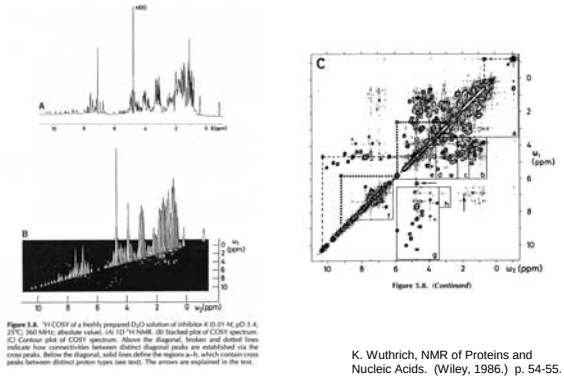
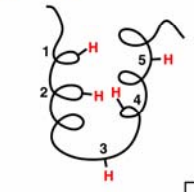
1D and 2D NMR spectra of inhibitor K (57 a.a.)



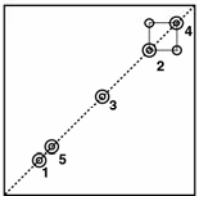
K. Wuthrich, NMR of Proteins and Nucleic Acids. (Wiley, 1986.) p. 54-55.

The nuclear Overhauser effect (NOE) identifies pairs of protons that are close to one another



Protons 2 and 4 are close together

Crosspeaks observed between 2 and 4



NMR structure determination

If you know where, along the diagonal, each proton's peak is, then the 2D NOE experiment tells you which pairs of protons are close in space.

This provides a set of distance constraints. The set of constraints can then be used to generate 3D structures consistent with these constraints. More constraints --> better structures!

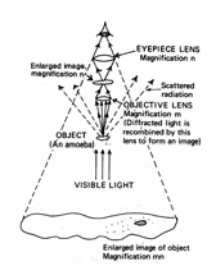
NMR structures are usually presented as families of structures, each of whose members satisfies the NOE distance constraints.

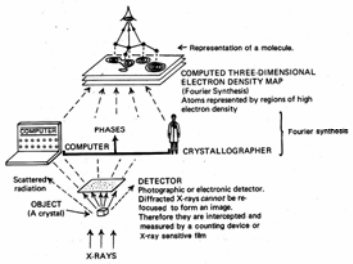
Cellulase (36 a.a.)

Ten superimposed structures that all satisfy the NMR distance constraints equally well

Branden & Tooze, Fig. 18.20

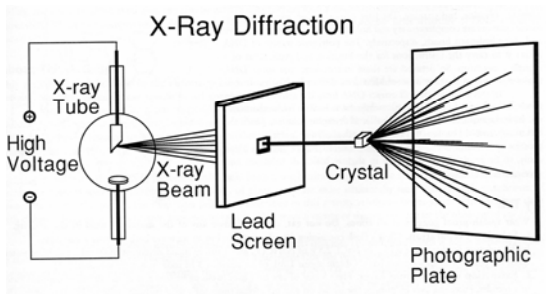
An Analogy



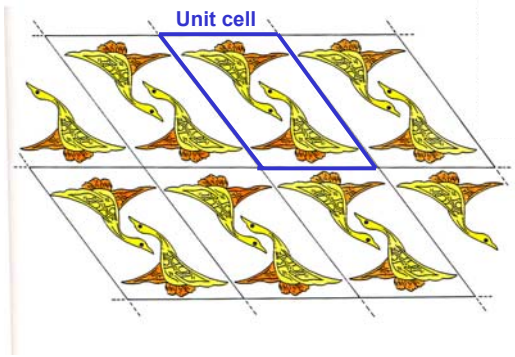


Light microscopy **X-ray crystallography**

J.P. Glusker & K.N. Trueblood, Crystal Structure Analysis: A Primer. (Oxford, 1985.) pp. 4-5

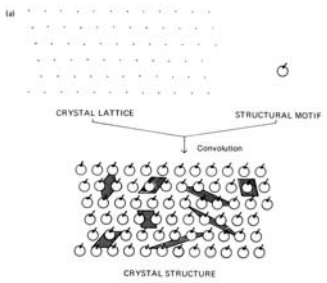


A crystal is built of identical units

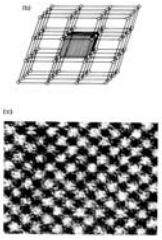


Branden & Tooze, Fig. 18.1

A crystal is the convolution of a lattice and a structural motif



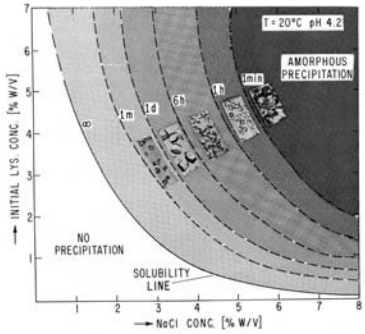
Unit cells



Electron micrograph of fumarase crystal

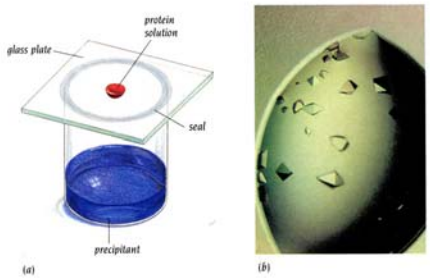
J.P. Glusker & K.N. Trueblood, Crystal Structure Analysis: A Primer. (Oxford, 1985.) pp. 12-13.

Lysozyme crystallization and precipitation



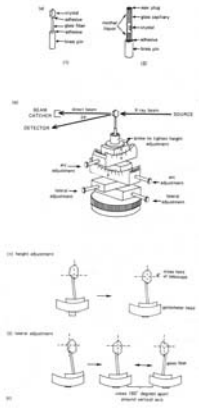
Feher G and Kam Z (1985) Nucleation and growth of protein crystals: general principles and assays. Meth. Enzymology 114, 77-112.

Hanging drop method for crystallization



Branden & Tooze, Fig. 18.4

Mounting crystals and centering them in the x-ray beam



J.P. Glusker & K.N. Trueblood, Crystal Structure Analysis: A Primer. (Oxford, 1985.) p. 44.

Cryoloop

Figure 1.14. A cryoloop, approximately the size of the crystal, is mounted in a metal capillary that can be fixed to a base. This base has a magnetic plate at the bottom to attach it quickly to a goniometer head. The base can also be screwed to a cap for transport and storage.

Jan Drenth, Principles of Protein X-Ray Crystallography. (Springer, 1999.) p. 18.

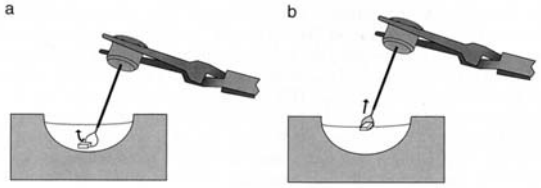
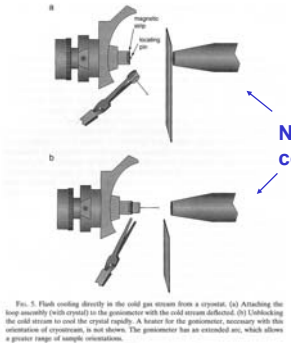


FIG. 4. Mounting a crystal in a loop. (a) Teasing the crystal to the surface of the liquid with the loop assembly. (b) Drawing the crystal into the loop. The illustration shows the crystal and cryoprotective harvest solution in a transparent well plate, which permits viewing with a stereomicroscope while mounting. The relative size of the loop has been exaggerated.

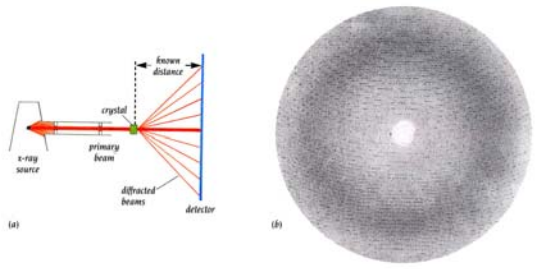
Rodgers DW (1997) Practical Cryocrystallography. Meth. Enzymology 276, 183-203.



Nozzle emitting cold gas stream

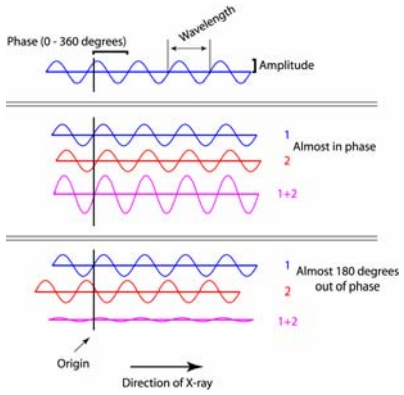
Rodgers DW (1997) Practical Cryocrystallography. Meth. Enzymology 276, 183-203.

Schematic view of x-ray diffraction experiment

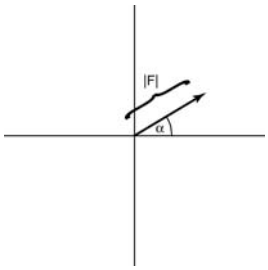


Branden & Tooze, Fig. 18.5

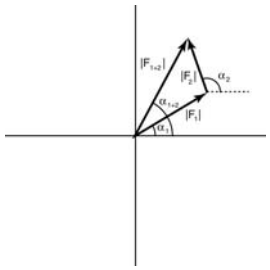
An X-ray behaves like a wave

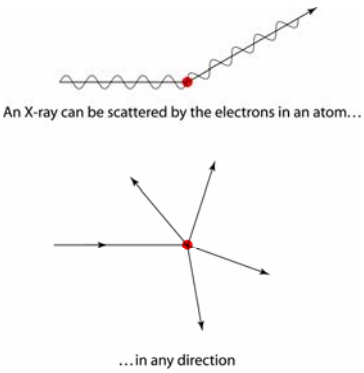


A sine wave with amplitude |F| and phase α can be represented as a vector

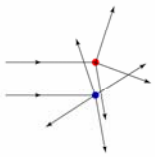


Then the sum of two sine waves (with the same wavelength) is represented by the vector sum!

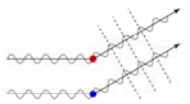




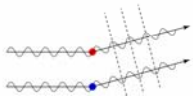
What about two atoms?



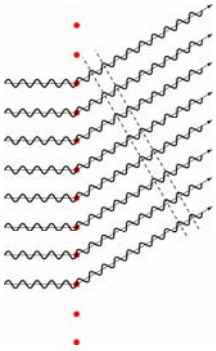
Consider some particular directions:



In this direction, the scattered X-rays are perfectly in phase and reinforce

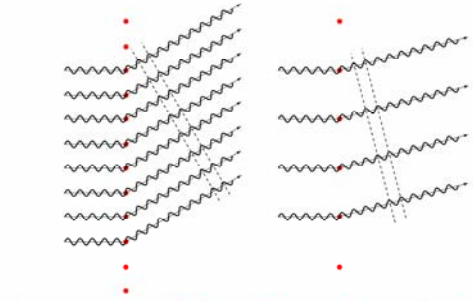


In this direction, the scattered X-rays are perfectly out of phase and cancel out



- Simplest 'crystal' (1D); each unit cell has one atom
- All X-rays scattered in this direction reinforce
- Scattering in certain special directions is millions of times stronger than scattering by a single atom
- If we consider a slightly different direction, scattered X-rays will be out of phase

Shown here is the smallest angle that displays diffraction



Larger spacing of objects leads to smaller spacing between diffraction angles

Take Home Messages

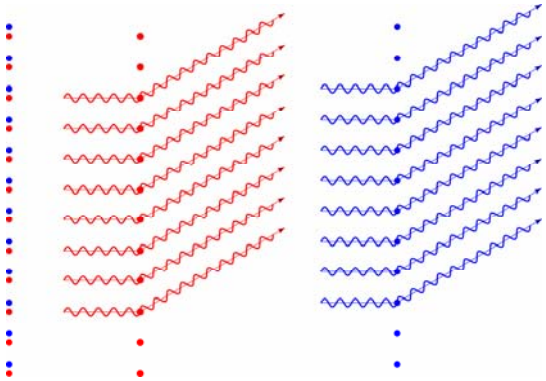
The unit cell is the building block that, repeated many times, makes up a crystal.

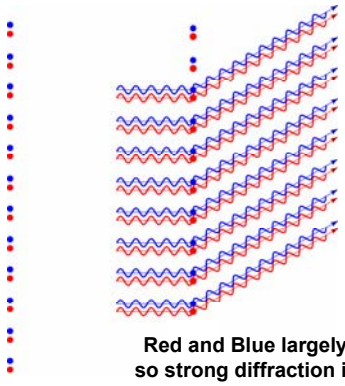
The dimensions of the unit cell determine the angles where strong diffraction can potentially be observed.

A very simple molecule (2 atoms) …

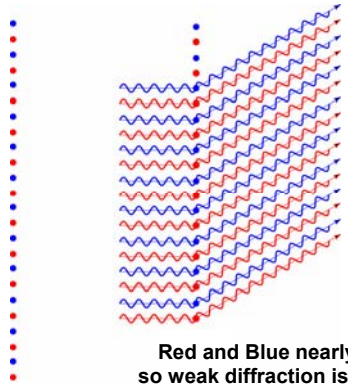
…crystallizes in a very simple lattice (1-dimensional)

Protein molecules have thousands of atoms, and real crystals have three dimensions. But the principles are exactly the same!





Red and Blue largely reinforce, so strong diffraction is observed



Red and Blue nearly cancel, so weak diffraction is observed.

Take Home Messages

The unit cell is the building block that, repeated many times, makes up a crystal.

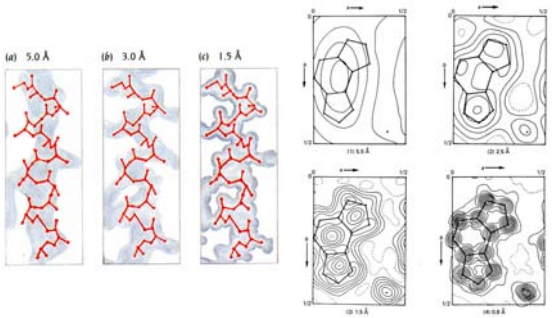
The dimensions of the unit cell determine the angles where strong diffraction can potentially be observed.

The arrangement of the atoms within each 'unit cell' determines how intense any particular diffraction 'spot' is.

As a result, the diffraction pattern can be mathematically analyzed to yield atomic structure.

- Even one atom per unit cell (the simplest possible crystal) gives a pattern of diffracted spots (sometimes called 'reflections')
- Adding additional atoms changes the intensity, but not the position, of these spots
- (Note that changing the dimensions of the unit cell changes the positions and spacing of the spots)
- The x-rays scattered in these selected directions can be thought of as a sum of sine waves over all the atoms in the unit cell. This sum is a new sine wave with a new amplitude and new phase.
- We saw this for two atoms/unit cell - it's just as true for a million!
- We can measure this new amplitude using x-ray film or a geiger counter: on x-ray film, larger amplitude gives darker spots.
- But we can't measure the new phase!
- Unfortunately, we need to know the amplitudes and phases to compute the electron density.

Electron density maps at various resolutions



Branden & Tooze, Fig. 18.11

J.P. Glusker & K.N. Trueblood, Crystal Structure Analysis: A Primer. (Oxford, 1985.) p. 134

So we do what we can - measure spot intensity.
~1000 spots per film x ~100 crystal orientations => ~100,000 spots
Each spot is "indexed" with its own h,k,l

h	k	l	I (intensity)	|F| (amplitude) $=\sqrt{I}$	α (phase)
0	0	1	94016	307	?
0	0	2	71552	267	?
	. . .				
10	27	38	37723	194	?
10	28	1	59923	244	?
10	28	2	5097	71	?
	. . .				
23	45	32	987	31	?

$\rho(x,y,z) = \text{electron density at some } x,y,z$

$$\rho(x,y,z) = \sum_{\text{all } h,k,l} \sum \sum |F| \cos(2\pi(hx + ky + lz) - \alpha)$$

So, if we knew α's (i.e. phases), we could compute ρ at all x,y,z

Three techniques for estimating α's
1. **Multiple isomorphous replacement (MIR)**
2. **Multiwavelength anomalous dispersion (MAD)**
3. **Molecular replacement (MR) - only works if your molecule resembles one whose structure is already known**

Measured |F|'s, estimated α's

↓ **Fourier transform**

Electron density map (see Branden & Tooze Figs. 18.11, 18.12)

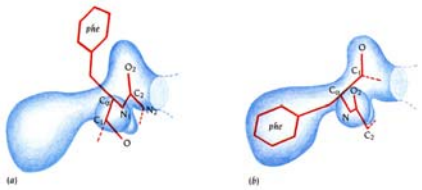
↓ **Computer graphics**

Model (structure)

↓ **Fourier transform**

Calculated |F|'s, calculated α's

Fitting a structural model into the electron density map



Branden & Tooze, Fig. 18.12

R-factor

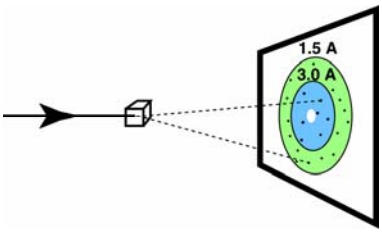
$$\text{R - factor} = \frac{\sum \left| F_{measured} - F_{calculated} \right|}{\sum F_{measured}}$$

A measure of how closely the model matches the observed data.

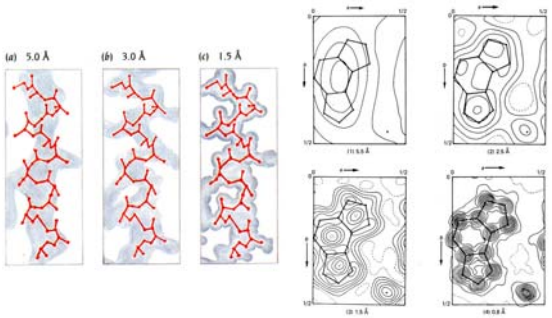
Perfect match:	**R = 0.00**
Completely random:	**R = 0.59**
Observed for protein structures:	**R = 0.15 - 0.25**

Resolution

Resolution: **Better crystals give spots at larger 2θ angles (2θ is the angle between the x-ray beam and the scattered x-rays)**
Higher resolution data (e.g. 1.5 Å) provides a more detailed and accurate electron density map than lower resolution data (e.g. 3.0 Å)
See Branden & Tooze, Fig. 18.11.



Electron density maps at various resolutions



Branden & Tooze, Fig. 18.11

J.P. Glusker & K.N. Trueblood, Crystal Structure Analysis: A Primer. (Oxford, 1985.) p. 134

Crystallography Web Sites

http://blackboard.princeton.edu,
click on External Links under MOL 504

All of the listed sites are interesting, but don't miss the "Book of Fourier".