mismatch with the bilayer or combinations of these effects. As a consequence, mechano-sensitivity may arise due to lipid bilayer properties that change with mechanical deformation such as bilayer dilation/ thinning, changes in the local membrane curvature, or lateral pressure profile. In this study, we tested the effect of induced-changes in lipid properties on MscL gating by using reconstituted MscL channels with different tension sensitivities (e.g. MscL mutants and MscL channels from different organisms). We could differentiate tension-sensitivity phenotypes among the channels from the same microorganism. However, our results on MscL channels from different organisms were surprising. Even though their tension-sensitivities were as expected when tested in patch clamp, the channels exhibited the opposite response to the lipid modifications in our assay system. The possible causes for this unexpected behaviour will be discussed.

2792-Symp
Mechanisms of Cholesterol Regulation of Kir Channels
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Numerous types of ion channels were shown to be suppressed by cholesterol but the mechanisms underlying these effects are only starting to emerge. Our studies focus on inwardly-rectifying K+ channels (Kir) that are ubiquitously expressed in a variety of tissues and play major roles in the maintenance of membrane potential and control of cellular excitability. We have found that Kir channels are suppressed by membrane cholesterol in multiple cell types including endothelial cells, progenitor cells, macrophages and cardiomyocytes. Furthermore, cholesterol-induced suppression of Kir is observed both in vitro and in vivo. Our earlier studies suggested that cholesterol-induced suppression of Kir results from stabilizing the channels in the closed state and that this effect is due to specific cholesterol-protein interactions. Analysis of all Kir subfamilies (Kir1-7) showed that most of the Kir channels are suppressed by cholesterol although to a different degree. More recently, focusing on Kir2.1 channels as a model for Kir, we showed that cholesterol sensitivity of Kir critically depends on a specific set of cytosolic residues that form a ''cholesterol sensitivity belt'' around the cytosolic gate of the channels. However, this region does not constitute a cholesterol binding site but rather interferes with cholesterol sensitivity of the channels by affecting Kir gating. In parallel, we used a purified bacterial homologue of Kir channels, KirBac1.1 to show that purified KirBac channels are also suppressed by cholesterol when incorporated into liposomes demonstrating that cholesterol inhibits the channels directly without a need for any intermediates. Furthermore, cholesterol-KirBac binding is required for the inhibitory effect providing the first direct evidence for cholesterol-ion channel binding. Most recently, we identified a two-way molecular switch that regulates cholesterol sensitivity of the channels through distant cytosolic residues. This switch appears to be a common mechanism for cholesterol and PIP2 regulation of the channels.

2793-Symp
Mechanosensitive Channels as Sensors for Lipophilic Drug Partitioning
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Membrane permeability is a crucial parameter for development of new drugs, but currently no direct technique exists to predict the partitioning of a lipophilic or amphipathic substance into the native bacterial membrane. In this study we use the endogenous mechanosensitive channel MscS residing in the cytoplasmic membrane of *Escherichia coli* as a lateral pressure sensor reporting on the asymmetric presence of extrinsic amphipaths in the surrounding bilayer. The unique asymmetric location of the channel gate relative to the mid-plane of the lipid bilayer suggests that the channel is especially sensitive to pressure changes in the inner leaflet of the membrane. Upon testing of several types of amphipaths, including three parabens, on excised membrane patches of *E. coli,* we found that right shifts in the activation curve of MscS correlate with the surface activity and more strongly with extra lateral pressure imposed by the same substances through intercalating into Langmuir monolayers. Similar correlations were found for bacterial quorum sensing substances AI-1 and AI-2 and their synthetic analogs. We recently characterized the partitioning capacity for several dialkylamine analogs of the antibiotic platensimycin, which were synthesized with the identical dihydroxyl benzoate pharmacophore, but varied in sidechain structure. QD-11, the analog containing two myrtenal groups, had the highest propensity for membrane intercalation compared to analogs with aliphatic and aromatic sidechains and had strongest antibiotic effect. This new principle of measuring lateral pressure perturbations in membranes using endogenous mechanosensitive channels provides a foundation for a probe-based techniques that track the partitioning of biologically active substances directly into the bacterial membrane and monitors the process of permeation in a species-specific way.

Symposium: Light-driven Reactions and Physiology

2794-Symp
Protein Tuning of Ligand Pathways and Metal Centers in Heme-Copper Oxidases
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Knowledge of how the protein environment can tune ligand pathways and metal-based prosthetic group(s) is fundamental for understanding the catalytic functions of metallo-enzymes. The structural and functional similarities and differences among the heme-copper oxidases, which play a key role in energy production of aerobic organisms, make them an ideal system for exploring the effect of the protein environment on enzymatic function. For example, the catalytic subunit of the *Rhodobacter sphaeroides* (*Rs*) and *Paracoccus denitrificans* (*Pd*) aa_3 oxidases has high sequence homology to its mitochondrial counterpart, while that of other oxidases, including *Thermus thermophilus* (*Tt*) ba_3, is much lower. To establish possible relationships between the structural diversity of these enzymes and their ligand binding dynamics, we used photolabile O_2 and NO complexes to monitor the kinetics of O_2 and NO reacting with reduced wild-type *Thermus thermophilus* (*Tt*) ba_3 and the bovine heart aa_3 oxidase. Time-resolved optical absorption measurements show that O_2 and NO bind to reduced heme a_3 in *Tt*ba_3 with a second-order rate constant of $1x10^9$ $M^{-1}s^{-1}$. This rate is 10-times faster than observed for the mammalian enzyme, suggesting that inherent structural differences affect ligand access in the two enzymes. To test this hypothesis, we used x-ray crystallography, time-resolved optical absorption spectroscopy, and theoretical calculations to study ligand access in *Tt* ba_3 mutants, in which tyrosine and/or threonine in the O_2-channel of *Tt* ba_3 were replaced by the corresponding bulkier tryptophan and phenylalanine residues of the aa_3 enzymes. The results are consistent with a constriction point and hydrophobic pocket in the O_2-channel of the bovine aa_3 impeding access of NO and O_2 to the active site in this enzyme but not in *Tt* ba_3. Such structural differences may reflect evolutionary adaptation of the two enzymes to different environments.

2795-Symp
Interaction of Antenna Carotenoid and Retinal in the Light-Driven Pumps of Salinibacter Ruber and Gloeobacter Violaceus
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Xanthorhodopsin is a light-driven proton pump like bacteriorhodopsin, but contains a second chromophore, salinixanthin, a carotenoid. Action spectra for transport and fluorescence of the retinal upon excitation of salinixanthin, as well as femtosecond kinetics of carotenoid fluorescence, indicate that the carotenoid functions as an antenna to the retinal, with ca. 50% efficiency for excited-state energy transfer. The resulting charge redistribution in the retinal and the local electric field produced, in the excited state and in the primary stable photoproduct K, in turn, causes distinct electrochromic shifts in the carotenoid spectrum. The close interaction of carotenoid and retinal suggests that the two chromophores are in proximity. Tight binding of the carotenoid, as indicated by its sharpened vibration bands and intense induced circular dichroism in the visible, is removed in the absence of the retinal, and restored upon reconstitution with retinal or retinal analogues. The crystallographic structure of the xanthorhodopsin at 1.9 Å resolution identifies the location of the chromophores. The structural model from x-ray diffraction reveals that the ring moieties of the retinal and the carotenoid are at nearly van der Waals distance from one another. Salinixanthin and one of the native carotenoids of Gloeobacter, echinenone, bind to gloeorhodopsin expressed in E. coli, and the complex shows excited-state energy exchange much like xanthorhodopsin. Protein sequences and mutational studies in Gloeobacter indicate that binding of the carotenoid depends on a conserved glycine at the keto-ring, which is a tryptophan in bacteriorhodopsin and other archaeal rhodopsins. On this basis, numerous otherwise unrelated eubacterial rhodopsins are predicted to contain light-harvesting carotenoids.