respond differently to the presence of the TMDs. The extents to which headgroups are pulled in and lipid tail order is disturbed will be discussed in relation to the extent by which these model TMDs promote fusion and induce lipid flip.
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**3166-Pos Board B27**
**The Autotransporter β Domain: Insights into Structure and Function through Molecular Dynamics Simulations**
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The autotransporters are a family of bacterial outer membrane proteins that are characterised by three functional domains: an N-terminal signal sequence, a central passenger domain, and a C-terminal transmembrane β-barrel domain. Using molecular dynamics simulations we explore the structural dynamics and membrane interactions of the β-barrel domains from six autotransporters: BrkA, NalP, EspP, EstA, Hia and Hbp. The β-barrel domain has been proposed to aid in the translocation of the passenger domain across the outer membrane. Extended timescale simulations are performed to investigate the flexibility of the β-barrel domains from the six autotransporters in lipid bilayers designed to capture the complexity of the bacterial outer membrane. We propose a model of the missing pore α-helix from the crystal structure of the *Bordetella pertussis* autotransporter, BrkA, and we explore the dynamics of the ubiquitous hydrophobic cavity that is required for translocation of the passenger domain. Furthermore, we explore the structure-function relationship of EstA from *Pseudomonas aeruginosa* by simulating the translocator domain *and* the associated passenger domain. Thus our simulations provide unprecedented details regarding the membrane interactions and dynamics of the autotransporter proteins we have studied.

**3167-Pos Board B28**
**Langevin Dynamics Simulations of Protein Fencing of $PIP_2$**
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Anionic phosphotidylinositol 4,5-*bis*phosphate ($PIP_2$) occupies only 2~3% of the phospholipids in the inner leaflet of the plasma membrane, but participates in numerous cellular processes. Concentrated pools of $PIP_2$ on the membrane surface appear to be the source for such PIP2-involved cellular processes, and recent experimental observations indicate that a ''protein fence'' prevents $PIP_2$ from diffusing out of the pool. Here the necessary properties of the protein fence are examined using Langevin dynamics simulation with effective potential maps. $PIP_2$ molecules are modeled as explicitly diffusing spheres, and the rest of the system represented by steric and electrostatic potentials. Simple models of charged or porous rods, as well as rigid proteins are considered. Retardation by electrostatic forces from a rod is only significant when the charges are highly concentrated and close to the diffusion plane; charge densities and geometries of proteins are insufficient to effectively block diffusion. Hence, fencing appears to be dominated by steric effects, though even a small ($> 1\%$) opening in a porous rod in contact with the surface results in substantial leakage. The three protein fence candidates, actin, human septin, and yeast septin, are known to self-assemble into filaments on the cell surface and to interact with $PIP_2$. Simulations reveal that actin is a poor fence for all depths and orientations simulated because its arch-type shape leaves passageways for diffusion. In contrast, the septins effectively block $PIP_2$ diffusion when buried in the membrane to 10~15 Å from the lipid surface. Free energy calculations using an implicit membrane model support the possibility of burial of septins at these depths. It is also possible that the septins are not buried as deeply, but associate with other proteins or peptides to make a fence.

**3168-Pos Board B29**
**Computational Approach to Study Membrane Protein Topology**
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Membrane proteins represent an important class of proteins with a variety of biological functions and a major fundamental and pharmaceutical interest. The protein-conducting channel translocon is responsible for protein-membrane integration. Despite significant progress it is unknown how membrane proteins achieve different topologies. Experimental studies suggest that the location of the positive charges on the signal peptide may influence its orientation (so-called ''positive-inside'' rule). In addition the topology of the membrane proteins may be affected by the prl (protein localization) mutations on the translocon.
In this study we tried to estimate the barrier for the polypeptide insertion into the translocon using our renormalization approach (1). In this approach the insertion dynamics is first simulated with coarse grain model (2) where the whole insertion profile is divided on intermediate states and the time dependence response of each step is obtained. The second step involves the simulation with an implicit Langevin Dynamics (LD) model of reduced dimensions. By adjusting the friction and the barrier of the implicit LD model we can get the agreement between the time dependence responses of both models. By combining the barriers from different intermediate steps we can get the barrier for the whole insertion process.
The renormalization approach allowed us to compare the barriers for different signal peptides, as well as to study the effect of mutations of the translocon on the orientation of the signal peptide.
(1) Kamerlin et al., Annual Reviews, 2010.
(2) Rychkova et al., PNAS, 2010.

**3169-Pos Board B30**
**Rationale Membrane Protein Design of a Beta-Barrel**
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Bacterial outer membrane proteins have robust beta-barrel structures. Therefore, they are targeted for engineering biological nanopores as stochastic biosensing elements. We used membrane protein engineering and single-channel electrical recordings to explore the ferric hydroxamate uptake component A (FhuA) as a target for redesigning membrane proteins. FhuA is a monomeric 22-stranded beta-barrel protein from the outer membrane of Escherichia coli. FhuA has a lumen with cross section of 3.1 X 4.4 nm that is filled by a globular N-terminal cork domain. We investigated various redesigned FhuA proteins, which had either single, double, or multiple deletions of the large extracellular loops and the cork domain. Analysis of the electrical signatures of these initial exploratory redesigned FhuA proteins led to the identification of four large extracellular loops that partially occlude the lumen when the cork domain is removed. Accordingly, we removed the cork along with the extracellular loops, FhuA delta C/delta 4L, resulting in the deletion of almost one third of the total number of amino acids of the wild-type FhuA protein. Remarkably, the newly redesigned protein forms an open pore in planar lipid bilayers, with a measured unitary conductance of ~4.8 nanosiemens in 1 M KCl at pH 7.4, a value that has not been recorded previously with other engineered FhuA protein channels. We show several advantages and prospects of using such an engineered outer membrane protein in fundamental studies of membrane protein folding and design, and the mechanisms of ion conductance and gating. Further, FhuA delta C/delta 4L can be a platform for customized engineering in applicative areas of single-molecule sensing and analysis of proteins, nucleic acids and their ensembles.

**3170-Pos Board B31**
**Rhodopsin Simulations in Detergent Micelles Characterize Meta I and Meta II Photointermediates**
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G protein-coupled receptors (GPCRs) are prominent pharmaceutical targets due to their prevalence in the human genome. Membrane proteins such as GPCRs have been historically difficult to characterize by crystallographic methods, severely limiting development of therapeutical applications. A commonly used *in vitro* method for studying GPCRs is detergent solubilization, which can retain protein activity but affects thermodynamics and conformational flexibility. We used molecular dynamics (MD) to simulate the canonical GPCR rhodopsin in micelles containing the detergent DDM or CHAPS. The Meta I ↔ Meta II activation mechanism of rhodopsin is forward-shifted in a DDM micellar system [1], whereas it is back-shifted when solubilized in CHAPS. Previous MD simulations of rhodopsin could not reach Meta II due to the long timescale required (ms); in addition crystal structures of the activated state have been lacking [2-4]. However, detergents such as DDM accelerate Meta II kinetics to the s timescale [1], making it possible to reach the Meta II state in a DDM micelle. Using the recently published crystal structure of a putative Meta II state [5], we are able to show how the forward- and back-shifting of the Meta I ↔ Meta II equilibrium takes place in DDM and CHAPS detergent environments, respectively. These results provide important structural insights into rhodopsin activation, which can be extended to other GPCRs in a membrane mimetic environment, such as the $A_{2A}$ adenosine receptor. We have introduced a powerful