Advances in Mass Spectrometry:
Laser activation & Spectroscopy

Background literature:
- Polfer and Dugourd, Lecture Notes in Chemistry 83, Springer (2013)
- Rijs and Oomens, Top. Curr. Chem. 364, 1-42 (2015)

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Free Electron Lasers for
Infrared eXperiments

Master course Advances in MS - lecture 6

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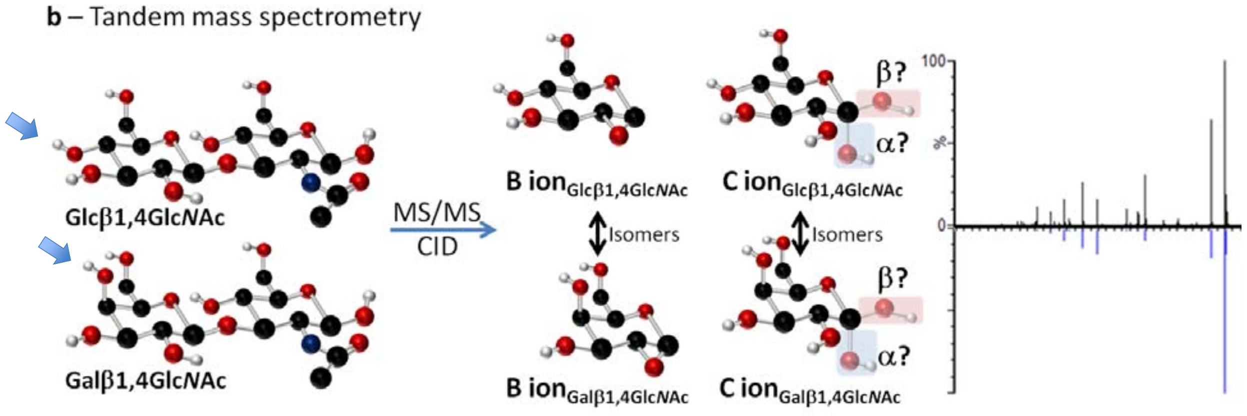
When tandem-MS is not enough….



C. J. Gray, B. Schindler, L. G. Migas, M. Pičmanová, A. R. Allouche, A. P. Green, S. Mandal, M. S. Motawie, R. Sánchez-Pérez, N. Bjarnholt, B. L. Møller, **A. M. Rijs**, P. E. Barran, I. Compagnon, C. E. Eyers, and S. L. Flitsch, *Anal. Chem.* **89**, 4540−4549 (2017).

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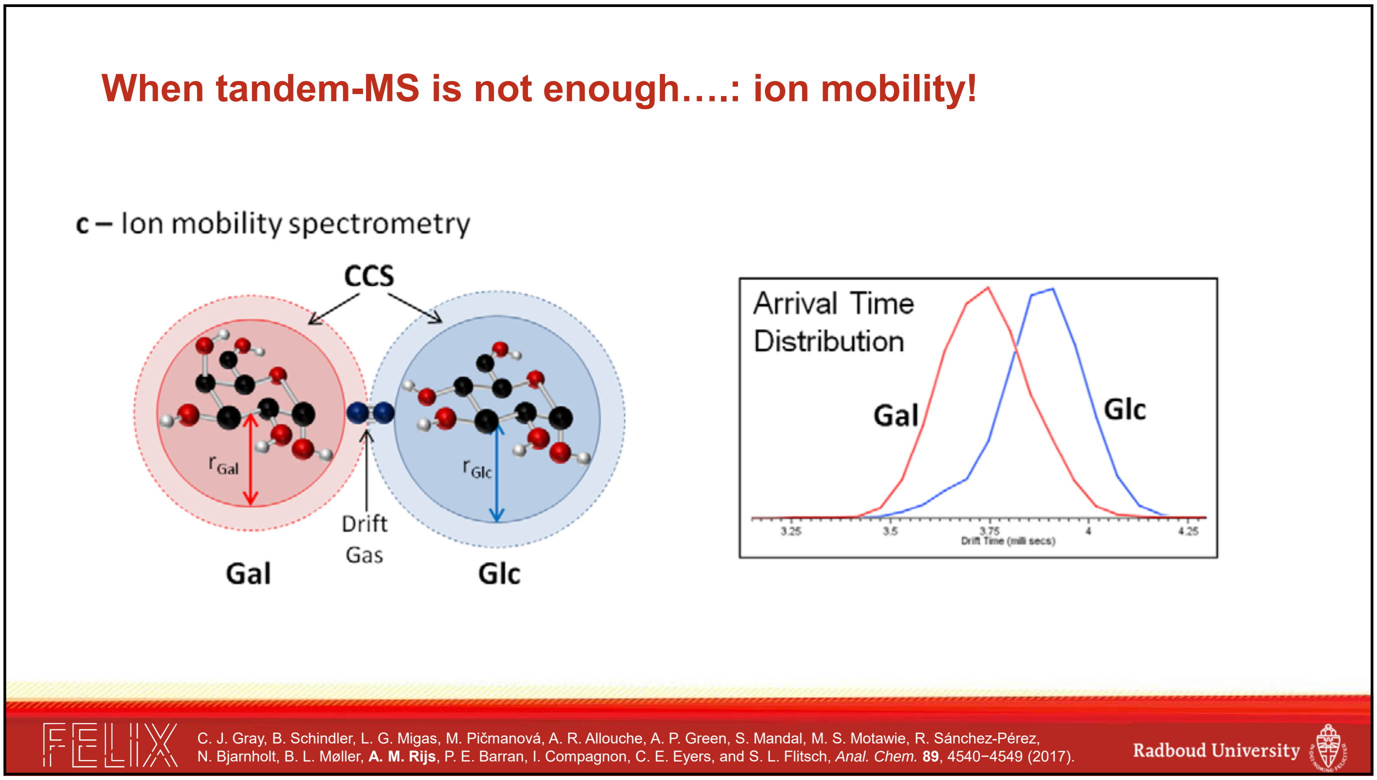

When tandem-MS is not enough….: ion mobility!

c – Ion mobility spectrometry

C. J. Gray, B. Schindler, L. G. Migas, M. Pičmanová, A. R. Allouche, A. P. Green, S. Mandal, M. S. Motawie, R. Sánchez-Pérez, N. Bjarnholt, B. L. Møller, **A. M. Rijs**, P. E. Barran, I. Compagnon, C. E. Eyers, and S. L. Flitsch, *Anal. Chem.* **89**, 4540−4549 (2017).

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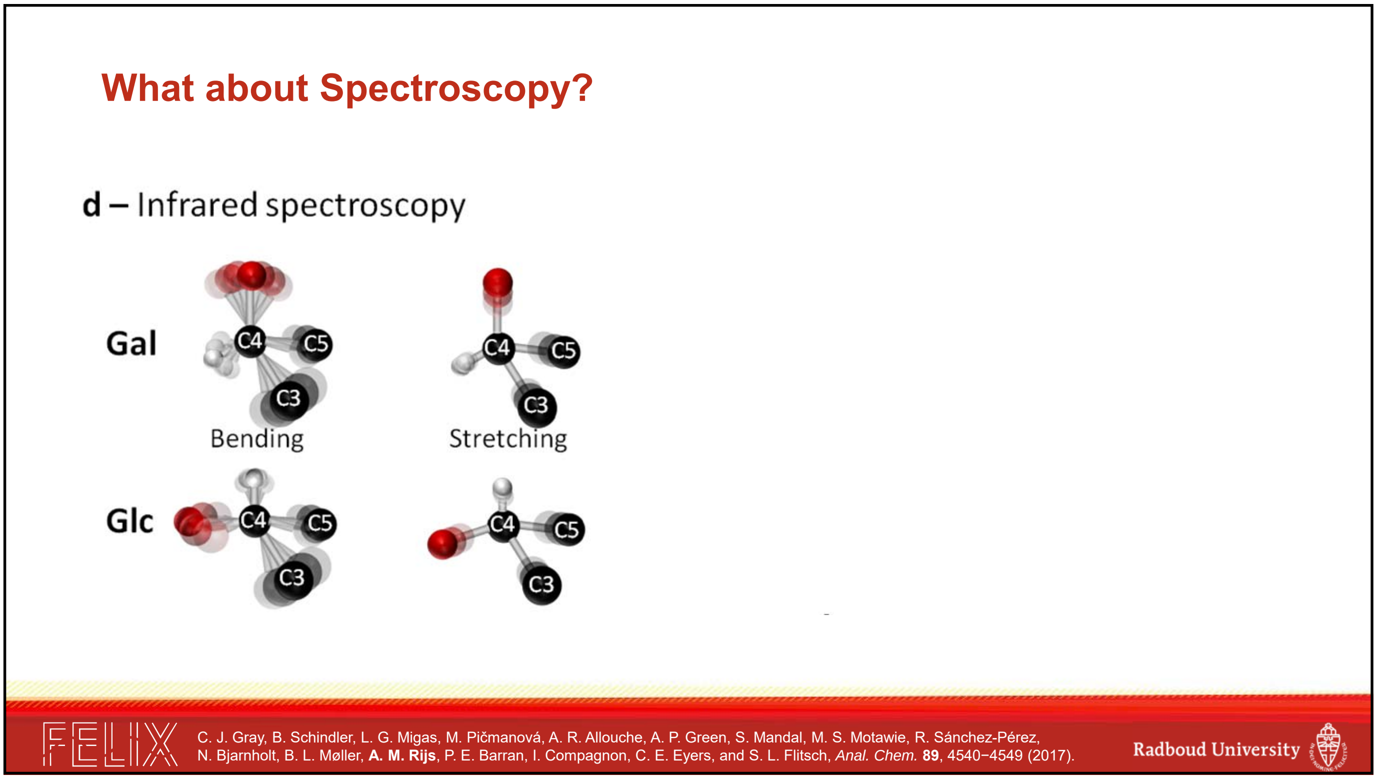

What about Spectroscopy?

d – Infrared spectroscopy

C. J. Gray, B. Schindler, L. G. Migas, M. Pičmanová, A. R. Allouche, A. P. Green, S. Mandal, M. S. Motawie, R. Sánchez-Pérez, N. Bjarnholt, B. L. Møller, **A. M. Rijs**, P. E. Barran, I. Compagnon, C. E. Eyers, and S. L. Flitsch, *Anal. Chem.* **89**, 4540−4549 (2017).

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Course Layout – Spectroscopy & Mass Spectrometry

1) **Background into Spectroscopy**
   - i. fundamental principles
   - ii. direct vs action spectroscopy
   - iii. instrumentation

2) IR spectroscopy
   - i. IRMPD (single wavelength) and IR ion spectroscopy
   - ii. Sources
   - iii. Scientific example

3) UV spectroscopy
   - i. UVPD (single wavelength)
   - ii. Photoabsorption and fragmentation spectroscopy
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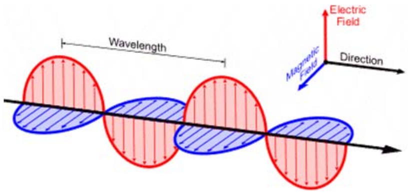
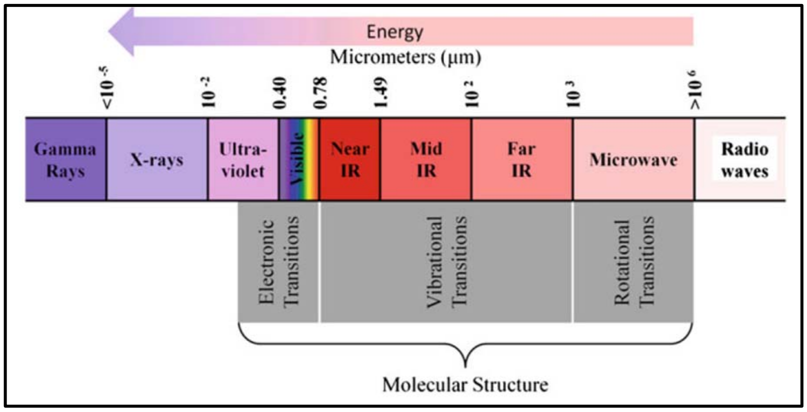
Spectroscopy – the electromagnetic spectrum



- E-field and M-field component (perpendicular)
- EM wave: traveling wave with constant velocity (speed of light)
- **Wavelength** = distance between 2 maxima
- **Frequency =** number of maxima per second
- $E = h\nu = \frac{hc}{\lambda} = hc\tilde{\nu}$



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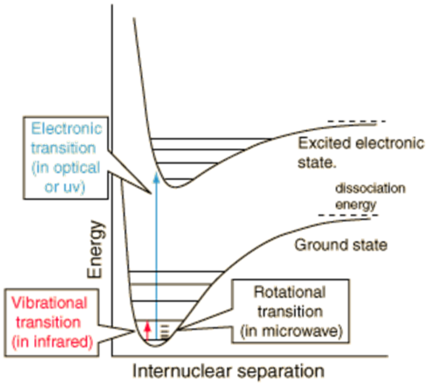
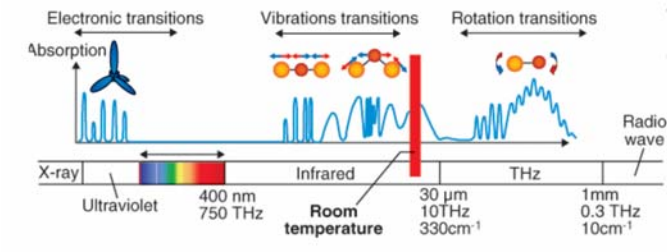
Spectroscopy and MS spectrometry: molecular structure

Q: Can every type of spectroscopy be combined with MS spectrometry?



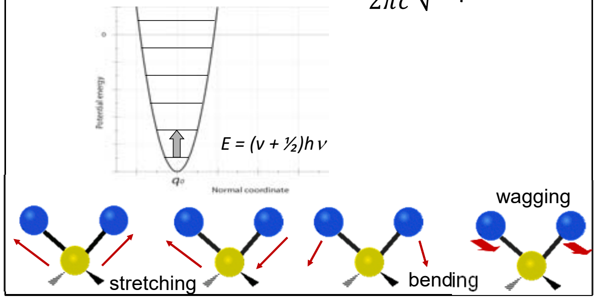


Spectroscopy and MS spectrometry: molecular structure

Q: Can every type of spectroscopy be combined with MS spectrometry?

IR Spectroscopy (vibrational)

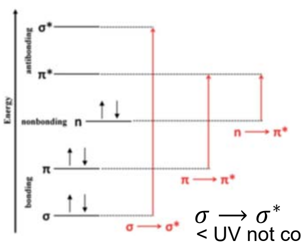
- Vibration are assumed to be harmonic oscillations
- Vibrational wavenumber $\tilde{\nu} = \frac{1}{2\pi c}\sqrt{k/\mu}$



UV Spectroscopy (electronic)

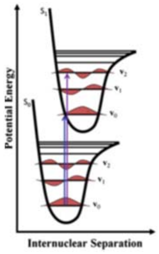
$A + h\nu \longrightarrow A^*$

- Electronic transitions
- HOMO to LUMO
- Fast time scales (10^{-15} to 10^{-18} s)
- Franck Condon principle / vertical transitions



UV-VIS:
$n \longrightarrow \pi^*$
$\pi \longrightarrow \pi^*$

$\sigma \longrightarrow \sigma^*$
< UV not considered



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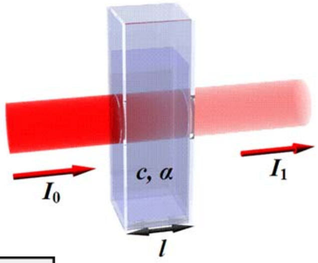
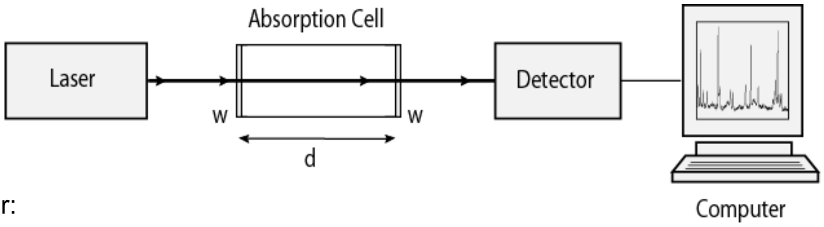
Direct Absorption Spectroscopy



- Provides absolute concentrations or absolute cross-sections
- Measures **attenuation of light** traveling through a sample
- Transmitted intensity follows the Beer-Lambert law

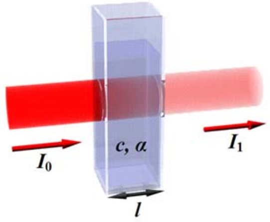


Lambert-Beer:

$$-log\frac{I}{I_0} = \epsilon Lc$$

I_0	Intensity of the incident light
L	length of the sample
ε	Molar absorption coefficient

Direct Absorption Spectroscopy



UV spectroscopy

$$-\log\frac{I}{I_0} = \epsilon L c$$

Typical numbers for Tryptophan:

- $\varepsilon = 5690\ M^{-1}cm^{-1}$
- $\lambda = 280$ nm
- L= 1 cm
- Detection efficiency $I/I_0 = 0.1\%$
- required concentration: c= 75 nM
- ~$4.6 \cdot 10^{13}$ ion Trp per ml

How many ions are there typically in an ion trap??

10^6-10^7 ions

UV spectroscopy in a trap

$$I = I_0 e^{-\alpha L}$$
$$\alpha(\nu) = \Delta N \sigma(\nu)$$

Typical numbers:

- $\sigma = 10^{-16}$-10^{-17} cm^2/molecule (UV)
- L= 0.1 cm
- $\Delta N = 10^8$ molecules/cm^3
- ratio #absorbed photons 10^{-9}-10^{-10}
- Irradiation time 1s
- 1 out of 10^9-10^{10} photons are absorbed

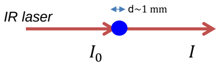
What does this mean for our light source to have detectable quantities?

10^{16}-10^{17} photons cm^2 s^{-1}

light bulb ☺

I_0	Intensity of the incident light
L	length of the sample
α	absorption coefficient sample
ΔN	population difference
$\sigma(\nu)$	cross section
ν	frequency of the light

Molecules in the gas phase - IR absorption



Absorbance: $A(\nu) = \log_{10}\left(\frac{I_0}{I}\right)$ ⟶ Molar absorption coefficient:

$$\varepsilon(\nu) = \frac{A(\nu)}{Cd} = \frac{1}{Cd}\log_{10}\left(\frac{I_0}{I}\right)\ [\mathrm{L \cdot mol^{-1} \cdot cm^{-1}}]$$

Assuming Lorentz shape of the IR absorption band, the molar absorption coefficient at resonance frequency is:

$$\varepsilon(\nu_0) = 27.7\frac{I_{IR}}{w}$$

where I_{IR} is the IR intensity of the band in km/mol, and w is FWHM of the absorption band in cm^{-1}.

If for example IR intensity of the band is 100 km/mol, and w=10 cm^{-1}, this gives:

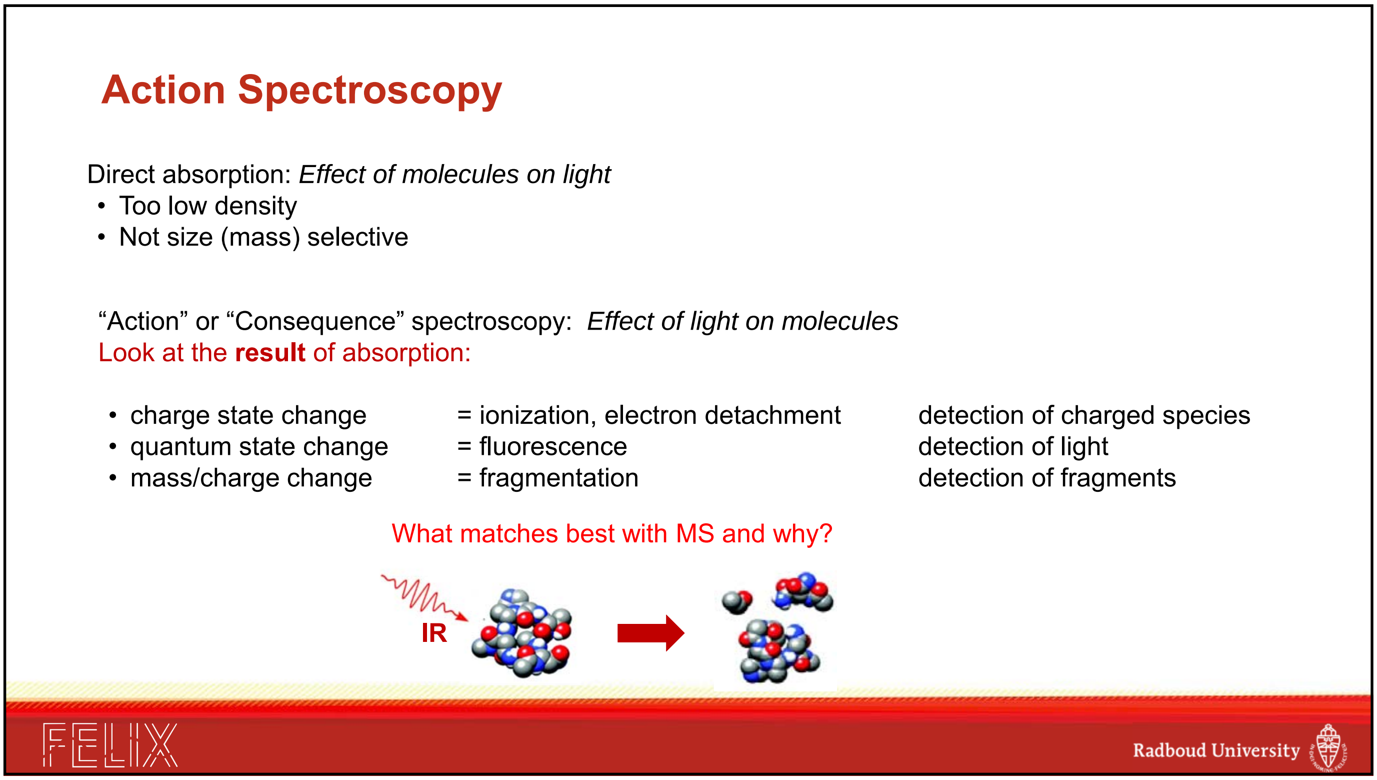
$$\varepsilon(\nu_0) = 27.7\frac{100}{10} = 277\ [\mathrm{L \cdot mol^{-1} \cdot cm^{-1}}]$$

$C \sim 10^7\ cm^{-3} = 10^{10} L^{-1}$ (number of ions)
d ~1 mm = 0.1 cm
$N_A = 6.022 \cdot 10^{23}$ mol^{-1}

$$A(\nu) = \varepsilon(\nu_0) \cdot Cd = 277 \cdot 10^{10} \cdot 0.1 \left[\frac{\mathrm{L \cdot mol^{-1} \cdot cm^{-1} \cdot cm}}{\mathrm{L}}\right] = \frac{2.77 \cdot 10^{11}}{6.022 \cdot 10^{23}} = 4.6 \cdot 10^{-13}$$

Changes in the transmission:

$$\Delta T = \frac{I_0 - I}{I_0} = 1 - 10^{-A(\nu)} = \sim 10^{-12} \Rightarrow 10^{-10}\ \%$$



Action Spectroscopy

Direct absorption: *Effect of molecules on light*

- Too low density
- Not size (mass) selective

“Action” or “Consequence” spectroscopy: *Effect of light on molecules*
Look at the **result** of absorption:

• charge state change	= ionization, electron detachment	detection of charged species
• quantum state change	= fluorescence	detection of light
• mass/charge change	= fragmentation	detection of fragments

What matches best with MS and why?

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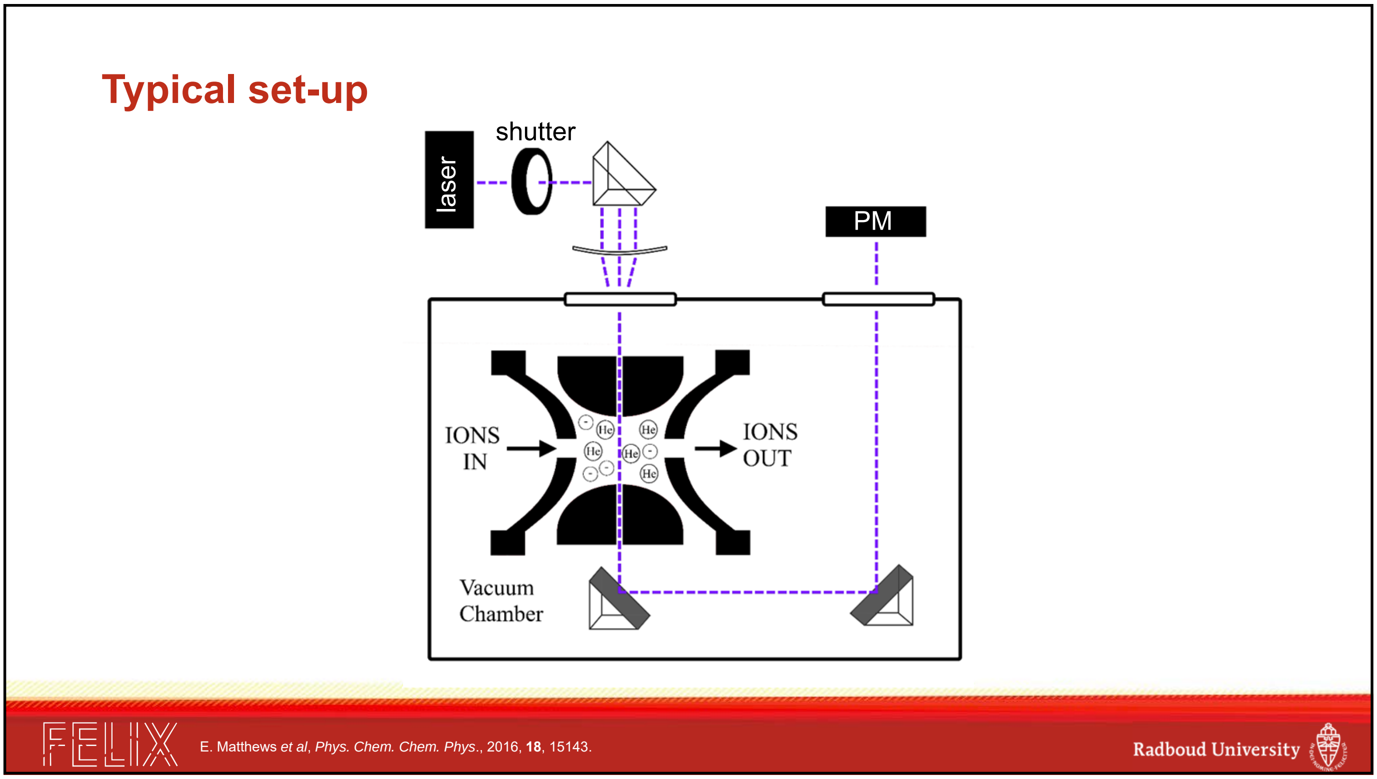

Typical set-up

E. Matthews *et al*, *Phys. Chem. Chem. Phys.*, 2016, **18**, 15143.

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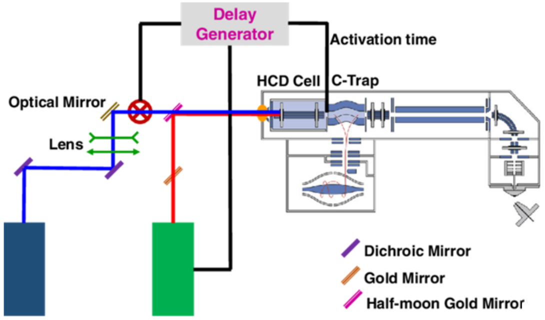
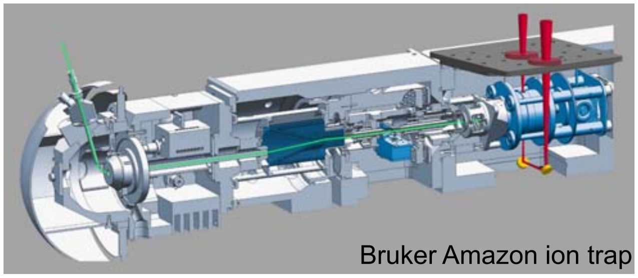
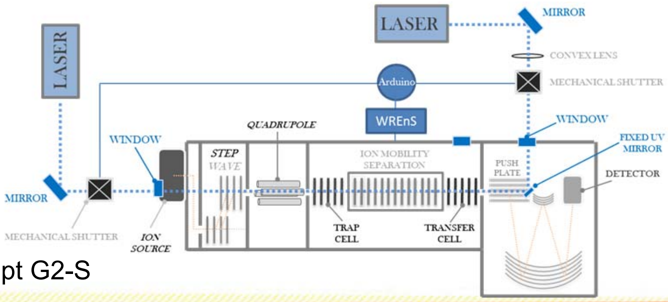
Combined with commercial mass spectrometers



Thermo-Fisher orbitrap (fusion lumos)



Bruker Amazon ion trap



Waters Synapt G2-S

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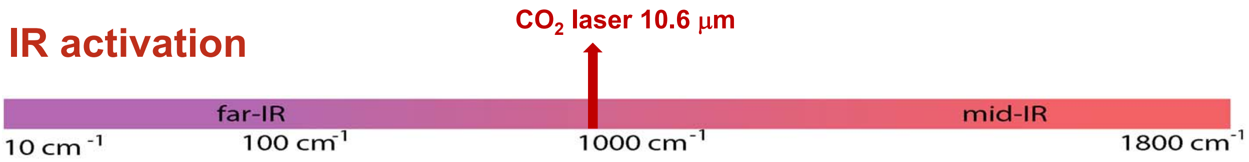
IR activation

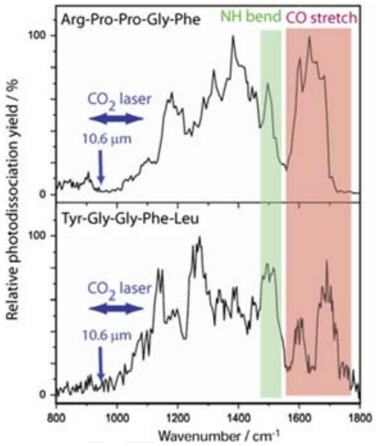


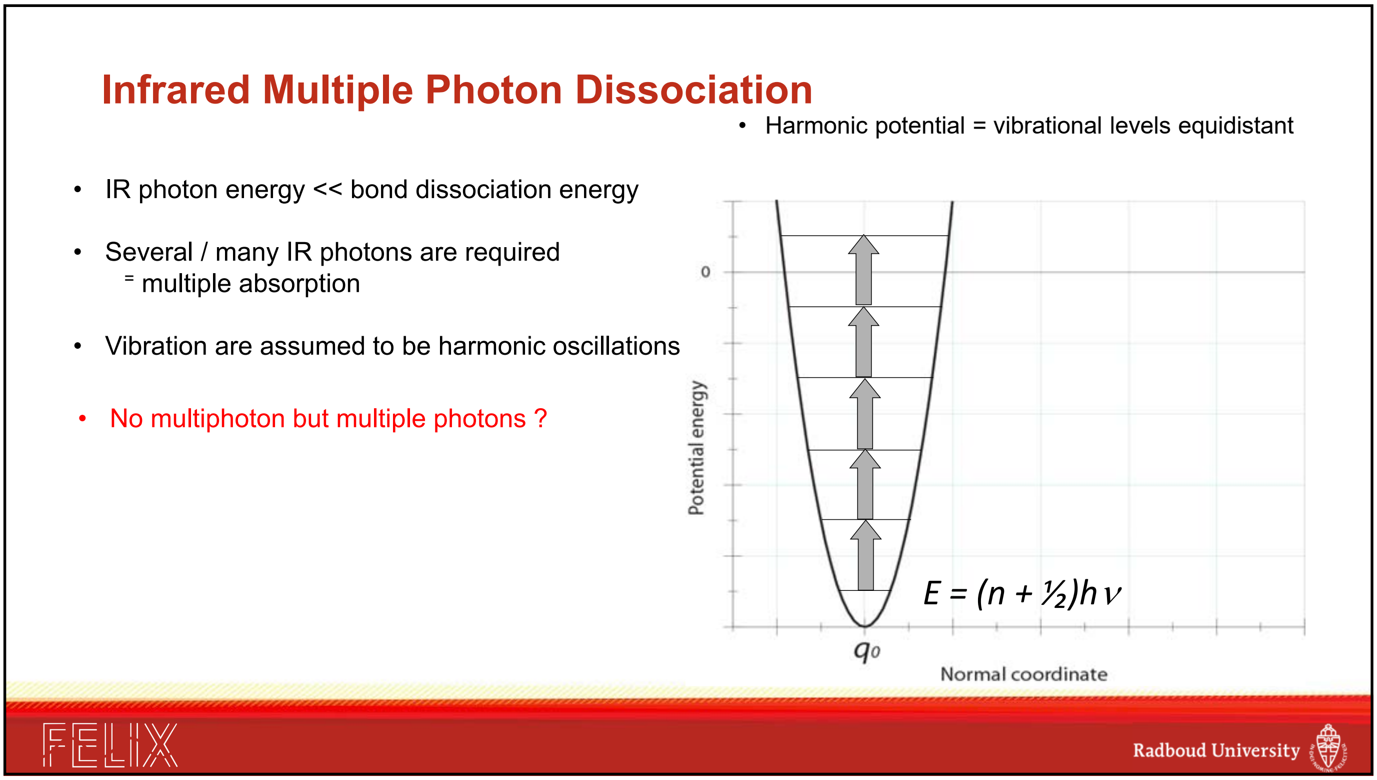
Why 10.6 μm (=940 cm^{-1})?

- High power laser (typically 50 W)
- CW
- Fluence 100x FEL
- Slightly tunable (900-1100nm)

- in tail of absorption of peptides, still enough due to high fluence
- phosphate groups and glycans do absorb: post-translational modifications

- CID-like process

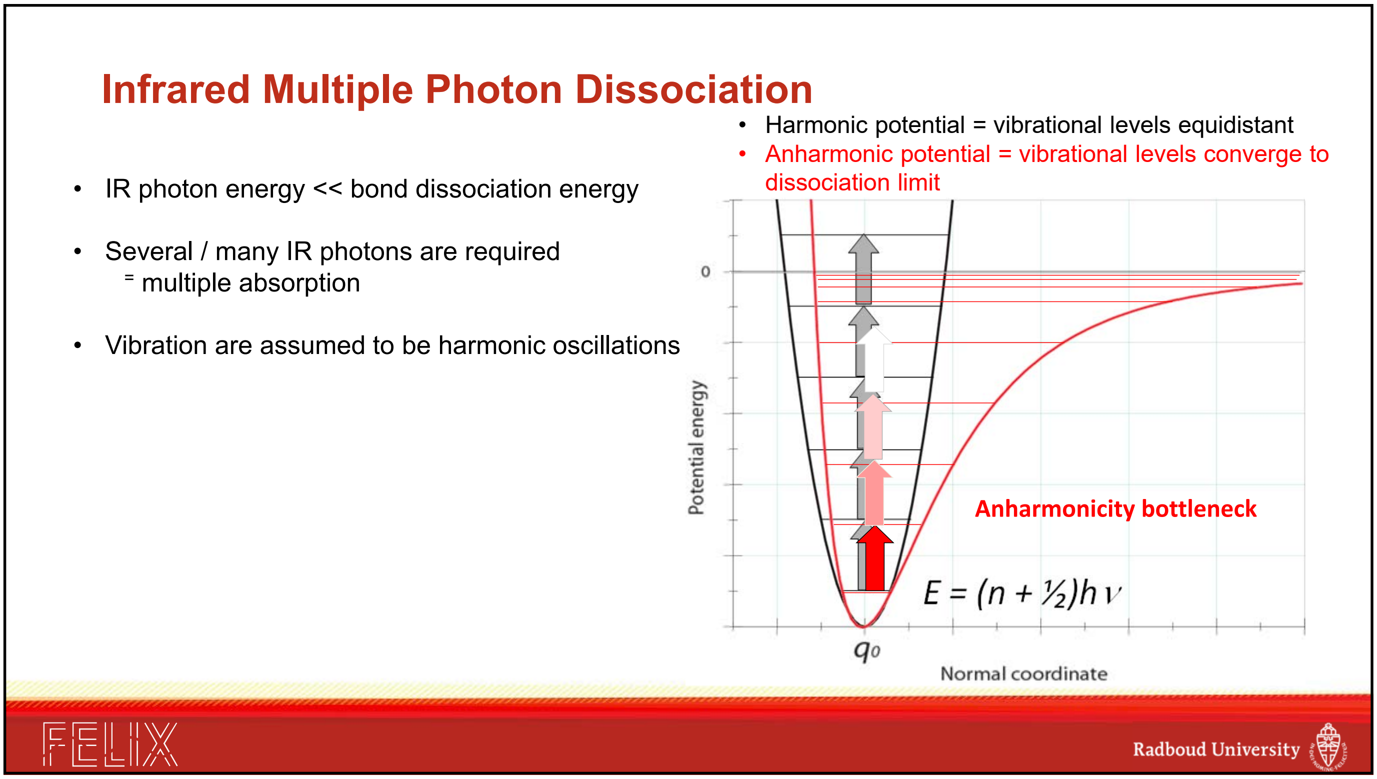




Infrared Multiple Photon Dissociation

- IR photon energy << bond dissociation energy
- Several / many IR photons are required
  = multiple absorption
- Vibration are assumed to be harmonic oscillations
- No multiphoton but multiple photons ?

- Harmonic potential = vibrational levels equidistant



Infrared Multiple Photon Dissociation

- IR photon energy << bond dissociation energy
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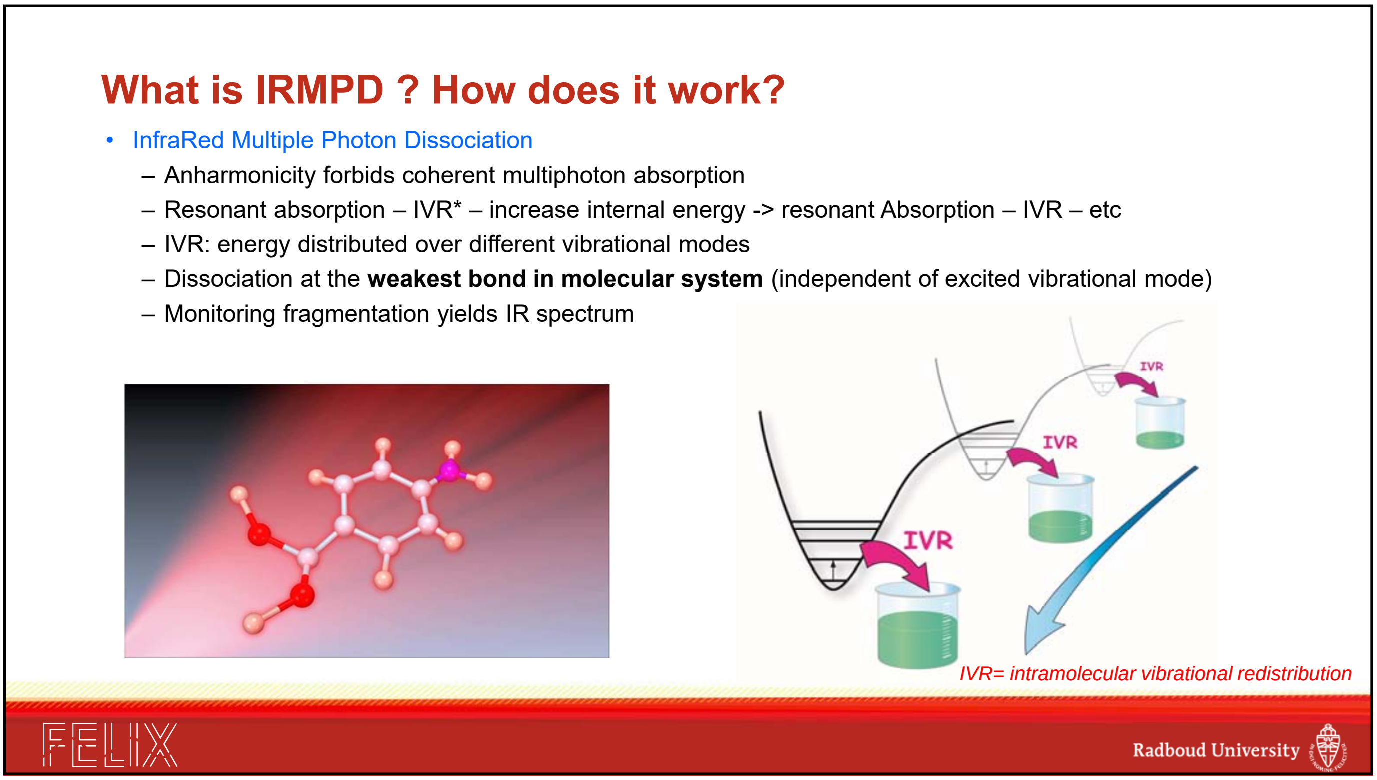
  = multiple absorption
- Vibration are assumed to be harmonic oscillations

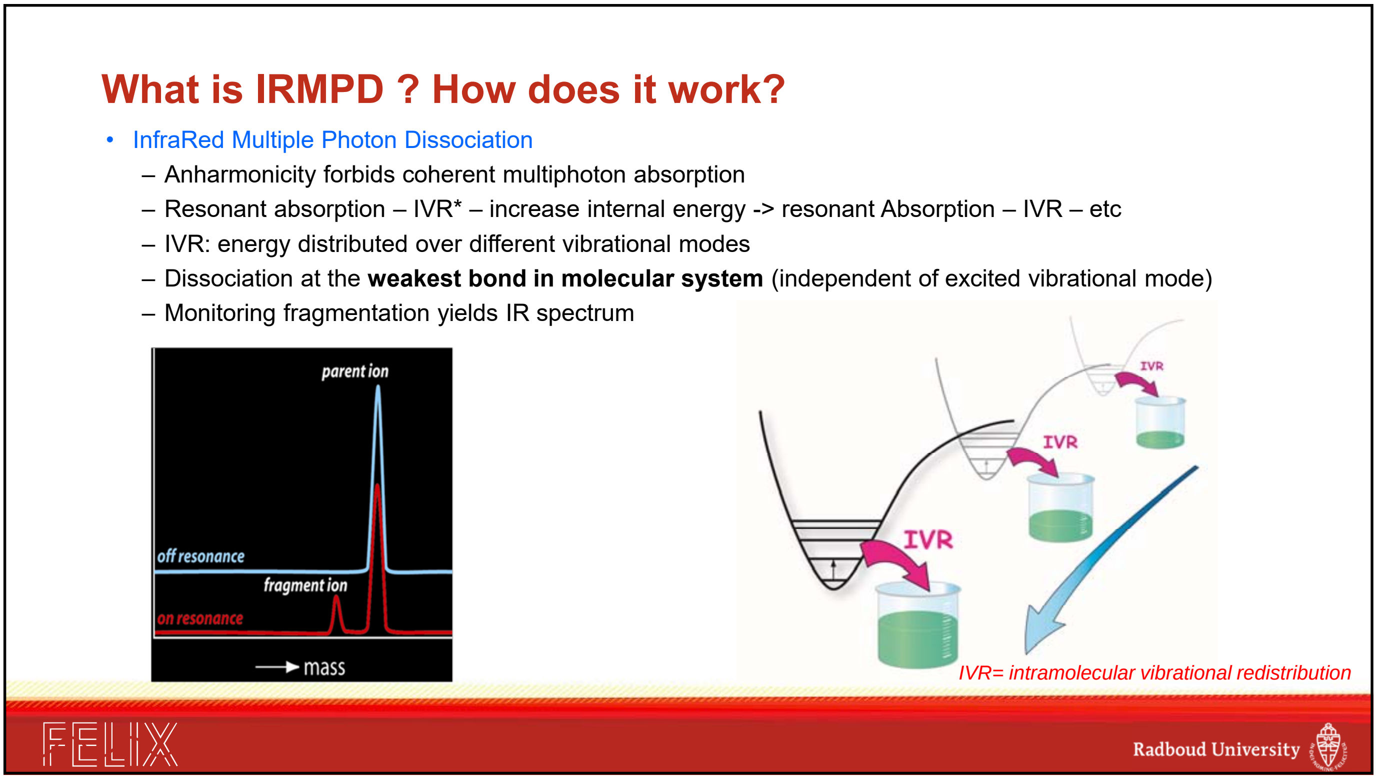
- Harmonic potential = vibrational levels equidistant
- Anharmonic potential = vibrational levels converge to dissociation limit



What is IRMPD ? How does it work?

- InfraRed Multiple Photon Dissociation
  - Anharmonicity forbids coherent multiphoton absorption
  - Resonant absorption – IVR* – increase internal energy -> resonant Absorption – IVR – etc
  - IVR: energy distributed over different vibrational modes
  - Dissociation at the **weakest bond in molecular system** (independent of excited vibrational mode)
  - Monitoring fragmentation yields IR spectrum

IVR= intramolecular vibrational redistribution



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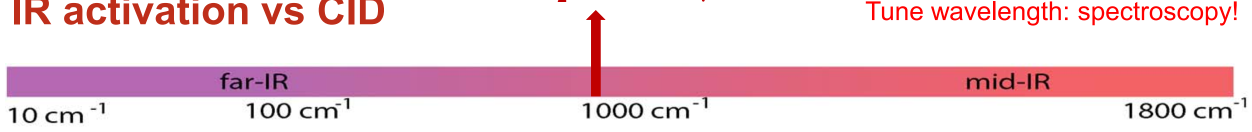
IVR= intramolecular vibrational redistribution

IR activation vs CID

CO_2 laser 10.6 μm

Tune wavelength: spectroscopy!



CID-like behavior:
- multiple IR photons = gradual heating as collisions with CID

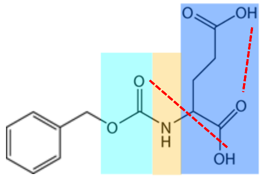
IRMPD:
- broader m/z trapping range possible than for CID
- allows to reduce the radio frequency (rf) trapping voltage during ion activation
  (with CID this would result in decrease in energy deposition associated with lower rf trapping voltages)
- promotes secondary dissociation of primary product ions due to continuous irradiation
  = formation of a more diverse array of diagnostic ions compared with CID

Challenges:
- balance between activation and cooling
- performance is hindered by the failure to provide adequate fragmentation at the standard pressure
- No wavelength depended information, only fragmentation

IR ion spectroscopy (vibrational spectroscopy)

What does on IR spectrum tell you?

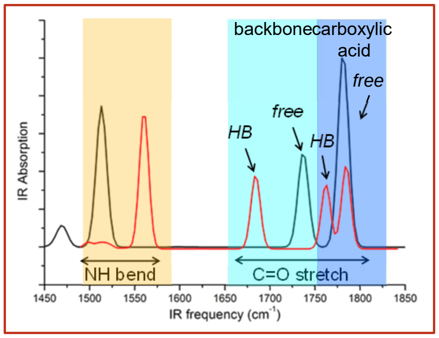
IR spectroscopy direct view on:
- Conformational changes
- Hydrogen bond interactions
- Van der Waals interactions



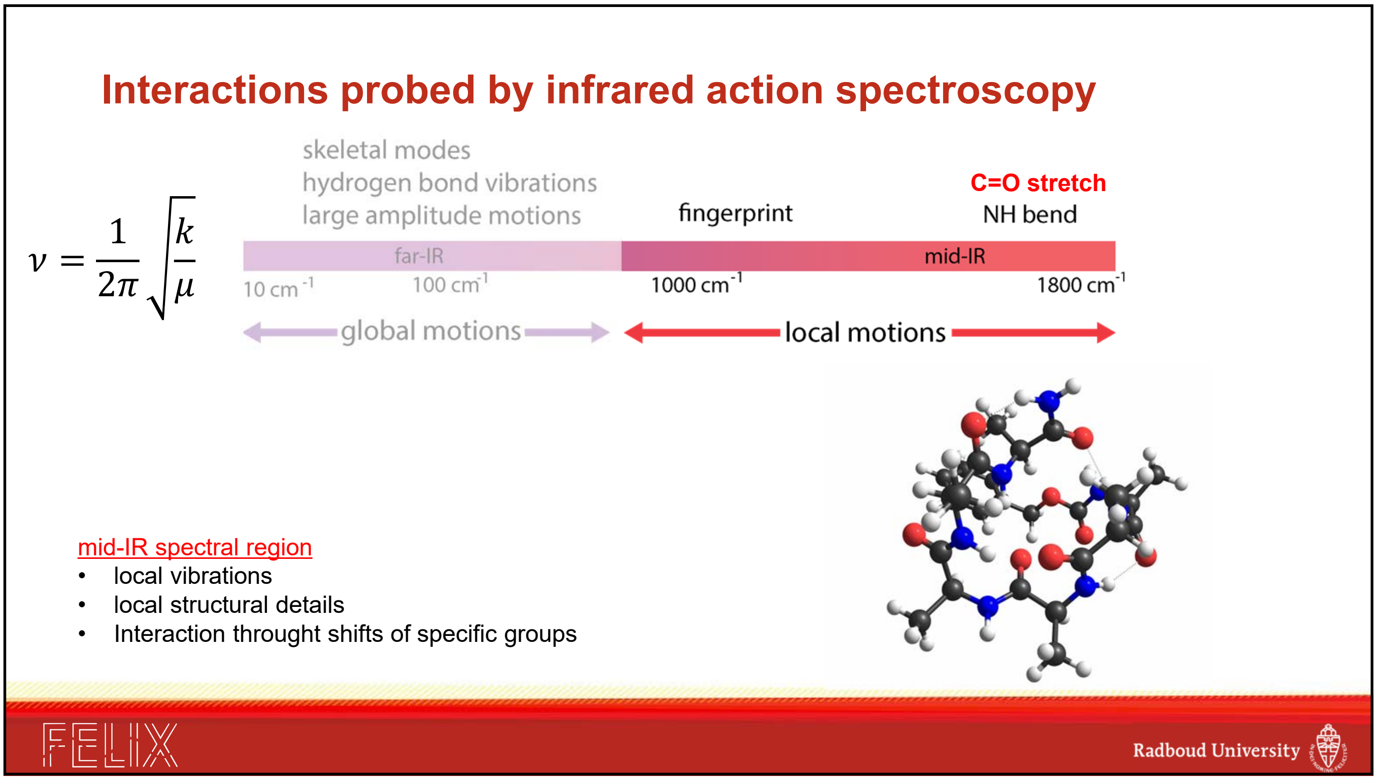
Amide A - NH stretch, OH stretch: 3300-3600 cm^{-1}

Amide I - C=O stretch: 1700-1800 cm^{-1}

Amide II – NH bend: 1500 cm^{-1}



Experiment + quantum chemical calculations = structure



Interactions probed by infrared action spectroscopy

$$\nu = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}}$$

skeletal modes
hydrogen bond vibrations
large amplitude motions

fingerprint

C=O stretch
NH bend

far-IR
mid-IR

$10\ cm^{-1}$ $100\ cm^{-1}$ $1000\ cm^{-1}$ $1800\ cm^{-1}$

global motions
local motions

mid-IR spectral region

- local vibrations
- local structural details
- Interaction throught shifts of specific groups

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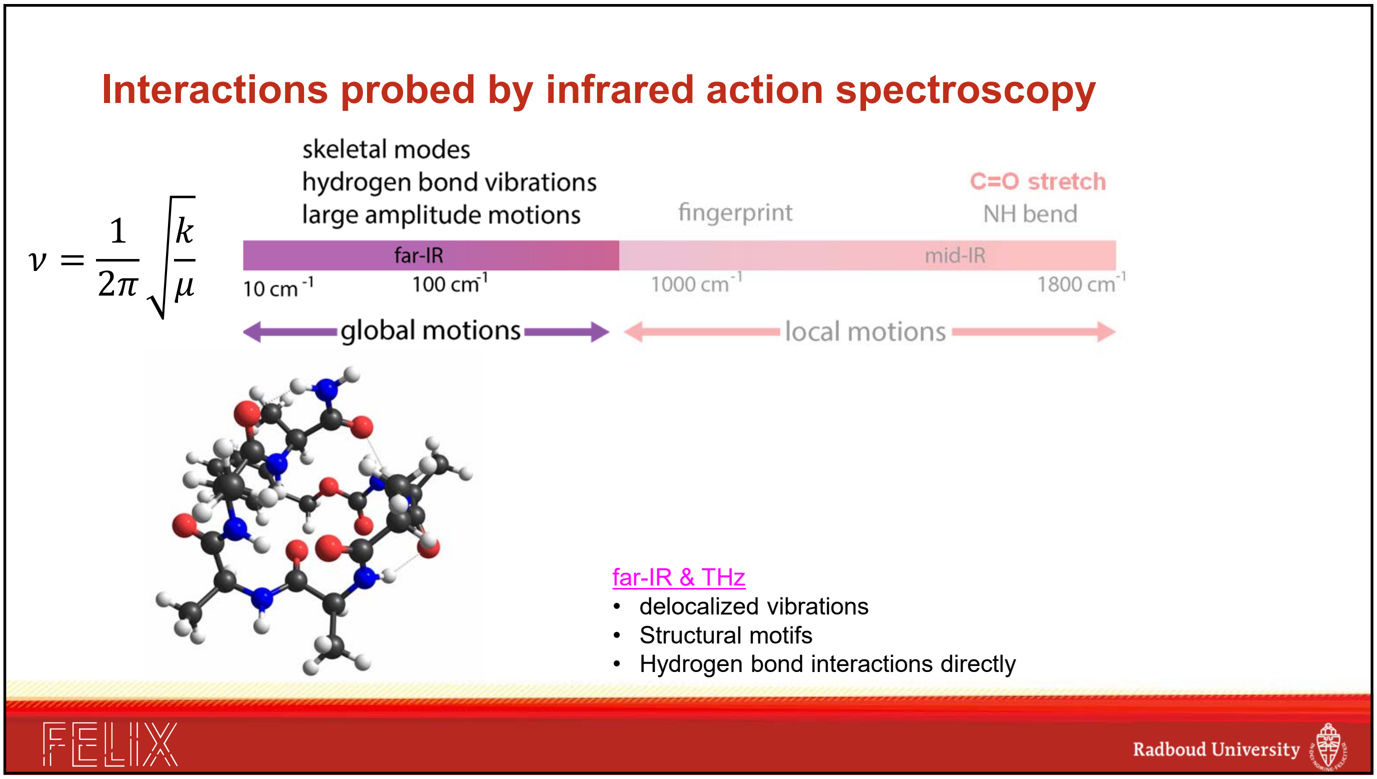

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$$\nu = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}}$$

skeletal modes
hydrogen bond vibrations
large amplitude motions

fingerprint

C=O stretch
NH bend

far-IR
mid-IR

$10\ cm^{-1}$ $100\ cm^{-1}$ $1000\ cm^{-1}$ $1800\ cm^{-1}$

global motions
local motions

far-IR & THz

- delocalized vibrations
- Structural motifs
- Hydrogen bond interactions directly

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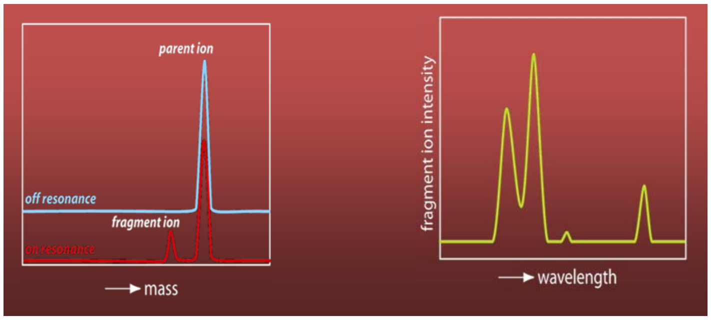
IR ion spectroscopy

- Infrared absorption
  = dissociation at the **weakest bond in molecular system** (independent of excited vibrational mode)
- Monitoring fragmentation as function of the wavelength yields IR spectrum



$$Yield = \frac{\sum I_{fragments}}{I_{parent} + \sum I_{fragments}}$$

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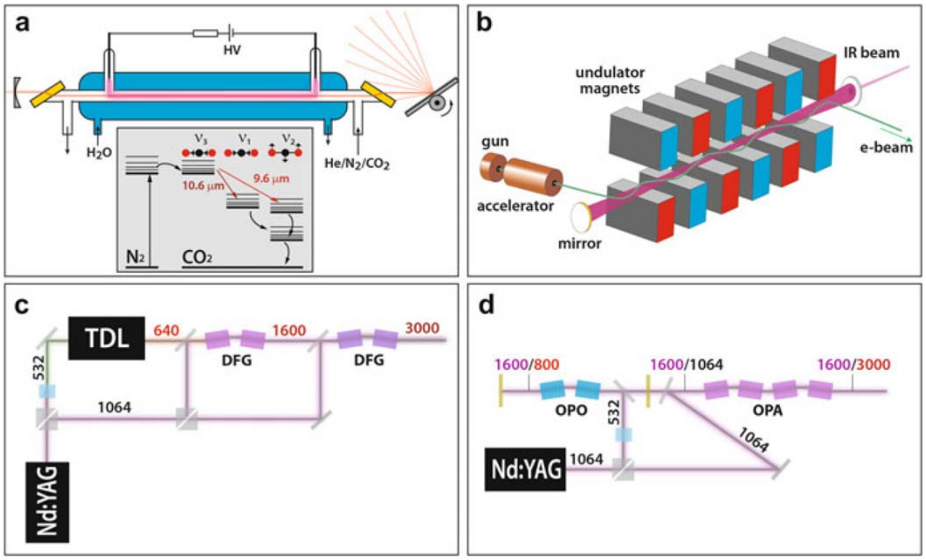
IR sources



Rijs and Oomens, Top. Curr. Chem. 364, 1-42 (2015)

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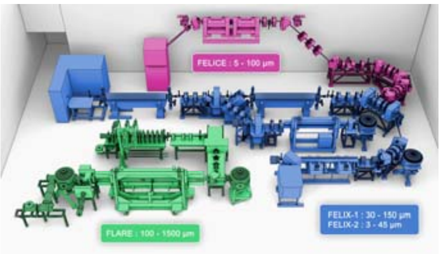
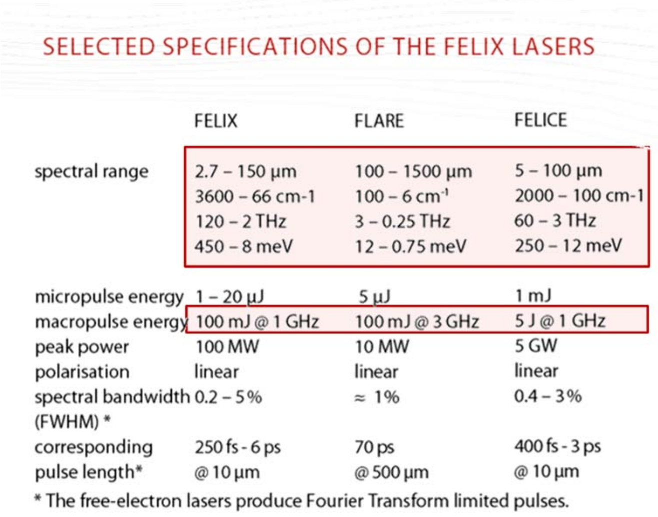
The free electron lasers at the FELIX laboratory





SELECTED SPECIFICATIONS OF THE FELIX LASERS

	FELIX	FLARE	FELICE
spectral range	2.7 – 150 µm	100 – 1500 µm	5 – 100 µm
	3600 – 66 cm-1	100 – 6 cm^{-1}	2000 – 100 cm-1
	120 – 2 THz	3 – 0.25 THz	60 – 3 THz
	450 – 8 meV	12 – 0.75 meV	250 – 12 meV
micropulse energy	1 – 20 µJ	5 µJ	1 mJ
macropulse energy	100 mJ @ 1 GHz	100 mJ @ 3 GHz	5 J @ 1 GHz
peak power	100 MW	10 MW	5 GW
polarisation	linear	linear	linear
spectral bandwidth (FWHM) *	0.2 – 5%	≈ 1%	0.4 – 3%
corresponding pulse length*	250 fs - 6 ps @ 10 µm	70 ps @ 500 µm	400 fs - 3 ps @ 10 µm

* The free-electron lasers produce Fourier Transform limited pulses.

Interested in beam time: www.ru.nl/felix

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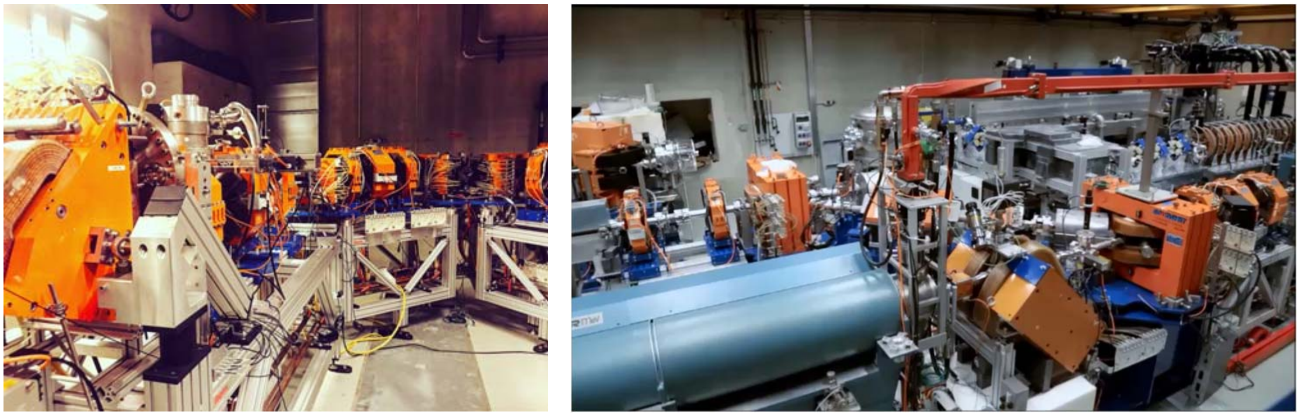
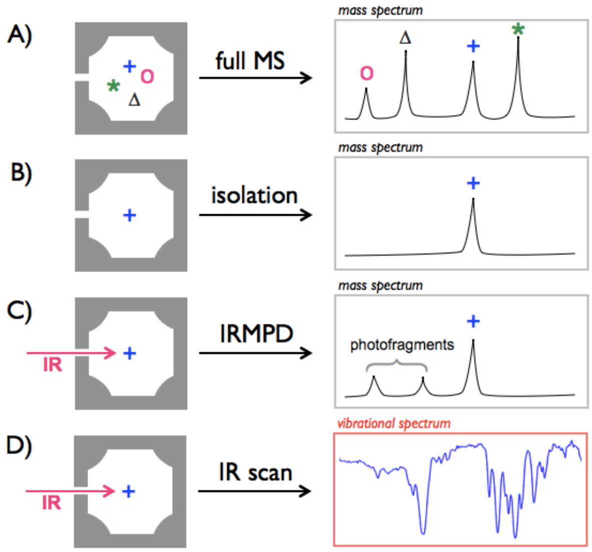
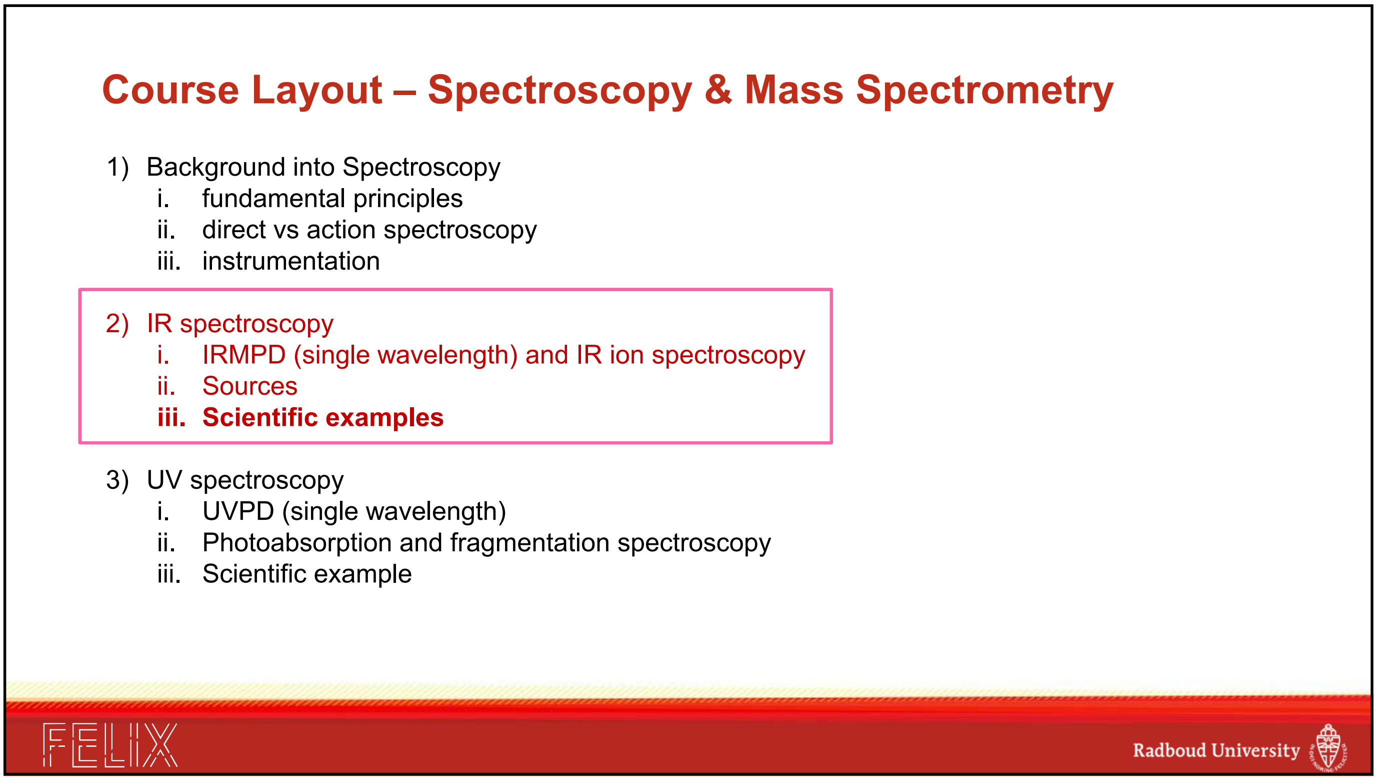
The FELIX Laboratory



Type of experiments





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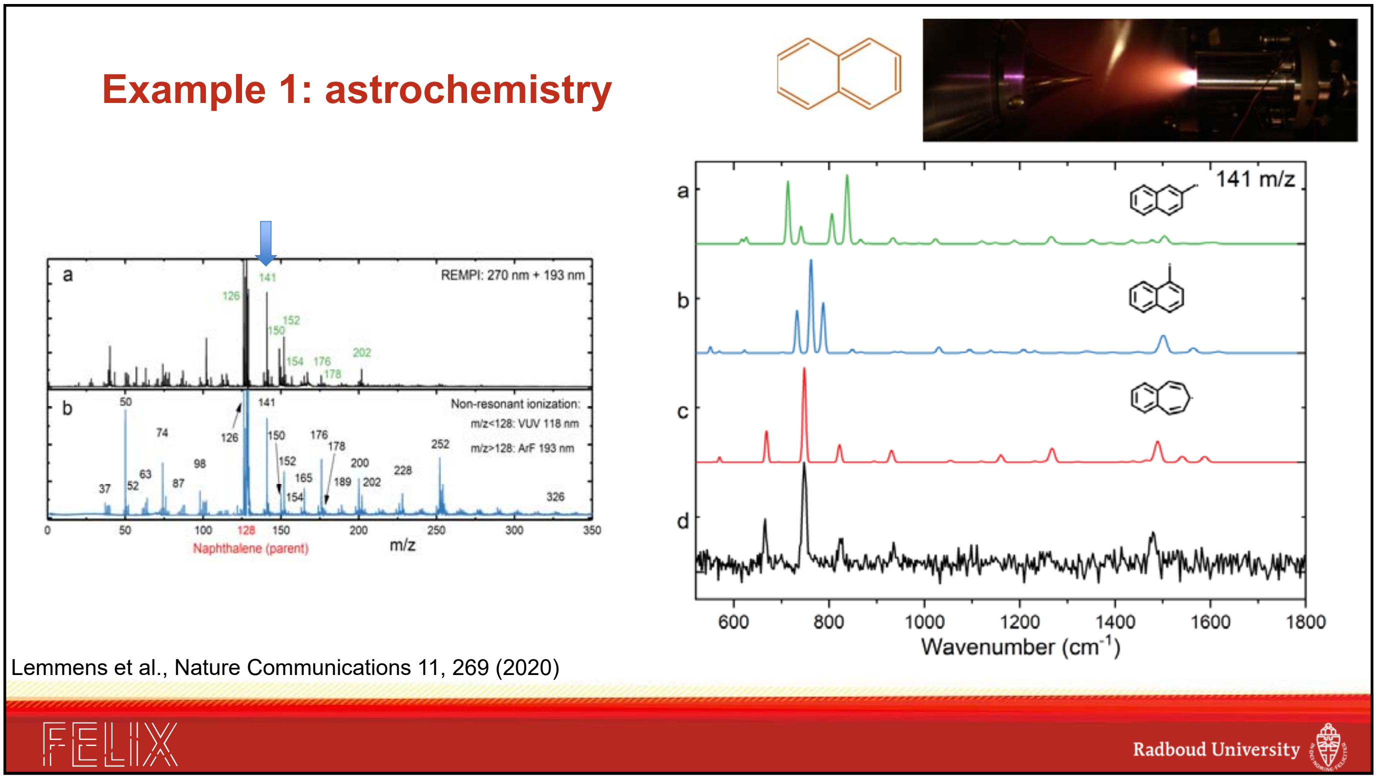


Example 1: astrochemistry

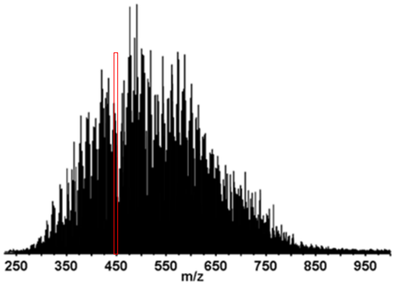
Lemmens et al., Nature Communications 11, 269 (2020)

Example 2: identification in complex mixtures

Metabolomics - profiling of small-molecule metabolites: **identification** and **quantitation**

Possible identification methods

- **NMR** has seen wide spread application
  = gives structure, no selectivity, low sensitivity
- **(LC)-Mass spectrometry**
  = mass selective and sensitive, no structures
- **IR spectroscopy**
  = Structurally diagnostic, not selective
- **Combine MS and IR**: IR spectra with selectivity and sensitivity of MS



Application of IR-MS to biomarker discovery in metabolomics

ARTICLES

nature genetics

NANS-mediated synthesis of sialic acid is required for brain and skeletal development

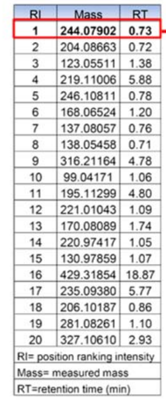
Clara D M van Karnebeek[1,2,28], Luisa Bonafé[3,28], Xiao-Yan Wen[4,5,28], Maja Tarailo-Graovac[2,6], Sara Balzano[7], Beryl Royer-Bertrand[3,7], Angel Ashikov[8], Livia Garavelli[9], Isabella Mammi[10], Licia Turolla[11], Catherine Breen[12], Dian Donnai[12], Valerie Cormier[13], Delphine Heron[13], Gen Nishimura[14], Shinichi Uchikawa[15], Belinda Campos-Xavier[3], Antonio Rossi[16], Thierry Hennet[17], Koroboshka Brand-Arzamendi[4,5], Jacob Rozmus[1], Keith Harshman[18], Brian J Stevenson[19], Enrico Girardi[20], Giulio Superti-Furga[20,21], Tammie Dewan[1], Alissa Collingridge[1], Jessie Halparin[1], Colin J Ross[1,2,6], Margot I Van Allen[6], Andrea Rossi[22], Udo F Engelke[23], Leo A J Kluijtmans[23], Ed van der Heeft[23], Herma Renkema[23], Arjan de Brouwer[24], Karin Huijben[23], Fokje Zijlstra[23], Thorben Heisse[25], Thomas Boltje[25], Wyeth W Wasserman[2,6], Carlo Rivolta[7], Sheila Unger[26], Dirk J Lefeber[8,23], Ron A Wevers[23,29] & Andrea Superti-Furga[3,27,29]

Patient and Controls

Next Generation Metabolic Screening Cerebrospinal fluid (CSF)

a

High-resolution QTOF results



RI	Mass	RT
1	**244.07902**	**0.73**
2	204.08663	0.72
3	123.05511	1.38
4	219.11006	5.88
5	246.10811	0.78
6	168.06524	1.20
7	137.08057	0.76
8	138.05458	0.71
9	316.21164	4.78
10	99.04171	1.06
11	195.11299	4.80
12	221.01043	1.09
13	170.08089	1.74
14	220.97417	1.05
15	130.97859	1.07
16	429.31854	18.87
17	235.09380	5.77
18	206.10187	0.86
19	281.08261	1.10
20	327.10610	2.93

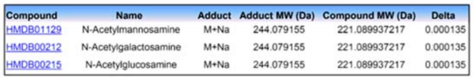
RI= position ranking intensity
Mass= measured mass
RT=retention time (min)

b

MS intensity plot of m/z 244.07902 in CSF of controls (blue) and the patient with NANS deficiency (red).

c

HMDB MS search results for 244.07902 m/z: multiple compounds possible

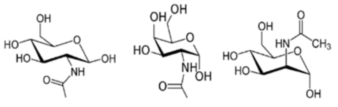


Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta
HMDB01129	N-Acetylmannosamine	M+Na	244.079155	221.089937217	0.000135
HMDB00212	N-Acetylgalactosamine	M+Na	244.079155	221.089937217	0.000135
HMDB00215	N-Acetylglucosamine	M+Na	244.079155	221.089937217	0.000135

Three database hits are indistinguishable by both CID MS/MS and LC retention time

Identification of inborn errors of metabolism

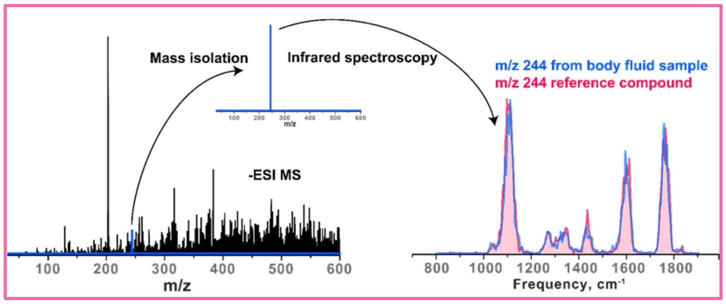
Possible database matches
identified by metabolic screening



N-Acetylglucosamine [M+Na]+ - m/z 244.07916
N-Acetylgalactosamine [M+Na]+ - m/z 244.07916
N-Acetylmannosamine [M+Na]+ - m/z 244.07916

~~MS~~, ~~MS/MS~~, ~~LC~~, NMR…

NMR undesirable:
Large sample volumes
Low sensitivity - only viable for few cases



Martens, et al., *Sci. Rep.* **2017**, *7*, 3363

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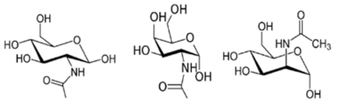
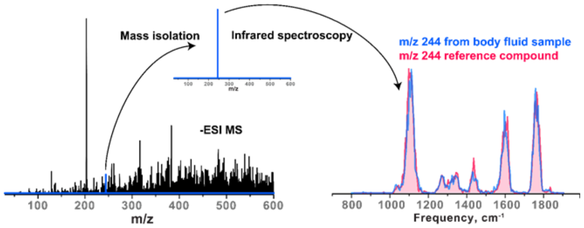
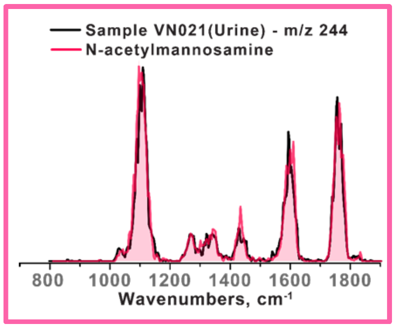
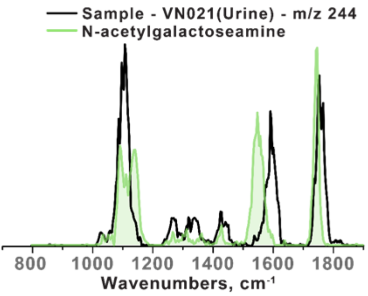
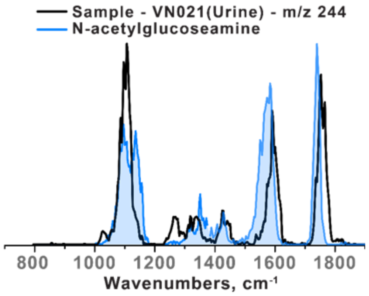


N-Acetylglucosamine [M+Na]+ - m/z 244.07916
N-Acetylgalactosamine [M+Na]+ - m/z 244.07916
N-Acetylmannosamine [M+Na]+ - m/z 244.07916









Martens, et al., *Sci. Rep.* **2017**, *7*, 3363

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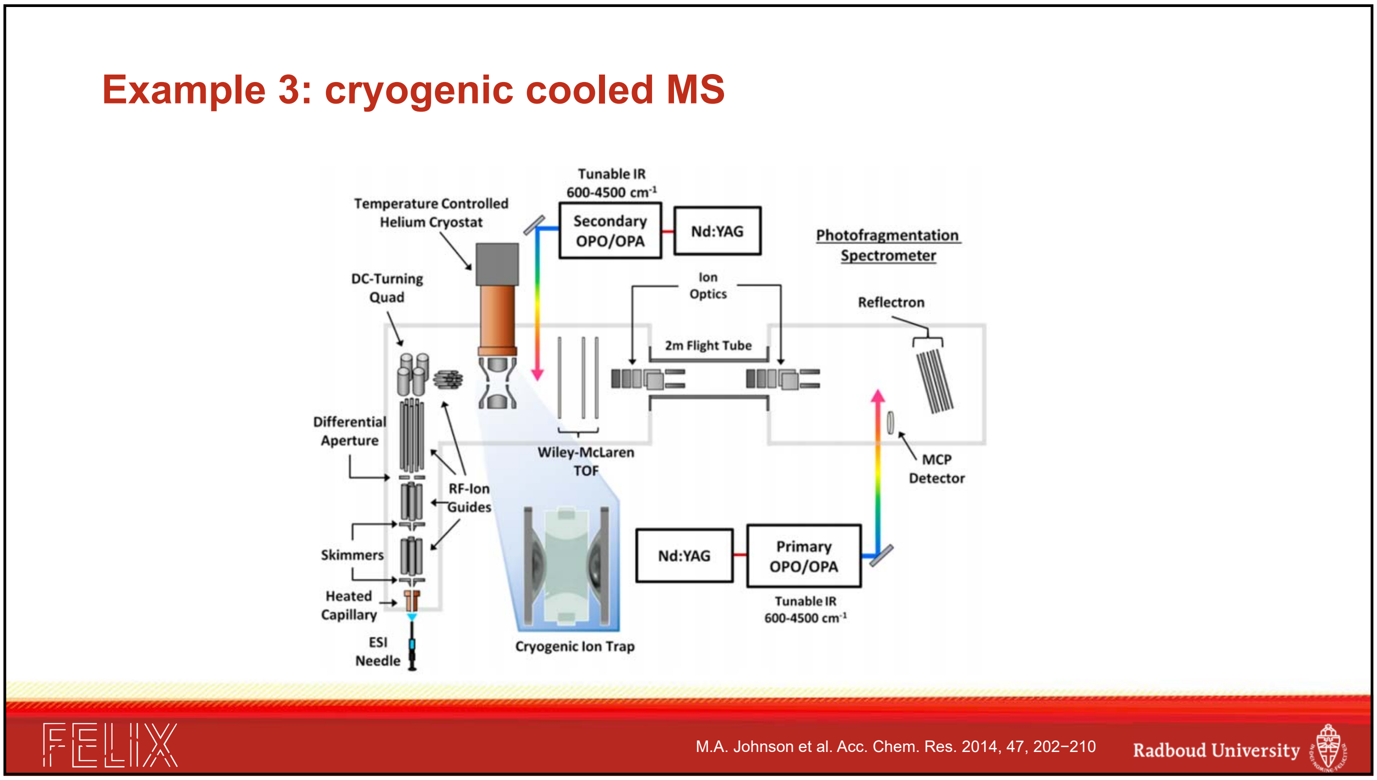
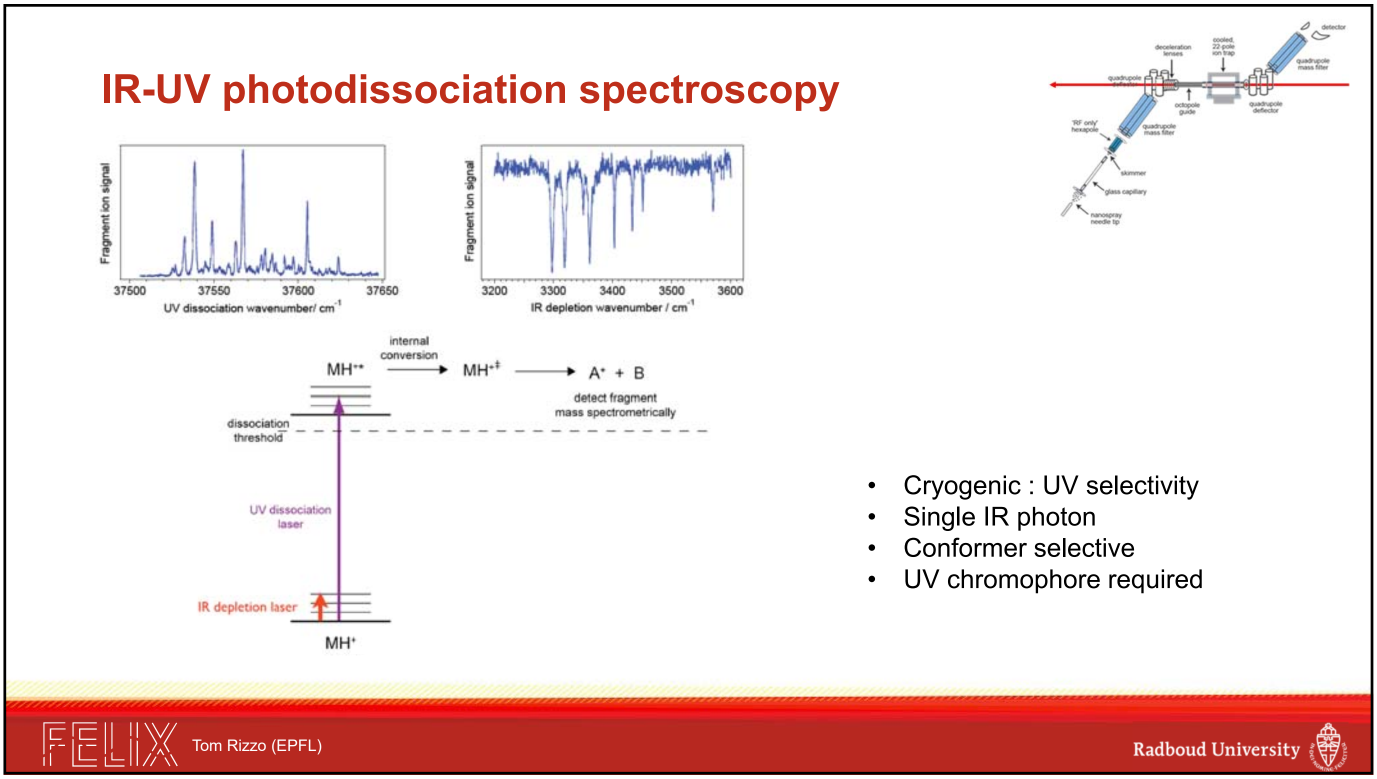


Example 3: cryogenic cooled MS

M.A. Johnson et al. Acc. Chem. Res. 2014, 47, 202–210



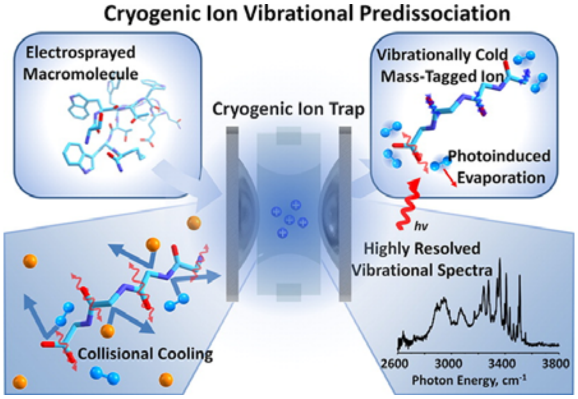
IR-UV photodissociation spectroscopy

- Cryogenic : UV selectivity
- Single IR photon
- Conformer selective
- UV chromophore required

Tom Rizzo (EPFL)

Messenger Spectroscopy – predissociation spectroscopy

- Tag is attached to analyte ions via weak vdWaals interactions at cryogenic temperatures
- the tag is detached upon resonant absorption of a photon by the analyte ion
- Tag: He, Ne, Ar, H_2, N_2
- temperatures depends on tag
  slightly stronger binding tags, less low temperatures
- tag can cause a distortion in the ions IR spectrum



methodology is applicable to any molecule, as IR modes of the molecule are probed

FELIX M.A. Johnson et al. Acc. Chem. Res. 2014, 47, 202−210 Radboud University

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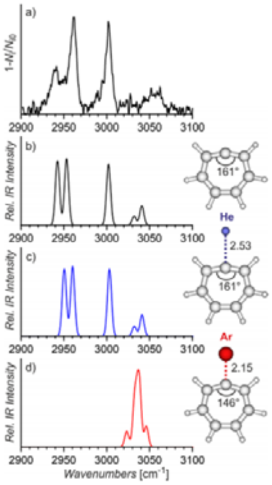
Messenger Spectroscopy – predissociation spectroscopy

- **The ideal tag:**
  - causes no to minimal distortion to IR response of ion
    - least polarizable and therefore the lowest binding energy
    - helium = ideal choice but challenging (ask Jana!), He tags at extremely low temperatures (<<10K)
  - Large enough mass difference
    - To be able to isolated m/z
    - the tagged ions are extremely fragile, any collisional activation will lead to loss of the tag
    - N_2 (28 Da) – easy mass ejection of the untagged ions and minimized activation of tagged ions.



methodology is applicable to any molecule, as IR modes of the molecule are probed

FELIX Roithová et al. Acc. Chem. Res. 2016, 49, 223−230 M.A. Johnson et al. Acc. Chem. Res. 2014, 47, 202−210 Radboud University

Course Layout – Spectroscopy & Mass Spectrometry

1) Background into Spectroscopy
    - i. fundamental principles
    - ii. direct vs action spectroscopy
    - iii. instrumentation

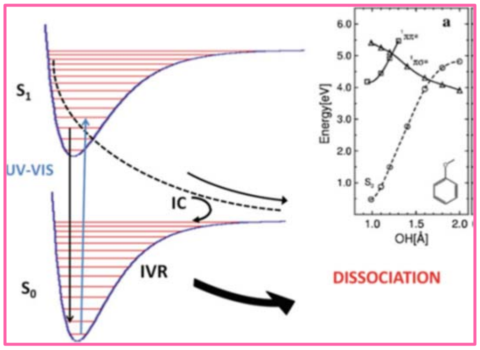
2) IR spectroscopy
    - i. IRMPD (single wavelength) and IR ion spectroscopy
    - ii. Sources
    - iii. Scientific examples

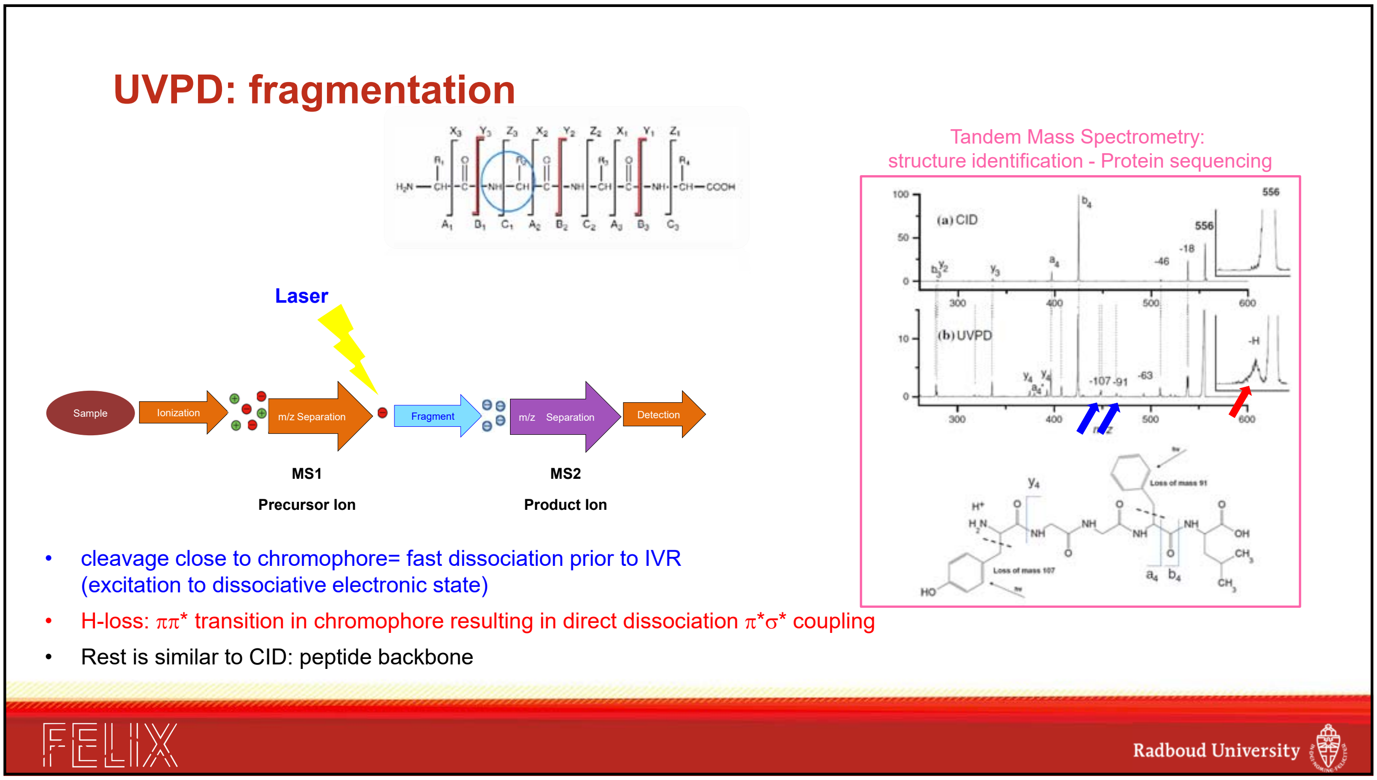
3) UV spectroscopy
    - **i. UVPD (single wavelength)**
    - **ii. Photoabsorption and fragmentation spectroscopy**
    - iii. Scientific example

Electronic Excitation – UV-VIS activation

UV-VIS : Electronic excitation of valence electrons

Linear excitation (absorption of single photon):
- Energy is well-defined in your system
- After population excited state, various de-excitation pathways:
    - Emission of a photon (fluorescence)
    - Internal Conversion (IC) from electronic energy to vibrational energy followed by IVR
    - Fragmentation in electronic excited state
    - Electron emission (depending on photon energy)





UVPD: fragmentation

Tandem Mass Spectrometry:
structure identification - Protein sequencing

- cleavage close to chromophore= fast dissociation prior to IVR (excitation to dissociative electronic state)
- H-loss: $\pi\pi^*$ transition in chromophore resulting in direct dissociation $\pi^*\sigma^*$ coupling
- Rest is similar to CID: peptide backbone

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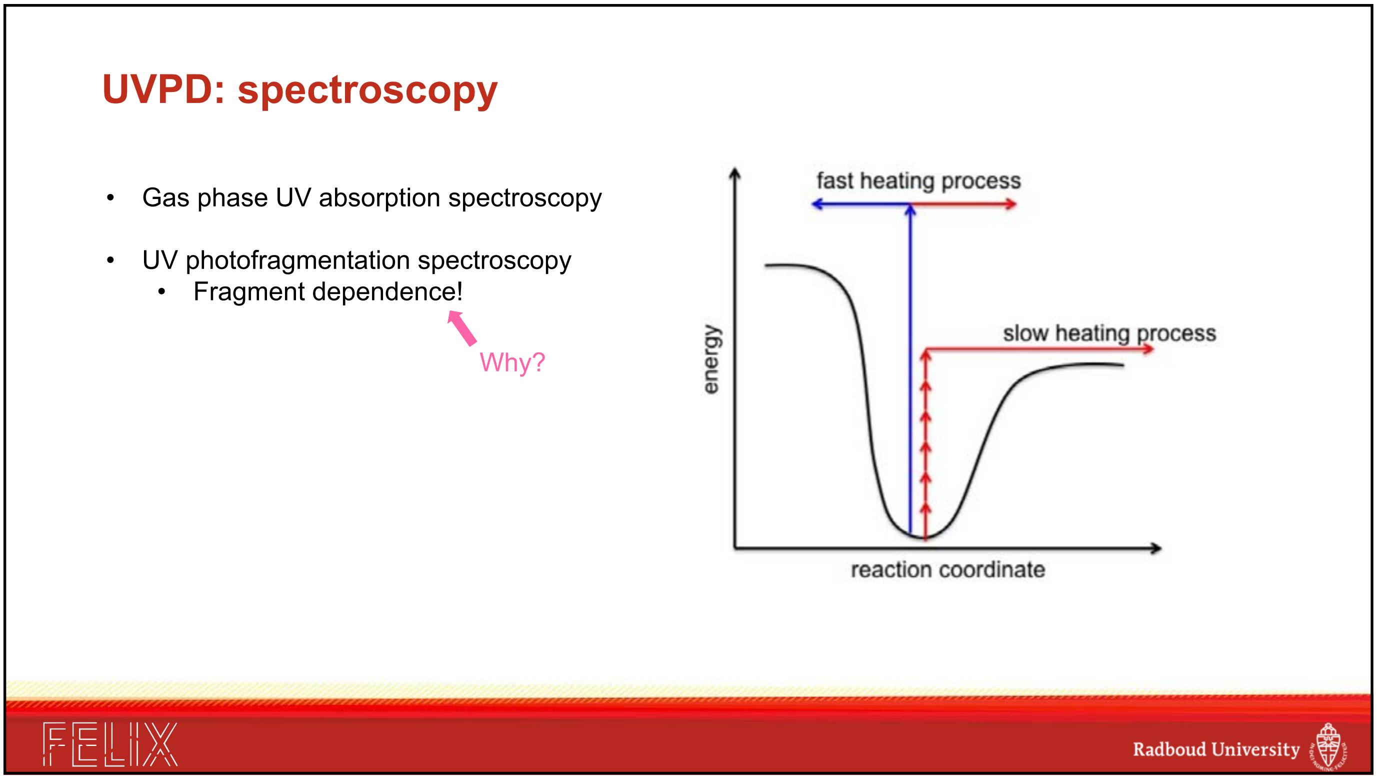

UVPD: spectroscopy

- Gas phase UV absorption spectroscopy
- UV photofragmentation spectroscopy
  - Fragment dependence!

Why?

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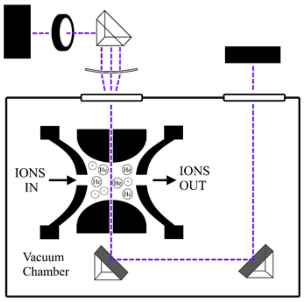
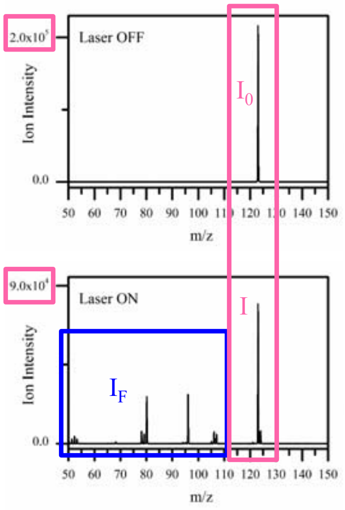
Laser-interfaced Mass Spectrometry



Typical UV sources

- Fixed wavelength:
  ArF (193 nm) F_2 (157 nm), solidstate (213 nm)
- Tunable:
  Nd:YAG pumped OPO
  (variable between 193 – 2700 nm)



Photodepletion (Absorption) (*PD*)

$$\boxed{PD = \frac{Ln\left(\frac{I_0}{I}\right)}{\lambda\, P}}$$

Photofragment Production (*PF*)

$$\boxed{PF = \frac{\left(\frac{I_F}{I_0}\right)}{\lambda\, P}}$$

λ is the wavelength
P is the average laser power

Set-up: Caroline Dessent (University of York-UK)

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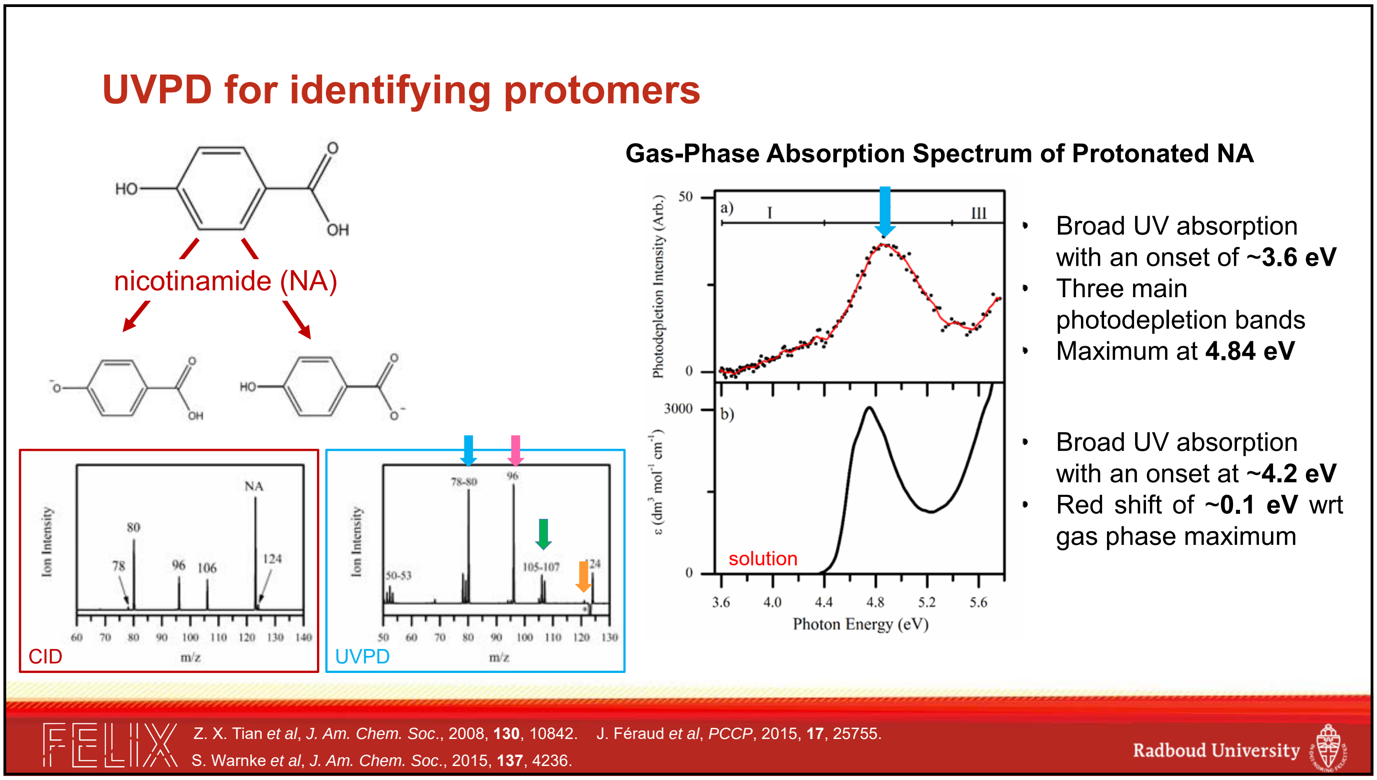

UVPD for identifying protomers

Gas-Phase Absorption Spectrum of Protonated NA

- Broad UV absorption with an onset of ~**3.6 eV**
- Three main photodepletion bands
- Maximum at **4.84 eV**

- Broad UV absorption with an onset at ~**4.2 eV**
- Red shift of ~**0.1 eV** wrt gas phase maximum

Z. X. Tian *et al*, *J. Am. Chem. Soc.*, 2008, **130**, 10842. J. Féraud *et al*, *PCCP*, 2015, **17**, 25755.
S. Warnke *et al*, *J. Am. Chem. Soc.*, 2015, **137**, 4236.

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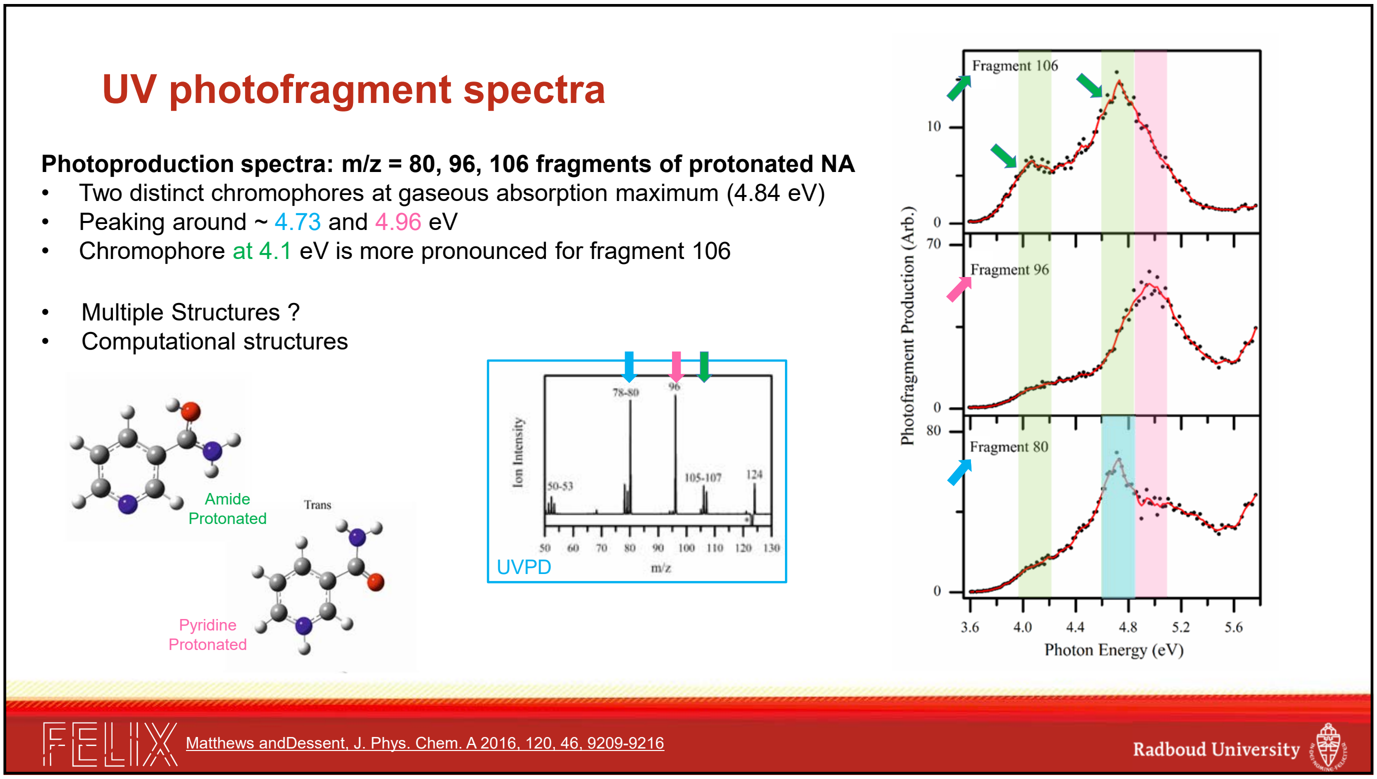

UV photofragment spectra

Photoproduction spectra: m/z = 80, 96, 106 fragments of protonated NA

- Two distinct chromophores at gaseous absorption maximum (4.84 eV)
- Peaking around ~ 4.73 and 4.96 eV
- Chromophore at 4.1 eV is more pronounced for fragment 106

- Multiple Structures ?
- Computational structures

Matthews andDessent, J. Phys. Chem. A 2016, 120, 46, 9209-9216

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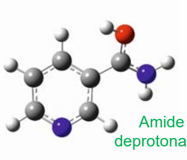
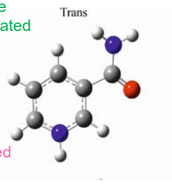
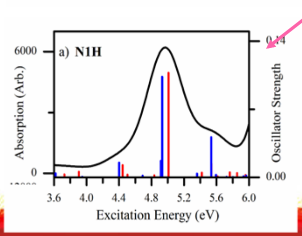
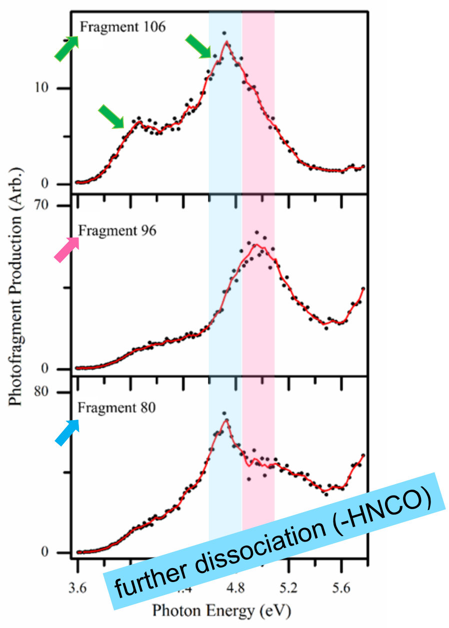

Amide deprotonated



Pyridine deprotonated





further dissociation (-HNCO)

Matthews andDessent, J. Phys. Chem. A 2016, 120, 46, 9209-9216

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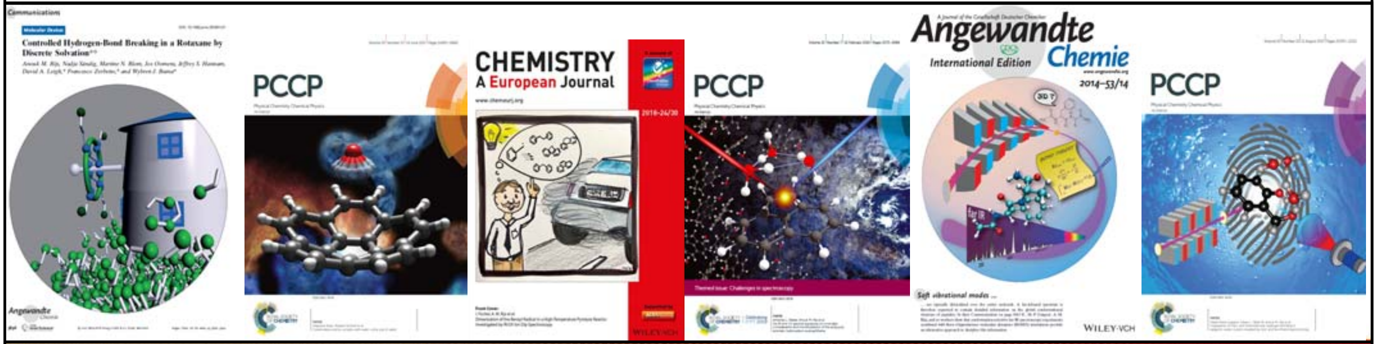
Summary

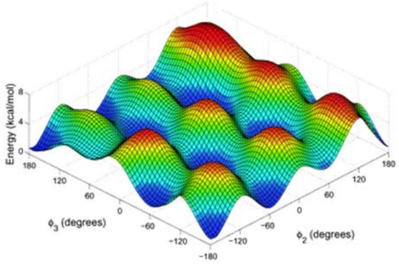
- Spectroscopy another orthogonal addition to mass spectrometry
- Spectroscopy provides both structural information as photochemical/photophysical insights
- Various types of IR and UV experiments

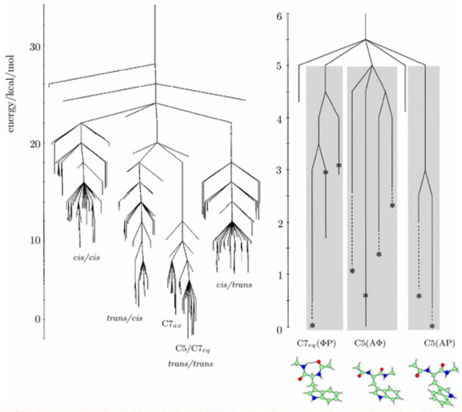


Structural assignment

Procedure:

- Extensive exploration of potential energy surface
- Set of possible low energy conformations





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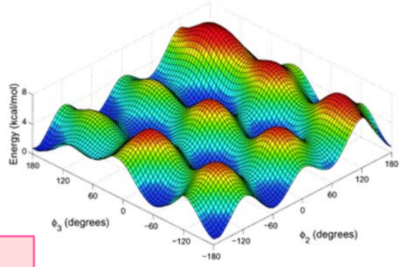
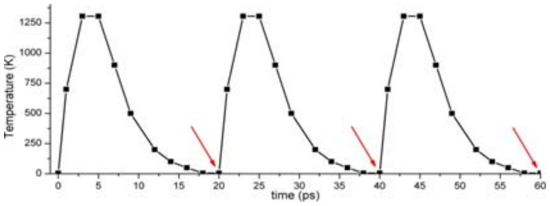
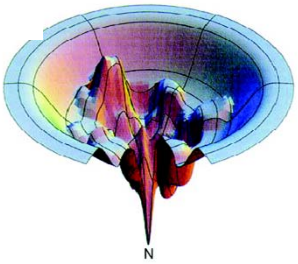


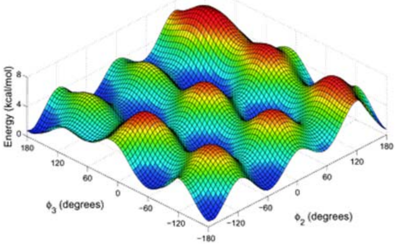
- Simulated annealing
  - Max T: 1300 K
  - Simulation time: 10 – 20 ns
  - # structures: 500 – 1000

Resulting structures:
Global and many local minima
on potential energy surface





Structural assignment



Procedure:

- Extensive exploration of potential energy surface
- Set of possible low energy conformations
- Calculate energy and vibrational frequencies

- Solve the Schrödinger Equation
- Energy Eigen values determine the vibrational frequencies
  - Born-Oppenheimer approximation
    - *Electronic terms to be considered for a fixed set of nuclear positions*
    - *Nuclear-nuclear potential calculated separately as a constant*

$$\begin{aligned} \psi(\mathbf{R},\mathbf{r}) &= \psi_e(\mathbf{R},\mathbf{r})\psi_n(\mathbf{R}) \\ H(\mathbf{R},\mathbf{r}) &= H_e(\mathbf{R},\mathbf{r}) + H_n(\mathbf{R}) \end{aligned}$$

$$\Bigg(\underset{= \text{neglegible}}{\cancel{-\sum_j \frac{\hbar^2}{2M_j}}}\nabla^2_{\mathbf{R}_j} - \sum_k \frac{\hbar^2}{2m_e}\nabla^2_{\mathbf{r}_k} - \sum_j \sum_k \frac{Z_j e^2}{4\pi\varepsilon_0 |\mathbf{R}_j - \mathbf{r}_k|^2}$$

$$+ \frac{1}{2}\sum_k \sum_{k' \neq k} \frac{e^2}{4\pi\varepsilon_0 |\mathbf{r}_{k'} - \mathbf{r}_k|^2} + \underset{= \text{constant}}{\cancel{\frac{1}{2}\sum_j \sum_{j' \neq j} \frac{Z_j Z_{j'} e^2}{4\pi\varepsilon_0 |\mathbf{r}_{j'} - \mathbf{r}_j|^2}}} \Bigg) \psi = E\psi$$

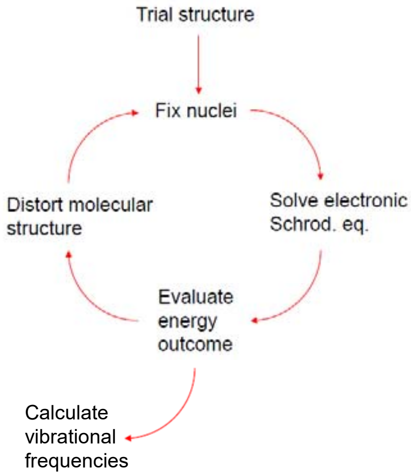
Structural assignment

Procedure:

- Extensive exploration of potential energy surface
- Set of possible low energy conformations
- Calculate energy and vibrational frequencies

- Solve the Schrödinger Equation
- Energy Eigen values determine the vibrational frequencies
  - Born-Oppenheimer approximation
    - *Electronic terms to be considered for a fixed set of nuclear positions*
    - *Nuclear-nuclear potential calculated separately as a constant*

- Construct trial wavefunction of molecular orbitals, solve SE via variational principle
- Approximation of electron-electron correlation
  - *Hatree-Fock based: MP2*
  - *Wavefunction based: DFT*
- Commercial packages
  - *Gaussian, Turbomole, ADF*



Trial structure

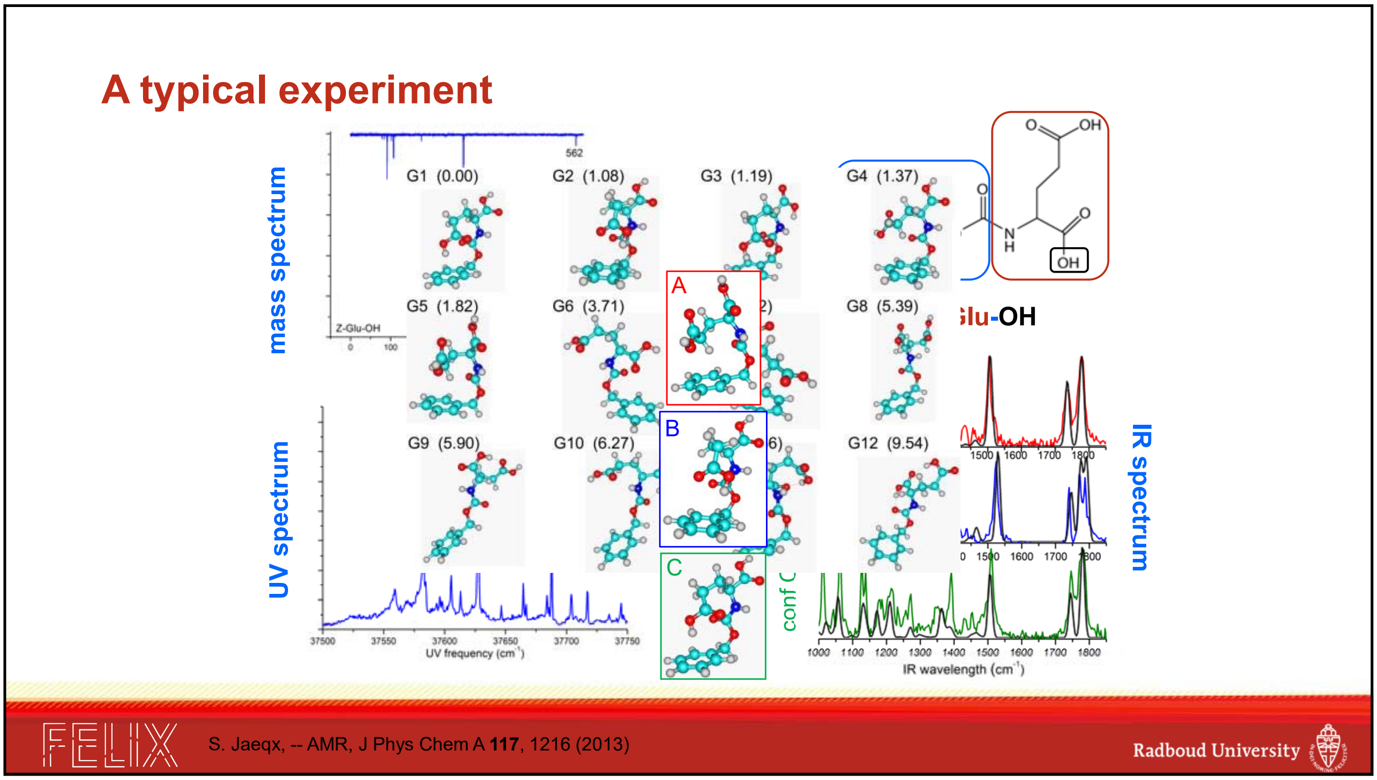
Fix nuclei

Solve electronic Schrod. eq.

Evaluate energy outcome

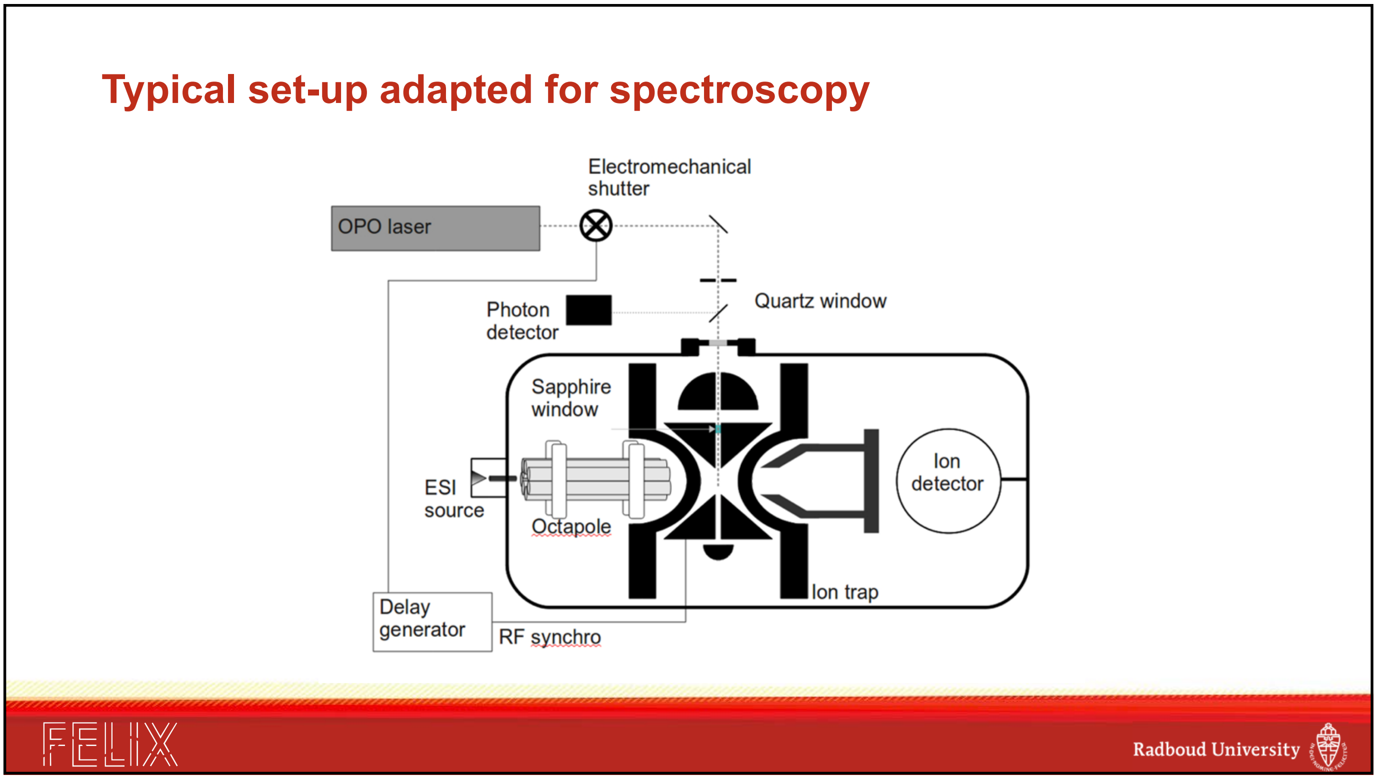
Distort molecular structure

Calculate vibrational frequencies



A typical experiment

S. Jaeqx, -- AMR, J Phys Chem A **117**, 1216 (2013)



Typical set-up adapted for spectroscopy