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 Huang, Soumya Jagannathan, James Turkson, Peilin Yue.

Many intracellular biochemical processes are triggered by the assembly of proteins into macromolecular complexes, providing a means to control the myriad of intracellular biochemical processes. The detection and analysis of protein complexes is extremely important for understanding molecular mechanisms of disease. We herein present a new technology, NanoDLSay™, 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. NanoDLSay™ 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 nanostucture formed in vivo at the inner membrane. Results for the flagellar proteins FlgI and FlgJ 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 metal adducts of gramicidin A monomer, as well as the monomerization and conformer 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 metals 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 conformer 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 solutions 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, Melanet 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 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. 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 hydrophobic-hydrophilic 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 force for protein conformational transitions in ordered monolayers.
force for adsorption to the interface (Engin et al. 2010). By comparing the stability and surface tension of a series of alternative packing structures, we determine the internal structure of the monolayer, identify key residues for the stability, and make a relation between the structural organization and thermodynamics of the systems.

**2092-Pos Board B78**

**Peptide Nanovesicles: Supramolecular Assembly of Branched Amphipathic Peptides**

Sushanith Gudlur, Xiao Yao, Yasuki Hiromasa, Takeo Iwamoto, John M. Tomich.

Research on non-lipid based carriers show promise in replacing lipid based delivery systems in delivering drugs. Peptide vesicles are one such class. We have designed and synthesized a set of (15, 19 and 24 residue), branched, amphipathic peptides that self-assemble into nanovesicles - 50 - 200 nm in diameter as determined by TEM and dynamic light scattering. Pairs of peptides with different lengths are mixed as helical monomers. After drying and redissolving in water, they undergo supramolecular assembly. CD studies show the assembled peptides adopt a predominantly beta-like conformation. Analytical ultracentrifugation data suggest that the peptide assemblies have a weighted average size of 8. In addition to encapsulating and delivering both anionic and cationic fluorescent probes, we have recently been able to deliver a 4.7 kbp plasmid (EGFP-N3), into MCF-7 cells grown on coverslips and observed expression of GFP. Peptide vesicles (Figs) are shown undergoing fusion, a property associated with lipid vesicles. We are currently exploring how to control size and stability by altering the ratios of the different chain lengths, as well as the ability to deliver plasmons of different sizes. These are potential drug delivery vehicles for targeted delivery.

**2093-Pos Board B79**

**Tuning the Neurofilament Hydrogel Network - a Synchrotron X-Ray Scattering Study of Salt Dependent Response**

Joanna Deek, Roy Beck, Cyrus R. Safinya.

Neurofilaments (NFs) are the cytoskeletal intermediate filament protein class expressed in neuronal cells and play a major role in the maintenance and mechanical integrity of neuronal processes (i.e. the axon and dendrites). NFs assemble into flexible bottlebrushes from 3 different molecular weight subunits (NF-Low (NF-L), NF-Medium (NF-M), NF-High (NF-H)) with compositions that are specific for the axon and dendrites. The main variation in the subunits is the charge distribution of their unstructured C-termini sidearms. The sidearms are polyampholytes (i.e. containing both cationic and anionic amino acid residues), which enables interpenetration and interfilament attraction. We examine the strength and range of the electrostatic interfilament interactions by varying the salinity of the in vitro buffer. Reassembled (in vitro) binary system NF-hydrogels have revealed the different contributions of individual subunits to interfilament interactions and to network interfilament spacings [1-2]. This network tunability parallels with variable in vivo subunit expression in axons versus dendrites that results in variable network packing. We describe synchrotron x-ray scattering experiments that have allowed us to quantitatively study the changes in the microscopic structure of the NF gels as a function of salt and sidearm density. At high weight ratios of NF-M and NF-H, and as a function of increasing salt concentrations, NF gels exhibit an unexpectedly abrupt (most likely electrostatically driven) transition from a weakly oriented (nearly isotropic) low filament density gel with interfilament spacing d ~ 1000Å to a highly oriented liquid crystalline gel with high filament density and d ~ 500Å (NF-M) and ~ 700Å (NF-H). Funded by DOE DE-FG-02-06ER46314, NSF DMR-0803103, NIH R01-059288, HFSP.


**2094-Pos Board B80**

**Analysis of Bundle Formation in Biofilaments**

Osman N. Yogurtcu, Sean X. Sun.

Bundles of biofilaments are ubiquitous in cells with functions ranging from force transmission to cellular protection and thus understanding the self-assembly of the biofilaments into bundles is crucial. The conformation of a bundle is dictated by a small number of mechanical and chemical parameters. In this work, given a set of parameters, our aim is to find the most favorable bundle configuration and the number of filaments within. The filaments are treated as discrete elastic rods. The final bundle structure is reached as a result of the competition between the elastic energy and favorable chemical interaction among individual filaments within the bundle. The results indicate that there is a variety of different size bundles with different conformations attainable, from dimers to hexagonal-closed-packed. We discuss our results along with case studies of important biofilaments.

**2095-Pos Board B81**

**The Role of Bending Stiffness on the Rheology of Fibrin Networks**

Huayin Wu, Louise Jawerth, David Weitz.

During the formation of a blood clot, fibrinogen is converted to fibrin which in turn polymerizes to form a biopolymer network whose mechanical properties are partially imparted to the clot itself. When such a network is deformed, it has an initial, low strain linear regime followed by a nonlinear, high strain regime. In the body, blood clots must function under a large range of stresses. For this reason, both the linear and nonlinear properties of a fibrin network are important for proper function. Previous work has studied the relation between calcium concentration and the linear modulus and fiber radius. Here we extend this work by also characterizing the effect of calcium concentration on the nonlinear rheology of the fibrin clot, as well as on the hydrated state of the fibrin clot using microscopy. Unlike previous work, using this information, we can also directly test model predictions of the role of bending stiffness on both the linear and nonlinear moduli.

**2096-Pos Board B82**

**Structural Basis of Bispecific Protein:Protein Interactions**

Poorni Adikaram, Dorothy Beckett.

The ability of the *Escherichia coli* protein, BirA, to act as both an enzyme and a transcription repressor allows communication between metabolism and gene expression. BirA forms a hetero-dimer for the essential post-translational biotin addition to acetyl-CoA carboxylase and forms a homodimer to bind site-specifically to DNA and repress transcription initiation at the biotin biosynthetic operon. A single surface on BirA is used for both protein:protein interactions. However, the extent to which the structural information on this surface is functionally shared by the two interactions is not known. Multiple loops on the surface are located in both dimer interfaces. In this work alanine substitution variants of two loops, composed of residues 140-146 and 193-199, have been constructed and the proteins have been purified. Measurements of homo- and hetero-dimerization energetics of loop variant proteins using sedimentation equilibrium indicate that several residues in the two loops contribute to both interactions. Thus, structural information in both loops was important for evolution of bifunctionality in BirA. Supported by NIH grants R01-GM46511 and S10-RR15899.

**2097-Pos Board B83**

**Linkage Between SecA Dimerization and Ligand Binding**

Andy J. Wowor, Dongmei Yu, Debra A. Kendall, James L. Cole.

The general secretion (Sec) pathway, found in bacteria, archaea, and eukaryotes, transports preproteins across membranes. The SecA protein mediates preprotein translocation through the SecYEG channel linked to ATP hydrolysis. Several studies suggest that SecA exists in a monomer-dimer equilibrium. Although self-association of SecA has been intensively studied, the oligomeric state of SecA, especially during preprotein transport, remains controversial. SecA dimerization is reported to be sensitive to salt concentration, temperature and ligand binding. We have characterized the energetics of SecA dimerization as a function of salt concentration, temperature and binding of a signal peptide using sedimentation velocity analytical ultracentrifugation. We employ fluorescence-detection to enhance sensitivity at low protein concentrations. SecA was labeled with Alexa Fluor 488 at the N-terminus. Labeling does not affect the dimer dissociation constant. Lower salt concentrations and higher temperatures greatly enhance dimerization. The dimer dissociation constants measured at 20°C range from 40 μM in 500 mM KCl to 14 nM in 100 mM KCl. Linkage analysis indicates that SecA dimerization is accompanied by the release of 5 ions. In addition, SecA dimerization is reduced upon binding of signal peptide, indicating that SecA oligomerization and ligand binding are thermodynamically linked.