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Membrane translocation is the first step required for drug action on internal bacterial targets. One of the main mechanism through which bacteria exhibit resistance to antibiotics is reduced drug accumulation. Influx of antibiotics into the periplasm of gram negative bacteria is facilitated by porins that form channel in the outer membrane. We investigate the permeation pathways of Beta-lactams and fluroquinolone antibiotics into bacteria by reconstitution of a single porin into an artificial lipid bilayer and measuring the binding of antibiotic molecules through the time-resolved modulation of a small ion current. Temperature dependent antibiotic interaction through porin is measured in the range from 0°C to 55°C revealed that increasing temperature reduces the antibiotic residence time and leads to faster binding events. Combining these results with microbiological assays, molecular dynamics simulation, fluorescence spectroscopy, we conclude that efficiency of permeation for antibiotics depends strongly on their association rate constant with bacterial pores. Given the similar structure within these antibiotic classes, and the detail of the MD simulations, this is also an ideal empirical model system to confirm analytical models for the effect of an affinity site on the flux through a nanopore. Deciphering antibiotic translocation provides new insights for the design of novel drugs that may be highly effective at passing through the porin passport control.

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Detecting Conformational Changes In The Bacterial Glutamate Transporter Homolog GltPh Using EPR Spectroscopy

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Abnormal glutamate transporter function is implicated in Parkinson's disease, Alzheimer's disease, cerebral ischemia, epilepsy, and amyotrophic lateral sclerosis, underscoring the importance of understanding how these transporters function. Our research is centered on elucidating the structural and functional properties of glutamate transporters to reveal novel approaches for treating these various neuropathological conditions. A major advance in this field was the elucidation of the crystal structure of a bacterial glutamate transporter homolog, Pyrococcus horikoshii (GltPh) (Yernool et al., 2004). More recently, evidence for conformational changes in the putative extracellular gate (hairpin loop 2: HP2) was provided by crystallizing GltPh in the presence of the non-transportable blocker, TBOA (Boudker et al., 2007), rendering HP2 unable to properly close due to steric restrictions. In addition, in both the glutamate-bound and TBOA-bound crystal structures, excess non-protein electron density was found occluded in a pocket between hairpin 1 (HP1; putative internal gate), transmembrane domain 7a (TM7A), and transmembrane domain 8 (TM8). This was interpreted as being trapped solvent, and suggested that the trapped solvent was the result of the fact that the putative internal gate (HP1) was closed in both structures. Further conformational change was speculated to expand this solvent-filled cavity, providing a pathway for glutamate to reach the cytoplasm, potentially along the polar face of TM8. Therefore, using site-directed spin-labeling electron paramagnetic spectroscopy (SDSL-EPR) on GltPh, we are working to define the conformational changes that occur in both the extra- and intracellular gates during the glutamate transport process, and to define the pore-like region that allows glutamate access to the cytosol.

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Uncovering an Analytical Description of the Transmembrane Voltage Bistability at Low Extracellular Potassium Concentrations

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In a hypokalemic medium with extracellular potassium concentrations ($[K^+]_{out}$) between about 1.5 mM and 3.5 mM, the transmembrane potential of muscle cells is observed to have two stable steady states: a hyperpolarized state (~ -90 mV) and a depolarized state (~ -60 mV). By varying the potassium concentration and traversing the bistable region back and forth, one can make the system trace out a hysteresis loop. Essential for the bistability are the inwardly-rectifying potassium channels. The open-closed ratio of these channels depends on the transmembrane potential and on ionic concentrations.
By adding isoprenaline to the medium we can create constant potassium permeability. For that case, we no longer observe bistability. We construct a model involving sodium channels, potassium channels, and the Na,K-pump. By solving steady-state equations, i.e. demanding no net flow of sodium and potassium, we can find an analytical expression for the potential as a function of $[K^+]_{out}$. For the isoprenaline case, the model agrees well with the experimental data and indeed shows no bistability.
Solving the model equations is more complicated when the inwardly-rectifying potassium channels are involved. Numerical solutions for that case clearly show the bistability and the model agrees well with experimental observations. We manipulate the equations and also obtain an approximate analytical expression for where on the $[K^+]_{out}$-axis the bistable region is located.

Membrane Dynamics & Bilayer Probes I

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Solid-State 2H NMR Spectroscopy Reveals Micromechanics of Raft-Like Ternary Lipid Membranes Containing Sphingomyelin and Cholesterol

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Much interest has been focussed recently on sphingomyelin as an essential component of a variety of biological membranes. Using solid-state ^{2}H NMR spectroscopy, we investigated the micromechanical effect of varying concentrations of cholesterol in ternary mixtures composed of *N*-palmitoylsphingomyelin (PSM), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), and cholesterol in unoriented multilamellar bilayers. The hydrocarbon chains of PSM or POPC were ^{2}H labeled which enabled us to investigate the distribution and the order profiles of the individual lipid components in the mixtures [1]. A mean torque potential model [2] was employed to characterize the structural properties and map the existence of lipid domains in these mixtures. By calculating the average hydrocarbon thickness, area per lipid, and structural parameters such as chain extension and thermal expansion coefficients, we were able to further characterize the structural properties of these domains. We then measured R_{1Z} relaxation rates, which in combination with order parameter profiles gave a signature square-law dependence corresponding to the mechanical properties of the respective lipid membranes on a mesoscopic length scale [3]. The slope of the square-law plots of relaxation rates and order parameter were found to decrease progressively with the mole fraction of cholesterol, due to a stiffening of the membrane. Different membrane domains thus gave distinctively different micromechanical signatures which indicated that the modes contributing to R_{1Z} relaxation rates are on a length scale comparable to the lipid domain size.
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Implementation of Two Photon Excitation Fluorescence Microscopy Techniques in Langmuir Films

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Most of the reported fluorescence microscopy applications on lipid films at the air/water interface (Langmuir films) are focused in obtaining fluorescence images of the lipid film using particular fluorescence probes. In this type of experiments the probes are generally utilized to obtain "contrast" between different membrane regions (lipid domains) displaying dissimilar physical properties. This information largely depends on the preferential partition of the fluorescent probes for the existing membrane regions and provides only details about shape and size of these lipid domains. However, fluorescence properties associated with the fluorescent probes are almost unexplored in this type of experiments. Examples of the aforementioned parameters are fluorescence lifetimes, fluorescence emission shift, polarization (anisotropy) or eventually probe local diffusion. These parameters are highly sensitive to the physical state of the lipid membrane and can be further used to characterize and correlate structural and dynamical properties of the lipid film. With the aim to measure some of the aforementioned parameters we have setup a specially designed NIMA® film balance on top of a custom built multiphoton excitation fluorescence microscope. This particular setup allows measuring for example LAURDAN GP images (1), polarization (anisotropy), fluorescence lifetimes of UV excited fluorescent probes and probe diffusion using fluorescence correlation spectroscopy. The obtained results using single phospholipids systems demonstrated the high potentialities of this approach in order to fully characterize structure and dynamics of Langmuir films.
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Three-dimensional Dynamic Structure Of Phospholipid Bilayers Saturated With Cholesterol

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Membranes made of synthetic phospholipids, as well as total phospholipids extracted from the eye lenses of young and old animals and containing saturating amounts of cholesterol (close to or exceeding the cholesterol solubility threshold), were investigated using conventional and saturation-recovery EPR spin-labeling methods. Profiles of the order parameter and hydrophobicity were obtained from conventional EPR spectra. Profiles of the oxygen transport