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.

**767-Pos Board B646**

**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.

**768-Pos Board B647**

**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

**769-Pos Board B648**

**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 $^2H$ 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 $^2H$ 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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[2] Petrache, H.I. *et al* (2000) *Biophys. J.* **79**, 3172-3192.
[3] Brown, M.F. *et al* (2002) *J. Am. Chem. Soc.* **124**, 8471-8484.

**770-Pos Board B649**

**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.

1) L.A. Bagatolli 2006, Biochim. Biophys. Acta 1758:1541-556

**771-Pos Board B650**

**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

parameter (oxygen diffusion-concentration product) were obtained from saturation-recovery curves. All of these profiles provide unique information about the depth-dependent physical properties and the three-dimensional dynamic organization of the membrane. Additionally, saturation-recovery measurements allow discrimination of membrane domains because the collision rate of molecular oxygen with the nitroxide spin label may differ in these domains. All membranes saturated (but not oversaturated) with cholesterol are homogenous on the EPR timescale. When properties of the phospholipid-cholesterol membrane are monitored with phospholipid analogue spin labels (by measuring the alkyl chain order parameter), the membrane shows high rigidity that decreases gradually toward the membrane center. However, when membrane properties are measured by monitoring movement and/or concentration of small molecules like molecular oxygen or water, the monitored properties change abruptly between the C9 and C10 positions (depth to which the rigid cholesterol ring-structure is immersed), showing low membrane fluidity and hydrophobicity to the depth of the ninth carbon and high membrane fluidity and hydrophobicity in the membrane center. Based on these observations, it can be concluded that the bulk physical properties of membranes saturated with cholesterol (with a cholesterol-to-phospholipid mole ratio close to one) are mainly determined by the presence of the saturating amount of cholesterol and are practically independent on membrane phospholipid composition.

**772-Pos Board B651**

**Comparative Characterization of Lateral Organization and Packing Properties of Lipids in Pulmonary Surfactant Membranes and Interfacial Films**

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The main function of the lipid-protein complex pulmonary surfactant is to stabilize the respiratory surface by formation of a surface active interfacial film on top of the thin aqueous layer that covers the alveoli. From synthesis in the pulmonary epithelium, until formation of the functional film, surfactant complexes adopt different lamellar structures during its storage and secretion. The functional structure of surfactant makes Langmuir monolayers especially useful for the analysis of structure-function correlations in the pulmonary surfactant system, although they are also widely used as a model to obtain structural information about biological membranes. However, the exact correspondence between lateral organization and molecular packing in bilayers and monolayers is still a matter of controversy.

The fluorescent probe laurdan (6-dodecanoyl-2-dimethylaminonaphthalene) is a lipophilic dye used to analyze the structure of membranes due to its spectral characteristics. Once inserted in membranes, the fluorescence emission of laurdan is very sensitive to the level of hydration of the phospholipid headgroups. Changes in packing or lateral organization of the membrane produce a shift of the emission maximum of the label from 440 nm in ordered membranes to 490 nm in disordered membrane phases. Taking advantage of the properties of this probe, films from pulmonary surfactant lipids containing laurdan were prepared and compressed to obtain surface pressure-area isotherms. Surface pressure and area occupied per molecule along compression were obtained in parallel with the generalized polarization function (GPF) of laurdan -as calculated from its interfacial fluorescence emission spectra- and compared with the fluorescence of laurdan in multilamellar suspensions of the same surfactant lipids at different temperatures.

**773-Pos Board B652**

**Nystatin Action On POPC/sterol Membranes Along Its Phase Diagram**

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Nystatin is a membrane active polyene antibiotic that has been studied for a long time due to its permeabilization activity. Its clinical use is based on the different potency in membranes containing ergosterol (fungi) vs membranes containing cholesterol (mammal). It has been proposed that the mechanism of permeabilization is through the formation of transmembrane pores constituted of several monomers of the drug in a barrel stave configuration. The greater selectivity in ergosterol containing membranes as compared with those containing cholesterol has been proposed to be due to greater pore stability produced by ergosterol. This hypothesis has been questioned over the time and alternative models have been suggested. Experimental evidence has shown that sterol is not a requirement for membrane permeabilization, then the proposal of an indirect role of sterol through the effect on membrane structure. This idea has been supported by experimental evidence showing a different action in different phases of the membrane. In order to evaluate the effect of phase changes of the membrane on the pores formed by Nystatin, single channel experiments were performed along the phase diagram of 1-palmitoyl-2-oleoyl-sn-glycero-phophocholine (POPC) membranes containing ergosterol (erg) or cholesterol (chol). The results show that for POPC-erg membranes there is a region of maximum permeabilization consistent with the liquid-ordered (lo) + liquid disordered (ld) mixed region. For the POPC-chol membranes the maximum permeabilization occurs also in the mixed region but at lower temperatures. These results are taken as strong evidence supporting the idea that the phase of the membrane is determinant for the activity of polyene antibiotics.

**774-Pos Board B653**

**Changes in Membrane Fluidity of Blood Platelets in Myeloid Neoplasm**

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In this study we evaluate the alterations in membrane fluidity of blood platelets in patients with various entities of myeloid malignancies.

The clinical history of myeloproliferative and myelodysplastic disorders is often complicated by thromboembolic or hemorrhagic events.The mechanism of these major complications remains unclear. Since there is a weak correlation between the risk of these life threatening complications and the number of blood platelets, our research is focusing on qualitative defects of platelets.

Membrane fluidity is an important parameter which influences many of the unique cellular functions and which is strongly correlated (among other factors) to the membrane lipid composition. We try to correlate changes in cell membrane fluidity with the clinical status of the patient disease.

32 patients with various entities of myeloid neoplasm were selected (Department of Hematology, Emergency University Hospital of Bucharest). They were diagnosed according to the WHO criteria. 11 normal healthy volunteers, non smokers, drug-free, were used as controls.

Membrane fluidity was assessed by fluorescence anisotropy measurements. The platelet membrane shows to be more rigid compared with controls/normal regardless of the clinical type of myeloproliferative disorder. However patients with severe clinical status due to acute myeloid leukemia have a more fluid membrane compared to the same patients found previously in a better state. Thus, the activity (or severity) of the disease correlates with the increase in membrane fluidity, as other studies revealed on lymphocytes. We consider that detection of these modifications may be useful for a better insight in cell abnormalities occurring in this pathology.

**775-Pos Board B654**

**NSAIDs Alter Lipid Bilayer Mechanical Properties**

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Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are a class of widely prescribed medications that have analgesic, antipyretic, anti-thrombotic and anti-inflammatory properties. Their primary mechanism of action is through non-selective inhibition of the Cyclo-oxygenase enzymes (COX-1 and COX-2), which mediates many of the clinical - and side - effects of NSAIDs. Other effects are mediated through COX-independent mechanisms, however. Given that NSAIDs are amphiphiles, that they modulate the function of many different, structurally unrelated membrane proteins, and that the lipid bilayer serves as a gate-keeper/regulator for many different cell functions, we tested whether NSAIDs could alter lipid bilayer material properties. To measure such changes in bilayer material properties, we used gramicidin A (gA) channels as molecular force transducers. We found that salicylate, ibuprofen, diclofenac, sulindac sulfide and flurbiprofen are potent modifiers of bilayer properties. At pH 7, NSAIDs were found to increase both the lifetime and appearance rate of channels formed by both short (13-residue) and long 15-residue gramicidin analogues, with the larger effects on the shorter channels - the channels with the larger hydrophobic mismatch, which shows that NSAIDs decrease lipid bilayer stiffness by increasing the bilayer elasticity. These effects were achieved at the high end of clinically relevant concentrations. This suggests that in both the clinical and research setting, NSAIDs may have effects that arise from modulation of lipid bilayer mechanical properties.