used solid-state ^{2}H NMR spectroscopy which gives site-specific orientational order parameters of the lipid segments to study structural fluctuations and deformations of phospholipid bilayers due to osmotic stress [1]. Model lipid membrane systems (DMPC and POPC) were subjected to osmotic pressure and temperature to establish their sensitivity to environmental changes in the liquid-crystalline state. Osmotic stress was applied by addition of osmolytes (polyethylene glycol) as well as by gravimetric dehydration. We observed very large changes in segmental order parameters with the application of osmotic pressures in the biological range. The NMR order parameters represent the area/lipid and show large changes in mean-square fluctuations of the lipid structure [3,4]. Stresses from these pressures are thermodynamically equivalent because changing chemical potential when transferring water from the interlamellar space to the bulk water phase corresponds to an induced pressure, as verified experimentally [1]. By employing mean-torque analysis of the NMR observables [3] we calculated the mean area per lipid and the volumetric bilayer thickness, which change up to 20% upon introduction of osmotic stress. These ^{2}H NMR studies [4] show striking bilayer deformation due to the application of osmotic pressure. They distinguish molecular-level force regimes associated with lipids that can play a significant role in biological processes.
[1] K.J. Mallikarjunaiah *et al*. (2011) *BJ* **100**, 98-107. [2] A. Leftin *et al*. (2011) *BBA***1808**, 818-839. [3] H.I. Petrache *et al*. (2000) *BJ***79**, 3172-3192. [4] K.J. Mallikarjunaiah *et al*. (2012) to be submitted to *PCCP*.

173-Plat
Water Channel Formation and Ion Transport in Linear and Branched Lipid Bilayers
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The composition of natural lipid membranes varies greatly depending on the type of organism, and it has been observed that small variations in lipid composition affect dramatically the membrane properties, such as structural stability and solute permeability. One of the variations is the methyl-branched lipids commonly found in archaea and bacteria. Using molecular dynamics simulations, we studied the influence of methyl branching on the electric-field induced formation of water channels in lipid bilayers and ion transports through them. We employed a double lipid bilayer setup to create within a periodic box two water compartments separated by those two bilayers. One of the compartments contains an excess of cations, while the other an equal excess of anions. This setup creates an initial transmembrane potential controlled by the ion concentration in each water compartment. We compared the response of diphytanoylphosphatidylcholine (DPhPC) lipid bilayers that have multiple methyl-branches with that of the straight-chain dipalmitoylphosphatidylcholine (DPPC) lipids. We found that compared to the straight-chain DPPC lipids, branched DPhPC lipids require a higher critical transmembrane potential and a longer time for the membrane to break down, followed by water channel formation, and transport of anions and cations through the channel. We demonstrated that while adding methyl branches reduces the lateral diffusion of the lipids, different transport properties of branched lipids are mainly due to the bulkiness of the branched lipid tails resulting in different water channel morphologies. The transmembrane potential creates toroidal pores in the straight-chain lipid bilayers, but barrel stave pores in the branched-lipid bilayers the formation of which requires a higher transmembrane potential. Our results provided a deeper understanding of the ion transport process through lipid bilayer membranes and shed light on the transport of various molecules across the lipid membranes.

Symposium: Proton Channels

174-Symp
Selectivity of the Voltage Gated Proton Channel H_V1
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The voltage gated proton channel, H_V1, triggers the flash in bioluminescent dinoflagellates, aids calcification by coccolithophores, and in humans, regulates pH in the respiratory tract, sperm capacitation, histamine secretion, the phagocyte respiratory burst, and B lymphocyte signaling. On the down-side, H_V1 exacerbates breast cancer metastasis and brain damage from ischemic stroke. All of these functions require extreme proton selectivity, because $[H^+]$ is 10^6 lower than that of other ions. We studied proton selectivity in H_V1 from humans and *Karlodinium*, a dinoflagellate, which share only 15% sequence identity. A crucial aspartate in the S1 transmembrane domain of each (Asp^{112} in hH_V1, Asp^{51} in kH_V1) is essential for proton selectivity; neutralizing mutations result in anion permeability or abolish conduction (Musset et al, 2011, *Nature* 480:273-277; Smith et al, 2011, *Proc. Natl. Acad. Sci.U.S.A*. 108:18162-18168). That the two proteins exhibit the same phenomenology despite limited sequence identity indicates that the selectivity mechanism is highly conserved. A combination of approaches, including accessibility studies of the three Arginine residues in the S4 helix, provides a picture of the open human H_V1 channel, in which Arg^{205} (R1) is accessible to the external solution, Arg^{208} (R2) forms a salt bridge with Asp^{112}, and Arg^{211} (R3) is accessible to the internal solution. Further studies of the molecular requirements for proton permeation and selectivity are underway.
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175-Symp
Biophysical Studies of the Voltage-Dependent Proton Channel
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Voltage-dependent H^+ (Hv) channels conduct protons across the cell membrane. They are important in immunity and fertility. In contrast to other voltage-dependent cation channels, Hv channels do not have a separate voltage sensor and pore. Instead, their amino acid sequence corresponds only to that of a voltage sensor. Mutagenesis studies suggest that the voltage sensor is itself the proton pore. By purifying and reconstituting Hv channels into synthetic lipid membranes we have shown that no other structural components are required for proton conduction. We have also sought to find conditions under which Hv channels can be stabilized for structural and biophysical studies. Our findings together with preliminary structural analyses will be presented.

176-Symp
H_V1 Structure and Function: Insights from Evolutionary Information
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The voltage gated proton channel, H_V1, is the most selective ion channel known, and plays an important role in innate immunity, human sperm maturation, tumorigenesis, and dinoflagellate bioluminescence, to name a few examples. When the gene was identified in 2006, H_V1 was found to be homologous to the voltage sensor domains of classic voltage gated cation channels; these homologous domains do not conduct ions. Phylogenetic analysis of VSDs showed that H_V1, voltage sensing phosphatases, and c15orf27 are three twigs that occupy a branch distinct from other voltage gated ion channels, and that this branch is part of the larger group comprising voltage gated sodium and calcium channels, which split from potassium channels at least a billion years ago. We used a combination of sequence and phylogenetic analysis, structural modeling, and electrophysiological experimentation to identify the proton selectivity filter of H_V1, an important milestone in elucidating the functional components of the protein. We proposed a ''signature sequence'' that defines all presently known H_V1, and used it to identify the first dinoflagellate H_V1, despite only 15% identity with hH_V1. We combined molecular dynamics and sampling methods with other approaches, to help us choose and experimentally validate a refined model that we are using to help us discover the determinants of proton specific conduction.

177-Symp
Proton Channels in Normal and Malignant B Cells
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HVCN1 is the only mammalian voltage-gated proton channel, highly expressed in B cell cancers such as lymphoma and chronic lymphocytic leukaemia (CLL). In normal B cells, we showed that HVCN1 sustains ROS production by the NADPH oxidase, required for optimal B Cell Receptor (BCR) signalling. In malignant B cells, HVCN1 appears to sustain tumour growth, since HVCN1 downregulation impairs cell survival. We are currently addressing whether HVCN1 supports cancer cells via maintaining ROS production and BCR signalling. During our initial investigation on HVCN1 in B cells, we identified a shorter translational isoform of 253 aa, translated from a second initiation site 20 aa downstream from the first ATG. HVCN1 Short is specifically expressed by malignant B cells: we have evidence that it has different electrophysiological properties, such as conducting larger currents with faster activation kinetics after PMA stimulation. In addition, while HVCN1 Long is internalised with the BCR upon stimulation, HVCN1 short is not, indicating it could mediate proton currents constitutively, thus further helping tumour cells maintain their aberrant pH and sustaining BCR signalling.
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