Fluorescence signal amplification using engineered peroxidase (FLEX) for correlative light and electron microscopy imaging approach

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Electron Microscopy is the most reliable technique for identification of protein localization. However, for large scale sample, EM application becomes tedious for visualizing target proteins. Hence fluorescence microscopy in combination with electron microscopy (CLEM) is used to visualize the region of interest in large sample size. Here we devised a genetically encodable enzymatic fluorescence signal amplification for electron microscopy using engineered peroxidase (FLEX). For this method we tested various fluorescent APEX substrates for signal amplification. Utilizing this probe generated a restricted signal amplification of protein of interest in respect to conventional AmplexRed. Furthermore, our probe was resistant to osmium staining and Epon embedding. This property enables correlative light and electron microscope (CLEM) imaging approaches using APEX. With various APEX-POI targeted to the organelles, we observed their localization via FLEX imaging with well preserved ultra-structures. Overall, our method combines the genetic targeting of APEX-POI along with resistant fluorescent signal amplification for CLEM with broader multi cellular application.