Striking structural variations have been reported for the receptors in different detergents and for the receptors prepared from native and sf9 cells by the same research groups, suggesting that biochemical preparations of the receptors might have had significant variations and that the heterogeneity in the samples could be a limiting factor in reaching accordant results. To resolve such discrepancies, we developed a three- layered strategy to enhance biochemical homogeneity and structural agreements. We collected two cryoEM datasets of the receptors in the absence and presence of IP3 and Ca^{2+} and calculated two separate reconstructions. The two structures not only agree with each other in many aspects, but also reveal a conformational change at the top of the cytosolic domain that may lead to some reorganization of the channel pore. In order to verify the structural details and solidify the conformational changes in the pore domain, we need higher resolution structures. We are using the high-end facilities with direct electron detectors to collect near-atomic resolution data in order to further improve the resolutions of our 3D reconstructions.

1714-Plat
Multiple Closed States of the Ryanodine Receptor Determined by CRYOEM
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Ryanodine receptors (RyRs) are intracellular ion channels involved in Ca^{2+} release from internal stores in excitable cells. These channels are the largest channels known and are homotetramers, sizing ~2,26 MDa. The 3D structure of RyR1 in it open and close states was determined previously, revealing that the ion gate opening mechanism rely on long-range conformational changes over 100 Å. The RyR gating properties are highly regulated by Ca^{2+}, Mg^{2+}, ATP, and FKBP12. The native conformation of RyR1 in presence of physiological concentrations of Mg^{2+} and ATP is unknown. Here we determine the 3D structure of RyR1 in non-activating conditions (submicromolar Ca^{2+}) in the presence of Mg^{2+} and an ATP analog, but in a flexible conformation by absence of FKPBP12. This new structure was determined using cryoEM and image processing.
The resulting 3D structure is in the closed conformation when compared to 3D reconstructions of RyR1 in open and closed conditions in presence of FKBP12 determined previously. In addition, from the comparison among several 3D reconstructions, we establish new conformation-function correlations. We find that the rhomboid structures formed by domains 2-4-5-6 situated far away from the ion gate move as a whole during gating, and define a ''flexion angle'' that appears to be correlated with the degree of opening of the channel, whereby the flexion angle after adding Mg^{2+} and ATP shifts by 3 degrees towards the closed state. In conclusion this research suggests that physiological concentrations of Mg^{2+} and ATP shift the RyR1 conformation toward the closed conformation and also suggests that the closed conformation encompasses sub-states.

1715-Plat
Crystal Structures of the Ryanodine Receptor SPRY2 Domain
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The SPRY2 domain is one of three repeats of the same fold that are present within the RyR. It has been suggested as a key protein interaction site with dihydropyridine receptors to mediate excitation-contraction coupling in skeletal muscle tissue. RyR1 and RyR2 SPRY2 domains were crystallized and reveal differences with what was thought to be SPRY2 and with several other known SPRY domain structures. Our RyR1 SPRY2 construct (rabbit, 1070-1246) is 43% larger than a previously reported construct (1085-1208), consists of multiple modules, is highly soluble, and is monomeric in nature. Docking of the RyR1 SPRY2 structure places it in between the central rim and the clamp region. The structure of RyR2 SPRY2 (mouse, 1080-1253) and a loss-of-function disease mutant (human, T1107M) causing cardiomyopathy were also determined. The T1107M mutation is buried and causes a large ~10°C decrease in thermal stability as compared to wildtype and also causes local misfolding of the domain. Finally, RyR1 SPRY2 binding to the DHPR II-III loop is undetectable by isothermal titration calorimetry.

Platform: Membrane Structure

1716-Plat
Interaction of HIV-1 Gag Protein's Ma Membrane Binding Domain with Membrane Mimics Probed by Low- and Wide-Angle X-Ray Scattering
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The structural Gag protein from the HIV-1 virus is required for assembling new virus particles within an infected T-cell; Gag's binding to the inner leaflet of the host plasma membrane is the first step in this process. In an effort to understand the molecular and energetic details of this binding, we studied the N-terminal 31 amino acids of Gag's MA membrane binding domain with lipid membrane mimics: PC, PC:PE, PC:PS, PC:PI, PC:PIP, PC:PIP_2, PC:PE:PI, PC:PE:PIP, PC:PE:PIP2, PC:PE:PIP2:cholesterol and PC:PE:PS:cholesterol in various molar ratios. Oriented, fully hydrated lipid mimics with/without the myristoylated (MA_{myr}) and non-myristolyated (MA) peptide were X-rayed at the Cornell High Energy Synchrotron Source. We found that MA_{myr} lowered K_C (softened) pure POPC membranes more than did MA; in general, both peptides lowered K_C for all 18 mimics. MA_{myr} increased slightly S_{xray} (chain order) of PS-containing membrane mimics, but both peptides decreased S_{xray} slightly with increasing concentrations of PI, PIP or PIP_2 when mixed with POPC. When POPC:PEPE was mixed with PI, PIP or PIP_2 (5:3:2), PI decreased, PIP had no effect and PIP_2 increased chain order with either peptide compared to controls. The head-to-head spacing (D_{HH}) was decreased by both peptides (1-3 Å) in most mimics. In pure POPC, or when PI, PIP or PIP_2 was mixed with POPC, modeling suggested a penetration of MA_{myr} into the hydrocarbon region, compared to an interfacial headgroup position for MA. Therefore the effect of MA peptides on membrane structure and properties depends on the composition of the lipid mimics as well as on the myristoyl group.
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1717-Plat
Influence of Domain Size on Structure and Elastic Fluctuations in Complex Lipid Mixtures
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Lipid-only domains are well-established mimetic systems for membrane rafts enabling the study of their physical properties under strictly controlled conditions. Of particular interest are four component lipid mixtures entailing the variation of lipid domain size from micron regime down to a few nanometers [1]. Applying our recently developed small-angle x-ray scattering data analysis technique, we have studied changes of membrane thickness, lateral lipid packing and bending fluctuations for coexisting liquid-ordered (Lo) and liquid-disordered (Ld) phases in DOPC/POPC/DSPC/cholesterol mixtures along this domain-size trajectory, including the melting of Lo domains as a function of temperature. Bending fluctuations for coexisting Lo domains were found to be significantly lower than for single Lo phases at the boundary of the Lo+Ld regime. In turn, little variation was observed when domains exceeded sizes of 160 nm. Further, we found that the melting of Lo domains as a function of temperature is controlled by thickness differences between Lo and Ld and the associated domain line tension.
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[1] F.A. Heberle et al., J. Am. Chem. Soc. 135 (18), 6853-6859 (2013)

1718-Plat
Phase Coexistence in Lipid Membranes Induced by Buffering Agents and Charged Lipid Headgroups
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Recent literature has shown that buffers affect the interaction between lipid bilayers through a mechanism that involves van der Waals forces, electrostatics, hydration forces and membrane bending rigidity. We endeavour to show phase coexistence as an effect of charges from the aqueous boundary layer on the mixed chain 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) lipid bilayers. We will present data that suggests one phase dehydrates below the value in pure water while the other phase swells as the concentration of buffer is increased. However, since the two phases must be in osmotic equilibrium with one another, this behavior challenges theoretical models of lipid interactions and introduces new variables to consider for the Gibbs phase rule. This model of lipid charging was then applied to explain the mechanisms behind phase separation in lipid mixtures containing phosphatidylinositols.
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