Proteomics, Metabolomics & other Omics

MMG 835, SPRING 2016
Eukaryotic Molecular Genetics

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Proteomics

Proteomics

Central Dogma of Molecular Biology

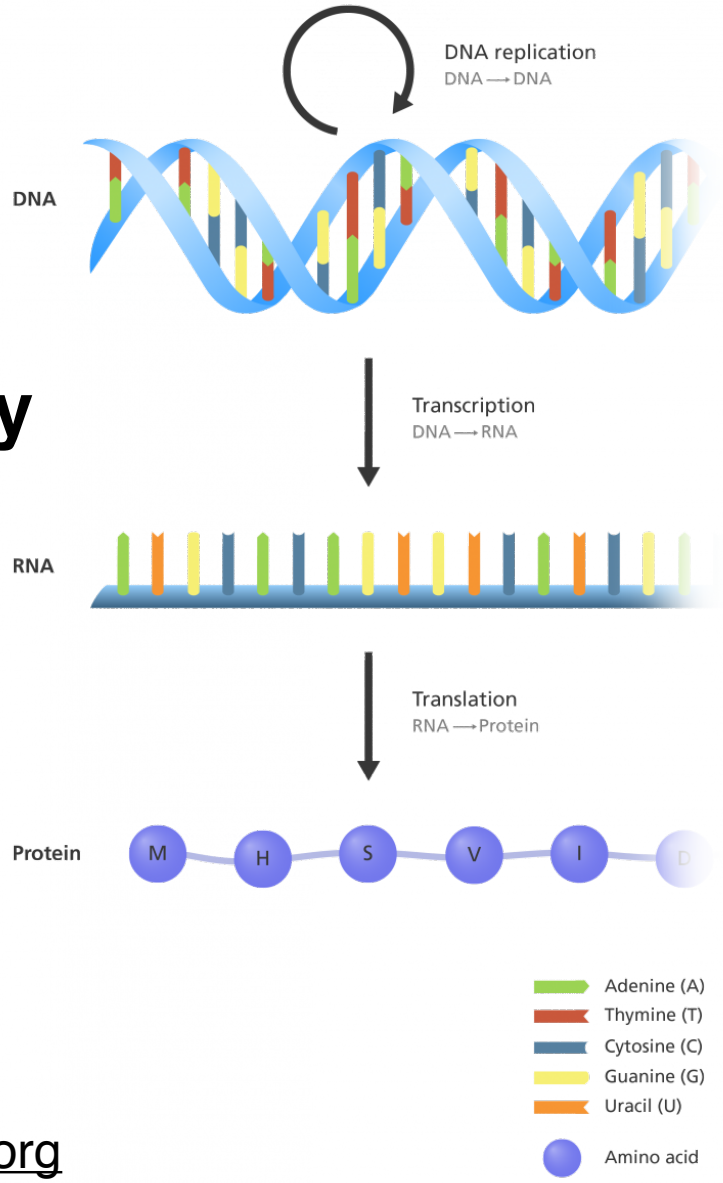


Image credit: Genome Research Limited From yourgenome.org

Proteomics

First letter of codon (5′ end) ↓ / Second letter of codon →	U		C		A		G	
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys
	UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys
	UUA	Leu	UCA	Ser	UAA	Stop	UGA	Stop
	UUG	Leu	UCG	Ser	UAG	Stop	UGG	Trp
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg
	CUC	Leu	CCC	Pro	CAC	His	CGC	Arg
	CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
	CUG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser
	AUC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
	AUA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
	AUG	Met	ACG	Thr	AAG	Lys	AGG	Arg
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly
	GUC	Val	GCC	Ala	GAC	Asp	GGC	Gly
	GUA	Val	GCA	Ala	GAA	Glu	GGA	Gly
	GUG	Val	GCG	Ala	GAG	Glu	GGG	Gly

Figure 27-7
Lehninger Principles of Biochemistry, Sixth Edition

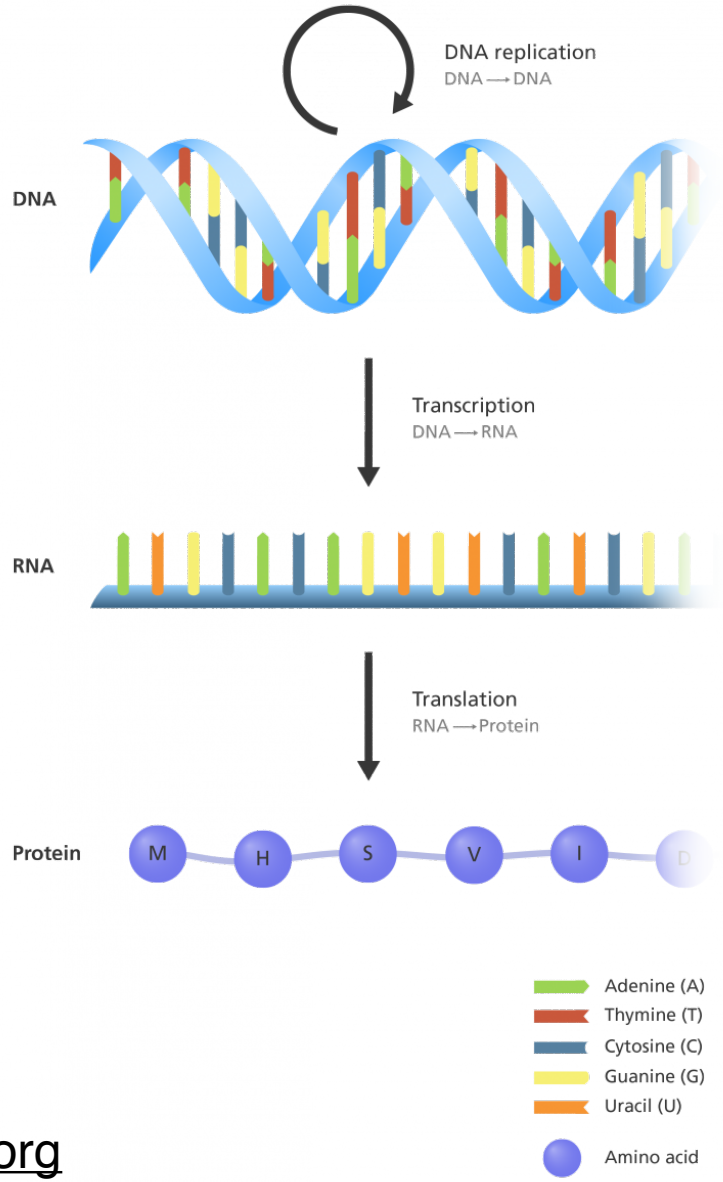


Image credit: Genome Research Limited From yourgenome.org

Proteomics

- DNA (static)
- RNA (dynamic)
- Protein (dynamic)
  - All proteins encoded by the genome
  - Multiple modifications possible (e.g. phosphorylation)

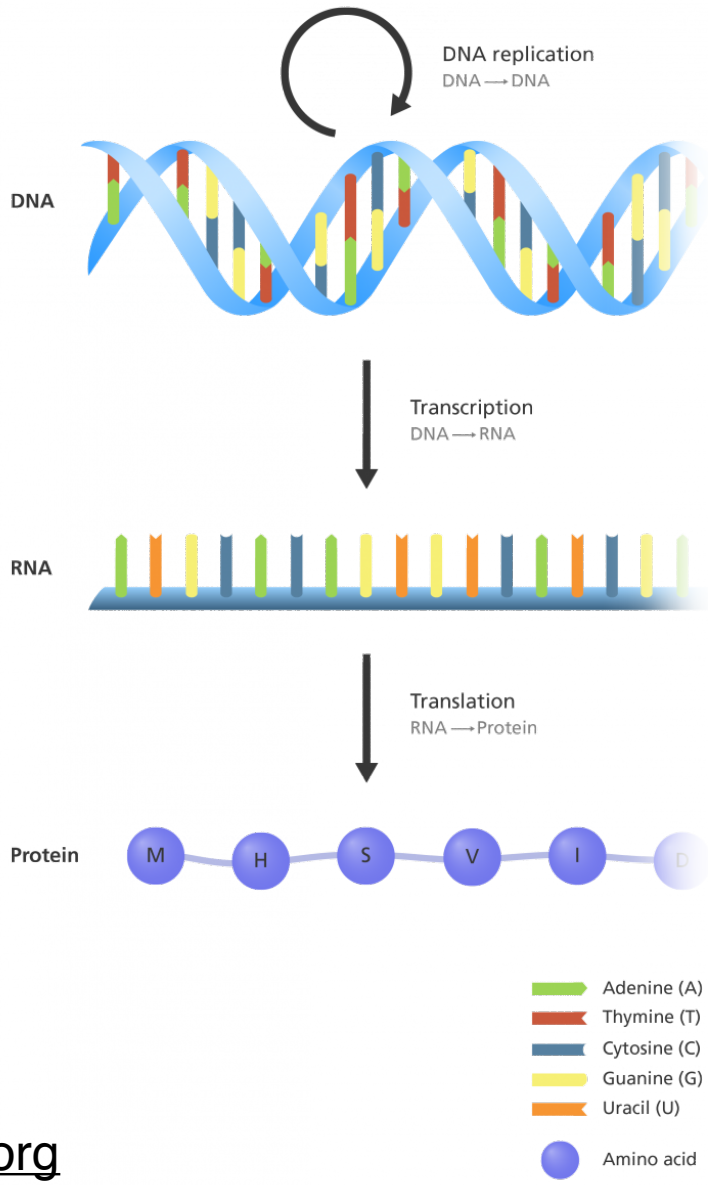
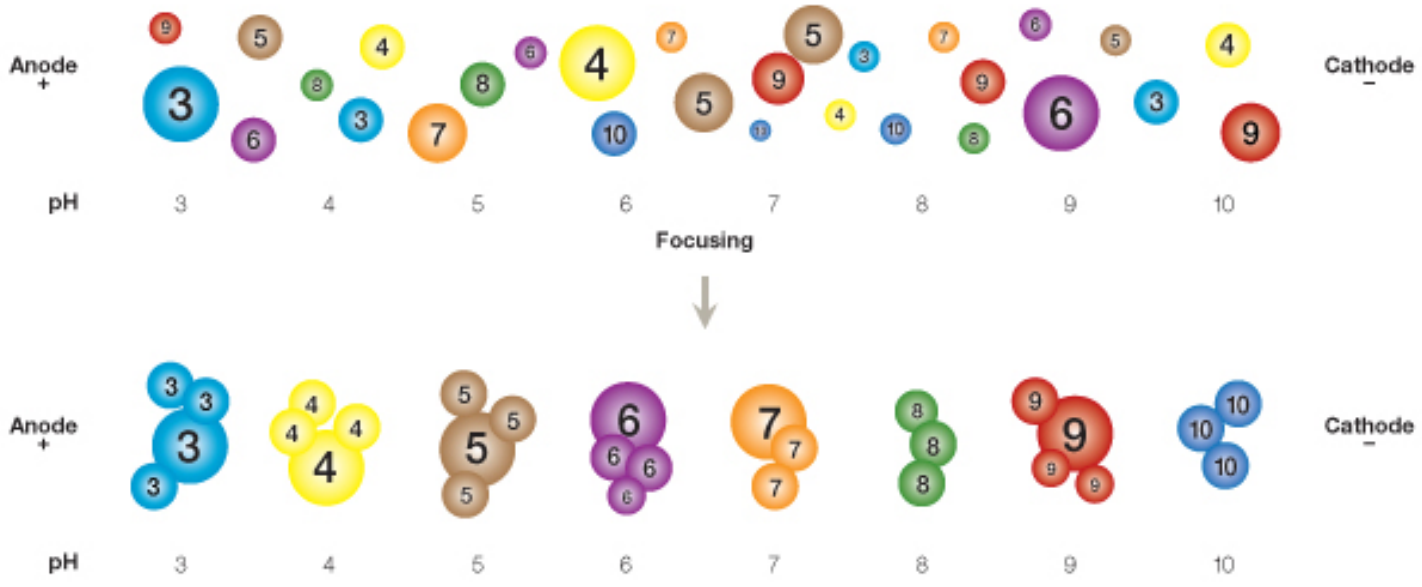


Image credit: Genome Research Limited From yourgenome.org

2D-SDS PAGE GEL

1st Dimension:separation of proteins according to their isoelectric point (pI) by isoelectric focusing (IEF).

- Proteins mixture resolved on a pH 3–10 IPG strip according to each protein's pI and independently of its size.
- Net protein charge is 0 at the pI, so at that point it will no longer respond to the electric field



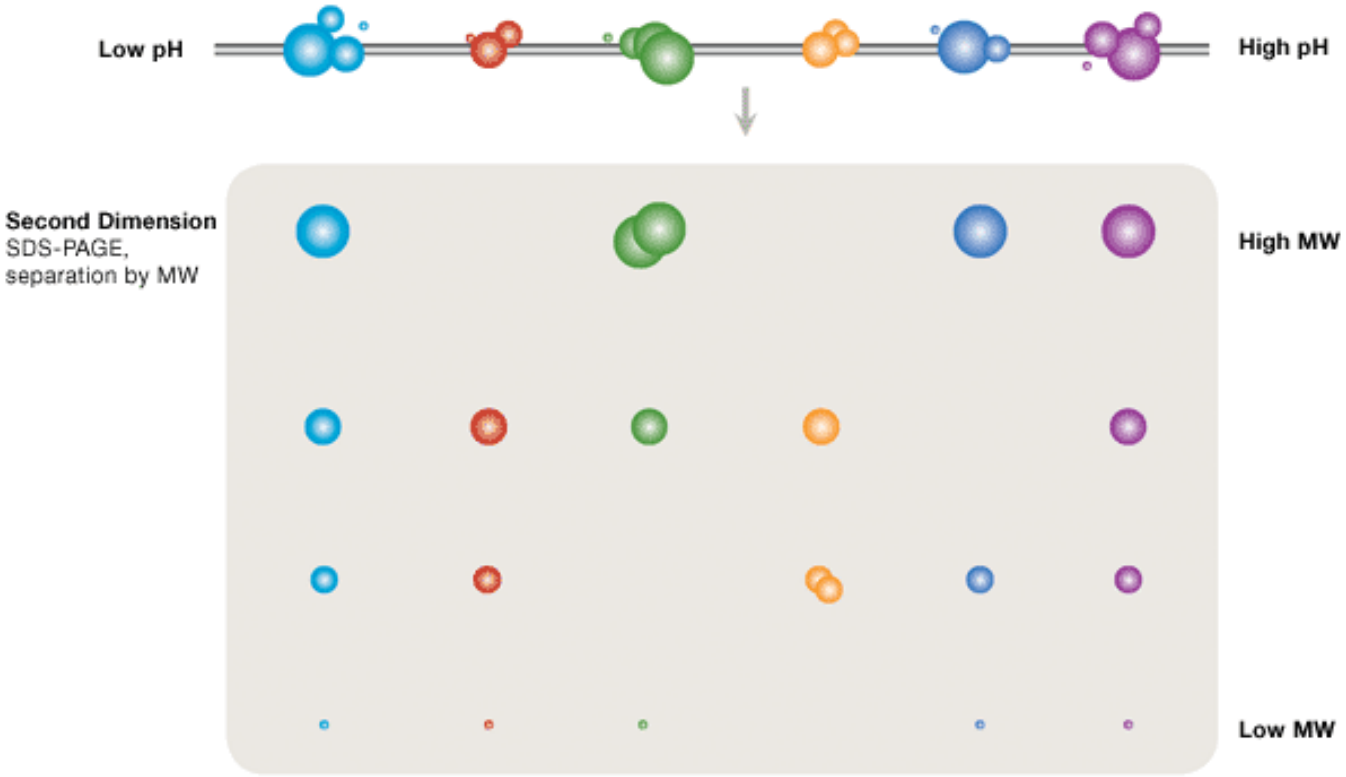
immobilized pH gradients (IPG) strips

www.bio-rad.com/en-us/applications-technologies/isoelectric-focusing-2d-electrophoresis

2D-SDS PAGE GEL

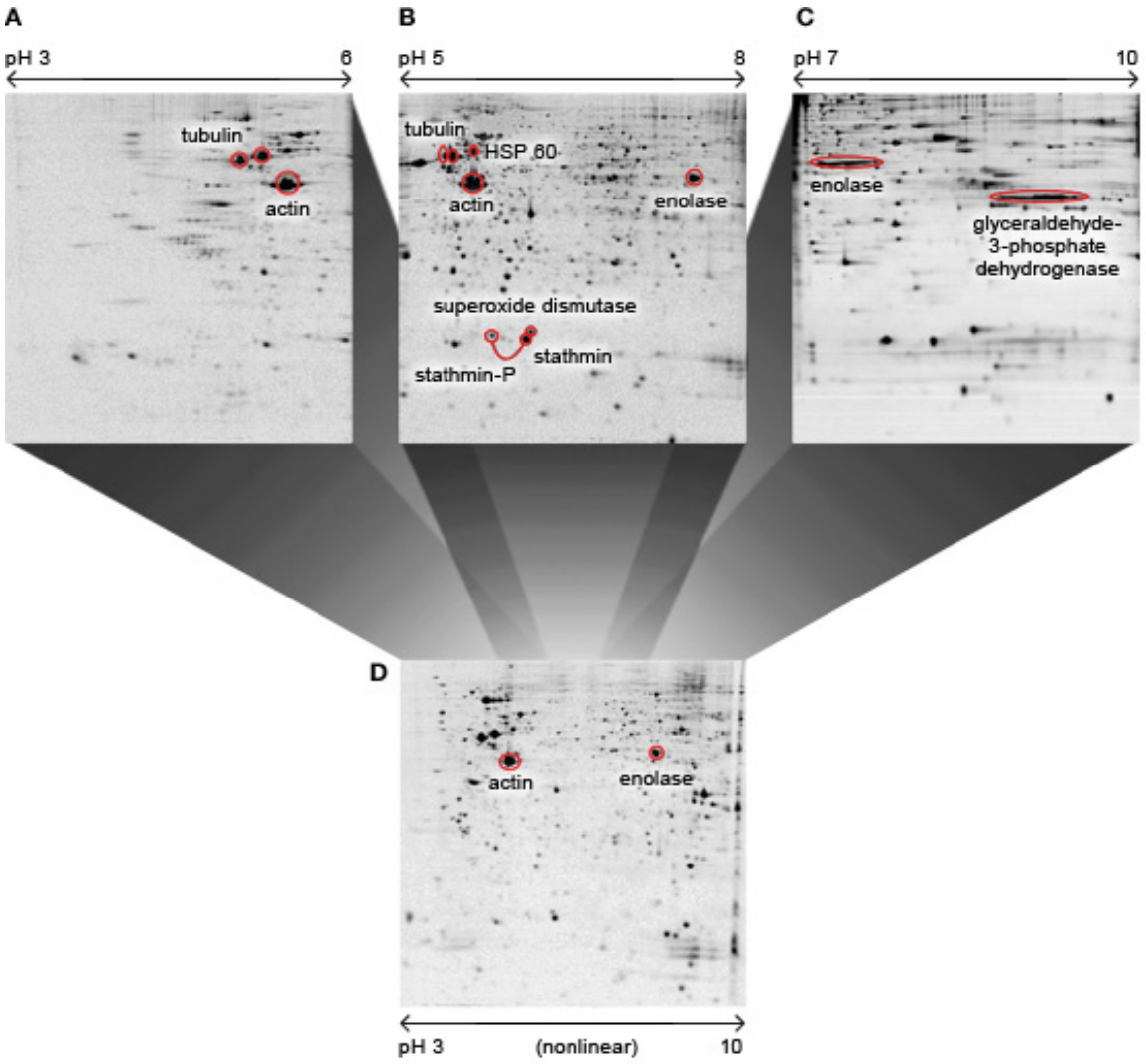
Second **D**imension **S**eparation of proteins according to their molecular weight

- polyacrylamide gel electrophoresis (PAGE)
- Proteins denatured
- SDS coats all proteins with a negative charge essentially in proportion to their mass
- mobility related logarithmically to mass



http://www.bio-rad.com/en-us/applications-technologies/second-dimension-separation

2D-SDS PAGE GEL



www.bio-rad.com/en-us/applications-technologies/isoelectric-focusing-2d-electrophoresis

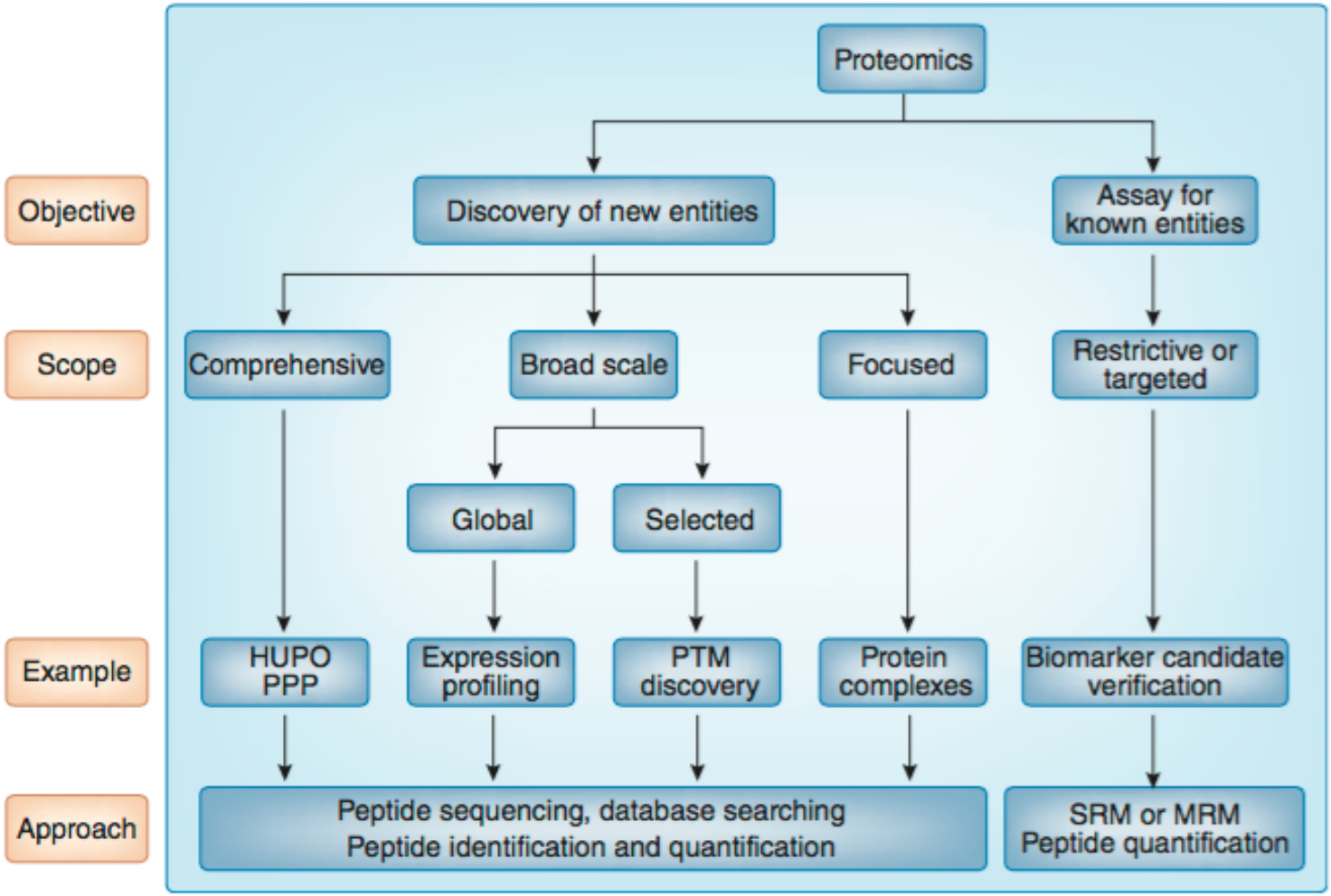
Proteomics

- Proteomics Used in
  - Functional studies
  - Drug Studies
  - Epigenetics
  - Biomarker Search
  - Signaling Pathways

Mallick & Kuster Nature Biotechnology 28, 695–709 (2010)

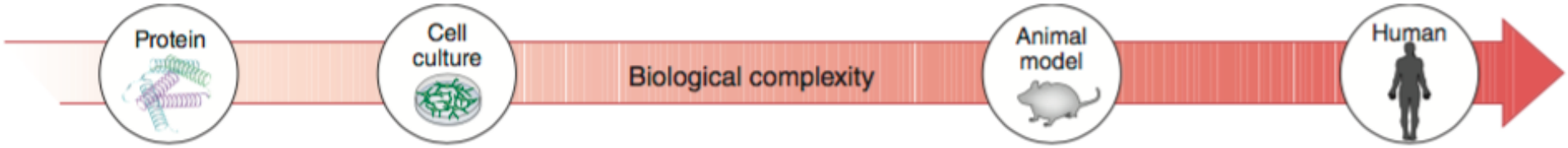
Proteomics

organization of proteomic experiments



Mallick & Kuster Nature Biotechnology 28, 695–709 (2010)

Proteomics



Technical expertise

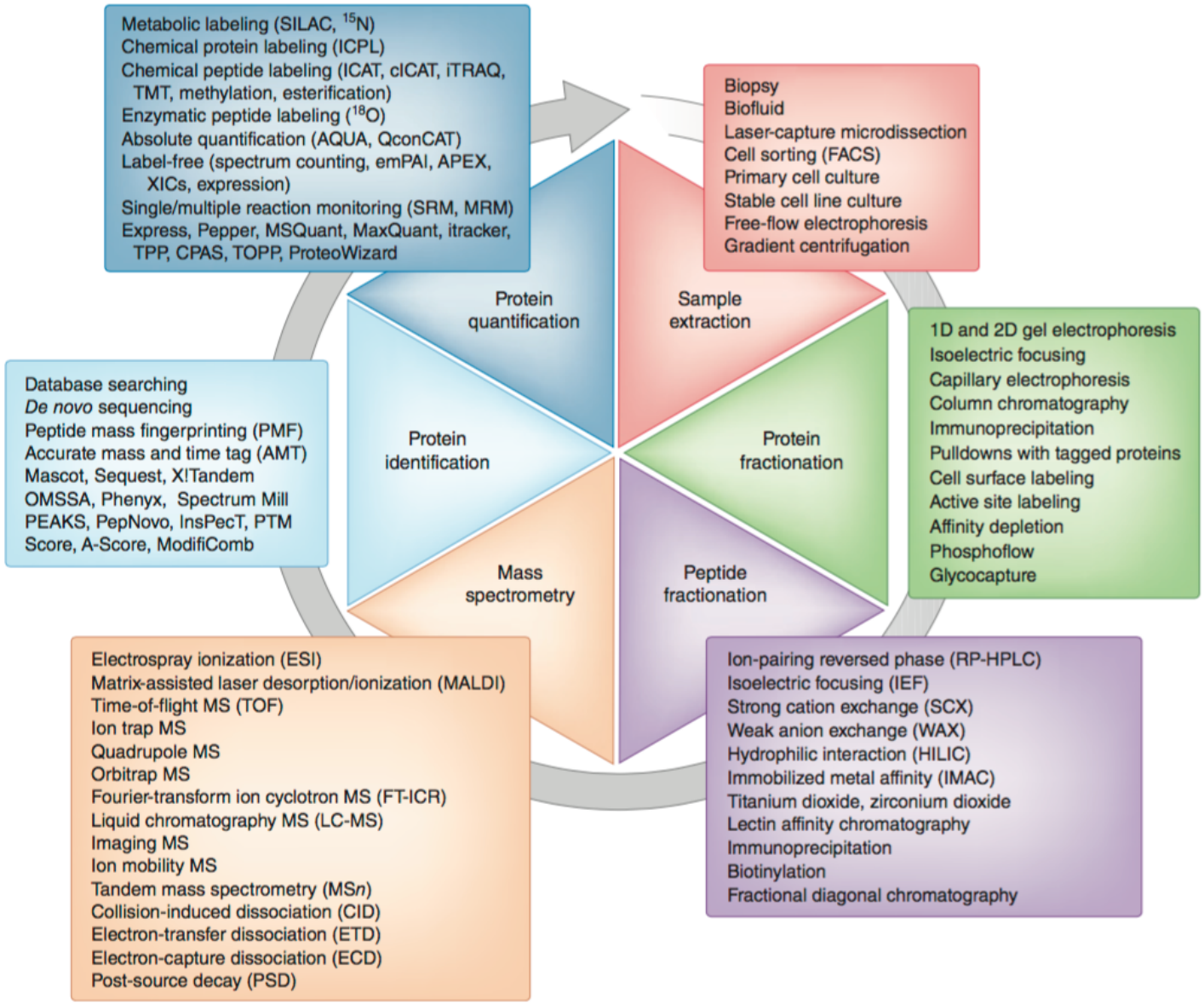
Protein analysis *In vitro* biochemistry	Protein complexes *In vitro* biology	Protein networks *In vitro* biology	Cell culture models *In vitro* target or marker discovery	Translational studies *In vivo* marker discovery	Population proteomics *In vivo* marker discovery or verification
Purity and identity	Pairwise interactions	Interaction screening	Cell composition	Biopsy composition	Genetic variation
Single PTM	Complex composition	Network composition	PTM discovery	Xenograft composition	Marker verification
Quantitative PTM	PTM analysis	Pathway crosstalk	Organelle composition	Perturbation analysis	PTM characterization
Multiple PTM	Complex dynamics	Network dynamics	Expression profiling	Cross model analysis	Patient stratification
PTM stoichiometry	Complex stoichiometry	Comprehensive PTM	Protein activity	Biofluid composition	Marker discovery
Splicing, SNPs	Spatial organization			MALDI imaging	

Applications of proteomic technologies

Mallick & Kuster Nature Biotechnology 28, 695–709 (2010)

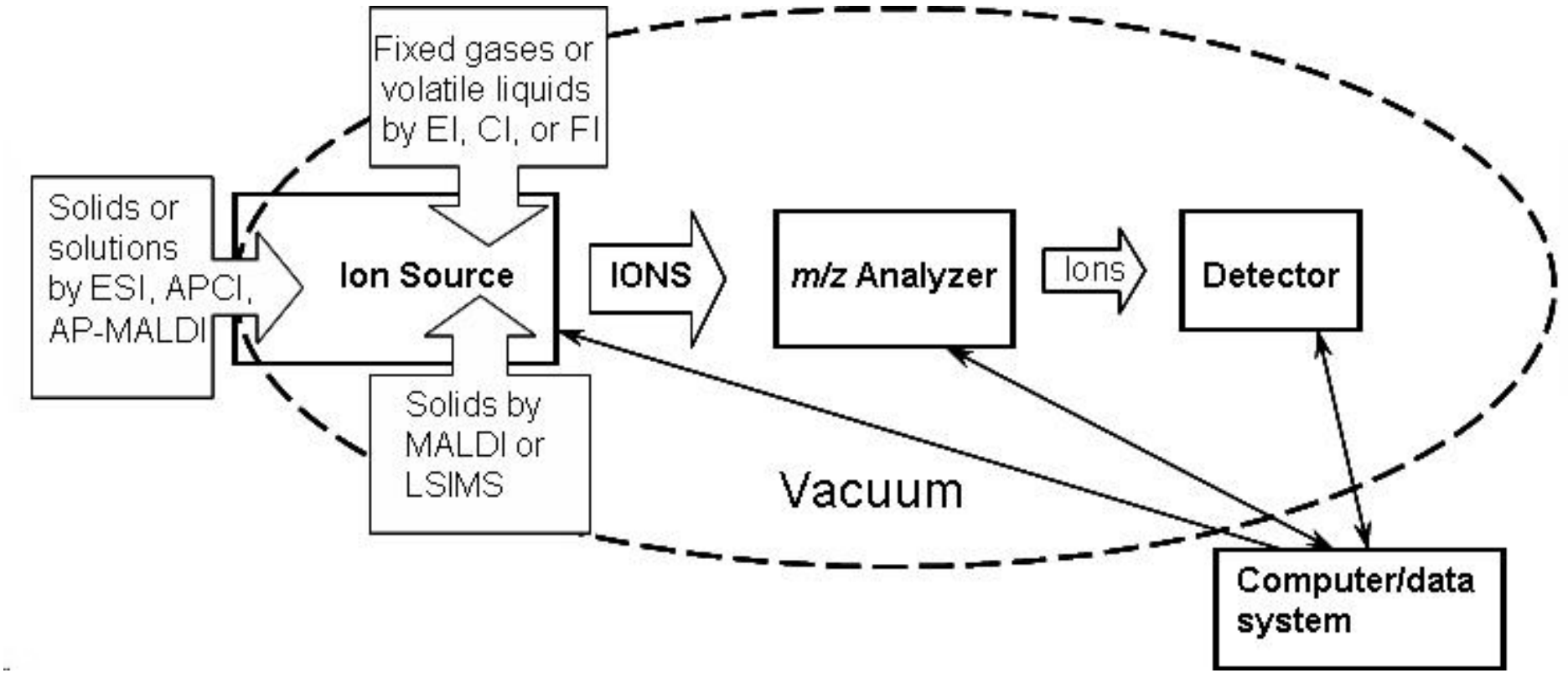
Proteomics

Technologies for proteomics



Mallick & Kuster Nature Biotechnology 28, 695–709 (2010)

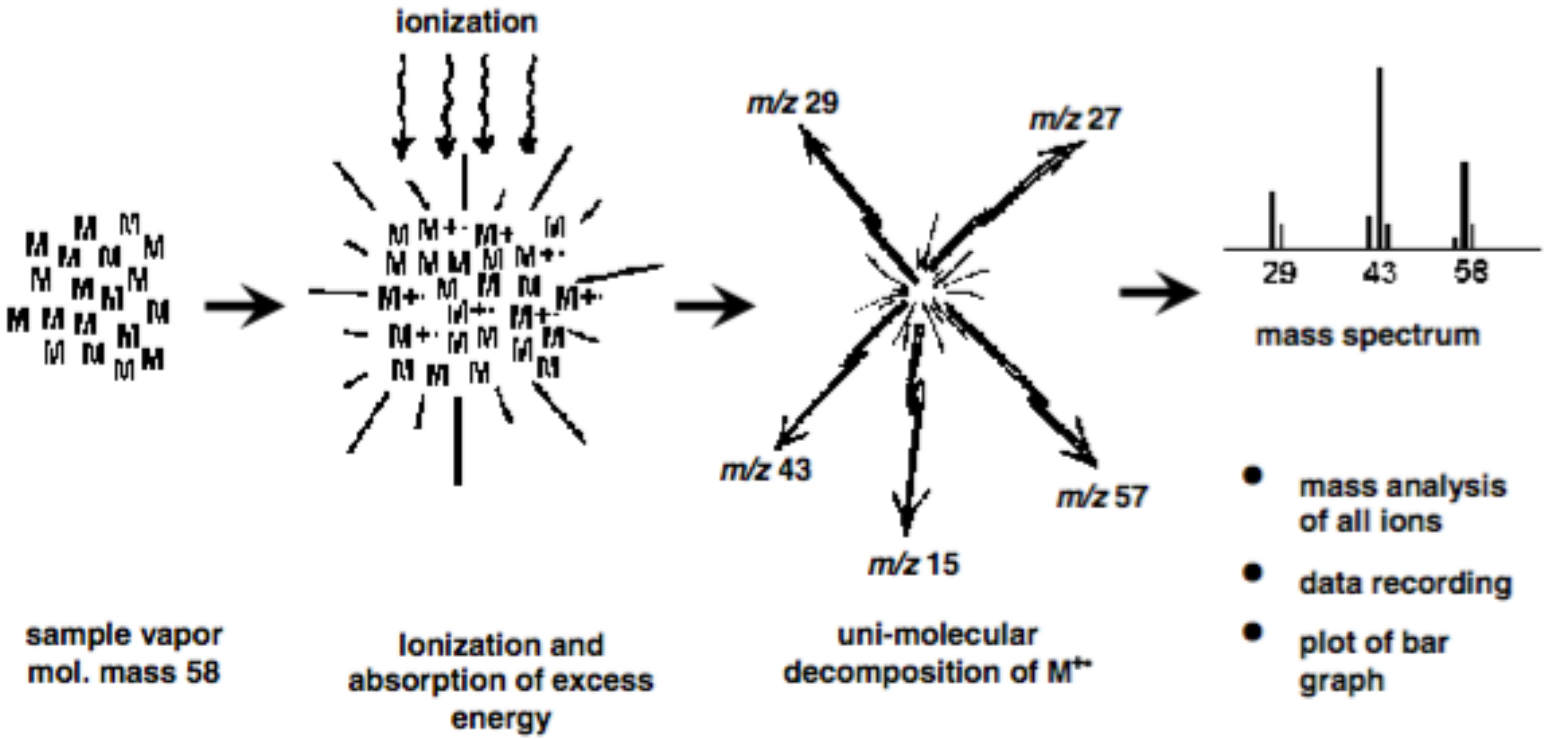
Conceptual Proteomics



Applications of proteomic technologies

Introduction to Mass Spectrometry: Instrumentation, Applications, and Strategies for Data Interpretation; Fourth Edition. J.T. Watson & O.D. Sparkman, John Wiley & Sons, Ltd (2007)

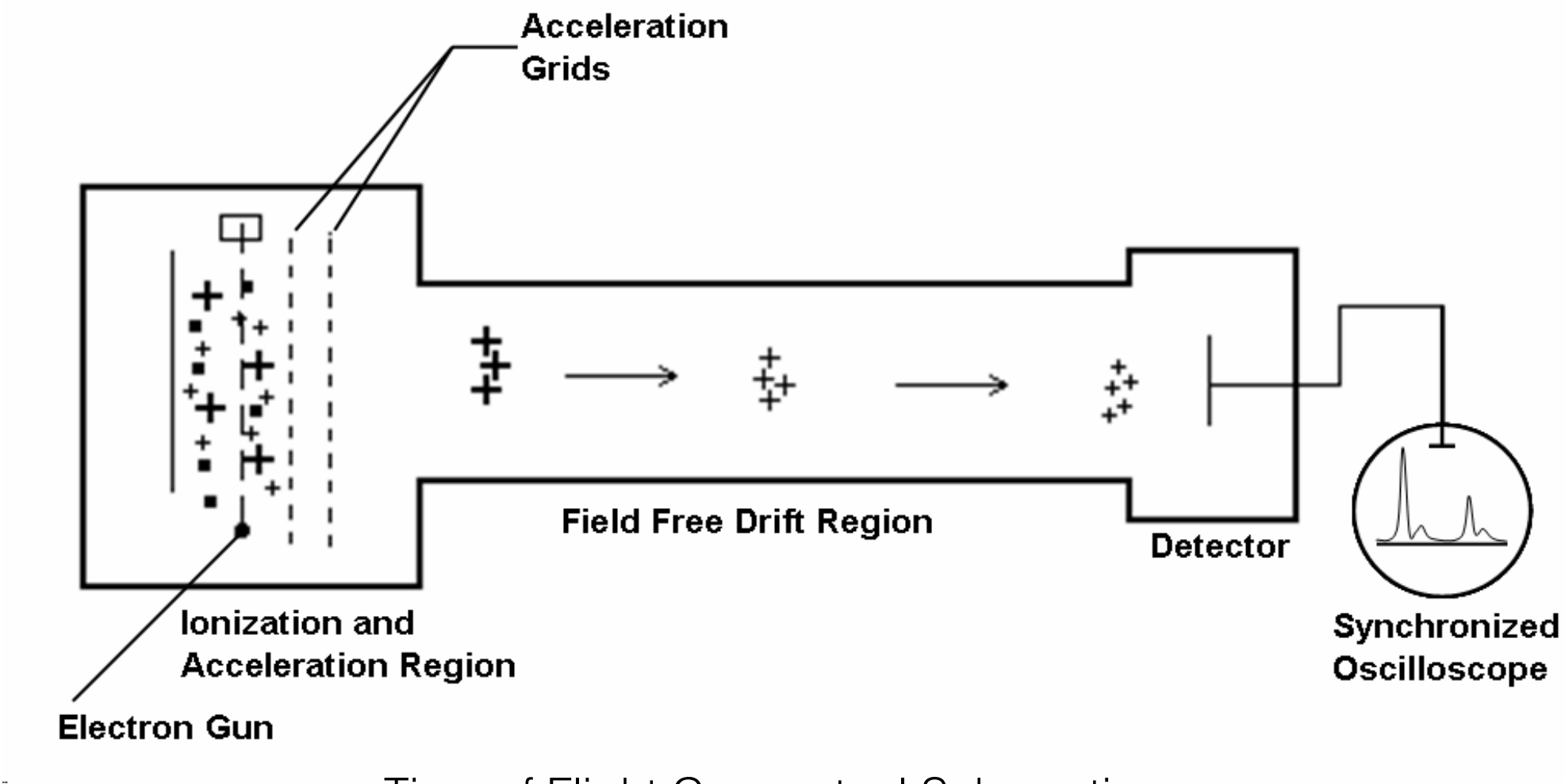
Conceptual Proteomics



Applications of proteomic technologies

Introduction to Mass Spectrometry: Instrumentation, Applications, and Strategies for Data Interpretation; Fourth Edition. J.T. Watson & O.D. Sparkman, John Wiley & Sons, Ltd (2007)

Time of Flight (TOF)



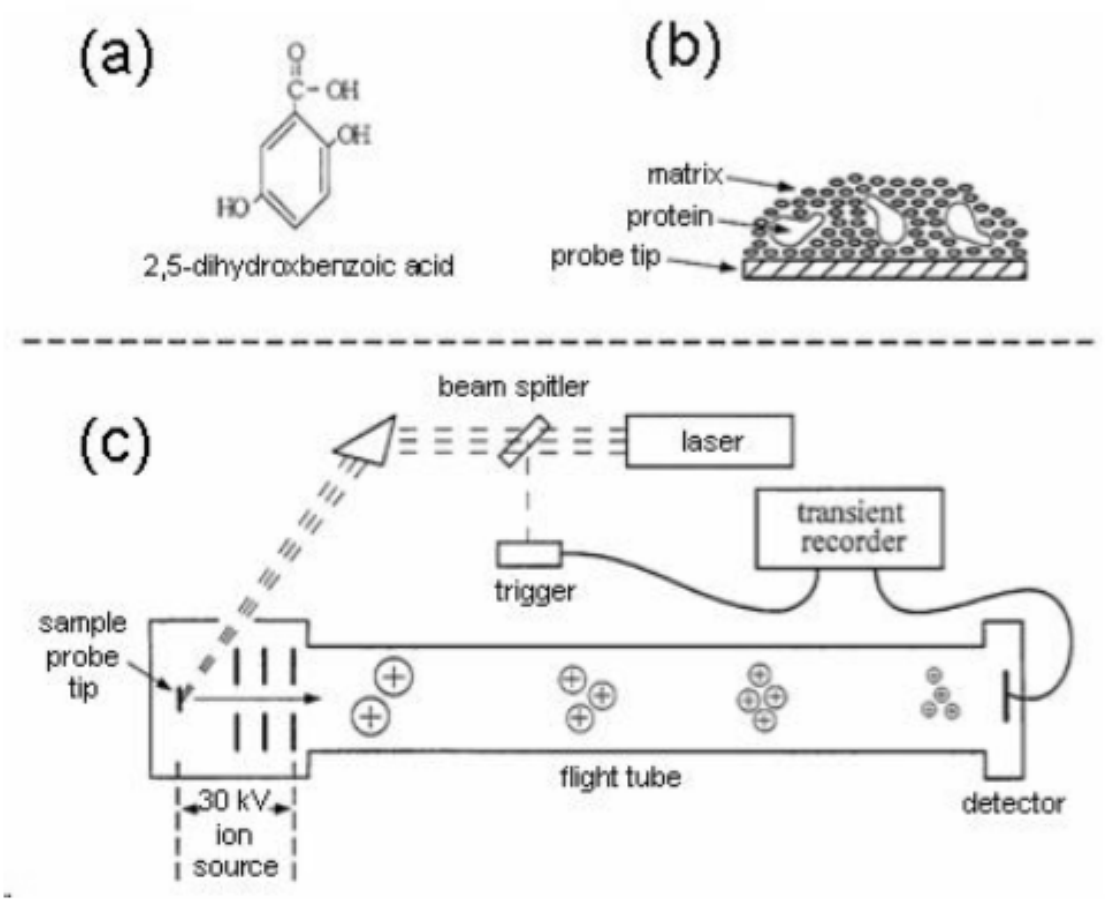
Time of Flight Conceptual Schematic

Introduction to Mass Spectrometry: Instrumentation, Applications, and Strategies for Data Interpretation; Fourth Edition. J.T. Watson & O.D. Sparkman, John Wiley & Sons, Ltd (2007)

MALDI TOF

Matrix **A**ssisted **L**aser **D**esorption **I**onization

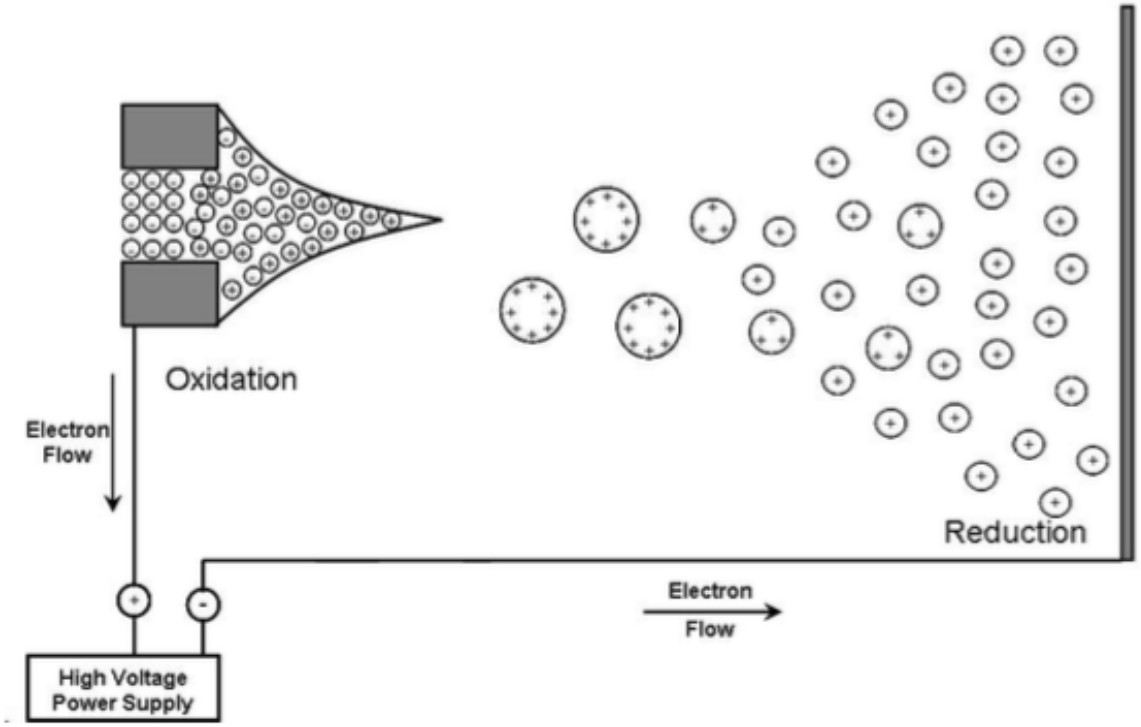
- Sample spotted in matrix 2,5-dihydroxybenzoic acid (DHB)
- Vaporization by laser
- Fast
- Most analytes +1 charge
- Can breaks analytes



Introduction to Mass Spectrometry: Instrumentation, Applications, and Strategies for Data Interpretation; Fourth Edition. J.T. Watson & O.D. Sparkman, John Wiley & Sons, Ltd (2007).

Electrospray Ionization

- Sample liquid through charged needle
- Sprayed into evaporation chamber
- Gas phase +ve charge analytes
- Can have multiple charges (deconvolution issues)



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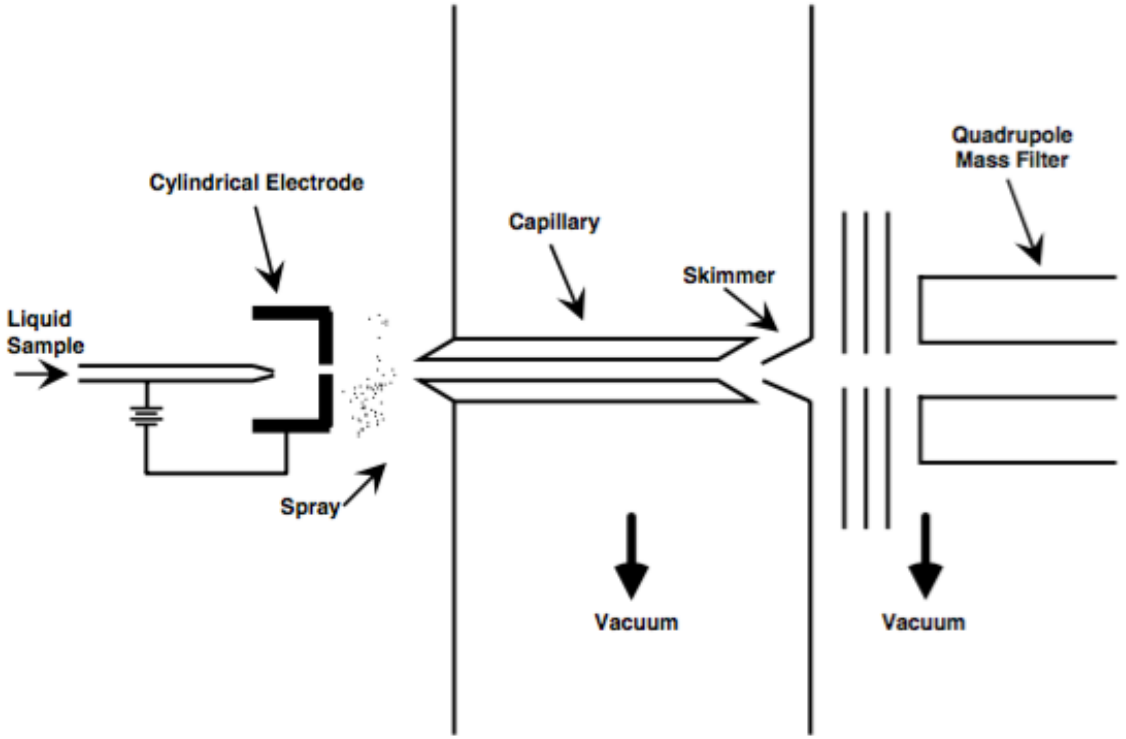
Electrospray Ionization



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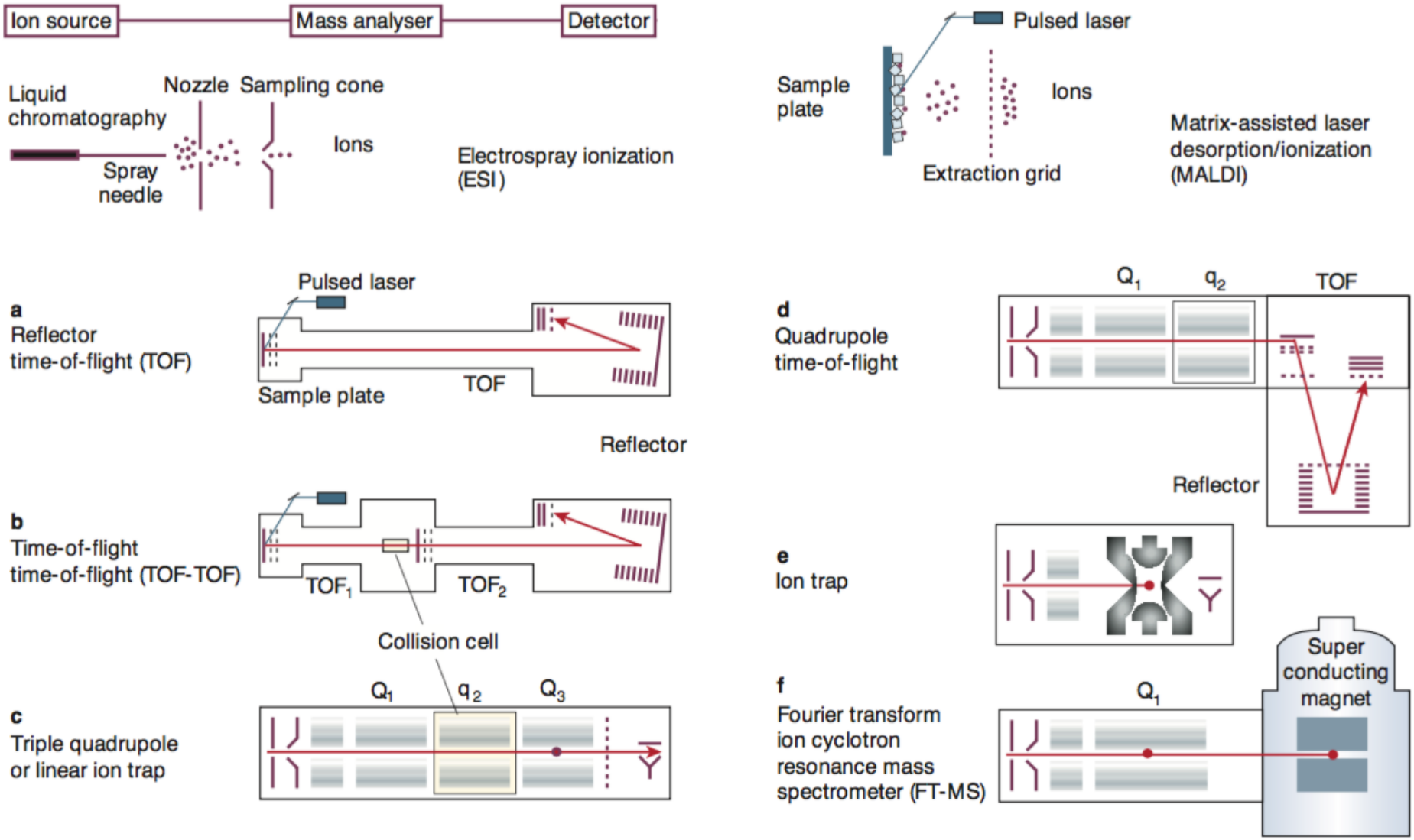
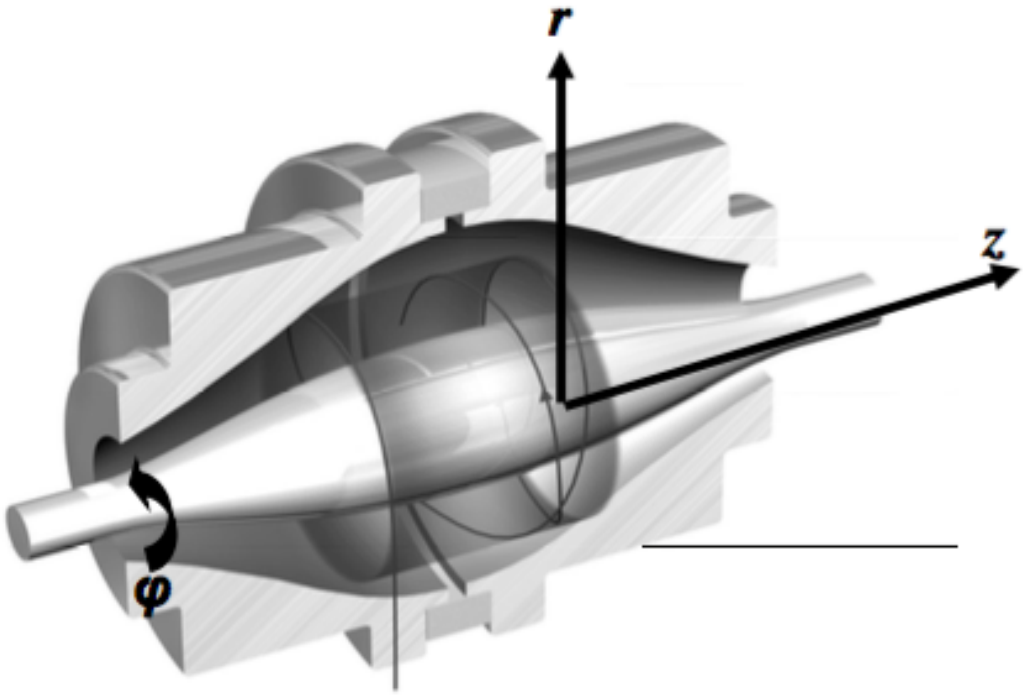
Mass Spectrometers



Aebersold & Mann, Nature 422 p. 198 (2003)

Orbitrap



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thermo.com

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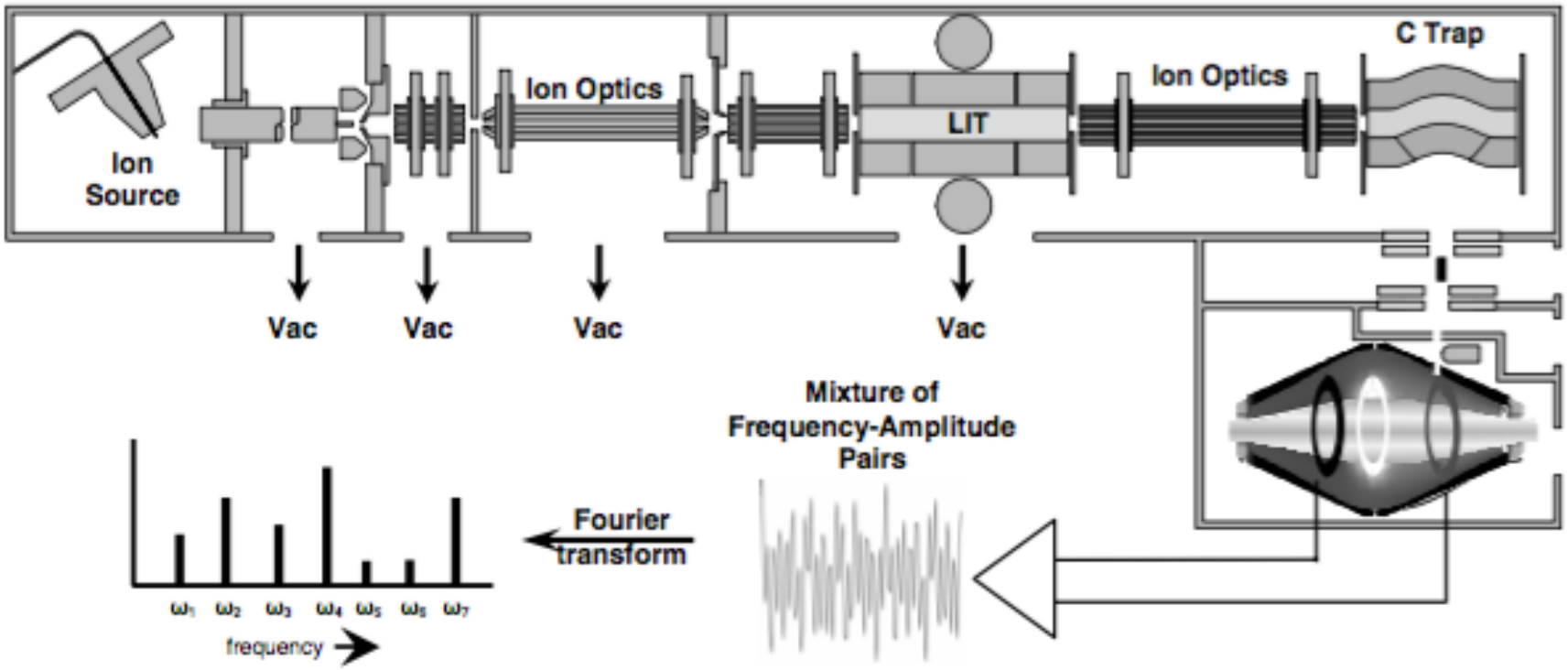

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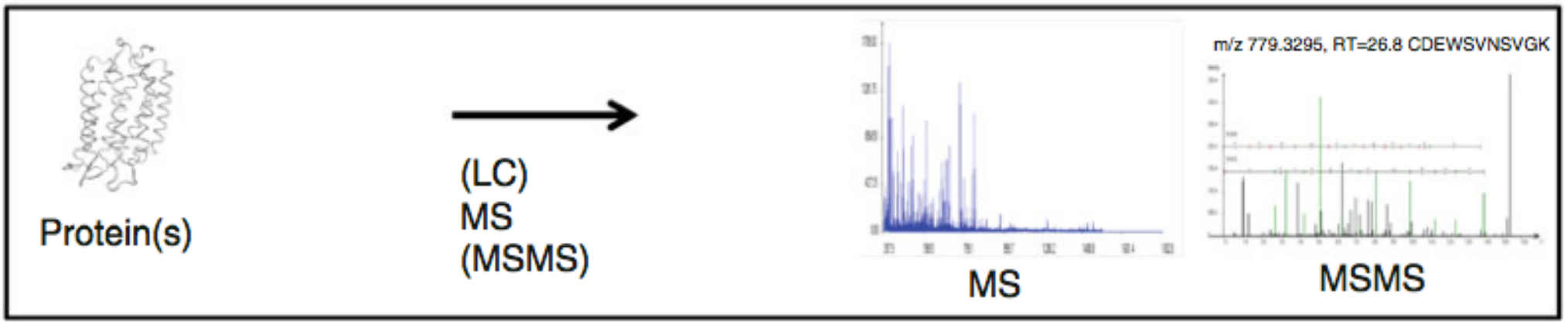
Proteomics Approaches

Top-down

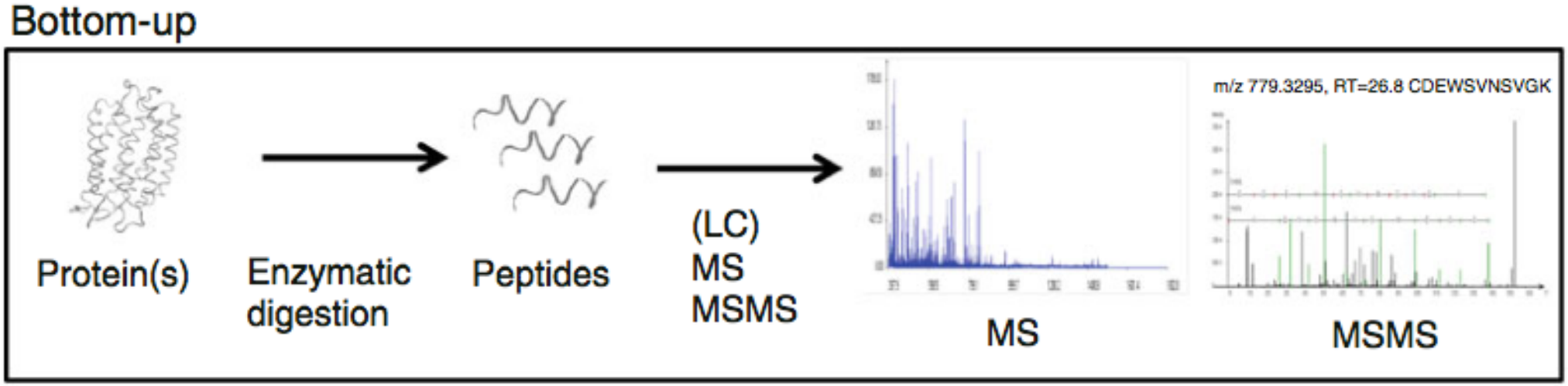


Top-down Proteomics

Entire Proteins

Rune Matthiesen (ed.), Mass Spectrometry Data Analysis in Proteomics, Springer (2013)

Proteomics

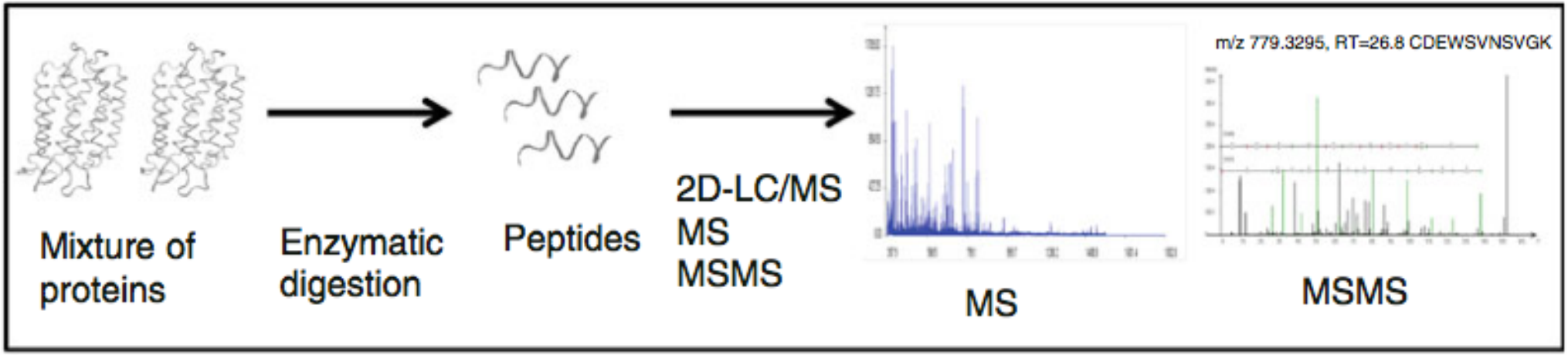


Digest to Peptides (typically trypsin)

Rune Matthiesen (ed.), Mass Spectrometry Data Analysis in Proteomics, Springer (2013)

Proteomics

Shotgun



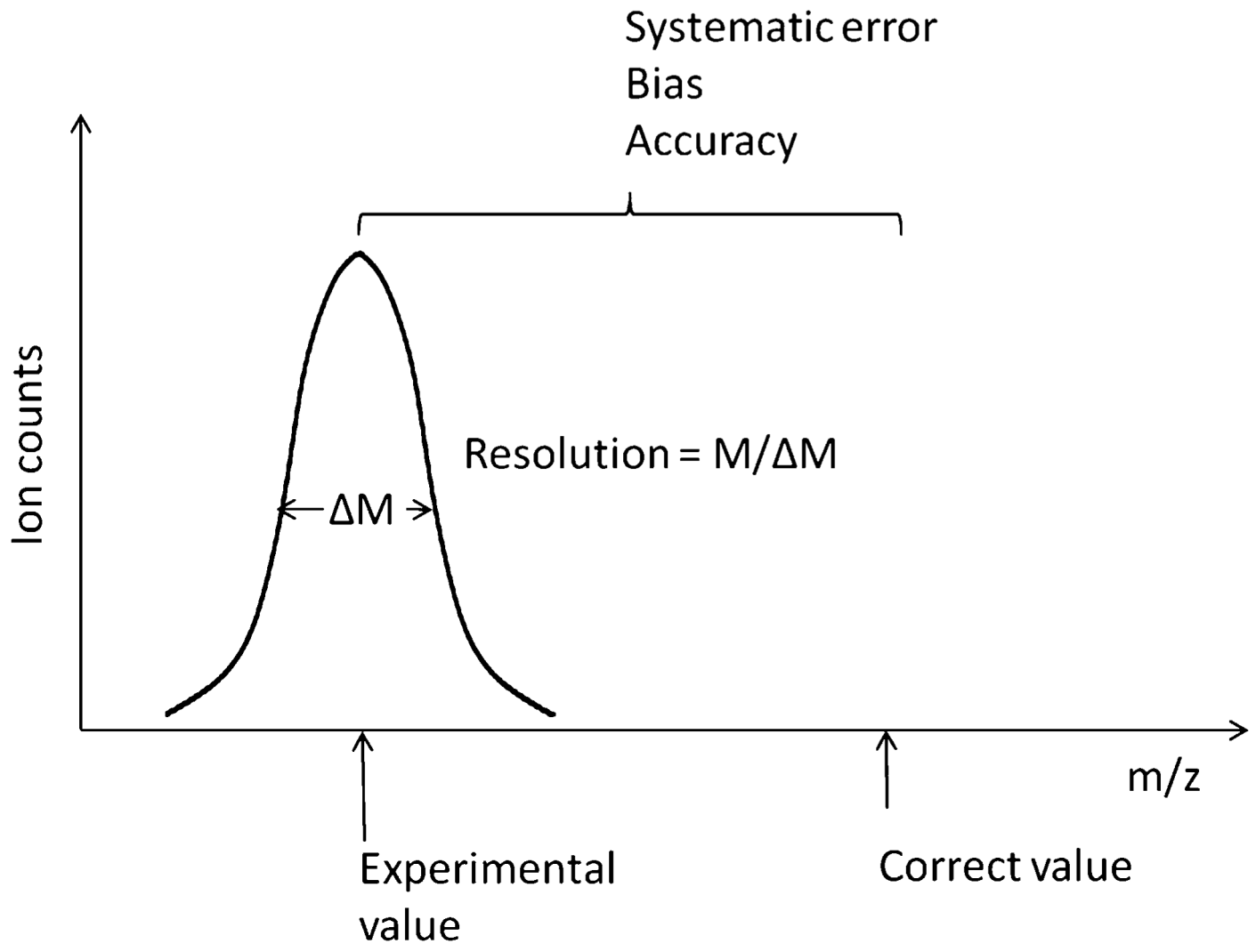
Many Proteins

Digest to Peptides

Reconstruct

Rune Matthiesen (ed.), Mass Spectrometry Data Analysis in Proteomics, Springer (2013)

Accuracy and resolution

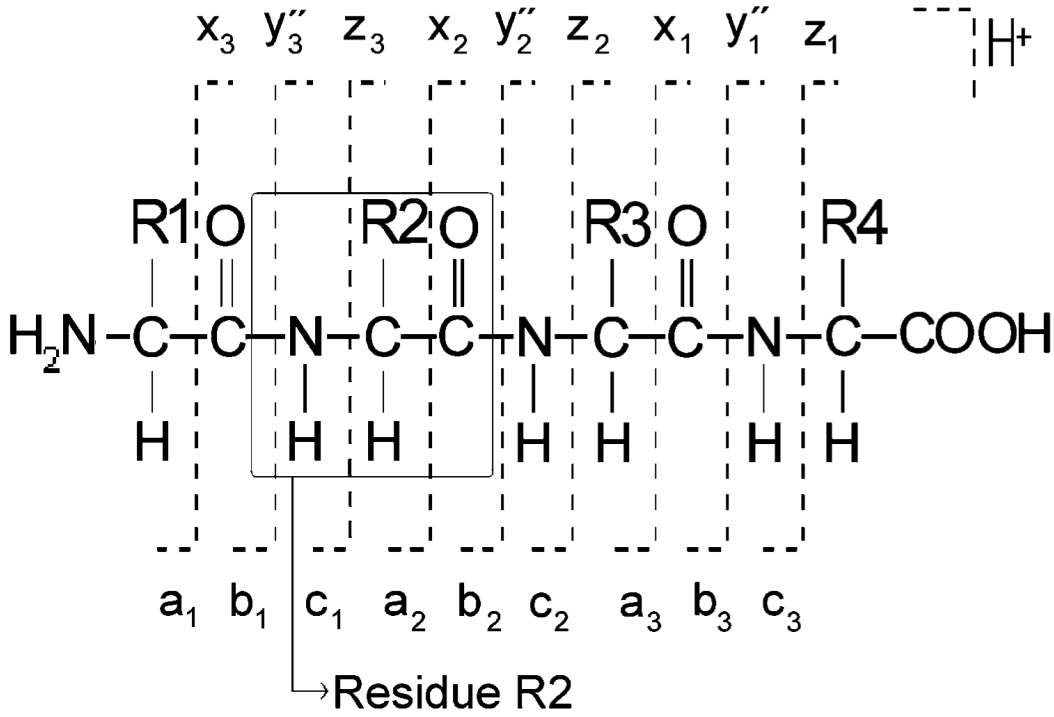


Rune Matthiesen (ed.), Mass Spectrometry Data Analysis in Proteomics, Springer (2013)

Proteomics

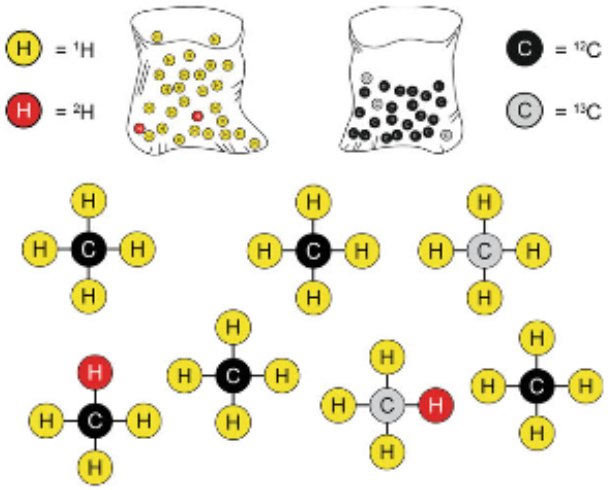
- a, b, and c ions are charged fragments containing the N-terminal part of the fragment.
- x, y, and z the C-terminal part of the fragment.
- Box indicates the composition of residue R2.
- Residue mass for R2 is given by summing the masses of the elements of residue R2

Nomenclature for peptide fragment ions



Rune Matthiesen (ed.), Mass Spectrometry Data Analysis in Proteomics, Springer (2013)

Proteomics



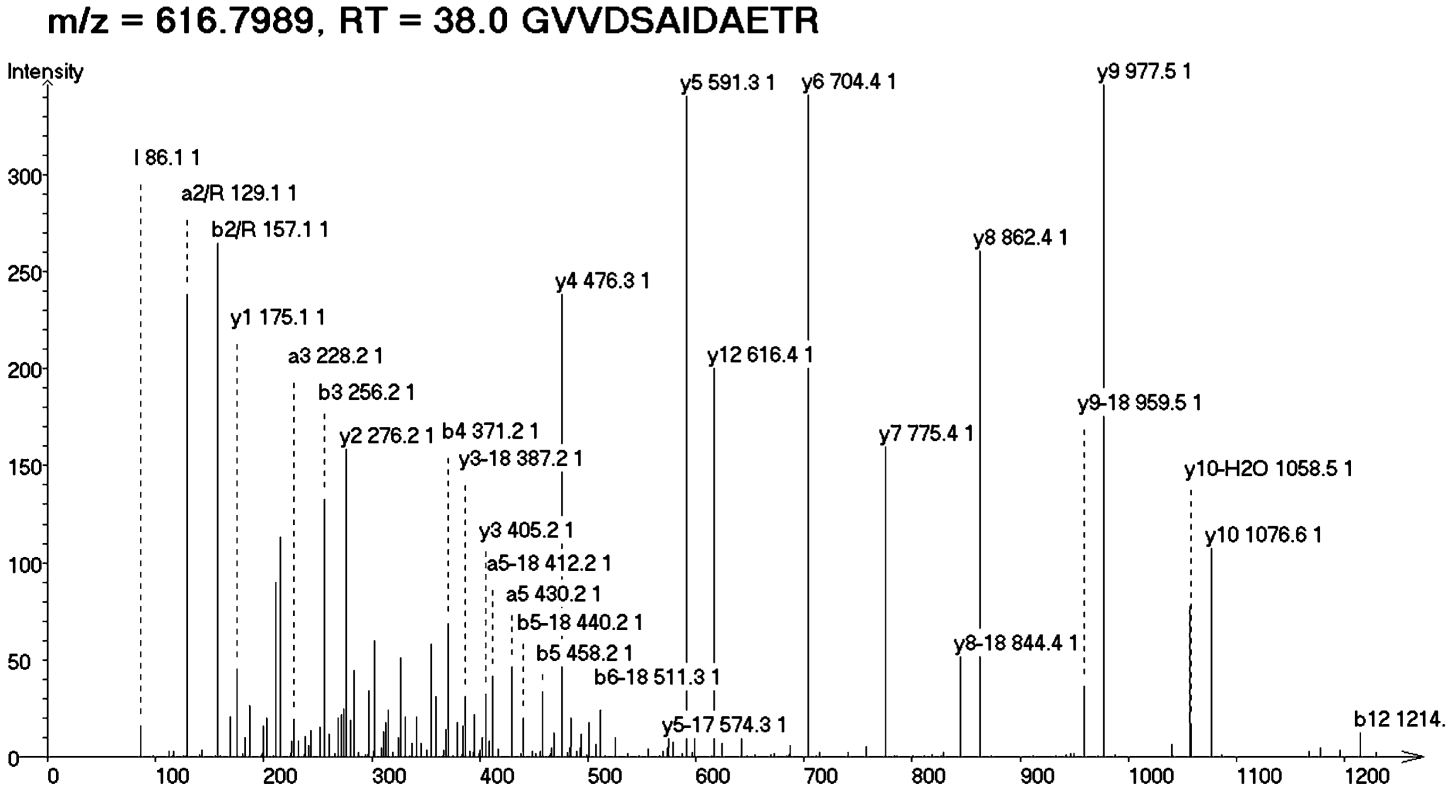
Isotopic distributions are a combinatoric problem

Rockwood & Palmblad in Rune Matthiesen (ed.), Mass Spectrometry Data Analysis in Proteomics, Springer (2013)

Proteomics

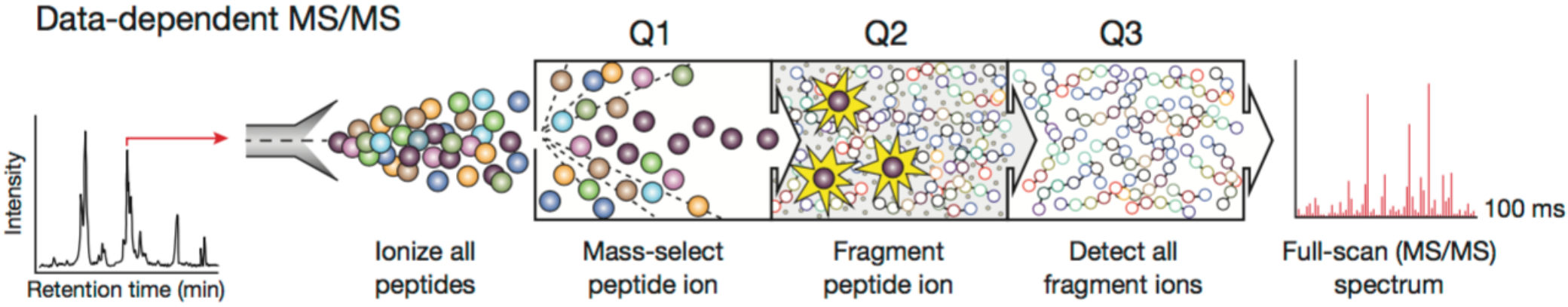
tryptic peptide
GVVDSAIDAETR

Example MS/MS

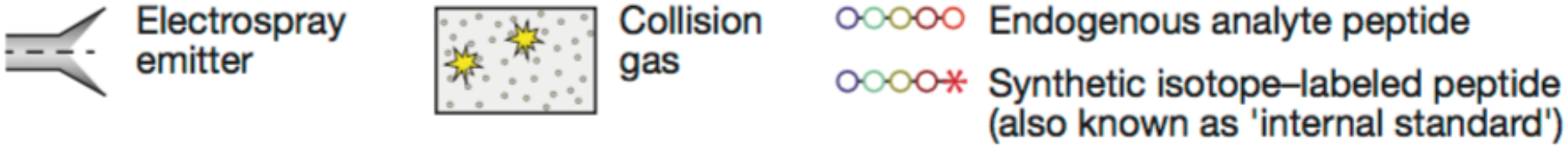


Rune Matthiesen (ed.), Mass Spectrometry Data Analysis in Proteomics, Springer (2013)

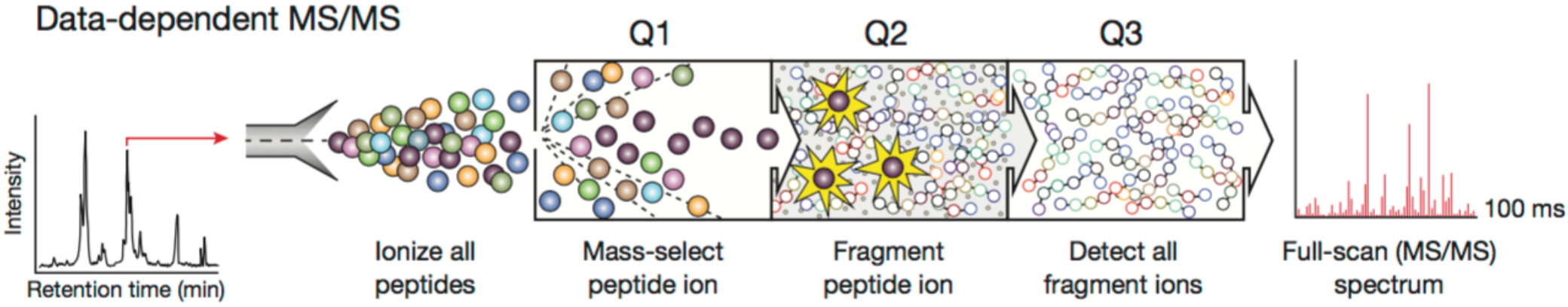
Multiple Reaction Monitoring



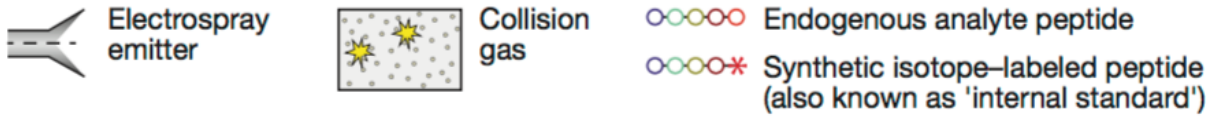
Ions automatically selected for MS/MS based on intensity in preceding MS spectrum.



Multiple Reaction Monitoring

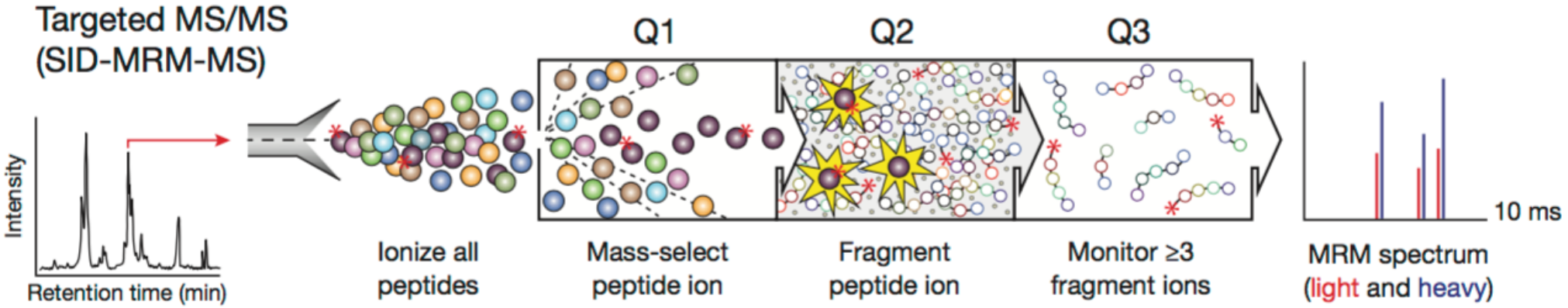


- In chromatographic separation 10-100s peptides eluting.
- 4-10 ions in MS spectrum are automatically selected for MS/MS.

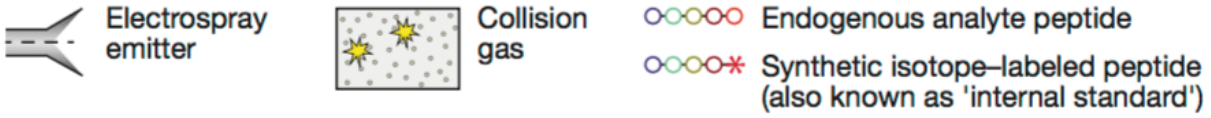


Gillette & Carr Nature Methods 10, 28–34 (2013)

Multiple Reaction Monitoring



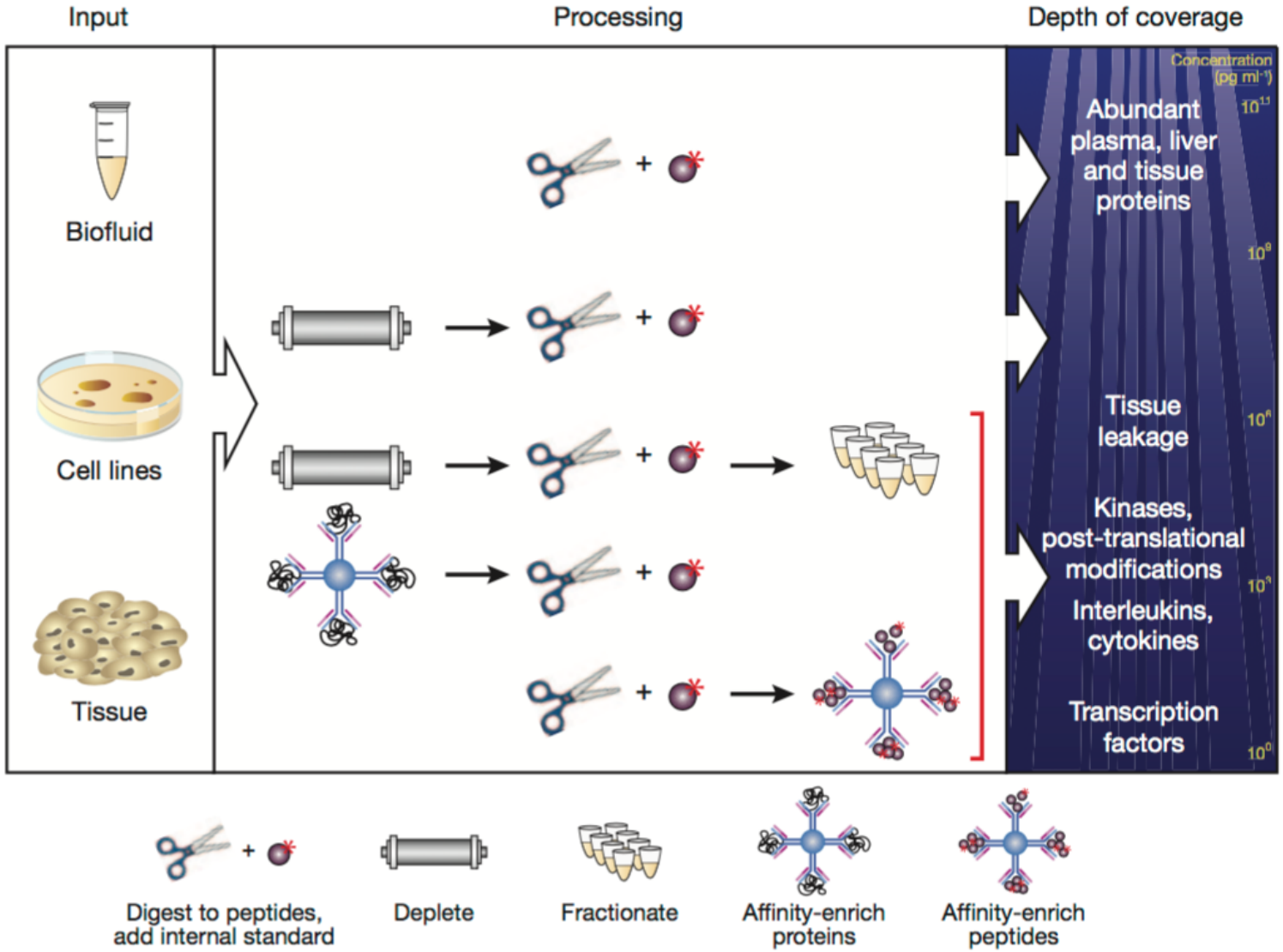
- Proteotypic peptides uniquely representing proteins of interest predefined together with their most informative fragment ions.
- User-specified list of targeted precursor-fragment pairs ('transitions') for fragmentation.
- Synthetic peptides with stable-isotope labels possible spike-in standards
- Comparing labeled to unlabeled peak area for precise relative quantification of endogenous analyte.



SID, stable isotope dilution
MRM - Multiple Reaction Monitoring

Gillette & Carr Nature Methods 10, 28–34 (2013)

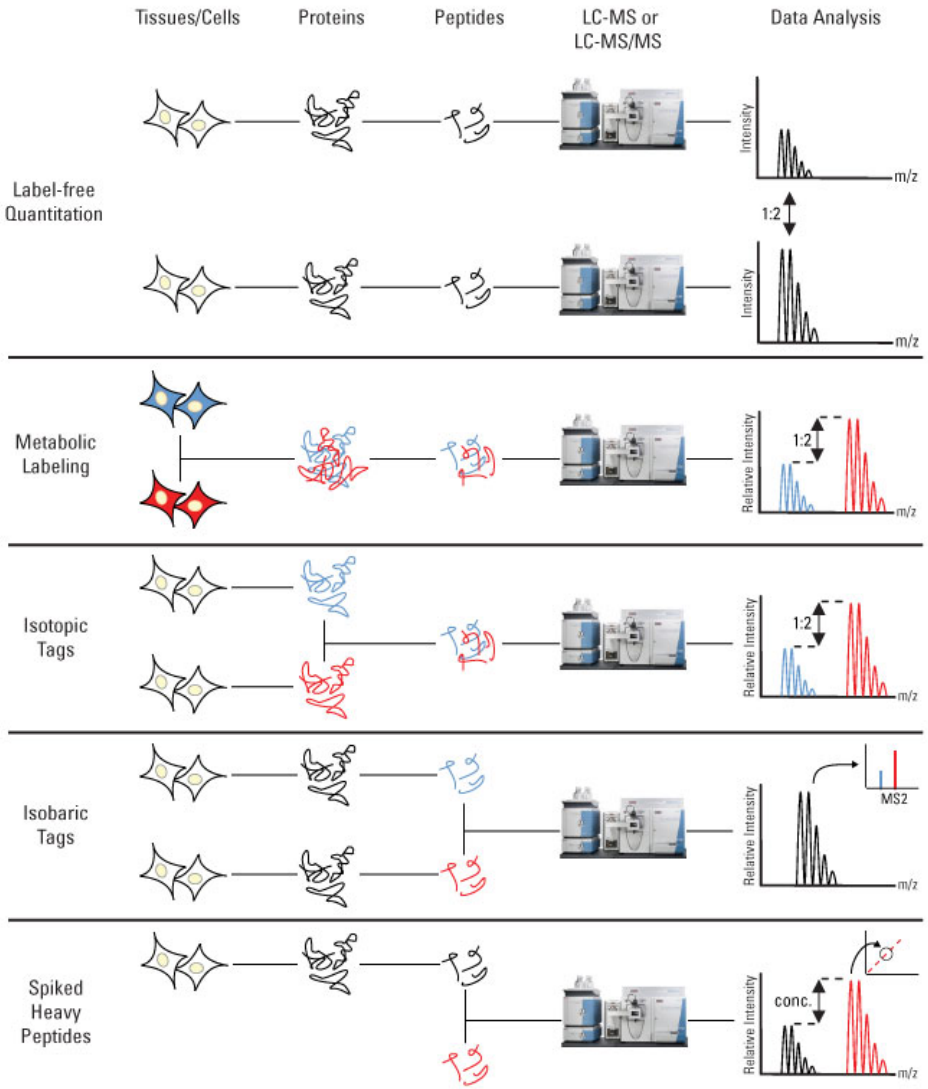
Multiple Reaction Monitoring



Gillette & Carr Nature Methods 10, 28–34 (2013)

SID, stable isotope dilution
MRM - Multiple Reaction Monitoring

Quantitative Proteomics



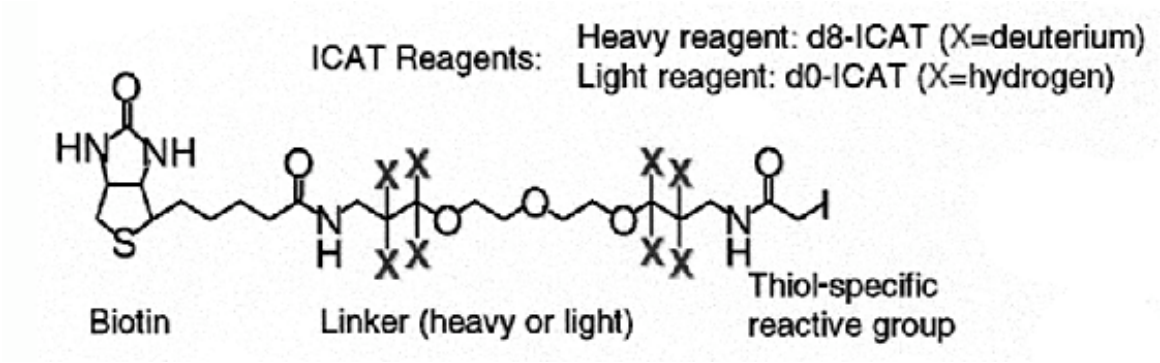
thermofisher.com

Quantitative Methods

- **I**sotope **C**oded **A**ffinity **T**agging (**ICAT**)
  - Light Vs. Heavy
  - Hydrogen Vs Deuterium (Δm = 8 Daltons)
- **I**sobaric **T**agging for **R**elative and **A**bsolute **Q**uantitation (**iTRAQ**)
  - 4 Isobaric Tags
  - Reporter Ions
- **T**andem **M**ass **T**ags (**TMT**)
  - 6-10 Isobaric Tags
  - Reporter Ions
- **S**table **I**sotope **L**abeling of **A**mino acids in **C**ell culture (**SILAC**)
  - Cells grown in isotope-enriched media
  - ^{13}C Glucose, ^{15}N Ammonium, ^{2}H Water
- Label Free Quantitation

Isotopic Tags

- **I**sotope-**C**oded **A**ffinity **T**ag (ICAT)

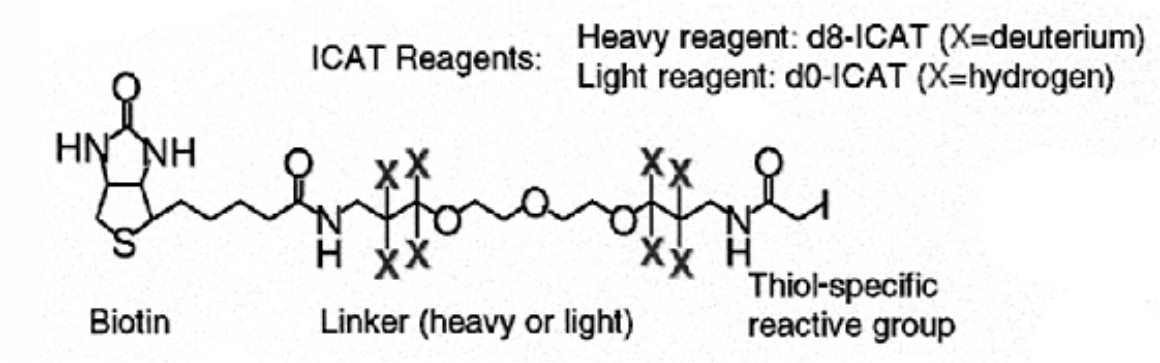


8 Dalton difference (hydrogen vs deuterium)

Gygi et al., Nature Biotechnology 17, 994 - 999 (1999)

Isotopic Tags

- **I**sotope-**C**oded **A**ffinity **T**ag (ICAT)



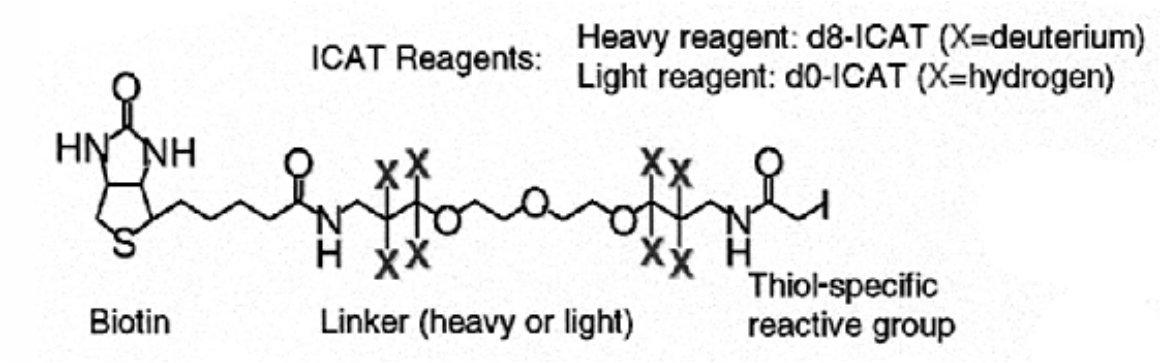
8 Dalton difference
(hydrogen vs deuterium)

binds to
cysteine
(alkylation)

Gygi et al., Nature Biotechnology 17, 994 - 999 (1999)

Isotopic Tags

- **I**sotope-**C**oded **A**ffinity **T**ag (ICAT)



8 Dalton difference (hydrogen vs deuterium)

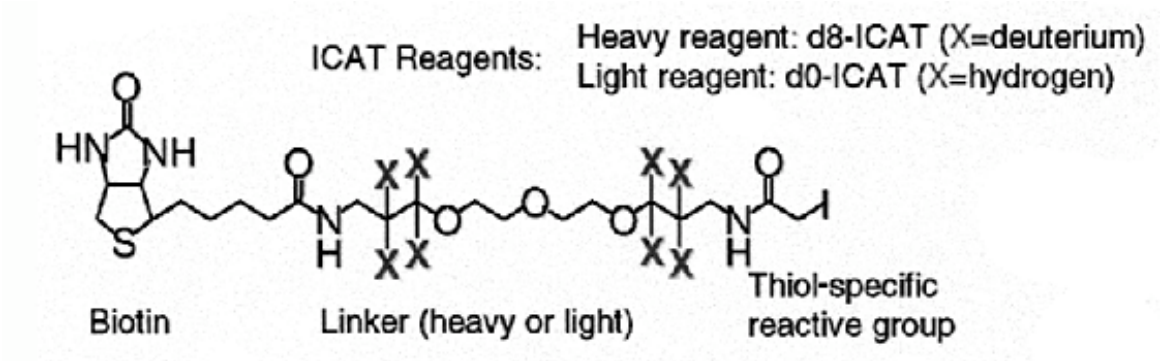
affinity capture

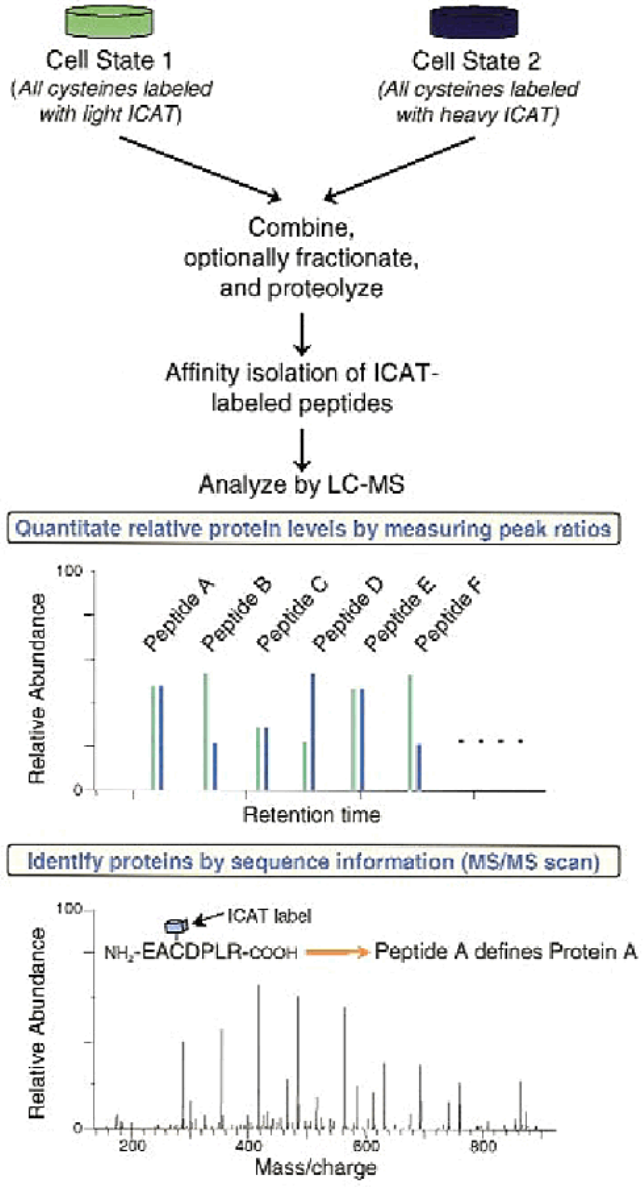
binds to cysteine (alkylation)

Gygi et al., Nature Biotechnology 17, 994 - 999 (1999)

Isotopic Tags

- **I**sotope-**C**oded **A**ffinity **T**ag (ICAT)





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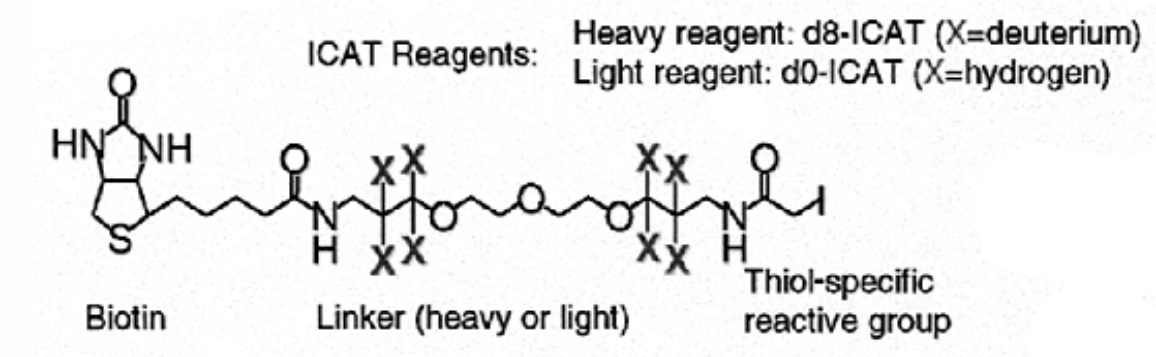
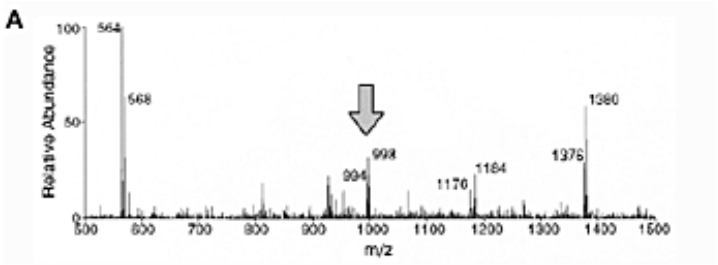
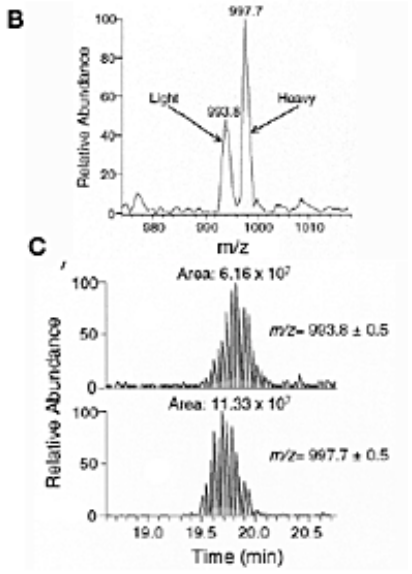
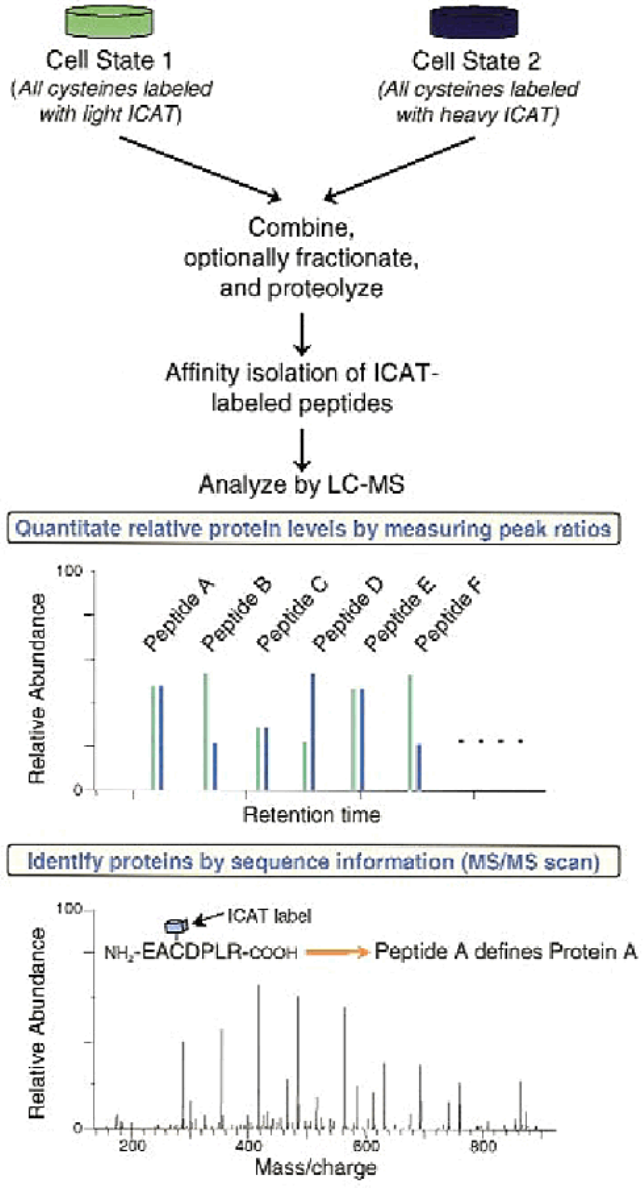
- **I**sotope-**C**oded **A**ffinity **T**ag (ICAT)





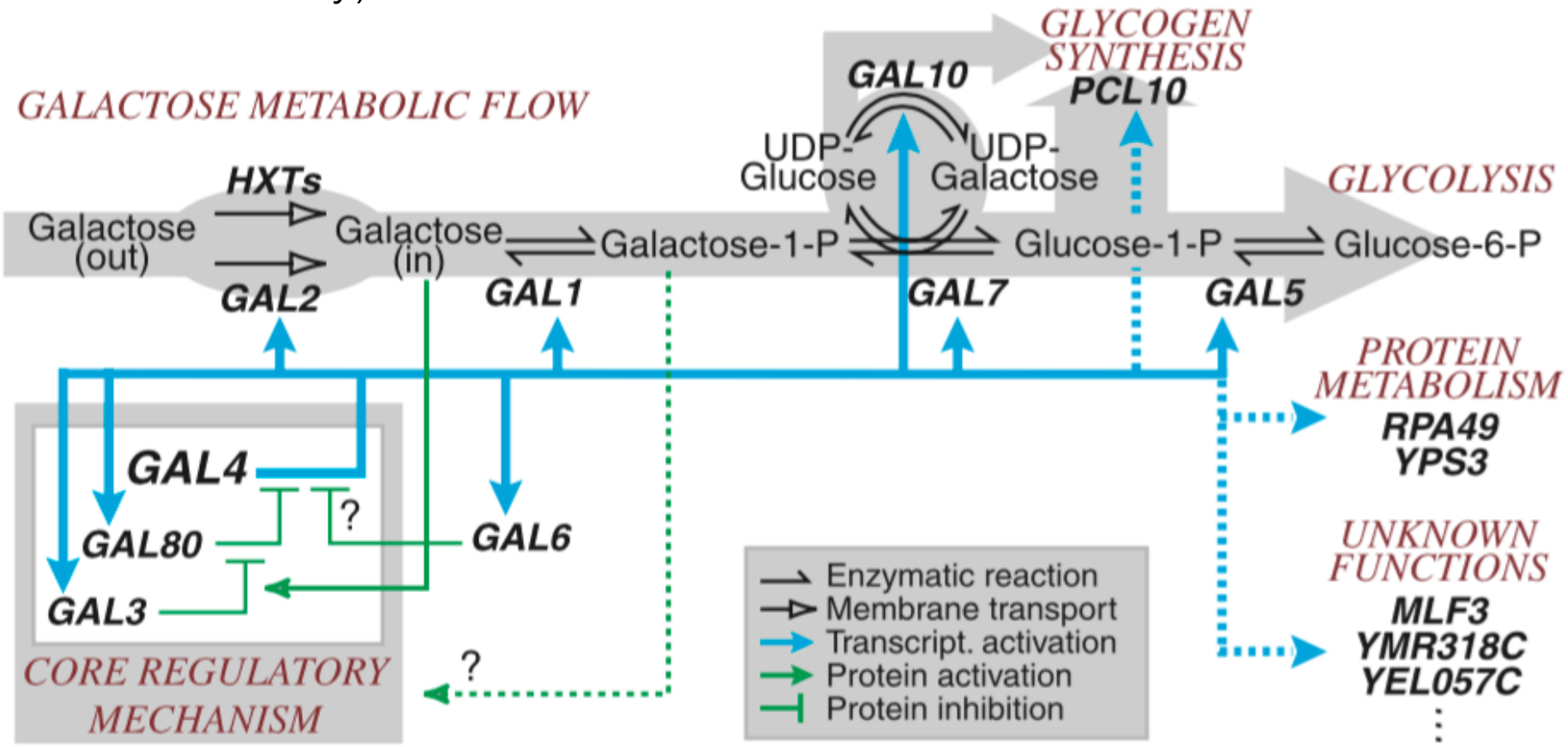




Gygi et al., Nature Biotechnology 17, 994 - 999 (1999)

Protein Vs mRNA

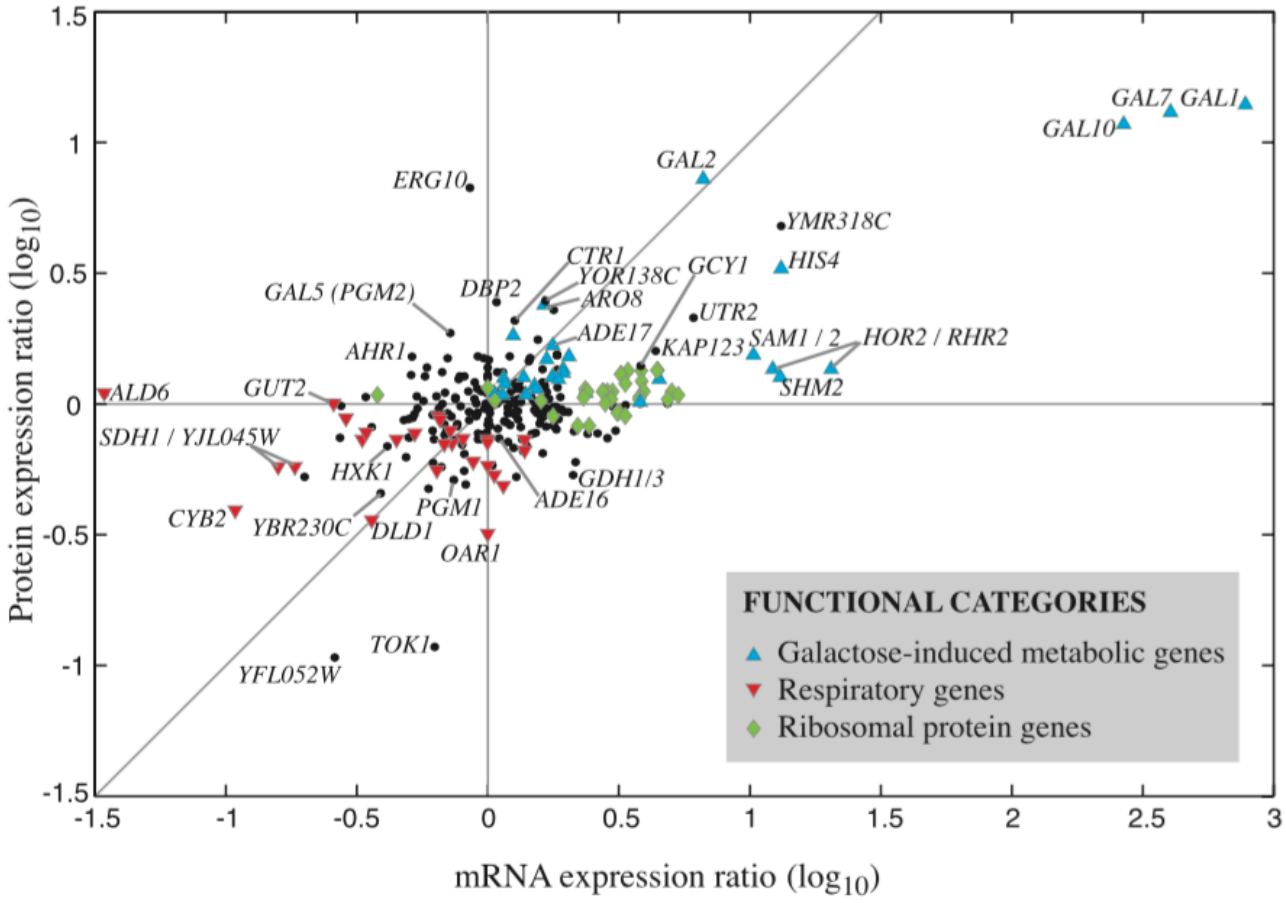
Model of galactose utilization
(perturbation study)



Ideker et al., Science 292(5518):929-34, (2001).

Protein Vs mRNA

- Strains in presence or absence of 2% galactose (+gal vs. -gal)
- Ratios of wt+gal to wt-gal protein expression
- 289 genes using (ICAT) & tandem mass spectrometry (MS/MS)
- Corresponding mRNA expression ratios measured by microarray.

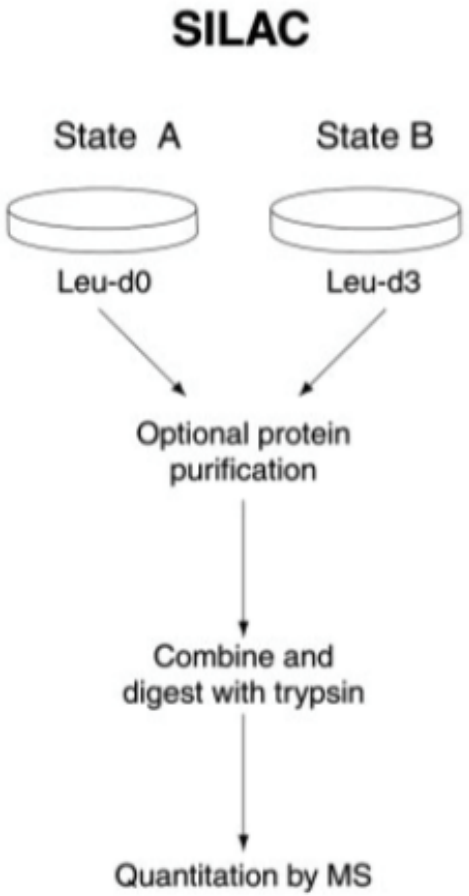


Ideker et al., Science 292(5518):929-34, (2001).

SILAC

Stable **I**sotope **L**abeling by **A**mino **A**cids in **C**ell Culture (SILAC)

- *in vivo* incorporation of specific amino acids into all mammalian proteins.
- cell lines are grown in media lacking a standard essential amino acid but supplemented with a non-radioactive, isotopically labeled form of that amino acid, in this case deuterated leucine (Leu-d3).
- Normal leucine or Leu-d3 media at the start of the experiment
- Isotopic state information is already "encoded" into the amino acid sequences.
- No chemical modification or affinity steps



Ong et al., Molecular & Cellular Proteomics, 1, 376-386 (2002).

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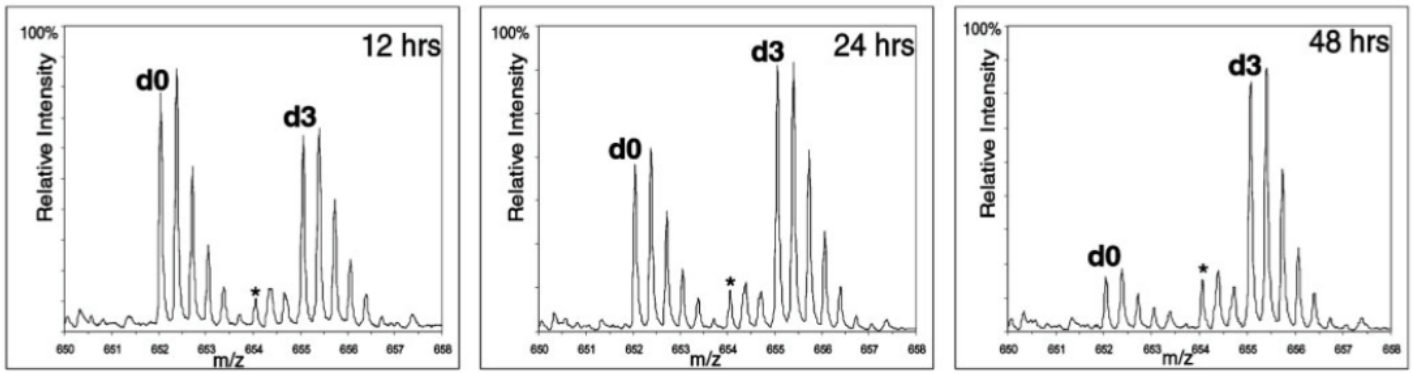
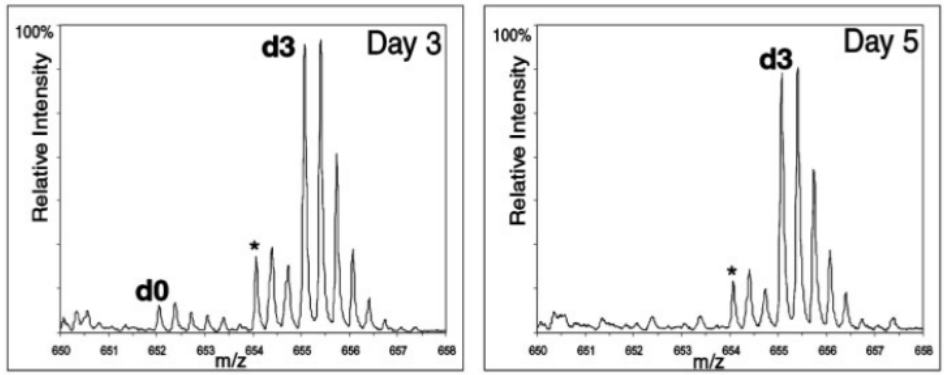
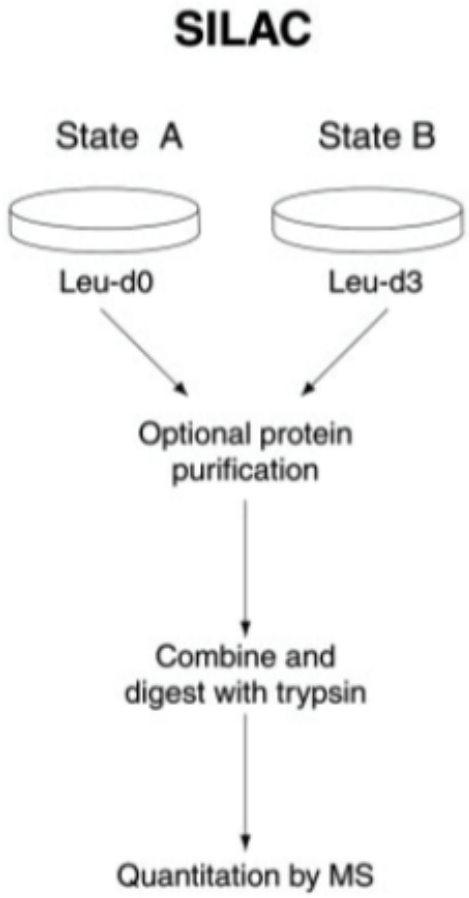




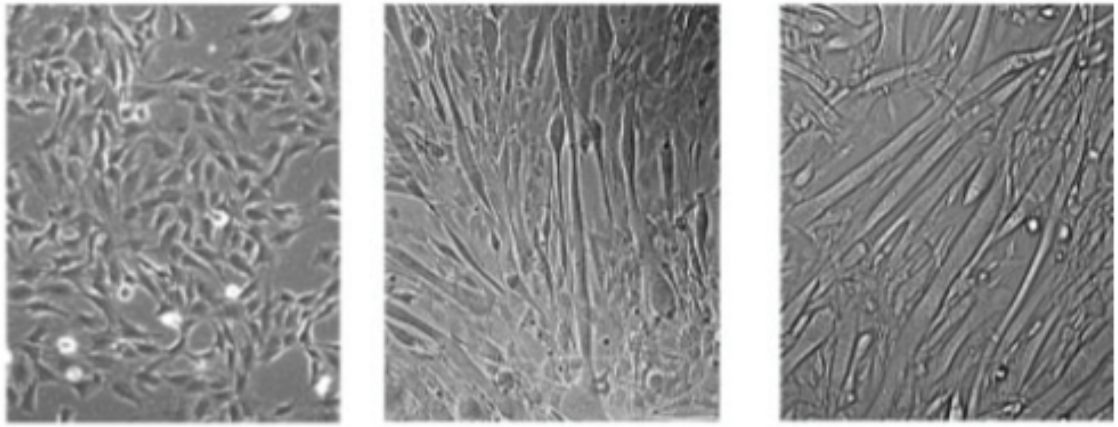


Ong et al., Molecular & Cellular Proteomics, 1, 376-386 (2002).

SILAC

Stable **I**sotope **L**abeling by **A**mino **A**cids in **C**ell Culture (SILAC)

- Mouse C2C12 cells as they differentiate from myoblasts into myotubes
- Muscle differentiation induced by a 5-fold reduction in serum



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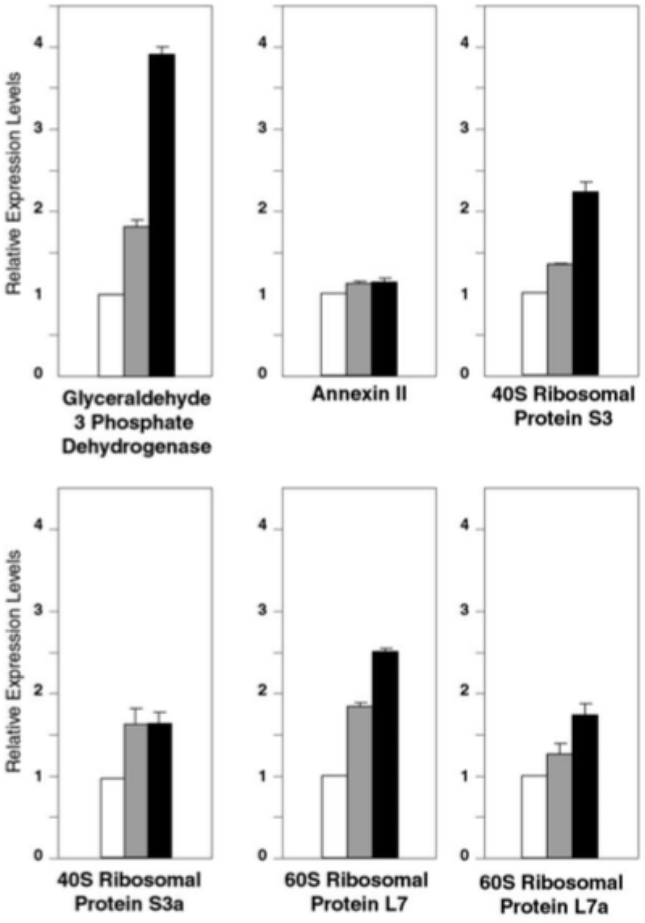
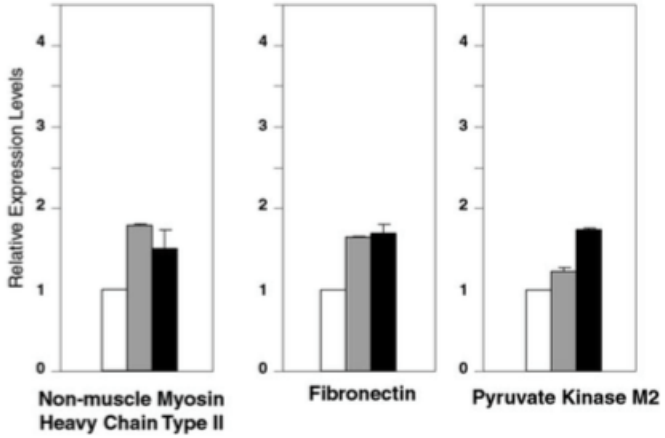


Day 0 (white)

Day 2 (gray)

Day 5 (black)



- Glucose metabolism-related enzymes upregulated on days 2 & 5 of muscle differentiation relative to day 0.
- Protein synthesis-related factors such as ribosomal proteins upregulated up to 2.5-fold, in accordance with increased protein synthesis during the conversion process.
- Levels of fibronectin, (major component of extracellular matrix essential for myogenesis)

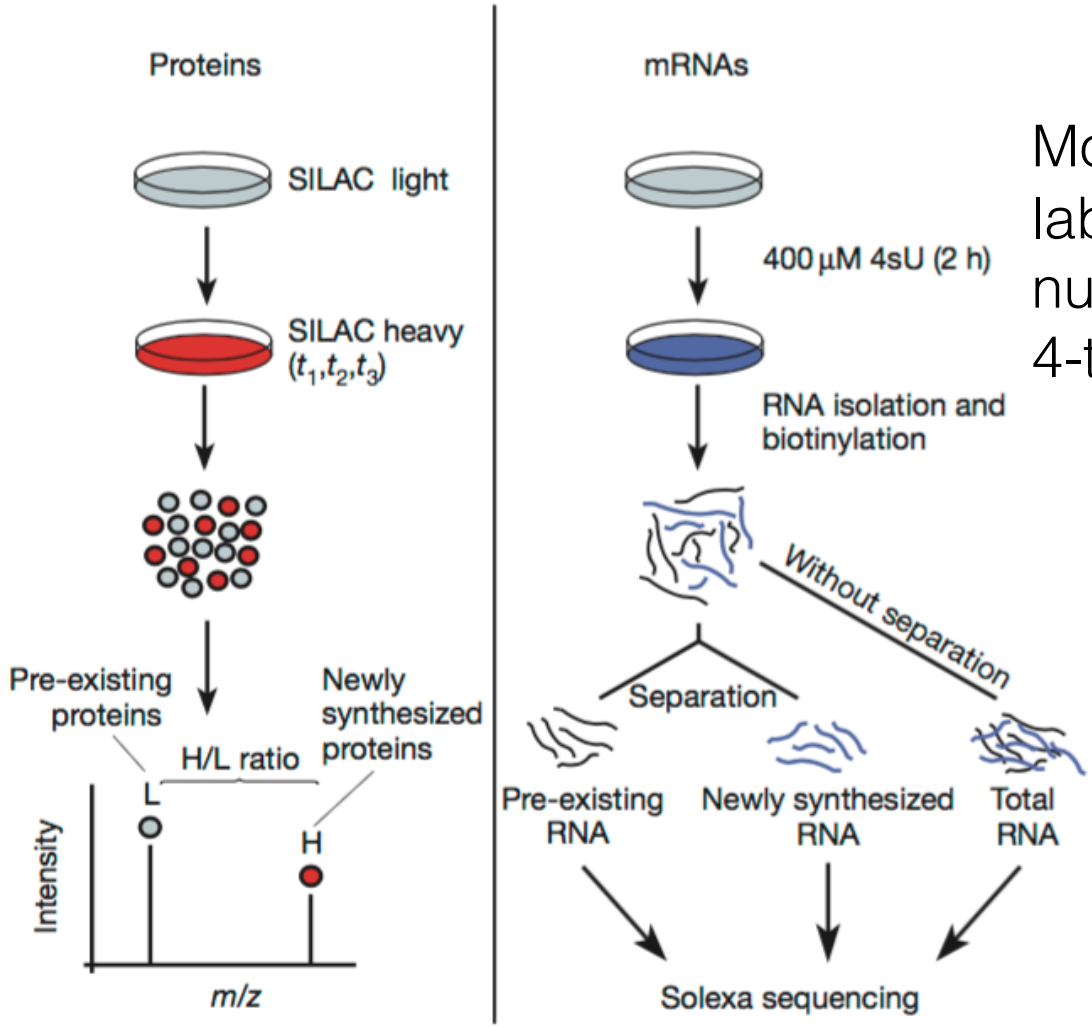
Ong et al., Molecular & Cellular Proteomics, 1, 376-386 (2002).

Protein Vs mRNA

Mouse fibroblasts pulse labelled with heavy amino acids

3 timepoints

1.5, 4.5, 13.5 h

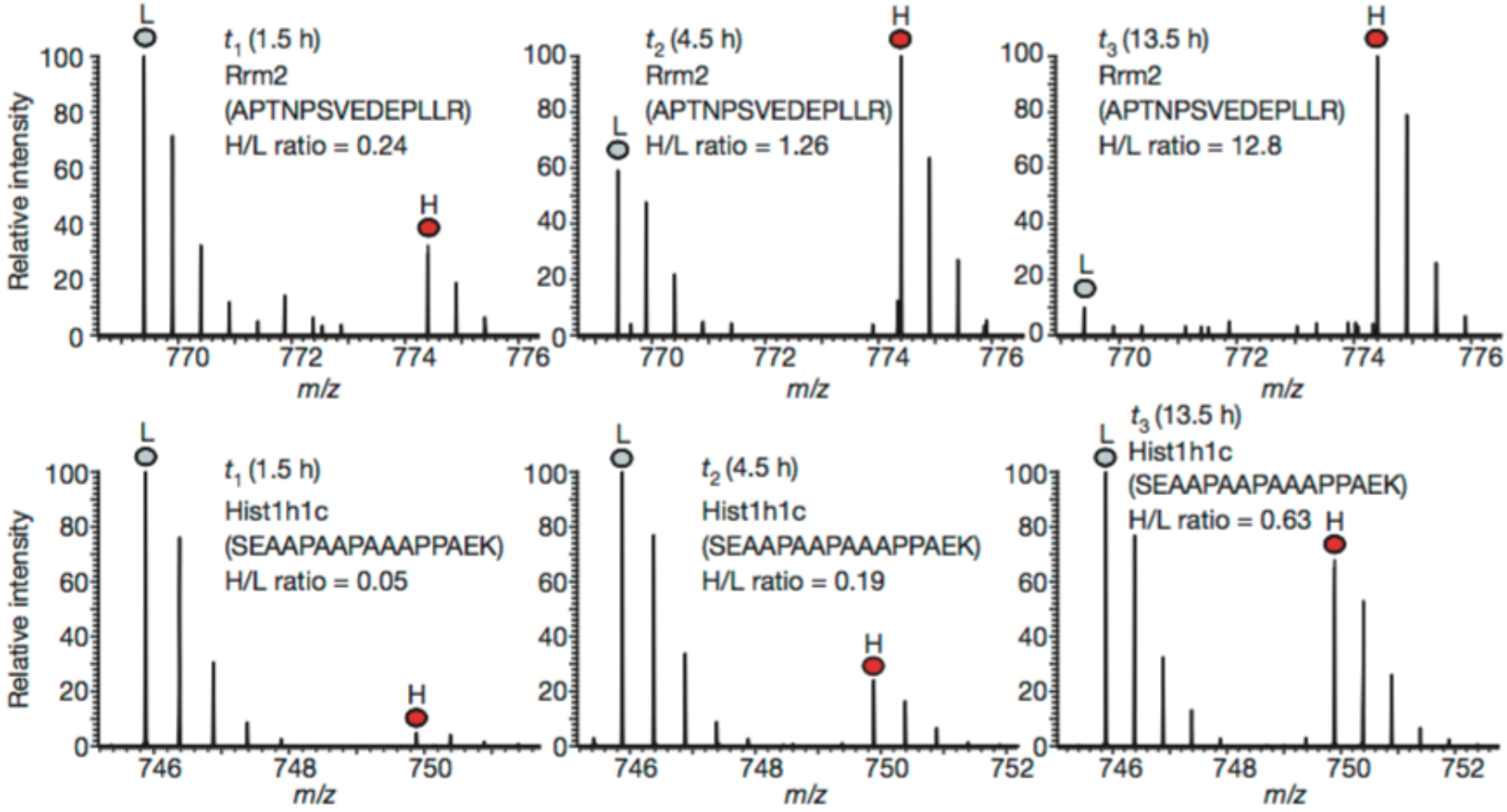


Mouse fibroblasts labelled with nucleoside 4-thiouridine (4sU)

Schwanhäusser et al., Nature 473(7347):337-42, (2011) & Corrigendum Nature 495, 126–127 (2013)

Protein Vs mRNA

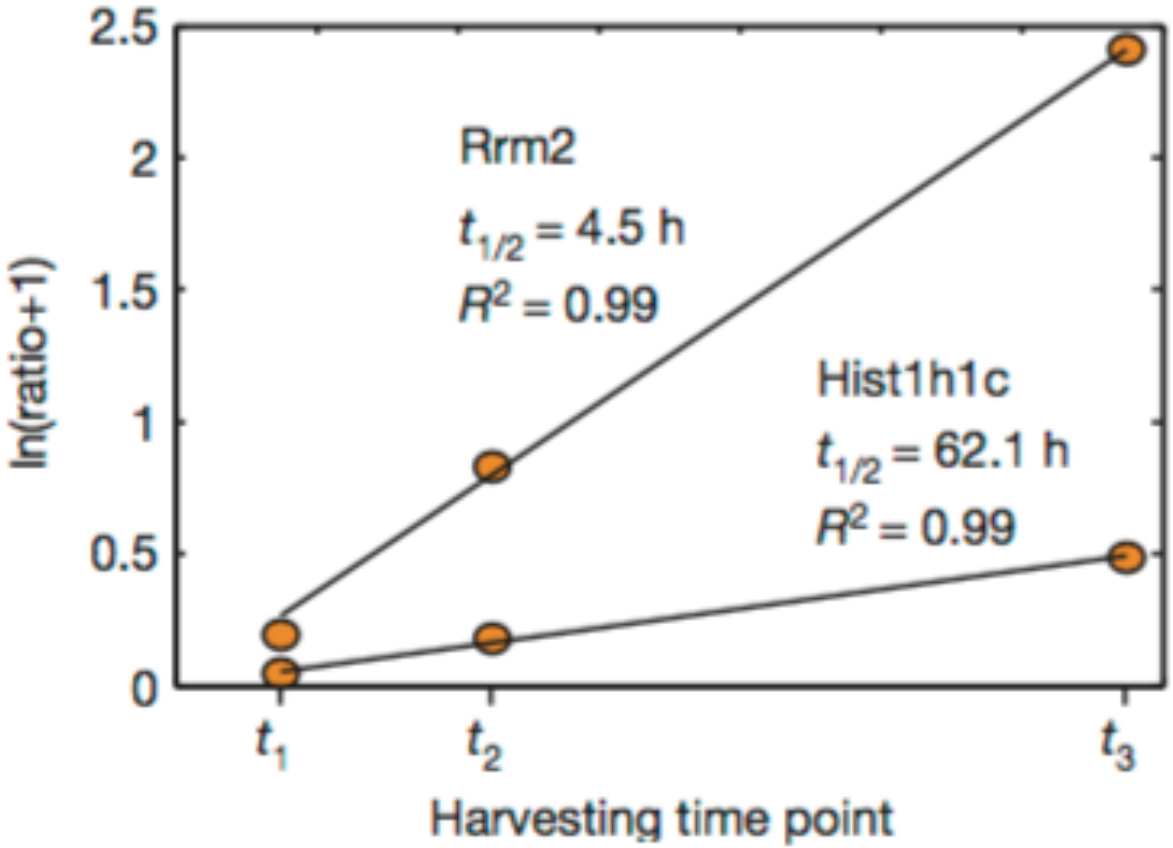
Increased H/L ratios over time



Schwanhäusser et al., Nature 473(7347):337-42, (2011) & Corrigendum Nature 495, 126–127 (2013)

Protein Vs mRNA

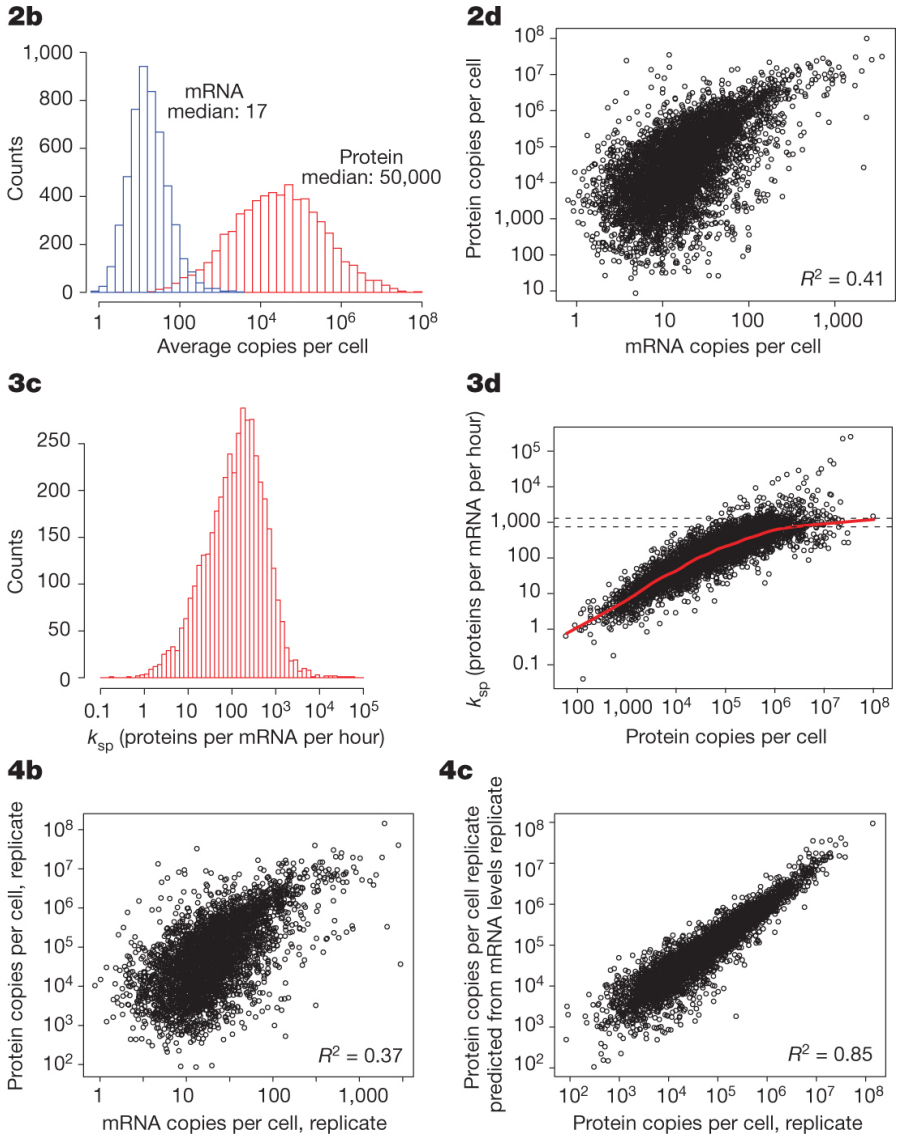
Protein half-lives based on log H/L ratios over 3 points



Schwanhäusser et al., Nature 473(7347):337-42, (2011) & Corrigendum Nature 495, 126–127 (2013)

Protein Vs mRNA

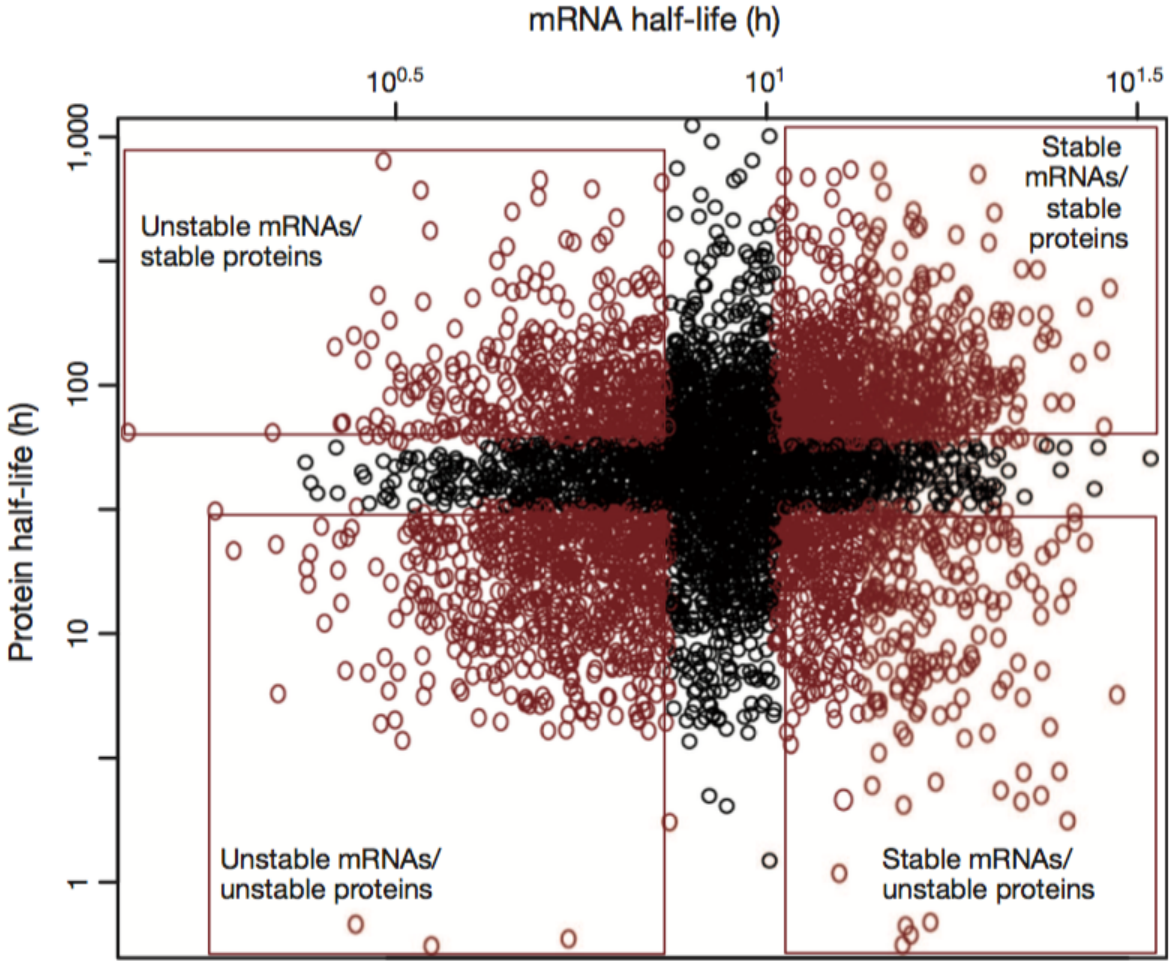
- Proteins more stable
- Proteins have greater dynamic range



Schwanhäusser et al., Nature 473(7347):337-42, (2011) & Corrigendum Nature 495, 126–127 (2013)

Protein Vs mRNA

Functional characteristics of genes with different mRNA and protein half-lives



Schwanhäusser et al., Nature 473(7347):337-42, (2011) &
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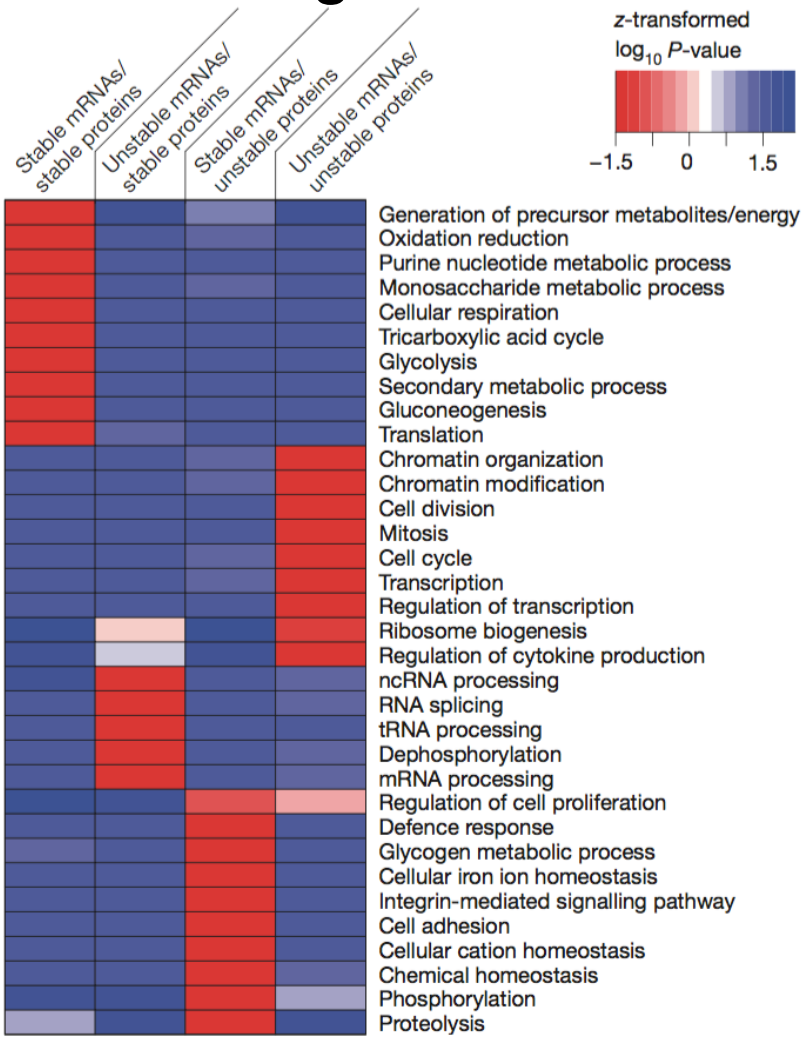
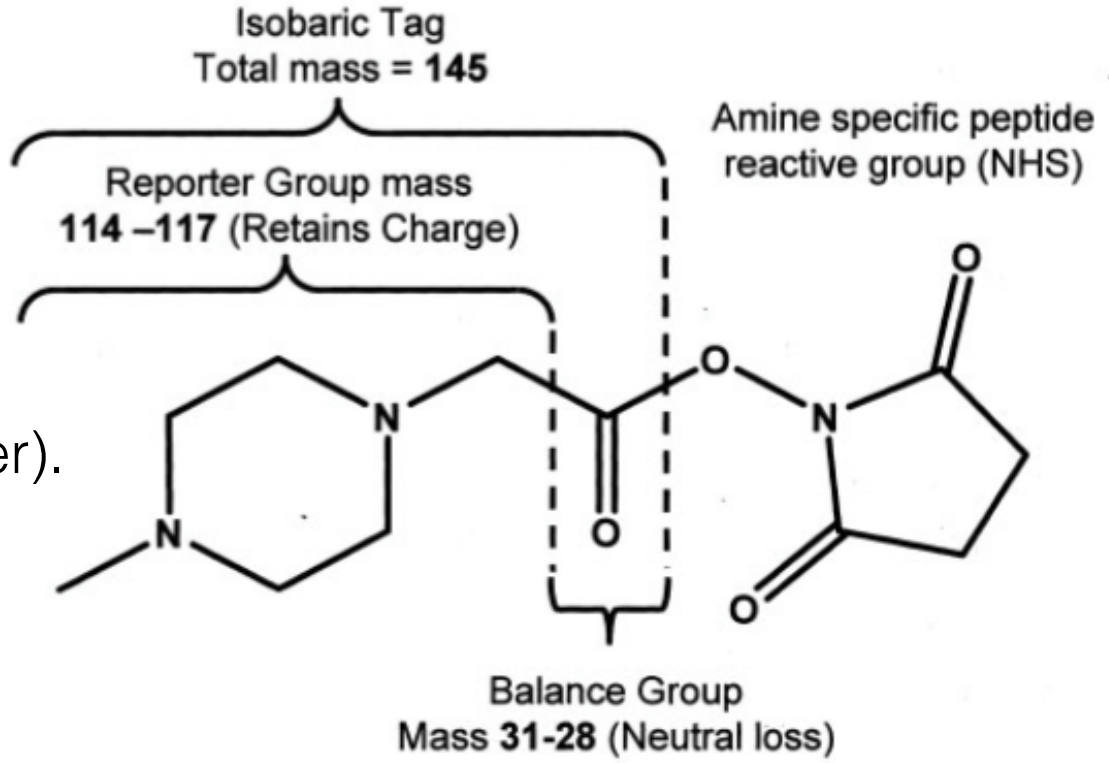


Schwanhäusser et al., Nature 473(7347):337-42, (2011) &
Corrigendum Nature 495, 126–127 (2013)

iTRAQ

isobaric **T**agging for **R**elative and **A**bsolute **Q**uantitation (iTRAQ)

- reporter group (based on N-methylpiperazine)
- mass balance group (carbonyl)
- peptide-reactive group (NHS ester).

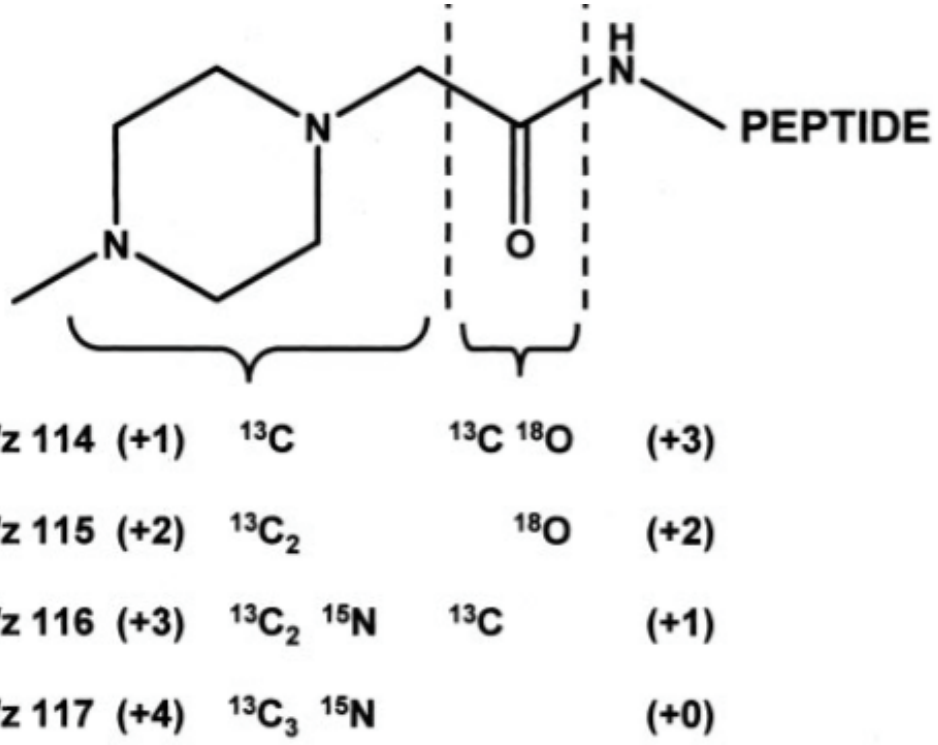


Ross, P. L. et al. Mol Cell Proteomics 3(12): 1154-69, (2004)

iTRAQ

isobaric **T**agging for **R**elative and **A**bsolute **Q**uantitation (iTRAQ)

- 4 Labels
- The overall mass of reporter and balance components of the molecule are kept constant using differential isotopic enrichment with ^{13}C, ^{15}N, and ^{18}O atoms
- Avoids problems with chromatographic separation seen with enrichment involving deuterium substitution



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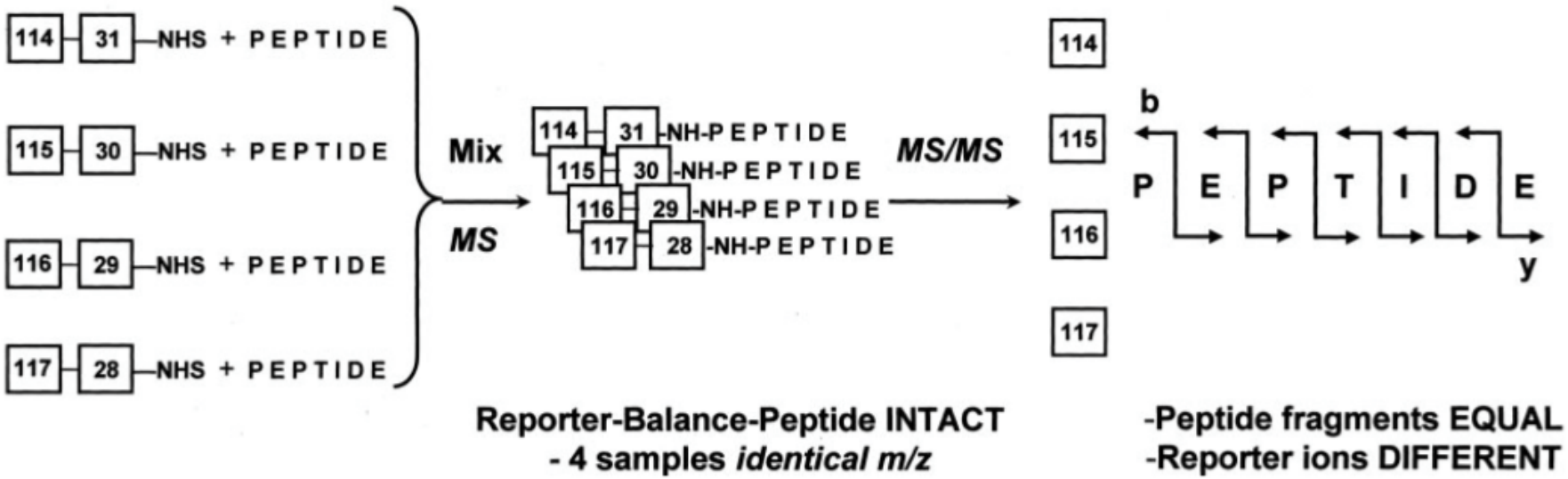


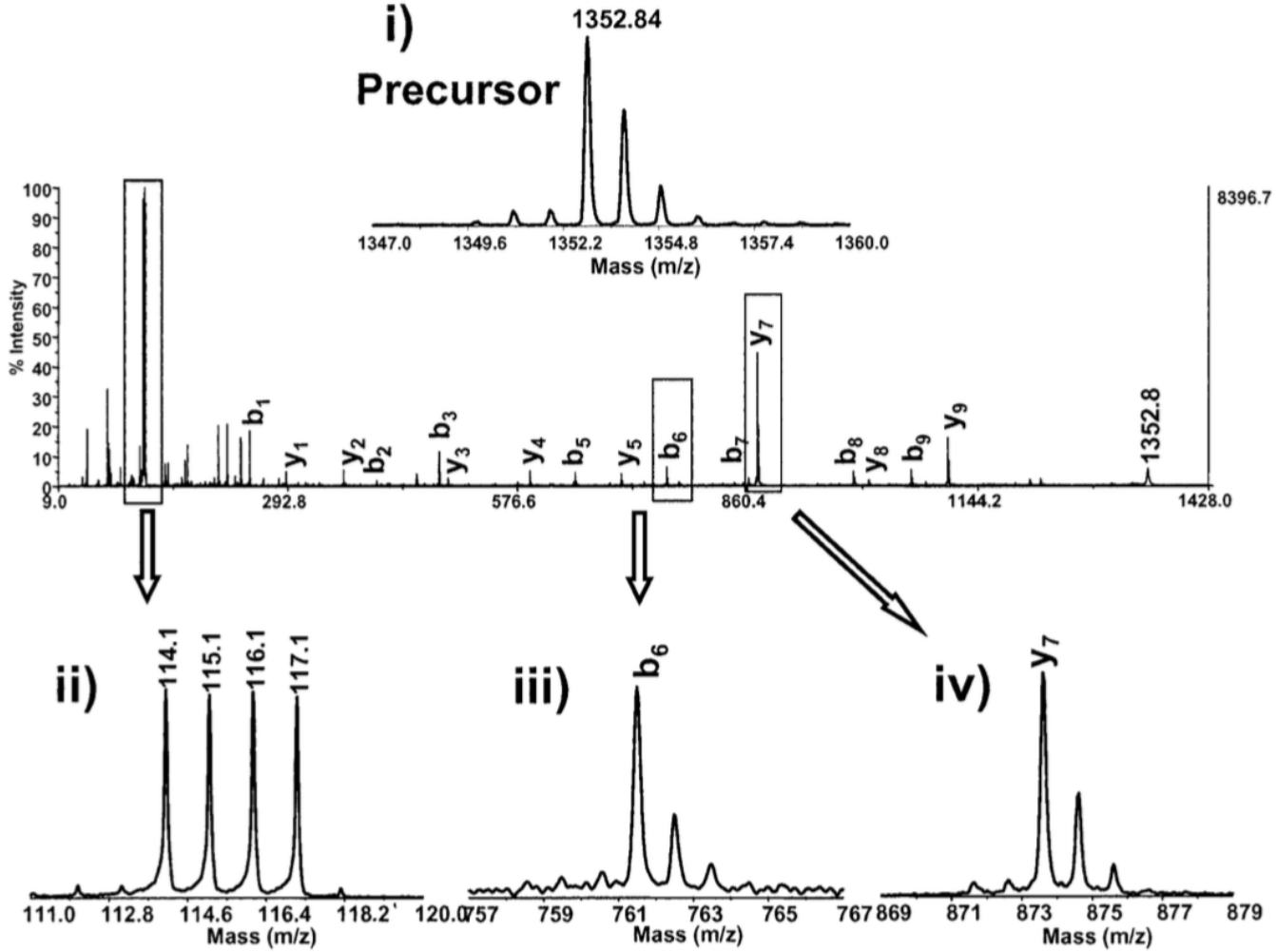
Compare to ICAT quantitation at the MS/MS stage Vs MS

Ross, P. L. et al. Mol Cell Proteomics 3(12): 1154-69, (2004)

iTRAQ

isobaric **T**agging for **R**elative and **A**bsolute **Q**uantitation (iTRAQ)

- E.g. MS/MS spectrum of peptide TPHPALTEAK
- Protein digest mixture of 4 separate digests 1:1:1:1 ratio



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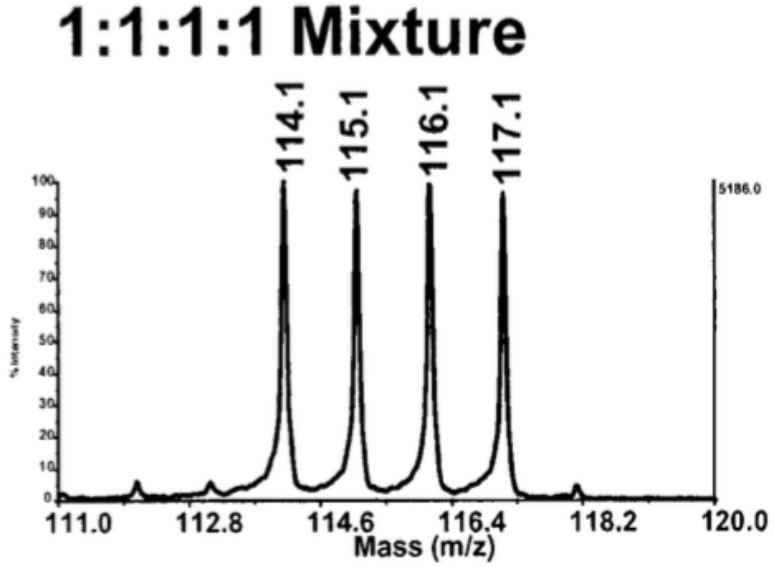
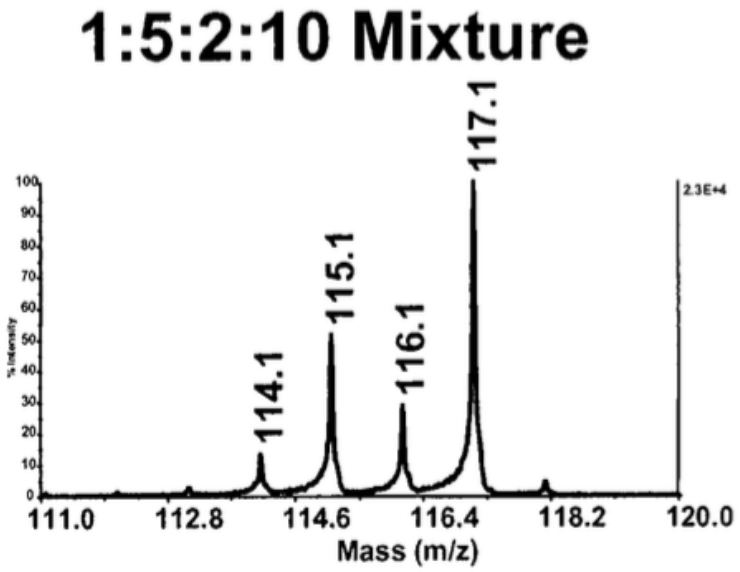
iTRAQ

isobaric **T**agging for **R**elative and **A**bsolute **Q**uantitation (iTRAQ)

- 1:1:1:1 ratio Vs
- 1:5:2:10

Ratio	Mean	SD
1:1	1.03	0.16
1:2	0.514	0.12
1:5	0.204	.045
1:10	0.097	0.023





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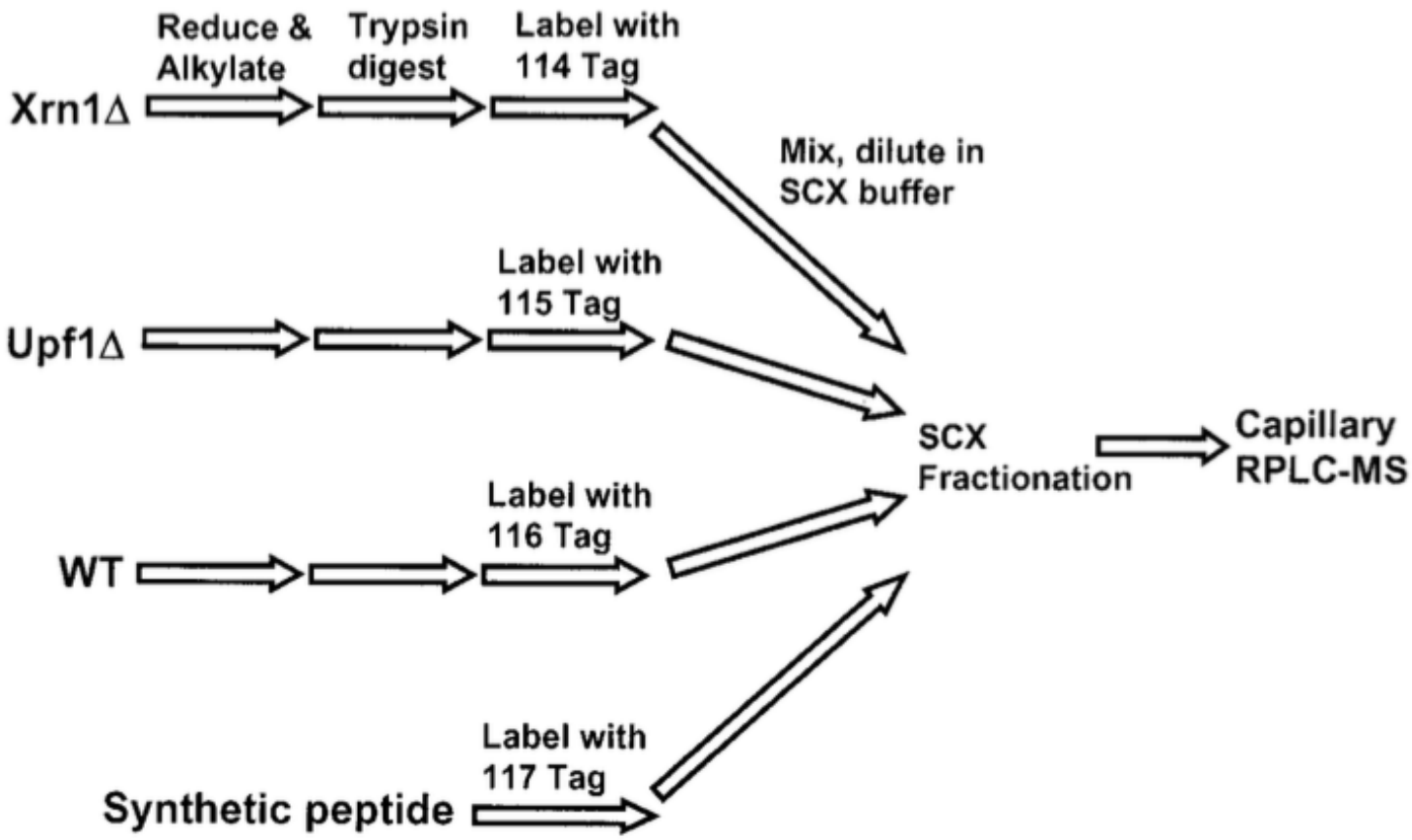
iTRAQ

isobaric **T**agging for **R**elative and **A**bsolute **Q**uantitation (iTRAQ)

- Yeast
- WT, Upf1Δ & Xrn1Δ strains.
- Standards



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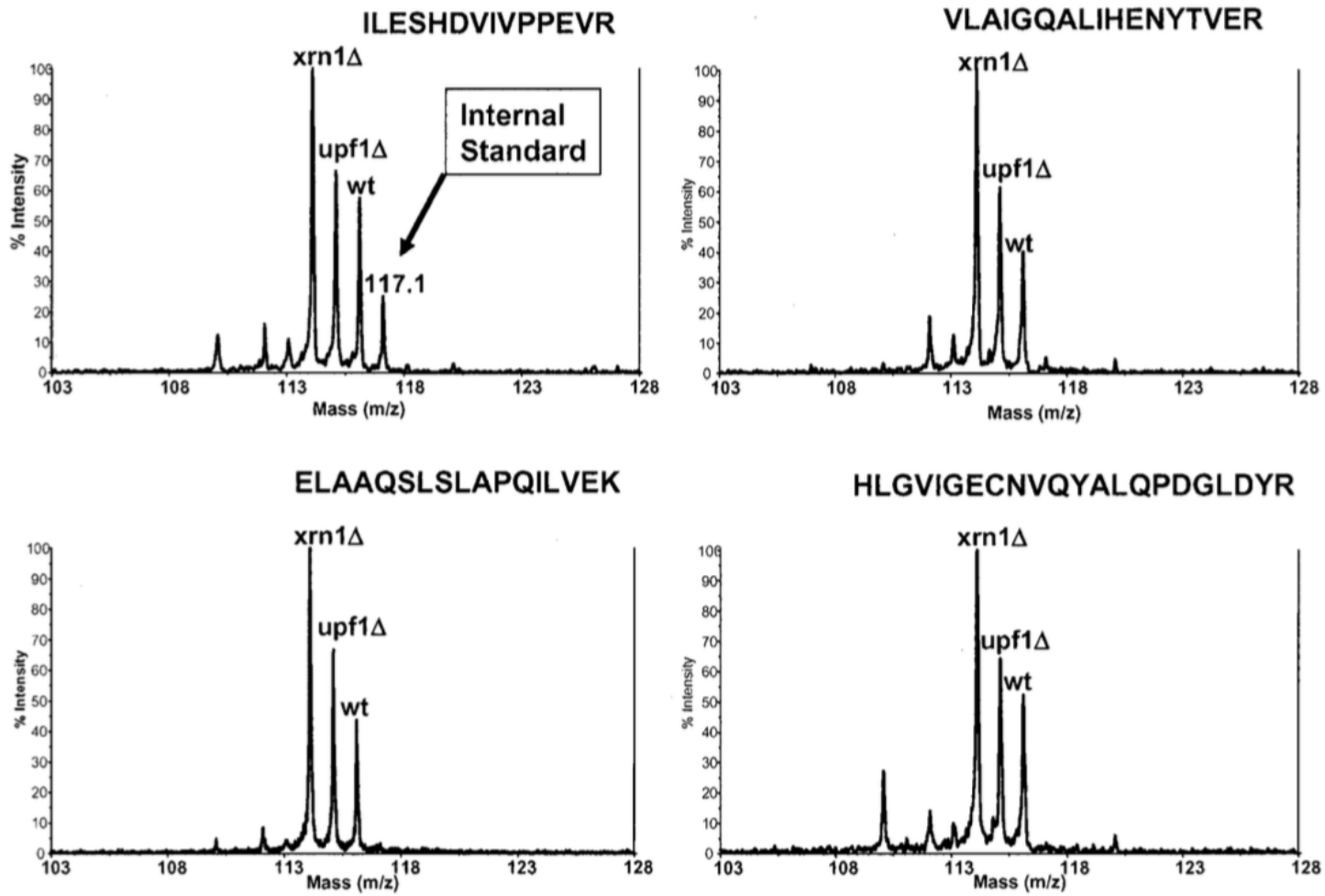

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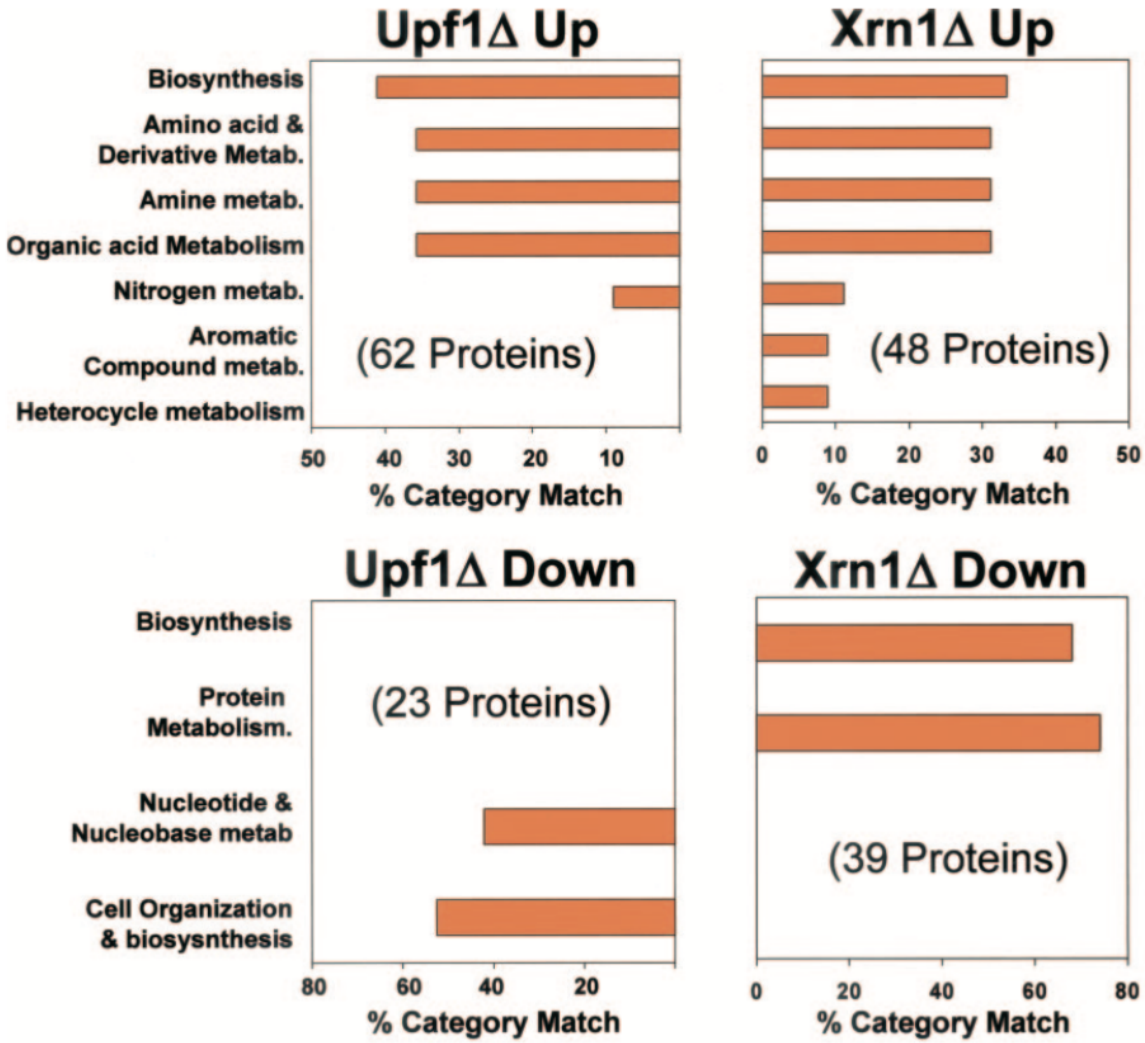
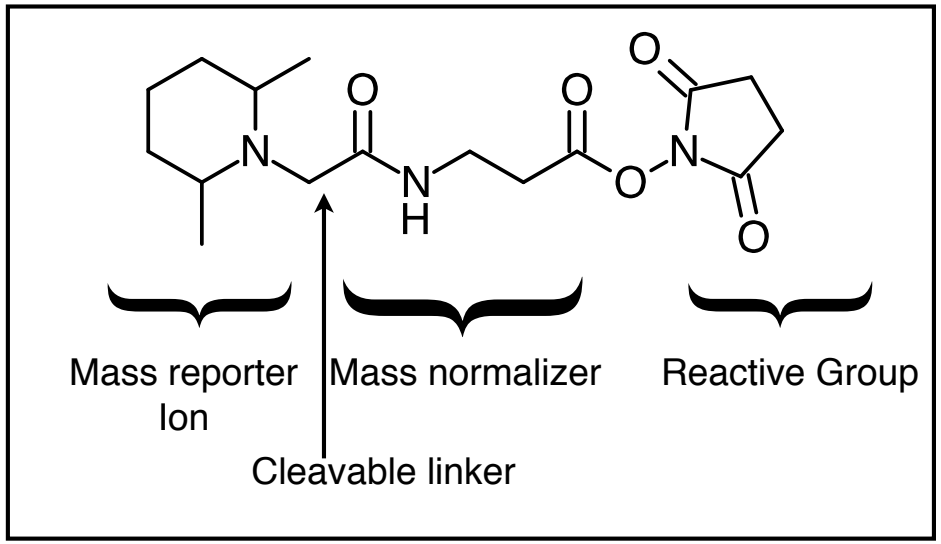


Ross, P. L. et al. Mol Cell Proteomics 3(12): 1154-69, (2004)

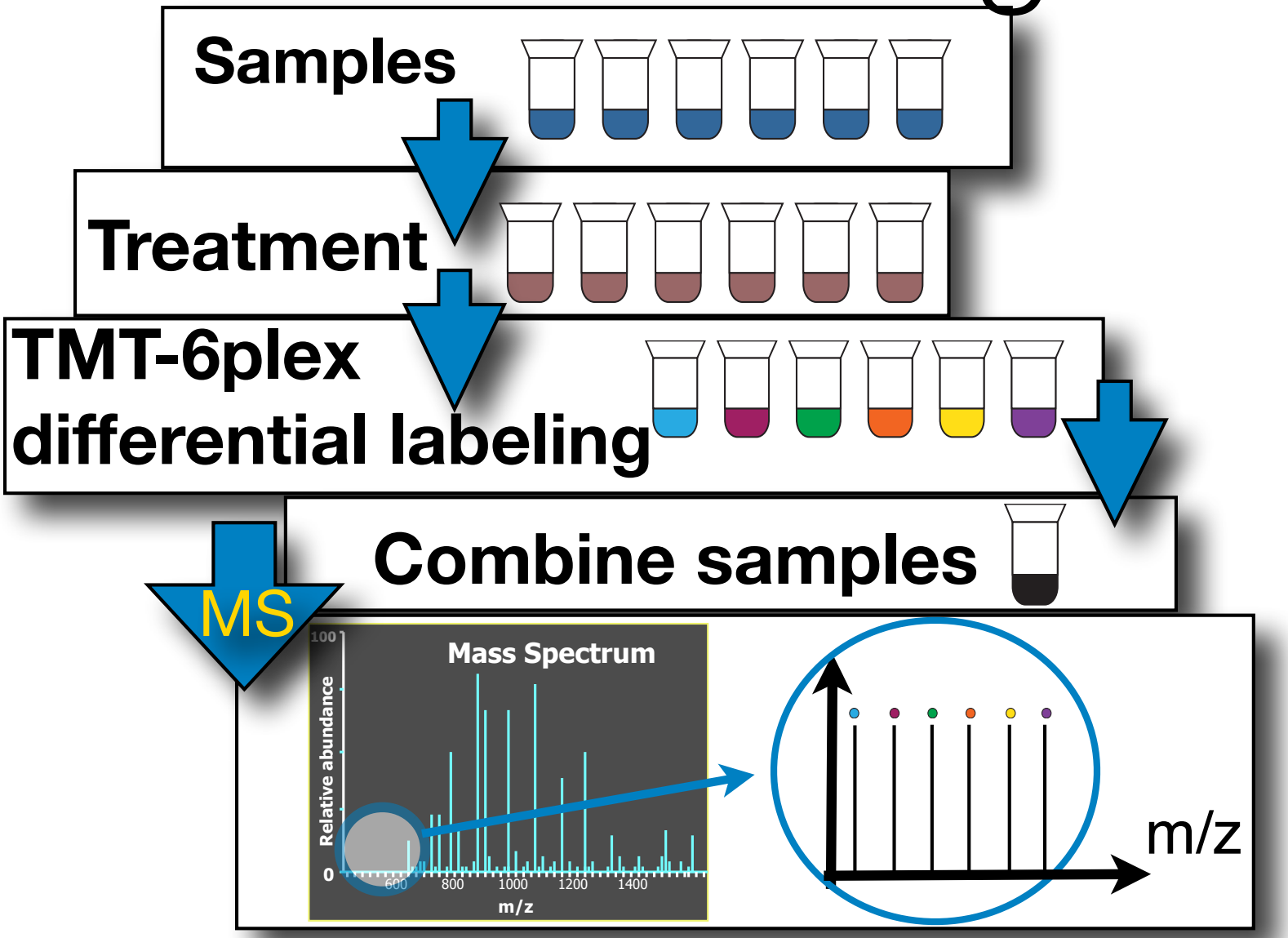
Tandem Mass Tags

- Tracking Different Timepoints with Chemical Labeling (TMT 6plex [or 10-plex])
  - Mass normalizer: Peptides from different sample have same peak
  - Reporter ion ratio
  - for quantitation



http://www.thermo.com

Isobaric Labeling



http://www.thermo.com

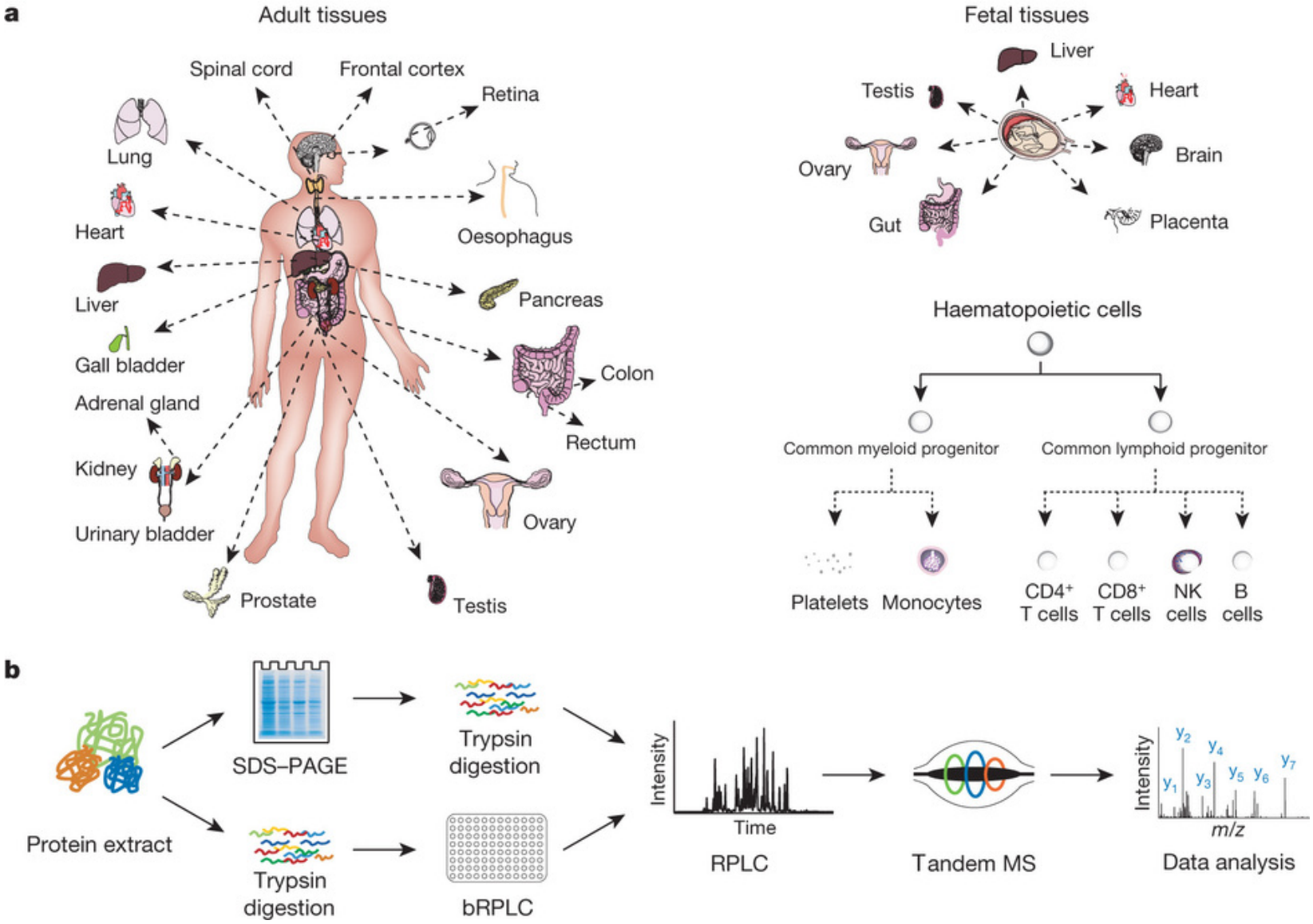
Label Free Quantitation

Compare

- signal intensity
- Acquired Spectral counts

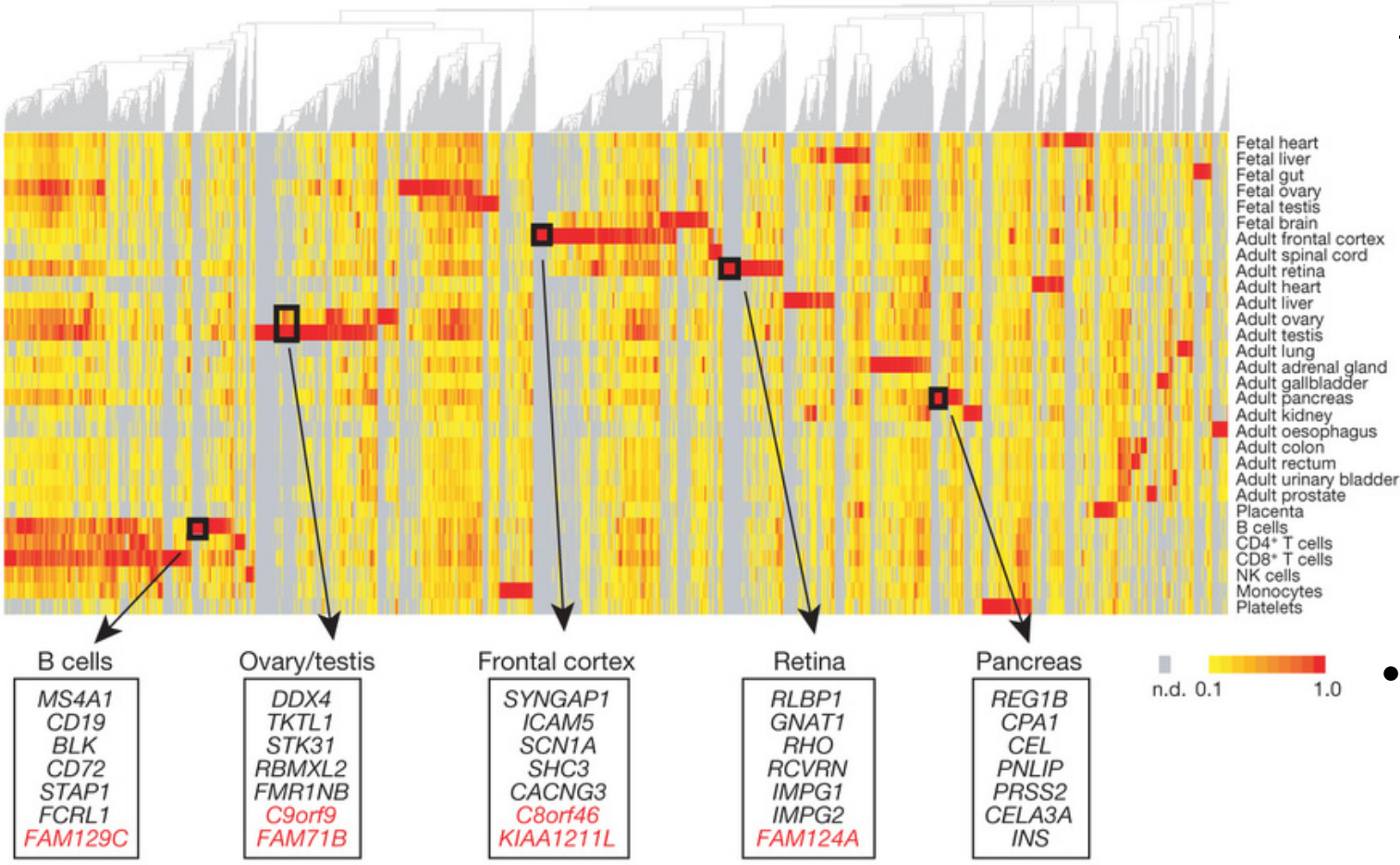
Human Proteome

A draft map of the human proteome



Kim et al., Nature 509, p. 575 (2014)

Human Proteome



Tissue-supervised hierarchical clustering

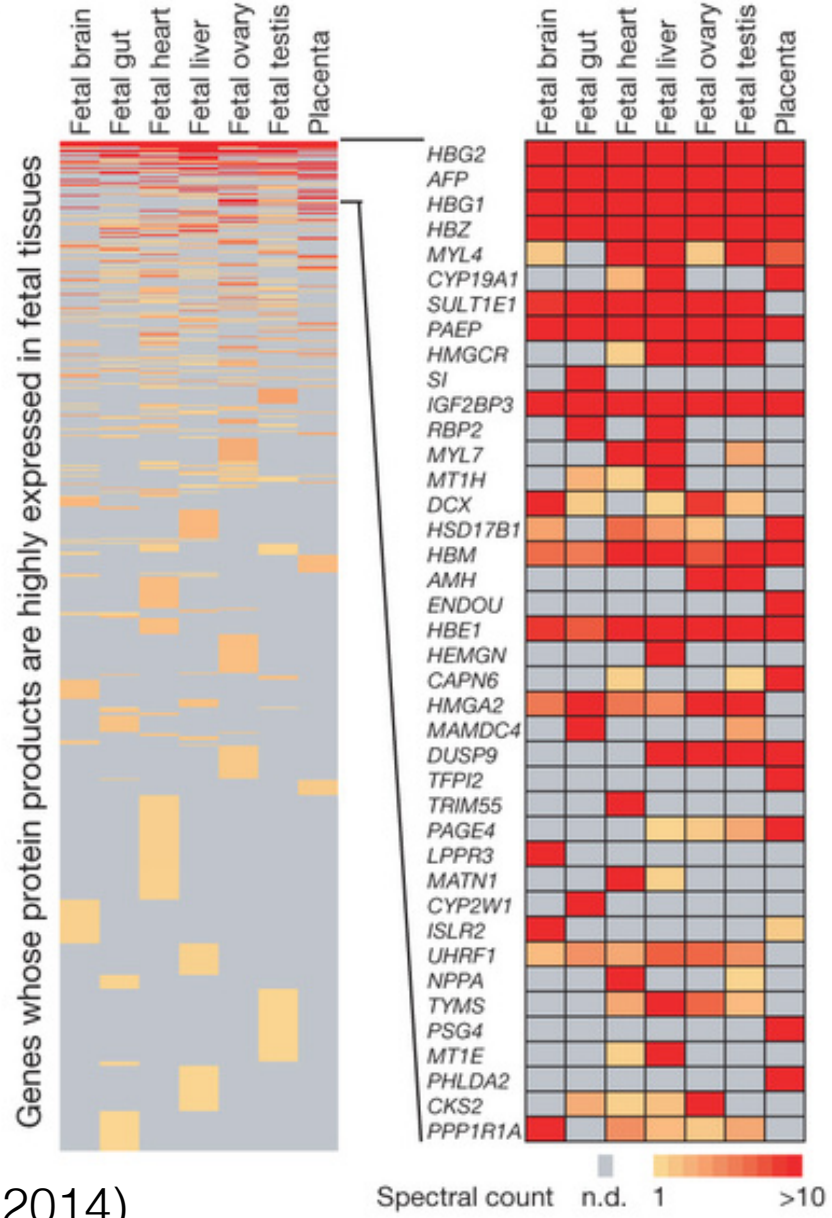
- Normalized spectral counts per gene detected across the tissues.
- n.d., not determined.

- well-studied genes (black)
- hypothetical proteins of unknown function (red)

Kim et al., Nature 509, p. 575 (2014)

Human Proteome

Tissue expression of fetal tissue-restricted genes ordered by average expression across fetal tissues



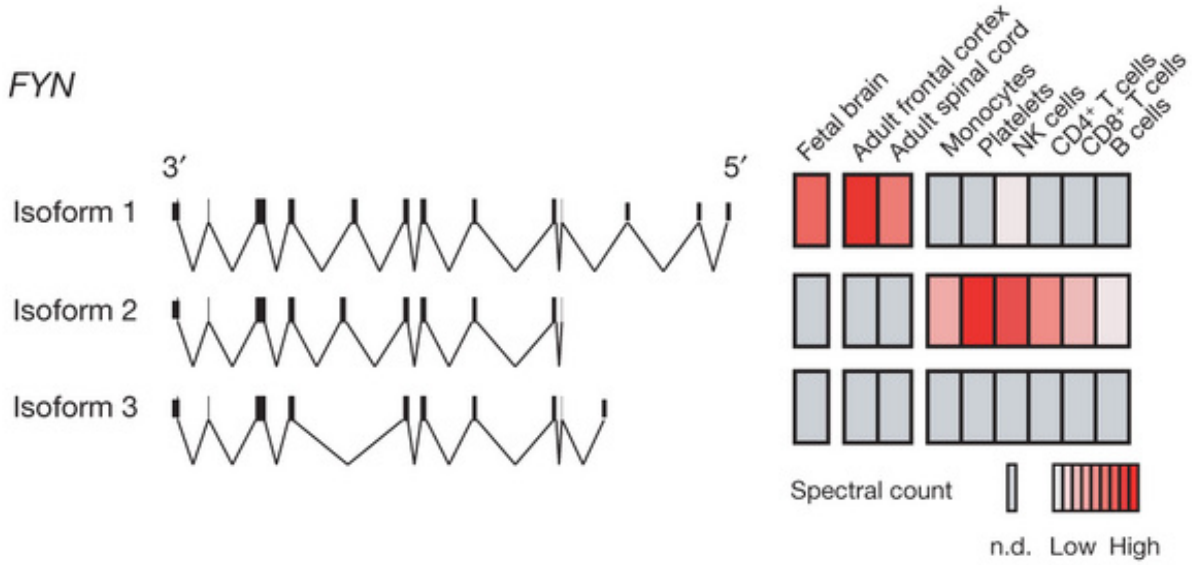
top 40 most abundant genes

Kim et al., Nature 509, p. 575 (2014)

Human Proteome

Isoform Specific Expression for *FYN*

(FYN protein tyrosine kinase)



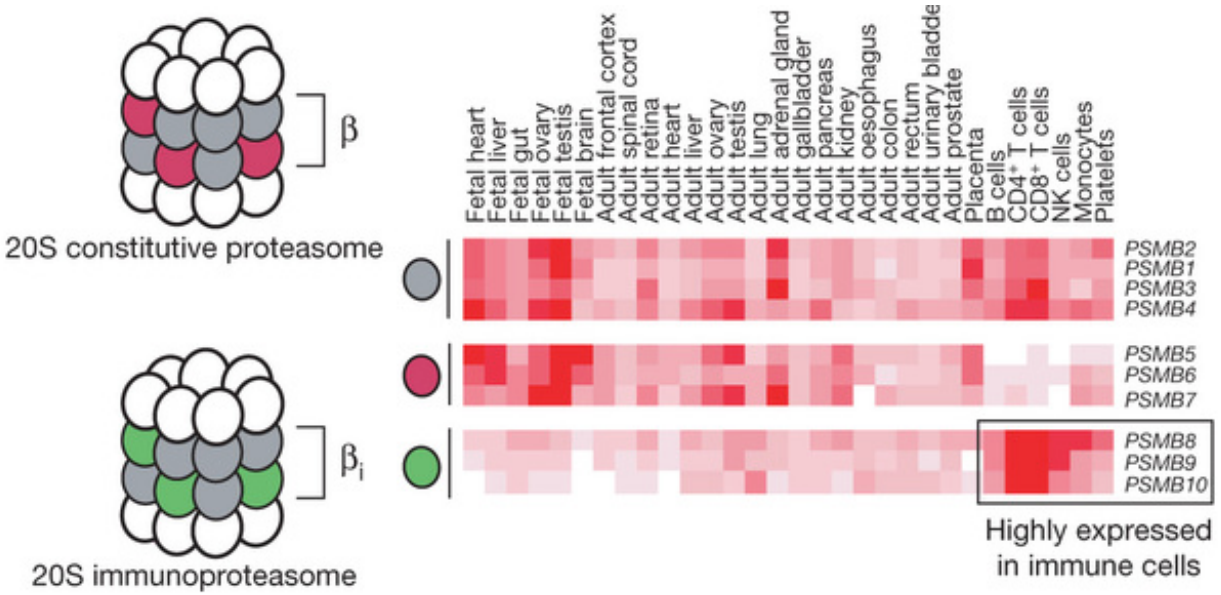
Kim et al., Nature 509, p. 575 (2014)

Human Proteome

Three of the subunits in 20S constitutive proteasome (PSMB5, PSMB6 and PSMB7; coloured **red**)

known to be replaced by three other subunits

(PSMB8, PSMB9 and PSMB10; coloured **green**) in the 20S immunoproteasome

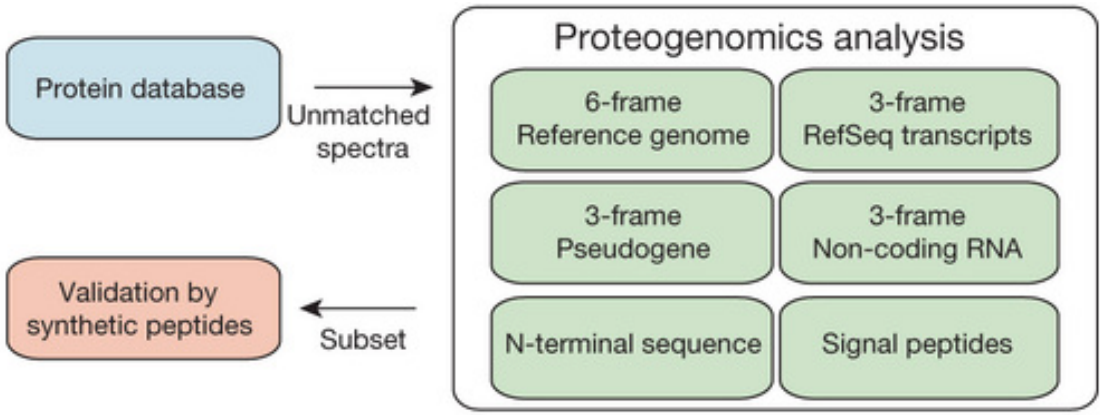


Kim et al., Nature 509, p. 575 (2014)
www.humanproteomemap.org

Human Proteome

Proteogenomics analysis

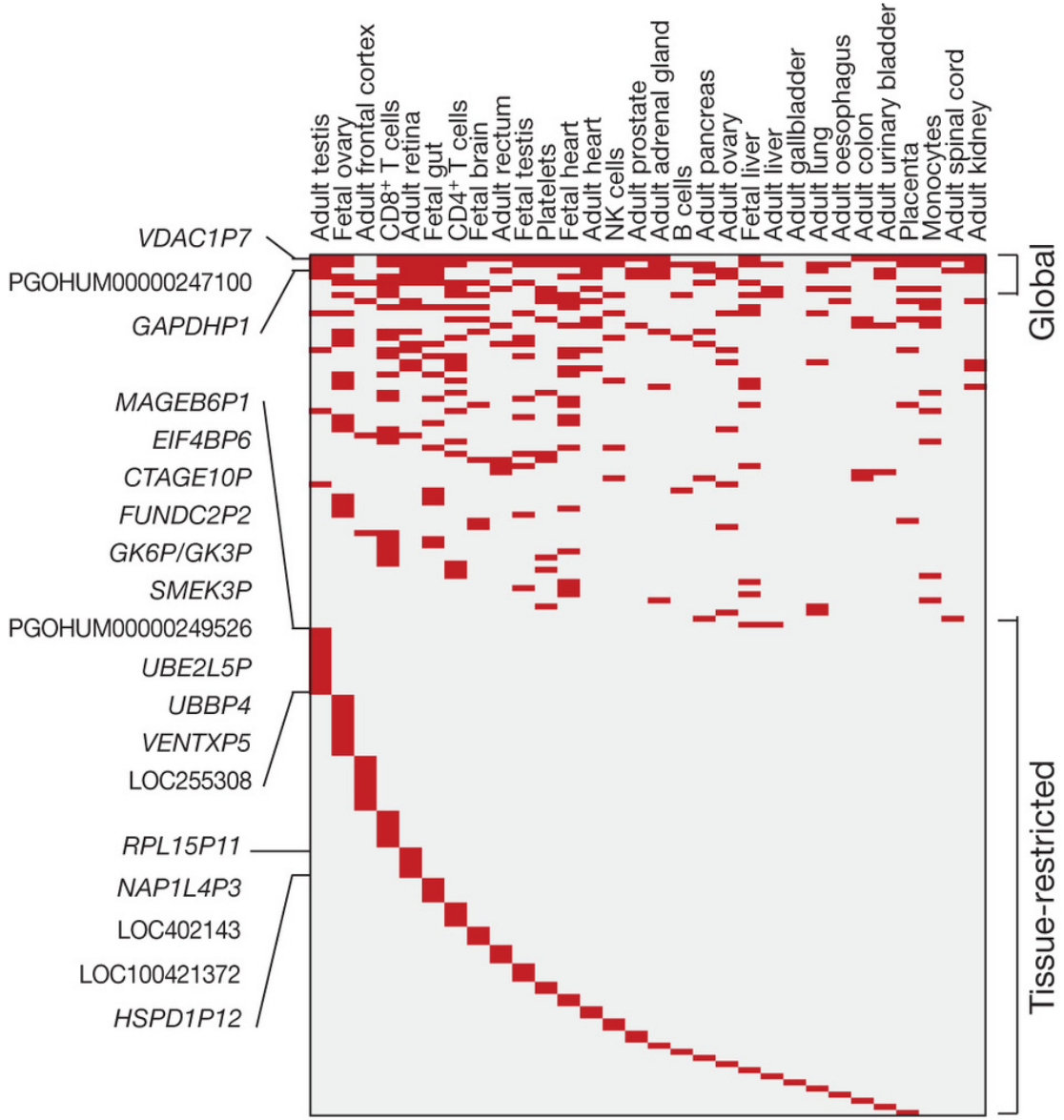
- 16 million MS/MS spectra did not match known protein annotations.
- Compared against conceptually translated
  - human genome reference,
  - RefSeq transcript sequences
  - non-coding RNAs
  - pseudogenes
- Searched against theoretical amino termini & predicted signal sequences



Kim et al., Nature 509, p. 575 (2014)

Human Proteome

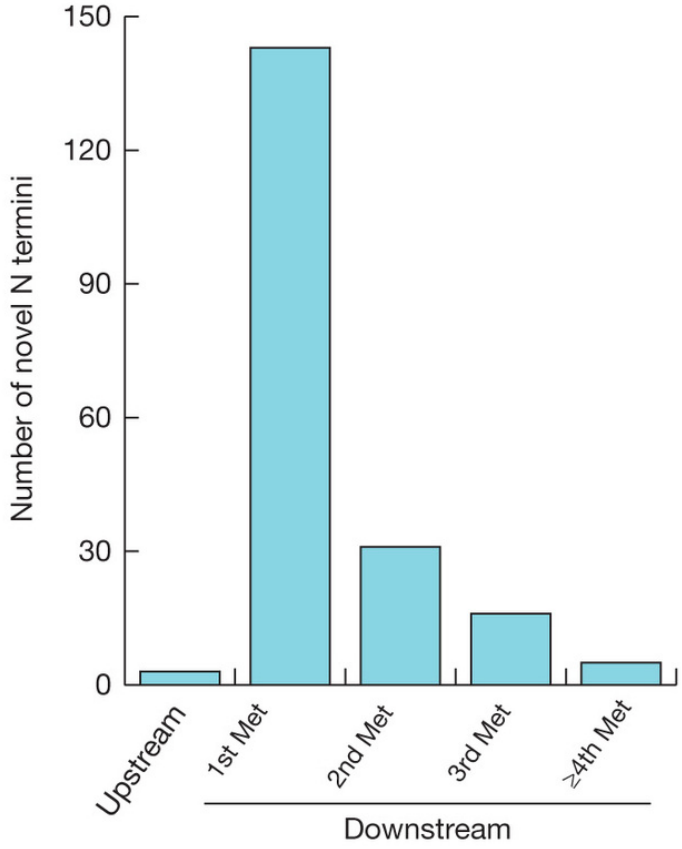
- Expression of pseudogenes across the analysed cells/ tissues.
- Some pseudogenes such as *VDAC1P7* and *GAPDHP1* found to be globally expressed.



Kim et al., Nature 509, p. 575 (2014)

Human Proteome

- The distribution of novel N termini detected with N-terminal acetylation is shown with respect to the location of the annotated translational start site.
- sites in the 5′ UTR are labelled upstream
- sites located downstream of the annotated AUG start sites labelled as 1st Met, 2nd Met and so on.
- Compared to current annotated N-terminus
  - 3 cases in which the N-terminal peptide mapped upstream.
  - 195 cases where it mapped downstream



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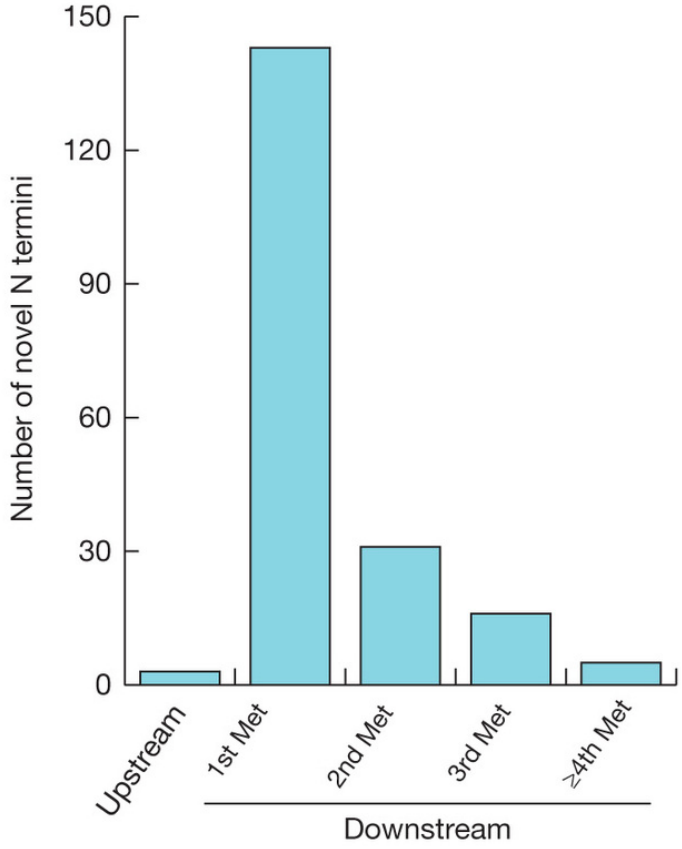

Kim et al., Nature 509, p. 575 (2014)

Human Proteome

ARTICLE

doi:10.1038/nature13319

Mass-spectrometry-based draft of the human proteome

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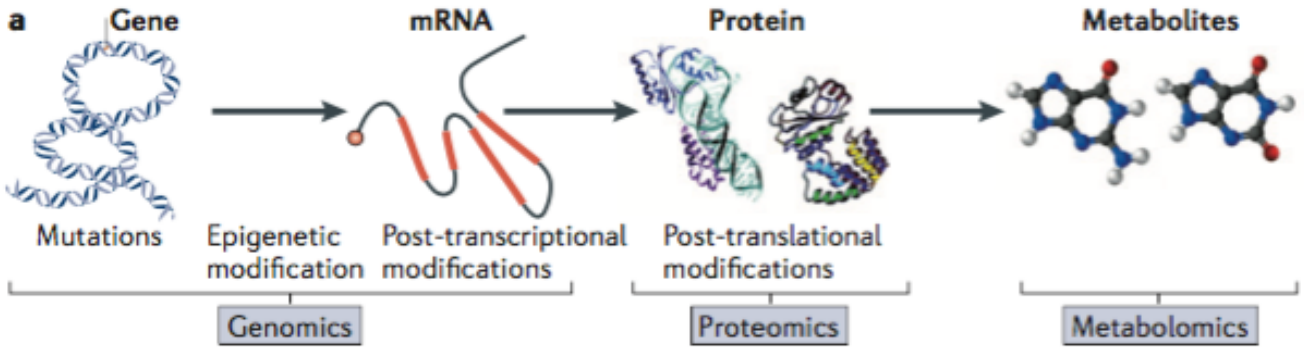
Proteomes are characterized by large protein-abundance differences, cell-type- and time-dependent expression patterns and post-translational modifications, all of which carry biological information that is not accessible by genomics or transcriptomics. Here we present a mass-spectrometry-based draft of the human proteome and a public, high-performance, in-memory database for real-time analysis of terabytes of big data, called ProteomicsDB. The information assembled from human tissues, cell lines and body fluids enabled estimation of the size of the protein-coding genome, and identified organ-specific proteins and a large number of translated lincRNAs (long intergenic non-coding RNAs). Analysis of messenger RNA and protein-expression profiles of human tissues revealed conserved control of protein abundance, and integration of drug-sensitivity data enabled the identification of proteins predicting resistance or sensitivity. The proteome profiles also hold considerable promise for analysing the composition and stoichiometry of protein complexes. ProteomicsDB thus enables navigation of proteomes, provides biological insight and fosters the development of proteomic technology.

Assembled protein evidence for 18,097 genes in ProteomicsDB

Wilhelm et al., Nature Nature 509, p. 582 (2014).

Metabolomics



Patti, Yanes & Siuzdak, Nature Reviews Molecular Cell Biology 13, 263-269 (2012)

Metabolomics

- Biochemistry of pathways
- Biomarkers for disease
  - Alzheimers
  - Inflammation
- Toxicity (especially liver toxicity) in new drugs.
- Drug Tests

Metabolomics

thermofisher.com

NMR

Other varieties

agilent.com

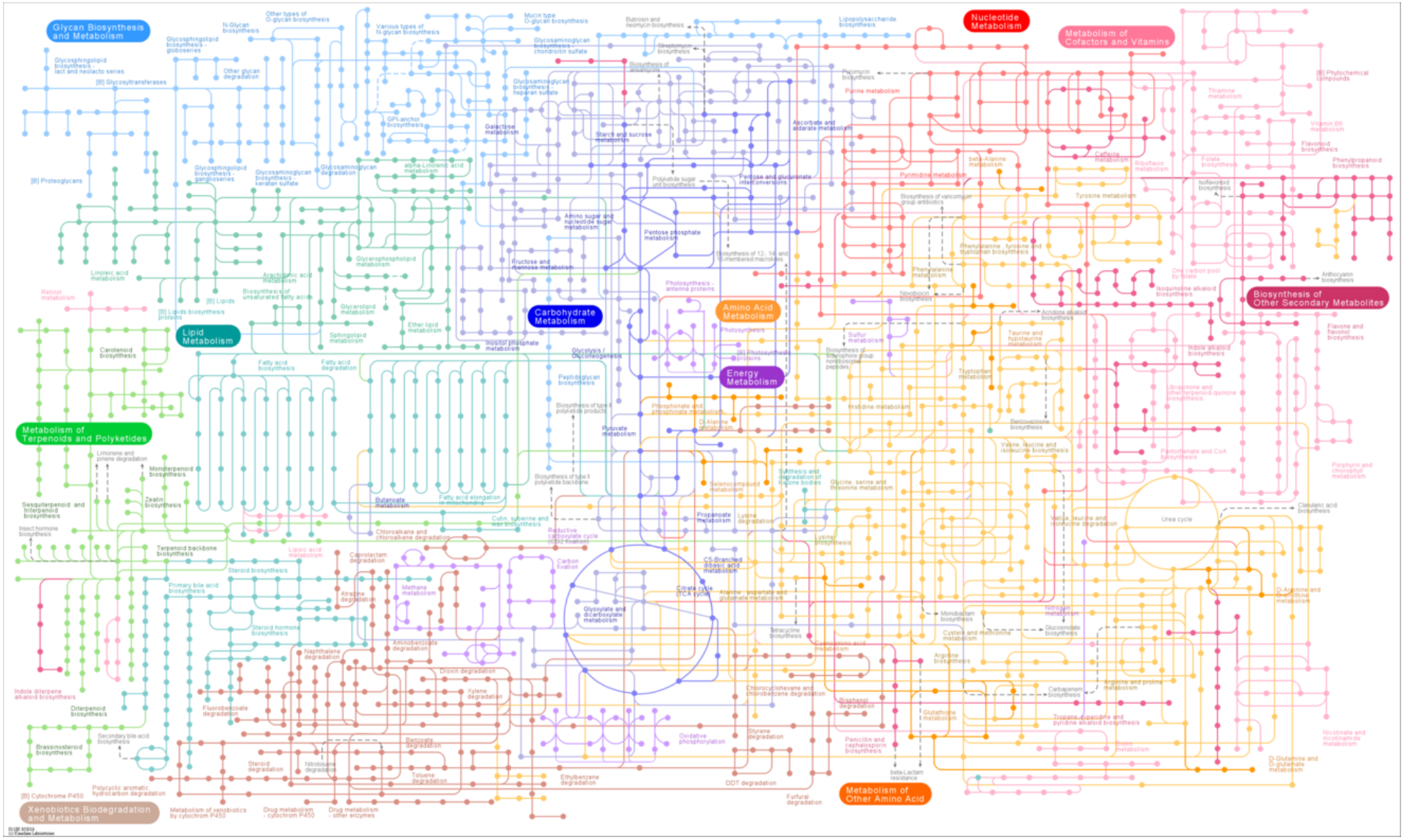
all metabolites in cells

- small molecules
- lipids
- peptides
- amino acids
- nucleic acids
- organic acids

Lipidomics

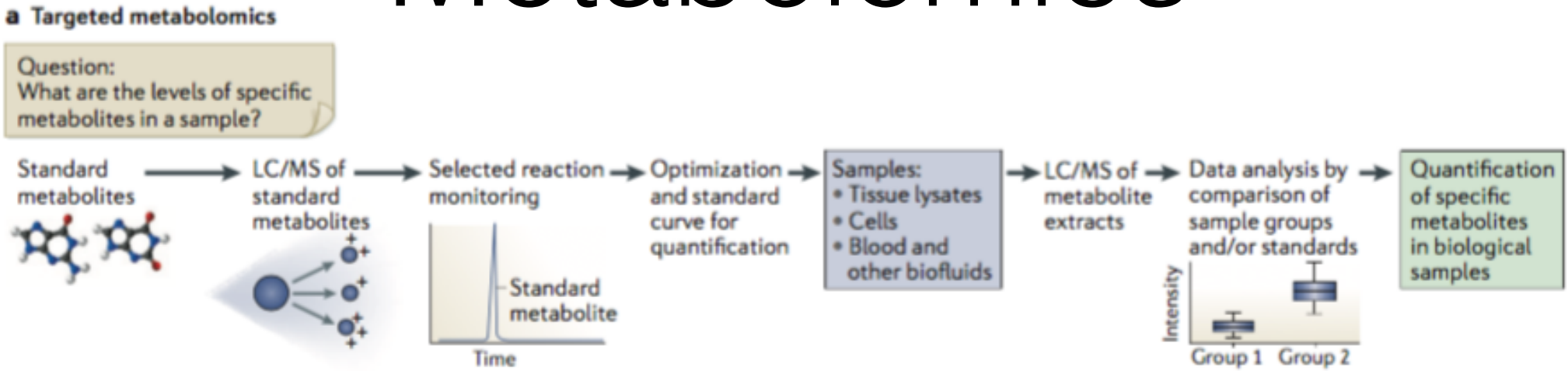
- lipids
- metabolic signaling
- energy storage
- cell proliferation
- cell migration
- apoptosis
- cellular membrane

Metabolomics



KEGG, http://www.genome.jp/kegg-bin/show_pathway?map01100

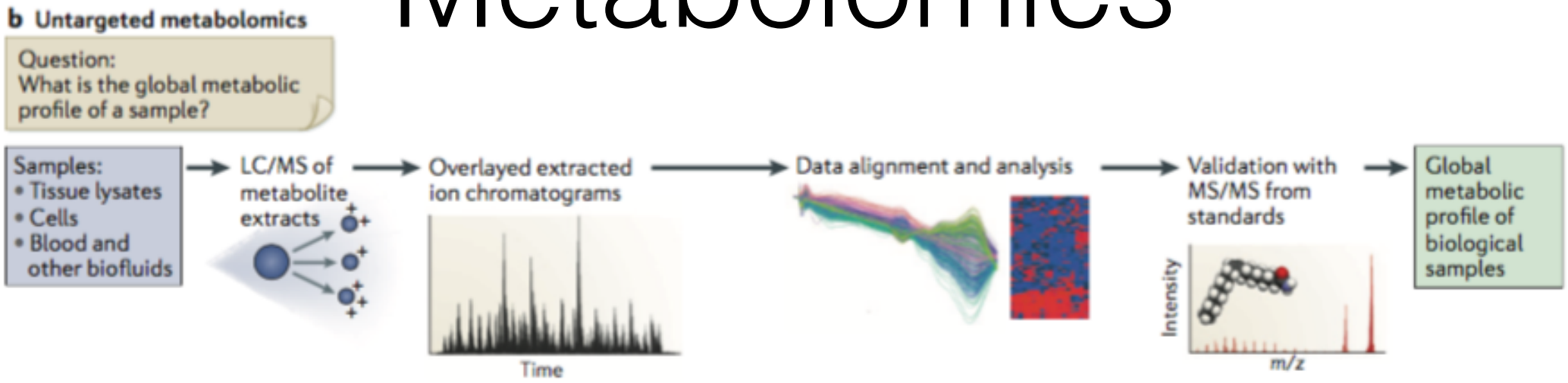
Metabolomics



- Standard compounds for the metabolites of interest are first used to set up selected reaction monitoring methods.
- Optimal instrument voltages are determined and response curves are generated for absolute quantification.
- After the targeted methods have been established on the basis of standard metabolites, metabolites are extracted from tissues, biofluids or cell cultures and analysed.
- The data output provides quantification only of those metabolites for which standard methods have been built.

Patti, Yanes & Siuzdak, Nature Reviews Molecular Cell Biology 13, 263-269 (2012)

Metabolomics



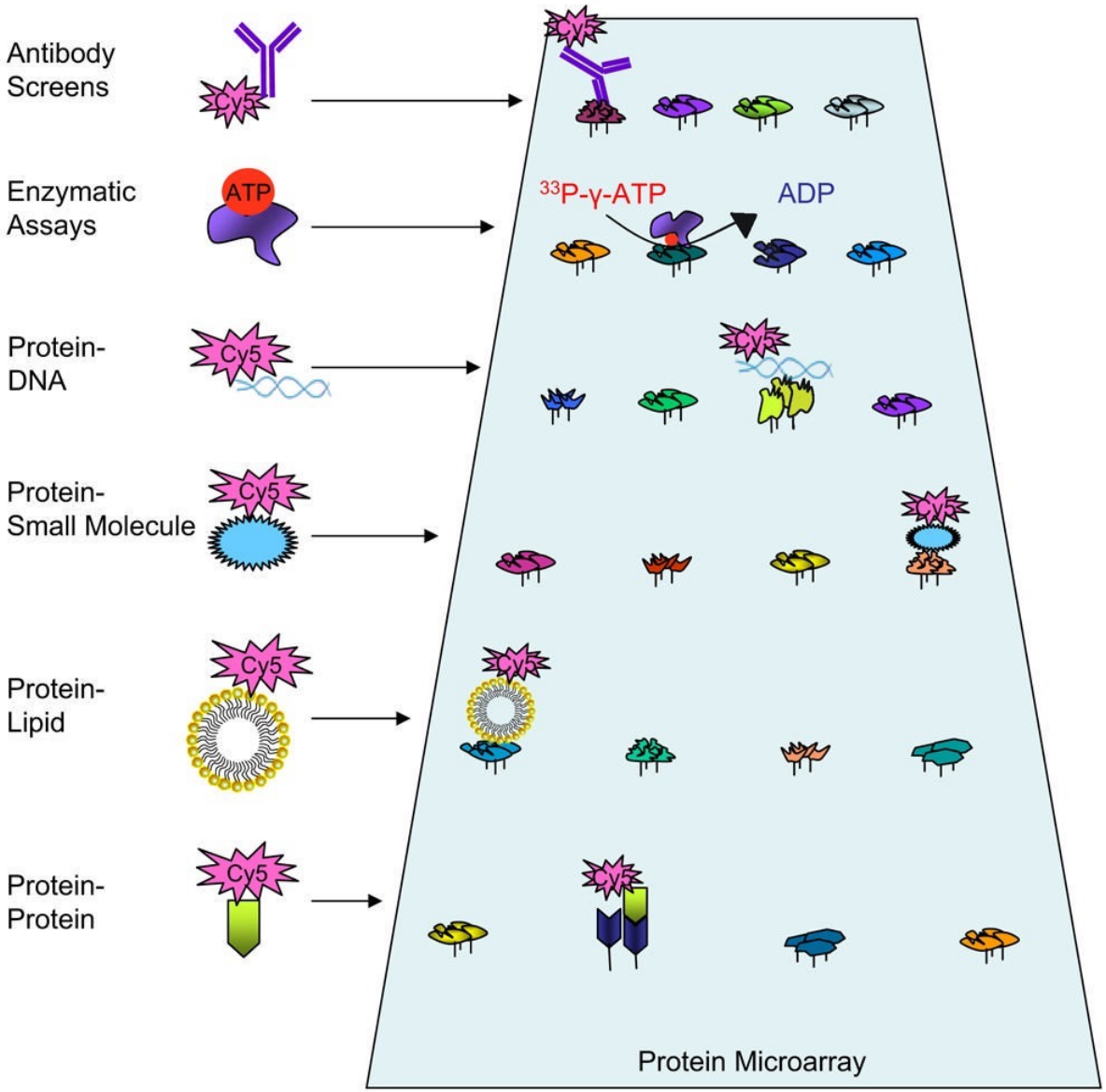
- Metabolites isolated from biological samples .
- Liquid chromatography followed by mass spectrometry (LC/MS).
- Process data (e.g. XCMS) to perform nonlinear retention time alignment.
- Identify changing peaks.
- Use *m/z* values for peaks of interest to search metabolite databases to obtain *putative* identifications.
- Confirm *putative* identifications by comparison to (MS/MS) data & retention time data to standard compounds.

Patti, Yanes & Siuzdak, Nature Reviews Molecular Cell Biology 13, 263-269 (2012)

Protein Interactions

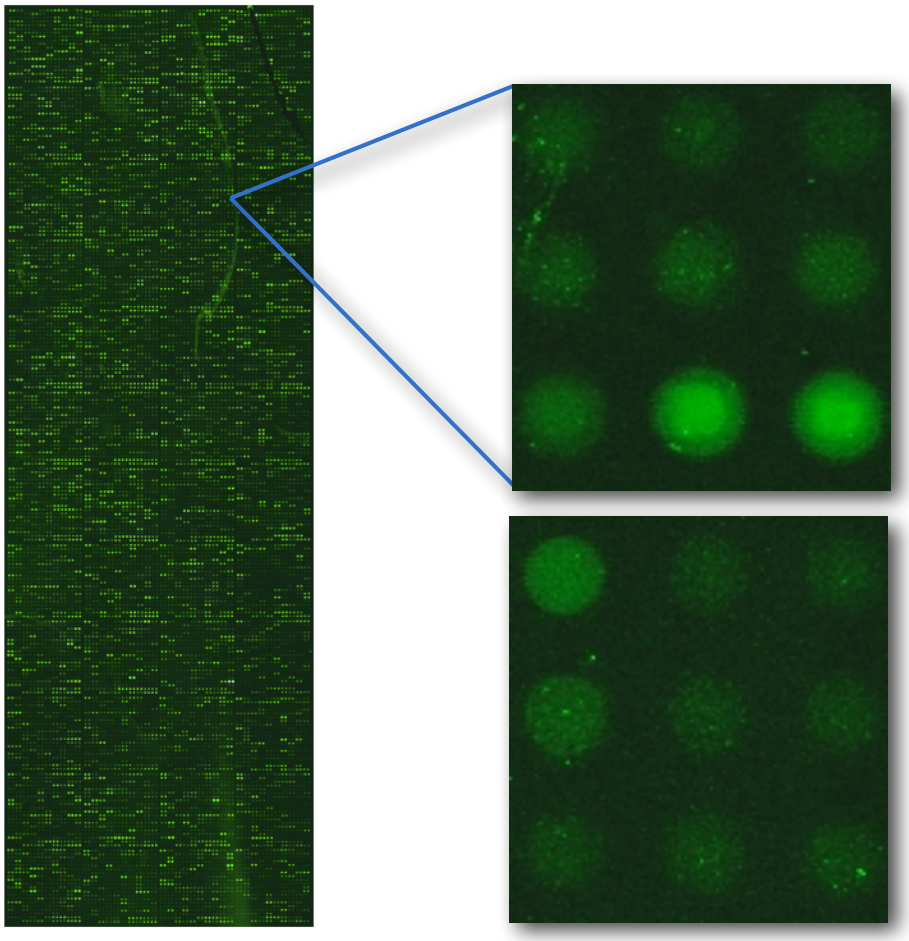
Protein Microarrays

- Different Assays.
- Proteins immobilized at high spatial density onto a microscope slide.
- Many fluorophores may be used for detection.



Hall, Ptacek & Snyder, Mech Ageing Dev. 128(1): 161–167 (2007)

Protein Microarrays



ProtoArrays
(Invitrogen)

9483 unique
human proteins in
duplicate

532 nm channel
example

(also 635 nm used)

Chen*, Mias GI*, Li-Pook-Than*, Jiang* et al Cell 148, 1293–1307 (2012)
Mias, Chen et al. Scientific Reports 3, Article number: 3311 (2013)

Protein Microarrays

- Myelodysplastic Syndrome (MDS)
- Plasma IgG

2 Stage Approach

(I) *Exploratory stage* Multiple patient samples and proteins were tested for Immunoglobulin G (IgG) reactivity,

(II) *Validation stage* using a smaller, high-interest subset of the proteins identified in Stage I based on the retrospective classification, and expanded to a larger cohort.

Mias, Chen et al. Scientific Reports 3, Article number: 3311 (2013)

Protein Microarrays

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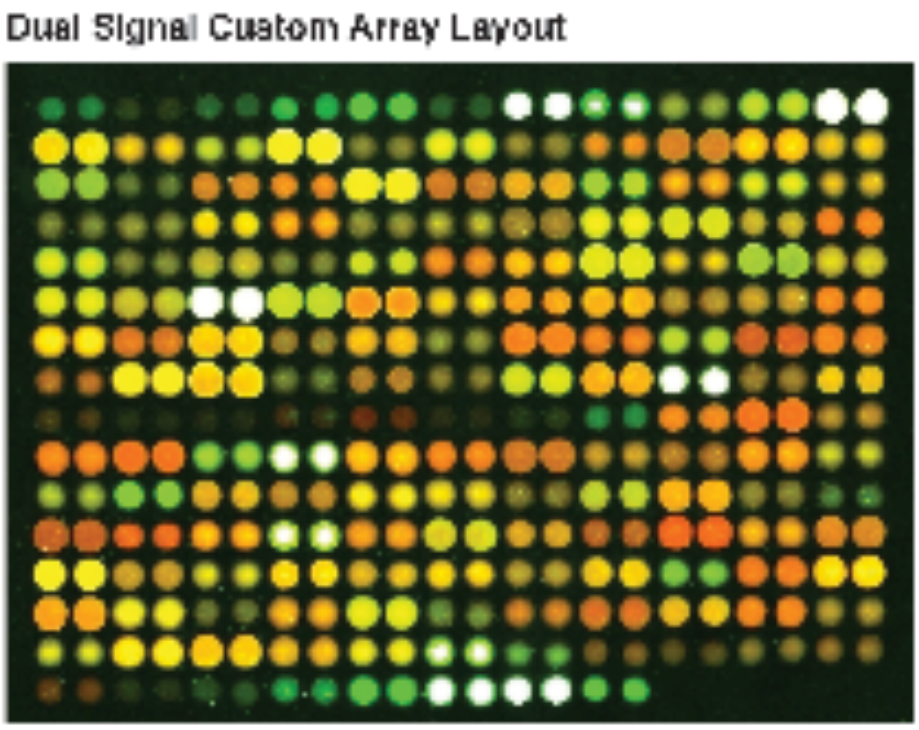
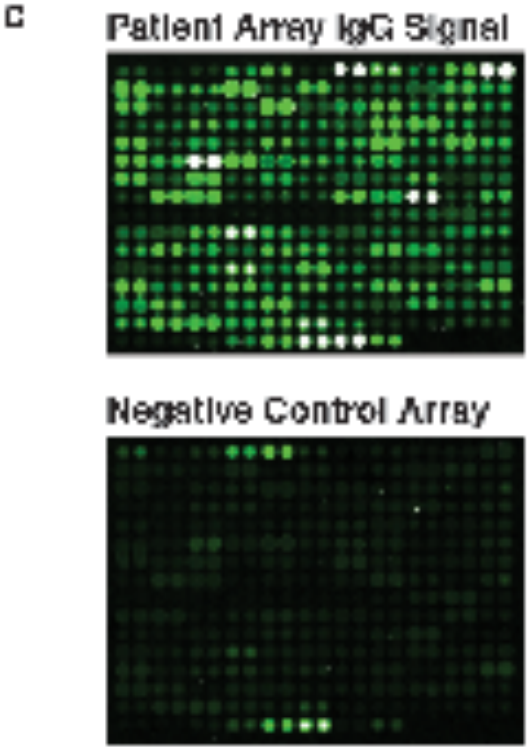
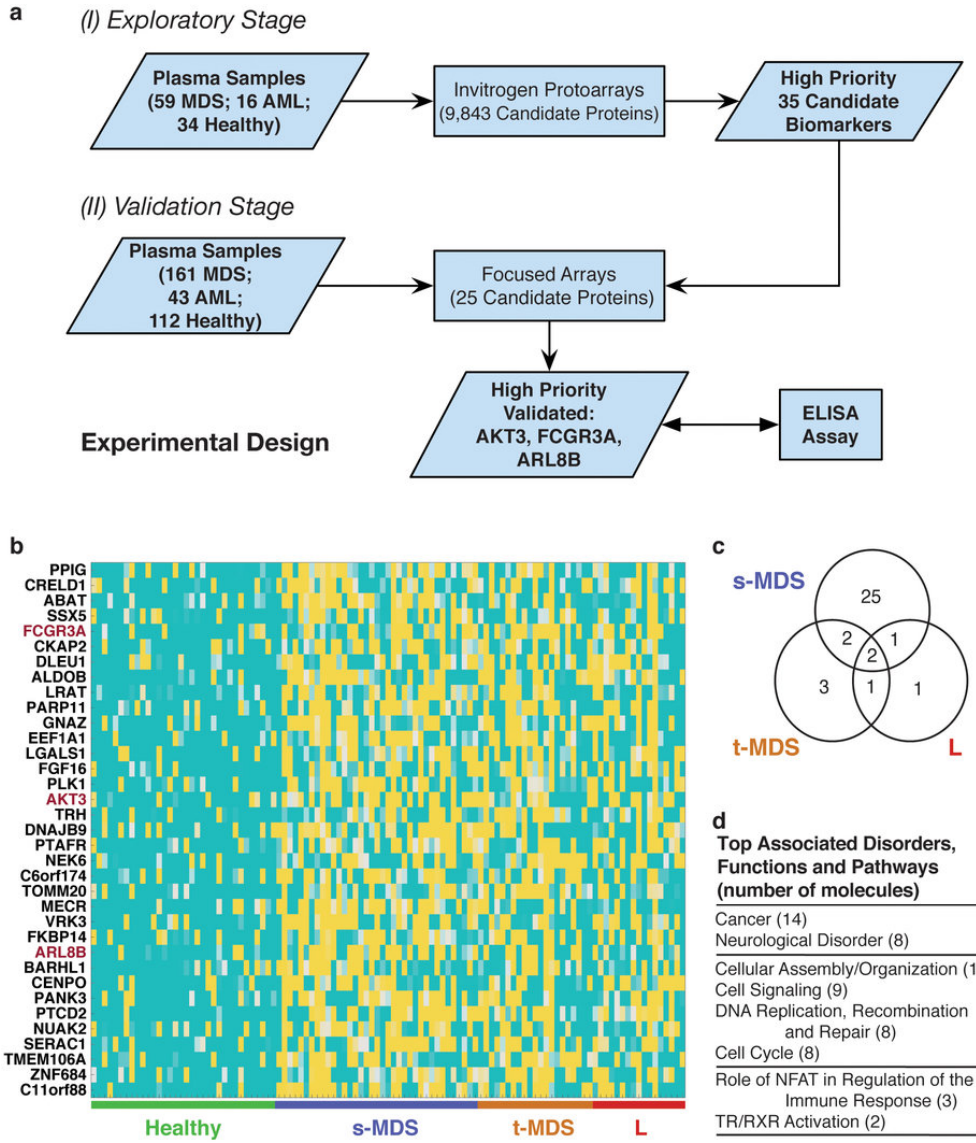




Mias, Chen et al. Scientific Reports 3, Article number: 3311 (2013)

Protein Microarrays

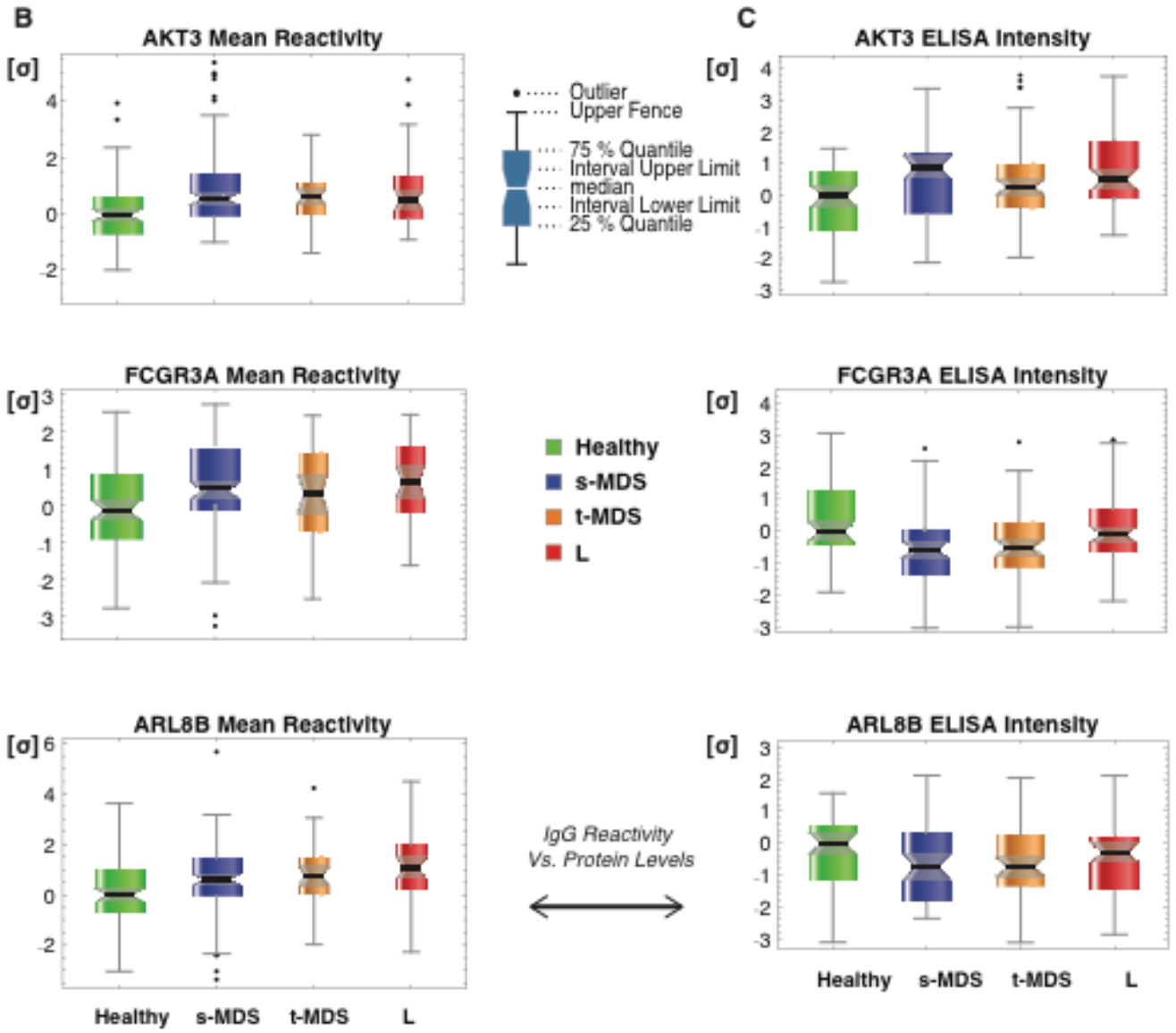
- 3 Proteins Validated
- AKT3
- FCGR3A
- ARL8B



ELISA, Enzyme-linked Immunosorbent Assays

Mias, Chen et al. Scientific Reports 3, Article number: 3311 (2013)

Protein Microarrays



Mias, Chen et al. Scientific Reports 3, Article number: 3311 (2013)

High throughput PPI

technology	brief summary	advantages	disadvantages
protein arrays	generate ORF expression libraries, purify panels of proteins, make array, test binding to labeled query protein	once made, can do lots of tests quick and cheap, binding of compounds other than proteins, too	lots of start-up work, need binding assays, array conditions may not be physiological
Yeast two hybrid method (Y2H) (numerous variants also possible)	interaction between hybrid protein domains required to positively regulate reporter gene expression	test occurs *in vivo* transient ppi detected, high resolution, can use protein domains	hard to scale up, takes place in nucleus, non-physiological, verification often tedious
mass spectrometry of purified protein complexes (AP/MS)	protein complexes purified using affinity tags in recombinant proteins, then analyzed by MS	physiological, modest throughput, detects indirect interactions in complexes	interactions must be stable to purification, weak ones lost, tags could interfere
antibody co-immunoprecipitation	protein complexes purified using specific antibodies, then analyzed by MS or other methods	physiological, detects indirect interactions in complexes	need antibody for each bait, lower throughput, antibody binding site must still be open
co-expression (co-localization, etc.)	measure expression under multiple conditions, assume common expression reflects interaction	fast, lots of data already out there and easy to get more, physiological	basic assumption is tenous, unreliable predictor, requires follow-up
synthetic lethality	mutations in interacting genes are synergistic, e.g., two non-lethal mutations are lethal together	physiological, depending on species can be high throughput	need mutant collection, high start-up effort, not all positives due to ppi

von Merling et al., Nature 417:399-403 (2002), courtesy of Jerry Dodgson