Introduction to Proteomics

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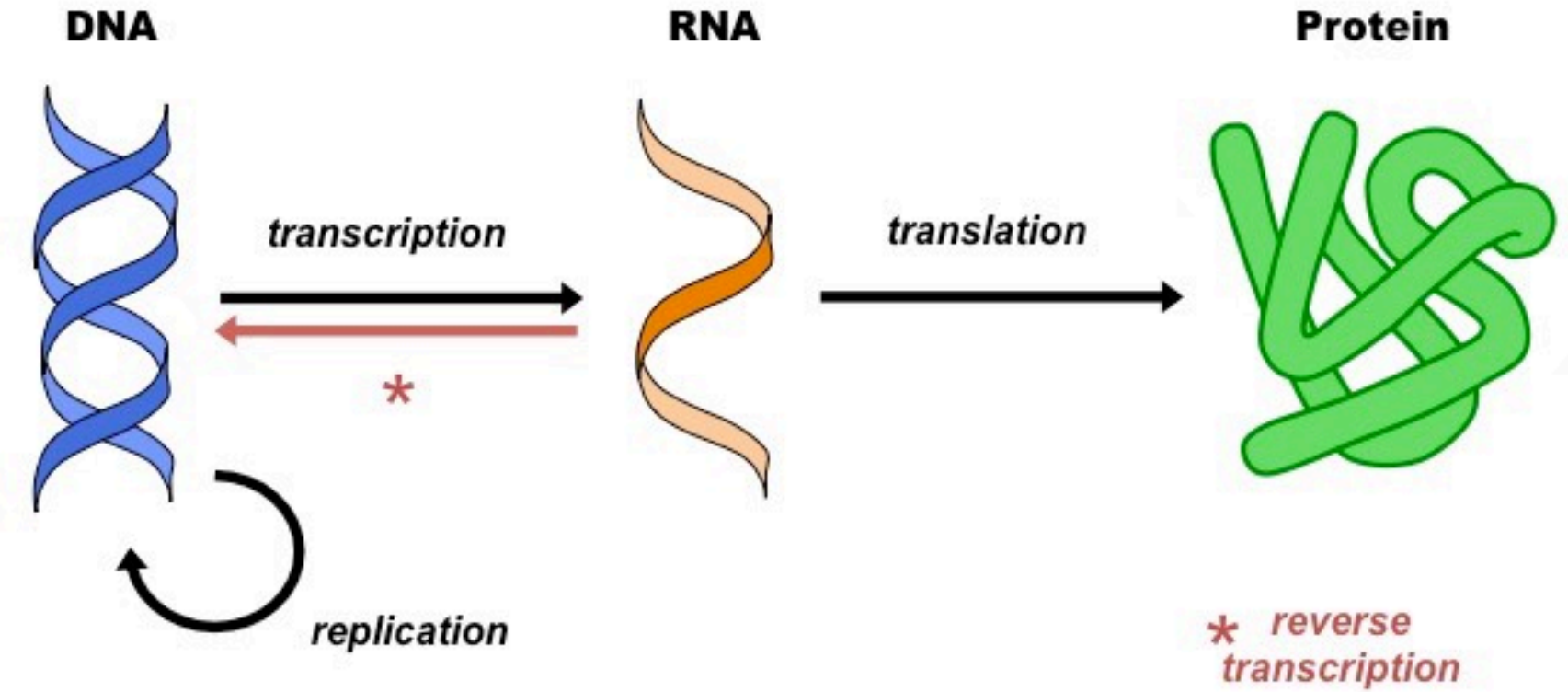
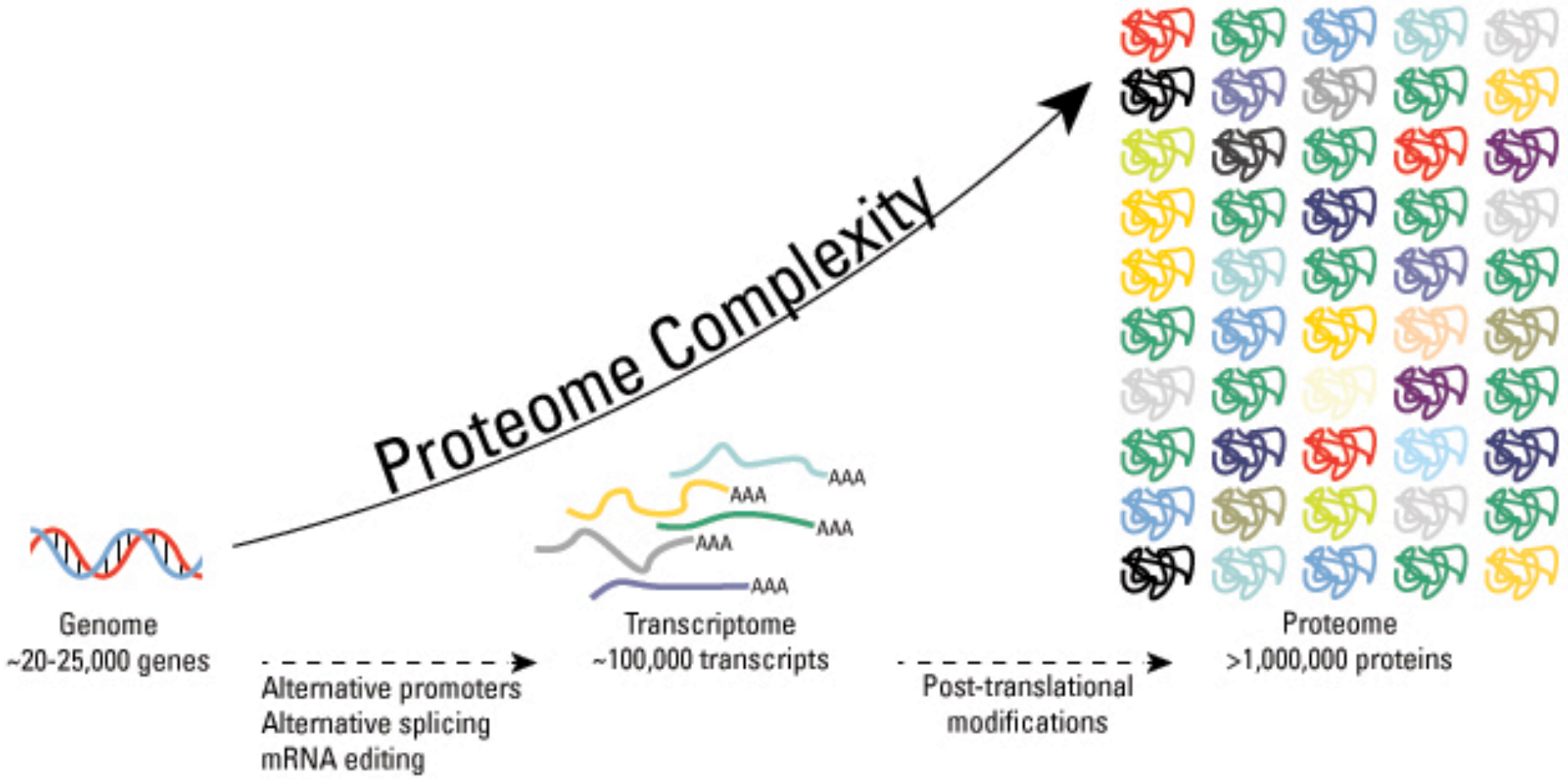
CCBB Short Course
April 25, 2018

Outline

- Introduction
- Protein Separations
- Protein Identification by Mass Spectrometry
- Quantitative Proteomics
- Protein Interactions
- Protein Arrays
- Post-translational Modifications
- Structural Proteomics
- Cellular Localization
- Imaging Mass Spectrometry



http://ib.bioninja.com.au/standard-level/topic-2-molecular-biology/27-dna-replication-transcri/central-dogma.html



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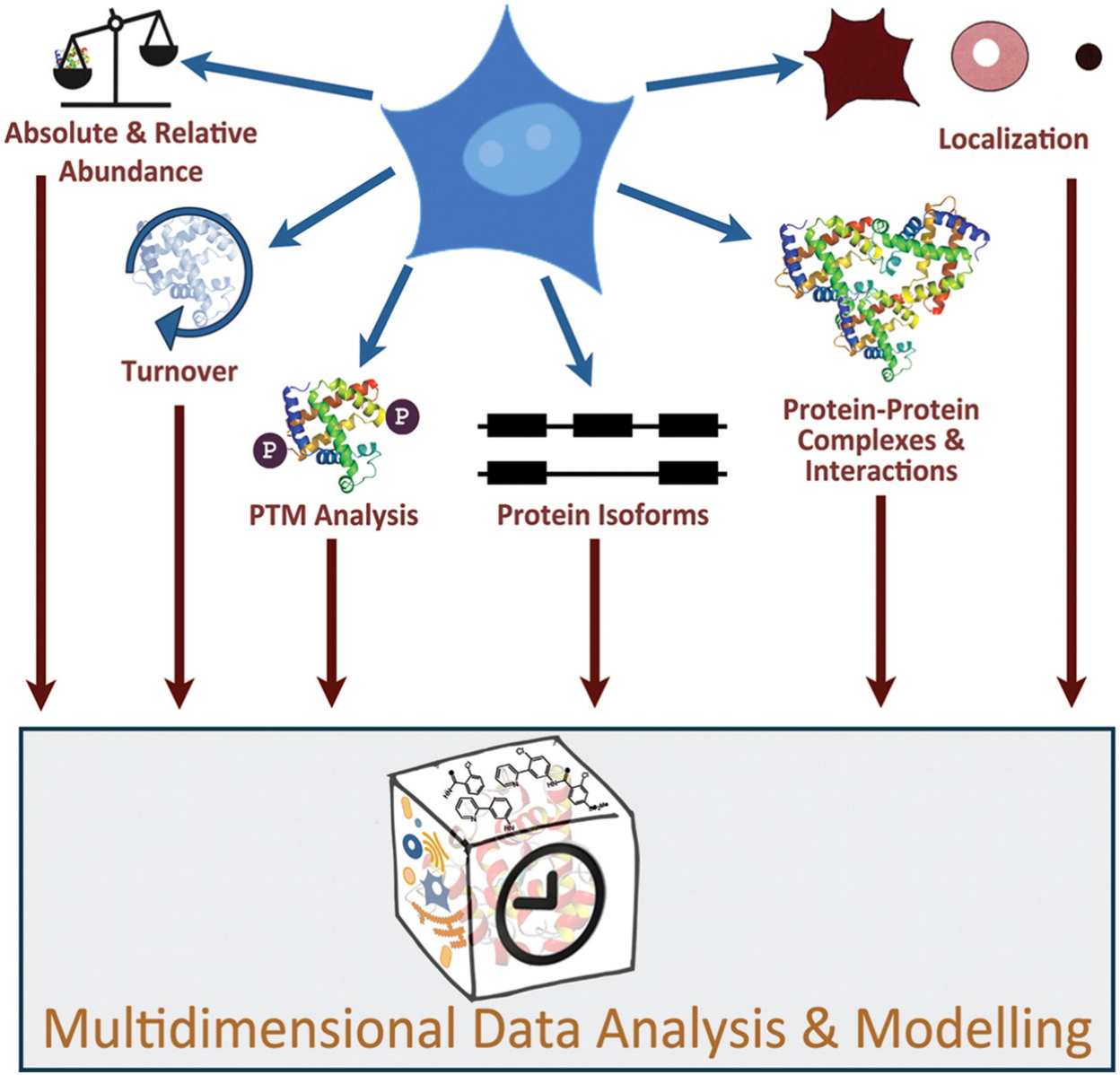
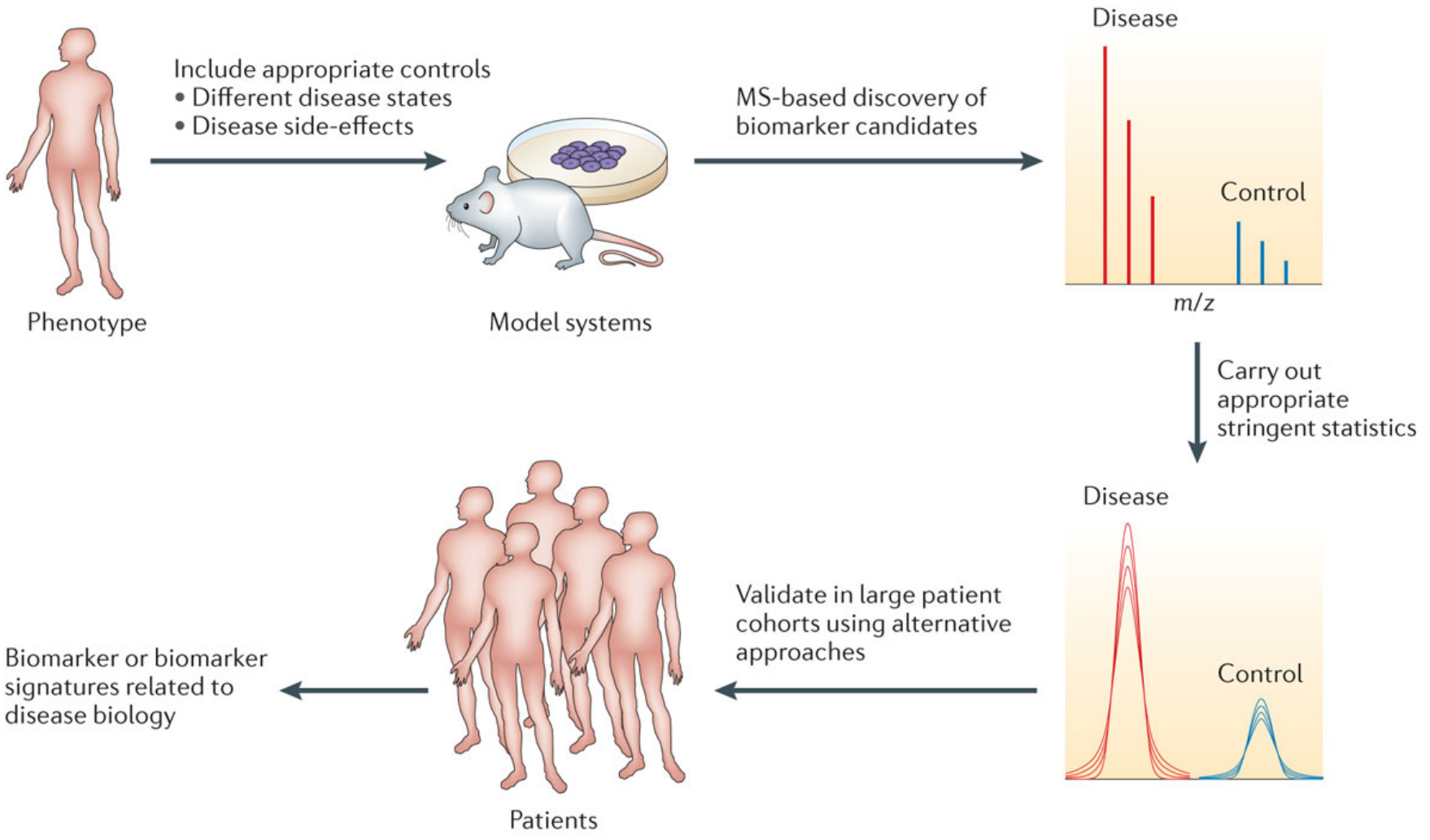
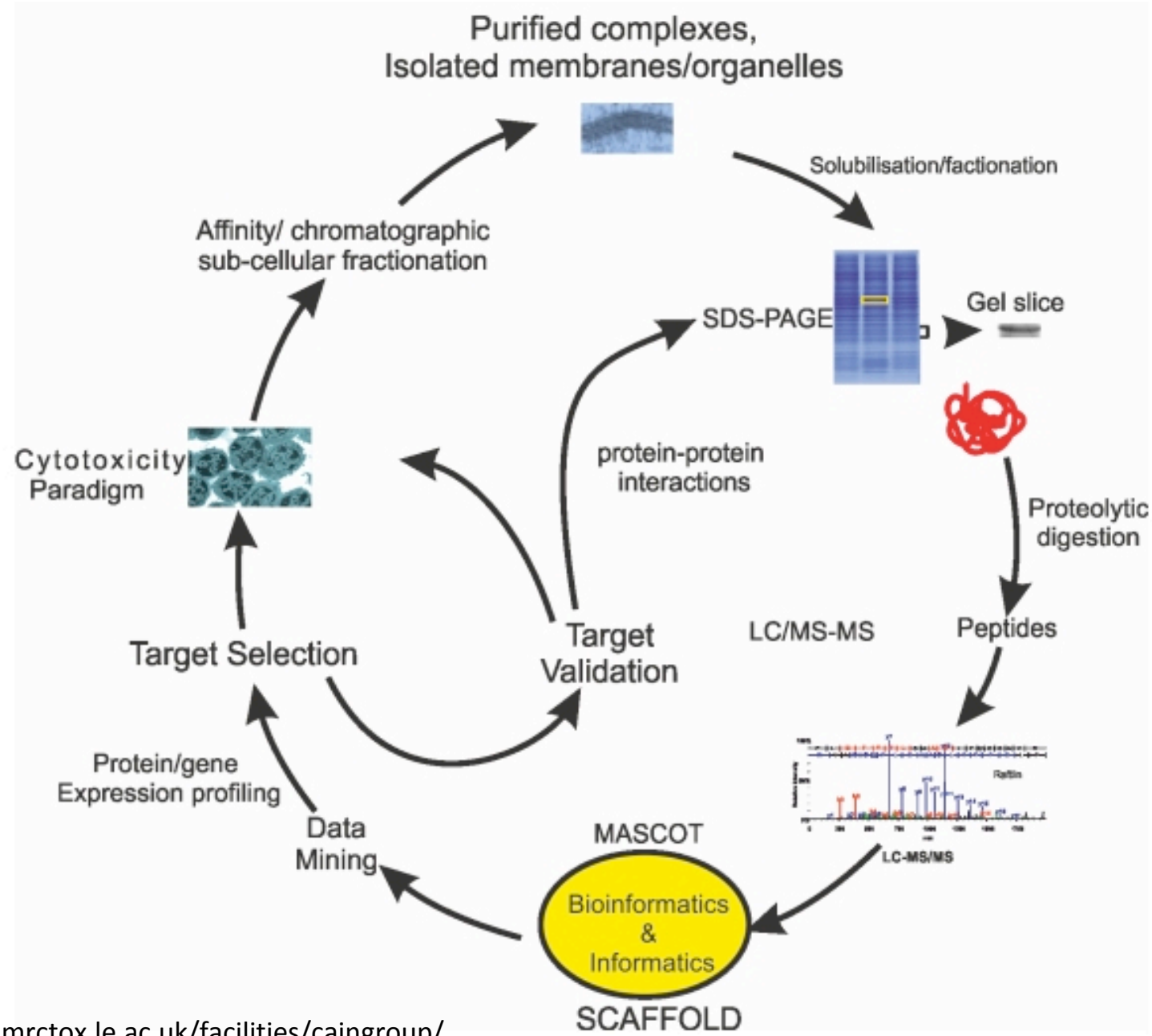


Lamond A I et al. Mol Cell Proteomics 2012;11:O112.017731



Altelaar et al. Nat Rev Genet. 2013 Jan;14(1):35-48. doi: 10.1038/nrg3356.

Workflow to identify new cytotoxicity targets with quantitative proteomics



http://www.mrctox.le.ac.uk/facilities/caingroup/

Proteomics Sample Preparation

Mass spectrometry requires buffer free samples:

- Run a gel, then can use in-gel digest to remove unwanted buffer components
- TCA or acetone precipitation and wash lysate
- Ziptip / membrane centrifugation / dialysis / Sep Pack to remove salts, esp. Na or K or phosphate
- Avoid use of polymers and detergents, i.e. Triton-X, NP-40, SDS, glycerol; use urea and mass spec friendly detergents instead or remove with Pierce detergent removal kit
- separate and purify components—HPLC

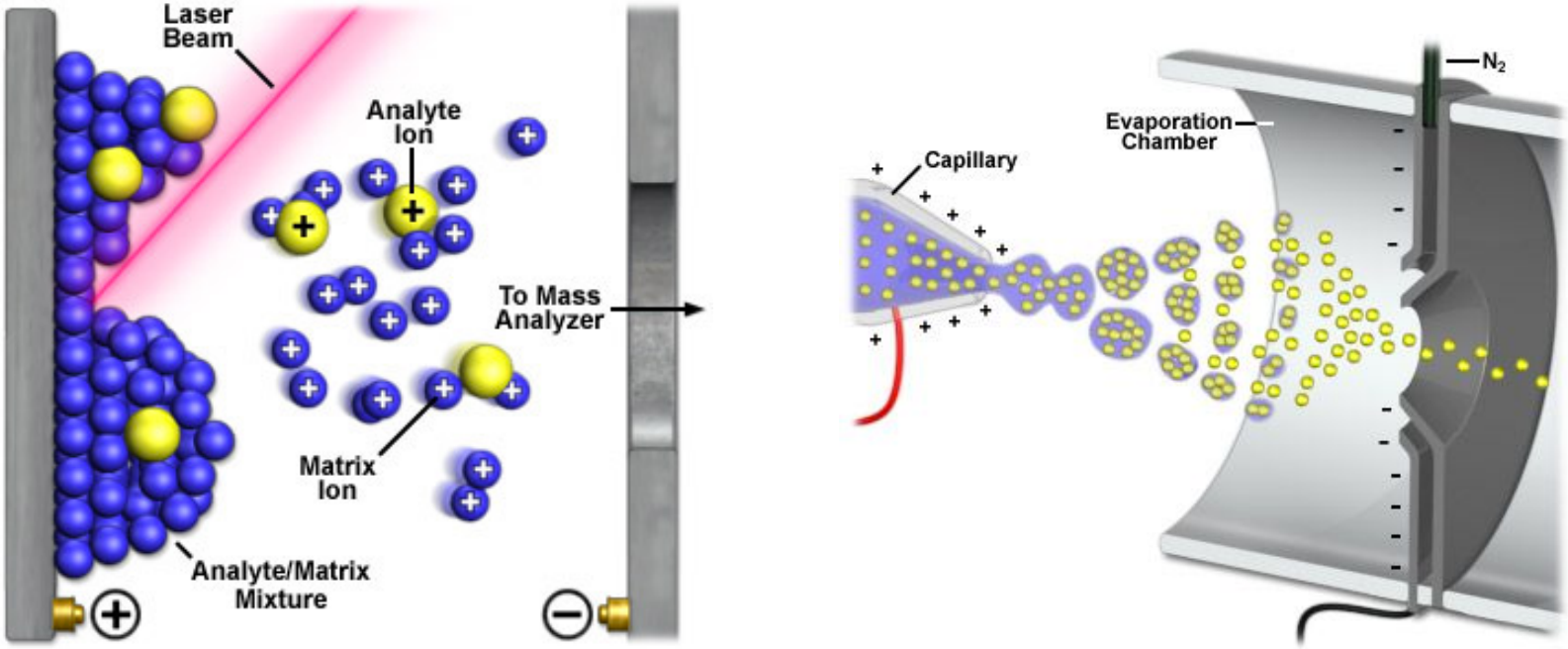
Separation of proteins and peptides

- Immobilized: gel electrophoresis, isoelectric focusing
- Ultra High Pressure Liquid chromatography: better/faster separation achieved at high P
- Gel filtration/Size Exclusion (SEC), Ion exchange Chromatography (IEX) Reversed phase (RP), HILIC
- Affinity chromatography

Methods combined for 2D separation: MudPIT (SCX-RPLC of peptides) high pH/low pH RP/RP, 2DGE, GeLC (1D gel protein RPLC peptides)

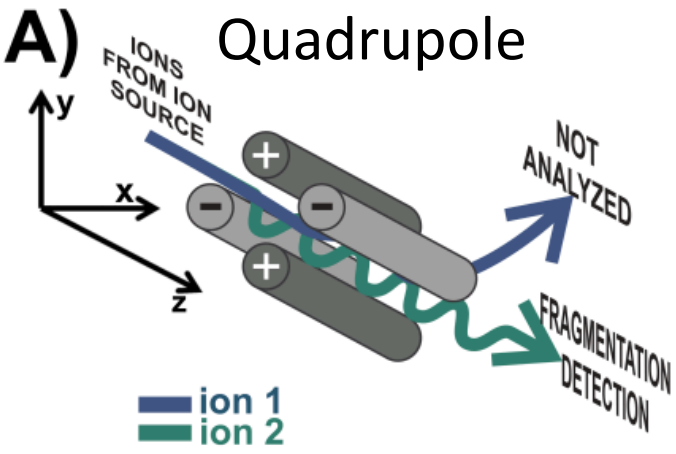
Mass Spectrometry

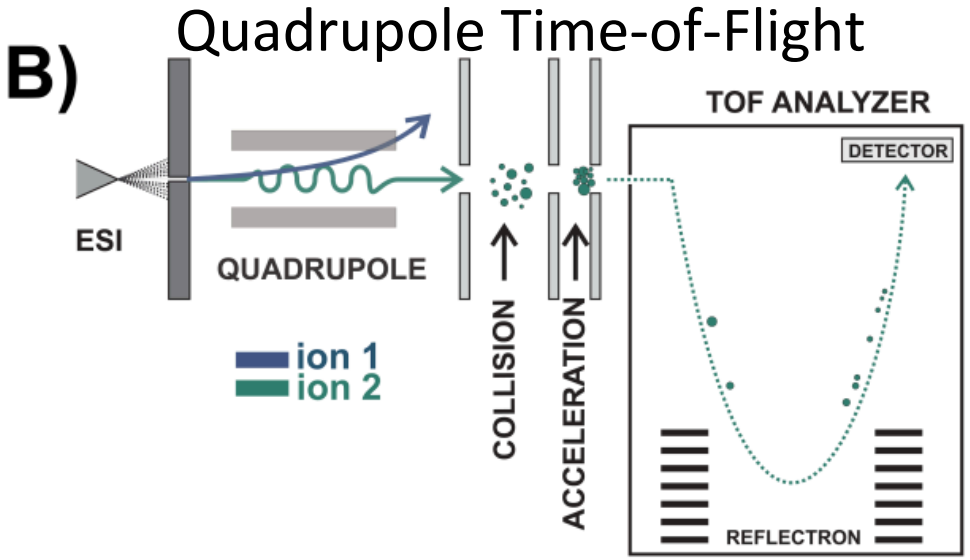
- Mass spectrometry is a widely used analytical tool for measuring the mass/charge (*m/z*) ratio of ions in the gas phase
- The mass spectrometer consists of an ionization source, mass filter and ion detector
- While MS originated 100 years ago, development of MALDI and ESI ionization sources made it possible to detect peptides and proteins

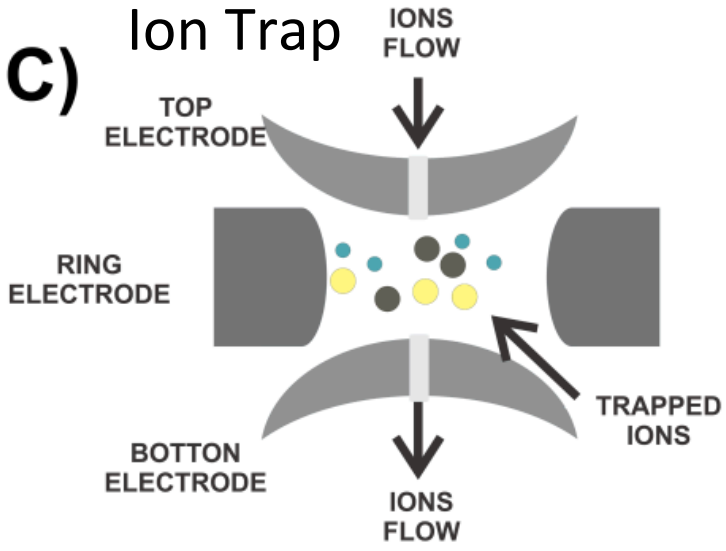


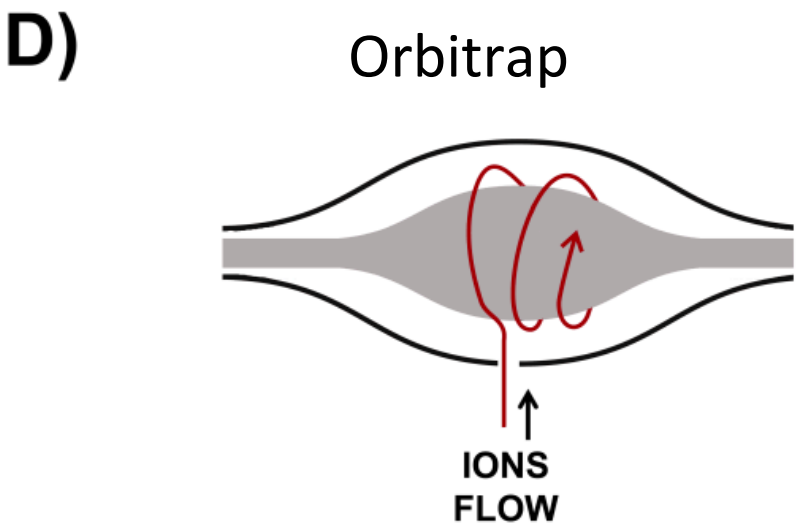
https://nationalmaglab.org/user-facilities/icr/techniques/maldi

Common Types of Mass Filters



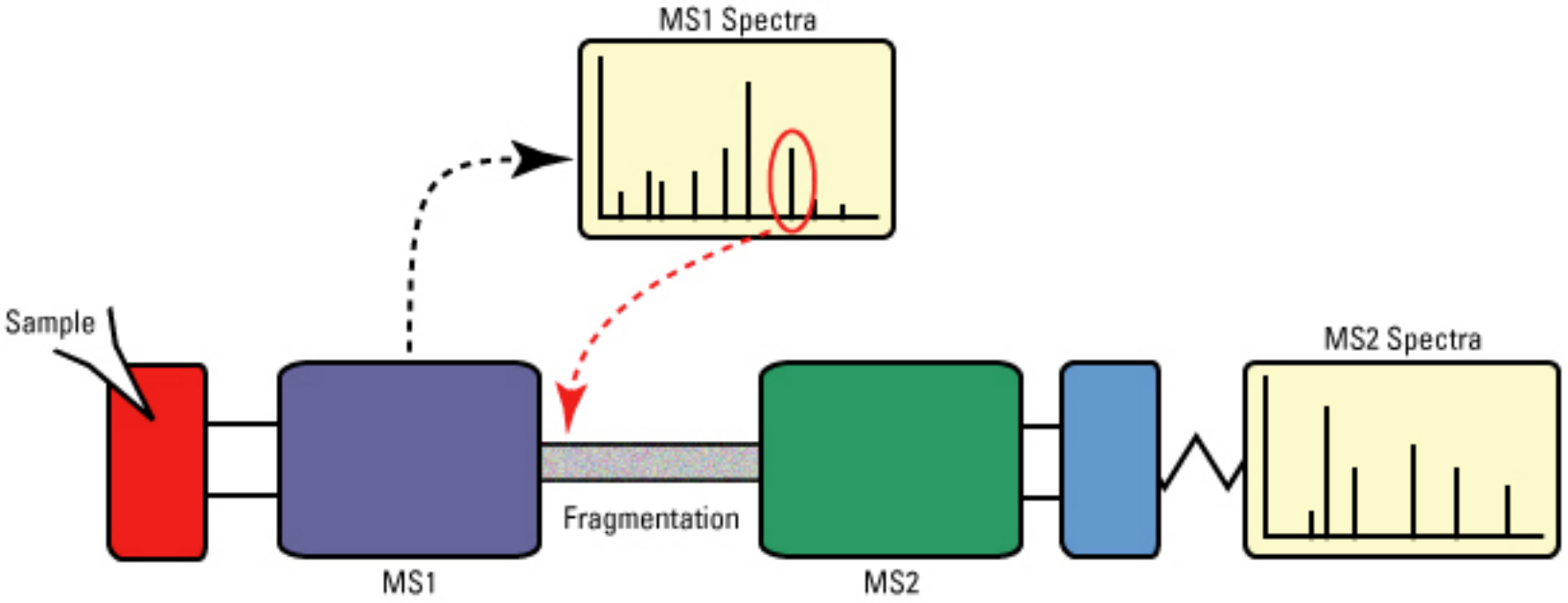






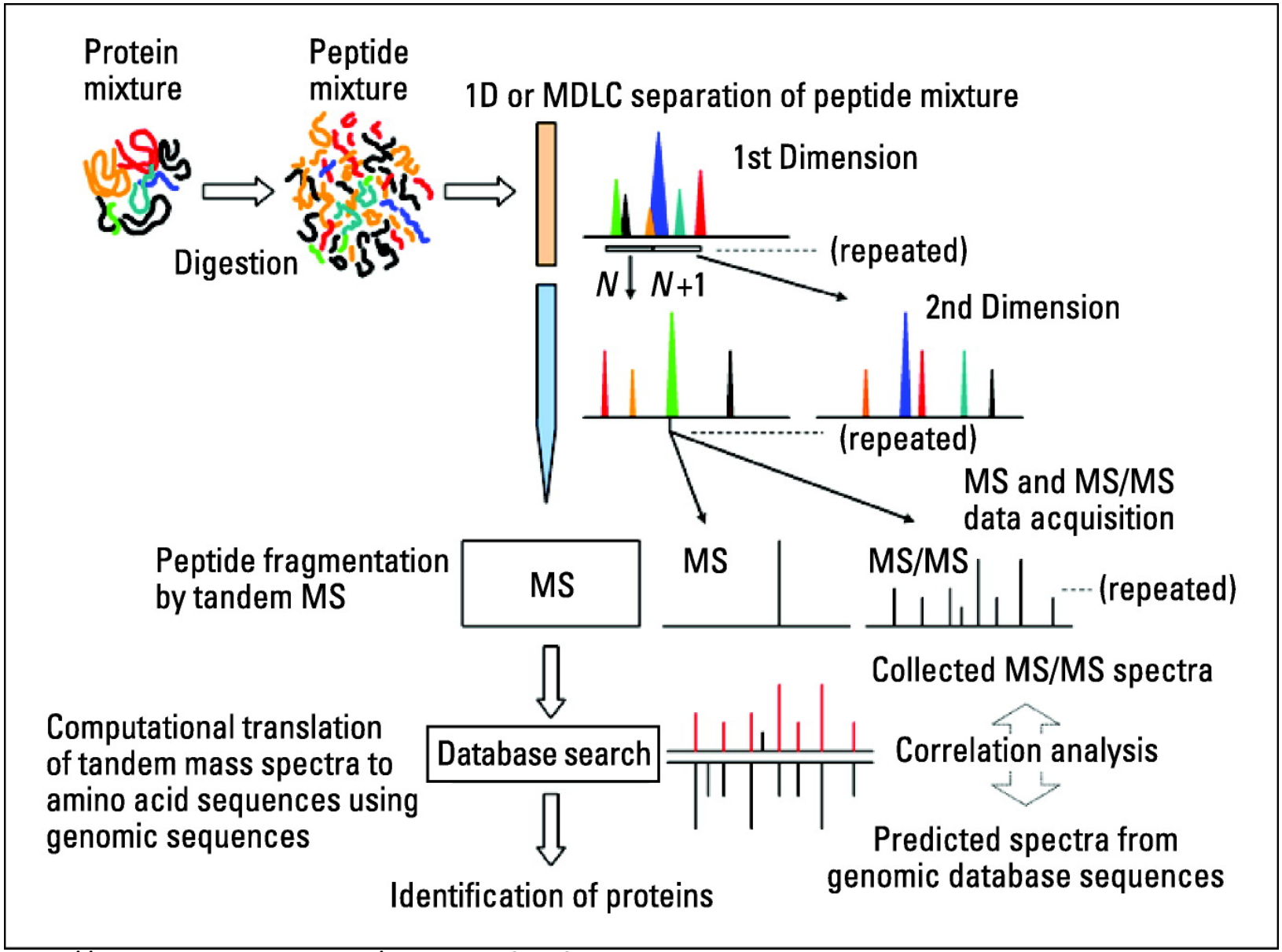
A Short Overview of the Components in Mass Spectrometry Instrumentation for Proteomics Analyses *By Diogo Ribeiro Demartini DOI: 10.5772/54484*

Tandem Mass Spectrometry



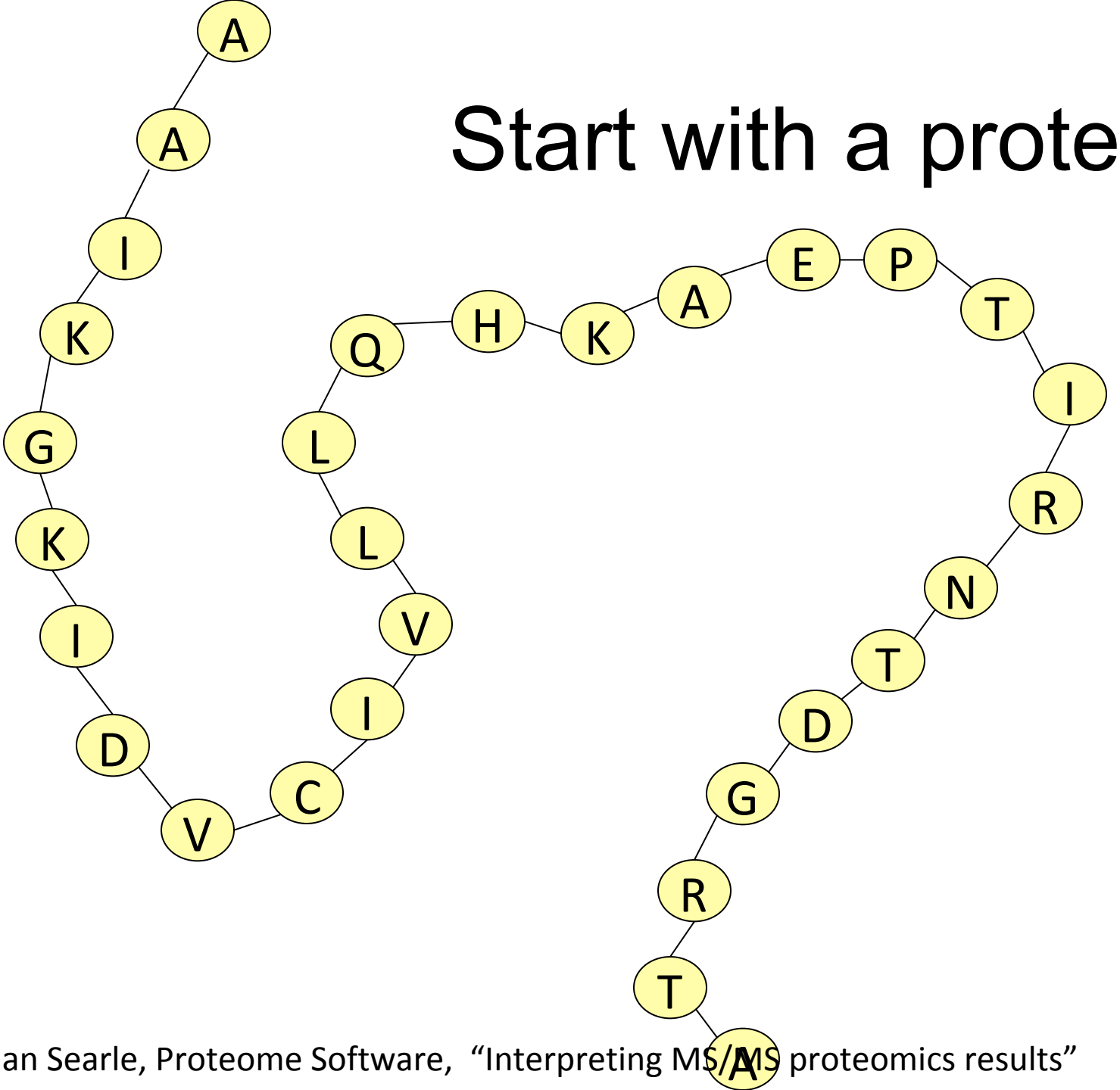
Proteomics Facility has two high sensitivity, high resolution Orbitrap Fusion mass spectrometers

Protein Identification Process



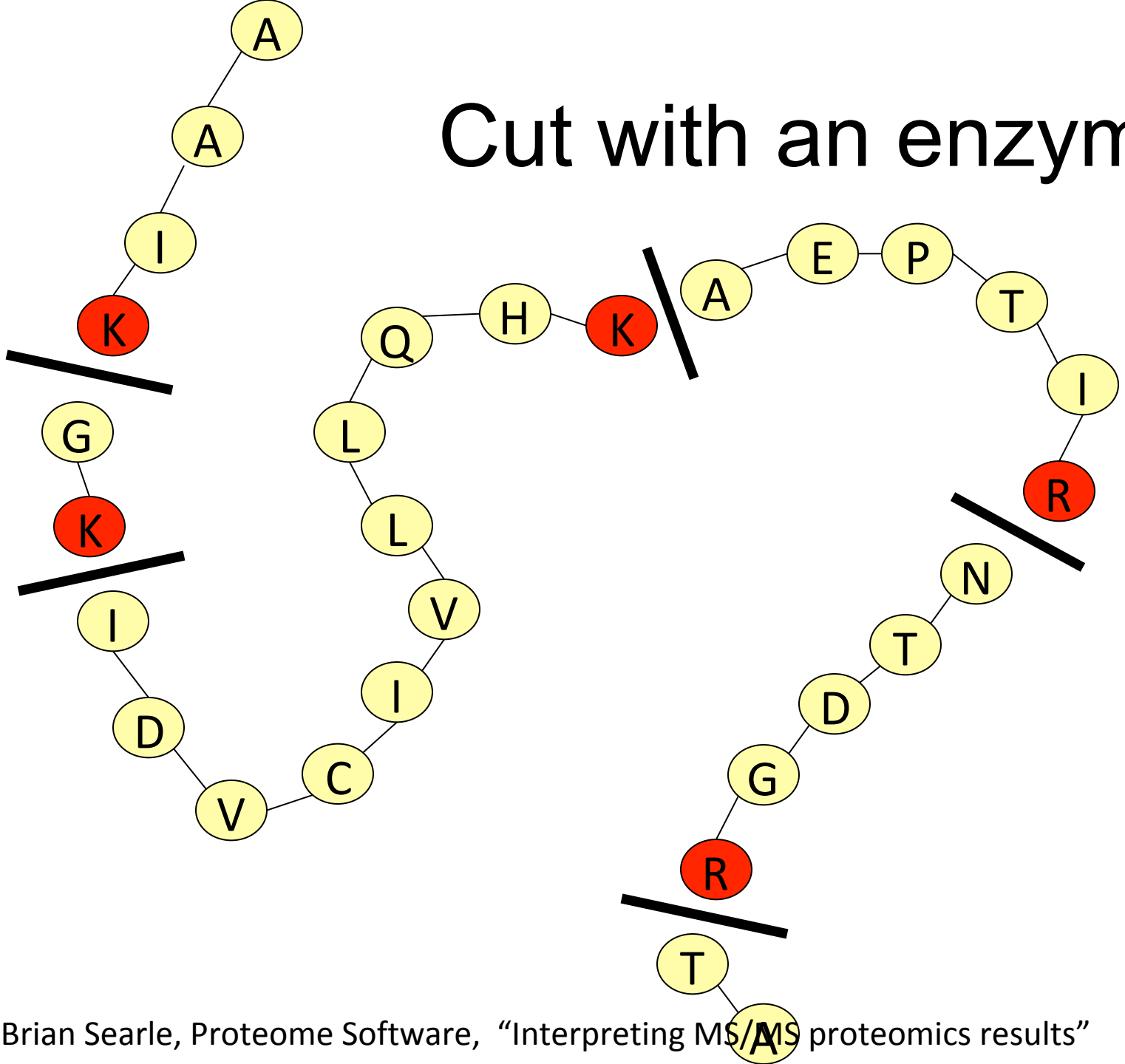
http://www.piercenet.com/browse.cfm?fldID=7CE3FCF5-0DA0-4378-A513-2E35E5E3B49B

Start with a protein



Brian Searle, Proteome Software, "Interpreting MS/MS proteomics results"

Cut with an enzyme



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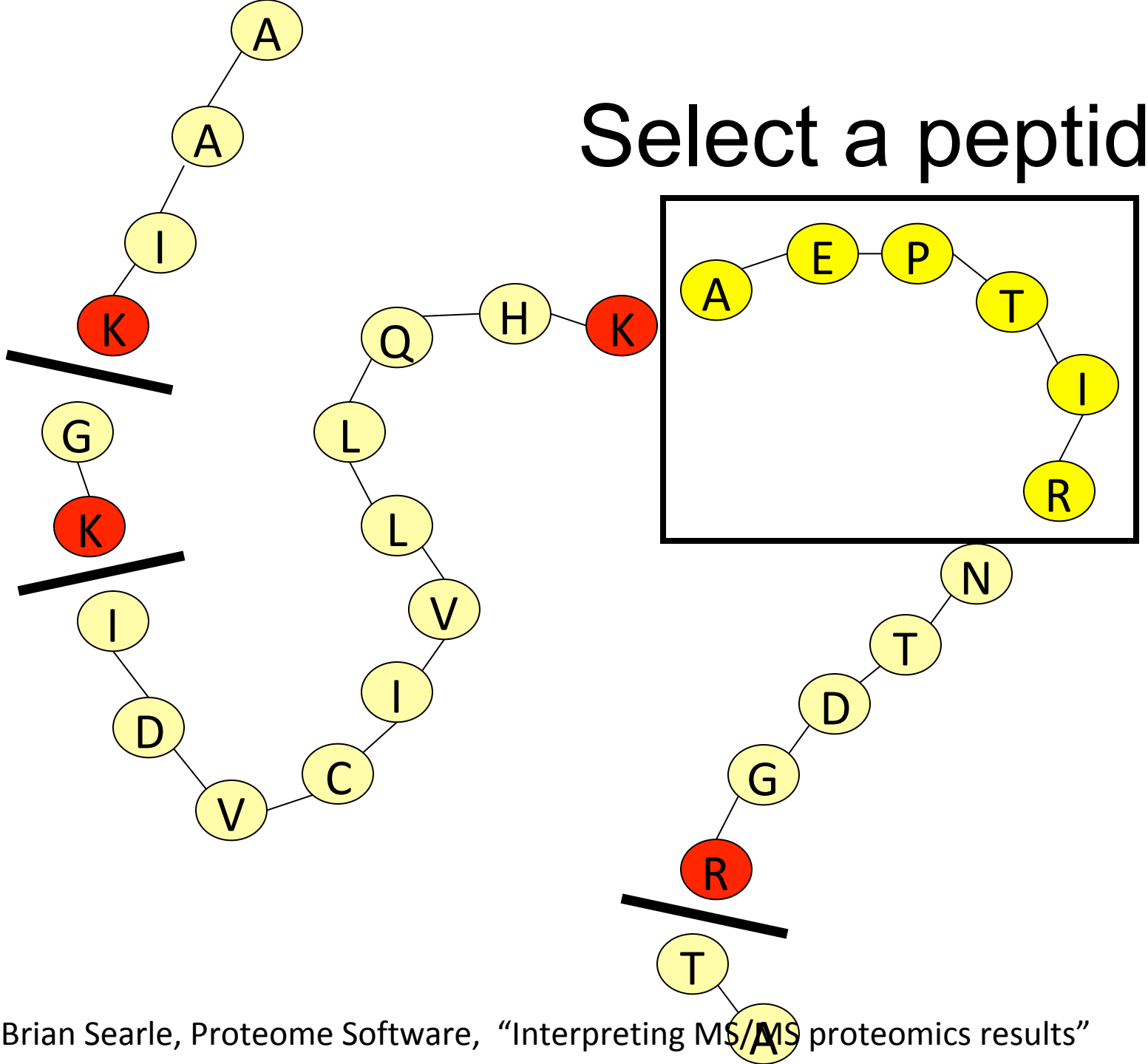
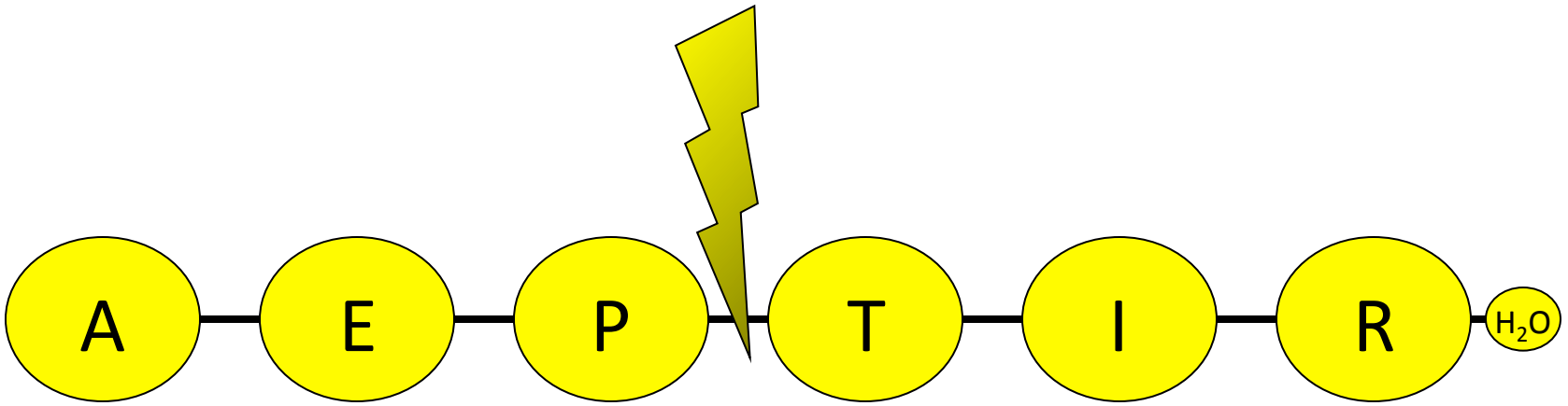


Brian Searle, Proteome Software, "Interpreting MS/MS proteomics results"

Impart energy in collision cell



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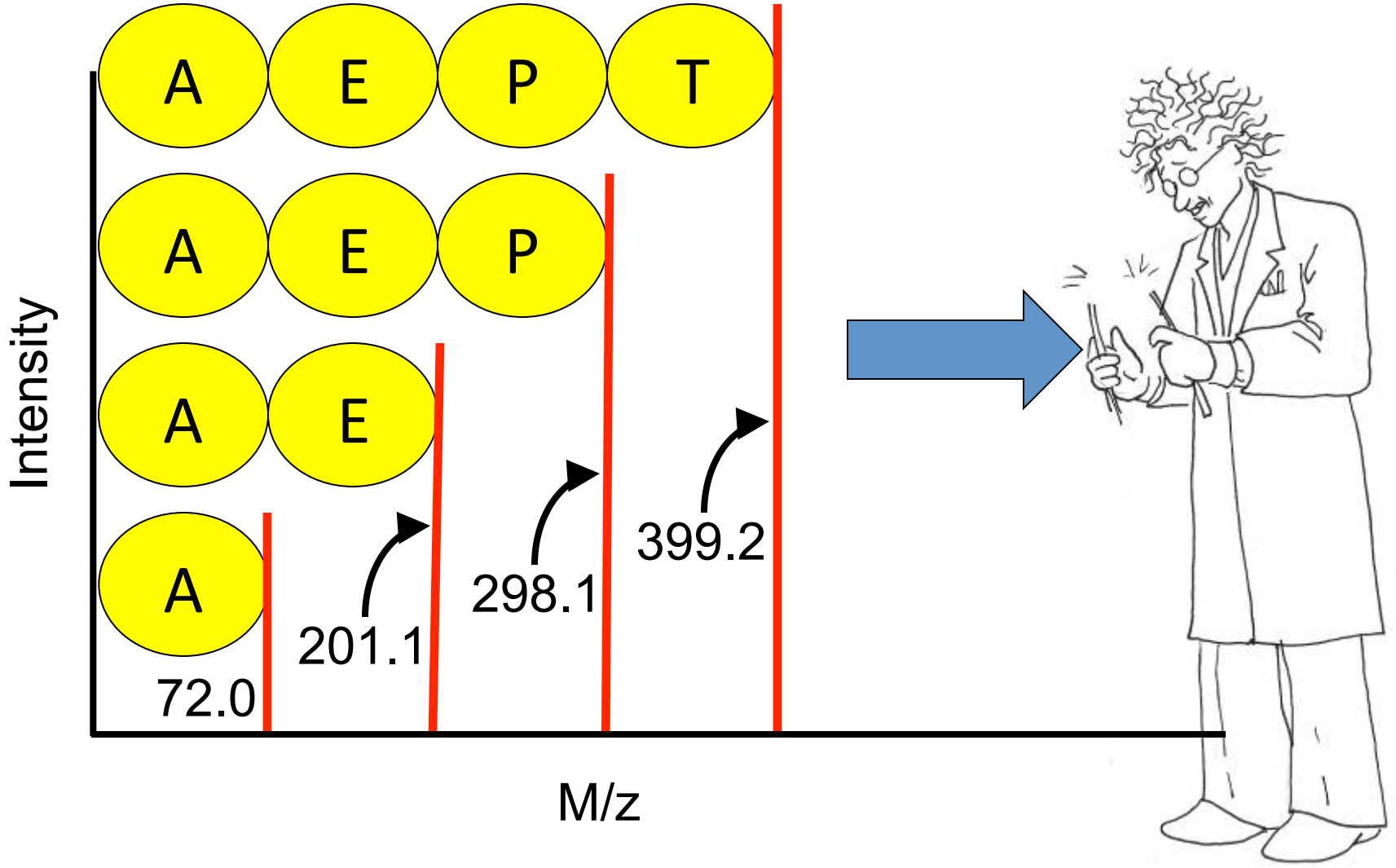
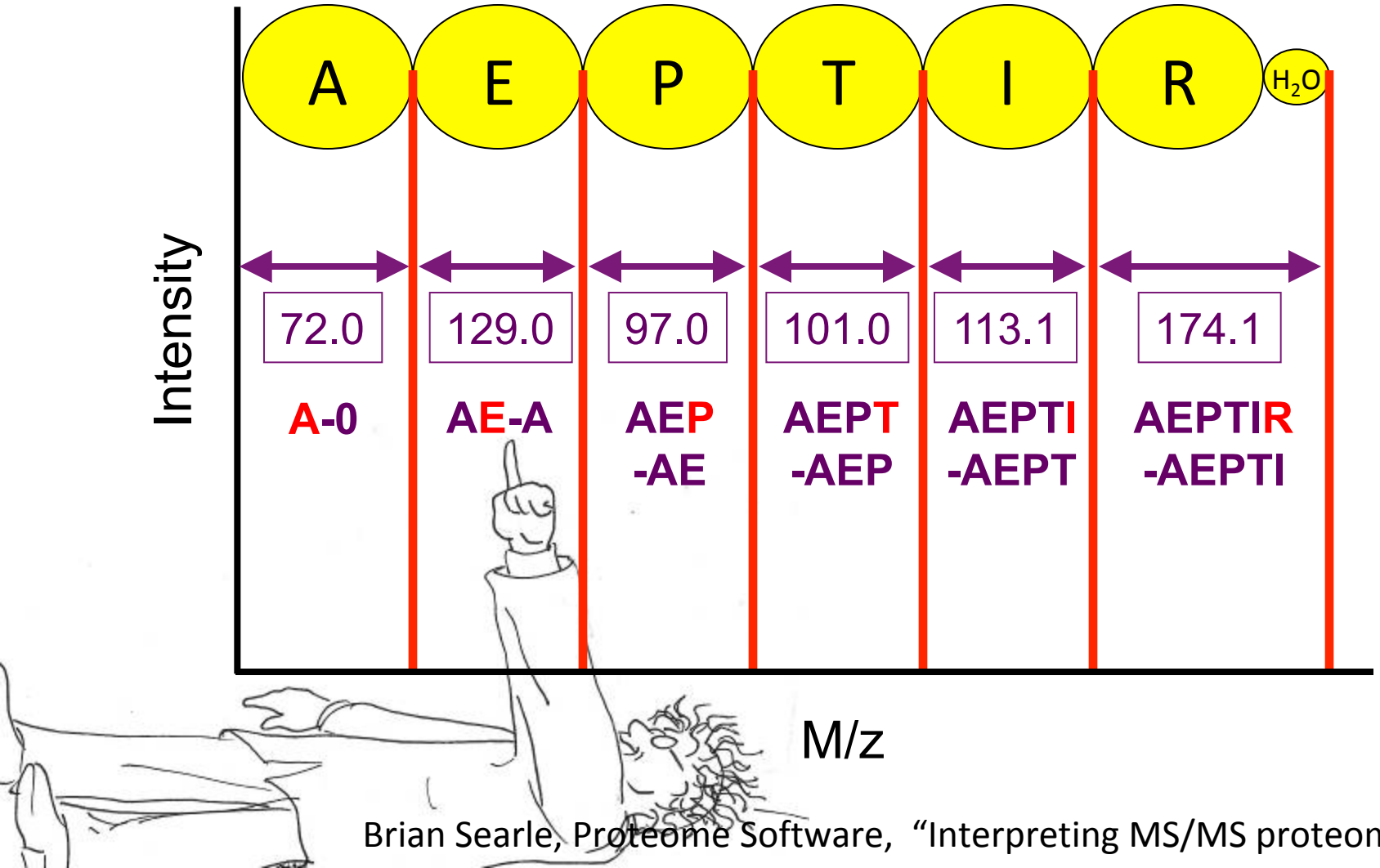
Measure mass of product ions



Brian Searle, Proteome Software, "MS/MS based peptide identification by database searching"

B-type Ions



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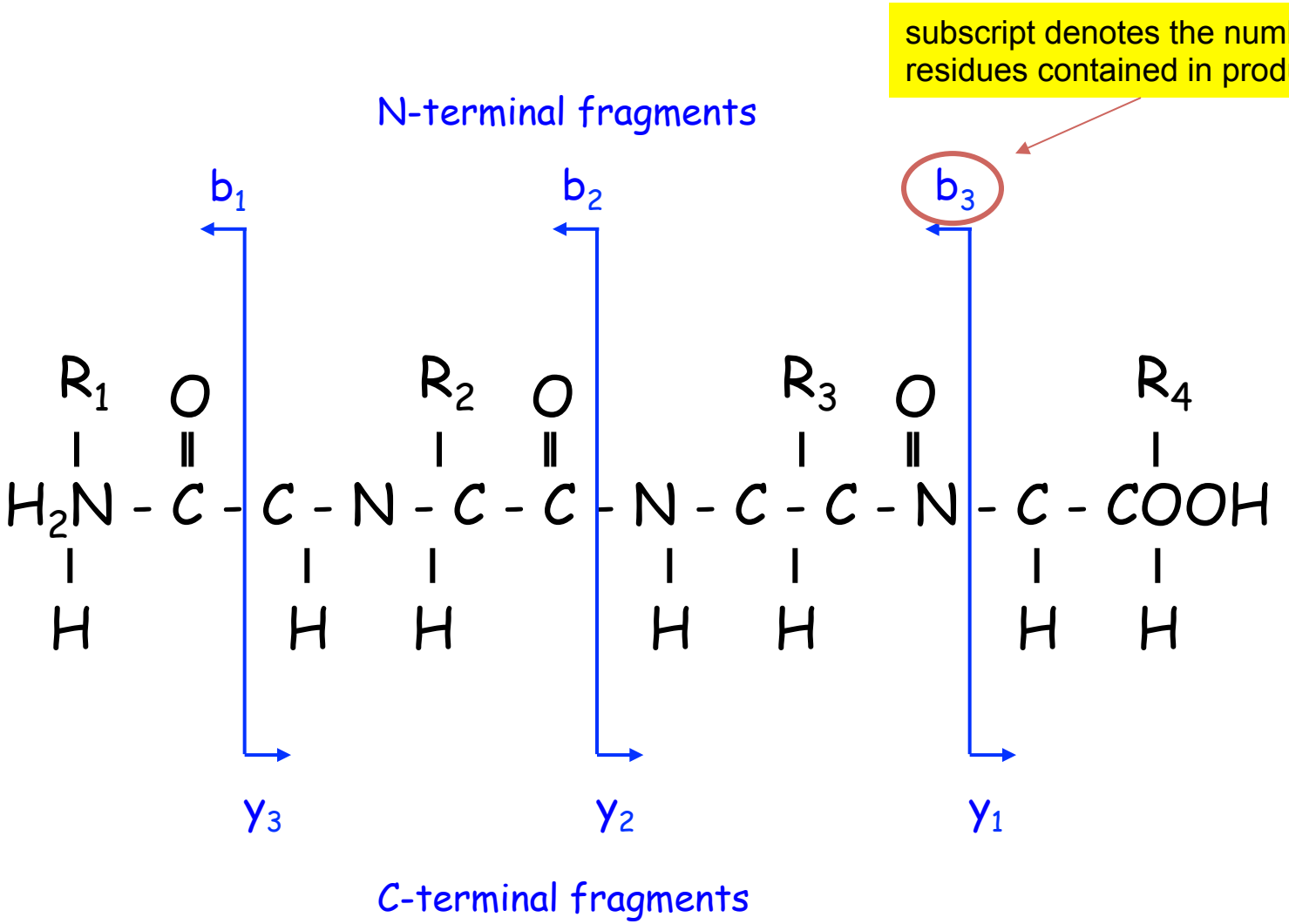
Nomenclature for MS Sequencing of Peptides



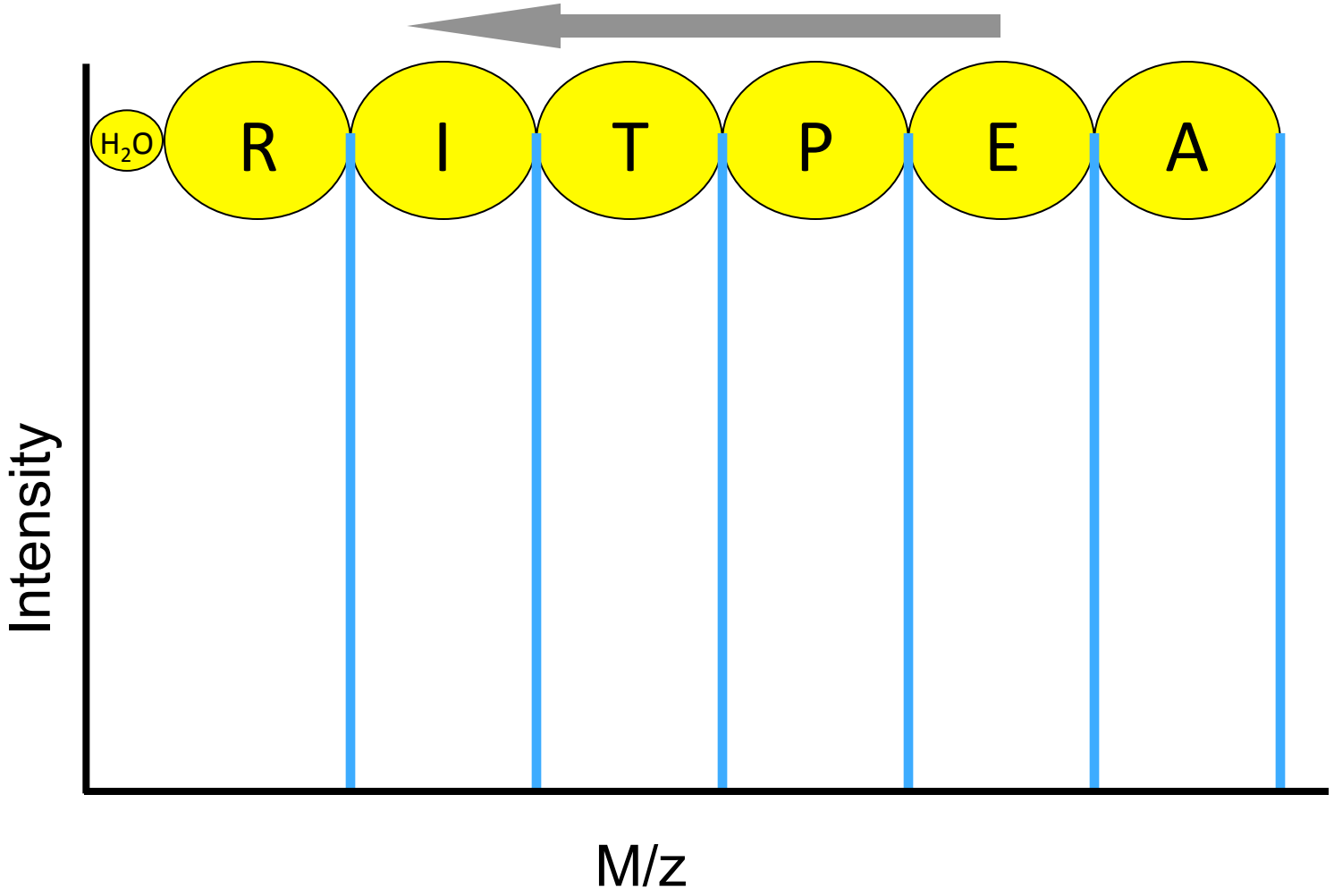
Joseph A. Loo, "*Mass Spectrometry for Protein Quantification and Identification of Posttranslational Modifications*"

Amino Acid Residue Masses.

Amino Acid	*3 Letter Code*	*1 Letter Code*	*Residue Mass*	
			Monoisotopic	Average
Glycine	Gly	G	57.02147	57.052
Alanine	Ala	A	71.03712	71.079
Serine	Ser	S	87.03203	87.078
Proline	Pro	P	97.05277	97.117
Valine	Val	V	99.06842	99.133
Threonine	Thr	T	101.04768	101.105
Cysteine	Cys	C	103.00919	103.144
Isoleucine	Ile	I	113.08407	113.160
Leucine	Leu	L	113.08407	113.160
Asparagine	Asn	N	114.04293	114.104
Aspartic Acid	Asp	D	115.02695	115.089
Glutamine	Gln	Q	128.05858	128.131
Lysine	Lys	K	128.09497	128.174
Glutamic Acid	Glu	E	129.04260	129.116
Methionine	Met	M	131.04049	131.198
Histidine	His	H	137.05891	137.142
Phenylalanine	Phe	F	147.06842	147.177
Arginine	Arg	R	156.10112	156.188
Tyrosine	Tyr	Y	163.06333	163.170
Tryptophan	Try	W	186.07932	186.213
Homoserine Lactone			83.03712	83.090
Homoserine			101.04768	101.105
Pyroglutamic acid			111.03203	111.100
Carboxyamidomethyl Cysteine			160.03065	160.197
Carboxymethylcysteine			161.01466	161.181
Pyridylethylcysteine			208.06703	208.284

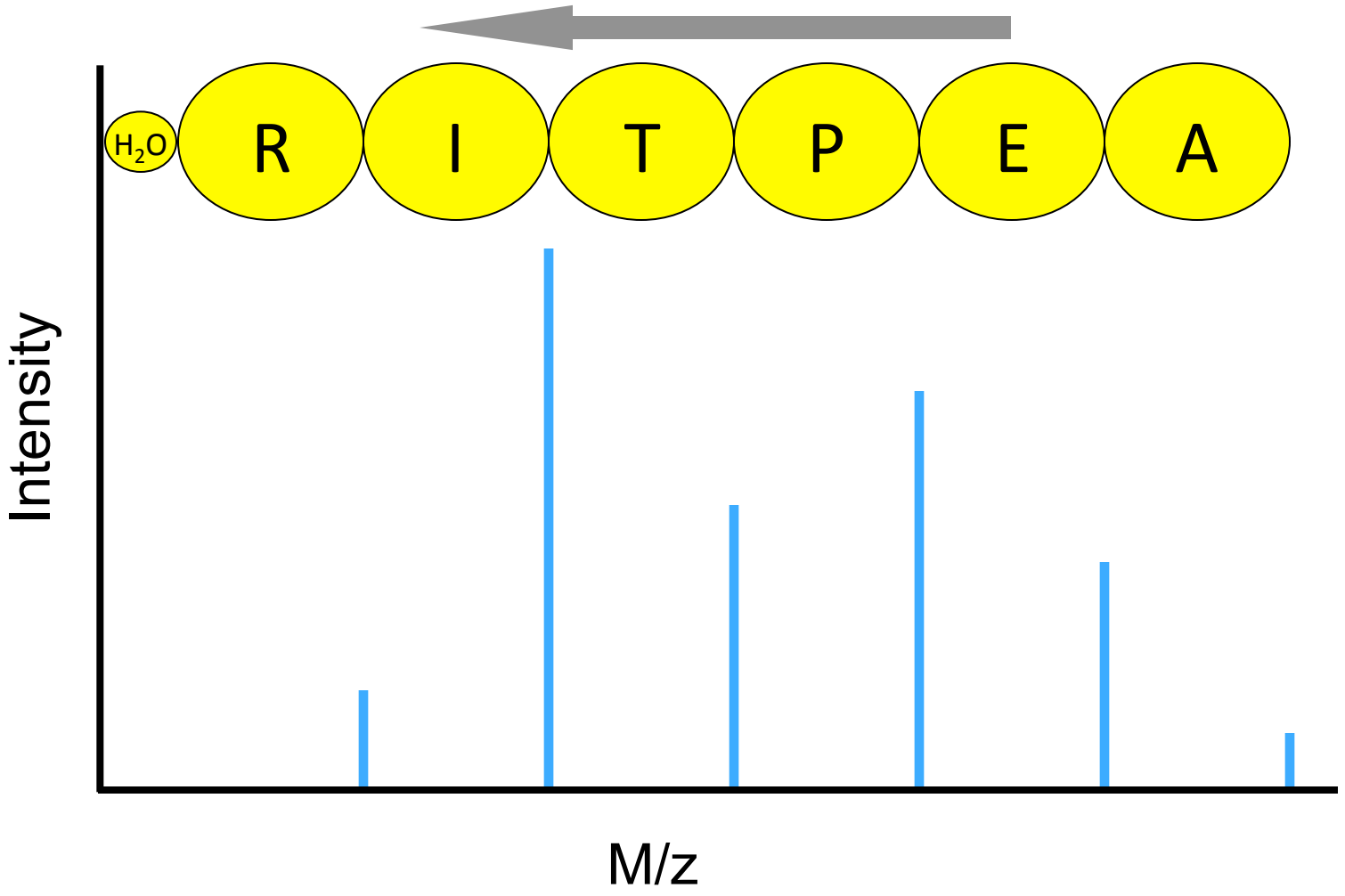
http://www.its.caltech.edu/~ppmal/sample_prep/work3.html

Y-type Ions



Brian Searle, Proteome Software, "Interpreting MS/MS proteomics results"

Y-type Ions



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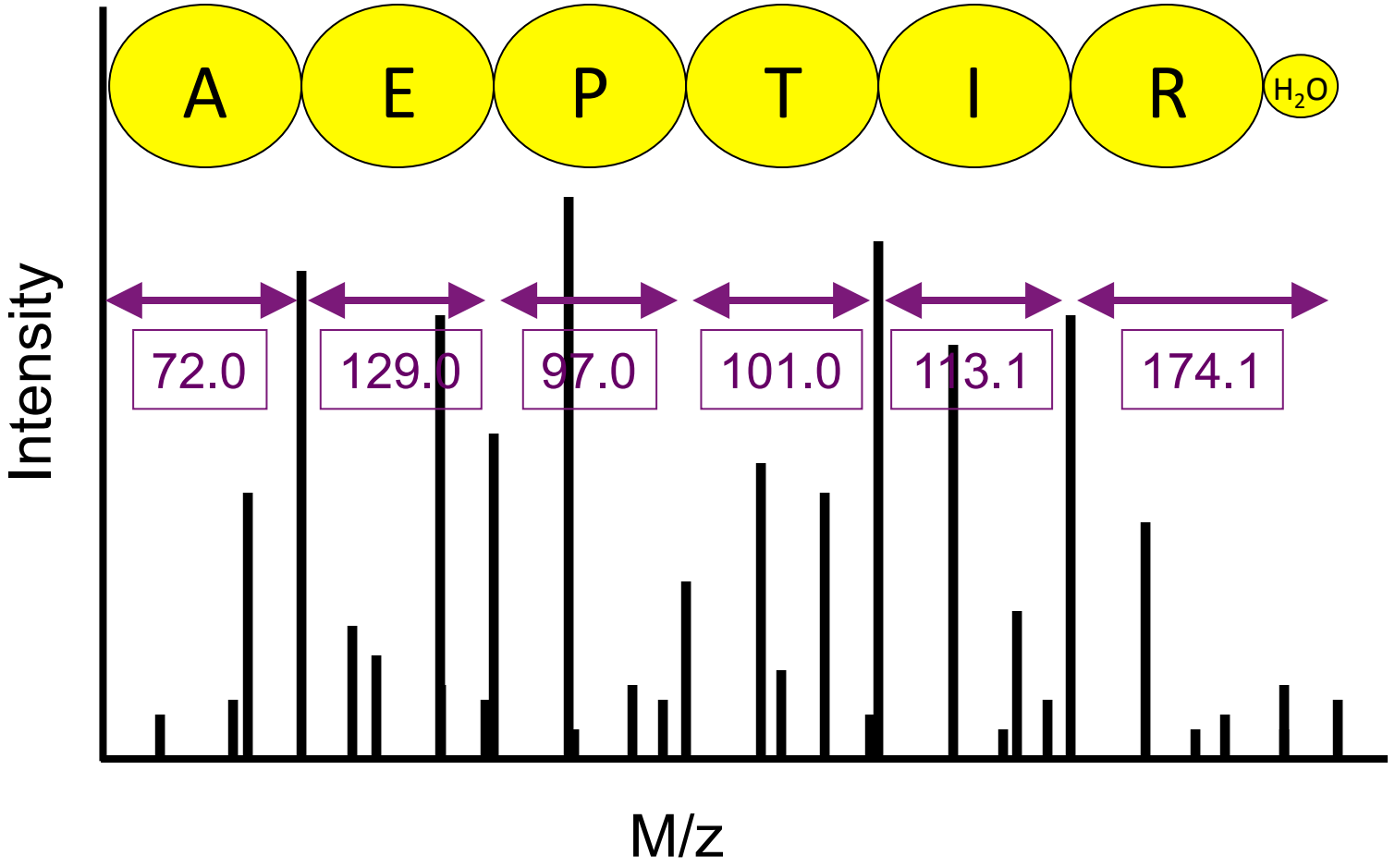

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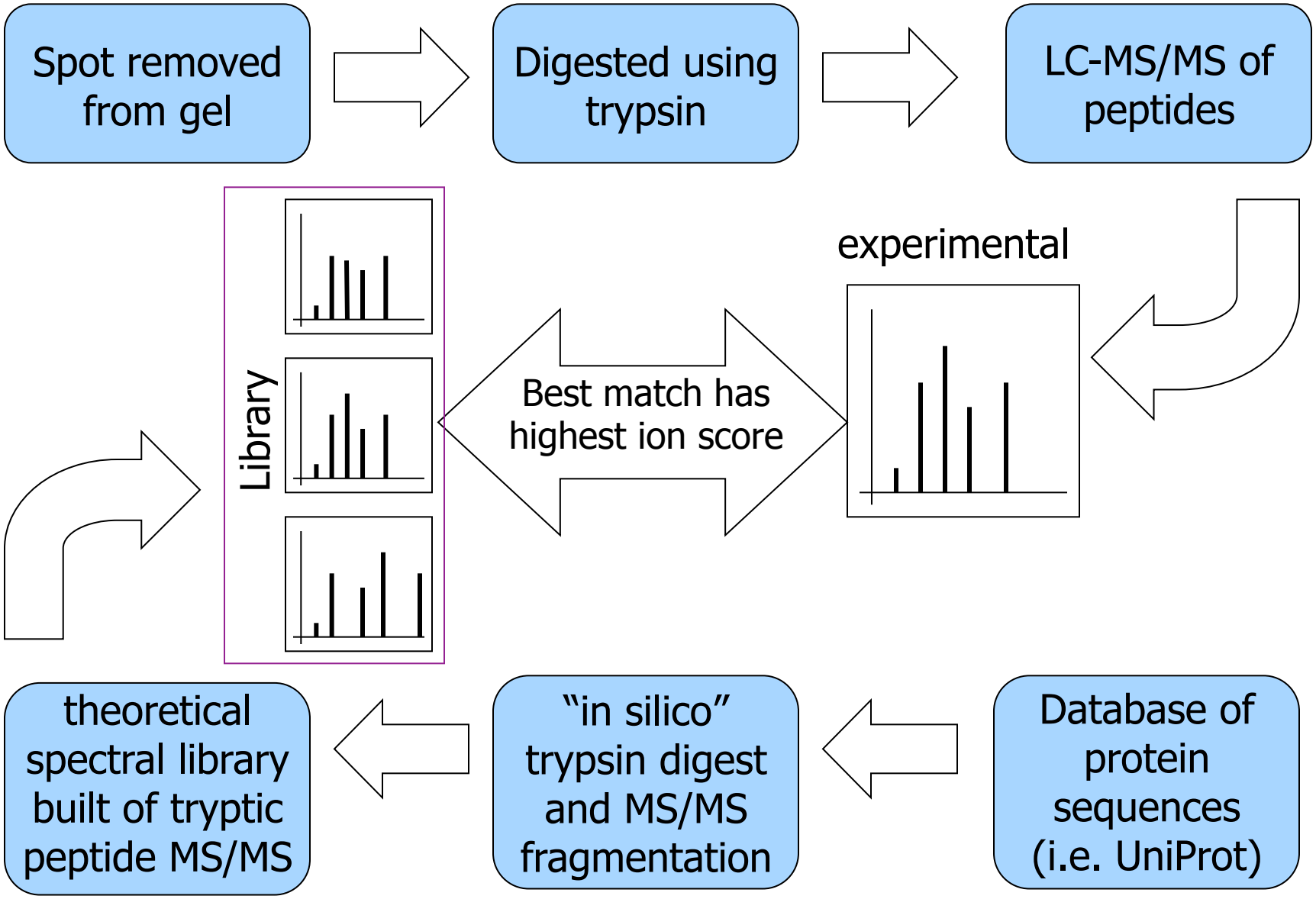
Peptide ID by Spectral Matching Process



Robert Britton, Tom Schmidt, Pat Venta www.msu.edu/course/mmg/433/lecturesS2005/

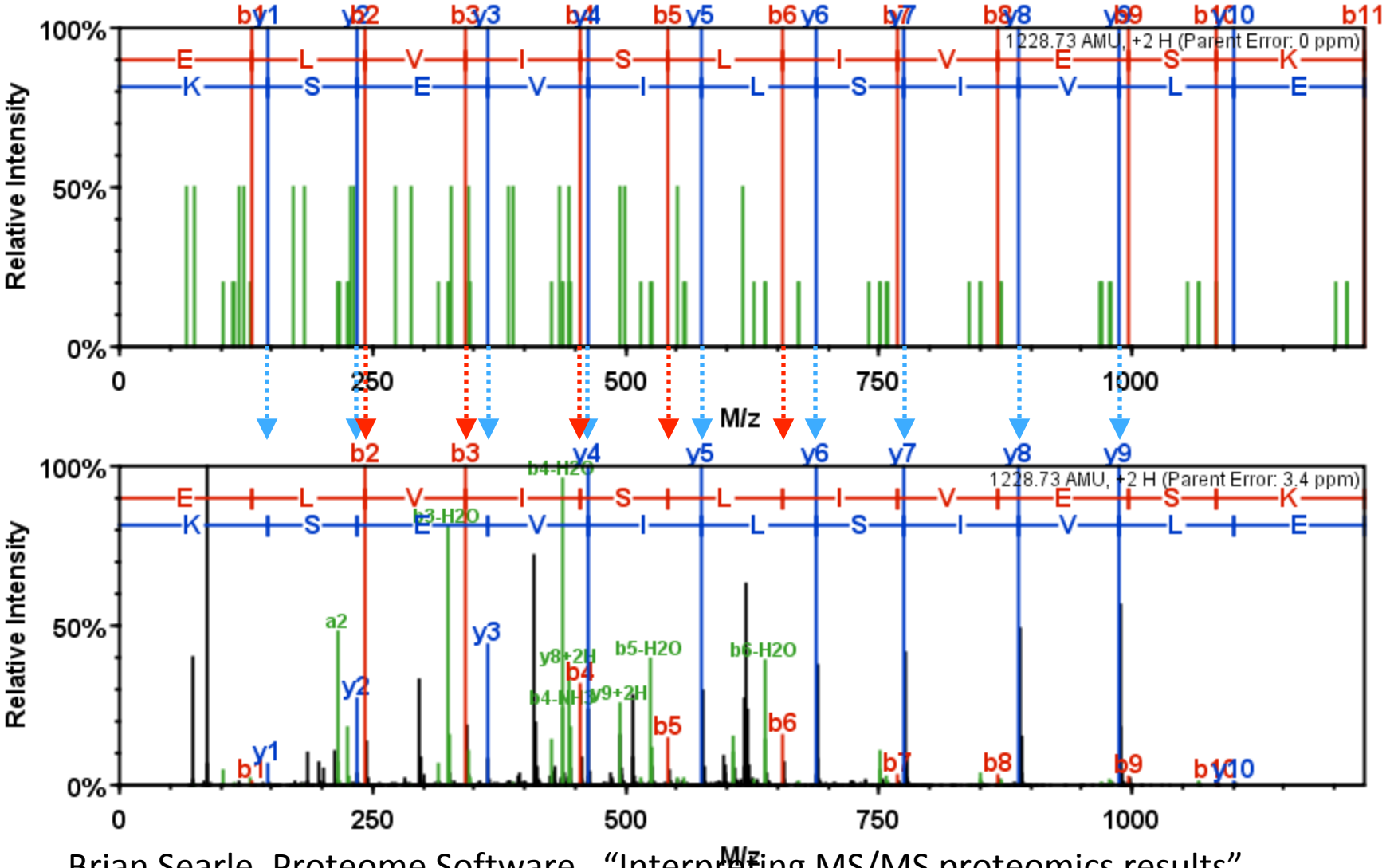
Proteomic Databases:

- UniProt–SwissProt + TrEMBL
- NCBI

MS/MS Search Engines:

- MASCOT (Matrix Science)
- SEQUEST (J. Eng & J. Yates, Scripps)
- SEQUEST HT (Thermo)
- ProteinProphet (R. Aebersold, ISB)
- OMSSA (NCBI)
- X!Tandem (thegpm)
- MS-Amanda (K. Mechtler, IMP, IMBA & GMI)
- Andromeda (M. Mann, Max Planck Institute)
- Scaffold (Proteome Software) validation only

SEQUEST search engine measures overlap between library spectrum and experimental spectrum



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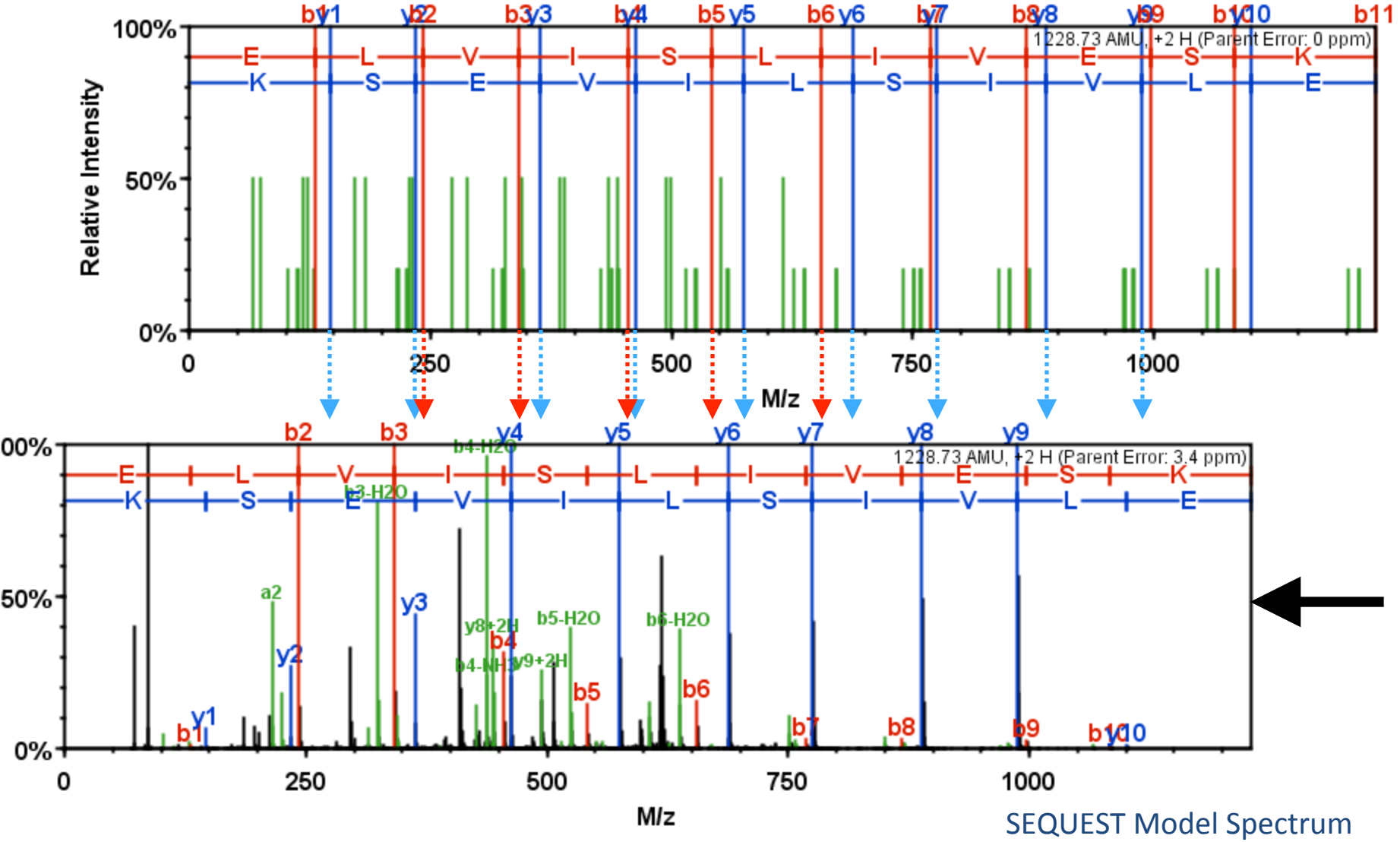
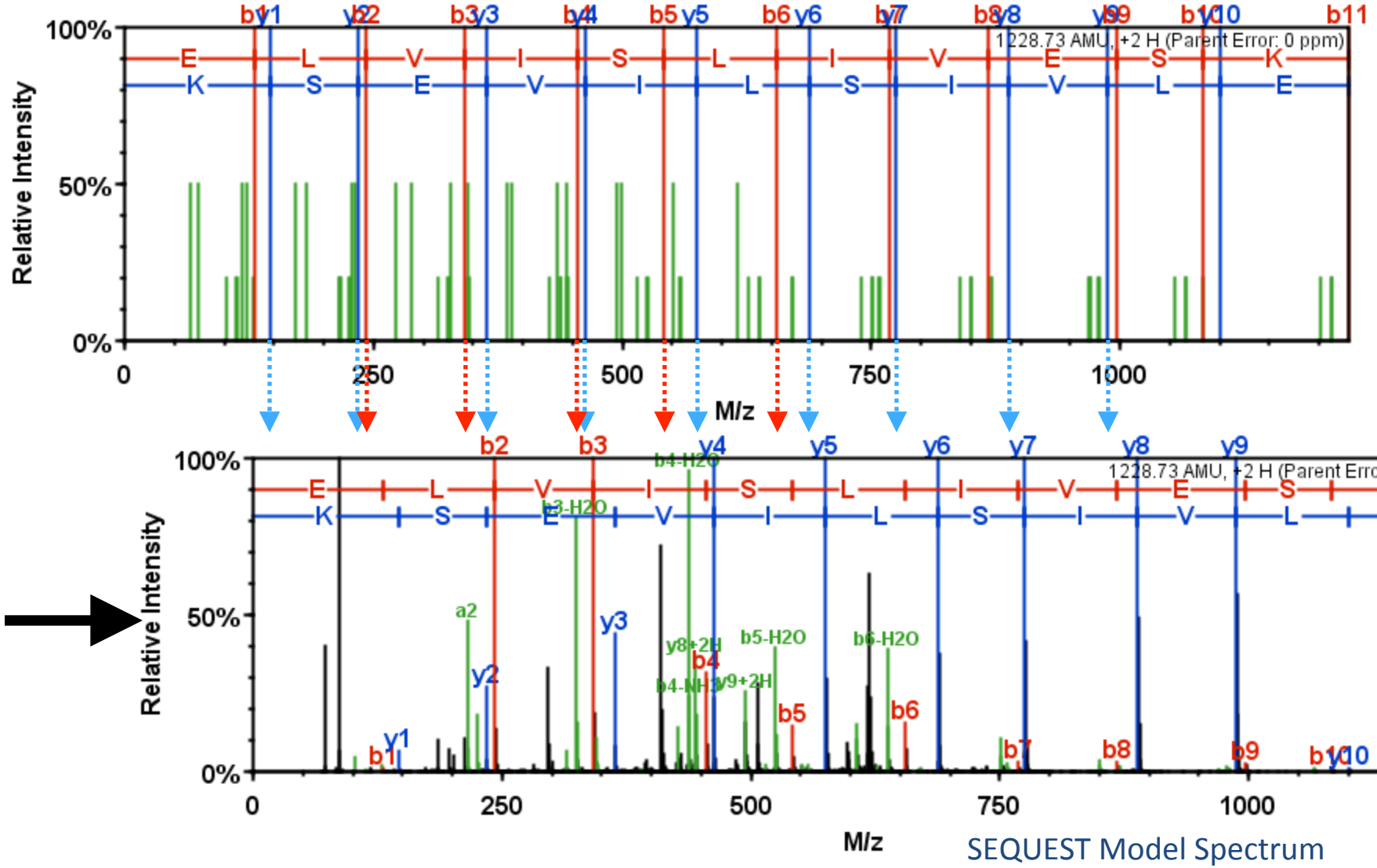
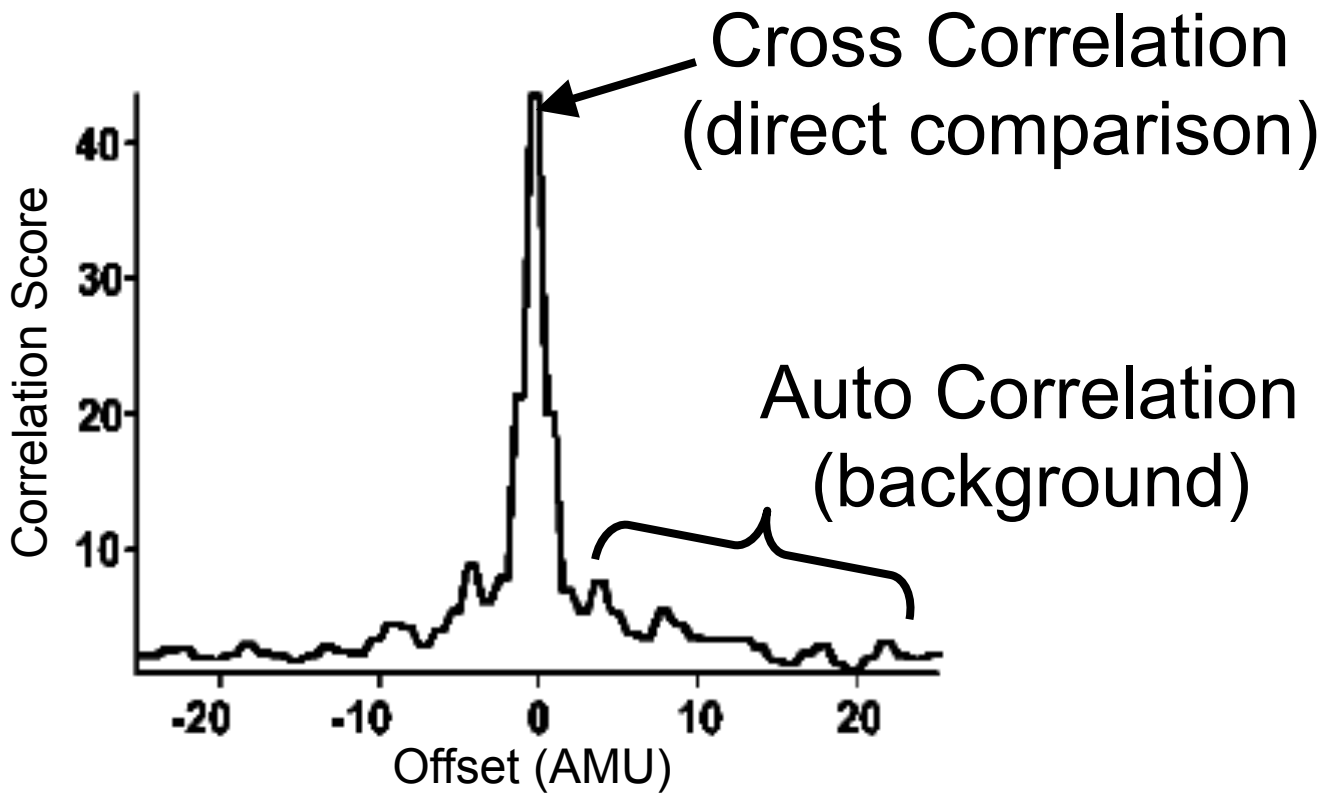


SEQUEST Model Spectrum



SEQUEST Model Spectrum

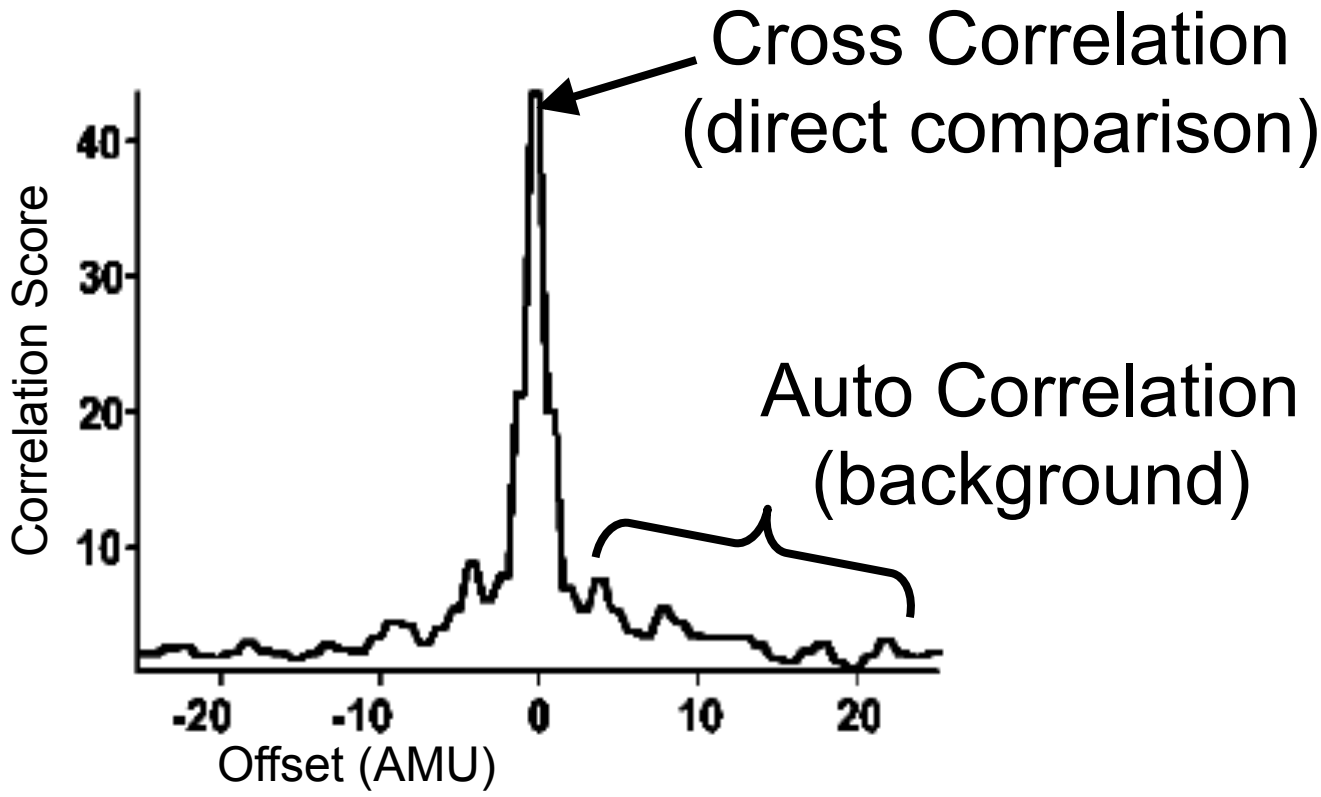
SEQUEST XCorr



Gentzel M. et al *Proteomics* **3** (2003) 1597-1610

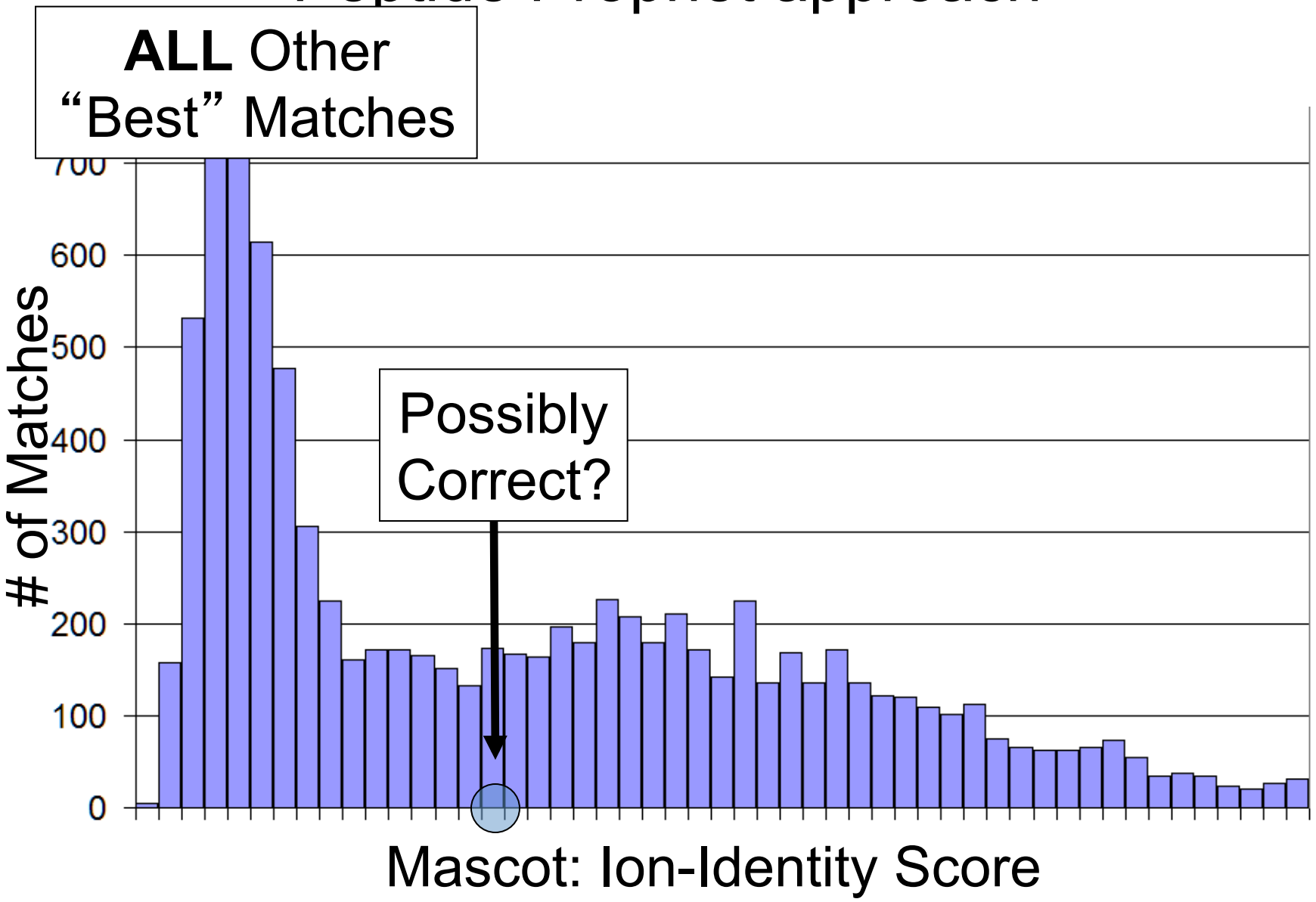
Brian Searle, Proteome Software, "Interpreting MS/MS proteomics results"

SEQUEST XCorr



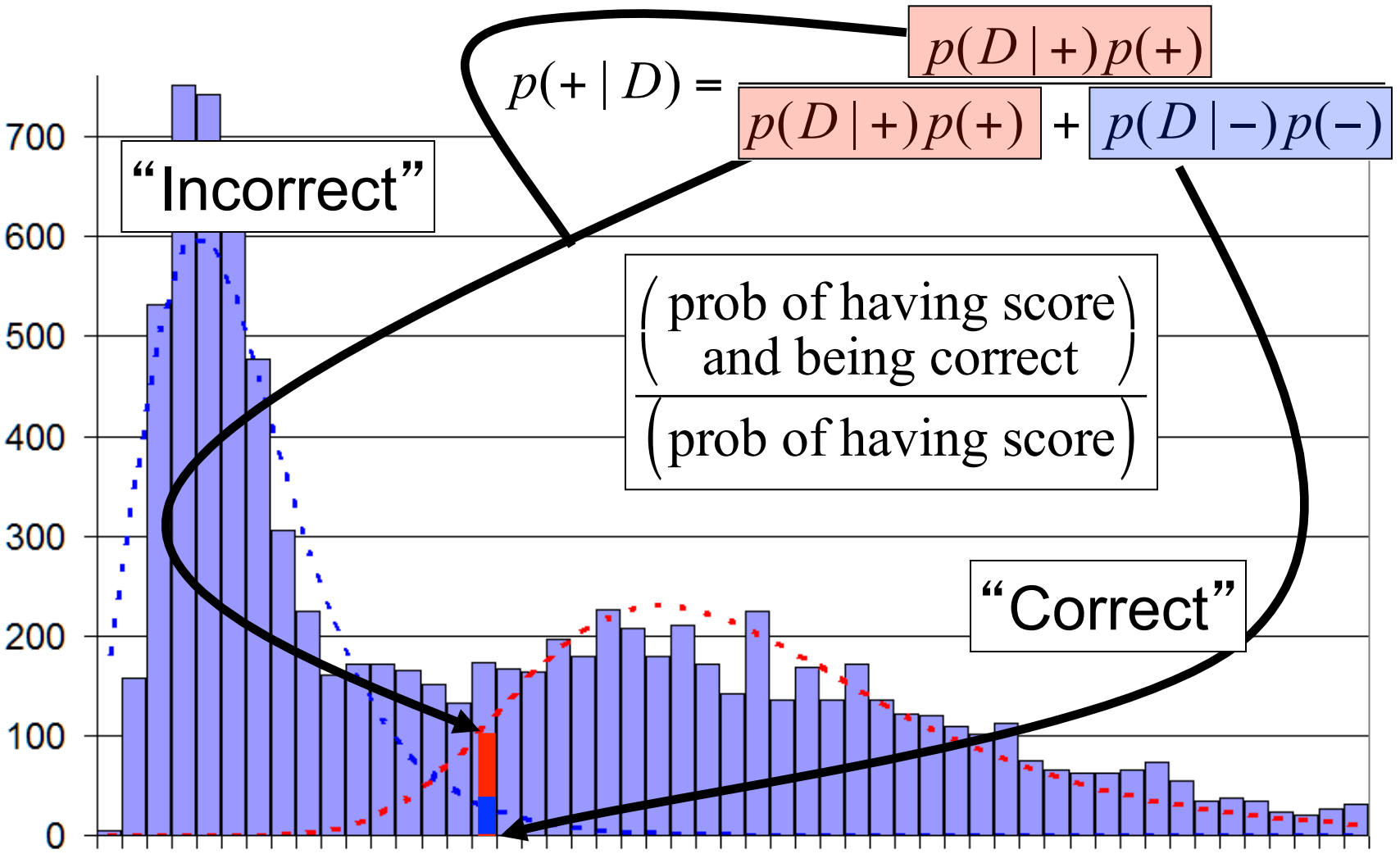
$$\text{XCorr} = \frac{CrossCorr}{avg\left(AutoCorr_{\text{offset=-75 to 75}}\right)}$$

10 Protein Control Sample (Q-ToF)
Peptide Prophet approach



Keller, A. et al*Anal. Chem.* 74, 5383-5392

10 Protein Control Sample (Q-ToF)



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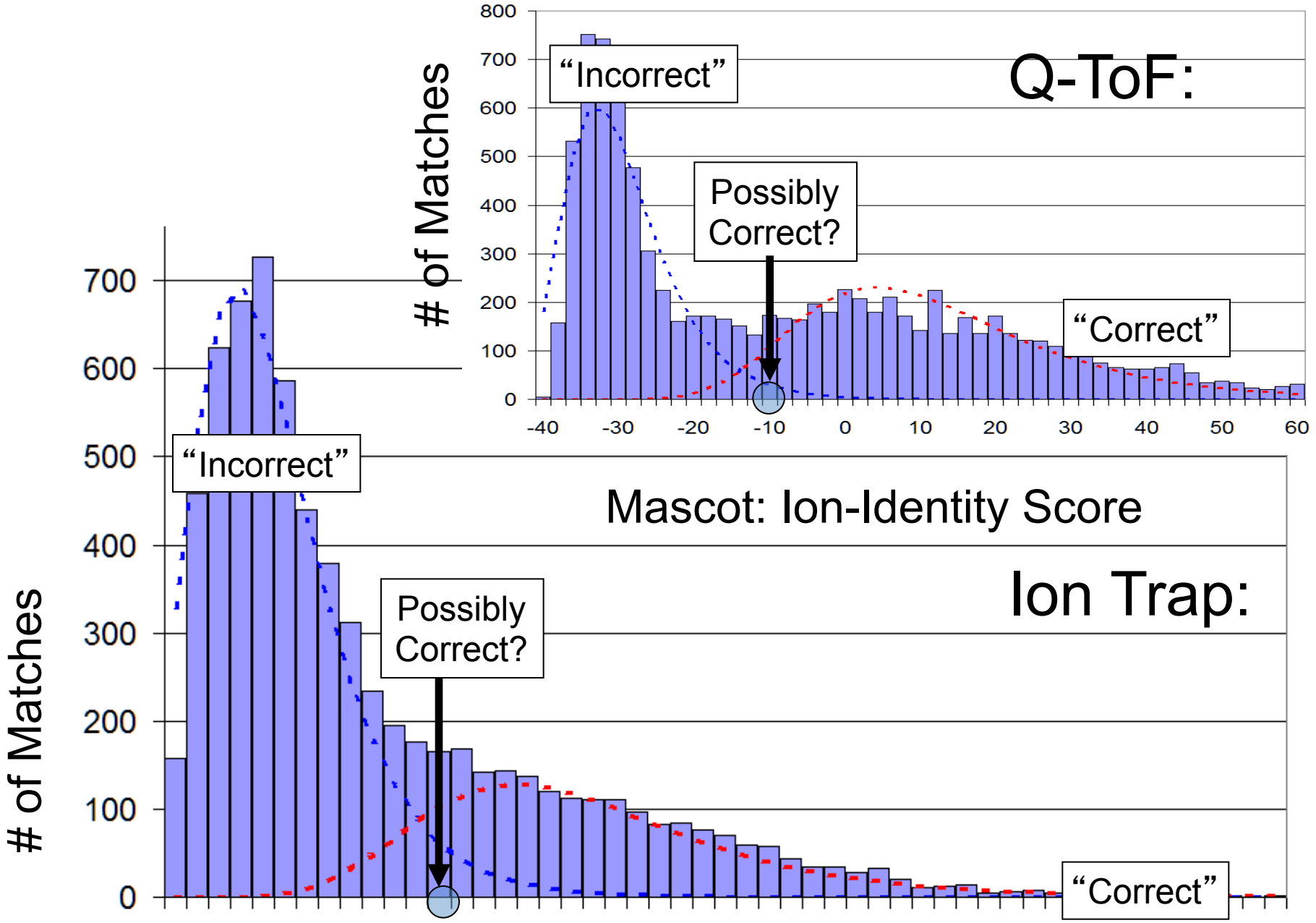

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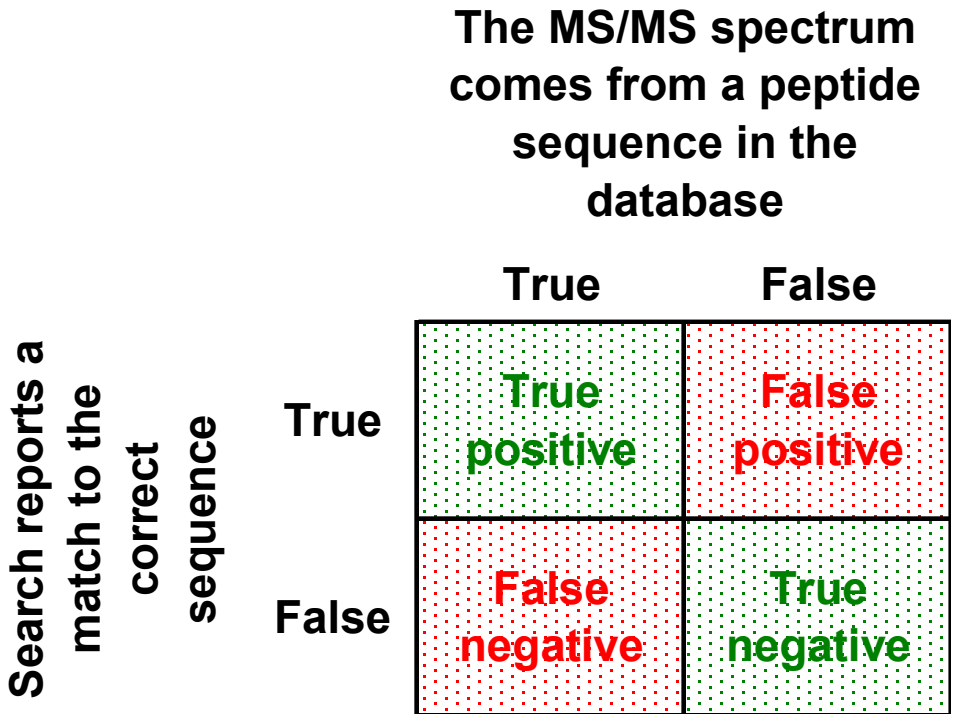
False Discovery Rate calculated by searching the data with a decoy DB to provide statistical confidence measure for peptide identifications

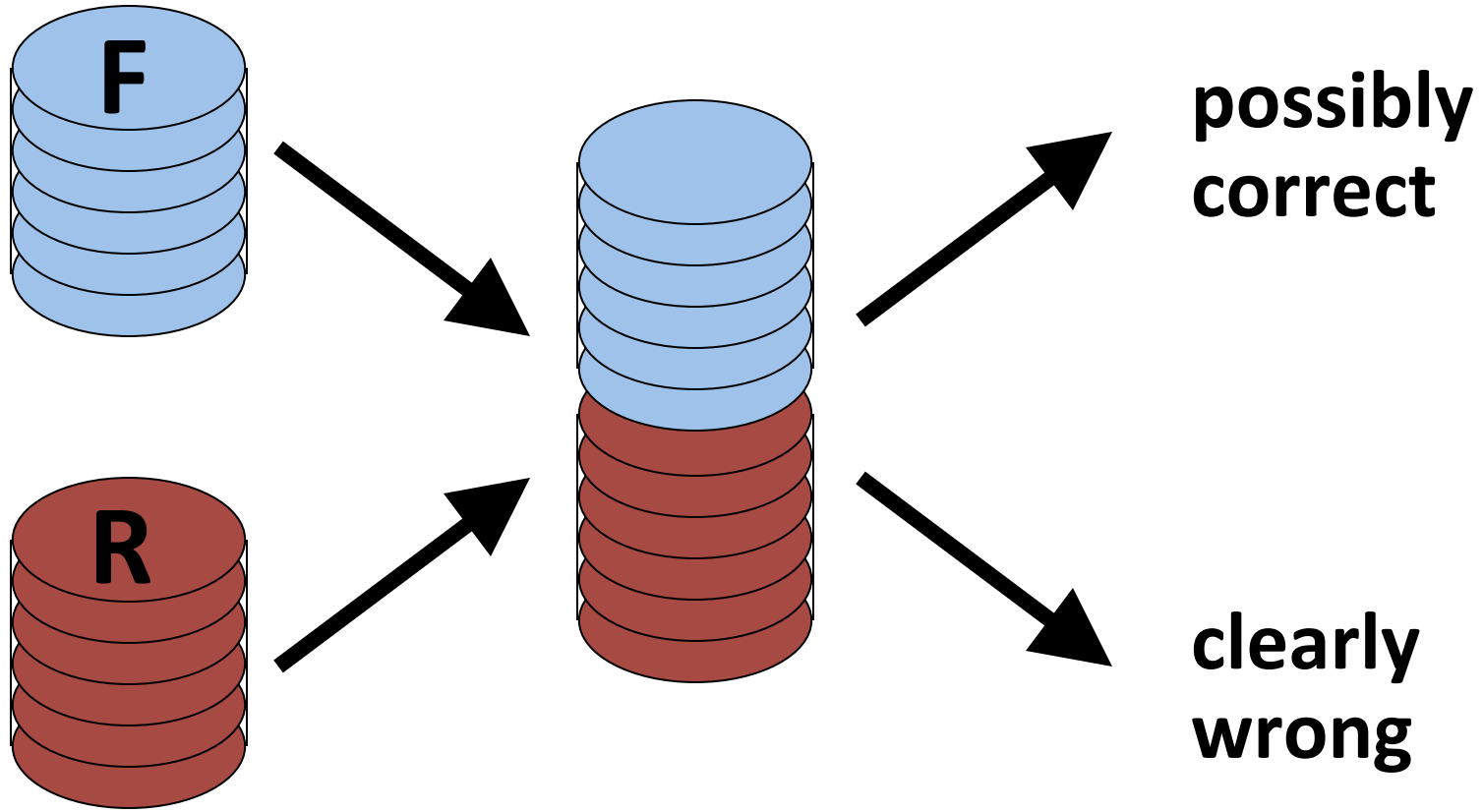


False Discovery Rate
= FP / (FP + TP)

True Positive Rate
= TP / (TP + FN)

False Positive Rate
= FP / (FP + TN)

Decoy DB for False Discovery Rate:



Elias JE, Gygi SP. Nat Methods. 2007 Mar;4(3):207-14.

Brian Searle, Proteome Software, "Reporting protein identifications with MS/MS results"

#	Spectrum	Accession	Peptide	Score
1	scan 3632	P35908	GFSSGSAVVSGGSR	4.6
2	scan 3609	P0AFY8	FSAASQPAAPVTK	3.7
3	scan 3629	P0A940	GFQSNTIGPK	3.0
4	scan 3635	P0A6F9	STRGEVLAVGNGR	2.2
5	scan 3636	P0A870	ELAESEGAIER	2.1
6	scan 3607	P0A799	ADLNVPVKDGK	1.9
7	scan 3626	P0ABC7	EAEAYTNEVQPR	1.6
8	scan 3602	P0A853	IRVIEPVKR	1.4
9	scan 3623	P38489	KLTPEQAEQIK	0.9
10	scan 3616	P00448	GTTLQGDLK	0.8
11	scan 3621	P09546	LLPGPTGER	0.4
12	scan 3615	P0AFG8	AFLEGR	0.2
13	scan 3624	P14565	SAADVAIMK	0.0
14	scan 3613	rev_P06864	EGSLAVNVQGDAAIR	-0.4
15	scan 3604	P36562	DPEEVVGIGANLPTDK	-0.7
16	scan 3606	P0A9C5	IPVVSSPK	-0.7
17	scan 3611	P0ABB0	ASTISNVVR	-0.7
18	scan 3614	rev_Q2EEU2	KFVALTCDTLLLGER	-0.8
19	scan 3620	rev_P0ACL5	NNESAALMKEYCR	-0.9
20	scan 3633	rev_P37309	SDGSCNQRALNR	-0.9
21	scan 3627	P32132	VEETEDADAFRVSGR	-1.0
22	scan 3618	P37342	ILTQDEIDVR	-1.0
23	scan 3610	rev_P0ADK0	IANVSDVVPR	-1.2
24	scan 3601	P0AG93	LGMKREHMLQQK	-1.3

Brian Searle, Proteome Software, “Reporting protein identifications with MS/MS results”

FDR=2x decoy/total PSMs above threshold

#	Spectrum	Accession	Peptide	Score
1	scan 3632	P35908	GFSSGSAVVSGGSR	4.6
2	scan 3609	P0AFY8	FSAASQPAAPVTK	3.7
3	scan 3629	P0A940	GFQSNTIGPK	3.0
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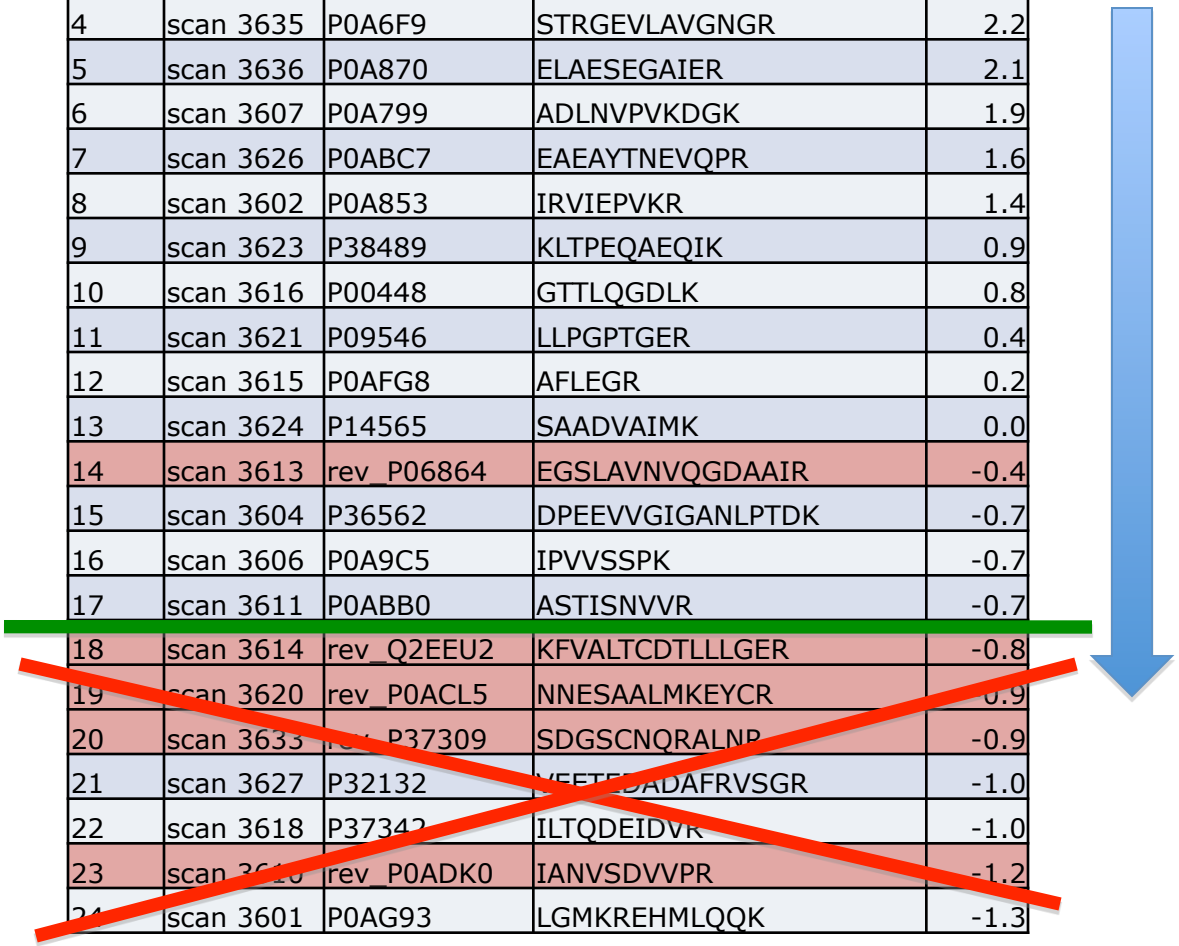
14	scan 3613	rev_P06864	EGSLAVNVQGDAAIR	-0.4
15	scan 3604	P36562	DPEEVVGIGANLPTDK	-0.7
16	scan 3606	P0A9C5	IPVVSSPK	-0.7
17	scan 3611	P0ABB0	ASTISNVVR	-0.7
18	scan 3614	rev_Q2EEU2	KFVALTCDTLLLGER	-0.8
19	scan 3620	rev_P0ACL5	NNESAALMKEYCR	-0.9
20	scan 3633	[illegible]_P37309	SDGSCNQRALNR	-0.9
21	scan 3627	P32132	[illegible]ADAFRVSGR	-1.0
22	scan 3618	P37342	ILTQDEIDVR	-1.0
23	scan [illegible]	rev_P0ADK0	IANVSDVVPR	-1.2
24	scan 3601	P0AG93	LGMKREHMLQQK	-1.3



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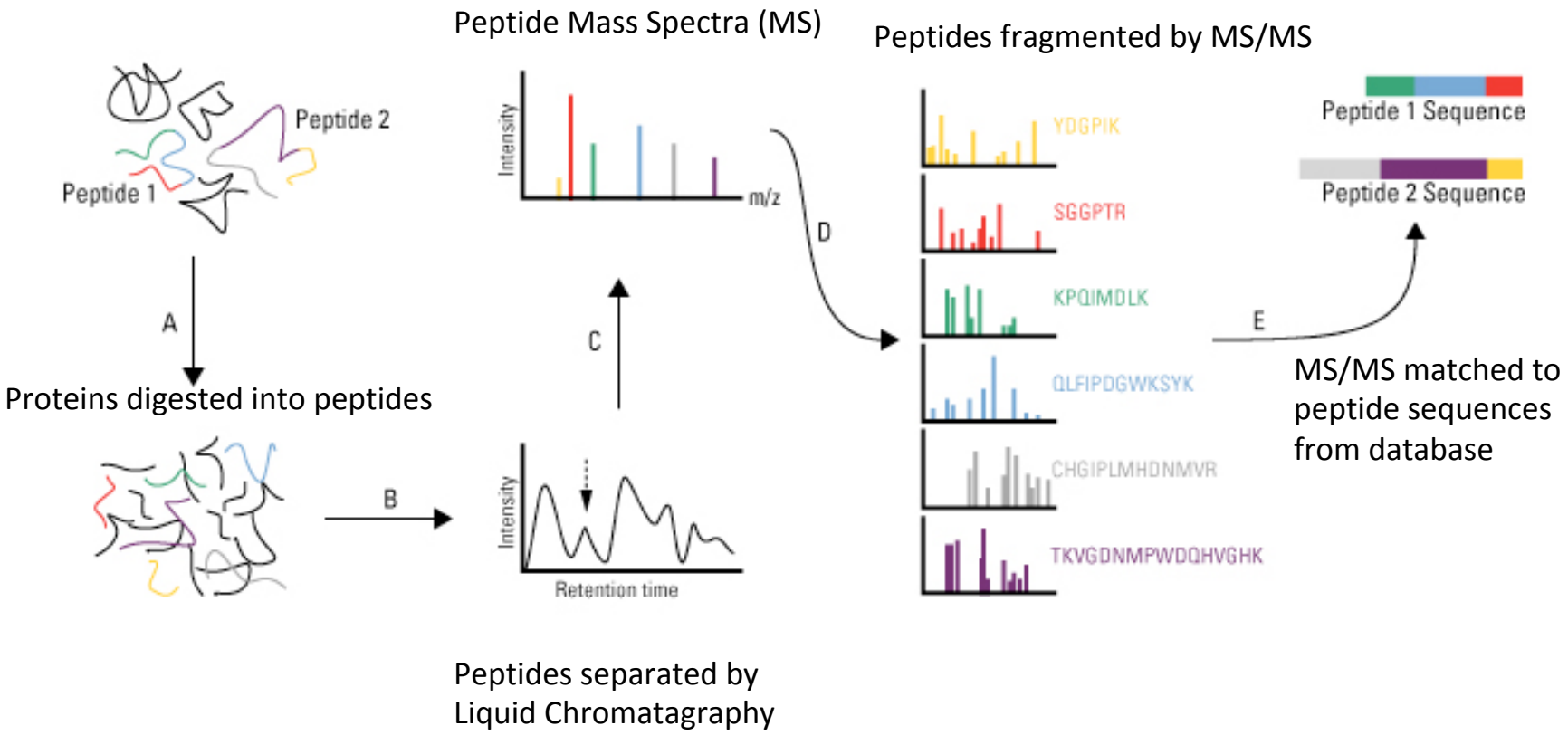
LC-MS/MS Protein Identification

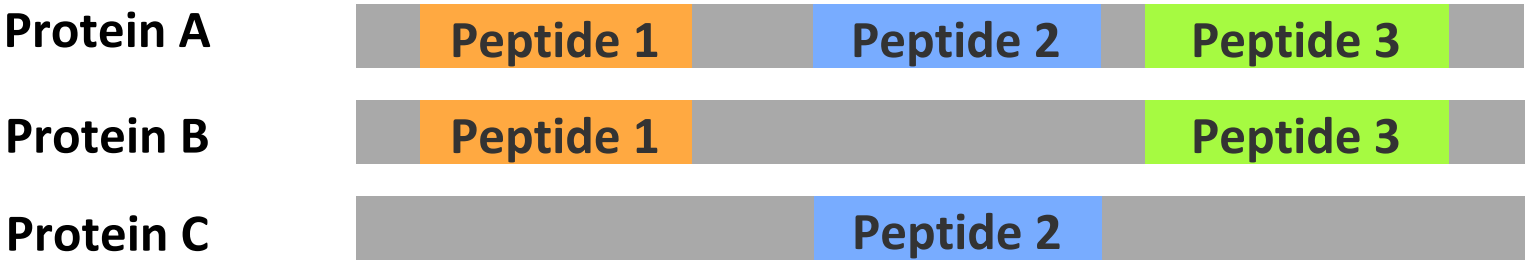


http://www.piercenet.com/browse.cfm?fldID=754E41C1-8B46-444E-AEE8-E33E5D37A1DE

Protein Inference

General approach is to create a minimal list of proteins.

“Principal of parsimony” or “Occam’s razor”



Protein Inference

Peptides identified:

1	TIGGGDDSFNTFFSETGAGK	5	IHFPLATYAPVISAEK	9	VGINYQPPTVVPGGDLAK
2	AVFVDLEPTVIDEVR	6	AYHEQLSVAEITNACFEPANQMVK	10	AVCMLSNTTAIAEAWAR
3	QLFHPEQLITGKEDAANNYAR	7	YMACCLLYR	11	LDHKFDLMYAK
4	NLDIERPTYTNLNR	8	SIQFVDWCPTGFK		

Assignment of peptides to proteins:

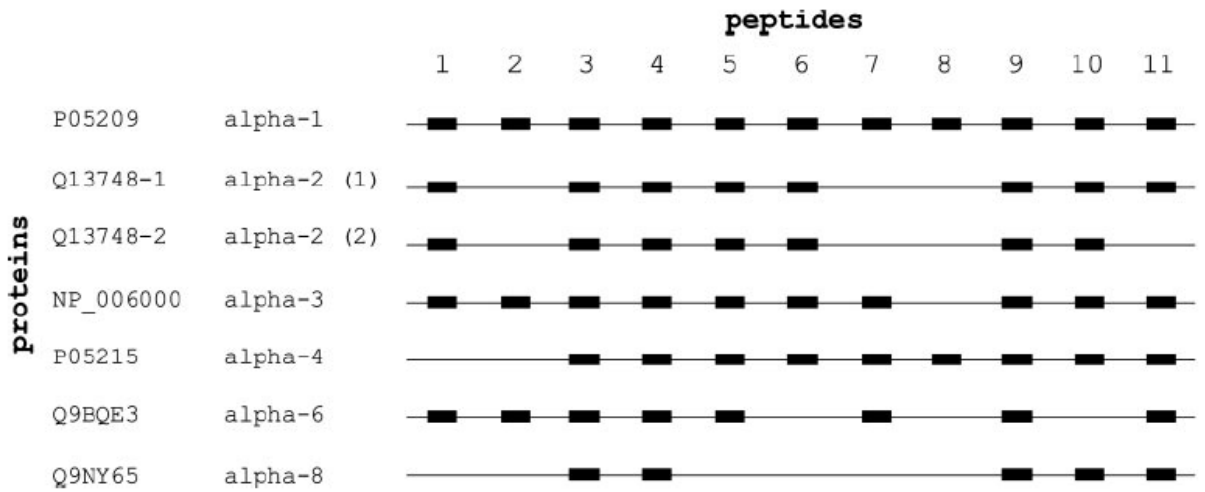
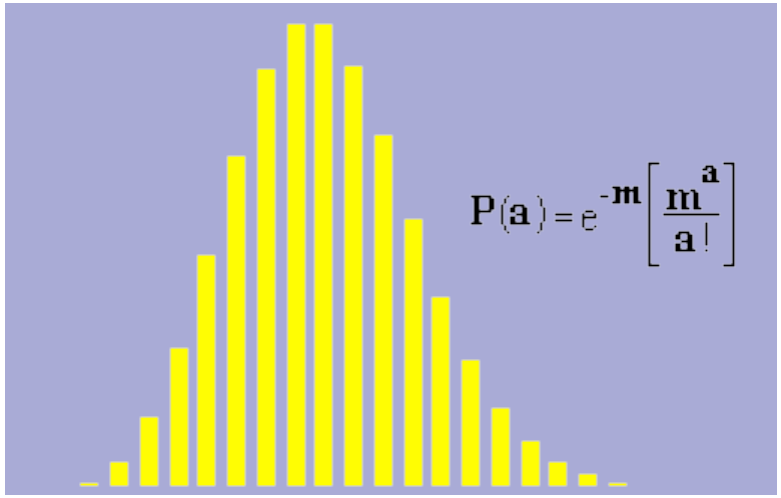


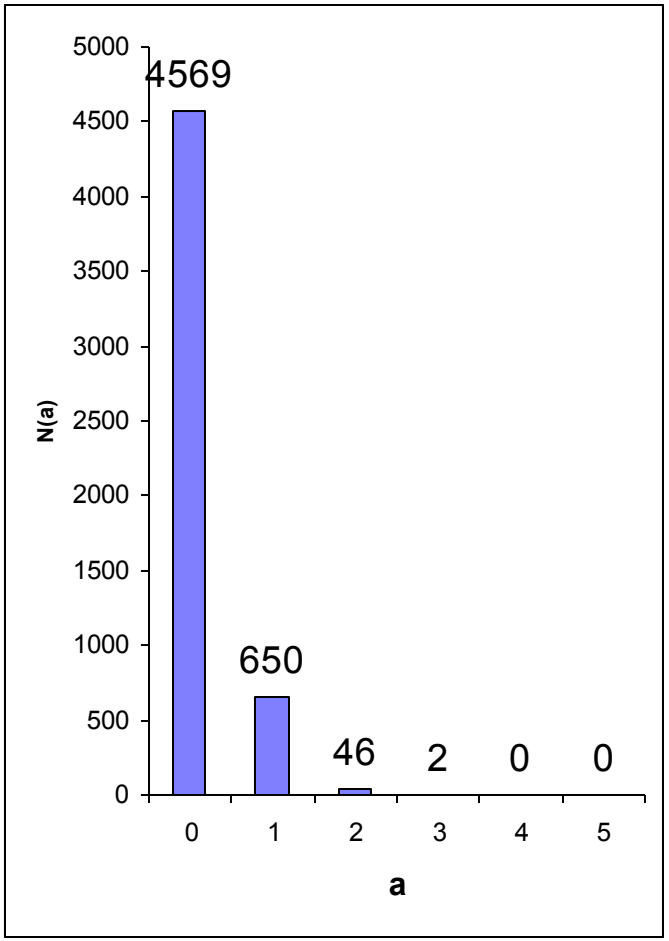
FIG. 3. **An example of a protein family.** Eleven tryptic peptides are identified that are shared between the members of the α-tubulin family. None of the proteins is identified by a peptide that is unique to it, thus making it impossible to determine which particular member(s) of the family is present in the sample.

- **Nesvizhskii, A. I. and Aebersold, R. (2005). Interpretation of shotgun proteomic data - The protein inference problem. Mol. & Cellular Proteomics, 4, 1419-1440.**

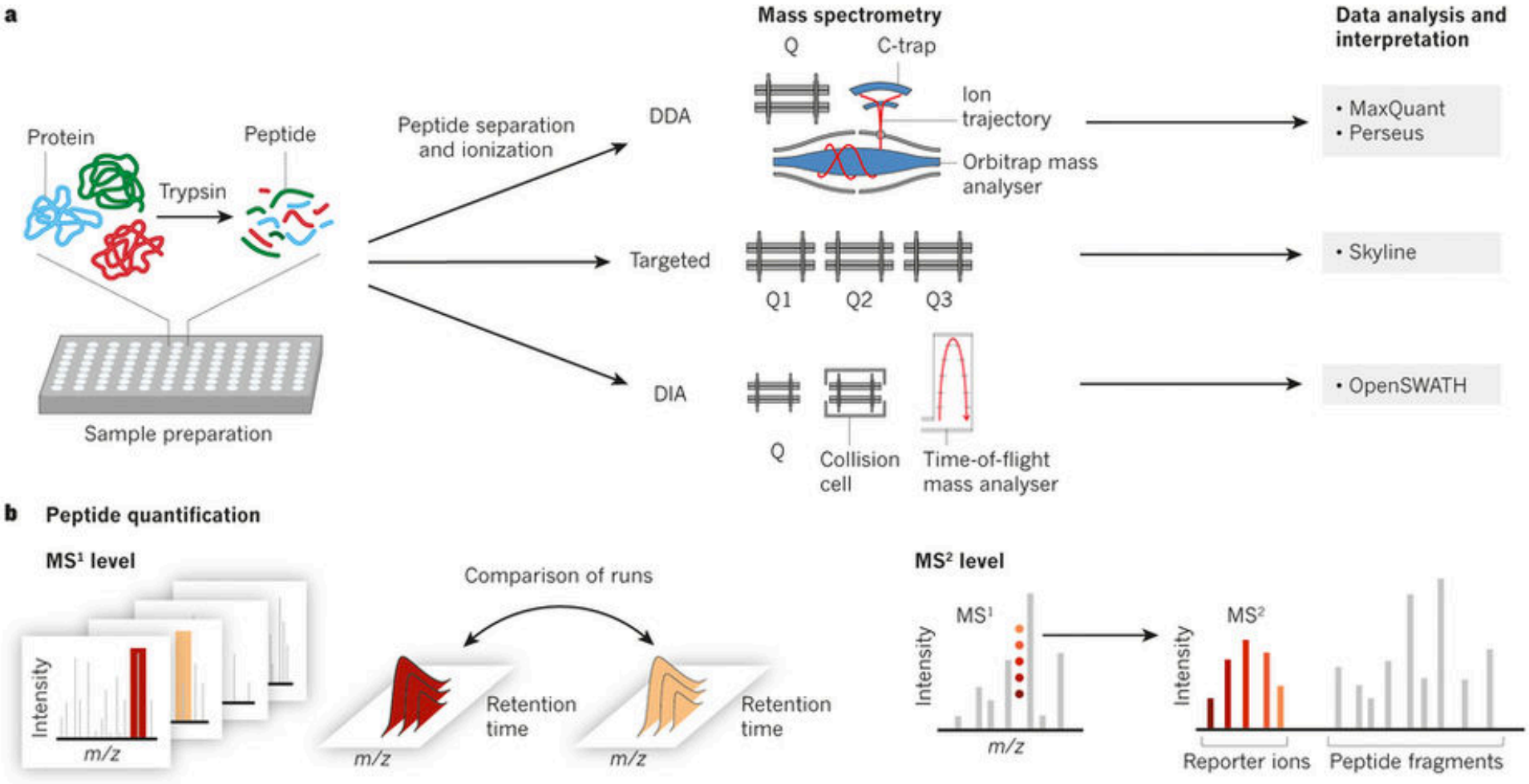
One Hit Wonders



- Huge MudPIT data set
- Search Swiss-Prot using drosophila taxonomy filter (5268 entries)
- 75,000 matches with 1% FDR
- i.e. 750 false matches

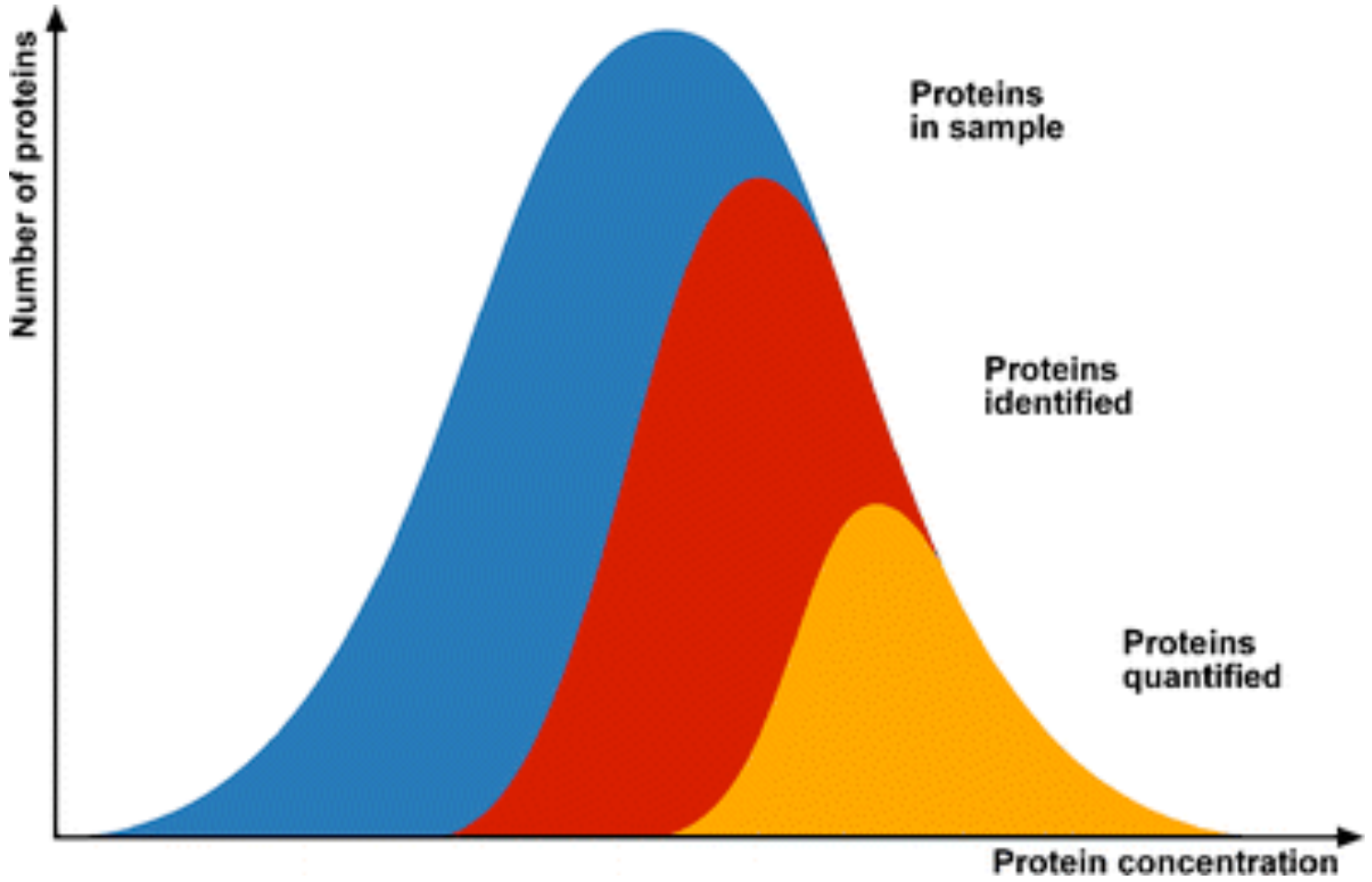


Quantitative Proteomics



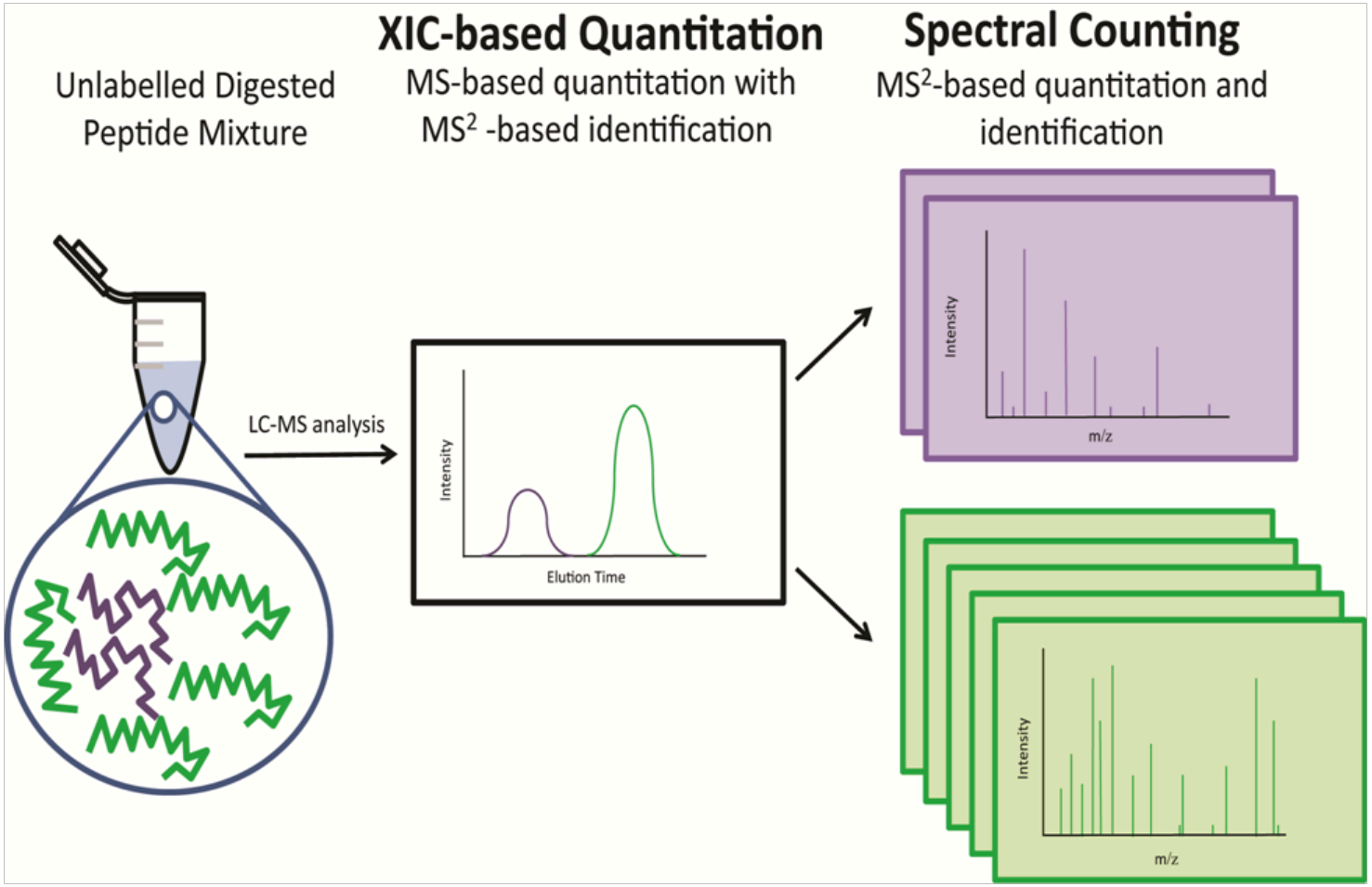
Nature **537**, 347–355 (15 September 2016) doi:10.1038/nature19949

Quantifiable Proteins Are Subset of Proteome



Marcus Bantscheff et al., Anal Bioanal Chem (2012) 404:939-965

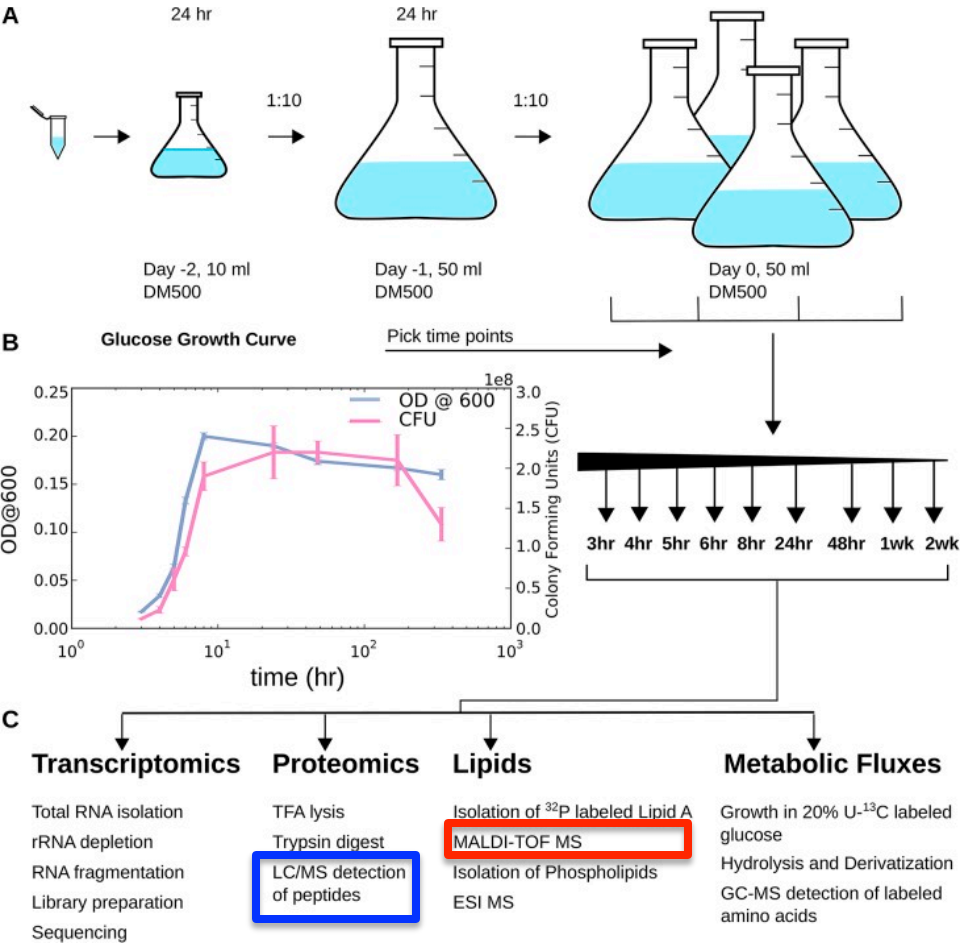
Quantitation uses peptide peak intensity or counts number of MS/MS



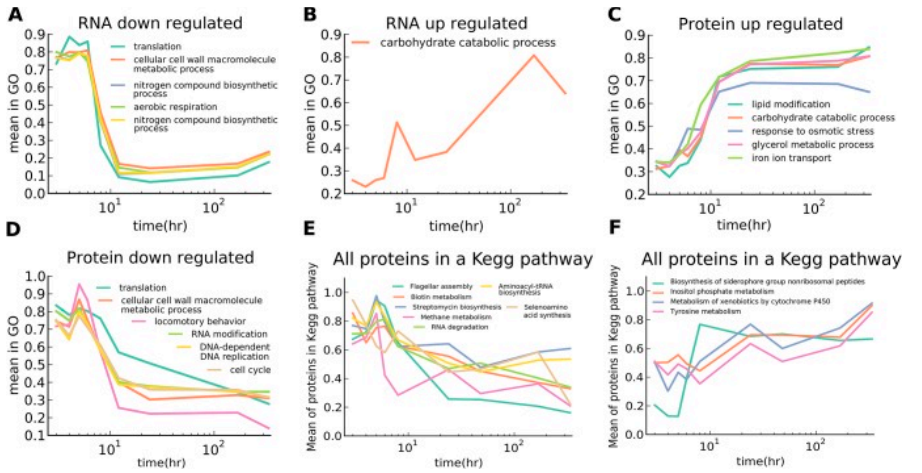
https://www.bioanalysis-zone.com/2016/02/08/chapter-5-modern-techniques-in-quantitative-proteomics/ Smith and Macklin from the series Advanced LC-MS applications for proteomics

Systems study of E. coli glucose starvation

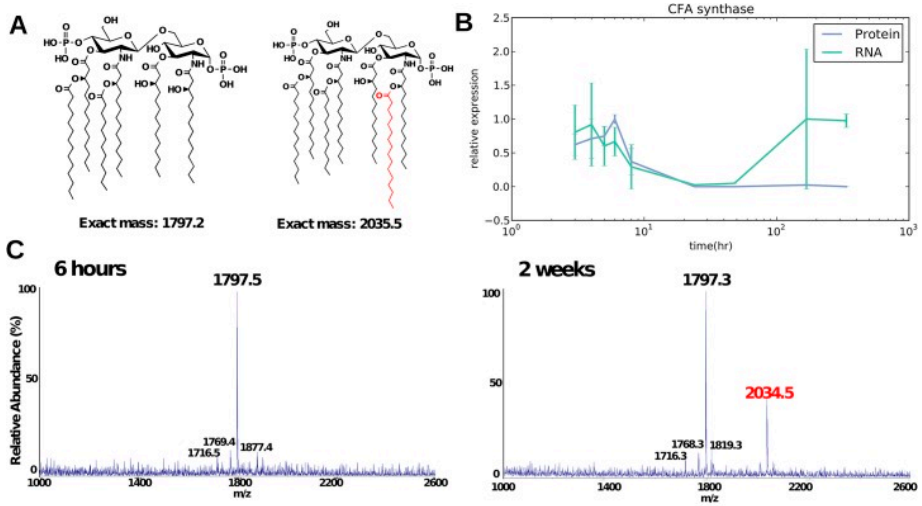
Wilke, Marcotte, Barrick and Trent labs



Quantitative proteomics by spectral counting

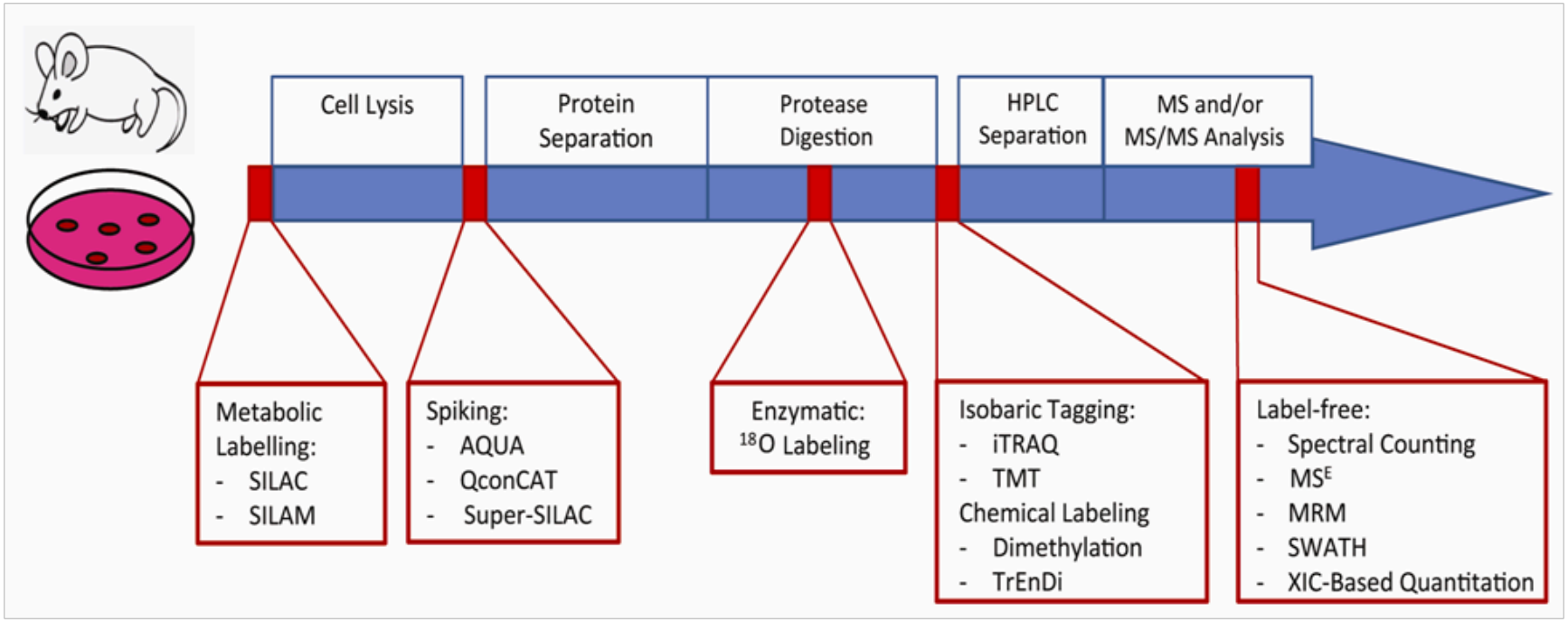


Lipid A analysis on MALDI-TOF



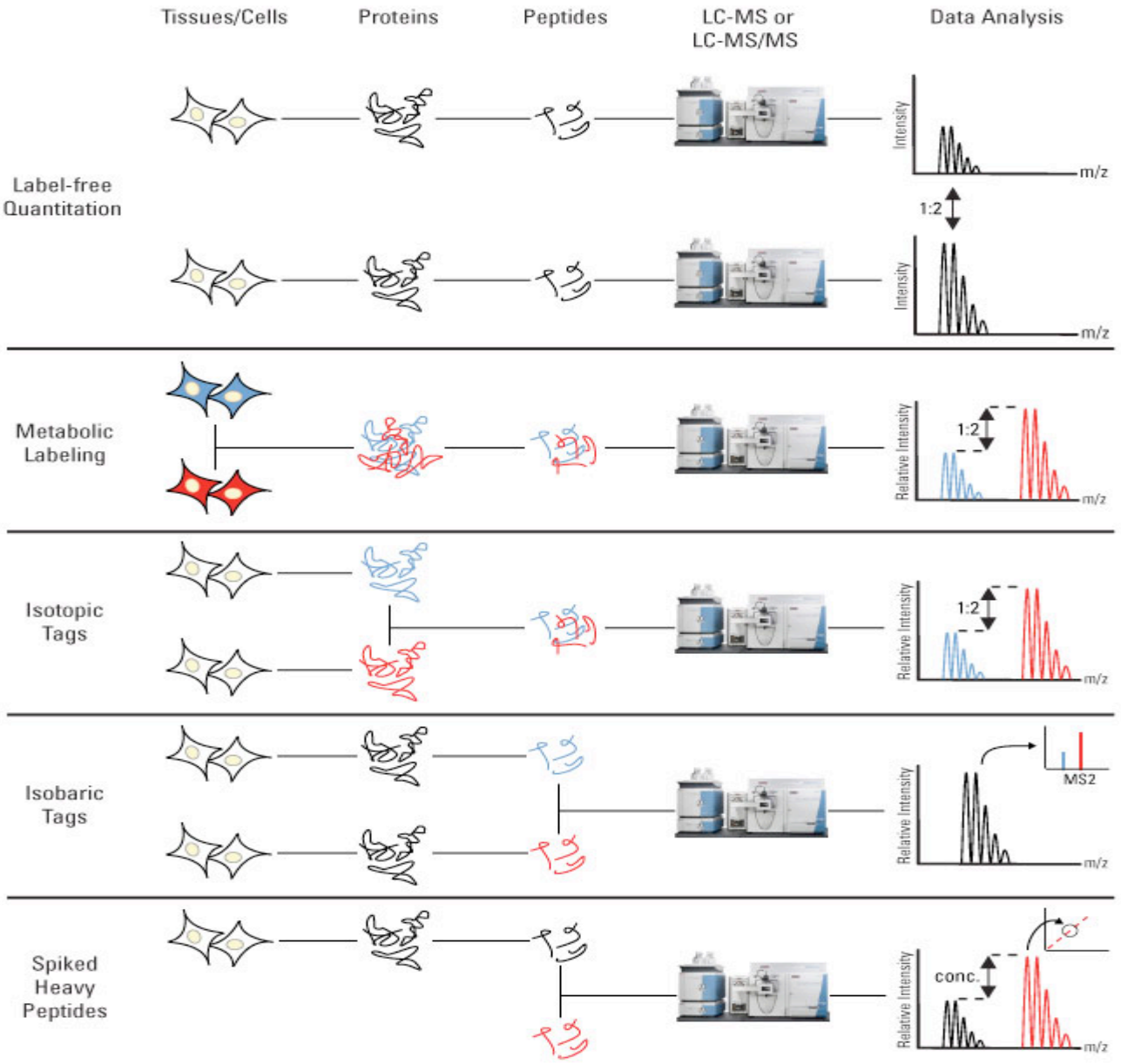
PLoS Comput Biol. 2015 Aug 14;11(8):e1004400

Experimental stages for initiating quantitation protocol



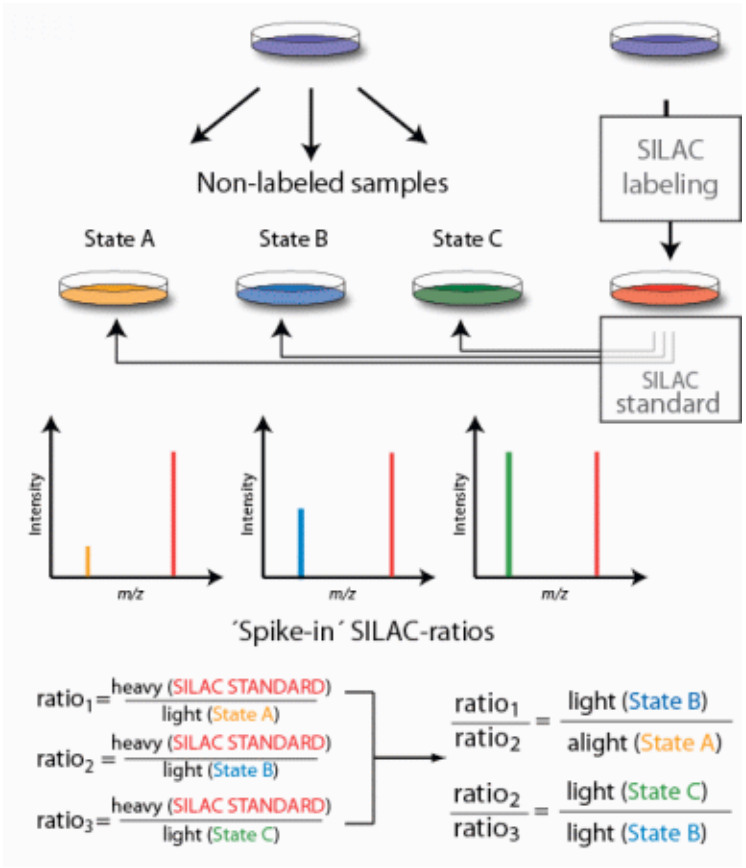
Smith and Macklin, https://www.bioanalysis-zone.com/2016/02/08/chapter-5-modern-techniques-in-quantitative-proteomics/

Quantitation Methods

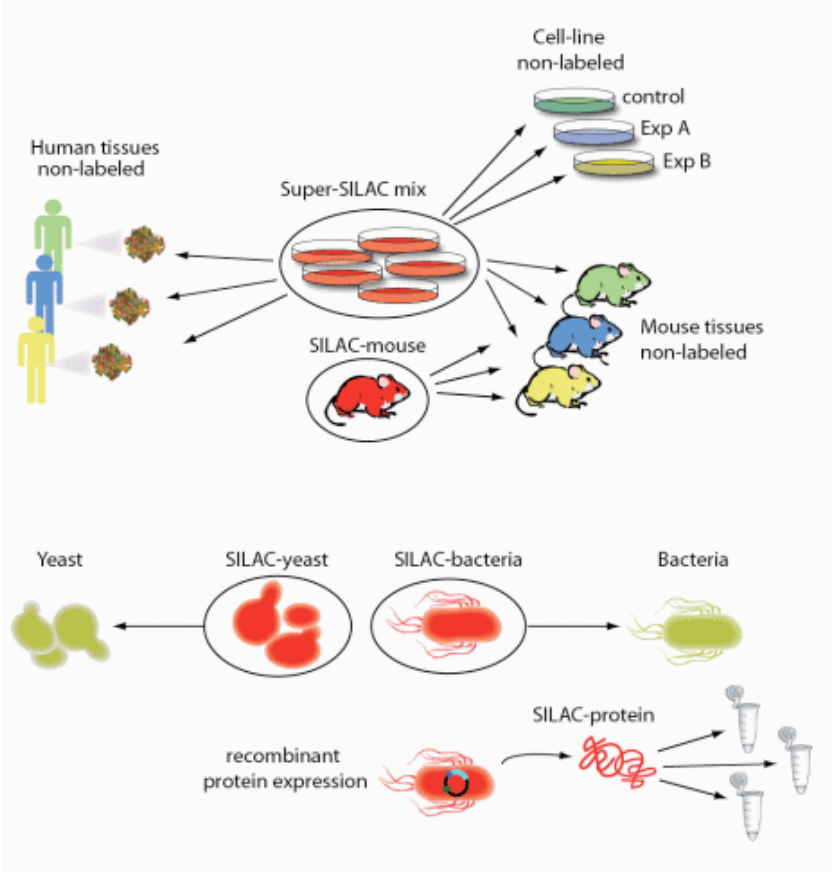


http://www.piercenet.com/method/quantitative-proteomics

Spike-in SILAC standard

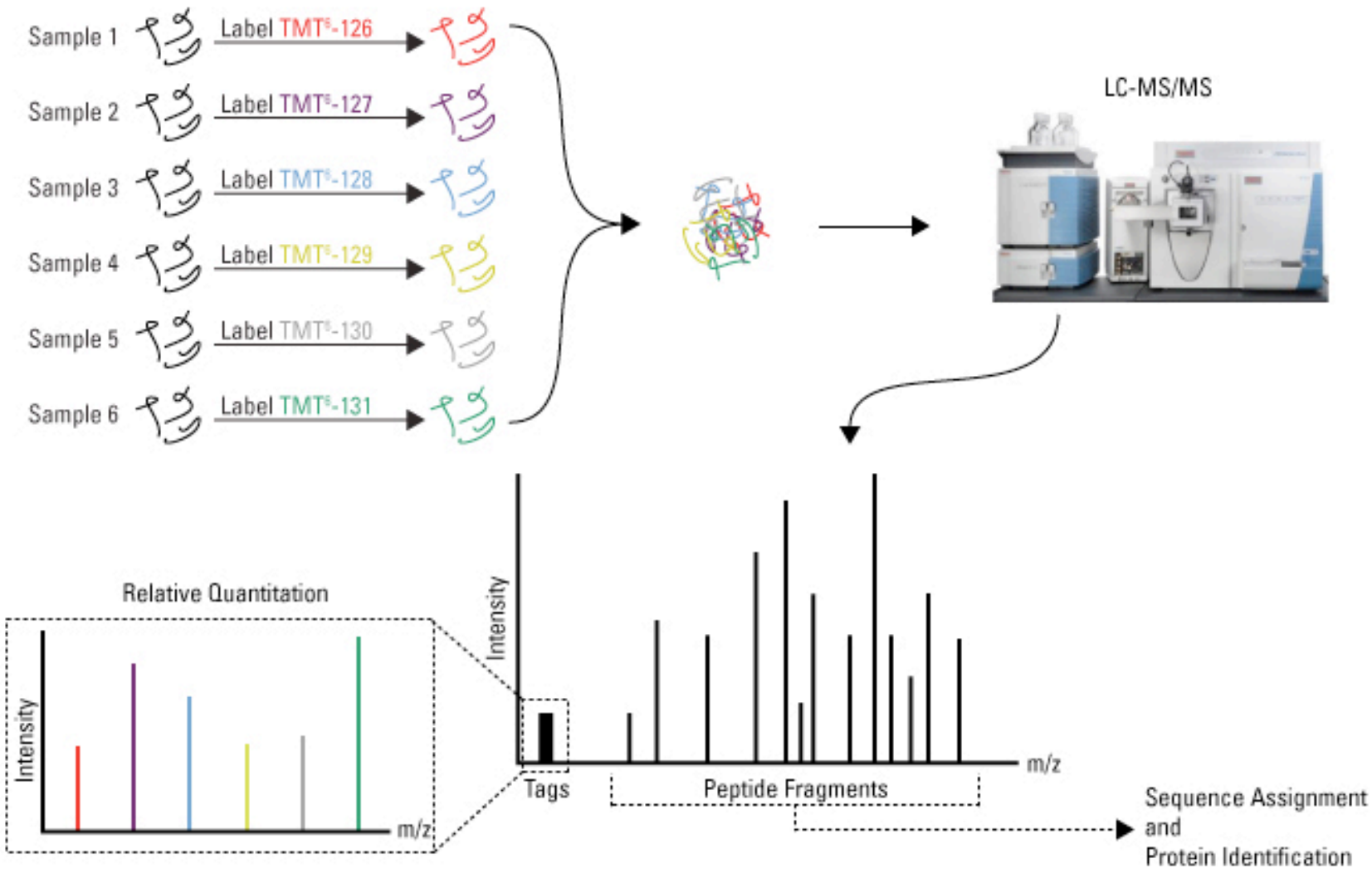


Super SILAC for Tissue quantitation



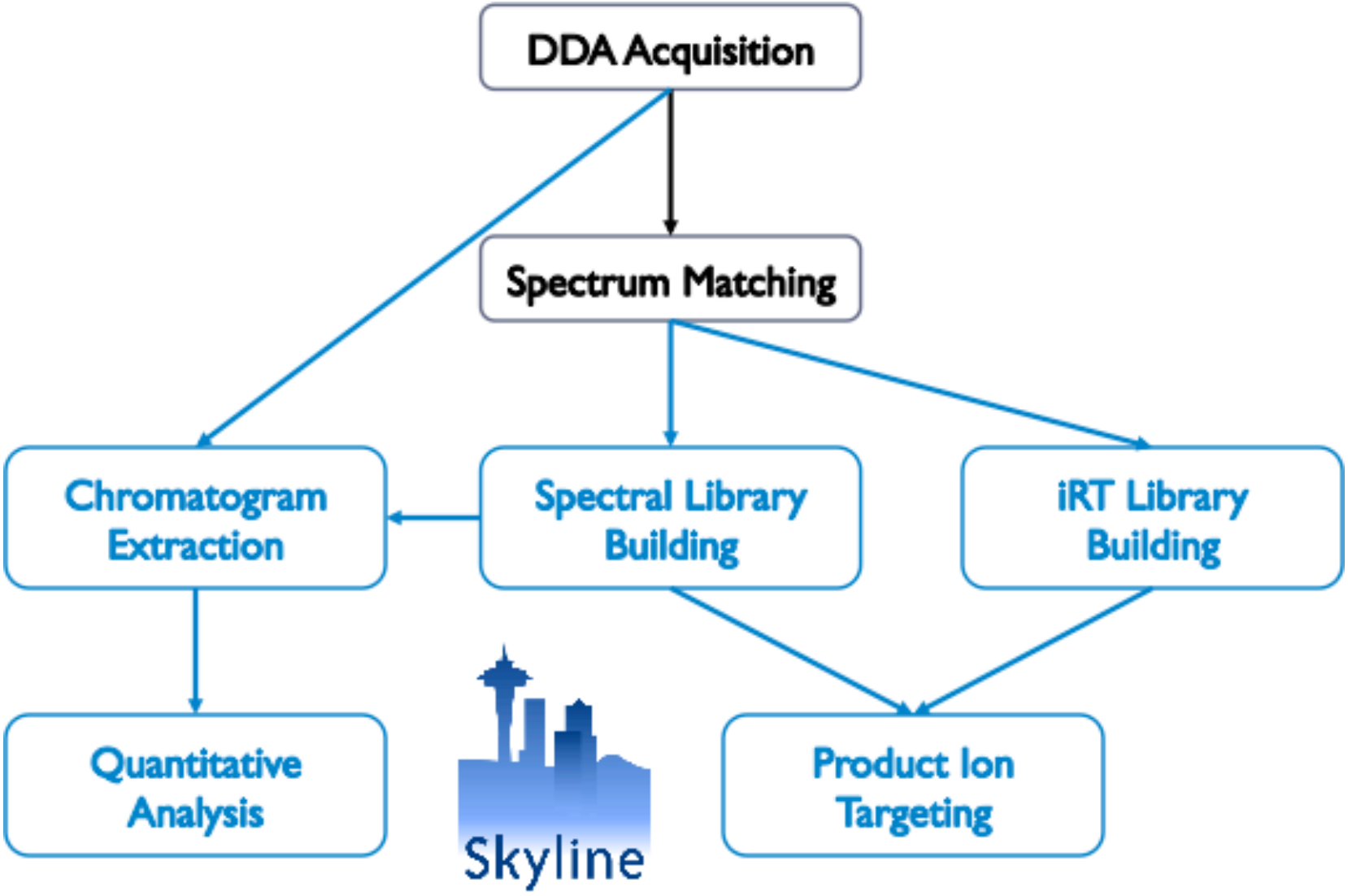
Matthias Mann, Max Planck Institute for Biochemistry
http://www.biochem.mpg.de/mann/SILAC/index.html

Isobaric Tagging: iTRAQ/TMT



http://www.piercenet.com/method/quantitative-proteomics

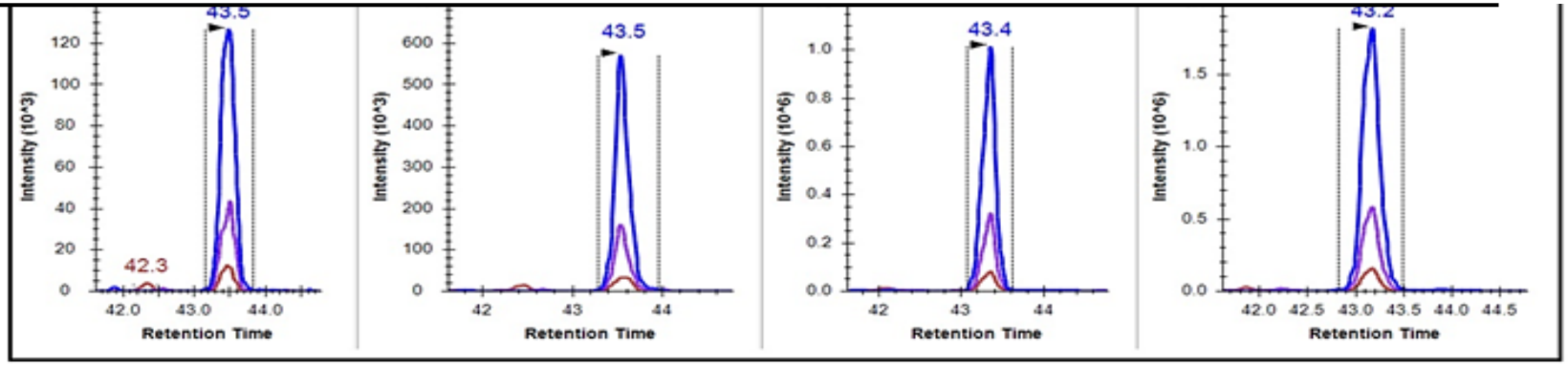
Targeted Quantitation

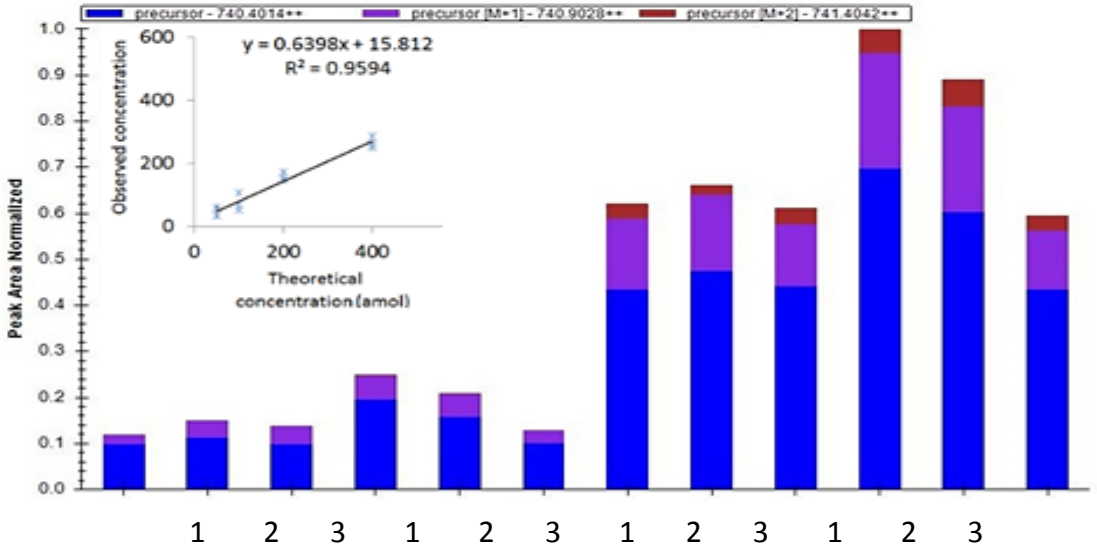


Targeted Quantitation using Skyline

BSA

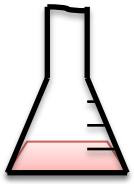
50 amol 100 amol 200 amol 400 amol



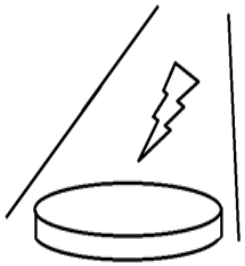


Replicate peak areas

Lydia Contreras Quantitation Workflow

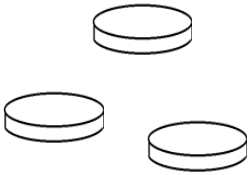


D. radiodurans were cultured to exponential (OD = 1) or stationary (OD = 3) phase in 30 degree shaker.

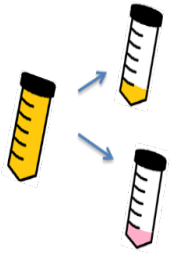


Cells were kept cold on ice and irradiated under 0, 2, 5 & 15 kGy (250Gy/s) with a 10 MeV, 18 kW LINAC β ray source.

Cells were diluted 4-5 fold to OD ~1 and recovered in fresh culture (TGY) medium for 2 hours at 30° C.



Cells were plated on TGY plates and incubated at 30 degree to measure survival rate (CFU).



Total RNA and protein were prepared from recovered cells. Cells were sonicated and treated with lysozyme to obtain the protein lysate.

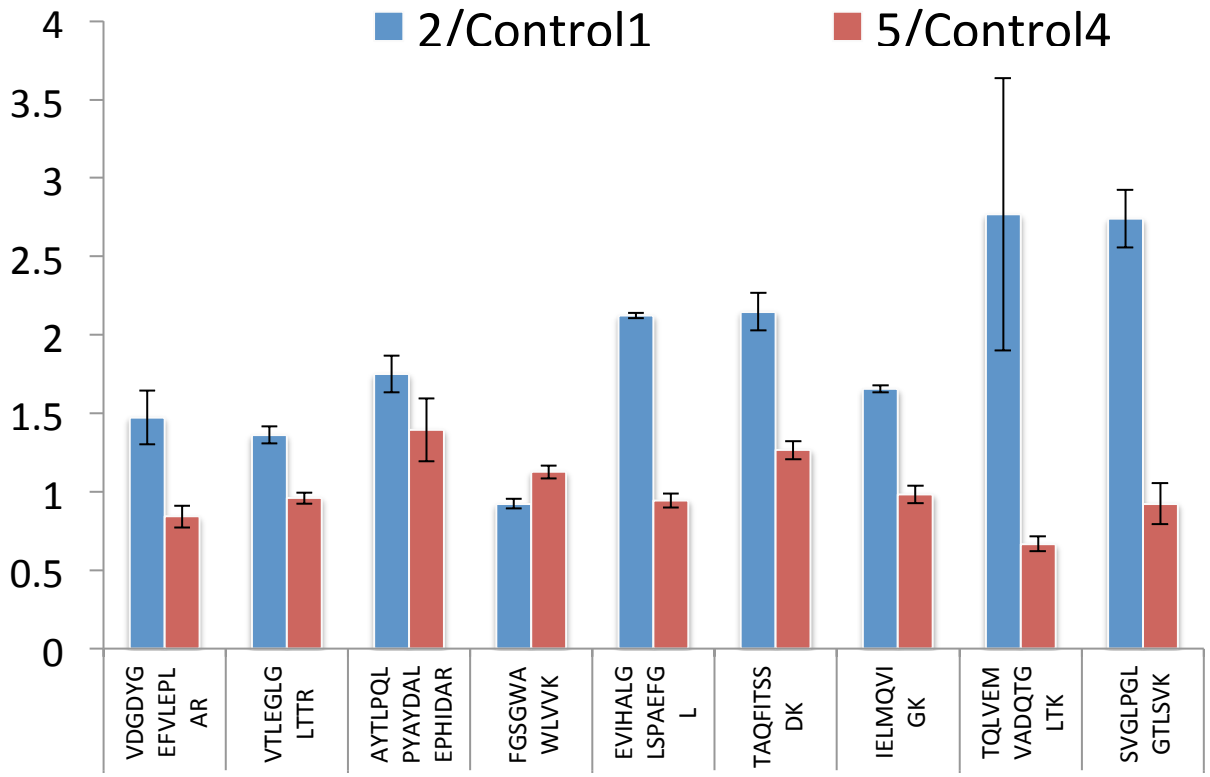
The protein lysates were digested with trypsin and analyzed with UPLC-MS/MS on the Orbitrap Elite.

Differential protein ID

High fold change proteins under 15 kGy irradiation in log phase

Protein	Fold change
Serine esterase, GN=DR_0657	162
Succinate-semialdehyde dehydrogenase [NADP(+)], GN=ssdA	99
Fibronectin/fibrinogen-binding protein, GN=DR_0559	33
Alkaline shock protein-related protein, GN=DR_2068	14
N utilization substance protein B homolog, GN=nusB	14
Response regulator, GN=DR_0743	12
D-3-phosphoglycerate dehydrogenase, GN=DR_1291	10

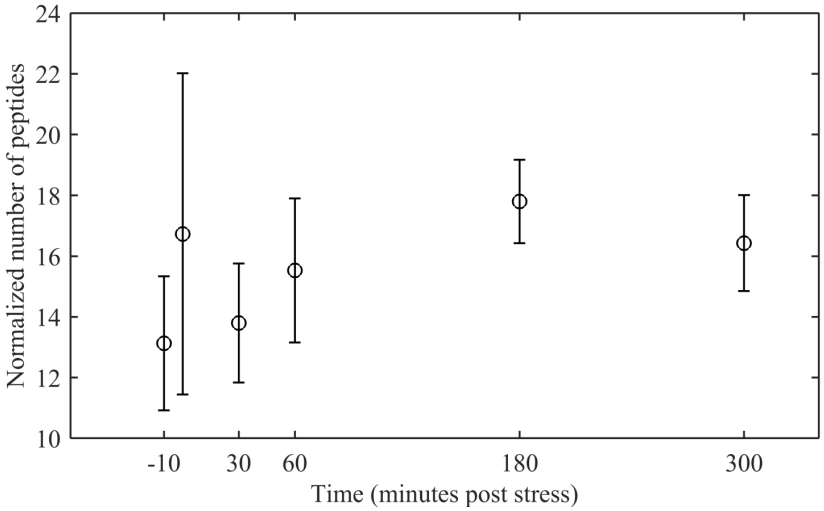
Quant with Synthetic Peptides



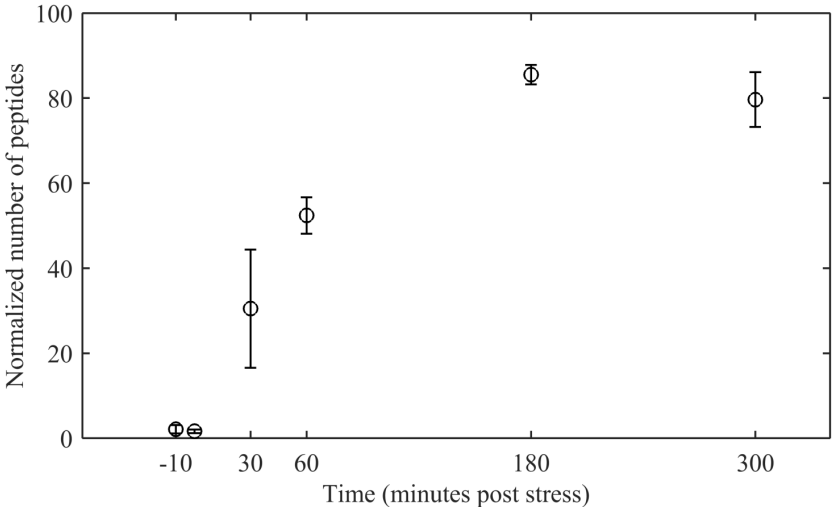
- *Samples were treated with low (2) and high (5) kGy*
- *Peak area from the targeted peptide is normalized against synthetic peptide*
- *Ratios obtained by comparing to non-irradiated controls*

Temporal proteomics used to measure time dependent protein expression

Protein regulator



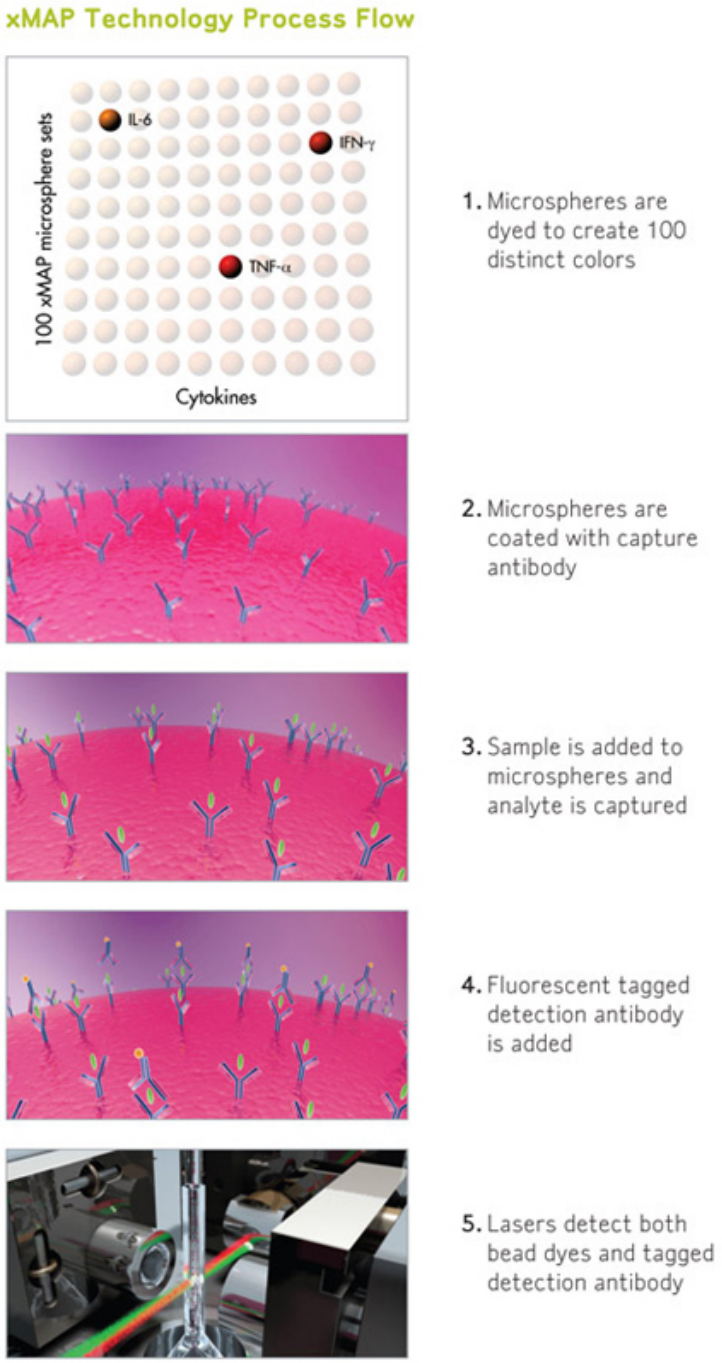
Putative ABC transporter periplasmic-binding protein YdcS



Sowa et al. Nucleic Acids Res. 2017 Feb 28;45(4):1673-1686. doi: 10.1093/nar/gkx048.

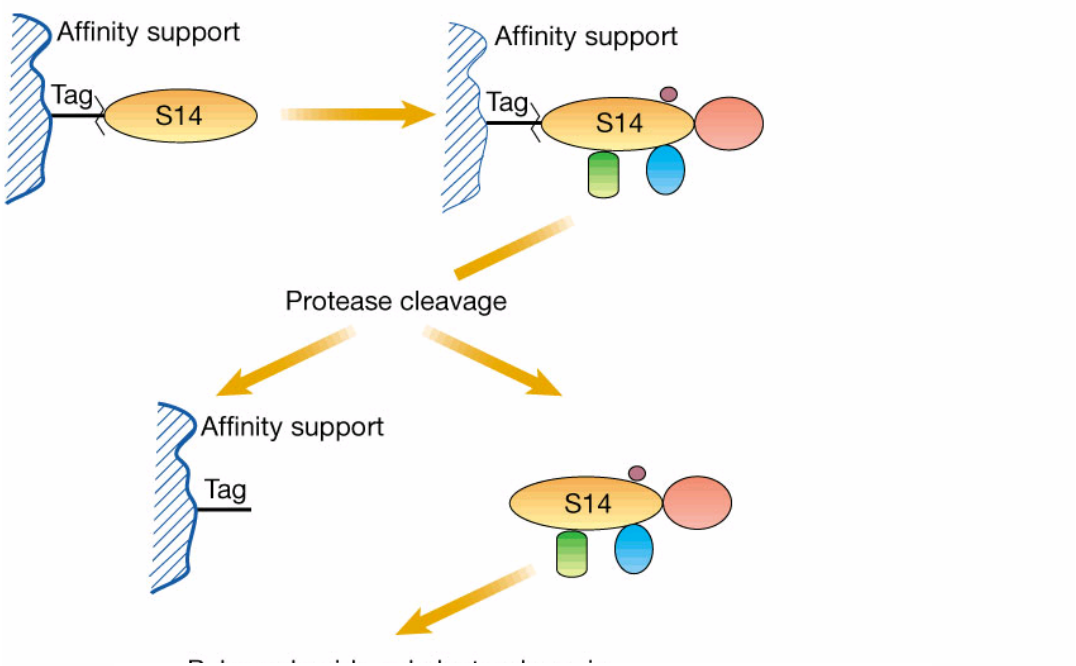
Luminex xMAP technology

Systems using xMAP technology perform a variety of bioassays including immunoassays on the surface of fluorescent-coded beads known as microspheres, which are then read in a compact analyzer. Using two lasers and high-speed digital-signal processors, the analyzer reads signals on each individual microsphere particle. The capability of adding multiple conjugated beads to each sample results in the ability to obtain multiple results from each sample. Open-architecture xMAP technology enables multiplexing of many types of bioassays reducing time, labor and costs over traditional methods.



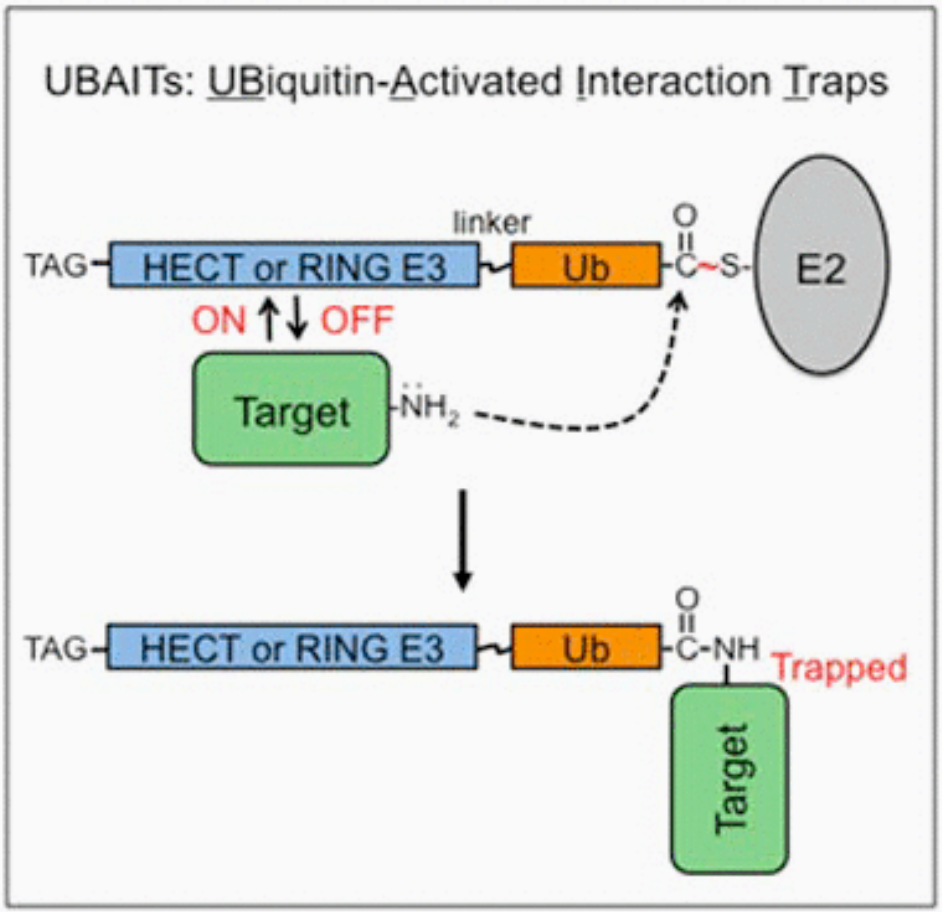
Protein interactions

- Yeast-2 hybrid
- Bait protein capture and MS identification



Pandey and Mann, *Nature* 405 *6788* 837 - 846 (2000)

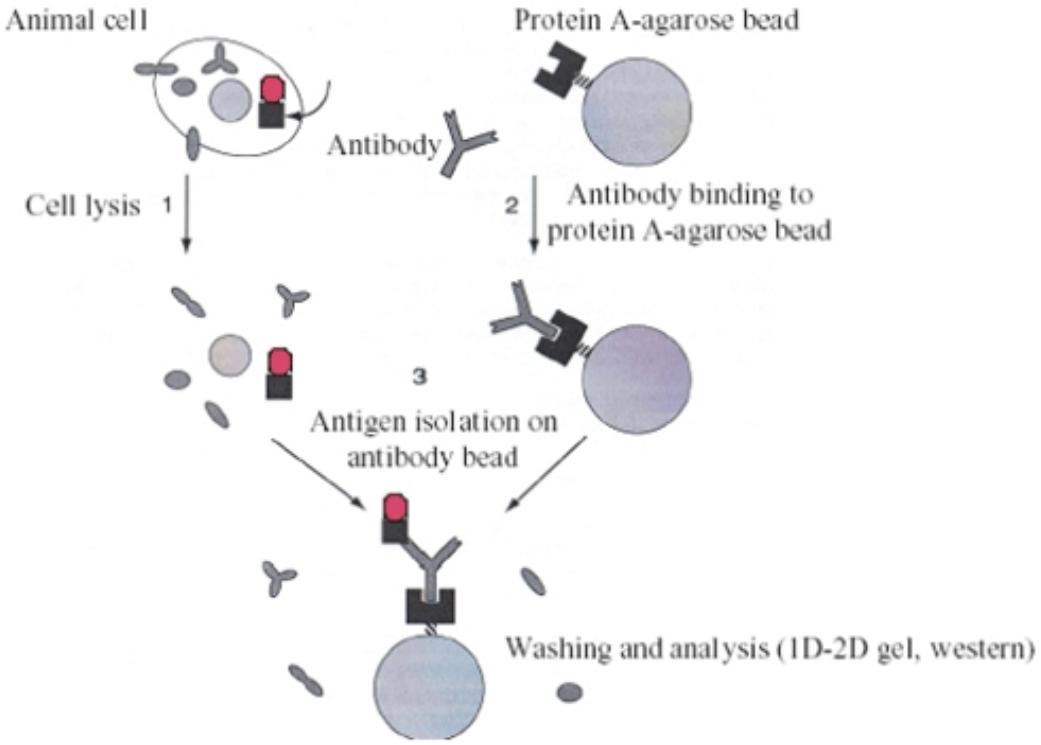
Huibregtse lab develops UBAIT method



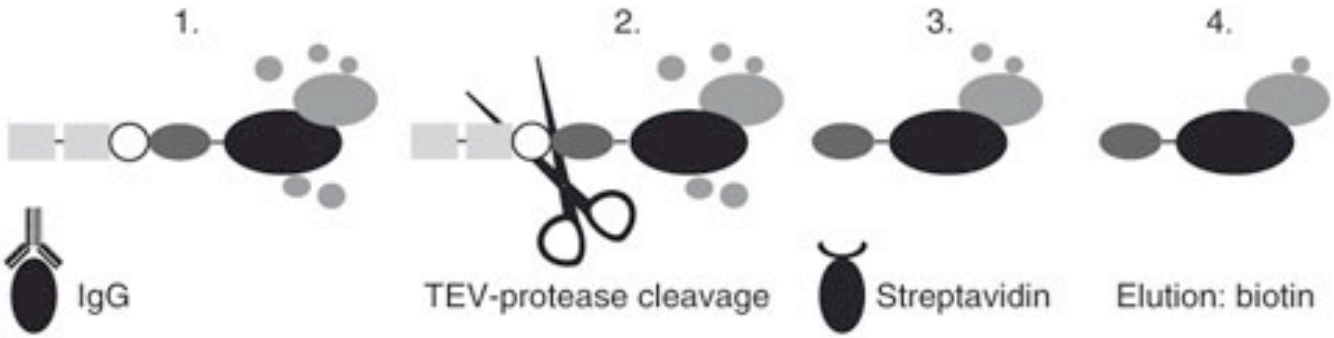
LC-MS/MS identifies known and novel E3 ligase interacting proteins from transient interactions

EMBO Rep. 2015 Dec;16(12):1699-712. doi: 10.15252/embr.201540620. Epub 2015 Oct 27.

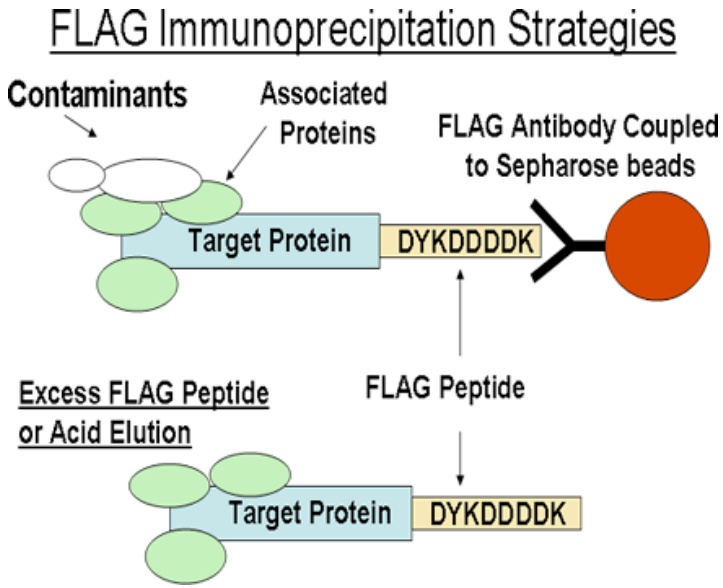
Co-Immunoprecipitation: Protein specific antibodies



Tandem Affinity Purification TAP tag

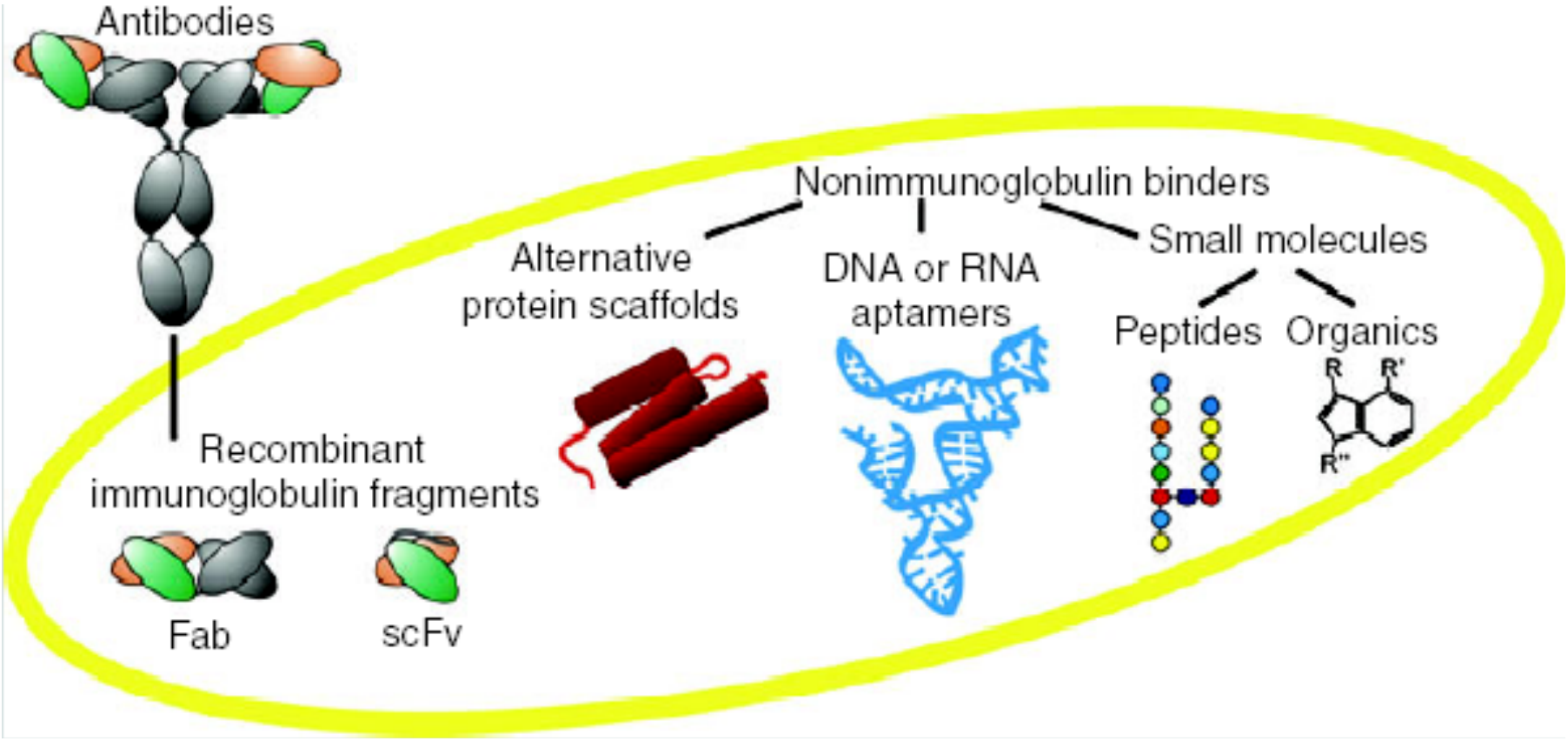


Most common epitope tags are:
His-tag
Flag-tag
V5-tag
Myc-tag
HA-tag



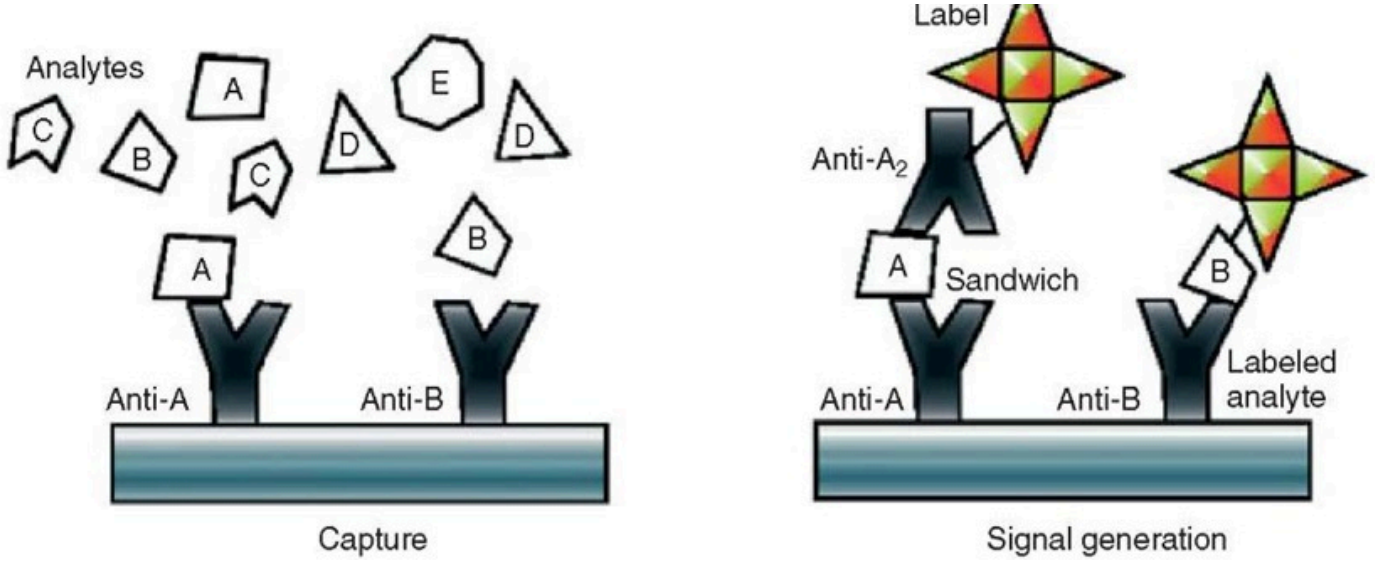
Problems – antibody cross-reactivity.

Different Types of Affinity Binders

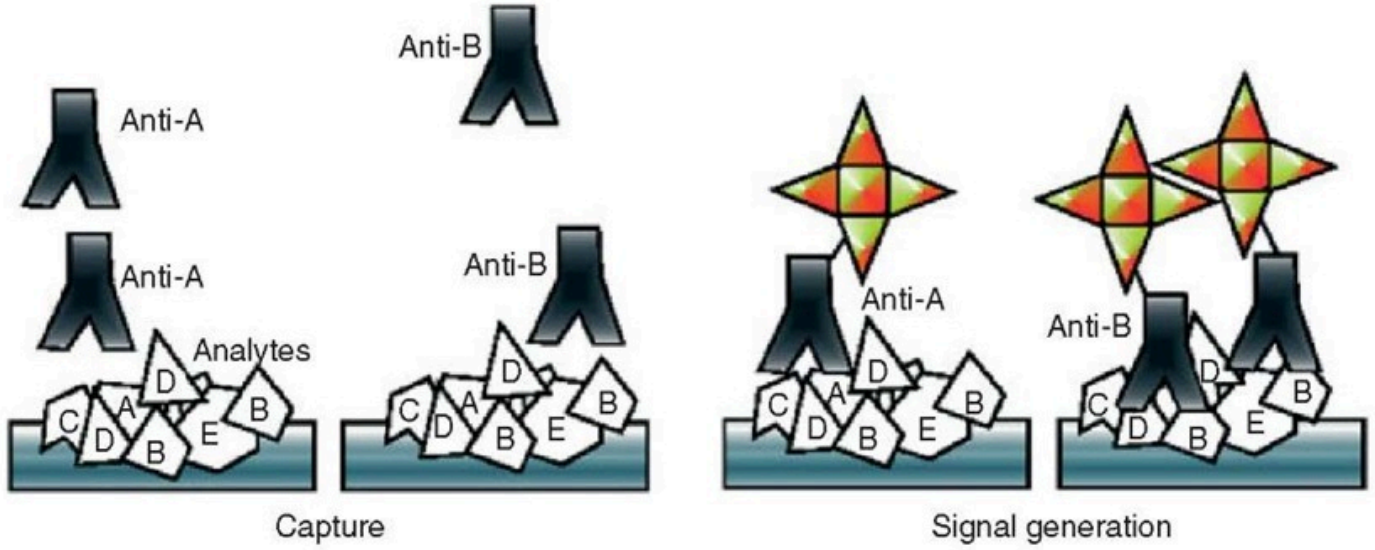


Nature Methods - **4**, 13 - 17 (2007)
Systematic generation of proteome binders by EU consortium

Forward Phase Protein Microarray Spots Antibody and Probes with Multiple Samplesc

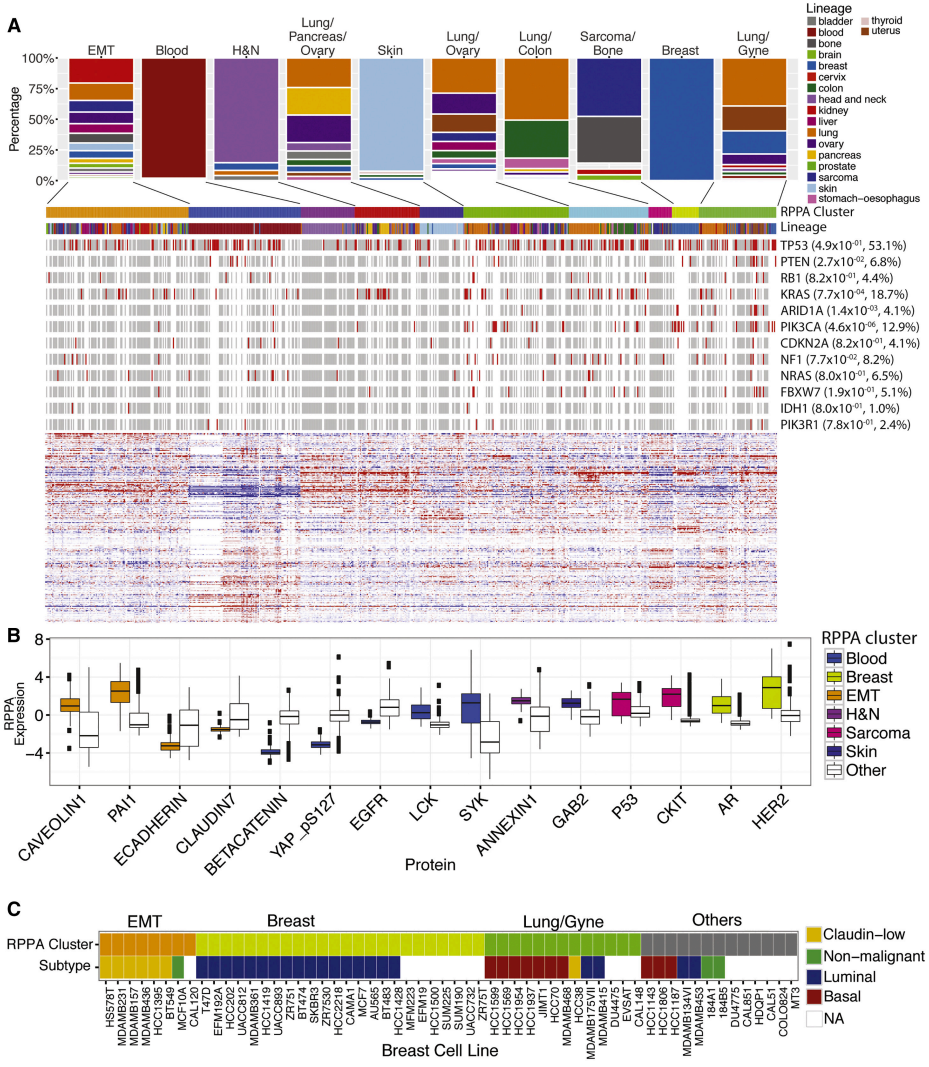


Reversed Phase Protein Microarray Spots Many Lysates and Probes with an Antibody



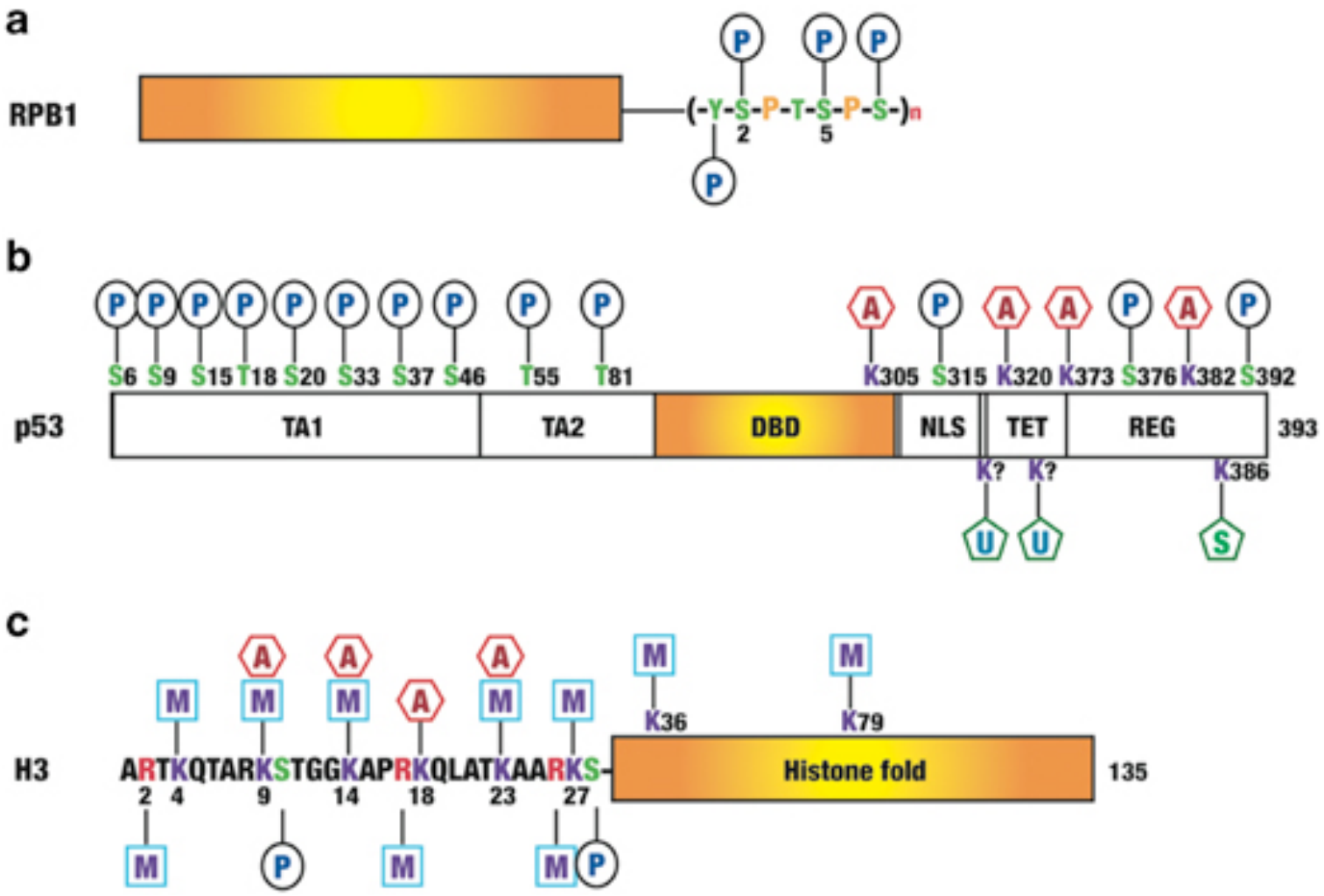
http://what-when-how.com/proteomics/basic-techniques-for-the-use-of-reverse-phase-protein-microarrays-for-signal-pathway-profiling-proteomics/

Clustered Heatmap of Human CancerI Cell Lines Based on RPPA Protein Expression Data



Li et al., Cancer Cell, 31: 225-239 2017. DOI: 10.1016/j.ccell.2017.01.005

Modifications Determine Protein Interactions, Localization & Function



P in oval, phosphorylation; A in hexagon, acetylation; U in pentagon, ubiquitination; S in pentagon, sumoylation; M in square, methylation

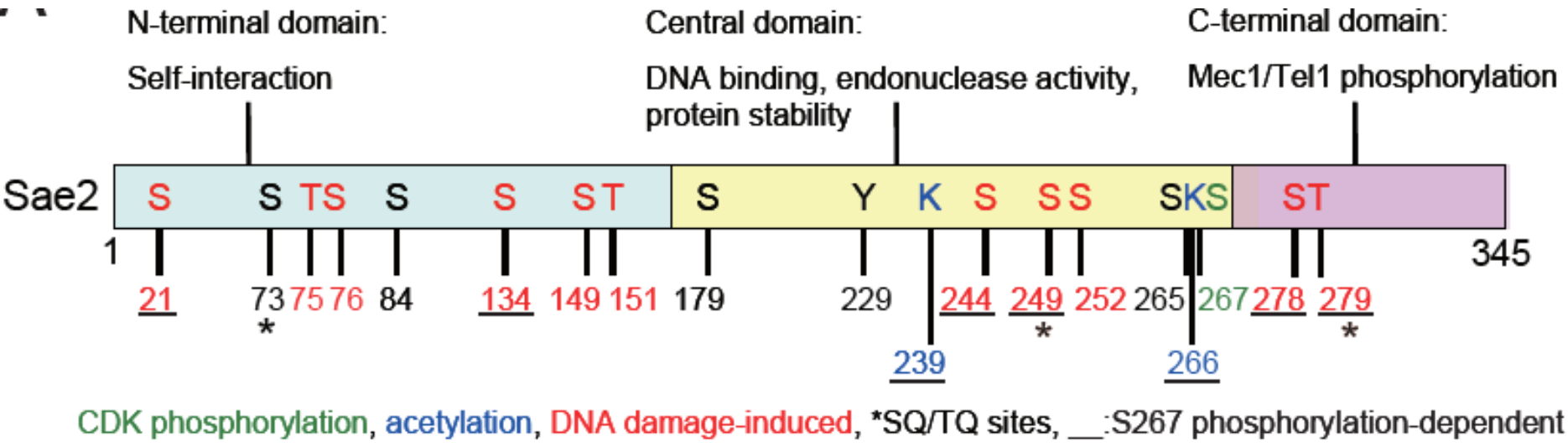
Xiang-Jiao Yang, **Multisite protein modification and intramolecular signaling** Oncogene (2005) 24, 1653–62

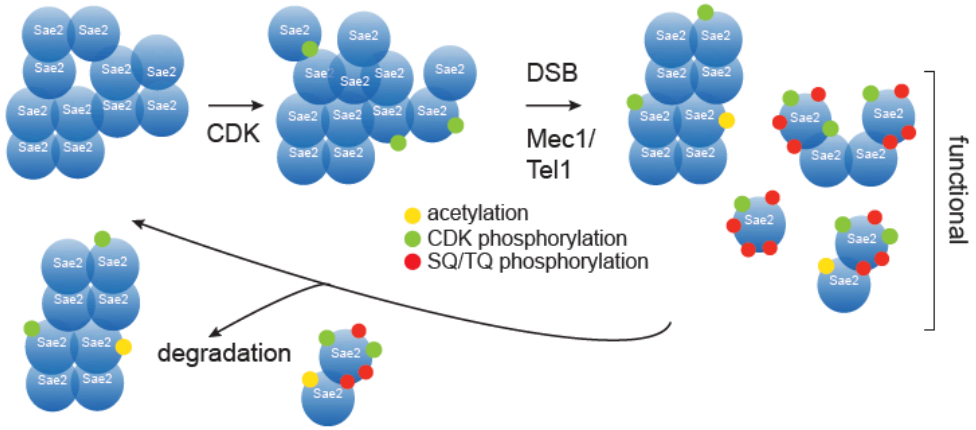
Detecting Modifications by MS

- Start with microgram levels of single protein or mg of complex sample
- Use modification enrichment: affinity chromatography, antibody pulldown, biotinylation, click chemistry
- Purify protein/protein complex
- Use multiple proteases to increase coverage
- Try targeted MS/MS on modified peptide
- Use Ascore to asses site localization
- Validate with synthetic modified peptide standard or antibody

Single Protein: PTM controls activation of Sae2

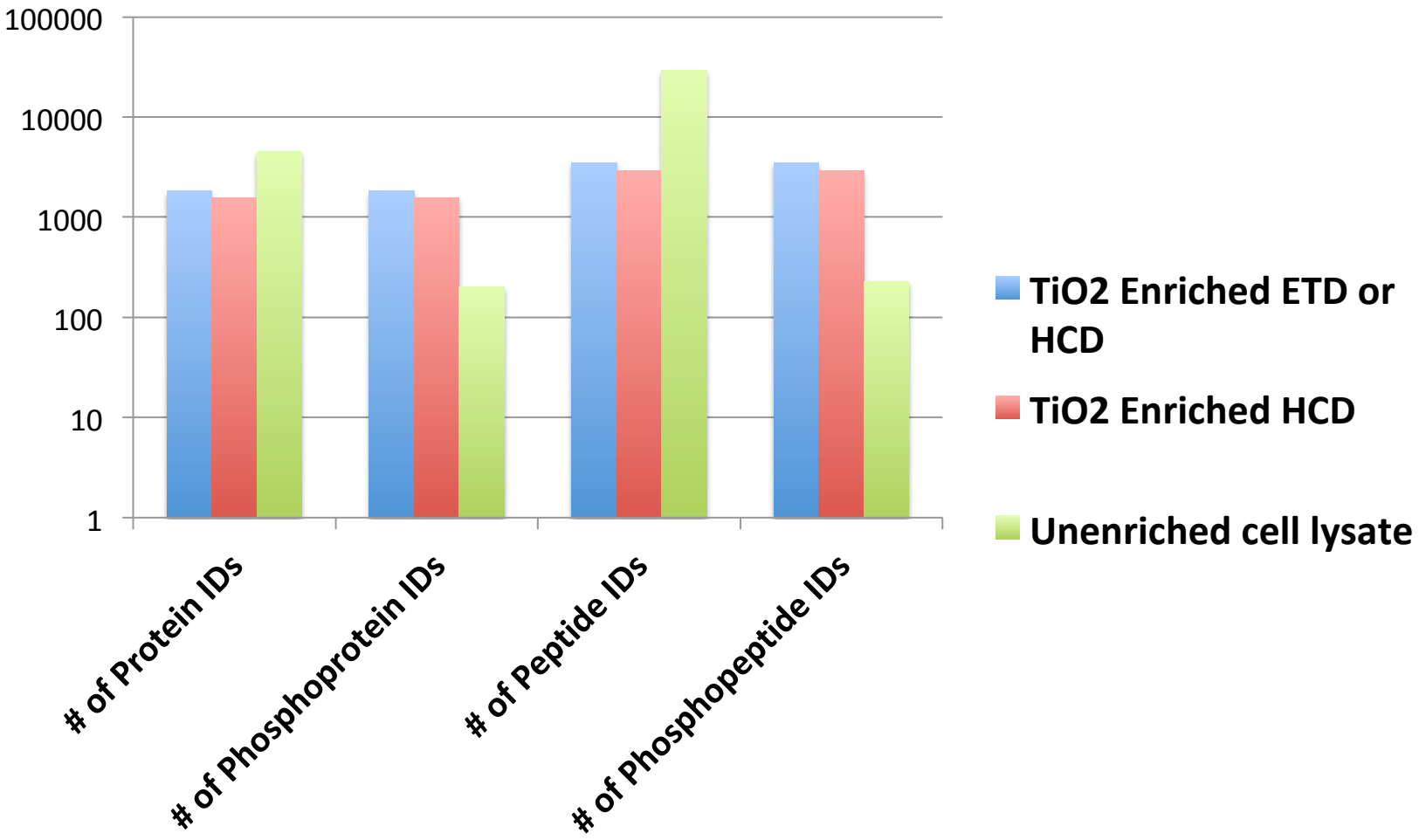
LC-MS/MS using multiple proteases map modifications of Sae2



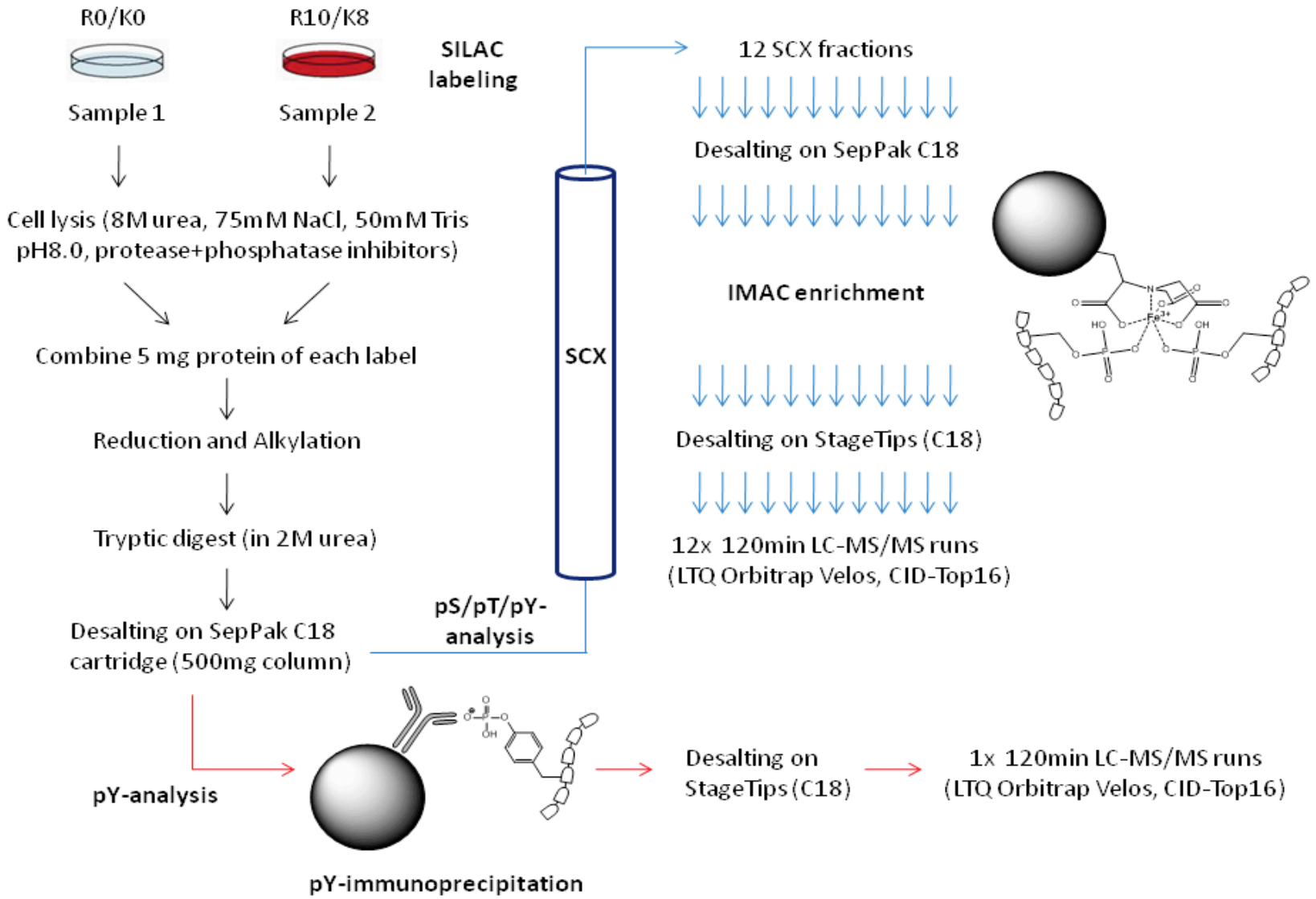


Fu et al. Mol Cell Biol. 2014 Mar;34(5):778-93. doi: 10.1128/MCB.00963-13.

Proteome wide: phosphopeptide enrichment

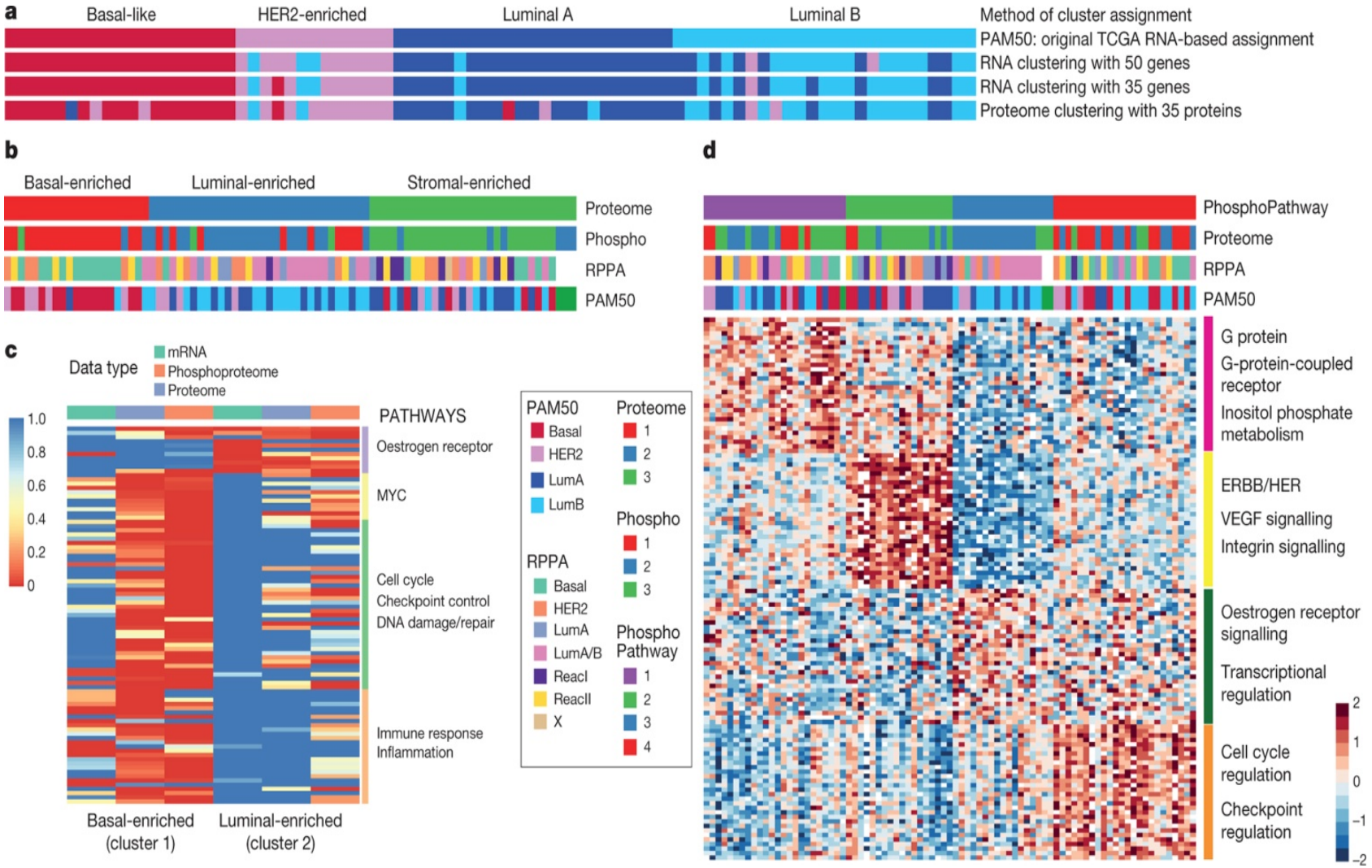


Phosphoproteomics



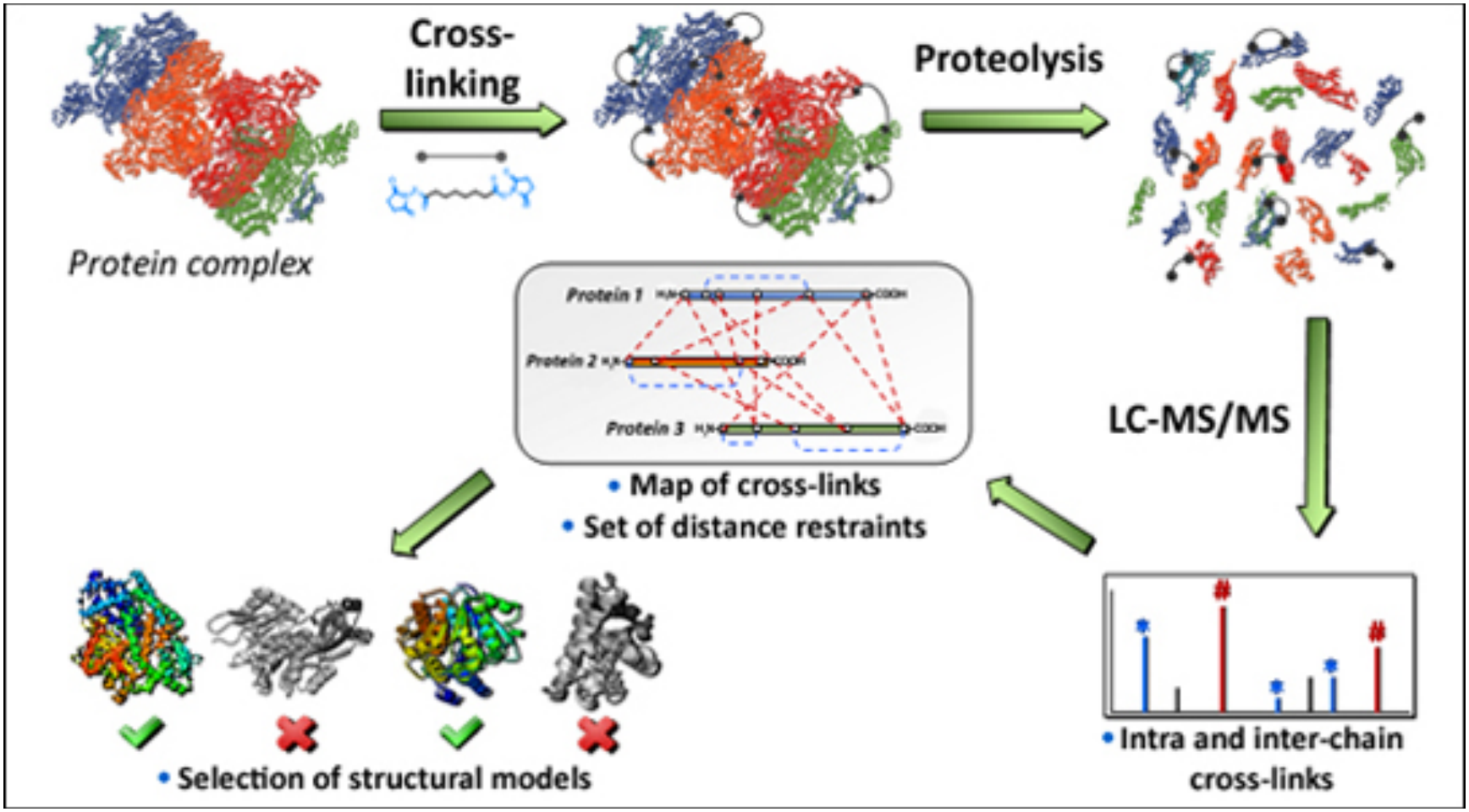
http://www.broadinstitute.org/scientific-community/science/platforms/proteomics/phosphoproteomics

Proteomic and phosphoproteomic subtypes of breast cancer identified



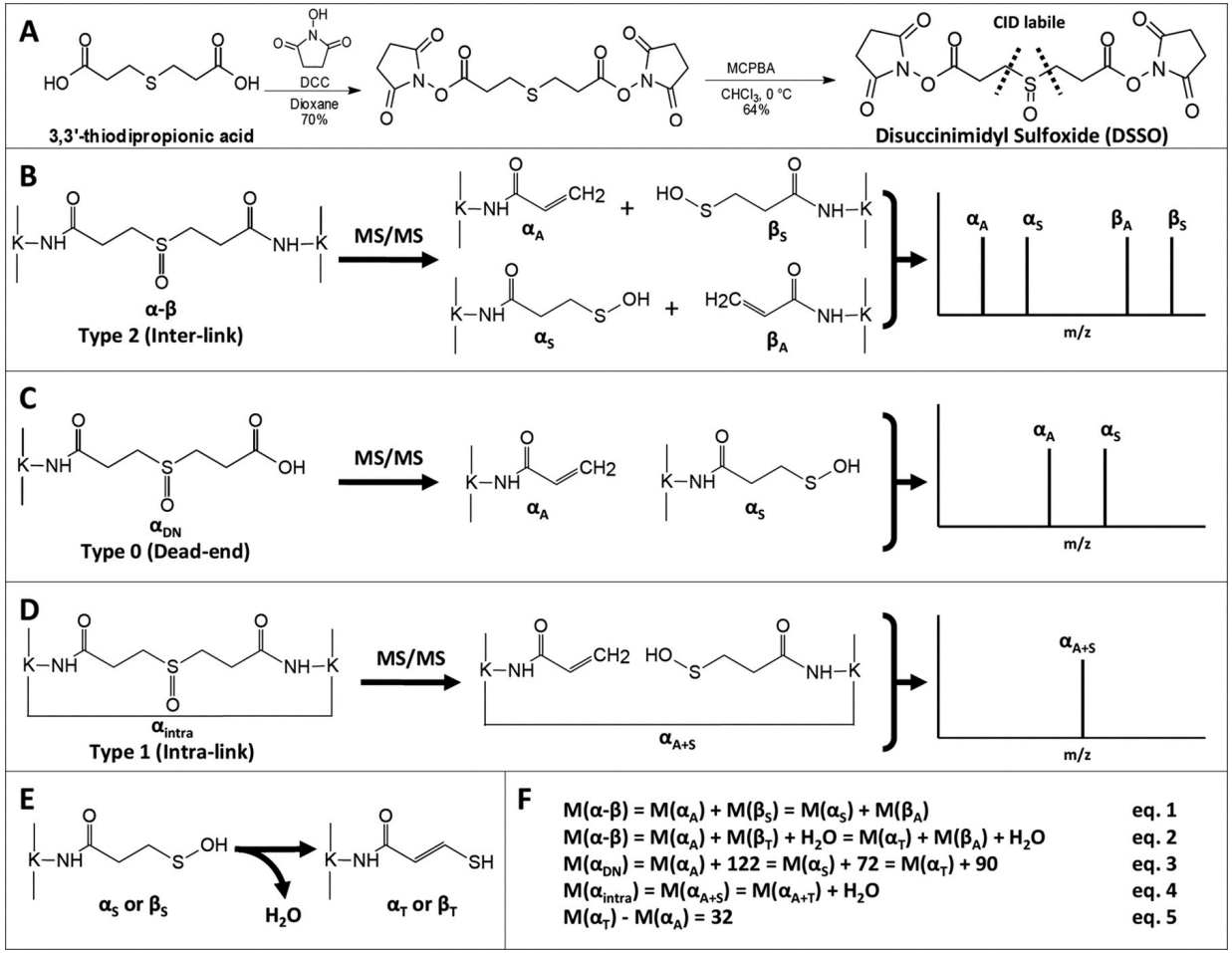
P Mertins *et al. Nature* 1–8 (2016) doi:10.1038/nature18003

Structural Proteomics
Crosslinking MS

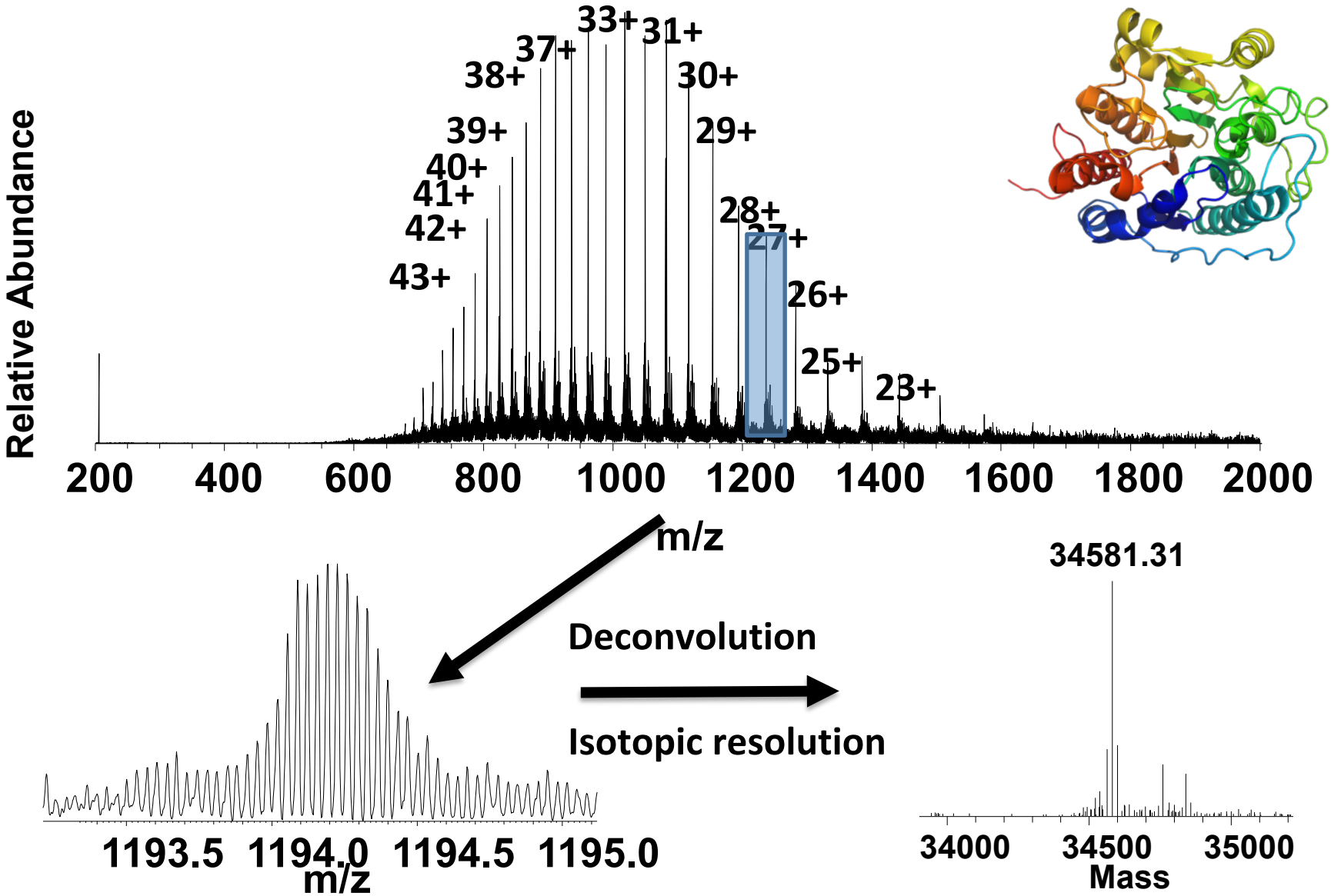


http://daltonlab.iqm.unicamp.br/research.html Fabio Gozo Dalton Mass Spectrometry Lab

DSSO is a MS2 cleavable crosslinker

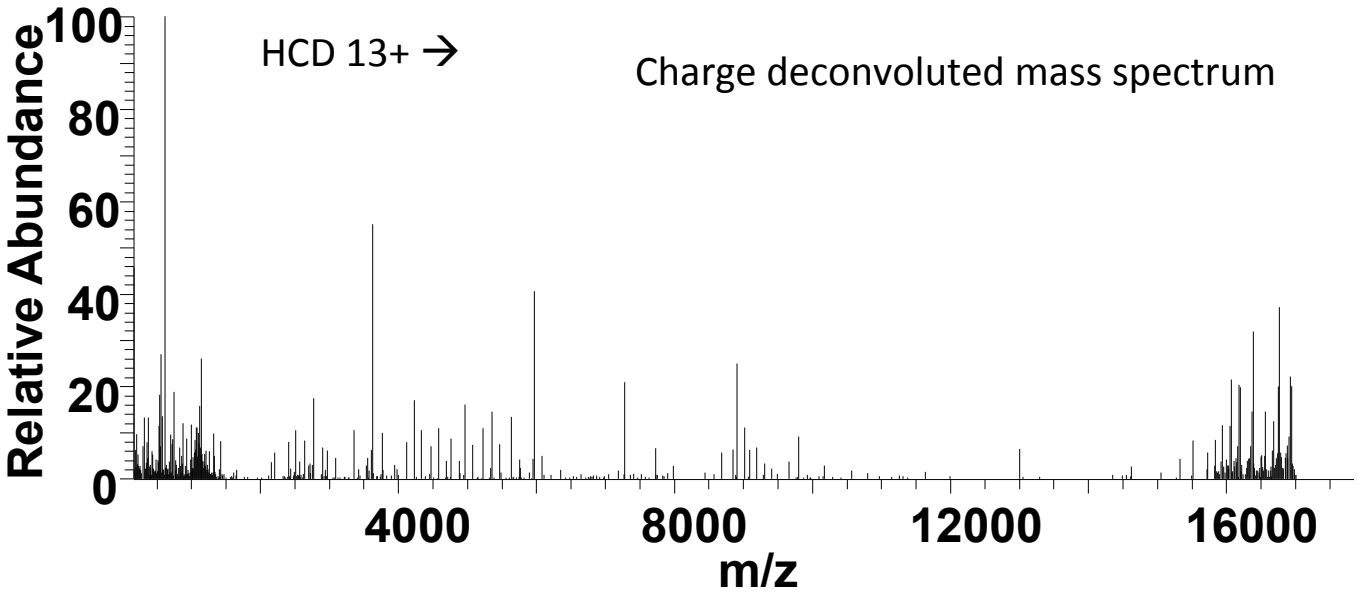


High Resolution Protein Analysis



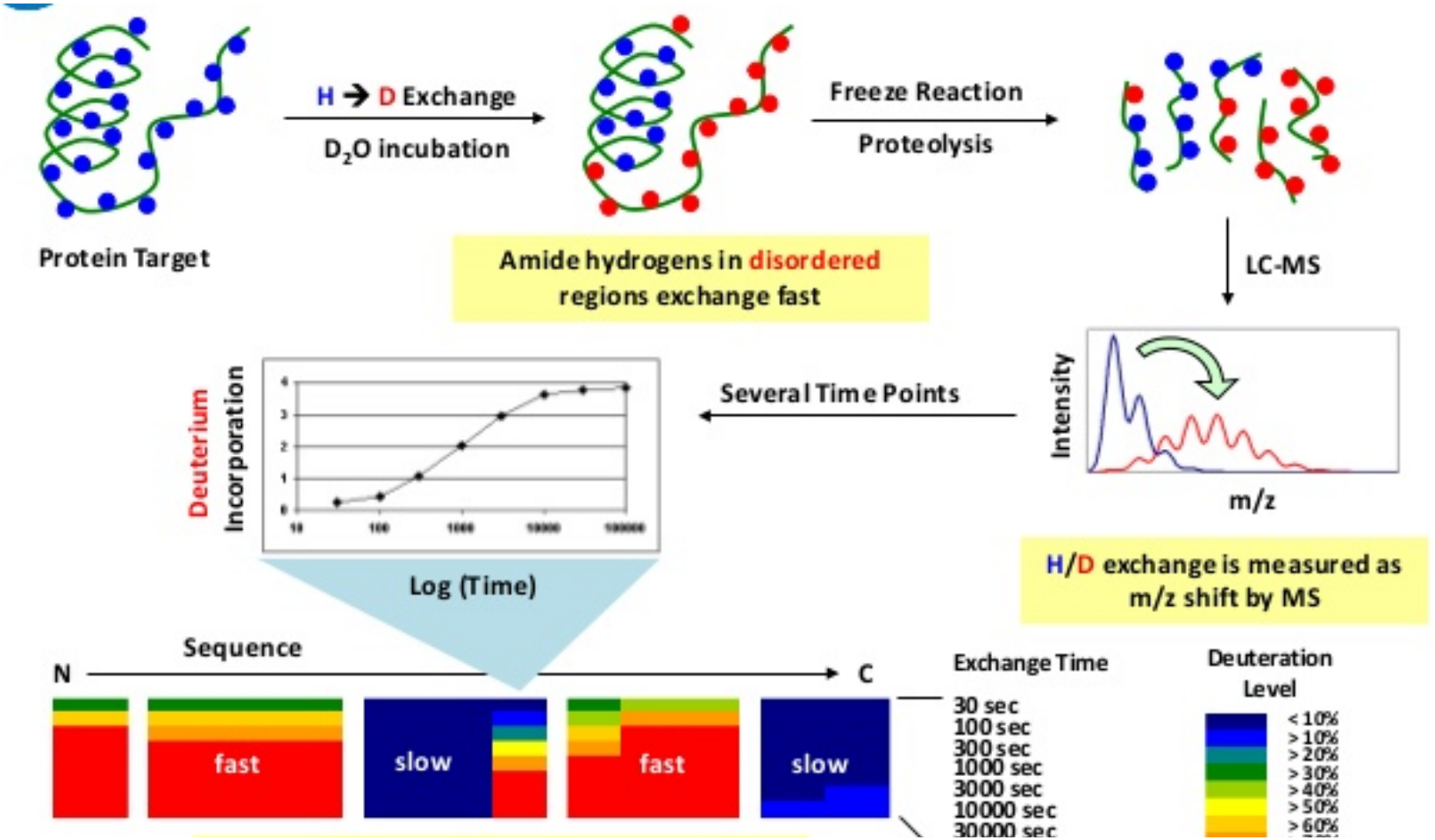
Top-Down Protein MS/MS: Myoglobin

Selected protein m/z fragmented with HCD for MS/MS in Orbitrap



N G L S D G E W Q Q V L N V W G K V E A D I A G H G 25
26 Q E V L I R L F T G H P E T L E K F D K F K H L K 50
51 T E A E M K A S E D L K K H G T V V L T A L G G I 75
76 L L K K K G H H E A E L L K P L A Q S H A T K H K I P 100
101 I K Y L E F I S D A I I H V L H S K H P G D F G A 125
126 D A Q G A M T K A L E L F R N D I A A K Y K E L G 150
151 F Q G C

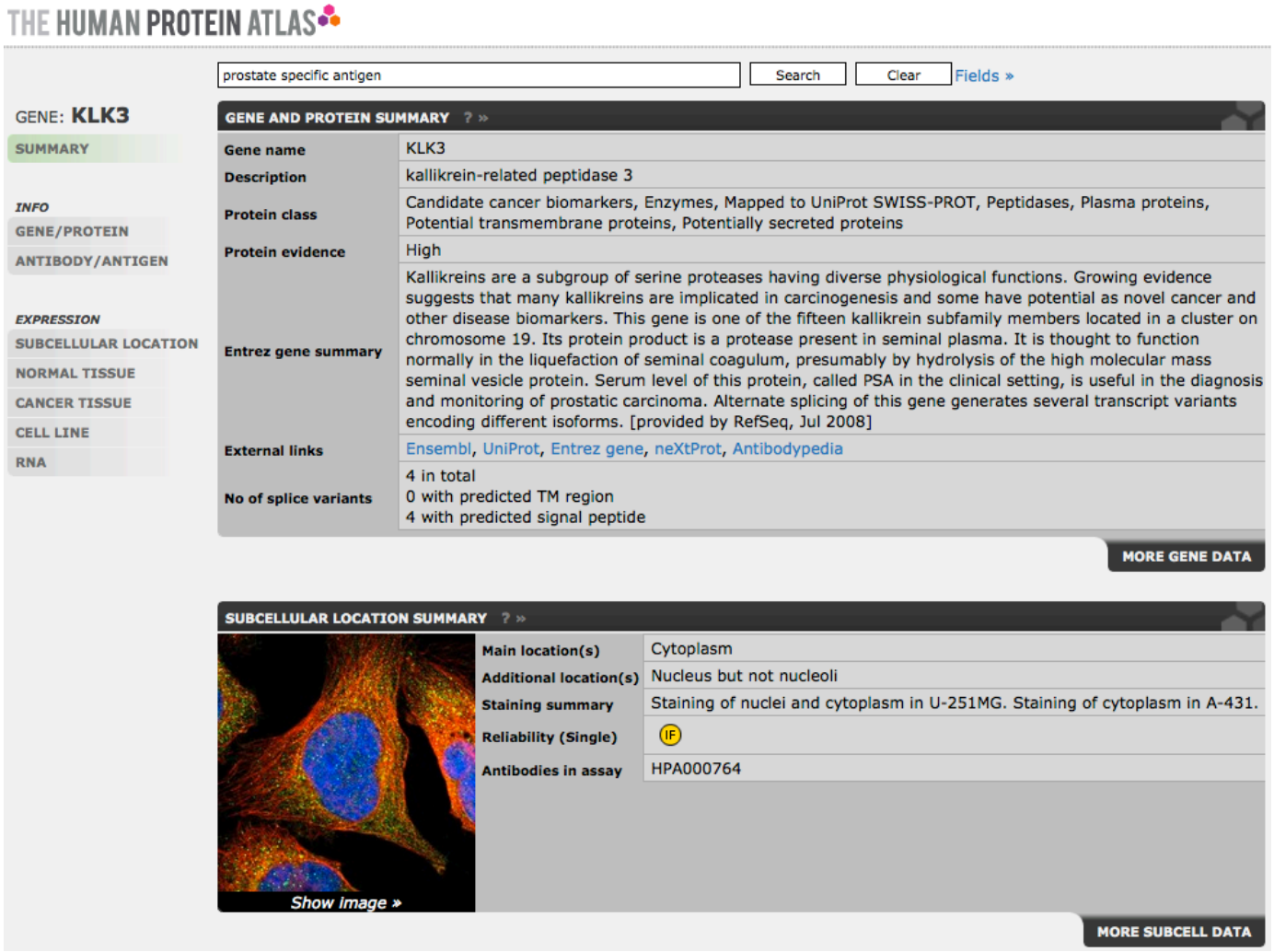
Hydrogen Deuterium Exchange



Hamuro http://www.slideshare.net/Chrom_Solutions/biopharmfocusonhdxappswebinar

Cellular Maps using Tissue Array and Omics Human Protein Atlases: Tissue, Cell and Pathology

v16 with more than 25,000 antibodies, targeting proteins from 17,000 human genes

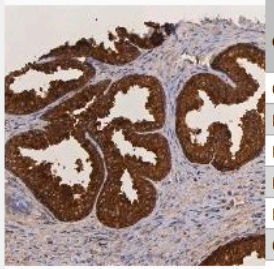


http://www.proteinatlas.org/

PSA localized in prostate tissue and expressed in prostate cancer

NORMAL TISSUE & ORGAN SUMMARY

Expression summary	Cytoplasmic expression exclusively in prostate. Caution: Based on antibodies targeting proteins from multiple genes.
Tissue specificity	Expressed in 1 out of 82 cell types
Reliability (APE)	High
Antibodies in assay	CAB000070, HPA000764



Show image »

Organ	No of cell types	Protein expression
CNS (brain)	11	
Hematopoietic (blood)	8	
Liver and pancreas	5	
Digestive (GI-tract)	13	
Respiratory (lung)	4	
Cardiovascular	1	
Female tissues	13	
Placenta	2	
Male tissues	5	
Urinary tract (kidney)	3	
Skin and soft tissues	14	
Endocrine tissues	3	

Level of annotated protein expression: High, Medium, Low, None

MORE TISSUE DATA

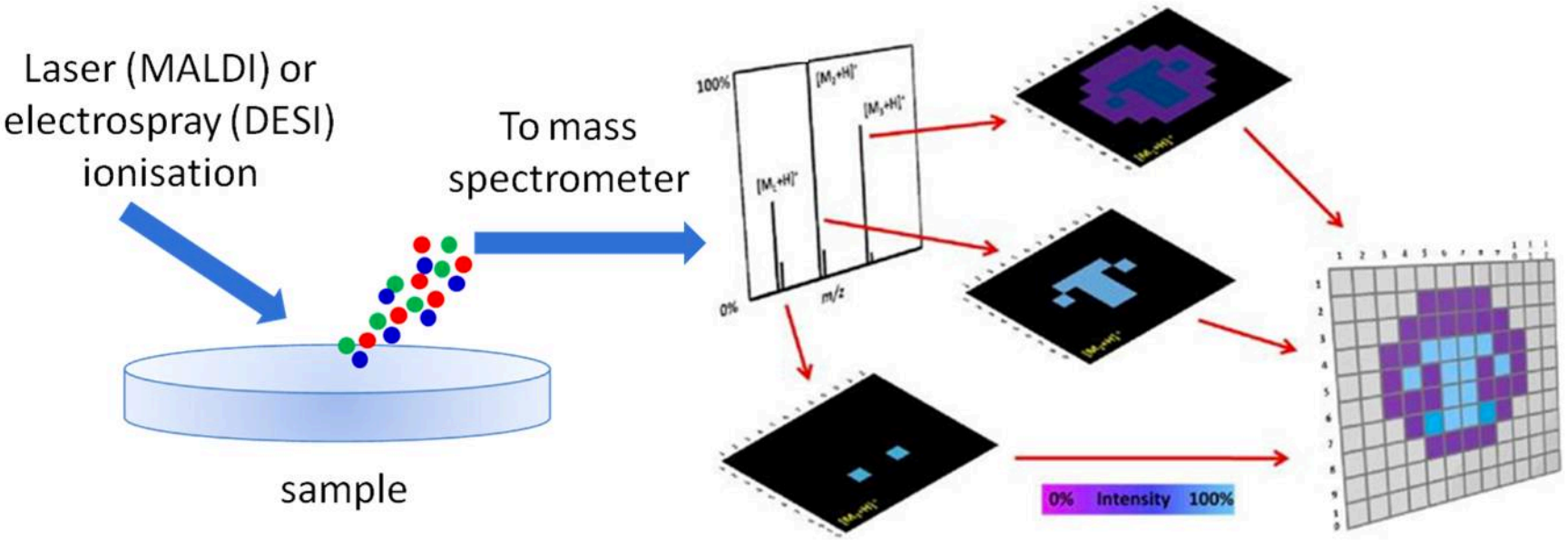
CANCER TISSUE SUMMARY

Staining summary	Antibody staining in 5% of the cancers
Antibodies in assay	CAB000070, HPA000764

Tissue	Cancer staining	Protein expression of normal tissue	Tissue	Cancer staining	Protein expression of normal tissue
Breast cancer			Melanoma		
Carcinoid			Ovarian cancer		
Cervical cancer			Pancreatic cancer		
Colorectal cancer			Prostate cancer		
Endometrial cancer			Renal cancer		
Glioma			Skin cancer		
Head and neck cancer			Stomach cancer		
Liver cancer			Testis cancer		
Lung cancer			Thyroid cancer		
Lymphoma			Urothelial cancer		

Level of antibody staining: Strong, Moderate, Weak, Negative

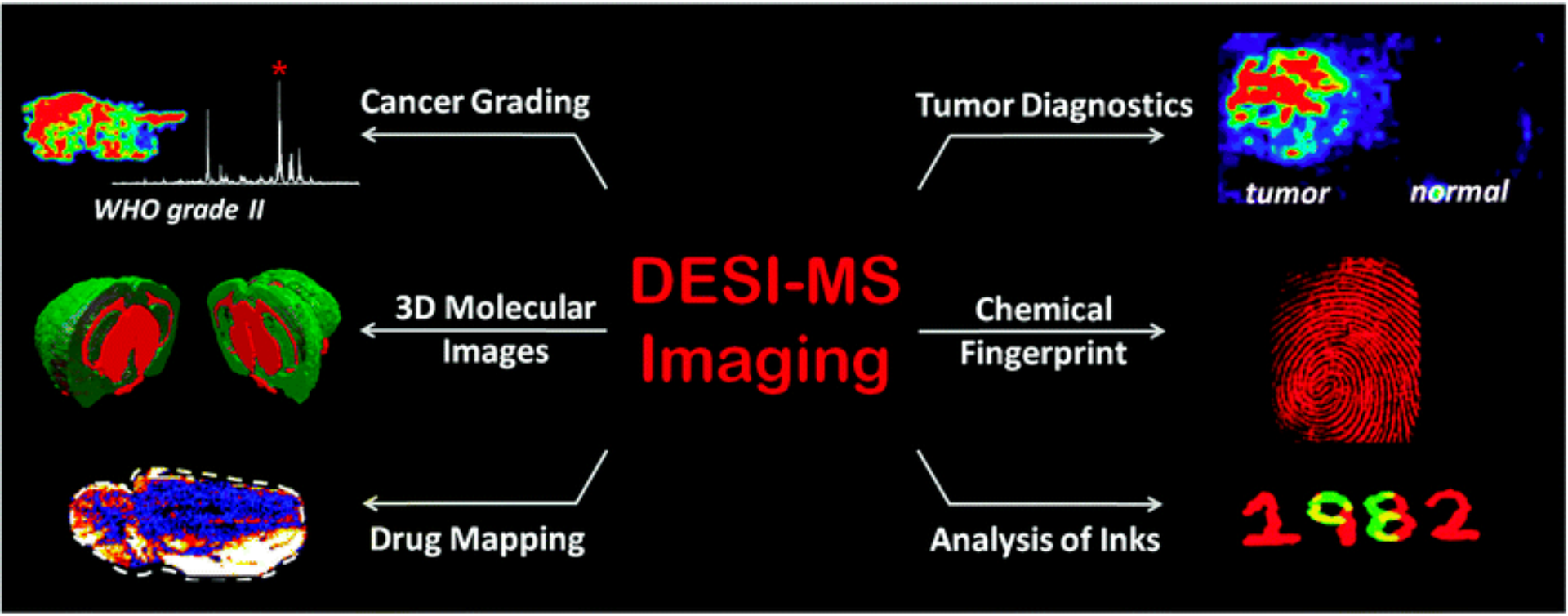
Imaging Mass Spectrometry (IMS)



- MS from tissue sections generate multiple images based on m/z from selected biomolecules

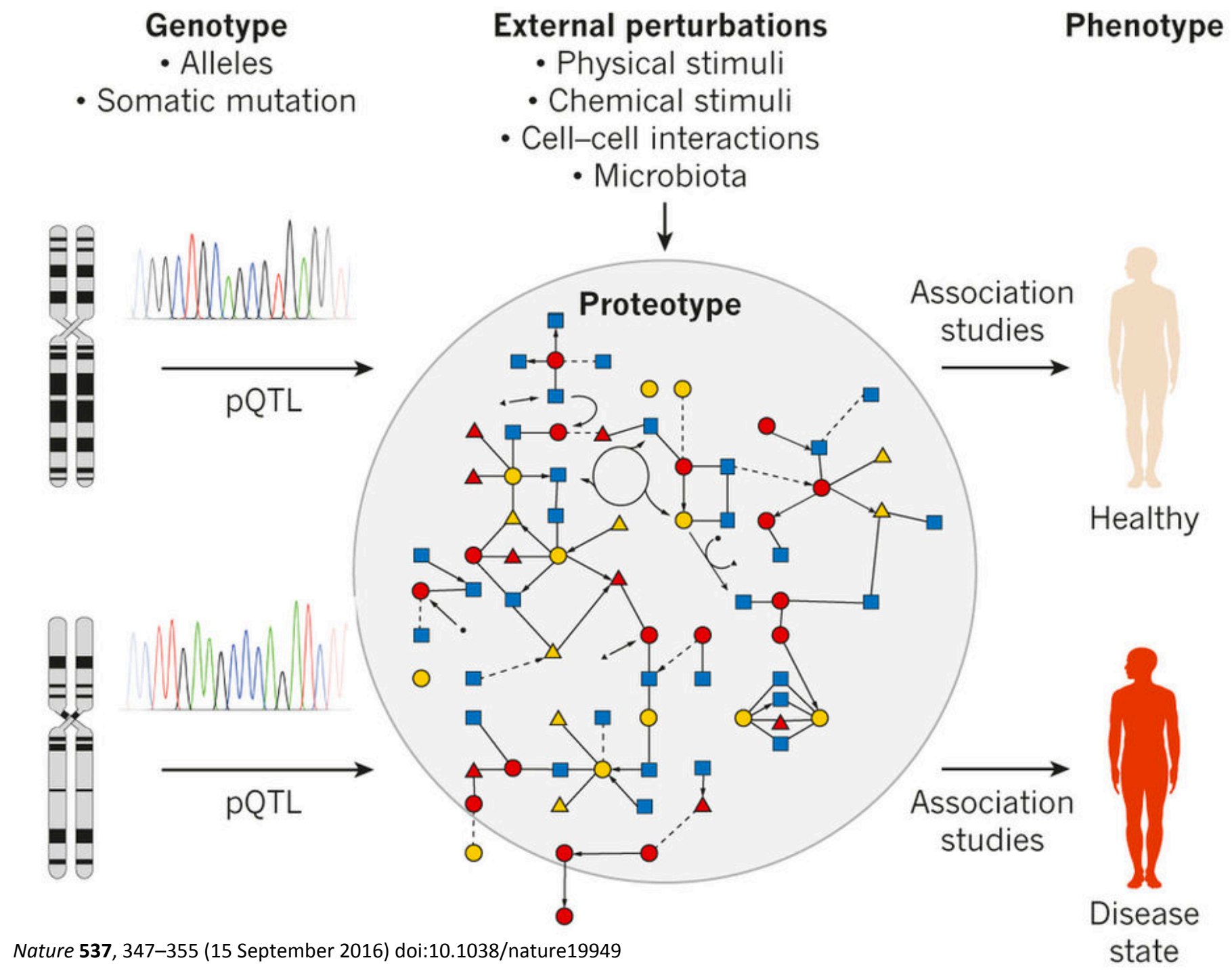
http://blog.waters.com/do-you-see-what-we-see-mass-spectrometry-imaging-is-revealing-insights-in-biomedical-research

Applications of IMS



- [Eberlin lab develops MasSpecPen for cancer diagnosis]

Chem. Commun., 2011, 47, 2741–2746



Nature **537**, 347–355 (15 September 2016) doi:10.1038/nature19949