Methods for the examination of biomolecular structure and dynamics.

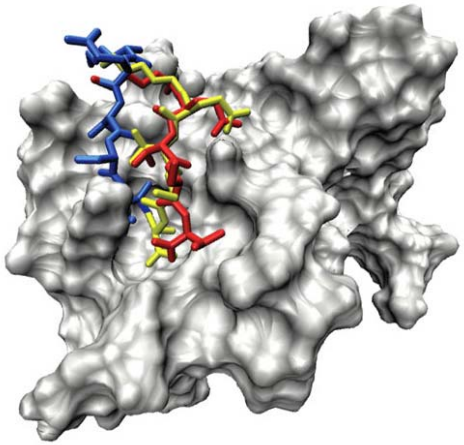
X-ray diffraction,
mass spectrometry,
infrared spectroscopy.

László Smeller

Overview of the methods for the examination of biomolecular structure and dynamics

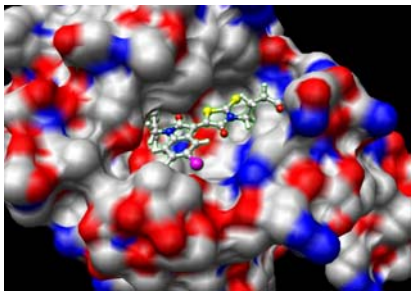
Structure	***Dynamics***
X-ray crystallography	Fluorescence lifetime
NMR spectroscopy	Fluorescence polarization
Mass spectrometry	ESR (EPR) spectroscopy
Infrared spectroscopy	Dynamic light scattering
Luminescence spectr.	FCS (Fluorescence correlation spectroscopy
…	…

What is the relevance of the structural information?



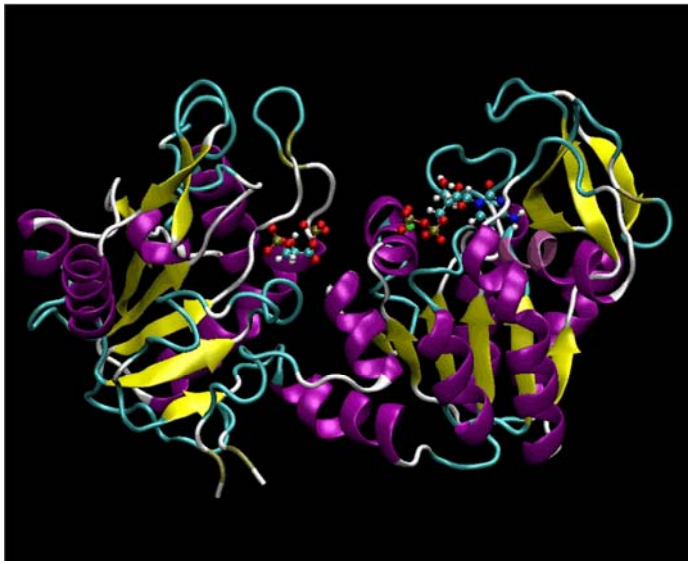
From:http://www.cipsm.de

Understanding the enyzmatic function,
Docking of small molecules
…



http://en.academic.ru

Structure - dynamics



X-ray diffraction

X-ray diffraction

- **What** (kind of information will it provide)?
  - 3D structure of the molecules.
  - x,y,z coordinates of all (heavy) atoms.
- **Why** is it interesting/useful in medicine?
  - Understanding of biological processes
    - e.g. DNA transcription, enzymatic function, molecular recognition, effect of mutations
  - Designing drug molecules (binding, docking)
- **How**?

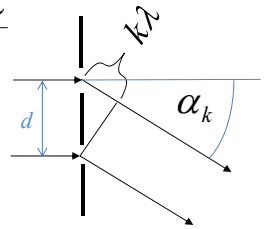
Reminder: diffraction of light

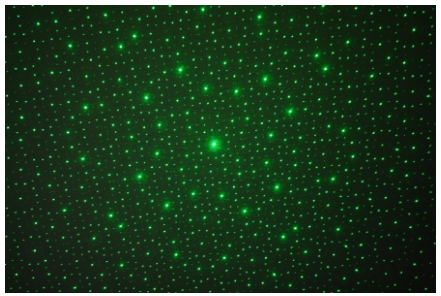
Diffraction of light on an optical grating:

Grating constant:

$d \gtrsim \lambda$

$$\sin \alpha_k = \frac{k\lambda}{d}$$





measure α_k if λ is known

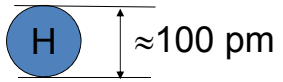
$\Rightarrow d$ can be calculated

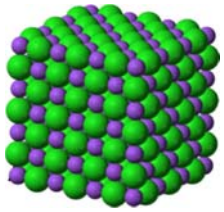
X-ray diffraction

Which grating (lattice) fits to the x-ray?

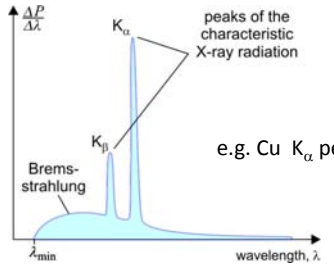
$d \gtrsim \lambda$

$\lambda_{\text{x-ray}}$ 10-200 pm

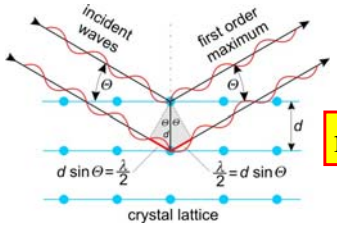




NaCl lattice constant: 564 pm

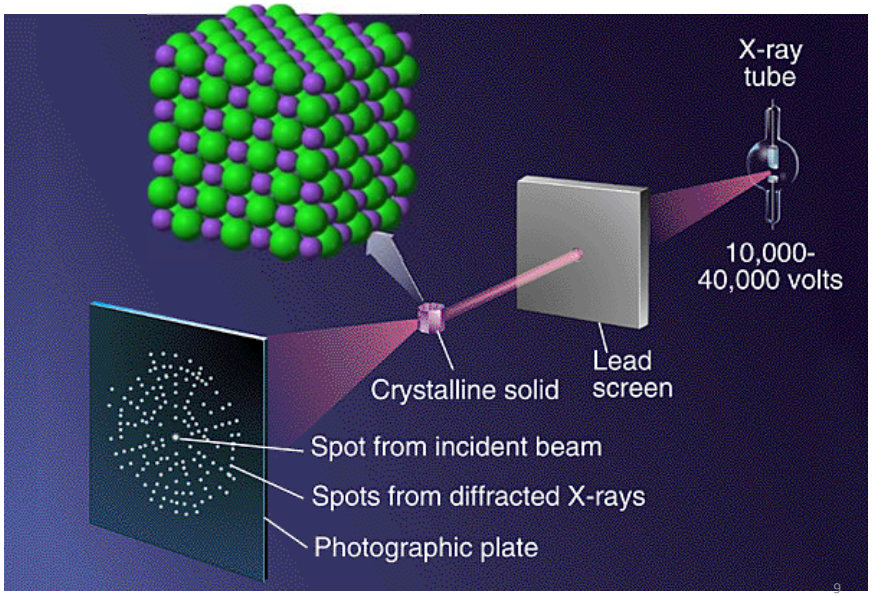


e.g. Cu K_α peak: λ=154 pm



$n\lambda = 2d \sin\Theta$

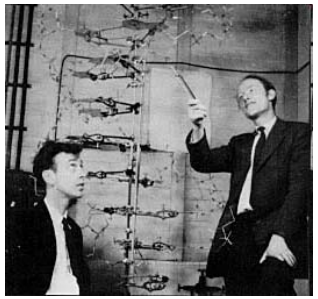
Scematic structure of the x-ray diffractometer



Investigation of 3D structure of macromolecules by x-ray diffraction

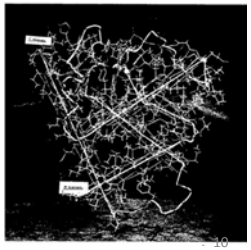
Nobel prize 1962
Globular protein
M. F. Perutz,
J. C. Kendrew

mioglobin:~1200 atoms

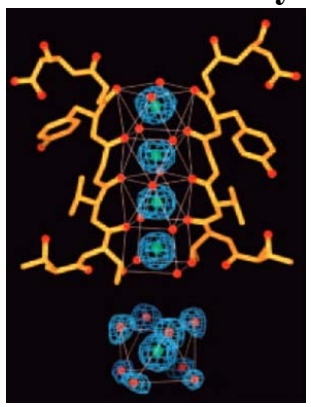


Nobel prize 1962
Structure of DNA
Francis Crick
James Watson
Maurice Wilkins

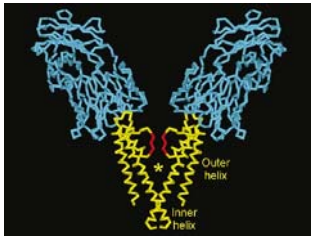
Rosalind Franklin



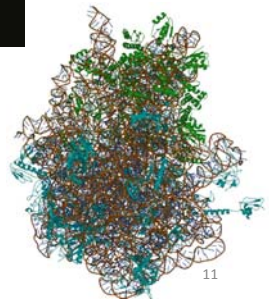
Investigation of 3D structure of macromolecules by x-ray diffraction

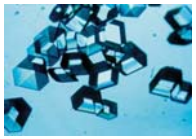


Nobel prize 2003
Memebrane channel
Roderick MacKinnon

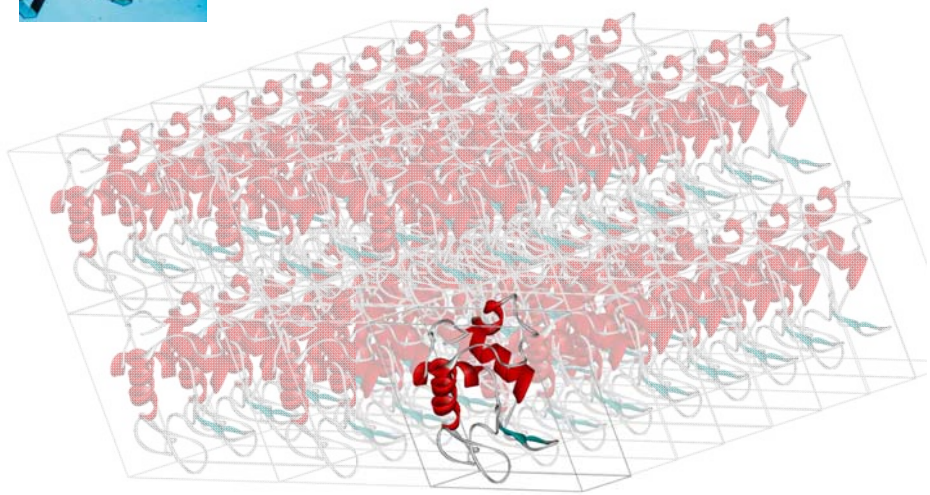


Nobel prize 2009
Ribosome
V. Ramakrishnan, T. A. Steitz, A. E. Yonath
30S subunit: ~35000 atoms,
50S subunit:~64000 atoms

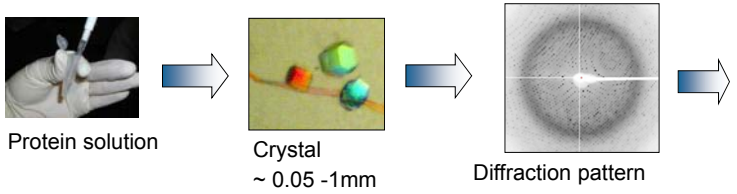


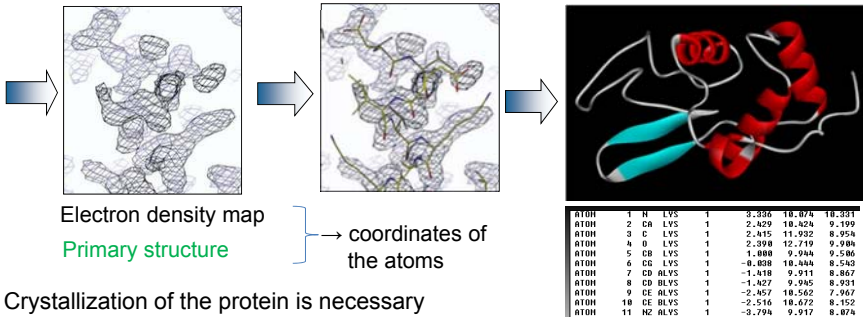


Protein crystals

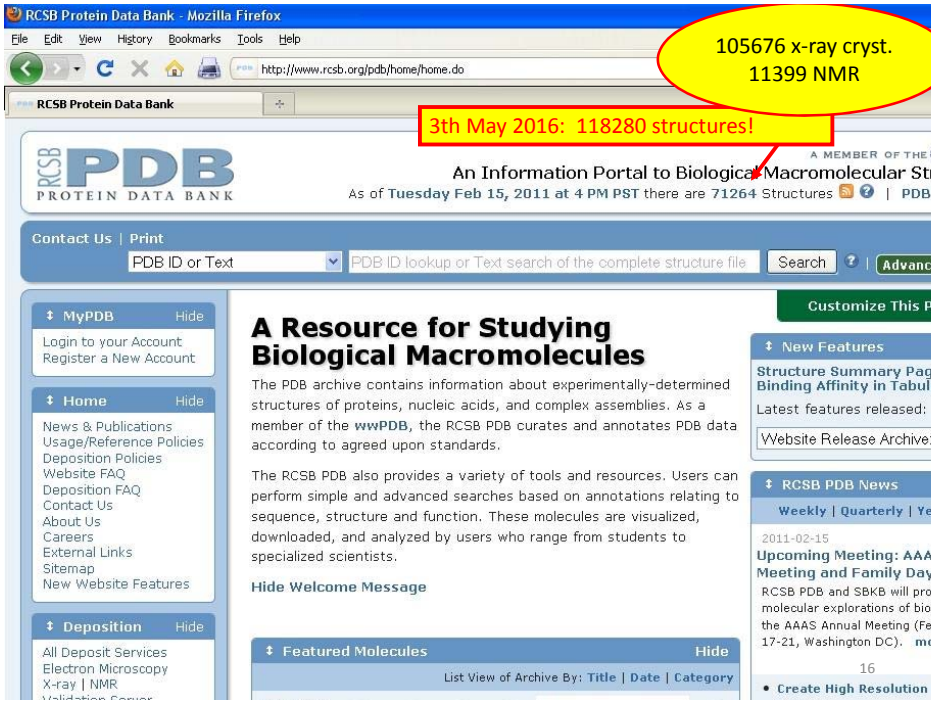


Determination of 3D structure of proteins by x-ray diffraction





- Crystallization of the protein is necessary
  - Proteins with stable conformations can be investigated (globular proteins)
- The time and space average of the **electron density map** is obtained experimentally

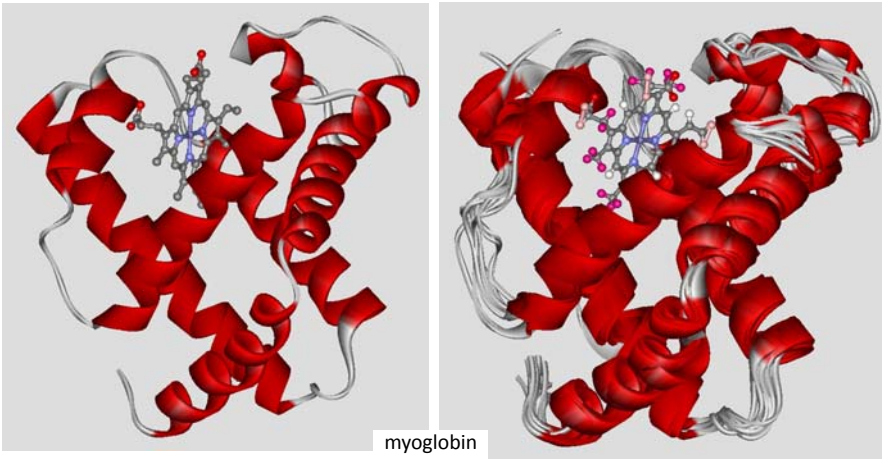


Protein data bases

- **PDB** (http://www.rcsb.org)
  Protein Data Bank
  3D structures (>100 thousand)
  from x-ray and
  NMR spectroscopic measurements
- **Swiss-prot** (http://www.expasy.org/)
  Primary structure (sequence)
  Proteomic tools to estimate
  the secondary structure (homology modelling)
  the chemical parameters (e.g. isoelectric point…)
  or to compare different sequences
  …

X-ray crystallography ↔ NMR

Is the crystal structure relevant for the proteins in physiological solutions?



myoglobin

Mass spectrometry

Mass spectrometry

- **What** (kind of information will it provide)?

  Sorting of the molecules (or their fragments) on the basis of their mass (exactly mass/charge ratio m/z).

  Identifying the fragment and the molecules

- **Why** is it interesting/useful in medicine?

  Proteomics, diagnostics, screening, intraoperative pathology

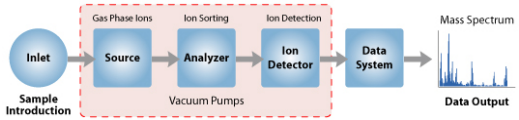
- **How**?

*z: the electric charge of the molecule in elementary charge units

The principle of the mass spectrometer

Important steps:

1. Producing gaseous molecules from the sample
2. Ionization
3. Fragmentation (break the molecules into smaller fragments)
4. Alalysis (sorting)
5. Detection
6. Data evaluation



Producing gaseous ions from the sample

Mostly used methods:

ESI (electrospray ionisation)

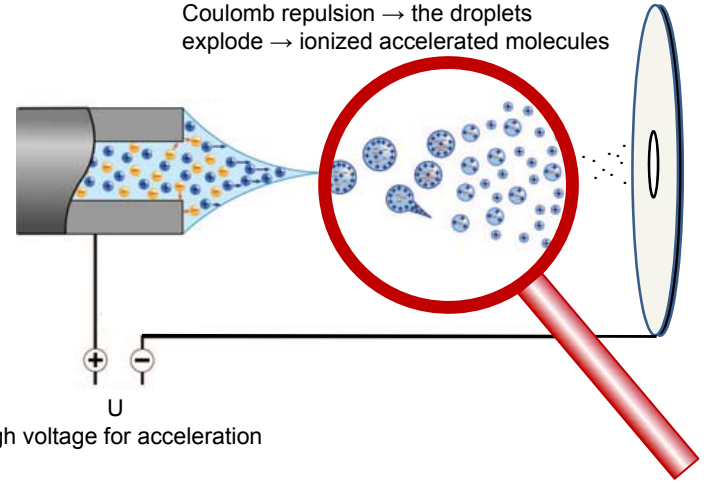
Electron ionization

Laser desorption

MALDI (matrix assisted laser desorption ionisation)

Principle of the electrospray ionization

Evaporation of the solvent → smaller droplet → increased surface charge → Coulomb repulsion → the droplets explode → ionized accelerated molecules

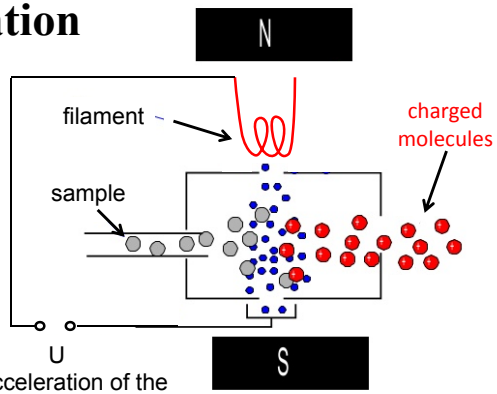


U

High voltage for acceleration

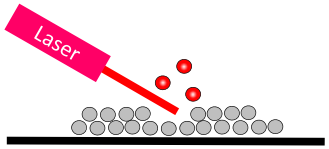
Electron ionization

The evaporated sample molecules collide with electrons



U
for acceleration of the electrons

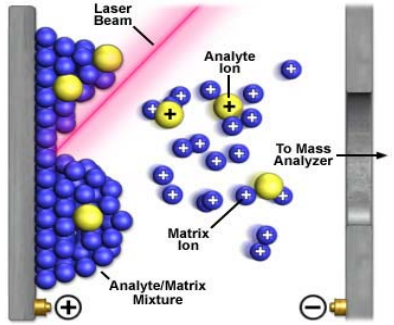
Laser desorption



MALDI
matrix assisted laser desorption ionisation

The laser light will be absorbed by the atoms (or molecules of the matrix (not the sample!)

Ideal for big molecules.



Analysis (Sorting)

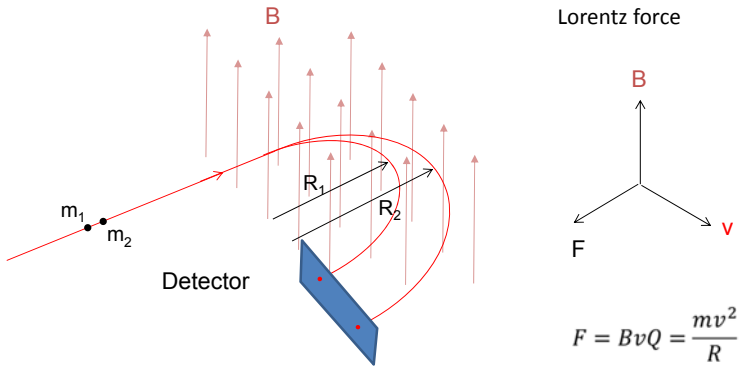
Magnetic

Quadrupole

TOF

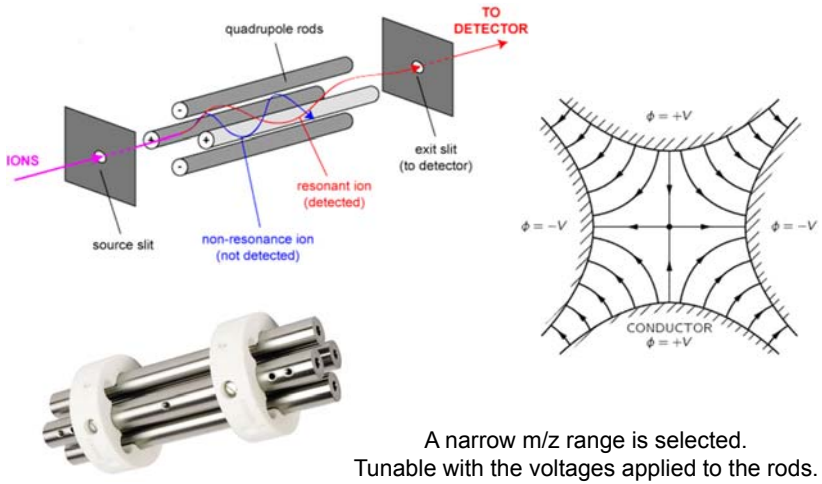
…

Magnetic mass analyzer



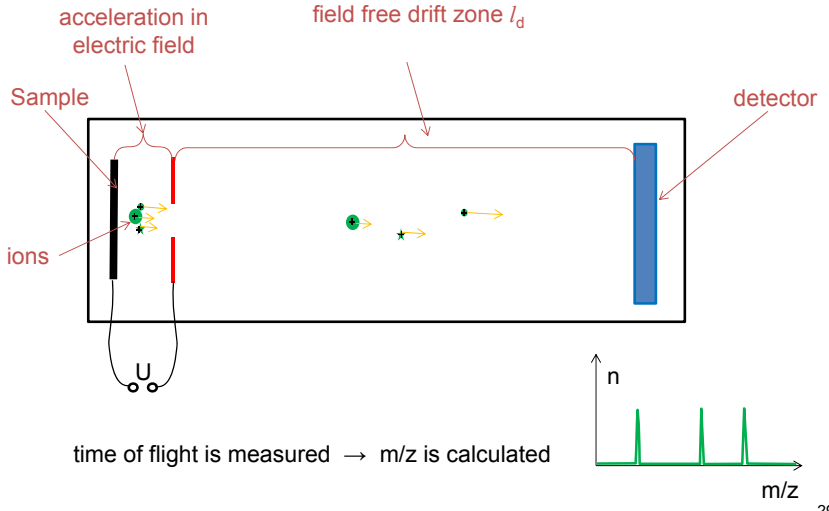
Outdated due to the high mass and high energy consumption of the magnet

Quadrupole mass analyzer



A narrow m/z range is selected.
Tunable with the voltages applied to the rods.

Principle of the *Time of Flight (TOF)* analyzer



time of flight is measured → m/z is calculated

Time of flight MS

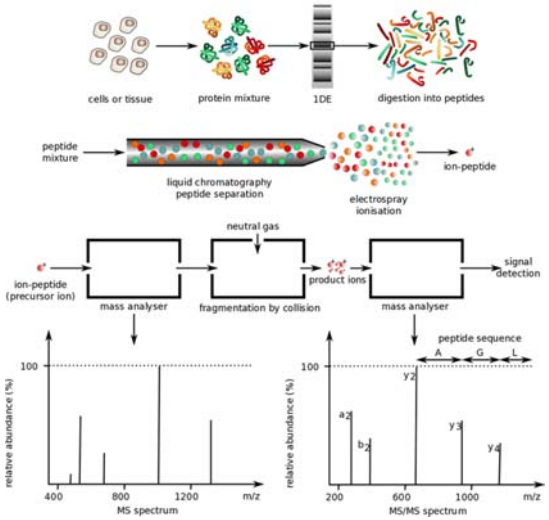
The time of flight:

The velocity:

m/z ratio:

Pulsed ionisation is needed (e.g. laser desorption)

Applications: proteomics



Applications: diagnostics

^{13}C-urea breath test to detect *Helicobacter pylori* (causing gastric ulcer)

^{13}C-urea pill (stable isotopic labelling)

After 30 min the increase of the $^{13}CO_2$ in the exhalated air indicates the presence of urease enzym (produced by *Helicobacter pylori*).

Mass spectrometry is one of the methods to determine the amount of $^{13}CO_2$ from the breath sample.

Applications: screening

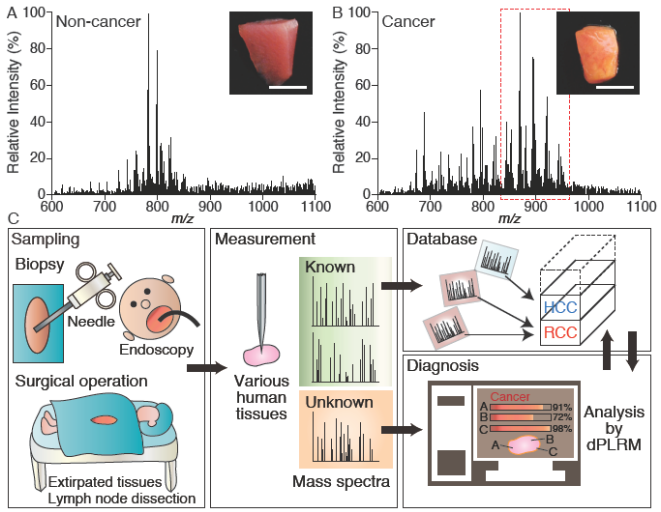
Newborn screening for metabolic diseases

TABLE 1. Metabolic disorders detectable in newborns aged 1–5 days by using tandem mass spectrometry

Disorder	Primary metabolic indicator
Amino Acids	
Phenylketonuria	Phe
Maple syrup urine disease	Leu/Ile, Val
Homocystinuria (cystathione synthase deficiency)	Met
Hypermethioninemia	Met
Citrullinemia	Cit
Argininosuccinic aciduria	Cit
Tyrosinemia, type I	Tyr
Fatty Acids	
Medium-chain acyl-CoA dehydrogenase deficiency	C8, C10, C10:1, C6
Very-long-chain acyl-CoA dehydrogenase deficiency	C14:1, C14, C16
Short-chain acyl-CoA dehydrogenase deficiency	C4
Multiple acyl-CoA dehydrogenase deficiency	C4, C5, C8:1, C8, C12, C14, C16, C5DC
Carnitine palmitoyl transferase deficiency	C16, C18:1, C18
Carnitine/acylcarnitine translocase defect	C16, C18:1, C18
Long-chain hydroxy acyl-CoA dehydrogenase deficiency	C16OH, C18:1OH, C18OH
Trifunctional protein deficiency	C16OH, C18:1OH, C18OH
Organic Acids	
Glutaric acidemia, type I	C5DC
Propionic acidemia	C3
Methylmalonic acidemia	C3
Isovaleric acidemia	C5
3-hydroxy-3-methylglutaryl CoA lyase deficiency	C5OH
3-methylcrotonyl CoA carboxylase deficiency	C5OH

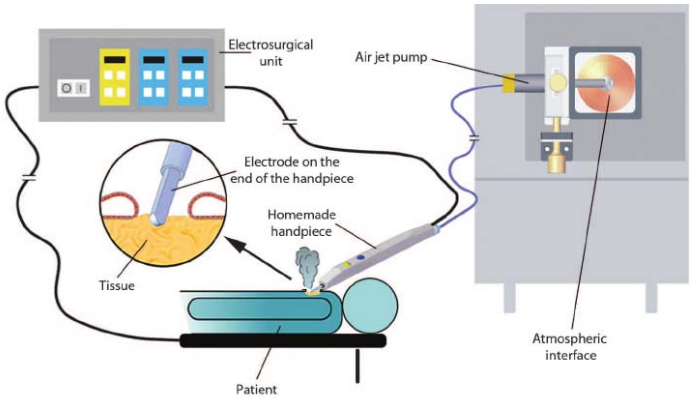
Notes: The list of primary metabolic indicators is not all-inclusive and serves only as a guideline. It is based o results obtained from laboratories experienced in tandem mass spectrometry technology and that serve as diagnos tic metabolic laboratories in the United States and other countries. The identified disorders have been detected fron analyses of dried blood-spot specimens collected during the newborn period. Certain disorders require comple metabolic profiles and intermetabolic relation to detect disease with low false-positive and no false-negative rate

Applications: cancer diagnostics



Takeda et. al.: Innovations in Analytical Oncology - *Status quo* of Mass Spectrometry-Based Diagnostics for Malignant Tumor *Journal of Analytical Oncology*, 2012, *1*, 74-80

Applications: intraoperative MS



IR spectroscopy

Infrared spectroscopy

- **What** (kind of information will it provide)?
  - vibrational frequencies of the molecules
  - conformation, configuration of the molecules.
- **Why** is it interesting/useful in medicine?
  - Identification of molecules (drugs)
  - Detection of the changes in the molecular structure
  - Diagnostics
- **How**?

Infrared spectroscopy

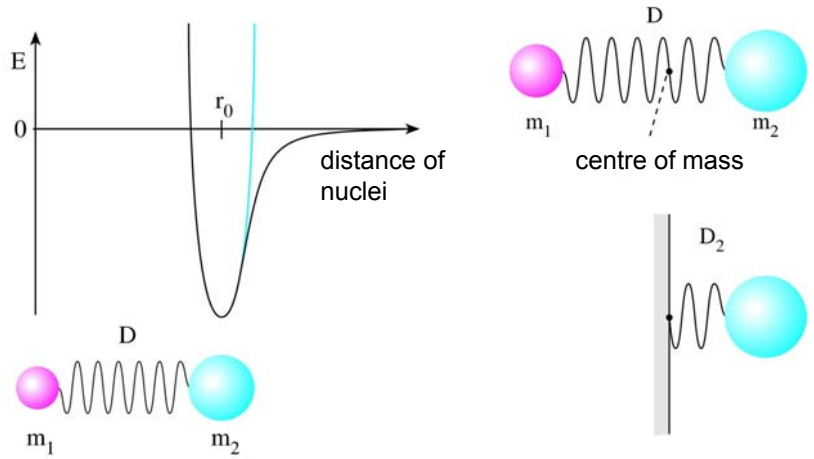
- Infrared light: λ=800 nm - 1 mm
  - MIR (mid-infrared) : 2,5-50 μm
- absorption spectroscopy
- the absorbed infrared radiation excite molecular vibrations
- very specific for the structure of the molecule
- special method for detection:
  - FT spectrometer

Molecular vibrations

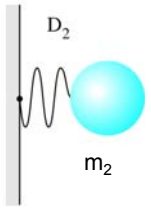
The electrons are light (m_e<<$m_{nucleus}$), they can follow the movements of the nuclei easily, therefore the movements of the nuclei are independent of the movements of the electrons.

Classical physical description: the chemical bond is represented by a spring

Molecular vibrations:

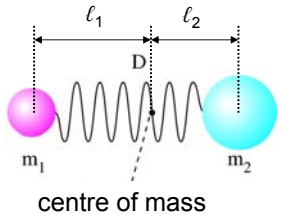


known from elementary mechanics:



$$f = \frac{1}{2\pi}\sqrt{\frac{D_2}{m_2}}$$

$$\frac{m_2}{m_1} = \frac{\ell_1}{\ell_2}$$

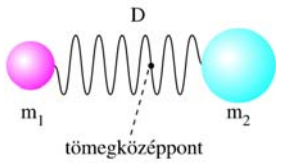


$$\frac{D_2}{D} = \frac{F/D}{F/D_2} = \frac{\Delta\ell}{\Delta\ell_2} = \frac{\ell}{\ell_2} =$$

$$= \frac{\ell_1 + \ell_2}{\ell_2} = \frac{\ell_1}{\ell_2} + 1 = \frac{m_2}{m_1} + 1 = \frac{m_1 + m_2}{m_1}$$

$$F = D\Delta\ell$$

substituting $\frac{m_1 + m_2}{m_1} = \frac{D_2}{D}$ into $f = \frac{1}{2\pi}\sqrt{\frac{D_2}{m_2}}$



frequency of the vibration:

$$f = \frac{1}{2\pi}\sqrt{\frac{D(m_1 + m_2)}{m_1 m_2}}$$

$$m_{red} = \frac{m_1 m_2}{m_1 + m_2}$$ is called as reduced mass

Frequency with the reduced mass: $f = \frac{1}{2\pi}\sqrt{\frac{D}{m_{red}}}$

The wavelength of an electromagnetic wave with frequency f is:

$$\lambda = \frac{c}{f} = 2\pi c\sqrt{\frac{m_{red}}{D}}$$

In the IR spectroscopy the wavenumber (ν) is used, which is the reciprocal of λ:

$$\nu = \frac{1}{\lambda} = \frac{1}{2\pi c}\sqrt{\frac{D}{m_{red}}}$$

ν: number of waves in a unit length [cm^{-1}]

Example: CO

The measured wavenumber: ν= 2143 cm^{-1}

⇨ λ=4,67μm ⇨ f=6,43 10^{13} Hz

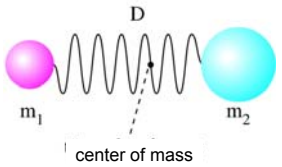
m_C=2·10^{-26} kg, m_O=2,7·10^{-26} kg } ⇨ D=1875 N/m

if ν is known, D can be calculated

if D is known, ν can be calculated

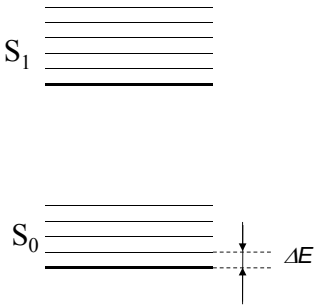
Classical vs. quantum physics

Classical physical picture



$$f = \frac{1}{2\pi}\sqrt{\frac{D}{m_{red}}}$$

Quantum mechanical picture



Vibrations of the large molecules

Molecule consisting of N atoms:

- 3N degree of freedom,

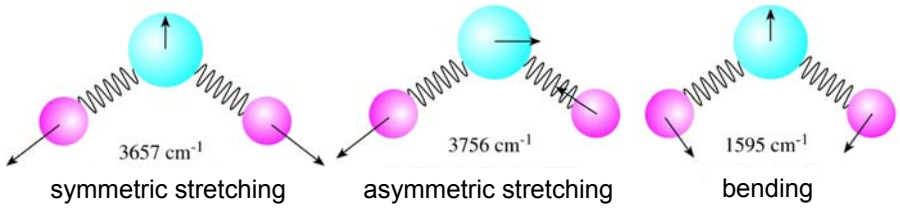
  3-3 are the rotations and translations of the whole molecule
- 3N-6 vibrational degree of freedom (3N-5 for the linear molecules)
- 3N-6 independent normal vibrations

Normal vibrations

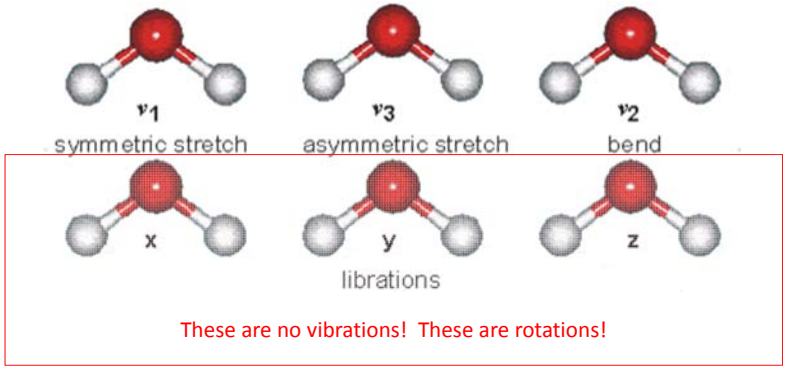
All the atoms vibrate

- with the same frequency but
- with different amplitude and
- in different direction.

Example: water

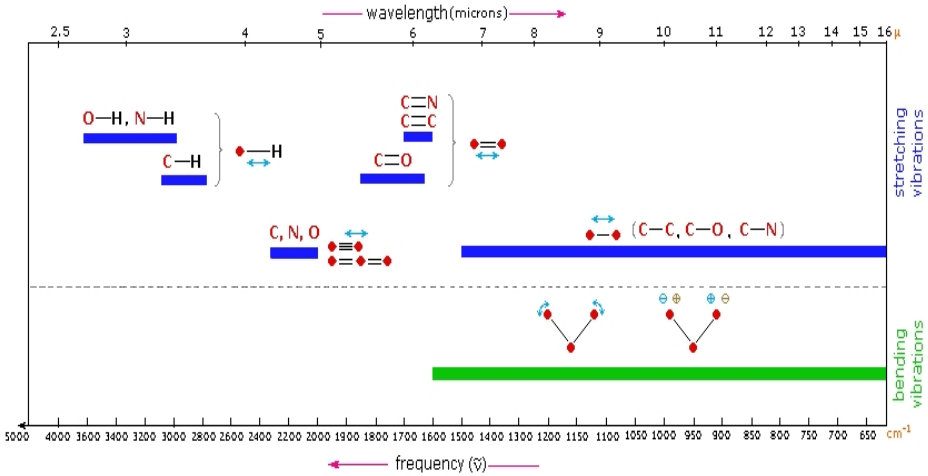


Normal vibrations of water

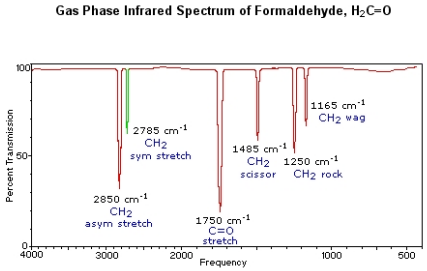


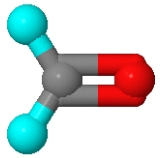
These are no vibrations! These are rotations!

Typical vibrational frequencies (wavenumbers)



Example: Formaldehyde



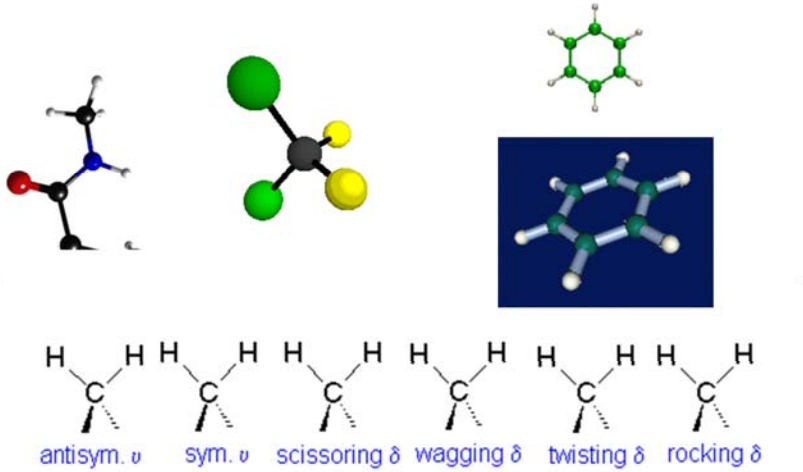


O View CH_2 Asymmetric Stretch
O View CH_2 Symmetric Stretch
O View C=O Stretch
O View CH_2 Scissoring
O View CH_2 Rocking
[O] View CH_2 Wagging

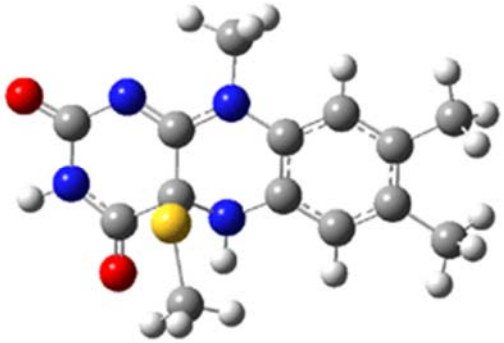
O Ball&Stick Model
O Spacefill Model
O Stick Model
[O] Motion Off

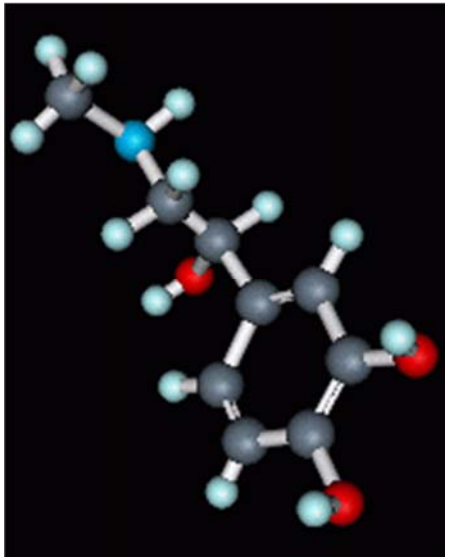
http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/InfraRed/infrared.htm

A few vibrations for illustration



Flavin





Vibrations of the macromoleclues

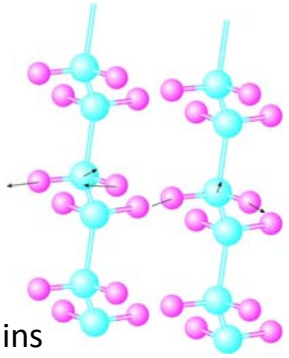
Complex global vibrations

Localised vibrations:

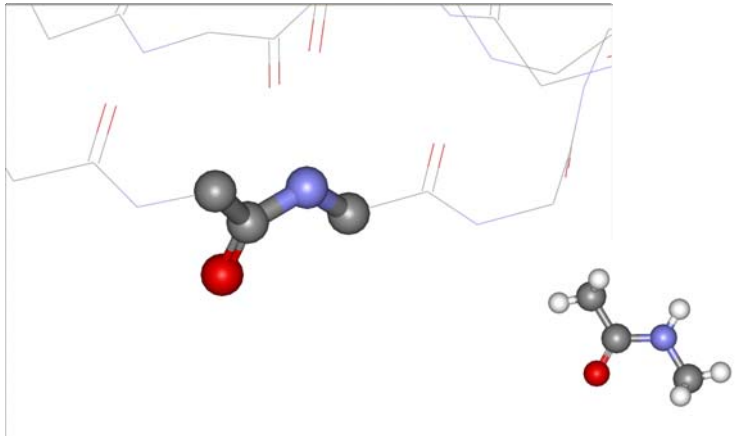
Infromation about the environment of the vibrating group

e.g.:

CH_2 vibrations of the lipid fatty acid chains

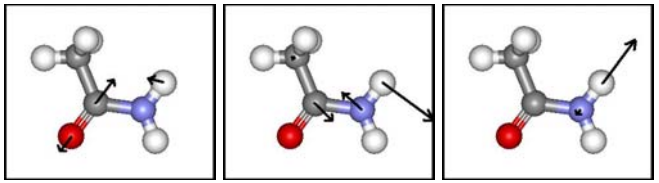


Infrared spectroscopy of the proteins



Confromation sensitive protein vibrations

Amid vibrations

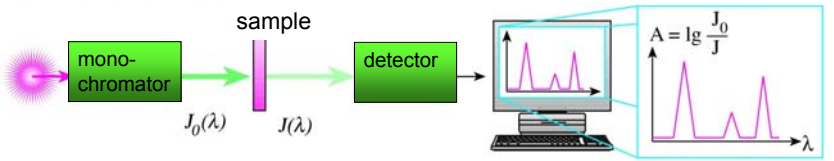


Sensitive to the

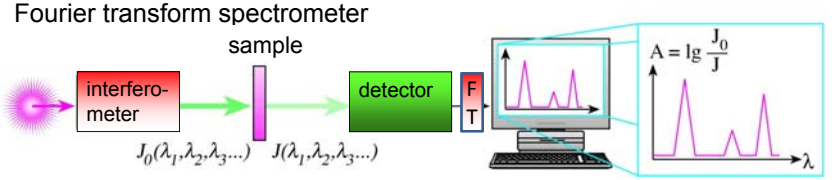
secondary and tertiary structures

The technique of the measurement : Fourier transform spectrometer (FTIR)

conventional (dispersion) spectrometer

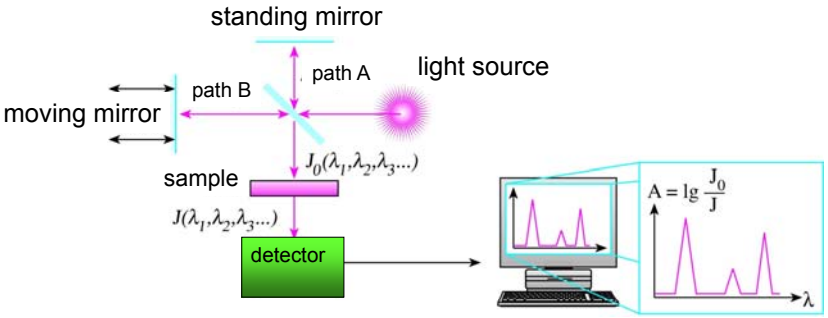


Fourier transform spectrometer



book 6.17

The function of the interferometer

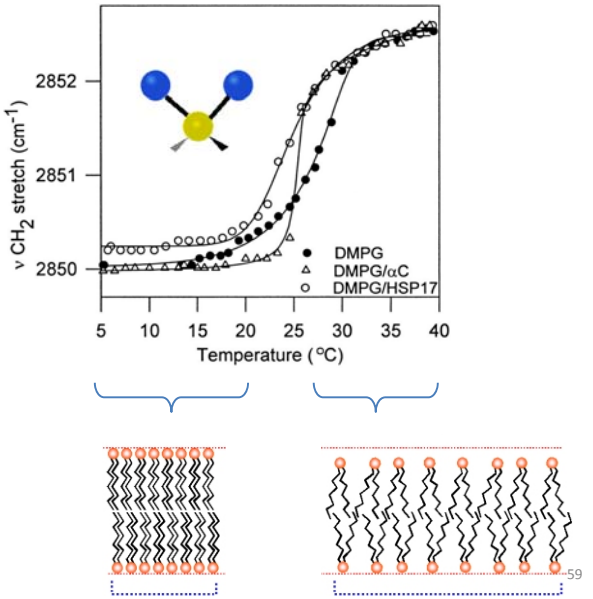


book 6.18

Applications

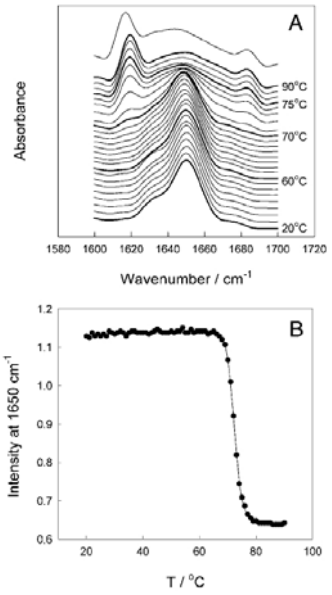
phase transitions
of a lipid bilayer

Interaction of
heat-shock proteins
with the lipid layer



Applications

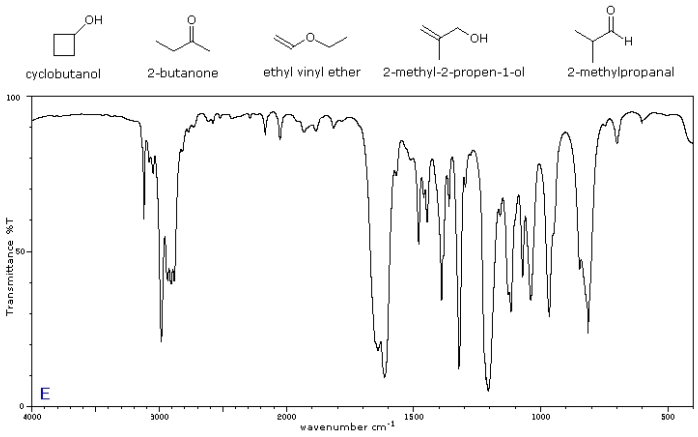
Protein denaturation



Meersman et al. Biophys J.

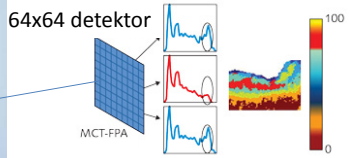
Identification of molecules

C_4H_8O

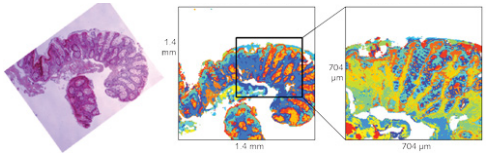


http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/InfraRed/infrared.htm

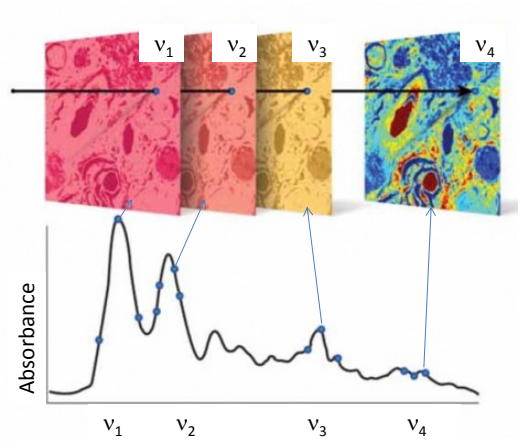
Infrared microscopy

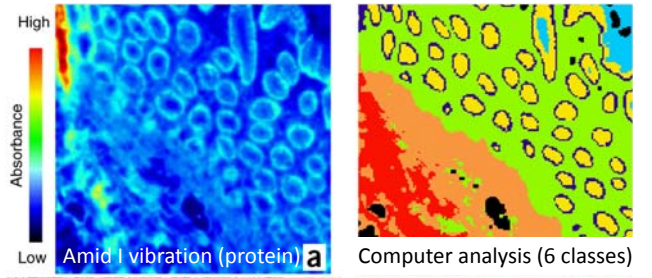


the whole spectrum
is measured in each
pixel of the picture.

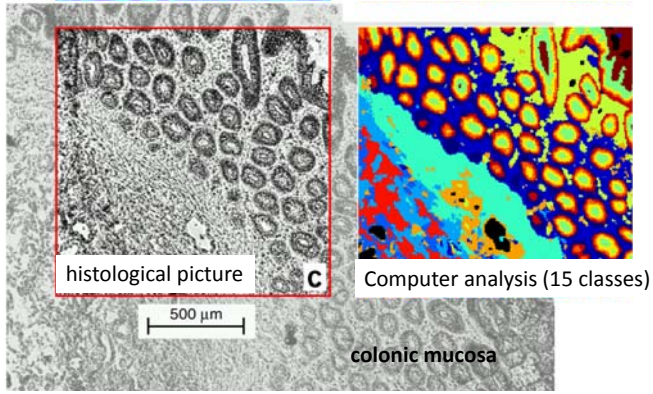


Infrared microscopy





Computer analysis (6 classes)



Computer analysis (15 classes)

Classification of kidney stones using IR microspectroscopy