Structural Biology, Biochemistry & Biophysics

RE-EXAMINATION OF THE STRUCTURE AND ELASTICITY OF THE TITIN IG65-70 SEGMENT. Rachel A. Policke, Chris E. Berndsen & Nathan T. Wright, Department of Chem. and Biochem., James Madison Univ., Harrisonburg VA. 22807. Previously published crystal structure data of 6 tandem domains of titin, Ig65-70, suggest that the region is rigid in structure with discrete hinge points in a "carpenter's ruler" model. However, this model does not agree with the more recent, modified wormlike model (mWLM), in which the linkers between semi-rigid Ig domains are hinge points capable of bending. In order to reconcile the differences between these two models, we re-analyzed the crystal structure data. We found that the apparent rigid regions of I6 could be explained through crystal packing interactions rather than intermolecular interactions. Thus, while domain-domain and domain-linker interactions may contribute minimally to titin elasticity, most of this elasticity must be accomplished through intradomain stretching and an entropic spring-like motion.

HIGH RESOLUTION STRUCTURE OF TITIN ZIG10. Allyn Letourneau & Nathan T. Wright, Dept. of Chemistry and Biochemistry, James Madison University, 800 South Main St, Harrisonburg, VA 22807. Titin domains ZIg9/10 bind to obscurin domains Ig58/59 during myofibrillogenisis. Mutations in this region lead to hypertrophic cardiomyopathy (HCM) in humans. While the cellular consequences of this interaction are well characterized, the molecular determinants governing this structure are unknown. Previous work from our lab has solved the high-resolution structure of the obscurin domains of the complex. Here, we describe the purification and complete structure characterization of titin domain ZIg10. (Supported by Jeffress Memorial Fund, Research Corporation Cottrell College Grant, NSF-REU (CHE-1461175)).

COMPARING THE TEMPORAL DYNAMICS OF CASPASE ACTIVITY DURING THE INTRINSIC AND EXTRINSIC PATHWAYS OF APOPTOSIS. S. Morris, P. Stuckey & R. Reif, Department of Chemistry, University of Mary Washington, 1301 College Ave., Fredericksburg VA 22401. Apoptosis, a process in which a cell systematically triggers its own death in response to DNA damage or external stimuli, is widely utilized in the body in both aging and cancer prevention. There are several known pathways that execute apoptosis utilizing a family of enzymes called caspases, with the intrinsic and extrinsic pathways undergoing separate initiation mechanisms and caspases, before converging to a conserved cascade. The goal of this experiment was to elucidate and compare the temporal dynamics of apoptosis with respect to the onset and duration of caspase

activity for both the intrinsic and extrinsic pathways. This was done through a novel combination of microfluidics and fluorescence microscopy. The intrinsic pathway showed consistent caspase activation around 3.5 hours after induction, with a consistent duration of two hours. The extrinsic pathway showed varying onset times with an average around 4 hours post induction, and a duration that exceeded the 6 hour window of this experiment. By timing the major events of apoptosis through both pathways, the overall apoptotic process can be more clearly understood.

OPTIMIZATION OF RNA SELEX PARAMETERS. K. Kerns, S. Clark & R. Reif, Department of Chemistry, University of Mary Washington, 1301 College Ave., Fredericksburg VA 22401. Systematic Evolution of Ligands by Exponential Enrichment, or SELEX, is a method of selectively purifying and amplifying stereospecific, high affinity RNA aptamers (oligonucleotides) for certain target ligands. First, a random DNA sequence is obtained, purified, amplified, and reverse transcribed into RNA. Then, the target is exposed to this random sequence RNA, and the RNA that binds to the target is extracted and amplified. This procedure is repeated for every successive step of SELEX isolating RNA with the highest affinity for the target. Because there are many different methods for conducting PCR, RNA transcription, reverse transcription, and RNA and DNA purification, it is essential to develop a standard, efficient method by which the SELEX experiment can be conducted. This experiment focuses on the optimization of the RNA transcription process as well as the filtration and purification of the aptamer-protein complex through a nitrocellulose membrane; focusing specifically on the extraction and amplification of the aptamer sequence. This is designed to create an abbreviated and efficient procedure which future researchers can follow to expedite the RNA SELEX process.

SEQUENCE VERSUS STRUCTURE: A LOOK AT HOW THE GENETIC CODE DIRECTS PROTEIN FOLDING. John T. Bedford, Jason C. Collins, Brittney L. Ruedlinger & Lesley H. Greene, Department of Chemistry and Biochemistry, Old Dominion University, Norfolk VA 23529-0126. The immunoglobulin-binding domain of protein G (GB1) is a relatively small protein consisting of 56 amino acid residues. GB1 is, although thermostable at high temperatures, classified as a mesophilic protein. A structural alignment was created using the DaliLite server. One of the proteins that was identified was the small archaeal modifier protein 1 (SAMP1) from the extremophile *Haloferax volcanii*. A long-range interaction network was constructed for each protein and a betweeness centrality measure was applied. Several residues were identified as having high betweeness centrality in each protein. When comparing these residues with the alignment three

of them correspond in location. We propose that these residues are important in the formation and/or stability of the ubiquitin-like β-grasp fold found in both proteins. The results of computational studies will be presented in the context of structure, stability, conservation, and folding.

MECHANISTIC INSIGHTS INTO UBC13-CATALYZED UBIQUITINATION. Aaron G. Davis[1], Walker M. Jones[1], Serban Zamfir[2] & Isaiah Sumner[1], [1]Department of Chemistry and Biochemistry, James Madison University, Harrisonburg VA 22807 and [2]Department of Chemistry, Virginia Commonwealth University, Richmond VA 23284. Ubc13 is an E2 enzyme that catalyzes lysine ubiquitination, a type of protein post-translation modification. Ubiquitinating a protein can signal for its degradation, affect its activity and also plays a role in DNA repair and inflammatory response. Defects in this process are linked to different disorders including cancer and Parkinson's and Alzheimer's diseases. The accepted mechanism for Ubc13-catalyzed ubiquitination is a stepwise mechanism that proceeds through an oxyanion intermediate. This intermediate is hypothesized to be stabilized by a nearby asparagine residue, which is known as the "oxyanion hole." However, the validity of the accepted mechanism has recently come into question. In our study, we use a combination of simulation techniques including classical molecular dynamics, *ab initio* molecular dynamics and hybrid Quantum Mechanics/Molecular Mechanics to examine this hypothetical mechanism. Our calculations indicate that several different intermediates are possible and that water may stabilize the intermediate, whereas the asparagine serves to stabilize a random coil near the active site.

STRUCTURAL AND FUNCTIONAL BASIS OF THE LYSOSOMAL PROTEIN PHAFIN2. Tuo-Xian Tang, Amy Jo, Iulia Lazar, Richey Davis & Daniel G. S. Capelluto. Department of Biological Sciences, Biocomplexity Institute, Virginia Tech, Blacksburg VA 24061. The lysosomal protein Phafin2 is one of the fourteen members of the Phafin protein family, which all contain a N-terminal PH (pleckstrin homology) domain followed by a C-terminal FYVE (Fab 1, YOTB, Vac 1, and EEA 1) domain. Phafin2 was found to interact with the serine/threonine kinase Akt and forms the Akt-Phafin2 complex, which plays an important role in the induction of autophagy. It was demonstrated that after the induction of autophagy, Akt-Phafin2 complex accumulates on the lysosome via the interaction of Phafin2 with lysosomal phosphatidylinositol 3-phosphate (PtdIns(3)P). Our study focuses on the elucidation of the structural and functional properties of Phafin2 using NMR and other biophysical techniques, and its interaction with PtdIns(3)P. Phafin2 has been purified to homogeneity and exhibits an elongated monomeric α/β structure of

about 6.5 nm with modest thermal stability. NMR data also indicates that the protein presents substantial random coil regions in its structure. Using surface plasmon resonance, we show that Phafin2 interacted with PtdIns(3)P-enriched liposomes with high affinity. By studying the structural properties of Phafin2, we can better understand its molecular interactions at high resolution.

STRUCTURAL BASIS OF LIGAND RECOGNITION BY THE ENDOSOMAL ADAPTOR PROTEIN TOM1. Wen Xiong, Xiaolin Zhao, Michael Hodge, Phillip Choi & Daniel G. S. Capelluto, Department of Biological Sciences, Biocomplexity Institute, Virginia Tech, Blacksburg, VA 24061. Tom1 (target of Myb 1) plays a role in membrane trafficking by serving as an endosomal sorting complex required for transport (ESCRT) component, or at least be associated with the ESCRT machinery. A model has been proposed in our recent study in which Tom1 interaction with the Toll-interacting protein (Tollip) induces the unfolded TBD domain of Tollip to fold, and then this folding modulates lipid binding of Tollip, mediating its dissociation from phosphatidylinositol-3-phosphate (PI(3)P) and committing Tollip to cargo trafficking. However, there are still some underlying questions about this model that need to be addressed as follows: **(i)** how does the Tom1 distinguish between structurally distinct polyubiquitin chains which represent functionally distinct intracellular signals; **(ii)** what the structural basis of Tom1 VHS interactions. Recently, Tom1 VHS domain has been shown to serve as a new PI(5)P effector at signaling endosomes through its VHS domain delaying epidermal growth factor receptor degradation in a bacterial infection model; Tom1 VHS domain also binds ubiquitin and form a complex with Tollip through the Tom1 GAT domain for receptor degradation; therefore, we hypothesize that the ubiquitin and PI(5)P compete each other for Tom1 VHS binding. Addressing these questions will unveil the role of Tom1 in endosomal protein trafficking and the integration of membrane trafficking with cellular processes.

ESTIMATING EFFICIENCY IN RNA-MIRNA BINDING PREDICTION SOFTWARE. O. M. Plotnikova[1], A. Baranova[2,3], D. A. Zubtsov[1] & M. Y. Skoblov[1,2]. [1]Moscow Institute of Physics and Technology, Moscow, 117303, Russia, [2]Centre for Medical Genetics, Federal Agency for Scientific Organizations, Moscow, 115478, Russia, [3]School of Systems Biology, George Mason University, Manassas, VA, 20110, USA. miRNA play a key roles in regulation of gene expression as guide molecules in the RNA-induced silencing complex (RISC). There are now more than 2,500 identified human's miRNAs, but the question about the mechanism of interaction between mRNA and miRNA is still open. A theory of canonical interactions is considered that the 2-8nt seed region at the 5’ end of miRNA interacts with 3'UTR

end of mRNA according to the complementary Watson-Crick pairing rule. There are five widely used predictive algorithmes for searching miRNA-mRNA interactions: TargetScan, Pictar2, PITA, RNA22, and miRanda. Recently, CLASH method was designed for revealing miRNA-mRNA binding sites in HEK293 cell line. Thus we have the opportunity to assess the effectiveness of all five algorithms. We developed a multiparameter analysis of prediction and function of software including estimation of sensitivity and positive predictive value. Analysis of what region of mRNA interacted with miRNA revealed different types of "canonical", "nocanonical", and "energy class" interactions. We conducted a qualitative comparison of predicted miRNA and miRNA from the experimental data-set. The activity of binding mRNA was analyzed and compared experimental data with the complete level of expression of mRNA in HEK 293 using the FANTOM5 project data. According to our analysis, we have established a low level program ability to predict real miRNA-mRNA interactions.

EXPLORING NOVEL FLAVIN-DEPENDENT CHEMISTRY: THE MECHANISM OF OLEATE HYDRATASE FROM *ELIZABETHKINGIA MENINGOSEPTICA*. Madeleine Marcus, Kim Harich, Julia S. Martin del Campo & Pablo Sobrado, Department of Biochemistry, Virginia Tech, Blacksburg, VA 24060. Oleate hydratase (OhyA; E.C.4.2.1.53) from *Elizabethkingia meningitis,* is an flavin-dependent enzyme that catalyzes the conversion of oleic acid to 10-hydrostearic acid. OhyA has become the focus of studies due to its ability to introduce hydroxyl groups without wasted energy on cofactor recycling. By converting oleic acid to (R)-10-hydroxysteric acid, oleate hydratase provides a product that has potential use as a surfactant, a lubricant, in cosmetic applications, and a possible initiation to greater polymer chemistry. The hydration of unsaturated fatty acids is believed to be a detoxification mechanism and a survival strategy. The reaction catalyzed by OhyA does not involve a net redox changes, however the enzyme requires the flavin cofactor in the reduced form for activity. Our hypothesis is that the flavin cofactor in OhyA functions as an acid, which represents a novel role in nature. OhyA was expressed as a recombinant protein in *Escherichia coli* and purified with FAD bound (Figure 1). Overall purification resulted in 68.3 mg of purified OhyA per 1 liter of growth media, with FAD incorporation of 49 %. Activity assays were performed in the presence of oleic acid and the coenzymes: NADH, NADPH under both anaerobic and aerobic conditions. OhyA was active only under anaerobic and reduced conditions. We present the stopped-flow kinetic characterization of OhyA and propose a role for the reduced FAD in this non-redox reaction. (supported in part by NSF-CHEM 1506206.)