

# Exploring the effects of isotope depletion on proteins by native mass spectrometry and cryogenic electron microscopy

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Isotope depleted protein samples have successfully addressed various challenges in native mass spectrometry (MS), notably by enhancing the signal-to-noise (S/N) ratio- an advantage particularly beneficial for high molecular weight protein analysis. In this study, we explore the broader impact of isotope depletion on reducing sample heterogeneity, and enhancing mass spectral quality in MS, as well as in improving imaging resolution in cryogenic electron microscopy (cryo-EM). Together, these two techniques offer a more detailed visualization of higher-order molecular structure of proteins with high spatial and mass resolutions.

We successfully expressed and purified test proteins of varied MW, in isotope depleted media. Native mass spectra of these samples showed a distinctive shift towards monoisotopic peaks and a reduction in mass window as compared to the proteins grown in normal media, confirming effective isotope depletion. The improved S/N ratio also enhanced sequence coverage in top-down proteomic analysis in isotope depleted proteins compared to the normal ones. The study extends to cryo-EM to assess the potential improvements in imaging resolution and any structural alterations induced by isotope depletion.

## User consent

yes

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