structures. This method is unique in providing ''local'' structural information in solution for gaining insight into dynamic processes involving, large RNA-protein and protein-protein assemblies on biologically relevant timescales. The method also can uniquely probe the ''local'' structure of large complexes poised at equilibrium for functional states of interest, and has been extended to *in vivo* studies.

Beamline X28C is located at the National Synchrotron Light Source of Brookhaven National Laboratory. An expanding set of user groups utilize this national resource funded by the National Institute of Biomedical Imaging and Bioengineering of the National Institutes of Health. The facility is operated by the Center for Synchrotron Biosciences and the Center for Proteomics and Bioinformatics of Case Western Reserve University. The facility supports both onsite and offsite user access. Beam time is allocated online through peer reviewed user proposal system. Examples of recent research projects are provided.

**343-Pos Board B222**

**Expression and Purification of the Myxoma Virus Leukemia Associated Protein Zinc Finger Domain**

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Myxoma Virus Leukemia Associated Protein (MV-LAP) is a protein encoded by Myxoma virus, a poxvirus responsible for the lethal disease, myxomatosis in the European Rabbit. MV-LAP has developed a ''stealth'' mechanism to evade the host immune system by reducing the expression of major histocompatibility complex (MHC)-1 molecules, responsible for identifying self vs. non-self antigens. In order to help understand this stealth mechanism, the N-terminal domain (NTD; 94 residues) of MV-LAP will be characterized structurally using NMR. The current project builds upon the purification of the MV-LAP-Maltose Binding Protein fusion protein system and the purification of MV-LAP NTD. Purification of the fusion protein occurred via the use of a $Co^{2+}$ Immobilized Metal ion Affinity Chromatography (IMAC) column. Several buffer systems allow for the purification of a soluble fusion protein. In addition, several buffer systems were evaluated for the use in the purification of MV-LAP NTD. The purification of a soluble form of MV-LAP NTD and the preparation of a sample for NMR analysis is currently underway.

**344-Pos Board B223**

**Expression and Purification of Zinc Finger Antiviral Protein**

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Ever since the discovery of viruses so many years ago there have been researchers of many different fields racing to find the perfect inhibitor. Any and all discoveries of viral resistance have been taken into a spotlight to discover the mechanisms. Zinc Finger Antiviral Protein (ZAP) is found in the kidney and liver cells of *Rattus norvegicus*; this protein makes these cells more resistant to viral infection. Gao et al concluded that ZAP facilitates inhibition of the antiviral gene expression, one of the major propagation steps (rather than inhibition of infection ZAP affects viral expression) by binding viral mRNA. Since then ZAP has been shown to increase resistance against Moloney Murine Leukemia virus, Sindbis virus, Ebola virus, and Marburg virus. Activity of ZAP containing four CCCH zinc fingers seems to be dependent on the integrity of the second and fourth CCCH zinc fingers. With HIV in mind, which is also a retrovirus, we have concluded that ZAP will have profound and influential implications.

Our goal is to determine the structure of the zinc-binding domain of ZAP using Nuclear Magnetic Resonance. Zap was expressed as a fusion protein in *E. coli* with several different cleavage conditions and purified using Immobilized Metal Ion Affinity Chromatography (IMAC) were screened and found unsuccessful in that the cleaved ZAP was insoluble. However, we are currently working on the smaller ZAP proteins that only contain two zinc fingers; their constructs show promising results in terms of solubility after cleavage.

**345-Pos Board B224**

**Troponin T Deletion 96 Related to Restrictive Cardiomyopathy Ablates the Effects of Cardiac Troponin I PKA Pseudo-Phosphorylation on $Ca^{2+}$ Sensitivity of Force Development**

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The human cardiac TnT deletion 96 (HcTnT3-ΔE96) has been linked to Restrictive Cardiomyopathy and causes a dramatic increase in $Ca^{2+}$ sensitivity of force development (J Biol Chem 283(4):2156-66). Here, we present further biochemical and functional studies to assess whether there are additional factors contributing to the severity of the phenotype. To determine whether the RCM mutation interferes with the cTnI-PKA phosphorylation signal, that decreases $Ca^{2+}$ sensitivity of the myofilament upon $\beta$-adrenergic stimulation, we utilized a cTnI phosphorylation mimetic. The $Ca^{2+}$ sensitivity of force development was evaluated in porcine cardiac skinned fibers using cTnT displacement (HcTnT3-WT or HcTnT3-ΔE96) followed by reconstitution with the binary complex containing pseudo-phosphorylated cTnI (where serines 23, 24 were replaced with aspartic acid - $cTnI_{SS/DD}$.cTnC) or the non-phosphorylatable control cTnI (where serines 23, 24 were mutated to alanine - $cTnI_{SS/AA}$.cTnC). Fibers displaced with HcTnT3-WT and reconstituted with $cTnI_{SS/DD}$.cTnC showed a decrease in the $Ca^{2+}$ sensitivity compared to the control reconstituted with $cTnI_{SS/AA}$.cTnC. In contrast, fibers displaced with HcTnT3-ΔE96 and reconstituted with $cTnI_{SS/DD}$.cTnC or $cTnI_{SS/AA}$.cTnC showed similar $Ca^{2+}$ sensitivities. These results indicate that the mutation may ablate the effects of cTnI PKA phosphorylation. Additionally, Circular dichroism (CD) studies using 0.5M NaF showed a decreased $\alpha$-helical content of HCTnT3-ΔE96 compared to HCTnT3-WT. CD thermal denaturation measuring the $\alpha$-helical content at 222 nm revealed that the mutant unfolded earlier and at a drastically reduced $T_m$ compared to WT. Furthermore, Troponin complexes containing cTnC-IAANS labeled at both Cys 35 and 84 with HcTnT3-D96 did not show alterations in the apparent $Ca^{2+}$ affinity of the cTnC low affinity site II compared to troponin complex containing HcTnT3-WT. Work supported by NIH HL-042325 (J.D.P.) and Postdoctoral Fellowship AHA 0825368E (J.R.P.).

**346-Pos Board B225**

**Structural Comparison Of A Diabetes Drug Target, Mitoneet, A 2Fe-2S Cluster Protein To Its More Stable Mutant, H87C**

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MitoNEET, a recently discovered 2Fe-2S containing outer mitochondrial protein (1), was identified as a binding target for pioglitazone, an insulin-sensitizing drug of the thiazolidinedione class used in the treatment of type 2 diabetes (2). MitoNEET possesses a unique dimeric structure, with a new fold (3). The pH sensitive lability of the 2Fe-2S cluster was attributed to protonation of the conserved solvent accessible His87 (Figure). Its replacement with Cys increased the stability of the cluster ~10-fold (1). The crystal structure of the H87C mutant (1.8Å, Rfactor = 18%) shows that the Sγ of Cys87 remains at a similar position to the Nδ of His87 in the native (Figure). The only other change was a reorientation of Lys55. Thus, the increased stability of the HC87 mutant is attributed to the specific change in the ligation of the 2Fe-2S cluster, not a more global conformational change.

(1) Wiley et al. (2007) J Biol Chem. 282, 23745-23749.
(2) Colca et al. (2004) Am J Physiol Endocrinol Metab 286, E252-E260.
(3) Paddock et al. (2007) Proc Natl. Acad. Sci USA 104, 14342-14347.
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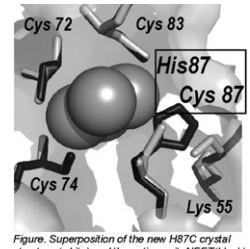

*Figure. Superposition of the new H87C crystal structure (white) and the native mitoNEET(black) showing the 2Fe-2S cluster (spheres), a semitransparent view of the protein and its surface and specific amino acids (as labeled) that interact with the cluster.*

**347-Pos Board B226**

**Crystallographic Structure And Structural Stability Of Vertebrate Digestive Lysozyme**

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C-type lysozyme catalyzes the hydrolysis of peptidoglycan of bacterial cell wall. Most of C-type lysozymes express to protect body against bacterial infection. However, ruminants and some leaf-eating animals have evolved their lysozyme as digestive enzyme. They recruit bacteria which ferment cellulose in the foregut, and digest the bacteria by lysozyme in the true stomach to obtain nutrient. Digestive lysozyme has acquired some properties, such as low optimal pH, and resistance to protease and acid hydrolysis. The structural basis for these properties still remains unclear. In this investigation, we have obtained the crystallographic structure of bovine stomach lysozyme (BSL). This is the first report on the structure of vertebrate digestive lysozyme. We have carried out the denaturant-unfolding experiment and revealed that BSL has high structural stability at acidic pH compared to non-digestive (hen egg-white) lysozyme. The structural stability in acidic solution would be related to the pepsin resistance