enhance the intrinsic ability of membranes to promote lateral organization of lipidated proteins in highly curved areas.

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93-Plat

Molecular Simulations of the Membrane-Associated State of the PTEN Tumour-Suppressor Protein

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PTEN is a tumour-suppressor protein responsible for regulating the phosphatidylinositol 3-kinase (PI3K) signalling pathway. The action of PI3K generates PI(3,4,5)P3, which goes on to promote several downstream processes such as cell proliferation and survival through its ability to recruit protein kinase B or Akt (PKB/Akt) and other proteins containing pleckstrin homology (PH) domains to the cytoplasmic leaflet of the plasma membrane. Negative regulation of PI3K signalling by PTEN is achieved through degradation of PI(3,4,5)P3 to PI(4,5)P2 by hydrolysis of the D3 phosphate, thereby controlling the plasma membrane concentration of PI(3,4,5)P3. However, inactivating mutations of PTEN that impair its lipid phosphatase activity can result in accumulation of PI(3,4,5)P3 and lead to uncontrolled downstream PI3K signalling and subsequent tumourigenesis. PTEN possesses a two-domain architecture, with a phosphatase domain responsible for PI(3,4,5)P3 hydrolysis and a C2 domain. In an effort to better understand the nature of the membrane-associated state of PTEN, we used molecular modelling to generate a model of the protein-membrane complex based on the crystal structure of cytosolic PTEN (PDB 1D5R) and explored its interactions with PI(3,4,5)P3 and the surrounding membrane lipids using molecular dynamics simulations at both coarse-grained and atomistic resolution. The predicted location of the protein/membrane interface was robust to changes in both resolution of the simulations and in bilayer lipid composition. We identified several key membrane-interacting amino acid residues lining this interface, some of which correspond to the locations of established disease-causing mutations, and demonstrated that mutation of these residues in silico can reduce the degree of membrane interaction.

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Amphetamine Actions Rely on the Availability of Phosphatidylinositol-4,5-Bisphosphate

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Neuronal functions, such as excitability or endo- and exocytosis, require phosphatidylinositol-4,5-bisphosphate (PIP2) since ion channels and other proteins involved in these processes are regulated by PIP2. Monoamine transporters control neurotransmission by removing monoamines from the extracellular space. They also display channel properties, but their regulation by PIP2 has not been reported. The psychostimulant amphetamine acts on monoamine transporters to stimulate transporter-mediated currents and efflux and thereby increases the levels of extracellular monoamines. Direct or receptor-mediated activation of phospholipase-C (PLC) reduced membrane PIP2 and amphetamine-evoked currents through recombinant serotonin transporters; extracellular application of a PIP2-scavenging peptide mimicked this effect. PLC activation also diminished amphetamine-induced reverse transport without altering transmitter uptake. Inhibition of reverse transport by PLC activation was also observed in brain slices and with recombinant dopamine and noradrenaline, but not GABA transporters; rises in intracellular Ca2+ or activation of protein kinase C were not involved in these effects. These data demonstrate for the first time PIP2-dependence of reverse transport and current in monoamine transporters.

95-Plat

Nucleation of Lipid Domain by Neuroligin-1 during Oligomerization

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The neuronal membrane protein neuroligin-1 (Nlg-1) is best known for its interaction with neurexin, and its ability to trigger presynaptic differentiation in a neurexin-expressing axon. Here, we are reporting that nlg-1 over-expression in developing neurons triggers extensive morphology changes. We show that similar changes are observed in HEK-293 cells when we co-expressed nlg-1 with psd-95, a scaffolding protein which binds nlg-1 PDZ domain. Co-transfected cells exhibited long expansions resembling dendritic branches, as well as a significant increase in cell surface area. These branches could be destabilized by adding a PI3K inhibitor, or by depleting the membrane cholesterol suggesting that the formation of these branches is due to membrane lipid domain formation around nlg-1 clusters. We have exploited the photophysical properties of cerulean, a fluorescent protein, to assess environment and conformational changes in nlg-1. Fluorescence lifetime anisotropy imaging showed that neuroligin-cerulean fusion protein environment is not uniform along these branches, and that broad changes in life time anisotropy are observed upon addition of PI3K inhibitor. These measurements suggest that neuroligin tight clustering is involved in changing cell membrane phosphoinositol lipid composition enabling the recruitment of PI3K, which in turns promotes the growth and the maintenance of these expansions.

96-Plat

Molecular Mechanism of T Cell Signaling

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T cell activation begins with the formation of signaling complexes at the cell surface involving among others, the T cell antigen receptor (TCR), the Src family kinase Lck and the adaptor protein, linker for activation of T cells (LAT). We have established photo-activatable localization microscopy (PALM), stochastic optical reconstruction microscopy (STORM), dual channel PALM/direct STORM (dSTORM), live cell PALM and quantitative statistical cluster analysis to determine how TCR engagement reorganizes the T cell membrane on the molecular scale.

In quiescent T cells, LAT is already segregated into clusters on the cell surface raising the question how TCR triggering initiates signaling through these pre-existing domains. We demonstrated that pre-existing LAT domains are neither phosphorylated nor laterally transported to the TCR activation site suggesting that these clusters do not participate in TCR signaling. Instead, TCR activation results in the recruitment, and phosphorylation of new LAT clusters from sub-synaptic vesicles. Our data suggests that TCR ligation preconditions the membrane for vesicle recruitment and bulk activation of the LAT signaling network. We next demonstrated that TCR triggering re-organizes Lck into distinct signaling clusters. Lck clustering, driven by self-association was independent of the kinase activation state but TCR activation segregated Lck clusters from clusters of the phosphatase CD45. In addition, de-phosphorylation of Lck, either at Tyr 394 or Tyr 505, resulted in larger but fewer clusters. Phosphorylated TCR and Lck co-clustered at the centre of the activation area suggesting that molecular re-organization may result in unique TCR signaling domains in the plasma membrane.

In conclusion, single-molecule imaging provides insights into T cell signaling that could not have been obtained by any other means.

Symposium: Fluorescence Correlation Spectroscopy: Applications to Biophysics

97-Symp

Using FCS to Study Protein Disorder and Aggregation

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Fluorescence correlation spectroscopy (FCS) is powerful approach for studying the dynamic properties of fluorescently labeled biomolecules including diffusion, binding, and conformational dynamics. We have applied FCS to the study of membrane binding, dynamics, and aggregation of two intrinsically disordered proteins. The first, α-synuclein, is a small, neuronal proteins whose aggregation is implicated in Parkinson's disease. Its native functions are poorly understood, but are thought to involve binding to cellular membranes. Here we have measured the free energy of binding of α-Synuclein to lipid membranes using FCS. Our results show that the binding energy can be strongly altered by changes to the bilayer curvature and composition, suggesting that even modest changes to lipid components in vivo may be able to exert significant effects on α-Synuclein function. Furthermore, modifications to the C-terminus of α-Synuclein, which does not directly interact with the lipid bilayer but exerts control of the aggregation of the protein, is also able to modulate binding interactions. Measurements of intrachain dynamics indicate that modifications alter the flexibility of the C-terminus as well as the conformational ensemble populated by the protein. The second protein, tau, is a microtubule binding protein that is found in aggregated paired-helical filaments in Alzheimer's and other