Protein Crystallography

Part I

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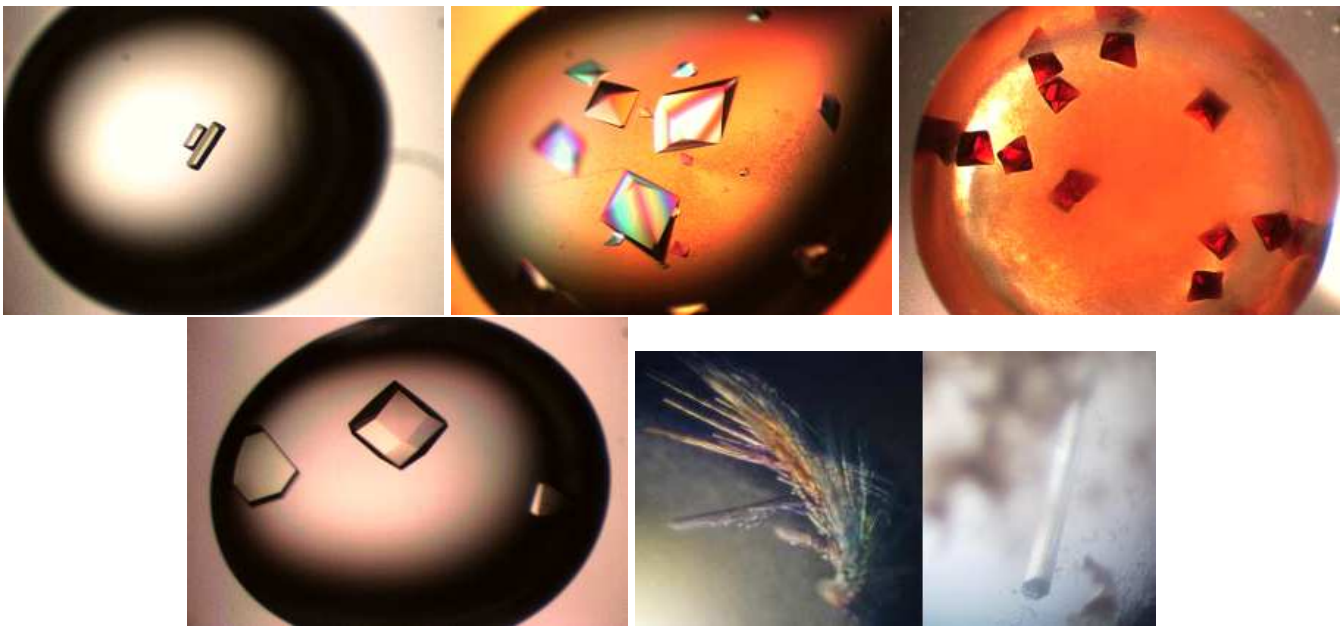
Overview

- Crystal Lattice and Symmetry
- Growing Protein Crystals
- X-ray diffraction
- Bragg's Law
- Resolution
- Electron Density

Kirsten Böttcher et al.

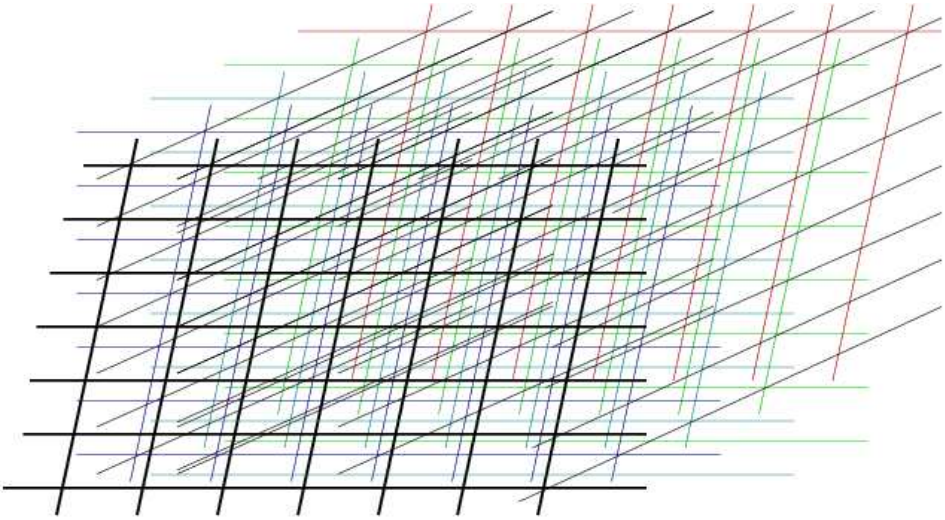


Crystals

A crystal consists of an infinite number of copies of one object. This object can be as small as a single atom or ion (*e.g.* $NaCl$ or diamond crystals) or as big as a protein-RNA complex as the ribosome.

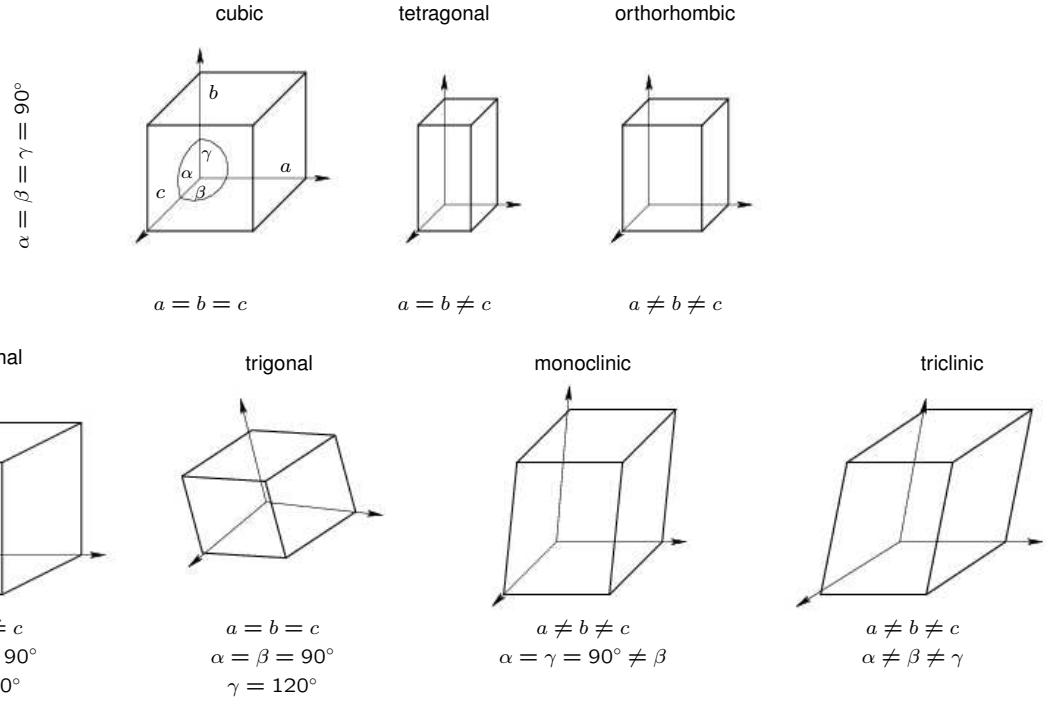
The objects must form a regular pattern, the “crystal lattice”.

The main characteristic of a lattice is its translational invariance: If you move from one lattice point to another one but keep looking in the same direction, you can tell no difference.



Seven Lattice Types

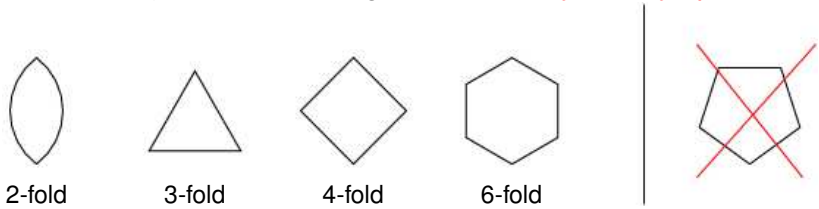
The lattice is an infinite repetition of one “box”, the unit cell. It is defined by the lengths of three edges a, b, c, and the angles α, β, and γ. There are 7 different lattice types that allow to fill an infinite space.



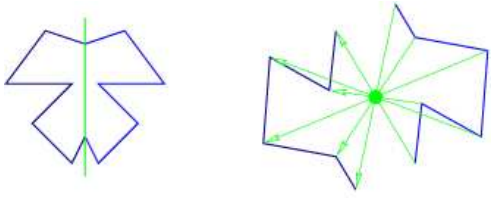
Crystal Symmetry

Only a limited number of “operations” are allowed to go from one object to the next one. These operations can be translations by a vector $(h \cdot a, k \cdot b, l \cdot c)$ with h, k, l integers, but also symmetry operations, e.g.

- rotations (only 2-, 3-, 4-, and 6-fold axes possible)



- mirrors and inversion centres

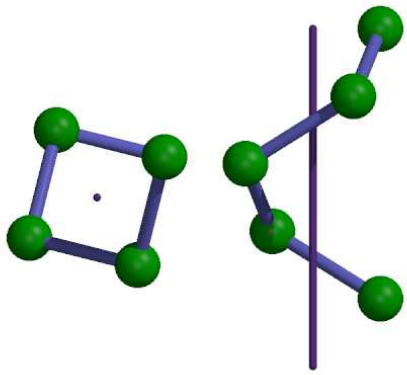


Space Groups

In a three dimensional lattice, symmetry operations cannot be combined arbitrarily. Together with translations, there are 230 allowed combinations, the 230 space groups. Of those, only 65 are chiral, i.e. suitable for macromolecules like proteins, RNA, or DNA.

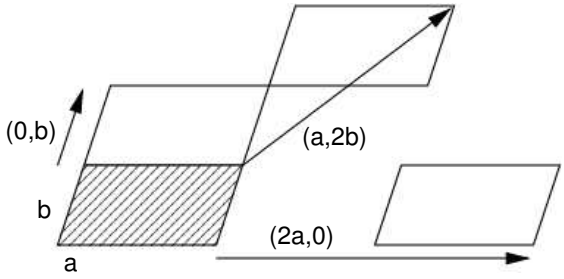
An important example of combining two symmetry elements is the screw axis: rotation with simultaneous translation along an axis.

The example shows a 4_1 axis. A rotation of $1/4 \cdot 360°$ accompanied by a translation of $1/4$ of the length of the axis.

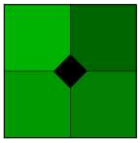


Unit cell and asymmetric unit

In every crystal there is always a smallest box, defined by its edges a, b, and c, and the angles they enclose, α, β, and γ, from which one can create the whole crystal solely by integer translations along its sides. This is called the unit cell.



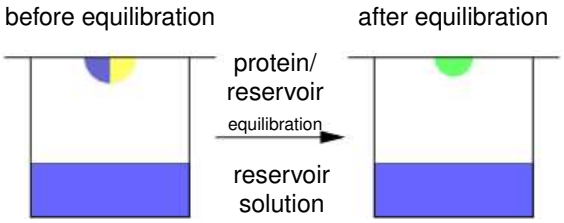
A unit cell consists itself of a smallest unit from which one can create the unit cell by applying all symmetry operators that belong to the crystals space group. This is called the asymmetric unit.



Growing Crystals

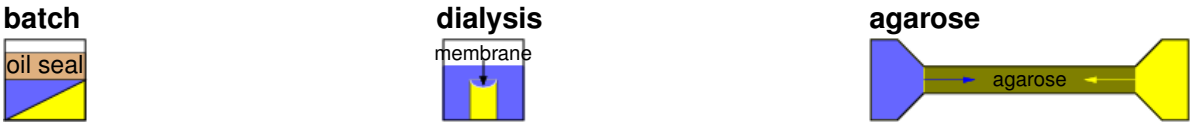
Protein crystals are usually grown from solution. The probably most common way is to apply the “vapour diffusion” method.

The protein must be very pure ($\geq$ 90–95%) and highly concentrated (several mg/ml). The reservoir contains a buffer, a precipitant (salts, PEG's with $M_r^{\varnothing}$ = 400–20,000, organic solvents) and additives (small molecules that help packing, e.g. $MgCl_2$).



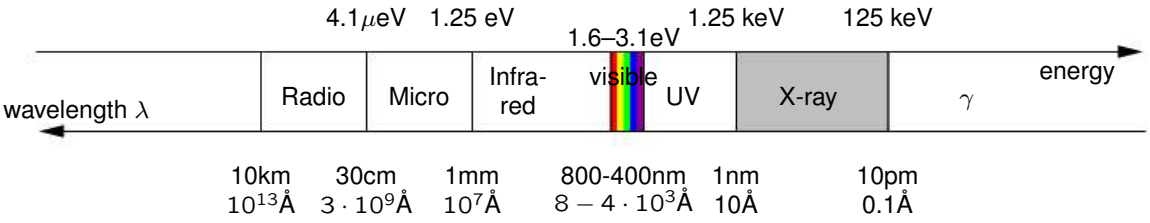
This method is very fast and one can perform a large number of experiments in a rather short time. Robots are used in some laboratories, especially in companies, that can set up 100 drops in 1/2 hour.

Other techniques include

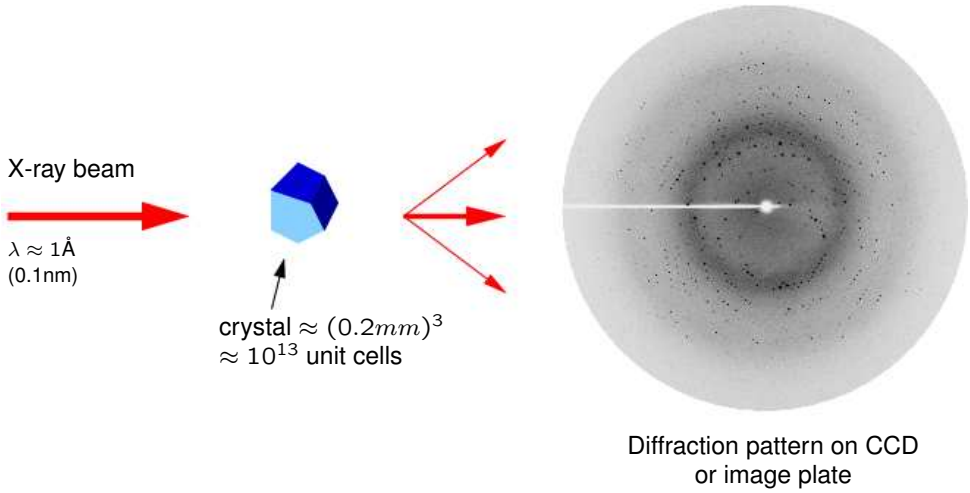


What are X-rays?

X-rays are electromagnetic waves, as is ordinary light or radio waves. They have much more energy or, which is equivalent, a much shorter wavelength than light.



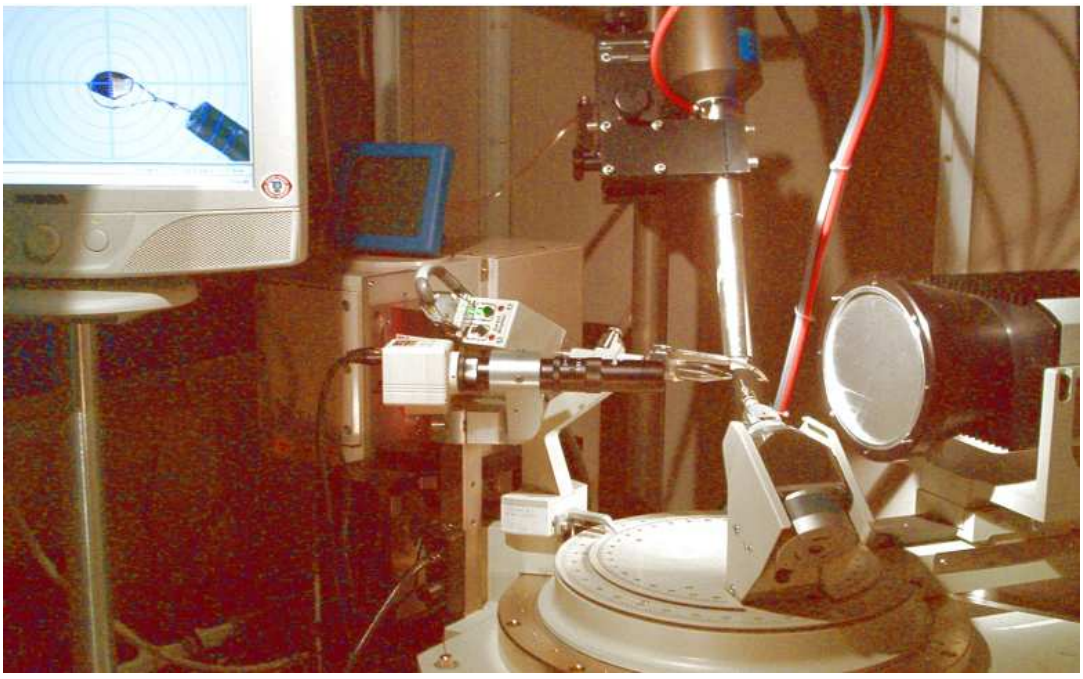
X-ray diffraction



T. Schneider

X-ray sources — inhouse

The SMART 6000



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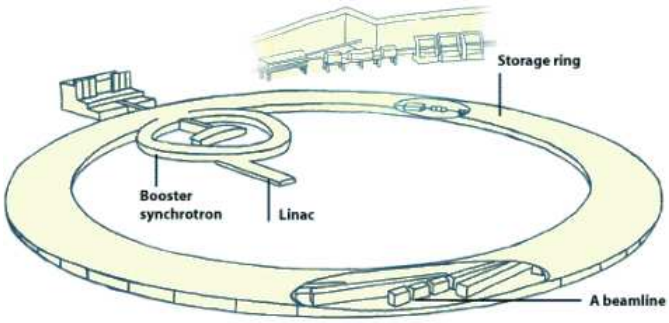
X-ray sources — synchrotrons

The ESRF (European Synchrotron Radiation Facility) Grenoble



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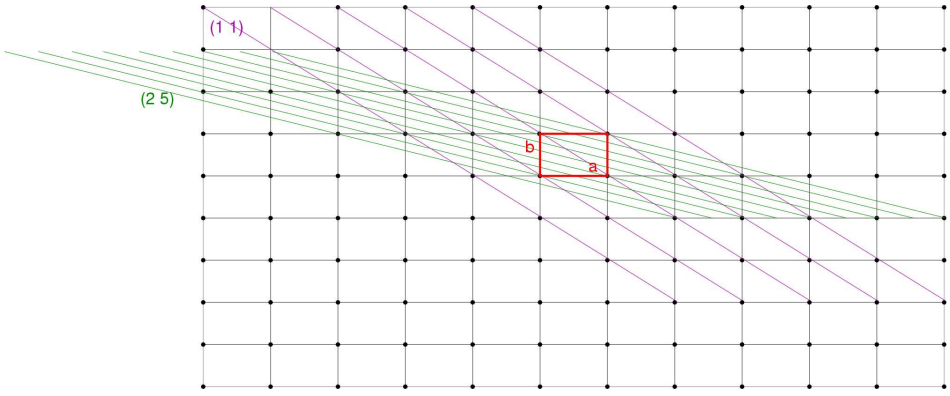
Bragg's Law — Crystal Planes

The lattice points in the crystal define sets of parallel planes. In 3 dimensions the planes are defined by three integers (h, k, l), the so-called "Miller indices". h denotes the number of intersections of the a-axis of the crystal's unit cell by the set, etc.



Each set of parallel planes runs through lattice points. The distance d between two planes is given by

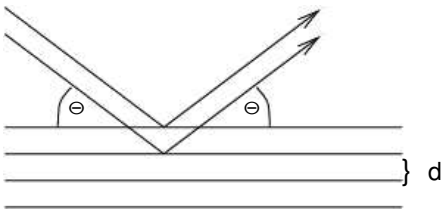
$$\frac{1}{d^2} = \frac{h^2}{a^2} + \frac{k^2}{b^2} + \frac{l^2}{c^2}$$

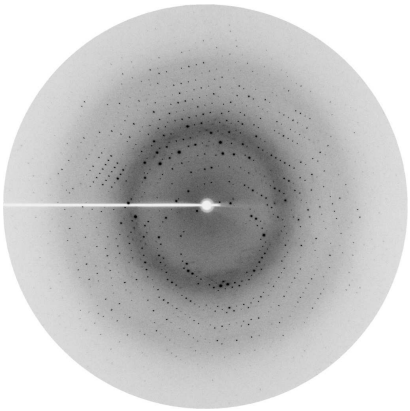
Bragg Reflections

The X-ray beam is reflected at sets of planes in the crystal. Due to interference, a plane does only produce a reflection spot, if Bragg's law is met:

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

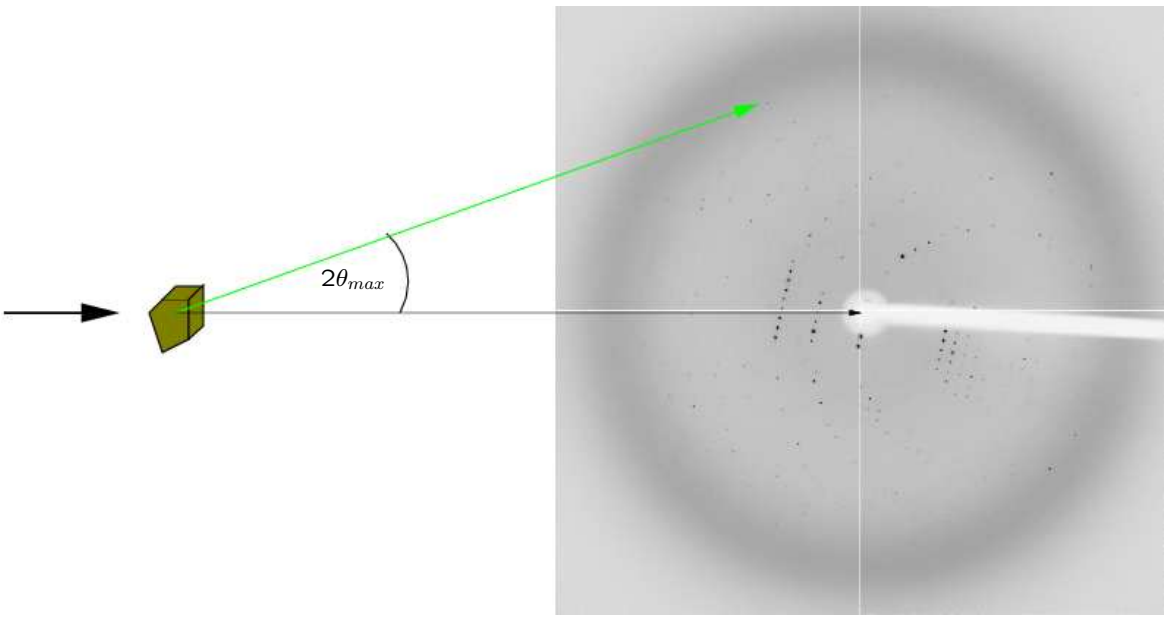
λ is the wavelength of the beam, d the distance between the planes of one set.
Every spot that can be seen on a diffraction image originates from one set of planes that is in diffracting orientation according to Bragg's law. The spot can be indexed with the same (h, k, l) values that describe the set of planes.





(Bragg's law is actually $n\lambda = 2d \sin\theta$, but $n > 1$ refers to higher order reflections that are too weak to be recorded for "normal" crystals.)

Concept of Resolution I



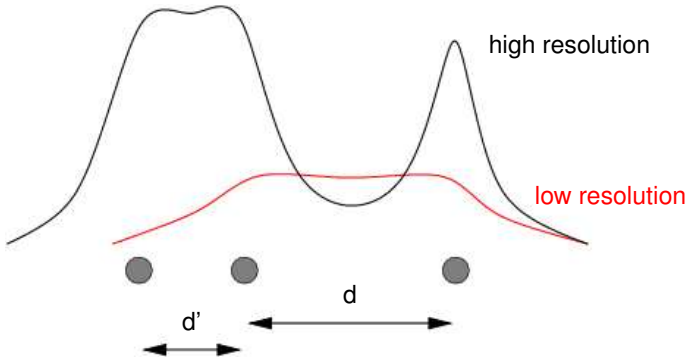
The Concept of Resolution II

The resolution of data collected by X-ray diffraction is a measure for how much detail can be seen. It is related with the plane distance d through Bragg's law by

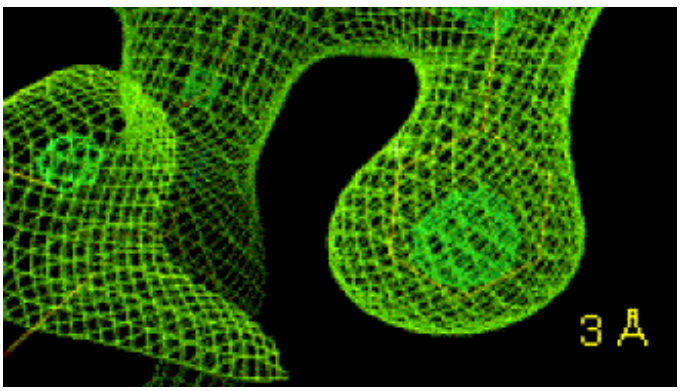
$$d = \frac{\lambda}{2 \sin \theta_{max}}$$

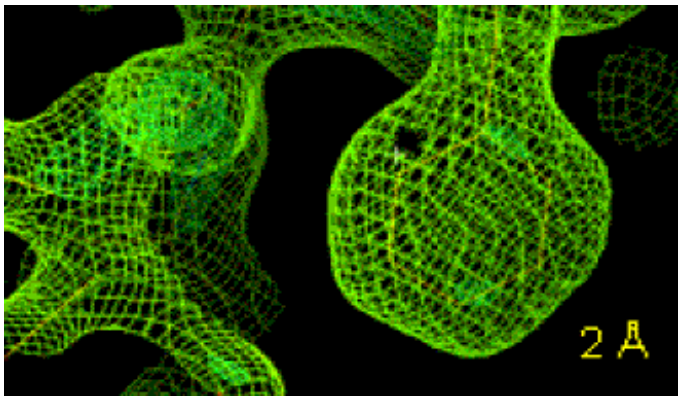
θ_{max} is the maximum angle to which data could be collected.

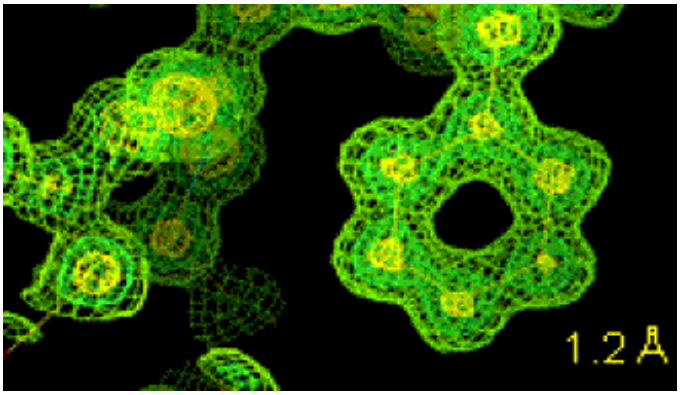
The resolution corresponds quite well to the minimum distance between two atoms that can still be resolved in the electron density map.



Resolution — Examples







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X–ray diffraction → Electron Density → Model !?

The intensities of the reflections measured by an X-ray diffraction experiment are proportional to the square modulus of the structure factors.

Every reflection marked by (h, k, l) has its origin in a structure factor $F(h, k, l)$. The structure factors are related to the electron density distribution within the unit cell by an expression called Fourier transformation:

$$\rho(x, y, z) = \frac{1}{V_{unitcell}} \sum_{h,k,l=-\infty}^{h,k,l=\infty} F(h, k, l) \cdot e^{-2\pi i(hx+ky+lz)}$$

and its inversion

$$F(h, k, l) = \int_{V_{unitcell}} d^3x \; \rho(x, y, z) e^{2\pi i(hx+ky+lz)}$$

If we knew all structure factors, we could calculate the electron density in the whole unit cell.

A major effort of crystallography lies in the determination of as many and as accurate structure factors as possible.

Limitations of Data Collection

Every X-ray data collection has a limited resolution. There are various reasons for this:

1. it is not possible to determine an infinite number of reflections
   ⇒ resolution limit, truncation errors in Fourier transform

2. due to Bragg's law, even a perfect crystal has a resolution limit

3. crystals have limited size, i.e. there are only a finite number of planes. This becomes an issue for small crystals with a large unit cell

4. Crystal imperfections, mostly mosaicity — it leads to a broadening of the signal so that weak reflections vanish in the background noise