OPTICAL NANOTOOLS TO INVESTIGATE THE SPATIO-TEMPORAL ORGANIZATION OF THE CELL MEMBRANE AT THE NM SCALE.

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The ability to study the structure and function of cell membranes and membrane components is fundamental to understanding cellular processes. This requires the use of methods capable of resolving structures with nanometer-scale resolution in intact or living cells. Although fluorescence microscopy has proven to be an extremely versatile tool in cell biology, its diffraction-limited resolution prevents the investigation of membrane compartmentalization at the nanometer scale. In our group we are applying a combination of near-and far-field optical techniques equipped with single molecule detection sensitivity to gain deeper insight on the spatio-temporal organization of the cell membrane at the nm scale. On the one hand, Near-field scanning optical microscopy (NSOM) combines both enhanced spatial resolution and simultaneous measurement of topographic and optical signals. Because of the very small near-field excitation volume, background fluorescence from the cytoplasm is effectively reduced, enabling the visualization of nano-scale domains on the cell membrane at physiologically relevant packing densities. On the other hand, epi/TIRF microscopy can provide exquisite temporal information of the cell membrane at the level of single molecules.

In this contribution I will show our current efforts towards the investigation of “lipid rafts” as local organizers of the cell membrane using NSOM imaging in *aqueous* conditions on intact cells. Lipid rafts (domains within the membrane enriched in cholesterol and glycosphingolipids) are believed to play a key role in many membrane related processes like immune cell signaling and viral entry. Their existence is however rather controversial, since evidence for the presence of lipid rafts in native cell membranes can only be obtained via indirect methods. We have used single molecule NSOM to investigate the nano-scale organization of both lipid and protein domains on cells of the immune system. Most recent results will be presented and discussed on the basis on distinct nano-scale compartmentalization.

Furthermore, I will show most recent results concerning the spatio-temporal organization of the adhesion receptor LFA-1 on living monocyte cells. Using epi/TIRF microscopy in combination with micro-fabricated biofunctionalized patterned surfaces we have discovered that the dynamics and functional binding of LFA-1 to its ligand ICAM-1 is determined by both the conformation sate of LFA-1 and its spatial distribution on the cell membrane. These studies will shed light on the role of affinity *vs.* avidity of LFA-1 to its ligand ICAM-1 on a single molecule basis.