In conclusion, aquaporin co-expression widens and enhances regulatory properties that control adjustment of water movements which might be of great importance to react to variable osmotic and pH stress.

1935-Pos
Membrane Transport of Hydrogen Sulfide: No Facilitator Required

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Hydrogen sulfide (H2S) has emerged as a new and important member in the group of gaseous signalling molecules. However, the molecular transport mechanism has not yet been identified. Prediction of its actual membrane permeability, PM, according to Overton’s rule (1) is hampered by the fact that the partition coefficient into the organic phase is not known. Because of structural similarities with H2O, it was hypothesized that aquaporins may facilitate HS transport across cell membranes. We tested this hypothesis by reconstituting the archael aquaporin AFAQP from sulfide reducing bacteria Archaeoglobus fulgidus into planar membranes and by monitoring the resulting facilitation of osmotic water flow and H2S flux. To measure H2O and H2S fluxes, respectively, sodium ion dilution and buffer acidification by proton release were recorded in the immediate membrane vicinity. Both [Na+] and pH were measured by scanning ion selective microelectrodes. A lower limit of PM,H2S > 5 ± 0.4 cm/s was calculated by numerically solving the complete system of differential reaction diffusion equations and fitting the theoretical pH distribution to experimental pH profiles. Even though reconstitution of AFAQP significantly increased water permeability through planar lipid bilayers, PM,H2S remained unchanged. The fact that cholesterol and sphingomyelin reconstitution did not turn these membranes into a H2S barrier indicates that H2S transport through epithelial barriers, endothelial barriers and membrane rafts also occurs by simple diffusion and does not require facilitation by membrane channels (2).


1936-Pos
Protein Transport Through the Anthrax Toxin Channel: Molecular Mechanisms

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Bacillus anthracis, the causative agent of anthrax, produced a toxin composed of a translocase heptameric channel, (PA63)7, which allows its two substrate proteins, lethal and edema factor (LF and EF), to translocate across a host cell’s endosomal membrane, disrupting the cell’s normal function. Protein translocation through the channel, reconstituted in lipid bilayers, is driven (N-terminal end first) by a proton electrochemical potential gradient. The (PA63)7 channel structure favors the entry of negatively charged charged residues on proteins, and hence the acidic side chains on LF, (the N-terminal 263 residues of LF) enter the trans. These protons are released into the trans solution upon exiting the channel, thereby making this a proton-protein symporter. Consistent with this idea, a single SO3−, which is essentially not titratable, introduced at most positions in LF, drastically inhibited voltage-driven LF translocation. The lumen of the (PA63)7 14-strand β barrel is ~15 A wide and can barely accommodate an alpha-helix with its side chains. Translocation through the lumen thus requires the substrates to unfold. Here we present an approach using biotin-streptavidin chemistry to determine the length of the translocating polypeptide chain within the channel as it traversing the (PA63)7 lumen, with the goal of shedding light on the structure of the polypeptide chain as it crosses the channel. We created a stopper at the LF C terminus and attached a biotin at the N terminus. Translocation proceeds until the C terminus reaches the channel’s cis entrance, and binding of the N-terminal biotin with streptavidin added to the trans side of the membrane, locks the polypeptide chain within the channel. By reducing the distance between the N-terminal biotin and the C-terminal stopper by deletion constructs, we can determine the minimum length that allows streptavidin to grab the N-terminal biotin. 

1933-Pos
Molecular Dynamics Simulations Reveal TolC Flexibility in the AcrB Interface Region

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Over-production of multi-drug efflux pumps is a prominent example of how bacteria gain resistance against antibiotics. In Escherichia coli the AcrA/B-ToLC efflux pump is capable to expel a broad range of drugs, using the energy of proton-motive force. The detailed functional mechanism of this efflux system is not fully understood yet. While AcrB is the engine in this system, the outer membrane protein ToLC acts as an efflux duct that also interacts with a numerous other inner membrane translocases. TolC occurs in at least two states, one that is impermeable for drugs and one where drug passage is possible. To gain insight into TolC ground state dynamics, we performed a series of 5 independent, unbiased 150ns MD simulations of closed state wild type ToLC (PDB ID 1E9K) in a phospholipid/water environment at 0.15M NaCl concentration. Simulations were performed using GROMACS 4.0.3 and G53a6-GROMOS96 force field. While TolC remains closed between a “bottleneck region” outlined by Asp374 &371 and above, we observe opening and closing motions in the AcrB interface region near Gly-365. This local flexibility could be of functional relevance in the AcrB-ToLC complex formation. In all simulations the Asp371&374 aspartate ring region was stable, displaying no fluctuations in the cross-sectional area of the TolC channel. Whereas previous studies found potassium ions to bind frequently, stabilizing a closed TolC conformation in the AcrB interface region, we observe frequent and unhindered passage of sodium ions. However, in one simulation a consecutive binding event of two sodium ions occurs between Gly-365 and Asp-374, stabilizing a similarly closed conformation for more than 15 ns. We introduce a new tool to analyze protein internal cavities and record pore profiles based on time-averaged water & protein residence probabilities.


1934-Pos
Plant Aquaporins Co-Expression Senses Differentially the Intracellular pH
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