Project A3: Controlled deformations and modifications of model and cellular lipid membranes

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Current state of the research. A consequence of the diversity and dynamics of the lipids in membrane bilayers is their self-organization in transient, highly ordered domains referred to as “rafts”. Although membrane properties are of key importance, their investigation is difficult: The width of the membrane is only 4-5 nm, i.e. two orders of magnitude below the resolution of optical microscopes. Similarly, the fast dynamics and small sizes of the “rafts” (< 100 nm) have hampered their direct observation. Recently, important progress has been made by the design of tailored probes precisely located in the membrane and sensitive to site-selective properties, such as hydration, polarity, lipid phase, and electric fields. Progress has also been made in the development of quantitative high-resolution microscopy techniques, e.g. fluorescence correlation spectroscopy (FCS) or FRET/FLIM. These developments can now be harnessed to address questions of high interest, such as the question of how proteins can influence the lipid composition and deformation of model and cell membranes.

Contributions of the principal investigators. The Mély group has developed environment-sensitive probes tailored to be located in the membrane at a defined depth and orientation and to respond to changes in the environment by changes in emission spectrum. These probes were successfully applied to characterize “raft” domains in giant unilamellar vesicles (GUV). The group has also developed various quantitative high-resolution fluorescence microscopy techniques which will be used in this project. The Römer group has expertise in the study of the initial steps of the uptake of diverse human pathogens (bacteria, viruses) and pathogenic products (toxins) in non-phagocytic cells, in particular by using a combination of fluorescence and atomic force microscopy.

Research project and collaborations. During infection by *P. aeruginosa*, interactions of bacterial lectins with membrane glycosphingolipids probably induce cluster formation that destabilizes the membrane. To monitor these processes on model membranes, we will apply a combination of advanced fluorescence microscopy techniques, atomic force microscopy and membrane probes to characterize the cluster size, their topology and the membrane dynamics. In a first step, these interactions will be investigated on GUV and supported lipid bilayers (planned collaboration with the Marques group). We will test ligands of different complexity ranging from wild-type lectins and binding-site mutants, over lectin-coated beads of various sizes and occupancy rate, to bacteria bearing the lectins at their outer membrane. Since we expect the lipid composition to strongly influence the membrane dynamics, we will test different lipid compositions. Finally, our conclusions will be validated on live cells.

Work plan. This PhD project is split into two parts: *i)* Freiburg (18 months): Preparation of giant unilamellar liposomes and solid supported bilayers. Reconstitution of glycosphingolipids (or glycoproteins) as receptor molecules. Study of lectin-induced domain formation and membrane invagination by confocal fluorescence microscopy using different ligands (lectins, lectin-coated beads, bacteria), receptor molecules (varying in lipid chain length, saturation degree) and lipid compositions. Investigation of domain size, domain topology of lectin-induced clusters by atomic force microscopy. *ii)* Strasbourg (18 months): Integration of membrane probes in the above mentioned model systems in order to image lipid phases at high resolution. Detailed study of the effect of lectin-induced lipid clustering, preceding membrane invagination by FCS and FLIM. Investigation of membrane order and dynamics, in response to lectin binding. Study of the mechanical properties of model membranes in the presence of the different ligands with the Marques group. Study of membrane dynamics with super-resolution fluorescence microscopy using fluorescent receptor molecules.