Quantification in MS proteomics

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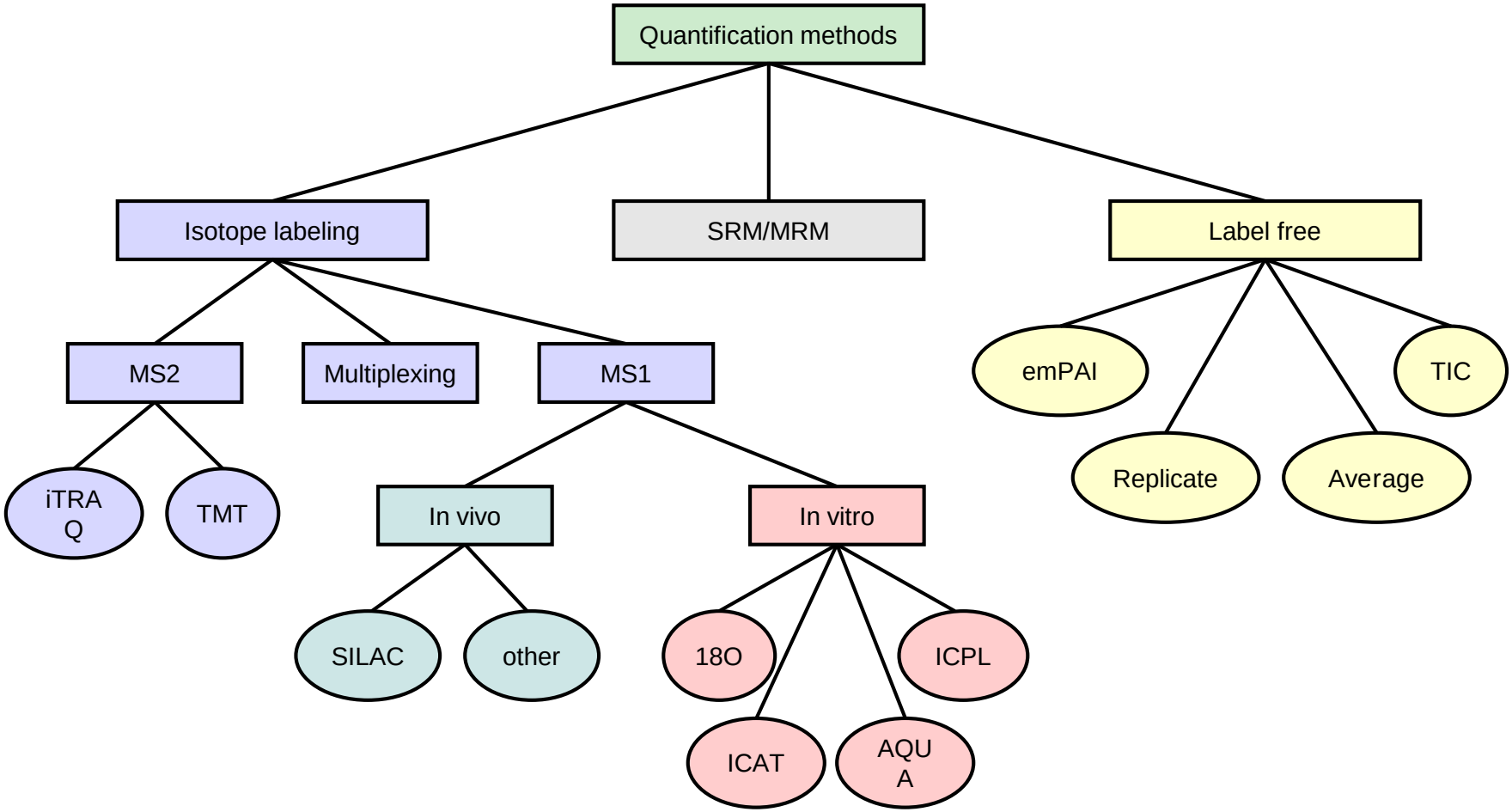
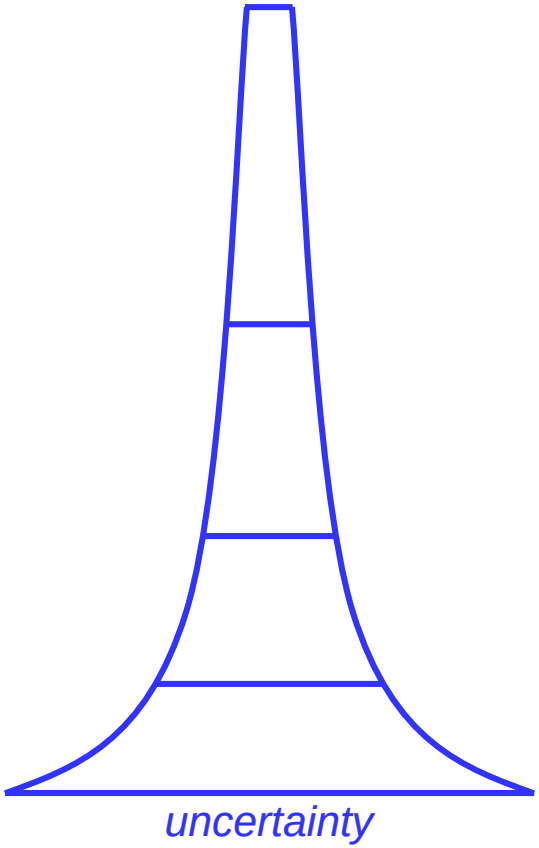
Protein quantification by MS in one slide



Vaudel, Proteomics, 2010

Data processing introduces uncertainty

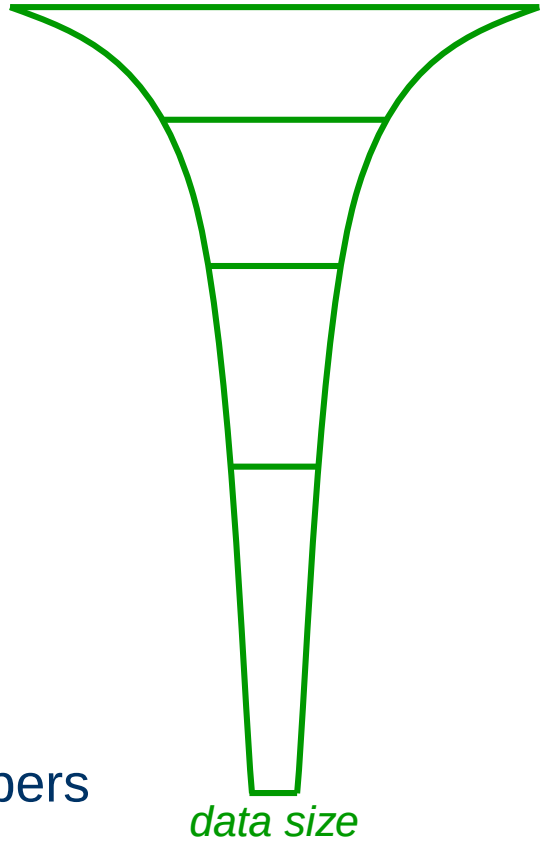


Raw data

Peaklists

Peptide sequences

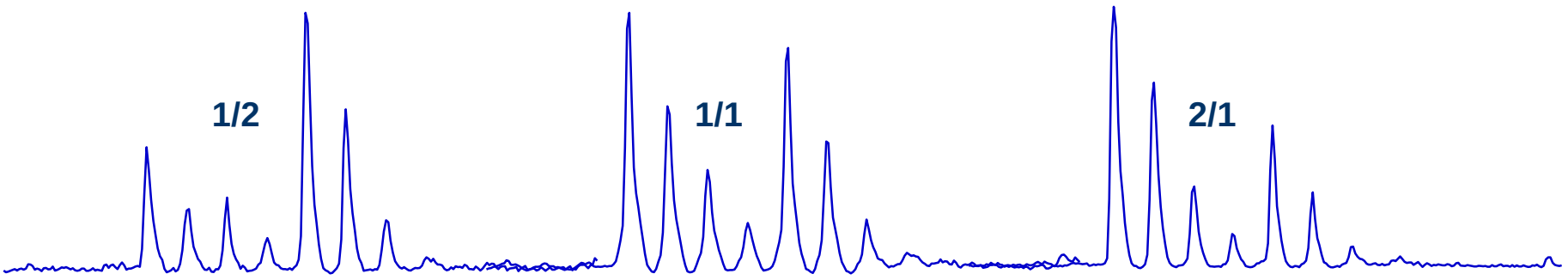
Protein accession numbers



Martens and Hermjakob, Molecular BioSystems, 2007

The primary principles in quantitation

- Make each sample distinguishable
  - ✓ *fluorescent markers with different excitation wavelengths* (1)
  - ✓ *introduce mass differences between the samples* (2)
  - ✓ *perform distinct experimental runs for each sample* (3)
- Measure the intensity of the signal for each analyte in each sample
- Statistically process the accumulated information



Techniques: overview

SILAC (2), cell cultures, relative

2D PAGE spot intensity (1), proteins, relative

ICAT (2), proteins, relative

ICPL (2), proteins, relative

LC peak area (3), peptides, relative, absolute

Trypsin-mediated ^{18}O incorporation (1), peptides, relative

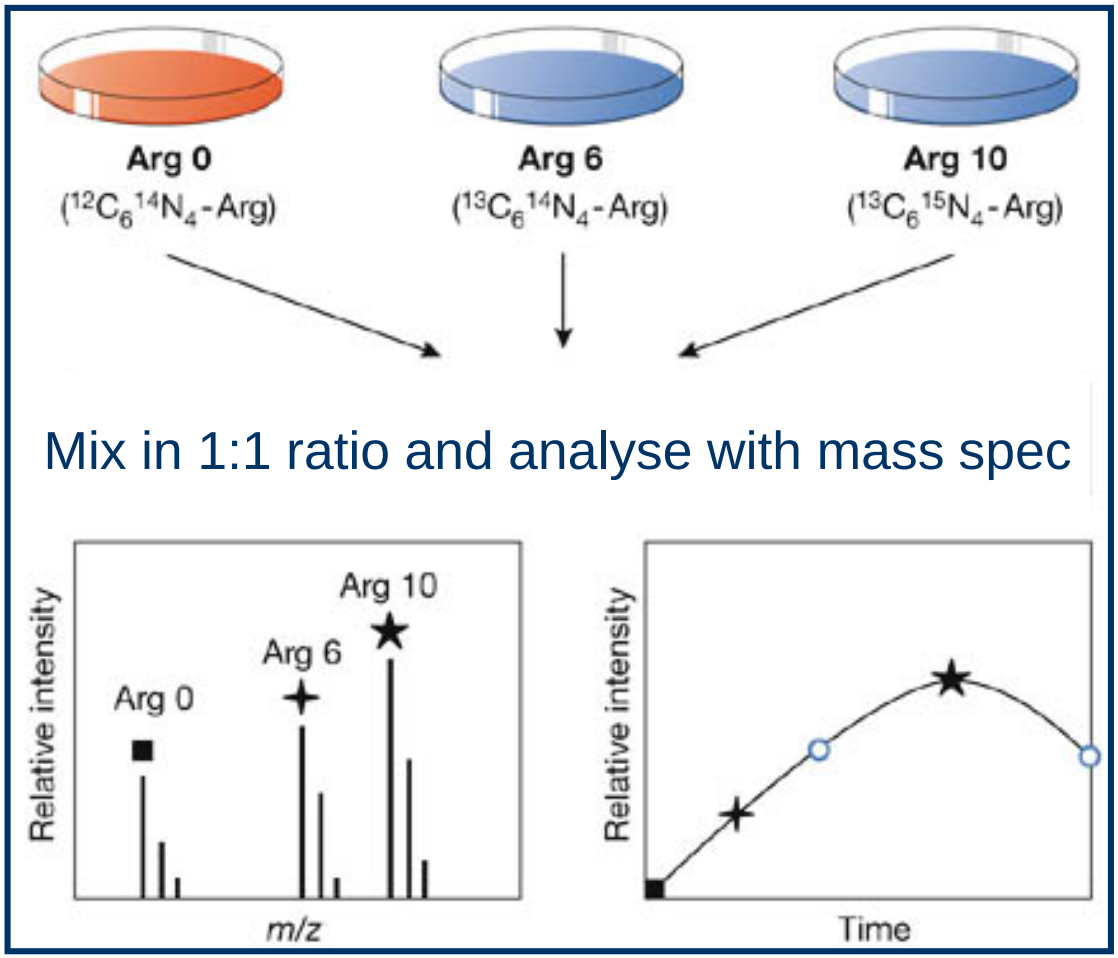
iTRAQ (2), peptides, relative

Spiked peptides (eg. AQUA) (2), peptides, absolute

Label-free approaches (3), peptides, peptide fragments, relative, *absolute*

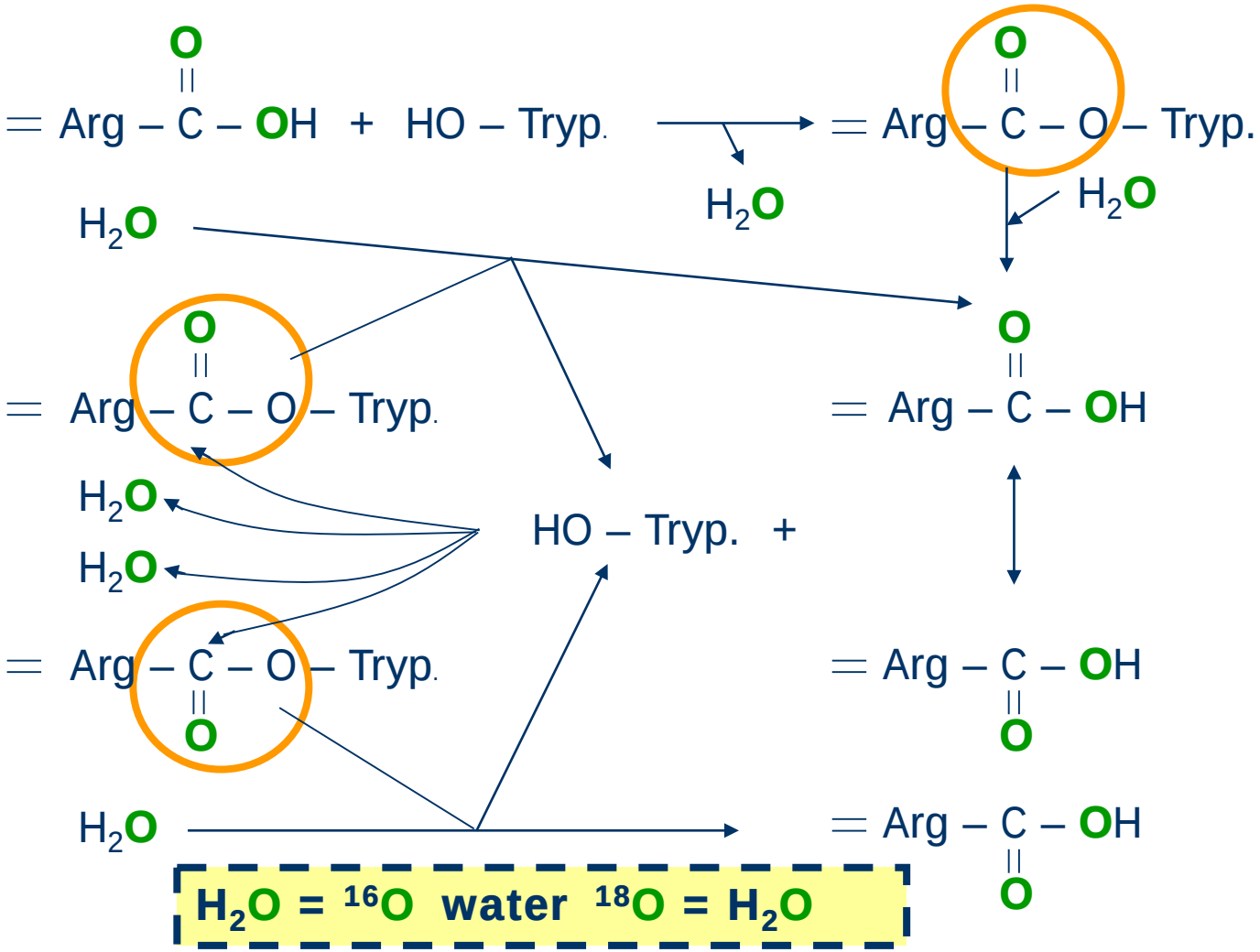
MRM (2, 3), peptide fragments, relative[2]

SILAC



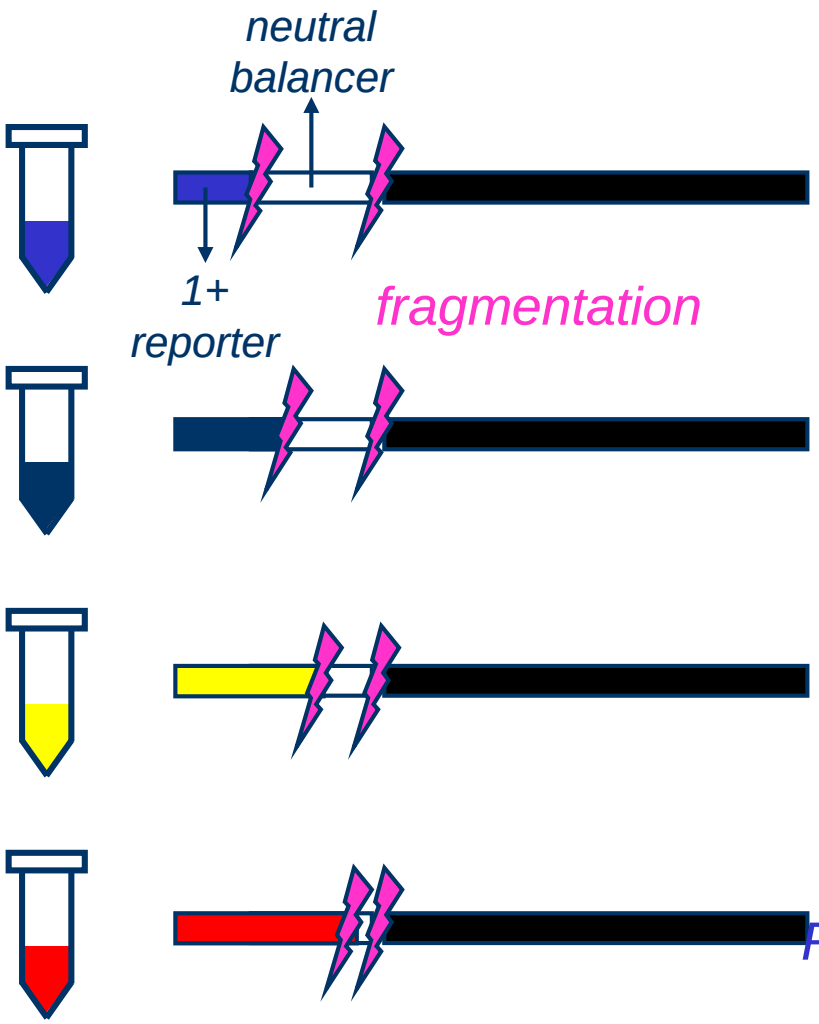
Andersen and Mann, EMBO Reports, 2006

^{16}O – ^{18}O labelling

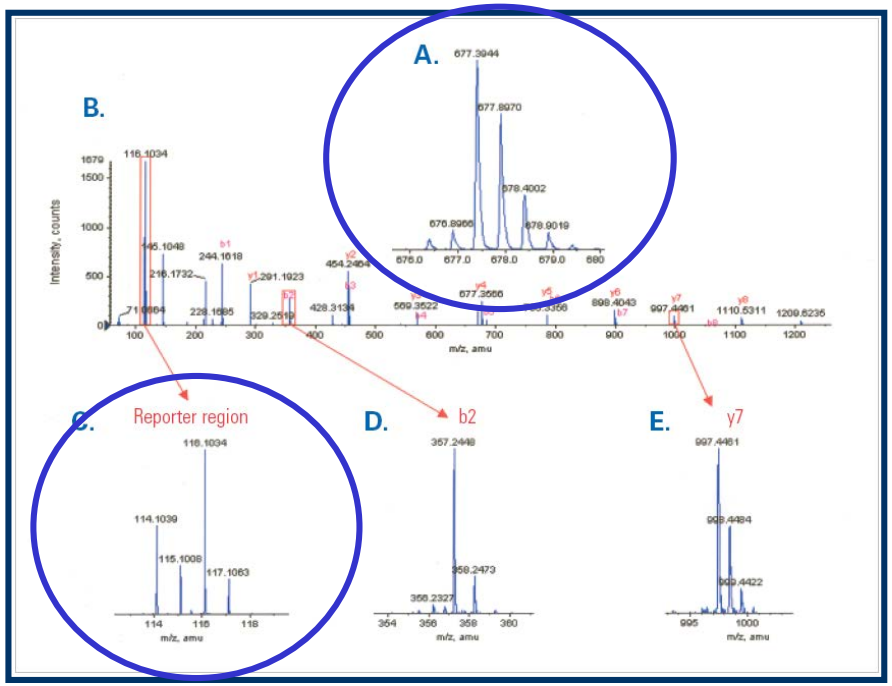


Staes, Journal of Proteome Research, 2004

iTRAQ / TMT



precursors

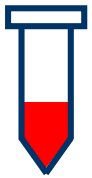


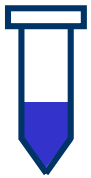
Reporter fragments

Applied Biosystems, Product Bulletin iTRAQ™ Reagents

AQUA

Aimed at absolute quantitation



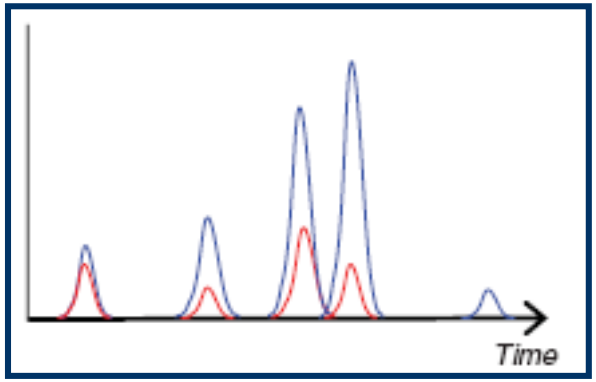


sample
unlabelled peptides
unknown abundance

internal standard
labelled, synthesized peptides
known abundance

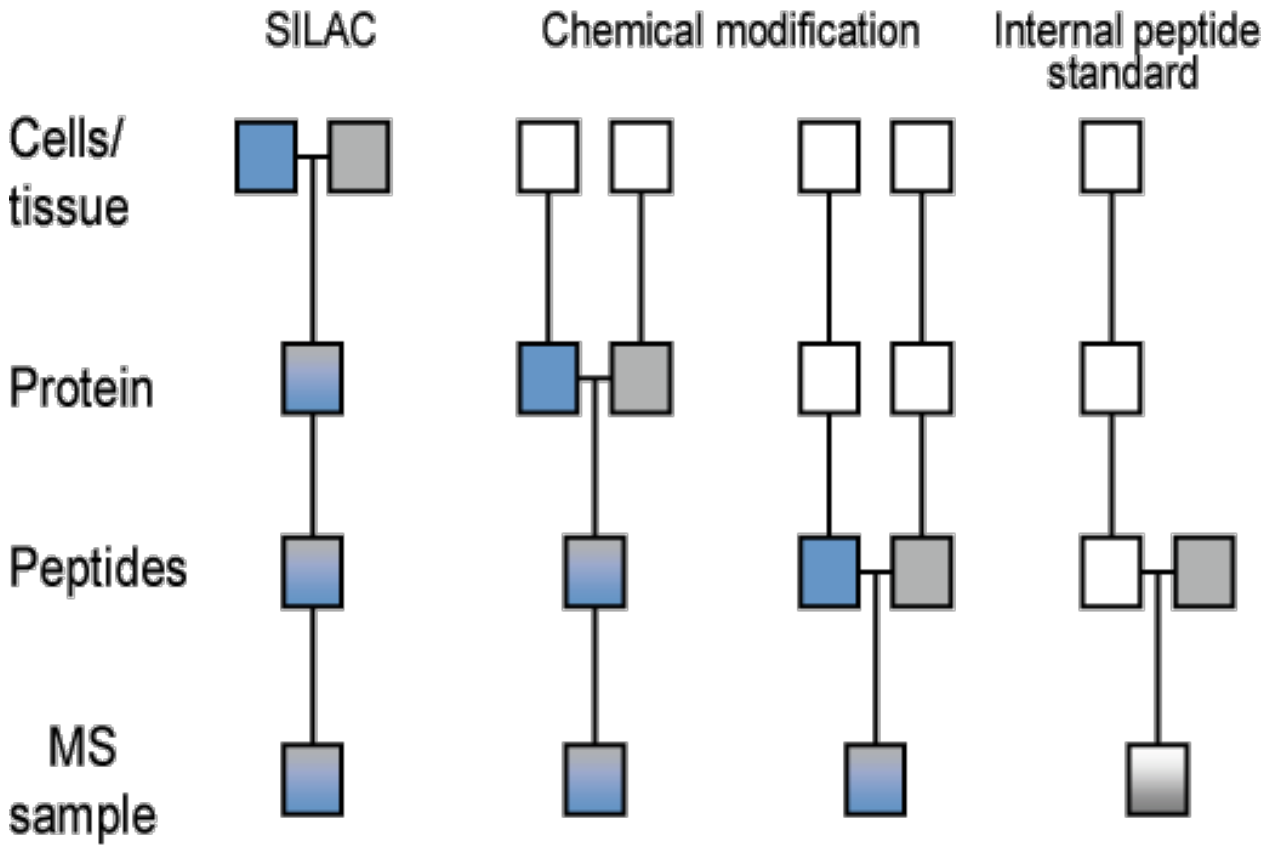
Mix in desired ratio

MS analysis



Compare signal intensities
Derive absolute quantitation

Moment of labelling matters



http://www.biochem.mpg.de/en/research/rd/mann/approaches/silac/silac_intro/index.html

Thank you!

Questions?