

# Lecture 2: Basic MALDI and Electrospray Theory

## 1. Desorption/Ionization and its Mechanisms

- a. chemical ionization processes
- b. preformed ions
- c. matching the charge sign

## 2. Matrix-Assisted Laser Desorption/Ionization (MALDI)

- a. basic mechanisms
- b. pulsed lasers and their wavelengths
- c. laser optics and instrumentation
- d. matrices
- e. mass spectra of proteins
- f. MALDI discourages the formation of multiply-charged ions; survivor theory
- g. fragmentation nomenclature for peptides
- h. masses of the 20 common amino acid “residues”

## 3. Electrospray Ionization (ESI)

- a. basic instrumentation and theory of operation
- b. mass spectra of proteins

### *3. Electrospray Ionization (ESI) continued.*

- c. ionization and fragmentation of carbohydrates
- d. fragmentation nomenclature for carbohydrates
- e. masses of the common carbohydrate residues
- f. N-linked and O-linked glycosylation
- g. examples of ESI mass spectra of carbohydrates

### *4. MALDI and Electrospray: when to use each.*

### *5. MS and MS/MS: observing fragmentation.*

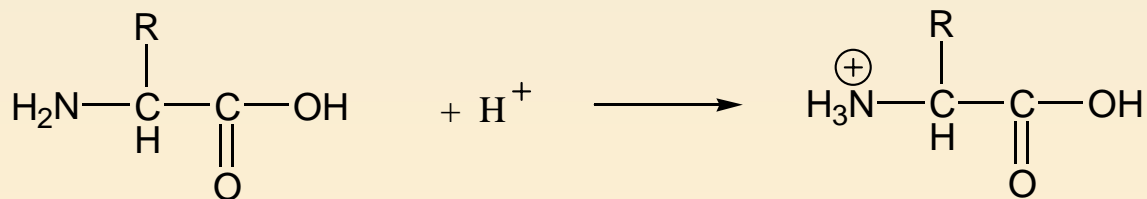
# 1. Desorption/Ionization and its Mechanisms

Both *electron impact* and *chemical ionization* required that a sample molecule be first volatilized and then ionized (as a separate event) in the gas phase. Thus, these methods are limited to relatively small volatile molecules.

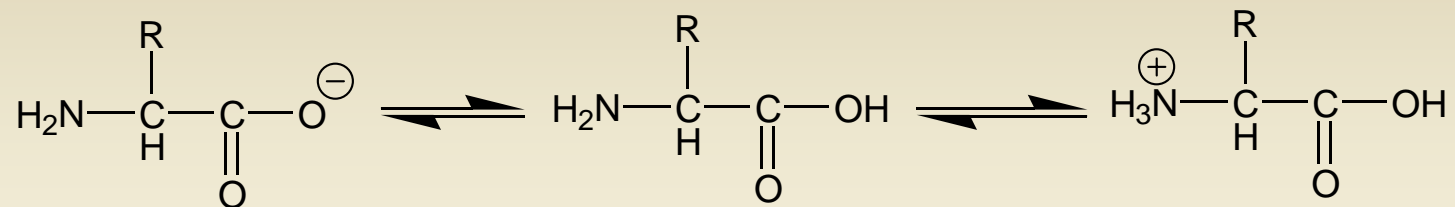
In *desorption/ionization* methods the movement of molecules from the condensed (either solid or solution) phase to the gas phase and ionization are (operationally) indistinguishable events. On a microscopic scale, ions may pre-exist in the condensed phase prior to desorption (*preformed ions*) or be ionized immediately upon entering the gas phase (*chemical ionization*).

## a. chemical ionization processes

Amino acids or peptides entering the gas phase would encounter an ample number of proton donors originating from the matrix (MALDI) or solution (ESI) to be chemically ionized:

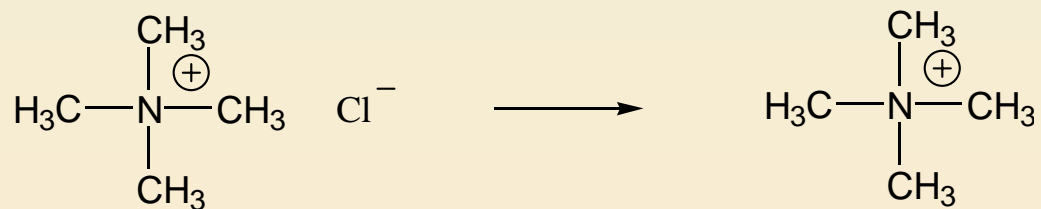


However, amino acids and peptides exist as zwitterions in solution and, since either positive (protonated) and negative (deprotonated) species can be recorded by a mass spectrometer, one may be simply desorbing ions already formed:



### *b. preformed ions*

Quaternary ammonium ions are clearly preformed. Basic residues (such as arginine and lysine) in peptides are also likely to result in direct desorption of preformed ions.



Most likely, both preformed and chemical ionization processes occur, as well as chemical reactions in the condensed phase/gas phase interface (selvedge).

### *c. matching the charge sign*

Mass spectrometers can be made to record either positive or negative ions by making the source voltage positive or negative, respectively. The recording mode should match the charge sign of the analyte:

**peptides** are generally best analyzed as positive ions, particularly those containing arginine or lysine; because they are zwitterions they are best analyzed at low pH

**phosphorylated** or **sulfated peptides** may be analyzed in the -ve ion mode

**oligonucleotides**, which contain a large number of anionic phosphate groups may nonetheless be best analyzed as positive ions at low pH, in order to promote the formation of singly-charged species

**fatty acids** are best analyzed as fatty acyl anions

**carbohydrates** are more easily protonated than deprotonated, although they may be observed as their oxyanions

In general ions are all even-electron and follow the fragmentation patterns typical of chemically ionized species

## 2. Matrix-Assisted Laser Desorption/Ionization

The technique which we now know as *matrix-assisted laser desorption/ionization* (MALDI) was developed simultaneously in two laboratories in 1987. The first report of high mass ions (above  $m/z$  10,000) was a paper presented by Koichi Tanaka of the Shimadzu Corporation (Kyoto, Japan) at the *Second Japan-China Joint Symposium on Mass Spectrometry*, held September 15-18, 1987 in Takarazuka, Japan. Using a pulsed  $N_2$  laser (337 nm) and a time-of-flight mass spectrometer equipped with a coaxial reflectron, they recorded molecular ions at  $m/z$  34,529 from carboxypeptidase-A dissolved in a slurry of glycerol and an ultra-fine metal powder. In addition, they reported a mass spectrum of lysozyme (MW 14,307) containing multimeric ions up to the pentamer recorded at  $m/z$  71,736. At the same time, Michael Karas and Franz Hillenkamp from the University of Muenster (Germany) had developed a matrix-assisted technique using a frequency-quadrupled (266 nm) Q-switched Nd:YAG laser to desorb intact molecular ions from proteins dissolved in matrix solution containing nicotinic acid. Their first high mass results were reported at the *International Mass Spectrometry Conference* (IMSC) in Bordeaux, France in August 1988, and included molecular ions for bovine serum albumin observed in their mass spectrum at  $m/z$  66,750. Results from both of these groups were first published in 1988, followed by a number of other reports by Hillenkamp and Karas for proteins with molecular weights in excess of 100 kDa.

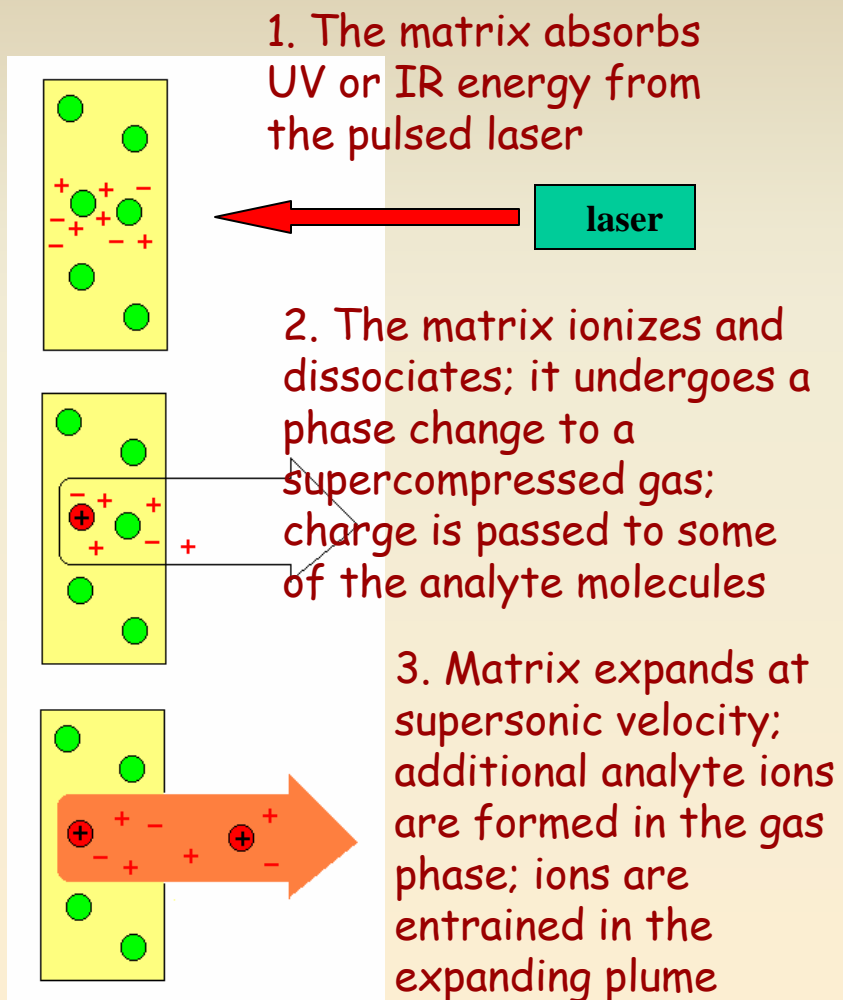
Tanaka, K.; Waki, H.; Ido, Y.; Akita, S.; Yoshida, Y; Yoshida, T., *Rapid Commun. Mass Spectrom.* **2** (1988) 151-153.

Karas, M.; Hillenkamp, F., *Anal. Chem.* **60** (1988) 2299-2301.

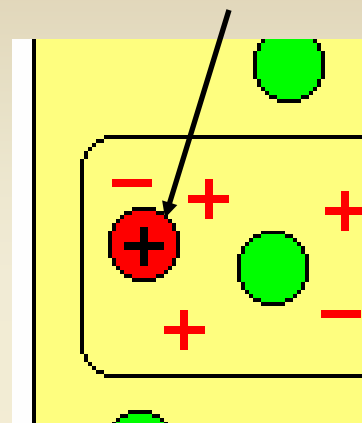
Cotter, R.J., *Time-of-Flight Mass Spectrometry: Instrumentation and Applications in Biological Research*, American Chemical Society, Washington, DC (1997) p. 128.

## 2. Matrix-Assisted Laser Desorption/Ionization

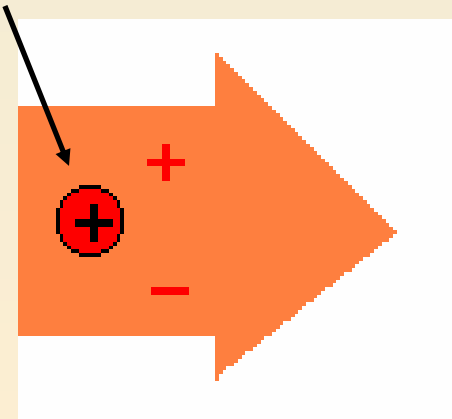
### a. basic mechanisms



Ions may be preformed and released by the expanding plume



And, the matrix supplies proton donors for gas-phase chemical ionization



## b. pulsed lasers and their wavelengths

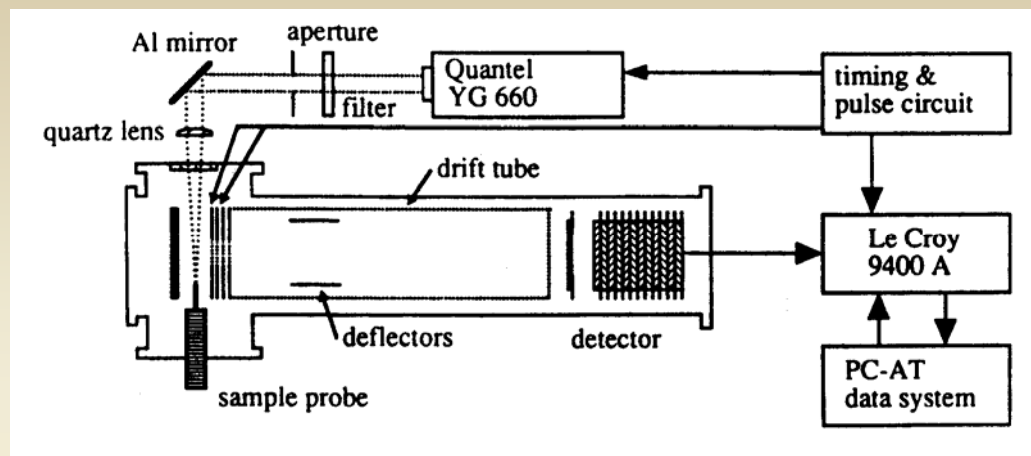
IR	10.6 microns	CO <sub>2</sub>	-
IR	2.94 microns	Er:YAG	- recently suggest for oligos (4)
IR	1.06 microns	Nd:YAG	-
VIS	532 nm	Nd:YAG (2 <sup>nd</sup> harmonic)	-
UV	355 nm	Nd:YAG (3 <sup>rd</sup> harmonic)	- common prior to use of N <sub>2</sub> (2)
UV	337 nm	N <sub>2</sub>	- most popular MALDI laser (3)
UV	266 nm	Nd:YAG (4 <sup>th</sup> harmonic)	- original MALDI laser (1)

AP MALDI  
with aqueous  
matrix

**New!**

- (1) Karas, M.; Bachmann, D.; Bahr, U.; Hillenkamp, F., *Int. J. Mass Spectrom. Ion Processes* 78 (1987) 53-68
- (2) Beavis, R.C.; Chait, B.T., *Rapid Commun. Mass Spectrom.* 3 (1989) 432-435.
- (3) Chevrier, M.; Cotter, R.J., *Rapid Commun. Mass Spectrom.* 5 (1991) 611-617.
- (4) Overberg, A.; Karas, M.; Bahr, U.; Kaufmann, R.; Hillenkamp, F., *Rapid Commun. Mass Spectrom.* 4 (1990) 293.

### c. laser optics and instrumentation



Laser optics usually includes:

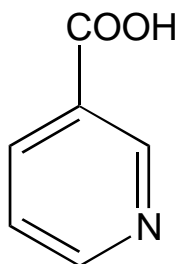
- variable attenuator, generally rotating optical density filter
- mirror
- lens (material dependent on wavelength) with 1-5 inch focal length
- window to vacuum chamber (material dependent on wavelength)

Laser pulse width: 300 ps to 3 nanoseconds

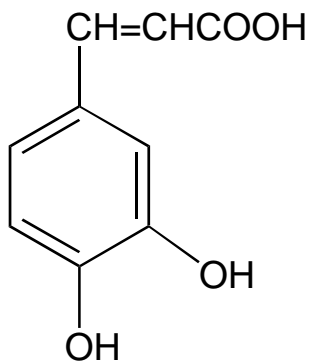
Laser energy: 10 microjoules to 10 millijoules (depending on pulse width)

Laser power density:  $10^6 - 10^7$  watts/cm<sup>2</sup>

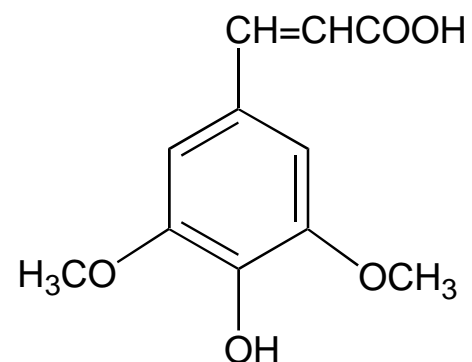
## d. matrices



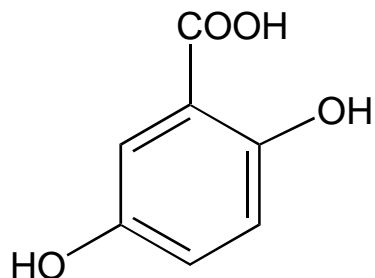
nicotinic acid (NA)



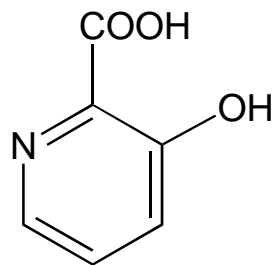
caffeic acid (CA)  
3,4-dihydroxycinnamic acid



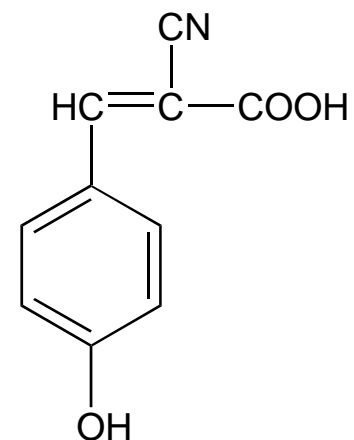
sinapinic acid (SA)  
3,5-dimethoxy-4-hydroxycinnamic acid



gentisic acid (DHBA)  
2,5-dihydroxybenzoic acid



3-hydroxypicolinic acid (HPA)



$\alpha$ -cyano-4-hydroxycinnamic acid  
(CHCA)

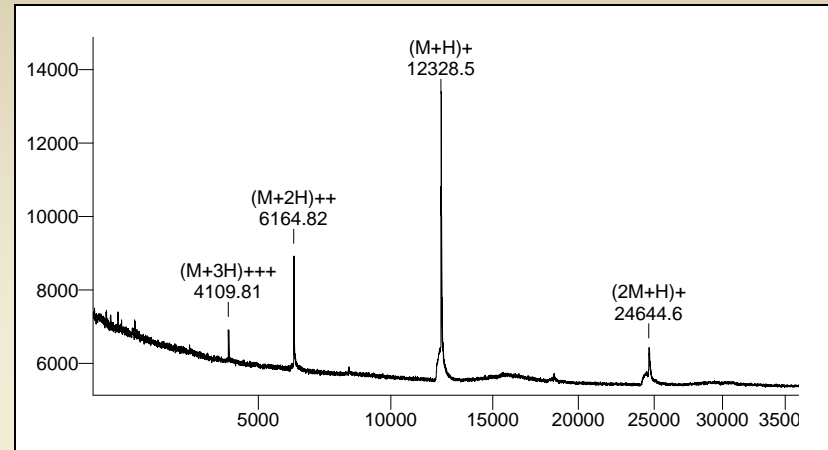
### *e. mass spectra of proteins*

MALDI mass spectra of proteins obtained on a 4-inch *endcap* time-of-flight mass spectrometer. Mass resolution  $\approx 500$

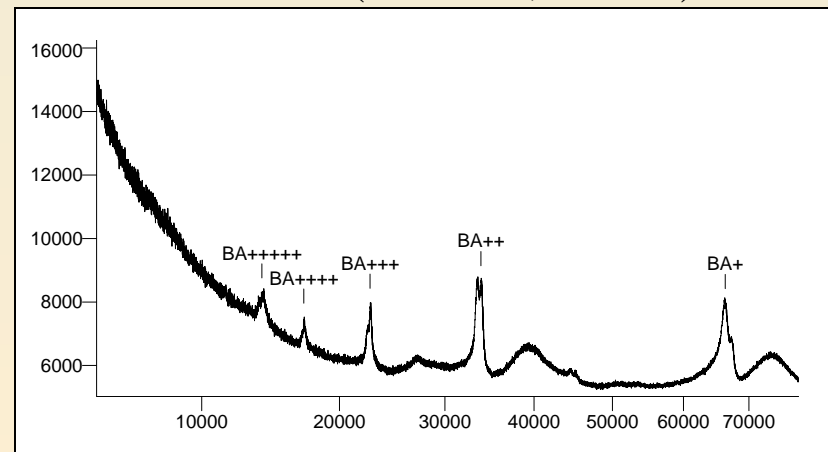
### *f. MALDI discourages the formation of multiply-charged ions; survivor theory*

- multiply-charged ions less stable in gas phase, though charge-state rises with increasing mass
- multiply-charged ions less easily desorbed from the surface because of electrostatic attraction
- charge-state of desorbed ions are reduced by collision in expanding plume. We see the surviving ions.

### IR MALDI mass spectrum of cytochrome C (MW=12,327 Da)



### UV MALDI mass spectrum of bovine serum albumin (MW=66,429 Da)

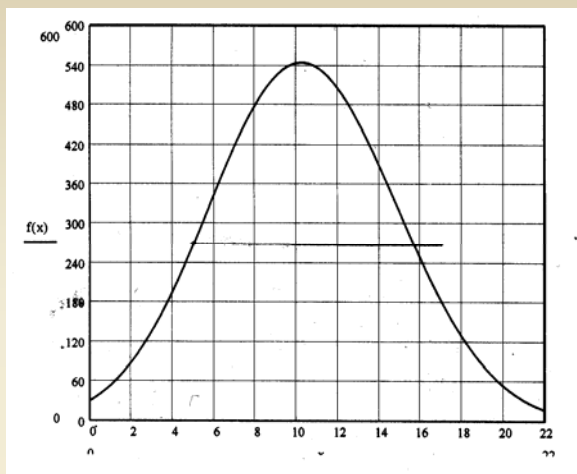


## e. mass spectra of proteins

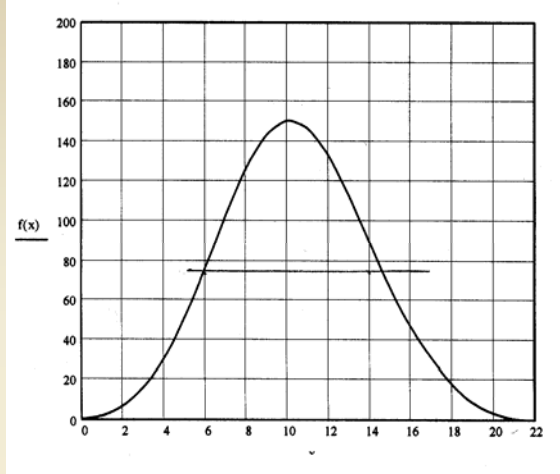
How much resolution do you need?

Theoretical peak shapes for a peptide with an elemental composition of  $C_{900}H_{1323}O_{294}N_{247}S_6$  with a molecular weight = 20,488.5

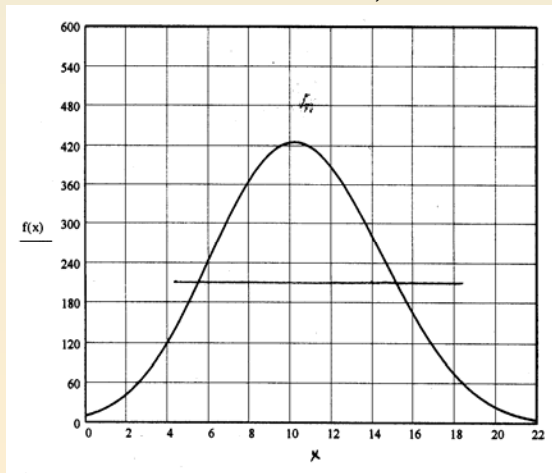
Resolution = 500



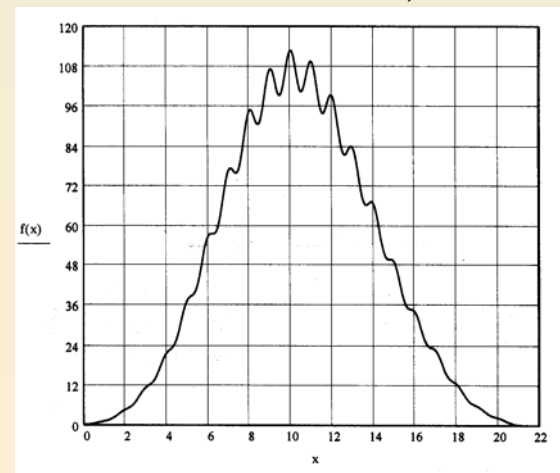
Resolution = 10,000



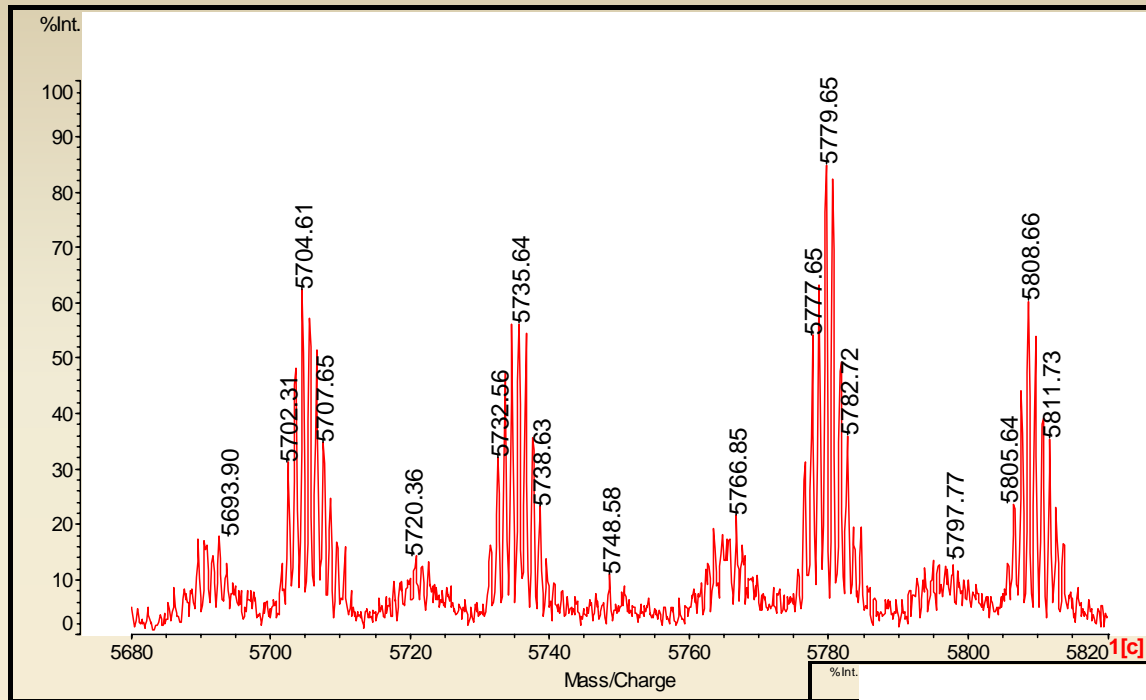
Resolution = 1,000



Resolution = 20,000

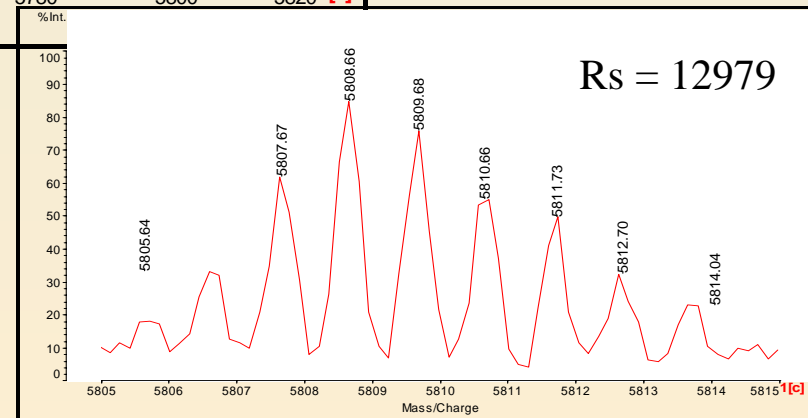


## e. mass spectra of proteins



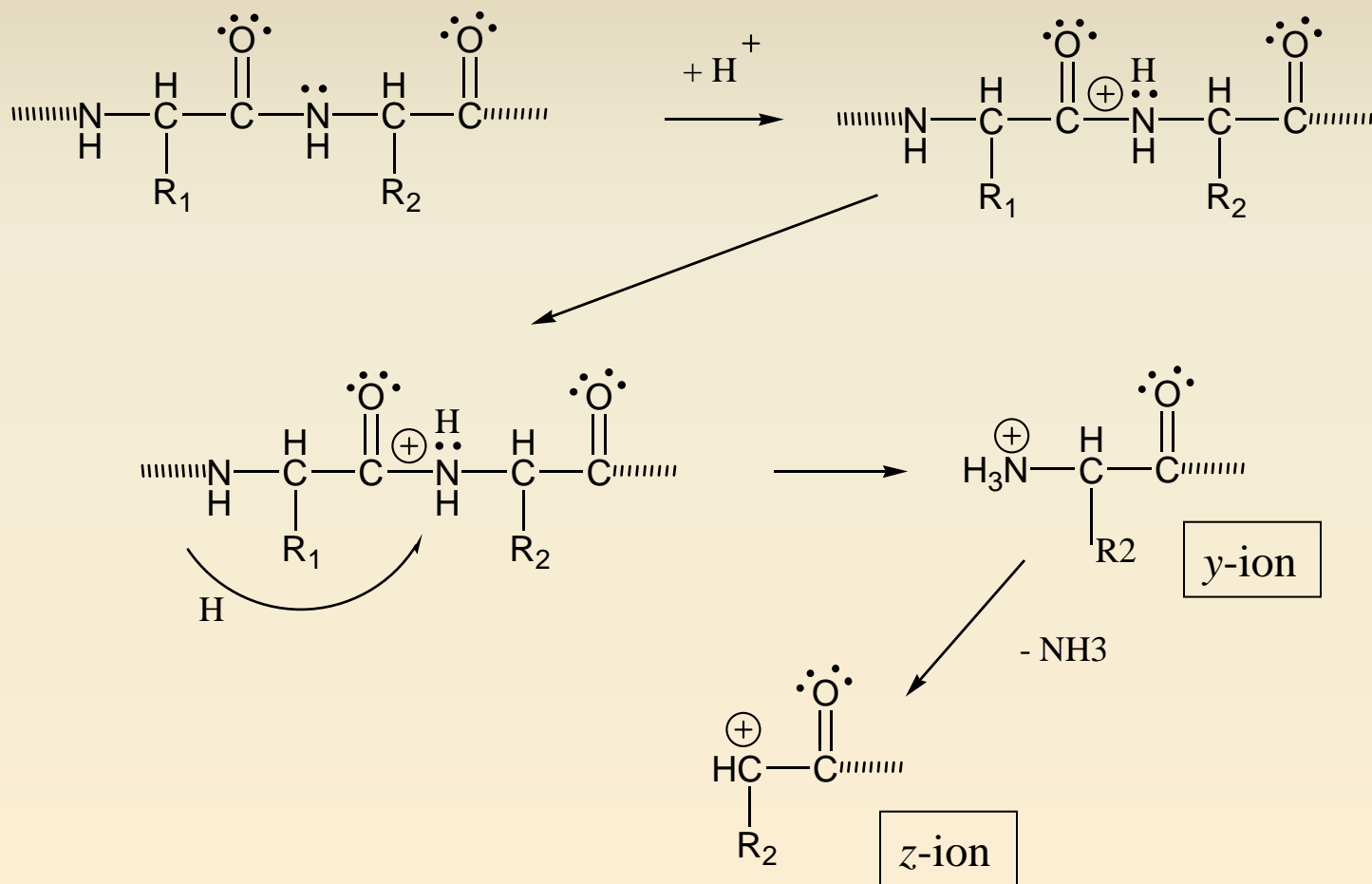
Mixture of sheep, bovine, porcine and human insulins

High resolution mass spectrum obtained on a Kratos AXIMA time-of-flight mass spectrometer with curved-field reflectron, with expansion of molecular ion region for human insulin.



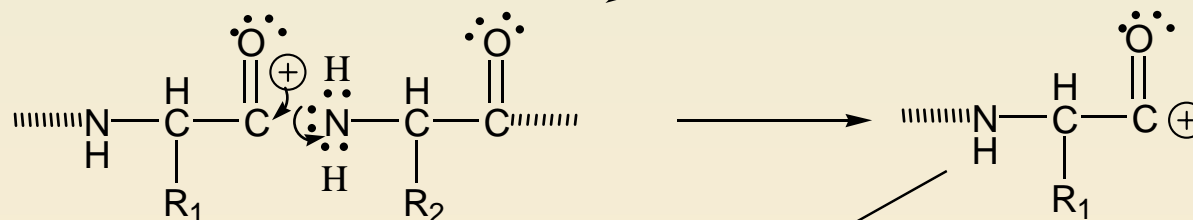
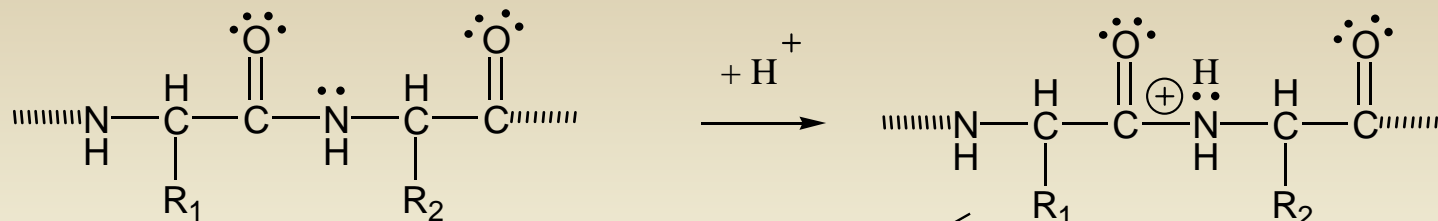
## g. fragmentation nomenclature for peptides

C-terminal ions: charge is retained on the carboxy terminal fragment

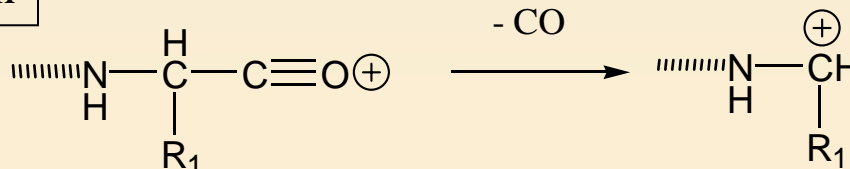


## g. fragmentation nomenclature for peptides

N-terminal ions: charge is retained on the amino terminal fragment



**b-ion**

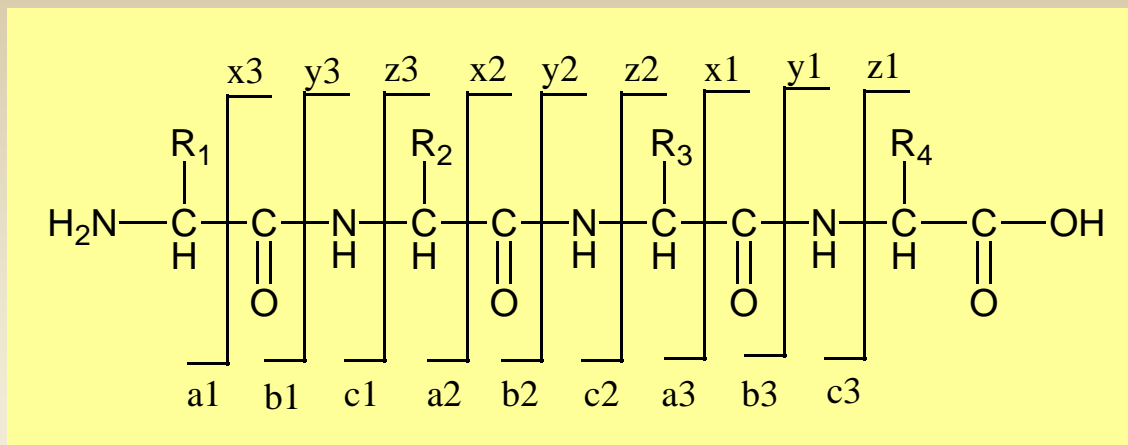


**a-ion**

### *h. masses of the 20 common amino acid “residues”*

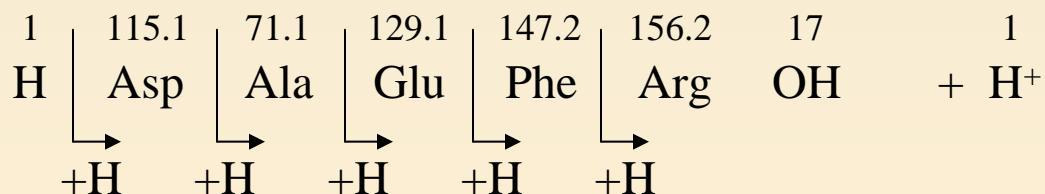
<i>Amino acid</i>	<i>Three letter code</i>	<i>Single letter code</i>	<i>Nominal mass</i>	<i>Monoisotopic mass</i>	<i>Average mass</i>
<i>Alanine</i>	Ala	A	71	71.037	71.079
<i>Arginine</i>	Arg	R	156	156.101	156.188
<i>Asparagine</i>	Asn	N	114	114.043	114.104
<i>Aspartic acid</i>	Asp	D	115	115.027	115.089
<i>Cysteine</i>	Cys	C	103	103.009	103.143
<i>Glutamic acid</i>	Glu	E	129	129.043	129.116
<i>Glutamine</i>	Gln	Q	128	128.059	128.131
<i>Glycine</i>	Gly	G	57	57.021	57.052
<i>Histidine</i>	His	H	137	137.059	137.141
<i>Isoleucine</i>	Ile	I	113	113.084	113.160
<i>Leucine</i>	Leu	L	113	113.084	113.160
<i>Lysine</i>	Lys	K	128	128.095	128.175
<i>Methionine</i>	Met	M	131	131.040	131.197
<i>Phenylalanine</i>	Phe	F	147	147.068	147.177
<i>Proline</i>	Pro	P	97	97.053	97.117
<i>Serine</i>	Ser	S	87	87.032	87.078
<i>Threonine</i>	Thr	T	101	101.048	101.105
<i>Tryptophan</i>	Trp	W	186	186.079	186.214
<i>Tyrosine</i>	Tyr	Y	163	163.063	163.176
<i>Valine</i>	Val	V	99	99.068	99.133

## g. fragmentation nomenclature for peptides



Biemann, K., *Biomed. Mass Spectrom.* 16 (1988) 99; Biemann, K. in *Methods in Enzymology 193: Mass Spectrometry*, McCloskey, J.A., Ed.; Academic Press, San Diego (1990) pp. 886-887.

**y-ions** for the peptide *DAEFR* are calculated by summing the masses to the right of each cleavage point including the mass of a hydrogen ion:



$$115.1 + 1 + 71.1 + 129.1 + 147.2 + 156.2 + 17 + 1 \longrightarrow m/z = 637.7$$

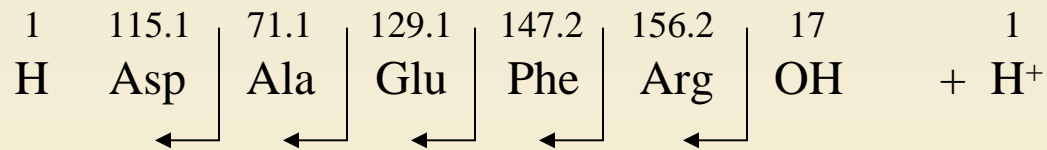
$$71.1 + 1 + 129.1 + 147.2 + 156.2 + 17 + 1 \longrightarrow m/z = 522.6$$

$$129.1 + 1 + 147.2 + 156.2 + 17 + 1 \longrightarrow m/z = 451.5$$

$$147.2 + 1 + 156.2 + 17 + 1 \longrightarrow m/z = 322.4$$

$$156.2 + 1 + 17 + 1 \longrightarrow m/z = 175.2$$

**b-ions** for the peptide *DAEFR* are calculated by summing the masses to the left of each cleavage point:



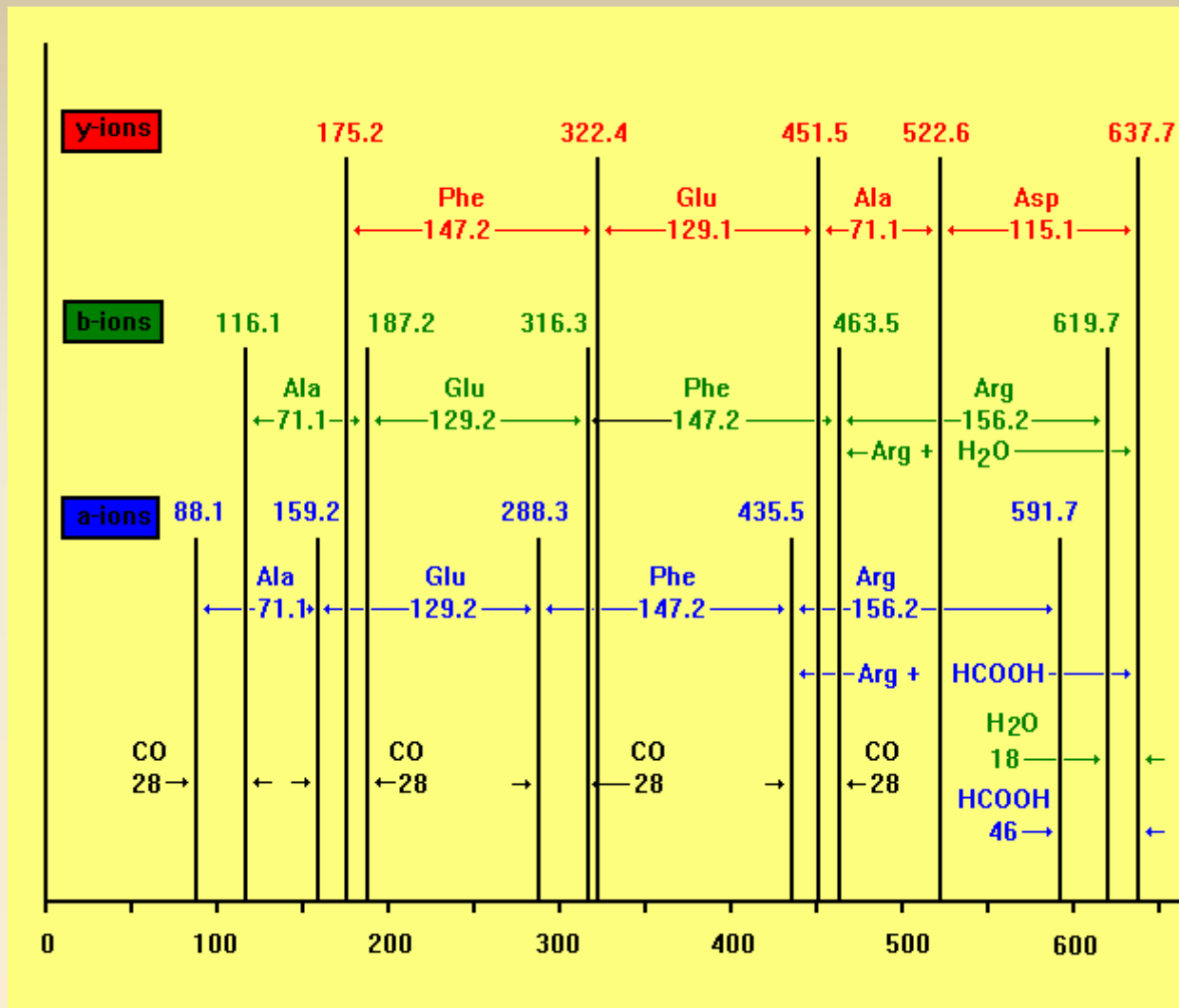
$$1 + 115.1 + 71.1 + 129.1 + 147.2 + 156.2 \longrightarrow m/z = 619.7$$

$$1 + 115.1 + 71.1 + 129.1 + 147.2 \longrightarrow m/z = 463.5$$

$$1 + 115.1 + 71.1 + 129.1 \longrightarrow m/z = 316.3$$

$$1 + 115.1 + 71.1 \longrightarrow m/z = 187.2$$

$$1 + 115.1 \longrightarrow m/z = 116.1$$

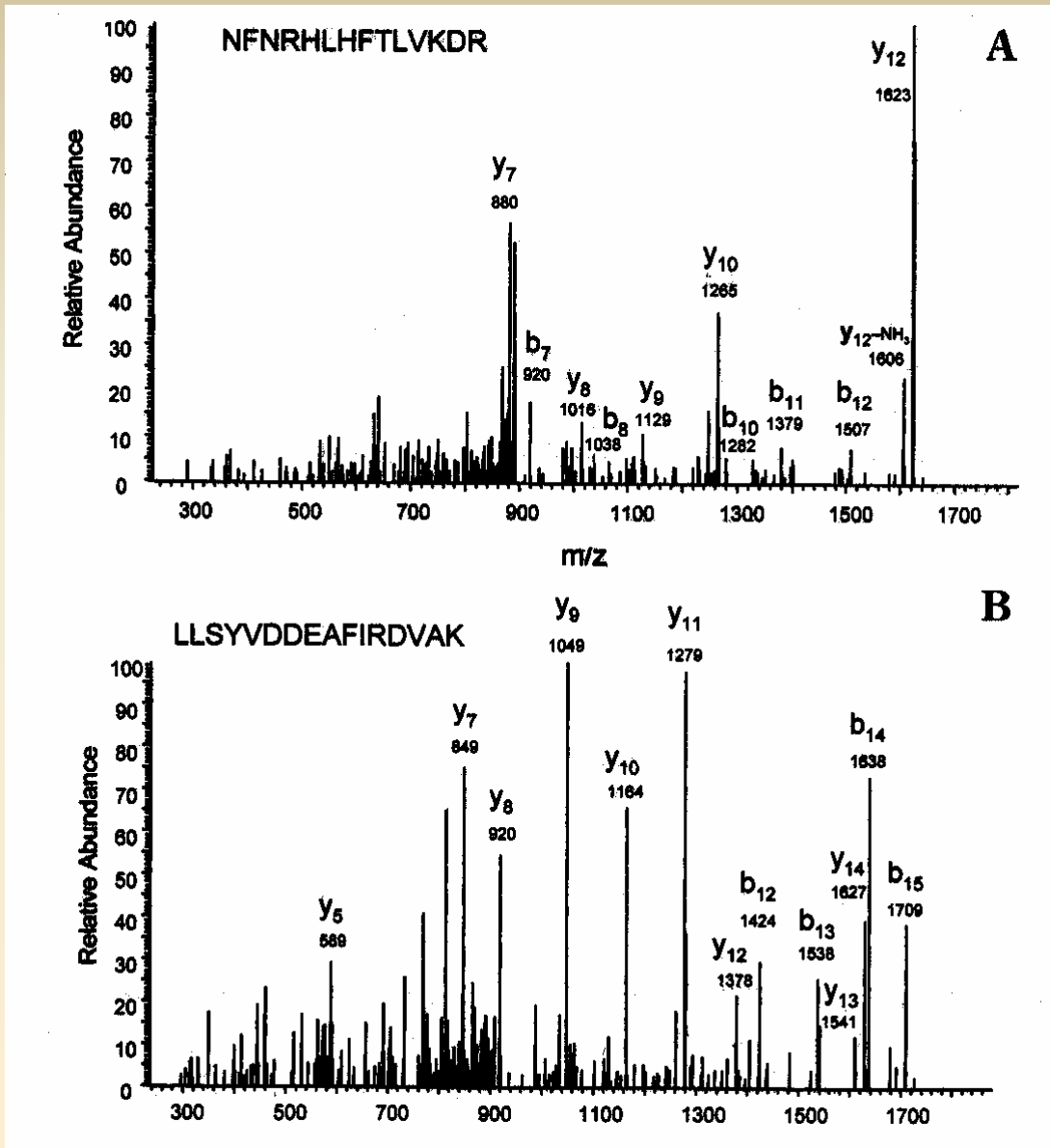


**a-ions** for the peptide *DAEFR* are **b-28**:

CID mass spectra of peptides:  
NFNRHLHFTLVKDR and  
LLSYDDEAFIRDVAK

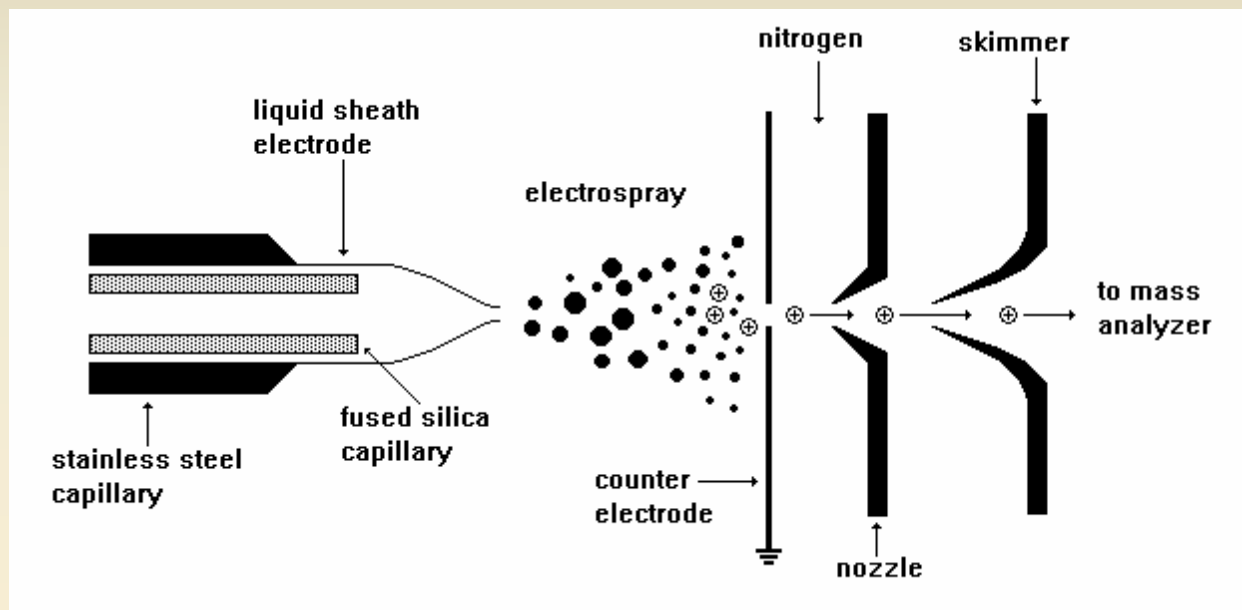
Timperman, A.T.; Aebersold, R., *Anal. Chem.* **72** (2000) 4115-4121.

*Homework problem:  
calculate the masses of b  
and y ions for the two  
peptides shown and  
compare with results  
obtained in their mass  
spectra.*



### 3. Electrospray Ionization (ESI)

#### a. basic instrumentation and theory of operation



Introduced by John Fenn:

Yamashita, M.; Fenn, J.B., *J. Phys. Chem.* 88 (1984) 4451.

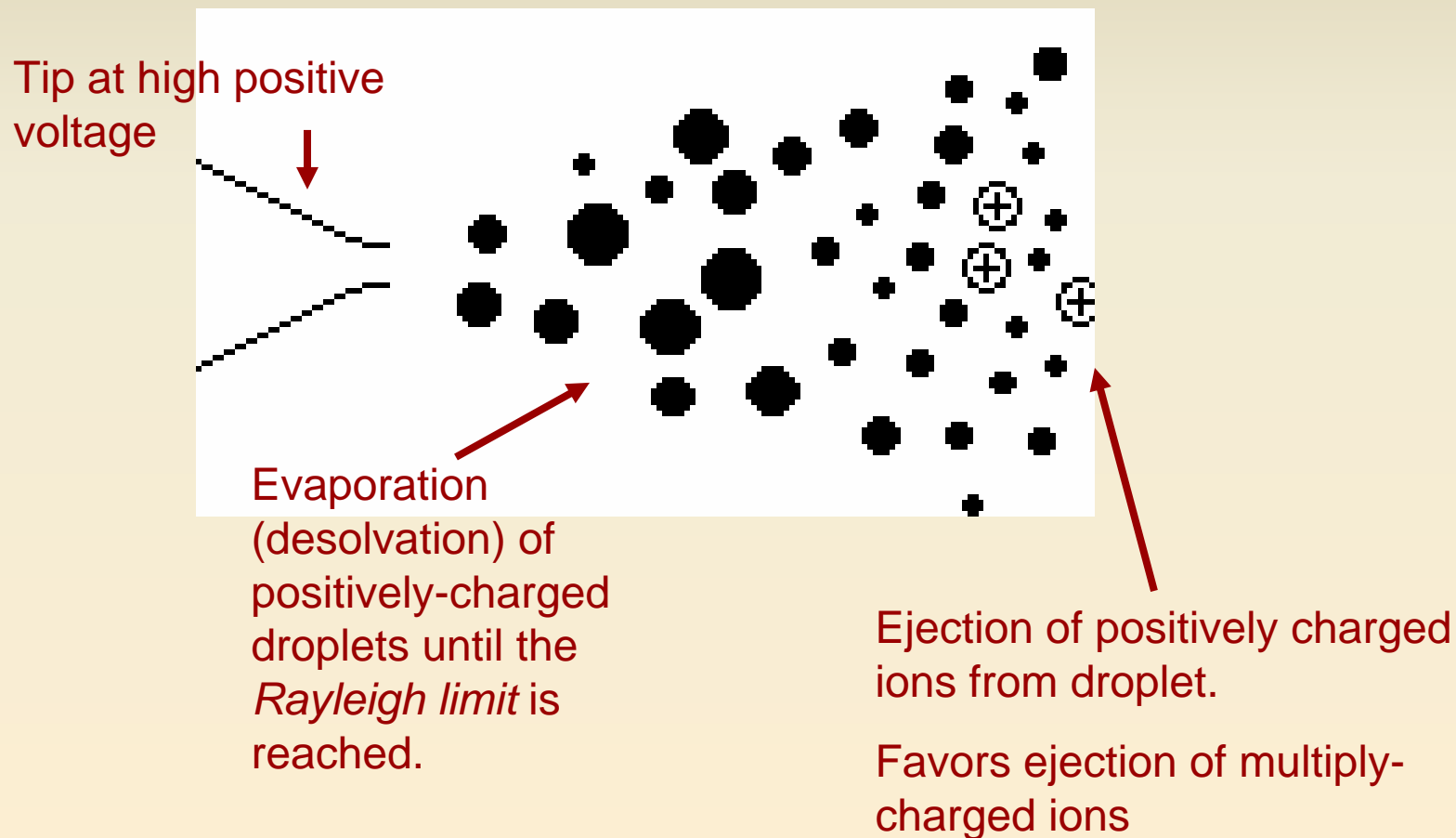
Whitehouse, C.M.; Dreyer, R.N.; Yamashita, M.; Fenn, J.B., *Anal. Chem.* 57 (1985) 675.

Fenn, J.B.; Mann, M.; Meng, C.K.; Wong, S.F.; Whitehouse, C.M., *Science* 246 (1989) 64.

Based on an ion evaporation model of Iribarne and Thomson:

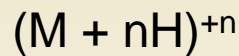
Iribarne, J.V.; Thomson, B.A., *J. Chem. Phys.* 64 (1976) 2287.

Thomson, B.A.; Iribarne, J.V., *J. Chem. Phys.* 71 (1979) 4451.



## b. mass spectra of proteins

Multiply-charged ions are favored in ESI mass spectra, with ions having the formula:



Average  $m/z$  are around 1000, which is compatible with quadrupole mass spectrometers.

Edmonds, C.G.; Smith, R.G. in *Methods in Enzymology* 193: *Mass Spectrometry*, McCloskey, J.A., Ed.; Academic Press, San Diego (1990) pp. 412-431.

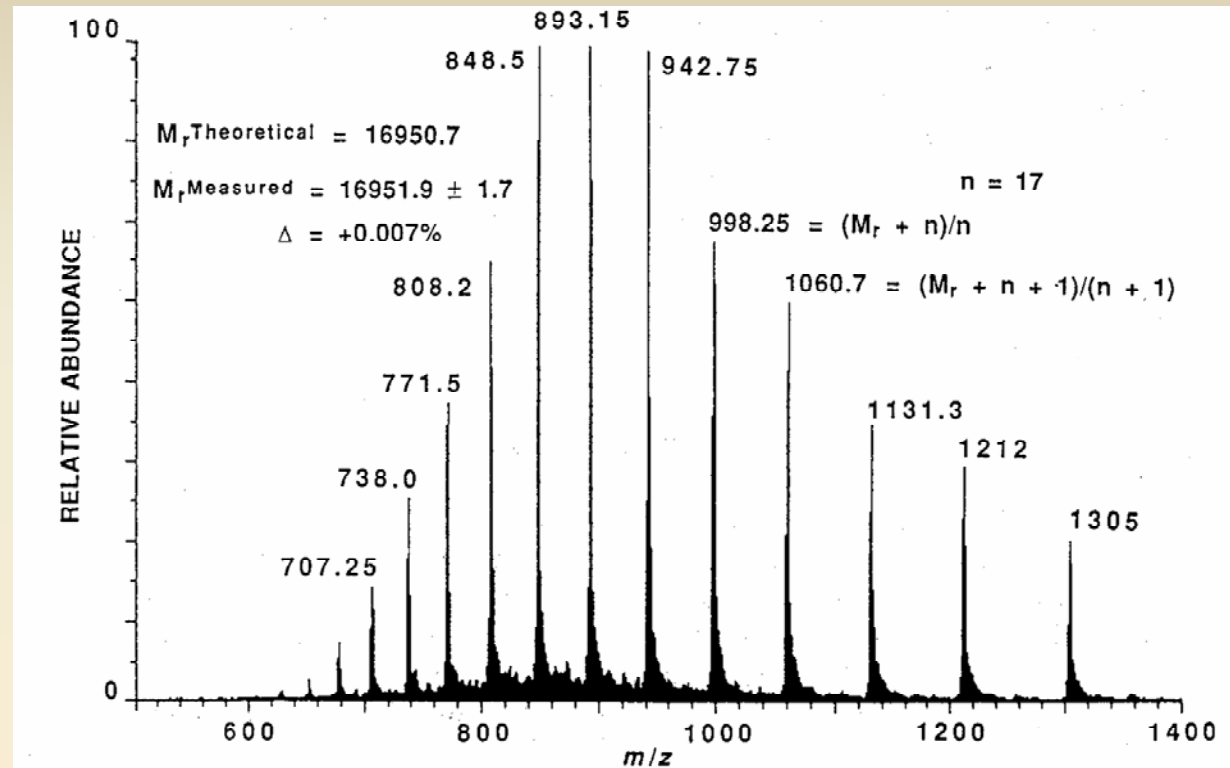
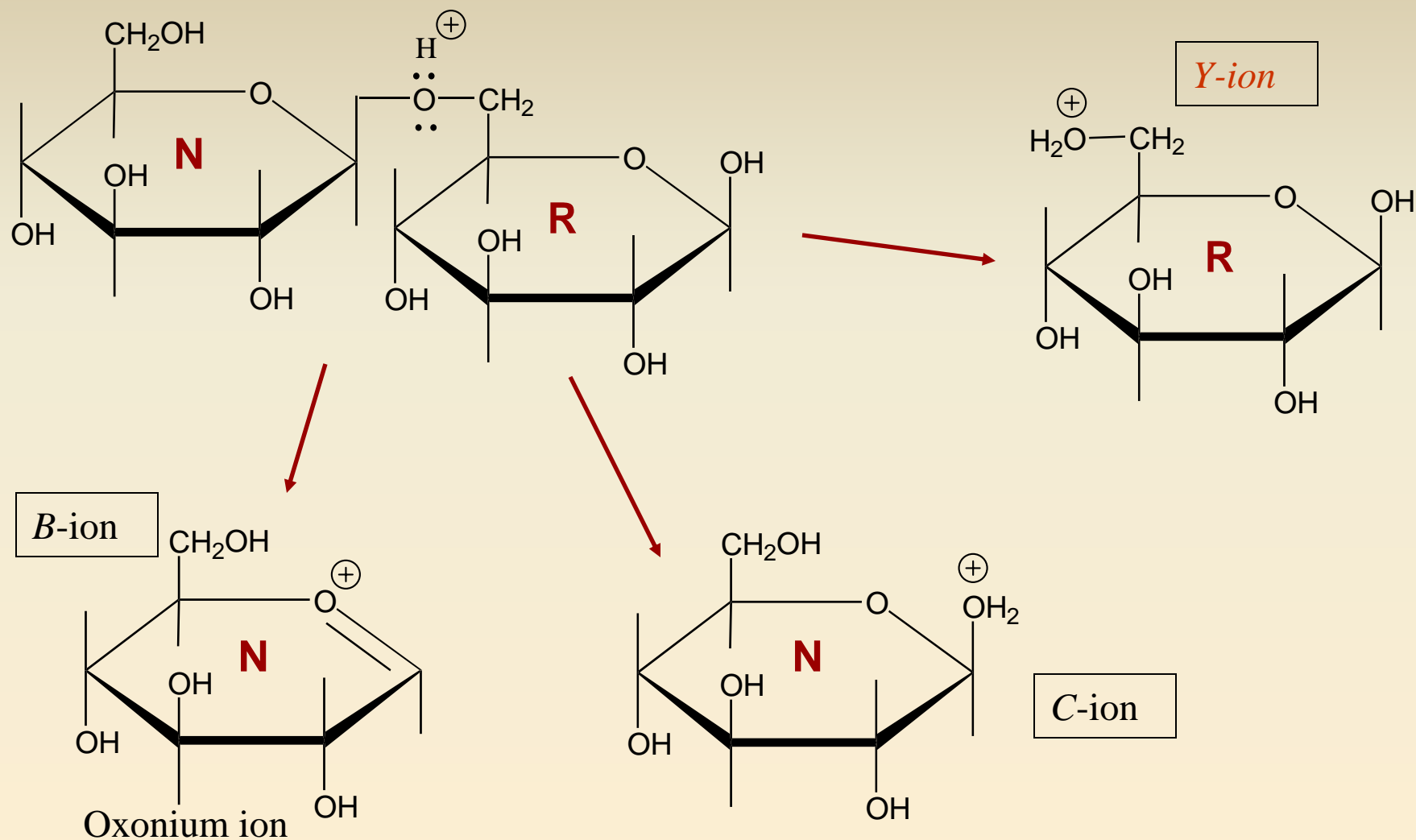
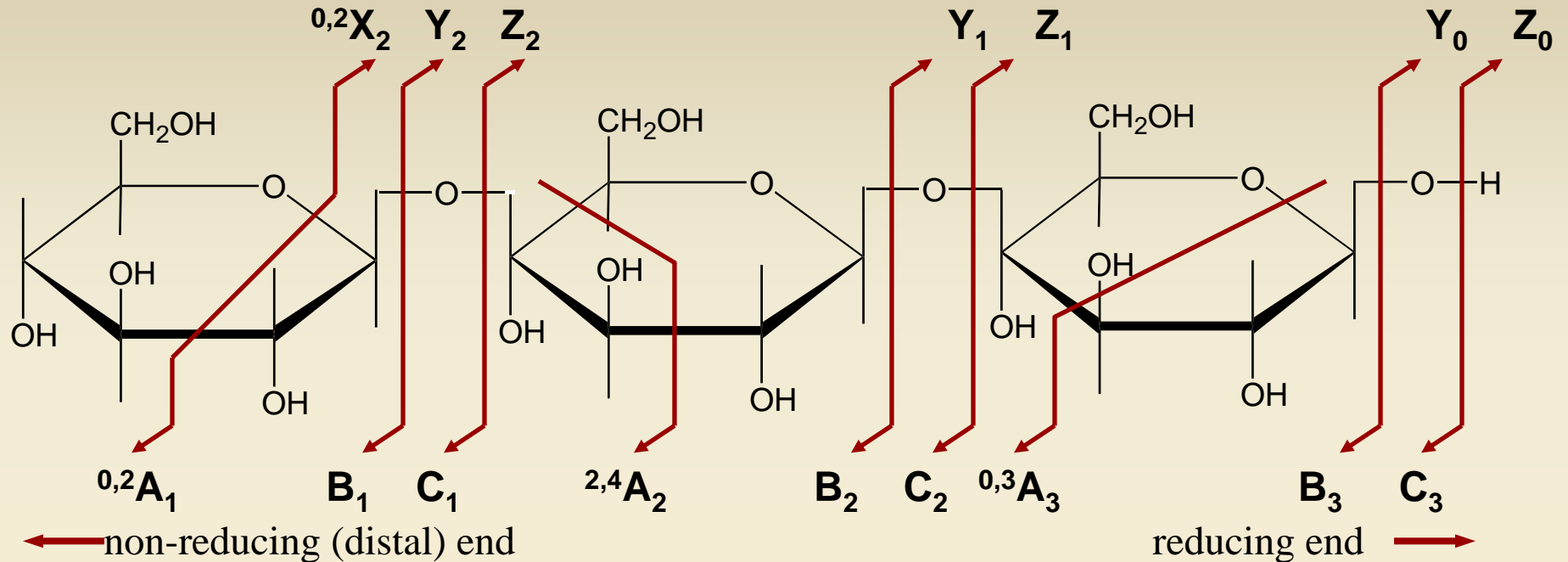


FIG. 3. ESI mass spectrum of equine myoglobin. Peaks  $m/z$  707.25 through  $m/z$  1305 are centroid values for these multiply protonated (24+ through 13+) ions. The simultaneous relation of one pair,  $m/z$  998.25 and  $m/z$  1060.7, is illustrated where the integer value of  $n$  is 17. Calculation of  $M_r$ , as discussed in the text.

### c. ionization and fragmentation of carbohydrates



### d. fragmentation nomenclature for carbohydrates



Nomenclature suggested by: Domon, B.; Costello, C., *Glyconj. J.* 5 (1988) 397-409

### e. masses of the common carbohydrate residues

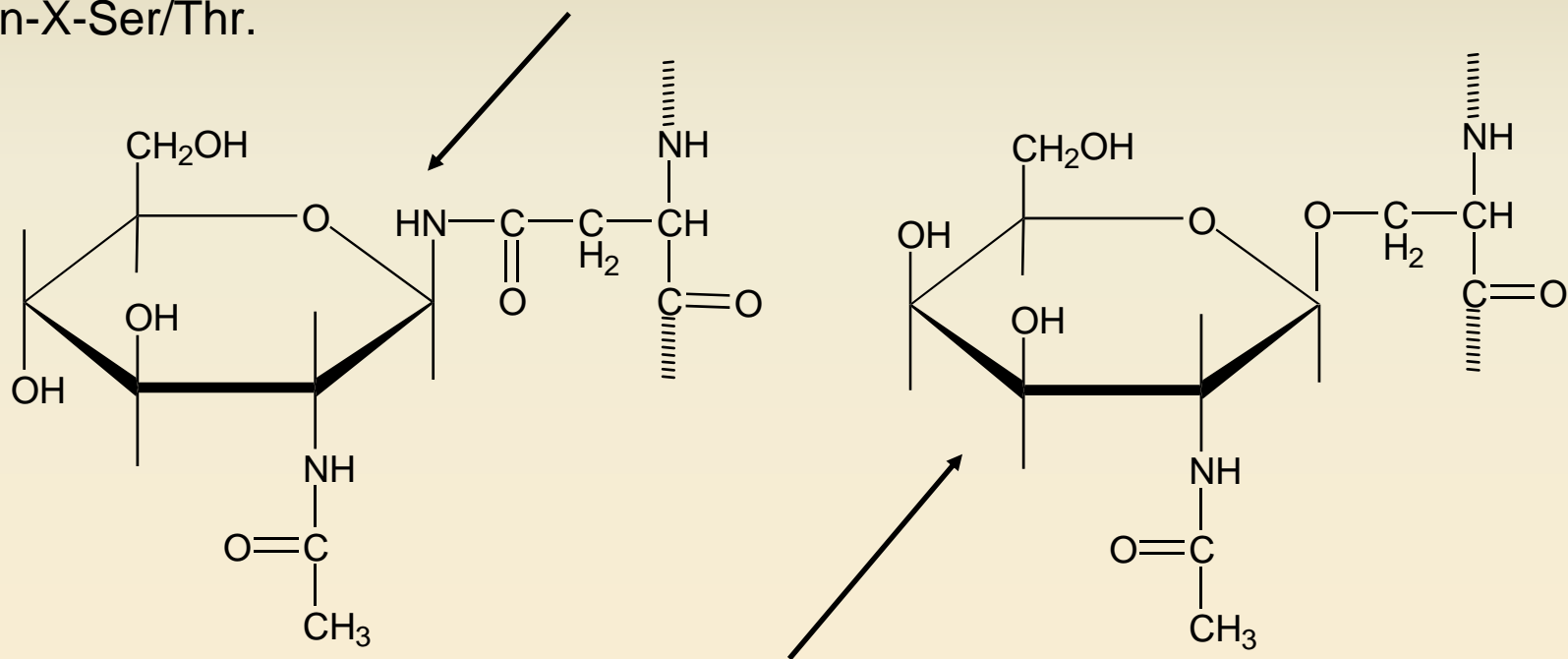
Similar to the amino acids, these residue masses are the masses of monomers minus water. The mass of a single water is added to any collection of residues (sugar, peptide, phosphate, etc.) for a biological molecule.

*e. masses of the common carbohydrate residues*

<i>Monosaccharide</i>	<i>Abbreviations</i>	<i>Monoisotopic mass</i>	<i>Average mass</i>
<i>D-fructose</i>	Fru	162.052	162.143
<i>L-fucose</i>	Fuc      F	146.058	146.140
<i>D-galactose</i>	Gal      GL	162.052	162.143
<i>N-acetyl-D-galactosamine</i>	GalNAc	203.079	203.190
<i>D-glucose</i>	Glc      G	162.052	162.143
<i>D-glucuronic acid</i>	GlcA	176.032	176.126
<i>N-acetyl-D-glucosamine</i>	GlcNAc	203.079	203.190
<i>Hexose (non-specific)</i>	Hex	162.052	162.143
<i>D-mannose</i>	Man      M	162.052	162.143
<i>N-acetyl-D-mannosamine</i>	ManNAc	203.079	203.190
<i>N-acetylneuraminic acid</i>	NANA      NeuAc	291.095	291.260
<i>Sialic acid</i>	SA		

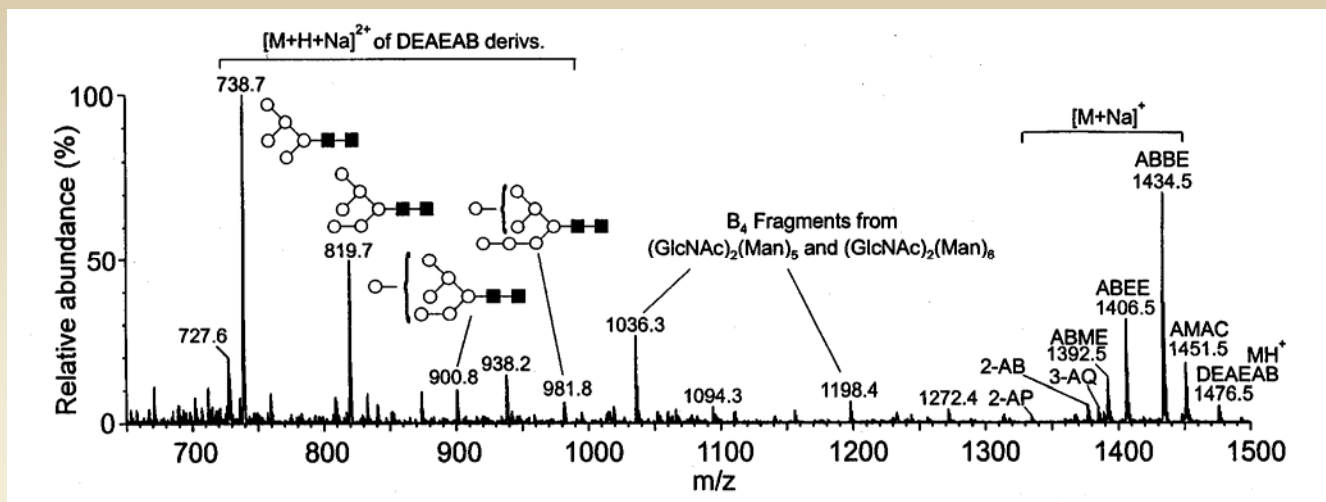
## f. N-linked and O-linked glycosylation

Glycopeptides: glycosylation is one of the most common post-translational modifications of peptides. N-linked oligosaccharides are generally attached via a terminal GlcNAc to an asparagine residue found in the consensus sequence: Asn-X-Ser/Thr.



O-linked oligosaccharides are attached to serine or threonine residues by an acyl linkage formed between two hydroxyl groups (loss of  $H_2O$ ), such as the linkage between GalNAc and serine above.

## g. examples of ESI mass spectra of carbohydrates



Electrospray ionization mass spectrum of high mannose sugars derivatized with seven different amines.

Harvey, D.J., *J. Amer. Soc. Mass Spectrom.* 11 (2000) 900-915.

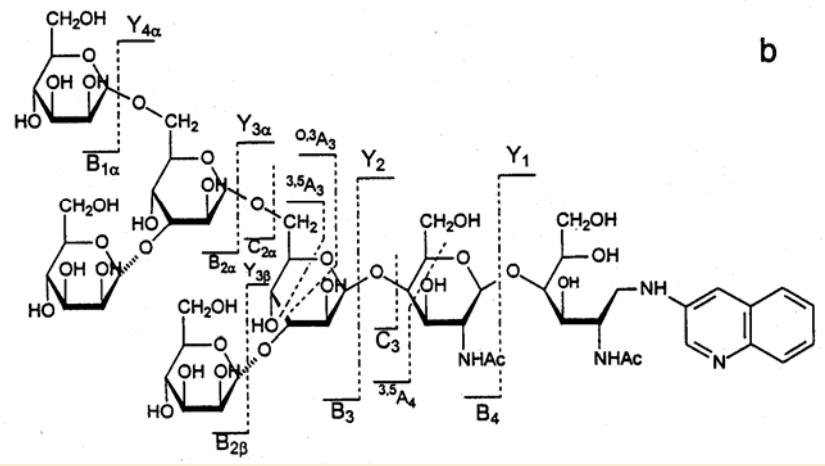
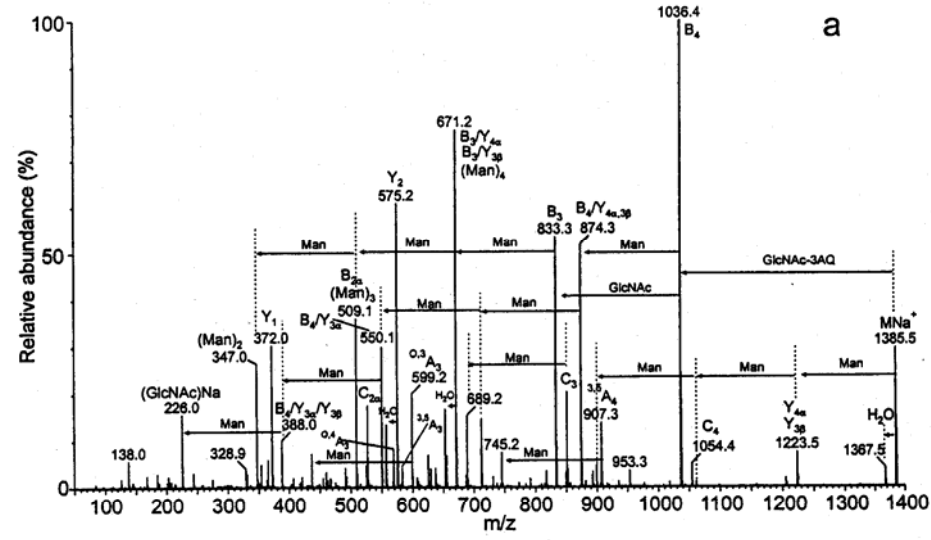
Table 1. Structures of the amines used for the preparation of derivatives

No.	Amine	Abbreviation	Mass Increment	Structure
1	2-Aminobenzamide	2-AB	120	
2	2-Aminopyridine	2-AP	78	
3	2-Aminoacridone	2-AMAC	194	
4	3-Aminoquinoline	3-AQ	128	
5	4-Aminobenzoic acid methyl ester	ABME	135	
6	4-Aminobenzoic acid ethyl ester	ABEE	149	
7	4-Aminobenzoic acid n-butyl ester	ABBE	176	
8	4-amino-N-(2-diethylaminoethyl)benzamide	DEAEAB	219	

*g. examples of ESI mass spectra of carbohydrates*

Electrospray ionization  
CID mass spectrum of the  $[M+Na]^+$  ion from the  
3-AQ derivative of  
 $(GlcNAc)_2Man_3$ .

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## 4. MALDI and Electrospray: when to use each.

MALDI: singly-charged ions best used with a high mass range analyzer such as the TOF mass analyzer. Singly-charged ions are an advantage where there is a complex mixture accompanied by significant fragmentation; an example is microheterogenous mixtures of glycopeptides.

ESI: multiply charged ions; can be used with quadrupoles and quadrupole ion traps. These instruments are more readily configured as tandem mass spectrometers for mass-selecting and fragmenting single components of a mixture.

MALDI: a solid-phase technique that will be utilized for high throughput microarrays on silicon chips, imaging of tissue or selection of individual cells or microorganisms

ESI: a liquid techniques compatible with on-line chromatographic (reversed-phase HPLC, anion exchange, etc.) chromatography and capillary electrophoresis.

## 5. MS and MS/MS: observing fragmentation.

Fragmentation in normal MS: structurally informative fragmentation is sometimes observed in normal mass spectra, but generally only for relatively small compounds; cannot distinguish fragmentation from mixtures.

In-source decay: in TOF instruments observable fragmentation is increased by lengthening the time ions spend in the source; again, cannot distinguish fragmentation from mixtures.

Post-source decay: in TOF instruments this provides the opportunity to mass-select molecular ions and to observe fragmentation specific to each molecular ion.

Tandem mass spectrometer: quadrupoles, ion traps, Fourier transform mass spectrometers and hybrids (such as the QTOF) provide the opportunity for mass-selection of molecular ions and observation of their specific fragment ions; in addition, they increase the extent of fragmentation through *collision-induced dissociation* (CID).