Engineered heme peroxidases as genetically encoded probes with diverse applications in cell biology

Fluorescent proteins such as GFP have been revolutionary in allowing specific proteins to be tracked within living cells, but the resolution of fluorescence microscopy (~200 nm) is often inadequate for determining precise subcellular localization. Electron microscopy (EM), on the other hand, offers far superior resolution (<10 nm), but no genetic tag equivalent to GFP has been developed for EM. I will describe the development of enhanced ascorbate peroxidase (APEX), a genetic tag that generates EM contrast in all cellular compartments, and the application of APEX for EM studies of previously uncharacterized mammalian proteins. I will also demonstrate the utility of APEX for applications beyond EM, including spatially-resolved proteomic mapping in living cells. Finally, I will describe the development of a split horseradish peroxidase (HRP) protein complementation assay for detection of intercellular protein-protein interactions and visualization of neurological synapses.

References