

Scattering Lecture

February 24, 2014

Structure Determination by Scattering

- Waves of radiation scattered by different objects interfere to give rise to an observable pattern
- The wavelength needs to be close to the separation of the objects (Typical C-C bond is about 1.2Å)
- Ready source of radiation needs to be available
- Interact with matter but not complete absorption.
- Three types of radiation are useful for this:
 - X-rays of wavelength about 1 Å,
 - electrons of wavelength about 0.01 Å,
 - neutrons of wavelength about 0.5--10 Å.

Neutron Scattering

- Neutrons are scattered by atoms
- Scattering is considered to be from point scatterers
- Biological samples hydrogen (^1H) is the main scatterer of neutrons
- Heavier elements do not dominate neutron scattering and the scattering power of different isotopes of the same element can be very different
- Differences between ^1H and deuterium (^2H or D) is of particular interest in structural biology.
- This is extremely useful for labeling experiments of biological samples.
- What protons would be exchange with D on a protein? What processes can be studied with this?

X-ray Scattering

- X-ray are scattered by electron clouds, which are comparable to the wavelength of X-rays
- More electrons the better the scattering, unlike neutrons
- X-ray scattering decreases as a function of angle divergence from the beam position
- Heavy atoms or metals have been extremely important in X-ray crystallography.
- Hydrogens have one electron and freely lose it
- Hydrogens can be placed in cases of extremely high resolution (1.0Å resolution) or tightly coordinated.

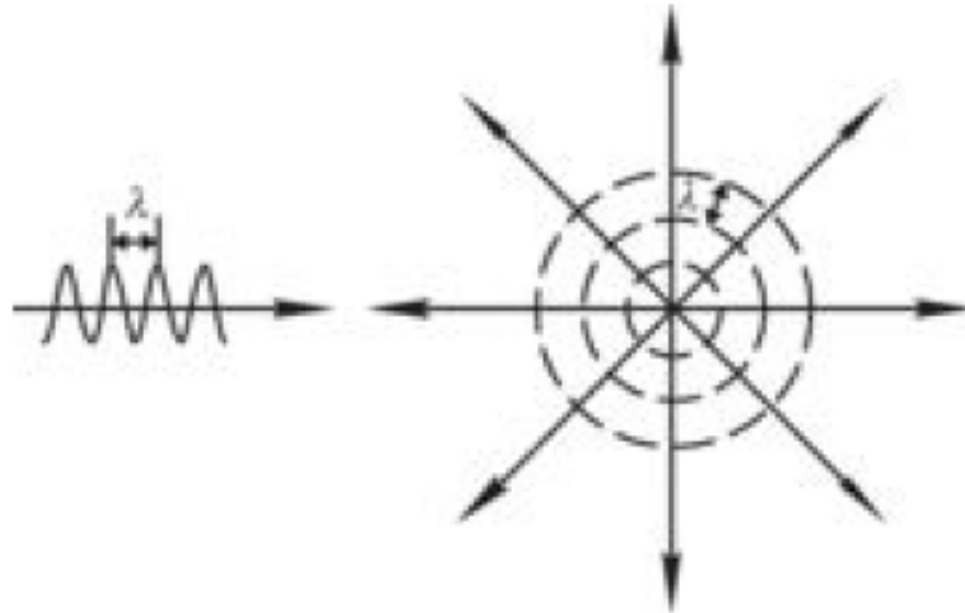
X-ray Absorption

- X-rays can be absorbed by exciting electrons to higher energy levels
- Absorption is energy- (and wavelength-) dependent
- Almost all atoms have a particular wavelength that absorb X-rays
- X-ray absorption leads to non-negligible radiation damage in the sample that should be corrected for in diffraction experiments.
- Absorption is severe for wavelengths above 2.5 \AA , where even air in the beam path absorbs significantly.
- The excited electron can return to ground state by fluorescence.
- Fluorescence is the release of EM of lower energy than input.
- X-ray absorption will permit detailed location of the atom.

X-ray Absorption Edges

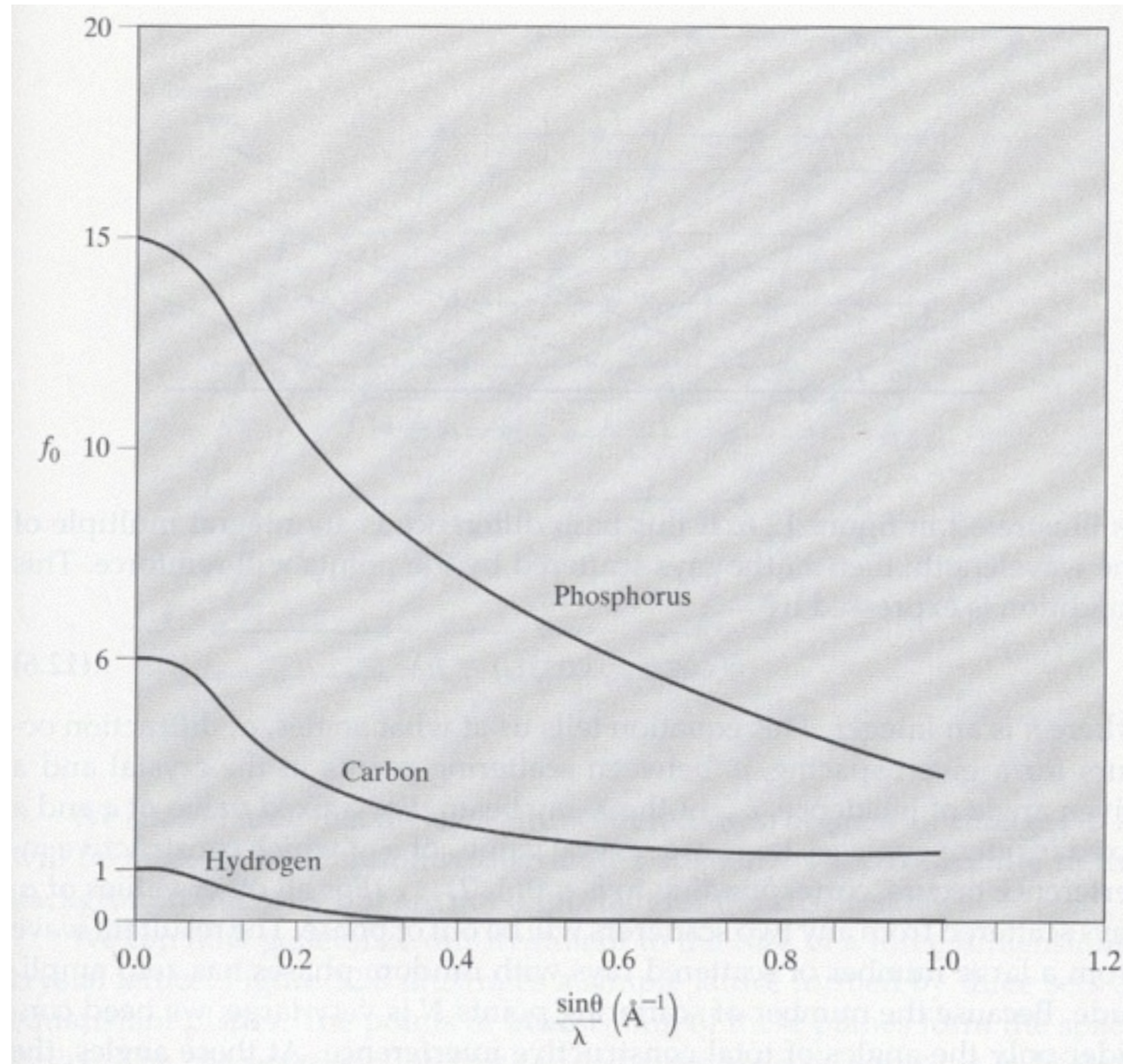
1a	2a	3b	4b	5b	6b	7b	8	1b	2b	3a	4a	5a	6a	7a	0		
1 H															2 He		
3 Li	4 Be									5 B	6 C	7 N	8 O	9 F	10 Ne		
11 Na	12 Mg									13 Al	14 Si	15 P	16 S	17 Cl	18 Ar		
19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
55 Cs	56 Ba	57 La	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
87 Fr	88 Ra	89 Ac	104 Rf	105 Ha													
Lanthanides		58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb	71 Lu		
Actinides		90 Th	91 Pa	92 U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr		

Point Scattering



- isotropic means scattering is equal in all directions
- however, for X-rays there is an angle dependence on scattering

Scattering Factors

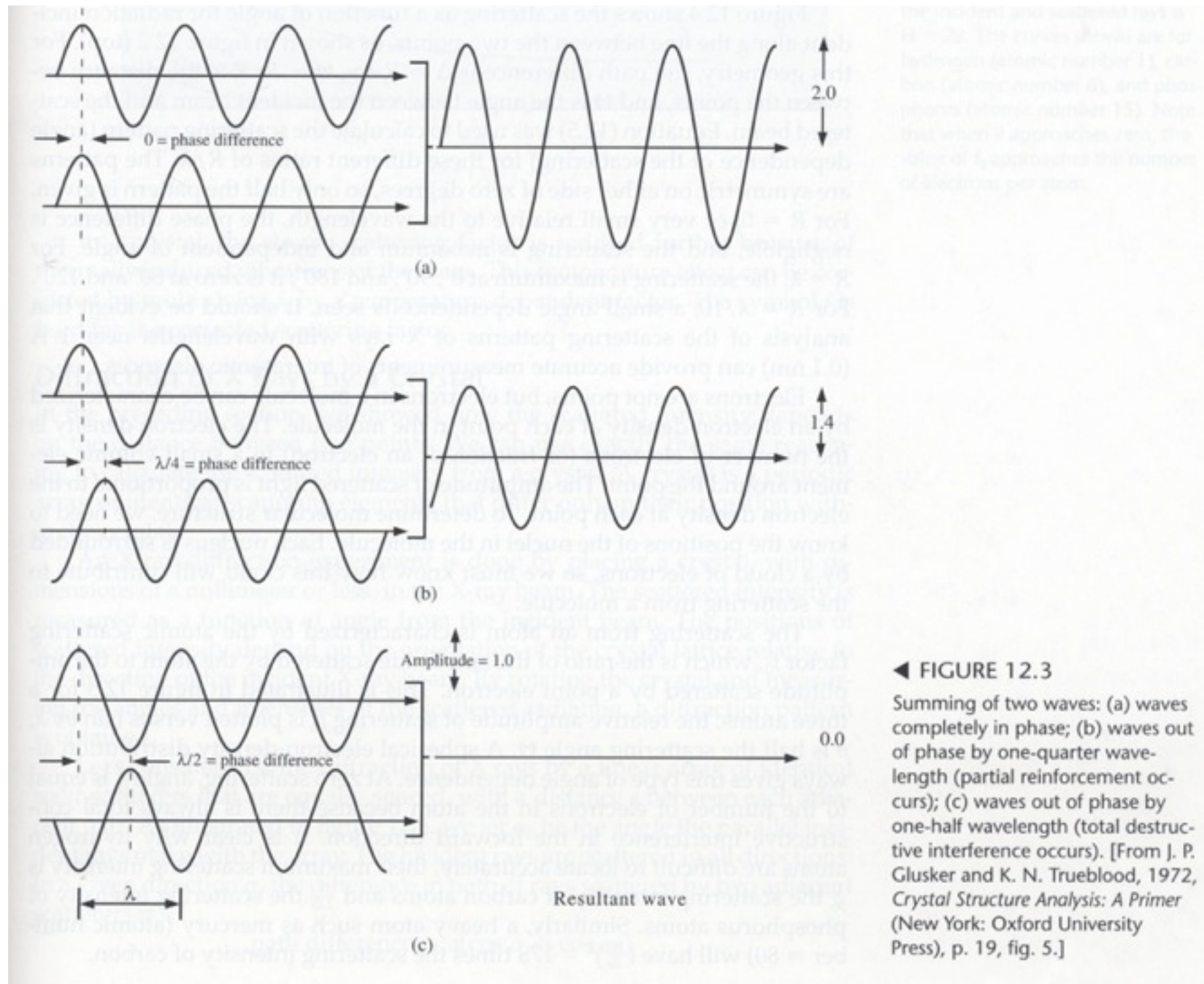


◀ FIGURE 12.5

Dependence of atomic scattering f_0 on $(\sin \theta)/\lambda$. The angle between the incident and scattered rays is $\Theta = 2\theta$. The curves shown are for hydrogen (atomic number 1), carbon (atomic number 6), and phosphorus (atomic number 15). Note that when θ approaches zero, the value of f_0 approaches the number of electrons per atom.

- Scattering factor (f_0) ratio of the amplitude scattered by an atom to the amplitude scattered by a point electron

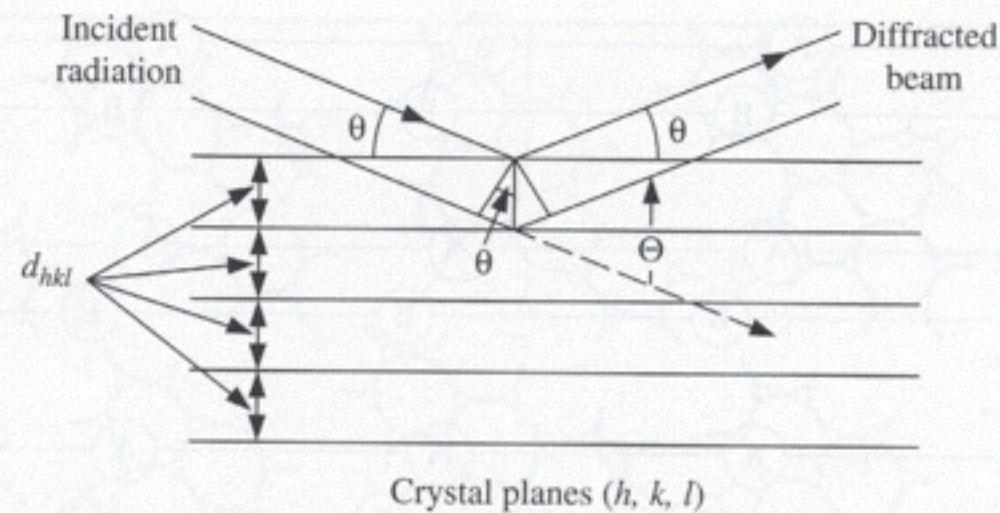
Summing of Waves



◀ FIGURE 12.3

Summing of two waves: (a) waves completely in phase; (b) waves out of phase by one-quarter wavelength (partial reinforcement occurs); (c) waves out of phase by one-half wavelength (total destructive interference occurs). [From J. P. Glusker and K. N. Trueblood, 1972, *Crystal Structure Analysis: A Primer* (New York: Oxford University Press), p. 19, fig. 5.]

Bragg's Law



$$\text{Bragg condition: } n\lambda = 2d_{hkl} \sin \theta$$

▲ FIGURE 12.12

Diffraction of radiation from a crystal. The parallel lines represent planes of atoms with Miller indices h , k , and l . The Bragg condition for diffraction is for the incident beam and the diffracted beam to make an angle $\theta = \sin^{-1}(n\lambda/2d_{hkl})$ with the planes. Note that the scattering angle (Θ) between incident and scattered beams (defined before in figure 12.2) is 2θ . Simple geometric construction shows that the extra path length traveled by the ray scattered by the top layer relative to the next layer down is $2d_{hkl} \sin \theta$.

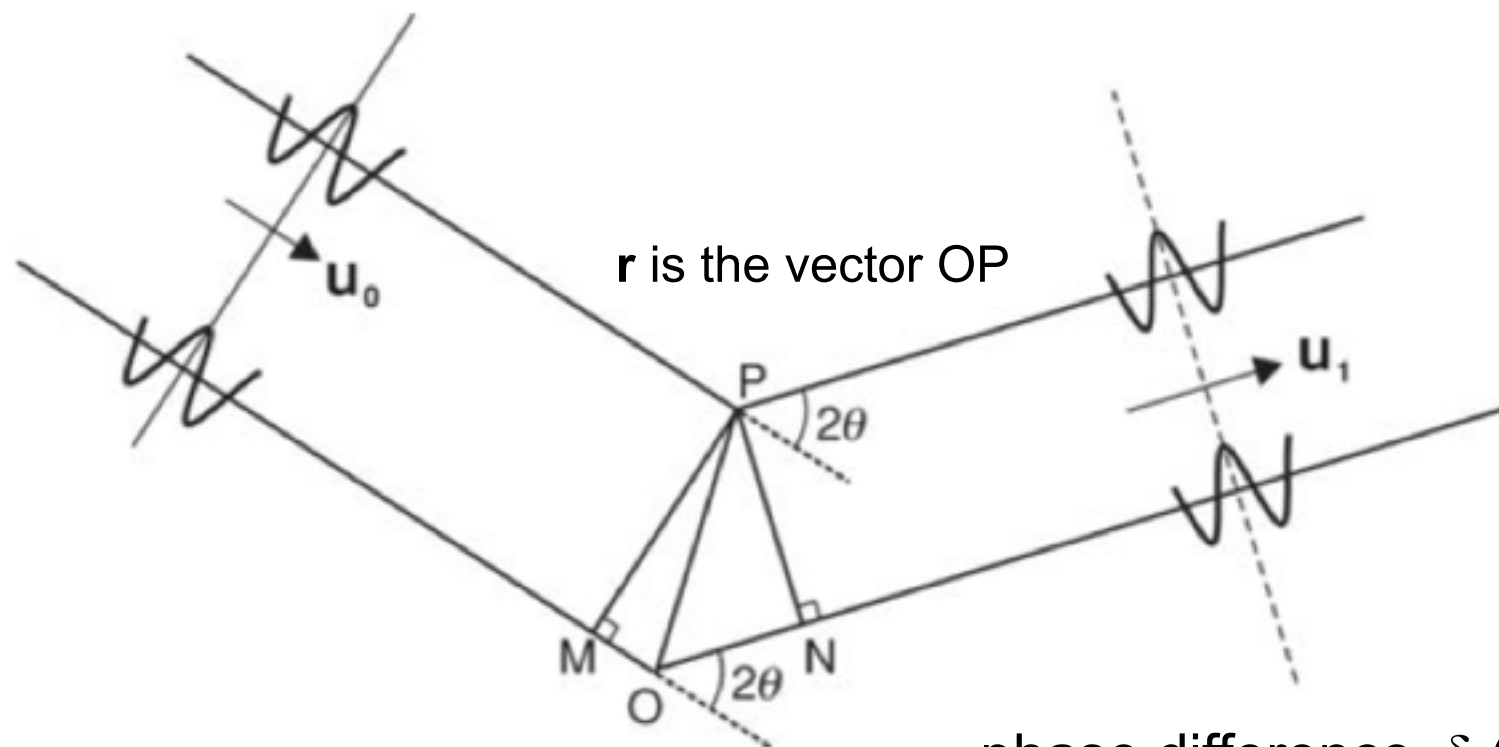


Fig. G1.3. Diffraction from two points.

phase difference, δ (in radians)
 $\delta = (\Delta/\lambda) 2\pi$

A wave front W can be defined perpendicular to the wave propagation as joining points of equal phase. PM is the wave front of the incident wave when it touches P ; PN is the wave front of the wave scattered in the direction 2θ . The path difference, Δ , between the waves scattered by P and those scattered by O is $ON - OM$. We now write this in vector notation,

$$OM - ON = \Delta = \mathbf{r} \cdot \mathbf{u}_1 - \mathbf{r} \cdot \mathbf{u}_0 = \mathbf{r} \cdot (\mathbf{u}_1 - \mathbf{u}_0)$$

(G1.3)

Scattering Vector

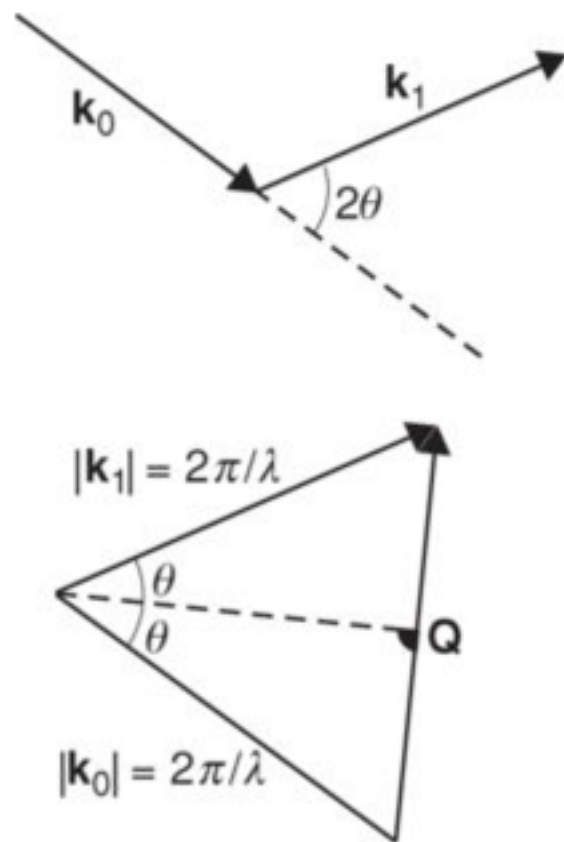


Fig. G1.4. Definition of the scattering vector.

$$k_0 = (2\pi/\lambda)u_0$$

$$k_1 = (2\pi/\lambda)u_1$$

$$Q = k_1 - k_0$$

$$Q = (4\pi \sin\theta)/\lambda$$

Scattering from an atom

$$A = f \exp(i\mathbf{Q} \cdot \mathbf{r})$$

where f is the scattering amplitude of the atom

- Scattering vector essentially defines the magnification that can be achieved in the diffraction experiment
- Waves from atoms separated by a vector \mathbf{r} are out of phase by one cycle (2π phase angle or λ path length),
- constructive interference occurs when the scattering vector \mathbf{Q} is parallel to \mathbf{r} and Q equal to $2\pi/r$.
- Q and r have an reciprocal relationship
- smaller values of r , we need to go to larger values of Q to obtain the same path difference.
- Geometric space represented by Q is called reciprocal space
- real space is represented by r
- In order to 'resolve' shorter distances in a diffraction experiment it is necessary to go to larger scattering vector values
- Increase the path difference
- The *resolution* of an experiment is the minimum distance between points that can be observed separately

Scattering from Many Atoms

- Scattering from individual atoms is very weak as X-rays don't interact well with matter
- Need many atoms in the exact orientation and distance in order to achieve a better signal
- In order to achieve coherent scattering there must be a plane of atoms properly arranged

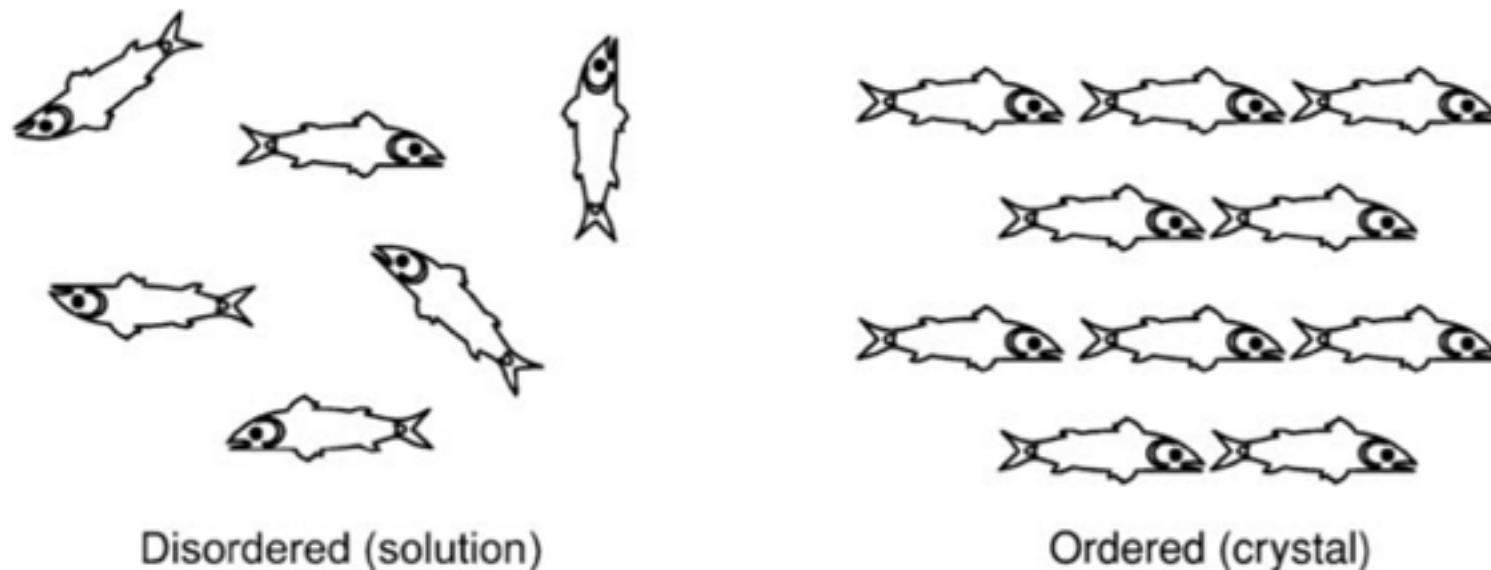


Fig. G1.6. Samples from order to disorder.

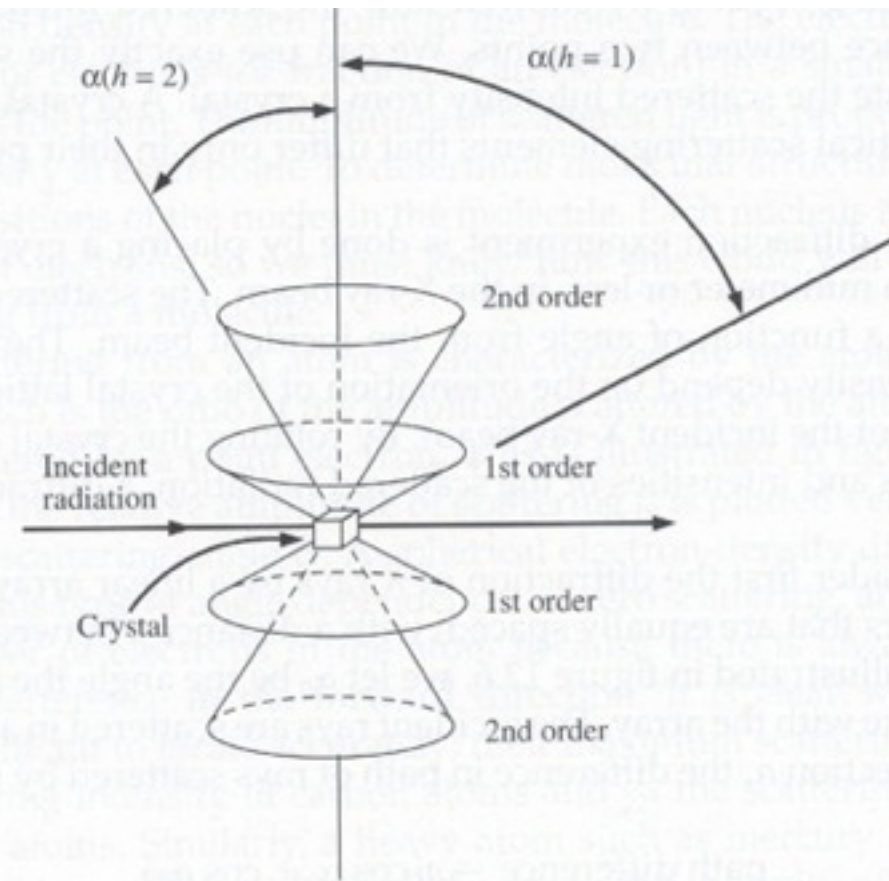
Phase Problem

- Scattering from an individual atom $A = f \exp(i\mathbf{Q}\cdot\mathbf{r})$
- Scatter from an assembly of atoms $F(\mathbf{Q}) = \sum_j f_j \exp(i\mathbf{Q}\cdot\mathbf{r}_j)$
- f_j , \mathbf{r}_j are respectively the scattering amplitude and position vector with respect to the origin, of atom j
- $F(\mathbf{Q})$ is a complex number. It defines the scattered wave for scattering vector \mathbf{Q} in terms of two quantities: its amplitude and its phase.
- However, all that is measured is the Intensity of the reflection
 $I = |F(\mathbf{Q})|^2$
- All phase information is lost during data collection
- Therefore, phases must be estimated.

Diffraction from a Lattice

► FIGURE 12.7

Equidistant point scatterers arranged in the vertical direction at the apices of the cones. With an incident angle $\alpha_0 = 90^\circ$, Eq. (12.6) reduces to $a \cos \alpha = h\lambda$, or $\alpha = \cos^{-1}(h\lambda/a)$. Cones with $h = 1$ (first order) and $h = 2$ (second order) are shown. Note that $\cos \alpha = \cos(-\alpha)$; thus, in this particular case, for each order there are two cones 180° apart. The zero-order diffraction ($h = 0$) is not shown.



Fiber Diffraction

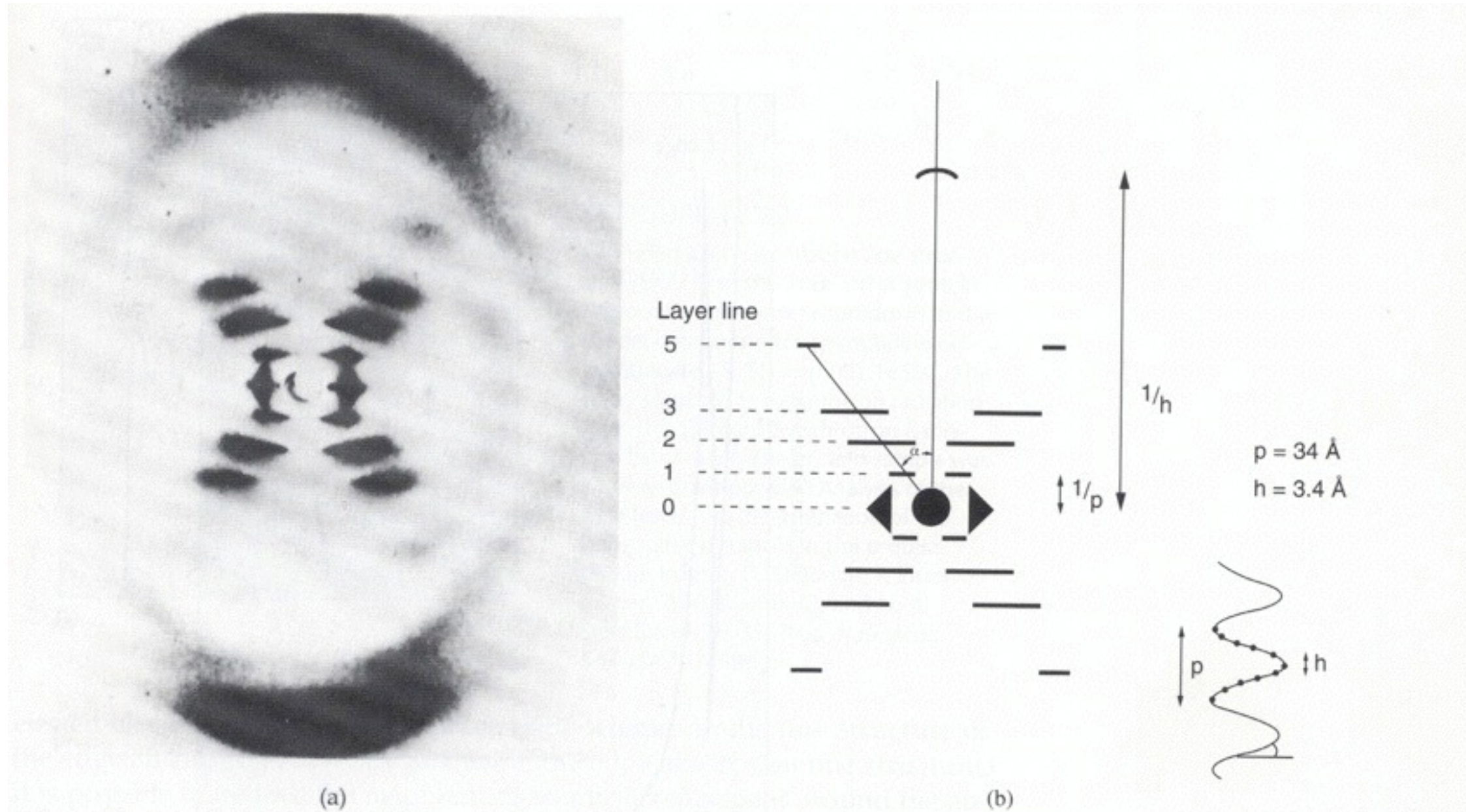
Fiber Diffraction

- Can X-ray diffraction provide structural information on molecules that do not form ordered three-dimensional crystalline arrays?
- Yes, IF there is inherent symmetry within the molecule to cause constructive and destructive interference of X-rays.
- This can provide low resolution structural information. Will not achieve atomic resolution.

Fibers

- If the molecule has regular structure then the repeating unit can give rise to constructive interference.
- Fibers can have some alignment in one dimension
 - Usually along the length of the fiber
- For helices, the helical axis will be parallel to the long axis of the fiber
- Difference between fibers and crystals:
 - Crystals have order in all three dimensions
 - Fibers will be ordered in one direction, but will be randomly rotated between molecules

Fiber Diffraction of DNA



▲ FIGURE 12.20

(a) X-ray diffraction pattern of a NaDNA fiber in the B-helix conformation. Note the characteristic cross and the very strong meridian spots. (b) Line drawings of the pattern above, illustrating the relation between the helix dimensions (lower right) and the layer lines and meridian spots. The DNA helix has repeating distances of $h = 3.4 \text{ \AA}$, the distance between successive base pairs, and pitch, $p = 34 \text{ \AA}$, the distance between successive turns. The angle α characterizes the ratio of the pitch to the radius; $\tan \alpha \cong \text{pitch}/2\pi \text{ radius}$. [From J. P. Glusker and K. N. Trueblood, 1972, *Crystal Structure Analysis: A Primer* (New York: Oxford University Press), p. 137, fig. 39b.]