Multiplexed Nominal Mass and Sub-Da Isotopologue Quantitative Proteomic Reagents

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Summary

We have assembled a versatile molecular scaffold from simple building blocks to create binary and multiplexed stable isotope reagents for quantitative mass spectrometry. The basic idea is to mix peptides from a protein expressed under one set of conditions and labeled with diglycine containing $^{13}C_2$, with peptides from a protein expressed under another set of conditions and labeled with diglycine containing $^{15}N_2$. The two labeled peptides thus differ in mass by ~0.0127 Da per primary amine. The relative amounts of the two peptides (and thus the proteins from which they derive) may thus be measured from a single ultrahigh-resolution Fourier transform ion cyclotron resonance mass spectrum.

These Protected Amine Label (PAL) reagents offer multiple analytical figures of merit including, (i) robust targeting of peptide N-termini and lysyl side chains, (ii) optimal mass spectrometry ionization efficiency through regeneration of primary amines on labeled peptides, (iii) an amino acid-based mass tag that incorporates heavy isotopes of carbon, nitrogen, and oxygen to ensure matched physicochemical and MS/MS fragmentation behavior among labeled peptides, and (iv) a molecularly efficient architecture, in which most of the heteroatom centers can be used to synthesize a variety of nominal mass and sub-Dalton isotopologue stable isotope reagents. We demonstrate the performance of these reagents in traditional MS-based quantitation strategies whereby isotopomers are separated by 4 Da, as well as novel approaches in which peptide isotopologues differ in mass based on the nuclear binding energy in carbon and nitrogen nuclei, enabling quantification in MS or MS/MS spectra.

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References

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