Taken together, this result allowed to propose molecular mechanisms of permeation and selectivity of UT-B for urea permeability.

581-Pos Board B350
Repeated Perfusion of Droplet-Interface Bilayers
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Repeated Perfusion of Droplet-Interface Bilayers
The droplet-interface bilayer (DIB) is a recently-developed model cell membrane that is created at the contact between two sub-microliter lipid-encased aqueous droplets. The DIB is a promising approach in mechanistic studies of membrane transport, allowing the translocation of material to be monitored and quantified[1]. However, it is limited by the inability to introduce new reagents once the droplets are formed. Recently, Thompson and coworkers added reagents to a droplet through injection, after the bilayer formed between this droplet and a monolayer on a hydrogel[2]. Nevertheless, reagents could not be imported and removed repeatedly. Herein, we designed a fluid exchange device that replaces one of the droplets with a flowing system that allows reagents to be introduced and perfused continuously without rupturing the DIB. As a proof of concept, α-hemolysin (αHL), which assembles to form heptameric pores on cell membranes, and γ-cyclodextrin (γCD) that binds non-covalently to the lumen of αHL were used in the device. Specifically, αHL was placed in the droplet and γCD was added to and removed from a microliter, perfused volume to act as a representative membrane phenomenon to test the device. The observation of successive γCD blocking event upon introduction and the cessation of blocking events when the volume was perfused with buffer demonstrated that the fluid exchange on DIB could be completed repeatedly. We anticipate that this approach will be widely applicable and will broaden the utility of studies that require reconstituted membranes.
(1) Huang, J.; Lein, M.; Gunderson, C.; Holden, M. A. *Journal of the American Chemical Society* **2011**, *133*, 15818-15821.
(2) James R. Thompson; Bríd Cronin; Hagan Bayley; Wallace*, a. M. I. *Biophysical Journal* **2011**, *101*, 2679–2683.

582-Pos Board B351
Structure and Selectivity of a Bacterial Concentrative Nucleoside Transporter
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Concentrative nucleoside transporters (CNTs) are membrane-bound solute carrier proteins that are responsible for the ion-coupled translocation of nucleosides across cell membranes. Along with nucleosides, CNTs are also capable of transporting many widely used anticancer and antiviral nucleoside-derived drugs into the cell. We have solved the crystal structures of a bacterial CNT ortholog bound to several different nucleosides and nucleoside-analog drugs. These structures, along with equilibrium-binding and transport studies, provide key insight into the origins of permeant selectivity, the role of sodium coupling, and the possible mechanism of nucleoside transport. These studies not only serve to further our understanding of nucleoside-related physiological processes, but they also lay the framework for the design of nucleoside-analog drugs that can better reach their cellular targets.

583-Pos Board B352
Translocation of TonB Dependent Colicins
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Colicins are protein toxins produced by *E. coli* to kill closely related competitor *E. coli*. Colicins initially attach to target bacteria by binding to outer membrane receptors, most of which are TonB-dependent nutrient transporters—22-stranded -barrels plugged by their amino-terminal domains. One group of colicins, which use the Tol proteins, TolA,B,Q,R, for uptake, have been shown to use OmpF or TolC as their translocators after the initial step when the colicin binds to its receptor. The N-terminal translocation domain of the colicin actually threads into the translocator (Housden *et al*. (2010) PNAS: **107:** 20412). Until recently, no translocator or ''second receptor'' had been identified for the other family of colicins, those that use TonB and ExbB and D for uptake. Recently, colicin Ia was shown to use a second copy of its primary receptor, Cir, as its translocator (Jakes, K.S. and Finkelstein, A. (2010) Mol. Micro.: **75:** 567). In an attempt to determine whether other TonB-dependent colicins also use a second copy of their primary receptor as a translocator, I have made chimera constructs with colicin M, substituting the receptor-binding domain of colicin E3 for that of colicin M, and also deleting the entire receptor-binding domain. These constructs were insoluble and went into inclusion bodies. Dissolving the inclusion bodies in 8M urea, renaturing by dilution and dialysis, and purifying by nickel chelation yielded a small amount of pure protein that had no *in vivo* killing activity. However, the chimera blocks killing by colicin E3, demonstrating that it binds the E3 receptor, BtuB. Osmotic shock to bypass receptor binding and translocation showed that the enzymatic moiety of the chimera is also active.
These results suggest that colicin M translocates differently than colicin Ia and may not normally use a translocator remote from its primary binding site.

584-Pos Board B353
Like Fish Out of Water: Membrane Proteins in Detergent Micelles
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Biophysical studies of structure-function relations of membrane proteins are often done in detergent micelles. However, the scarce molecular understanding of the way in which such micelles form and organize around guest proteins makes it difficult to evaluate the relation between the experimental models and the properties of the membrane proteins in their native environment. Because experimental conditions were shown to have a major effect on structure-function relations of the leucine transporter (LeuT), a prokaryotic homolog of the mammalian neurotransmitter:sodium symporter proteins and a prototype for their study, we investigated LeuT in dodecyl-β-maltoside (DDM) detergent micelles. Atomistic molecular dynamics (MD) simulations revealed the formation of a constant-sized ''detergent core'' within 4Å of LeuT consisting of ~120 DDM molecules regardless of the DDM-to-protein number ratio. However, we found that the aggregation number of the protein-detergent complex (i.e., the number of DDMs associated with the micelle surrounding the protein) depends on detergent concentration. Notably, this aggregation number appears to determine the extent of detergent penetration into the LeuT extracellular vestibule. Thus, we observed DDM penetration into LeuT via two pathways, but the penetration was detected only in the constructs with high detergent content. In constructs with low DDM concentration, any detergent penetration was at most transient. Entering from the ''side'' of LeuT, a DDM molecule passes between the extracellular segments of transmembrane helix 6 (TMH6) and TMH10, and interacts with TMH1 residues Arg30 and Gln34 in the secondary substrate-binding site (S2) of LeuT. When insertion is ''from the top'', the DDM passes by extracellular loop 4 (ECL4) before it penetrates the S2 site at the level of Phe320 (in ECL4) and Leu400 (in TMH10). These findings are discussed in light of experiments that established a modulatory effect of DDM concentration on LeuT activity.

Polysaccharides

585-Pos Board B354
Effects of Lytic Polysaccharide Monooxygenase Oxidation on Cellulose Structure and Binding of Oxidized Cellulose Oligomers to Cellulases
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Cost-effective enzymatic breakdown of recalcitrant polysaccharides such as cellulose and chitin to constituent sugars has tremendous potential for industrial applications for the production of fuels and high-value chemicals. Research towards that goal has been aided recently by the discovery and classification of polysaccharide mono-oxygenase enzymes (GH61s), which conduct oxidation of glycosidic linkages and thus represent a new enzymatic paradigm in carbohydrate deconstruction from the standard glycoside hydrolases. Recent experimental studies suggest that GH61s cleave chains oxidatively in crystalline regions of cellulose and chitin, exposing further ends for processive degradation, which leaves an oxidized end exposed to solution. Using molecular dynamics and free energy calculations, we explore what impact potential oxidations have on the overall structure of the cellulose crystal, including their impact on the free energy of fibril decrystallization. Particularly noteworthy differences appear in the solvent-accessible surface area and decrystallization free energy changes of particular oxidation states, suggesting that these states are more accessible to processive cellulose-degradation enzymes.
In addition, the soluble deconstruction products of oxidation will potentially bind to the active sites of cellulases. To examine this effect, we have used thermodynamic cycles to compute ΔΔG between the hydrolyzed and oxidized products in the active site of the Family 7 and Family 6 glycoside hydrolases from Trichoderma reesei, which are key industrial enzymes and commonly used model systems for fungal cellulases. Our analysis shows that oxidation brings about changes in the hydrogen bonding pattern within the cellulases, and thus changing the binding affinity and product inhibition depending on the oxidation state.