ion and photon interactions with trapped biomolecular ions

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university of groningen / faculty of mathematics and natural sciences / zernike institute for advanced materials

this talk

1. motivation
2. experimental
3. mass spectra and soft X-ray spectra
   - leucine enkephalin as a typical system
4. the role of protein size
   - from small peptides to larger proteins
5. secondary structure
   - soft X-ray spectroscopy and gas-phase structure
6. soft X-ray photoionization of oligonucleotides

why energetic ions & photons?

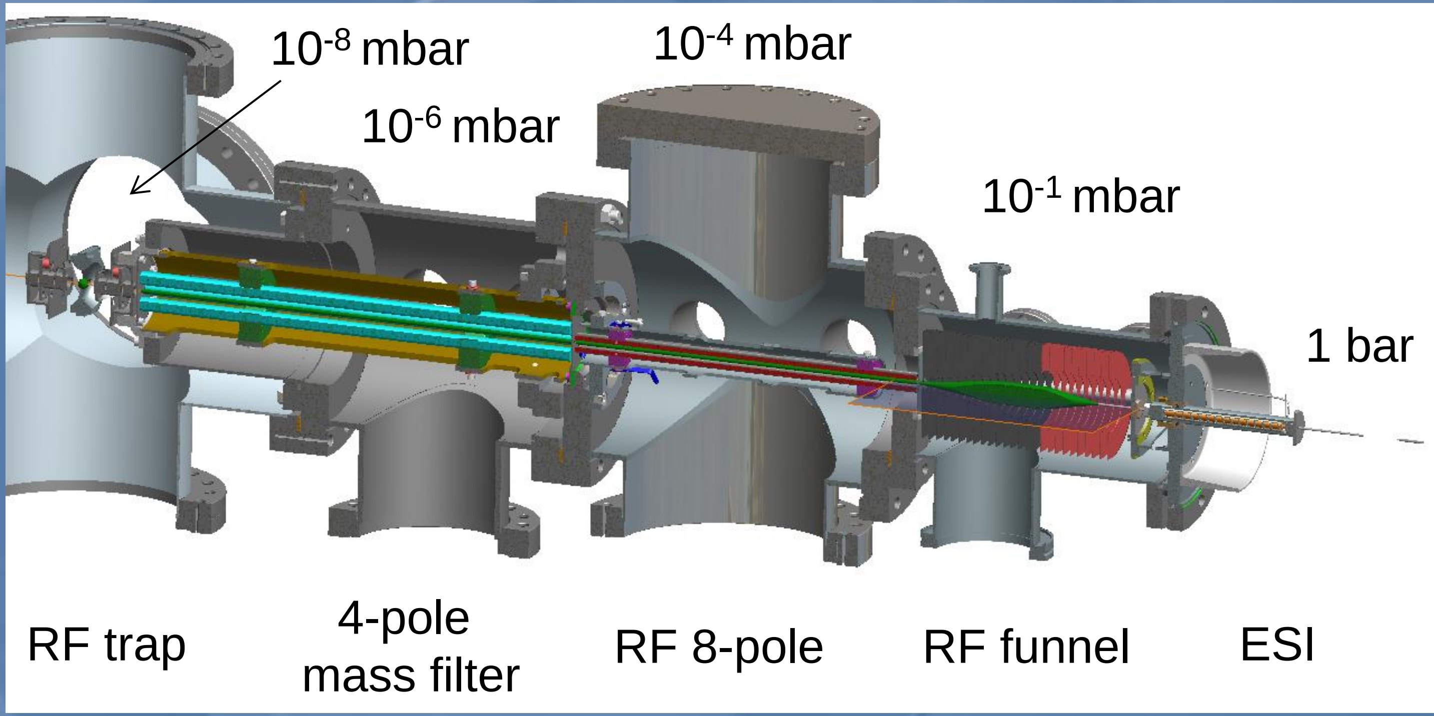
driving forces:

- molecular movies of protein dynamics with atomic resolution at X-ray free electron lasers
- mass spectrometry
  - CID, multiphoton IR dissociation: slow heating and scission of weak bonds
  - UV photoionization: dissociation via excited states
  - keV ion and soft X-ray photoionization: fs deposition of > 10 eV
- site sensitivity, electronic structure sensitivity - geometric structure sensitivity?
- biological radiation damage

key questions

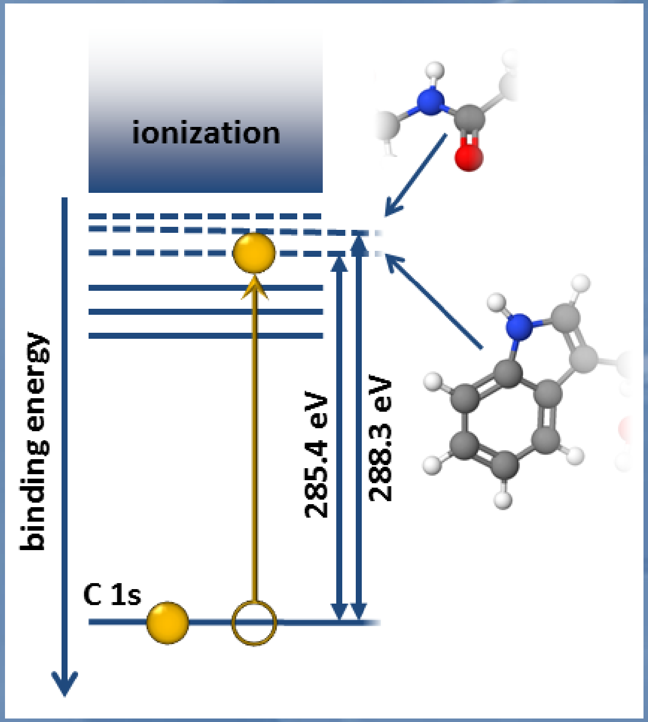
- is soft X-ray or ion induced fragmentation proceeding via IC/IVR or is it fast and localized?
- how are excitations and charges migrating through the system?
- is soft X-ray absorption spectroscopy sensitive to gas-phase protein structure?

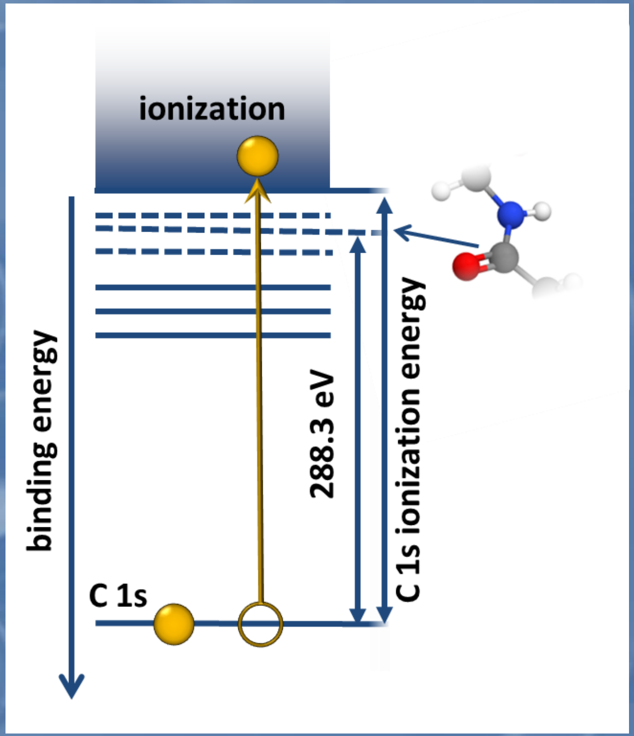
the Groningen RF ion trap



- high fluence electrospray source (>100 pA mass selected protonated peptides)
- pulsed operation (ion accumulation within RF 8-pole, transfer to 3D RF-trap)
- exposure of trap content to photon beam
- TOF mass spectrometry of trap content

C K-edge X-ray absorption



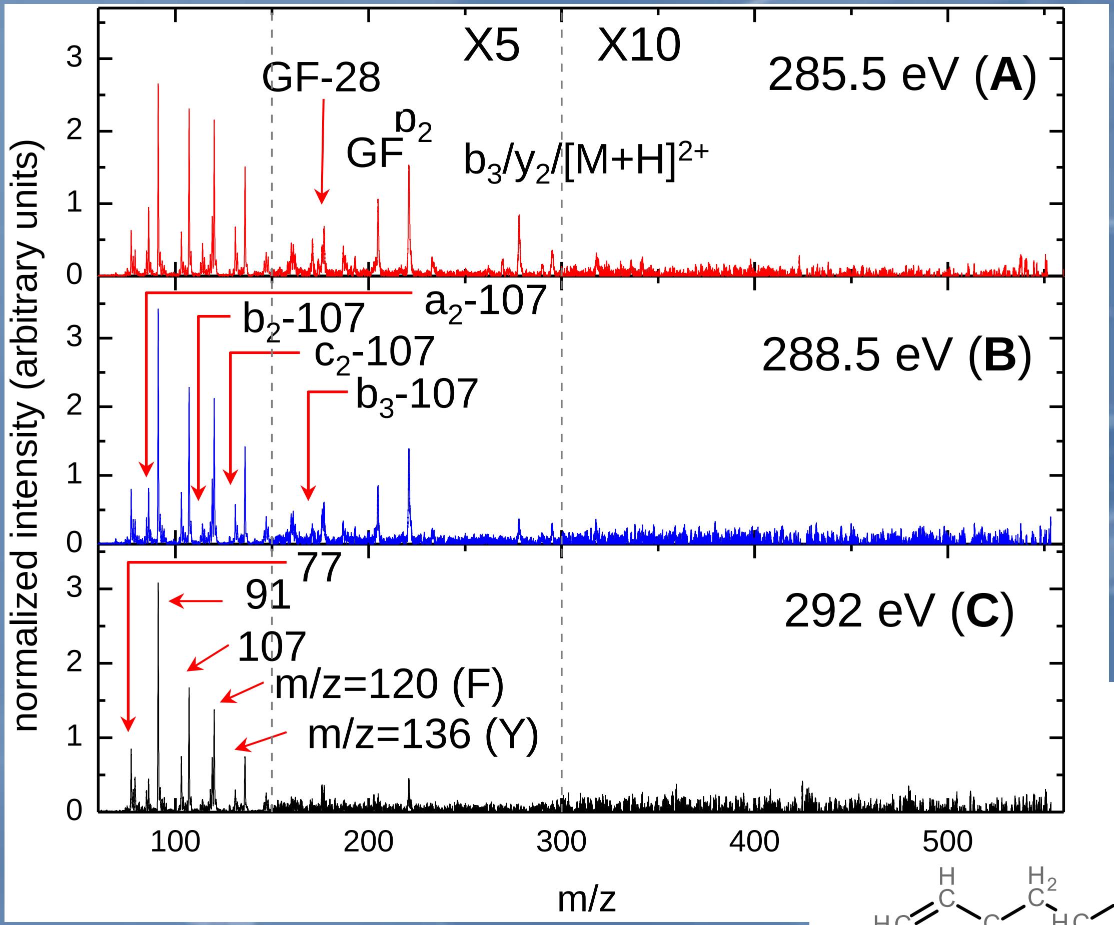


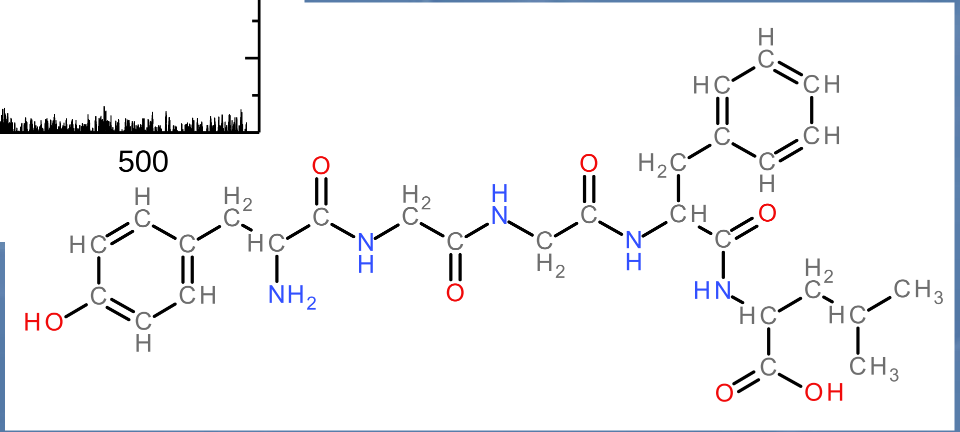
core excitation
followed by Auger ionization
→ mainly single ionization

core ionization
followed by Auger ionization
→ mainly double ionization

resulting excitation energy distribution is broad around ~20 eV

C K-edge X-ray absorption

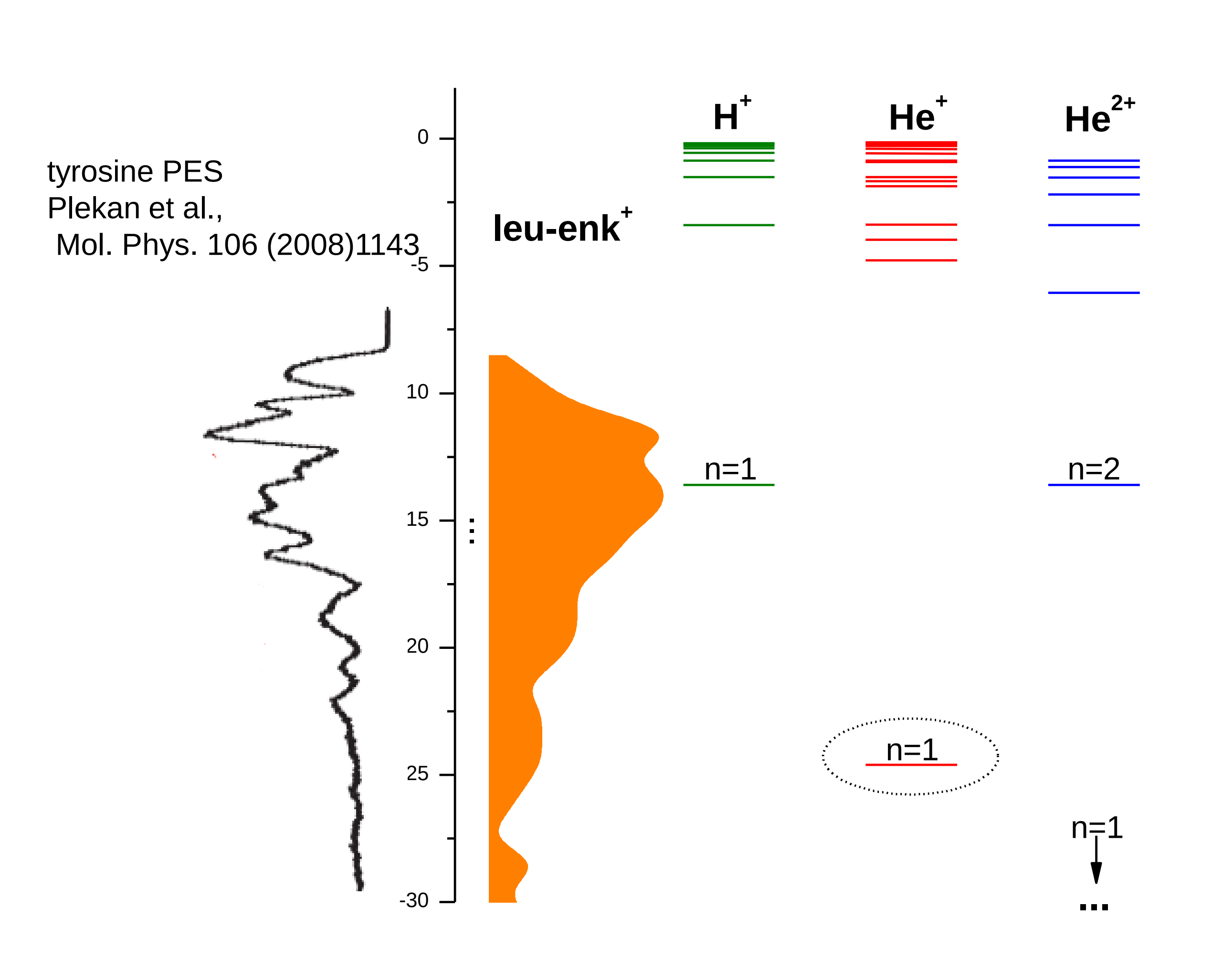


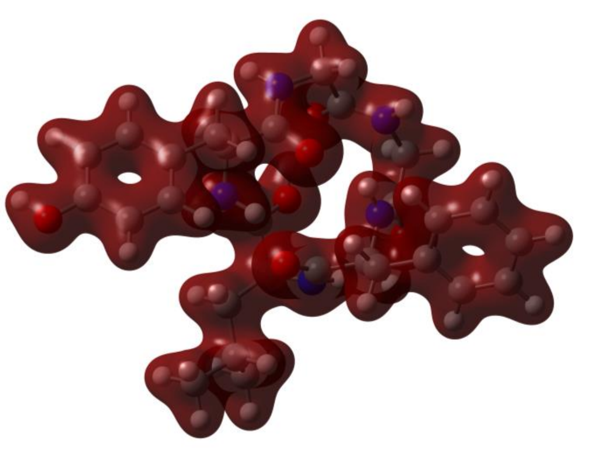


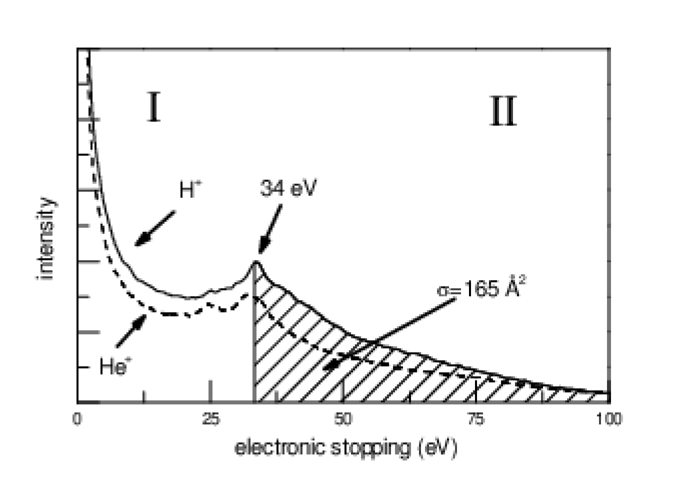
photofragmentation exhibits clear photon energy dependence

*O. González-Magaña, G. Reitsma, M. Tiemens, L. Boschman, R. Hoekstra, and T. Schlathölter, JPCA **116** (2012) 10745*

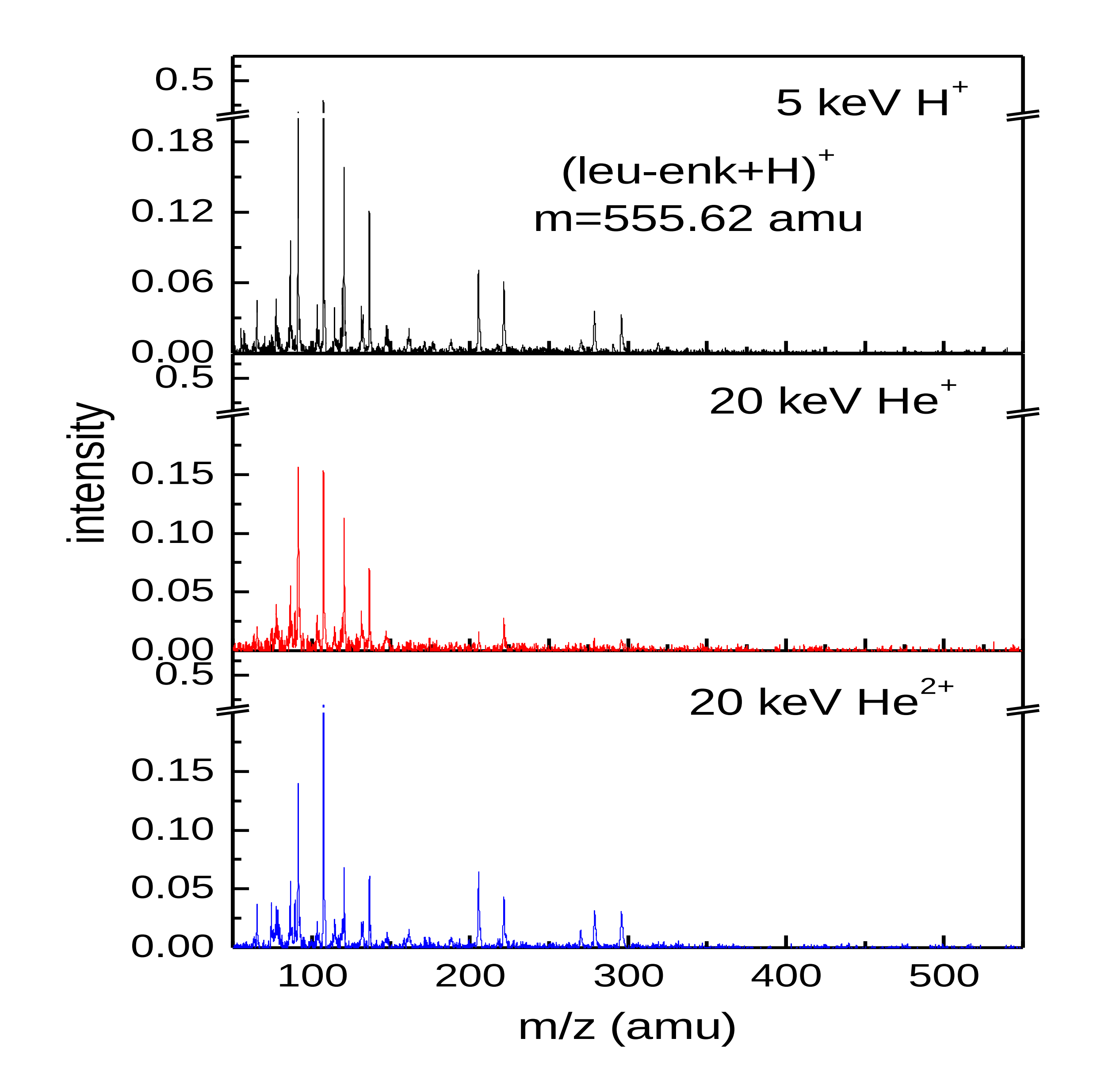
and for keV ions …







and for keV ions …

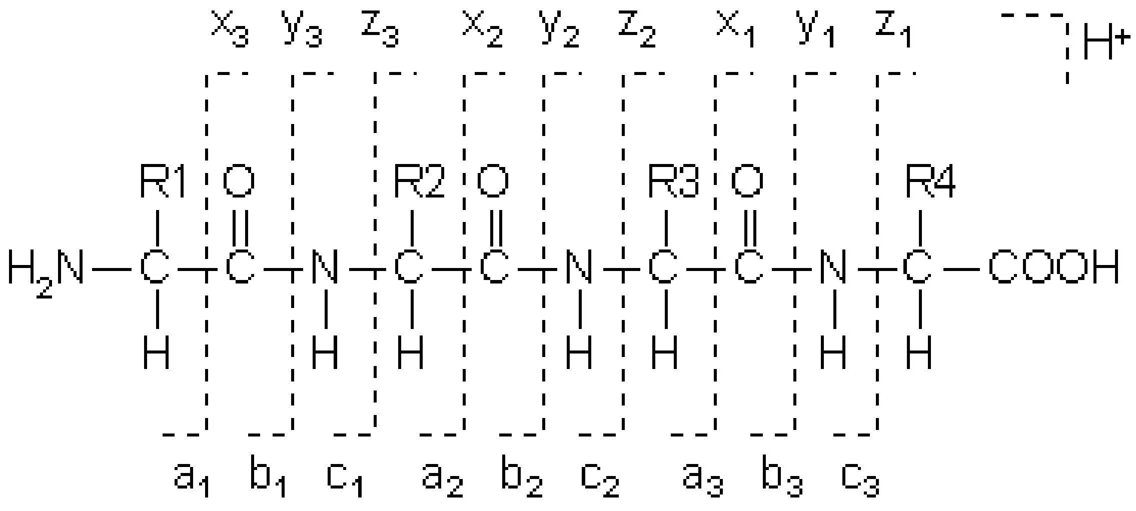


- similar fragmentation pattern
- same dependence on excitation energy

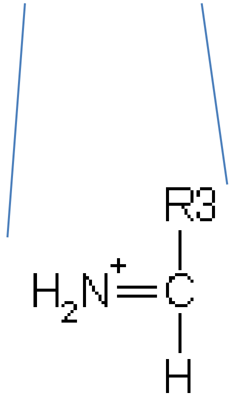
S. Bari, R. Hoekstra, and T. Schlathölter, *Phys. Chem. Chem. Phys.* 12 (2010) 3360

peptide fragmentation

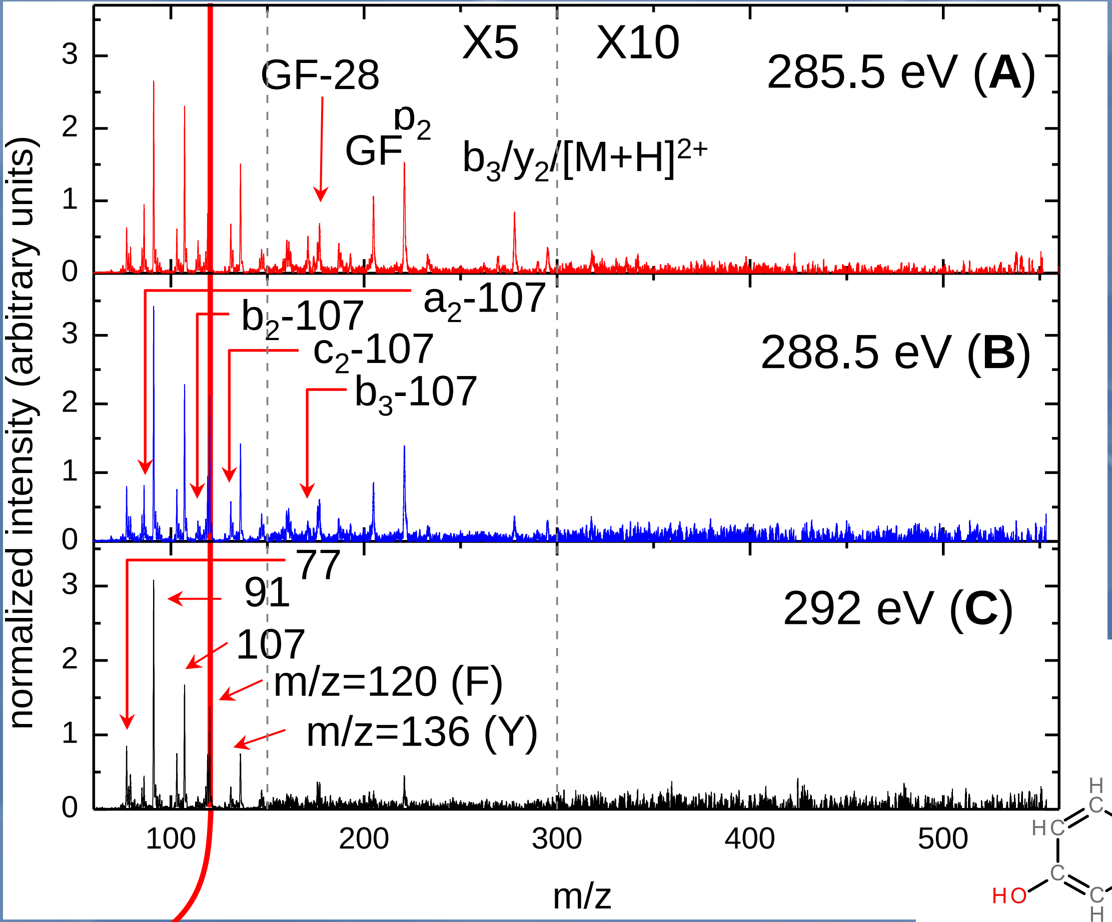
sequence ions



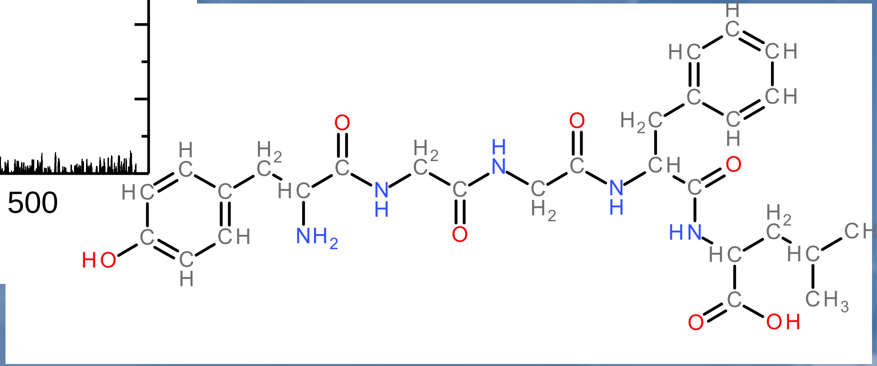
immonium ion



C K-edge X-ray absorption



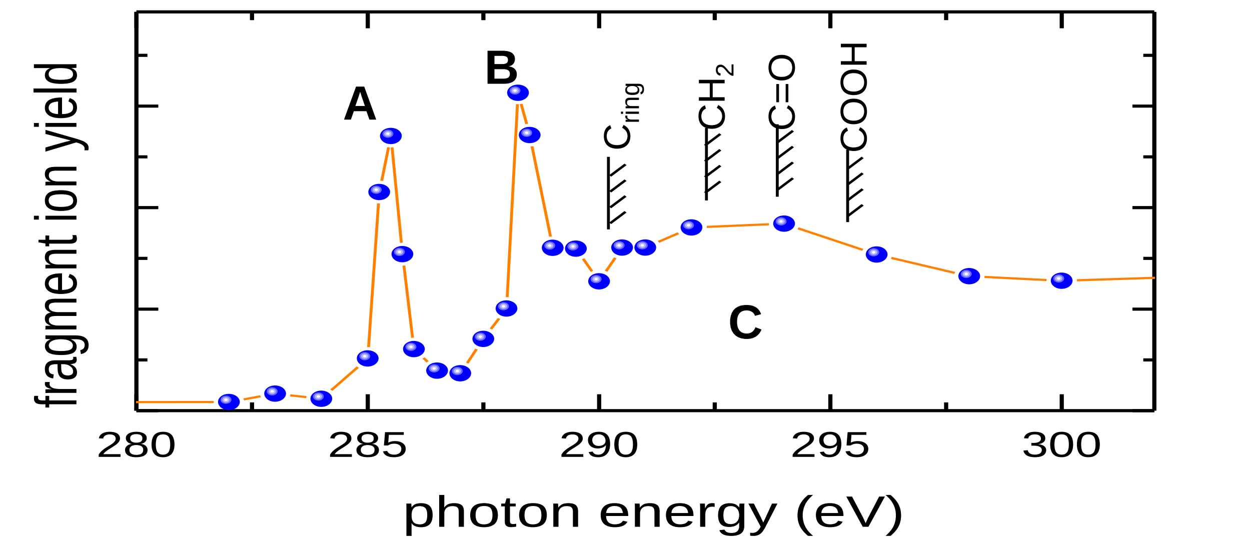
photofragmentation exhibits clear photon energy dependence



photofragment yield can be recorded as function of photon energy

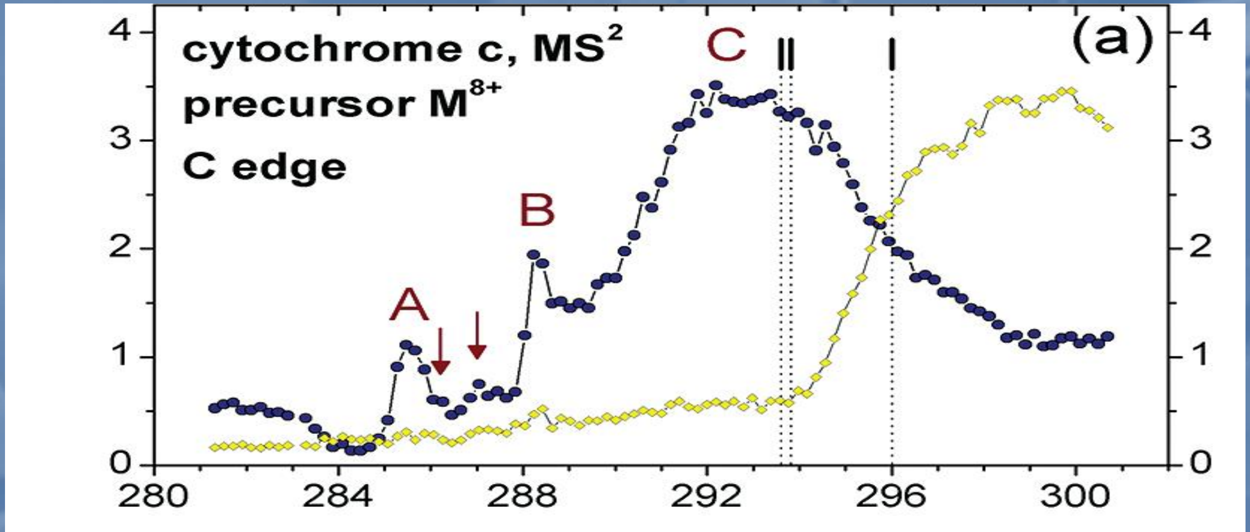
*O. González-Magaña, G. Reitsma, M. Tiemens, L. Boschman, R. Hoekstra, and T. Schlathölter, JPCA **116** (2012) 10745*

C K-edge X-ray absorption



partial ion yield spectroscopy
leu-enk (YGGFL)
m/z=120 (F)
BESSY II, Germany

O. González-Magaña et al., *JPCA* ***116*** *(2012)* 10745



partial ion yield spectroscopy
cytochrome C, 105 amino acids
M=11833
non-diss. single/double ioniziation
SOLEIL, France

A. R. Milosavljević et al., *JPCL 3 (2012)* 1191

high quality data for smaller biomolecules – for instance from K. Prince's group - and for thin films

C K-edge X-ray absorption

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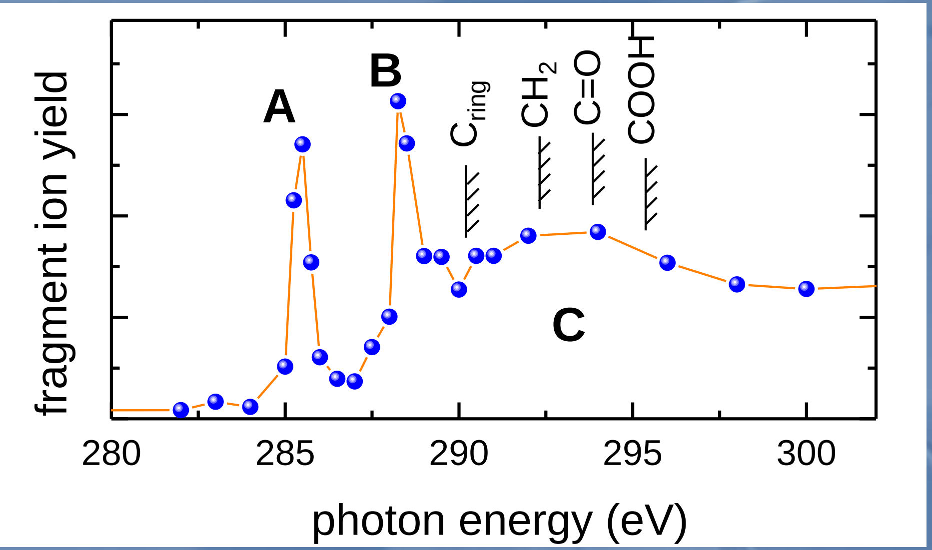
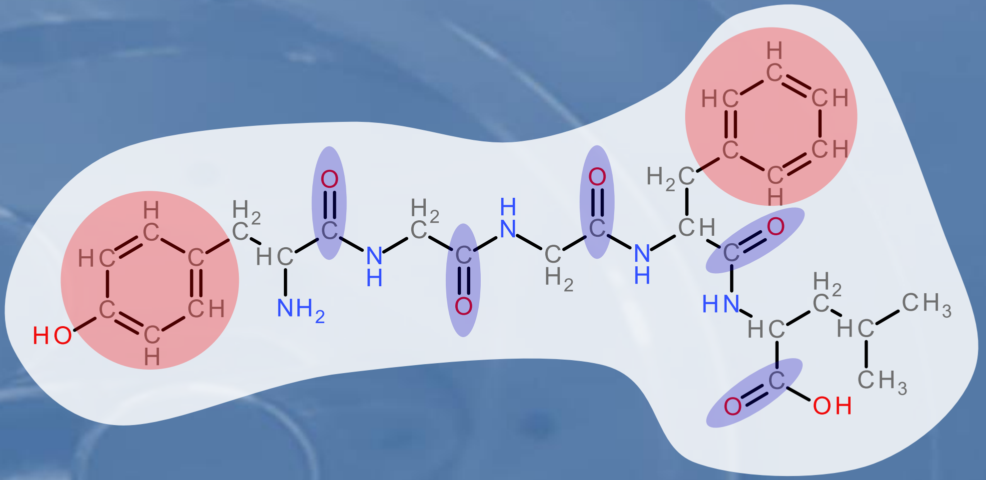




A: C(1s)-π^* transitions in the aromatic F and Y sidechains

B: C(1s)-$\pi^*_{C=O}$ amide group transitions – NO specificity

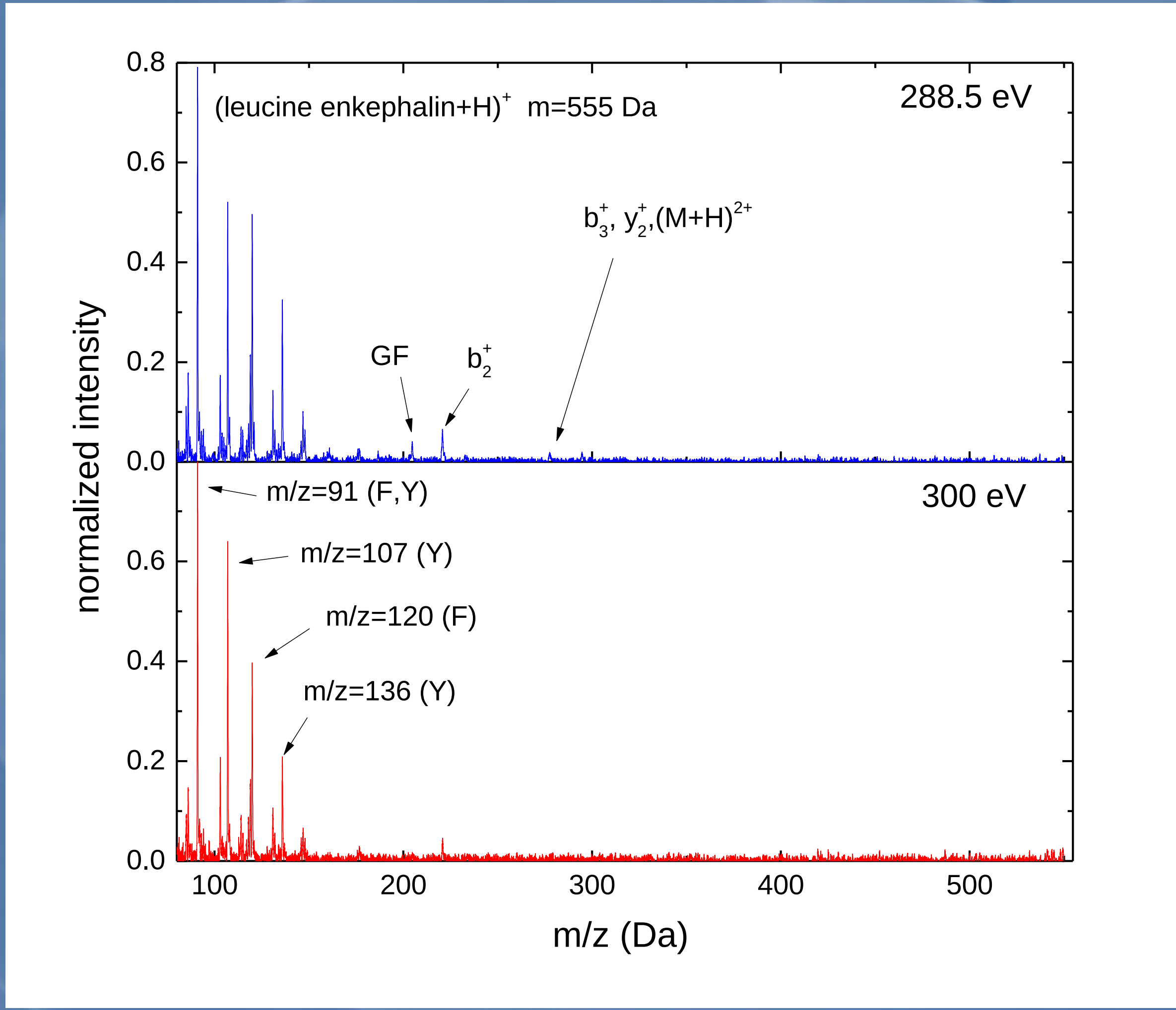
C: C(1s)-σ^* transitions, Rydberg transitions, ionization

A and **B** deexcite non-radiatively by Auger processes → ionization

C (ionization part) followed by Auger de-excitation → double ionization

protein size

university of groningen



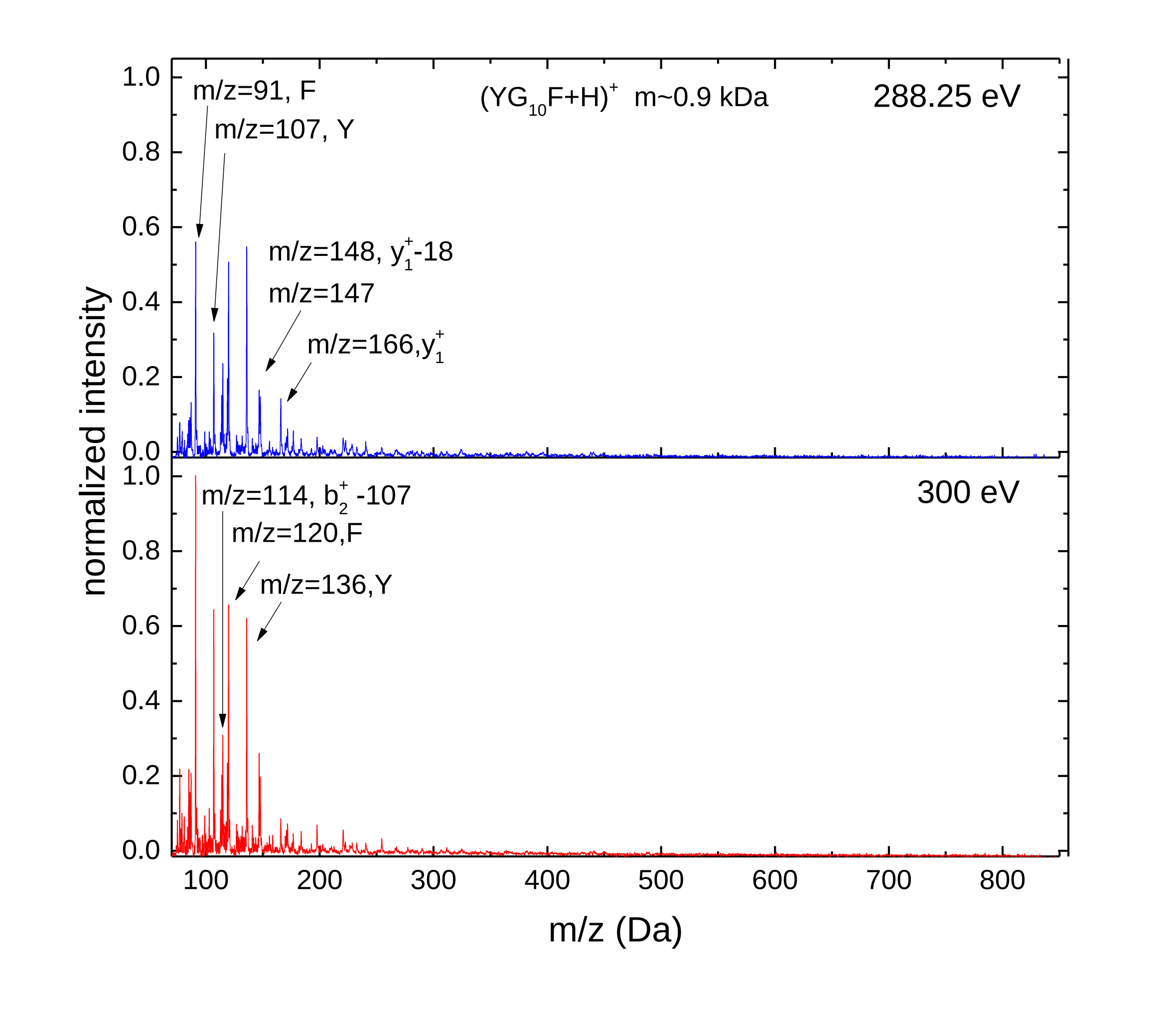
immonium ions and small sequence ions

YGGFL

D. Egorov, L. Schwob, M. Lalande, R. Hoekstra, and T. Schlathölter, PCCP ***28*** *(2016)* 26213

protein size

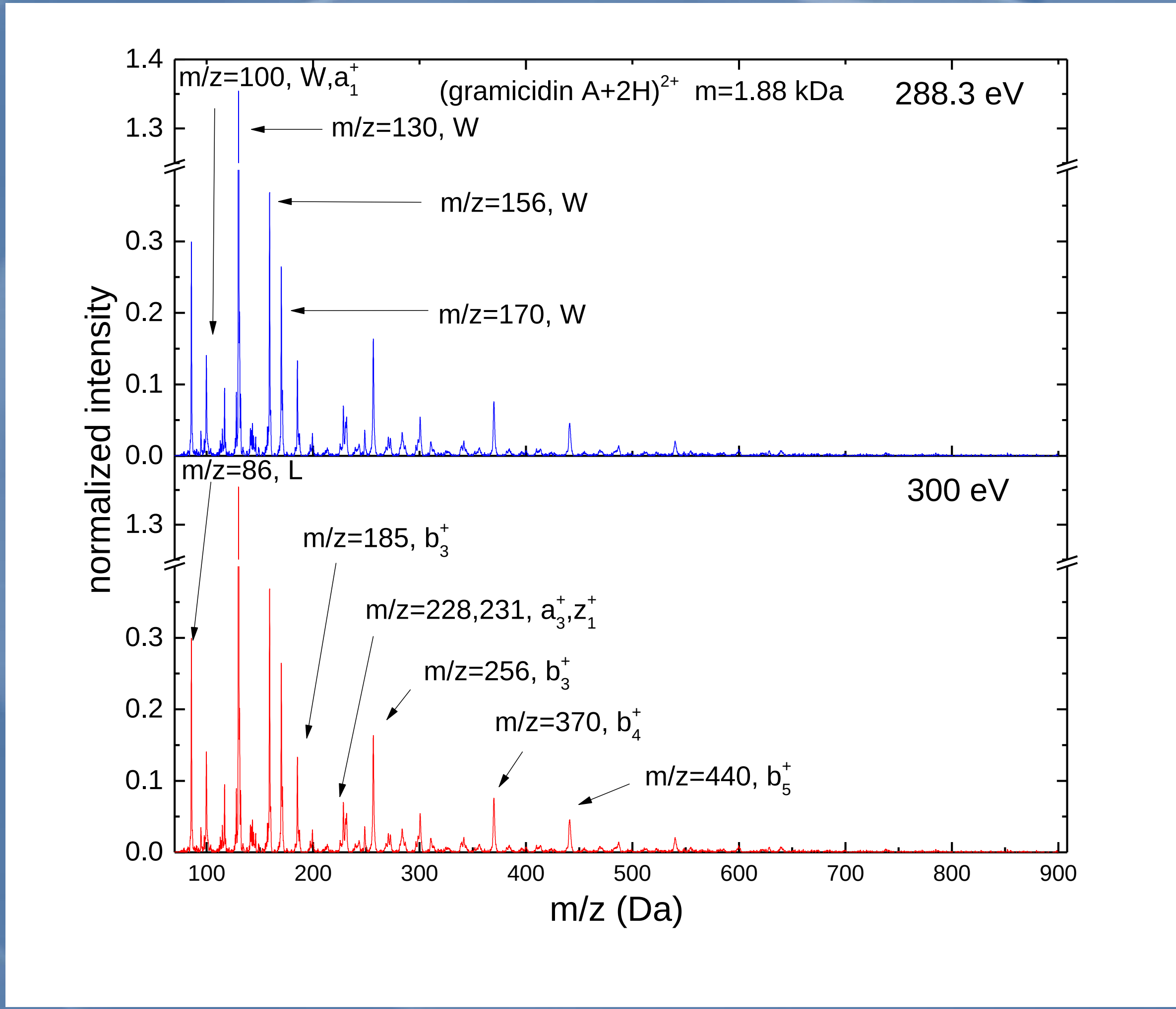
university of groningen



immonium ions and small sequence ions

YGGGGGGGGGGF

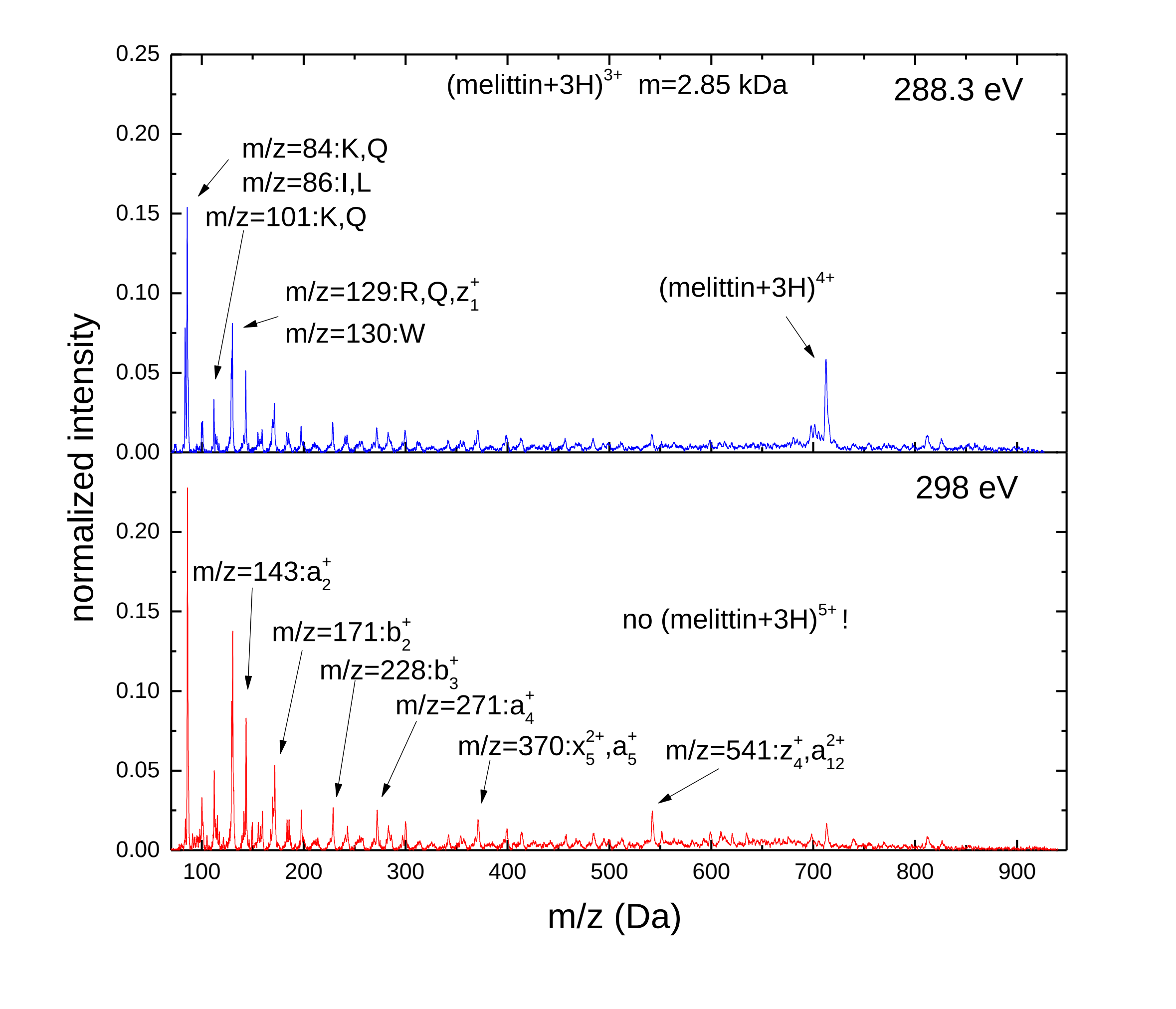
protein size

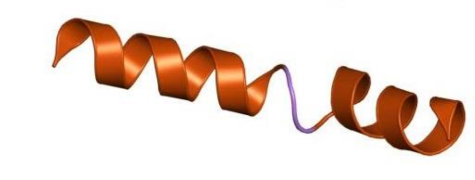


immonium ions and larger sequence ions

VGALAVVVWLWLWLW

protein size

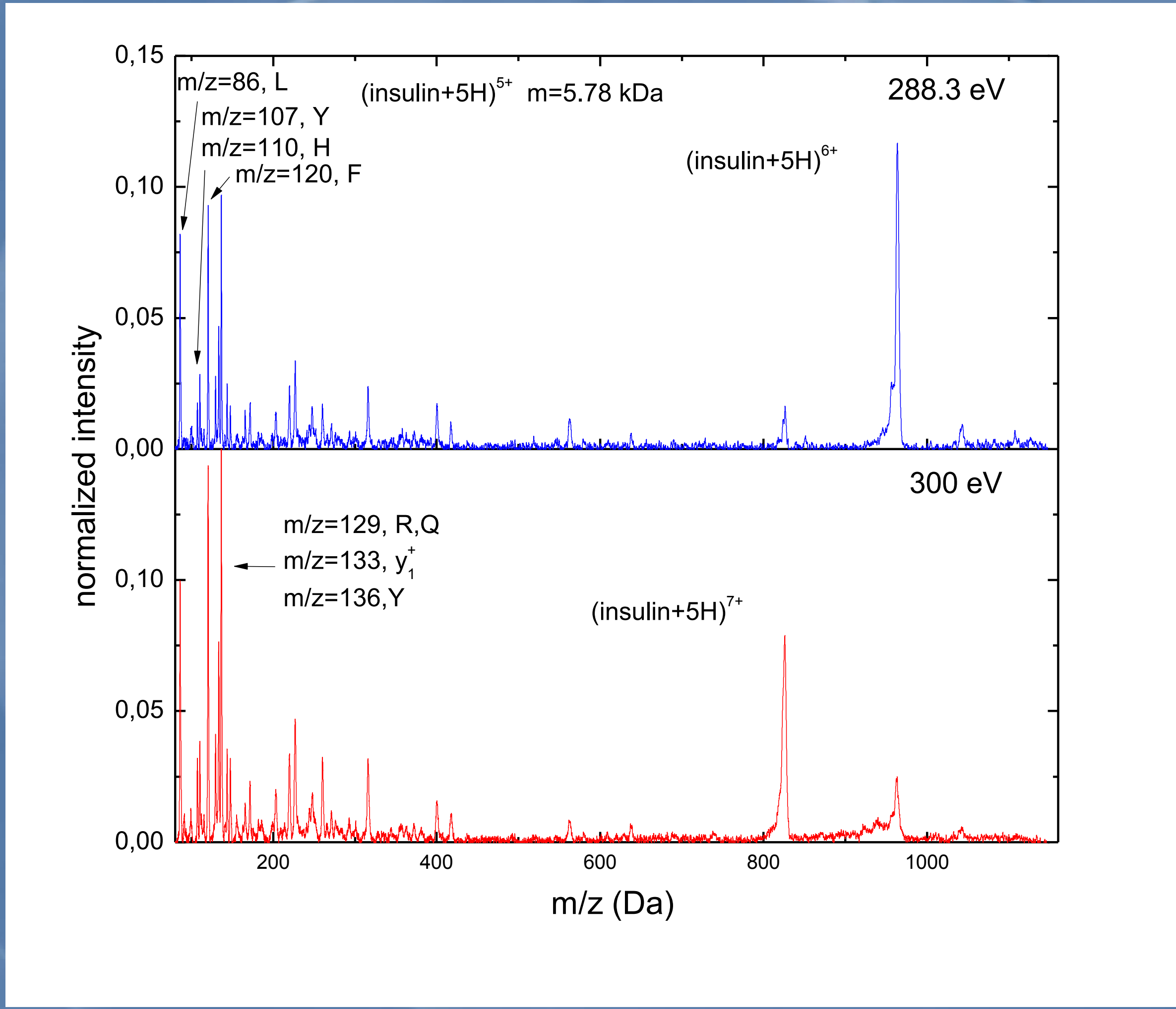


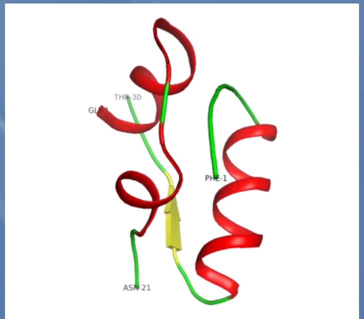


immonium ions, larger sequence ions and non-dissociative ionization

GIGAVLKVLTTGLPALISWIKRKRQ

protein size

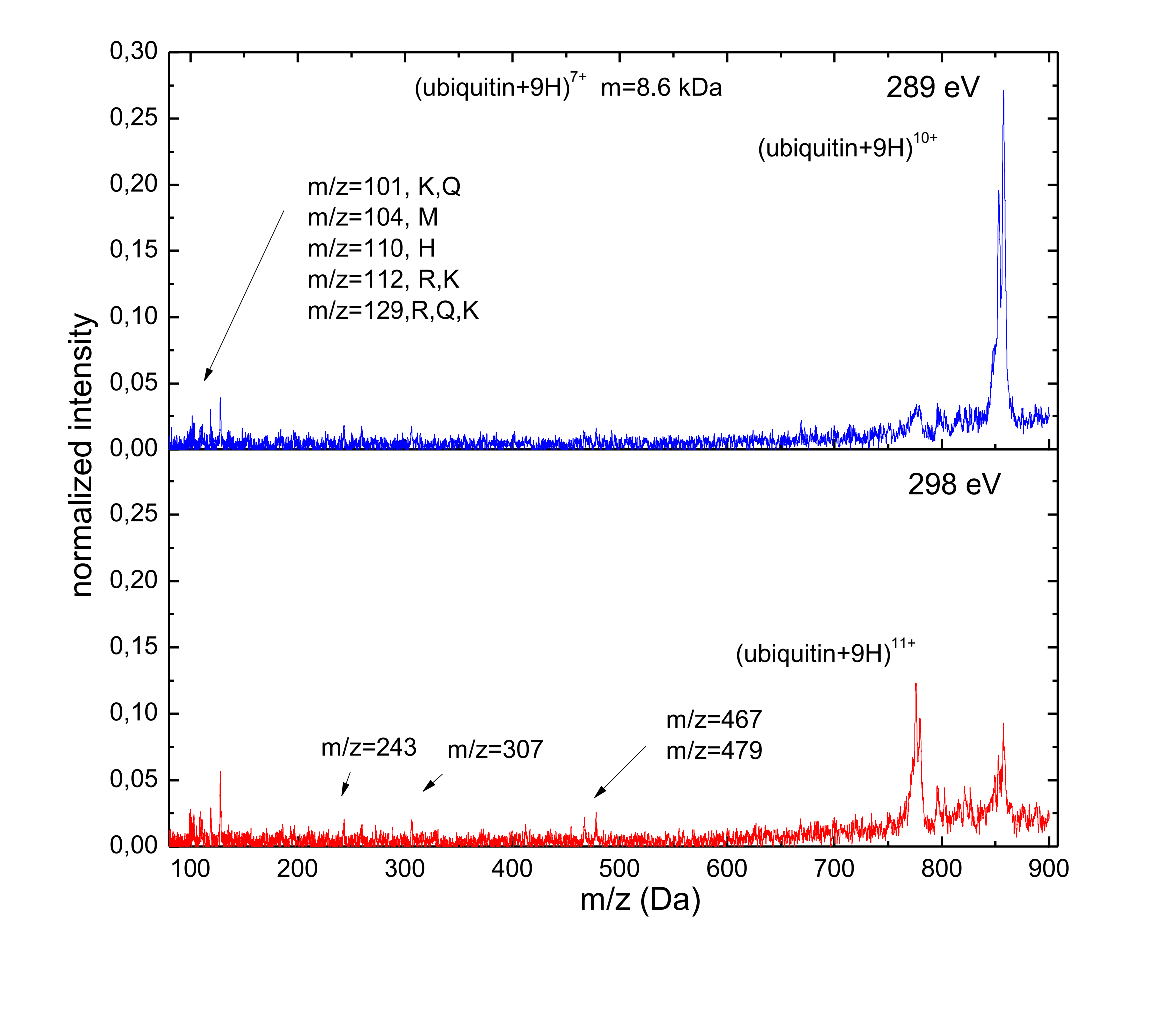


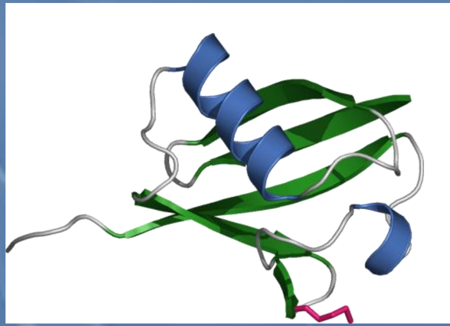


immonium ions, larger sequence ions and non-dissociative ionization

GIVEQCCTSICSLYQLENYCN-FVNQHLCGSHLVEALYLVCGERGFFYTPKT

protein size



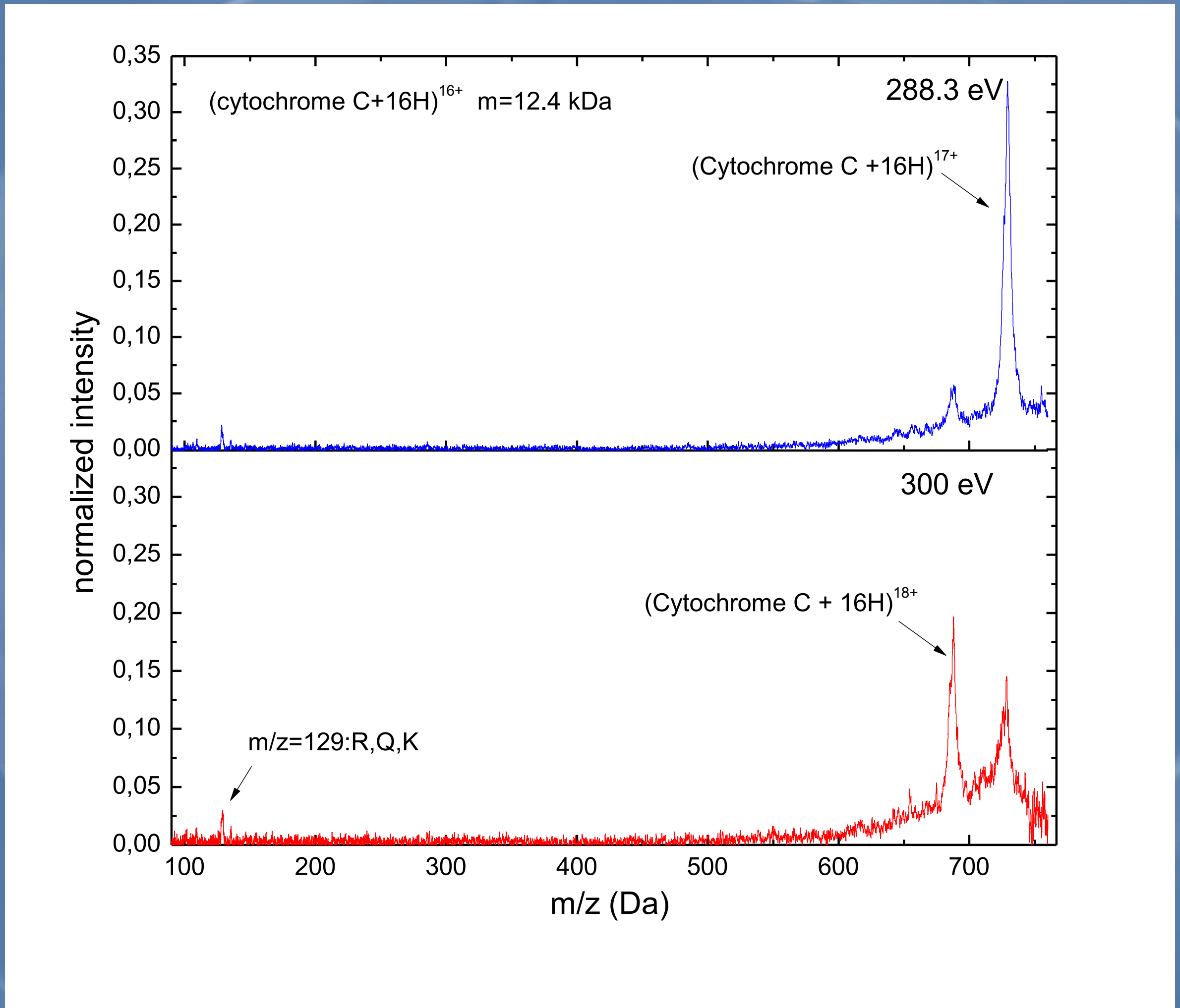


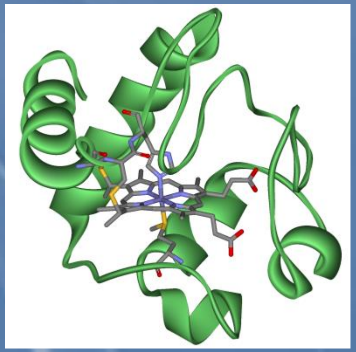
immonium ions, few larger sequence ions non-dissociative ionization, neutral losses

MQIFV**K**TLTG**K**TITLEVEPSDTIENV**K**A**K**IQD**K**EGIPPDQQRLIFAG**K**QLEDGRTLSDYNIQ**K**ESTLHLVLRLRGG

protein size

university of groningen





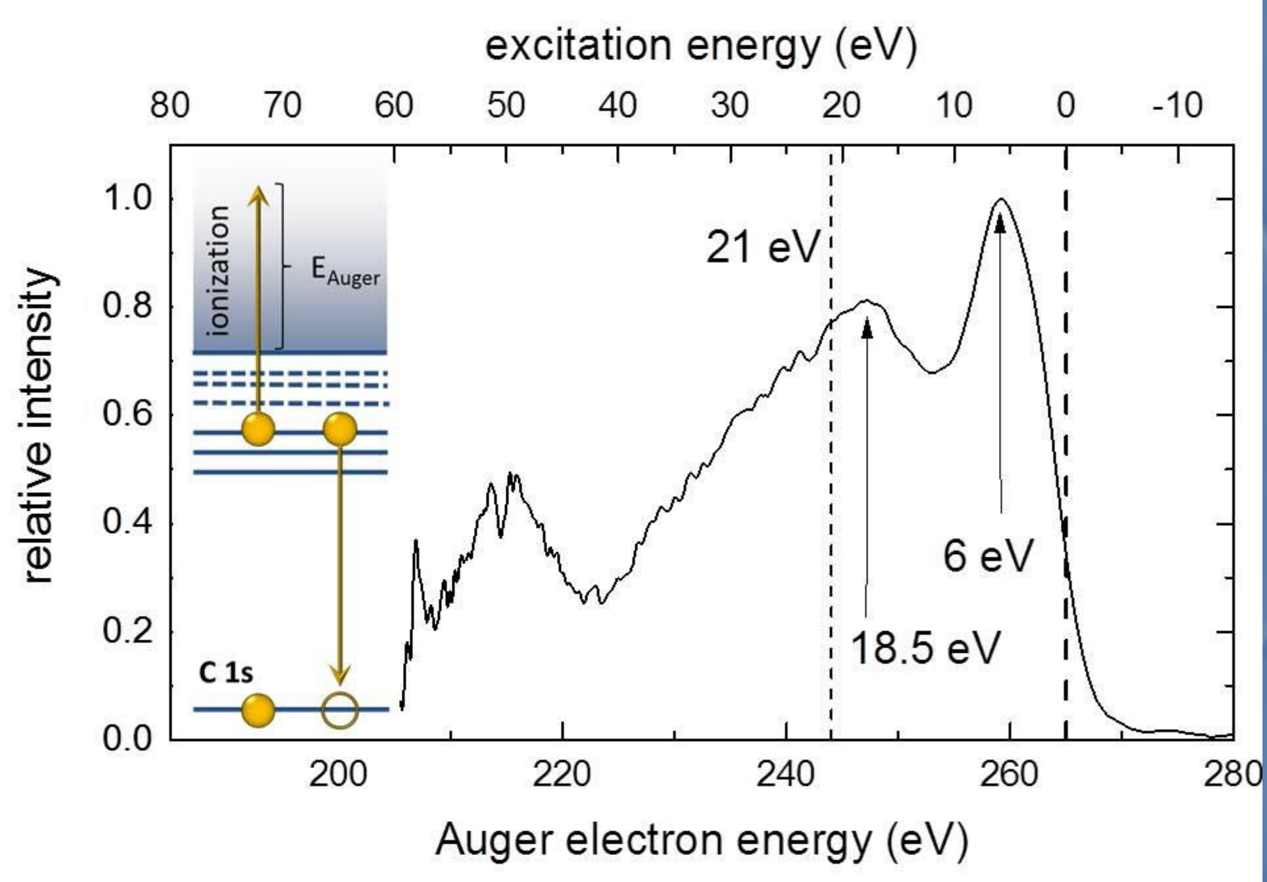
immonium ions, non-dissociative ionization, neutral losses

MGDVEKGKKIFVQKCAQCHTVEKGGKHKTGPNLHGLFGRKTGQAPGFTYTDANKNKGITWKEETLMEYLE
NPKKYIPGTKMIFAGIKKKT EREDLIAYLKKATNE + heme

protein size

university of groningen

core ionization of gas-phase glycine @ LCLS – AMO



from the Auger spectrum, typical exciation energies can be obtained

A. Sanchez-Gonzalez, et al., *J. Phys. B.: At. Mol. Opt. Phys*, 2015, **48**, 234004

protein size

assume deposition of 18.5 eV in the different systems

$$T = \frac{E_{exc}}{c(T,\nu)sk}$$

K. Vekey, J. Mass Spectr., 1996, **31**, 445-463

degrees of freedom

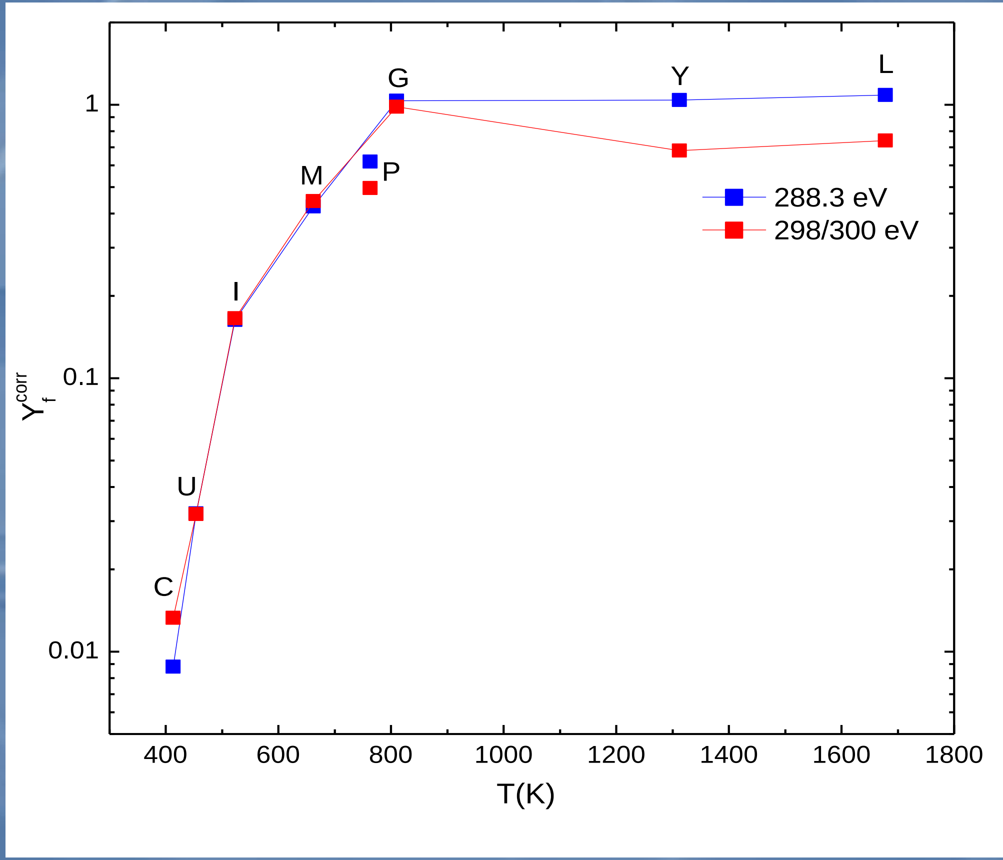
temperature and frequency dependence

→ approximate T as a function of number of degrees of freedom
(harmonic oscillator model)

$$c_{peptide}(T) = 5.61 \times 10^{-4} K^{-1} T - 1.24 \times 10^{-7} K^{-2} T^2$$

protein size

fragmentation yield as a function of T



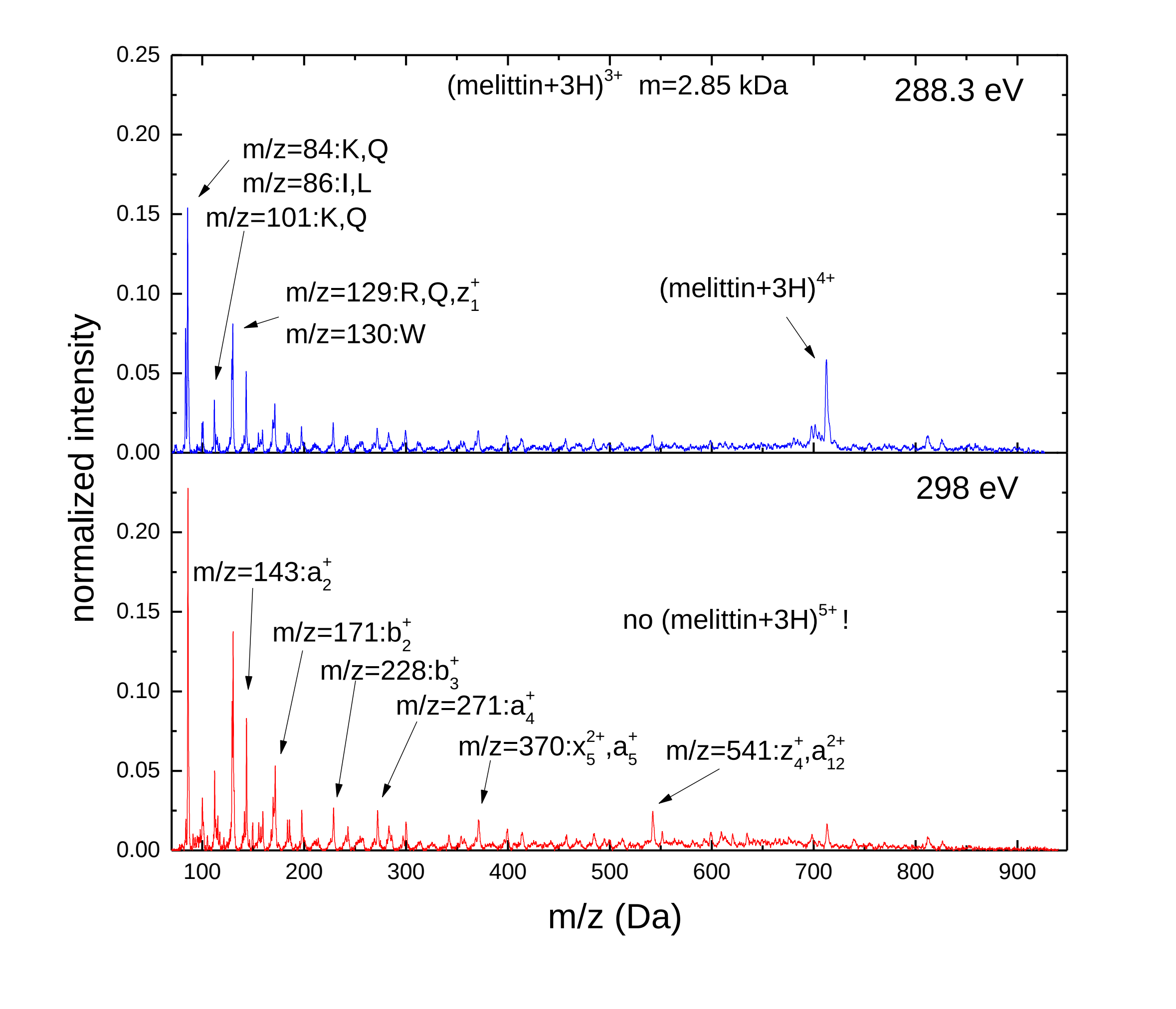
similar trend as for direct heating, CID, SID but onset at lower T

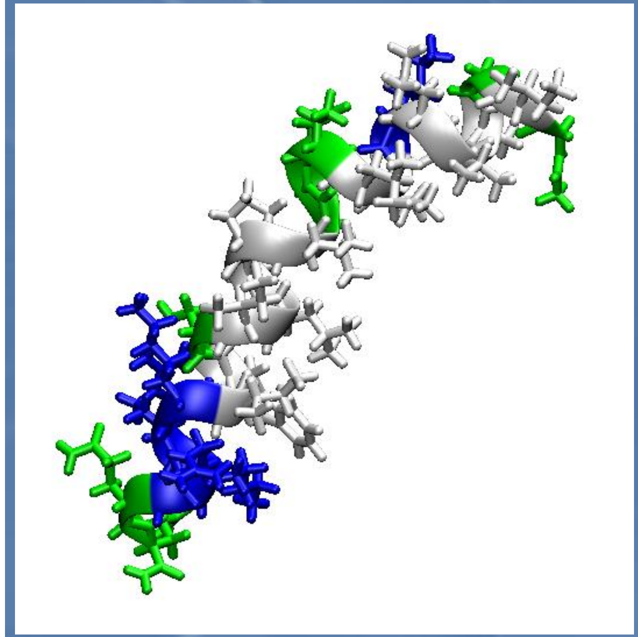
D. Egorov, L. Schwob, M. Lalande, R. Hoekstra, T. Schlathölter, Phys. Chem. Chem. Phys. 18 (2016) 26213-26223

protein size

- excitation energy dissipates in large proteins
- even from the largest molecules, immonium ions can be formed
- IVR competes with fast local dissociation channels (repulsive states?)
- radiation damage in thin films (soft X-ray microscopy) might only be an issue for small peptides!

back to melittin



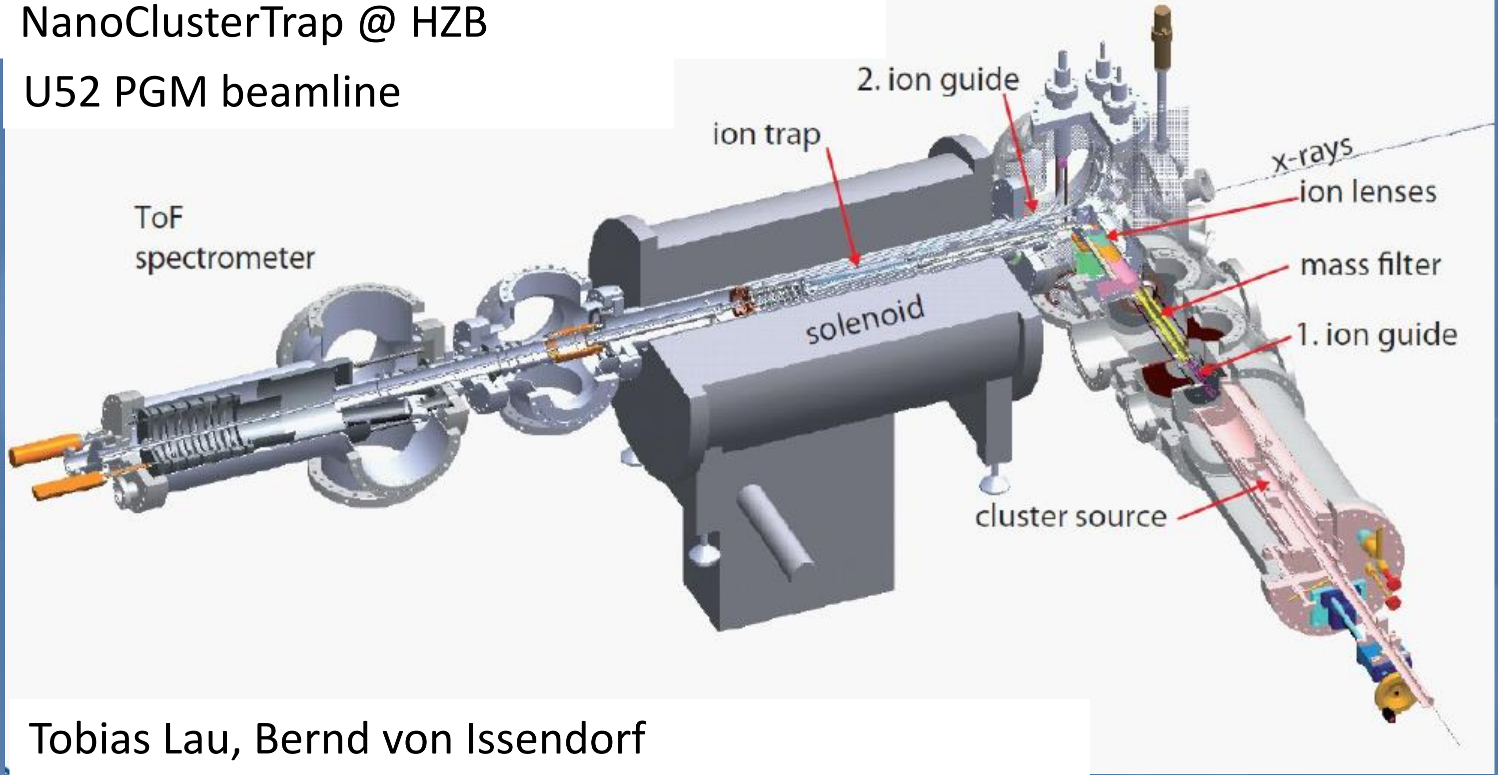


GIGAVLKVLTTGLPALISWIKRKRQ

BESSY II @ Helmholtz Zentrum Berlin

NanoClusterTrap @ HZB

U52 PGM beamline



Tobias Lau, Bernd von Issendorf

linear trap geometry → much larger capacity
LHe cooling → T~10K
mass dependent transmission → good for partial ion yield scans, bad for mass spectra

melittin – conformation effects

university of groningen

- resolution:

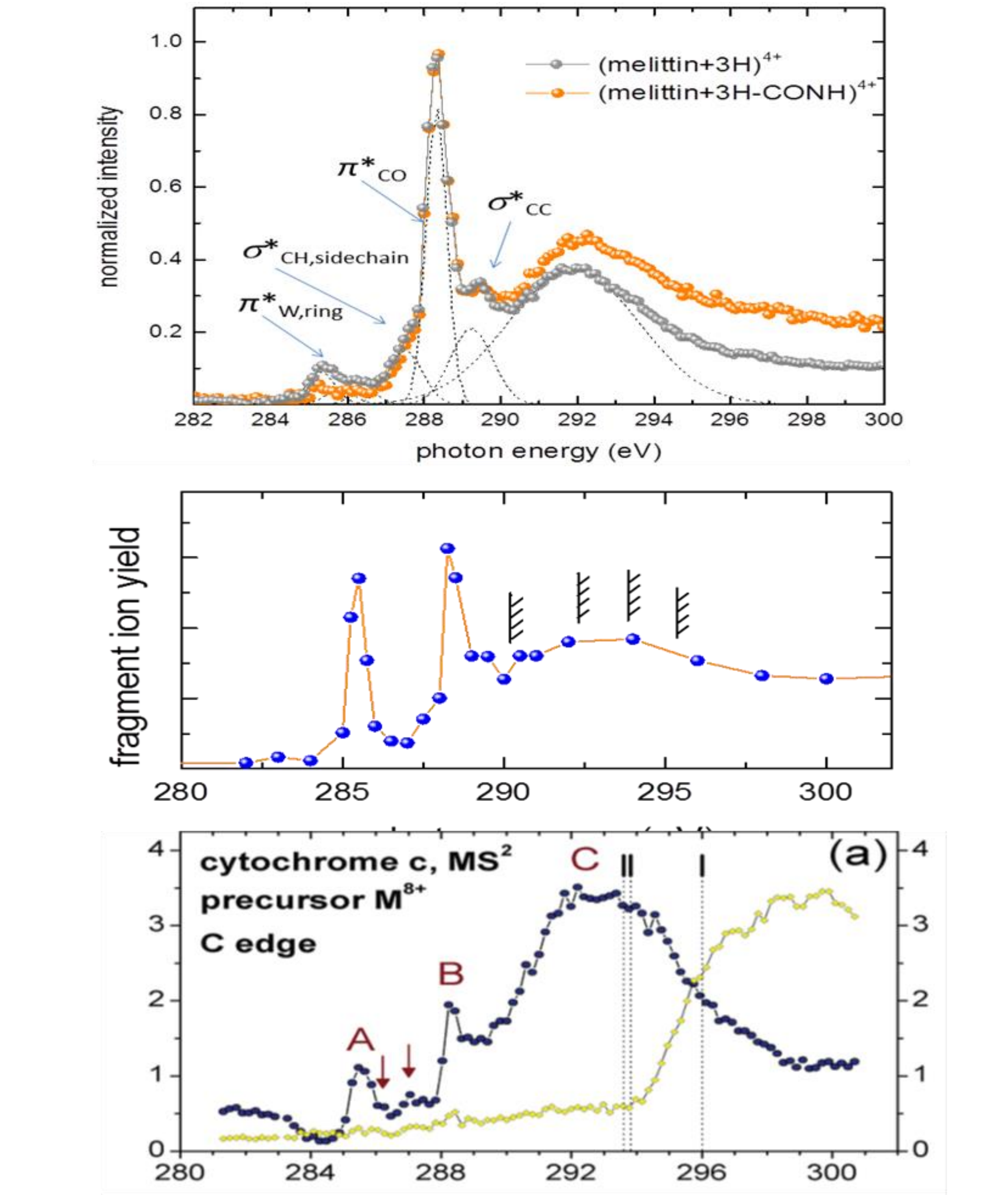
similar as for gas-phase amino acids
*Plekan et al., JPCA, **111** (2007) 10998*

- problem:

averaging over residues with slightly shifted peaks

- advantage:

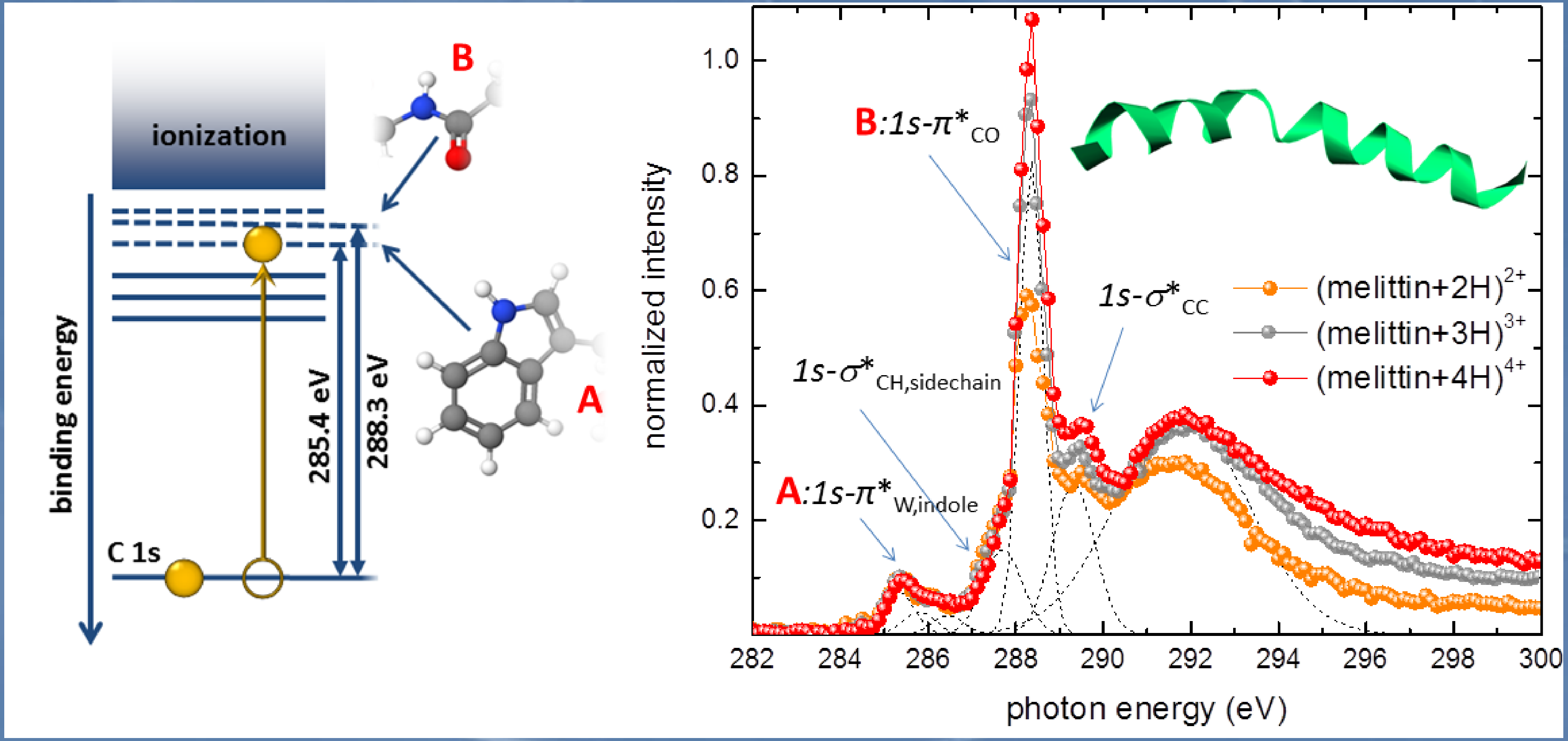
T=10K
→ less thermal broadening
→ non-diss ionization stronger



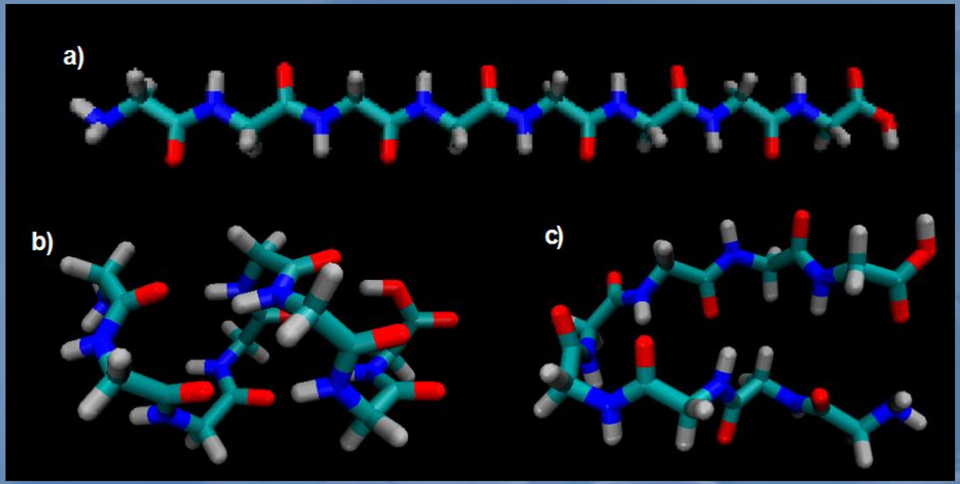
melittin – conformation effects

gas phase structure:
(melittin +2H)$^{2+}$: helical
(melittin +3H)$^{3+}$: mainly helical
(melittin +4H)$^{4+}$: not helical (relaxation due to Coulomb repulsion)

H. V. Florance, A. P. Stopford, J. M. Kalapothakis, B. J. McCullough, A. Bretherick and P. E. Barran, Analyst, 2011, 136, 3446



melittin – TD-DFT



hydrogen bonds induce out of plane distortion of the backbone

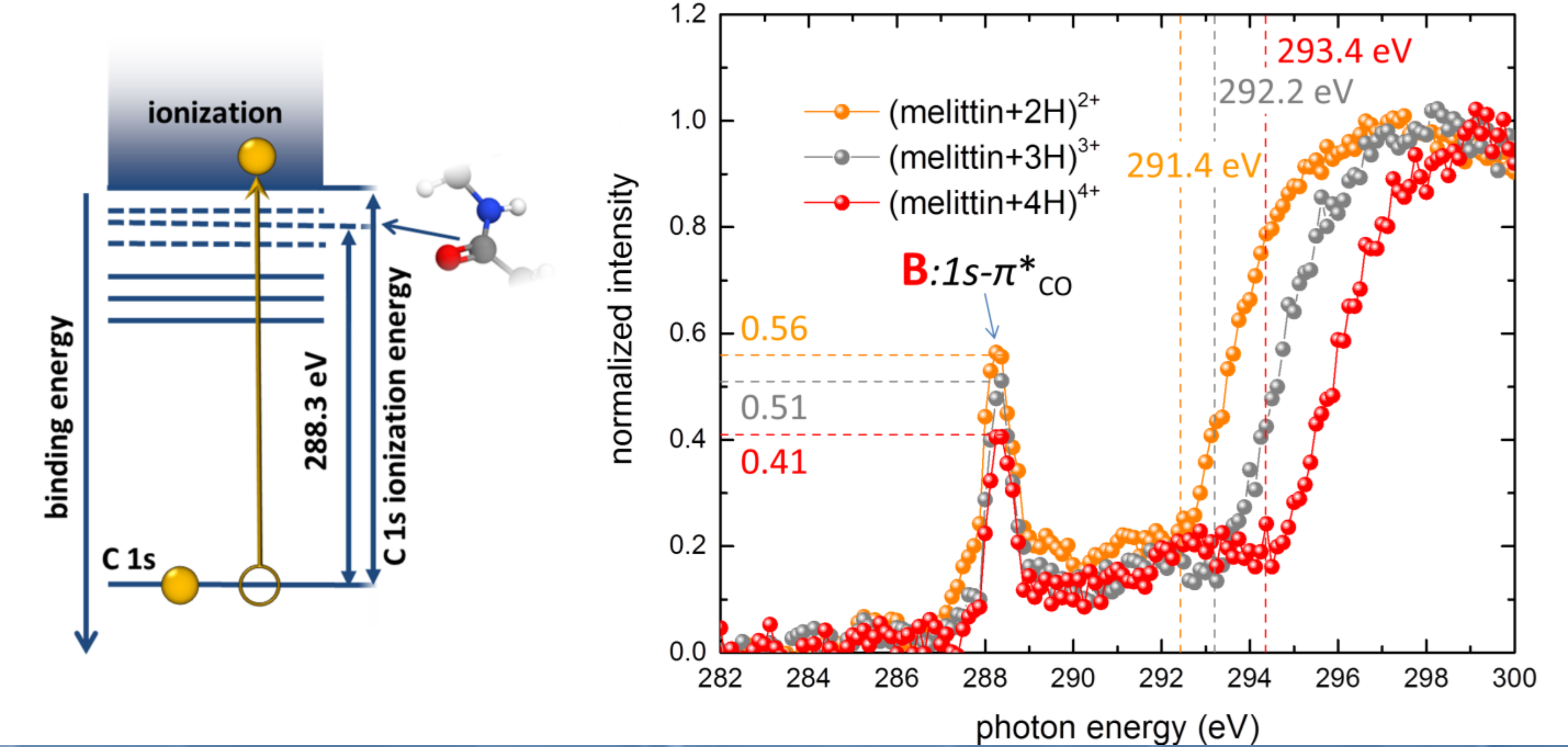
symmetries of the transition densities are broken

(too) small reduction in the transition dipoles

very sensitive to the structure

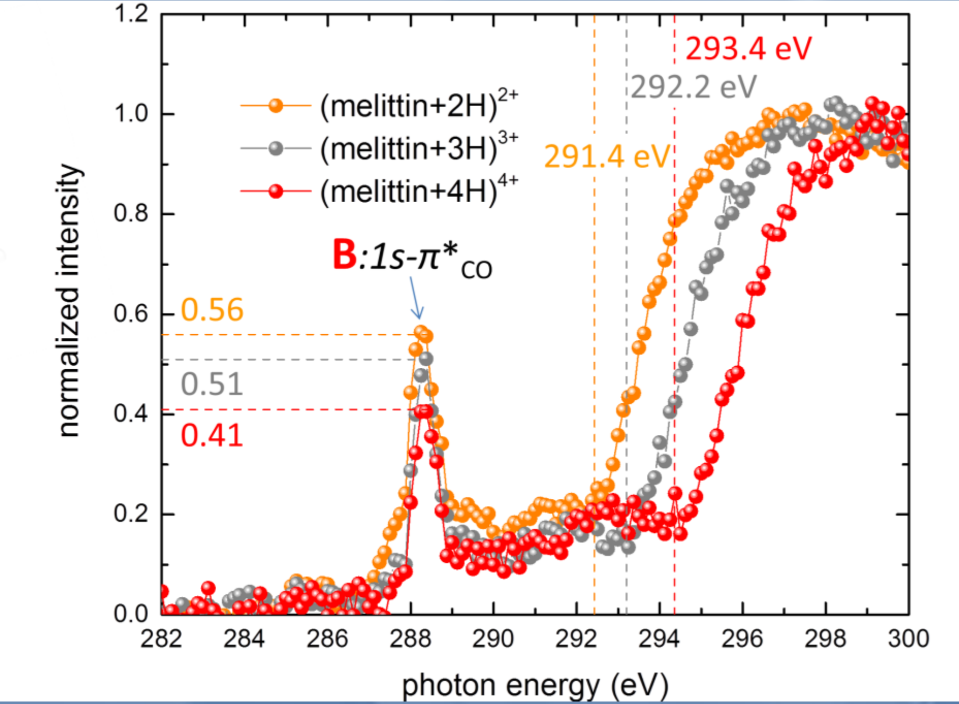
	Chain		α-helix			β-hairpin		
site	E (eV)	f_{os}	E(eV)	f_{os}	ratio	E(eV)	f_{os}	ratio
1	277.63	0.0589	277.48	0.0585	1.006	277.46	0.0583	1.010
2	277.78	0.0608	277.48	0.0569	1.069	277.52	0.0575	1.058
3	277.79	0.0606	277.48	0.0561	1.080	277.42	0.0586	1.034
4	277.79	0.0606	277.47	0.0547	1.108	277.45	0.0555	1.091
5	277.79	0.0606	277.44	0.0496	1.223	277.68	0.0593	1.023
6	277.79	0.0607	277.50	0.0479	1.268	277.67	0.0623	0.974
7	277.79	0.0610	277.48	0.0504	1.21	277.49	0.0576	1.060
8	278.14	0.0655	277.89	0.0424	1.078	277.78	0.0611	1.073
8’	-	-	277.88	0.0184	-			

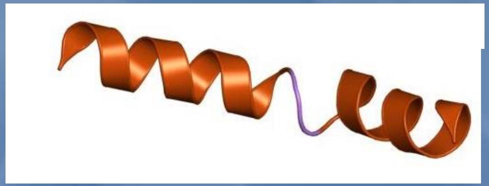
melittin – conformation effects

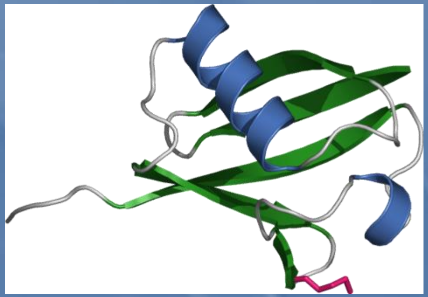


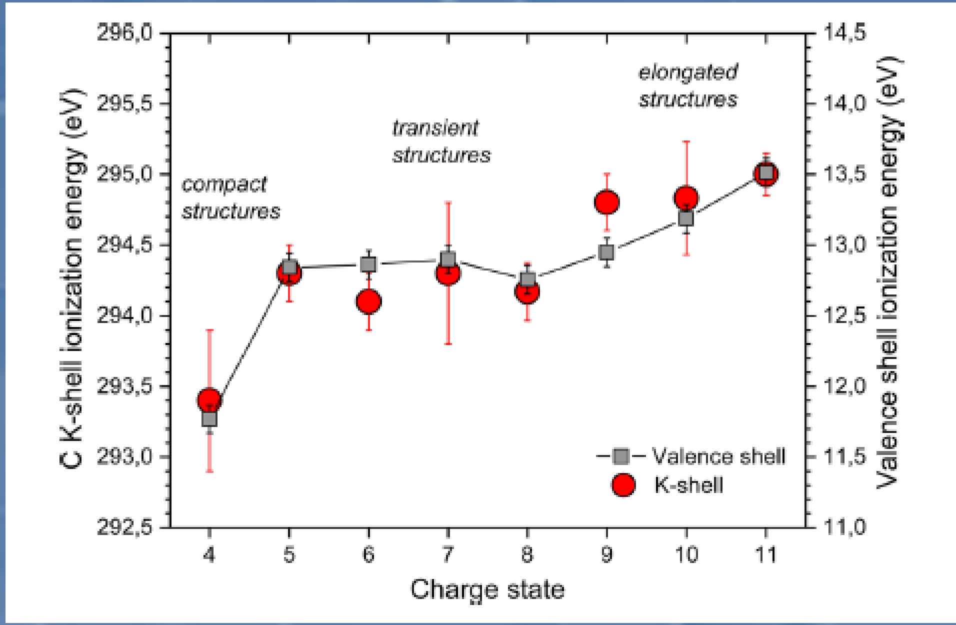
1s ionization energy increase and conformational relaxation at the same time

melittin – conformation effects









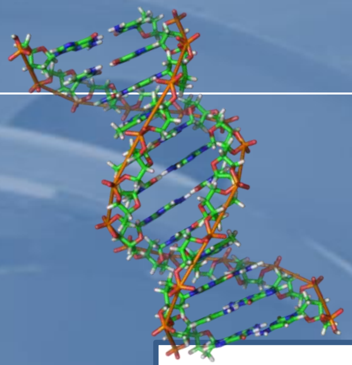
at variance with previous data on ubiquitin

A. R. Milosavljevic, C. Nicolas, M. L. J. Rankovic, F. Canon, C. Miron and A. Giuliani, *J. Phys. Chem. Lett.*, 2015, **6**, 3132-3138

melittin – conformation effects

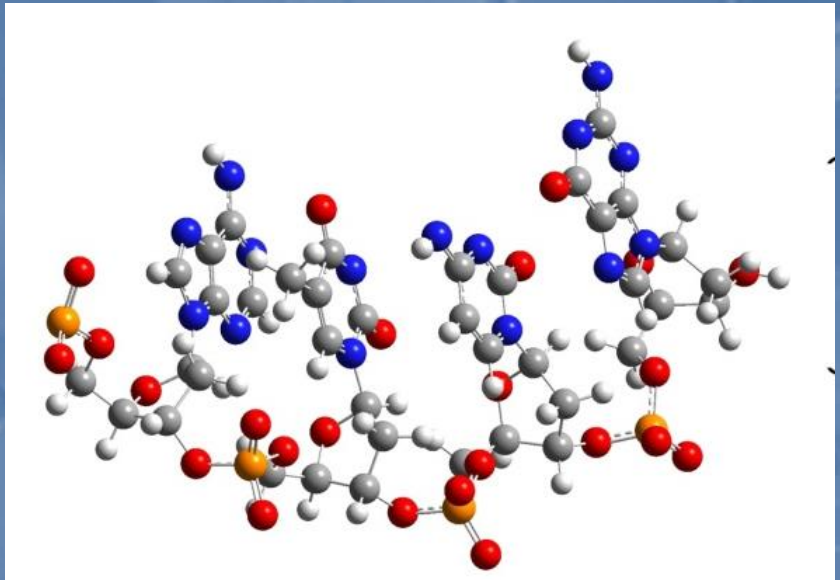
- DFT calculations (T.Jansen): helicity reduces the main resonance, but the effect is (too?) weak
- there is no circular dichroism observed for any channel (within the accuracy of the experiment)
- other explanations?
- future: IMS!

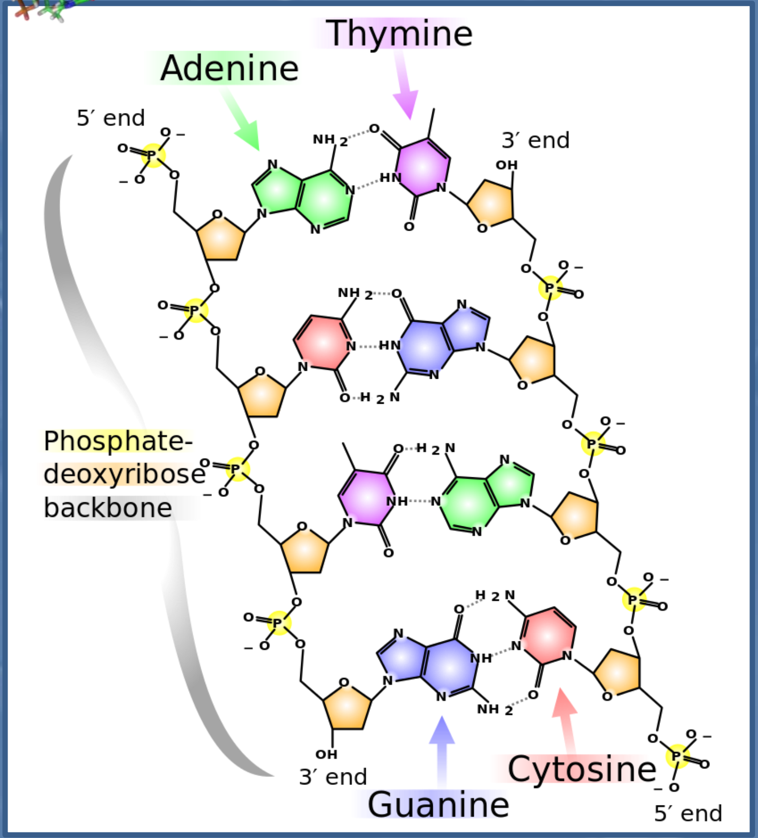
exciting DNA



DNA interactions with energetic photons

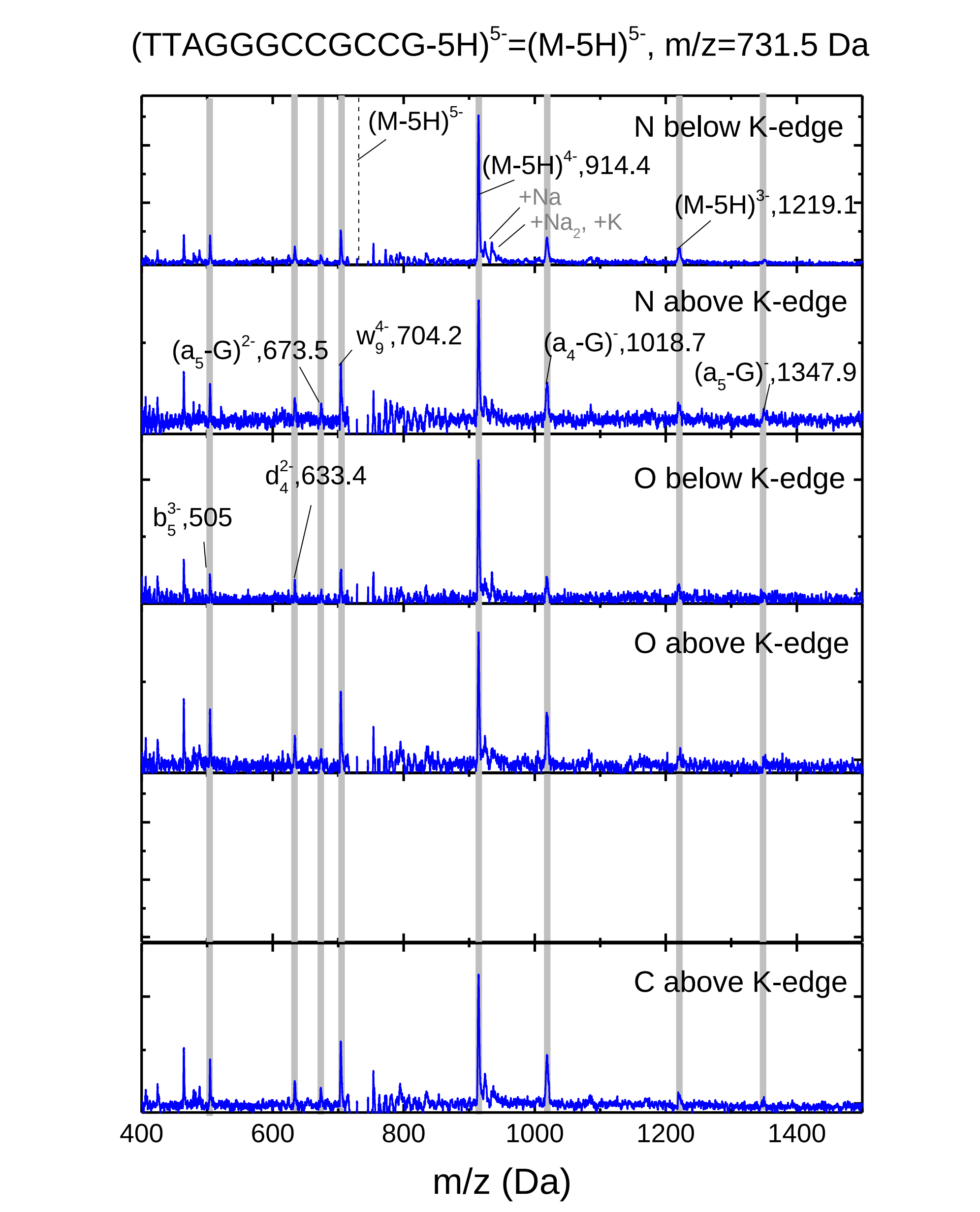
- **biological radiation action / radiotherapy**
- **charge transport in DNA**
- **interactions with aqueous environment**

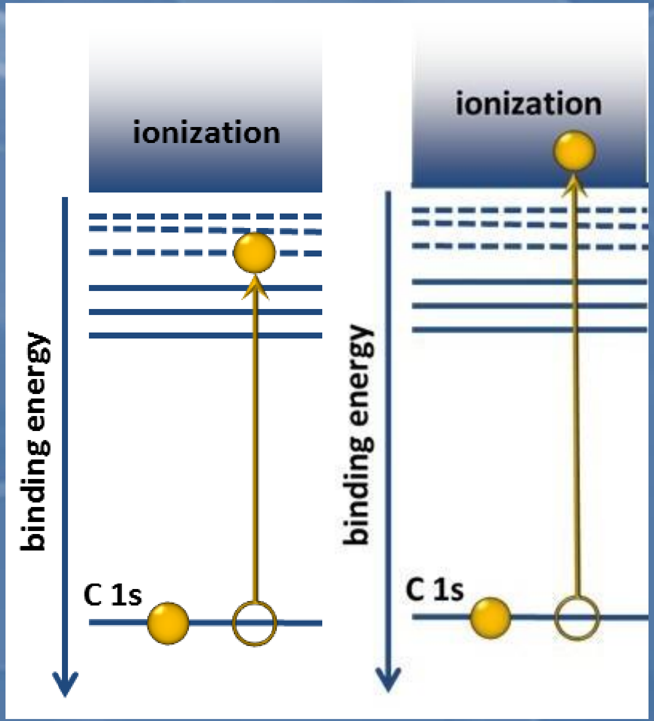




negatively charged in solution

telomers as hole traps?



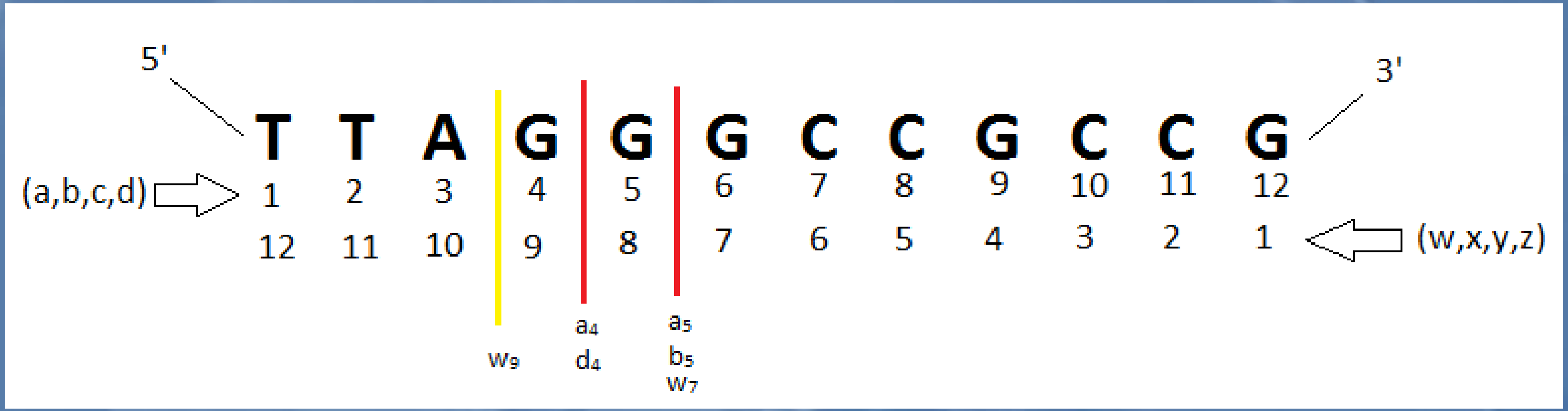


Moller-Plesset perturbation theory:
the telomere sequence TTAGGG can
function as a profound hole trap.

E. Cauët, Journal of Biomolecular Structure & Dynamics 29 (2011) 557

single/double ionization of *deprotonated*
telomer containing oligos induced by soft
X-ray absorption

telomers as hole traps?



all major fragmentation channels involve strand breakage somewhere in the GGG sequence!

thanks

ZIAM, Groningen

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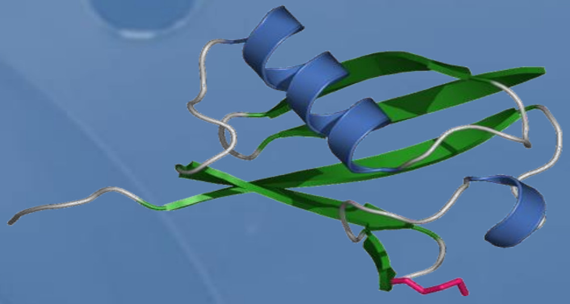
Georg Schmid
Claus Dieter Schröter
Robert Moshammer

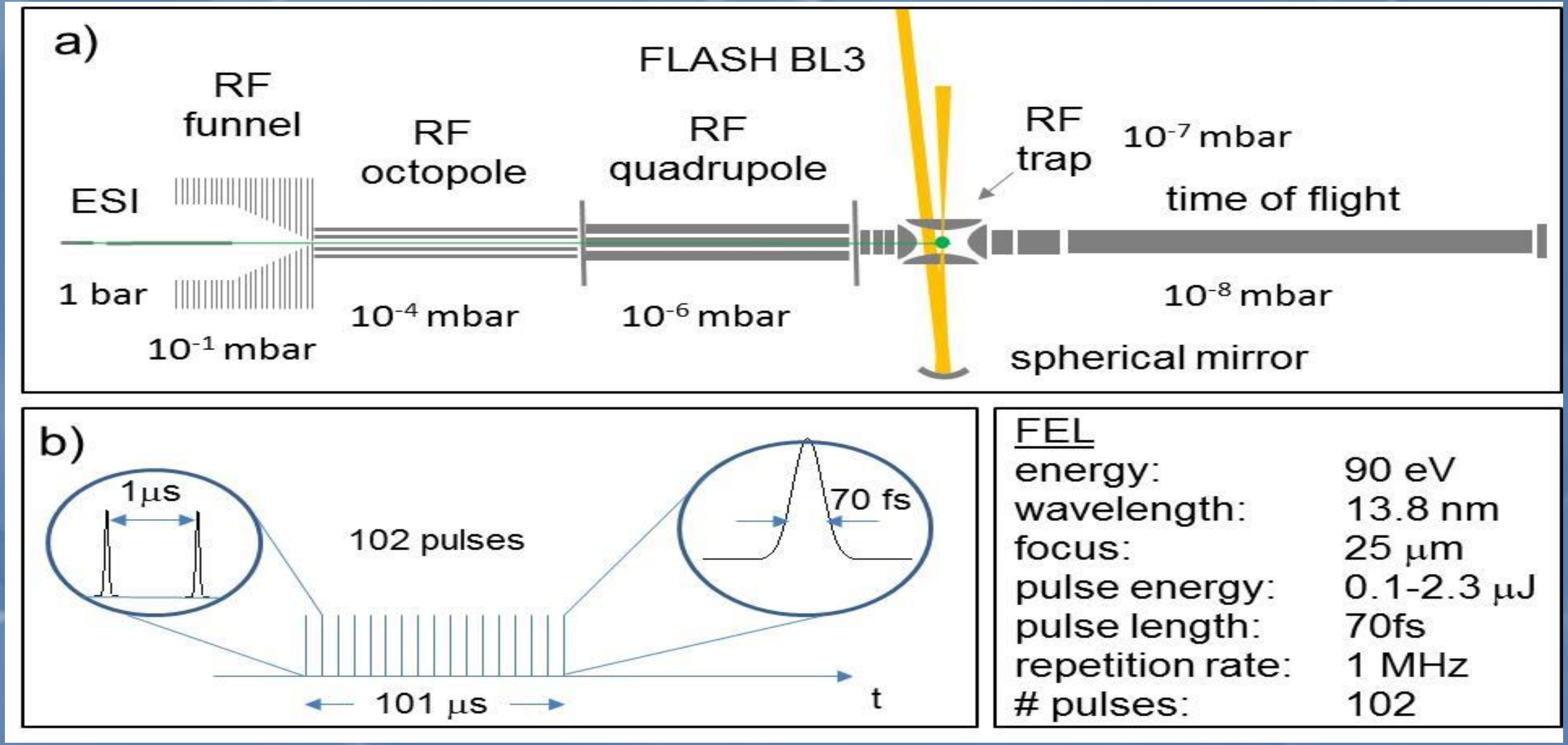
large protein–many photons



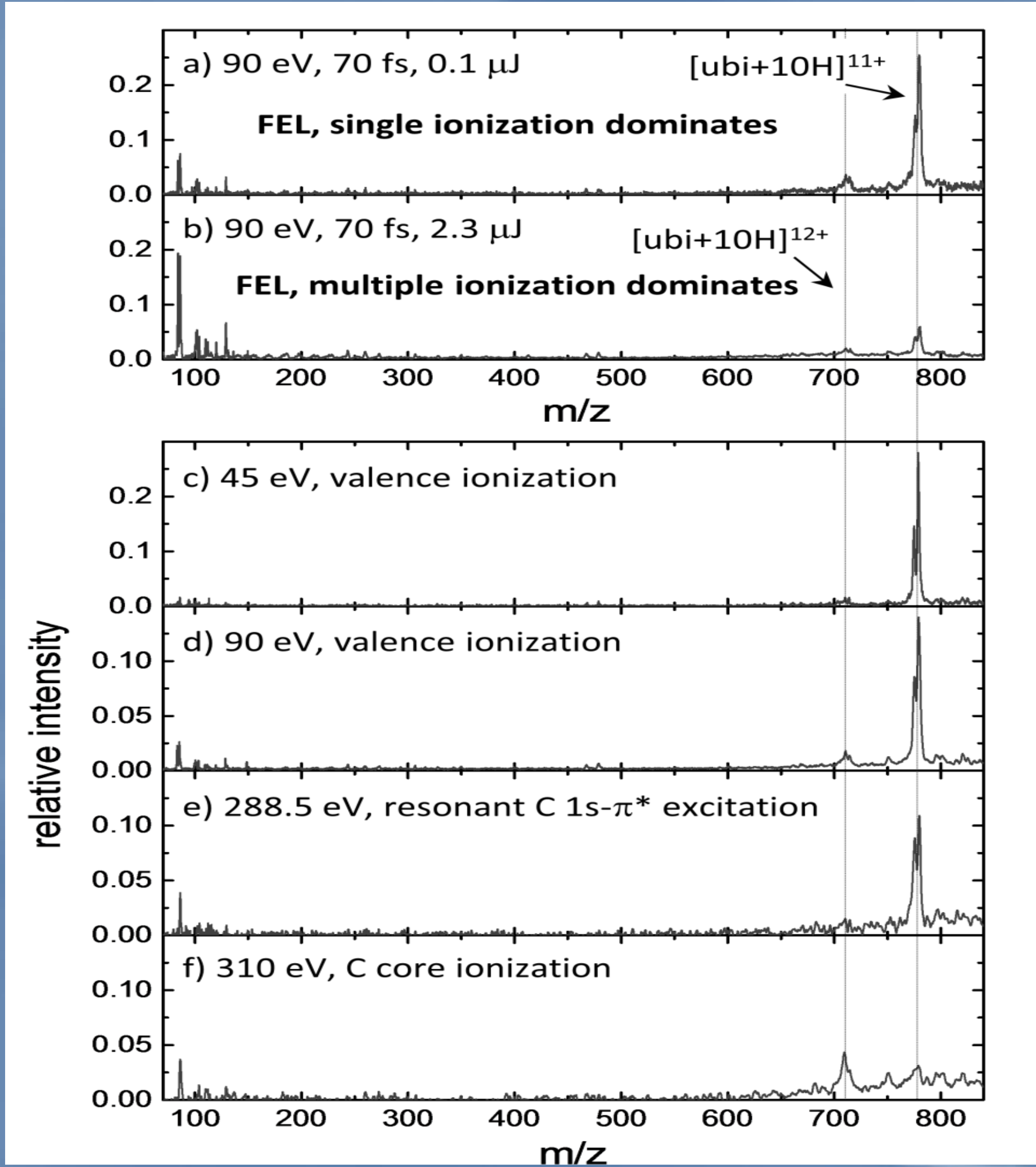
next step:

- **large protein (ubiquitin+10H)$^{10+}$**
- **many photons**

→ FLASH

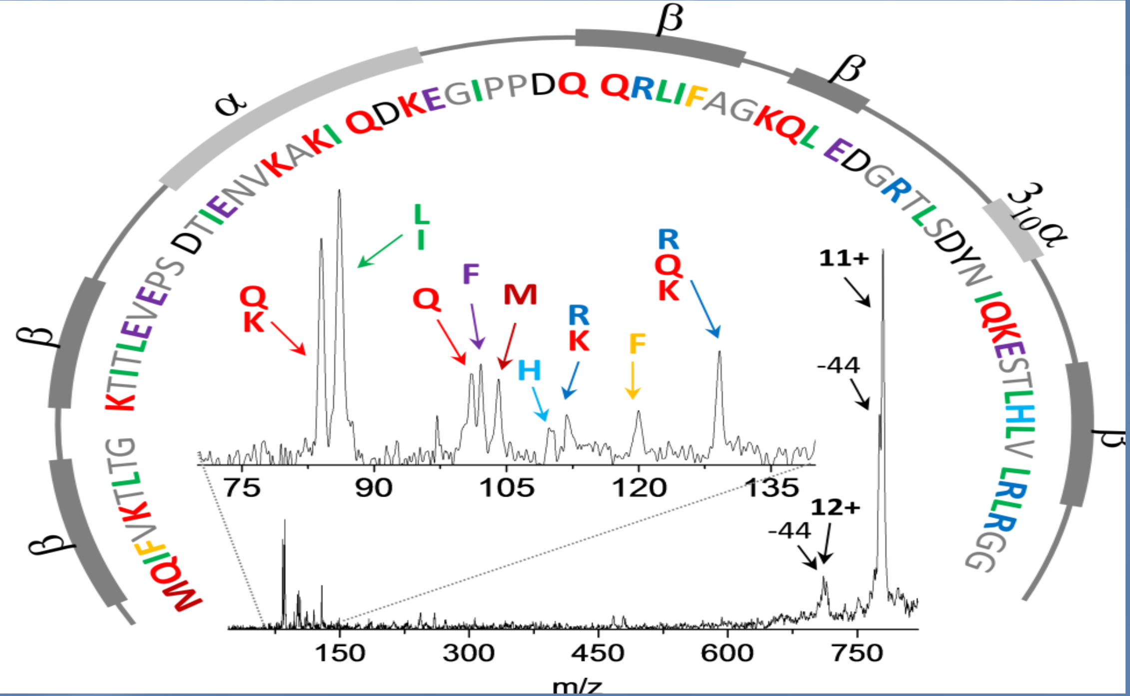


large protein–many photons



single photon conditions:
- **non-dissociative ionization**
- **immonium ions weak**

multiphoton conditions:
- **immonium ions dominate**



large protein–many photons

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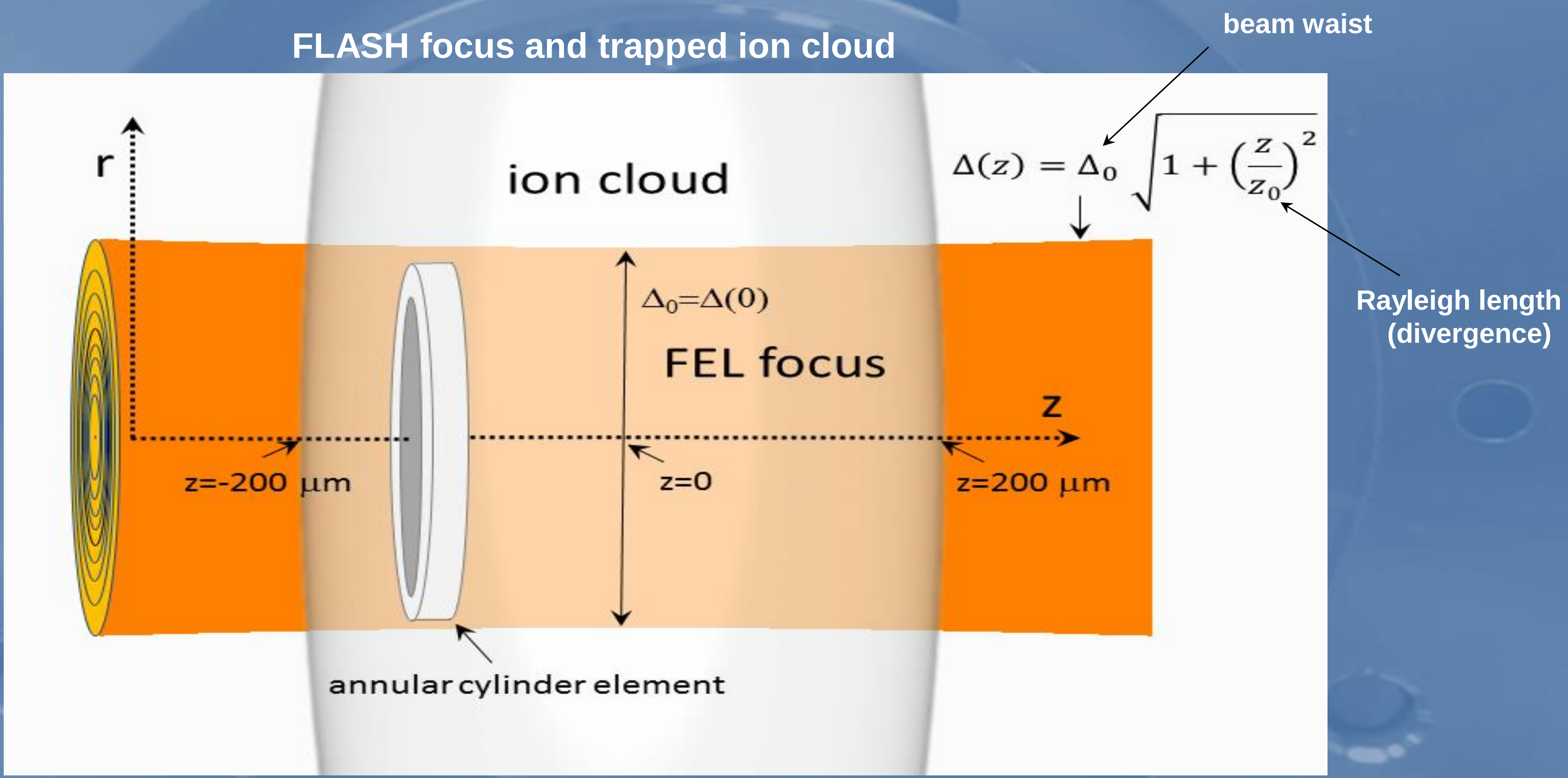
FLASH focus and trapped ion cloud



$$I(r,z) = I_0 \frac{4\,ln2}{\pi\Delta(z)^2} exp\left(-\frac{4ln2}{\Delta(z)^2} r^2\right)$$

ubiquitin – multiphoton

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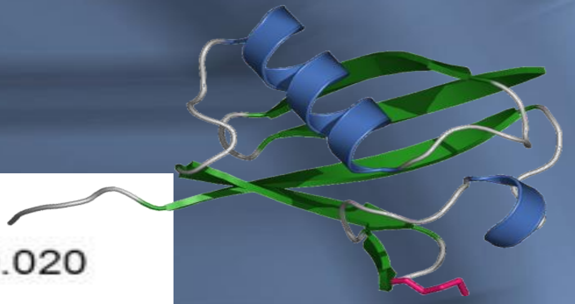
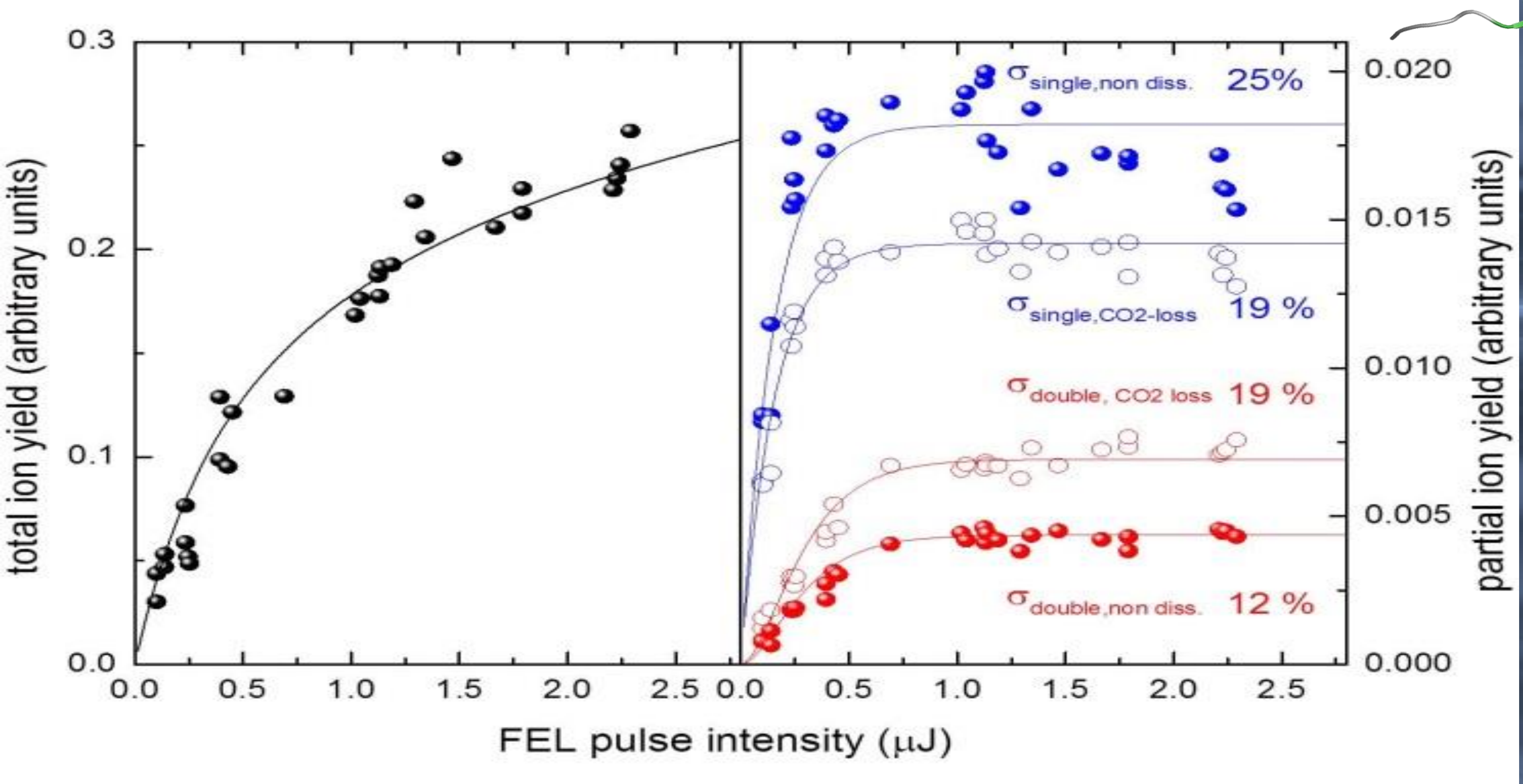
σ_{total}(90 eV) ~6.7 x 10^{-18} cm^2 from summation of atomic data

J. J. Yeh, *Atomic Calculation of Photoionization Cross-Sections and Asymmetry Parameters*, Gordon and Breach Science Publishers, Langhorne, PE, USA, **1993**.





non-diss processes due to single/double ioninzation in the "halo" of the FEL beam

MQIFV**K**TLTG**K**TITLEVEPSDTIENV**K**A**K**IQD**K**EGIPPDQQRLIFAG**K**QLEDGRTLSDYNIQ**K**ESTLHLVLRLRGG

ubiquitin – multiphoton

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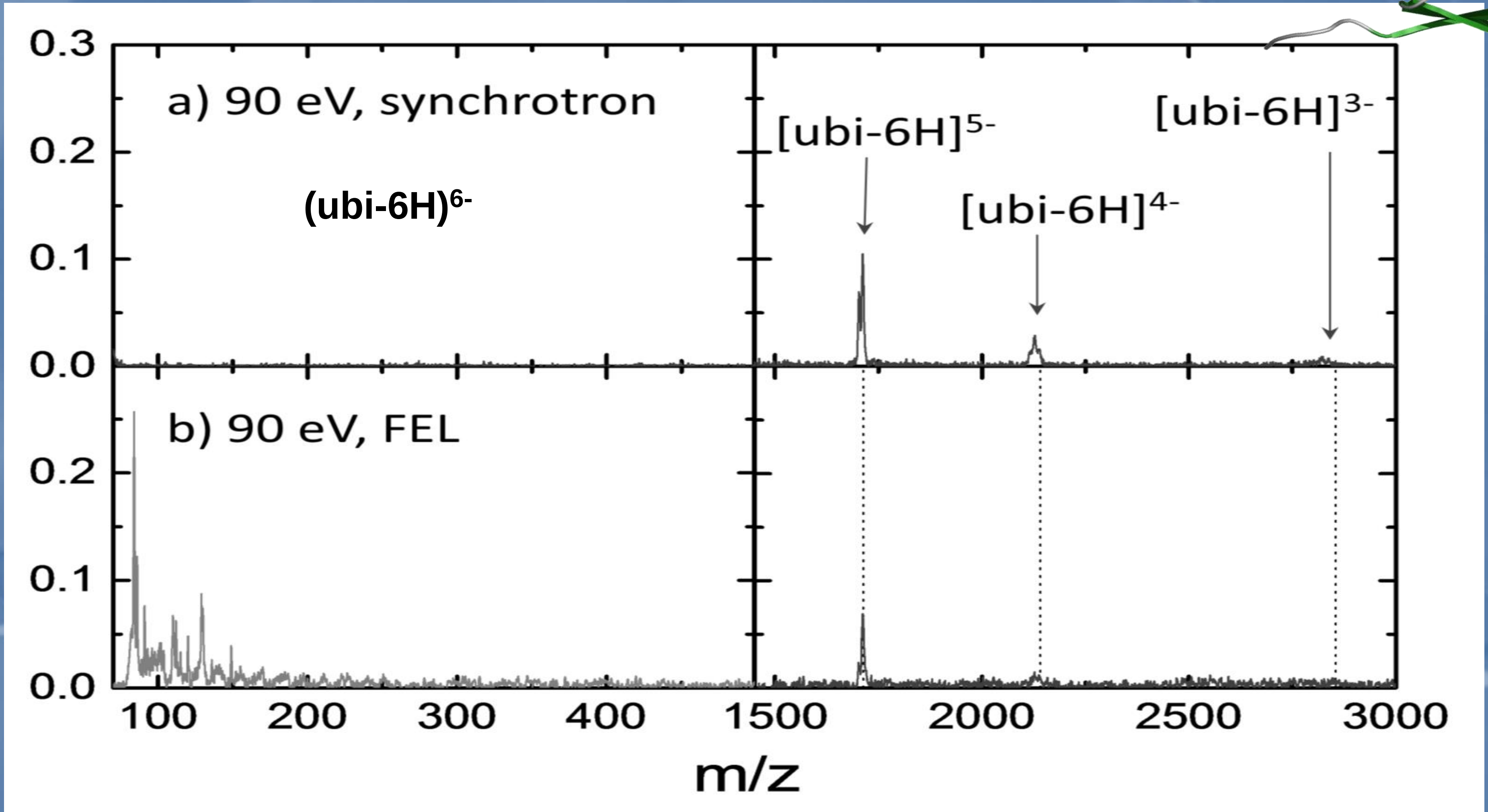
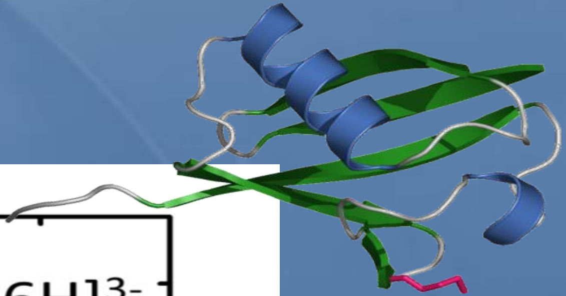
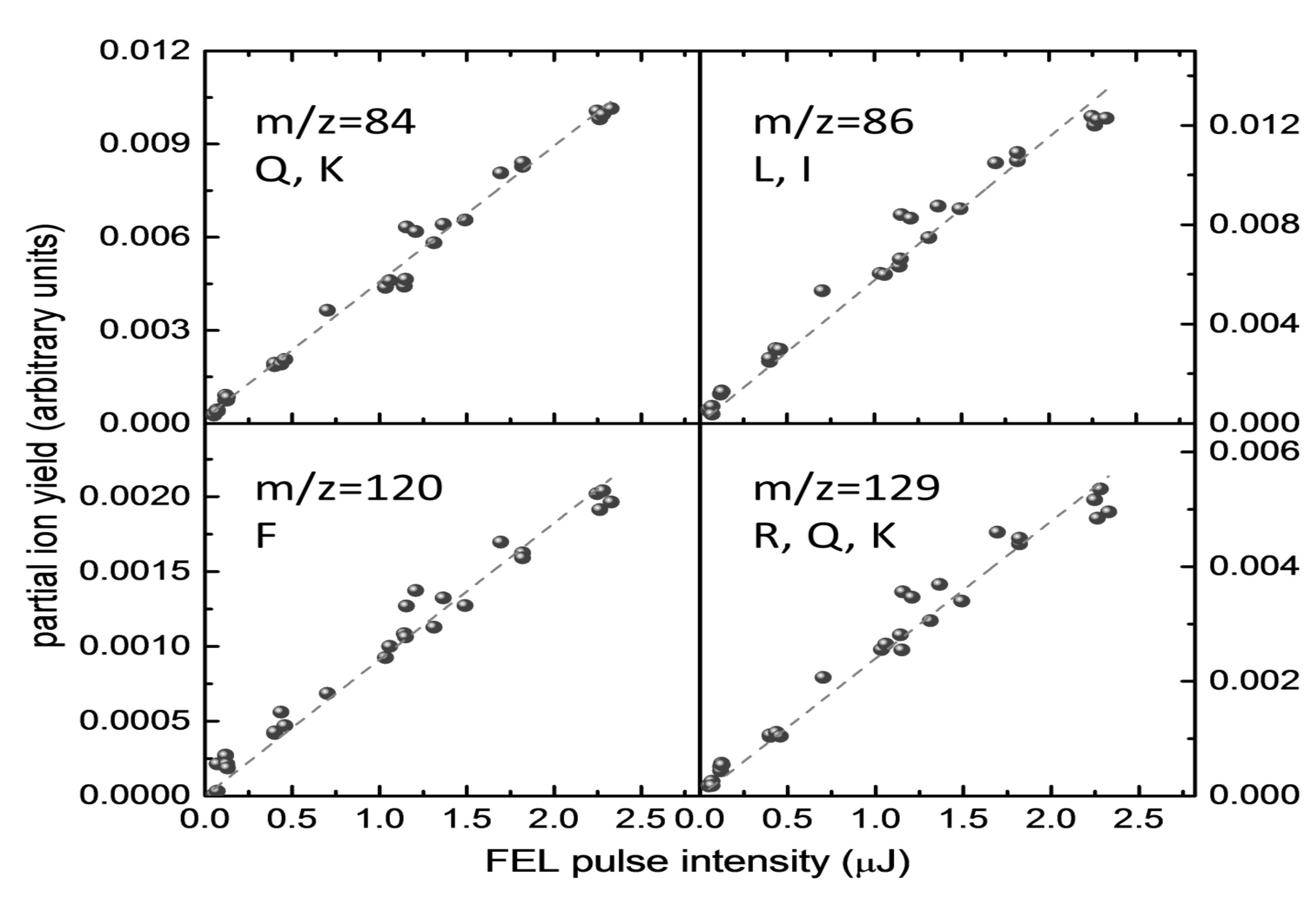
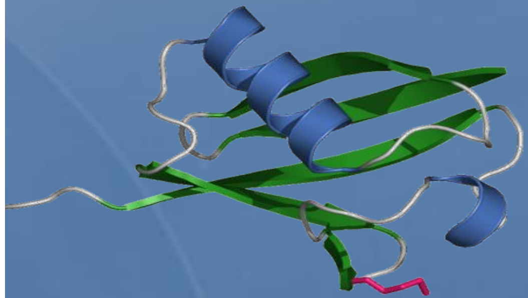
immonium ion formation upon multiphoton absorption
NOT
due to initial charge state!





ubiquitin – multiphoton





T. Schlathölter, G. Reitsma, D. Egorov, O. Gonzalez–Magaña, S. Bari, L. Boschman, E. Bodewits, K. Schnorr, G. Schmid, C. D. Schröter, R. Moshammer, and R. Hoekstra. Angewandte Chemie – International Edition 55 (2016) 10741

ubiquitin – multiphoton

linear increase of immonium yields

protein behaves as ensemble of free amino acids or peptides

fast local structural response