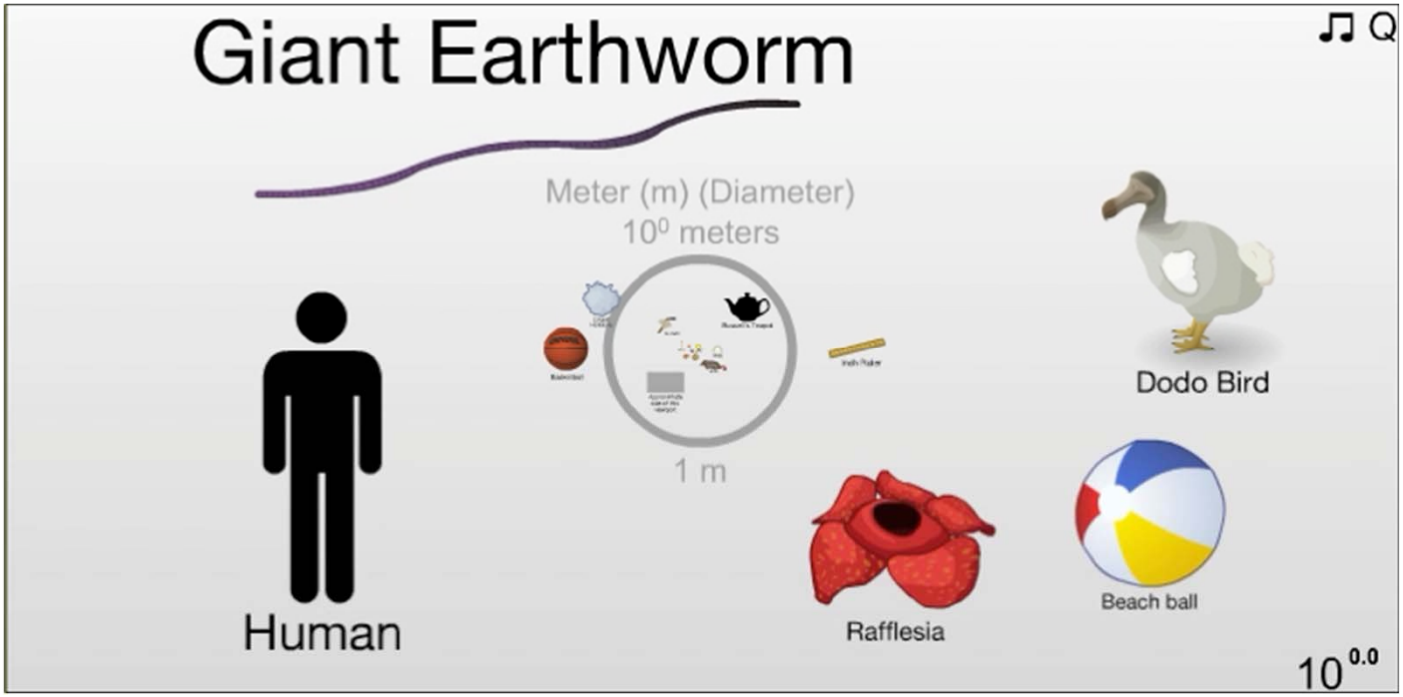
Structure and dynamics of biomolecular systems

mass spectrometry, IR spectrometry, X-ray diffraction, MD simulation

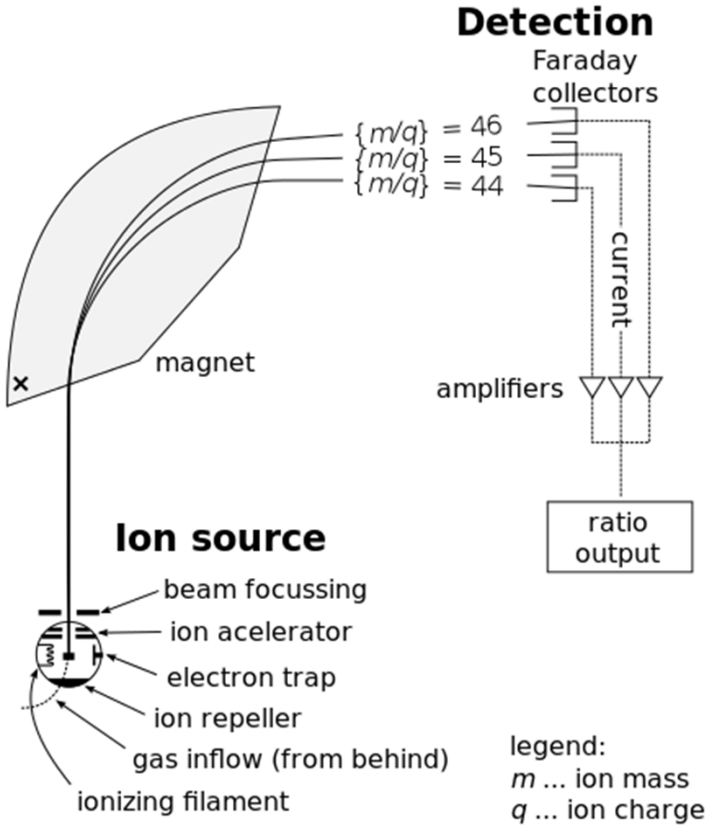
Erika Balog


Giant Earthworm
Meter (m) (Diameter)
10^0 meters
1 m
Human
Rafflesia
Beach ball
Dodo Bird
$10^{0.0}$

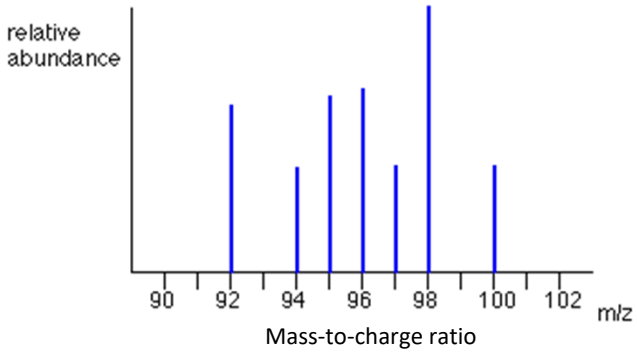
Mass spectrometry

- analytical technique producing spectra of the masses of the atoms or molecules in a sample. The spectra are used to determine the elemental or isotopic signature, thereby elucidating the chemical structures of molecules.

Steps:
1. Ionization
2. Acceleration
3. Deflection
4. Detection



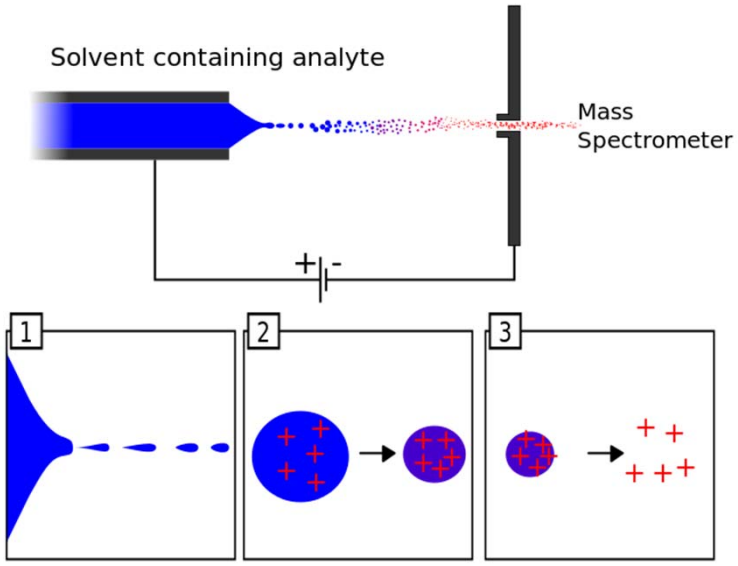
Result: "Stick" diagram



Spectrum is compared with structure database

Ionization of biological samples

Electrospray ionization

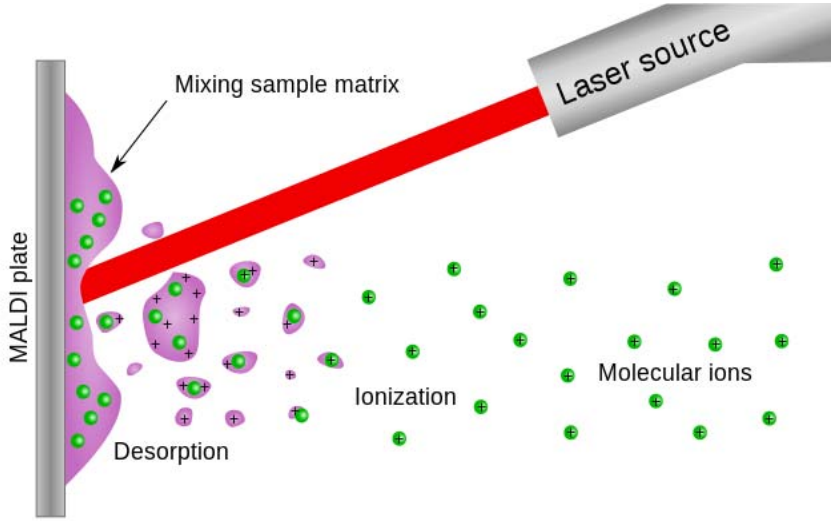


(1) decompositions to droplets,

(2) solvent evaporation → smaller droplet
→ greater surface charge,

(3) Coulomb repulsion → droplets explode → ionized, accelerated molecules .

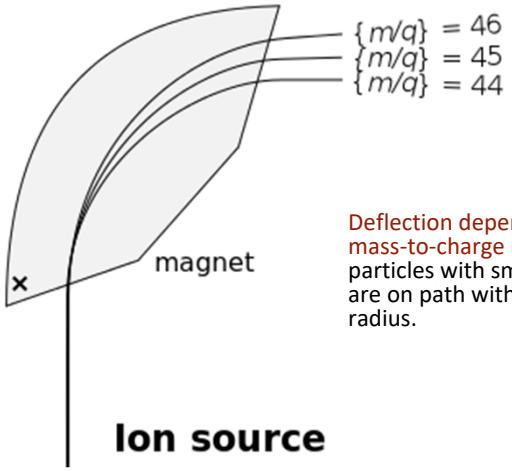
MALDI: “matrix-assisted laser desorption/ionization”



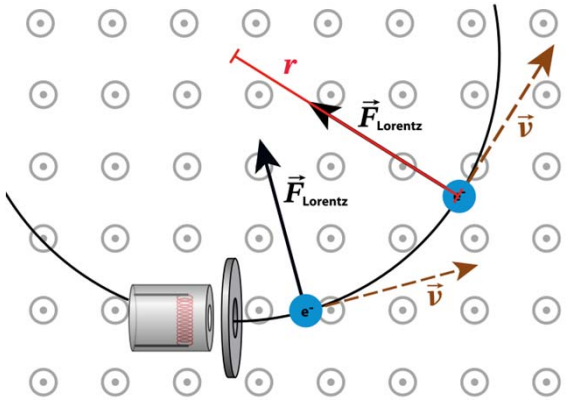
- the laser light is absorbed by the atoms/molecules of the matrix.
- used for investigating large molecules.

Methods of mass analysis 1.

Magnetic method



Deflection depends on mass-to-charge ratio (m/q): particles with smaller mass are on path with smaller radius.



$$\vec{F}_{Lorentz} = q\left(\vec{E} + \vec{v} \times \vec{B}\right)$$

E=electric field, *v*x*B*=vectorial product of speed and magnetic induction

$$\vec{F}_{Lorentz} = \vec{F}_{centrip}$$

$$qvB = \frac{mv^2}{r}$$

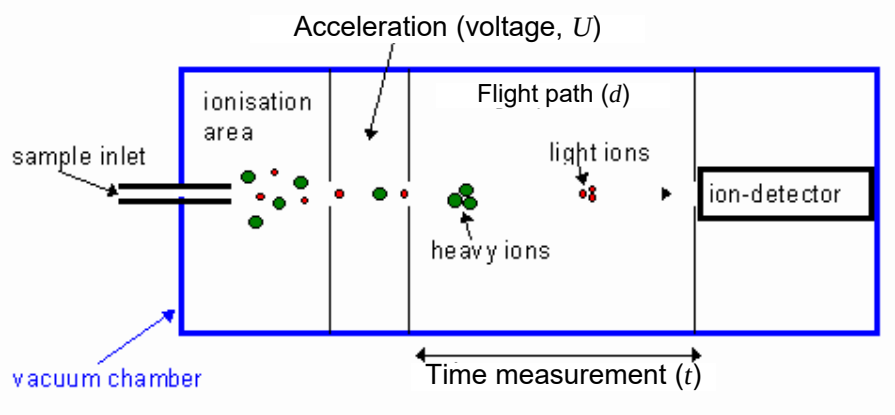
$$r = \frac{m}{q}\,\frac{v}{B}$$

from which the mass-charge ratio (m/q) can be determined.

instead of *m/q* usually *m/z* is used, where *z=q/e* (dimensionless number).

Methods of mass analysis 2.

"Time-of-flight" method



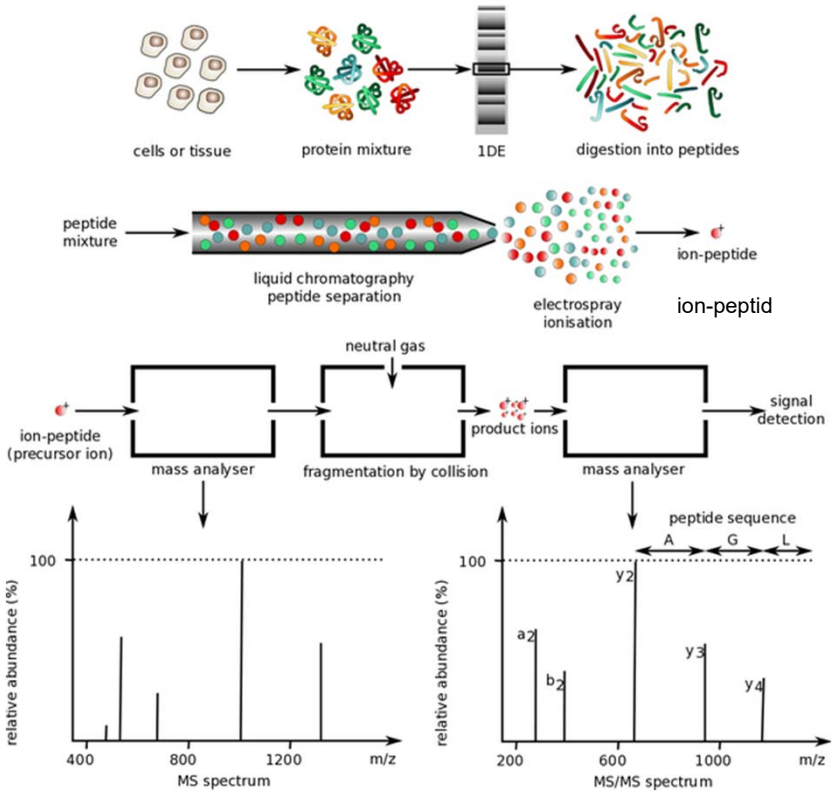
$$qU = \frac{1}{2}mv^2 = \frac{1}{2}m\left(\frac{d}{t}\right)^2$$

$$t = \frac{d}{\sqrt{2U}}\sqrt{\frac{m}{q}} = k\sqrt{\frac{m}{q}}$$

from which the mass-charge ratio (m/q) can be determined.

Applications of mass spectrometry

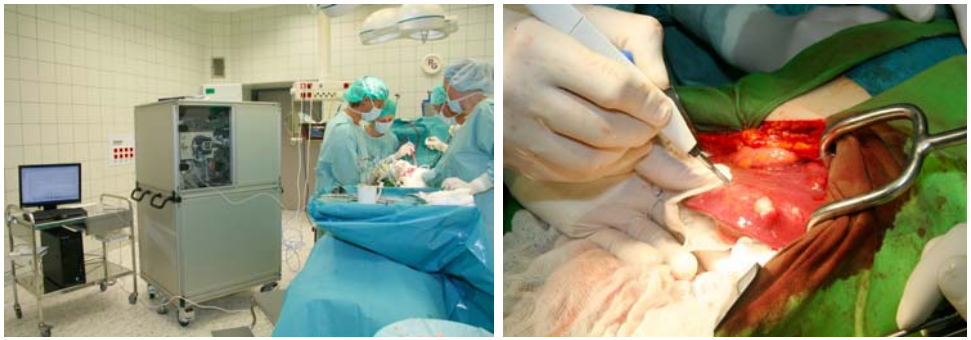
1. Protein analytics (proteomics)

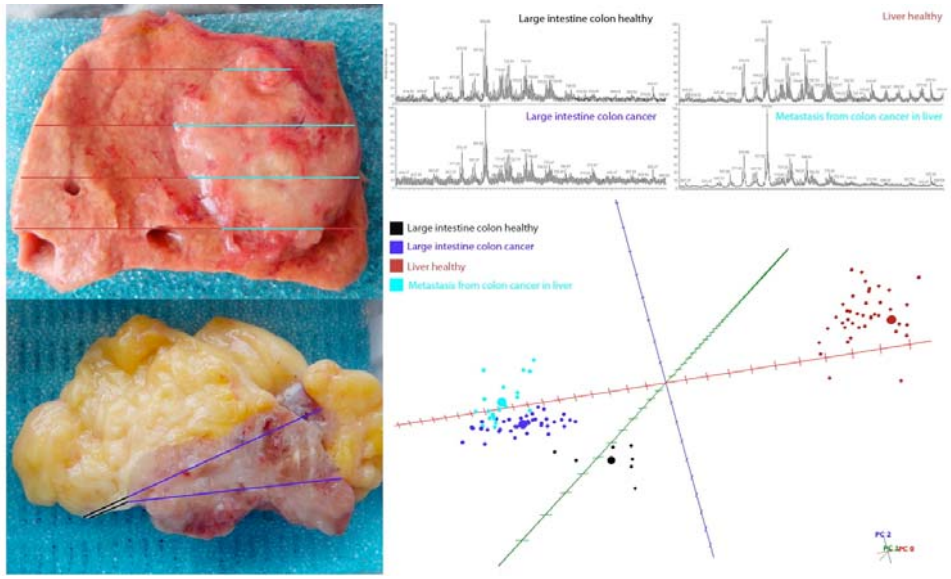


2. Diagnostic screening:

Metabolic diseases (from 1 drop of blood)
e.g., phenylketonuria (PKU)

3. Real-time tissue analysis (“onco-knife”)



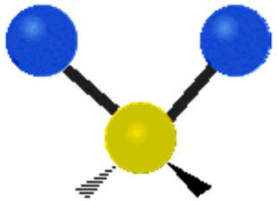


Infrared (IR) spectroscopy

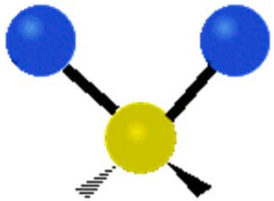
- measures vibrations of molecules.

Vibration: periodic motion along the axis of the covalent bond
Rotation: periodic motion around the axis of the covalent bond

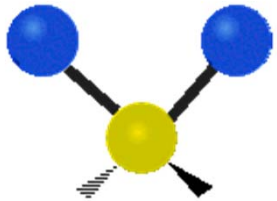
Examples of vibrational motion in the triatomic methylene group (-CH2-):



Asymmetric stretching



Symmetric stretching

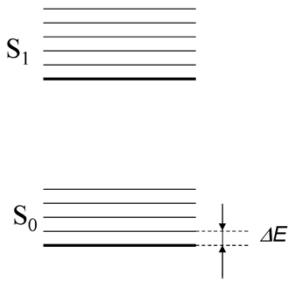


Scissoring

Energy of a molecule: Born-Oppenheimer approximation

$$E_{total} = E_e + E_v + E_r$$

- Types of energy states are independent (not coupled).
- Energy states are non-continuous, but discrete.
- Transition between states involves packets (quanta) of energy.
- Scales of transition energies between different states are different.



Scales of transition energies:

~100x ~100x

$E_e > E_v > E_r$

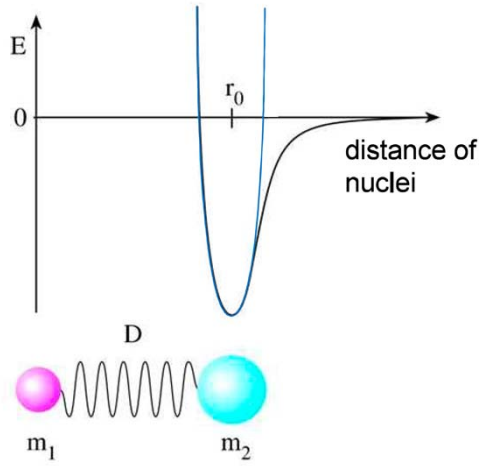
~$3x10^{-19}$ J (~2 eV) > ~$3x10^{-21}$ J > ~$3x10^{-23}$ J

UV/VIS > mid IR > far IR

Molecular vibrations

Molecule: mass connected by a spring

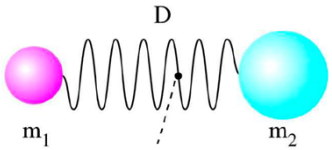
- two-atomic molecule (e.g., CO)
- masses (m1, m2): atomic nuclei ($m_e << m_{nucleus}$)
- spring: covalent bond connecting the atoms
- distance-depedence of interaction energy: can be approximated with a parabola
- r_0: equilibrium inter-nuclear distance
- D: spring constant



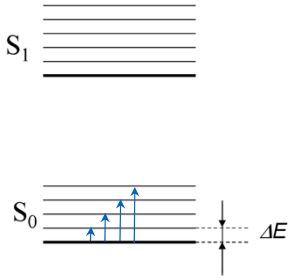
$$f = \frac{1}{2\pi}\sqrt{\frac{D}{m_{red}}} = \frac{\Delta E}{h}$$ (see: Resonance lab)

where: $$m_{red} = \frac{m_1 m_2}{m_1 + m_2}$$

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



In IR spectroscopy, the wavenumber (ν) is used: $$\nu = \frac{1}{\lambda} = \frac{1}{2\pi c}\sqrt{\frac{D}{m_{red}}}$$



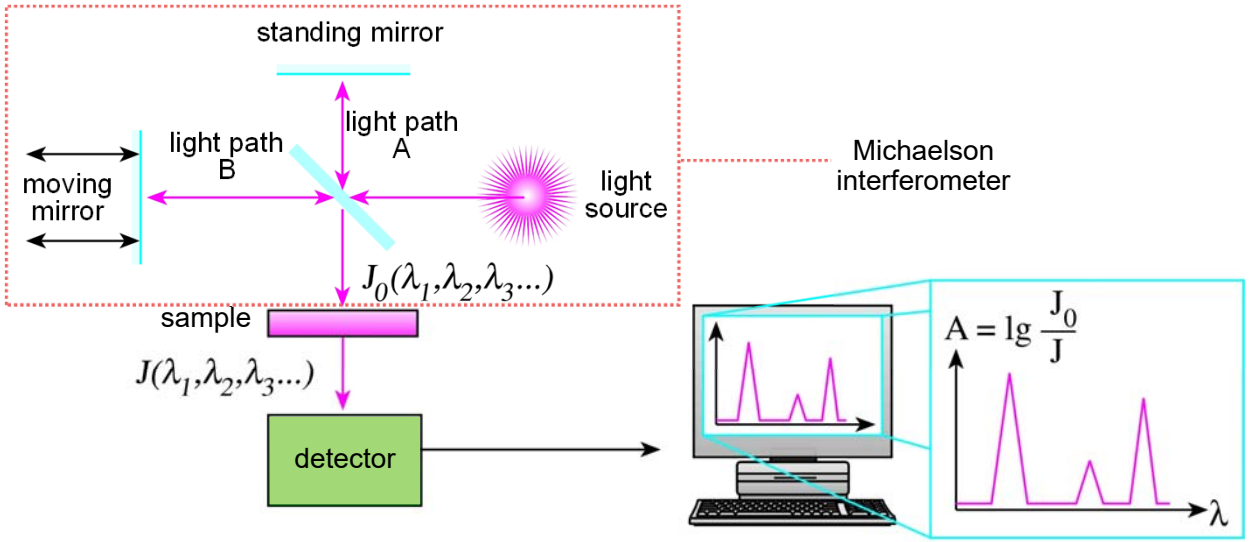
Values for the CO molecule: measured wavenumber, ν =2143 cm^{-1}

λ =4,67 µm, f=6,43x10^{13}Hz (64,3 THz), D=1875 N/m

IR spectroscopy - measurement

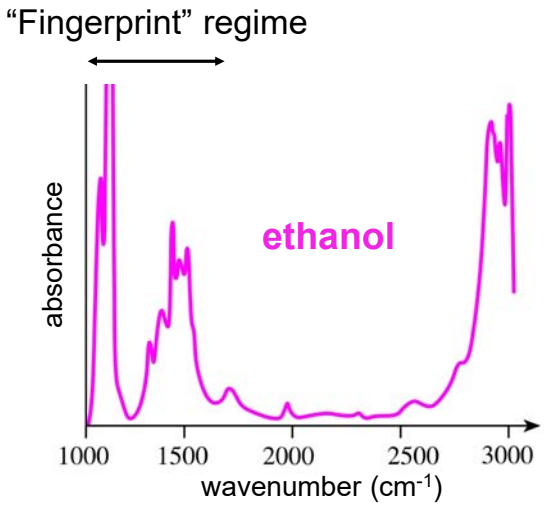
Fourier Transform Infrared (FTIR) Spectroscopy:

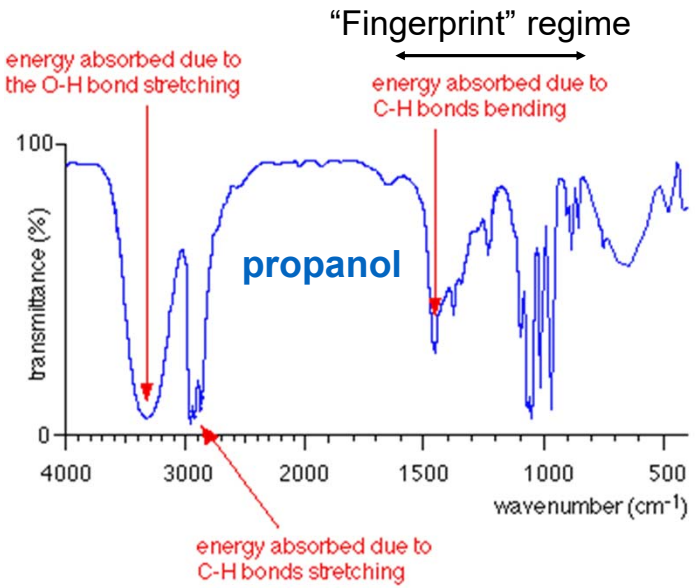
- multiple wavelengths are generated (with a Michaelson interferometer)
- Intensities at multiple wavelengths are converted to wavelength-dependent intensities.



IR spectrum:

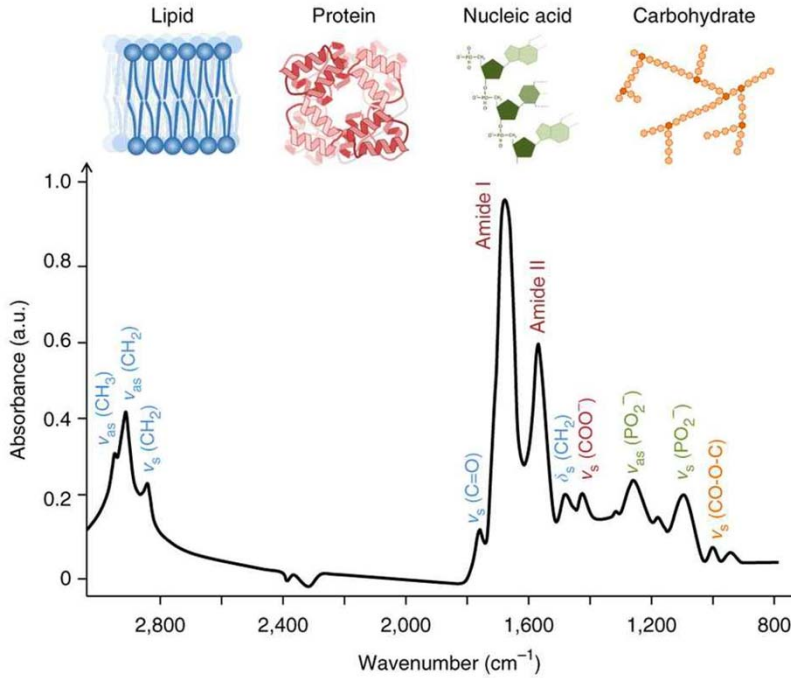
- very rich information about molecular structure and vibrational properties
- absorbance versus wavenumber
- transmittance versus wavenumber

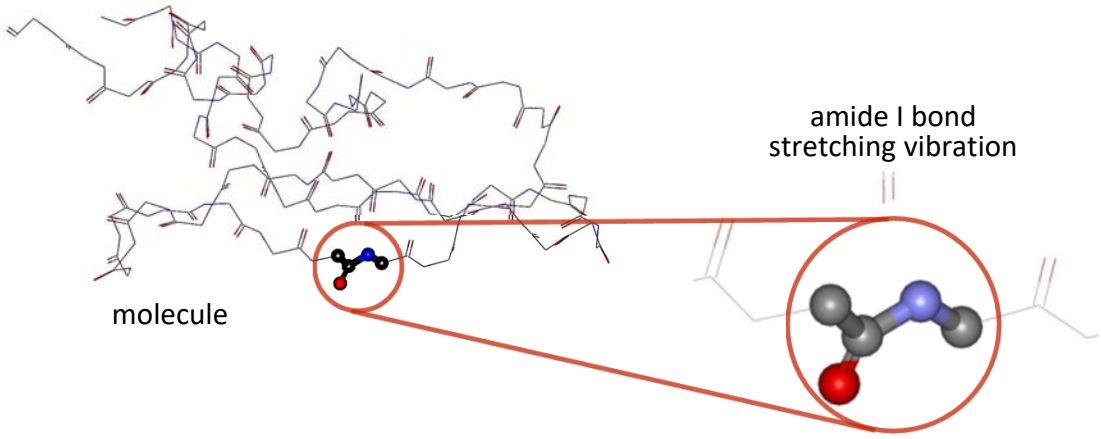




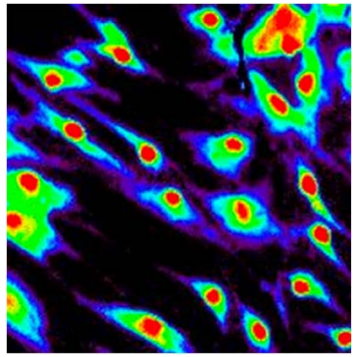
Applications of IR spectroscopy

- Identification of chemical species (e.g., intermediate and end products of reactions)
- Determination and verification of molecular structure
- Detection of metabolites
- In proteins, both backbone (amide vibrations) and side chain (ligand binding) behavior can be followed (e.g., denaturation, folding, aggregation)
- In nucleic acids, the bases, the sugar and phosphate components can be studied independently
- In lipids, phase transitions (e.g., order-disorder) can be followed
- N.B.: in aqueous samples, due to water absorption, heavy water (D2O) is used instead.





FTIR microscopy

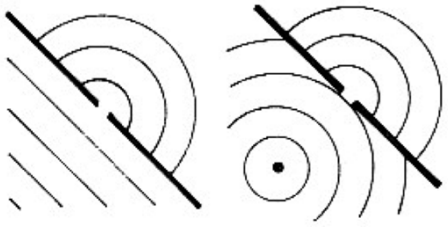


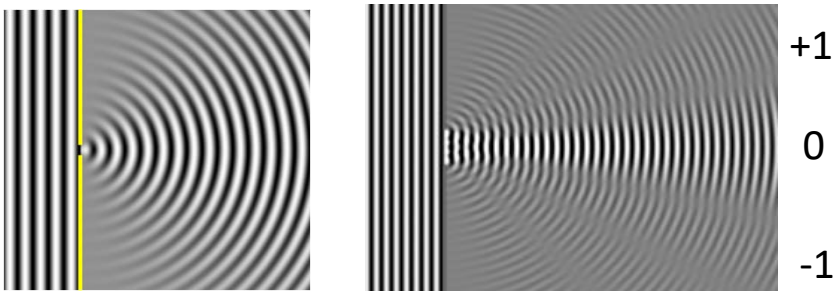
Dermal fibroblasts imaged at 1224 cm^{-1}

X-ray diffraction, crystallography

Foundations: wave diffraction and interference

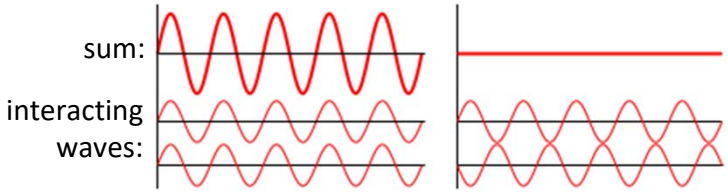
Slit smaller or comparable with the wavelength

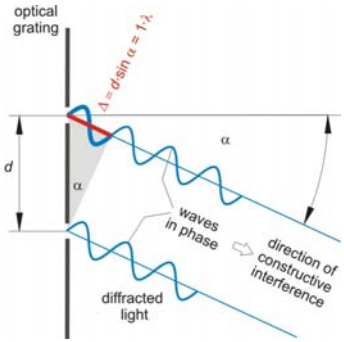




Waves in phase (φ=0): amplification

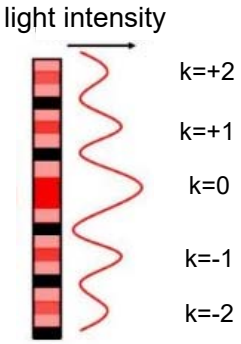
If $\varphi=\pi$: destruction



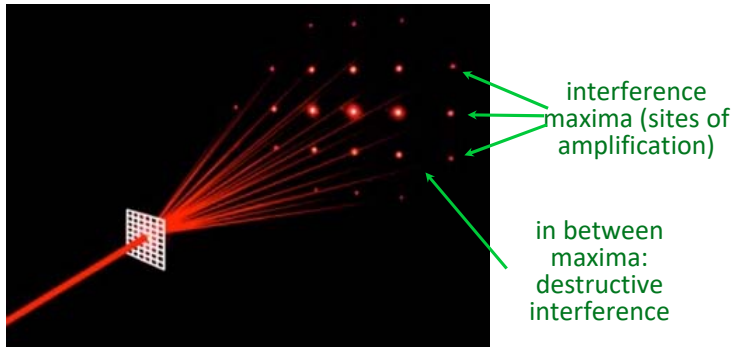


Lattice spacing (d) and wavelength (λ) are comparable

Diffraction pattern of a 1D optical grating



Diffraction pattern of a 2D optical grating

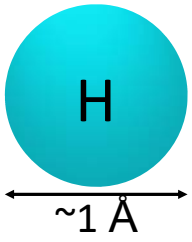


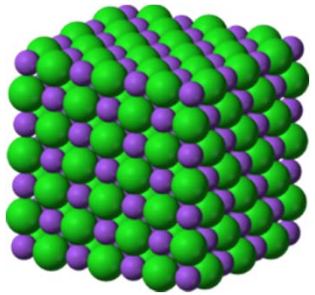
Condition of interference maxima:

$$d \sin\alpha = k \lambda$$ (see: Microscopy II lab)

$$d = k \frac{\lambda}{\sin\alpha} \quad k = 0, \pm 1, \pm 2 \ldots$$

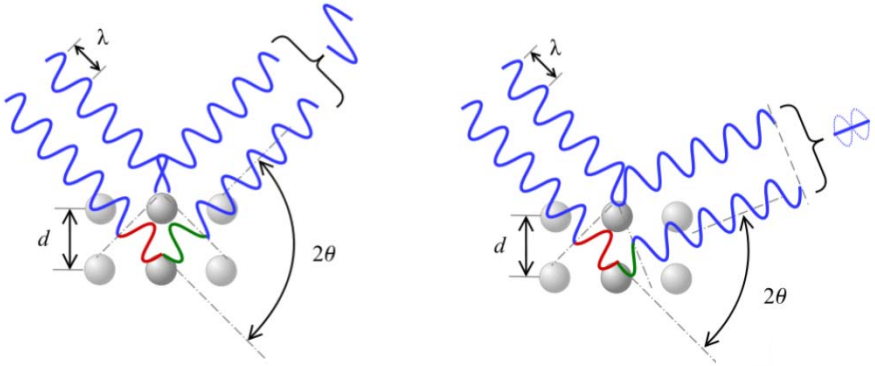
Molecular structure

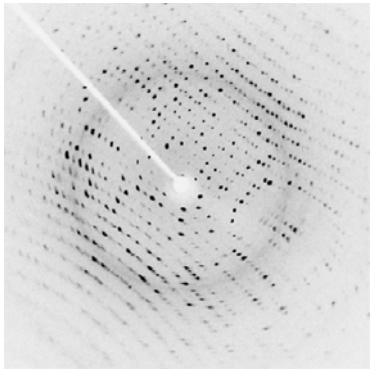


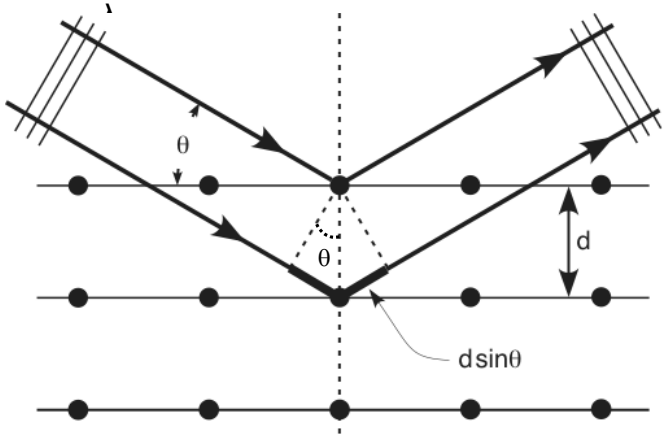


d_{NaCl}: 5.64 Å

Which kind of wave should be used for a molecular lattice? 0.01-10 nm
$\lambda_{X\text{-}ray}$: 0.01-10 nm = 0.1-100 Å



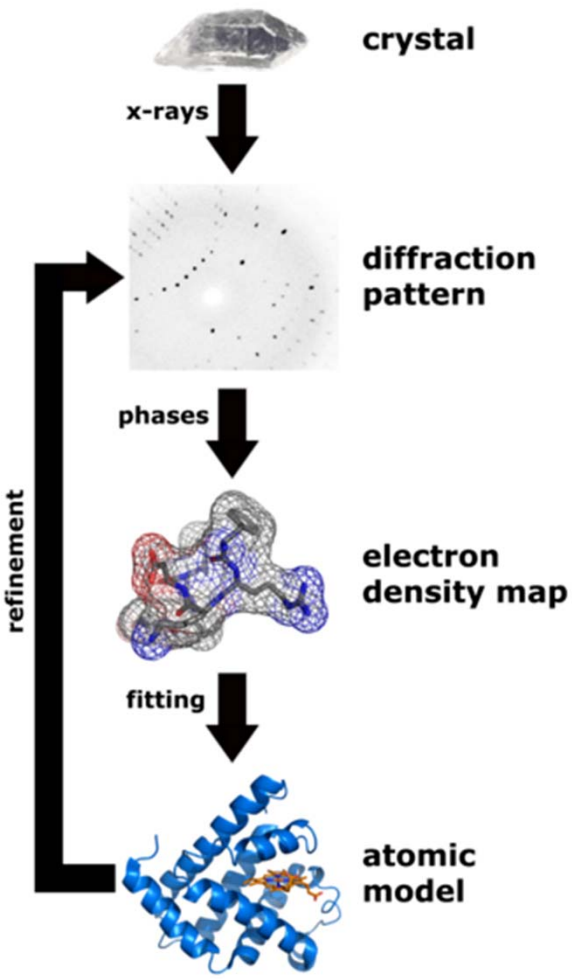


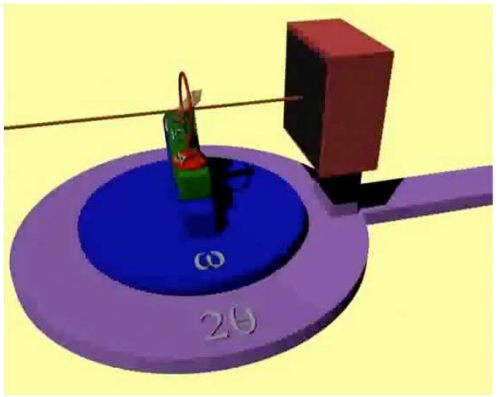


$2d\ sin\theta = k\ \lambda \longrightarrow d = \ldots$

more difficult…

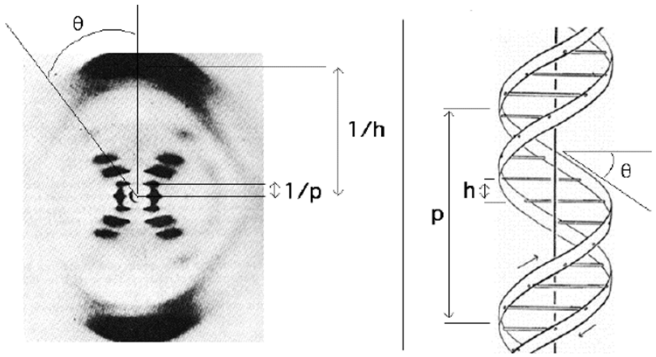
from the X-ray interference pattern:
spatial coordinates of atoms ⟶ spatial structure of the molecule


crystal
x-rays
diffraction pattern
phases
electron density map
fitting
atomic model
refinement


ω
2θ

Solving molecular structure with x-ray crystallography

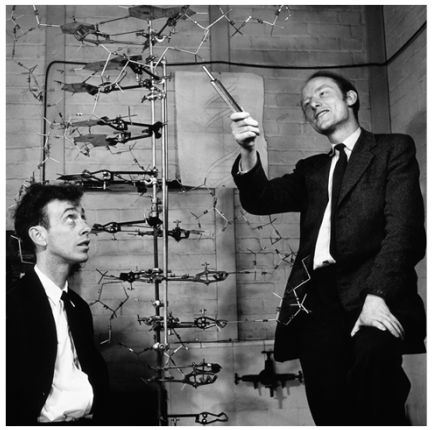
dsDNA



θ tilt of helix

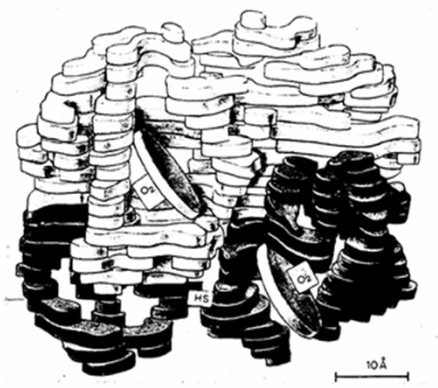
h = 3.4 Å distance between bases

p = 34 Å repeat unit of helix (one pitch)



J.D. Watson and F. Crick
Nobel-prize 1962

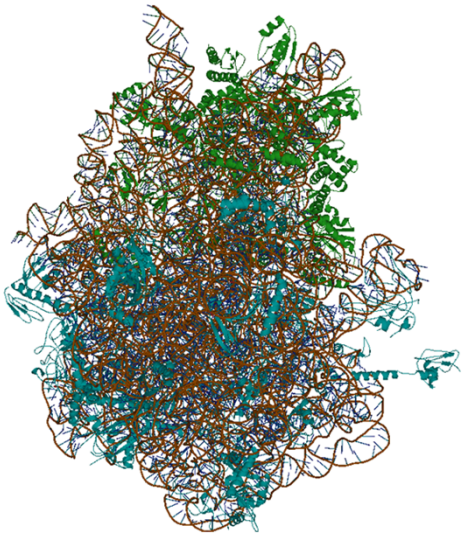
Globular protein: myoglobin



~1200 atoms

M. F. Perutz, J. C. Kendrew
Nobel-prize 1962

Molecular complex: ribosome



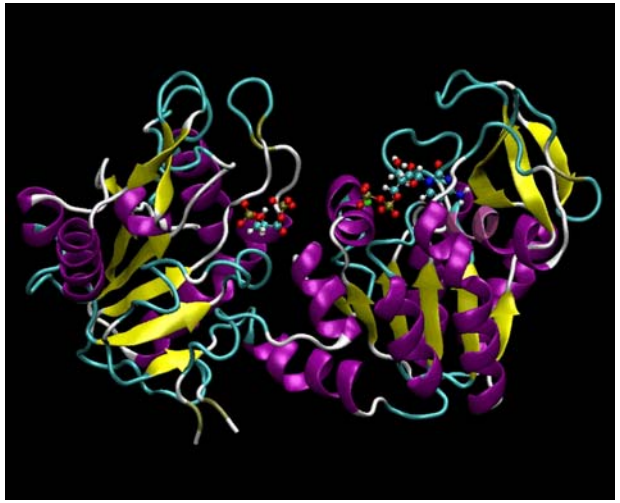
30S subunit: ~35000 atoms,
50S subunit:~64000 atoms

V. Ramakrishnan, T. A. Steitz, A. E. Yonath
Nobel-prize 2009

Structure - Function

X-ray crystallography:

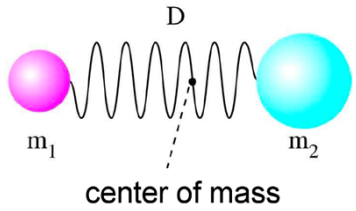
3D *structure of the* molecule – static image



Functional motions of the molecule?

FTIR:

bond *vibrations*



atomic coordinates

spring constants

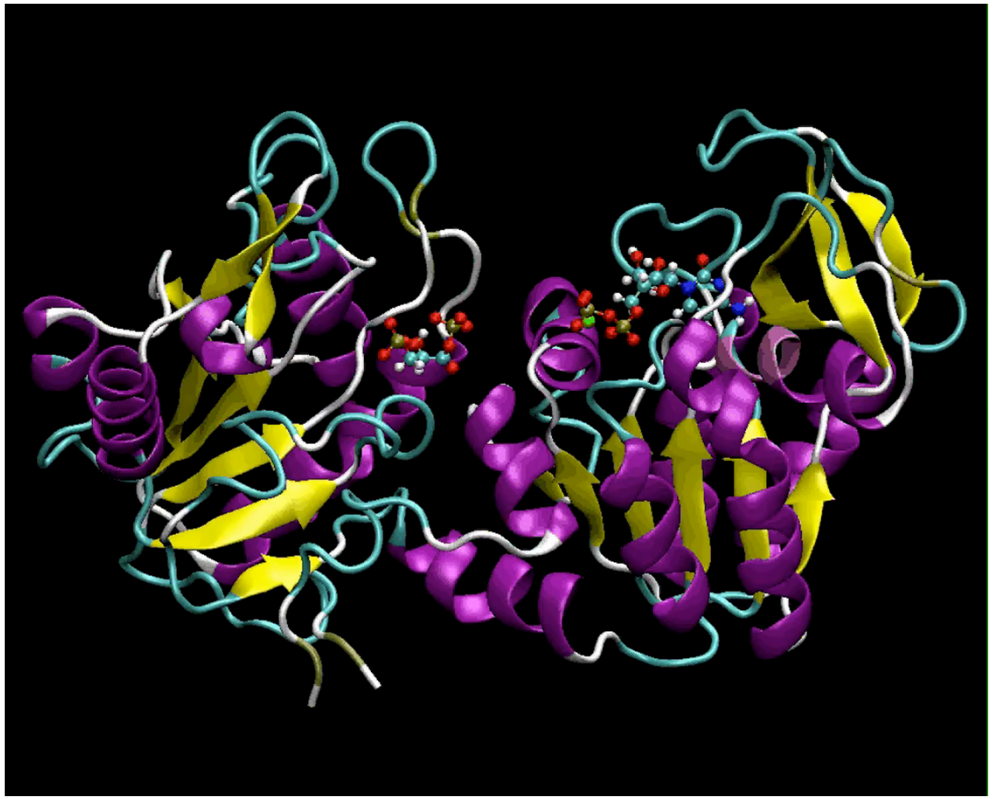
Molecular Dynamic (MD) simulations

calculates internal motions of the molecule

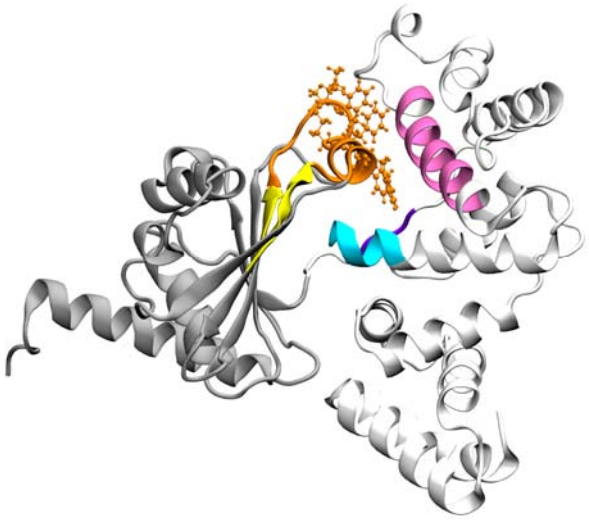
Molecular dynamic simulation

Aim:

- starting from experimental data to map the internal motion of macromolecules (to understand their function),
- to give atomic interpretation to experimental results.



Phosphoglycerate kinase (PGK)



RalF:

- effector of the virus causing legionella disease (sever pneumonia).

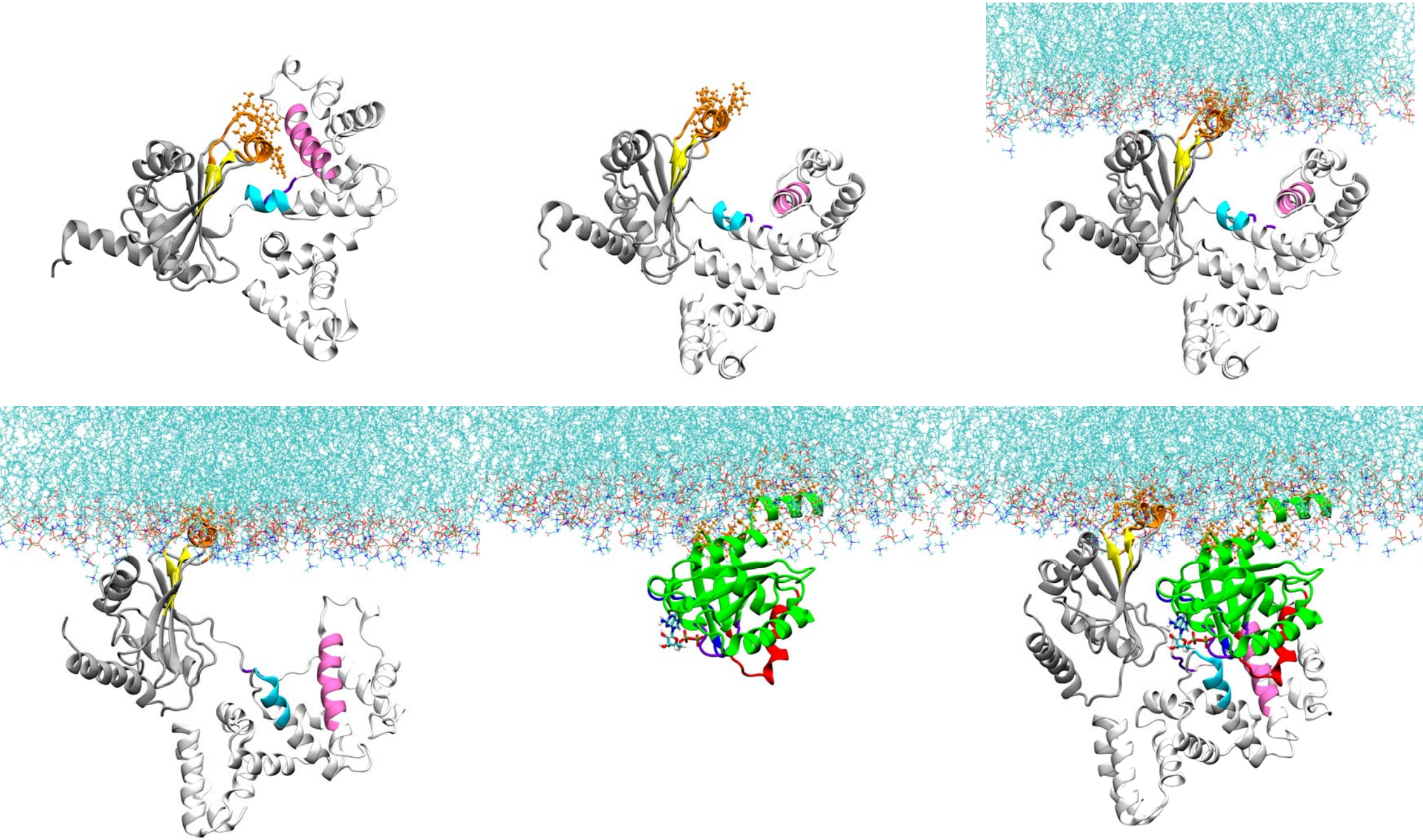
Experiment:

- inactive crystal structure,
- it gets activated by attaching to the membrane (aa denoted by orange).

But: proteins attached to the membranes can not be crystalized.
The structure of the active form can not be crystalized.

How does it work?

Simulation



complementarity of experiment and simulation