Mass spectrometry-based proteomics

BMI/CS 776

www.biostat.wisc.edu/bmi776/

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Goals for lecture

Key concepts

- Benefits of mass spectrometry
- Generating mass spectrometry data
- Computational tasks
- Matching spectra and peptides

Mass spectrometry uses

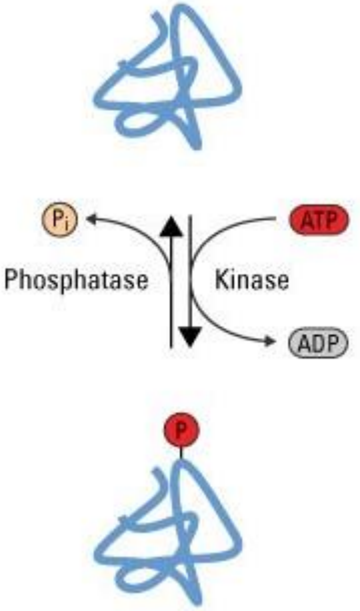
- Mass spectrometry is like the protein analog of RNA-seq
  - Quantify abundance or state of all (many) proteins
  - No need to specify proteins to measure in advance

- Other applications in biology
  - Targeted proteomics
  - Metabolomics
  - Lipidomics

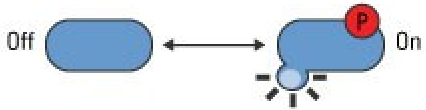
Advantages of proteomics

- Proteins are functional units in a cell
  - Protein abundance directly relevant to activity

- Post-translational modifications
  - Change protein state

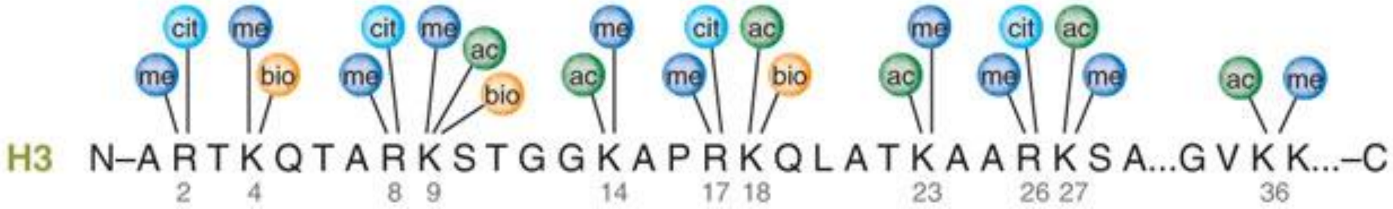


Phosphorylation in signaling



Thermo Fisher Scientific

Histone modifications



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H3 N–A R T K Q T A R K S T G G K A P R K Q L A T K A A R K S A...G V K K...–C
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26
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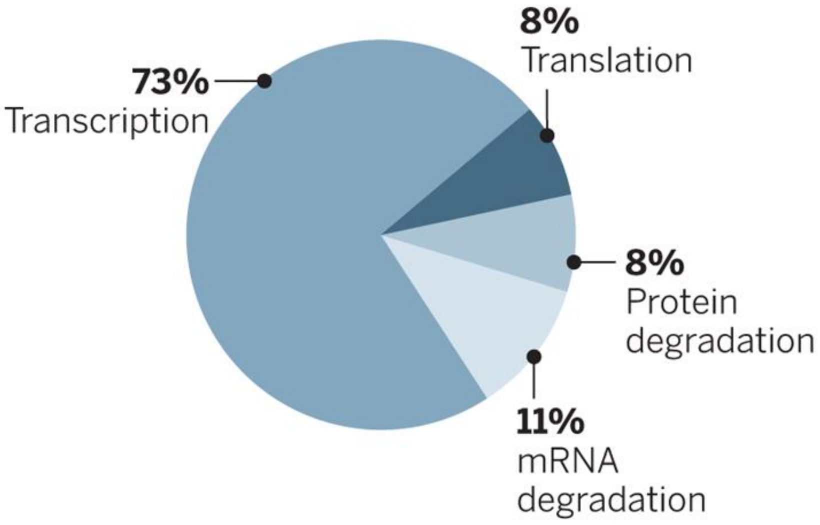
36

Latham *Nature Structural & Molecular Biology* 2007; Katie Ris-Vicari

Estimating protein levels from gene expression

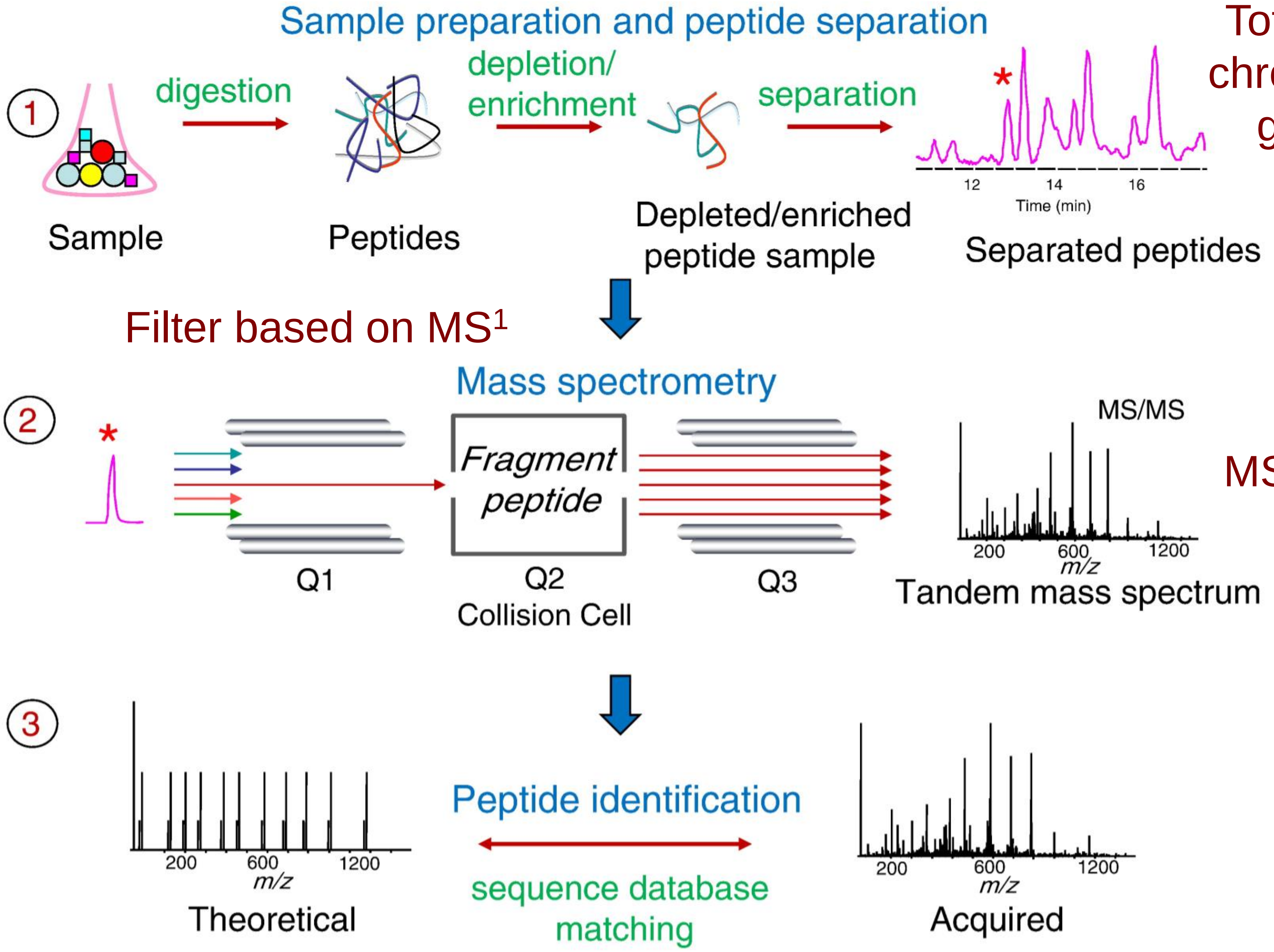
- Correlation between gene expression and protein abundance has been debated
- Gene expression tells us nothing about post-translational modifications

Contribution to protein levels



Li and Biggin *Science* 2015

Mass spectrometry workflow



Total ion chromato-gram

Filter based on MS^1

MS^2

Nesvizhskii *Journal of Proteomics* 2010

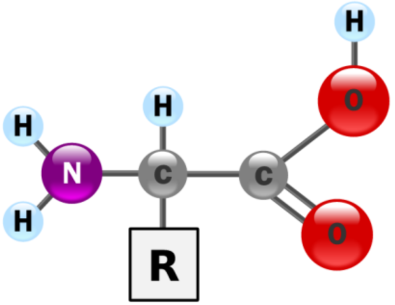
Amino Acids

- 20 amino acids
- Building blocks of proteins
- Known molecular weight

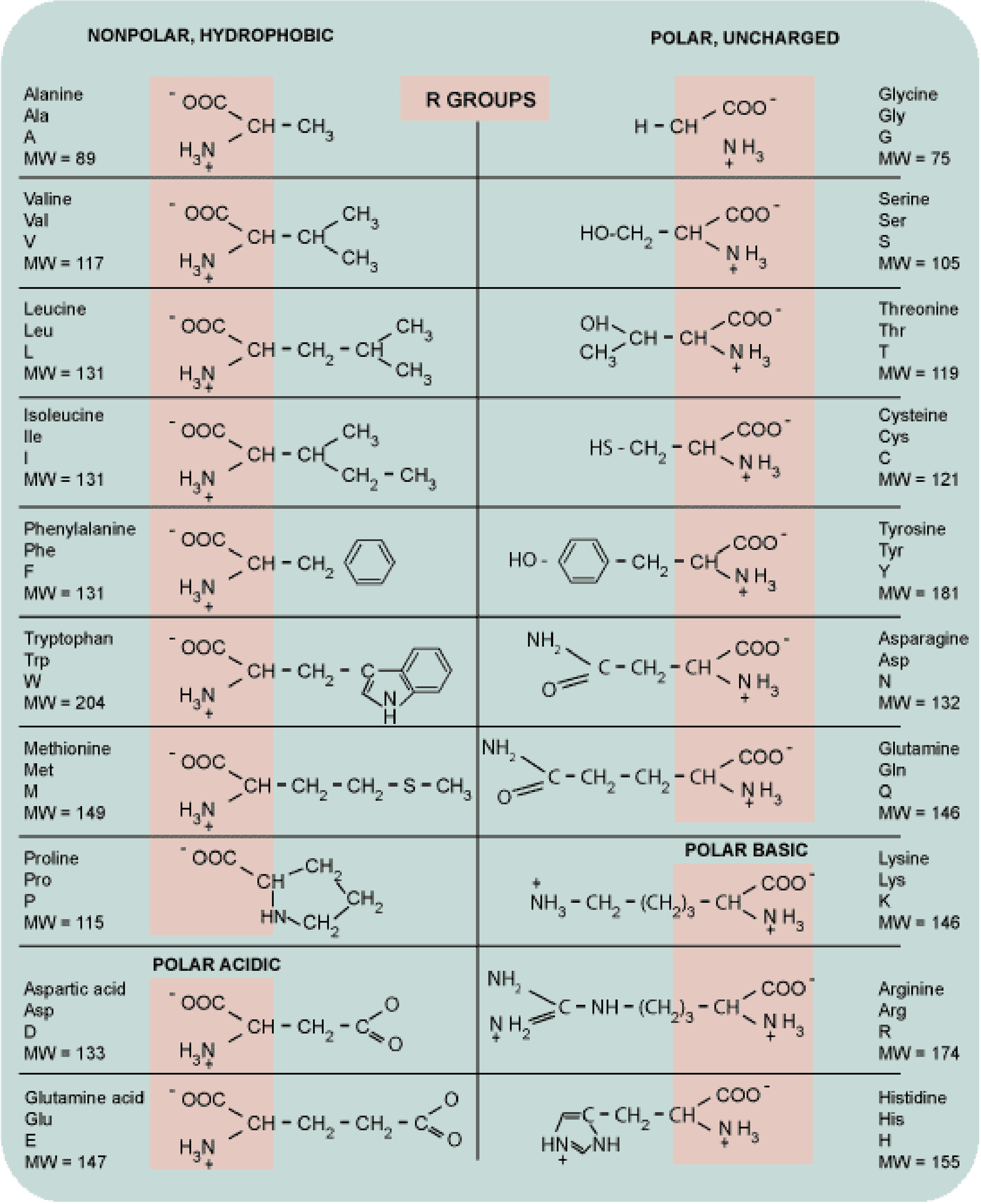
- Common template

Amino-terminal

Carboxy-terminal

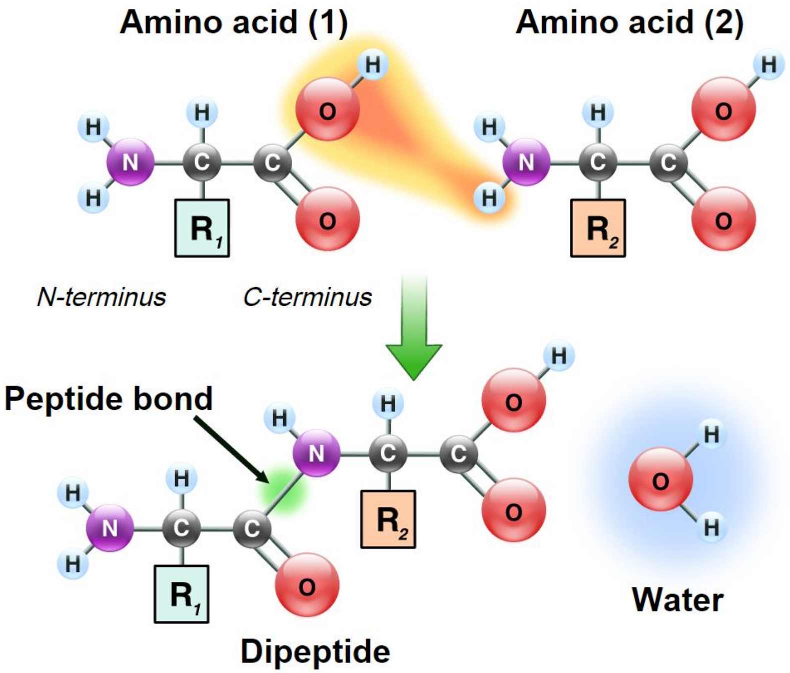


Wikipedia, Yassine Mrabet



Peptide fragmentation

Peptide bond

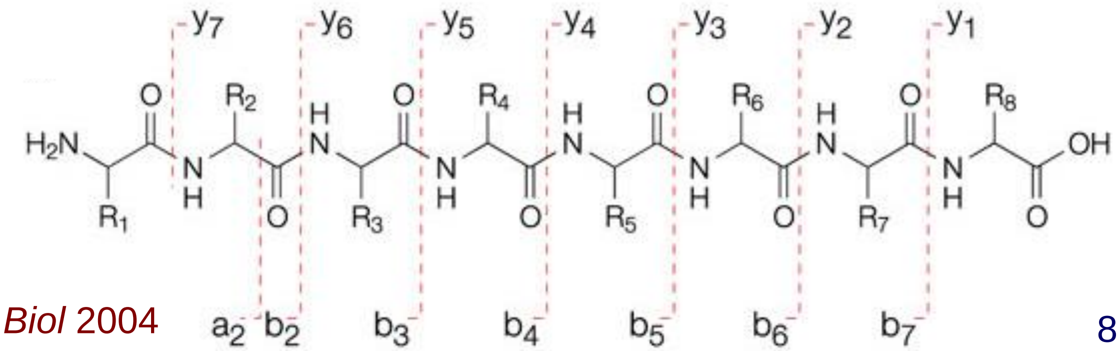


Wikipedia, Yassine Mrabet

- Select similar peptides from MS^1
- Fragment with high energy collisions
- Break peptide bonds

Charge on amino-terminal (b) or carboxy-terminal fragment (y)

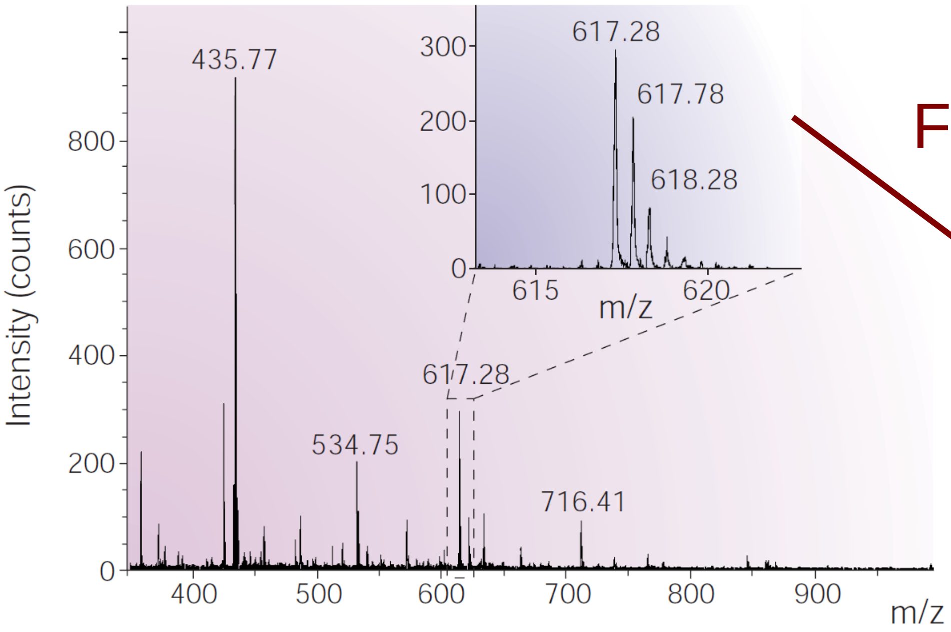
Subscript = # R groups retained



Steen and Mann *Nat Rev Mol Cell Biol* 2004

Mass spectra

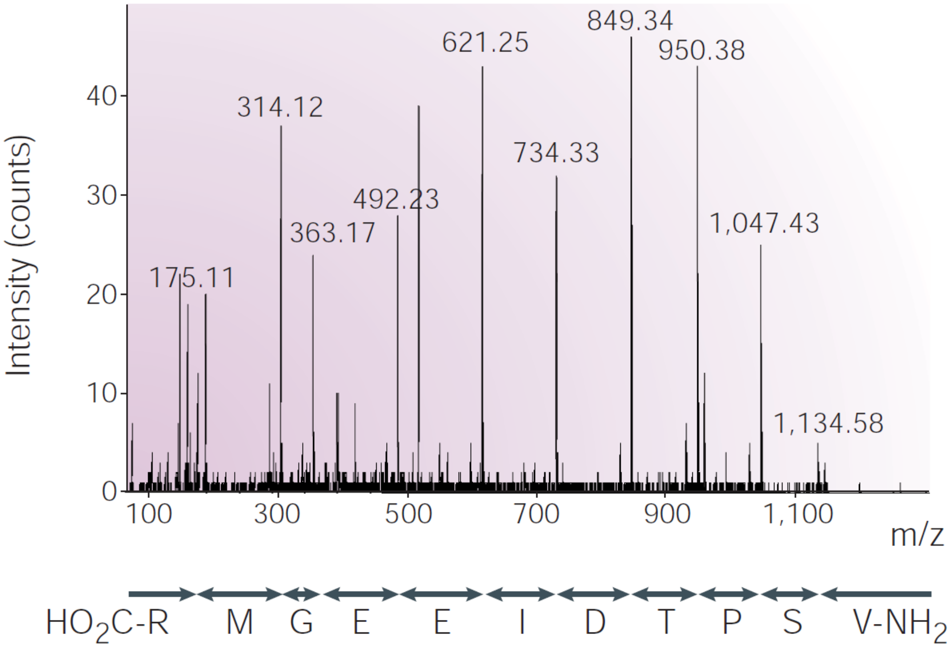
MS^1



Steen and Mann *Nat Rev Mol Cell Biol* 2004

Fragment and analyze one precursor ion

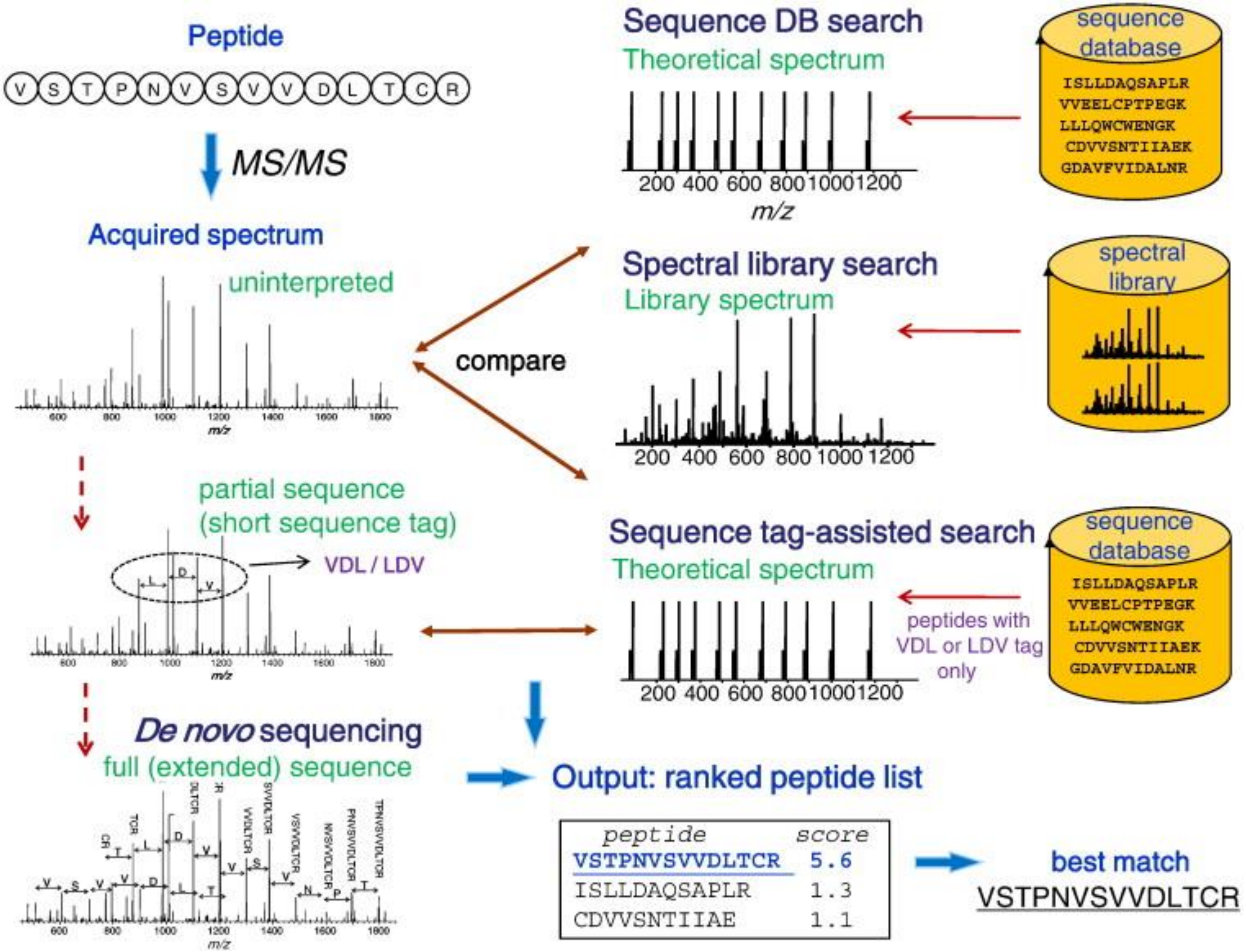
MS^2



Mass-to-charge ratio

Spectrum contains information about amino acid sequence, fragment at different bonds

From spectra to peptides



Peptide

MS/MS

Acquired spectrum

uninterpreted

partial sequence
(short sequence tag)

VDL / LDV

De novo sequencing
full (extended) sequence

compare

Sequence DB search
Theoretical spectrum

sequence database

ISLLDAQSAPLR
VVEELCPTPEGK
LLLQWCWENGK
CDVVSNTIIAEK
GDAVFVIDALNR

Spectral library search
Library spectrum

spectral library

Sequence tag-assisted search
Theoretical spectrum

peptides with VDL or LDV tag only

sequence database

ISLLDAQSAPLR
VVEELCPTPEGK
LLLQWCWENGK
CDVVSNTIIAEK
GDAVFVIDALNR

Output: ranked peptide list

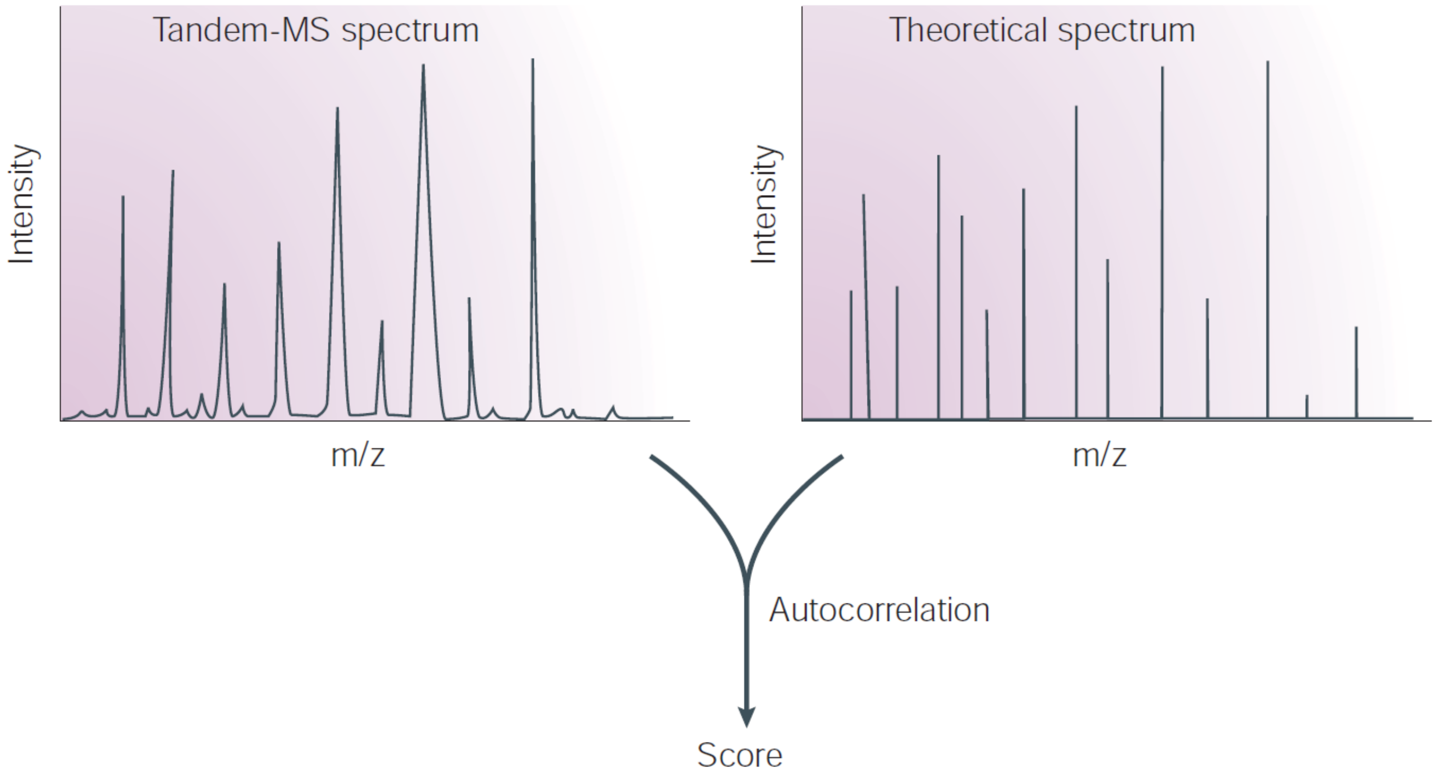
peptide	score
VSTPNVSVVDLTCR	5.6
ISLLDAQSAPLR	1.3
CDVVSNTIIAE	1.1

best match
VSTPNVSVVDLTCR

Nesvizhskii *Journal of Proteomics* 2010

Sequence database search

- Need to define a scoring function
- Identify peptide-spectrum match (PSM)



Steen and Mann *Nat Rev Mol Cell Biol* 2004

SEQUEST

- Cross correlation (xcorr)
- Similarity between theoretical spectrum (x) and acquired spectrum (y)
- Correction for mean similarity at different offsets

Offsets

$$\text{xcorr} = R_0 - \left(\sum_{\tau=-75}^{\tau=+75} R_\tau \right) \Bigg/ 151$$

Actual similarity

$$R_\tau = \sum x[i] \cdot y[i + \tau]$$

Theoretical

Acquired

Eng, McCormack, Yates *J Am Soc Mass Spectrom* 1994

Fast SEQUEST

- SEQUEST originally only applied to top 500 peptides based on coarse filtering score

$$\mathrm{xcorr} = x_0 \cdot y_0 - \left(\sum_{\tau=-75}^{\tau=+75} x_0 \cdot y_\tau \right) \Big/ 151$$

$$\mathrm{xcorr} = x_0 \cdot \left(y_0 - \left(\sum_{\tau=-75}^{\tau=+75} y_\tau \right) \Big/ 151 \right)$$

$$\mathrm{xcorr} = x_0 \cdot y' \quad \text{where} \quad y' = y_0 - \left(\sum_{\tau=-75,\tau\neq 0}^{\tau=+75} y_\tau \right) \Big/ 150$$

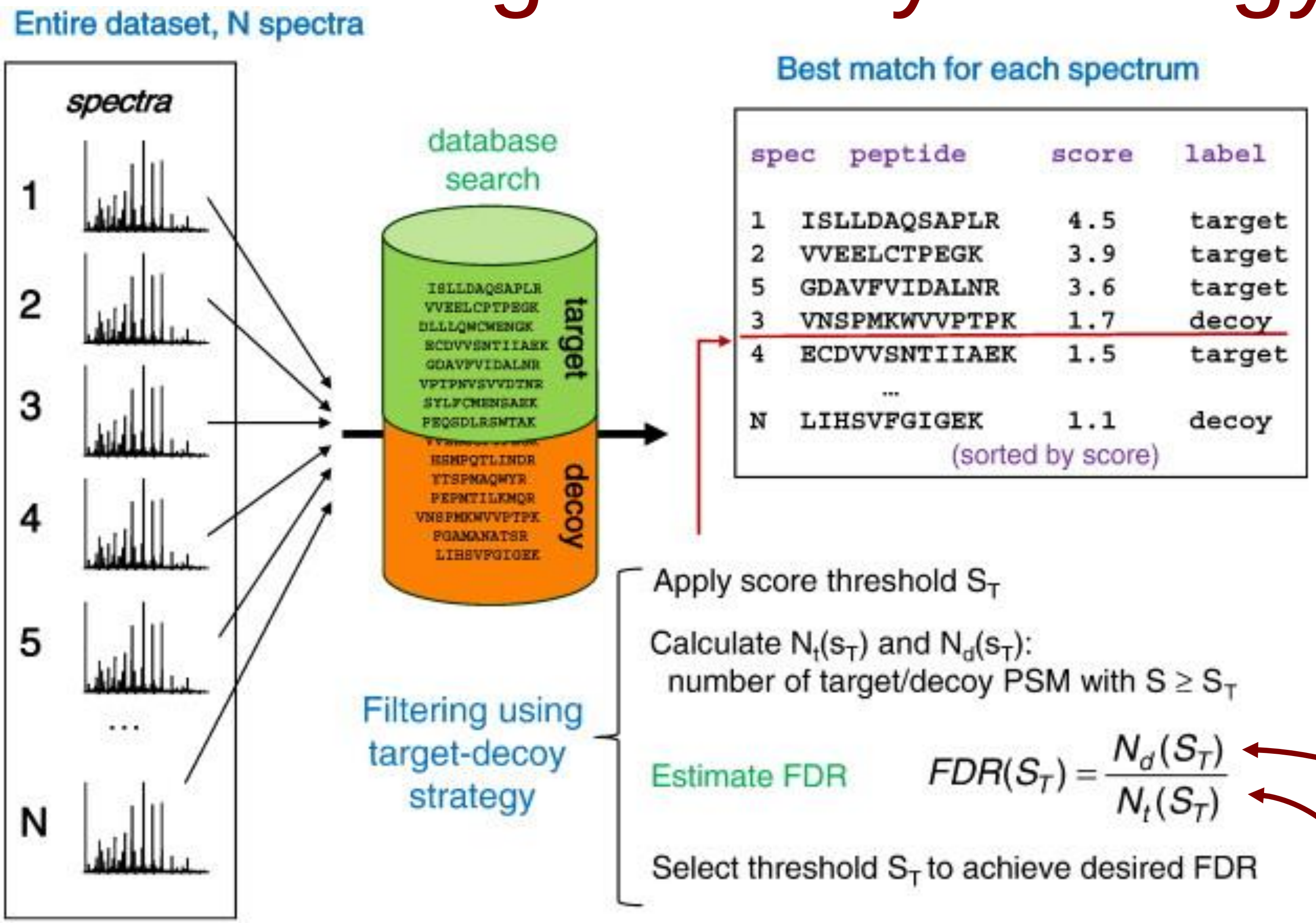
Skip the 0 offset

Eng et al *J Proteome Res* 2008

PSM significance

- E-value: expected number of null peptides with score ≥ observed score
- Compute FDR from E-value distribution
- Add decoy peptides to database
  - Reversed peptide sequences
  - Used to estimate false discoveries

Target-decoy strategy



Best match for each spectrum

spec	peptide	score	label
1	ISLLDAQSAPLR	4.5	target
2	VVEELCPTPEGK	3.9	target
5	GDAVFVIDALNR	3.6	target
3	VNSPMKWVVPTPK	1.7	decoy
4	ECDVVSNTIIAEK	1.5	target
	...		
N	LIHSVFGIGEK	1.1	decoy

(sorted by score)

Apply score threshold S_T

Calculate $N_t(s_T)$ and $N_d(s_T)$: number of target/decoy PSM with $S \geq S_T$

Estimate FDR $$FDR(S_T) = \frac{N_d(S_T)}{N_t(S_T)}$$

Select threshold S_T to achieve desired FDR

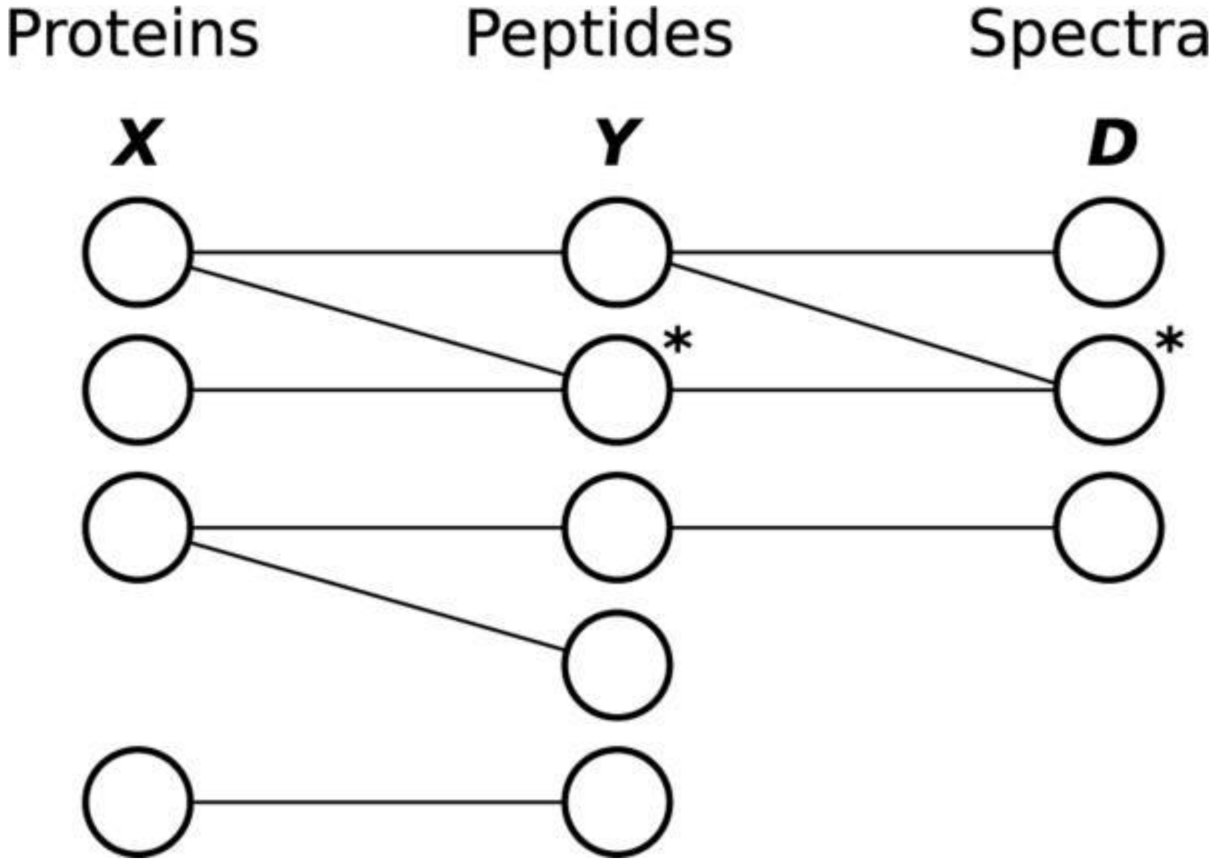
Nesvizhskii *Journal of Proteomics* 2010

target PSMs above score threshold = $N_t\,(S_T)$

decoy PSMs above score threshold = $N_d\,(S_T)$

Identifying proteins

- Even after identifying PSM, still need to identify protein of origin



Serang and Noble *Stat Interface* 2012

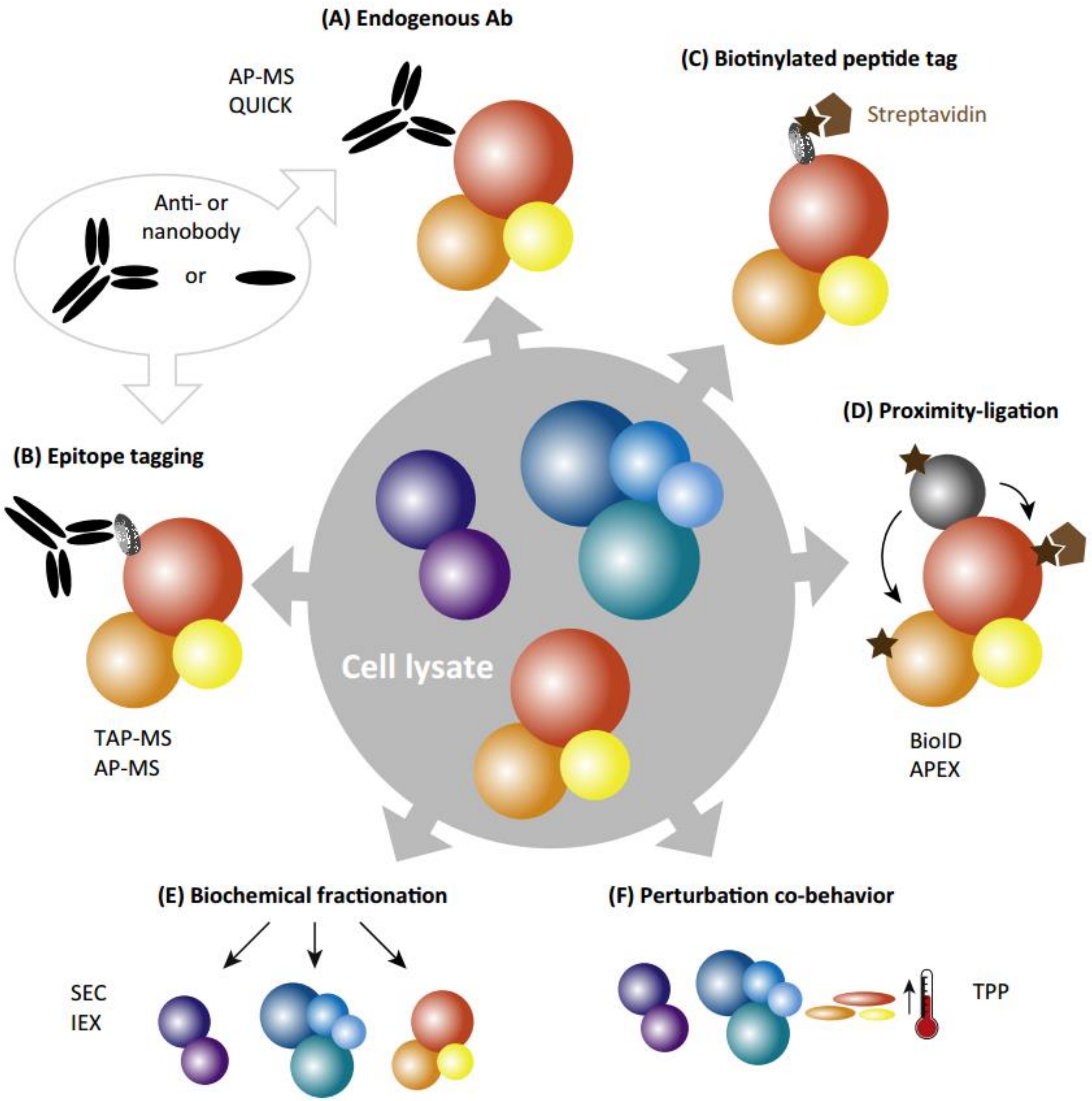
Mass spectrometry versus RNA-seq

- RNA-seq
  - Transcript → RNA fragment → paired-end read

- Mass spectrometry
  - Protein → peptides → ions → spectrum

- Mapping spectra to proteins more ambiguous than mapping reads to transcripts
- Spectra state space is enormous

Protein-protein interactions

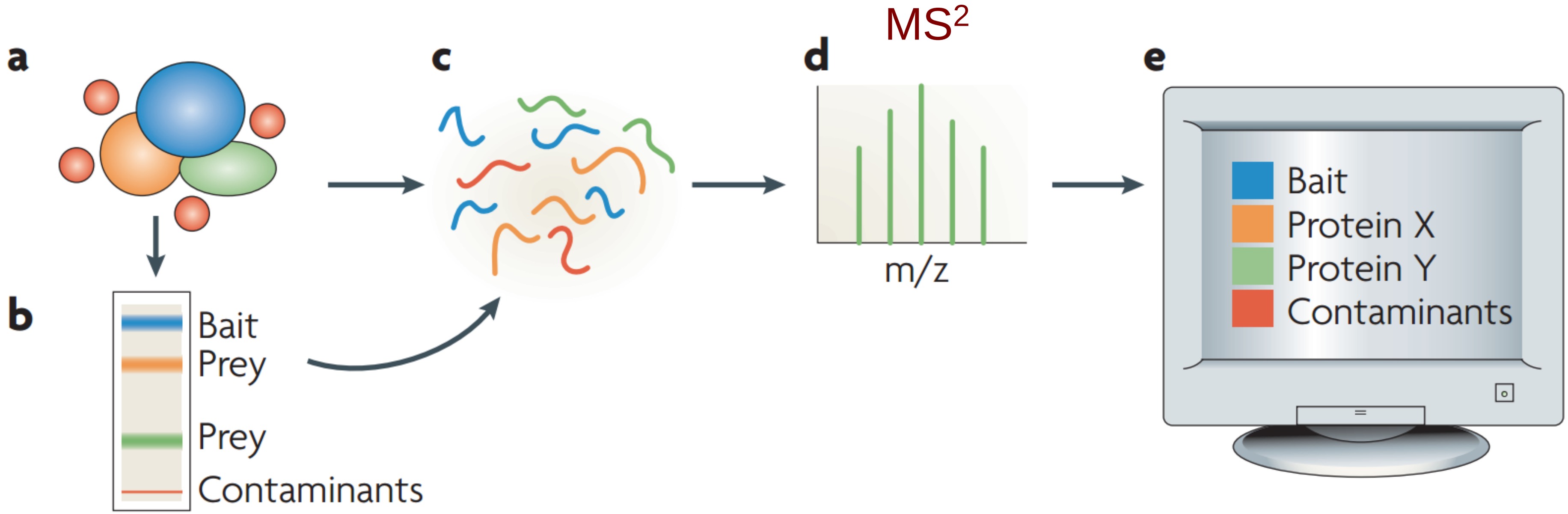


Smits and Vermeulen T*rends in Biotechnology* 2016

- Affinity-purification mass spectrometry
- Purify protein of interest, identify complex members

Protein-protein interactions

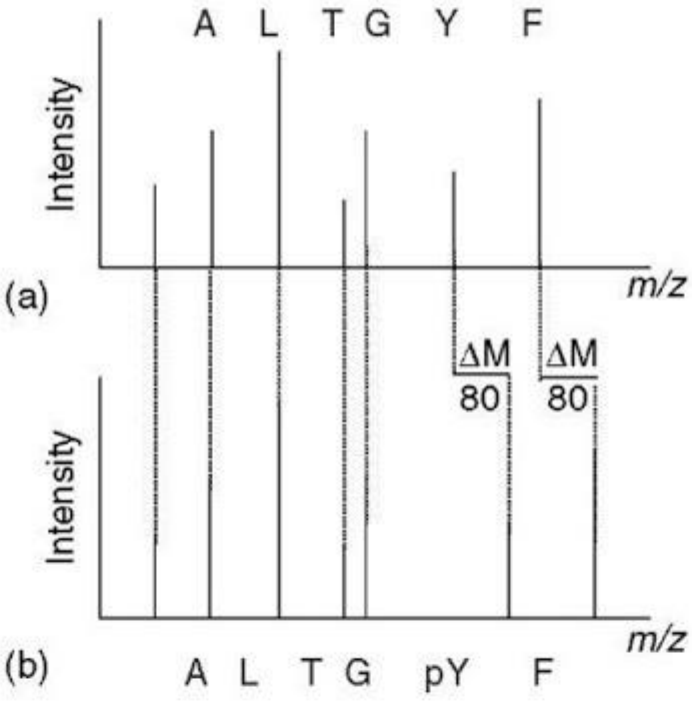
- Mass spectrometry identifies proteins in the complex
- Must control for contaminants



Gingras et al *Nature Reviews Molecular Cell Biology* 2007

Post-translational modifications (PTMs)

- Shift the peptide mass by a known quantity



what-when-how

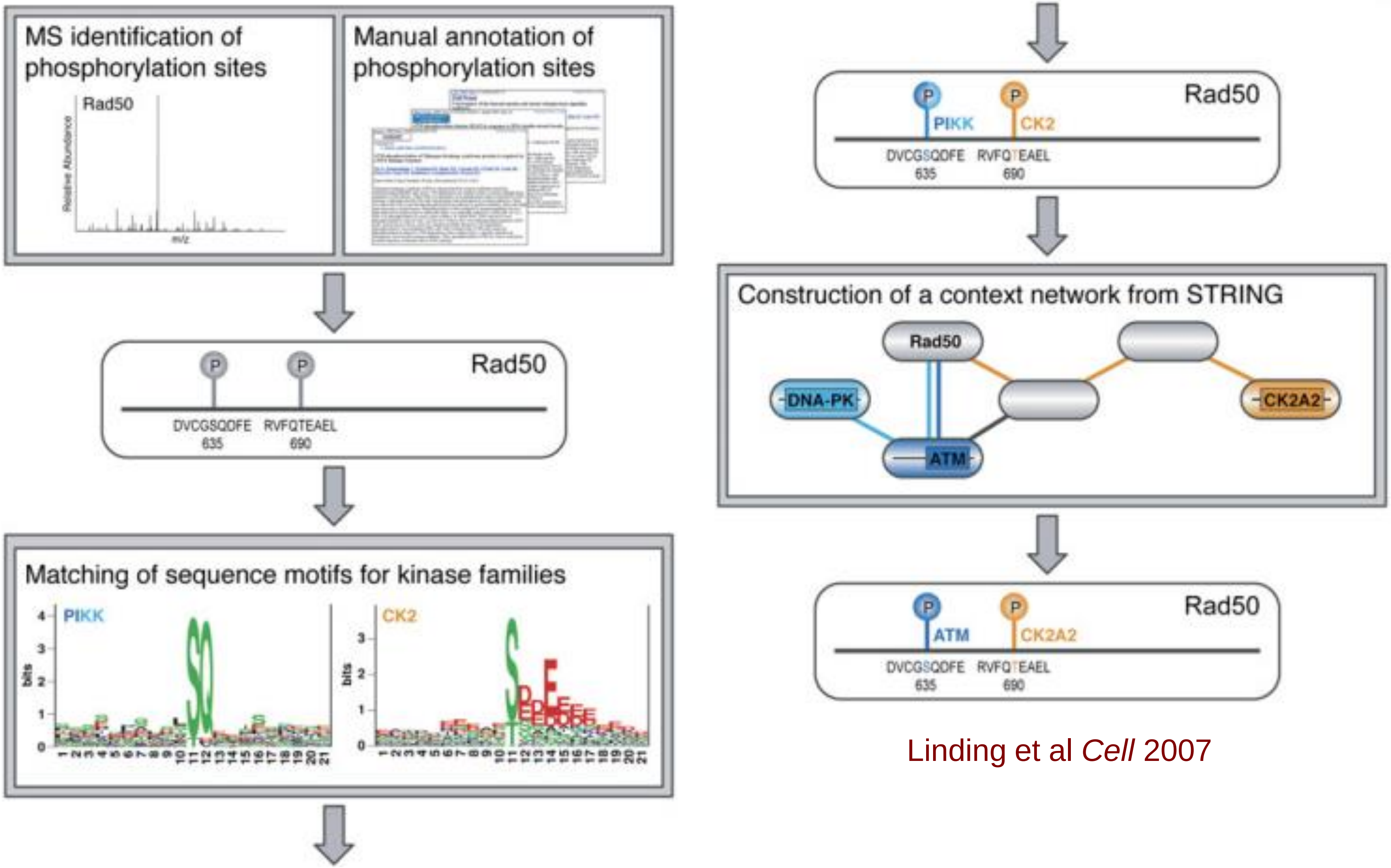
Phosphoproteomics example

Gene	Modified Site	Peptide	Phosphorylation (Treatment / Control)
AGRN	S671	AGPC[160.03]EQAEC[160.03]GS[167.00]GGSGSGEDGDC[160.03]EQELC[160.03]R	4.54
ADAMTS10	S74	RGTGATAES[167.00]R	0.30
CABYR	T16	T[181.01]LLEGISR	0.37
TTC7B	T152	VIEQDET[181.01]R	5.97
STAT3	Y705	K.n[305.21]YC[160.03]RPESQEHPEADPGSAAPY[243.03]LK[432.30].T	4.50

Sychev et al *PLoS Pathogens* 2017

Phosphoproteomics interpretation

- Predict kinases/phosphatases for phospho sites


MS identification of phosphorylation sites
Rad50
Relative Abundance
m/z
Manual annotation of phosphorylation sites
P
P
Rad50
DVCGSQDFE
635
RVFQTEAEL
690
Matching of sequence motifs for kinase families
PIKK
bits
CK2
bits
P
PIKK
P
CK2
Rad50
DVCGSQDFE
635
RVFQTEAEL
690
Construction of a context network from STRING
Rad50
DNA-PK
ATM
CK2A2
P
ATM
P
CK2A2
Rad50
DVCGSQDFE
635
RVFQTEAEL
690

Linding et al *Cell* 2007

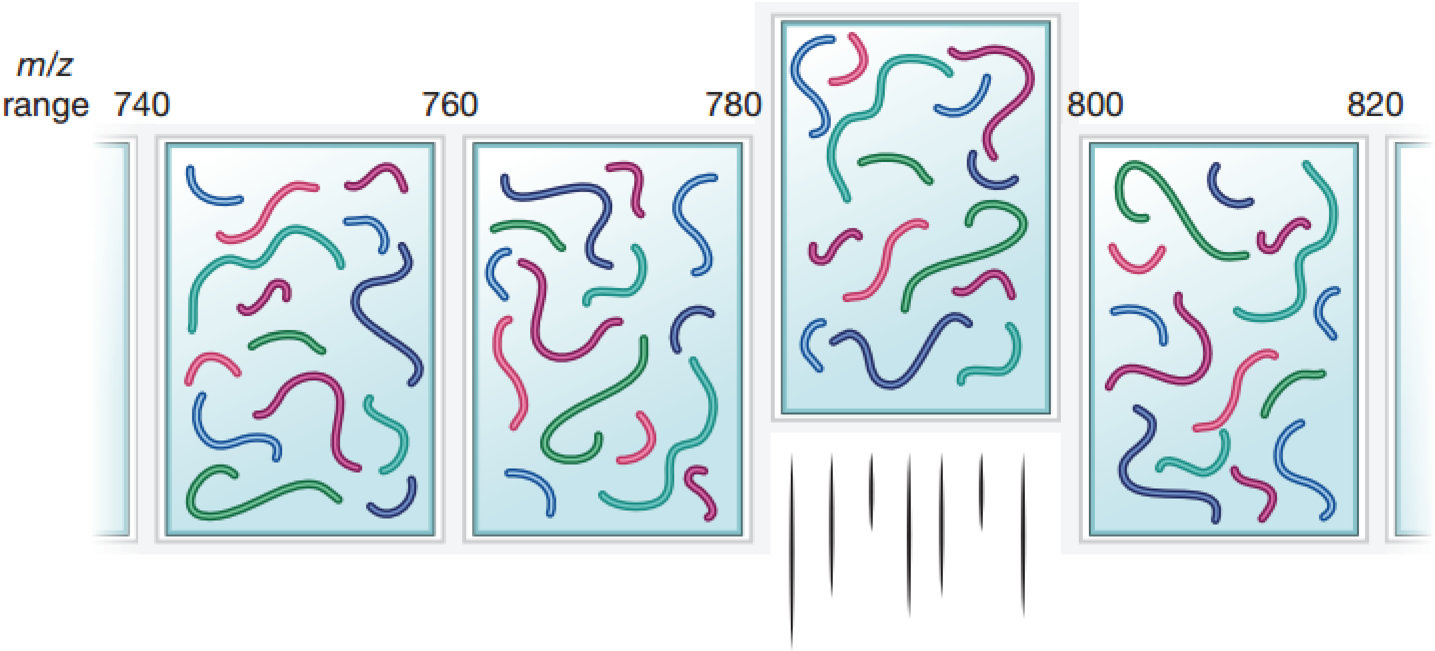
Mass spectrometry replicates

- Doesn’t identify all proteins in the sample
  - Data dependent acquisition has low overlap across replicates
  - Partly due to biological variation
  - New protocols to overcome this

- Phosphorylation PTMs are especially variable
  - Grimsrud et al *Cell Metabolism* 2012
    - 5 biological replicates
    - 9,558 phosphoproteins identified
    - 5.6% in all replicates

Data independent acquisition

- Not dependent on most abundance signals in MS^1
- Sliding *m*/*z* window



Doerr *Nature Methods* 2015

Mass spectrometry summary

- Incredibly powerful for looking at biological processes beyond gene expression
  - Protein abundance
  - Post-translational modifications
  - Metabolites
  - Protein-protein interactions
- Typically reports relative abundance
- Labeling strategies for comparative analysis
  - Compare relative abundance in multiple conditions
- Missing data was a big problem, but improving
- Fully probabilistic analysis pipelines are not the most popular tools
  - Arguably greater diversity in software than RNA-seq