EU FT-ICR MS

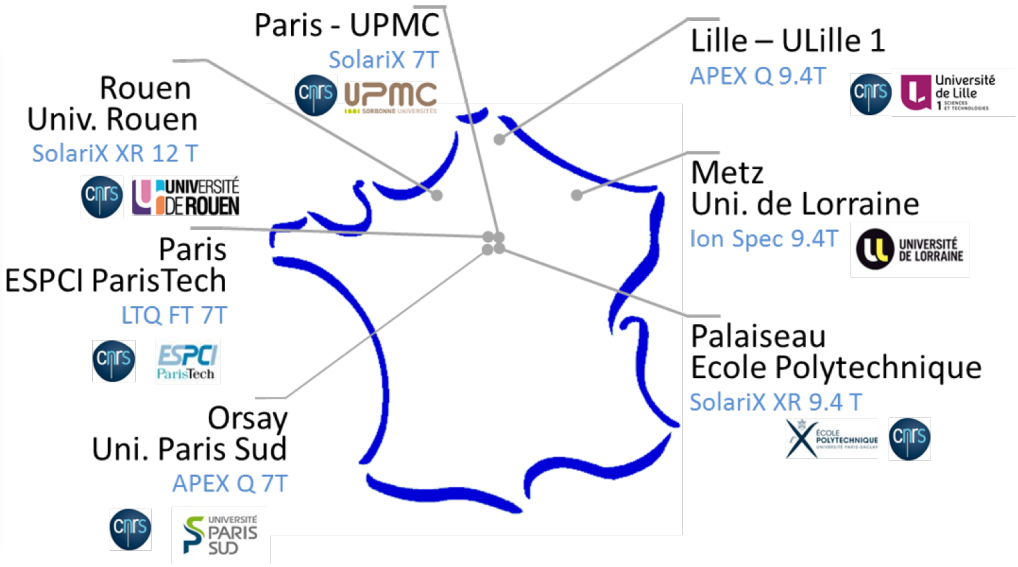
UNIVERSITÉ PARIS SUD

Comprendre le monde, construire l'avenir®

METHODS FOR ION ACTIVATION

Guillaume van der Rest
Laboratoire de Chimie Physique
Université Paris Sud

National network of high field FT-ICR mass spectrometers (FR 3624)



Informations and proposal submission :

www.fticr.org

A national research infrastructure, **open** to the scientific community, accessible **at no additional cost** after scientific assessment of the proposals.

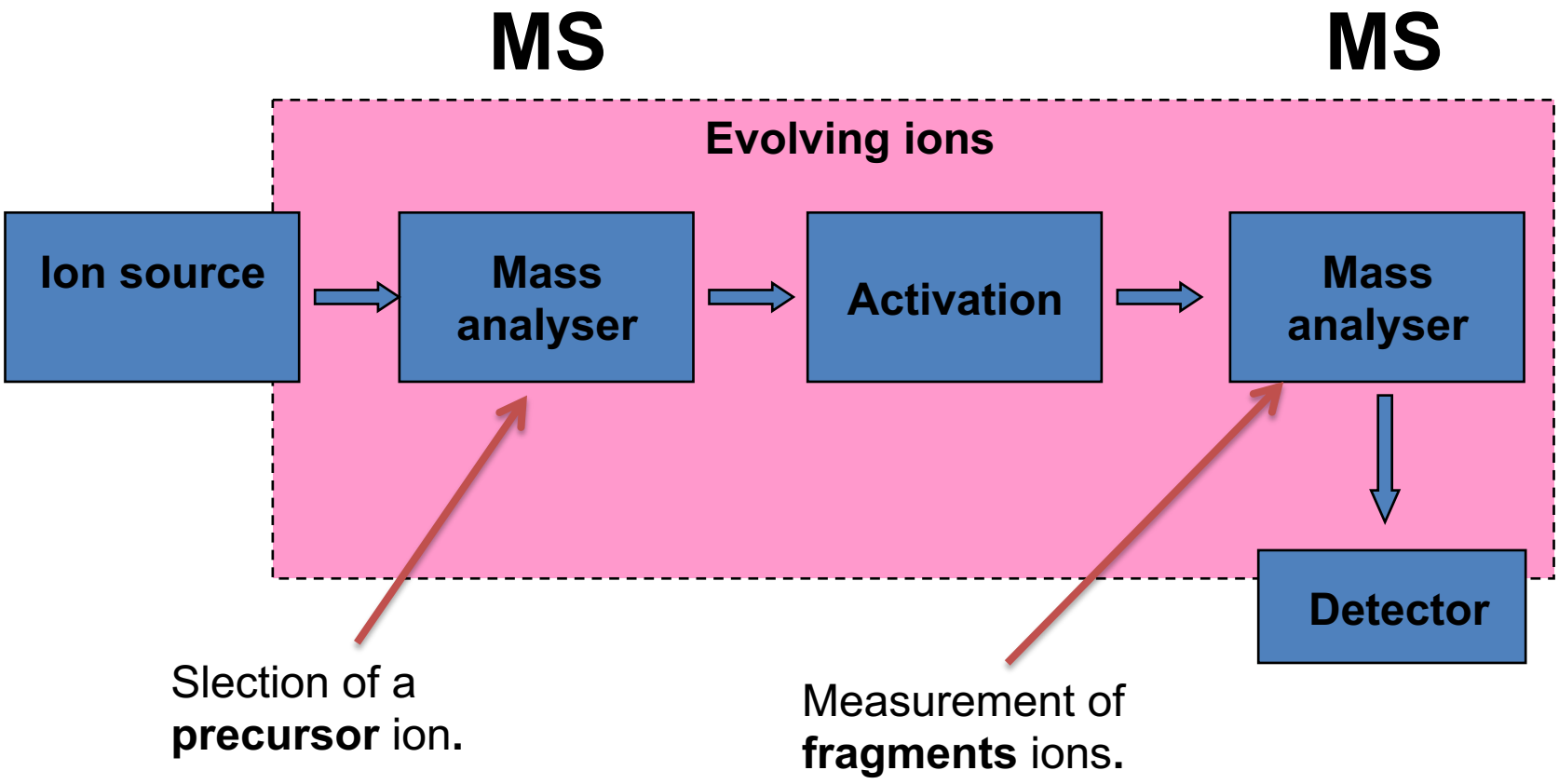
WP2 US 21 T access

- EU_FTICR_MS Project includes access to US facilities, on the basis of 10 travel / accomodations for the 4 years period.

- Projects are intended to either:
  a) Prepare a bid for a top-line instrument installation in Europe
  b) Provide help to access these world class instruments for users in the EU perimeter

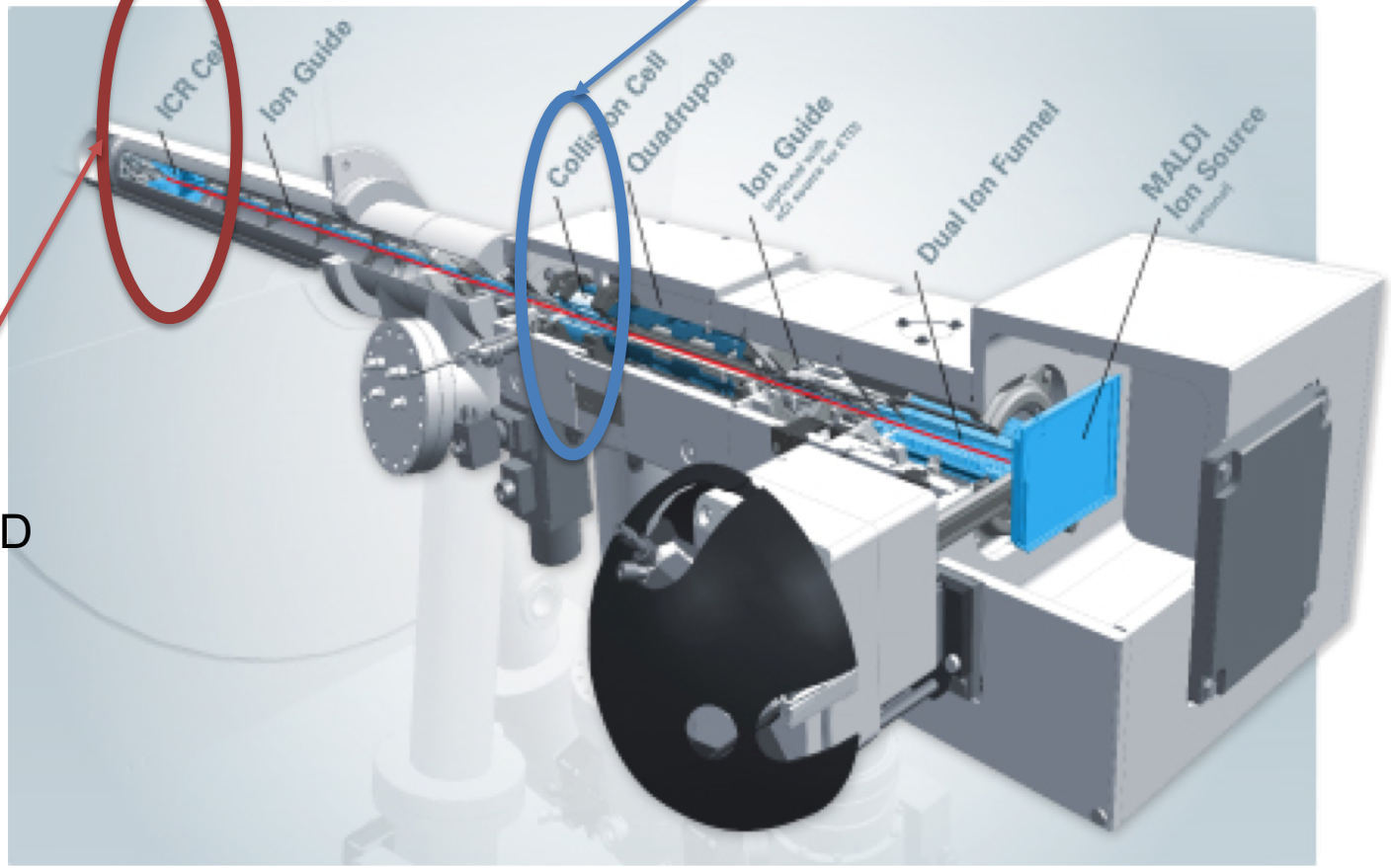
- First call for proposals starting now, ending at the end of Septemeber, and put on the website, with an ephasis for proposals aimed at gathering data for preparing the IFRA-DEV call in 2019, but other projects will also be examined.

Tandem mass spectrometry



Ion activation in FT-ICR instruments

Collision cell = CID / ETD / Reactivity



ICR Cell :
(SORI–)CID
ExD
IRMPD
UVPD
BIRD
…

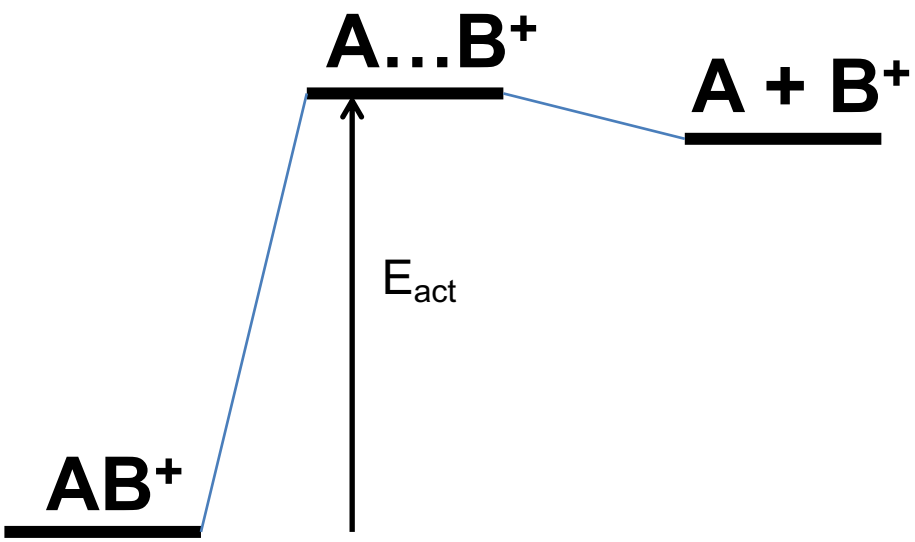
FT-ICR specificity of activation techniques

- Prior to the FT-ICR cell:
  - No direct specificity of the FT-ICR analyser on the fragmentation techniques
  - Gain in the ultra-high MS resolution and accuracy, useful for top-down protein analysis, simultaneous fragmentation of many precursors within a selection window
- Using the FT-ICR cell properties:
  a) Ion trapping properties (up to hours, ions aligned along the cell axis
     Photon activation, BIRD, ExD, Reactivity
  b) Ion manipulation using radiofrequency pulses
     CID, SORI-CID, MS^n

Outline

1. General considerations on ion activation
2. FT-ICR cell for collisional activation
3. FT-ICR cell as a trap
    1. Interaction with photons
    2. Interaction with reactive gas
    3. Interaction with electrons

Kinetics and energetics of fragmentation



Fragmentation : generally an endothermic reaction.

Thermodynamics point of view :

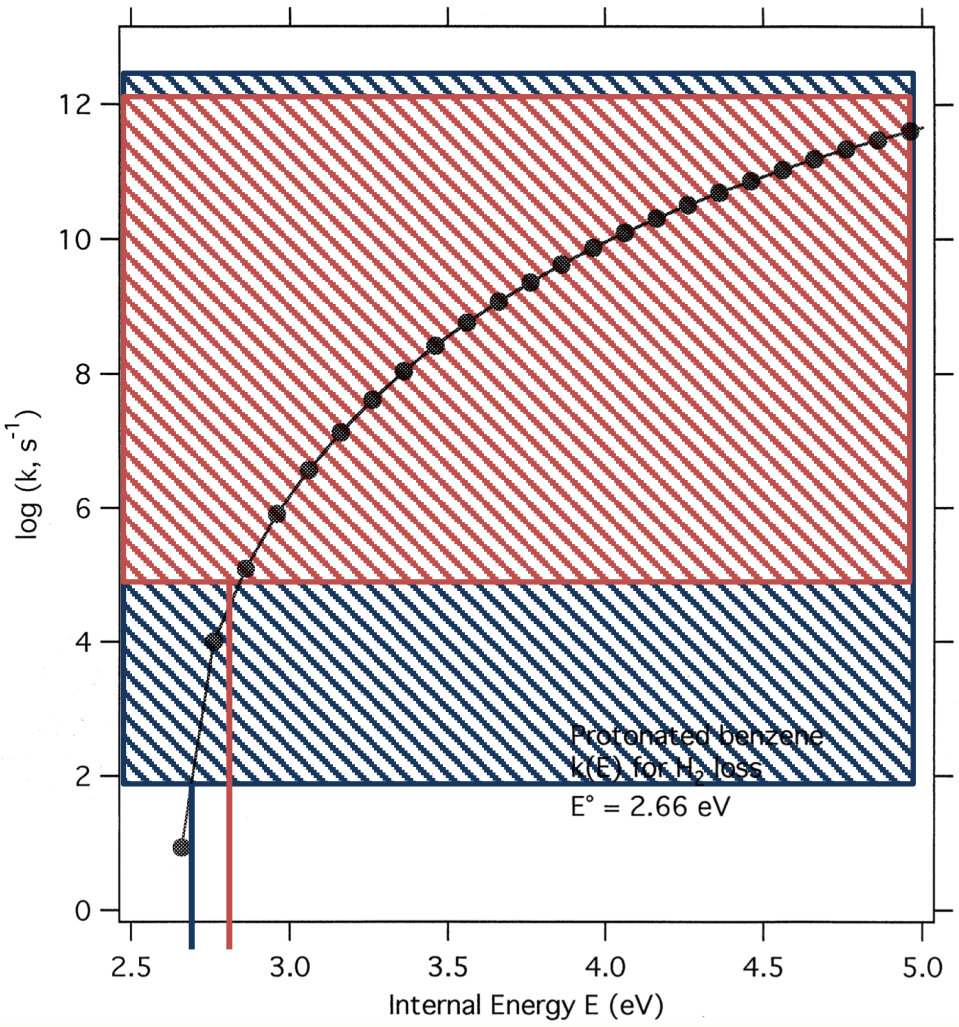
$E_{int} < E_{act}$: No fragmentation

$E_{int} > E_{act}$: Fragmentation possible with a variable speed.

Kinetics point of view :
Unimolecular dissociation rate constant depends on the excess energy and transition state

Characteristic times can vary a lot depending on MS conditions.

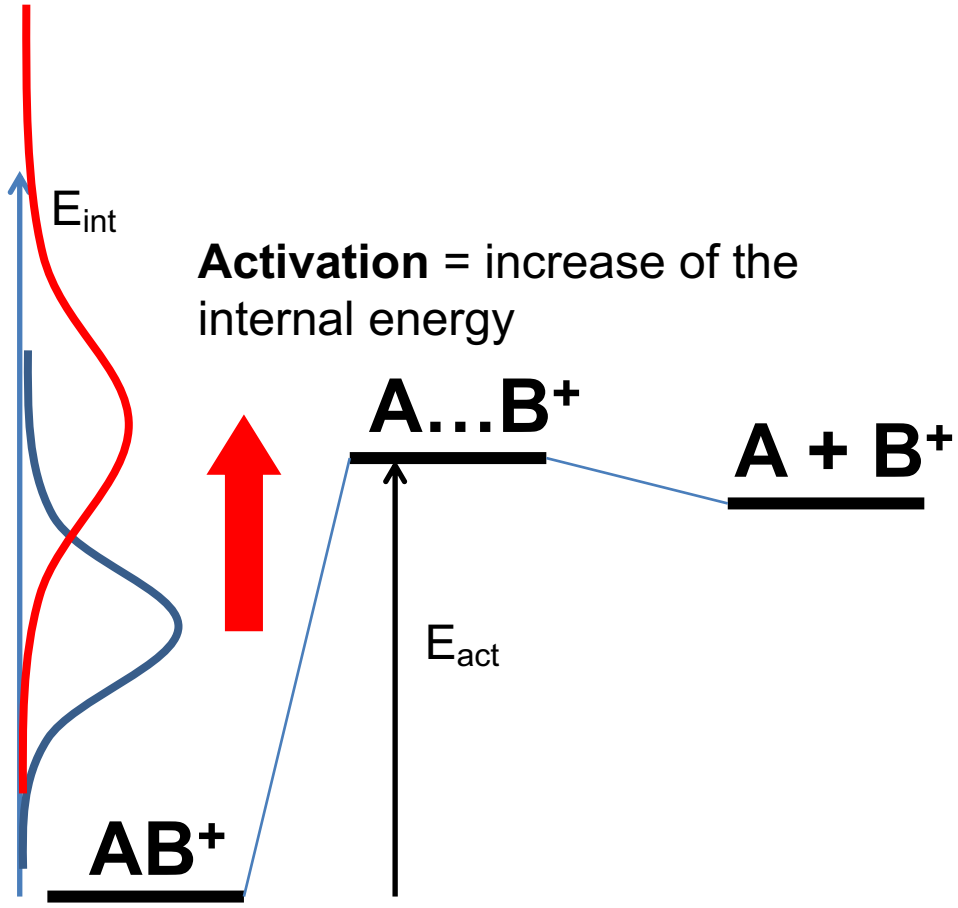
Kinetic shift and the observation time window



« Beam » type experiments.

« Ion trap » type experiments.

Precursor ion activation



Activation methods:

Collision with an inert gas (CID)

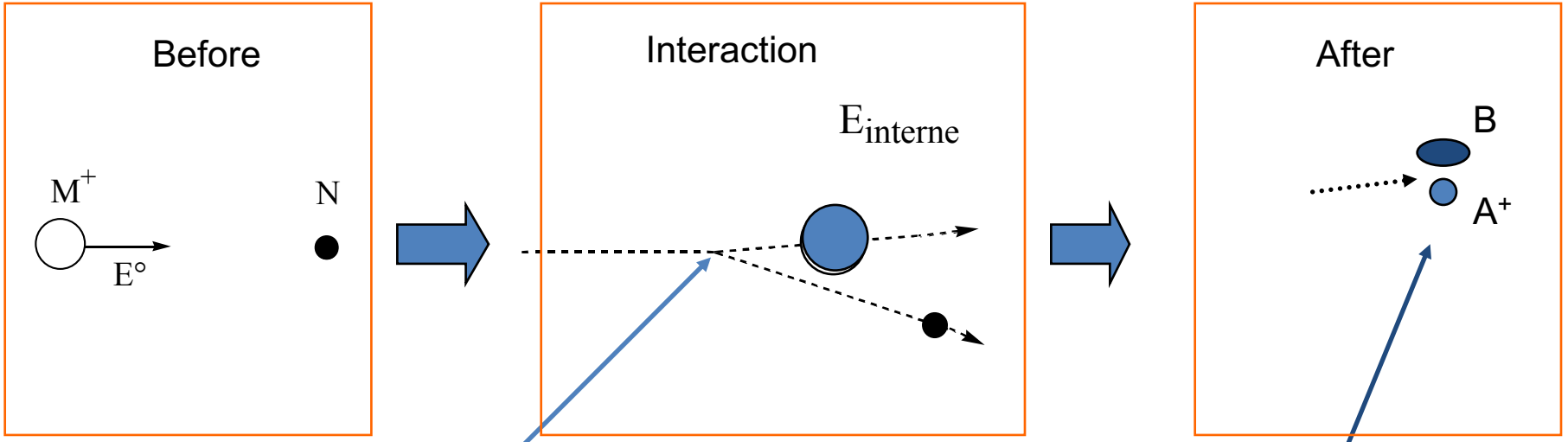
Collision with a surface (SID)

Photon absorption :
- **UV (UVPD)**
- **IR (IRMPD)**

Reactive activations :

Reactive collisions with a gas
Cation / anion reaction
Interaction with electrons.

Collision induced dissociation



Collision: a fraction of the kinetic energy E°
Is converted into internal energy M^+

Activated M^+ ion fragments
at a given kinetics:
$M^+ \rightarrow A^+ + B$

Factors controlling the conversion into internal energy in the M^+ ion:

1°) Mass of M (the highest, the lowest conversion is possible in inelastic conditions),
2°) Mass of the neutral N (ex: 28 for N_2, 4 for He)
3°) Initial kinetic energy E°

Collision activation conditions

Classical gases in collision cells :

Ar, N_2, Xe, He

Control of the collision energy :

Acceleration of the ion before reaching activation region (HCD, Multipole cell)

rf excitation at secular frequency (ion trap)

The maximum amount of energy that can be transferred in a single collision is :

$$E_{int\ max} = [\ m(N)/(m(M) + m(N))\ ]\ E^{\circ}$$

Collision energy regimes

- High energy (> keV) : unusual in FT-ICR.
  - Very fast ion / neutral interaction times
  - Within a molecule, the nuclei are fixed
  - Electronic excitation

- Low energy (eV – 100 eV)
  - Interaction times allow rearrangement of the nuclei as the electron clouds collide.
  - A « hard sphere » model is reasonnable
  - Mostly vibrational energy.

Number of collision events in an activation process

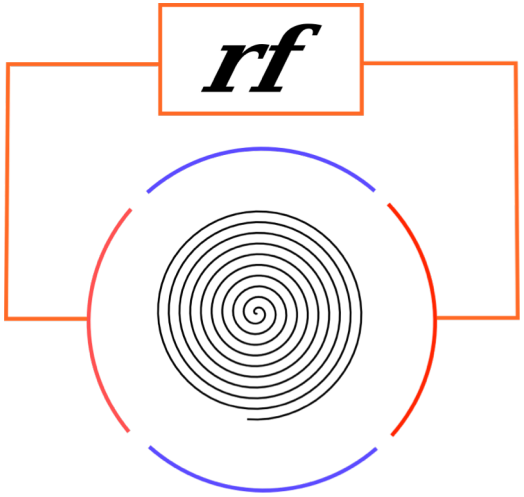
- Single collision : a single collision leads to fragmentation
  - Directly limited by the center of mass energy
  - Requires high kinetic energies and / or small molecules.
  - Low pressure cells.
- Multiple collisions : kinetic energy is progressively transformed into internal energy by successive collisions
  - Allows reaching higher internal energies
  - Competition between adding energy through a collision and other relaxation processes (fragmentation, photon emission, thermalisation) becomes an issue.

COLLISION INDUCED DISSOCIATION IN FT-ICR CELLS

CID in an FT-ICR cell

On resonance excitation :

The ion kinetic energy can be controlled through the excitation duration and amplitude :

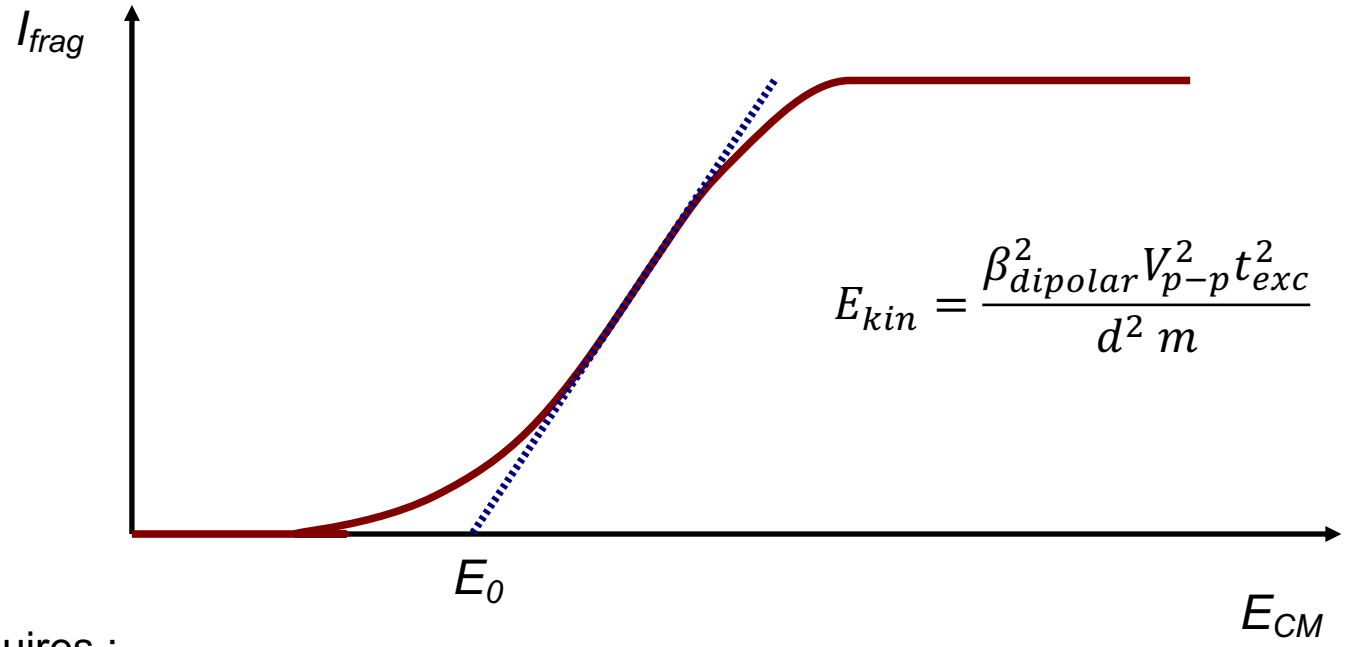


$$r = \frac{\beta_{dipolar} V_{p-p} t_{exc}}{2\ d\ B_0}$$

$$E_{kin} = \frac{\beta_{dipolar}^2 q V_{p-p}^2 t_{exc}^2}{d^2\ m}$$

Some numerical values, considering B = 7T, m/z 1000, a cylindrical cell of r = 3 cm
At r_{max} : E_{kin} 2125 eV
At 10% r_{max} : E_{kin} 21,3 eV
Considering collision gas Ar (40 Da) :
E_{CM} at 10% r_{max} : 0,82 eV

Single collision conditions: measurement of threshold fragmention energies



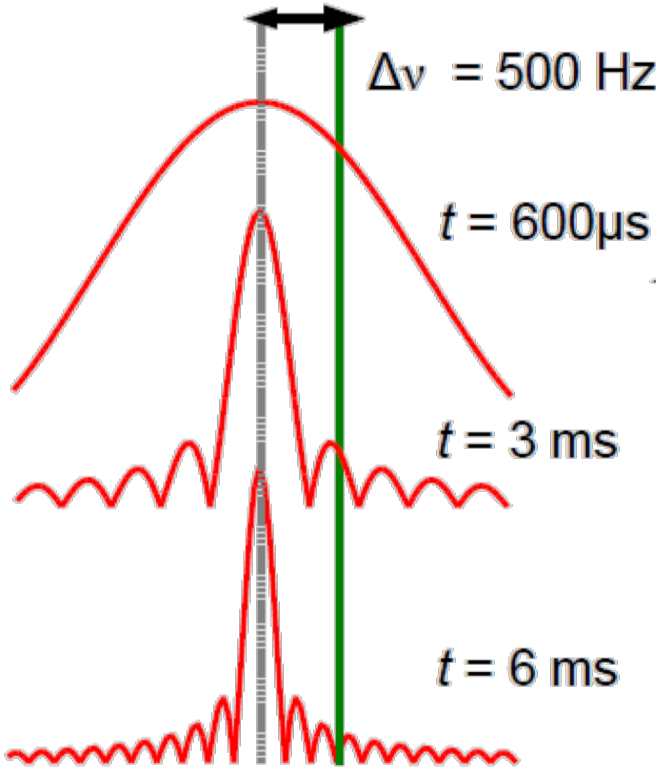
Requires :

- Checking that gas pressure is low enough to allow for a single collision to occur in the experimental time frame.
- Measurement of the $V_{p\text{-}p}$ or calibration with a reference fragmentation pathway if absolute values are to be derived.

Limitations :

- Maximal radius has to be kept low in order to not perturb excitation / detection stage.

Sustained off resonance CID SORI-CID

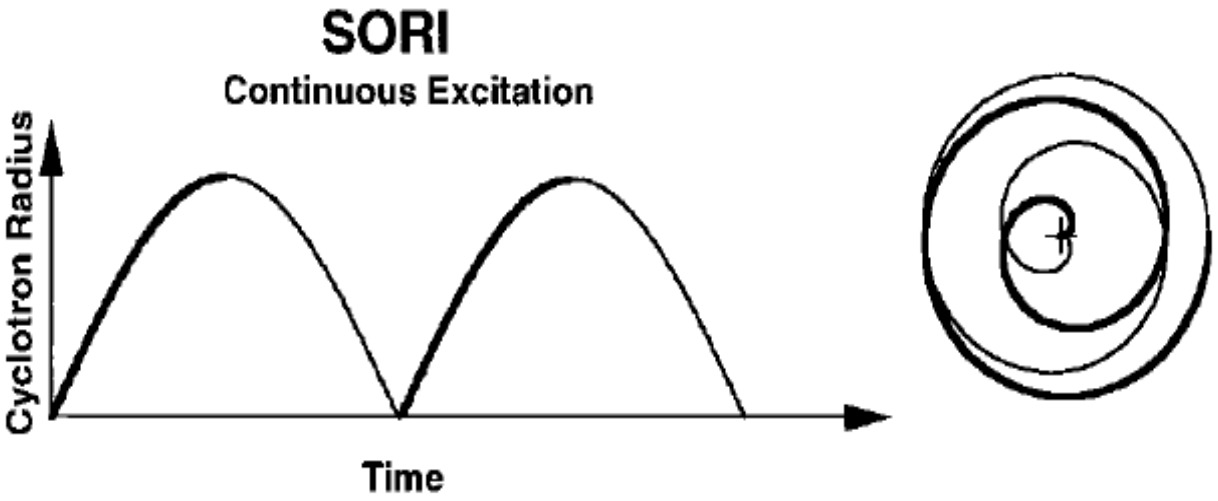


$$r(\Delta\nu, t_{exc}) = \frac{\beta_{dipolar} V_{p-p}}{2\, d\, B_0} \frac{\sin(\pi\, \Delta\nu\, t_{exc})}{\pi\, \Delta\nu}$$

$$E_{kin}(\Delta\nu, t_{exc}) = \frac{\beta_{dipolar}^2 q^2 V_{p-p}^2}{d^2\, m} \left(\frac{\sin(\pi\, \Delta\nu\, t_{exc})}{\pi\, \Delta\nu} \right)^2$$

- Radius and kinetic energy will oscillate as the excitation period progresses.
- Maximal radius will remain limited.

SORI-CID



- Kinetic energy is brought to the ion as long as fragmentation (change in mass) has not occured.
- Although each collision is not very energetic, internal energy can be accumulated in the ion through mutliple collisions with the gas, allowing multiple collision activation.

In-cell CID summary

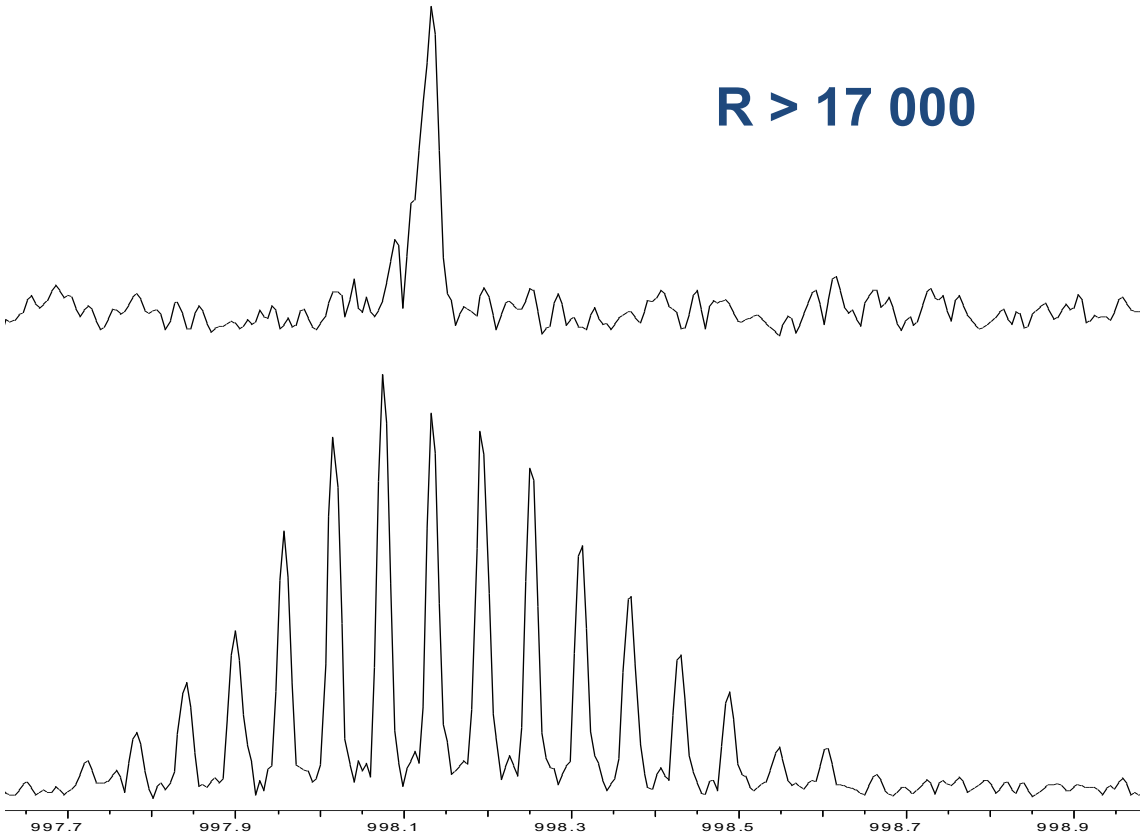
- Control on the internal energy deposited in the excitation process can be achieved.
- Large molecules can be activated using SORI-CID activation.
- Precursor ion selection can use the cell rf selection, potentially up to resolutions > 10 000.
- Multiple stages of activation can be performed (MS^n experiments, with a practical limit at n ≈ 4)

- Need to introduce a collision gas in the FT-ICR cell (decrease in resolution or long pumping delays to restore the base pressure)
- SORI activation can be time consuming (~ 0.5 to 1 s activation times)
- Overall, limited to low collision energies
  - SORI mode : only the lowest fragmentation pathways will be sampled

High resolution in the in-cell precursor ion selection

Broadband ion selection + 800 ms selection pulses on the isotopic peaks.

Broadband ion selection



Native myoglobin (10 µM – ESI) – zoom on the $[M, 17H]^{17+}$ charge state.

Performing surface induced dissociation in an FT-ICR cell

- Replacing the collision gas with a surface (SID) allows to perform collisions with greatly improved energy transfer.

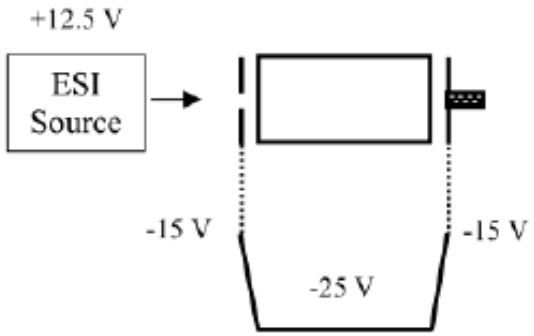
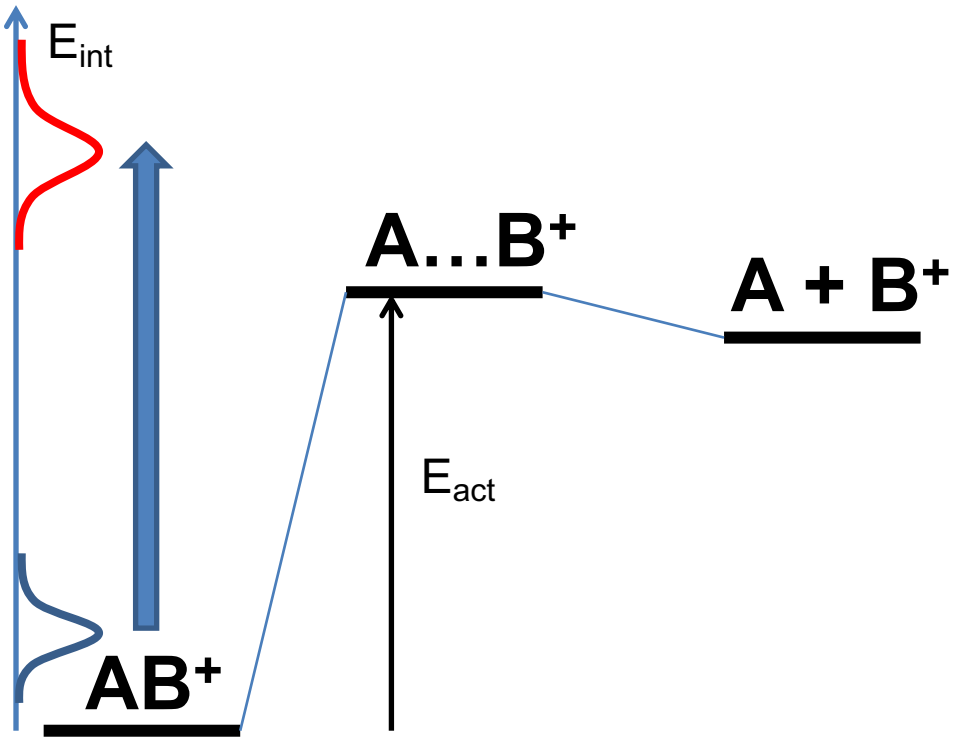


Figure 5. Schematic representation of the continuous SID experiments. The surface is in contact with the rear trapping plate of the ICR cell. Both trapping plates are at −15 V and the cell offset is at −25 V for trapping positive ions.

Laskin et al., *Anal. Chem.* **74**, 3255 (2002)

PHOTON INDUCED DISSOCIATIONS IN FT-ICR CELLS

Photon activation (UV – Visible)



Single photon activation :

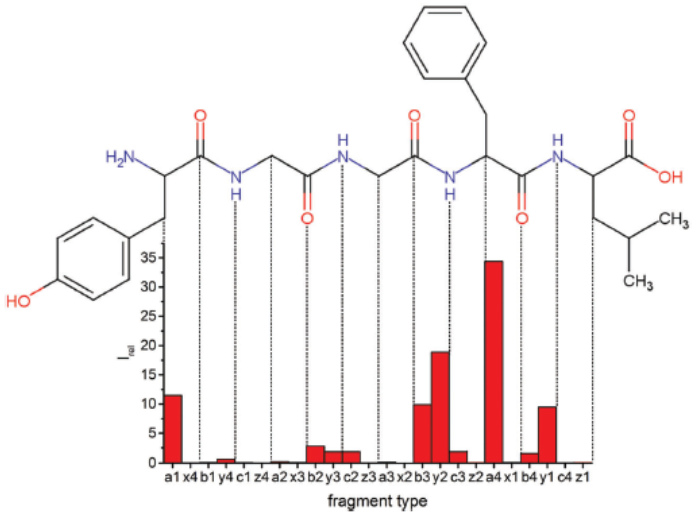
- Requires a photon with sufficient energy : ~ eV
- Requires an absorption band

- Proceeds through an electronic excited state, which can either directly dissociate or redistribute energy through intersystem crossing.

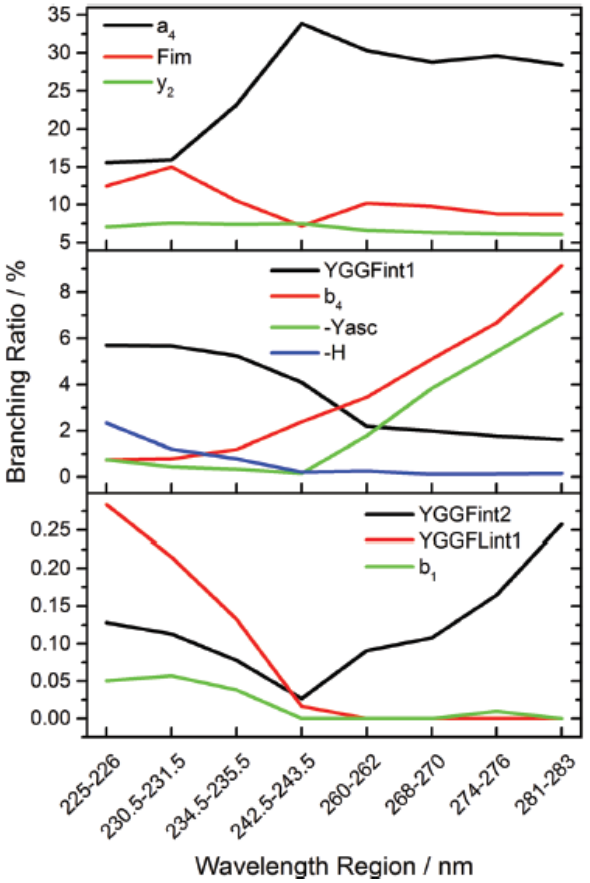
UV-PD and FT-ICR

- Old story:
  - 1990 – 2000 : many groups worked on UV activation in an FT-ICR cell. Mostly at 355 nm, 266 nm and 193 nm.
  - Ease to focus a laser beam along the main axis of the ICR cell
- Hardly a general method:
  - For physical chemistry: ions are at room temperature in an ICR cell, leads to line broadening.
  - 355 nm and 266 nm require a chromophore group in the molecule.
- Ion spectroscopy can be achieved through recording action spectra.

Tunable UV and FT-ICR



- Fragmentation favored close to the aromatic residues.
- Some dependence of the pathways on the laser wavelength.



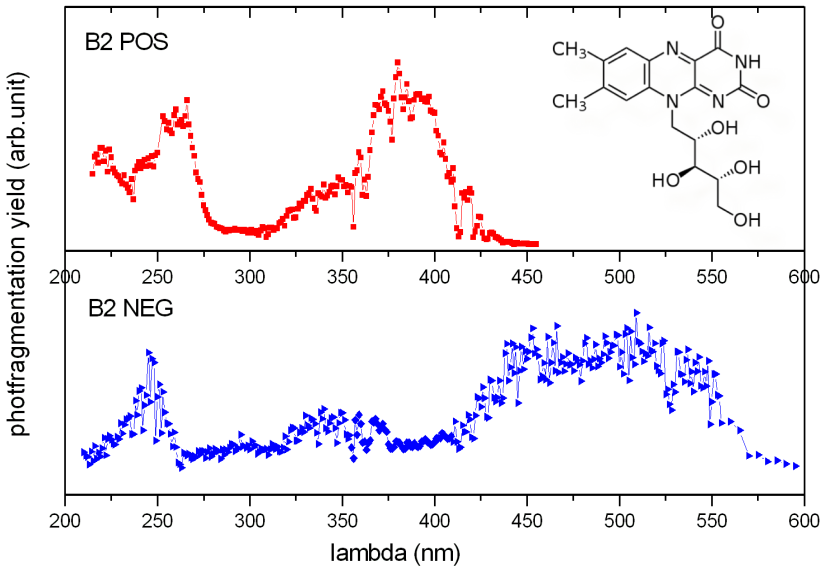
A. Herburger, C. van der Linde, M. Beyer, Phys. Chem. Chem. Phys. **19** 10786 (2017)

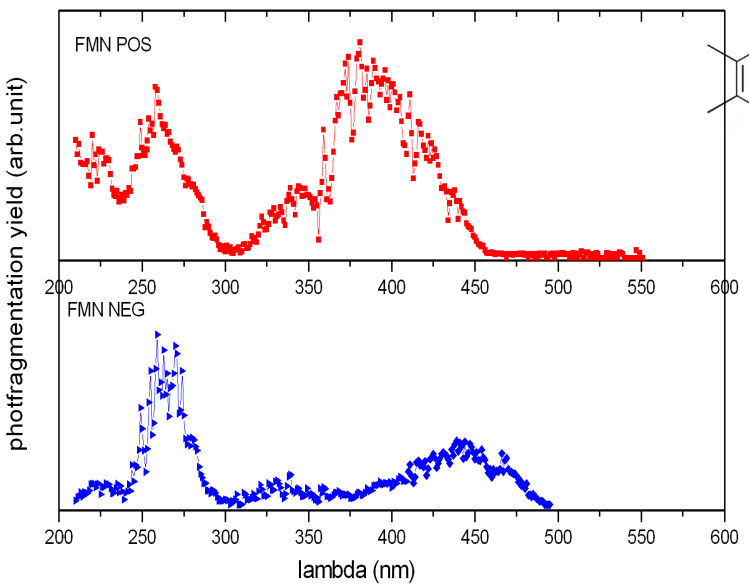
Tunable UV-PD

- FT-ICR trap is quite ideal for alignment with a laser and long activation periods due to a low photon flux.

Validation on small systems: flavin co-factors of flavoproteins

OPO UV-Vis laser @ Ecole Polytechnique





The optical response depends on the **chemical environment**

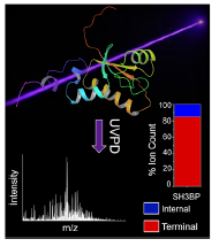
The optical response depends on the **charge environment**

UVPD at 213 nm

The Ups and Downs of Repeated Cleavage and Internal Fragment Production in Top-Down Proteomics

Yana A. Lyon,[1] Dylan Riggs,[1] Luca Fornelli,[2] Philip D. Compton,[2] Ryan R. Julian[1]

[1]Department of Chemistry, University of California, Riverside, 501 Big Springs Road, Riverside, CA 92521, USA
[2]Departments of Chemistry and Molecular Biosciences, and the Proteomics Center of Excellence, Northwestern University, N. Sheridan Road, Evanston, IL 60208, USA

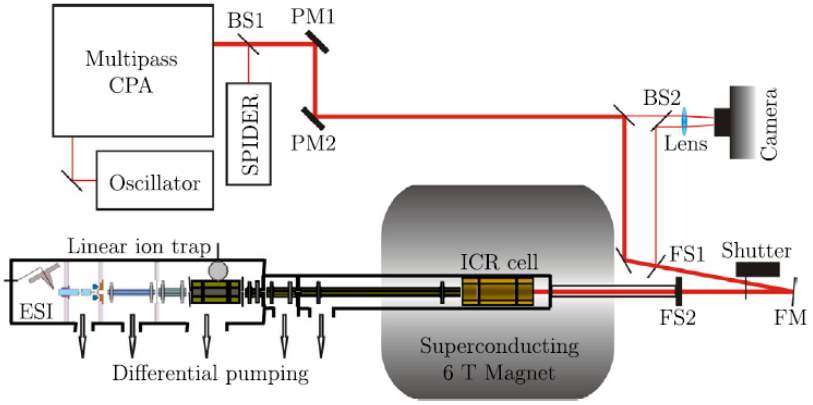


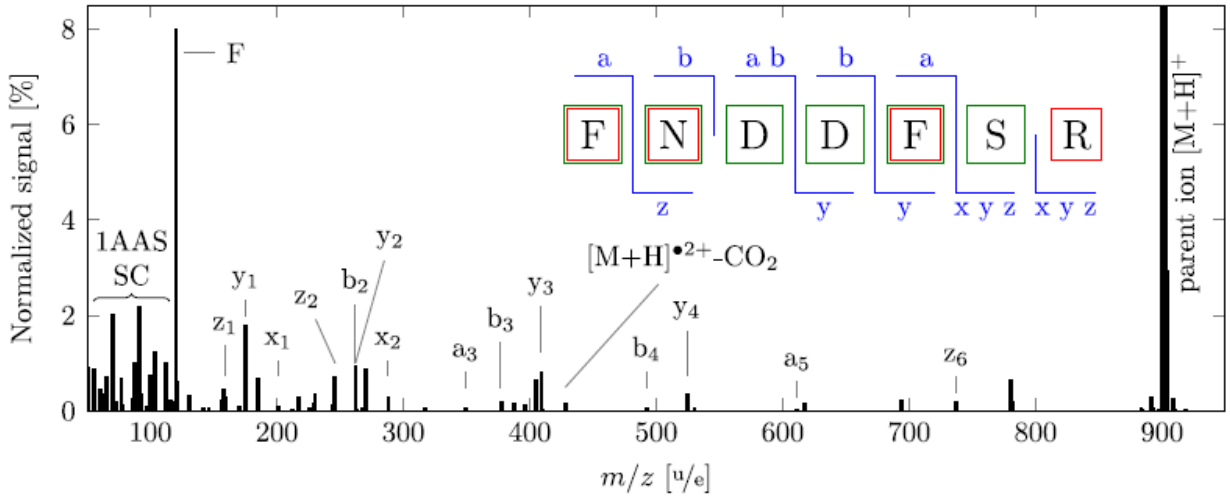
Abstract. Analysis of whole proteins by mass spectrometry, or top-down proteomics, has several advantages over methods relying on proteolysis. For example, proteoforms can be unambiguously identified and examined. However, from a gas-phase ion-chemistry perspective, proteins are enormous molecules that present novel challenges relative to peptide analysis. Herein, the statistics of cleaving the peptide backbone multiple times are examined to evaluate the inherent propensity for generating internal versus terminal ions. The raw statistics reveal an inherent bias favoring production of terminal ions, which holds true regardless of protein size. Importantly, even if the full suite of internal ions is generated by statistical dissociation, terminal ions are predicted to account for at least 50% of the total ion current, regardless of protein size, if there are three backbone dissociations or fewer. Top-down analysis should therefore be a viable approach for examining proteins of significant size. Comparison of the purely statistical analysis with actual top-down data derived from ultraviolet photodissociation (UVPD) and higher-energy collisional dissociation (HCD) reveals that terminal ions account for much of the total ion current in both experiments. Terminal ion production is more favored in UVPD relative to HCD, which is likely due to differences in the mechanisms controlling fragmentation. Importantly, internal ions are not found to dominate from either the theoretical or experimental point of view.
Keywords: UVPD, HCD, Statistical analysis, Internal ion

- Nd-YAG 5th harmonic
- Commercial device by Thermo
- Applied for top-down proteomics on Orbitrap systems

Femto-second laser induced dissociation

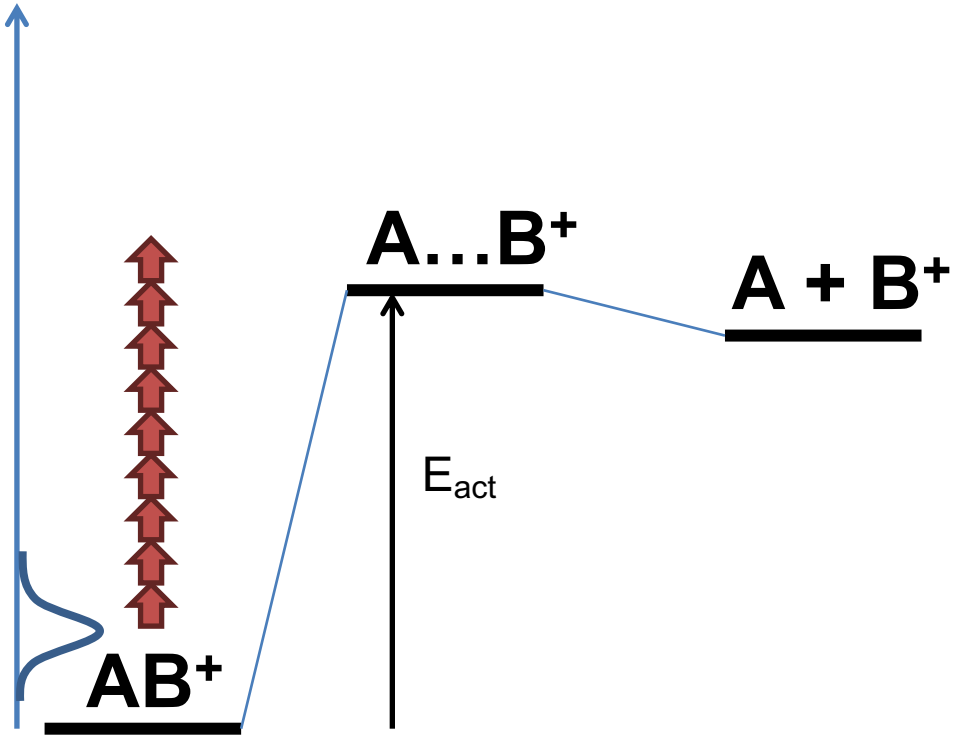
Technique introduced by G.E. Reid in 2009 for peptide fragmentation analysis, using an activation in a rf ion trap device.





C. Neidel et al. Chem. Phys. In press (2018)

Infra-red Multiphoton dissociation

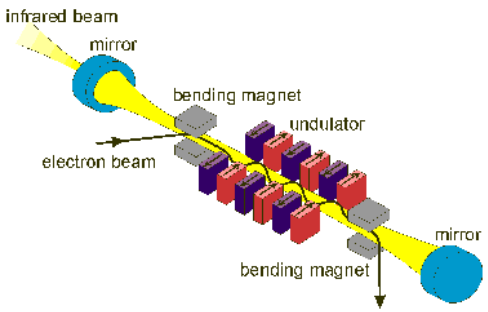


Multiple photon activation:

- IR photon ~ 0,1 eV
- Absorption of a number of photons is required to lead to fragmentation
  ***I**nfra-**R**ed **M**ulti-**P**hoton **D**issociation (IRMPD)*

- As for UVPD, an absorption band is required to observe fragmentation.

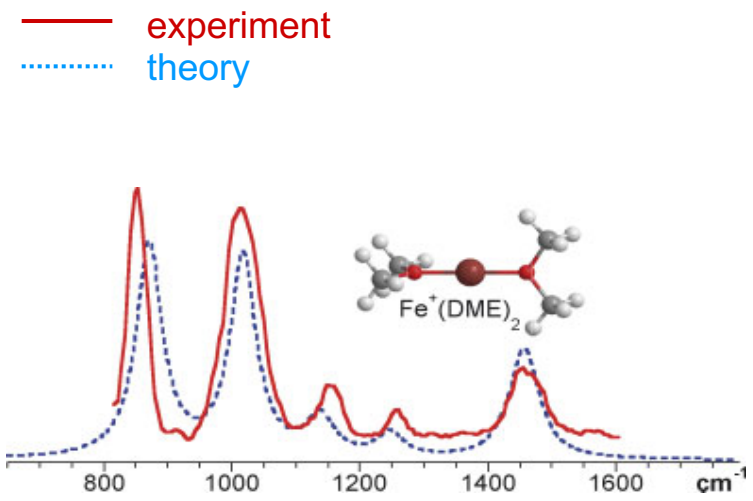
Photon sources for IRMPD



- Tunable lasers :
  - FEL Lasers (CLIO facility @ Orsay):
    - 1 W from 500 – 2000 cm^{-1}
    - Time structure ideal for IRMPD activation
  - OPO / OPA table-top lasers (@ Orsay also)
    - 2000 – 4000 cm^{-1}
    - ~ 0.1 W at 10 Hz
- CO_2 laser : fixed wavelength (10,6 µm)
  - High power (up to 25 W)
  - Low absorption bands are sufficient to lead to fragmentation.

Tunable IRMPD spectroscopy

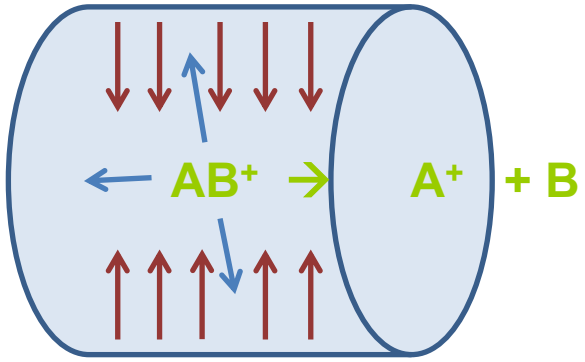
- Action spectroscopy: the fragment ion intensities are plotted as a function of wavelength.



BIRD

- Radiative equilibrium between ions and cell walls.

Presure ~10^{-6}-10^{-8} mbar
t ~ 10s of seconds



T_{walls}

Measurement of fragmentation kinetics at variable temperatures:

$$k(T) = A\, e^{-\frac{E_a}{RT}}$$

Access to kinetic parameters (A and E_a)

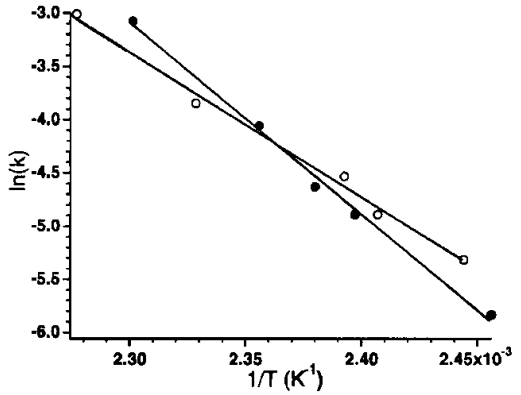


Figure 2. Arrhenius plot for dissociation of ubiquitin 5+ (○) (E_a = 1.2 eV; $A = 10^{12}$ s^{-1}) and 11+ (●) (E_a = 1.6 eV; $A = 10^{17}$ s^{-1}).

Application to a protein – ligand system

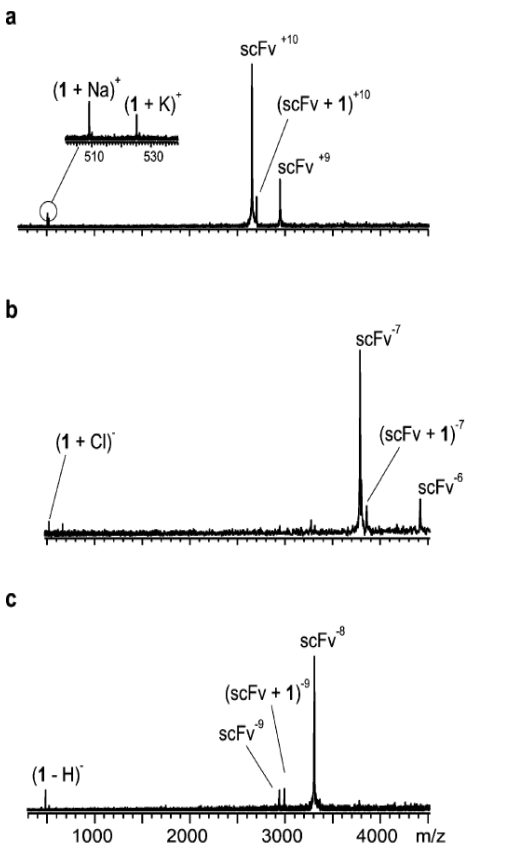
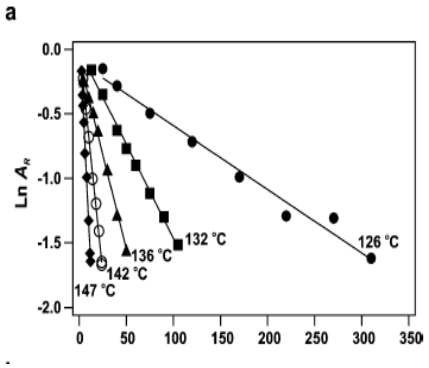


Figure 4. BIRD mass spectra obtained for protonated and deprotonated (scFv +**1**)$^{n+/-}$ ions (a) $n = +10$, 154 °C, 6 s; (b) $n = -8$, 147 °C, 5 s; (c) $n = -9$, 147 °C, 5 s.



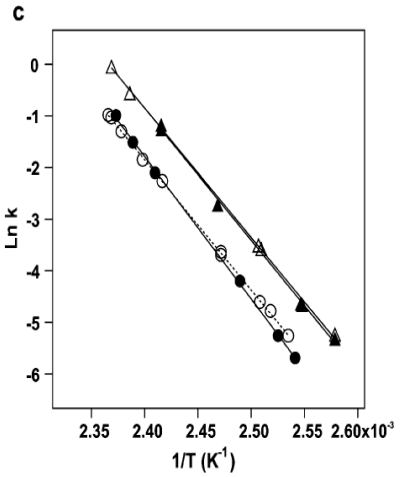
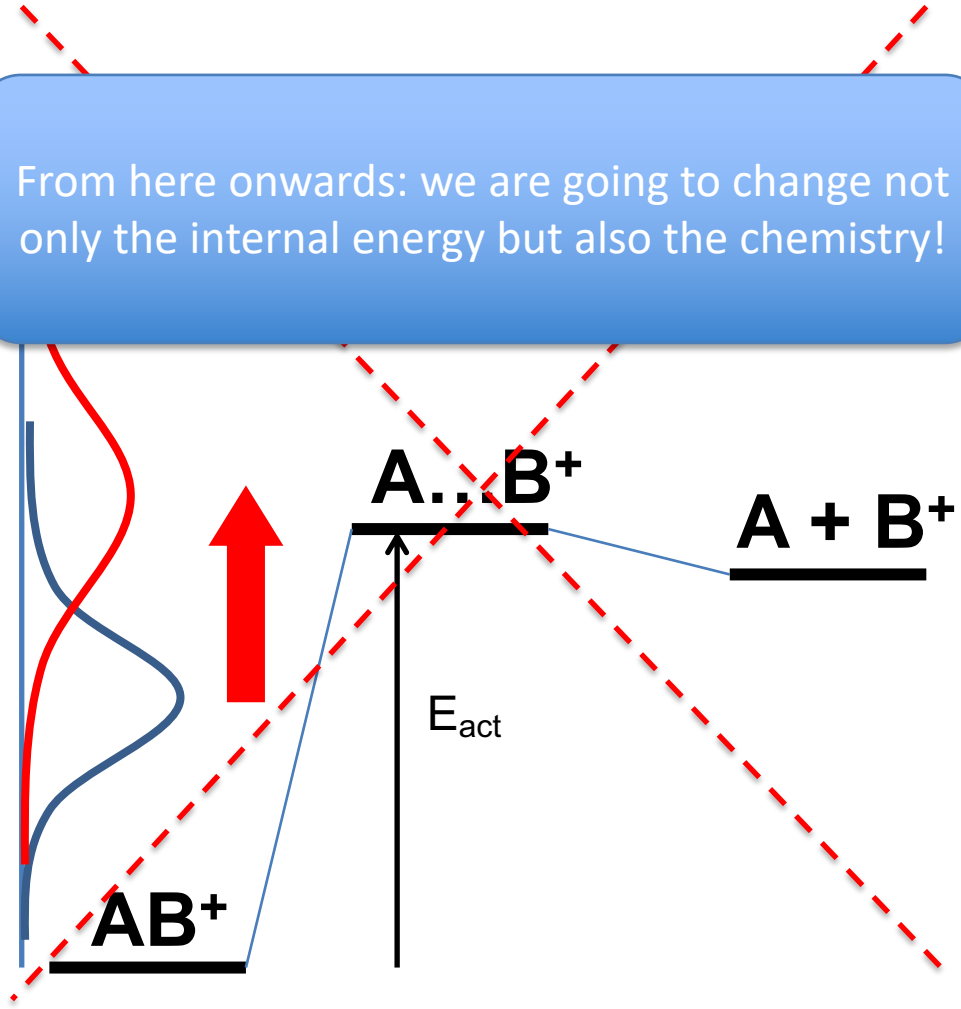


Figure 6. Arrhenius plots obtained for the loss of neutral L from the (P + L)$^{n+/-}$ ions: (a) L = **1**, P = scFv, +6 (◆), +7 (◇), +8 (△), +9 (▲), +10 (●), +11 (■), +12 (○), +13 (×), (b) L = **1**, P = scFv, −6 (□), −7 (▼), −8 (▽); (c) $n = +10$, L = **1**, P = scFv (●), L = **1**, P = His^{H101}Ala (○), L = **4**, P = scFv (▲), L = **4**, P = His^{H101}Ala (△).

*E.N. Kitova et al, J. Am. Chem. Soc **130**, 1214 (2008)*

Precursor ion activation

From here onwards: we are going to change not only the internal energy but also the chemistry!



Activation methods:

Collision with an inert gas (CID)

Collision with a surface (SID)

Photon absorption :
- **UV (UVPD)**
- **IR (IRMPD)**

Reactive activations :

Reactive collisions with a gas
Cation / anion reaction
Interaction with electrons.

FT-ICR FOR ION MOLECULE REACTION MONITORING

Ion-molecule reactions

- If one wants to study a reaction between an ion and a gas:

$$I^+ + N \rightarrow P^+ + R$$

- The FTICR cell will allow to trap ions for long and variables times and to measure reaction products.
- From the kinetics, one can derive rate constants. If the pressure of the neutral is known or measured through an other experiment, bimolecular rate constants can be derived.
- Conversly, if the rate constants are known, absolute partial pressures can be derived.

Some examples of kinetics

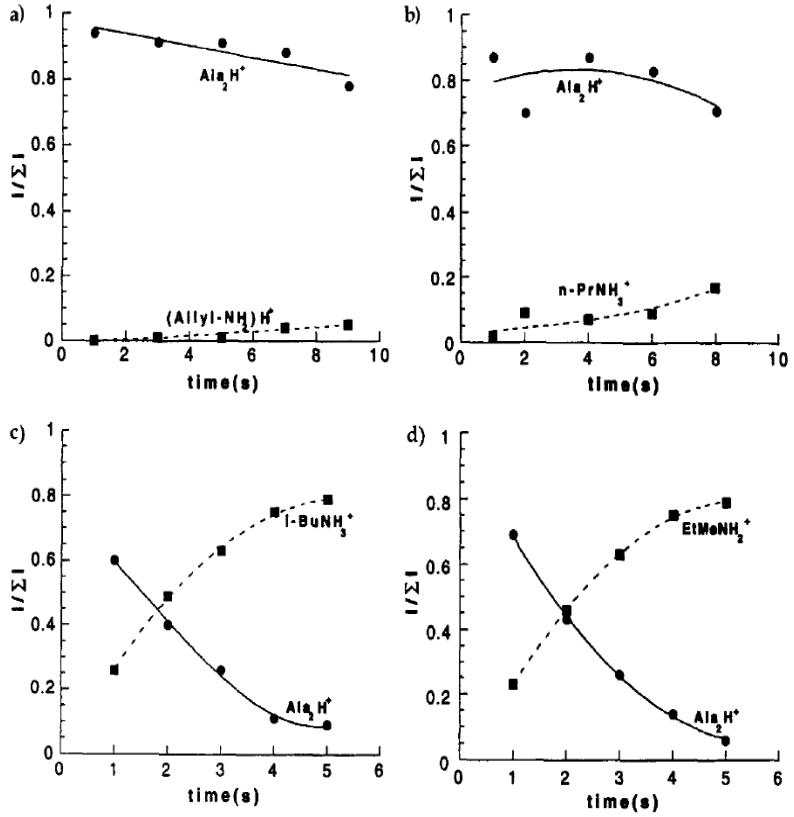
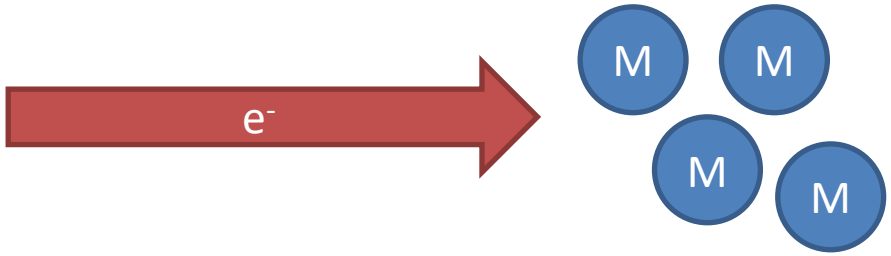


Figure 2. Relative intensity profile of protonated dialanine reacting with (**a**) allylamine, GB = 207.9 kcal/mol (1.4×10^{-8} torr), (**b**) *n*-propylamine, 210.1 (1.3×10^{-8} torr), (**c**) *i*-butylamine, 211.1 (1.2×10^{-8} torr), and (**d**) ethylmethylamine, 215.1 (1.1×10^{-8} torr). The symbol $I/\Sigma I$ represents the intensity of the ion over all ion intensities. No reactions or extremely slow reactions are observed in (a) and (b), whereas rapid reactions are observed in (c) and (d). The GB of dialanine therefore lies between the GB of *n*-propylamine and *i*-butylamine.

ORIGINS OF ELECTRON ACTIVATION TECHNIQUES

Variations on electron activation (ExD)

- Direct interaction with the electron flux



Parameters :

- Electron kinetic energy (eV), which plays on the interaction cross section and energy available in the system.
- Flux of the electron beam
- Irradiation time

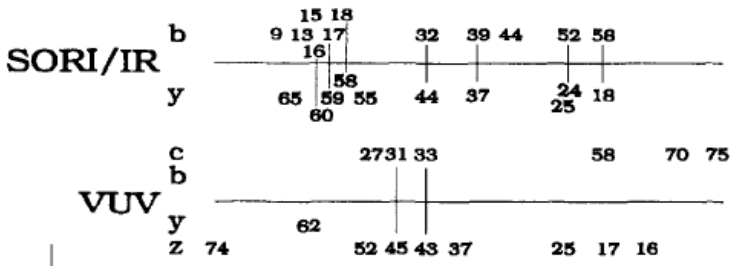
- Electron transfer between two molecules

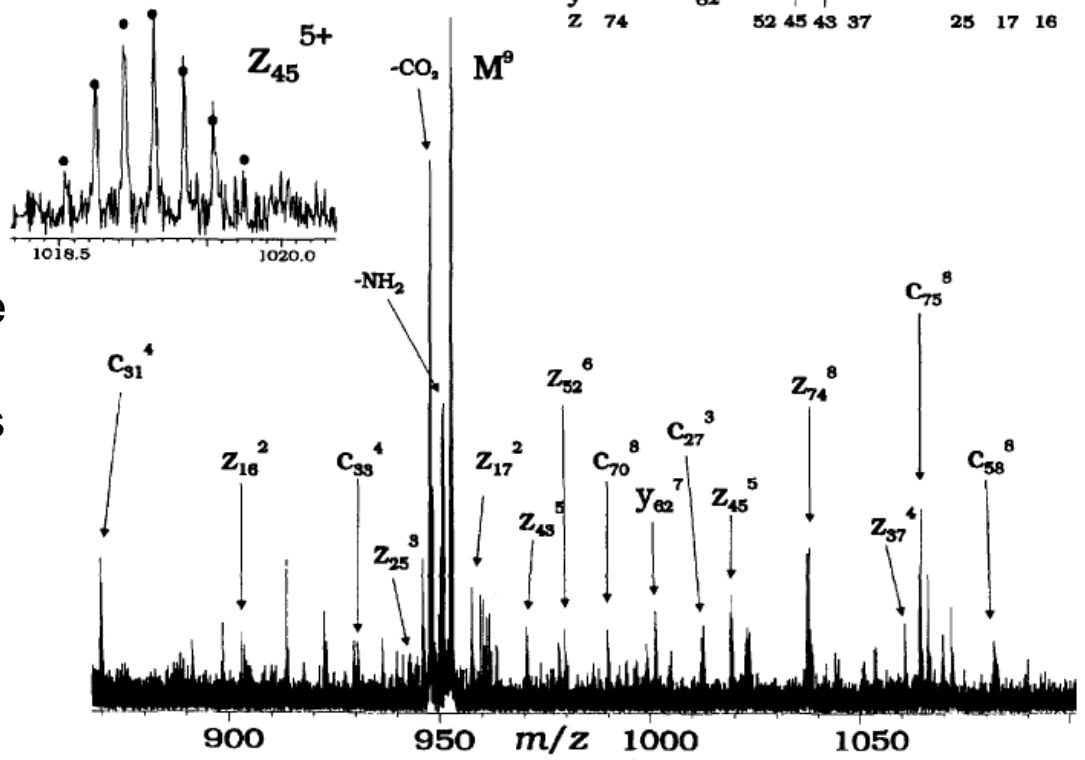
Origins of electron capture dissociation

ELSEVIER International Journal of Mass Spectrometry and Ion Processes 157/158 (1996) 357–364

193 nm photodissociation of larger multiply-charged biomolecules

Ziqiang Guan, Neil L. Kelleher, Peter B. O'Connor, David J. Aaserud, Daniel P. Little, Fred W. McLafferty*





Before ECD was even conceived, the authors were marked by:

- Formation of *c* and *z* ions
- Fragmentation of large size systems

Formation of *c* and *z* ions

Electron Capture Dissociation of Multiply Charged Protein Cations. A Nonergodic Process

Roman A. Zubarev, Neil L. Kelleher, and Fred W. McLafferty*

J. Am. Chem. Soc. **1998,** *120,* 3265−3266

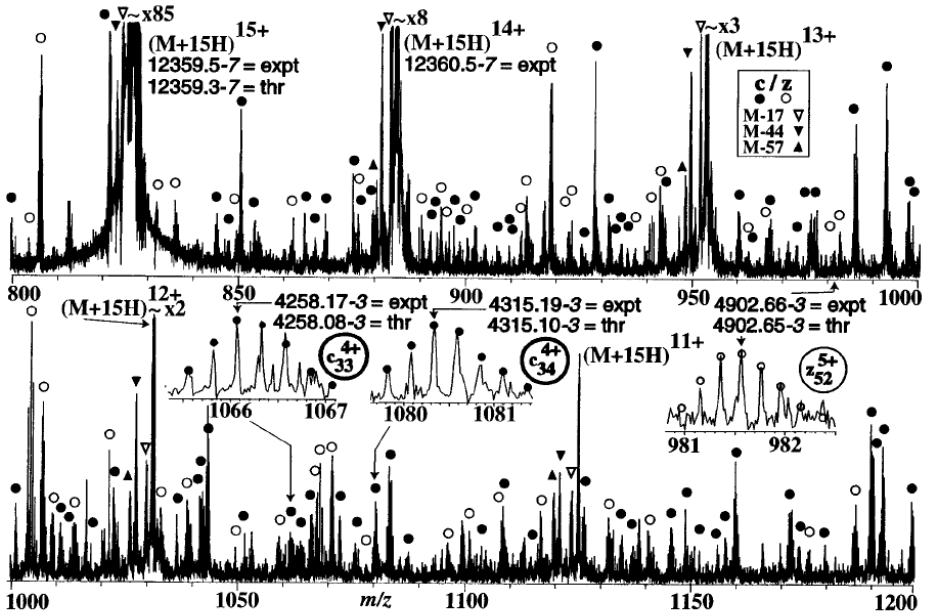
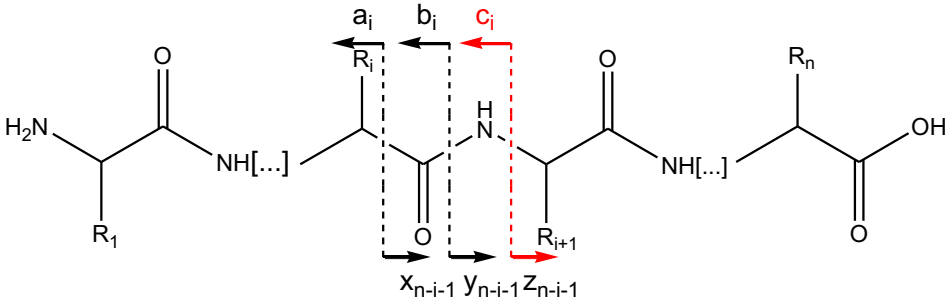


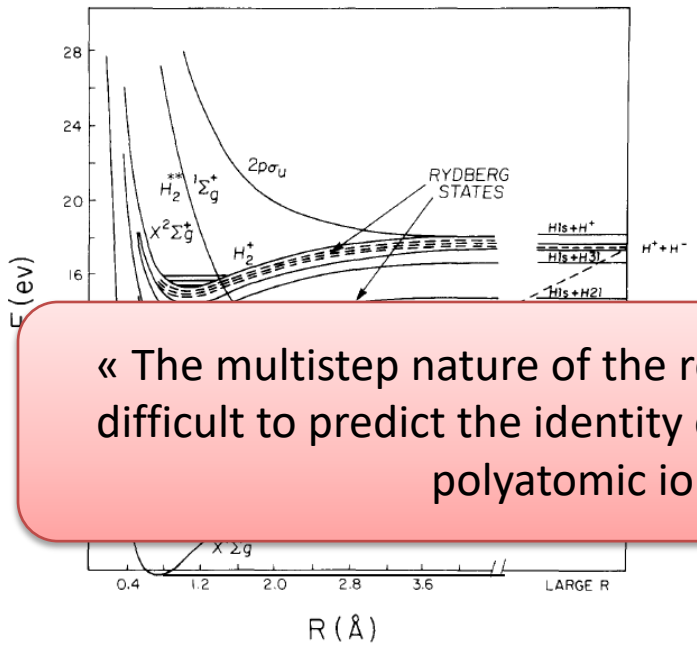
Figure 2. ECD spectra of 15+ ions of cytochrome *c* (Fe^{III}),[16] 75 scans: ▽, −17 Da; ▼, −44 Da; ▲, −59 Da; ●, *c* ions, ○, *z* ions. Mass values are for the neutral molecule's most abundant isotopic peak; the mass difference (units of 1.0034 Da) between this and the monoisotopic peak is shown by the italicized final digit.[6] Circles, as in Figure 1.



Dissociative recombination: one of the origins of ECD

- Started in the early 1980s.
- Mainly for systems of astrophysical or planetary interest.

$$M^{\bullet+} + e^- \rightarrow M^* \rightarrow N_1 + N_2$$



Theories elaborated for DR serve as a basis for the understanding of ECD:

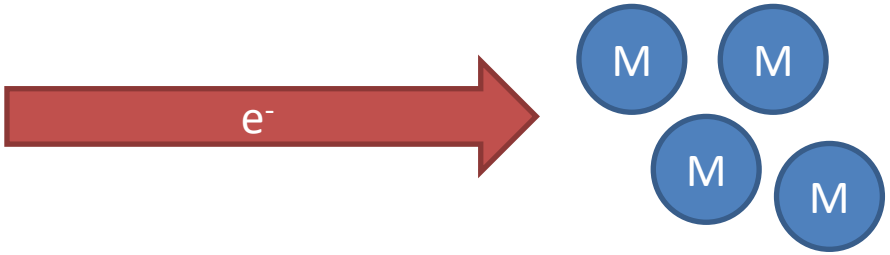
- Direct capture in a dissociative state versus indirect capture in a Ryberg state
- Electron energy dependence in $1/E_c$

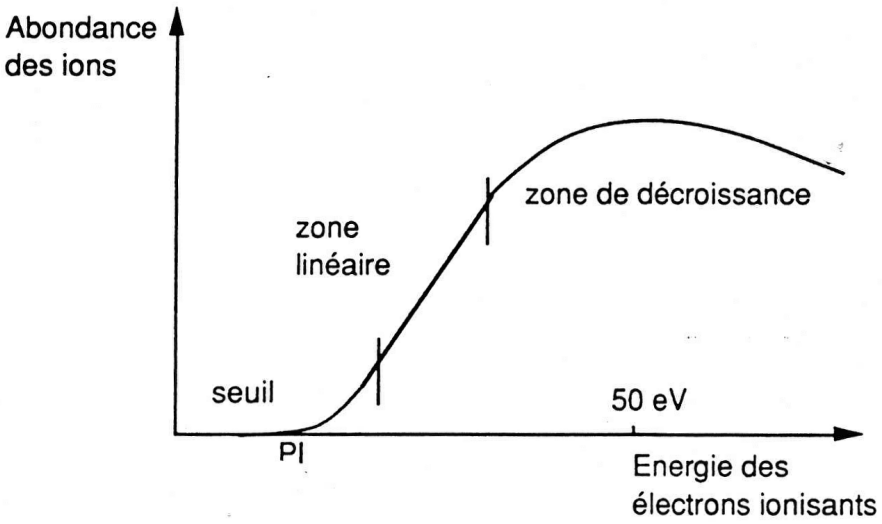
→ Requirement to work at low electron

« The multistep nature of the recombination process means that it is very difficult to predict the identity of the products. This is particularly true for polyatomic ions. » J.B.A. Mitchell, 1990

Review: Mitchell, *Physics Reports* 1990.

Some parallels with the much better known electron ionization





Organic molecules: IE ~ 8-12 eV for neutrals, higher for charged species.

Abundant fragmentation induced by electron ionization at 50 eV.

The neutral form is not always ionized: interaction with an electron can lead to an internal energy increase without ionization.

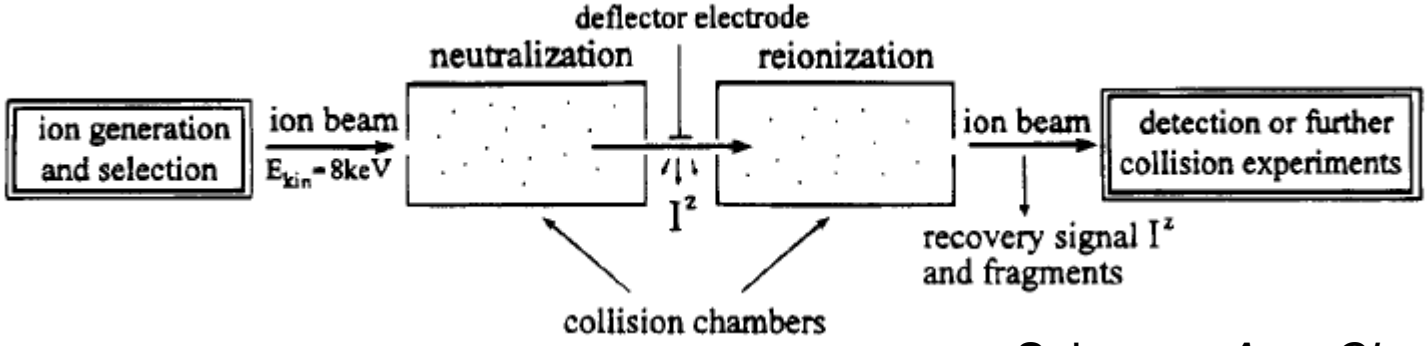
Electron capture dissociation

- Nowadays, Electron Capture Dissociation (ECD) applied to the charge reduction of a positive multiply charged precursor ion:

$$(\mathrm{P}, n\mathrm{H})^{n+} + e^{-} \rightarrow (\mathrm{P}, n\mathrm{H})^{(n-1)+\bullet *} \rightarrow \mathrm{F}_1^{i+} + \mathrm{F}_2^{(n-1-i)+\bullet}$$

- Reaction products are directly measured by mass spectrometry and ECD can be considered as the other activation methods in tandem mass spectrometry.
- Other terms and acronyms are to be used for other variations on ion / electron interactions.

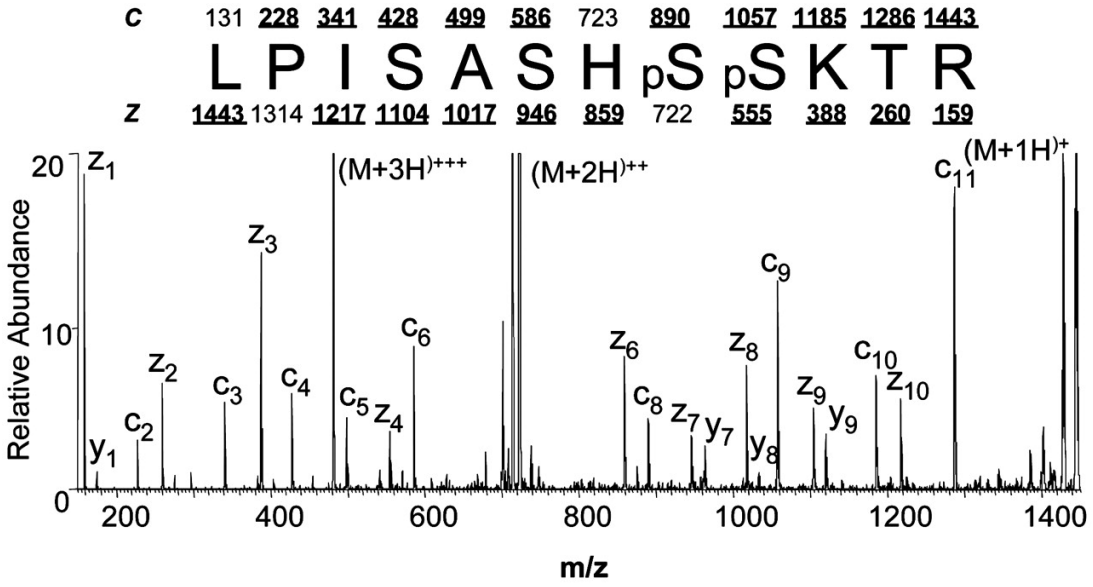
Neutralisation – reionisation for sector mass spectrometers (NRMS)



Schwarz, *Acc. Chem. Res.* 1994

- Neutralisation :
  - Various gases can be used (Xe, Hg, Na, Zn, NH_3, NO, …)
  - Electron transfer to / from the ion in the beam
- Reionisation :
  - In general O_2 leading to a positively charged product.
- The kinetic energy of the ions / molecules brings the necessary energy to achieve the endothermic reactions if necessary (usually the case for reionisation).

Electron transfer dissociation

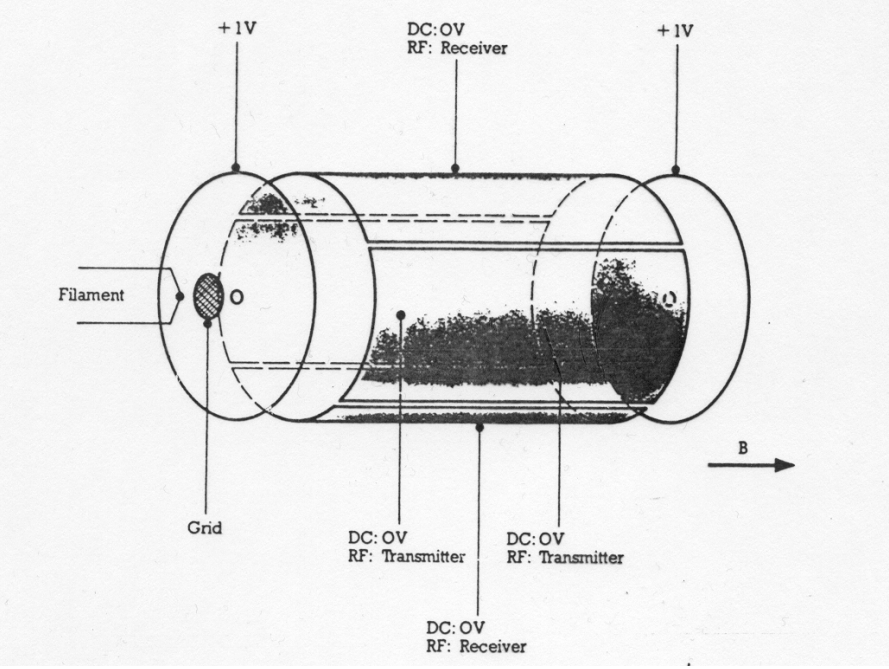


$$(M, 3H)^{3+} + A^{-} \rightarrow (M, 3H)^{2+\bullet *} + A^{\bullet} \rightarrow F_1^{+} + F_2^{+\bullet} + A^{\bullet}$$

A = $C_4H_9^-$ / $C_4H_{11}^-$ formed in a negative CI source (CH_4)

Syka et al, PNAS, 2004

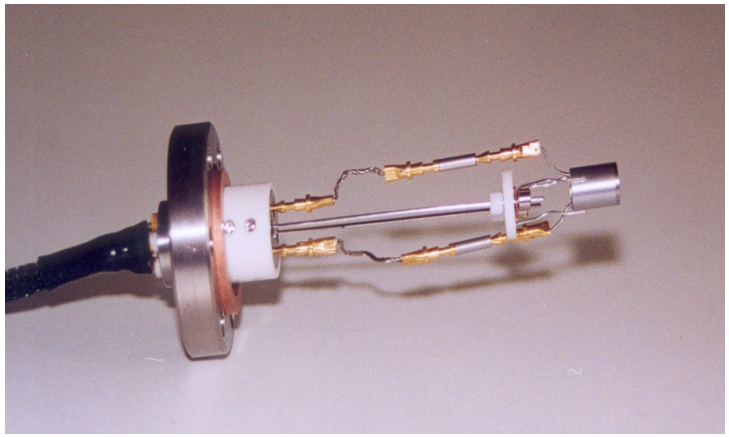
Reusing the electron ionisation filament of the ion cell

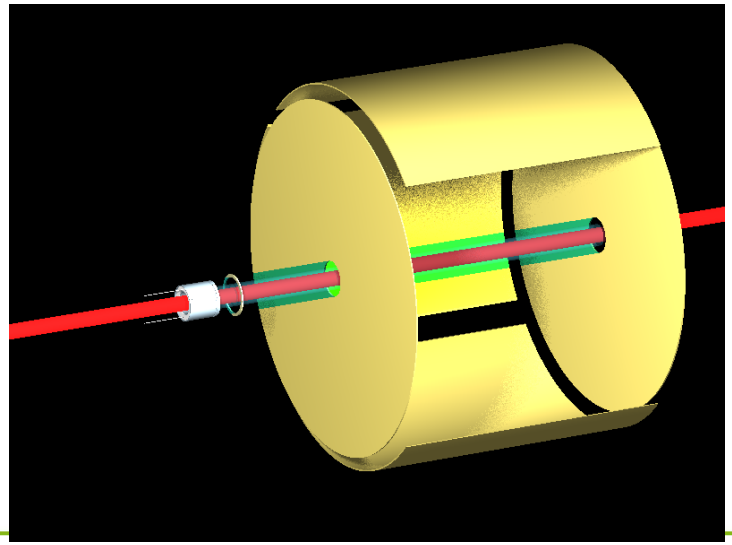


Issues:

- Low electron flux
- Low overlap of the electron beam from the filament with the ion cloud in the cell.
- Main axis of the instrument is also used by the external ion introduction / laser for activation.

Cathode and hollow cathodes

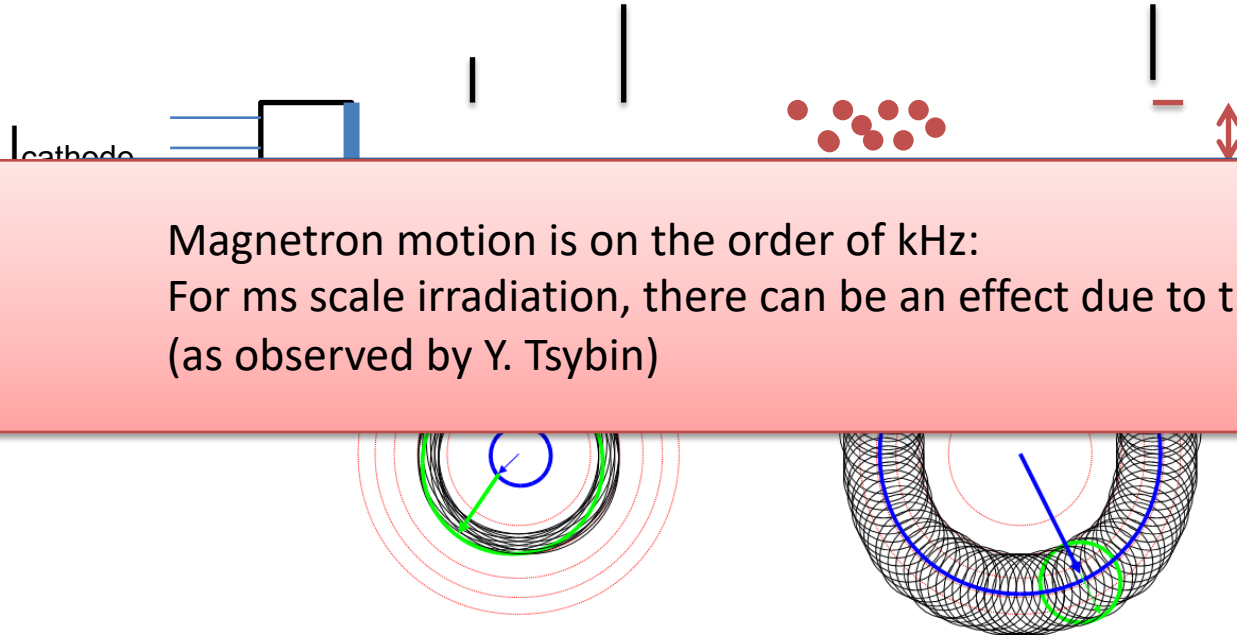




Indirectly heated cathodes have replaced the filament:

- Higher electron current
- Improved overlap of the electron beam and ion cloud.
- Additional extraction lens allows to contron electron energy independently from the electron flux.
- Hollow cathode allows combination with laser activation methods.

ECD parameters: overlap with the ion cloud



Magnetron motion is on the order of kHz:
For ms scale irradiation, there can be an effect due to this motion!
(as observed by Y. Tsybin)

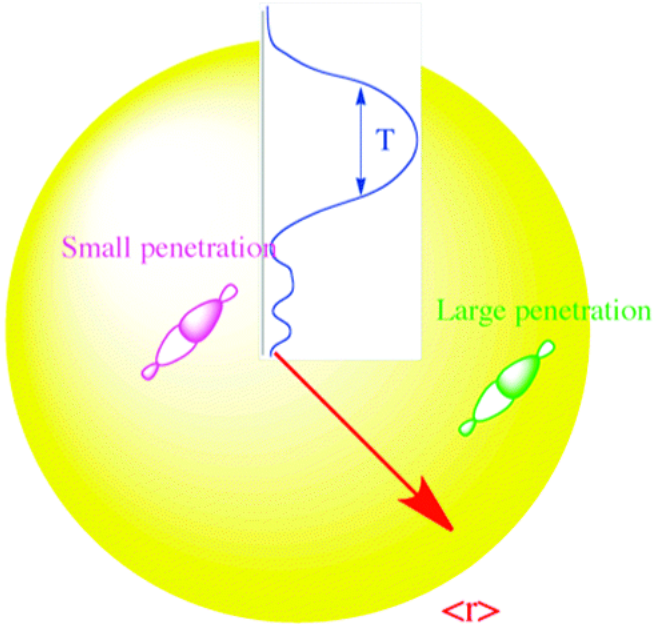
Full cathode:

Ideal: $r_c + r_M < r_{cathode}$

Hollow cathode :
More complex. Ideal case would be
$|r_M - r_C| > r_{inside}$
and
$r_c + r_M < r_{outside}$

Molecular level reactions

- Initial electron arrival in the charged molecule
  - Initial attachment to a high Rydberg state.
  - Formalised by J. Simons.

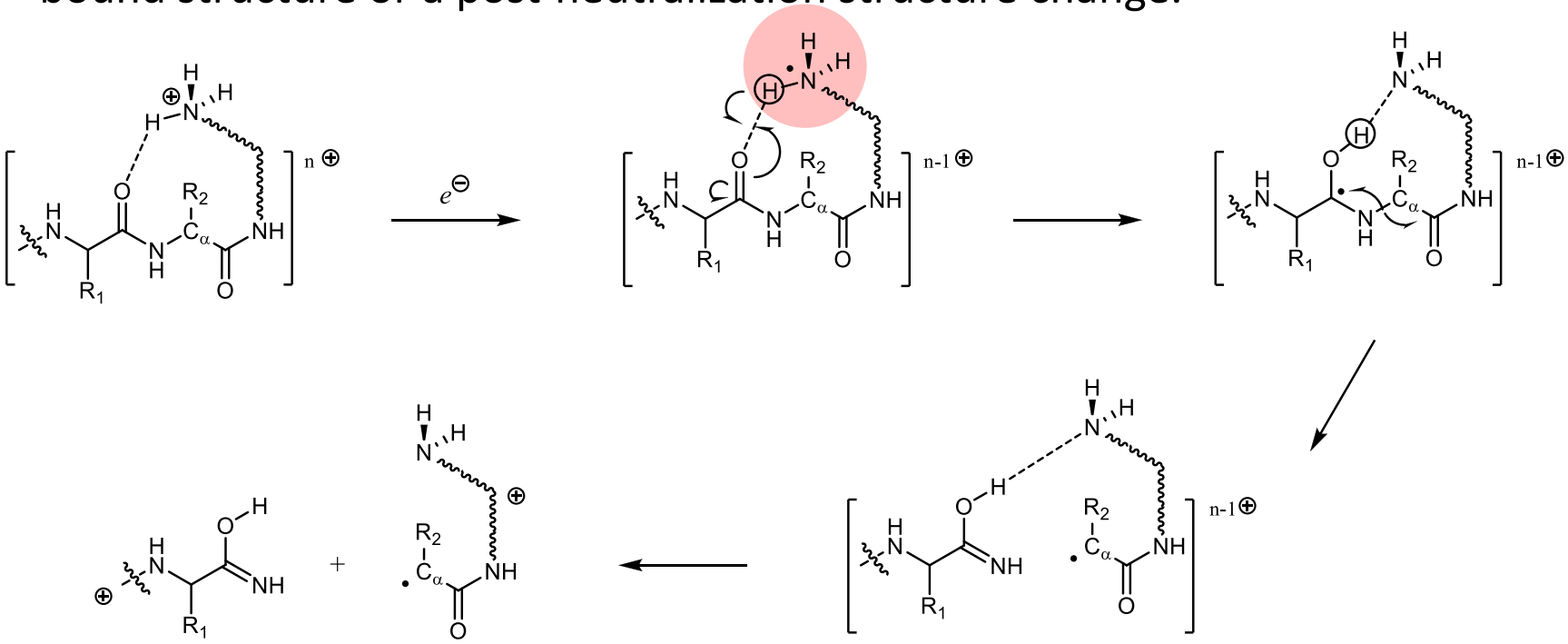


The overlap beteen this loose Rydberg orbital and the tighter orbital towards which the electron will ingress allows a transfer to the various regions of a singe polypeptide provided that the Rydberg quantum state is sufficiently high.

J. Am. Chem. Soc. **132** 7074 (2010)

Cornell-like mechanism (localized H atom transfer)

Electron capture on an ammonium group followed by transfer of a hydrogen atom towards a carbonyl group. Could be either a preformed hydrogen bound structure or a post-neutralization structure change.

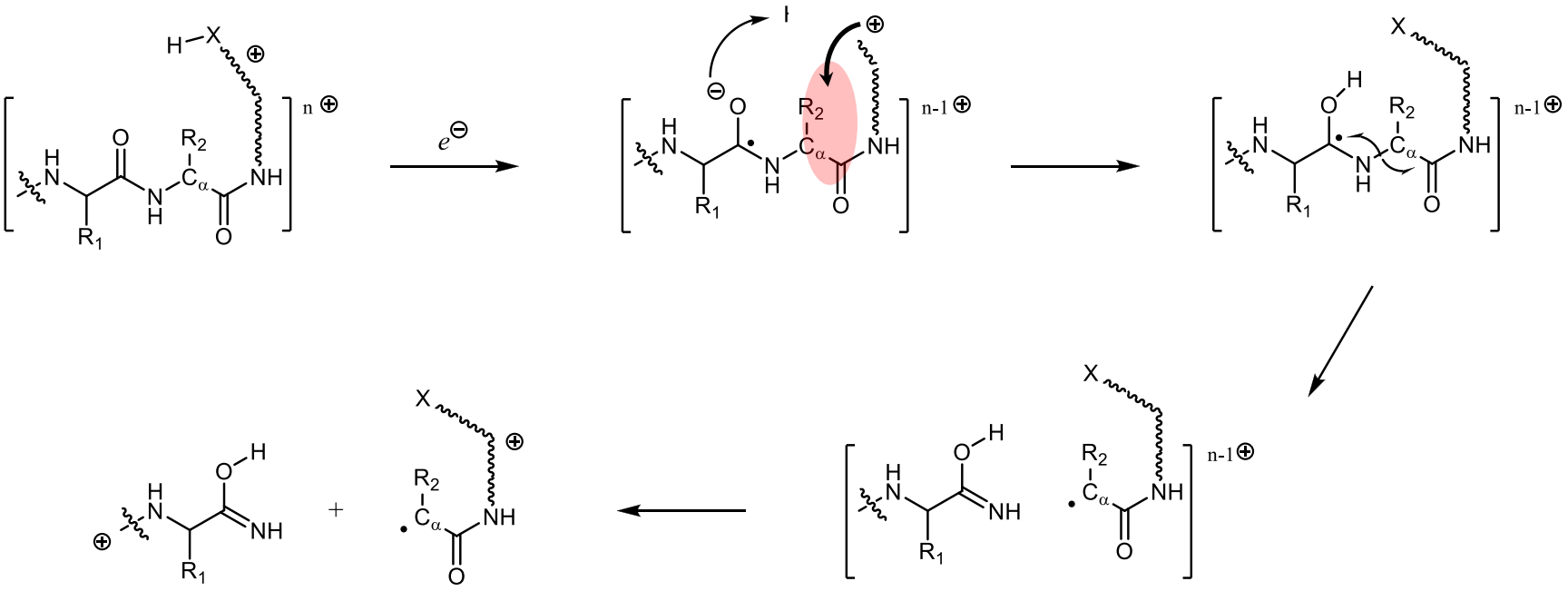


Zubarev , Kelleher, Mc. Lafferty *J. Am. Chem. Soc.* (1998)
Zubarev, *Eur. J. Mass Spectom.* (2002)

Electron capture in a carbonyl orbital a. Followed by a proton transfer

Electron goes to a carbonyl π* orbital (of which the electron affinity has been decreased by charge proximity), followed by 1. a proton transfer 2. N-C_α bond cleavage.

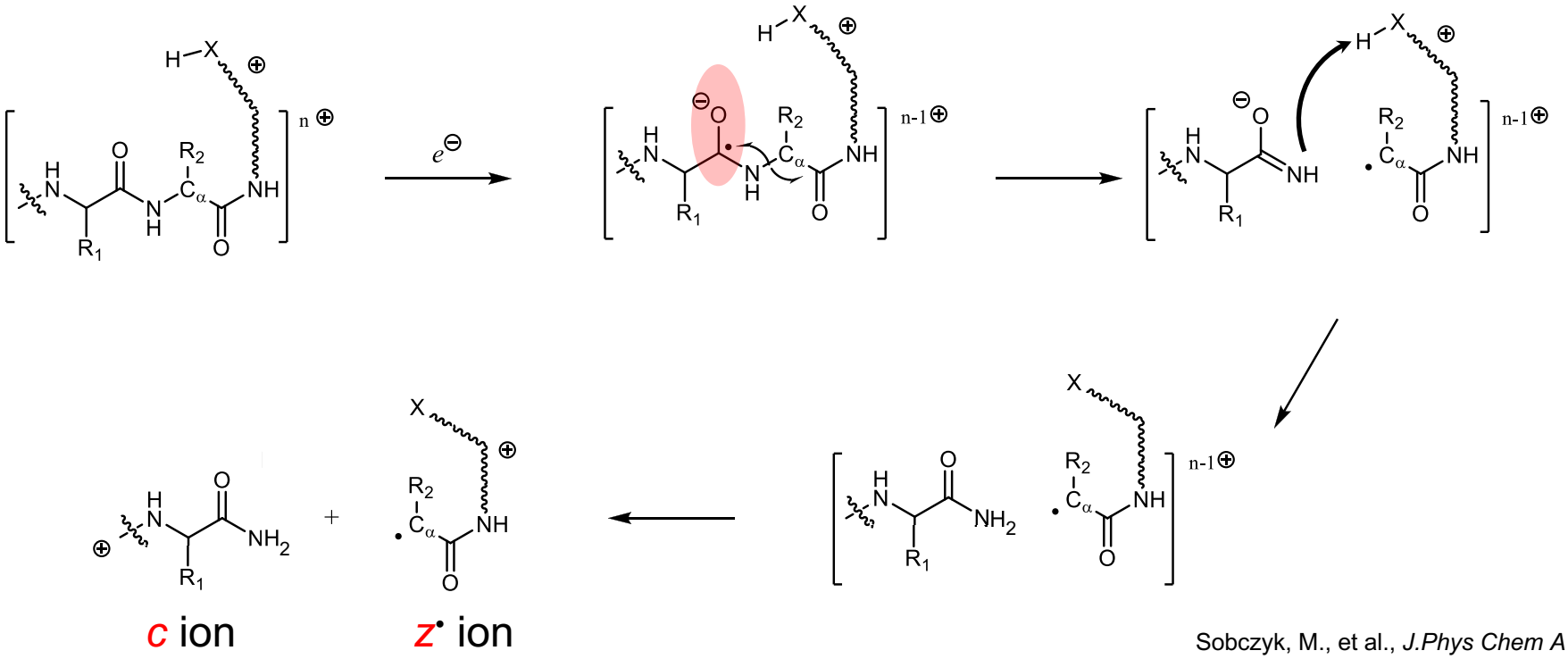
Very close to the previous mechanism if in a hydrogen bond network.



Tureček, Syrstad, *J. Am. Chem. Soc.* (2003)

Electron capture in a carbonyl orbital a. Followed by N-C_α bond cleavage

Electron goes to a carbonyl π^* orbital followed by 1. N-C_α bond cleavage, 2. proton transfer

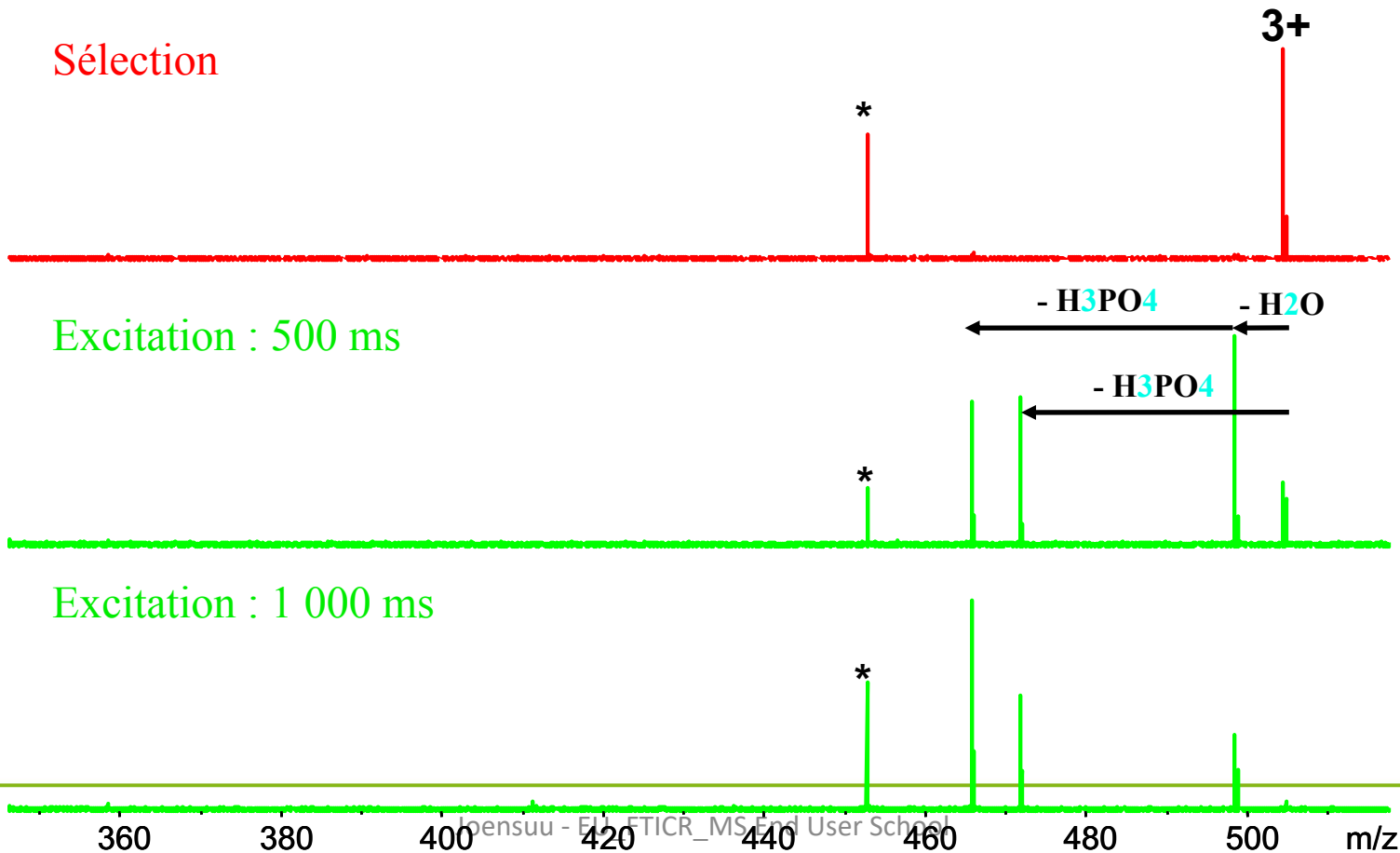


Sobczyk, M., et al., *J.Phys Chem A* (2004)

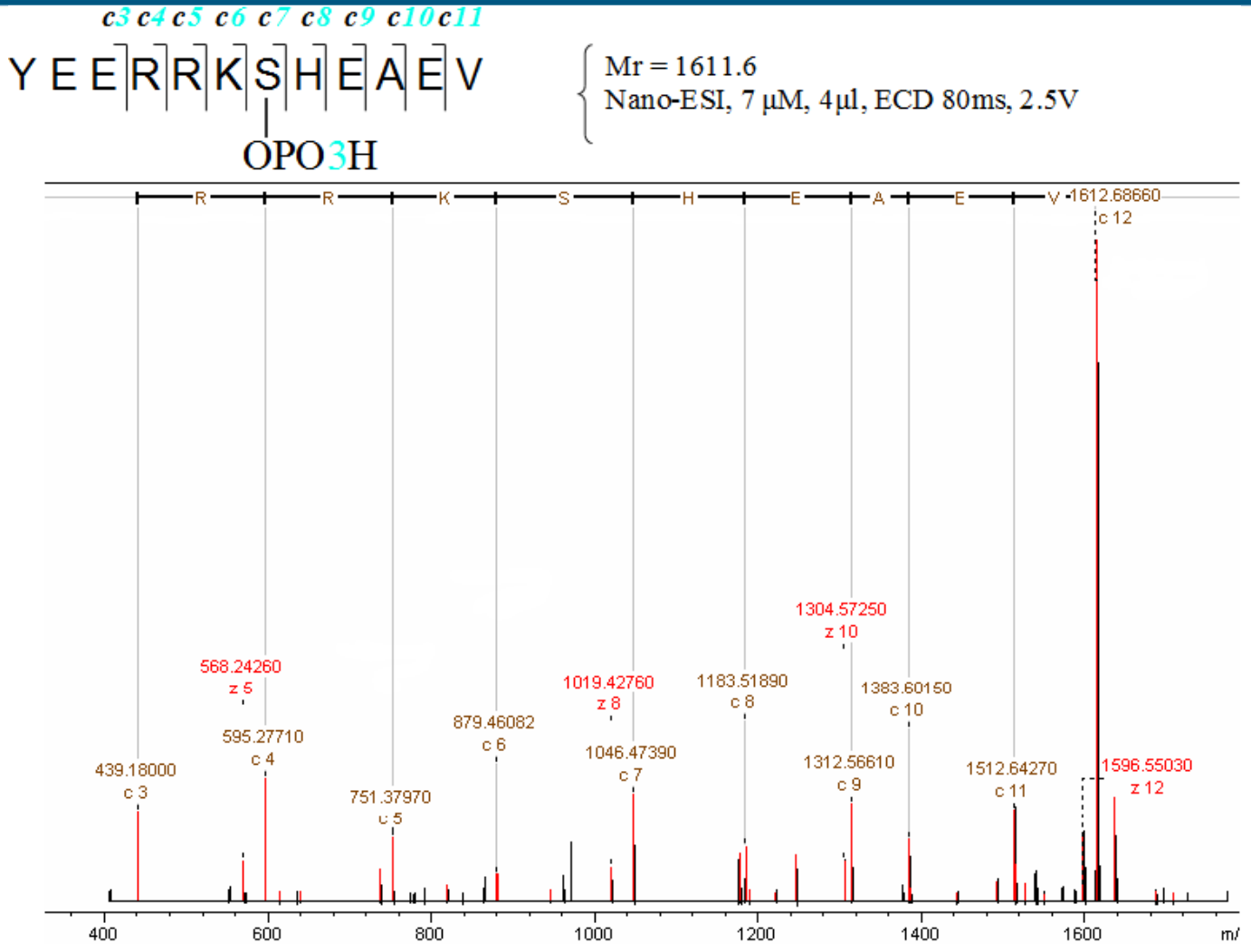
SORI-CID of a phosphorylated peptide

YEERRKSHEAV
|
OPO_3H

Mr = 1611.6
Nano-ESI, 7 µM, 4µl, SORI-CID / Xe



ECD of a phosphorylated peptide



Application to non-covalent complexes

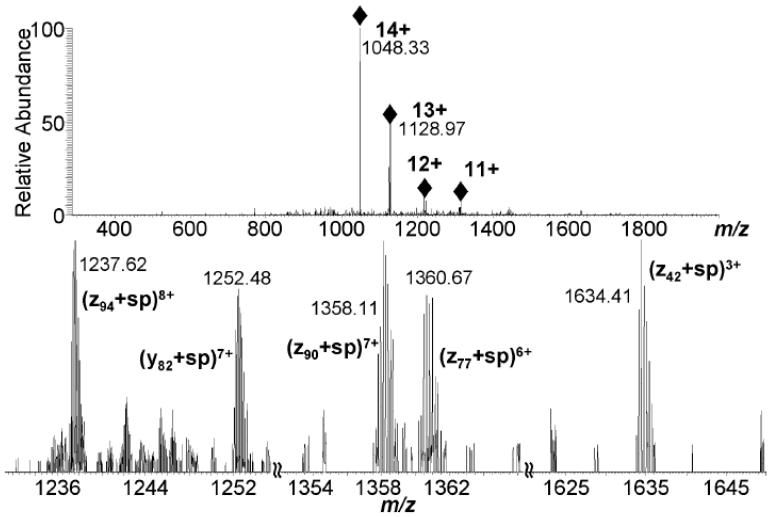


Figure 1. ECD mass spectrum of the 14+ 1:1 α-synuclein−spermine complex (*m/z* 1048; top), with expanded regions shown (bottom).

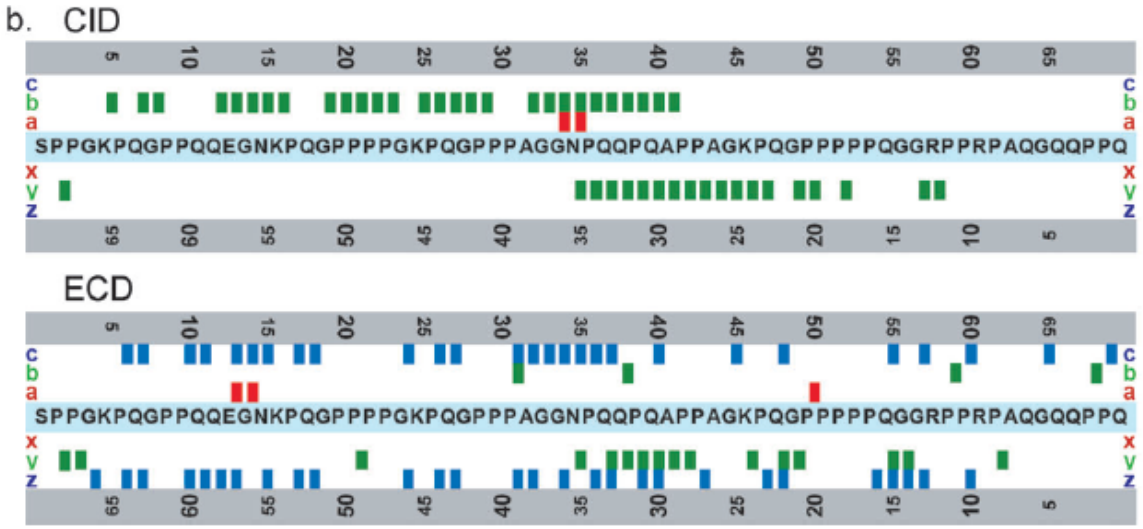
Figure 2. ECD generated products from the 14+-charged 1:1 α-synuclein−spermine complex. Product ions that retain spermine binding are indicated by the extra line underneath the fragments (e.g., ⌊).

- Hydrogen bonds between a protein and its ligand can be conserved whereas peptide bonds are cleaved.

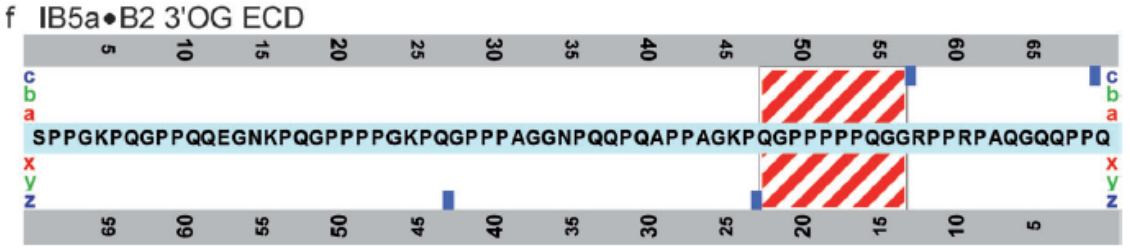
Y. Xie, *J. Am. Chem. Soc.* 14432 (2006)

Typical access project: localisation of tanin binding sites with IB-5 salivary protein.

Without 3'-OG

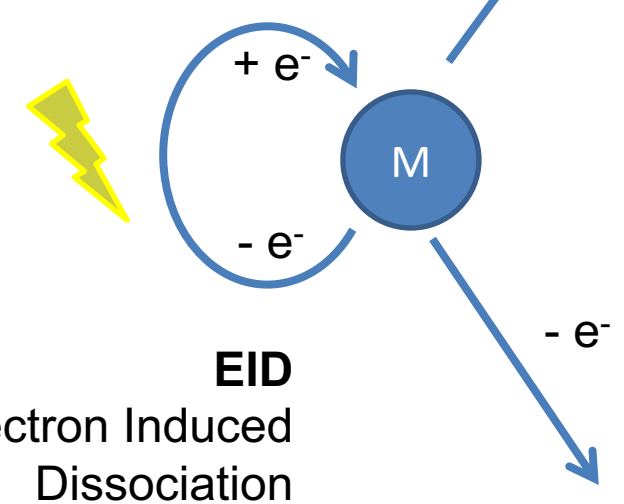


With 3'-OG, fragments containing the 3'-OG moiety



Canon, *Angew. Chem. Int. Ed.* 8377 (2013)

Other electron activation modes



EID
Electron Induced
Dissociation

Positives

ECD
Electron
Capture
Dissociation

ETD
Electron Transfer
Dissociation
$A^{\bullet -}$

EI-ID
Electron Ionization
Induced Dissociation

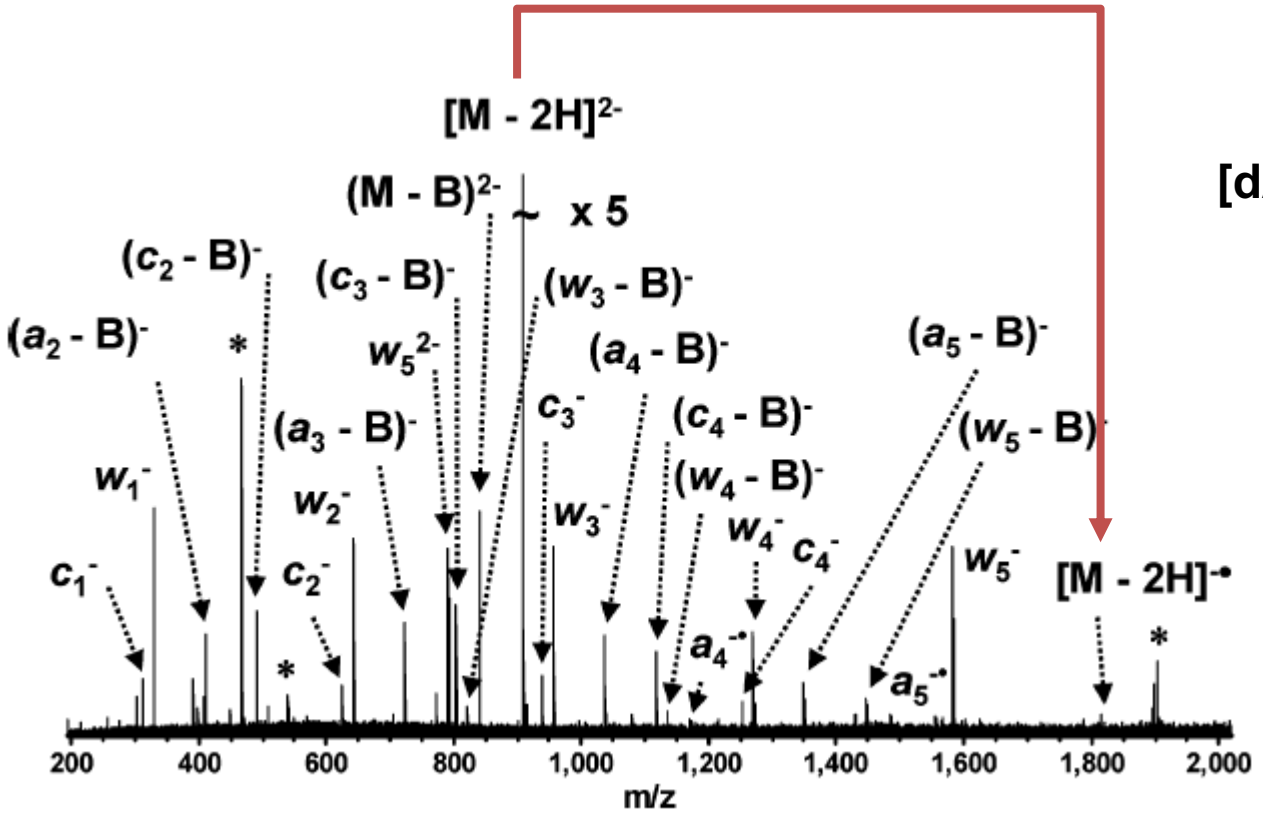
Négatifs

ni-ECD
Negative Ion
Electron
Capture
Dissociation

ni-ETD
Nagative Ion
Electron Transfer
Dissociation
$A^{\bullet +}$

EDD
Electron Detachment
Dissociation

EDD on oligonucleotides

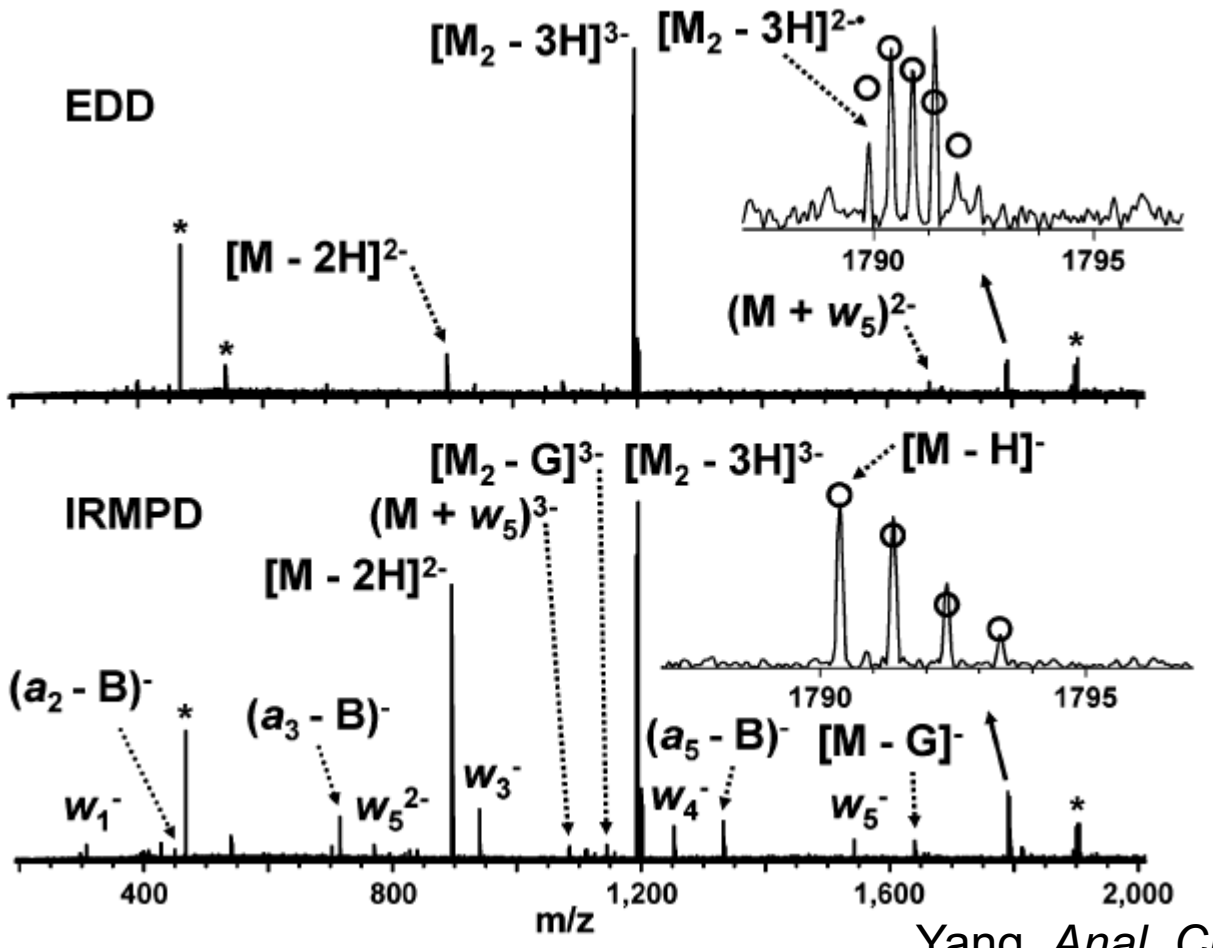


$[dA_6 - 2H]^{2-}$

1s irradiation, 17 eV

Yang, *Anal. Chem.* 1876 (2005)

Comparison between EDD and IRMPD on oligonucleotides



Duplex :
M = d(GCATGC)

EDD : 16 eV, 1s

IRMPD : 10 W, 150 ms

Yang, *Anal. Chem.* 1876 (2005)

Electron induced dissociation (EID)

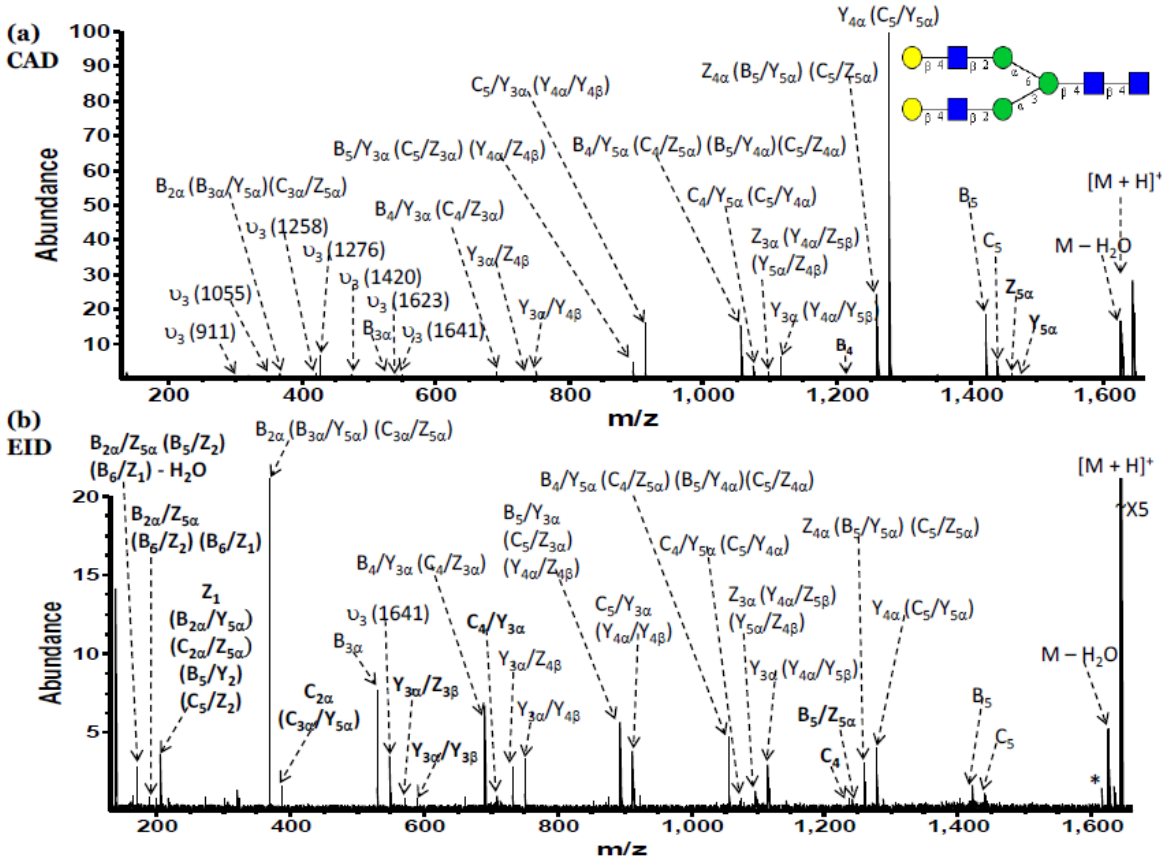


Figure 4.9 CAD and EID of an asialo, galactosylated, biantennary glycan (NA2): (a) CAD, (b) EID. For both spectra, precursor ions are labeled $[M + H]^+$. *: noise; ν_3: third harmonic peaks. Bold font indicates fragments that are unique to CAD or EID.

Bias : -11 to -20 V
Time: 0,3 – 0,5 s

Gao, PhD Thesis, Univ. Of Michigan, 2013

Which mechanisms ?

- EDD : Radical process, through the formation of a H• lacking species
  - Potentially several detachment sites, initiating radical reaction from these sites.
  - Combined with the deposit of energy which leads to CID-like fragmentation in parallel to the radical induced fragmentation.
- EID: Either electron ejection / recapture or electronic excitation of the molecule.