Gas Phase IR spectroscopy:

towards large biomolecules
&
far-IR wavelengths

Anouk M. Rijs

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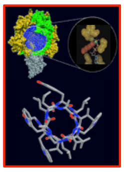
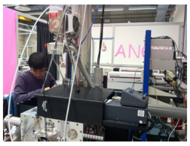
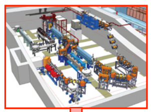
Institute for Molecules and Materials

FELIX Facility

Molecular and Biophysics







Layout topics to discuss

Introduction
- Why, o why?
- What & how?

Experimental methods
- Cooling methods
  - Why cooling
  - Supersonic expansions
  - Collisional cooling
- Molecule production --> larger & larger
  - heating
  - Laser desorption
  - Molecular beams & laser desorption

Spectroscopic Techniques
- UV + mass selective detection
- IR
- Table top IR vs Free electron lasers
- Combined methods:
  - IR-UV, UV-UV
  - Combination with VUV sources

Applications
- Molecular motors: functioning of rotaxanes
- Biomolecules
  - Local environment
  - Biological environment
  - Larger and larger….

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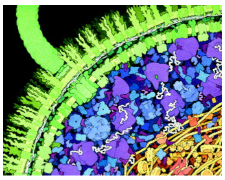
Biomolecules in the gas phase: **Why??**

The biological medium is very complex
- Molecular crowding

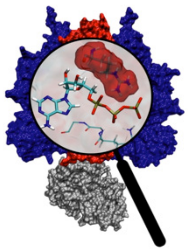


cross-section of part of E.coli cell

Questions that arise…

- Who does what?
- Does it do it by itself?
- Can the environment affect the molecule's role or functioning
- What can go wrong?
  car engine - living system
  gears - isolated molecule
- What are the minimum requirements to do the job?

Biomolecules in the gas phase: **Why??**

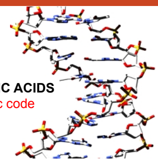


If we want to **understand the details** how the whole thing function we need to **undertand its components**

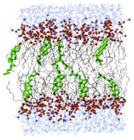
The **best environment** to study intrinsic properties of a molecular system* is **in vacuo** (no interactions, no collisions)

*(of limited size)

The four families of biomolecules

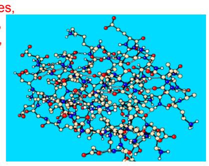


NUCLEIC ACIDS
• genetic code

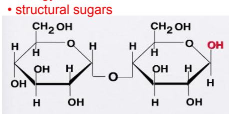


LIPIDS
• energy storage
• cells membranes

PROTEINS
• body structure
• functions : enzymes, hormones, poisons, transport, digestion, immunity



CARBOHYDRATES
• energy source
• structural sugars



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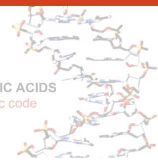

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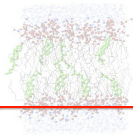

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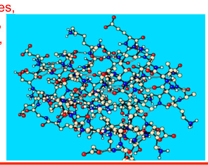

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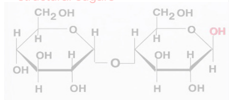
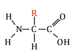
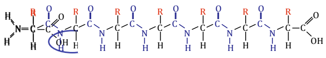


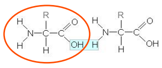
The structure of peptides

amino acid ………………….. dipeptide …………………..… polypeptide

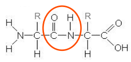




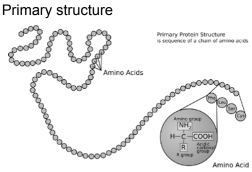
Peptide formation



Peptide bond



Primary structure



3D structure and function

Primary structure
linear sequence of amino-acids

1 KIPNFVVPGK CASVDRNKLW AEQTPNRNSY AGVWYQFALT
51 VRNEYSFDGK QFVIKSTGIA YDGNLLKRNG KLYPNPFGEP HLSIDYENSF
101 AAPLVILETD YSNYACLYSC IDYNFGYHSD FSFIFSRSAN LADQYVKKCE
151 AAFKNINVDT TRFVKTVQGS SCPYDTQKTV

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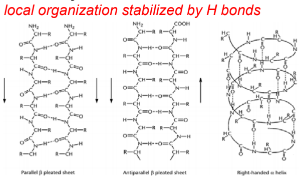
Secondary structure
local organization stabilized by H bonds



Early folding events: femto to microseconds

3D structure and function

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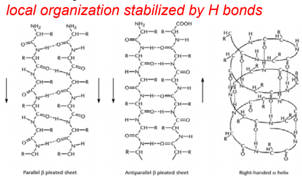
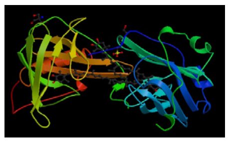


Tertiary structure
3 dimensional form "native protein

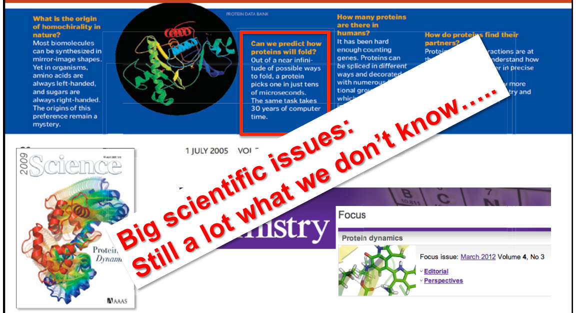


5 helices
9 beta strands
22 reverse turns
(3 disulfide bonds)

The function can only be carried out if the protein is in its native structure

Early folding events: femto to microseconds   folding in 3D structure: ~ 1 sec.

Protein folding & protein dynamics

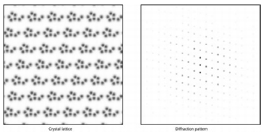


Understanding conformations

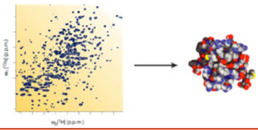
Nobel prize 1972: **Afinsen**, Moore and Stein

Methods to study : static

X-ray crystallography : *myoglobin 1959*



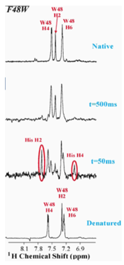
Nuclear magnetic resonance : *1984*



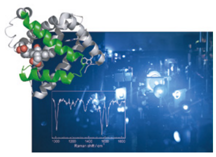
10 % of 100,000 human proteins

Methods to study : dynamic

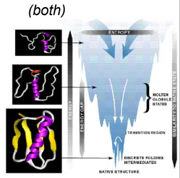
real time NMR→ slow dynamics



Time-resolved methods → fast dynamics

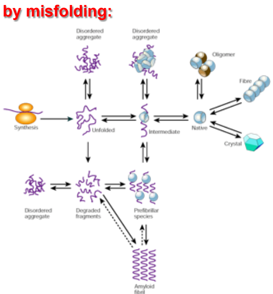


Theoretical studies *(both)*



Methods to study : conformation selective

diseases induced by misfolding:



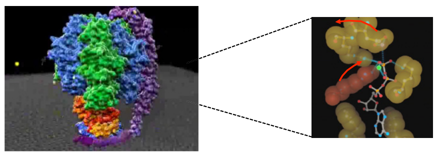
conformation selective processes + local information

Flexibility and function

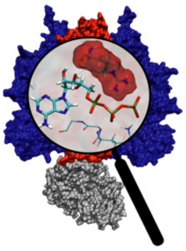
Biomolecular motors

⇨ Biomolecular motion induced by conformational changes at the active site

⇨ Initiated by ATP association and hydrolysis



Biomolecules in the gas phase: **Why??**



If we want to **understand the details** how the whole thing function we need to **undertand its components**

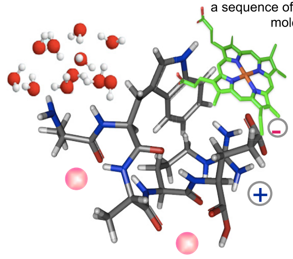
The **best environment** to study **intrinsic properties** of a molecular system* is **in vacuo** (no interactions, no collisions)

*(of limited size)

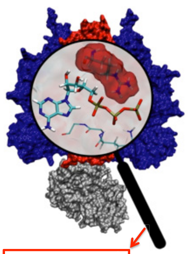
Methods to study : conformation selective

HYPOTHESIS

a complex biological process
=
a sequence of unit processes at the molecular level



Biomolecules in the gas phase: **What can we learn??**



IR spectroscopy

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Conformation selective & local information

Who does what

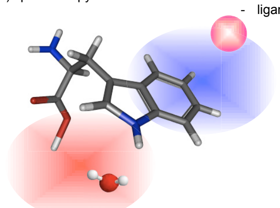
Influence local and biological environment

*(of limited size)

Conformation selective spectroscopic techniques

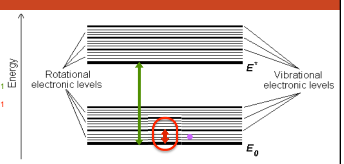
methods
Gas phase spectroscopy
Molecular beams = neutral molecules
Mass spectrometry (neutrals/ions)
Electronic (UV) spectroscopy
Vibrational (IR) spectroscopy

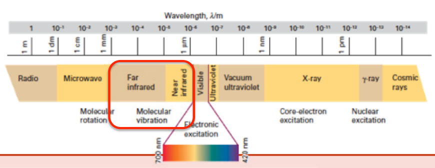
objects
isolated biomolecules
biomolecular interactions
- neutrals vs local charges
- water
- metals
- ligands



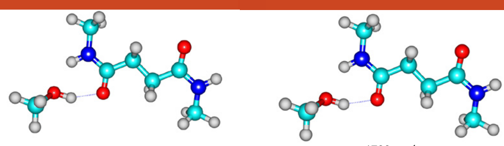
IR Spectroscopy

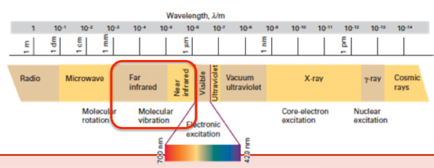
Orders of energies:
$E_1 \leftarrow E_0$ 10^{-18} J /10 eV / ± 80 000 cm^{-1}
$v+1 \leftarrow v$ 10^{-20} J /100 meV / ± 800 cm^{-1}
$J+1 \leftarrow J$ 10^{-22} J /1 meV / ± 8 cm^{-1}





IR Spectroscopy





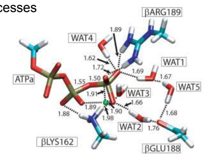
What can gas phase IR spectroscopy tell?

⇨ - direct view on **hydrogen bond interactions** (shift in IR frequency)

- provides detailed structural information
- influence of local and biological environment
- + calculations: reveal the secondary structure

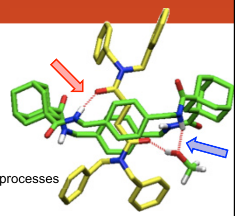
hydrogen bonding plays a central role in all biological processes

- non covalent interaction between polar groups in a molecular system
  - Hydrogen bonding
  - Dispersion interaction
  - Charge – dipole interaction (metal ion)
  - Salt bridges (zwitterions)



Hydrogen bonding

- **INTRA**molecular H-bond
- **INTER**molecular H-bond



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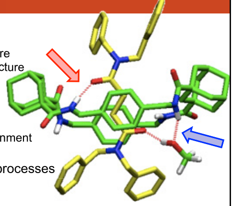
Hydrogen bonding

- **INTRA**molecular H-bond
  - *structure stabilization* { conformational structure, folding into native structure
- **INTER**molecular H-bond
  - *promotes interactions* { molecular recognition, adaptation to the environment



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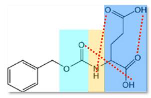
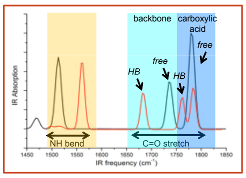
What does an IR spectrum tell you: H-bonding!

IR spectroscopy direct view on the hydrogen bond network present in your molecule



Amide I - C=O stretch: 1700-1800 cm^{-1}
Amide II – NH bend: 1500 cm^{-1}



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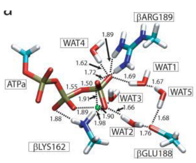

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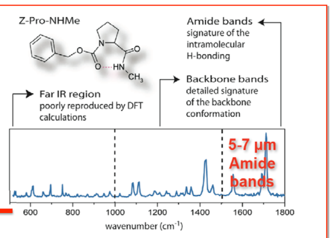
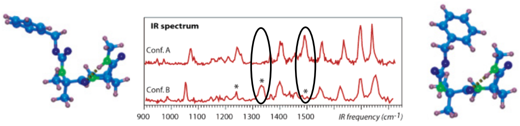


FIR / THz
collective motions

Example

- direct view on **hydrogen bond interactions** (shift in IR frequency)

⇨ - provides detailed structural information



two conformations of a capped di-peptide
distinguished by gas phase IR-UV spectoscopy

What can gas phase IR spectroscopy tell?

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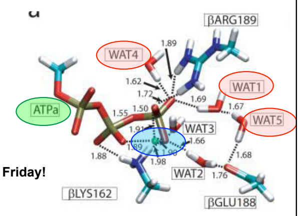
⇨ - influence of local and biological environment

- local: neigbouring amino acids
- biological:
  - water
  - metal ions /atoms
  - other molecules



Discuss in more detail on Thursday / Friday!

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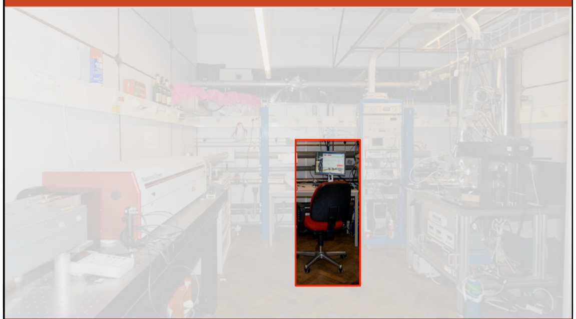
theoreticians toolbox



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Spectroscopist's toolbox

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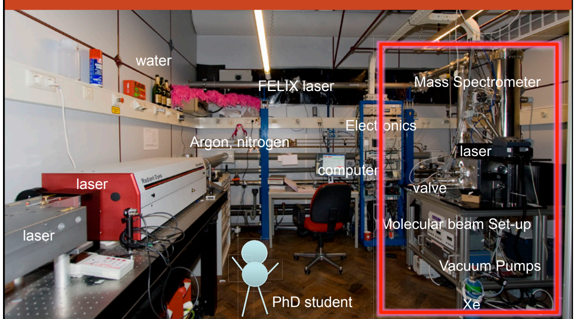
Spectroscopists toolbox



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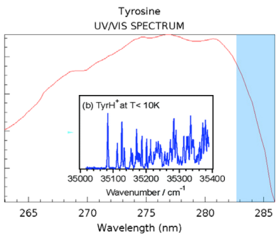
Cooling molecules

As you know...
Biological processes take place at room temperature or in our case at 37ºC

Question:
Why do we -spectroscopists- want to cool down molecules (or ions) to temperatures down to a few Kelvin??

Cooling molecules

Question:
Why do we -spectroscopists- want to cool down mocules (or ions) to temperatures down to a few Kelvin??



JACS 128, p. 2816, 2006

Cooling molecules

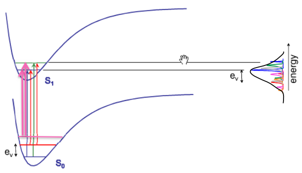
As you know...
Biological processes take place at room temperature or in our case at 37ºC

Question:
Why do we -spectroscopists- want to cool down molecules (or ions) to temperatures down to a few Kelvin??

Answer:
To be able to analyze the spectra !!

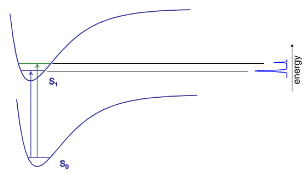
Cooling molecules

How does cooling simplify spectra?



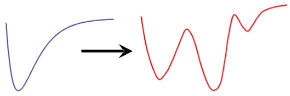
Cooling molecules

How does cooling simplify spectra?



Cooling molecules

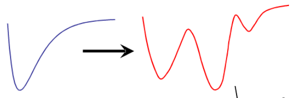
Besides simplifying the spectra, what are the effects of cooling of large biomolecules



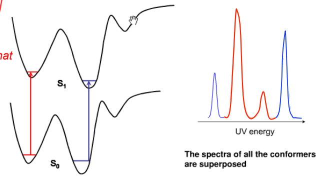
Biomolecules are large, floppy molecules that can adapt several conformations

Cooling molecules

Besides simplifying the spectra, what are the effects of cooling of large biomolecules

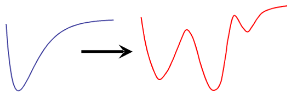


Biomolecules are large, floppy molecules that can adapt several conformations



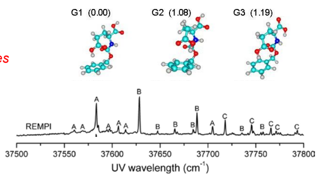
Cooling molecules

Besides simplifying the spectra, what are the effects of cooling of large biomolecules



Biomolecules are large, floppy molecules that can adapt several conformations

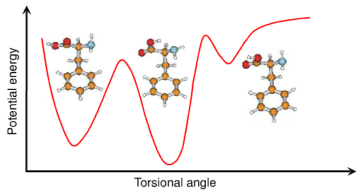
UV-UV hole-burning spectroscopy
to locate origins of conformers



Cooling molecules

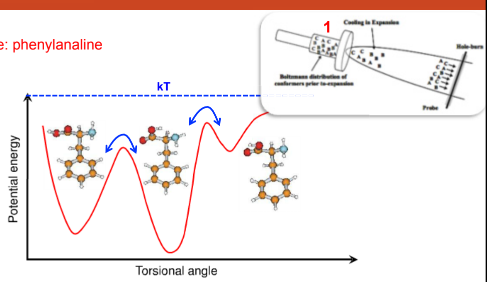
Example: phenylanaline

Considering pro vs pre expansion conditions
- Average internal energy prior to expansion
- Cooling rate
- Relative energy of minima
- Barrier heights



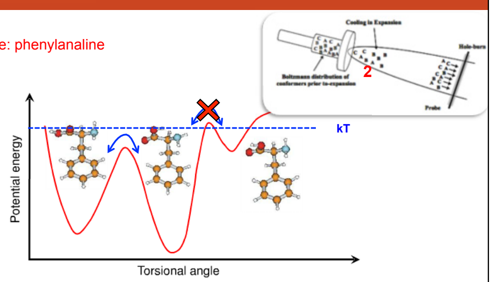
Cooling molecules

Example: phenylanaline



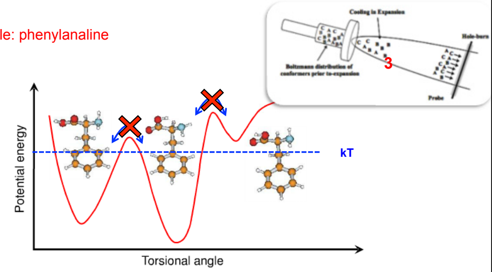
Cooling molecules

Example: phenylanaline



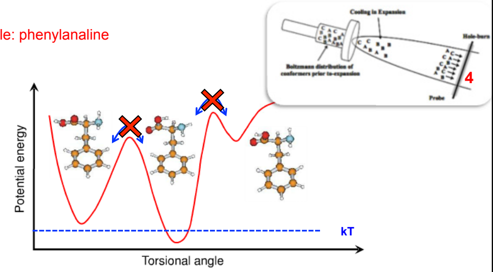
Cooling molecules

Example: phenylalanine



Cooling molecules in a fast refrigerator ?!

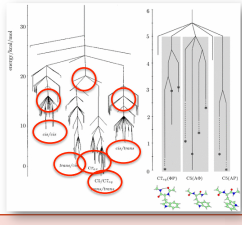
Example: phenylalanine



Cooling fast = population pro-expansion = pre-expansion

Cooling molecules

- When one studies cold molecules, it may or may not be the same systems as the ones at room temperature
- Depending on the cooling rate, we can end up with different conformational distributions

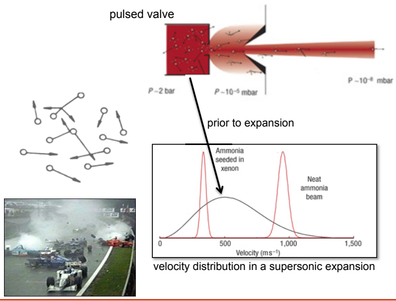


Nodal points

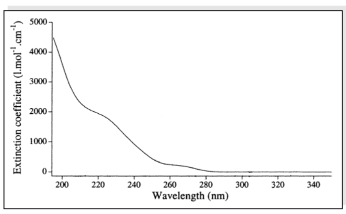
Conformational minima

164 (local) minima
714 transition states

Supersonic expansion as a fast refrigerator



Cold & Isolated Molecules

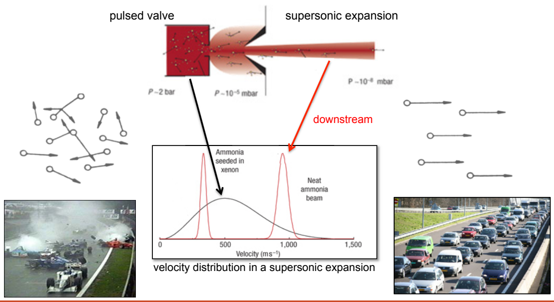


Thermal energy
→ molecules in different energy levels

Intermolecular interactions
→ energy levels for each molecule different

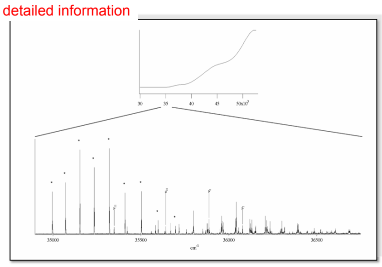
F. Zerbetto *et al.*, *J. Am. Chem. Soc.* 102, 149 (2002)

Supersonic expansion as a fast refrigerator



Cold & Isolated Molecules

Unveiling detailed information

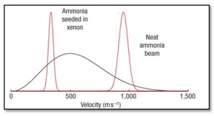


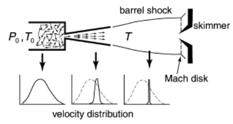
F. Zerbetto *et al.*, *J. Am. Chem. Soc.* 102, 149 (2002)

Supersonic Expansion as a fast refrigerator

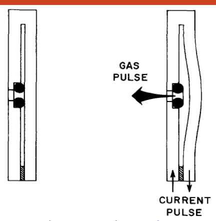
Supersonic expansions

- Internal energy is converted to kinetic energy
  - Nearly monokinetic beam
  - Cooling of internal degrees of freedom
  - Carrier gas acts as a cooling bath for impurities (= molecules of interest)
- Zone of silence
  - No interference with background gas
  - Molecules studied in collision free regime
- High collision rate
  - Formation and stabilization of weakly bound complexes
    - *Water clusters, metal ions, etc*



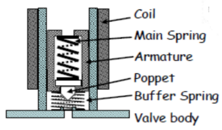


Used types of valves



Precursor to the present-day *Jordan valve*: based on the principle of magnetic repulsion between two metal strips through which a large opposing current (3-5 kA) was pulsed.

W. R. Gentry and C. F. Giese, Rev. Sci. Instrum. **49**, 595 (1978)



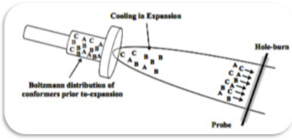
General Valve

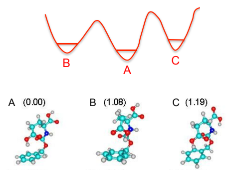
magnetically activated plunger valve

L. Abad, D. Bermejo, V. J. Herrero, J. Santos, and I. Tanarro, Rev. Sci. Instrum. 66, 3826 (1995)

Supersonic Expansion applied to flexible biomolecules

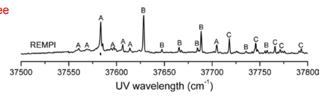
Cool conformationally flexible molecules into the zero-point levels of low energy conformations



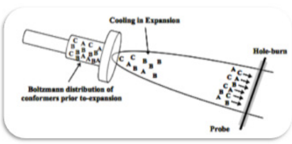


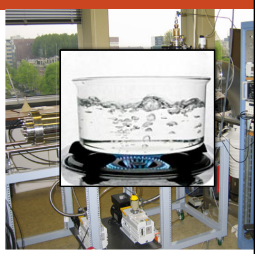
Spectroscopic challenge: Record the IR and UV spectra of single conformations! Free from interference from one another!

IR-UV ion dip spectroscopy



Biomolecules in the gas phase: heating





Heating:
- volatile
- stable
- up to 300 C

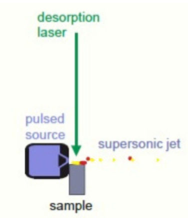
- limited
- fragmentation?

From the 20 natural amino acids, only one (maybe two) possible to study via heating

Laser desorption

Involatile biomolecules

Laser desorption combined with jet cooling

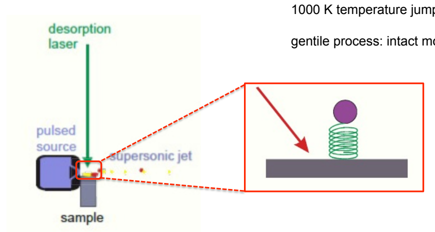


= Laser desorption

Laser desorption combined with jet cooling

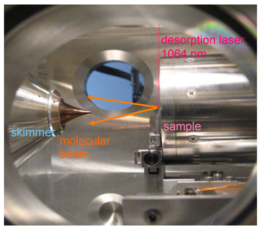
1000 K temperature jump in 10 ns

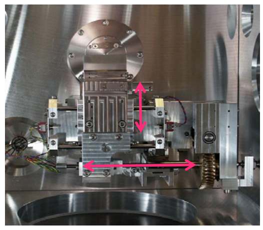
gentile process: intact molecules



Experimental Method: molecular beam + laser desorption

laser desorption:
Creation gaseous molecules





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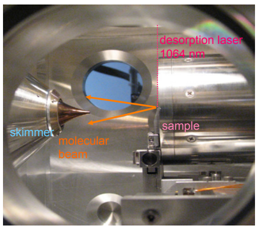
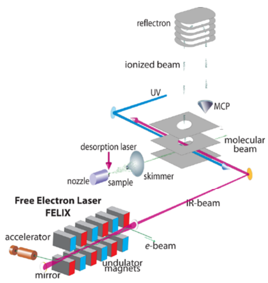
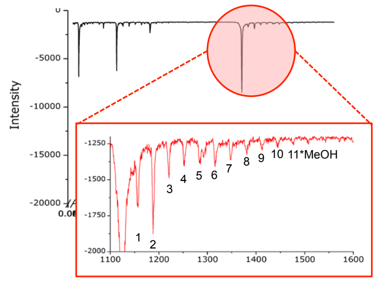
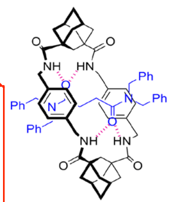




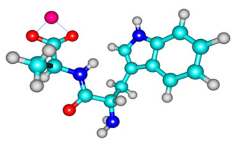
Controlled addition of the environment: solvent

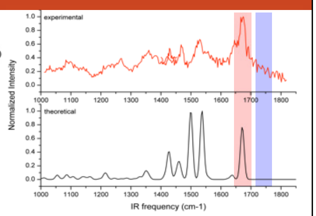




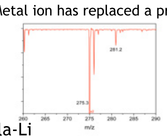
neutral rotaxanes

Controlled addition of the environment- metal ions





Mass spectra:
Metal ion has replaced a proton



TrpAla-Li

IR-UV spectra:
Carboxylic C=O is missing
Gas phase salt

Layout

Introduction
- Why, o why?
- What & how?

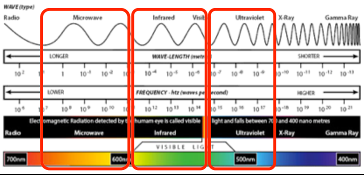
Experimental methods
- Cooling methods
  - Why cooling
  - Supersonic expansions
  - Collisional cooling
- Molecule production --> larger & larger
  - heating
  - Laser desorption
  - Molecular beams & laser desorption

Spectroscopic Techniques
- ~~History~~
- Use of lasers
- Experimental approach
  - UV, IR spectroscopy
- Free electron lasers
- Other methods:
  - IR-UV, UV-UV
  - Combination with VUV sources

Applications
- Molecular motors: functioning of rotaxanes
- Biomolecules
  - Local environment
  - Biological environment
  - Larger and larger….

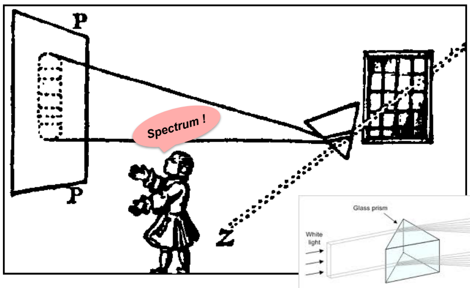
Spectroscopy

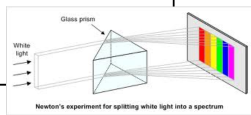
Interaction of molecules / atoms with electromagnetic radiation



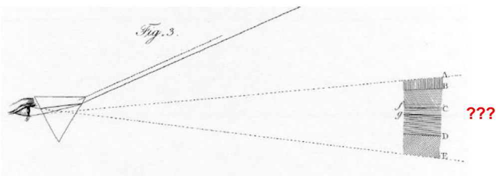
unity	microwave	IR	Vis/UV
wavelength	1 m - 1 mm	1 mm - 750 nm	750 nm - 390 nm / 390 -100 nm
Wavenumbers (cm^{-1})	10^{-2} - 10	10 - 10^4	10^4 - 10^6
Frequency (Hz)	300 MHz - 300 GHz	300 GHz - 405 THz	405 THz - 790 THz - 30 PHz
Energy (J)	10^{-25} - 10^{-22}	10^{-22} - 10^{-19}	10^{-19} - 10^{-17}
Energy (eV)	1,24 µeV - 1,24 meV	1,24 meV - 1,7 eV	1,7 eV - 3,3 eV - 124 eV

History of Spectroscopy: Newton – the year 1666



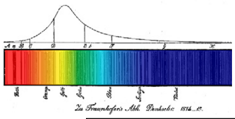


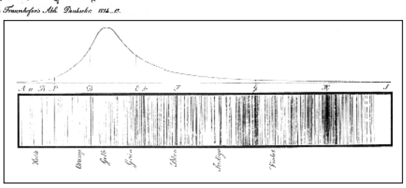
History of Spectroscopy: Wollaston – the year 1802



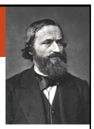
First observation of dark lines in the solar spectrum

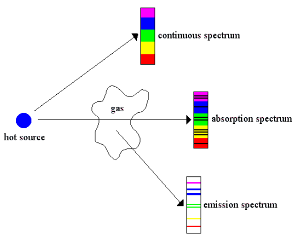
History of Spectroscopy: Fraunhofer – the year 1814





History of Spectroscopy: Kirchhoff's law - 1859



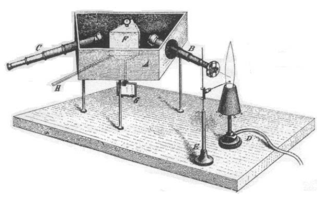


1) Body irradiates
--> continuous spectrum

2) passes through a gas
--> absorption spectrum with specific lines removed

3) Radiating gas
--> emission spectrum of discrete lines

History of Spectroscopy: Kirchhoff & Bunsen - 1860



Spectroscopy in 2012

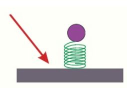
the lab …

Rijnhuizen

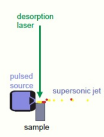
Nijmegen

Modern experimental approach

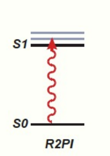
Laser desorption



Jet cooling

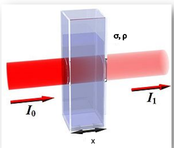


UV (or IR) spectroscopy

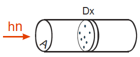


✔ (1) Pick them up ✔ (2) Put them together (3) Hold them up against the light

Classical spectroscopy



Beer's law



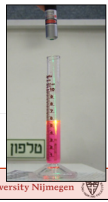
$$\Delta I = -\frac{\sigma}{A} N I = -\sigma \frac{N}{A \cdot \Delta x} \Delta x \cdot I = -\sigma \frac{N}{V} \Delta x \cdot I = -\sigma \rho \Delta x \cdot I$$

$$\rightarrow \frac{\Delta I}{\Delta x} = \frac{dI}{dx} = -\sigma \rho I$$

$$\rightarrow I(x) = I_0 e^{-\sigma\rho x}$$

$$\frac{I_t}{I_0} = e^{-\sigma\rho x}$$

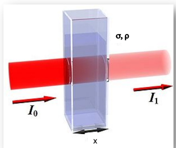
NB: $\sigma[cm^2]\rho\left[\frac{1}{cm^3}\right]x[cm]$



but:

"Classical" spectroscopy: Requires optical depth (10^{-3})
➔ selected experiments

Restrictions



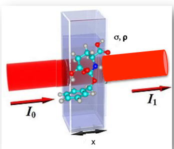
almost every lab has an absorption spectrometer

- identification
- structure
- geometry
- quantitative analysis
- .....

but:

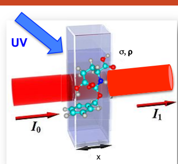
"Classical" spectroscopy: Requires optical depth (10^{-3})
➔ selected experiments

Restrictions



$I_1 \approx 0$ ➔ $I_1 \approx I_0$, no absorption signal visible

Action Spectroscopy



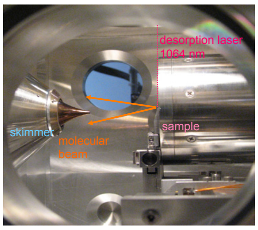
Dissociation
Fragmentation
Ionization
Electron Loss
....

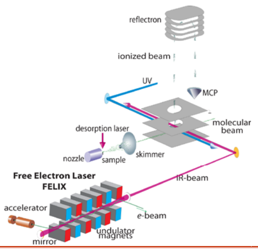
main applications:
gas phase studies
molecules, clusters, complexes
neutral, ionic species
molecular beams, ion traps

- IR-UV ion-dip spectroscopy
  - provides detailed structural information / conformation selective
  - direct view on hydrogen bond interactions (shift in IR frequency)

Experimental Method: Molecular Beam

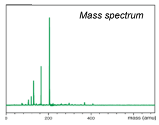
laser desorption: Creation gaseous molecules → Molecular Beam: Cooling neutral molecules → Spectroscopic Techniques: IR / UV excitation → Mass selective Detection

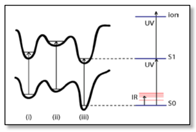




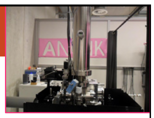
The experiment

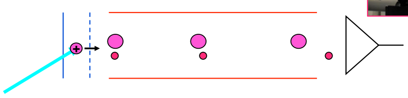
laser desorption: Creation gaseous molecules → Molecular Beam: Cooling neutral molecules → Spectroscopic Techniques: IR / UV excitation → Mass selective Detection





The time-of-flight spectrometer





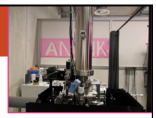
ion optics — time-of-flight tube — detector

creation and extraction of the ions

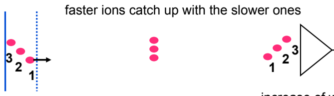
separation according their mass:
- light ions travel faster than heavier ions
- ions separated by mass number

detection:
- conversion of ions into data peaks

TOF-spectrometer: ***spatial distribution***



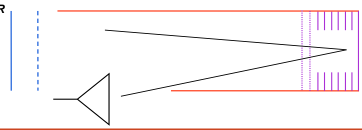
faster ions catch up with the slower ones



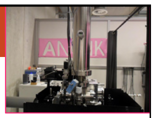
1) leaves first, will move slower
2) leaves later, move slight faster
3) leaves last, moving faster

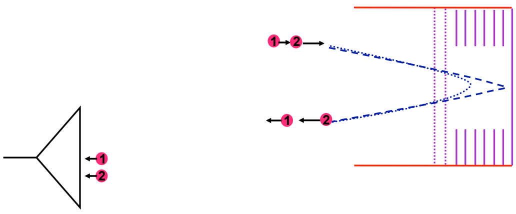
increase of width of ion packets (limitation of the resolution)

Extend resolution: ***REFLECTOR***



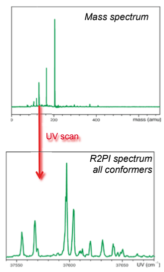
TOF-spectrometer: ***the reflector***

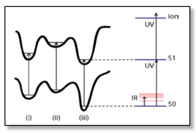




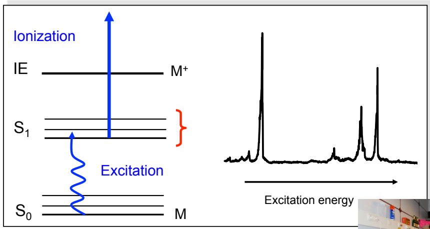
The experiment

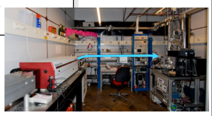
laser desorption: Creation gaseous molecules → Molecular Beam: Cooling neutral molecules → Spectroscopic Techniques: IR / UV excitation → Mass selective Detection





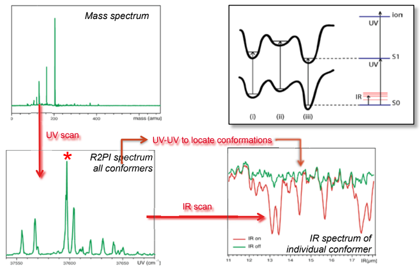
REMPI (resonant enhanced multiphoton ionization)



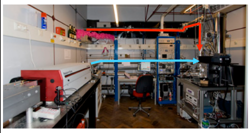


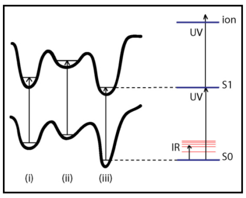
The experiment

laser desorption: Creation gaseous molecules | Molecular Beam: Cooling neutral molecules | Spectroscopic Techniques: IR / UV excitation | Mass selective Detection

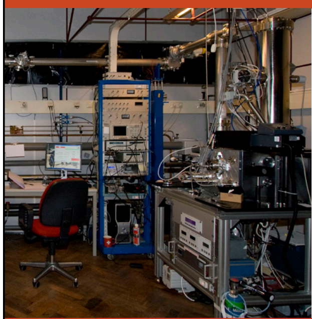


IR spectroscopy





Molecular beam + laser desorption



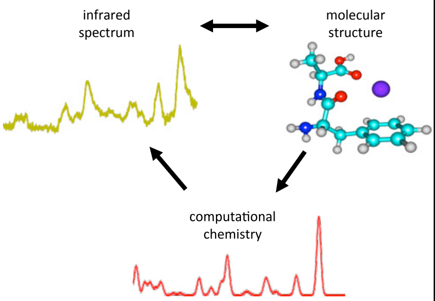
Molecular beam expansion

- laser desorption source
- neutral molecules
- cooled molecules ≈10 K
- mass selective (TOF)
- double resonance spectroscopy

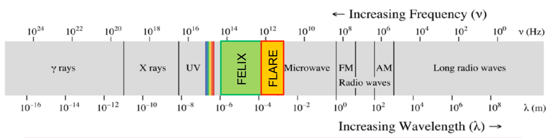
√ **spectroscopic resolution**
√ **mass selective**
√ **conformer selective**
√ **+IR= structural analysis**
√ **limitation molecular size**
√ **UV chromophore required**

Infrared spectroscopy – molecular structure

Why IR:
- identification
- structure
- geometry
- interaction
- .....



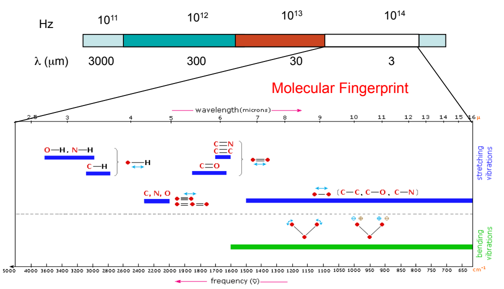
Why is the IR / terahertz region interesting?



1 THz –1 psec – 300 μm – 33 cm⁻¹ – 4.1 meV – 47.6 K – 0.39 kJ/mol – 0.094 kcal/mol

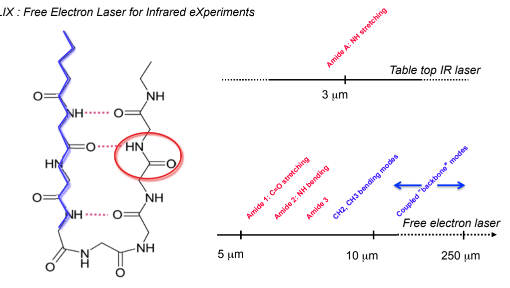
Why IR:
- identification
- structure
- geometry
- interaction
- .....

The fingerprint region



The fingerprint of a peptide: need for FELIX

FELIX : Free Electron Laser for Infrared eXperiments



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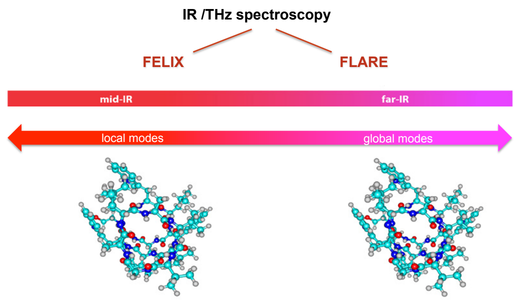
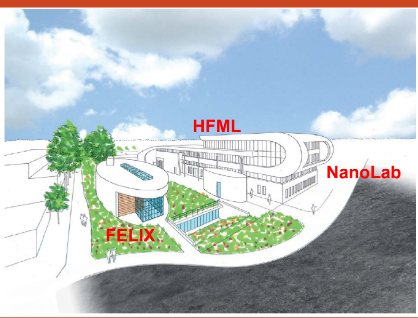
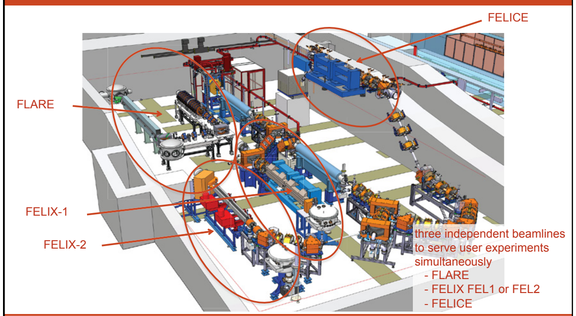


The merge: new FELIX facility Nijmegen

The new FELIX facility Nijmegen



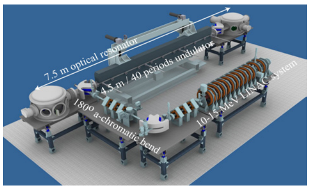
FELIX facility: Infrared Sources



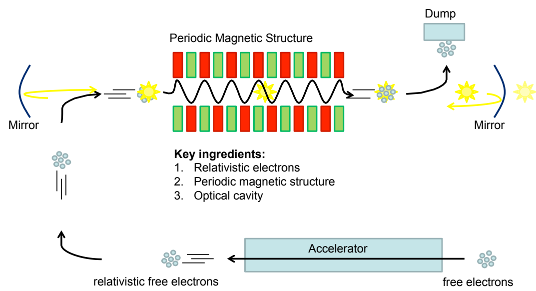
three independent beamlines to serve user experiments simultaneously
- FLARE
- FELIX FEL1 or FEL2
- FELICE

What is a Free Electron Laser (FEL)

A laser light source where free electrons generate the light.
In everyday-life lasers the electrons are bound in the lasing medium (gasses, dyes, solid state diodes)



How an FEL works



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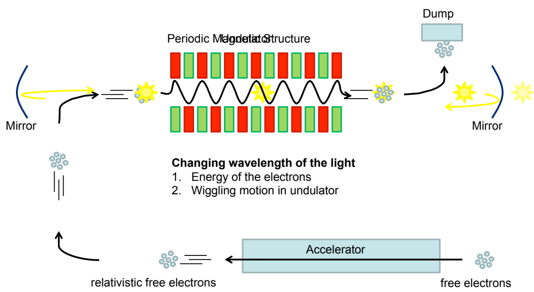
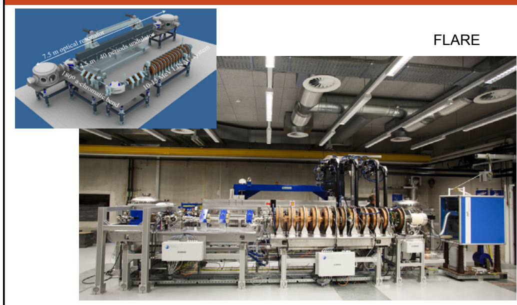


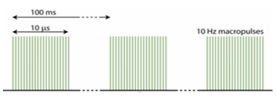
Free electron Laser for Advanced spectroscopy and high Resolution Experiments

FLARE



FLARE – specifications

- Spectral range: 0.1 – 1.5 mm (0.2 – 3 THz / 6 – 100 cm^{-1})
- Repetition rate: 10 Hz
- Intense pulses of light:
  - Typically microJoules in a picosecond pulse (~100 kW)
  - Train of picosecond pulses that lasts for 10 microseconds
  - 0.5 – 2% bandwidth



- Specialty:
  - Narrow bandwidth operation (10^{-5} – 10^{-6})

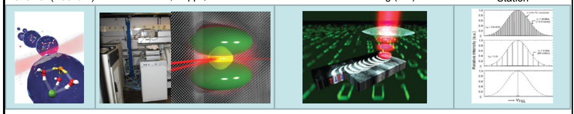
FELIX Facility: Specs of the Infrared Sources

SPECS:	FELIX:	FLARE:	FELICE:
e-beam energy	50/45 – 15 MeV	15 – 10 MeV	50/45 – 18 MeV
spectral range	2.7 - 150 micron 3600 - 66 cm^{-1} 120 - 2 THz 450 - 8 meV	100 - 1500 micron 100 - 6 cm^{-1} 3 – 0.25 THz 12 – 0.75 meV	5 - 100 micron 2000 - 100 cm^{-1} 60 - 3 THz 250 - 12 meV
pulse structure	micro / macropulse	micro / macropulse	micro / macropulse
rep. rate	25 MHz/1 GHz@10 Hz	3 GHz/20 MHz@10 Hz	16 MHz/1GHz@10 Hz
micropulse energy	1- 20 µJ	≈ 5 µJ	max. 1 mJ
macropulse energy	≤ 100 mJ @ 1 GHz	≤ 100 mJ @ 1 GHz	max. 5 J @ 1 GHz
peak power	≤ 100 MW	≤ 10 MW	≤ 5 GW
polarisation	linear	linear	linear
spectral bandwidth (FWHM)	0.2 – 5%	≤ 1%* * spectral mode ≤10^{-4}	0.4 – 3%
continuous tunability	200 – 300%	? %	200 – 300 %

FELIX facility @ Nijmegen: User Laboratories

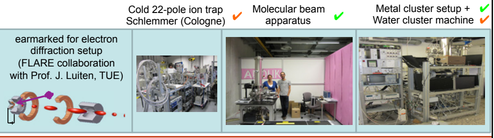
User laboratory 1 – FLARE & FELIX

He-droplet machine Havenith (Bochum) ✔
EPR spectrometer EPSRC, Aeppli, Murdin ✔
Ultrafast laser system Kimel & Rasing (RU) ✔
FLARE Diagnostic Station ✔



Cold 22-pole ion trap Schlemmer (Cologne) ✔
Molecular beam apparatus ✔
Metal cluster setup + Water cluster machine ✔ ✔

multi-purpose station with optical table
earmarked for electron diffraction setup (FLARE collaboration with Prof. J. Luiten, TUE)



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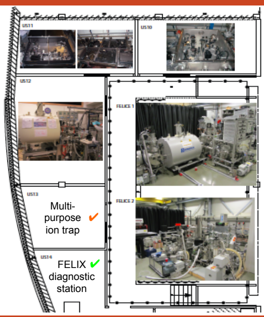
FELIX facility @ Nijmegen: User Laboratories

Ultrafast laser systems ✔
Versatile FTICR mass spectrometer ✔
Non-linear optics laboratory ✔
FELICE FTICR mass spectrometer ✔
FELICE cluster apparatus ✔
Multi-purpose ion trap ✔
FELIX diagnostic station ✔



User laboratory 2 – FELIX & FELICE

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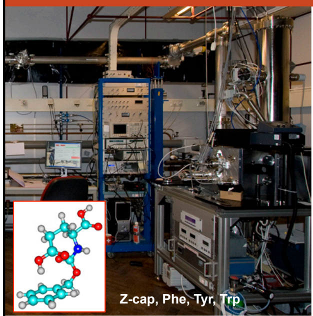
Molecular beam + laser desorption



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✓ **conformer selective**
✓ **+IR= structural analysis**
✓ **limitation molecular size**
✓ **UV chromophore required**

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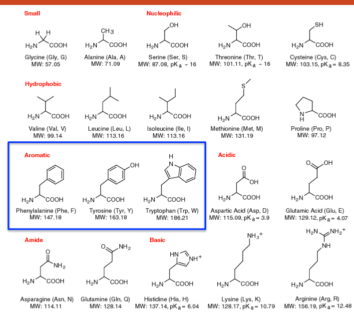
Natural amino acids



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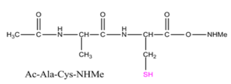
VUV: 1-photon ionization

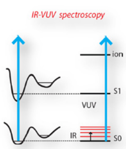
Why VUV?

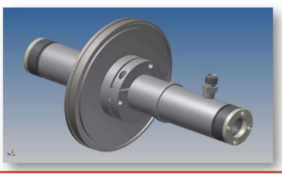


Ac-Ala-Cys-NHMe

Without UV-chromophore

VUV single photon ionization





355 nm frequency tripling

Xe:Ar (1:10), 200 Torr

118 nm (10.6 eV)

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Layout

Introduction
- Why, o why?
- What & how?

Experimental methods
- Cooling methods
  - Why cooling
  - Supersonic expansions
  - Collisional cooling
- Molecule production --> larger & larger
  - heating
  - Laser desorption
  - Molecular beams & laser desorption

Spectroscopic Techniques
- History
- Use of lasers
- Experimental approach
  - UV, IR spectroscopy
- Free electron lasers
- Other methods:
  - IR-UV, UV-UV
  - Combination with VUV sources

Applications
- Biomolecules
  - Local environment
  - Biological environment
  - Larger and larger….
- Molecular motors: functioning of rotaxanes

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