on their potential combined effects. Standard statistical methods suffer from multicollinearity effects due to the complex correlation structure among the components. We have developed novel statistical methods to determine empirical weights that identify subsets of components most associated with a response variable. The results are weighted indices of body burden and nutrients – i.e., simple to understand and visualize indices that may be easily combined with other behavioral data for additional analyses. Characterization of the approach will be described through simulation studies. The methods will be demonstrated using NHANES data.

Structural Biology, Biochemistry, and Biophysics

UBIQUITIN MODULATES TOLLIP'S LIPID-MEDIATED ENDOSOMAL MEMBRANE BINDING. S. Mitra, A. Traughber, S. Gomez, & D. G. S. Capelluto. Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061. Ubiquitylation is a highly controlled post-translational modification of proteins, in which proteins are conjugated either with monoubiquitin or polyubiquitin chains. Ubiquitin modifications on target proteins are recognized by ubiquitin-binding domains, which are found in several effector proteins. In this study, we describe for the first time how ubiquitin controls the function of the Toll-interacting protein (Tollip), which is an effector protein in the innate immune signalling pathway and an adaptor protein for endosomal trafficking. We have demonstrated that the central C2 domain of Tollip preferentially interacts with phosphoinositides. Remarkably, we have observed an ubiquitin dose-dependent inhibition of binding of Tollip to phosphoinositides and it does so specifically by blocking Tollip C2 domain-phosphoinositide interactions. This led us to discover that the Tollip C2 domain is a novel ubiquitin-binding domain. In addition, we have biophysically characterized the association of the Tollip CUE domain to ubiquitin and compared it with Tollip C2 domain-ubiquitin binding. We have also found that ubiquitin binding to dimeric Tollip CUE domain induces a drastic conformational change in the protein, leading to the formation of a heterodimeric Tollip CUE-ubiquitin complex. These data suggest that ubiquitin binding to the Tollip C2 and CUE domain and ubiquitin-mediated dissociation of CUE dimer reduces the affinity of Tollip protein for endosomal phosphoinositides, allowing Tollip's cytoplasmic sequestration for its cytosolic commitments.

MECHANISTIC STUDIES OF A FLAVIN DEPENDENT LYSINE MONOOXYGENASE FROM *NOCARDIA FARCINICA*. R.M. Robinson, P. Rodriguez, N. Keul, & P. Sobrado, Department of Biochemistry, Virginia Tech, Blacksburg VA 24061. The kinetic and chemical mechanism of the lysine monooxygenase Nocobactin G (NbtG) from *Nocardia farcinica* was investigated using both steady-state and rapid reaction kinetics. NbtG hydroxylates both L- and D-lysine, a unique characteristic among members of the *N*-hydroxylating monooxygenases (NMOs), which are typically selective for the L-stereoisomer of their respective substrate. NbtG is mostly uncoupled (~75%) and produces superoxide and hydrogen peroxide as oxidative by-products. O_2 consumption increases from 0.581 s^{-1} in the absence of lysine to 1.09 s^{-1} and 3.03 s^{-1} when saturating concentrations of L- and D-

lysine are present, respectively. NbtG has a ~8-fold higher k_{cat}/K_m value for L-lysine suggesting it is the preferred substrate. Reduction of NbtG by NADPH is independent of both lysine stereoisomers and occurs in two exponential phases with rates of 1.00 s^{-1} and 0.119 s^{-1} for the first and second phase, respectively. Primary kinetic isotope effect studies with pro-(*R*)-NADPD indicate a ${}^{D}k_{red}$ value of ~1.5 where both phases of reduction are isotopically sensitive. The rate of flavin oxidation is affected by lysine and increases from 3.1 x 10^3 $M^{-1}s^{-1}$ in the absence of lysine to 3.9 x 10^3 $M^{-1}s^{-1}$ and 5.4 x 10^3 $M^{-1}s^{-1}$ when saturating concentrations of L- and D-lysine are present, respectively. Solvent kinetic isotope studies yield a ${}^{D2O}k_{cat}$ value of ~2.4. These results indicate the active site of NbtG is loosely stereospecific for lysine and hydride transfer is only partially rate-limiting, but a chemical step that is influenced by proton exchange with solvent is rate-limiting. This work was supported by a grant from the National Science Foundation MCB-1021384.

A CONSERVATIVE ISOLEUCINE TO LEUCINE MUTATION CAUSES MAJOR REARRANGEMENTS AND COLD-SENSITIVITY IN KLENTAQ DNA POLYMERASE. E. Y. Wu[1], E. P. Kornberg[1], B. Zielinski[1], W. M. Barnes[2], & M. B. Kermekchiev[3], [1]Dept. of Biology, University of Richmond, Richmond, VA 23173, [2]Dept. of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO 63110, & [3]DNA Polymerase Technology, Inc., St. Louis, MO 63104. Assembly of PCRs at room temperature can lead to low yields or unintentional products due to mispriming. A conservative mutation of isoleucine 707 to leucine in DNA polymerase I from *Thermus aquaticus* substantially decreases its activity at room temperature without compromising its ability to amplify DNA. To understand why such a small change located 24Å from the active site can have a large impact on enzyme activity at low temperature, we solved the X-ray crystal structure of the large fragment of *Taq* DNA polymerase containing the cold-sensitive mutation (Cs3C KlenTaq) in the closed, ternary (E-DNA-ddNTP) and open, binary (E-DNA) complexes. The Cs3C KlenTaq ternary complex was identical to the wild-type except for the mutation and a rotamer change in a nearby phenylalanine 749, suggesting that the enzyme should remain active. However, soaking out the nucleotide substrate at low temperature results in an altered binary complex made possible by the rotamer change at F749 near the tip of the polymerase O-helix. Surprisingly, two adenosines in the 5′-template overhang fill the vacated active site by stacking with the primer strand, thereby blocking the active site at low temperature. These results explain the cold sensitivity phenotype of the I707L mutation in KlenTaq and serve as an example of a large conformational change affected by a conservative mutation.

EVOLUTION AND NETWORK ANALYSIS OF LIPOCALIN PROTEINS USING COMPUTATIONAL APPROACHES. Nardos F. Sori & Lesley H. Greene. Dept. of Chemistry and Biochemistry, Old Dominion University, Norfolk Va. 23529. The lipocalins are a functionally diverse and divergent superfamily of proteins that share a common three- dimensional structure which consists of an antiparallel β-barrel and a C-terminal α-helix. Lipocalins are found in various organisms with a wide range of functions such as pheromone activity, lipid transport and coloration. Even though lipocalins are found widely in nature, the first indication of its existence in plants was observed in late 20th century by the sequence analysis of Violaxanthin de-epoxidase

and Zeaxanthin epoxidase, which were shown to be lipocalin-like proteins. Recently lipocalins from Wheat and Arabidopsis were identified as true lipocalins through the elucidation of three structurally conserved regions. The study of these lipocalins is vital as these proteins are believed to help plants tolerate oxidative stress and extreme conditions which broadens our understanding of plant sustainability in different environments. Protein structure homology modeling was used to construct a preliminary structure of the temperature induced wheat lipocalin. Furthermore, conservation of amino acids through evolution was analyzed by using modified Shannon entropy parameter with 20 divergent lipocalins. It revealed that three positions have conservation that have greater than 0.45 which indicates high conservation.

THERMOTOLERANCE IN MAMMALIAN CELLS. Ovidiu Lipan, Dept. of Phys., Univ. of Richmond, Richmond VA, 23173. The heat-shock response network controls the adaptation and survival of the cell against environmental stress. A key element of the heat-shock network is the heat-shock transcription factor-1 (HSF1), which is transiently activated by elevated temperatures. The HSF1 homotrimers bind to the heat shock element on the DNA and control the expression of the hsp70 gene. The Hsp70 proteins protect cells from thermal stress. The modulation of the activity of the hsp70-promoter by the intensity of the input stress is thus critical for cell's survival. The promoter transient activity starts from a basal level and rapidly increases once the stress is applied, reaches a maximum level and attenuates slowly back to the basal level. The molecular construct used as a measure of the response to thermal stress is a Hsp70-GFP fusion gene transfected in Chinese hamster ovary (CHO) cells. The time profile of the GFP protein depends on the transient activity, of the heat shock system. The GFP time profile is recorded using flow cytometry measurements, which allows a quantitative measurement of the fluorescence of a large number of cells (10^4). The GFP responses to one and two heat shocks were measured for 261 conditions of different temperatures and durations. We found that: (i) the response of the cell to two consecutive shocks depends on the order of the input shocks. This phenomenon is thermotolerance without recovery time in between shocks; (ii) the responses may be classified as mild or severe, depending on the temperature level and the duration of the heat shock and (iii) the response is highly sensitive to small variations in temperature.

INHIBITION OF SIDEROPHORE BIOSYNTHESIS BY TARGETING *A. FUMIGATUS* ORNITHINE HYDROXYLASE: A STRUCTURE-BASED VIRTUAL SCREENING STUDY. S. Badieyan[1] & P. Sobrado[1,2], [1]Department of Biochemistry, Virginia Tech, Blacksburg, VA 24061 & [2]Virginia Tech Center for Drug Discovery, Virginia Tech, Blacksburg, VA 24061. Siderophore A (SidA) from *Aspergillus fumigatus* is a flavin-dependent monooxygenase that catalyses hydroxylation of L-ornithine. N5-hydroxyl-ornithine is subsequently incorporated into hydroxamate-containing siderophores. The pathogenicity of *A. fumigatus* in mammals is dependent on the availability and function of siderophores. Thus, inhibition of the siderophore biosynthetic pathway may significantly affect the virulence of this fungus. SidA plays a central role in this pathway and is considered the key target for inhibitory study. Availability of several high-quality structures of SidA in complex with different ligands allowed the in-silico screening of a large library of drugs, natural products, and synthetic compounds. The screening results were sorted by the absolute and normalized

binding energies and then filtered based on the formation of at least one hydrogen bond to the part of the ornithine binding site with several hydrogen bond donors. The top hits have average molecular weights of 200 Da and ΔG bindings of -7.5 kcal/mol. They are highly soluble nitrogen- or oxygen-rich compounds that make several hydrogen bonds with residues in the active site of SidA. This study was supported by NSF grant MCB 1021384 and by the Virginia Tech Biodesign and Bioprocessing Center.

A NOVEL TANK-BINDING KINASE 1 (TBK1) SUBSTRATE AND ITS ENDOGENOUS CONTROL OF A CRITICAL CATALYTIC HUB IN THE TYPE-I INTERFERON RESPONSE. James D. Marion, Jr. & Jessica K. Bell. Virginia Commonwealth University, Richmond, VA 23298. To defend against pathogen challenge, multi-cellular organisms mount an immune response that recognizes, sequesters and eradicates invading infectious agents. In response to receptor-mediated pathogen detection, several signaling pathways converge to activate TBK1, a kinase which phosphorylates substrates that lead to the production of pro-inflammatory cytokines and type-I interferons. Increasingly, TBK1 dysregulation has been linked to autoimmune disorders and cancers, heightening the need to understand its regulatory controls. Here, we describe the mechanism by which Suppressor of IKKe (SIKE) inhibits the TBK1-mediated phosphorylation of IRF3, a transcription factor essential to type-I interferon production. Kinetic analysis revealed that SIKE not only inhibits IRF3 phosphorylation, but is also a high-affinity, TBK1 substrate. Further analysis determined that TBK1 phosphorylation of IRF3 and SIKE displayed negative cooperativity. Biophysical experiments revealed that TBK1 directly phosphorylated SIKE on 6 C-terminal residues that mediated TBK1:SIKE associations and exhibited striking homology to the IRF3 phosphorylation motif. Taken together, our findings demonstrate that SIKE functions as a TBK1 substrate that regulates this critical catalytic hub, not through direct repression of activity, but by redirection of catalysis through substrate affinity.

MOLECULAR MECHANISM OF MEMBRANE TARGETING BY ENDOSOMAL ADAPTOR PROTEINS. Shuyan Xiao & Daniel G. S. Capelluto, Protein Signaling Domains laboratory, Department of Biological Sciences, Virginia Tech, Blacksburg VA, 24061. The Toll-interacting protein (Tollip) is an effector protein in the innate immune signaling pathway and an adaptor protein for endosomal trafficking. Tollip mediates protein sorting by association with Tom1, polyubiquitinated cargo, and clathrin. Tollip is modular in architecture with an N-terminal Tom1-binding domain (TBD), a central C2 domain, followed by a C-terminal CUE domain. Tom1 is a member of a protein family characterized by the presence of the VHS (Vps27/Hrs/STAM) domain at the N terminus, and a central GAT domain. In this study, we identified that Tollip-TBD directly binds to Tom1-GAT with a very high affinity. The solution structure of the complex formed by the Tollip-TBD and Tom1-GAT domain reveals that Tollip-TBD undergoes a coil/helix folding transition upon binding to GAT domain, forming an anti-parallel β-sheet followed by a short α helix structure. The amphipathic helix α1 and α2 of GAT play a key role in the interacting with TBD. We also demonstrated that Tom1 GAT binds to Tollip TBD and ubiquitin in a mutually exclusive manner. Overall, our study provides the structural and molecular basis of Tollip-TBD and Tom1-GAT binding interactions, which will aid in

understanding the role of Tollip in endosomal trafficking. This work was supported by the American Heart Association.

STRUCTURAL BASIS OF PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE (PIP2) RECOGNITION BY THE TIRAP PIP2-BINDING MOTIF. Xiaolin Zhao, Shuyan Xiao, Ayana Stukes & Daniel G. S. Capelluto, Department of Biological Sciences, Virginia Tech., Blacksburg VA. 24061. TIRAP is an adaptor molecule essential to the TLR2 and TLR4 signaling pathways. TIRAP contains a TIR domain, which is responsible for association with another adaptor protein, MyD88; and a N-terminal PIP2 binding domain (PBD) that binds to plasma membrane PIP2. The association of MyD88 and TLR is stabilized by the binding of TIRAP to the plasma membrane. Remarkably, a minimal region (residues 15-35) within PBD is sufficient to associate to the plasma membrane. We show that this region, that we called the PIP2-binding motif (PBM), adopts a helical conformation in the presence of the membrane mimic dodecylphosphocholine micelles and, more importantly, binds PIP2. NMR data indicates that TIRAP PBM binds PIP2 in a fast exchange regime, suggesting a moderate affinity for lipid binding. According to the TIRAP PBM resonance assignments, we found that conserved residues Lys16, Leu18, Lys20, Lys31, and Lys32 play important roles in association with PIP2. We are in the process to precisely identify the PIP2-binding site in TIRAP PBM and to obtain the solution structure of the peptide to understand the progressive mechanism and regulation of membrane insertion of the protein.

PROBING CONFORMATIONAL CHANGES WITH MULTIWAVELENGTH COLLISIONAL QUENCHING. Ellis Bell & Chun Li, Laboratory for Structural Biology, Biophysics & Bioinformatics, Dept. of Chemistry, University of Richmond, Richmond VA 23173. To probe the local environments of tryptophans in proteins we have developed a novel analysis of fluorescence collisional quenching using multiple emission wavelengths, combined with differently charged quenchers (acrylamide: neutral, Iodide: negative & Cesium: positive). With N Acetyl tryptophan, similar Stern-Volmer constants are obtained at all wavelengths, as expected. With glutamate dehydrogenase, (4 tryptophans per subunit), 4 distinct families of constants are obtained with the highest occurring at long wavelengths and progressively decreasing as the wavelength band analyzed decreases showing this approach separates the effects on individual tryptophans in the protein. We have explored nucleotide effects on accessibility and charge environment of the 4 tryptophans. Results indicate that overall, nucleotide ligands NADH, NADPH and ADP significantly lower accessibility particularly in the region of subunit interfaces while GTP has less effect and in the case of W72 and W281 increases accessibility. Acrylamide quenching gives Stern-Volmer plots concave upwards, which become linear when lifetime measurements are used. With NAD(P)H, the charged quenchers, Iodide and Cesium (but not the neutral Acrylamide) give negative slopes which appear to result from differential effects on tryptophan-NAD(P)H resonance energy transfer. Funded in part by NSF Grant MCB 0448905 to EB.

SYNTHETIC BIOLOGY: SYNTHETIC CIRCUITS AS A TOOL FOR BIMOLECULAR SYSTEMS EXPLORATION. Warren C. Ruder, Dept. of Biological

Systems Engineering, Virginia Tech, Blacksburg VA, 24061. Professor Ruder joined Virginia Tech's Biological Systems Engineering department as an assistant professor in 2012. His expertise in synthetic biology, cellular biomechanics, and lab-on-a-chip systems complements Virginia Tech's strengths in biomolecular, biomedical, and environmental engineering. Most recently, he spent two and a half years in the synthetic biology laboratory of James Collins, a Howard Hughes Medical Investigator and NAE and IOM member based at Boston University and the Harvard Wyss Institute for Biologically-Inspired Engineering. Professor Ruder began his academic career studying civil and environmental engineering at MIT and graduated with an S.B. in 2002 with a focus in mechanics. He spent two years as a research assistant at Harvard Medical School studying cell calcium and cAMP signaling systems. He then spent four years at Carnegie Mellon University, where he received an M.S. in Mechanical Engineering and a Ph.D. in Biomedical Engineering. His thesis work focused on the design of micromechanical systems to explore cell biomechanics.

Posters

WHERE DID THE SYNUCLEINS COME FROM? A COMPUTATIONAL LOOK INTO THE PAST. Agatha Munyanyi, John Bedford & Lesley H. Greene, Department of Chemistry & Biochemistry, Old Dominion University, Norfolk VA 23529. α-, β- and γ-Synucleins have been described as physiologically unstructured proteins primarily expressed in the brain tissue of vertebrates. Very recently α-synuclein has been shown to form a helical tetramer which is resistant to fibril formation under physiological conditions. This folded form of α-synuclein may be its natural state. A bioinformatics investigation was conducted to construct a synuclein superfamily to facilitate understanding their physiological structure, function and evolutionary history. The results identified similarity to an endoglucanase enzyme from bacterium *Acetobacter pomorum* , a CRE-DUR-1 protein from a nematode and a putative protein from the Tasmanian Devil. The Tasmanian Devil protein in turn identified a cytochrome c from the bacterium *vibrio cholera*. In conclusion however, the synucleins most surprisingly seem to be orphan proteins in the vertebrate line.

MOLECULAR DOCKING AND VIRTUAL SCREENING OF PPAR-δ AGONISTS. K. E. Ascetta, S. N. Lewis & D. R. Bevan, Department of Biochemistry, Virginia Tech, Blacksburg VA 24061. Peroxisome proliferator-activated receptor subtype delta (PPAR-δ) is a transcription factor that causes increased fatty acid transport and oxidation in white adipose tissue and skeletal muscle. It also plays a role in carbohydrate catabolism and fat synthesis in the liver. All of these processes require activation by agonists, which can be developed as treatments for metabolic disorders like type II diabetes and heart disease. One way to identify viable PPAR-δ agonists is through virtual screening. Screening requires a 3D model that best represents the receptor. In this study eighteen ligand-bound PPAR-δ crystal structures were tested with docking to determine which ones could serve as representative models. The fitness of these structures was evaluated by measuring root mean squared deviation (RMSD) and assessing protein-ligand interactions that match crystal structure references. The four fittest models were then used to screen a small group of published known PPAR-δ agonists that are not present in crystal structures. Here, only protein-ligand interactions

were used as measures for successful docking. Of the fittest models, 3SP9 and 1GWX faired the best in re-docking and cross-docking, while 3DY6 and 3SP9 most accurately predicted the protein-ligand interactions of the non- crystalized agonists. Presently, 3SP9 appears to be the model best fit for virtual screening to identify PPAR-δ agonists.

VIRGINIA TECH CENTER FOR DRUG DISCOVERY SCREENING LABORATORY. P. Sobrado[1], N. J. Vogelaar[1] & D. G. I. Kingston[2], [1]Department of Biochemistry, [2]Department of Chemistry, & [1,2]Virginia Tech Center for Drug Discovery, Virginia Tech, Blacksburg, VA 24061. A new high-throughput screening laboratory has been established at Virginia Tech and is available for use by the scientific community. The facility instrumentation for microplate preparation includes a versatile liquid dispenser and a robot capable of high-accuracy plate-to-plate transfers. Detection of assay results can be done with UV-Vis absorbance, fluorescence, time-resolved fluorescence, glow luminescence, or through use of a UPLC. The facility has over 35,000 compounds available for screening use. The application of high-throughput screening methodologies was demonstrated in an assay designed to identify inhibitors of SidA (*Af*SidA) from *Aspergillus fumigatus*. In a preliminary screening of 640 compounds from the ChemBridge Diverset compound library, N-(5-chloro-2-methylphenyl)-N-[(4-methylphenyl)sulfonyl]glycine was identified as a potential inhibitor and found to have a SidA-binding constant of 14 ?M. Further screening of the ChemBridge library is underway. The Virginia Tech Center for Drug Discovery is supported by the Fralin Life Science Institute, the Virginia Tech College of Science, and the Virginia Tech College of Agricultural and Life Sciences.

CHARACTERIZATION OF A FLAVIN-DEPENDENT –MONOOXYGENASE FROM *CUPRIAVIDUS TAIWANENSIS*. Aaron. Gringer[1], Isabel Da Fonseca[1] & Pablo Sobrado[1,2,3] , [1] Department of Biochemistry, [2] Virginia Tech Center for Drug Discovery & [3] Fralin Life Science Institute, Virginia Tech, Blacksburg, VA 24061. Microbial iron acquisition depends on complex steps and many of which are not shared by higher eukaryotes. Inhibition of siderophore biosynthesis; a specific set of microbial reactions critical for iron acquisition and storage, is therefore a potential antimicrobial strategy. One essential enzyme for this process is a flavin-dependent N-monooxygenase (NMO). Seeking for more insights of how this enzyme class works, we cloned, purified and initially characterized the L-ornithine-5-monooxygenase from Cupriavidus taiwanensis. The codon optimized gene was cloned into pVP56K and expressed in E. coli BL21(DE3). The recombinant target protein with MBP fusion was purified using affinity column and digested to remove MBP by treatment with Tev protease. The k_{cat} and K_M values with L-ornithine are $0.382 \pm 0.009\ s^{-1}$ and $55 \pm 7\ \mu M$, and with NADPH are $0.30 \pm 0.04\ s^{-1}$ and $46 \pm 2\ \mu M$, respectively. Supported by NSF grant MCB 1021384.

COMPUTATIONALLY CHARACTERIZING THE STRUCTURAL DYNAMICS AND INTERACTIONS OF IRISIN D. R. Maddox, A. M. Brown, & D. R. Bevan, Department of Biochemistry, Virginia Tech, Blacksburg VA 24061. Obesity is an epidemic in today's society with many negative effects, including type II diabetes, heart disease, and stroke. Due to this public health problem, the study of adipose tissue is central to the understanding of metabolic abnormalities associated with the

development of obesity. Irisin is a protein cleaved from the fibronectin type III domain containing 5 (FNDC5) protein in the skeletal muscle. It affects white adipose tissue by acting as a metabolic mediator. This effect is known as the "browning" of white fat where white adipose tissue is physiologically converted into brown adipose tissue. We applied computational techniques to investigate the characteristics of irisin by studying proteins homologous to irisin. Homology modeling with Modeller, Dali Server/LOMETS structure searches, and molecular dynamics simulations performed with GROMACS have been utilized to study the way irisin acts in comparison to the well-known homologous proteins. As a preliminary result, irisin acts much like the neural cell adhesion molecule 2 (NCAM2), which is thought to bind to the alpha-2,8-polysialic acid surface receptor in neural cell membranes. NCAM2 shares secondary and tertiary structure similarity to irisin, which suggests the proteins may share surface receptor types. Exploring the structural characteristics of the NCAM2 receptor may improve understanding of the role of irisin in metabolism.

TOWARDS THE STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF THE HFQ HOMOLOG FROM *AQUIFEX AEOLICUS*. Kimberly Stanek, Jennifer Patterson, & Cameron Mura, Department of Chemistry, University of Virginia, Charlottesville, VA 22904. Bacterial survival requires careful regulation of mRNA turnover. The host factor 'Hfq' is a bacterial RNA chaperone that functions in mRNA turnover and related pathways, by modulating mRNA-sRNA interactions. As the bacterial branch of the 'Sm' superfamily, Hfq homologs tend to assemble into cyclic oligomers that associate with RNA. An Hfq has been identified in the genome of the deep-branching thermophile *Aquifex aeolicus* (*Aae*), but little is known about its structure or activity. Earlier work has demonstrated that *Aae* Hfq cannot complement Hfq-deletion strains of *Salmonella enterica*, implying that different species have distinct RNA-binding profiles in vivo. To elucidate the structure and function of *Aae* Hfq, the protein has been over-expressed, purified, and crystallized; *Aae* Hfq oligomers have also been studied by cross-linking. In addition, *Aae* Hfq has been shown to co-purify with small endogenous RNAs. The binding of *Aae* Hfq to FAM-U6 and FAM-A18 has been studied via fluorescence polarization assays and binding constants have been determined.

COMPARISON OF ATOMISTIC MOLECULAR MECHANICS FORCE FIELDS FOR SIMULATING THE ALZHEIMER'S AMYLOID β-PEPTIDE. S. R. Gerben, J. A. Lemkul, & D. R. Bevan, Department of Biochemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0308. Macromolecular function arises from structure, and many diseases are associated with misfolding of proteins. Molecular simulation methods can augment experimental techniques to understand misfolding and aggregation pathways with atomistic resolution, but the quality of these predictions is a function of the parameters used for the simulation. There are many biomolecular force fields available, but most are validated using stably folded structures. Here, we present the results of molecular dynamics simulations on the amyloid b-peptide (Ab), whose misfolding and aggregation give rise to the symptoms of Alzheimer's disease. Because of the link between secondary structure changes and pathology, being able to accurately model the structure of Aβ would greatly improve our understanding of the disease, an outcome with implications for studying other

protein misfolding disorders. To this end, we compared five popular atomistic force fields (AMBER03, CHARMM27, GROMOS96 53A6, GROMOS96 54A7, and OPLS-AA) to determine which could best model the structure of Ab. By comparing secondary structure and calculated NMR shifts to available experimental data, we conclude that AMBER03 and CHARMM27 over-stabilize helical forms of Ab, whereas OPLS-AA, GROMOS96 53A6, and GROMOS96 54A7 produce very similar results that agree well with experimental observables.

CHARACTERIZATION OF UDP-ARABINOPYRANOSE AS A SUBSTRATE OF EUKARYOTIC UDP-GALACTOPYRANOSE MUTASES. William T. Ryan, Karina Kizjakina & Pablo Sobrado, Department of Biochemistry, Virginia Tech., Blacksburg VA 24061. UDP-Arabinofuranose is a precursor for L-arabinofuranose, an important constituent in plant cell walls. In plants, UDP-arabinopyranose (UDP-Ara*p*) is converted into UDP-arabinofuranose (UDP-Ara*f*) by the enzyme UDP-arabinopyranose mutase (UAM). UDP-galactopyranose mutases (UGMs) interconvert UDP-galactopyranose (UDP-Gal*p*) into UDP-galactofuranose (UDP-Gal*f*), a reaction important for virulence in the human pathogen *Aspergillus fumigatus*. The substrates for both classes of mutases are very close in their chemical composition, yet these enzymes perform their function through seemingly different mechanisms. While UGM family requires a flavin cofactor for catalysis, UAM does not require this cofactor for activity. Herein, we investigate the activity of eukaryotic UGMs with UDP-Ara*p*/*f* as a substrate. It was determined that *A. fumigatus* UGM was able to utilize UDP-Ara*f* only when the flavin was in the reduced state. A k_{cat} value of 0.013 s^{-1} was determined. This is ~5000-fold lower than with UDP-Gal*f*. The K_m value for UDP-Ara*f* was 65 µM, which is 1.72-fold lower than for UDP-Gal*f*. Fluorescence polarization binding assay revealed that the K_d value for UDP-Ara*p* was 347 µM and 1179 µM in the reduced and oxidized state, respectively. Supported by NIH grant R01 GM094469.

SOLUTION CONDITIONS AND OXIDATION STATE OF METHIONINE-35 AFFECT THE AGGREGATION PROPERTIES OF THE AMYLOID β-PEPTIDE (1-40). A. M. Brown, J. A. Lemkul, N. Schaum, & D. R. Bevan, Department of Biochemistry, Virginia Tech, Blacksburg VA 24061. The amyloid β-peptide (Aβ) is a 40-42 residue peptide that is the principal toxic species in Alzheimer's disease (AD). With AD being the primary causes of senile dementia in the United States, understanding the mechanism by which Aβ aggregates is essential in therapeutic design. The conversion of Aβ from a helical configuration in the membrane to a β-strand configuration is essential for aggregation. The oxidation of methionine-35 (Met35) to the sulfoxide form ($Met35^{ox}$) has been identified as potential modulator of Aβ aggregation. Experimental studies disagree on the role $Met35^{ox}$ plays in Aβ neurotoxicity, which may be due to inconsistent solution conditions (pH, buffer, temperature). We applied atomistic molecular dynamics (MD) simulations as a means to probe the dynamics of the 40-residue alloform of Aβ ($A\beta_{40}$) containing Met35 or $Met35^{ox}$. We found that Met35 oxidation decreases the β-strand content of the C-terminal hydrophobic region (residues 29-40), with a specific effect on the secondary structure of residues 33-35, thus potentially impeding aggregation. Further, there is an important interplay between oxidation state and solution conditions, with pH and salt concentration augmenting the effects of oxidation. The results presented here serve to

rationalize the conflicting results seen in experimental studies and provide a fundamental biophysical characterization of Aβ_{40} dynamics in both reduced and oxidized forms. Ultimately, understanding the characteristics of Aβ that are important for aggregation, such as the oxidation state of Met35, can provide insight into the biochemical mechanism of AD.

CHARACTERIZATION OF AN IMP CYCLOHYDROLASE IN *HALOFERAX VOLCANII*. Michelle L. Pasier & Cathy A. Sarisky, Dept. of Chem., Roanoke College, Salem VA 24153. Purine biosynthesis is a fundamental metabolic pathway present in essentially all life forms. The domain Archaea, unlike bacteria and eukaryotes, does not universally conserve enzymes across this pathway. Archaeal purine biosynthetic enzymes are also not necessarily homologous to non-archaeal enzymes. The final reaction in *de novo* pathway is the conversion of 5-formamidoimidazole-4-carboxamide ribotide (FAICAR) to inosine-5'-monophosphate dehydrogenase (IMP). In *Haloferax volcanii*, a halophilic archaeon, this reaction is catalyzed by an IMP cyclohydrolase. The genome of *H. volcanii* is sequenced, but the characterization of its genes is not yet completed. The gene HVO_0011 encodes a protein with a high amino acid sequence similarity to TK0430's, an archaeal IMP cyclohydrolase. HVO_0011 was inserted into *E. coli* cells in the vector pMal-c5e. These cells were induced to express the protein of interest. This protein was extracted from the induced cells by sonication and purification. The protein of interest was then separated from *E. coli* proteins using size exclusion and placed in a buffer simulating *H. volcanii* cellular conditions. An assay of the protein of interest and FAICAR was shown to produce IMP by its presence in the assay solution by HPLC chromatography. This confirms the protein encoded by HVO_0011 as an IMP cyclohydrolase.

DISABELED-2 (DAB-2) MODULATES PLATELET- CANCER CELL INTERACTIONS THROUGH ITS SULFATIDE BINDING DOMAIN. K. J. Andreano, X. Fu, D. G. S. Capelluto, C. V. Finkielstein. Dept. of Biol. Sci., Virginia Tech, Blacksburg, VA 24060. Platelets' role in cancer progression and metastasis has 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 that it is manifested as a hypercoagulable state. We have identified a tumor suppressor (Dab-2) that is released upon platelet activation and that modulates the extent of blood clotting. Our results show Dab-2 is released from platelet alpha-granules to the membrane surface where it binds to the integrin receptor, inhibiting platelet aggregation. Dab-2 binding to integrins is modulated by sulfatides, glycosphingolipids that accumulate in platelet membrane and effectively compete for Dab-2. We found that the N-terminal region of Dab-2 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 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. Thus, we hypothesize that

manipulation of Dab-2 function in response of platelet activation will impact emboli formation offering an alternative route for therapeutic strategies aimed to control metastatic processes.

THE ROLE OF ENTROPY IN THE REGULATION OF 3-PHOSPHOGLYCERATE DEHYDROGENASE. C. Meehan, A. Magnuson, C. Roach & E. Bell, Laboratory for Structural Biology, Biophysics & Bioinformatics, Dept. of Chemistry, University of Richmond, Richmond VA 23173. Proteins can take on an array of conformations determined by regional flexibility of the protein. This may play critical roles in catalysis and allosteric regulation. The relationship between structure and function was studied to determine the contributions particular amino acids in 3phospho-glycerate dehydrogenase (3pGDH), may play in flexibility and function of the protein. Based upon available crystal structures a number of amino acids were identified by analysis of the temperature factors that may show altered flexibility in response to serine binding. Addtionally, in conjunction with analysis of the temperature factors of bound cofactor (NAD(H)) we observe that two of the 4 central loops in the tetramer structure are more flexible than the other two and that this correlates with increased affinity for the cofactor. This creates an entropy sink that can be used to harness flexibility changes for catalysis and allosteric regulation. Various point mutations of specific amino acid residues involved in this "entropy sink" were created and the expressed proteins were purified using Ni-NTA affinity chromatography. Preliminary results suggest significant effects on activity. Our observations suggest the presence of an entropy sink, which contributes to cofactor binding cooperation and possibly catalysis. This work is supported by NSF Grant MCB 0448905 to EB.

ANS AS A PROBE OF LIGAND INDUCED CHANGES IN OLIGOMERIC DEHYDROGENASES. G. Kingdom[1], S-H. Kim[2], C. Guzman[1], C. Metz[1] and E. Bell[1], [1]Laboratory for Structural Biology, Biophysics & Bioinformatics, Dept. of Chemistry, University of Richmond, Richmond VA 23173 & [2]Maggie Walker Governor's School, Richmond, VA 23220. 8-anilino-1-naphthalenesulfonic acid (ANS) fluorescence in the presence or absence of 3 oligomeric dehydrogenases has been used to assess ligand-induced changes in conformational plasticity. We have explored cofactor and carboxylic acid induced changes in various dimeric malate dehydrogenases including porcine mitochondrial and cytosolic forms and watermelon glyoxysomal forms. With 3-phosphoglycerate dehydrogenase, a tetrameric enzyme found in higher eukaryotes including humans and in prokaryotes such as E. coli, we have compared the E coli and human forms using NSD mutants (containing the cofactor and substrate binding domains and the critical cofactor domain dimerization interface where a conserved tryptophan crosses the interface). Human NSD enhances ANS fluorescence more than E coli NSD suggesting increased conformational plasticity. Alpha-ketoglutarate appears to enhance indicators of conformational flexibility. With hexameric glutamate dehydrogenase, the effects of the substrate Glutamate and a non catalytically active analog glutarate on ANS fluorescence suggest that, while glutarate has little effect on the overall conformational plasticity of the enzyme, reduced cofactors result in a greatly enhanced blue shift in ANS fluorescence, suggesting enhanced binding to newly exposed hydrophobic regions of the protein. Funded in part by NSF Grant MCB 0448905 to EB.

THE ROLE OF CONFORMATIONAL FLEXIBILITY IN NITRIC OXIDE SYNTHASE. Rachel Jones[1], & Ellis Bell[2], [1]Clover Hill High School, Midlothian VA, 23112, [2]Laboratory for Structural Biology, Biophysics & Bioinformatics, Dept. of Chemistry, University of Richmond, Richmond VA 23173. Nitric Oxide Synthase (NOS) plays a critical role in a variety of signaling pathways and responses to cellular challenge. Although the enzyme is well studied, the role of conformational flexibility in Nitric Oxide Synthase (NOS) activity has received little attention. NOS exists in three isoforms, inducible Nitric Oxide Synthase (iNOS), associated with malignant disease; neuronal (nNOS), and endothelial (eNOS). Each consists of the N-terminal oxygenase domain which binds Arginine and NADPH and the C-terminal reductase domain that binds flavin adenine dinucleotide, and calmodulin. To understand the role of conformational flexibility, experiments using 8-anilino-1-naphthalenesulfonic acid (ANS) fluorescence, and multi-wavelength collisional quenching with differently charged quenchers were conducted in the presence/absence of Arginine to determine how exposed hydrophobic regions of the protein were changed in iNOS. The data suggests that Arginine increases the number of ANS binding sites per protein molecule; implying an increase in iNOS non-polar regions. The collisional quenching data suggests that Arginine increases the exposure of non-polar regions, suggesting that Arginine increases the flexibility of the protein. Analysis of the amino acid sequence of iNOS suggests that these changes in flexibility could be related to the regulation of disordered regions of the protein. A comparison of the effects of a number of inhibitors of iNOS on both ANS fluorescence or the multi-wavelength collisional quenching suggest different structural basis for their inhibitory effects.

EXPLORING THE STRUCTURAL BASIS FOR PIP_2 SENSITIVITY OF INWARDLY RECTIFYING POTASSIUM CHANNELS. Trevor Larry, Kalen Hendra, Arco Paul & Linda M. Boland, University of Richmond, Department of Biology, Richmond, VA 23114. All vertebrate inwardly rectifying potassium (Kir) channels are activated by phosphatidyl-inositol 4,5-bisphosphate (PIP_2). Dynamic changes in PIP_2 concentrations impact how Kir channels control the cellular resting membrane potential. This project investigates a novel Kir channel (AqKir) that was cloned from the marine sponge *Amphimedon queenslandica*. Study of the sponge channels may provide insight into key changes in structure and function that occurred during animal evolution. To probe the requirement for PIP_2 in the maintenance of AqKir, we reduced PIP_2 concentrations in three ways: (1) application of wortmannin (at mM concentrations) to inhibit the kinase that phosphorylates PI to PIP; (2) activation of a co-expressed muscarinic acetylcholine receptor to activate a G protein-coupled phospholipase C; (3) activation of a co-expressed voltage–sensing phosphatase (CiVSP). The results confirm that PIP_2 regulates AqKir but the sponge channel requires much less PIP_2 to remain active when compared to vertebrate homologs. We used mutagenesis to identify two key residues that are important for higher sensitivity to PIP_2 of the vertebrate Kir channels.

TO TRANSLATE OR NOT TO TRANSLATE: HOW DED1, A DEAD-BOX ATPASE, REGULATES MRNA TRANSLATION. Angie Hilliker, Dept. of Biology, University of Richmond, Richmond, VA 23173. Translation regulation is important, especially during stress, in neurons, and in early development, where many mRNAs

are stored in translationally repressed states. These repressed mRNAs can be returned to translation, but this process is poorly understood. We have previously shown evidence that the RNA helicase, Ded1, performs two sequential functions to return a repressed mRNA back to translation in an ATP-dependent manner. First, Ded1 promotes the formation of a "pre-48S mRNP" that includes the initiation factors eIF4F and Pab1. Then, Ded1 hydrolyzes ATP, allowing the mRNA to become translated. We hypothesize that Ded1 acts as an ATP-dependent switch to return mRNAs back to the translating pool. We show that Ded1 interacts directly with eIF4G, while others have shown that the mammalian ortholog of Ded1, DDX3, interacts with both eIF4E and Pab1. We find that Ded1 helps recruit eIF4E and Pab1 to the m7G cap in vitro. We are currently testing the roles of polyA binding protein and the polyA tail in helping Ded1's first role in forming the pre-48S intermediate. Additionally, genetic screens have revealed factors that may modulate Ded1's first or second function via post-translational modifications. These studies will help us understand how Ded1 is regulated, which will illuminate how mRNAs return to from a repressed state back into translation.

INDUCTION OF APOPTOSIS BY NONSTRUCTURAL PROTEINS OF THE SINDBIS VIRUS IN XENOPUS LAEVIS EMBRYOS. Kaitlyn Childs, Jacob Graham, Kevin M. Myles, & Carla V. Finkielstein, Virginia Polytechnic Institute and State University, Blacksburg VA 24060. Viral infections are of interest as is related to virotherapy and host-virus interaction studies for mechanism construction that may result in knockout therapies to reduce or diminish virulence. *Sindbis* virus, an Alphavirus of the Togaviridae family, is transmitted by mosquitos; the endogenous strain is opportunistic, infecting immunocompromised individuals, elders and young children. The viral genome is divided into nonstructural (ns) and structural open reading frames. The nonstructural region encodes a polyprotein consisting of nsP1, nsP2, nsP3, and nsP4. In this study, we have found that the nonstructural protein nsp3 has the ability to induce apoptosis in an heterologous system and that this property is restricted to its N-terminus domain. Interestingly, whereas the nonstructural polyprotein is able to trigger apoptosis in *Xenopus* embryos when a polycistronic mRNA is injected in one-cell stage, neither nsp1, 2 nor 4 was able to accomplish this form of cell death on their own. Apoptosis was confirmed by measuring caspase-3 activity, visualization of membrane blebbing, and cleavage of cyclin A2, a known caspase substrate in *Xenopus* early embryogenesis. Next, we investigated whether nsp3 pro-apoptotic activity was inhibited by interacting with anti-apoptotic members of the Bcl-2 family. Thus, *Xenopus laevis* embryos were injected with various nsp3 constructs in one-cell, collected at different times before MBT and will be analyzed for binding by immunoprecitation. We hope to elucidate the mechanism behind apoptosis induction of the *Sindbis* virus in *Xenopus laevis* embryos in this way.

DOWN-REGULATION OF THE CIRCADIAN FACTOR PERIOD 2 BY THE ONCOGENIC E3 LIGASE Mdm2: EVIDENCE OF THE PRESENCE OF CIRCADIAN AND CELL CYCLE CROSSTALK MECHANISMS. Jing-Jing Liu, Tetsuya Gotoh, Marian Vila-Caballer, Carlo S. Santos, Jianhua Yang & Carla V. Finkielstein, Integrated Cellular Responses Laboratory, Department of Biological Sciences, Virginia Tech, Blacksburg, VA. The circadian rhythm and cell cycle are the

two main oscillatory systems in cells. How cells sense time and decide what is the best time for growing, proliferating or apoptosis? One possibility is that there are crosstalks between these two systems. Based on the fact that Period 2 (Per2) also plays essential role in DNA damage response, Per2 is supposed to connect circadian rhythm and cell cycle, which makes Per2 work as a tumor suppressor. We found Per2 regulating p53 pathway but little is known about how Per2 itself is regulated. One interesting finding is that independent of transcriptional regulation, overexpressed Per2 protein also oscillates; this implies posttranslational modifications are essential for sustaining Per2 protein oscillation. Per2 binds to Mdm2, a well-known E3 ubiquitin ligase, both *in vitro* and *in vivo*. Mdm2 induces Per2 ubiquitination *in vitro*, but further experiments are needed to verify Mdm2 is an E3 ligase for Per2 *in vivo*.

USE OF ELECTROSTATIC POTENTIALS TO EVALUATE PPARα FOR DRUG DISCOVERY. F. Buzzalino, N. Lewis & D. Bevan, Dept. of Biochemistry, Virginia Tech 24060. It is estimated that in the United States, approximately 600,000 deaths per year are attributed to cardiovascular disease. Peroxisome proliferator activating receptors (PPARs), transcription factors that pertain to the nuclear receptor family, play an important role in maintaining metabolism. PPARα, particularly responsible for the catabolism of fatty acids, resides in the liver, heart, kidneys, and skeletal muscle. In recent years Fibrates, synthetic ligands used to treat hyperlipidemia via specific activation of PPARα, have demonstrated effectiveness in reducing cardiovascular disease. Our goal is to identify unknown ligands that interact with PPARα in a similar manner as fibrates. Prior to performing studies to identify unknown ligands, an appropriate PPARα structure must be chosen. In our studies we have analyzed the binding of 11 PPARα crystal structures with the 11 crystalized agonists via docking experiments (re-docking, and cross-docking). Our analysis of the residue interactions between the 11 PPARα structures and the agonists reveal that four residues – His440, Tyr314, Tyr464, Ser280 – are associated with agonist binding. Docking of 544 compounds with three PPARα for the presence of the four residues was performed. The PPARα structure 2P54 demonstrated to be representative of a structure that can bind varying compounds. Our results lead us to believe that the most appropriate PPARα structure for docking of unknown ligands is 2P54. Identification of PPARα selective ligands may help produce fibrates in hopes of reducing the number of casualties associated with cardiovascular disease.