

Fragmentation in MALDI-TOF-TOF: beyond proteomics studies

Gérard Bolbach, UPMC, UMR 7203 and Plate-Forme Mass spectrometry and proteomic

- MALDI-TOF, TOF-TOF : ion analysis in time window, ion stability

- 3 examples: - very fragile peptides

- detection of D-residue

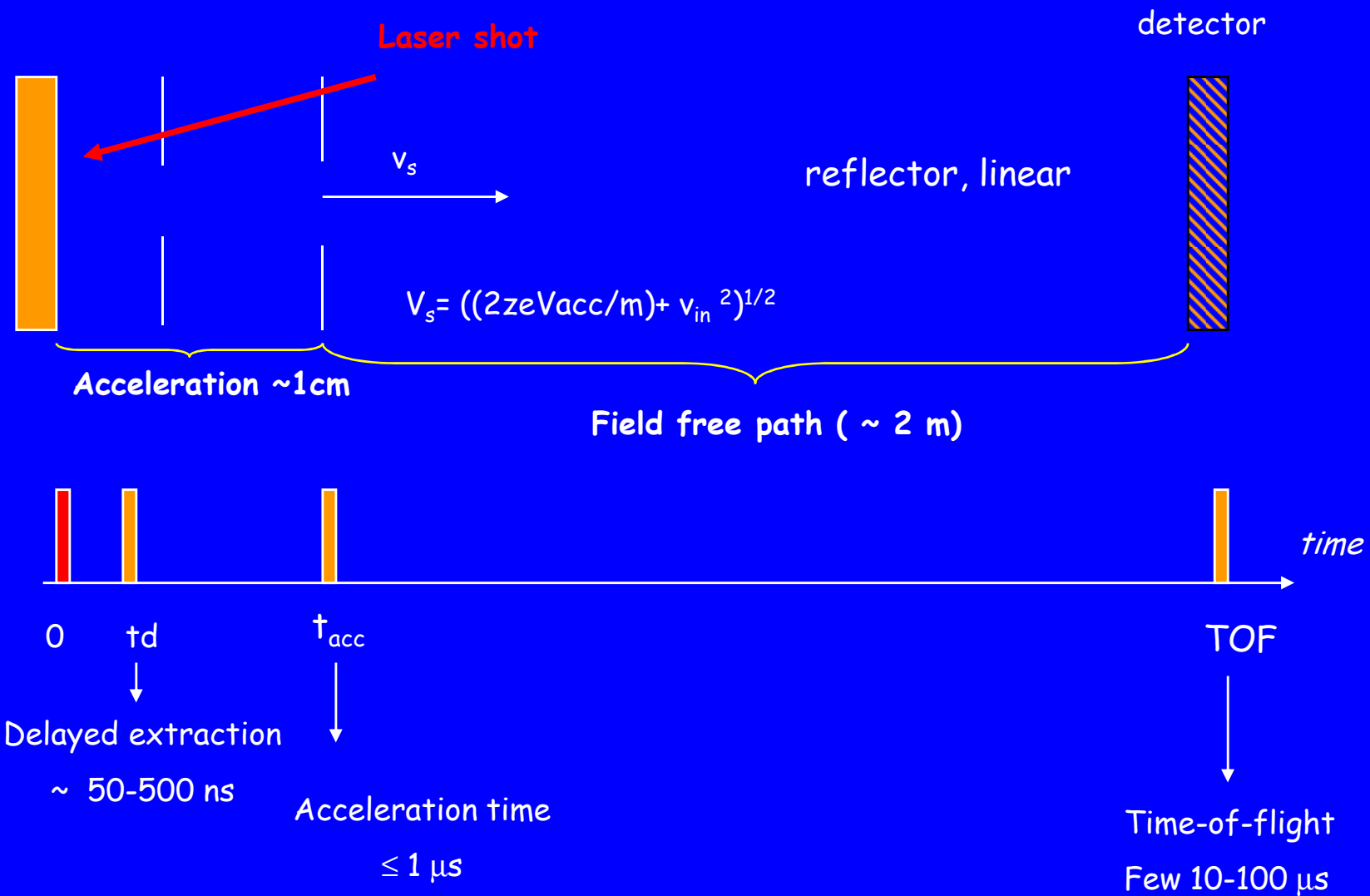
- Na cationized Val, Leu oligopeptides

very prompt fragmentation

metastable decomposition

high energy collision

MALDI-TOF: time analysis of produced ions, ion stability



Ion stability



$\tau(E_{\text{int}}) = 1/k(E_{\text{int}})$



$\tau < t_d$

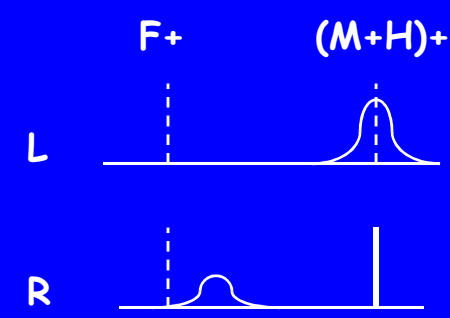
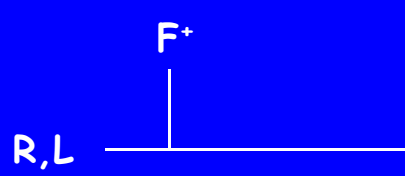
F^+ ISD (c, z)

$t_{\text{acc}} < \tau < \text{TOF}$

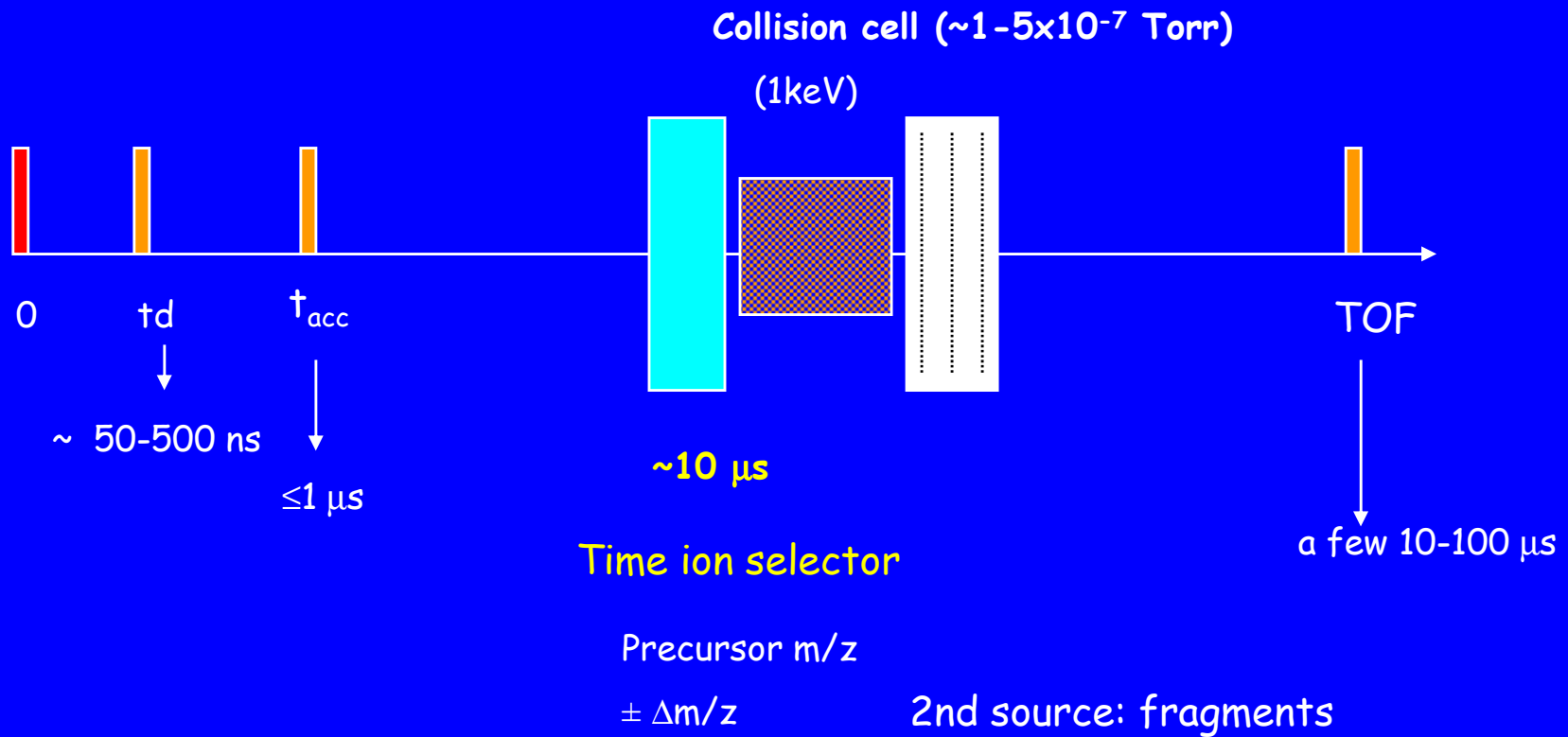
Metastable decay (b, y)

$\tau > \text{TOF}$

Stable ion



MALDI-TOF-TOF



4700 Proteomic Analyzer

First example: very fragile peptide in MALDI-TOF and TOF-TOF (Y. Jacquot)

✓ Ac-DAEPPILYSEY-NH₂, proline rich with no basic site

Miming the active region of oestrogen receptor

Synthesis and purification: 1 peak elution



✓ MS analysis: main features

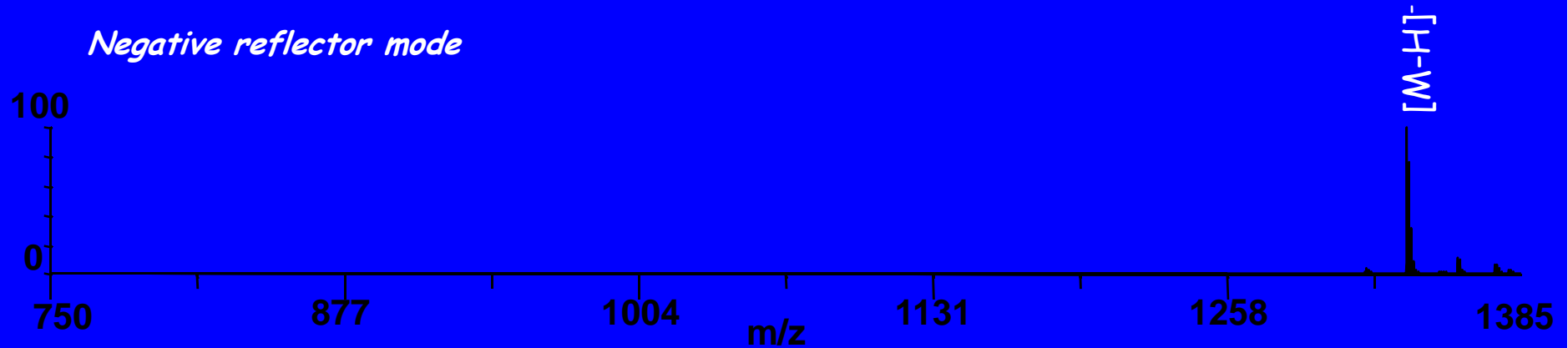
MALDI-TOF: amazing behaviors

Sachon et al Anal. Chem. 2009, in press

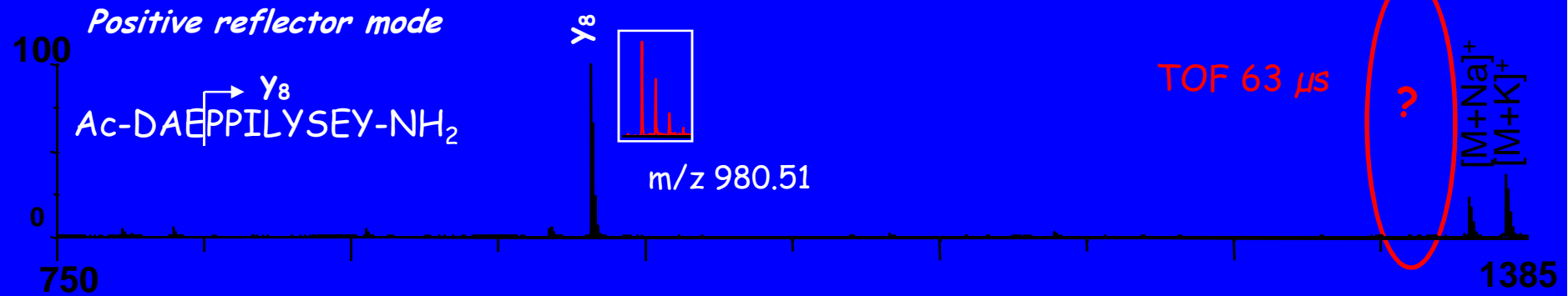
CHCA matrix, 4700 Proteomics Analyzer

Ac-DAEPPILYSEY-NH₂

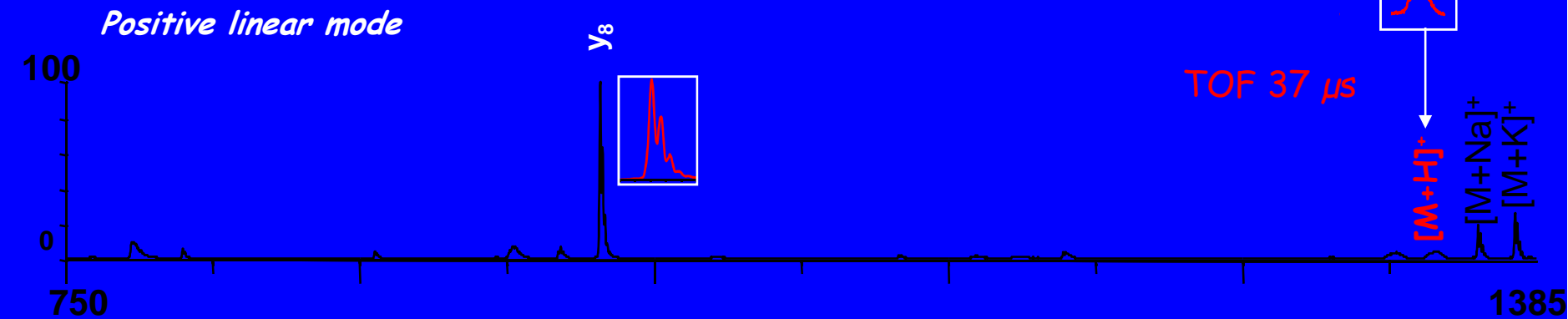
Negative reflector mode



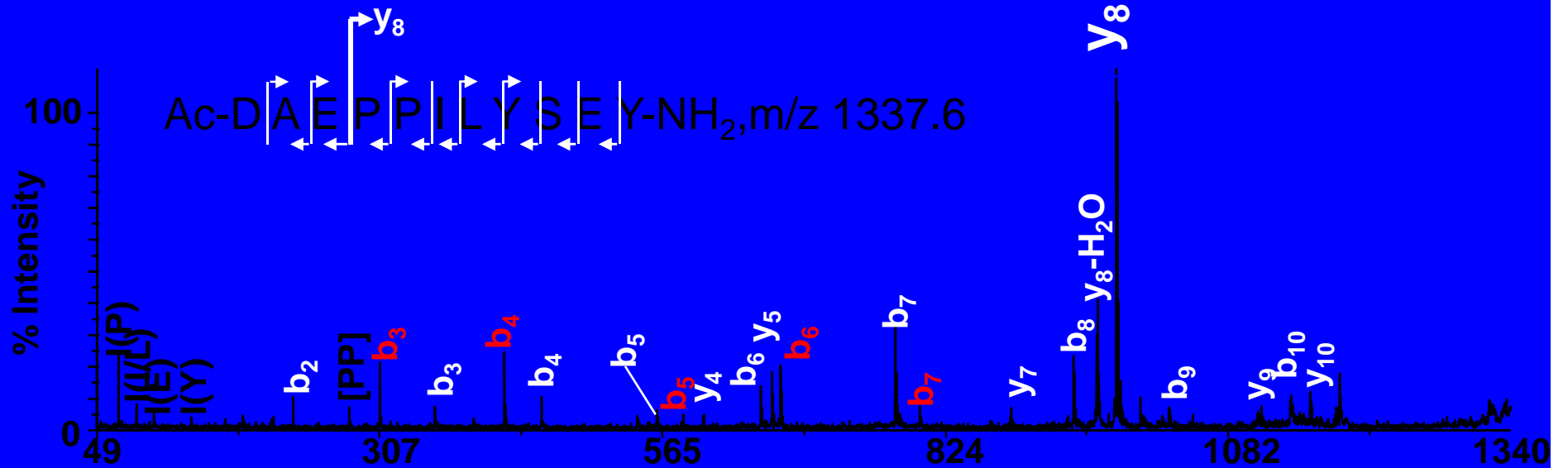
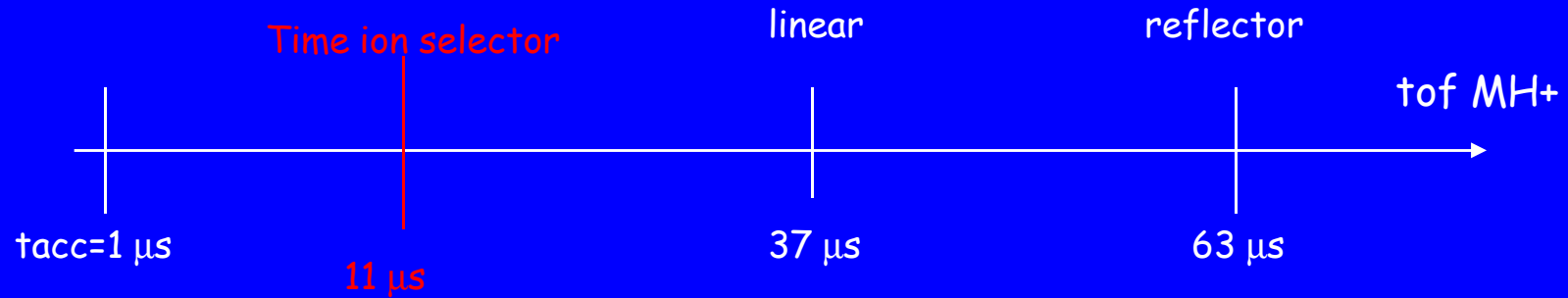
Positive reflector mode



Positive linear mode



MALDI-TOF-TOF of $(M+H)^+$: HCCA, gas 2×10^{-7} Torr N_2 , E coll 1keV



P P I L Y S E Y-NH₂ y_8 : m/z 980.5

Ac-DAEPPILYSEY-NH₂ very fragile peptide

Two peptide populations { Population I: very fast fragmentation → y₈
Population II: (M+H)⁺ fast metastable decay

Effects of

- the delay time: delay time=0 (continuous extraction)-2000ns

y₈ major ion: fragmentation not in the gas phase but in the target (no classical ISD)

-the peptide sequence: Ac-DAEPPILYSEY-NH₂, H₂N-DAEPPILYSEY-NH₂, H₂N-DAEPPILYSER-NH₂

population I >> population II: only one Pro (P effect), no free N-term, no basic residue

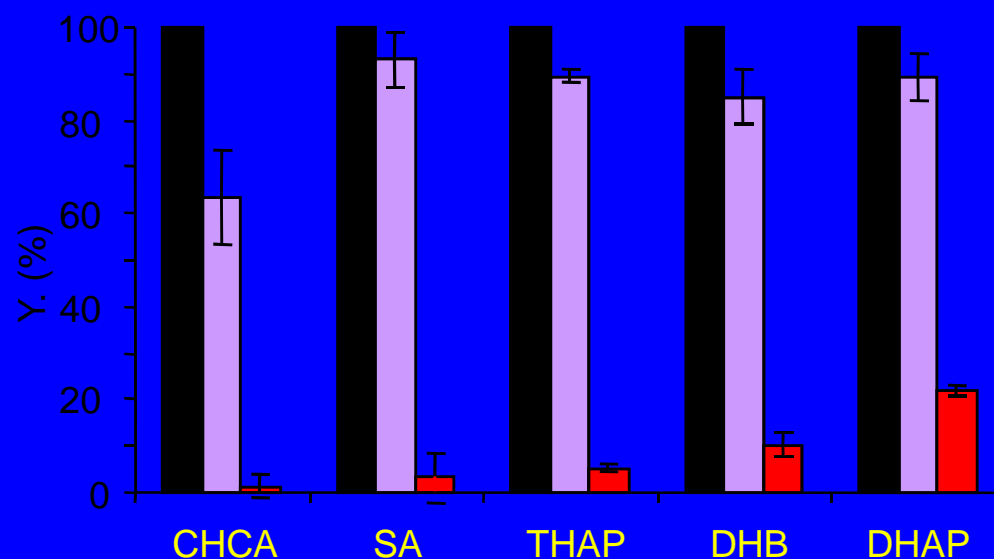
population I ~ population II : free NH₂ at N-term

population II only: basic residue (R)

-the nature of the matrix
Ac-DAEPPILYSEY-NH₂ : y₈
H-DAEPPILYSEY-NH₂ : y₈ et (M+H)⁺
H-DAEPPILYSER-NH₂ : (M+H)⁺

$$y = \frac{I(MH^+)}{I(MH^+) + I(y8)} \quad \% \text{ population II}$$

- Ac-DAEPPILYSEY-NH₂ : y8
- H-DAEPPILYSEY-NH₂ : (M+H)⁺ and y8
- H-DAEPPILYSER-OH : (M+H)⁺



Correlation with PA?

PA (kJ/mol)	CHCA	SA	THAP	DHB	DHAP
840	874	891	854	870	

Correlation with initial velocity ?
(relative to CHCA)

1	1.16	1.38	2.3	2.46
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role of the collision rate in early step of ablation : v_{in} low \rightarrow high collision rate

- a) Population I (y8): produced by Pro effect in the target, first step of ablation proton on/in the vicinity of the peptide (preformed ion, crystallisation process)
- b) Population II (low stability MH⁺): proton transfer in the plume expansion+ Pro effect

MALDI-TOF analysis of proline-rich peptide: carried out with extreme cautions

Second example: detection of peptides with D-residue, metastable decomposition

- inversion from L- to D-configuration:

⇒ post-translational modification (peptidylaminoacyl-L/D isomerization)

opioid peptides: antimicrobial activity only for one inversion L to D (dermorphin)

greater stability to proteases

- challenge for mass spectrometrists to detect such PTM

. classically : LC, ESI MSMS (kinetic method, metal cationization, ECD...)

. D/L : changes in thermochemical properties of ions in gas phase

. comparison: D- peptide/ all-L peptide

MALDI-TOF-TOF?

Collaboration D Soyez et al Biogenèse des Systèmes Peptidiques (UPMC)

primary structure	(M+H) ⁺ calcd <i>m/z</i>
Y <u>D</u> AFGYPS-NH ₂	803.37
YAFGYPS-NH ₂	803.37
Ac-Y <u>D</u> AFGYPS-NH ₂	845.38
Ac-YAFGYPS-NH ₂	845.38
<hr/>	
ASAWFTN	796.36
A <u>D</u> SAWFTN	796.36
AS <u>D</u> AWFTN	796.36
AS <u>A</u> DWFTN	796.36
ASAW <u>D</u> FTN	796.36
Ac-ASAWFTN	838.37
Ac-A <u>D</u> SAWFTN	838.37
<hr/>	
ASAWFTNDEC	1143.44
ASAWFTNDEC	1143.44

Dermorphin

opioid peptide, D-A in position 2

N-term Vitellogenesis Inhibiting Hormone (VIH)

Neuropeptide (lobster) D-W in position 4 and all-L

Heptapeptide miming endoproteinase Asp-N cleavage of VIH

ASAWFTN all-L

ADSAWFTN

ASDAWFTN

ASAWDFTN (*)

ASAWFTN

MALDI-TOF: MH⁺ and (M+C)⁺ C=Na and K

Sachon et al Anal. Chem. 2009, 81,4839

Vitellogenesis Inhibiting Hormone

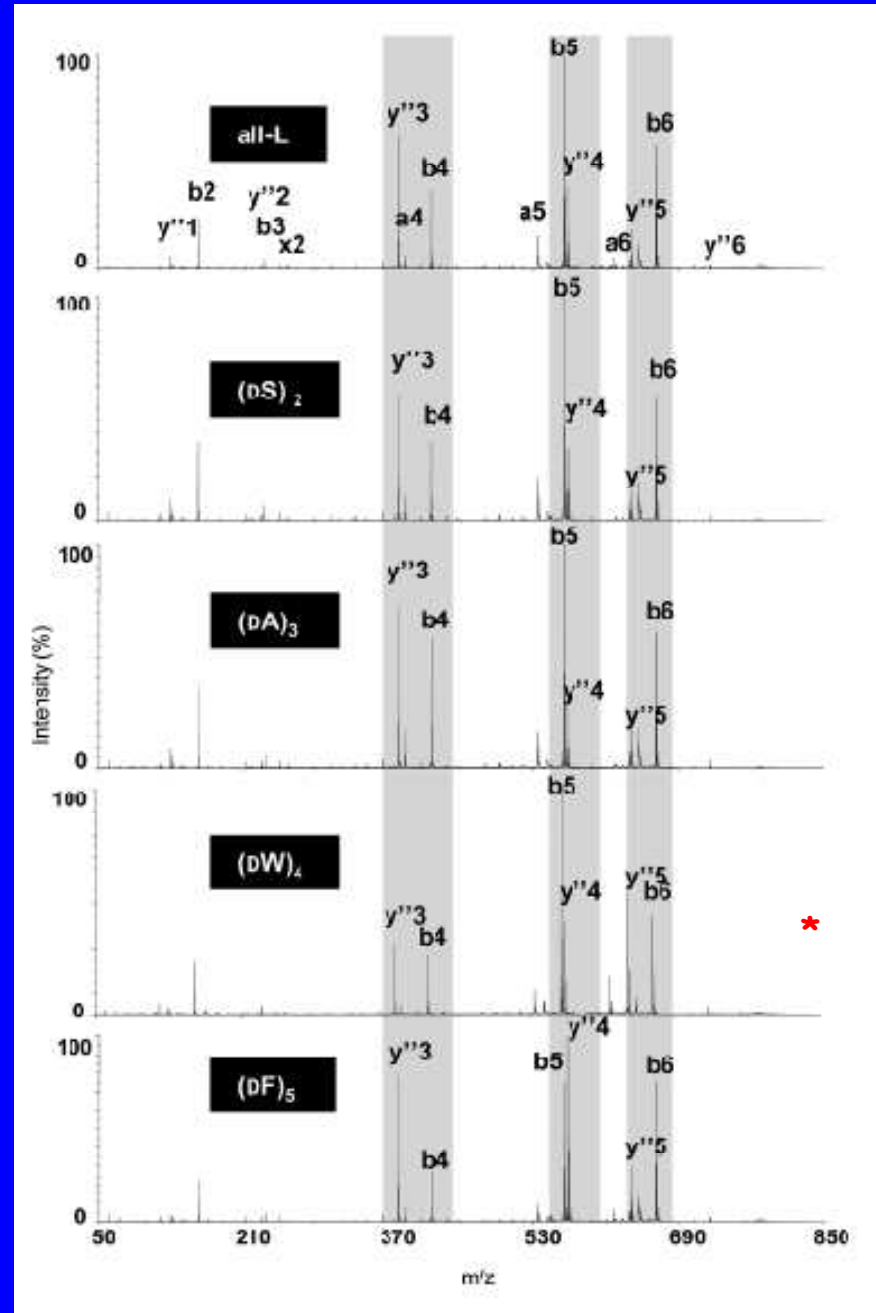
ASAWFTN

Heptapeptide miming
endoproteinase Asp-N cleavage
of VIH

MALDI-TOF-TOF (M+H)⁺

With gas: $2.6 \cdot 10^{-5}$ Pa

Same variation without gas



Discrimination of peptides isomers

ASAWFTN all-L

A^{DS}AWFTN

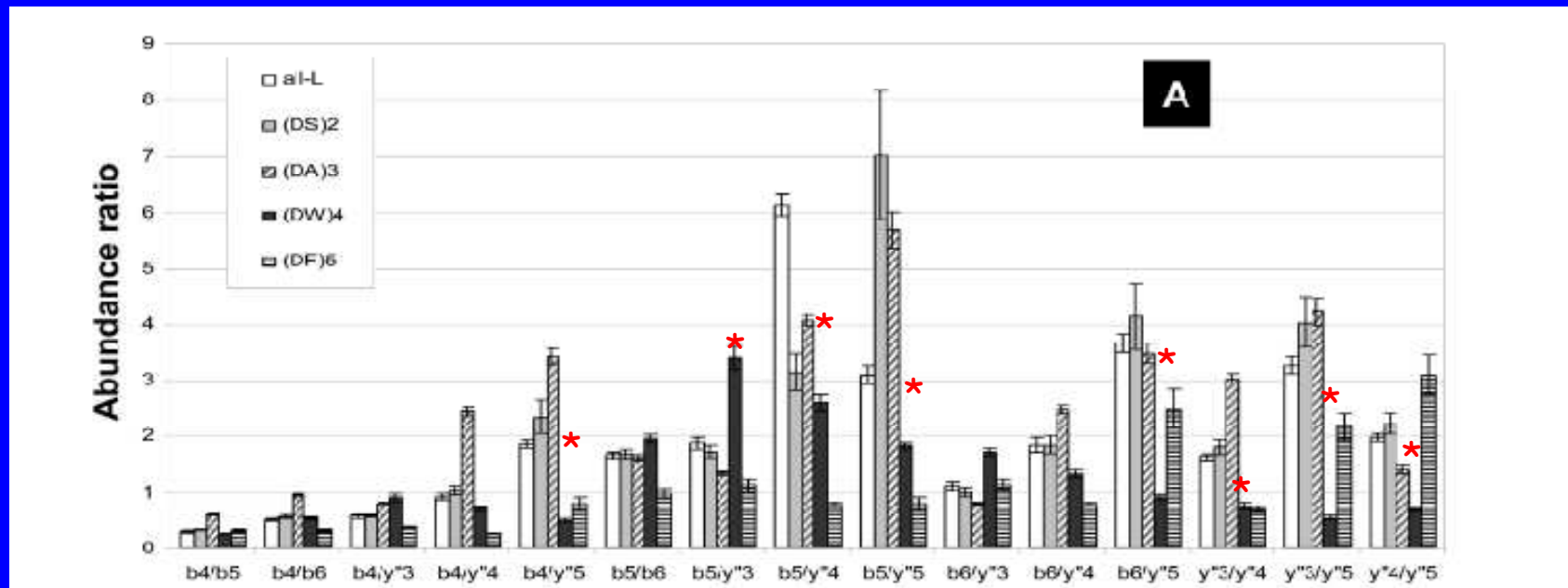
AS^DAWFTN

ASAD^WFTN (*)

ASAW^DFTN

N-term region of Vitellogenesis Inhibiting Hormone

8 different samples, same laser fluence



Many couple of fragments are fingerprints allowing the discrimination

Metastable & CAD results: very similar

- Chiral Recognition factor: $R_c = (F_i/F_j)_D / (F_i/F_j)_L$ on $(M+H)^+$ $R_c > 1$ or $R_c < 1$

$R_c = 0.7$ (dermorphin, b_5/γ''_5) 1.58 (Ac-dermorphin, b_5/γ''_5)

$R_c = 1.6-2.5$ (VIH heptapeptide D in position 2, 3, 4, 5)

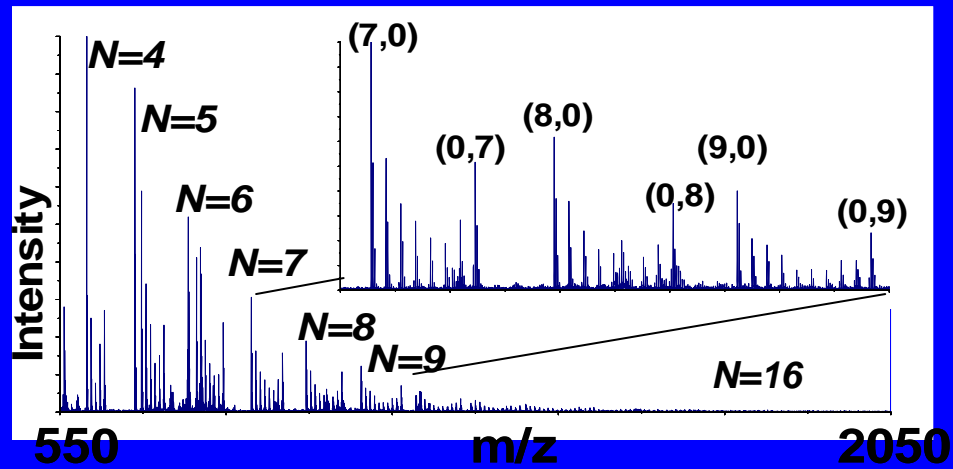
$R_c = 7.12$ (VIH decapeptide (D-W)₄, γ''_8/γ''_6)

- No discrimination between isomers with MSMS $(M+Na)^+$

- Quantification of isomers in mixture: possible

limitation: difference of cation affinity between the two isomers

MALDI-TOF: distribution of Val oligopeptides (N=3-20)



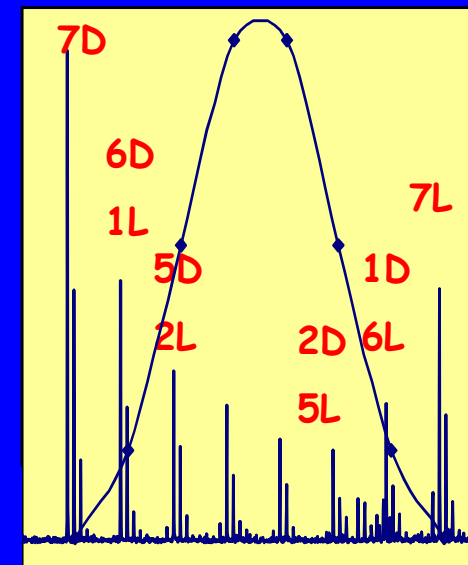
m/z of the oligopeptide: composition in D and L but not the sequence

D-Val-NCA:L(d8) Val-NCA Co-crystals 1:1 in water

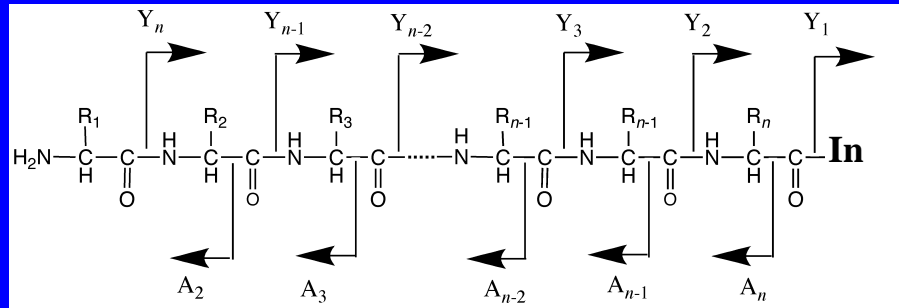
Initiator L-PheOMe (50%)

Homochiral species (N,0) and (0,N) the most abundant

N=7 residues



Fragmentation of Na cationized oligopeptides: very weak metastable decomposition,
CAD (1keV, 2×10^{-5} Pa) requisite



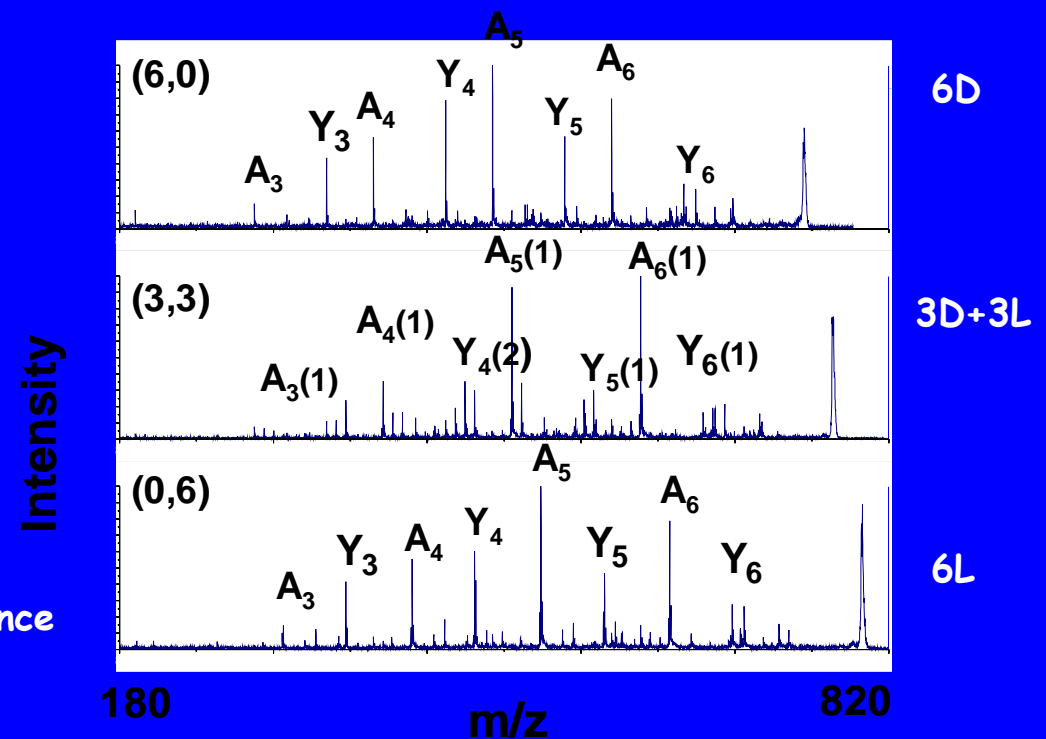
$(M+Na)^+$

Y and A series Na cationized

Polymerization quasi-racemic
DL(d8) Val-NCA in aqueous solution;
initiator: L-ValOMe

(3,3): 20 combinations of L and D Val

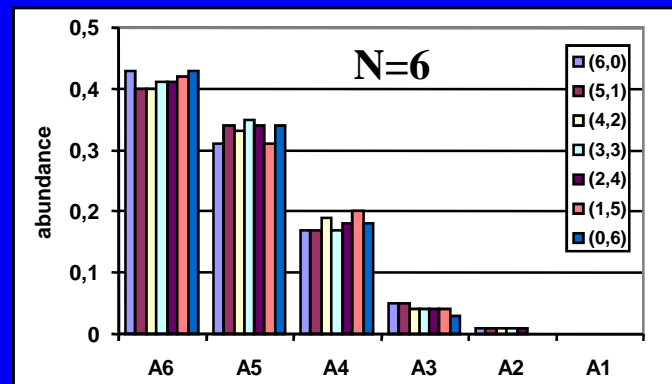
Quantitative analysis of fragments abundance



Quantitative analysis of fragment abundance and possible sequences

→ residues configuration does not affect the fragmentation ratio.

-the total intensity of a given fragment is constant for the oligopeptides of the same length
a few hundreds samples and thousands of MSMS:



DL(d8)-ValNCA with n-butylamine

- role of Na cationization: Na chelated with a folding of the oligopeptide around it

Comparison experimental fragments abundance: abundance if random distribution

Are some sequences more overexpressed or underexpressed?

-All results on oligopeptides showing a strong enhancement of homochirality:
sequence with two blocks of residues of opposite configuration are overexpressed

exemple: DDDLLL-Initiator overexpressed
LLLDDDD-Initiator
LLLDDDD-Initiator D

DLDLDL-Initiator: underexpressed
LLDLDDL- Initiator

→ Results in agreement with the model of polymerization

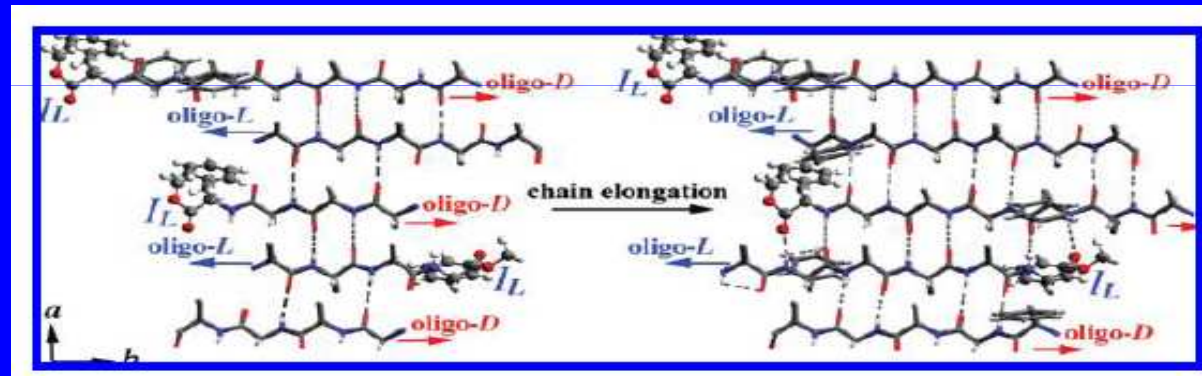
short isotactic oligopeptides+enantiomeric blocks oligopeptides

↓ Self-assembly

β-sheets (colloidal aggregates)

↓

Steric control in the
formation of long isotactic
peptides



Conclusions:

- MALDI-TOF et TOF -TOF : large capability to explore various ion stabilities
combination of linear and reflector modes (+ and - ions), effects of delayed extraction
- Fragile peptide (Proline effect): atypical ISD processes → fragments in MS
fragmentation in target
not taken into account for data base searching (non trypsin digestion)
- Peptide with one D residue
discrimination of isomers using metastable decay of $(M+H)^+$
quantification: possible artefacts (cation affinity of isomers)
- Complex peptides with many D- and L-residues as $(M+Na)^+$
collisions at high energy: need many experiments for characterizing sequence
 Na^+ « erases » the nature D and L of the residues

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