Protein Sequencing Using Mass Spectrometry
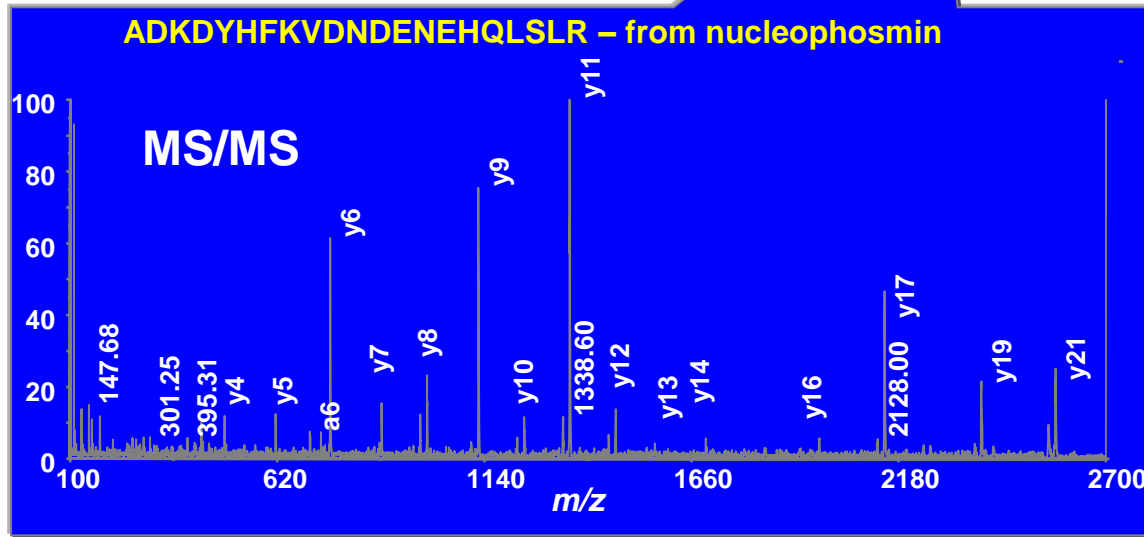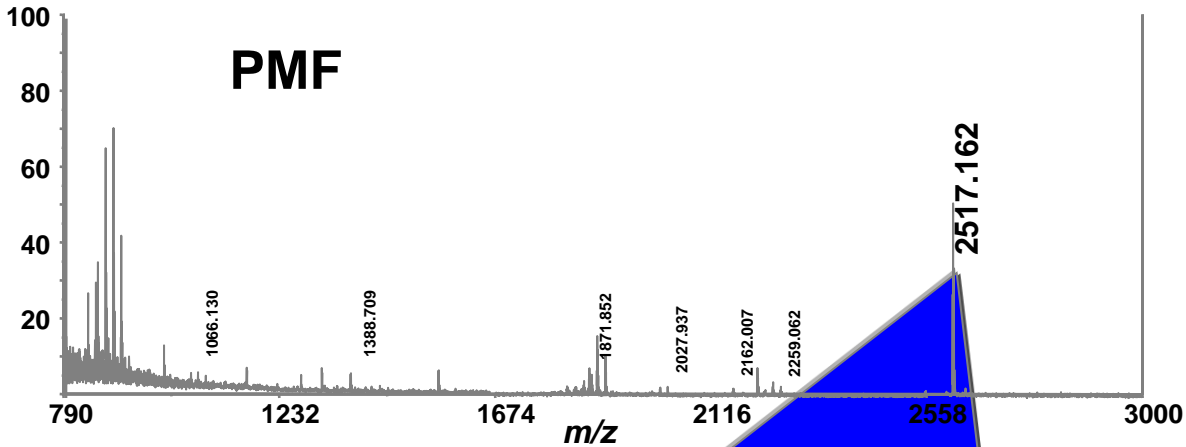
PMF

m/z spectrum with peaks labeled: 1066.130, 1388.709, 1871.852, 2027.937, 2162.007, 2259.062, **2517.162**

ADKDYHFKVDNDENEHQLSLR – from nucleophosmin

MS/MS

m/z spectrum with peaks labeled: 147.68, 301.25, 395.31, y4, y5, a6, y6, y7, y8, y9, y10, 1338.60, y11, y12, y13, y14, y16, y17, 2128.00, y19, y21

Browser screenshot:
Sample ID (comment): Apo A-1 1040 AKPVLEDLR
Database searched: NCBInr.10.28.2001
Full Molecular Weight range: 787946 entries.
Full pI range: 787946 entries.
Species search (HUMAN MOUSE) selects 74037 entries.
Pre searches select 74037 entries.
Ion Types Considered: a b B y n h

Search Mode	Peptide Masses are	Digest Used	Max. # Missed Cleavages	Cysteines Modified by	Peptide N terminus	Peptide C terminus
identity	monoisotopic	Trypsin	2	acrylamide	Hydrogen (H)	Free Acid (O H)

Number of sequences passing through parent mass filter: 1045
MS-Tag search selects 11 entries.

Parent mass: 2573.3000 (+/- 0.5000 Da)
16 Fragment Ions used in search: 430.34, 488.40, 616.50, 753.50, 882.50, 979.60, 996.60, 1125.70, 1240.70, 1338.70, 1354.70, 1470.80, 1696.90, 1982.05, 2145.10, 2388.30 (+/- 0.20 Da)
Max # Unmatched Ions = 11

Result Summary

Rank	# Unmatched Ions	Sequence	MH+ Calculated (Da)	MH+ Error (Da)	Protein MW (Da)/pI	Species	NCBInr.10.28.2001 Accession #	Protein Name
1	5/16	(K)ADKDYHFKVDNDENEHQLSLR(T)	2573.2021	0.1	75314.9 / 6.44	HOMO SAPIENS	609342	(U04946) nucleophosmin-anaplastic lymphoma kinase fusion protein
1	5/16	(K)ADKDYHFKVDNDENEHQLSLR(T)	2573.2021	0.1	30938.4 / 4.71	HOMO SAPIENS	825671	(X16934) B23 nucleophosmin (280 AA)
1	5/16	(K)ADKDYHFKVDNDENEHQLSLR(T)	2573.2021	0.1	62574.4 / 5.85	HOMO SAPIENS	1314308	(U41742) nucleophosmin-retinoic acid receptor alpha fusion protein NPM-

In this example the PMF contained only three peaks from the major protein component not yielding a significant match. Only MS/MS data could identify this protein unambiguously

MALDI MS/MS has been applied successfully to identify protein components of the nucleopore complex in yeast. (Rout *et al.*, *J. Cell Biol.*, **2000**, *148*, p. 635-651)
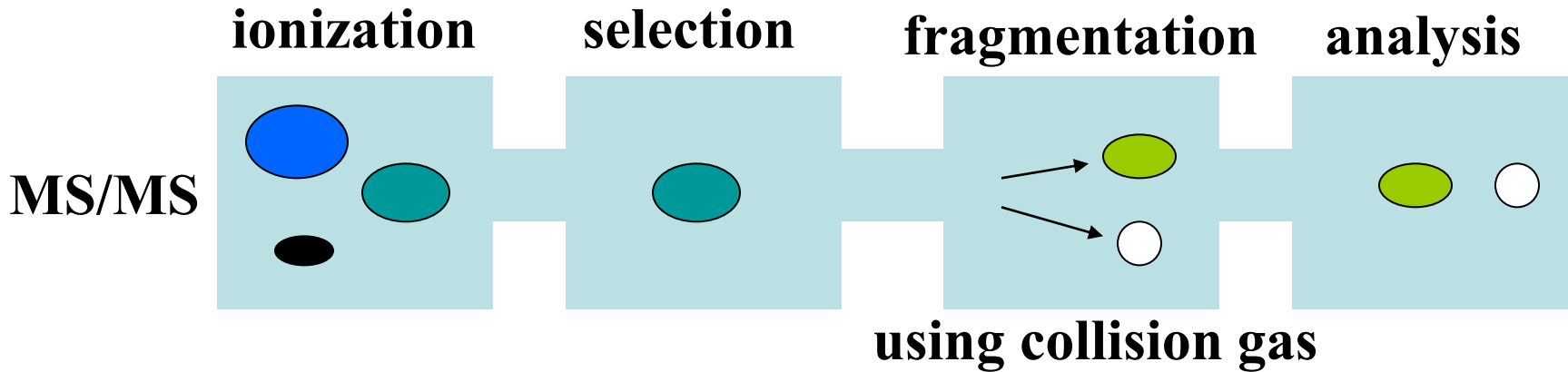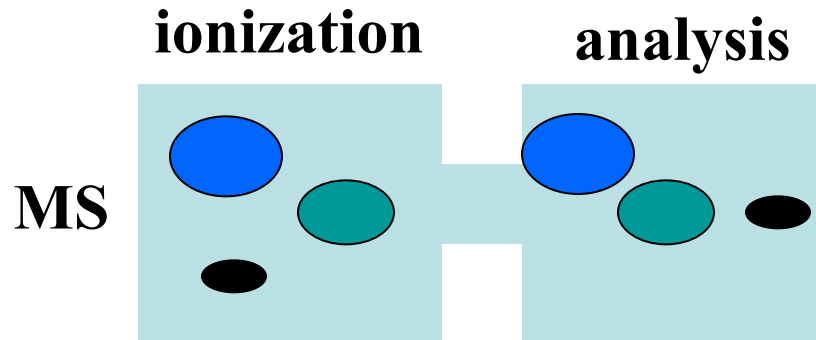
What is MS/MS?

MS/MS means using two mass analyzers (tandem in space) or one ion trap mass analyzer (tandem in time) to select an analyte (ion) from a mixture, then generate fragments from it to give structural information.

Mixture of ions · Single ion · Fragments

Ion source → MS-1 → MS-2 →
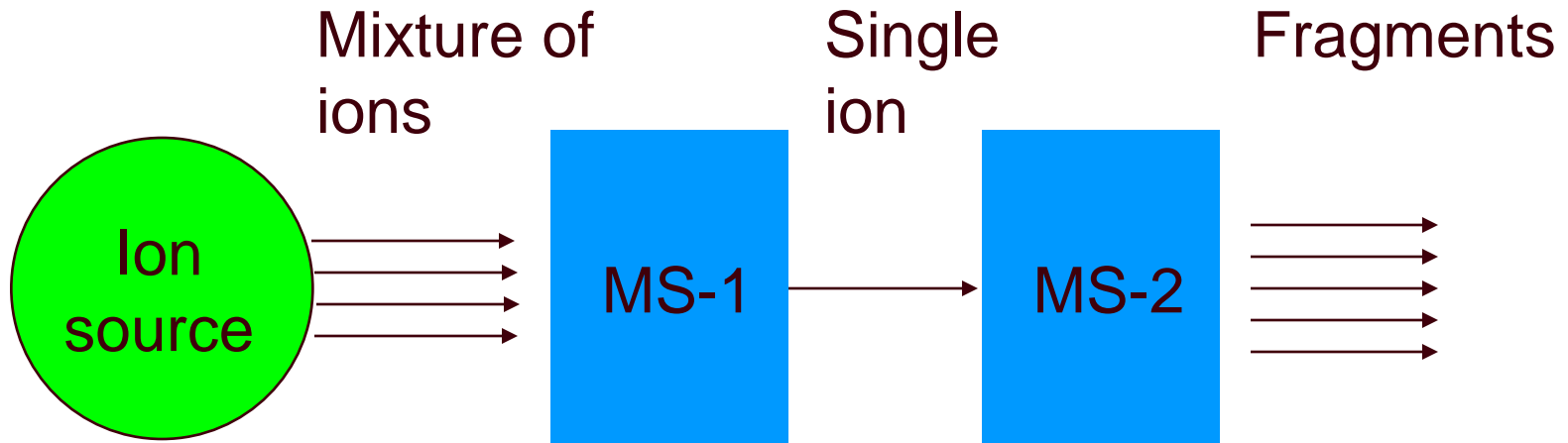
Peptide fragmentation

Peptide: S-G-F-L-E-E-D-E-L-K

	ion			ion	
88	b_1	S	GFLEEDELK	y_9	1080
145	b_2	SG	FLEEDELK	y_8	1022
292	b_3	SGF	LEEDELK	y_7	875
405	b_4	SGFL	EEDELK	y_6	762
534	b_5	SGFLE	EDELK	y_5	633
663	b_6	SGFLEE	DELK	y_4	504
778	b_7	SGFLEED	ELK	y_3	389
907	b_8	SGFLEEDE	LK	y_2	260
1020	b_9	SGFLEEDEL	K	y_1	147

Peptide Fragmentation

88	**145**	**292**	**405**	**534**	**663**	**778**	**907**	**1020**	**1166**	**b ions**
S	**G**	**F**	**L**	**E**	**E**	**D**	**E**	**L**	**K**	
1166	**1080**	**1022**	**875**	**762**	**633**	**504**	**389**	**260**	**147**	**y ions**

HPLC – MS/MS Ion Trap

UV Trace or TIC Chromatogram

MS at 25.2 Min

MS-MS of m/z 1574.2
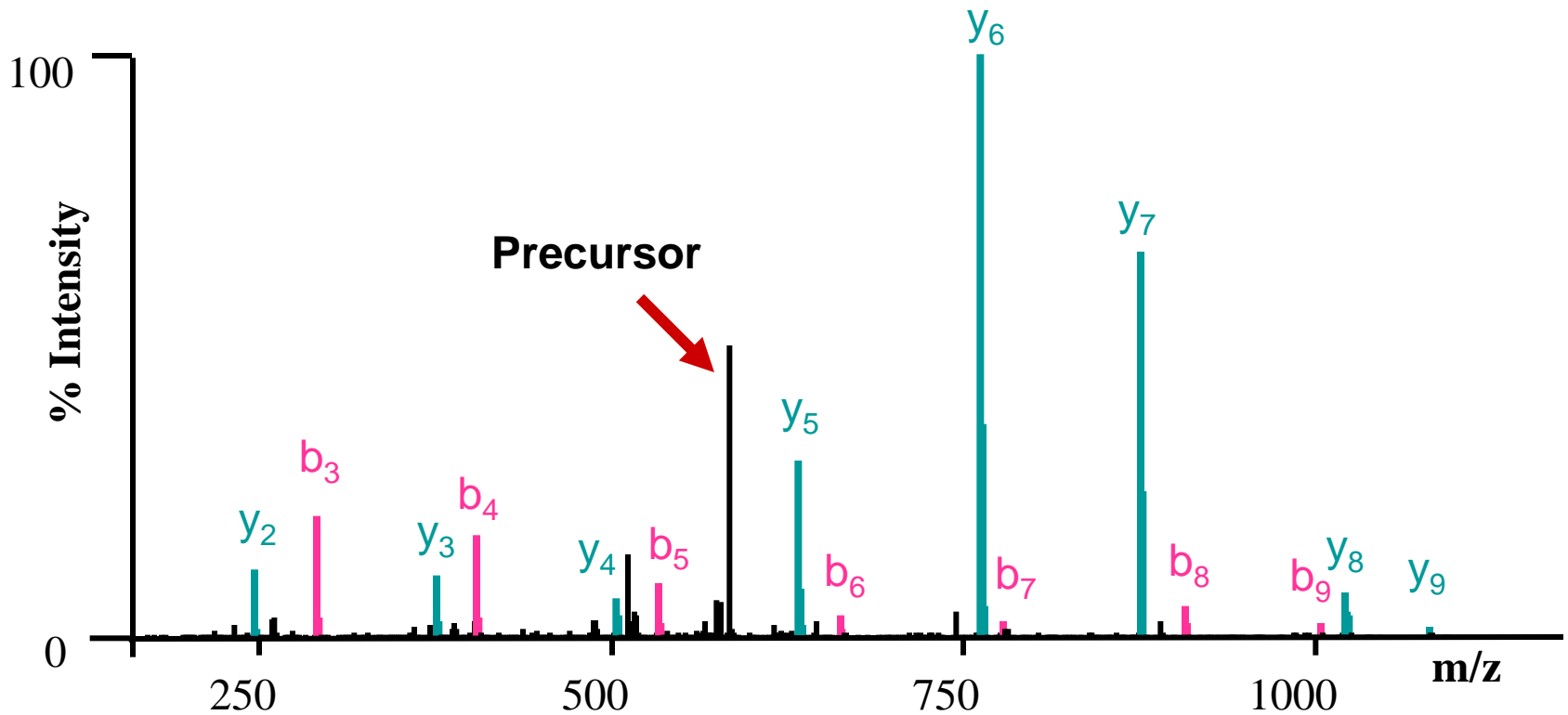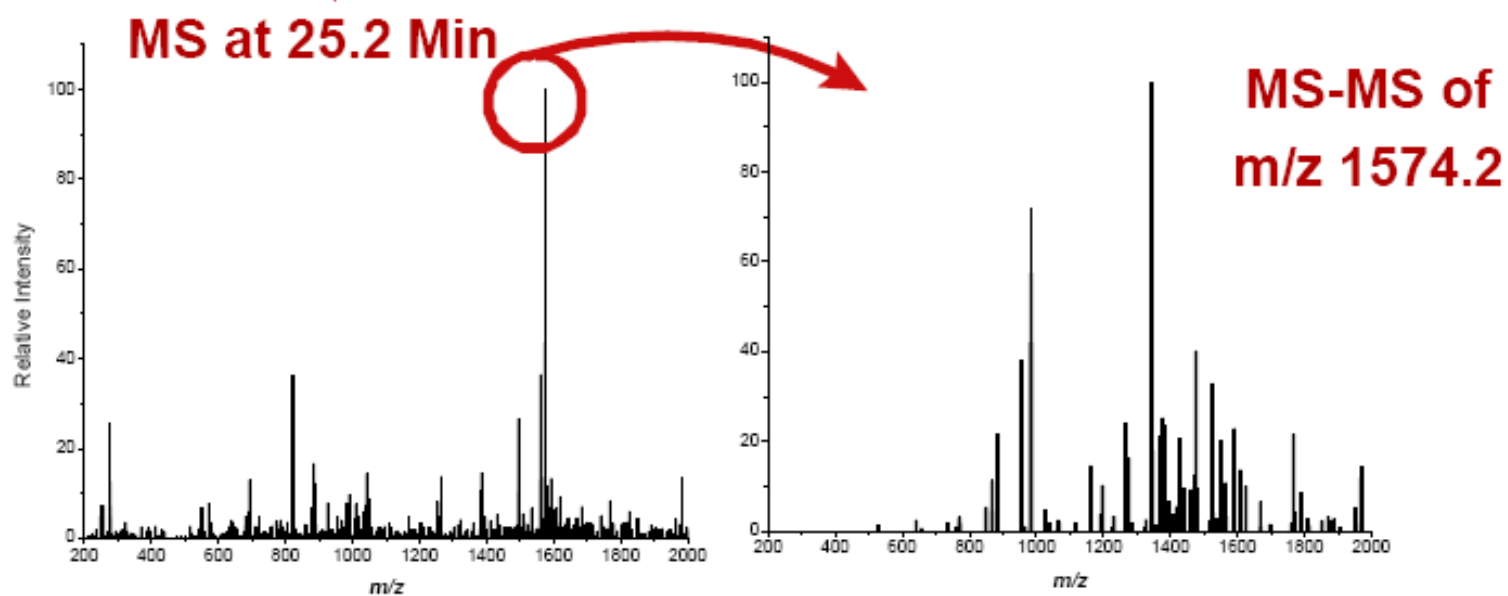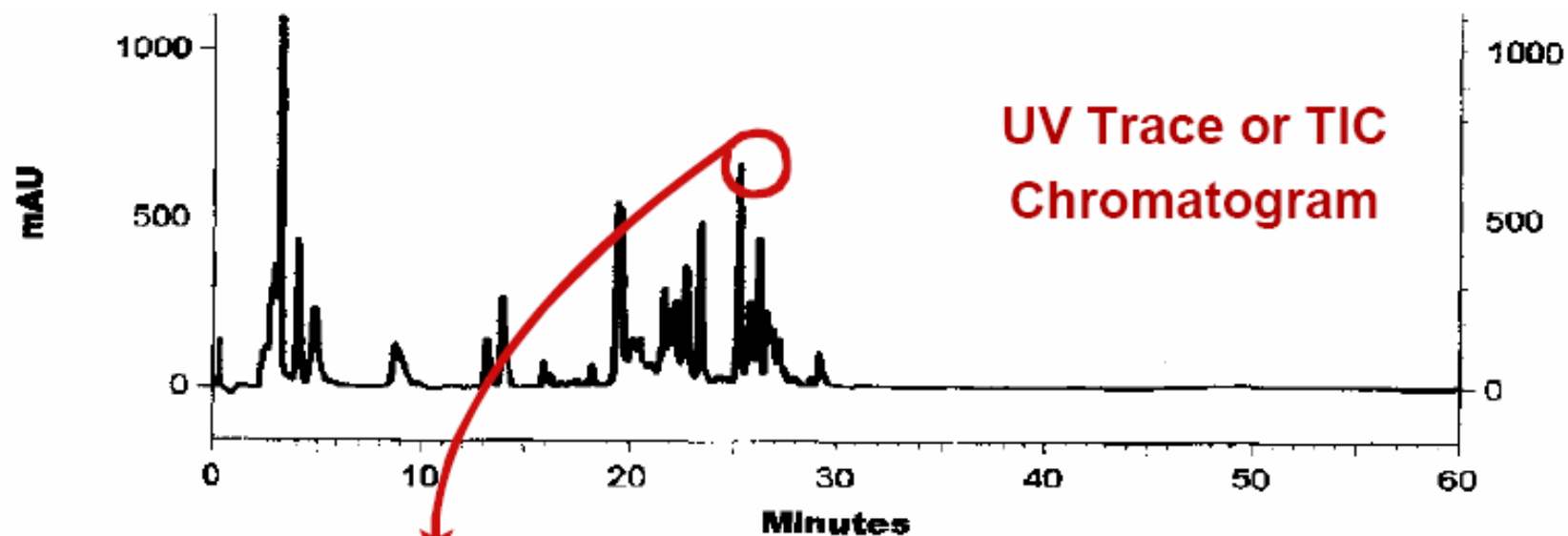
Data Analysis for MS/MS method

Peptide

mass ~ 1004.28

S E Q U E N C E

b ion

MS/MS fragmentation

S/E/Q/U/E/N/C/E

y ion

MS/MS spectrum

Peptides of correct mass

SEQUE
SEQUEN
SEQUENC
SEQUENCE (1004.279)
SEQUENCES
SEQUENCESE
SEQUENCESEQ

Theoretical b and y ions

S- 1008.279
E- 918.26
Q- 789.21
U- 661.15
E- 494.16
N- 365.11
C- 251.07
E- 148.06

Database Search

Theoretical MS/MS spectrum
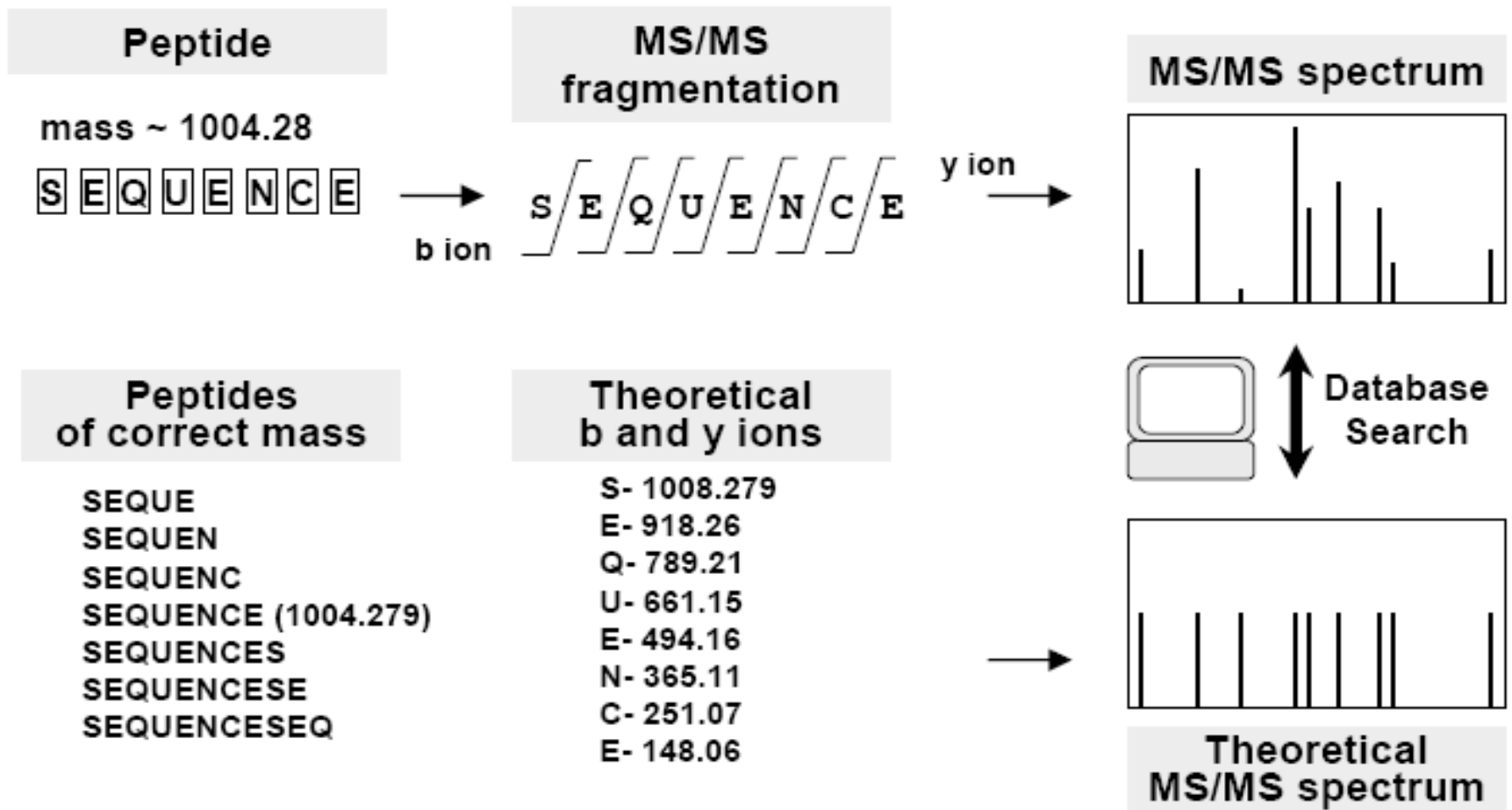
- a DB search will search for precursor peptide masses
- then align theoretical b/y ion masses with observed masses
- a probability algorithm will determine if the match is good
- %I for b/y ions NOT calculated!

Mascot search engine

{MATRIX SCIENCE}

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We look forward to meeting you at booth 621

ABRF 2004

Portland, OR February 28 to March 2

This site features **Mascot**, a powerful search engine that uses mass spectrometry data to identify proteins from primary sequence databases. To assist you, the help text for Mascot forms a substantial knowledge base concerning protein identification by MS.

If this is your first visit, please check for browser compatibility and read the small print. If you include results from Mascot in a publication, please cite either this URL or Electrophoresis, **20 (18)** 3551-67 (1999) (abstract).

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Mascot MS/MS ions search

Your name	[ ]	**Email**	[ ]
Search title	[ ]		
Database	MSDB ▼		
Taxonomy	All entries ▼		
Enzyme	Trypsin ▼	**Allow up to**	1 ▼ missed cleavages

Fixed modifications
```
AB_old_ICATd0 (C)
AB_old_ICATd8 (C)
Acetyl (K)
Acetyl (N-term)
Amide (C-term)
```

Variable modifications
```
AB_old_ICATd0 (C)
AB_old_ICATd8 (C)
Acetyl (K)
Acetyl (N-term)
Amide (C-term)
```

Protein mass	[ ] kDa	**ICAT**	☐
Peptide tol. ±	2.0	Da ▼	**MS/MS tol. ±** 0.8 Da ▼
Peptide charge	2+ ▼	**Monoisotopic** ◉ **Average** ○	
Data file	[ ] Browse...		
Data format	Mascot generic ▼	**Precursor**	[ ] m/z
Instrument	Default ▼		
Overview	☐	**Report top**	20 ▼ hits

Start Search ...   Reset Form

Mascot MS/MS search results

1. Q9XZJ2   **Mass:** 79480   **Score: 286**   **Peptides matched:** 4

    HEAT SHOCK PROTEIN 70.- Crassostrea gigas (Pacific oyster).

   ☐ Check to include this hit in error tolerant search

	Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
☑	1	671.90	1341.78	1341.73	0.06	0	95	1.3e-06	1	DAGTISGLNVLR
☑	2	808.30	1614.58	1613.76	0.83	0	75	0.00012	1	TTPSYVAFTDTER
☑	3	973.90	1945.78	1945.92	-0.14	0	90	2.9e-06	1	NQVAMNPNNTIFDAK
	4	1084.90	2167.78	2168.17	-0.39	1	30	2.7	3	IINEPTAAAIAYGLDKK

Proteins matching the same set of peptides:

Q94805   **Mass:** 79333   **Score: 283**   **Peptides matched:** 4

HSC70.- Trichoplusia ni (Cabbage looper).

Mascot MS/MS search results
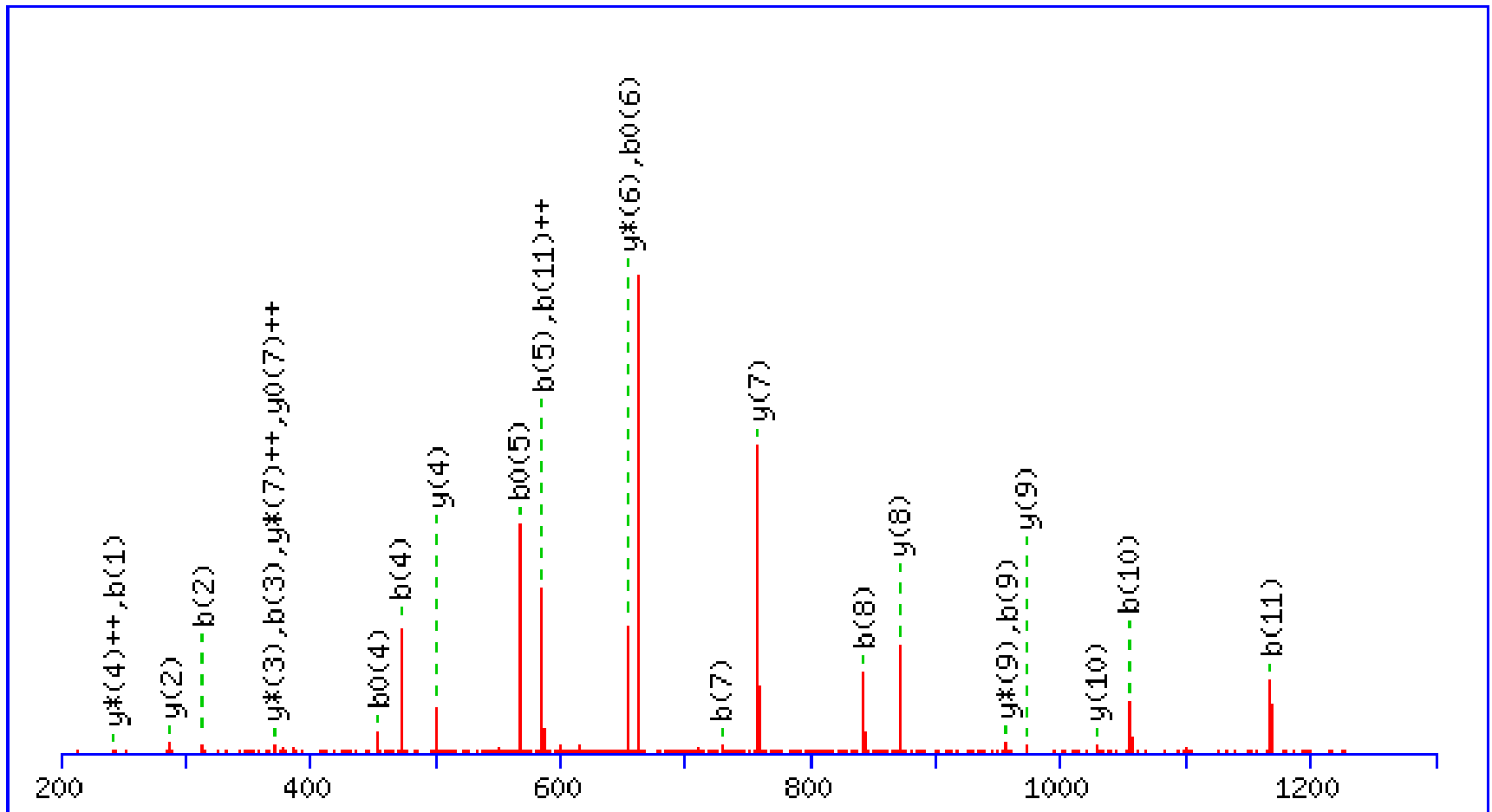
Mascot MS/MS search results

#	b	b^{++}	b^{*}	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y^{*}	y^{*++}	y^{0}	y^{0++}	#
1	243.10	122.05			225.09	113.05	D							12
2	314.13	157.57			296.12	148.57	A	1100.64	550.82	1083.62	542.31	1082.63	541.82	11
3	371.16	186.08			353.15	177.08	G	1029.61	515.31	1012.58	506.79	1011.59	506.30	10
4	472.20	236.61			454.19	227.60	T	972.58	486.80	955.56	478.28	954.57	477.79	9
5	585.29	293.15			567.28	284.14	I	871.54	436.27	854.51	427.76	853.53	427.27	8
6	672.32	336.66			654.31	327.66	S	758.45	379.73	741.43	371.22	740.44	370.72	7
7	729.34	365.17			711.33	356.17	G	671.42	336.21	654.39	327.70			6
8	842.43	421.72			824.41	412.71	L	614.40	307.70	597.37	299.19			5
9	956.47	478.74	939.44	470.22	938.46	469.73	N	501.31	251.16	484.29	242.65			4
10	1055.54	528.27	1038.51	519.76	1037.53	519.27	V	387.27	194.14	370.24	185.63			3
11	1168.62	584.81	1151.59	576.30	1150.61	575.81	L	288.20	144.61	271.18	136.09			2
12							R	175.12	88.06	158.09	79.55			1