Table 2.1.3.4. *The seven crystal systems*

Crystal system	Conditions imposed on cell geometry	Minimum point-group symmetry
Triclinic	None	1
Monoclinic	Unique axis b: $\alpha = \gamma = 90°$	2
Orthorhombic	$\alpha = \beta = \gamma = 90°$	222
Tetragonal	$a = b$; $\alpha = \beta = \gamma = 90°$	4
Trigonal	Hexagonal axes: $a = b$; $\alpha = \beta = 90°$; $\gamma = 120°$	3
	Rhombohedral axes: $a = b = c$; $\alpha = \beta = \gamma$ *	
Hexagonal	$a = b$; $\alpha = \beta = 90°$; $\gamma = 120°$	6
Cubic	$a = b = c$; $\alpha = \beta = \gamma = 90°$	23

* A rhombohedral unit cell can be regarded as a cube extended or compressed along the body diagonal (the threefold axis) (see Fig. 2.1.3.2).

occur either as primitive unit cells or as centred unit cells (Section 2.1.1). A total of 14 different types of unit cell exist, depicted in Fig. 2.1.3.3. Their corresponding crystal lattices are commonly called Bravais lattices.

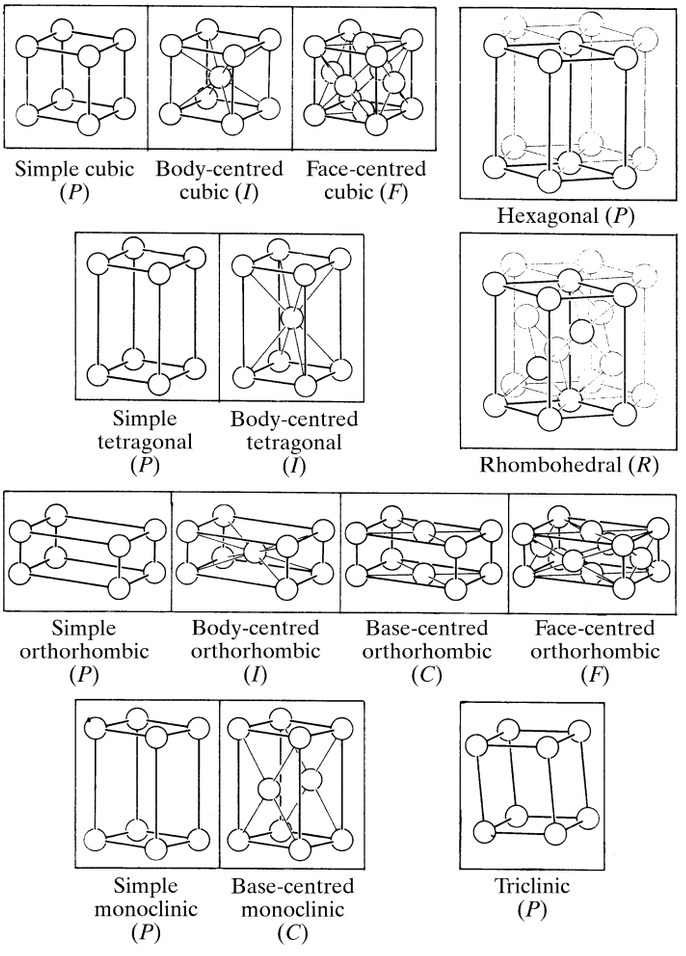

Fig. 2.1.3.3. The 14 Bravais lattices. Reproduced with permission from Burzlaff & Zimmermann (1995). Copyright (1995) International Union of Crystallography.

2.1.4. Basic diffraction physics

2.1.4.1. *Diffraction by one electron*

The scattering of an X-ray beam by a crystal results from interaction between the electric component of the beam and the electrons in the crystal. The magnetic component has hardly any effect and can be disregarded.

If a monochromatic polarized beam hits an electron, the electron starts to oscillate in the direction of the electric vector of the incident beam (Fig. 2.1.4.1). This oscillating electron acts as the aerial of a transmitter and radiates X-rays with the same or lower frequency as the incident beam. The frequency change is due to the Compton effect: the photons of the incident beam collide with the electron and lose part of their energy. This is inelastic scattering, and the scattered radiation is incoherent with the incident beam. Compton scattering contributes to the background in a diffraction experiment. In elastic scattering, the scattered radiation has the same wavelength as the incident radiation, and this is the radiation responsible for the interference effects in diffraction. It was shown by Thomson that if the electron is completely free the following hold:

(1) The phase difference between the incident and the scattered beam is π, because the scattered radiation is proportional to the displacement of the electron, which differs by π in phase with its acceleration imposed by the electric vector.

(2) The amplitude of the electric component of the scattered wave at a distance r which is large in comparison with the wavelength of the radiation is

$$E_{\rm el} = E_o \frac{1}{r} \frac{e^2}{mc^2} \sin\varphi,$$

where E_o is the amplitude of the electric vector of the incident beam, e is the electron charge, m is its mass, c is the speed of light and φ is the angle between the oscillation direction of the electron and the scattering direction (Fig. 2.1.4.1). Note that $E_o \sin\varphi$ is the component of E_o perpendicular to the scattering direction.

In terms of energy,

$$I_{\rm el} = I_o \frac{1}{r^2} \left(\frac{e^2}{mc^2}\right)^2 \sin^2\varphi. \qquad (2.1.4.1a)$$

The scattered energy per unit solid angle is

Copyright © 2006 International Union of Crystallography

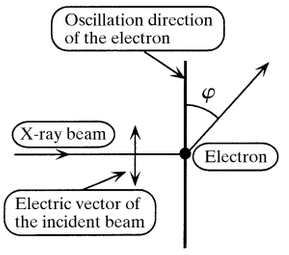

Fig. 2.1.4.1. The electric vector of a monochromatic and polarized X-ray beam is in the plane. It hits an electron, which starts to oscillate in the same direction as the electric vector of the beam. The oscillating electron acts as a source of X-rays. The scattered intensity depends on the angle φ between the oscillation direction of the electron and the scattering direction [equation (2.1.4.1)]. Reproduced with permission from Drenth (1999). Copyright (1999) Springer-Verlag.

$$I_{\rm el}(\Omega = 1) = I_{\rm el} r^2. \qquad (2.1.4.1b)$$

It was shown by Klein & Nishina (1929) [see also Heitler (1966)] that the scattering by an electron can be discussed in terms of the classical Thomson scattering if the quantum energy $h\nu \ll mc^2$. This is not true for very short X-ray wavelengths. For $\lambda = 0.0243$ Å, $h\nu$ and mc^2 are exactly equal, but for $\lambda = 1.0$ Å, $h\nu$ is 0.0243 times mc^2. Since wavelengths in macromolecular crystallography are usually in the range 0.8–2.5 Å, the classical approximation is allowed. It should be noted that:

(1) The intensity scattered by a free electron is independent of the wavelength.

(2) Thomson's equation can also be applied to other charged particles, *e.g.* a proton. Because the mass of a proton is 1800 times the electron mass, scattering by protons and by atomic nuclei can be neglected.

(3) Equation (2.1.4.1*a*) gives the scattering for a polarized beam. For an unpolarized beam, $\sin^2\varphi$ is replaced by a suitable polarization factor.

2.1.4.2. *Scattering by a system of two electrons*

This can be derived along classical lines by calculating the phase difference between the X-ray beams scattered by each of the two electrons. A derivation based on quantum mechanics leads exactly to the same result by calculating the transition probability for the scattering of a primary quantum $(h\nu)_o$, given a secondary quantum $h\nu$ (Heitler, 1966, p. 193). For simplification we shall give only the classical derivation here. In Fig. 2.1.4.2, a system of two electrons is drawn with the origin at electron 1 and electron 2 at position **r**. They scatter the incident beam in a direction given by the vector **s**. The direction of the incident beam is along the vector $\mathbf{s}_o$. The length of the vectors can be chosen arbitrarily, but for convenience they are given a length $1/\lambda$. The two electrons scatter completely independently of each other.

Therefore, the amplitudes of the scattered beams 1 and 2 are equal, but they have a phase difference resulting from the path difference between the beam passing through electron 2 and the beam passing through electron 1. The path difference is $p + q = \lambda[\mathbf{r} \cdot (\mathbf{s}_o - \mathbf{s})]$. Beam 2 lags behind in phase compared with beam 1, and with respect to wave 1 its phase angle is

$$-2\pi\lambda[\mathbf{r} \cdot (\mathbf{s}_o - \mathbf{s})]/\lambda = 2\pi\mathbf{r} \cdot \mathbf{S}, \qquad (2.1.4.2)$$

where $\mathbf{S} = \mathbf{s} - \mathbf{s}_o$.

From Fig. 2.1.4.3, it is clear that the direction of **S** is perpendicular to an imaginary plane reflecting the incident beam at an angle θ and that the length of **S** is given by

$$|\mathbf{S}| = 2\sin\theta/\lambda. \qquad (2.1.4.3)$$

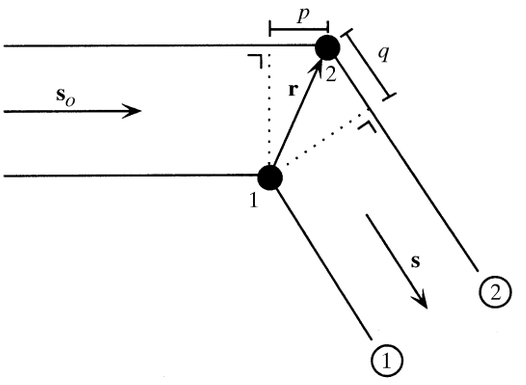

Fig. 2.1.4.2. The black dots are electrons. The origin of the system is at electron 1; electron 2 is at position **r**. The electrons are irradiated by an X-ray beam from the direction indicated by vector $\mathbf{s}_o$. The radiation scattered by the electrons is observed in the direction of vector **s**. Because of the path difference $p + q$, scattered beam 2 will lag behind scattered beam 1 in phase. Reproduced with permission from Drenth (1999). Copyright (1999) Springer-Verlag.

The total scattering from the two-electron system is $1 + 1 \times \exp(2\pi i\mathbf{r} \cdot \mathbf{S})$ if the resultant amplitude of the waves from electrons 1 and 2 is set to 1. In an Argand diagram, the waves are represented by vectors in a two-dimensional plane, as in Fig. 2.1.4.4(*a*).* Thus far, the origin of the system was chosen at electron 1. Moving the origin to another position simply means an equal change of phase angle for all waves. Neither the amplitudes nor the intensities of the reflected beams change (Fig. 2.1.4.4*b*).

2.1.4.3. *Scattering by atoms*

2.1.4.3.1. *Scattering by one atom*

Electrons in an atom are bound by the nucleus and are – in principle – not free electrons.

However, to a good approximation, they can be regarded as such if the frequency of the incident radiation ν is greater than the natural absorption frequencies, ν_n, at the absorption edges of the scattering atom, or the wavelength of the incident radiation is shorter than the

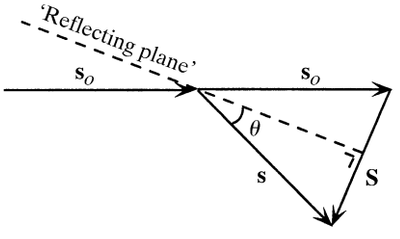

Fig. 2.1.4.3. The direction of the incident wave is indicated by $\mathbf{s}_o$ and that of the scattered wave by **s**. Both vectors are of length $1/\lambda$. A plane that makes equal angles with **s** and $\mathbf{s}_o$ can be regarded as a mirror reflecting the incident beam. Reproduced with permission from Drenth (1999). Copyright (1999) Springer-Verlag.

* The plane is also called the 'imaginary plane'. The real part of the vector in an Argand diagram is along the horizontal or real axis; the imaginary part is along the vertical or imaginary axis. Note also that $\exp(2\pi i\mathbf{r} \cdot \mathbf{S}) = \cos(2\pi\mathbf{r} \cdot \mathbf{S}) + i\sin(2\pi\mathbf{r} \cdot \mathbf{S})$. The cosine term is the real component and the sine term is the imaginary component.

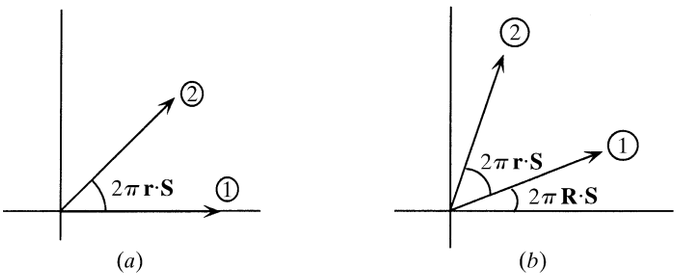

Fig. 2.1.4.4. An Argand diagram for the scattering by two electrons. In (*a*), the origin is at electron 1; electron 2 is at position **r** with respect to electron 1. In (*b*), electron 1 is at position **R** with respect to the new origin, and electron 2 is at position $\mathbf{R}+\mathbf{r}$.

absorption-edge wavelength (Section 2.1.4.4). This is normally true for light atoms but not for heavy ones (Table 2.1.4.1).

If the electrons in an atom can be regarded as free electrons, the scattering amplitude of the atom is a real quantity, because the electron cloud has a centrosymmetric distribution, *i.e.* $\rho(\mathbf{r})=\rho(-\mathbf{r})$.

A small volume, $\mathrm{d}v_r$, at **r** contains $\rho(\mathbf{r})\times\mathrm{d}v_r$ electrons, and at $-\mathbf{r}$ there are $\rho(-\mathbf{r})\times\mathrm{d}v_r$ electrons. The combined scattering of the two volume elements, in units of the scattering of a free electron, is

$$\rho(\mathbf{r})\mathrm{d}v_r\{\exp(2\pi i\mathbf{r}\cdot\mathbf{S})+\exp[2\pi i(-\mathbf{r})\cdot\mathbf{S}]\}=2\rho(\mathbf{r})\cos(2\pi\mathbf{r}\cdot\mathbf{S})\mathrm{d}v_r;$$

this is a real quantity.

The scattering amplitude of an atom is called the atomic scattering factor *f*. It expresses the scattering of an atom in terms of the scattering of a single electron. *f* values are calculated for spherically averaged electron-density distributions and, therefore, do not depend on the scattering direction. They are tabulated in *IT* C (1999) as a function of $\sin\theta/\lambda$. The *f* values decrease appreciably as a function of $\sin\theta/\lambda$ (Fig. 2.1.4.5). This is due to interference effects between the scattering from the electrons in the cloud. In the direction $\theta=0$, all electrons scatter in phase and the atomic scattering factor is equal to the number of electrons in the atom.

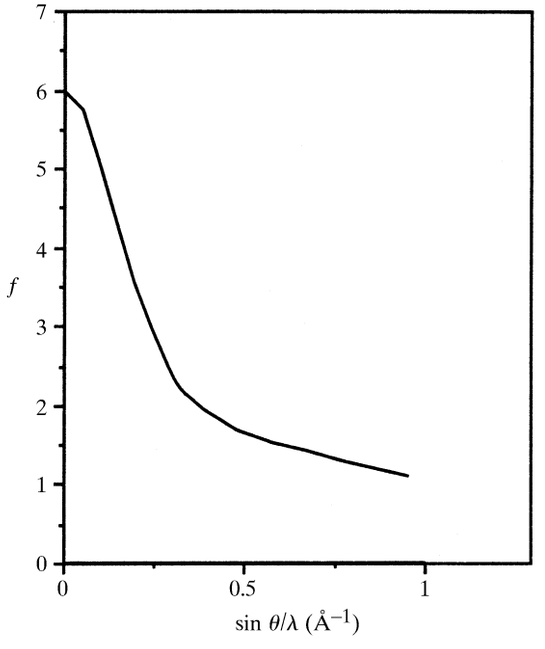

Fig. 2.1.4.5. The atomic scattering factor *f* for carbon as a function of $\sin\theta/\lambda$, expressed in units of the scattering by one electron. Reproduced with permission from Drenth (1999). Copyright (1999) Springer-Verlag.

Table 2.1.4.1. *The position of the Kα edge of different elements*

Atomic number	Element	*Kα* edge (Å)
6	C	43.68
16	S	5.018
26	Fe	1.743
34	Se	0.980
78	Pt	0.158

2.1.4.3.2. *Scattering by a plane of atoms*

A plane of atoms reflects an X-ray beam with a phase retardation of $\pi/2$ with respect to the scattering by a single atom. The difference is caused by the difference in path length from source (*S*) to atom (*M*) to detector (*D*) for the different atoms in the plane (Fig. 2.1.4.6). Suppose the plane is infinitely large. The shortest connection between *S* and *D via* the plane is *S*–*M*–*D*. The plane containing *S*, *M* and *D* is perpendicular to the reflecting plane, and the lines *SM* and *MD* form equal angles with the reflecting plane. Moving outwards from atom *M* in the reflecting plane, to *P* for instance, the path length *S*–*P*–*D* is longer. At the edge of the first Fresnel zone, the path is $\lambda/2$ longer (Fig. 2.1.4.6). This edge is an ellipse with its centre at *M* and its major axis on the line of intersection between the plane *SMD* and the reflecting plane. Continuing outwards, many more elliptic Fresnel zones are formed. Clearly, the beams radiated by the many atoms in the plane interfere with each other. The situation is represented in the Argand diagram in Fig. 2.1.4.7. Successive Fresnel zones can be subdivided into an equal number of subzones. If the distribution of electrons is sufficiently homogeneous, it can be assumed that the subzones in one Fresnel zone give the same amplitude at *D*. Their phases are spaced at regular intervals and their vectors in the Argand diagram lie in a half circle. In the lower part of Fig. 2.1.4.7, this is illustrated for the first Fresnel zone. For the second Fresnel zone (upper part), the radius is slightly smaller, because the intensity radiated by more distant zones decreases (Kauzmann, 1957). Therefore, the sum of vectors pointing upwards is shorter than that of those pointing downwards, and the resulting scattered wave lags $\pi/2$ in phase behind the scattering by the atom at *M*.

2.1.4.4. *Anomalous dispersion*

In classical dispersion theory, the scattering power of an atom is derived by supposing that the atom contains dipole oscillators. In

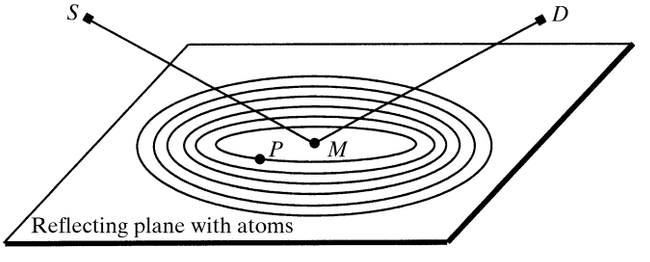

Fig. 2.1.4.6. *S* is the X-ray source and *D* is the detector. The scattering is by the atoms in a plane. The shortest distance between *S* and *D via* a point in the plane is through *M*. Path lengths *via* points in the plane further out from *M* are longer, and when these beams reach the detector they lag behind in phase with respect to the *MD* beam. The plane is divided into zones, such that from one zone to the next the path difference is $\lambda/2$.

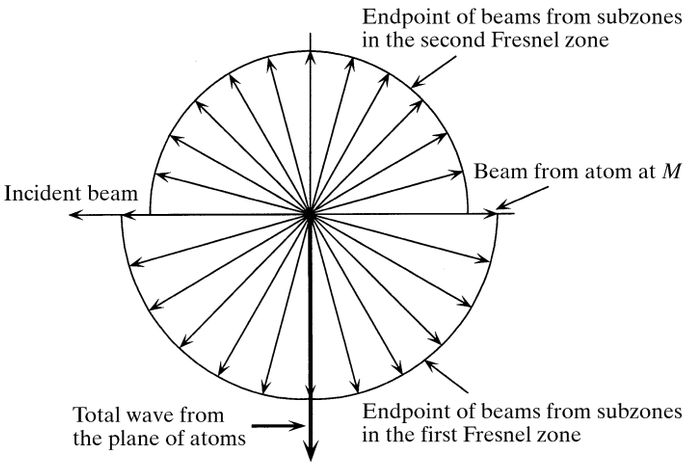

Fig. 2.1.4.7. Schematic picture of the Argand diagram for the scattering by atoms in a plane. All electrons are considered free. The vector of the incident beam points to the left. The atom at *M* (see Fig. 2.1.4.6) has a phase difference of π with respect to the incident beam. Subzones in the first Fresnel zone have the endpoints of their vectors on the lower half circle. For the next Fresnel zone, they are on the upper half circle, which has a smaller radius because the amplitude decreases gradually for subsequent Fresnel zones (Kauzmann, 1957). The sum of all vectors points down, indicating a phase lag of $\pi/2$ with respect to the beam scattered by the atom at *M*.

units of the scattering of a free electron, the scattering of an oscillator with eigen frequency ν_n and moderate damping factor κ_n was found to be a complex quantity:

$$f_n = \nu^2/(\nu^2 - \nu_n^2 - i\kappa_n\nu), \qquad (2.1.4.4)$$

where ν is the frequency of the incident radiation [James, 1965; see also *IT* C (1999), p. 244]. When $\nu \gg \nu_n$ in equation (2.1.4.4), f_n approaches unity, as is the case for scattering by a free electron; when $\nu \ll \nu_n$, f_n approaches zero, demonstrating the lack of scattering from a fixed electron. Only for $\nu \cong \nu_n$ does the imaginary part have an appreciable value.

Fortunately, quantum mechanics arrives at the same result by adding a rational meaning to the damping factors and interpreting ν_n as absorption frequencies of the atom (Hönl, 1933). For heavy atoms, the most important transitions are to a continuum of energy states, with $\nu_n \geq \nu_K$ or $\nu_n \geq \nu_L$ *etc.*, where ν_K and ν_L are the frequencies of the *K* and *L* absorption edges.

In practice, the complex atomic scattering factor, $f_{\text{anomalous}}$, is separated into three parts: $f_{\text{anomalous}} = f + f' + if''$. f is the contribution to the scattering if the electrons are free electrons and it is a real number (Section 2.1.4.3). f' is the real part of the correction to be applied and f'' is the imaginary correction; f'' is always $\pi/2$ in phase ahead of f (Fig. 2.1.4.8). $f + f'$ is the total real part of the atomic scattering factor.

The imaginary correction if'' is connected with absorption by oscillators having $\nu_n \cong \nu$. It can be calculated from the atomic absorption coefficient of the anomalously scattering element. For each of the *K*, *L etc*. absorption edges, f'' is virtually zero for frequencies below the edge, but it rises steeply at the edge and decreases gradually at higher frequencies.

The real correction f' can be derived from f'' by means of the Kramers–Kronig transform [*IT* C (1999), p. 245]. For frequencies close to an absorption edge, f' becomes strongly negative.

Values for f, f' and f'' are always given in units equal to the scattering by one free electron. f values are tabulated in *IT* C (1999) as a function of $\sin\theta/\lambda$, and the anomalous-scattering corrections for forward scattering as a function of the wavelength. Because the anomalous contribution to the atomic scattering factor is mainly due to the electrons close to the nucleus, the value of the corrections diminishes much more slowly than f as a function of the scattering angle.

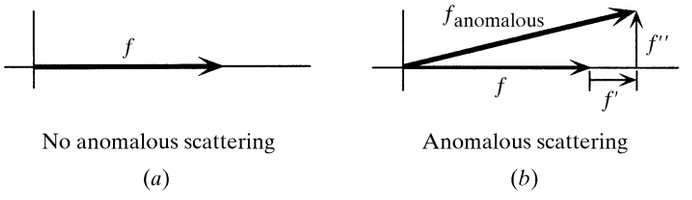

Fig. 2.1.4.8. The atomic scattering factor as a vector in the Argand diagram. (*a*) When the electrons in the atom can be regarded as free. (*b*) When they are not completely free and the scattering becomes anomalous with a real anomalous contribution f' and an imaginary contribution if''. Reproduced with permission from Drenth (1999). Copyright (1999) Springer-Verlag.

2.1.4.5. *Scattering by a crystal*

A unit cell contains a large number of electrons, especially in the case of biological macromolecules. The waves scattered by these electrons interfere with each other, thereby reducing the effective number of electrons in the scattered wave. The exception is scattering in the forward direction, where the beams from all electrons are in phase and add to each other. The effective number of scattering electrons is called the *structure factor F* because it depends on the structure, *i.e*. the distribution of the atoms in the unit cell. It also depends on the scattering direction. If small electron-density changes due to chemical bonding are neglected, the structure factor can be regarded as the sum of the scattering by the atoms in the unit cell, taking into consideration their positions and the corresponding phase differences between the scattered waves. For *n* atoms in the unit cell

$$F(\mathbf{S}) = \sum_{j=1}^{n} f_j \exp(2\pi i \mathbf{r}_j \cdot \mathbf{S}), \qquad (2.1.4.5)$$

where $\mathbf{S}$ is a vector perpendicular to the plane reflecting the incident beam at an angle θ; the length of $\mathbf{S}$ is given by $|\mathbf{S}| = 2\sin\theta/\lambda$ [equation (2.1.4.3) in Section 2.1.4.2].

The origin of the system is chosen at the origin of the selected unit cell. Atom *j* is at position $\mathbf{r}_j$ with respect to the origin. Another unit cell has its origin at $t \times \mathbf{a}$, $u \times \mathbf{b}$ and $v \times \mathbf{c}$, where *t*, *u* and *v* are whole numbers, and $\mathbf{a}$, $\mathbf{b}$ and $\mathbf{c}$ are the basis vectors of the unit cell. With respect to the first origin, its scattering is

$$F(\mathbf{S}) \exp(2\pi i t\mathbf{a} \cdot \mathbf{S}) \exp(2\pi i u\mathbf{b} \cdot \mathbf{S}) \exp(2\pi i v\mathbf{c} \cdot \mathbf{S}).$$

The wave scattered by a crystal is the sum of the waves scattered by all unit cells. Assuming that the crystal has a very large number of unit cells $(n_1 \times n_2 \times n_3)$, the amplitude of the wave scattered by the crystal is

$$W_{\text{cr}}(\mathbf{S}) = F(\mathbf{S}) \sum_{t=0}^{n_1} \exp(2\pi i t\mathbf{a} \cdot \mathbf{S}) \sum_{u=0}^{n_2} \exp(2\pi i u\mathbf{b} \cdot \mathbf{S}) \times \sum_{v=0}^{n_3} \exp(2\pi i v\mathbf{c} \cdot \mathbf{S}). \qquad (2.1.4.6)$$

For an infinitely large crystal, the three summations over the exponential functions are delta functions. They have the property that they are zero unless

$$\mathbf{a} \cdot \mathbf{S} = h, \ \mathbf{b} \cdot \mathbf{S} = k \text{ and } \mathbf{c} \cdot \mathbf{S} = l, \qquad (2.1.4.7)$$

where *h*, *k* and *l* are whole numbers, either positive, negative, or zero. These are the Laue conditions. If they are fulfilled, all unit

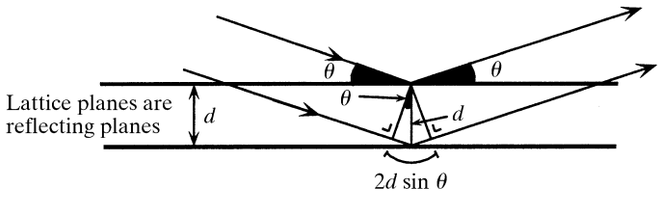

Fig. 2.1.4.9. X-ray diffraction by a crystal is, in Bragg's conception, reflection by lattice planes. The beams reflected by successive planes have a path difference of $2d\sin\theta$, where d is the lattice-plane distance and θ is the reflecting angle. Positive interference occurs if $2d\sin\theta = \lambda$, 2λ, 3λ *etc.*, where λ is the X-ray wavelength. Reproduced with permission from Drenth (1999). Copyright (1999) Springer-Verlag.

cells scatter in phase and *the amplitude of the wave scattered by the crystal is proportional to the amplitude of the structure factor F*. Its intensity is proportional to $|F|^2$.

S vectors satisfying equation (2.1.4.7) are denoted by **S**(*hkl*) or **S(h)**, and the corresponding structure factors as $F(hkl)$ or $F(\mathbf{h})$.

Bragg's law for scattering by a crystal is better known than the Laue conditions:

$$2d\sin\theta = \lambda, \qquad (2.1.4.8)$$

where d is the distance between reflecting lattice planes, θ is the reflecting or glancing angle and λ is the wavelength (Fig. 2.1.4.9). It can easily be shown that the Laue conditions and Bragg's law are equivalent by combining equation (2.1.4.7) with the following information:

(1) Vector **S** is perpendicular to a reflecting plane (Section 2.1.4.2).

(2) The Laue conditions for scattering [equation (2.1.4.7)] can be written as

$$\frac{\mathbf{a}}{h}\cdot\mathbf{S} = 1; \quad \frac{\mathbf{b}}{k}\cdot\mathbf{S} = 1; \quad \frac{\mathbf{c}}{l}\cdot\mathbf{S} = 1. \qquad (2.1.4.9)$$

(3) Lattice planes always divide the unit-cell vectors **a**, **b** and **c** into a number of equal parts (Section 2.1.1). If the lattice planes divide the **a** vector of the unit cell into h equal parts, the first index for this set of planes is h. The second index, k, is related to the division of **b** and the third index, l, to the division of **c**.

From equation (2.1.4.9) it follows that vector **S**(*hkl*) is perpendicular to a plane determined by the points **a**/h, **b**/k and **c**/l, and according to conditions (3) this is a lattice plane. Therefore, scattering by a crystal can indeed be regarded as reflection by lattice planes. The projection of **a**/h, **b**/k and **c**/l on vector **S**(*hkl*) is $1/|\mathbf{S}(hkl)|$ (Laue condition), but it is also equal to the spacing $d(hkl)$ between the lattice planes (see Fig. 2.1.1.3), and, therefore, $|\mathbf{S}(hkl)| = 1/d(hkl)$. Combining this with equation (2.1.4.3) yields Bragg's law, $2d\sin\theta = \lambda$ [equation (2.1.4.8)].

2.1.4.6. *The structure factor*

For noncentrosymmetric structures, the structure factor,

$$F(\mathbf{S}) = \sum_{j=1}^{n} f_j \exp(2\pi i\mathbf{r}_j\cdot\mathbf{S}),$$

is an imaginary quantity and can also be written as *

$$F(\mathbf{S}) = \sum_{j=1}^{n} f_j\cos(2\pi\mathbf{r}_j\cdot\mathbf{S}) + i\sum_{j=1}^{n} f_j\sin(2\pi\mathbf{r}_j\cdot\mathbf{S}) = A(\mathbf{S}) + iB(\mathbf{S}).$$

It is sometimes convenient to split the structure factor into its real part, $A(\mathbf{S})$, and its imaginary part, $B(\mathbf{S})$. For centrosymmetric structures, $B(\mathbf{S}) = 0$ if the origin of the structure is chosen at the centre of symmetry.

The average value of the structure-factor amplitude $|F(\mathbf{S})|$ decreases with increasing $|\mathbf{S}|$ or, because $|\mathbf{S}| = 2\sin\theta/\lambda$, with increasing reflecting angle θ.

This is caused by two factors:

(1) A stronger negative interference between the electrons in the atoms at a larger scattering angle; this is expressed in the decrease of the atomic scattering factor as a function of **S**.

(2) The temperature-dependent vibrations of the atoms. Because of these vibrations, the apparent size of an atom is larger during an X-ray exposure, and the decrease in its scattering as a function of **S** is stronger. If the vibration is equally strong in all directions, it is called isotropic, and the atomic scattering factor must be multiplied by a correction factor, the temperature factor, $\exp[-B(\sin^2\theta)/\lambda^2]$. It can be shown that the parameter B is related to the mean-square displacement of the atomic vibrations, $\overline{u^2}$:

$$B = 8\pi^2\overline{u^2}.$$

In protein crystal structures determined at high resolution, each atom is given its own individual thermal parameter B.† Anisotropic thermal vibration is described by six parameters instead of one, and the evaluation of this anisotropic thermal vibration requires more data (X-ray intensities) than are usually available. Only at very high resolution (better than 1.5 Å) can one consider the incorporation of anisotropic temperature factors.

The value of $|F(\mathbf{S})|$ can be regarded as the effective number of electrons per unit cell scattering in the direction corresponding to **S**. This is true if the values of $|F(\mathbf{S})|$ are on an absolute scale; this means that the unit of scattering is the scattering by one electron in a specific direction. The experimental values of $|F(\mathbf{S})|$ are normally on an arbitrary scale. The average value of the scattered intensity, $\overline{I(\text{abs.},\mathbf{S})}$, on an absolute scale is $\overline{I(\text{abs.},\mathbf{S})} = |F(\mathbf{S})|^2 = \sum_i f_i^2$, where f_i is the atomic scattering factor reduced by the temperature factor. This can be understood as follows:

$$\begin{aligned} I(\text{abs.},\mathbf{S}) &= F(\mathbf{S})\cdot F^*(\mathbf{S}) = |F(\mathbf{S})|^2 \\ &= \sum_i\sum_j f_i f_j \exp\big[2\pi i(\mathbf{r}_i - \mathbf{r}_j)\cdot\mathbf{S}\big]. \end{aligned} \qquad (2.1.4.10)$$

For a large number of reflections, **S** varies considerably, and assuming that the angles $[2\pi(\mathbf{r}_i - \mathbf{r}_j)\cdot\mathbf{S}]$ are evenly distributed over the range 0–2π for $i \neq j$, the average value for the terms with $i \neq j$ will be zero and only the terms with $i = j$ remain, giving

$$\overline{|F(\mathbf{S})|^2} = \overline{I(\text{abs.},\mathbf{S})} = \sum_i f_i^2. \qquad (2.1.4.11)$$

Because of the thermal vibrations

$$f_i^2 = \exp\big(-2B_i\sin^2\theta/\lambda^2\big)(f_i^o)^2,$$

where i denotes a specific atom and f_i^o is the scattering factor for the atom i at rest.

It is sometimes necessary to transform the intensities and the structure factors from an arbitrary to an absolute scale. Wilson (1942) proposed a method for estimating the required scale factor K and, as an additional bonus, the thermal parameter B averaged over the atoms:

* For convenience, we write $F(\mathbf{S})$ when we mean $F(hkl)$ or $F(\mathbf{h})$, and **S** instead of **S**(*hkl*) or **S(h)**.

† Do not confuse the thermal parameter B with the imaginary part $B(\mathbf{S})$ of the structure factor.

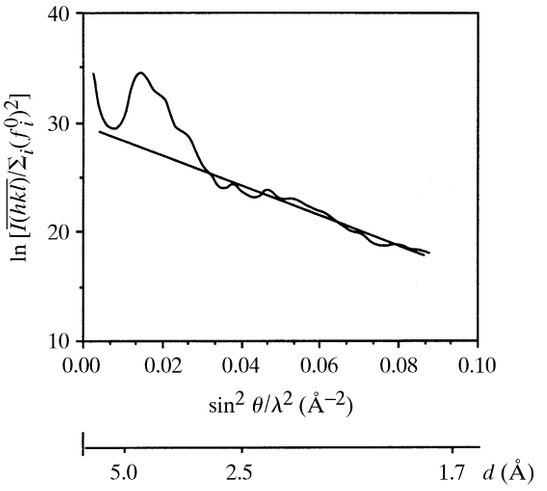

Fig. 2.1.4.10. The Wilson plot for phospholipase A_2 with data to 1.7 Å resolution. Only beyond 3 Å resolution is it possible to fit the curve to a straight line. Reproduced with permission from Drenth (1999). Copyright (1999) Springer-Verlag.

$$\overline{I(\mathbf{S})} = K\overline{I(\text{abs.},\mathbf{S})} = K\exp(-2B\sin^2\theta/\lambda^2)\sum_i (f_i^o)^2. \quad (2.1.4.12)$$

To determine K and B, equation (2.1.4.11) is written in the form

$$\ln[\overline{I(\mathbf{S})}/\sum_i (f_i^o)^2] = \ln K - 2B\sin^2\theta/\lambda^2. \quad (2.1.4.13)$$

Because f_i^o depends on $\sin\theta/\lambda$, average intensities, $\overline{I(\mathbf{S})}$, are calculated for shells of narrow $\sin\theta/\lambda$ ranges. $\ln[\overline{I(\mathbf{S})}/\sum_i (f_i^o)^2]$ is plotted against $\sin^2\theta/\lambda^2$. The result should be a straight line with slope $-2B$, intersecting the vertical axis at $\ln K$ (Fig. 2.1.4.10).

For proteins, the Wilson plot gives rather poor results because the assumption in deriving equation (2.1.4.11) that the angles, $[2\pi(\mathbf{r}_i - \mathbf{r}_j)\cdot\mathbf{S}]$, are evenly distributed over the range 0–2π for $i \neq j$ is not quite valid, especially not in the $\sin\theta/\lambda$ ranges at low resolution.

As discussed above, the average value of the structure factors, $F(\mathbf{S})$, decreases with the scattering angle because of two effects:

(1) the decrease in the atomic scattering factor f;

(2) the temperature factor.

This decrease is disturbing for statistical studies of structure-factor amplitudes. It is then an advantage to eliminate these effects by working with normalized structure factors, $E(\mathbf{S})$, defined by

$$E(\mathbf{S}) = F(\mathbf{S})\Big/\left(\sum_j f_j^2\right)^{1/2}$$

$$= F(\mathbf{S})\exp\left(B\sin^2\theta/\lambda^2\right)\Big/\left[\sum_j (f_j^o)^2\right]^{1/2}. \quad (2.1.4.14)$$

The application of equation (2.1.4.14) to $\overline{|E(\mathbf{S})|^2}$ gives

$$\overline{|E(\mathbf{S})|^2} = \overline{|F(\mathbf{S})|^2}\Big/\sum_j f_j^2 = \overline{|F(\mathbf{S})|^2}\Big/\overline{|F(\mathbf{S})|^2} = 1. \quad (2.1.4.15)$$

The average value, $\overline{|E(\mathbf{S})|^2}$, is equal to 1. The advantage of working with normalized structure factors is that the scaling is not important, because if equation (2.1.4.14) is written as

$$E(\mathbf{S}) = \frac{F(\mathbf{S})}{(\overline{|F(\mathbf{S})|^2})^{1/2}},$$

a scale factor affects numerator and denominator equally.

In practice, the normalized structure factors are derived from the observed data as follows:

$$E(\mathbf{S}) = F(\mathbf{S})\exp\left(B\sin^2\theta/\lambda^2\right)\Big/\left(\varepsilon|F(\mathbf{S})|^2\right)^{1/2}, \quad (2.1.4.16)$$

where ε is a correction factor for space-group symmetry. For general reflections it is 1, but it is greater than 1 for reflections having **h** parallel to a symmetry element. This can be understood as follows. For example, if m atoms are related by this symmetry element, $\mathbf{r}_j\cdot\mathbf{S}$ (with j from 1 to m) is the same in their contribution to the structure factor

$$F(\mathbf{h}) = \sum_{j=1}^{m} f_j\exp(2\pi i\mathbf{r}_j\cdot\mathbf{S}).$$

They act as one atom with scattering factor $m\times f$ rather than as m different atoms, each with scattering factor f. According to equation (2.1.4.11), this increases $F(\mathbf{h})$ by a factor $m^{1/2}$ on average. To make the F values of all reflections statistically comparable, $F(\mathbf{h})$ must be divided by $m^{1/2}$. For a detailed discussion, see *IT* B (2001), Chapter 2.1, by A. J. C. Wilson and U. Shmueli.

2.1.5. Reciprocal space and the Ewald sphere

A most convenient tool in X-ray crystallography is the reciprocal lattice. Unlike real or direct space, reciprocal space is imaginary. The reciprocal lattice is a superior instrument for constructing the X-ray diffraction pattern, and it will be introduced in the following way. Remember that vector $\mathbf{S}(hkl)$ is perpendicular to a reflecting plane and has a length $|\mathbf{S}(hkl)| = 2\sin\theta/\lambda = 1/d(hkl)$ (Section 2.1.4.5). This will now be applied to the boundary planes of the unit cell: the *bc* plane or (100), the *ac* plane or (010) and the *ab* plane or (001).

For the *bc* plane or (100): indices $h = 1$, $k = 0$ and $l = 0$; $\mathbf{S}(100)$ is normal to this plane and has a length $1/d(100)$. Vector $\mathbf{S}(100)$ will be called $\mathbf{a}^*$.

For the *ac* plane or (010): indices $h = 0$, $k = 1$ and $l = 0$; $\mathbf{S}(010)$ is normal to this plane and has a length $1/d(010)$. Vector $\mathbf{S}(010)$ will be called $\mathbf{b}^*$.

For the *ab* plane or (001): indices $h = 0$, $k = 0$ and $l = 1$; $\mathbf{S}(001)$ is normal to this plane and has a length $1/d(001)$. Vector $\mathbf{S}(001)$ will be called $\mathbf{c}^*$.

From the definition of $\mathbf{a}^*$, $\mathbf{b}^*$ and $\mathbf{c}^*$ and the Laue conditions [equation (2.1.4.7)], the following properties of the vectors $\mathbf{a}^*$, $\mathbf{b}^*$ and $\mathbf{c}^*$ can be derived:

$$\mathbf{a}^*\cdot\mathbf{a} = \mathbf{a}\cdot\mathbf{a}^* = \mathbf{a}\cdot\mathbf{S}(100) = h = 1.$$

Similarly

$$\mathbf{b}^*\cdot\mathbf{b} = \mathbf{b}\cdot\mathbf{S}(010) = k = 1,$$

and

$$\mathbf{c}^*\cdot\mathbf{c} = \mathbf{c}\cdot\mathbf{S}(001) = l = 1.$$

However, $\mathbf{a}^*\cdot\mathbf{b} = 0$ and $\mathbf{a}^*\cdot\mathbf{c} = 0$ because $\mathbf{a}^*$ is perpendicular to the (100) plane, which contains the b and c axes. Correspondingly, $\mathbf{b}^*\cdot\mathbf{a} = \mathbf{b}^*\cdot\mathbf{c} = 0$ and $\mathbf{c}^*\cdot\mathbf{a} = \mathbf{c}^*\cdot\mathbf{b} = 0$.

Proposition: The endpoints of the vectors $\mathbf{S}(hkl)$ form the points of a lattice constructed with the unit vectors $\mathbf{a}^*$, $\mathbf{b}^*$ and $\mathbf{c}^*$.

Proof: Vector **S** can be split into its coordinates along the three directions a^*, b^* and c^*:

$$\mathbf{S} = X\cdot\mathbf{a}^* + Y\cdot\mathbf{b}^* + Z\cdot\mathbf{c}^*. \quad (2.1.5.1)$$

Our proposition is true if X, Y and Z are whole numbers and indeed they are. Multiply equation (2.1.5.1) on the left and right side by **a**.

references