Biological membranes exhibit a large degree of lateral heterogeneity. Membrane rafts, that is, small and highly dynamic yet distinct regions in the membrane, are supposed to play important roles for cellular processes such as signaling, trafficking, and membrane protein structure, function, and clustering. The study of the atomistic structural dynamics that governs these processes however, was hitherto impeded by the limited resolution of experimental techniques.
We studied the sorting and clustering of synthetic WALP transmembrane peptides in heterogeneous model membranes with two coexisting fluid domains that resemble membrane rafts. To this end, we combined large-scale molecular dynamics simulations (using both coarse-grained and all-atom models) with confocal fluorescence microscopy experiments. In particular, we focused on how the interplay between peptide- and membrane-mediated forces determines the processes, and studied the role of hydrophobic mismatch between the peptide and the membrane. On the multi-microsecond timescale accessed by our simulations, the peptides prefer the liquid-disordered over the liquid-ordered membrane domain, irrespective of the mismatch. Free energy calculations provide a deeper understanding of the underlying physical processes and reveal how a delicate balance between entropic and enthaplic contributions determines the sorting of peptides in the membrane domains. Our study is a first step towards understanding the driving forces for protein sorting in heterogeneous membranes, which might ultimately enable a rational design of raft proteins.

**319-Pos**
**Theory of the Solubility of Protein Crystals**
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We present a theory describing the solubility of protein crystals as a function of pH, salt concentration, and temperature. There are four terms in the model. The neutral terms arise from 1) the translational entropy of the soluble proteins, and 2) H-bond and hydrophobic attractive interactions which we obtain from a fitting procedure. The two electrostatic terms are a result of counterions confined in the crystal to satisfy charge neutrality. These counterions contribute 3) an entropic penalty from the trapping of ions in the crystal, and 4) a favorable enthalpy from the interaction of each protein with its counterion cloud. This theory quantitatively describes the solubility of tetragonal and orthorhombic lysozyme crystals as determined by Pusey et al. According to the theory, the reduced solubility at high salt concentrations comes, not from increased screening, but from a reduced entropy of counterion confinement. The theory correctly describes the weak pH dependence of the solubility, which is a result of the compensating effects of the two electrostatic terms. We discuss the implications of this theory for crystal nucleation and the success of the ''crystallization slot''.

**320-Pos**
**Dynamics of In Vitro Bacterial S-Layer Crystallization**
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S-layer proteins form crystalline lattices on the outsides of certain bacteria. While the structures of many S-layers are known, the dynamics of their formation is poorly understood. In an effort to provide such understanding, the DeYoreo and Bertozzi groups at the Molecular Foundry have used atomic force microscopy to image in real time the deposition of a certain S-layer protein on a supported lipid bilayer. This protein forms a square crystal lattice whose dynamics of assembly are strikingly complex: proteins first aggregate into amorphous clusters on the membrane; clusters subsequently crystallize and grow via the addition of tetramers at the cluster edge.
Similar 'two-step' crystallization mechanisms have been observed in computer simulations of globular proteins [1], polymer melts [2] and Lennard-Jones particles [3-5], and inferred experimentally from the observation, via dynamic light scattering, of dense liquid droplets present in solution prior to lysozyme crystallization [6]. Here we explore the origin of two-step crystallization in the S-layer system via a simple computer model of associating monomers on a substrate. Dynamical simulation reveals that phase separation induced by nonspecific monomer-monomer interactions facilitates phase ordering driven by directional binding. Our results suggest that the interplay of non-specific attractions and site-specific binding are crucial in driving crystallization in the S-layer system.
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**321-Pos**
**Proteomic Scale Small Angle X-ray Scattering (SAXS):applications and Implications**
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High throughput solution structural analyses by small angle X-ray scattering efficiently enables the characterization of shape and assembly for nearly any purified protein. Crystallography has provided a deep and broad survey of macromolecular structure. Shape and assembly from SAXS in combination with available structures is often enough to answer critical mechanistic questions both enhancing the value of a structure and obviating larger crystallographic projects. Moreover, SAXS is a solution based technique, sample requirement are modest and compatible with many other biophysical methods. Here we present our high throughput SAXS data collection and analysis pipeline as applied to structural genomics targets, and metabolic pathways. Our goals of metabolic engineering and understanding protein mediated reactions rely on knowing the shape and assembly state of reactive complexes under an array of conditions. Given the number of gene products involved in metabolic networks, SAXS will play an important role in characterizing the structure of each individually, in complex with partners, and in various contexts. SAXS is well positioned to bridge the rapid output of bioinformatics and the relatively slow output of high resolution structural techniques.

**322-Pos**
**(his)6-Tag-Specific Optical Probes For Analyses of Proteins and Their Interactions**
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The hexahistidine (His6)/Nickel (II)-Nitrilotriacetic Acid (Ni2+-NTA) system is a rapid and efficient tool for affinity purification of recombinant proteins. The NTA group has many other valuable applications, including surface immobilization of (His)6-tagged proteins and the attachment of chromophores and fluorophores to His6-tagged proteins. Here we explore several applications of the NTA-derivative fluorescent probe, (Ni2+-NTA)2-Cy3. This molecule binds (His)6-tagged proteins N-ethylmaleimide Sensitive Factor (NSF) and O6-alkylguanine-DNA alkyltransferase (AGT) with moderate affinity (KD 200-300 nM) and without detectable effect on the assayable functions of these proteins. High specificity makes this interaction suitable for detecting a (His)6-tagged protein in the presence of a large excess proteins that do not carry (His)6-tags, allowing (Ni2+-NTA)2-Cy3 to be used as a probe in crude cell extracts and as a (His)6-specific gel stain. (Ni2+-NTA)2-Cy3 binding is rapidly reversible in 10 mM EDTA or 500 mM imidazole but in the absence of these agents the probe exchanges slowly between (His)6-tagged proteins (kexchange $\sim 5 \times 10\text{-}6$ s-1 with 0.2 μM labeled protein in the presence of 1μM (His)6-peptide). Labeling a protein with (Ni2+-NTA)2-Cy3 allows characterization of hydrodynamic properties by fluorescence anisotropy or analytical ultracentrifugation under conditions (such as high ATP concentration) that would interfere with direct detection of protein by absorbance or fluorescence in the near UV. In addition, FRET between of (Ni2+-NTA)2-Cy3-labeled protein and a suitable donor or acceptor provides a convenient assay for binding interactions and has the potential to allow accurate measurements of donor-acceptor distance.
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**323-Pos**
**Structural Determination of Macromolecular Machines Guided By Proteomics and Electron Microscopy**
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Models of macromolecular assemblies are essential for a mechanistic description of cellular processes. Low-resolution density maps of these assemblies are increasingly obtained by electron-microscopy techniques. In addition, interactions between subunits in these assemblies can be systematically mapped by proteomics techniques.
We have developed MultiFit [1], a method used for simultaneously fitting atomic structures of components into their assembly density map at resolutions as low as 25 Å. The method was benchmarked on large assemblies of known structures. It generally finds a near-native configuration in one of the 10 top scoring solutions. The component positions and orientations are optimized with respect to a scoring function that includes the quality-of-fit of components in the map, the protrusion of components from the map envelope, as well as the shape complementarity between pairs of components. The scoring function is optimized by our exact inference optimizer DOMINO that efficiently finds