Abstract

Membrane rafts (also referred as nanodomains) are membrane structures responsible for many cell processes. Their characterization is challenging because of the transparency, dynamics and small size of those structures. Moreover, high variability of cells makes their study even more complicated. In order to simplify the studies of membrane processes including the formation of those rafts often model membranes like Giant Unilamellar Vesicles (GUVs) and Supported Phospholipid Bilayers (SPBs) are used. In this Thesis new fluorescent tools for studying such membrane processed were developed, tested, or improved.

Specifically, the phasor plot an approach applicable to the analysis of the fluorescence lifetime data, was theoretically and experimentally tested and afterwards applied to the characterization of the membrane nanodomains in GUVs. First, we introduced the phasor plots to the excitation state processes like solvent relaxation and Förster resonance energy transfer (FRET) in lipid vesicles. We also employed the phasor plots in protein-ligand interaction, protein folding and denaturation studies. Finally, the phasor plot analysis of FRET data in combination with Fluorescence Correlation Spectroscopy (FCS) was used in characterization of membrane nanodomains in terms of the size, mobility and mechanisms of formation. We succeeded for the first time to determine the size and dynamics of nanodomains in GUVs smaller than 20 nm in radius. Our findings allowed us to make general conclusion on the mechanism of raft formation. The lateral diffusion on SPBs of a weakly bound blood coagulation protein was the second membrane process being studied. By developing a 2-color z-scan FCS approach using pulsed interleaved excitation, we were able to simultaneously characterize this protein diffusion and lipid diffusion. Although the protein diffusion is about two times slower that the lipid diffusion, the lipid composition and protein concentration dependencies suggest that both processes are to some extend coupled.