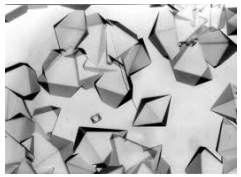


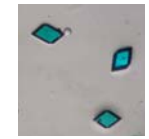
Single Crystal Diffraction

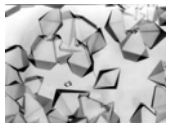
Cheiron School, Spring8, 2012

Claire Wilson

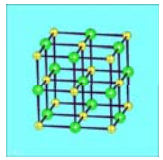
diamond

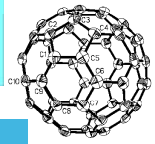
Our aim

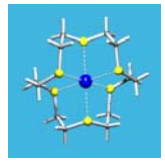




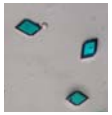
Xray diffraction



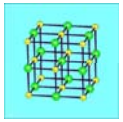




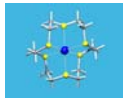
- To explore getting from a crystal to a structural model



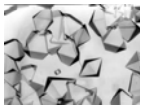
Single Crystals



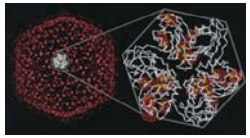
Material in solid state with 3-d translational symmetry



- Crystals may be:
  - elements (e.g. diamond), minerals, inorganic salts, molecular materials (organic, metal complexes), extended lattice structures (MOFs), macromolecules (proteins, DNA, viruses)

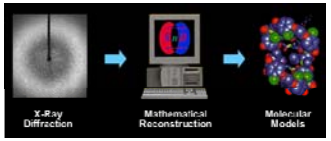


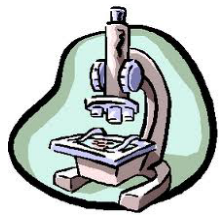
For synchrotron single crystal diffraction experiments crystals are typically 10 – 100 microns in size (1μm= $1x10^{-6}$m=10,000Å)

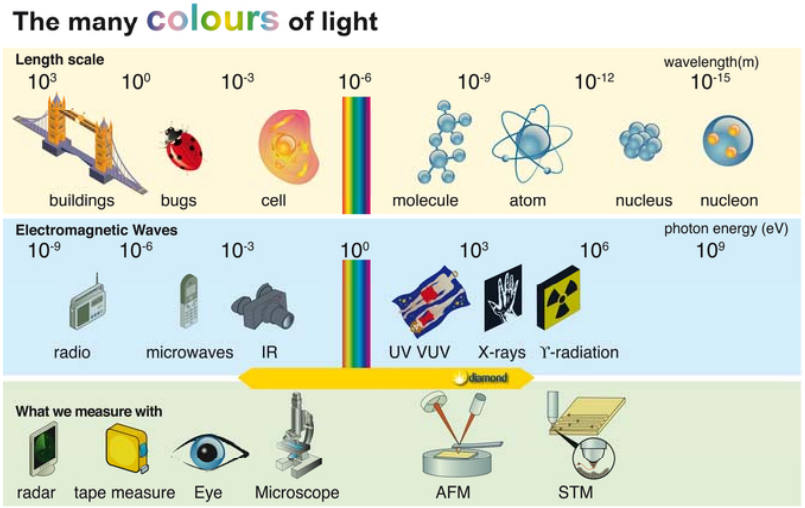


Microscope analogy

- With visible light look at objects similar size to wavelength (400-700nm)
- For atoms and molecules radiation with wavelength of similar size is xrays
- Reform image mathematically and not in real time

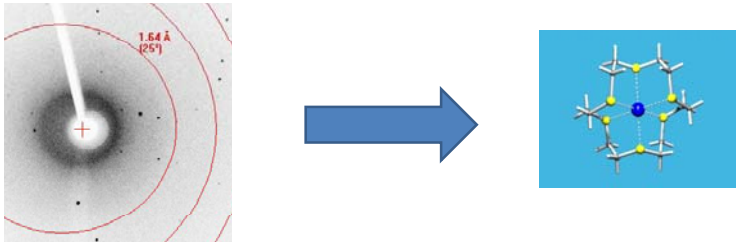






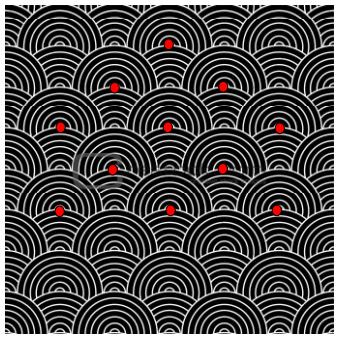
Why do we need a crystal?

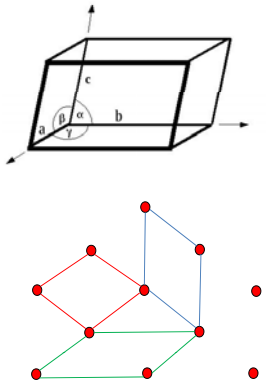
- Identical repeat unit repeated infinitely in 3 dimensions leads to diffraction
- Measure position and intensity of diffracted beams and work back to the structure



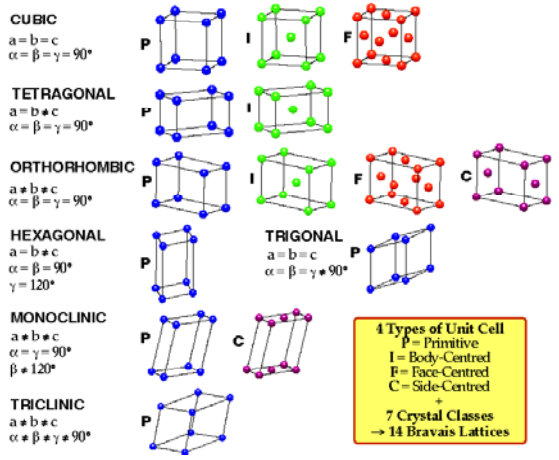
Lattice

- Array of points equivalent by translation – lattice
- Unit cell describes geometry of lattice





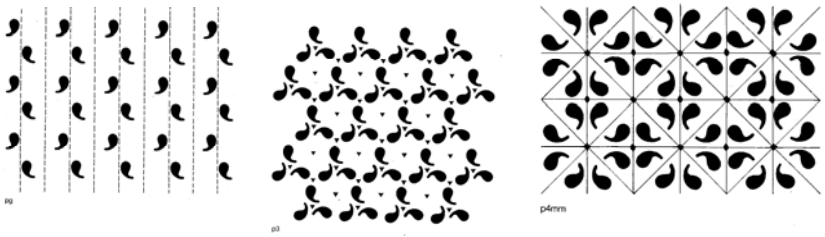
Crystal Systems



Symmetry

Definition

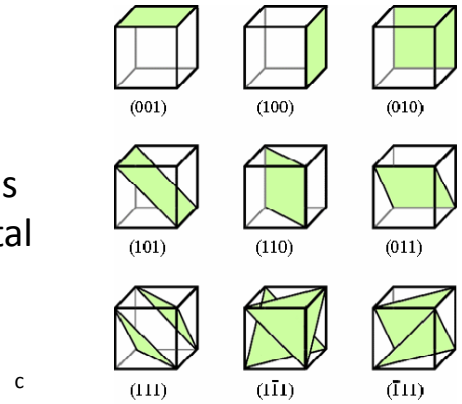
– A **crystallographic symmetry operation** is a symmetry operation, which maps a (periodic) crystal structure onto itself.



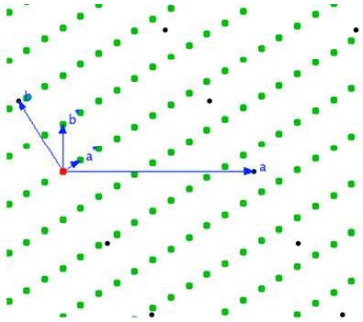
http://www.york.ac.uk/depts/maths/histstat/symmetry/welcome.htm

Miller indices hkl

- Description of orientation of planes slicing through crystal



Reciprocal lattice



- Valuable construct in diffraction
- Reciprocal geometric relationship between crystal lattice and its diffraction pattern

http://escher.epfl.ch/rlattice/

Reciprocal lattice relationships

- **a***.**b** = **a***.**c** = **b***.**a** = **b***.**c** = **c***.**a** = **c***.**b** = 0
- **a***.**a** = **b***.**b** = **c***.**c** = 1
- **a*** = (**b** × **c**) / *V*(4)
- **b*** = (**c** × **a**) / *V*(5)
- **c*** = (**a** × **b**) / *V*(6)
- *V** = **a**.**b** × **c**

Reciprocal lattice vector

h = h**a*** + k**b*** + l**c***

a x **b** = |a||b|sinγ **a.b** = |a||b|cosγ

Diffraction

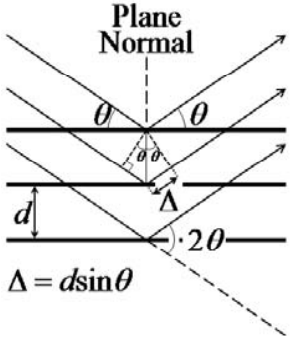
- Radiation scattered in all directions
- only measurable intensity where constructive interference – scattered waves are in phase
- xrays scattered by different points must have path length differences equal to nλ
- Laue Conditions – 3 conditions in 3D must be simultaneously satisfied to have diffraction

The Bragg Equation

- Consider diffraction as if reflection from sets of planes passing through lattice points
- Path length difference of the scattered rays from adjacent planes is 2Δ

$$n\lambda = 2d_{hkl}\sin\theta$$

Usually ignore n and consider n=2 as scattering from planes 2h,2k,2l



Scattering vector

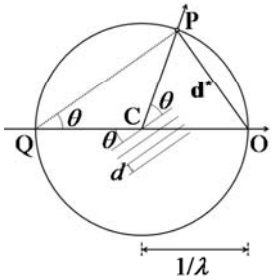
- In plane of and bisecting the incident and diffracted beams with magnitude 1/d or d*
- Rearrange Bragg equation

$$\sin\theta = (\lambda/2)\,(1/d)$$

scattering angle related to 1/d

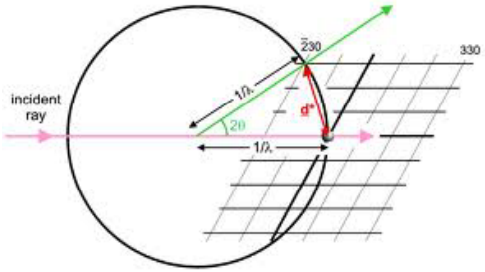
Ewald sphere

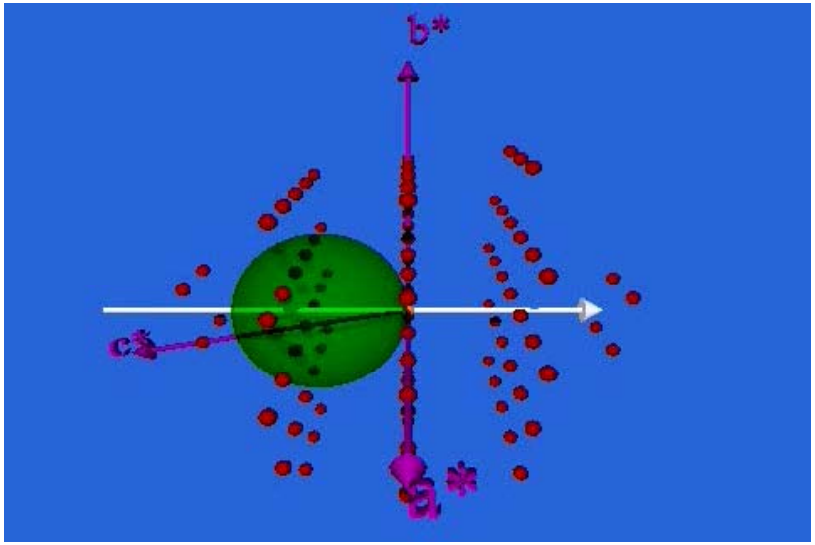
- For monochromatic beam - sphere radius 1/λ centered on crystal (C)
- Incident beam enters at Q
- Origin of reciprocal space at O
- Bragg equation is satisfied when node P on surface of sphere the sinθ =OP/QO = d*/(2/λ) = λ¥2d



Ewald sphere

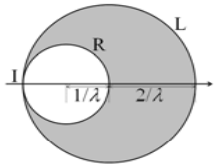
- Crystal rotated to bring reciprocal lattice points into diffracting position
- Multiple diffraction
- Lorentz Factor
- Blind regions





Limiting Sphere

- Boundary between observable and unobservable reflections for a given wavelength, λ
- Long wavelengths – small limiting sphere – lower resolution data obtainable



L is the limiting sphere radius $2/\lambda$,
R the reflecting sphere radius $1/\lambda$

Intensity of Diffracted beams

- Lattices and unit cells describe geometry of diffraction
- Diffracted intensities differ for different reflections – depend on orientation of plane in crystal
- Weak and strong intensities – both contain information
- Weaker at high scattering angles – atomic scattering factor and temperature factor
- Depend on unit cell contents – atom types and positions
- $I(\mathbf{h}) = k\,|F(\mathbf{h})|^2$

Structure factor F(**h**)

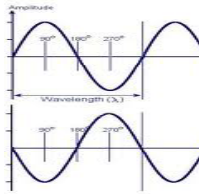
$$F(\mathbf{h}) = \sum_{j=1}^{n} f_j \, e^{2\pi i \, \mathbf{h}\mathbf{x}_j} = |F(\mathbf{h})| \, e^{i\phi(\mathbf{h})}$$

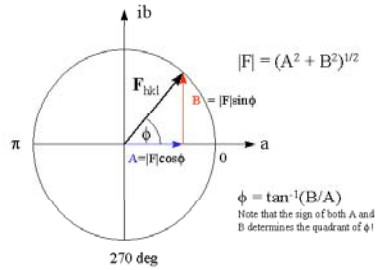
- Structure factor for a given plane or reflection (**h**)
- Summed over all *j* atoms in unit cell, with positions x_j and that have scattering power fj – atomic scattering factor
- $\mathbf{x}_j = x_j\mathbf{a} + y_j\mathbf{b} + z_j\mathbf{c}$ position of atom j
- $\mathbf{h} = h\mathbf{a}^* + k\mathbf{b}^* + l\mathbf{c}^*$
- $\mathbf{h}\mathbf{x}_j = hx_j + ky_j + lz_j$

Structure factor representation

$$F(\mathbf{h}) = |F(\mathbf{h})| \, e^{i\phi(\mathbf{h})} = A(\mathbf{h}) + iB(\mathbf{h}) =$$
$$|F(\mathbf{h})|(\cos\phi(h) + \sin\phi(h))$$

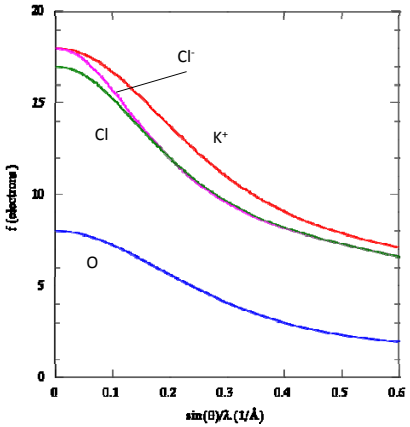
- Each reflection (diffracted wave) has amplitude |F| and phase ϕ



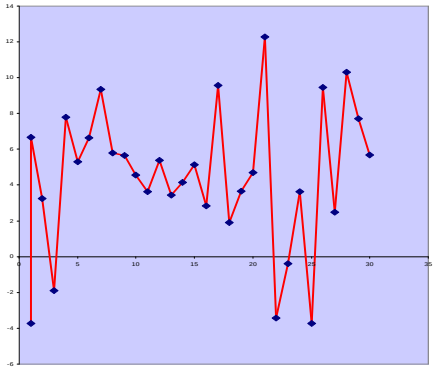


Xray atomic scattering factors

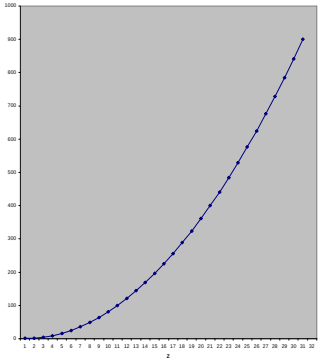
- Not point scatterer – electron cloud
- $2\theta=0°$ electrons scatter in phase = atomic number



Neutron scattering



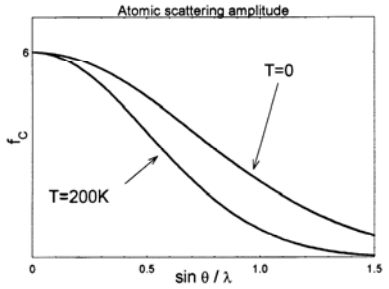
Neutron scattering length (fm) – random variation with Z



scattered intensity for X-rays – proportional to Z^2

Atomic scattering factors and atomic displacements

- Atoms in crystals not stationary but vibrating
- Intensity reduced at higher T and higher angles by temperature or Debye-Waller factor, B
- U, mean displacement of atom



$$f_B = f \cdot e^{-B(\sin\theta/\lambda)^2}$$

$B=8\pi^2\langle u\rangle^2$

Structure factor

$$F(\mathbf{h}) = \int_{cell} \rho(\mathbf{x})\, d\mathbf{x}\, e^{2\pi i\, \mathbf{hx}} = \sum_{j=1}^{n} f_j\, e^{2\pi i\, \mathbf{hx}_j}$$

- F(**h**) integrated electron density ρ at every point **x** in the unit cell
- Or summation atomic scattering factors f_j (all j atoms in unit cell)

- Structure factor (phase and amplitude) - Fourier transform of the electron density

Electron density

$$\rho(\mathbf{x}) = \frac{1}{V} \sum_{\mathbf{h}} F(\mathbf{h})\, e^{-2\pi i\, \mathbf{hx}}$$

V = unit cell volume

- ρ(**x**) inverse Fourier transform of F(**h**)
- With all F(**h**) (phase and amplitude) - complete description of unit cell contents

The 'Phase problem'

$$\rho(\mathbf{x}) = \frac{1}{V} \sum_{\mathbf{h}} F(\mathbf{h})\, e^{-2\pi i\, \mathbf{hx}}$$

But do not have all information to reconstruct ρ(x)

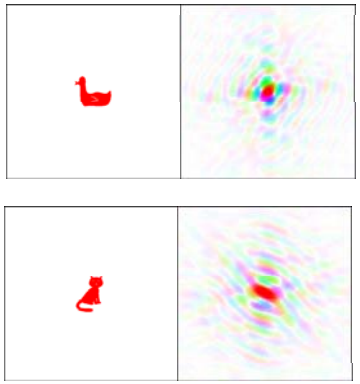
$$F(\mathbf{h}) = \sum_{j=1}^{n} f_j\, e^{2\pi i\, \mathbf{hx}_j} = |F(\mathbf{h})|\, e^{i\phi(\mathbf{h})}$$

✓ V - unit cell volume

✓ |F(**h**)| - structure factor amplitude (proportional to measured intensities)

X ϕ(**h**) – phase information – don't measure

Is the phase important?

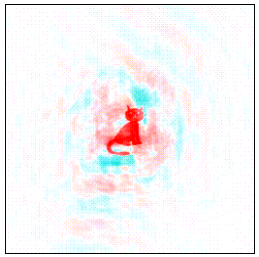


A duck and its Fourier transform

and a cat and its Fourier transform

From Kevin Cowtan's Book of Fourier
http://www.ysbl.york.ac.uk/~cowtan/fourier/magic.html

Is the phase important?



- Take amplitudes (magnitudes) from duck transform with the phases from the cat transform and when you reconstruct an image it is recognisable as a cat
- The image that contributed to the magnitudes has gone

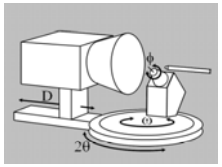
From Kevin Cowtan's Book of Fourier
http://www.ysbl.york.ac.uk/~cowtan/fourier/magic.html

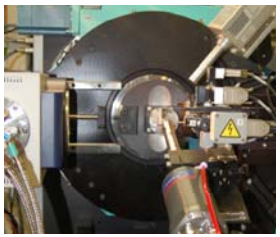
Is the phase important?

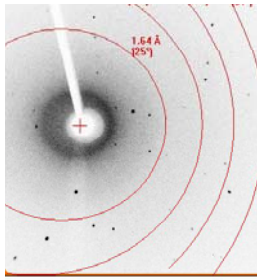
- The phase contains much more information about the atomic positions than the amplitudes
- Need some way to obtain estimated values for the phase and to 'solve' the structure

Data collection

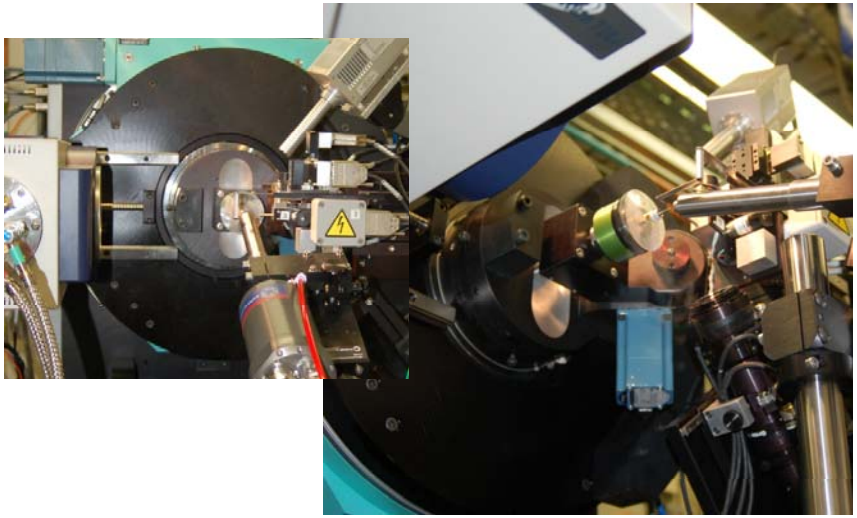
- Xray source
- Diffractometer – circles to move crystal
- Detectors - CCD, image plate, pixel detectors to measure position and intensity of diffracted intensities







Diffractometer



http://phillips-lab.biochem.wisc.edu/xrayviewuse.html

Experimental considerations

- Some examples of experimental choices:
  - choice of crystal
  - wavelength to use
  - sample environment e.g. temperature
  - exposure time
  - resolution of data
- Depending on material and nature of study

Data reduction

- Convert measured intensities to $|F_{obs}|$ - 'observed structure factor amplitudes' with associated standard uncertainty, $\sigma(F_{obs})$
- Corrections applied e.g.
  - Lorenz-polarisation effects
  - Absorption correction – absorption by crystal depends on path length through crystal for a given reflection, unit cell contents, wavelength
- Unit cell parameters
- Space group determination
- Result – list of reflections as *h, k, l,* $|F_{obs}|$, $\sigma(F_{obs})$

Structure Solution methods

- Direct methods
- Heavy-atom (Patterson) methods
- Charge flipping
  - Fragment searches
  - Inference
  - isostructural relationships

Result may be one heavy atom site or complete non-hydrogen structure depending on method and success level

Model development

$$F(\mathbf{h}) = \sum_{j=1}^{n} f_j e^{2\pi i \mathbf{h}\mathbf{x}_j} = |F(\mathbf{h})| e^{i\phi(\mathbf{h})}$$

- Calculate amplitudes $|F_{calc}(\mathbf{h})|$ and phases as now 'know' some atom positions $\mathbf{x}_j$ from trial structure
- Fourier transform $|F_{obs}(\mathbf{h})|$ with ***calculated*** phases – **new** information - model density plus additional atoms not included in model
- Difference Fourier maps using $|F_{obs}(\mathbf{h})| - |F_{calc}(\mathbf{h})|$ missing atoms/misassigned atoms clearer

Model development and structure refinement

- Include 'new' atoms in model and repeat until all atoms located
- Model improvements – displacement parameter modeling – isotropic-anisotropic, disorder, hydrogen atoms
- Least squares refinement - minimise $|F_{obs}(\mathbf{h})| - |F_{calc}(\mathbf{h})|$ to give best fit of model

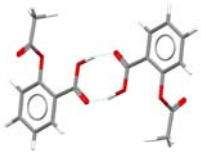
Structure model

Typically consists of

- Atom type e.g. C, N, Fe (which atomic form factor to use)
- Atom positions in fractional coordinates x,y,z
- Site occupancy
- Atomic displacement parameters – usually isotropic Uiso or anisotropic Uij
- Unit cell and space group

Results and interpretation

- Result - electron density map
- Refined model
  - coordinates of atoms, x,y,z with estimated error, at centre of electron density peaks
  - Atomic displacement parameters
  - Unit cell parameters and space group



- Interpret - bond lengths and angles for 'bonded' atoms, torsion angles
- Intermolecular distances e.g. H-bonding.

Web resources

- Kevin Cowtan
  http://www.ysbl.york.ac.uk/~cowtan/
- Gervais Chapuis
  http://escher.epfl.ch/eCrystallography/
- Xrayview http://phillips-lab.biochem.wisc.edu/xrayviewuse.html

- Joe Reibenspies
  http://xray.tamu.edu/courses.php