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.

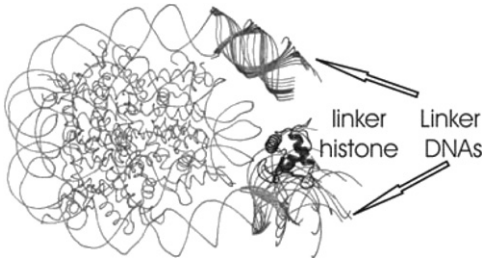
430-Pos Board B309

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

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

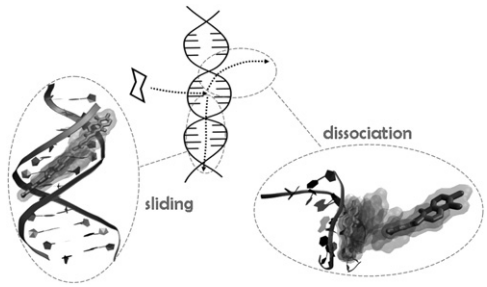
In the cell nucleus, DNA wraps around histone proteins, forming nucleosome particles, and packs into a highly negatively charged structure, the chromatin.

The linker histone is a protein that binds to the nucleosome and determines how the nucleosomes are linked to each other. To simulate the nucleosome-linker histone interactions, we applied a Brownian Dynamics (BD) technique together with normal mode analysis (NMA). NMA of the nucleosome revealed the most prominent modes of motion of its two linker DNAs. The results were used to generate conformations of the linker DNAs which were used in BD simulations of docking of a linker histone and its mutants to the nucleosome. From the simulations, two distinct binding sites on the linker histone were identified. The residues found to be most important for binding in the simulations with the linker histone mutants are consistent with experimental data. Moreover, a unique binding mode of the linker histone to the nucleosome was found for a wide range of conformations of the linker DNAs. As well as providing insights into the determinants of linker histone-nucleosome binding, the results are valuable for higher-order modelling of the chromatin.



431-Pos Board B310
Exploring The Spatiotemporaloral Dynamics of DNA Binding and Cleavage by Restriction Endonucleaes
Wei-Ting Wang, Jing-Shin Tsai, Chien-Ting Hsu, Tzu-Sen Yang*.
Taipei Medical University, Taipei, Taiwan.
Using restriction endonucleases to catalyze the double-stranded DNA (dsDNA) breakage at certain recognition sequences is an important molecular biology technique. The restriction endonucleases constitute an important defense mechanism of bacteria against viral attacks; this mechanism is to destroy invading foreign DNA molecules via cleaving a specific site (phosphodiester bond) of a dsDNA. By cleaving recognition sites on dsDNA with extraordinary specificity can lead to the DNA double strand breaks (dsb). We presented a novel single-molecule approach to investigate the interaction between DNA and restriction endonucleases, including DNA recognition and cleavage. To elucidate how fast restriction endonucleases recognize and cleavage DNA sequence, we constructed a high resolution dual-beam laser tweezers system to manipulate single DNA molecule, together with the site-specific restriction enzymes, namely, EcoRI (one-site endonuclease) and Cfr9I (two-site endonuclease), conjugated to nanometer-sized fluorescence particle. Because most endonucleases work in the presence of magnesium ions, we will apply optically based reaction mechanism to control and synchronize the restriction endonuclease activity in this study. Furthermore, both laser tweezers and fluorescence particle imaging will be used to probe whether the DNA double strand breaks occurred due to the molecular cutting. Hence, this single-molecule approach allows us to directly observe and visualize the spatiotemporal dynamics of DNA binding and cleavage by restriction endonucleases, and can be further applied to determine the DNA cleavage rate due to the presence of EcoRI and Cfr9I. Finally, we extend this approach, together with the light-induced molecular cutting, to investigate the DNA binding and cleavage by restriction endonucleases under tension at different temperatures.

432-Pos Board B311
Molecular Recognition Routes Of DNA By Anticancer Ligands: Mechanisms and Free Energies Explored Via Molecular Dynamics Simulations
Attilio Vittorio Vargiu[1,2], Alessandra Magistrato[3], Paolo Carloni[3], Paolo Ruggerone[1].
[1]Universitá di Cagliari, Cagliari, Italy, [2]CNR-INFM-SLACS, Cagliari, Italy, [3]SISSA/ISAS, Trieste, Italy.
Molecular recognition of the DNA minor groove is a multi-route process which can involve many steps before the formation of the most stable adduct. In particular, many studies have pointed out the importance of events like sliding along the groove and dissociation (which is a relevant step in the translocation among different sequences) for the affinity and the specificity of minor groove binders.
In this contribution we present our recent work on the subject. Umbrella sampling and metadynamics were used to characterize mechanisms and free energy profiles of molecular recognition routes by the antitumoral agents anthramycin, duocarmycin and distamycin. Our results are in very good agreement with the available experimental data, and provide insights on the influence of factors like size, charge and flexibility on the molecular recognition process.



433-Pos Board B312
Computational Studies of Substrate Binding and Conformational Change in the Glycine Betaine Symporter BetP
Kamil Khafizov, Christine Ziegler, Lucy Forrest.
Max-Planck Institute of Biophysics, Frankfurt am Main, Germany.
The glycine betaine symporter BetP is an important protein for regulation of osmotic pressure in the microbe *Corynebacterium glutamicum*, a bacterium used extensively in biotechnology. BetP responds to changes in external osmolality by regulation of its transport activity. The recently solved X-ray crystallographic structure of this protein reveals that it is a homotrimer and that each monomer possesses its own substrate binding pocket. Available structural data for several secondary transporters suggest that these proteins may adopt one of several structurally-distinct states, namely outward- and inward-facing state conformations, as well as a so-called occluded state. Since the monomers in the BetP X-ray structure do not show any apparent substrate pathways, they are likely to represent an occluded state. To identify structural features of alternate states, which may have relevance for a range of secondary transporters, we constructed 3D models of outward- and inward-facing states of BetP using secondary transporters of known structure as templates and validated the modelling results through the rigid-body fitting of these models to low-resolution cryo-EM maps. In addition, to address several other remaining questions, including: the location of glycine betaine and Na^+ binding sites; the effect of the headgroup size and net charge of lipid molecules; and the importance of the trimeric state of the protein, we have performed all-atom molecular dynamics simulations of BetP. Finally, we combined the results of structural and simulation studies with those from sequence analysis of BCCT transporters in order to identify structural and functional roles for several important residues. The results of our computational studies may lead to a better understanding of key events in the transport cycle and they are being validated experimentally.

434-Pos Board B313
Substrate Binding Directs the Functional Hinge Bending Motion of Human 3-Phosphoglycerate Kinase
Erika Balog[1], Zoltan Palmai[1], Laurent Chaloin[2], Corinne Lionne[2], Judit Fidy[1], David Perahia[3].
[1]Semmelweis University, Budapest, Hungary, [2]CNRS–Université Montpellier 1—Université Montpellier 2, Montpellier, France, [3]Université Paris-Sud, Orsay, France.
3-Phosphogycerate kinase (PGK) is a two domain enzyme, with a binding site of the 1,3-bisphosphoglycerate on the N-domain and of the ADP on the C-domain. In order to transfer a phosphate group the enzyme has to undergo a hinge bending motion from open to closed conformation to bring the substrates to close proximity. Molecular dynamics simulation was used to elucidate the effect of ligand binding onto the domain motions of this enzyme. The simulation results indicate the presence of a relatively small amplitude hinge bending motion of ns timescale in the apo form while the time period of the hinge bending motion of the complex form is clearly over the 20 ns simulation time. Upon binding the ligands, the hinge bending shows more directed characteristics with one dominant hinge point in the vicinity of the substrates while the apo form exhibits several hinge points that contribute to the hinge bending motion. The correlation of interdomain atomic movements also increased upon substrates binding.

435-Pos Board B314
Molecular Dynamics Simulation Study of T Cell Receptor Molecular Recognition of Peptide-Major Histocompatibility Complexes
Ghalib A. Bello, Michael E. Paulaitis.
The Ohio State University, Columbus, OH, USA.