vectored Env expression induces epithelial tumors in several animals. Previous studies showed that the region containing the short 46 amino acid C-terminal cytoplasmic tail (CT) of JSRV Env is essential for the ability of JSRV to transform cells. Residues in the cytoplasmic tail include a tyrosine (Y590), which is present in a consensus motif YXXM, which could potentially bind the regulatory subunit of phosphatidyl inositol 3-kinase (PI3K) if the Y590 is phosphorylated. Alanine scanning mutagenesis on the JSRV TM cytoplasmic tail has been conducted. Mutation of some residues abolished Env transformation potential, while mutation of other residues had not effect or partial effects. To further understand the mechanism of JSRV transformation, structure-function analysis of the TM cytoplasm tail (CT) is important. We have determined the structure of the JSRV CT using NMR spectroscopy. These data allow us to interpret the alanine scanning mutagenesis, and allow better understanding of previous studies. Interestingly, using both CD and NMR, we find that the CT is only structured in the presence of a phosphocholine surface. The results validated some aspects of the predicted structure, and they also provided a basis for evaluating models of transformation.
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1356-Pos Board B126
Studies on Co-Translational Import of Bacteriorhodopsin into Lipid Bilayer Systems
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A huge literature exists on in-vitro protein refolding of water soluble proteins after unfolding. However, these studies cannot provide a deeper understanding of protein folding at a ribosome in a cell. Besides many possible interactions of the nascent polypeptide chain with cellular components, it is obvious that the kinetics of protein folding in a cell is largely influenced by the rate of polypeptide synthesis. Cell-free transcription/translation systems offer a valuable approach to study protein folding in a cell-like environment. Membrane protein folding implicates an additional complication as the nascent chain should either directly integrate into a membrane during synthesis (co-translational folding and import) or chaperons prevent the nascent chain from misfolding followed by an integration of the full length protein into a membrane (post-translational folding and import). A study of Kalmbach et al. [JMB 371, 639. 2007] showed that the membrane protein bacteriorhodopsin (bR) synthesized in a cell-free system was integrated into lipid vesicles which were supplied to the system. Was this folding and import process co- or post-translational? To answer this question ensemble experiments with three different commercially available in-vitro transcription/translation systems for bR synthesis and import into discoidal artificial membrane particles (nanodiscs) where executed. The positive bR incorporation into these nanodiscs in a cell-free expression system without chaperons indicated a co-translational import of bR into the lipid bilayer. Fluorescence microscopy offers the possibility for an in-vitro protein synthesis experiment on a single molecular level, which will verify the co-translational import mechanism.

1357-Pos Board B127
Utilizing the Extraordinary Optical Transmission of Metallic Microarray Mesh for Enhanced Molecular Dynamic and Protein Interactions Within a Supported Bilayer Lipid Membrane In Vivo by Infrared Microscopy
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Self-assembling monolayers are physisorbed on metal mesh as a supporting struture for a protein being analyzed. A protein is embedded into a supported phospholipid bilayer membrane that is stacked between two microarray square-holed meshes of the same metal (either copper or nickel). Fourier transformed infrared (IR) microscopy and spectroscopy was used to observe the molecular vibration of protein dynamic and interaction by measuring the absorption of light that passes through the square holes of the mesh. This phenomenon of the extraordinary optical transmission (EOT) has been observed in the visible and in the IR. An enhanced amount of light is transmitted through the mesh due to propagating surface plasmons that are formed when photons hit and excite the conducting electrons on the surface of the metal creating surface waves. This extra amount of light was used to study the protein interaction structurally and dynamically while embedded within a lipid bilayer membrane. Work with liposomes and extract rabbit powder has been done but not within a bilayer lipid membrane to give an extremely similar example of how the protein actually occurs in vivo. Preliminary results were obtained by observing the interaction of globular actin as it changes to fibrous actin as it begins to form the microtubules of the cytoskeleton. Further studies are being conducted on L-Glutathione, a tripeptide protein residue of the anti-freeze protein. Further studies of the residue will give a good example of the anti-freeze protein dynamics in the membrane and may lead to understanding its role in protecting a cell from becoming cancerous.

1358-Pos Board B128
Amphipols Stabilize a Membrane Protein Vaccine Against Chlamydia
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Chlamydia trachomatis infections are widespread; it is the most common sexually transmitted bacterial pathogen in advanced countries, and in poor sanitary conditions, leads to trachoma and lymphogranuloma venereum. Although antibiotic therapy can be implemented with early detection, a majority of the infections are asymptomatic. To address this problem the search for a vaccine against Chlamydia was initiated.
Characterization of C. trachomatis identified the major outer membrane protein (MOMP) as a potential immunogen, a surface-exposed protein constituting 60% of the mass of the bacterial outer membrane. However, MPs are difficult to handle in vitro because of their insolubility in water and a limited stability once extracted from biological membranes. Classically, MPs are extracted from membranes and handled in aqueous solution using detergents, which ultimately tend to destabilize MPs and lead to inactivation. A new class of polymers called amphipols (APols) has been specially designed that can substitute for detergents to keep MPs soluble and stable.
Ensuring maintenance of protein structure and optimization of exposure of the most effective antigenic regions can avoid costly vaccination trials with misfolded or inactive protein. Circular dichroism (CD) provides an easy measure of secondary structure content, allowing direct comparison of the structure of the protein in various environments. CD data indicate that APols maintain MOMP native secondary structure. CD can be used to monitor thermal and long-term stability. Long-term stability is important because a vaccine preparation that initially contains native, soluble MOMP may lose its efficacy if the protein denatures upon storage. Thus, we are determining the thermal stability of nMOMP in detergents and APols by assaying secondary structure content over a period of months for solutions kept at a range of temperatures. Preparations found to be most protective will be validated for long shelf-life and thermal stability.

Physical Chemistry of Proteins & Nucleic Acids

1359-Pos Board B129
Thermal Melting of Duplex DNA in the Slow Exchange Regime
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The thermal melting of small DNA duplexes usually occurs as a two-state transition in which the duplex and its component single strands are co-populated throughout the transition. Since a duplex and its single-stranded forms can be resolved by free solution capillary electrophoresis, this technique can be used to monitor the kinetics of denaturation. When a duplex and its component single-strands are in fast exchange, a single peak is observed in the electropherograms, with a mobility that is proportional to the weighted average of the mobilities of the duplex and its component single strands at various temperatures. However, if melting occurs in the slow exchange regime, resolved peaks are observed for the duplex and its component single strands. The area under the peak corresponding to the duplex progressively diminishes with increasing temperature, while the area under the peak(s) characteristic of the single strands progressively increases. Although the melting of small DNA hairpins and duplexes often occurs in the fast exchange regime, the melting of DNA duplexes containing internal loops occurs in the slow exchange regime. The equilibrium and kinetic parameters accompanying the melting of DNA duplexes with internal loops will be reported.

1360-Pos Board B130
Why Proteins Crystallize (or not): The Role of Non Specific Protein Interactions
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X-ray crystallography remains the gold standard for determining protein structures. Yet obtaining high-quality protein crystals often relies on experimental trial and error. Understanding the physical principles underlying protein crystallization would thus help inform the experimental approach to the problem. Here, we use a soft matter methodology to study the crystallization mechanism of a model family of proteins. We perform atomistic simulations to characterize the surface contribution to protein pair interactions. The results indicate that the interaction varies significantly from one crystal contact to the other both in