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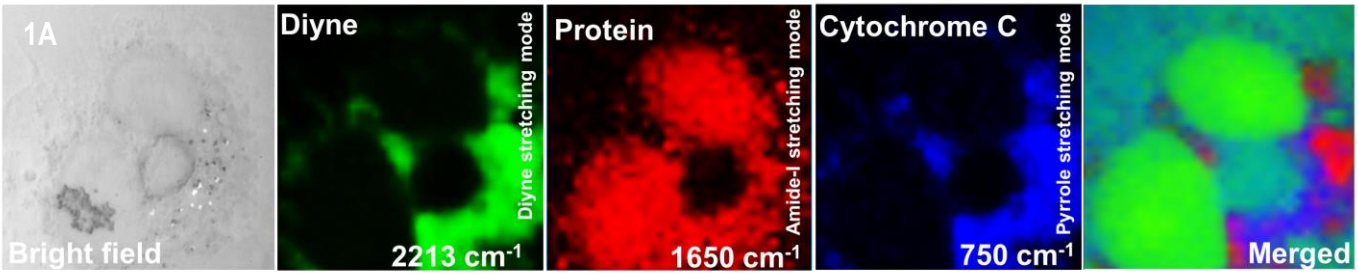
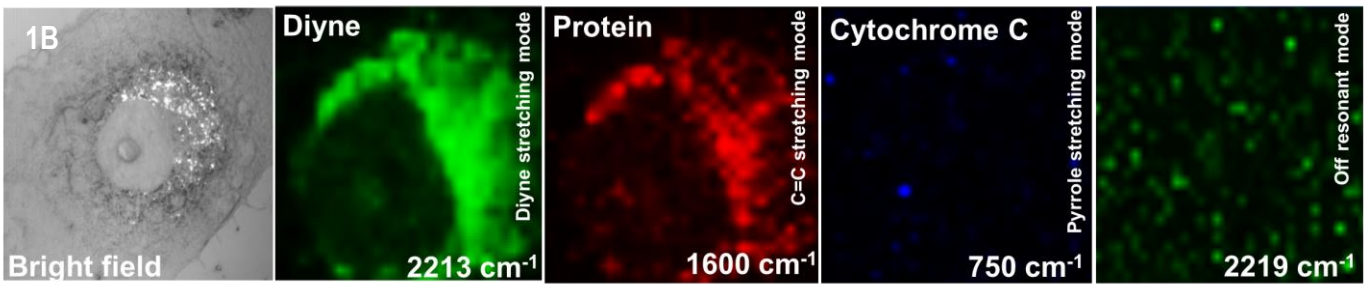
A genetically encoded Raman probes for organelle-specific labeling: unleashing the power of Raman microscopy for bioimaging

Raman microscopy, with its spectral sensitivity to local electric fields, holds promise for probing intracellular environments. Yet, the weak Raman scattering signal, about 10^{10} times weaker than fluorescence emission, has limited its use in molecular profiling of intracellular organelles. To overcome this, we combined proximity labeling with spontaneous Raman microscopy, using APEX2 for site-specific labeling of the Raman probe. This approach precisely locates new diynes probes within organelles, offers a strong signal in the cell silent window (1800-2800) cm^{-1}, and maintains photostability. It enables rapid imaging, outperforming conventional confocal Raman microscopy in acquisition time. Organelle-specific Raman images are generated in under 5 minutes, relying on the 2213 cm^{-1} peak intensity of polyyne probe. Colocalization with the pyrrole breathing mode of cytochrome C validates Raman mapping specificity (**Figure 1A**). The diyne stretching mode signal is an order of magnitude stronger than cytochrome C pyrrole breathing mode and EdU alkyne mode, resulting in shorter acquisition times and lower laser power for live cell imaging **(Figure 1B)**.

Figures



Acquisition time: 1s, Accumulation 2, laser power: ~10 mW/μm², exposure time: 42 m.



Acquisition time: 0.25s, Accumulation 2, laser power: ~ 3.25mW/μm², exposure time: 11:42 m

Figure 1: Bright field images and Raman images show colocalization of both diyne stretching mode and the pyrrole breathing mode of cytochrome C at mitocondria and confirm the strenth of diyne signal at very short measuring time