生物动力系统模拟

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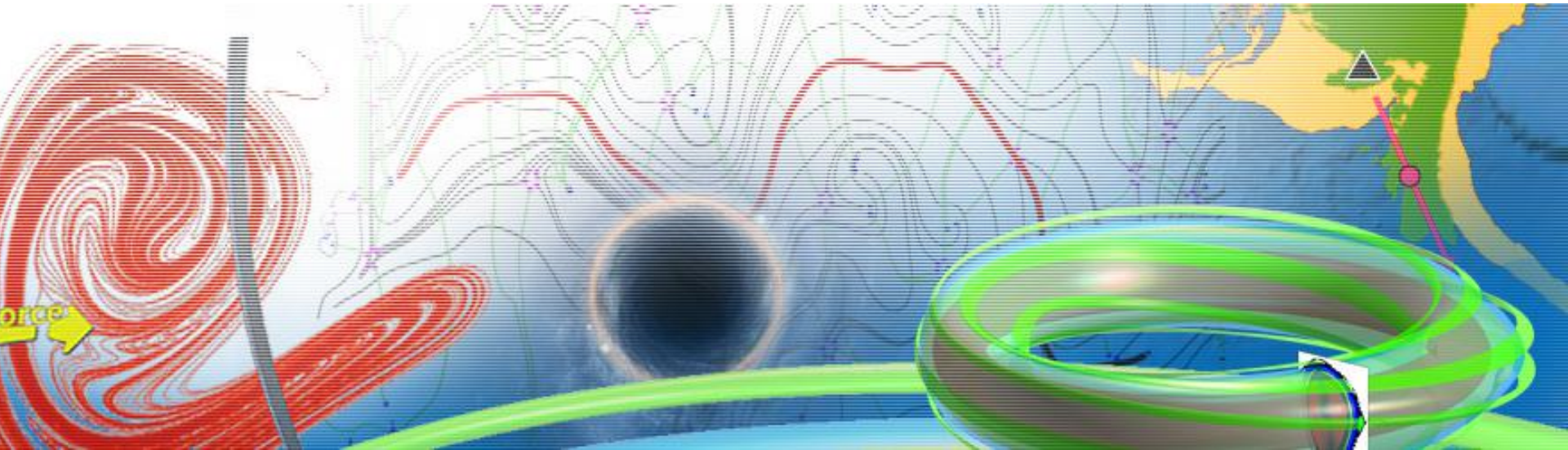


Methods for Determining Atomic Structures

X-ray Crystallography:

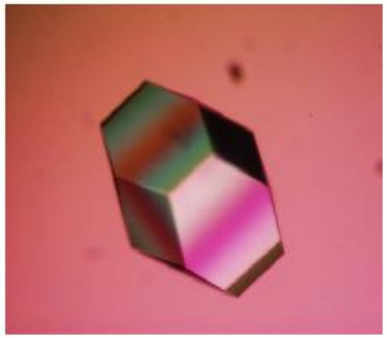
Protein purification

Protein crystallization : The protein solution becomes supersaturated
so that individual protein molecules can pack
a repeating array, held together by noncovale

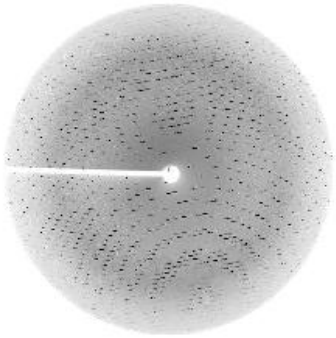
Crystals of proteins grown on
the U.S.
Space Shuttle or Russian Space
Station

Shortcomings: it is difficult to obtain crystals, especially those large, co

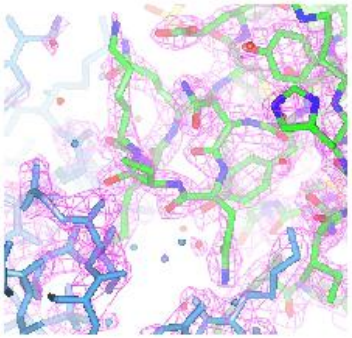
X-ray diffraction: The proteins in the crystal diffract the X-ray beam int
characteristic pattern of spots, which are then analyzed (with some tricky
determine the phase of the X-ray wave in each spot) to determine the dis
electrons in the protein. The resulting map of the electron density is then
determine the location of each atom.



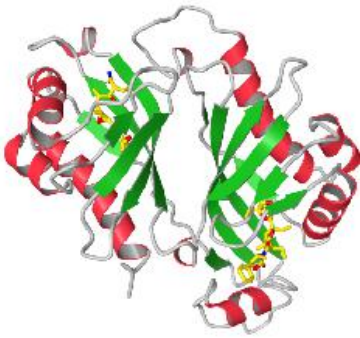
Crystal



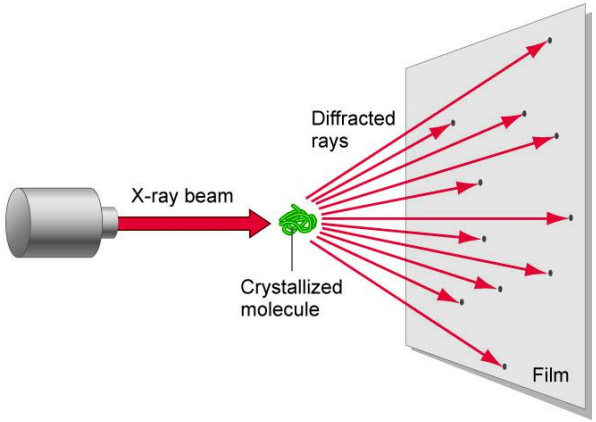
Diffraction pattern

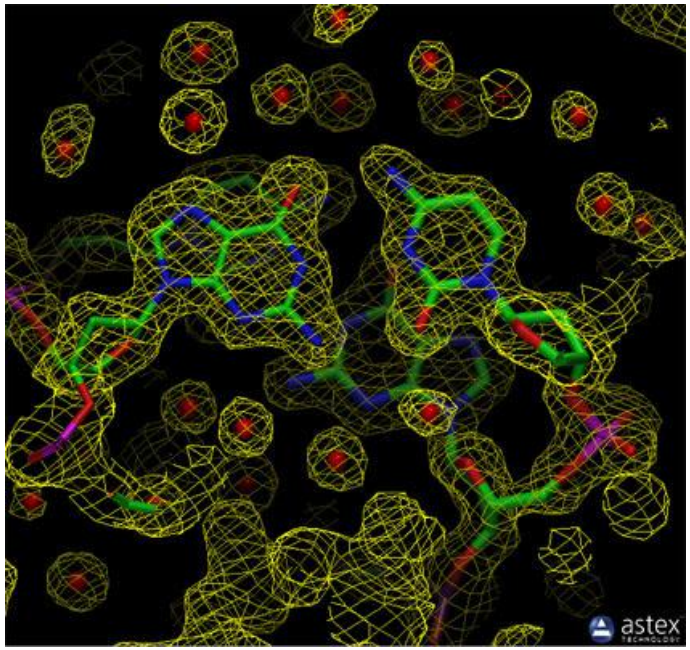


Electron density map



Protein model





NMR Spectroscopy

NMR Spectroscopy (comparison with X-ray)

Sample: proteins in solution vs crystal

> A major advantage of NMR spectroscopy is that it provides information on proteins in solution, as opposed to those locked in a crystal or bound to a microscope grid, and thus, NMR spectroscopy is the premier method for studying the atomic structures of flexible proteins.

Probing matter: radio wave vs X-ray

Resultant pattern: atomic resonance pattern vs diffraction pattern

Advantages: flexible vs fixed

Disadvantages: small proteins vs Larger proteins

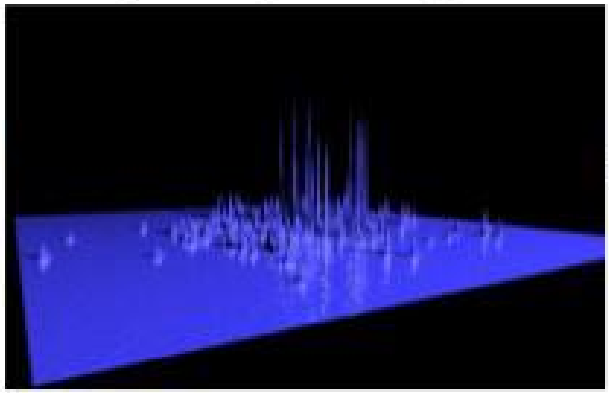
↓

large proteins present problems with overlapping peaks in the NMR spectra.

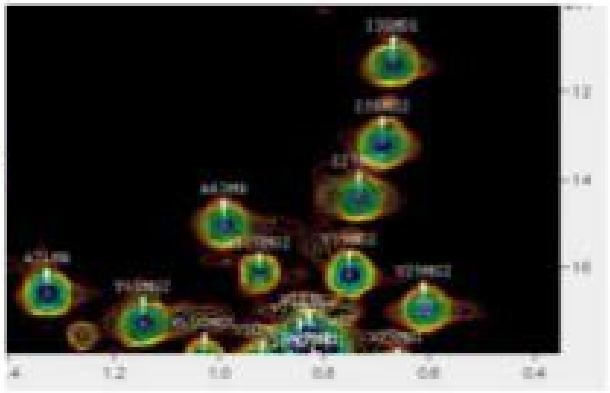
Summary of solution NMR spectroscopy

Experiment

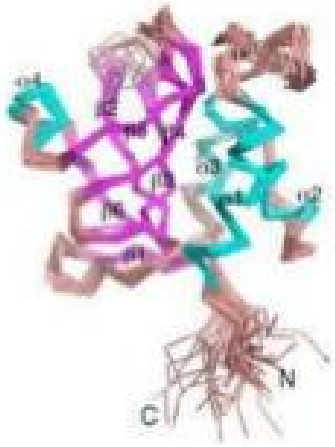
Spectra processing



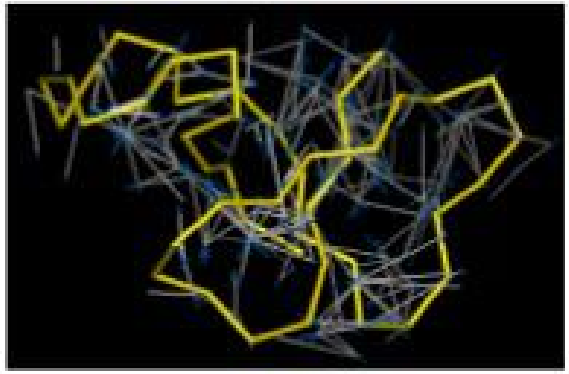
Spectra assignment



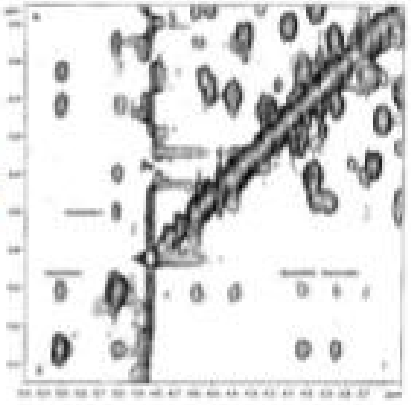
Model generation



Distance restraints



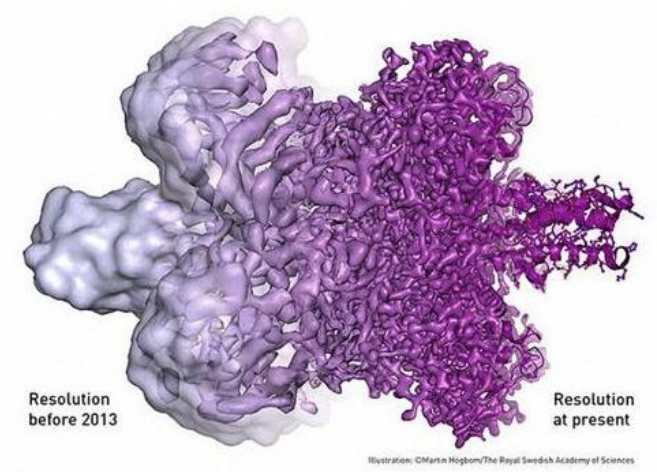
NOE assignment



Cryo-electron microscopy

1. The basic principle is diffraction, now we use electrons instead of X-r

2. High resolution

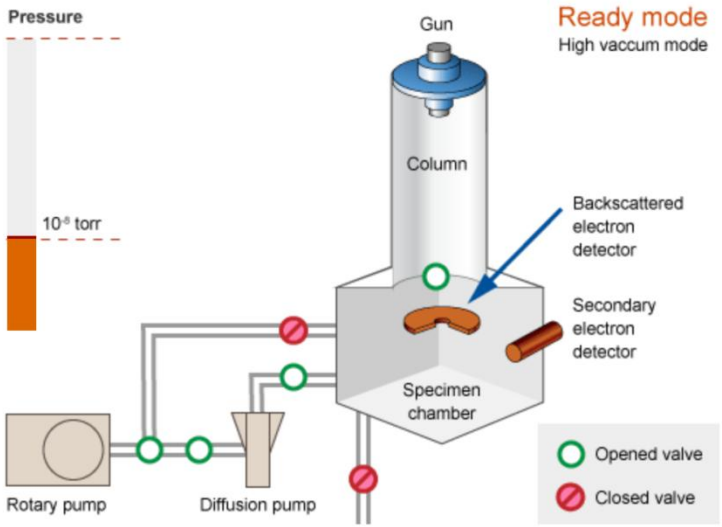


3. Why high resolution

Because high resolution corresponds to small wavelength

Electrons are more like particles, having very small wavelengt

3. Why under cryogenic temperatures ?



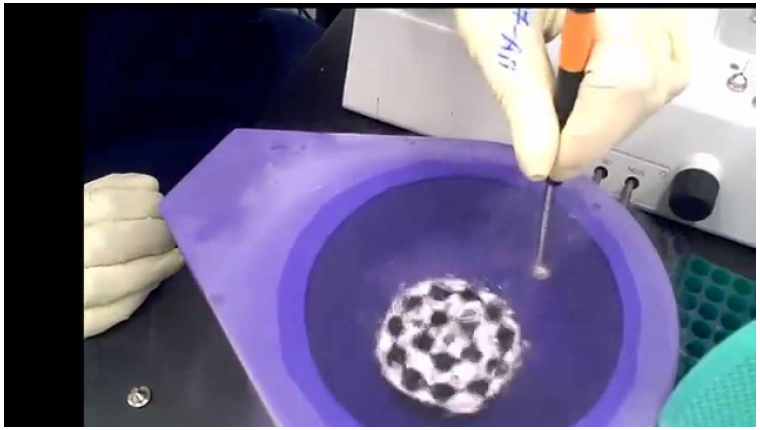
Vacuum is required in the Column to avoid electrons being scattered.

But high vacuum would damage the spe
vaporizing molecules.

The solution is to freeze the sample

Slow freezing does not solve the problem,
because water becomes ice (crystal) which distort the protein molecule

The solution is “flash freezing”, so fast that
The sample is frozen even before the re-organization of water molecules into crystall.



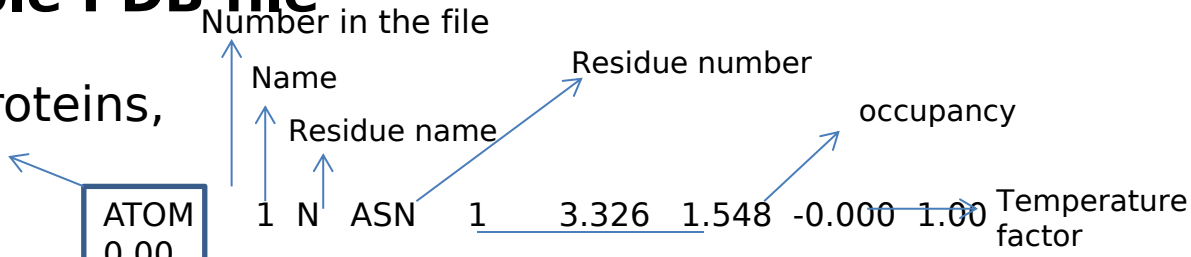
Introduction to PDB files

The PDB archive is a repository of atomic coordinates and other information describing proteins and other important biological macromolecules. Structural biologists use methods such as X-ray crystallography, NMR spectroscopy, and cryo-electron microscopy to determine the location of each atom relative to each other in the molecule. They then deposit this information, which is then annotated and publicly released into the archive by the wwPDB.

you can go to the PDB archive to find structures for ribosomes, oncogenes, drug targets, and even whole viruses. However, it can be a challenge to find the information that you need, since the PDB archives so many different structures. You will often find multiple structures for a given molecule, or partial structures, or structures that have been modified or inactivated from their native form.

An example PDB file

Atoms in proteins, DNA, RNA

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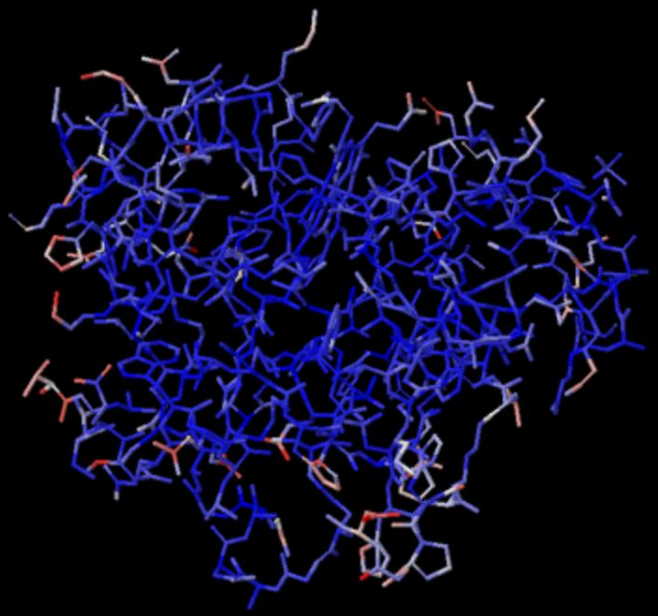
Atoms in non-standard molecules, including: inhibitors, cofactors, ions, and solvent (e.g. H2O)

Temperature factor (B factor)

It measures uncertainty in the atom’s position

If the value is high, the atom is probably moving a lot, and the coordinate in the PDB file are only one possible snapshot of its location.



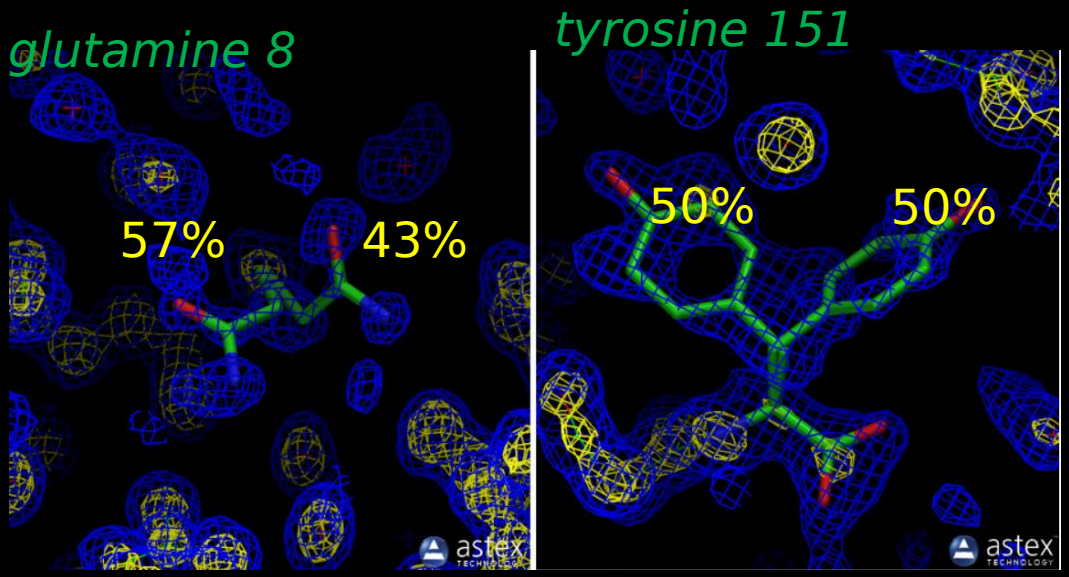
It shows the whole molecule, with the atoms colored by the temperature factors. High values, indicating lots of motion, are in red and yellow, and low values are in blue. Notice that the interior of the protein has low B-values and the amino acids on the surface have higher values.

Occupancy

A crystal does not contain a single molecule, it contains many repetitions

Differences may exist between these repetitive molecules:

- A side chain may change orientations
- A substrate may bind in different orientations
- A metal ion may be bound to only a few of the molecules
- ...

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