Abstract

The complexity of cell membranes is far from being only a simple assembly of lipids and proteins separating cells from the surrounding environment. Each of the thousands of different membrane components performs its specific role in cellular functions, since a multitude of biological processes is mediated by membranes. The understanding of the molecular basis of these processes is one of the important aims of current biological research. Our research employing single-molecule fluorescence methods (e.g. FCS, FCCS, FLIM-FRET) has made a contribution to the knowledge of membrane lateral organization or mechanism of membrane fusion. Furthermore, we revealed the mechanism of membrane activity of a small natural compound. As native cell membranes are very complex structures, we performed the experiments on simplified model lipid membranes that allow studying lipid-lipid or lipid-protein interactions at the molecular level in a controlled way.

The first part of this thesis deals with the mode of action of a membrane active secondary metabolite didehydroroflamycoin (DDHR). We demonstrated that DDHR is a pore-forming agent and that this activity is influenced by the presence of cholesterol. Direct visualization of intrinsic fluorescence of DDHR revealed its preferential partitioning into membrane areas with higher lipid order.

The second part concentrates on the membrane lateral heterogeneity close to the phase separation boundary. Membrane heterogeneity plays an important role in multiple cellular processes, but its nature is controversial. Although conventional fluorescence microscopy techniques do not allow direct visualization of these sub-microscopic structures, we were able to detect them by various single-molecule approaches. We identified approximately 9 nm sized fluid nanodomains in GUVs composed of ternary DOPC/Chol/SM and even in binary DOPC/SM lipid compositions. Furthermore, we showed that also ganglioside GM1 clusters into nanoscale domains and that its availability for binding by cholera toxin B subunit is influenced by GM1 density as well as by the presence of cholesterol.

The third part is focused on investigation of complementary coiled-coil forming lipopeptides CP_nK_4 and CP_nE_4 that serve as a model system for membrane fusion. Single-molecule fluorescence techniques were employed to study their roles in the initial steps of the fusion process mediated by these lipopeptides. Our research revealed the asymmetrical nature of this fusion system. We proposed a model where the peptide moiety of the lipopeptide CP_nE_4 acts as a "handle" for positively charged peptide moiety of CP_nK_4 resulting in liposome docking, while the peptide K_4 interacts with the membrane causing local deformations, which enhances the fusion process.