Sub-mitochondrial map of intermembrane space bridging components

Our work reports on generally applicable tools for the biological community that are now allowing researchers to address one of the most commonly asked questions in biomedical research, “What is interacting with or in proximity with a molecule or organelle of interest?” Protein engineering and chemical synthesis were used to create a suite of tools that push the boundaries of protein imaging in cells. MiniSOG and APEX2 are introduced as types of genetically encoded tags for electron microscopy. They reveal the nanometer-scale localization of specific tagged proteins and can now be used for proximity proteomics. For the first time, the transformative technology of high-resolution imaging through multi-tilt tomography driven by unique TxBR software has mapped the sub-mitochondrial 3D locations of miniSOG and APEX2-tagged mitochondrial Intermembrane space bridging (MIB) proteins.

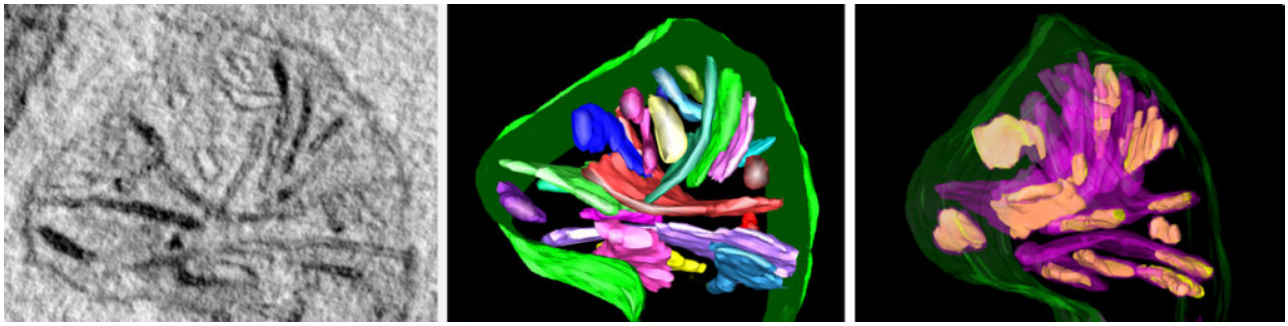

Fig. 1. Left: A 1-nm slice through the center of a tomographic volume from a HL-1 cell showing Mic19-miniSOG labeling (dark stain) that partially lines the inside of the cristae. Middle: The segmented and surface-rendered volume of the mitochondrion. The outer mitochondrial membrane is green and the cristae are in various colors. HL-1 cells are densely packed with cristae. Right: Same orientation but showing the Mic19-positive domains (green) inside the cristae (translucent magenta).

MIB composition and function has been a timely and prolific line of investigation given its emerging role in mitochondrial organization. Perhaps the most important components of the large MIB complex are Mic60 (also called mitofilin), which resides in the inner mitochondrial membrane, Mic19 (also called ChChd3), which is tethered in the mitochondrial intermembrane space and Sam50, which is found in the outer mitochondrial membrane. The key conceptual advance of our work is the deciphering of the sub-compartmental locations of Mic19, Mic60 and Sam50 in the environment of structural landmarks such as cristae and crista junctions (CJs).

Key Results

Tagged Mic19 (Fig. 1) and Mic60 (Fig. 2) are located at all CJs, distributed in a network pattern along the mitochondrial periphery, and also enriched inside cristae.
The Mic19 and Mic60 labeled regions do not fill the cristae volumes; the MIc19 regions were in one discrete shape yet the Mic60-positive domains had several smaller shapes distributed throughout that did not fill the cristae volumes to the same extent as Mic19.
Mic19 associates with cytochrome c oxidase subunit IV.
Tagged Sam50 is not uniformly distributed in the outer mitochondrial membrane and appears to incompletely overlap with Mic19 or Mic60-positive domains, most notably at the CJs.

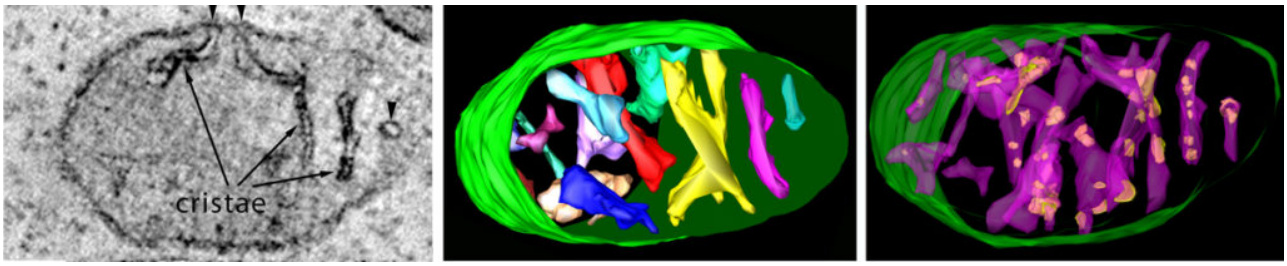

Fig. 2. Left: A 1-nm slice through the center of a tomographic volume from a human primary astrocyte showing a patchy pattern of Mic60-APEX2 labeling inside the cristae. Middle: The segmented and surface-rendered volume of the mitochondrion. The outer mitochondrial membrane is green and the cristae are in various colors. Right: Same orientation but showing the 3D patchy pattern of Mic60-positive domains (green) inside cristae (translucent blue).

These findings help to determine the MIB's physiological function and interaction partners.

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