

Osnova

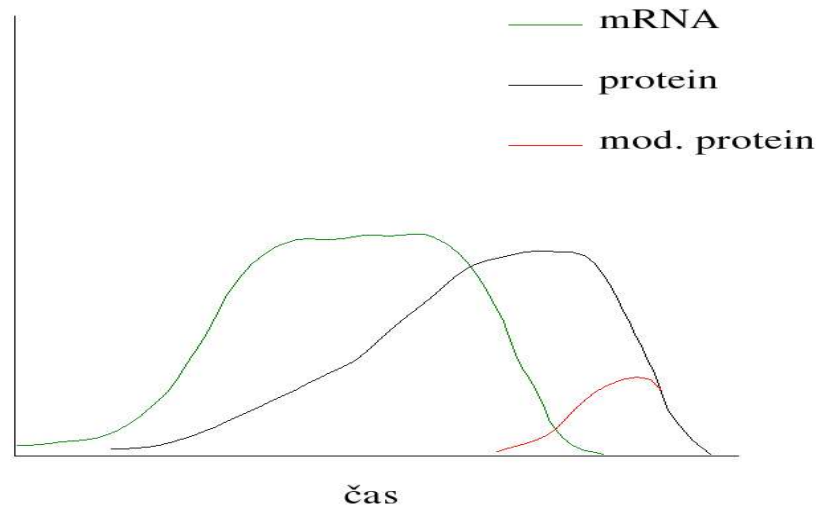
- Proteomika
 - 2-D gely
 - Hmotnostní spektrometrie
 - Proteinová pole (protein array)

GENOM, TRANSKRIPTOM, PROTEOM, METABOLOM

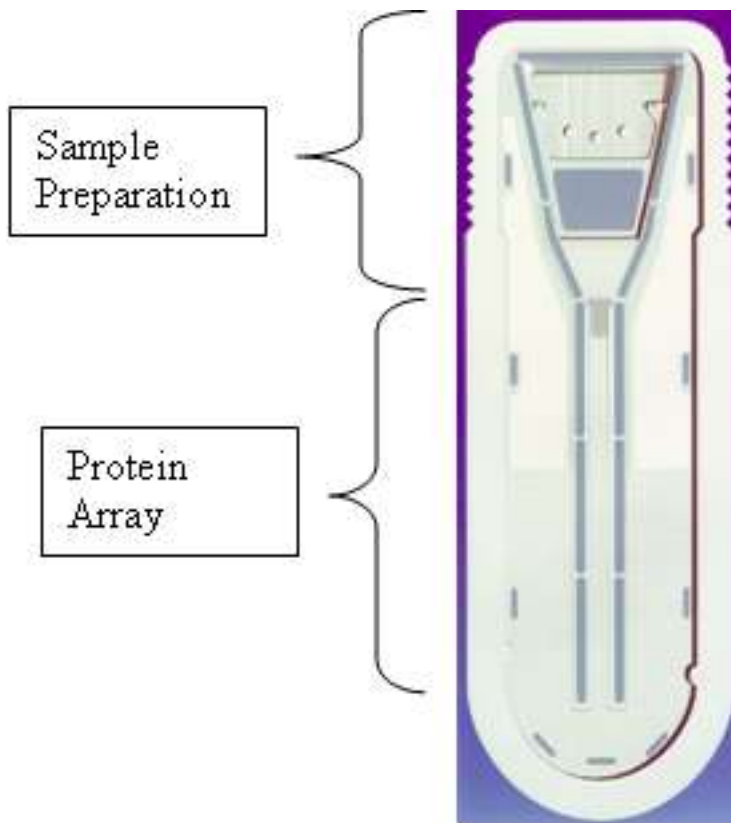
G	Sekvence DNA	statická data	sekvenátor
T	Populace mRNA	dynamická data	microarray
P	Populace proteinů	dynamická data	2-D gely, MS
M	Populace molekul	vysoce dyn. data	chromatografie spektrometrie

http://www.expasy.org/tools/findmod/findmod_masses.html

Proč nestačí “microarray” ?



GENOMIKA <-> PROTEOMIKA



Komerční model
proteinového čipu pro
analýzu vzorků krve

Princip identifikace proteinů

Běžné charakteristiky celých molekul nedokážou jednoznačně identifikovat protein

- molekulová hmotnost
- zastoupení aminokyselin
- elektrický náboj (pI)

N	NAME	%
237795	L	Leu 9.4
199138	A	Ala 7.8
192381	G	Gly 7.6
191458	S	Ser 7.5
175970	V	Val 6.9
154267	E	Glu 6.1
146184	T	Thr 5.7
137735	I	Ile 5.4
135069	K	Lys 5.3
133582	R	Arg 5.2
124916	D	Asp 4.9
115228	P	Pro 4.5
105957	F	Phe 4.1
102780	N	Asn 4.0
95080	Y	Tyr 3.7
93296	Q	Gln 3.6
56348	M	Met 2.2
50486	H	His 1.9
37544	C	Cys 1.4
36530	W	Trp 1.4
3202	X	- 0.1

Zastoupení aminokyselin v
sekvencích 10000 proteinů z
databáze OML

Výsledky hledání pomocí aminokyselinového složení, pI a Mw

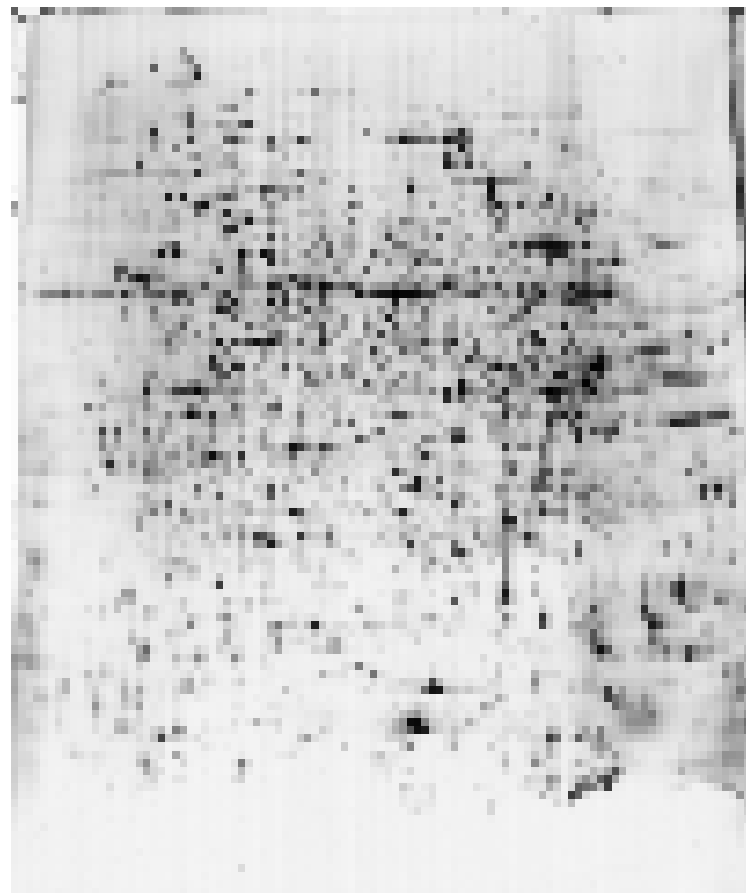
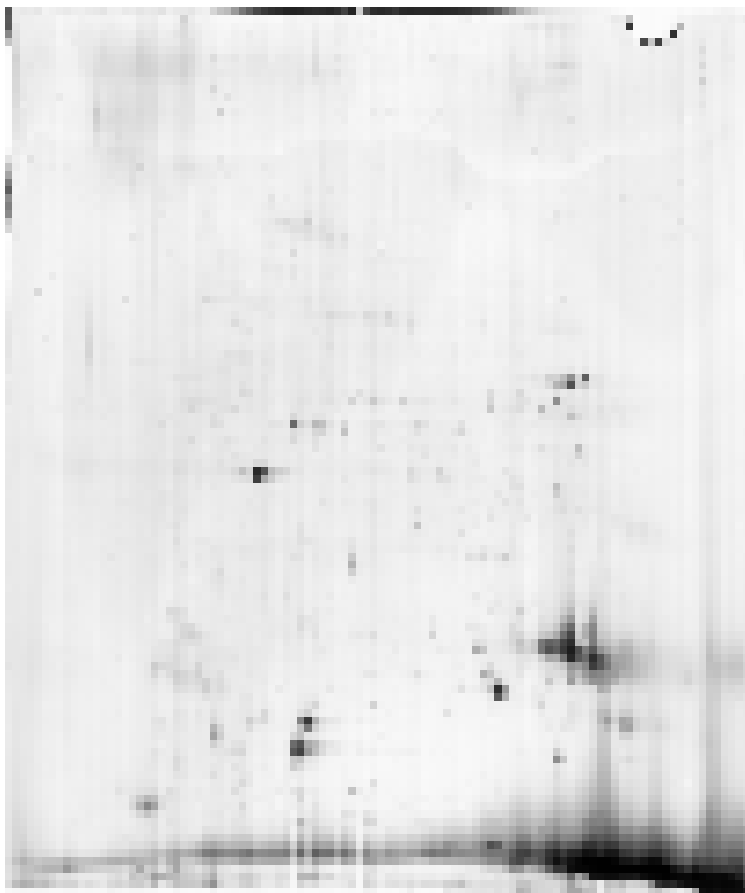
Rank	Score	Protein	(pI	Mw)	Description
1	5	GRIA4_RAT	7.59	98421	Glutamate receptor 4.
2	5	GRIA4_MOUSE	7.59	98295	Glutamate receptor 4.
3	6	GRIA4_HUMAN	8.21	98489	Glutamate receptor 4.
4	6	POL1_ARMVN	6.44	71899	NTP-binding protein (Potential).
5	7	TYDC4_PETCR	6.24	56771	Tyrosine decarboxylase 4 (EC 4.1.1.25).
6	7	HMCS1_CHICK	5.41	57559	Hydroxymethylglutaryl-CoA synthase,
7	7	ACOX5_CANTR	6.09	74106	Acyl-coenzyme A oxidase 5 (EC 1.3.3.6)
8	7	TYDC2_PETCR	6.29	57450	Tyrosine decarboxylase 2 (EC 4.1.1.25).
9	8	LOX1_LENCU	6.01	96639	Lipoxygenase (EC 1.13.11.12).
10	8	VIRD4_AGRTU	7.12	75950	Protein virD4.
11	8	TYDC3_PETCR	6.80	57633	Tyrosine decarboxylase 3 (EC 4.1.1.25).
12	8	ACOX3_ARATH	7.70	71909	Acyl-coenzyme A oxidase 3.

<http://www.expasy.org/tools/multiident/>

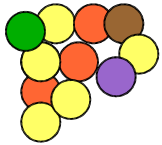
Fibroblasty – WT v. Downuv syndrom



Červené krvinky v. rostlina *Arabidopsis*



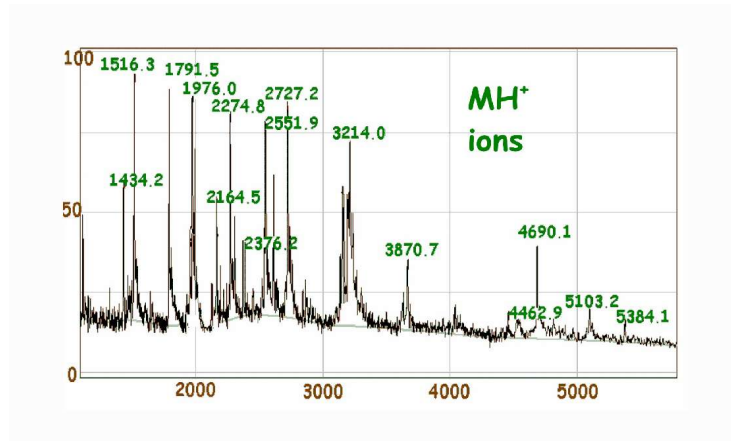
Hmotnostná spektrometria



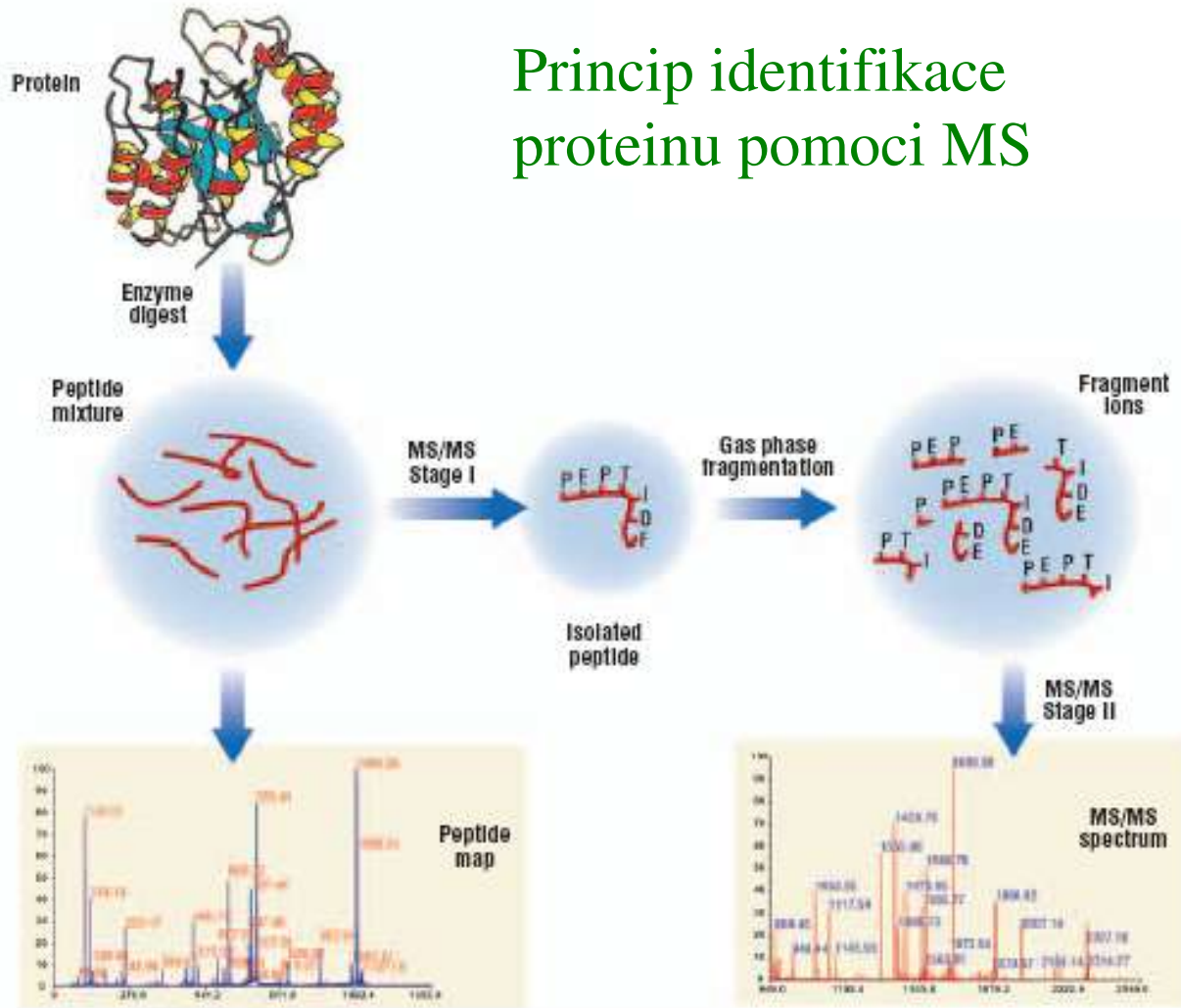
PROTEIN

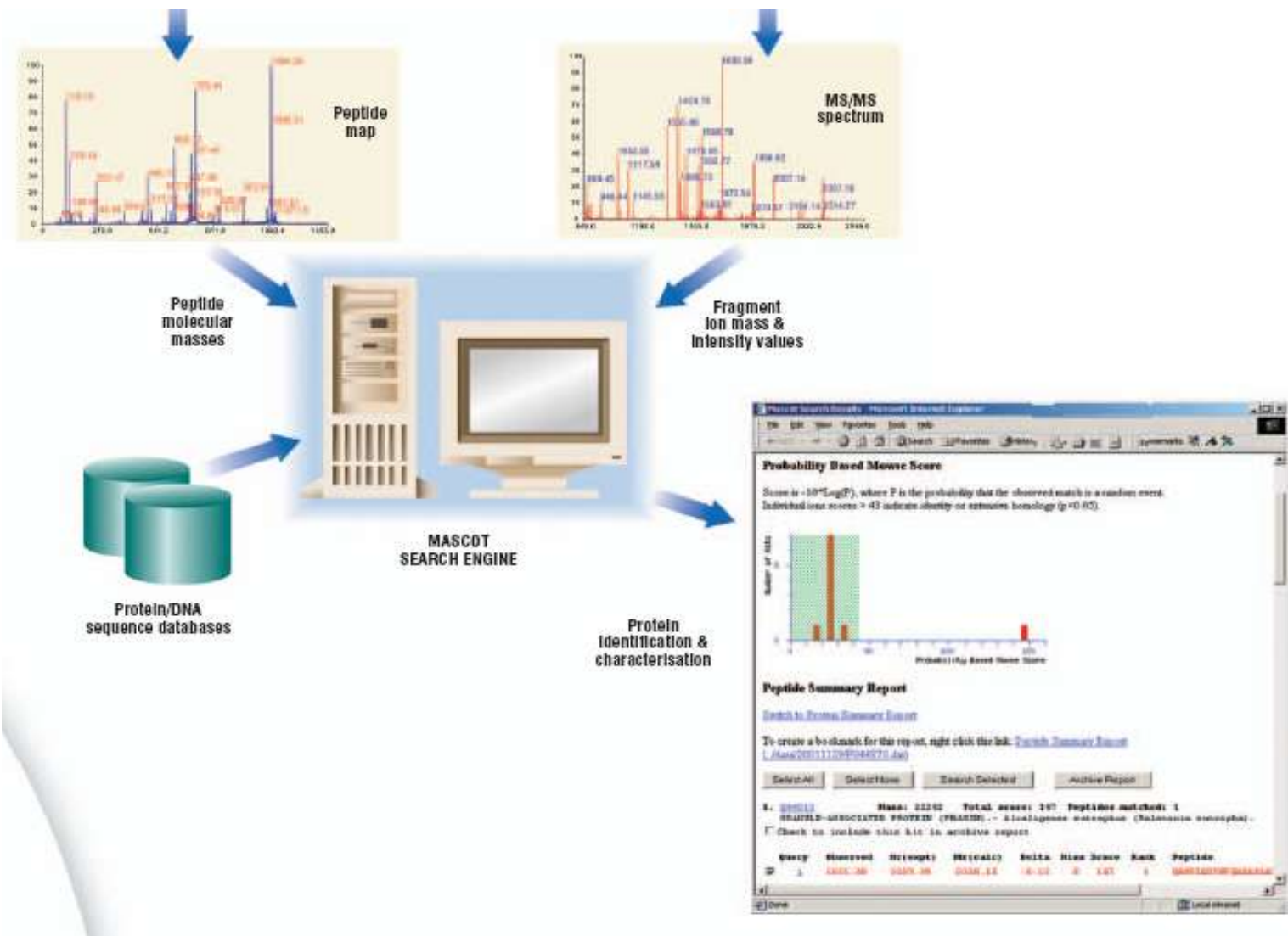


SPEKTRUM



Princip identifikace proteinu pomoci MS





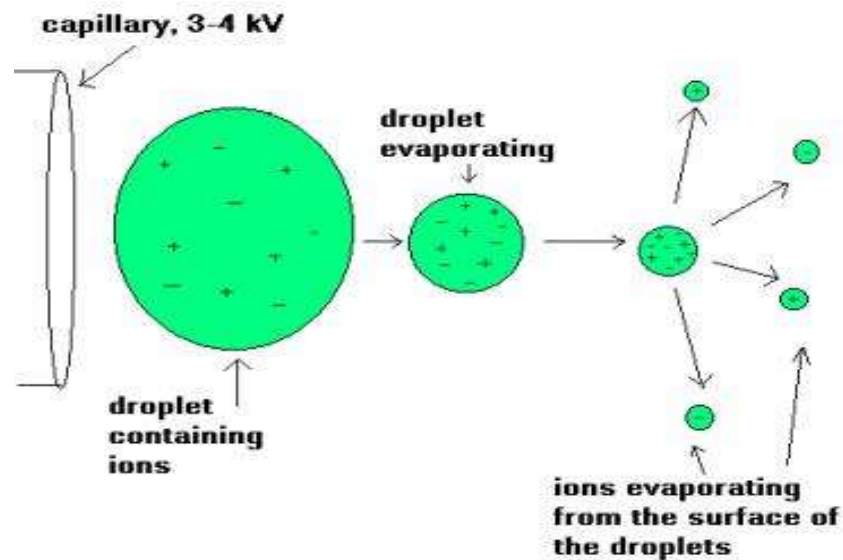
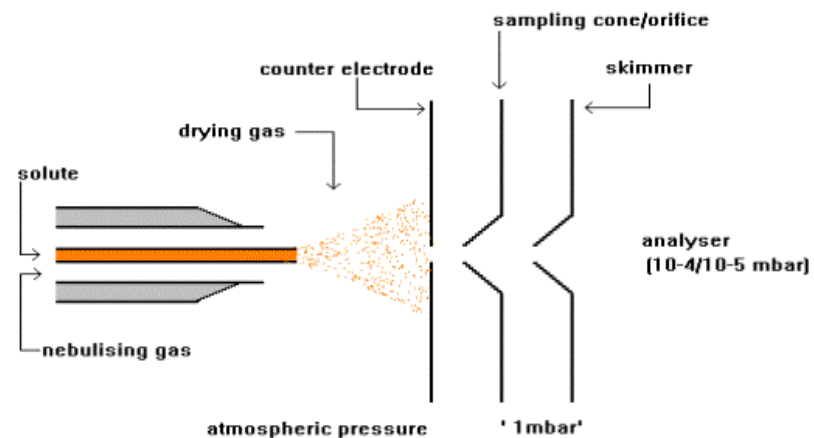
MS – Peptide Mass Fingerprinting(ESI)

MS – Peptide Mass Fingerprinting
(MALDI-TOF)

MS/MS (tandem MS) – Ion Search

Hmotnostná spektrometria

MS (ESI)



Hmotnostná spektrometria

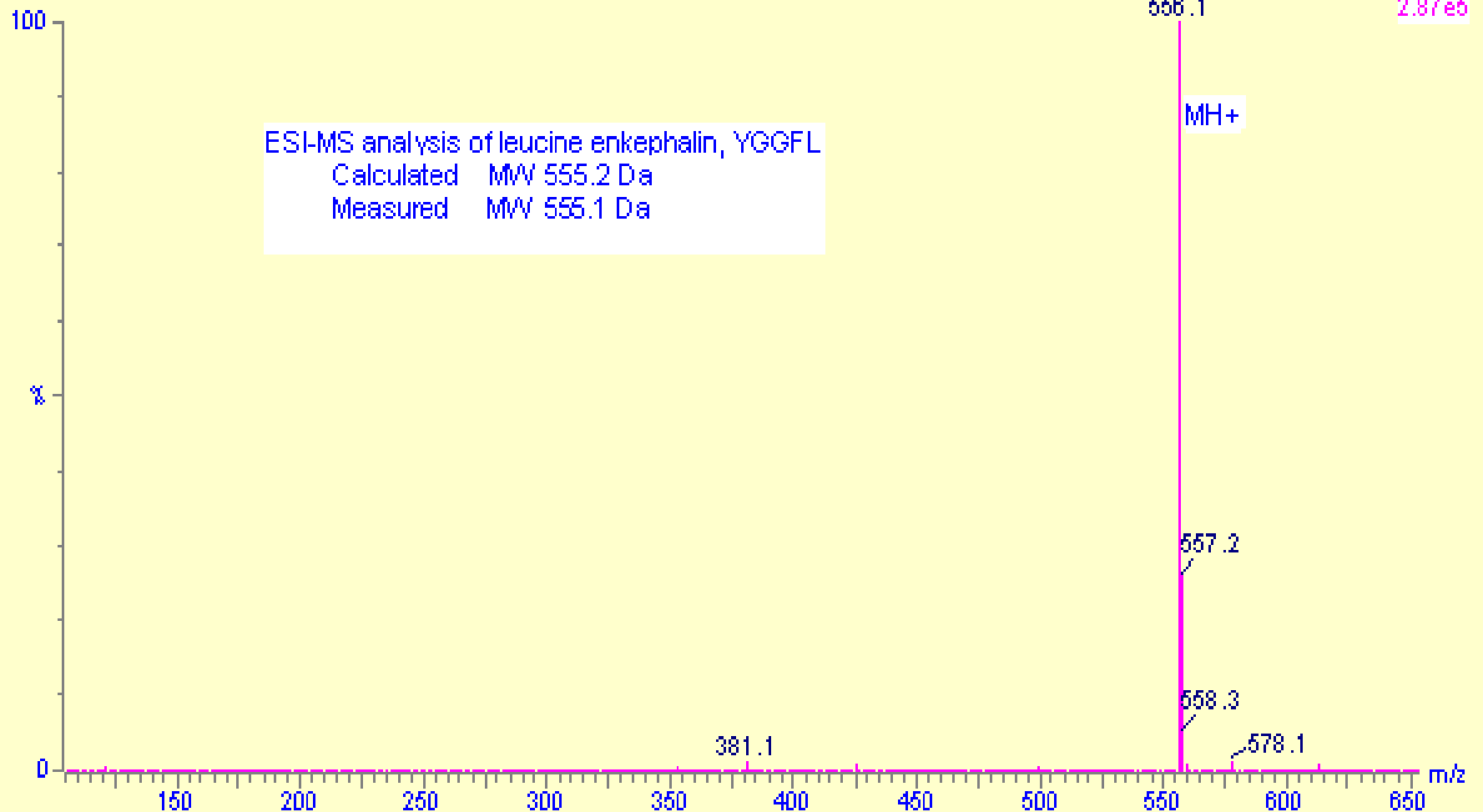
leucine enkephalin

Platform II, BMB, The University of Leeds

04-Oct-1999 10:12:26

TESTD1 32 (1.679) Cm (3:34)

Scan ES+
2.87e5



Hmotnostná spektrometria

hen egg lysozyme

Platform II, BMB, The University of Leeds

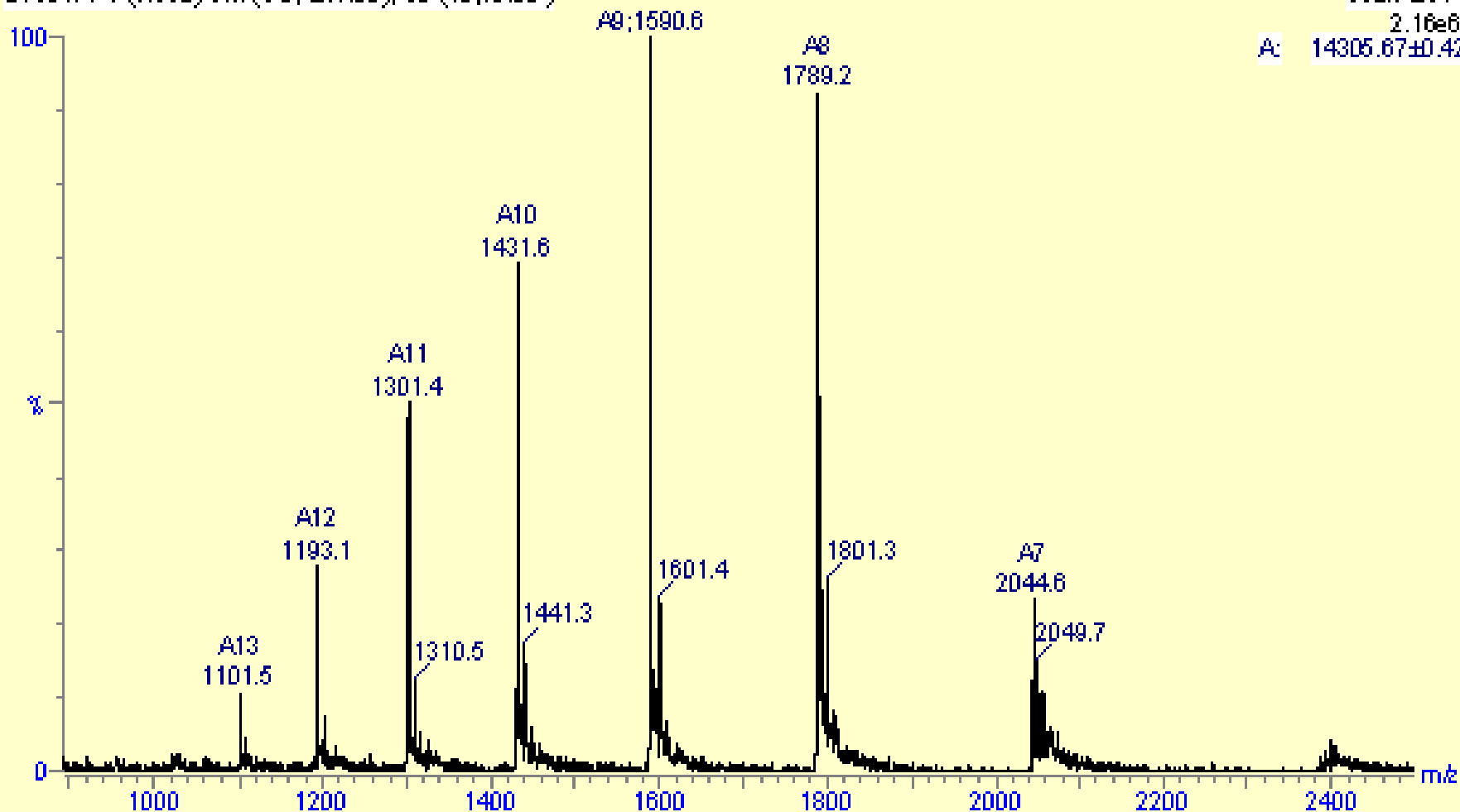
25-Jan-2000 10:00:37

LYSD1A_1 (1.392) Sm (SG, 2x1.00); Sb (10,10.00)

Scan ES+

2.16e6

A: 14305.67±0.42



Hmotnostná spektrometria

apo-pseudoazurin + Cu

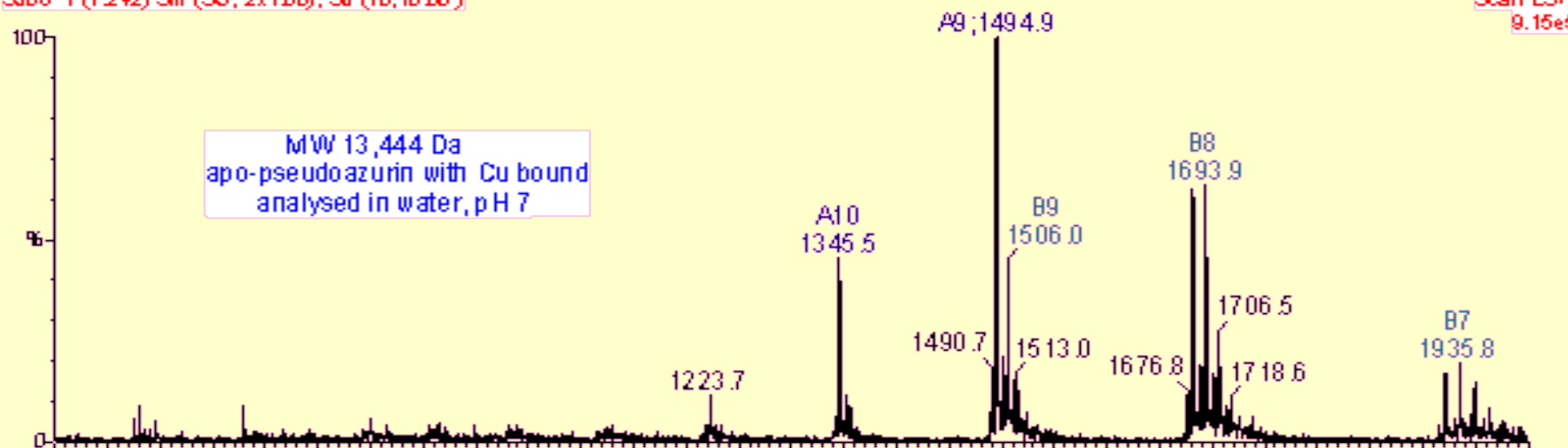
SJ06 1 (1.242) Sm (SG, 2x1.00); Sb (10, 10.00)

BMB, The University of Leeds

30-Oct-1998 14:50:44

Scan ES+
9.15e5

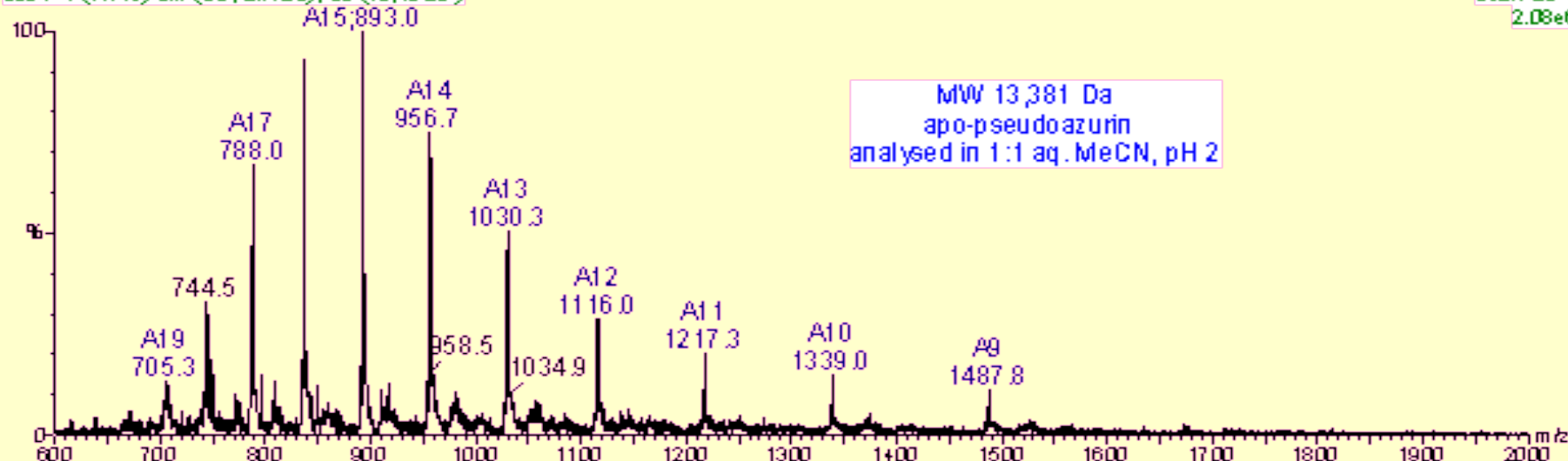
MW 13,444 Da
apo-pseudoazurin with Cu bound
analysed in water, pH 7



SJ0+ 1 (1.415) Sm (SG, 2x1.00); Sb (10, 10.00)

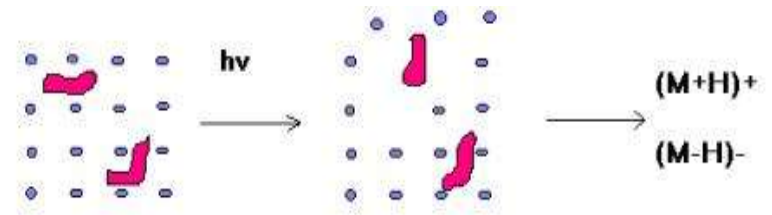
Scan ES+
2.08e6

MW 13,381 Da
apo-pseudoazurin
analysed in 1:1 aq. MeCN, pH 2

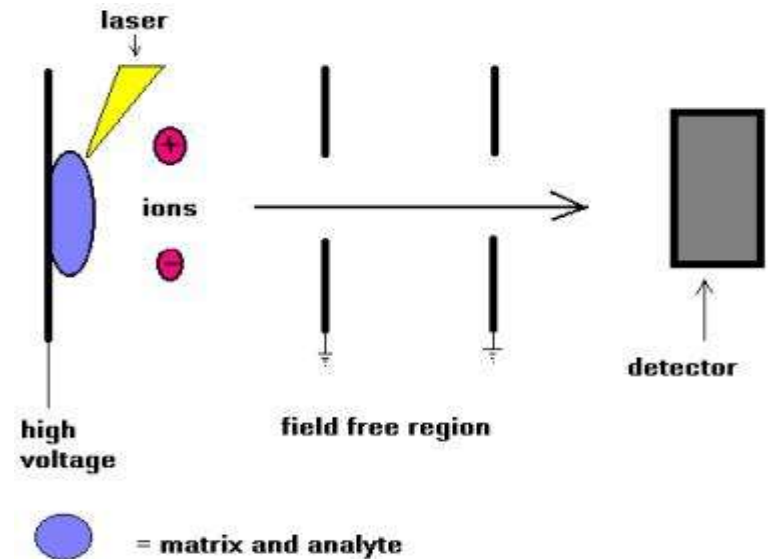


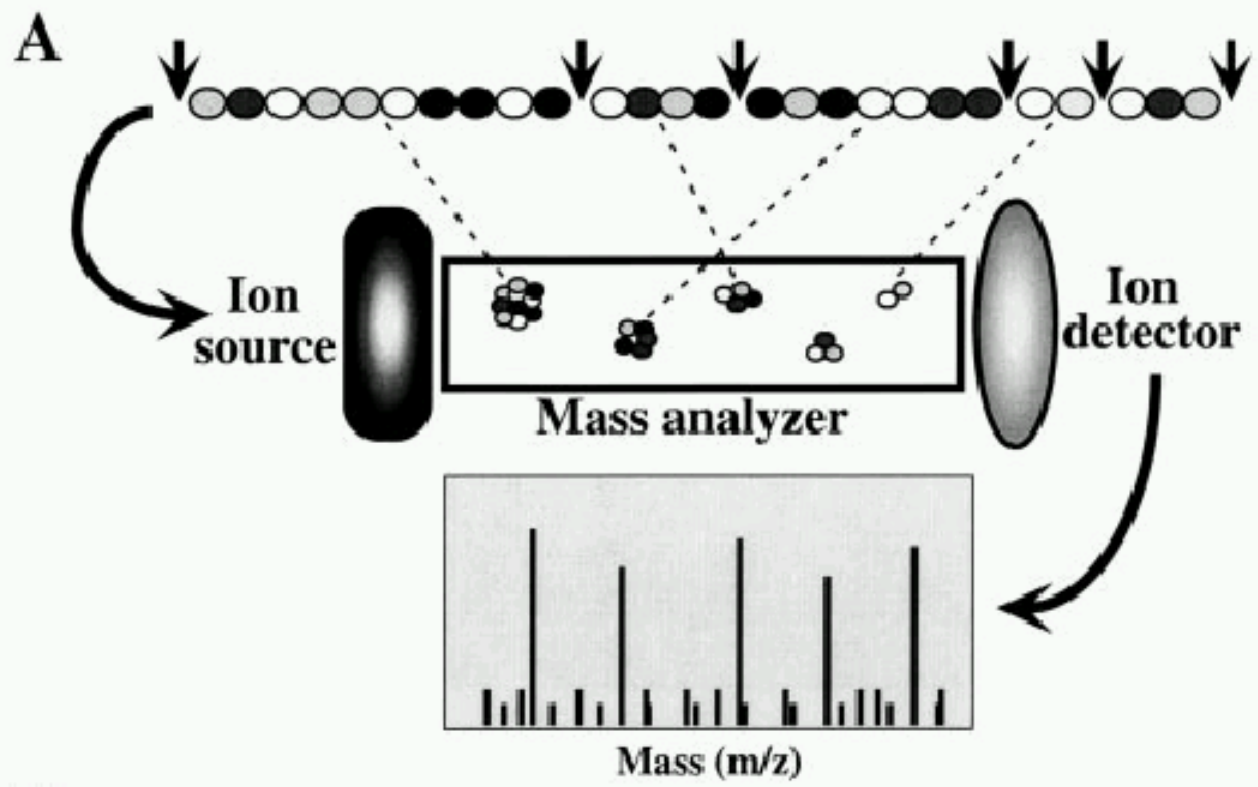
Hmotnostná spektrometria

MS (MALDI-TOF)



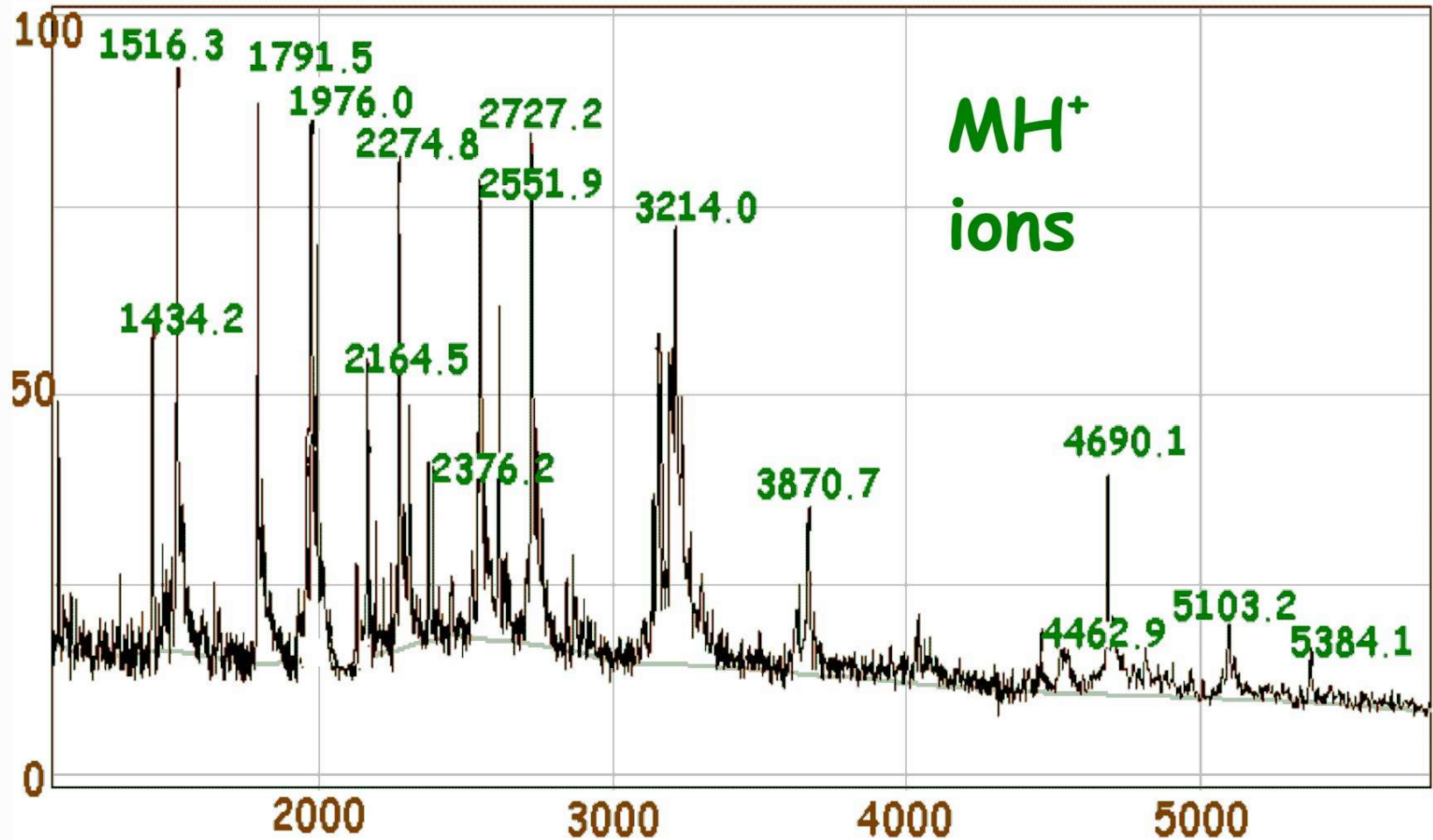
 = matrix
 = analyte

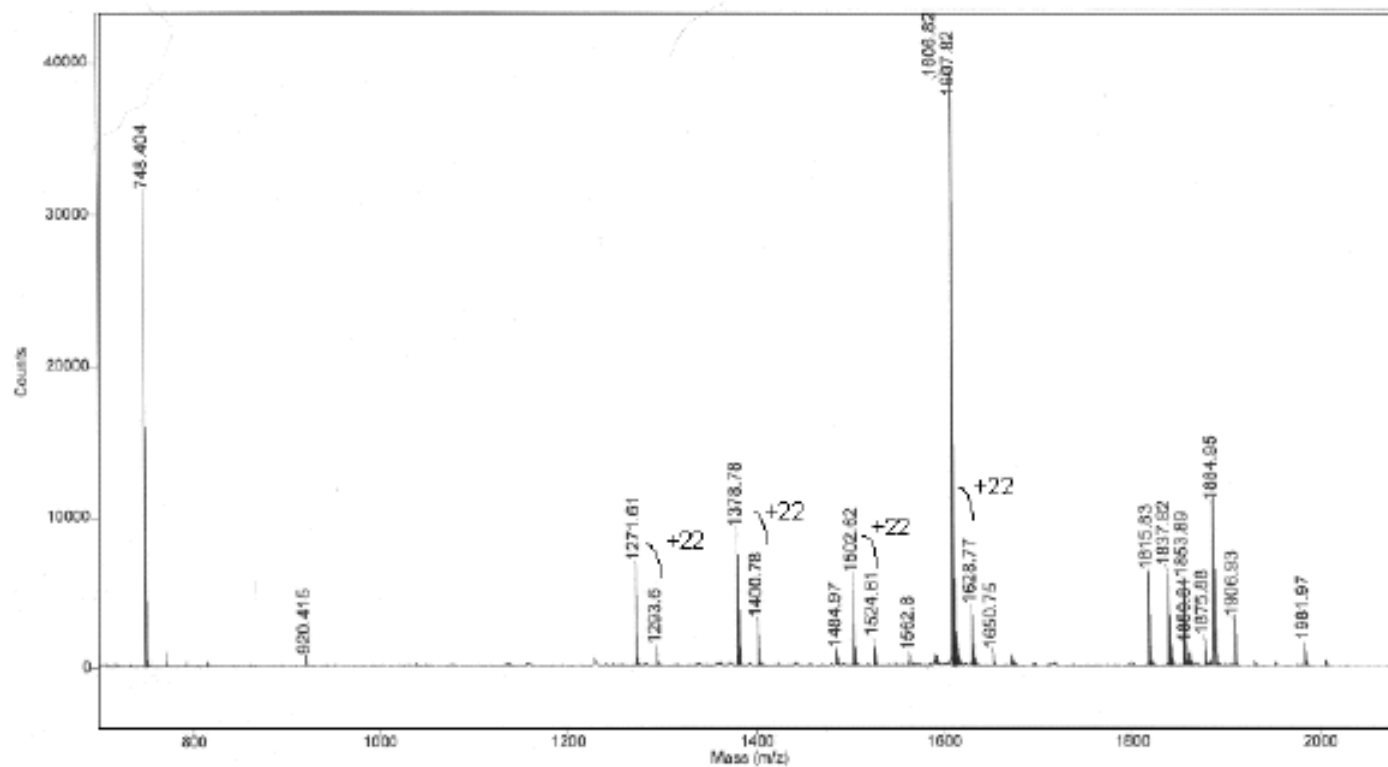




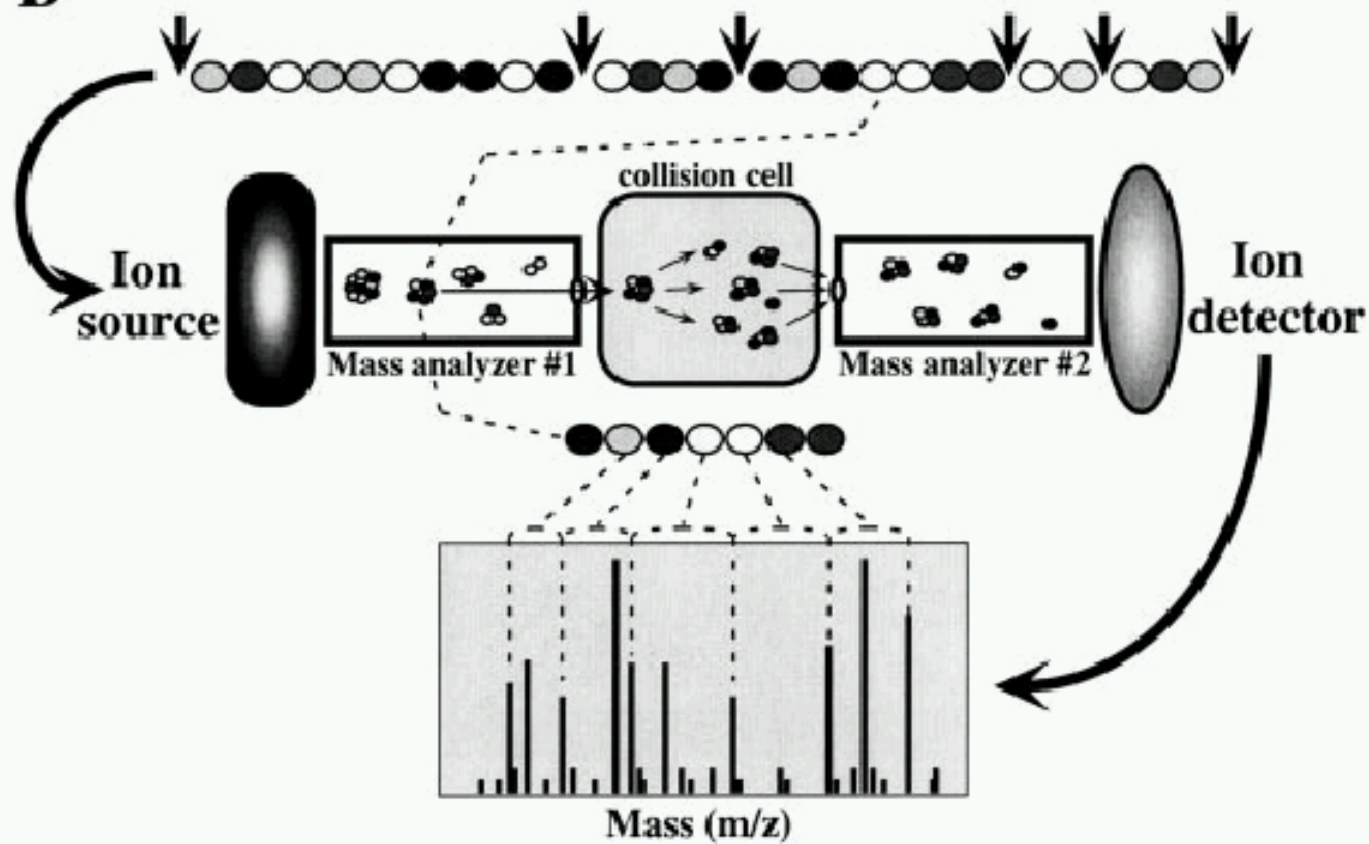
D

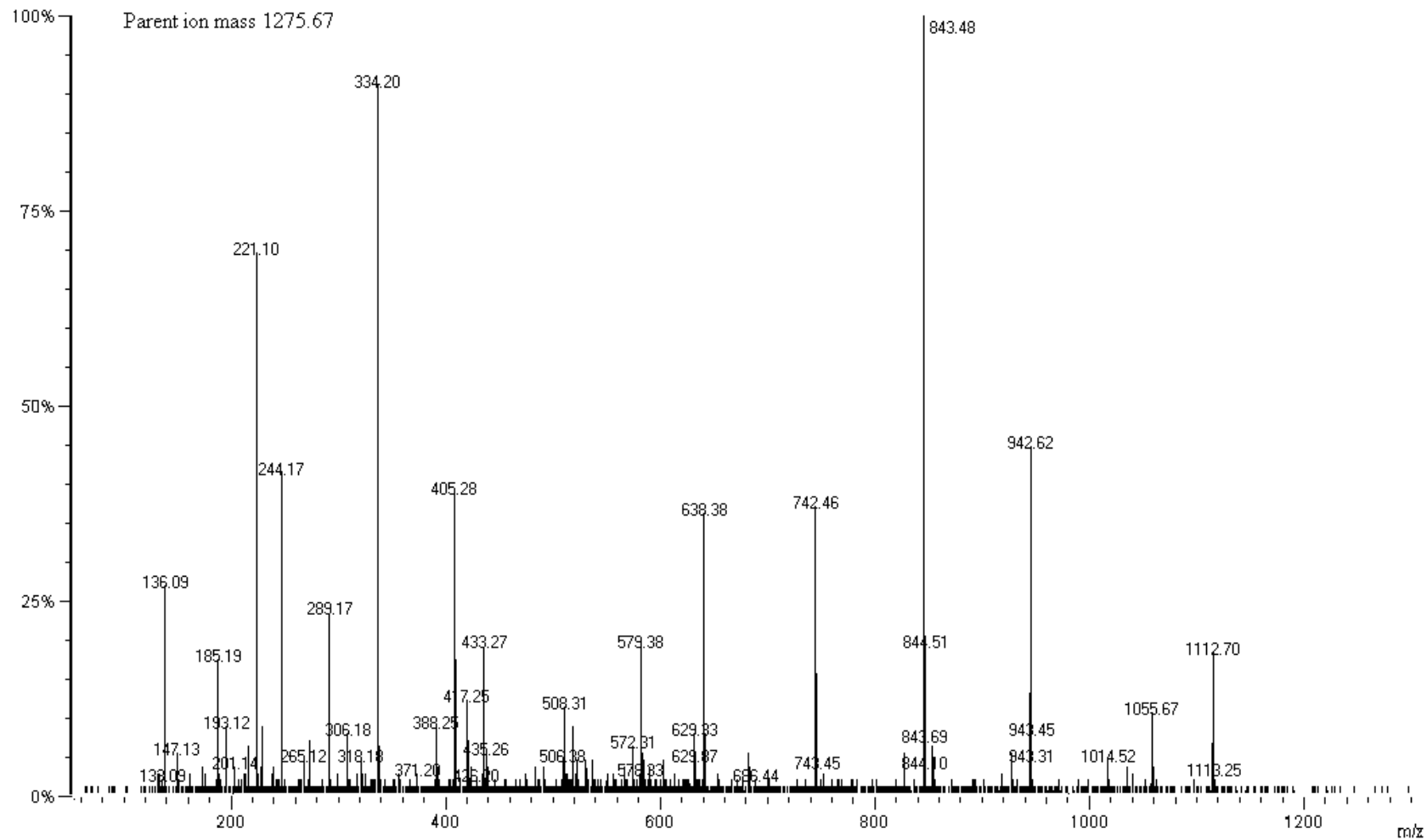
Hmotnostná spektrometria





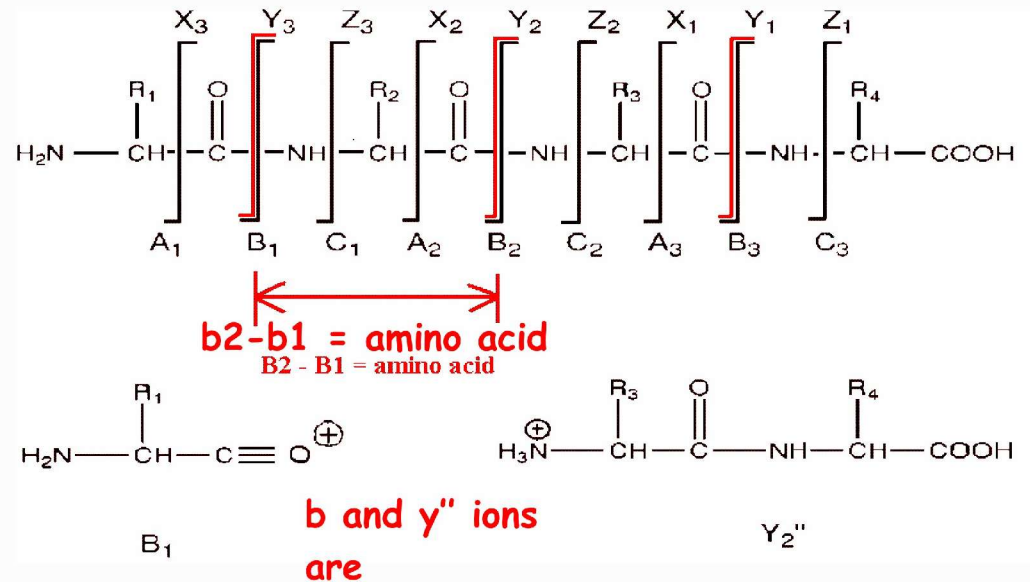
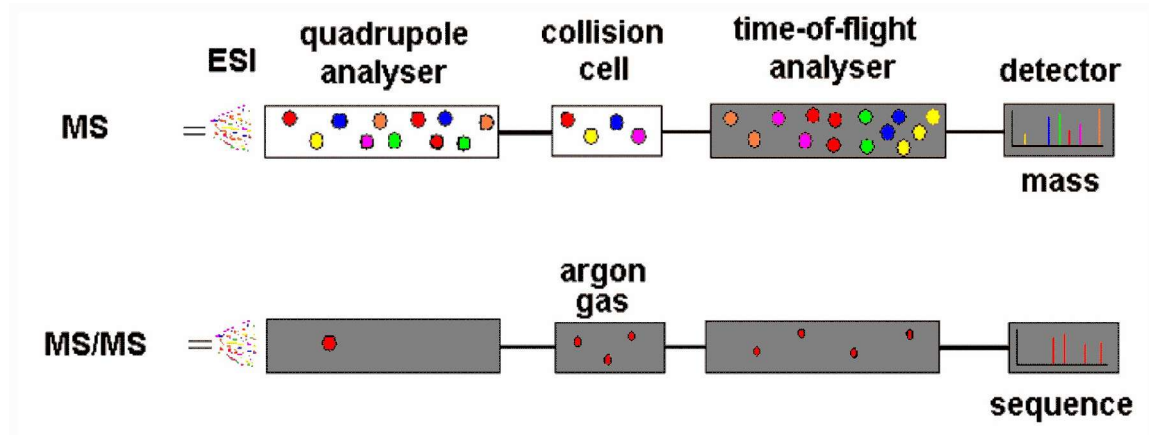
B



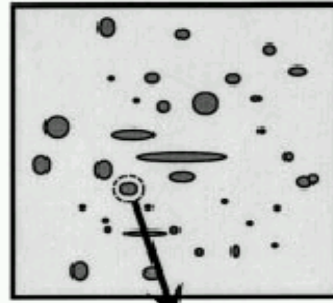


Hmotnostná spektrometria

MS/MS (tandem Q-TOF)

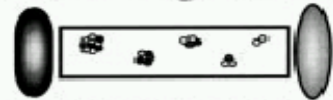


Two Dimensional Gel Electrophoresis (2D-GE)



**Spot Excision
and Proteolysis**

Time-of-flight (TOF) Tandem Mass Spectrometry (MS/MS)



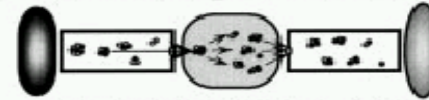
peptide mass mapping

Pros:

- speed of analysis
- more compatible with automation

Cons:

- mixture analysis
- more sensitive to quantity and quality of database information



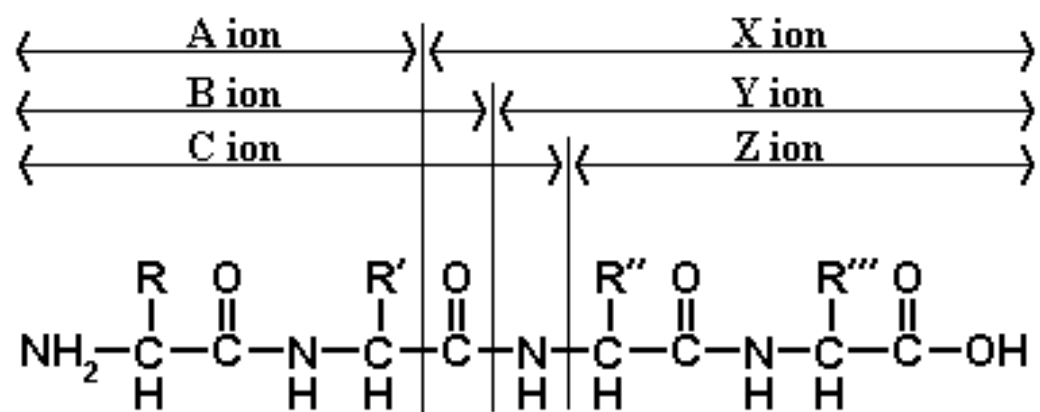
**amino acid sequence from
tandem mass spectra**

Pros:

- robust for mixture analysis
- identification from limited database information

Cons:

- more labor intensive
- less compatible with automation

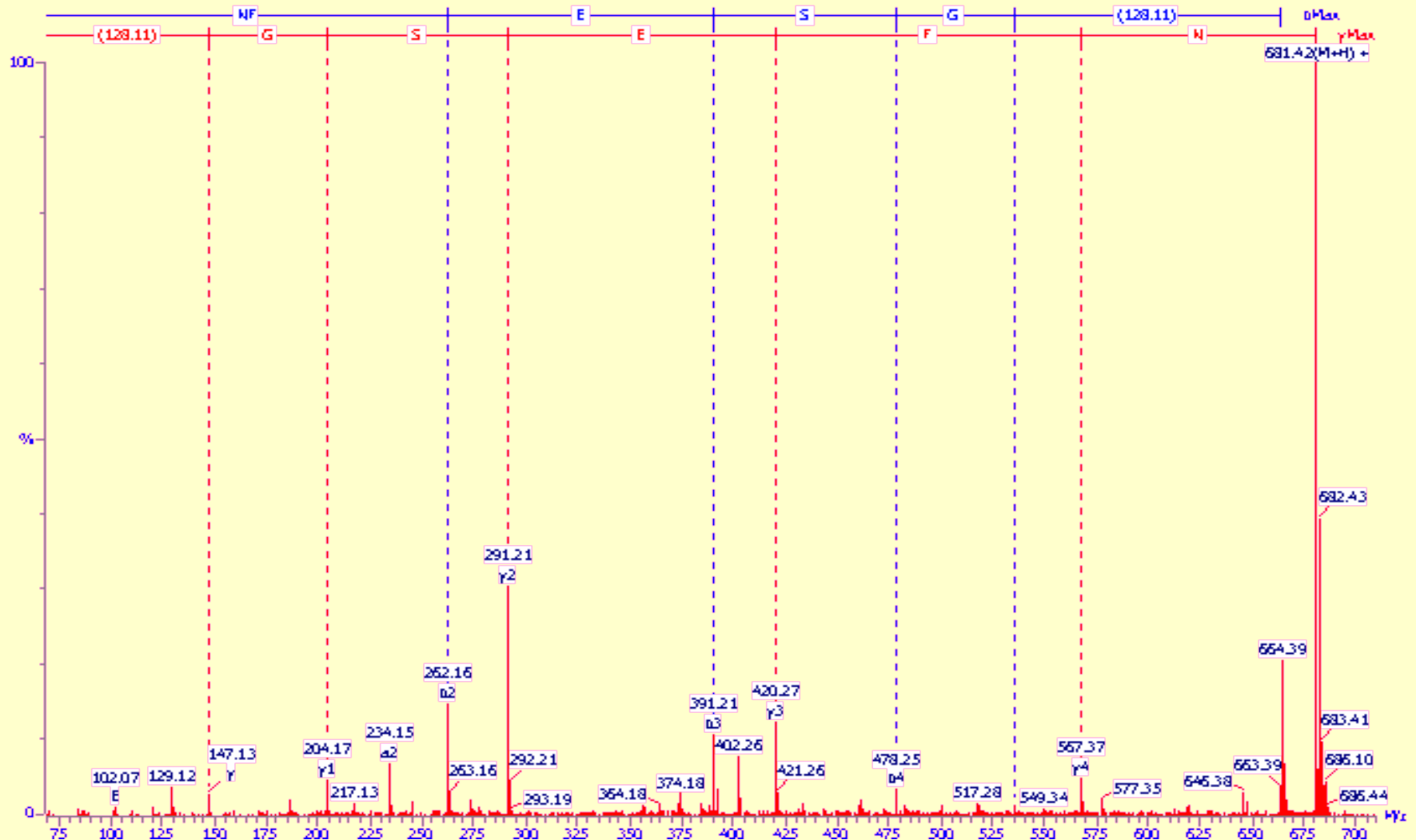


Hmotnostná spektrometria

1:1000; D of 681.3

snap09 AccMass 3 (Top,2, H,5000.0,0.00)

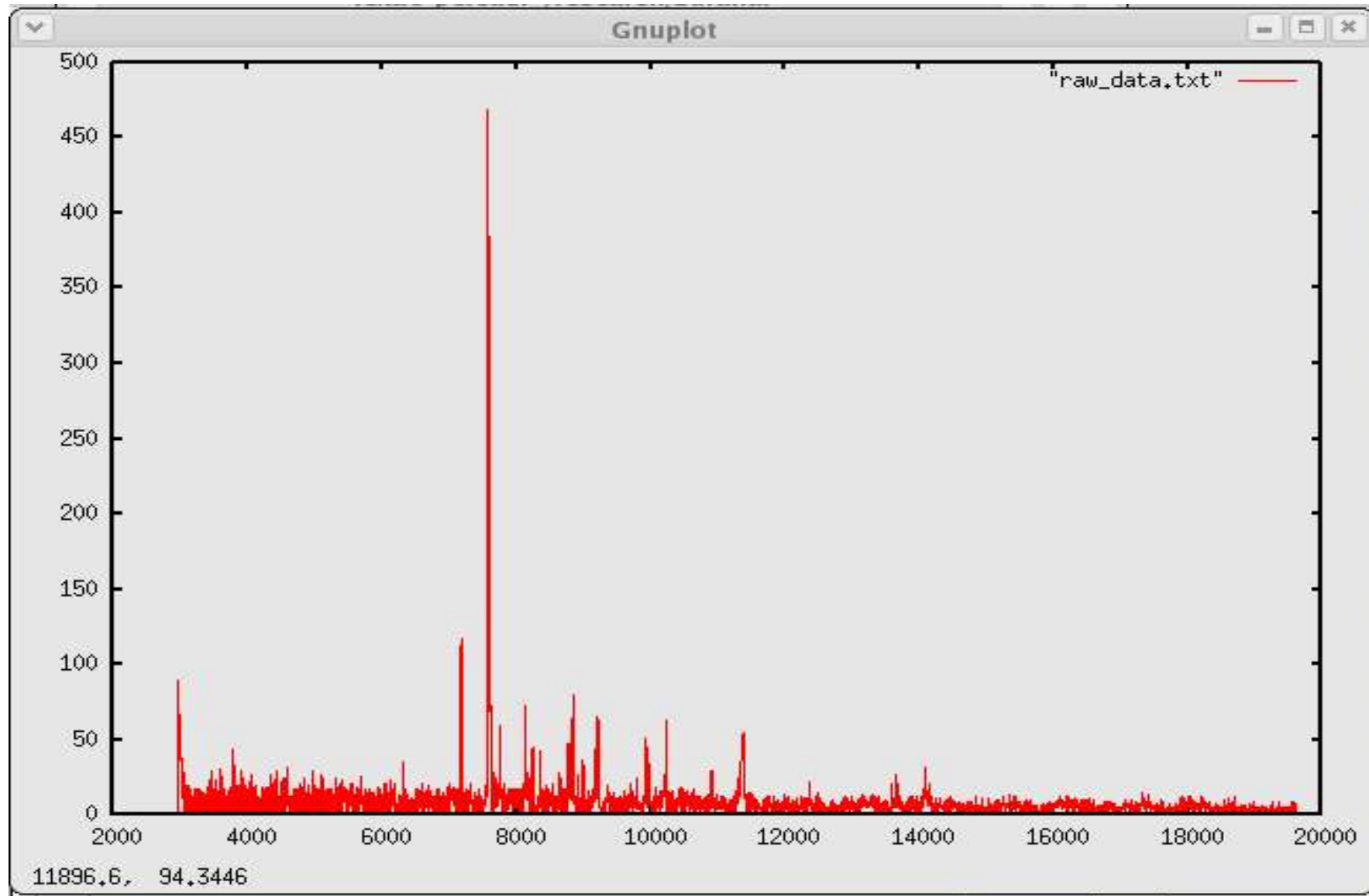
1: TOF MSMS 681.30ES+



Využitie MALDI pre klasifikáciu vzoriek a hodnotenie ich vzájomnej podobnosti

- * *identifikácia pík*
- * *porovnanie dvoch alebo viac spektier*
- * *zhlukovanie*

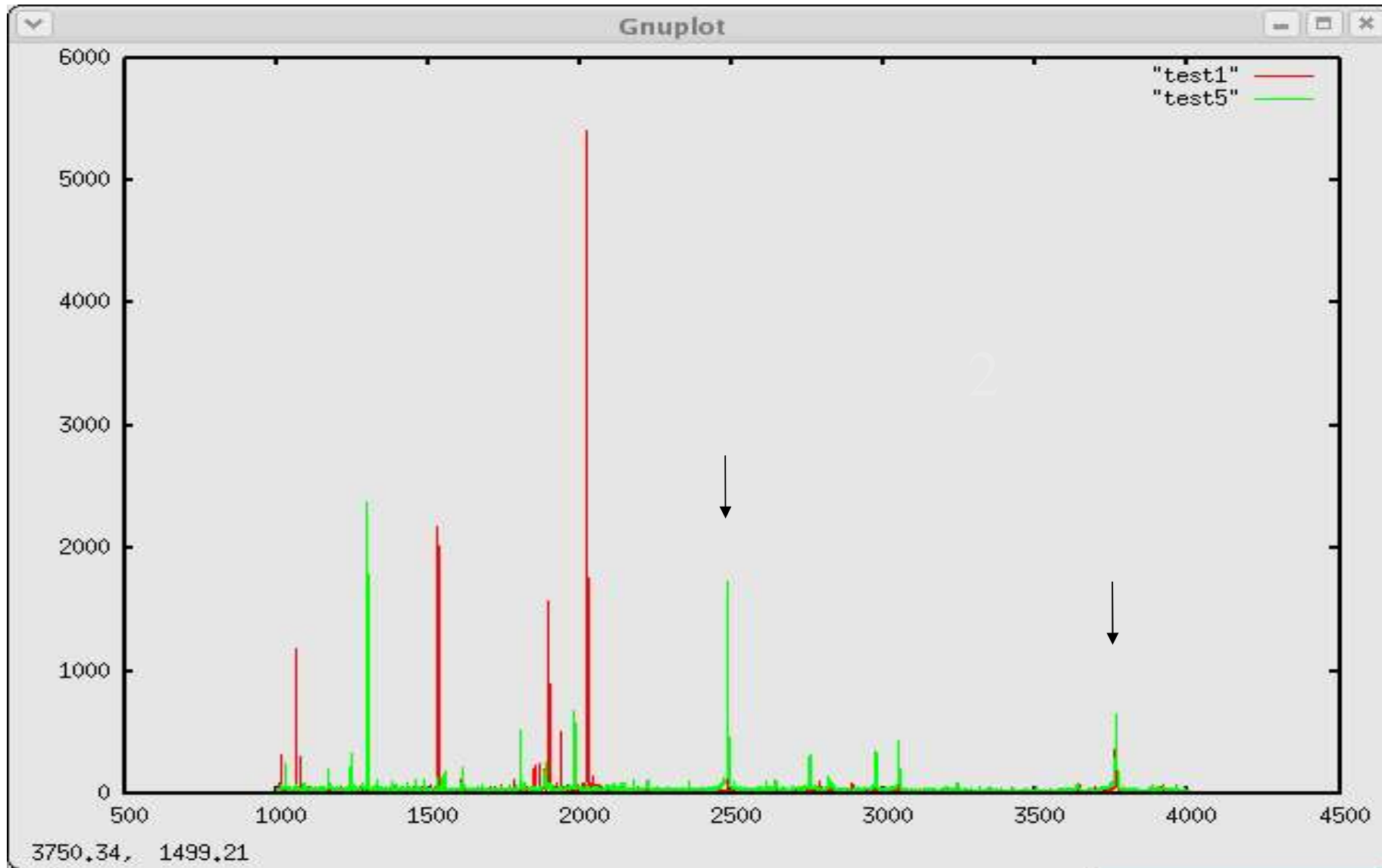
MALDI-TOF spektrum mikrobiologickej kultúry



Možnosti hodnotenia podobnosti spektier

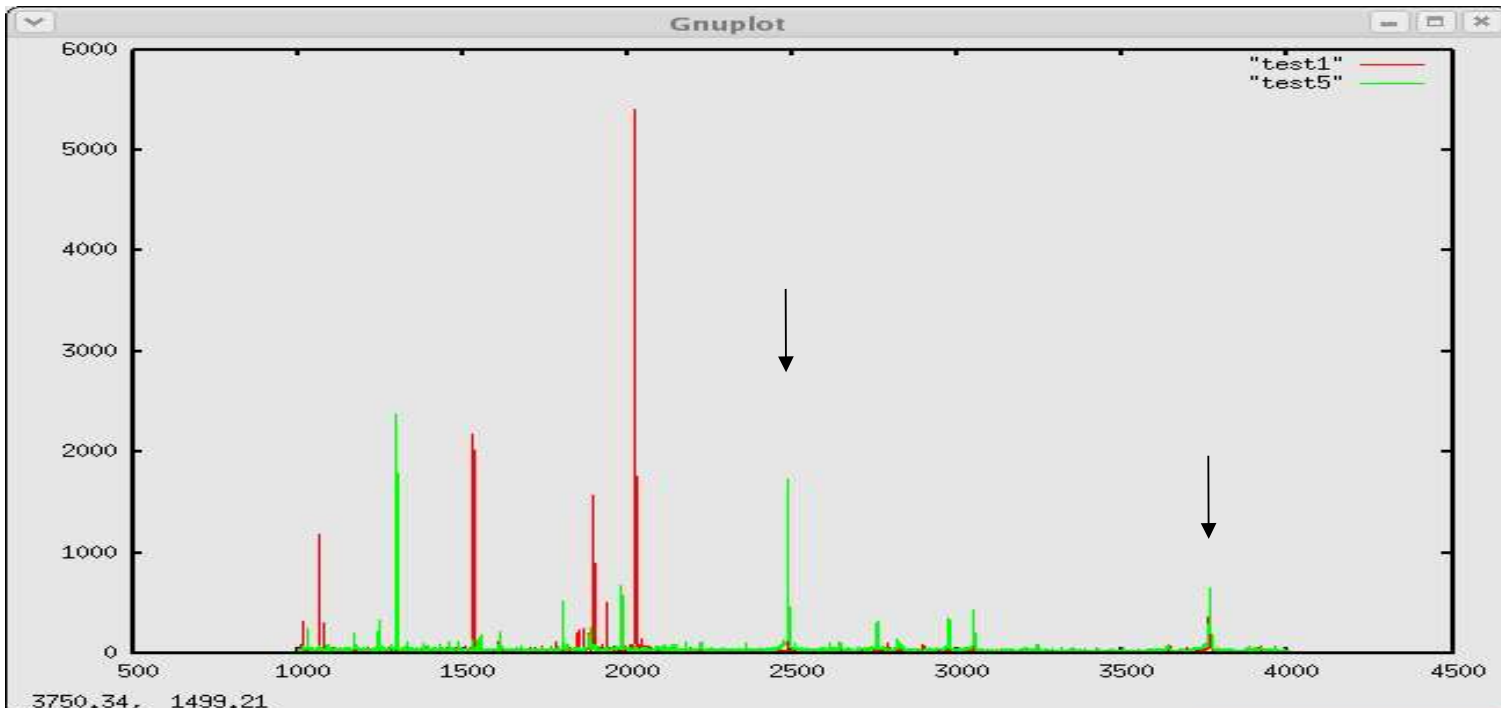
- * počet identických pík
- * vektorový súčin
- * euklidovská vzdialenosť vektorov
- * korelácia
- * vzájomná informácia

Možnosti hodnotenia podobnosti spektier



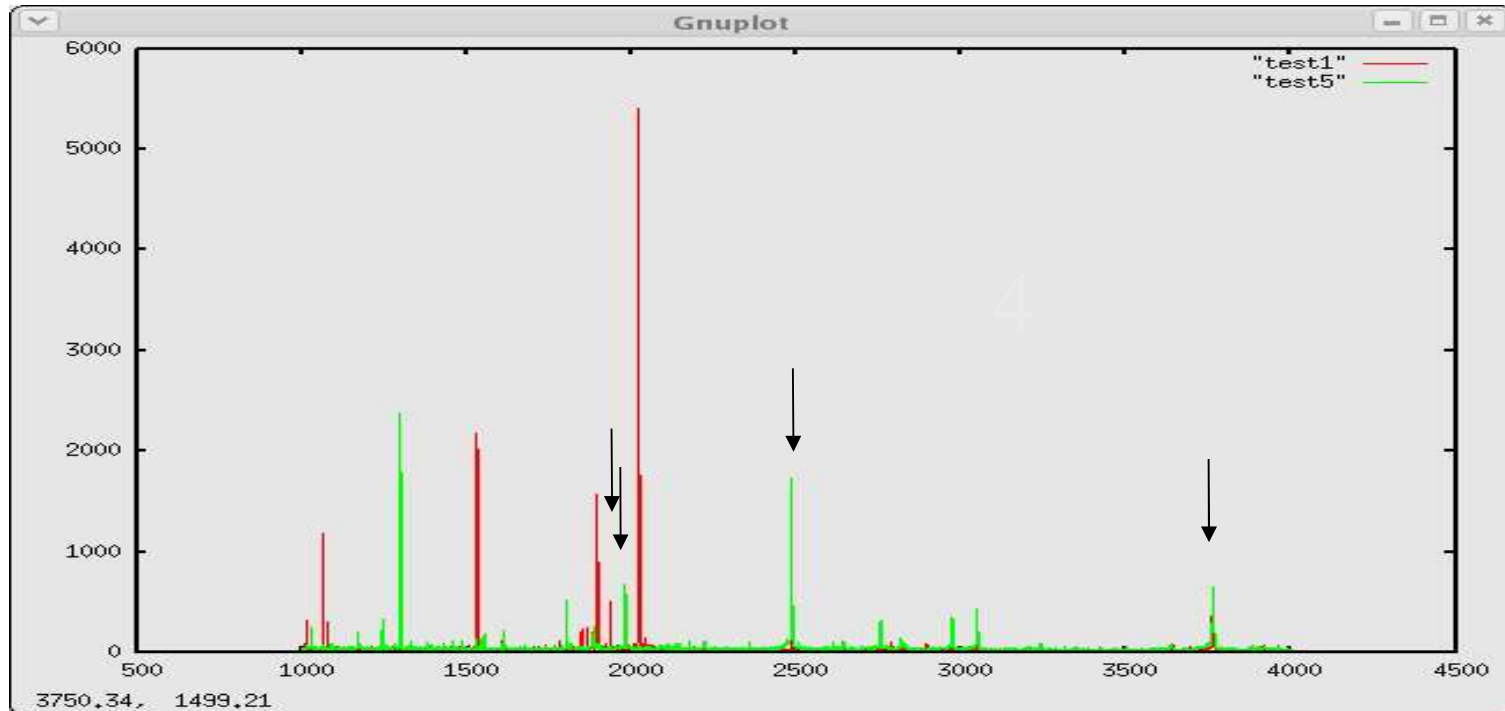
Možnosti hodnotenia podobnosti spektier

$$\begin{aligned} &(1,0,1,1,0,0,0,1,0,1,1,1,1,0,1,1,0,0,0,0,1) \\ &\quad \times \\ &(0,1,0,0,1,1,1,0,1,0,0,0,0,1,0,1,1,1,1,1,1) \\ &\quad = \\ &\quad \mathbf{0.00001} \end{aligned}$$

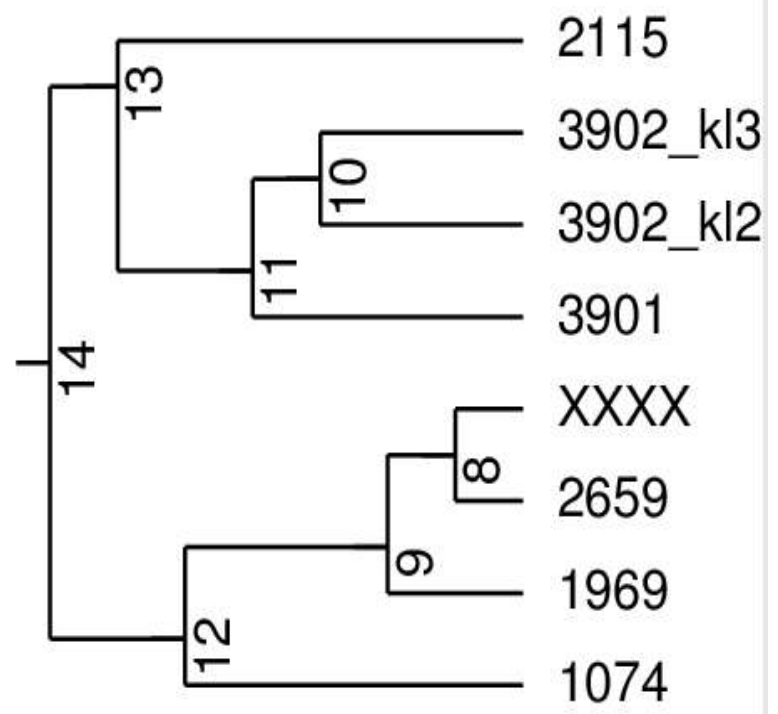
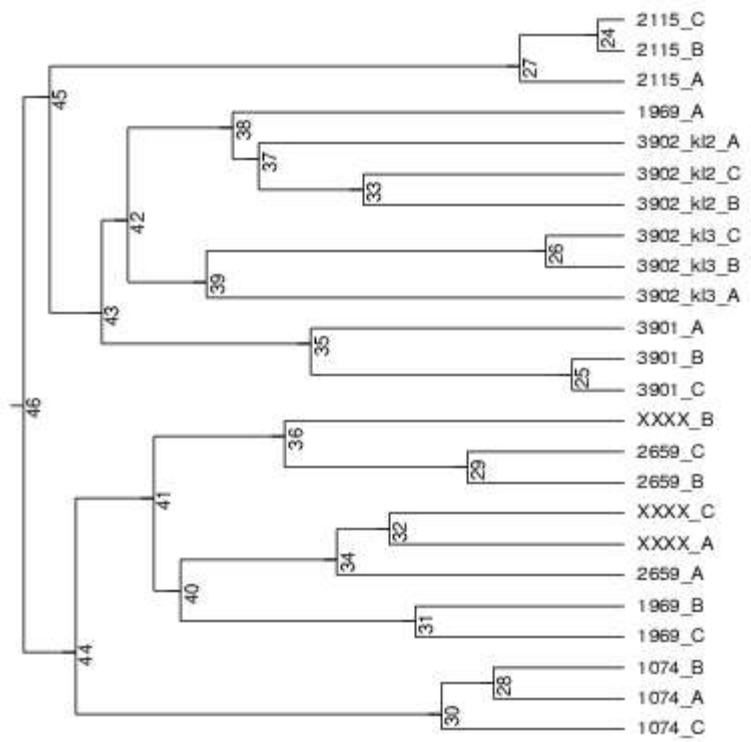


Možnosti hodnotenia podobnosti spektier

```
const float ptm[NUM_PTM] = {  
42.0106,  
541.0610,  
-1.0316,  
-0.9840,  
634.6628,  
45.9877118,  
226.0776,  
77.9105,  
72.0211,  
368.3443,  
294.1831,  
0.9840276,  
162.052823,  
15.9949146,  
31.9898292,  
31.9721,  
0.9840,
```



Zhluková analýza získaných spektier s použitím vektorizácie spektier a hodnotenia podobnosti vektorovým súčinom (program CLUTO)





ProteinProspector <http://prospector.ucsf.edu>



GPM <http://gpmdb.thegpm.org>

