Title: Proteomic strategies for comparative and quantitative analysis based on stable isotope labeling and mass spectrometry

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The use of stable isotope labeling in biological mass spectrometry has extended the use of the technique from structural characterization and identification to precise quantitative measurements and comparisons. In our lab, we have developed two methods based on stable isotope labeling that address different analytical questions.

The first method addresses an often-encountered problem: how to distinguish which protein components are unique to one protein population out of two or more. The method is based on enzymatic 18O-labeling of proteolytic peptides, followed by LC-MALDI MS analysis. An example is given in which this method was used in protein-protein interaction experiments to distinguish interacting protein ligands isolated by affinity pull-down, from non-specific interactants present in control samples.

The second method presented allows one to monitor, on a proteomic scale, changes in protein synthesis and degradation in response to an induced systemic perturbation. The onset of the perturbation of the cell culture is coupled to the exchange of the culture growth medium to a medium enriched in the stable isotope 15N. Proteins, isolated from cell cultures harvested at time points following the onset of the perturbation, are analyzed by mass spectrometric peptide mass fingerprinting. By measuring the intensity ratios of the mass spectrometric signals corresponding to the 15N- and 14N-incorporated peptide forms relative to the ratios obtained for unperturbed control cell cultures, it is possible to detect alterations in protein synthesis and degradation resulting from the perturbation. Examples are shown for HeLa cells subjected to heat stress.