Extended Abstract
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Stable isotope probing coupled Raman microscopy: An efficient way to study single cell biochemistry

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Abstract

Lipid droplets have been hypothesized to be intimately associated with intracellular proteins. However, there is little direct evidence for both spatiotemporal and functional relations between lipid droplets and proteins provided by molecular -level studies on intact cells. To elucidate the interplay between them at the single cell level, Raman microscopy was coupled with a very powerful strategy, namely, stable isotope labeling. Here, I present in vivo time lapse Raman imaging, coupled with stableisotope (13C) labeling, of single living Schizosaccharomyces pombe cells. Our results show that the proteins newly synthesized from incorporated 13C-substrate are localized specifically to lipid droplets as the lipid concentration within the cell increases. Lipids, which help to store energy in a compact form, have variety of roles in biological systems and their metabolism is central to life. Here, we show that combination of stable isotope probing (SIP), Raman micro-spectroscopy and multivariate curve resolution analysis can serve as a valuable approach in metabolomics research. We studied ergosterol biosynthesis in single living fission yeast cells, grown in mixtures of normal (12C) and 13C-glucose as the sole carbon source.

Microbial communities are essential to many ecosystems and interact in complex ways with almost all eukaryotes. Therefore, a detailed understanding of the functioning of such communities is a basic requirement for biologists, including microbiologists and microbiome researchers. Using a single Raman microspectroscopy cell, the biochemical fingerprints of cells by other microbials can be obtained without the label and without injury. When combined with stable isotope testing (SIP), Raman spectroscopy can directly detect the activities of microorganisms in their natural environment. This review provides an overview of the various SIP methods that should be integrated with the various Raman distribution processes and shows how a single Raman SIP cell can be directly linked to omics-centric analysis pipelines investigating viral communities.

By carefully looking into the biosynthetic pathways and by comparing the observed peak positions with calculation results on isotope-substituted ergosterol, it is possible to understand how 13C is incorporated in the conjugated C=C moiety of the molecule. The multivariate spectral data analysis revealed intrinsic spectra and their relative abundances of all isotopomers.

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