Dual-color CLEM imaging for genetically encodable enzymatic fluorescence signal amplification method using APEX (FLEX)

Dr. Min Kyo Jung[1], Dr. Nirmali Sharma[2,3], Dr. Pratyush Kumar Mishra[2], Prof. Hyun-Woo Rhee[2,4], Dr. Ji Young Mun[1]
[1]Neural Circuits Research Group, Korea Brain Research Institute, Seoul, Korea, [2]Department of Chemistry, Seoul National University, Seoul, Korea, [3]Department of Chemistry, Ulsan National Institute of Science and Technology (UNIST), Ulsan, Korea, [4]School of Biological Sciences, Seoul National University, Seoul, Korea

Background
Correlative Light and Electron Microscopy (CLEM) is a useful imaging approach that allows us to obtain two types of different imaging information from the same sample. Fluorescence microscopy (FM) provides multi-color information on different proteins of interest (POIs), while electron microscopy (EM) reveals the ultrastructure of cellular organelles. As a result, CLEM, which has become an essential tool in cell biology and neuroscience, enables a comprehensive understanding of POIs and their connections to intracellular structures or subcellular organelles1,2. For fluorescence-mediated visualization of intracellular regions of interest, fluorescent proteins (FPs) are commonly used. However, the fluorescence of intracellular FPs is often lost, especially during harsh EM sample preparation, such as fixation, dehydration, heating and resin embedding3. Especially for highly dynamic organelles such as mitochondria and lysosomes, the precise localization of these organelles can change as they are observed live in the light microscope and are actively moving even during the short delay time for fixation. To overcome this problem, we developed a fluorescent reporter that can effectively maintain its fluorescence signal under harsh EM sample processing conditions and developed a method to obtain distortion-free CLEM images using it.

Methods
Given the difficulty of maintaining the fluorescence signal during EM sample preparation with conventional fluorescent proteins, we use a two-colour CLEM technique that integrates small molecule probe-based signal amplification using genetically encoded peroxidases with mEosEM4, which retains fluorescence even in strong fixatives. In particular, based on our previous findings on peroxidase-based fluorescent substrates, we synthesised and applied to CLEM imaging a newly designed JF-induced probe based on the styryl-benzothiazolium phenol probe genfluor (JF), known for its fluorescent signal amplification by peroxidase5.

Results
As a results, we developed a fluorescent probe (JFT1) that exhibits a fluorescence-amplified signal when APEX2 is expressed. Unlike conventional fluorescent labels or fluorescent proteins that lose their fluorescence upon OsO4 pretreatment, JFT1 produces a well-retained and restricted fluorescence pattern in both OsO4 pretreated and OsO4 post-treated samples. The application of FLEX targeting to lysosomes in conjunction with mito-mEosEM facilitated the visualization of interactions between lysosomes and mitochondria. It also revealed distinct contacts between these two organelles in response to the lysosomal stressors bafilomycin and U18666A. Furthermore, the interactions observed under the influence of bafilomycin and U18666A revealed distinct mechanisms of action as indicated by CLEM imaging. Taken together, we propose that our FLEX approach is a very useful way to target different APEX-POI combinations for fluorescence and high-resolution EM imaging.

Keywords:

CLEM. APEX, Proximity-labeling, Fluorescent probe

Reference:

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