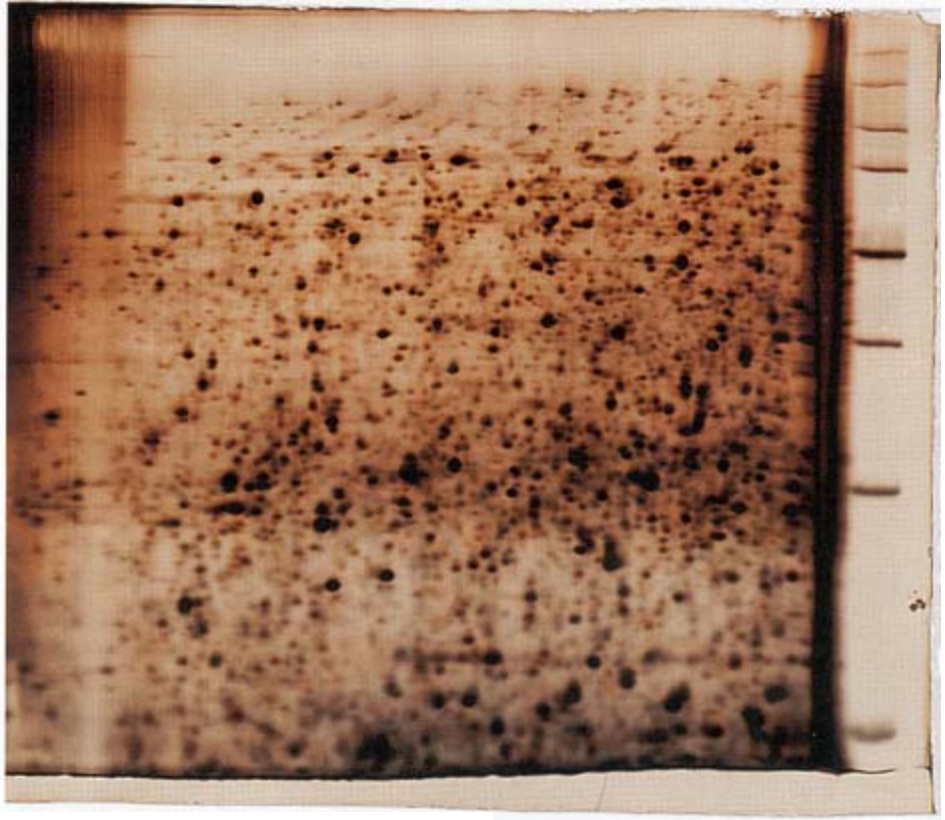
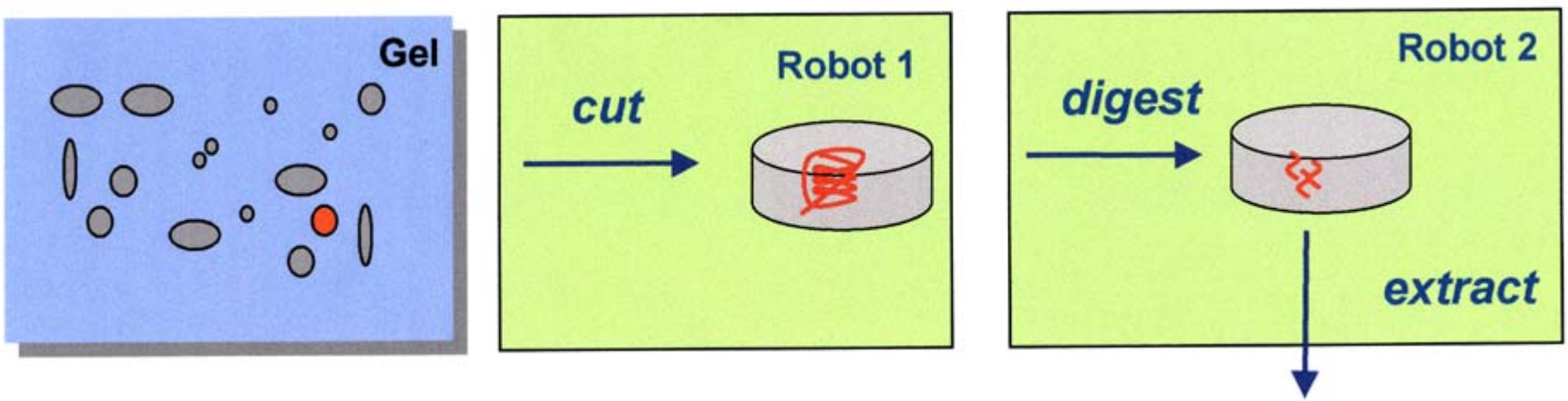
Proteòmica en el I.B.V.

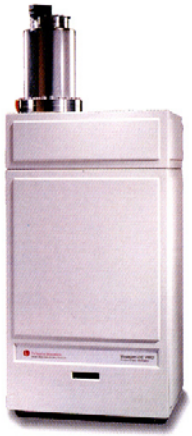




Mass Analysis + Database search

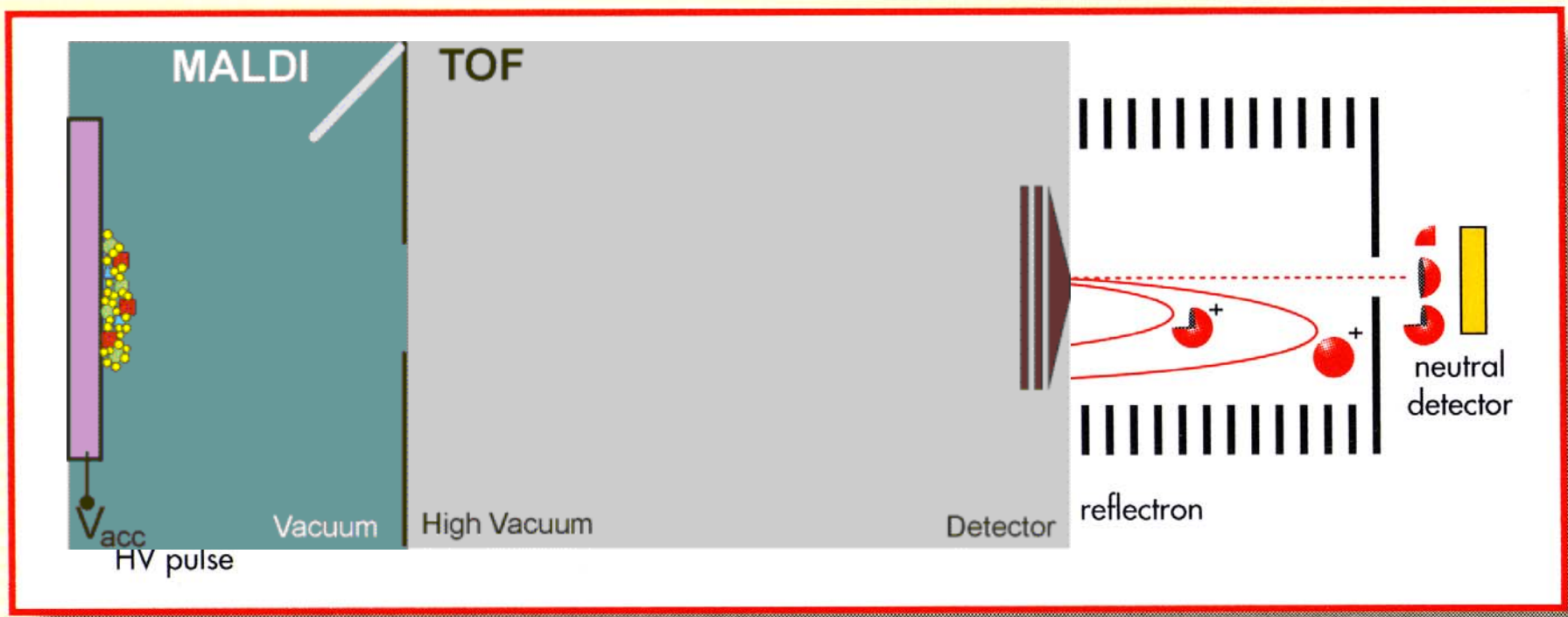
INVESTIGATOR™ PROGEST

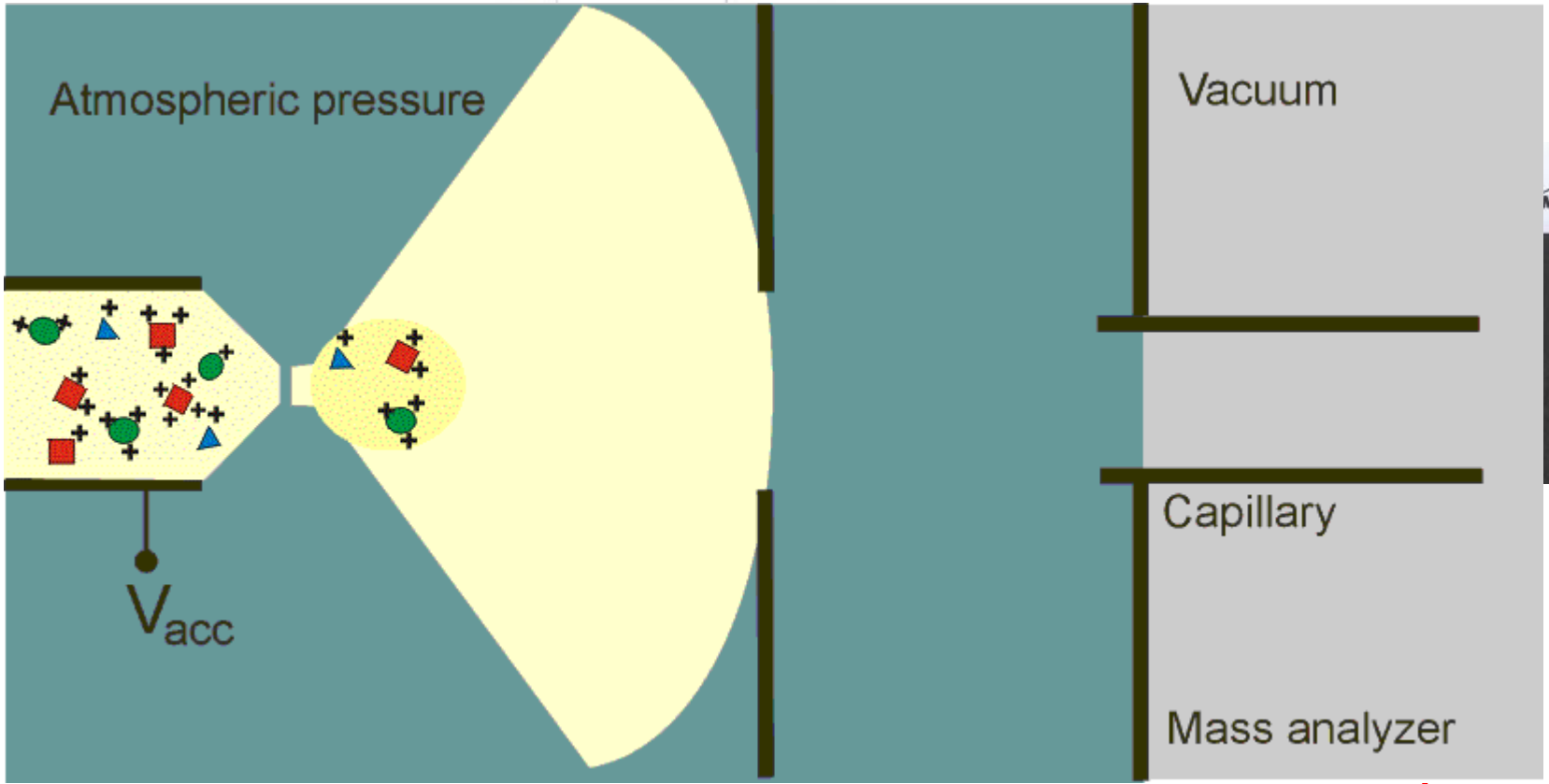
Voyager-DE™ PRO
Biospectrometry™
Workstation

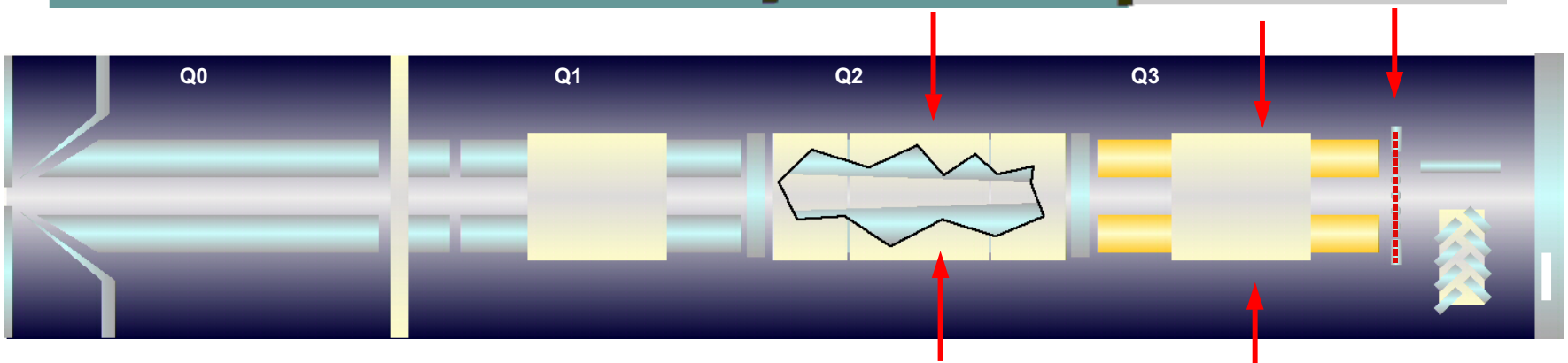


Mass fingerprint analysis

Post-source decay of metastable ions

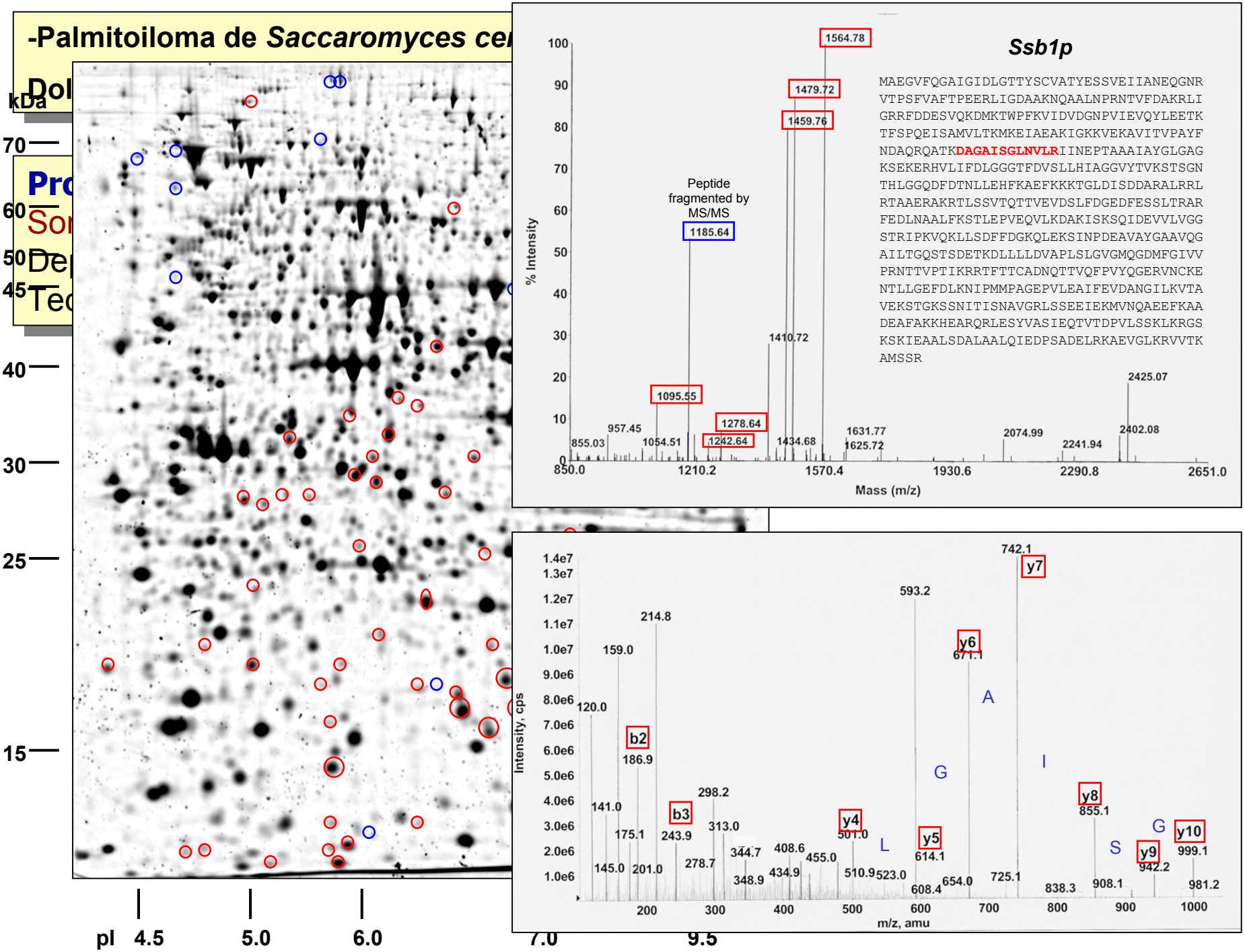


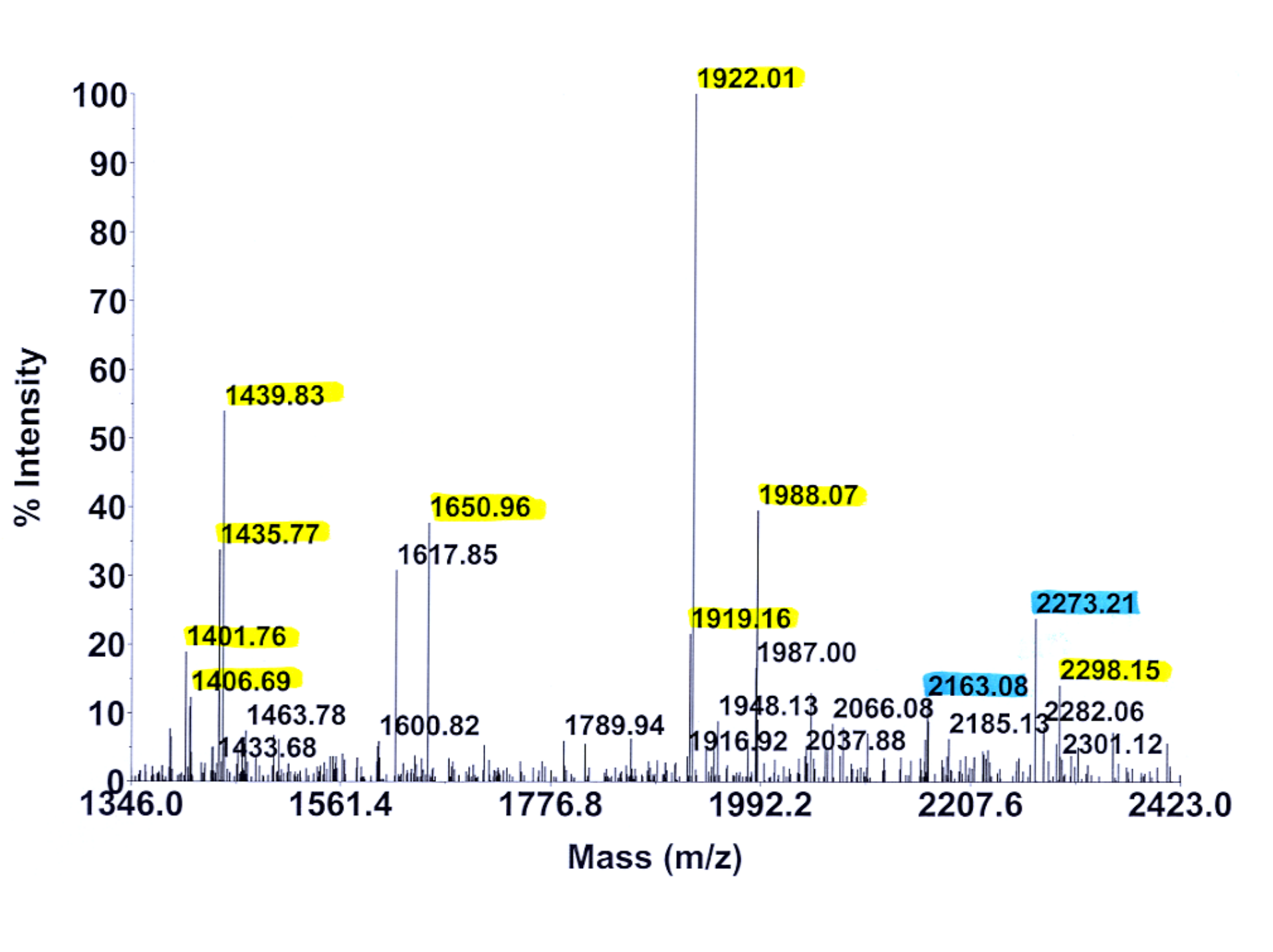




Precursor ion selection

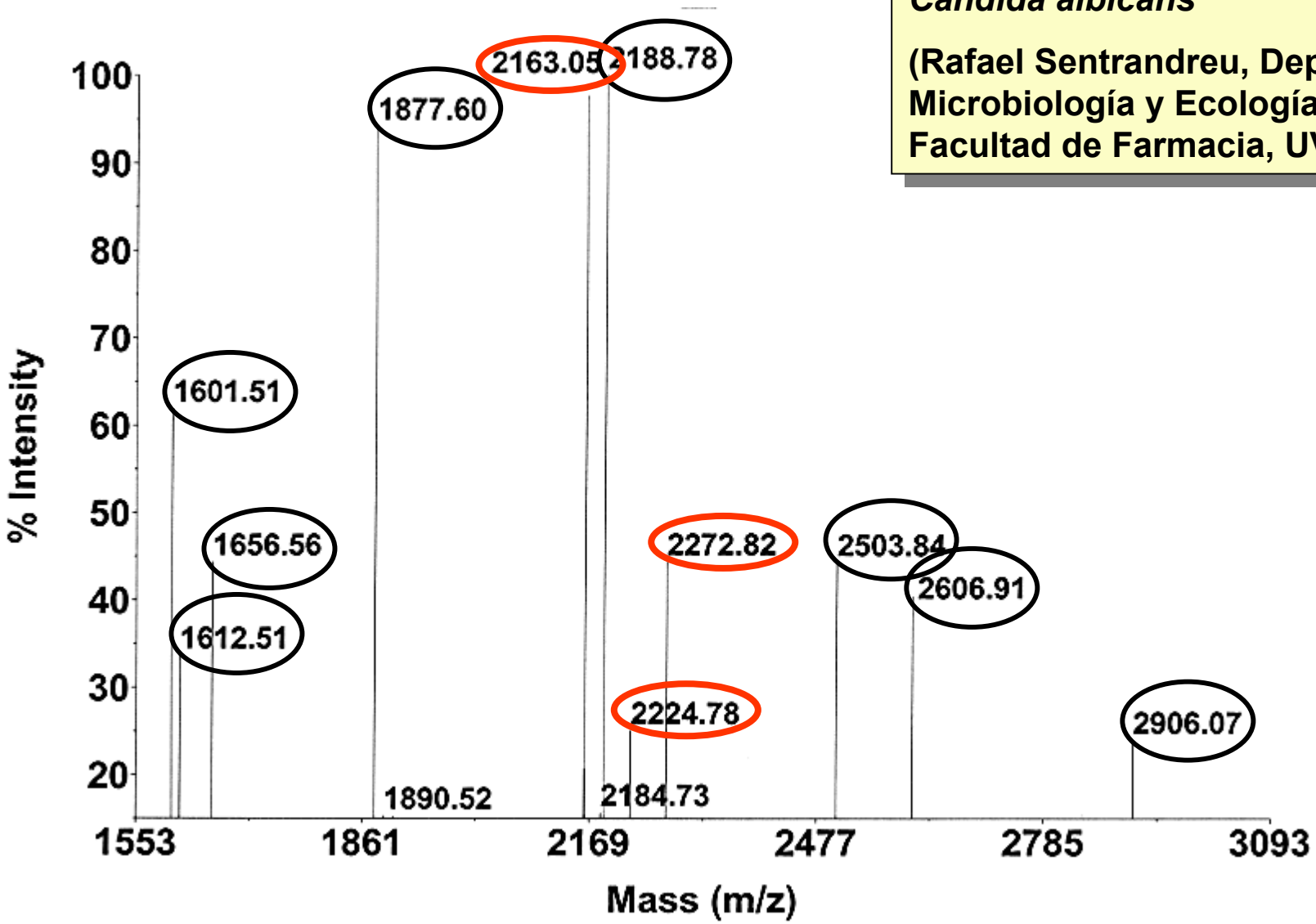
linear ion trap 3x10⁻⁵ Torr

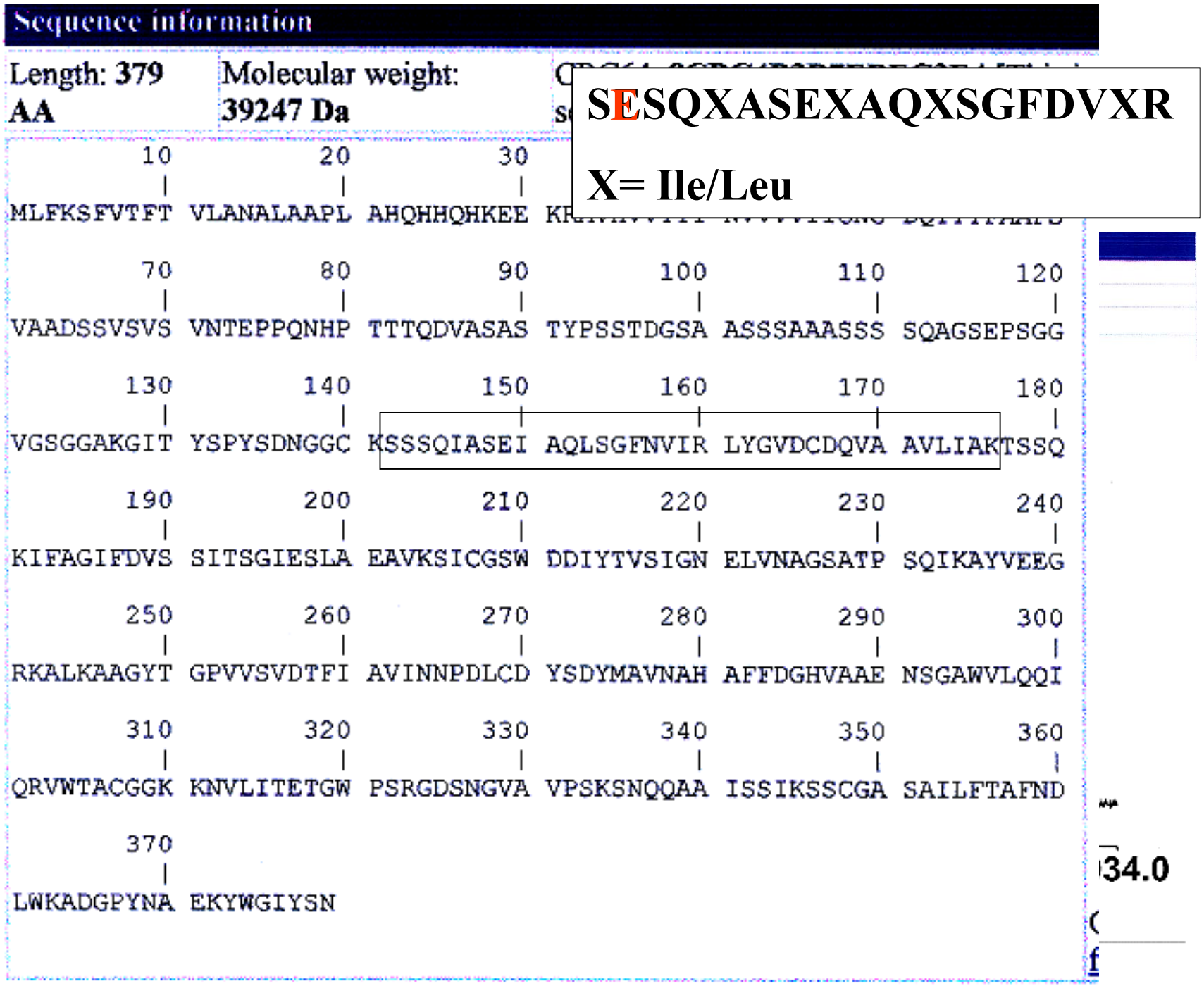

-Palmitoiloma de Saccaromyces cer
kDa
70
60
50
45
40
30
25
15
pI 4.5
5.0
6.0
7.0
9.5
Ssb1p
MAEGVFQGAIGIDLGTTYSCVATYESSVEIIANEQGNR
VTPSFVAFTPEERLIGDAAKNQAALNPRNTVFDAKRLI
GRRFDDESVQKDMKTWPFKVIDVDGNPVIEVQYLEETK
TFSPQEISAMVLTKMKEIAEAKIGKKVEKAVITVPAYF
NDAQRQATKDAGAISGLNVLRIINEPTAAAIAYGLGAG
KSEKERHVLIFDLGGGTFDVSLLHIAGGVYTVKSTSGN
THLGGQDFDTNLLEHFKAEFKKKTGLDISDDARALRRL
RTAAERAKRTLSSVTQTTVEVDSLFDGEDFESSLTRAR
FEDLNAALFKSTLEPVEQVLKDAKISKSQIDEVVLVGG
STRIPKVQKLLSDFFDGKQLEKSINPDEAVAYGAAVQG
AILTGQSTSDETKDLLLLDVAPLSLGVGMQGDMFGIVV
PRNTTVPTIKRRTFTTCADNQTTVQFPVYQGERVNCKE
NTLLGEFDLKNIPMMPAGEPVLEAIFEVDANGILKVTA
VEKSTGKSSNITISNAVGRLSSEEIEKMVNQAEEFKAA
DEAFAKKHEARQRLESYVASIEQTVTDPVLSSKLKRGS
KSKIEAALSDALAALQIEDPSADELRKAEVGLKRVVTK
AMSSR
Peptide fragmented by MS/MS
1185.64
1564.78
1479.72
1459.76
1410.72
1095.55
1278.64
1242.64
957.45
855.03
1054.51
1434.68
1631.77
1625.72
2074.99
2241.94
2425.07
2402.08
% Intensity
Mass (m/z)
Intensity, cps
m/z, amu
742.1
y7
593.2
214.8
159.0
y6
671.1
A
120.0
b2
186.9
G
I
298.2
b3
141.0
243.9
313.0
y4
501.0
y5
614.1
L
y8
855.1
G
y10
999.1
S
y9
942.2
175.1
145.0
201.0
278.7
344.7
348.9
408.6
434.9
455.0
510.9
523.0
608.4
654.0
725.1
838.3
908.1
981.2

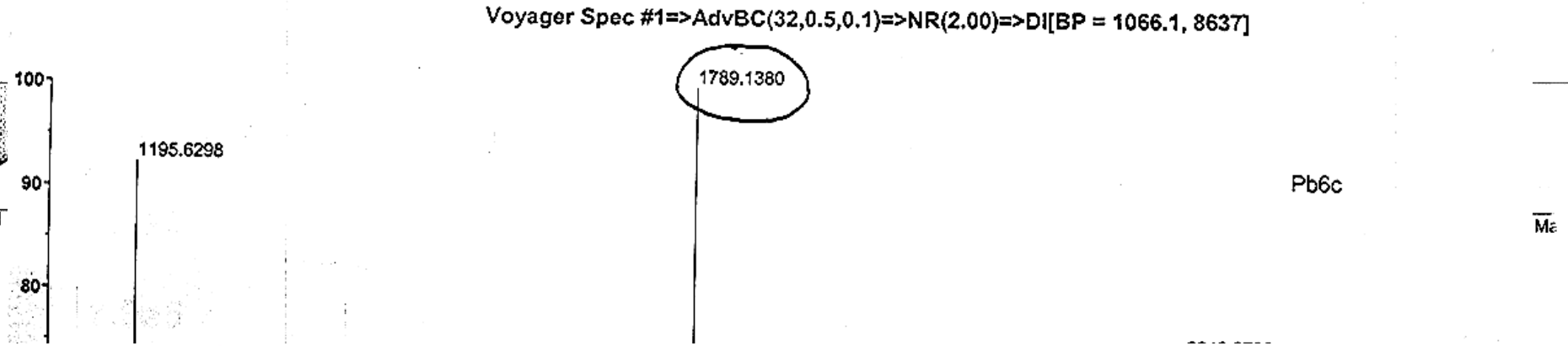

1922.01
1439.83
1650.96
1988.07
1435.77
1617.85
1919.16
2273.21
1401.76
1987.00
1406.69
2163.08
2298.15
1948.13
2066.08
1463.78
1600.82
1789.94
2185.13
2282.06
1433.68
1916.92
2037.88
2301.12
100
90
80
70
60
50
40
30
20
10
0
% Intensity
1346.0
1561.4
1776.8
1992.2
2207.6
2423.0
Mass (m/z)

Proteínas de pared celular de *Candida albicans*

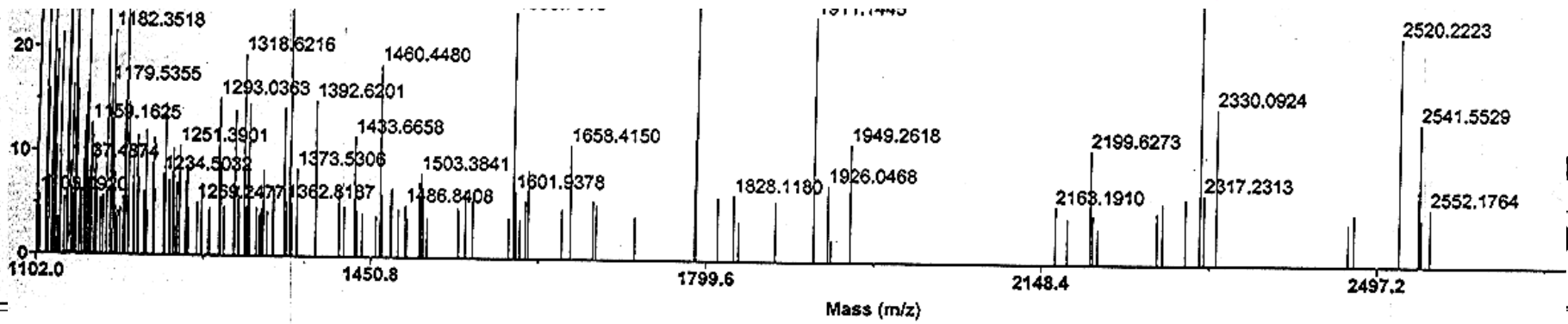
(Rafael Sentrandreu, Dept. Microbiología y Ecología, Facultad de Farmacia, UV)




Sequence information
Length: 379 AA
Molecular weight: 39247 Da
SESQXASEXAQXSGFDVXR
X= Ile/Leu
10 20 30
MLFKSFVTFT VLANALAAPL AHQHHQHKEE
70 80 90 100 110 120
VAADSSVSVS VNTEPPQNHP TTTQDVASAS TYPSSTDGSA ASSSAAASSS SQAGSEPSGG
130 140 150 160 170 180
VGSGGAKGIT YSPYSDNGGC KSSSQIASEI AQLSGFNVIR LYGVDCDQVA AVLIAKTSSQ
190 200 210 220 230 240
KIFAGIFDVS SITSGIESLA EAVKSICGSW DDIYTVSIGN ELVNAGSATP SQIKAYVEEG
250 260 270 280 290 300
RKALKAAGYT GPVVSVDTFI AVINNPDLCD YSDYMAVNAH AFFDGHVAAE NSGAWVLQQI
310 320 330 340 350 360
QRVWTACGGK KNVLITETGW PSRGDSNGVA VPSKSNQQAA ISSIKSSCGA SAILFTAFND
370
LWKADGPYNA EKYWGIYSN
% Intensity
34.0

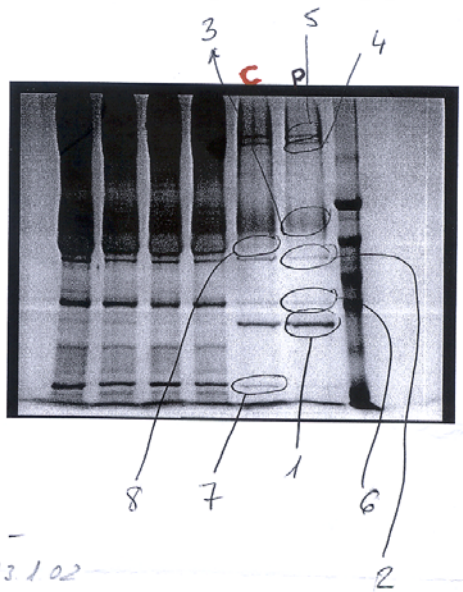


ION	z	SECUENCIA	PROTEÏNA
621.3	2+	**QYFNLS(226)EL**	Similar to Q8H7F4
750.2	2+	**LNKYGPPPLGCTIK**	Rubisco large chain
792.3	2+	**ALLSDPVFRPLVEK**	Ascorbate peroxidase
631.3	2+	**QVTLGFVD(I/L)(I/L)R**	Rubisco large chain
858.7	2+	**SKATNSINDASNSSYR**	Protein tyrosine kinase
691.3	2+	**EHNSSPGYYDGR**	Rubisco small chain
983.8	2+	**FETLSYLP(1015)**	Rubisco small chain
739.0	2+	**LPLFGATDSSQVLK**	Rubisco small chain
942.6	2+	**IVDTFPGQSIDFFGALR**	Rubisco activase
697.5	3+	**VPIIVTGNDFSTLYAPLIR**	Rubisco activase
880.8	2+	**DGIDYAAVTVQLPGGER**	33 kDa polypeptide of oxygen-evolving complex in Photosystem II
482.7	2+	**VPFLFT(I/L)K**	33 kDa polypeptide of oxygen-evolving complex in Photosystem II



Proteína de paciente

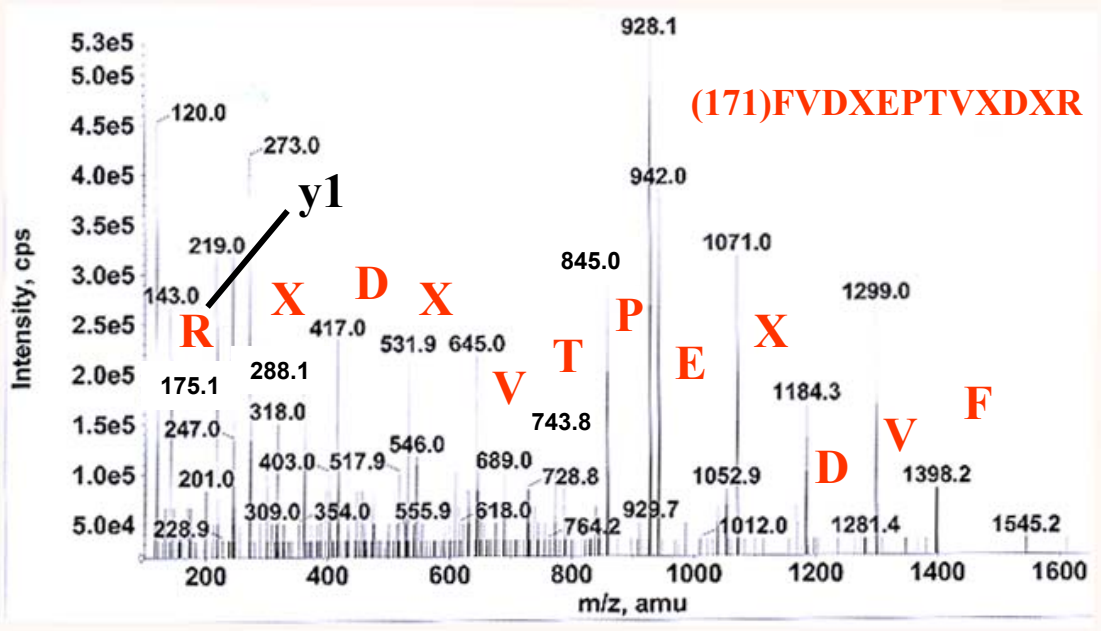
(Vicente Felipo, FVIB)

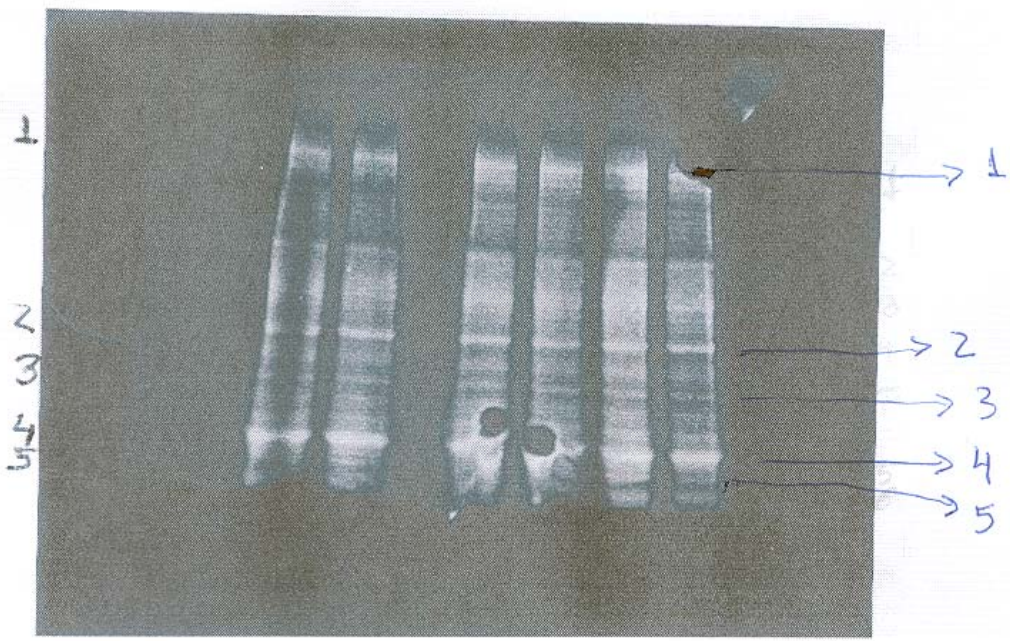


(R265) LTVADALEPVQFEDGQK (1859.8)
(R 98) GAISAEVYTEEDAASYVR (1930.9)
(R318) LGPSDYFGEIALLMNRPR (2048.9)
(K282) IVVQGEPGDEFFIILEGSAAVLQR (2587.3)

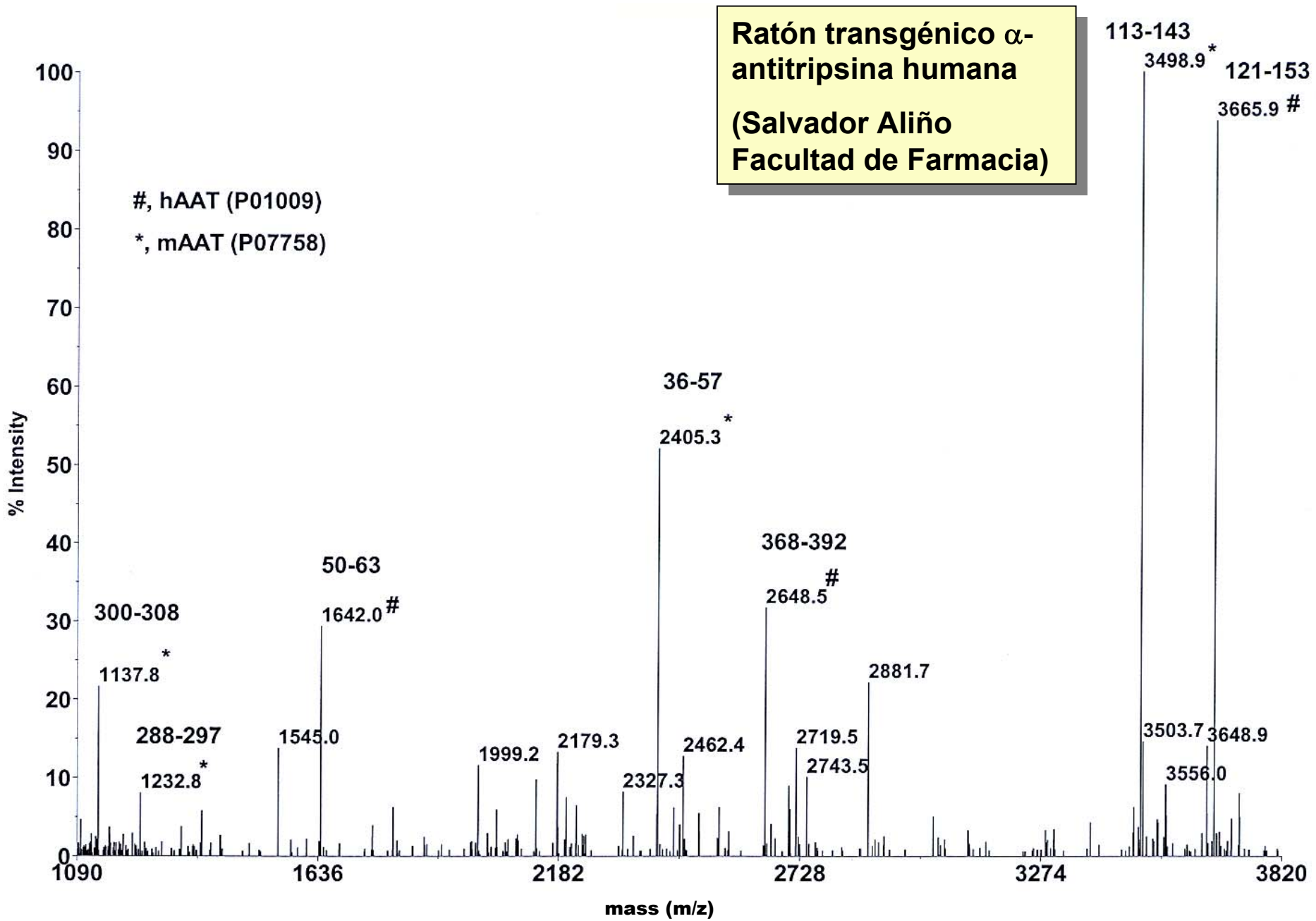
Proteína de cerebelo de rata/NMDA (anti-Pser/Thr)

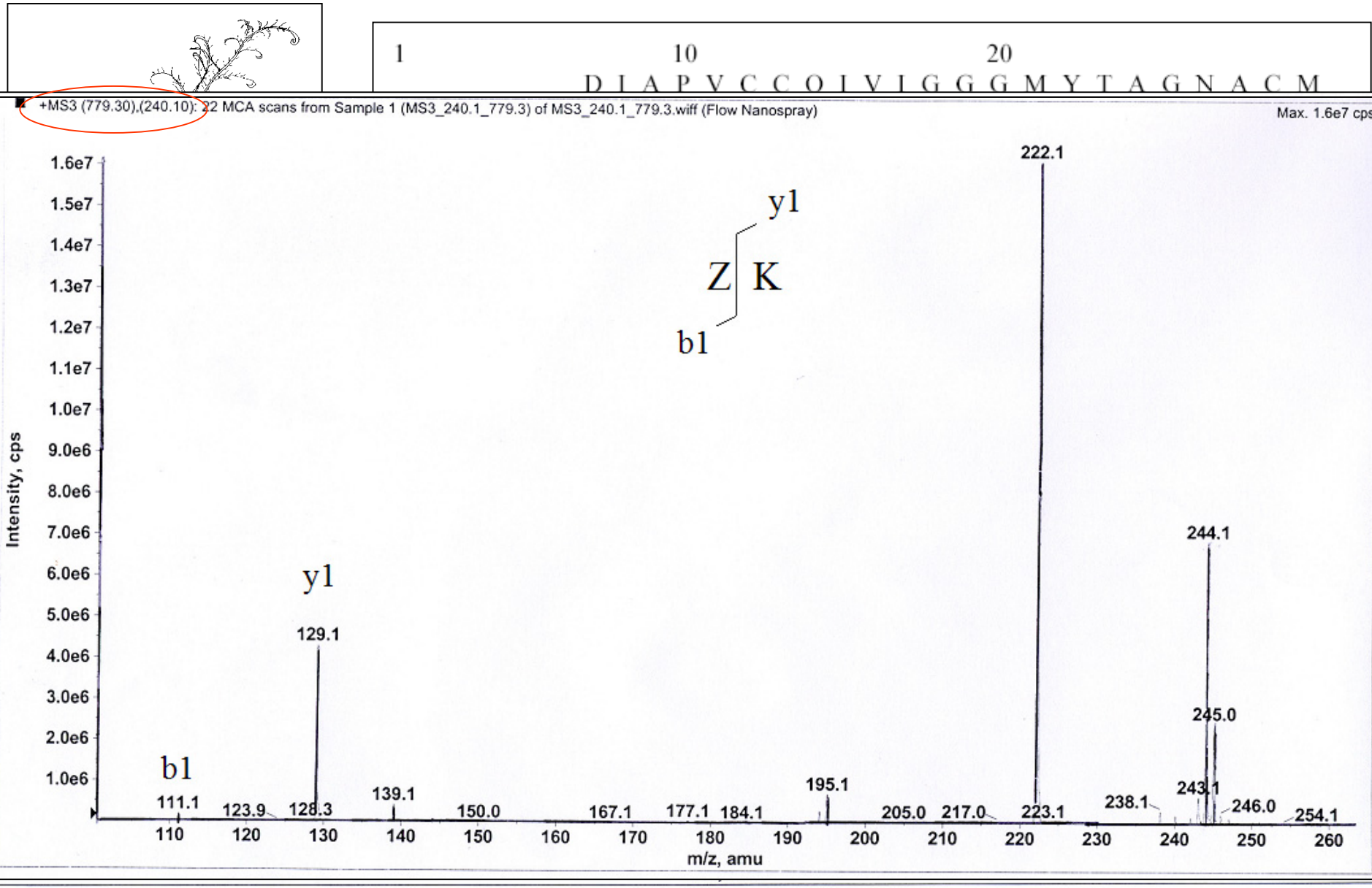
(Vicente Felipo, FVIB)





Tubulin α2


Ratón transgénico α-antitripsina humana
(Salvador Aliño Facultad de Farmacia)
#, hAAT (P01009)
*, mAAT (P07758)
% Intensity
mass (m/z)
100
90
80
70
60
50
40
30
20
10
0
1090
1636
2182
2728
3274
3820
113-143
3498.9 *
121-153
3665.9 #
36-57
2405.3 *
368-392
2648.5 #
50-63
1642.0 #
300-308
1137.8 *
288-297
1232.8 *
1545.0
1999.2
2179.3
2327.3
2462.4
2719.5
2743.5
2881.7
3503.7
3556.0
3648.9


1 10 20
D I A P V C C Q I V I G G G M Y T A G N A C M
+MS3 (779.30),(240.10): 22 MCA scans from Sample 1 (MS3_240.1_779.3) of MS3_240.1_779.3.wiff (Flow Nanospray)
Max. 1.6e7 cps.
y1
Z K
b1
222.1
y1
129.1
244.1
245.0
b1
111.1
123.9
128.3
139.1
150.0
167.1
177.1
184.1
195.1
205.0
217.0
223.1
238.1
243.1
246.0
254.1
Intensity, cps
1.6e7
1.5e7
1.4e7
1.3e7
1.2e7
1.1e7
1.0e7
9.0e6
8.0e6
7.0e6
6.0e6
5.0e6
4.0e6
3.0e6
2.0e6
1.0e6
110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260
m/z, amu

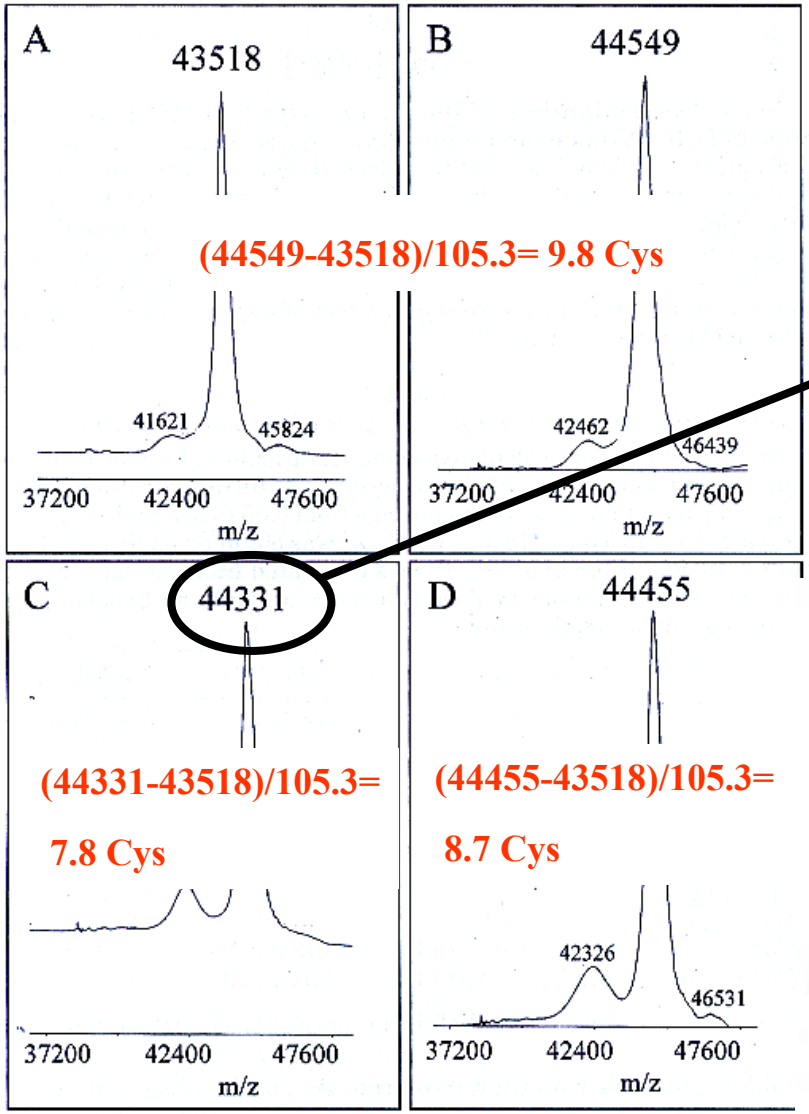


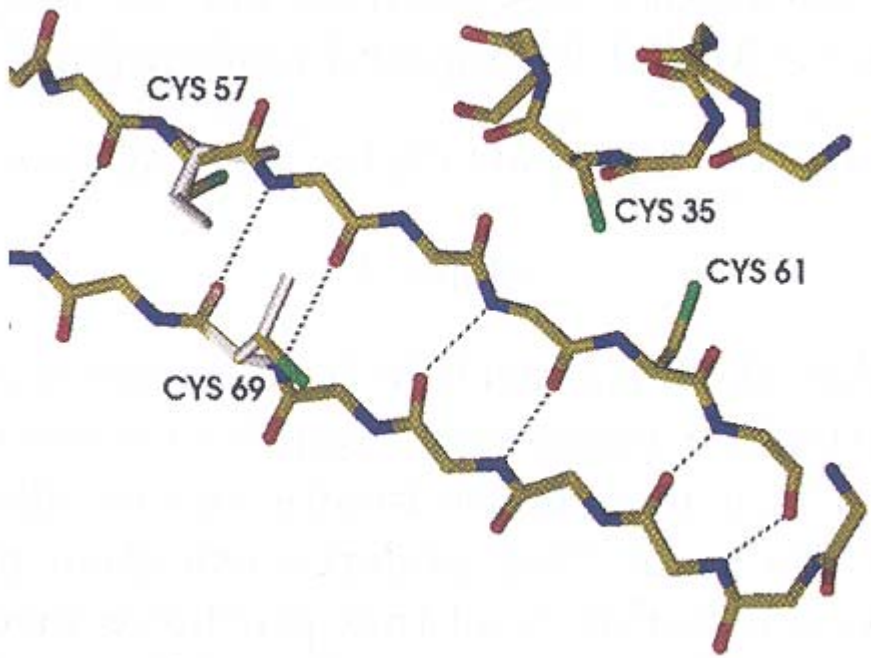
FIG. 5. **MALDI-TOF mass spectrometry.** Mass spectrometric d termination of the molecular masses of DTT-refolded MAT before (and after (*B*) treatment with vinylpyridine under denaturing but nc reducing conditions is shown. Molecular masses of GSH/GSSG-refold MAT I and III (*C*) and of C35S and C61S MAT mutants (*D*) up... treatment with vinylpyridine under denaturing but nonreducing conditions are also included.

TABLE IV

Results of the tryptic digestion analyzed by MALDI-TOF mass spectrometry

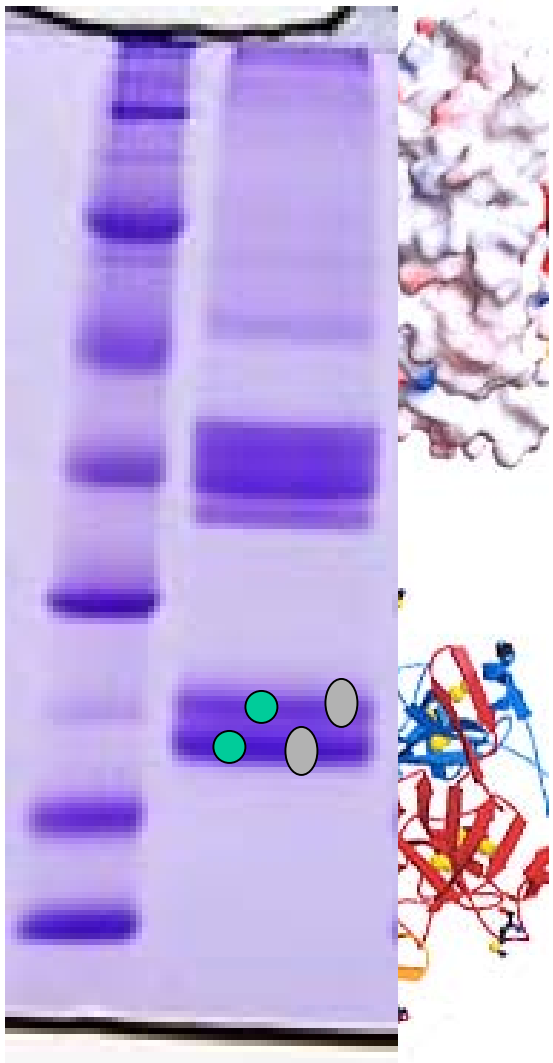
Fragments assigned by MALDI-TOF mass spectrometry in GSH/GSSG-refolded MAT III digested with trypsin under denaturing but nonreducing conditions in the presence of 10 mM iodoacetamide.

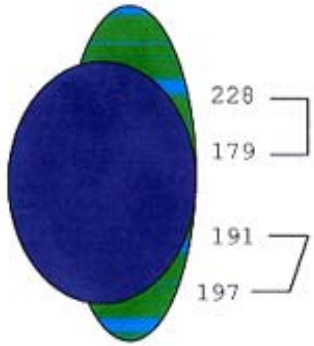
M + H$^+$	Fragment	Sequence modifications
Da		
2551.9	34–48 55–62	1 CM-C; 1 S-S
6588.3	2–62	2 CM-C; 1 S-S; M20-ox
672.7	49–54	
2308.6	63–82	CM-C69; 1 M-ox
2264.8	63–82	C69-red; 2 M-ox
2233.1	63–82	C69-red
1006.0	80–98	
3261.1	99–126	CM-C105; CM-C121
2553.9	104–126	C105 and C121 red

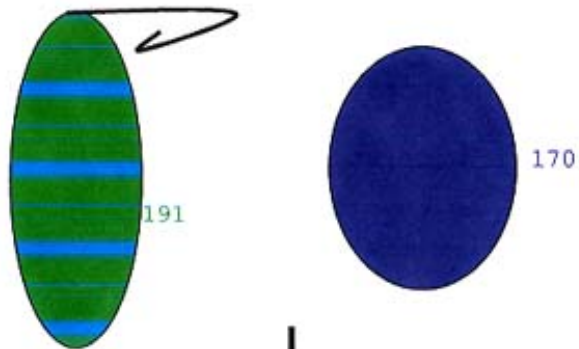


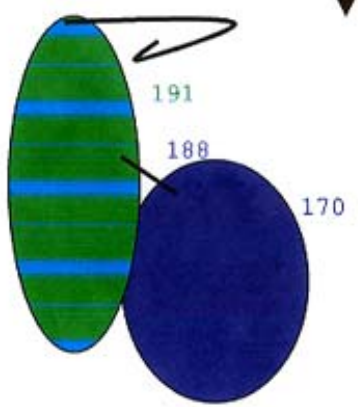
α3 Colágeno IV y enfermedad autoinmune de Goodpasture

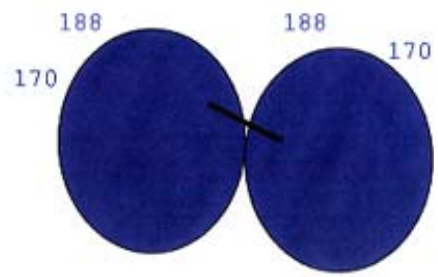
Juan Saus, Quique Pérez-Payá (FVIB)

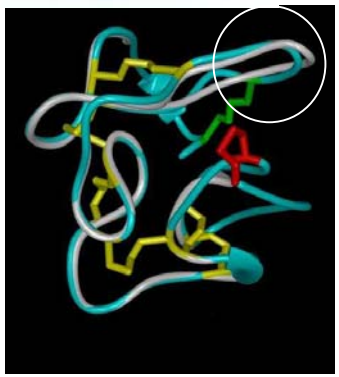


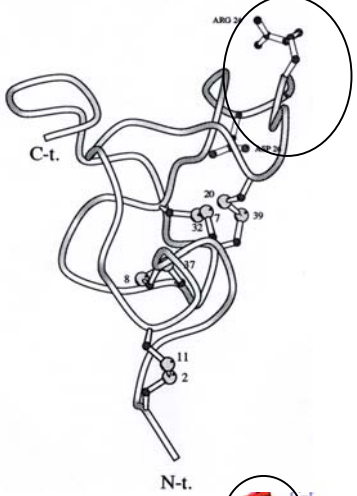


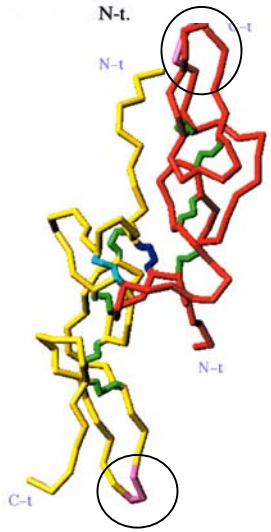


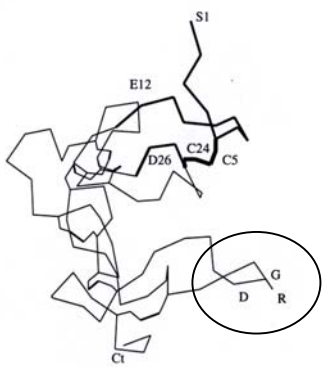


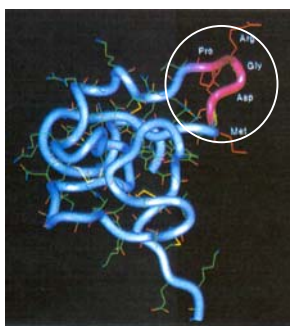


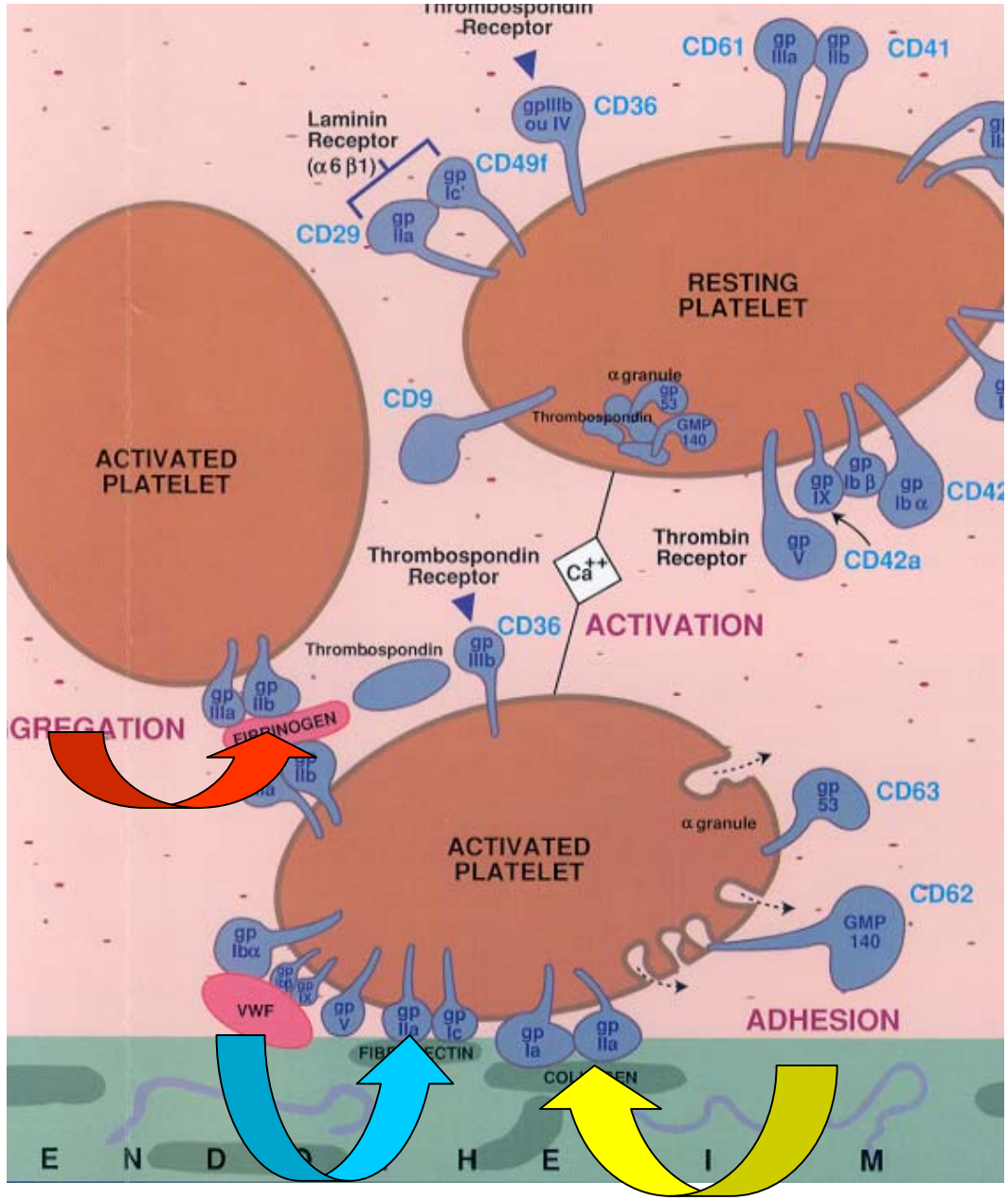



C-t.
N-t.


N-t
C-t
N-t
C-t


S1
E12
D26
C24
C5
G
D
R
Ct


Pro
Arg
Gly
Asp
Met


Thrombospondin Receptor
CD61
gp IIIa
gp IIb
CD41
gpIIIb ou IV
CD36
Laminin Receptor (α6 β1)
CD49f
gp Ic'
CD29
gp IIa
RESTING PLATELET
α granule
gp 53
Thrombospondin
GMP 140
CD9
ACTIVATED PLATELET
gp Ib β
gp Ib α
gp IX
gp V
CD42a
Thrombin Receptor
Thrombospondin Receptor
Ca++
CD36
ACTIVATION
Thrombospondin
gp IIIb
gp IIIa
gp IIb
FIBRINOGEN
GREGATION
CD63
α granule
ACTIVATED PLATELET
CD62
GMP 140
gp Ibα
VWF
gp V
gp IIa
gp Ic
gp Ia
gp IIa
ADHESION
E N D H E I M

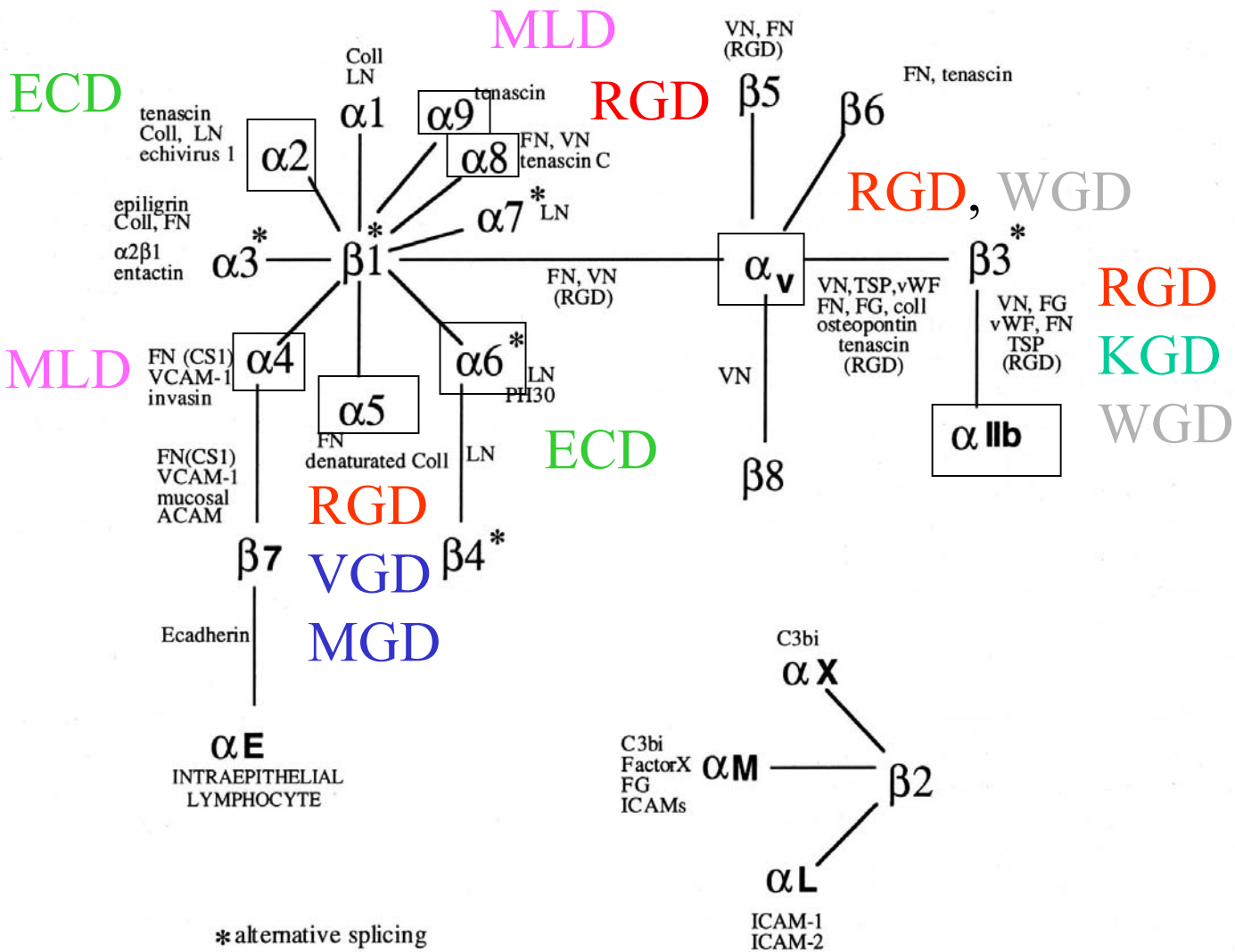


Fig 2. Diagram of the different $\alpha\beta$ integrin heterodimers and of their ligands. coll, collagen; FN, fibronectin; VN, vitronectin; LN, laminin; TSP, thrombospondin; vWF, von Willebrand factor; FG, fibrinogen.

[CANCER RESEARCH 63, 2020–2023, May 1, 2003]

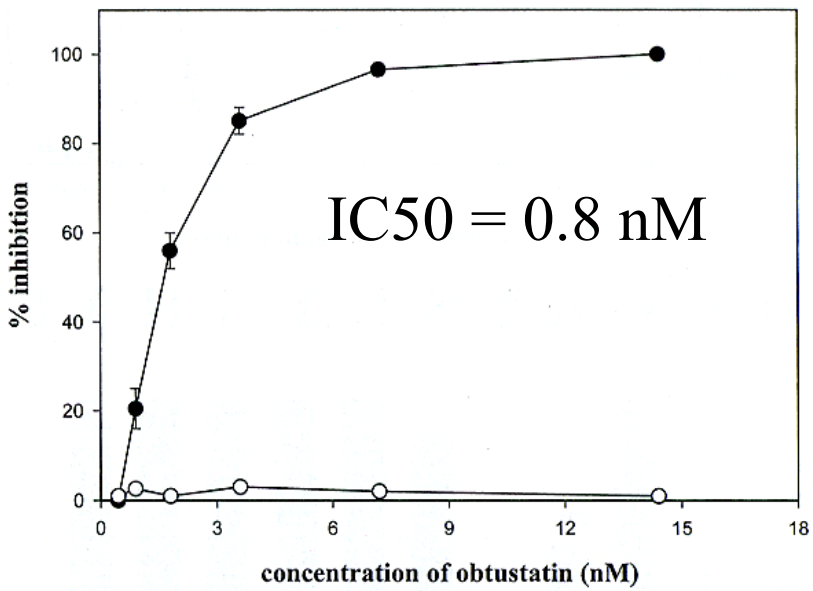
Advances in Brief

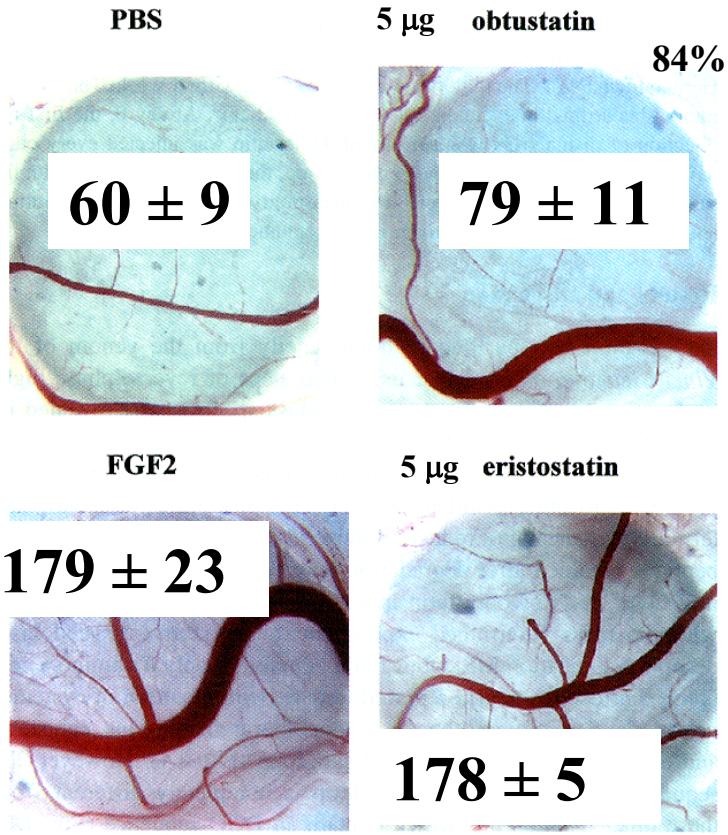
Obtustatin: A Potent Selective Inhibitor of α1β1 Integrin *in Vitro* and Angiogenesis *in Vivo*[1]

Cezary Marcinkiewicz,[2] Paul H. Weinreb, Juan J. Calvete, Dariusz G. Kisiel, Shaker A. Mousa, George P. Tuszynski, and Roy R. Lobb

Temple University, School of Medicine, Thrombosis Research Center, Philadelphia, Pennsylvania 19140 [C. M., D. G. K., G. P. T.]; Biogen, Inc., Cambridge, Massachusetts 02142 [P. H. W., R. R. L.]; Instituto de Biomedicina, C.S.I.C., 46010 Valencia, Spain [J. J. C.]; and Albany College of Pharmacy and PRI at Albany, Albany, New York 12208 [S. A. M.]

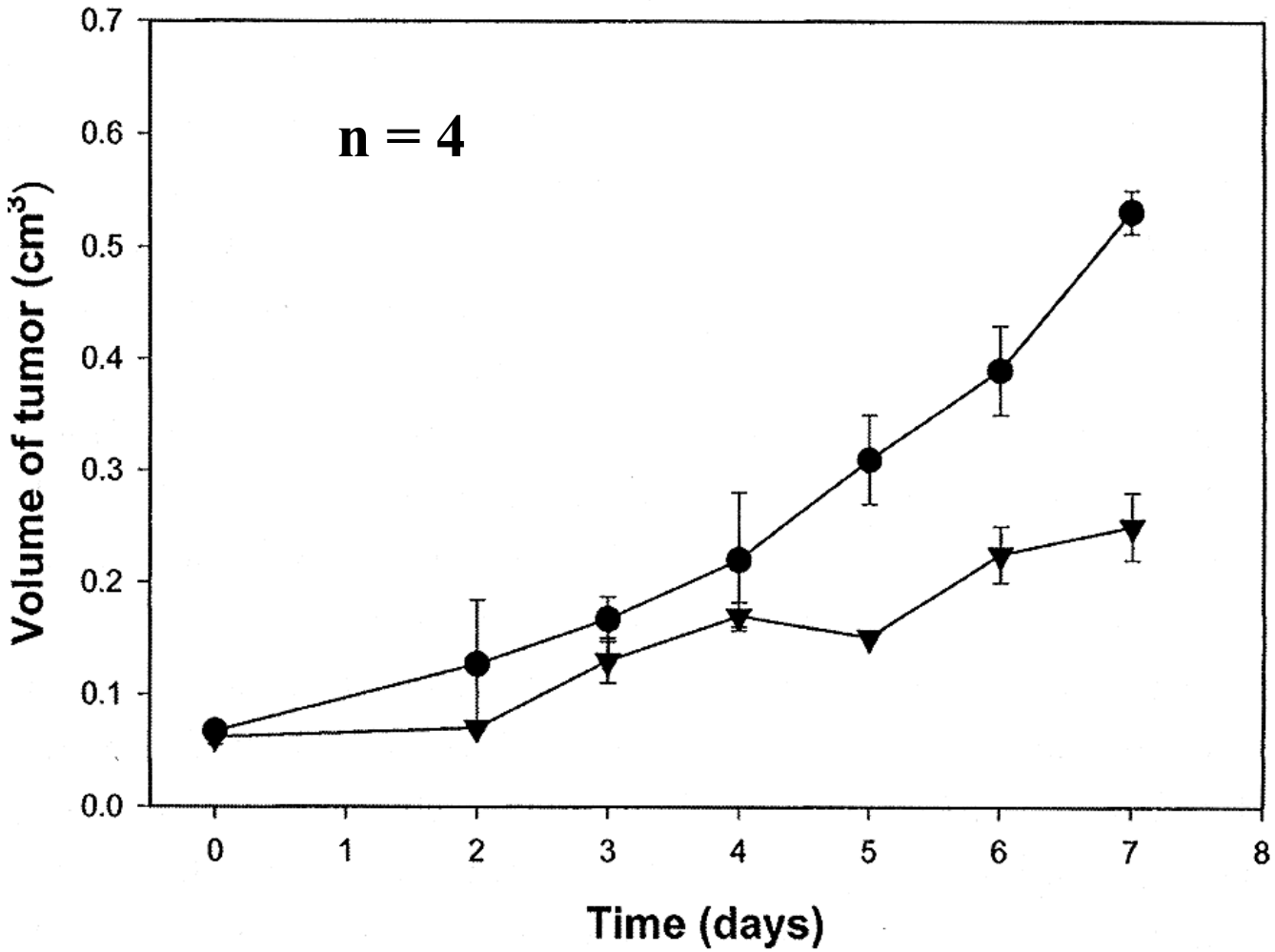
Effect on the adhesion of α1-K562 to coll IV (●) and α2-K562 to coll I (○).





AI, Angiogenesis index = nº vessel branch points

Effect of obtustatin (▼) and PBS (●) on Lewis lung carcinoma growth in C57BL/ 6 mice



FOR THE RECORD

Amino acid sequence and homology modeling of obtustatin, a novel non-RGD-containing short disintegrin isolated from the venom of *Vipera lebetina obtusa*

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4395.3

Sequence of peptide	IC_{50} (μM)	
CWKTSLTSHYC	586	
CAKTSLTSHYC	612	
CWATSLTSHYC	1185	K
CWKASLTSHYC	>2000	T
CWKTALTSHYC	916	S
CWKTSATSHYC	698	
CWKTSLASHYC	603	
CWKTSLTAHYC	632	
CWKTSLTSAYC	658	
CWKTSLTSTAC	669	

doi:10.1016/S0022-2836(03)00371-1 J. Mol. Biol. (2003) 329, 135–145

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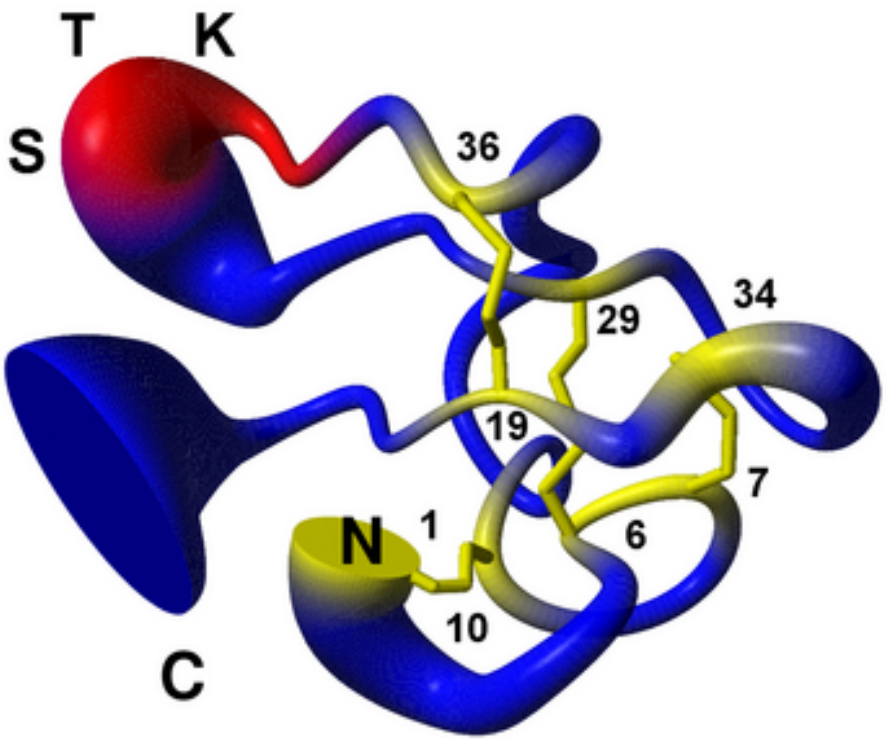
NMR Solution Structure of the Non-RGD Disintegrin Obtustatin

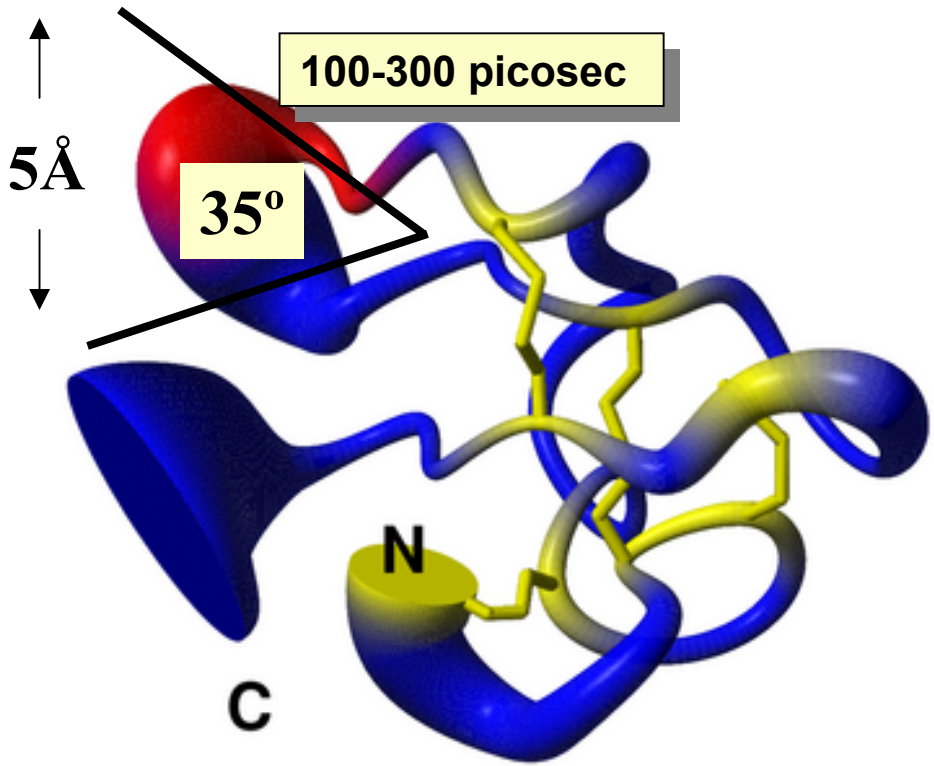
M. Paz Moreno-Murciano[1,2], Daniel Monleón[1], Cezary Marcinkiewicz[3] Juan J. Calvete[2*] and Bernardo Celda[1*]

Monleón,D., Moreno-Murciano,M.P., Kovacs,H., Marcinkiewicz,C., Calvete,J.J. & Celda,B.

Concerted motions of the integrin-binding loop and the C-terminal tail of the non-RGD disintegrin obtustatin

J. Biol. Chem. (2003) in press

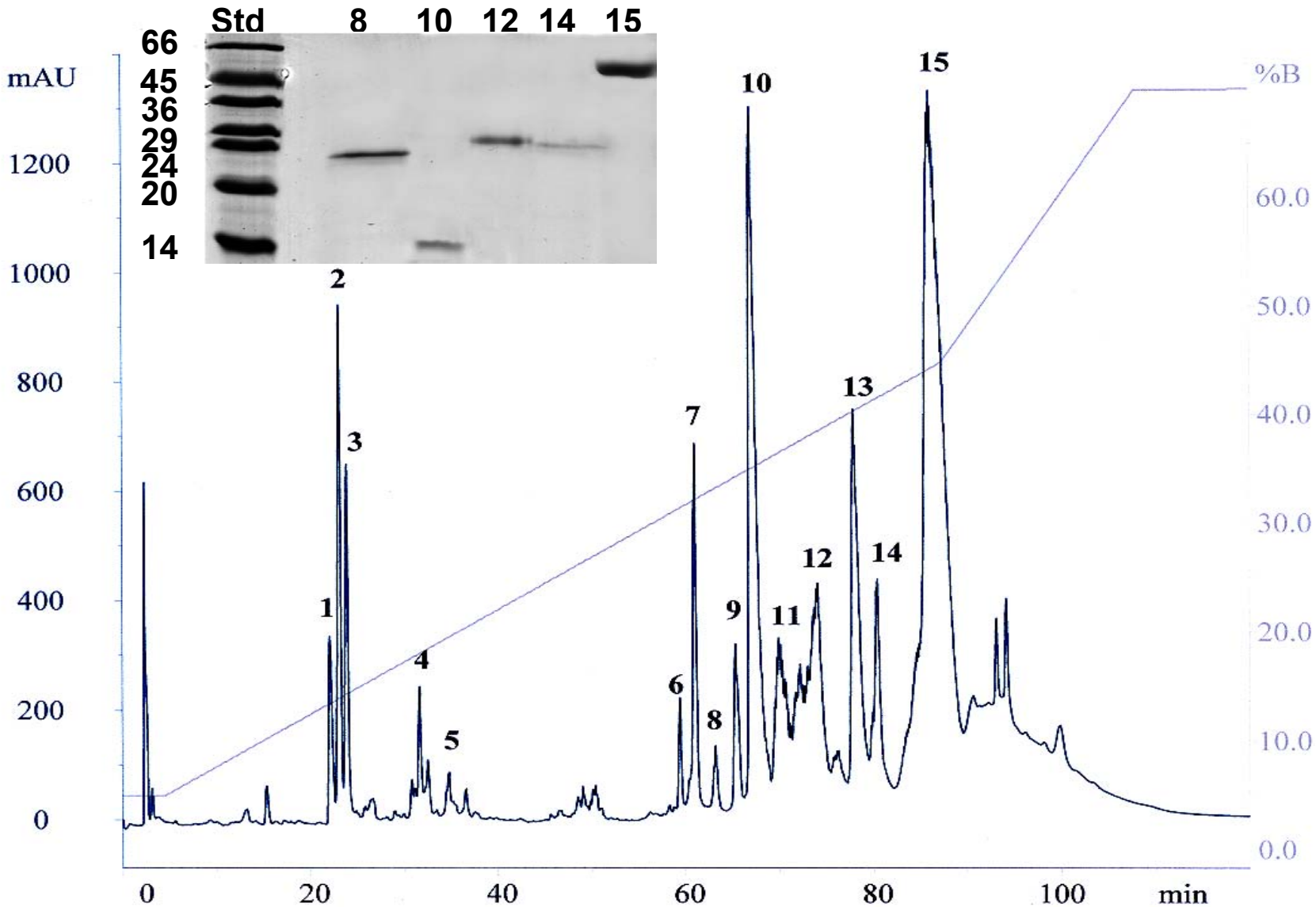


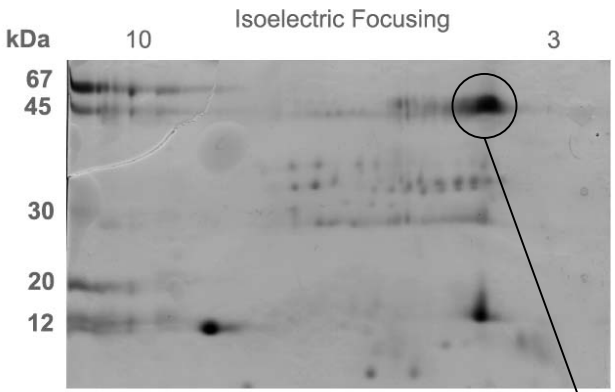


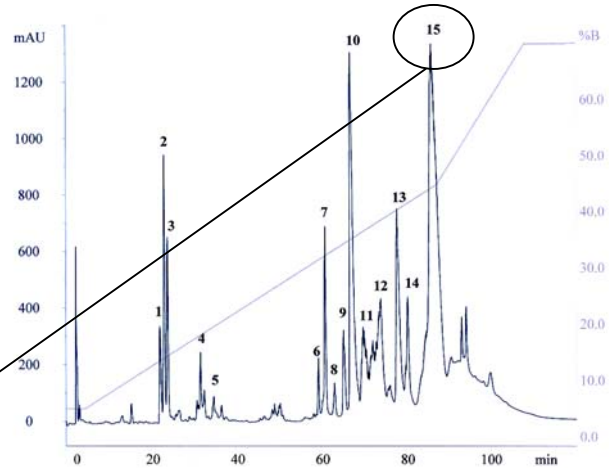
Venómica

Paula Juárez, Libia Sanz
IBV

Characterization of the protein components of the venom of *Sistrurus barbouri*

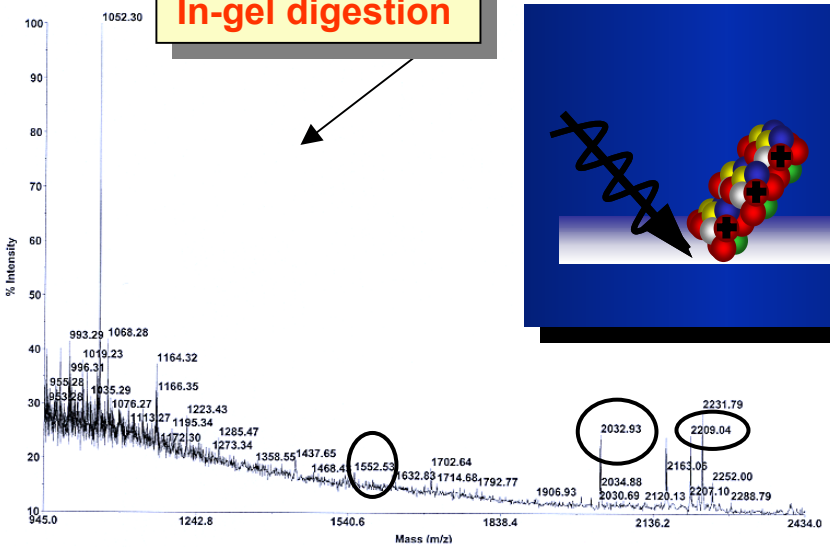




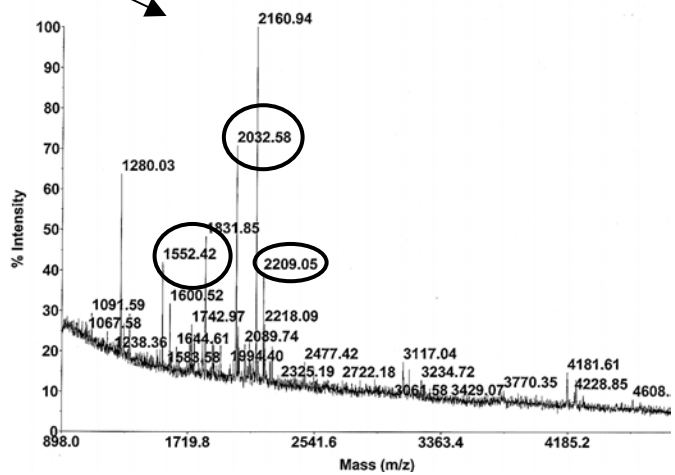


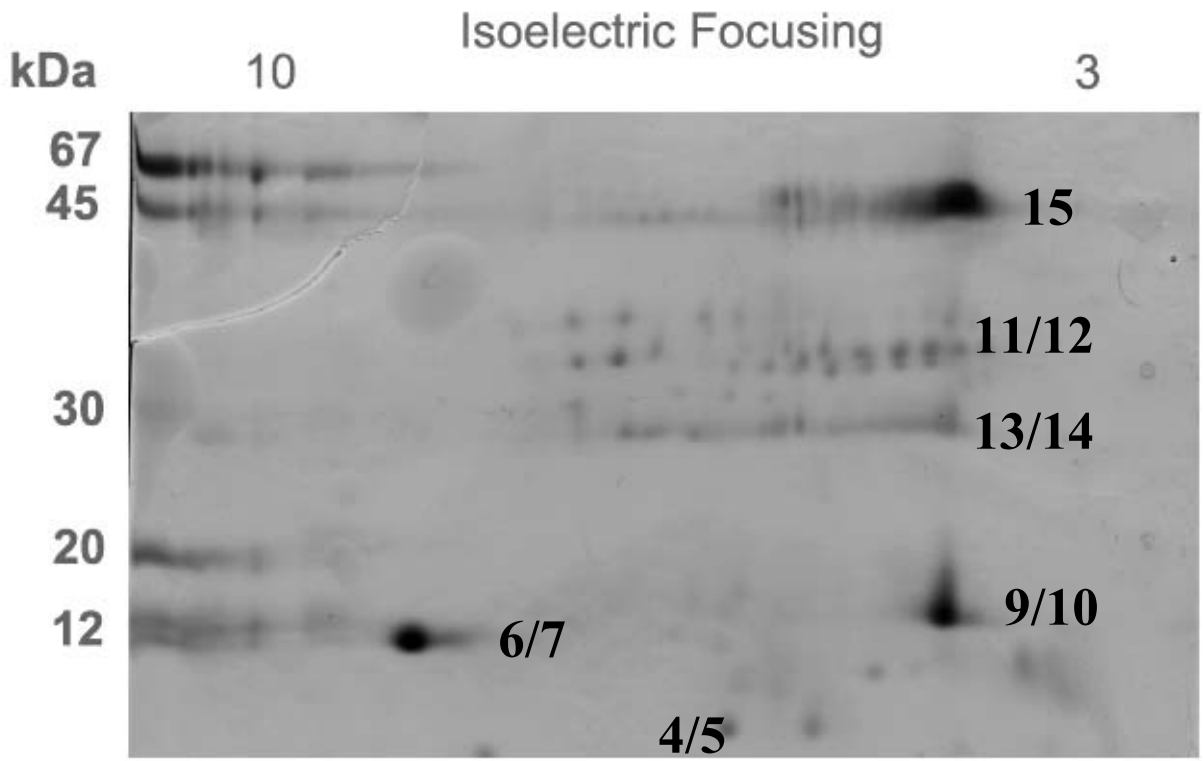
MALDI-TOF mass fingerprinting

In-gel digestion



In solution



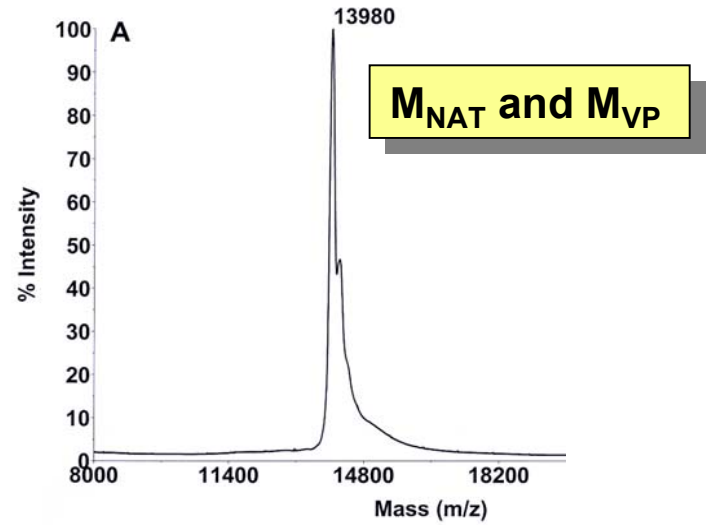

Isoelectric Focusing
kDa
10
3
67
45
30
20
12
15
11/12
13/14
9/10
6/7
4/5

QUANTITATION OF FREE CYSTEINE RESIDUES AND DISULPHIDE BONDS

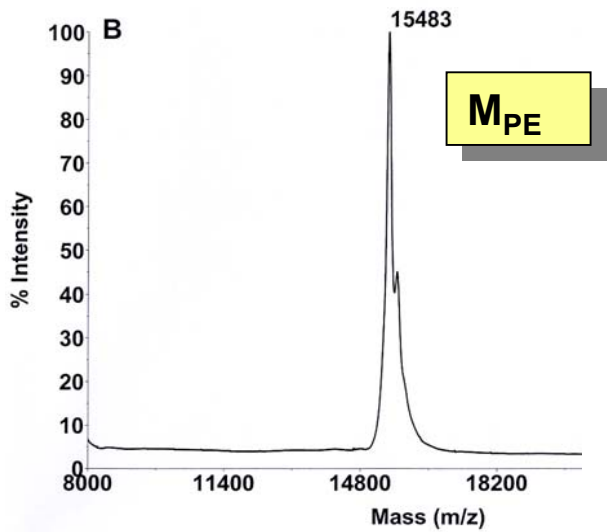
No free cysteine residue

(15483-13980)/106= 14.1 Cys

14/2 = 7 Disulphide bonds



M_{NAT} and M_{VP}



M_{PE}

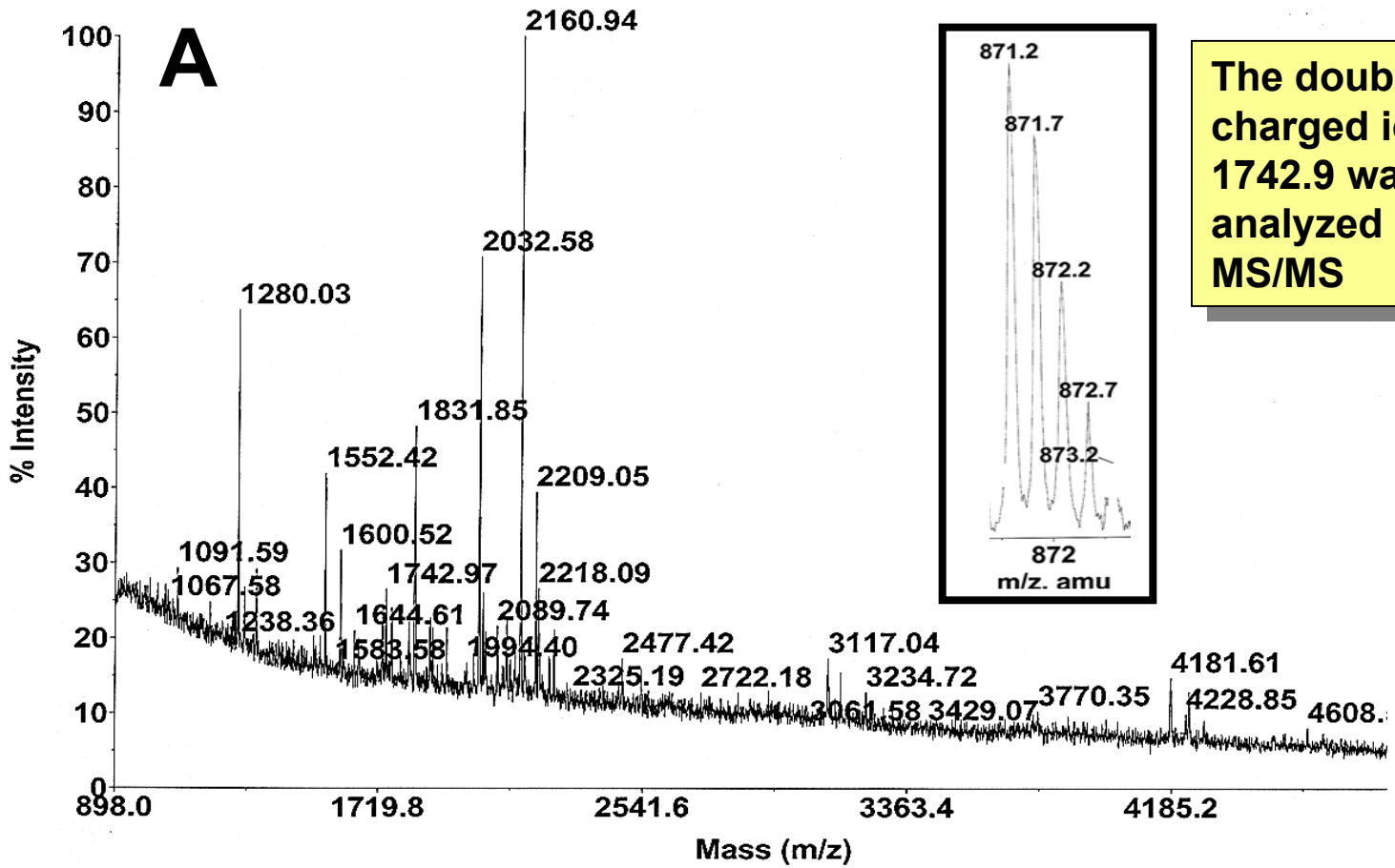
HPLC fraction	N-terminal sequence	MALDI-TOF MS [Da]			N° of cysteines*			Protein family
		M_{Nat}	M_{VP}	M_{PE}	Free SH	Total Cys	S-S	
4	AGEECDCGSP GEECDCGSPE EECDCGSPEN	7501	7502	8762	-	12	6	Disintegrin
5	GEECDCGSPE EECDCGSPEN					12	6	Disintegrin
6						14	7	PLA_2
7						14	7	PLA_2
8						16	8	CRISP
9						14	7	PLA_2
10	HLITFEQLIMKIAGRSGVFW	13980	13983	15483	-	14	7	PLA_2
11						12	6	Ser-proteinase
12						12	6	Ser-proteinase
13	NPEHQRYVELFIVVDHGM	23187	23293	23921	1	7	3	metalloproteinase
14	NPEHQRYVELFIVVD	23356	23375	24089	1	7	3	metalloproteinase
15	Blocked	48555	48664	52241	1	35	17	

Protein 15 contained a blocked N-terminus.

The protein contained 1 free cysteine and 17 disulphide bonds

This suggested that protein 15 might be a metalloproteinase of the ADAM family

The proteins in the major peaks were identified by combination of N-terminal sequencing and mass spectrometric determination of molecular masses and cysteine content



The doubly-charged ion of 1742.9 was analyzed by MS/MS

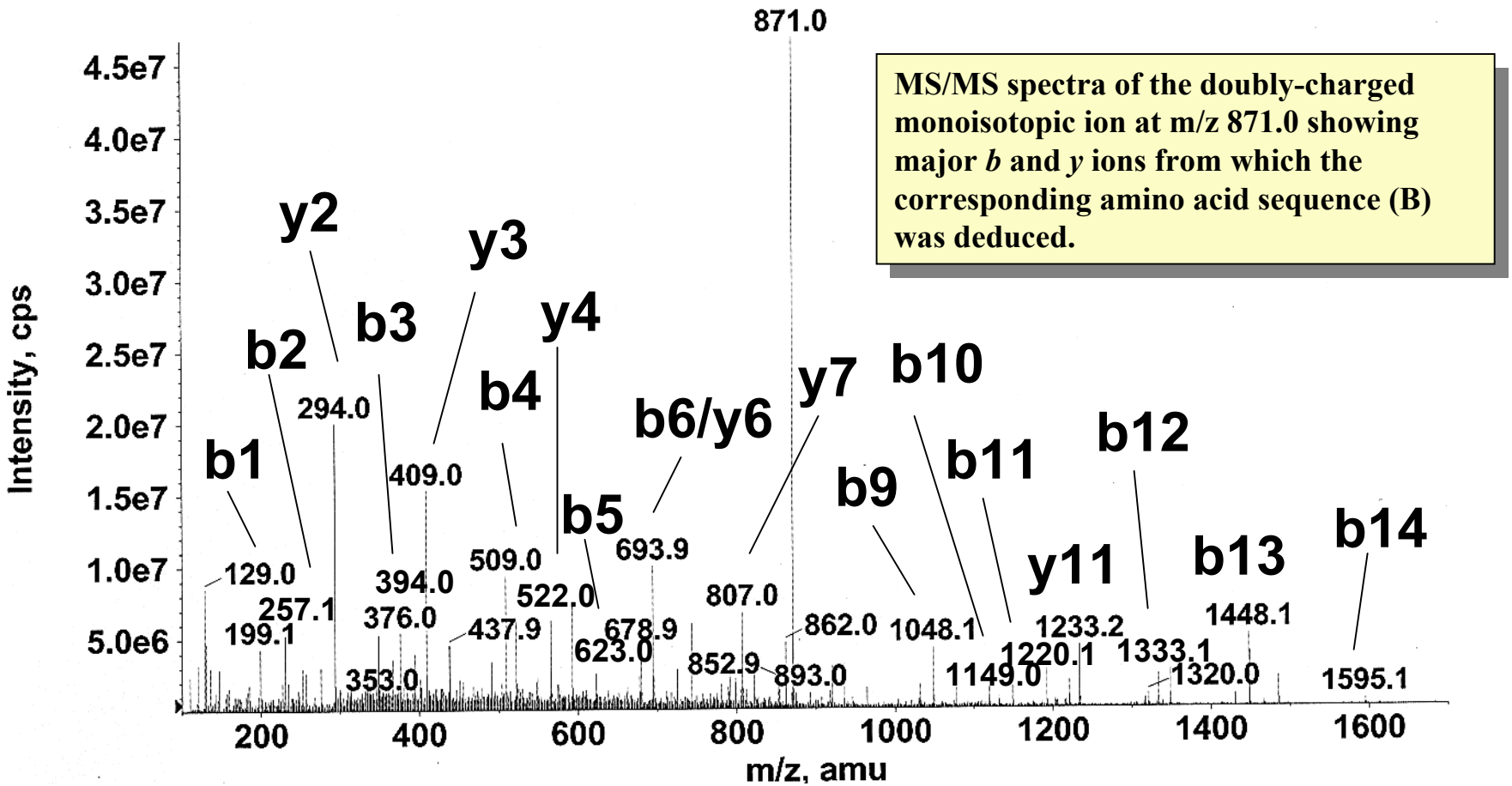
MALDI-TOF tryptic mass fingerprint of the carboxyamidomethylated-protein 15 in solution.

B

K	K	H	D	N	A	Q	I/L	I/L	T	A	I/L	D	F	K

y14 y13 y12 y11 y10 y9 y8 y7 y6 y5 y4 y3 y2 y1

(b1) b2 b3 b4 b5 b6 b7 b8 b9 b10 b11 b12 b13 b14



MS/MS spectra of the doubly-charged monoisotopic ion at m/z 871.0 showing major *b* and *y* ions from which the corresponding amino acid sequence (B) was deduced.

The sequence of peptide at m/z 871.0:

K K H D N A Q I/L I/L T A I/L D F K

shows strong homology to peptide 43-58 of jararhagin:

K K H D N A Q L L T A I D F N

Further indicating that protein 15 of *Sistrurus barbouri* venom is a jarrarhagin-like metalloproteinase

CONCLUSIONS

Although the lack of genome sequences of snake species is a handicap for the identificacion of venom proteins by MALDI-TOF mass fingerprinting, MS/MS fragmentation of selected ions yielded sufficient sequence information to identify an homologue protein from a related snake venom metalloprotease.

The venom proteome of the pigmy rattlesnake *Sistrurus barbouri* is composed of proteins belonging to only a few known proteins families, including:

- **Phospholipases A2**
- **PI Zn^{2+} metalloproteinases**
- **Disintegrins**
- **CRISP**
- **Serine proteinases**
- **ADAM**