Plasma membrane (PM) contains a huge variety of lipids and proteins which are dynamically and spatially organized into lateral membrane domains, an example of which are resting state rafts. Membrane rafts are highly order nanoscale subdomains (<200 nm), particularly enriched in cholesterol, sphingolipids, gangliosides and certain proteins such as stomatin and flotillins. The latter assemble into oligomers to form molecular scaffolds that regulate the clustering at the PM and activity of many receptors, including EGFR, WNT, and HER2, src family kinases, G proteins and other components. Several signal transduction processes involved in cell adhesion, migration and in the formation of sorting platforms for targeted protein trafficking are dependent on precise membrane domain organization.

Neoplastic cell metastases from the primary tumor to distant organs require not only formation of tumor neovasculature to provide in nutrients and oxygen, but also directed migration and transendothelial migration (TEM), enhanced cellular motility through an epithelial-to-mesenchymal transition (EMT). EMT is a biological process which allows a polarized epithelial cell, which normally interacts with basement membrane, to undergo multiple biochemical changes that enable it to assume a mesenchymal cell phenotype, which includes enhanced migratory capacity, invasiveness, elevated resistance to apoptosis, and greatly increased production of extracellular matrix (ECM) components. In many cancers, including breast cancer, EMT is related with metastatic expansion and generation of tumor cells with stem cell properties which play a major role in the resistance to cancer treatment.

In recent years it has been shown, that the EMT is accompanied by numerous changes in various signaling pathways that are initiated at the PM, and that lipid raft disruption by cholesterol depleting agents impaired EMT in cancer.

As the major function of the membrane rafts is modulation of signaling pathways initiated at the PM, we hypothesize that changes observed during EMT could be correlated with alterations in membrane raft organization and regulation.

Considering the role of flotillins in promoting the local co-assembly of specific activated proteins on the cell surface, we expect to observe differences in the network of flotillin-binding proteins in case of cells of epithelial vs mesenchymal phenotype represented by MCF-7 and MDA-MB-231 breast cancer lines, respectively. Therefore the question we ask in this project is whether the differences present in the mentioned cells types correlates with alterations in PM domain organization.

To address this issue we propose here three experimental approaches: 1. Analysis of lateral organization of membrane rafts including biochemical (cholesterol and protein content) and biophysical plasma membrane order) characteristic and 2. comparative analysis of the flotillins interactome via *Proximity*-dependent biotin identification (BioID) in MCF-7 vs MDA-MB-231 cells together with 3. further kinetic in vitro studies of the most prominent interactions. Comparative analysis of flotillins interactome in epithelial vs mesenchymal cells, should provide valuable data that help understanding the role flotillin-dependent rafts domains in metastatic cascades, specifically those accompanying EMT. Understanding the relationship between specific biophysical properties of membrane rafts with EMT may be crucial for future therapeutic discoveries aiming to limit prometastatic processes.