

Vibrational Imaging of Lipid-Associated Enzymes Using Bioorthogonal Small-Molecule Probes

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Abstract

Understanding the spatial organization and regulation of enzymes involved in lipid metabolism is essential for deciphering cellular signaling pathways yet remains challenging due to the limited availability of minimally perturbative imaging tools. Conventional fluorescence-based approaches often rely on bulky labels that can alter molecular behavior, while label-free techniques lack target specificity.

Vibrational imaging methods, such as Raman and stimulated Raman scattering (SRS) microscopy, offer chemical specificity without the need for fluorescent tags, but their application to selective enzyme visualization has been limited.¹⁻⁴ Understanding the spatial organization of enzymes involved in lipid metabolism remains a major challenge due to the lack of minimally perturbative imaging tools.

Here, we present a bioorthogonal alkyne-tagged small-molecule probe that enables vibrational imaging of monoacylglycerol lipase (MAGL), a key enzyme in the endocannabinoid system, inside cells. The alkyne reporter generates a distinct Raman signal in the cellular Raman-silent region, allowing detection by spontaneous Raman and stimulated Raman scattering (SRS) microscopy without the need for fluorescent labels. Vibrational imaging reveals efficient cellular uptake of the probe and its enrichment in lipid-rich intracellular regions, consistent with the known lipid-associated function of MAGL. SRS microscopy enables rapid, high-contrast imaging with subcellular resolution, while correlative fluorescence lifetime imaging microscopy using a

matching fluorescent MAGL probe confirms target engagement and demonstrates compatibility of vibrational and fluorescence readouts.

Furthermore, we have obtained results from several complementary vibrational probe designs, including covalent inhibitors and activity-dependent turn-on probes, demonstrating the generality of this approach for enzyme visualization and functional imaging in complex cellular environments. Together, these results highlight the versatility of bioorthogonal vibrational probes for studying lipid-associated enzymatic processes in cells.

Acknowledgement

S.F.E.-M. is grateful to Hoffmann-La Roche for funding (RPF-638).

References

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