Multicolor Activatable Raman Probes for Simultaneous Detection of Plural Enzyme Activities

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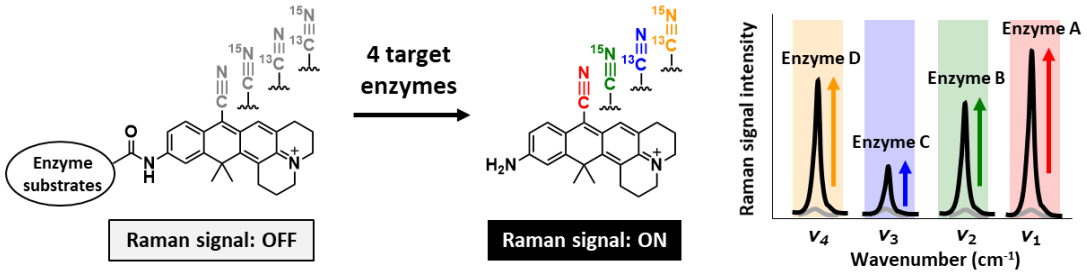
Raman microscopy, which can characterize molecular species by detecting the vibrations of chemical bonds, has been developed as an important imaging modality to characterize cellular biochemical constituents without labeling. In the past decade, various Raman tags, including alkyne, nitrile and the carbon–deuterium bond, which show characteristic vibrational frequencies in the cell-silent region (1800–2800 cm^{-1}), have been developed for bioorthogonal imaging in live cells and tissues. However, most of the pre-existing Raman probes show constant Raman signal intensity ("always-on" Raman probes), and thus their application has been limited to labeling tags.

In this study, we present a general strategy to prepare activatable Raman probes that show enhanced Raman signals due to electronic pre-resonance (EPR) upon reaction with enzymes under physiological conditions. We identified a xanthene derivative bearing a nitrile group at position 9 (9CN-JCP) as a suitable scaffold dye, and synthesized four types of activatable Raman probes, which are targeted to different enzymes (three aminopeptidases and a glycosidase) and tuned to different vibrational frequencies by isotope editing of the nitrile group. We validated the activation of the Raman signals of these probes by the target enzymes and succeeded in simultaneous imaging of the four enzyme activities in live cells. As our design strategy should be generally applicable for developing activatable Raman probes targeted to other specific biomolecules or biological processes, we believe multiplex imaging with these functionalized Raman probes will be a powerful tool for studying complex biological and pathological phenomena.



1) H. Fujioka, et al. *J. Am. Chem. Soc*. **2020**, *142*, 20701–20707.