be critical for proper biological function, and serve as markers for various disease states. To study the effects of these important mutations, we have prepared constructs of the caveolin protein that encompass the transmembrane domain. Both Caveolin(99-137) and caveolin(122-142) were overexpressed and isotopically labeled in E.coli, purified to homogeneity, and incorporated into dodecylphosphocholine micelles. The three-dimensional structure of the constructs will be revealed using a combination of circular dichroism and NMR spectroscopy. Moreover, comparison of the wild-type structure with that of the constructs containing the proline mutations will give valuable insight into the unusual nature of the caveolin-1 transmembrane domain.

2082-Pos Board B68
Reconstitution and Topological Analysis of Caveolin-1 in Phospholipid Vesicles
Kyle Root
One of the greatest challenges of working with membrane proteins is their reconstitution into native bilayers. The extreme insolubility of integral membrane proteins renders them incompatible with most common reconstitution methods. It has been shown that perfluorooctanoic acid (PFOA) is a very favorable detergent for highly hydrophobic peptides and proteins. Therefore, a method has been developed using PFOA to reconstitute proteins into egg lecithin unilamellar vesicles. Using dynamic light scattering and electron microscopy, vesicles generated by this method are spherical and have a hydrodynamic radius of 14 nm. In addition, the entrapment of glucose by the vesicles demonstrates that they are indeed bilayered and have a hollow center. The membrane interacting domain of caveolin-1 and has been incorporated into these vesicles. Using the Stern-Volmer analysis methodology, fluorescence quenching studies of the tryptophan residues in the caveolin-1 membrane interacting domain have given qualitative structural information of how caveolin-1 interacts with the lipid bilayer. In addition, the topology of caveolin-1 has been explored using proteolysis. The construct contains one enterokinase cleavage site at the N- and C- termini. Both termini were cleaved with the enterokinase enzyme confirming the "horseshoe" orientation of the caveolin-1 membrane interacting domain in the bilayer. We believe these studies will provide further insight into the caveolin-1 transmembrane domain structure in a lipid bilayer. These experiments begin to address the long-standing uncertainties surrounding the topology of the caveolin protein.

Protein Assemblies

2083-Pos Board B69
Automated Prediction of Protein-Protein Association Rate Constants
Sanbo Qin, Huan-Xiang Zhou.
The association of two proteins generally occurs in two steps [1]. The first is the formation of a transient complex, in which the two proteins have near-native separation and relative orientation. In the subsequent step, the proteins undergo conformational rearrangement to form the native short-range interactions. One of the biggest challenges of the transient complex limit is formation to a "basal" rate constant \( k_{a0} \approx 10^5 \text{ M}^{-1}\text{s}^{-1} \), but long-range electrostatic attraction can enhance the rate by three to four orders of magnitude [1]. In our recently developed transient-complex theory [1], the electrostatically enhanced association rate is predicted as

\[
k_{a} = k_{a0} \exp(-U_{di}*/k_{T})
\]

where \( k_{a0} \) is the basal rate constant and \( U_{di}/* \) is the average electrostatic interaction energy between the proteins in the transient complex. The predictive power of the transient-complex theory has been demonstrated over a large number of protein-protein and protein-RNA complexes [1-3]. To make our method widely accessible for predicting protein association rate constants, here we developed it into a web server. The “TransComp” server is available at [http://rnp.sc.fsu.edu/transcomp/](http://rnp.sc.fsu.edu/transcomp/), with the structure of the native complex formed by two proteins as input. The generation of the transient complex and the calculation of \( k_{a0} \) and \( U_{di}/* \) are done automatically. Test run of the server on 14 protein-protein complexes was carried out to demonstrate its capability. Association rate constants calculated by the server for these complexes agree well with experimental data, which range from 5.5 \( \times 10^5 \) to 2.4 \( \times 10^7 \) \( \text{ M}^{-1}\text{s}^{-1} \). We expect that the TransComp server will become a valuable tool for kinetic characterization of protein-protein and protein-nucleic acid association.


2084-Pos Board B70
Prediction of Protein-Protein Interactions at Genome Scale
Nathan Tunchag, Arttia Gursoy, Ruth Nussinov, Ozlem Keskin.
Construction of the structural protein interaction network is of crucial importance since it is a prerequisite for understanding how the proteome, and thus the cell, function. Yet, predicting, on the proteome scale, which proteins interact and how they interact is a daunting task. Structural predictions of protein interactions are frequently carried out via ‘docking’. However, in the absence of additional biochemical data, docking is challenging on the proteome scale because there are many favorable ways for proteins to interact. An alternative strategy is knowledge-based, using a protein-protein interface database. Using such dataset is efficient because the number of architectures, in single chain proteins and in protein-protein interfaces is limited in nature, and structurally different protein pairs can use the same (preferred) binding architectures. This suggests that using structural alignment of each side of known interfaces against the entire surfaces of all monomers can predict protein associations: a protein whose surface matches one side of the interface can bind a protein whose surface matches the complementary side. Yet, on their own knowledge-based methods may not be sufficient for proteome modeling because they disregard flexibility and energetics. Here, for the first time, we combine the two methods, leading to a powerful combinatorial multi-scale strategy to predict functional associations of the proteome. As examples, we present the tumor suppressor protein p53 interaction network, focusing on the nucleotide excision repair and cyclin dependent kinase subsystems. The validated examples demonstrate the power of this strategy. New interactions are also predicted for NFkB, p27 and Skp2.

2085-Pos Board B71
Monte Carlo Study of the Molecular Mechanisms of S-Layer Protein Self-Assembly
Christine Horejs, Mithun K. Mitra, Dietmar Pum, Uwe B. Sleytr, Murugappan Muthukumar.
Self-assembly is one of nature’s strategies to organize matter on the large scale and thereby create order from disorder. The process is ubiquitous for a great variety of biological molecules. Proteins, however, tend to stay soluble in solution or to aggregate into various structures rather than self-assemble into defined patterns. This is because of their complex structure exhibiting different conformations and a close-knit relationship between structure and function. Aggregation into three-dimensional composites thus generally leads to a loss of functionality. However, Surface-layer proteins, which constitute the crystallized outermost cell envelope of a great variety of bacterial cells, represent a remarkable exception to this general trend. The crystallization of this kind of proteins facilitates their function rather than forming a nonfunctional state. Using a combination of structural information and a Monte Carlo method with a coarse-grained model, we have studied the functional protein self-assembly into S-layers. The molecular mechanisms guiding the self-assembly of proteins into functional or pathogenic large-scale structures can be only understood by studying the correlation between the structural details of the monomer and the eventual mesoscopic morphologies. Among the myriad structural details of protein monomers, we seek to identify the most crucial set of structural features necessary for the spontaneous selection of desired morphologies. We discover that only few and mainly hydrophobic amino acids, located on the surface of the monomer, are responsible for the formation of a highly ordered anisotropic protein lattice. In addition to elucidating the molecular mechanisms and explaining experimental findings, the present work offers a tool, which is chemical enough to capture details of primary sequences and coarse-grained enough to explore morphological structures with thousands of protein monomers, to promulgate design rules for spontaneous formation of specific protein assemblies.

2086-Pos Board B72
The Importance of Cell Lysis Methods in Measuring Proteasome Activity
Susan T. Nguyen, Shannamar Dewey, Qian Xu, Jasdeep S. Chahal, Aldrin V. Gomes.
Proteasome dysfunction has been implicated in a wide range of diseases, including neurodegenerative disorders such as Alzheimer’s or Parkinson’s diseases, as well as cardiomyopathies and diabetes. The role of the proteasome is critical to the normal functioning of the body’s degradation pathways. Numerous methods of isolating and measuring proteasome activity in cell lysates are currently being used by different groups. In particular, the homogenization and lysis buffers vary considerably between different laboratories. In this study, we investigated how different cell lysis procedures and buffers affect both 20S and 26S proteasome proteolytic activity. Our results suggest that the inclusion of glycerol in the lysis buffer is important for optimal 20S proteasome activity. We also found that in comparison to cell lysis buffers
containing 1% Triton X-100, buffers containing NP-40 as the detergent resulted in up to a 50% decrease in beta5 20S proteasome activity. For 26S proteasome measurements, many labs utilize varying freeze-thawing methods for cell lysis. We found that brief cycles of freeze-thawing at −20°C resulted in higher beta5 26S proteasome activity compared to freezing cells overnight at −80°C or shaking cells vigorously at 4°C for 1 hour. Optimization of cell lysis techniques is important for helping future studies investigate smaller changes in proteasome activity by allowing the same amount of protein to show significantly higher activity. It also allows proteasome activity to be measured using less protein sample. Our results indicate that the type of cell lysis buffer used as well as the procedure used to disrupt cells is important for optimal proteasome activity measurements. This research is partially supported by NIH grant HL096819.

2087-Pos  Board B73
Gold Nanoparticle Coupled with Dynamic Light Scattering for Protein Complex Detection and Analysis
Qun Hua, Sounya Jagannathan, James Turkson, Peibin Yue.
Many intracellular biochemical processes are triggered by the assembly of proteins into macromolecular complexes, providing a means to control the myriad of biochemical processes for the efficient management of vital biochemical responses. The detection and analysis of protein complexes is extremely important for understanding molecular mechanisms of diseases. We herein present a new technology, NanoDLSayTM, for protein complex detection and analysis using gold nanoparticles coupled with dynamic light scattering (DLS). Gold nanoparticles are conjugated with antibody to form nanoparticle immunoprobes. Upon binding of the gold nanoparticle immunoprobes with target protein and protein complexes in the sample solution, the nanoparticle size will increase. Such a particle size increase can be readily detected by DLS and used to extract information on protein-protein interaction and protein complex binding partners. Using this technology, we recently discovered a novel protein complex formed between EGFR, Src and Stat3 protein in the nucleus of a cancer cell line, Panc-1. This is a novel finding with potential major clinical implications. NanoDLSayTM is a label-free and solution-based biomolecular assay. Other important applications of NanoDLSay™ as a general tool for biomolecular research will be discussed briefly as well in this presentation.

2088-Pos  Board B74
Studying Rapidly Reversible Protein-Protein Interactions by Sedimentation Velocity Analytical Ultracentrifugation
Huaying Zhao, Patrick H. Brown, Peter Schuck.
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Studies on protein-protein interactions are of considerable interest in the fields of macromolecular recognition, signal transduction and cellular regulation. With the introduction of modern instrumentation and computational methods, sedimentation velocity (SV) analytical ultracentrifugation has reemerged in the last decade as a powerful technique for characterizing binding equilibria. For rapidly reversible systems of interacting macromolecules when the lifetime of a complex is short relative to the transport time of a SV experiment, complicated transport patterns arise even for simple bimolecular reactions, when all species migrate at different velocities in the solution. Their physical origin of these patterns has been poorly understood, and this has limited fully exploiting all aspects of SV for rapid protein interactions. Recently, we have reported new solutions (effective particle theory, EPT) to the transport equations for rapidly reacting systems, which describe the average sedimentation coefficients and the composition of undisturbed and reaction boundaries with simple analytical expressions and provide a physical picture of the phenomenon of combined transport and reacting processes. In this work, we apply EPT to characterize several protein-protein interactions and demonstrate how the prediction of the transport patterns helps to quantify the assembly energetic interacting systems.

Key words: protein interactions; transport; sedimentation; signal transduction

2089-Pos  Board B75
Quantitative Study of Membrane Protein Self-Assembly Using Cell-Free Expression
Jerome Chalmeau.
We have developed a quantitative method, based on cell-free expression, to study in real-time the self-assembly of membrane proteins (MPs) in vitro. An E. coli transcription/translation extract is used to express MPs either in phospholipid vesicles or on supported phospholipid bilayers. The MPs self-assembly process is studied by fluorescence microscopy and with a quartz crystal microbalance with dissipation (QCMD).

This new approach, which links the information flow to the self-assembly process, was first used to study α-hemolysin, a pore-forming protein produced by Staphylococcus aureus. Two different clones, one labelled with eYFP and the other one with eCFP, were expressed simultaneously inside synthetic phospholipid vesicles. Self-assembly of the heptamers was studied by Forster resonance energy transfer (FRET) between the two fluorophores. In addition, a QCMD was used to study the pore formation in a supported phospholipid bilayer. The cell-free reaction producing the toxin was directly incubated inside the QCMD chamber on the sensor while both frequency and dissipation signals were recorded. The kinetic constant of adsorption was determined.

We are now using this method to study the basal body of the E. coli flagellum, a complex multiprotein nanostructure formed in vivo at the inner membrane. Results for the flagellar proteins FlIF and FlIG will be presented.

2090-Pos  Board B76
Conformation and Self-Assembly of the Transmembrane Peptide Gramicidin A: Insights from ion Mobility Spectrometry and Molecular Dynamics
Lixiu Chen, Yi-Qin Gao, David H. Russell.
Gramicidin A which is composed of alternating L- and D-amino acids is a naturally occurring pentadecapeptide from Bacillus brevis known to form monovalent metal ion channels in lipid membranes. The active form is a noncovalently bound dimer. The conformation and self-assembly behavior of gramicidin A highly depends on the solution environment. In this presentation, we report the use of electrospray-ion mobility-mass spectrometry to study the conformation of alkali metals adducts of gramicidin A monomer, as well as the monomerization and conformation interconversion equilibria of gramicidin A dimer as a function of the solvent. The conformation of gramicidin A monomer vary significantly upon binding different metal adducts. Enhanced sampling molecular dynamics simulations are performed on alkali metal adducts to provide thermodynamics information of different conformers and gain insights of the interaction of different metal ions with the monomer. The kinetics of the monomerization and conformation interconversion processes of dimer in various alcohol solutions (Ethanol, 1-Propanol and, Isobutanol) are monitored by using the ion mobility profile of the monomer and the dimer. The rate constants and the temperature dependence of the rate constants of the monomer compare well with literature values which were obtained by using fluorescence. Furthermore, we found that the water content in the alcohol solution greatly influences the self-assembly process significantly. The role of water in catalyzing the conformer interconversion is being investigated further. Ion mobility spectrometry (IMS) combined with molecular dynamics simulations is a merging technique for conformational analysis of gas-phase low-lying energy level structures of biomolecules. In this study, we will demonstrate that this gas phase technique can also be of utility in studying a solution phase structural dynamics problem.

2091-Pos  Board B77
Organization and Thermodynamics of Peptidic Amphiphiles at the Air/Water Interface
Ozge Engin, Melumat Sayar.
Peptidic oligomers play an essential role as model compounds for identifying key motifs in protein structure formation and protein aggregation. The spontaneous assembly of these molecules leads to a variety of structures ranging from one dimensional aggregates, like ribbons or cylindrical micelles, to highly organized monolayers in the presence of an interface. Here, we present our results from extended molecular dynamics simulations of self-assembly of amphiphilic peptides at the air/water interface. Experimental results show that these molecules with an alternating sequence of hydrophobic and hydrophilic residues, spontaneously form ordered monolayers at the air/water interface adopting a beta-hairpin like structure within the film. Our results reveal that the beta-hairpin structure can be observed both in bulk and at the air/water interface. However, the presence of an interface significantly shortens the folding time and increases the stability of the hairpin, which is mainly maintained by hydrogen bonds. The adsorption free energy of a single beta-hairpin at the air/water interface is highly negative suggesting that the process is favorable. Decomposition of the free energy into its enthalpic and entropic constituents shows that it is favorable in terms of the first contribution, whereas it is unfavorable in terms the second contribution due to geometric confinement of the peptides at the interface. The alternating hydrophilic-hydrophobic residue sequence provides the main driving force for surface adsorption of these molecules, in agreement with our previous results which show that de-solvation of hydrophobic groups is the main driving