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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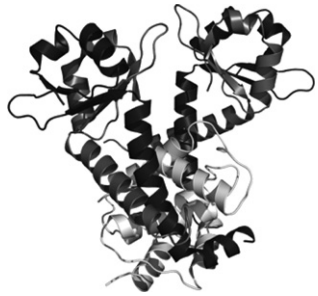
Regulation of gene expression is a fundamental process that allows a cell to respond to changes in its environment. At the molecular level, expression is tuned by the concerted action of both activators and repressors whose activity is typically linked to external or internal stimuli. Bacterial toxin-antitoxin (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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