surfactant, and DPPC/POPG/Palmitic Acid (68:22:9) (lipidTA), a system widely used as a basis for clinical surfactants. We found similar equilibrium surface tensions after 5 min of adsorption of all the samples, regardless the protein and lipid system, although SP-B exhibited somehow slower initial adsorption in lipidTA. Significant differences were found in SP-B activity under quasi static compression-expansion cycling for the two lipid systems tested. In lipidTA, SP-B allowed reaching tensions near 2mN/m, whereas in lipidS surface tension did not fall below 20mN/m. However, SP-B-containing samples produced similarly low tensions, within the two lipid compositions, once cycled dynamically at physiologically relevant compression-expansion rates. Analysis of film stability under mechanical perturbations showed that SP-B introduces a significant resistance of the films to relaxation, which is particularly remarkable in lipidS samples. This stability was maximal in the simultaneous presence of SP-B and SP-C.

297-Pos

The NHERF2 Dependent Dynamic Ca2+/LPA Regulation of NHE3 Mobility and Interaction At the Epithelial Brush Border

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Na+/H+ exchange 3 (NHE3) plays an essential role in NaCl absorption intestine and kidney. NHE3 rapidly cycles between the plasma membrane and recycling endosomal compartment under basal conditions. Those regulations require PDZ domain containing NHERF proteins. Especially NHERF2 is required both in Ca2+ inhibitory and LPA stimulatory regulations in NHE3 activity. In this study, using the FRET technique, the dynamic binding between NHERF2 and NHE3 at the brush border was investigated in the presence of Ca2+ or LPA. Zeiss 510 Meta confocal microscopy was used to perform FRET (acceptor photobleaching) between NHE3-YFP and CFP-NHERF2 on the apical brush border in polarized epithelial kidney OK cells.
We observed that NHERF2 and NHE3 exhibited 10-20% FRET signaling at the microvilli and not at the juxtanuclear region under basal conditions. As a negative control, there was no FRET signaling between CFP-NHERF2 and YFP-GPI. With treatment of Ca2+ ionophore, A23187(0.5μM) or LPA(100μM), FRET signaling was transiently abolished within one minute for A23187 and within 30min for LPA and recovered at 1 hr later.
The dynamic interactions between NHE3 and NHERF2 by LPA and Ca2+ in OK cell microvilli were quantified by FRET. We conclude that the dissociation of NHEFR2 from NHE3 at the microvillus leads to NHE3 activity inhibition by A23187 by increasing the NHE3 endocytosis and leads to stimulation of NHE3 activity by LPA by increasing NHE3 translocation to the brush border.

Protein Assemblies

298-Pos

Structural Survey of Large Protein Complexes in Desulfovibrio Vulgaris

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Large protein complexes purified by a tagless strategy have been analyzed for an unbiased survey of the stable, most abundant multi-protein complexes in Desulfovibrio vulgaris Hildenborough (DvH) that are larger than Mr ~400 k. The quaternary structures were determined for 8 out of 16 complexes by single-particle reconstruction of negatively stained specimens. The success rate of getting structure was about 10 times greater than that of previous ''proteomic'' screens. For the remaining complexes, the subunit compositions and stoichiometries were analyzed by biochemical methods. Our results show that the structures of large protein complexes vary to a great extent from one microorganism to another. None of the complexes except for GroEL and the ribosome could not be modeled from the previously known homologous structures due to organism dependent variation of quaternary structures. This result indicates that the interaction interfaces within large, macromolecular complexes are much more variable than has generally been appreciated. As a consequence, the quaternary structures for homologous proteins may not be sufficient to understand their role in another cell of interest. The diversity of subunit stoichiometries and quaternary structures of multiprotein complexes that has been observed in our experiments with DvH is relevant to understanding how different bacteria optimize the kinetics and performance of their respective biochemical networks. It is further anticipated that imaging the spatial locations of such complexes, through the analysis of tomographic reconstructions may also be important for accurate computational modeling of such networks. While templates for some multi-protein complexes such as the ribosome or GroEL could be derived from previously determined structures, it is quite clear that single-particle electron microscopy should be used to establish the sizes and shapes of the actual complexes that exist in a new organism of interest to prepare valid templates.

299-Pos

Ranolazine Preserves the Integrity of Mitochondrial Supercomplexes

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Mitochondrial respiratory complexes are known to exist in multi-complex assemblies (respirasomes). These respirasomes and their constituents are known to be damaged during ischemia reperfusion (IR) injury. In the present study we examined if ranolazine, a late sodium current blocker, and also a partial fatty acid oxidation inhibitor, preserves these assemblies after cardiac IR injury. Guinea pig hearts (n=6) were isolated and perfused with Krebs Ringer buffer (KR) and exposed to one of the following three protocols: 1) KR perfusion for 30 min, (time control), 2) 30 min global ischemia, or 3) ranolazine (10 μM) perfusion for 10 min just before 30 min global ischemia. Mitochondria were isolated by differential centrifugation and then subjected to blue native Polyacrylamide Gel Electrophoresis (BN-PAGE) to examine for damage to the respirasomes. We observed that there is a loss of protein bands after electrophoresis at 720 kDa and at 250 kDa in the untreated ischemic group. These bands were restored in the ranolazine treated group. These proteins will be subject to identification. Our results indicate that cardiac ischemia causes a loss of integrity of respiratory complexes, which is restored partially by ranolazine. A candidate for ranolazine's protective effect is cardiolipin, which stabilizes the respiratory chain supercomplexes, and which may be less oxidized after ranolazine treatment.

300-Pos

A Biophysical Investigation of the Non-Classical Release Complex of Fibroblast Growth Factor-1

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Fibroblast Growth Factor-1 (FGF-1) is a potent angiogenic agent that is released via the non-classical protein secretion pathway. Angiogenesis, the process of formation of new blood vessels, is vital to the formation of tumors, and is also responsible for tumor metastasis, as cancer cells travel from one part of the body to another through the newly formed vessels. Export of FGF-1 is based on the Cu2+ -dependent structure of multi-protein complexes, which involves the S100A13, a Ca2+ binding protein belonging to the family of S100 protein. The goal of this study is to characterize the structure of the FGF-1/S100A13 Data will be presented analyzing the interaction between FGF-1 and an S100A13 peptide that has been designed to mimic the binding region of FGF-1 on S100A13. The binding interaction was characterized using various biophysical techniques including ITC, DSC, proteolytic digestion, and multi-dimensional NMR spectroscopy. Characterization of the binding FGF-1/S100A13 interface is expected to shed light on the molecular mechanism(s) underlying the non-classical secretion of FGF-1.

301-Pos

The Monomerization of a Dimeric, Calcium-Binding Protein Involved in the Non-Classical Export of Fibroblast Growth Factor 1

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The non-classical secretion of fibroblast growth factor 1 (FGF1) is a poorly understood process. FGF1 is known to interact with the calcium-binding protein S100A13, which escorts FGF1 to the cytoplasmic surface of the cell membrane. The dimeric, highly alpha-helical structure of S100A13 has been well characterized. In addition to binding to Ca^{2+}, S100A13 has been shown to bind to Cu^{2+}. Binding of Cu^{2+} to S100A13 is believed to be crucial for the formation of the FGF1 release complex. In order to gain a better understanding of the structural forces involved in the organization of the multi-protein FGF1 release pathway, we have embarked on the determination of the 3D structure of the FGF1 release complex in solution using multi-dimensional NMR spectroscopy techniques. As a first step toward achieving this objective, we have designed an S100A13 monomer through site-specific mutations at the S100A13 dimeric interface. Results on the characterization of the S100A13 monomer using ITC, DSC, CD spectroscopy, and multi-dimensional NMR spectroscopy will be presented.

302-Pos

Reconstructing the Neisseria Type IV Pilus System in E.coli

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Type IV Pili, long, thread-like structures found on the surface of many species of bacteria, are important virulence factors involved in motility, DNA/phage uptake, biofilm formation, and adhesion. Energy for the system is supplied by a set of cytoplasmic, hexameric ATPases which interact with proteins within the bacterial membrane to traffic pilin monomers to and from the pilus. The

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

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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

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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

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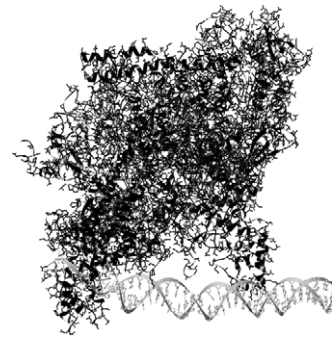
Bacterial RNA polymerase (RNAP) is responsible for transcribing genes into messenger-RNAs. RNAP is a multi-components protein complex consisting of α, β, β', Ω-subunits. RNAP, together with one or more initiation factor(s), binds to promoter DNA to yield an RNAP-DNA initiation complex. While many attempts 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

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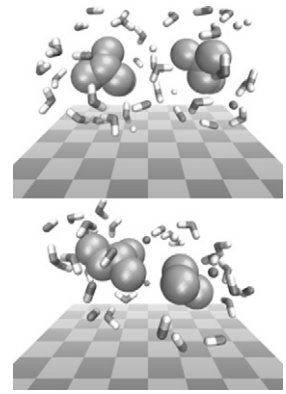
The structure and dynamics of large (membrane)protein complexes is an important challenge for biophysical sciences. The formation of such complexes plays a major role in many biological pathways. Despite of their importance, until recently molecular dynamics simulations of protein complexes were impossible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

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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.