

2.5. ELECTRON DIFFRACTION AND ELECTRON MICROSCOPY IN STRUCTURE DETERMINATION

the polymer-structure analyses cited above, as well as the layer-packing analysis of some phospholipids (Dorset, 1987).] While attempts at *ab initio* modelling of three-dimensional crystal structures, by searching an n -dimensional parameter space and seeking a global internal energy minimum, has remained an active research area, most success so far seems to have been realized with the prediction of two-dimensional layers (Scaringe, 1992). In general, for complicated unit cells, determination of a structure by trial and error is very difficult unless adequate constraints can be placed on the search.

Although Patterson techniques have been very useful in electron crystallography, there are also inherent difficulties in their use, particularly for locating heavy atoms. As will be appreciated from comparison of scattering-factor tables for X-rays [IT C (2004), Chapter 6.1] with those for electrons [IT C (2004), Chapter 4.3], the relative values of the electron form factors are more compressed with respect to atomic number than are those for X-ray scattering. As discussed in Chapter 2.3, it is desirable that the ratio of summed scattering-factor terms, $r = \sum_{\text{heavy}} Z^2 / \sum_{\text{light}} Z^2$, where Z is the scattering-factor value at $\sin \theta / \lambda = 0$, be near unity. A practical comparison would be the value of r for copper (DL-alanine) solved from electron-diffraction data by Vainshtein *et al.* (1971). For electron diffraction, $r = 0.47$ compared to the value 2.36 for X-ray diffraction. Orientation of salient structural features, such as chains and rings, would be equally useful for light-atom moieties in electron or X-ray crystallography with Patterson techniques. As structures become more complicated, interpretation of Patterson maps becomes more and more difficult unless an automated search can be carried out against a known structural fragment (Chapter 2.3).

2.5.8.2. Direct phase determination from electron micrographs

The 'direct method' most familiar to the electron microscopist is the high-resolution electron micrograph of a crystalline lattice. Retrieval of an average structure from such a micrograph assumes that the experimental image conforms adequately to the 'weak phase object' approximation, as discussed in Section 2.5.5. If this is so, the use of image-averaging techniques, *e.g.* Fourier filtration or correlational alignment, will allow the unit-cell contents to be visualized after the electron-microscope phase contrast transfer function is deconvoluted from the average image, also discussed in Section 2.5.5. Image analyses can also be extended to three dimensions, as discussed in Section 2.5.6, basically by employing tomographic reconstruction techniques to combine information from the several tilt projections taken from the crystalline object. The potential distribution of the unit cell to the resolution of the imaging experiment can then be used, *via* the Fourier transform, to obtain crystallographic phases for the electron-diffraction amplitudes recorded at the same resolution. This method for phase determination has been the mainstay of protein electron crystallography.

Once a set of phases is obtained from the Fourier transform of the deconvoluted image, they must, however, be referred to an allowed crystallographic origin. For many crystallographic space groups, this choice of origin may coincide with the location of a major symmetry element in the unit cell [see IT A (2005)]. Hence, since the Fourier transform of translation is a phase term, if an image shift $[\delta(\mathbf{r} + \mathbf{r}_0)]$ is required to translate the origin of the repeating mass unit $\varphi(\mathbf{r})$ from the arbitrary position in the image to a specific site allowed by the space group,

$$g(\mathbf{r}) = \varphi(\mathbf{r}) \otimes \delta(\mathbf{r} + \mathbf{r}_0) = \varphi(\mathbf{r} + \mathbf{r}_0),$$

where the operation ' \otimes ' denotes convolution. The Fourier transform of this shifted density function will be

$$G(\mathbf{s}) = F(\mathbf{s}) \exp(2\pi i \mathbf{s} \cdot \mathbf{r}_0) = |F(\mathbf{s})| \exp[i(\phi_s + 2\pi i \mathbf{s} \cdot \mathbf{r}_0)].$$

In addition to the crystallographic phases ϕ_s , it will, therefore, be necessary to find the additional phase-shift term $2\pi i \mathbf{s} \cdot \mathbf{r}_0$ that will access an allowed unit-cell origin. Such origin searches are carried out automatically by some commercial image-averaging computer-software packages.

In addition to applications to thin protein crystals (*e.g.* Henderson *et al.*, 1990; Jap *et al.*, 1991; Kühlbrandt *et al.*, 1994), there are numerous examples of molecular crystals that have been imaged to a resolution of 3–4 Å, many of which have been discussed by Fryer (1993). For π -delocalized compounds, which are the most stable in the electron beam against radiation damage, the best results (2 Å resolution) have been obtained at 500 kV from copper perchlorophthalocyanine epitaxially crystallized onto KCl. As shown by Uyeda *et al.* (1978–1979), the averaged images clearly depict the positions of the heavy Cu and Cl atoms, while the positions of the light atoms in the organic residue are not resolved. (The utility of image-derived phases as a basis set for phase extension will be discussed below.) A number of aromatic polymer crystals have also been imaged to about 3 Å resolution, as reviewed recently (Tsuji, 1989; Dorset, 1994b).

Aliphatic molecular crystals are much more difficult to study because of their increased radiation sensitivity. Nevertheless, monolamellar crystals of the paraffin n -C₄₄H₉₀ have been imaged to 2.5 Å resolution with a liquid-helium cryomicroscope (Zemlin *et al.*, 1985). Similar images have been obtained at room temperature from polyethylene (Revol & Manley, 1986) and also a number of other aliphatic polymer crystals (Revol, 1991).

As noted by J. M. Cowley and J. C. H. Spence in Section 2.5.1, dynamical scattering can pose a significant barrier to the direct interpretation of high-resolution images from many inorganic materials. Nevertheless, with adequate control of experimental conditions (limiting crystal thickness, use of high-voltage electrons) some progress has been made. Pan & Crozier (1993) have described 2.0 Å images from zeolites in terms of the phase-grating approximation. A three-dimensional structural study has been carried out on an aluminosilicate by Wenk *et al.* (1992) with thin samples that conform to the weak-phase-object approximation at the 800 kV used for the imaging experiment. Heavy and light (*e.g.* oxygen) atoms were located in the micrographs in good agreement with an X-ray crystal structure. Heavy-atom positions from electron microscopic and X-ray structure analyses have also been favourably compared for two heavy-metal oxides (Hovmöller *et al.*, 1984; Li & Hovmöller, 1988).

2.5.8.3. Probabilistic estimate of phase invariant sums

Conventional direct phasing techniques, as commonly employed in X-ray crystallography (*e.g.* see Chapter 2.2), have also been used for *ab initio* electron-crystallographic analyses. As in X-ray crystallography, probabilistic estimates of a linear combination of phases (Hauptman & Karle, 1953; Hauptman, 1972) are made after normalized structure factors are calculated *via* electron form factors, *i.e.*

$$|E_{\mathbf{h}}^2| = I_{\text{obs}} / \varepsilon \sum_i f_i^2, \text{ where } \langle |E|^2 \rangle = 1.000.$$

(Here, an overall temperature factor can be found from a Wilson plot. Because of multiple scattering, the value of B may be found occasionally to lie close to 0.0 Å².) The phase invariant sums

$$\psi = \phi_{\mathbf{h}_1} + \phi_{\mathbf{h}_2} + \phi_{\mathbf{h}_3} + \dots$$