

The lateral membrane organization is maintained by raft domains, dynamic protein-lipid structures, which essential role was shown in a variety of biological processes. Despite many years of research, it is still unknown what are the regulating mechanisms of raft domains formation. It is considered, that raft domains formation is the result of numerous lipid-protein, lipid-lipid and protein-protein interactions. The current hypothesis assumed that these interactions initiate the coalescence of little assemblies, created by several proteins and lipids, into bigger, more stable, and functional raft domains. The presence of proteins in raft domains is predominantly regulated by their modification with fatty acids, and one of the most common is palmitoylation. Recent studies have shown that palmitoylation is responsible not only for protein localization in raft domains, but it occurs to be required for the process of raft domains formation. The objective of our research is palmitoylated MPP1 (Membrane Palmitoylated Protein 1), a peripheral membrane protein that belongs to MAGUK (Membrane Associated Guanylate Kinase) family. It has been shown, that MPP1 acts as a molecular switch regulating the organization of functional raft domains in erythroid cells. Silencing of *MPP1* gene expression in erythroid precursor cells led to a dramatic decrease in order parameters of both plasma membranes (PM) of intact cells and PM-derived vesicles, and most significantly affected signaling via the raft-dependent receptor. Similar results were obtained under the inhibition of cellular palmitoylation processes suggesting a possible role of MPP1 palmitoylation in raft domains organization mechanism. Further studies on MPP1 role in raft domains formation led to the characterization of flotillin 1 and flotillin 2, the protein markers of raft domains, as direct partners for MPP1. Based on this observation we proposed that palmitoylation of MPP1 may be crucial for its direct binding to the plasma membrane and subsequent assembly of functional pre-existing flotillins-associated complexes to initiate signaling cascades. However, the unexplained issue that remains is whether MPP1 palmitoylation itself participates in raft domain formation or acts only as a membrane raft targeting signal. MPP1 was indicated as the major palmitoylated protein of the erythrocyte membranes. MPP1 contains four cysteine residues in positions 94, 179, 242, and 454, and cysteine 242 was supposed to be a potential modification site. However, our recent preliminary data indicates that MPP1 has several palmitoylated cysteine residues. These results raise the intriguing question of whether the palmitoylation of individual naturally modified residues and/or their various configuration within MPP1 protein has an impact on the physicochemical properties of the membranes. Another interesting issue is whether palmitoylation of all cysteines is necessary for raft domains formation process or maybe modification of individual residue impacts differently the MPP1 function. Therefore, the main goal of this project is to characterize in detail the role of MPP1 palmitoylation in the context of its raft domain-organizing potential in cell-based and model membrane systems. The first step of the cell-based study will be the identification of MPP1 palmitoylation sites by preparing a set of C/A mutants with changed cysteine residues for alanine residues and their overexpression in cells with silenced endogenous MPP1 expression. Next, using APE (Acyl-PEG Exchange) and Acyl-RAC (Resin-Assisted Capture) methods, their palmitoylation level will be analyzed. In turn, the binding ability of each mutant to membrane/raft domains will be elucidated by analysis of their localization in isolated DRM (Detergent Resistant Membranes) fractions and microscopic imaging of their colocalization with specific membrane phases in GPMVs (Giant Plasma Membrane Vesicles). Based on these results, selected mutants will be used in further studies of membrane fluidity of live cells as well as isolated GPMVs using the FLIM (Fluorescence Lifetime Imaging Microscopy) technique. The second part of the project will be performed using a recombinant MPP1 protein, its C/A mutants and model membrane such like LUVs (Large Unilamellar Vesicles). The binding properties to liposomes of different lipid compositions upon MPP1 palmitoylation will be checked using floatation assay and BLI (Bio-Layer Interferometry). The last step will be the analysis of changes in physicochemical properties of the model membrane by calculation of general polarization (GP) of fluorescence probes sensitive to changes in the lipid packing order. Taking into account the essential function of raft domains and palmitoylation in a variety of cellular processes, we proposed a research plan, which aims to understand the role of MPP1 palmitoylation in its function as a membrane organizing molecule. Due to the fact that many cellular processes depend on membrane compartmentalization and the presence of functional raft domains, therefore the problem concerning their formation is extremely important. Furthermore, understanding the mechanism of raft domain formation, and the role of protein palmitoylation in this process could open the way for discovering effective therapies of different diseases related to abnormalities of protein palmitoylation (and thus raft organization) such as neurodegenerative diseases or cancers.