

# 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



Human



Dodo Bird



Rafflesia



Beach ball

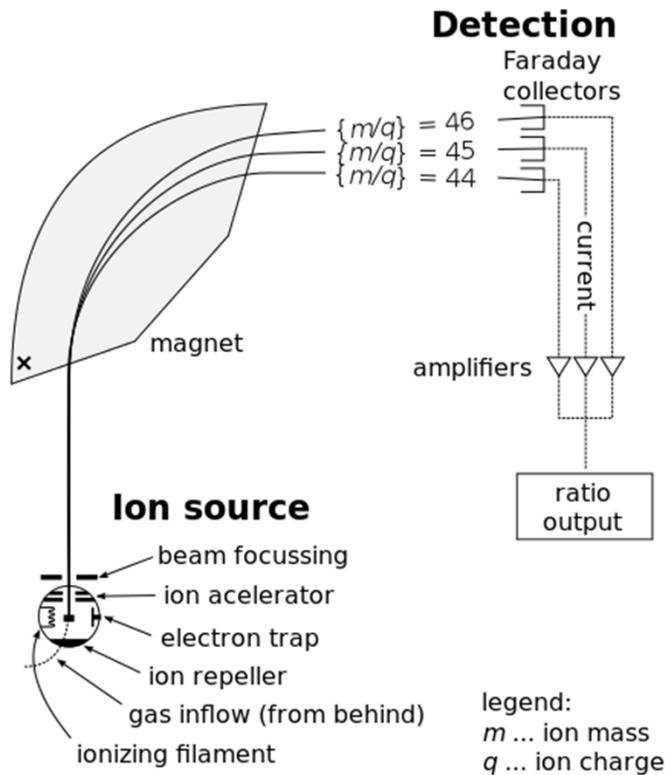
$10^{0.0}$

# Mass spectrometry

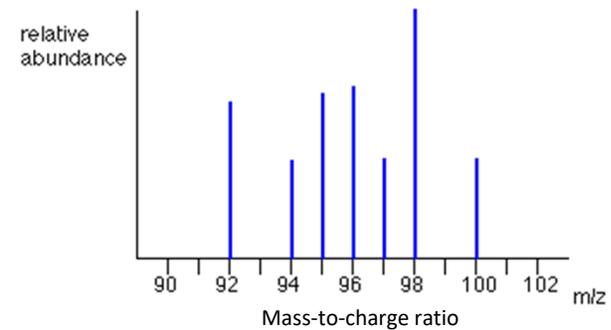
- analytical technique producing spectra of the masses of the atoms or molecules in a sample. The spectra are used to determine the elemental or isotopic signature, thereby elucidating the chemical structures of molecules.

Steps:

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



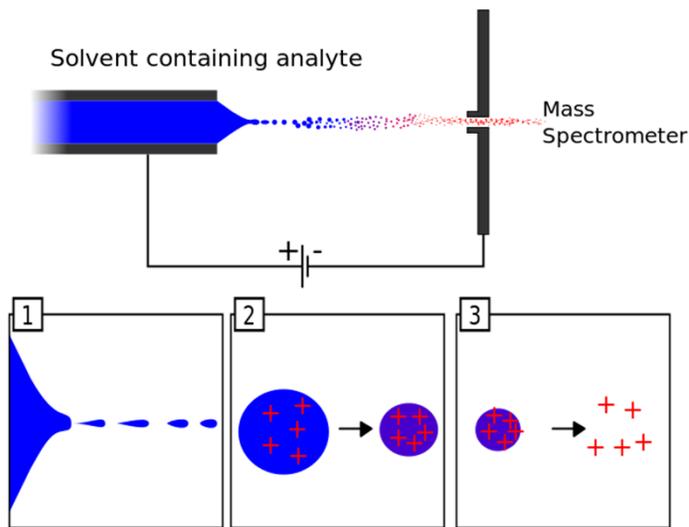
Result: "Stick" diagram



Spectrum is compared with structure database

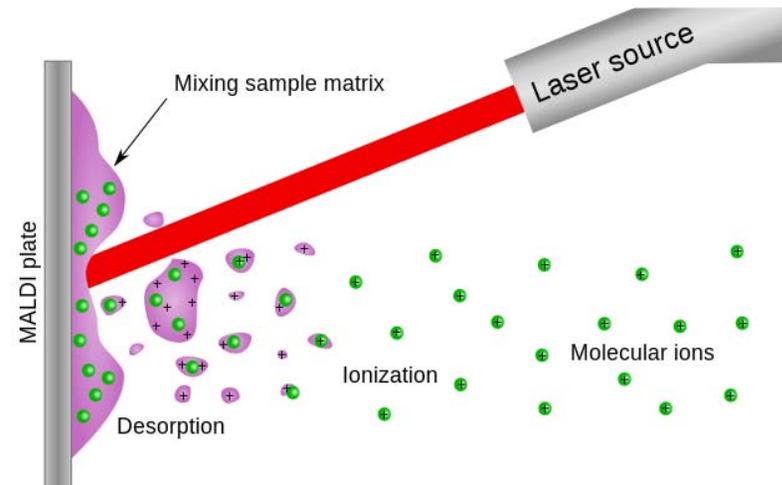
# Ionization of biological samples

## Electrospray ionization



- (1) decompositions to droplets,
- (2) solvent evaporation → smaller droplet  
→ greater surface charge,
- (3) Coulomb repulsion → droplets explode →  
ionized, accelerated molecules .

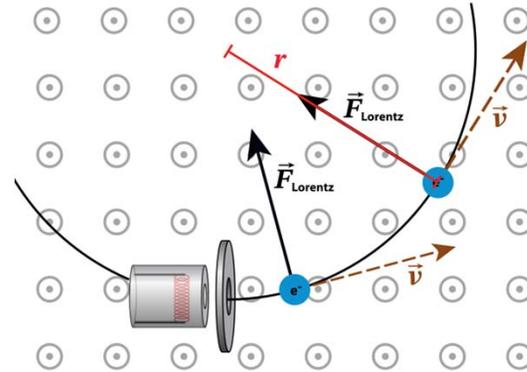
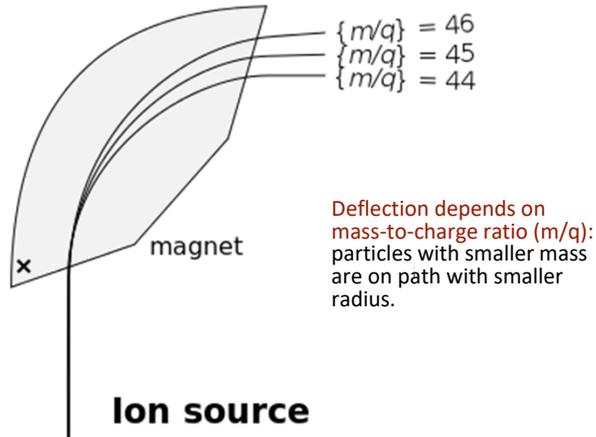
## MALDI: “matrix-assisted laser desorption/ionization”



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

# Methods of mass analysis 1.

## Magnetic method



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

$E$ =electric field,  $v \times B$ =vectorial product of speed and magnetic induction

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

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

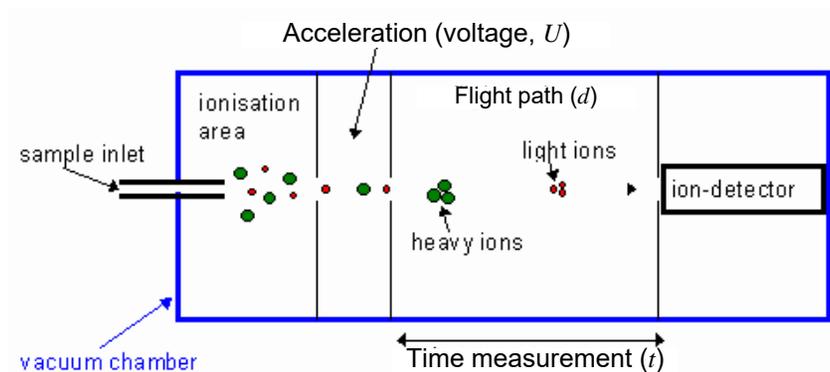
$$r = \frac{m}{q} \frac{v}{B}$$

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



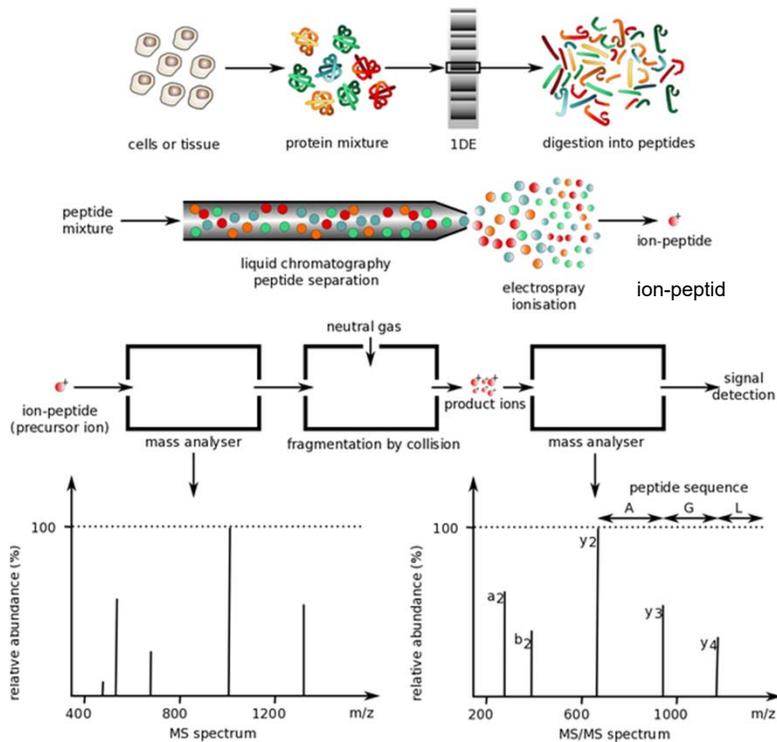
$$qU = \frac{1}{2}mv^2 = \frac{1}{2}m\left(\frac{d}{t}\right)^2$$

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

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

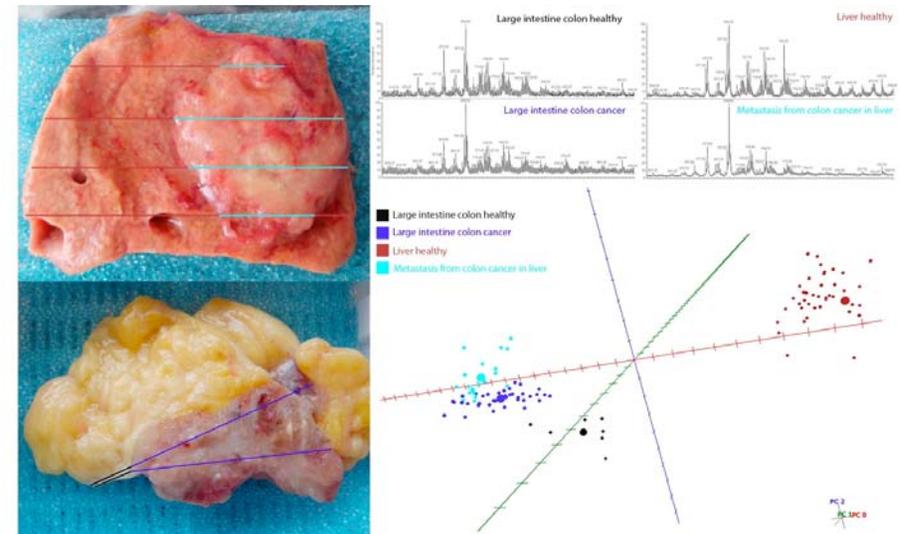
# Applications of mass spectrometry

## 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”)

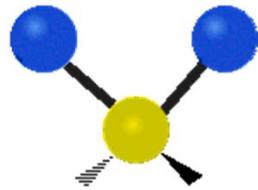


# Infrared (IR) spectroscopy

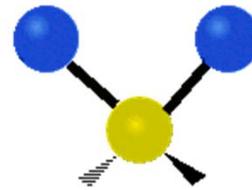
- measures vibrations of molecules.

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

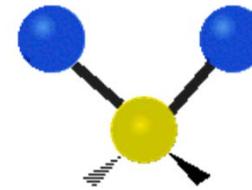
Examples of vibrational motion in the triatomic methylene group (-CH<sub>2</sub>-):



*Asymmetric stretching*



*Symmetric stretching*

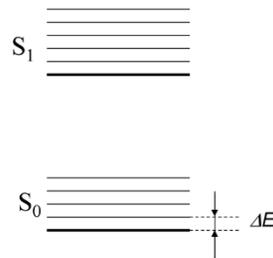


*Scissoring*

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:

$$E_e \overset{\sim 100x}{>} E_v \overset{\sim 100x}{>} E_r$$

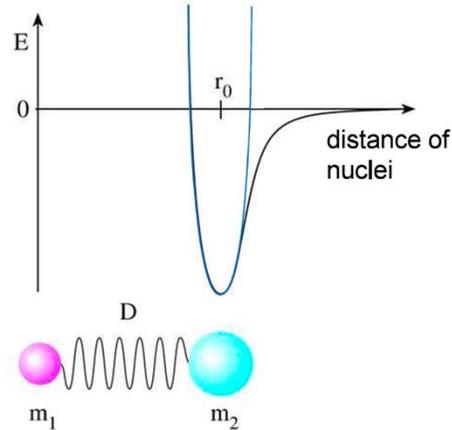
$$\sim 3 \times 10^{-19} \text{ J } (\sim 2 \text{ eV}) > \sim 3 \times 10^{-21} \text{ J} > \sim 3 \times 10^{-23} \text{ J}$$

$$UV/VIS > mid IR > far IR$$

# Molecular vibrations

Molecule: mass connected by a spring

- two-atomic molecule (e.g., CO)
- masses ( $m_1, m_2$ ): atomic nuclei ( $m_e \ll m_{\text{nucleus}}$ )
- 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



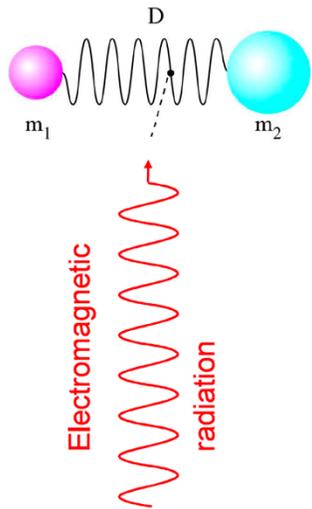
$$f = \frac{1}{2\pi} \sqrt{\frac{D}{m_{red}}} = \frac{\Delta E}{h}$$

(see: Resonance lab)

where:

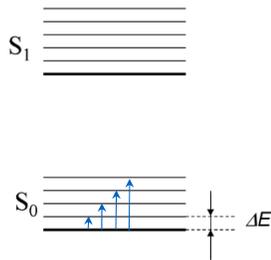
$$m_{red} = \frac{m_1 m_2}{m_1 + m_2}$$

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



In IR spectroscopy, the wavenumber ( $\nu$ ) is used:

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



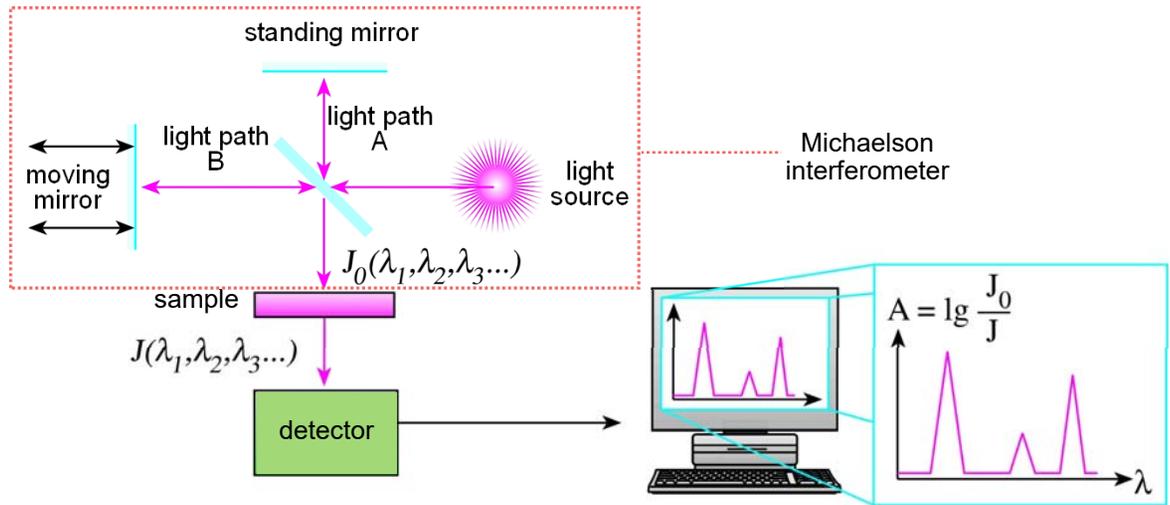
Values for the CO molecule: measured wavenumber,  $\nu = 2143 \text{ cm}^{-1}$

$\lambda = 4,67 \text{ }\mu\text{m}$ ,  $f = 6,43 \times 10^{13} \text{ Hz}$  (64,3 THz),  $D = 1875 \text{ N/m}$

# IR spectroscopy - measurement

## Fourier Transform Infrared (FTIR) Spectroscopy:

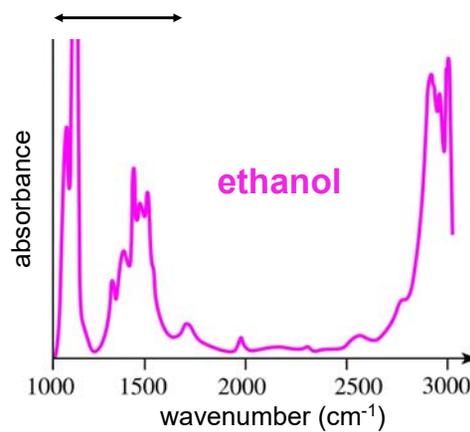
- multiple wavelengths are generated (with a Michelson interferometer)
- Intensities at multiple wavelengths are converted to wavelength-dependent intensities.



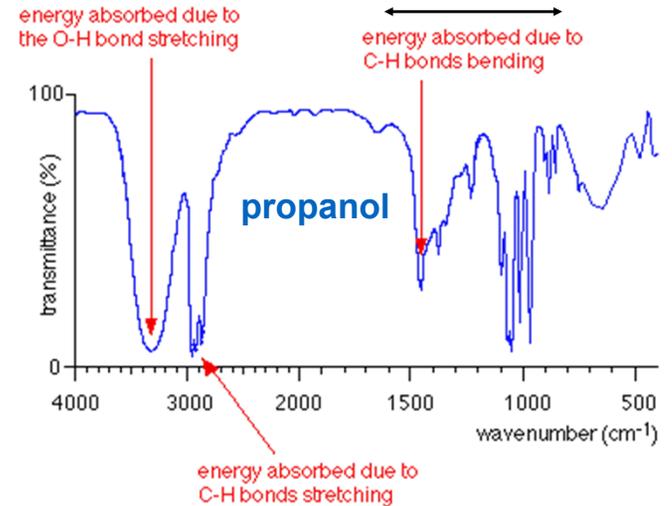
## IR spectrum:

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

“Fingerprint” regime

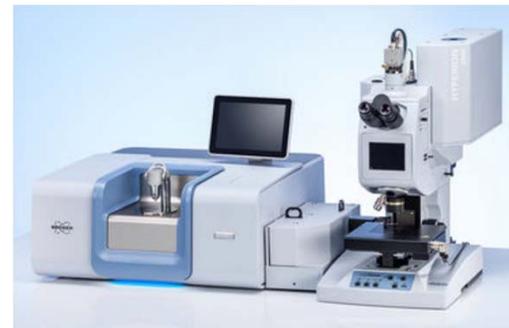
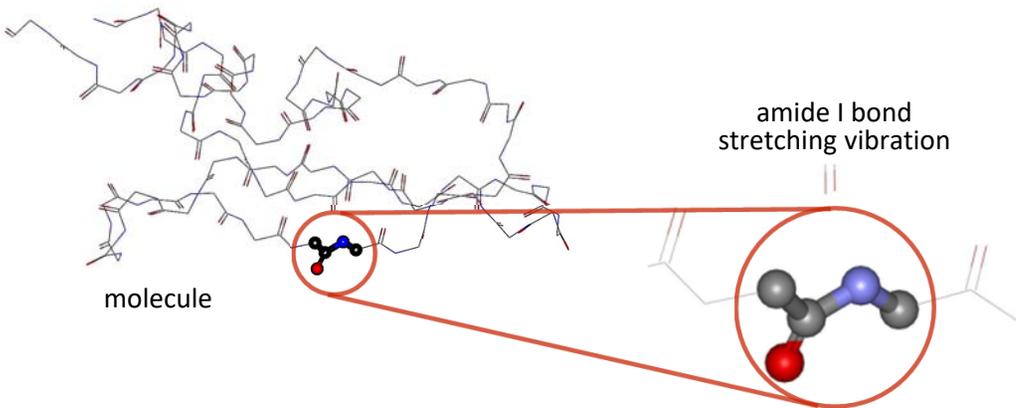
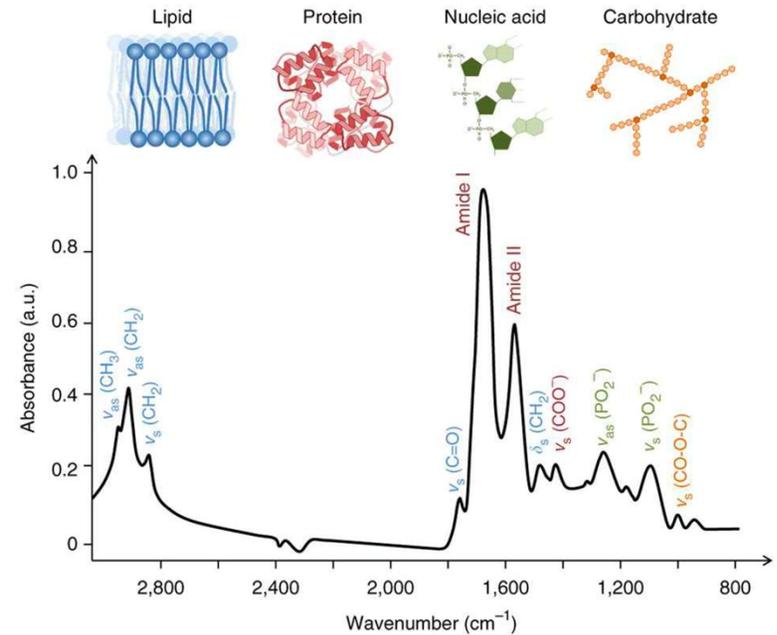


“Fingerprint” regime

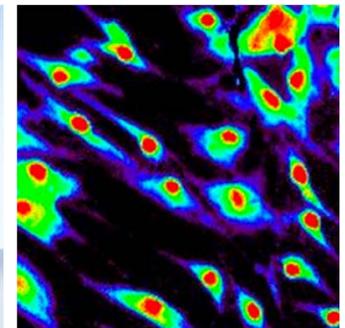


# Applications of IR spectroscopy

- 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 (D<sub>2</sub>O) is used instead.



FTIR microscopy

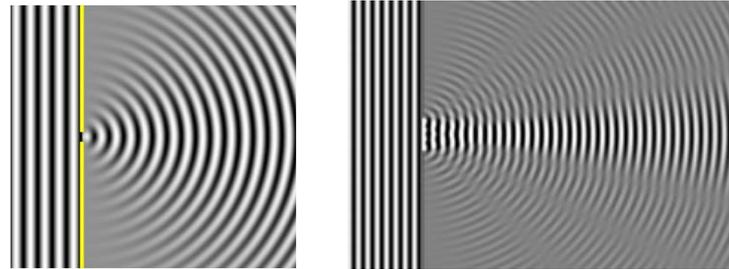
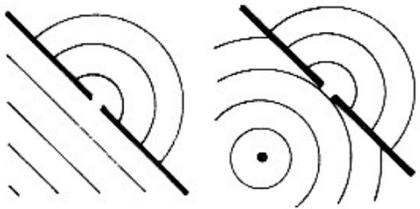


Dermal fibroblasts imaged at 1224 cm<sup>-1</sup>

# X-ray diffraction, crystallography

## Foundations: wave diffraction and interference

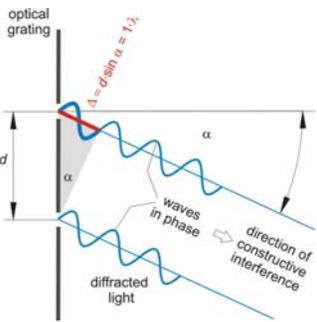
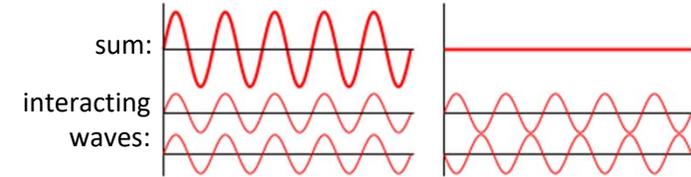
Slit smaller or comparable with the wavelength



+1  
0  
-1

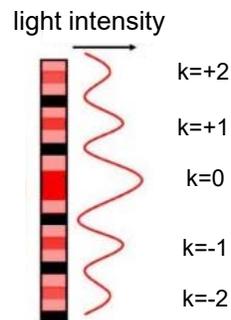
Waves in phase ( $\varphi=0$ ):  
amplification

If  $\varphi=\pi$ :  
destruction

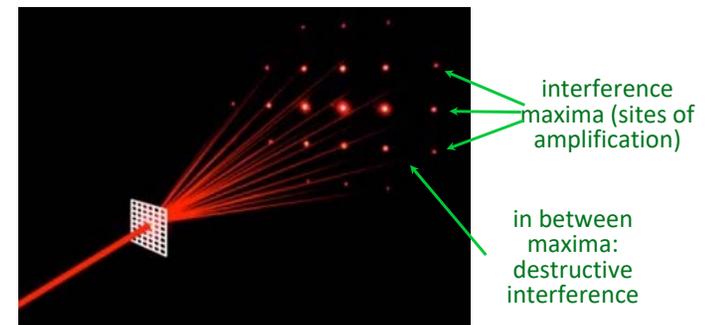


Lattice spacing ( $d$ ) and wavelength ( $\lambda$ ) are comparable

Diffraction pattern of a 1D optical grating



Diffraction pattern of a 2D optical grating

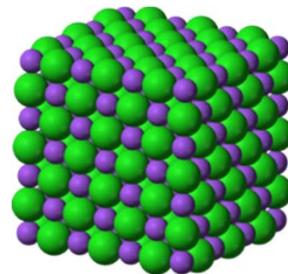
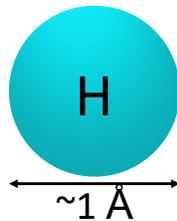


Condition of interference maxima:

$$d \sin \alpha = k \lambda \quad (\text{see: Microscopy II lab})$$

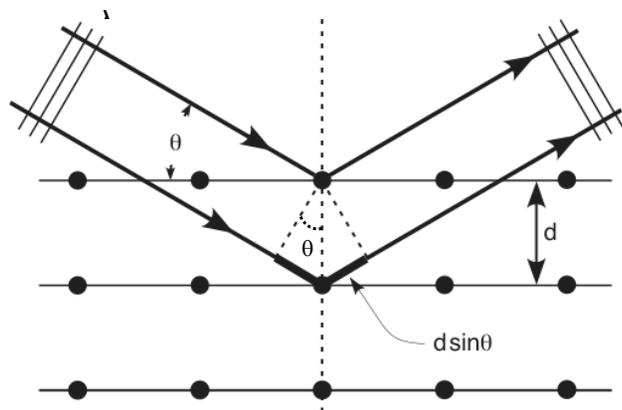
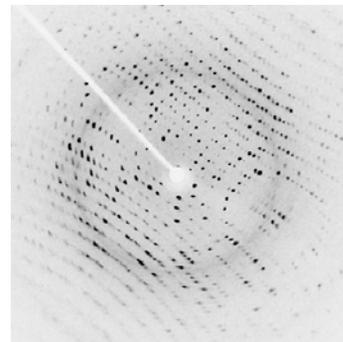
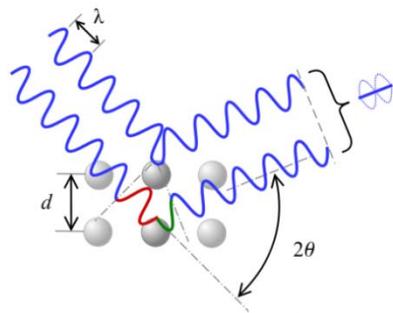
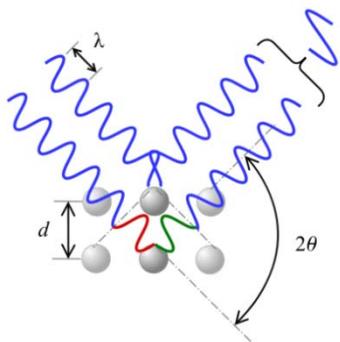
$$d = k \frac{\lambda}{\sin \alpha} \quad k = 0, \pm 1, \pm 2 \dots$$

# Molecular structure



$d_{\text{NaCl}}: 5.64 \text{ \AA}$

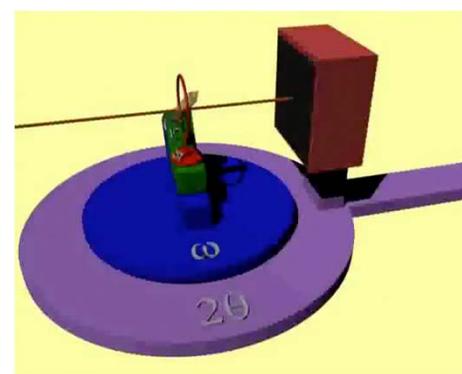
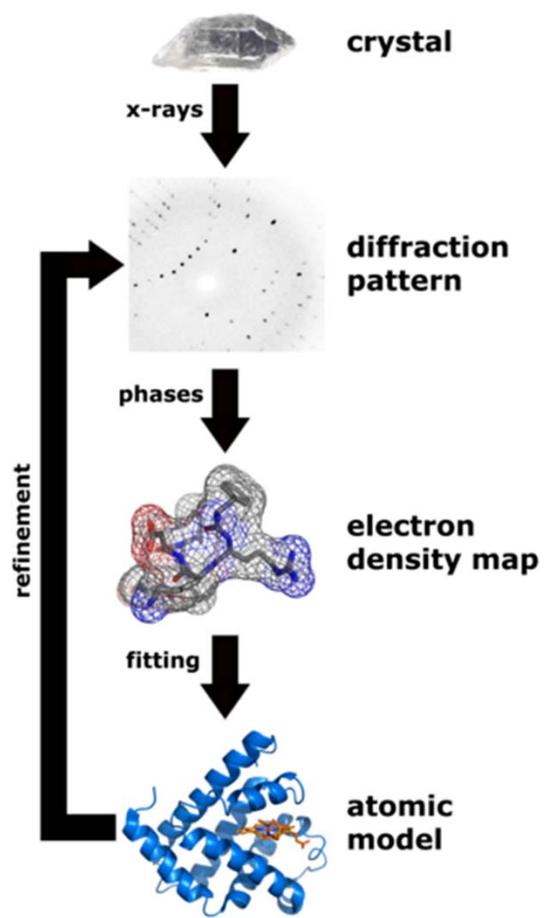
Which kind of wave should be used for a molecular lattice? 0.01-10 nm  
 $\lambda_{\text{X-ray}}: 0.01-10 \text{ nm} = 0.1-100 \text{ \AA}$



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

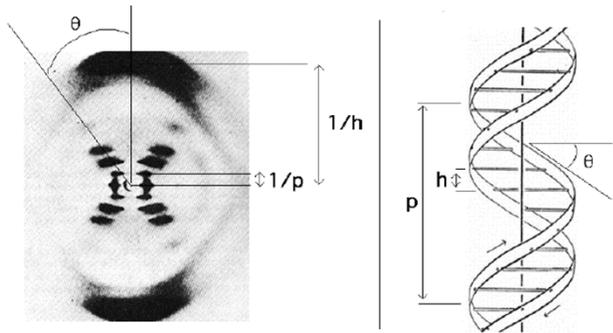
more difficult...

from the X-ray interference pattern:  
 spatial coordinates of atoms  $\longrightarrow$  spatial structure of the molecule

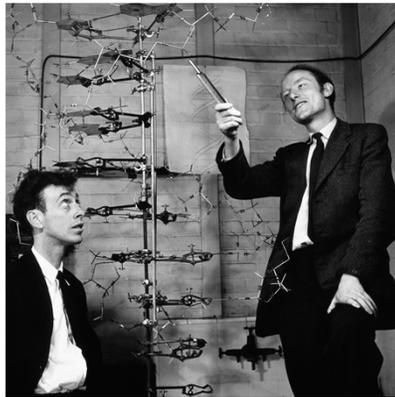


# Solving molecular structure with x-ray crystallography

dsDNA



$\theta$  tilt of helix  
 $h = 3.4 \text{ \AA}$  distance between bases  
 $p = 34 \text{ \AA}$  repeat unit of helix (one pitch)



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

Globular protein:  
 myoglobin

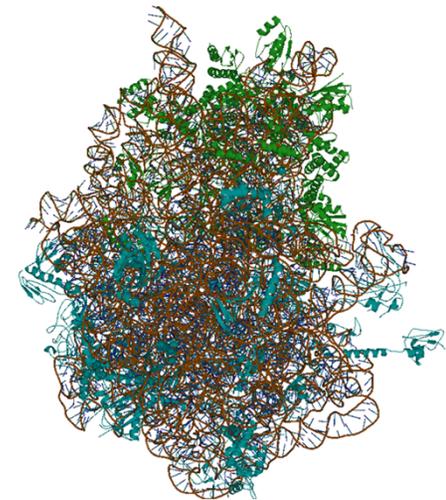


~1200 atoms



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

Molecular complex:  
 ribosome



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

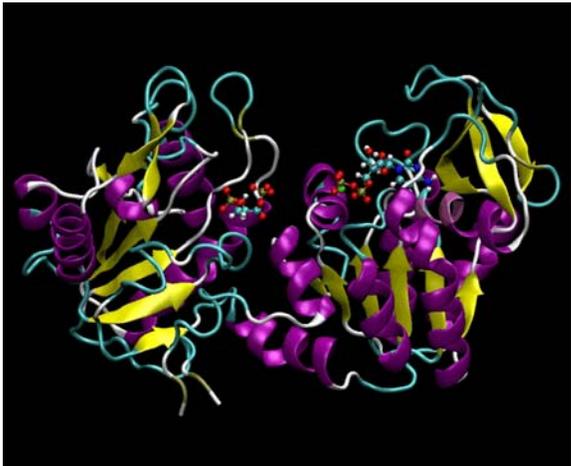


V. Ramakrishnan, T. A. Steitz, A. E. Yonath  
 Nobel-prize 2009

# Structure - Function

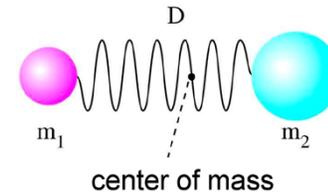
X-ray crystallography:

3D *structure of the molecule* – static image



FTIR:

bond *vibrations*



Functional motions  
of the molecule?

atomic coordinates

spring constants

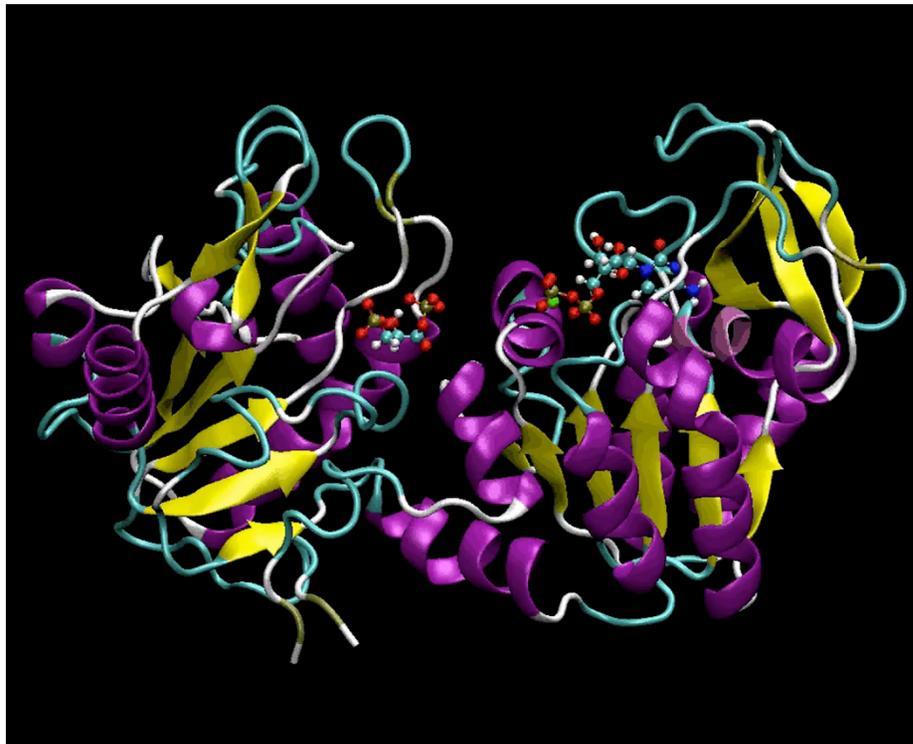
Molecular Dynamic (MD) simulations

calculates internal motions of the molecule

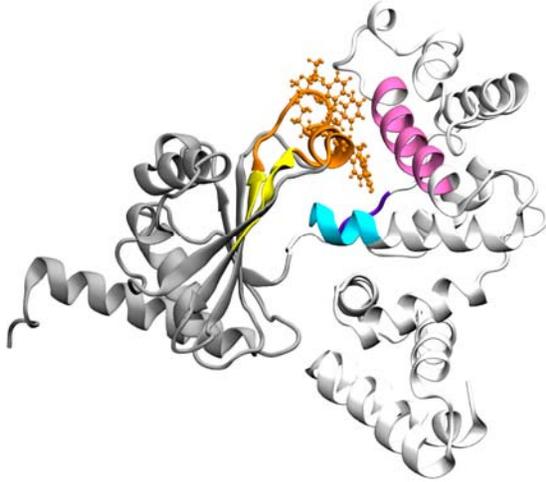
# Molecular dynamic simulation

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).

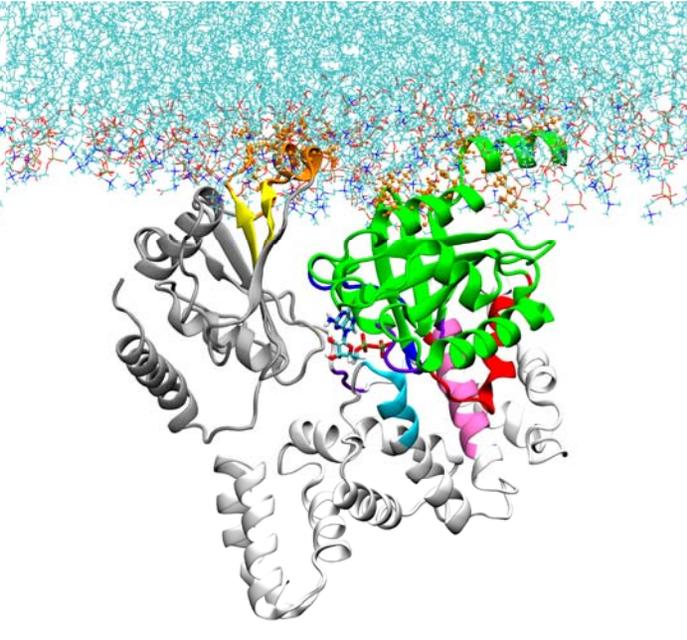
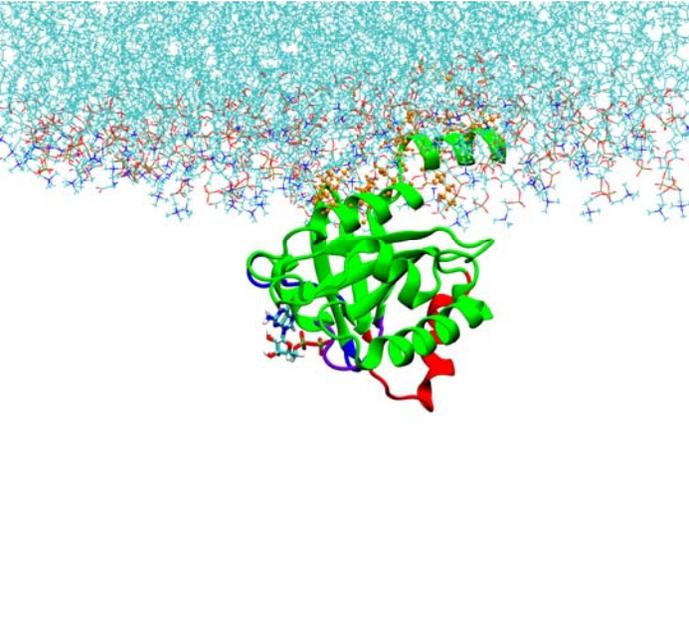
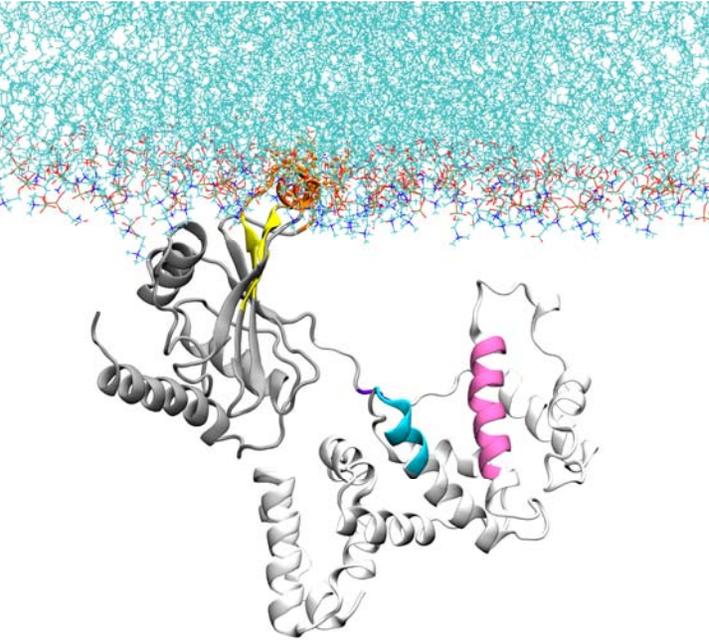
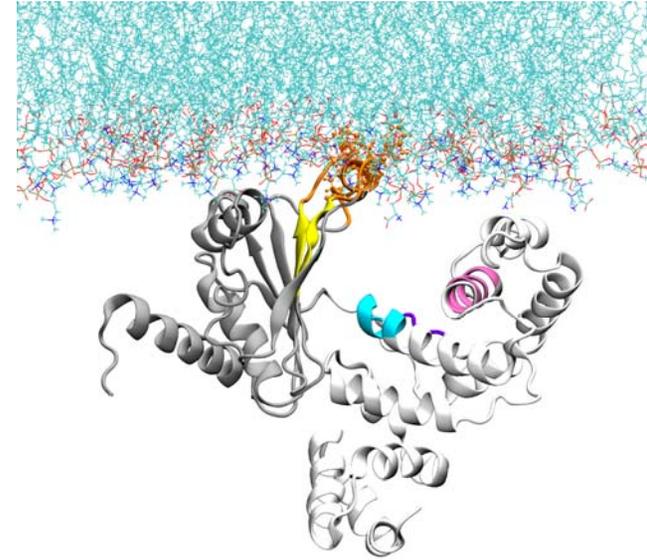
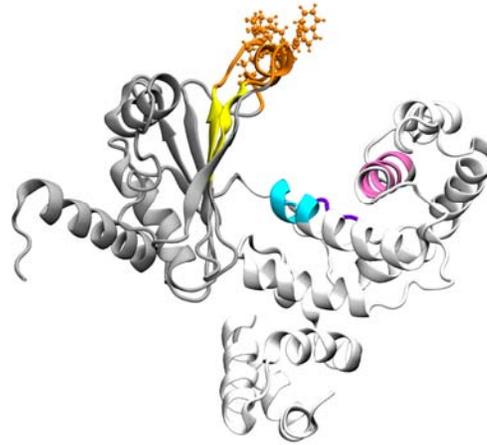
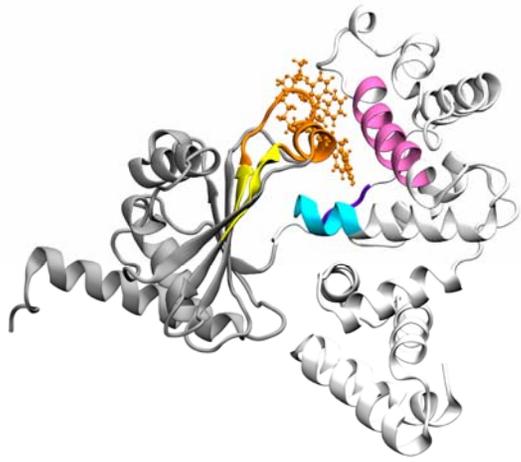
### Experiment:

- 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*