Engineered ascorbate peroxidase (APEX)-mediated proteomic mapping and fingerprinting

Yasunori Kajiki

2016/11/04

Abstract:

Obtaining complete protein inventories for subcellular regions is a challenge that often limits our understanding of cellular function, especially for regions that are impossible to purify and are therefore inaccessible to traditional proteomic analysis. Previously, the authors developed a method relies on a genetically targetable engineered ascorbate peroxidase (APEX) that biotinylates nearby proteins, which are subsequently purified and identified by MS. They used this approach to identify 495 proteins within the human mitochondrial matrix, including 31 not previously linked to mitochondria. Recently, they use APEX-generated molecular labeling patterns to provide information regarding intracellular microenvironments in living cells. Using APEX labeling of specific proteins, they uncovered subcellular localization at subcompartmental resolution and successfully elucidated the membrane protein topology of HMOX1 and sub-mitochondrial localization of recently identified mitochondrial proteins. This method can be expanded to confirm sub-mitochondrial localization and membrane topologies of previously identified mitochondrial proteins.

References:

1. Song-Yi Lee, Myeong-Gyun Kang, Jong-Seok Park, Geunsik Lee, Alice Y. Ting, and Hyun-Woo Rhee. APEX Fingerprinting Reveals the Subcellular Localization of Proteins of Interest. Cell Reports 15, 1837 (2016).
2. Hyun-Woo Rhee, Peng Zou, Namrata D. Udeshi, Jeffrey D. Martell, Vamsi K. Mootha, Steven A. Carr, Alice Y. Ting. Proteomic Mapping of Mitochondria in Living Cells via Spatially Restricted Enzymatic Tagging. Science 339, 1328 (2013).
3. Jeffrey D Martell, Thomas J Deerinck, Yasemin Sancak, Thomas L Poulos, Vamsi K Mootha, Gina E Sosinsky, Mark H Ellisman & Alice Y Ting. Engineered Ascorbate Peroxidase as a Genetically Encoded Reporter for Electron Microscopy. Nat. Biotech. 30, 1143 (2012).