Symposium: Nanoclustering of Membranes and Membrane Proteins

1796-Symp
Structure and Function of Membrane-Remodeling ESCRT-III Assemblies
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The ESCRT pathway mediates a series of important cellular membrane remodeling and fission events, including cytokinetic abscission. During these processes, ESCRT-III family proteins, including CHMP1B and IST1, form filaments that appear to constrict membranes and facilitate fission. Here, we report the first structure of ''open'' and assembled ESCRT-III proteins. Near-atomic resolution electron cryomicroscopy reveals that filaments comprise a copolymeric assembly of an open conformation inner strand and, unexpectedly, a closed conformation outer strand.

1797-Symp
In Vivo-Studies of GPCR Conformational Changes using Fluorescence-Based Assays
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Activation of and signaling by GPCRs involves conformational changes in the receptors themselves as well as downstream signaling proteins and also the rearrangement of protein complexes. We have developed a number of optical assays based on fluorescence resonance energy transfer (FRET) which allow the measurement and imaging of GPCR activation and signaling in intact cells. These assays are based on fluorescently labeled receptors and/or downstream signaling proteins and can be used in intact cells as well as in entire organisms such as Drosophila larvae. They permit the analysis of GPCR activation mechanisms, their amplitudes and kinetics, as well as an analysis of the spatio-temporal patterning of receptor activation and signaling. We have also used single particle tracking to elucidate the movements of these proteins on the cell surface and the monitoring of their interactions. Our data show that these processes are highly dynamic and that they can occur in temporally and spatially restricted patterns. This dynamic behavior begins with the high mobility of the components in the cell membrane and their dynamic and reversible interaction and ends with temporally and spatially regulated activation and signaling. Supported by grants from the Deutsche Forschungsgemeinschaft (SFB487 and SFB688), the European Research Council (Grants ''Topas'' and Fresco), the Humboldt Foundation and the European Molecular Biology Organization (EMBO).

1798-Symp
Lipid Organization of the Plasma Membrane
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The detailed organization of cellular membranes remains rather elusive. In this lecture, I provide a high-resolution view of the lipid organization of a plasma membrane (PM) that is based on large-scale molecular dynamics simulations using the Martini force field [1]. Our mammalian PM model consists of 63 different lipid species, combining 14 types of headgroups and 11 types of tails asymmetrically distributed across the two leaflets [2]. The complexity of the PM mixture gives rise to an enrichment of cholesterol in the outer leaflet and a general non-ideal lateral mixing of the different lipid species. Transient domains with liquid-ordered character form and disappear on the microsecond time scale. These domains are coupled across the two membrane leaflets. In the outer leaflet, distinct nanodomains consisting of gangliosides are observed. Additional simulations of membrane proteins embedded in the PM reveal preferential interactions with specific lipids. The in-silico data provide a key view on the lateral organization of lipids and proteins in one of life's fundamental structures, the cell membrane.
[1] S.J. Marrink, D.P. Tieleman. Perspective on the Martini model. Chem. Soc. Rev., 42:6801-6822, 2013.
[2] H.I. Ingólfsson, M.N. Melo, F.J. van Eerden, C. Arnarez, C.A. López, T.A. Wassenaar, X. Periole, A.H. De Vries, D.P. Tieleman, S.J. Marrink. Lipid organization of the plasma membrane. JACS, 2014. http://dx.doi.org/10.1021/ja507832e

1799-Symp
Differential Phosphatidylserine Recognition by the Tim Family of Immune Regulatory Receptors
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Recognition of phosphatidylserine (PS) lipids exposed on the extracellular leaflet of plasma membranes is implicated in both apoptotic cell removal and immune regulation. Using a combination of interfacial x-ray scattering, molecular dynamics simulations, and membrane binding assays, we examined how different members of the T-cell immunoglobulin and mucin-domain-containing (Tim) family of PS receptors recognize PS in the context of a lipid bilayer. Our findings demonstrate that in addition to the known Ca^{2+}-coordinated, single-PS binding pocket, the different Tim proteins have different number of weaker sites of potential ionic interactions with PS lipids, and show different levels of hydrophobic insertion. These subtle differences in PS recognition likely contribute to the differences in immunological function among the Tim proteins.

Symposium: Extremophiles: Testing the Physical Limits of Living Systems

1800-Symp
Protein Folding at Extreme Temperatures: Current Issues
Georges Feller.
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The range of temperatures compatible with life is currently estimated from −20°C, as exemplified by metabolically active bacteria between sea ice crystals, and up to 122°C in hydrothermal vents as exemplified by the archaeon *Methanopyrus kandleri*. Microbial life under these extreme environmental temperatures obviously requires a vast array of adaptations at all cellular levels.
In the context of protein folding, as soon as a polypeptide emerges from the ribosome, it is exposed to the effects of the environmental temperatures. Recent investigations have addressed some essential questions: *i*) what is the effect of extreme environmental temperatures on the protein folding rate; *ii*) how do PPIases catalyze prolyl isomerization, a rate-limiting step in protein folding; *iii*) the ''trigger factor'' is the first chaperone interacting with nascent chains: how does it help protein folding at extreme temperatures and *iv*) what are the properties of the final native state of proteins adapted to these temperatures?
The available results that will be summarized here open new perspectives for the study of life in extreme environments.

1801-Symp
Using Single Molecule Force Spectroscopy to Probe Proteins from Extremophiles
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Extremophiles are organisms which survive and thrive in the most extreme chemical and physical conditions on Earth. The proteins from extremophilic organisms play a key role in enabling them to survive and function in specific environmental extremes. These proteins are of great interest as they have the ability to retain their folded structure and to possess the necessary flexibility to be functional under conditions which normally denature proteins. For this reason, they offer attractive, model systems in which to explore the origin of protein structure and dynamics under different, extreme environmental conditions. We use single molecule force spectroscopy to measure the mechanical stability and flexibility of proteins derived from extremophile organisms. We have characterised the mechanical stability of a cold shock protein from the hypethermophilic organism, *Thermotoga maritima* in the temperature range 5-40°C. We measure temperature-dependent changes in features of the unfolding energy landscape of this protein by studying the pulling speed dependence of the unfolding force with temperature in combination with Monte Carlo simulations. We find that the position of the transition state to unfolding shifts away from the native state with increased temperature, reflecting a reduction in the spring constant of the protein and an increase in the malleability of the structure. The mechanical robustness and malleability of this cold shock protein over the temperature range studied, provides an insight into the dynamical properties of hyperthermophilic proteins. To gain further insight into the kinetic stability and adaptation strategies of this protein we are examining structural homologues from mesophilic organisms and mutants of the cold shock protein. These will provide a deeper understanding of the adaptations found in hyperthermophilic proteins, and will enable the rational design of proteins for bionanotechnological applications.