2689-Plat Role of Luminal Ca and Calsequestrin in Controlling Local Ca Release in Cardiac Myocytes

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Recently, we showed that ectopic expression of certain variants of cardiac CASQ2 linked to sudden cardiac death exert either gain of function or loss of function effects on intracellular Ca release by either disrupting CASQ2 polymerization required for high capacity Ca binding or impairing CASQ2 interaction with the RyR channel complex. To gain further insights in the role of CASQ2 in SR Ca signaling, we compared the effects of expression of two such mutants, $CASQ2^{G112+5X}$ and $CASQ2^{R33Q}$, on local cytosolic Ca release events (sparks) and associated SR depletion signals ("blinks") measured simultaneously in rat ventricular myocytes. Overexpression of $CASQ2^{WT}$ increased both the amount of Ca released during a Ca spark and the time required for refilling of individual SR cisternae following local Ca release as compared to control, indicative of enhanced Ca buffering within this structure. At the same time, disrupting CASQ2 polymerization by expressing $CASQ2^{G112+5X}$ resulted in reduced local Ca release and accelerated SR refilling with Ca, consistent with reduced Ca buffering inside SR. However, despite profound differences in SR Ca buffering strengths, local Ca release terminated at the same free luminal [Ca] in the control, $CASQ2^{WT}$ and $CASQ2^{G112+5X}$ groups, suggesting that a decline in [Ca]SR is a signal for RyR2 closure. Importantly, by expressing $CASQ2^{R33Q}$ that impairs interaction of CASQ2 with the RyR2 complex, we were able to alter (lower) the [Ca]SR threshold for Ca release termination. We conclude that CASQ2 aggregated in the SR determines the magnitude and duration of Ca release from each SR cisternae by providing a local source of releasable Ca and by mediating the effects of luminal Ca on the RyR2 channels.

2690-Plat Loss Of Cardiac Triadin Decreases Junctional SR Of Cardiac Muscle And Increases Free Calcium In The SR Lumen

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Triadin binds both to the cardiac ryanodine receptor Ca release channel (RyR2) and to calsequestrin, forming a quaternary complex that also includes junctin. It has been postulated that triadin serves a dual role as anchor for calsequestrin in the junctional SR (jSR), and as a regulator of RyR2 channels. We previously reported that deletion of triadin causes a dramatic reduction of triadin's partner proteins RyR2 (−50%), calsequestrin (−60%) and junctin (−90%) in triadin knock-out mice (KO). Paradoxically, SR Ca content of triadin KO cardiomyocytes is significantly increased, and SR Ca fractional release is significantly reduced. Here we used electron microscopy and measurements of free [Ca] in the SR lumen using MagFura2AM to further probe the role of triadin in cardiac muscle. We find that the overall SR surface area and SR volume of triadin KO hearts are unchanged relative to wild type (WT) hearts (SR vol/vol [%] KO 1.55±0.65 vs WT 1.42±0.58). However, the extent to which the jSR forms feet-bearing junctions with the T tubule is reduced by 50% (jSR extent [A.U.] KO 42±13 vs WT 96±27, $p<0.05$). The decrease in feet bearing junctions is strikingly similar to the reduction in RyR2 protein of triadin KO hearts. Interestingly, free $[Ca]_{SR}$ is significantly increased in KO cardiomyocytes (MagFura2 ratio KO 0.085±0.030 vs 0.062±0.039, n=18 each, $p<0.05$). These data demonstrate that triadin is an important regulator of cardiac Ca release unit structure and function. It remains to be determined whether direct regulation of RyR2 channels by triadin, or the alterations in jSR structure are responsible for the increased luminal $[Ca]_{SR}$.

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Platform BH: Membrane Structure, Rafts, Domains and Phase Separation

2691-Plat Neutron Diffraction Studies of Lipopolysaccharide Bilayers

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Lipopolysaccharides (LPS) are a major class of macromolecules populating the surface of Gram-negative bacteria. They contribute significantly to the bacterium's surface properties and play a crucial role in regulating the permeability of its outer membrane. We report on neutron diffraction studies performed on aligned, self-assembled bilayers of LPS isolated from *Pseudomonas aeruginosa* PAO1. This LPS system comprises of a mixture of rough and smooth A-band and B-band LPS, similar to that naturally found in *P. aeruginosa*. From the construction of one-dimensional scattering length density profiles, we found that water penetrates into the hydrocarbon region up to and including the center of liquid crystalline Na^+-LPS bilayers. This permeability to water also extends to bilayers in the continuous phase transition region and could have far-reaching implications as to how small molecules penetrate the outer membrane of Gram-negative bacteria. Most recently, we have studied the permeability of LPS bilayers to water in the presence of Mg^{2+} and Ca^{2+} cations. We found that, compared to Na^+ and Mg^{2+}, Ca^{2+} causes the bilayers to be less permeable to water.

2692-Plat Membrane Shape Fluctuations and Hydration Forces Observed by Solid-State ^{2}H NMR Spectroscopy

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Membrane shape fluctuations arise under physiological conditions and the determination of the modes of deformation provides insight into the role of shape in biomolecular function. The ^{2}H NMR experimental study consists of measuring acyl-chain order parameters S_{CD} and relaxation rates R_{1Z} of randomly oriented DMPC dispersions at various hydration levels in the liquid-crystalline state. Following spectral de-Paking and subsequent data analysis we gain insight into the structure and dynamics of multilamellar lipid membranes [1]. Both S_{CD} and R_{1Z} values are sensitive to hydration, indicating that inter-membrane separation (and implicitly contacts) affect membrane deformations at all length scales. A square-law functional relationship between R_{1Z} and S_{CD} suggests that a highly coupled distribution of fluctuations within a 3-D multilamellar sample exists. In this instance the measured change in slope of R_{1Z} versus ${S_{CD}}^2$ reflects the constraints on molecular motions and membrane shape imposed by hydration. Similarly, small-angle X-ray scattering detects a reduction in inter-bilayer spacing corresponding to a decrease of fluctuation amplitude and a smaller lipid cross-sectional area [2]. In a continuum approximation for collective motions of the lipid molecules [3] the change of membrane shape and frequency of molecular motions is expressed as a change in material properties of the membrane. The alteration of local bilayer rigidity and dependence of the inter-membrane coupling on continuum fluctuations as observed in this study parallels conclusions made for membranes in the presence of cholesterol [4].

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2693-Plat Ternary Mixture of Sphingomyelin, POPC, and Cholesterol - Lateral Fluctuations at Higher Temperatures

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Ternary mixtures of sphingomyelin, unsaturated phosphaditylcholines, and cholesterol represent a model system for the outer leaflet of eukaryotic cell membranes, characterized by the formation of lateral lipid domains, called "rafts".

While domain formation in lipid membranes at low temperature has been well established it remains open whether such inhomogeneities also exist at higher, physiologically more relevant temperatures, where no phase separation can be detected by optical methods. For the understanding of "rafts" in biological membranes it is also important to determine the properties of each phospholipid in the ternary mixture at such temperatures. Further, it is unclear how the distribution of cholesterol in the ternary mixture changes upon temperature variations.

In this study, the ternary mixture of palmitoyl-sphingomyelin (PSM), palmitoyloleoyl-phosphatidylcholine (POPC), and cholesterol was studied. Upon decrease in temperature, a redistribution of cholesterol into large liquid-ordered PSM/cholesterol domains and depletion of the sterol from liquid-disordered POPC domains was observed by ^{2}H NMR and confocal fluorescence microscopy. However, there is no complete segregation of cholesterol into the liquid-ordered phase and also POPC rich domains contain the sterol in the phase coexistence region.

We further compared order parameters and packing properties of deuterated PSM or POPC in binary and raft mixtures at temperatures above the main phase transition of the two phospholipids. PSM shows significantly larger ^{2}H NMR order parameters in the ternary mixture than POPC at 313 K. This can be explained by an inhomogeneous distribution of cholesterol between the two lipids and the mutual influence of the phospholipids on each other. From the prerequisite that order parameters are identical in a completely homogeneously mixed membrane, we can determine a minimal microdomain size of 45–70 nm in PSM/POPC/cholesterol mixtures above the main phase transition of the two phospholipids.

2694-Plat Coupling of Cholesterol-Rich Lipid Phases in Asymmetric Bilayers

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We showed previously that cholesterol-rich liquid-ordered domains with lipid compositions typically found in the outer leaflet of plasma membranes could induce liquid-ordered domains in adjacent regions of asymmetric lipid bilayers with apposed leaflets composed of typical inner leaflet lipid mixtures (Biophys. J. 91:3313–26 [2006]). To further examine the nature of transbilayer couplings in asymmetric cholesterol-rich lipid bilayers, the effects on the lipid phase behavior of different combinations of asymmetric lipid bilayers were investigated. We established several systems with natural extracted and synthetic lipid combinations that exhibited coexisting liquid-ordered (l_o) and liquid-disordered (l_d) domains in a supported bilayer format. We found that l_o phase domains were induced in all quaternary inner leaflet combinations composed of PCs, PEs, PSs, and cholesterol. Ternary mixtures of PCs/PEs/Chol, PCs/PSs/Chol also exhibited l_o phases on top of outer leaflet l_o phases. However, with the exception of brainPC extracts, binary PC/Chol mixtures were not induced to form l_o phases by adjacent outer leaflet l_o phases. Higher melting lipid adducts of PEs and PSs are needed for l_o phase induction in the inner leaflet. It appears that the phase behavior of the inner leaflet mixtures is dominated by their intrinsic melting temperatures, rather than by their specific head-

group class. In addition, similar studies with synthetic, completely saturated lipids plus cholesterol show that lipid oxidation is probably not a factor in the observed behavior.

2695-Plat Cholesterol Dependent Phosphoinositide Domain Formation

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Phosphoinositides act as signaling molecules by recruiting critical effectors to specific subcellular membranes to regulate cell proliferation, apoptosis and cytoskeletal reorganization, which requires a tight regulation of phosphoinositide generation and turnover as well as a high degree of compartmentalization. In order to investigate the processes that lead to the formation of phosphoinositide-enriched microdomains under different physicochemical conditions, domain formation in fluorescently labeled giant unilamellar vesicles (GUVs) composed of either phosphatidylcholine(PC)/phosphatidylinositol-4,5-biphosphate(PI(4,5)P_2) or PC/PI(4,5)P_2/cholesterol were studied by fluorescence microscopy. GUVs were developed in different salt concentrations (no salt, 50 mM and 100 mM NaCl) by using the slow hydration method. For binary PC/PI(4,5)P_2 (80:20) mixtures, the formation of macroscopic domains was observed only after cooling of the mixture to 10 °C. In contrast, in the presence of cholesterol (PC/PI(4,5)P_2/cholesterol 60:20:20) macroscopic domain formation was observed even at room temperature (20 °C, pH 7.0 buffer with 50mM NaCl or 100mM NaCl). Most importantly, this domain formation was accompanied with a pronounced curvature change of the PI(4,5)P_2 rich domain, leading to a "bulging" of this part of the vesicles. The ionic strength of the solution is an important factor for the observed domain formation and the observed microdomains are presumably stabilized by hydroxyl/hydroxyl as well as hydroxyl/phosphodiester interactions.

2696-Plat Macroscopic Domain Formation In The Platelet Plasma Membrane: An Issue Of Low Cholesterol Content

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There has been ample debate on whether cell membranes can present macroscopic lipid domains as predicted by three-component phase diagrams obtained by fluorescence microscopy. Several groups have argued that membrane proteins and interactions with the cytoskeleton inhibit the formation of large domains. In contrast, some polarizable cells do show large regions with qualitative differences in lipid fluidity. It is important to ask more precisely, based on the current phase diagrams, under what conditions would large domains be expected to form in cells. In this work we study the thermotropic phase behavior of the platelet plasma membrane by FTIR, and compare it to a POPC/Sphingomyelin/Cholesterol model representing the outer leaflet composition. We find that this model closely reflects the platelet phase behavior. Previous work has shown that the platelet plasma membrane presents inhomogeneous distribution of DiI18:0 at 24°C, but not at 37°C, which suggests the formation of macroscopic lipid domains at low temperatures. We show by fluorescence microscopy, and by comparison with published phase diagrams, that the outer leaflet model system enters the macroscopic domain region only at the lower temperature. In addition, the low cholesterol content in platelets (~15 mol%), appears to be crucial for large domain formation during cooling.

2697-Plat Preparation and Properties of Asymmetric Model Menbrane Vesicles That Mimic Eukaryotic Cell Membrane: Implications for Lipid Raft Formation

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Eukaryotic cell membranes contain asymmetric lipid bilayers with different lipid compositions in the outer and inner leaflet. To aid studies of the structure and function of eukaryotic membranes and their asymmetry a procedure to prepare asymmetric model membrane vesicles was developed. Small unilamellar vesicles with stable asymmetric lipid compositions in the inner and outer leaflets were created by methyl-β-cyclodextrin (mβCD) induced lipid exchange, and the asymmetry was confirmed by novel spectroscopic assays. Asymmetric vesicles could be prepared with sphingomyelin (SM) as the predominant lipid in the outer leaflet, and unsaturated phospholipids such as dioleoylphosphatidylcholine (DOPC), 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC), and 1-palmitoyl-2-oleoyl phosphatidylserine (POPS) by itself or mixed with 1-palmitoyl-2-oleoyl phosphatidylethanolamine (POPE) in the inner leaflet. Transmembrane helices, and a high concentration of cholesterol could be incorporated into these vesicles with maintenance of asymmetry. Analysis of the physical properties of asymmetric vesicles composed of SM outside, DOPC inside (SMo/DOPCi) or SM outside, 2:1 POPE:POPS inside (SMo/2:1 POPE-POPSi) demonstrated that the outer leaflet SM could form an ordered state while the inner leaflet lipids remained in a disordered state. The thermal stability of the ordered state in SMo vesicels was similar to that in pure SM vesicles, and higher than that of symmetric vesicles with the same lipid composition as the asymmetric vesicles. This was also true when cholesterol was introduced into the vesicles. The physical state of the inner leaflet in asymmetric vesicles appeared to be more ordered than in vesicles composed of just inner leaflet lipids. This suggests that the outer leaflet physical state can alter that of the inner leaflet. The availability of lipid vesicles with controlled asymmetry should provide a more useful model membrane system for comparison to real biological membranes.

2698-Plat Different Lipid Anchors Recruit Proteins into Distinct Membrane Domains: A FCCS Study on Activated T Cells

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While the heterogeneity of the plasma membrane of eukaryotic cells is by now a well-established fact, the precise architecture of this important cellular structure is still seriously debated. Here, we focus on the role of specific lipid anchor motifs in the organization of T-cell plasma membranes into distinct domains of particular composition. To that end we generated a combinatorial library of protein constructs by fusing different lipid-modification sites of lipid anchored proteins with one of two fluorescent proteins. Two of these constructs that encode for either myrestilation, palmytilation, geranylation or glycosylphosphatidylinositol (GPI) elaboration and are labeled with either enhanced green fluorescent protein (EGFP) or monomeric red fluorescence protein 1 (mRFP1) were co-expressed in each cell. We used dual color fluorescence cross-correlation spectroscopy (FCCS) to exploit co-movement of the same or different lipid anchors as a signature of spatial cluster formation, thereby circumventing the limitations of direct imaging of nanometer sized membrane structures. Our FCCS studies on activated T-cell membrane sheets and membranes of whole T-cells show that most anchors only co-localize with themselves, while different anchors move independently from each other. These findings not only suggest that the plasma membrane is composed of a variety of different domains (each with specific protein content), but also that the lipid anchor structure plays a key role in the specific recruitment of proteins into their target domains.

Protein Folding Stability - I

2699-Pos An All-Atom Structure-Based Model for Protein Folding: Interplay Between Geometry and Energetics

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Board B1

The funneled energy landscape is dominated by a protein's native state and gives a theoretical foundation for minimally frustrated structure-based models. For simulation, the protein is typically reduced to a one bead per residue representation. These simulations reproduce a folding mechanism in agreement with experiments. In order to get a more detailed description of a protein, one can add layers of complexity. One possibility is including all atoms of a protein in structure-based simulations to better capture the process of side-chain packing. We introduce an all-atom, structure-based model to explore the interplay between geometry and energy during folding. The model is applied to several proteins. It is shown that the folding mechanism stays stable over a wide range of parameters, while the energetic properties like folding temperature and folding barrier vary. Further, several definitions for the native contacts are evaluated and a simple contact map definition is proposed.

2700-Pos Fold Specific Knowledge-based Potential Functions for Protein Structure Prediction

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Board B2

In protein structure prediction, potential functions are used to guide the conformational search or to select the native structure from a set of candidate structures. Therefore, an accurate and efficient potential function is crucial in structure prediction. Quasi-chemical knowledge-based potential functions derived from known protein structure databases have been proven to be quite effective in structure selection. However, it has also been showed that knowledge-based potential functions might be training database dependent due to some unrealistic assumptions, such as the use of sequence independent reference state. In this study, we exploit the dependence of knowledge-based potentials on training databases. A set of *fold-specific* knowledge-based potential functions instead of a universal one is derived. We trained these potential functions at the fold level of SCOP classification and found that such fold-specific knowledge-based potentials are more sensitive and can discriminate almost all native structures from the decoys in both 4State reduced and LMDS decoy sets if the fold can be correctly predicted in advance. Even with a much simpler function than some sophisticated potential functions, the performance of our fold-specific potential functions is quite competitive. The results thus not only verify the dependence of the quasi-chemical knowledge-based potentials on the training databases, but also demonstrate that the sensitivity of the quasi-chemical knowledge-based potentials can be improved by applying a proper fold-specific potential, if the fold of the protein to be predicted is known. The conditional clause can be dropped by incorporating with a reliable fold prediction server, in which case the applicability of these fold-specific knowledge-based potential functions can be extended even to those proteins with unknown folds.

2701-Pos Calculations of Protein Thermodynamics via Distance Constraints: Refined Account of Hydration Effects

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