of the respective membrane structure is not directly accessible by the diffraction limited resolution of conventional far-field optical microscopes. We report the detection of the membrane heterogeneities in nanosized areas in the plasma membrane of living cells using the superior spatial resolution of stimulated emission depletion (STED) far-field nanoscopy. By combining a (tunable) resolution of down to 30 nm with tools such as fluorescence correlation spectroscopy (FCS), we obtain new details of molecular membrane dynamics. Sphingolipids are transiently (~ 10 ms) trapped on the nanoscale in cholesterol-mediated molecular complexes, while glycerophospho-lipids diffuse freely. The results are compared to STED experiments on model membranes, which highlight that the nanoscale trapping in cells is not correlated with liquid order partitioning in model systems. The novel observations shine new light on the distribution and interaction of lipids and proteins in the plasma membrane with respect to the lipid raft hypothesis.

164-Plat
Multivalent Chelator Lipids for Targeting and Manipulation of Proteins in Membrane Nanodomains
Oliver Beutel[1], Joerg Nikolaus[2], Alf Honigmann[3], Christian Eggeling[3], Andreas Herrmann[2], Jacob Piehler[1].
[1]University of Osnabrück, Department Biology, Division of Biophysics, Osnabrück, Germany, [2]Humboldt University Berlin, Department Biology/ Molecular Biophysics, Berlin, Germany, [3]Max-Planck-Institut für biophysikalische Chemie Göttingen, Göttingen, Germany.
Membrane nanodomains based on phase-segregation of lipids have emerged as a key organizing principle of the plasma membrane. They have been shown to play important roles in signal transduction and membrane trafficking. We have developed lipid-like probes carrying multivalent nitrilotriacetic acid (tris-NTA) head groups for selective targeting of His-tagged proteins into liquid ordered or liquid disordered lipid phases. The stable, non-covalent interaction of His-tagged proteins to the tris-NTA moiety can be employed not only for efficient specific tethering of spectroscopic probes, but also for versatile manipulation of membrane nanodomains. In giant unilamellar vesicles strong partitioning of tris-NTA lipids into different lipid phases was observed. For a saturated tris-NTA lipid, at least 10-fold preference for the liquid ordered phase was found. In contrast, an unsaturated NTA lipid shows a comparable preference for the liquid disordered phase. Simular partitioning of the tris-NTA lipids was observed in solid-supported membranes on mica. Partitioning into submicroscopic membrane domains formed in solid-supported membranes was confirmed by superresolution imaging techniques (FPALM, STED). Single molecule tracking of His-tagged proteins tethered to solid-supported phase-separating membranes revealed clear differences in the diffusion behavior of the different NTA-lipids. By using vesicles as a carrier, multivalent NTA lipids were efficiently incorporated into the plasma membrane of live cells. After formation of giant plasma membrane vesicles (GPMV), efficient partitioning of the lipid probes into the respective membrane phases was confirmed. We have employed these probes for exploring lipid diffusion, morphology and spatiotemporal dynamics of membrane nanodomains in *vitro* and live cells by single molecule tracking and STED FCS.

165-Plat
Model of Self Reproducing Vesicles
Yuka Sakuma, Masayuki Imai.
Ochanomizu University, Tokyo, Japan.
Evolution of molecular assemblies toward a cellular life in prebiotic world is fascinating research filed in soft matter science. So far extensive investigations have been performed to construct molecular model system which expresses similar dynamics as those of a life system using well-defined organic molecules. Especially modeling of a self-reproducing vesicular system is a key concept to understand origin of the cellular life.
In typical self-reproducing vesicle systems, membrane precursors are converted into membrane molecules with the help of a catalyst and the membrane molecules induce the formation of daughter vesicles. The self-reproducing vesicle systems have two pathways for the topology transition, the birthing and the budding. In the birthing pathway, new synthesized membrane molecules form daughter vesicles inside a mother vesicle. When the daughter vesicle grows to a certain size, it extrude through the membrane of the mother vesicle to the environment. On the other hand, in the budding pathway, the mother vesicle deforms to pear-like shape and is divided into two independent vesicles.
We established a model self-reproducing vesicle system without the membrane molecule synthesis route. The model vesicle composed of cylinder- and inverse-cone-shaped lipids formed inclusion vesicles inside the mother vesicle and the inclusion vesicles were then expelled by a temperature cycling. By changing the vesicle composition, the mother vesicles showed the budding type self-reproduction pathway. A key concept of this system is the coupling of the main-chain transition and the shape of lipids.

166-Plat
Lipid Membrane Deformation in Response to a Local pH Modification
Anne-Florence Bitbol[1], Nicolas Puff[2], Yuka Sakuma[3], Masayuki Imai[3], Jean-Baptiste Fournier[1], Miglena I. Angelova[2].
[1]Matière et Systèmes Complexes CNRS UMR 7057, Université Paris 7, Paris, France, [2]UFR925-Physics Dept-University Paris 6, Matière et Systèmes Complexes CNRS UMR 7057, Université Paris 7, Paris, France, [3]Dept of Physics, Faculty of Science, Ochanomizu University, Tokyo, Japan.
During cell life, membranes are subjected to a variable and inhomogeneous environment. These local variations can be strongly related to biological processes. For instance, in the inner membrane of mitochondria, ATP synthesis is tightly coupled to the local pH. It is therefore interesting to study the response of a lipid membrane to a local modification of its environment.
In our previous works (Khalifat et al., Biophys. J., 2008; Fournier et al., PRL, 2009; Bitbol et al., J. Phys.: Condens. Matter, 2011), we designed an experiment where the local pH at an artificial membrane (lipid giant unilamellar vesicle) is modified using microinjection. We showed that modifying the local pH induced a dynamic membrane deformation. We also developed a theoretical description of the dynamics of a membrane subjected to a local modification affecting its physical properties. It involved elaborating a local version of the area-difference elasticity model and accounting for the friction between the two monolayers of the bilayer membrane.
We have now extended our theoretical model to account for the diffusion of the membrane-modifying reagent in the surrounding solution. We solved numerically the equations describing the dynamics of the membrane in this case. We showed that the effect of diffusion from the local reagent source is generally important, but that there are experimentally accessible cases where the dynamics is dominated by processes intrinsic to the membrane, such as the relative sliding of its two monolayers. We compared the predictions of our extended theoretical model to experimental results regarding the dynamics of lipid (PC/PS-) membrane deformation during and just after the local delivery of a basic solution. Moreover, we used a pH-sensitive fluorescent membrane marker to have a direct experimental visualization of the membrane pH profile together with the deformation.

167-Plat
The Investigation of Lipid Membrane Deformation in Giant Unilamellar Vesicles using Microfluidic Technology
Tom Robinson, Petra Dittrich.
ETH Zurich, Zurich, Switzerland.
Membrane curvature is known to play an important role in cell growth, division and movement. Different lipid and membrane proteins can influence the cell's shape by changing the membrane curvature. Developing new technologies is vital for investigating and understanding the processes involved when membranes deform. We present an approach to study membrane curvature in giant unilamellar vesicles (GUVs) using microfluidic technology. Micron-sized channel networks in microfluidic devices are ideally suited for the manipulation and imaging of biological structures. The ability to confine liposomes in highly controlled environments allows them to be exposed to precise mechanical stresses for membrane analysis. The device presented here was fabricated in polydimethylsiloxane (PDMS) and consists of microchannels where GUVs can be introduced or grown within using the electro-swelling method. They can be immobilised at specific positions using a biotin-PEG-cholesterol linker patterned on to the glass bottom of the channel, then subjected to mechanical forces using an actuated PDMS membrane the above the channel. Initial experiments show that the GUVs are stable when subjected to a force from the PDMS micro-stamp allowing them to be imaged. To obtain high resolution 3-D images of the GUVs as they are deformed, we have combined established imaging methods with integrated optical components. More precisely, a silver coated micro-mirror inside the PDMS wall allows objects within the channels to be imaged from the side as well as from below. This enables high-resolution 3-D imaging in the axial plane using a confocal microscope. With the aim to study the effects of curvature on phase separation, we have created lipid domains in GUVs using a ternary lipid mixture of SM/DOPC/Cholesterol and DLPC/DPPC/Cholesterol. The device has the potential for investigating the mechanosensitivity of membrane bound proteins and whole cells.