

best-worst attribute-level pair, but we include covariates that are class depending. The class allocation is fitted under a mixed logit model. Such heterogeneous classification generalizes the choice impact of consumers. An example is developed and given as illustration.

LISA 2020: CREATING A NETWORK OF STATISTICAL COLLABORATION LABORATORIES. Eric Vance, Department of Statistics, Virginia Polytechnic Institute and State University, Blacksburg VA 24061. To increase the global impact of statistics as a discipline useful for helping researchers answer research questions, and sponsored by a Google Research Award, LISA—The Laboratory for Interdisciplinary Statistical Analysis at Virginia Tech—is partnering with universities and individuals around the world to create a network of 20 new statistical collaboration laboratories in developing countries by 2020. LISA and its partners will educate and train statisticians from developing countries to communicate and collaborate with non-statisticians and then support these statisticians to create statistical collaboration laboratories in their home countries to help researchers, government officials, local industries, and NGOs apply statistical thinking and data science to make better decisions through data. At LISA and elsewhere, we will unlock the collaborative potential of technically sound statisticians who will in turn unlock the research potential of their collaborators and teach other statisticians to do likewise. These local research collaborations, now with the power of statistical thinking and data science open to them, will be key to improving human welfare worldwide. This talk will focus on the steps of the LISA 2020 plan, including how LISA trains statisticians to become interdisciplinary collaborators, how statistical collaboration laboratories create knowledge, and how we are building a mentoring network to assist statisticians in developing countries to enable and accelerate research by making statistics more practical in solving real world problems.

Structural Biology, Biochemistry, and Biophysics

EVOLUTION OF THE SYNUCLEIN PROTEINS: ORPHANS OR SUPERFAMILY MEMBERS. Lesley Greene, Agatha Munyanyi, John Bedford, & Zeinab Haratipour, Department of Chemistry and Biochemistry, Old Dominion University, Norfolk, VA 23529. The synucleins are composed of three related proteins: α , β and γ . The functions and structures of these proteins in the brain remain unresolved. Computational approaches such as bioinformatics can be highly informative and advance theories about the structure and function of proteins long before lengthy experimental work is completed. Computational research can also inform and direct experimental directions. In line with this perspective we conducted bioinformatics and modeling research to facilitate understanding the structure and function of the synucleins. Using PSI-BLAST searches we identified four proteins which contain large regions of significant sequence identity and similarity with the synucleins. These proteins are an endoglucanase enzyme from *Acetobacter pomorum*, a CRE-DUR-1 protein from a nematode, a cytochrome c from a spiral bacterium and a putative protein from the Tasmanian Devil. Three-dimensional molecular modeling indicates that these proteins share a common helix-turn-helix structure with the membrane-bound form of

the α -synuclein monomeric NMR structure. This data and their evolutionary status will be presented. We thank Old Dominion University for funding to LG and from the American Association of University Women to AG for this work.

NEW FUNCTIONS OF FLAVIN DEPENDENT ENZYMES: THE MECHANISM OF 2-HALOACRYLATE HYDRATASE. Yumin Dai & Pablo Sobrado, Department of Biochemistry, Virginia Polytechnic Institute & State University, Blacksburg, VA, 24061. The flavin-dependent 2-haloacrylate hydratase (2-HAH) catalyzed conversion of 2-chloroacrylate, a major component in manufacturing of acrylic polymers, to a non-toxic pyruvate through a double bond hydration and subsequent chloride elimination. The enzyme from *Pseudomonas* sp. YL has been cloned, recombinantly expressed and purified from *E. coli*. The enzyme, which is monomer in solution, contains FAD and is active only in the reduced state, although the reaction is redox neutral. Enzyme activity was recorded after incubation of the enzyme with 2 mM NADH under light for 10 min ($k_{\text{cat}} = 1.35 \pm 0.02 \text{ s}^{-1}$; $K_M = 2.01 \pm 0.19$). Interestingly, reaction with NADH is slow in the absence of light. When the enzyme is exposed to light, the reduction occurs ~ 30 fold faster, indicating the light activation is unique to 2-HAH. Mechanism of 2-HAH was studied, and the results indicated FAD radicals, both neutral and anionic semiquinones were involved during the catalysis. This work was supported by NIH grant R01 GM094469.

STRUCTURAL BASIS OF PHOSPHOINOSITIDE (PIP) RECOGNITION BY THE TIRAP PIP-BINDING MOTIF. Xiaolin Zhao, Shuyan Xiao, & Daniel G. S. Capelluto, Department of Biological Sciences, Virginia Tech., Blacksburg VA 24061. TIRAP is an adaptor molecule required for both plasma membrane and endosome TLR signaling in response to viral infection. TIRAP contains a TIR domain, which is responsible for association with another adaptor protein, MyD88; and a N-terminal PIP binding domain (PBD) that allows TIRAP's promiscuous binding to various lipid targets, such as phosphatidylinositol 4,5-bisphosphate (PIP_2) at the plasma membrane and phosphatidylinositol 3-phosphate (PI_3P) on the endosomes, resulting in functional formation of myddosome in both locations. A minimum region (residues 15-35) in PBD is sufficient to associate to plasma membrane. We show that this region, we called the PIP-binding motif (PBM) adopts a helical conformation in the presence of artificial membrane, such as dodecylphosphocholine micelles, and more importantly, monodispersed PIP_2 and PI_3P can induce helical structure of TIRAP PBM, but neither monodispersed phosphatidylinositol nor inositol trisphosphate (head group of PIP_2 , IP_3) induce TIRAP PBM's helical structure. Resonance assignments of TIRAP PBM NMR spectra reveal that conserved basic residues Lys16, Lys31, and Lys32 play important roles in association with PIPs at either plasma membrane or endosome. Mutagenesis studies identified key- PIP_2 interacting residues of TIRAP PBM. We are in the process to precisely identify the PIPs binding site in TIRAP PBM and to obtain the solution structure of it in the free and PIP_2 -bound states to understand the progressive mechanism and regulation of membrane association of the protein.

STRUCTURAL STUDIES OF A DUAL-DOMAIN SYSTEM FROM THE GIANT MUSCLE PROTEIN OBSCURIN (Ig58/50). N. T. Wright, L. C. Meyer, M. C. Oehler & T. A. Caldwell, Dept. of Chemistry and Biochemistry, James Madison University.

Obscurin is a giant protein (800-900 kDa at its largest) that serves to link the contractile apparatus to the surrounding membrane structure in muscle cells. This protein is highly modular in nature, and mostly consists of tandem Ig domains. Each of these domains has a unique sequence, and each domain that has been extensively studied has unique binding partners. Two of these domains- Ig58 and Ig59- are both required to bind to the giant muscle protein titin at ZIg9 and ZIg10. An R->Q mutation in the obscurin Ig58 domain is associated with cardiomyopathy, presumably through disruption of obscurin/titin binding. Here, we present the high resolution structures of Ig58 and Ig59. Both domains fold in a typical Ig fold, but Ig58 has an extensively charged surface. NMR analysis shows that no structural deformations occur with the R4344Q mutation, however MD simulations indicate that the surface electrostatic interactions change significantly. SAXS data and NMR data suggest that Ig58 and Ig59 do not extensively interact in solution. These findings suggest a molecular mechanism of how obscurin interacts with its targets.

OLIGOMERIC STATES OF AN ADENOVIRUS RECEPTOR, CD46, DIFFER DUE TO ALTERNATIVE SPLICING. Emily L. Romanoff & Eugene Y. Wu, Dept. of Biology, 28 Westhampton Way, University of Richmond, Virginia 23173. CD46 is an integral membrane protein expressed on the surfaces of many human cells. CD46 is alternatively spliced into BC (~60-65 kDa) and C (50-55 kDa) isoforms; the BC isoform contains an extra serine-threonine-proline rich (STP) domain that is highly O-linked glycosylated. Adenovirus type 37 (Ad37), a species D adenovirus, can use the C isoforms of CD46 as a receptor to cause ocular and genital tract infections, but Ad37 does not bind A549 lung epithelial cells efficiently. Based on existing structural data, we hypothesized that the extra B STP domain of CD46 could prevent oligomerization at the cell surface. To explore whether adenovirus tropism is linked to CD46 isoform expression, we treated HeLa cervical carcinoma cells, which are known to express both BC and C isoforms, with a chemical crosslinker that does not cross the cell membrane. Western blotting of the cell lysate showed no substantial change in the migration of the BC isoforms of CD46 after crosslinking, but the C isoforms exhibited a large change in molecular weight to > 250 kDa. Western blotting of crosslinked A549 cells, which only express the 60-65 kDa isoforms, also showed no substantial change in molecular weight of CD46. These results indicate that alternative splicing of CD46 produces two different extracellular domains that can be differentiated by their oligomeric states. The absence of CD46 C isoforms on lung epithelial cells correlates with low binding and infection by Ad37, which may preferentially bind oligomeric CD46. These results may have implications for the tropisms of many human pathogens that use CD46 as a receptor.

IONOTROPIC GLUTAMATE RECEPTOR DYNAMICS. D. Holley¹, S. Holmes¹, A. Hahn², & C. A. Parish¹, ¹Department of Chemistry, University of Richmond, Richmond VA 23173 & ²Harvard School of Dental Medicine, Harvard University, Boston, MA 02115. Ionotropic glutamate receptors (iGluRs) are tetrameric, ligand-gated ion channels that initiate post-synaptic, fast, excitatory neurotransmission in the mammalian central nervous system. The gating mechanism involves a conformational change that arranges the transmembrane region into a pore, allowing ions to move across the neuronal membrane. Glutamate, which is released as a neurotransmitter by

pre-synaptic neurons, induces this conformational change by binding the iGluR ligand-binding domain (LBD), forming bonds that stabilize the clamshell-like structure of the LBD into a sustained, occluded conformation. The ligand-induced, sustained closure of the LBD may instigate the pore-forming conformational change in the transmembrane domain. Understanding LBD structural dynamics is a necessary step in deconstructing the mechanism that leads to glutamate binding. Here, we use 400 and 600 ns all-atom molecular dynamics (MD) simulations to measure the stability and inherent motions of the isolated, solvated, apo iGluR ligand binding domains for three iGluR subfamily-specific subunits: GluR2, NR2A, and NR2B. As no crystal structures are available for NR2B, we constructed a homology model based on NR2A sequence similarity (PDB code 2A5S). We show that a majority of the large-scale LBD dynamics can be described by three different eigenvectors, and that the first eigenvector correlates to the opening and closing of the LBD, which we estimate occurs every 60-150ns. Our results also indicate that LBD hinged movement may be constrained by LBD dimer interactions, suggesting that the flexibility of the isolated LBD monomer reported in several recent MD studies may in part represent an artifact of monomeric systems.

GLUTAMATE INDUCED CHANGES IN CONFORMATIONAL FLEXIBILITY IN THE LIGAND BINDING DOMAIN IONOTROPIC GLUTAMATE TRVR[YPTD]. Philip Varnes, Forest Barkdoll-Weil, Carlos Metz, & Ellis Bell, Chemistry Dept., University of Richmond, Richmond, VA. Ionotropic Glutamate receptors (iGluRs) are tetrameric proteins with 4 domains, an amino terminal domain, the ligand binding domain, the transmembrane ion channel and a largely disordered intracellular C terminal region. The ligand binding domain binds glutamate, a major fast excitatory neurotransmitter, which activates the receptor channels. There are three main families of iGluRs, AMPA, NMDA, and kainite which among other things differ in their responses to two endogenous neurosteroids, pregnenolone sulfate (PS) and pregnenolone sulfate (PREGAS). To investigate the role that conformational flexibility of the ligand binding domain plays in response to either glutamate or the neurosteroids (in the presence or absence of glutamate) we have used Multiwavelength Collisional Quenching (MWCQ), a novel approach to collisional quenching using multiwavelength analysis of spectral data collected at two different excitation wavelengths which reports on the exposure and charge environment of tyrosines and tryptophans in the protein. While Glutamate has different effects on flexibility depending upon the family of receptor, the use of differently charged quenching molecules shows glutamate induced changes in the charge environment that can be correlated with steroid binding ability. This work was supported by NSF Grants MCB-104995 and MCB 0448905 to EB.

A COMPUTATIONAL MODEL FOR THE IRON HOMEOSTATIS IN *E. Coli*. Ovidiu Lipan, Dept. of Physics, Univ. of Richmond, Richmond VA, 23173. In order to establish an infection in a human organism, bacteria must grow and multiply effectively. Bacteria need iron for growth and for that reason a homeostatic system evolved to control iron acquisition and the intracellular iron levels. Iron is one of the important factors intimately connected to the synthesis of some of the virulence determinants. *E. Coli* bacteria are highly used as an experimental model for understanding the adaptation of bacteria to different iron environments. A couple of deterministic models for the iron homeostatic system appeared in the literature.

However they do not incorporate the abrupt probabilistic discontinuities that appear at the end of the bacteria's cell cycle. At division, the number of molecules is split between the mother and the daughter cell. Also, the moment in time for division is not strictly periodic and depends on the maturity of the bacteria. Following an idea of von Foerster and Rubinow we present a computational model for the Iron homeostatic system in *E. Coli* that combines a deterministic approach for the regulatory system with a probabilistic model for the bacteria's growth and division processes. We present the method to obtain the partial differential equation and a numerical approach to solve for the time evolution.

EXPLORING THE STEROID BINDING SITE OF iGLURS BY LIMITED PROTEOLYSIS AND HYDROGEN DEUTERIUM EXCHANGE. Emily Bartle & Ellis Bell, Dept. of Biochemistry, University of Richmond, Richmond VA 23060. AMPA, NMDA, and kainate receptors belong to the ionotropic glutamate (iGluR) family of receptors located in the post-synaptic neural membrane. These receptors bind glutamate, a major fast excitatory neurotransmitter, which activates the receptor channels. Consequently, the receptor's proper function plays an important role in synaptic plasticity, memory and learning. Mis-regulation of these receptors has been linked to many neurodegenerative diseases, such as Alzheimer's and Parkinson's disease. Thus, our research focuses on understanding the receptors' activity and regulation to ultimately develop therapeutic applications. Additionally, two endogenous neurosteroids, pregnenalone sulfate (PS) and pregnenalone sulfate (PREGAS), are known to contribute to regulating the potentiating and inhibiting effects of iGluRs on neuronal activity. Therefore, the primary aim of this branch of the project is to determine the exact locations for the binding sites of these two neurosteroids to the receptors. Using molecular docking studies and other computational methods, several potentially significant residues have been suggested by our collaborator and subsequently tested via sited directed mutagenesis. Because the site directed mutagenesis has yet to produce significant changes in binding affinity, we are attempting to answer the same question through alternative methods, such as limited trypsin proteolysis and hydrogen/deuterium exchange protection assays.

INVESTIGATION INTO THE OPENING MECHANISM FOR DNA POLYMERASE USING MOLECULAR DYNAMICS SIMULATIONS. Bill R. Miller III^{1,2}, Carol A. Parish² & Eugene Y. Wu¹, ¹Dept. of Biol., Univ. of Richmond & ²Dept. of Chem., Univ. of Richmond, Richmond VA 23173. Crystal structures of DNA polymerase, a key enzyme for DNA replication in organisms, have shown an important α -helix on the enzyme, called the O-helix, plays a crucial role in binding the incoming dNTP. The O-helix can form an "open", "ajar", and "closed" conformation in the presence or absence of certain substrates in the active site. Although crystal structures have given insight into these conformations, traditional biochemistry experimental techniques are unable to capture the details of the transition between these states and how the polymerase traverses the potential energy surface from open to closed and back again. In this study, we have utilized recent computational advances to simulate the opening of the fingers domain starting from the closed and ajar conformations of *Bacillus stearothermophilus* DNA polymerase Klenow fragment using dynamics on the microsecond time scale, and also simulated the open state for comparison. We have fully characterized the opening

process that occurs after catalysis, and determined the key events and movements that are critical to O-helix opening. The transition from ajar to open is quick (<20 ns), while the full transition from closed to open was observed taking nearly 300 ns. We observed a key intermediate step in the pathway from closed to open involving a salt bridge between an arginine side chain on the O-helix and an aspartate in the thumb domain. These simulations aid in the elucidation of the O-helix opening mechanism for DNA polymerase on an atomistic level not currently available with experimental measures.

EXAMINING THE IMPACT OF MUTATIONS IN THE X-LINKED MENTAL RETARDATION PROTEIN CASK. Leslie E. W. LaConte, Vrushali Chavan, Jeffrey Willis, & Konark Mukherjee, Virginia Tech Carilion Research Institute, Roanoke VA 24016. Mutations in the protein CASK (calcium/calmodulin-activated Ser-Thr kinase) are associated with a range of severe phenotypes, including pontocerebellar hypoplasia, microcephaly, optic nerve hypoplasia, intellectual disabilities, and autistic characteristics. CASK is widely expressed, but is most highly expressed in the central nervous system. Although a number of roles have been proposed (scaffolding, trafficking, transcription factor, kinase), no single function has been definitely identified as responsible for the severe phenotypes, although a CASK hypomorph mouse phenocopies the human condition quite well. A careful phylogenetic, structural, computational, and in vitro analysis of five known pathogenic mutations of CASK reveals that in high conserved regions, mutations are structural, whereas in less conserved regions, mutations are functional (less likely to lead to protein unfolding). Predicting the pathogenic nature of a sequence variant remains challenging to do computationally, but a simple cell-based assay helped quickly identify CASK mutations that led to protein misfolding and aggregation. This aggregation was reversible with the addition of glycerol to cell culture. More detailed future studies of CASK mutations will offer further insight into the function of CASK.

INTRINSIC DISORDER IN ADAPTOR PROTEINS INCREASES COMMITMENT TO ENDOSOMAL PROTEIN TRAFFICKING. Daniel G. S. Capelluto, Dept. Biol. Sci., Virginia Tech, Blacksburg VA 24061. Adaptor proteins are often committed to cellular processes that involve cargo internalization from the plasma membrane. Ubiquitinated cargo is internalized by endocytosis and delivered to early endosomes *via* intracellular vesicles. Adaptor proteins, such as Tollip and Tom1, facilitate cargo sorting through their ubiquitin-binding domains. Tollip is localized to early endosomes, through binding to phosphatidylinositol 3-phosphate (PtdIns(3)P). Tom1 can also bind ubiquitin-conjugated cargo and is recruited to the endosomal membranes through its association with Tollip. In this work, we demonstrate that binding of Tollip to PtdIns(3)P is negatively modulated by interaction with Tom1. Structural studies determine that the Tom1-binding domain (TBD) of Tollip is intrinsically disordered and folds upon binding to the Tom1 GAT domain, which also undergoes a conformational change upon binding. Intermolecular NOEs of the Tollip TBD-Tom1 GAT complex indicate that association is mainly driven by hydrophobic contacts with high affinity. Ubiquitin binds to the Tom1 GAT domain at a site that does not overlap with that for the Tollip TBD, allowing their simultaneous binding. Using fluorescence microscopy, we show that mutations in the binding interphase of the Tom1 GAT and Tollip TBD complex leads to a dissociation of the proteins and triggers cytosolic

localization of Tom1. Accordingly, we propose that association of Tom1 to Tollip helps to release Tollip from endosomal membranes, allowing Tollip to commit to endosomal ubiquitinated cargo trafficking.

Posters

DISABLED-2 MODULATES ERYTHROCYTIC INTERACTIONS THROUGH ITS SULFATIDE BINDING DOMAIN. Kaitlyn J. Andreano¹, Mi Song Kim¹, Daniel G. S. Capelluto², & Carla V. Finkielstein¹, ¹Integrated Cellular Responses Laboratory, ²Protein Signaling Domains Laboratory, Department of Biological Sciences, Virginia Polytechnic Institute and State University. Platelets' role in cancer progression and metastasis has largely been attributed to platelet-mediated enhancement of tumor cell survival, extravasation, and angiogenesis. Correlations exist between the tumor cells' ability to aggregate platelets in vitro and their metastatic potential in vivo which is manifested as a hypercoagulable state found in most cancer patients. We have identified a tumor suppressor molecule (Dab-2) that is released upon platelet activation and that modulates the extent of blood clotting. Our results show Dab-2 is released from alpha-granules in the platelet to the membrane surface where it binds to the integrin receptor, thus inhibiting platelet aggregation. Binding of Dab-2 to integrins is modulated by sulfatides, a glycosphingolipid that accumulates in platelet membrane and effectively competes for Dab-2 levels. We found that Dab-2's N-terminal region binds to sulfatides through two conserved sulfatide-binding sites. Upon activation, sulfatides protect Dab-2 from thrombin cleavage, facilitate its internalization and modulate the surface expression of P-selectin, a coagulation protein needed for stabilization of platelet aggregates. P-selectin also mediates tumor cell adhesion to vascular endothelial cells and the interaction between activated platelets and cancer cells (emboli) during metastasis. Our data show that Dab-2/sulfatide recognition influences the stability of platelet aggregates heterotypically with cancer cells through sulfatide binding and, indirectly, by controlling P-selectin levels.

RECOGNITION MAPPING OF BIMOLECULES ON SURFACES USING ATOMIC FORCE MICROSCOPY. Congzhou Wang & Vamsi K. Yadavalli, Department of Chemical and Life Science Engineering, Virginia Commonwealth University. Atomic force microscopy based adhesion force measurements have emerged as a powerful tool in the biophysical analysis of biological systems. Such measurements can now be extended to mapping biomolecules on biosurfaces via integrated imaging and force spectroscopy techniques. Critical to these experiments is the choice of the biomolecular recognition probe. We demonstrate how oligonucleotide aptamers can be used as versatile probes to image and spatially locate specific targets on surfaces. The unique advantage of this technique is its capability of localizing specific biomolecules via simultaneous topographic and force information in a short time. We focus on two structurally distinct proteins - human alpha thrombin and vascular endothelial growth factor (VEGF165) relevant to the clotting cascade. Via AFM-recognition mapping using their specific DNA aptamers, a clear consistency between height and force map obtained simultaneously on same areas is shown. The aptamer specificity and ability to distinguish their targets is shown through positive and negative controls. In addition, changes in binding due to blocking by free heparin shows the ability to study

fluctuating biological systems in real time. These two channels may be overlaid to form high resolution maps to identify proteins at the single molecule level. These results show that this approach has the capability to detect the presence of specific biomolecules which can increase our understanding of the fundamental organization, mechanics, interactions and processes on complex surfaces such as cells.

ASSESSING STUDENT UNDERSTANDING OF FOUNDATIONAL CONCEPTS OF PROTEIN STRUCTURE AND FUNCTION. Ellis Bell, Department of Chemistry, University of Richmond, Richmond, VA. A number of reports emphasize the importance of student understanding of foundational concepts and core competencies in the context of both disciplinary and interdisciplinary content and skills. For biochemistry and molecular biology, the content knowledge, skills and allied fields necessary for the discipline have been discussed in recent papers. Protein Structure and Function was identified as one of the five essential content areas. Principles of scientific teaching and backward design that have been used to align six critical components of understanding protein structure function relationships with focused assessment of student understanding and potential teaching strategies are illustrated. Of the six, two align with key features of understanding structure (Bonding & Dynamics), two with understanding biological function (Catalysis & Regulation), while two (Interactions & Evolution) clearly bridge the other concepts. Integrated with these core concepts of protein structure and function are the essential interdisciplinary concepts of Modularity, Energy, Change over time, Stochasticity and the Use of Mathematical models. Using a modified Bloom's taxonomy potential, assessments aligned to each concept of protein structure and function are discussed. In addition to assessing student understanding, the goal of such assessments is to incorporate key aspects of both the allied fields and the skills necessary for student success in biochemistry and molecular biology. Finally, potential student centered teaching strategies for introductory, gateway or capstone courses will be presented. This work was funded by NSF RCN-UBE Grant 0957205 to EB.

INVESTIGATION OF ENZYME KINETICS OF N-TERMINAL RCC1 PROTEIN METHYLTRANSFERASE REVEALS A DISTRIBUTIVE RANDOM ORDERED BI-BI MECHANISM. Stacie L. Richardson¹, Gang Zhang¹, Pahul Hanjra¹, Yunfei Mao¹, Darrell L. Peterson², & Rong Huang¹, ¹Department of Medicinal Chemistry & ²Department of Biochemistry and Molecular Biology, Virginia Commonwealth University, Richmond VA 23219. Human protein N-terminal RCC1 methyltransferase 1 (NRMT1) methylates the N-terminal alpha-amino group of its protein substrates. This is the first example of a mammalian methyltransferase responsible for protein N-terminal methylation. NRMT1 is overexpressed in several types of cancer, including gastric, breast, head, and neck cancers, making it a potential therapeutic target. To understand its kinetic mechanism, we developed an assay for direct ratiometric quantitation by MALDI mass spectrometry. We have characterized the kinetic mechanism of recombinant NRMT1 using a MALDI-MS method for monitoring the various levels of methylation of its substrate peptides. We found that NRMT1 proceeds via a random ordered bi bi mechanism through Lineweaver-Burk analysis of the inhibitory patterns of the natural and dead-end products. Results of the processivity studies indicate that NRMT1 proceeds via a distributive mechanism for multiple

methyations. Based on our results, a bisubstrate inhibitor that mimics the structure of the ternary complex could be an effective selective inhibitor of NRMT1. In our ongoing studies we will investigate the inhibitory activity and mechanism of inhibitors designed in our lab against NRMT1 and its substrates.

INVESTIGATION OF SUPPRESSOR OF IKK-EPSILON FUNCTION THROUGH STRUCTURAL ANALYSIS. Sean W. McKinley¹ & Jessica K. Bell², ¹Department of Microbiology & Immunology, ²Department of Biochemistry & Molecular Biology, Virginia Commonwealth University, Richmond, VA 23298. During viral infections, Toll-like receptor 3 (TLR3) stimulation initiates signaling to activate transcription of pro-inflammatory cytokines and type-1 Interferons. Suppressor of IKK ϵ (SIKE) interacts with two kinases in the signaling pathway, IKK ϵ and TANK binding kinase 1 (TBK1), inhibiting the transcription of type I interferons. Recently, we discovered that SIKE blocks TBK1-mediated activation of type I interferons by acting as a high affinity, alternative substrate of TBK1. To further characterize SIKE's function within the antiviral response, this study focused on defining the overall SIKE structure through X-ray crystallography. Using recombinant protein expressed from *E. coli* and purified via immobilized metal affinity chromatography, SIKE crystals were obtained from a sample concentrated to 15 mg/ml under several crystallization conditions. Yet, reproducing these results has been difficult. In this study, we have modified the purification scheme to remove an *E. coli* contaminant, SlyD. Purification under denaturing conditions, native conditions, incorporation of ion exchange and different IMAC resins has been tested. For each scheme, size exclusion chromatography and SDS-PAGE/Coomassie/silver stain were used to assess purity. Crystallization trials for samples from each purification scheme were completed. Supported by CCTR Endowment Fund.

USING COLOCALIZATION ASSAYS TO DEFINE SUPPRESSOR OF IKK-EPSILON FUNCTION. Kenneth F. Lawrence¹ & Jessica K. Bell², ¹Department of Microbiology & Immunology, ²Department of Biochemistry & Molecular Biology, Virginia Commonwealth University, Richmond, VA 23298. The innate immune system is the body's first line of defense against pathogens. Signaling pathways initiated by pattern recognition receptors, such as Toll-like receptor 3 (TLR3), communicate the danger signal recognized by the receptor into the cell to mediate a rapid host defense. Suppressor of IKK-epsilon (SIKE) functions downstream of TLR3. Initially identified as an inhibitor of the TANK binding kinase 1 (TBK1)-mediated interferon response, we discovered that SIKE is a substrate of TANK-binding kinase 1 (TBK1). Co-immunoprecipitation assays of SIKE from epithelial and myeloid cells lines were analyzed by tandem mass spectrometry to identify interaction partners. These studies suggested that the SIKE interaction network impinged upon the cytoskeleton and RNA transport. We hypothesized that SIKE links TLR3 signaling to formation/resolution of RNA granules. To further delineate SIKE's role in RNA transport and cytoskeletal rearrangements, we used immunofluorescence assays to identify colocalization between SIKE and cellular markers in epithelial and myeloid cell types. Colocalization was quantified either at the level of whole cell or region of interest. SIKE was found to colocalize with cytoskeleton components (actin and tubulin), endosomal and plasma membrane markers (Rab11, LAMP-1, and LC3), and

ribosomes (S6). These results are consistent with our hypothesis that SIKE plays a role in trafficking related to the anti-viral innate immune response. Supported by CCTR Endowment Fund and NIH R21AI107447.

DESIGN, PREPARATION, AND VALIDATION OF AN ACTIVITY-BASED PROBE OF PROTEIN KINASE A. Robert A. Coover & Keith C. Ellis, Dept. Med. Chem., Va. Commonwealth Univ., Richmond VA 23298. Activity-based protein profiling is an important technique for analyzing the activity of proteins in disease states and validating their pharmacological modulation. The cAMP-dependent protein kinase (PKA) is the prototype kinase and its activity is regulated by multiple endogenous mechanisms, which include phosphorylation, binding to regulatory domains, localization by anchoring proteins, and redox regulation by glutathiolation. These mechanisms directly compete with or reduce substrate binding and/or modulate the conformation of the activation loop, a key motif in PKA activity. Using the crystal structure of PKA, we have developed a selective peptidomimetic covalent probe of PKA that utilizes high affinity portions of the PKI pseudosubstrate inhibitor. PKI is a pseudosubstrate inhibitor that preferentially binds to PKA when the activation loop is ordered and the enzyme is not bound to the regulatory subunit. We have prepared fluorophore-labeled PKI analogues that contain a halomethylketone at the C-terminus (P+2 position). We have evaluated our probe for specificity of labeling (confirmed by gel and MS), selectivity (lysates), and preliminary activity-based experiments and will present these data accordingly. This study was funded in part by the American Cancer Society Institutional Research Grant IRG-73-001-37 and the VCU Massey Cancer Center.

MOLECULAR DYNAMICS STUDIES OF IONOTROPIC GLUTAMATE RECEPTORS. Stephen Holmes, Dave Holley, Alex Hahn, Lisa Gentile, & Carol Parish, Department of Chemistry, Gottwald Center for the Sciences, University of Richmond, Richmond, VA 23173. Ionotropic glutamate receptors, iGluRs, are a family of ligand gated channels located in the post-synaptic neural membrane. These receptors serve a major role in the excitation of the neural cells, which is important for all neurotransmissions in the central nervous system. There are three subfamilies of iGluRs: NMDA, AMPA, and kainite receptors. NMDA receptors mediate the fast component of the excitatory post synaptic potential. AMPA receptors mediate fast synaptic transmission in the central nervous system. Kainate receptors have a major role in excitatory neurotransmission. A conformational change that opens a membrane pore is caused by the binding of the neurotransmitter glutamate to the extracellular ligand binding domain on these receptors, allowing cations to flow into the post-synaptic neural cell. Because glutamate functions as a major excitatory neurotransmitter in the central nervous system, the level of activity of iGluRs is strictly controlled. Neurodegenerative diseases such as schizophrenia and Alzheimer's, Parkinson's, and Huntington's diseases have been linked to mis-regulation of glutamate. Long time scale molecular dynamics studies using molecular dynamics were performed on a homology model for the S1S2 domain of the NMDA iGluR receptor. The resulting stable trajectories were subjected to exhaustive analysis. Dynamical results will be presented that shed light on the structure and function of these glutamate receptors.

X-RAY CRYSTALLOGRAPHY & SMALL ANGLE X-RAY SCATTERING STUDIES OF INTERFERON REGULATORY FACTOR 4. Soumya Govinda Remesh & Carlos R. Escalante, Department of Physiology & Biophysics, Virginia Commonwealth University, VA 23298-0613 USA. Interferon (IFN) regulatory factor family member (IRF4) is a transcription factor that serves specific roles in transcriptional regulation of IFN responsive genes is limited to the immune system. Generally, members of IRF family, like IRF3 and IRF5, have carboxy terminal auto-inhibitory regions that are phosphorylated to generate a transcriptionally active dimer. IRF4 also has a carboxy terminal auto-inhibitory region but it gets activated by binding to multiple different partners. We have determined the crystal structure of the C-terminal activation domain of IRF4 and carried out small angle X-ray scattering (SAXS) studies to generate *ab initio* models for the full-length protein to obtain insights into the autoinhibitory mechanism. The data suggests that the putative linker of IRF4 connecting the N- and C-terminus is most likely a folded well-structured domain that interacts with the auto-inhibitory carboxy tail indicative of a different mechanism of activation for IRF4 compared to the other members of the family. We are currently trying to further our understanding of the activation process for IRF4 that will aid in highlighting its uniqueness within the IRF family. Information obtained by the current study will help in development of novel therapeutics in disease states that are mediated by IRF4 like multiple myeloma, cardiac hypertrophy and certain autoimmune diseases.

ASSEMBLY OF AAV2 REP68 ON AAVS1 DNA REQUIRES COOPERATIVITY OF INDIVIDUAL DOMAINS AND OLIGOMERIZATION. Clayton M. Bishop, Francisco Zarate-Perez, Faik N. Musayev, & Carlos R. Escalante, Department of Physiology and Biophysics, Virginia Commonwealth University, 1101 East Marshall Street, Richmond, VA 23298-0551 USA. Multiple DNA transactions are at the center of almost all processes regulating AAV life cycle. A common feature shared by all transactions is the binding of the large AAV Rep proteins Rep78/Rep68 onto DNA sites harboring multiple GCTC repeats. AAV mediated site-specific integration is contingent upon the formation of a productive complex between Rep78/Rep68 and the AAVS1 site located at chromosome 19. In order to understand the mechanistic details of the initial assembly process we carried out equilibrium binding experiments of Rep68 and its individual domains with a 42-mer AAVS1 site. Results show that although Rep68 binds AAVS1 with high affinity (69 nM), both the OBD and helicase individual domains bind DNA weakly with affinities of $\gg 60\mu\text{M}$ and $22\mu\text{M}$ respectively under our experimental conditions. Mutant Rep68 proteins that have a defective oligomerization interface bind DNA poorly suggesting that productive binding requires both the concerted interaction of the individual domains with DNA and oligomerization. Moreover, we show that a minimal number of two repeats is required to form a stable complex.

STRUCTURAL AND FUNCTIONAL BASIS OF TOLLIP ASSOCIATION TO THE ENDOSOMAL ADAPTOR PROTEIN TOM1. Mary K. Brannon¹, Shuyan Xiao¹, Geoffrey Armstrong³, Kristen Fread¹, Carla V. Finkielstein², & Daniel G. S. Capelluto¹, ¹Protein Signaling Domains and ²Integrated Cellular Responses Laboratories, Department of Biological Sciences, Virginia Tech, Blacksburg VA, 24061 & ³Department of Chemistry and Biochemistry, Boulder CO, 80309. Adaptor proteins

facilitate cellular cargo sorting through their ubiquitin-binding domains, such as Tollip and Tom1. The interaction of these two proteins is proposed to be involved in the lysosomal degradation of polyubiquitinated cargo. In this work, we demonstrate that binding of Tollip to PtdIns(3)P is negatively modulated by interaction with Tom1. Structural studies determined that the Tom1-binding domain (TBD) of Tollip is intrinsically disordered and folds upon binding to the Tom1 GAT domain, which also undergoes a conformational change upon binding. Intermolecular NOEs of the Tollip TBD-Tom1 GAT complex indicate that association is mainly driven by hydrophobic contacts with very high affinity. Ubiquitin binds to the Tom1 GAT domain at a site that does not overlap with that for the Tollip TBD, but the binding events are mutually exclusive and are driven by conformational changes in the GAT domain. Endosomal localization of Tom1 depends on the presence of Tollip in this compartment. Using fluorescence microscopy, we show that mutations in the binding interphase of the Tom1 GAT and Tollip TBD complex leads to a dissociation of the proteins and triggers cytosolic localization of Tom1. Consequently, we propose that association of Tom1 to Tollip helps to release Tollip from endosomal membranes, allowing Tollip to commit to endosomal ubiquitinated cargo trafficking.

ELECTRON MICROSCOPY STUDIES OF REP68-AAVS1 COMPLEX. Francisco Zarate-Perez, Montserrat Samso, & Carlos R. Escalante, Department of Physiology and Biophysics, Virginia Commonwealth University, 1101 East Marshall Street, Richmond, VA 23298-0551. Rep68 is a multifunctional protein of the Adeno-associated virus (AAV), a parvovirus widely used as a gene therapy vector. Recognition of the Rep-binding site (RBS) by the N-terminal origin-binding domain (OBD) leads to the assembly of a Rep78/Rep68-DNA complex in a process that is highly cooperative. The stoichiometry and nature of this complex is not known. In this work we used a combination of Electron microscopy (EM), single reconstruction, and image analyses to determine the three-dimensional structure of Rep68-AAVS1 complex. We obtained a model that suggests a 7-ring shape structure, which exhibit defined monomer units conforming the molecule. The rings exhibit dimensions of 180 Å X 140-150 Å, and a 34-Å diameter central cavity that appears to be constant at both sides of the particle and is enough wide for the DNA insertion of the DNA. We perform a combination of modeling and fitting using both OBD and Helicase crystallographic structures into the EM model to analyze the EM structure at an atomic level, thus allowing a localization of each domain in the individual monomers. The structure illustrates the remarkable flexibility of Rep proteins to form a diverse number of oligomeric complexes.