STRUCTURE AND DYNAMICS OF BIOMOLECULAR SYSTEMS

MASS SPECTROMETRY , X-RAY DIFFRACTION

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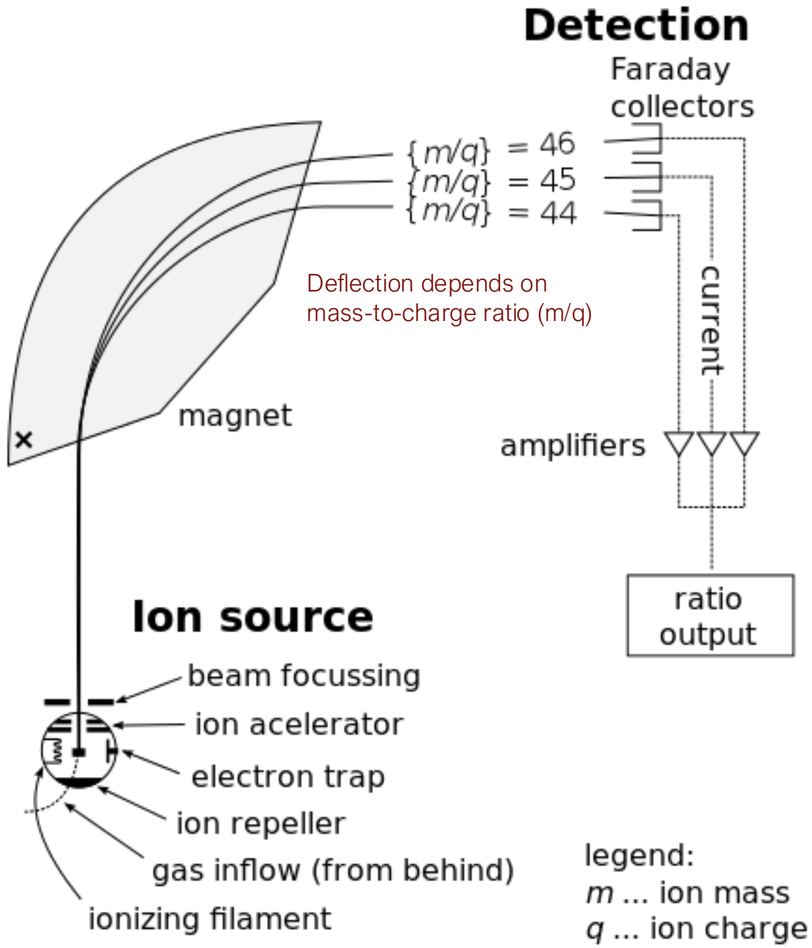
MOLECULAR COMPOSITION: MASS SPECTROMETRY

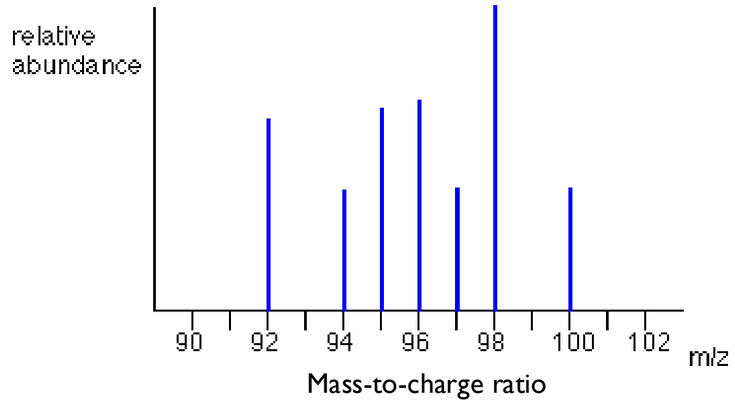
Mass spectrometry (MS): analytical technique producing spectra of the masses of the atoms or molecules in a sample. The spectra are used to determine the elemental or isotopic signature, thereby elucidating the chemical structures of molecules.

Steps:

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



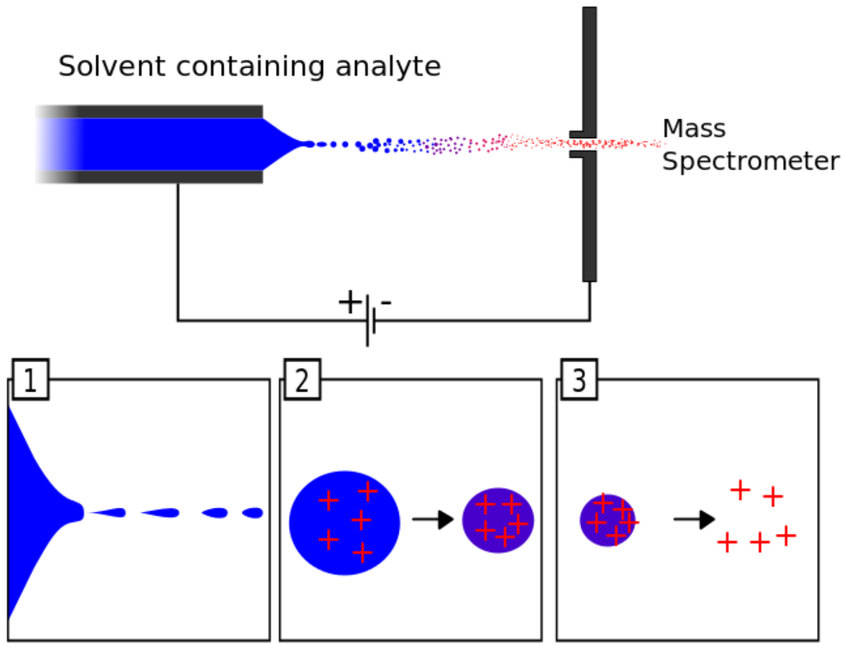
Result: "Stick" diagram



Spectrum is compared with structure database

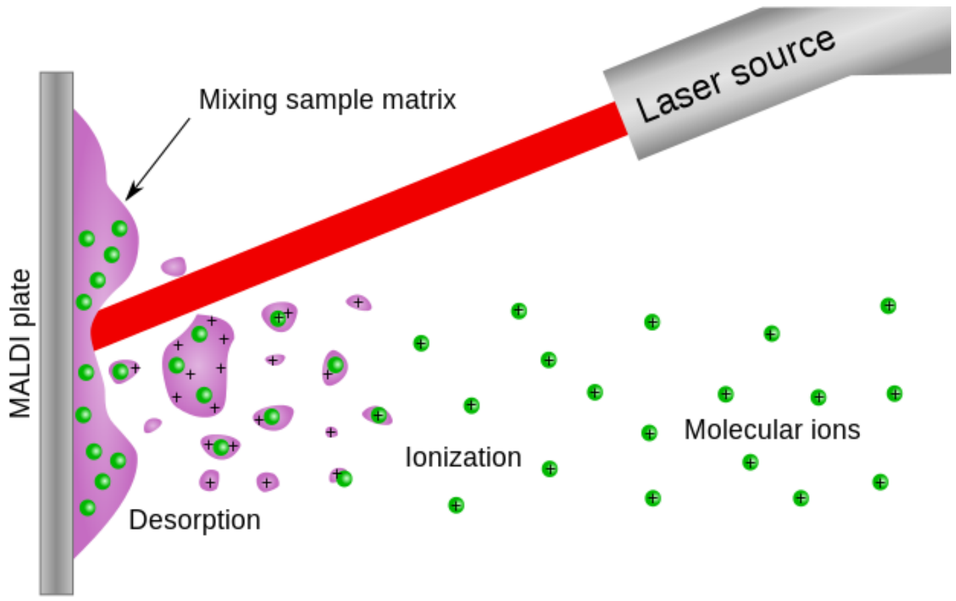
IONIZATION OF BIOLOGICAL SAMPLES

Electrospray ionization



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

MALDI: “matrix-assisted laser desorption/ionization”



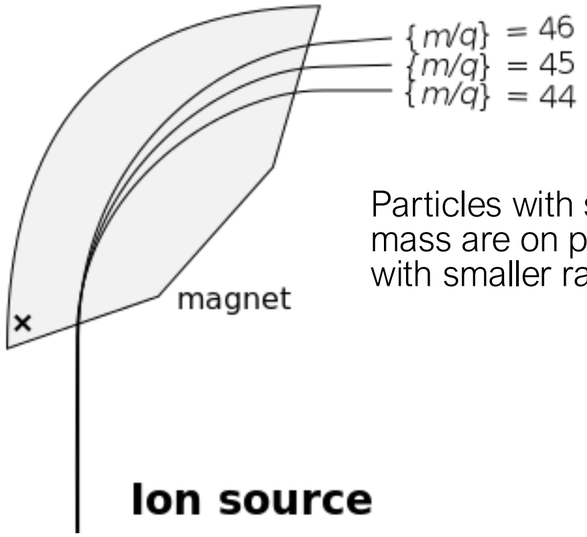
Protein sample is mixed with organic substance (matrix) which absorps laser light and volatile

Laser light is absorbed by the atoms/molecules of the matrix.

Ideal for investigating large molecules.

METHODS OF MASS ANALYSIS

Magnetic method



Particles with smaller mass are on path with smaller radius.

Lorentz force accelerates (a) particles of mass m and charge q:

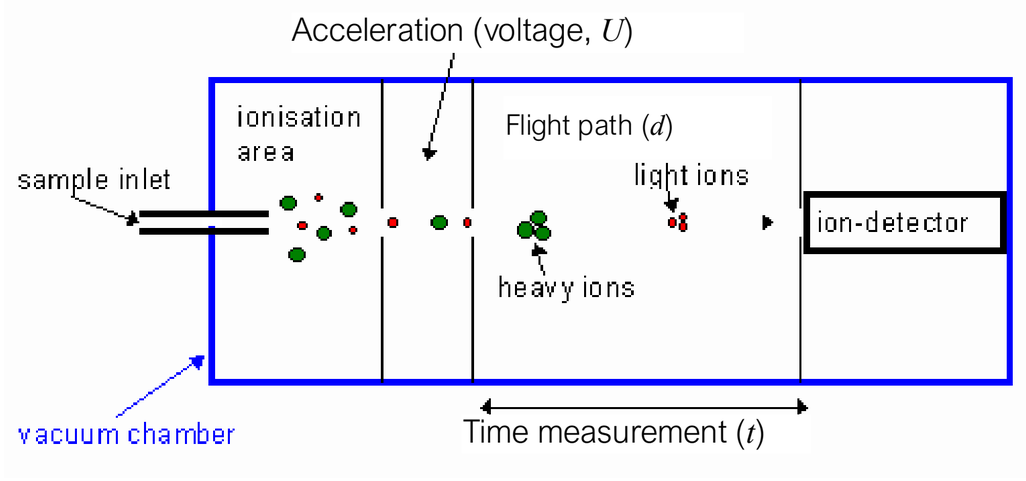
$$q\left(E + v \times B\right) = ma$$

E=electric field
vxB=vectorial product of speed and magnetic induction

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

$$\frac{m}{q} = \frac{E + v \times B}{a}$$

"Time-of-flight" method



Potential energy of charged particle (qU) is converted into kinetic energy:

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

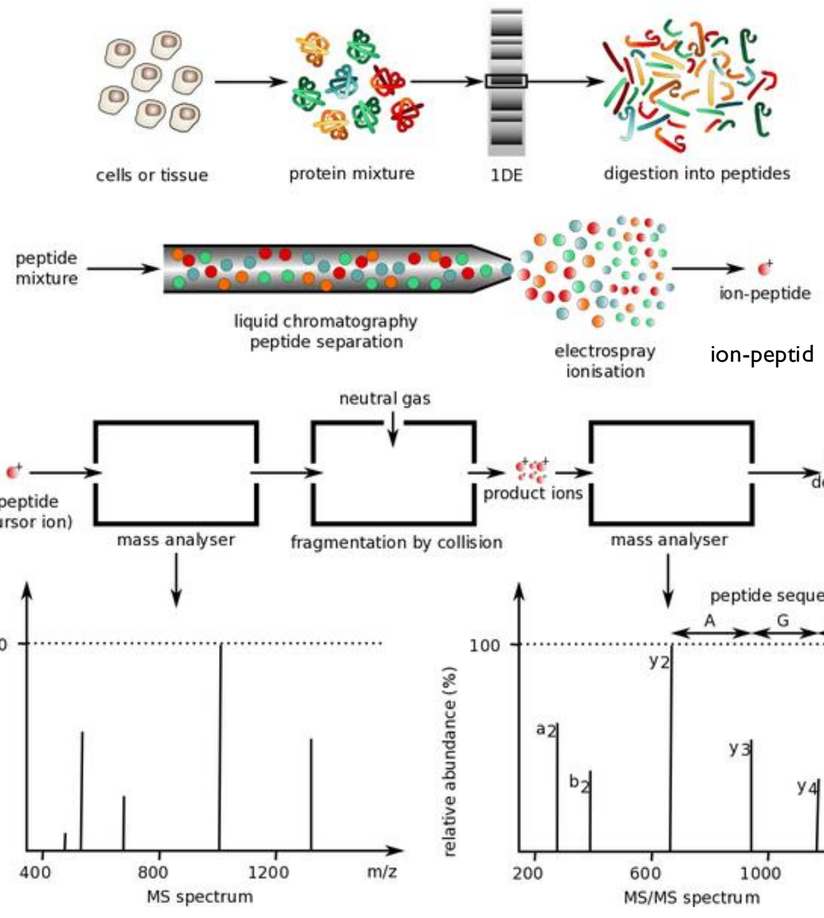
from which time (t) and hence m/q can be calculated:

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

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

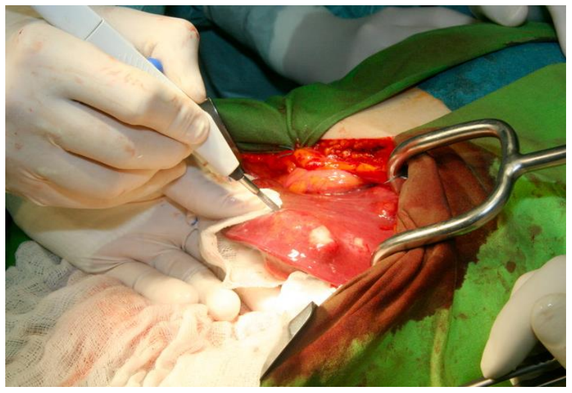
MASS SPECTROMETRY APPLICATIONS

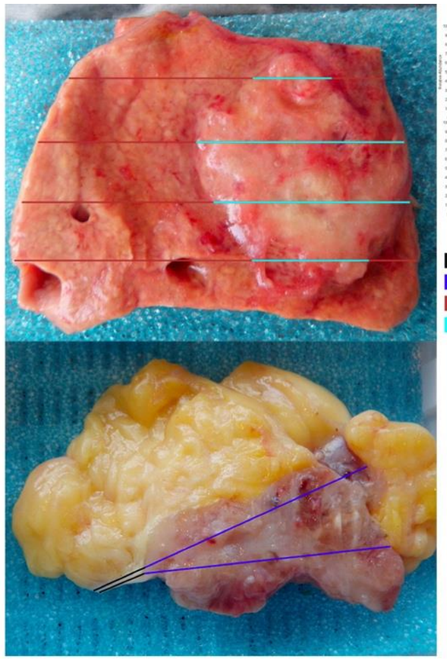
1. **Protein analytics** (proteomics)

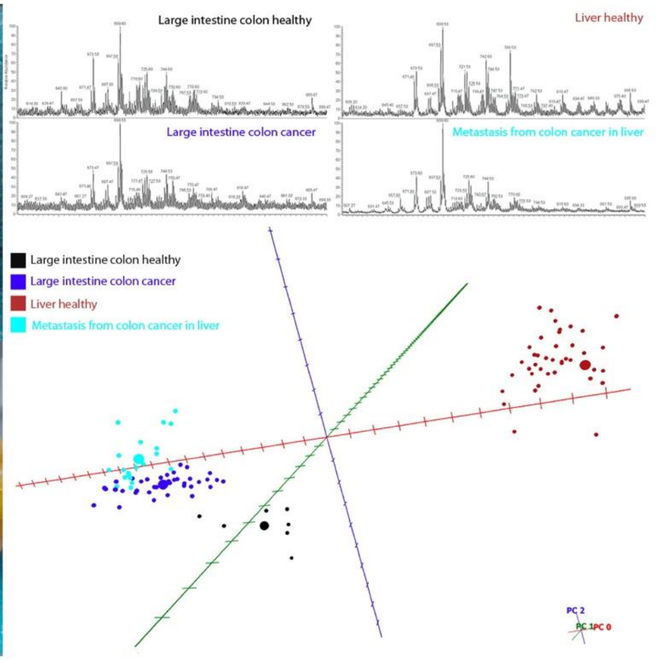


2. Diagnostic screening:
Metabolic diseases (from 1 drop of blood)
e.g., phenylketonuria (PKU)

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



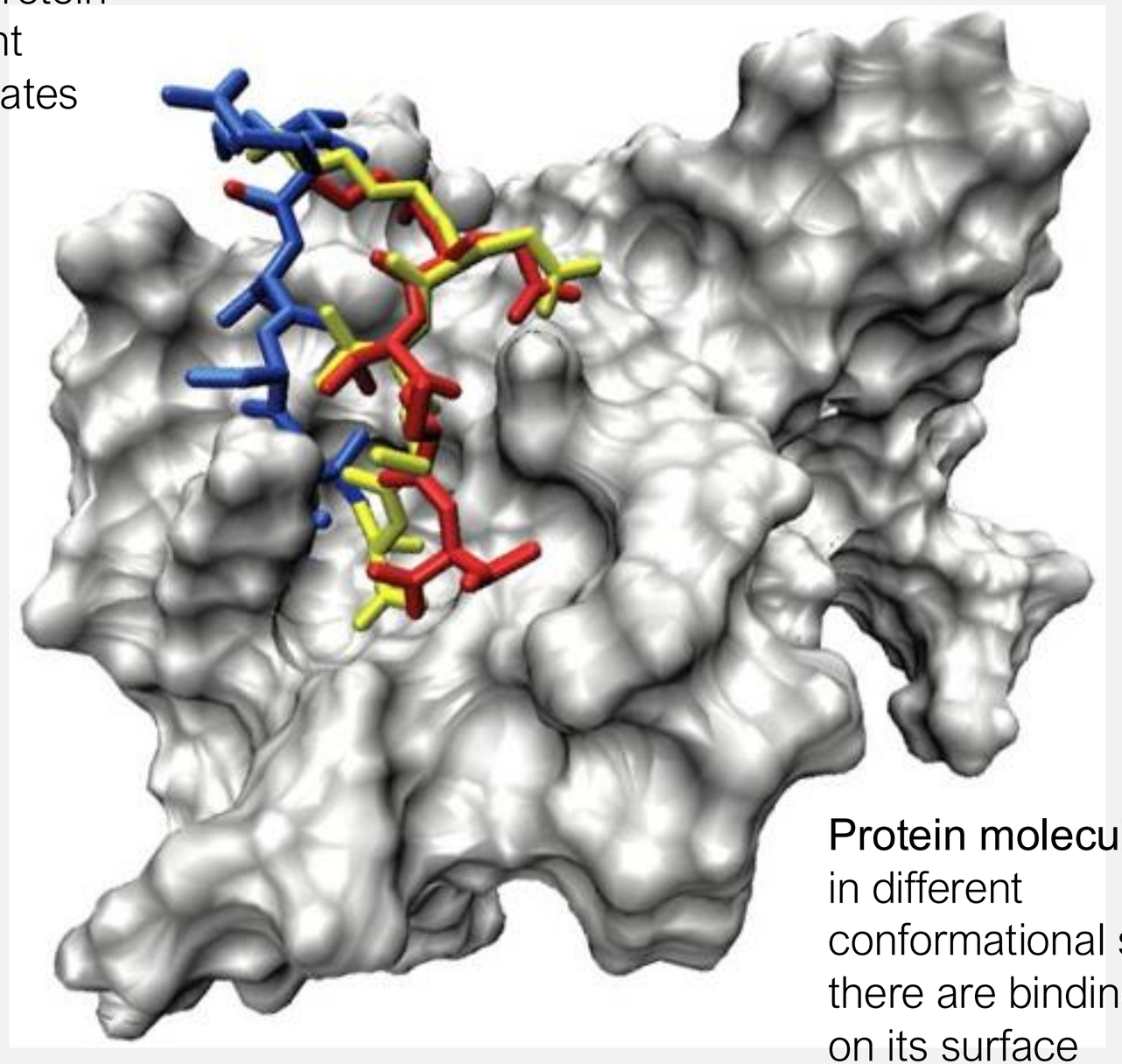




- **Electrosurgical cutting**: The surgeon uses the knife to cut tissues with electric current. This generates heat, which vaporizes part of the tissue—producing smoke.
- **Smoke analysis**: The smoke contains the molecular fingerprints of the tissues, which the onkoknife directs into a mass spectrometer.
- **Real-time diagnosis**: The device compares the obtained spectrum with a database and immediately provides feedback on whether the cut tissue is cancerous or healthy.

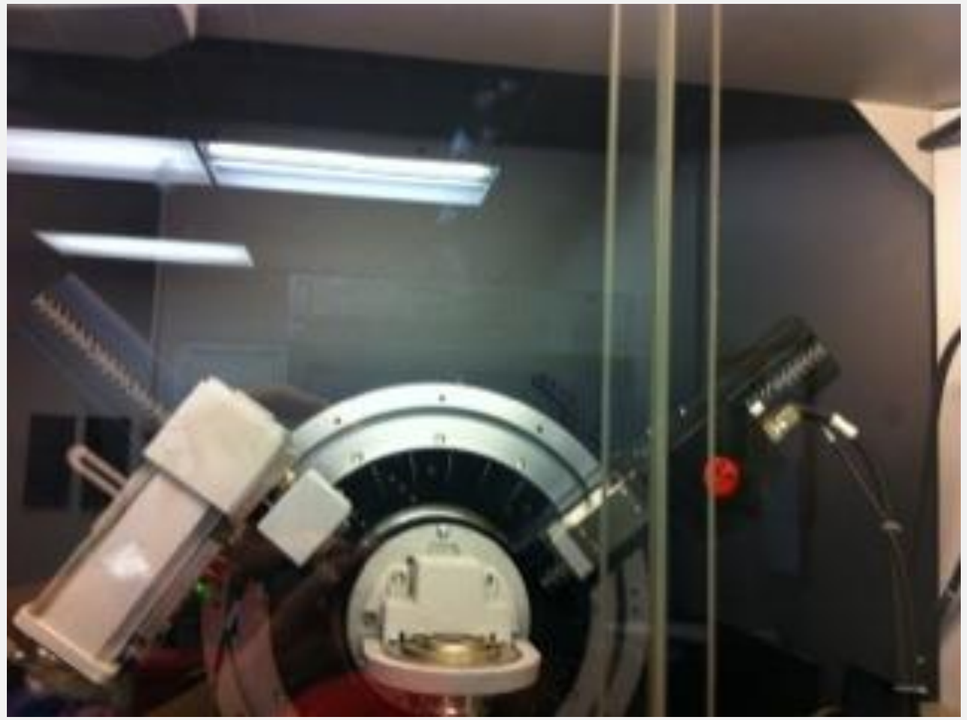
STRUCTURE

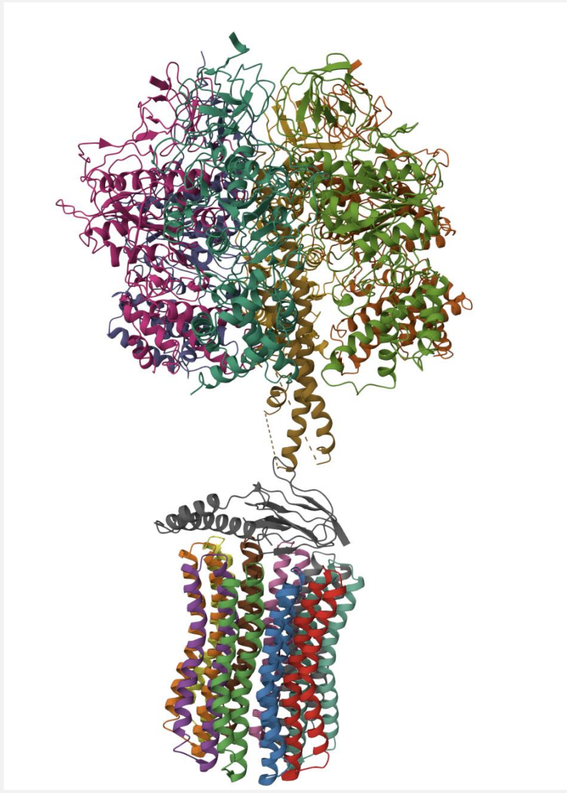
Small molecule (ligand):
may bind to the protein
surface in different
conformational states



Protein molecule:
in different
conformational states;
there are binding sites
on its surface

X-RAY DIFFRACTION / PROTEIN CRYSTALLOGRAPHY





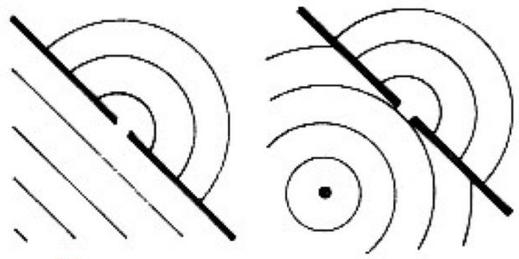
Obtained information: atomic coordinates of heavy atoms

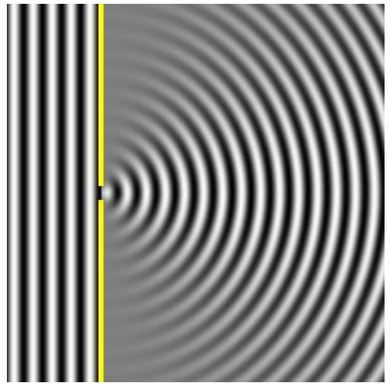
Main obstacles:

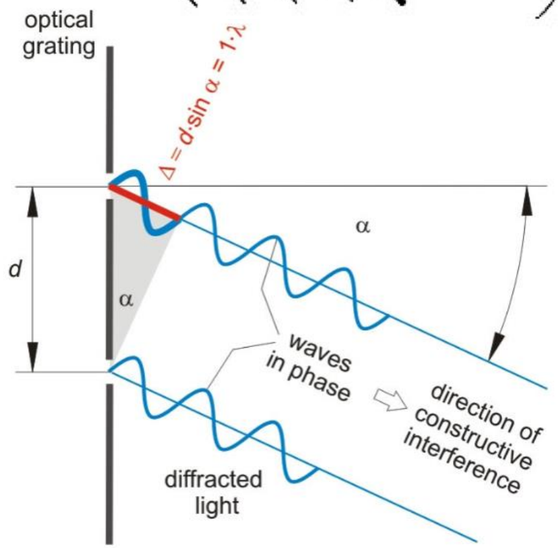
- Hydrogen is not „visible” due to low electrondensity
- Phase problem (see slide 11)

Diffraction on optical grating

slit smaller than wavelength







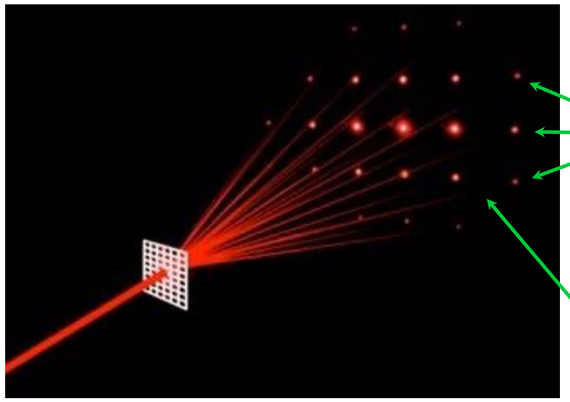
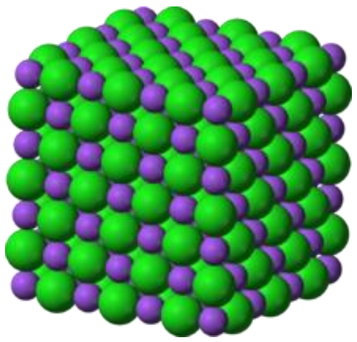


Fig. 2. *Appearance of the path difference (Δ) and formation of the fist order maximum.*

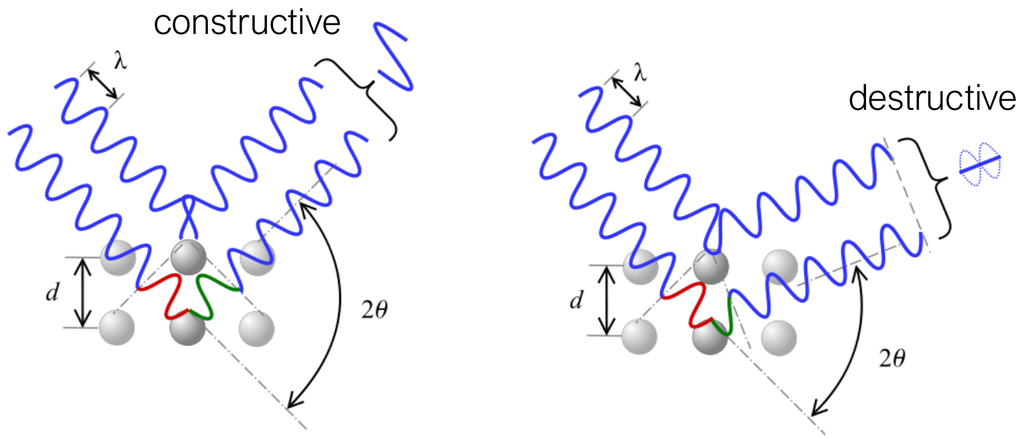
What grating is comparable with x-ray?
$\lambda_{\text{X-ray}}$: 10-200 pm

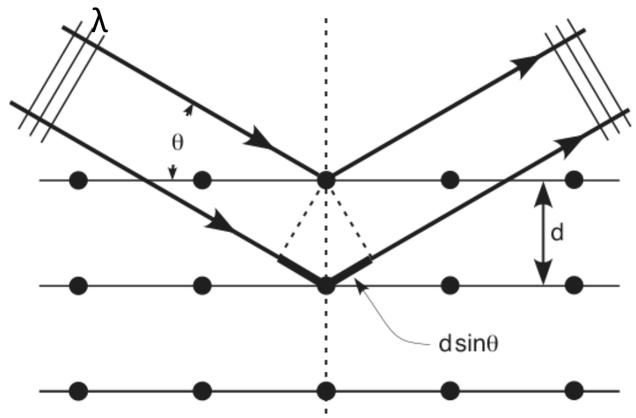


d_{NaCl}:
564 pm

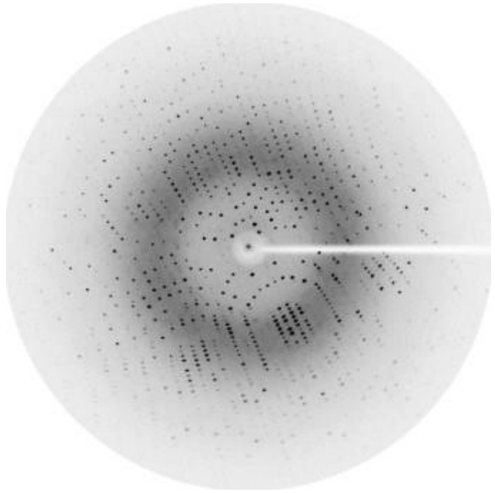
Information obtained with x-ray diffraction: spatial coordinates of atoms → spatial structure of the molecule

Diffraction on crystal

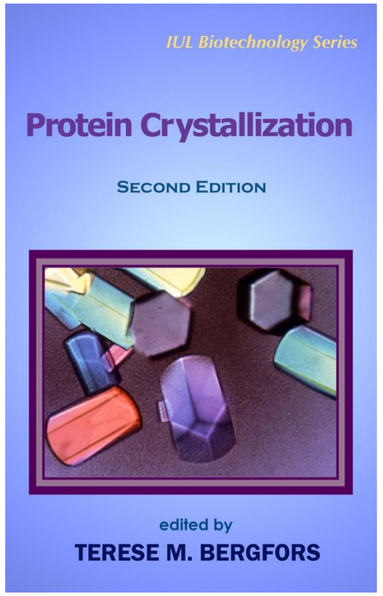




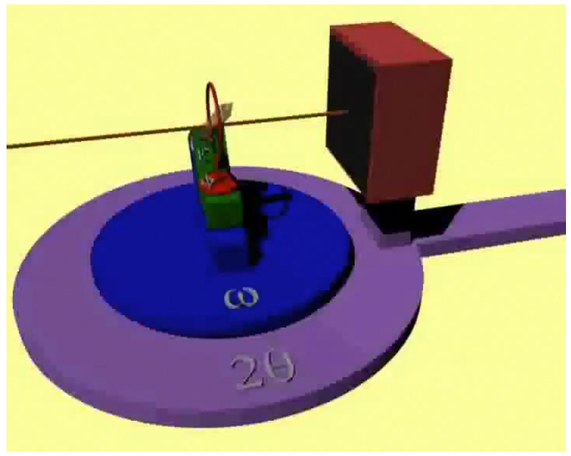
Condition of constructive interference: **Bragg's equation** $2d\sin\theta = n\lambda$

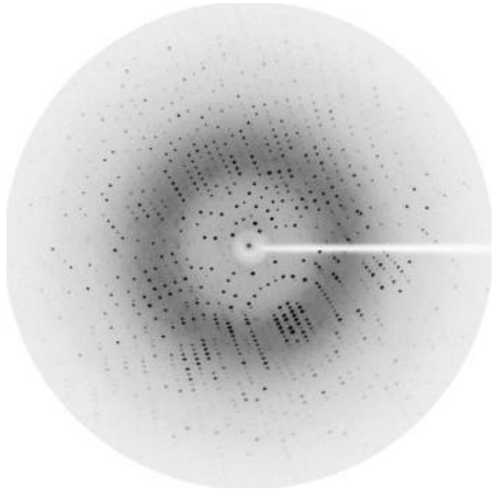


1st step: protein crystallization



2nd step: data collection





3rd-4th step: trick the phase problem

We can **measure the brightness** (intensity) of each spot — that's easy.
BUT: each spot has **two pieces of information**:
1.Amplitude (how strong it is — we get this from the brightness)
2.Phase (how the wave is shifted in time — we do **not** get this)

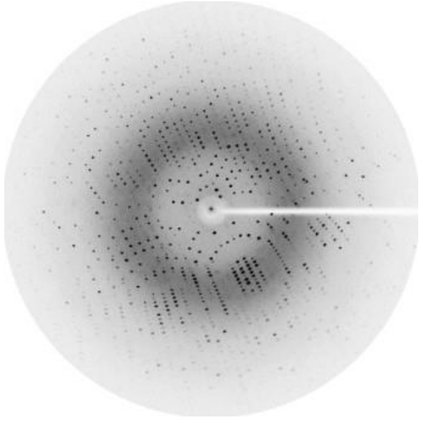
So what's the problem?
The **phase** is essential to figure out the exact **shape and position of atoms**.
But:

❗**Detectors only measure intensities, not phases**.

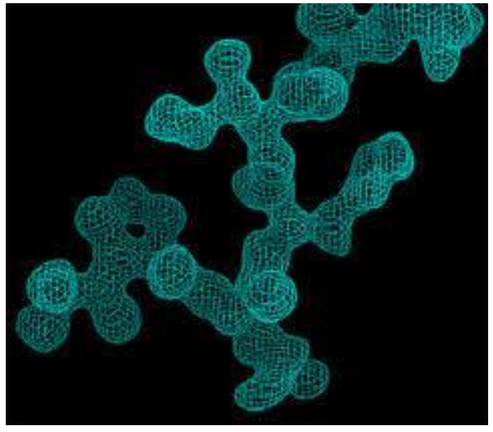
So we lose half the information we need to build the 3D structure.

We can't directly measure the phases, so we can't immediately know what the atomic structure is — even though we have the diffraction pattern.

Solution:

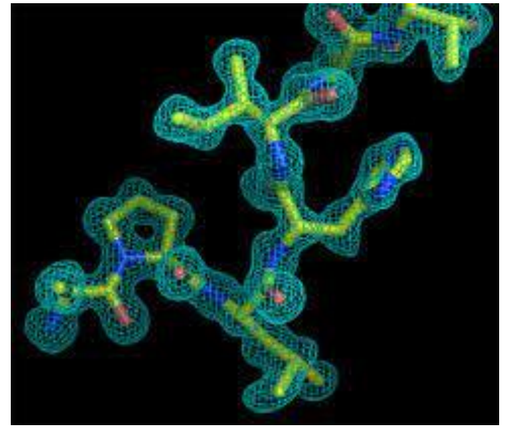


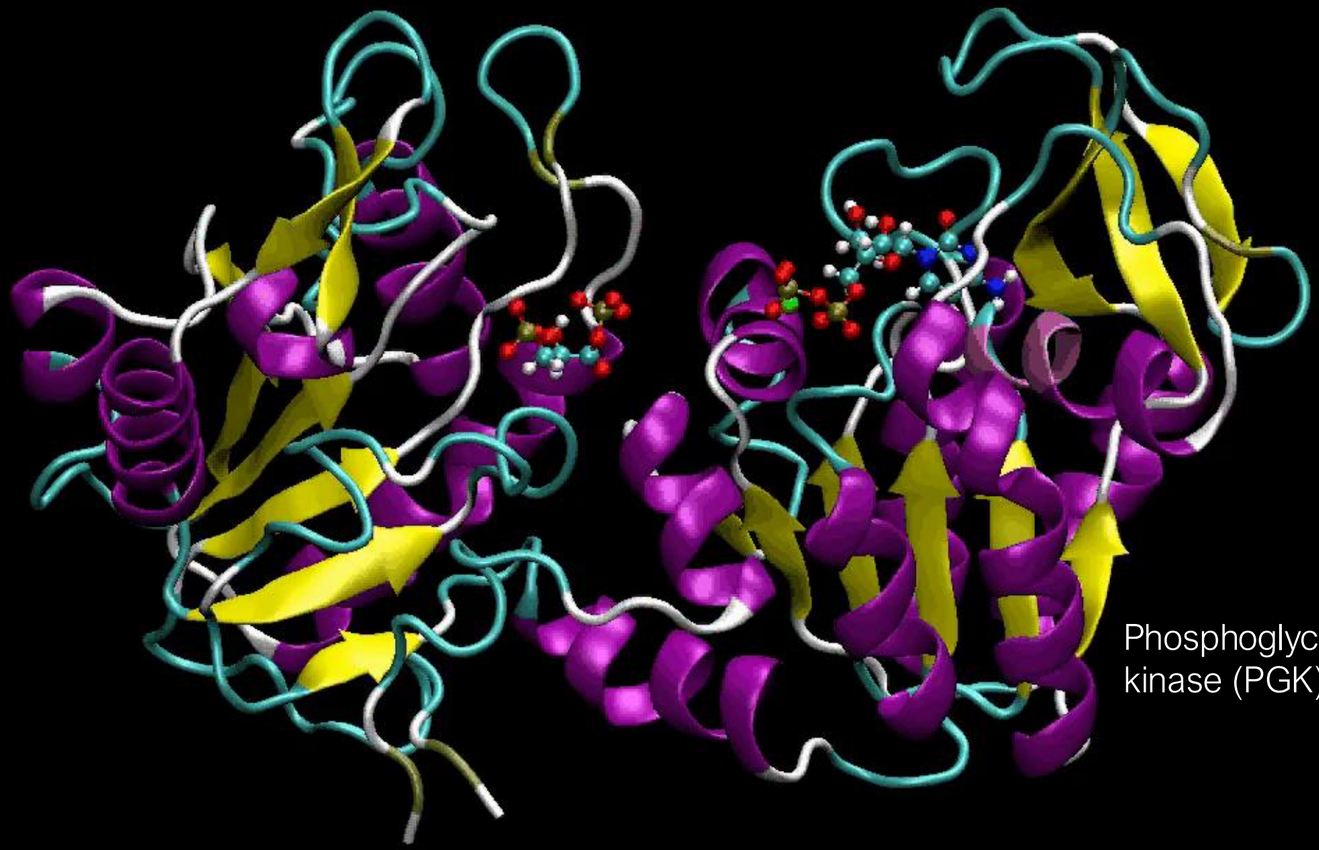
Converting diffraction pattern to electron density map →



Fit molecule into the map (similar to fitting a trendline on data) →

Refinement



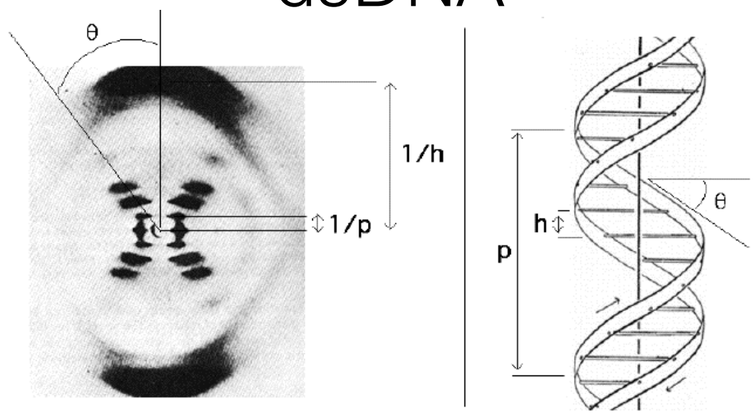


Phosphoglycerate
kinase (PGK)

Molecules are in constant rapid motion. In complex molecules (e.g., proteins) the hierarchy of different dynamic modes (e.g., vibration, rotation) results in extremely complex motions. Certain global motions are related to function of the molecule (e.g., domain rotation in a motor protein).

SOLVING MOLECULAR STRUCTURE WITH X-RAY CRYSTALLOGRAPHY

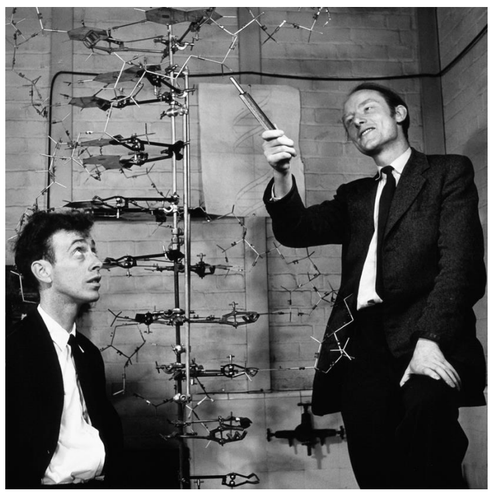
dsDNA



θ tilt of helix

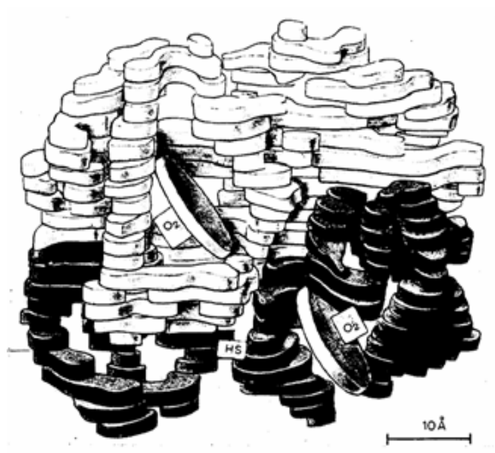
h = 3.4 Å distance between bases

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



J.D. Watson and F. Crick, 1953
Nobel-prize1962

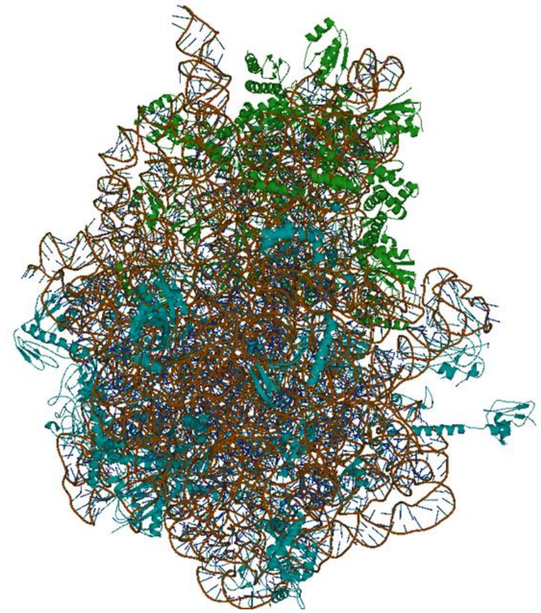
Globular protein: myoglobin



~1200 atoms

M. F. Perutz, J. C. Kendrew
Nobel-prize1962

Molecular complex: ribosome



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

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

THANK YOU FOR YOUR ATTENTION.

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