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

the global minimum in a discrete sampling space. DOMINO decomposes the set of optimized variables into relatively uncoupled but potentially overlapping subsets that can be sampled independently form each other, followed by efficiently gathering the subset solutions into the global minimum.
We have further extended MultiFit for modeling the architecture of macromolecular assemblies by aligning proteomics data into electron-microscopy density maps. The method facilitated the structural modeling of the AAA-ATPase/20S core particle sub-complex of the 26S proteasome [2].
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324-Pos
Multifunctional Gfp Tag: A Useful Tool For Isolation of Protein Complexes
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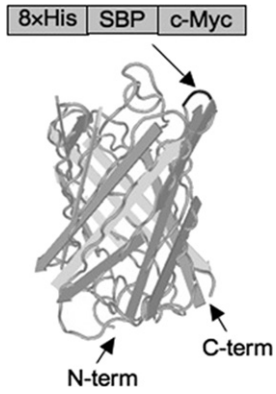
Protein complexes are functional units essential for virtually all cellular processes. To understand molecular mechanisms of the functions, it is necessary to identify and characterize the protein complexes involved. Protein tags are genetically encoded tags and useful tools for detection and isolation of protein complexes. So far, many kinds of protein tags have been developed. We have recently reported a novel multifunctional green fluorescent protein (mfGFP) tag which can be used for cellular localization, composition, and structure of the protein of interest (Kobayashi et al. PLoS ONE, 3, e3822, 2008). mfGFP was engineered by inserting several peptide tags (8×His, SBP, and c-Myc) in tandem into a loop of GFP. In the present study, we developed several variations of mfGFP having different tag systems, which are optimized for isolating various levels of protein complexes from small proteins to large organelles. The mfGFP will be a useful tool for isolation of protein complexes.



325-Pos
Effect of Kinetics on Sedimentation Velocity Profiles and the Role of Intermediates
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We have previously presented a tutorial on direct boundary fitting of sedimentation velocity data for kinetically mediated monomer-dimer systems (Correia & Stafford, 2009). We emphasized the ability of Sedanal to fit for the k_{off} values and measure their uncertainty at the 95% confidence interval. We concluded for a monomer-dimer system the range of well determined k_{off} values is limited to 0.005 to 10^{-5} sec^{-1} corresponding to relaxation times of ~70 to ~33000 sec. More complicated reaction schemes introduce the potential complexity of low concentrations of an intermediate that may also influence the kinetic behavior during sedimentation. This can be seen in a cooperative ABCD system (A+B->C; B+C->D) where C, the 1:1 complex, is sparsely populated ($K_1 = 10^4 M^{-1}$, $K_2 = 10^8 M^{-1}$). Under these conditions a $k_{1,off} < 0.01$ sec^{-1} produces slow kinetic features. The low concentration of species C contributes to this effect while still allowing the accurate estimation of $k_{1,off}$ (although $k_{2,off}$ can readily compensate and contribute to the kinetics). More complex reactions involving concerted assembly or cooperative ring formation with low concentrations of intermediate species also display kinetic effects due to a slow flux of material through the sparsely populated intermediate states. This produces a kinetically limited reaction boundary with partial resolution of individual species during sedimentation. Cooperativity of ring formation drives the reaction and thus separation of kinetics and energetics can be challenging. This situation is experimentally exhibited by systems that form large oligomers or rings, formation of micelles and various protein aggregation diseases including formation of β-amyloid and tau aggregates. Simulations, quantitative parameter estimation by direct boundary fitting and diagnostic features for these systems are presented with an emphasis on the features available in Sedanal to simulate and analyze kinetically mediated systems.

326-Pos
Determining Thermodynamic Parameters of Protein Interactions By Global Analysis of Data From Multiple Techniques
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When studying macromolecular interactions, the thermodynamics and stoichiometry of binding are of considerable interest because they indicate the physical-chemical nature of the biological mechanism. Since a single biophysical technique is limited in the number of observable properties and may provide only insufficient information for more complex systems, one promising approach is the simultaneous consideration of data from multiple biophysical methods. In the past, we have developed a robust computational framework (SEDPHAT) for this purpose which has been widely used in the biophysical community. However, the best strategy for assembling individual data sets into a global analysis has not been explored. It requires understanding of the limitations and consideration of possible systematic errors for each method. In this work, we have performed experiments on a model system (α-chymotrypsin binding to soybean trypsin inhibitor) to study the detailed compatibility of data from calorimetry (ITC), surface binding (SPR), sedimentation (SV) and fluorescence anisotropy. The significance of each data set from the different techniques has been explored through both individual and global analysis with detailed error surface projection using the program, SEDPHAT. We propose a rational strategy for global analysis that deviates from the purely statistical point of view, by rescaling the weights of each data set such that all techniques can make significant contributions. This allows a more detailed picture of the interaction to emerge.

327-Pos
Information Extraction From Simulations-Based Data Fitting of Distributions of Fret Efficiencies from Donors and Acceptors in the Cytoplasm of Living Cells
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Fluorescence Resonance Energy Transfer (FRET) has evolved to the point where the efficiency of energy transfer at each pixel in an image may be obtained after only one scan of the sample and without recourse to photobleaching or external calibration of acceptor excitation. With this method it is now possible to obtain entire distributions of FRET efficiencies in populations of proteins self-associating into oligomeric complexes. To exploit this opportunity, it is necessary to develop tools for analysis of such data. Here we present comparative results from Monte-Carlo simulations for FRET in homogeneous and inhomogeneous spatial distributions of molecules. The FRET efficiencies were interpreted in terms of both average value (as it would be obtained from wide-field microscopy) and statistical distributions of values (as if obtained from scanning optical microscopy). The advantage of an analysis based on the distribution of FRET efficiencies is that it enables one to discriminate between constitutive oligomers and random collisions between diffusing donors and acceptors. We next evaluated the approach based on the distribution of FRET efficiencies with regard to its potential to provide stoichiometric information from whole distributions of FRET efficiencies by using simulation-based data fitting. The experimental FRET data were obtained from a system of donors and acceptors that reside in the cytoplasm of yeast cells (*S. cerevisiae*) and which appear to interact transiently.

DNA Replication, Recombination, & Repair

328-Pos
Molecular Traffic Jams on DNA Highways: Single Molecule Observation of Collisions Between RecBCD Helicase and DNA Binding Proteins
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DNA helicases, polymerases, and other translocases must proceed along a substrate crowded with other DNA-binding proteins. The outcomes of these molecular collisions play a crucial role in shaping multiple metabolic pathways, such as DNA replication and repair. To address the question of how a translocase proceeds along a congested DNA substrate, we have established a high-throughput single molecule assay to observe the motion of RecBCD on individual DNA molecules. RecBCD is a heterotrimeric helicase and exonuclease that initiates homologous DNA recombination at the free dsDNA ends in E. coli. RecBCD is a processive motor enzyme that uses the energy of ATP hydrolysis to digest both strands of dsDNA until the protein encounters the regulatory