Abstracts

– Imaging and Biospectroscopy –

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PI(4,5)P2 acts as a lipid calcium sensor in the presence of physiological calcium concentrations

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The localized enrichment of $PI(4,5)P_2$ in the plasma membrane at particular sites and timings is essential for the regulation of several cellular functions. During Ca^{2+}-triggered exocytosis, $PI(4,5)P_2$ is known to interact and co-segregate with specific synaptic proteins. The role of Ca^{2+} in this process is still not well understood. Ca^{2+} has already been shown to induce $PI(4,5)P_2$ clustering at non-physiological concentrations of Ca^{2+} and/or $PI(4,5)P_2$, or within membranes under high surface pressure. Here, we aimed to understand if physiological $[Ca^{2+}]$ are able to modulate $PI(4,5)P_2$ lateral organization. Using several different approaches which included information on fluorescence quantum yield, polarization, spectra and diffusion properties of a fluorescent derivative of $PI(4,5)P_2$ (TopFluor(TF)-$PI(4,5)P_2$), we show for the first time that Ca^{2+} promotes $PI(4,5)P_2$ clustering in bilayers at physiological concentrations of both Ca^{2+} and $PI(4,5)P_2$. The data is consistent with an average cluster size of ~15 $PI(4,5)P_2$ molecules. Additionally, calcium mediated $PI(4,5)P_2$ clustering was more pronounced in liquid ordered (l_o) membranes. These results suggest that $PI(4,5)P_2$ functions as a lipid calcium sensor in the plasma membrane.

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Dynamic submicroscopic signaling zones revealed by TALM and image correlation analysis

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By orthogonally labeling the type I IFN receptor subunit IFNAR1 and IFNAR2 expressed at endogenous level with monofunctional quantum dots, the dynamic formation of the individual IFN ternary complex was probed by dual color single molecule fluorescence imaging over extended time period. By tracking and localizing individual receptor subunits, TALM (Tracking And Localization Microscopy) images were obtained with spatial resolution of ~15 nm together with temporal resolution ranging from 0.5 ms to seconds. Image correlation analysis of such TALM images was implemented to quantitatively explore the temporal and spatial dynamics of the receptors and cytosolic effector protein. Long-term tracking of individual ternary complexes was established based on time-lapse particle correlation and pair correlation of TALM (pcTALM). To this end, the life-time of the signaling ternary complex over 15 s was confirmed, as well as a submicron confinement of the ternary complex in plasma membrane where transient recruitment of the cytosolic effector protein STAT2 was observed. Our results highlight the role of microcompartmentation for the assembly and stability of signaling complexes in the plasma membrane.

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Direct observation of supported lipid bilayer formation with interferometric scattering microscopy

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Supported lipid bilayers (SLB) are commonly used to study processes associated with or mediated by lipid membranes. The mechanism by which SLBs form is a matter of debate, largely due to the experimental difficulty associated with observing the adsorption and rupture of individual vesicles. Here, we use interferometric scattering microscopy (iSCAT) to directly visualize membrane formation from nanoscopic vesicles in real time. We observe a number of previously proposed phenomena such as vesicle adsorption, rupture, movement and a wave-like bilayer spreading. By varying the vesicle size and the lipid-surface interaction strength, we can rationalize and tune the relative contributions of these phenomena. Our results are in agreement with a model where the interplay between bilayer edge tension and the overall interaction energy with the surface determine the mechanism of SLB formation. The unique combination of sensitivity, speed and label-free imaging capability of iSCAT provides exciting prospects not only for investigations of SLB formation, but also for studying assembly and disassembly on the nanoscale with previously unattainable accuracy and sensitivity.

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Therapeutic protein encapsulation in isasome type lipid nanocarriers

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Advances in protein nanoencapsulation have led to studies of nanostructured lipid particles as multicompartment carriers. Nanostructured formulations for therapeutic protein delivery include internally self-assembled (isasome) particles, which display inner structure of nanochannels. They are formed by fragmentation of liquid crystalline phases of hydrated non-lamellar lipids using amphiphilic dispersing agents. Defining an appropriate delivery strategy for therapeutic proteins, based on isasome carriers, requires knowledge of their hierarchical organization, which determines the loading properties. The purpose of our work was to reveal the structural features of protein-loaded isasome nanocarriers by means of high resolution small-angle X-ray scattering and cryogenic transmission electron microscopy. The obtained results demonstrate that the effects of entrapped a-chymotrypsinogen A and brain-derived neurotrophic factor are concentration dependent. This conclusion is in agreement with previous studies showing that guest species smaller than the mesophase periodicity of the lipid carriers are confined within the aqueous channels and influence the lipid hydration, whereas larger proteins are expelled and partition at grain boundaries of subdomains formed in the carriers.