761-Pos   Board B640

Roles Of The Membrane Protein CD36 In Fatty Acid Transport And Metabolism

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Obesity has become a major public health concern and represents a predisposition factor for the development of cardiovascular-related diseases and non-insulin dependent diabetes mellitus. Therefore, deciphering the molecular mechanisms of fatty acid (FA) uptake will provide new insights for dietary and other therapeutic interventions for managing diseases associated with obesity. FA uptake into cells occurs by multiple mechanisms, including transport and metabolism, beginning at the plasma membrane. Understanding the contributions of passive diffusion and facilitated transport by plasma membrane proteins, such as FAT/CD36, requires experimental approaches that separate biophysical and metabolic mechanisms. In previous experiments with protein-free lipid bilayers, we have used multiple fluorescence assays to show that FA bind to, and diffuse through, phospholipid bilayers very rapidly but desorb more slowly from the lipid into the aqueous phase. Here we apply these methods to HEK293 cells engineered to stably express CD36. FA movement across the plasma membrane occurred rapidly (within sec) with or without expression of CD36. HEK293 cells without CD36 exhibit very slow conversion of FA into acetylated products. However, incorporation of 14C-labeled oleic acid into triglycerides occurred more rapidly and was significantly increased in HEK293 cells overexpressing CD36. Formation of small lipid droplets was observed after incubation with a fluorescent FA analog BODIPY-FA. Thus, FA transport through plasma membranes occurred by the mechanism of diffusion without a requirement for CD36. It appears that this protein enhanced metabolism by an as yet unknown mechanism.

762-Pos   Board B641

Analytical Description Of The Diffusion Coefficient And Current Of A Single Particle On A Two-dimensional Corral Model

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The study of lateral dynamics in corrals has attracted atention since 1983 when Sheetz introduced the corral model. In this project, we propose a simple model consisting of a two-dimensional lattice containing periodically distributed flashing walls. These walls are represented by finite asymmetrical flashing potentials which isolate a portion of the lattice to represent semipermeable corrals. Contigous corrals share a wall creating an arrangement of similar compartments on the lattice. We included the presence of a constant external field to account for the effect of gradients, adding another degree of complexity to the dynamics of a single particle in this medium. We derive general analytical expressions to describe the diffusion coefficient (D) and the current (J) of a single particle moving on this medium. We use a formulation based on a single particle microscopic model and a diffusion relaxation condition to derive our equations as a function of the corral's size (and concentration), the time between flashes, and the strength of the external field. We compare our theory against Monte Carlo simulations.

763-Pos   Board B642

Phase Transitions in Single Nano-Vesicles

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Phase transitions in nano-scale lipid vesicles are believed to be influenced by the morphology as well as the finite size of the system. Both the effect of finite size and the asymmetric lateral stresses in the bilayer are believed to broaden the phase transition in small vesicles which has been confirmed by bulk studies. However, it still remains to be resolved whether the measured phase transition of the ensemble results from a broadening of the phase transition in individual vesicles or to heterogeneities among single vesicles exhibiting sharp phase transitions. We investigate the transition from gel (ordered) to fluid (disordered) state in single nano-sized lipid vesicles, composed of only $\sim 10^4$ molecules, by measuring the anomalous permeability which peaks at the phase transition. We study single vesicles by immobilizing them on functionalized substrates. The vesicle size and efflux/influx for each vesicle is accurately quantified which allows us to study the effect of curvature on phase transitions. To assess the width of the phase transition we probe the pore size at different temperatures using chromophores of different molecular radii. Our data suggest a revaluation of the currently accepted concepts about the nature of permeation at Tc and of phase transitions in nano-scale systems.

764-Pos   Board B643

Intracellular Water Lifetime Depends on Cellular Energetic State

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Intra- and extracellular water undergoes equilibrium exchange *via* mechanisms that include passive diffusion across the membrane and movement through channels, like aquaporin. Longitudinal ^1H_2O NMR relaxography can employ extracellular relaxation agent GdDTPA^{2-} to distinguish intra- and extracellular ^1H_2O signals by creating a difference in their relaxation time constant (T_1) values. Inverse Laplace transform of relaxation decay produces the relaxogram, the T_1 distribution, which reports *apparent* water populations, $p_i{}'$ and $p_e{}'$. The *true* water fractions, p_i and p_e - measures of the volume fractions v_i and v_e, are determined if the kinetics of transcytolemmal water exchange (the mean intracellular water life time τ_i) are quantified using two-site-exchange analysis. The "well-mixed" kinetic expression is: $\tau_i^{-1} = P_W(A/V)$, where P_W is the water permeability coefficient; A and V are the *individual* cell surface area and volume, respectively. Furthermore: $v_i/\tau_i = P_WS$, where S is the *total* cell surface area. We determined the relationship between τ_i and cellular energetics in suspensions of *Saccharomyces cerevisiae*. Under aerobic conditions τ_i was 333 ($\pm$ 4) ms; under anaerobic conditions τ_i was 670 ($\pm$ 62) ms. Changing from aerobic to anaerobic conditions also resulted in a decrease of ATP content. In the yeast strain MR6, which requires adenine supplementation to grow, ATP content increased with medium adenine concentration. As ATP was thus increased, the τ_i was found to inversely correlate with ATP content ($R^2 = 0.96$). Therefore, τ_i is a sensitive indicator of the cellular energetic state and energetics affects the kinetics of transcytolemmal water exchange. The aerobic P_WS was greater than the anaerobic P_WS. The underlying mechanism(s) are not yet clear. Experiments with baker's yeast stored in an anaerobic state found that inhibition of protein synthesis prevented the anaerobic-to-aerobic τ_i decrease and that aquaporin is not responsible.
1

765-Pos   Board B644

Interaction Of Enrofloxacin With Model Membrane Systems. Implications In The Permeation Pathways, Revealed By Fluorescence And Conductance Studies

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With the actual increasing menace of bacterial resistance, the antibiotic permeation mechanisms have been the centre of attention, in many research fields. OmpF is a pore forming protein, found in Gram-negative bacteria's outer membrane, known to have an important role in the uptake of nutrients and antibiotics (e.g. quinolones), towards the interior of the bacterial cell. Although several studies have been published of protein-drug interaction in the complex microbiological environment, an understanding of this mechanism at a molecular level is still lacking. Quinolones are one of the most prescribed classes of antibiotics, both in human and veterinary medicine, and this wide use seems to be the main cause for bacterial resistance. Fluoroquinolones were developed to increase quinolone antibacterial activity by changes in the quinolone structure. Enrofloxacin is a second generation fluoroquinolone being the first one to be introduced in Veterinary medicine. Its human analogue is the well known Ciprofloxacin. The main purpose of this work was to study Enrofloxacin permeation pathways. Permeation or interaction of antibiotics with lipid bilayers (liposomes) is of great importance in order to clarify the lipid component function's in membrane permeation. A first approach was the determination of partition coefficients Enrofloxacin/liposomes, which allowed the quantification of this interaction. After, OmpF was reconstituted in liposomes (proteoliposomes) by two different reconstitution methods (Gel exclusion chromatography and using detergent adsorbing beads) in order to compare size, homogeneity and protein orientation in proteoliposomes. Proteoliposomes quenching studies, using water soluble quencher KI and acrylamide, as well as ion conductance measurements were performed to establish the site of interaction/translocation of Enrofloxacin.

766-Pos   Board B645

The Porin passport control - Conductance measurements and biological relevance

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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.

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 ^{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.
[1] Bartels, T. *et al* (2008) *J. Am. Chem. Soc.*, in press.
[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.

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

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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