SEPTEMBER 15, 2022

ECSI 2022

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POSTDOCTORAL PROGRAM

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SCHEDULE

CHECK-IN & POSTER SETUP **COFFEE & CONTINENTAL BREAKFAST**	**8:30 AM – 9:00 AM**
MORNING SESSION	
WELCOME & INTRODUCTION	**9:00 AM – 9:10 AM**
SESSION #1 **POSTDOC & GRADUATE STUDENT TALKS** 12 MINUTE TALKS + 3 MINUTE Q&A	**9:15 AM – 10:30 AM**
COFFEE BREAK	**10:30 AM – 10:50 AM**
KEYNOTE SPEAKER **SUSAN BUCHANAN, NIDDK, NIH** 45-50 MINUTE TALK + 10-15 MINUTE Q&A	**11:00 AM – 12:00 PM**
LUNCH BREAK	**12:00 PM – 1:00 PM**

SCHEDULE

AFTERNOON SESSION

POSTER SESSION	**1:15 PM – 2:00 PM**
SESSION #2 **POSTDOC AND GRADUATE STUDENT TALKS** 12 MINUTE TALKS + 3 MINUTE Q&A	**2:15 PM – 3:45 PM**
CAREER SESSION YANXIN LIU, UNIVERSITY OF MARYLAND/IBBR JAMES STEINHARDT, ASTRAZENECA	**4:00 PM - 4:45 PM**
CLOSING REMARKS	**4:45 PM – 5:00 PM**
NETWORKING RECEPTION	**5:00 PM – 6:00 PM**

KEYNOTE SPEAKER

SUSAN BUCHANAN

Susan is Chief of the Section on Structural Biology of Membrane Proteins in the National Institute for Diabetes and Digestive and Kidney Diseases, at the National Institutes of Health. She received her Ph.D. from the Johann-Wolfgang-Goethe Universität in Frankfurt, Germany in 1990. She completed postdoctoral fellowships at the MRC Laboratory of Molecular Biology, Cambridge, UK, and at UT Southwestern Medical Center, Dallas, before returning to the UK to establish a research group at Birkbeck College, London in 1998. She joined the NIDDK as a tenure track investigator in 2001 and is currently a Senior Investigator, Deputy Scientific Director, and Chief of the Laboratory of Molecular Biology, NIDDK.

KEYNOTE SPEAKER ABSTRACT

SUSAN BUCHANAN, NIDDK, NIH, "Structural insight into the biogenesis of beta barrel membrane proteins"

β-barrel membrane proteins are essential for nutrient import, signaling, motility, and survival. In Gram-negative bacteria, the β-barrel assembly machinery (BAM) complex is responsible for the biogenesis of β-barrel membrane proteins, with homologous complexes found in mitochondria and chloroplasts. Structures of BamA, the central and essential component of the BAM complex, were determined from two species of bacteria: Neisseria gonorrhoeae and Haemophilus ducreyi. BamA consists of a large periplasmic domain attached to a 16-strand transmembrane β-barrel domain. Three structural features speak to the mechanism by which BamA catalyzes β-barrel assembly. The first is that the interior cavity is accessible in one BamA structure and conformationally closed in the other. Second, an exterior rim of the β-barrel has a distinctly narrowed hydrophobic surface, locally destabilizing the outer membrane. And third, the β-barrel can undergo lateral opening, evocatively suggesting a route from the interior cavity in BamA into the outer membrane. Recent structures of the BAM complex illustrate how the BamC, BamD, and BamE lipoproteins assemble on the BamA periplasmic domain and provide further evidence for lateral opening of the β-barrel.

Mitochondria are essential eukaryotic organelles and play a vital role in many cellular processes, including ATP production, lipid synthesis, and apoptosis. The majority of the mitochondrial proteome is translated in the cytosol and imported into the mitochondria as unfolded precursors. Outer membrane β-barrel proteins are imported by the translocase of the outer membrane (TOM complex) then folded and inserted into the membrane by the sorting and assembly machinery (SAM complex). The SAM complex is composed of three subunits: a β-barrel core (Sam50) that spans the mitochondrial outer membrane, and two accessory subunits (Sam35 and Sam37) that associate on the cytosolic side of the membrane. We recently solved cryoEM structures of the SAM complex from Thermothelomyces thermophilus in detergent and lipid nanodiscs to resolutions of 3.0Å and 3.4Å, respectively. These structures confirm that Sam50 is a sixteen strand β-barrel and that Sam35 and Sam37 are located on the cytosolic side of the membrane. Similarities and differences for the bacterial and mitochondrial folding machineries will be discussed.

ABSTRACTS - ORAL PRESENTATIONS

RILEY METCALFE, University of Melbourne, "Structures of the interleukin 11 signaling complex reveal dynamics of gp130 extracellular domains and the inhibitory mechanism of a cytokine variant"

The cytokine interleukin (IL)-11 is a validated therapeutic target in several diseases, most notably in solid cancers and fibrotic complications. Despite this therapeutic relevance, a structural understanding of IL-11 and the mechanism of action of existing inhibitors is lacking. IL-11 is an IL-6 family cytokine member that signals through the shared cell-surface receptor glycoprotein (gp) 130. Our laboratory has previously solved the structure of IL-11 and IL-11Rα, which has provided insights into the mechanism of cytokine engagement by IL-11Rα. We have solved both the cryoEM and crystal structures of the ~170 kDa hexameric IL-11 signalling complex. The complex has 2:2:2 stoichiometry consisting of two copies each of IL-11, IL-11Rα the shared receptor gp130, and is formed by ten coupled binding interfaces. Our structure allows us to map the interfaces in the complex in detail and reveal mechanistic differences in receptor engagement between IL-11 and other cytokines, particularly in the engagement of the shared receptor gp130. These structural insights are complemented by extensive biophysical analysis, which describe the thermodynamic mechanisms underpinning complex formation. We additionally demonstrate the structural basis of inhibition by a lead IL-11 signalling inhibitor, 'IL-11 Mutein'. We show that structural shifts in IL-11 Mutein underlie inhibition by altering cytokine binding interactions at all three receptor-engaging sites and abrogating the final gp130 binding step, which has implications for the development of novel inhibitors for IL-11 signalling and IL-6 cytokine family signalling more broadly. Our results reveal the structural basis of IL-11 signalling, define the molecular mechanisms of an inhibitor, and advance understanding of gp130-containing receptor complexes, with applications in therapeutic development.

ABSTRACTS - ORAL PRESENTATIONS

DANIEL BIRTLES, University of Maryland, "SARS-CoV-2 contains A Structurally Unique Fusion Domain that Preferentially Initiates Fusion Within the Endocytic Pathway"

Since the discovery of SARS-CoV-2 in late 2019 over half a billion people have been infected worldwide and no single viral component has been more heavily investigated than the spike glycoprotein. Despite extensive research, one area of interest that remains poorly understood is that of membrane fusion, the process by which the virus delivers its genetic information into the target cell. Viral glycoproteins contain a short stretch of highly conserved and predominantly hydrophobic residues known as the fusion domain (FD), which is widely accepted as the initiator of fusion. We found that the FD of SARS-CoV-2 is unique as it consists of two structurally distinct domains, an N-terminal fusion peptide (FP: S816-G838) and an internal fusion loop (FL: D839-F855). The helix-turn-helix motif within the FP is the primary site of membrane interaction yet requires the disulfide bonded FL, which contains no discernible secondary structure to initiate fusion. Furthermore, as SARS-CoV-2 can utilize both plasma and endosomal membrane pathways, we investigated how pH may impact the FD. We discovered that in a low pH environment (pH5.0) the FD undergoes a minor conformational change that results in a dramatic increase in fusogenicity. In conclusion, our findings suggest that the novel FD of SARS-CoV-2 preferentially fuses at the low pH environment found in the endosomal pathway.

ABSTRACTS - ORAL PRESENTATIONS

JINGYU ZHAN, Laboratory of Cell Biology, NIH-NCI, "How to Transport Folded Protein Across the Membrane Bcs1: Concerted or Sequential"

AAA (ATPases Associated with diverse cellular Activities) proteins are ubiquitously present in all kingdoms of life to participate in diverse cellular processes, such as DNA replication, membrane fusion, and protein homeostasis. Canonical AAA proteins share a highly conserved ATP-binding domain, usually forming a hexametric ring with a central pore which engages with protein or DNA substrates via the conserved pore loops, usually unfolding the protein or unwinding the DNA substrates. Through sequential ATP-hydrolysis powered conformational change of each protomer around the ring, the AAA ring operates in a spiral staircase fashion moving along the peptide/DNA string, resulting in stepwise substrate translocation.
Bcs1, bc1 synthesis protein 1, is a AAA protein anchored to the mitochondrial inner membrane that facilitates the assembly of the respiratory Complex III, also known as the cyt bc1 complex, by translocating the fully assembled iron-sulfur protein subunit (ISP) across the mitochondrial inner membrane. Recent Cryo-EM and X-ray structures of mouse and yeast Bcs1 in different nucleotide states (Apo, ATPgS and ADP) revealed that, unlike typical AAA proteins, Bcs1 forms a homo-heptameric ring that lacks the conserved pore loop. The Apo/ADP bound Bcs1 ring displayed a central cavity opening towards the mitochondrial matrix, which is sufficiently large to accommodate the fully folded ISP. This cavity collapsed to one-third the size when Bcs1 were uniformly bound with ATPgS and could no longer accommodate folded ISP, thus the ATPgS-bound state were deemed as the post-substrate translocation state.

Although the ISP translocation cycle can be speculated from the Bcs1 conformations in three different nucleotide states, mechanistic details remain elusive: whether ATP-hydrolysis coupled conformational change of Bcs1 is sequential or concerted when translocating ISP remains contentious. To investigate this, we captured Bcs1 conformations during its active translocatiobenjamn cycle by incubating Bcs1 with ATP in the presence or absence of substrate for a given time period before freezing sample and analyzing using Cryo-EM. Our results show that heptameric Bcs1 transits uniformly between ATP and ADP conformations with no co-existing nucleotide states, which strongly suggest that Bcs1 acts in a concerted mechanism.

ABSTRACTS - ORAL PRESENTATIONS

SAIF YASIN, UMBC, Howard Hughes Medical Institute, "Monitoring HIV-1 Genome Dimerization: Development of NMR and FRET Methods to Study Dimeric RNA Assemblies"

Like nearly all retroviruses, HIV-1 selectively packages two copies of its full-length genome, a requirement for strand-transfer mediated recombination during reverse transcription. Genome dimerization is not only essential for selective packaging, but also critical in modulating translation, splicing, and reverse transcription. Current studies support a mechanism mediated by the untranslated region of the genome (5'-Leader), involving an initial loop-loop interaction that triggers dimerization leading to a “kissing” conformation. Subsequently, extensive intermolecular base pairing are proposed to form to stabilize the dimer in an extended conformation. Questions remain regarding the mechanism of this process and the impact the structural rearrangement has on replication.

We have developed NMR and FRET based approaches to allow discrimination of kissing and extended conformers both in vitro and in vivo. Using 2H-edited NMR we can directly probe for intermolecular interactions in the full-length, dimeric HIV-1 5′-leader (>230 kDa), and have measured relative populations of kissing and extended conformers for two HIV-1 strains. Our FRET-based approach relies upon molecular beacons designed such that FRET transfer efficiencies will help differentiate between the formation of kissing and extended dimers. We have validated our ability to accurately quantify these proportions in vitro, and are comparing this to the populations present in cells and virus.

We are applying these tools to investigate how dimeric structure varies in the presence of sequence variation, RNA trafficking, and viral maturation. Our model system should elucidate the importance of dimer structure to subsequent processes including packaging efficiency, capsid maturation, and reverse transcription. The approaches developed here should also be generally applicable for characterization of RNA structure.

ABSTRACTS - ORAL PRESENTATIONS

ADAM CATCHING, NIAID, NIH, "A Tradeoff between Enterovirus A71 Particle Stability and Cell Entry"

Virus capsids protect the viral genome from the harsh extracellular environment while allowing for the release of the viral genome when in the presence of tuned cell entry factors. The trade-off between stability and efficiency of cell entry is balanced to maximize the number of surviving viral particles between hosts while allowing for the release of the genome in susceptible cells. In this study we genetically perturb this balance in a non-enveloped virus, enterovirus A71. We isolated a single-point mutation variant with increased particle thermotolerance and a decrease in cell entry efficiency. Using cryo-electron microscopy and molecular dynamics simulations we determined that the thermostable native particles have acquired an expanded conformation with a significant increase in protein dynamics. Particle heat-treatment uncovered several uncoating intermediate states resolved for the first time, suggesting an entry pathway, whereas the lipid pocket factor is first released, followed by internal VP4, and finally the viral RNA.

ABSTRACTS - ORAL PRESENTATIONS

AISHWARYA IYER, University of Maryland, School of Medicine, "Investigating the Molecular Pathogenesis of a Novel MYBPC1 Duplication Mutation Linked to Myopathy with Tremor"

Our group identified a novel Leu266Lys267Arg268 (LKR) duplication in the MYBPC1 gene encoding the slow skeletal myosin binding protein-C (sMyBP-C), a critical sarcomeric protein that plays key structural and regulatory roles in striated muscle contraction. Starting in infancy, the resulting clinical phenotype is associated with progressive generalized muscle weakness, skeletal deformities, dysmorphia, and a unique myogenic tremor. The molecular mechanism underlying these pathological manifestations is elusive, and currently no therapeutics exist for this emerging sarcomeric myopathy. I intend to characterize the LKR duplication on a molecular level and delineate the structural and functional alterations that it elicits. Interestingly, the LKR duplication localizes to the sMyBP-C N-terminus, specifically the highly conserved M-motif region responsible for dynamic interactions with myosin S2Δ and actin to regulate crossbridge cycling, the essential mediator of muscle contraction. I therefore hypothesize that the duplicated LKR residues alter the biochemical properties of the M-motif, disrupting the sMyBP-C N-terminus structure and function. Molecular dynamics simulations, validated by circular dichroism experiments, suggest that the LKR duplication stabilizes the terminal α-helix present in the M-motif. 2D-1H-1H nuclear magnetic resonance spectroscopy experiments of the M-motif indicate major changes in proton electronic environment and structure in the presence of the mutation. Additionally, isothermal titration calorimetry experiments demonstrate significant strengthening of the M-motif–myosin S2Δ binding affinity from a micromolar to low nanomolar range due to the LKR duplication. In vitro motility assays reveal that the mutant M-motif uniquely modulates crossbridge formation and actin movement by dampening actin velocity. Through in-silico and in vitro assays, we have shown, for the first time, that this mutation alters the folding of the skeletal M-motif region and augments myosin binding. Future biophysical and structural studies will illuminate the molecular and functional basis of this novel myopathy, ultimately aiding in the development of targeted therapeutics.

ABSTRACTS - ORAL PRESENTATIONS

MUDDASSAR IQBAL, University of Maryland, Baltimore, "Galectin-3 disrupts the airway epithelial integrity during influenza A virus infection"

Influenza A virus (IAV) infects the airway and alveolar epithelia, and in severe cases causes acute respiratory distress syndrome (ARDS) resulting from increased alveolar permeability due to the disruption of cell-cell tight junctions. The detailed mechanisms involved, however, remain elusive. Galectin-3 (Gal3) is a member of a β-galactose-binding lectin family (galectins), which owing to its unique structure can oligomerize at cell surfaces to cluster receptors, modulating cell signaling that is implicated in diverse cellular functions. In previous studies on a mouse model, we showed that IAV infection enhances Gal3 secretion in the bronchoalveolar fluid. In the present study, we investigated in vitro the potential role of Gal3 in airway epithelial integrity during IAV infection. Our results indicate that at 8 hours post-infection, the surface of the airway epithelial cell line A549 is significantly de-sialylated, exposing the sub-terminal β-galactose ligands, with an increase in the binding of recombinant Gal3 (rGal3). We detected the potential Gal3 receptors β1-integrin, CD147 and MUC1 on the surface of A549 cells, and observed an increase in the secretion of matrix metalloproteinases MMP2 and MMP9 upon exposure of these cells to rGal3. Lastly, exposure of A549 cell monolayer to rGal3 disrupted the surface distribution of tight junction proteins occludin and ZO-1, while also enhancing the intercellular permeability. Therefore, we propose that during IAV infection, Gal3 secreted into the airway causes disruption of cell-cell tight junctions, and an increase in alveolar permeability to cause ARDS. Our current studies are aimed at investigating the genes differentially expressed, and signaling pathways modulated by the enhanced binding of Gal3 to the de-sialylated receptors on the A549 cell surface during IAV infection, that lead to these above cellular effects.

ABSTRACTS - ORAL PRESENTATIONS

JULIANA MARTINEZ FIESCO, NCI, NIH, "Structural insights into the BRAF monomer-to-dimer transition mediated by RAS binding"

The RAF kinases are key intermediates in the RAS pathway, functioning in the transmission of signals that regulate cell proliferation, differentiation, and survival. Under most signaling conditions binding to activated RAS is required for RAF dimerization and activation; however, the structural details for how RAS binding allows autoinhibited RAF monomers to assume an active dimer conformation has remained unclear. We determined cryo-electron microscopy structures of full-length BRAF complexes obtained from mammalian cells: autoinhibited, monomeric BRAF:14-3-32:MEK and BRAF:14-3-32 complexes, and an inhibitor-bound, dimeric BRAF2:14-3-32 complex. The autoinhibited BRAF structures reveal the structural determinants for BRAF inhibition. They also show that prior to signaling events, BRAF and its substrate MEK can exist as a preassembled BRAF:14-3-32:MEK complex; however, the interaction with MEK is not required for BRAF to maintain the autoinhibited conformation. In the dimeric BRAF2:14-3-32 complex, both BRAF protomers are bound to an ATP-competitive BRAF inhibitor and the kinase domains assume the active conformation, suggesting the active sites of both protomers may bind ATP simultaneously to promote catalysis. In both autoinhibited, monomeric structures, the RAS binding domain (RBD) of BRAF is resolved, revealing the position and orientation of this critical domain and providing insights regarding how RAS binding facilitates the BRAF monomer to dimer transition.

ABSTRACTS - ORAL PRESENTATIONS

DAVID BLOODGOOD, Xiao Lab - University of Maryland, College Park, IBBR, **"Understanding Why Plant GPCR-like Mildew Locus O (MLO) Proteins are Essential for Pathogenesis of Powdery Mildew"**

Powdery mildew (PM) fungi are obligate biotrophic pathogens that strictly require living hosts to survive and thrive. Loss of one or several Mildew Locus O (MLO) genes in host plants leads to complete suppression of PM pathogenesis. Results from our recent genetic analyses indicate that three Arabidopsis MLOs (MLO2/6/12) are essential host susceptibility factors of PM fungi in Arabidopsis. Consistent with this notion and early findings, we observed MLO2-GFP's focal accumulation at the fungal penetration site. However, why PM fungi require host MLO proteins for pathogenesis remains unknown. To determine if the type-I C-terminal calmodulin-binding domain of MLO2 specifies its focal accumulation and role in PM pathogenesis, we performed domain swapping between MLO1 (which contains a type-II calmodulin-binding domain and is localized to the plasma membrane but not involved in fungal pathogenesis) and MLO2 and found that stable Arabidopsis transgenic plants expressing MLO2Ct-MLO1 showed plasma membrane localization similar to MLO1 and enabled fungal growth. This result confirmed the role of the C-terminal calmodulin-binding domain of MLO2 in polarized trafficking. To identify working partners of MLO2, we used a multiplex CRISPR system to target several candidate gene families involved in calcium signaling or calcium-regulated processes. Excitingly, we found that loss of function mutations in multiple Synaptotagmins (SYTs) abolished MLO2-GFP's focal accumulation and fungal pathogenesis, suggesting that MLO2 may be involved in SYT-assisted membrane fusion and sealing at the fungal penetration site, which is essential for fungal invasion. More plants expressing chimeric MLO genes or multiplex CRISPR DNA constructs targeting other gene families are being generated and will be used to further elucidate the molecular functions of MLOs in plant development and fungal pathogenesis.

ABSTRACTS - ORAL PRESENTATIONS

ROISIN DONNELLY, University of Delaware, "Studying Intrinsic Dynamics of the monoclonal antibody with SANS and NSE"

Hydrogen deuterium exchange, (HDX) is of increasing interest for characterization of protein dynamics in solution, which can inform therapeutic stability and efficacy. Due to very large difference of the neutron scattering cross section between H and D, small angle neutron scattering (SANS), is a very sensitive technique to study the HDX in solution. SANS allows for the continual measurement of HDX over time and is a non-invasive technique to investigate the HDX in solution. By using SANS to study temperature, we probed the HDX of monoclonal antibody, NISTmAb RM 8670, over the course of a couple of days. Understanding the dynamics of NISTmAb in solution is also possible through technique, neutron spin echo, which can be used to deduce molecular translational, rotational, and internal dynamics. The aim of this work is to explore the correlation between HDX, (SANS), and dynamics (NSE). The detailed analysis of both SANS and NSE data will be discussed. The exchange kinetics and the spatial distribution of exchangeable protons are determined using SANS. And the effect of the sample conditions, such as the temperature and buffer conditions, on the HDX and dynamics will be presented.

ABSTRACTS - ORAL PRESENTATIONS

DINENDRA ABEYAWARDHANE, University of Maryland, School of Medicine, IBBR, **"Calcium-mediated Pore Formation by Clostridioides difficile Binary Toxin"**

Clostridioides difficile infection (CDI) is a high-risk nosocomial disease prevalent in immunocompromised patients exposed to long-term antibiotic treatments and/or cancer therapy. C. difficile bacterial invasion reduces symbiotic gut microbiota causing severe diarrhea and pseudomembranous colitis in patients. Clinical solutions of this healthcare-related threat is in demand due to hypervirulence and recurrence. Although therapeutic developments targeting the large clostridial toxins produced by C. difficile are available, antitoxins for the C. difficile transferase (CDT) binary toxin are not identified. CDT consists of an enzymatic component (CDTa), and a pore-forming binding component (CDTb). Cytotoxicity of the host cell is initiated by the formation of the CDTa-CDTb binary toxin complex and subsequent cell entry of CDTa triggered by the conformational shift in the CDTb, and results in the destruction of the host cell cytoskeleton through ADP-ribosylation of the actin filaments.

Molecular structure of the CDTb has been determined and the functional unit of the translocase is a heptameric assembly which in solution forms a further di-heptamer assembly. Unlike it's homologues, CDTb includes two C-terminal receptor binding domains (RBD1/RBD2). RBD1 contains a calcium binding site, which is important for the stability of CDTb. We constructed a double mutant (D623/734A) targeting the Ca2+ binding site in RBD1. CryoEM analysis of CDTb D623/734A revealed a drastic structural transition from the di-heptamer to a single heptamer with an extended β-barrel assembly. Removal of RBD1-Ca2+ increased the flexibility of both RBDs and therefore we investigated the structural changes of WT CDTb after depletion of Ca2+ in the system by dialysis. Two distinct classes of single heptamer pore states are detected and the key difference was the degree of RBD2 flexibility. Since RBD2 is essential for receptor binding, these structures could be intermediate states in the transition from prepore to pore state. To understand the toxin mechanism, we monitored the pore formation at the lipid bilayer by different CDTb variants and Ca2+-depleted WT CDTb inserted pores by increasing the membrane fluidity.

CDTb pore formation was expected to be induced by acidic pH in endosomes as similar to membrane insertion mechanism of related anthrax toxin. However, experimental evidence is lacking to support this hypothesis. Coincidently, Ca2+ concentration in endosomes is decreased along with pH. We propose that low Ca2+ drives β-barrel extension of CDTb, transitioning the prepore to pore state. While systematic Ca2+ depletion and subsequent instability of RBD1 can lead to channel formation, low pH may influence the CDTa translocation. Our discovery of unraveling the distinctive conformational features in the mechanistic pathway of CDT can be beneficial in fine-tuning potential therapeutic agents.

ABSTRACTS - POSTERS

ADIT ALREJA, University of Maryland, IBBR, "An investigation on the molecular basis for dimerization of Streptococcus pneumoniae endolysin Cpl-1 for antimicrobial applications"

Streptococcus pneumoniae is the most common cause of community-acquired bacterial pneumonia. Despite vaccines, pneumococcal disease is still a problem, and compounding the issue, antibiotic resistant strains have risen in the last 20 years. Endolysins are bacteriophage-encoded peptidoglycan hydrolases and are an attractive alternative to antibiotics. Cpl-1 is one of the most active endolysins targeting S. pneumoniae and this study dissects the molecular mechanisms governing dimerization of Cpl-1 in the presence of choline. Analytical gel filtration on a Superose 12 column and analytical ultracentrifugation were conducted in the presence or absence of choline. Antimicrobial bacteriolytic/bactericidal activity was quantified by means of turbidity reduction assays and log-fold killing assays. We have found the F328 residue critical in dimer formation in the presence of choline. This mutant also displayed less bacteriolytic activity compared to wild-type Cpl-1. Furthermore, choline titration experiments suggest an apparent KD for wild-type Cpl-1 of ~5 mM. We have also shown, for the first time, dimerization in phosphocholine, the native form of choline on the pneumococcal cell surface. Finally, we identify a consensus sequence (FXXEPDGLIT) and show that choline-dependent dimerization is a wide-spread phenomenon occurring in many novel pneumococcal endolysins.

ABSTRACTS - POSTERS

SEONGMIN KIM, National Institute of Standards and Technology, “High-Sensitivity Mid Infrared Absorption Spectroscopy for Proteins in Aqueous Solutions”

Infrared absorption spectroscopy is a powerful analytical tool to identify and quantify primary biochemical structures and elucidate their higher-order structures. However, in the conventional approaches, the strong IR absorption of water has made it difficult to measure aqueous protein solutions with high concentration sensitivity. In this presentation, we introduce an external cavity quantum cascade laser (EC-QCL) IR spectroscopy with a solvent absorption compensation (SAC) unit helps it to improve the concentration sensitivity and increase the path length availability. A series of aqueous protein solution measurements show a clear linear concentration dependance of Amide I and II bands from 100 mg/ml to 0.02 mg/mL without postprocessing. The sensitivity demonstrated with this new approach is >100 greater than conventional FT-IR spectroscopy. This highly-sensitive spectroscopic approach will make IR absorption spectroscopy an innovative tool for quantitation of the integrity of low-concentration drugs and characterization of subtle changes in the higher-order structure of biomolecules in aqueous solutions.

ABSTRACTS - POSTERS

PATRICK KEATING, University of Maryland, College Park, "pH-dependent structural changes in the transmembrane domain of Lassa virus glycoprotein complex"

Membrane fusion of Lassa virus (LASV) is a vital process of the infection cycle, and it is facilitated by the glycoprotein complex (GPC), the sole proteins found on the surface of the viral membrane. Previous studies have shown a dramatic structural change of the soluble domain of the GPC in membrane fusion. However, the membrane interacting domains, including the transmembrane domain (TM), are still uncharacterized. For LASV to perform membrane fusion efficiently, the transmembrane domain interacts with the unusual stable signal peptide (SSP), which resides next to the TM and is critical for GPC assembly and membrane fusion. To fully understand the fusion mechanism, we must understand this interaction at the molecular level. Here, we successfully expressed and purified the TM and showed that the TM undergoes a conformational change in a pH-dependent manner. During purification, a detergent screening revealed that LMPG was the most suitable detergent for structural studies. CD spectroscopy suggests the TM contains a helical structure and slightly increases helical content at lower pH, mimicking the fusion environments. Although CD suggests a subtle change, NMR shows TM exhibits a significant change during fusion events. Upon completion of the backbone assignments and chemical shift indexing analysis, a slight elongation at the N-terminus of the helix was revealed in TM. Furthermore, relaxation measurements were performed, but no significant changes were observed. All these results suggest that the helical elongation leads to a change in the tertiary structure of TM that could be important for the interaction with SSP in the membrane to promote membrane fusion.

ABSTRACTS - POSTERS

VARVARA FOLIMONOVA, NCI, NIH, "Structural Studies and Optimization of CAR Hinge Regions"

Chimeric antigen receptors (CARs) are an innovative tool for developing novel T-cell-based therapies against cancer, which include the FDA-approved CTL-019 (KYMRIAH, Novartis Pharmaceuticals Corp.) and axicabtagene ciloleucel (YESCARTA, Kite Pharma, Inc.). CAR T-cell therapies are profoundly successful in treating refractory or relapsed B-cell malignancies, but they carry risk of T-cell hyperactivation syndrome-related toxicity in patients. Recently, a 4-1BB-based CAR was developed with a degron motif that allows its activity to be reversibly controlled by lenalidomide-dependent interactions with the CRL4CRBN E3 ubiquitin ligase.1 These types of innovative implementations to CAR therapies continue to be developed, and better understanding of the structural and dynamic properties of CAR molecules will facilitate their improvement. CAR constructs are engineered to target tumor-specific antigens and are developed by using modified domains from single-pass cell surface receptors (SPMRs). Generally, CARs are composed of an extra-cellular antigen recognition domain and intracellular signal transduction domains, with an intervening linker region and transmembrane domain. Whereas structural information is available for the CAR antigen recognition, signal transduction and transmembrane domains, the hinge domain and the overall CAR structure has proven difficult to study due to their dynamic nature. Recently, we applied biophysical methods including nuclear magnetic resonance (NMR) spectroscopy to identify dynamic exchange driven by proline isomerization in a CD8alpha linker that is intrinsically disordered.2 In this poster, we expand these studies to other hinge regions to gain insight into the impact of varying the hinge on CAR activity.

ABSTRACTS - POSTERS

XIAORAN SHANG, University of Maryland, Baltimore, IBBR, "Functional and Structural Characterization of Monoclonal Antibodies Isolated from a Convalescent COVID-19 Patient Reveals Neutralizing Epitopes with Broader Variant Coverage"

Neutralizing antibodies (nAbs) can block viral entry, clear pathogens, and play an essential role in long-term immunity. The study of nAbs elicited to respiratory syndrome coronavirus 2 (SARS-CoV-2) by natural infection and vaccination significantly contributes to understanding B cell response and developing pan-SARS-CoV-2 vaccines. Utilizing antigen-specific B cell sorting and antibody cloning approach, we have isolated a panel of monoclonal antibodies recognizing the spike (S) protein of SARS-CoV-2 from a convalescent COVID-19 patient. Here, we have characterized seven nAbs from this antibody panel, which target the receptor binding domain (RBD) or the N-terminal domain (NTD) of S protein. One of the RBD targeting nAbs, MC89, displays extremely high neutralization potency which neutralizes the early D614G-variant, as well as the Alpha, Delta, and Epsilon variants with half-maximal inhibitory concentrations (IC50) at sub-picomolar level. Moreover, we found that another RBD nAb, MC25 displaying pan-SARS-CoV-2 variant neutralization breadth including the highly immune-evading Omicron BA.1 and BA.2 variants, binds to a conserved but cryptic RBD epitope. Furthermore, we have delineated the structure of a NTD-targeting nAb, MC5 by Cryo-EM that reveals a novel neutralizing epitope distinct from the prototypical NTD neutralization supersite described by previous work, consistent with the expanded virus neutralization breadth of MC5 compared with prototypical NTD–targeting nAbs. These neutralizing epitopes with broader variant coverage could be exploited as COVID vaccine and therapeutic targets for future work.

ABSTRACTS - POSTERS

JOE CZEKNER, National Institute of Standards and Technology, "Quantitation of Enantiomeric Excess in an Achiral Environment using Trapped Ion Mobility Mass Spectrometry"

Obtaining enantiopure products is often a goal in pharmaceutical synthesis. For example, D-ethambutol is used in the treatment of tuberculosis while L-ethambutol can cause blindness in patients. Here, we present a novel, straightforward method to determine enantiomeric excess (ee) of tryptophan (Trp) and N-tert-butyloxycarbonyl-O-benzyl-serine (BBS) solutions without chiral additives. For this, lithium carbonate, sodium carbonate, or silver acetate were added to solutions of Trp or BBS. Singly negatively charged dimer and trimer clusters were then formed by electrospray ionization (ESI) and analyzed using Trapped Ion Mobility Spectrometry (TIMS) and Time-of-Flight Mass spectrometry (TOFMS). When a solution contains both enantiomers, homo- and heterochiral clusters are generated which can be separated in the TIMS-tunnel based on their different mobilities using a nitrogen buffer gas. The ratio of homochiral to heterochiral clusters shows a binomial distribution and can be calibrated with solutions of known ee to yield measurements of samples with better than 1% accuracy. Samples can be prepared rapidly and measurements are completed in less than five minutes. Current instrumental limitations restrict this method to rigid molecules with large functional groups adjacent to the chiral centers. Nevertheless, we expect this method to be applicable to many pharmaceuticals and provide the example of 1-methyl-tryptophan to demonstrate this.

ABSTRACTS - POSTERS

SHARANBASAPPA KARADE, University of Maryland, IBBR, "Accelerating Antiviral drug discovery: Structure Guided Antivirals Could be the Key to Preventing Another Pandemic"

Most enveloped viruses rely on the host cell endoplasmic reticulum (ER) quality control (QC) machinery for proper folding of glycoproteins. The key ER α-glucosidases (α-Glu) I and II of the ERQC machinery are attractive targets for developing broad-spectrum antivirals. We synthesized a series of N-substituted derivatives of valiolamine, the iminosugar scaffold of type 2 diabetes drug voglibose. To understand the basis for up to 100,000-fold improved inhibitory potency, we determined high resolution crystal structures of mouse ER α-GluII in complex with valiolamine and 10 derivatives. The structures revealed extensive interactions with all four α-GluII subsites. We further showed that N-substituted valiolamines were active against dengue virus and SARS-CoV-2 in vitro. This study introduces valiolamine-based inhibitors of the ERQC machinery as candidates for developing potential broad-spectrum therapeutics against the existing and emerging viruses.

ABSTRACTS - POSTERS

HITENDRA NEGI, NCI, NIH, "Exploring The Base Subcomplex Of The 26s Proteasome For Ubiquitin-Binding Sites"

The 26S proteasome is a megadalton complex that tightly regulates the lifespan of countless proteins in the cellular pool. It is formed by a 19S regulatory particle (RP) and 20S core particle (CP), the latter of which proteolyzes proteins into peptides. The RP has non-ATPases subunits, including a lid subcomplex, and AAA-ATPase subunits that are part of a base sub-complex. Ubiquitinated substrates bind the 26S proteasome by base subunits Rpn1 and Rpn13 as well as Rpn10, which is at the lid-base interface1. Substrates are unfolded by the AAA-ATPases as they translocate from the RP to CP for cleavage2. Rpt5/PSMC3 is one of the six ATPase subunits with a C-terminal tail that interacts with the CP and the molecular chaperone Nas2; loss of these interactions causes production of faulty proteasome in cells3. In contrast to the C-terminus of Rpt5, not much information is available for its N-terminal region, which is absent from all available cryo-EM structures of the 26S proteasome complex. However, earlier studies by Dr. Cecile Pickart's group4 provided evidence for Rpt5 contributing to ubiquitin binding at the 26S proteasome. We studied the structure and binding properties of the N-terminal tail (1-43 aa residues) of Rpt5.

ABSTRACTS - POSTERS

MISTI CARTWRIGHT, UMBC, "Biophysical Characterization of Mus musculus ATE1-1"

Post-translational modifications are polypeptide alterations that affect protein function, solubility, and activity, among other characteristics. Some post-translational modifications involve the addition and/or removal of an amino acid. One such modification is arginylation, a post-translational modification that uses the enzyme arginyltransferase 1 (ATE1) to catalyze the post-translational addition of the amino acid Arg to a eukaryotic protein. Arginylation has been shown to be essential to eukaryotic cellular homeostasis and overall human health. Currently there are structures for yeast ATE1s, but there are no known structures of higher-order eukaryotic ATE1s. In this work, we have cloned, overproduced, and purified mouse (Mus musculus) ATE1 isoform 1 (ATE1-1) for structural and biophysical studies. Purified mouse ATE1-1 produced high yields of monomeric protein. Crystallization trials of this protein are currently ongoing, and small-angle X-ray scattering (SAXS) was performed. The experimental SAXS data suggest that mouse ATE1-1 may be composed of two separate, ordered domains, in contrast with its AlphaFold prediction. Comparisons of the mouseATE1-1 SAXS profile and ab initio envelope to prokaryotic amino-acyl transferases such as the S. auerus FemA enzyme, which exhibits an additional coiled-coil domain of unknown function, suggest a structural similarity. Future work will include additional SAXS analyses and crystallization studies to probe whether an additional structured domain is indeed a feature of higher-order eukaryotic ATE1s.

ABSTRACTS - POSTERS

JOSHUA LUCKER, University of Maryland, Institute for the Physical Sciences and Technology, "A United Atom Representation for Sphingolipids in the CHARMM Force Field"

With the development of the CHARMM molecular dynamics force field family in the 1970s and 1980s came a computer program that could model the complexity of biomolecular processes. Since then, CHARMM has expanded and improved, now being able to model the intricacies of the plasma membrane. Sphingolipids have been of increasing interest in these cell membranes. Originally found in brain extracts in the 1870s, these lipids have since been found to be important components in mammalian plasma membranes. These lipids have been implemented into CHARMM in recent years. However, there is still more to be included. One such inclusion that is currently being implemented by the presenter and his PI is the inclusion of a united-atom representation of these sphingolipids. Currently, lipids in CHARMM can be modeled using an 'all-atom' representation, in which all of the atoms are modelled as separate entities, or using a 'united-atom' representation, in which the hydrogens on certain atoms are combined for computational ease. Although an all-atom force field is useful in many cases, a united-atom force field can also be beneficial, such as in modeling large membrane systems in a shorter amount of time. Thus, the goal of the presenter and his PI is to create such a representation and implement the representation into CHARMM. The presenter and PI hope that this representation will be used to further study the functions and properties of cellular membranes in order to create solutions in the biophysical and biomedical field surrounding cellular membranes.

ABSTRACTS - POSTERS

NATHANIA DABILLA ALVES SILVA, NIAID, NIH, "Identifying pan-enteroviral therapeutic targets with pooled CRISPRi screens"

Viruses of the genus Enterovirus cause an estimated 15 million infections in the U.S. annually and are a significant cause of disease globally. HEVs include the prototypical pathogenic enterovirus, poliovirus, as well as emerging pathogens, including Coxsackievirus A and B (CVA and CVB), enterovirus A-71 (EV-A71) and enterovirus D-68 (EV-D68).HEV infection primarily affects infant and adolescent populations, causing a wide range of clinical manifestations that commonly include respiratory illness and mucocutaneous lesions, or Hand, Foot, and Mouth Disease (HFMD). In rare cases, HEV infections can progress to life-threatening pathologies including acute flaccid myelitis, cardiomyopathy, encephalitis, and aseptic meningitis. There are no treatments for enteroviral disease, emphasizing the need for a better understanding of enterovirus infection and pathogenesis. Moreover, the host genes promoting and restricting replication of HEVs have not been fully described, limiting our understanding of potential therapeutic opportunities. The proposed study will be conducted in collaboration the Functional Genomics Lab (FGL) of the National Center for Advancing Translational Science (NCATS). The goal of this project is: (i) Perform genome-wide CRISPRi screen for pro-HEV host factors; (ii) Characterize role of proviral host factors in viral infection at single-cell resolution using Perturb-seq and (iii) Evaluation and validation of putative pan-enterovirus therapies. This project will deepen our understanding of host factors involved in HEV replication and identify points of therapeutic intervention targeting identified host factors and cellular pathways. By focusing on a broad collection of prototype pathogens within the EV genus, these data will enhance preparedness for potential HEV pandemics.

ABSTRACTS - POSTERS

NABA KRISHNA DAS, UMBC, "Crystal structure of Coxsackievirus replication platform"

Coxsackievirus B3 (CVB3) represents a major human pathogen that causes acute and chronic viral pancreatitis and myocarditis. It belongs to the Enterovirus genus of the Picornaviridae family. The extreme 5' untranslated region of the CVB3 (+)-sense RNA genome contains a cloverleaf-like (CL) RNA domain that forms an essential platform for viral genome replication. It is known to interact with the host PCBP and viral 3CD proteins through its stem-loop subdomains, B and D, respectively. Despite being essential and highly conserved among enteroviruses, high-resolution structures of the 5'CLs remain known. Here, we have reported a crystal structure of intact CVB3 5'CL at 1.9 Å resolution in complex with an antibody chaperone. The crystal structure assumes a compact, H-type antiparallel 4-way junction fold composed of one stem and three stem-loop regions. The subdomains assemble into two sets of co-axially stacked helices, with each coaxial stacking forming almost a continuous A-form helix. The A helix stacks on the D helix and the B helix on the C helix. Remarkably, the crystal structure also revealed unprecedented tertiary interactions between the C-loop and the D helix's Py-Py region. These interactions agree well with our NMR results, confirming that these structural features observed in the crystal also exist in the solution. The phylogenetic and 3C protein binding analyses suggest that our crystal structure likely represents a conserved architecture of the enteroviral 5'CL domains, including the sC-loop and Py-Py interactions. As our structure represents the first high-resolution structural determination of the enteroviral 5'CLs, our research will set up a stage for further studies into the mechanism of enteroviral genome replication and the development of antiviral drugs that target this RNA-centric platform.

ABSTRACTS - POSTERS

MEGAN DUNAGAN, NIAID, NIH, "The role of activating FcγRs during Mayaro infection"

Alphaviruses are mosquito-borne, single-strand, positive-sense RNA viruses that have caused explosive outbreaks worldwide. Individuals infected with an alphavirus can manifest arthritic or encephalitic symptoms, depending on the virus. Mayaro virus (MAYV), an emerging alphavirus, can cause fever, rash, myalgia, and severe polyarthritis and polyarthralgia. In a subset of infected individuals, long-term joint pain can persist for months to years, leading to a substantial social and economic cost following outbreaks. While there are no approved vaccines or therapeutics against acute or chronic MAYV disease, antibodies have been shown to be critical for protection. Previous studies evaluating the efficacy of monoclonal antibodies against MAYV determined that Fc-effector functions were necessary for protection. However, the impact of antibody Fc-Fc gamma receptor (FcgR) interactions during MAYV infection has not been evaluated. To address this question, we assessed the role of Fc-mediated effector functions during MAYV infection using mice that lack activating FcgRs (KO). Preliminary results showed that the KO mice had prolonged foot swelling and delayed viral clearance from joint-associated tissue compared to WT mice. Interestingly, there was an early and robust neuro-invasion of MAYV in the brain of KO mice, with infectious virus detected as late as 10 days post-infection. These results suggest a role of FcgRs in both dissemination and clearance of MAYV. Our current research aims to identify immune cell mediators that may be affecting MAYV tropism and clearance.

ABSTRACTS - POSTERS

HASAN AL BANNA, UMBC, "Development of synthetic anti-RNA scFvs for RNA imaging in cells"

Visualization of coding and noncoding RNAs in cells is essential to elucidate their functions in different biological processes. The antibody derivatives such as single-chain variable fragments (scFvs) for peptides and proteins have revolutionized the imaging and quantification of cellular proteins. Still, there is a lack of such antibody-based tools and analogous strategies for RNA imaging. We are developing anti-RNA scFvs as new RNA probes for visualization, tracking, and quantifying RNAs in cells. Our approach relies on transforming the existing anti-RNA Fabs into the scFv formats and developing their scFv-GFP (Green Fluorescence Protein) fusions. The scFv probes bind with the target RNA, whereas GFP allows visual detection. Our preliminary results are promising. We have so far developed three scFvs and their scFv-GFP fusions based on the existing BL3-6, HCV2, and HCV3 Fabs. These scFv proteins have been expressed in E. coli and purified using standard chromatographic methods. We have also illustrated that these scFvs with and without GFP tags bind their cognate RNA targets with affinities similar to their parent Fabs. The scFv binding studies in cell lysates and structural studies of the scFvs and scFv-RNA complexes are underway. Our long-term goal is to use these probes to visualize RNA in living cells. We anticipate that these new tools will bring great opportunities to track mature RNAs, capture folding dynamics of nascent RNAs, and analyze conformational changes and Spatio-temporal dynamics of various RNA molecules in living cells.

ABSTRACTS - POSTERS

WILLIAM SEXTON, University of Maryland, College Park, "An ESCRT-Interacting Deubiquitinase (AMSH1) Is Involved in Arabidopsis Nonhost Resistance Against Powdery Mildew Fungi"

Plant nonhost resistance (NHR) is a phenomenon in which all genotypes of a plant species are immune to all strains of a phytopathogen. While there is great interest in understanding NHR to facilitate engineering robust pathogen resistance in crops, its molecular basis is poorly characterized. Based on our current understanding, plant immunity consists of two intertwined branches: MAMP-triggered immunity (MTI) and effector triggered immunity (ETI). MTI is induced upon recognition of microbe-associated molecular patterns (MAMPs) (e.g., chitin, flagellin) by cell-surface receptors and is generally sufficient to suppress non-adapted pathogens. Better adapted pathogens secrete effectors to inhibit MTI. Detection of a pathogen effector by intracellular receptors induces ETI which is often accompanied by the hypersensitive response (HR), which is a type of programmed cell death at the infection site. Past research has revealed that MTI and ETI can contribute to NHR; however, further elucidation of NHR is inherently difficult due to its lack of intraspecific variations.

To circumvent this difficulty, we first made an immunocompromised Arabidopsis mutant in which five immunity genes are knocked out. This eds1-pad4-sid2-pen1-pen2 (epsp1p2) mutant has compromised resistance against non-adapted dicot powdery mildews but remains resistant to barley powdery mildew, Blumeria graminis f. sp. hordei (Bgh). We then performed a genetic screen with EMS-mutagenized epsp1p2 seeds for mutants that are susceptible to non-adapted Bgh (snab). The first mutant we isolated (snab1) supports profuse sporulation of Bgh, indicating considerable breakdown of NHR. Bulked segregant analysis was used to map the snab1 causal mutation to ASSOCIATED MOLECULE WITH THE SH3 DOMAIN OF STAM1 (AMSH1). AMSH1, a deubiquitinase, interacts with ESCRT machinery, which is involved in several cellular processes, including degradation of membrane proteins and autophagy. Preliminary data suggest AMSH1 is required for HR associated with classic ETI in Arabidopsis, thus implicating the ESCRT machinery in regulation of plant immunity.

ABSTRACTS - POSTERS

SURUCHI SINGH, University of Maryland, School of Medicine, IBBR, “Insights into maturation of immuno-modulatory glycans in the SARS-CoV-2 spike protein”

Genetic vaccines have been critical tools in combating the COVID-19 pandemic. These mRNA and adenovirus-based DNA vaccines encode the SARS-CoV-2 spike gene, which is translated into the spike protein in the rough endoplasmic reticulum (ER) in the host cell secretory pathway. This is followed by recycling of the spike protein between ER and Golgi before export to the plasma membrane for immune presentation. This ER-Golgi trafficking is associated with spike protein glycan maturation that modulates interactions with immune effectors such as antibodies. However, little is known about the mechanistic details by which spike glycans undergo compartment-specific maturation in the secretory pathway, which has direct implications on spike immunogenicity. A major obstacle has been the production and structure-determination of spike constructs that are arrested in distinct stages of ER-Golgi trafficking, and thus of glycan maturation. To address this question, we have generated constructs with single-site mutations in trafficking motifs of the spike protein, thereby controlling their localization and recycling. This has been validated by immuno-fluorescence microscopy and cleavage assays of the spike protein for trans-Golgi specific protease, furin. Using these novel spike maturation intermediates, we are employing a combinatorial approach using single particle cryoEM to determine the structural characteristics of these spike intermediates and mass spectrometry to map glycan maturation states. This investigation will enable a fundamental understanding of spike protein biogenesis that underlies the acquisition of immunogenicity. Since this ER-Golgi-plasma membrane pathway is utilized for the biogenesis of membrane-associated glycoproteins in viral pathogens such as HIV, HCV, Ebola virus, and Zika virus, this investigation will serve as a platform for future studies of viral glycoprotein biogenesis in the context of infections and genetic anti-viral vaccines.

ABSTRACTS - POSTERS

MANJU OJHA, UMBC, “The crystal structure of an RNA domain from a cactus virus that binds human eIF4E”

The cap-independent translation enhancers (CITEs) found within the 3' untranslated regions of tombusvirus genomes promote genome translation via cap-independent mechanisms. These structures are known to bind translation initiation factors or the ribosomal subunits. They have been purposed to interact with the 5'-end to circularize the viral genome, thus priming the genome for translation. However, no high-resolution crystal structures exist for various classes of 3'-CITEs, except for a few computational models and biochemically-derived secondary structures. Here we have crystallized and determined the structure of the Saguaro Cactus Virus (SCV) 3'-CITE that binds the eukaryotic cap-binding protein, eIF4E. The structural determination was made possible by the complexation of the RNA with a Fab chaperone. The SCV 3'CITE RNA folds into a T-shaped three-way junction with a pseudoknot formation involving a G-rich bulge within the first stem and the unpaired nucleotides within the three-way junction. Within the bulge flips out a G to mimic the 5'-cap for eIF4E binding. Our mutagenesis studies suggest that SCV 3'CITE RNA pre-organizes its bulge structure, forcing a single nucleotide to flip out. Many genotypes of the tombusviridae family contain 3'-CITEs with sequences potential to fold similar to the SCV 3'-CITE structure, suggesting that these RNAs adopt a common topology, mimicking the mRNA 5'-cap for initiating their genome translation.

ABSTRACTS - POSTERS

VICTORIA CALLAHAN, Laboratory of Viral Diseases, NIH-NIAID, "Modulation of the innate immune response in the presence of a broadly neutralizing alphavirus antibody during Ross River virus infection in mice"

Ross River virus (RRV) belongs to the family Togaviridae and genus Alphavirus which includes other emerging alphaviruses such as chikungunya virus and Mayaro virus. The disease associated with infection, Ross River Fever, is characterized by maculopapular rash, flu-like symptoms, myalgia, and polyarthritis that may be long-lasting. Importantly, RRV remains to be an endemic and circulating virus in Australia, with over 5,000 cases reported annually with significant economic impacts. Numerous studies have highlighted the significance of antibody-mediated protection during alphavirus infection. A cross-reactive anti-chikungunya virus monoclonal antibody, CHK-265, moderately neutralized RRV in vitro and reduced clinical disease in a mouse model of RRV arthritis and musculoskeletal dysfunction. Prophylaxis administration of CHK-265 reduced viral load early, but RRV rapidly escaped from CHK-265 neutralization resulting in viral dissemination. Interestingly, tissues proximal to the inoculation site (e.g., ipsilateral ankle) remained protected through a type I interferon-dependent pathway even in the presence of the neutralization escape virus. However, the mechanism of antibody-mediated site-specific protection remains undefined. In this study, we evaluated CHK-265-mediated effects, as well as antibody-independent effects, on the early innate immune response near the inoculation site following RRV infection in mice. C57BL/6 mice were administered CHK-265 or an isotype control antibody one day before subcutaneous RRV infection in the rear footpad and the proximal ankle tissue was harvested at multiple time points. CHK-265 reduced viral RNA at early time points post-infection and blockade of the type I interferon receptor prevented CHK-265-mediated viral RNA reduction. To determine how CHK-265 treatment could impact the type I interferon response, we analyzed early time points for innate immune signatures. Following the identification of critical time points, the expression of innate immune genes in RRV-infected ankle tissue with or without CHK-265 treatment was analyzed via Nanostring technology and RT-qPCR. At 8 hpi, CHK-265 increased interferon stimulatory gene (ISG) expression in ankle skin compared to isotype controls, but there were minimal differences in ISG expression between the treatment groups in the ankle tissue. These results suggest that CHK-265 enhances the innate immune response within the skin rather than the underlying tissue. To focus on skin-associated immune signatures, we are developing an intradermal RRV infection model in the ear which we will use to better understand the early immune modulation of CHK-265.

ABSTRACTS - POSTERS

DEBAJIT DEY, University of Maryland, School of Medicine, IBBR, "Insights into SARS CoV-2 spike and COPI residues that control binding and release during retrograde trafficking"

β-Coronaviruses such as SARS-CoV-2 hijack coatomer protein-I (COPI) for spike protein retrograde trafficking to the progeny assembly site in endoplasmic reticulum-Golgi intermediate compartment (ERGIC). However, limited residue-level details are available into how the spike interacts with COPI. Here we identify a novel extended COPI binding motif in the spike that encompasses the canonical K-x-H dibasic sequence. This motif demonstrates selectivity for αCOPI subunit. Guided by an in silico analysis of dibasic motifs in the human proteome, we employ biophysical and cell biology techniques to show that the spike motif terminal residues are critical modulators of complex dissociation, which is essential for spike release in ERGIC. αCOPI residues critical for spike motif binding are elucidated by mutagenesis and crystallography and found to be conserved in the zoonotic reservoirs, bats, pangolins, camels, and in humans. Collectively, our investigation on the spike motif identifies key COPI binding determinants with implications for retrograde trafficking.

ABSTRACTS - POSTERS

JUN ZHANG, University of Maryland, IBBR, "Establishing an in-planta Papaya-Liberibacter interaction pathosystem for studying the devastating citrus greening disease"

Huanglongbing, or citrus greening, is the most destructive diseases of citrus in the world. It is caused by a phloem-restricted bacterial pathogen Candidatus Liberibacter asiaticus (CLas). CLas cannot be cultured in vitro; hence, research on pathogenesis mechanisms of CLas is inherently difficult. Liberibacter crescence (Lcr) , which was isolated from babaco mountain papaya, is culturable in the media and can be genetically modified. Because Lcr is a close relative to CLas, it has been used as a surrogate of CLas for studying pathogenicity mechanisms of Liberibacter. However, Lcr has never successfully been introduced back to its original host, which makes in-planta study of Lcr or its genetically modified variants or relatives such as CLas impossible. Supported by a USDA-funded project, we have explored three different methods, namely, dodder-mediated transmission, bark flap-delivery, and leaf-vein puncture, to try to establish infection of Papaya with a GFPuv labeled Lcr strain. Our results demonstrate that (i) dodder can absorb Lcr from a test tube and transmit it to papaya plants that are parasitized by dodder; (ii) Lcr can be delivered to leaf vein by bark flap inoculation and (iii) Lcr can also be successfully delivered inot vascular cells of papaya by needle puncture. Papaya plants inoculated with Lcr using either of the three approaches developed extensive leaf necrosis associated with the presence of GFPuv-tagged Lcr, suggesting successful proliferation of Lcr in its original host. This is the first report to demonstrate that Lcr could be re-inoculated back to its host plant and cause disease symptoms. Results from this study will provide a powerful tool for investigating pathogenicity mechanisms of Liberibacter bacteria including CLas, thereby accelerating development of innovative strategies for control of CLas-caused citrus greening.

ABSTRACTS - POSTERS

SULIMAN SHARIF, University of Maryland, School of Pharmacy, “The Pythonic Common Chemical Universe”

The virtual chemical universe is expanding rapidly as open access titan databases Enamine Database (20 Billion), Zinc Database (2 Billion), PubMed Database (68 Million) and cheminformatic tools to process, manipulate, and derive new compound structures are being established. We present our open source knowledge graph, Global-Chem, written in python to distribute dictionaries of common chemical lists of relevant to different sub-communities out to the general public i.e What is inside Food? Cannabis? Sex Products? Chemical Weapons? Narcotics? Medical Therapeutics? To navigate new chemical space we use our natural chemical names data as a reference index as to help us keep track of common patterns of interest and help us explore new structures that could be theoretically real. In our talk, we will present the chemical data, the rules governing the data and it's integrity, and how to use our tools to understand the chemical universe with python.

ABSTRACTS - POSTERS

NELE HOLLMANN, UMBC, Howard Hughes Medical Institute, "Structure determination of the highly selective HIV-1 Gag-CES complex"

To replicate and infect a new cell, HIV-1 must assemble infectious particles that contain the viral genome. Like all retroviruses, HIV-1 packages two copies of its genome, which gets trafficked from the cytoplasm to the membrane by a small number of viral Gag proteins. Later additional Gag proteins assemble and budding occurs1,2,3,4,5. The 5'-leader is the most conserved region of the HIV-1 genome and is responsible for regulating multiple activities during viral replication, including RNA recognition and packaging during assembly6,7. Although viral RNA represents only a small proportion in the cell, over 95% of assembled particles are packaged with HIV-1 RNA8. Individual structures of both the minimal required packaging signal of the 5'-leader (core encapsidation signal; CES) and the Gag polyprotein are solved9. However, a complex structure showing the interplay of both and thereby explaining the highly selectivity towards viral RNA during packaging is still missing. By using analytical SEC and ITC measurements we could show that about 10-12 monomeric proteins form a tight and homogenous complex on the RNA, making future structural studies promising. An integrated approach of cryogenic electron microscopy and nuclear magnetic resonance using specific labeling schemes that allow the study of larger molecular assemblies, shall reveal the complex structure. Moreover, gaining structural information will allow to determine the features contributing to the selective packaging of the viral genome.

ABSTRACTS - POSTERS

KARNDEEP SINGH, UMBC, Department of Chemistry and Biochemistry, "Exposing the Interactions between the HIV-1 5'-Leader and the cellular cap-binding protein, eIF4E IBBR, University of Maryland Baltimore, "Effect of Excipient Concentration determined by Water Magnetic Resonance (wNMR)"

The human immunodeficiency virus type-1 (HIV-1) retrovirus is the causative agent for the acquired immunodeficiency syndrome. Although antiretroviral therapies exist, side effects and drug resistance remain global challenges. Our laboratory's research is focused on a highly conserved region of the HIV-1 RNA genome known as the 5'-Leader (5'-L), which adopts two conformations dependent on the transcriptional start site usage: 5'-capped RNAs beginning with one guanosine (Cap1G) adopt the dimeric conformation and 5'-capped RNAs beginning with two or three guanosines (Cap2G and Cap3G, respectively) adopt a monomeric conformation. Published work from our laboratory revealed that Cap1G leader RNAs sequester the 5'-cap through coaxial stacking of the two 5'-hairpins, preventing its binding to eIF4E – the initial recognition step in cap/eIF4E-dependent translation of the HIV-1 mRNAs. For monomeric transcripts, the 5'-cap is exposed and accessible for the recruitment and binding of eIF4E. While it is well-established that cap-dependent translation serves as a primary mechanism of HIV-1 genome translation in eukaryotes, the molecular nature of interactions between the monomeric 5'-L and eIF4E are unknown and, in general, whether the structured RNA body affects binding of eIF4E remains to be determined. I hypothesize that elements of the monomeric 5'-L facilitate binding to eIF4E. I present preliminary evidence for significantly interactions between eIF4E and segments of the RNA body in vitro. Nuclear magnetic resonance (NMR) titration experiments using a selectively methyl labeled (Ile, Leu and Val) eIF4E and a HIV-1 Cap3G RNA oligo reveal additional chemical shift perturbations (CSPs) of eIF4E compared to CSPs observed when a 5'-cap analog was titrated. Isothermal titration calorimetry experiments reveal that eIF4E binding to a Cap3G RNA oligo exhibit ~fourfold tighter binding compared to a 5'-cap analog. These findings suggest novel interactions of cap-dependent translational machinery with the monomeric 5'-L and justify the following structural determination of the RNA-protein complex.

ABSTRACTS - POSTERS

PALLAVI GUHA BISWAS, University of Maryland, Baltimore, IBBR, "Effect of Excipient Concentration determined by Water Magnetic Resonance (wNMR)"

Vaccines and therapeutic drugs consist of compounds inert substances along with the active drug molecule known as excipients. These excipients play a central role in the drug development process in formulating stable dosage forms and their administration. However, depending on the concentration of the excipient in, the drug could be active, leading to allergic reactions or other adverse effects in patients. The effect of increasing concentrations of excipients is studied by water magnetic resonance, known as wNMR, a noninvasive analytical technique. This presentation also shows the correlation between wNMR and viscosity to determine the adequate concentration of excipients to be used in the formulation of the drugs.

ABSTRACTS - POSTERS

BRIAN GROSSMAN, UMBC, "Nuclear magnetic resonance studies of the HIV-1 packaging signal"

The 5'-Leader (5'-L) of the HIV-1 RNA genome contains conserved elements that direct selective packaging of the unspliced, dimeric genomic RNA into assembling viral particles. Studies of selective HIV-1 packaging have revealed the main facilitators for RNA genomic recognition to be the Gag polyprotein (Gag) and a core region of the 5'-L referred to as the core encapsidation signal (CES). Solution-state nuclear magnetic resonance (NMR) of a monomeric form of the CES (CESm) from the NL4-3 strain of HIV-1 (NL4-3) showed that the NL4-3-CESm adopts a tandem three-way junction structure that directs genomic RNA packaging by exposing guanosine residues that are essential for high-affinity binding to Gag. However, owing to size limitations with NMR, the previous NL4-3 NMR studies required the introduction of mutations and the use of fragmentation to prevent RNA dimerization, reduce nucleotide signal overlap, and detect long-range interactions. The relevance and conservation of the tandem three-way junction structure have yet to be confirmed in other strains of HIV-1 and there are putative models based on chemical probing, including recent models, that appear to be incompatible with NMR data obtained for fragments and mutants of the NL4-3 5'-L. To address the structural disagreements between NMR and chemical probing techniques, we utilized a different strain of HIV-1, the MAL strain, that does not require the introduction of mutations for collecting excellent quality NMR data, and the use of a novel RNA labeling technique that allows for the structural characterization of the intact HIV-1 5'-L packaging signal.

ABSTRACTS - POSTERS

FARID GHELICHKHANI, University of Delaware, Department of Chemistry and Biochemistry,
"Selenoprotein S interacts with the replication and transcription complex of SARS-CoV-2 by binding nsp7"

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) uses endoplasmic reticulum membranes and associated human proteins for its replication and to evade detection. One human protein recruited by several viral proteins is selenoprotein S, which takes part in the endoplasmic reticulum protein degradation pathway, NFkB signaling, and cytokines secretion. A key interaction was reported with SARS-CoV-2 non-structural protein (nsp7), which is essential for virus replication. However, it was unclear whether selenoprotein S and nsp7 interact directly and whether the interaction is possible when nsp7 forms a complex with the other components of the virus's replication machinery. We used biochemical assays to show that selenoprotein S binds nsp7, including when nsp7 is in complex with the coronavirus's RNA-dependent RNA polymerase. This places selenoprotein S at the heart of the coronavirus's replication complex and marks it as the first human protein shown to directly interact with the viral replication complex. Cross-linking experiments were employed to map the interactions of selenoprotein S and nsp7 in the replication complex. We show that the hydrophobic segment of selenoprotein S is essential for binding nsp7. This arrangement leaves an extended helix and the intrinsically disordered region of selenoprotein S exposed and free to recruit additional proteins to the complex.

ABSTRACTS - POSTERS

ELISSA MOLLER, University of Maryland, College Park, "Electrostatics of the MscS cytoplasmic cage domain optimizes fast electroneutral osmolyte release from bacterial cells"

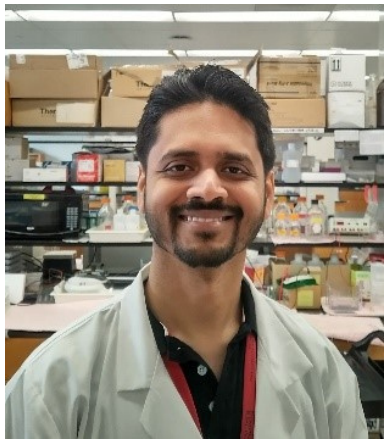
Free-living microorganisms have little control of their environment and are often subjected to changes in osmolarity. Therefore, bacteria have evolved robust osmoadaptation, evading lysis by efficiently ejecting metabolites through mechanosensitive channels from the MscL and MscS families. The larger MscL is non-selective, but the smaller MscS is slightly anionic (2K+: 3Cl-). Substitution of chloride for acetate, a more prevalent intracellular anion, renders MscS non-selective, implying that it is designed to pass carboxylic substances together with counterions. We reason that these channels should select for dispensable intracellular osmolytes to offset the metabolic cost of release. Further, a drastic ionic preference would generate a Donnan potential, which would preclude further transport and be detrimental to efflux. Electrostatic analysis of MscS structures prompted investigation of the positive 'trim' around the windows in the cytoplasmic cage domain as the basis of selectivity. Charge reversing and neutralizing mutations were introduced at residues R156, K161, R184, R185, R224 and R238 and substitution for glutamines at all positions (6xQ) produced a decisive change in selectivity (3K+: 1Cl-). 6xQ still expresses comparably to WT and has similar unitary conductance; however, it provides no rescuing function in osmotic viability assays. Additionally, the mutant shows a slightly higher activation midpoint, faster kinetics and prolonged inactivation indicating an allosteric effect of the selectivity filter on gating. Molecular dynamics simulations corroborated the experimental data and provided further insight into ion flux and channel permeability. Stopped-flow light scattering experiments of 6xQ show slower osmolyte release rates and a higher fraction of permeable osmolytes. Based on metabolomic analysis of shock fluids, 6xQ mediates decreased release of carboxylic compounds and increased release of basic amino acids compared to WT. Clearly, the cytoplasmic domain of MscS acts as a release filter, with electrostatics finely tuned to the repertoire of intracellular osmolytes allowing for electroneutral transport.

ABSTRACTS - POSTERS

LUCIA RODRIGUEZ, UMBC, "Characterizing the interactions between the HIV-1 Rev response element and the viral proteins Rev and Gag"

The human immunodeficiency virus (HIV) affects millions of individuals each year, and current treatments face challenges due to several reasons, including high toxicity, unwanted side effects, and potential for drug resistance. One potential target for drug development is the Rev response element (RRE), a highly conserved, structured RNA element responsible for regulating nuclear export of unspliced and incompletely spliced transcripts through interaction with the viral protein Rev. The RRE can form a 4- or 5-stem loop structure, with three Rev binding sites that have been previously described, on stem 1A, stem 2B, and on the stem 2 junction site. In addition to Rev, another HIV protein has been identified which binds to the RRE, which is the viral protein Gag. Gag is the major structural protein of HIV and is mainly involved in viral genome packaging, so it is unexpected that it would bind to the RRE, which regulates nuclear export. While it is known that Gag binds to the RRE on stem 5 through the 3' end of stem 1, mainly on the upper region of stem 1, a specific binding location is still not known. This work investigates the interactions between the RRE and Rev as well as between the RRE and Gag, by applying a combination of techniques including electrophoretic mobility assays (EMSAs), isothermal titration calorimetry (ITC), and nuclear magnetic resonance (NMR). These studies will provide valuable information regarding RRE protein binding sites and how the RRE interacts with Rev and Gag, which will in turn contribute to novel drug developments targeting the RRE and aid in improving current HIV treatments that are available.

ORGANIZING COMMITTEE



DEBAJIT DEY
CO-CHAIR

Debajit is a postdoctoral research associate in Dr. Saif Hasan's lab at CBT, IBBR. His current research is elucidating the molecular determinants of interaction between SARS-2 spike and the host coatomer complex (COPI) that control binding and release during retrograde trafficking. His work includes expression and purification of recombinant proteins as well as biophysical and structural techniques like BLI and crystallography. He received his PhD in Virology from the Indian Institute of Technology, Delhi. Outside of science, Debajit loves to travel and try out local cuisines.

JOSHUA IMPERATORE
CO-CHAIR

Joshua is a postdoctoral research associate in Dr. Robert Brinson's lab at the National Institute of Standards and Technology (NIST)/IBBR. His current research focuses on using SHAPE-MaP metrology to structurally profile mRNA vaccine constructs. Specifically, he is interested in using this technology to highlight structural variations within key regulatory elements of the mRNA caused by nucleotide modifications, such as N1-ethylpseudouridine substitutions. Joshua received his Ph.D. in Chemistry at Duquesne University. Outside of the lab, he enjoys running, working on his Jeep, and spending time with family and friends.

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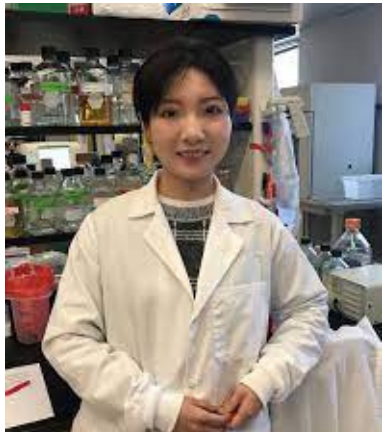

XIAORAN SHANG
TREASURER

Xiaoran is a fourth-year postdoctoral research associate in Dr. Yuxing Li's Lab at IBBR. Currently, she is investigating the B cell response to viral infection and antibody engineering for immunotherapy. Xiaoran received her PhD in Cellular and Molecular Biology from University of Maryland. In her free time, she enjoys spending time with family, reading, and hiking.

TSEGA SOLOMON
TREASURER

Tsega is a postdoctoral research associate in Dr. Robert Brinson's lab at the National Institute of Standards and Technology (NIST)/IBBR. Her postdoctoral research focuses on the application of high-resolution NMR spectroscopy methods for critical quality attribute (CQA) assessment of biotherapeutics and higher order structure characterization. Tsega's recent work demonstrated the utility of combining 2D NMR fingerprinting with other analytical and functional methods to measure structural changes in therapeutic monoclonal antibodies that are relevant to CQA in the common pharmaceutical accelerated stability study of oxidation. She received her Bachelor of Science in Chemistry from the University of Virginia and PhD in Biochemistry from the University of Maryland. Outside the lab, Tsega enjoys traveling, hiking and reading.

ORGANIZING COMMITTEE

SURUCHI SINGH

Suruchi is a postdoctoral research associate in Dr. S. Saif Hasan's lab at IBBR. Her research focuses on understanding the atomic basis of SARS-CoV-2 spike protein biogenesis and glycan maturation in infected host cells. She uses Cryo-EM and BLI in her research to achieve the goals. Suruchi received her PhD in Protein Structure Biology from India. Outside the lab, she loves to go for nature walks with her family.

SALMAN SHAHID

Salman is a postdoctoral research associate in Dr. Roy Mariuzza's lab at IBBR. Salman's expertise is in structural biology and his work focuses on mapping the epitopes on hepatitis C virus (HCV) envelop proteins using various human monoclonal antibodies (HMAbs) through X-ray crystallography and cryogenic electron microscopy. He received his PhD in Structural Biology from India. He loves to hang out with friends and also likes reading Urdu novels and poetry.

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DINENDRA ABEYAWARDHANE

Dinendra is a postdoctoral research associate in Dr. David J. Weber's lab at CBT, IBBR. Her research background is in bioinorganic chemistry and protein structural biology. She currently utilizes the state-of-the-art cryoEM technique to analyze the structural details of disease-relevant proteins to assist computer-aided drug design. She is involved in several collaborative projects conducted in CBT and her main project focuses on understanding the cell entry mechanism of binary protein toxin associated with Clostridioides difficile infectious disease. Dinendra received her PhD in Chemistry at Virginia Commonwealth University. In her free time, Dinendra enjoys cooking, baking, sightseeing, and watching movies.

SHARANBASAPPA KARADE

Sharanbasappa is a fifth year postdoctoral research associate in Dr. Roy A. Mariuzza's lab at IBBR. His research work involves developing small-molecule inhibitors to treat the virus. Sharan's research focus is on structural studies of small-molecule drug candidates, which inhibit the α-glucosidase enzymes of the endoplasmic reticulum (ER) translation quality control (QC) pathway. The small molecules developed against these glucosidases have demonstrated broad-spectrum antiviral activity. He also works on structural aspects of IL, IFN and complexes with inhibitory proteins. Sharan received his PhD in Structural Biology at Central Drug Research Institute, India. He loves to travel and explore new places, playing Ping-Pong and Cricket.

ORGANIZING COMMITTEE

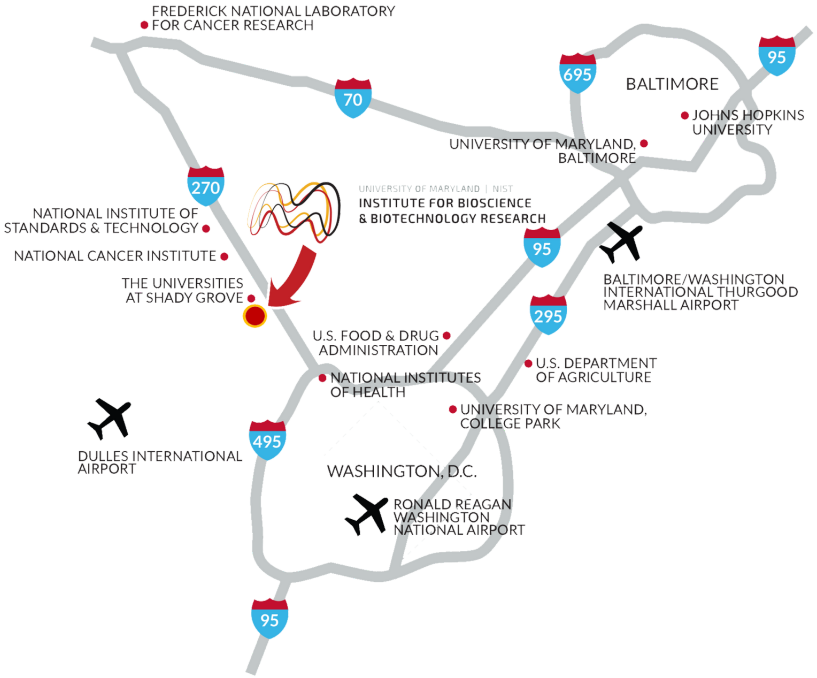
S. SAIF HASAN
FACULTY ADVISOR

Saif is an assistant professor in the Department of Biochemistry and Molecular Biology, and the Center for Biomolecular Therapeutics, University of Maryland School of Medicine, and a Group Leader at IBBR. His research focuses on atomic-level investigations of evolutionary forces that shape coronavirus spike proteins for hijacking of host trafficking machinery. He utilizes X-ray crystallography and single particle cryoEM as the major tools for his investigations. He completed his PhD and post-doctoral training at Purdue University in structural biology, and loves to cook and play basketball in his free time.

LOCATION

ADDRESS:
9600 GUDELSKY DRIVE
ROCKVILLE, MARYLAND 20850

EVENT PARKING:
SHADY GROVE GARAGE
9621 GUDELSKY DRIVE
ROCKVILLE, MARYLAND 20850



CAMPUS MAP

The Universities AT SHADY GROVE

1 **THE UNIVERSITIES AT SHADY GROVE**
9630-9640 Gudelsky Drive

UNIVERSITY OF MARYLAND | NIST
INSTITUTE FOR BIOSCIENCE & BIOTECHNOLOGY RESEARCH

2 **UMD INSTITUTE FOR BIOSCIENCE & BIOTECHNOLOGY RESEARCH**
9600 Gudelsky Drive

3 **SHADY GROVE GARAGE**
9621 Gudelsky Drive

Walking directions from Shady Grove Garage to IBBR

UNIVERSITY OF MARYLAND | NIST

INSTITUTE FOR BIOSCIENCE & BIOTECHNOLOGY RESEARCH

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