA model for the dsDNA. We have used protein NColE7 which exhibits a non-specific interaction with the DNA as the case study to parameterize the proposed model.

We gratefully acknowledge the Robert A. Welch Foundation (H-0037) and the National Institutes of Health (GM- 066813) for partial support of this work.

A Data-Driven Statistical Field Theory of Active Matter

Borzou A[1], Schwarz JM[1]

1. Department of Physics, Syracuse University

Corresponding authors: Ahmad Borzou & Jennifer M. Schwarz, Physics Department, Crouse Dr., Syracuse, NY, E-mail: aborzou@syr.edu

One of the major challenges in modeling biological systems is that some of the key players and their interactions are not fully understood. With the advent of high throughput experiments, and in the age of big data, data-driven methods are on the rise. However, although machine-learning approaches have been useful so far, they do not necessarily shed light on the underlying principles of such systems. To begin to illuminate the underlying principles, I will present a data-driven approach to infer the statistical field theory for active matter---a leading candidate for quantifying living systems. The method is first developed analytically. The parameters of the model will be learned from observations of the biological system of interest. I validate the approach with several simulations.

Observing a Polymerase Incorporate a Wrong Nucleotide

Chang C[1], Gao Y[1]
1. Department of BioSciences, Rice University

Corresponding author: Caleb Chang, Department of BioSciences, Rice University, 6100 Main St, Houston, TX, E-mail:cc109@rice.edu.

Error-free replication of DNA is essential for all life. Despite the proofreading capabilities and conformational selections innate to replicative, high-fidelity DNA polymerases, intrinsic polymerase fidelity is two to three times higher than what can be provided by base-pairing energies. Although researchers have studied this long standing question with steady-state and pre steady-state kinetics, structural studies, and computational simulations, the structural basis of intrinsic polymerase fidelity remains elusive. An atomic resolution visual of the whole process may provide new, direct mechanistic insights into polymerase fidelity.

Previous time-resolved results show that during the incorporation of a correct nucleotide, the nucleotidyl reaction is promoted by 3 magnesium ions that control alignment of the 3'-OH of the primer terminus and α-phosphate of the incoming nucleotide, fixation of nucleotide-phosphate groups, and coordination of substrate and product. However it is unknown if the same events occur during misincorporation or the incorporation of an incorrect nucleotide. To fully understand the mechanism that governs polymerase fidelity, we seek to elucidate the mechanism of DNA misincorporation at atomic level with X-ray time-resolved crystallography. We will use polymerase η, a Mg^{2+} dependent translesion polymerase, which follows similar kinetic pathways leading up to the nucleotidyl reaction of replicative polymerases and whose catalytic process can also be easily captured in *crystallo* with atomic resolution.

We are currently performing kinetic single-base extension incorporation assays to determine if nucleotide binding or nucleotidyl-transfer is affected during DNA misincorporation. Our preliminary results suggest that alignment of substrate and the position of magnesium binding near the primer terminus, but not the reaction process, are affected during DNA misincorporation.

This work is supported by CPRIT (RR190046), The Welch Foundation (C-2033-20200401), and a fellowship from the Houston Area Molecular Biophysics Program (NIH Grant No. T32 GM008280, Program Director Dr. Theodore Wensel).

Measuring the Mechanical Forces of Elongation Factor G Mediated Translocation

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 GTPases Elongation Factor G (EF-G) and Elongation Factor-Tu (EF-Tu). EF-G is responsible for catalyzing tRNA and mRNA translocation on the ribosome, while EF-Tu is responsible for shuttling aminoacyl-tRNAs to the ribosome and catalyzing their addition to growing peptides. During the last step of translocation, EF-G binds to the ribosome and completes translocation, allowing the ribosome to continue translating. Along with its' proteins, the ribosome is capable of translating mRNA into protein with high fidelity and correct reading frame maintenance. This mechanism, however, for the movement during translation remains elusive. The mechanism by which EF-G drives translocation is also not well understood. The focus of this project, therefore, is to characterize the effector loop in the GTPase center of EF-G and understand how it relates to the mechanism of EF-G mediated translocation. This study will, therefore, help to gain a better understanding of the mechanism of translocation and how the ribosome can efficiently translate mRNA into protein, while maintaining the correct reading frame.

Funded by the National Institutes of Health grant T32GM008280 to Baylor College of Medicine

Structural and Functional Studies of Colletotrichum Camelliae Filamentous Virus 1 Methyltransferase and RNA-dependent RNA polymerase

Zihan L[1], Yizhi T[1]
1. Department of Biosciences, Rice University
Corresponding author: Zihan Lin, Department of Biosciences, Rice University, 6100 Main St., Houston, TX, E-mail: zl36@rice.edu

Colletotrichum Camelliae Filamentous Virus 1 (CCFV-1) is a filamentous shape double-strand RNA virus. CCFV-1 infects the *Colletotrichum Camelliae* to inhibit its infection on *Camelliae sinensis*, commonly known as a tea leaf. Surprisingly, CCFV-1 is the only dsRNA virus encapsidated by filamentous capsid protein.

Seldom study has been done on any CCFV-1 viral protein further than functional prediction. ORF-1, ORF-3, and ORF-4 are predicted as RNA-dependent RNA polymerase (RdRp), methyl-transferase (MTase), and capsid protein (C protein) respectively. RdRp plays an important role on RNA replication and viral mRNA transcription. MTase is the key enzyme of the 5'RNA cap synthesis. Viral RNA with 5'RNA cap mocks host cell mRNA to translate viral protein and avoid host cell defense mechanism. Monomeric capsid protein oligomerizes into a flexuous capsid, which has more space for viral RNA and protein than helical virus. However, sequence alignment in protein data bank didn't provide any high conserved structure to any of these viral proteins. The enzymatic functions of them have yet to confirm. Therefore, high resolution crystal structures with some biochemistry assay will make a big impact to understand these proteins.

The RdRp and MTase of the CCFV-1 are very good potential target protein for X-ray crystallography. They are both likely to express and functionalize under cytosol. Meanwhile, the preliminary data indicates good solubility of them. Capsid protein, however, tends to form huge oligomer in its native form, which is difficult to crystallize in general. The crystal structures of them will not only provide a better understanding on their enzymatic function, but also give better clue on their closed relative viral protein. These data may reveal to the evolutionary track of the CCFV-1.

Viral MTase methylates the guanine for 5'RNA cap synthesis. It potentially continues to methylate the ribose forming a different cap structure. The CCFV-1 MTase has conserved active site but low sequence identity to other MTases or methyltransferase domains in RdRps. According to the preliminary test, the viral MTase is well-expressed but aggregated eventually. It means the protein is not stable under its current condition. A couple methods are designed to overcome the problem but have not been tested yet due to the delay of package delivery. If the well behaved MTase protein were obtained, it would be used to crystallize and perform an enzymatic activity test.

Viral RdRp catalyzes viral RNA replication and transcription. It presumably interacts with viral MTase during the RNA packaging process. Indeed, both RdRp and MTase require occurring in CCFV-1 particle, because the CCFV-1 need to produce many capped viral RNAs. In consonance with preliminary test, RdRp behaves stable so far but suffer from expression yield. Currently, the protein yield has slightly increased by adding extra IPTG for the induction. Tiny crystals are observed from the recently crystal screening. Hopefully a more concentrated protein sample can lead to bigger crystals for X-ray crystallography.

This research is supported by Welch Foundation and NIH.

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

Lu X[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, which was recently discovered in 2012, is a distant relative of the previously characterized influenza C virus. It can be propagated among living stocks such as pigs and goats, but bovines are believed to be the natural reservoir. Influenza D virus can infect human cell lines, although its ability to transmit among human population has yet to be assessed. Influenza D virus has single-strand, negative-sense RNA genome with seven genomic RNA segments. The RNA segment 6 of the influenza D virus expresses two mRNAs: an unspliced mRNA that encodes a 387aa protein called DM and a spliced mRNA that is translated into the matrix protein DM1. The function and structure of DM is unknown, but previous studies on the influenza C virus suggest that DM is a protein precursor, which may be processed by a host signal peptidase during maturation to generate DM2, the putative viral proton channel. Our preliminary results indicate that DM can be expressed as a soluble protein in E. coli with a well-defined oligomeric structure. By solving the atomic structure of DM, we expect to obtain a better understanding of its function – how it is processed to generate DM2 and whether it has a separate function other than being 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 and its structure has been mapped through X-ray synchrotron and is under modification. For full length DM1, the gel filtration data shows that it can form dimer at natural pH and has the tendency to aggregate. For DM, the size exclusion profile indicates an apparent molecular weight (MW) in the range of 440 to 669 kD, suggesting the formation of possible octamers, decamers or dodecamers. The sample was taken for negative staining transmission electron microscopy (TEM) and the size of DM was measured to be ~10 to 15 nm, consistent with our theoretical calculation. Based on the uniform morphology of those particles, DM seems to be a good target for cryo-EM and is under the cryo-EM analysis.

Funding source: The Welch Foundation

Optogenetic Engineering to Probe the Molecular Choreography of Cell Signaling

Ma G, Zhou Y*

Center for Translational Cancer Research, Institute of Biosciences and Technology, Texas A&M University, Houston, TX, 77030, USA

*Corresponding author: Yubin Zhou, Center for Translational Cancer Research, Institute of Biosciences and Technology, Texas A&M University, 2121 W Holcombe Blvd Suite 925, Houston, TX, 77030, USA. E-mail: yubinzhou@tamu.edu

Genetically encoded photoswitches have enabled spatial and temporal control of cellular events to achieve tailored functions in living cells, but their applications to probe the structure-function relations of signaling proteins are still underexplored. We report herein the design of a series of synthetic optogenetic tools to dissect the structure-functional relationship of a signaling protein (STIM1), and to reconstruct key molecular steps involved in the activation of calcium channels in mammalian cells. The chemical biology toolkit derived from engineered STIM1 can find broad applications by permitting light-inducible gating of calcium channels, overcoming intramolecular autoinhibition in a signaling protein, screening cancer-associated mutant libraries, and controlling dynamic protein-microtubule interactions, as well as reversible assembly of inter-organellar membrane contact sites in living cells at real time.

Compared with traditional biophysical and biochemical methods, the optogenetic method rivals by non-invasiveness, reversibility and high spatiotemporal precision. By using STIM1 as an example, we have illustrated the prowess of optogenetic engineering to study protein actions and cell signaling. Although our study presented herein is focused on STIM1, similar optogenetic engineering approaches can be widely adopted by many laboratories, and broadly extended to probe structure-function relations of other signaling biomacromolecules, with the goal of achieving remote control over various physiological processes with high spatiotemporal resolution.

Funding sources: This work was supported the Welch Foundation (BE-1913-20190330) and the American Cancer Society (RSG-16-215-01-TBE)

Characterization of the ORF2 Protein in Recombinant Human Picobirnavirus (hPBV)

Ouyang Y[1], Tao YJ[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). Diarrhea is a common complication in immune-compromised patients and the second most common cause of death in infants. HPBV is proposed to be a possible gastroenteritis cause or to play a synergistic role in the infection associated with the primary enteric cause. 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 cell culture or animal model in the laboratory. Besides, the recent analyses 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.

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. To test our hypothesis, the viral RNA segments were expressed in *E. coli* Rosetta cells. Interestingly, the viral proteins were successfully detected from the expression of ORFs under the predicted bacterial RBS on viral RNA gene segments. The purified recombinant hPBV virus particles were then characterized for its protein and RNA composition. Unexpected, the ORF2 protein was detected to be packaged inside the purified recombinant hPBV. Our characterization of ORF2 proteins showed that it is insoluble unless co-expressing with the hPBV capsid protein. The package of ORF2 in hPBV viral particles were then confirmed by TEM and Cryo-EM images (Figure 1). Since ORF2 is insoluble, various ORF2 truncates were co-expressed with the capsid protein to map the interaction region of ORF2 to capsid proteins. Our results would be first time to report the observation of the predicted ORF2 proteins in hPBV viral particles.

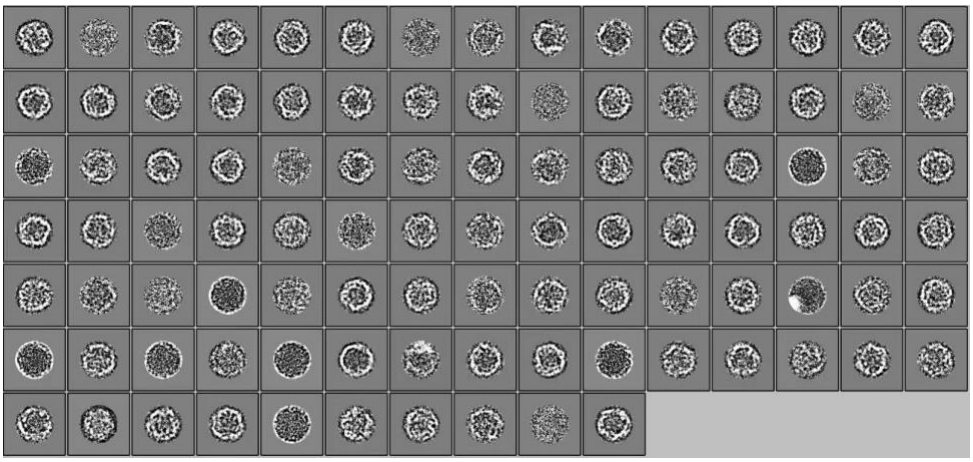


Figure 1: Cryo-EM images of capsid protein subtract. The samples are hPBV recombinant VLPs composed of capsid protein and ORF2. By subtracting the capsid signals, the ORF2 was underlying the capsid layer, which indicated ORF2 was packaged inside the hPBV capsid structure.

Funding Sources: National Institutes of Health (Grant AIO67638 to YJT) and Welch Foundation (C-1565 to YJT)

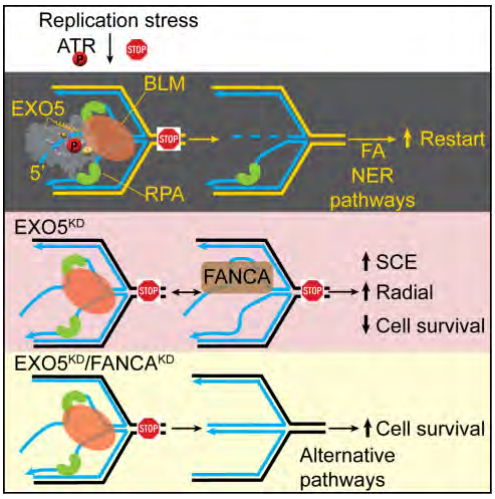
EXO5-DNA Structure and BLM Interactions Control DNA Resection for Replication Restart

Tsai CL[1], Hambarde S[2,3], Pandita RK[2], Bacolla A[1], Charaka V[1], Hunt CR[2], Kumar R[5], Limbo O[6], Le Meur R[7], Chazin WJ[7], Russell P[6], Pandita TK[2,4],Tainer JA[1]

[1]Departments of Cancer Biology and of Molecular and Cellular Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030, USA
[2]Department of Radiation Oncology, The Houston Methodist Research Institute, Houston, TX 77030, USA
[3]Department of Neurosurgery, The Houston Methodist Research Institute, Houston, TX 77030, USA
[4]Departments of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX 77030, USA
[5]School of Biotechnology, Shri Mata Vashino Devi University, Katra, Jammu and Kashmir, 182320, India
[6]Department of Molecular Medicine, The Scripps Research Institute, La Jolla, CA 92037, USA
[7]Departments of Biochemistry and Chemistry, and Center for Structural Biology, Vanderbilt University, Nashville, TN, USA.

ABSTRACT

Stressed replication fork restart orchestrated by ATR kinase, BLM helicase, and structure-specific nucleases enables replication, cell survival, and genome stability. It is unclear if ATR directly regulates nucleases for DNA end resection. Here we unveil human exonuclease V (EXO5) as the ATR-regulated structure-specific nuclease and BLM partner for restart of SMARCAL1-regressed replication forks. We find higher *EXO5* transcription in tumors correlates with increased mutation loads and poor patient survival, suggesting EXO5 upregulation is oncogenic. EXO5 and EXO5-DNA complex crystal structures reveal structure-specific single-stranded DNA binding. ATR phosphorylation neighboring the DNA-binding channel regulates functional EXO5-BLM roles. The EXO5 phospho-mimetic mutant rescues the restart defect from DNA damaged cells establishing EXO5 as a keystone nuclease for ATR orchestration of replication restart. Intriguingly, protein depletions/deletions suggest EXO5 and SMARCAL1 epistasis and show EXO5 and BLM epistasis that is evolutionarily conserved: EXO5 depletion increases sister chromatid exchange and rescues survival by FANCA-deficient cells. Thus, an EXO5 axis connects ATR and BLM in directing replication fork restart.



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Expression and Characterization of the Human Astrovirus (HAstV) VP90 Capsid Protein for Investigating the 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. Liposome infiltration assays have been used to verify a newly discovered disruption-function found in the viral capsid, which may change the model of HAstV cell entry. This research is funded by the Welch Foundation (C-1565 to YJT) and (HAMBP T32GM008280 to MY)