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Symposium 4: Membrane Protein Structure and Interactions

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Chair: Frances Separovic

Seeing spots: miscibility transitions in lipid/cholesterol membranes

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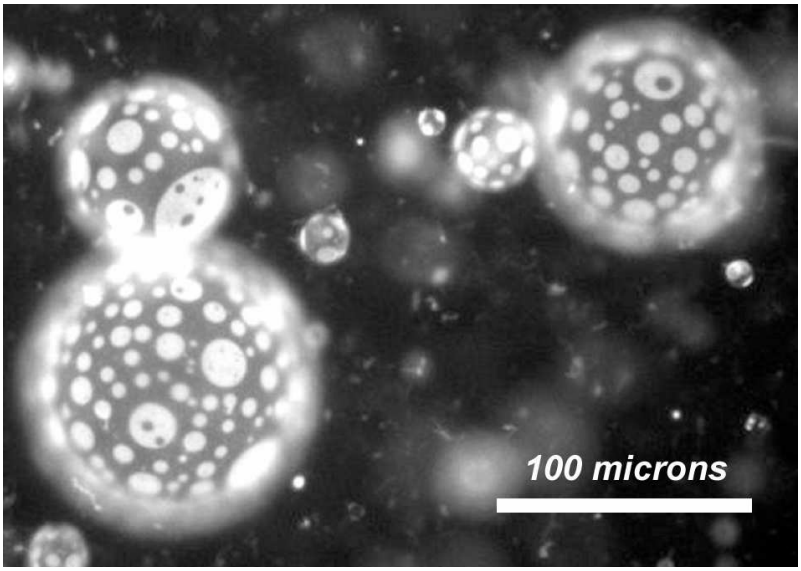
Mammalian cells are surrounded by an outer wall or "plasma membrane" of proteins and lipids arranged in opposing leaflets of a bilayer. There is growing evidence that this membrane is not uniform, but instead laterally phase separates into "raft" domains rich in particular lipids and proteins.

We study a simpler physical model of cell membranes, giant unilamellar vesicles (GUVs). Using fluorescence microscopy, we can directly observe liquid domains in free-floating vesicles containing three components: a lipid with high melting temperature (e.g. a saturated lipid), a lipid with low melting temperature (e.g. an unsaturated lipid), and a “membrane active” sterol (e.g. cholesterol). Liquid domains in vesicles exhibit interesting behavior. They collide and coalesce until only one bright domain and one dark domain remain on each vesicle. Domains also finger into stripes near the critical point, and can bulge out of or into the vesicle (Veatch & Keller, 2003, Veatch & Keller, 2005).



By recording miscibility transition temperatures for many lipid compositions, we have mapped ternary phase diagrams. Our fluorescence microscopy studies give us qualitative tie-lines across the phase diagram. These tie-lines run from a region that is rich in the unsaturated lipid to a region rich in the saturated lipid, with little change in cholesterol. Applying this statement to the figure above, the bright domains are rich in unsaturated lipid, and the dark domains are rich in the saturated lipid, and only to a lesser extent in cholesterol. Using NMR (in collaboration with Klaus Gawrisch’s laboratory at the National Institutes of Health, Bethedsa, MD, USA), we have quantitatively verified the direction of the tie-lines, and have then estimated free energies to transfer lipids between phases, which are at most a few k_BTs (Veatch *et al.*, 2004).

In other studies, we have captured domains in lipid layers on glass substrates, and found that they assume static, noncircular shapes. We have substituted different sterols for cholesterol, and found that those which are structurally similar to cholesterol produce coexisting liquid domains in vesicles, just as cholesterol does (Beattie *et al.*, 2005). Finally, we have compared the phase diagrams of bilayer systems to monolayer systems and found them very different (Stottrup *et al.*, 2005).

Beattie, M.E., Veatch, S.L., Stottrup, B.L. & Keller, S.L. (2005) *Biophysical Journal* in press.
Stottrup, B.L., Stevens, D.S. & Keller, S.L. (2005) *Biophysical Journal* 88, 269-276.
Veatch, S.L. & Keller, S.L. (2003) *Biophysical Journal* 85, 3074-3083.
Veatch, S.L. & Keller, S.L. (2005) *Biochimica et Biophysica Acta* in press.
Veatch, S.L., Polozov, I.V., Gawrisch, K. & Keller, S.L. (2004) *Biophysical Journal* 86, 2910-2922.

Role of the plasma membrane in amyloid formation and toxicity

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A number of neurodegenerative diseases are caused by the aggregation and deposition of amyloid in the nervous system. For example, the deposition of β-amyloid peptide (Aβ) is considered to be a key event in the pathogenesis of the Alzheimer's disease (AD), which is the prototypic amyloidosis. Another example is Transthyretin (TTR), a plasma protein produced in the liver and the choroid plexus that can form amyloid. TTR is the predominant component of the amyloid fibrils in familial amyloidotic polyneuropathy (FAP), a hereditary disorder characterized by systemic extracellular deposition of amyloid fibrils, mainly in the peripheral nervous system. Native TTR consists of four identical subunits that form an extensive β-sheet structure, which is prone to misfolding. So far nearly eighty mutations have been identified in TTR, most of which are amyloidogenic. It is believed that structural modifications by these mutations destabilize the native tetrameric conformation and favour its dissociation into monomeric structure, which is the building block of TTR amyloid fibrils. While the mechanism by which amyloidogenic proteins cause neurotoxicity is unclear, it is now emerging that the cytotoxicity of amyloids is a direct consequence of binding to the plasma membrane. We have therefore used surface plasmon resonance (SPR) for the study of Aβ- and TTR-membrane interactions to determine whether this binding could explain the toxic effects of Aβ and TTR seen in cell culture.

Our results show that Aβ and TTR bind to the lipids of the plasma membrane through electrostatic interactions and that the amount of binding is increased upon aggregation. We also show that the amount of Aβ and TTR binding to the plasma membrane correlates with the degree of cytotoxicity observed in cell culture. Finally, we demonstrate that binding of Aβ and TTR amyloid to the plasma membrane alters membrane fluidity, providing a possible explanation for the cytotoxic effect. Overall, the results strongly support the view that both Aβ and TTR toxicity is a direct consequence of binding to lipids in the membrane. However, there are specific differences in the factors that affect this interaction. In particular, binding of Aβ was strongly influenced by the concentration of cholesterol in the membrane but did not affect TTR binding. This presentation demonstrates the application of SPR to the study of the molecular interactions associated with AD and FAP and how this information enhances our molecular understanding of neurodegenerative diseases

Are chloride intracellular ion channel proteins (CLICs) really channels? Exploring their membrane structure

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Most proteins adopt a well-defined three-dimensional structure, however, it is increasingly recognized that some proteins can exist with at least two or more stable conformations. Recently, a class of Chloride Intracellular ion Channel proteins (CLICs) has been shown to exist in both soluble and integral membrane forms. Members of this class of ion channels have a CLIC domain of approximately 240 amino acids in length and vary widely in their cellular and sub-cellular distribution. They are associated with a variety of intracellular membranes and are involved in numerous diverse physiological processes including cell cycle regulation, bone re-absorption, tubular formation and apoptosis. However, the function of CLICs as ion channels is still controversial because of their unusual dual-environmental nature and because none of the family members show a clear identifiable transmembrane domain.

Our group has now determined the structure of the soluble conformation/s for several members of this family. Despite this knowledge and because of their auto-inserting nature, the membrane channel structure is still proving difficult to determine using traditional atomic resolution structural techniques. It is therefore necessary to establish how these ubiquitous, soluble proteins can unfold, insert into membrane bilayers and refold to form ion channels. Furthermore, the processes that control this mechanism in the cell also require clarification but may include regulation by oxidation and disulphide bond formation, as in the case of CLIC1. The CLIC family members may also be modulated by pH, alteration in lipid composition, divalent cations, phosphorylation and interaction with other proteins.

A combination of structural studies including Electron Paramagnetic Resonance (EPR) and fluorescence spectroscopy, with functional studies performed in parallel, was used to investigate the insertion of CLIC1 into the membrane bilayer. The results demonstrate a role for the conserved cysteine residue at position 24 for insertion of CLIC1 into the membrane and subsequent chloride channel activity. The transmembrane region has been confirmed as comprising residues 24-46 in the N-domain of CLIC1. EPR experiments also show that insertion is likely to involve a large conformational re-arrangement of the C-terminal domain of CLIC to allow the N-domain to span the membrane bilayer.

Structure and dynamics of cellular components using fluorescence and X-ray diffraction techniques

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The techniques of Fluorescence Recovery After Photobleaching (FRAP) and Fluorescence Correlation Spectroscopy (FCS) can be employed using the optics of a confocal microscope to examine the organization and dynamics of different components in cellular systems. For these studies the protein of interest is generated as a chimera with the green fluorescent protein (GFP) and expressed in transfected live cells. We have prepared constructs encoding GFP appended to N-terminal fragments of a series of exported malaria parasite proteins, including the major cytoadherence antigen, PfEMP1. We have used FRAP techniques to examine PfEMP1-GFP dynamics in live cells and have found that the chimera exhibits a half-time for fluorescence recovery of a few seconds indicating that it is trafficked to the host cell membrane as a protein complex. These measurements are at the limit of the accessible time domain using FRAP analysis, therefore we have explored the use of FCS as a means of monitoring the rapid motion of GFP-labelled proteins. For FCS measurements, fluctuations in fluorescence intensity are measured as molecules move in and out of a small illuminated region (volume ~0.5 fL). Analysis of the fluctuations as a function of time provides information about the diffusion of the labelled species and has enabled us to measure the diffusion coefficient diffusion of GFP in the cytoplasm of the malaria parasite.

A new Centre of Excellence in Coherent X-ray Science has been established to develop techniques for imaging cellular architecture with greatly increased resolution and to develop methods for determining the structures of membrane protein without the need for crystallisation. The methods employ a highly coherent curved beam and imaging in the far field with iterative Fourier transformation protocols to extract phase image information. Soft X-rays have wavelengths of about 1-10 nm; these wavelengths allow imaging at high spatial resolution and will be used to study the intracellular structures of *P. falciparum*-infected erythrocytes with a resolution of down to 10 nm.