Quantification in the world of proteomics

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Proteomics workshop
September 12, 2006

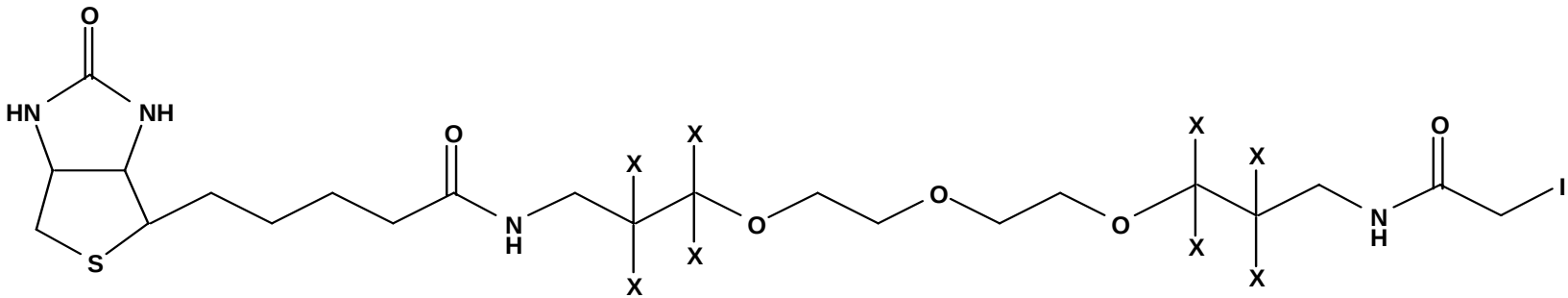
Quantitative proteomics

Use of isotopes

- **ICAT (d_o/d_8) and ICAT $^{13}C_0/^{13}C_8$**
- **d_0/d_{10} propionic anhydride (N-terminal labeling**
- **$^{15}N/^{14}N$ (whole cell labeling)**
- **$^{18}O/^{16}O$ (trypsin)**

- **Non-isotopic methods**
  - **iTRAQ labelling**
  - **Peptide coverage**
- **Classical triple quadrupole methods**

Isotope-coded affinity technology (ICAT)



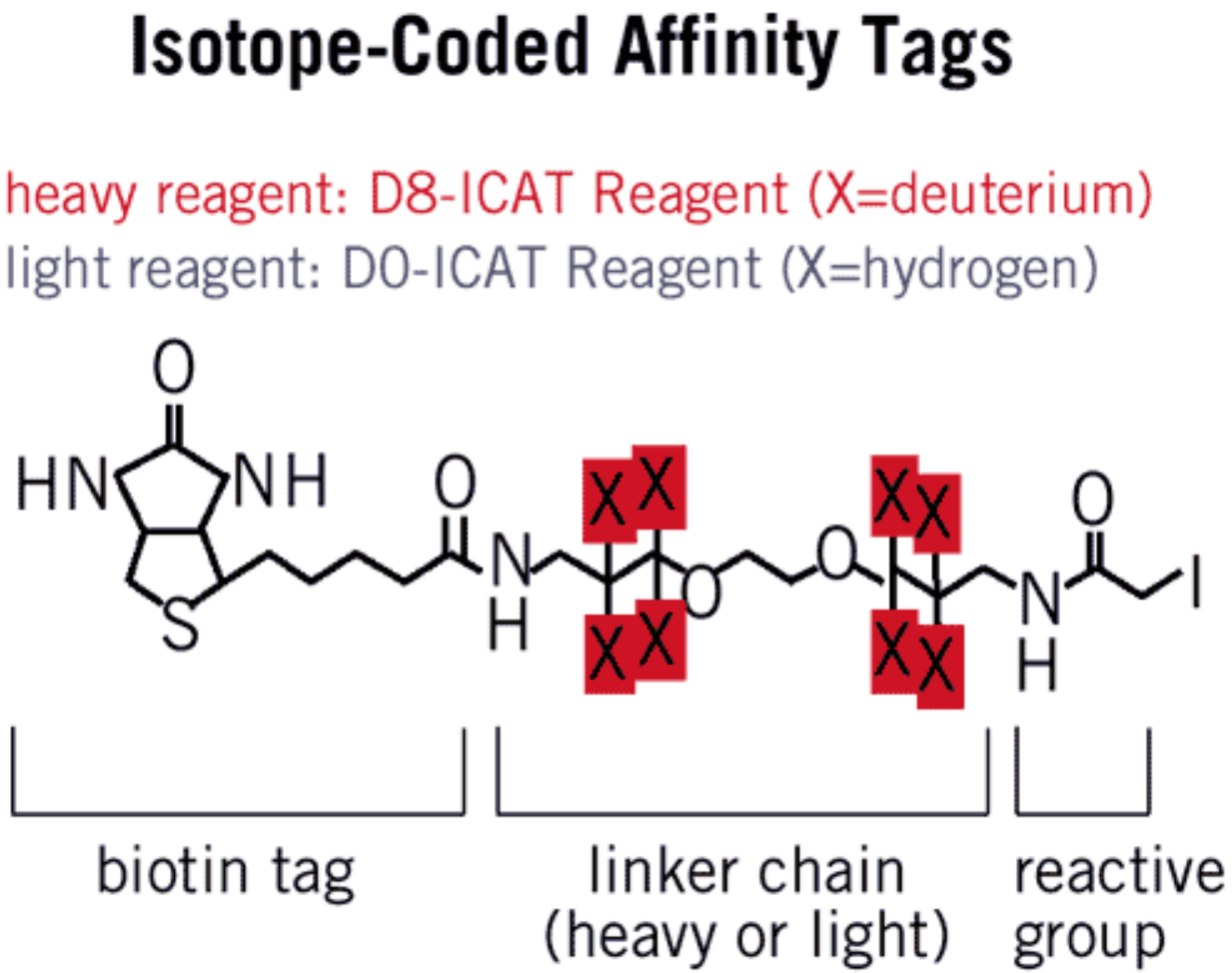
biotin **Linker region (heavy or light)** **Thiol-specific reactive group**

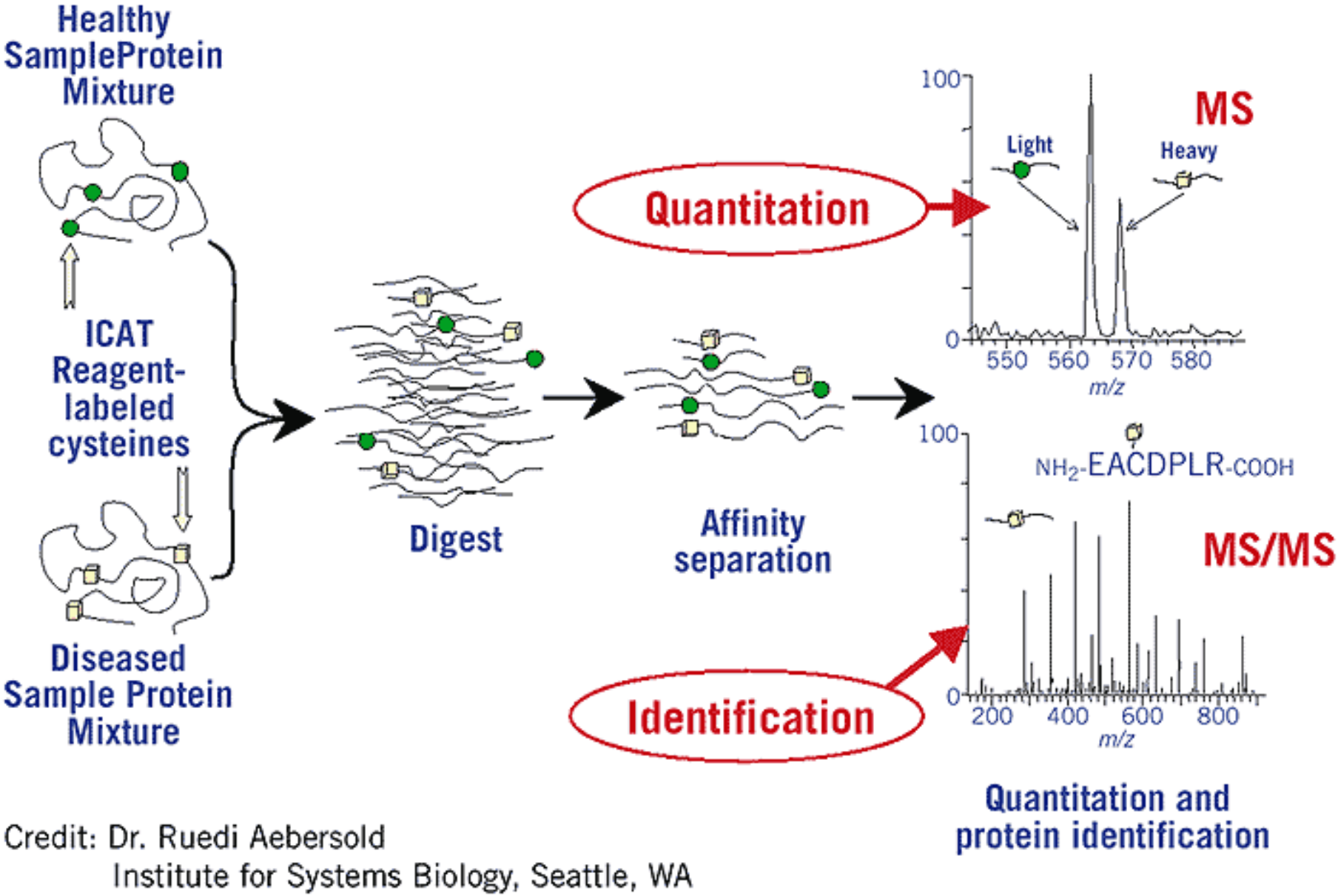
This reagent reacts with cysteine-containing proteins (80-85% of proteome)

Labeling can be replacement of hydrogens (X) with deuterium, or better to exchange ^{12}C with ^{13}C in the linker region (this avoids chromatography issues)

Available from ABI-Sciex

Cysteine reacting agents

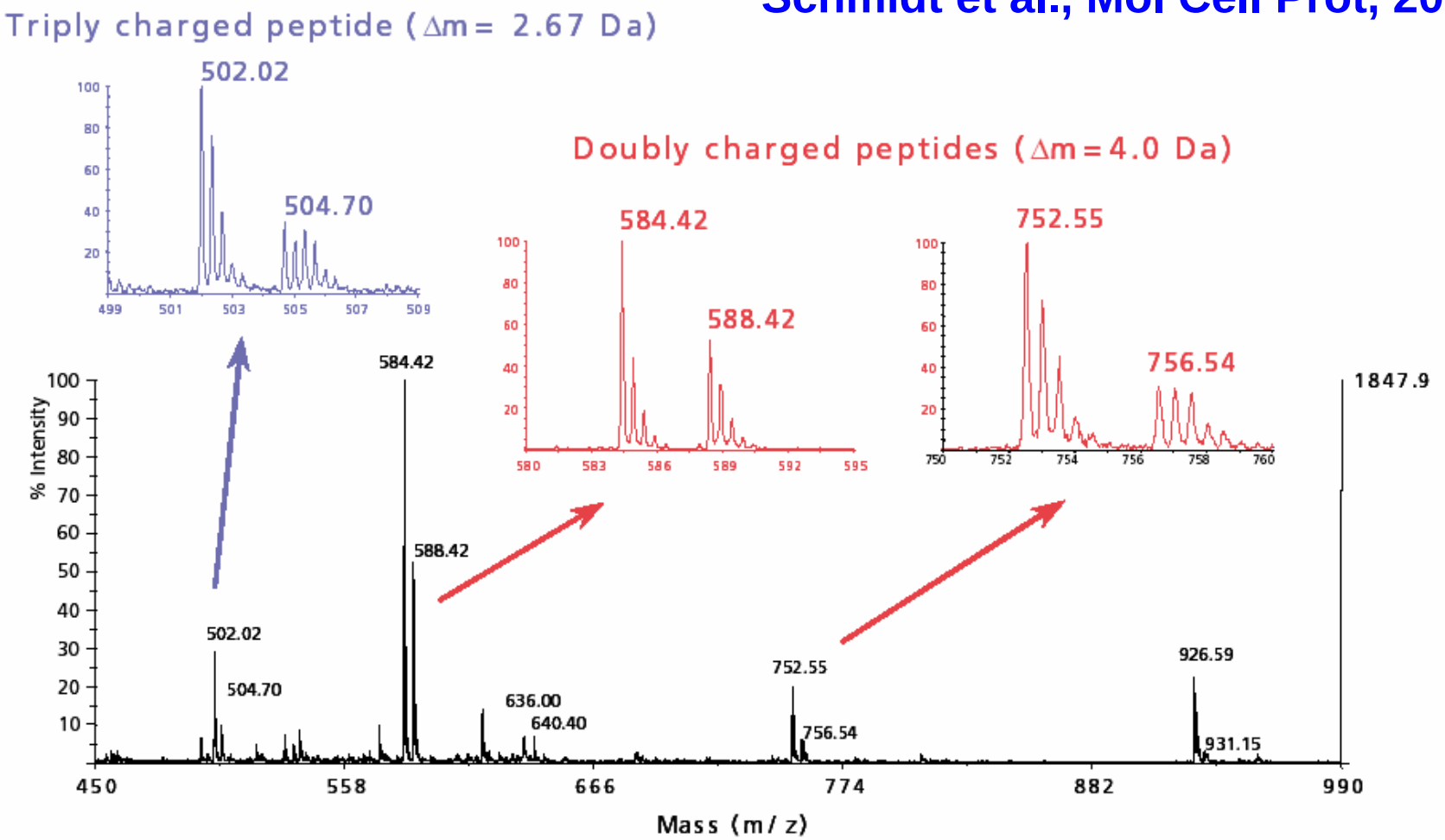




Credit: Dr. Ruedi Aebersold
Institute for Systems Biology, Seattle, WA

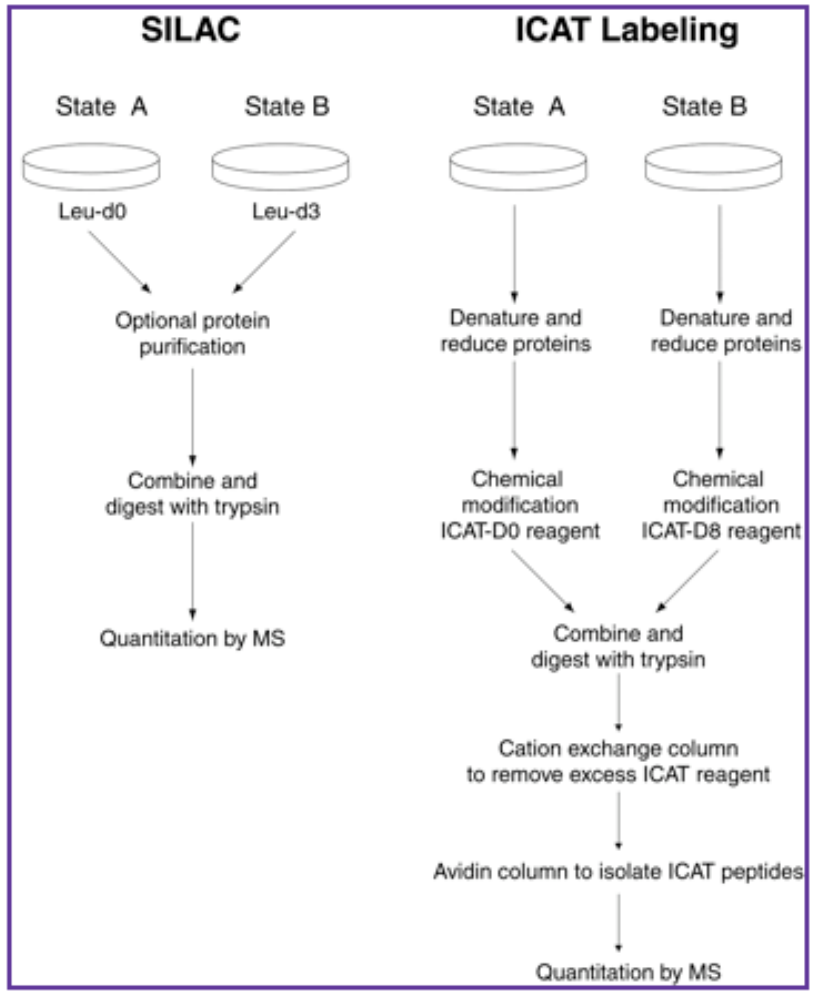
Quantitation from ESI-mass spectrum

Schmidt et al., Mol Cell Prot, 2003



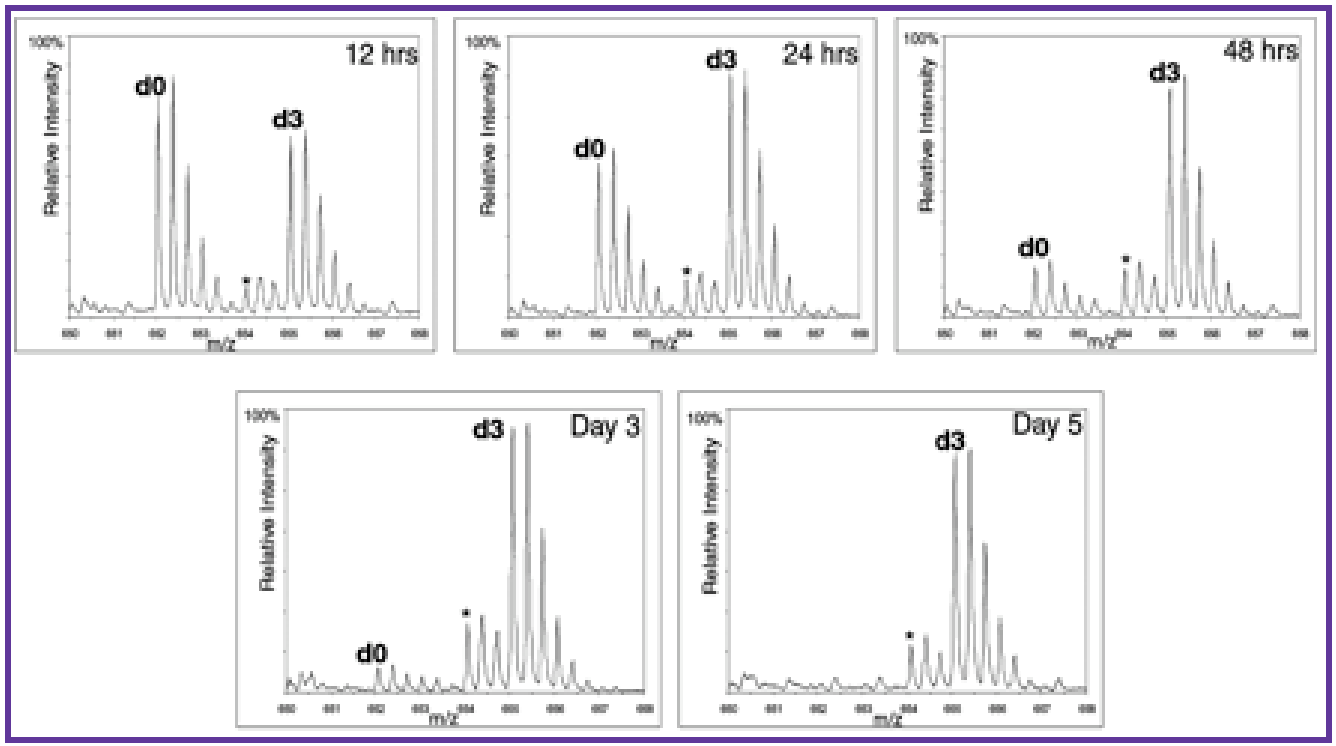
SILAC

- **SILAC, stable isotope labeling by amino acids in cell culture, is being used to quantify proteins.**



Ong et al., MCP 1:367, 2002

Time-dependent leucine incorporation with SILAC



The cells are pre-labeled with leucine-d_0. Leucine-d_3 is added to the medium and cells sampled at various times later. The peaks annotated with d0 and d3 are the triply charged peaks of the peptide VAPEEHPVLLTEAPLNPK, which contains three leucines.

Ong et al., MCP 1:367, 2002

^{18}O-labeling

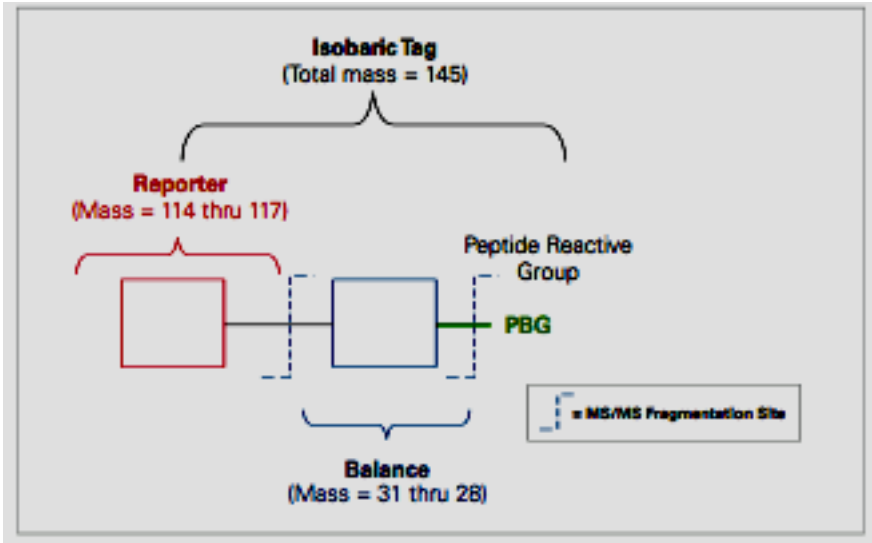
- Trypsin catalyzes the transfer of ^{18}O in ^{18}O-enriched water to both the carboxylate oxygens of the C-terminus of tryptic peptides

$$R\text{-}COOH \longrightarrow R\text{-}C^{18}O_2H$$

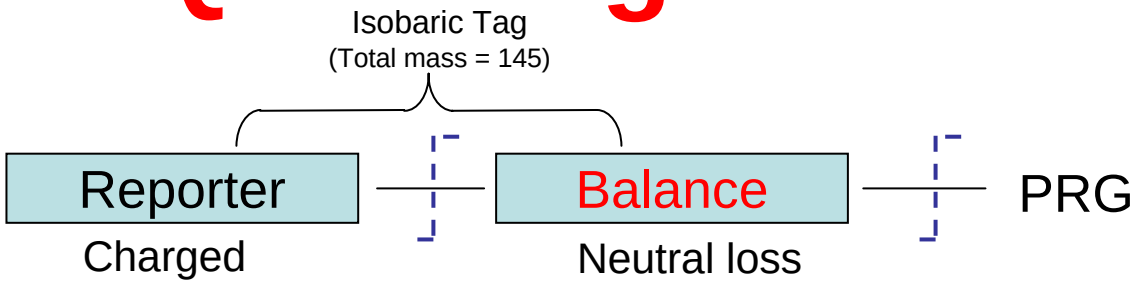
- The peptides have an increase in mass of 4 Da
- Generally not considered a large enough mass difference

Non-isotopic quantitation

- **The iTRAQ™ reagents**
  - **React with Lys amino groups and each one adds 145 Da to the molecular weight of the peptide**
  - **Fragmentation produces reporter ions from *m/z* 114, 115, 116 and 117**
  - **New iTRAQ kit contains 8 forms with reporter fragment ions of *m/z* 114, 115, 116, 117, 118, 119 and 121**



iTRAQ™ Reagent Design

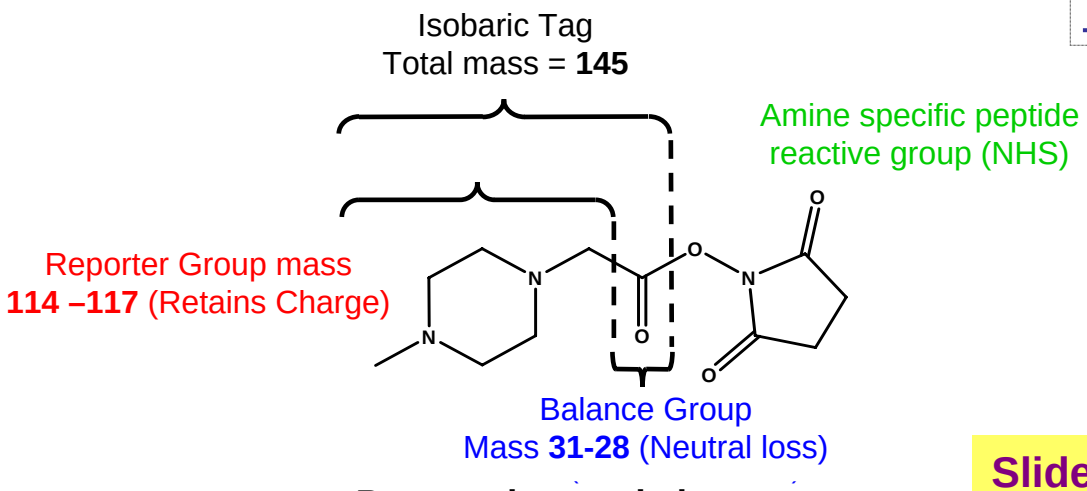


Gives strong signature ion in MS/MS
Gives good b- and y-ion series
Maintains charge state
Maintains ionization efficiency of peptide
Signature ion masses lie in quiet region

Balance changes in concert with reporter mass to maintain total mass of 145
Neutral loss in MS/MS

Amine specific

= MS/MS Fragmentation Site



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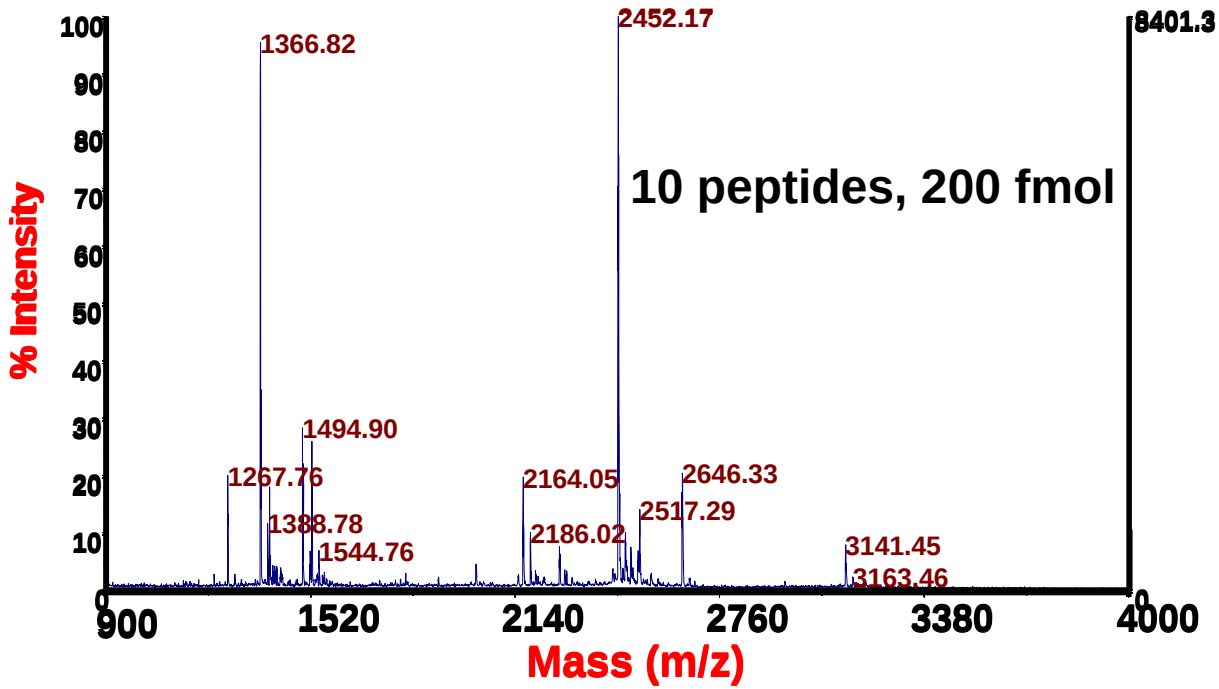
Slide provided by Applied Biosystems

Other non-isotopic quantitative methods in proteomics

The coverage (the number of peptides observed for a protein) is sensitive to the amount of the protein

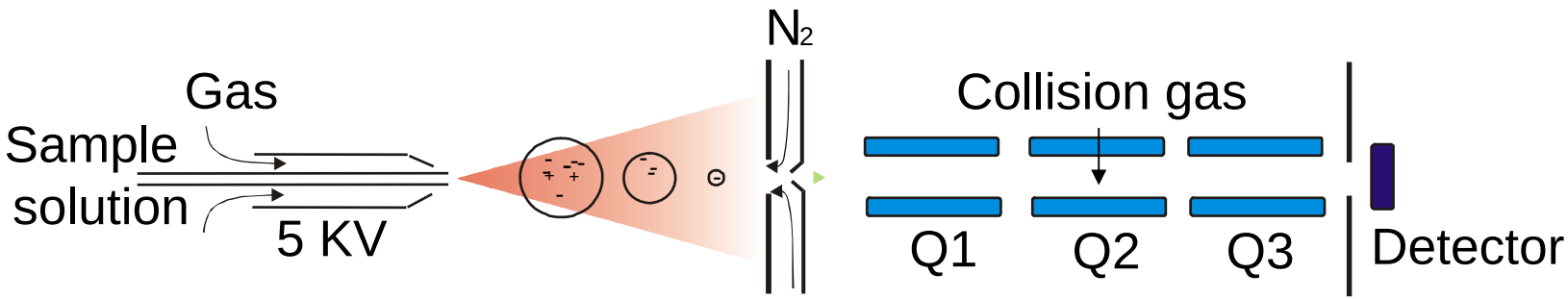
- **This can be used to calculate whether a treatment affects the abundance of a protein where fold-change > 2**
- **Applies to LC-MS (MUDPIT methods)**



Triple quad MRM analysis

Peptides of interest can be analyzed like small molecules

- **Choose the parent molecular ion, collide with argon gas and select a unique fragment**



- **Multiple reaction ion scanning**

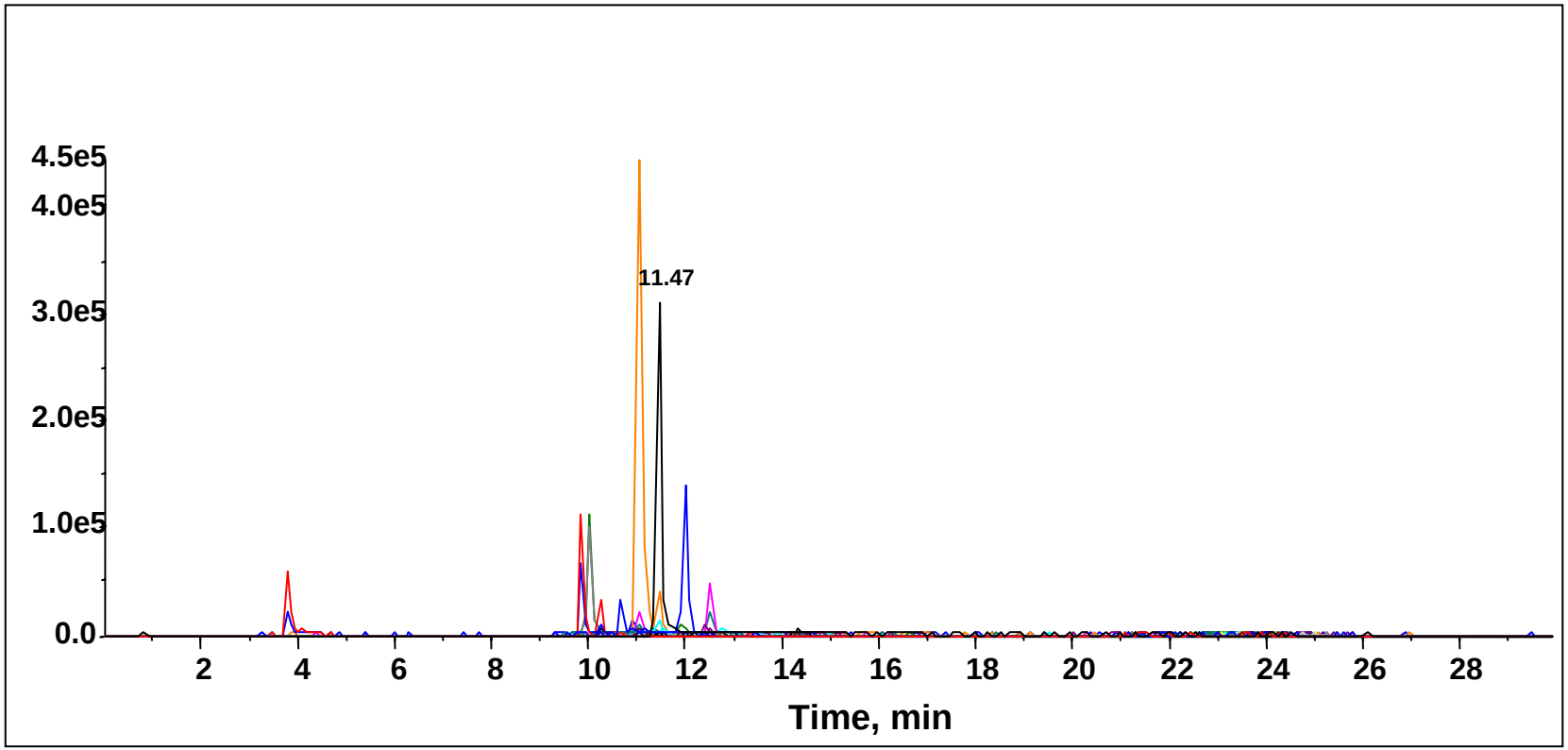
  First filter the [M-H]- molecular ion of the analyte (Q1)

  Fragment the molecular ion with N_2 gas (Q2)

  Select a specific (and unique) fragment ion (Q3)

Quantitation experiment for biotinylated cytochrome c peptides

MRM analysis monitored in 50 channels



Each colored peak represents a different biotinylated peptide

Application of LC-MRM-MS to the plasma proteome

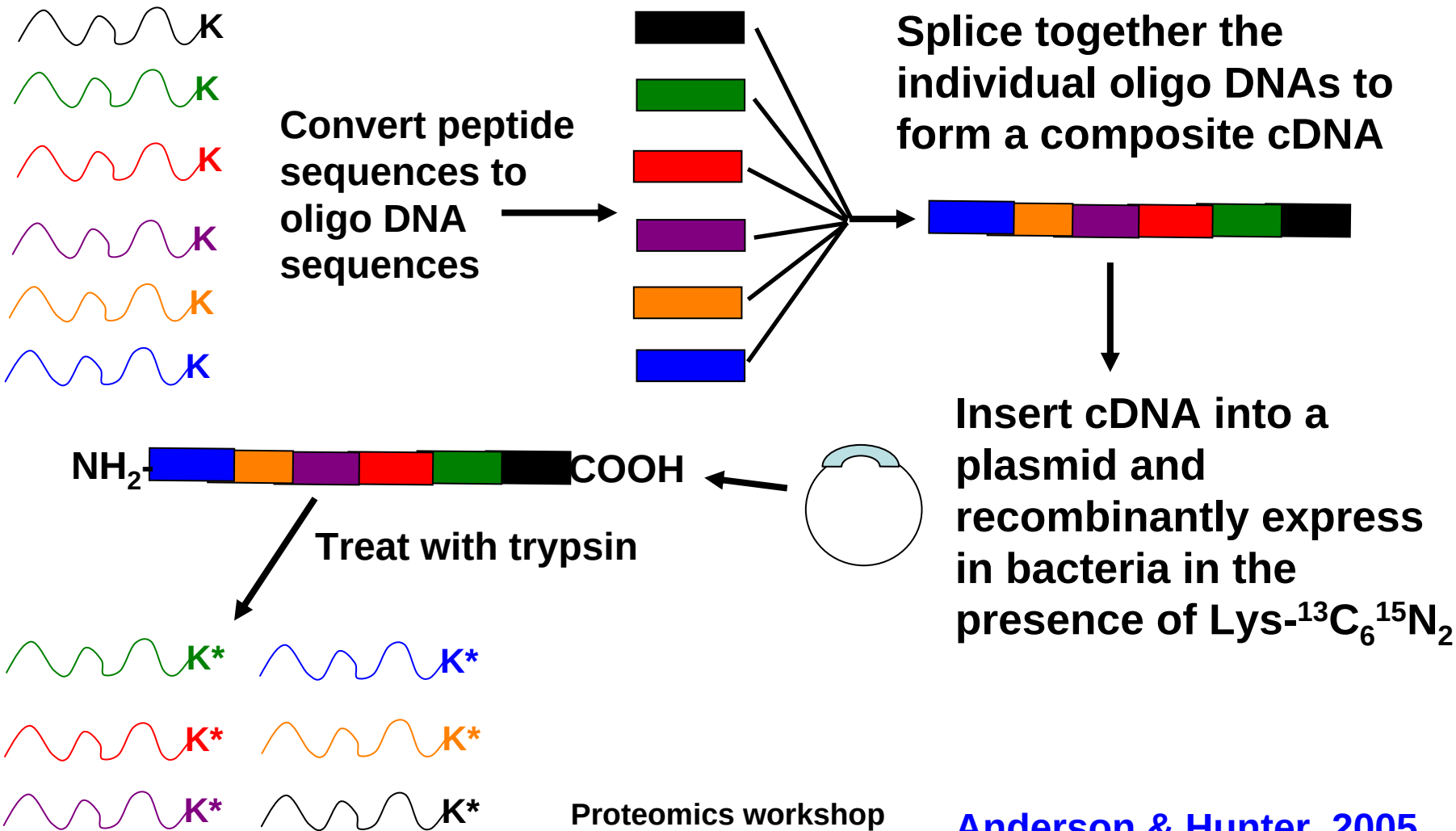
Identify the proteins of interest → **In silico, generate the tryptic peptides from each protein**

↓

Determine the expected y ions for each peptide and compare to y ions of all other tryptic peptides of known human proteins that have masses within ± 1Da

← **Select the best combination of parent peptide and fragment y ion**

Slick way of making ^{13}C-labeled peptide internal standards



Anderson & Hunter, 2005

Quantitative peptide MRM-MS

- **The albumin-depleted plasma proteome is mixed with the composite $^{13}C,^{15}N$-labeled protein internal standard and then treated with trypsin**
- **The molecular ions (doubly charged) and the specific y ions for each peptide and its labeled form are entered into the MRM script one channel at a time**
- **A single run may consist of 30 peptides in 60 channels**
- **Sensitivity is compromised by “sharing out” measurement time, but can be compensated for by carrying out nanoLC**

Advantage of a C-terminal labeled lysine

	186	301	448	505	642	755	886	987	1115	b ions
A	D	E	F	G	H	I	M	T	K	
1133	1062	948	833	686	629	492	379	248	147	y ions

With the labeled lysine at the C-terminus, only the b_{10} ion contains the isotope atoms

	186	301	448	505	642	755	886	987	1123	b ions
A	D	E	F	G	H	I	M	T	K*	
1141	1070	956	841	694	637	500	387	256	155	y ions

Bibliography

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- **Gruhler A, Schulze WX, Matthiesen R, Mann M, Jensen ON. Stable isotope labeling of *Arabidopsis thaliana* cells and quantitative proteomics by mass spectrometry. *Molecular & Cellular Proteomics. 4:1697-1709, 2005.***
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