SUPER HIGH RESOLUTION MICROSCOPY TO REVEAL THE NANO-LANDSCAPE OF LIPIDS AND PROTEINS ON THE MEMBRANE

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The membrane of a cell is seen as a mosaic of lipids and proteins. Cholesterol enriched membrane domains, known as lipid rafts, are dynamic small platforms involved in signaling pathways. Additional biochemical assays revealed that these lipid rafts are enriched in glycosylphosphatidylinositol anchored proteins (GPI-APs) and glycosphingolipids. It has been postulated that proteins can be either in lipid rafts or in the remaining non-raft part of the membrane. Spatial co-localisation of proteins with the 10-200 nm sized raft domains will then have functional implications for the protein under investigation. Unfortunately, these length-scales are not available for conventional light microscopy, which is diffraction limited to ~300 nm, showing the need for state-of-the-art high resolution techniques.

Near-field scanning optical microscopy (NSOM) is a shear-force based scanning probe technique that uses a sub-wavelength aperture probe to locally excite fluorophores. The size of the aperture dictates the lateral resolution which is below 100 nm. Due to the evanescent character of the light emerging from the tip-end, high axial resolution is also obtained, making the NSOM ideally suited to investigate the cell membrane. Here, we have used two-color excitation/detection NSOM in aqueous conditions to map with nm accuracy the heterogeneity of membrane domains on the surface of monocytic-like cells.

The decay accelerating factor, DAF (CD55), is a well established model GPI-AP and therefore often used as a marker for membrane rafts. To study the potential heterogeneity within rafts we used as raft markers both cholera toxin-β labeling to the glycosphingolipid GM1 and FLAER labeling targeted to GPI-APs. In addition, the CD55 has been also labeled via specific antibodies. Surprisingly, high resolution NSOM imaging did not show significant co-localisation of CD55 with either raft marker. However, inter-domain distance analysis with 3nm localization accuracy, between the CD55 domains and raft revealed a preferential proximity of CD55 to rafts, irrespective of the raft marker used.

These results suggest that the cell membrane contains spatially segregated regions actively held in close proximity. In addition, our results support the notion of distinct raft-like domains occupying different compartments on the cell membrane.