P. Moretto-Cappelle and A. Le Padellec

Irradiation of biomolecules: experimental investigations

Interactions of biomolecules with multiply (singly) charged ions

# Fragmentation of adenine under energy control

The CIDEC method merges two well-known techniques, the double charge transfer spectroscopy and the mass spectrometry

$\Rightarrow$ the fragmentation of adenine dication is studied as a function of the excitation energy of the molecule. The method is based on the measurement of the kinetic energy loss of the scattered anion projectile Cl- formed by double electron transfer during the interaction with the molecule.

In (a), the mass spectrum is plotted as a function of the analyzer voltage, leading to the map of the internal energy of the Ade2+ parent molecules. (b) shows the mass spectrum integrated over the analyzer voltage. (c) displays an overview of the population distributions of Ade2+ parent ions as a function of the excitation energy for each peak of the mass spectrum, obtained by projection onto the horizontal axis of (a).
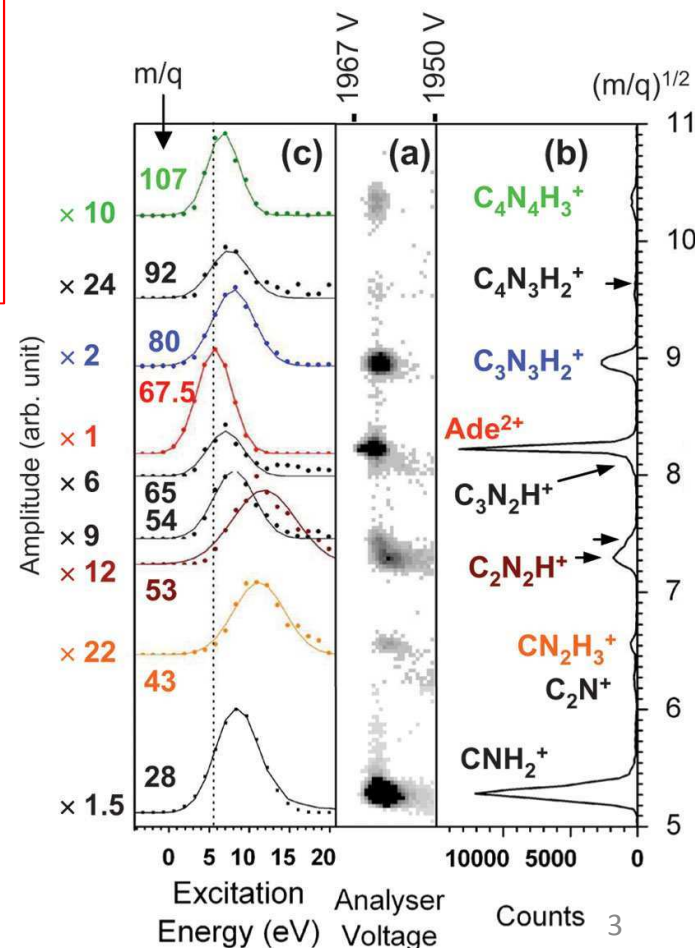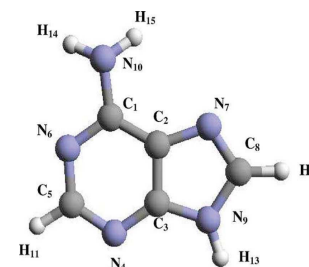
⇒ analysis of the correlation mass spectrum and the measurement of the kinetic energy release reveal details on the fragmentation scheme of the molecule
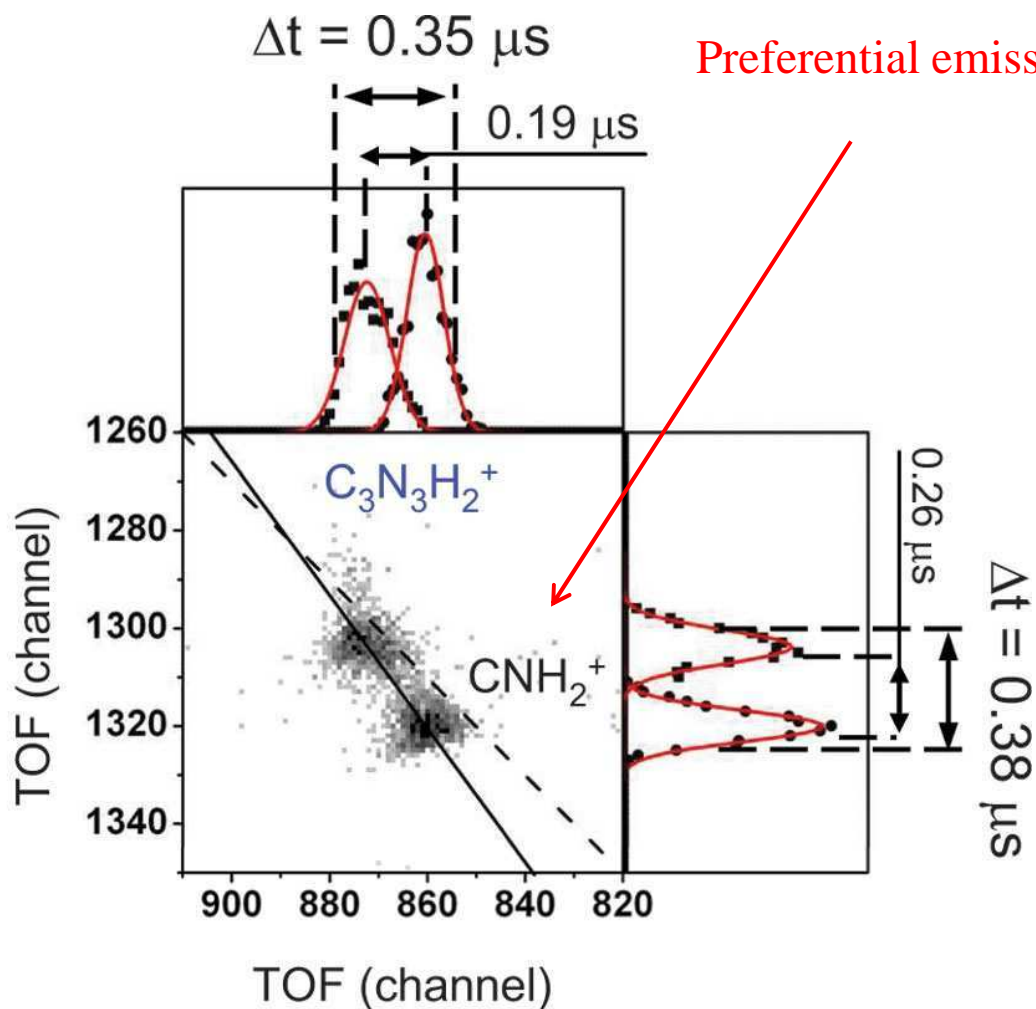
Preferential emission of mass 28

Correlation spot corresponding to C3N3H2+ m/q=80 and HCNH+ m/q=28 fragments with X top panel and Y right panel projection. The dashed line corresponds to a 1 slope two-body breakup. The plain line corresponds to the slope −1.360.03 indicating a two-step process: Emission of CNH2+ followed by the evaporation of HCN.

□t: Time difference between the fragments emitted in the forward and the backward direction for KER measurement.
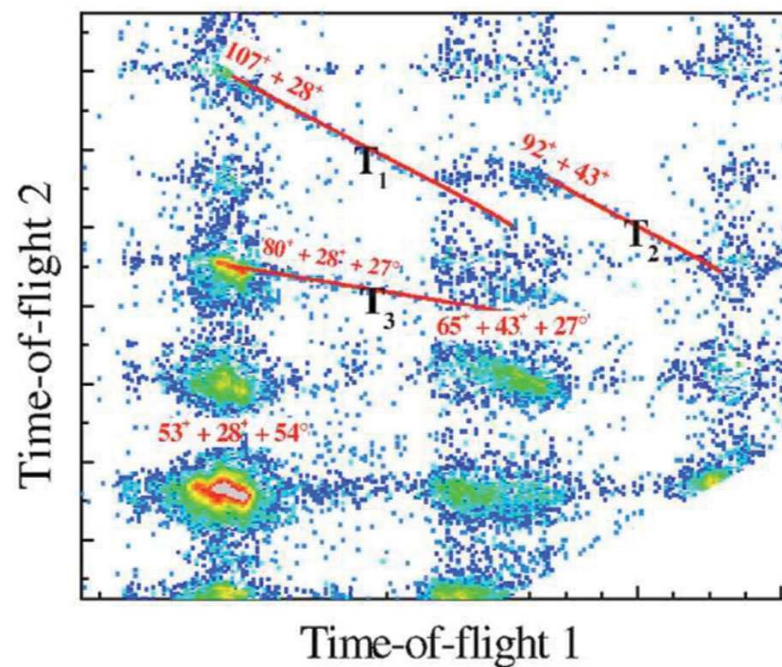
Energetics and metastability of the adenine dication observed in proton - adenine collisions

Moretto-Capelle *et al* THE JOURNAL OF CHEMICAL PHYSICS 127, 234311 2007


(a)

(b)
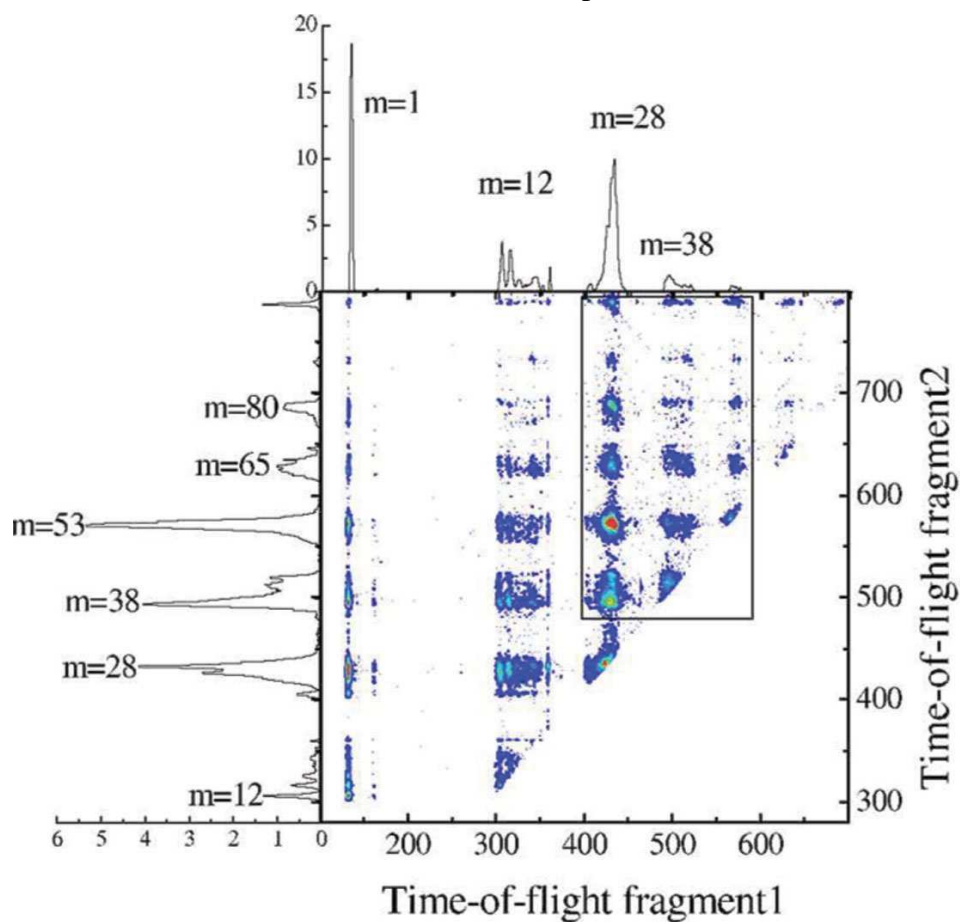
(a) Total correlated two-ion time-of-flight spectrum.
(b) Inset of the region of interest

→ KER values were evaluated for several channels. Combining these findings with calculations that use the GAMESS code, activation energies in the 5–6.5 eV range—with respect to the ground-state of the adenine dication—were deduced for three-body fragmentation processes

→ observation of long-lived states, i.e., metastability in the 100–200 ns range

→ in the specific case of the two-body breakup that involves mass 28, the transition states as well as the fission barriers for the two competing pathways could be identified, with preferential emission of the H–C–N–H+ ion over the C–N–H2+ ion.

→ estimation of the internal temperature of the adenine molecule from the lifetime data via transition state theory
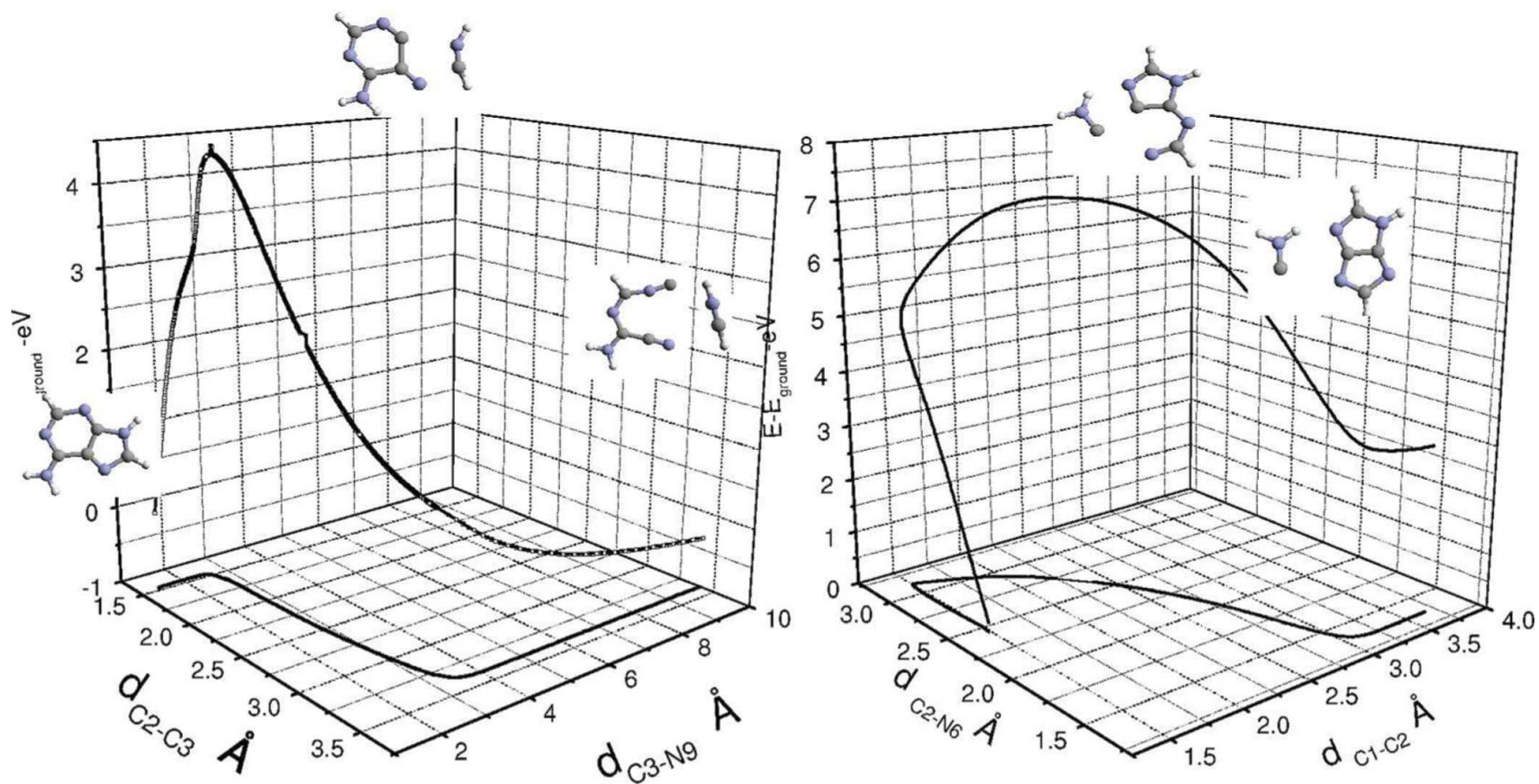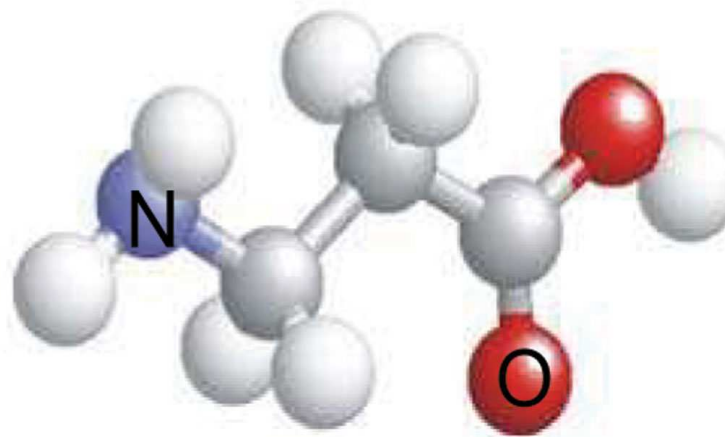
Energy relative to the ground state of the internal reaction coordinates in Å. Geometry of the initial, transition state, and final products are shown for emission of (a) H–C–N–H fragment and (b) C–N–H2 fragment.

Isomeric effects in ion-induced fragmentation of α- and β-alanine Sobocinski *et al* Journal of Physics: Conference Series **101 (2008) 012006**
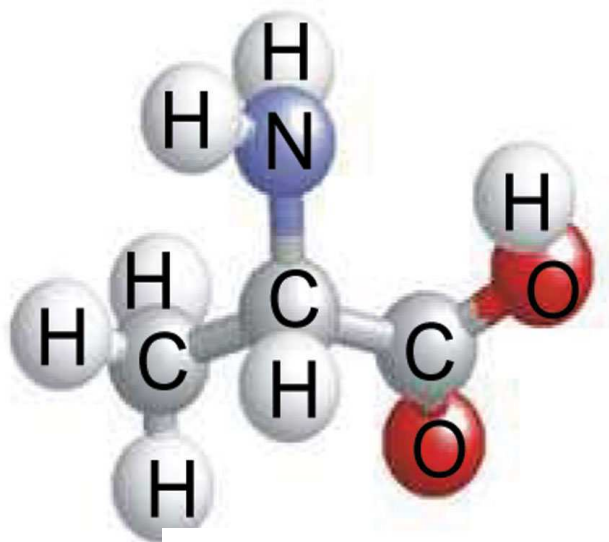

Geometries of α- and β- alanine: two commercially available isomers !

Study of the ion-induced dissociation of α- and β- alanine by means of mass spectrometry over projectile charge states ranging from 1 to 20

β-alanine   50 keV O⁵⁺

$NH_2(CH_2)_2^+$

d-ala⁺

α-alanine

$NH_2CH_2^+$

$NH_2CH_3CH^+$

intensity

m/q (amu)
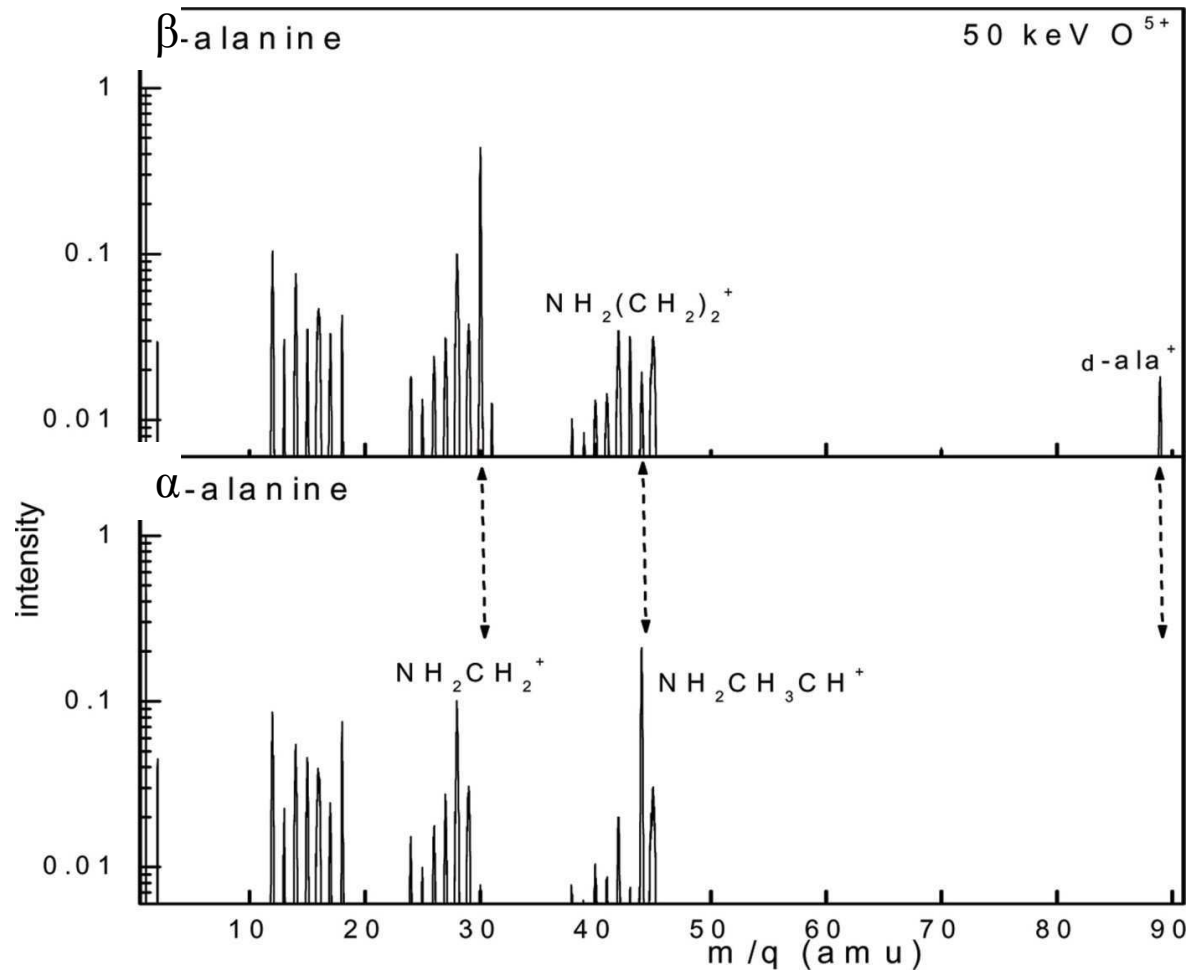
Mass spectra of β-alanine (top) and α- alanine measured following 50 keV O5+ impact. The dashed arrows located at m/q = 30, 44 and 89 amu indicate the most pronounced differences

→ strong geometry-dependence of the fragmentation process: the mass spectra exhibit significantly different peak intensities at m/q = 30 and 44 amu.
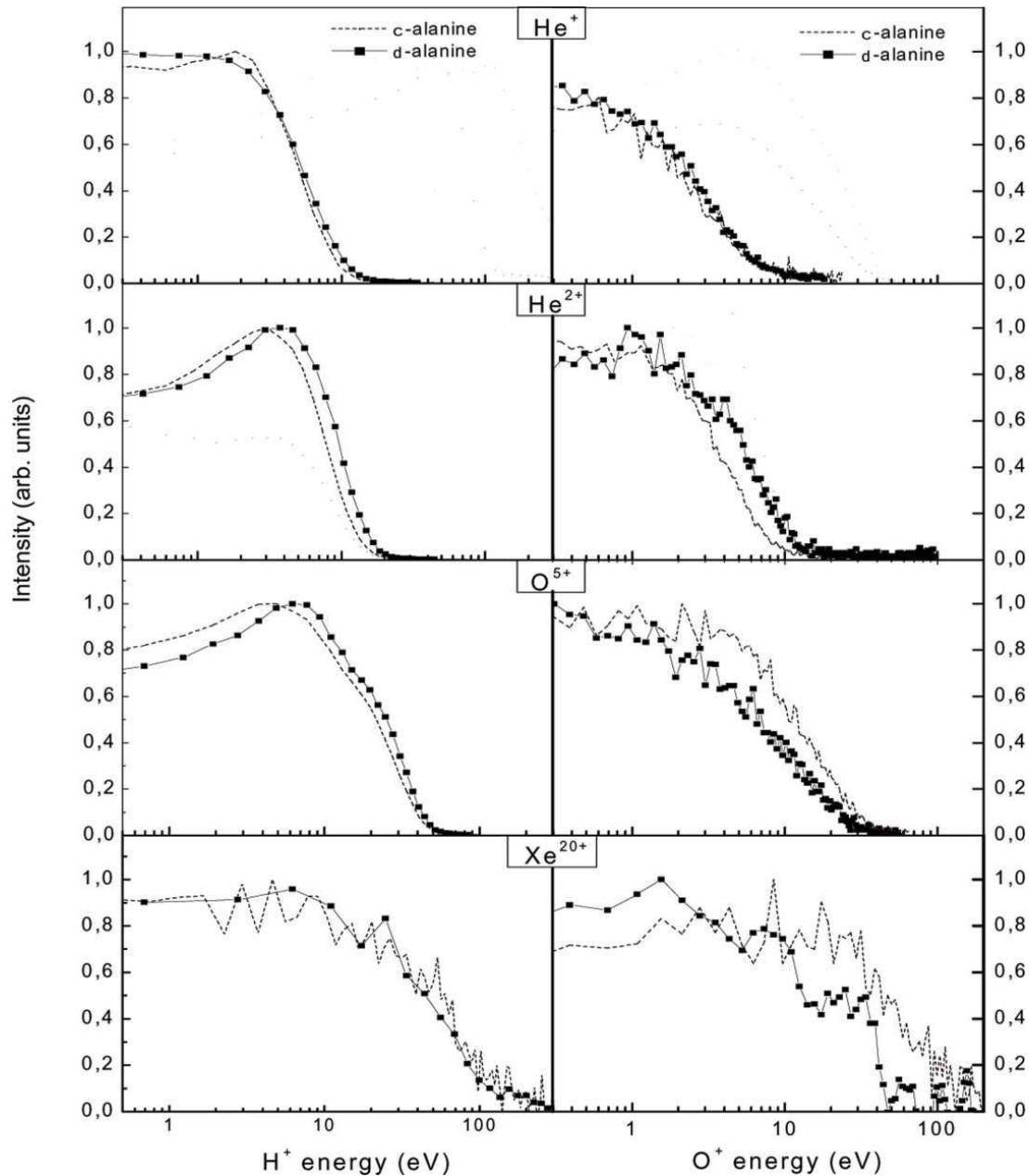
Fragment energy distribution (left column: H+ fragments, right column: O+ fragments) following impact of He+, He2+, O5+ and Xe20+ projectiles on α- and β- alanine.

→ energy of a given fragment depends not only on the fragmentation channel but also on the isomer geometry

→ some common features. In particular, O+ and H+ energies largely exceed 30 eV, which indicates that fragmentation of aminoacids generates secondary ions which can, in turn, induce severe biological damage.

# Precise Determination of 2-Deoxy-D-Ribose Internal Energies after keV Proton Collisions
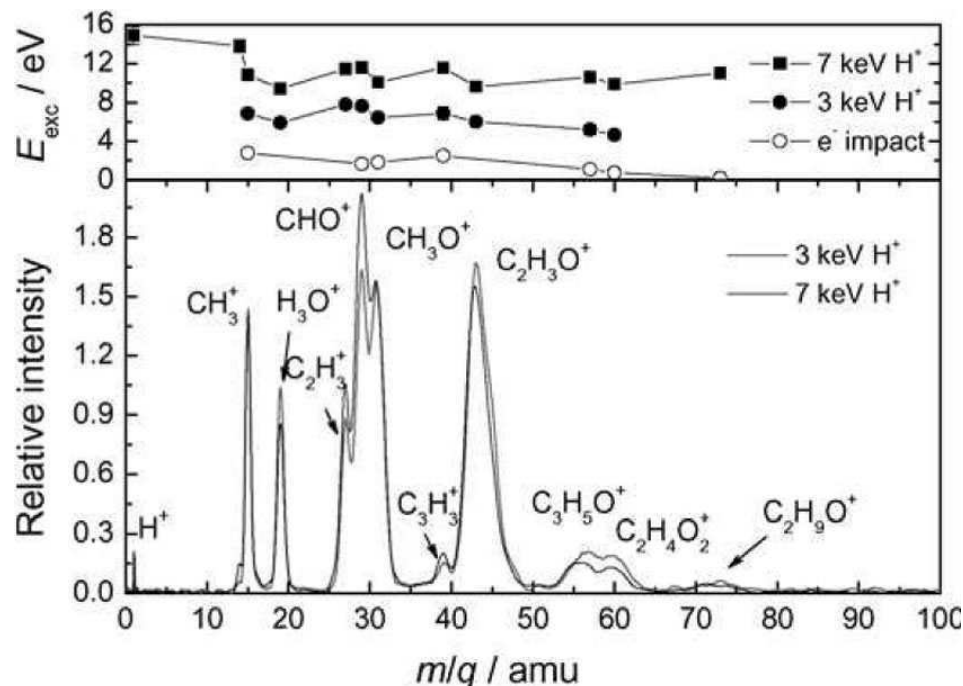
Lower panel: Fragment ion mass spectra from collisions of 3 and 7 keV protons with 2-deoxy-d-ribose. Upper panel: Most probable excitation energy Eexc associated to major fragments for different proton impact energies (3 keV and 7 keV) and excitation energy obtained by electron impact ionization.

Two obvious conclusions can be drawn:

1) The mass spectrum is only weakly dependent on the kinetic energy of the proton projectile for the energy range under study.

2) The most probable excitation energy is only weakly dependent on the fragment mass, but depends strongly on the projectile energy. On average, 6.5 eV and 11 eV are deposited for 3 keV and 7 keV proton impacts, respectively.
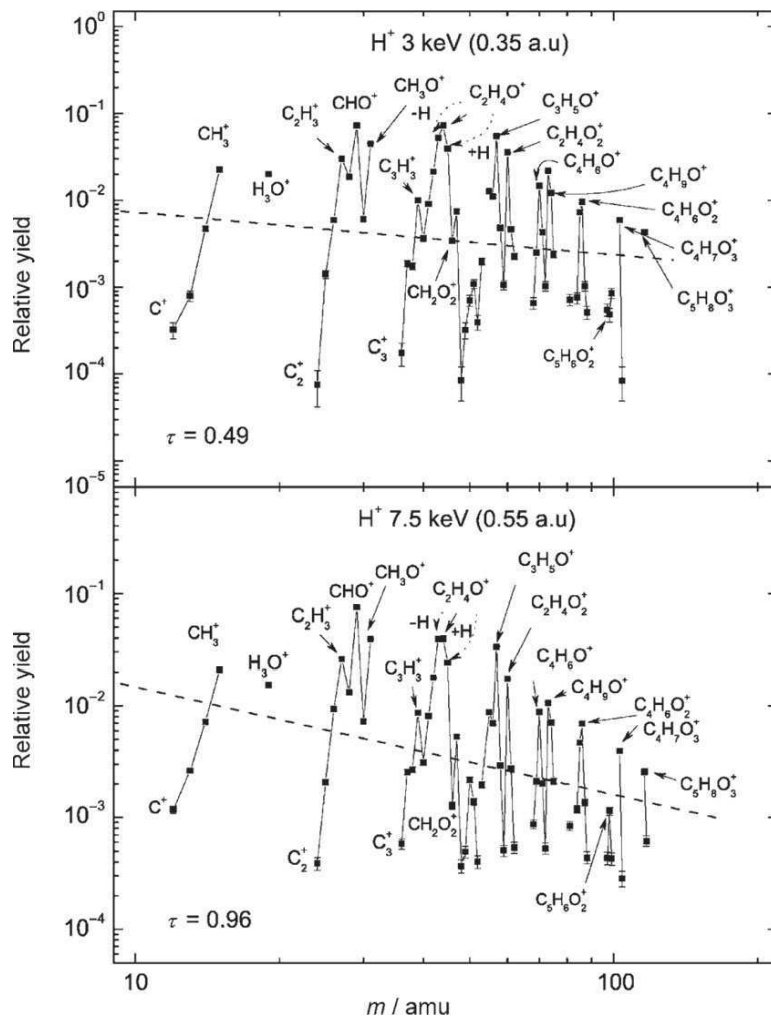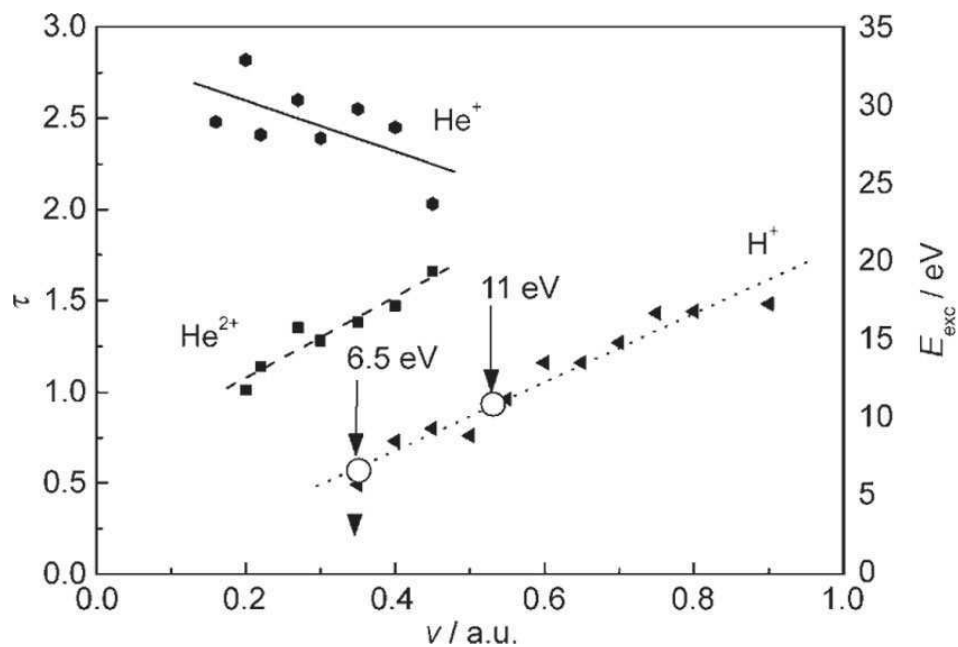
Peak integrals of integral fragment ion mass spectra from collisions of 3 and 7.5 keV protons (v=0.35 a.u. and v=0.55 a.u.) with 2-deoxy-dribose.

⇒ clear indications of statistical fragmentation processes where the deposited excitation energy redistributes over the vibrational degrees of freedom before the excited molecular complex eventually breaks apart. The inclusive fragment ion distributions follow a power law $n(M) \approx M^{-\tau}$, with n being the number of detected fragments, M the fragment mass and τ the characteristic exponent.

⇒ deviations from the fit are due to different stabilities, appearance energies and ionization potentials of the various fragment cations.

⇒ such power-law trends of fragmentation patterns with characteristic exponents around $\tau=2$ are found on various scales, ranging from nuclear fragmentation to cluster fragmentation: signatures of statistical fragmentation

Value of the characteristic parameter τ for deoxyribose spectra after collisions with different projectile ions at different velocities v

$\Rightarrow$ for the proton projectiles, the nearly linear dependence of the characteristic exponent τ on the collision velocity correlates fairly well with the variation in the measured excitation energy

$\Rightarrow$ tight relation between these two parameters

Ionization and fragmentation of tetraphenyl iron (III) porphyrin chloride induced by slow multiply charged ion impact Bernigaud *et al* Eur. Phys. J. D 51, 125–130 (2009)
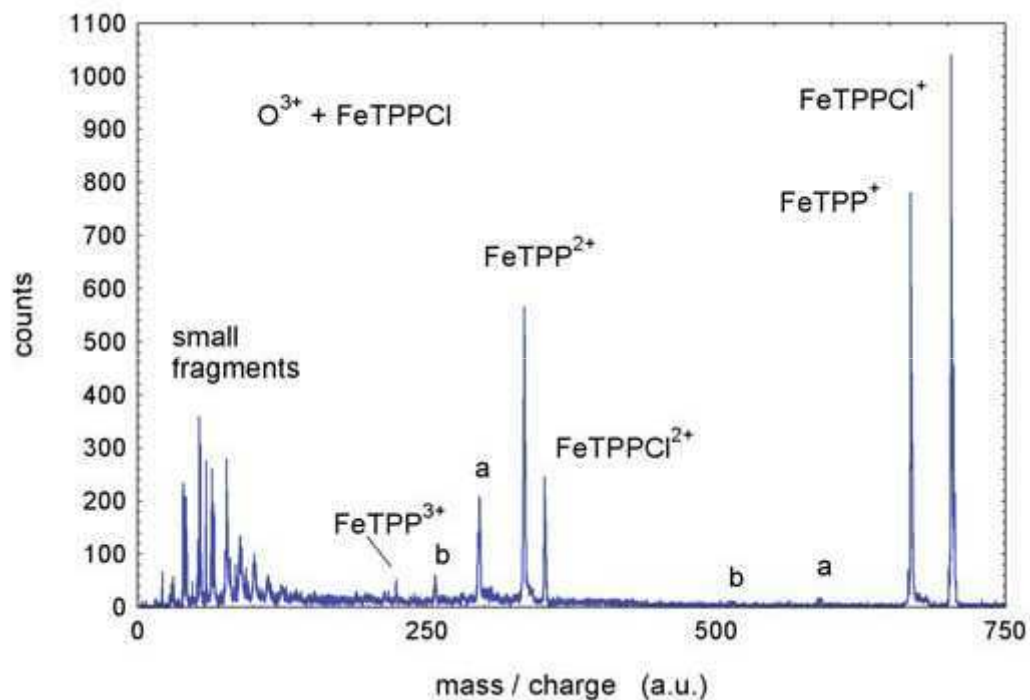
Sketch of the molecular structure of FeTPPCl ($C44H28ClFeN4$)
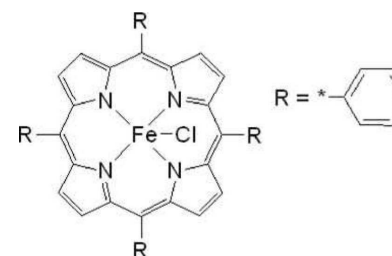
$\Rightarrow$ ionizing process much less violent than collisions with slow electrons (70 eV)

$\Rightarrow$ the loss of neutral Cl-atoms represents the dominant decay channel

Typical fragmentation spectrum obtained in 30 keV-collisions of O3+ ions with FeTPPCl molecules. The peaks, characterized by a and b correspond to the loss of 1 and 2 phenyl groups in addition to the Cl-atom, respectively.
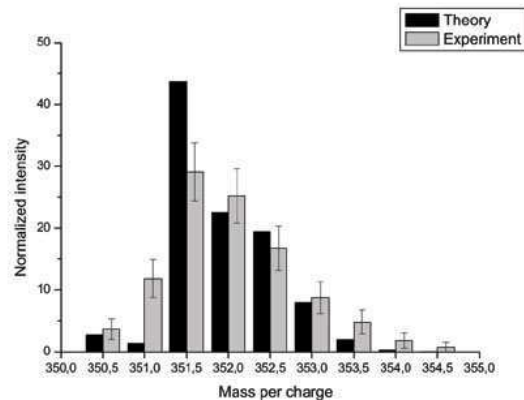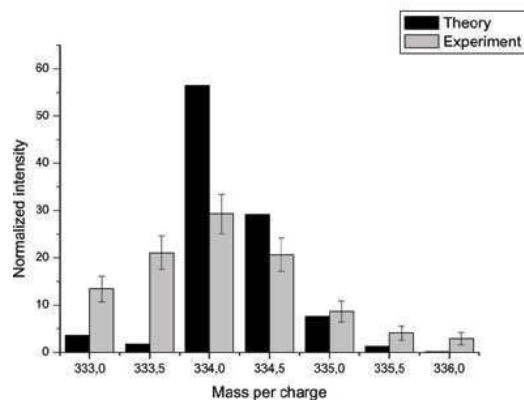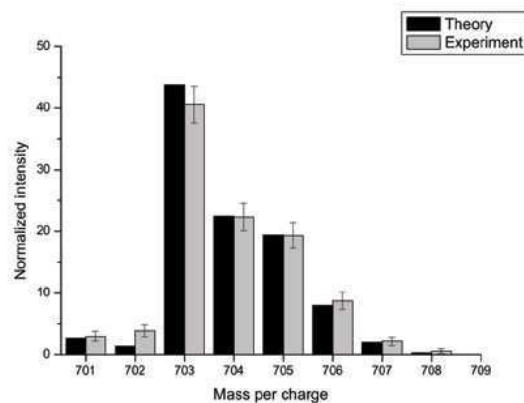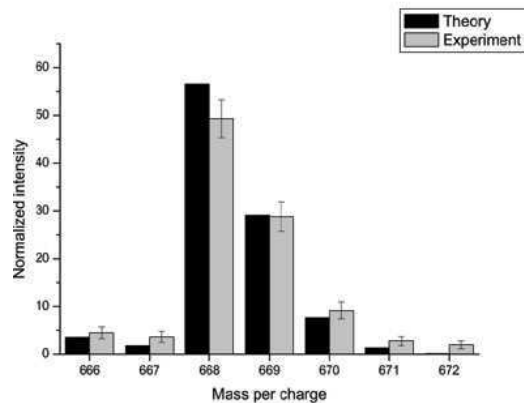
Isotopic contributions for singly (upper part: FeTPPCl+ (right) and FeTPP+ (left)) and doubly charged (lower part) ions. Comparison of the experimental results with distributions obtained by taking into account the natural isotopic contributions to the different peaks. Collision system: O3+ + FeTPPCl at 30 keV.

$\Rightarrow$ singly charged ions: general agreement experiment / theory. In the experimental spectra the intensity of the most prominent peak is slightly reduced in favor of the masses 701/702 and 666/667, respectively: one or two hydrogen atoms are emitted after single ionization with low probability.

$\Rightarrow$ doubly charged ions: hydrogen loss becomes more important.

…prompt and delayed processes
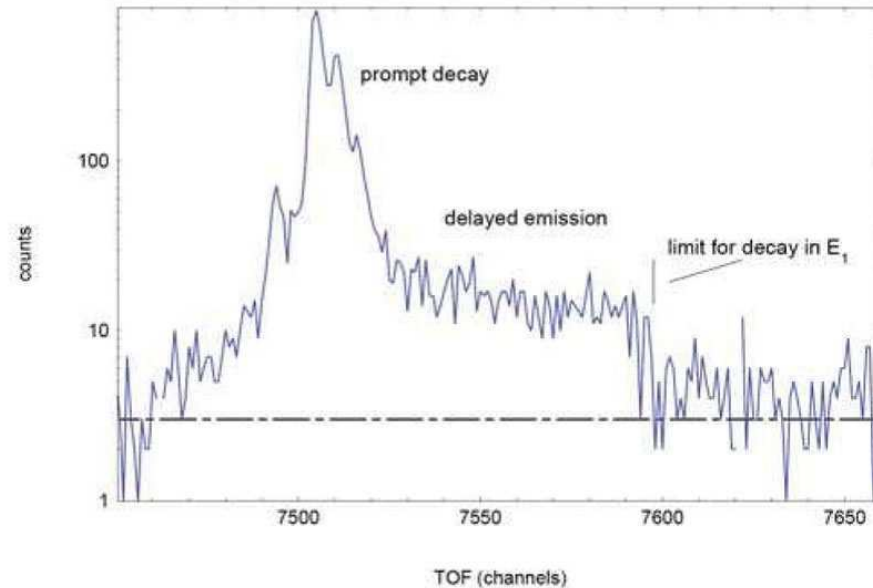
Shape of the FeTPP+ peak form created by a prompt decay (< ns) and delayed decay (<10 μs) of the intact molecule by emitting a neutral Cl-atom.

⇒ a tail towards longer drift times: contributions due to a decay which occurs within the extraction region of the Wiley- McLaren –TOF-system.

⇒ from the intensity of the observed tail they could calculate the relative fraction of the delayed Cl-emission with respect to the prompt process, as well as the characteristic lifetime, 10% and 8 *μs, respectively*

+ (not shown here) emission of negative charges, either as delayed electrons or possibly as negative anions is observed increasing the initial positive charge of the ionized system.

Ion-Induced Biomolecular Radiation Damage: From Isolated Nucleobases to Nucleobase Clusters Schlathçlter *et al* Chem. Phys. Chem 2006, 7, 2339 – 2345
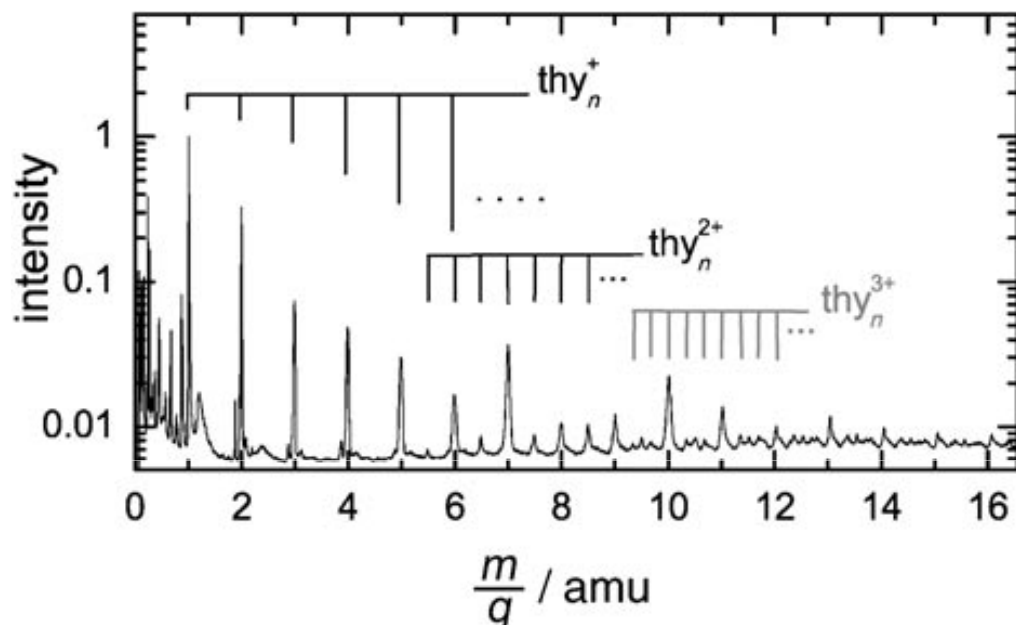

Wide-scale mass spectrum for collisions of 50 keV O5+ with neutral thymine clusters. The masses are given in multiples of the thymine mass. Peak series due to singly, doubly, and triply charged clusters are indicated.

⇒ characteristic additional fragmentation channels are observed in the cluster case: the most important of these channels are the loss of O and H.
⇒ the fragmentation was compared to existing data on condensed-phase thymine, where the respective characteristic channel was observed as well.
⇒ these are probably due to the hydrogen bonding between O and H atoms in thymine, that is, the clusters apparently favor planar geometries over stacked ones.

Electron spectroscopy in proton collisions with dry gas-phase uracil base Moretto-Capelle *et al* PHYSICAL REVIEW A 74, 062705 2006

→ measurement of the DDCS for the emission of secondary electrons in proton-dry uracil collisions, at a fixed angle of 35°
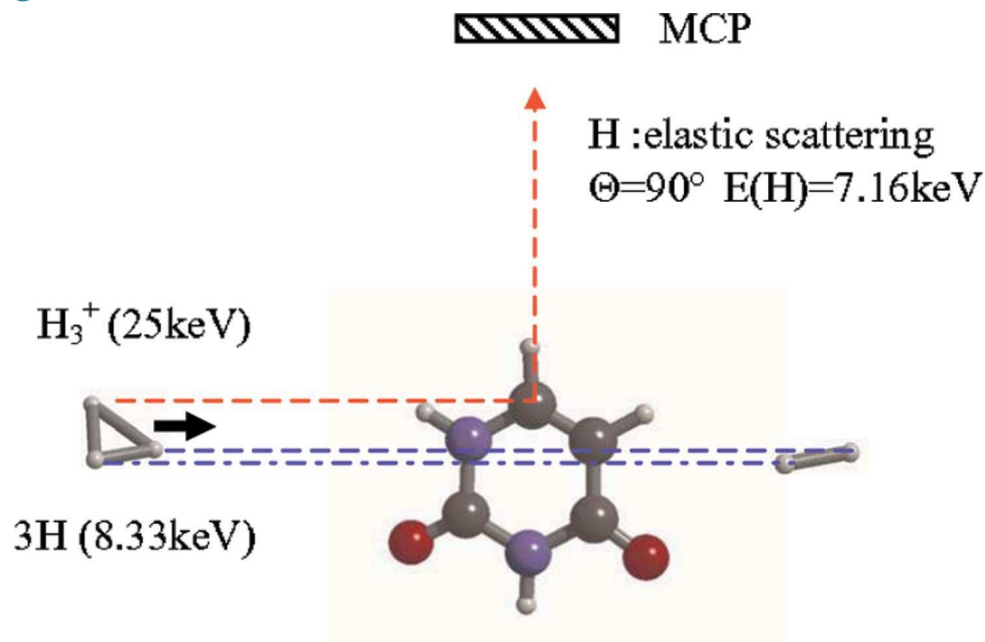
Schematic of the normalization procedure. Elastic scattering, at right angle, in H3 + (25 keV) + uracil collisions. The scattered particles are identified by time-of-flight spectrometry and detected by a multichannel plates package.
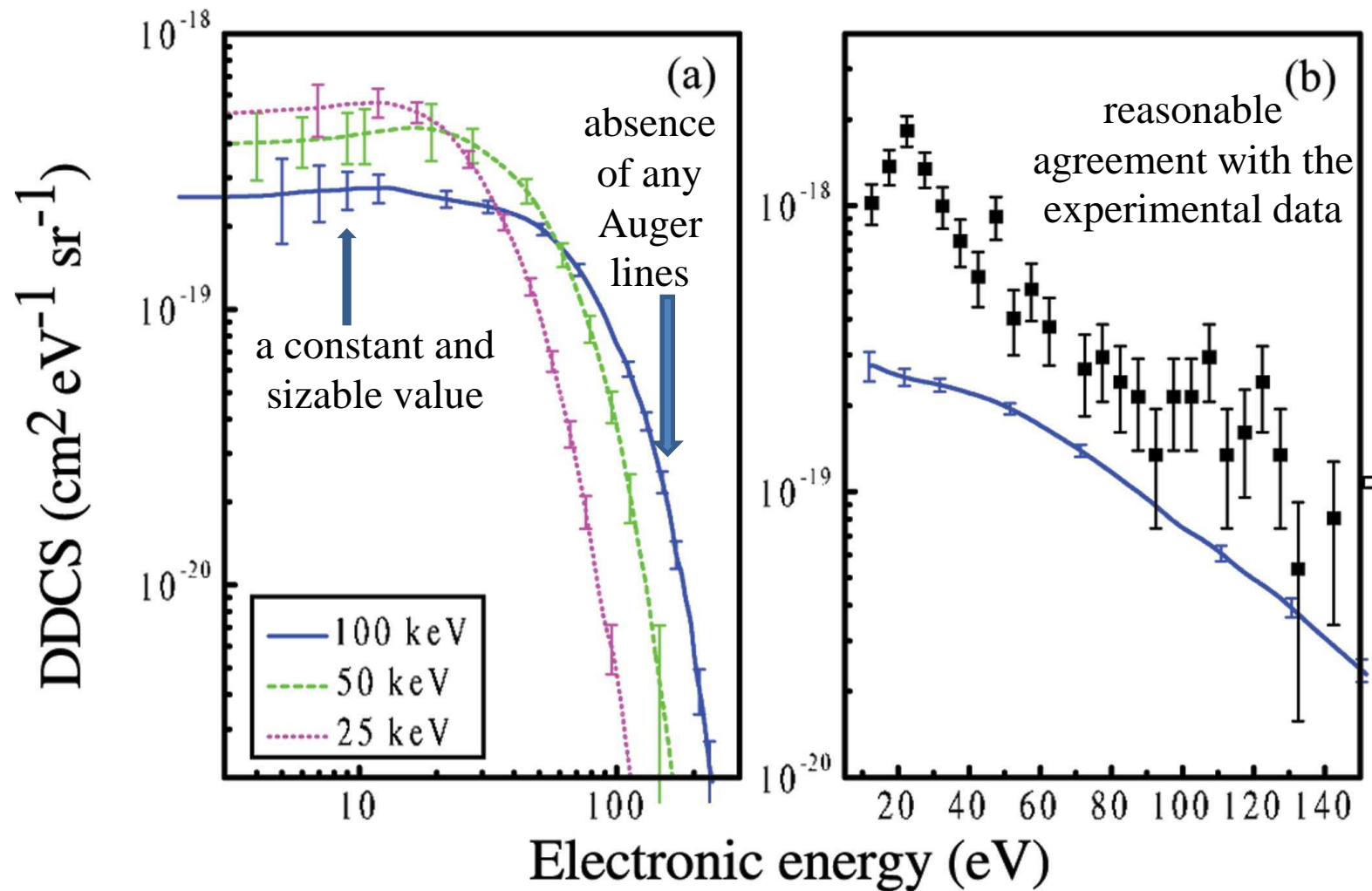
(a) Left: DDCS versus electronic energy. Three sets of data are presented for incident proton energies of 100, 50, and 25 keV, in solid, dashed, and dotted lines, respectively.

(b) Right: Comparison between CTMC computation in filled squares and experimental data in solid line.

Interactions Biomolecules - atoms

Electron capture-induced dissociation of AK dipeptide dications: influence of ion velocity, crown-ether complexation and collision gas

→ this work deals with collisional electron transfer between dipeptide dications and a rare gas

⇒ electron capture leads to three dominant channels, H loss, NH3 loss, and N–C bond breakage to give either c+ or z+ fragment ions.
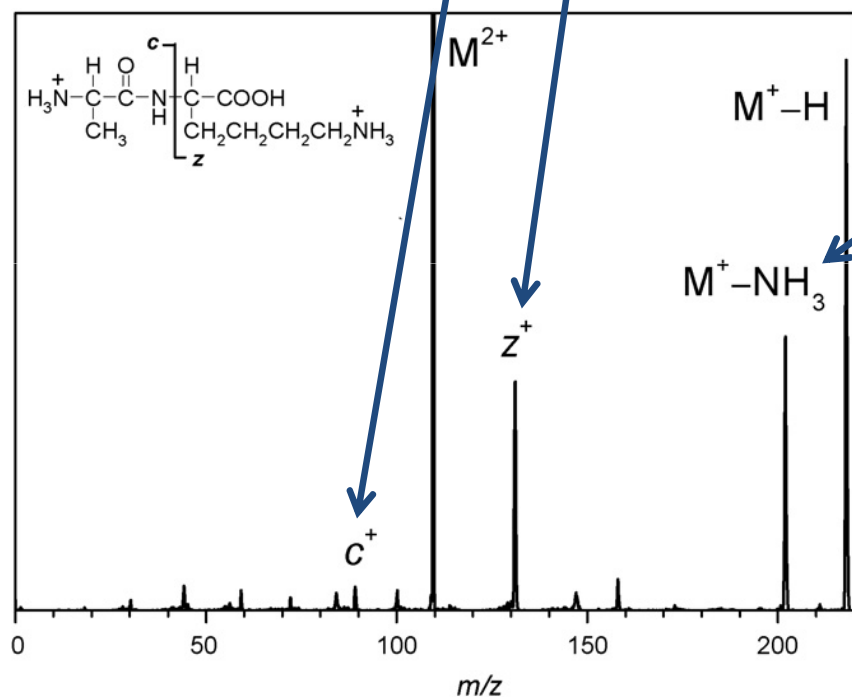
ECID spectrum of M2+ = [AK + 2H]2+ obtained after electron capture from Na
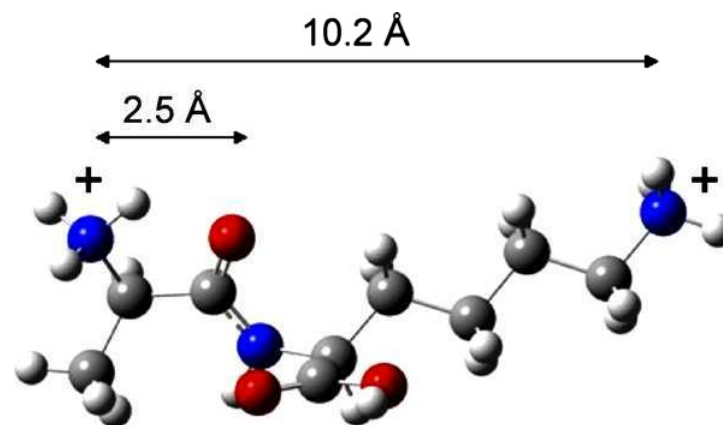
One possible structure of [AK + 2H]2+ obtained at the B3LYP/6-31+G(d) level of theory.

The relative importance of these channels has been explored as a function of ion velocity, the degree of complexation with crown ether, and collision gas.
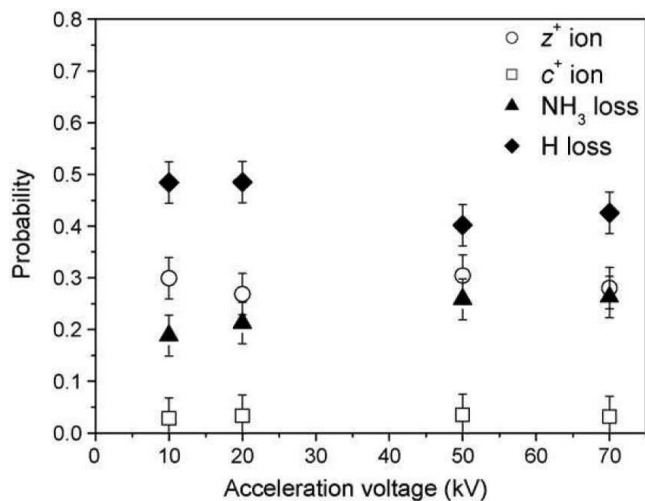
Probability of formation of the different ECID ions of [AK + 2H]2+after electron capture from Na as a function of the acceleration voltage.
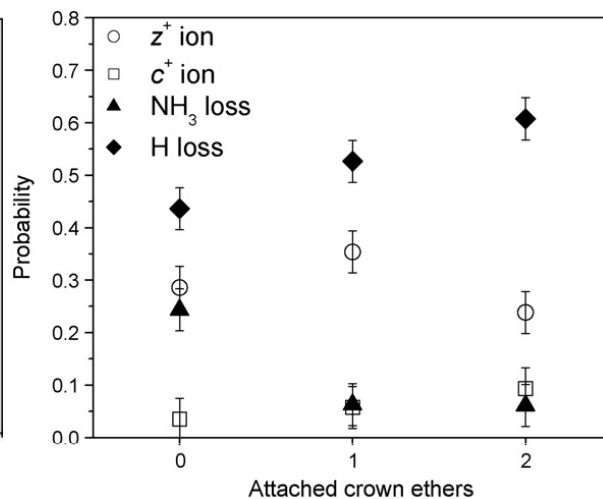
Probability of formation of the different ECID ions after electron capture from Na as a function of the number of attached crown ethers to [AK + 2H]2+.
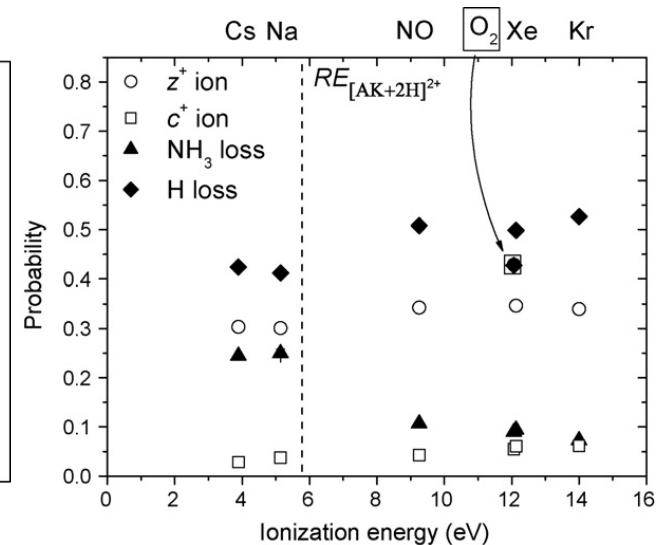
Probability of formation of the different ECID ions after electron capture to [AK + 2H]2+ as a function of the ionization energies of the target gases.

⇒ H loss and NH3 loss are competing channels whereas the probability of N–C bond breakage is more or less constant.

$\Rightarrow$ Interactions with charged ions and with atoms

$\Rightarrow$ In the gas phase or in complexation with crown ether

$\Rightarrow$ Different types of targets: nucleobases and small systems, larger molecules, clusters / effect of the isomerization

$\Rightarrow$ Different processes: (prompt and/or delayed) fragmentation, ionization (electron spectroscopy/DDCS)

$\Rightarrow$ Different parameters: excitation energy of the target, correlation spectra, KER, activation energies, fission barriers, transition states

Electron biomolecules interactions

Ionizing radiation $\Rightarrow$ generation of secondary electrons
Problem of Transport of electrons in (bio) matter

A-'Scattering' processes
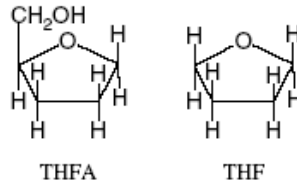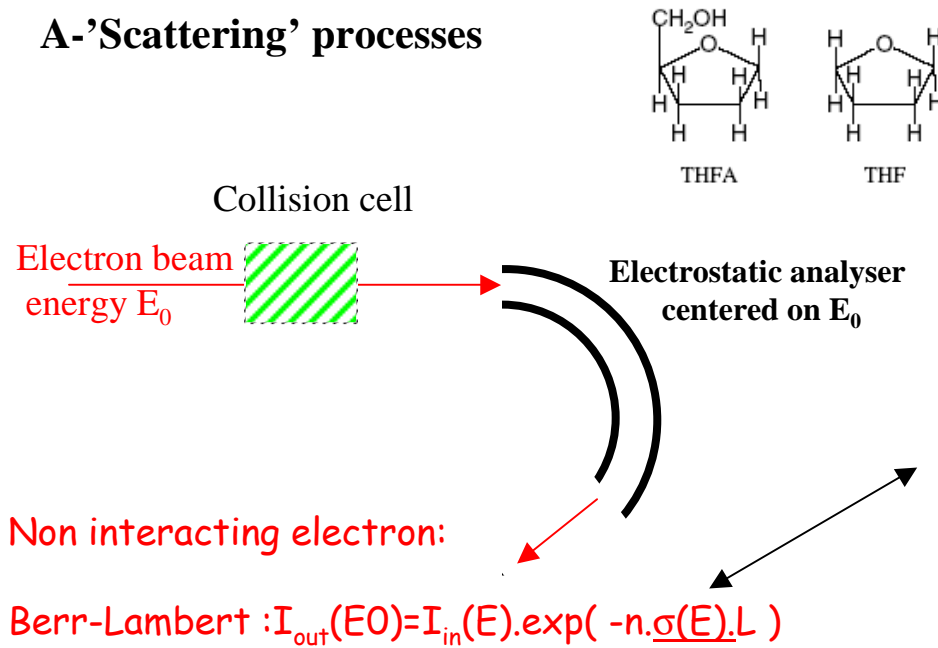
**Tetrahydrofurfuryl alcohol, Tetrahydrofuran simplest analogue of the DNA deoxyribose**

Collision cell

Electron beam
energy E_0

**Electrostatic analyser
centered on E_0**

Resonances ?

Non interacting electron:

Berr-Lambert : $I_{out}(E0) = I_{in}(E) . \exp(-n.\underline{\sigma(E)}.L)$
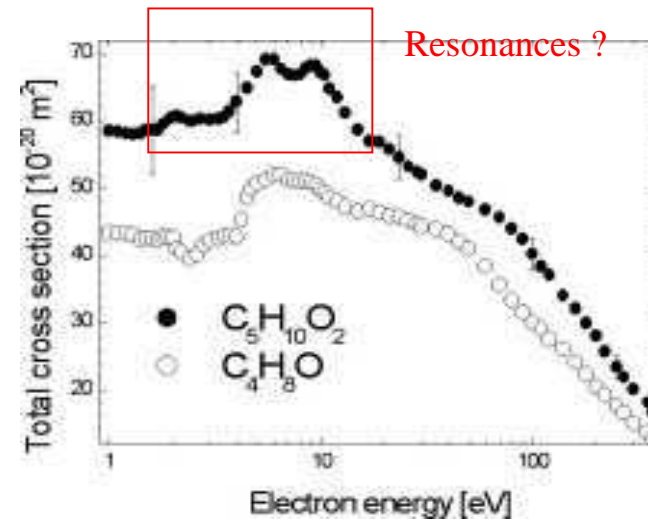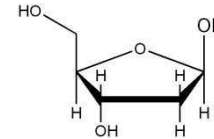
Scattering angle: differential cross sections
Transport of electrons….

Angular dependence of absolute
DCSs for elastic electron scattering
from THFA molecule at different
incident energies: (•), experiment; (−),

Other molecules
Calculation in **I**ndep. **A**toms **M**odel
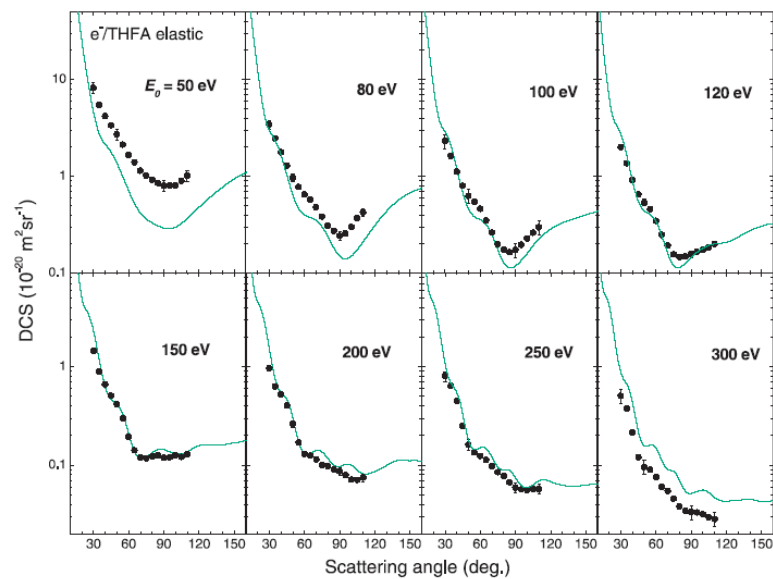
Milosavljevic, et al,
Eur. Phys. J. D 35, 411-416 (2005).
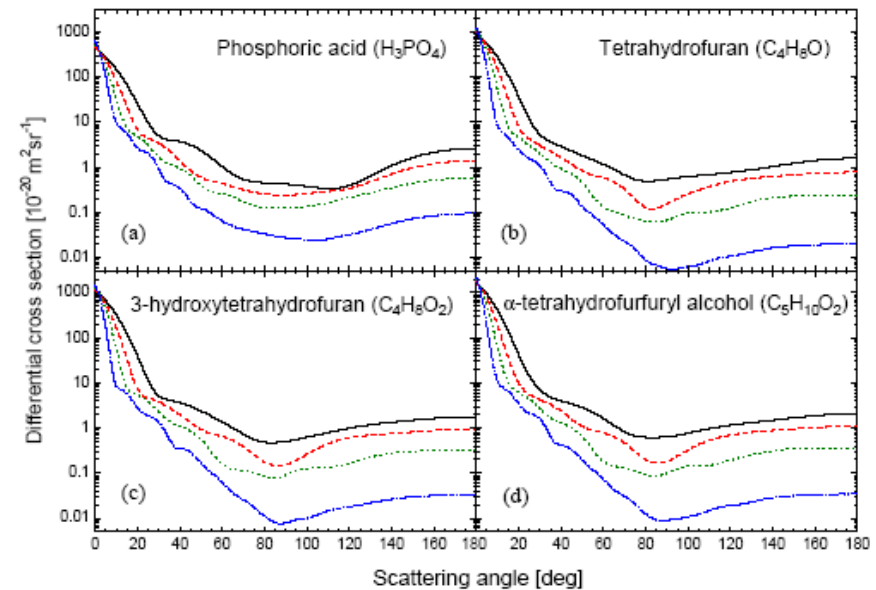
ross section for elastic electron collisions with studied targets at: (—) 50 eV, (− − −) 100 eV

Mozeiko and Sanche
Rad.Phys.Chem 73 (2005),77

B- Excitation/Fragmentation processes

Action of electrons on neutral molecules depends on the incident electron energy …
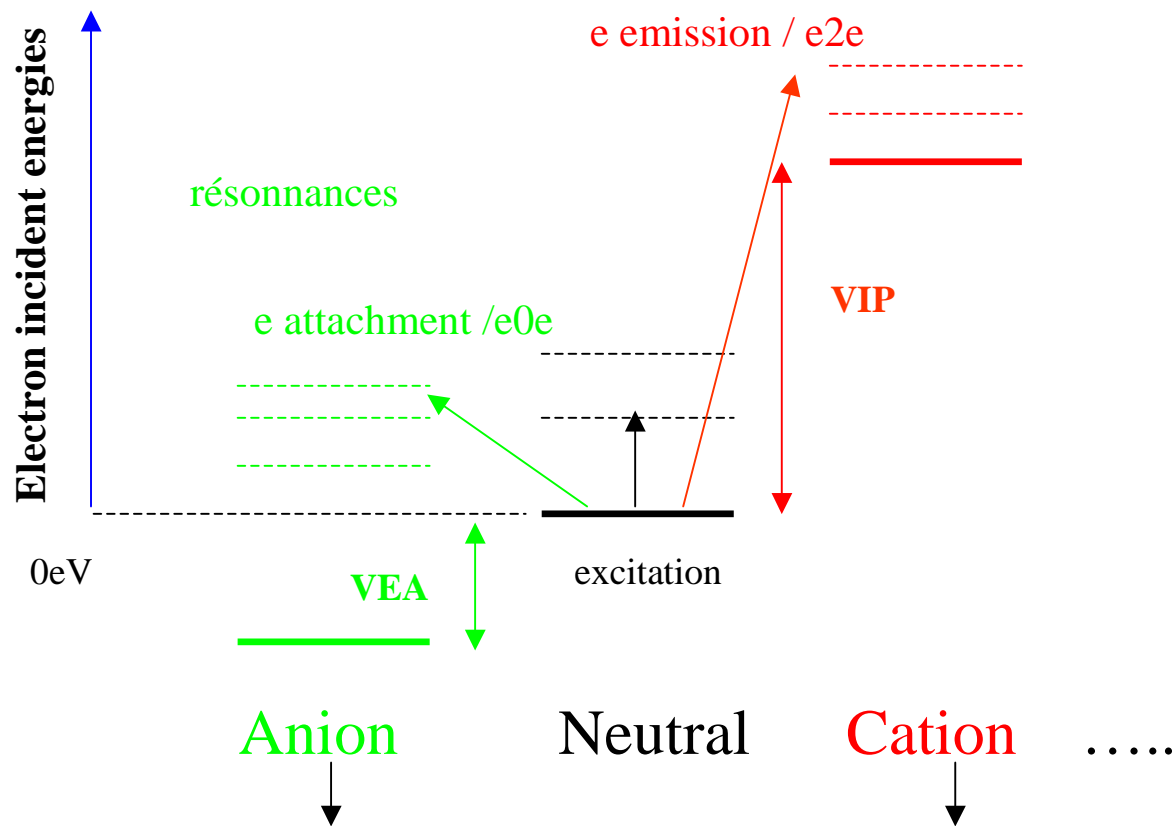
Investigation of initially charged molecule: mass spectrometry

1-Electronic and vibrational excitation: **E**lectron **E**nergy **L**oss **S**pectrometry

Einc

Neutral molecule

$E^* = Einc - Eexc$

θ

Excited state

Ground state
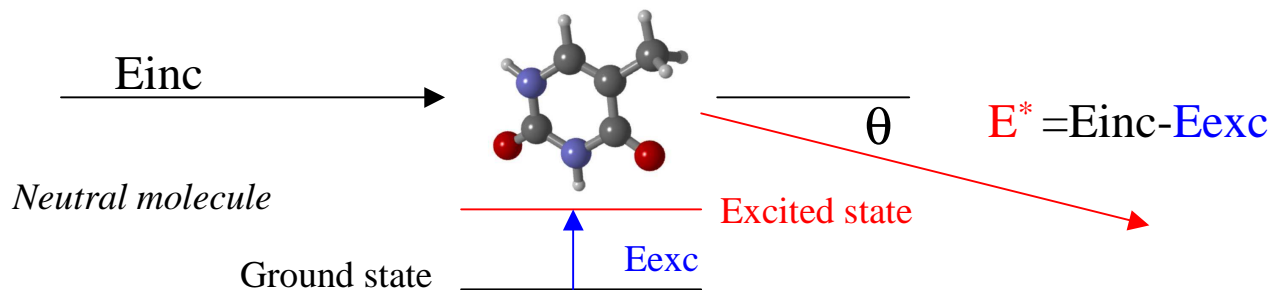
Eexc

Einc>>Eexc: no electron exchange: dipole allowed transition ie **Singlet states**≡**VUV absorption**

Einc≈Eexc: electron exchange can occur : also population of **Triplet states**

Singlet states

Abouaf et al,
Chem.Phys.Lett 381(2003) 486
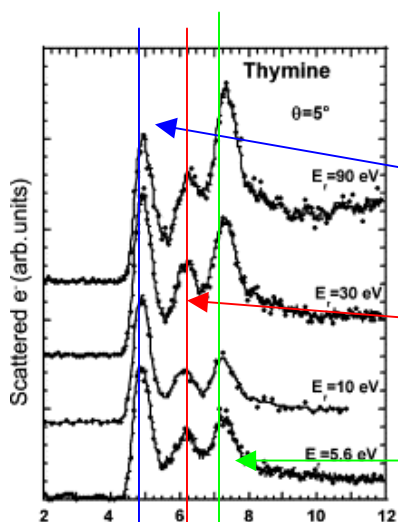
TABLE 3: INDO/S-Predicted $\pi \to \pi^*$ and Polarizations of Thymine and Uracil Observed Spectra[a]

	INDO			CASPT2 (ref 4)		
	E	f_{osc}	Θ^{DT}	E	f_{osc}	Θ^{DT}
				Thymine		
	40.2	0.386	−5	39.4	0.17	15
I	(39.8)	(0.395)	(−4)			
II	46.9	0.231	85	47.4	0.17	−19
	(45.4)	(0.248)	(86)			
III	52.8	0.261	−25	49.2	0.15	67
	(53.6)	(0.310)	(−26)			
IV	57.8	0.576	−29	57.5	0.85	−35
	(58.4)	(0.580)	(−27)			

x1000cm^{-1}

Broo et al, J.Phys.Chem A 101 (1997),3589

…+Triplet states

Comparison between VUV optical absorption and EEL spectroscopy of formamide

Synchrotron radiation source
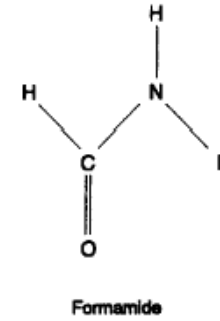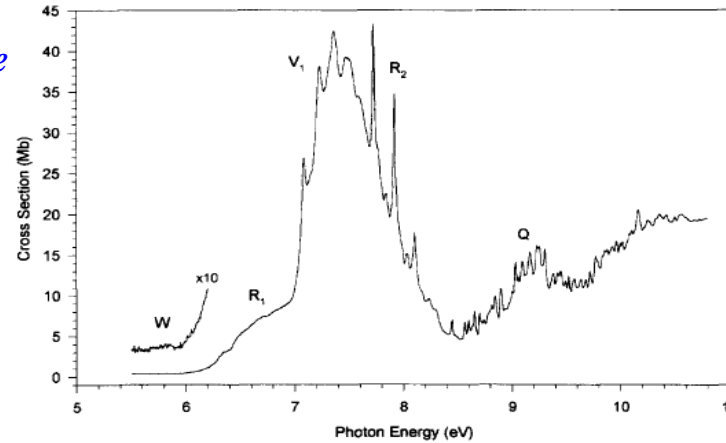
'Table top' experiment

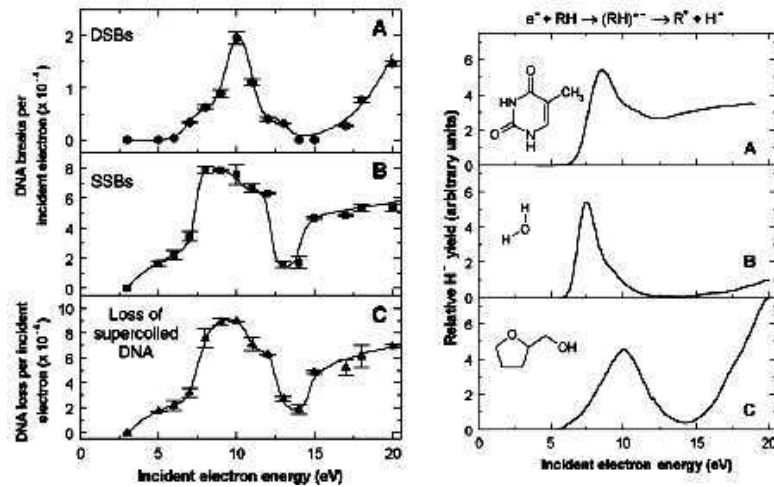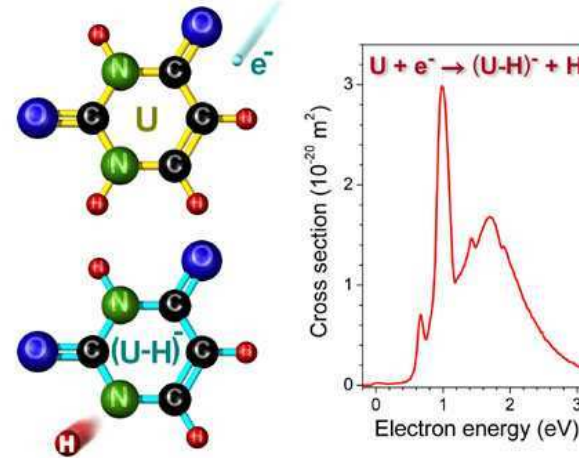**E=150eV, FWHM=50meV**

Gingell et al Chem.Phys. 220(1997),191
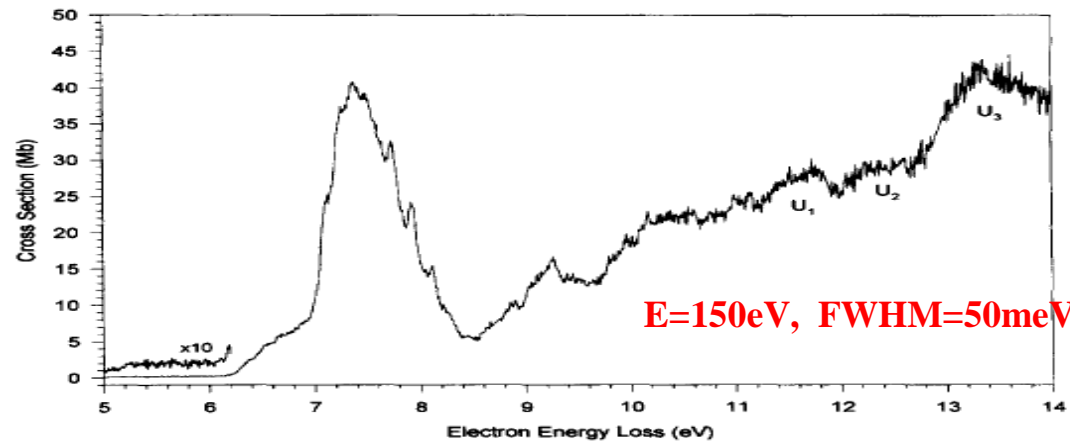
2-Low energy part: Dissociative Electron Attachment

'Big system: DNA'   *'Molecule'*

'It Doesn't Take Much to Break Up Uracil !'

Boudaiffa et al, Science 287 (2000)1653

$U + e^- \rightarrow (U-H)^- + H$

G. Hanel et al Phys.Rev.Lett 90(2003)

Caracterization of resonances through electron scattering calculation

Differents resonances

↓

Different dissociation patterns
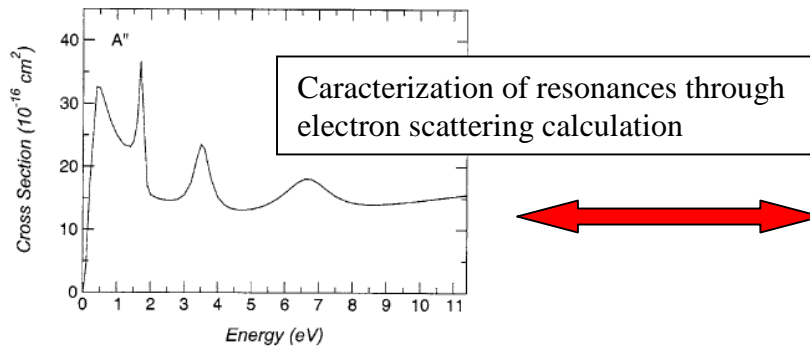
Gianturco et al J. Chem. Phys. **128**, 174302 2008

3-'High' energy part: Electron Induced Dissociation

Scanning of incident electron energy

$$e^- + M \rightarrow M^{+*} + 2e^-$$

Fragments

Fig. 3. Mass spectrum obtained by electron impact ionization of uracil at the electron energy of 70 eV.
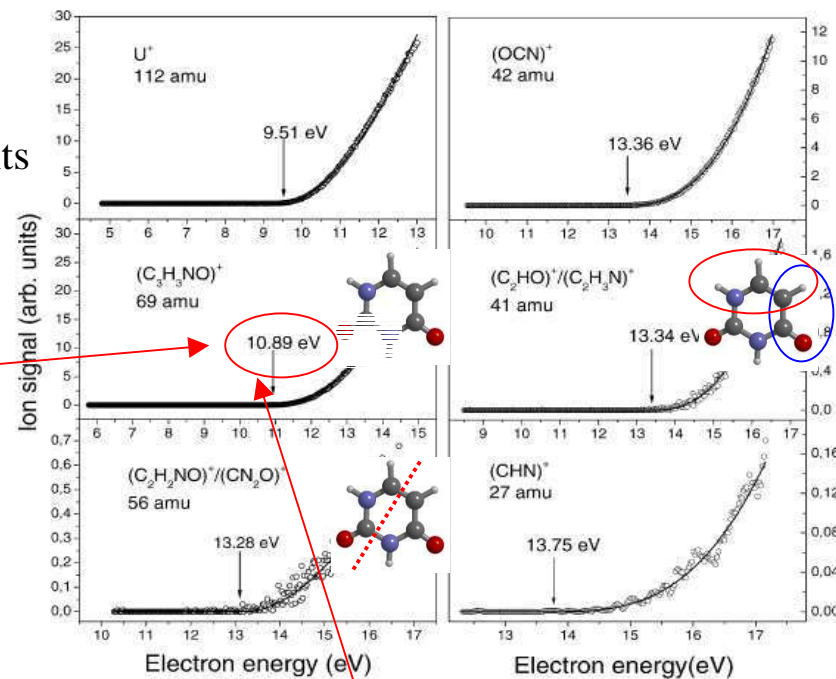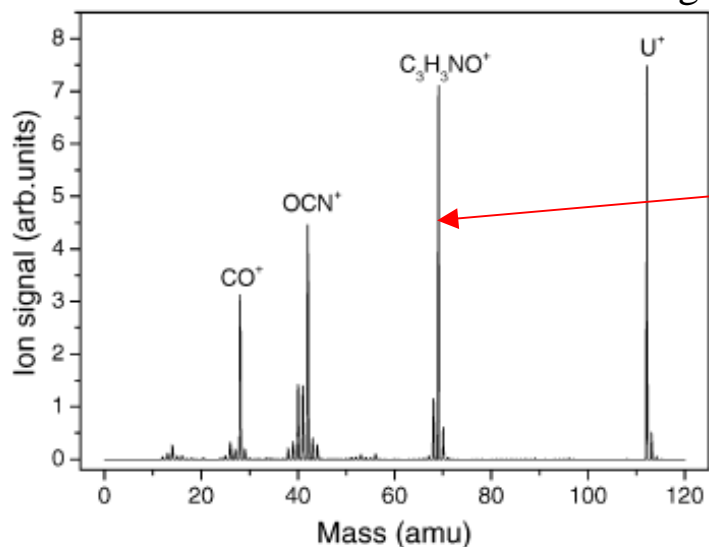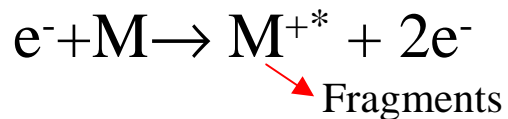
Denifl et al Int.J Mass Spect 238 (2004) 47–53

Theoretical considerations:
Emission of neutral OCNH
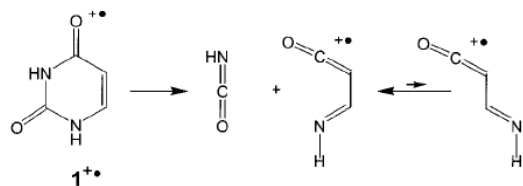
Turecek* and Wolken, *J. Phys. Chem. A 105 (*2001), p 8740

reaction	B3LYP/ 6-31+ G(d,p)	B3LYP/ 6-311+ G(2d,p)	B3-PMP2/ 6-311+ G(2d,p)
1 → 1⁺•	889 (9.21)ᵇ	889 (9.21)	891 (9.23)
1⁺• → (E)-syn- HN=CH−CH=C=O⁺• + HNCO	193	172	168
1⁺• → (E)-anti- HN=CH−CH=C=O⁺• + HNCO	203	182	182
	11.21	10.99	10.97 eV
	11.31	11.09	11.11eV

Other example: e⁻ + 5ClU

Loss of OCNH

Loss of mass 70??
Proton transfer

or

keto-enol
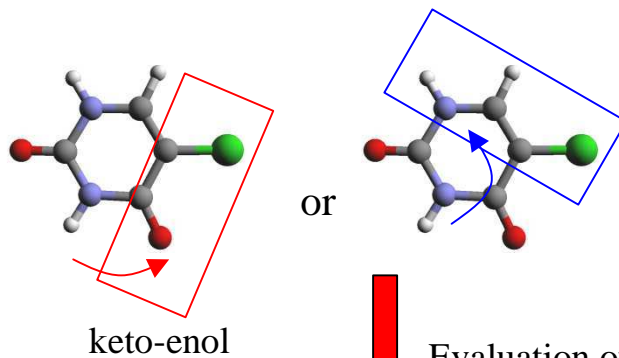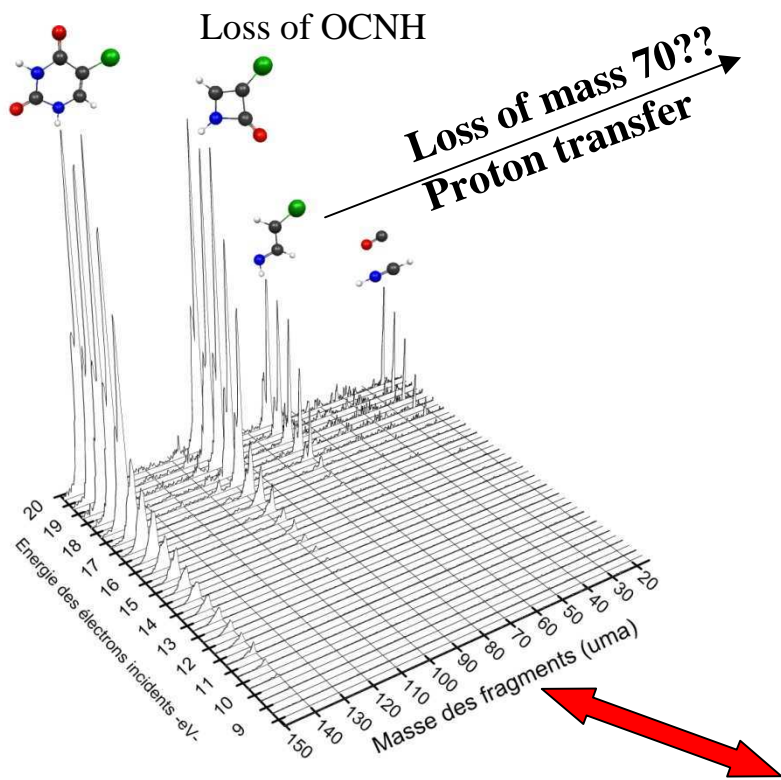
Evaluation of dissociation limits

Calcul B3LYP 6-31G(d,p)

cation

neutre
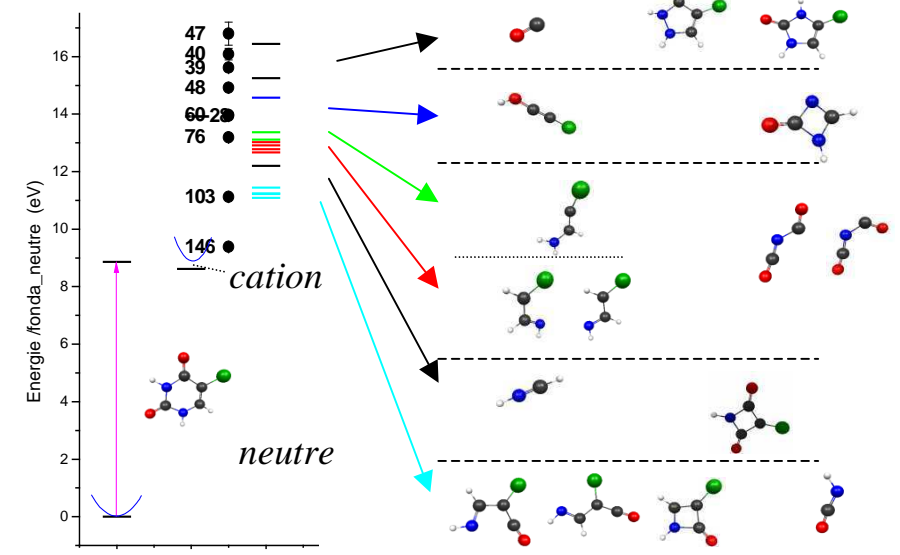
Comparaison with experimental thresholds

Structure of fragments

EXAMPLE OF SET-UP:

Innsbruck:

Quadripole mass spectrometer
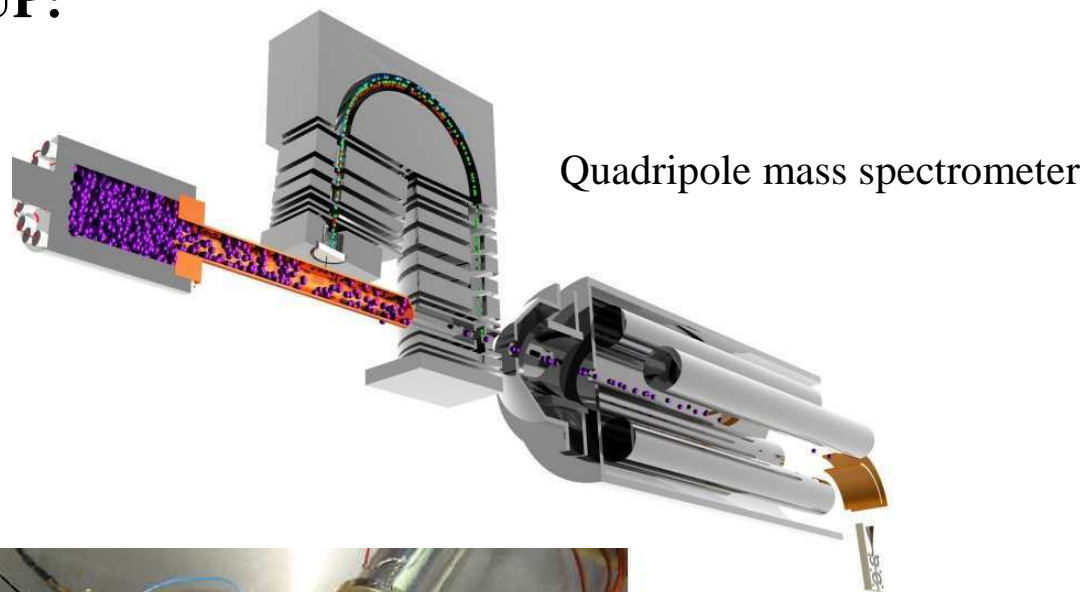
Time of flight cell
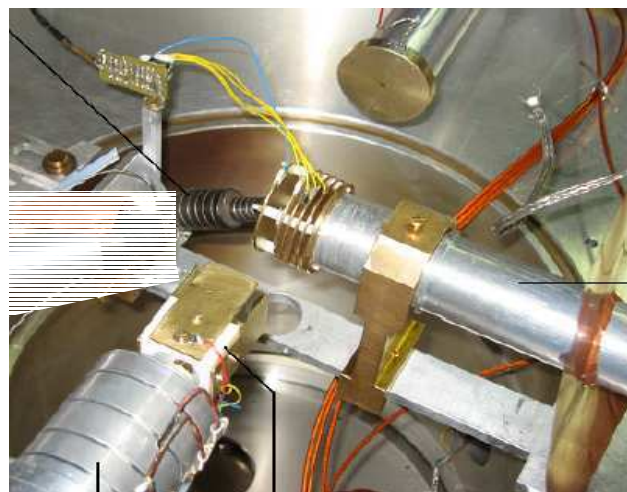
: Toulouse

canon à électrons
(5 électrodes)

déflecteurs

TOF

Photon biomolecules interactions

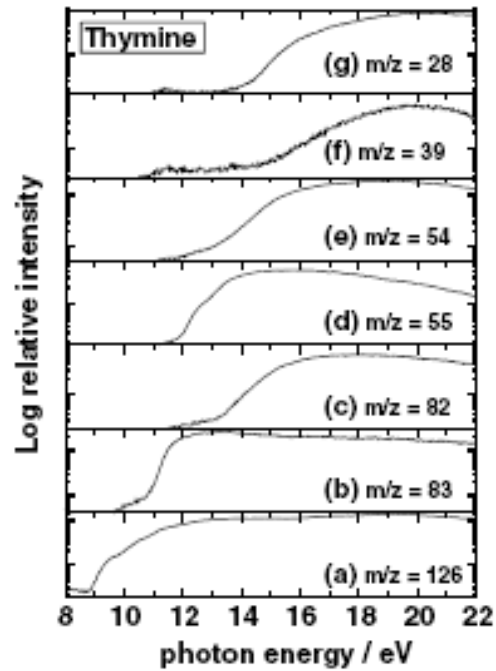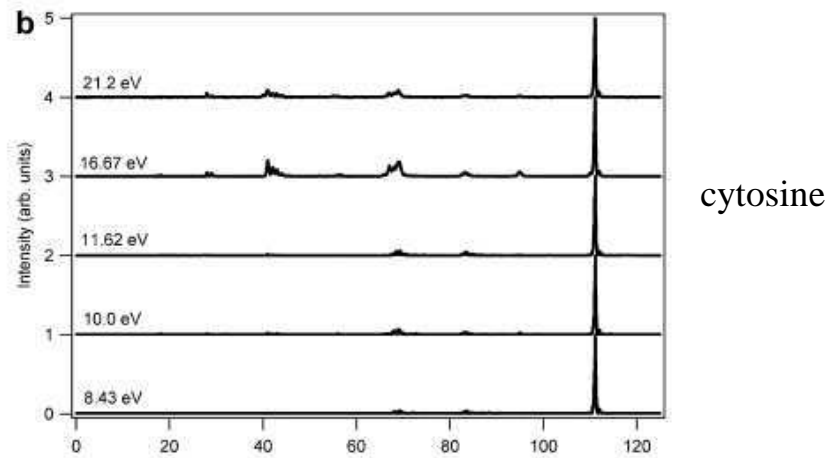Synchrotron Radiation sources : photo dissociation

Fig. 6. Selected ion yield curves of thymine.

cytosine

Same kind of experiment / electrons: better resolution

Photoelectron spectroscopy

Access to the electronic structure

Valence shells

A study of the valence shell electronic structure of uracil and the methyluracils
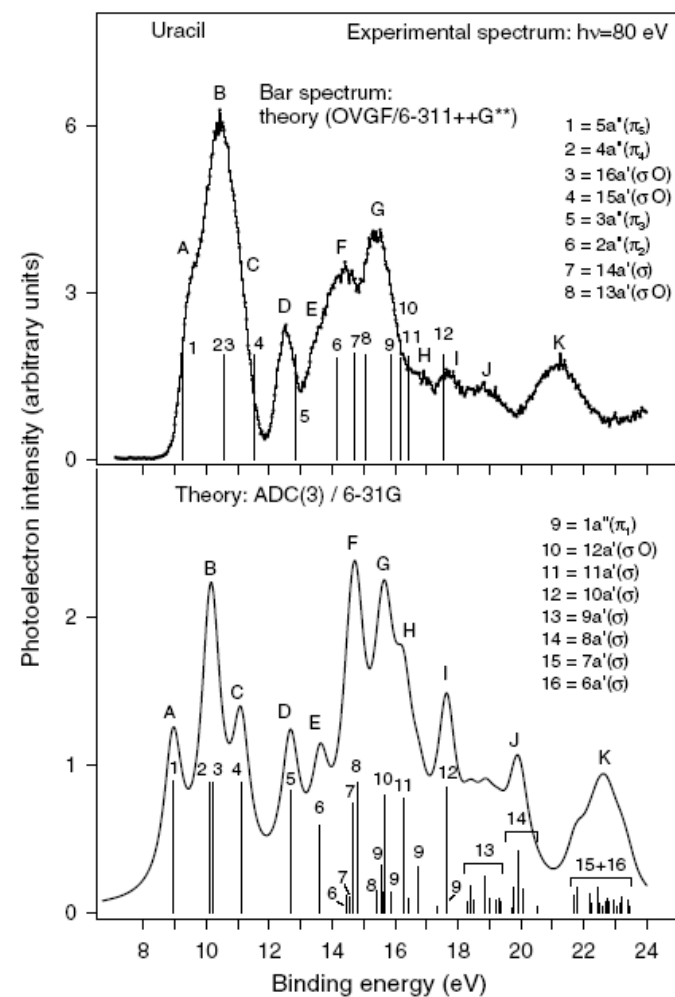D.M.P. Holland[a] el al
Chemical Physics 353 (2008) 47–58
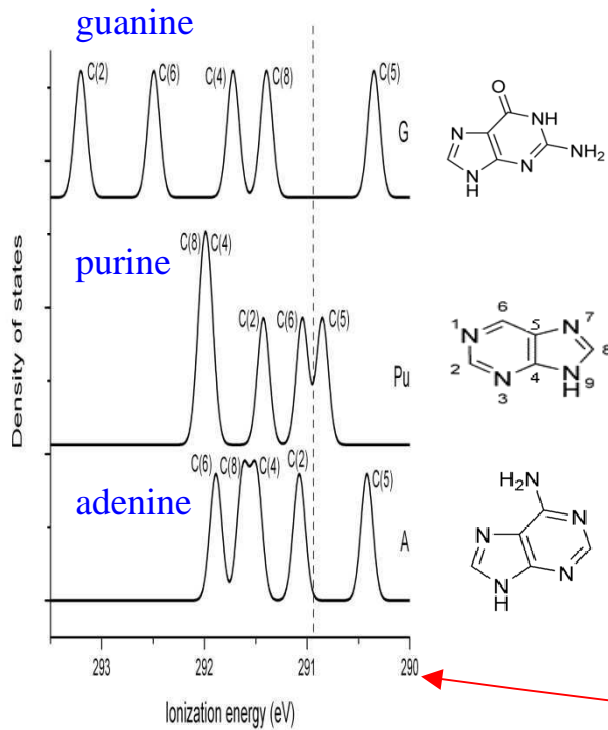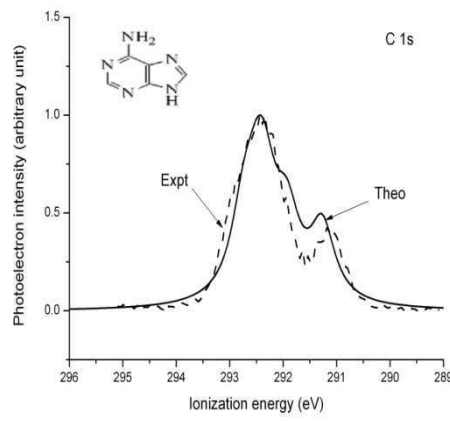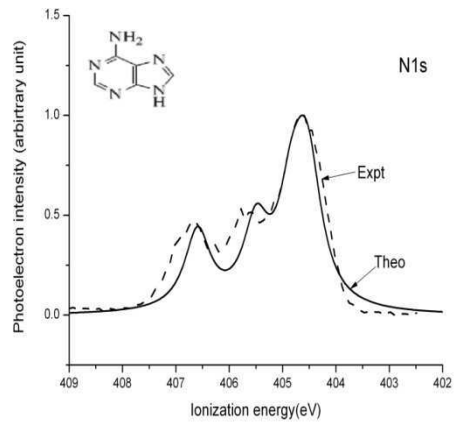

Fig. 2. Upper frame: The valence shell photoelectron spectrum of uracil recorded at a photon energy of 80 eV for $\theta = 0°$. The bar spectrum represents the results obtained using the OVGF approach. Lower frame: The theoretical photoelectron spectrum of uracil obtained using the ADC(3) method.

Inner shells spectroscopy.   1s electron

Radiation synchrotron
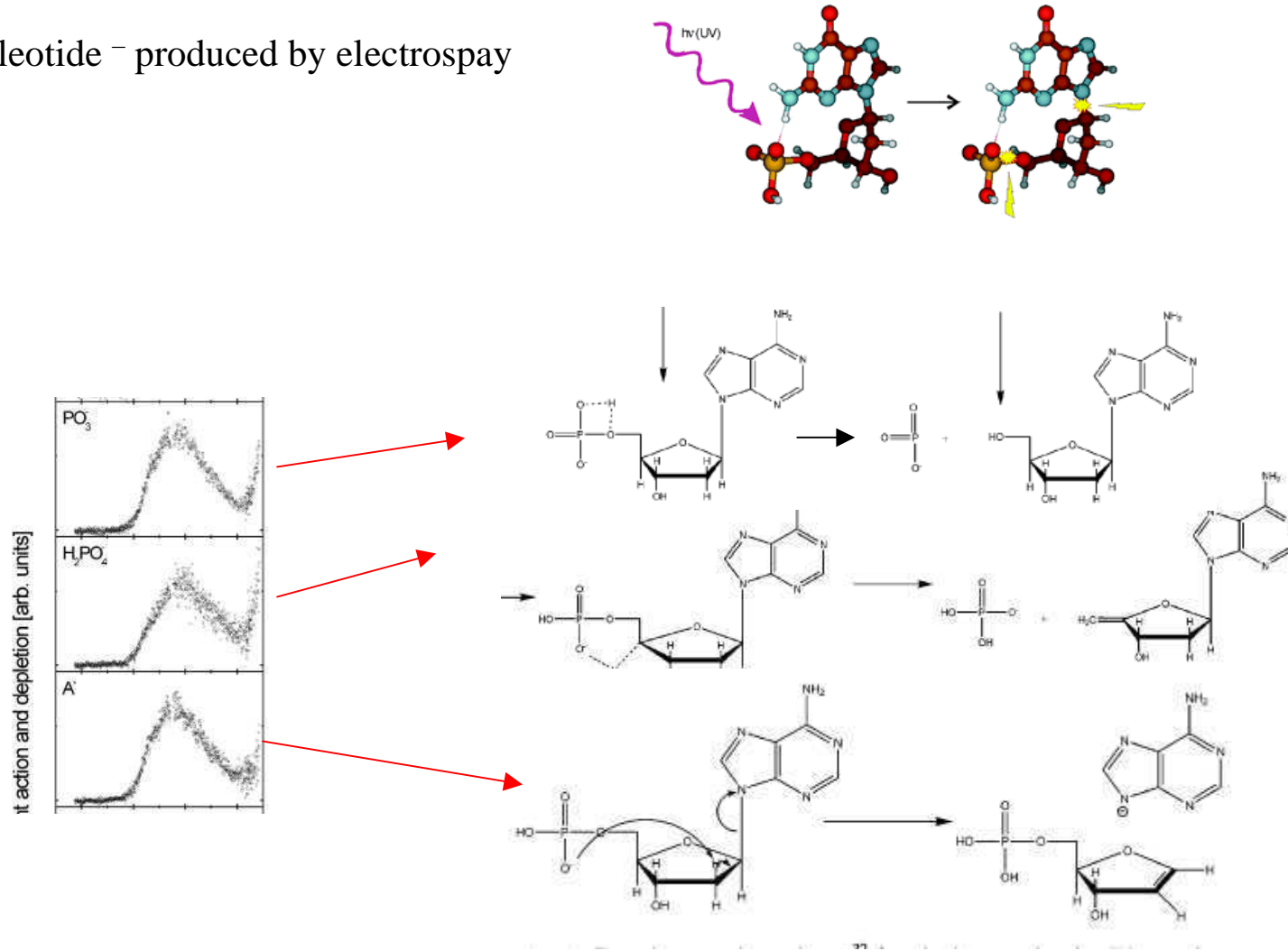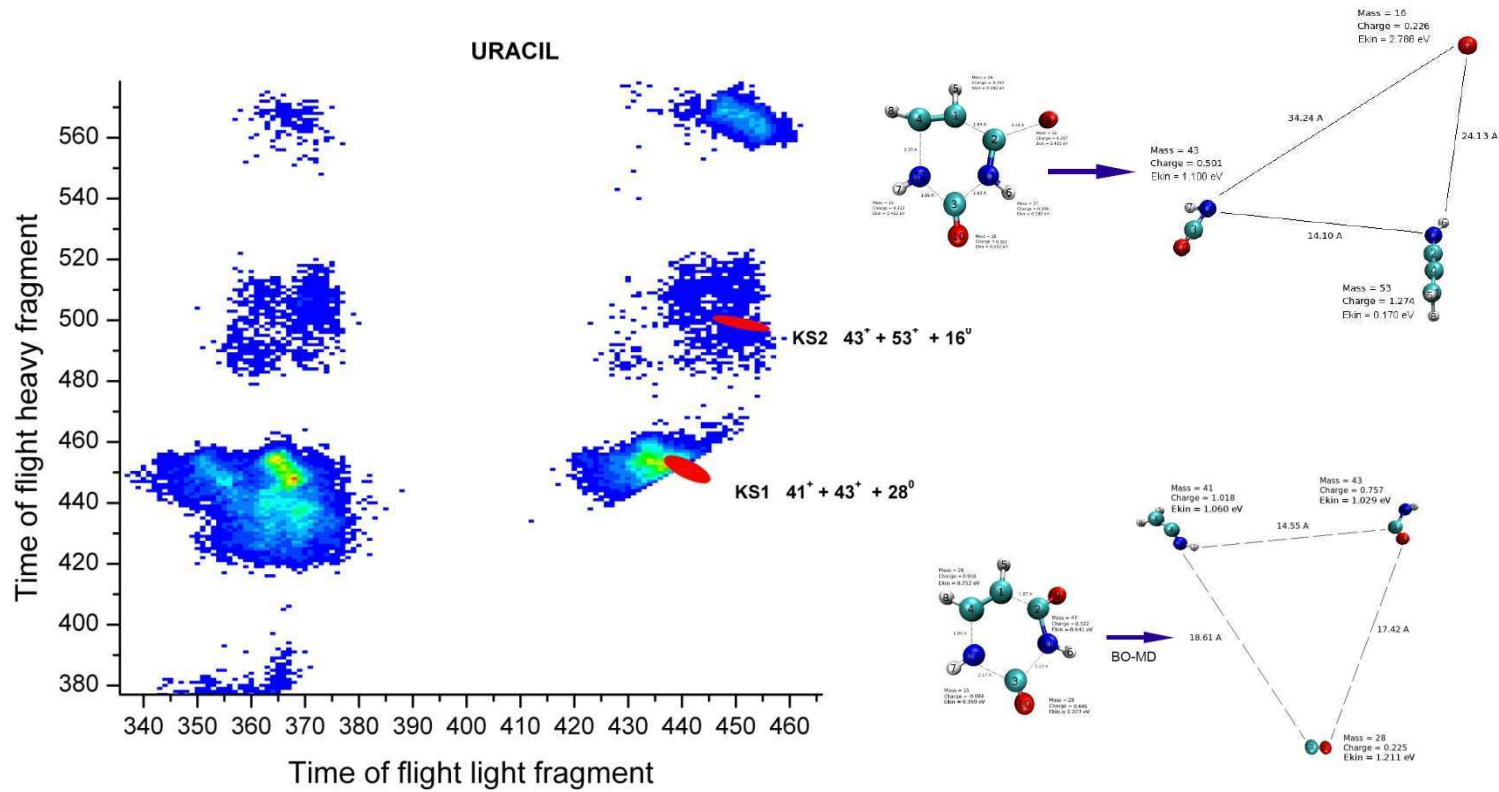**E_{photon}>350eV**

1s levels: sensitive to conformation

Photodamage to isolated mononucleotides—photodissociation spectra and fragment channels

Marcum J.et al *Phys. Chem. Chem. Phys.*, 2009, **11**, 1740-1751

Nucleotide ⁻ produced by electrospay

Double Ionization of molecule and fragmentation of dication
Access to the dynamic of dissociation

Theory: Two vacancies in valence orbitals Evolution CPMD+BO-MD + classical mecanism
MF Politis MP Gaigeot MA Hervé Le Penhoat R.Vuillemier F-Martin, P.Lopez…..

Concluding remarks:

Experiment acces to a large panel of phenomena often complex

But interpretation of results….sometimes difficult

NEEDING OF THEORETICAL INVESTIGATIONS