

Quantification in MS proteomics

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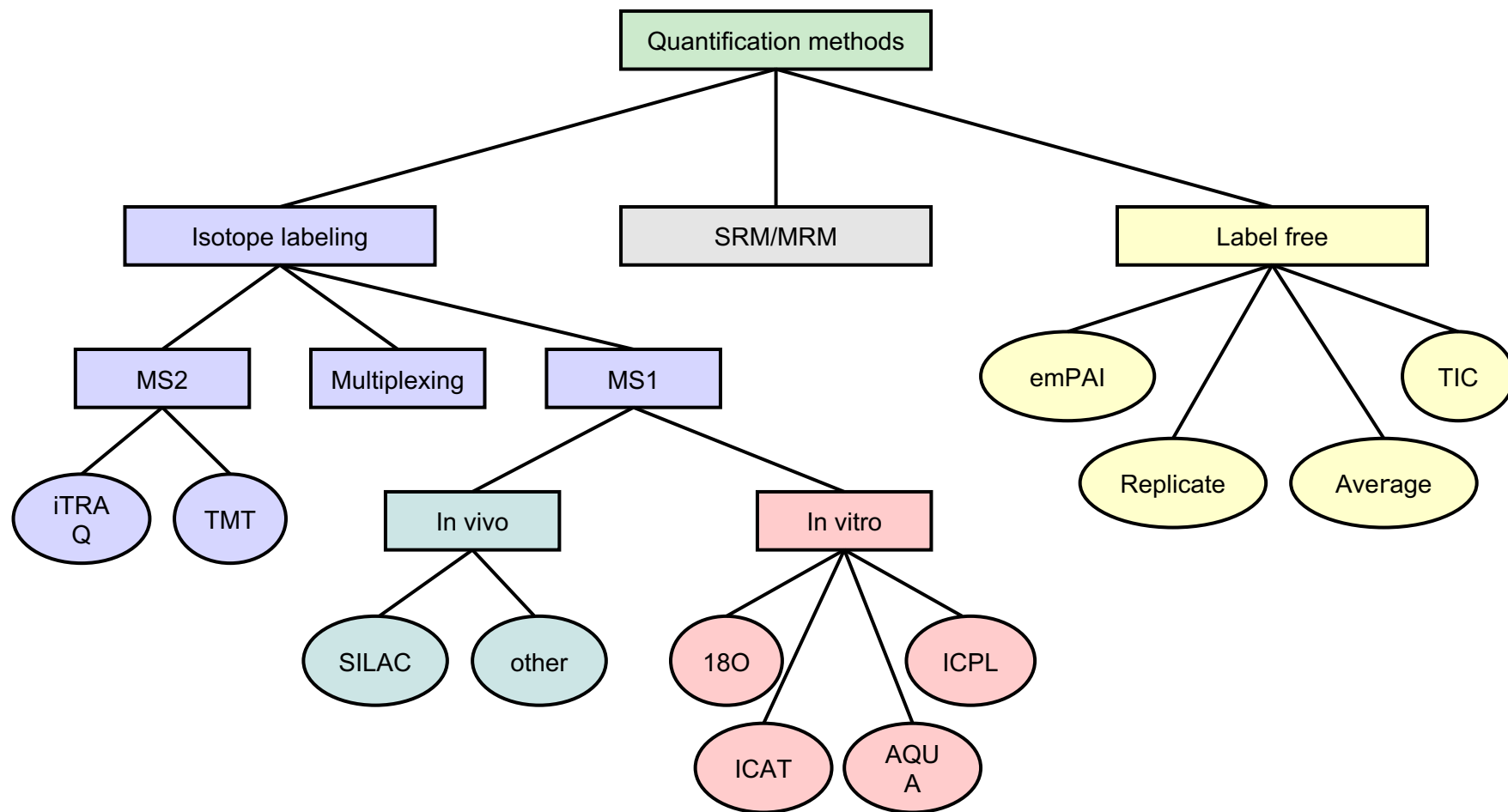
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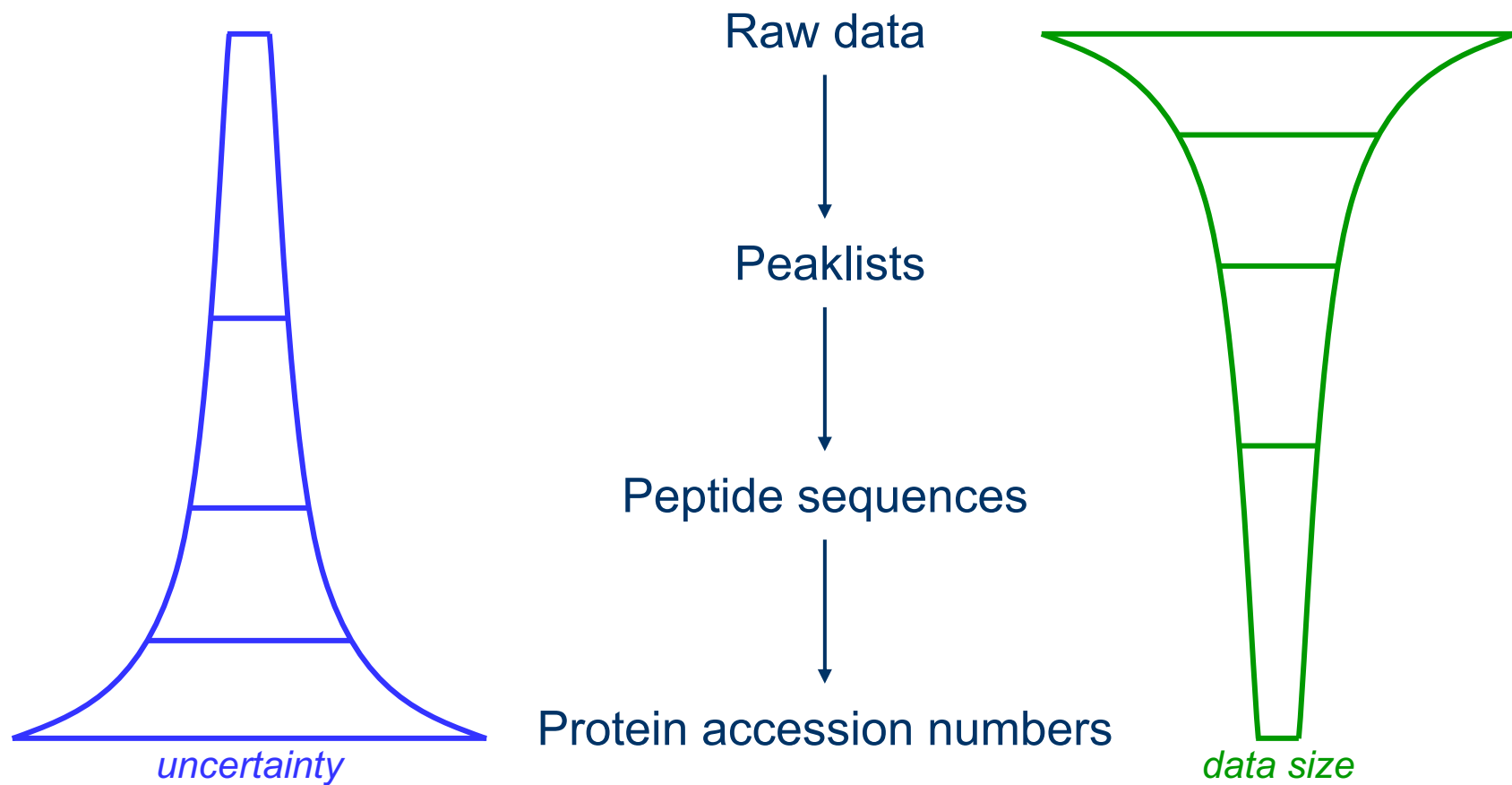


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Protein quantification by MS in one slide

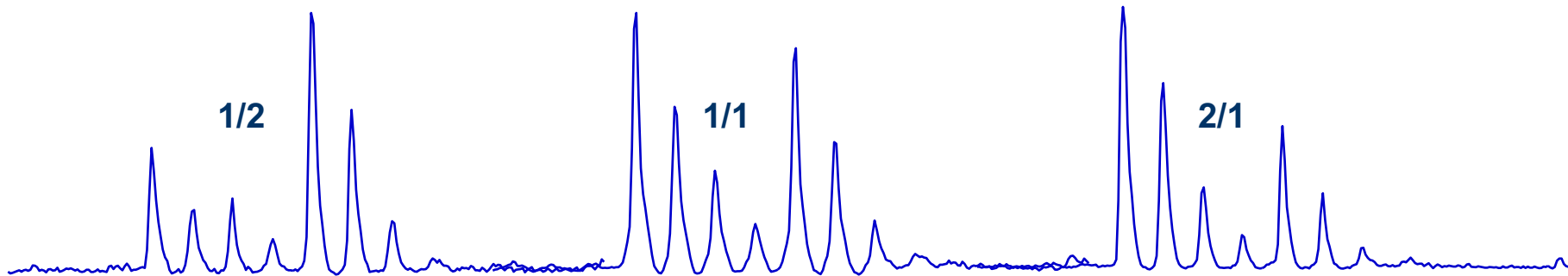


Data processing introduces uncertainty



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

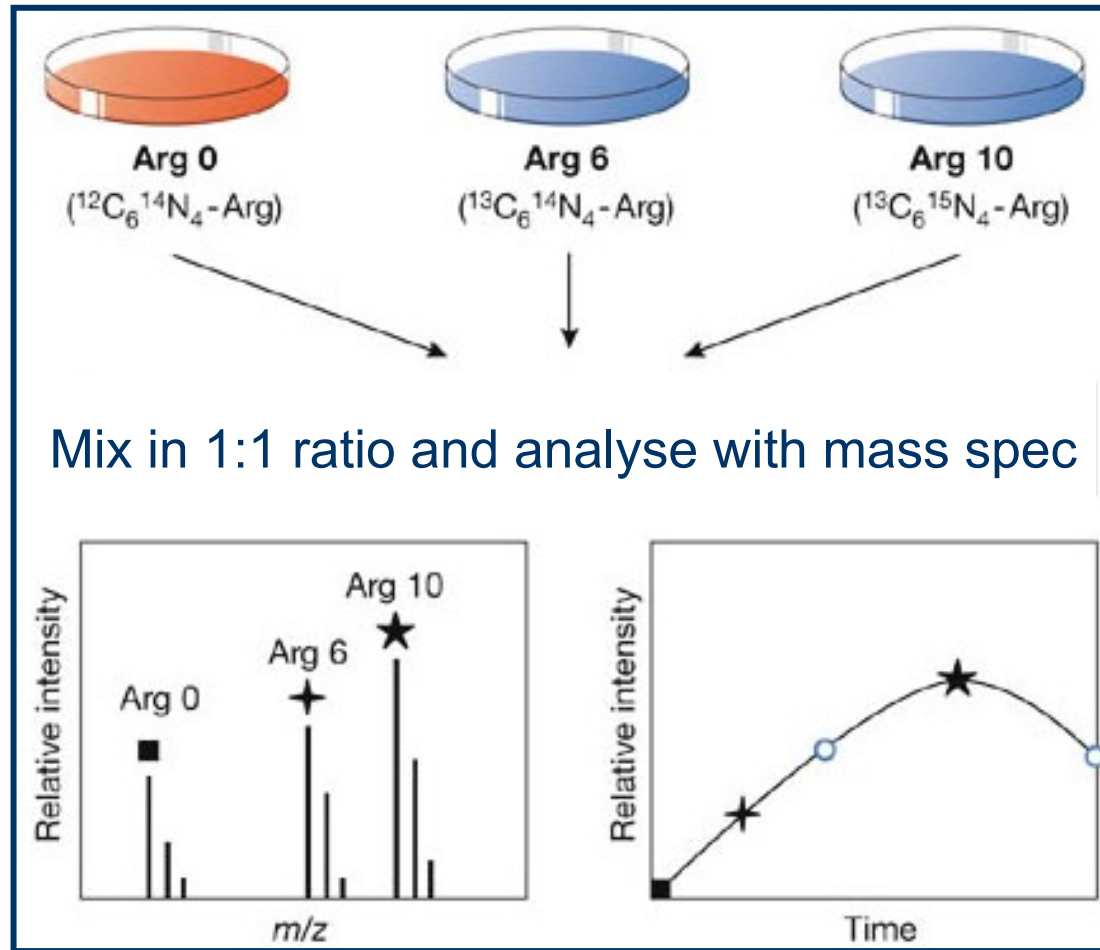
iTRAQ (2), peptides, relative

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

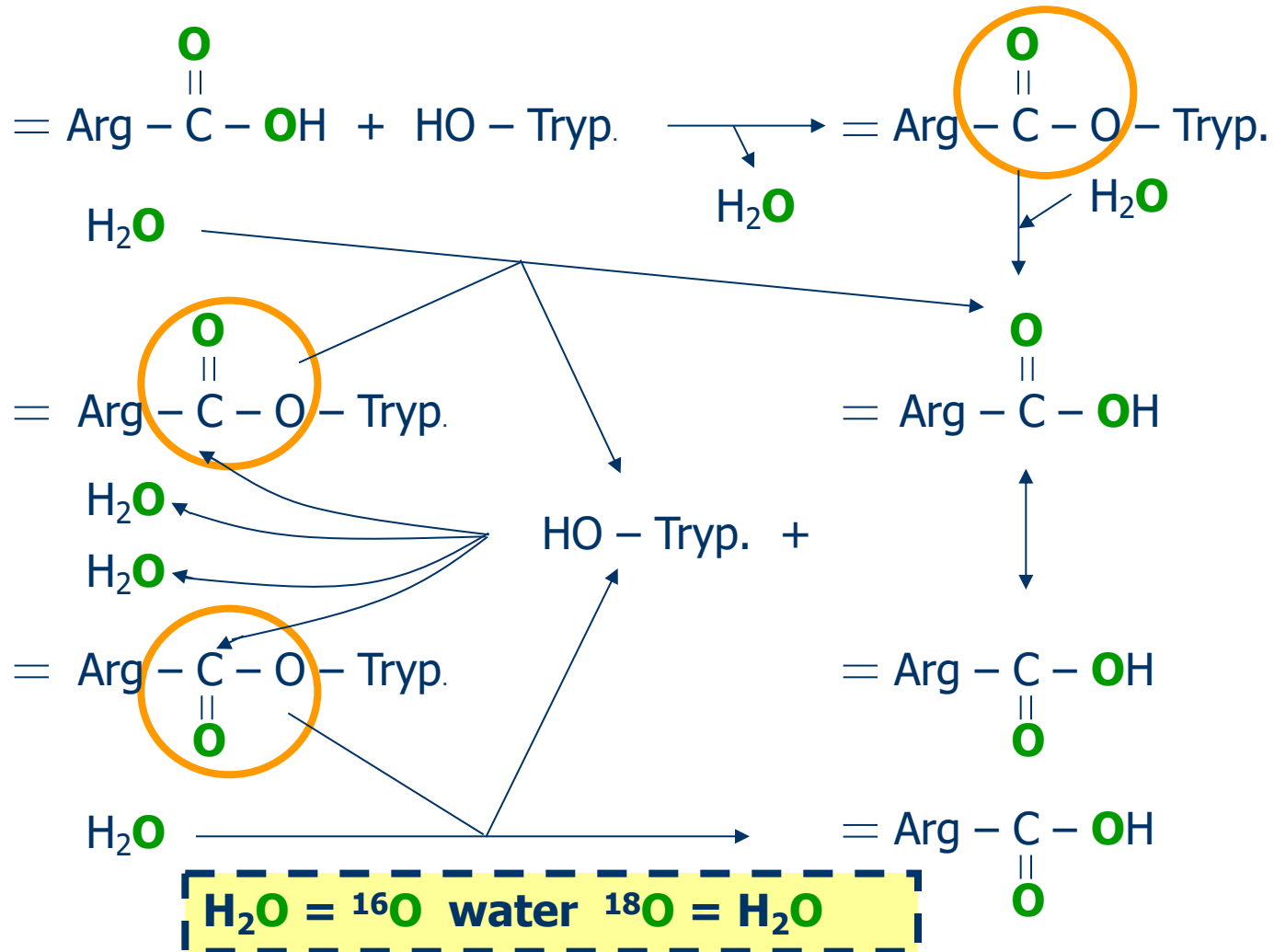
Label-free approaches (3), peptides, peptide fragments, relative, *absolute*

MRM (2, 3), peptide fragments, relative²

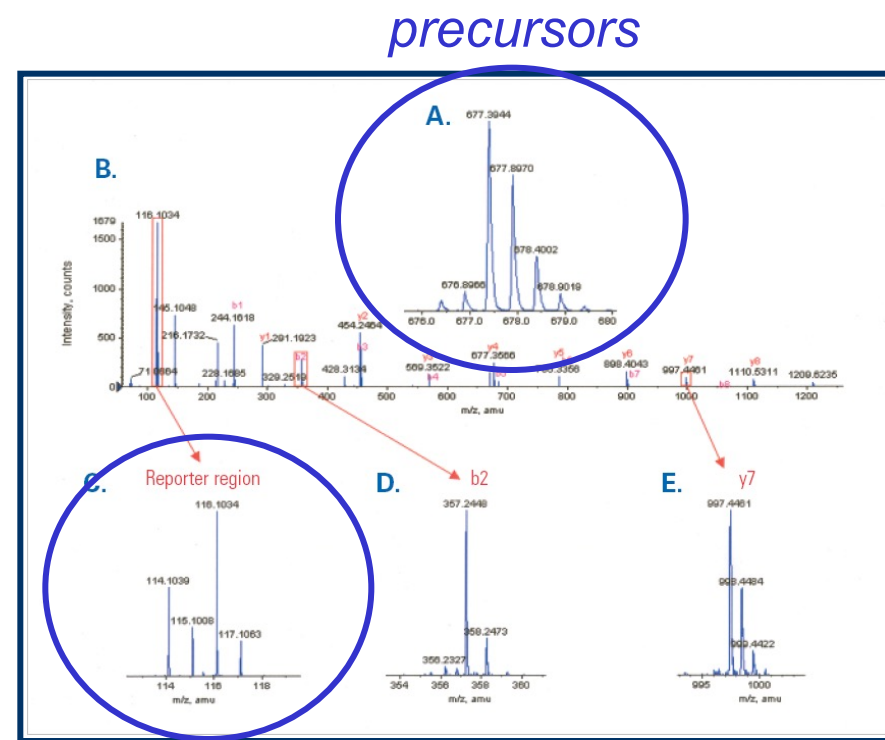
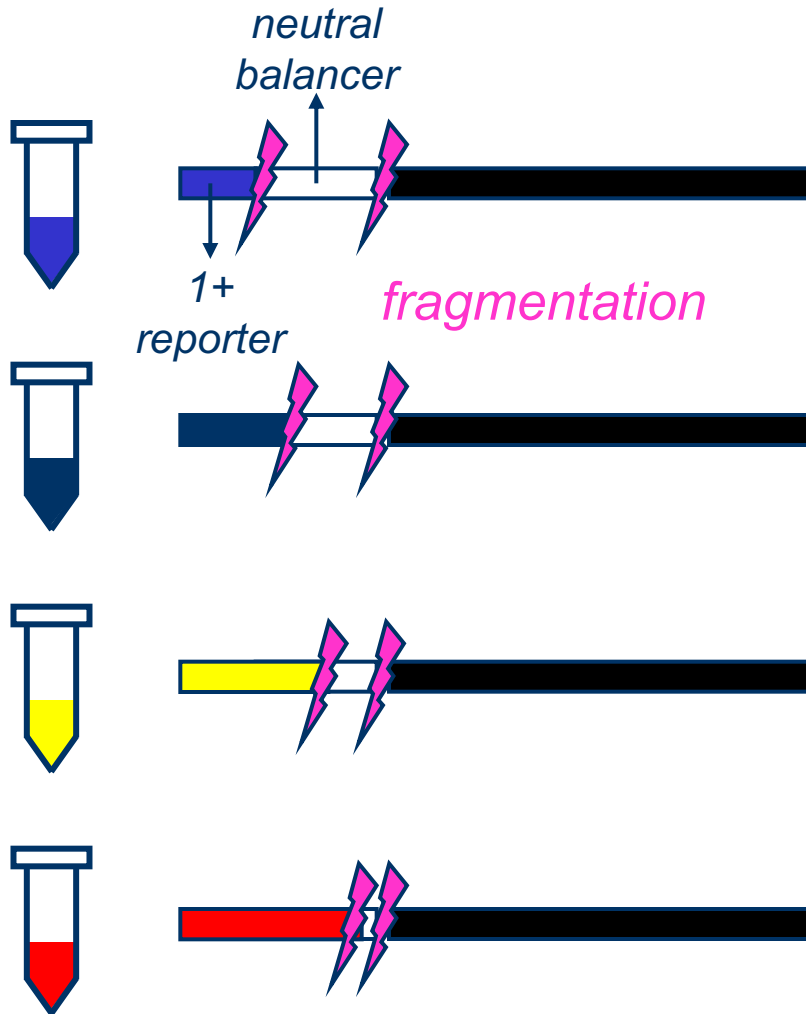
SILAC



^{16}O – ^{18}O labelling



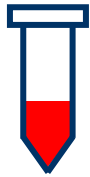
iTRAQ / TMT



Reporter fragments

AQUA

Aimed at absolute quantitation

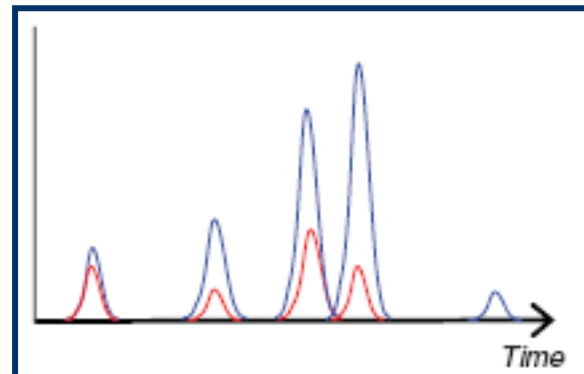


sample
unlabelled peptides
unknown abundance

internal standard
labelled, synthesized peptides
known abundance

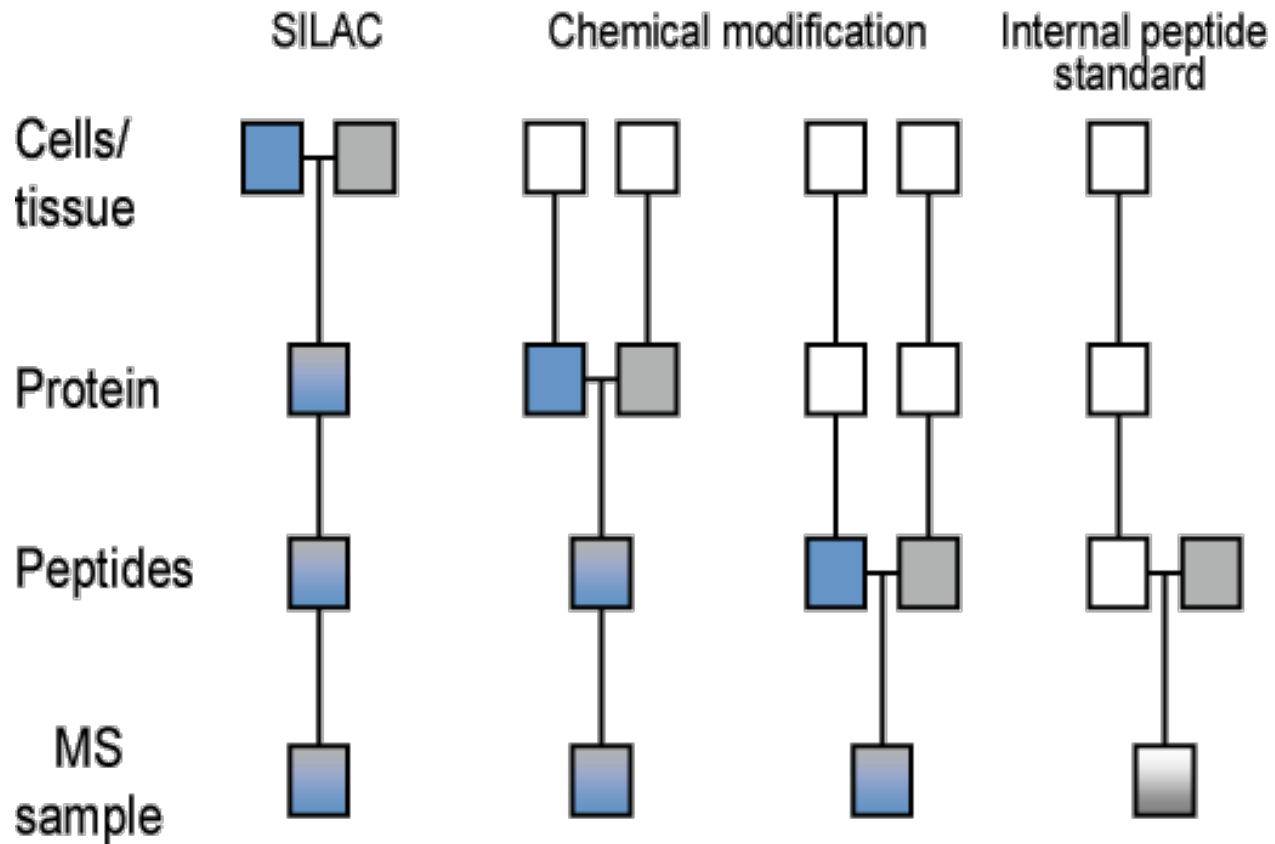
Mix in desired ratio

MS analysis



Compare signal intensities
Derive absolute quantitation

Moment of labelling matters



Thank you!

Questions?