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.

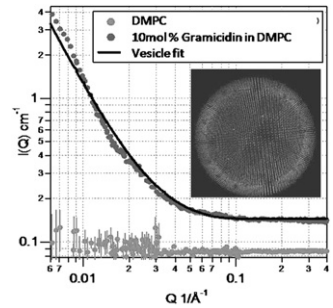

Figure 1: SANS from a pure lipid vesicle and a lipid vesicle containing 10 mol% Gramicidin, vesicle fit shown as black line.
(Inset) 3D shape representation of a lipid vesicle containing protein clusters.

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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