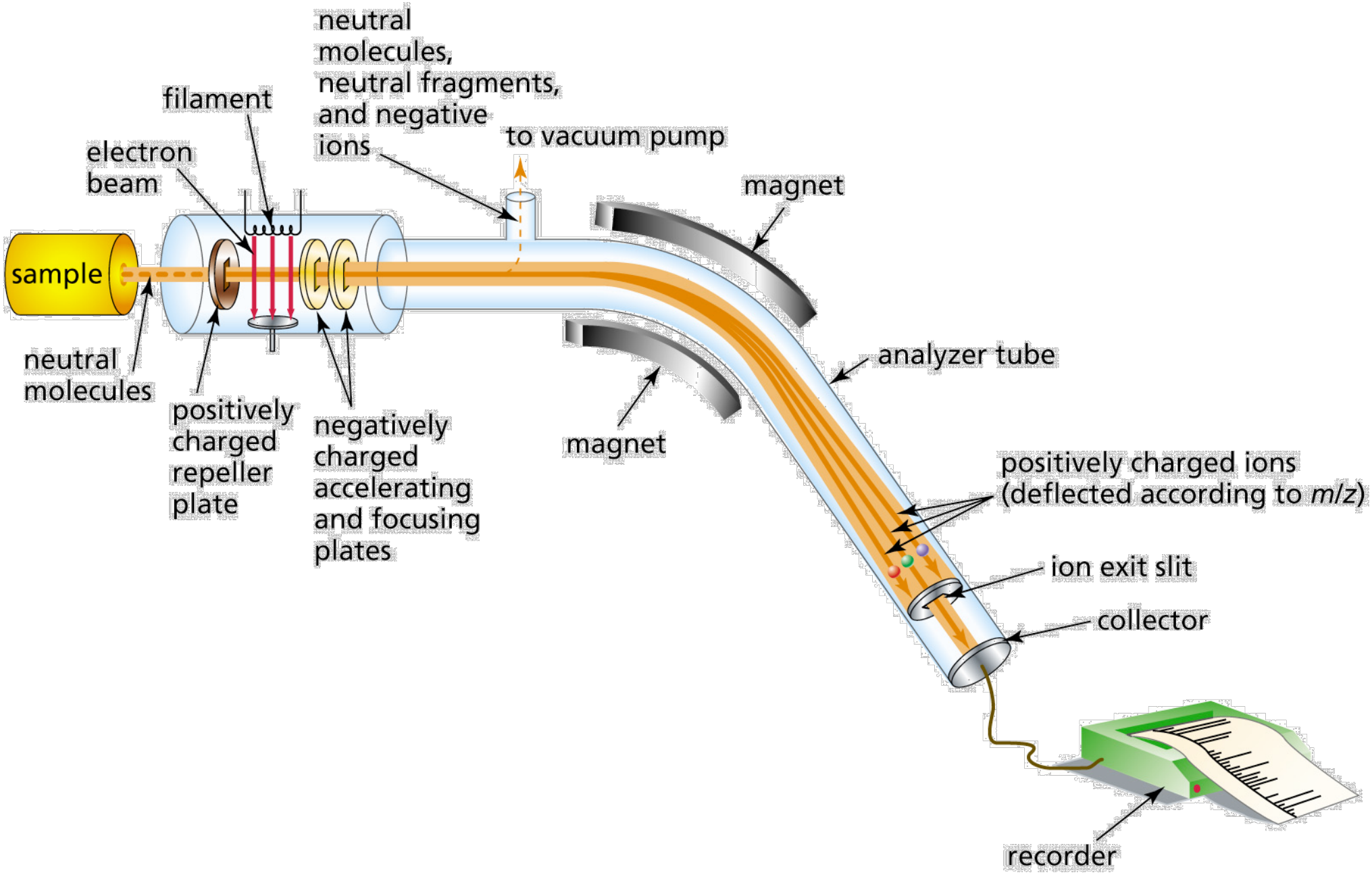
Making Elephants Fly*

*"Well, in homely terms, we learned how to make elephants fly, as it were."
John B. Fenn during an interview on *News Hour with Jim Lehrer*, October 9, 2002

Mass Spectrometry



MS for Small Organic Molecules

Small molecules are easily vaporized

Electron beam ionization (10,000–20,000 V) conveniently ionizes organic molecules

Fragmentation pattern caused by electron beam ionization provides clues about the structure of the molecule

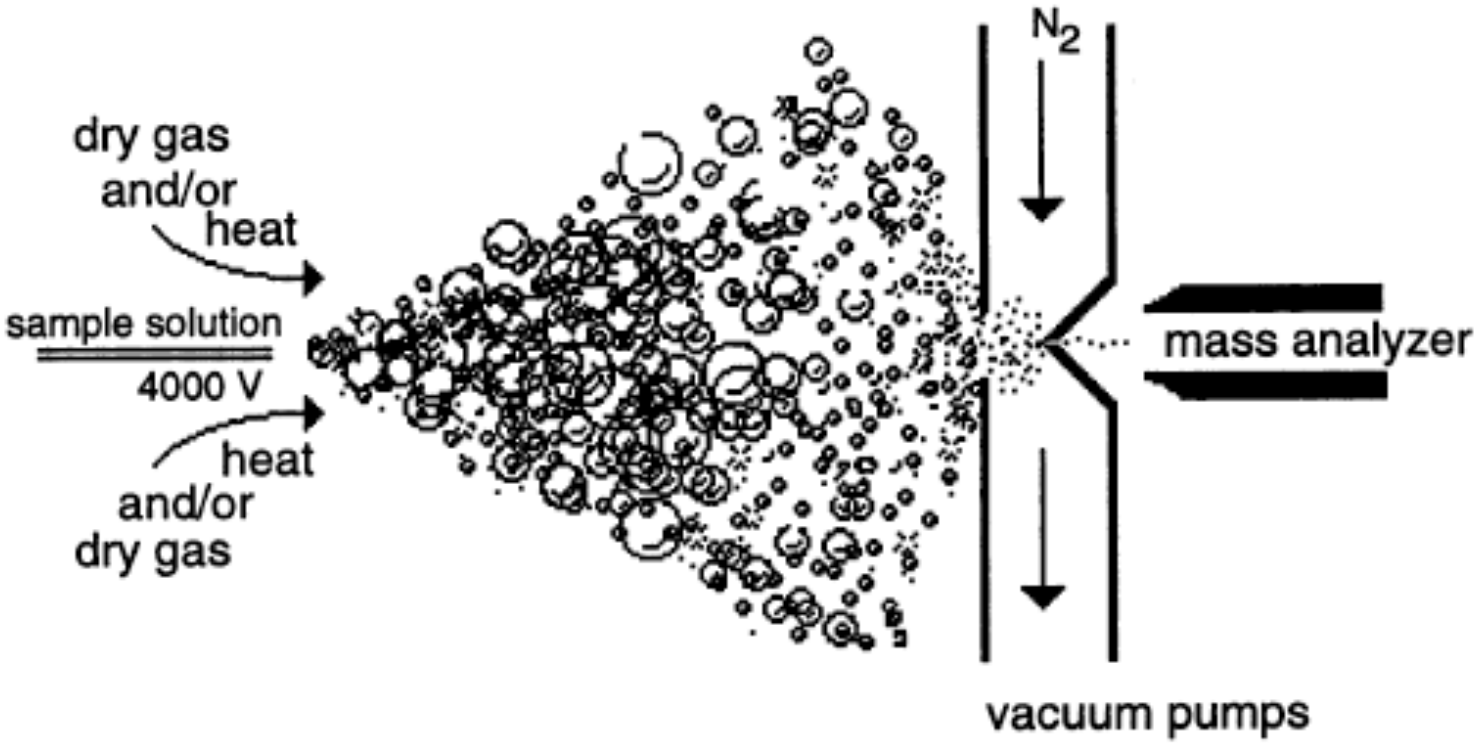
MS for Proteins

Proteins are not easily vaporized

Electron beam ionization (10,000–20,000 V) easily ionizes (cooks) proteins…

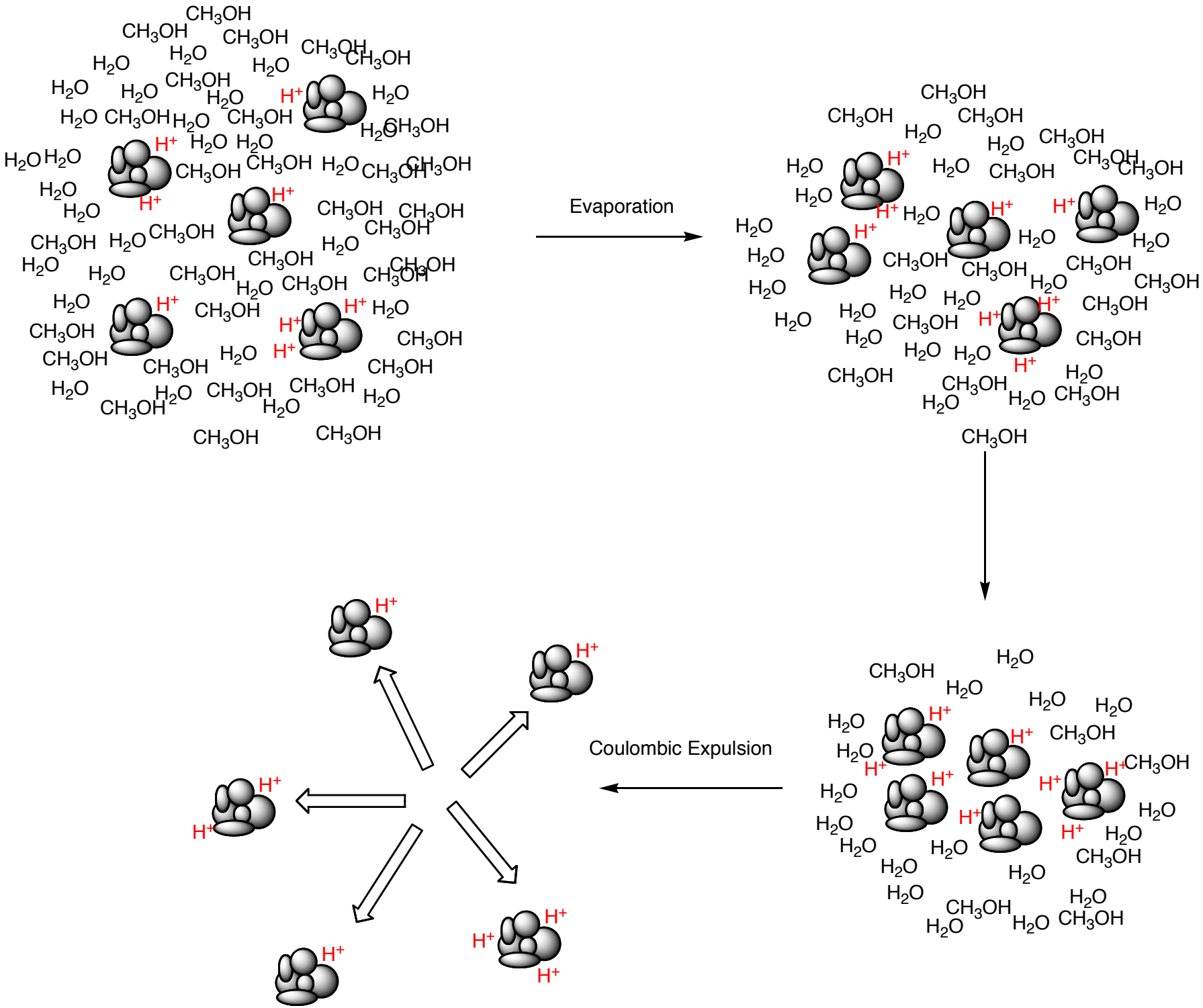
Fragmentation patterns produces by electron beam ionization are too complicated to analyze

Electrospray Ionization (ESI)

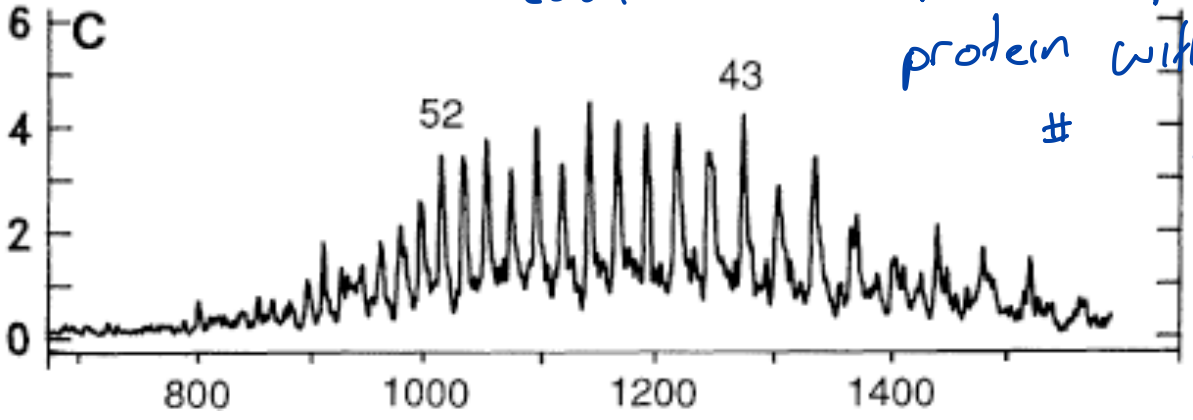


Gary Siuzdak

Proceedings of the National Academy of Sciences of the United States of America, Vol. 91, No. 24. (Nov. 22, 1994), pp. 11290-11297.

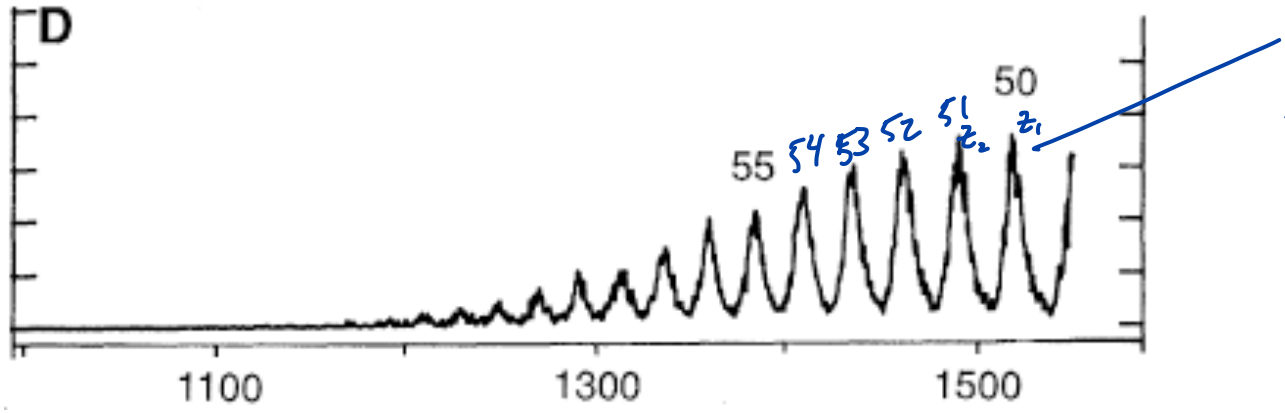

Evaporation
Coulombic Expulsion

each of these peaks represents the protein with a different # of H^+'s bonded to it ... a different ⊕ charge



α-analyse: 54,700 da

We don't know the charge, but we know that adjacent peaks are separated by a charge of +1



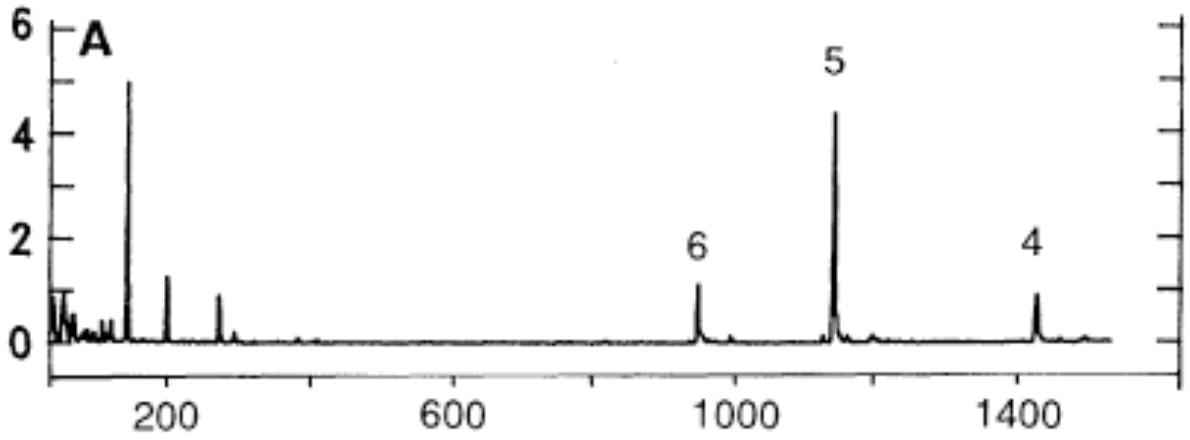
$z_1 = ?$

$z_2 = z_1 + 1$

conalbumin: 76,000 da

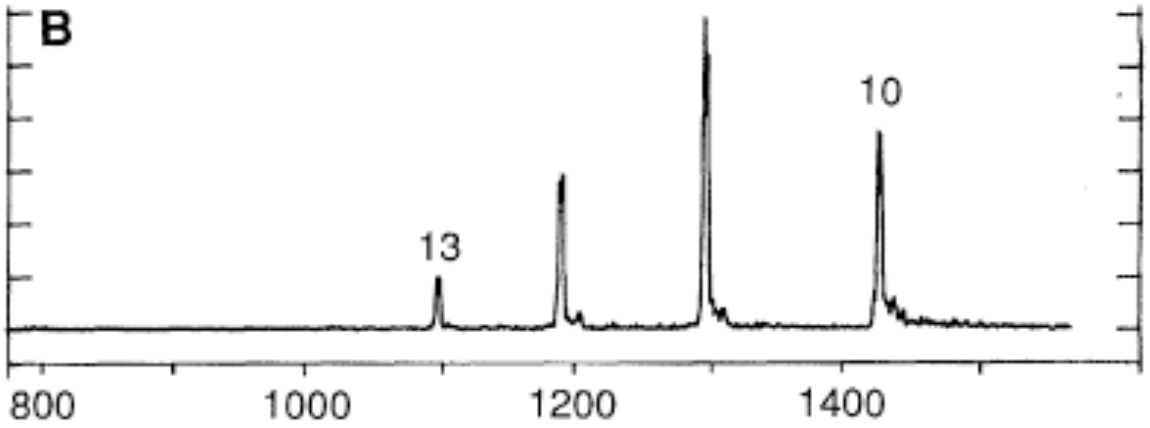
John B. Fenn; Matthias Mann; Chin Kai Meng; Shek Fu Wong; Craig M. Whitehouse

Science, New Series, Vol. 246, No. 4926. (Oct. 6, 1989), pp. 64-71.



m/z	m/z x z
1433	1433 * 4 = 5732
1146	1146 * 5 = 5730
955	1433 * 6 = 5730

Insulin: 5730 da



m/z	m/z x z
1430	1430 * 10 = 14,300
1300	1300 * 11 = 14,300
1192	1192 * 12 = 14,304
1100	1100 * 13 = 14,300

Lysosome: 14300 da

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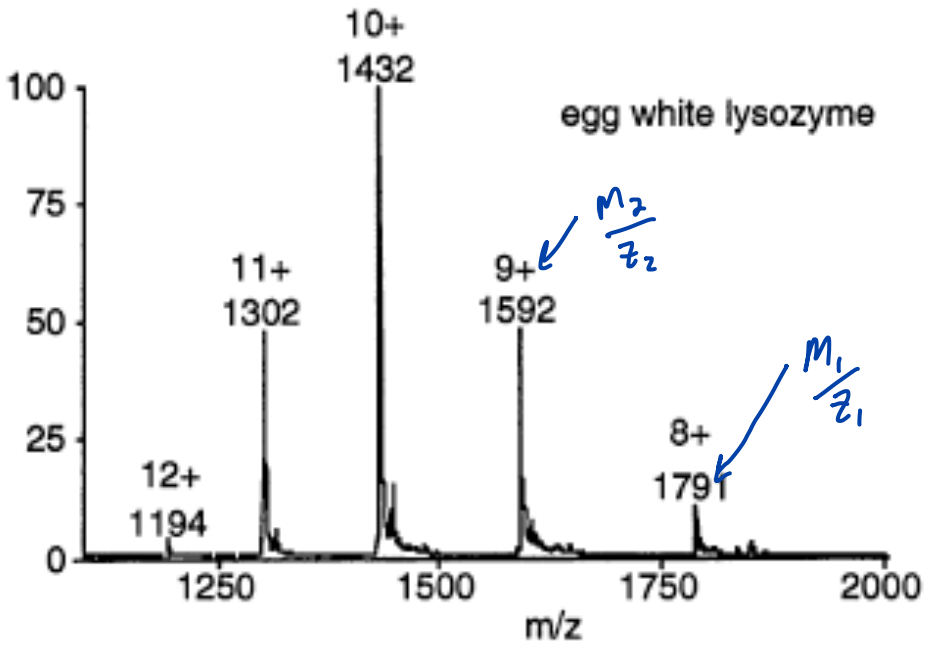

$(m/z_1)_1 z_1 = m$ — mass of the molecule

$(m/z_2)_2 z_2 = m$

$1791\ z_1 = m$

$1592\ z_2 = m$

$$1592\ z_2 = 1791\ z_1$$

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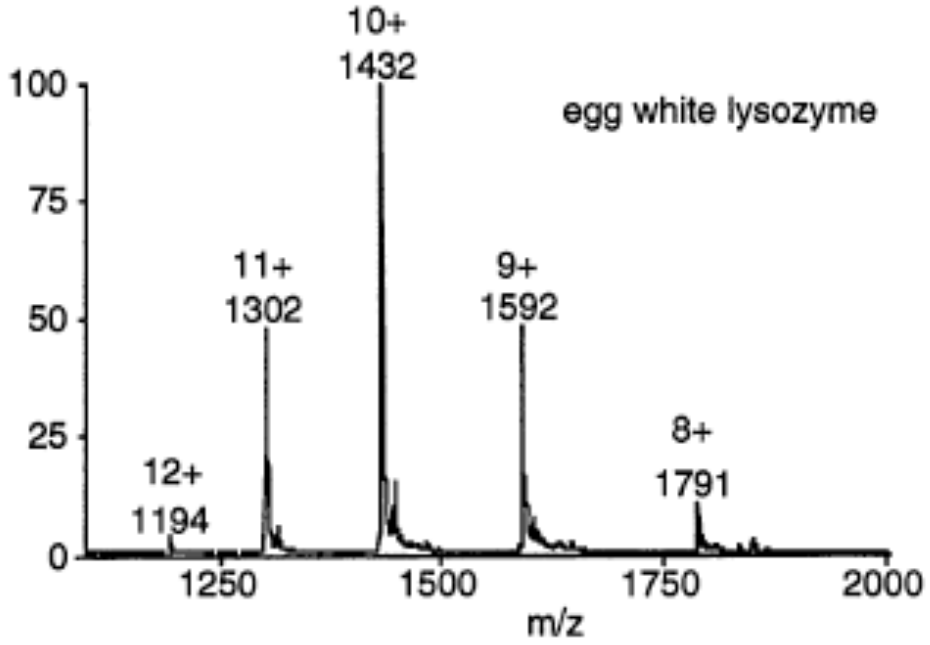

$$(m/z_1)_1\, z_1 = m$$

$$(m/z_2)_2\, z_2 = m$$

$$z_1 + 1 = z_2$$

$$1791\ z_1 = m$$

$$1592\ z_2 = m$$

$$1592\ z_2 = 1791\ z_1$$

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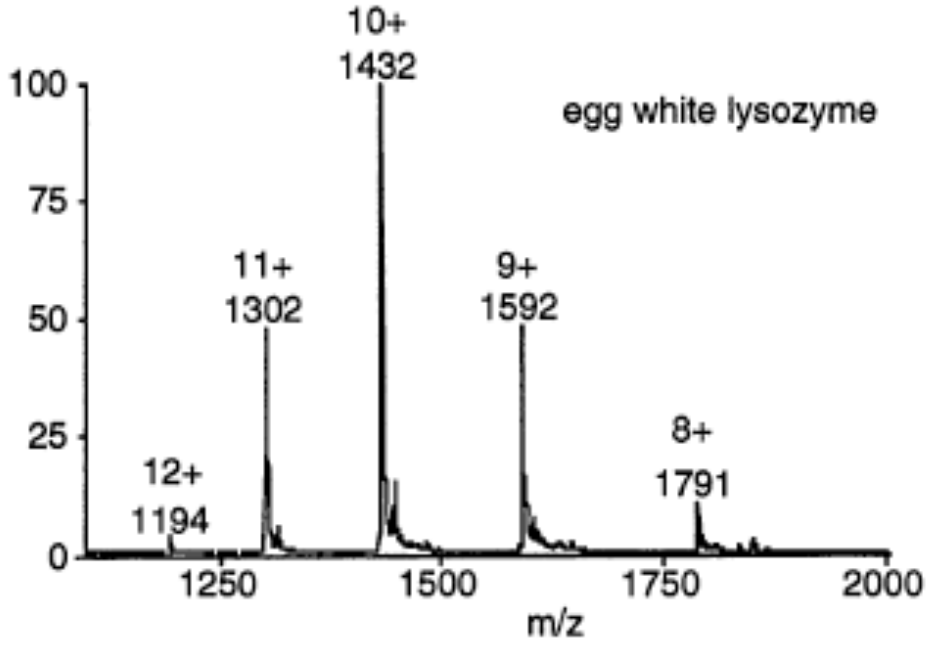

$$(m/z_1)_1 \, z_1 = m$$

$$(m/z_2)_2 \, z_2 = m$$

$$z_1 + 1 = z_2$$

$$1791 \; z_1 = m$$

$$1592 \; z_2 = m$$

$$1592 \, z_2 = 1791 \; z_1$$

$$1592 \, (z_1 + 1) = 1791 \; z_1$$

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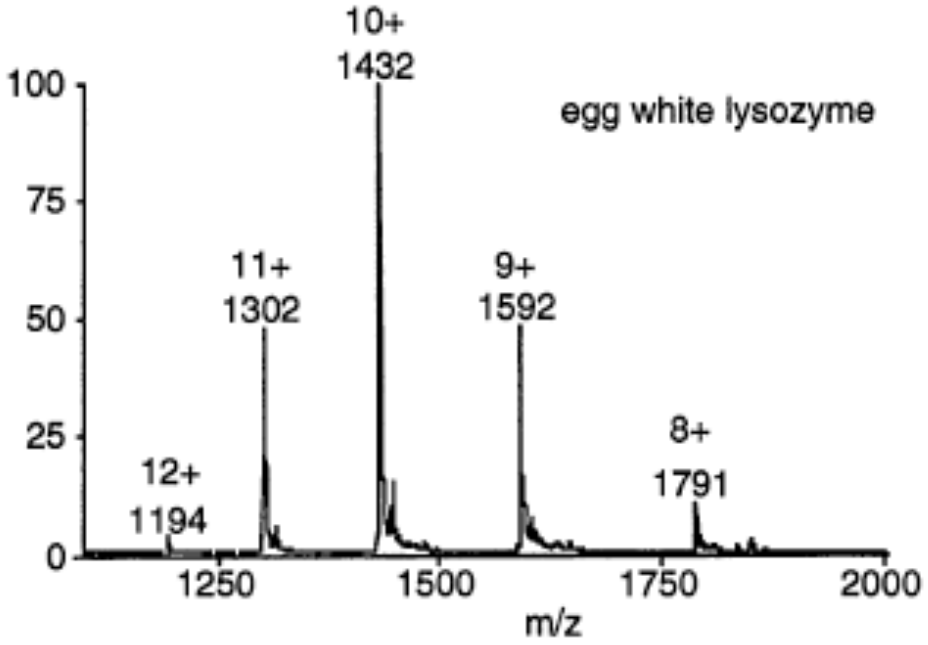

$$(m/z_1)_1\, z_1 = m$$

$$(m/z_2)_2\, z_2 = m$$

$$z_1 + 1 = z_2$$

$$1791\ z_1 = m$$

$$1592\ z_2 = m$$

$$1592\ z_2 = 1791\ z_1$$

$$1592\ (z_1 + 1) = 1791\ z_1$$

$$-1592\, z_1 + 1592\, z_1 + 1592 = 1791\, z_1 - 1592\, z_1$$

$$1592 = 199\, z_1$$

$$8 = z_1$$

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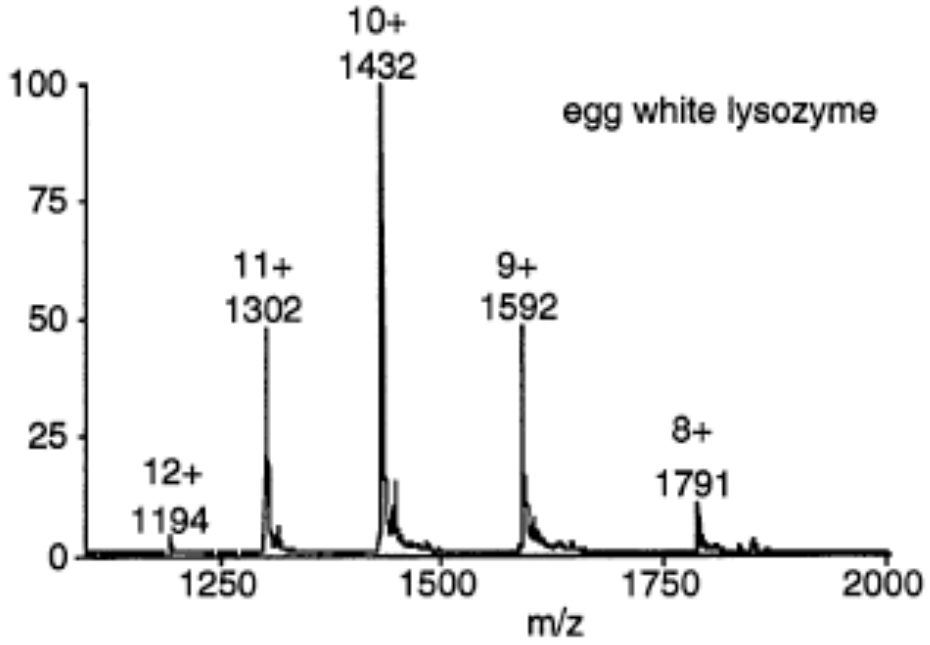

$$(m/z_1)_1 \, z_1 = m$$

$$(m/z_2)_2 \, z_2 = m$$

$$z_1 + 1 = z_2$$

$$1791 \; z_1 = m$$

$$1592 \; z_2 = m$$

$$1592 \, z_2 = 1791 \; z_1$$

$$1592 \, (z_1 + 1) = 1791 \; z_1$$

$$1592 \, z_1 + 1592 = 1791 \, z_1$$

$$1592 = 199 \, z_1$$

$$8 = z_1$$

$$1791 \, (8) = m$$

$$14{,}328 = m$$

Gary Siuzdak

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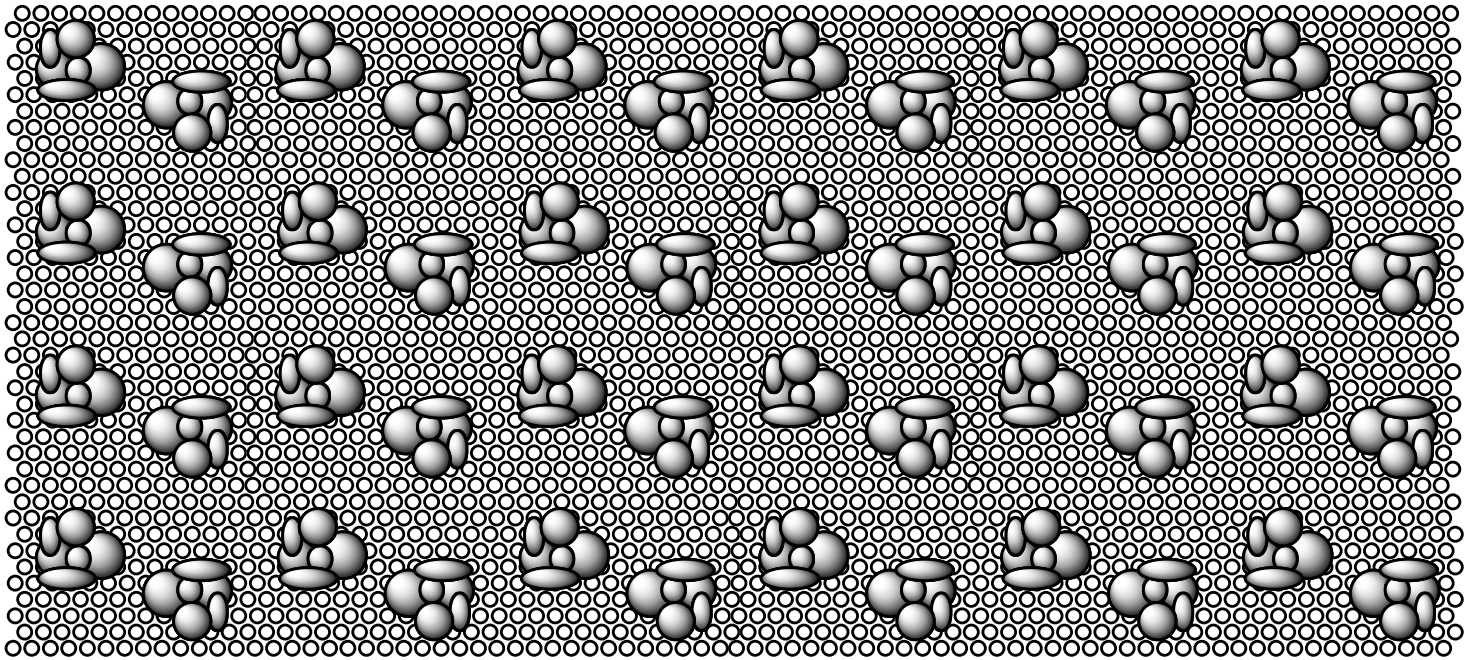
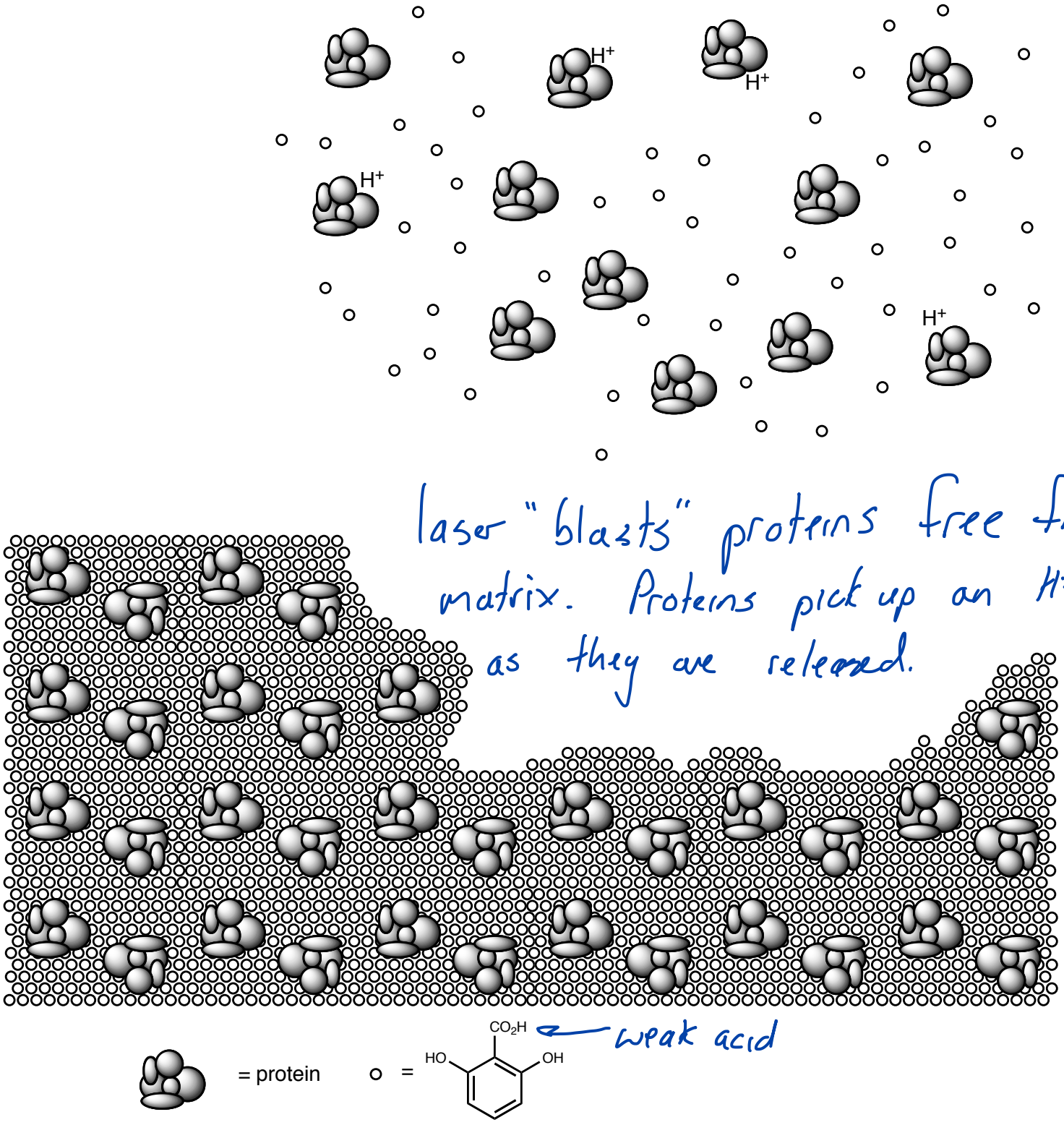
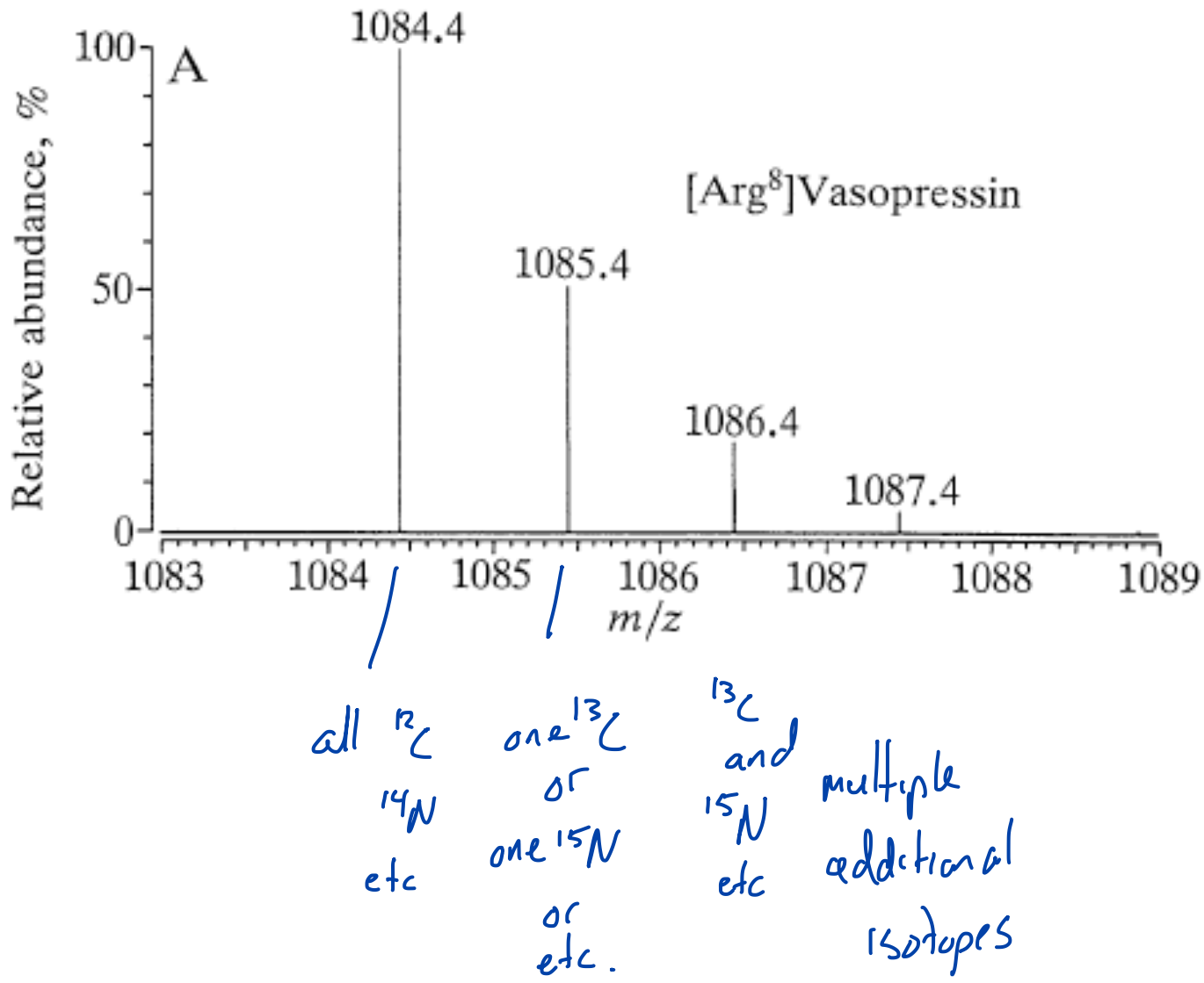
MALDI

Matrix Assisted Laser Desorption Ionization




H+
H+
H+
H+
laser "blasts" proteins free from matrix. Proteins pick up an H+ as they are released.
= protein
o =
CO2H
HO
OH
← weak acid



Robert T. McIver, Jr.; Yunzhi Li; Richard L. Hunter

Proceedings of the National Academy of Sciences of the United States of America, Vol. 91, No. 11. (May 24, 1994), pp. 4801-4805.

Protein Ladder sequencing

1. 5% phenylisocyanate 95% phenylisothiocyanate
2. Trifluoroacetic acid
3. repeat

[Glu1]fibrinopeptide

PC-Glu-Gly-Val-Asn-Asp-Asn-Glu-Glu-Gly-Phe-Phe-Ser-Ala-Arg

PC-Gly-Val-Asn-Asp-Asn-Glu-Glu-Gly-Phe-Phe-Ser-Ala-Arg

PC-Val-Asn-Asp-Asn-Glu-Glu-Gly-Phe-Phe-Ser-Ala-Arg

PC-Asn-Asp-Asn-Glu-Glu-Gly-Phe-Phe-Ser-Ala-Arg

PC-Asp-Asn-Glu-Glu-Gly-Phe-Phe-Ser-Ala-Arg

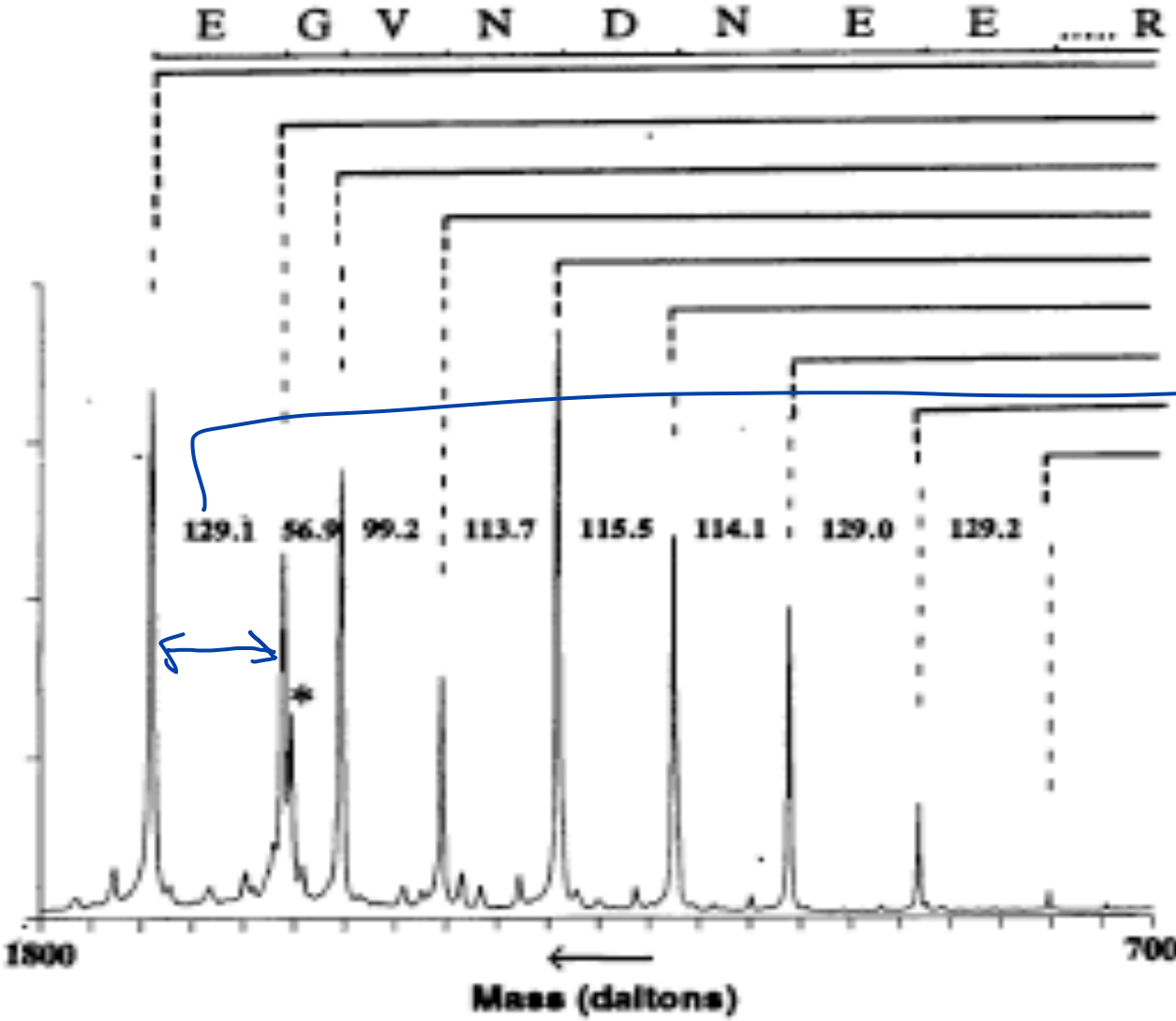
PC-Asn-Glu-Glu-Gly-Phe-Phe-Ser-Ala-Arg

- glu lose mass of 1 glutamate AA
- gly
- val

PC = phenylisocyante

glu gly val asn asp asn glu glu arginine

It's the difference that matters!



this difference tell us the mass of the amino acid that was lost and we know the masses of the amino acids so we can determine which ones are lost thus the sequence

Brian T. Chait; Rong Wang; Ronald C. Beavis; Stephen B. H. Kent

Science, New Series, Vol. 262, No. 5130, Genome Issue. (Oct. 1, 1993), pp. 89-92.