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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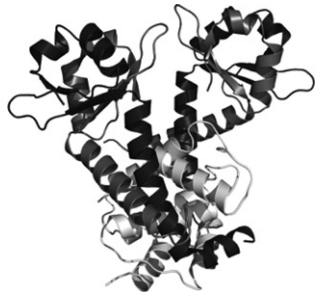
Regulation of gene expression is a fundamental process that allows a cell to respond to changes in its environment. At the molecular level, expression is tuned by the concerted action of both activators and repressors whose activity is typically linked to external or internal stimuli. Bacterial toxin-antitoxin (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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SERT a 12-helix presynaptic plasma membrane protein terminates synaptic transmission by Na+ symport of the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) from the extracellular mileu of synapse into the cell.
Crystal structures of Leucine transporter (LeuT), the bacterial homologue of SERT, represent a substrate-bound occluded conformation in which the the intracellular part of the transporter is closed and packed.
We have constructed an experimentally-validated homology model of SERT based on the LeuT structure and cognate information about other transporters in this family and performed SMD simulations by pulling serotonin from the primary binding site towards the cytoplasm, to explore the alternative inward-facing conformation and reveal the cytoplasmic permeation pathway. The initial state of the SERT model has 5-HT both in the primary binding site and the newly discovered secondary site (L. Shi et al, Mol Cell, 2008). Local structural rearrangements associated with substrate movement reveal changes in water solvent accessibility that explain the energetic drive of the transport mechanism and provide data for validation against experimentally determined accessibilities measured with the substituted cysteine mutagenesis accessibility method (SCAM). Interactions of the moving substrate in SMD identify the residues of SERT which line the transport pathway and their role in stabilizing the inward-open or inward-closed state of SERT. These states involve large-scale helix movements triggered by changes in the binding sites of the substrate and Na ions, and enabled by solvent stabilized changes in the states of an ionic interaction network at the cytoplasmic end of SERT. While the detailed representation of the process provided by SMD simulations at atomic resolution offers specific new hypotheses for experimental probing of the proposed mechanism, it also reveals new insights and mechanistic aspects that are not achievable in other ways.

354-Pos Board B233
Solvent Effects On Protein Mechanical Stability: A Steered Molecular Dynamics Study
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The solvent is an integral component in all cellular processes and solvent composition is actively regulated in the living cell. Changes in solvent environment constitute a chemical signal that is transduced into a mechanical response: a chemically induced change of mechanical properties of a protein system. In this study, steered molecular dynamics simulations are used to stretch mechanical proteins set in non-aqueous solvent environment to reveal details and mechanism of protein - solvent interactions. We explore the atomic level mechanism and measure the effect of solvent substitution on the mechanical properties of proteins. We investigate the distance to the transition state during force induced unfolding as a function of solvent molecule size and the atomistic detail and timescale of participation of solvent molecules in the transition state structure. Resulting constant velocity and constant force extension profiles showed increased stability and resistance to unfolding force by proteins solvated in deuterium oxide vs. proteins solvated in water. Solvent molecules were found present the transition state structure and involved in forming a stabilizing bridge between the force-bearing sheer topology elements. Features of the simulations were also matched with previously reported experimental results.

355-Pos Board B234
Fluorescence Resonance Energy Transfer Reveals Key Binding Domains of Neurotrophin Receptor-Interacting Melanoma-Associated Antigen Homolog in Bone Morphogenetic Protein-Mediated Apoptosis
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Apoptosis, one form of programmed cell death, is used by tissues to develop normally and maintain homeostasis. Lack of apoptosis underlies many diseases such as cancer, while an excess can cause neurodegenerative disorders. Understanding the molecular events that initiate either condition is necessary for the development of treatments. Bone morphogenetic proteins (BMP) play profound roles in development, such as regulation of neural progenitor apoptosis and glial differentiation. Binding of ligand to the BMP receptors triggers the canonical and non-canonical pathways involving Smad and TAK1 activation respectively. In the non-canonical pathway, p38 mitogen activated kinase ($p38^{MAPK}$) is upregulated in P19 cells, a model line of neural progenitors, triggering a signal cascade leading to apoptosis. X-linked inhibitor of apoptosis protein (XIAP) functions as a positive mediator linking TAK1 to the BMP receptors through TAB1. Neurotrophin receptor-interacting MAGE (NRAGE) homolog binds with XIAP in the XIAP-TAB1-TAK1 complex and is necessary in the non-canonical pathway for apoptosis. We have measured fluorescence resonance energy transfer (FRET) between enhanced green fluorescent protein attached to NRAGE deletion mutants and DsRed-monomer attached to XIAP. Results show that the interaction is direct and is facilitated by a unique tryptophan-glutamine-X-proline-X-X (WQXPXX) repeat in NRAGE. We have continued using FRET with enhanced cyan and yellow fluorescent proteins (FPs) in designs with the FP fused to the amino or carboxy termini of NRAGE deletion mutants and XIAP to compare the FRET efficiencies and verify that the FP placement does not affect the NRAGE-XIAP interaction. Additionally, we compare the FRET of overexpressed NRAGE-XIAP with that of the endogenous interaction using antibodies conjugated to Alexa Fluors.

356-Pos Board B235
Conformational Dynamics of Antithrombin III With its Allosteric Activator Heparin
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Antithrombin III (ATIII) is a serpin that is involved in the regulation of blood coagulation through the inhibition of blood clotting enzymes. Heparin is an allosteric activator of ATIII that binds to helix-D and causes a conformational change in the reactive center loop (RCL), expelling it from its position partially inserted into beta-sheet A. RCL expulsion in turn increases ATIII activity toward fXa several hundred-fold. Hydrogen/deuterium exchange and mass spectrometry were used to probe the dynamics of ATIII in the presence and absence of a synthetic heparin pentasaccharide (Fondaparinux). Results of our initial hydrogen/deuterium exchange mass spectrometry experiments provide direct, solution phase evidence that heparin cofactor binding alters conformational dynamics in four specific regions of the antithrombin molecule. (1) Helix D – Heparin binding reduced H/D, consistent with hD extension upon cofactor binding. (2) Breach region – Beta strands 3A and 5A, flanking the site for RCL insertion into sheet A, showed reduced H/D exchange, consistent with increased rigidity of the breach region and stabilization of a loop-expelled form. (3) Proximal RCL and hinge region – H/D exchange for residues 376-387, which includes the N-terminal hinge region of the RCL, increased in the presence of heparin, indicating greater solvent exposure and expulsion from beta sheet A. (4) Distal RCL – Deuterium exchange for 388-402, which includes s1C and the distal side of the RCL, decreased significantly in the presence of heparin, suggesting that s1C extension-mediated stabilization on the C-terminal side of RCL contributes to exposure of the proximal its end upon cofactor binding. Thus, dynamic H/D exchange studies of free and heparin-bound antithrombin molecules in solution will be useful for validating and refining models of ATIII heparin activation inferred from crystal structures.

357-Pos Board B236
Structural Evaluation of the Effects of Disulfide Bond Eliminations on Scorpion Toxin κ-Hefutoxin1 from *Heterometrus Fulvipes*
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κ-Hefutoxin1, a novel weak potassium-ion-channel toxin present in the venom of the scorpion *Heterometrus fulvipes*, is a 22-residue peptide which has a unique spatial fold consisting of two parallel helices linked by two disulfide bridges without any β-sheets. In order to evaluate the structural contribution of the disulfide bonds, wild and three mutant forms of κ-Hefutoxin1 were cloned, expressed, purified and structurally analyzed. To do so, synthetic genes encoding wild and three mutant forms of k-Hefutoxin1 were designed using appropriate codons and synthesized using overlapping primers. In the mutant forms, alternative pairs of cystein residues, which participate in the formation of disulfide bonds, were replaced by serine (Mut1: C4S, C22S; Mut2: C8S, C18S; Mut3: C4S, C22S, C8S and C18S). To facilitate cloning into expression vector, EcoRI and BamHI restriction sites were inserted to the flanking ends of the genes. Moreover, Tev cleavage site was added to the N-terminal part of the genes. The amplified k-Hefutoxin1 genes of wild and mutant forms were cloned into pET32a vector, followed by transformation into *E.coli* host strain DH5α. The positive colonies with recombinant plasmid were first screened by PCR analysis and finally confirmed by sequencing. Then, the correct recombinant plasmid was transformed into *E.coli* host strain BL21 in which protein expression was induced by IPTG. After assuring protein expression by polyacrylamide gel electrophoresis, the cells containing wild and mutant forms of