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Super-resolution analysis of Caveolae and Cav1 scaffolds in Prostate Cancer Cells

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Upregulation of caveolin-1 (Cav1) correlates with reduced survival of prostate cancer patients. Cav1 is a key structural component of caveolae, 50-100 nm omega-shaped invaginations of the plasma membrane formed with the assistance of another protein, cavin-1 (or polymerase I and transcript release factor, PTRF). High levels of Cav1 and no PTRF in prostate cancer epithelium support a non-caveolar role for Cav1 in prostate cancer progression.

However, defining the domain distribution of Cav1 is difficult as both caveolae and non-caveolar Cav1 scaffolds are below the diffraction limit (~200 nm) for fluorescent microscopy and indistinguishable by standard optical microscopy approaches. Particle localization super-resolution microscopy is based on the stochastic activation (blinking) of small numbers of discrete fluorophores (using, for instance, ground state depletion or GSD) whose precise localization is determined using a Gaussian fit of the point-spread function. These approaches generate images with an X-Y resolution of ~20 nm and Z resolution of ~25-50 nm.

We applied GSD microscopy to Cav1-labeled PC3 cells, a metastatic prostate cancer cell line that expresses Cav1 but lacks PTRF and caveolae, and PC3 cells stably transfected with PTRF (PC3-PTRF) that form cell surface caveolae. For analysis, we represented the GSD 3D particle locations as nodes in a network, with distance-weighted inter-particle links, then calculated network measures e.g. density, degree. Our preliminary analysis has identified significant differences in Cav1 particle distribution and clustering between PC3 and PC3-PTRF cells. This approach will contribute to further understanding the structures and functions of different Cav1 membrane domains.