185-Plat
Quantification of Membrane Free Area and Dynamic Undulations using Fluorescence Lifetime and Autocorrelation of Fluorescence Lifetime Fluctuations
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Through extensive characterization in solution and lipid bilayers, we report that fluorescence lifetime of DiI (a popular membrane probe) can be used to quantitatively determine lipid free-area in giant vesicles and nanoliposomes. The method is sensitive to subtle changes in lipid packing on the order of 1 $Å^2$. We measured the areas-per-lipid in bilayers of different compositions and show that our method matches values obtained from NMR/X-ray/ or neutron scattering. We further characterized free-area changes induced by temperature and lateral tension in nanoliposomes, to determine their thermal expansivity coefficients and free-area compressibility moduli, respectively. Second, we used the autocorrelation of DiI fluorescence lifetime fluctuations to capture dynamic changes occurring in membrane packing due to thermally-driven membrane undulations. We demonstrate that fluctuation frequencies from 10Hz to 1KHz can be detected under confocal mode to gain spatially refined determination of membrane dynamics.

186-Plat
Controlled Labeling of Lipids with Single Gold Nanoparticles on Living Cell Membranes
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The cellular membrane is the physical boundary of cells where numerous cellular processes occur. Gold nanoparticles (Au NPs) are attractive optical tools to investigate living cell membranes due to their chemical stability and unique optical properties.[1] Direct utilization of weakly stabilized Au NPs often results in NP aggregation in biological media and in their uncontrolled attachment to biological membranes. Controlled strategies, however, are typically based on protein-mediated binding, which restricts the use of the Au NPs to protein-specific studies, and therefore limits their spectrum of sensing and optothermal applications.
Here we present a general, versatile and controlled strategy to bind individual Au NPs to lipids in living cell membranes, while preserving the sensing and optothermal capabilities of the original colloid [2]. Our approach is based on the controlled and selective binding of Au NPs to phospholipids prior to cell incubation. First, CTAB-capped gold nanospheres are PEGylated and maleimide derivatized for conjugation to thiol-ended lipids in liposomes. Thereafter the Au-labeled lipids are incorporated in the cellular membrane of living cells via liposome/cell membrane fusogenesis. As a result of lipid binding, the diffusion of the Au NPs on the cell membrane is rather slow and spatially-limited. In this presentation we will show different strategies to manipulate the diffusion of those Au NPs on the cell membrane, which enables us to gain further insight into the membrane structure.
References:
[1] R.A. Sperling et al., Chem.Soc.Rev., 37, 1896 (2008).
[2] Haojin Ba et al., Nano Lett., 10, 3006 (2010).

187-Plat
The Facultative Role of Lipid Rafts and Membrane Microdomains in Controlling the Assembly of the Store-Operated Channel Complex
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Calcium influx is an important factor in a wide variety of cellular functions. The signal initiating calcium influx may originate from the activation of G-protein coupled receptors, or arise from inside the cell, via maneuvers that can lead to depletion of intracellular calcium storage compartments. While the role of members from the Transient Receptor Potential Cation (TRPC) family of channels as receptor-operated channels (ROC) is well established and supported by abundant studies, the role of this family of channels as store-operated channels (SOC) has been the focus of a heated controversy over the last few years. In the present study, we have explored the modulation of STIM1 on human TRPC1 channel. We have found that the association of STIM1 to TRPC1 favors the insertion of this channel into lipid rafts, where TRPC1 functions as a SOC in association to Orai channels forming a complex we have named SOCIC (store-operated calcium influx complex). In the absence of STIM1, TRPC1 associates to other members from the TRPC family of channels to form ROCs. A novel imaging method implemented for the present study based on multicolor LightGuide Total Internal Reflection Fluorescence Microscopy (LG-TIRFM), illustrates the relevance of the dynamic association between STIM1 and TRPC1 for the activation of SOC and the lipid raft localization of the STIM1-TRPC1-Orai complex. This study provides new evidence about the dual activity of TRPC1 (forming ROC or SOC) and the partners needed to determine TRPC1 functional fate. It highlights also the role of plasma membrane microdomains (lipid rafts) and ER-PM junctions in modulating TRPC1 channel function. Using LG-TIRFM, we identified new partners of the SOCIC, and studied the dynamics of SOCIC assembly and disassembly in real time.

WORKSHOP 1: X-Ray Free Eectron Lasers and Biophysics

189-Wkshp
Hard X-Ray Free Electron Lasers: How They Work, Photon Characteristics Important to Biology and Possibilities for the Future
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Accelerator based photon sources have played a key role in the understanding of protein structures at the atomic scale. The next generation of these sources, free electron lasers, now operate with Angstrom scale wavelengths and open unique opportunities for biophysics. How these lasers work, the photon properties they yield and a look toward future upgrades will be discussed using LCLS, the world's first operating hard x-ray free electron laser as an example.

190-Wkshp
Fermtosecond Coherent X-Ray Nanocrystallography at LCLS
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DESY, Hamburg, Germany.
The ultrafast pulses from X-ray free-electron lasers have opened up a new form of protein nanocrystallography. The X-ray pulses are of high enough intensity and of sufficiently short duration that individual single-shot diffraction patterns can be obtained from a sample before significant damage occurs. This ''diffraction before destruction'' method may enable the determination of structures of proteins that cannot be grown into large enough crystals or are too radiation sensitive for high-resolution crystallography. Ultrafast pump-probe studies of photoinduced dynamics can also be studied. We have carried out experiments in coherent diffraction from protein nanocrystals, including Photosystem I membrane protein, at the Linac Coherent Light Source (LCLS) at SLAC. The crystals are filtered to sizes less than 2 micron, and are delivered to the pulsed X-ray beam in a continuously flowing liquid jet. Millions of diffraction patterns were recorded at the LCLS repetition rate of 30 Hz with pnCCD detectors, in a instrument designed and built by the Max Planck Advanced Study Group at CFEL. Tens of thousands of the single-shot diffraction patterns have been indexed, and combined into a single crystal diffraction pattern, which can be phased for structure determination and analysed for the effects of pulse duration and fluence. Details of these first LCLS experiments and analysis will be discussed.

191-Wkshp
Free-Electron Lasers - emerging Opportunities for Structural Biology
Ilme Schlichting.
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Electron microscopy and X-ray crystallography provide unique insight into the architecture of cells, molecular assemblies and (macro)molecules. Ultimately, both methods are limited by radiation damage. Compared to synchrotron sources, free-electron lasers (FEL) provide orders of magnitude brighter and shorter X-ray pulses that have been proposed to yield diffraction patterns of biological samples before the onset of significant radiation damage (''diffraction before destruction''). Recently, the Linac Coherent Light Source (LCLS) has become accessible to users, accessing the hard X-ray regime, thereby allowing Ångstrom-resolution studies with femtosecond time resolution. This enables a plethora of new experiments, including femtochemistry and the structural analysis of complex materials, warm dense matter, and biological samples. The latter include single particles such as viruses. Recent results will be described that were obtained by a collaboration consisting of the Max Planck Advanced Study Group at CFEL, CFEL DESY, Arizona State University, SLAC, Uppsala