identity of these proteins and how they cooperate to transmit the energy of ATP hydrolysis is yet unclear. Up to fifteen proteins are often considered necessary for pilus function; however, as few as six proteins may be sufficient for pilus assembly the rest being involved in the disassembly process and/or regulation of the system. To better understand the assembly mechanism and to definitively identify the components of the assembly apparatus, a library of plasmids containing from one to nine component genes of the Type IV Pilus system from Neisseria gonorrhoeae has been constructed using a combinatorial assembly method. Expression of these sets of proteins in non-piliated E. coli is currently being tested to identify those sufficient for pilus assembly and to enable further mechanistic studies.

303-Pos

Assembly Models of P7 Protein From HCV

Yi Ting Wang, Hao-Jen Hsu, Wolfgang Fischer.
Institute of Biophotonics, School of Biomedical Science and Engineering, NYMU, Taipei, Taiwan.

Hepatitis C virus (HCV) encodes for a transmembrane protein p7 which is known to alter electrochemical gradients across the lipid membrane by forming a channel. This task is assumed to be essential for the life cycle of HCV, since p7-deletion mutants cannot survive. Therefore p7 depicts a very promising target for antiviral therapy.

The p7 protein has two transmembrane domains, TM1 and TM2, which are connected via a short basic loop. In this study novel computational models of p7 are presented made by a fine grained search protocol which enables to cover extensive conformational space of (i) the monomeric units of TM1 and TM2, and consequently the conformational space of (ii) hexa- and heptameric assemblies of the monomeric untis. The affect of different force fields and dielectric on the assembly is investigated. Models with the lowest energies are due to multi nano second molecular dynamics simulations to evaluate structural integrity. In most low energy models the formally reported motif of histidines facing the lumen of the pore is conserved. The models may serve for structure based drug development.

304-Pos

Defining the Interaction Between S100A13 and Annexin II Peptide: Insight into Non-Classical Secretion

Anna E. Daily, T.K.S. Kumar.
University of Arkansas, Fayetteville, AR, USA.

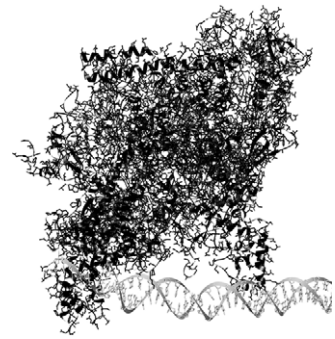
S100A13 is a calcium binding chaperone protein that is known to be involved in the non-classical export of signal peptide-less proteins, such as fibroblast growth factor (FGF-1) and interleukin-1α,. It has also been shown that the interaction of S100A13 with Annexin II, which exhibits an inducible flip-flop mechanism across the cell bilayer, helps the multiprotein release complex to traverse the membrane bilayer. The interaction of S100A13 and Annexin 2 has been characterized using various biophysical techniques including Isothermal Titration Calorimetry (ITC), Differential Scanning Calorimetry (DSC), and multidimensional NMR spectroscopy. Results of the Isothermal titration calorimetry (ITC) experiments show that holo-S100A13 exhibits preferential binding to Annexin II with high affinity in the low micro molar range compared to apo-S100A13. Equilibrium guanidine hydrochloride denaturation monitored by steady-state fluorescence and limited trypsin digestion analysis both reveal holo-S100A13 to be stabilized upon binding with the Annexin II peptide. ANS (8-anilino-1-napthalene sulfonate) binding experiments indicate that the presence of Annexin II peptide does not increase the solvent accessiblity of hydrophobic residues in holo-S100A13, which is a unique characteristic of S100A13. 1H-15N- HSQC NMR experiments reveal the binding site of the Annexin II peptide on holo-S100A13 to be distinctly different from other S100/Annexin interactions. In order to define the specificity of S100A13 for Annexin II specifically, the interaction of S100A13 with other Annexin peptides was characterized by ITC. The information gained from this study provides valuable information on the interaction between S100A13 and the Annexin II peptide and gives much needed insight into the mysterious pathway of non-classical release of the signal peptide-less proteins.

305-Pos

Structural Modeling of a Bacterial RNAP/DNA Complex to Understand Functions of the Prokaryotic Transcription Machinery

Chang-Shung Tung, **Paul Fenimore**, Benjamin McMahon.
Los Alamos National Laboratory, Los Alamos, NM, USA.

Bacterial RNA polymerase (RNAP) is responsible for transcribing genes into messenger-RNAs. RNAP is a multi-components protein complex consisting of α, β, β', Ω-subunits. RNAP, together with one or more initiation factor(s), binds to promoter DNA to yield an RNAP-DNA initiation complex. While many attempts were made to solve structures of various components of the transcription complex, the detailed structure of the RNAP-transcription factor-DNA complex is still not available. We have engaged in an exercise to develop a detailed model structure for an RNAP-σ^{70}-DNA complex. The detailed structure of the complex (as shown in the attached figure) is modeled using a structure and sequence-based approach developed in our laboratory utilizing the low-resolution crystal structure of *T. aquaticus* RNAP- σ^{70}-DNA (PDB: 1L9Z) as a scaffold and information derived from high-resolution crystal structures of the polymerases (minus the σ-subunit and the promoter DNA duplex) from *T. aquaticus* (PDB: 1HQM) and *T. thermophilus* (PDB: 2O5J). The physical constraints for the C-terminal domains of the α-subunits binding to DNA with and/or without other factors (e.g., MarA, CAP) in the presence of the RNAP-σ^{70}-DNA are discussed.

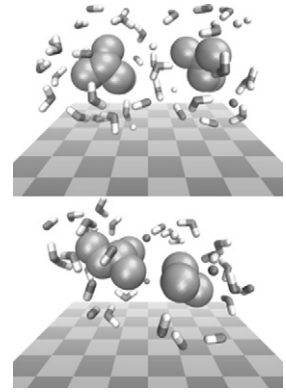


306-Pos

Towards Molecular Dynamics Simulations of Large Protein Complexes

Djurre de Jong, Xavier Periole, Siewert Jan Marrink.
Rijks Universiteit Groningen, Groningen, Netherlands.

The structure and dynamics of large (membrane)protein complexes is an important challenge for biophysical sciences. The formation of such complexes plays a major role in many biological pathways. Despite of their importance, until recently molecular dynamics simulations of protein complexes were impossible because of their large size.



Coarse grained molecular dynamic force fields, like the Martini force field [Monticelli, 2008], are potentially powerful tools to investigate protein complex formation. Therefor it is important to probe there performance. To assess the performance of the Martini force field on protein interactions, we calculated the binding free energies of pairs of amino acid side chains in different solvents. The binding free energies were calculated from both equilibrium and PMF-simulations. The results were compared to results for united-atom (Gromos53) and all-atom (OPLS-AA) force fields.

307-Pos

Replica Exchange Simulations For Macromolecular Crowding Effects on Multiprotein Binding

Youngchan Kim[1], Robert B. Best[2], Jeetain Mittal[3].
[1]Naval Research Laboratory, Washington, DC, USA, [2]University of Cambridge, Cambridge, United Kingdom, [3]Lehigh University, Bethlehem, PA, USA.

Protein-protein interactions play an essential role in most of the biological processes inside a cell. The cellular medium is crowded with an ensemble of macromolecules, e.g., proteins, nucleic acids, sugars and lipids. These macromolecules can occupy as high as 30% of the cell volume, thereby affecting the stability and kinetics of multiprotein complexes. We present results from molecular simulations for the effect of macromolecular crowding on the formation of multiprotein complexes, using a residue-level coarse-grained protein model and repulsive spherical crowder particles. The protein model has already been shown to yield thermodynamics and structures of various weakly binding protein complexes in good agreement with experimental data. We performed replica exchange Monte Carlo simulations on two distinct protein complexes, ubiquitin-UIM1 and cytochrome *c*-cytochrome *c* peroxidase. For crowders of sizes 12-24 Å in radius, we find that crowding has a modest stabilization effect on the formation of both complexes, lowering the binding free energy by up to 2 k_BT as the volume fraction of crowding increases to 30%. This modest stabilization is consistent with experimental observations on macromolecular crowding effects. More importantly, crowders increase the population of native complexes by destabilizing transient encounter complexes. All the simulation data are well described by the scaled particle theory for single size crowders as well as mixed crowders.