2. RECIPROCAL SPACE IN CRYSTAL-STRUCTURE DETERMINATION

resolution of the electron-microscope image, if one seeks phases for diffraction data in reciprocal-space regions where the objective lens phase contrast transfer function $|C(s)| \leq 0.2$, the method proves to be successful. The method is also quite effective for phase extension from 2 Å to 1 Å diffraction resolution, where the low-angle data serve as a large initial phase set for the tangent formula. However, no useful results were found from an *ab initio* phase determination carried out solely with the electron-diffraction structure-factor magnitudes. Similar results were obtained when *RANTAN* was used to phase experimental data from this compound (Fan *et al.*, 1991), *i.e.* the multisolution approach worked well for phase extension but not for *ab initio* phase determination. Additional tests were subsequently carried out with *QTAN* on an experimental *hk*0 electron-diffraction data set collected at 1200 kV (Dorset, McCourt, Fryer *et al.*, 1994). Again, *ab initio* phase determination is not possible by this technique. However, if a basis set was constructed from the Fourier transform of a 2.4 Å image, a correct solution could be found, but not at the lowest value of NQEST. This figure of merit was useful, however, when the basis set was taken from the symbolic addition determination mentioned in the previous section.

2.5.8.5. Density modification

Another method of phase determination, which is best suited to refining or extending a partial phase set, is the Hoppe–Gassmann density modification procedure (Hoppe & Gassmann, 1968; Gassmann & Zechmeister, 1972; Gassmann, 1976). The procedure is very simple but also very computer-intensive. Starting with a small set of (phased) $F_{\mathbf{h}}$, an initial potential map $\varphi(\mathbf{r})$ is calculated by Fourier transformation. This map is then modified by some real-space function, which restricts peak sizes to a maximum value and removes all negative density regions. The modified map $\varphi'(\mathbf{r})$ is then Fourier-transformed to produce a set of phased structure factors. Phase values are accepted *via* another modification function in reciprocal space, *e.g.* $E_{\text{calc}}/E_{\text{obs}} \geq p$, where p is a threshold quantity. The new set is then transformed to obtain a new $\varphi(\mathbf{r})$ and the phase refinement continues iteratively until the phase solution converges (judged by lower crystallographic R values).

The application of density modification procedures to electron-crystallographic problems was assessed by Ishizuka *et al.* (1982), who used simulated data from copper perchlorophthalocyanine within the resolution of the electron-microscope image. The method was useful for finding phase values in reciprocal-space regions where the transfer function $|C(s)| \leq 0.2$. As a technique for phase extension, density modification was acceptable for test cases where the resolution was extended from 1.67 to 1.0 Å, or 2.01 to 1.21 Å, but it was not very satisfactory for a resolution enhancement from 2.5 to 1.67 Å. There appear to have been no tests of this method yet with experimental data. However, the philosophy of this technique will be met again below in the description of the maximum entropy and likelihood procedure.

2.5.8.6. Convolution techniques

One of the first relationships ever derived for phase determination is the Sayre (1952) equation:

$$F_{\mathbf{h}} = \frac{\theta}{V} \sum_{\mathbf{k}} F_{\mathbf{k}} F_{\mathbf{h}-\mathbf{k}},$$

which is a simple convolution of phased structure factors multiplied by a function of the atomic scattering factors. For structures with nonoverlapping atoms, consisting of one atomic species, it is an exact expression. Although the convolution term resembles part of the tangent formula above, no statistical averaging is

implied (Sayre, 1980). In X-ray crystallography this relationship has not been used very often, despite its accuracy. Part of the reason for this is that it requires relatively high resolution data for it to be useful. It can also fail for structures comprised of different atomic species.

Since, relative to X-ray scattering factors, electron scattering factors span a narrower range of magnitudes at $\sin\theta/\lambda = 0$, it might be thought that the Sayre equation would be particularly useful in electron crystallography. In fact, Liu *et al.* (1988) were able to extend phases for simulated data from copper perchlorophthalocyanine starting at the image resolution of 2 Å and reaching the 1 Å limit of an electron-diffraction data set. This analysis has been improved with a 2.4 Å basis set obtained from the Fourier transform of an electron micrograph of this material at 500 kV and extended to the 1.0 Å limit of a 1200 kV electron-diffraction pattern (Dorset *et al.*, 1995). Using the partial phase sets for zonal diffraction data from several polymers by symbolic addition (see above), the Sayre equation has been useful for extending into the whole *hk*0 set, often with great accuracy. The size of the basis set is critical but the connectivity to access all reflections is more so. Fan and co-workers have had considerable success with the analysis of incommensurately modulated structures. The average structure (basis set) is found by high-resolution electron microscopy and the 'superlattice' reflections, corresponding to the incommensurate modulation, are assigned phases in hyperspace by the Sayre convolution. Examples include a high T_c superconductor (Mo *et al.*, 1992) and the mineral ankangite (Xiang *et al.*, 1990). Phases of regular inorganic crystals have also been extended from the electron micrograph to the electron-diffraction resolution by this technique (Hu *et al.*, 1992).

In an investigation of how direct methods might be used for phase extension in protein electron crystallography, low-resolution phases from two proteins, bacteriorhodopsin (Henderson *et al.*, 1986) and halorhodopsin (Havelka *et al.*, 1993) were extended to higher resolution with the Sayre equation (Dorset *et al.*, 1995). For the noncentrosymmetric bacteriorhodopsin *hk*0 projection a 10 Å basis set was used, whereas a 15 Å set was accepted for the centrosymmetric halorhodopsin projection. In both cases, extensions to 6 Å resolution were reasonably successful. For bacteriorhodopsin, for which data were available to 3.5 Å, problems with the extension were encountered near 5 Å, corresponding to a minimum in a plot of average intensity *versus* resolution. Suggestions were made on how a multisolution procedure might be successful beyond this point.

2.5.8.7. Maximum entropy and likelihood

Maximum entropy has been applied to electron crystallography in several ways. In the sense that images are optimized, the entropy term

$$S = -\sum_i P_i \ln P_i,$$

where $P_i = p_i / \sum_i p_i$ and p_i is a pixel density, has been evaluated for various test electron-microscope images. For crystals, the true projected potential distribution function is thought to have the maximum value of S. If the phase contrast transfer function used to obtain a micrograph is unknown, test images (*i.e.* trial potential maps) can be calculated for different values of Δf_{trial}. The value that corresponds to the maximum entropy would be near the true defocus. In this way, the actual objective lens transfer function can be found for a single image (Li, 1991) in addition to the other techniques suggested by this group.

Another use of the maximum-entropy concept is to guide the progress of a direct phase determination (Bricogne & Gilmore, 1990; Gilmore *et al.*, 1990). Suppose that there is a small set H of known phases $\phi_{\mathbf{h} \in H}$ (corresponding either to origin

definition, or the Fourier transform of an electron micrograph, or both) with associated unitary structure-factor amplitudes $|U_{\mathbf{h}\in H}|$. [The unitary structure factor is defined as $|U_{\mathbf{h}}| = |E_{\mathbf{h}}|/(N)^{1/2}$.] As usual, the task is to expand into the unknown phase set K to solve the crystal structure. From Bayes' theorem, the procedure is based on an operation where $p(\text{map}|\text{data}) \propto p(\text{map})p(\text{data}|\text{map})$. This means that the probability of successfully deriving a potential map, given diffraction data, is estimated. This so-called posterior probability is approximately proportional to the product of the probability of generating the map (known as the prior) and the probability of generating the data, given the map (known as the likelihood). The latter probability consults the observed data and can be used as a figure of merit.

Beginning with the basis set H, a trial map is generated from the limited number of phased structure factors. As discussed above, the map can be immediately improved by removing all negative density. The map can be improved further if its entropy is maximized using the equation given above for S. This produces the so-called maximum-entropy prior $q^{\text{ME}}(X)$.

So far, it has been assumed that all $|U_{\mathbf{h}\in K}| = 0$. If large reflections from the K set are now added and their phase values are permuted, then a number of new maps can be generated and their entropies can be maximized as before. This creates a phasing 'tree' with many possible solutions; individual branch points can have further reflections added *via* permutations to produce further sub-branches, and so on. Obviously, some figure of merit is needed to 'prune' the tree, *i.e.* to find likely paths to a solution.

The desired figure of merit is the likelihood $L(H)$. First a quantity

$$\Lambda_{\mathbf{h}} = 2NR\exp[-N(r^2 + R^2)]I_o(2NrR),$$

where $r = |^{\text{ME}}U_{\mathbf{h}}|$ (the calculated unitary structure factors) and $R = |^{o}U_{\mathbf{h}}|$ (the observed unitary structure factors), is defined. From this one can calculate

$$L(H) = \sum_{\mathbf{h}\notin H} \ln \Lambda_{\mathbf{h}}.$$

The null hypothesis $L(H_o)$ can also be calculated from the above when $r = 0$, so that the likelihood gain

$$LLg = L(H) - L(H_o)$$

ranks the nodes of the phasing tree in order of the best solutions.

Applications have been made to experimental electron-crystallographic data. A small-molecule structure starting with phases from an electron micrograph and extending to electron-diffraction resolution has been reported (Dong *et al.*, 1992). Other experimental electron-diffraction data sets used in other direct phasing approaches (see above) also have been assigned phases by this technique (Gilmore, Shankland & Bricogne, 1993). These include intensities from diketopiperazine and basic copper chloride. An application of this procedure in protein structure analysis has been published by Gilmore *et al.* (1992) and Gilmore, Shankland & Fryer (1993). Starting with 15 Å phases, it was possible to extend phases for bacteriorhodopsin to the limits of the electron-diffraction pattern, apparently with greater accuracy than possible with the Sayre equation (see above).

2.5.8.8. *Influence of multiple scattering on direct electron-crystallographic structure analysis*

The aim of electron-crystallographic data collection is to minimize the effect of dynamical scattering, so that the unit-cell

potential distribution or its Fourier transform is represented significantly in the recorded signal. It would be a mistake, however, to presume that these data ever conform strictly to the kinematical approximation, for there is always some deviation from this ideal scattering condition that can affect the structure analysis. Despite this fact, some direct phasing procedures have been particularly 'robust', even when multiple scattering perturbations to the data are quite obvious (*e.g.* as evidenced by large crystallographic residuals).

The most effective direct phasing procedures seem to be those based on the Σ_2 triple invariants. These phase relationships will not only include the symbolic addition procedure, as it is normally carried out, but also the tangent formula and the Sayre equation (since it is well known that this convolution can be used to derive the functional form of the three-phase invariant). The strict ordering of $|E_{\mathbf{h}}|$ magnitudes is, therefore, not critically important so long as there are no major changes from large to small values (or *vice versa*). This was demonstrated in direct phase determinations of simulated *n*-beam dynamical diffraction data from a sulfur-containing polymer (Dorset & McCourt, 1992). Nevertheless, there is a point where measured data cannot be used. For example, intensities from *ca* 100 Å-thick epitaxically oriented copper perchlorophthalocyanine crystals become less and less representative of the unit-cell transform at lower electron-beam energies (Tivol *et al.*, 1993) and, accordingly, the success of the phase determination is compromised (Dorset, McCourt, Fryer *et al.*, 1994). The similarity between the Sayre convolution and the interactions of structure-factor terms in, *e.g.*, the multislice formulation of *n*-beam dynamical scattering was noted by Moodie (1965). It is interesting to note that dynamical scattering interactions observed by direct excitation of Σ_2 and Σ_1 triples in convergent-beam diffraction experiments can actually be exploited to determine crystallographic phases to very high precision (Spence & Zuo, 1992, pp. 56–63).

While the evaluation of positive quartet invariant sums (see Chapter 2.2) seems to be almost as favourable in the electron diffraction case as is the evaluation of Σ_2 triples, negative quartet invariants seem to be particularly sensitive to dynamical diffraction. If dynamical scattering can be modelled crudely by a convolutional smearing of the diffraction intensities, then the lowest structure-factor amplitudes, and hence the estimates of lowest $|E_{\mathbf{h}}|$ values, will be the ones most compromised. Since the negative quartet relationships require an accurate prediction of small 'cross-term' $|E_{\mathbf{h}}|$ values, multiple scattering can, therefore, limit the efficacy of this invariant for phase determination. In initial work, negative quartets have been mostly employed in the NQEST figure of merit, and analyses (Dorset, McCourt, Fryer *et al.*, 1994; Dorset & McCourt, 1994*a*) have shown how the degradation of weak kinematical $|E_{\mathbf{h}}|$ terms effectively reduced its effectiveness for locating correct structure solutions *via* the tangent formula, even though the tangent formula itself (based on triple phase estimates) was quite effective for phase determination. Substitution of the minimal function $R(\phi)$ for NQEST seems to have overcome this difficulty. [It should be pointed out, though, that only the Σ_2-triple contribution to $R(\phi)$ is considered.]

Structure refinement is another area where the effects of dynamical scattering are also problematic. For example, in the analysis of the paraelectric thiourea structure (Dorset, 1991*b*) from published texture diffraction data (Dvoryankin & Vainshtein, 1960), it was virtually impossible to find a chemically reasonable structure geometry by Fourier refinement, even though the direct phase determination itself was quite successful. The best structure was found only when higher-angle intensities (*i.e.* those least affected by dynamical scattering) were used to generate the potential map. Later analyses on heavy-atom-containing organics (Dorset *et al.*, 1992) found that the lowest kinematical *R*-factor value did not correspond to the chemically correct structure geometry. This observation was also made in the

references