Nano-Domain Organization of Plasma Membrane Proteins and Their Association to Lipid Rafts Studied With Near-Field Scanning Optical Microscopy

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The cell plasma membrane of eukaryotic cells is a lipid bilayer that forms the link between cell cytosol and the extracellular environment. The composition and organization of proteins and lipids within this bilayer have a direct impact on many cellular processes. 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 [1]. 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. Recent experimental evidence points to the fact that both protein and lipid domains are thought to be in the range of 50-100nm in diameter [2] well below the diffraction-limited resolution of fluorescence microscopy, and too densely packed to be resolved individually.

In here we discuss the use of high-resolution near-field scanning optical microscopy (NSOM) to directly visualize lipid rafts, enriched in glycosphyngomyelin (GM1) in immature dendritic cells (imDC) and human monocytes (THP1) under *aqueous* conditions. NSOM is a surface sensitive technique that has a lateral and axial resolution of ~70nm and few nm respectively, being ideal for investigation of the cell membrane at the nm scale [3]. Furthermore, our set-up is equipped with single molecule detection sensitivity, enabling accurate quantification of monomeric *vs.* clustered type of organization. Remarkably, on both imDC and THP-1 cells, GM1 organizes in nano-domains that appear to be smaller than 100nm in size. While on imDCs, about 50% of the domains binds to only 10 CTxB molecules, on THP1 cells, up to 25 CTxB molecules per domain have been identified.

Furthermore, we also investigated the organization of different protein receptors with a spatial resolution better than 100nm. We have focused on two non-raft associated protein (CD71 and CD46) and have discovered that while the tranferrin receptor CD71 appears randomly organized on THP1 cells, CD46 forms nano-domains on imDCs. Interestingly, we also found that the GPI anchored protein CD55, a commonly used raft marker, does not cluster on both imDC and THP1 cells, but rather organizes on a random fashion. These results indicate that clustering of protein receptors occurs even when they are not associated to rafts. Conversely, raft partitioning does not necessary implies clustering. Consequently, these results suggest a greater complexity for protein organization than their mere association to lipid rafts.

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