An osmium acid- and Epon embedding-resistant fluorescent protein for superresolution CLEM

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Superresolution correlative light and electron microscopy (SR-CLEM) is a powerful tool for localizing a specific molecular context under electron microscopy (EM) beyond the diffraction limit of light microscopy (LM). The right sampling method and labeling fluorophore are critical to maintain both bright fluorescent signals for LM and ultrastructure of the EM images. Epon epoxy resin is superior to other resins for conventional EM chemical fixation because it preserves the cellular ultrastructure and has better sectioning properties. However, no fluorescent protein (FP) was reported to survive Epon embedding after osmium tetroxide (OsO4) fixation for use in SR-CLEM.

Here, we have developed an OsO4 fixation-resistant photoconvertible FP (PCFP), PCEM, which not only is much brighter than mEos4b after OsO4 treatment but also retains its fluorescence and photoswitching property after Epon embedding. PCEM and Epon-embedding-based SR-CLEM microscopy (ESR-CLEM) enable the visualization of biomolecules and their cellular context in the same section.

Fig.1 Epon-embedding-based SR-CLEM using the same-section approach
Scale bar (main image), 2 μm. Scale bars (zoomed in images), 1 μm.

We labeled mitochondria and nuclear laminar structure with mEosEM in CHO cells, and imaged them with conventional electron microscopy and light microscopy, then we realized super-resolution correlative light and electron microscopy (Fig.1).

REFERENCES