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 β-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 α-helical content of HCTnT3-ΔE96 compared to HCTnT3-WT. CD thermal denaturation measuring the α-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.
*Supported by NIH (GM41637, GM54038 and DK5441).

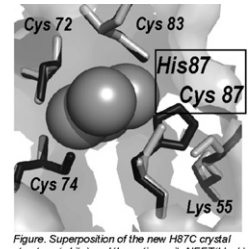

Figure. Superposition of the new H87C crystal structure (white) and the native mitoNEET(black) showing the 2Fe-2S cluster (spheres), a semitransparent view of the protein and its surface and specific amino acids (as labeled) that interact with the cluster.

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

which was previously reported for BSL. The crystal structure of BSL suggested that negatively charged surfaces, a shortened loop, and salt bridges should provide the structural stability.

348-Pos Board B227
Structural Determination of the Carboxyl Terminal Domain from the Gap Junction Protein Connexin45
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Gap junctions are intercellular channels that enable ions, small molecules, and second messenger metabolites to travel between adjacent cells. Gap junctions provide a pathway for molecules involved in growth, regulation, and development. In the cardiac conduction system, they are critical for impulse propagation. Alterations in the gap junction proteins or connexins (Cx) are associated with life threatening arrhythmias. The major connexins in the heart are Cx40, Cx43, and Cx45. Previous studies have shown that these connexins are able to interact causing the formation of heteromeric gap junction channels, which have different biophysical properties than homomeric channels. The mechanisms involved in the regulation of these heteromeric channels are still largely unknown, but recent evidence supports involvement of their carboxyl terminal (CT) domains. Our laboratory has focused biophysical studies on the Cx43CT and Cx40CT domains, and here we have extended our studies to the Cx45CT. Using different biophysical methods, including NMR spectroscopy and Circular Dichroism, we have found that the Cx45CT is predominately unstructured, like the Cx43CT and Cx40CT domains. Ongoing studies are focused on identifying if hetero-CT domain interactions are involved in the regulation of heteromeric channels.

349-Pos Board B228
Structural and Functional Basis for (*S*)-allantoin Formation in the Ureide Pathway
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The ureide pathway, which mediates the oxidative degradation of uric acid to (*S*)-allantoin, represents the late stage of purine catabolism in most organisms. The details of uric acid metabolism remained elusive until the complete pathway involving three enzymes was recently identified and characterized. However, the molecular details of the exclusive production of one enantiomer of allantoin in this pathway are still undefined. Here we report the crystal structure of 2-oxo-4-hydroxy-4-carboxy-5-ureidoimidazoline (OHCU) decarboxylase, which catalyzes the last reaction of the pathway, in a complex with the product, (S)-allantoin. The homodimeric helical protein represents a novel structural motif, and reveals that the active site in each monomer contains no cofactors, distinguishing this enzyme mechanistically from other cofactor-dependent decarboxylases. On the basis of structural analysis, along with site-directed mutagenesis, a mechanism for the enzyme is proposed in which a decarboxylation reaction occurs directly, and the invariant histidine residue in the OHCU decarboxylase family plays an essential role in producing (S)-allantoin through a proton transfer from the hydroxyl group at C4 to C5 at the re-face of OHCU. These results provide molecular details that address a longstanding question of how living organisms selectively produce (S)-allantoin.

350-Pos Board B229
A Folding Switch Regulates the Phd/doc Operon by Conditional Cooperativity
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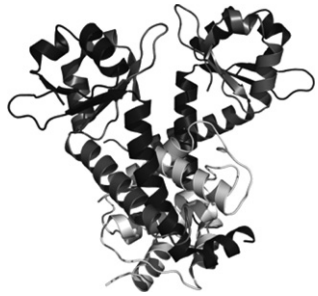
Regulation of gene expression is a fundamental process that allows a cell to respond to changes in its environment. At the molecular level, expression is tuned by the concerted action of both activators and repressors whose activity is typically linked to external or internal stimuli. Bacterial toxin-antitoxin (TA) operons are repressed under unrestrained growth conditions and activated during episodes of nutritional stress.
The auto-regulation of TA operons has remained enigmatic. They all share the general feature that the antitoxin acts as an auto-repressor. The toxin modulates this repressor activity by acting either as a co-repressor or as a co-activator depending on the molar ratio of toxin over antitoxin, a phenomenon recently termed conditional cooperativity.
The structural and thermodynamic basis for conditional cooperativity is unknown. We have solved the crystal structure of bacteriophage P1 Phd, unbound and in complex with the toxin Doc. The complex shows two Phd dimers sandwiching a monomeric Doc. The crystal of the free antitoxin imprisons two distinct folding states of the protein. Together these structures suggest a model for the operator DNA complex of Phd/Doc and explain conditional cooperativity for the auto-repression of the *phd/doc* operon.



351-Pos Board B230
Double Hexamer Structure Of The Archaeal Helicase MCM From Methanobacterium Thermoautotrophicum
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Hexameric DNA helicases are key enzymes in the replicative machinery. They are also an example of flexible proteins which undergo conformational changes related to their function. The minichromosome maintenance factor MCM of Methanobacterium thermoautotrophicum (mtMCM) is at present the best characterized archaeal replicative helicase, as well as a useful experimental model for the more complex eukaryotic helicases. Biochemical and crystallographic evidence indicate that the double hexamer is the functional form of mtMCM, but previous EM reports have detected assembly as single heptamer, single hexamer, or other stoichiometries.
In our studies, we have observed not only 6-fold and 7-fold structures, but also open rings and double rings in the same wild type mtMCM preparation. This is an indication of polymorphism in the assembly of mtMCM, and possibly of equilibrium between these various forms. It is not clear at present, what is the functional relevance of each of the structural arrangements, although a hexameric form would correlate best with the presence of six MCM components in the eukaryotic MCM2-7 complex. The presence of open ring forms, reported here for the first time, suggests that loading of mtMCM onto DNA might be achieved through a ring opening mechanism. Such an MCM loading mechanism would be similar to that proposed for the T7 helicase or the bacterial Rho terminator, and different to that of the SV40 large T antigen, where monomers assemble around the DNA to form the hexameric rings.
We also present the first three-dimensional reconstruction of the MCM double hexamer from negatively stained samples. The map allows direct observation of the dodecameric complex for the first time, and highlights characteristics similar to those found for SV40 large T antigen, such as the existence of side channels in each hexamer.

Protein Dynamics I

352-Pos Board B231
Dynamics and Statistical Properties of Disordered Proteins
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To understand the full picture of protein folding, structural and dynamic properties of the unfolded ensemble of a protein under folding conditions need to be elucidated. This could give clues to the early stages and events in the folding process. In this work, we examined the end-to-end intramolecular contact formation rates by the technique of tryptophan triplet quenching by cysteine, and numerically modeled the conformational distributions that agree with the experimental results using Szabo, Schulten and Schulten (SSS) theory, which treats intramolecular diffusion as diffusion on a one dimensional potential of mean forces. We performed numerous all-atom implicit-solvent molecular dynamics simulations with different stating configurations. The preliminary results performed in AMBER9 using the ff99 force field suggests a rather non-ergodic conformational space sampling by protein L and a relatively ergodic sampling by apocytochrome C. This observation appears to be consistent with our results of experimental data analysis and the calculated diffusion coefficients for protein L and apocytochrome C.

353-Pos Board B232
Probing the Cytoplasmic Substrate Permeation Pathway of the Serotonin Transporter with Steered Molecular Dynamics Simulation
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