X-ray crystallography

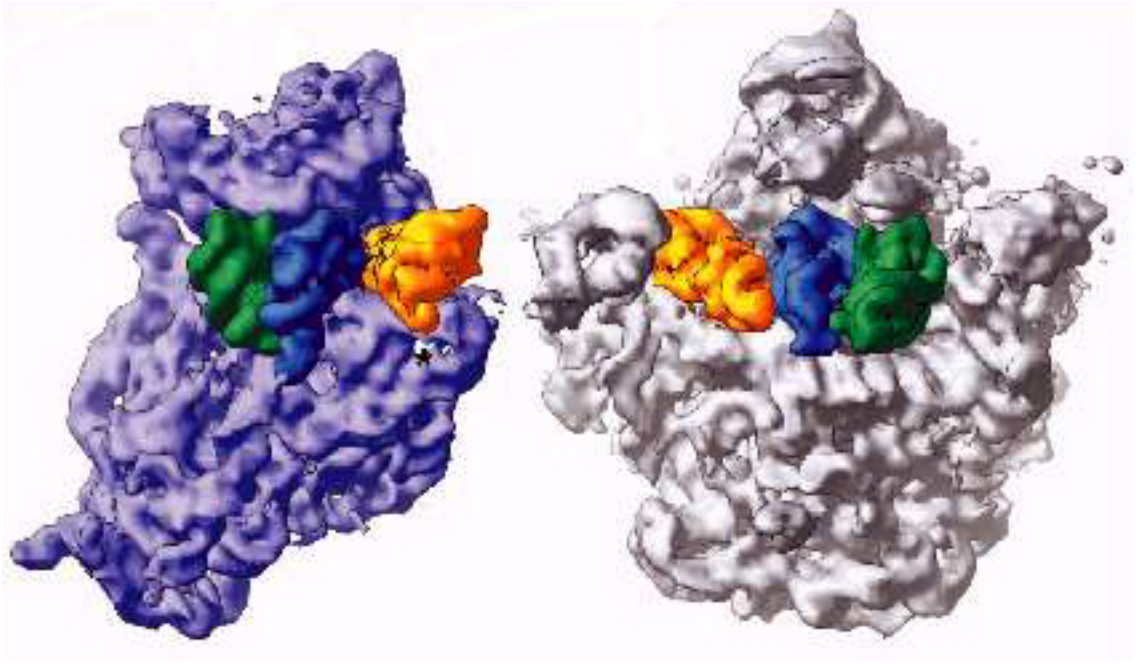
In this final section of the course, we will learn a bit about x-ray crystallography.

- Like NMR, x-ray crystallography allows us to obtain three-dimensional structures of biomolecules.

- Unlike MRI, it is not an imaging technique. Rather it is a direct method, with no limitation on the size of the biomolecule being studied.

- Only limited information on dynamics.

- Requires at least two-dimensional crystals



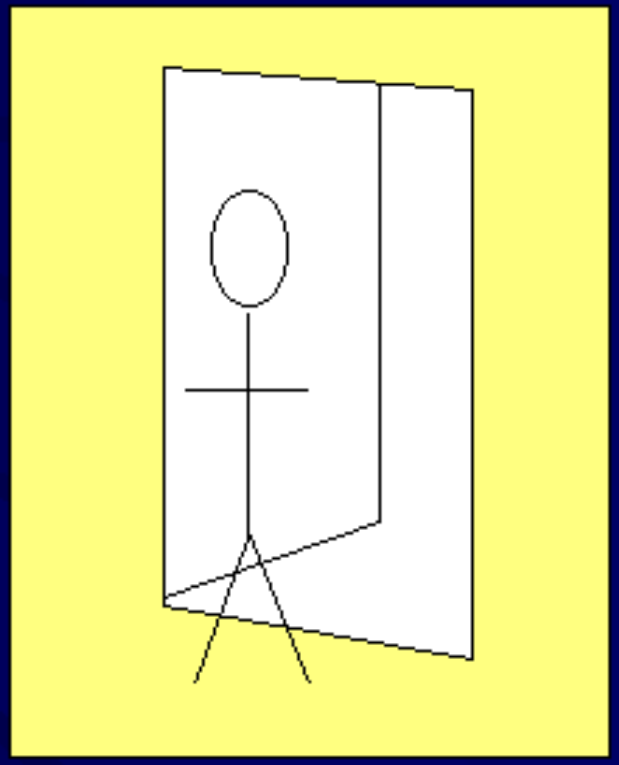
Ribosome: three RNA, 50 proteins, 2.5 million daltons!!!!

How does it work? How can be generate these structures?

X-ray Diffraction Theory

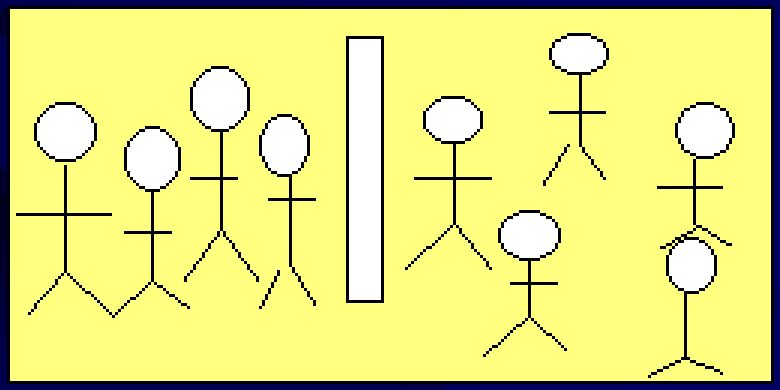
What is diffraction?

- Imagine a person going through a door. If alone and not carrying anything bulky s/he can walk straight through the door without any problem.



ref: http://www.ukc.ac.uk/physical-sciences/cmg/openday/

- Now, imagine a crowd of people trying to go through this door at the same time. The door is not wide enough and so the people bounce off (i.e. interfere with) each other to try and get through. The result is that people emerge from the other side of the door from many directions. The direction at which they emerge will depend upon the angle at which they came to the door.



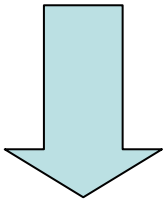
This 'interference' is known as diffraction

Another example: Diffraction of light

http://micro.magnet.fsu.edu/primer/java/diffraction/basicdiffraction/index.html

-see what happens when you keep the colour of the light fixed but vary the aperture

-conversely, see what happens when you keep the aperture fixed and vary wavelength.

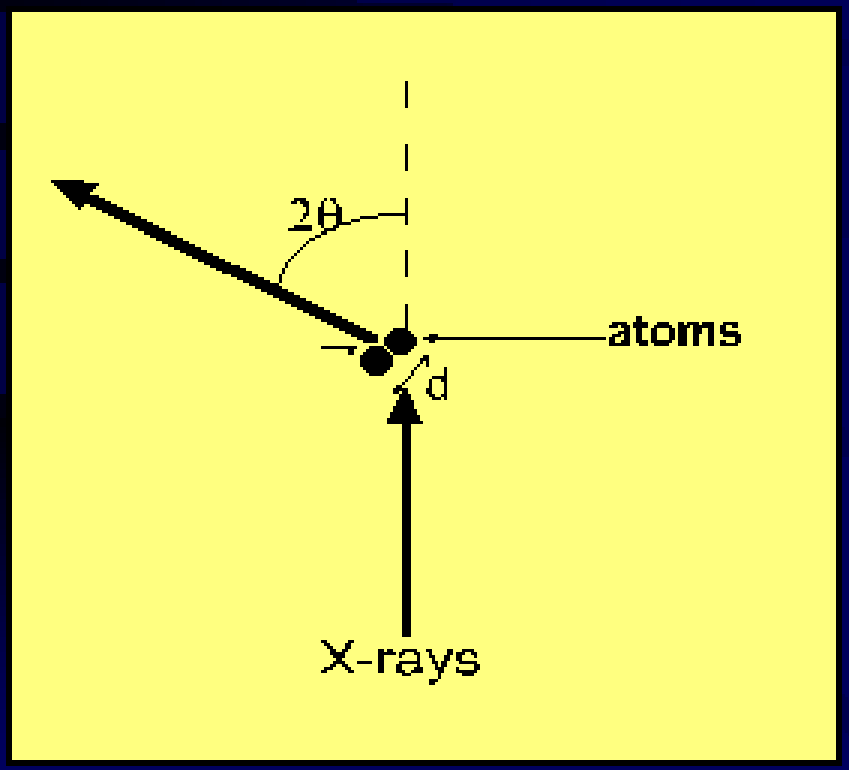


Diffraction occurs when the incident light and the size of the grating (i.e.“size of the door”) are similar.

Applying this to X-rays

- Consider an X-ray beam passing through this door. Obviously, the X-rays will easily fit through it and so will continue in a straight line beyond the door.
- But what if the X-ray beam was to try and pass through something a similar size to itself, for instance between two atoms in a solid? Just as the crowd of people had to interfere with each other to get through the door, the X-rays have to interfere with the electrons surrounding these two atoms to get between them.

- The angle at which the X-rays emerge from the other side of the two atoms will depend upon the incoming angle of the X-rays relative to the two atoms, according to the following equation:

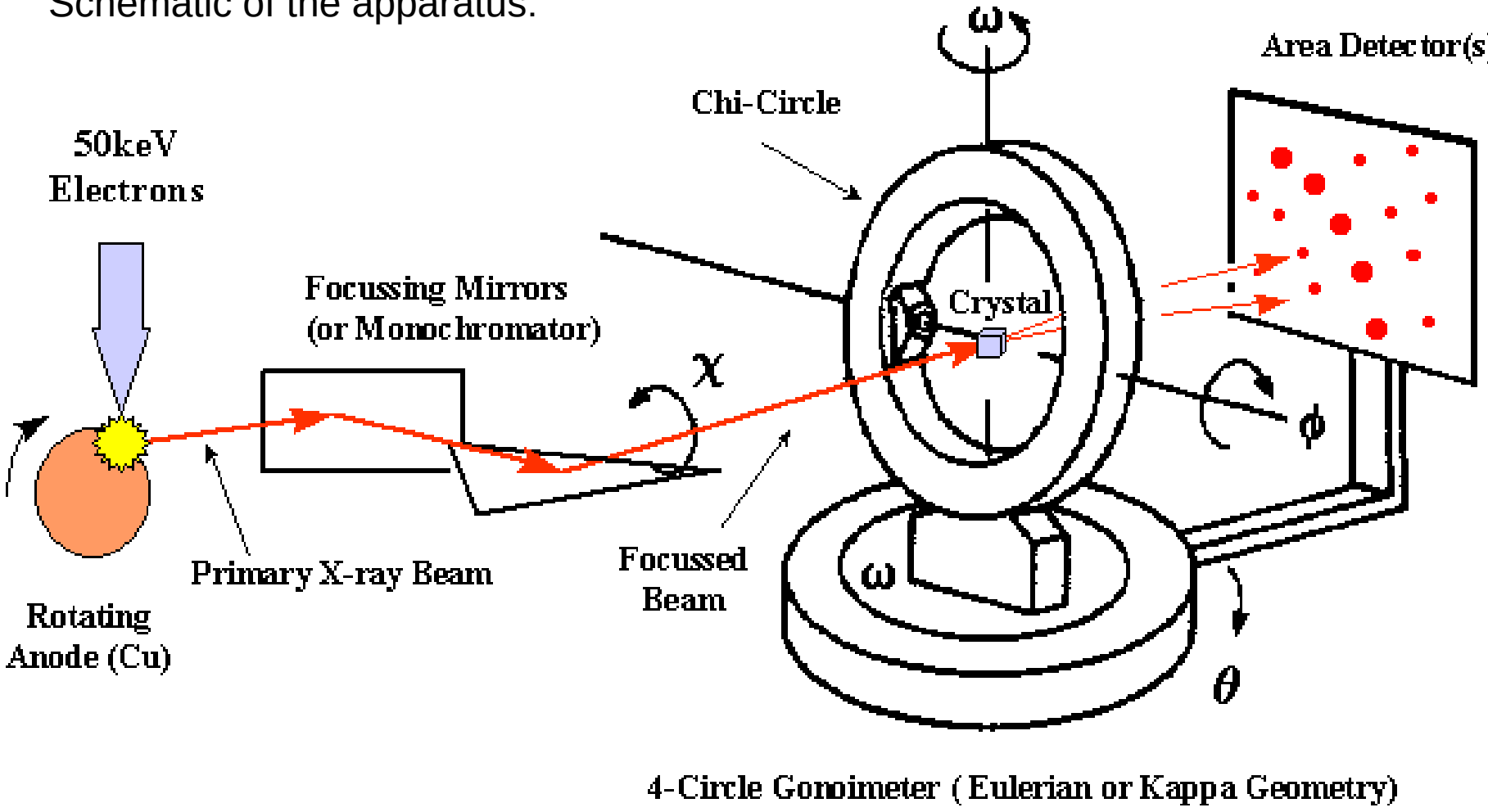


$$\lambda = 2d\sin\theta$$

Bragg's equation

Where λ is the wavelength of the X-ray and d is the distance between the two atoms.

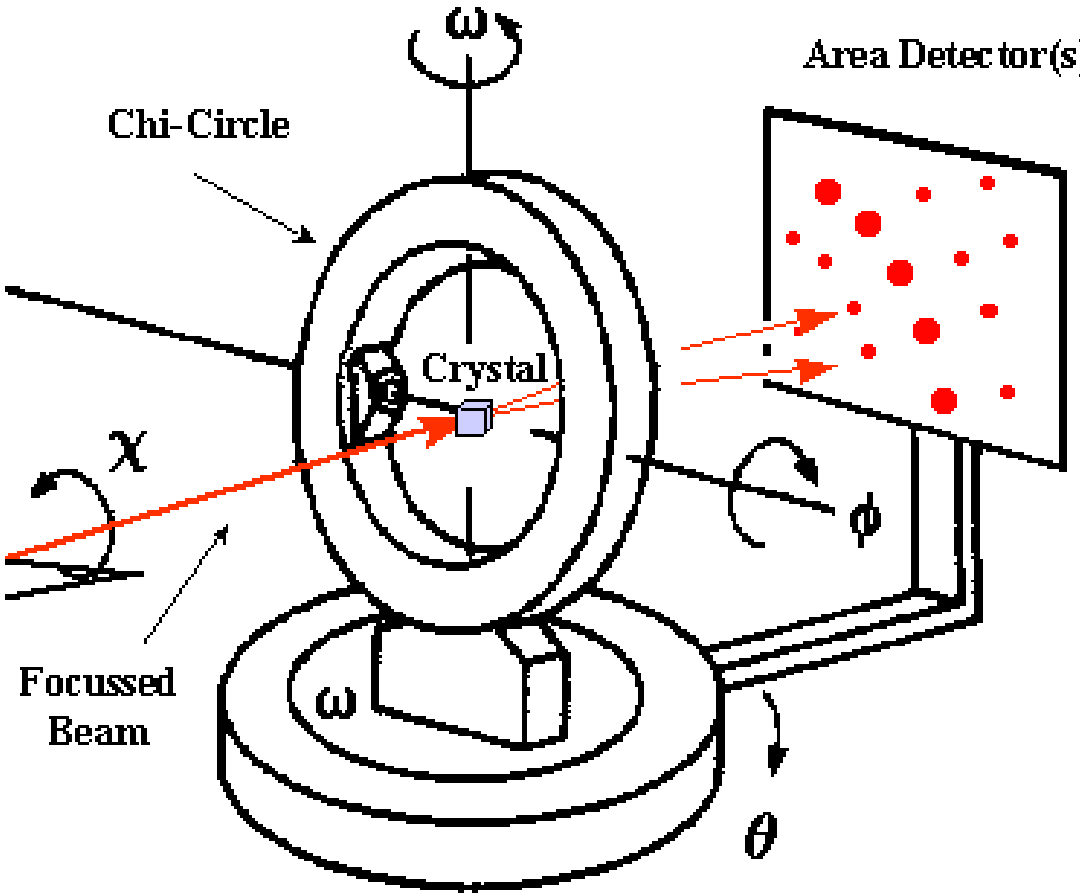
Schematic of the apparatus:



Two things can move: 1) the sample holder, which can rotate by ω
2) the detector, which can rotate by θ

http://www-structure.llnl.gov/Xray/101index.html

The brighter spots which appear on the detector arise because of the following basic principles:



4-Circle Goniometer (Eulerian or Kappa Geometry)

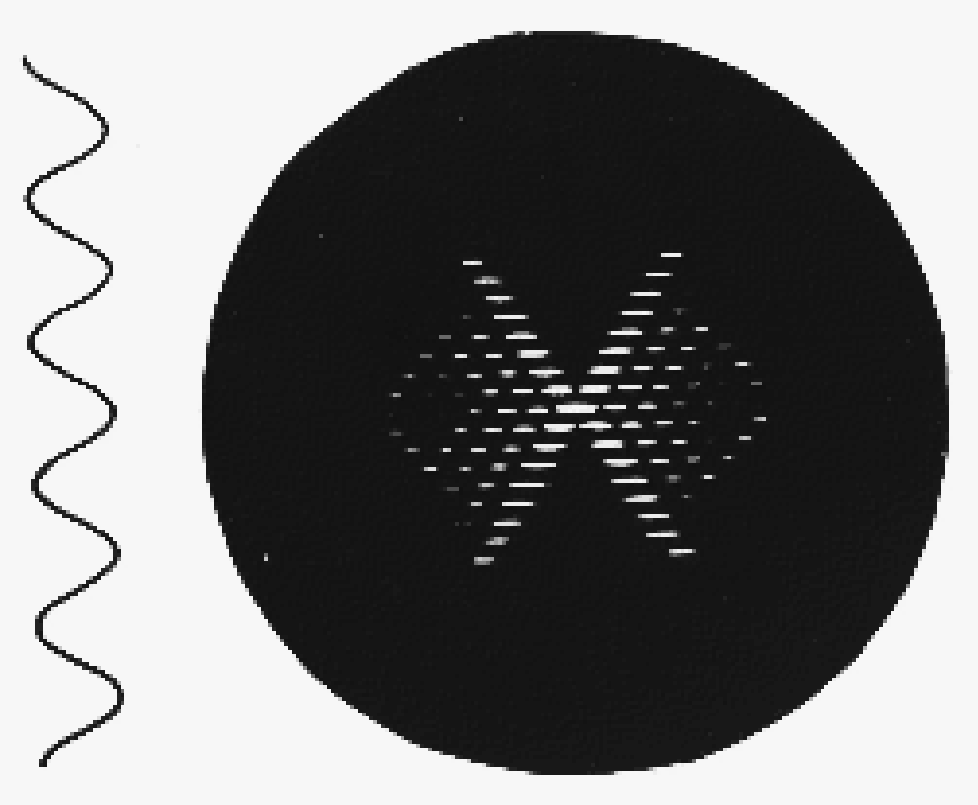
1) electrons diffract x-rays – the amplitude of the diffracted x-ray is proportional to its number of electrons.

   e.g. C diffracts 6 times more strongly that H

2) the diffracted waves recombine constructively if they are in phase and destructively if they are out of phase

3) the way in which the diffracted waves recombine depends on the arrangement of the atoms.

The intensity of the diffracted x-rays are measured as a function of angular movement of both the detector (θ) and the sample holder (ω). This is a lot like what we saw previously in MRI – we need to measure in different directions in order to get a 3D picture.



In order to get this regular array of spots (called reflections), we need a crystal lattice.

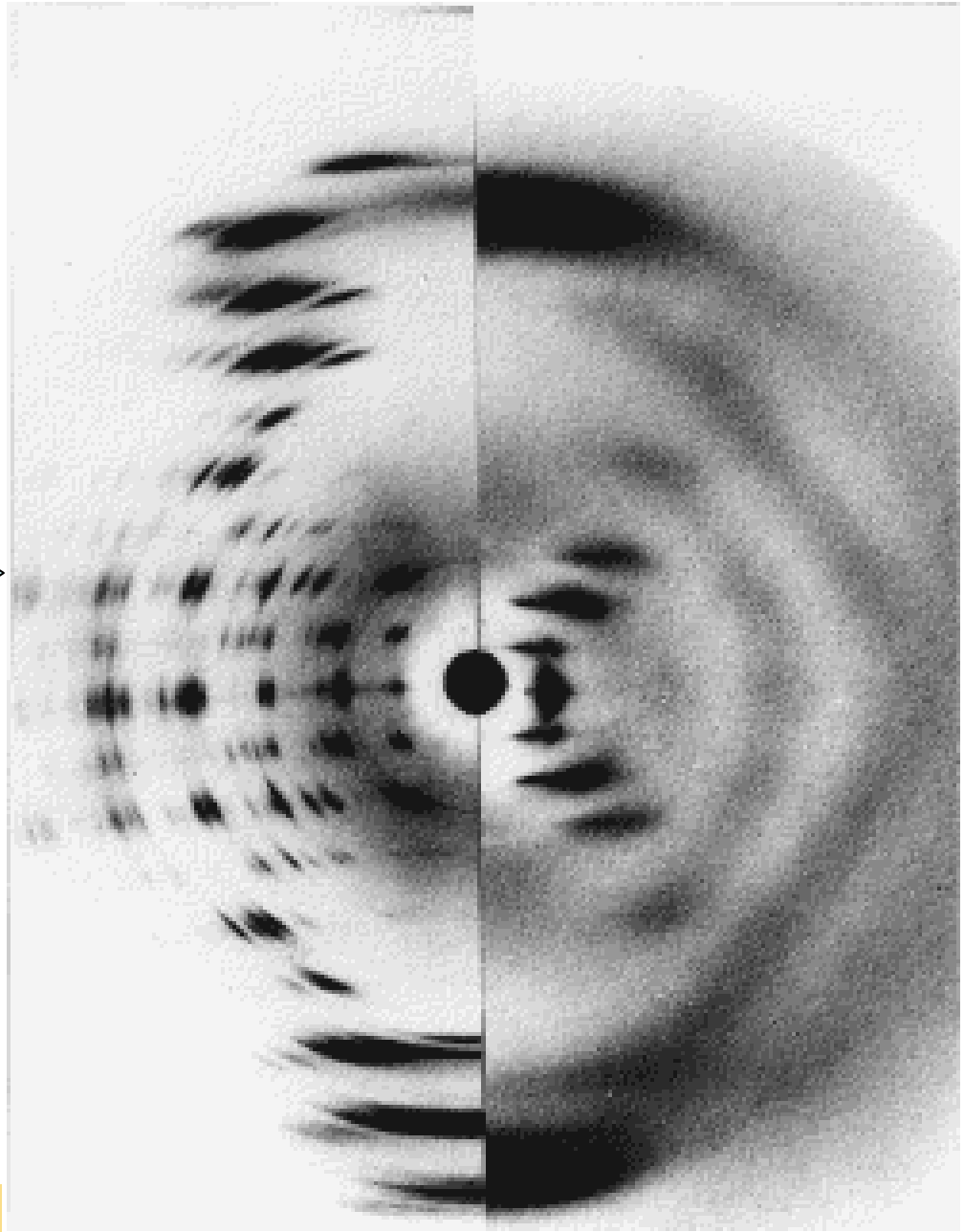
Some definitions:

crystal → solid where each element is periodic in three-dimensions.

lattice → regular arrangement of an object

A-form of DNA

(crystalline)

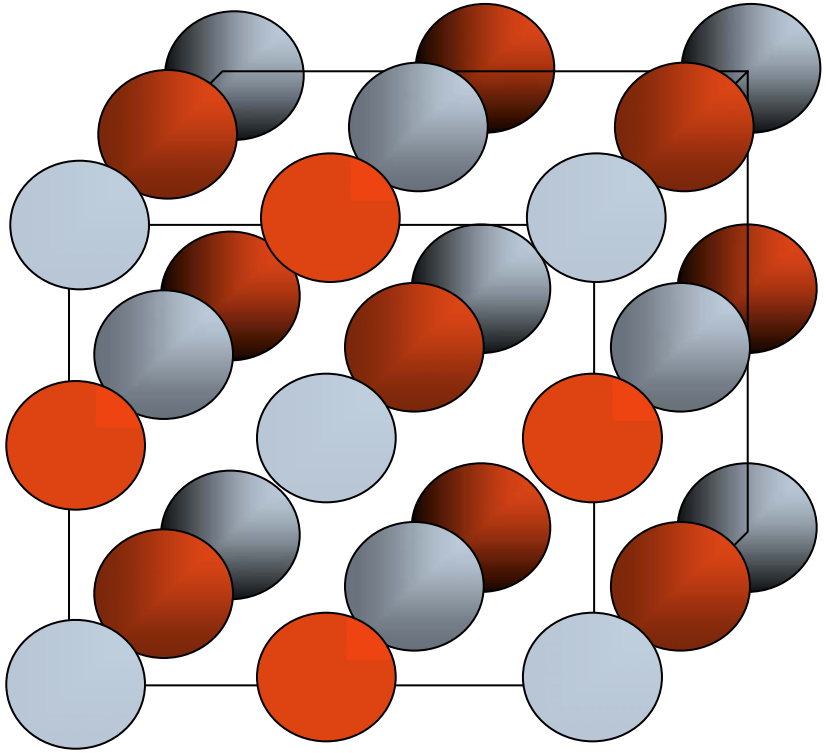


B-form of DNA

(non-crystalline)

http://www.mpimf-heidelberg.mpg.de/~holmes/fibre/branden.html

Crystal lattice



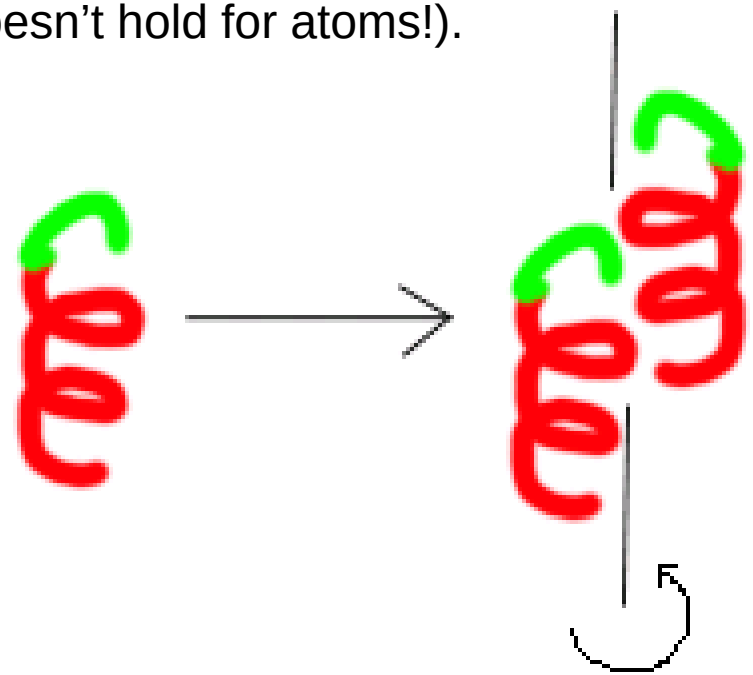
sodium chloride

What if the “object” we are trying to arrange in a lattice has a different shape, such as

A motif can be a single atom, a small molecule, a protein or any combination thereof.

We can simply repeat this motif in three-dimensions. This will result in a simple crystal, like the NaCl crystal.

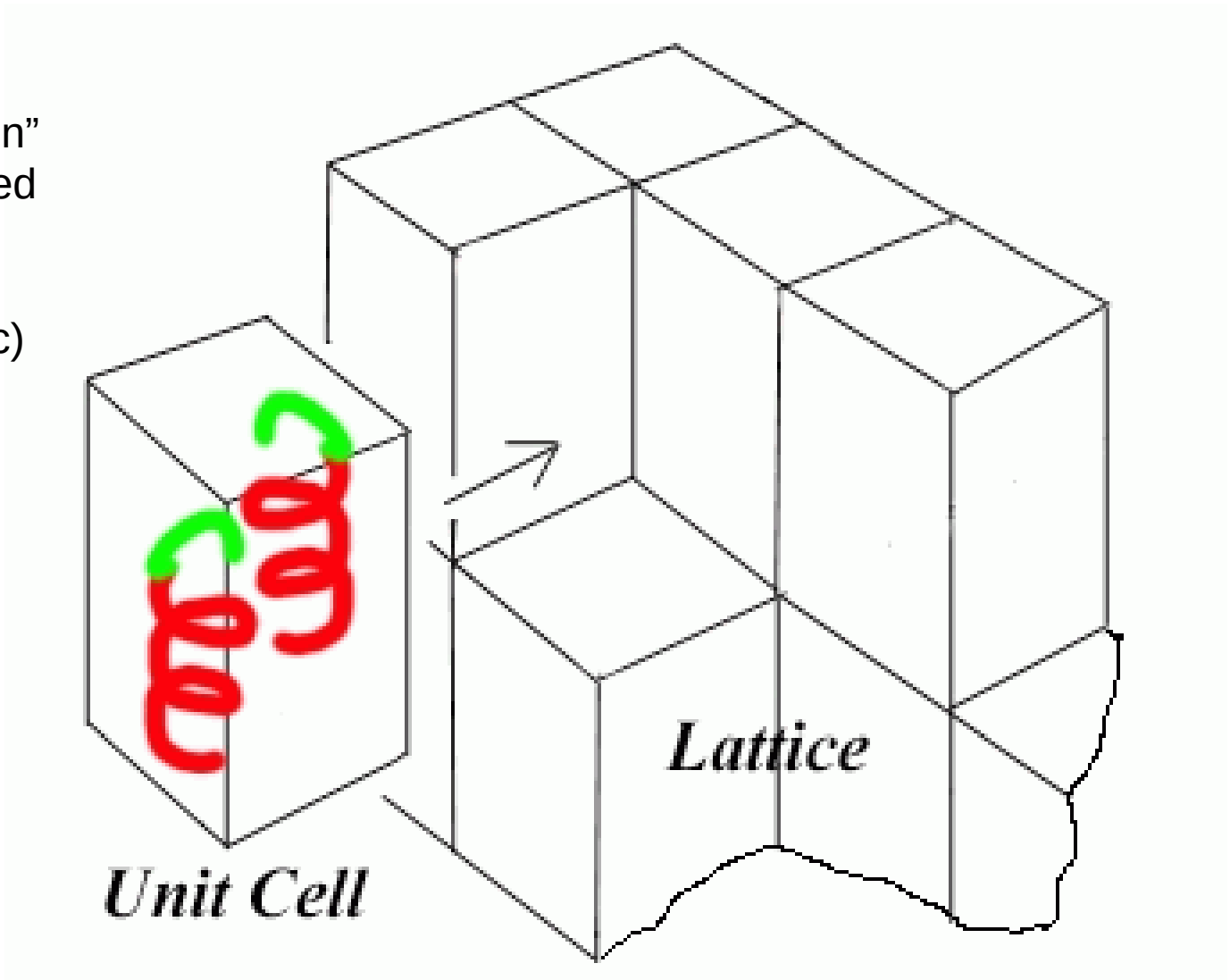
Often, however, motifs can arrange in differently oriented copies (this of course doesn’t hold for atoms!).

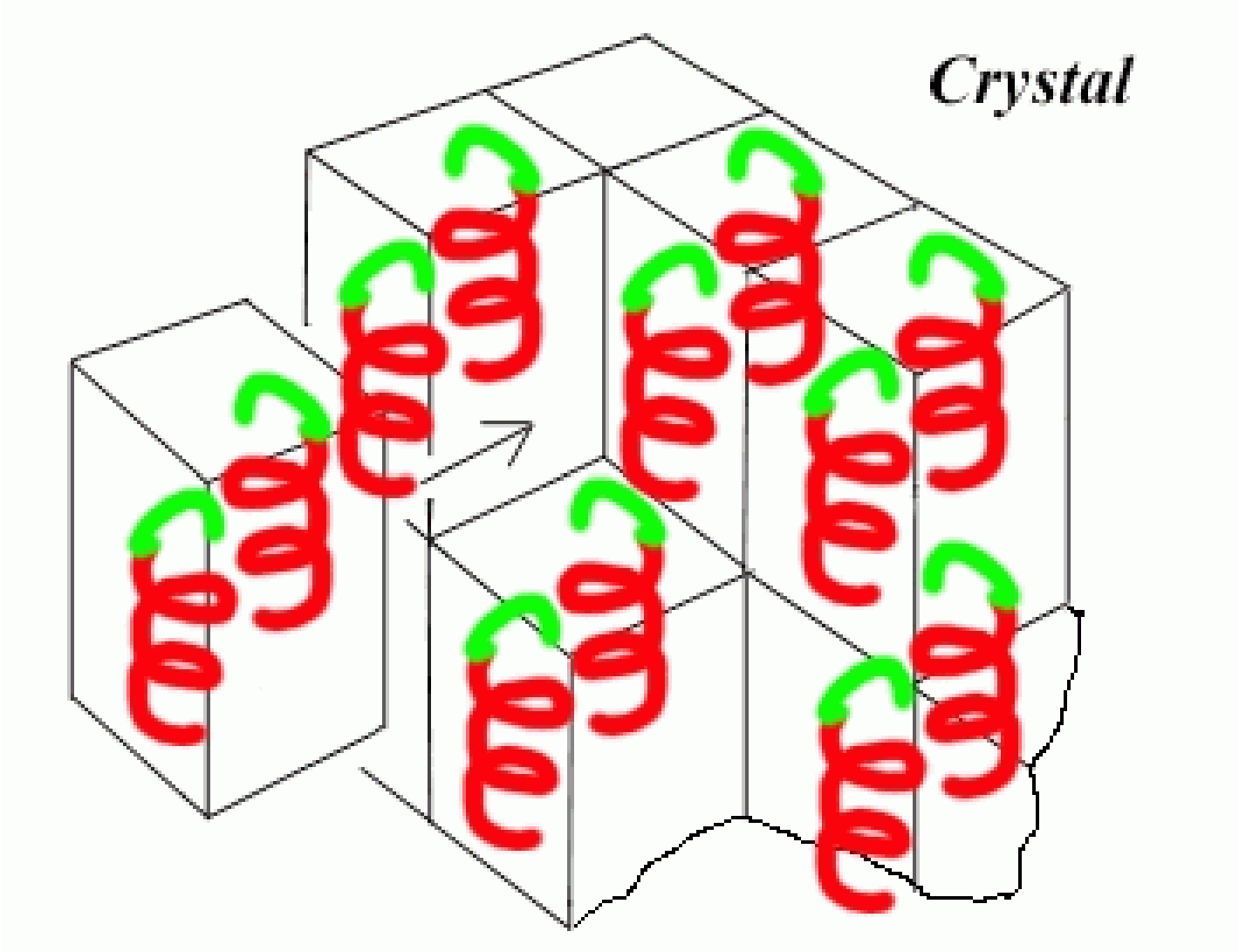


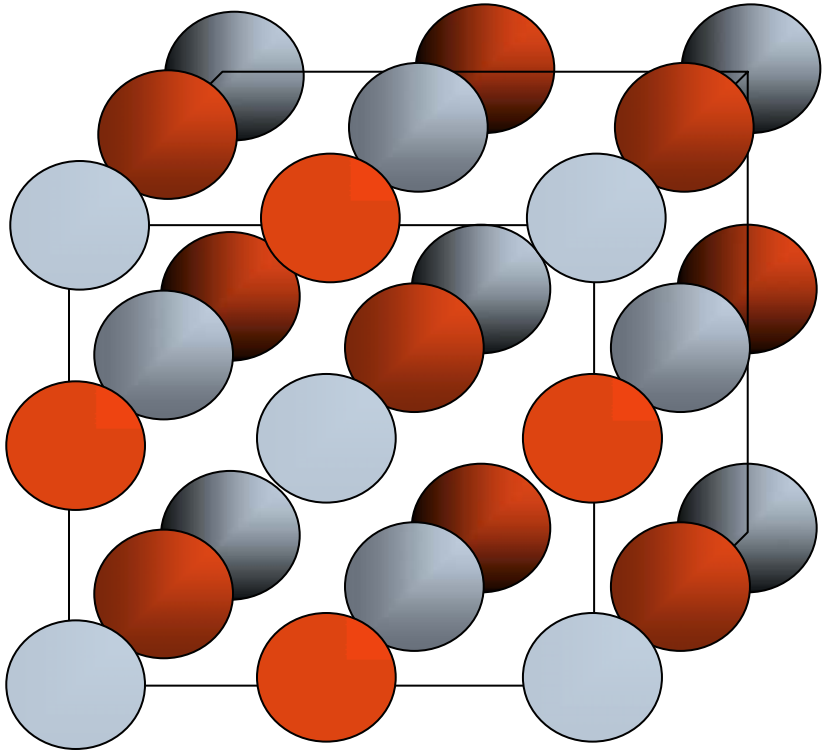
Unit cell:

- smallest “collection” of symmetry related objects

- dimensions: (a,b,c)




Crystal

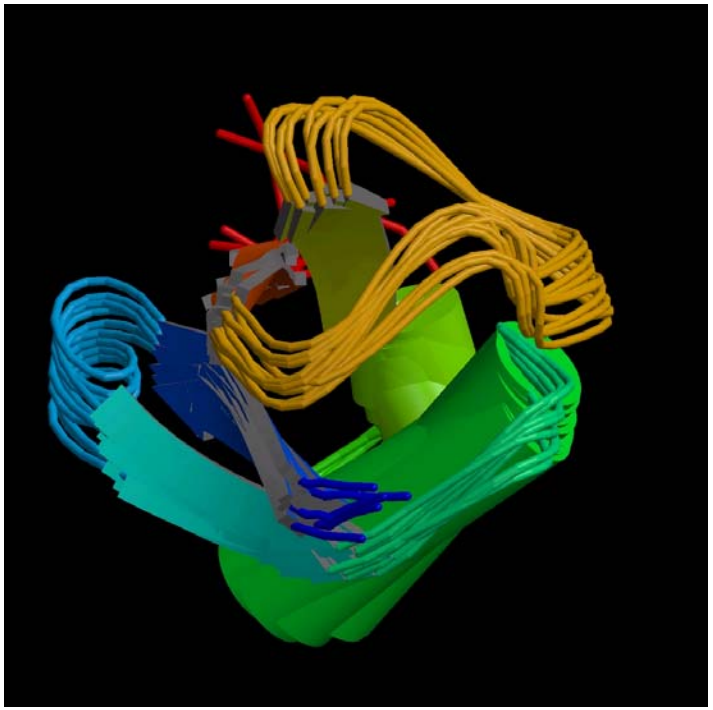


Sodium chloride

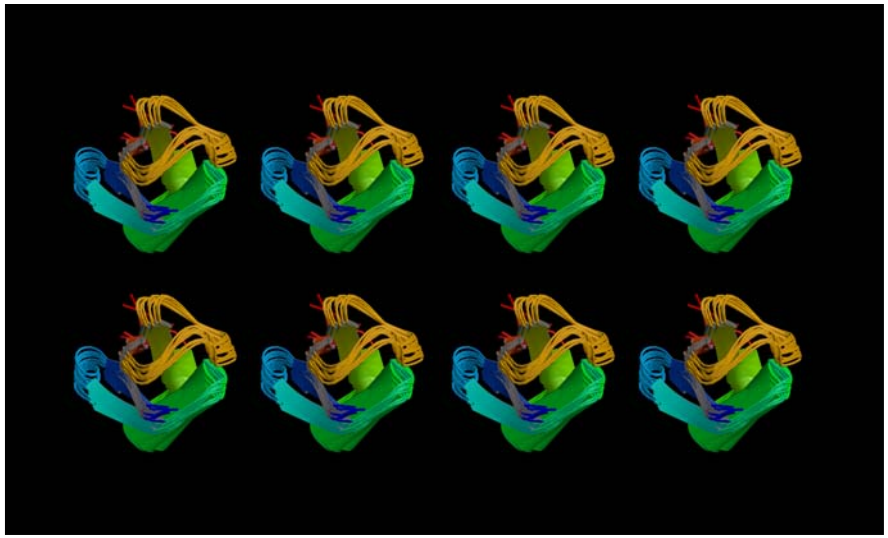
- both types of atoms are charged
- ionic interaction

What about proteins?

- irregular shapes
- “soft”
- mobile



Can protein crystals be formed?



Yes! But they are fragile.

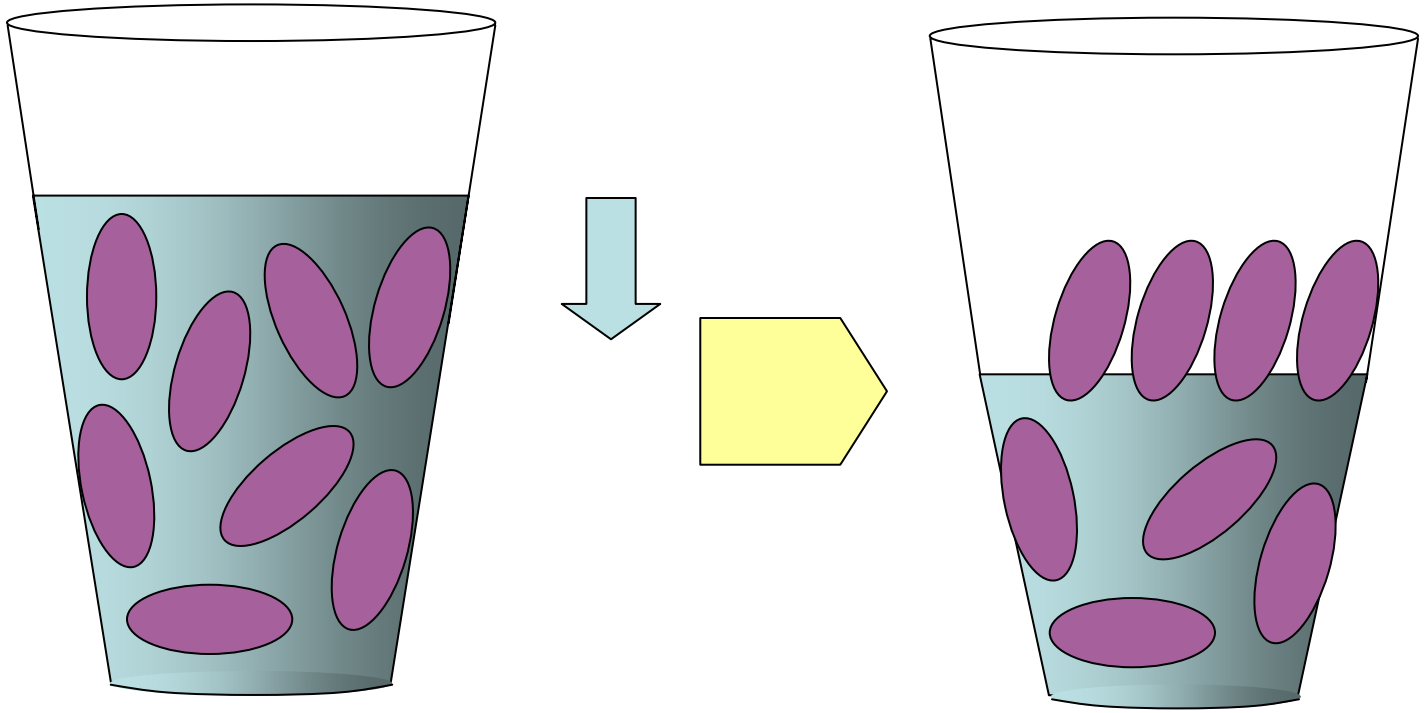
Sodium chloride is a rock…
Protein crystals are like jelly.

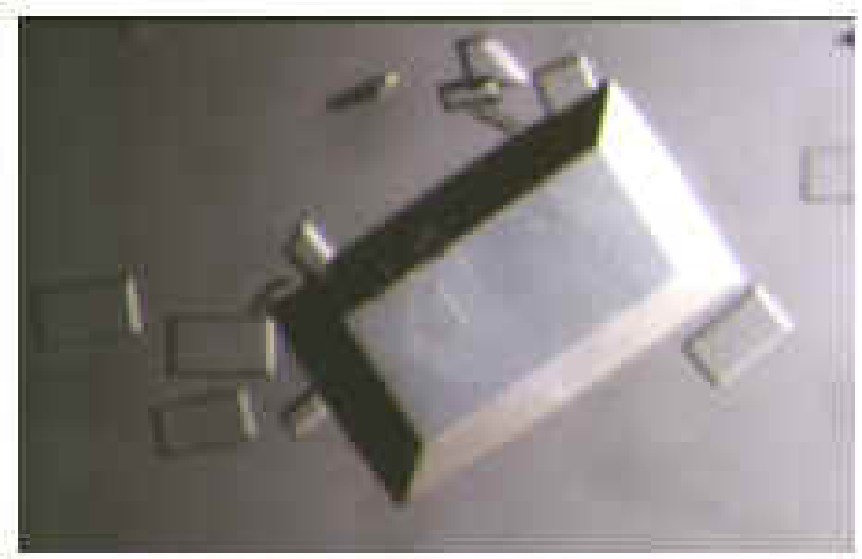
The reason for this is that
protein crystals contain on average 50% solvent, mostly in large channels between the stacked molecules on the crystal. (Example: periplasmic reductase).

The interactions holding proteins together in a crystal are weak: hydrogen bonds, salt bridges, and hydrophobic interactions.

In comparison, sodium chloride and other mineral crystals are held together strongly via covalent or ionic interactions.

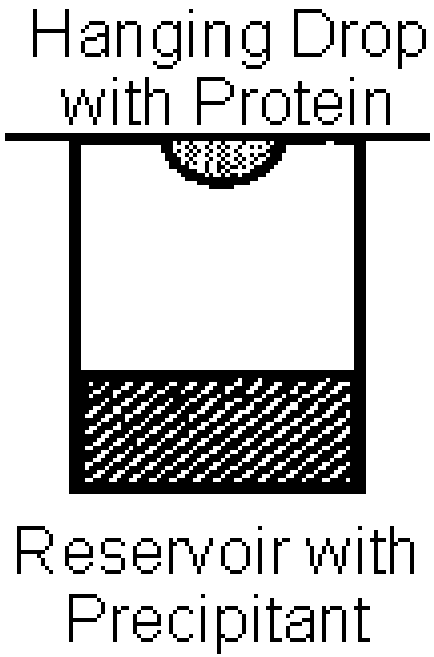
To form protein crystals, we have to find conditions so that the proteins assemble in a periodic lattice. This is done by having a highly concentrated solution of the protein and adding reagents to reduce the solubility.





Things to consider to obtain good crystals:

-the process must not be too rapid
(precipitation yields powders!).



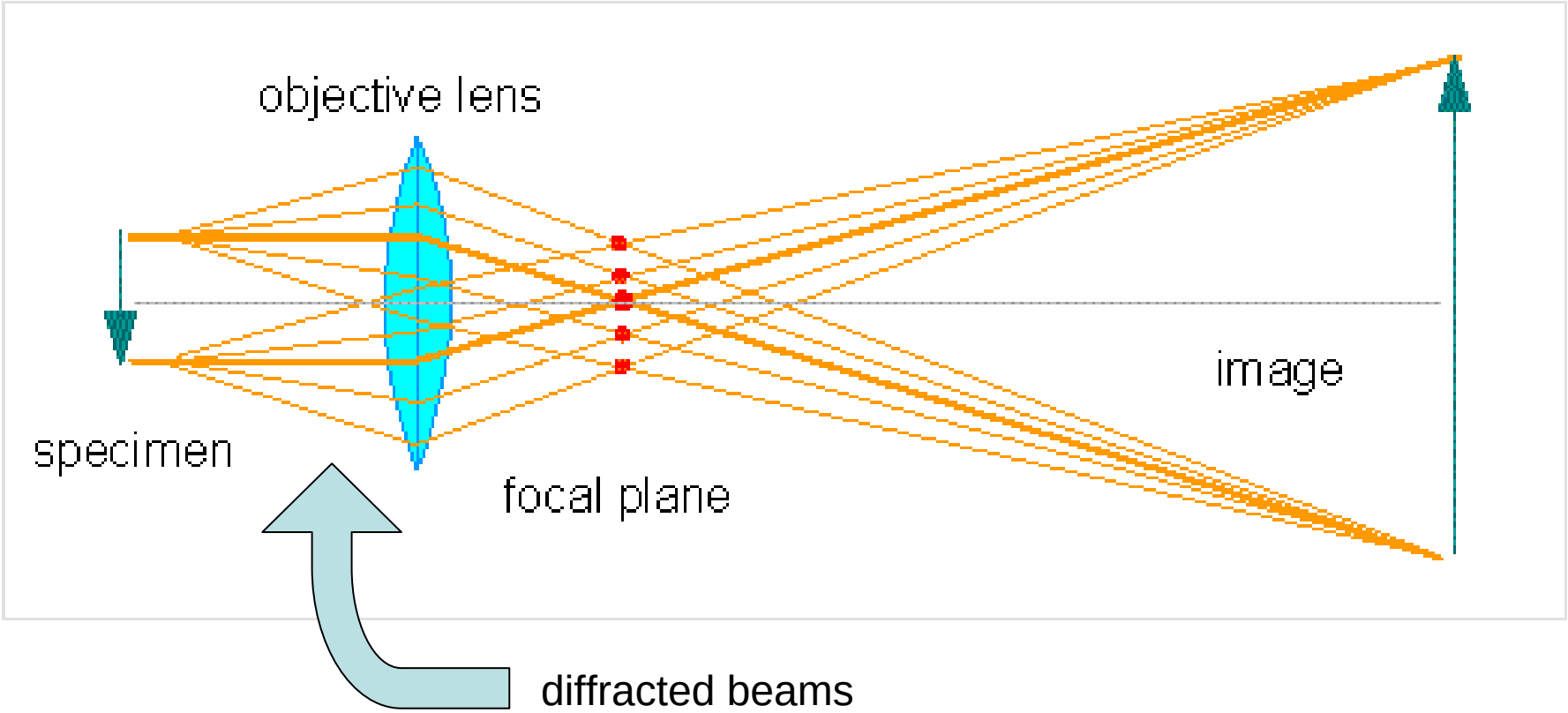
Typical precipitants:

- ammonium sulfate
- polymers (e.g. polyethylene glycol (PEG))
- polyalcohols
- organic solvents
- saccharides

Obtaining good quality crystals is an art!

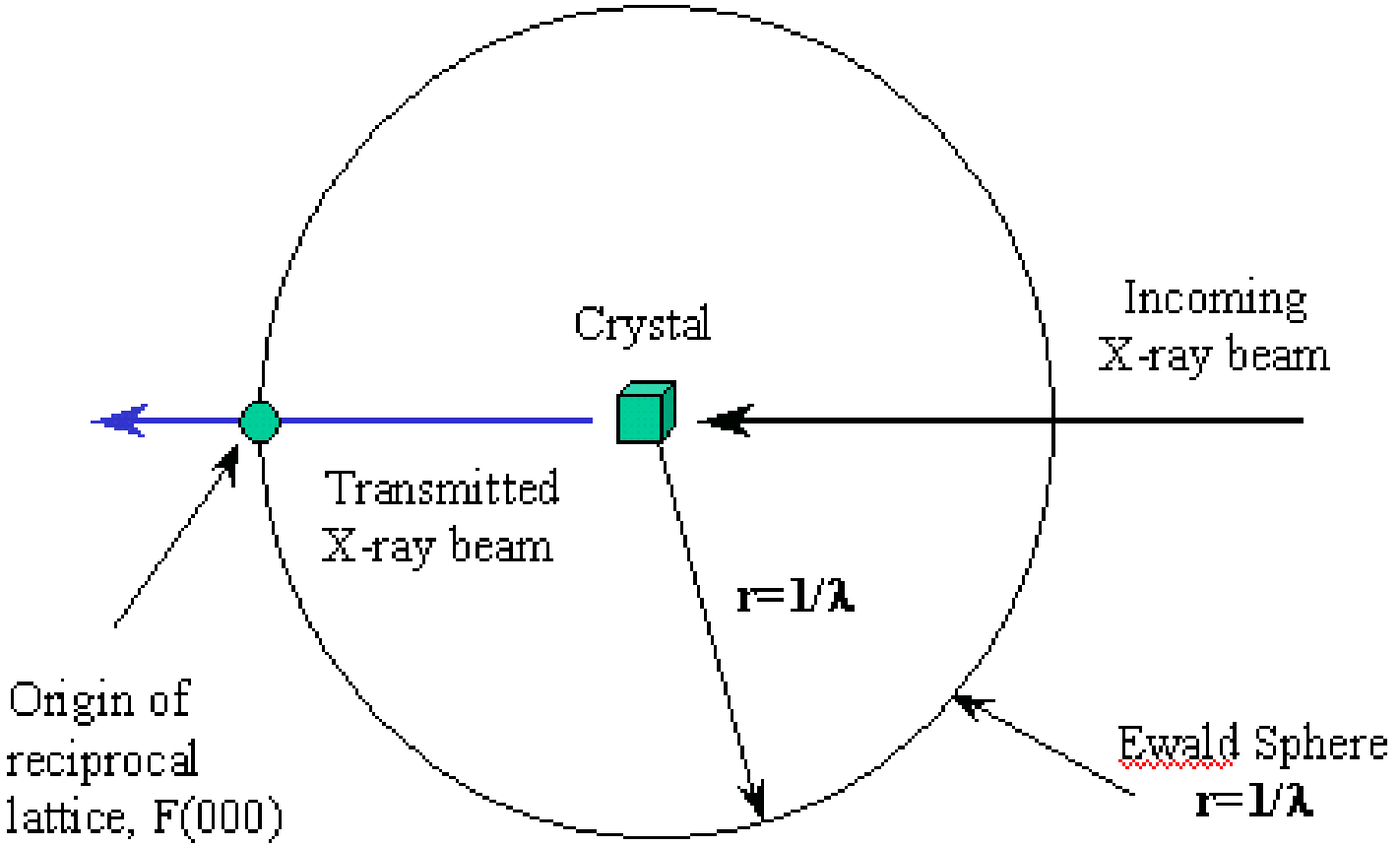
With crystals in hand, x-ray crystallographers measure the intensities of all of the reflections obtained. Their next task is to reconstruct an image of the protein from the intensities.

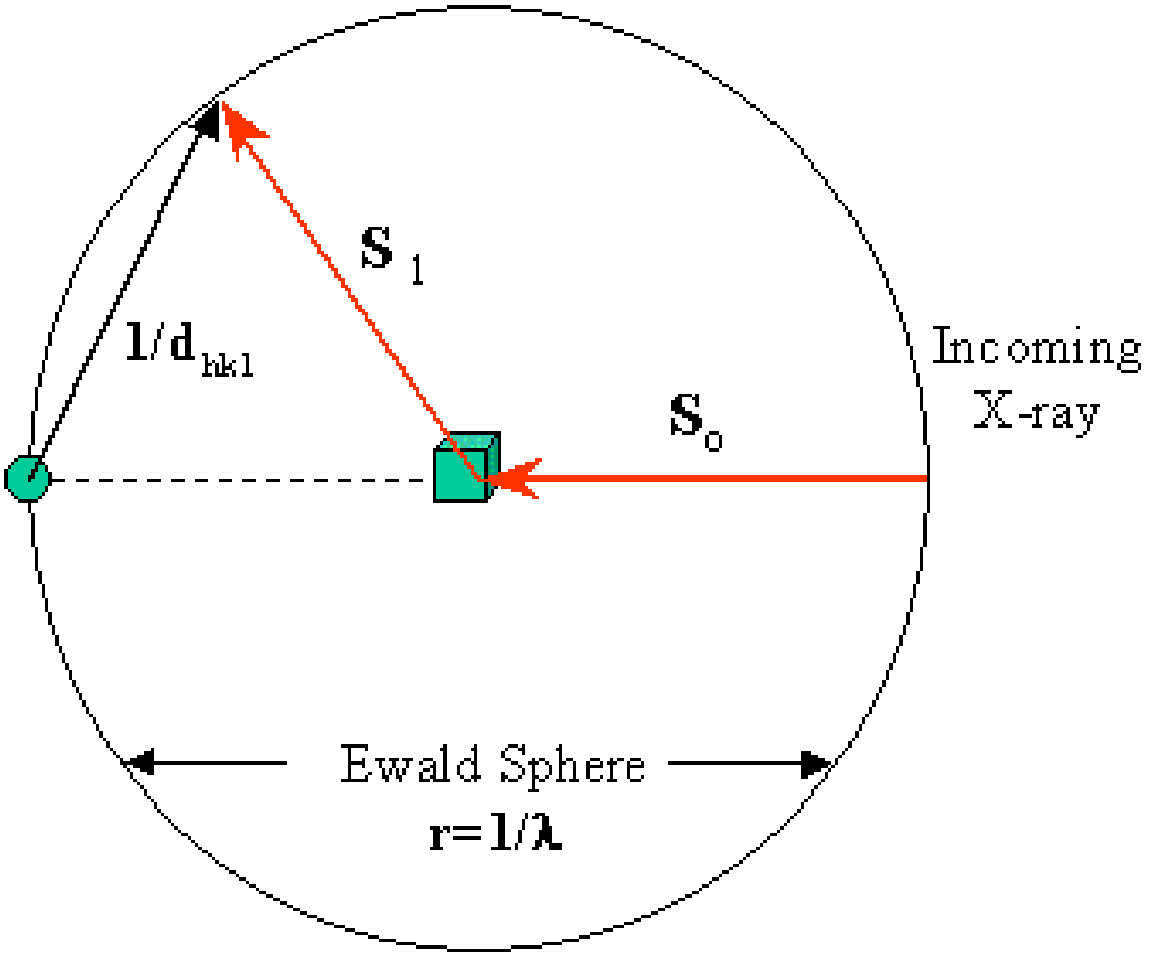
Analogy to microscopy:

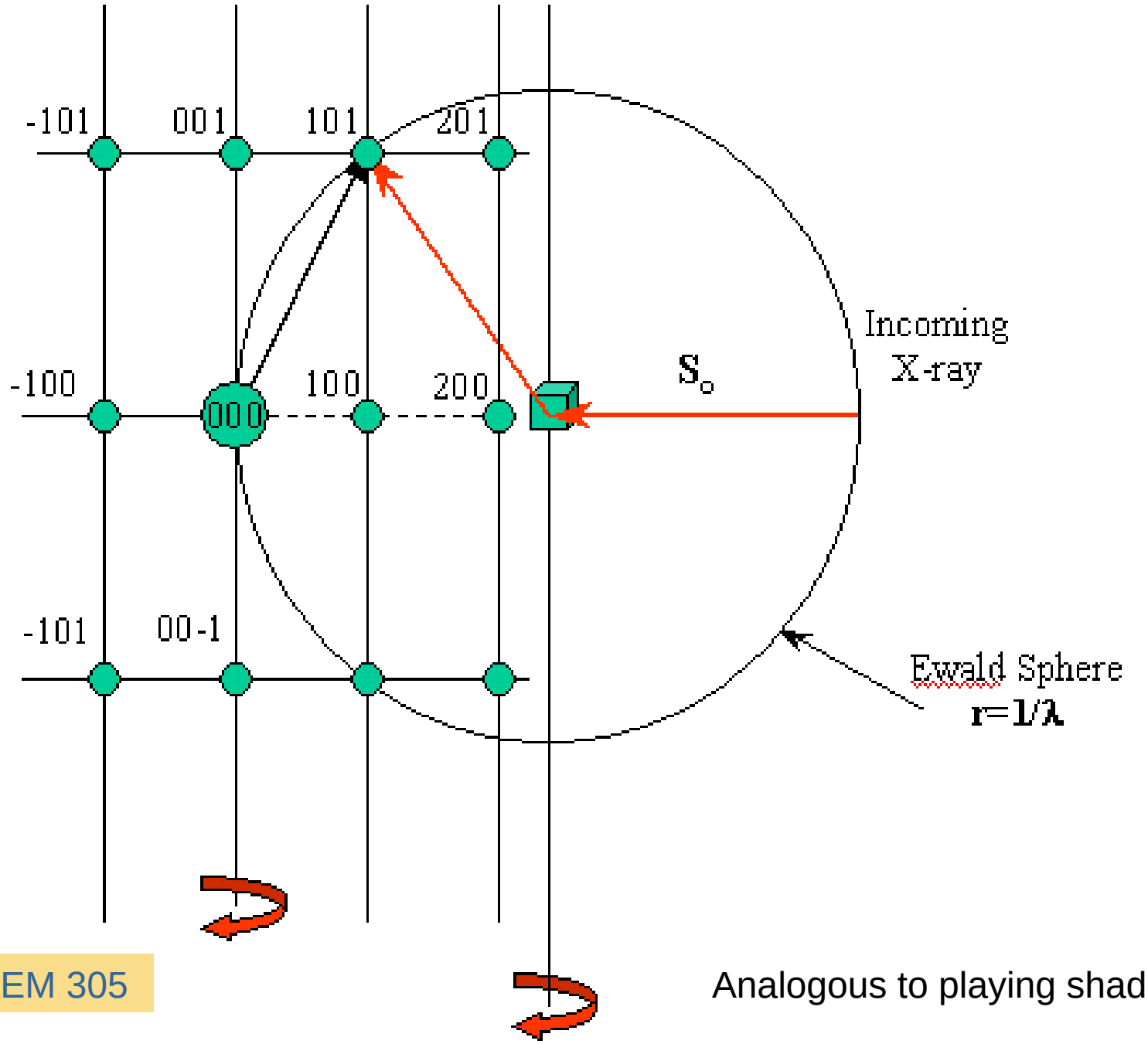


There exists no physical lens to refocus x-rays!

Instead we use a “mathematical” lens, which works with Fourier transforms. First we need to construct a reciprocal lattice. This is done using the Ewald construction.

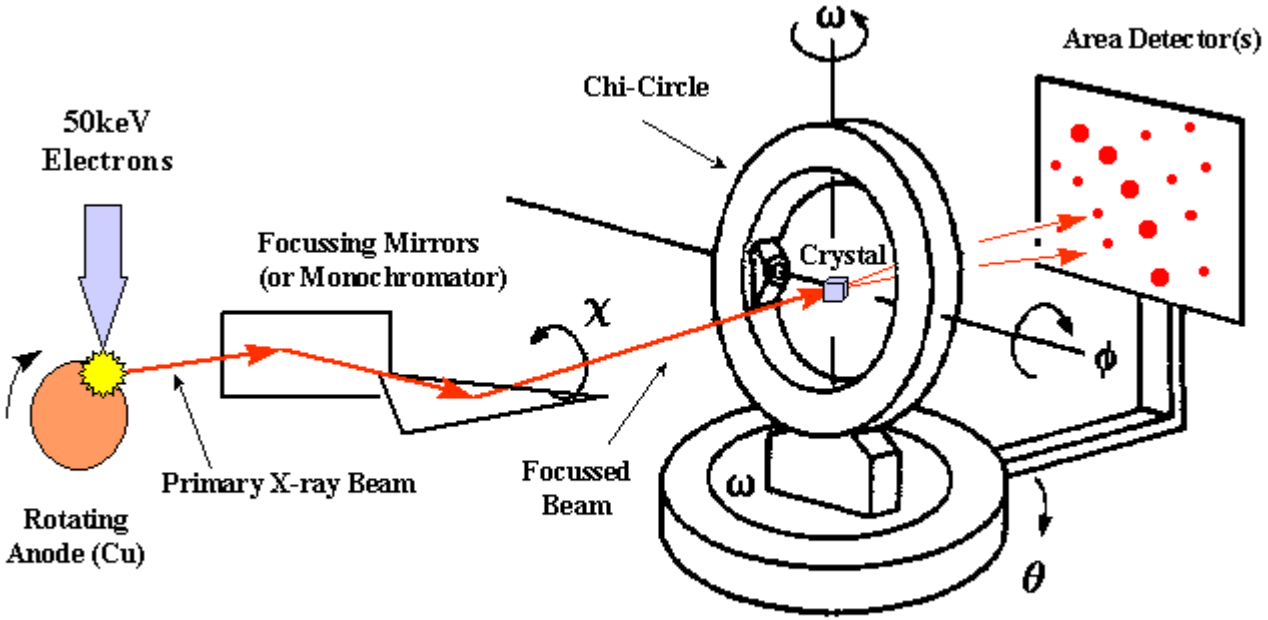



$\mathbf{s}_1$
$1/d_{hkl}$
$\mathbf{s}_o$
Incoming
X-ray
Ewald Sphere
$r=1/\lambda$



Analogous to playing shadows.

Summary: X-ray diffraction relies on the principle of diffraction between atoms.

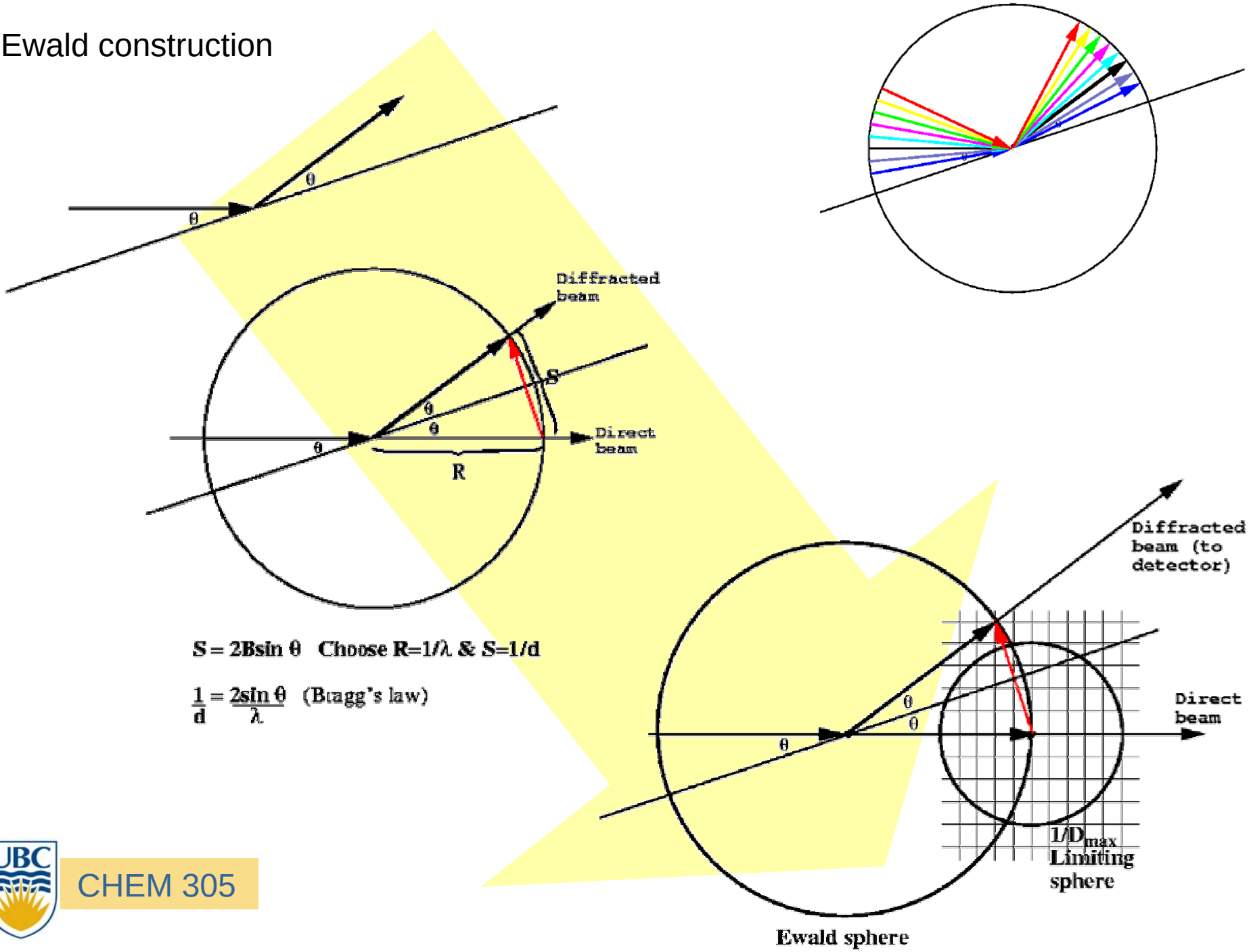


4-Circle Goniometer (Eulerian or Kappa Geometry)

In order to diffract the x-rays, crystals are required, i.e. regular 3D arrangements of molecules.

How do we reconstruct the image detected into a 3D structure?

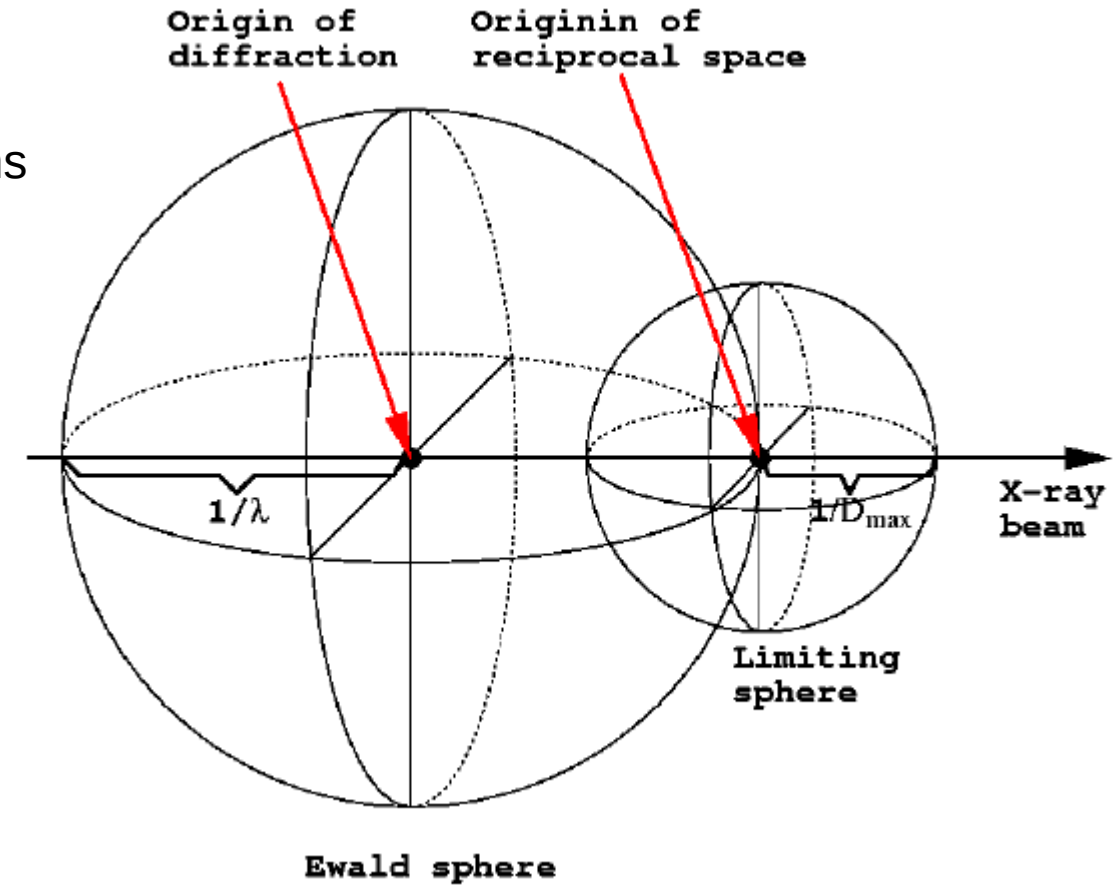
Ewald construction



$S = 2B\sin\theta$ Choose $R=1/\lambda$ & $S=1/d$

$\frac{1}{d} = \frac{2\sin\theta}{\lambda}$ (Bragg's law)

In three-dimensions



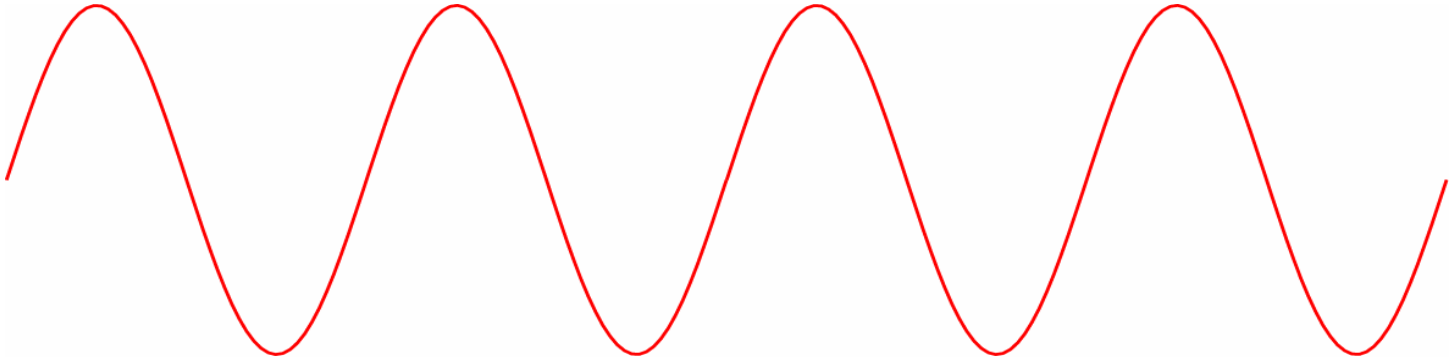
- The Ewald sphere allows you to visualize the diffraction experiment
- Diffraction only occurs when a reciprocal lattice point intersects the Ewald sphere
- The radius of the Ewald sphere is 1/λ

http://eagle.mid.med.ualberta.ca/highlights.html

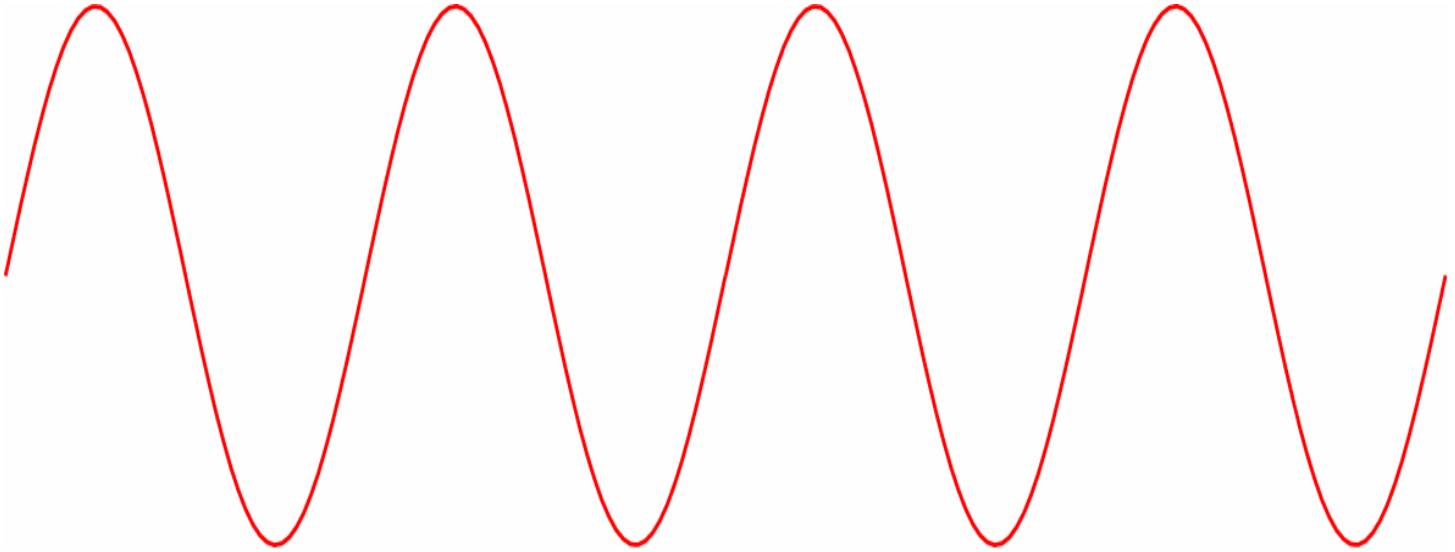
Each spot is then Fourier transformed to yield a wave of electron density with

- amplitude proportional to the square root of the intensity of the spot
- phase

e.g. at 001



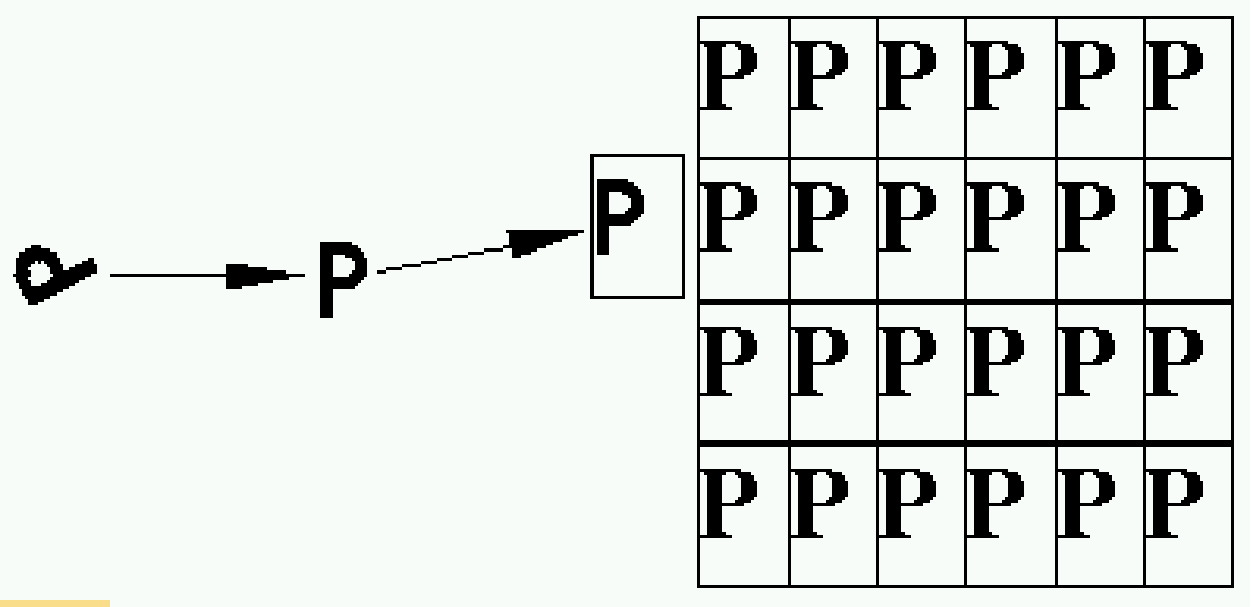
at -101



The phase problem

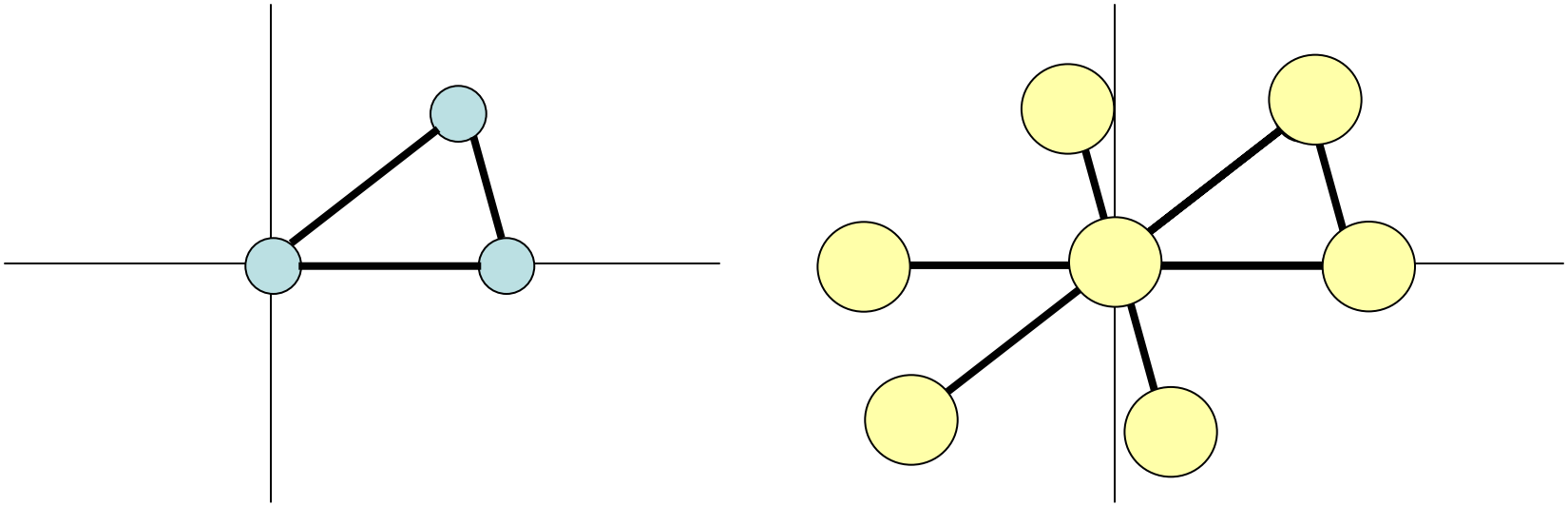
The phases we obtain from the Fourier transformations are not referenced to a specific point in space….

To solve the problem, we introduce a “heavy atom” such as uranium or mercury at specific sites in the protein – for example, by soaking the protein crystals. The diffraction patterns for these “heavy atoms” are well known, so they can be used as references.



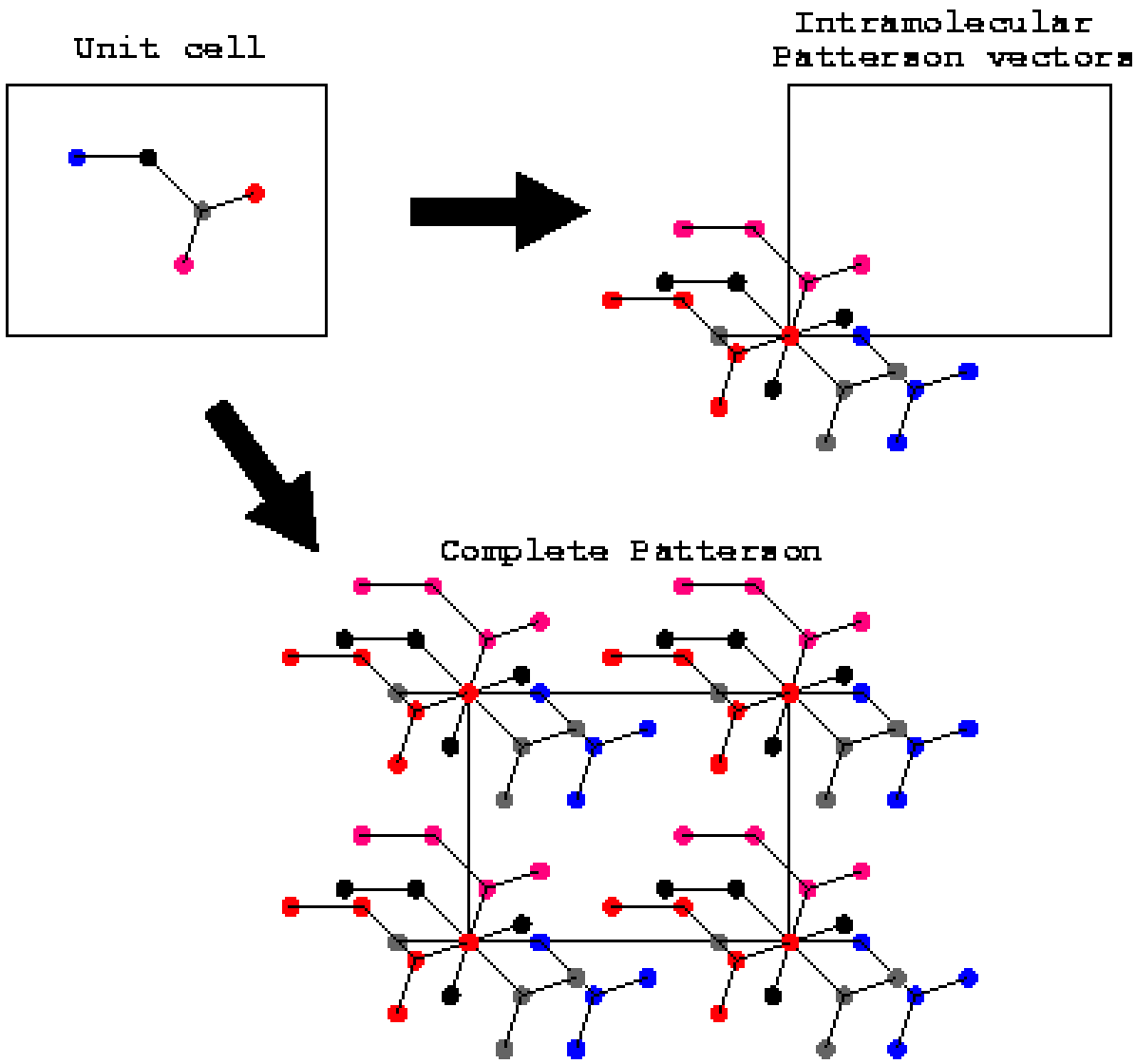
Relating reflections to atom position using Fourier maps

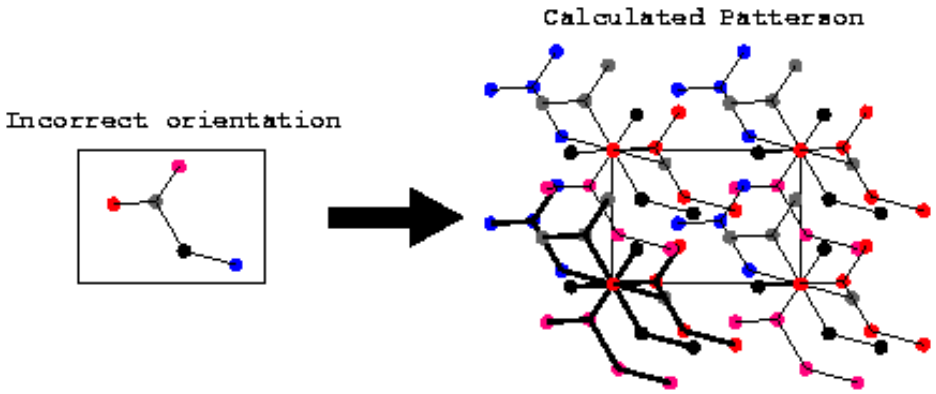
- Special case: Patterson map (used for determining heavy atom positions)
- Generating a Patterson map for a tri-atomic molecule

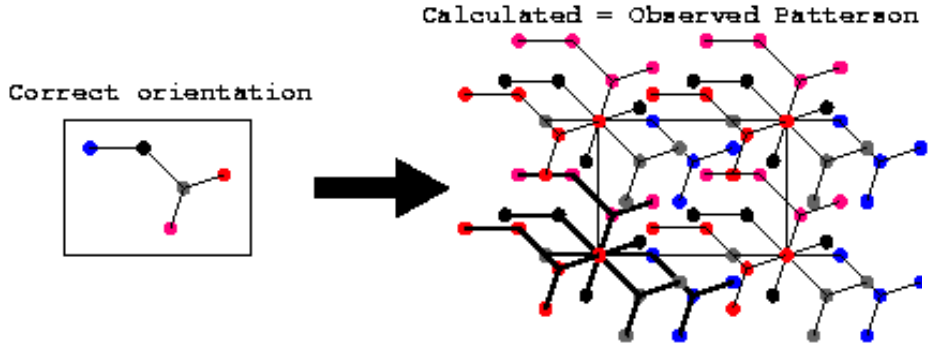


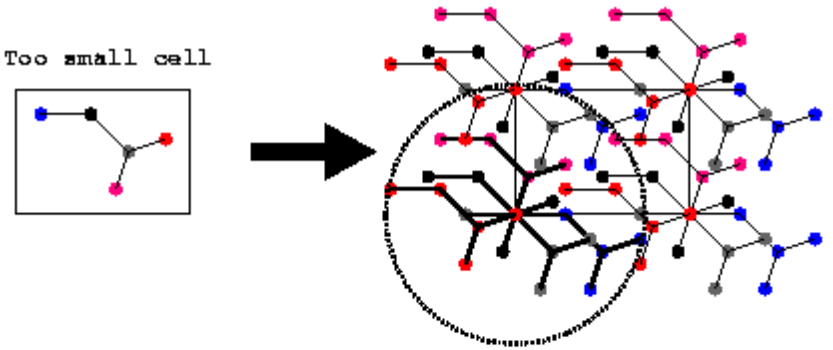
Unit cells of different symmetry will result in different Patterson maps

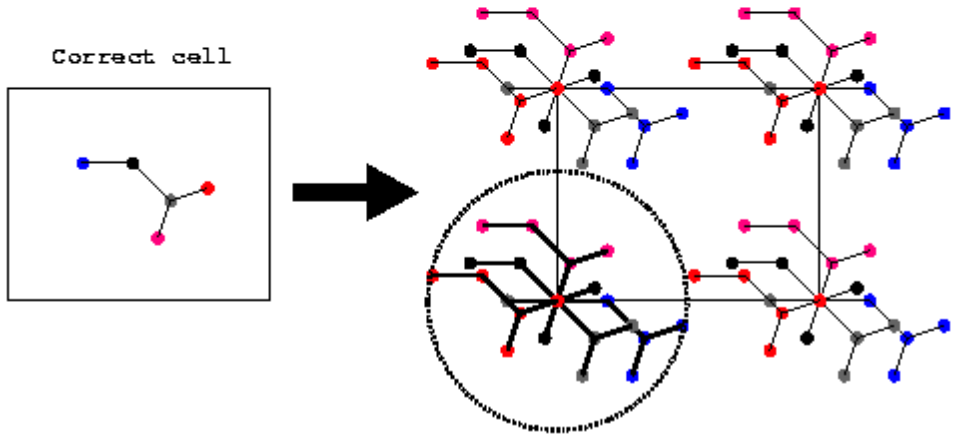
e.g. P1





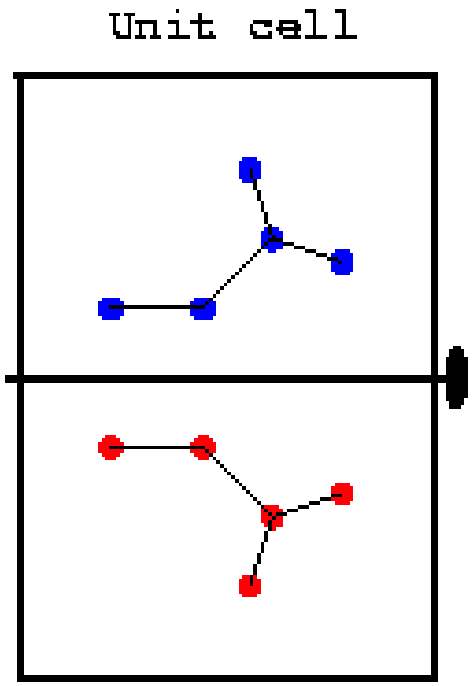


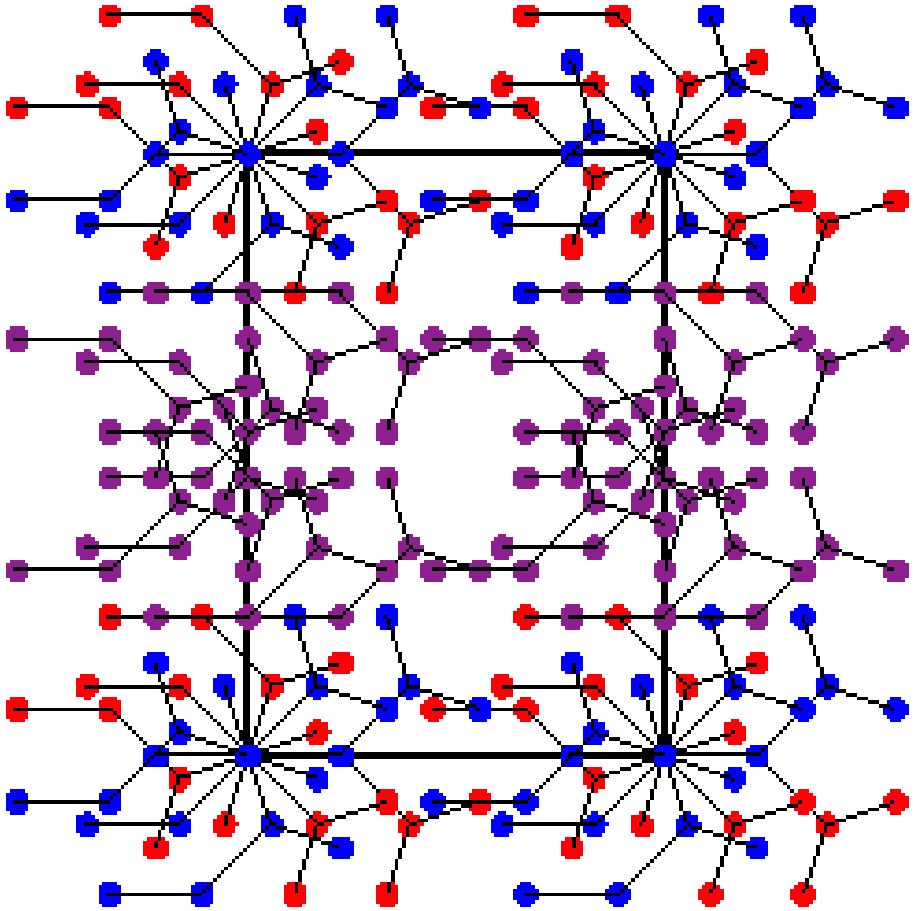




Patterson maps are sensitive to the orientation and cell size.

P2 symmetry



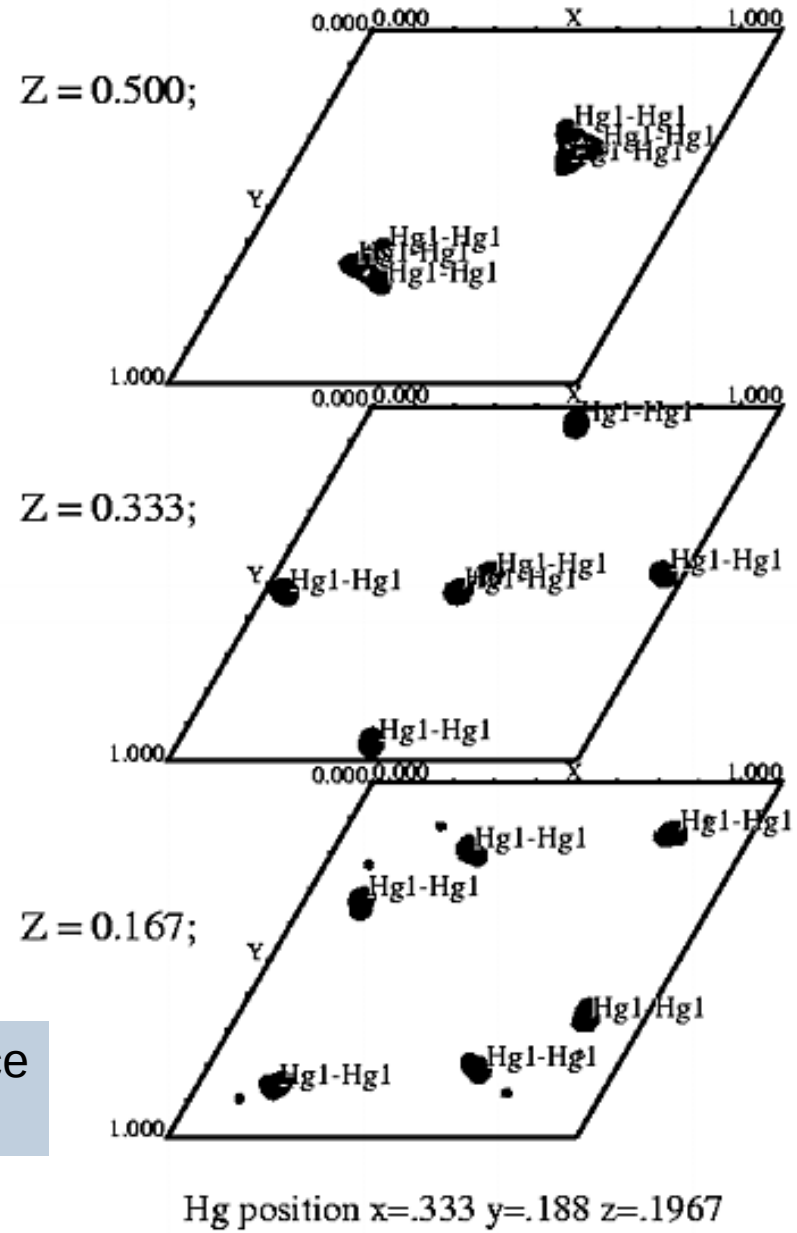


If it is this complicated for a small molecule – then it must be even more complex for a protein!!! And what about our heavy atom located in the protein?

By taking the difference between the Patterson map one would obtain for the protein with a Patterson map obtained for the protein + heavy atoms, we can get a “difference Patterson”.

These are simpler to interpret and can be used to locate the heavy atom.

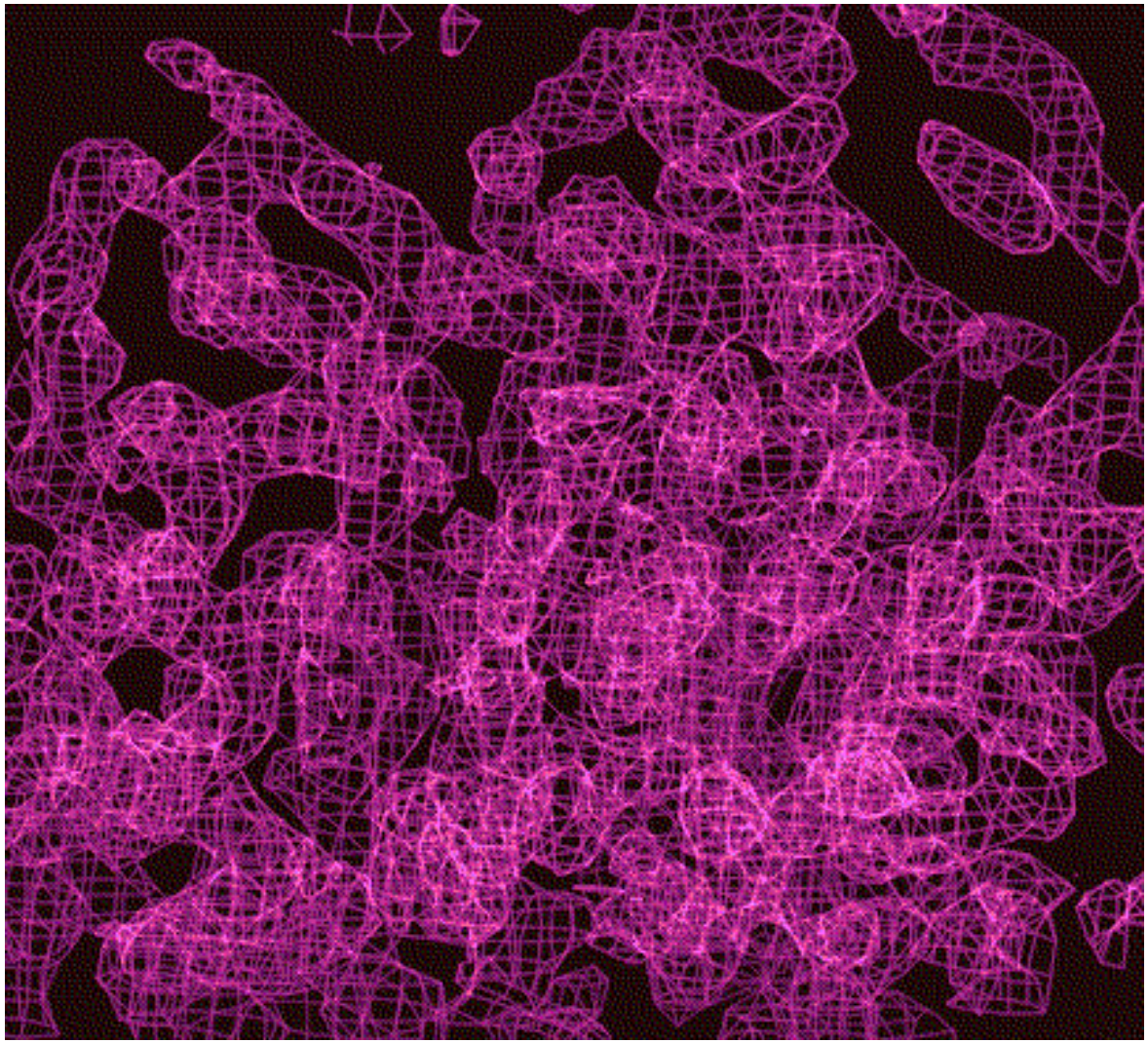
Consequence: Must measure diffraction patterns for a minimum of two crystals! (Why would we want to measure more?)

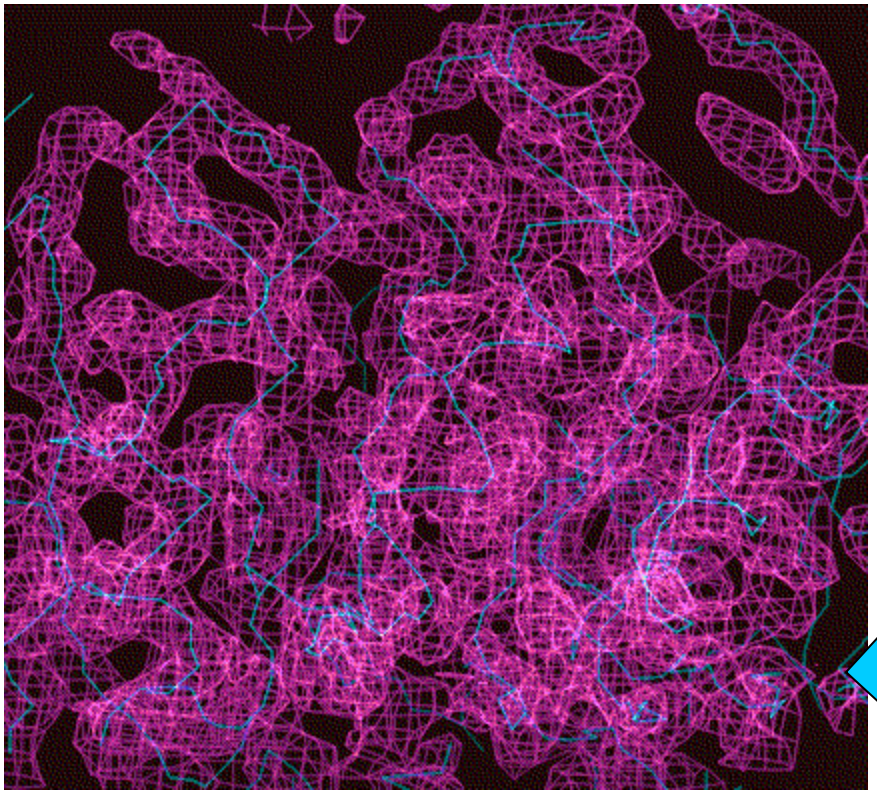


Hg position x=.333 y=.188 z=.1967

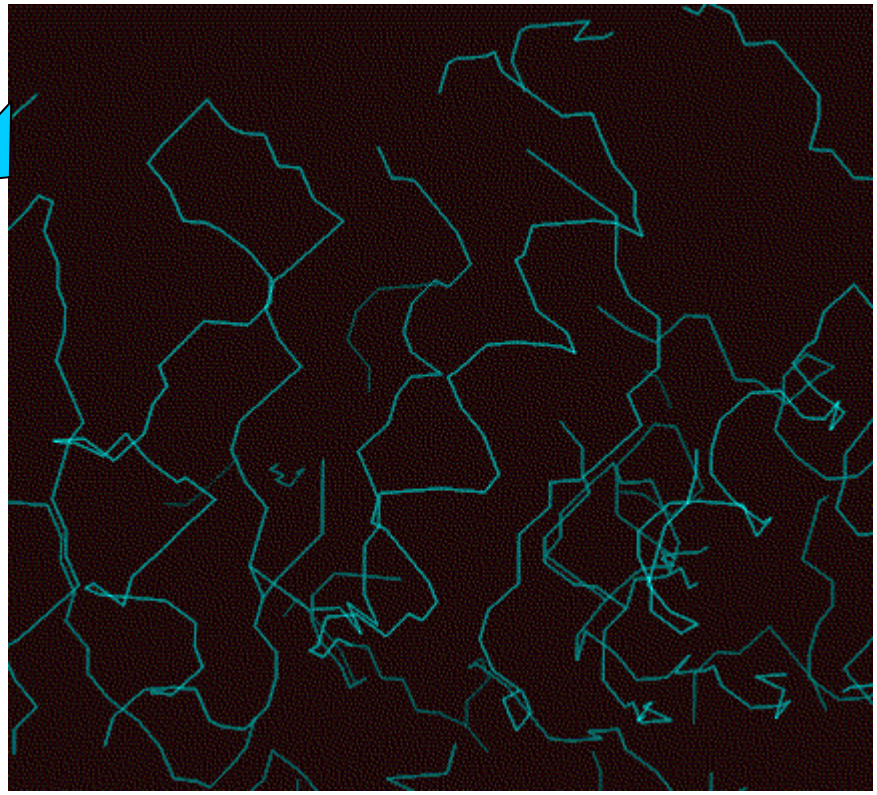
Isomorphous difference Patterson maps

At this point, we obtain a trial electron density map and can start building models of the structure.





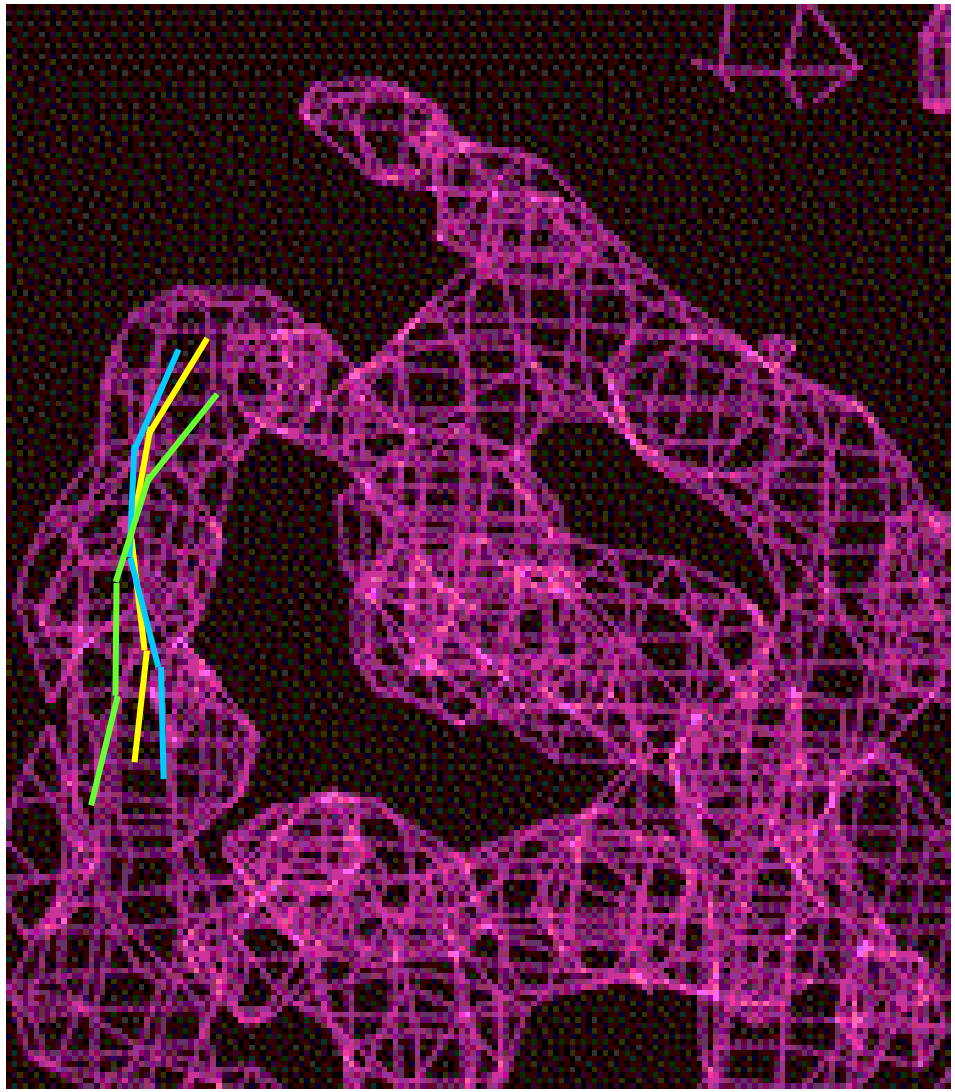
Given that you know the sequence of the protein, you can generate a model and fit it within the density (using a number of computer programs). First, you start with the backbone.



Then you can fit the side-chains

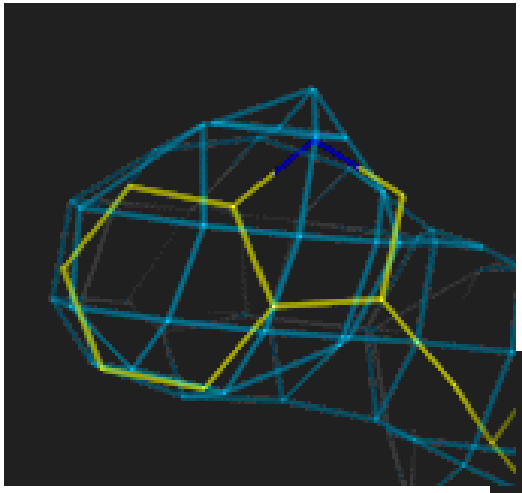
E.g. http://perch.cimr.cam.ac.uk/Course/Basic_refinement/Refinement.html

In the example above, we show one way of drawing in the backbone for the protein to fit the density. When first starting this process, we can find a number of possible solutions. Analogous to a monkey in a cage!

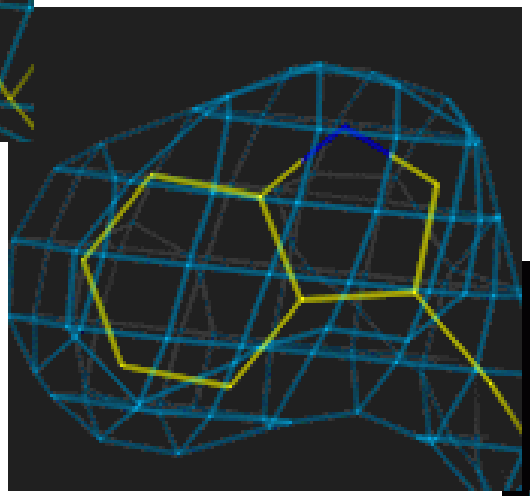


At this stage, the structure is poorly resolved. We say that the structure has a resolution of e.g. 8 Å.

Since we want atomic resolution (i.e. on the order of 1 Å), we need to *refine* the structure.

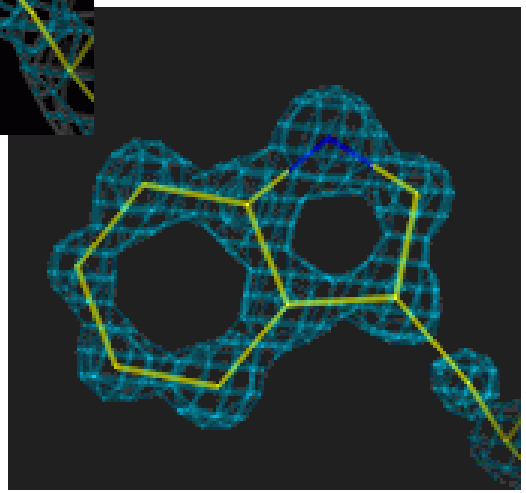


4.0 Å



3.0 Å

2.5 Å



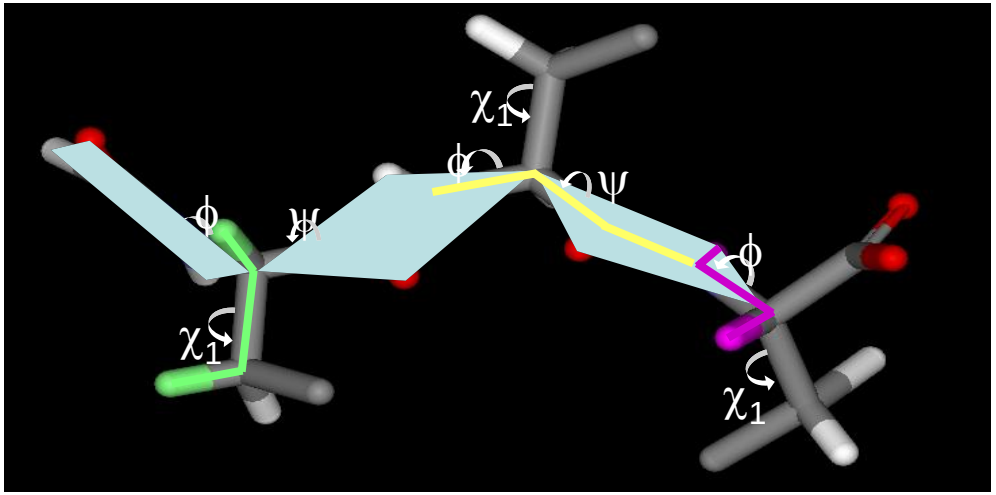
1.0 Å

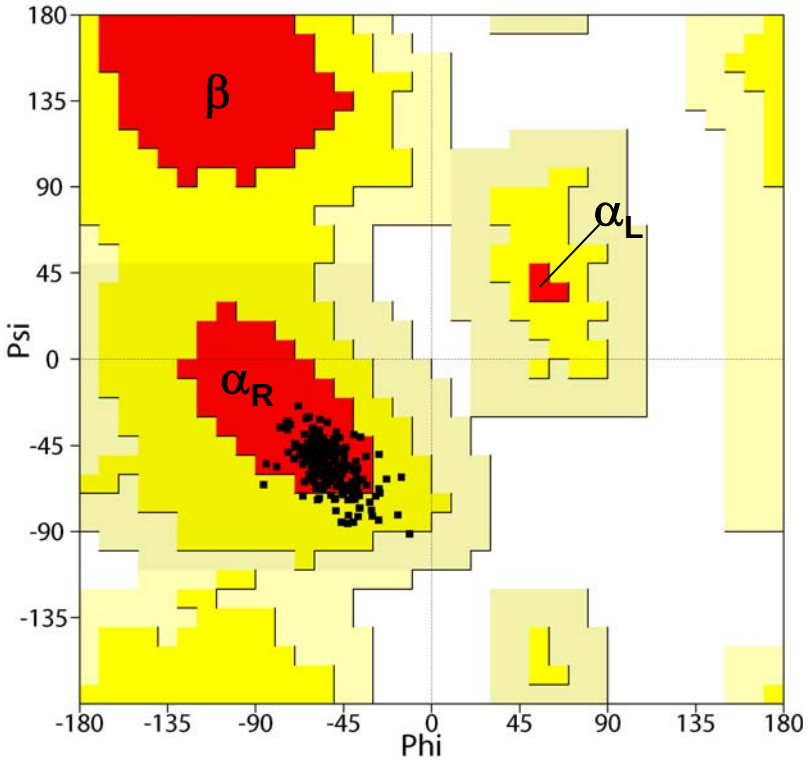
Refinement

This is achieved by back-calculating the diffraction data using the model we just generated, comparing to experimental data, and tweaking the model until we get a better match between the two.

For proteins, this process alone would require a lot of time, because the data we acquire is incomplete. Therefore, other tools are needed, which keep the geometry of the molecules restrained within a reasonable range:

- bond distances
- torsion angles





Ramachandran plot