strategies is the use of small solute molecules called osmolytes that most often confer stability to folded proteins by preferential exclusion from macromolecular surfaces. Recent evidences indicate that modest changes in environmental conditions set by osmolytes and other cosolutes can have profound effects on protein and peptide conformation and aggregation. Such aggregation processes constitute a hallmark of neurodegenerative pathologies, including Alzheimer's, Huntington's, and Parkinson's diseases. This study examines the effect of natural osmolyte on a model peptide that can fold from a ''random coil'' to β-hairpin, or aggregate into fibrils. We use Fluorescence and Circular Dichroism measurements as well as perform Molecular Dynamic simulations to determine the mechanism by which osmolytes control the structure and thermodynamic stability of the peptide, and to follow changes in peptide aggregation kinetics. We find that excluded osmolytes such as sugars and polyols cause peptides to favor a more compact (folded) structure relative to more extended (unfolded) conformations, and that this stabilization sensitively depends on the osmolyte used. Water structuring in close proximity to peptide surfaces crucially affects this process. Understanding the role of osmolytes in regulation will not only allow to predict the action of osmolytes on macromolecular interactions in stressed and crowded environments typical of cellular conditions, but will also provide insights on how osmolytes may be involved in pathologies or in their prevention.

426-Pos Board B305

The Effects of Reduction Potential and Number of Disulfide Bonds on the Correct Folding of Lin-12/Notch Repeats (LNRs) Using Human Notch 1 LNRA as a Model System

Lauren Choi, Didem Vardar Ulu.
Wellesley College, Wellesley, MA, USA.

Notch receptors are multi-domain trans-membrane proteins that are important for cell-cell communication and development. Deregulated Notch signaling has been linked to many human diseases such as sclerosis, artereopathy and leukemia. The extra-cellular domain of the Notch Receptor contains the Ligand Binding Domain and the Negative Regulatory Region (NRR), which includes three Lin-12/Notch Repeats (LNR), small disulfide-rich sequences of 35 residues. It has been previously shown that the first LNR from human Notch1, hN1LNRA, requires Ca2+ and a certain reduction potential that ensures the correct formation of three specific disulfide bonds believed to be critical for LNR structure and function. However, the first LNR in human Notch 4 and some of the LNRs found in PAPP (pregnancy-associated plasma protein-A), only possess four cysteines thereby can only form two disulfide bonds.

In this work we present our findings on the effect of various reduction potentials as well as the elimination of the first disulfide bond in the in vitro folding of hN1 LNRA through a comparative analysis. The kinetics of the folding process for both the wild-type and the four-cysteine mutant form of hN1LNRA is studied by trapping various folding intermediates in a time-course manner, which is possible due to the slow rate of disulfide bond formation. Our results indicate that even though the wild-type hN1LNRA is very tolerant to variations in the specific redox potential in obtaining its ultimate correct folding, the its folding kinetics is significantly impacted. This is in contrast to the mutant form, which does not fold into a single species under identical refolding conditions.

427-Pos Board B306

Helical Flexibility Governed by the Placement of Alanine Residues in a Series of Aib-Rich Model Peptides

Matthew Cocchiola, Valentine Sackmann, **Adrienne P. Loh**.
University of Wisconsin - La Crosse, La Crosse, WI, USA.

It has been established that peptides composed primarily of the amino acid Aib (α-aminoisobutyric acid) fold into 3_{10}-helices. Aib is structurally similar to alanine but with an additional methyl group at the α-carbon. The α,α-dialkylation creates significant steric hindrance, which is responsible for the helical preference of Aib. We are studying the effects of steric hindrance on the flexibility of Aib-rich helices. ^{1}H NMR spectra of peptides dissolved in a deuterated solvent (CD_3OD) are obtained as a function of time and temperature. Rate constants for amide proton/solvent deuteron exchange are found using a pseudo first order model. Activation energies are obtained using the Arrhenius equation. Larger activation energies suggest stronger intramolecular H-bonds and a more rigid helix. Preliminary results on an Aib octamer (known to form a regular 3_{10}-helix) show similar activation energies for all but the first two solvent-exposed amides, suggesting that the helix is fairly rigid in solution. When alanines are substituted at the fourth and fifth positions (4,5-AA), the exchange rates at Ala4 and Aib6 *decrease* relative to the other hydrogen-bonded amides, while that at Ala5 *increases*. Thus, the reduction in steric hinderance at Ala4 and Ala5 creates a local compression in the helix, opening one face of the helix and pinching the other. FTIR spectra of 4,5-AA shows a broader distribution of helical conformations than observed for the Aib octamer. Placement of the two alanines instead at positions three and six (3,6-AA) results in a narrow conformational distribution by FTIR similar to that of the Aib octamer. NMR data also suggest a more regular 3_{10}-helical conformation for 3,6-AA than for 4,5-AA. Thus the positioning of the less hindered Ala residues is a significant driving force in determining the helix flexibility.

Molecular Recognition in Silico

428-Pos Board B307

Free Energy Calculations of Sparsomycin Analogs Binding to the Ribosome with Molecular Dynamics Simulations

Xiaoxia Ge[1,2], Benoit Roux[2].
[1]Weill Medical College of Cornell University, New York, NY, USA, [2]The University of Chicago, Chicago, IL, USA.

The accurate calculation of absolute binding free energy is one of the holy grails of computer-aided drug design. The emerging successes reported in computing the binding free energy of small ligands to proteins using molecular dynamics (MD) simulations indicated that such physics-based approaches hold the promise of expediting the rational drug discovery process. Among numerous receptor-ligand systems, ribosome-antibiotic binding provides an important paradigm for studying the molecular recognition of RNAs by small molecules. The interactions of the 50S bacteria ribosomal subunit with antibiotic sparsomycin and its derivatives have been studied through the calculation of the binding free energy and the characterization of conformational dynamics. The standard binding free energies of the complexes were calculated using free energy perturbation (FEP) method. Restraining potentials affecting the orientational, translational and conformational freedom of the ligand and receptor were applied and then removed during the simulations to enhance the sampling and the convergence. The loss of ligand conformational entropy upon binding was estimated with Umbrella Sampling method by calculating the Potential of Mean Force as a function of the RMSD relative to the reference conformation of the ligand. Due to the large size of the ribosome, the Generalized Solvent Boundary Potential method was used to reduce the computational cost of MD/FEP calculations. For a deeply buried binding pocket in the ribosome, the fluctuation of solvent occupancy during the alchemical free energy calculation was also characterized by combining the MD with Grand Canonical Monte Carlo simulation. This computational study further revealed the mechanism of ribosome-antibiotics interactions and shed light on the design of ribosomal drugs. With the above stated developments, the evaluation of the binding free energies has become computationally more appealing for large systems.

429-Pos Board B308

Computational Discovery Of The Electronegative Channel In RNA Loop-loop Interactions

Andrey Semichaevsky, Abhishek Singh, **Yaroslava G. Yingling**.
North Carolina State University, Raleigh, NC, USA.

The most common motifs found in nature and used in bionanotechnology are hairpin loops which consist of a helical part and a loop with unpaired residues. The unpaired residues in these elements can lead to further super-assembly of RNA structures via formation of the loop-loop interactions. These loop-loop interactions regulate biological functions in both prokaryotic and eukaryotic organisms such as gene expression in different viruses and are also actively used in bionanotechnology for self-assembly of RNA building blocks into novel nanostructures. It has been observed that the super-assembly of RNA directly depends on the presence and specific concentration of ions. In order to understand the role of ions in loop-loop formation and stability, we conducted a series of explicit solvent atomistic molecular dynamics simulations of distinct kissing loops elements taken from various organisms. In our simulations we varied the concentration of different ions (such as Na+, K+, Mg2+, and Cl−) from zero to 1M solution and examined known destabilizing mutations. We discovered that in most organisms the loop-loop assembly process depends on the presence of electronegative and hydration channel. The properties of this channel are independent of the concentration and the type of ions. The size of this channel and RNA sequence determines the stability. We also examined the formation of the channel during self-assembly and discovered the critical threshold for the channel formation.

430-Pos Board B309

Docking of a Linker Histone to The Nucleosome With Flexible Linker DNAs

Georgi V. Pachov, Rebecca C. Wade.
EML Research gGmbH, Heidelberg, Germany.

In the cell nucleus, DNA wraps around histone proteins, forming nucleosome particles, and packs into a highly negatively charged structure, the chromatin.