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Permeation Of Beta-lactam Antibiotics Through E. Coli Ompf Altered By Constriction Zone Mutations

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Accelerated growth of resistance of pathogenic gram-negative bacteria to various antibiotics makes evaluation of highly efficient assay for antibiotic screening an important task. Control of the outer membrane permeability and/or increase of the antibiotic efflux via various efflux pumps prevent the antibiotic penetration into the cell. It was found earlier that beta-lactam antibiotics translocate into bacteria mainly via nonspecific porins like E. coli OmpF. Some of the recent studies showed that the mutations in the OmpF alter the translocation rate of antibiotics and this mechanism is still vigorously investigated. Data indicate that the structural and functional effect of each mutation should be taken into account separately and is individual for each antibiotic. It is expected that the constriction region of the porin play a very important role in antibiotic passage. This zone is characterized by a strong electric field, where negatively charged residues Asp113, Glu117 face a cluster of positively charge residues Arg42, Arg82, and Arg132. In the present study OmpF mutants were incorporated into the planar lipid bilayer and ionic current through the channels was analyzed in the presence of beta-lactams. As an additional method to investigate the process of permeation we employed liposome swelling assay technique that has been applied previously to such problems with success. The advantage of this technique is that penetration rate of antibiotic in proteoliposomes generally mimic that into the intact cells and swelling rates are directly proportional to the permeability of antibiotic in vivo. Finally, molecular dynamic simulations were used to study the event of translocation through OmpF in molecular level. Thus is become possible to obtain the objective picture of permeation mechanism which later can be applied in evaluation of highly efficient antibacterial drugs.

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Calculation of Current-Voltage properties of Biological Ion Channel, using Poisson-Nernst-Planck (PNP) Theory

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Biological ion channels are integral membrane proteins that are usually formed by relatively large proteins. Studying current-voltage (I-V) characteristics of channels is commonly used to understand how channels function and estimate effectiveness of existing and potential drugs. The current - voltage characteristics of a channel depend on the channel's structure and conformation. Current conduction through a channel in the protein is a slow process, which makes the traditional methods for theoretical modeling of proteins (such as molecular dynamics or Monte-Carlo simulations) practically inapplicable for direct modeling of channel function. Therefore other simplified theoretical methods have been developed. One such method is the Poisson-Nernst-Plank (PNP) theory of electrodiffusion, where a set of partial differential equations (the Poisson and the Nernst-Plank equations) are solved self-consistently. We are developing the PNP equations solver (PNPS) for calculating current-voltage properties of ion channel proteins. To improve computational efficiency the solver was parallelized. The new solver has been applied to predict ion conductance properties of the α-Hemolysin channel, a robust and well studied pore forming heptameric protein complex. Because of the asymmetry of the protein structure its position with respect to the lipid bilayer has not been well determined. We performed a - series of calculations in which the membrane position has been varied. pKa calculations show that the protonation state of some residues depends on the membrane position. The results of PNP calculation are compared with experimental data on channel conductance, ion selectivity, reverse potential, rectification properties. Such detailed analysis allowed us to pinpoint position of the protein in the membrane. Several methods for setting diffusion coefficients were tested. We have also investigated models for interaction of a permeant ion with the protein and its mobility in the constricted environment of the protein pore.

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Computational Study on the Ion Selectivity of Modified Alpha-Hemolysin Channels

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Alpha-hemolysin (AHL) monomers are secreted by the bacterium Staphylococcus aureus. They self-assemble into heptameric beta-barrel AHL channels, which act as exotoxins by forming wide pores in the outer membrane of cells. AHL channels can be successfully engineered for applications in biotechnology such as stochastic sensing of molecules and DNA sequencing. Ion permeation through AHL can be manipulated via mutations and by lodging molecular adapters such as the cyclic polysaccharide beta-cyclodextrin (BCD) in the pore. In order to clarify how BCD and BCD derivatives can alter the permeability and ion selectivity of AHL, we have performed potential of mean force (PMF) calculations and grand canonical Monte Carlo/Brownian dynamics (GCMC/BD) simulations on the basis of the x-ray structures of wild type AHL and two AHL mutants. The computed current-voltage curves and reversal potentials with and without BCD bound reproduce the experimentally observed increase in anion selectivity after adding BCD. The PMF free energy profiles of single and multiple ions along the channel axis show that BCD reduces the ionic and dielectric shielding of positively charged residues and, thus, amplifies the weak anion selectivity of AHL. Our results for a positively charged BCD derivative predict a further increase in anion selectivity and also more current compared to BCD.

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Vectorial Ion Transport by Channelrhodopsin-2

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Channelrhodopsins represent a third member of microbial-type rhodopsins and have gained considerable attention in neurobiology as a tool to control the excitability of neurons. The other photoreceptors act either as light-driven ion pumps like bacteriorhodopsin (BR) or as part of a relay system like the sensory rhodopsins (SR). In contrast, channelrhodopsins are light-gated ion channels that allow the passive permeation of cations over the membrane barrier after light activation. Previously, we could follow the spectral characteristics of different photointermediates and relate them to the functional states of the channel. In the gating process, the Schiff-base undergoes a deprotonation reaction before it relaxes into a red-shifted species. The red-shifted photointermediate is characteristic for the open state with a lifetime of 10 ms.
So far, the role of the protonation reactions is not fully understood. Channelrhodopsins possess a glutamate residue at the homologue position of the proton acceptor D85 in BR. The main difference among the microbial-type rhodopsins is found in the nature of the proton donor at the opposite side of the retinal moiety towards the cytoplasm: In BR, an aspartic acid reprotonates the Schiff-base in a fast manner as expected for an efficient proton pump, while in SR an aromatic residue leads to a long-lived deprotonated state of the Schiff-base. In channelrhodopsins, the homologue residue is a histidine that could allow a reprotonation of the observed deprotonated Schiff-base from the cytoplasmic side. As a net result, one proton would be transported per photocycle. Here we show that one can indeed observe such a vectorial ion translocation under illumination of purified and proteoliposome reconstituted channelrhodopsin-2. Therefore, the mechanism of channelrhodopsin-2 shows similar features as other rhodopsins, i.e. control of the accessibility to the Schiff-base by light-induced isomerization of the retinal and vectorial ion transport.

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Directional Ion Selectivity In An Ion Channel With Bipolar Charge Distribution

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The selectivity of the bacterial porin OmpF from E. coli to small inorganic ions has been investigated by single channel experiments. In a recent study, we showed that the OmpF channel may function as a pH-regulated, biological, nanofluidic diode (J. Phys. Chem. B 110 (2006) 21205). Here we show that Reversal potential measurements done under asymmetric conditions of pH and salt concentration provide valuable information about the channel fixed charge distribution that cannot be extracted from the rectification displayed in current-voltage curves. We find that the pH gradient imposed across the pore induces an asymmetric fixed charge distribution that resembles the structure of a synthetic bipolar membrane (a composite of an anion-exchange membrane and a cation-exchange membrane used to split water under reverse polarization conditions). This particular arrangement demonstrates that the ionic selectivity of a non-uniformly charged pore is not an intrinsic property of the system but depends crucially on several external factors. Amazingly, changing the direction of the salt concentration gradient can turn a cation selective channel into an anion selective one.

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Relative Dielectric Permittivity And Resting Membrane Potential In Living Cells Suspensions: An Experimental Approach

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We present a method to obtain the resting membrane potential ($\Delta\Psi$) from the dielectric behavior of a suspension of living cells by the use of dielectric spectroscopy. Since cells behave as conducting particles surrounded by low-conducting shells with surface charge densities, we can apply this technique to record the dielectric permittivity ϵ and conductivity σ of the suspension as a function of frequency. A previous theoretical model has correlated the relative dielectric permittivity ϵ of the suspension with resting membrane potential in the very low radio frequency regime (alpha). We use this model with our experimental results to obtain $\Delta\Psi$ for bacteria (E.Coli K12) and mammalian cell suspensions from HEK293-hERG line. We compare our value for $\Delta\Psi$ and its changes with the traditional methods-voltage sensitive dyes and patch clamping. For E. Coli measurements, resting membrane potential is changed by KCl addition to the suspension bath. As for mammalian cells, $\Delta\Psi$ changes are triggered by the use of various pharmaceutical compounds that act as HERG K^+ channel blockers and IC_{50} values are computed for each compound. Precise measurements of the dielectric permittivity ϵ and conductivity σ of live cells suspensions in the alpha frequency regime require prior elimination of the polarization errors. Polarization errors are caused by the ionic content of a buffer, and they affect the total impedance in the low frequency interval. We hereby present our approach of measure the polarization impedance then remove it by fitting both real and imaginary experimental curves with an ideal impedance $Z=d/i\omega\epsilon^*S$, where $\epsilon^*=\epsilon+1/i\omega\sigma$.

3423-Pos Board B470

Continuum Multi-dielectric Treatment Of Fluctuations And Breakdown In Membranes With Embedded Charges

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Stabilization of protein charges due to their interaction with membrane fluctuations is a subject of growing interest, especially because of its possible implications for voltage gating. Two complementary mechanisms governing charge-fluctuation interactions are considered: (1) the *electroelastic mechanism (EM)* [Partenskii, et al., *Israel J. Chem.* **47**, 385 (2007)], where the membrane is treated as an elastic slab (smectic bilayer model); (2) the *hydrophobic mechanism* (*HM*), which accounts for water penetration into the membrane's hydrophobic core with a corresponding interfacial tension contribution. In both cases the linear Poisson-Boltzmann equation is solved using a multi-dielectric continuum model with arbitrarily shaped membrane-water interfaces and a point charge surrounded by a ''Born sphere'' of low dielectric constant. The *EM* often leads to large membrane thickness perturbations, far larger than are consistent with elastic model descriptions. We demonstrate that switching from *EM* to *HM* becomes energetically advantageous at intermediate perturbation amplitudes. We apply kinetic Monte Carlo Reaction Path Following [Miloshevsky & Jordan. *J. Chem. Phys.* **122**, 214901 (2005)] using the water dimple's amplitude or the z-coordinate of the charge as the reaction coordinate for determining the shape of the solvation cavity. The resulting picture confirms that of recent MD studies.

3424-Pos Board B471

Phosphatidylserines Transduce Cell-Penetrating Peptides

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Certain short polycations, such as TAT and polyarginine, rapidly pass through cell membranes and pervade all intracellular compartments by an unknown mechanism called transduction. These cell-penetrating peptides (CPPs) when fused to biologically active peptides promise to be medically useful. I offer a simple model in which phosphatidylserine (and possibly other anions) transduce CPPs. The model also involves surface tension and the electrostatic field across the membrane of the cell. The model is consistent with the empirical upper limit on the cargo peptide of about 35 amino acids. More importantly, it also fits experimental data on how the transduction of a polyarginine-fluorophore into mouse C2C12 myoblasts depends on the number of arginines in the CPP and on the CPP concentration.

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Electrical Relaxation Experiments With Bilayer Lipid Membranes In The Presence Of Cationic Quinones

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Mitochondria-targeted antioxidants consisting of a quinone part conjugated with a lipophilic cation via a hydrocarbon linker were previously shown to prevent oxidative damage to mitochondria *in vitro* and *in vivo*. In the present work, we studied the permeation of a series of compounds of this type across a planar bilayer phospholipid membrane. For this purpose, relaxation of the electrical current after a voltage jump was measured. All compounds studied exhibited slow relaxation kinetics in the time range from seconds to minutes. With respect to the characteristic time of the relaxation, hydrophobic cations can be ranked in the following series: 10(plastoquinonyl) decylrhodamine 19 (SkQR1) > 10-(6′-plastoquinonyl) decyltriphenylphosphonium (SkQ1) > 10-(6′-methylplastoquinonyl) decyltriphenylphosphonium (SkQ3) > 10-(6′-ubiquinonyl) decyltriphenylphosphonium (MitoQ). The relaxation was strongly dependent on the redox state of the quinone part of the molecule being substantially suppressed in the reduced form. Surprisingly, the kinetics of relaxation of several compounds depended not only on the phospholipid composition of the bilayer but also on the pH of the bathing solution.

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Impact Of $Na_V1.7$-PEPD Missense Mutations That Slow The Rate Of Inactivation On Sensory Neuronal Resurgent Sodium Currents

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Voltage-gated sodium ($Na_v1.1$-9) channels are dynamic transmembrane proteins that, in response to changes in the potential across the lipophilic cell membrane, undergo specific conformational (gating) modifications, between ion-conducting (open) and non-conducting (closed and inactivated) states, to selectively conduct sodium ions through their aqueous pore. Importantly, changes in these voltage-dependent gating properties can impact action potential (AP) characteristics. TTX-sensitive sodium channels in cerebellar neurons can produce resurgent currents (Raman & Bean, 1997), intriguing currents that are reactivated during intermediate repolarizations following strong, but short, depolarizations. We observe resurgent currents in some DRG neurons and found that wild-type $Na_v1.6$ but not wild-type $Na_v1.7$ channels can generate resurgent currents in DRG neurons (Cummins *et al.*, 2005). It has been demonstrated that, in cerebellar neurons from $Na_v1.6$-null mice, slowing inactivation of the remaining Na_v current can induce resurgent currents (Grieco & Raman, 2004). Interestingly, single-point missense mutations in the SCN9A gene that encode for $Na_v1.7$, implicated in paroxysmal extreme pain disorder (PEPD), slow the rate of $Na_v1.7$ inactivation (Jarecki *et al.*, 2008). Therefore, we hypothesized that slowing of $Na_v1.7$ by PEPD mutations might induce abnormal resurgent currents, thus altering AP properties. To explore this hypothesis, we transiently transfected adult rat DRG neurons with a TTX-resistant form of human $Na_v1.7$-wild-type or PEPD mutant cDNA and rat $Na_v1.8$-targeted shRNA. Voltage-dependent properties were observed using whole-cell voltage-clamp electrophysiology and AP generation was tested using current-clamp electrophysiology. Recordings were made in the presence and absence of extracellular TTX. These experiments should yield insight into (1) the mechanism of resurgent sodium current generation in DRG neurons, (2) a potential additive effect in channel dysfunction observed in PEPD, and (3) how these mutant channels contribute to alterations in AP characteristics.

Cardiac Electrophysiology II

3427-Pos Board B474

Effects Of Mitochondrial Depolarization On Cardiac Electrical Activity In An Integrated Multiscale Model Of The Myocardium

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Metabolic or oxidative stress can trigger the abrupt collapse or oscillation of mitochondrial membrane potential, which activates KATP current and alters the cardiac action potential. This mechanism can introduce both temporal and spatial dispersion of electrical excitability in the form of a ''metabolic sink'', leading to heterogeneous conduction in the tissue, reentrant tachyarrhythmias, or fibrillation. To quantitatively study this mechanism, a 2D monodomain model of the myocardium (5×5cm2; 200 micron resolution) was developed, comprised of 63,000 nodes, each representing an integrated cellular model of cardiac excitation-contraction coupling, mitochondrial energetics, and ROS-induced ROS release (ECME-RIRR). Oxidative stress was initiated in a central circular zone of the tissue by increasing the fractional mitochondrial ROS production (shunt) during oxidative phosphorylation from 2% to 10%. Model simulations show that mitochondrial dynamics bifurcate during stimulation at 1 Hz and complete depolarization of $\alpha\Psi m$ ensues in the sink zone. Within the metabolic sink, sarcolemmal KATP currents increase, action potentials dramatically shorten, and the refractory period is abbreviated. These effects are enhanced by increasing the KATP density. In addition, fast and irregular electric activity (ventricular fibrillation) in the electrically paced tissue is observed when an S2 stimulus is introduced within or near the border of the