X-ROS is the novel signaling pathway seen in ventricular myocytes1 and skeletal muscle2 that arises when cell stretch triggers local ROS (reactive oxygen species) production by NADPH oxidase 2. A sustained (10-20s) stretch produces a transient rise in ROS with a half-time of decay of ~5 seconds. In cardiomyocytes, this ROS sensitizes local Ca^{2+} release channels (ryanodine receptors) in the sarcoplasmic reticulum. In normal heart cells, this produces a transient burst of Ca^{2+} sparks and ensures the fidelity of excitation-contraction coupling, but can trigger arrhythmogenic Ca^{2+} waves in diverse pathologies.
In reports to date, X-ROS has been characterized in cells that were stretched and held at a constant length. However, this is not how a cardiomyocyte is stretched in situ - instead it is stretched (during diastole) and shortens (during systole) in rhythmic fashion. Additionally, this stretch is regulated by two factors that vary with changing physiologic demand: 1) preload (the amount of blood that fills the ventricles during diastole) grades the magnitude of cell stretch, and 2) heart rate varies the frequency of cell stretch. Thus we investigated how the magnitude and frequency of stretch affect X-ROS signaling. Briefly, we find a critical effect: rhythmic stretch elevates the steady state level of ROS production in the cell, and this level is graded by both the magnitude and frequency of stretch. In turn, the elevated ROS proportionately modulates Ca^{2+} spark rate. Thus our findings hold the critical implication that the redox state and Ca^{2+} signaling sensitivity of cardiomyocytes is coupled to mechanical changes and graded by both preload and heart rate.
1. Prosser et al. ''X-ROS signaling: Rapid mechano-chemo transduction in heart.'' Science. 333, 1440 (2011 9 Sept).
2. Khairallah et al. ''Microtubules underlie dysfunction in Duchenne muscular dystrophy.'' Sci. Signal. 5, ra56 (2012).

208-Plat
Pivoting between Calmodulin Lobes in the Calmodulin/Kv7.2 Complex Triggered by Calcium
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Mammalian KCNQ genes encode five Kv7 potassium channel subunits (Kv7.1-Kv7.5). Kv7.2 and Kv7.3 are expressed in the nervous system, being the principal molecular components of the slow voltage gated M-channel, which exert a strong control in neuronal excitability.
Like all Kv channels, the Kv7 α subunits share a common core structure of six transmembrane segments with a voltage-sensing domain (S1-S4), a pore domain (S5-S6) and intracellular N- and C-terminal regions. The C-terminus harbours four regions that present a high probability of adopting an alpha helix configuration (helices A-D). This region binds some lipids and several proteins, including the ubiquitous calcium binding protein calmodulin (CaM).
CaM mediates inhibition of Kv7.2 channels and is required for the channels to exit the endoplasmic reticulum. Both processes are enhanced by Ca^{2+}, but the molecular details of how Ca^{2+} trigger channel trafficking or the reduction of M-current are unknown. The aim of this study was to explore the molecular events within CaM triggered by Ca^{2+} using two complementary approaches. In one, we have performed a fluorimetric assay using dansylated calmodulin (D-CaM) to characterize the interaction of individual lobes to the Kv7.2 CaM binding site. The association of the Kv7.2 with CaM was also explored using NMR spectroscopy, employing 15N-labeled CaM as a reporter. Our data show interdependency of the N- and C-lobes in the interaction and suggest that Ca^{2+} causes the contacts with CaM to pivot between EF-1 in the N-lobe and EF-4 in the C-lobe. In addition, Ca^{2+} makes CaM binding to the channel more difficult, and the channel makes CaM binding to Ca^{2+} more difficult.

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Free Ca^{2+} Initiates and Regulates Malaria Parasite Egress Program in Infected Erythrocytes
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Malaria parasite egress from infected erythrocytes finalizes the asexual cycle of this organism and leading to parasite dissemination and disease progression. The egress mechanism is not fully understood, but several tightly coordinated steps and pathways were described recently. Mature parasites breach two sets of membranes to escape an infected host cell. We investigated the role of free calcium in the initiation and control of individual steps in the egress program of Plasmodium falciparum. The entire egress pathway (tens of minutes before release) is independent of extracellular free calcium, using rather intracellular calcium accumulated within the parasite during the trophozoite-schizont stages of parasite development. Based on experiments with calcium chelators and ionophore, inhibitors of the parasite endoplasmic reticulum, fluorescence microscopy and morphological analysis of mature parasites we propose that the schizont endoplasmic reticulum is the calcium source for egress initiation and that several post-initiation steps in the egress program are affected by increased intracellular Ca^{2+}. We also reveal a new calcium-dependent step in the parasite egress mechanism: swelling of the parasitophorous vacuole (PV). This major event in parasite egress leads to the rupture of critically swelled vacuoles and mature parasite extrusion from the host cell. We found that this vacuolar swelling is exaggeratedly slow in dehydrated sickle cells, leading to a severe defect in parasite egress, probably due to low levels of ions conducting osmotic driven water translocation from erythrocyte cytoplasm to PV. Parasite egress can be accelerated in mature schizonts by pharmacological intervention; the mechanism of this phenomenon involves PV swelling. However, the same treatment of immature schizonts leads to immediate parasite death within the host cell due to erythrocyte hemolysis of a critically expanded PV. Exploring this stage-dependent parasite killing may be useful for development of antimalarials.

Platform: Membrane Protein Structure & Function I

210-Plat
The Role of Internal Water in GPCR Complexes
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Many protein complexes possess a substantial number of internal waters some observed as bridging between polar groups and other filling internal voids. The thermodynamics of these waters and their effect on the stability and dynamics of the protein complexes are not well understood. Using MD and MC simulations and an enhanced inhomogenous fluid solvation theory in canonical and grand canonical ensembles we obtain the free energy of the internal waters and their contribution to the stability and dynamics of protein complexes. Recent x-ray structures allow us to focus on the Adenosine-2A GPCRs in complex with agonists and antagonists in an attempt to characterize the properties of waters in the active and inactive states. Results from MC Grand Canonical simulations indicate that the number of waters in the active state is smaller than in the inactive form and their occupancies are reduced on the average by 0.3 waters. The free energies of the waters are also less negative by approximately 3 kcal/mol on the average. To assess the role of this change on the dynamics of the GPCRs, we will present results from MD simulation of the complexes emphasizing the difference in receptor dynamics and in the free energies of the waters between the different states.

211-Plat
Model-Free Spectral Density Mapping Applied to Dynamics of Rhodopsin in Solid-State NMR Spectroscopy
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Crystal structures of rhodopsin are available, yet details of the activation mechanism remain unknown [1,2]. We applied solid-state ^2H NMR to investigate structural and dynamical changes occurring in the process of rhodopsin activation. From the ^2H NMR spectra, molecular mobility can be obtained by calculating the segmental order parameters from the residual quadrupolar couplings (RQCs). Moreover ^2H nuclear spin relaxation rates related to the dynamics can be measured together with the RQCs [2]. Site-specific ^2H labels were introduced into different methyl groups of retinal, and relaxation rate measurements were performed as a function of temperature (-30 to -150°C) [3]. Model-free analysis employed an irreducible representation of the combined ^2H NMR line shape and relaxation data. Fluctuations of the irreducible components with respect to the average values are characterized by the individual spectral densities of motion evaluated at characteristic frequencies: $J_0(0)$, $J_1(\omega_0)$, and $J_2(2\omega_0)$, where ω_0 is the nuclear resonance frequency [4]. Differences in the spectral densities manifest details of the methyl group motions within the retinal binding pocket at low temperature. At the high temperature limit, $J_1(\omega_0)$ and $J_2(2\omega_0)$ are insensitive to details of motion and collapse to a universal curve, thus substantiating the validity of the model-free analysis. Further analysis of