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that TatA forms larger complexes upon substrate binding in the presence of a membrane potential.

275-Pos
Solvation and Binding of the Membrane Enzyme PagP By Detergents and Lipids
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The solvation of membrane proteins by detergents is a necessary step for structure determination by NMR and X-ray crystallography, and yet this process remains poorly understood. The severe under-representation of membrane proteins amongst proteins of known structure is a direct consequence of the difficulties associated with the solubilization of the large hydrophobic faces presented by this important class of proteins. The prominence of these membrane proteins as important drug targets provides a strong impetus for the rational design of new detergents or cofactors to assist the solubilization of hydrophobic faces while maintaining structural integrity. To this end, we begin by investigating the process of detergent self-aggregation, utilizing molecular dynamics simulations to characterize the atomic interactions that underlie the dynamic aggregation of detergents in aqueous solution. We determine the critical micelle concentration and the equilibrium aggregation number based on generalized ensemble methods and, separately, thermodynamic cycles involving non-physical order parameters that are more computationally efficient.

Next, we apply similar methods to study the aggregation of detergents around the bacterial outer membrane enzyme and virulence factor PagP. These studies shed light on the process of protein self-aggregation in solution and how this process may prevent protein precipitation at high concentrations. Finally, we draw functional conclusions for PagP, an acyltransferase that binds and catalyses its own lipid solution and the atomistic mechanisms by which detergents may prevent efficient. Next, we apply similar methods to study the aggregation of detergents based on generalized ensemble methods and, separately, thermodynamic

Electrophysiology of Viral Envelope Protein Ion Channels in Lipid Membranes Across Apertures in Polystyrene and Silicon
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Viral ion channels, such as the coronavirus envelope proteins (E protein), belong to a family of channels that have attracted a considerable amount of interest during recent years. However, not many studies on the electrophysiology have been performed; mainly due to the fact that these channels are membrane proteins that do not easily express in the outer membrane of bacteria. Moreover, the channel currents are small (on the order of 10-30 pS) when compared with bacterial outer membrane channels. In our studies, we reconstituted the full-length channel-forming E protein from murine hepatitis coronavirus (MHV-A59) into 3:1:1 POPE:POPS:POPC lipid bilayers that were suspended either across 150 μm diameter apertures in polystyrene cups or across 50 μm diameter aperture in silicon. Lipid bilayers were formed using the painting method on silicon wafers, resulting in reproducible Gigaseal formation. The aperture in silicon was prepared using photolithography and dry reactive ion etching, resulting in excellent reproducibility of the pore geometry. The surface was coated hydrophobically to allow lipid bilayer attachment. Bilayers created in the presence of E-protein in solution showed reproducible ion channel activity, independent of the substrate used. We were able to identify the signature conductance steps of E ion channels. Similar to what has been shown previously using the OmpF channel of E. coli, the ion channel activity on the silicon substrate was identical to that measured using the polystyrene cup, indicating the feasibility of the silicon substrate for the investigation of ion channels with conductances in the range of tens of picosiemens. Using silicon apertures for ion channel reconstitution experiments in array geometry provides an opportunity to increase measurement throughput.

279-Pos
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280-Pos
Single Amino Acid Substitutions Change the Sodium/Iodide Symporter (NIS) Selectivity and Stoichiometry
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The Na+/I- symporter (NIS) is a key plasma membrane protein that mediates active I- transport in the thyroid and such other tissues as salivary glands, stomach, and lactating breast. NIS-mediated I- uptake is the first step in thyroid hormone biosynthesis. NIS mediates the inward simultaneous movement of Na+ and I- with a 2:1 stoichiometry, thus resulting in a net transfer of positive charge into the cell (i.e., electrogenic transport). We recently reported that NIS translocates different anion substrates with different stoichiometries, as Na+/perchlorate (or perrhenate) transport is electroneutral. Neutral mechanistic information on NIS has been obtained by the characterization of NIS mutants that cause congenital I- transport defect in patients. Here we provide a detailed study of the G93R NIS mutant. As we substituted neutral amino acids at this position, we observed that the longer the side chain of the substituted residue, the lower the protein’s activity. G93T and G93N NIS exhibited significantly higher Km values for I- than WT NIS, the first time that such a change has been observed in any NIS mutants. Strikingly, we show by kinetic analysis that G93T-mediated Na+/perrhenate symport is electrogenic with a 2:1 stoichiometry, a discovery confirmed by the detection of currents elicited by perrhenate (or perchlorate) in G93T NIS-expressing X. laevis oocytes in electrophysiological experiments. These observations demonstrate that a single amino acid substitution at position 93 converts NIS-mediated Na+/perchlorate (or perrhenate) transport stoichiometry from electroneutral to electrogenic. Based on the 3-D structure of the bacterial Na+/galactose transporter, we built a 3-D homology model of NIS and we propose a mechanism in which changes from an outwardly open to an inwardly open conformation during the transport cycle use G93 as a pivot.