Bioinformatics D: Proteomic quantitation

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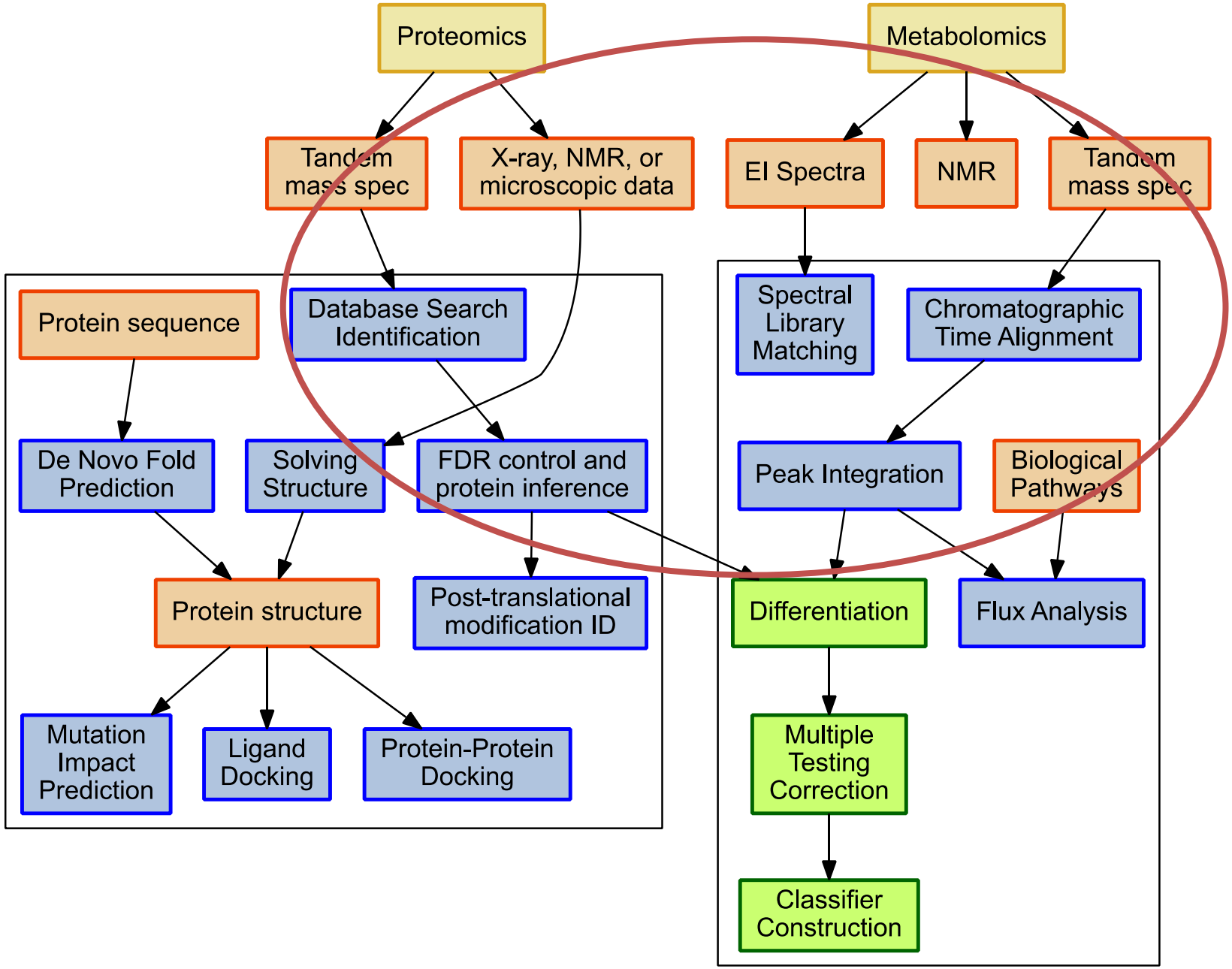
Overview

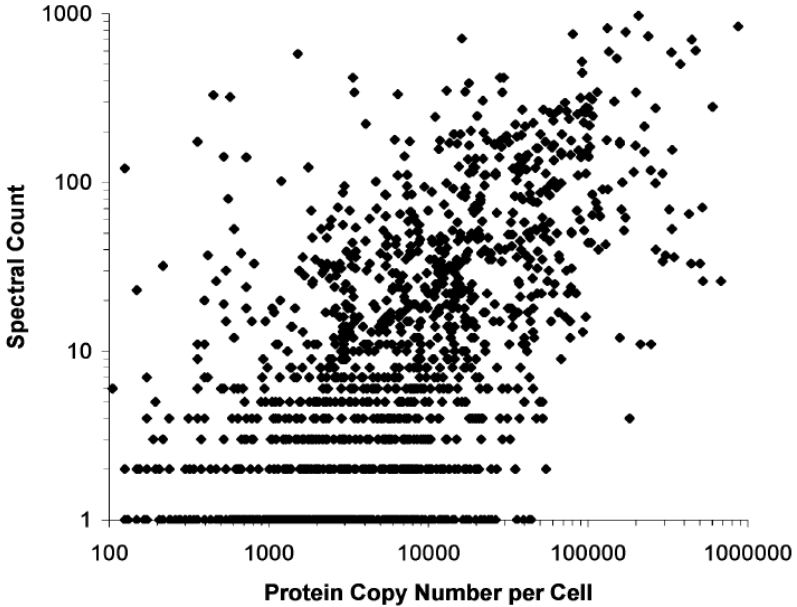
- Proteomic quantitation
  - Spectral counting
  - Extracted ion chromatogram integration
  - Constituitive or isobaric labeling
  - Selected Reaction Monitoring



Today we stay in the ellipse.

Label-Free: Spectral counting

- More concentrated proteins match to larger numbers of tandem mass spectra.
- If the same protein is observed in different samples, the number of spectra that match it are predictive of its relative concentration.
- Shotgun experiments cause ions to compete for MS/MS selection, favoring abundant proteins.



Liu et al (2004) *Anal. Chem*. 76: 4193-4201.
Tabb et al (2010) *J. Proteome Res*. 9: 761-776.

Why does spectral counting work?

- The potential sites of trypsin digestion for each protein yield different probabilities of cutting.
- A peptide competes with others for ionizing protons in nanoelectrospray ionization.
- Every proteomics sample contains too many peptides for the mass spectrometer to produce a comprehensive set of MS/MS scans.
- MS/MS scans may fail in identification due to low signal or co-isolated peptides.

Outcome: peptides “compete” for identification; those from abundant proteins have an advantage.

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What proteins changed in level?

Protein	r1	r2	r3	r4	r5	y1	y2	y3	y4	y5
P02931	54	61	74	57	61	83	99	101	104	99
P0A6N1	51	53	56	55	46	89	99	103	86	95
P0A6M8	47	60	55	45	53	53	57	60	52	57
P05825	53	55	51	51	51	59	50	54	57	56
P09373	49	51	48	52	52	51	58	58	52	57
P0A853	52	55	59	51	46	52	51	56	48	50
P0AFG8	51	50	43	46	54	43	47	51	50	48
P0A6F5	66	80	77	67	69	23	26	20	26	25
P0A8V2	42	54	49	49	47	45	43	42	48	47
P00968	46	50	45	51	42	50	48	45	42	41
P0A6Y8	39	45	45	41	43	46	42	41	44	42
P0A8T7	44	44	46	39	45	39	39	42	40	39
P0A9B2	33	42	43	34	36	49	46	42	42	42
P63284	42	46	30	40	39	38	48	39	37	48
P61889	36	39	38	41	37	37	34	38	37	38
P0A910	44	37	33	38	41	35	34	37	34	35

Tables like these are common in biomarker studies. Each number reports how many spectra were observed for a particular protein in five replicates of two different cohorts.

Data from ABRF iPRG 2009 study

Key concept: *chromatogram*

- Technologies for separation are often called "chromatography."
- In LC-MS, we can measure intensity for ions as a function of chromatographic time.
- This record of intensity over time is called a "chromatogram."

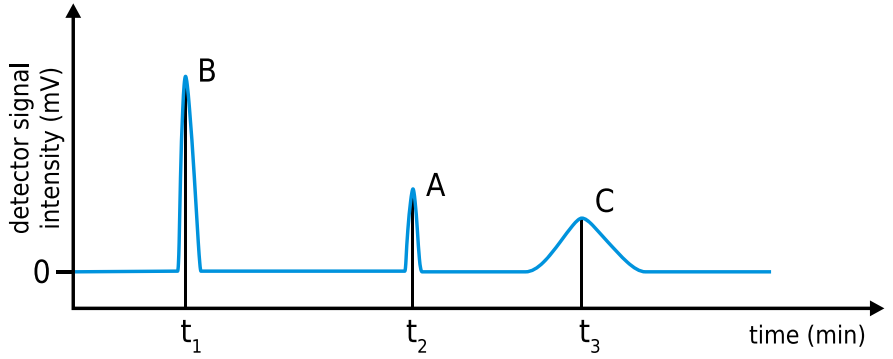
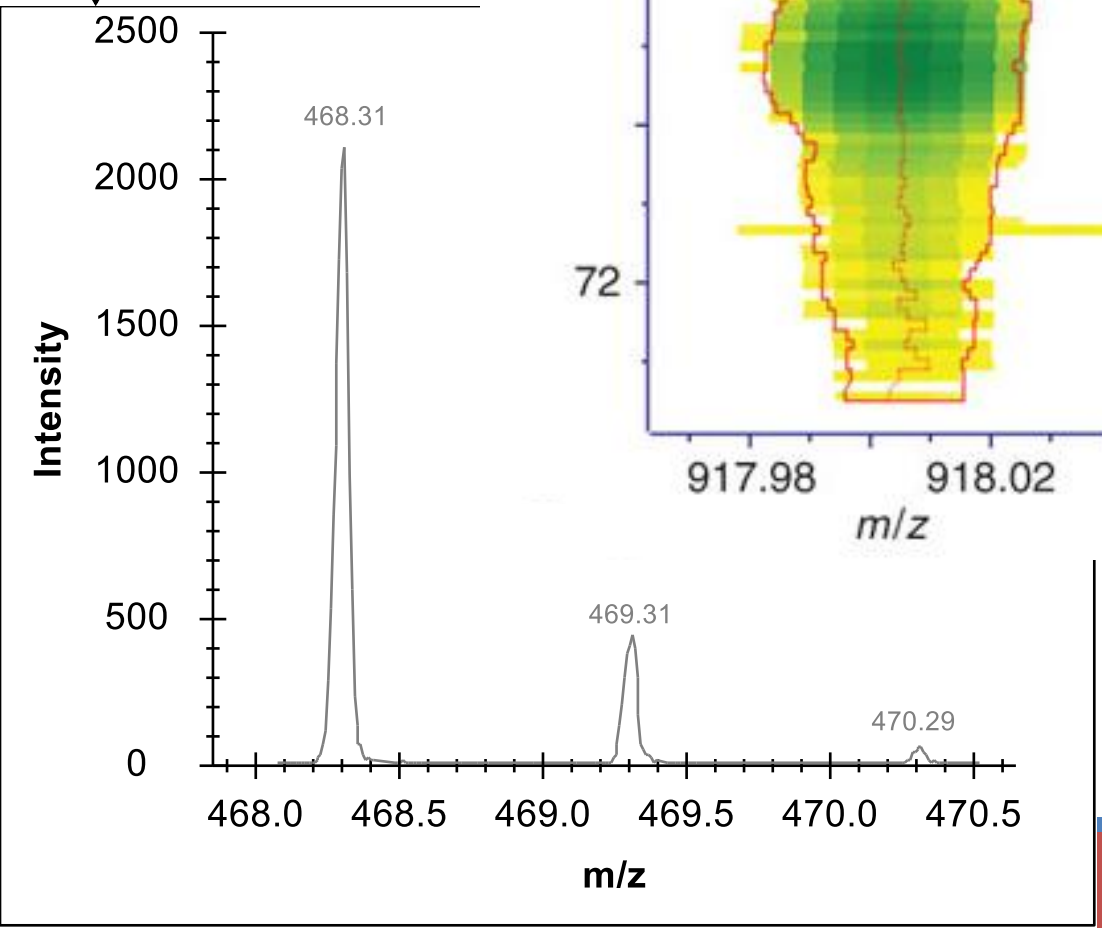


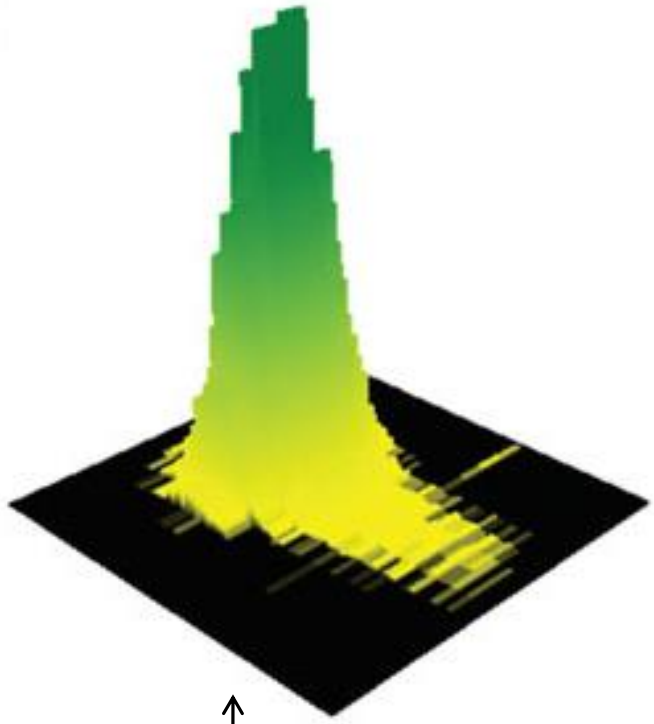
Image by Heliagon from Wikimedia Commons

MaxQuant:
MS Intensity as quantitative data

Close-up of isotopic packet



Peak through time



3D volume reconstruction

Upper image from Cox (2008) *Nat. Biotech.* 26: 1367-1372.

Principles of isotopic labeling

- Extra neutrons do not change chemical behavior, but MS differentiates them.
- Mixing labeled and unlabeled samples enables absolute quantitation when concentration of internal standard is known.
- Incomplete labeling can distort isotopic packets and complicate quantitation.

Isotopic labeling

- Metabolic labeling replaces all carbons or nitrogens with “heavies” in growing cells.
  Wu et al (2004) *Anal. Chem*. 76: 4951-4959.
- Incorporation of a labeled amino acid changes only its mass (SILAC).
  Ong et al (2002) *Mol. Cell. Proteomics* 1: 376-386.
- Attaching isobaric tags (iTRAQ, TMT) to peptides yields MS/MS signal only.
  Ross et al (2004) *Mol. Cell. Proteomics* 3: 1154-1169.

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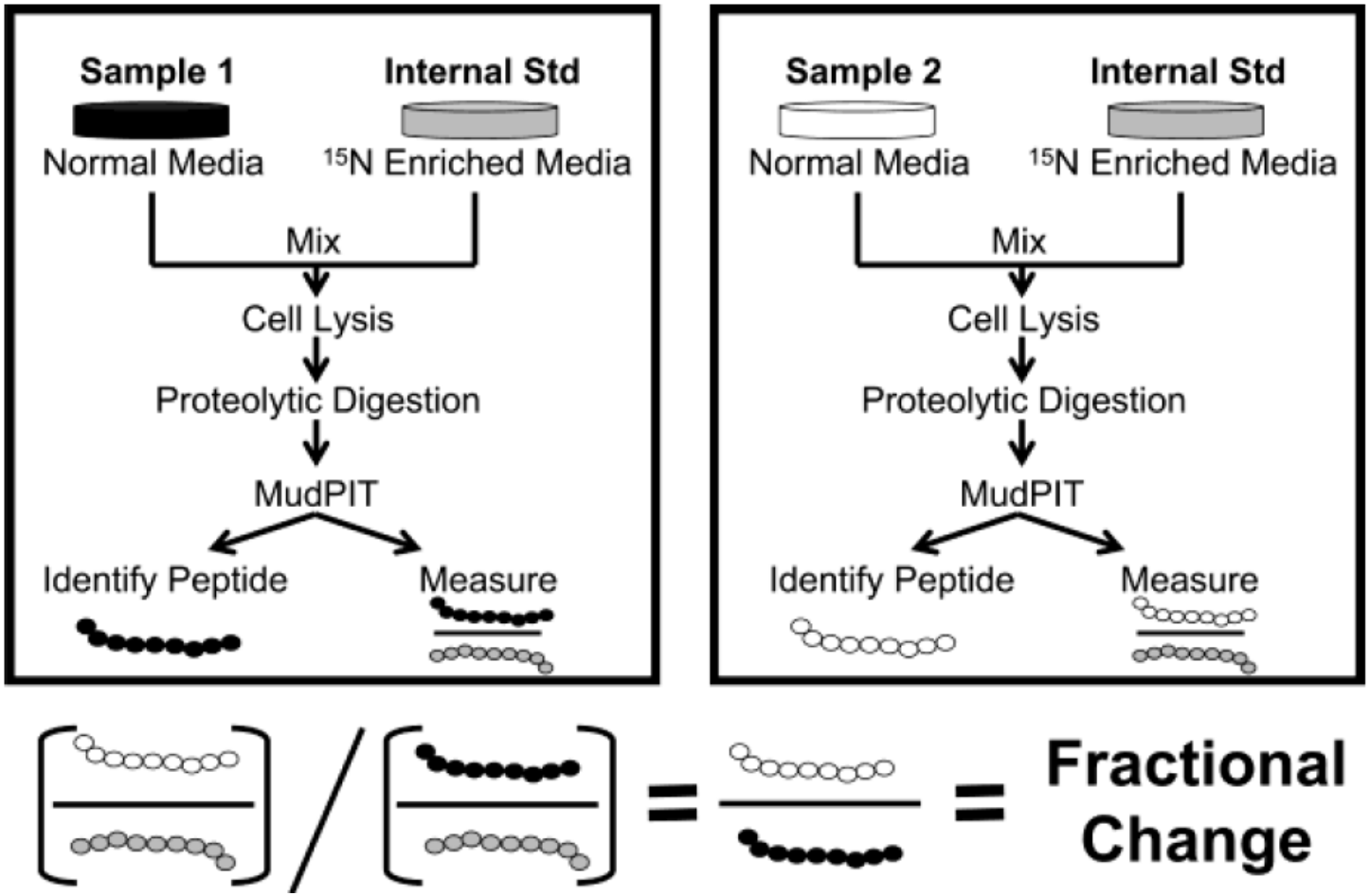
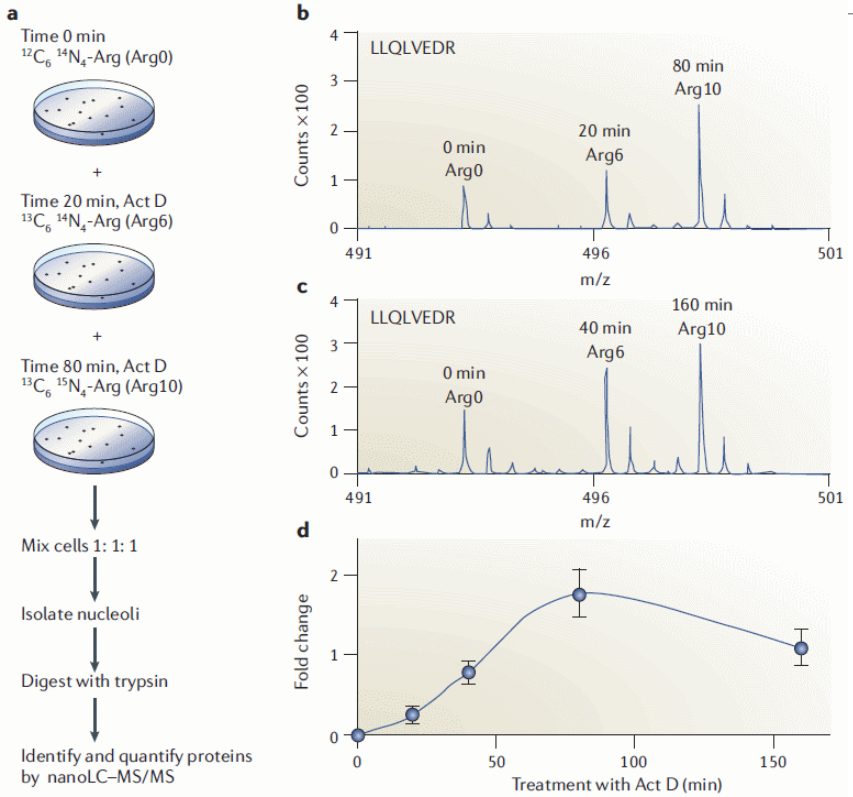
Label-based quantitation



MJ MacCoss et al. (2003) *Anal. Chem.* 75: 6912-6921

SILAC for individual amino acids

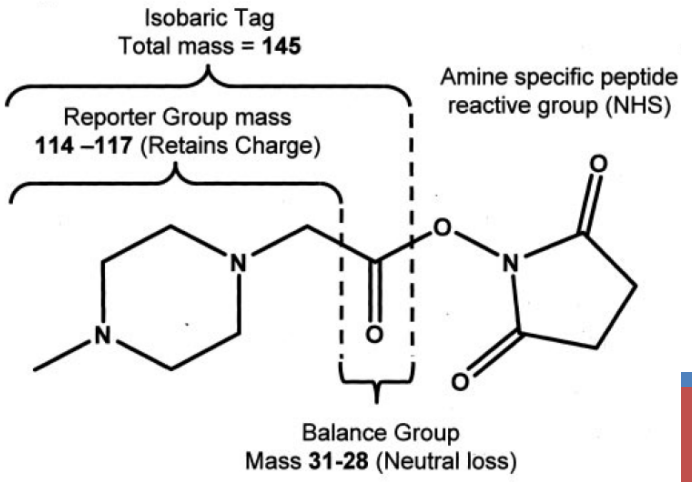


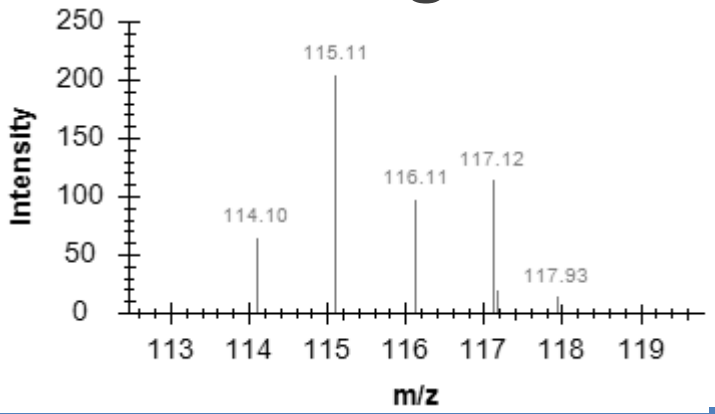
- Specific amino acids are available in different isotopic masses.
- Here, three labeled forms of Arg allow the comparison of five different time points.

M Mann, *Nature Rev. Mol. Cell. Bio*. (2006) 952-958.

iTRAQ / TMT isobaric labeling

- After digestion, amino groups of peptides can be attached to isobaric tagging reagents.
- These tags fragment in MS/MS to produce different reporter ions.
- AB Sciex sells 4- and 8-channel reagents.



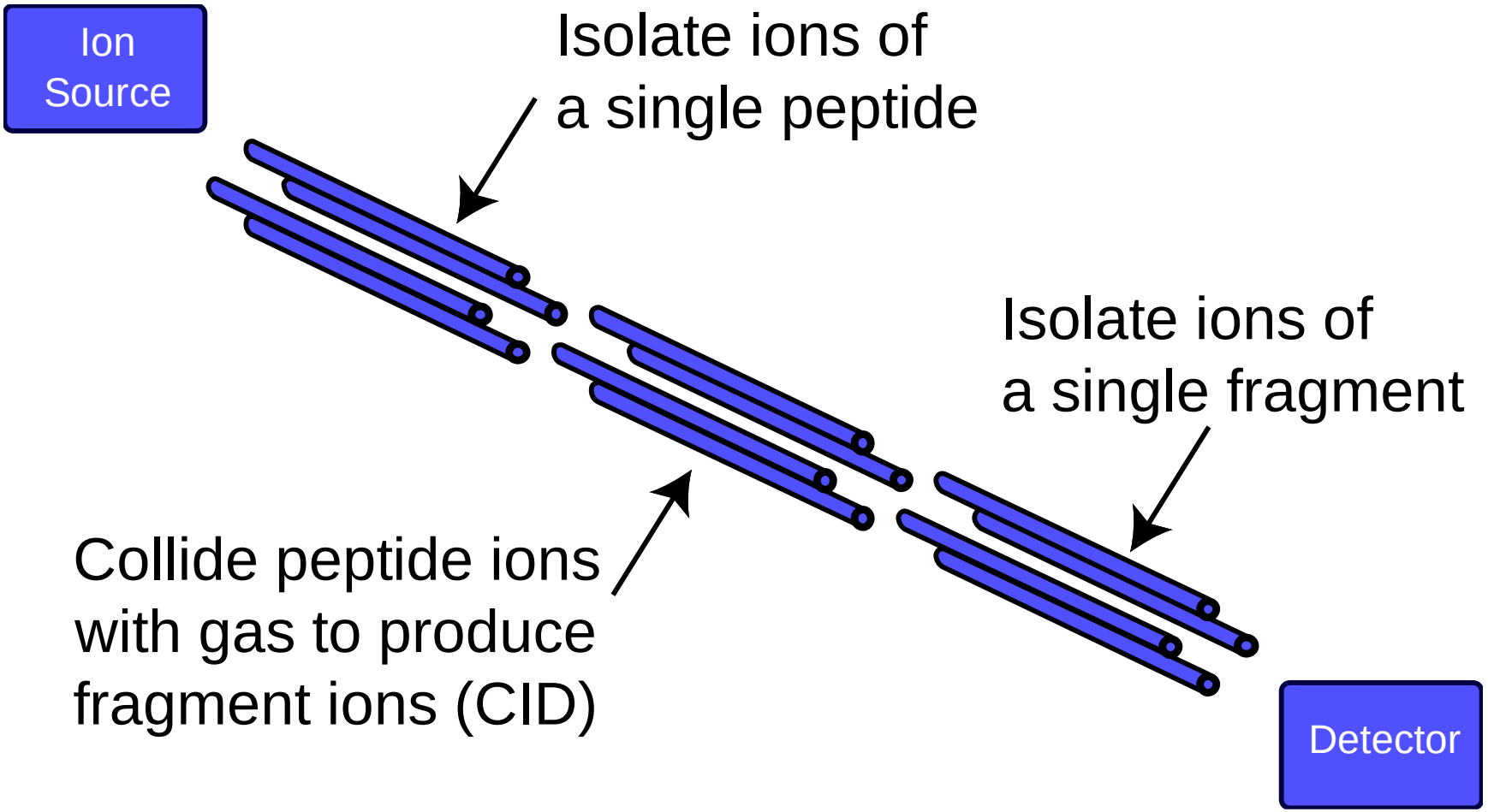


Ross et al (2004) *Mol. Cell. Proteomics* 3: 1154-1169.

Why targeted proteomics?

- Interesting proteins are low in concentration and may be masked by dominant proteins.
- Shotgun methods detect any given peptide with low probability.
- Targeting a peptide achieves higher sensitivity and generates a intensity profile.
- *Few journals will publish proteomic differences without orthogonal confirmation.*

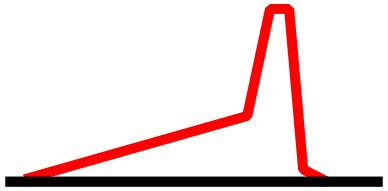
Triple quads for Selected Reaction Monitoring (SRM)



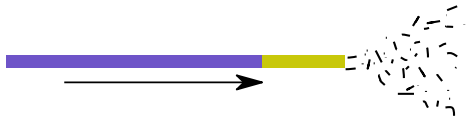
Liebler and Zimmerman. *Biochemistry* (2013) 52: 3797.

Targeted Proteomics

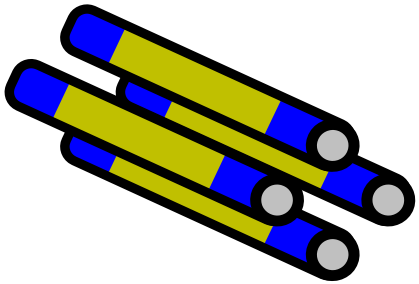
Peptide Mixture



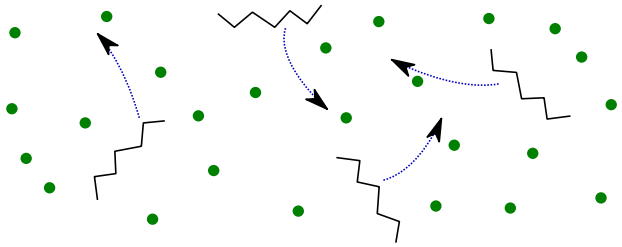
Liquid Chromatography



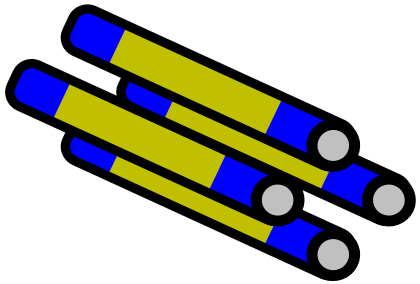
Electrospray Ionization



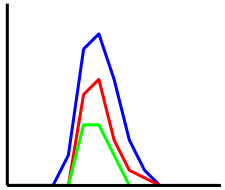
Screen out all but Target Mass



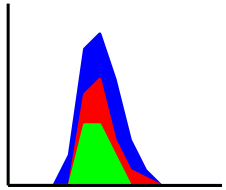
Collide Ions to Dissociate



Screen out all but Fragment Mass



Find Peaks from Related Traces



Integrate Peak Areas

Compare Areas to Reference Areas

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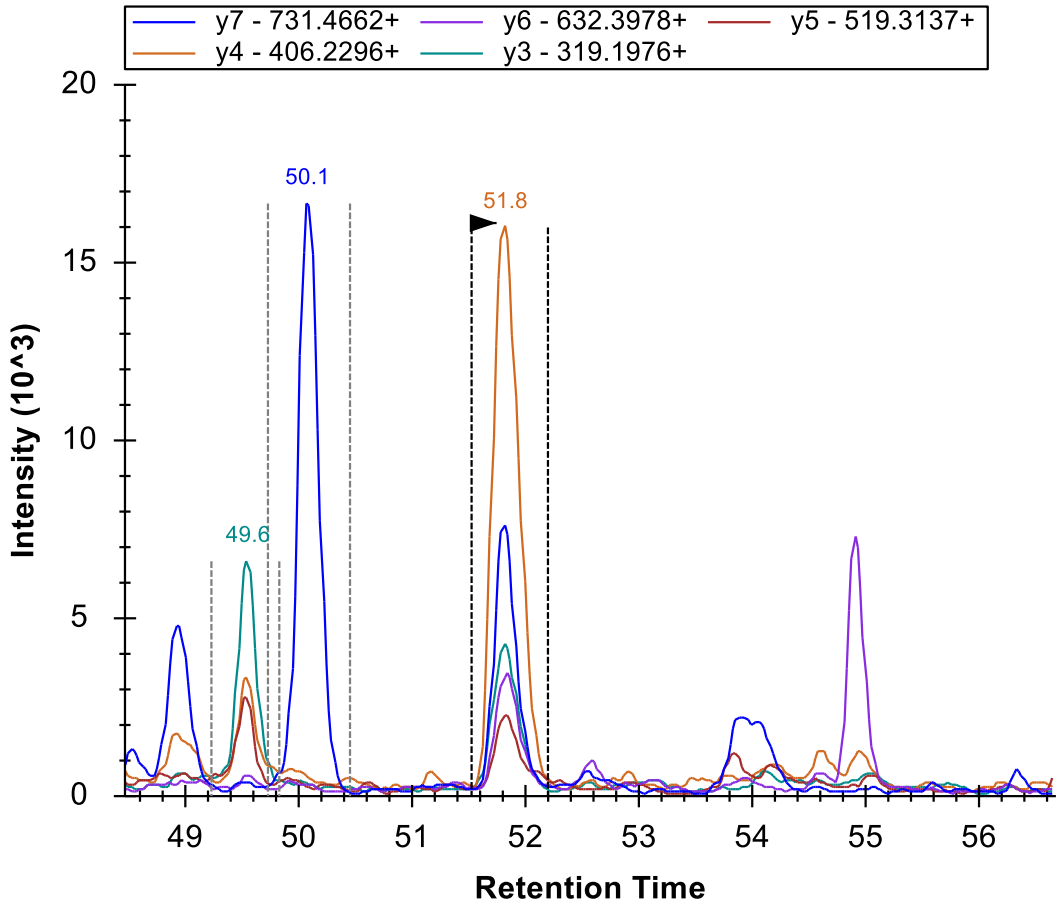
Chromatogram areas yield peptide quantities



Tallest may not be correct peak; look for correspondence among traces.

Skyline

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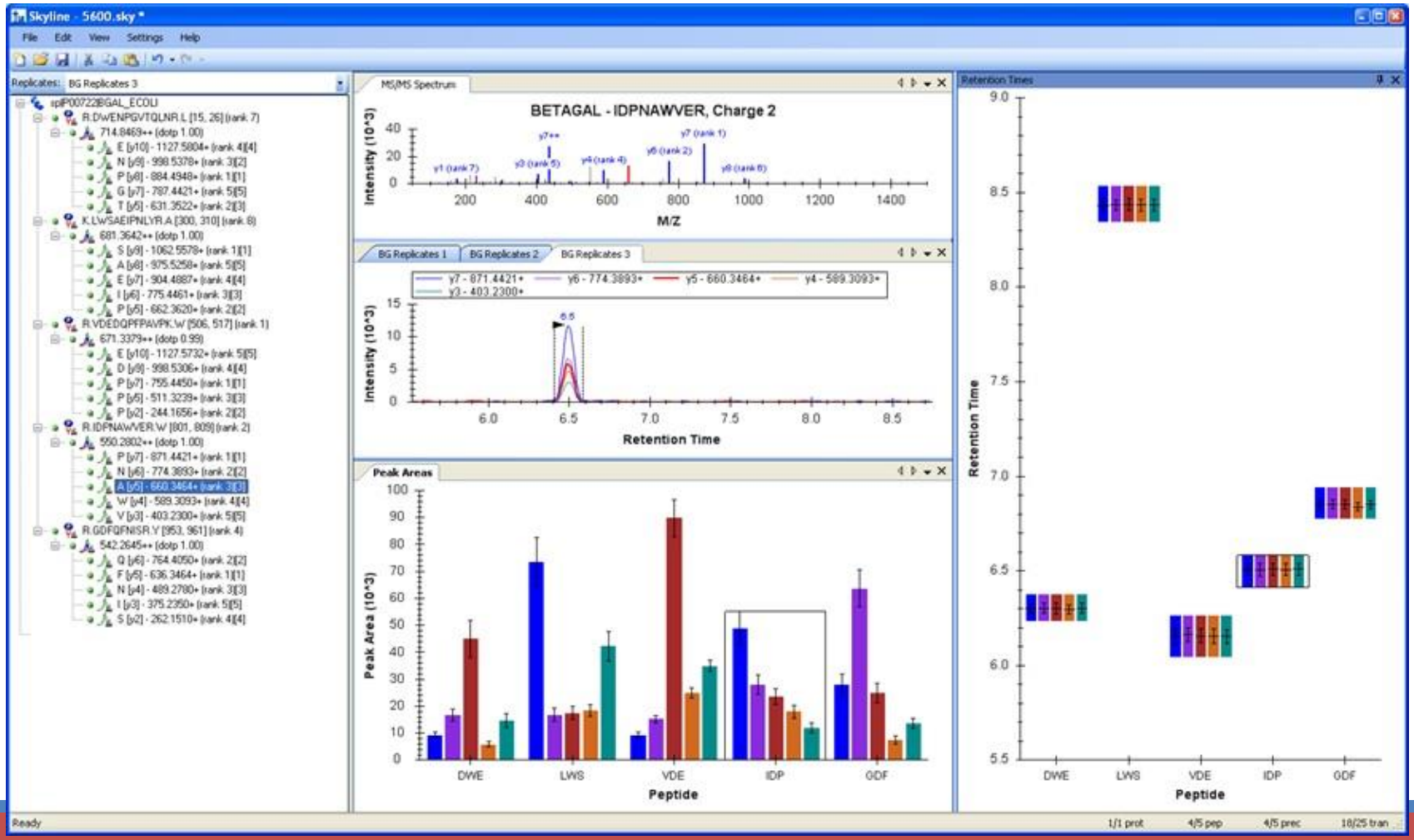
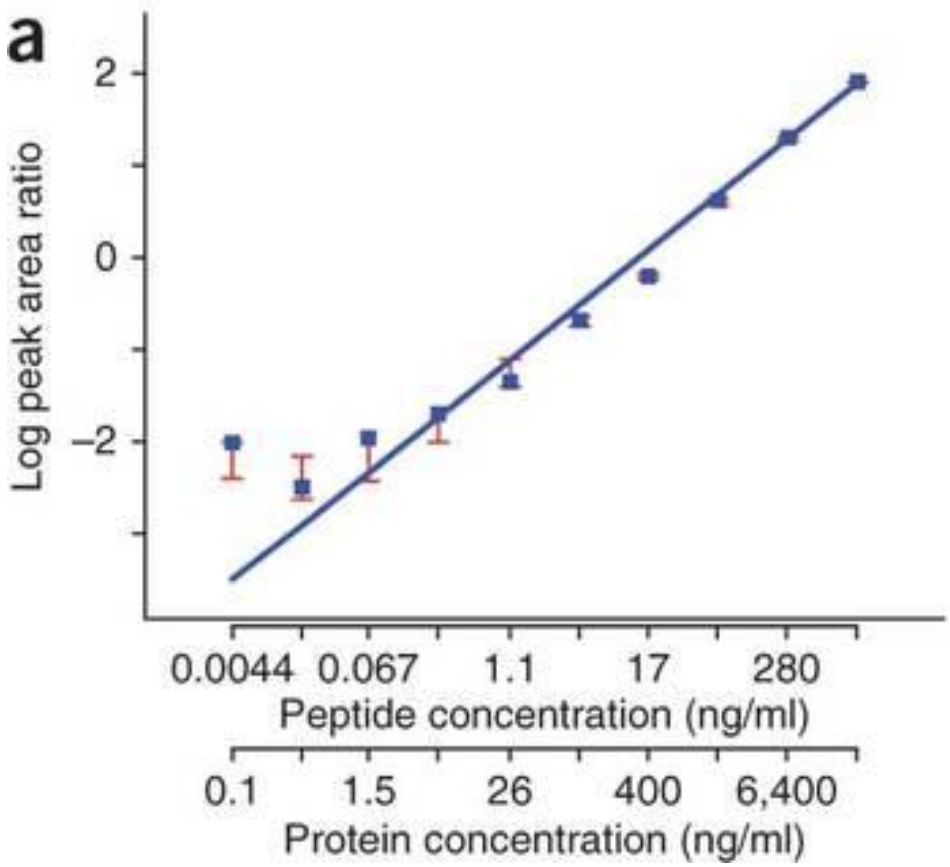
Standard quantitative workbench



Images from https://skyline.ms/ and https://sciex.com/products/software/skyline-software

Calibration curves relate measurement to true concentration



- Limit of detection (LOD): lowest conc. for non-zero measurement
- Limit of quantitation (LOQ): lowest conc. for suitable precision and accuracy in quantitation
- Linear response range: area where increase in conc. results in proportionate increase in measurement.

Whiteaker et al. *Nature Biotechnology* (2011) 29: 625–634

"Masters of the x-axis"

- Mass spectrometers offer very high precision for m/z values, with FT mass analyzers varying well within 10 ppm for mass measurement.
- Intensity measurement, however, is far more variable, especially in low signal-to-noise ions.
- Intralaboratory variation for targeted quantitative experiments generally range up to 10% CV.

COEFFICIENT OF VARIATION:
Standard deviation divided by mean

Addona et al. *Nature Biotech*. (2009) 27: 633

Takeaway messages

- Your quantitative experiment may change depending on whether you need to know *what changed* or by *how much*.
- Label-free, isotopically-labeled, isobaric tagging, and targeted quantitation are all legitimate strategies for quantifying, with different strengths and weaknesses.