

This diploma thesis deals with the analysis of structural and dynamic organization of thyrotropin releasing hormone receptor (TRH-R) and δ -opioid receptor (DOR) within plasma membrane (PM) in relation to the specific sub-compartments of PM denominated as domains or membrane rafts. Modern fluorescence microscopy techniques FLIM, FRAP and RICS were used for this purpose. The experiments were performed on the live cells derived from HEK293 cell line. To reach the main goal of this work, the integrity of PM structure was altered by depletion of cholesterol which was performed by incubation of cells with β cyclodextrin. Results clearly support our previously suggested idea that the vast majority of TRH-R is localized in non-raft regions of plasma membrane. This work also compared different modes of performance of FRAP and results obtained by FRAP and RICS because these methods are to some extent analogous. This is one of the first works that used the RICS approach to characterize the G protein-coupled receptors. In the second part of this work, the setup of transient transfection of the HEK293 cells with DOR-ECFP and DOR EYFP constructs was established. Simultaneously, the functionality of these constructs, i.e. the ability of DOR to activate the cognate G protein was determined.