

Comparison of our model with other competing methods and practical applicability is illustrated using real world consumer preference data.

SIMULATING DEPENDENT BINARY DATA WITH RANDOM EFFECTS. Aobo Wang, Roy T. Sabo, Department of Biostatistics, Virginia Commonwealth University, Richmond, Virginia 23298-0032. Dependent binary data can be simply simulated using the multinomial sampling method. We extend this method to simulate dependent binary data with clustered random effect structures. Several distributions are considered for constructing random effects among cluster-specific parameters and effect sizes, including the normal, uniform and beta distributions. We present results from simulation studies to show proof of concept for the multinomial sampling method in creating data sets of repeated-measure binary outcomes with clustered random effect structures in various scenarios. The simulation studies show that multinomial sampling method can be successfully adapted to simulate dependent binary data with desired random effect structures.

COMPOSITIONAL DATA ANALYSIS. Theodore Chang, Department of Statistics, University of Virginia, Charlottesville, VA 22904-4135. Suppose we have a sample of rocks and x_1, \dots, x_p is the weight of p minerals within the rock. Usually we assume that $x_1 + \dots + x_p$ represents the total weight of a sample element, so x_p might be 'other'. Then x_1, \dots, x_p are called the 'open' variables. Let $y_i = x_i / (x_1 + \dots + x_p)$ denote the proportions, or 'closed' variables, such that $y_1 + \dots + y_p = 1$. This is an example of compositional data. Another example might be data on time allocation: e.g. x_1 is the time spent eating, x_2 the time spent watching TV, etc.. John Aitchison (JRSS B 1982) proposed an approach to analyzing this type of data. We examine the geometry of moving from open to closed variables and, in that light, the mathematical attractiveness of the Aitchison approach.

Structural Biology, Biochemistry, and Biophysics

BIOPHYSICAL CHARACTERIZATION OF NATURALLY OCCURRING TITIN M10 MUTATIONS. Michael W. Rudloff, Alec N. Woosley & Nathan T. Wright. Department of Chemistry & Biochemistry, James Madison University, Harrisonburg, Virginia 22807. The giant proteins titin and obscurin are important for sarcomeric organization, stretch response, and sarcomerogenesis in myofibrils. The extreme C-terminus of titin (the M10 domain) binds to the N-terminus of obscurin (the Ig1 domain) in the M-band. The high-resolution structure of human M10 has been solved, along with M10 bound to one of its two known molecular targets, the Ig1 domain of obscurin-like. Multiple M10 mutations are linked to limb-girdle muscular dystrophy type 2J (LGMD2J) and tibial muscular dystrophy (TMD). The effect of the M10 mutations on protein structure and function has not been thoroughly characterized. We have engineered all four of the naturally occurring human M10 missense mutants and

biophysically characterized them *in vitro*. Two of the four mutated constructs are severely misfolded, and cannot bind to the obscurin Ig1 domain. One mutation, H66P, is folded at room temperature but unfolds at 37 °C, rendering it binding incompetent.

The I57N mutation shows no significant structural, dynamic, or binding differences from the wild-type domain. We suggest that this mutation is not directly responsible for muscle wasting disease, but is instead merely a silent mutation found in symptomatic patients. Understanding the biophysical basis of muscle wasting disease can help streamline potential future treatments.

MOLECULAR DYNAMICS STUDIES OF THE UBIQUITIN CONJUGATION MECHANISM. Serban Zamfir & Isaiah Sumner, Department of Chemistry & Biochemistry, James Madison University, Harrisonburg, Virginia 22807. Post-translational modification of proteins can have drastic effect on their structure and function. One such modification involves the attachment of a small protein, ubiquitin. An important function of ubiquitination is to signal proteins for cellular degradation. This process occurs in three enzymatic steps. In the second step, ubiquitin transfers to a conjugating enzyme, called E2, which then transfers ubiquitin to a lysine in the target protein. However, the mechanistic details for this final transfer remain obscured. Although it is clear that ubiquitin does bind, there are no studies that show exactly how this happens. The two most favored proposals involve a step-wise mechanism with a tetrahedral oxyanion intermediate and concerted mechanism. This work probes the accuracy of the oxyanion hypothesis. In particular, if the oxygen on the observed carbonyl carbon can form a stable hydrogen bond with the hydrogen on the nitrogen of the asparagine side chain, then oxyanion intermediate is plausible. By using molecular dynamics (MD), combined with umbrella sampling, a free energy profile of the formation of the breaking and forming of the hydrogen bond is constructed to see if its creation is thermodynamically favorable. Furthermore, information about the hydrogen-bonding environment in the active site is extracted.

STRUCTURAL BASIS OF PHOSPHOINOSITIDE (PIP) RECOGNITION BY THE TIRAP PIP-BINDING MOTIF. Daniel G. S. Capelluto¹, Xiaolin Zhao¹, Shuyan Xiao¹, & Geoffrey Armstrong², ¹Department of Biological Sciences, Virginia Tech., Blacksburg VA. 24061 & ²Department of Chemistry & Biochemistry, University of Colorado, Boulder CO 80309, USA. Toll-like receptors (TLRs) provide early immune system recognition and response to infection. TLRs activated by pathogens initiate a cytoplasmic signaling cascade through adaptor proteins, the first being the modular TIR domain-containing adaptor protein (TIRAP). TIRAP contains a C-terminal TIR domain, which is responsible for association with TLRs and other adaptors. Membrane recruitment of TIRAP is mediated by its N-terminal PIP- binding motif (PBM). Upon ligand-mediated activation, TLRs are recruited to the PIP-enriched regions where TIRAP resides. To understand the mechanism of membrane targeting of TIRAP and the basis for its regulation, we functionally and structurally characterized its PBM. TIRAP PBM adopts a folded conformation in membrane mimics, such as

dodecylphosphocholine micelles, and binds PIPs. Structural rearrangements of TIRAP PBM were influenced by membrane interaction, with monodispersed PIPs inducing helical structure in the peptide. In contrast, monodispersed phosphatidylinositol and inositol trisphosphate did not promote structural changes in TIRAP PBM. NMR spectra reveals that TIRAP PBM binds PIPs in a fast exchange regime with a moderate affinity through two conserved basic regions. Solution NMR structure of TIRAP PBM shows a central short helix, and paramagnetic studies indicate that this region is close to the micelle core. Thus, we proposed that two sets of basic residues contact both the head group and the acyl chains of PIPs, whereas the central helix is responsible for membrane insertion.

CHONDROCYTES: A SHOCKING REVELATION. Anthony J. Asmar, Michael W. Stacey, & Frank Reidy, Research Center for Bioelectrics & Department of Biological Sciences, Old Dominion University, Norfolk, VA 23508. Ion channels are typically associated with excitable cells such as neurons and muscle (skeletal and cardiac). However, chondrocytes also utilize ion channels, so to what extent are they electrically active? Chondrocytes are the cells that produce and maintain the extracellular matrix in cartilage. They are able to endure a variety of mechanical and biochemical stresses using ion channels. In this study, we establish a gene expression profile of ion channels in chondrocytes. Although many ion channels have been uncovered previously, we detected several more from various gene families. Not only do we observe a diverse population of channels, but we found the ion channel expression profile of chondrocytes to be similar to cardiomyocytes.

ADAPTING HYPERTHERMOPHILIC PROTEINS INTO SWITCH-BASED BIOSENSORS. Jonathan D. Dattelbaum, University of Richmond, Department of Chemistry, Richmond, VA 23173. The analytical detection of small molecules is an important theme in the molecular life sciences. Our studies here focus on the design of reagentless fluorescence protein biosensors using two members of the periplasmic binding protein superfamily recently identified from the hyperthermophile, *Thermotoga maritima*. This Gram-negative bacterium is found primarily in hot springs and hydrothermal vents. Proteins isolated from *T. maritima* are thus likely to exhibit a high degree of physicochemical stability, making them useful targets for manipulation and utilization in biosensor design. Using site-directed mutagenesis, we created single cysteine variants of TmArgBP (*T. maritima* arginine binding protein) and TmMBP (*T. maritima* maltose binding protein). Thiol-reactive fluorophores were covalently attached to the protein and used to probe ligand binding. Changes in fluorescence were used to determine dissociation constants that typically range in the low micromolar level. Because these proteins exhibit large conformational alterations upon ligand binding, we propose a theoretical framework that provides a rational approach to the design of switch-based biosensors. Stable protein biosensors may provide a sensitive and specific platform for small molecule testing, particularly in complex cellular matrices.

HOFMEISTER ION AND CO-SOLVENT EFFECTS ON THE STRUCTURE, AGGREGATION, AND SOLVATION OF RECA. Taylor P. Light, Karen M. Corbett, Michael A. Metrick & Gina MacDonald, Department of Chemistry & Biochemistry, James Madison University, Harrisonburg VA 22801. RecA is an *Escherichia coli* protein that catalyzes the strand exchange reaction utilized in DNA repair. Previous studies have shown that the presence of salts influence RecA activity, aggregation, and stability. Here we utilized attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy and circular dichroism (CD) to further investigate how various Hofmeister salts and co-solvents alter RecA structure, aggregation, and solvation. Spectroscopic studies performed in water and deuterium oxide suggest that salts alter amide I (or I') and amide II (or II') vibrations arising from the protein backbone. Specific IR vibrations that may arise from protein-solvent interactions were identified. IR vibrations that correlate with protein desolvation were observed in the presence of strongly hydrated SO_4^{2-} anions. The vibrations that correlate with protein solvation were observed in the presence of weakly hydrated Cl^- and ClO_4^- anions. An increase in the IR frequency of amide I (or I') correlated with increasing concentrations of trifluoroethanol (TFE) and sucrose. This result suggests an increase in desolvation of the amide backbone with an increase in the concentration of co-solvents. Additionally, increasing concentrations of TFE resulted in an increase in RecA aggregation. These results show that salts and co-solvents alter the solvation water surrounding proteins and influences overall structure and aggregation. This research was supported by NSF grants NSF-RUI CHE-0814716 and NSF-REU CHE-1062629.

SMD SIMULATIONS OF THE M-BAND TITIN-OBSCURIN INTERACTION. Tracy A. Caldwell, Isaiah C. Sumner & Nathan T. Wright, Department of Chemistry & Biochemistry, James Madison University, Harrisonburg, VA 22807. Titin and obscurin, two giant muscle proteins, bind to each other in an antiparallel Ig-Ig fashion at the M-band. This interaction must be able to withstand the mechanical strain that the M-band typically experiences and remain intact. The mechanical force on these domains is likely exerted along one of two axes: a longitudinal axis, resulting in a 'shearing' force, or a lateral axis, resulting in a 'peeling' force. Here we present molecular dynamics data suggesting that these forces result in distinct unraveling pathways of the titin-obscurin complex and that peeling the domains apart requires less work and force.

MECHANISM OF UFM1 CONJUGATION: STUDIES OF UFC1. Emily A. Todd, Nathan, Wright & Christopher E. Berndsen, Dept. of Chemistry & Biochemistry, James Madison University, Harrisonburg, VA 22807. Ufm-ylation is the process by which the ubiquitin-fold modifier (Ufm1) is transferred to a substrate lysine. This process is crucial for morphology changes in many parasites and is linked to regulation of ER stress and breast cancer. There are two known enzymes in the pathway, Uba5, the Ufm1 activating enzyme and Ufc1 the conjugating enzyme. The Ufm-ylation pathway is similar to the ubiquitin conjugation pathway however many of the structural

characteristics of ubiquitin conjugating enzymes are not apparent in Ufc1. Previous studies on the E2 enzyme Ubc13 showed that the HPN motif and an active site loop are critical for activity. However, none of these conserved characteristics are present in Ufc1. To elucidate the function of Ufc1 structural and functional differences we present NMR and molecular modeling studies of Ufc1. Future studies include understanding the structural characteristics of Ufc1 in substrate and Uba5 binding and the biochemical mechanism of Ufc1 conjugation.

THE IMPORTANCE OF THE DISULFIDE BONDS WITHIN BST-2 FOR STRUCTURE AND VIRAL TETHERING. Kelly E. Du Pont, Aidan M. McKenzie, Oleksandr Kokhan, Isaiah C. Sumner & Christopher E. Berndsen, Department of Chemistry & Biochemistry, James Madison University, Harrisonburg, VA 22807. Human BST-2/tetherin is a host factor that inhibits release of enveloped viruses, including HIV-1, HIV-2, and SIV, from the cell surface by tethering viruses to the host cell membrane. BST-2 consists of an N-terminal cytoplasmic domain, a transmembrane domain, an alpha helical ectodomain, and a C-terminal membrane anchor. In cells, BST-2 forms disulfide-linked dimers between the ectodomains of each monomer forming a coiled-coil. The N-terminal half of the ectodomain contains three cysteine residues which can participate in disulfide bond formation and are critical for viral tethering. The role of the disulfides in viral tethering is unknown, but proposed to be for maintenance of the dimer. We explored the role of the disulfides in maintaining BST-2 structure in order to propose the molecular basis for the requirement for disulfides in viral tethering. We found that the disulfide bonds strengthen the coiled-coil structure of BST-2 but do not appear to affect the dimeric state of the protein. To further explore the role of the disulfides, we chose to take a novel approach and simulated viral tethering and observe changes in BST-2 structure. These simulations of viral tethering revealed that the disulfides alter the unfolding pathway of BST-2 and that the disulfide-linked dimer of BST-2 is more resistant to virus induced stretching. These data provide new insights into viral tethering by the innate immune system and have generated novel models of viral tethering a previously inaccessible biological phenomenon.

STRUCTURAL ELUCIDATION OF THE IG58, IG59, AND IG58/59 DOMAINS OF OBSCURIN. Rachel A. Policke, Tracy Caldwell, Logan Meyer & Nathan T. Wright, Department of Chemistry & Biochemistry, James Madison University, Harrisonburg, VA 22807. Obscurin (800-900 kDa) is a giant muscle protein vital to muscle cell maintenance and organization. It is the only known linker between the contractile apparatus and the sarcoplasmic reticulum of myocytes and may also bind to cytoskeletal, signaling, or membrane-associated proteins. One such interaction is the binding of obscurin domains Ig58/59 to titin domains ZIG9/10. This binding, which requires all four domains to be present, is hypothesized to stabilize the sarcomeric cytoskeleton. Mutations in this region of obscurin lead to malformed muscle architecture and may lead to hypertrophic cardiomyopathy (HCM). In order to better

understand the molecular underpinnings of this disease, we used X-ray crystallography and NMR to solve high-resolution structures of the Ig58 and 59 domains of obscurin individually as well as in tandem.

IDENTIFICATION AND CHARACTERIZATION OF *THERMOTOGA MARITIMA* Hfq PROTEIN BINDING PARTNERS. Thushani Nilaweera, Lissa Anderson, Jennifer Patterson, Donald Hunt & Cameron Mura, Department of Chemistry, University of Virginia, Charlottesville, VA 22904 USA. The host factor for bacteriophage Q β (Hfq) is the bacterial branch of Sm family proteins which are involved in RNA metabolism. In bacteria, Hfq acts a global post-transcriptional regulator, affecting the stability and translation of messenger RNA (mRNA) by facilitating the annealing between small regulatory RNAs (sRNA) and their target mRNAs. Hfq has also been linked to proteins involved in general cellular RNA metabolism (RNA synthesis and degradation). An Hfq homolog is present in *Thermotoga maritima* (*Tma*), an early-branching anaerobic thermophile. In previous work, a novel class of nanoRNAs (.5-6 nts) was found to co-purify with recombinant *Tma* Hfq expressed in *Escherichia coli*. NanoRNAs are regulated by oligoribonuclease (Orn), and a putative homolog of this enzyme can be detected in *Tma* based on sequence similarity. We have successfully cloned, expressed and purified the recombinant *Tma* Orn in *E.coli*. We are investigating the putative function of *Tma* Orn and potential interactions between *Tma* Hfq and nanoRNA degradation pathways. Additionally, we are attempting to isolate endogenous *Tma* Hfq protein binding partners using Co-immunoprecipitation (CoIP) and using liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. By providing a map of *Tma* Hfq *in vivo* interactions, this work will lay a foundation for understanding the roles of Hfq as a global ribo-regulator.

BIOLOGICAL QUEUEING: A FRAMEWORK FOR PROTEIN SYNTHESIS AND DEGRADATION NETWORKS. Nicholas Butzin, Philip Hochendoner, Curtis Ogle & W.H. Mather, Dept. of Physics, Virginia Tech, Blacksburg VA, 24061. It is known that all biological cells must cope with limited resources, but less appreciated is the impact of finite *processing* resources behind a myriad of tasks, including not only metabolism but also protein production, degradation, and modification. I will discuss the experimental and theoretical development of a biological queueing theory, which fundamentally assumes limited processing resources. In particular, recent results demonstrate that shared degradation machinery can form a strong effective mutual activation between protein species, which forces a rewriting of regulation diagrams currently assumed for many gene networks. Complementing this work, limited translational resources can lead to “riboqueueing,” establishing mutually repressive interactions between gene activities. I conclude with very recent developments concerning competition within polysomes.

Posters

IDENTIFICATION OF NOVEL ALLOSTERIC MODULATOR BINDING SITES IN NMDA RECEPTORS: A MOLECULAR MODELING STUDY. Lucas T. Kane¹ & Blaise M. Costa^{1,2} ¹Edward Via Virginia College of Osteopathic Medicine & ²Department of Biochemistry, Virginia Tech, Blacksburg VA, 24061. The dysfunction of N-methyl-D-Aspartate receptors (NMDARs), a subtype of glutamate receptors, is correlated with schizophrenia, stroke, and many other neuropathological disorders. However, not all NMDAR subtypes equally contribute towards these disorders. Since NMDARs composed of different GluN2 subunits (GluN2A-D) confer varied physiological properties and have different distributions in the brain, pharmacological agents that target NMDARs with specific GluN2 subunits have significant potential for therapeutic applications. In our previous research, we have identified a family of novel allosteric modulators that differentially potentiate and/or inhibit NMDARs of differing GluN2 subunit composition. To further elucidate their molecular mechanisms, in the present study, we have identified four potential binding sites for novel allosteric modulators by performing molecular modeling, docking, and *in silico* mutations. The molecular determinants of the modulator binding sites (MBS), analysis of particular MBS electrostatics, and the specific loss or gain of binding after mutations have revealed modulators that have strong potential affinities for specific MBS on given subunits and the role of key amino acids in either promoting or obstructing modulator binding. These findings will help design higher affinity GluN2 subunit-selective pharmaceuticals, which are currently unavailable to treat psychiatric and neurological disorders.

INVESTIGATING THE ENZYMATIC ACETYL TRANSFER REACTION PATHWAY. B. Hamilton Young & Christopher. E. Berndsen, Dept. of Chemistry & Biochemistry, James Madison University, Harrisonburg, VA 22807. Protein acetylation, which involves the transfer of an acetyl group to the side chain of a lysine residue, serves many vital cellular purposes. Defects in enzymatic acetylation have been linked to diseases such as cancer, insomnia, and anemia. Despite decades of research into the biological function of protein acetylation, the enzymatic mechanism of acetyl transfer is unknown. We aim to determine the mechanism of catalysis for acetyl transfer using the yeast protein acetyltransferase GCN5. Using a fluorescent activity assay, we have found a normal deuterium solvent isotope effect for this reaction, suggesting that proton transfer occurs during the acetyl transfer reaction. This information is crucial for understanding the enzyme mechanism. Upon investigation of an inhibitor-bound GCN5 X-ray crystallography structure, the presence of multiple steric and geometric anomalies were observed. Molecular dynamics was used to investigate the validity of these published structures. Future experiments will determine the number of protons being transferred, measure the kinetic isotope effects on the GCN5 acetyl transfer reaction, and investigate the mechanism of acetyl transfer using molecular dynamics. We aim to compare data obtained on the GCN5 reaction

mechanism to that of the ubiquitin transfer reaction catalyzed by the enzyme Ubc13, which performs the same basic reaction using a vastly different structure. This comparison will allow for determination of whether the mechanism of acetyl transfer is conserved between the two enzymes.

AN ARGININE CLAW IN PHOSPHORYLATED DESMOPLAKIN SUGGESTS A MECHANISM FOR CONTROL OF INTERMEDIATE FILAMENT BINDING.

Charles E McAnany & Cameron Mura, Department of Chemistry, University of Virginia, Charlottesville, VA, 22904. Cellular adhesion is governed by desmosomes, large transmembrane complexes that tether the intermediate filaments of separate cells. It has recently been shown that the serine-rich C-terminus of desmoplakin governs binding to intermediate filaments. Several phosphorylation sites on the C-terminal region have been shown to regulate the strength of the binding. Here, we show that the phosphorylation of S2849 leads to the formation of an arginine claw that is not present in the non-phosphorylated protein. We show that the methylation of R2834 causes the protein to adopt a structure suited to processive phosphorylation. This suggests that the conformation of desmoplakin acts as a switch to control the action of GSK3, a kinase noted for its low substrate specificity. We further show that the site of these modifications is not free in solution, but contacts other parts of desmoplakin, suggesting a binding competition mechanism for the regulation of intermediate filament adhesion.

COMPARISON OF KINETIC PROPERTIES OF AROMATIC DESULFINASES.

Ianna Hutchinson-Lundy, Austin Crithary, Jonathan Schmitz & Linette M. Watkins, Department of Chemistry & Biochemistry, James Madison University, 901 Carrier Dr. Harrisonburg, VA 22807. Dibenzothiophene (DBT) and its derivatives comprised up to 60% of the organosulfur contamination of crude oil. The enzyme 2-(2'-hydroxyphenyl) benzenesulfinate desulfinate (DszB) catalyzes the carbon-sulfur bond cleavage in the final, and rate-limiting step in the biodesulfurization of DBT. The DszB enzymes from *Nocardia asteroides* A3H1 and *Rhodococcus erythropolis* IGTS8 were overexpressed in *E. coli*, purified and characterized kinetically. The stability of the enzyme was measured under various storage conditions and increased stability was observed upon immobilization of the enzyme to CNBr-activated Sepharose beads.

EFFECT OF IMMOBILIZATION ON THE STABILITY AND SPECIFICITY OF CHOLINE OXIDASE.

Jonathan Schmitz & Linette M. Watkins, Department of Chemistry & Biochemistry, James Madison University, 901 Carrier Dr. Harrisonburg, VA 22807. Choline oxidase catalyzes the conversion of choline into glycine betaine and hydrogen peroxide. Choline oxidase has two roles in industry and medicine, as a sensor of choline, and in the synthesis of glycine betaine. For optimal utility in industrial synthesis pathways choline oxidase must have a broad specificity while maintaining activity over a range of pH and temperature. The specificity, pH optimum, temperature optimum and temperature stability of choline oxidase from three different

sources was determined. In order to address low stability at elevated temperatures, choline oxidase was immobilized on CNBr-activated Sepharose beads. Immobilized choline oxidase showed enhanced stability over broader range of temperature and pH. The turbidity of the immobilized enzyme prevents the use of continuous spectrophotometric assays to test the specificity and alternative methods for testing specificity are under investigation.

INFRARED STUDIES OF SALT-INDUCED EFFECTS OF AMINO ACIDS.

Elizabeth P. Mortimer, Elijah T. Johnson, Taylor P. Light & Gina MacDonald, Department of Chemistry & Biochemistry, James Madison University, Harrisonburg VA 22801. Infrared spectroscopy was used to study glycine at different pH and salt conditions. The addition of various Hofmeister salts was studied to determine how these ions influence glycine infrared spectra. Changes in pH affect the protonated or deprotonated state of the amino and carboxyl groups suggesting altered vibrational spectra. The presence of a kosmotropic salt should desolvate the amino acid while the chaotropic salts should solvate the amino acid. These studies aimed to determine if infrared spectroscopy could identify if the presence of salts alters glycine absorptions. The spectrum of glycine was obtained at pH's of 1.5, 7, and 12.5 in phosphate buffer in deuterium oxide. Glycine spectra were also obtained at pH 7.0 in sodium chloride, sodium perchlorate, and sodium sulfate. Glycine infrared spectra show clear pH and salt induced shifts in infrared vibrations. Comparison of the various infrared spectra will be discussed. This research was supported by NSF grants NSF-RUI CHE-0814716 and NSF-REU CHE-1062629.

ASSESSING THE RNA-BINDING SITES OF Hfq VIA COMPUTATIONAL DOCKING.

Matthew A. Cline & Cameron Mura, Department of Chemistry, University of Virginia; Charlottesville, VA 22904 USA. The Sm superfamily of proteins bind RNA and are found in all domains of life. The RNA binding characteristics have yet to be fully characterized, particularly for the Sm-like archaeal proteins (SmAPs). In principle, molecular docking may be used to determine RNA binding sites; while in practice, docking force fields have been parameterized for use with small organic compounds rather than for RNA. Therefore, we must first examine the suitability of our docking approach (using AutoDock 4 and AutoDock Vina) for the prediction of RNA binding sites. We began explorations in the bacterial branch of the Sm superfamily, Hfq, where the binding has been experimentally characterized. Hfq is known to chaperone RNA-RNA interactions, thus regulating translation of mRNA. To validate the use of docking for the prediction of RNA binding sites on Hfq, NTPs were docked to *Pseudomonas aeruginosa* Hfq and compared to known crystal structures of *Pseudomonas aeruginosa* Hfq bound to NTPs. Preliminary results suggest that AutoDock 4 does not predict accurate RNA binding pockets of Hfq reliably, while AutoDock Vina is capable of predicting binding pockets.

INTERPLAY AMONG E3 UBIQUITIN LIGASES REGULATES TIMELY DEGRADATION OF THE CIRCADIAN FACTOR PERIOD 2. JingJing Liu & Carla V. Finkielstein, Integrated Cellular Responses Laboratory, Department of Biological Sciences, Virginia Tech. Circadian rhythms are mechanisms that measure time on a scale of about 24 h and that adjusts our body to external environmental signals. Core circadian clock genes are defined as genes whose protein products are necessary components for the generation and regulation of circadian rhythms. Oscillatory rhythms are generated as result of the activation of positive and negative transcriptional feedback loops in which post-translational modifications, shuttling, and degradation are common themes. Ubiquitin E3 ligases-mediated protein degradation is critical to sustain physiological rhythms. Here we show beside the F-box β -TrCP1/2 targeting PER1 and PER2 for degradation via ubiquitination, the mouse double-minute 2 homolog (Mdm2) RING finger E3 ligase binds to PER2 and promotes PER2 polyubiquitination occurring both *in vitro* and in cells. Overexpression Mdm2 or down-regulation Mdm2 by siRNA, results in decreasing or increasing PER2 stability reciprocally. Furthermore, we found Mdm2-dependent post-translational modification of PER2 directly influences the amplitude of the circadian oscillatory response in synchronized cells and that both, β -TrCP and Mdm2 target PER2 at different circadian times. Overall, our findings support a model in which, the rate of nuclear degradation of PER2 leads to slow accumulation of the protein in the nucleus and, later in the day, to a temporal switch in which β -TrCP takes control over PER2 degradation. Thus, the interplaying between these two E3 ligases is indispensable for sustaining robust circadian oscillation.