A Modular Covalent Labeling System for Ground-Truth Localization of Synaptic Proteins by Cryo-electron Tomography

Richard Held[1], Maia Azubel[2], Jiahao Liang[2], Axel Brunger[1]
[1]Howard Hughes Medical Institute, Stanford University, [2]Stanford University
rgsheld@stanford.edu

Neuronal synapses are micron sized cell-cell contacts that serve as sites of information transfer in the nervous system. Single synapses are densely crowded, with thousands of proteins taking part in dozens of signaling pathways. To regulate sensitivity, selectivity, and crosstalk between these pathways, synapses control the nanometer-scale topography of their proteome. Cryo-electron tomography (cryoET)—in combination with focused ion beam milling and sub-tomogram averaging—is a powerful technique to determine in situ protein structures. The positions and orientations of particles contributing to the averaged structure can then be mapped back to the original tomogram to gain insight into protein topography. However, synapses and other crowded cellular environments present challenges to accurately identifying a protein of interest. This is further exacerbated by the presence of structurally similar non-target proteins and the low resolution of individual subtomogram volumes. To realize the potential of cryoET for visualization of nanoscale protein topography, we have developed a modular system to covalently label endogenously-expressed membrane proteins with gold nanoparticles (AuNPs). AuNPs are homogenous, can be tuned in size, and are amenable to monomeric functionalization to target extracellular terminal ends or loops. We apply our labeling system to localize AMPA-type glutamate receptors within tomograms of neuronal synapses, revealing a distinctive clustered receptor topography with defined offsets from sites of neurotransmitter release. Furthermore, our system facilitates in situ quantification of receptor copy number and subunit stoichiometry—hallmarks of visual proteomics—and enables accurate particle picking for subsequent sub-tomogram averaging of receptors and associated scaffolding proteins. Our method is broadly applicable to target proteins with extracellular epitopes, can be readily implemented in most wetlabs, and should enhance cryoET as a method for both in situ structural biology and the mapping of nanoscale protein topography.