CHARACTERIZATION OF CHOLESTEROL CRYSTAL NUCLEATION AND GROWTH FROM BIOLOGICAL MEMBRANES IN MACROPHAGE FOAM CELLS

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Atherosclerosis, the major precursor of cardiovascular disease, is characterized by the deposition of excessive cholesterol in the arterial intima.\textsuperscript{1} Atherosclerotic plaques build up in arteries in a slow process that initiates with uptake of LDL particles by macrophage cells, leading to deposition of cholesterol monohydrate crystals and cell death.\textsuperscript{1} Precipitation of cholesterol crystals is a crucial part of the pathological progression.\textsuperscript{2}

We suggested that the initial step in atherosclerosis development may be from cholesterol domains segregating in cell membranes and serving as nucleation sites for the formation of 3-dimensional (3D) cholesterol crystals.\textsuperscript{3,4} To verify whether this process can be relevant to in vivo processes, we have developed a high resolution correlative method combining cryo-soft X-ray tomography (cryo-SXT) and stochastic optical reconstruction microscopy (STORM) (see Figure).\textsuperscript{5} The approach provides 3D information on large cellular volumes at 70 nm resolution.\textsuperscript{5} Cryo-SXT morphologically identifies and localizes aggregations of carbon-rich materials, while STORM identifies specific markers on the desired epitopes, enabling colocalization between the identified objects and the cellular environment. Using a specific antibody (MAB 58B1) which labels cholesterol crystals,\textsuperscript{6} we identify and image crystals at a very early stage (200-400 nm) on the cell plasma membrane and in intracellular locations. This technique can in principle be applied to other biological samples where specific molecular identification is required in conjunction with high resolution 3D-imaging.

References:


Correlative Microscopy Workflow

Figure 1: Step1: Cells were grown on gold finder grids with fiducial markers that allow navigation to desired locations on the grid. The cells were incubated for 48 h with acLDL. Step2: Cells were fixed and incubated with primary and secondary antibodies. The super-resolution fluorescence signal was resolved by STORM. Step3: Grids were vitrified. Step4: X-ray tomograms were taken of the same cells that were analyzed by STORM. Data were reconstructed (field of view 18 μm³). Step 5: Overlay of the data in xy and z. Step 6: (A): 3D reconstruction of the same cell from a side view. The localization map of the corresponding resolved super-resolution image (red spots) is 1 μm in thickness and is located on the upper part of the cell, above the nucleus. Black rectangles indicate clusters of STORM signals. (B): Side orientation of the superimposed data combined with a perpendicular slice. (C1-F1) and (C2-F2) show magnifications of the crystals indicated by 1 and 2 in A, viewed in SXT, in STORM and superimposed, respectively.