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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 1EK9) 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 Asp-374 & 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 Asp-371&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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The plant plasma membrane (PM) expresses two types of aquaporins: PIP1 and PIP2. These PIP are characterized by: i- the faculty to reduce water permeation through the pore after cytosolic acidification as a consequence of gating process, ii- the ability to modulate membrane water permeability by co-expression of both types.

We investigated if these functional characteristics of PIP can act together to give a new and relevant modulation response to acidification. To test our hypothesis we used PIP1 and PIP2 from different plant sources (Beta vulgaris roots and Fragaria x ananassa fruits). The experimental approach used was to perform a functional study of PIP by means of the heterologous expression system *Xenopus* oocytes and analyzed the oocyte PM water permeability coefficient (Pf) when PIP are injected.

Briefly, the Pf was increased ten-fold by PIP2, but it remained low for both control oocytes and PIP1 injected ones. Moreover, when oocytes expressed PIP2, a partial (70%) pH inhibitory response under cytosolic acidification (pH 6) was detected.

When PIP1-PIP2 co-expression was assayed, Pf was enhanced seven-fold in comparison with Pf obtained by PIP2 expression alone. Furthermore, the pH dependent behavior showed that PIP1-PIP2 co-expression accounts for different pH sensitivity by shifting the EC50 of the inhibitory response from pH 6.1 to pH 6.9, compared to PIP2.

Our results show that: i- PIP co-expression impacts on the membrane water permeability not only by modulating the water transport capacity but also the pH regulatory response, improving in this way membrane plasticity, ii- this PIP behavior is not a tissue specific and/or species-dependent response but a more general one.

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, P_M , 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 H₂S transport across cell membranes. We tested this hypothesis by reconstituting the archeal 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 $P_{M,H_2S} > 0.5 \pm 0.4$ 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, P_{M,H_2S} 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).

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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 strongly disfavors the entry of negatively charged residues on proteins, and hence the acidic side chains on LF_N (the N-terminal 263 residues of LF) enter protonated; 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 titrateable, introduced at most positions in LF_N, drastically inhibited voltage-driven LF_N translocation. The lumen of the (PA₆₃)₇ 14-strand β barrel is ~15 Å 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 is traversing the (PA₆₃)₇ channel 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_N 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.