Computational Lipidomics and the Lipid Organization of Cell Envelopes

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Analysis of PI(4,5)P2 Lateral Organization at the Plasma Membrane of Living Cells Through FRET

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Phosphatidylinositol (4,5)-bisphosphate (PI(4,5)P2) is an important component of the inner leaflet of the plasma membrane of eukaryotic cells. Despite the fact that it only comprises approximately 1 mol% of the total membrane phospholipids, this phosphoinosite has been associated with many different cells functions including membrane trafficking, actin cytoskeleton remodeling and cell motility, among others. The physiological functions of this lipid seem to depend on localized concentration fluctuations within the plasma membrane. In fact, the distribution of this lipid in the plasma membrane has been proposed to be heterogeneous, and PI(4,5)P2 clustering is detected on model membranes under specific conditions. Domains highly enriched in PI(4,5)P2 were also reported at the plasma membrane of specific cell types. However, for most cellular models, scarce evidence has been found for PI(4,5)P2 segregation/clustering in the plasma membrane.

In this context, our main goal was to study the distribution of PI(4,5)P2 molecules in cells lines where no heterogeneity in PI(4,5)P2 lateral distribution had been previously observed. The distribution of PI(4,5)P2 was assessed from FRET microscopy measurements with pleckstrin homology (PH) domains tagged with different fluorescent proteins. We applied a FRET methodology capable of discriminating between FRET from aggregates/clusters from non-interacting molecules within the plasma membrane of living cells. Our results clearly show distinct PI(4,5)P2 local densities in different cellular models, suggesting different patterns of PI(4,5)P2 lateral distributions within the plasma membrane. In addition, the effect of cholesterol removal on PI(4,5)P2 lateral organization is significantly different in distinct cell lines, suggesting that the role of cholesterol in the formation of PI(4,5)P2 enriched domains varies considerably.

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Impact of PI(3,4,5)P3-Mediated Beta-Arrestin-1 Recruitment on Structure of Asymmetric Lipid Bilayers

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The membrane spanning G-protein coupled receptors (GPCRs) facilitate crucial physiological responses to a variety of extracellular ligands, such as hormones, neurotransmitters, ions, photons, and other stimuli [1], thus 50% of drugs available in the market today target GPCRs [2]. The biological mechanisms behind GPCR-signaling involves several other biomolecules, including the key protein group of β-arrestins. These cytosolic adaptor proteins regulate signaling through several different pathways, classically by removing receptors from the plasma membrane via clathrin-mediated endocytosis [3].

In membrane binding experiments using both confocal fluorescence microscopy and quartz crystal microbalance, we found that β-arrestin-1 binds specifically to supported lipid bilayers (SLBs) containing phosphatidylinositol trisphosphate lipids, PI(3,4,5)P3. We have consistently shown, using both methods, that such binding is lipid specific and not driven by membrane charge. We have also shown that the membrane-protein interaction depends on protein concentration and membrane composition, and that β-arrestins can induce membrane curvature, which may play a role in the endocytic pathway. This is for the first time quantified with respect to dynamics and respective contributions of specific vs non-specific binding.

Having established an assay for PI(3,4,5)P3-mediated recruitment of β-arrestin-1, we conducted a series of neutron reflection experiments to study the protein-lipid interaction on a structural level. Both hydrogenated and deuterated protein was used in reflectometry experiments with diverse contrast matching of the bulk media. We present here the detailed structures of asymmetric lipid bilayers containing PI(3,4,5)P3, and demonstrate that β-arrestin-1 not only binds but also reorganizes the membrane structure.

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Computational Lipidomics and the Lipid Organization of Cell Envelopes

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The detailed lipid organization of cellular membranes remains elusive. A typical plasma membrane contains hundreds of different lipid species that are actively regulated by the cell. Currently over 30,000 biologically relevant lipids have been identified and specific organisms often synthesize thousands of different lipid types. This is far greater diversity than is needed to maintain bilayer barrier properties and to solvate membrane proteins. Why organisms go through the costly progress of creating and maintaining such a large diversity of lipids is one of the big open questions in biology. What is the individual role of these lipids, and how do they interact and organize in the membrane plane?

To start to address these questions we optimized and developed the Martini coarse-grained force field lipidome. We systematically explore over a hundred different lipid types using the Martini model. Bulk properties of each individual lipid type (e.g. bilayer thickness, area per lipid, diffusion, order parameter and area compressibility) were analyzed and overall trends compared to experimental values. Using pre-existing Martini lipids and the newly characterized ones, idealized plasma membranes containing dozens of different lipid types asymmetrically distributed between the membrane leaflets were constructed and simulated on the multi microsecond time scale. In terms of lipid composition these are by an order of magnitude the most complex simulations to date. These large-scale simulations provide a high-resolution view on the lipid organization of plasma membranes at an unprecedented level of complexity and allow us to analyze a variety of plasma membrane physicochemical properties, including: lipid-lipid interactions, bilayer bulk material properties, domain formation and coupling between the bilayer leaflets. Overall, the plasma membranes show global non-ideal mixing of different lipid species at different spatiotemporal scales.

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Phase Behavior of Synaptosomal Membranes: The Effect of Lipid Composition and Temperature

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The phase behavior of synaptosomal membranes isolated from mammalian rat and marine invertebrate squid intact nerve endings was compared. Homogeneous liquid disordered (Lα) phase was observed at their body temperatures of 37 and ~20°C for the rat and squid, respectively, using fluorescence microscopy and ^{1}H-MAS NMR. Temperature decrease resulted in co-existence of an ordered phase with onset temperatures of 24°C for rat and 8°C for squid. Comprehensive analyses of the lipid composition by LC/MS explained the significant difference in the onset temperature: >2x higher content of 18:0 and 18:1 lipid chains in rat, which contributes to a higher transition temperature. The amount of ω-3 chains was ~3x higher in squid than in rat due to a difference in the amounts of 22:6 and 20:5: squid membranes contained 2x more total 22:6 in major phospholipids (PC, PE, and PS) and cardiolipin (17:0,22:6,22:6,22:6-species). While prominent in rat, 20:4 was not found in squid. It is known for Lα phase PC that 16:0,22:6 is more ordered and thicker with a smaller area/lipid than 16:0,20:4. The high content of 22:6 in squid may contribute to order in the Lα phase at the body temperature (~20°C) in contrast to the high content of 20:4 in rat at a higher body temperature (37°C). Fluorescence microscopy data showing the temperature-dependent phase behavior using giant unilamellar vesicles prepared with lipid compositions based on the synaptosomal measurements above will be discussed. Synaptosomal preparations of squid with body temperatures differing by ~9°C will be compared.

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Morphology Induced Receptor Trapping in Artificial Dendritic Spines

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Memory and learning are believed to be regulated by the strength of the connections in the synapses of the 100 billion neurons in the human brain. In the synapse the signal is transmitted between the presynaptic axon and the dendritic spine by neurotransmitters. The number of receptors in the membrane of the dendritic spine defines the strength of a synapse. The persistent increased local concentration of receptors however, is contradicted by the finding of high receptor mobility within the synapse, dependent on spine morphology. The