Peptide fragmentation : sequence and size effects on peptide fragmentation

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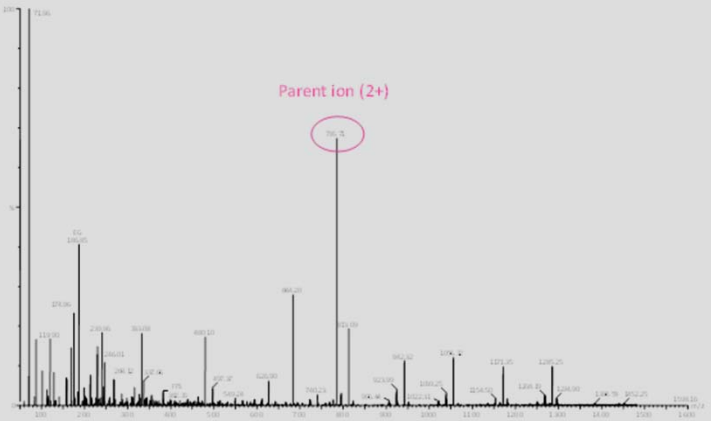
Why fragmentation rules are essential



- Protein database
- Spectrum interpretation (*de novo*)

Rules

Peptide sequence

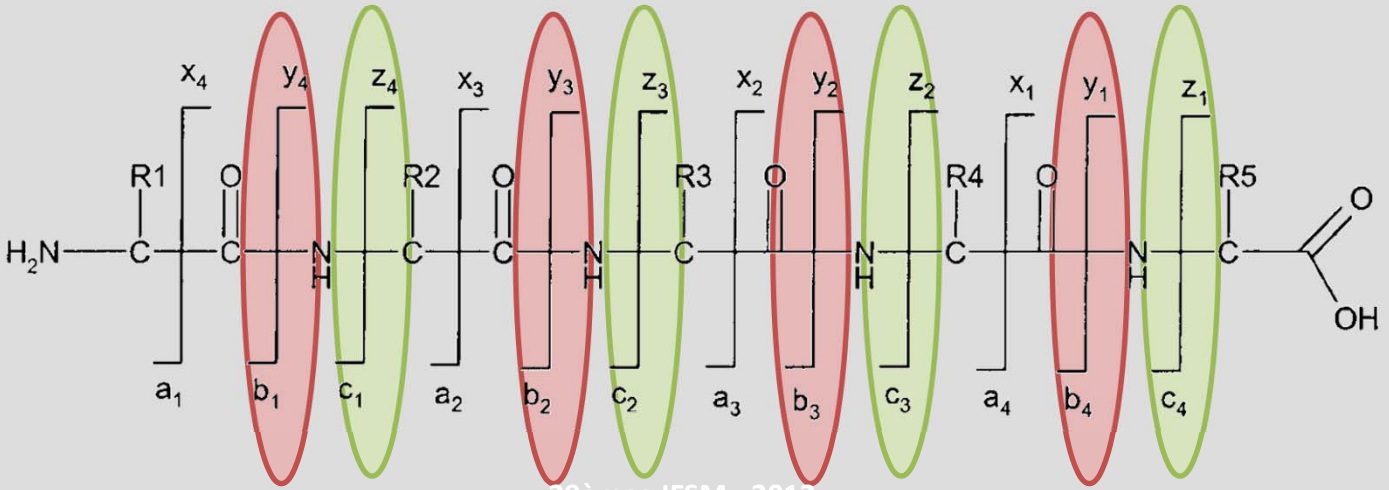
How efficient are we at:

- **Predicting peak m/z ?**
- **Predicting intensities?**

Rules can be simple…

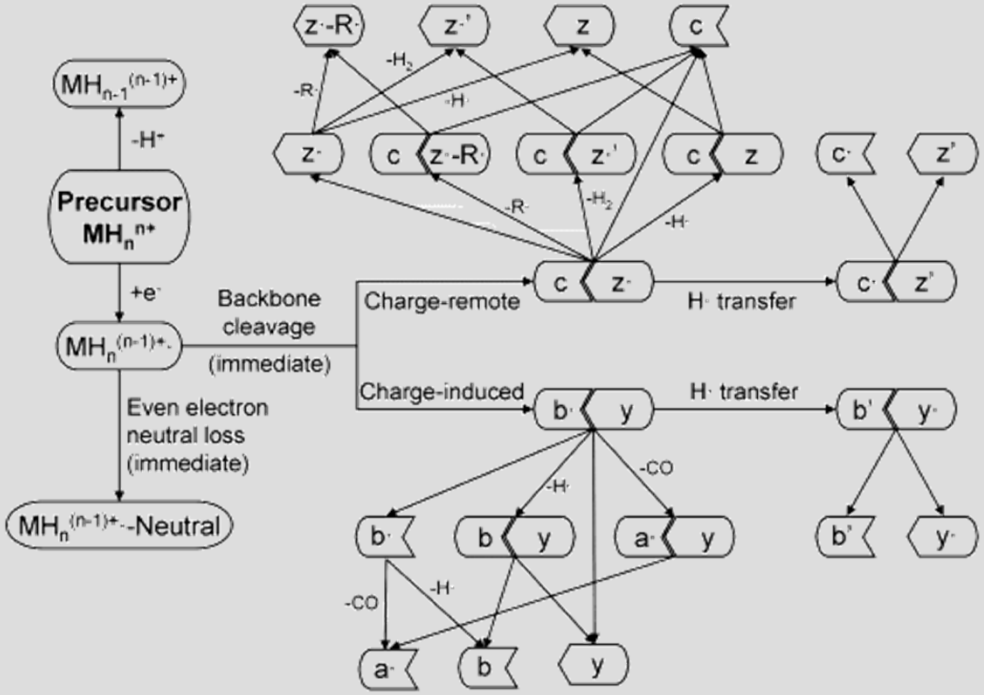
- In CID :
  - Fragmentation of peptides lead to *b* and *y* type ions

- In ECD :
  - Fragmentation of peptides lead to *c* and *z* type ions



Or include a high number of parameters

- Reflect the diversity of protein and peptide compositions



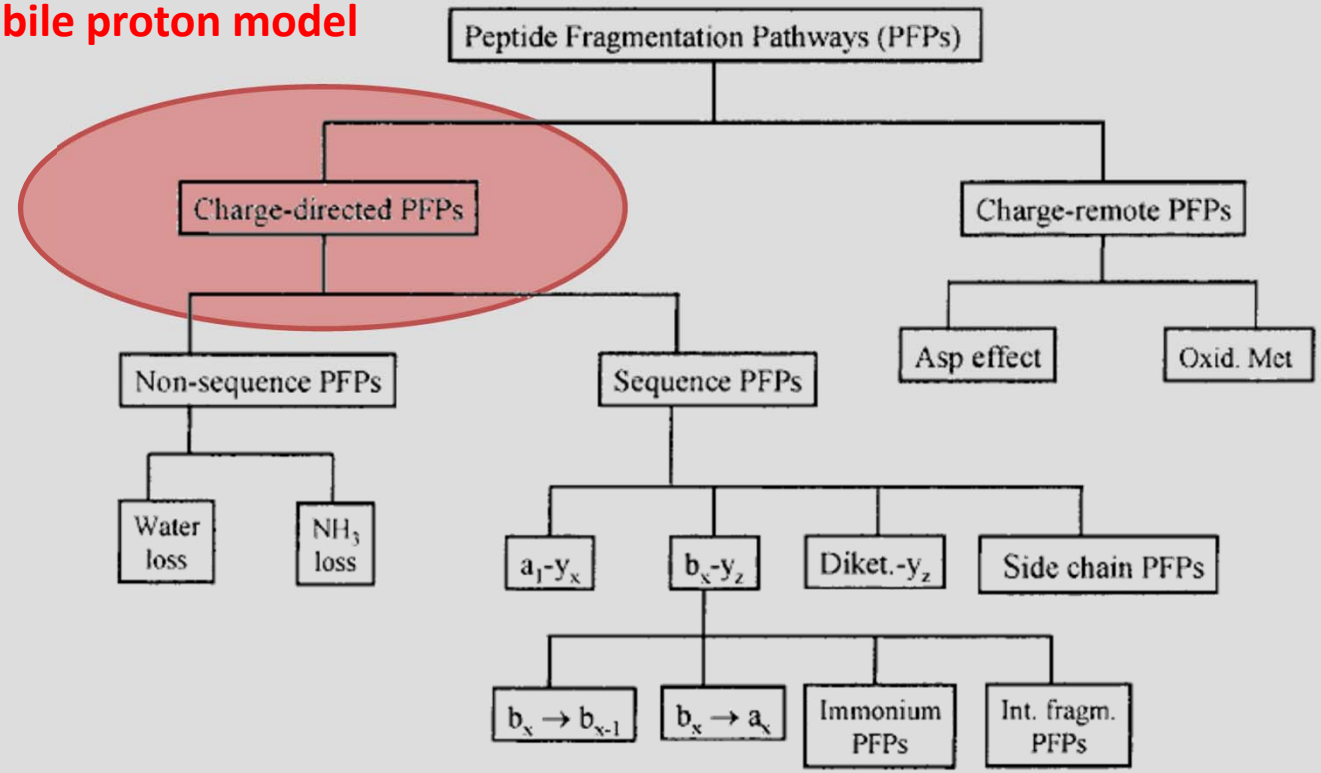
280 parameters, including

- Gas-phase basicity of residue n
- Hydrogen affinity of residue n
- Same for residue n+1

Z. Zhang, *Anal. Chem.* 1990 (**2010)**

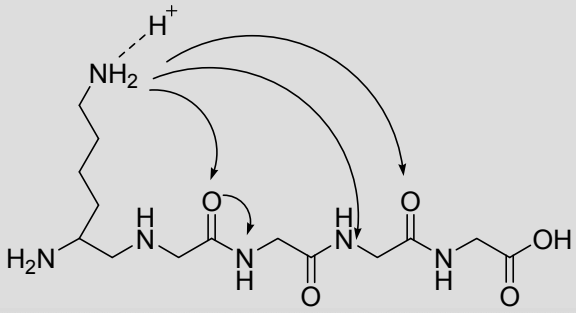
Low-energy CID of peptides

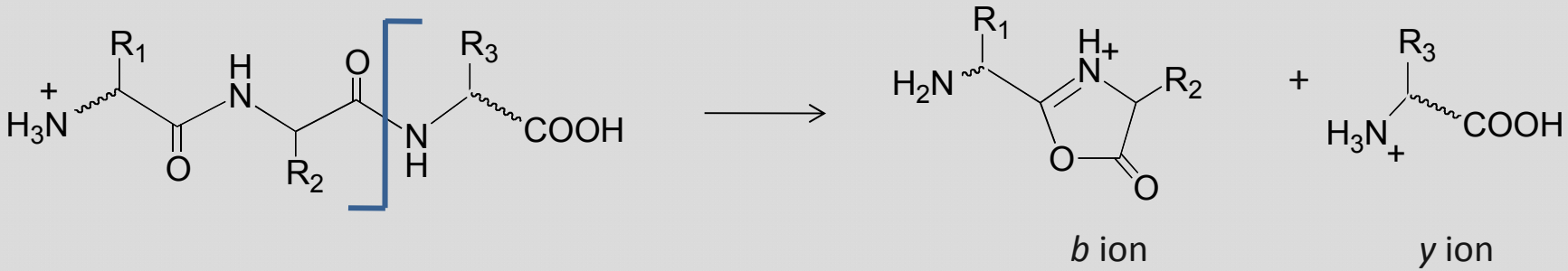
Mobile proton model



Paisz, Suhai, *Mass Spectrom. Rev.* 508 (2005)

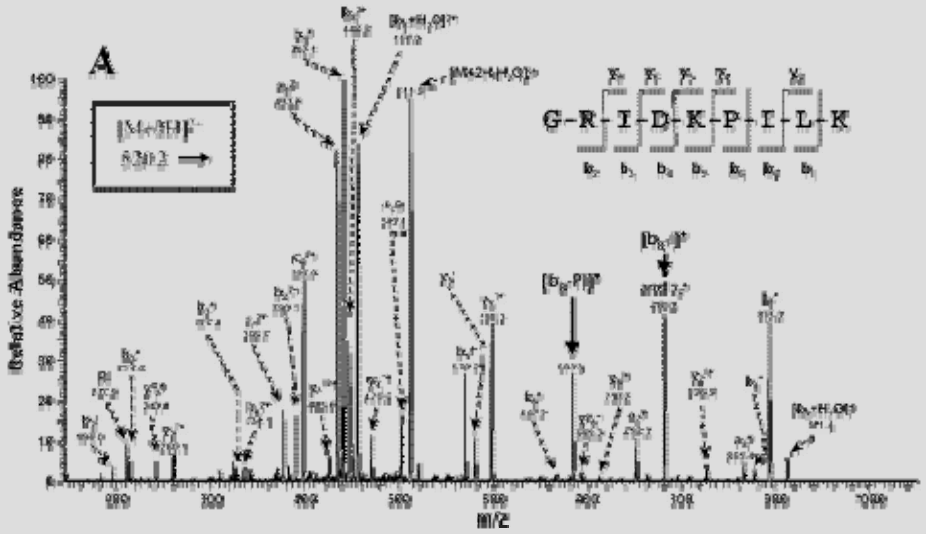
The mobile proton model



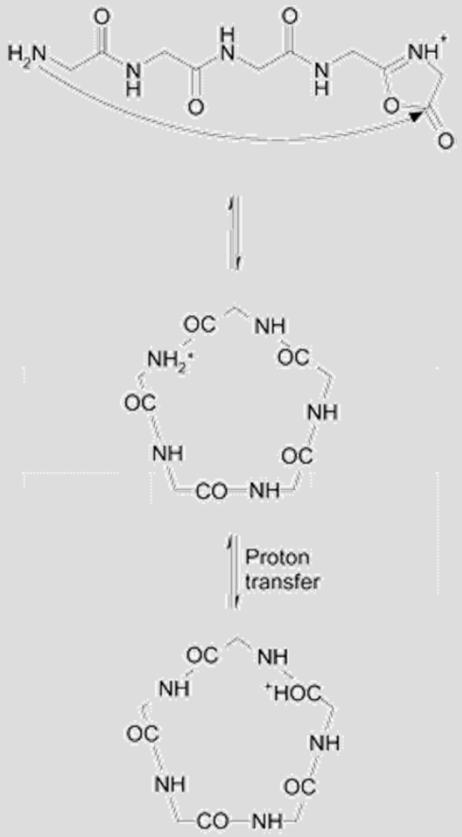


Dongré, *J. Am. Chem. Soc.* (1996)
Tsaprailis et al. , *J. Am. Chem. Soc.* (1999)

Small intensity ions: a large number of origins



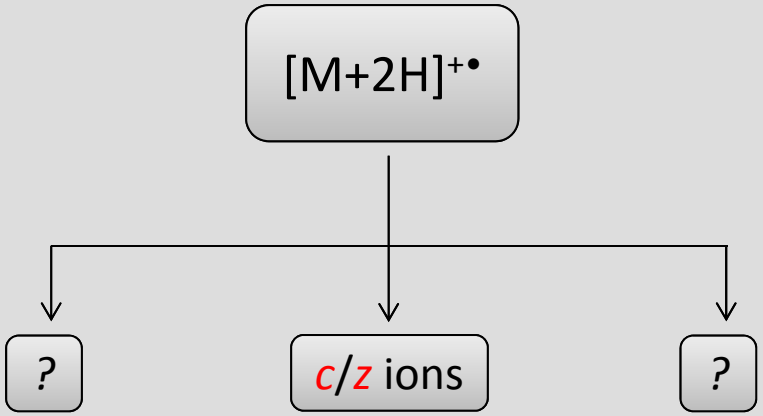
J. Yague *et al. Anal. Chem.*, 1524 (2003)



U. Erlekam, *J. Am. Chem. Soc.,* 11503 (2008)

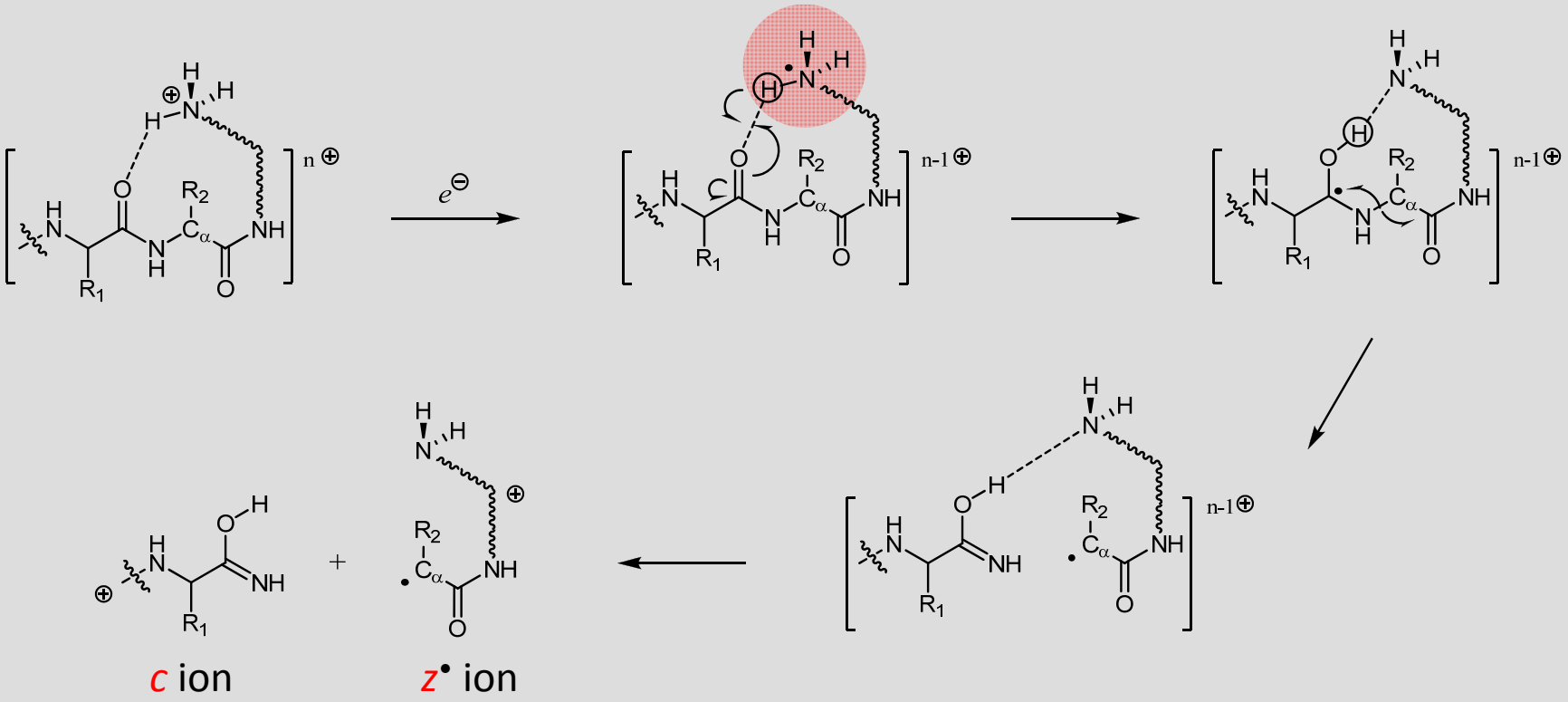
On the mechanism of ECD

- Other pathways than *c/z* fragmentation are observed.
  - Formation of *b*, *y*, *a*, *w* ions has been described.
- Little is known of the parameters favoring a given pathway.



Positive charge directed fragmentations (Cornell-like)

Electron capture at ammonium followed by H• transfer to a carbonyl.
Variants depend on structural changes before H• transfer.



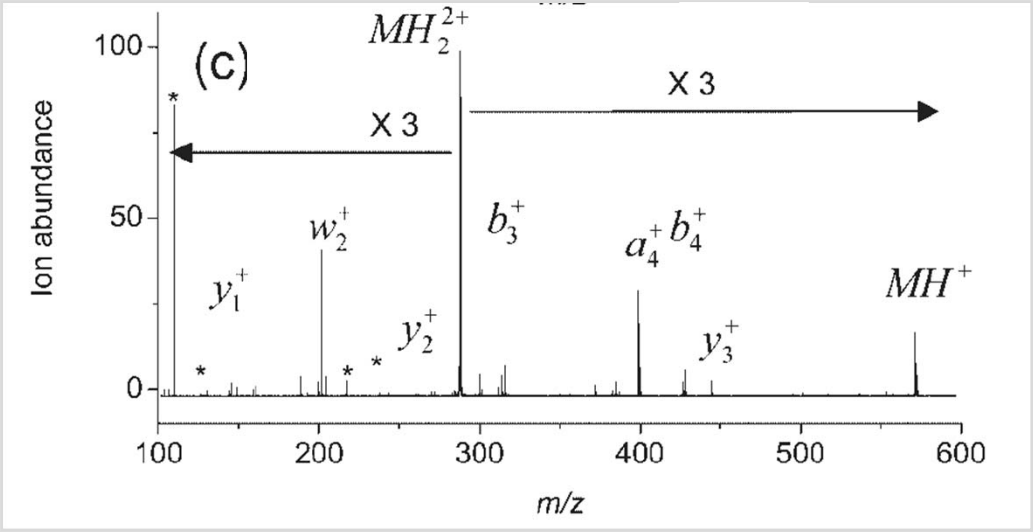
Zubarev , Kelleher, Mc. Lafferty *J. Am. Chem. Soc.* (1998)
Zubarev, *Eur. J. Mass Spectom.* (2002)

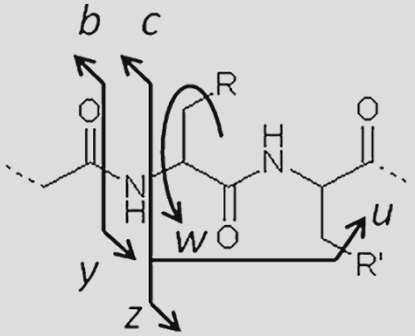
After covalent bond breaking

- $H^{\bullet}$ transfers
  - within the $z^{\bullet}$ fragment
    - Secondary fragmentation (side-chain loss, amine loss, …)
  - Between *c* and $z^{\bullet}$ fragments
    - Formation of $[c+H]^{\bullet}$ or [*z*-H] depending on the intermediate $[c, z^{\bullet}]$ lifetime.
- Separation of the *c* / *z* moieties
  - Not systematic, can lead to a stable $[M, nH]^{(n-1)\bullet+}$ reduced species.

Three independent pathways in ECD fragmentation

- ECD de $[AGWLK+2H]^{2+}$





R. Antoine et al. *RCMS* 2006

- *Pas de fragments c/z !*
- *Essentiellement des fragments w, b et y*

b ions in ECD

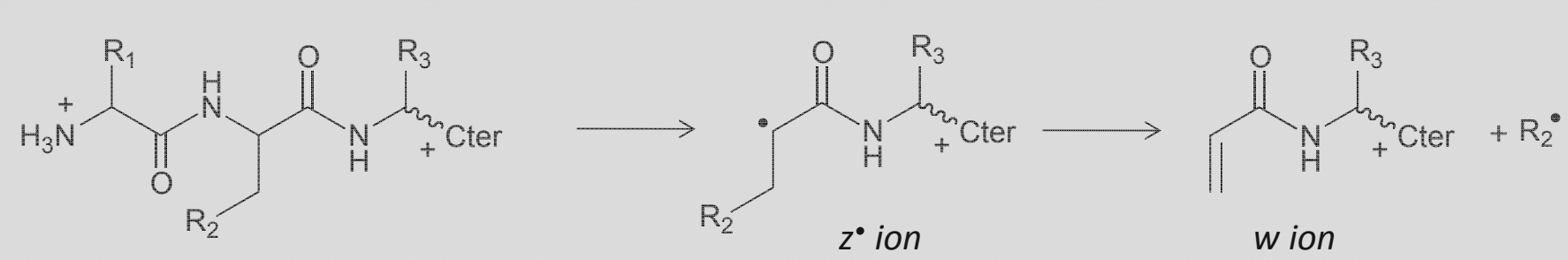
- *b* are frequently present in ECD spectra

- Possible origins for the formation of *b* ions:
  - Vibrational excitation of the $[M+nH]^{n+}$ precursors
  - Secondary fragmentation of *c* ions
  - $H^{\bullet}$ loss after electron capture leading to a $[M+(n-1)H]^{(n-1)+}$ ion leading to *b*/*y* ions.

Liu and Hakansson. *JASMS* 2007

w ions

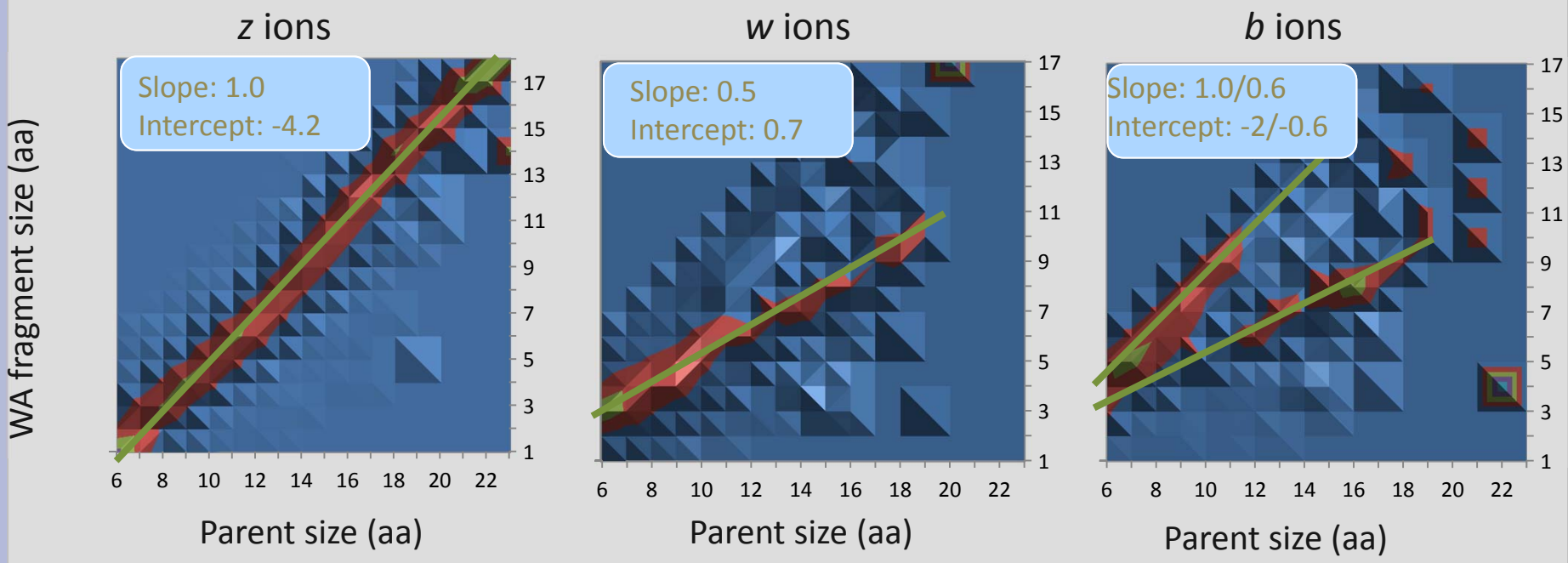
- Generally, *w* ions are considered secondary fragments of *z* ions
  - But no *z* ions are present in our ECD spectra.



R. Zubarev, *J. Am. Chem. Soc.* (2003)
R. Zubarev, *Anal. Chem.* (2003)
M. Savitski, *Anal. Chem.* (2007)

Analysis of the SwedECD database

- Average size of *z*, *w* and *b* fragments as a function of parent peptide size.



- *Independance of the c/z, b/y and w pathways.*

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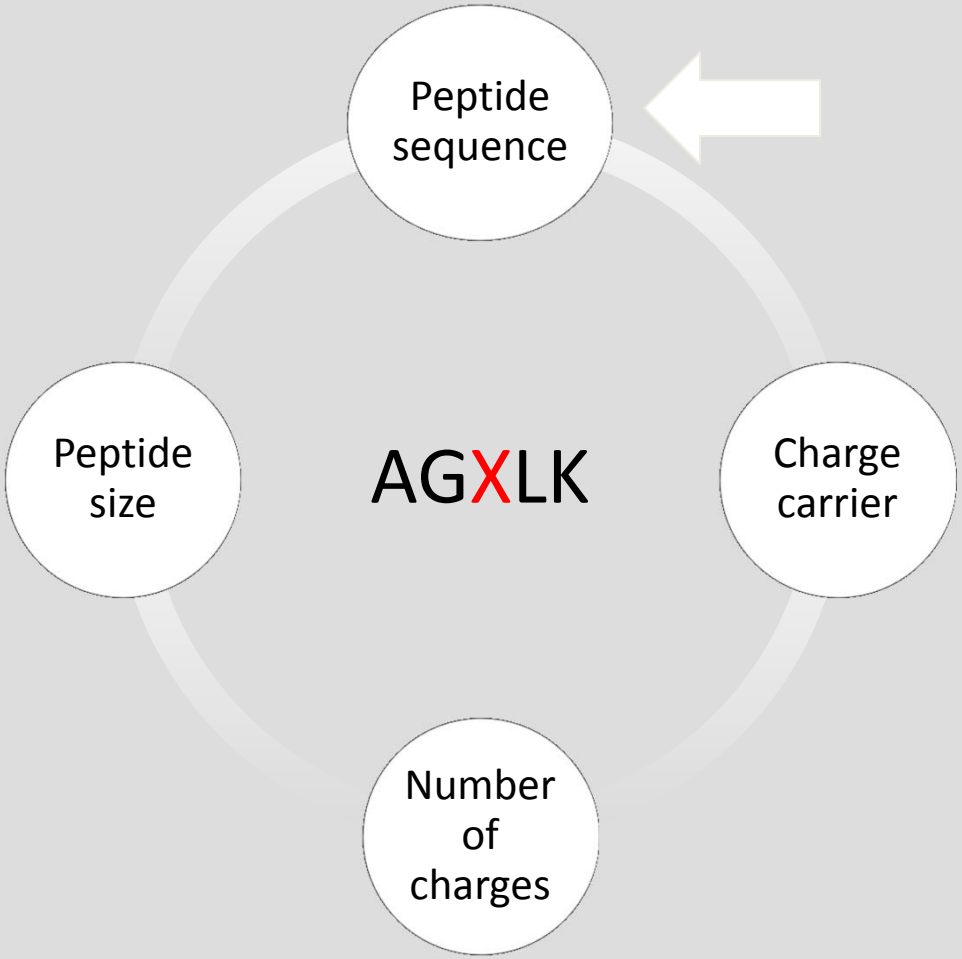
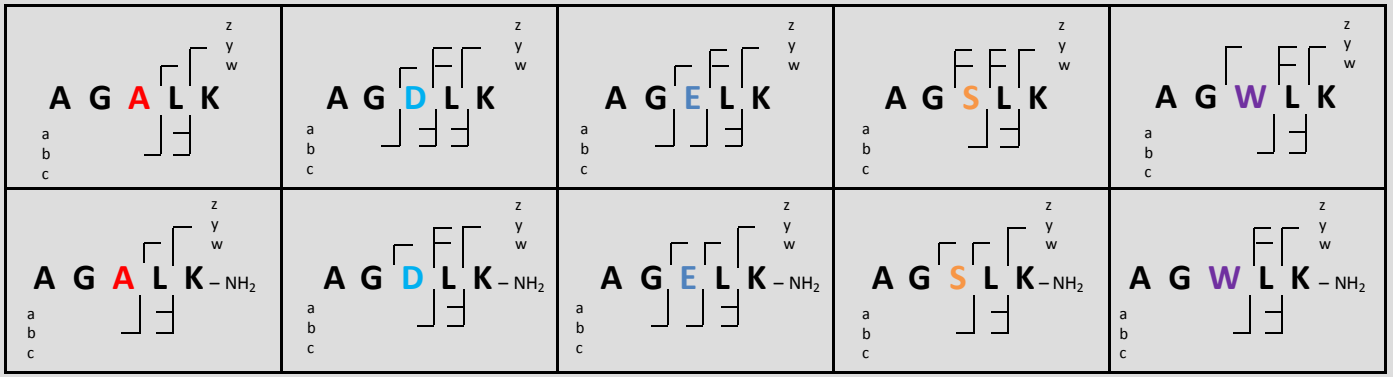
The AGXLK peptide series



The $(AGXLK)H_2^{2+}$ series

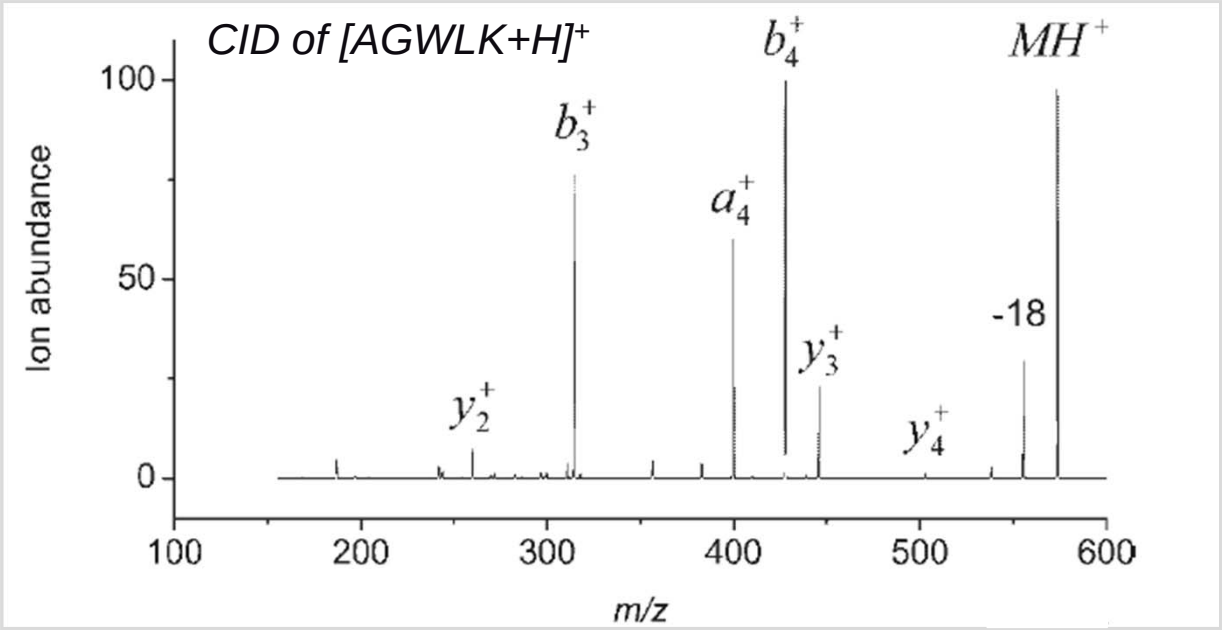


Peptides	*b/y*	*c/z*	*w*	*Peptides*	*b/y*	*c/z*	*w*
AGALK	73	0	27	AGALK-NH_2	65	0	35
AGDLK	59	0	41	AGDLK-NH_2	35	0	65
AGELK	55	0	45	AGELK-NH_2	48	0	52
AGSLK	53	0	47	AGSLK-NH_2	64	0	36
AGWLK	76	0	24	AGWLK-NH_2	68	0	32

- *Large b/y and w ions.*
- *The nature of X does not open the c/z pathway.*
- *w ions are increased when the side-chain of X can lead to stable radicals.*

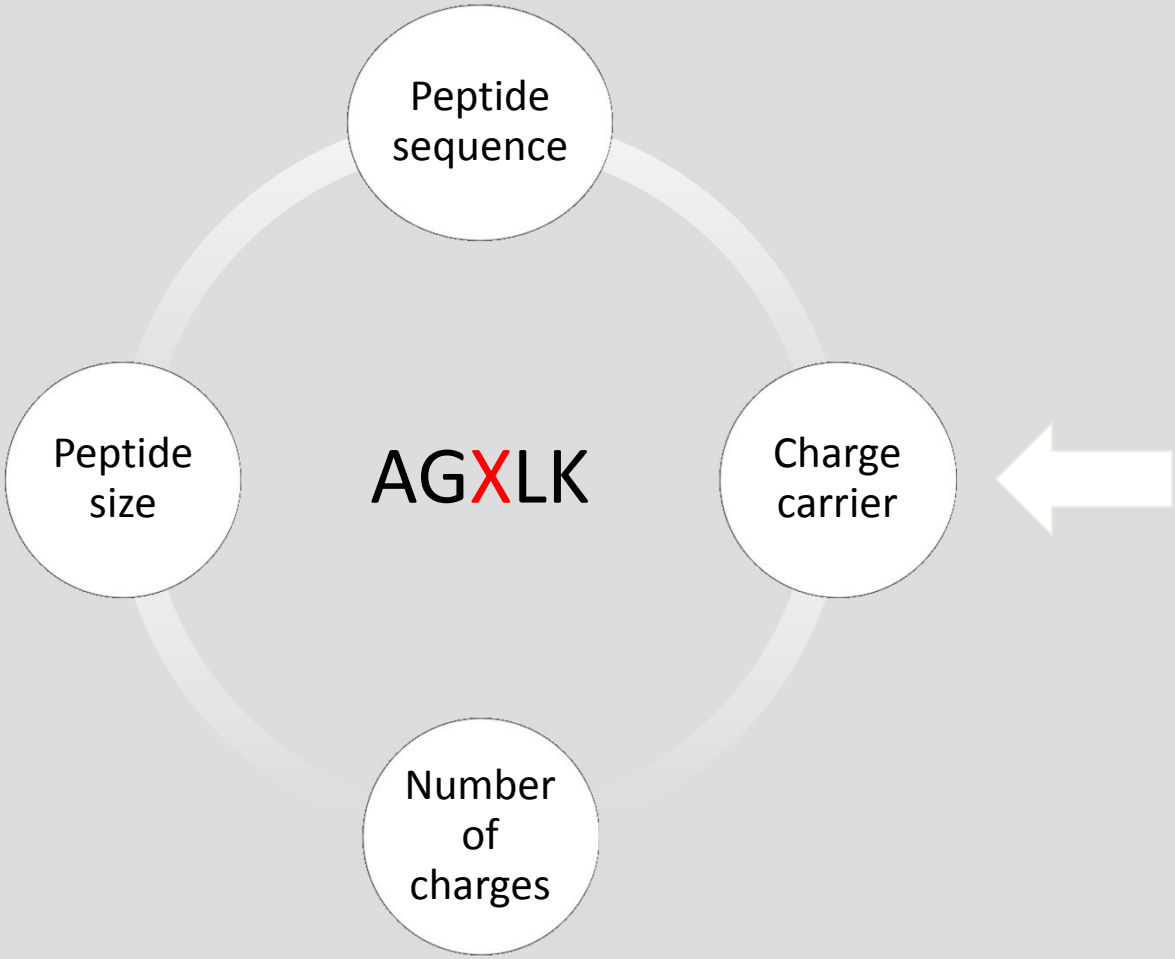
High energy CID spectra

- High and low energy CID of $[M+H]^+$ leads to fragments similar to ECD, except for the *w* ions.



➢ *Could b/y ions be produced from the fragmentation of $[M+H]^+$ precursors?*

The AGXLK peptide series



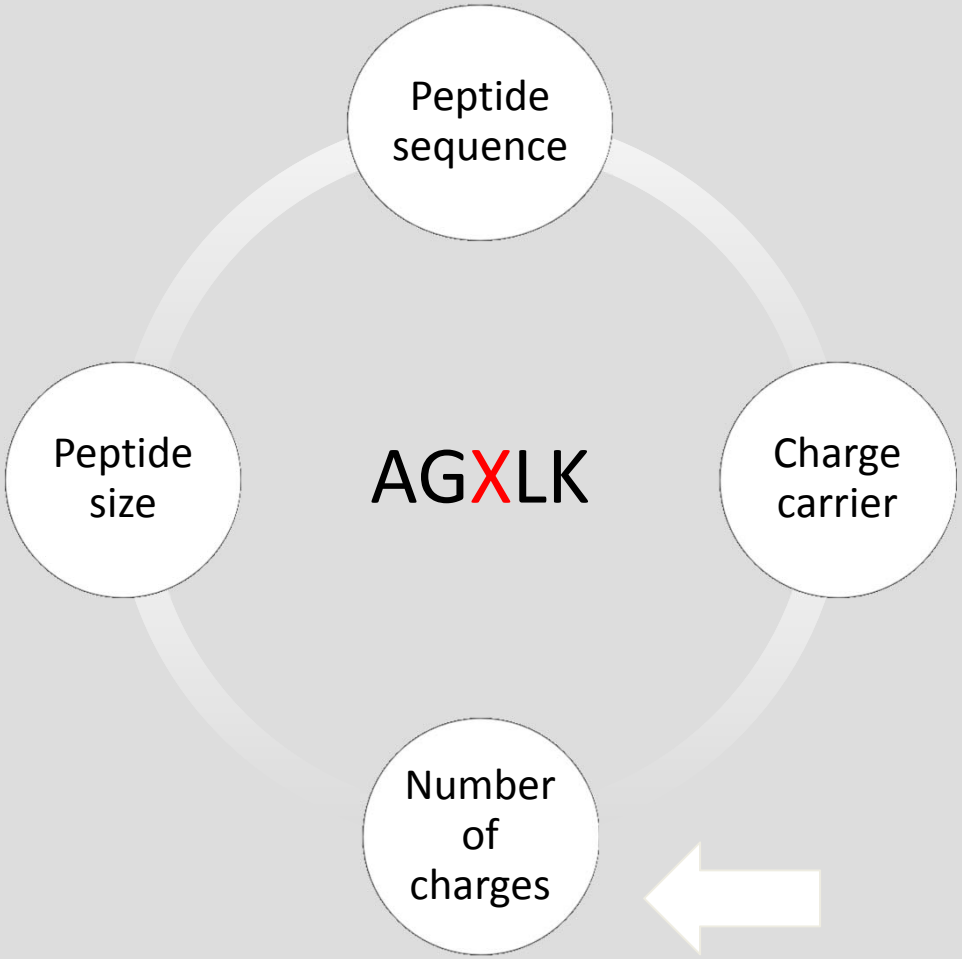
Nature and position of the charged sites

Lys	*b/y*	*c/z*	*w*	*Arg*	*b/y*	*c/z*	*w*
AGELK	52	0	48	AGELR	41	10	49
AGEKL	72	18	10	AGERL	60	14	26
AGKEL	100	0	0	AGREL	67	33	0
AKGEL	100	0	0	ARGEL	61	39	0
KAGEL	88	12	0	RAGEL	41	59	0

- Lys and Arg containing peptides behave differently Only Arg leads to significant *c/z* ions.
- Major differences which could account for this behavior:
  - Recombination energies (GK: 3.3 eV, GR 2.98 eV)
  - Hydrogen bonding network and charge solvation

➢ *What factor between energetics and structure is most important?*

The AGXLK peptide series



Number of charged groups

- Comparing 2+ vs 3+ for XGELX peptides (X= Lys or Arg)

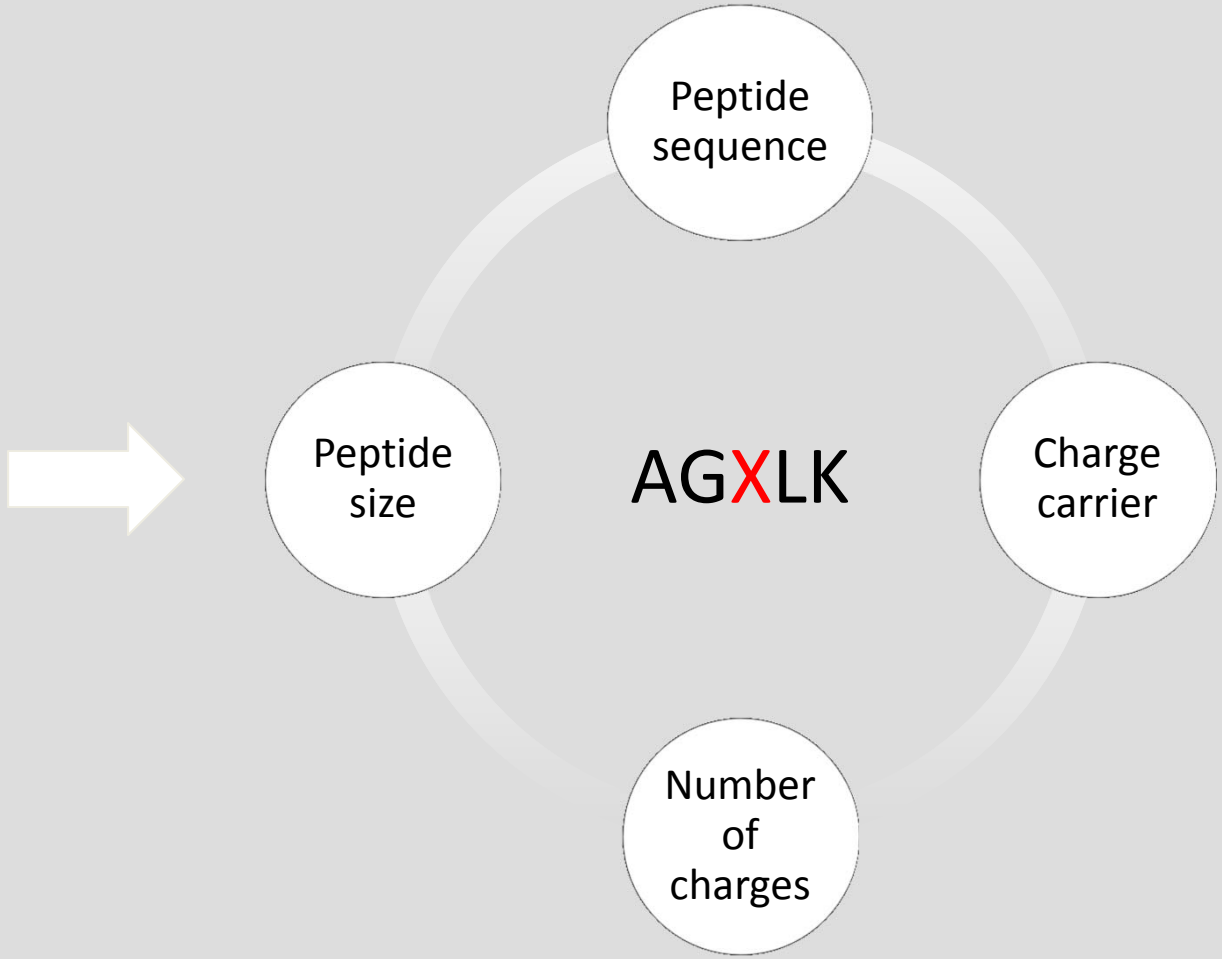
Peptides(2+)	*b/y*	*c/z*	*w*	*Peptides(3+)*	*b/y*	*c/z*	*w*
KGELK	16	42	42	KGELK	72	0	28
KGELR	12	19	69	KGELR	64	0	36
RGELK	15	85	0	RGELK	76	3	21
RGELR	13	74	13	RGELR	68	2	30

- c/z observed for (2+) but not for (3+)
  - Coulomb repulsion stronger for 2+ than 3+
    - Change in recombination energies
    - Change in structure

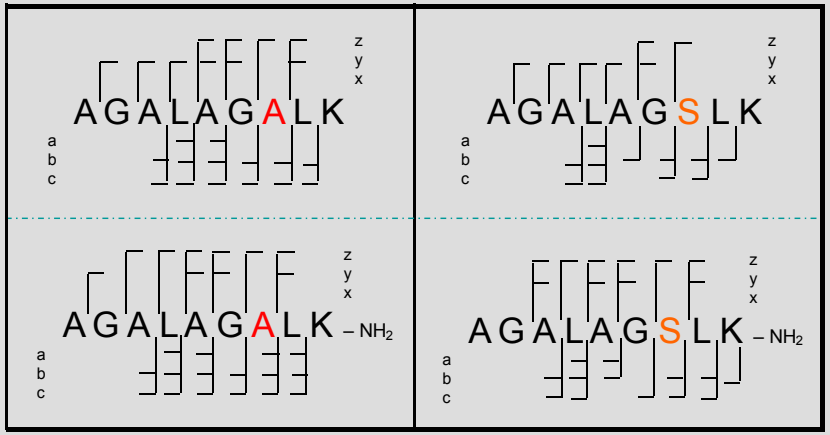
➢ *Peut-on tenter de séparer ces deux contributions?*

➢ *Que se passe-t-il pour des peptides plus grands, qui auront une structure plus compacte à charge égale?*

The AGXLK peptide series

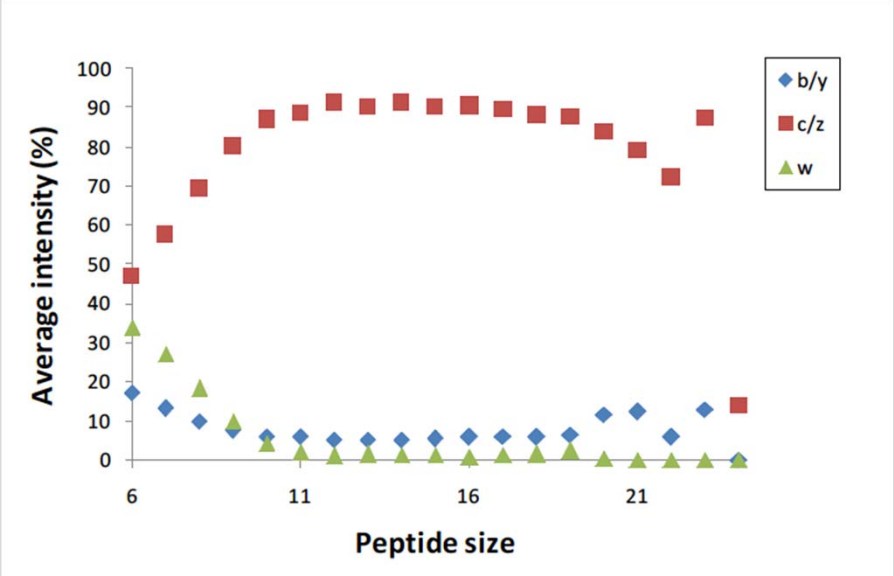


Peptide size



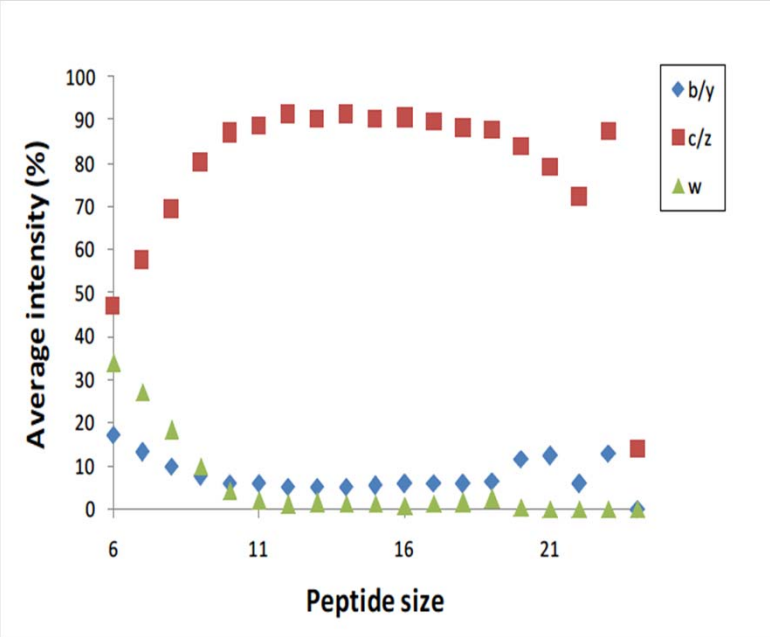
Peptides	*b/y*	*c/z*	*w*	*Peptides*	*b/y*	*c/z*	*w*
AGALAGALK	69	23	8	AGALAGALK-NH_2	86	11	3
AGALAGSLK	71	14	15	AGALAGSLK-NH_2	64	25	11

Back to the SwedECD database

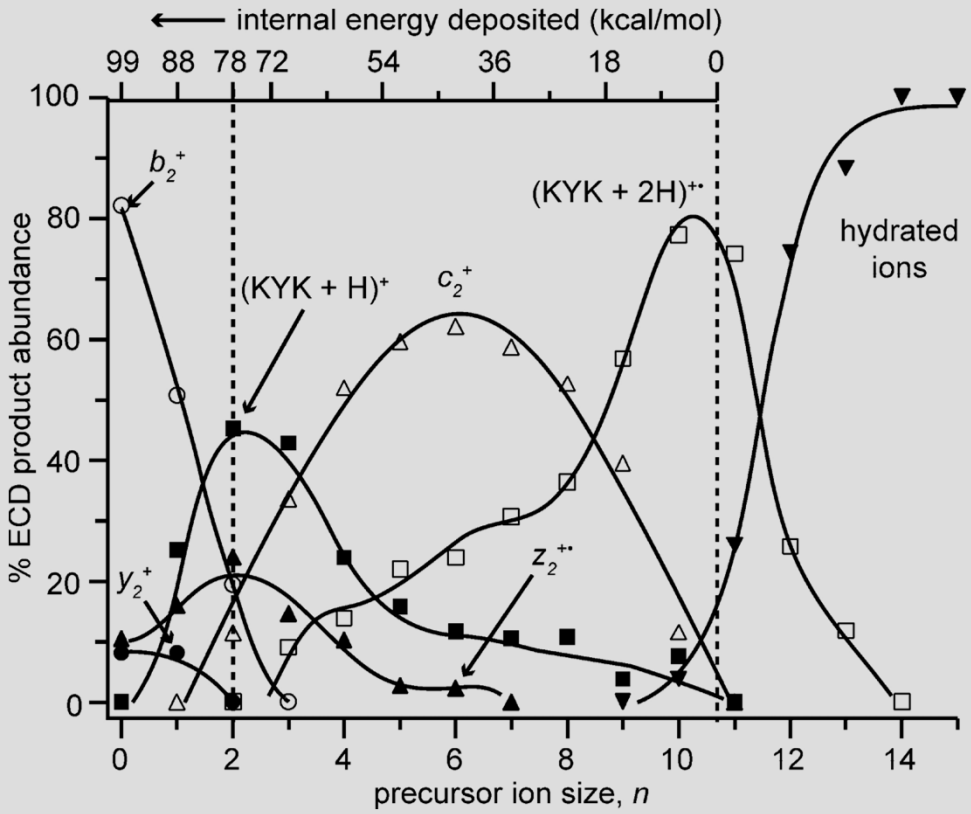


- Branching ratios strongly depend on peptide size:
  - For small peptides: dominant *b/y* and *w* fragments
  - For large peptides: dominant *c/z* fragments.

Comparison with nanocalorimetry experiments



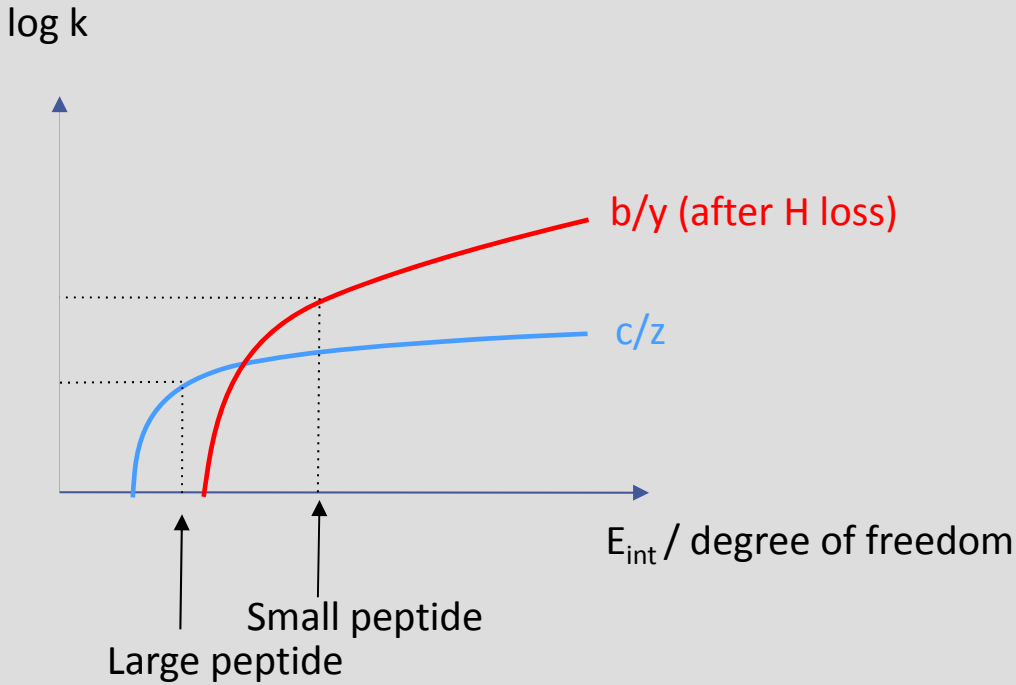
$[KYK, 2\ H, n\ H_2O]^{2+}$



J.S. Prell; *et al. J. Am. Chem. Soc.* 12680-1268 (2008)9

Energy based model

- Branching ratios depend on the energy density (energy per degree of freedom)

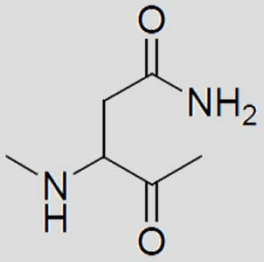


➢ *Can we build a model peptide to test this hypothesis?*

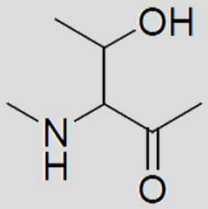
Model peptides based on Asn vs Thr

- Asparagine (Asn) will present a carbonyl group that can strongly interact with the charge
  - Should favor the c/z pathway in the structural model.

- Comparison between peptides with the same number of Asn and Thr groups



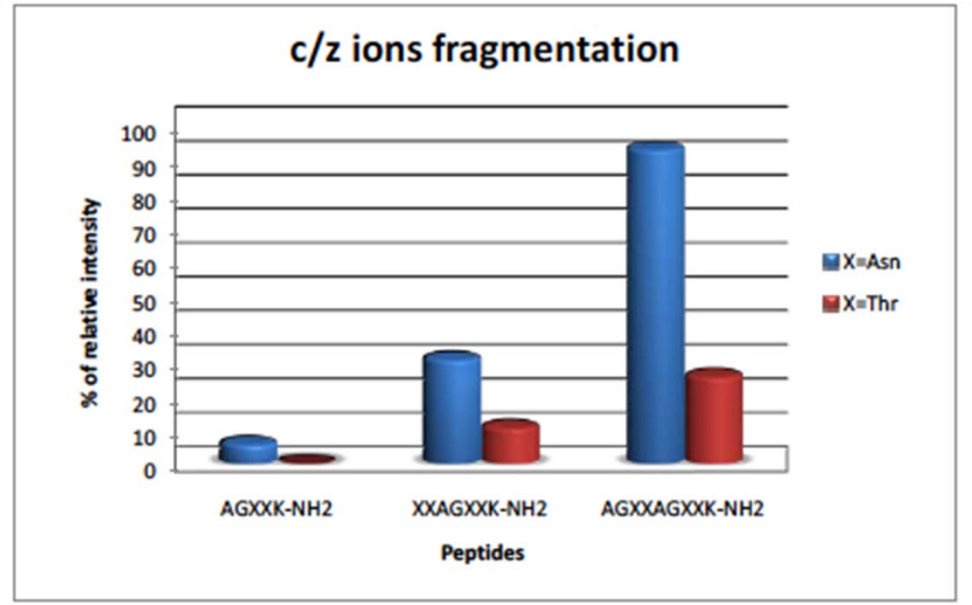
Asparagine (N)



Threonine (T)

Asn/Thr comparison

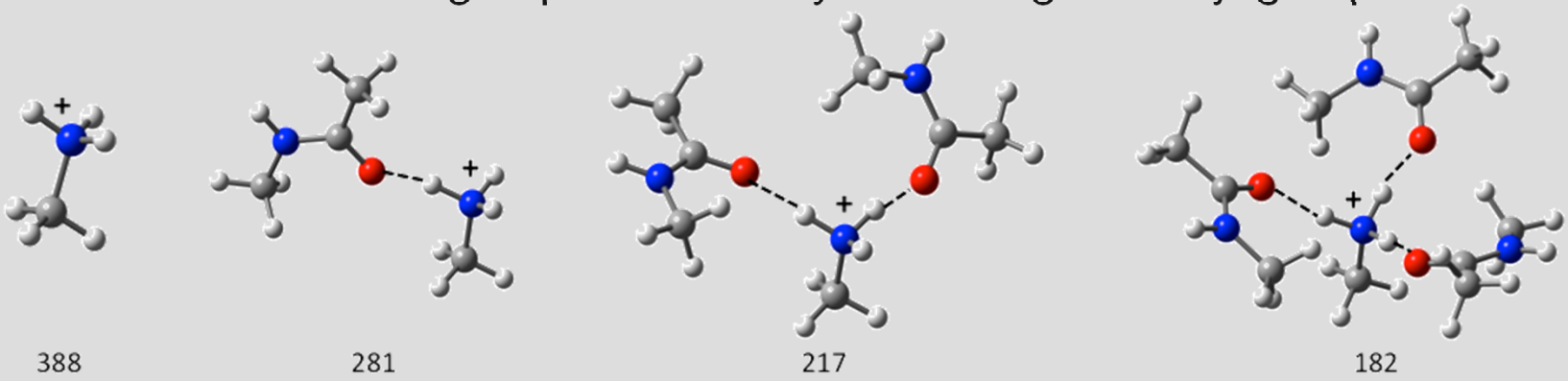
Peptides	*b/y*	*c/z*	*w*	*Peptides*	*b/y*	*c/z*	*w*
AGNNK-NH_2	78	6	16	AGTTK-NH_2	88	0	12
NNAGNNK-NH_2	42	31	27	TTAGTTK-NH_2	70	11	19
AGNNAGNNK-NH_2	2	93	5	AGTTAGTTK-NH_2	74	26	0



- *c/z pathway increases with the number of asparagine groups.*

Effect of solvation on recombination energies

- RE of ammonium groups solvated by increasing carbonyl groups

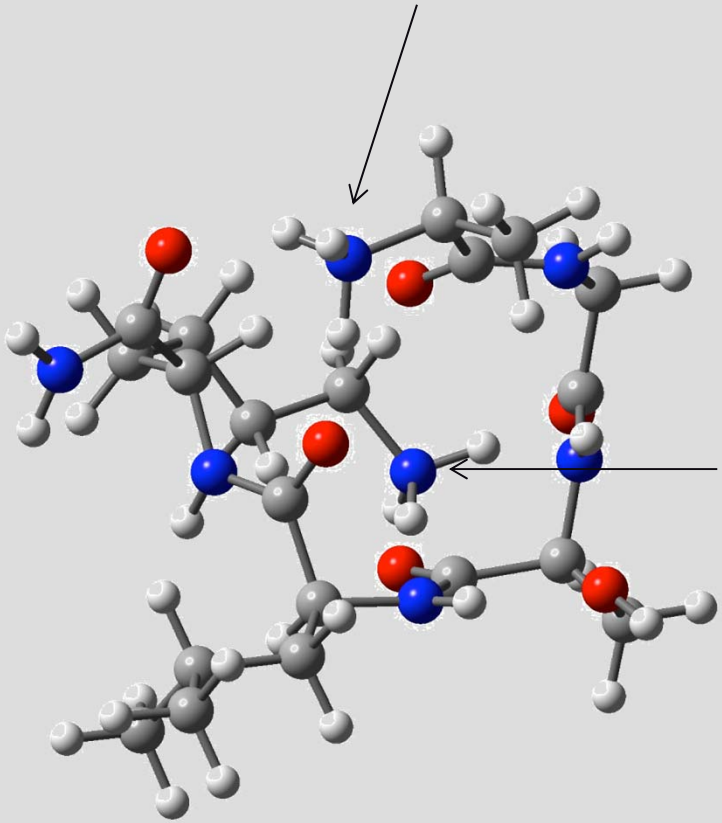


Computed adiabatic recombination energies in kJ/mol. LC-BLYP/6-311++G(2d,p)

- H bonding can reduced RE up to 2 eV

➢ *Secondary structure and energetics are linked through RE !*

Our current model for peptide fragmentation



$[M+2H]^{2+}$

e- captured by a free ammonium

→ H loss

→ $[M+H]^{+}$

→ b/y ions

e- captured by a solvated ammonium

→ H transfer to carbonyl

→ c/z fragments

Ont participé à ce travail :

J. Chamot - Rooke
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P. Maître
J-M. Ortega
B. Rieul
V. Steinmetz
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