

## **CryoAPEX: inception, growth and evolution of the method**

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The application of electron tomography in the functional dissection of membrane remodeling within the subcellular space requires localization of candidate proteins in 3D space. The precise localization of membrane proteins at nanometer resolution requires an electron microscopy based robust detection technology coupled with sample preparation that confer superior ultrastructural preservation. Here, I provide step-by step detail of our method, cryoAPEX, which couples chemical fixation and high-pressure freezing of cells with peroxidase tagging (APEX) to allow precise localization of membrane proteins in the context of a well-preserved subcellular membrane architecture. The superior membrane preservation obtained by the cryoAPEX method makes it amenable to electron tomography, arming the versatile APEX tagging technology to answer questions in the realms of organelle biogenesis and in situ membrane remodeling. Question based evolution of the method in its application in virus cell biology will be discussed.