^{18}O Labeling

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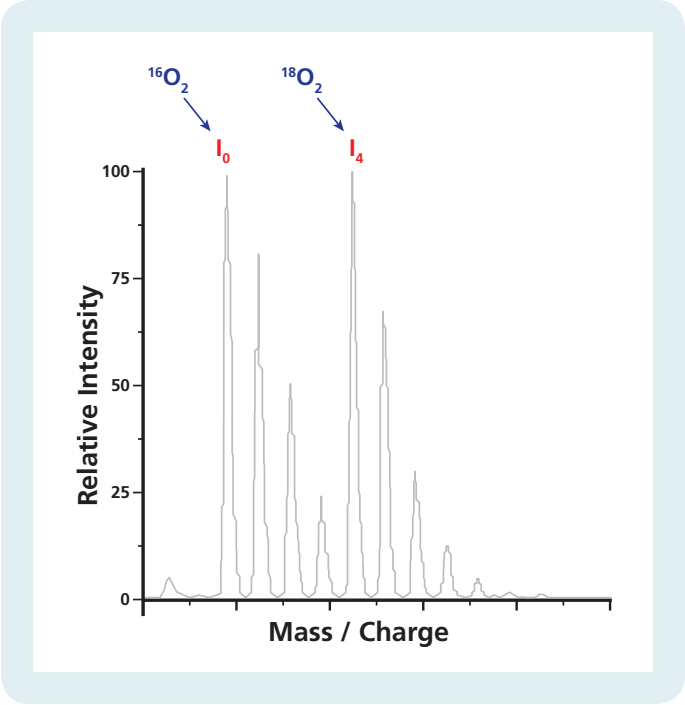
$^{18}O_2$-labeling is the Linux of isotope-labeling methods. Any laboratory can buy Water (^{18}O, 99%) (OLM-240-99) and adapt the method to its own applications. It offers a universal strategy for uniform labeling of all peptides from any kind of protein, including modified proteins.[1] It is used to label clinical samples with unrivaled sensitivity.[2, 3] The only byproduct is water, and the immobilized catalytic enzyme can be removed mechanically. Labeling with $^{18}O_2$ is limited to binary comparisons or series thereof, and it requires a workflow with minimal manipulation of proteins since the light and heavy samples are combined at the peptide level.

Two atoms of ^{18}O are introduced into the carboxylic acid group of every proteolytic peptide in a protein pool that has been catalyzed by members of the serine protease family, which includes trypsin, Glu-C protease, Lys-C protease and chymotrypsin. In the binding site of each protease, the residue of choice is covalently bound in a tetrahedral intermediate, which is then disrupted by nucleophilic attack by a water molecule, cleaving the protein. The C-terminal residue in each peptide product is re-bound by the protease, *e.g.* Arginine and Lysine in the case of trypsin, and released by hydrolysis. If the peptide products are incubated with the catalytic enzyme in Water (^{18}O, 99%) the level of ^{18}O in the peptides will eventually equilibrate with the level of ^{18}O in the solvent, preferably >95%. Peptide binding by the protease offers the advantage that cleavage of the protein can be optimized and carried out separately from labeling the peptide.[4]



Each heavy peptide weighs 4 Da more than its $^{16}O_2$ light analog. After labeling, the mixtures of heavy and light peptides are mixed, and isotope ratios of peptide pairs are determined by LC/MS. Concurrent MS/MS measurements and appropriate computer algorithms can provide peptide identification along with quantitation.

References

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