ligand binding affinity of integrins is central to cell migration, extracellular matrix assembly, immune response, and hemostasis. Much of the current thought on the mechanism of integrin activation is inferred from crystal structures of the β3 integrin ectodomains. All of these structures show the receptor to adopt a ''bent'' conformation, where the headpiece containing the ligand binding site is pointed in the same direction as the tail.
Our results provide direct structural evidence that both the ''on'' and ''off'' forms of integrin $\alpha_{IIb}\beta_3$ are distributed across multiple conformations, ranging from a compact nodule to a fully upright stance. The experimental system employed provides the means to examine the structure of full-length receptors in the presence or absence of their activators and thus does not rely on the ''computational ligation'' of independently determined structures of extracellular face and cytoplasmic domains. Here, in this study we identify the density associated with a cytoplasmic activator (talin) juxtaposed with the membrane where it associates with the C-terminal region of integrin while embedded in the membrane. Consequently, here we show that integrin extension does not equate to activation of ligand binding function and thus extension is not synonymous with activation, nor is the activation state of the integrin correlated with any particular global conformation.

1169-Plat
Structural Analysis of a Membrane Protein by Small Angle X Ray Scattering : The Example of the Aquaporin 0
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Membrane proteins are involved in many biological processes and allow for example the transport of small solutes, ions or water across the lipidic membrane. Membrane proteins are also the target of many therapeutic agents. Despite this importance, the number of atomic structure of membrane proteins is very low compared to soluble proteins. In the present work, we propose to use Small Angle X rays Scattering (SAXS) applied to membrane proteins in order to monitor the conformational changes of membrane proteins. SAXS technique is very powerful and has been widely used to obtain structural information of soluble proteins.
In the case of membrane proteins, the presence of detergent in solution but also around the protein greatly complicates the analysis of scattering curves. Accordingly, obtaining structural information of membrane proteins was up to now quite complicated.
In the present work, we propose a new methodology to model the organization of detergents around the membrane protein using data from SAXS. Our experiments were performed at the synchrotron SOLEIL SWING beamline. The main advantage of this beamline is that just prior to the SAXS experiment, the sample is passed through a network connecting chromatography column and a refractometer.
Using the membrane protein aquaporin-0 as a model system, we successfully managed to model the SAXS curves allowing us to accurately determine the arrangement of detergent molecules around the aquaporin-0.
The ability to model the organization of detergents around membrane proteins opens the route for studies of their conformational changes.

1170-Plat
MD For MS: Simulations of the Transfer of Membrane Protein-Detergent Complexes from Solution to Vacuum in Electrospray Ionisation Mass Spectrometry (ESI-MS)
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Recent developments in mass spectrometry have allowed membrane proteins in complex with detergent micelles to be studied in the gas phase - previously thought only possible for soluble proteins. This development paves the way for future studies of membrane protein stability, structure and oligomerisation. The gas phase environment is very different to that of a cell membrane, and questions have been raised over the conformation of proteins in the gas phase. Molecular dynamics (MD) simulations have been used extensively to explore the evolution of gas phase structures of soluble proteins, with some evidence that the native state may be stabilised in vacuum (although the timescale of this stability remains unclear). However, membrane protein-detergent complexes have been less well studied by simulations.
We have used a combination of non-equilibrium and equilibrium MD simulations to elucidate some of the processes that occur during transition of a protein-detergent complex from aqueous solution to vacuum (thus mimicking electrospray ionisation). We compare two membrane protein architectures (an α-helical bundle vs a β-barrel) and different detergents with respect to protein-detergent complex dynamics and stability.

1171-Plat
Lipid Mediated Packing of Transmembrane Helices
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The factors that affect packing of Transmembrane (TM) helices are vast. Previous research studying packing focused on the role of direct helical interactions but ignored the role of the membrane. Studies typically rely on a single configuration snapshot (e.g. X-ray crystallographic structure) and address factors that affect only the minimal energy structure such as specific side-chain interactions. In previous work (1) we have showed that the membrane has profound effects on the interactions between proteins. In this work we study the effect of the membrane on the packing of model TM helices and show that closed packing and crossing angle distribution are largely affected by the presence of the lipid surroundings as well as by the helical geometry. We study a vast range of hydrophobic mismatches and show that the role of the membrane is in ensuring a tilted configuration as well as in minimizing the area of disturbed lipids around the helix pair. These driving forces combine together to create a mismatch-specific cross-angle distribution that reproduces the qualitative features of the naturally occurring helical cross-angle distribution. Specific interactions are not essential for obtaining an ensemble of configurations with tightly packed crossed helix pairs. We study those effects using a coarse grained model with Dissipative Particle Dynamics (DPD) (2).
(1) FJM de-Meyer, M Venturoli, B Smit; Biophys J, 95 (2008) 1851-1865.
(2) FJM de-Meyer, A Benjamini, JM Rodgers, Y Misteli, B Smit; JPC B 114 (2010) 10451-10461.

1172-Plat
Association-Dissociation Dynamics of Transmembrane Helices as Detected by Single-Molecule Fluorescence Microscopy
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Conformational fluctuations of helical membrane proteins among active-inactive conformations are crucial for their functions. Not only the amino acid sequence of the protein but also the composition of surrounding lipids should significantly affect the fluctuation, although the fundamental principles are mostly unknown. Experimental systems using model transmembrane helices and lipid bilayers have been proposed to be useful for direct measurements of elementary processes that determine the folding and conformational change of membrane proteins, such as insertion of the helix into lipid bilayers and helix-helix interaction in the bilayers. Here we show that real-time detection of self-association and dissociation of the transmembrane helix (AALALAA) 3 is possible by single molecule FRET detection using the Cy3B- and Cy5-labeled helices incorporated into large unilamellar vesicles (LUVs, diameter ~100 nm) composed of POPC. The LUVs were fixed on a glass surface via biotin-avidin interaction and observed under a total internal reflection fluorescence microscope. After simultaneous observation of Cy3B and Cy5 fluorescence with 10-20-ms time resolution, LUVs that had incorporated only one Cy3B- and one Cy5- labeled helices were selected based on the number of photobleaching steps, and single-molecular FRET from Cy3B to Cy5 was analyzed. FRET time course revealed that the helices fluctuate between monomer and dimer with a time scale of subseconds in the presence of cholesterol in the LUVs. The above experimental system will be useful for measurement of kinetics of helix-helix interactions in membranes with various lipid compositions.

1173-Plat
Highly Accurate Quantification of the Oligomerization of the β2 Adrenergic Receptor using FRET
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The oligomerization of G-protein coupled receptors, GPCRs, has in many cases shown to be important for transmembrane protein structure and function [1,2], however achieving quantitative parameters describing the process that governs oligomerization is difficult. Traditionally oligomerization is quantified by preparing distinct samples that carry different concentrations and by monitoring bulk spectroscopic signals e.g FRET efficiencies [3], still the limited amount of samples does not allow very accurate determination of the experimental parameters.
Here we developed a nanoscale assay [4,5] that allows a highly accurate quantification of the interaction forces between transmembrane molecules. As