was 66.7% male and 83.3% Caucasian. Results indicated that diagnostic agreement between the two instruments was less than "good" based on weighted kappa. Item response theory analyses suggested that abuse and dependence diagnostic criteria lie on a single dimension, removal of the "legal problems" criterion would not alter item parameters of other criteria, and the proposed "craving" criterion is associated with severe inhalant use disorder. The findings also suggest that inhalant use is better conceptualized on one continuum (as proposed for DSM-5), than as separate abuse/dependence diagnoses (as in DSM-IV). This research was funded by grants from the National Institute on Drug Abuse (R01 15984; P50 10075; T32 017629).

EVALUATING INDIVIDUAL DIFFERENCES IN MOTION RESEARCH. Brittany N. Neilson, J. Christopher Brill, Jessica L. Habermehl & Monique S. Henderson, Department of Psychology, Old Dominion University, Norfolk VA 23529. The purpose of the present investigation is to address the importance of accounting for individual differences in susceptibility when conducting motion research. Motion sickness susceptibility is a multidimensional construct involving, but not limited to, initial sensitivity to motion, rate of adaptation to motion, and the ability to retain motion adaptation long-term (i.e., habituation or desensitization). Additionally, susceptibility to motion sickness presents differently across various forms of motion. Predictors of individual differences in susceptibility have also been identified: Women, greater than 6 years of age, Asian race, and more incidences of motion sickness among biological relatives. Previous research has assessed susceptibility factors using questionnaires to measure previous responses to sickening motion (i.e., motion sickness and simulator sickness/vection), previous responses to mild motion (i.e., sopite syndrome), and perceptual style. Another potential contributor to differences in susceptibility may be psychological processing. Individuals with high motion sickness susceptibility endorse significantly higher levels of trait anxiety. The relationship between trait anxiety and susceptibility to motion sickness should be explored further to determine if anxiety is a significant predictor. Furthermore, it is suggested that susceptibility be assessed in research using multiple measures to either categorize susceptibility groups or measure susceptibility as a covariate. If individual differences are not taken into account, then researchers may fail to find effects that are in fact present.

Structural Biology, Biochemistry and Biophysics

SULFATIDES PARTITION DISABLED-2 IN RESPONSE TO PLATELET ACTIVATION. Karen E. Drahos[1,2], John D. Welsh[2], Julia L. Button[2], Carla V. Finkielstein[2], & Daniel G. S. Capelluto[1]. [1] Protein Signaling Domains Laboratory, Virginia Polytechnic Institute and State University and [2] Integrated Cellular Responses Laboratory, Virginia Polytechnic Institute and State University. Platelets contact each other at the site of vascular injury to stop bleeding. One negative regulator of platelet aggregation is Disabled-2 (Dab2), which is released to the extracellular surface upon platelet activation. Dab2 inhibits platelet aggregation through its phosphotyrosine-binding (PTB) domain by competing with fibrinogen for aIIb3 integrin receptor

binding by an unknown mechanism. Using protein-lipid overlay and liposome-binding assays, we identified that the N- terminal region of Dab2, including its PTB domain (N-PTB), specifically interacts with sulfatides. Moreover, we determined that such interaction is mediated by two conserved basic motifs with a dissociation constant (Kd) of 0.6 mM as estimated by surface plasmon resonance (SPR) analysis. In addition, liposome-binding assays combined with mass spectroscopy studies revealed that thrombin, a strong platelet agonist, cleaved N-PTB at a site located between the basic motifs, a region that becomes protected from thrombin cleavage when bound to sulfatides. Sulfatides on the platelet surface interact with coagulation proteins, playing a major role in haemostasis. Our results show that sulfatides recruit N-PTB to the platelet surface, sequestering it from integrin receptor binding during platelet activation. This is a transient recruitment that follows N-PTB internalization by an actin-dependent process.

STRUCTURAL AND FUNCTIONAL ANALYSIS OF *TRYPANOSOMA CRUZI* UDP-GALACTOPYRANOSE MUTASE. Jacob Ellerbrock[1], Michelle Oppenheimer[1], Richa Dhatwalia[2], John J. Tanner[2], and Pablo Sobrado[1] [1]Virginia Tech, Department of Biochemistry, Blacksburg, VA 24060. [2]University of Missouri, Department of Chemistry, Columbia, MO 65211. Trypanozoma cruzi is the protozoan parasite that causes Chagas disease. Chagas disease is found predominantly in Latin America, where 8-11 million are infected and 50,000 people die from this disease every year. Galf is a sugar found on the cell surface of T. cruzi where it plays a role in host-parasite interaction. Because Galf is not found in humans, enzymes in its biosynthetic pathway are targets for drug design. UDP-galactopyranose mutase (UGM) is a flavin-containing enzyme responsible for the conversion of UDP-galactopyranose to UDP-galactofuranose (UDP-Galf), the precursor to Galf. UGM is a unique flavoprotein as the flavin must be reduced to catalyze the non-redox formation of UDP-Galf. Our group has previously shown that the flavin remains in the reduced state in order to perform approximately 1000 reactions, before it gets oxidized. Recently, the structure of T. cruzi UGM (TcUGM) was solved and several residues have been proposed to be important to stabilize the reduced form of the flavin and for substrate binding. We present the structure-function analysis of three residues important for TcUGM activity. Specifically we show that G61 and H62 play a role in maintaining the reduced state of the flavin. This work is important for understanding how TcUGM is able to maintain the reduced state and perform this unique reaction. Funding provided by NIH grant R01 GM094468.

HUMAN BETA-SYNUCLEIN FORMS A TETRAMERIC ALPHA-HELICAL COMPLEX. Agatha Munyanyi, Jason C. Collins & Lesley H. Greene, Department of Chemistry & Biochemistry, Old Dominion University, Norfolk VA 23529. Alpha, beta and gamma- synucleins are cytoplasmic proteins expressed primarily in the neurons. Alpha- synuclein shares 78% and 60% identity with beta- and gamma- synuclein respectively. While alpha and beta -synuclein colocalize in the presynaptic axon terminals of the central nervous system including the dopaminergic neurons of the substantia nigra, gamma-synuclein is expressed in the neurons of the peripheral nervous

system. The N-terminal domains of the three proteins predominantly consist of several degenerate 11 amino acid repeats containing a conserved KTKEGV sequence, while the C-terminal domains are acidic and vary between the proteins. The expression of recombinant human beta-synuclein yielded a high molecular weight form which was confirmed by running analytical gel filtration chromatography. The high molecular weight form eluted at the same location as a 55.6 kDa protein marker, glutamic dehydrogenase, indicating a tetrameric complex of the 14 kDa beta-synuclein monomer. Far-UV circular dichroism spectra indicated a helical secondary structure for the beta-synuclein complex. The ultimate aim is to solve the three-dimensional structure of this important complex which may be the natural physiological and functional form of the protein in vivo.

CHARACTERIZATION OF THE OLIGOMERICSTATES OF HFQ FORM *THERMOTOGA MARITIMA* ALLUDES TO A COMPLEX REGULATORY INVOLVING TWO DISTINCT OLIGOMERIC STATES. Jennifer Patterson & Cameron Mura. University of Virginia, Charlottesville, VA 22904. RNA-based regulatory pathways and their effect on gene expression enable bacteria to rapidly adapt to various environments. For instance, mRNA levels for a particular protein depend on rate of transcription and half-life; rapid degradation of mRNAs is essential to the ability of bacteria to respond to their environments. The host factor 'Hfq' is involved in regulation of bacterial gene expression via its role as an RNA chaperone. The functional form of Hfq in *Escherichia coli* and other bacterial species have been previously determined to be a homohexamer, but our studies of the Hfq from *Thermotoga maritima* suggest an equilibrium exists between a homohexamer and a homododecamer. Data suggests that the functional form is the homohexamer, which is able to interact with adenosine and uracil rich sequences, suggesting an additional level of regulation in this already complex system.

CAPTURING RNA-DEPENDENT PATHWAYS FOR CRYO-EM. J. Tanner[1], K. Degen[2] & D. F. Kelly[1], [1]Virginia Tech Carilion Research Institute, Virginia Tech, Roanoke, VA 24015 and [2]Department of Biomedical Engineering, University of Virginia, Charlottesville, VA 22908. Transmission Electron Microscopy (TEM) performed at cryo temperatures is the technique of choice for gathering structural details of large macromolecular complexes. To withstand the TEM vacuum system, specimens are frozen at high velocity and preserved in a thin film of vitreous ice. While this procedure is highly effective at maintaining the structural integrity of biological complexes, it also traps active assemblies in the midst of performing their duties. This results in a static snapshot of dynamic processes. Here we present a novel strategy to visualize multiple components of cellular pathways within a functionally relevant framework. We used the bacterial protein synthesis machinery as a model system in conjunction with modified EM Affinity Grids. In doing so, we were able to recruit multiple protein assemblies bound to nascent strands of mRNA. Our system reveals the native interactions of transcription-translational coupling events that control gene expression in prokaryotes. The combined use of Affinity Capture technology and single particle EM provide the basis for visualizing RNA-dependent pathways in a remarkable new way.

CRYSTALLOGRAPHIC SCREENING OF METABOLITE COCKTAIL BINDING AS A TOOL FOR DETERMINATION OF UNKNOWN PROTEIN FUNCTION. I.A. Shumilin[1,3], M. Cymborowski[1,3], O. Chertihin[1], K.N. Jha[1], J.C. Herr[1], S.A. Lesley[2], A. Joachimiak[3] & W. Minor[1,3], [1]Univ. of Virginia, Charlottesville, VA, [2]Joint Center for Struct. Gen., [3]Midwest Center for Struct. Gen.. An estimated 30-40% of sequenced bacterial genes, and an even higher fraction of archaea and eukaryotes genes, encode for proteins that lack an assigned function. Identification of unknown protein functions is a high-priority task in our efforts to understand cellular processes. We applied crystallographic screening to assess the binding of compounds in a metabolite library to proteins in two families. The two families are the YjeF_N family of unknown function, represented mouse apolipoprotein-I binding protein and the N-terminal domain of Tm0922 from *Thermotoga maritima*, and the PF01256 family, previously annotated as a family of small molecule kinases, represented by YxkO from *Bacillus subtilis* and the C-terminal domain of Tm0922. Selective binding of several structurally-related compounds observed for each protein provided the basis for identification of the likely ligands. Subsequently, PF01256 proteins were shown to catalyze ATP-dependent NAD(P)H-hydrate dehydration. The YjeF_N proteins interacted with an adenosine diphosphoribose-related (ADPR) substrate and most likely serve as ADPR transferases. Metabolite library screening may be used as an efficient tool for the functional studies of uncharacterized proteins. Funding provided by U01-HD060491, U54-GM74492, U54-GM074942 and R01-GM53163 (NIH).

EXPRESSION, PURIFICATION, AND PRELIMINARY CHARACTERIZATION OF MEMBERS OF THE *N*-HYDROXYLATING MONOOXYGENASE FAMILY. Nick Keul, Reeder Robinson & Pablo Sobrado, Department of Biochemistry, Virginia Tech, Blacksburg, 24061. Microbial *N*-hydroxylating monooxygenases (NMOs) are a family of flavin-containing monooxygenases that catalyze the hydroxylation of amines to be incorporated into hydroxamate-containing siderophores. This class of enzymes is relatively uncharacterized, mainly due to difficulties in the production of soluble and stable recombinant enzymes. We have identified novel NMOs that might be amenable for *in vitro* studies. Two selections include the putrescine monooxygenase AlcA from *Bordetella pertussis* and the L-lysine monooxygenase NbtG from *Nocardia farcinica*. These enzymes are involved in the biosynthesis of alcaligin and nocobactin, respectively. Both AlcA and NbtG were expressed as fusions to maltose-binding protein and purified to homogeneity with a bound flavin cofactor. Both were found to be active by detection of hydroxylated product and preliminary kinetic values were obtained with NbtG. The L-lysine hydroxylase NbtG was found to have k_{cat} values for oxygen consumption of 1.06 ± 0.02 s^{-1} and 1.19 ± 0.02 s^{-1} for NADPH and NADH, respectively. The K_m values for NADPH and NADH obtained were 257 ± 20 μM and 465 ± 34 μM, respectively. Comparison with other studied NMOs will be presented as well. Further advancement of these studies will lead to a better understanding of the mechanism of action of the NMO family of flavin-containing monooxygenases. This research was funded by a grant from NSF (MCB-1021384).

CONVERSION OF AN ALL α-HELICAL GREEK-KEY PROTEIN INTO HIGHLY ORDERED AMYLOID FIBRILS COMPOSED OF β-SHEET STRUCTURE. Jason C. Collins & Lesley H. Greene, Department of Chemistry & Biochemistry, Old

Dominion University, Norfolk VA 23529. The transition of native proteins into amyloid fibrils is associated with many prevalent diseases. The investigation into the determinants of protein misfolding and fibril formation is an increasingly expanding and important field of research. The transformation of a functional folded protein into a fibril-like polymer composed of β-sheet structure is a fascinating transition. Proteins can be induced to alternatively fold into amyloid fibrils *in vitro* using a variety of factors such as primary sequence polymorphisms, agitation, temperature and solution conditions. In this talk we present the identification of a specific set of conditions required to induce the transition of the all α-helical Fas-associated death domain, a nonamyloidogenic protein, into a fibril-like morphology composed of a rich β-sheet structure. The transition to fibrils is monitored using a broad range of spectroscopic techniques which include atomic force microscopy, fluorescence spectroscopy and circular dichroism. In summary, we show that out of ninety-five conditions attempted only one condition produced fibril-like structures which indicates, that proteins are robust against the amyloid conformation.

UBIQUITIN MODULATES TOLLIP FUNCTION IN THE ENDOCYTIC PATHWAY. S Mitra[§], A Traughber[§], S Gomez[§], C. V. Finkielstein[⊥], L Li[||] & D. G. S. Capelluto[§], [§]Protein Signaling Domains Laboratory, [⊥]Integrated Cellular Responses Laboratory and [||]Laboratory of Innate Immunity and Inflammation, Department of Biological Sciences. Mono or polyubiquitylation of proteins mediate plethora of cellular processes such as signaling, histone modification, proteosomal degradation, and receptor-endocytosis. Ubiquitin modifications on target proteins are recognized by ubiquitin binding domains that are found in several effector proteins. In this study, we describe the function of Toll-interacting protein (Tollip), which is an effector protein found in mammalian innate immune signaling pathway. We demonstrate for the first time ubiquitin and lipid binding regulate Tollip's role as an endocytic adaptor protein. We propose that ubiquitin binding to Tollip induces a conformational change leading to a closed conformation, in which it cannot interact with endosomal membrane phosphatidylinositol 3-phosphate. Release of ubiquitin from Tollip induces an open conformation suitable for endosomal membrane targeting. Overall, our findings will provide the structural and molecular basis to understand how Tollip, as an endocytic adaptor protein, is modulated by ubiquitin and determines the fate of polyubiquitinated cargo for endosomal degradation.

COLOCALIZATION OF ROBO1 AND ALCAM IN MOUSE BRAINS. Caroline Owen & Amanda G. Wright, Department of Biology and Physical Sciences, Marymount University, Arlington, VA 22207. It is shown here that Activated Leukocyte Cell Adhesion Molecule (ALCAM), a member of the immunoglobin superfamily, and ROBO1, the receptor for the SLIT family of ligands, colocalize in postnatal day 0 (P0) mouse brains. This experiment was done using co-immunofluorescence staining of cryopreserved mouse brain sections. Fluorescent microscopy, paired with Hoescht staining, was used to analyze location of the two proteins within individual cells. The results showed that ROBO1 and ALCAM do colocalize in postnatal day 0 mouse brain and suggests a likely interaction of the two

proteins to potentially regulate axon guidance. Supported by Marymount University's DISCOVER program and the Clare Boothe Luce Foundation.

A WINDOW INTO THE YEAR 2100: THE EFFECT OF HIGH pCO_2 ON GENE EXPRESSION IN THE CYANOBACTERIA *Trichodesmium erythraeum.* Nardos Sori[1], Ivy Ozman[2], Adele Kruger[3], Margaret Mulholland[2], Alice Hudder[4] & Lesley Greene[1]. [1]Department of Chemistry and Biochemistry, Old Dominion University, Norfolk, Va. [2]Department of Ocean, Earth and Atmospheric Sciences, Old Dominion University, Norfolk, Va. [3]School of Medicine, Wayne State University, Detroit, Michigan. [4]Institute of Environmental Health Sciences, Wayne State University, Detroit, Michigan. Increase in atmospheric carbon dioxide is a key factor in global climate change and can lead to alterations in ocean chemistry. It is estimated that by the year 2100 atmospheric carbon dioxide will exceed 700 ppm. Cyanobacteria are ubiquitous organisms believed to have originated about 2.7 billion years ago that can help in studying the biological response to increasing carbon dioxide. *Trichodesmium erythraeum* was chosen to understand the effect of global warming on cyanobacteria. Total RNA sequencing was used to quantify changes in gene expression in *Trichodesmium erythraeum* grown under present day and projected pCO_2 concentrations for the year 2100. A bioinformatics analysis indicates that there are a substantial number of genes affected by high pCO2. The results of our studies will be presented.

CIRCADIAN MODULATION OF ESTROGEN RECEPTOR ALPHA PROMOTER ACTIVITY. Kelly A. Barford, Linda M. Villa, Marian Vila & Carla V. Finkielstein, Integrated Cellular Response Laboratory Virginia Polytechnic Institute. The circadian rhythm is an important method of controlling the body's internal clock and adapts to environmental changes. When this clock is disrupted, various diseases have been shown to arise, including breast cancer. Period 2 (PER2), a circadian protein, is the key protein that drives the circadian clock. Cancers, such as breast and ovarian, have shown a loss of PER2 function. This connection between loss of PER2 and the development of estrogen-related tumors has led to a correlation between this protein and estrogen receptor alpha (ERα). ERα mRNA oscillates during the day, similar to PER2, but instead of peaking during the day, ERα peaks at night. Up-regulation of ERα has been shown to influence tumor growth, and if this mechanism can be determined it will lead to a better understanding of estrogen-related cancers, specifically breast cancer. The focus of this project is the effect of Per2 on ERα. This will be done through increasing the level of Per2 in MCF-7 cells and analyzing the expression of the downstream genes through qRT-PCR.

CONFORMATIONAL CHANGE OF THE RYANODINE RECEPTOR AT 10 ANGSTROMS RESOLUTIOn. Tyler W.E. Steele & Montserrat Samsó, Department of Physiology and Biophysics, Virginia Commonwealth University, Richmond VA 23298. The ryanodine receptor isoform 1 (RyR1) is an intracellular ion channel with an important role in depolarization-induced Ca^{2+} release and skeletal muscle contraction. Several of RyR1's molecular partners are distantly situated from its ion gate, suggesting that long-range conformational pathways play an important role in RyR1's function. We have compared two kinds of conformational changes by cryo

electron microscopy and analysis of the 3D reconstructions at 10 Angstroms resolution: those resulting from the transition of the closed to the open state and those resulting from the removal of FKBP12 under closed-state conditions. The vector representation indicates movement of different magnitudes and directions along the 3D structure of RyR1, delineating a long-range allosteric pathway, spanning more than 10 nm, that could couple the binding of RyR1's binding partners and the state of its ion gate.

CIRCADIAN AND CELL-CYCLE MEDIATED REGULATION OF PER2 LOCALIZATION IN COLON CANCER CELLS. Kaitlyn J. Andreano, Marian Vila-Caballer & Carla V. Finkielstein, Integrated Cellular Responses Laboratory, Department of Biological Sciences, Virginia Polytechnic Institute and State University. A key aspect of cancer research is identifying new regulatory pathways involved in proliferation and differentiation of a cancer cell. Disruption of circadian rhythm has recently emerged as a new potential risk factor in the development of cancer, pointing to the core gene *period 2* (*per2*) as a tumor suppressor. Immunofluorescence studies show endogenous Per2 localizes in both cytosol and nucleus in asychronized colon cancer cells. Initial experiments aimed to evaluate whether Per2 localization was cell cycle dependent. Accordingly, HCT116 colon cancer cells were arrested by treatment with nocodazole, hydroxyurea, and thymidine. Using immunoflourescence microscopy, we visualized the localization of endogenous Per2 when cells were arrested at either S or G2 phase as monitored by flow cytometry. Results show Per2 localizes in both nuclei and cytosol when cells are arrested in S but later shuttles to the cytosol during G2 phase. Further experiments aim to elucidate the various Per2 partners that drive its localization and the function of Per2 in the various compartments and among the different cell cycle phases.

HIGH THROUGHPUT MICROARRAY STUDY OF THE EFFECT OF HEME-MEDIATED REGULATION OF PER2 STABILITY FOR GENE EXPRESSION. R. Powles[1], J. Yang[1], R. V. Jensen[2] & C. V. Finkielstein[1] [1]Integrated Cellular Responses Laboratory, [1,2] Dept. of Biological Sciences, Va. Polytechnic Inst. & State Univ., Blacksburg VA 24061. Results from our group show that proteasome-mediated degradation of the human core circadian transcription factor period 2 (hPer2) results from heme, a metabolite that exhibits rhythmic oscillations and is crucial for maintaining cellular homeostasis. In vivo studies show heme levels directly control hPer2 stability and by extension influence the period length and phase-shifting properties of the biological clock in synchronized cells. Using microarray-based gene expression analysis of Aml-12 mouse liver cells to study the effects of hPer2 reduction either by hemin-treatment or by silencing using siPer2 , we identify novel pathways regulated by hPer2 using Pathway Analysis based on Gene Ontology (GO) classifications and the biomedical literature. Multiple transport and metabolic genes are found to be differentially modulated by heme-mediated hPer2 degradation and siPer2 treatment. Many of these genes are overexpressed in cancer cells or belong to major transcription factors involved in cellular proliferation. Results reveal multiple cell-wide pathways that act together in controlling normal cell physiology and, when de-regulated, lead to proliferative disorders.

IDENTIFICATION OF STRUCTURAL DETERMINANTS OF COENZYME SELECTIVITY IN EUKARYOTIC UDP-GALACTOPYRANOSE MUTASES. LuisMiguel Solano & Pablo Sobrado, Dept. of Biochemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061. UDP-galactopyranose mutase (UGM) is the key enzyme in the biosynthesis of galactofuranonose (Gal*f*), a sugar found in the cellular surface of many prokaryotic and eukaryotic pathogens including the fungus *Aspergillus fumigatus*. The chemical mechanism of the UGM from *A. fumigatus* has been recently elucidated indicating that the conversion of the substrate UDP-galactopyranose (UDP-Gal*p*) to UDP-Gal*f*, requires previous reduction of UGM enzymes by NAD(P)H. Here, we identify key amino acids in AfUGM involved in NAD(P)H binding based on structural and mechanistic studies. The role of the residues studied on coenzyme reactivity in AfUGM was determined by rapid reaction kinetics of purified mutants in a stopped-flow spectrophotometer. Measurements of the rate of reduction (k_{red}) of the flavin cofactor at 452 nm, shows that mutations on residues R91, S93 and R447 produce a significant effect on the enzyme kinetics. These mutants have diminished k_{red} in comparison with wild-type AfUGM. This work gives new insights into the mechanism of AfUGM by elucidating key residues involved in NAD(P)H binding. This work was supported by NIH grant R01 GM094468.

DAB2: COMPARATIVE STUDIES BETWEEN ANTI-AGGREGATORY DRUGS. Julia L. Button[1], Olutayo Sengura[2], Marian Vila[1], Daniel G.S. Capelluto[3], & Carla V. Finkielstein[1], [1]Integrated Cellular Responses Laboratory, Virginia Polytechnic Institute and State University, [2] Virginia College of Osteopathic Medicine and [3] Protein Signaling Domains Laboratory, Virginia Polytechnic Institute and State University. Cancer patients, especially those in the metastatic stage, have been found to have highly elevated platelet counts. The thrombocytosis (high platelet count) symptom has been associated with poor prognoses in many cancers. Platelets play a role in metastasis by forming platelet-tumor cell masses in the circulatory system preventing recognition and activation of an immune response. Anti-platelet drugs are supported to be potential cancer therapies by preventing the activation and aggregation of platelets in the bloodstream, thus preventing cancer cells from surviving and proliferating in target metastatic tissues. Disabled-2 (Dab2) is a protein that inhibits platelet aggregation by binding to sulfatides found on the outer-leaflets of the platelet cell membrane. The focus of this project is to compare the cytotoxic potential of currently prescribed anti-aggregatory drugs, including aspirin and plavix, to Dab2, a sulfatide-deficient form of the protein and a recombinant peptide comprising the sulfatide binding motifs. Our results show the N-terminus portion of the Dab2 exhibits less cytotoxic effect than current anti-aggregatory drugs, while inhibiting platelet/cancer cells association. These results provide basis for the use of Dab2 as part of an adjuvant therapy to treat cancer.

THE EFFECTS OF CITRATE ON GLYOXASOMAL MALATE DEHYDROGENASE. Jacqunae Mays[1], James D. Marion[2] & Ellis Bell[1], [1]Laboratory for Structural Biology, Biophysics & Bioinformatics, University of Richmond, Richmond, VA 23173, [2]Department of Biochemistry & Molecular Biology, Virginia Commonwealth University, Richmond, VA 23298. Malate Dehydrogenase plays critical roles in the cytosol, the mitochondrion and in plants also in glyoxysomes. Mitochondrial and gyloxysomal forms of malate dehydrogenase are thought to be

subject to allosteric regulation by citrate. To explore the mechanisms of citrate effects on the enzyme we have used a combination of initial rate kinetics, site directed mutagenesis, circular dichroism (in conjunction with thermal melts) to follow effects on secondary and tertiary structure, dynamic light scattering to follow effects on quaternary structure and limited proteolysis to explore local flexibility/accessibility changes. Citrate, which binds to only one of the two otherwise identical active sites, is a partial inhibitor under all circumstances examined (varied pH, forward or reverse reaction), has no effect on the quaternary structure of the protein but induces changes in local flexibility/accessibility in a loop near the subunit interface. Several mutations at the subunit interface (V195A, L269A, E256Q, H90Q)also impact citrate inhibition. Together these results suggest that citrate exerts its effects on activity by binding to the "empty" active site in a reciprocating subunit mechanism, eliciting altered subunit interactions that contribute to overall catalysis. This work is supported by NSF Grant MCB 0448905 to EB.

ENHANCING STUDENT ENGAGEMENT IN AN ADVANCED PROTEIN STRUCTURE, FUNCTION & BIOPHYSICS CLASS. Ellis Bell, University of Richmond, Richmond, VA 23173. Junior and Senior majors in a biochemistry and molecular biology major take advanced courses in the discipline yet have often only been exposed to traditional lecture based courses. As a result they are often ill-prepared to think about foundational concepts or apply them to new situations and are not adept at the various skill sets necessary for them to compete in graduate school or future professions. To counter these effects a dynamic and active classroom engages students more fully in their own education. In an upper level protein structure, function and biophysics course which revolves around three major themes, Protein Structure and Flexibility, Energy Barriers and ways to overcome them, and Function and Regulation, a variety of active learning strategies including small group discussion and peer teaching, literature discussion and presentation, original summary synthesis of topics from the literature, challenging problem sets containing real data, and a fully integrated laboratory experience where students have to design their own experiments and interpret data replace traditional lectures and labs. Although students take a while at the start of the course to adapt to such a different teaching and learning style, pre and post testing using tools designed to assess foundational knowledge and skills shows significant gains in student understanding. This work is supported by NSF Grant MCB 0448905 to EB.

INHIBITORY MECHANISM AND REGULATORY RELEASE OF SIKE FROM THE TBK1 KINASE COMPLEX. J.D. Marion & J.K. Bell, Dept. of Biochem. and Mol. Biol., Virginia Commonwealth University, Richmond, VA 23284. The innate immune system is the body's first line of defense against infectious agents. Essential to this response are cellular mechanisms that recognize, sequester and eradicate these invading organisms. Toll-like receptor 3 (TLR3), a transmembrane receptor found in endosomes, is stimulated by the viral genomic material double stranded RNA (dsRNA), initiating a signaling cascade that leads to the production of type I interferons and pro-inflammatory cytokines. Critical to this pathway is a proposed kinase complex, NAP1 (NAK associated protein 1) – TBK1 (TANK Binding Kinase 1) – IKKe (IkB kinase), which upon activation, leads to the phosphorylation of IRF3/7 (interferon regulatory

factor) and production of IFNb. To control this activity, SIKE (suppressor of IKKe) acts as a physiological inhibitor of IKKe and TBK1 through a previously undefined mechanism. These studies show SIKE to be a mixed-type inhibitor, regulating the kinases through both competitive and non-competitive modes of inhibition. Further studies have elucidated post-translational modifications that affect SIKE before and after dsRNA stimulation. We hypothesize that post-translational modifications mediate SIKE release from the NAP1-TBK1-IKKe complex allowing for IRF3/7 activation. Current studies are focused on 1) SIKE post-translational modifications, 2) how modifications may alter SIKE protein interactions, and 3) kinetic analysis of SIKE's effect on TBK1 activity. Funding provided by the Jeffress Memorial Trust.