University of Zurich[UZH]

Swiss Institute of Allergy & Asthma Research

Proteomics

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SIB Swiss Institute of Bioinformatics

After this lecture, you will be able:

- to recognize and describe the significance of protein characterization
- to explain the principle of a mass spectrometry-based experiment
- to apply the principle of *de novo* peptide spectrum matching
- to describe the principle of database-dependent peptide spectrum matching
- to calculate the false discovery rate based on a target decoy approach
- to describe various application methods of proteomics

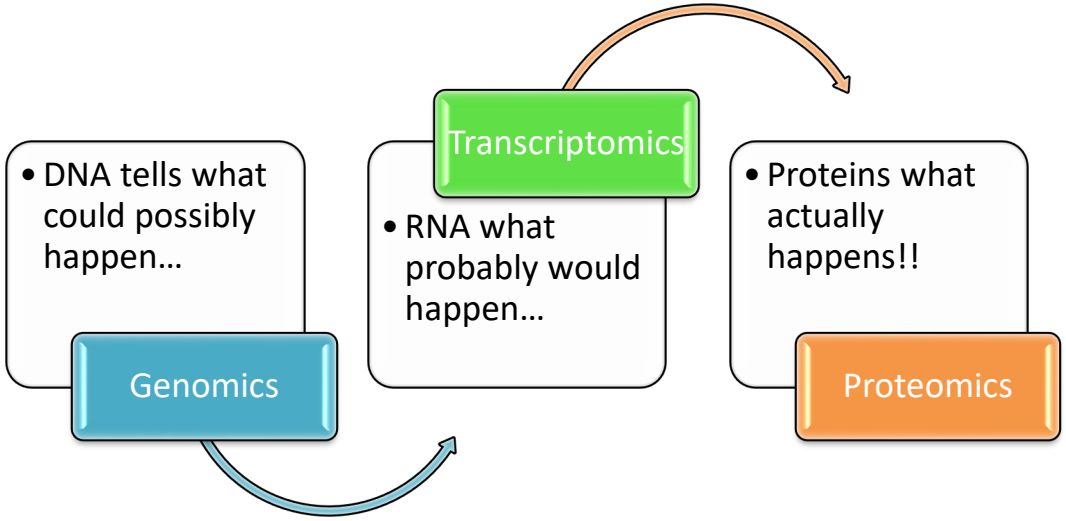
- Buzz group (7 min):

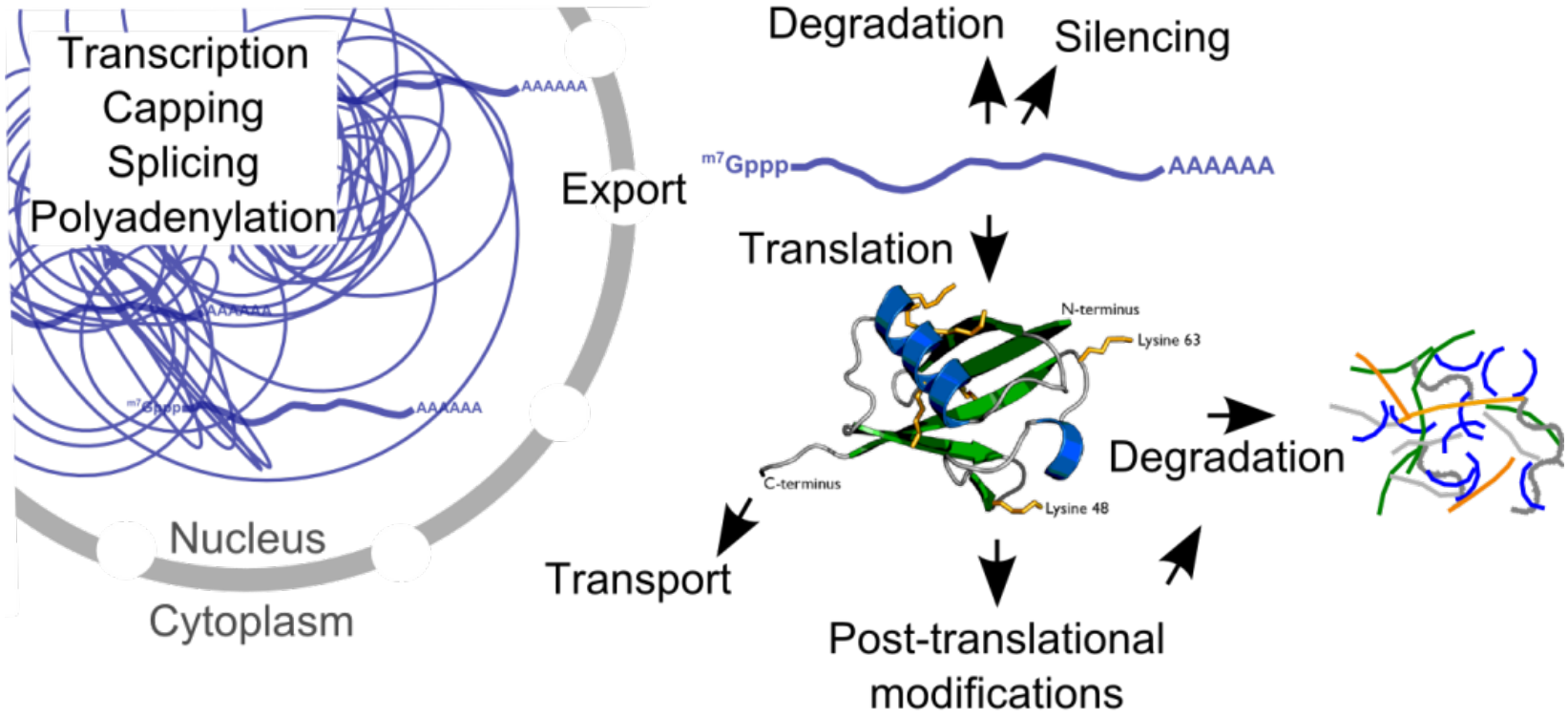
Discuss in groups of two or three why examining proteins is important and what information can be gained (only) through the direct characterization of proteins.

Proteomics is the large-scale study of proteins and the proteome, which is the entire set of proteins produced or modified by an organism or system.

Proteomics includes the study of:
- protein roles, structures, quantities, localization, and functions
- post-translational modifications (and their change)
- protein interactions with DNA, RNA, other proteins, etc.

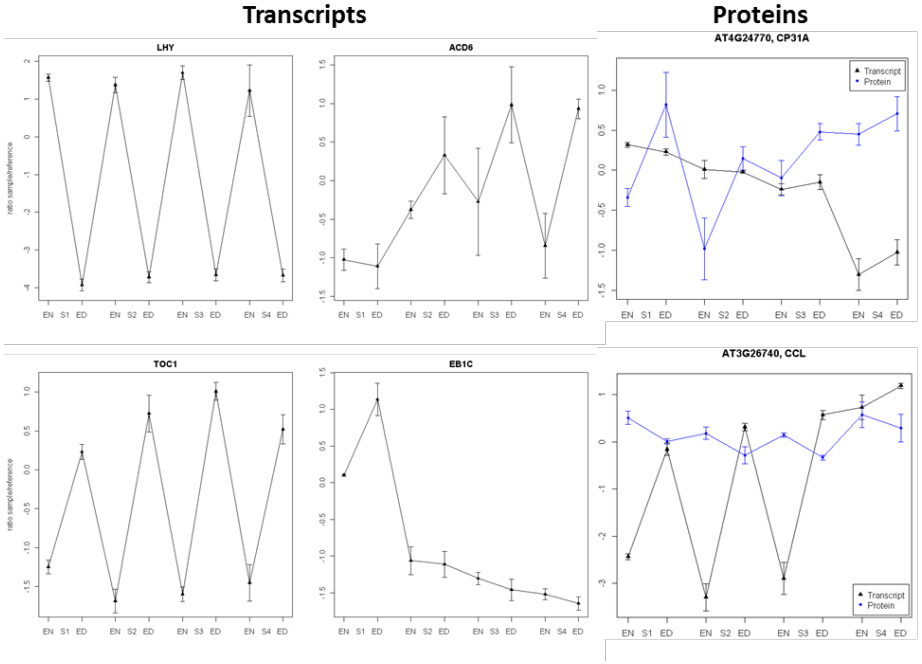
and how all these change in time, between conditions or in response to stimuli

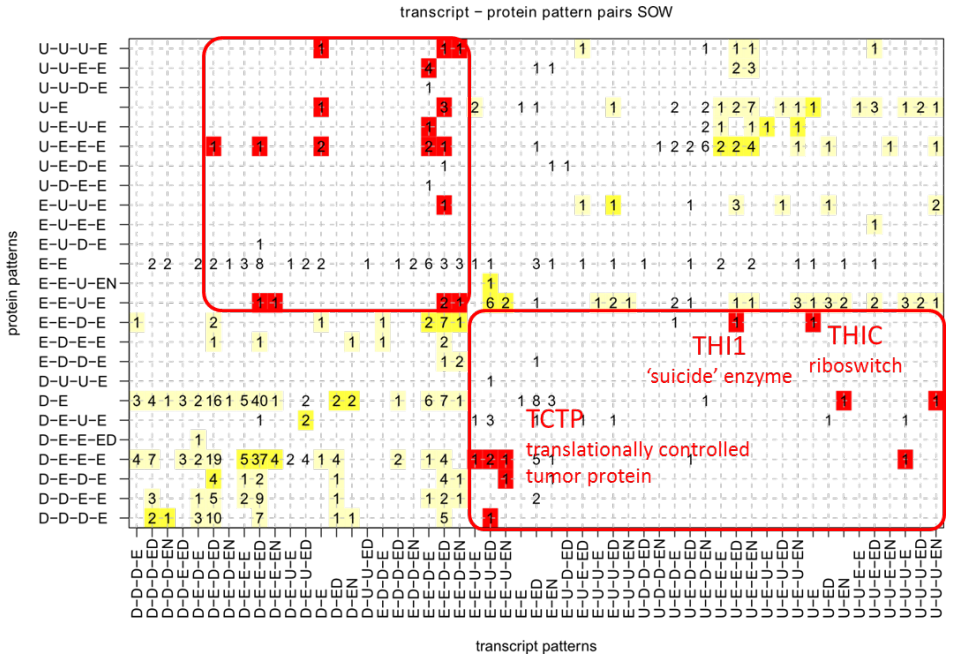




- It is primarily the proteins, their activities, modifications, subcellular localization, and interactions that are responsible for determining the appearance and state of a biological organism.
- Various processes determine protein levels and activities

The abundance and activitiy of proteins can not be automatically inferred from transcript levels due to the complexity of gene expresssion regulation.

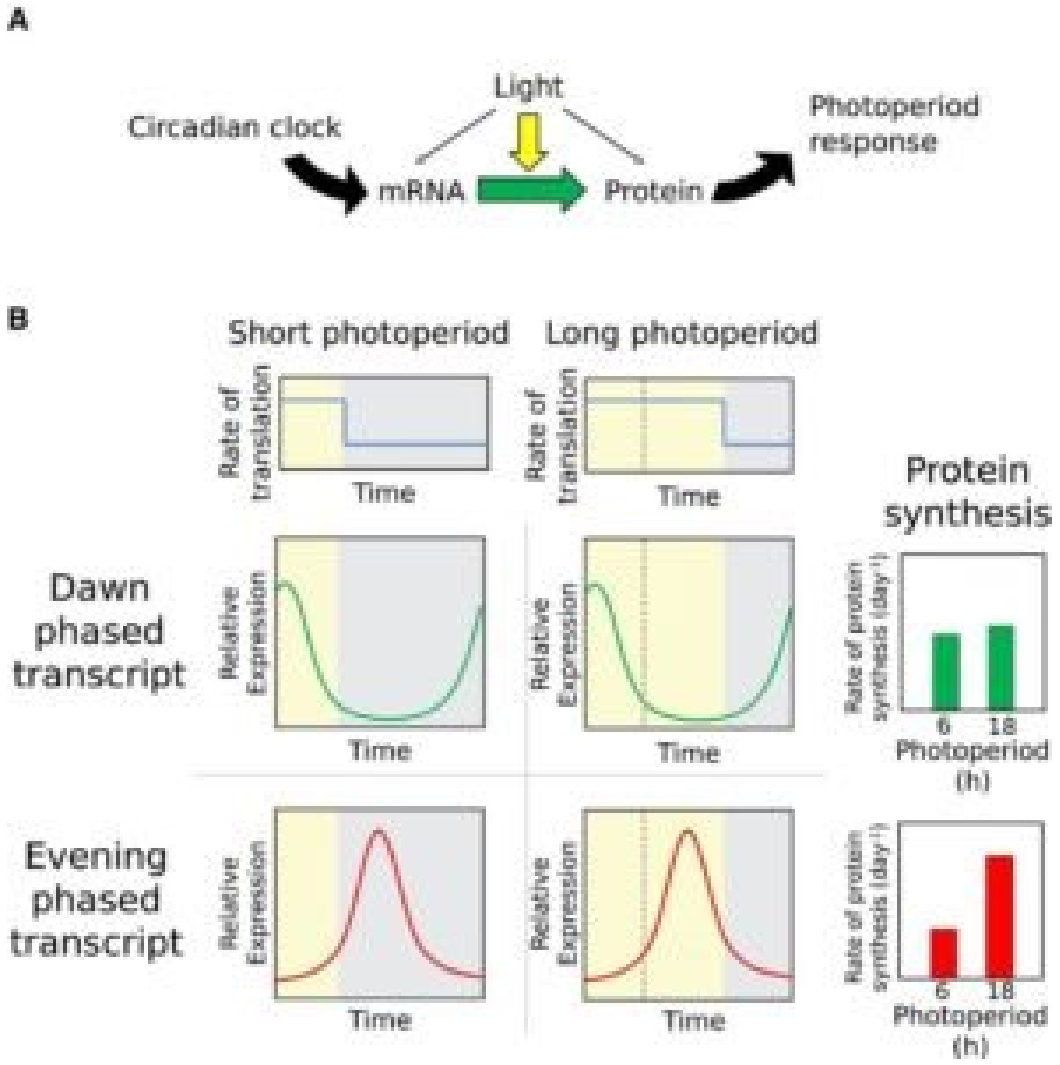




Baerenfaller *et al.*, 2012

https://www.youtube.com/watch?v=C5l2d-QXOnU

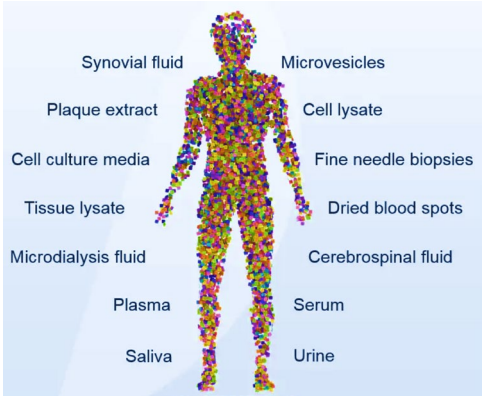
Translational coincidence mechanism

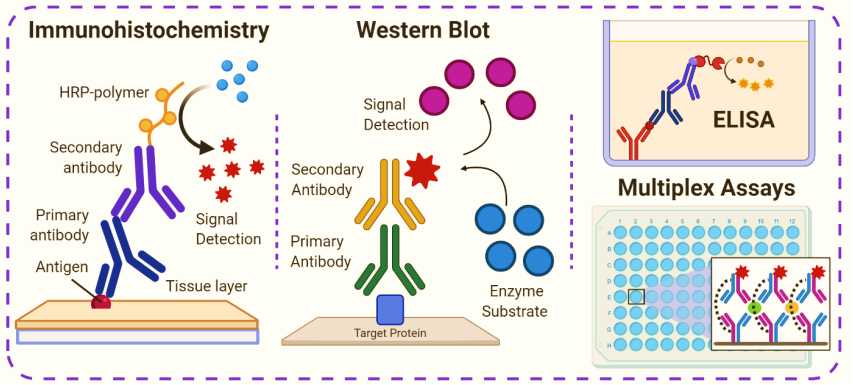


Seaton *et al.,* MSB, 2018

- Quantitative protein and transcript data of Arabidopsis in four different photoperiods have revealed that proteins with clock-regulated, evening-peaking RNAs tended to increase in abundance under longer daylengths, whereas proteins with morning-peaking RNAs did not.
- A simple, “translational coincidence” model predicted the experimental results, because high, light-induced translation rates will coincide with high levels of an evening-expressed RNA only under long days, not short days.
- Many clock-controlled genes might gain seasonal control of protein levels via translational coincidence.

An example of a non mass spectrometry-based proteomics approaches



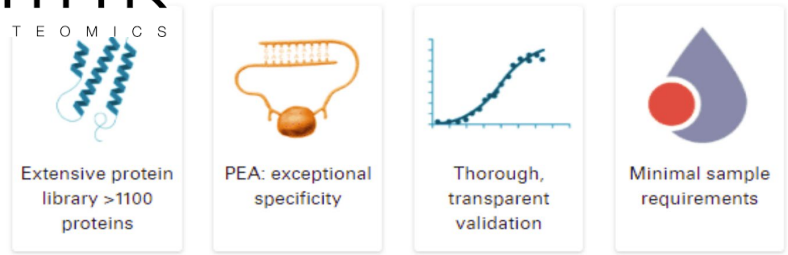


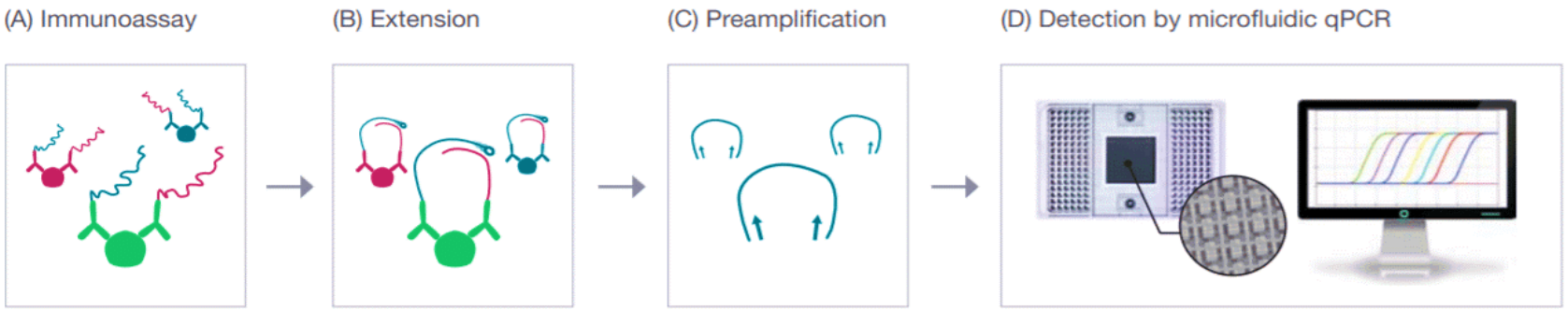
Olink: Targeted proteomics:

olink PROTEOMICS

High-throughput, multiplex immunoassays:
1µl → 92 proteins across 88 samples

Proximity Extension Assay technology:

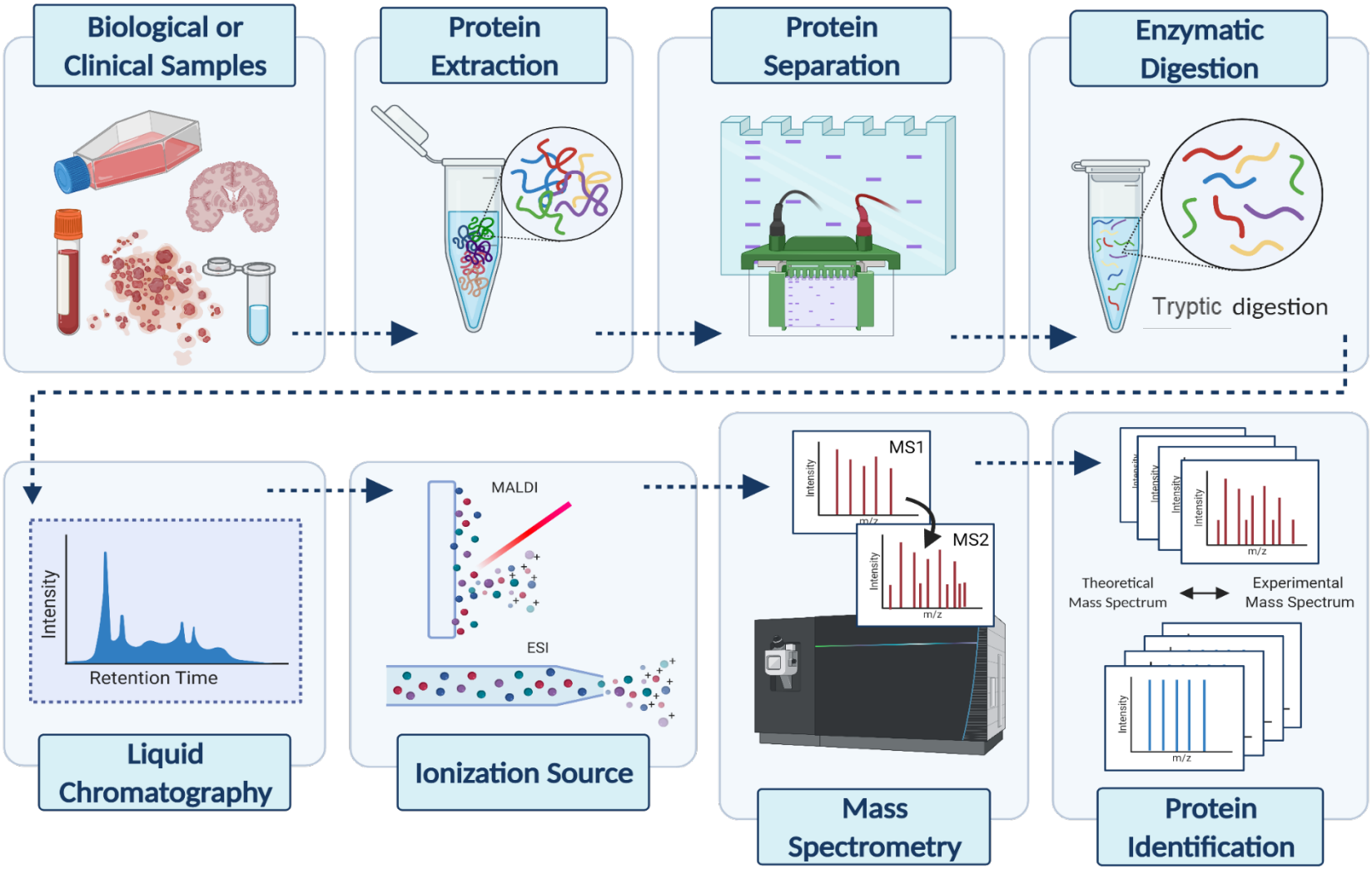


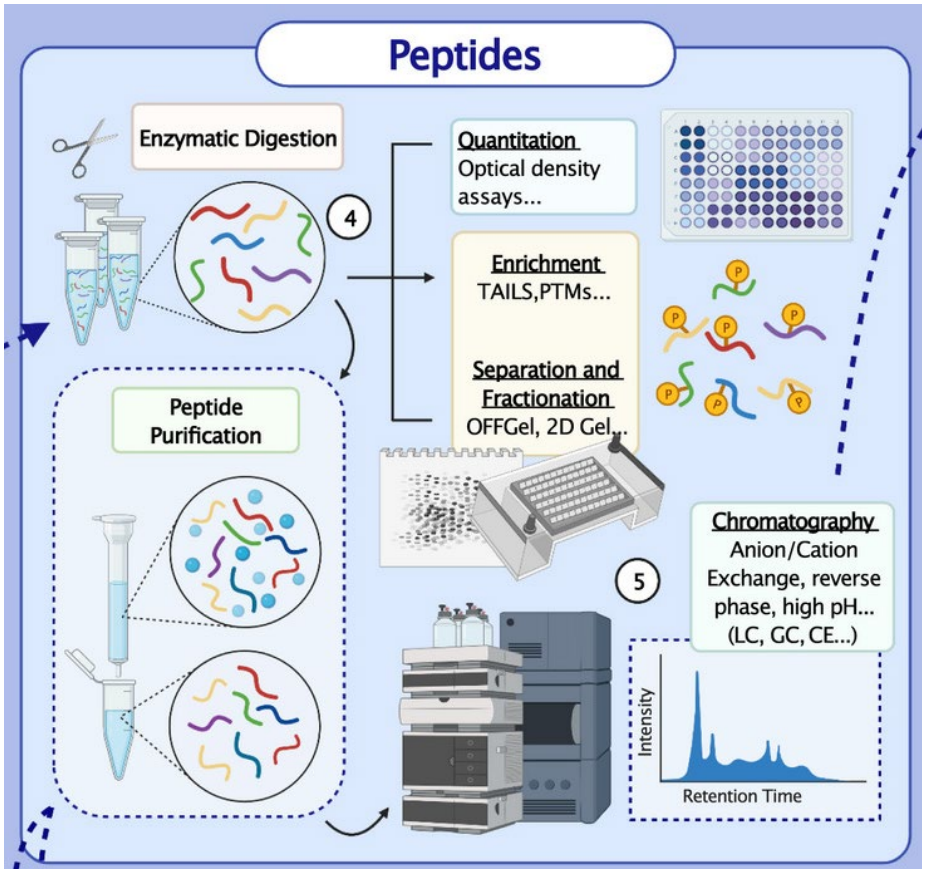


Mass spectrometry

Generic mass spectrometry-based proteomics experiment

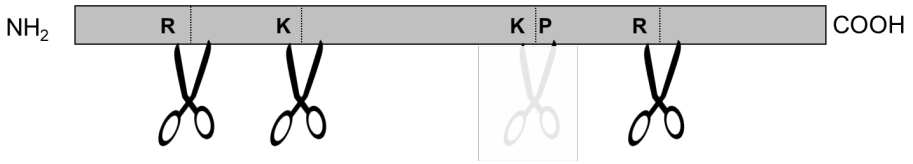
Elena Barletta





Radzikowska et al., *Omics technologies in allergy and asthma research: An EAACI position paper,* Allergy, 2022

- Before analysis, the proteins are typically digested with a site specific protease, most of the time with trypsin.
- Trypsin cuts after arginine or lysine, except when the cutting site is followed by proline, which leads to limited cleavage.

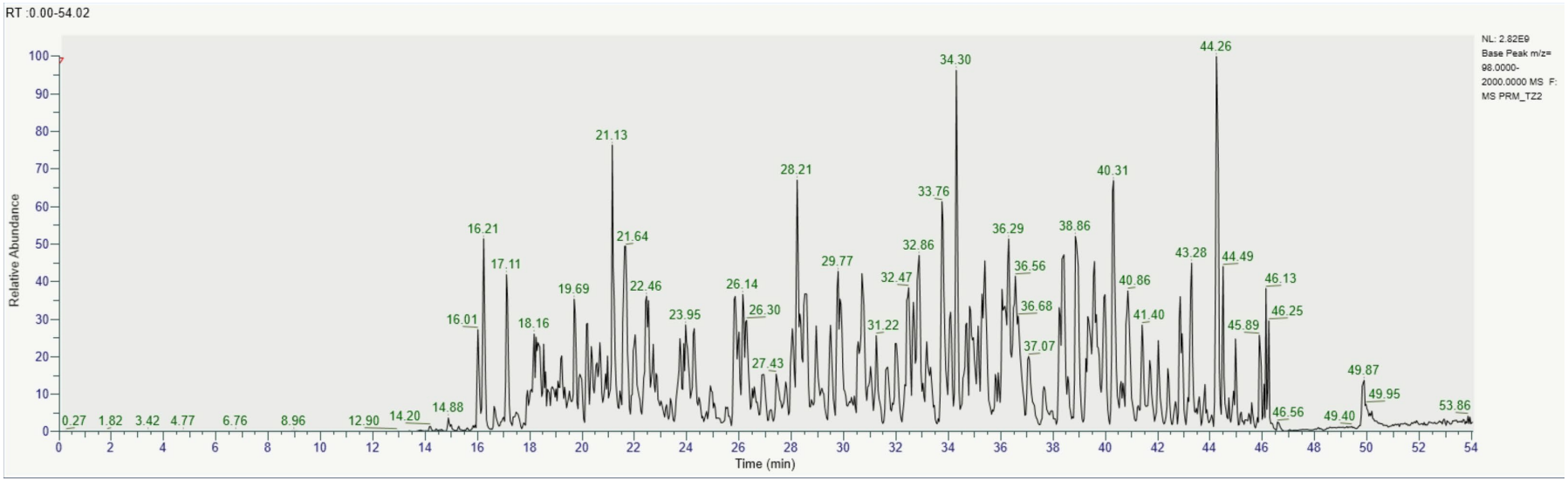


- After protein cleavage, the peptides need to be purified.
- The peptides are often fractionated or enriched for specific features such as specific post-translational modifications, internal cleavage sites, etc.

Liquid chromatography separates the mixture of peptides based on the principle of partitioning of solutes between two phases.

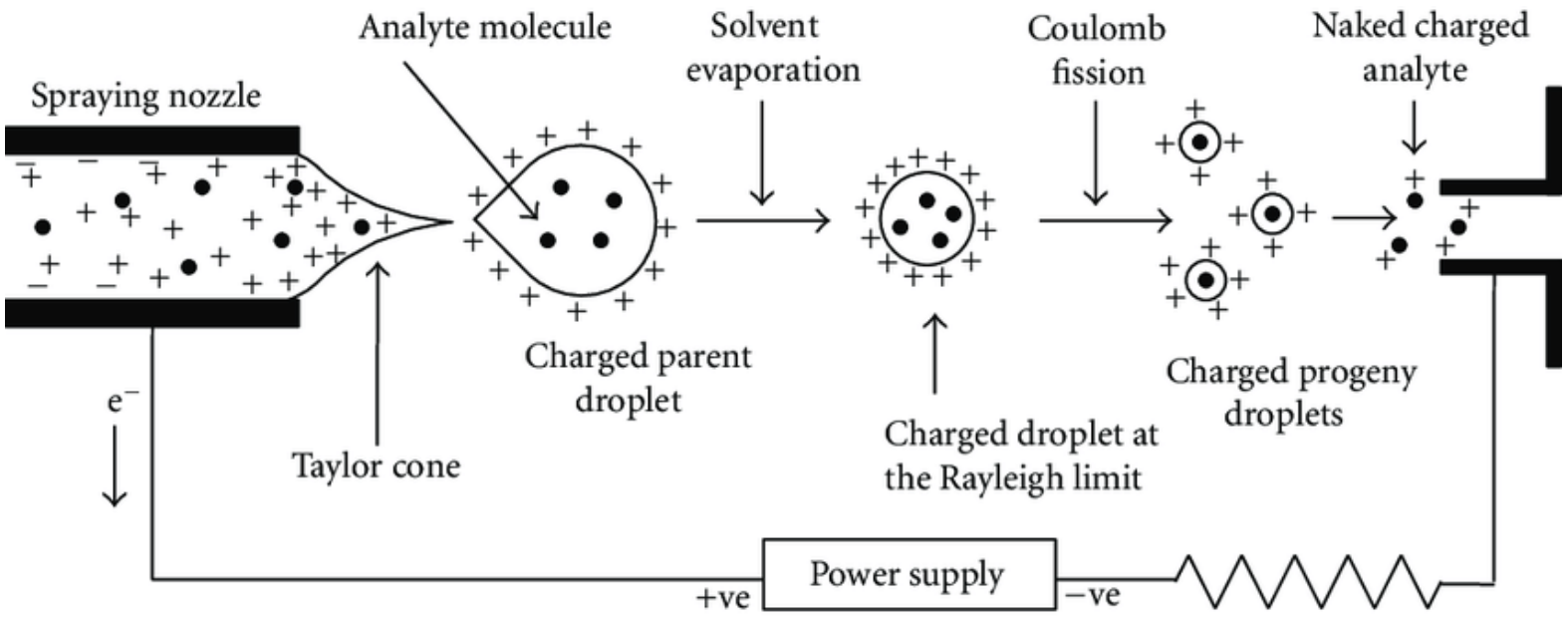
In reverse phase (RP) chromatography, the separation uses a column (non-polar stationary phase) and solvent (gradient of a mixture of a polar and a non-polar mobile phase); the components are separated from each other based on their affinity for the stationary phase depending on the polarity of the mobile phase that is changing over time.

The goal of the separation is to find the best compromise between resolving the components over time and having sharp and intense elution peaks.

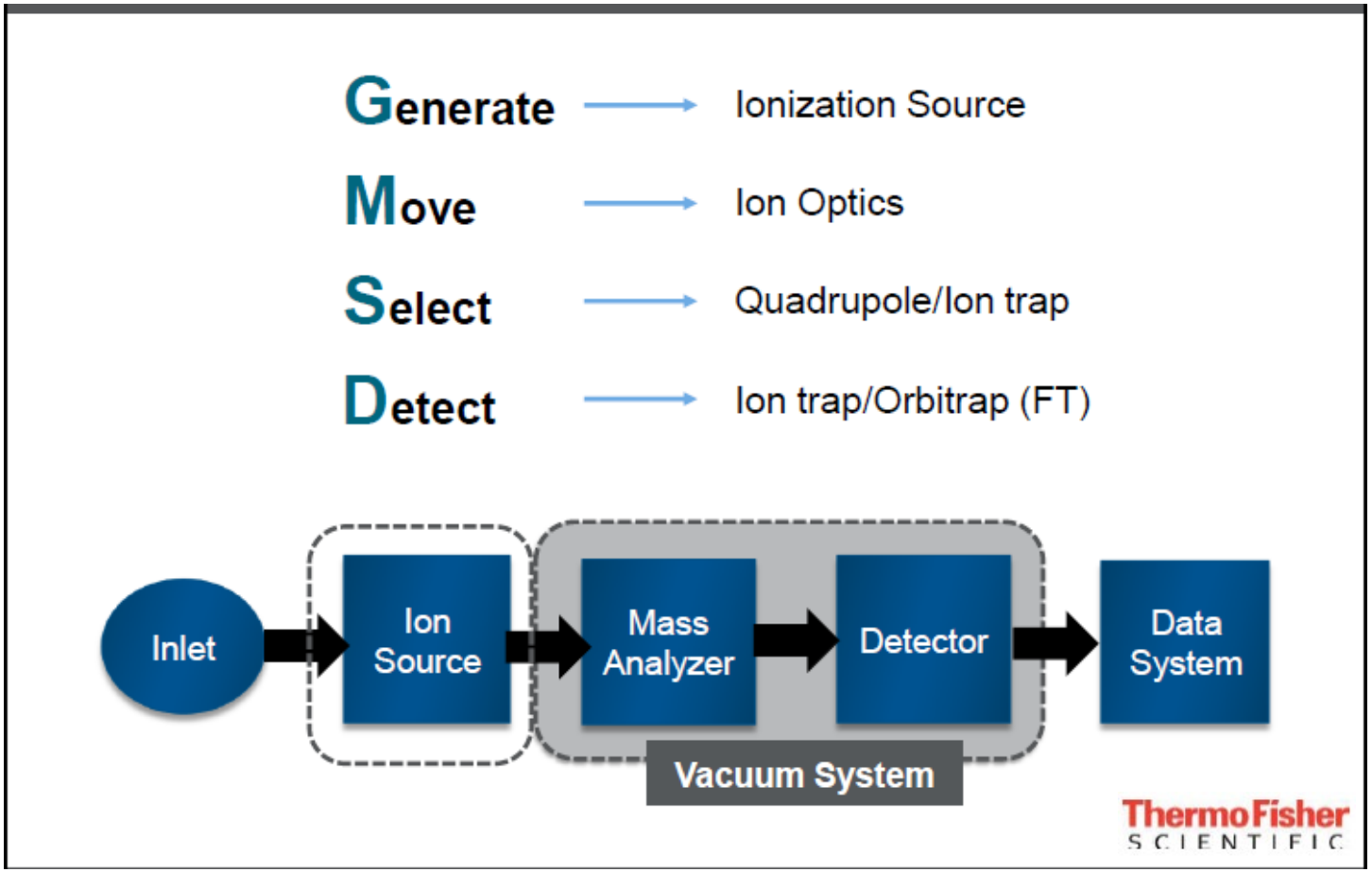


Chromatogram of a reverse phase column chromatography

Ion Generation - Electrospray Ionization (ESI)

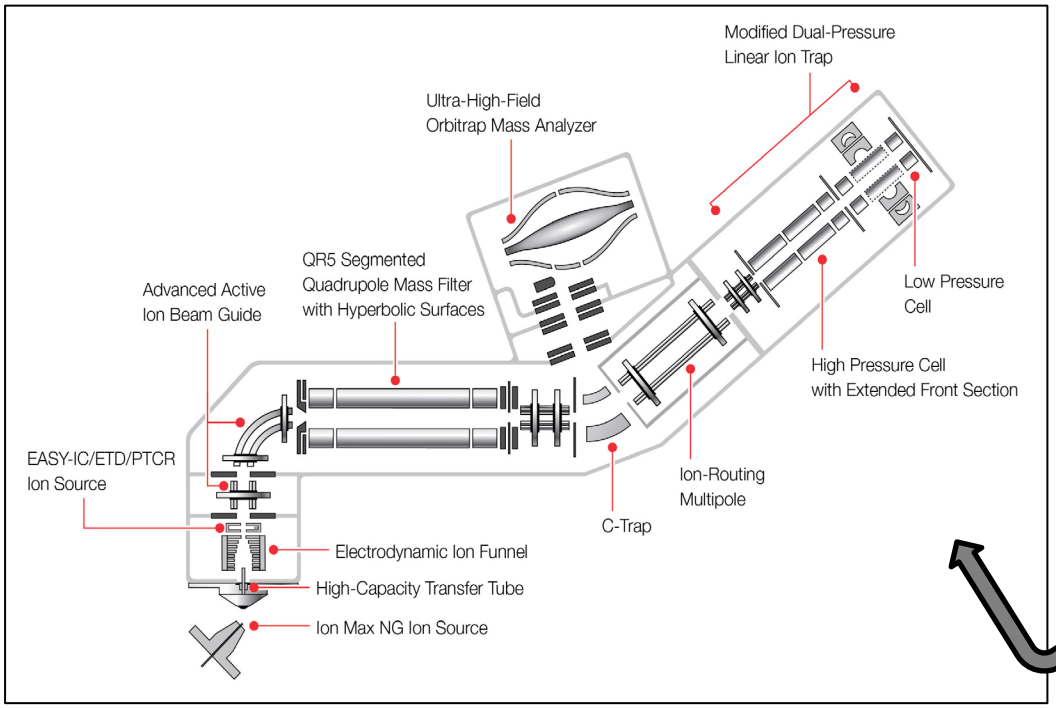


Banerjee and Mazumdar S. *IJAC*, 2012



Mass spectrometer types:

TOF = **T**ime **o**f **F**light
Quadrupole (Q)
Ion Trap
Orbitrap
FT-ICR = **F**ourier **T**ransform **I**on **C**yclotron **R**esonance



©Thermo Fisher Scientific - Orbitrap Eclipse™ Tribrid™ Mass Spectrometer

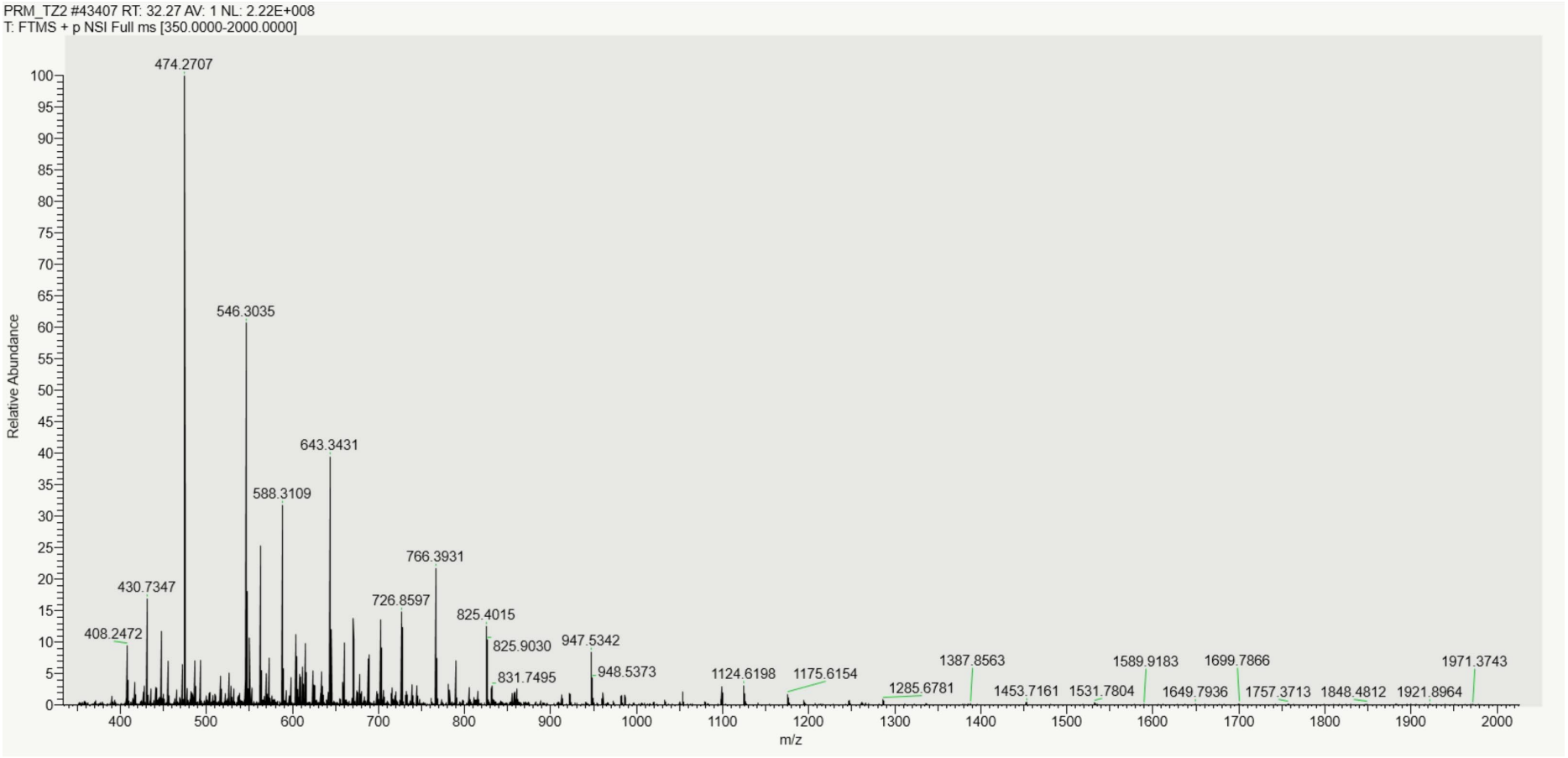
Generate

Move

Select

Detect

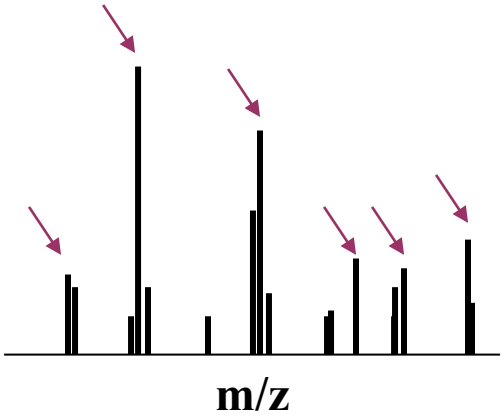
MS Spectrum



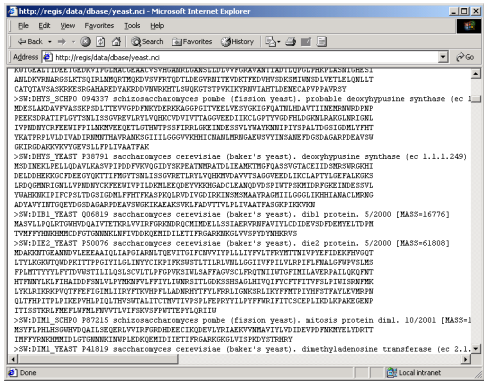
Mass spectrometry

Identifying peptides using an MS spectrum:

List of peptide masses from MS scan



Sequence database

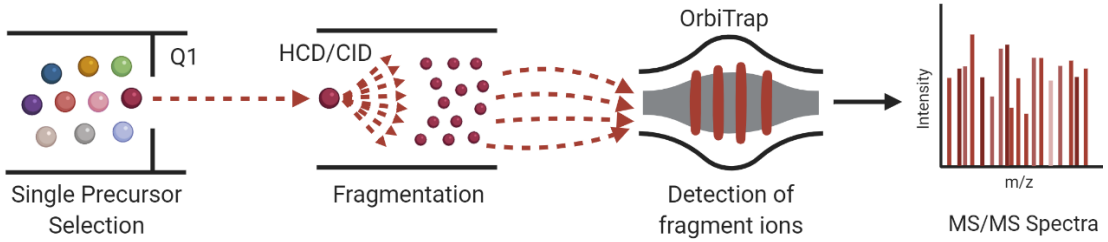


Search algorithm

Identified peptide/protein

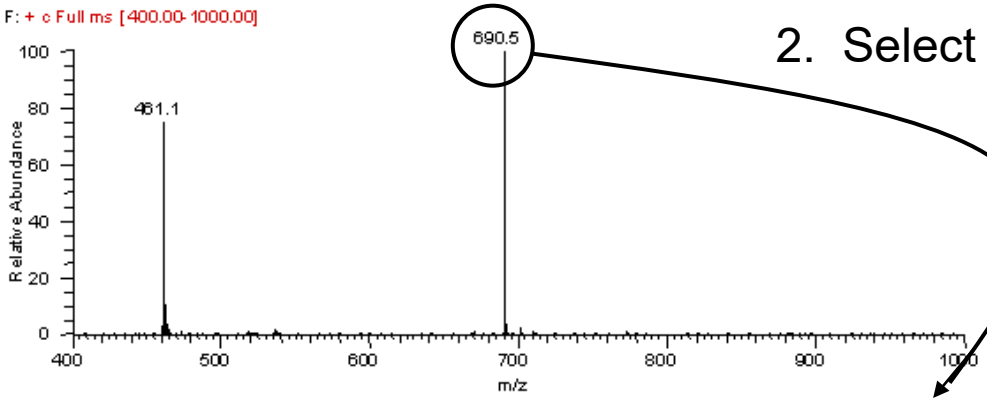
- Peptide spectrum assignment with Peptide Mass Fingerprinting is only advisable with samples of low complexity and small sequence databases, as the number of all possible peptides with a given mass over charge is huge in large sequence databases.

- Obtaining **sequence information** for a peptide ion:



1. Acquire full (MS) scan

Radzikowska et al., *Omics technologies in allergy and asthma research: An EAACI position paper,* Allergy, 2022

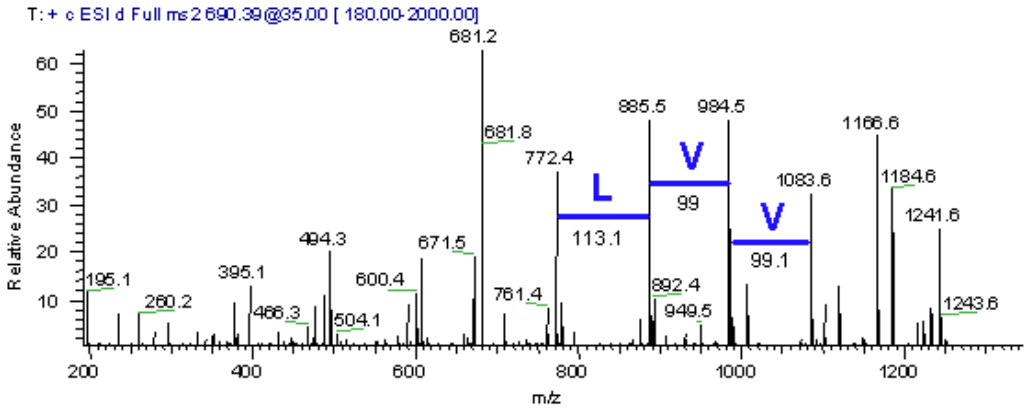


2. Select an ion

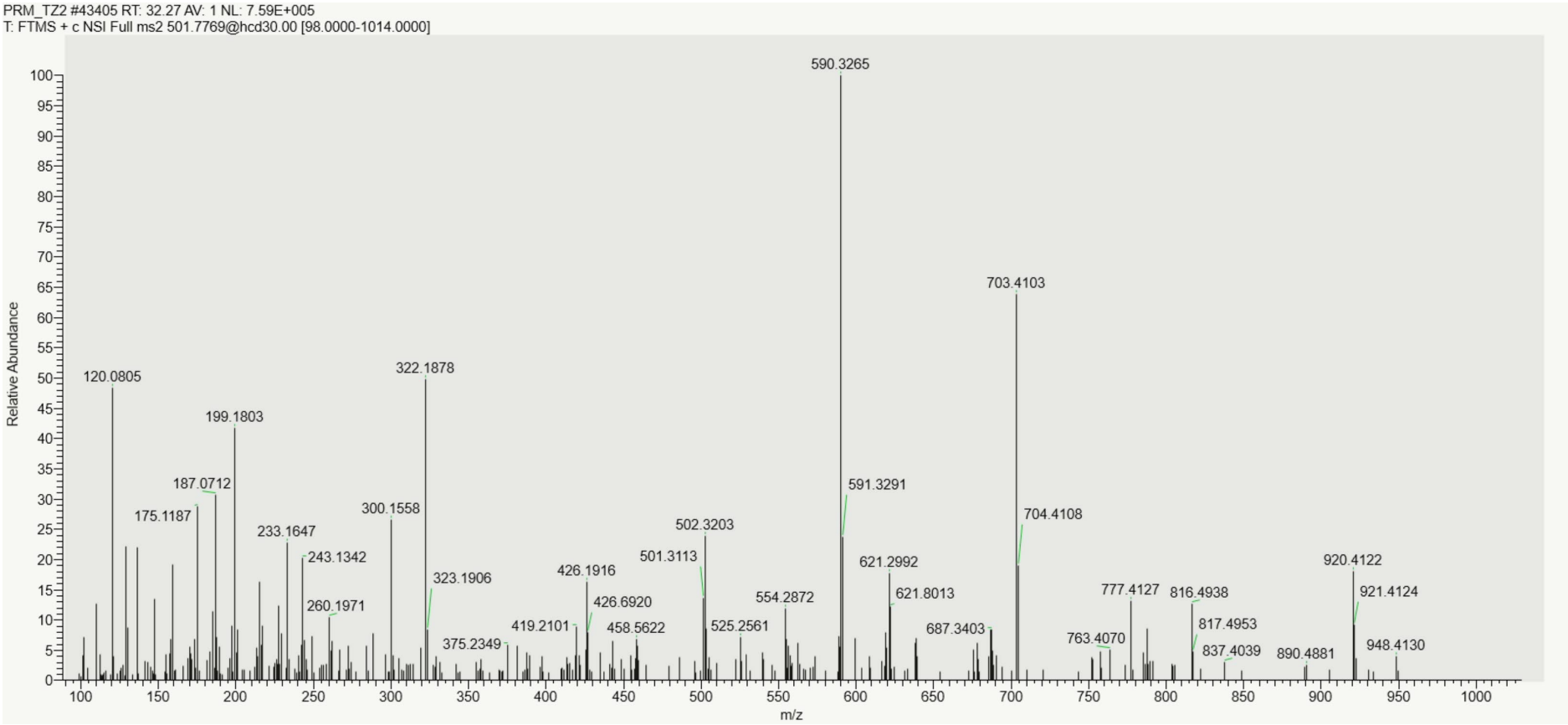
3. Isolate the ion

4. Fragment the ion

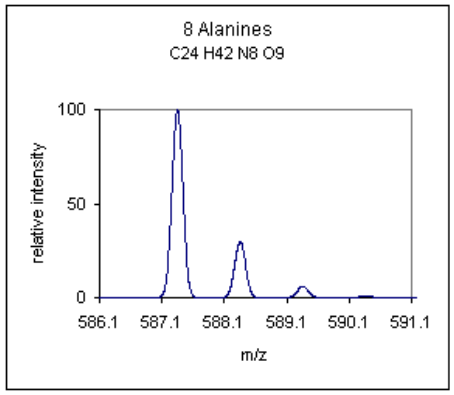
5. Acquire MS/MS scan

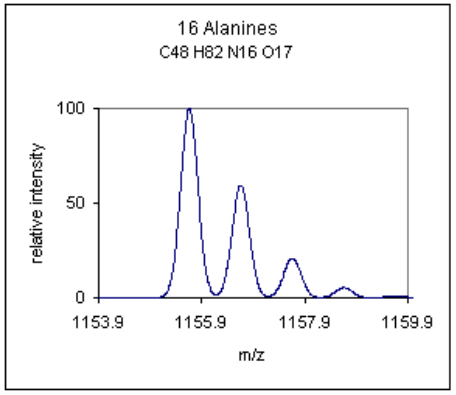


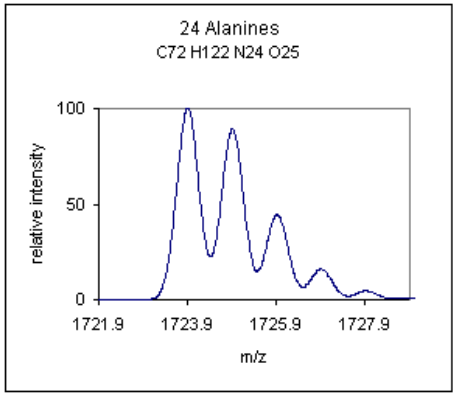
MS/MS spectrum

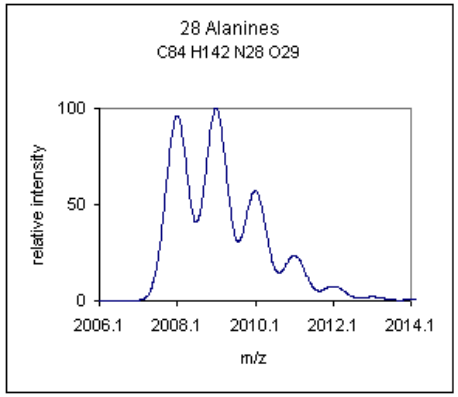


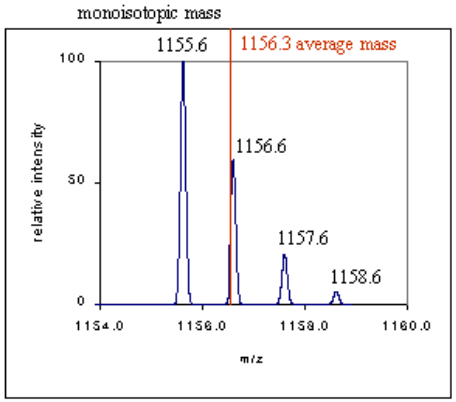
The isotope issue

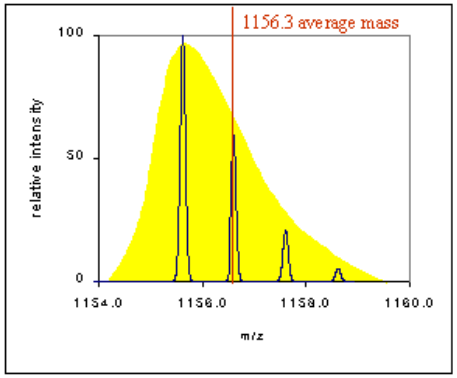






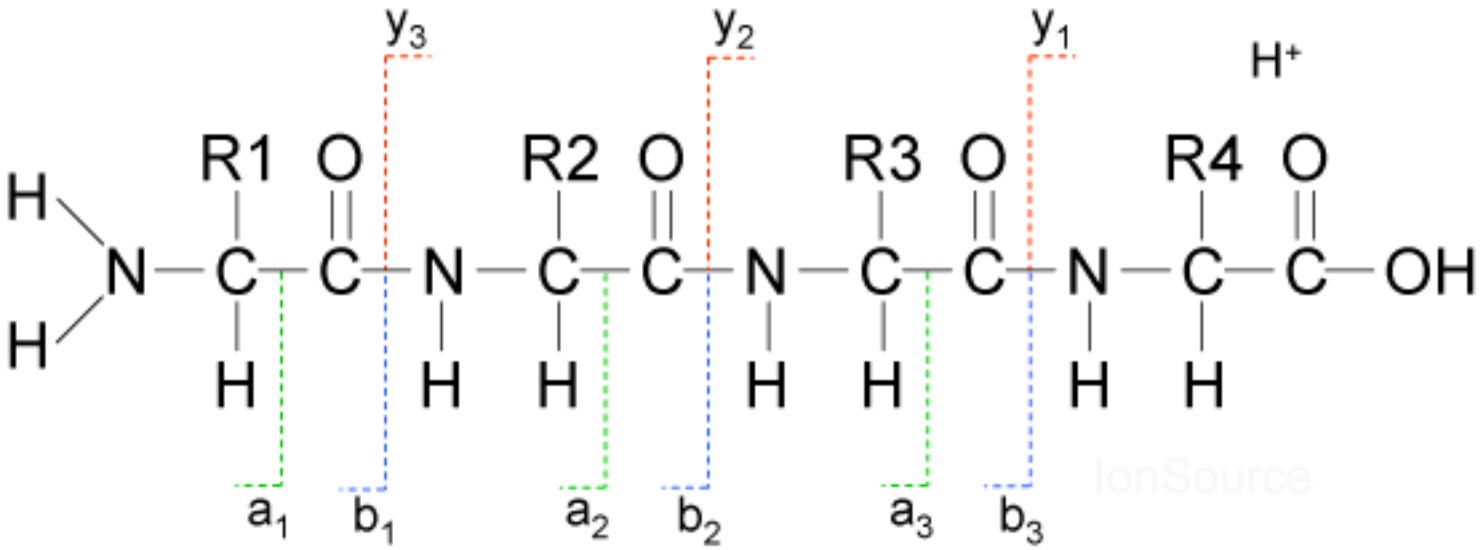






- 1/100 C atoms is C13
- The more atoms a peptide contains, the more probably it is that one to several C atoms are C13

a, b and y ions after CID



- The most common peptide fragments observed in low energy collisions are **a, b** and **y** ions.
- The **b** ions appear to extend from the amino terminus (N-terminus), and **y** ions appear to extend from the carboxyl terminus (C-terminus).
- **a** ions occur at a lower frequency and abundance in relation to **b** ions.

adjusted from http://www.ionsource.com/

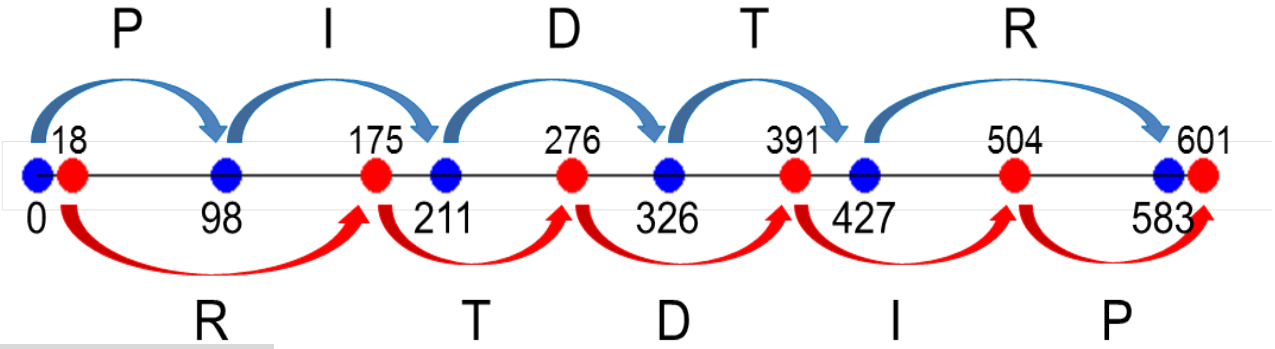
P – I – D – T – R

m/z = 601.31

Masses of b- and y- ions:

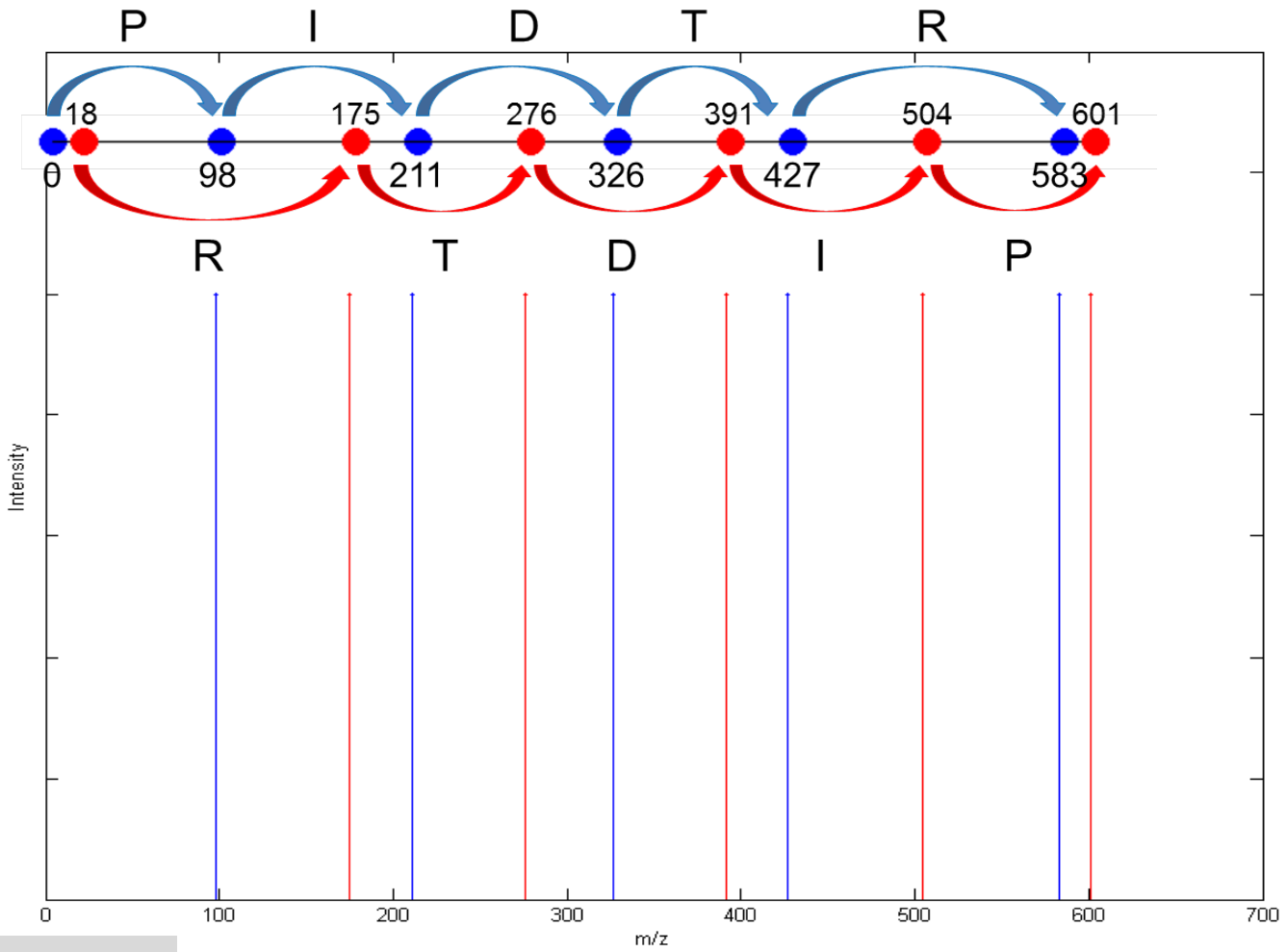
b-ions				y-ions
		PIDTR	601.31	y5
b1	98.05	P ----- IDTR	504.26	y4
b2	211.14	PI ----- DTR	391.18	y3
b3	326.16	PID ----- TR	276.15	y2
b4	427.21	PIDT ----- R	175.10	y1
b5	583.31	PIDTR		

Fragment masses aligned along a spectrum graph:

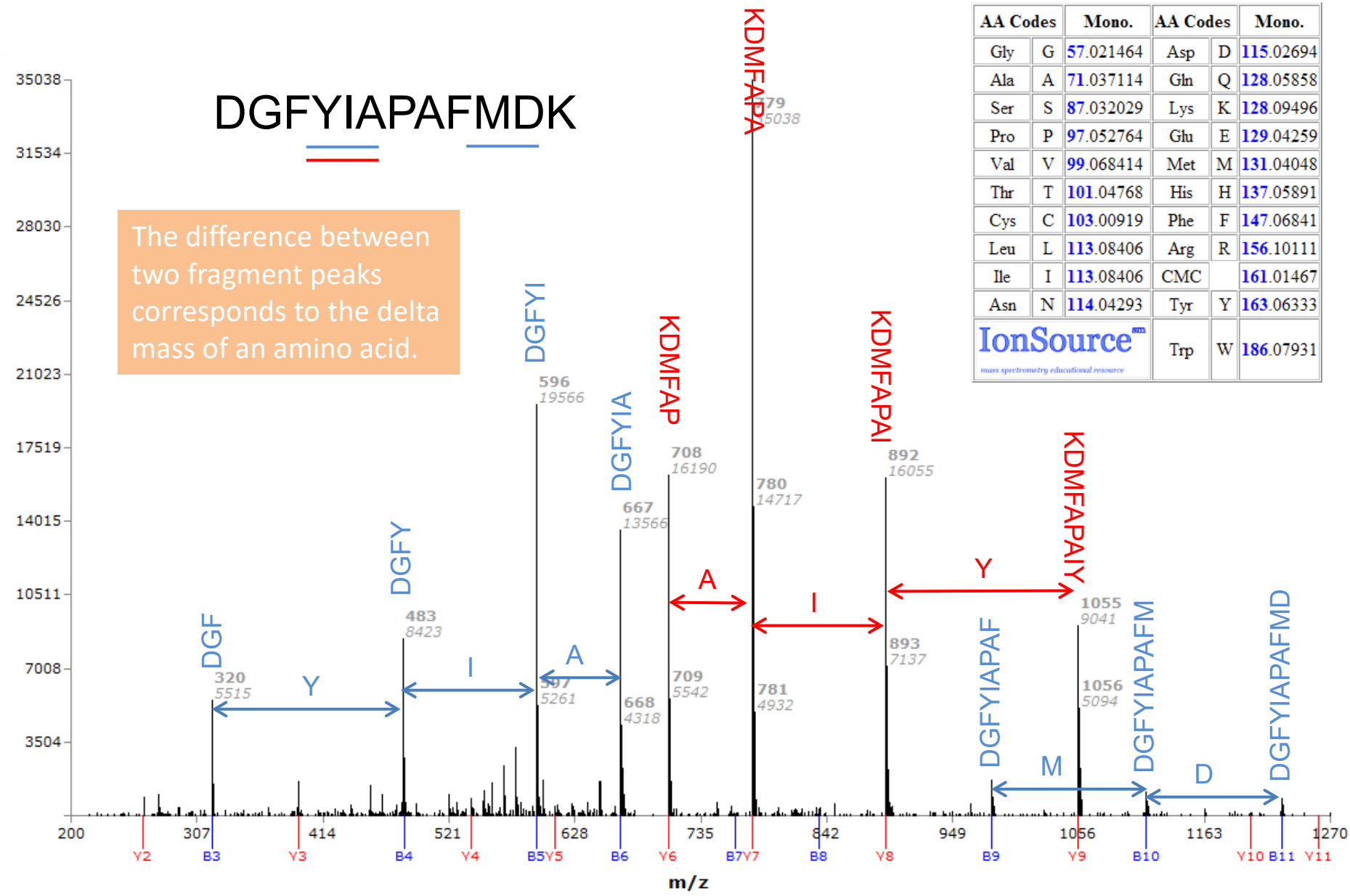


P – I – D – T – R

m/z = 601.31



Analysing a spectrum for the fragments



AA Codes		Mono.	AA Codes		Mono.
Gly	G	57.021464	Asp	D	115.02694
Ala	A	71.037114	Gln	Q	128.05858
Ser	S	87.032029	Lys	K	128.09496
Pro	P	97.052764	Glu	E	129.04259
Val	V	99.068414	Met	M	131.04048
Thr	T	101.04768	His	H	137.05891
Cys	C	103.00919	Phe	F	147.06841
Leu	L	113.08406	Arg	R	156.10111
Ile	I	113.08406	CMC		161.01467
Asn	N	114.04293	Tyr	Y	163.06333
IonSource sm mass spectrometry educational resource			Trp	W	186.07931

In *de novo* peptide sequencing, all information about the peptide sequence resides in the MS/MS spectrum itself. It is therefore database-independent and can be done manually or with algorithms.

Manual *de novo* peptide sequencing:

- M = Mass of the peptide
- **Precursor ion** $M_{Precursor} \cong \frac{(M + 2H)^{2+}}{2}$

- **Parent ion** $M_{Parent} \cong (M+H)^+$

$$M_{Parent} = M_{Precursor} * 2 - 1 = \frac{(M+2H)^{2+}}{2} * 2 - 1$$

- Monoisotopic mass of the parent ion

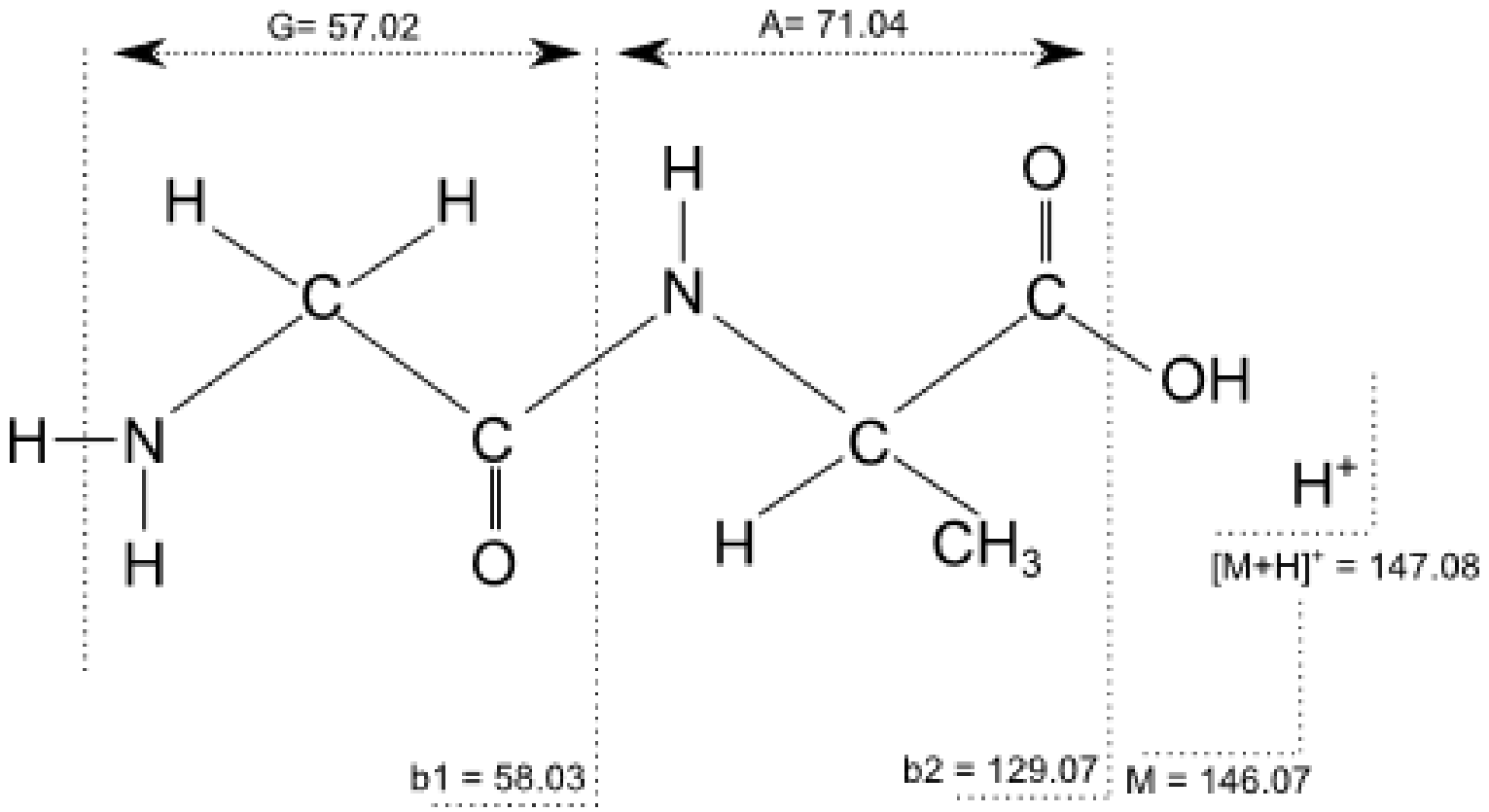
$$M_{Parent\ mono} = M_{Parent\ average} - \frac{M_{Parent\ average}}{1463}$$

- b- and y- ions: Zubarev and Bondarenko, 1991

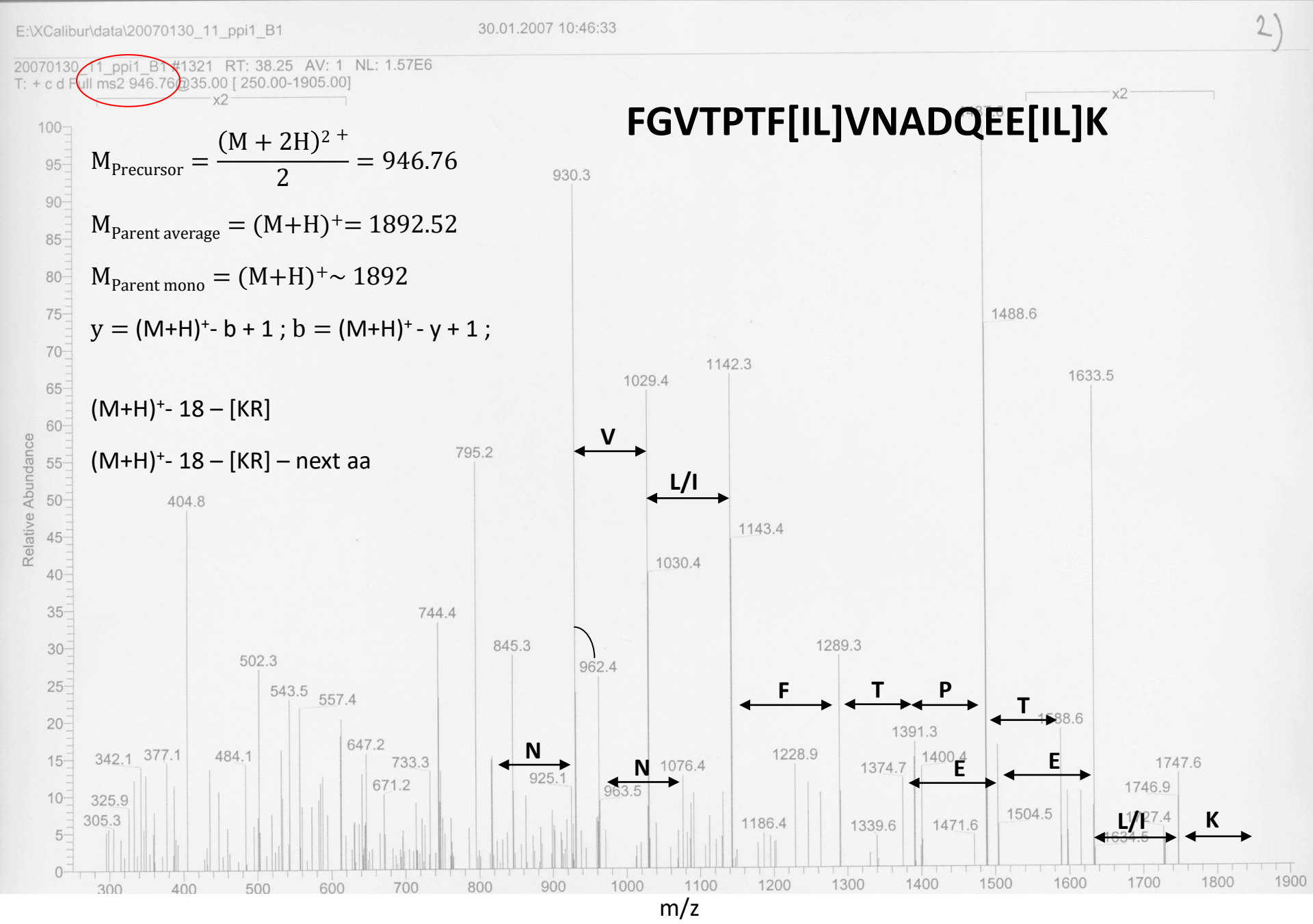
$$y = (M+H)^+ - b + 1 \ ; \ b = (M+H)^+ - y + 1$$

- Find the b-ion without the C-terminal K or R: $(M+H)^+$ - 18 ('lost' oxygen) – [KR]

AA Codes		Mono.	AA Codes		Mono.
Gly	G	57.021464	Asp	D	115.02694
Ala	A	71.037114	Gln	Q	128.05858
Ser	S	87.032029	Lys	K	128.09496
Pro	P	97.052764	Glu	E	129.04259
Val	V	99.068414	Met	M	131.04048
Thr	T	101.04768	His	H	137.05891
Cys	C	103.00919	Phe	F	147.06841
Leu	L	113.08406	Arg	R	156.10111
Ile	I	113.08406	CMC		161.01467
Asn	N	114.04293	Tyr	Y	163.06333
IonSource sm mass spectrometry educational resource			Trp	W	186.07931



- Upon peptide bond formation, H_2O gets released and this mass is therefore not included in the Δ mass for the individual amino acids
- The largest b-ion is mass of the parent ion – 18, because H_2O gets released and the positive charge resides at the C-terminus


E:\XCalibur\data\20070130_11_ppi1_B1
30.01.2007 10:46:33
2)
20070130_11_ppi1_B1 #1321 RT: 38.25 AV: 1 NL: 1.57E6
T: + c d Full ms2 946.76@35.00 [250.00-1905.00]
x2
x2
FGVTPTF[IL]VNADQEE[IL]K
$M_{Precursor} = \frac{(M + 2H)^{2+}}{2} = 946.76$
$M_{Parent\ average} = (M+H)^{+} = 1892.52$
$M_{Parent\ mono} = (M+H)^{+} \sim 1892$
y = (M+H)+- b + 1 ; b = (M+H)+ - y + 1 ;
(M+H)+- 18 – [KR]
(M+H)+- 18 – [KR] – next aa
Relative Abundance
m/z
V
L/I
F
T
P
T
N
N
E
E
L/I
K
930.3
1488.6
1142.3
1029.4
1633.5
795.2
404.8
1143.4
1030.4
744.4
845.3
962.4
1289.3
502.3
543.5
557.4
647.2
733.3
671.2
342.1
377.1
484.1
325.9
305.3
925.1
963.5
1076.4
1228.9
1391.3
1400.4
1374.7
1747.6
1746.9
1504.5
1186.4
1339.6
1471.6

Neutral losses and peptide modifications

- Neutral losses

- H_20 (-18 Da)
- NH_2 (-17 Da)
- CO (-28 Da)
- H_3PO_4 (-98 Da)

- Modifications

Oxidation: M +16 Da

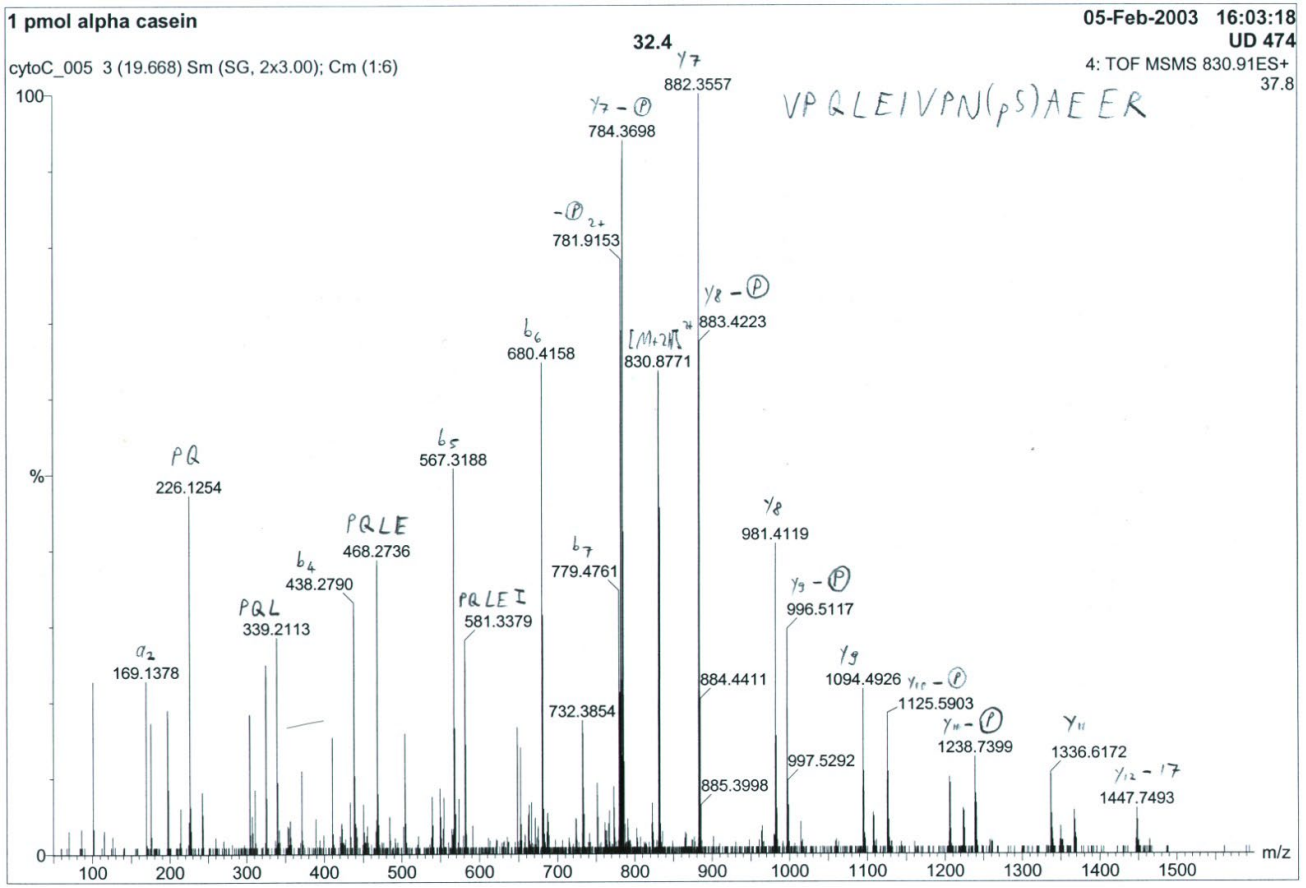
Deamidation: N → Q, -1 Da

Methylation: R +14 Da

Phosphorylation: S, T, Y +80 Da

Acetylation: K, R, N-terminus +42 Da

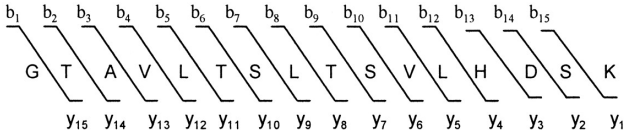
Carboxyamidomethylation: C +57 Da

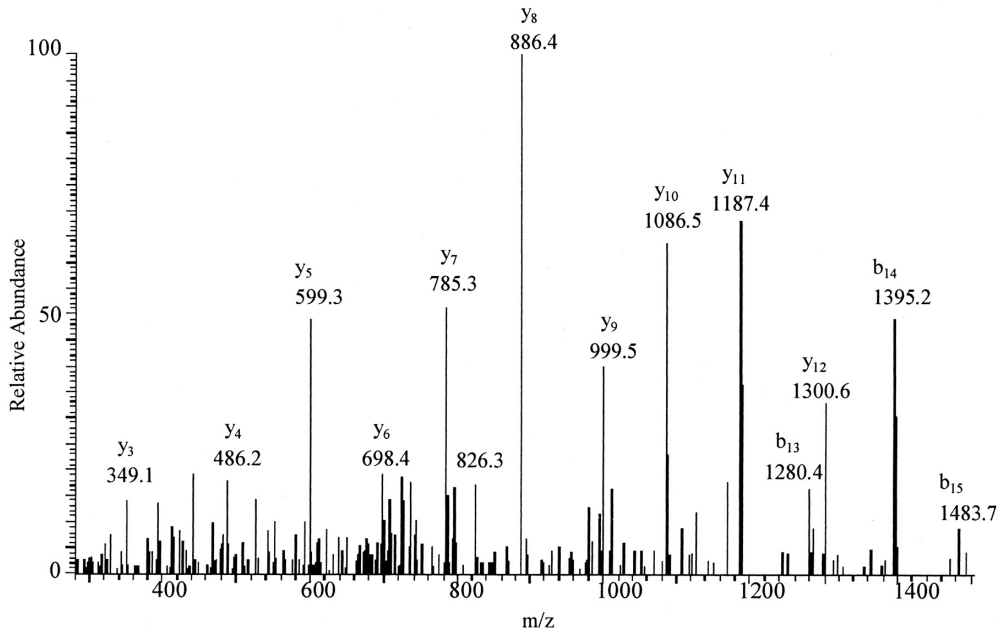


Peptide spectrum assignment

Given the following peptide with the indicated fragment ions,

1) What ions will change their m/z when Serin 7 will be phosphorylated?
2) What ions will change their m/z when the peptide N-terminus is acetylated?
3) What ions will change their m/z when the C-terminal lysine is isotopically labeled?





Nisar *et al., Drug Metabolism and Predisposition,* 2004
https://doi.org/10.1124/dmd.32.4.382

https://pwa.klicker.uzh.ch/join/kbaere

De novo sequencing with software

1. Dynamic programming
    - The algorithms suffer from ‘real life issues’ of peptide mass spectrometry, e.g. they are sensitive against noisy data
    - Requires pre-processing of the information in an MS/MS spectrum
2. Hidden Markov Models
    - Fully probabilistic
    - Can deal with noisy data

→ Studies comparing the performance of *de novo* sequencing algorithms revealed that the rate of exact peptide sequence identification is low with high error rate

→ Problems for *de novo* sequencing are limited mass accuracy of the mass spectrometers, missing ions, unknown identity of the peaks and additional, sequence-independent peaks

Different database-dependent peptide identification search algorithms:

- Sequest
- Mascot
- PepSplice
- OMSSA
- X!Tandem
- Phenyx
- ProteinPilot
- SpectrumMill
- ProbID
- PepFrag
- InSpect
- ...

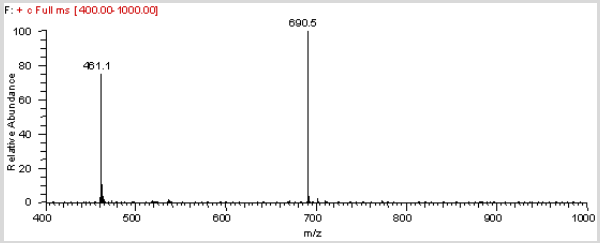
- Peptide sequences with one or more scores with which to evaluate the likelihood that the resulting sequence is correct.
- Even though each implementation is different, they operate under the same general principle.

Database-dependent peptide identification

Experimental

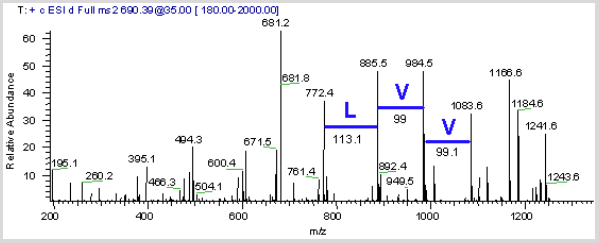
Protein sample

tryptic digest



Full Scan [MS]:
Mass measurement of full peptides

fragmen-tation



MS/MS-Scan:
Measurement of peptide fragments

1) Peptides from a protein database are matched to the measured mass

2) Theoretical spectrum is cross-correlated to the measured one

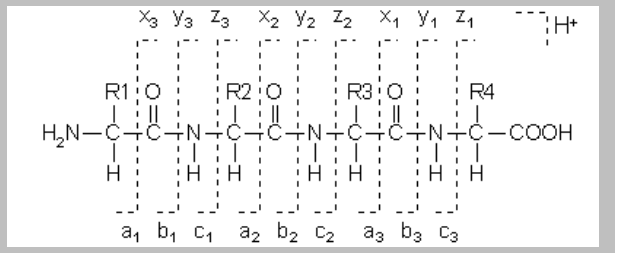
Protein database

in silico digest

Peptide database

Theoretical tryptic peptides
from a protein database

in silico dissociation



Theorectical spectra
assuming peptide bond breakage

In silico

Peptide spectrum assignment

Database-dependent peptide identification

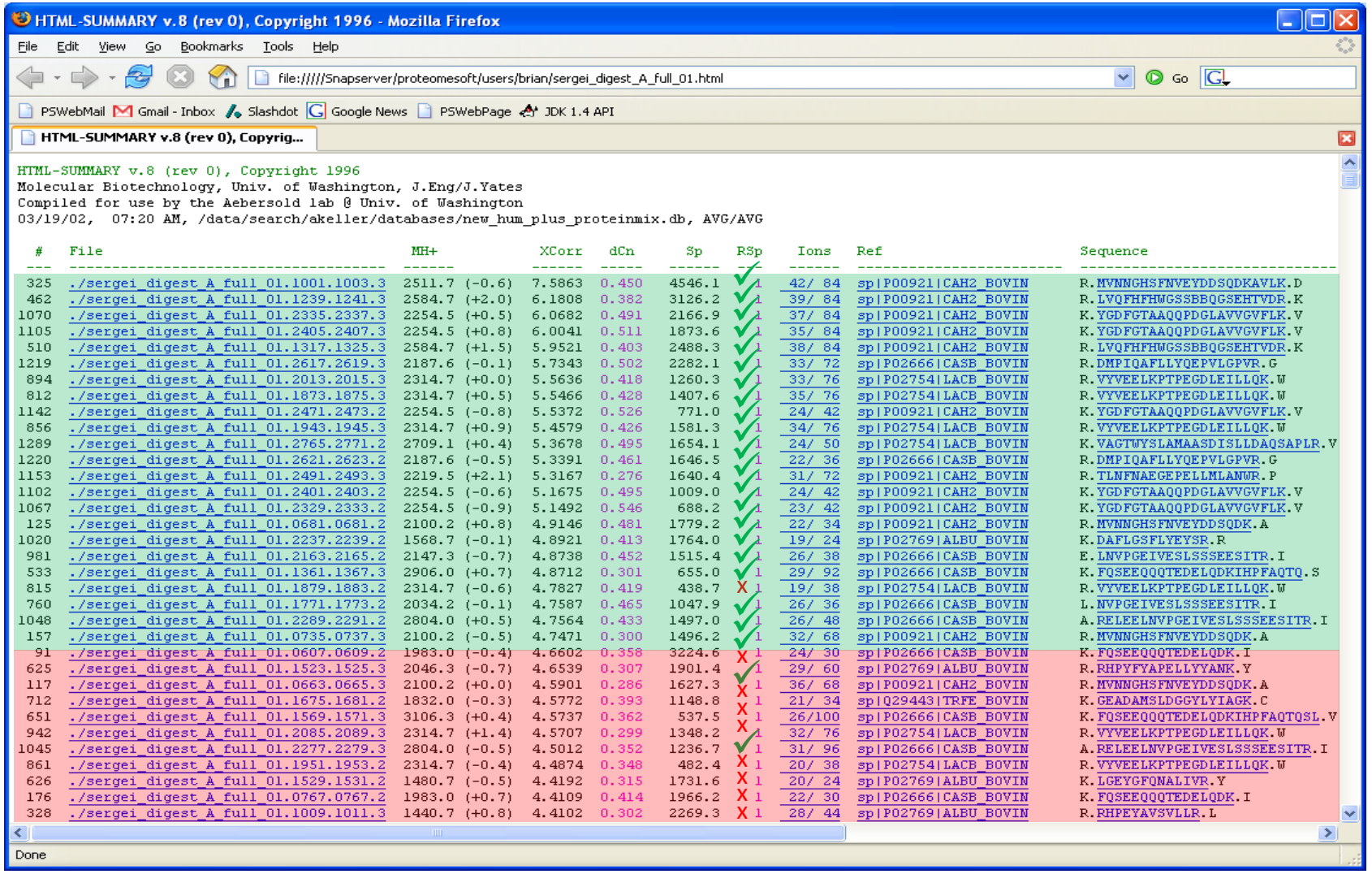
- The goal is to identify the best sequence match to the spectrum
- The details of this implementation differ among the algorithms. In addition, the methods used to assign scores are very different.
- Four basic approaches have been developed to model matches to the sequences: descriptive models, interpretative models, stochastic models and statistical and probability models (Sadygov, Cociorva and Yates, 2004)

Descriptive algorithms are based on a mechanistic prediction of how peptides fragment in a tandem mass spectrometer, which is then quantified to determine the quality of the match between the prediction and the experimental spectrum. Mathematical methods such as correlation analysis have been used to assess match quality.

- Sequest is an example of a program using a descriptive model:
  - S_p, sums the peak intensity of fragment ions matching the predicted sequence ions and accounts for the continuity of an ion series and the length of a peptide
  - Xcorr, is a cross-correlation score of the experimental and theoretical spectra
  - ΔCn gives the normalised difference of Xcorr values between the best sequence and lower-scoring matches and is useful to determine the uniqueness of the match

Sadygov, Cociorva and Yates, 2004

Empirical statistical model to estimate the accuracy of peptide identifications



#	File	MH+	XCorr	dCn	Sp	RSp	Ions	Ref	Sequence
325	./sergei_digest_A_full_01.1001.1003.3	2511.7 (-0.6)	7.5863	0.450	4546.1	✓ 1	42/ 84	sp|P00921|CAH2_BOVIN	R.MVNNGHSFNVEYDDSQDKAVLK.D
462	./sergei_digest_A_full_01.1239.1241.3	2584.7 (+2.0)	6.1808	0.382	3126.2	✓ 1	39/ 84	sp|P00921|CAH2_BOVIN	R.LVQFHFHWGSSBBQGSEHTVDR.K
1070	./sergei_digest_A_full_01.2335.2337.3	2254.5 (+0.5)	6.0682	0.491	2166.9	✓ 1	37/ 84	sp|P00921|CAH2_BOVIN	K.YGDFGTAAQQPDGLAVVGVFLK.V
1105	./sergei_digest_A_full_01.2405.2407.3	2254.5 (+0.8)	6.0041	0.511	1873.6	✓ 1	35/ 84	sp|P00921|CAH2_BOVIN	K.YGDFGTAAQQPDGLAVVGVFLK.V
510	./sergei_digest_A_full_01.1317.1325.3	2584.7 (+1.5)	5.9521	0.403	2488.3	✓ 1	38/ 84	sp|P00921|CAH2_BOVIN	R.LVQFHFHWGSSBBQGSEHTVDR.K
1219	./sergei_digest_A_full_01.2617.2619.3	2187.6 (-0.1)	5.7343	0.502	2282.1	✓ 1	33/ 72	sp|P02666|CASB_BOVIN	R.DMPIQAFLLYQEPVLGPVR.G
894	./sergei_digest_A_full_01.2013.2015.3	2314.7 (+0.0)	5.5636	0.418	1260.3	✓ 1	33/ 76	sp|P02754|LACB_BOVIN	R.VYVEELKPTPEGDLEILLQK.W
812	./sergei_digest_A_full_01.1873.1875.3	2314.7 (+0.5)	5.5466	0.428	1407.6	✓ 1	35/ 76	sp|P02754|LACB_BOVIN	R.VYVEELKPTPEGDLEILLQK.W
1142	./sergei_digest_A_full_01.2471.2473.2	2254.5 (-0.8)	5.5372	0.526	771.0	✓ 1	24/ 42	sp|P00921|CAH2_BOVIN	K.YGDFGTAAQQPDGLAVVGVFLK.V
856	./sergei_digest_A_full_01.1943.1945.3	2314.7 (+0.9)	5.4579	0.426	1581.3	✓ 1	34/ 76	sp|P02754|LACB_BOVIN	R.VYVEELKPTPEGDLEILLQK.W
1289	./sergei_digest_A_full_01.2765.2771.2	2709.1 (+0.4)	5.3678	0.495	1654.1	✓ 1	24/ 50	sp|P02754|LACB_BOVIN	K.VAGTWYSLAMAASDISLLDAQSAPLR.V
1220	./sergei_digest_A_full_01.2621.2623.2	2187.6 (-0.5)	5.3391	0.461	1646.5	✓ 1	22/ 36	sp|P02666|CASB_BOVIN	R.DMPIQAFLLYQEPVLGPVR.G
1153	./sergei_digest_A_full_01.2491.2493.3	2219.5 (+2.1)	5.3167	0.276	1640.4	✓ 1	31/ 72	sp|P00921|CAH2_BOVIN	R.TLNFNAEGEPELLMLANWR.P
1102	./sergei_digest_A_full_01.2401.2403.2	2254.5 (-0.6)	5.1675	0.495	1009.0	✓ 1	24/ 42	sp|P00921|CAH2_BOVIN	K.YGDFGTAAQQPDGLAVVGVFLK.V
1067	./sergei_digest_A_full_01.2329.2333.2	2254.5 (-0.9)	5.1492	0.546	688.2	✓ 1	23/ 42	sp|P00921|CAH2_BOVIN	K.YGDFGTAAQQPDGLAVVGVFLK.V
125	./sergei_digest_A_full_01.0681.0681.2	2100.2 (+0.8)	4.9146	0.481	1779.2	✓ 1	22/ 34	sp|P00921|CAH2_BOVIN	R.MVNNGHSFNVEYDDSQDK.A
1020	./sergei_digest_A_full_01.2237.2239.2	1568.7 (-0.1)	4.8921	0.413	1764.0	✓ 1	19/ 24	sp|P02769|ALBU_BOVIN	K.DAFLGSFLYEYSR.R
981	./sergei_digest_A_full_01.2163.2165.2	2147.3 (-0.7)	4.8738	0.452	1515.4	✓ 1	26/ 38	sp|P02666|CASB_BOVIN	E.LNVPGEIVESLSSSEESITR.I
533	./sergei_digest_A_full_01.1361.1367.3	2906.0 (+0.7)	4.8712	0.301	655.0	✓ 1	29/ 92	sp|P02666|CASB_BOVIN	K.FQSEEQQQTEDELQDKIHPFAQTQ.S
815	./sergei_digest_A_full_01.1879.1883.2	2314.7 (-0.6)	4.7827	0.419	438.7	X 1	19/ 38	sp|P02754|LACB_BOVIN	R.VYVEELKPTPEGDLEILLQK.W
760	./sergei_digest_A_full_01.1771.1773.2	2034.2 (-0.1)	4.7587	0.465	1047.9	✓ 1	26/ 36	sp|P02666|CASB_BOVIN	L.NVPGEIVESLSSSEESITR.I
1048	./sergei_digest_A_full_01.2289.2291.2	2804.0 (+0.5)	4.7564	0.433	1497.0	✓ 1	26/ 48	sp|P02666|CASB_BOVIN	A.RELEELNVPGEIVESLSSSEESITR.I
157	./sergei_digest_A_full_01.0735.0737.3	2100.2 (-0.5)	4.7471	0.300	1496.2	✓ 1	32/ 68	sp|P00921|CAH2_BOVIN	R.MVNNGHSFNVEYDDSQDK.A
91	./sergei_digest_A_full_01.0607.0609.2	1983.0 (-0.4)	4.6602	0.358	3224.6	X 1	24/ 30	sp|P02666|CASB_BOVIN	K.FQSEEQQQTEDELQDK.I
625	./sergei_digest_A_full_01.1523.1525.3	2046.3 (-0.7)	4.6539	0.307	1901.4	✓ 1	29/ 60	sp|P02769|ALBU_BOVIN	R.RHPYFYAPELLYYANK.Y
117	./sergei_digest_A_full_01.0663.0665.3	2100.2 (+0.0)	4.5901	0.286	1627.3	X 1	36/ 68	sp|P00921|CAH2_BOVIN	R.MVNNGHSFNVEYDDSQDK.A
712	./sergei_digest_A_full_01.1675.1681.2	1832.0 (-0.3)	4.5772	0.393	1148.8	X 1	21/ 34	sp|Q29443|TRFE_BOVIN	K.GEADAMSLDGGYLYIAGK.C
651	./sergei_digest_A_full_01.1569.1571.3	3106.3 (+0.4)	4.5737	0.362	537.5	X 1	26/100	sp|P02666|CASB_BOVIN	K.FQSEEQQQTEDELQDKIHPFAQTQSL.V
942	./sergei_digest_A_full_01.2085.2089.3	2314.7 (+1.4)	4.5707	0.299	1348.2	X 1	32/ 76	sp|P02754|LACB_BOVIN	R.VYVEELKPTPEGDLEILLQK.W
1045	./sergei_digest_A_full_01.2277.2279.3	2804.0 (-0.5)	4.5012	0.352	1236.7	✓ 1	31/ 96	sp|P02666|CASB_BOVIN	A.RELEELNVPGEIVESLSSSEESITR.I
861	./sergei_digest_A_full_01.1951.1953.2	2314.7 (-0.4)	4.4874	0.348	482.4	X 1	20/ 38	sp|P02754|LACB_BOVIN	R.VYVEELKPTPEGDLEILLQK.W
626	./sergei_digest_A_full_01.1529.1531.2	1480.7 (-0.5)	4.4192	0.315	1731.6	X 1	20/ 24	sp|P02769|ALBU_BOVIN	K.LGEYGFQNALIVR.Y
176	./sergei_digest_A_full_01.0767.0767.2	1983.0 (+0.7)	4.4109	0.414	1966.2	X 1	22/ 30	sp|P02666|CASB_BOVIN	K.FQSEEQQQTEDELQDK.I
328	./sergei_digest_A_full_01.1009.1011.3	1440.7 (+0.8)	4.4102	0.302	2269.3	X 1	28/ 44	sp|P02769|ALBU_BOVIN	R.RHPEYAVSVLLR.L

Peptide spectrum assignment

Empirical statistical model to estimate the accuracy of peptide identifications

Task:

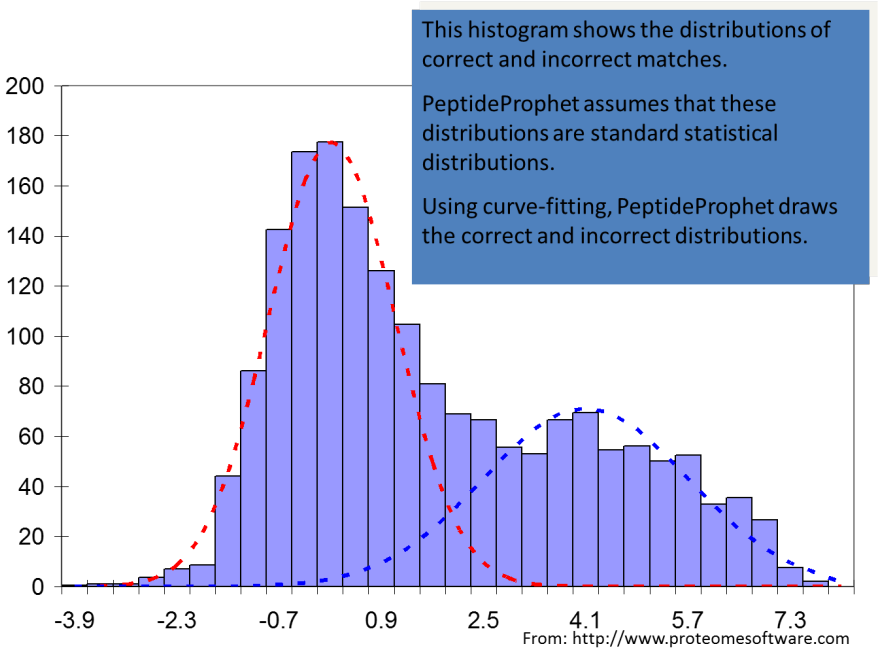
Derive a list of identified peptides from database search results carried out with a large number of MS/MS spectra.

- This entails distinguishing correct peptide assignments from false identifications.
- For high-throughput analysis and consistent data analysis a statistical model is needed to assess the validity of peptide identifications made by MS/MS database searches.

Keller et al., 2002

- **PeptideProphet** computes for each peptide assignment to a spectrum a probability of being correct.

- A discriminant function analysis is used to combine together any number of database search scores into a single discriminant score that best separates training data into correct and incorrect identifications. The discriminant score F is a weighted combination of the database search scores.



- Bayes' theorem gives the probability that a particular peptide assignment with a specific discriminant score is correct:

  $p(+|F) = p(F|+)p(+) / (p(F|+)p(+) + p(F|-)p(-))$

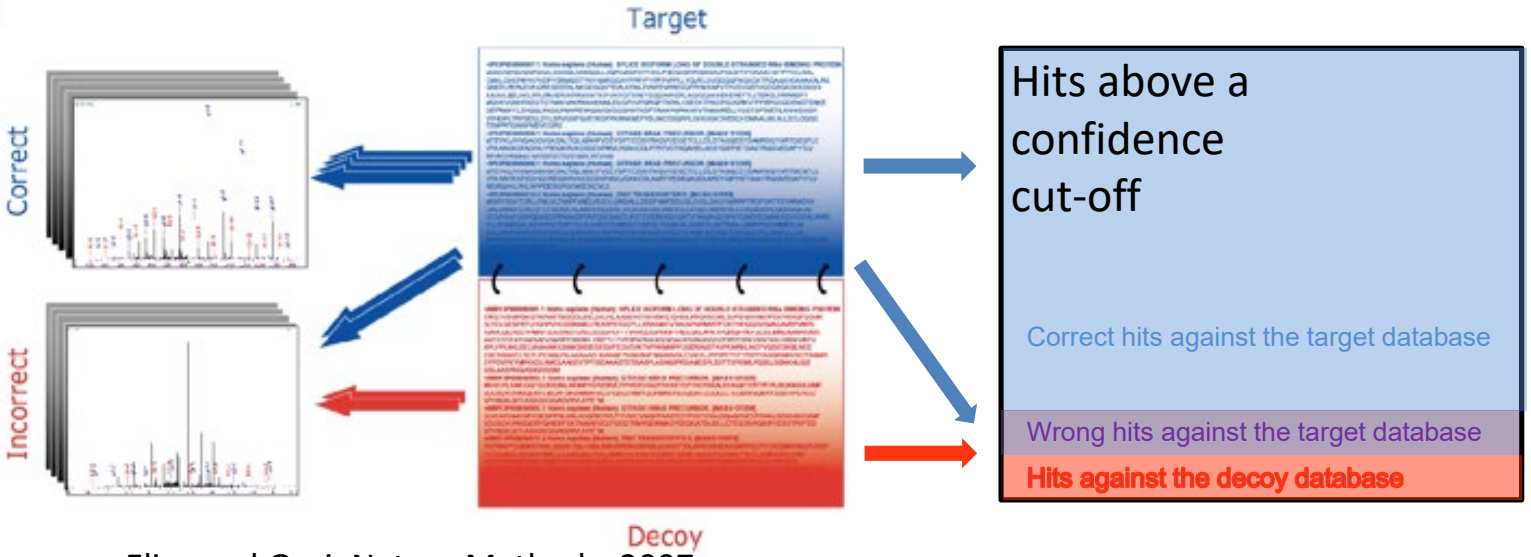
  where $p(+|F)$ = probability that the peptide assignment with discriminant score F is correct

Keller et al., 2002

False positives are a concern and can occur because:

- There is usually a mess of peaks observed in a fragment spectrum, which is a reflection of the population of fragment ions produced in the collision cell of a mass spectrometer (not only b and y ions are produced)
- Spectra can be single peptide ions, chemical noise, non-peptide molecules for mixtures of co-eluting isobaric peptides
- Peptides are often present at a wide range of concentrations in a sample, and peptides present at the limit of detection can produce poor quality spectra
- Chemistry of peptide fragmentation is not completely understood
- There are amino acid sequences that do not produce a unique fragmentation pattern but share enough of the same fragment ions to be indistinguishable from one another

1. All the spectra are searched against a database that consists of the target database concatenated to a decoy database (either randomized or reversed target database)



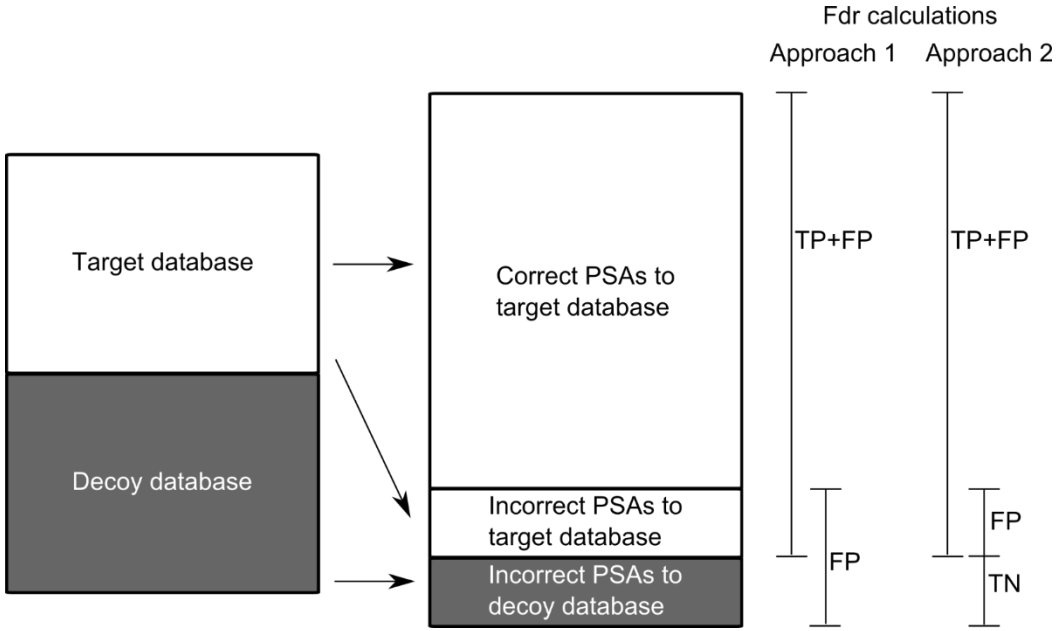
Elias and Gygi, Nature Methods, 2007

1. The hits against the decoy database are clearly wrong as these sequences don't exist

2. It can then be assumed that the number of noticeable wrong hits against the decoy database equals the number of non-noticeable wrong hits against the target database

Calculation of false discovery rates (fdrs)

- The estimation of the fdr is a requirement for the analysis and documentation of mass spectrometry data according to the Paris guidelines of Molecular and Cellular Proteomics (Bradshaw, Burlingame, Carr & Aebersold, 2006)



Svozil and Baerenfaller, MIE, 2017

- Global fdrs are calculated for the full dataset
- Local false discovery rates (lfdrs) can be calculated for a subset of the peptide spectrum matches, e.g. the spectra assigned to peptides carrying specific post-translational modifications, or spectra assigned to peptides in an alternative search database, etc.

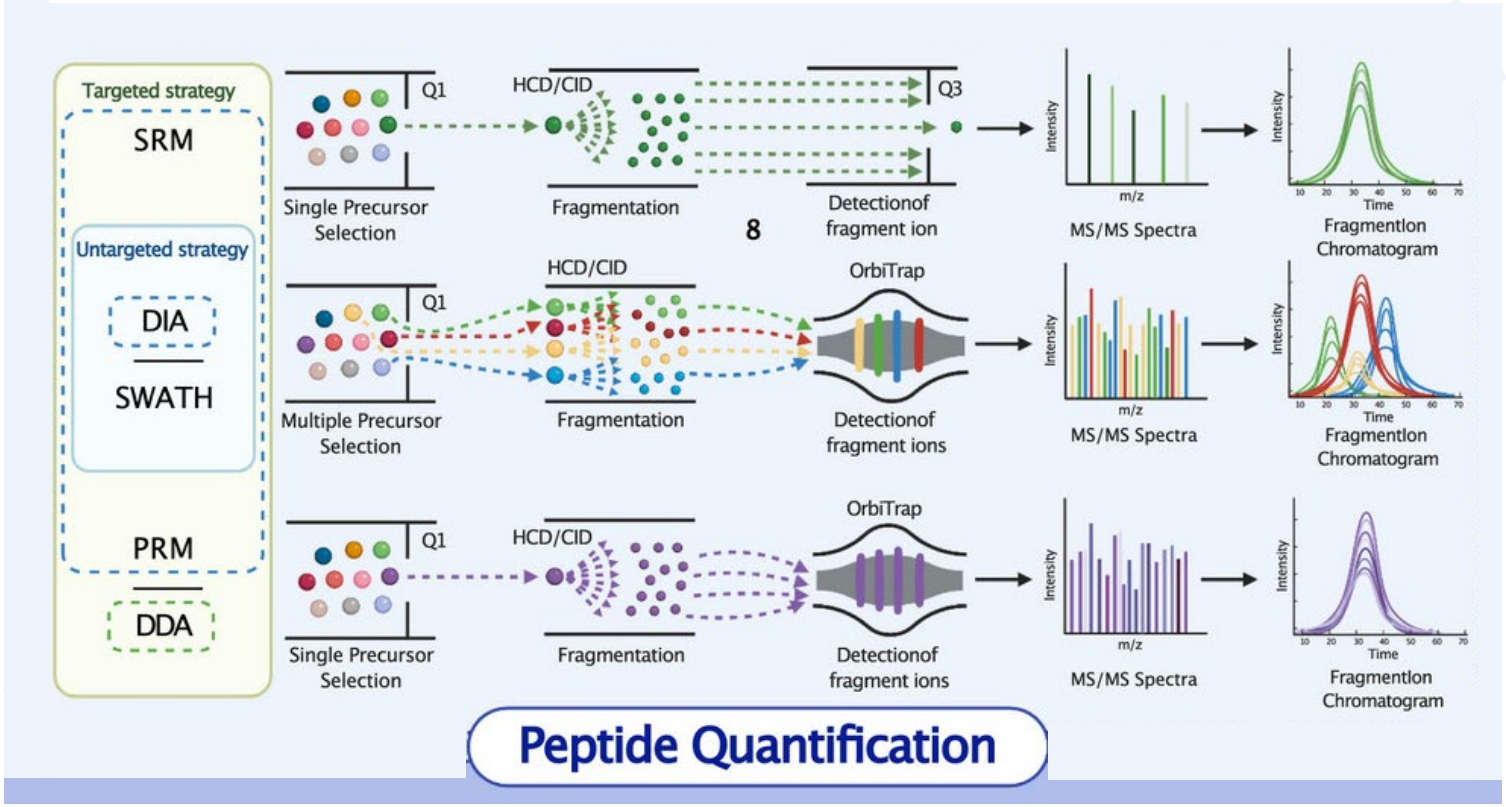
Target-decoy search strategy - Excercise

In a PeptideProphet Search applying a 95% probability cut-off, 1500 spectra were matched to peptides; of these, 30 were matched against peptides from the decoy database.

How many incorrect peptide spectrum matches against the target database do you expect?

https://pwa.klicker.uzh.ch/join/kbaere

Hypothesis-driven, targeted bottom-up proteomics approaches



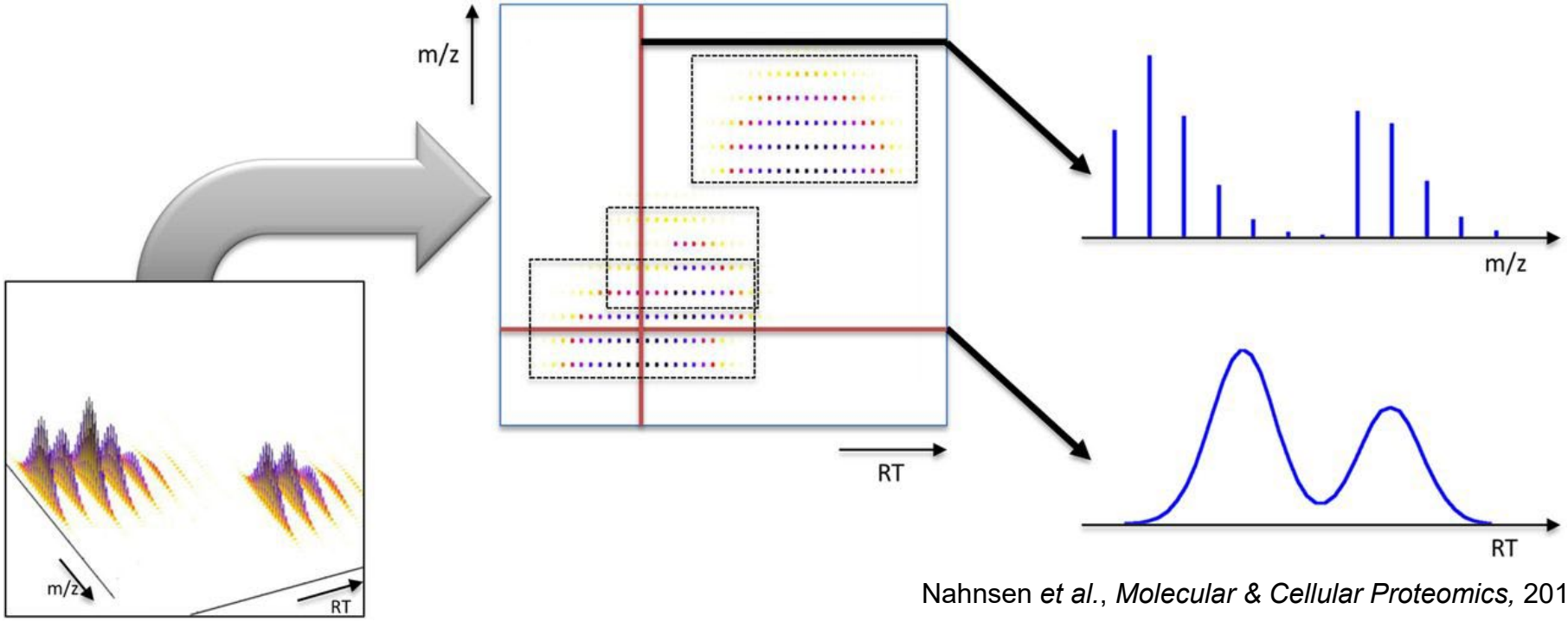
Radzikowska et al., *Omics technologies in allergy and asthma research: An EAACI position paper,* Allergy, 2022

S/MRM: Selected/Multiple Reaction Monitoring; the proteins are pre-selected and provide information on the characteristic peptide precursor and fragment ion signals (transitions)

DIA/SWATH: Data Independent Acquisition/Sequential Windowed Acquisition of All Theoretical Mass Spectra

PRM: Parallel Reaction Monitoring; similar to S/MRM, but all resulting fragment ion signals from a precursor ion are monitored

Label-free quantification (data dependent analysis DDA)



Nahnsen *et al.*, *Molecular & Cellular Proteomics,* 2013

LC/MS data consist of individual MS spectra accumulated over (retention) time. Stacked side by side, these spectra form two-dimensional maps.

→ In spectral counting the basic assumption is that protein abundance is proportional to the number of spectra (after normalization)
→ Quantification can also be based on the comparison of features, which can be defined as all mass-spectrometric signals (peaks) caused by the same peptide

Workflow for post-translational modifications (PTMs)

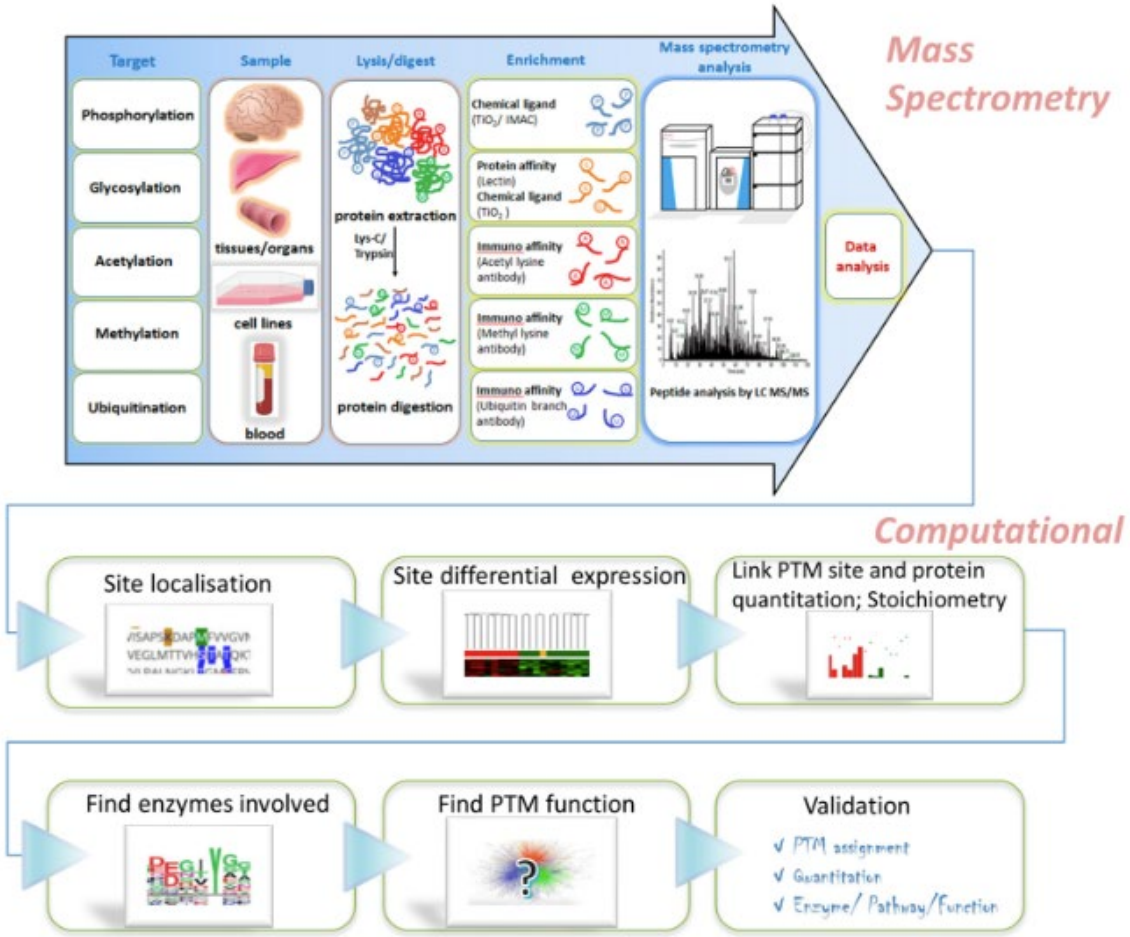
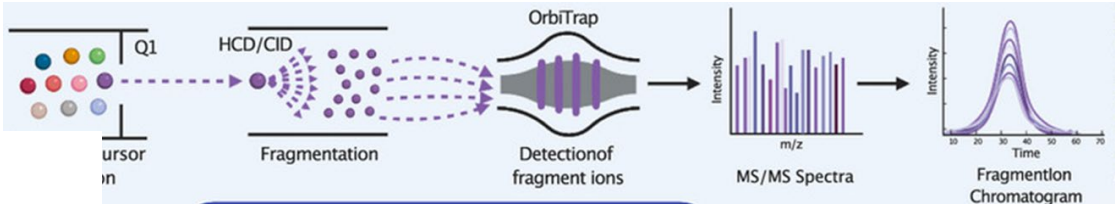
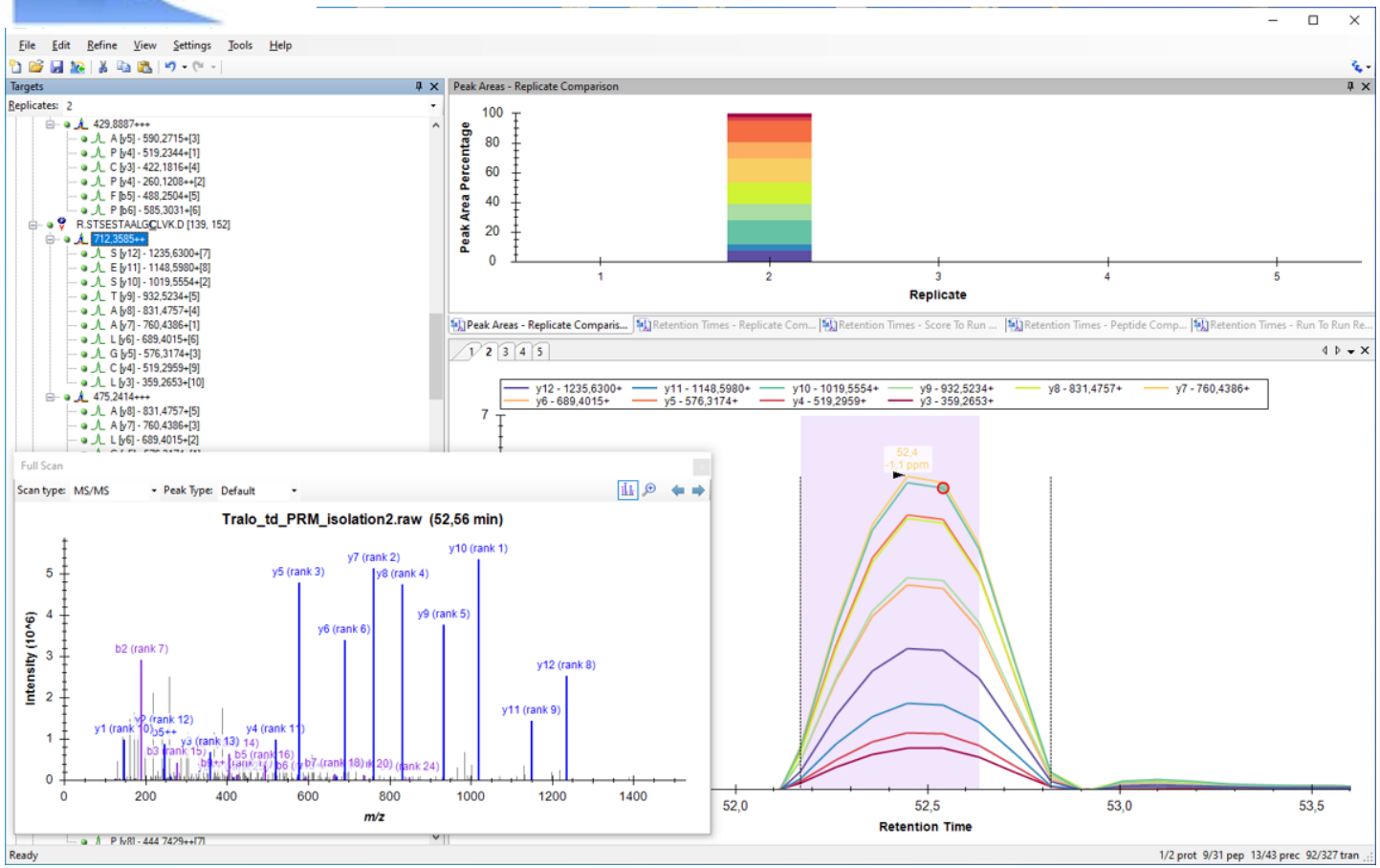


Figure 1 in Pascovici et al., Int. J. Mol. Sci. 2019, doi.org/10.3390/ijms20010016

- If PTMs are analysed, the modified proteins or peptides need to be enriched first, as the modified peptides are strongly in the minority
- Searching a lot of variable modifications in your peptide spectrum matching will result in a huge search space and a lot of false positive identifications, so only search for enriched or abundant modifications
- To claim changes in protein/peptide modifications, the PTM data need to be linked to measurements of the total proteome without PTM enrichment

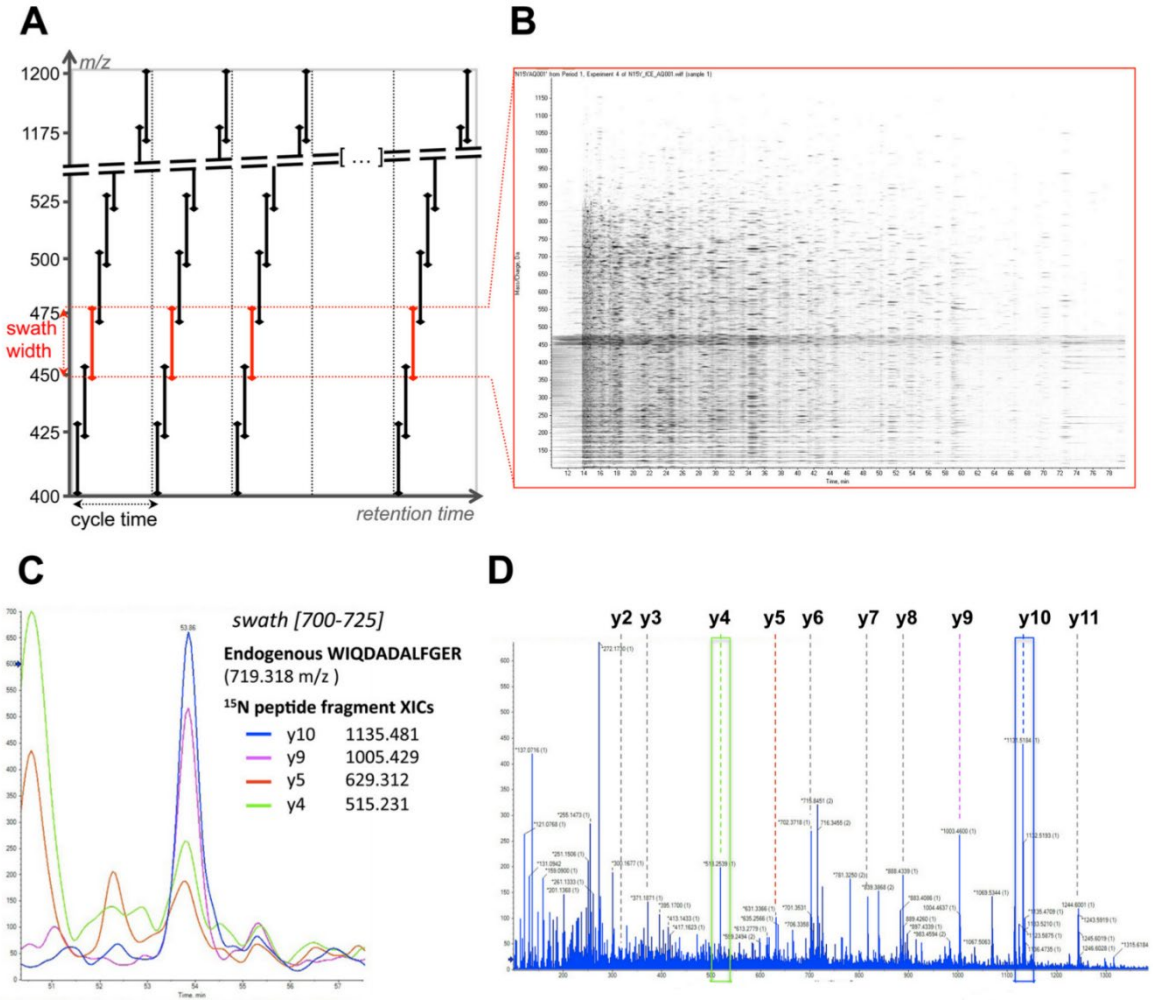
Peptide identification and quantification with Parallel Reaction Monitoring (PRM)





→ With PRM, a set of predefined proteins/peptides can be detected and quantified

SWATH-MS (data independent analysis DIA)



A) the mass spectrometer steps through a set of precursor acquisition windows

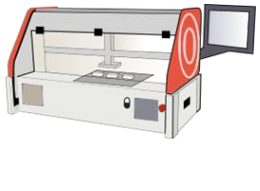
B) In each cycle it fragments all precursors from all the respective quadrupole isolation windows and in each isolation window it records a complete, high accuracy fragment ion spectrum of all precursors

C) The data are analyzed by reconstructing the lineage of precursor and fragment ions based on their chromatographic elution profile, and with software for automated targeted data analysis

@ http://www.imsb.ethz.ch/research/aebersold/research/swath-ms.html

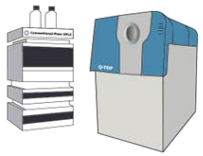
How to measure thousands of proteome profiles

Christoph Messner
Center for Precision Proteomics



Sample/Culture
(in 96 well plates)

Sample peparation:
96-well based semi-automated
Protein extraction, digestion
and SPE clean-up



Data acquisition:
- "High" flow LC
- "scanning" SWATH
- 1-20 minute gradient

Data processing with DIA-NN:
- based on neural networks
- open source
- github.com/vdemichev/diann

Throughput:

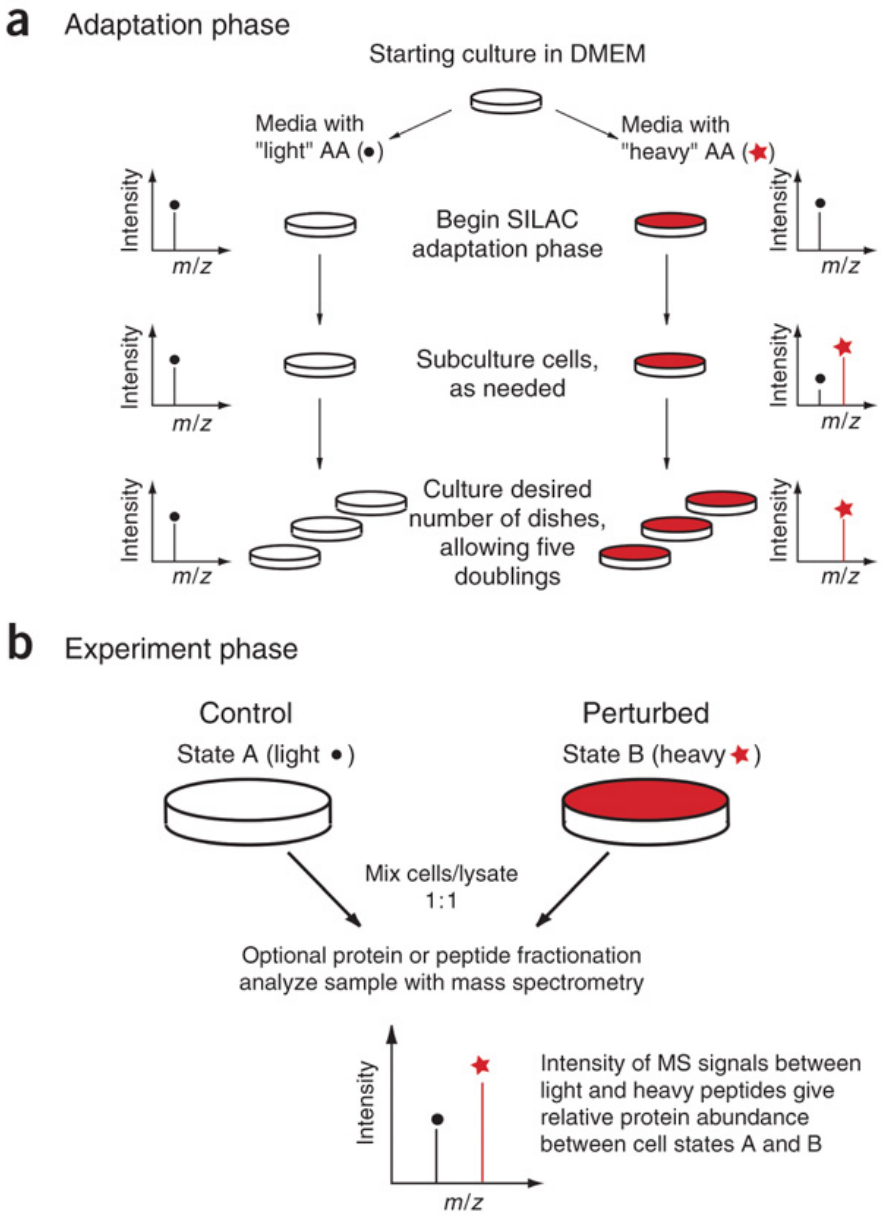
Up to 768 samples/day

> 100 samples/day
on one Instrument

> 2,000 samples/day

Messner et al, Nature Biotechnology, 2021
Messner et al, Cell Systems, 2020
Demichev, Messner et al, Nature Methods, 2020

Quantification with labelling: SILAC

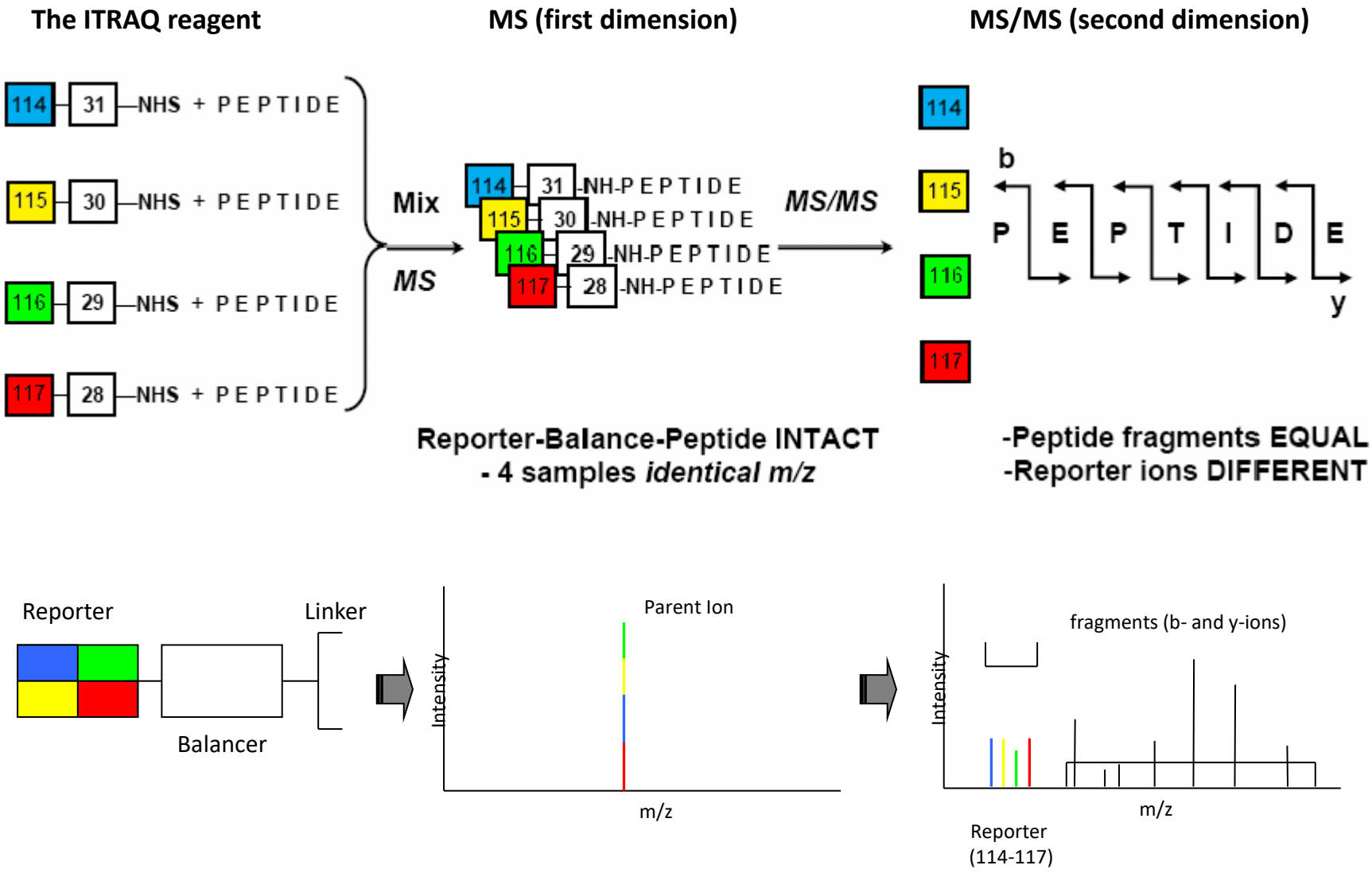


SILAC = stable isotope labeling by amino acids in cell culture

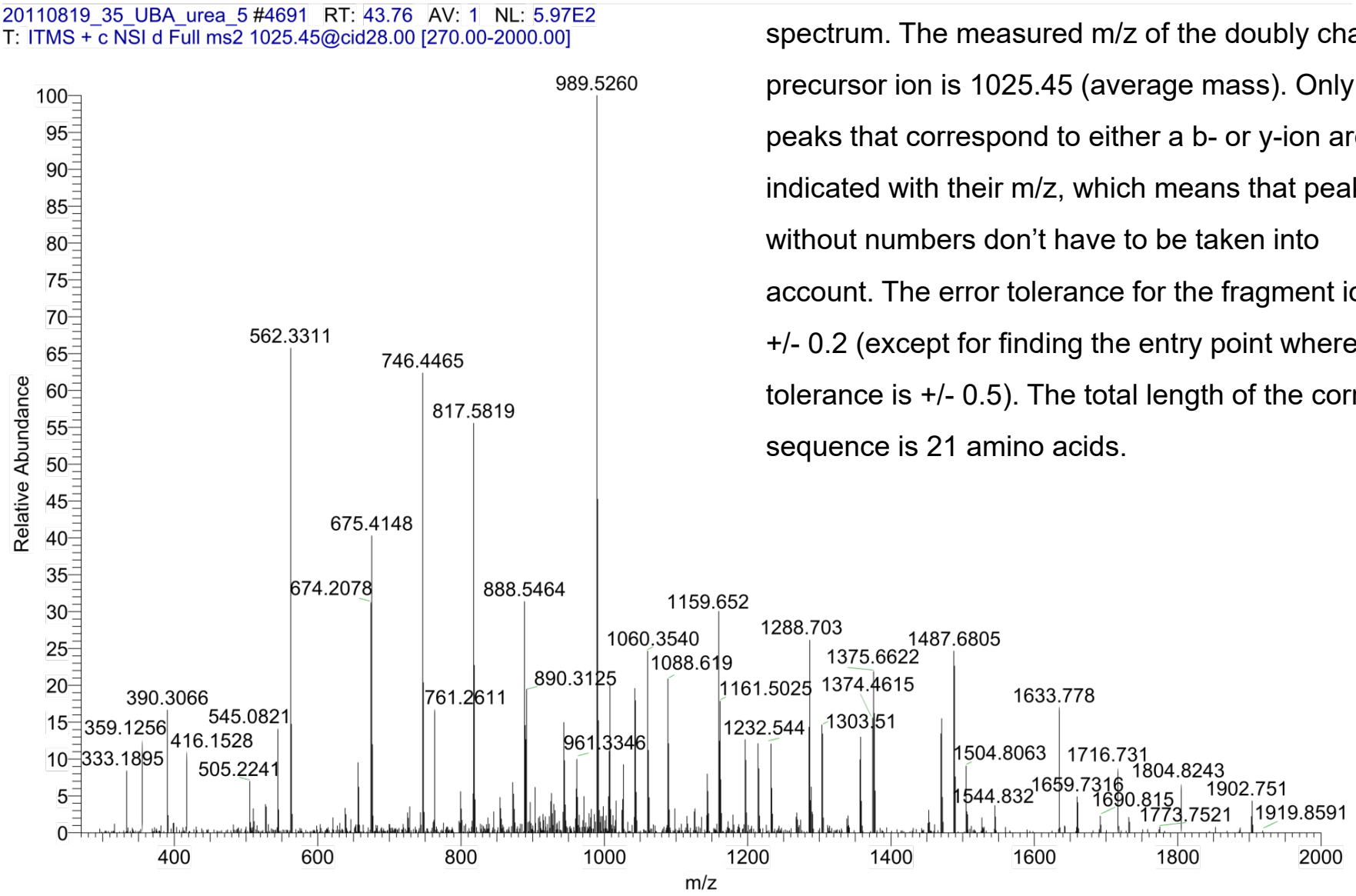
→ In the adaptation phase, cells are grown in light and heavy SILAC media until full incorporation of heavy amino acids
→ In the experimental phase, the two populations are treated differentially to induce changes in the proteome

Afterwards, the samples are mixed and processed; the peptide are analyzed by MS for protein identification and quantification

Ong and Mann, *Nature Protocols,* 2007


The ITRAQ reagent
MS (first dimension)
MS/MS (second dimension)
114 31 NHS + PEPTIDE
115 30 NHS + PEPTIDE
116 29 NHS + PEPTIDE
117 28 NHS + PEPTIDE
Mix
MS
114 31 -NH-PEPTIDE
115 30 -NH-PEPTIDE
116 29 -NH-PEPTIDE
117 28 -NH-PEPTIDE
MS/MS
b
P E P T I D E
y
Reporter-Balance-Peptide INTACT
- 4 samples identical m/z
-Peptide fragments EQUAL
-Reporter ions DIFFERENT
Reporter
Linker
Balancer
Intensity
Parent Ion
m/z
fragments (b- and y-ions)
Intensity
m/z
Reporter
(114-117)

De novo peptide sequencing – Exercise



Derive an amino acid sequence from the idealized spectrum. The measured m/z of the doubly charged precursor ion is 1025.45 (average mass). Only peaks that correspond to either a b- or y-ion are indicated with their m/z, which means that peaks without numbers don't have to be taken into account. The error tolerance for the fragment ions is +/- 0.2 (except for finding the entry point where the tolerance is +/- 0.5). The total length of the correct sequence is 21 amino acids.