Chapter 6 X-ray Diffraction

X-ray Diffraction

Picture is worth a thousand words

Visual image

Microscope

X-rays **cannot be focused** by lenses to form an image of a molecule.

Reflected from the surface of an object

Transmitted through the object

X-ray are **scattered** from a regular repeating array or molecule to give a **pattern** that represent the macromolecular order and structure.

The structure must be **reconstructed** using mathematics as the lens to transform the pattern back into the original structure.

Light Microscope

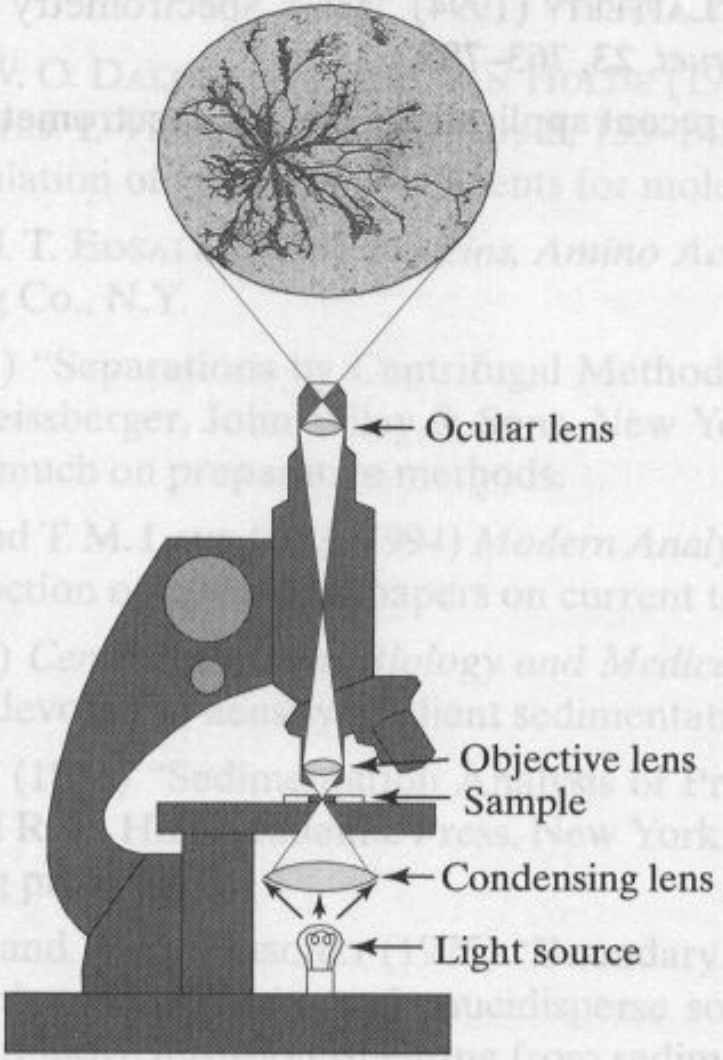
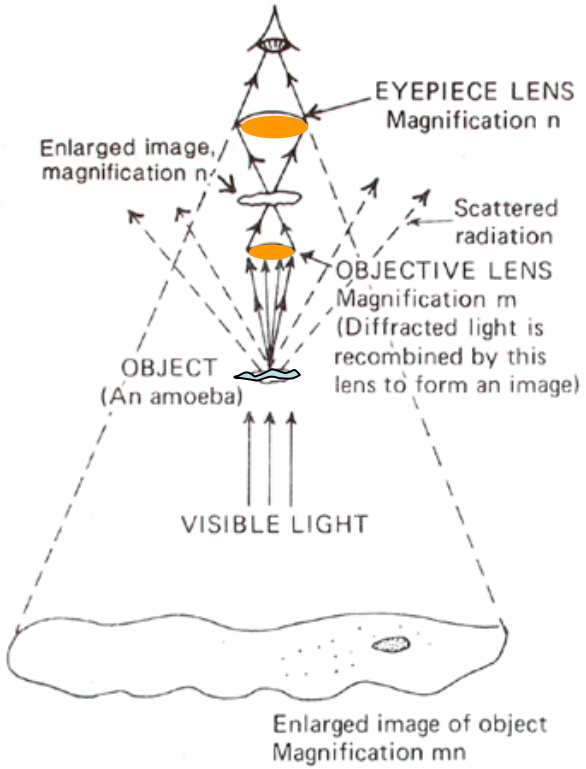
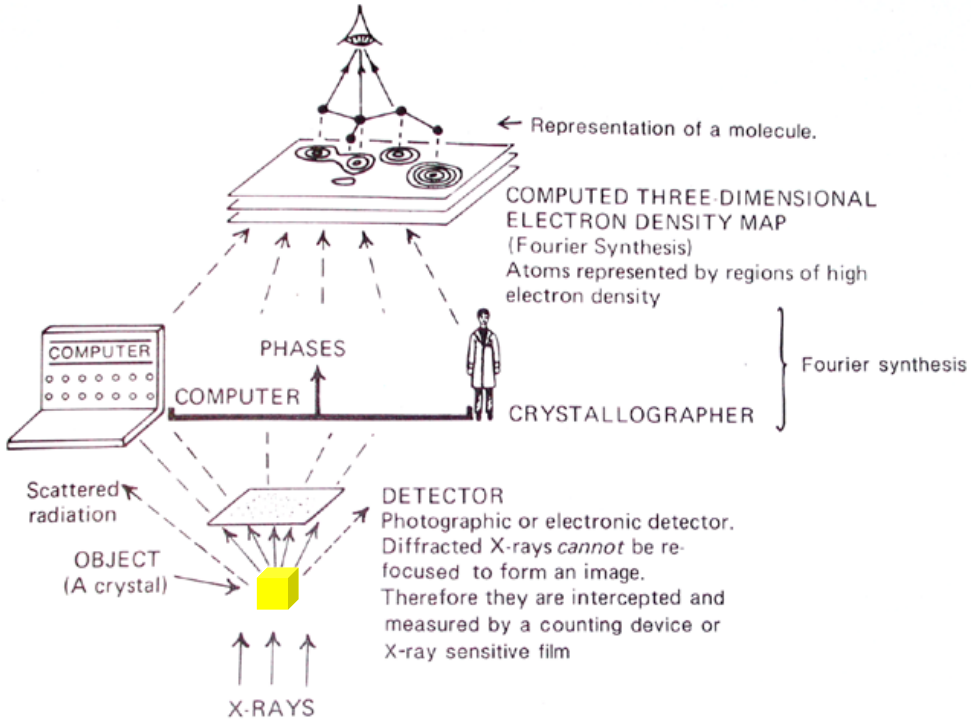


Figure 6.1 The light microscope. A microscope forms an image by focussing the light scattered from a sample, such as a melanophore on a slide, through a series of lens. The image can be magnified infinitely, but the resolution is limited by the wavelength of visible light to about 0.2 microns. [Courtesy of P. McFadden.]



(a) MICROSCOPE



(b) X-RAY DIFFRACTION

Analogies Between Light Microscopy and X-ray Diffraction.

Certain analogies between these two methods of using scattered radiation for determining structure are shown here. The object (sample) in both set-ups scatters some of the incident radiation into a diffraction pattern.

In the ordinary microscope there is no need to record the diffraction pattern because the scattered light can be focused by the objective lens to give a magnified image of the object under study. The closer this lens is to this object, the wider the angle through which scattered radiation is caught by the lens. Thus, if this distance is small, most of the diffracted light will be caught by the objective lens and focused to form an image. The rest of the radiation is lost to the surroundings.

With X rays the diffraction pattern has to be recorded electronically or photographically (as indicated schematically here), because X rays cannot be focused by any known lens. Therefore the recombination of the diffracted beams that is done by a lens in the microscope must, when X rays are used, be done mathematically by a crystallographer with the aid of a computer. As stressed later (Chapter 5), this recombination cannot be done directly because the phase relations among the different diffracted beams cannot usually be measured directly. However, once these phases have been derived, deduced, guessed, or measured indirectly (which is what this book is mostly concerned with), an approximate image of the scattering matter can be formed.

6.1 Structures at atomic resolution

- **This technique requires three distinct steps**

1) Growing **crystal**

2) Collecting X-ray **diffraction pattern** from the xtal

3) **Constructing** and **refining** a structure model to fit the X-ray diffraction pattern.

- **Atomic resolution:**

The positions of each atom can be **distinguished** from those of all other atoms in 3D space.

The closest distance between 2 atoms is “covalent bond” approximately 1.2Å.

- **Two limitations**

The atoms of its molecules held **rigidly**.

Each molecule in the system must have **identical conformations.**

Atomic Resolution

Any **fluctuation** in the positions of the atoms in the molecules or any significant **deviations** of molecule from a signal conformation

A **averaging** of the structure

Blur our vision/reduce the resolution

Limit of resolution

$$LR=\lambda/2$$

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

$$d=\lambda/2$$

Visible light (λ=400 - 800 nm/5-10 ev)

X-ray (λ= 0.1-10 nm/1-100Å /10^2 to 10^5 ev/)

X-ray diffraction

The **constructive & destructive interference** caused by scattering radiation from the regular repeating lattice of a single crystal to determine the structure of macromolecules

Resolving molecules to the atomic level

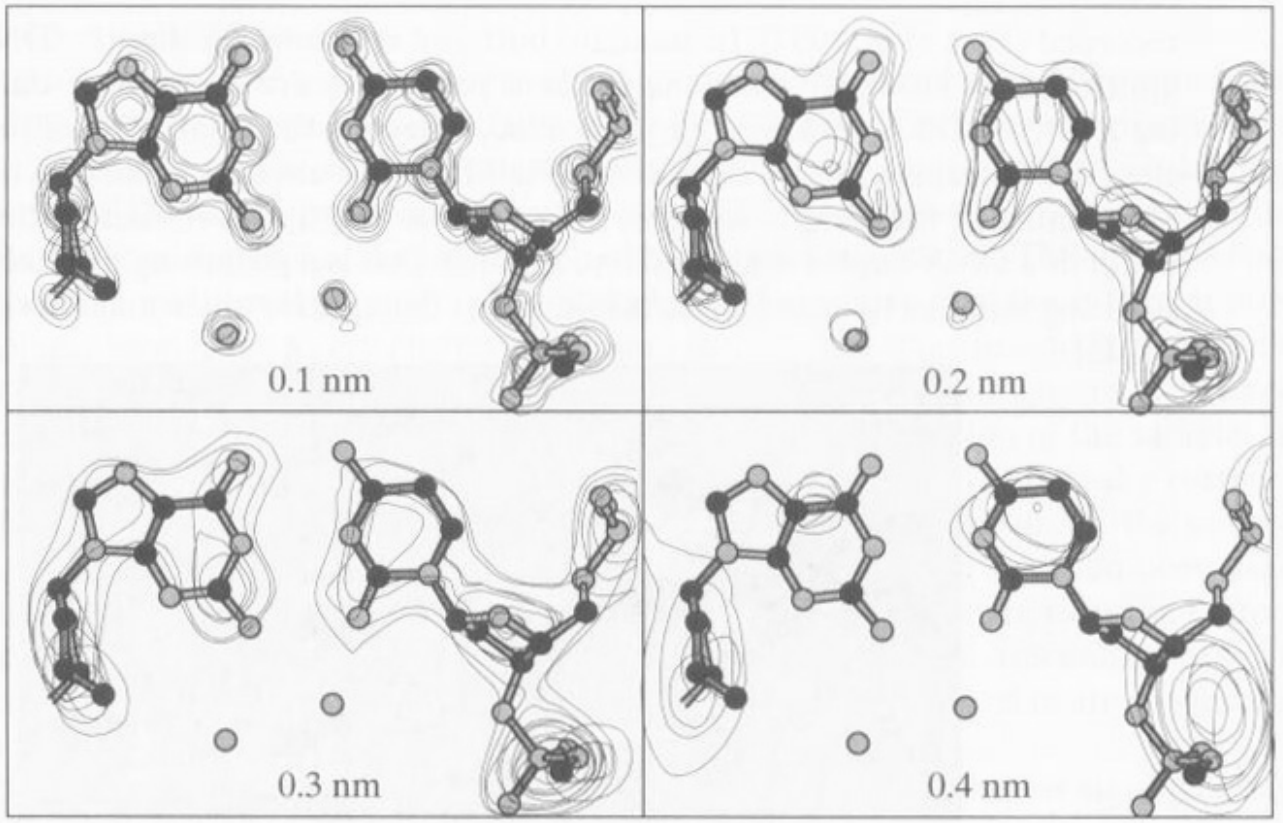


Figure 6.2 Resolving molecules to the atomic level. The information content increases as structures are determined to higher resolution (in this case, lower numbers are better). The 0.1 nm resolution structure of a dG·dC base pair in a crystal of a DNA fragment show details of each atom in the molecule, as well as the solvent structure surrounding the molecule. At 0.2 nm and 0.3 nm resolution, the structure of the nucleotides are still discernable, but by 0.5 nm resolution, only the presence of the strongly diffracting phosphates of the backbone can be unambiguously distinguished.

Electromagnetic

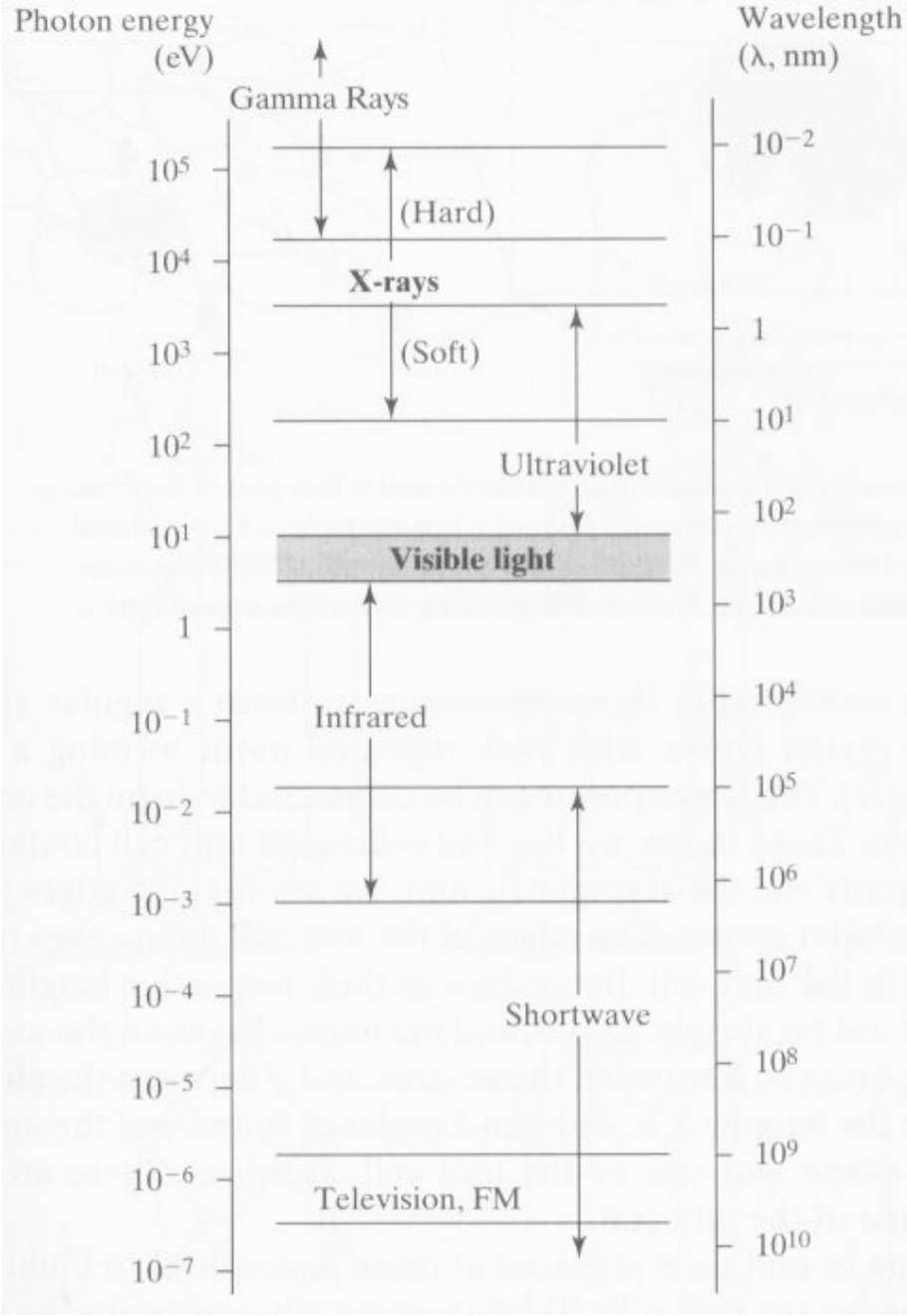


Figure 6.3 Electromagnetic spectrum. Visible light falls in the wavelength range of 400 to 800 nm, with corresponding energies of about 5 to 10 eV. X-rays are shorter wavelength (0.1 to 10 nm) and consequently higher energy (10^2 to 10^5 eV). [Adapted from J. A. Richards, Jr., F. W. Sears, M. R. Wher, and M. W. Zemansky (1960), *Modern University Physics*, Addison-Wesley, Reading, MA, p. 600.]

6.2 Crystals

6.2.1 What is a crystal?

Quartz & Glass

Quartz: order, regular, symmetric & repeating

Glass: amorphous solid, disorder

Xtal can be cleaved, **basic unit =unit cell**

Symmetry operators: translation/rotation

Determine the structure of a crystal

$\Rightarrow$ Determine the structure of **the least** symmetric component of the unit cell.

Unit cell: basic unit/all unit cells within the xtal are identical

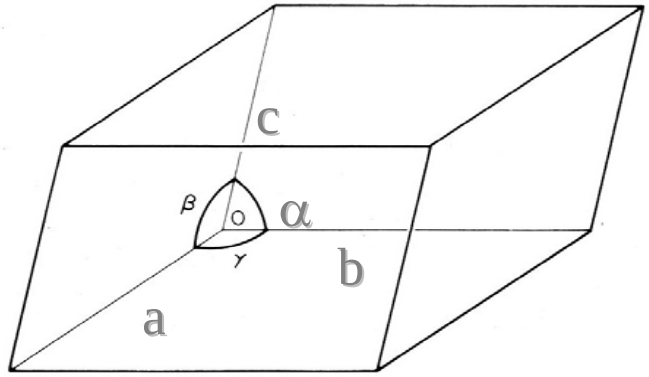
Asymmetric unit: no symmetry is aptly, ex: $\alpha\beta$-dimer of Hb tetramer

Cell dimension

Cell parameters

a, b, c

α, β, γ



- **The edges of the unit cell defines a set of unit vector axes, a, b, c**

- **These vectors need not be at right angles, and the angles between the axes are denoted as**
  - **α, between the bc-axes**
  - **β, between the ac-axes**
  - **γ, between the ab-axes**

Component of a Crystal

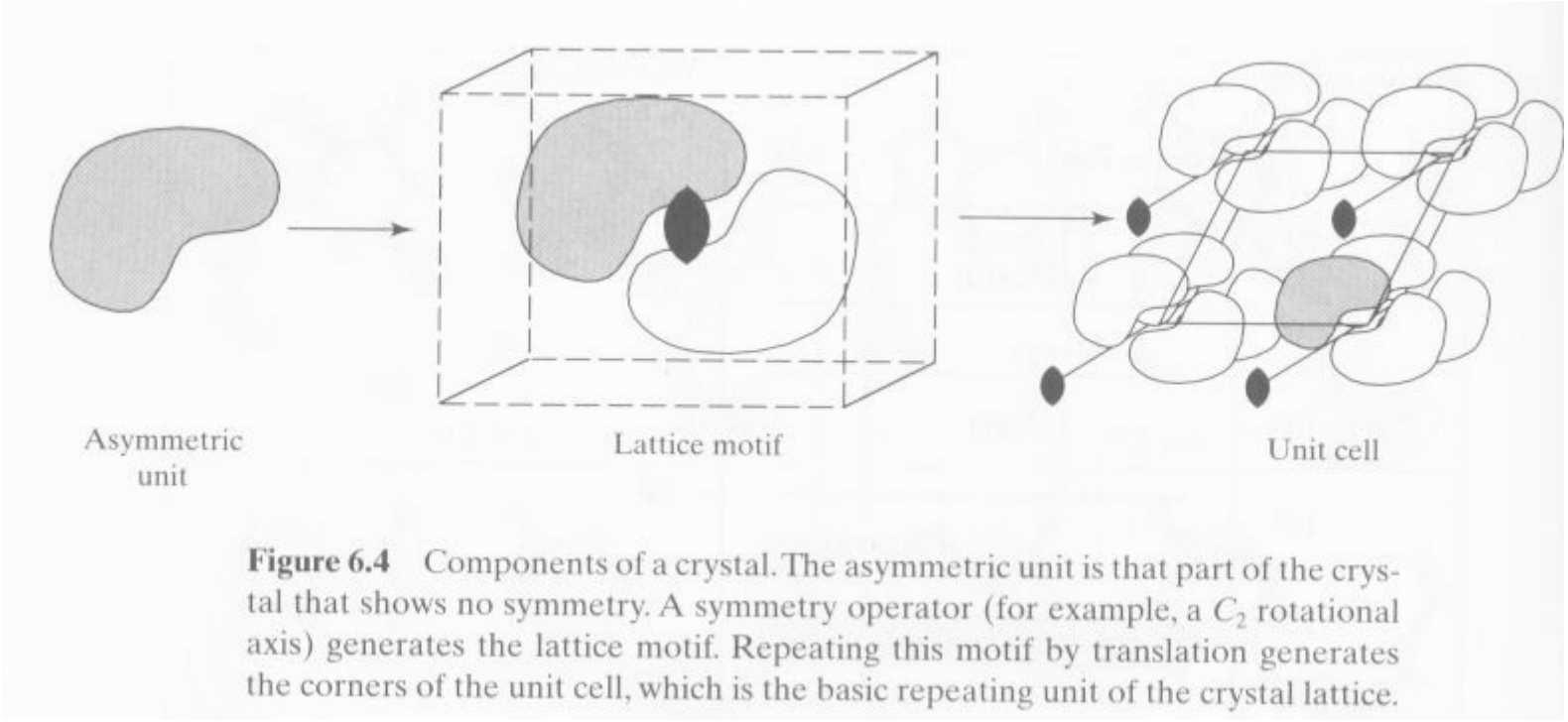


Figure 6.4 Components of a crystal. The asymmetric unit is that part of the crystal that shows no symmetry. A symmetry operator (for example, a C_2 rotational axis) generates the lattice motif. Repeating this motif by translation generates the corners of the unit cell, which is the basic repeating unit of the crystal lattice.

Each level of the crystal, with the exception of the asymmetric unit can be generated using mathematical **operators**

Solving a crystal structure requires **only** that we determine the conformation of the atoms in the **asymmetric unit**

- **Crystal Morphology**

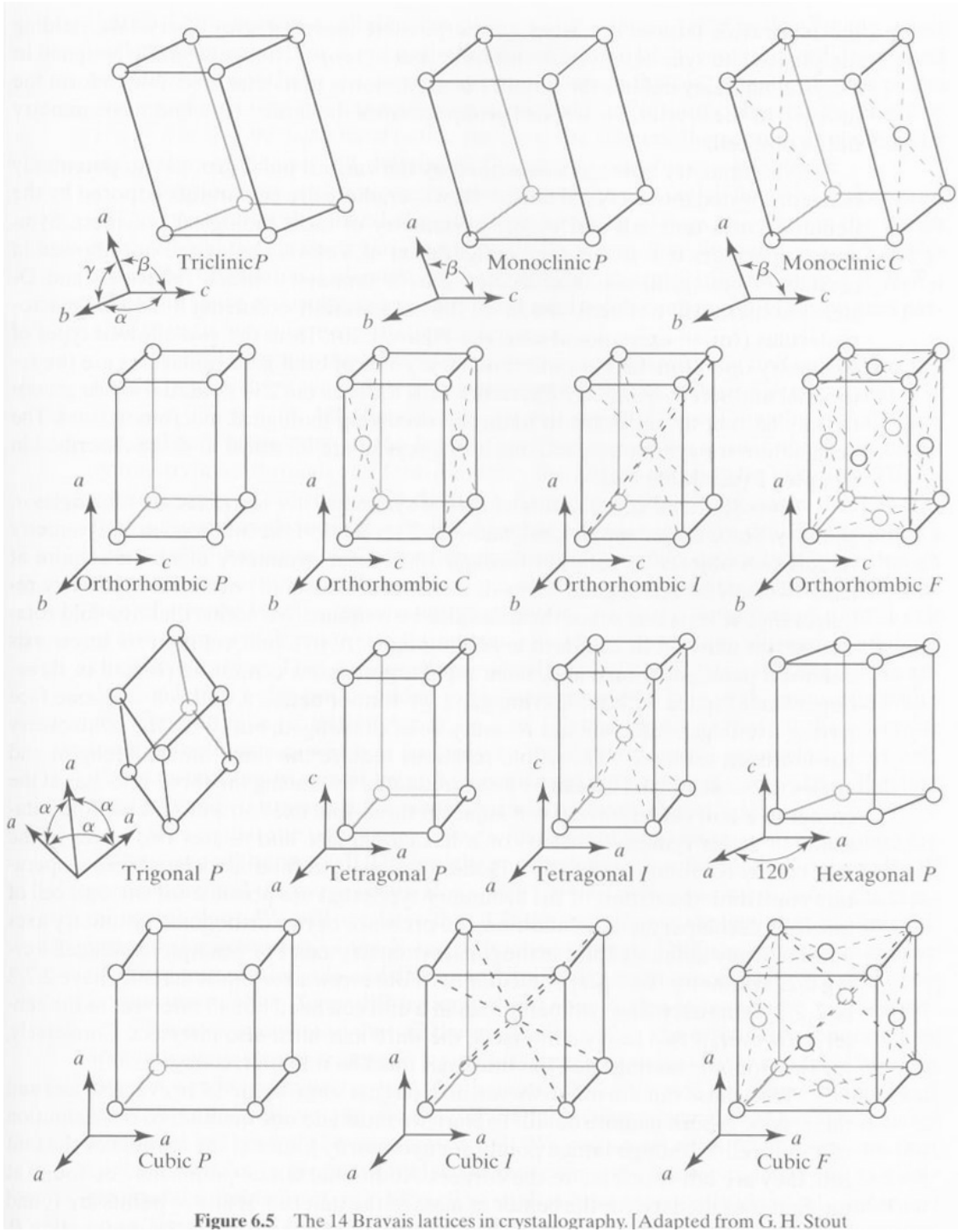


Figure 6.5 The 14 Bravais lattices in crystallography. [Adapted from G. H. Stout

Symmetry

- **P,C,F & I**

  P: Lattic points are found only at the corner
  C: Lattic points are found only at the corner & the one face
  F: Lattic points are found only at the corner & the 6 faces
  I: Lattic points are found only at the corner & the center of the unit cell

Five Fold
rotation or screw axis defines a pentagonal face and since regular pentagons cannot be packed in 3D without leaving gaps, we can not define a unit cell with one face having five edges

Invert the configuration of a **chiral center** are not allowed in crystal of biological macromolecules

Mirror symmetry with relates L & D molecules **stereoisomers** will not be found in crystals of naturally occurring biological macromolecules

Space Group

Two orthogonal symmetry axes automatically defines a **third** orthogonal symmetry axis

The symmetry axes in a unit cell need not all intersect in the center. However, if two axes do intersect, the third axes must also intersect $2_1 2_1$ (two perpendicular 2 fold screw axes)$\Rightarrow$ $2_1 2_1$ 2_1 or $2_1 2_1 2$

If two axes do nonintersecting, the third axes must also nonintersecting

Space Group /Shorthand abbreviation

L R_T R_T R_T

L: lattice type
R: rotation
T: translation
Ex: P 2_1 2_1 2_1

65 space groups

TABLE 6.1 SIXTY-FIVE POSSIBLE SPACE GROUPS IN MACROMOLECULAR CRYSTALS

Lattice Type	Possible Bravais Lattices	Crystal Shape	Possible Space Groups
Triclinic	P	$a \neq b \neq c$ $\alpha \neq \beta \neq \gamma \neq 90^\circ$	$P1$
Monoclinic	P, C	$a \neq b \neq c$ $\alpha = \gamma = 90^\circ, \beta \neq 90^\circ$	$P2, P2_1, C2$
Orthorhombic	P, C, I, F	$a \neq b \neq c$ $\alpha = \beta = \gamma = 90^\circ$	$P222, P2_12_12_1, P2_12_12, P222_1,$ $C222, C222_1, F222, I222,$ $I2_12_12_1$
Tetragonal	P, I	$a = b \neq c$ $\alpha = \beta = \gamma = 90^\circ$	$P4, P4_1, P4_2, P4_3, I4, I4_1,$ $P422, P42_12_1, P4_122, P4_12_12,$ $P4_222, P4_22_12, P4_32_12, P4_322,$ $I422, I4_122$
Trigonal	P	$a = b \neq c$ $\alpha = \beta = 90^\circ, \gamma = 120^\circ$	$P3, P3_1, P3_2,$ $P321, P312, P3_112, P3_121,$
	R (Rhombohedral)	$a = b = c$ $\alpha = \beta = \gamma < 120^\circ (\neq 90^\circ)$	$P3_212, P3_221,$ $R3, R32$
Hexagonal	P	$a = c \neq b$ $\alpha = \gamma = 90^\circ, \beta = 120^\circ$	$P6, P6_1, P6_2, P6_3, P6_4, P6_5,$ $P622, P6_122, P6_322, P6_322,$ $P6_422, P6_522$
Cubic	P, I, F	$a = b = c$ $\alpha = \beta = \gamma = 90^\circ$	$P432, P4_132, P4_232, P4_332,$ $F432, F4_132, I432, I4_132$

Space Group

The lattices type along with the symmetry of the unit cell define the **space group** of the unit cell.

The length & angles of the unit cell define the unit cell parameters, and the space group along the unit cell parameters define the **crystal morphology**.

Isomorphous

Different xtal that has **identical** unit cell lengths and angles

Their diffraction pattern should also appear to be very similar

A xtal is nothing more a single asymmetric unit, solve the structure of a xtal, we need **only** solve the structure of the **asymmetric unit**.

6.2.2 Growing Crystals

Crystallization is more an **art** than a science

Precipitate: bring the molecule out of solution

S^o : **intrinsic solubility**, dep. on temp, pressure, solvent
Supersaturation
Decreased the overall volume to less than half the original volume
Evaporating solvent from solution

Salting in & Salting out

Ionic strength

Salting in: increase ionic strength, increase the solubility

Sating out: increase ionic strength, decrease the solubility

Mechanism of Crystallization

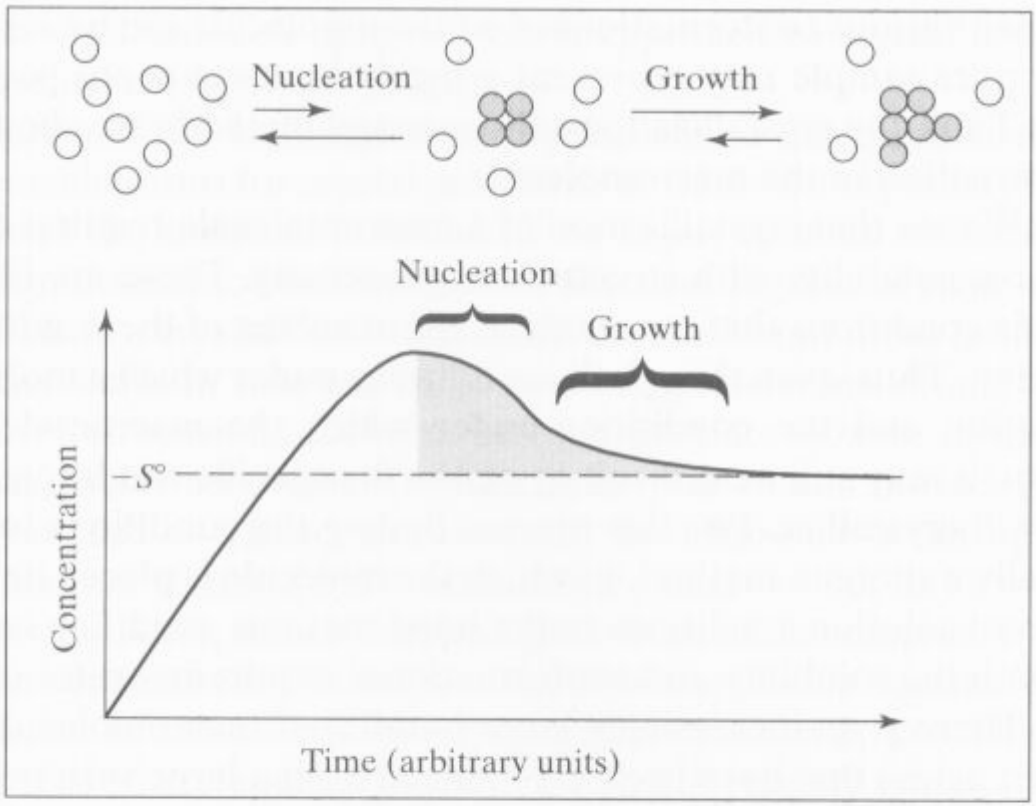


Figure 6.6 Mechanism of crystallization. The initial step in crystallization is the nucleation of a minimum crystal lattice. This is a low probability step that occurs in a supersaturated solution. The crystal grows by adding molecules to the surface of the seed, and occurs at concentrations close to the instrinsic solubility S° of the molecule.

Entropy difference

Highly ordered molecules in a crystal lattice have significantly lower entropy

Two molecules associate to nucleate the formation of a crystal lattice
The entropy difference between monomer and dimer states

$$\Delta S^o = -R \ln 2 = -5.8 \text{ J/mol}$$

At four unit cell must come together in a highly cooperative manner to form a stable and unique nucleation lattice.,
P212121 (4 equivalent positions),
The minumum for the formation of this nucleation lattice well be, 16 molecules and $\Delta S^o = -R \ln 16 = -23$ J/mol

Only a single conformation in the crystal
There is an additional **loss** in **conformational entropy** during crystallization
Difficult to estimate

A large driving force --- supersaturation, above the S^o, intrinsic solubility

Vapor pressure --- equilibrated

Reduce the solubility

6.2.3 Conditions for Macromolecular

Crystallization

- **Purity**

Biochemically pure---------- structure pure

- **Crystallization of macromolecule**

Shotgun
Different buffer/salt conditions

- **Crystallization methods**

Vapor diffusion
Microdialysis

Crystallization condition

TABLE 6.2 SCREENING SOLUTIONS IN SPARSE MATRIX METHODS FOR CRYSTALLIZING PROTEINS AND NUCLEIC ACIDS

Crystallization Solutions			
Salt	Buffer	Precipitant	Molecules Crystallized
Proteins			
None	0.1 *M* Tris	2 *M* Ammonium sulfate	Tropomysin *Eco*R1-DNA complex Monellin
0.2 *M* Na citrate	0.1 *M* Tris	30% Polyethylene glycol (PEG)	Lysozyme Myoglobin Ribonuclease A Insulin
0.2 *M* Na acetate	0.1 *M* Cacodylate	30% PEG	Lysozyme Pepsin
Nucleic acids			
12 mM Spermine, 20 mM Mg^{2+}, 80 mM Na^{+}	40 mM Cacodylate pH 7.0	10% 2-methyl-2,4-dimethylpentane diol (MPD)	$d(CG)^3$ Z-DNA G-quartet DNAs DNA-adriamycin
0.5 mM Spermine, 15 mM Mg^{2+}, 2 mM $BaCl_2$	pH 6.5	7% 2-Propanol	Phe-tRNA
2 mM $CaCl_2$, 10 mM Mg^{2+}	pH 7.0	15% MPD	Group I intron (from *Azoarcus*) 12-Base pair RNA

Vapor diffusion

Hanging drop

Sitting drop

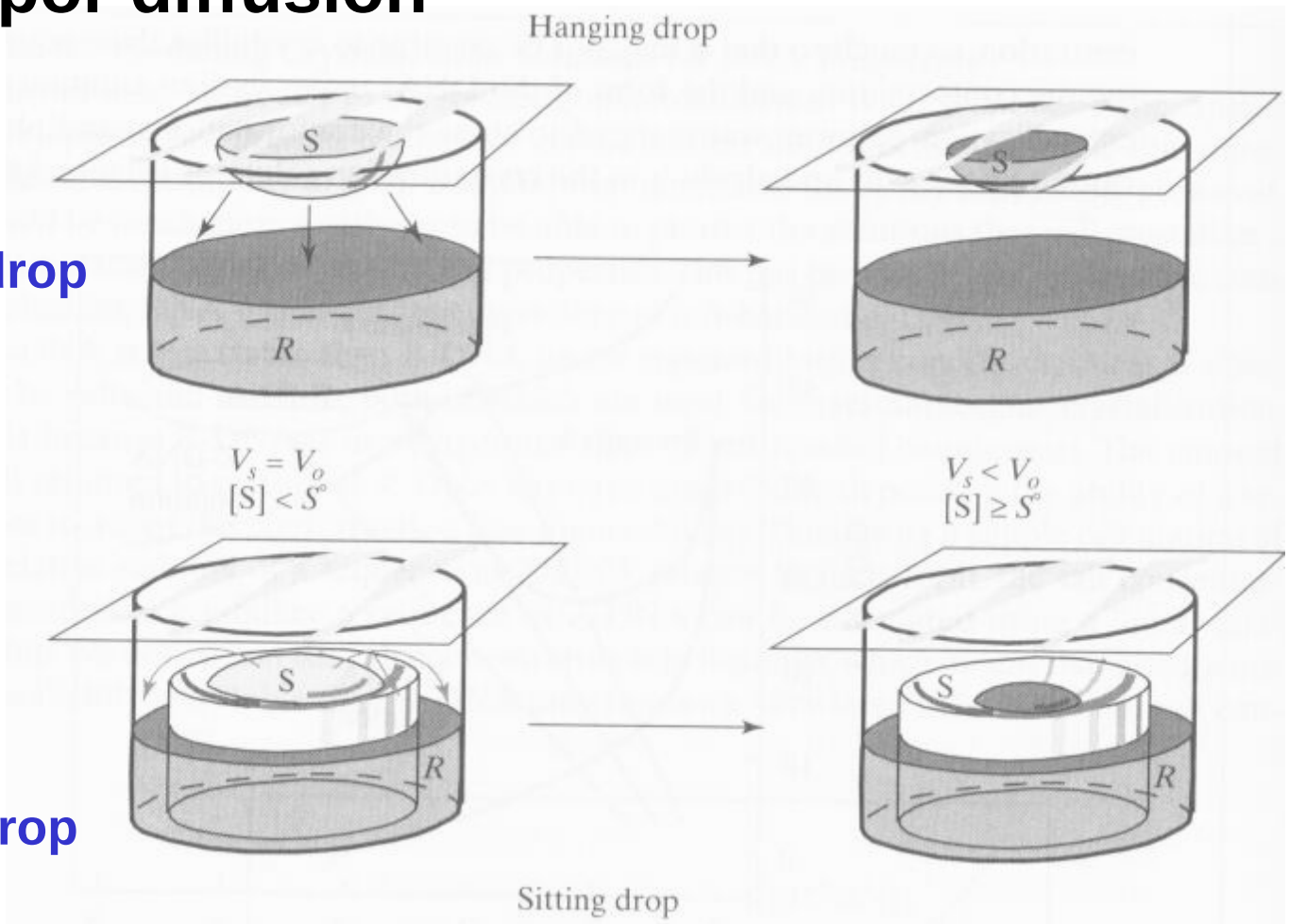
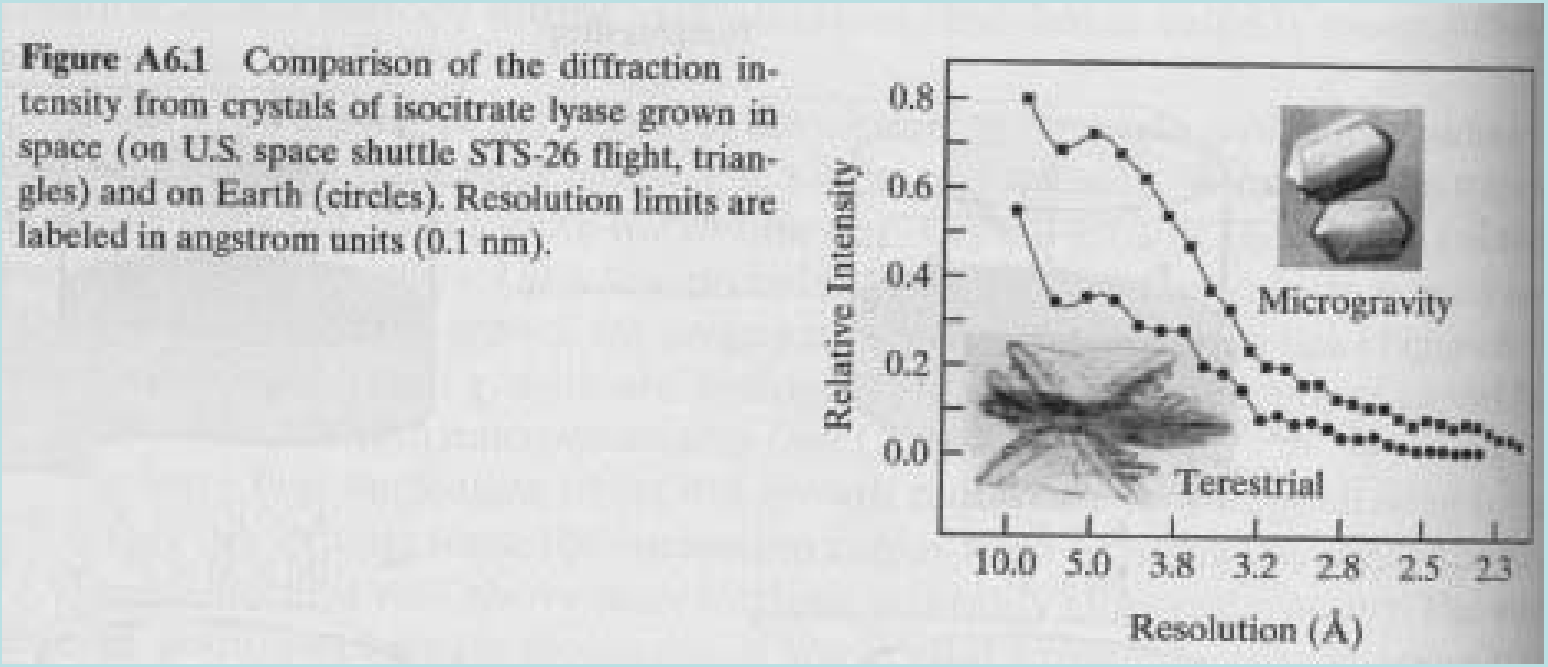


Figure 6.7 Vapor diffusion methods of crystallization. In the hanging drop method of vapor diffusion, a sample in solution is suspended above a reservoir, *R*, that contains a high concentration of a precipitant. The lower vapor pressure of the reservoir draws water from the sample solution, *S*, to reduce the volume of the sample, V_S, below its initial volume, V_o. Consequently, the concentration of molecules in the sample solution, [S], increases to above the intrinsic solubility S° of the molecule, resulting in precipitation or crystallization. In the sitting drop method, the sample solution sits in a well rather than hanging suspended, but

Crystal in space



How microgravity can improve the size and quality of protein crystals on the space shuttle STS-26 flight in 1988

6.3 Theory of X-ray Diffraction

X-ray radiation

Wavelength: 0.1-10nm

~ covalent bond =1.2nm

Quantum energy: 8000ev

~ the energy of electrons in their orbital

Electron interaction energy is responsible for the scattering of X-rays

Electron density:

the # of electron in a given volume of space

Determines how strongly an atom scatters X-rays

Diffraction:

The interference of the scattered X-rays leads the phenomenon of diffraction.

All electromagnetic radiation as "**waves**"

Scattering & **Interference**

Scattering: the ability of objects to **change** the direction of a wave

Ex: the reflection from a mirror, "plane"

Ex: an object place in the path of a light

Huygen's principle of diffraction

Every point along the wave front can be considered to be the **origin** of a new wave front

Objects placed in the path of a wave front act as points of propagation for new wave fronts.

The entirely new wave front is called a **scattered wave**.

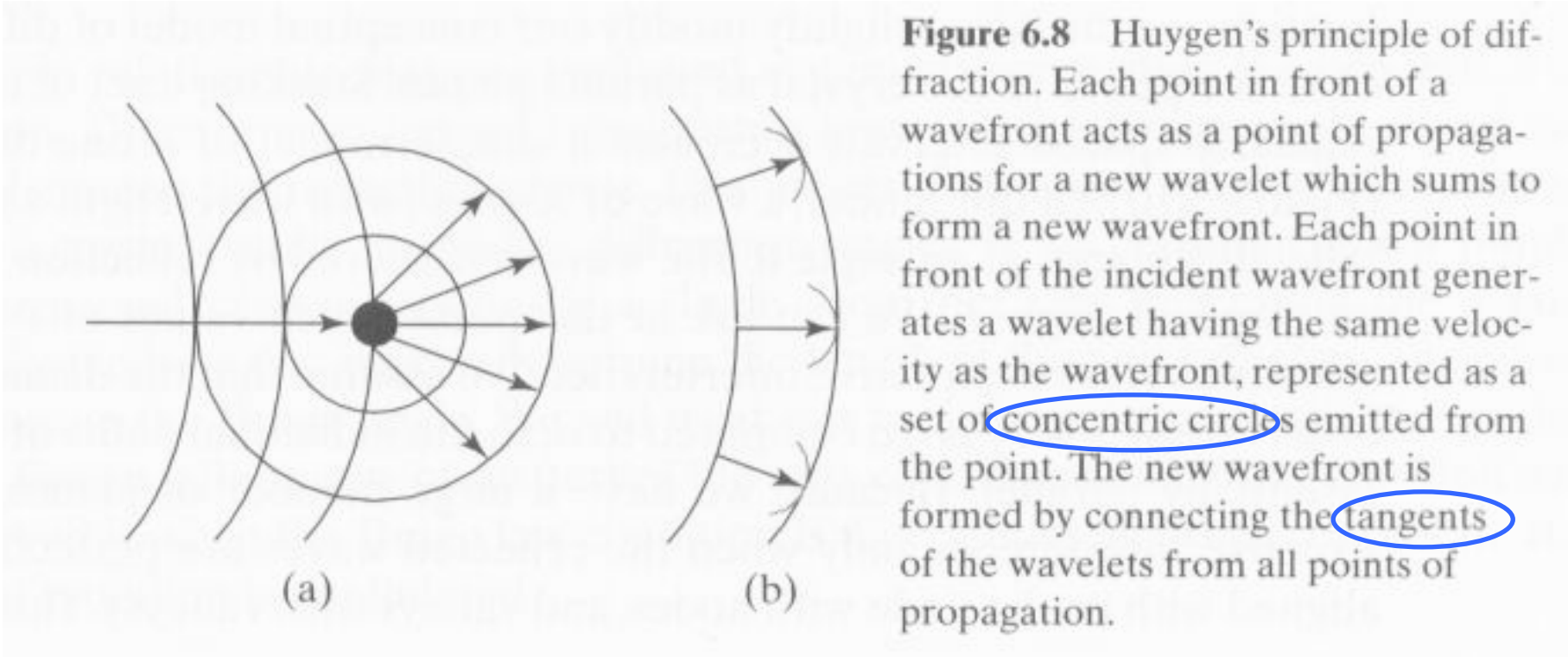


Figure 6.8 Huygen's principle of diffraction. Each point in front of a wavefront acts as a point of propagations for a new wavelet which sums to form a new wavefront. Each point in front of the incident wavefront generates a wavelet having the same velocity as the wavefront, represented as a set of concentric circles emitted from the point. The new wavefront is formed by connecting the tangents of the wavelets from all points of propagation.

Constructive & Destructive Interference of Scattered Waves

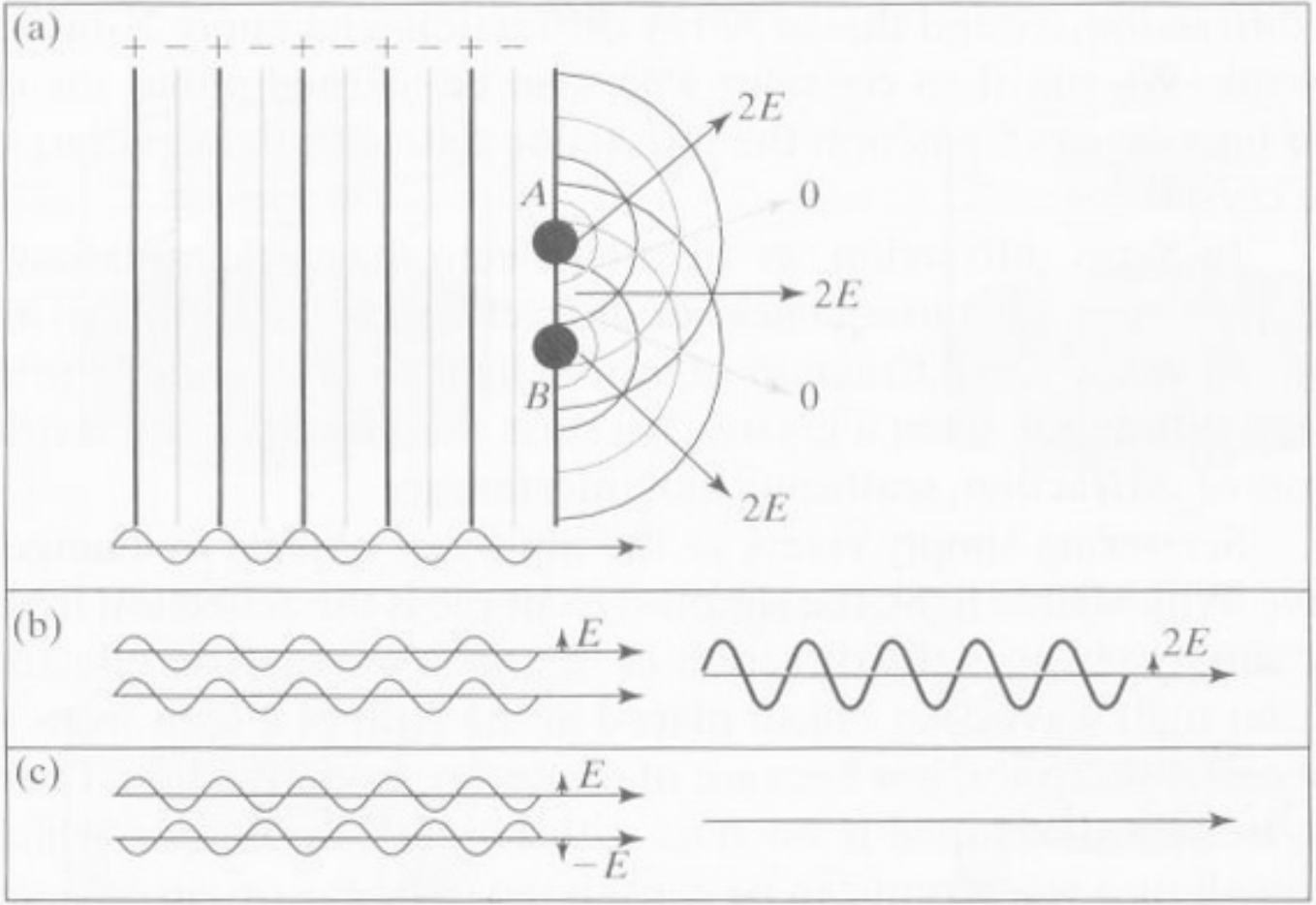


Figure 6.9 Constructive and destructive interference of scattered waves. (a) Two scattering points placed in front of an incident wavefront act as points of propagation. (b) The amplitudes E of the resulting wavelets from the scattering points can sum to form a new wave with twice the amplitude along vectors $2E$ in (a). (c) Waves that are 180° out of phase annihilate each other to give a net amplitude of zero along vectors 0 in (a).

How X-ray diffraction is used to solve the structure of molecules in crystals

The **sum** of the two waves propagated from **A** and **B** result in an **amplitude**

that is dependent on the relative positions of **A** and **B** and is also dependent on **where** the new wave fronts are being observed.

How the **positions** of atoms are determined by the diffraction of X-rays

6.3.1 Bragg's Law

Reflection plan
All parallel

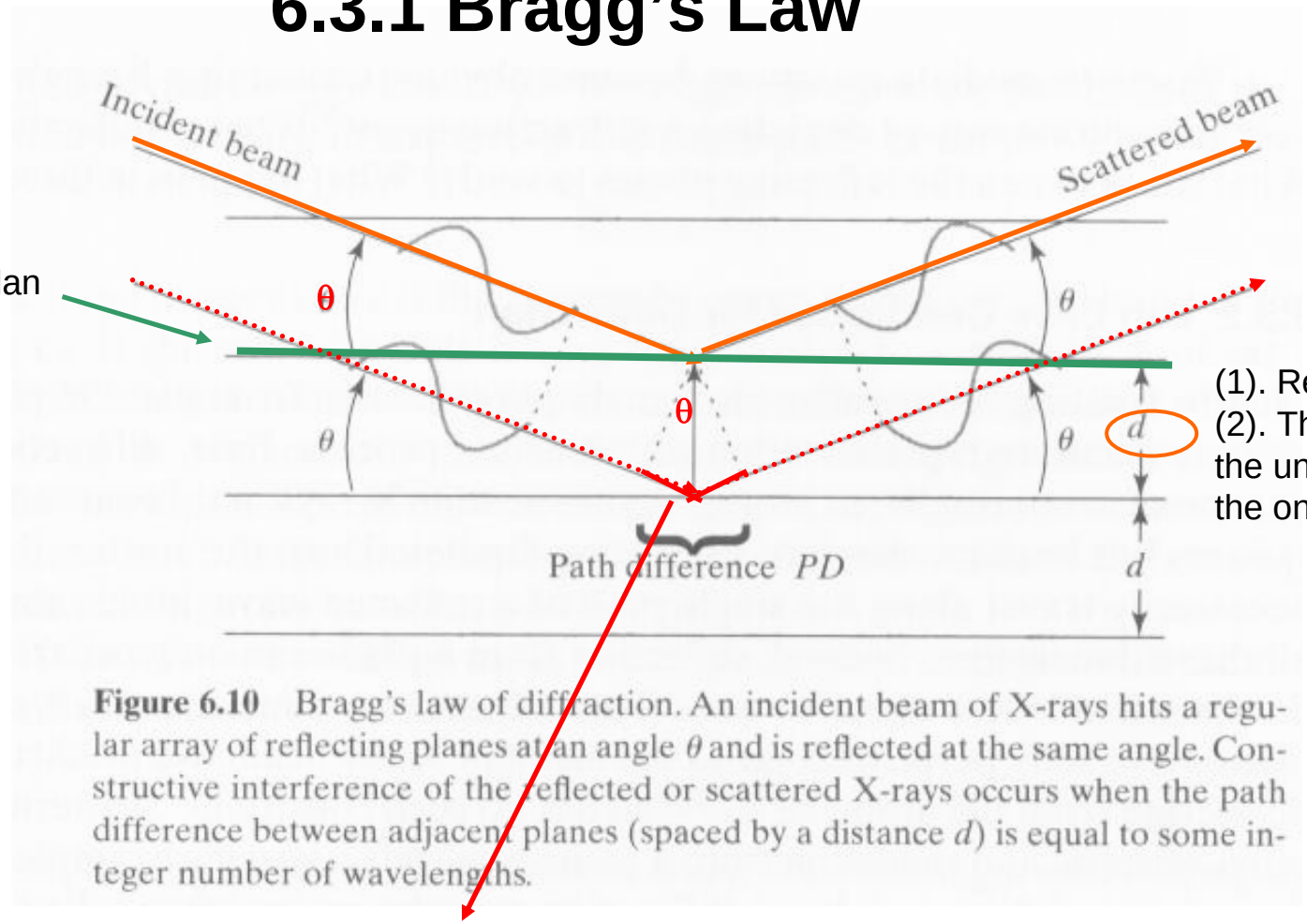


Figure 6.10 Bragg's law of diffraction. An incident beam of X-rays hits a regular array of reflecting planes at an angle θ and is reflected at the same angle. Constructive interference of the reflected or scattered X-rays occurs when the path difference between adjacent planes (spaced by a distance d) is equal to some integer number of wavelengths.

(1). Resolution
(2). The length of the unit cell alone the one axis

2 (dsinθ)

Path difference (PD)

$$2(d \sin\theta) = n\lambda$$ (d: space interval, θ: incident angle)

There is a **reciprocal relationship** between the Bragg angle (θ), and the **spacing** (**d**) between the reflecting planes

$$2 (d \sin\theta) = n\lambda$$

larger spacing of repeating units in a xtal, smaller diffraction angles

Determine the length of the unit cell along the axis by measuring the Bragg angle

6.3.2 von Laue condition for Diffraction

X-ray diffraction is not as **simple** reflection from planes

atoms scatter X-rays in all three dimensions

$\theta>0$

$\theta=0$, no reflections

$\theta=90°$, no reflections

Laue equation $l\lambda=c(\cos\gamma-\cos\gamma_0)$

$l\lambda = c\cos\gamma$, **(if γ equal to 90 degree)**

γ : angle between the scatted radiation and the row of the scatters

γ_0: angle between the incident beam and the row of the scatters

A set of scattering atoms arranged in a regular array

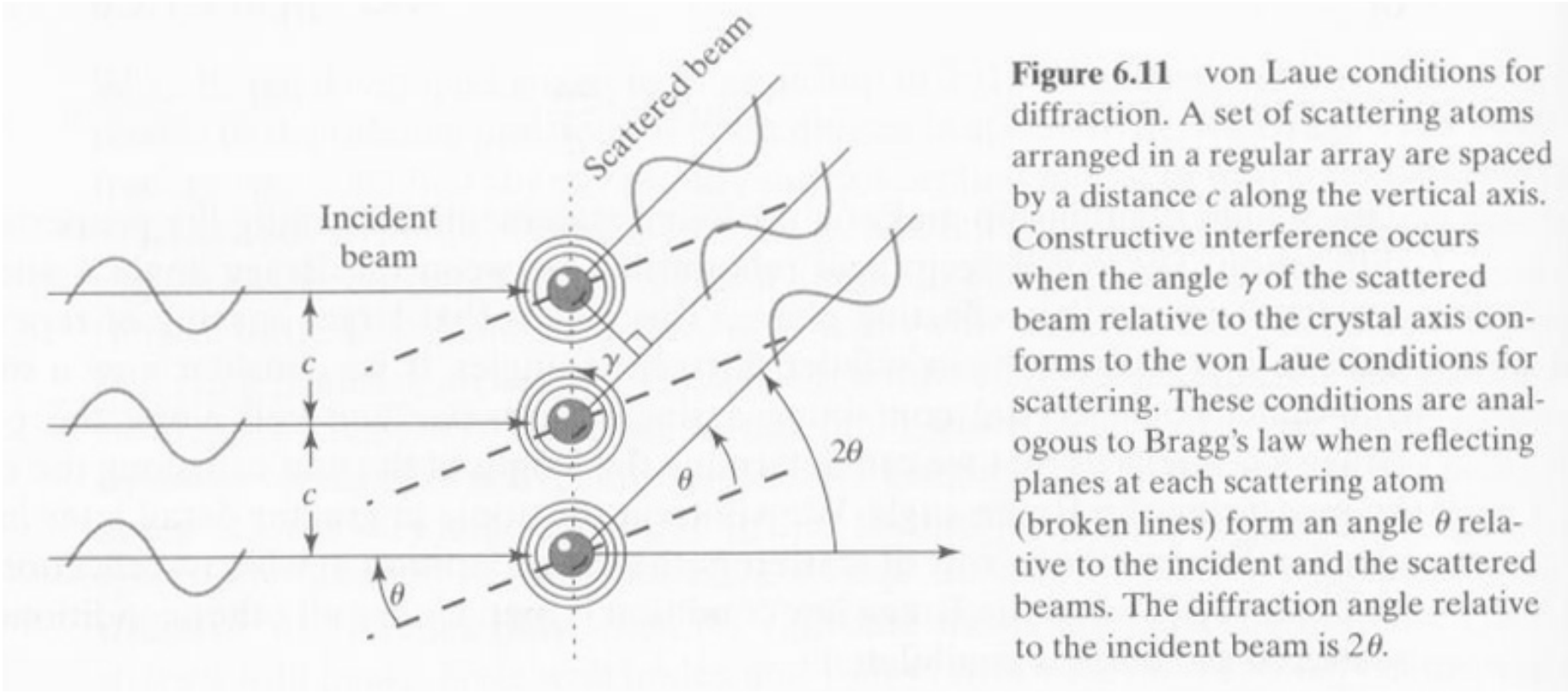


Figure 6.11 von Laue conditions for diffraction. A set of scattering atoms arranged in a regular array are spaced by a distance c along the vertical axis. Constructive interference occurs when the angle γ of the scattered beam relative to the crystal axis conforms to the von Laue conditions for scattering. These conditions are analogous to Bragg's law when reflecting planes at each scattering atom (broken lines) form an angle θ relative to the incident and the scattered beams. The diffraction angle relative to the incident beam is 2θ.

$$l\,\lambda = c\cos\gamma$$ (if γ_0 equal to 90 degree)

γ : angle between the scatted radiation and the row of the scatterers

γ_0: angle between the incident beam and the row of the scatterers

6.3.2 von Laue condition for Diffraction

Laue equation

$l\lambda = c \cos\gamma$, **(if γ equal to 90 degree)**

γ : angle between the scatted radiation and the row of the scatterers

γ_0: angle between the incident beam and the row of the scatterers

$$h\lambda = a(\cos\alpha - c\cos\alpha_0)$$

$$k\lambda = b(\cos\beta - \cos\beta_0)$$

$$l\lambda = c(\cos\gamma - \cos\gamma_0)$$

1D crystal

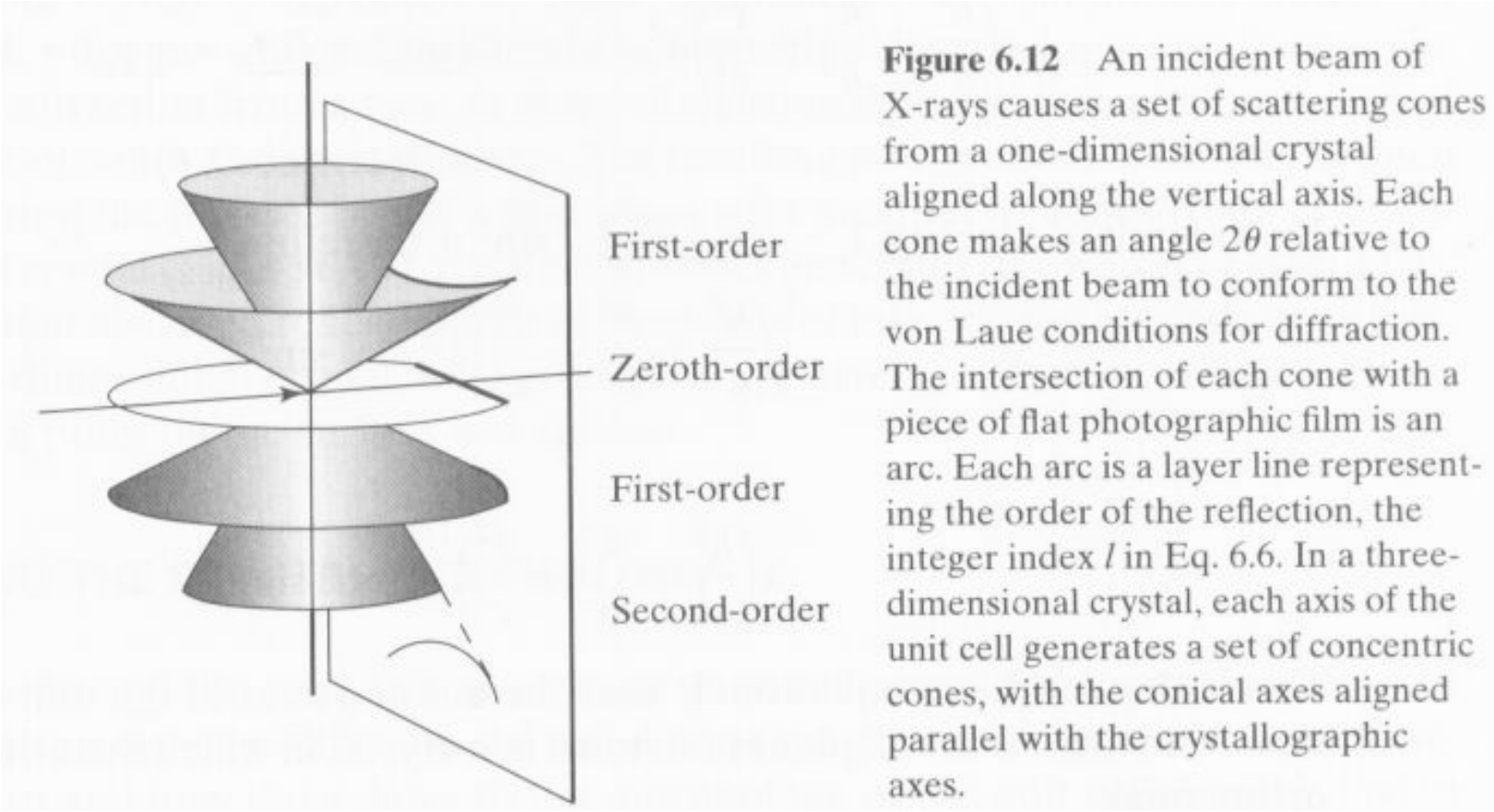


Figure 6.12 An incident beam of X-rays causes a set of scattering cones from a one-dimensional crystal aligned along the vertical axis. Each cone makes an angle 2θ relative to the incident beam to conform to the von Laue conditions for diffraction. The intersection of each cone with a piece of flat photographic film is an arc. Each arc is a layer line representing the order of the reflection, the integer index l in Eq. 6.6. In a three-dimensional crystal, each axis of the unit cell generates a set of concentric cones, with the conical axes aligned parallel with the crystallographic axes.

l = n

L = 0, conforms to the conditions for diffraction, and yields a plane of scattered X-ray, with $2\theta=0$

1D to 3D von Laue condition for diffraction

One-dimensional array

If the incident radiation makes an angle γ_0 other than 90º

$$l\,\lambda = c\,(\cos\gamma - \cos\gamma_0)$$

Expand to three-dimensional crystal

$$h\,\lambda = a\,(\cos\alpha - c\cos\alpha_0)$$
$$k\,\lambda = b\,(\cos\beta - \cos\beta_0)$$
$$l\,\lambda = c\,(\cos\gamma - \cos\gamma_0)$$

$$(h, k, l) \rightleftharpoons (a, b, c)$$

How do Bragg's and the von Laue conditions relate?

Fig 6.11 is there reinforcement of the scattered X-rays in this diffraction ?

If so, we have a reflection & the van Laue condition must be satisfied.

$$h\lambda = a(\cos\alpha - c\cos\alpha_0) \qquad (6.8)$$

square

$$h^2\lambda^2/a^2 = \alpha^2 - 2\alpha^2\alpha_0{}^2 + \alpha_0{}^2 \qquad (6.11)\ (\alpha = \cos\alpha\ ;\ \alpha_0 = \cos\alpha_0)$$

$$k^2\lambda^2/b^2 = \beta^2 - 2\beta^2\beta_0{}^2 + \beta_0{}^2$$

$$l^2\lambda^2/c^2 = \gamma^2 - 2\gamma^2\gamma_0{}^2 + \gamma_0{}^2$$

$$(h^2/a^2 + k^2/b^2 + l^2/c^2)\lambda^2 = 4\sin^2\theta$$

$$(h^2/a^2 + k^2/b^2 + l^2/c^2)^{1/2} = 2\sin\theta/\lambda$$

Bragg's and the von Laue conditions relate

$$(h^2/a^2 + k^2/b^2 + l^2/c^2)^{1/2} = 2\sin\theta/\lambda = n/\lambda$$

Miller indices, (h,k,l): define the integer number of wavelengths that result in an observed reflection from a 3D crystal.

A given set of Miller indices h,k & l , Bragg's law and the von laue equation are equal

Recording diffraction data using a photographic film

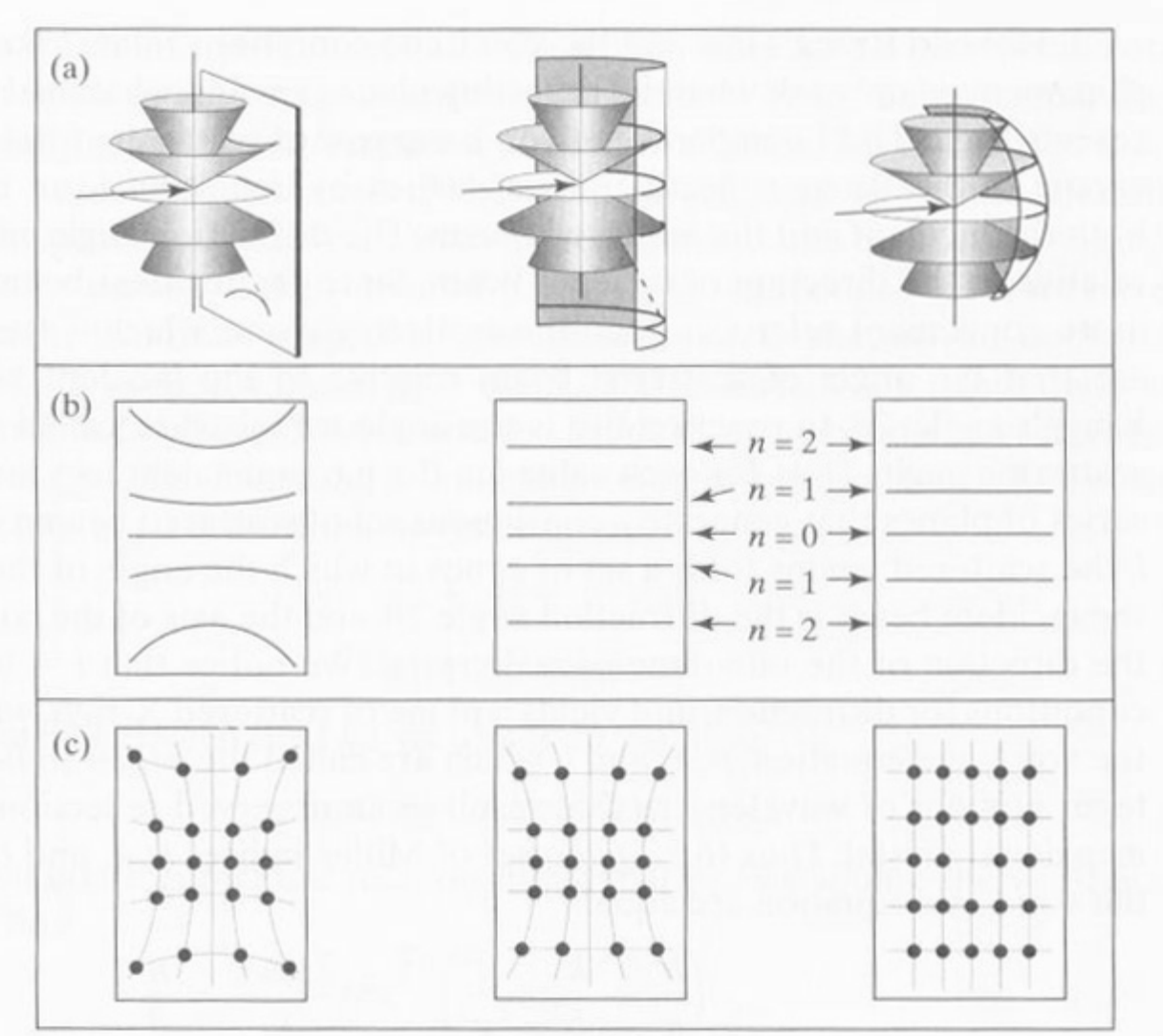


Figure 6.13 Recording diffraction data. The reflecting cones from a crystallographic axis can be recorded using a piece of photographic film that is flat, cylindrically wrapped around the cones, or spherical (shown in the three figures in (a)).

Bragg's and the von Laue conditions relate

As the crystal is expand to **3D**, each additional dimension yields a set of cones whose diffraction angle satisfies the von Laue conditions

The resulting points of resulting points of reflection can be seen by comparing the intersection of **a film plane** with each set of cones from a 2D crystal

Each **cones** generates its own set of **layer** lines.

A sphere of reflections where each reflection is a point on the **surface** of a sphere

6.3.3 Reciprocal space and Diffraction Patterns

Construction of a reciprocal (*) unit cell

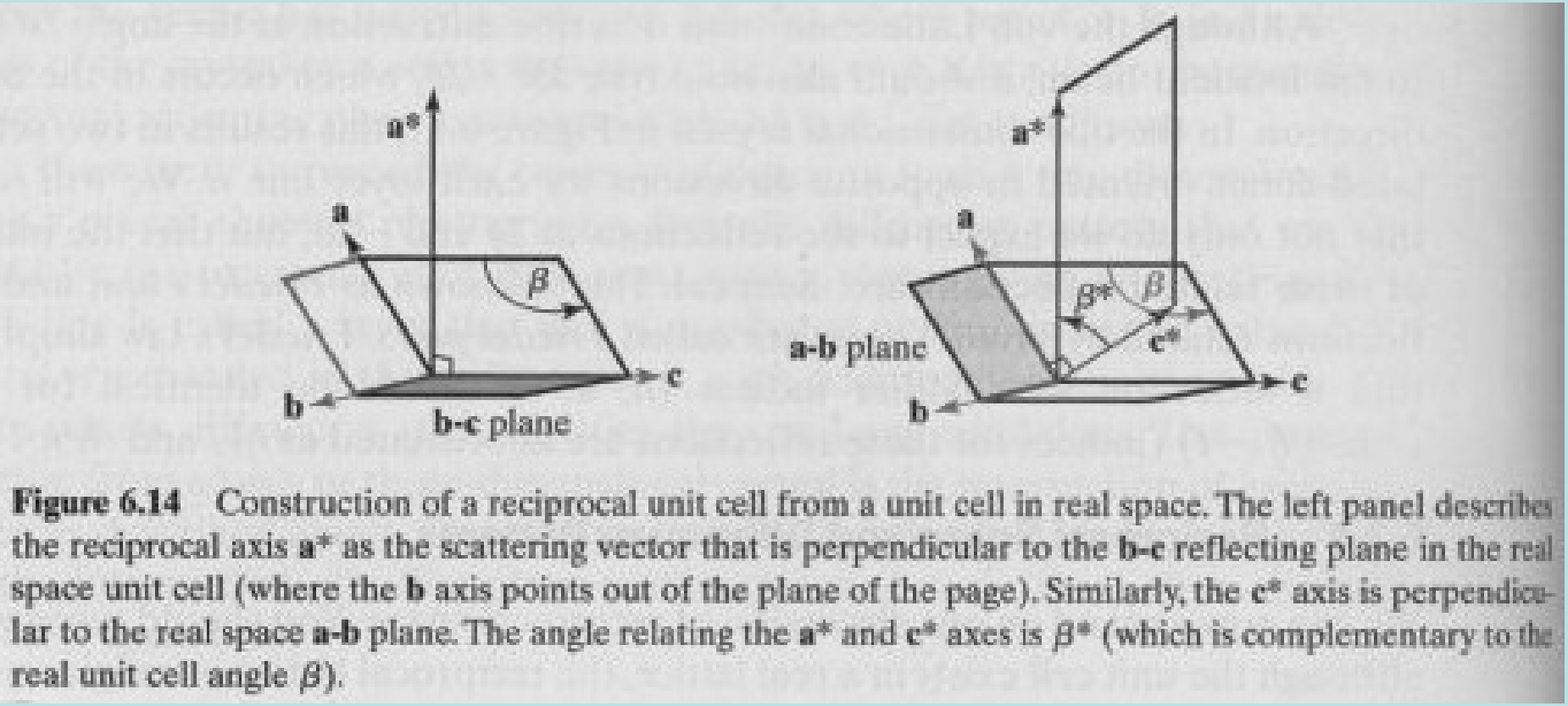
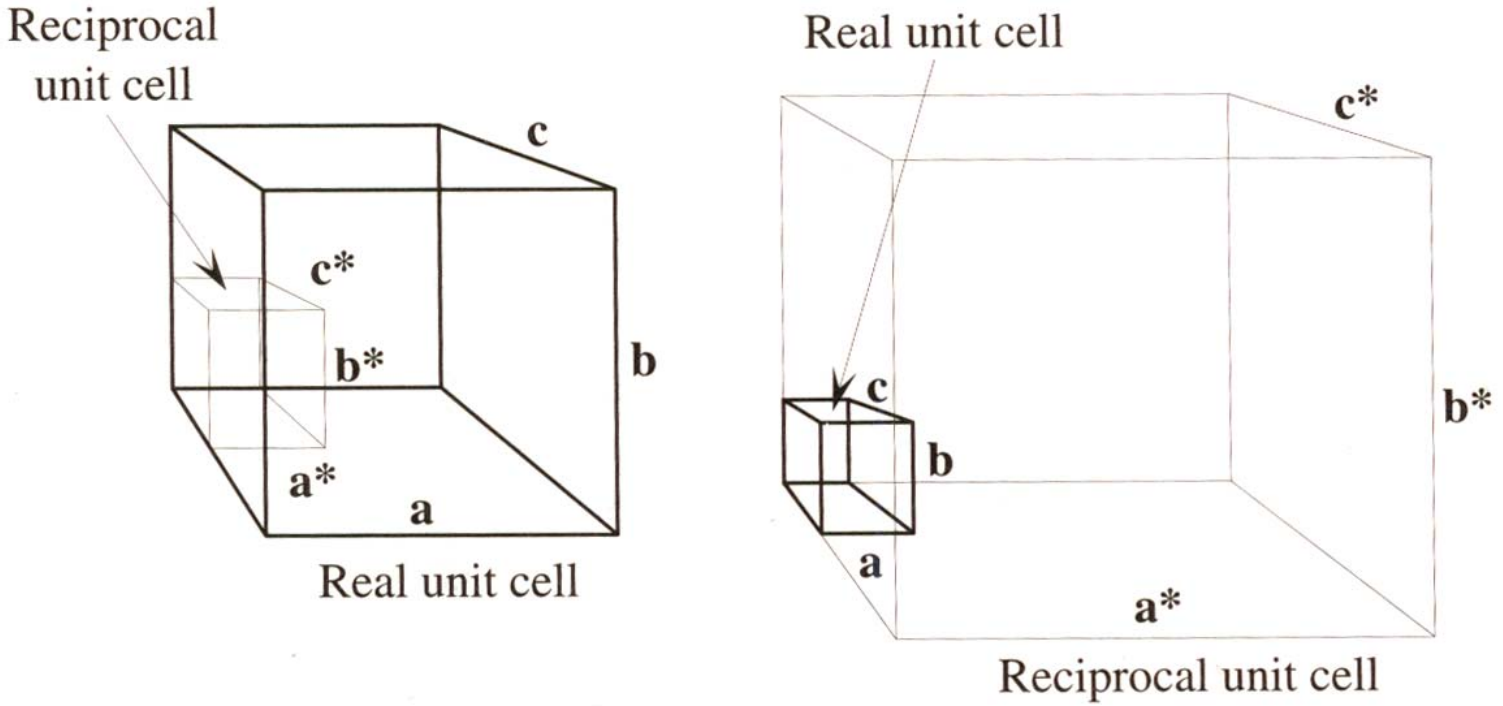


Figure 6.14 Construction of a reciprocal unit cell from a unit cell in real space. The left panel describes the reciprocal axis **a*** as the scattering vector that is perpendicular to the **b-c** reflecting plane in the real space unit cell (where the **b** axis points out of the plane of the page). Similarly, the **c*** axis is perpendicular to the real space **a-b** plane. The angle relating the **a*** and **c*** axes is β^* (which is complementary to the real unit cell angle β).

The reciprocal lattice is constructed using the scattering vector S (b*), which is perpendicular to the reflecting plane (ac plane) with with length "1/b"



Reciprocal unit cells of large and small real cells.

$\alpha = \beta = \gamma = 90^{o}$ ⟹ a* = 1/a, **a*** along **a**
b* = 1/b, **b*** along **b**
c* = 1/c, **c*** along **c**

Relationship between unit cell parameters in Real space & Reciprocal space

TABLE 6.3 RELATIONSHIP BETWEEN UNIT CELL PARAMETERS IN REAL SPACE AND RECIPROCAL SPACE

Lattice type	Real Space	Reciprocal Space
Orthorhombic and higher symmetry	a	$a^* = \frac{1}{a}$
	b	$b^* = \frac{1}{b}$
	c	$c^* = \frac{1}{c}$
	$\alpha = 90^\circ$	$\alpha^* = 90^\circ$
	$\beta = 90^\circ$	$\beta^* = 90^\circ$
	$\gamma = 90^\circ$	$\gamma^* = 90^\circ$
	V	$V^* = \frac{1}{V} = a^*b^*c^*$
Monoclinic		
	a	$a^* = \frac{1}{a \sin \beta}$
	b	$b^* = \frac{1}{b}$
	c	$c^* = \frac{1}{c \sin \beta}$
	$\alpha = 90^\circ$	$\alpha^* = 90^\circ$
	$\beta \neq 90^\circ$	$\beta^* = 180^\circ - \beta$
	$\gamma = 90^\circ$	$\gamma^* = 90^\circ$
	V	$V^* = \frac{1}{V} = a^*b^*c^* \sin \beta^*$
Triclinic		
	a	$a^* = \frac{bc \sin \alpha}{V}$
	b	$b^* = \frac{ac \sin \beta}{V}$
	c	$c^* = \frac{ab \sin \gamma}{V}$
	$\alpha \neq 90^\circ$	$\cos \alpha^* = \frac{\cos \beta \cos \gamma - \cos \alpha}{\sin \beta \sin \gamma}$
	$\beta \neq 90^\circ$	$\cos \beta = \frac{\cos \alpha \cos \gamma - \cos \beta}{\sin \alpha \sin \gamma}$
	$\gamma \neq 90^\circ$	$\cos \gamma = \frac{\cos \alpha \cos \beta - \cos \gamma}{\sin \alpha \sin \beta}$
	V	$V^* = a^*b^*c^*\sqrt{1 - \cos^2 \alpha^* - \cos^2 \beta^* - \cos^2 \gamma^* + 2 \cos \alpha^* \cos \beta^* \cos \gamma^*}$

A precession photography

mimics spherical film by rolling of processing a flat piece of film about the crystal axes.

This is **undistorted** diffraction pattern

A precession camera rotates both crystal and film in concert to give a photograph in which the spacing and the intensities of the diffraction pattern are recorded in an undistorted manner

Each precession photograph can be though of as a **slice** through the sphere of reflection.

Conditions for diffraction in reciprocal space

Ewald Sphere:

The sphere of reflections in the reciprocal space

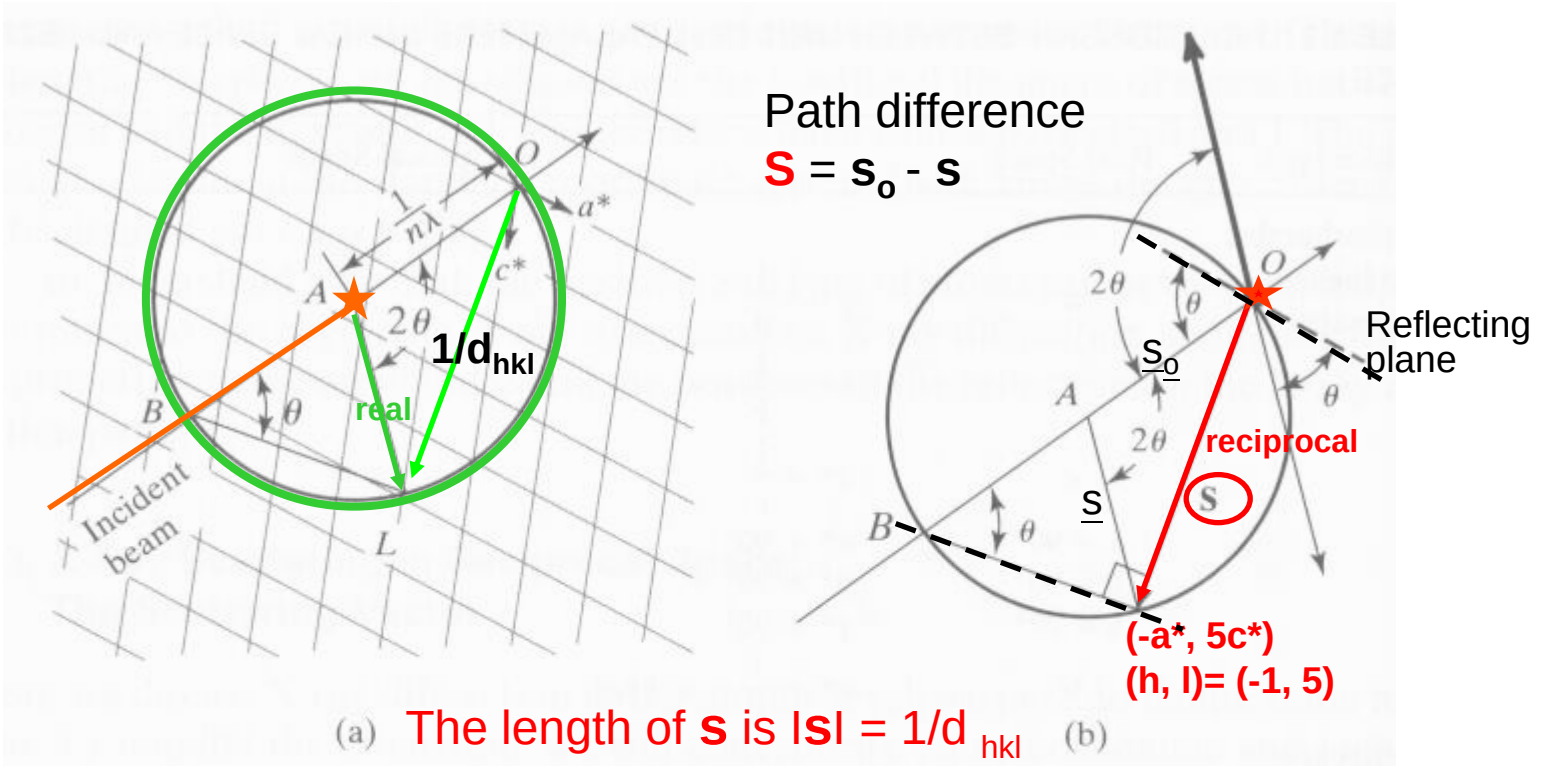


Fig 6.15 Conditions for diffraction in reciprocal space. A point of origin O for the scattered X-ray beam is defined at the origin of a unit cell of the reciprocal lattice. A point A is placed along the incident beam at a distance $1/n\lambda$ from O. A circle with a radius of $1/n\lambda$ is drawn with A at the center. The point where the circle intersects the incident beam is labeled point B. Any other lattice point L of the reciprocal lattice that intersects the circle represents a reflection in reciprocal space. Bragg's law is derived by defining the diffraction angle θ as the angle OBL, and the trigonometric relationship between the scattering vector **S** and the diameter of the circle. The vector LA is the direction of scattered beam in real space. This is shown in (b) as the bold arrow extending from the origin O and at an angle 2θ relative to the incident beam.

Ewald Sphere:

The sphere of reflections in the reciprocal space

With a radius of n/λ

Rotating the crystal allows a different set of lattice points to **intersect** with the sphere to cause scattering

The length of **S,** scalltering vector is $|\mathbf{S}| = 1/d_{hkl}$

In an X-ray diffraction experiment, the intensity of each reflections is given by the intensity of a single scattering vector $\boldsymbol{I(S)}$

The molecular structure defines the measured quantity $\boldsymbol{I(S)}$

The reflection sphere in reciprocal space

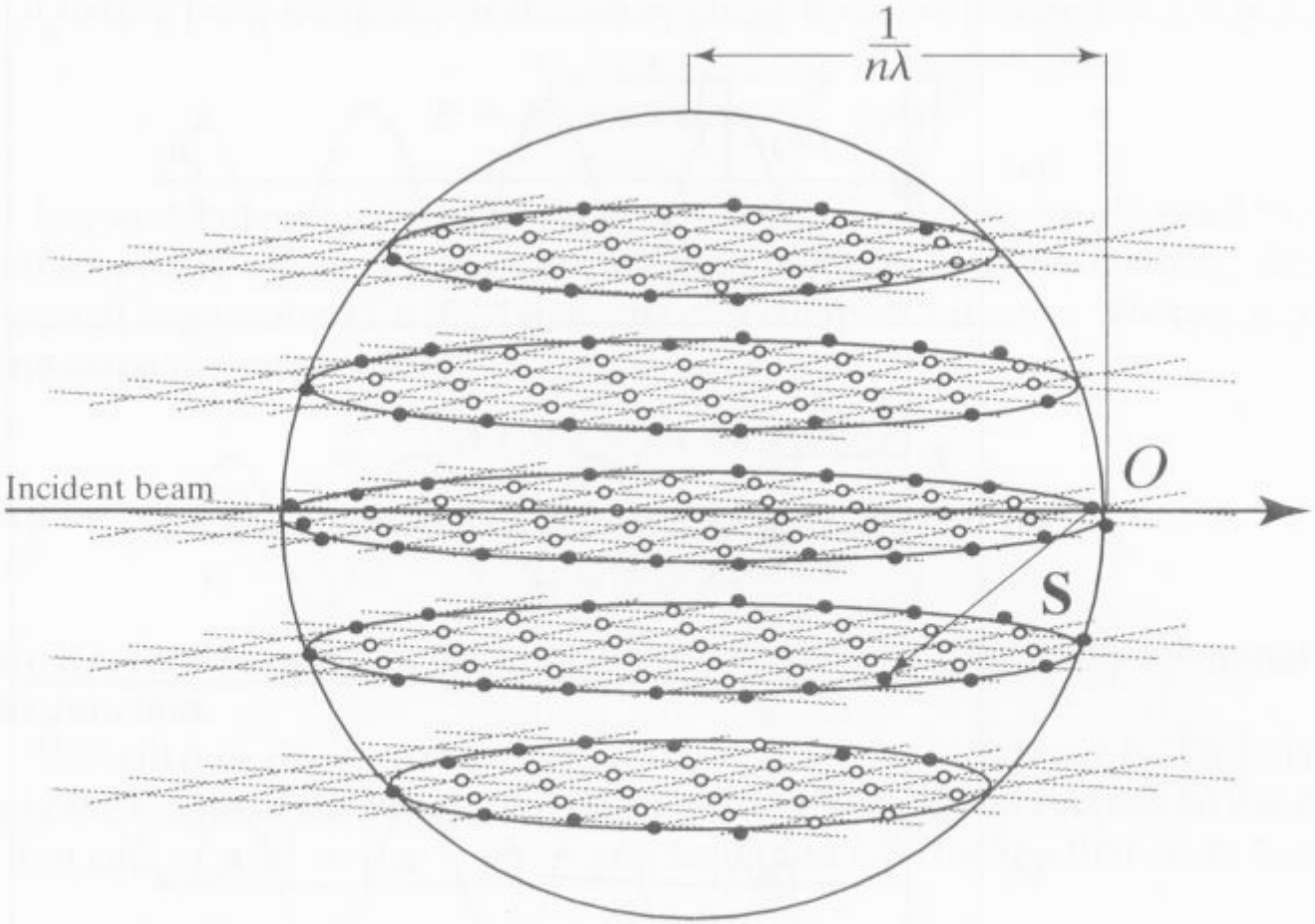


Figure 6.18 The reflection sphere in reciprocal space. The extension of the analysis in Figure 6.17 to a three-dimensional crystal is to draw a sphere with radius n/λ. Each reciprocal lattice point that intersects the surface of the sphere (filled points) is a reflection in reciprocal space. The points included in the volume of the sphere of reflections (open points) represent points along the surface of smaller concentric spheres.

The reflection sphere in reciprocal space

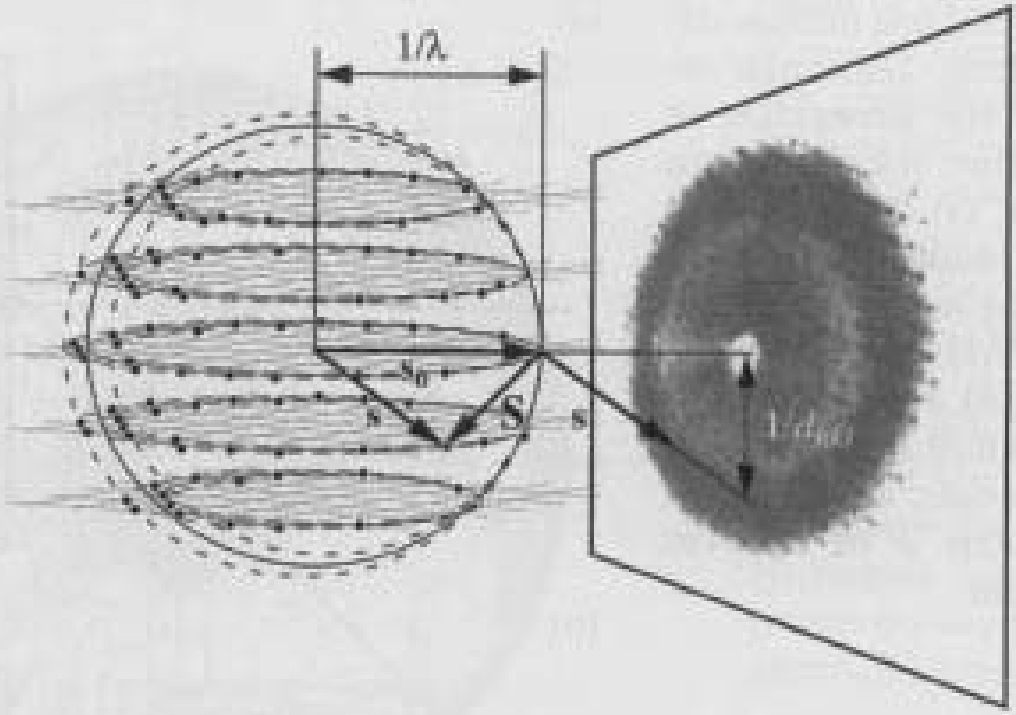


Figure 6.16 The reflection sphere in reciprocal space. The extension of the analysis in Figure 6.15 to a three-dimensional crystal is to draw a sphere with radius 1/λ. Each reciprocal lattice point that intersects the surface of the sphere (filled points) is a reflection in reciprocal space (we should note that since the X-ray is not entirely monochromatic, i.e., there is some spread Δλ, the surface of the sphere has some depth, as represented by the dotted surface, which allows more of the lattice points to intersect and thus to provide for more reflections to come under diffraction conditions). The points included in the volume of the sphere of reflections can come into diffraction condition as the crystal is rotated.

Still & Rotation Diffraction

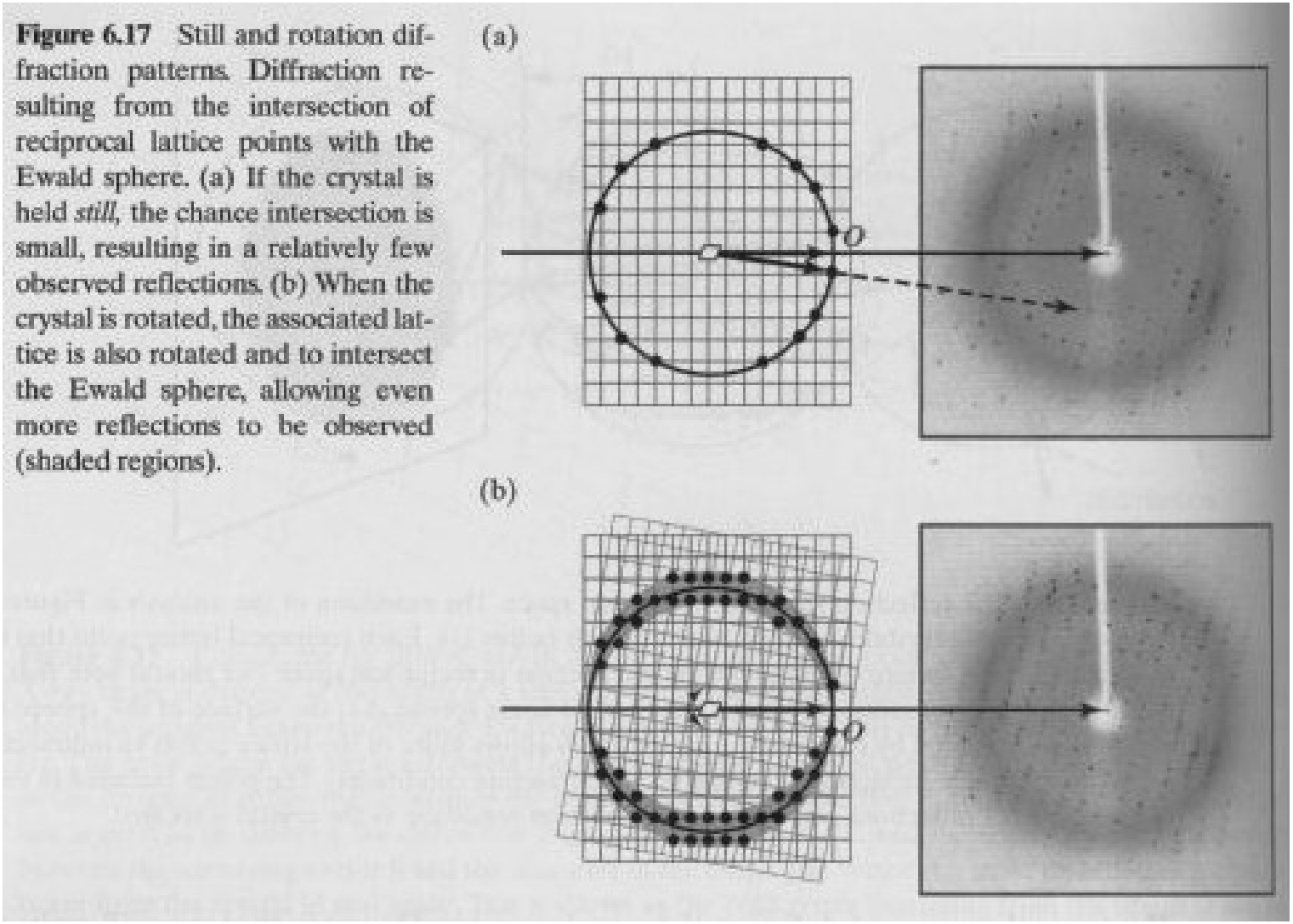


Figure 6.17 Still and rotation diffraction patterns. Diffraction resulting from the intersection of reciprocal lattice points with the Ewald sphere. (a) If the crystal is held *still*, the chance intersection is small, resulting in a relatively few observed reflections. (b) When the crystal is rotated, the associated lattice is also rotated and to intersect the Ewald sphere, allowing even more reflections to be observed (shaded regions).

6.4 Determining The Crystal Morphology

Observe the ***spacing*** and ***pattern*** of the reflections on the ***diffraction pattern***.

Determine the ***lengths*** and ***angles*** of the unit cell and space group.

Determine the ***symmetry*** or ***space group*** in the unit cell.

Define the ***morphology*** of the crystal.

Precession photography

Photographic film, a flat sheets of film

Rotates both the xtal & film in concert to give recorded in an undistorted manner

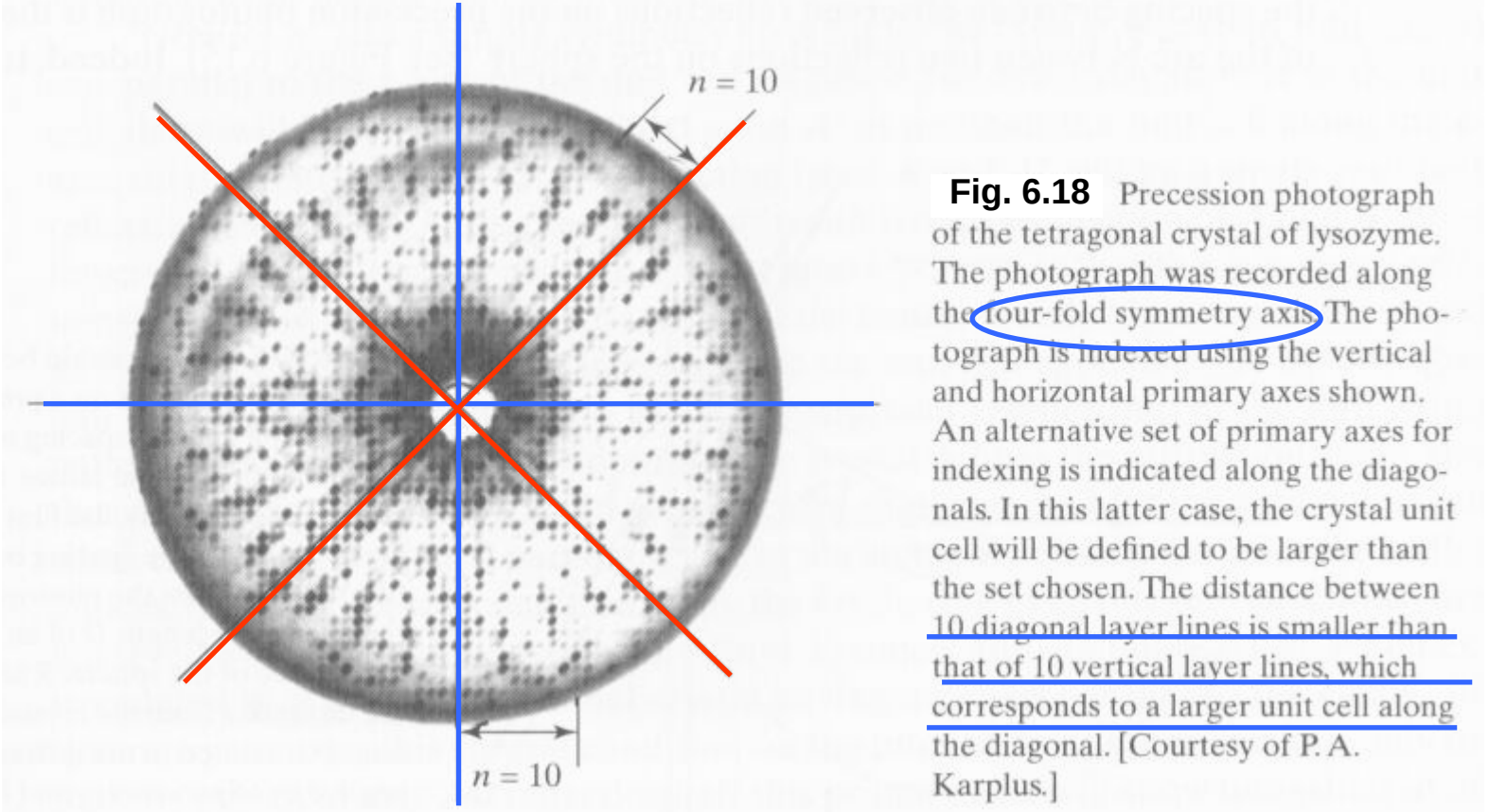
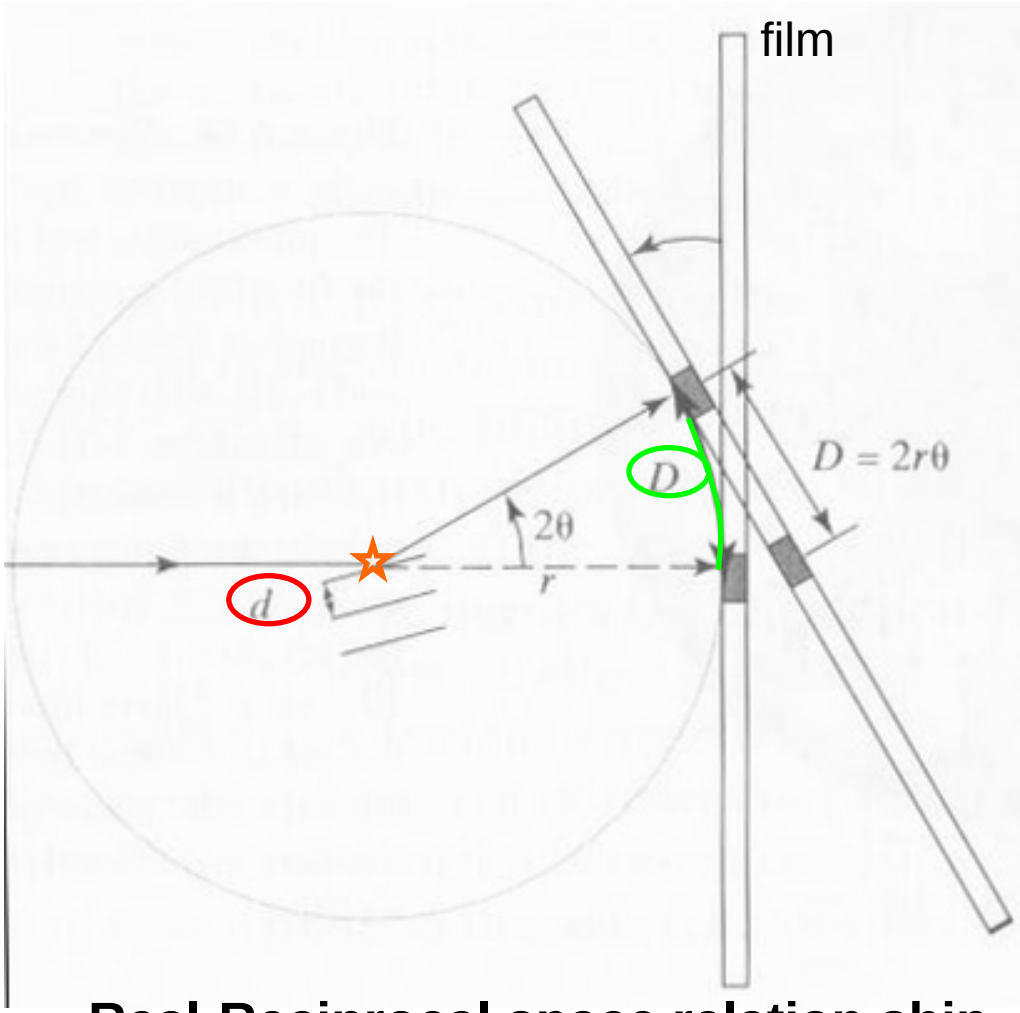


Fig. 6.18 Precession photograph of the tetragonal crystal of lysozyme. The photograph was recorded along the four-fold symmetry axis. The photograph is indexed using the vertical and horizontal primary axes shown. An alternative set of primary axes for indexing is indicated along the diagonals. In this latter case, the crystal unit cell will be defined to be larger than the set chosen. The distance between 10 diagonal layer lines is smaller than that of 10 vertical layer lines, which corresponds to a larger unit cell along the diagonal. [Courtesy of P. A. Karplus.]

$P4_32_12$ **Axis/ unit cell : Diagonal >Vertical (right)**

The spacing of reflections on a precession photograph & the spacing of reflecting planes in a crystal lattice.



$D = (2\pi r)(2\theta / 2\pi) = 2r\theta$

$\sin(D/2r) = \lambda / 2d$

$d = \lambda / 2 \sin(D/2r)$

Figure 6.15 Relationship between spacing of reflections on a precession photograph and the spacing of reflecting planes in a crystal lattice. In precession photography, flat film is rolled along a sphere. The spacing between two reflections on the photograph represents the length D of an arc at the surface of the sphere. Knowing the distance r from the crystal to the film, D is related to the diffraction angle θ by trigonometry and Bragg's law.

Real-Reciprocal space relation ship

d: real space

D: reciprocal

Friedel Law-Friedel pairs

The diffraction pattern will show mirror symmetry according to Frideel's law.
The reflection with Miller indices (h,k,l) should be identical for one at
(-h,-k,-l)
The two halves of the reflection sphere should be symmetry
I(h,k,l) = I(-h,-k,-l)

3 principal axes
(h 0 0)=a axis, (0 k 0)= b axis, (0 0 l)=c axis
(h k 0) ab plane, (0 k l) =bc plane, (h 0 l)=ac plane
origin (0 0 0)

Systematic absence **/observed & unobserved**

Ex: $P2_1$ l=2n observed , l=2n+1 unobserved (reciprocal, 1/2 --- 2)
Ex: $P2_12_12_1$ only even reflections can be observed, (h,k,l), h=2n, k=2n, l=2n

Systematic Absences

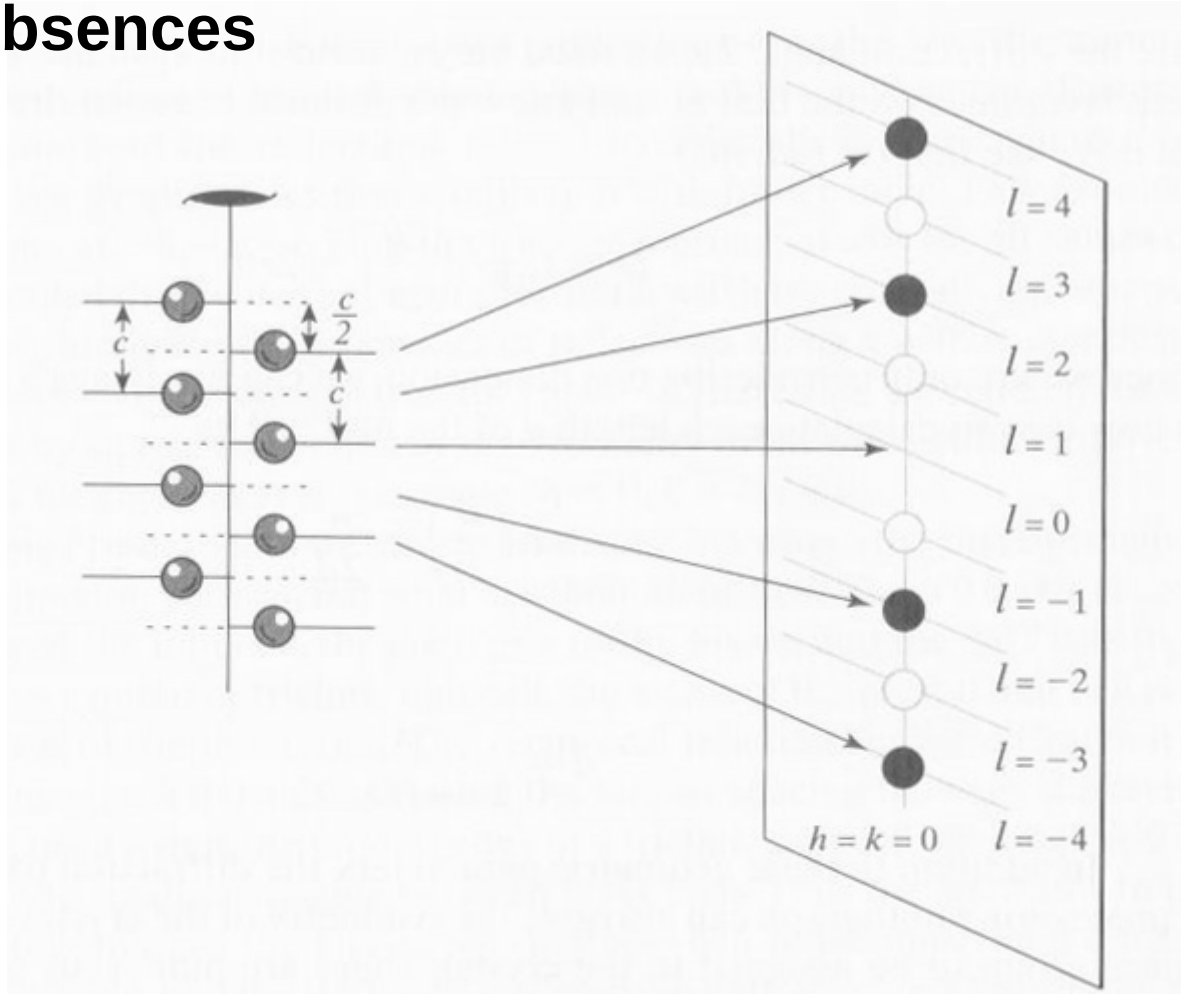


Figure 6.16 Systematic absences caused by a 2_1 axis along the crystallographic **c**-axis. A set of atoms A are spaced by a distance c along the vertical axis. A two-fold screw axis generates a set of symmetry related atoms A' that are rotated 180° and translated by $c/2$ relative to the atoms A. The resulting scattered beam appears to come from a unit cell that is half the length of the actual unit cell. The corresponding diffraction angle will be twice that expected from the unit cell and, therefore, the reflections along the principal axis 0 0 l will be spaced twice the distance expected. This appears as the absence of reflections at all odd values of l along this axis in the precession photograph.

6.5 Solving Macromolecular Structures By X-ray Diffraction

More than a single atom in a unit cell (upwards of 10,000 atoms in hemoglobin crystal).

Deconvolute each reflection into the phase and amplitude contributions from each atom in the molecule.

Atomic position: (x,y,z) coordinates.

Orthogonal Cartesian coordinate system

Fractional cell coordinates 0 to 1

6.5.1 The Structure Factor

Propagation of Waves

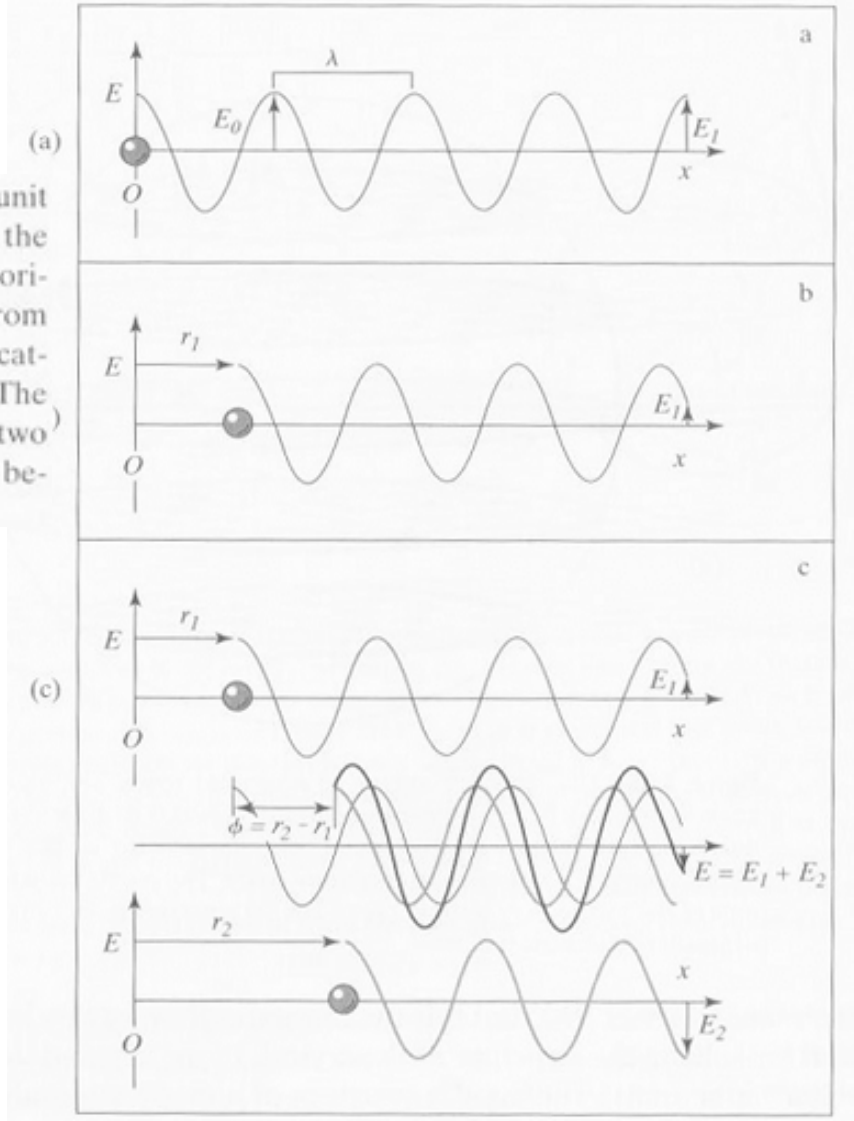


Fig. 6.20 Propagation of waves. A point placed at the origin O of the unit cell propagates a wave with a maximum amplitude E_o (a). At some point x, the instantaneous amplitude is observed as E_1. If the atom is displaced from the origin by a distance r_1, the amplitude of the wave is observed to be different from that propagated from the origin (b). The wave propagated from a second scatterer at a distance r_2 from the origin will have an observed amplitude E_2. The wave resulting from both scatterers has an amplitude that is the sum of the two waves ($E = E_1 + E_2$), which is dependent on the phase difference $\Delta\phi$ between the two scatterers.

The propagation of a wave as a cosine function

$E_1 = Eo \cos 2\pi (\upsilon t - x/\lambda)$

E_2: Shifted in phase by some fraction of a wave by the distance r_1
of a wave ϕ

$E = E_1 + E_2 = Eo \cos 2\pi (\upsilon t - x/\lambda + \phi)$

$\phi = r_1 - r_2$

$$E(x, t) = |E_o| \cos 2\pi (\upsilon t - x/\lambda) \quad (6.27)$$

A wave that is shifted in phase by some fraction of wave ϕ

$$E(x, t) = |E_o| \cos 2\pi (\upsilon t - x/\lambda + \phi) \quad (6.28)$$

Simplify by

Phase angle $\omega = 2\pi(\upsilon t - x/\lambda)$

Phase angle $\alpha = 2\pi\phi$

$$E(\omega) = |E_o| \cos(\omega + \alpha) \quad (6.29)$$

$$E(\omega) = \underline{|E_o| \cos\omega}\,(\cos\alpha + i \sin\alpha) \quad (6.32)$$

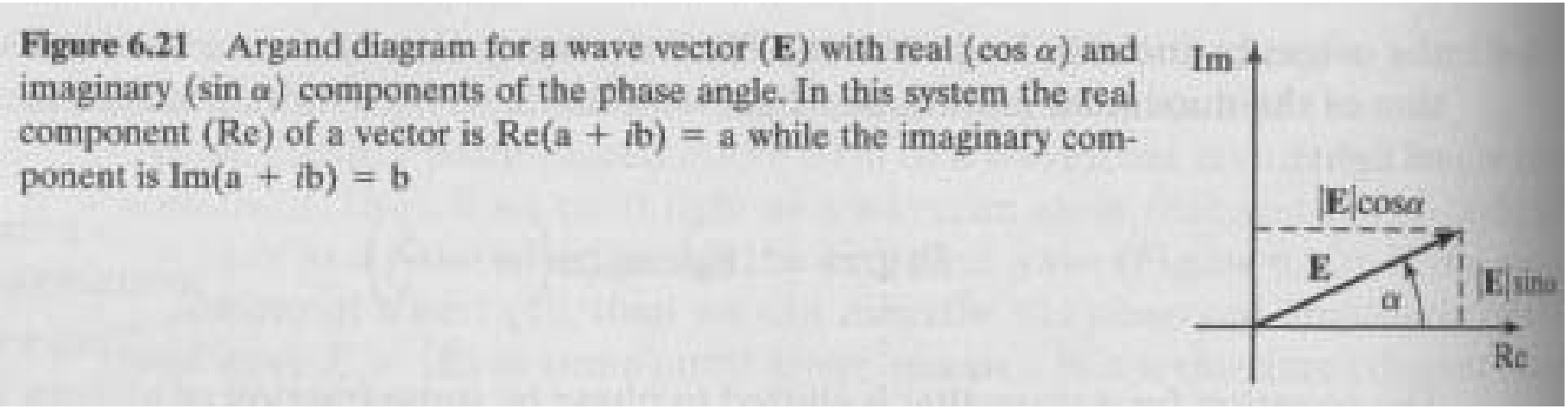
$$|E| = |E_o| \cos\omega$$

$$\boldsymbol{E = |E|\cos\alpha + i|E|\sin\alpha} \quad 6.33)$$

A wave can be represented vectorially in a system with one axis defined as the real component **cos**α and the orthogonal axis as the imaginary component **sin**α

Argand diagram

Argand Diagram



$$E = |E| \cos\alpha + i|E| \sin\alpha \qquad 6.33)$$

Express the scattering as the cosine & sine function in their exponential forms

$$|E| \cos\alpha + i|E| \sin\alpha = |E|\, e^{i\alpha} \qquad (6.36)$$

Propagation of Waves

$$|E|\cos\alpha + i|E|\sin\alpha = |E|\, e^{i\alpha} \qquad (6.36)$$

$$E1 = |Eo|\, e^{2\pi i (\upsilon t - x/\lambda + r1)} = |E|\, e^{2\pi i\, r1}$$

$$E2 = |Eo|\, e^{2\pi i (\upsilon t - x/\lambda + r2)} = |E|\, e^{2\pi i\, r2}$$

The relative positions of the two atoms in space can be defined as $\phi = |r_1 - r_2|$

$$E2 = |E_0|\, e^{2\pi i (r1 + \phi)}$$

$$= E_1\, e^{2\pi i \phi}$$

The observed amplitude for the scattering from the two atoms is simply the sum of the two waves

$$\boldsymbol{E = E_1 + E_2 = E_1 (1 + e^{2\pi i \phi})} \qquad (6.41)$$

ϕ =0 cycle, E = $2E_1$, in phase

ϕ =1/2 cycle, E = 0, out of phase

Atomic scattering factor (f)

If the two atoms are different types of elements, each atom will have a different number of electron occupying a given volume in space

Atomic scattering factor (f)

f_j defines the **maximum amplitude** of the scattered X-ray if that atom is placed at the origin of the unit cell (ϕ=0) and is **dependent only on the type of atom** that is scatterer.

$$f = f\, e^{2\pi i\, \delta}$$

r = $(x\,a + y\,b + z\,c)$) & the scattering vector (S) $\delta = \mathbf{S} \cdot \mathbf{r}$

$$f = f\, e^{2\pi i\, \mathbf{S}\cdot\mathbf{r}}$$

$$\delta = \mathbf{S} \cdot \mathbf{r}_j = (ha^*, kb^*, lc^*)\,(x_j\,a, y_j\,b, z_j\,c)$$
$$= (\,h\,x_j + k\,y_j + l\,z_j\,) \qquad (6.44)$$

Structure Factor (F)

For multiple atoms in a molecule of a unit cell, we simply add each of the atomic scattering vectors to give a summed vector called the molecular scattering factor "F"

Structure Factor (F)

The amplitude of each scattered beam of observed at specific values of the Miller indices (hkl).
The sum of the scattering by the separated atoms in the unit cell.
The total scattering from the unit cell.
It depends on the arrangement (structure) of the atoms in the unit cell.
A function of the scattering in reciprocal space
Written in terms of the electron densities in real space

$$F(hkl) = F(S) = \sum f_i = \sum f_i \, e^{[2\pi I \, r_j \cdot S]}$$
$$= \sum f_j [\cos(2\pi S \cdot r_j) + i \sin 2\pi (S \cdot r_j)]$$

f_j & F(S)

$$\alpha_{hkl} = \tan^{-1} \{ \Sigma f_j[\cos (2\pi S r_j)] / \Sigma f_j[\sin (2\pi S r_j)]\}$$

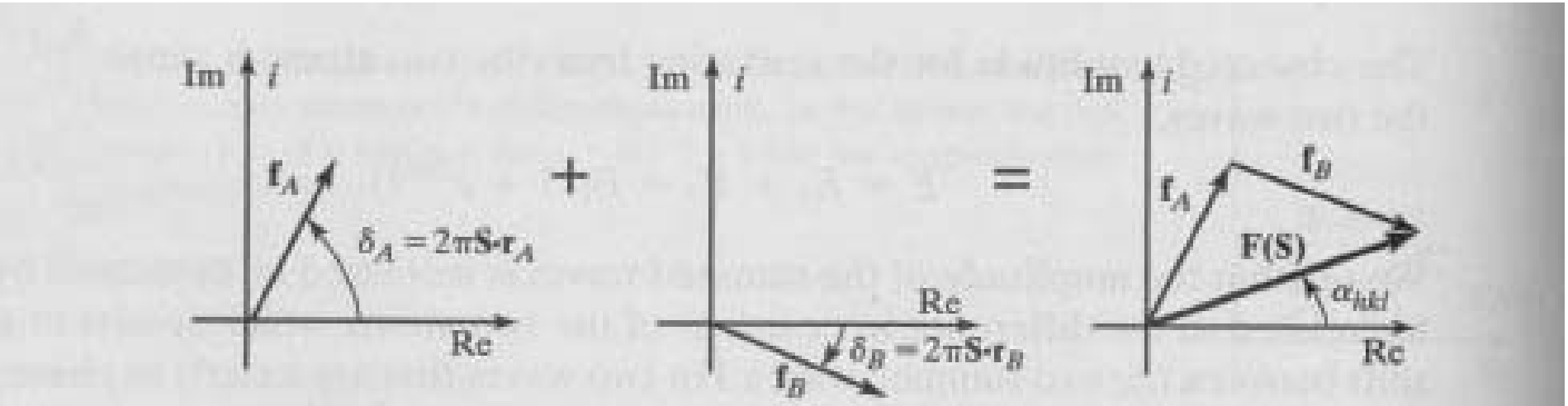


Figure 6.22 Adding the atomic scattering components of two atoms (f_A and f_B) to give the summed molecular structure factor, F(S). Both f_A and f_B have their own phase angles defined as δ_A and δ_B, and equal to $2\pi \mathbf{S} \cdot \mathbf{r}_A$ and $2\pi \mathbf{S} \cdot \mathbf{r}_B$. The resulting F(S) has an overall phase angle α_{hkl} that corresponds to the sum of the individual phases of f_j according to Eq. 6.48.

$$|F(hkl)| = [\Sigma f_j[\cos (2\pi S \cdot r_j)]^2 + [\Sigma f_j[\sin (2\pi S \cdot r_j)]^2$$

The real & imaginary components of individual ***f_j's*** can be summed separately to give the corresponding real & imaginary components of the overall ***F(S)***

Electron density, $\rho(\mathbf{r}) = \rho(x,y,z)$

X-ray are scattered by **electrons**

In quantum mechanics that electrons should be treated as a **probability** distribution in space

X-ray scattering is dep. on the electron density (r), **the number of electrons per unit volume**

At any point in the unit cell, **r**, there will be an electron density, $\rho(\mathbf{r}) = \rho(x,y,z)$

The electron density at any particular point in **real space**

Written in terms of the scattering vector in **reciprocal space**

Interpreting structural information from an electron density.

X-ray are scattered by electrons

, X-ray scattering is dep. on the electron density (ρ)

$$f_j = \int \int \int \rho(r)\, e^{2\pi i S r}\, dxdydz \qquad (6.49)$$

molecular structure factors is described by integrating over the volume (V) of the unit cell

$$F(S) = \int \int \int_V \rho(r)\, e^{2\pi i S r}\, dxdydz \qquad (6.50)$$

as a Fourier series

F(S) Is a function of the scattering in reciprocal space is written in terms of the electron densities in real space

Electron density (ρ) & Structure Factor (F)

X-ray are scattered by electrons, X-ray scattering is dep. on the electron density (ρ)

F(S) Is a function of the scattering in reciprocal space is written in terms of the electron densities in real space

$$F(S) = \int V \rho(r) \exp [2 \pi i \mathbf{S} \mathbf{r}] \partial V \qquad (6.50)$$

↓ **Fourier Transform**

$$\rho(r) = 1/V \int V^* \exp [-2 \pi i \mathbf{S} \mathbf{r}] F(S) \qquad (6.51)$$

ρ(r) Is a function that gives the electron density at any particular point in real space in terms of the scattering vector in reciprocal space

ρ(r)

Electron density at any particular point in real space

↔

F(S)

Scattering vector in reciprocal space

Electron density map (ρ)

$$\rho(r) = 1/V \int V^* \exp [-2 p i \mathbf{S} \mathbf{r}] \quad F(\mathbf{S}) \qquad (6.51)$$

V* is the volume element in reciprocal space and V is the real space volume of the unit cell

The electron densities can be calculated from a **sum** of the F(S) for all Miller indices (hkl).

$$\rho(\mathbf{r}) = 1/NV \sum \sum \sum \mathbf{F(hkl)} \ \exp [-2 \pi i \mathbf{S} \mathbf{r}] \qquad (6.52)$$

Electron density maps

Heme binding pocket of Myoglobin

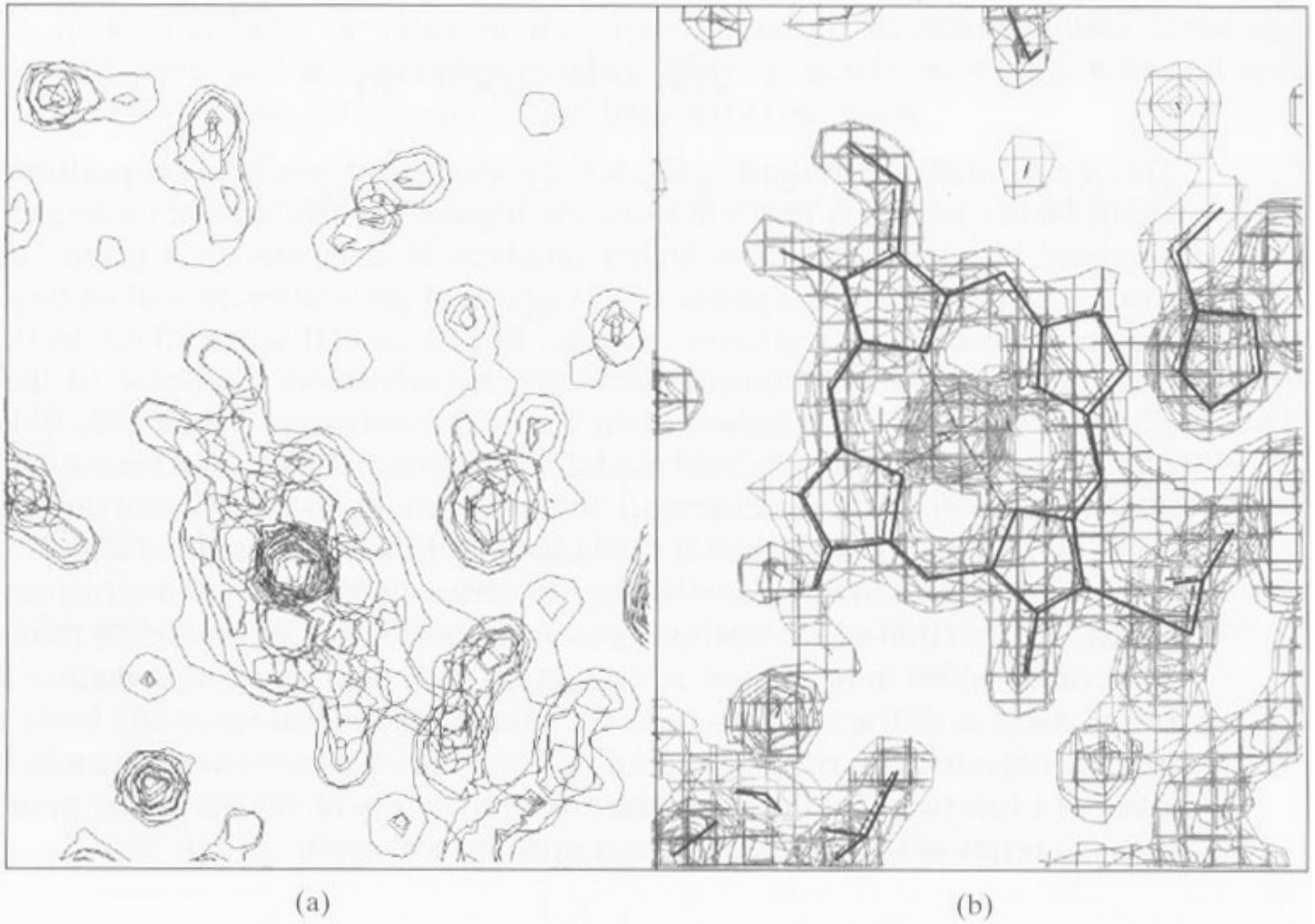


Figure 6.21 Electron density maps. The electron density calculated from the Fourier transform in Eq. 6.37 can be represented by (a) a contour map or as (b) a set of chicken wires. In this figure, the electron density of the heme binding pocket of myoglobin is shown. In (a), four sections of the contour map are overlapped to show the electron density at the heme and the surrounding amino acid residues. An enlarged view of this same set of electron densities are shown in (b) as surrounding the model of the heme (solid lines).

To plot the map as a set of contours, as in a geographical map

Each set of concentric contours represent peaks of electron density

6.5.2 The Phase Problem

Fourier series measured in the microwave region can be directly into the **NMR** spectrum.

Infarred absorption can be detected as a Fourier series that can be transformed directly into the **IR** spectrum.

Unfortunately, the devices that we have available to detect short-wavelength light measure total energy.

Intensity (I)

X-ray the intensity of a light wave is proportional to its amplitude E, **square**. Thus we have the amplitude information for each structure factor, but we **lost the phase information of the structure F(S)**

Intensity (I)

X-ray the intensity of a light wave is proportional to its amplitude E, **square**. Thus we have the amplitude information for each structure factor, but we **lost the phase information of the structure F(S), but not its direction**

$$\mathbf{I\,(hkl) = I(S) = |F(s)|^{2}}$$

$$\mathbf{= F(S)\,F^{*}(S)}$$

F*(S) is the complex conjugate of ***F(S)***

$$\boldsymbol{F^{*}(S)} = \sum f_i \, e^{-2\pi i \, S \, r_j}$$

The Phase Problem

$$I(hkl) = I(S) = |F(s)|^2$$

I(S) =I (-S)

A reflection at (hkl) has the same intensity as a reflection at (–h-k-l)

Determine F(S) from I (S), we lose critical information fro solving the structure of the molecule **$F(S) = |I(s)|^{½}$**

$|x|=4, \quad x= \pm 2$

We know the magnitude of x=2 but we do not know its sign this is known as the phase problem

Effect of shifting of the origin of the unit cell

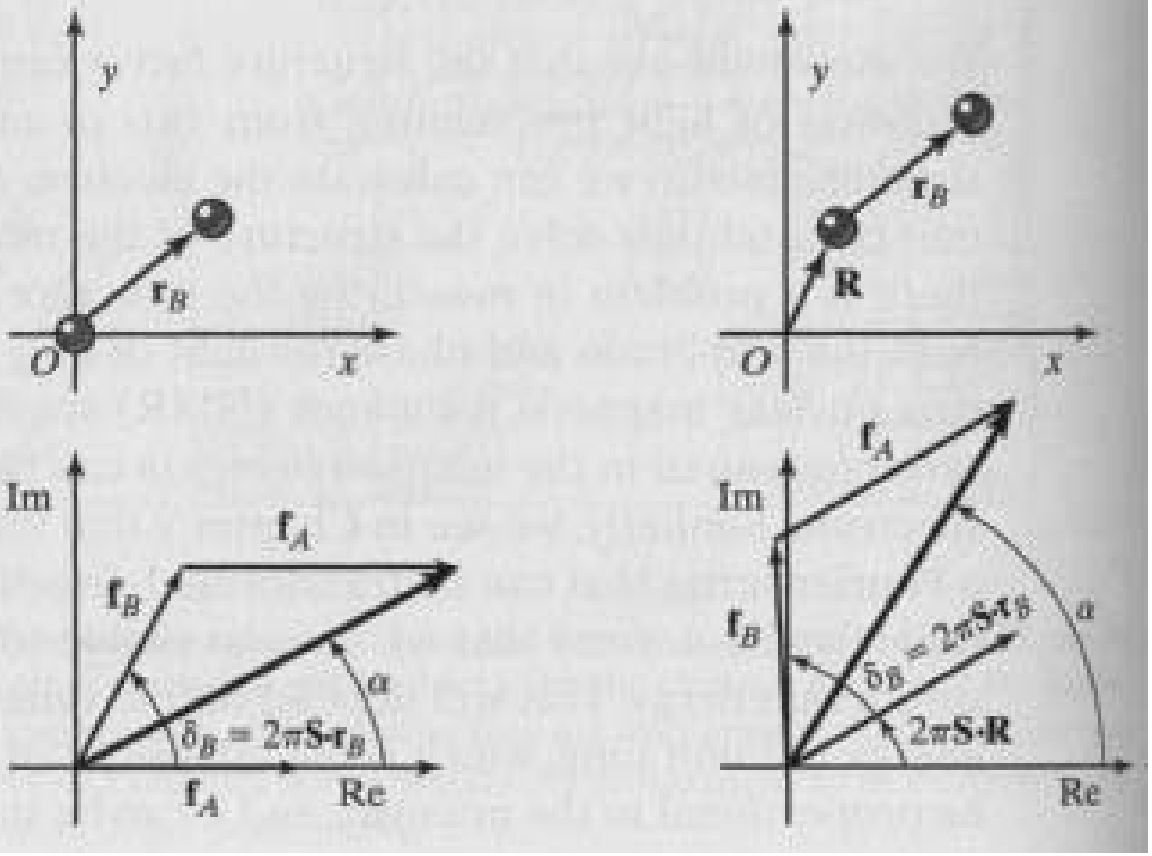


Figure 6.24 Effect of shifting the origin of the unit cell on the overall phase angle of two atoms. The left panels represent two atoms, with atom *A* at the origin of the unit cell and *B* displaced by **r** from *A* (*top left*), and the resulting atomic scattering vectors $\mathbf{f}_A$ and $\mathbf{f}_B$ to give a phase angle δ_B for atom *B* and α for the sum of the two atoms (*lower left*). When the origin of the unit cell is shifted, this is equivalent to shifting the position of the two atoms by some distance **R** (*upper right*). The result is that the phases of both atoms and the overall phase angle α are rotated by an additional angle $2\pi\mathbf{S}\cdot\mathbf{R}$ (*lower right*).

Origin shifting by an additional angles "2π S· R"

When the location of the unit cell is not known

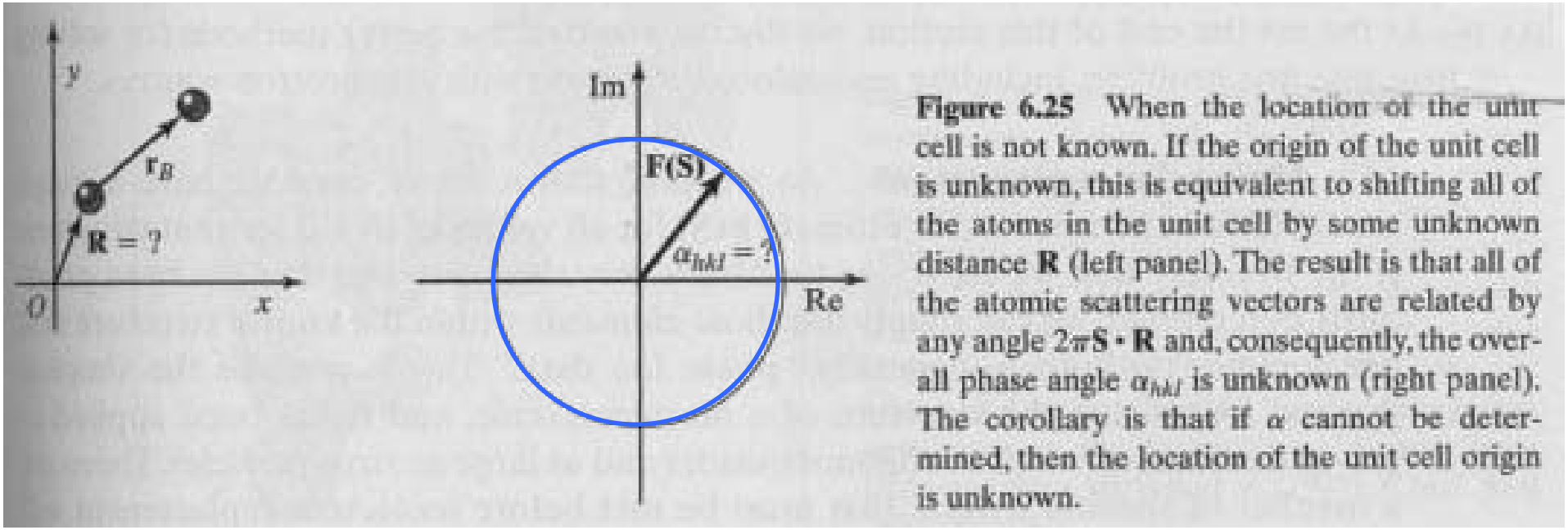
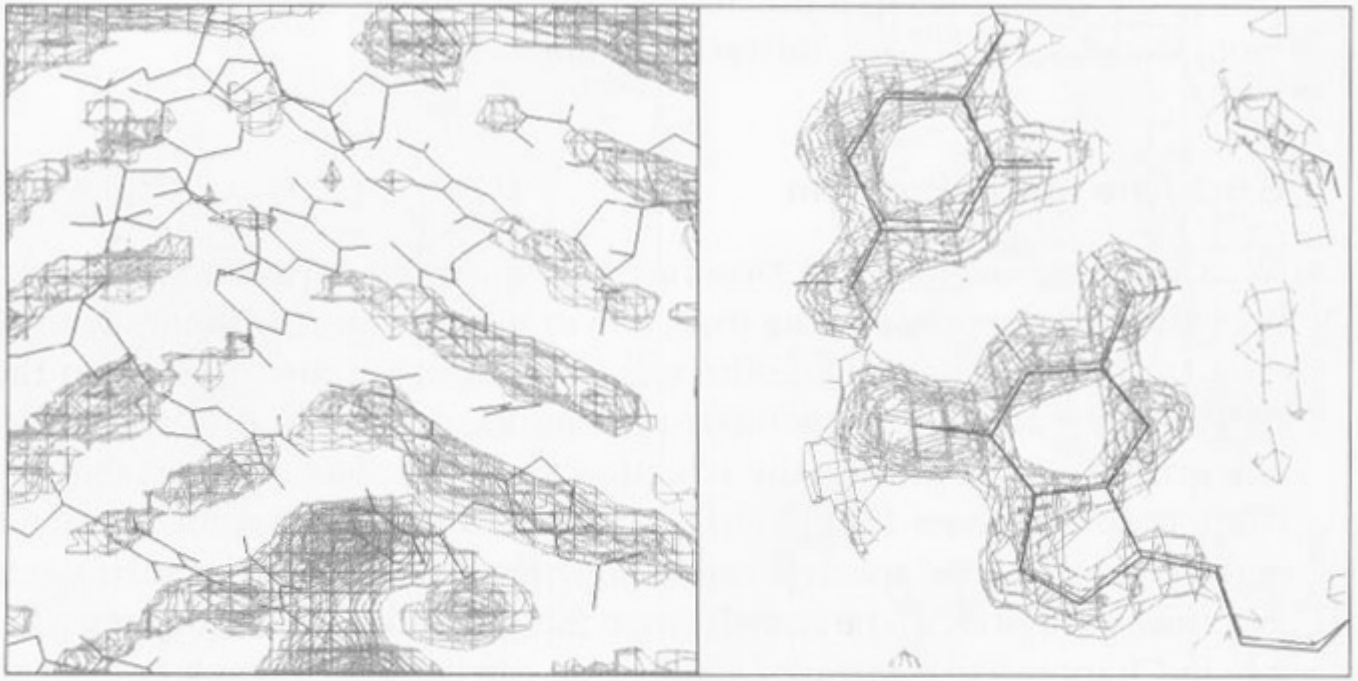


Figure 6.25 When the location of the unit cell is not known. If the origin of the unit cell is unknown, this is equivalent to shifting all of the atoms in the unit cell by some unknown distance **R** (left panel). The result is that all of the atomic scattering vectors are related by any angle $2\pi \mathbf{S} \cdot \mathbf{R}$ and, consequently, the overall phase angle α_{hkl} is unknown (right panel). The corollary is that if α cannot be determined, then the location of the unit cell origin is unknown.

Shifting all of the atoms in the unit cell by some unknown distance R and angle "**2π S · R**"

Electron density of dC-dG base pair

Wrong phase **Right phase**



(a) (b)

Fig 6.26 Electron density calculated from the two components of $F(h\ k\ l)$. In (a), the electron density of a DNA crystal was calculated using only $|F(h\ k\ l)|$ from the X-ray diffraction data. The map does not fit the model of the DNA structure, but resembles the pattern expected for the Patterson function. In (b), the same map was calculated using only the phase information for $F(h\ k\ l)$ with $|F(h\ k\ l)|$ set at 1.0 for all reflections. The resulting map very closely resembles the dC·dG base pair in the structure. This demonstrates the importance of the phasing information over the magnitude of the structure factor.

Methods for solving the phase problem

A. Direct method

B. Molecular replacement (MR)

C. Isomorphous replacement (MIR)

The Patterson Function

D. Multiple-wavelength anomalous dispersion (MAD)

(A) Direct method

(1) Trying all possible phase combination for each **S** and simply finding that combination that best fits the overall data to solve the structure

(2) Using the phase information for each atom inherent in the I data to retrieve some information concerning the relative positions of atoms in the crystal. (Patterson Function)

(3) Directly solve the structure of small molecules (100~300 atoms), the exponential growth in the phase problem as the size of the molecules increase.

(B) Molecular replacement

Using a model for a **known structure**, we can calculate F(**S**) for all values of (hkl) for that structure of **unknown** if both structures are **very similar** .

Accomplished by using a series of **rotation** and **translation functions** to fit the model to the electron density

•A mutant protein-native protein
•Homologous proteins from different species
•Double-helical Oligonucleotides

Omit map

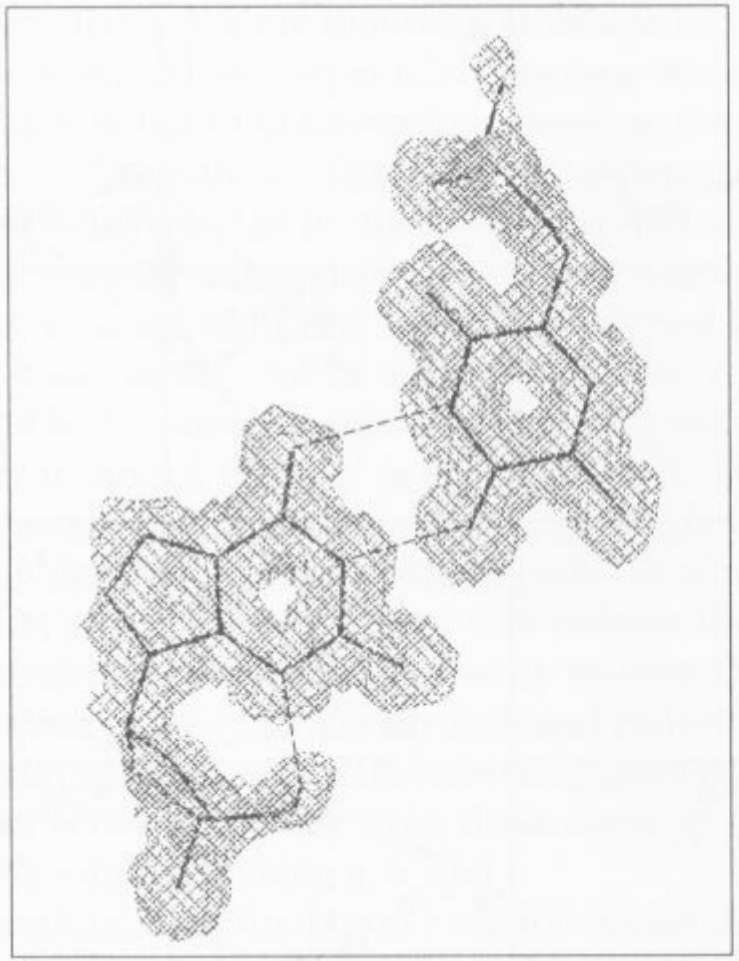


Fig. 6.27 The omit map calculated for the overhanging bases of the DNA fragment d(GCGCGCG)·d(TCGCGCG). The electron density was calculated using only the six dC·dG base pairs of the duplex region (underlined) to phase the data. The residual electron density that is not accounted for by the six base pairs is shown to be that of a dG·dT reverse-wobble base pair (the model is shown as solid lines, and the hydrogen bonds in the base pair as dashed lines). [Adapted from Mooers, et al. (1997), *J. Mol. Biol.* **269**, 796–810.]

It was calculated using only the 6 dC-dG base pair

The Patterson Function

Why do we not simply use the **observed intensities** to construct a fourier series that will be some function of the atomic position?

Correspond to the **vector difference** between the atomic positions

A very real indicator of this lost information is found in the

symmetry of a Patterson map

24 space group, removing all the **translational element** of the symmetry operators from the original crystal space group.

Centrosymmetric, therefore, there is always a symmetry axis at the origin

Patterson map corresponds to a **distance vector** separating two atoms

Usefully only for locating a small number of atoms within the unit cell

The Patterson Function

$$P(xyz) = 1/V \sum_h \sum_k \sum_l \mathbf{I(S)}\, e^{-2\pi i\, S\, rj}$$

$$= 1/V \sum_h \sum_k \sum_l \mathbf{F(S)\, F^*(S)}\, e^{-2\pi i\, S\, rj}$$

$$= 1/V \sum_h \sum_k \sum_l \mathbf{|F(hkl)|^2} \exp\left[-2\pi\, i(hx + ky + lz)\right]$$

If the transfrom of F(S) is $\rho(\mathbf{r})$

the transform F*(S) is $\rho(-\mathbf{r})$

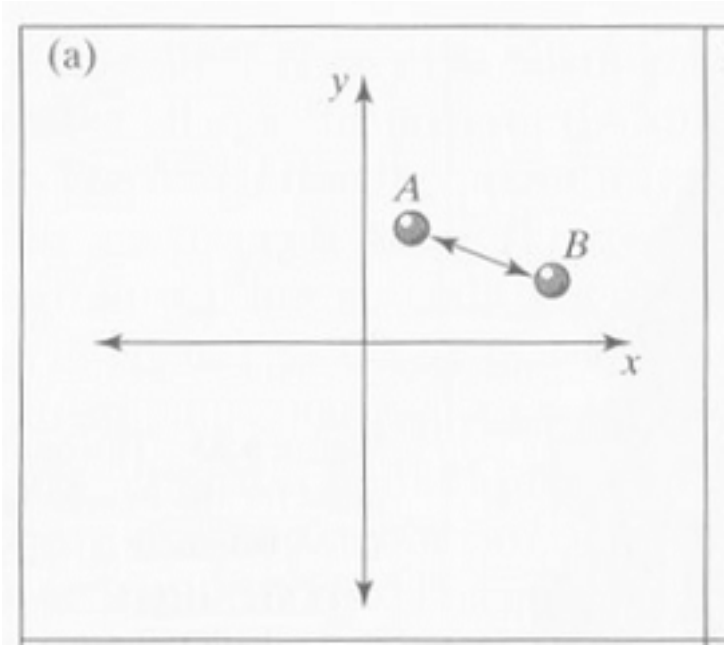
$$P(xyz) = \sum_j \sum_k \rho_j(r_j)\ \rho_k(-r_k)$$

Patterson Maps

2 atoms

Atom A, r_A

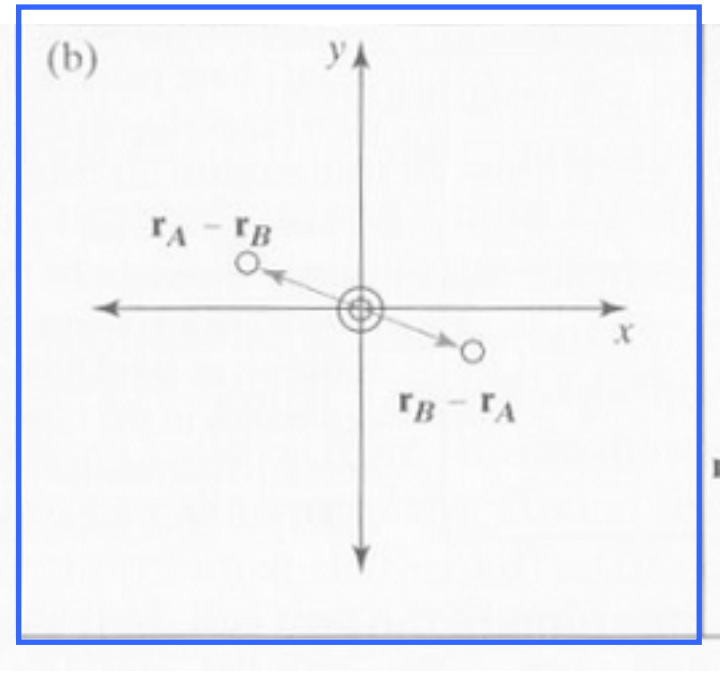
Atom B, r_B



2^2=4 peaks

2 for cross vectors

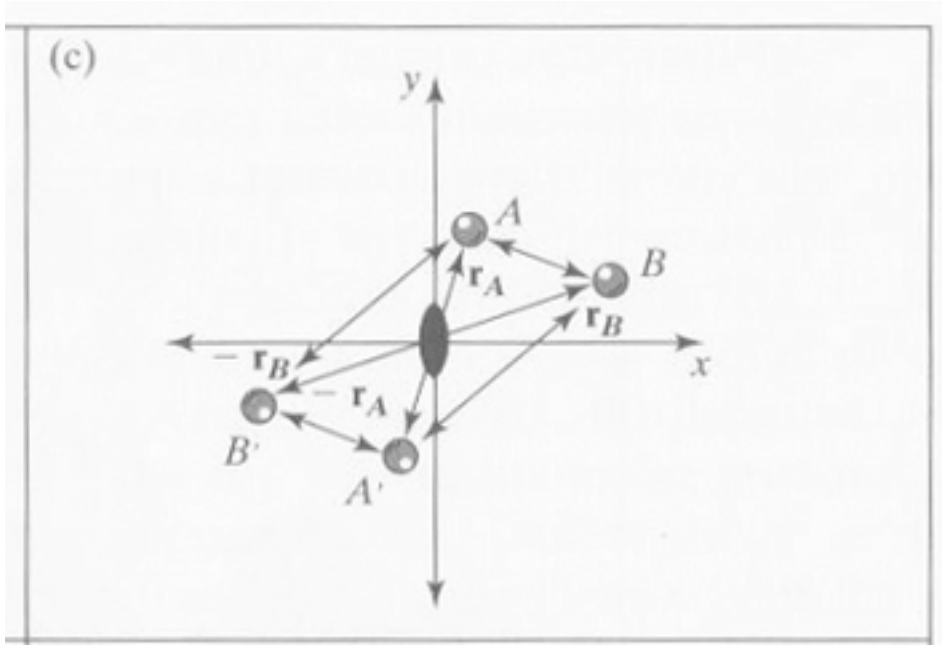
2 for self vectors



4 atoms

Atom A, r_A & A', r_A

Atom B, r_B & B', r_B

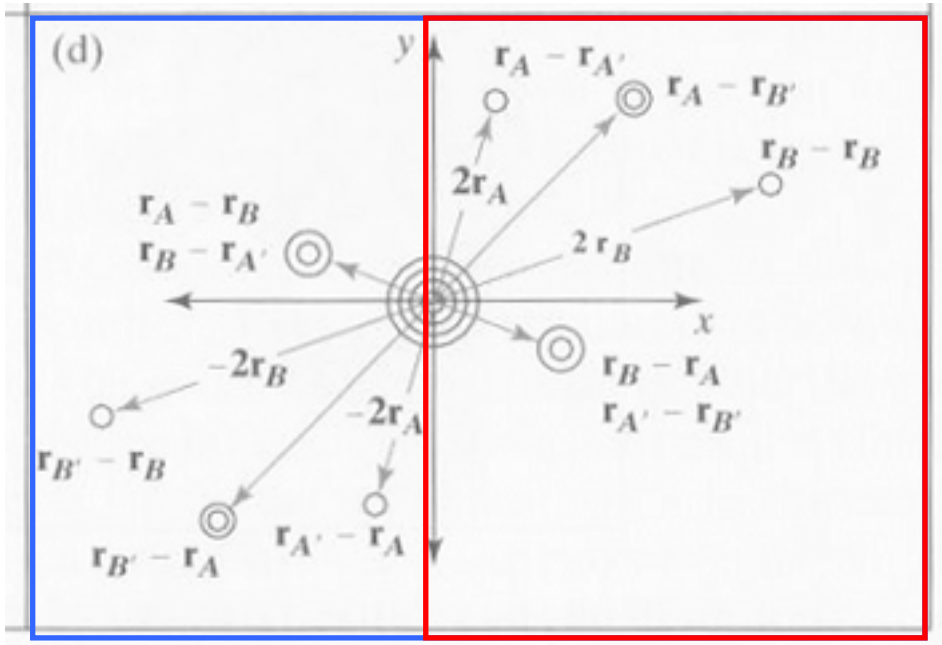


4^2=16 peaks

12 for cross vectors

1:1:2

4 for self vectors



Harker plane

Ex: **Two-fold screw, 2_1 , $(P\ 2_1)$**

A(x, y, z) & A'(x+1/2, –y, -z)

different vector of AA' is (**1/2**, 2y, 2z)

Patterson peak, (2y, 2z)

The absolute coordinates y and z of atom A can be determined directly from patterson peak in the **Harker plane** (x=1/2)

Patterson map of B-DNA

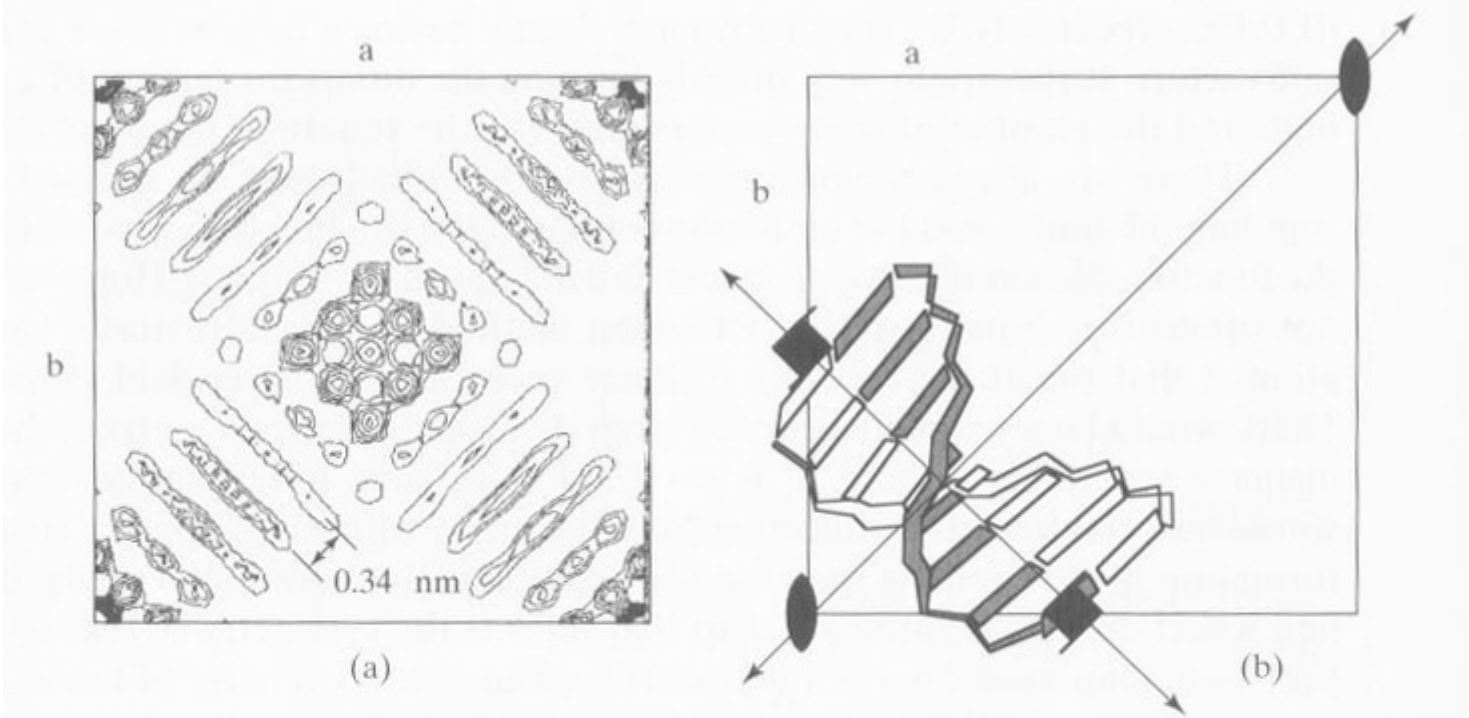
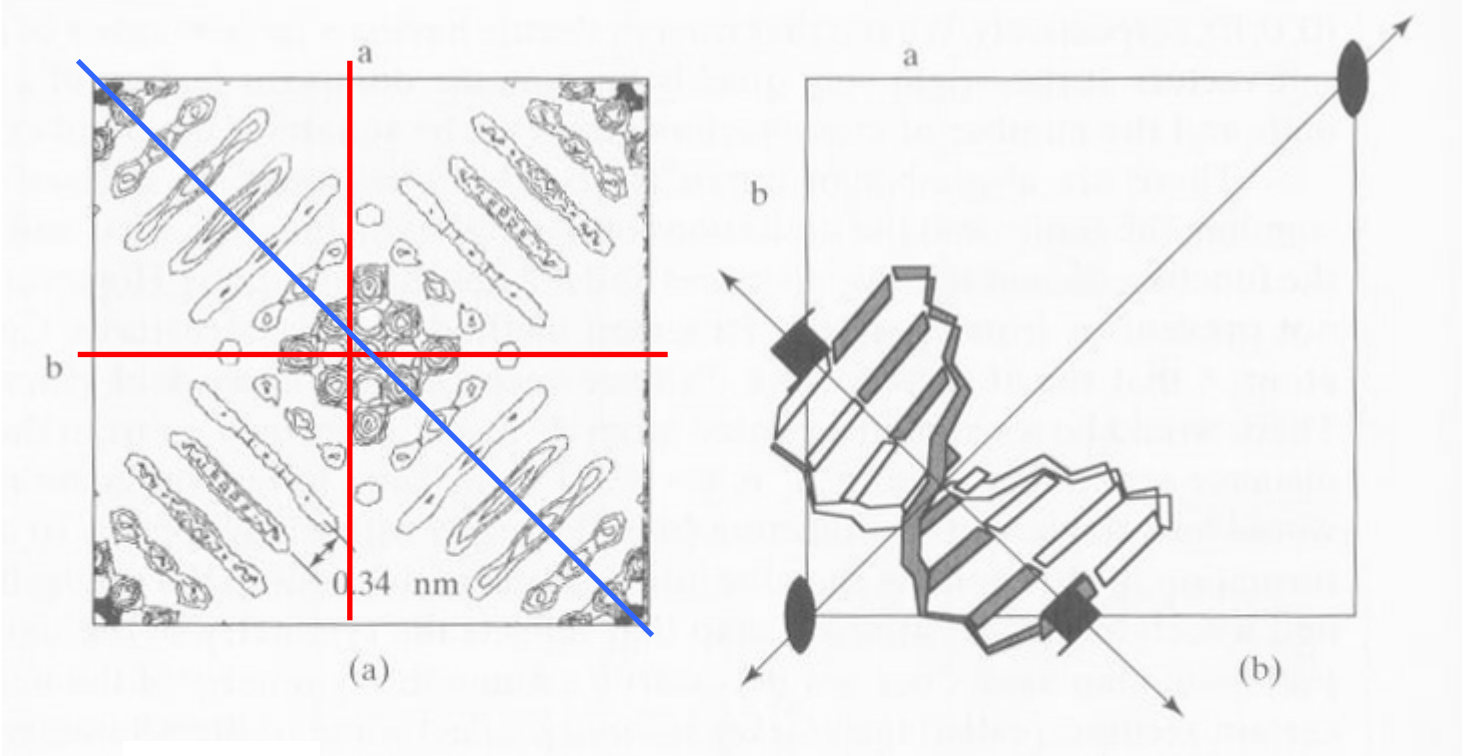


Fig. 6.29 Patterson map of B-DNA. (a) The Patterson map of an eight base pair duplex DNA in a tetragonal crystal shows regular densities spaced by 0.34 nm. The duplex must therefore be B-DNA, with the helical axis lying in the plane and aligned diagonal to the crystallographic axes **a** and **b**. (b) The asymmetric unit of this fragment is one strand of the duplex. The second strand is generated by two-fold rotation. This automatically places the asymmetric unit on the two-fold axis of the crystal and allows the structure to be solved entirely from the Patterson map and the symmetry of the crystal lattice.

Patterson map of B-DNA

8 base pair duplex DNA in tetragonal crystal



Regular densities of spacing of 0.34 A duplex B-DNA
The helical axis lyining the plan and aligned diagonal to the a & b axis
Asymmetric unit one strand of the duplex
The second strand is generated by 2 fold rotation
Allows the structure to be solved entirely fro mthe Patterson map and the symmetry of the crystal lattice

(C) Multiple Isomorphous Replacement (MIR)

Heavy atom method

Heavy atoms with high electron densities can strongly perturb the X-ray diffraction pattern.

Once the positions of these heavy atoms are located within the crystal, the overall phase of the original molecule can be estimated.

DNA or RNA fragments
Brominated or **iodinated** nucleotides (5-bromocytosine or 5-iodouridine)
Proteins
Soaking any heavy atoms

TABLE A6.3 CRYSTALLOGRAPHIC DATA AND RESULTS FOR UREASE

Crystal	Resolution of Data	Number of Unique Reflections	Final *R* factor	Nonhydrogen Protein atoms	Solvent Molecules
Native	2 Å	58,334	18.5%	6002	215
Apoenzyme	2.8 Å	20,532	18.4%	5944	157
$HOHgC_6H_4CO_2Na$	3.3 Å	11,027			
$EuCl_2$	3.3 Å	12,210			
$Hg_2(CH_3COO)_2$	2.5 Å	28,709			
$C(HgOOCCH_3)_4$	2.4 Å	29,672			
$(CH_3)_3Pb(CH_3COO)$	2.4 Å	23,486			
Se-Met	3.0 Å	20,332			

Data from Jabri et al. (1995).

Quaternary structure of Urease "αβγ"

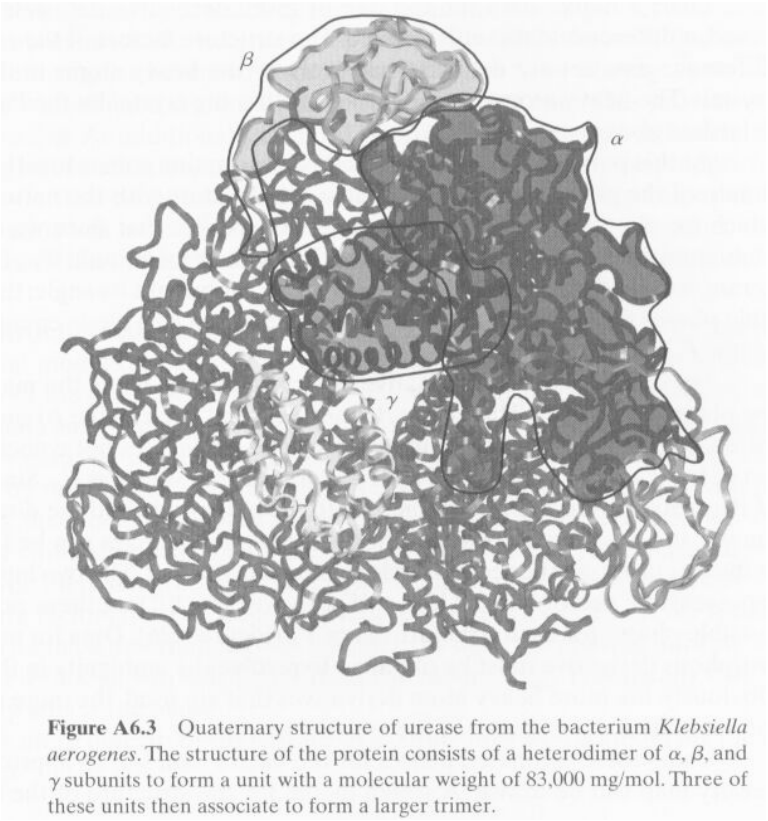


Figure A6.3 Quaternary structure of urease from the bacterium *Klebsiella aerogenes*. The structure of the protein consists of a heterodimer of α, β, and γ subunits to form a unit with a molecular weight of 83,000 mg/mol. Three of these units then associate to form a larger trimer.

TABLE 6.4 HEAVY ATOM DERIVATIVES FOR MACROMOLECULAR CRYSTALS

Heavy atom	Specificity
Proteins	
$AgNO_3$	His, Cys (minor)
$K_2Pd(Br \text{ or } Cl)_4$	Arg, His
Hg acetate	His, Cys
p-chloromercuric benzene sulphonate (PCMBS)	His
Se	Selenomethionine (incorporated during synthesis)
Nucleic Acids	
Cu	Guanine bases
Pt	Guanine bases
I	Iodouridine (incorporated during synthesis)
Br	Bromouridine (incorporated during synthesis)

(C) Multiple Isomorphous Replacement (MIR)

1. Native crystal, Native data set, $\mathbf{F_P}$
2. Isomorphous crystals-heavy atom derivative crystal, $\mathbf{F_{PH}}$
3. Make a difference data set, $\mathbf{F_H}$
4. The $\mathbf{F_H}$ are used to determine the positions and the phases of the heavy atoms in the unit cell
5. This process is repeated for at least one additional heavy atom derivative
6. The phases of at least two heavy atom derivatives are used to estimate the phase for the native data set to solve the structure of the macromolecule in the native crystal

Estimating phases from Multiple Isomorphous Replacement

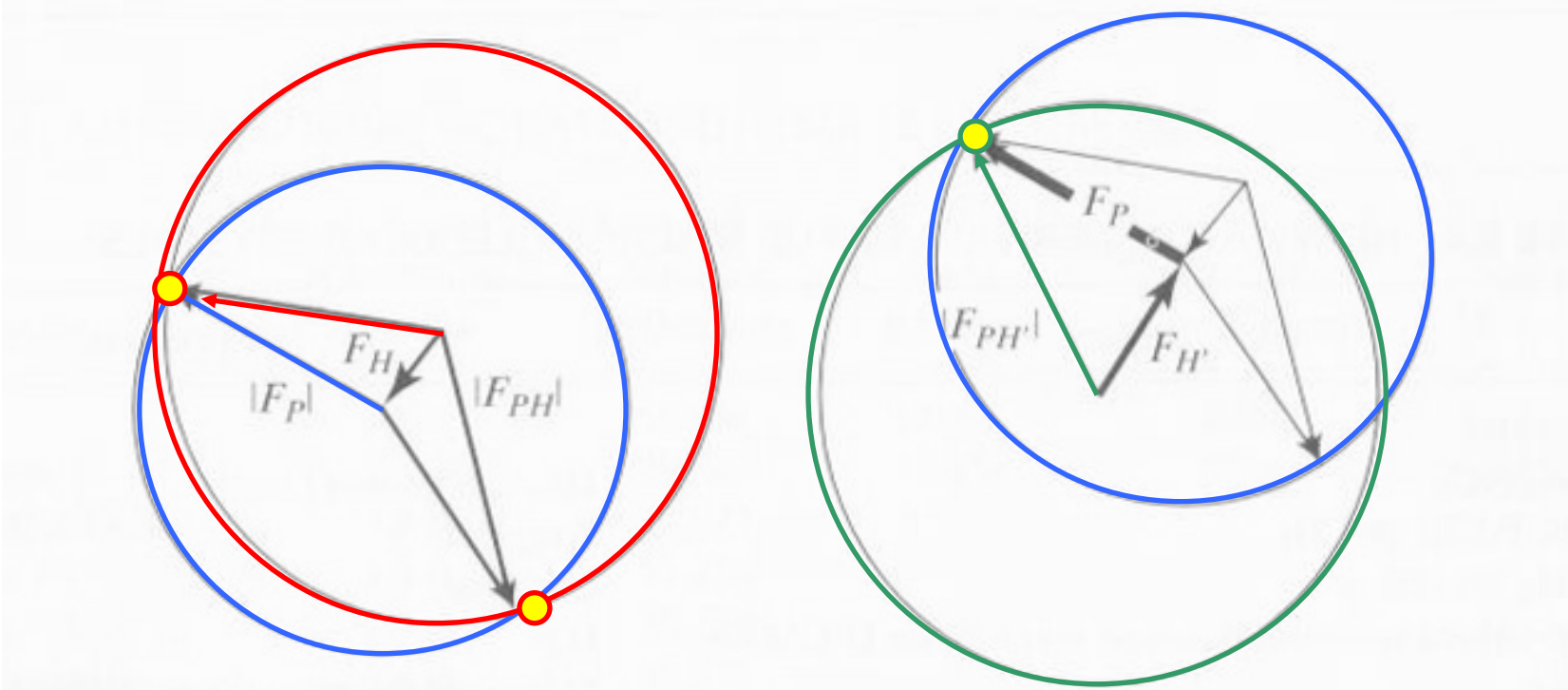


Fig 6.30 Estimating phases from multiple isomorphous replacement. The magnitude of the structure factors for the native protein $|F_P|$ and one heavy atom derivative $|F_{PH}|$ each define circles for all possible phases of the reflections. The structure factor F_H of the the heavy atom derivative shifts the two circles relative to each other. The intersection of the two circles defines two possibilities for F_P. A second heavy atom derivative H' is used to distinguish between the two possible phases for F_P.

R-factor: a criterion of a good fit of the molecule to the data

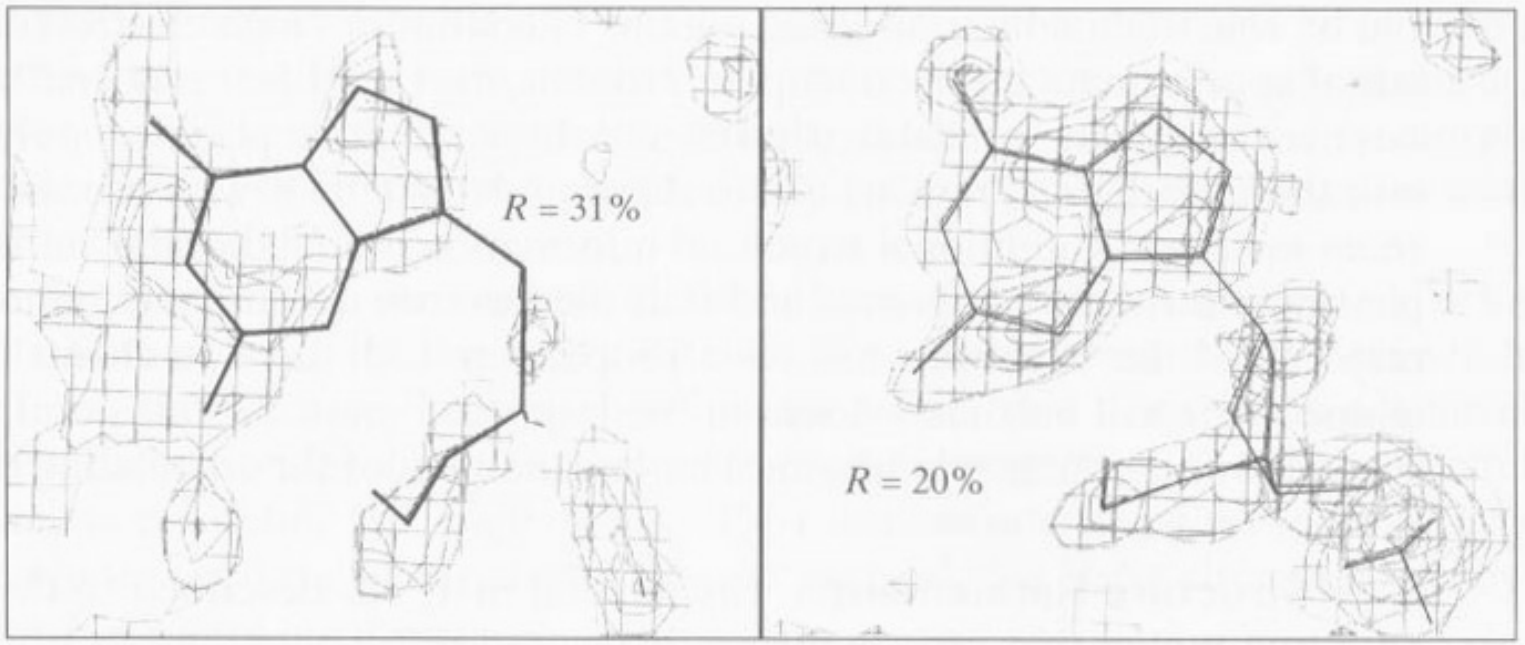


Fig.6.317 Effect of refinement on structure. The guanine nucleotide of a DNA fragment is shown with its electron density map prior to refinement and after refinement. Prior to refinement, the *R* factor is 31%. The structure is refined against the data to an *R* factor of 20%, which is the criterion of a good fit of the model to the data.

Structure Refinement

Initial model fits the measured diffraction data

Compare the observed & calculated structure factor

70% for a random fit, 0% for an ideal fit

For macromolecules, 20% indicates a good fit.

$$R = \frac{\sum \left| \left| F_{(hkl)} \right| - \left| F_{calc} \right| \right|}{\sum \left| F_{(hkl)} \right|}$$

Other Methods for Phasing X-ray Diffraction Data

*Anomalous dispersion

Atoms with high electron densities not only scatter X-ray, they also absorb X-rays, and it is near its absorption edge

Breakdown the Friedel's law ($\mathbf{f_+ = f_-}$), the difference in intensities between Friedel pairs can be used to determine the phase of heavy atoms

$$\mathbf{f_+ = f_o + f_+' + i\, f_+''}$$

$$\mathbf{f_- = f_o - f_-' - i\, f_-''}$$

$$\mathbf{f_+ \neq f_-}$$

Anomalous dispersion effects on the atomic scattering factor

$f_+ \neq f_-$

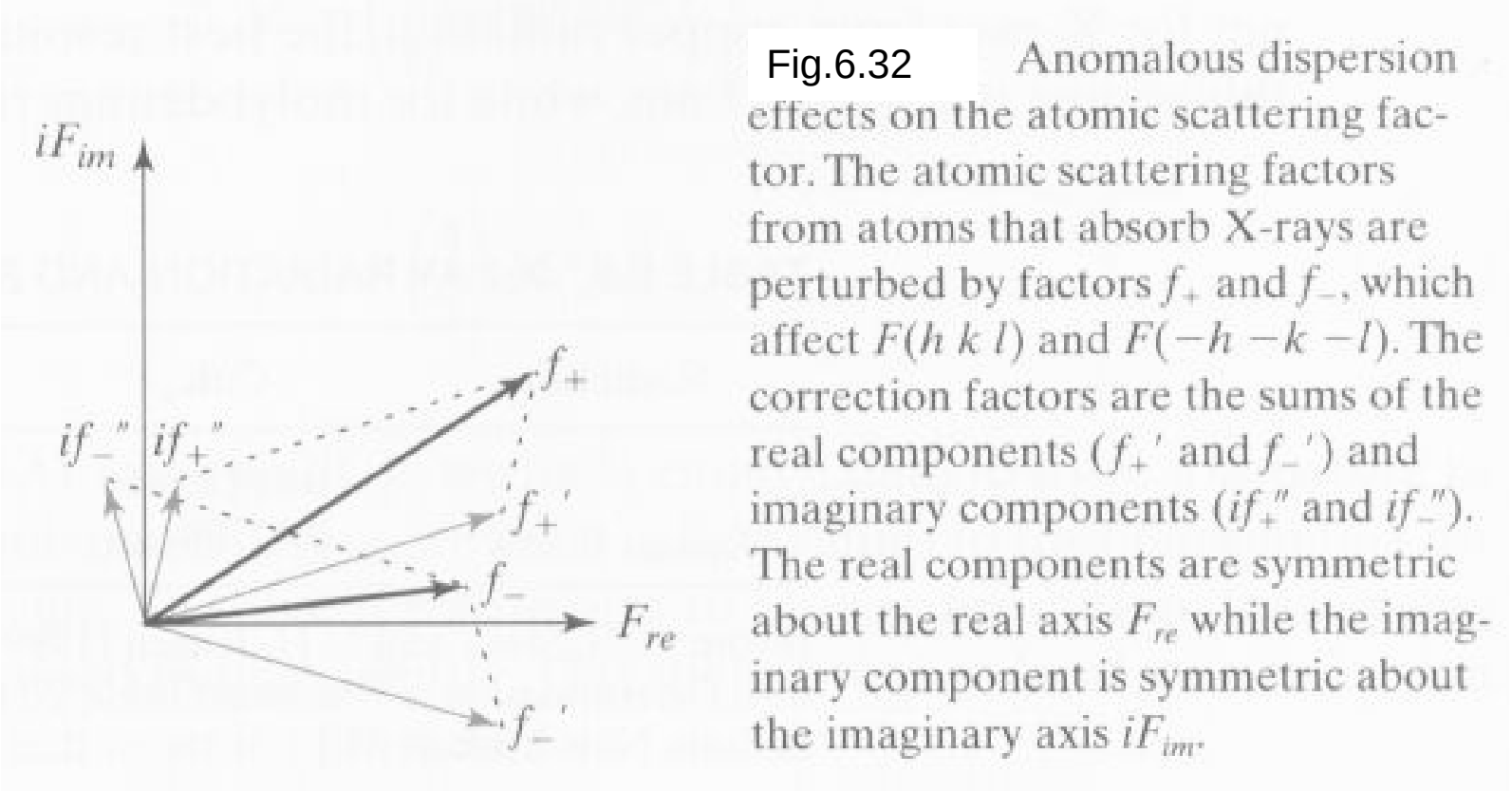


Fig.6.32 Anomalous dispersion effects on the atomic scattering factor. The atomic scattering factors from atoms that absorb X-rays are perturbed by factors f_+ and f_-, which affect $F(h\ k\ l)$ and $F(-h\ -k\ -l)$. The correction factors are the sums of the real components (f_+' and f_-') and imaginary components (if_+'' and if_-''). The real components are symmetric about the real axis F_{re} while the imaginary component is symmetric about the imaginary axis iF_{im}.

MAD experiment by Br at 1.6 Å

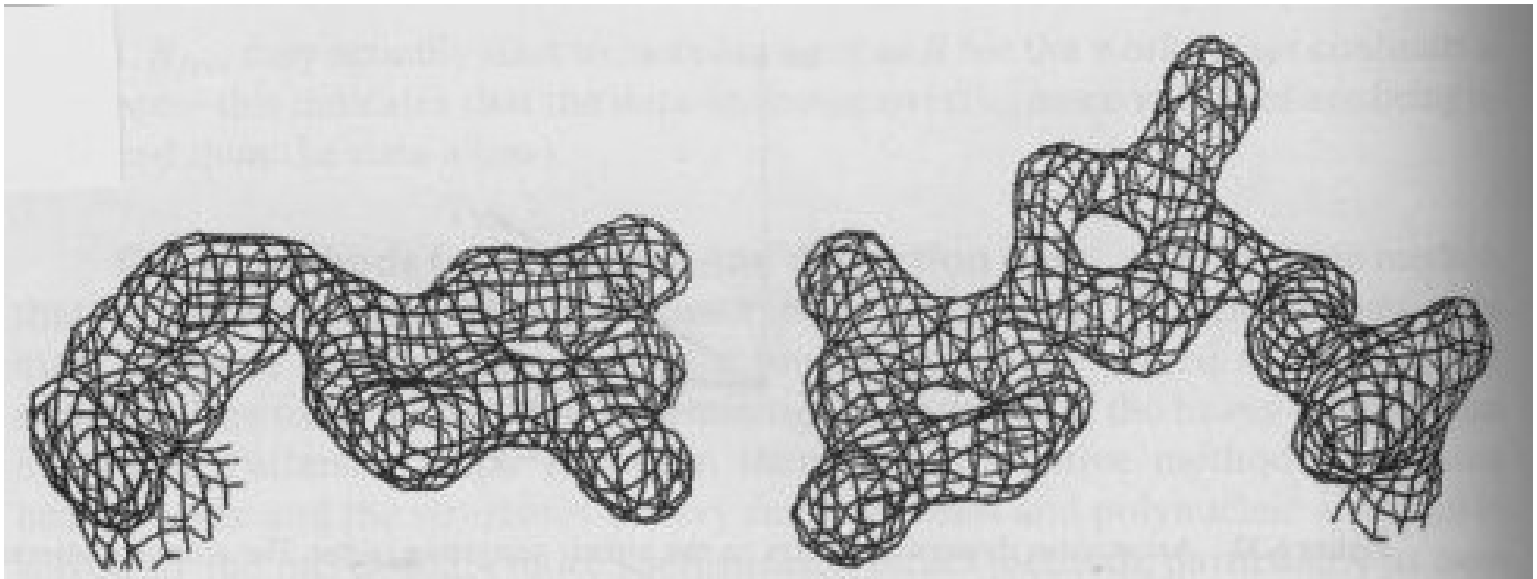


Figure 6.33 Experimental electron density map phased by a multiple-wavelength anomalous dispersion (MAD) experiment at 0.16 nm resolution. Crystals of the sequence d(GGbr5CGCC) was phased using the 5-bromocytosine in the sequence and wavelengths of 0.092052 nm (absorption of bromine), 0.092065 nm (inflection point for absorption), and 0.090836 nm (a wavelength that is remote from the absorption edge). The experimental electron density is shown for one of the 24 base pairs in the asymmetric unit of the crystal.

Absorption of Br at 0.092052 nm

Inflection for absorption of Br at 0.090836 nm

Remote from absorption of Br at 0.092065 nm

Multiple-wavelength anomalous dispersion (MAD)

1) The closer the wavelength of this radiation is to the **absorption edge** of the scattering atom, the stronger the anomalous dispersion.

2) Two different wavelengths result in 2 different values of **i f”** , which in turn gives us 2 different pieces of phase information from the heavy atom

3) This is the same as having **2 independent heavy atom derivatives**

4) The phase information is not as strong as with 2 derivatives with truly different atomic coordinates

Structure model and diffraction data

The best resolution $2d \sin\theta = n\lambda$ **(λ=1.54A , for CuKα radiation**)

Sin $\theta = 1$, $\theta = 90^o$, $2\theta = 180^o$, d = 0.077 nm,

The highest resolution cannot be collected

$2\theta \sim 110^o$, $\sin\theta = 0.82$, d=0.094 nm,

The highest resolution is 0.94 Å

Four parameters are need (x, y, z) & B temperature factor

B factor (temperature factor):

The thermal motion of the atom

Higher B, electron occupy a larger volume

Isotropic/anisotropic

<60

Partial occupancy (0-1)

Reflects the overall disorder of the atom.

6.5.4 resolution in X-diffraction

TABLE 6.5 X-RAY RADIATION AND RESOLUTION

Radiation	CuK_α	MoK_α
λ	0.15418 nm	0.07107 nm
$(d_{hkl})_{min} = \lambda/2$	0.07709 nm	0.03554 nm

[From G. H. Stout and L. H. Jensen (1989), *X-Ray Structure Determination, a Practical Guide,* 2d ed., John Wiley & Sons, New York, p. 37.]

How much data is required

$$N = (4/3)\,\pi\, V / d^3$$

Ex: 350 atoms, crystal volume of 6 nm^3, protein A, d = 0.26nm (2.6A) or $2\theta = 34^o$

N=1429 refs required

$2d\sin\theta = n\lambda$, $\theta = 17^o$, $2\theta = 34^o$

Ex: xtal B, crystal volume=25nm^3, at 0.1nm (1A) resolution,

104,720 refs are required for **P1**, the lowest symmetry,

unique refs=52,360 are required [unique refs, F(hkl)=F(-h,-k,-l)]

For higher symmetry, **P212121**

Unique refs: 52,360/**4**=13,090 are required

6.6 Fiber Diffraction

Fiber diffraction of B-DN

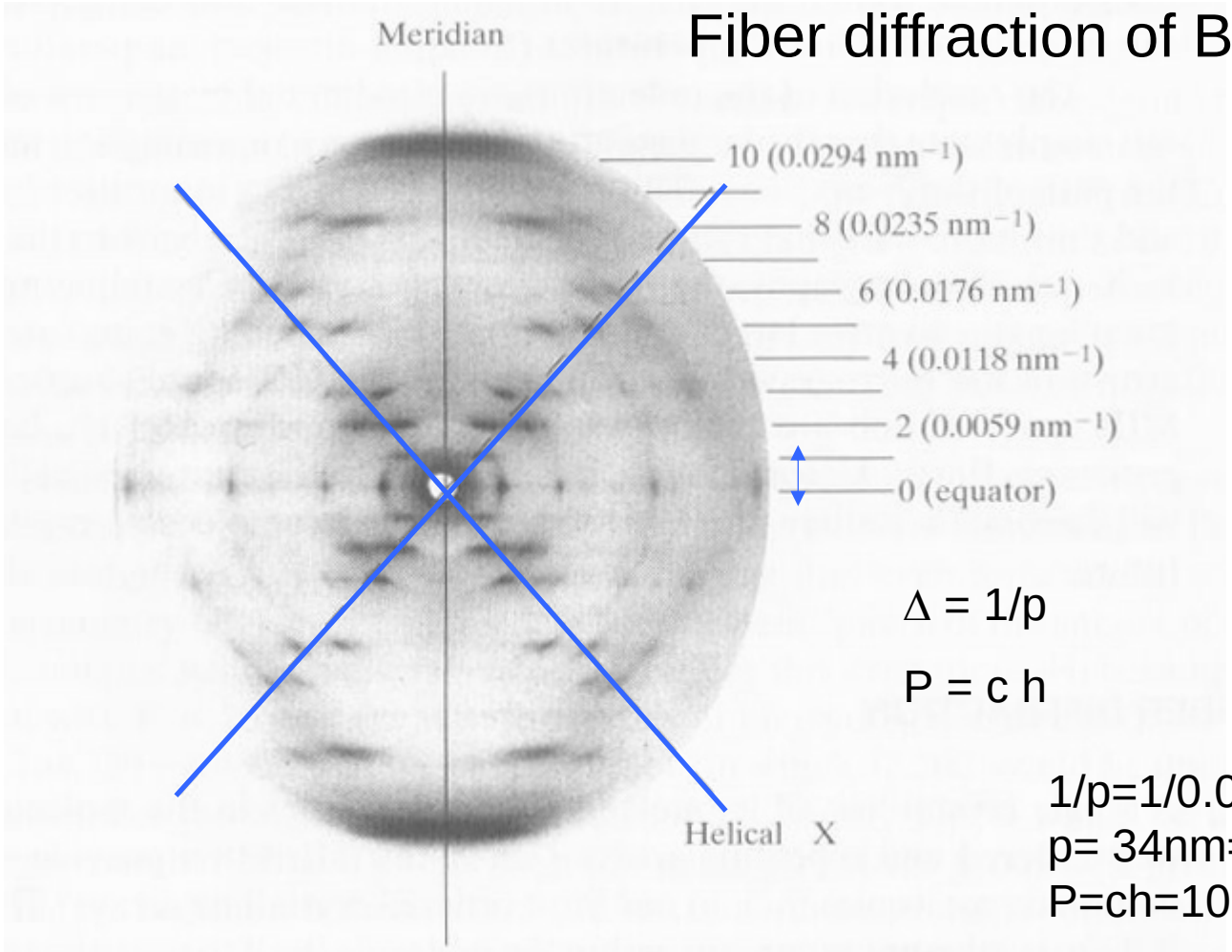


Fig.6.34

Figure 6.29 The fil n photograph of B-DNA. The diffraction photograph of the lithium form of a DNA fiber (recored at 66% humidity) shows the helical X expected for helical structures and 10 layer lines spaced according to n/P in nm^{-1} between the origin and the exact repeat of the pattern. This indicates that the fiber is B-DNA. [Courtesy of R. Langridge].

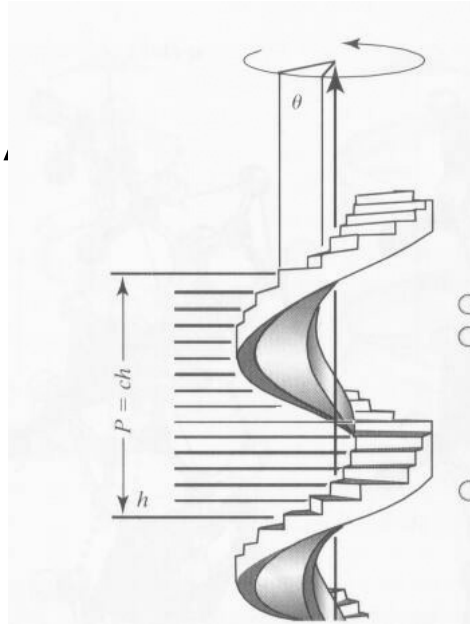


Figure 1.22 Spiral staircase and helix. A sp
discrete residues of a helix. Each step is ana
biopolymer helix, and can be described by
pitch (P), repeat (c), and twist (θ).

$\Delta = 1/p$

$P = c\ h$

$1/p=1/0.0294nm^{-1}$
p= 34nm=340Å
P=ch=10h, h=3.4Å

6.6.1 The Fiber Unit Cell

The packing of the symmetric unit (helices) in the fiber of a biopolymeris essentially the packing of infinitely long cylinders
A series of stacked repeating cylinders not a box
The unit cell in cylindrical coordinates

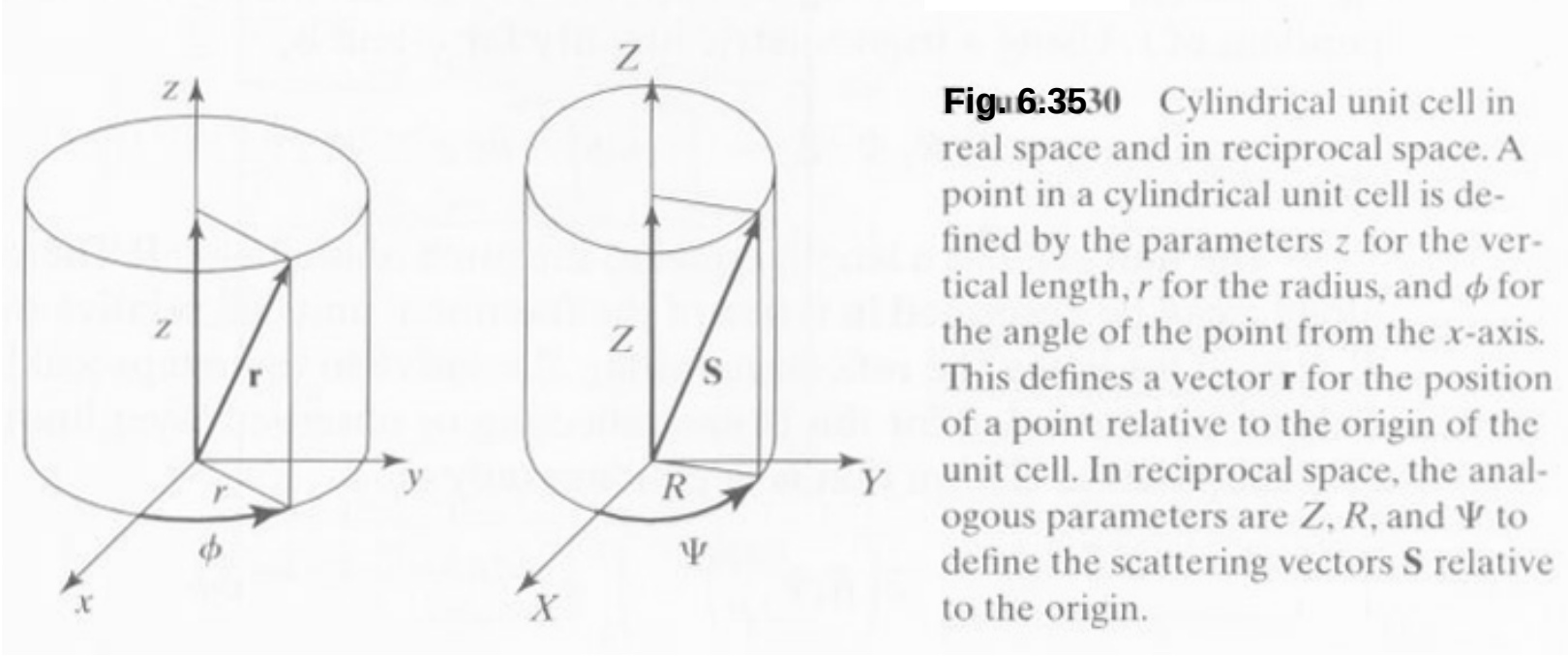


Fig. 6.35 Cylindrical unit cell in real space and in reciprocal space. A point in a cylindrical unit cell is defined by the parameters z for the vertical length, r for the radius, and ϕ for the angle of the point from the x-axis. This defines a vector **r** for the position of a point relative to the origin of the unit cell. In reciprocal space, the analogous parameters are Z, R, and Ψ to define the scattering vectors **S** relative to the origin.

Real Space

Reciprocal Space

6.6.2 Fiber Diffraction of Continuous Helices

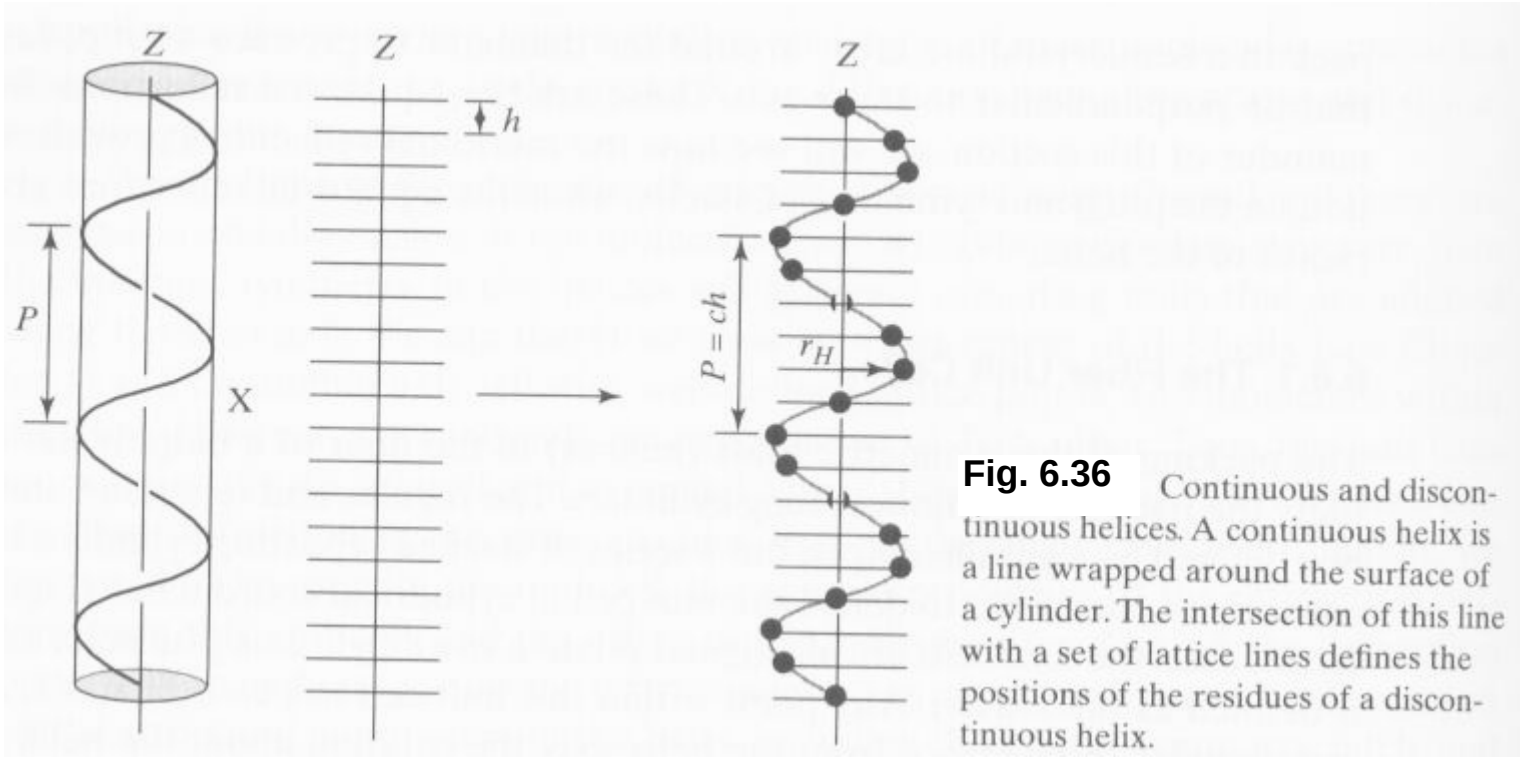


Fig. 6.36 Continuous and discontinuous helices. A continuous helix is a line wrapped around the surface of a cylinder. The intersection of this line with a set of lattice lines defines the positions of the residues of a discontinuous helix.

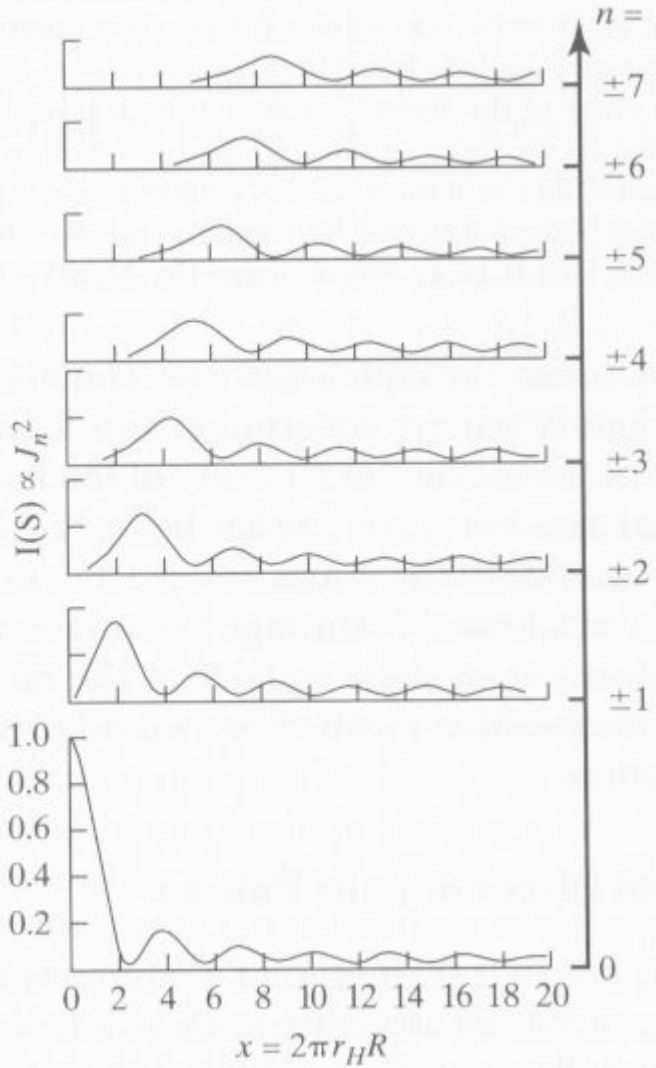


Fig. 6.37 Bessel functions, J_n. Bessel functions are shifted to higher values of x and are reduced in intensity J_n^2 as the order of the function n is increased. The intensity $I(\mathbf{S})$ along the layer lines in a fiber diffraction pattern is proportional to J_n^2.