Abstract

Rich structure of cell membranes raises broad number of questions regarding the mechanisms driving and regulating processes taking place on membranes. The thesis presents four articles investigating organization of lipid membranes and peptide-lipid interactions. The experiments were performed on model lipid membranes. These simplified systems that partially mimic cell membranes enable to study protein-lipid interactions at the molecular level and membrane physico-chemical properties in a controlled way. Advanced fluorescence techniques such as FCS, TDFS, FLIM and anisotropy were used for the system characterization. First publication describes newly designed fluorescence dyes based on boron dipyrromethene structure, the so-called molecular rotors, which are reported to be viscosity-sensitive probes. Detailed analysis of fluorescence lifetime of excited state of the molecular rotors inserted into lipid membranes showed diverse incorporation of dyes into membranes and their reorientation in membranes of different rigidity. The second part investigates existence of lipid nanodomains in membranes caused by the presence of a cross-linker. Even though standard fluorescence microscopy techniques do not allow direct visualization of the nanodomains, we were able to detect these structures by employment of FCCS and FLIM-FRET techniques supported by Monte-Carlo simulations. The study showed two mechanisms of nanodomain formation depending on the membrane lipid composition and concentration of the cross-linker. In the third part, TDFS method was newly applied to determine the peptide orientation with respect to the membrane normal. The results from the previous studies set the basis for the fourth paper investigating membrane dynamics in the presence of peptides. Experimental and computational results show that increasing amount of the peptide reduces diffusion rate of all membrane components due to temporal lipid acyl chain trapping on the rough surface of the peptide. In cholesterol-containing membranes, the sterol segregates from peptide surface. Due to the fact that rough surface is an intrinsic property of literally all integral membrane proteins the results of our work can be generalized to most eukaryotic cell membranes. Distribution of membrane proteins and cholesterol in cell membranes could therefore affect a variety of intermolecular interactions and reaction kinetics of cellular processes associated with membranes, thus affecting vital functions of living cells.