313-Pos
Role of Water in Mediating the Interaction Between Collagens
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Collagens are triple-helical molecules that self-assemble into higher order fibers forming the major component of extracellular matrix. We had previously reported the role of the first hydration layer in controlling the conformational behavior of the collagen triple helix. Here we perform explicit-water molecular dynamics simulations to elucidate the structural features of water in mediating the interaction between collagen triple helices (PDB ID: 1A3I, 2D3F). By dividing the simulation box into cells, we quantified local water density, diffusion coefficient, and water orientation at atomistic resolution. Around a single collagen triple helix the reduction in diffusion coefficient and density fluctuation extend up to 11 Angstroms from the collagen backbone, and the circumferential and radial orientation of water near hydrophobic and hydrophilic groups, respectively, were clearly distinguishable. When two triple helices were held at a radial separation that is a few Angstroms larger than their crystalline packing distance, water in between them had reduced diffusion coefficient and constrained angular orientation. This indicates that the experimentally observed attractive force between collagens at small distances may have an entropic origin. We also tested three-collagen systems where one collagen is radially translated from its original position in the crystal packing by 4 or 7 Angstroms, and found that it moves towards the other two within 3-ns of simulation, nearly restoring the crystal packing. These results illustrate the microscopic origins of water mediated attraction between collagen molecules.

314-Pos
Electrostatic Interactions Control the Permeability of Mucin Hydrogels
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Biological functional entities surround themselves with selective barriers which control the passage of certain classes of macromolecules while rejecting others. A prominent example of such a selective permeability barrier is given by mucus. Mucus is a biopolymer based hydrogel which lines all wet epithelial surfaces of the human body. It regulates the uptake of nutrients from our gastrointestinal system, adjusts itself with the menstrual cycle to control the passage of sperm, and shields the underlying cells from pathogens such as bacteria and viruses. In the case of drug delivery, the mucus barrier needs to be overcome for successful medical treatment. Despite its importance for both physiology and medical applications, the underlying principles which regulate the permeability of mucus remain enigmatic. Here, we analyze the mobility of microscopic particles in reconstituted mucus hydrogels. We show that electrostatic interactions between diffusing particles and mucin polymers set the permeability of reconstituted mucin hydrogels. As a consequence, various parameters such as particle surface charge, mucin density and buffer conditions such as pH and ionic strength can sensitively modulate the microscopic barrier function of the mucin hydrogel. Our findings demonstrate the wide range of permeability that operates in different compartments of our bodies, employing the very same biopolymer based hydrogel.

315-Pos
Fibrin Gel Ultrastructure
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Ischemic heart disease, which results from occlusion of one of the major coronary arteries as a consequence of thrombi and atherosclerotic plaque, continues to be the leading cause of morbidity and mortality in Western society, while stroke is the second leading cause of death worldwide. Nowadays, in addition to prevention, it is possible to treat atherosclerotic plaque by means of invasive endovascular procedures. With the advent of thrombolytic agents that favor clot lysis, treatment of patients suffering from thromboembolic diseases is greatly improved. Clots are composed of a three-dimensional fibrous network, known as fibrin gel; it is within the scaffold of this that platelets and other blood constituents get trapped, thus giving rise to the haemostatic plug. The structure of fibrin gel depends upon the polymerization conditions of fibrinogen, a glycoprotein present in the plasma of vertebrates. The thrombin-catalyzed polymerization process is usually modelled through the occurrence of a number of distinct steps that lead to the formation of fibrin monomers, which subsequently undergo polymerization to produce oligomers called protofibrils. Lateral aggregation of protofibrils forms fibers and the branching of fibers that takes place during the association of protofibrils creates the final fibrin network.
The chief factor responsible for clot lysis rate is the intrinsic permeability of the fibrin network and of the individual fibers to proteolytic agents. The diffusional access from outside to proteases involved in fibrinolysis is not yet fully understood. For this reason, further knowledge of fibrin network architecture and of the packing arrangement of protofibrils would be desirable.
Here we present the results of a combined Small Angle Neutron and X-ray scattering study of the packing arrangement of protofibrils. For the first time characteristic fibrils distance are related to the water trapped among fibrils and thus to space available to thrombolytic agents diffusion.

316-Pos
Protein Domain Formation in Lipid Membranes
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Clustering of Gramicidin within a DMPC membrane has been studied with Small Angle Neutron Scattering (SANS). Hydrogen and Deuterium scatter neutrons very differently, thus deuteration allows protein scattering to be studied independent of lipid and solvent scattering (when the lipid and solvent are contrast matched). Different protein to lipid ratios were probed and a strict protocol was followed to ensure uniform vesicle size with limited polydispersity. The experiments were performed above the melting temperature of DMPC.
A 100 nm deuterated lipid vesicle in deuterated solvent exhibits q-independent scattering showing that the two are truly contrast matched (see Figure 1). While the scattering obtained from lipid vesicles containing Gramicidin have significant q-dependent scattering. If the Gramicidin were uniformly distributed throughout the membrane the data should be well represented by vesicle scattering. However the vesicle fit of the data (also shown in Figure 1) clearly does not agree with the experimental scattering. Thus it is concluded that the protein is forming clusters within the lipid membrane giving rise to the difference in scattering. The system has been modeled and a 3D contrast map (inset Figure 1) has been generated. The map shows protein clustering within the membrane.

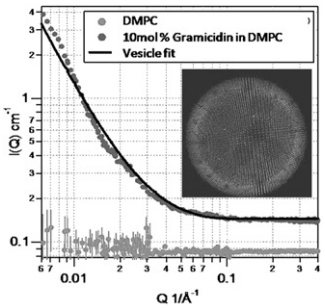

Figure 1: SANS from a pure lipid vesicle and a lipid vesicle containing 10 mol% Gramicidin, vesicle fit shown as black line.
(Inset) 3D shape representation of a lipid vesicle containing protein clusters.

317-Pos
Surfactant Sponge Phase Is a Versatile, Tunable and Biologically Relevant Medium To Study Membrane Protein Interactions
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We present an original approach that combines the Fluorescence Recovery After fringe Pattern Photobleaching (FRAPP) technique and the use of a versatile sponge phase that makes it possible to extract crucial informations about interactions between membrane proteins embedded in the bilayers of a sponge phase. The clear advantage lies in the ability to adjust at will the spacing between two adjacent bilayers. When the membranes are far apart, the only possible interactions occur laterally between proteins embedded within the same bilayer, whereas when membranes get closer to each other, interactions between proteins embedded in facing membranes may occur as well. The sponge phase is particulary well suited for the study of Gram negative bacteria possessing a double membrane such as P. aeruginosa.
However, such studies are relevant only if the sponge phase does alter neither the conformation nor the activity of anchored or transmembrane proteins. We have evaluated the conformation of the latter membrane proteins using circular dichroism (CD) spectroscopy and show that the overall structure of the proteins is similar whether the protein is solubilized in micelles or inserted into the sponge phase. We have also investigated the activity of several model transmembrane proteins inside the sponge phase such as the ATPase SERCA1a (the sarcoplasmic reticulum Ca2+-ATPase).
We provide evidence that the sponge phase maintains the properties of membrane proteins.

318-Pos
Sorting and Clustering of Transmembrane Helices in Coexisting Fluid Domains in Model Membranes
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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