24th Annual Sealy Center for Structural Biology Symposium

University of Texas Medical Branch
May 4, 2019
Sealy Center for Structural Biology and Molecular Biophysics Symposium
The University of Texas Medical Branch

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B. Montgomery Pettitt
Andrew Routh
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Lawrence Sowers
Stanley Watowich
Y. Whitney Yin
Scientific Program
University of Texas Medical Branch
Galveston, TX

7:30 - 8:50 am  Registration - Foyer
Poster Set-up - Foyer

8:50 - 9:00 am  Opening Remarks and Welcome address
Dr. Montgomery Pettitt
Director of SCSB
University of Texas Medical Branch

Morning Session

Session Chair:  Marc Morais

9:00 - 9:40   Michael Sheetz, Ph.D., Director
Mechanobiology Institute
Distinguished Professor, Dept of Biological Sciences
National University of Singapore
Nanometer Level Matrix Structures Control Adhesion
Clustering and Dynamics

9:40 - 9:50  Q & A

9:50 - 10:30  Thomas E. Cheatham, III, Ph.D.
Professor
Department of Medicinal Chemistry, College of Pharmacy
Director, Research Computing and CHPC, UIT
University of Utah
Convergence, Reproducibility and Accuracy – There are
Still Some Surprises in Modeling the Structure and
Dynamics of Nucleic Acids

10:30 - 10:40  Q & A

10:40 - 10:55  Break (Foyer)

11:00 - 11:40   Liang Tong, Ph.D.
William R. Kenan, Jr. Professor and Chair
Department of Biological Sciences
Columbia University
Structural studies of RNA 3’-end processing machineries

11:40 - 11:50  Q & A

11:50 - 12:55  Lunch – Levin Hall Dining Room

1:00 - 2:30  Posters/Judging – Foyer
Afternoon Session

Session Chair: Petr Leiman

2:30 - 3:10  Karen Maxwell, Ph.D.
Assistant Professor
Department of Biochemistry
University of Toronto
The Role of anti-CRISPRs in the Phage-Host Evolutionary Arms Race

3.10 - 3:20  Q & A

3:20 - 4:00  Matthieu G. Gagnon, Ph.D.
Assistant Professor
Department of Microbiology & Immunology
Sealy Center for Structural Biology & Molecular Biophysics
University of Texas Medical Branch
How RRF and tRNA Disassemble the Ribosome During Recycling

4:00 - 4:10  Q & A

4:10 - 4:20  Break

4:30 - 5:10  Edward H. Egelman, Ph.D.
Harrison Distinguished Professor
Department of Biochemistry & Molecular Genetics
University of Virginia
Cryo-EM of Helical Polymers at Near Atomic Resolution

5.10 - 5:20  Q & A

5:20 - 5:30  Poster Awards and Closing Remarks

6:00 pm   Cocktails & Banquet
Mario's Italian Restaurant
628 Seawall Blvd.
Galveston, TX  77550
Sealy Center for Structural Biology & Molecular Biophysics
Structure-based studies of proteins and other biological molecules are a key aspect to understanding the molecular basis for disease, as well as for designing drugs to treat disease. The Sealy Center for Structural Biology and Molecular Biophysics (SCSB) was established in 1995 to provide UTMB with state-of-the-art resources for structural and functional studies of biological macromolecules. However, unlike traditional structural biology centers, whose research efforts are directed almost entirely toward structure determination, SCSB was founded on the principle that the success of structural biology in medical research is predicated on an understanding of how structure is linked to function. Consequently, in addition to traditional research programs that use X-ray crystallography and nuclear magnetic resonance (NMR), recruitment in SCSB also included faculty whose expertise cover experimental and theoretical biophysics, as well as computational biology. Today the SCSB consists of 22 core members, 16 associate members and 6 managers (from 5 departments), and the breadth of research spans all aspects of molecular biophysics and biochemistry, addressing such fundamental issues as molecular recognition, signal transduction, allosteric regulation, protein folding, systems biology, and drug design.

The GCC delivers important advances in bioscience research and training by empowering individuals to go beyond the limitations of any single institution, discipline or degree program. Located in Houston's Texas Medical Center, GCC is one of the largest inter-institutional cooperatives in the world with a focus on building strong collaborative research groups and interdisciplinary training opportunities for PhD students and postdocs. GCC brings together the strengths of its member institutions to build interdisciplinary collaborative research teams and training programs in biological sciences at their intersection with the computational, chemical, mathematical, and physical sciences. GCC provides a unique, cutting edge collaborative training environment and research infrastructure beyond the capability of any single institution. Its mission is to train the next generation of biomedical scientists and to enable scientists to ask and answer questions that cross scientific disciplines to address the challenging biological issues of our time and, ultimately, to apply the resulting expertise and knowledge to the treatment and prevention of disease.

GCC is composed of seven prominent and geographically proximate Houston-Galveston area institutions: Baylor College of Medicine, Rice University, University of Houston, University of Texas Health Science Center at Houston, University of Texas Medical Branch at Galveston, University of Texas MD Anderson Cancer Center, Institute of Biosciences & Technology at Texas A&M Health Science Center.

The training arm of the GCC, the Keck Center, currently supports over 50 trainees through competitive grants from federal and state agencies, and has over 400 affiliated faculty. Within the Keck Center, the emphasis is on continuing its 25-year successful tradition of fostering interdisciplinary and multi-institutional training. The Keck Center provides a unique intellectual and physical setting in which to train the next generation of scientists with expertise in multiple disciplines, able to reach across boundaries to advance insight and understanding. The Keck Executive Committee formulates training policy in terms of didactic courses, seminars, workshops, retreats, selecting trainees, and advising Keck Fellows and mentors, while leaving individual program directors latitude to tailor the implementation of these to the unique needs of their program.
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SPONSORS
On behalf of everyone attending the Symposium, the Organizing Committee thanks those who have provided us with financial support. We are grateful to the following organizations for their generous assistance.

Keck Center for Interdisciplinary Bioscience Training

UTMB Health

Gulf Coast Consortia

Anatrace Molecular Dimensions

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Thermo Fisher Scientific

JEOL
“Nanometer Level Matrix Structures
Control Adhesion Clustering and Dynamics”

Michael Sheetz, Ph.D.
Distinguished Professor, Dept of Biological Sciences, National University of Singapore

Integrin-mediated cell matrix adhesions are key to sensing the geometry and rigidity of extracellular environments and influence vital cellular processes including growth, apoptosis or differentiation. In vivo, the extracellular matrix (ECM) is composed of fibrous arrays that are sensed and pulled by integrin-based adhesions that seem to have a standard structure at the level of 10s of nanometers. In particular, adhesions are made up of 100 nm clusters of about 50 integrin molecules and such clusters can form around 4 matrix ligands spaced by 60 nm. This implies that many integrins self-assemble in clusters around a few activated integrins. To understand the fiber geometries that support adhesion formation, we patterned nanolines of integrin binding ligand Arg-Gly-Asp (RGD) with various line widths and arrangements in single, crossing or paired arrays. Single thin lines (width ≤30nm) did not support cell spreading or formation of focal adhesions, despite the presence of a high density of integrin-binding ligands but wide lines (>40nm) did. Using super resolution microscopy, we observed stable, dense integrin clusters formed on parallel (within 100nm) or crossing thin lines (mimicking a matrix mesh) similar to those on continuous substrates. Integrin mutants unable to bind ligand co-clustered with ligand-bound integrins when present in an active extended conformation. Thus, functional integrin nanoclusters including unliganded integrins bridge between thin (≤30nm) matrix fibers to form stable integrin adhesions on dense fiber networks. Cryo-em studies are underway to determine how clusters are organized and if there is a specific structure of integrins in the clusters and in clusters of associated adhesion proteins.
"Convergence, Reproducibility and Accuracy – There are Still Some Surprises in Modeling the Structure and Dynamics of Nucleic Acids"

Thomas E. Cheatham III, Ph.D.
Professor, University of Utah

Researchers in the Cheatham lab make use of very large scale computational resources-- locally at the Center for High Performance Computing and nationally at both XSEDE and Blue Waters facilities-- to atomistically simulate the structure, dynamics and interactions of biomolecules in (close to) their native environment(s). The group both develops and applies the AMBER biomolecular simulation software mainly to study nucleic acids and proteins and their interactions with other molecules. Traditionally, if we run short simulations on an experimental structure, we well reproduce experimental properties. However, if we performed "longer" simulations, typically the simulations would move away from the experimental structure and deviate from known experimental properties. Recently, thanks to advances in the methods and "force fields", and also access to very large scale computational resources, we can demonstrate "convergence" in elucidating the conformational ensemble, "reproducibility" in the results from independent conditions, and are moving closer to reliably reproducing experimental properties. This has helped us better understand structure, dynamics, and drug interactions with proteins and nucleic acids, better refined NMR structures, and aided in the design of novel and potential therapeutics.
Distinct machineries are involved in the 3’-end processing of canonical premRNAs, metazoan replication-dependent histone pre-mRNAs, and snRNAs. Canonical pre-mRNAs are cleaved and then polyadenylated at their 3’-end, and their processing machinery is composed of the cleavage and polyadenylation specificity factor (CPSF, with CPSF-160, CPSF-100, CPSF-73, CPSF-30, Fip1, and WDR33 subunits), cleavage stimulation factor (CstF), poly(A) polymerase (PAP), symplekin, cleavage factor I (CF I), CF II, and other protein factors. CPSF-73 is the endoribonuclease for the cleavage reaction, and a crucial step in this processing is the recognition of an AAUAAA polyadenylation signal (PAS). Metazoan replication-dependent histone pre-mRNAs are cleaved at their 3¢-end but are not polyadenylated, and their processing machinery is composed of the U7 snRNP, stem-loop binding protein (SLBP), FLASH, CPSF-73, CPSF-100, symplekin, and other protein factors. The 3’-end processing machinery for Urich snRNAs includes the integrator complex, with 14 subunits (IntS1-IntS14), and IntS9 and IntS11 are homologs of CPSF-100 and CPSF-73, respectively. We have been studying the molecular basis for the functions of these machineries, and have determined the structures of several protein factors and their complexes over the years. More recently, we have determined the structure by cryo EM of a quaternary complex of human CPSF-160, WDR33, CPSF-30, and the AAUAAA PAS, providing the first molecular insight into how this hexanucleotide is recognized. The presentation will describe our most recent discoveries from our studies on these machineries.

Supported by grants from the NIH.
CRISPR-Cas systems provide bacteria with an adaptive immune system that protects them from the viruses that infect and kill them, known as phages. In response, phages have evolved anti-CRISPR proteins to inactivate CRISPR-Cas immunity. Anti-CRISPRs are widespread among bacteria, and have been identified against a variety of CRISPR-Cas systems. We discovered anti-CRISPR proteins that inhibit the Cas9 enzyme that is commonly used for genome editing purposes. Using a combination of structural and biochemical analyses, we have identified a variety of mechanisms of activity among this diverse group of inhibitors. Several of these anti-CRISPRs show activity against distantly related Cas9 proteins, and different anti-CRISPRs are able to inhibit the same Cas9 functional domain via independent means. These studies provide new insight into the mechanisms by which anti-CRISPRs function and their impact on the evolution and diversification of CRISPR-Cas systems.
Recycling of ribosomes into subunits is an essential step of protein synthesis. In bacteria, this step is mediated by two conserved proteins, elongation factor G (EF-G) and the ribosome recycling factor (RRF). The molecular basis for ribosome recycling by RRF, EF-G and tRNA is unknown. We have determined a 3.5-Å-resolution crystal structure of a Thermus thermophilus 70S ribosome containing RRF, EF-G, and deacylated tRNAs in both the peptidyl (P) and exit (E) sites. The deacylated tRNA in the P site has moved into a previously unsuspected state of binding (peptidyl/recycling; p/R) that is analogous to that seen during initiation. The terminal end of the p/R-tRNA forms non-favorable phosphate-phosphate contacts with the 23S rRNA, illuminating the active role of tRNA in ribosome splitting. The rearrangements in the p/R-tRNA propagate to the anticodon region, weakening the base pairing interactions with the mRNA. The RRF-mediated displacement of the deacylated P-site tRNA follows the reverse path of tRNA binding during initiation, providing a missing link in understanding the role of RRF and tRNA in ribosome recycling.
Large amounts (sometimes the majority) of protein in eukaryotic, bacterial and archaeal cells is often found in the form of helical polymers. Viruses infecting these cells can also be helical. We have been using electron cryo-microscopy (cryo-EM) to study the structure and function of many of these polymers. Since the introduction of direct electron detectors into transmission electron microscopes about six years ago, there has been a “resolution revolution” in cryo-EM where near-atomic levels of resolution can now almost routinely be achieved for many macromolecular complexes. While some of these complexes can, in principle, be crystallized, cryo-EM has emerged as the method of choice for structural studies of such complexes as it does not require crystallization, uses far less sample, and is much faster. But for helical polymers most can never be crystallized and cryo-EM is not only the preferred method but the only method available for reaching near-atomic resolution. I will describe applications of cryo-EM to a range of systems, from viruses that infect organisms living in nearly boiling acid, to an archaeal pilus that is nearly indestructible, to “microbial nanowires” that conduct electrons. All of these studies provide not only new understanding of biology and evolution, but yield insights into novel structures that can have applications to drug delivery, biomedical engineering and nanotechnology.
Map to the Banquet at Mario’s Italian Restaurant

1001-1099 Market St
Galveston, TX 77550

1. Head east on Avenue D toward 10th St

↑

2. Turn right onto 6th St/University Blvd

Continue to follow 6th St
Destination will be on the right

Mario’s Seawall Italian Restaurant
628 Seawall Blvd, Galveston, TX 77550
**Poster Presentations**

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| P-3  | Micah Castillo | Utilizing N-glycan mutations to develop aggregation resistant monoclonal antibodies. |
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| P-14 | Logan Sheffield | Molecular Dynamics Simulations Provide Insights into Stability of Hyperthermophilic Pro- |
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P-18 Yiyang Zhou Novel next generation sequencing techniques to probe chikungunya virus RNA structures related to defective-interfering RNA formation. Structural and Biochemical Studies of MurAA, an Enolpyruvate Transferase that Contribute to Daptomycin Resistance in Enterococcus faecium
Immunopeptidomics of cancer peptides reveals neglected HLA-binders

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Human Leukocyte Antigen (HLA) receptors are responsible for displaying intracellular peptides at the cell surface, allowing for the recognition of these peptide-targets by cells from the immune system. Therefore, computational prediction and experimental validation of peptides capable of binding HLA receptors are tasks of major importance for vaccinology and cancer immunotherapy. There are more than 10 thousand HLA variants in the population, being HLA-A*2402 (A24) the most prevalent worldwide. However, there is much more data available on HLA-A*0201 (A2), the second most prevalent. For instance, a search on IEDB shows 21,613 peptides restricted to A2, and only 7,900 peptides restricted to A24. The same is observed in other databases. In fact, even the computational prediction of peptides from a set of protein sequences would not generate even numbers of potential binders for both HLAs. To investigate this issue, we performed peptide elution and tandem mass-spectrometry of cell lines transduced to over-express A24. High confidence peptide sequences were submitted to a standard binding affinity prediction tool (NetMHC4), and validated through competitive binding assays. Our results show that a number of real binders were neglected by NetMHC4 predictions. Molecular dynamics of these complexes reveals the role of residue number 4 as an alternative anchor for peptide binding, establishing hydrogen bonds and Coulombic interactions with conserved HLA residues. We further corroborated these findings with mutational studies. These alternative interactions could compensate the lack of strong primary anchors and are not accounted for the prediction tools, potentially excluding viable targets for cancer immunotherapy.

dsDNA Packed inside Phage Capsids: Structure and Defects Emergence

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DNA packaging and ejection are two critical moments in dsDNA bacteriophages lifecycle and their understanding is decisive for the effective application of phages as an alternative to antibiotics. The forces needed to pack the DNA molecule to near crystalline density (~0.5g/ml) combined with the geometrical constrains of the phage capsid determine the conformation of the confined DNA. Most theoretical studies that have been performed to better understand how is the conformation of the DNA inside bacteriophage capsids considered DNA as a perfect elastic rod and predict highly ordered structures. However, the emergence of more disordered conformations exhibiting defects such as knots, kinks, loops, … that hinder both the insertion and extraction of the DNA molecule in the phage capsid is also plausible. This raises the central question of the present work - how much order and disorder is reasonable (or required) when DNA is confined inside the phage capsid? We have performed Molecular Dynamics simulations using oxDNA model for dsDNA and a purely repulsive harmonic wall representing the proteic capsid of the bacteriophage to mimic the packing process in phage ϕ29. We have thoroughly analyzed the DNA conformation by means of density profiles and correlation functions during packing finding different results depending on how fast the DNA is being inserted. DNA structure predicted by these simulations show patterns that agree with experiments, cryoEM and X-ray diffraction, but many features in a more realistic capsid model – presence of multivalent ions, torsional forces, and local attractive/repulsive sites in the capsid or an elongated shape - might contribute to the emergence of these or other characteristics.
P-3

Utilizing N-glycan Mutations to Develop Aggregation Resistant Monoclonal Antibodies.
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The development and commercialization of monoclonal antibodies (mAbs) comes with challenges related to maintaining their stability during synthesis, purification, formulation and pharmaceutical storage. In order to increase stability, different modifications such as polyethylene glycol (PEG)ylation and various methylations have been tested. However, often times this results in the alteration of affinity and effector functions in the mAb’s due to non-specific modifying chemistry at its complementarity determining region. We have shown that alteration of the glycosylation pattern of Herceptin® (trastuzumab) and Humira® (adalimumab) can change their stability over time, and therefore their viability as candidates in the biotherapeutics market could be negatively or positively affected. The advantage of the introduction of an N-linked glycan is the ability to target a specific region of the mAb, which does not have a role in antigen recognition and effector function. The aforementioned antibodies were previously mutated at computationally derived aggregation prone regions on their heavy chains. The effect of these mutations on the antibodies will be measured in order to confirm computational data, characterize their propensity to aggregate and compare their overall stability versus the wild-type. Briefly summarizing the proposed experimental design, changes in the secondary and tertiary structure of the mutants will be reviewed followed by their thermal stability and resistance to unfolding. Size exclusion chromatography will then be employed along with dynamic light scattering to measure the aggregate and fragment levels of the mAbs, as well as elucidate their size distribution in solution across different time points during an accelerated stability test.

P-4

The catalytic role of the Integrator complex endonuclease in cleaving nascent mRNAs to attenuate transcription
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The Integrator complex (INT) is a 14 subunit group of proteins involved in transcriptional regulation. INT has been shown to be essential for the 3’ end formation of both UsnRNA and eRNA. More recently, others and we have shown that INT also associates with paused RNAPII at the 5’ end of a diverse set of mRNA-encoding genes with an enrichment for those involved in immediate early response (IEG) pathways. While the IntS11 catalytic function as an endonuclease is defined in UsnRNA and eRNA production, the importance of IntS11 cleavage activity in regulating mRNA transcription is less understood.

Using a combination of molecular and biochemical approaches coupled with functional genomics, we show that the Integrator complex is recruited to the 5’ ends of many Drosophila protein-coding genes. The common feature of these genes is that they are expressed to very low levels normally despite having robust recruitment of RNAPII to their promoter region. Using RNA-seq, we find that depletion of IntS11 leads to de-attenuation of these INT-occupied genes resulting in their profound upregulation. PRO-seq analysis reveals that depletion of IntS11 results in significant release
of RNAPII from the promoter proximal region. Our work suggests a unifying and evolutionarily parsimonious model of Integrator function in which the Integrator complex is broadly used to drive RNAPII termination across the transcriptome and control nascent RNA fates.

P-5

Protein Structural Fluctuations at Criticality in the Temperature-pressure-crowding Folding Phase Diagram

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In the cell, proteins perform complex biological functions through large-scale motion, which are induced by slight environmental perturbations. This characteristic of having high susceptibility is similar to a physical system near a critical point. Indeed, recent experimental and computational findings demonstrate that protein folding transitions in the temperature (T), pressure (P), and crowding volume-fraction (ϕ) phase diagram point toward signatures of criticality, where distinct folding phases merge. Here, using coarse-grained molecular dynamics simulations, we theoretically show that at the critical regime, fluctuations exhibit high susceptibility and long-range correlations up to the size of the protein. Meaning that near criticality, the dynamics of each residue is influenced by each other residue even across the entire protein. We investigate the structural origin and the effect of macromolecular crowding on this critical behavior. Furthermore, this study leads us one step closer to developing universal principles of protein folding and function in vivo.

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P-6

The challenges of sampling and scoring when docking peptides to protein receptors

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Computationally docking large ligands, and especially peptides, to protein receptors is still considered a challenge in computational structural biology. The issue lies both in conformational sampling of a peptide, and in accurately scoring the binding modes of a protein-peptide complex produced by a molecular docking tool. In this study, we evaluate both parts of computational docking. First, we examine the impact of using parallelized and incremental paradigms on the accuracy and performance of conformational sampling when docking peptides. We use four datasets of protein-ligand complexes involving ligands that could not be accurately docked by classical protein-ligand docking tools in previous studies. We also evaluate several popular scoring functions in their ability to accurately rank the best protein-peptide complex conformations, based on the RMSD to the original crystal structure. Our evaluation of sampling shows that it is most efficient
to run several short instances of a docking tool in parallel and group the results together, as opposed to a more exhaustive sampling strategy. Even greater accuracy is achieved by our parallelized and incremental meta-docking tool, DINC. We conclude that the conformational sampling of large ligands should not be considered an issue anymore, as existing tools can solve it. On the other hand, our evaluation of scoring shows that existing scoring functions, though known to be adept at scoring drug-like ligands, are limited when it comes to peptides. Therefore, accurately scoring conformations from peptide docking should currently be regarded as the biggest unmet challenge in molecular docking.

P-7

Diffusion in Membraneless Organelles: A Simple Model
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Introduction: Membraneless organelles have garnered significant attention in the last decade for their roles in various cellular signaling functions. One of their unique properties is the ability of individual component proteins to dynamically diffuse into and out of the phase-separated aggregate, controlling how much protein or nucleic acid is available for a given cellular function. Certain mutations, however, may cause pathological aggregates, a characteristic of neurodegenerative diseases and cancers. It is not clear whether there is a relationship between solubility or hydrophobicity and the rates of diffusion of components in an aggregate. The goal of this project is to compute the diffusion coefficient of peptides in aggregates to elucidate the diffusive properties of different amino acid sequences.

Methods: Seven different equilibrated peptide aggregates were simulated in periodic boundary boxes with molecular dynamics for 38ns-46ns. The peptide motif was GGXGG, where X was glycine, valine, asparagine, glutamine, phenylalanine, aspartate, or arginine. For each of the seven peptide simulations, the average diffusion coefficient was computed for peptides while (1) in free solution, (2) in transition from aggregate to solution and vice versa, and (3) in the aggregate. Results: The diffusion coefficients calculated for peptides in aggregate show the following trend: GGGG > GGVGG > GQGQG > GGQNG > GGFGG. The diffusion coefficient of peptides in free solution was found to be on the order of 20 times higher than that in aggregate. Conclusion: Our results compared with previous solubility studies showed that the solubility predicts the ordering of diffusion rates. While the diffusion rates do not match traditional scales of hydrophobicity, they do accurately reflect observed biological phenomena, such as polar glutamine residues being a key factor in aggregation in Huntington’s diseases. This suggests that the classical hydrophobicity is insufficient to explain diffusion in aggregates.

P-8
Structure-Based Drug Design of Novel Bromodomain-Containing Protein 4 (BRD4) Inhibitors for the Treatment of Airway Inflammation

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Abstract: Disrupting the protein-protein interactions between bromodomain-containing protein 4 (BRD4) and acetylated lysine residues in histones has emerged as promising therapeutic targets for various human diseases including cancer and inflammation. Currently, a number of BRD4 inhibitors have been evaluated in different phases of human clinical trials (e.g. RVX-208, Phase III). Although small molecule BRD4 protein inhibitors have been widely reported, none are highly isoform selective over other members of the family. Our team has identified and validated BRD4 as a therapeutic target in viral and allergen induced airway diseases both in vitro and in vivo. Herein, we report the structure-based drug design, chemical synthesis, and pharmacological characterization of novel BRD4 inhibitors for the treatment of airway inflammation. Several novel potent and specific BRD4 inhibitors including ZL0454 and ZL0590 have been identified with nanomolar binding affinities. Further computational docking studies were performed to illustrate their interaction binding mode and selectivity towards BRD4. These compounds have much lower IC50s for the BRD4 bromodomains (BD1 and BD2) than JQ1 and RVX208. More importantly, these compounds not only exhibited significant suppression against inflammatory genes such as IL-6 and CIG5 in human small airway epithelial cells (hSAECs), but also displayed excellent in vivo efficacy in the mouse model of poly(I:C)-induced airway inflammation. In conclusion, we demonstrated the proof of efficacy for highly selective BRD4 inhibitors in viral and allergen induced airway diseases, indicating their therapeutic potential for preclinical development.

P-9

Disruption of IntS13 Interaction with Integrator Cleavage Module Contributes to Ciliopathy Disease

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The Integrator complex (INT) consists of at least 14 highly conserved subunits, and is associated with RNA polymerase II (RNAPII). Its key functions include the co-transcriptional cleavage of UsnRNA and eRNA, and regulating the activity of paused RNAPII at many genes critical for development. Cleavage of nascent RNA by INT is reliant on three subunits, IntS4/9/11, forming a ‘cleavage module’ with IntS11 housing the actual endonuclease activity. Not surprisingly, perturbation of INT function through mutation can cause developmental disorders in humans affecting multiple tissue types. This includes ciliopathies, which are caused by disrupted biogenesis of the primary cilium that is expressed in nearly all cells. We previously demonstrated that RNAi-mediated knockdown of INT subunits is sufficient to disrupt ciliogenesis through an unknown process.

Here, we describe two unrelated families with children diagnosed with a specific ciliopathy disease that have two distinct homozygous recessive mutations within the C-terminus of IntS13 – a previously uncharacterized Integrator subunit. Either mutation leads to decreased levels of IntS13 protein in patient cells, and cilia defects are recapitulated in Xenopus embryos treated with antisense morpholino oligos to IntS13. Using a modified yeast two-hybrid assay, we determined that IntS13 interacts with the IntS4/9/11 heterotrimer via its
C-terminus, and the patient mutations completely abolish this interaction. Co-immunoprecipitation and mass spectrometry data show that these mutations weaken INTS13’s association with the majority of INT subunits and RNAPII. We are currently using NGS approaches in patient cells and IntS13 knockdown cells to pinpoint the exact spectrum of ciliogenesis relevant genes whose transcription are disrupted under INT dysfunction.

P-10

Investigating The Role of N373 Residue of The αX Subunit on The Activation and Function of αXβ2 Integrin.
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Integrins are α/β heterodimeric proteins that integrate the extracellular and intracellular environments. Structural and functional studies have showed that integrins experience a transition from a bent conformation in the inactive state to an extended conformation in the active state caused by the extension of the headpiece, headpiece opening and leg domain separation. αXβ2 integrin has a restricted expression to leukocytes. When activated, αXβ2 binds to its ligands including complement component iC3b and mediates cellular trafficking, leukocytes adhesion, phagocytosis, and T cell proliferation. αXβ2 dysfunction is associated with inflammatory disease such as atherosclerosis and hypersensitivity. Both α and β integrin subunits are the major carriers of N-glycans. Studies showed that complex N-glycosylation stabilize the high-affinity extended open conformation of integrins. How does the glycans affect the activation of the integrin isn’t well understood. At position N373 of the α subunit of αXβ2 is a high mannose glycan that was resistant to enzyme hydrolysis. To study the role of this glycan, we mutated asparagine residue into serine (N373S variant). The effect of the mutation on the receptor-ligand affinity is assessed by erythrocyte rosetting, and the change in the conformation of the integrin is detected by monoclonal antibodies that identify structural epitopes. Molecular Dynamics studies will be done to measure the degree of freedom of the ligand binding domain, the αX I-domain and characterize the role of N373 in the integrin activation mechanism.

P-11

Identifying the effect of membrane depolarization on a model binary bilayer using molecular dynamics simulations
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Abstract: Electrophysiological analyses of numerous cell types implicate a role for plasma membrane (PM) charge depolarization in cell proliferation. Terminally differentiated cells possess a hyperpolarized transmembrane potential ($V_m$) (-50 to -100mV), whereas proliferating cells tend to be depolarized with a $V_m$ ~
-10 to -40mV. A similar effect has been observed in multiple cancers, with the cancer cells being more depolarized compared to their cognate non-transformed cells. PM lipids are believed to be responsive to such changes in $V_m$ and may undergo significant reorganization in their structure and lateral dynamics upon changes in $V_m$ induced by electric fields in computer simulations and experiments. Considering the large number of signaling proteins at the plasma membrane, whose function is determined by their interactions with PM lipids, this could be a mechanism by which cancer cells sustain proliferative signaling. To understand this in greater detail, we are investigating the effect of $V_m$ on PM lipid structure and lateral dynamics. We are performing detailed molecular dynamics (MD) simulations of a model binary bilayer using three variants of MD at three different resolutions, which we will be discussing.

**P-12**

**Designing a Broad-Spectrum Vaccine against Encephalitic and Arthritogenic Alphaviruses**

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Mosquito-borne alphaviruses, endemic in the U.S., cause periodic outbreaks horses and humans. Alphaviruses such as chikungunya (CHIKV) are known to cause debilitating arthralgia. Others, such as Venezuelan equine encephalitis virus (VEEV) can be fatal. For many alphaviruses, there are no vaccines or treatments approved for general use. Furthermore, clinical trials have encountered immune interference when alphavirus vaccines are given together or sequentially. Our objective is to develop a single, broad-spectrum vaccine that provides long lasting immune protection against both CHIKV and VEEV. The physicochemical properties (PCP) the amino acids in thousands of strains of VEEV and CHIKV are used to calculate a consensus E2 proteins with over 50% structural similarity to all alphaviruses. Alternatively, we have created in silico a chimeric E2 protein containing immunogenic epitopes of both VEEV and CHIKV. The constructs are tested for ability to bind antibodies raised against wild-type strains, generation of neutralizing antibodies determined by PRNT and protective ability against lethal challenge with diverse alphavirus strains. These E2 proteins are incorporated into an attenuated VEEV vaccine strain (IRESV1). IRESV1 expressing a VEEV consensus E2 protein was successfully rescued and shown to be protective against VEEV and Mucambo virus in a lethal mouse model. These new methods for in silico vaccine design can be the basis for broad-spectrum vaccines against diverse alphaviruses. This has the potential to allow for consolidation of several vaccines into a single antigen, significantly reducing the time and cost of production while avoiding any potential immune interference.
P-13

Osmotic Pressure Calculations in the Systems with the Highly Concentrated DNA and Polyethylen Glycol Polymers Solutions Using All-atoms Molecular Dynamic Simulations as a Way to Understand Biophysical Processes of DNA Extraction from Bacteriophages.

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Bacteriophages therapy is an alternative method to treat secondary infections. Some pathogenic bacteria develop resistance to antibiotics, bacteriophage can be used to treat such bacterial infections due to their ability to attach to the bacteria cell, extract DNA or RNA, carried in the protein capsid, into the host cell, disrupt bacteria metabolism, and destroy it. Sequence dependent mechanical coupling of the DNA to the thermodynamics of packing can change efficiency and possibly inhibit packing of some sequences. Understanding the biophysical basis of the biological process which transfers a viral genome to infect a cell is very important to the cellular machinery and many disease related fields central to the sequence design issue for reprogramming phages for emerging strains. We model the process of DNA extraction from the bacteriophage using all-atoms molecular dynamics combined with experimental data to model the structure and the thermodynamics of DNA confined by surfaces. The inside of the full capsid has a different concentration of species than the surrounding environment creating an osmotic pressure difference. We employ all-atoms molecular dynamics simulations to calculate osmotic pressure of one or multiple single strained DNA(s) in aqueous solution and concentrated polyethylene glycol (PEG) polymers solution, we discuss correlation between DNA conformations and osmotic pressure, we analyzed folded/unfolded DNA structures, hydrogen bonds formations in DNA, the ions distribution around the DNA strand(s) for the different salt and PEG concentrations, and dependence of osmotic pressure of DNA on the osmotic barrier proximity, and DNA molarity.

P-14

Molecular Dynamics Simulations Provide Insights into Stability of Hyperthermophilic Proteins

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It is well known that the cellular components of mesophiles and thermophiles are markedly disparate (e.g. differing membrane lipids and GC-content). Even still, microbes must rely on proteins capable of maintaining stability for the entire range of temperatures experienced within their environment, a characteristic termed thermostability. Enzymatic thermostability has become an important factor in industrial applications, where high-temperature reactions are often more efficient. One such example is in endoglucanases, enzymes that hydrolyze internal β-(1,4)-glycosidic bonds between the glucose monomers of cellulose. While this mechanism is used in various industrial applications such as paper production and food processing, it also
allows the production of biofuel from sources of cellulose rather than food-based sources such as sugar or corn. Unfortunately, this process is currently limited by the low thermostability of endoglucanases, which currently only allow for temperatures below 60°C. *Pyrococcus horikoshii* is a hyperthermophilic organism that has been shown to produce a highly thermostable β-(1,4)-endoglucanase (EGPh). It would be useful to compare this enzyme to similar enzymes of different thermostability levels in order to gain insight on what factors are responsible for the higher thermostability. In this study, molecular dynamics simulations are used along with various statistical methods to compare the behavior of both a mesophilic and a moderately thermophilic endoglucanase to that of the hyperthermophilic EGPh at various temperature levels. Simulations are carried about using crystal structures obtained from the Protein Data Bank. Results for each simulation are compared to look at differences between the enzymes of varying thermostabilities.

**P-15**

**Identification of Regulators of the Nav1.6 Channel Macromolecular Complex by High-Throughput Screening**

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Neuronal firing is a highly regulated process that depends on the integrity of ion channel macromolecular complexes, which in turn rely on the stability of cellular signaling networks. To characterize the specific cellular signaling mechanisms that regulate the Nav channel complex, we conducted an in-cell HTS against the FGF14:Nav1.6 complex using the split-luciferase complementation assay (LCA). We developed a double-stable HEK293 cell line expressing LCA constructs and miniaturized the assay from 96-well to 384-well plates by optimizing multiple parameters including cell density, media volume, incubation times, and enhancer/inhibitor controls to achieve $Z' \geq 0.5$. Compound toxicity was assessed in parallel using the CellTiter-Blue Cell Viability Assay, and effects on luciferase were assessed via counter-screening against full-length luciferase. Compounds were then ranked by a combination of % luminescence and individual Z-scores, which were calculated based on the mean and SD of respective plate controls (0.3% DMSO). Preliminary hits were identified using cut-offs of $Z \leq -3.5$ for inhibitors and $Z \geq 2$ for enhancers. Hits were then selected based on potency as assessed by an 8-point dose response. Rationally guided hit assessment revealed an over-representation of structurally diverse compounds targeting the JAK2 and Src tyrosine kinases, including TG101348 (IC₅₀ = 12.3 μM), WP1066 (IC₅₀ = 4.1 μM), Bosutinib (IC₅₀ = 9 μM), and Quercetin (IC₅₀ = 5.7 μM). Bioinformatic analysis revealed a probable JAK2 phosphorylation motif in FGF14 at Y158, a previously identified hot-spot at the FGF14:Nav1.6 interface, and *in vitro* phosphorylation studies confirmed phosphorylation of this site by JAK2.
P-16

Determining the Interaction Enthalpy of Side Chain and Backbone Amides in Polyglutamine Monomers and Fibrils
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Polyglutamine fibrils are implicated in the pathology of various neurodegenerative diseases, the most notorious of which is Huntington's disease. Understanding the formation of polyglutamine fibrils is key in developing a complete molecular picture of the pathology of these diseases. Here we present molecular dynamics (MD) results in which we calculate the interaction enthalpies of hydrogen bonds formed between various amide carbonyl groups present in D2Q10K2 peptides. We use several low energy monomeric D2Q10K2 conformations as well as an octameric amyloid-like fibril structure. Interaction enthalpies calculated from our MD simulations are compared with experimental results from UV resonance Raman spectroscopy experiments that relate the Amide I vibrational frequency to the interaction enthalpy of the carbonyl group. We find that inter-amide side chain side chain and side chain-peptide backbone interactions in fibrils and β-strand monomers are stronger than side chain-water interactions in monomers with polyproline II-like (PPII-like) solution conformations. In fibrils, we also find that interactions between side chain amides are enthalpically more favorable than interactions between peptide backbone amides. This work provides direct experimental validation that inter-amide side chain interactions play an important role in driving the formation and stability of polyQ fibrils.

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P-17

Computer simulations show key role stochasticity of replication fork speed plays in the dynamics of DNA replication
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Eukaryotic DNA replication is elaborately regulated to ensure that the genome is faithfully replicated in a timely manner. Replication initiates at multiple origins, from which replication forks emanate and travel bidirectionally. Activation of replication origins and fork speed are stochastic in individual cells, but reproducible population-wide. To study the complex spatio-temporal regulation of replication, models of DNA replication in S. cerevisiae have been developed, but none have considered stochastic replication fork speed. Here, we present Repli-Sim, the first model of DNA replication, which includes stochastic speed of the replication fork. Utilizing data from both wild-type and hydroxyurea-treated yeast cells, we show that Repli-Sim achieves more accurate results than models assuming constant fork speed. Due to the stochastic nature of replication, its completion in a timely fashion is a challenge. Previously proposed solutions promoted finishing replication by modifying replication initiation and origin activation, while we propose empirically-derived modification in replication speed based on distance to the approaching fork, which promotes completion of replication.
P-18

Novel next generation sequencing techniques to probe chikungunya virus RNA structures related to defective-interfering RNA formation.

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Viral defective RNA is truncated virus genome, commonly associated with almost every RNA virus family. The formation of defective RNA is a result of low fidelity of virus replicases and recombinational events. However, little is known about the relevance of viral RNA secondary structures to the processivity of RNA replication and the mechanism of RNA recombination, and therefore the contribution of RNA secondary structure to DI formation. Using a vaccine strain of chikungunya virus, we identified a defective RNA located exclusively in the sub-genomic RNA that is associated with in-silico predicted RNA hairpins that showed conservation with Mayaro virus.

Flock House virus (FHV) provides a simple, yet well characterized model system, with highly structured RNA in-particle organizations and well-characterized defective RNAs. With this proof-of-principle system, we have developed and demonstrated a series of novel generation sequencing techniques that can effectively and directly characterize viral RNA secondary structures. We use dimethyl sulfate (DMS) mutational profiling with sequencing (DMS-MaPseq) to probe the more flexible ssRNA regions, while a psoralens-enhanced cross-linking and ClickSeq provides a novel method to specifically target the conserved double stranded RNA regions. Using these techniques, we have successfully identified the constrained double stranded stems for FHV RNA packaging and sequences essential to RNA heterodimer formation. Here, we seek to extend the application of these complementary and high throughput methods to probe conserved RNA structures in chikungunya virus, and study potential RNA structure—DI formation relation.

P-19

Measuring the Mechanical Forces During Ribosome Translocation via EF-G Crosslinking

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The ribosome is the complex molecular machine found in all living cells that is responsible for the synthesis of protein. The ribosome is associated with various proteins, 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 observed. The question regarding the biological relevance of these conformational changes remains. To answer this, we have generated doublecysteine EF-G that was then internally crosslinked with various lengths of crosslinkers. If the large conformational change does occur in solution, then translocation will be affected by the crosslinking. To determine if crosslinking was successful, crosslinked samples were run on SDSPAGE gels until band separation was observed. A purification protocol for large scale preparation of crosslinked EF-G was developed. To purify crosslinked EF-G, we performed electro-elution with a 5% SDS-PAGE gel to collect crosslinked and non-crosslinked fractions. We have done preliminary biophysical measurements on the crosslinked EF-G that implies large conformational change in EF-G may indeed occur.
**P-20**

**Mutations in Small Heat-Shock Protein 27 Affect Phosphorylation Regulated Chaperone Activity**

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Molecular chaperones are proteins that aid in the refolding of denatured proteins; they are ubiquitously expressed and essential to life as they play various roles in health and disease. The family of small heat shock proteins are low molecular weight proteins that act in response to cellular stresses such as heat shock and oxidative stress. Small heat shock protein 27 (Hsp27) is an ATP independent chaperone that functions by preventing aggregation and shuttling partially denatured proteins to larger ATP dependent chaperonin complexes for refolding. It is speculated that the chaperone active form of Hsp27 is a large complex composed of subunits that reassemble into dimers upon phosphorylation by the mitogen-activated protein kinase activated protein kinase 2. The larger complex is in equilibrium with the smaller dimeric units and is controlled by phosphorylation at three serine residues, 15, 78, and 82. The shift in equilibrium of Hsp27 to the smaller subunits may have a role in regulation of chaperone activity by shuttling individual competent folding substrates to larger chaperonin complexes. Hydrophobic regions on the dimer interface favorably interact with the non-native proteins maintaining stability until transportation and reassembly can occur. There are currently five reported missense mutations of Hsp27 that manifest as Charcot-Marie-Tooth neuropathy, a neurodegenerative disorder that affects the motor and sensory neurons of the peripheral nervous system and is characterized by slow progressive weakness and atrophy of distal muscles. This study, therefore, aims to characterize the structure and activity of wild type Hsp27 in its dimeric and chaperone active oligomeric form as compared to the mutants.

**P-21**

**The Structural Arrangement at Intersubunit Interfaces in Homomeric Kainate Receptors**

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**ABSTRACT**

Kainate receptors are glutamate-gated cation-selective channels involved in excitatory synaptic signaling and are known to be modulated by ions. Prior functional and structural studies suggest that the dimer interface at the agonist-binding domain plays a key role in activation, desensitization, and ion modulation in kainate and closely related AMPA receptors. Here we have used fluorescence-based methods to investigate the changes and conformational heterogeneity at these interfaces associated with the resting, antagonist-bound, active,
desensitized, and ion-modulated states of the receptor. These studies show that in the presence of Na+ ions the interfaces exist primarily in the coupled state in the apo, antagonist-bound and activated (open channel) states. Under desensitizing conditions, the largely decoupled dimer interface at the agonist-binding domain as seen in the cryo-EM structure is one of the states observed. However, in addition to this state there are several additional states with lower levels of decoupling. Replacing Na+ with Cs+ does not alter the FRET efficiencies of the states significantly, but shifts the population to the more decoupled states in both resting and desensitized states, which can be correlated with the lower activation seen in the presence of Cs+.

P-22

Structural insights into the exonuclease activity and the movement of DNA between the two active sites in human mitochondrial DNA polymerase gamma

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Human mitochondrial DNA polymerase (Pol g) is the only replicase in mitochondria that is responsible for DNA synthesis and repair. Nucleoside Reverse Transcriptase Inhibitors (NRTIs), which act as chain terminators once incorporated by the HIV reverse transcriptase (RT), effectively inhibit viral replication. However, Pol g is an off-target of NRTIs, leading to mitochondrial DNA deletions and mutations that clinically manifest as neurological and cardiovascular disease. Although both are capable of polymerase (pol) activity, Pol g also has proofreading exonuclease (exo) activity as another line of defense against NRTIs. We have provided structural explanation behind drug discrimination in the pol site of Pol g by crystallography, yet the mechanism behind drug recognition and exonuclease activity in the exo site is unknown. Structural information of Pol g in proofreading mode can be exploited to design drug that can be removed by the exo site even if it escapes the discrimination in the pol site. Additionally, the structural comparison of the Pol g in replicating and editing modes will shed light on the coordination between the pol and the exo sites, which are separated from each other by 35 Angstroms, approximately. Here, we present the low-resolution models of Pol g in replicating and editing mode obtained by cryoEM, which provide global structural changes that can be used to gain some understanding behind DNA movement between the two active sites.
P-23

Different oligomerization states of *E. coli* hemolysin ClyA
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Pore-forming toxins (PFTs) are important bacterial virulence factors. Among the PFT family, hemolysin ClyA belongs to the α-helical subfamily, which can be found in *E. coli*, *Salmonella Typhi*, and *Shigella flexneri*. ClyA is shown to be a dodecamer in the crystal structure. Taking advantage of cryo-EM, we were able to observe that the ClyA pore complexes can exist as dodecamer, tridecamer, and tetradecamer. The reported resolutions for the three oligomeric complexes are 2.8 Å, 3.2 Å and 4.3 Å, respectively, allowing us to reveal the assembly mechanisms. We also show a stabilized intermediate state of ClyA during the transition process from soluble monomers to pore complexes. Unexpectedly, even without the formation of mature pore complexes, ClyA can permeabilize membranes and allow leakage of particles less than ~400 Daltons. In addition, we are the first to show that ClyA forms pore complexes in the presence of cholesterol within artificial liposomes. These findings provide new mechanistic insights into the dynamic process of pore assembly for the prototypical α-PFT ClyA.

P-24

Can Activation of Rho Factor Through RNA Binding-Induced Ring Closure be Identified by Cryo-EM?
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The rho transcription termination factor is an ATP-dependent, hexameric helicase responsible for terminating up to 50% of mRNA synthesis in *Escherichia coli*. Rho activates by binding to rut (rho utilization) sites on nascent RNA and translocating to the RNAP complex, thereby terminating transcription. Rho has been proposed to have a ‘lock-washer’ structure that is poised to load mRNA, and a closed-ring structure responsible for translocation. *rut* sites are known to be 70 to 80 nt in length and have a high content of cytosine residues but no consensus sequence has yet to be identified amongst the known rut sites. We hypothesize that the Rho protein binds cytosine at each of the six primary binding sites and that there must be a minimum of 11 nucleotides between each cytosine residue to properly load the mRNA. Rho is not necessarily an on/off switch, but a rheostat that preferentially terminates strong *rut* sites, based on the cytosine spacing on the RNA, that fully transition the protein into its closed-ring state. To that end, we have devised several synthetic RNAs with differing cytosine spacings that will be used to test the degree of ring closure by cryo-EM.
P-25

Characterizing Structural Mechanisms of Group III Metabotropic Glutamate Receptors
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G-protein coupled receptors are the largest, most diverse membrane-integrated proteins in our cells, agents in a myriad of functions and targeted by 40% of prescription drugs. In the nervous system, metabotropic glutamate receptors (mGluRs) are some of the most abundant GPCRs, key players in regulating neuron excitability, neuroprotection and plasticity. We propose to deliver a comprehensive model of the structure and dynamics of full-length mGluRs detailing the intra- and inter-domain mechanisms governing their function. We are particularly interested in the group III mGluR family (mGlu4/6/7/8) as they remain as some of the least understood GPCRs both structurally and functionally, highly relevant in multitude of pathologies including epilepsy, Parkinson’s disease, congenital night blindness, anxiety disorders and multiple sclerosis. Our lab has worked extensively in protocols for purifying mGluR6, which I will optimize for structural exploration of the mGlu4/6/7/8 receptors after reconstituting them in phospholipid bilayer anodiscs. Structures in the apo and ligand-bound states will be resolved using electron cryo-microscopy single particle reconstruction (cryo-EM SPR), employing techniques such as homology modelling and flexible fitting to obtain the anticipated full-length structures. High-resolution structures will allow us to determine important amino-acids for signal transmission and potential ligand-binding pockets. Strategies to solve disordered regions such as the C-termini will include local refinement, minimizing flexibility by antibody crosslinking or complexing the receptor with its secondary Gi/o-protein messenger. We will elucidate the conformational dynamics between different states of the group III mGluRs using total internal reflection microscopy (TIRF) coupled with single molecule Forster resonance energy transfer (smFRET). Fluorophore pairs will be introduced in various intracellular and extracellular domains of these receptors to characterize their rearrangement as a function of time and ligand-binding. Mutagenesis and functional G-protein coupling assays will be used to discern the critical amino-acids involved. With the resulting models, we will deliver a structural basis to mGluR function, detailing signal propagation across its domains.

P-26

A dynamic Norovirus capsid: bile salt driven collapse and receptor binding
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Human norovirus infections are a leading cause of gastroenteritis, with emergent strains capable of escaping herd immunity arising every 2-4 years causing world-wide epidemics. Noroviruses belong to the Calicivirus family and are plus strand ssRNA viruses. These non-enveloped viruses are composed of a single major capsid protein, VP1, with three structural domains - the N-terminus (N), the shell (S), and the protruding (P) domain. The P-domains form dimers, with the tip of the P-domains involved in antibody and receptor binding. The lack of a robust tissue culture system and a small animal model have made the study of the human norovirus life cycle and vaccine design difficult. In contrast, there is an infectious clone, tissue culture system, and small animal model for murine norovirus (MNV). Compared to the crystal structure of human norovirus virus, the P-domains in MNV appear to ‘float’ ~16Å above the shell. This was subsequently shown in three other genotypes as well. It has been recently shown that bile salts play an important role in MNV cell attachment. With our 3Å cryo-EM structure, we show here that the addition of bile salts causes the P-domain to rotate ~90° and collapse onto the shell, yielding a structure highly similar to the human
Norwalk X-ray structure. The binding site for bile is far from the P domain/shell contact surfaces and work is underway to understand how bile salts trigger P-domain collapse and what role this large conformational change plays in receptor binding.

P-27

**Giant Marine Virus Sample Preparation and Data Collection for Cryo-EM**

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Viruses play many important roles in marine eco-systems. To this end understanding their physical structure will shed light on virus entry and particle assembly, two critical processes in their life cycles. In the last two decades, cryo-electron microscopic (cryo-EM) image reconstruction has become a powerful tool to study giant virus structures, and in recent years atomic resolution has been achieved by cryo-EM. Nevertheless, marine giant viruses pose unique challenges to cryo-EM structural studies. The high concentration of salt in the ocean water where these viruses persist must be reduced before cryo-EM sample freezing to achieve vitrified clear ice. In addition, the presence of co-occurring bacteria and potentially low concentrations of giant virus particles within samples both require modifications of traditional cryo-EM image collection that normally rely on highly pure and concentrated samples. In this study, we have used two marine viruses, *Cafeteria roenbergensis virus* (CroV) and *Aureococcus anophagefferens virus* (AaV), to demonstrate protocols developed to tackle those aforementioned challenges. These method developments will pave the way for large cryo-EM data collection efforts that will resolve atomic structures of giant viruses.

P-28

**Membrane interaction of chikungunya virus capping enzyme (nsp1)**

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Alphavirus nonstructural protein 1 (nsp1) is a membrane associated viral protein that anchors the viral replication complex to the plasma membrane and thus is critical for viral replication. An amphipathic helix within nsp1 is proposed to be the membrane-association region, but how nsp1 interacts with membranes is not well understood. We used recombinant chikungunya virus (CHIKV) nsp1 to study the membrane association property of the protein. We show that CHIKV nsp1 is well folded even in the absence of membrane binding and that the amphipathic helix within nsp1 is important for interaction with membranes. CHIKV nsp1 and the amphipathic helix specifically bind liposomes containing negatively charged phosphatidylserine or phosphatidylglycerol, indicating that the interaction between the protein and membrane phospholipids is mainly electrostatic. Furthermore, we visualized the interaction between CHIKV nsp1 and liposomes by cryo-electron microscopy (cryo-EM) and show that nsp1 oligomerizes on membranes and interacts only with the outer leaflet of the lipid bilayer. Our results suggest that both the interaction of the amphipathic helix with the membrane bilayer and the cytoplasmic domain oligomerization are important for membrane association in alphavirus nsp1.
P-29

Probing the Impact of Tape Measure Protein on R-Type Pyocin Function
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Subjected to UV or mitomycin treatment Pseudomonas aeruginosa produces high-molecular weight bacteriocins called pyocins. While extensively researched in the mid-20th century, the study of pyocins was eventually subsided due to the rise of broad-spectrum antibiotics (e.g. penicillin). However, with the increased prevalence of bacteria resistant to all available antibiotics (MRSA, many strains of pathogenic E. coli, Acinetobacter, etc.), there is a renewed interest in the narrow-spectrum bacteriocins. R-type pyocins are structurally similar to “simple” contractile tails (e.g. phage P2 or Mu) and the spectrum of five types (R1 through R5) have been characterized. P. aeruginosa strain PAO1 produces R2 pyocins and they kill P. aeruginosa strain 13s. Similar to other bacteriophages (e.g. P2, PS17) the length of the R2 pyocin is determined by its tape measure protein (TMP). The length of this protein is functionally linked to the host attachment mechanism. Currently, the energetics of pyocin contraction is poorly understood and a model is needed for this process. Furthermore, the role of the TMP during penetration of the cellular membrane is clearly unknown. Here, we begin to explore the impact of the TMP on the R-type pyocin function by inducing an insertion mutation (TMP-INS(232)), and a deletion mutation (TMP-DEL(332)). We determined the impact of length on pyocin function, and on ability to contract.

P-30

State-specific localization of AcrAB-TolC in E. coli using cryoET and in situ subtomogram averaging
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Multidrug efflux pumps (MEP) expel a wide variety of toxic substances across the membranes of bacterial cells. In Gram-negative bacteria, MEP form tripartite complexes spanning the cellular envelope; however, the
in situ structure and assembly mechanism of many such pumps remain unknown. Using electron cryo-tomography (cryoET) and subtomogram averaging, we have solved the first in situ structure of the AcrAB-TolC tripartite complex and the bipartite precursor complex AcrAB at better than 2nm resolution. Here we discuss the computational workflow in the EMAN2 software suite used to obtain these two structural states and how these structures can be mapped back to the cell, facilitating state-specific localization in situ. In addition to demonstrating the current state of the art in cellular subtomogram averaging, our findings also uncover the assembly mechanism of this tripartite MEP complex in living bacterial cells, providing a structural basis for the design of MEP inhibitors.

P-31

CryoEM and X-ray structures of a homomeric ring ATPase that powers genome packaging in dsDNA viruses

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Bacteriophage φ29 has been used as a model system for viral because a functional viral-packaging system can be assembled from recombinantly expressed proteins and RNA. A highly coordinated mechanism for packaging DNA into the viral capsid has been proposed based on ensemble kinetics and single-molecule optical tweezer experiments. X-ray crystallography has provided atomic-level structures for some components of the motor, and lower resolution structures of the virus at different stages of genome packaging have been determined by cryoelectron microscopy. However, the most critical component of the motor, the homomeric ring ATPase that powers genome encapsidation, has only been crystallized as a monomer fragment. The full-length protein precipitates rapidly, making structural and biophysical analysis of the ring difficult. It has not been possible to visualize the functional ring to determine the molecular basis of inter-subunit coordination and DNA translocation. Homology searching of genetic databases produced a similar protein in phage ascc φ28. Analytical ultracentrifugation indicated the protein assembled as a decamer in solution. X-ray crystallography and cryo-electron microscope produced similar structures and showed that the decamer is arranged two pentameric rings related by D5 point group symmetry. EM images of packaging viruses show one of these five-membered rings is the functional form of the ATPase. This is the first atomic-resolution structure of the functional ring ATPase in a viral packaging motor, and provides insight into mechanisms for coordinating ATP hydrolysis and DNA translocation among the five ATPase subunits in the functional motor.

P-32

Allosteric Regulation of Glutamate Dehydrogenase

Zoe Hoffpauir, Hong Smith, and Dr. Thomas Smith

Glutamate dehydrogenase (GDH) catalyzes the oxidative deamination of Lglutamate to 2-oxoglutarate using NAD(P)+ as a coenzyme. In mammals, GDH is a critical branch point in multiple systems such as amino acid catabolism, insulin secretion, glial cell development, and tumor growth. Therefore, not surprisingly, GDH is heavily allosterically regulated by a wide range of metabolites. This complex allosteric regulation is made possible by a structural feature that is unique to mammalian GDH called the antenna, but the atomic details of this allosteric regulation is poorly understood. Because of the importance of GDH in these processes, it represents an important target for a wide range of diseases such as type II diabetes, hyperinsulinism/hyperammonemia syndrome (HHS), and cancer. We are combining structural studies with high throughput screening, not only to
P-33

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

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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. 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 post-translational modification through sequential proteolytic cleavages to form the mature infectious state. 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 nickel-NTA beads and size exclusion chromatography. Size-exclusion chromatography and transmission electron microscopy have indicated the capsid protein is forming two major states, a ~180 kD dimer and a rodshaped virus-like particle. The VP90 dimer was screened for the formation of protein crystals for use in X-ray crystallography, but no crystals have been observed. The formation of the rod-like oligomerization was unexpected, and is being investigated 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.

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P-34

Triple Resonance NMR backbone assignment of the $\alpha_x$ Integrin I domain

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The $\alpha_x\beta_2$ Integrin is a heterodimeric cell surface receptor exclusively expressed in leukocytes and plays a vital role in monocyte adhesion and atherosclerosis development. It functions in cellular trafficking, phagocytosis, and T-cell proliferation. Importantly, drugs targeted to $\alpha_x\beta_2$ could potentially have an anti-inflammatory effect without causing global immune suppression associated with steroids. The ligand binding domain of $\alpha_x\beta_2$, called the $\alpha X$ I-domain, transitions from closed/low affinity to open/high affinity states upon binding to a ligand. In order to design medications targeted to this protein and understand the mechanism of its transition between the open and closed conformational states, structural studies must be undertaken. Of
particular interest are structural studies of the ligand-binding αI-domain, which undergoes drastic conformational changes in the transition between open and closed states. While there are published crystal structures of the αI domain in the open and closed states, these structures fail to show how the transition between these two states occurs, and the flexibility of the domain. Nuclear Magnetic Resonance (NMR) studies can bridge this gap by allowing for probing molecular motions at the nanosecond timescale. Hence, as a preliminary project, we have conducted an NMR backbone assignment of the αI domain. The αX I-domain plasmids are transformed to *E. coli* Rosetta cells. 15N/13C/2H and 15N/13C labelled samples were purified by affinity and size exclusion chromatography. Differential Scanning Fluorimetry (DSF) was used to determine the NMR buffer in which the protein was most stable. Triple resonance experiments such as HNCACB, HNOCACB, HNCO, and HNCACO were used to assign residues in the HSQC spectra. 88.7% (165/186) were successfully assigned in the HSQC spectra.

**P-35**

**Universal Stress Protein 712 - Differential Scanning Flourimetry Analysis and Nuclear Magnetic Resonance Backbone Assignment of a Dimeric Protein.**

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The expression of Universal Stress Proteins in bacteria tend to increase during stress and are thought to be global regulators of protein expression. *Micrococcus luteus* genome encodes three Universal Stress Proteins; UspA-616, UspA-712, and UspA-184. Usp712 is a 12kD protein, known to be upregulated in the exponential growth phase of *Micrococcus luteus*. Usp712 knockout strains of *Micrococcus luteus* show an extended lag time in rich media compared to acetate minimal media. USP712 has been purified as a 24kD dimeric protein. Structural alignments have shown that it aligns with the C-terminal half of Usp616 of *Micrococcus luteus*. Differential scanning flourimetry & calorimetry experiments have been used to measure protein unfolding and melting temperatures thereby extracting the thermodynamic parameters of Usp712 protein unfolding. This method was used to extrapolate enthalpy and free energy values under increasing concentration of the Usp712 protein. It was found that increasing concentration of the Usp712 did not have any significant changes in melting temperatures, enthalpy and free energy values. As a preliminary experiment, HSQC spectra data have been obtained using 15N and triple labelled sample of our Usp712 protein which will be used for NMR backbone assignment. Future structural and dynamic studies will be carried out on this protein using varying concentrations of small molecules to identify interacting residues.
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Structural and Functional Characterization of Pheromone Binding Protein from *Ostrinia Furnacalis*
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Abstract:
*Ostrinia furnacalis*, an invasive agricultural pest, causes severe damage to economically important crops globally. Male insects have an extremely sensitive olfactory system that is capable of detecting sex pheromone emitted by females far away. The pheromone molecules are recognized by pheromone binding proteins (PBPs) present in male moth antennae. The PBP transports pheromone through aqueous lymph to sensory neuron initiating the mating process. Thus, mating and reproduction occur through this process of chemical communication in insects. However, the mechanistic detail of pheromone recognition, binding and release by the *O. furnacalis* PBP (OfurPBP) is not known. The current model for pheromone binding and release by PBPs of model lepidopteran species is that the PBP binds pheromone at high pH (of sensillar lymph) and releases it at the olfactory receptor neuron at low pH undergoing a dramatic conformational switch. The switch is controlled by two biological gates: histidine gate (His70 and His95) at one end of the hydrophobic pocket, and the C-terminus gate at the other end. In OfurPBP, the biological gates are quite different which may have an impact on the protein structure and function. The production of recombinant OfurPBP and its biophysical characterization with MALDITOF, circular dichroism (CD), fluorescence, small-angle X-ray scattering (SAXS), and NMR will be presented. Our pH titration studies with NMR indicate that OfurPBP does not release ligand at low pH suggesting a different mechanism of pheromone release for this insect pest.

P-37

Auto-inhibition as a Kinetic Booster of Target DNA Search by Proteins
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Regulation of genes requires transcription factors to be able to first locate their target sites in the genome. However, genomic DNA contains numerous sites that can strongly interact with proteins yet only a small fraction of these sites are functionally important. Nonfunctional high-affinity sites, which we call decoys, can sequester available proteins and prevent them from binding to functional targets. Our previous studies have shown that decoys can significantly impede target search processes of proteins. A major goal in our laboratory is to elucidate the mechanisms that allow proteins to efficiently locate functional target DNA. One possible mechanism for this could be by reducing the trapping effects of decoy DNA. We are testing a unique hypothesis whereby auto-inhibition of gene-regulatory proteins can allow acceleration of target DNA association. Auto-inhibition is a mechanism in which one region of a protein negatively regulates the function of another region, such as a DNA-binding domain. Although this may appear counterintuitive, protein auto-inhibition could actually accelerate the target DNA search by reducing the risk of proteins being trapped at decoy sites and this is supported by our kinetic simulation based on simple models. We use the nuclear DNA binding protein HMGB1 as a model system to examine the potential role of auto-inhibition as a kinetic booster of target search. This protein exhibits auto-inhibition through electrostatic interactions between the highly negatively charged C-terminal tail and the positively charged DNA-binding domains. We present some theoretical and experimental data suggesting that autoinhibition can accelerate target association in the presence of vast decoy DNA.
Completing the structure of a multicomponent viral genome packaging motor
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Double-stranded DNA viruses package their genomes into pre-assembled protein capsids, which is remarkable considering the various forces that resist compaction of DNA. Therefore, viruses must encode for highly efficient molecular motors that convert the energy released during ATP hydrolysis into DNA translocation. The packaging motor in bacteriophage phi29 is well-studied. Single molecule experiments show that it is one of the most powerful molecular motors in nature, and that it operates via a highly coordinated mechano-chemical cycle wherein 10-bp DNA translocation bursts are followed by dwells where no translocation occurs. In addition to the ATPase, the phi29 motor also consists of a portal protein, and a virally coded structural RNA (pRNA). The only motor component whose structure is currently unknown is the C-terminal domain of the ATPase (CTD). We thus determined the structure of the CTD by NMR spectroscopy. The structure is reminiscent of the RNase H-nuclease fold. While gp16 does not cleave its genome like other bacteriophages, this structural similarity suggests the CTD retained nuclease binding and translocating functions. Fitting the CTD into cryoEM density of the entire motor complex completes the first atomic structure of a functional DNA packaging motor. The CTD may simultaneously interact with DNA and pRNA via two clusters of basic residues on opposite sides of a central beta-sheet. Together with previous biochemical, biophysical, and single molecule results, this structure provides insight into how the activities of motor subunits are coordinated to translocate DNA, and how the motor transitions between burst and dwell phases.

Reduced Relaxation Matrix Analysis of STD NMR Initial Slopes Build-up Curves of Protein Ligand Complexes. Ridvan Nepravishtaa,b, Juan Carlos Muñoz Garciaa and Jesus Anguloa
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Saturation transfer difference NMR technique has revolutionized the study of the interaction of ligands with their receptors and structure determination of the complex. Its versatility and popularity is demonstrated by a myriad of approaches developed. As a general rule the intensities of the protons resonances of the ligand in the STD NMR spectra reflect the distance from the protein and form the so called “binding epitope”. In order to avoid artefacts due to T1 relaxation, kinetics of binding and extent of saturation the initial slopes of saturation build-up curves approach was introduced where the STD at time 0 is obtained from experimental data as: STD0 = ksat * STDmax. On the other hand the calculation of the STD NMR intensities from a structure for each proton of the ligand is of major interest in structure determination and evaluation of the ligand protein complexes in solution. Similarly to the initial slopes of build-up curves approach we purpose to calculate the STD0 instead of the STD intensity for each time point. This reduces the full relaxation and exchange matrix usually used for the calculation of the STD-NMR intensities to a reduced matrix $M$. $M$ is a general matrix term containing elements of both protein and ligand species in their bound and free form. In our approach the
STD0(calc) is equal to M. The results from our in house written python script show that the approach is valid and it can be used to calculate the STD0.

P-40

NMR-based investigation of counterions around DNA and their release upon protein-DNA association
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Due to a high density of negative charges on its surface, DNA attracts and condenses cations as counterions, which form the so-called ‘ion atmosphere’. Previously, the ion atmosphere around DNA was studied with small-angle X-ray scattering and ion-counting methods. However, there is a lack of experimental studies on dynamic aspects of the ion atmosphere. Moreover, although it has been considered that the release of counterions upon protein-DNA association could make a major contribution to the binding thermodynamics, this release remained to be confirmed though direct observation of ions. Here, we demonstrate that NMR diffusion-ordered spectroscopy allows us to characterize the ion atmosphere around DNA and to directly detect the release of counterions upon protein-DNA association. The NMR-based diffusion data reveal the highly dynamic nature of counterions within the ion atmosphere around DNA. Using the system of the Antp homeodomain and 15-bp DNA containing an Antp recognition sequence, we also show that our method accurately provides the number of counterions released upon protein-DNA association.

P-41

Towards Structural Models of Chemokine CXCL8 bound to CXCR1 and CXCR2 receptors of the GPCR class
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Chemokine CXCL8/IL-8 plays a crucial role in the pathophysiology of diverse human diseases from ARDS and sepsis to cancer. CXCL8 exists as monomers and dimers and exerts its function by activating the CXCR1 and CXCR2 receptors that belong to the GPCR class. CXCL8 monomers and dimers and CXCR1 and CXCR2 play nonredundant roles, as the affinity, activity, and physiological responses vary between monomer and dimer for each of the two receptors. Receptor interaction involves two distinct sites. The receptor N-terminal domain (Site-I) constitutes the initial docking site, and we propose that Site-I interactions trigger structural/dynamics changes both in the ligand and receptor that are essential for receptor activation, which occurs at a remote site located in the extracellular loops (Site-II). How Site-I and Site-II interactions determine selectivity and function are not known. Structure determination of the chemokine-receptor complexes is not possible due to
conformational plasticity and limitations of the current techniques. Therefore, we propose a hybrid approach, where we will solve the structures of Site-I complexes using solution NMR spectroscopy and characterize Site-II interactions by extended molecular dynamics (MD) simulations. We will staple the Site-I structure to the receptor structures, and characterize how CXCL8 bound at Site-I explores and binds Site-II residues. We discuss our current progress towards this goal. Our NMR studies have already shown that the Site-I interactions of the monomer for the two receptors are quite different and that structural plasticity mediates Site-I interactions.

P-42

Probing Stability of the Ligand Binding Site of Integrin αXβ2
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The migration of myeloid cells such as leukocytes contribute to inflammatory responses in many immune dysregulations including atherosclerosis, autoimmune encephalomyelitis, and arthritis. Recruitment of leukocytes from circulation to lesions is regulated by a heterodimeric cell surface receptor called integrin αXβ2. The significance of αXβ2 is illustrated by its mutation in leukocyte adhesion deficiency, a lethal disease. An extracellular domain of αXβ2, called the αX I-domain, is responsible for ligand binding— the phenomenon that initiates cellular signal transmission to the β2 subunit of αXβ2 and helps regulate αXβ2 activation. This research entails characterization of the αX I-domain via determination of its affinity to an antagonist, simvastatin, using Differential Scanning Fluorimetry (DSF) and Saturation Transfer Difference (STD)—concurrently showing their binding for the first time at molecular level. Along with the molecular dynamics and docking, we elucidate the fundamental interactions occurring between the Metal Ion Dependent Adhesion Site (MIDAS) of the αX I-domain and simvastatin. We characterized the binding mode of simvastatin, the first allosteric antagonist of the αX I-domain, which reveals the carboxylate moiety of simvastatin in hydroxy-form binds to the Mg2+ ion in MIDAS. This polar interaction is further buried with highly specific non-polar interactions. Furthermore, we observe that—despite the homology between the αX I-domain and its sister homolog aM I-domain —the key amino acid residues burying the carboxylate–Mg interactions differ. Thus, this observation proposes a potential for investigating ligands, such as small molecules or peptides, which could enhance or reduce selectivity and specificity for the αX I-domain.

P-43

Probing inter- and intramolecular translocation of HOXD9 in target DNA search by NMR paramagnetic relaxation enhancement
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Transcription factors (TFs) play a crucial role in gene regulations. To reach their target site, TFs first bind to
nonspecific sites stochastically and then move from one site to another. The target search process can be categorized into three main mechanisms: sliding, dissociation/reassociation and intersegment transfer. Translocations of proteins on DNA molecules between nonspecific sites provide a rapid mechanism for accelerating the target search processes in vivo. The intermediate states of a protein at different binding sites in fast exchanging systems can be detected by NMR paramagnetic relaxation enhancement (PRE). The observed intermolecular PRE at equilibrium are population weighted averages of the major and minor species, thereby permitting the extraction of structural information of the sparsely populated intermediates. In the current study, we utilized a discrete-state kinetic model in the analysis of PRE data for a sequence-specific TF, the homeodomain of HOXD9. In the presence of both the target DNA and competitors, translocations of the TF on DNA molecules result in unique PRE profiles depending on the location of the paramagnetic group centre. Using this approach, the inter- and intramolecular translocation of HOXD9 on DNA molecules are quantitatively characterized through collective analysis of PRE data at different conjugation sites. By incorporating microscopic kinetic states into the NMR master equation, our simulations are able to reproduce/predict experimental PRE profiles. This analysis is not only useful for gaining structural insights on protein-DNA complexes but also valuable for determining time scales of the target search mechanisms.

P-44

How different contractile injection systems solve their target cell membrane puncturing problem?

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Bacteriophages with contractile tails, R-type pyocins, type VI secretion system (T6SS) of Gram-negative bacteria, the Photorhabdus virulence cassette and the Serratia entomophila antifeeding prophage belong to the class of contractile injection systems (CIS) based on common ancestry. CIS class members use a specialized protein or protein complex to breach the target cell membrane. This complex termed as “the cell-puncturing device” was initially identified and characterized in phage T4. Later was discovered an identity of the small protein that stabilizes and sharpens the blunt end of the T4 gp5 beta-helix – gp5.4. Using bioinformatics we identified and structurally characterized a variety of tip proteins orthologous to gp5.4. These proteins belong to the superfamily of Proline-Alanine-Alanine-aRginine (PAAR)-repeat proteins that are characterized by a triplet of such repeats and share the same structural motif. A generic superfamily member is a monomeric protein that is folded into a triangular pyramid. The pyramid is formed by three loops of different length that wrap around each other. The protein is stabilized by a centrally positioned metal ion (iron or zinc), which is coordinated by three histidines and a cysteine originating from different loops. Metal ion coordinating residues are the only four amino acids that remain conserved among close gp5.4 orthologs. Distant gp5.4 orthologs like PVC PAAR do not have a metal binding site at all. Alternative approach to target cell membrane breaching is employed by phages like P2, phi92, OBP and SN. These phages employ a single trimeric protein that encompasses characteristics of all components comprising an above outlined cell-puncturing device. The b-helix of this type of central spikes is extended by an apex domain that is structurally similar to the PAAR tip proteins. Similar to PAAR tip proteins apex domain is also stabilized by an iron ion. However, due to the symmetric surrounding the metal binding site of the apex domain is composed by six histidines. These are organized into a double histidine motif HxH - two histidine residues separated by another residue type, which are contributed by every chain of the trimer, therefore creating an octahedral binding site. Despite different modality of the tip the architecture of the central spike proteins in all contractile systems is conserved. Structural conservation with low sequence identity as well as
the exceptional thermal stability in presence of denaturing agents suggest that these proteins interact with the host cell membrane non-specifically and most likely act as membrane-piercing drills.

P-45

**Oxidative Damage Diminishes Mitochondrial DNA Polymerase Fidelity**

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The human mitochondria contains it’s own circular genome, which carries the genes responsible for the electron transport chain. Maintenance and replication of this critical DNA is the function of Polymerase γ. Pol γ has both polymerase and exonuclease activity, allowing it to both extend nascent DNA and to excise errors. Deficiencies in these functions give rise to a diverse array of clinical phenotypes, as a result of dysfunction in the electron transport chain. The electron transport chain generates oxidative species, which can damage both DNA and enzyme. While oxidative damage to DNA has been extensively studied, the effects of this damage on enzymes are less well characterized. In the case of Pol γ, we have found that differences in the amino acid composition and solvent accessibility between the two active sites leads to a demonstrably higher inhibition of exonuclease activity as a result of local oxidative damage. This leads to decreased replication fidelity, which in turn will introduce mutations into the genome. We suggest that the mechanism by which oxidative stress induces DNA damage may be more intricate than convention states.

P-46

**Chloride ions play both a signaling and a structural role in the assembly of collagen IV scaffolds**

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Collagen IV is a major component of the basement membrane of metazoans. In mammals, this collagen is comprised of six homologous alpha chains, α1-α6, which assemble into three distinct triple helical protomers. Protomers oligomerize into a network by end to end association, forming a 7S domain, and head to head association, forming a NC1 hexamer. Recent studies revealed that Cl ions play a key role in the assembly of networks. Six Cl ions located at the trimer-trimer interface of the NC1 hexamer are associated with hexamer assembly. Yet, additional Cl ions may be involved. Here, we reevaluated the crystal structure of recombinant human α1α1α2 NC1 hexamer, solved at 1.9 Å, to determine the number of Cl ions. The findings reveal a total of 12 Cl ions are involved in hexamer assembly. Six Cl ions occur near the trimer-trimer interface and six at the interface. The former group initiates hexamer assembly by altering the trimer interface, which positions arginine residues for interacting with glutamate residues in opposing trimers. Finally, the latter group stabilizes hexamer by directly bridging the trimer-trimer interface. Our findings indicate that Cl ions play signaling and structural roles in the assembly of collagen IV scaffolds.
Developing a Therapeutic Option for MRSA via Directed Evolution
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Abstract:
One of the most widespread human pathogens, Methicillin-resistant Staphylococcus aureus (MRSA), contains the mecA gene that 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 are inhibited by protein-based inhibitors named β-lactamase inhibitory proteins (BLIPs) and BLIP-II inhibits class A β-lactamases with subnanomolar affinity. It was previously found that BLIP-II inhibits PBP2a in the low micromolar range (KD 1.5 µM), which is in contrast to BLIP-II’s potent inhibition of class A β-lactamases. This primarily is due to a ~44,000 times slower association rate of BLIP-II with PBP2a. Alanine scanning mutagenesis of BLIP-II revealed a majority of the mutations exhibited an increased association rate (ka) with PBP2a, while all mutations resulted in the normally fast dissociation rate (kd) to further increase. The alanine scan of BLIP-II identified binding hotspots for binding PBP2a and demonstrated that the binding interface between BLIP-II and PBP2a could be further optimized. A directed evolution approach with phage display affinity selection was used to identify a BLIP-II double mutation, N50A:Y113H, that enhanced the binding affinity to PBP2a 30-fold to a 50nM KD. An additional directed evolution cycle, starting with the tighter binding BLIP-II template, was then performed to drive the binding affinity down to low nanomolar range. To date, we have identified several mutations that have improved binding ~3-5-fold, while also identifying G205W and N246Y mutations that individually improved binding 50 -fold to a KD of ~1nM. These results suggest that BLIP-II can be further optimized and serve as a scaffold for developing potential PBP2a inhibitors. Funding by: NIH RO1 AI32956-25 to TP
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Interactions between the Zika Virus Polymerase NS5 and the RNA promoter, stem loop A (SLA)
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Zika virus has recently emerged as an important human pathogen which has spread to more than 60 countries. Infection with Zika virus in pregnant women might cause severe brain malformations in her child such as microcephaly or other birth defects. Despite the clear medical importance of Zika virus infection, the mechanism of viral replication, a process commonly targeted by antiviral therapeutics, is not well understood. Stem loop A (SLA) located in the 5’ untranslated region acts as a promoter for viral replication, and thus is critical for the viral polymerase NS5 to recognize the Zika genome. However, how NS5 engages SLA is not well understood. We have quantitatively examined the intrinsic affinities between Zika virus SLA and NS5 as well as its individual RNA-dependent RNA polymerase (RdRp) and methyltransferase (MTase) domains. Additionally, we identified the positively charged surface patches in NS5 that are involved in SLA binding. Substitutions in two areas, each in the RdRp and MTase domains show reduced SLA-binding affinity, indicating that they are part of the SLA-binding site. Furthermore, we performed kinetic analysis of Zika NS5-, RdRp- and MTase-SLA complex formation. This allowed us to identify the presence of intermediates during
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Cryo-trapping Crystal Studies of Photoreceptor PixJ Yield New Insights into its Photoconversion Mechanism
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Proteins are not the static models we create out of x-ray diffraction or cryo-EM data, but are dynamic, moving molecules that inhabit an ensemble of states and can change conformation to drive signaling cascades and catalyze reactions. To understand how these signaling cascades work at the atomic level, we are performing cryo-trapping experiments with crystals of the photosensing GAF domain from the blue/green-light photoreversible cyanobacterial photoreceptor PixJ. To observe intermediate structures along the photoconversion pathway, we are using two cryo-trapping techniques, temperature-scan cryo-crystallography and pump-quenching. The temperature-scan data shows small changes permitted at very low temperatures via Fo-Fo, which correspond to early intermediates during the photoconversion reaction, as PixJ transitions from its darkadapted Pb state to its activated Pg state. These smaller changes include the chromophore isomerization and reorientation in the binding pocket. Pump quench experiments allow us to observe larger changes and later intermediates that occur along the photoconversion pathway with time resolution, including binding pocket rearrangements. Together, these two techniques enable a more complete understanding of the photoconversion pathway as compared to studying the static Pb and Pg end point structures alone. Supported by NSF Science and Technology Center, BioXEL, Houston Area Molecular Biophysics Training Grant, and NIH. Data was collected at the Stanford Synchrotron Radiation Laboratory beamlines 12-2 and 9-2 and at the LS-CAT beamline 21-ID-D at the Advanced Photon Source.

P-50

Purification and Initial Characterization of a Hypothetical Membrane Protein
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Comparatively few structures of membrane proteins have been determined. Approximately 1% of structures in the protein database (PDB) belong to membrane proteins, despite the fact that 20-30% of predicted proteins and 50% of known drug targets being membrane proteins. This discrepancy is largely due to the difficulties presented by purifying and working with hydrophobic structures such as the transmembrane domains of membrane proteins. Previous data from our lab has identified a number proteins that are upregulated in the dormant state of Micrococcus luteus including a previously uncharacterized membraned protein (Hyp730). In this study we have used Ginzu and ROBETTA
server structure prediction, to computational predict the structure of Hyp730 based. We also identified a method of purifying Hyp730 using a mono Q anion exchange column. Once the protein was purified, we used CD spectroscopy to identify the secondary structure composition of Hyp730. The CD data corroborates our predicted structure, though further experiments will still be required fully elucidate the structure.

P-51

An atomic fulcrum for balancing fidelity and proofreading in mitochondrial DNA polymerase
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Human mitochondrial DNA polymerase γ (Polyγ) is responsible for both replication and repair of mitochondrial DNA. Consequently, mutations that affect the activity of Polyγ can have profound effects on human health. Patients with progressive external ophthalmoplegia with mitochondrial DNA deletions-1 (PEOB1) have mutated forms of Polyγ: R853W and R853Q. Polyγ contains polymerase and exonuclease active sites for DNA synthesis and proofreading, respectively. However, R853 does not directly contact the DNA and is not part of either active site. Despite this, we found mutations at this amino acid significantly reduce or completely abolish DNA synthesis activity of Polyγ, while the exonuclease activity remains intact. In order to gain insight into how these mutations exhibit the biased effect on Polyγ activities, we determined crystal structures of the R853A mutant and assayed the activities of the clinical mutants. The R853 mutants obliterate the structural stabilization for the polymerase catalytic core, hence significantly reducing the DNA synthesis ability of Polyγ. Our results suggest that residue R853 serves as a molecular switch between the polymerase and exonuclease active sites to balance DNA synthesis and error correction.

P-52

Effects of Inhibition of the Catalytic Domain of Histone Lysine Demethylase KDM5
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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-b 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.


P-53

Structures of flavivirus SLA suggest coevolution with viral polymerase NS5

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Flaviviruses are positive-strand RNA viruses that are globally emerging and cause significant human disease. They include important human pathogens such as Dengue, Zika, West Nile, Yellow Fever, and Japanese Encephalitis viruses. For example, Zika virus infection is associated with Guillain-Barré syndrome in adults and microcephaly in fetus during pregnancy. Flavivirus genome contains a stem loop A (SLA) at the 5’-end that functions as a promoter for viral genome replication and as a substrate for cap methylations. The predicted ‘Y’-shaped secondary structure of SLA is conserved in many flaviviruses, suggesting that flavivirus uses the common SLA-mediated RNA synthesis mechanism. We determined the first crystal structures of flavivirus SLAs from Zika and dengue virus. Both Zika and dengue virus SLAs, consisting of a top stem loop, a side loop, and a bottom stem, form a unique intermolecular three-way junction structure via side loop interactions with a neighboring SLA molecule. Zika and dengue SLAs differ in their relative orientations of the top stem loop to the bottom stem. In the NS5-SLA complex model based on mutational studies of NS5 proteins, the structural difference in SLAs correlates with the difference in the domain arrangement in viral polymerase NS5. Our results suggest that flavivirus SLA and NS5 polymerase may have coevolved to optimize their interactions during viral replication.

P-54
Targeting the *Leishmania* Hsp100 N Domain to Prevent Infective Parasite Stage Differentiation

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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.

P-55

Direct Phasing of Protein Structures with High Solvent Contents

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The phase problem in X-ray crystallography is a fundamental problem where the phases required to obtain an image of the electron density in the crystal are not directly recorded with the diffraction intensities and must be deduced by other means. Traditionally, in protein crystallography, the phases have come from MIR, MAD/SAD (where differences in intensity due to a subset of added or natively present heavier or anomalously scattering atoms are exploited) or molecular replacement (which requires a similar known structure be placed in the cell to obtain starting phases). While these techniques have been very successful, the phasing of new structures without sufficiently close homologs and for cases where it can be difficult to obtain heavy atom or anomalous scattering substitutions remains a problem. In the case of crystals with high solvent content, there has been progress using iterative transform phasing algorithms that have been developed in the fields of astronomy, coherent diffraction imaging and transmission electron microscopy. Several groups have had success starting from a low resolution protein masks. He & Su (2015, Acta Crystallogr. A71:92) reported the successful de novo phasing of a couple of structures with high solvent content using a hybrid input-output algorithm combined with a dynamically adjusted protein mask. Here we report on some of our on-going trials with this method and prospects to extend it to lower solvent contents.

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Study of a Heteromeric Kainate Receptor Gluk2/K5 by Probing Single-Molecule FRET

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Kainate receptors are ligand-gated ion channels that belong to the ionotropic glutamate receptor family. They are unique among glutamate receptors in being involved in both excitatory and inhibitory synaptic transmission. These receptors are homomeric and heteromeric assemblies of subunits GluK1-5 arranged as dimer of dimers. Each subunit consists of an extracellular aminoterminal domain, a ligand binding domain, a transmembrane domain and an intracellular Cterminal domain. Despite heteromeric GluK2/K5 receptors being the most predominantly expressed kainate receptor in the brain, there are currently no structures of the full length GluK2/K5. We have used smFRET measurements to determine the specific organization of the GluK2/GluK5 within the tetramer and additionally studied the structural changes associated with agonist binding, activation, and desensitization of the receptor. These results of the heteromeric receptors are compared to the homomeric receptors for which there are full length structures available.

P-57

The Role of MicroRNA-122 Binding to the 5’ Stem-loop 1 of Hepatitis C Virus in Promoting Viral Replication

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Hepatitis C virus (HCV) is an important human pathogen that is capable of persistent infections in hepatocytes. Maintenance of the persistent infection requires precise control over protein translation and RNA synthesis. Successful HCV replication is dependent on many host factors; one of importance, microRNA-122 (miR-122) has been found to interact with stem-loop 1 (SLI) of the 5’ untranslated region (UTR) of HCV. MiR-122 has been shown to promote HCV RNA synthesis although the mechanism is poorly understood. The HCV 5’ UTR contains two miR-122 binding sites near the first stem-loop (SLI). ITC data was used to characterize these binding sites and understand how the difference in thermodynamic properties of the two sites can regulate the levels of HCV RNA synthesis. The results indicate that the miR-122 binding sites are unequal, allowing for non-binary regulation by miR-122. Variants of the 5’ HCV UTR were also used in binding experiments to dissect the contributions of each site. Additionally, this work has helped design future endeavors to solve the structure of the HCV SLI bound to miR-122.

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Overuse of antibiotics has contributed to the evolution of multi-drug resistance. Vancomycin resistant enterococci (VRE) cause 20,000 infections in the United States each year and are an increasing concern for the biomedical community. DAP is a bactericidal cyclic lipopeptide antibiotic approved in 2003 that is used widely as a “rescue” drug against MDR Gram-positive organisms such as *Staphylococcus aureus*, *E. faecium* and *E. faecalis*. However, DAP resistant VRE have been reported clinically. Previous experimental evolution studies from our lab have suggested that DAP resistance in *E. faecium* in the gene *murAA* played a role in resistance when the common LiaFSR stress response pathway has been abrogated. MurAA is an enolpyruvate transferase that catalyzes the first committed step of peptidoglycan synthesis, transferring enolpyruvate from phosphoenolpyruvate (PEP) to UDP-N-acetylglucosamine (UNAG) to produce UDP-GlcNAc-enolpyruvate. Fosfomycin (FFQ) is a naturally occurring inhibitor that competes with PEP to bind to the active site of MurAA. We have solved the structure of MurAA in complex with both inhibitor, FFQ, and substrate, UNAG. The active site loop (Ala114-Ile126) adopts a closed conformation and the active site Cys119 is occupied by FFQ. Alternatively, the active site loop maintains a half-open state when the active site is covalently attached to the ligand in the absence of UNAG shown by the MurAA binary complex structure. Our enzymatic studies show that MurAA<sub>A149E</sub> is moderately less efficient than MurAA<sub>WT</sub>. The results suggest that MurAA<sub>A149E</sub> may modulate the cell wall composition. The effect of mutation on cell wall phenotype will be further tested.