STRUCTURE REFINEMENT AND STRUCTURAL INFORMATION

Part III

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Refinement of the trial structure

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When approximate positions have been determined for most, if not all, of the atoms, it is time to begin the refinement of the structure. In this process the atomic parameters are varied systematically so as to give the best possible agreement of the observed structure factor amplitudes (the experimental data) with those calculated for the proposed trial structure. Common refinement techniques involve Fourier syntheses and processes involving least-squares or maximum likelihood methods. Although they have been shown formally to be nearly equivalent—differing chiefly in the weighting attached to the experimental observations—they differ considerably in manipulative details; we shall discuss them separately here.

Many successive refinement cycles are usually needed before a structure converges to the stage at which the shifts from cycle to cycle in the parameters being refined are negligible with respect to their estimated errors. When least-squares refinement is used, the equations are, as pointed out below, nonlinear in the parameters being refined, which means that the shifts calculated for these parameters are only approximate, as long as the structure is significantly different from the "correct" one. With Fourier refinement methods, the adjustments in the parameters are at best only approximate anyway; final parameter adjustments are now almost always made by least squares, at least for structures not involving macromolecules.

Fourier methods

As indicated earlier (Chapters 8 and 9, especially Figure 9.8 and the accompanying discussion), Fourier methods are commonly used to locate a portion of the structure after some of the atoms have been found—that is, after at least a partial trial structure has been identified. Initially, only one or a few atoms may have been found, or maybe an appreciable fraction of the structure is now known. Once approximate positions for at least some of the atoms in the structure are known, the phase angles can be calculated. Then an approximate electron-density

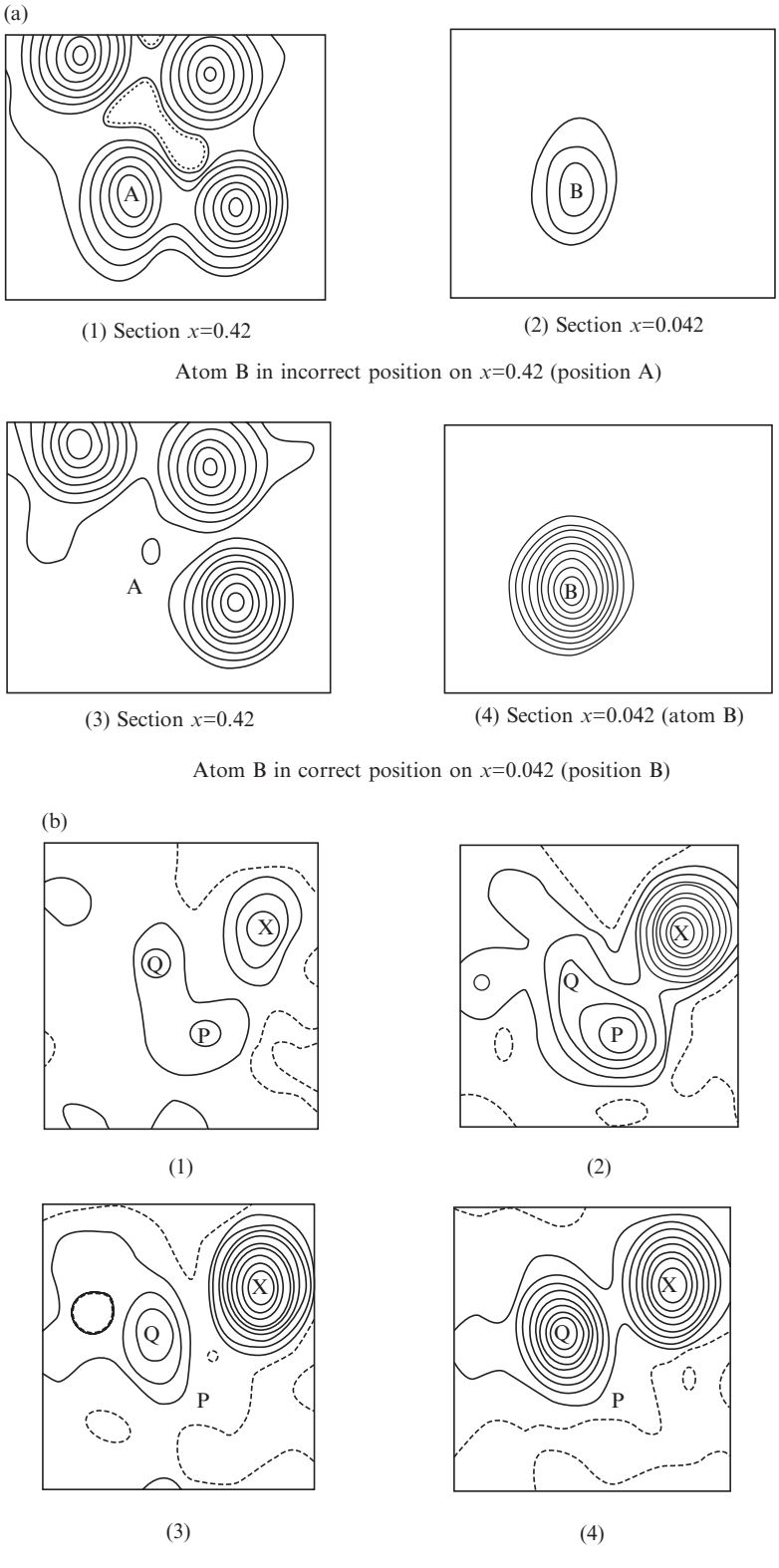

Fig. 11.1 Fourier maps phased with partially incorrect trial structures.

map calculated with *observed* structure amplitudes and *computed* phase angles will contain a blend of the true structure (from the structure amplitudes) with the trial structure (from the calculated phases). If the trial structure contains most of the atoms of the true structure, at or near their correct positions, the resulting electron-density map will contain peaks representing the trial structure, but, additionally, at other sites, peaks representing atoms that were omitted from the trial structure but that are really present. Conversely, if an atom in the trial structure has been incorrectly chosen, the corresponding peak in the electron-density map will usually be significantly lower than normal, so that its location will be questionable. Finally, if an atom was put into the calculation near, but not at, its correct position, the resulting peak in the electron-density map will usually have moved a slight amount from the input position towards (but not usually as far as) the correct position. Examples of these effects for a noncentrosymmetric structure are shown in Figure 11.1. In centrosymmetric structures, the phase angles are either 0° or 180° and a slight error in the structure may not have a large effect on most phase angles. Therefore, a map computed with observed $|F(hkl)|$ values and computed phase angles may be almost correct even if the model used was slightly in error. However, with noncentrosymmetric structures, for which the phase angles may have any value from 0° to 360°, there will be at least small errors in most of the phases, and consequently the calculated electron-density map will be weighted more in the direction of the trial structure used to calculate the phases than it would be if the structure were centrosymmetric.

It is usual, when most or all of the trial structure is known, to compute *difference maps* rather than normal electron-density maps. For difference maps, the coefficients for the calculation are $(|F_o| - |F_c|)$ and the phase

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(a) *The effect of an atom in the wrong position.* This example is from a noncentrosymmetric structure. In (1), one atom, B, was inadvertently included (an input typographical error) at the wrong position (marked by an A) in the structure factor calculation. The electron-density map phased with this incorrect structure contains a peak at the wrong position, but this peak is lower in electron density than the others near it. A small peak occurs in the correct position, B, shown in (2), although none was introduced there in the phasing. Corresponding sections of a correctly phased map are shown in (3) and (4); the spurious "atom" at A above has disappeared and the correct peak, B, is now a pronounced one.

(b) *The effect of an atom near but not at the correct position.* The appearance of a particular section in successive electron-density maps is shown as the structure used for phasing becomes more nearly correct. The map (1) was computed from the positions of two heavy atoms (positions not shown) and from this the location of atom X was correctly (as it turned out) deduced. But in (2) an atom was incorrectly placed at P; it can be seen that the peak for this atom is elongated in the direction of the correct position, Q. In (3) only atom X (of P, Q, and X) was included in the phasing and peak Q now is more clearly revealed. In (4) the peak at Q is now established as correct. A total of 2, 62, 54, and 68 atoms out of 73 were used in the phasing of maps (1), (2), (3), and (4), respectively.
From Hodgkin et al., 1959, p. 320, Figures 8 and 9.

angles are those computed for the trial structure. The difference map is thus the difference of an "observed" and a "calculated" map (both with "calculated" phases). In this map a positive region implies that not enough electrons were put in that area in the trial structure, while a negative region suggests that too many electrons were included in that region in the trial structure. For example, if an atom is included in the trial structure with too high an atomic number, a trough appears at the corresponding position; if it is included (at the correct position) with too low an atomic number or omitted entirely, a peak appears. Hydrogen atoms can be located from difference maps calculated from a trial structure that includes all the heavier atoms present (see Figure 11.2), although often hydrogen atoms are put at geometrically calculated positions and then refined. Another use of difference maps is in macromolecular structure determination, to locate the binding sites of inhibitors, substrates, or products.

Figure 11.3 shows some examples of further uses of difference maps for refinement of parameters. If an atom has been included near but not at the correct position, the location at which it was input will lie in a negative region, with a positive region in the direction of the correct position. The amount of the shift needed to move the atom to the correct position is indicated by the slope of the contours between the negative and positive regions. If an atom is left out of the trial structure (as in "omit maps") it will appear in the correct position as a peak, provided, of course, that the phase angles used in computing the electron-density map are approximately correct. If an atomic displacement factor is too small in the calculated trial structure, a trough will appear at the atomic position because the electrons in that atom have been assumed in the trial structure to be confined to a smaller volume than in fact they are, and hence to have too high a total electron density. Similarly, if the atomic displacement factor is too large in the trial structure, a peak will appear in the difference map. If the atom vibrates anisotropically, that is, different amounts in different directions, but has been assumed to be isotropic, peaks will occur in directions of greater motion and troughs in directions of lesser motion. In summary, if there is a positive area in a difference map, consider adding more electron density at that position; a negative area indicates too much electron density at that location in the trial structure.

The process of Fourier refinement can be adapted for automatic operation with a high-speed computer. Instead of evaluating the electron density at the points of a fixed lattice, we calculate it, together with its first and second derivatives, at the positions assumed for the atomic centers at this stage. The shifts in the atomic positions* and temperature-factor parameters can then be derived from the slopes and the curvatures in different directions. When this *differential-synthesis* method is used, it is normally applied to the difference density. In fact, however, the method is used much less extensively than least-squares refinement, for the latter is somewhat more convenient for computer application and has the advantage

* The shift required in x is

$$\Delta x = \frac{-\partial \Delta\rho}{\partial x} \Big/ \frac{\partial^2 \rho}{\partial x^2}$$

$$= \frac{-(\text{gradient of difference Fourier at } x_0)}{(\text{curvature of electron density at } x_0)}$$

where x_0 is the input position.

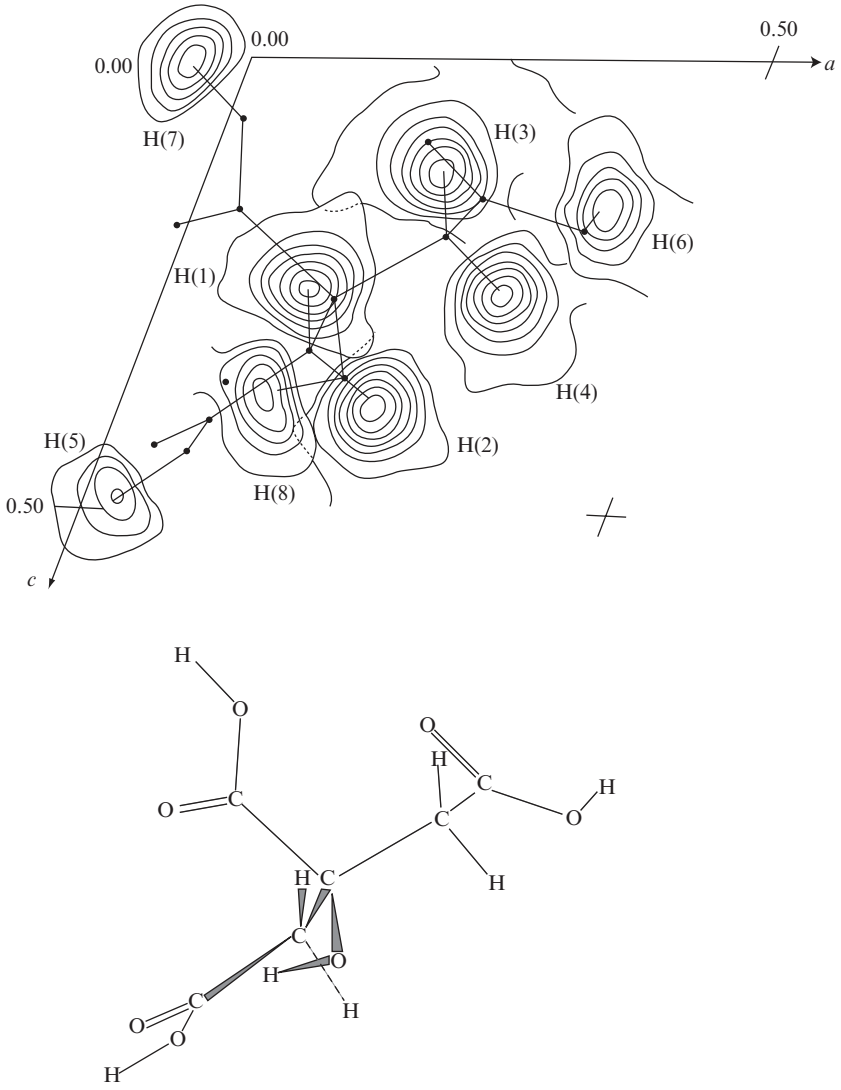

Fig. 11.2 Hydrogen atoms found from a difference map.

This is a composite map of sections of a difference map for a monoclinic structure, anhydrous citric acid, viewed down *b*. Eight sections containing hydrogen atoms are shown here. The contour interval is 0.1 electrons per cubic Å; the zero contour is omitted. Solid circles show the final positions of the heavier atoms that were used in the phase-angle calculation. Peaks occur in the map at positions in which not enough electron density has been included in the structure factor calculation, and thus at the positions of hydrogen atoms omitted from the phase-angle calculation. The molecular formula is shown below the map, on the same scale and in the same orientation.

From Glusker et al., 1969, *Acta Crystallographica* **B25**, p. 1066, Figure 1.

of a statistically sounder weighting scheme for the experimental observations.

One of the best criteria of a good structure determination is a flat difference map at the end of the refinement (because now the values of the observed and calculated structure amplitudes are approximately

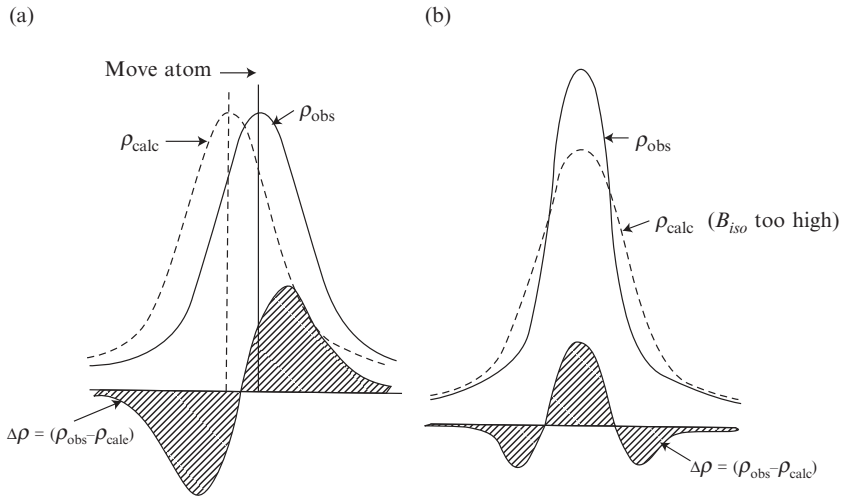

Fig. 11.3 Refinement by difference maps.

A difference map (the difference between the observed and calculated electron density, $\rho_{obs} - \rho_{calc}$) may be used to refine atomic positions and temperature factors. In a difference map a peak (a region of positive electron density) implies that not enough electron density was included in the model at that position, and a trough (a region of negative electron density) implies the opposite.

(a) *An error in the position of an atom.* The peak in ρ_{calc} shows the approximate position used in the calculation of structure factors. The peak in ρ_{obs} is nearer to the correct position. Therefore, the assumed atomic position should be moved (to the right) in the direction of the positive peak in the difference map.

(b) *Incorrect atomic displacement parameter.* If the displacement parameter exponent is too high in the model used to phase the map, the atom is vibrating through too large a volume. A peak surrounded by a region of negative density occurs at the atomic position, indicating that the exponent should be decreased to give a higher and narrower peak (and thus B should be decreased).

equal). It is possible to have a good average agreement of $|F_o|$ and $|F_c|$, and thus a low discrepancy index, R, and yet to have many ($|F_o| - |F_c|$) values contributing to a peak or trough in a given area of the map, indicating some error in the structure. Therefore, at the end of every structure determination, a difference map should be calculated and scanned for any peaks.

One question that always arises in discussions of Fourier refinement is: How good must the trial structure be, or how nearly correct must the phases be, for the process to converge? This question cannot be answered precisely. For an ordinary small-molecule structure analysis, if most of the atoms included are within about 0.3 Å (approximately half their radius) of their correct sites, then a few that are farther away and even one or two that may be wholly spurious can be tolerated. When the initial phases are poor, the first approximations to the electron density will contain much false detail (as illustrated in Figures 9.8 and 11.1b), together with peaks at or near the correct atomic positions. The sorting of the real from the spurious is difficult, especially with noncentrosymmetric structures; experience, chemical information, and a sound knowledge of the principles of structural chemistry are all desirable, and a good deal of caution is essential. A very astute or fortunate crystallographer may be able to recognize portions of a molecule of known

structure in a map produced from an extremely poor trial structure, but such perspicacity is uncommon.

Most investigators currently view electron-density and difference maps on a computer screen. There are several mouse-driven three-dimensional interactive programs such as O (Jones et al., 1991) and COOT (Emsley and Cowtan, 2004) that show electron densities as three-dimensional wire-frame entities. These can be rotated by the user to better view them, and a diagram of a three-dimensional trial structure can be overlaid on them. Some refinement can even take place at the computer screen as the trial structure diagram is moved to best fit the map. When the user is satisfied with the fit, the program will then generate the atomic coordinates of the new and better position of the model and these coordinates can be further refined.

The method of least squares

The method of least squares, first used by Legendre (1805), is a common technique for finding the best fit of a *particular assumed model* to a set of experimental data when there are more experimental observations than parameters to be determined. Parameters for the assumed model are improved by this method by minimizing the sum of the squares of the deviations between the experimental quantities and the values of the same quantities calculated with the derived parameters of the model. The method of least squares is often used to calculate the best straight line through a series of points, when it is known that there is an experimental error (assumed random) in the measurement of each point. The equation for a line may be calculated such that the sum of the squares of the deviations from the line is a minimum. Of course, if the points, which were assumed to lie on a straight line, actually lie on a curve (described very well by a nonlinear equation), the method will not tell what this curve is, but will approximate it by a straight line as best it may. It is possible to "weight" the points; that is, if one measurement is believed to be more precise than the others, then this measurement may, and indeed should, be given higher weight than the others. The weight $w(hkl)$ assigned to each measurement is inversely proportional to its precision, that is, the square of the standard uncertainty (formerly known as the estimated standard deviation).

The least-squares method has been extended to the problem of fitting the observed diffraction intensities to calculated ones (Hughes, 1946), and has been for more than six decades by far the most commonly used method of structure refinement, although this practice has not been without serious criticism.** Just as in a least-squares fit of data to a straight line (a two-parameter problem), the observed data are fitted to those calculated for a particular assumed model. If we let $\Delta|F(hkl)|$ be the difference in the amplitudes of the observed and calculated structure factors, $|F_o| - |F_c|$, and let the standard uncertainty of the experimental value of $F_o(hkl)^2$ be $[1/w(hkl)]$, then, according to the theory of

** These criticisms are based in part on the fact that the theory of the least-squares method is founded on the assumption that the experimental errors in the data are normally distributed (that is, follow a Gaussian error curve), or at least that the data are from a population with finite second moments. This assumption is largely untested with most data sets. Weighting of the observations may help to alleviate the problem, but it depends on a knowledge of their variance, which is usually assumed rather than experimentally measured. For a discussion of some of these points, see Dunitz's discussion of least-squares methods (Dunitz, 1996).

errors, the best parameters of the model assumed for the structure are those corresponding to the minimum value of the quantity[†]

$$Q = \Sigma w(hkl)[\Delta|F(hkl)^2|]^2 \tag{11.1}$$

in which the sum is taken over all unique diffraction maxima. In an analysis of the equations that define F_c, the effects of small changes in the atomic parameters are considered, and changes are found that will difference between F_o and F_c [and thus the sum in Eqn. (11.1)]. Since even the problem of fitting data to a two-parameter straight line involves much calculation, this method requires a high-speed, large-memory computer.

The variable parameters that are used in the minimization of Q in Eqn. (11.1) normally include an overall scale factor for the experimental observations; the atomic position parameters x, y, and z for each atom, j; and the atomic displacement parameters for each atom, *which may number as many as six*.[‡] Occasionally, when disorder is present, occupancy factors (varying from 0 to 1, and perhaps correlated with those of other atoms) may be refined for selected atoms. Thus *in a general case* there may be as many as (9N + 1) or even a few more parameters to be refined for a structure with N independent atoms.

If the total number of parameters to be refined is p, then the minimization of Eqn. (11.1) involves setting the derivatives of Q with respect to *each* of these parameters equal to zero. This gives p independent simultaneous equations. The derivatives of Q are readily evaluated. Clearly, at least p experimental observations are needed to define the p parameters, but, in fact, since the observations usually have significant experimental uncertainty, it is desirable that the number of observations, m, exceeds the number of variables by an appreciable factor. In most practical cases with three-dimensional X-ray data, m/p is of the order of 5 to 10, so that the equations derived from Eqn. (11.1) are greatly overdetermined.

Unfortunately, the equations derived from Eqn. (11.1) are by no means linear in the parameters, since they involve trigonometric and exponential functions, whereas the straightforward application of the method of least squares requires a set of linear equations. *If* a reasonable trial structure is available, then it is possible to derive a set of linear equations in which the variables are the *shifts* from the trial parameters, rather than the parameters themselves. This is done by expanding in a Taylor series about the trial parameters, retaining only the first-derivative terms on the assumption that *the shifts needed are sufficiently small that the terms involving second- and higher-order derivatives are negligible:*

$$\Delta|F_c| = \frac{\partial|F_c|}{\partial x_1}\Delta x_1 + \frac{\partial|F_c|}{\partial y_1}\Delta y_1 + \cdots + \frac{\partial|F_c|}{\partial b_{33,n}}\Delta b_{33,n} \tag{11.2}$$

The validity of this assumption depends on the closeness of the trial structure to the correct structure. If conditions are unfavorable, and Eqn. (11.2) is too imprecise, the process may sometimes converge to

[†] The equations can be formulated with $|F^2|$ rather than $|F|$, so that the equation parallel to Eqn. (11.1) then becomes

$$Q = \Sigma w(hkl)[\Delta|F^2(hkl)|]^2$$

Most crystallographers prefer refinement that involves F^2 for a variety of reasons, including ease of refining twinned structures, calculating weights for the least-squares refinement, and dealing with weak Bragg reflections (which may have negative values of F^2 from the nature of the measurement process).

[‡] These six vibration parameters, different for each atom j, are symbolized in various ways (see Chapter 12). Here we represent them as b^{11}, b^{22}, b^{33}, b^{12}, b^{23}, and b^{31}, with sometimes an additional subscript to denote the atom j. As mentioned later, more parameters may be needed to describe the atomic motion in extreme circumstances.

a false minimum rather than to the minimum corresponding to the correct solution or may not converge at all. Thus this method of refinement also depends for its success on the availability at the start of a reasonably good set of phases—that is, a good trial structure. Since the linearization of the least-squares equations makes them only approximate, several cycles of refinement are needed before convergence is achieved. However, the linear approximation becomes better as the solution is approached because the neglected higher-derivative terms, which involve high powers of the discrepancies between the approximate and true structures, become negligible as these discrepancies become small.

It is often desirable in a least-squares refinement to introduce various constraints or restraints on the atomic parameters to make them satisfy some specific criteria, usually geometrical. Constraints are limits on the values that parameters in a least-squares refinement may take. For example, they may relate two or more parameters, or may assign fixed values to certain parameters. As a result they reduce the number of independent parameters to be refined and are mathematically rigid with no standard uncertainty. For example, suppose that the structure is disordered in some way, or that the available diffraction data are of limited resolution. The individual atomic positions obtained by the usual least-squares process for some of the atoms will then have relatively high standard uncertainties and the geometrical parameters derived from these positions may not be of high significance. If geometrical constraints are introduced—for example, constraining a phenyl ring to be a regular hexagon of certain dimensions, or merely fixing certain bond lengths or bond angles or torsion angles within a particular range of values—the number of parameters to be refined will be significantly reduced and the refinement process accelerated. By contrast, restraints are assumptions that are treated like additional data that need to be refined against. For example, a phenyl group would be described as an "approximately regular hexagon" with a standard uncertainty within which it is supposed to be refined. Constraints remove parameters and restraints add data.

If the trial model used in a least-squares refinement is incorrect or partially incorrect, there are almost always indications that this is so. The discrepancy index R may not drop to an acceptable value, and the parameters may show certain anomalies. For example, if a false atom has inadvertently been included in the initial trial structure, it may move to a chemically unreasonable position, perhaps too close to another atom, and its temperature factor will increase strikingly to a value far higher than that normally encountered for any real atom. This corresponds physically to a very high vibration amplitude—that is, a smearing of the atom throughout the unit cell, an almost infallible sign that there is no atom in the actual structure at the position assumed in the trial structure.

At the conclusion of any least-squares refinement process, it is always wise to calculate a difference Fourier synthesis. If it is zero everywhere,

within experimental error, then the least-squares procedure is a reasonable one. If it is not, and the peaks in it are not attributable to light atoms that have been left out of the structure factor calculations or to some other understandable defect of the model, then it is distinctly possible that the least-squares procedure may have converged to a false minimum because the initial approximation (the trial structure) was not sufficiently good. Another plausible trial structure must be sought and refinement tried again.

The maximum likelihood method

Maximum likelihood estimation is an increasingly commonly employed statistical method that is used to refine a statistical model to experimental data, and thereby provide improved estimates of the parameters of this model (Murshudov et al., 1997; Terwilliger, 2000). It deals in conditional probability distributions, that is, probabilities that are conditional upon additional variables, and aims to maximize their likelihoods. For example, if we know that the probability of data A is dependent upon model B, we can find the likelihood of model B given the data A. Stephen Stigler compares maximum likelihood to the choice that prehistoric men made of "where and how to hunt and gather," that is, experience and acute observation which indicates how best to do something (Stigler, 2007). The likelihood function for macromolecular structures is proportional to the conditional distribution of experimental data when the parameters are known. The conditional probability distributions for each Bragg reflection are multiplied together and the result is the joint conditional probability distribution. This includes the experimental data plus any phase information and any experimental standard uncertainties that may be available. The aim of the method is to find those values of the parameters that make the observed data most likely. The necessary equations are contained in the program REFMAC (Vagin et al., 2004), which will minimize atomic parameters to satisfy either a maximum likelihood or a least-squares residual. The method has been used with great success, and, if the data have been measured to very high resolution, approaches least squares as a good refinement method.

The correctness of a structure

What assurance is there that the changes suggested by difference maps, least-squares methods, or maximum likelihood estimations are correct? Are the suggested changes really improvements that will make the trial structure more nearly resemble the actual distribution of scattering matter in the crystal? In fact, if the experimenter is injudicious or unfortunate, some changes may actually make the model worse, since an image formed with incorrect phases will always contain false

detail—for example, peaks that may seem to suggest atoms but that really arise from errors in the phases. If the model is altered in a grossly incorrect way (or if it was inadequate in the first place), the "refinement" process may converge to a quite incorrect solution. What then are the criteria for assessing the likely correctness of a structure that has been determined by the refinement of approximate phases? There are no certain tests, but the most helpful general criteria are the following. (A number of erroneous structures have been reported because of inadequate attention to these criteria.)

(1) The agreement of the individual observed structure factor amplitudes $|F_o|$ with those calculated for the refined model should be comparable to the estimated precision of the experimental measurements of the $|F_o|$. As stressed in Chapter 6, the discrepancy index, R [Eqn. (6.9)], is a useful but by no means definitive index of the reliability of a structure analysis.
(2) A difference map phased with the final parameters of the refined structure should reveal no fluctuations in electron density greater than those expected on the basis of the estimated precision of the electron density.
(3) Any anomalies in the molecular geometry and packing, or other derived quantities—for example, abnormal bond distances and angles, unusually short nonbonded intramolecular or intermolecular distances, and the like—should be scrutinized with the greatest care and regarded with some skepticism. They may be quite genuine, but if so they should be interpretable in terms of some unusual properties of the crystal or the molecules and ions in it.

If writers of crystallographic papers have done their work properly, the information needed for a reader to assess the precision and accuracy of the reported results will be given. The precision of an experimental result, usually expressed in terms of its standard uncertainty, is a measure of the reproducibility of the observed value if the experiment were to be repeated. Accuracy, on the other hand, gives the deviation of a measurement from the value accepted as true (if that is known). The standard uncertainties of the various observed results—distances, angles, and so on—can be *estimated* by statistical methods, using as a basis the estimated errors of the prime experimental quantities, the intensities and directions of the diffracted radiation and the instrumental parameters of the equipment used. The basic assumption involved in the estimation of standard uncertainties is that fluctuations in observed quantities are due solely to *random* errors, which implies that the fluctuations are about an average value that agrees with the "true value." However, it is very important to recognize that there may be *systematic* errors, too, arising from failure to correct for various effects, which may be either known—for example, the effect of absorption on the intensities—or unknown—for example, inadequacies of the model because of lack of knowledge of the way in which molecular motion occurs in the crystal. Uncorrected systematic errors can cause

the reported values to differ from the "true" values by considerably more than would be estimated on the basis of the precision; that is, the accuracy may be low even if the precision is high. As in any experiment, it is far harder to assess the accuracy than the precision, because many systematic errors are unsuspected; the best way to detect systematic errors is to compare many distinct measurements of the quantity of interest, under different experimental conditions and by different methods if possible.§

§ A classic example of this approach led to the discovery of the noble gases by Rayleigh and Ramsey through a comparison of highly precise measurements of the density of nitrogen prepared from various pure nitrogen-containing compounds with that of a sample obtained by fractionation of liquid air.

If the distribution of errors is normal, statistical tables can be used to assess the probability that one observation or derived quantity is "significantly" different from another—that is, that the difference arises not merely from random errors but rather is one that further sufficiently precise measurements could verify. If two measurements differ from one another by twice the standard uncertainty (s.u.) of either, the probability is about 5 percent that the difference between them represents a random fluctuation; if they differ by 2.7 times the s.u., the probability is only about 1 percent that the difference represents a random fluctuation—in other words, there is about 99 percent probability that they represent two distinct values, which further precise measurements would verify as being different. It is a matter of taste what one accepts as being "significantly different"; some people accept the 2 s.u. (or "95 percent confidence") level, while those who are more conservative may choose the 2.7 s.u. (or "99 percent confidence") level, or an even higher one. Because systematic errors are so difficult to eliminate, the standard uncertainties calculated on the assumption that only random errors are present are usually quite optimistic as estimates of the *accuracy* of the results, however valuable they may be as measures of *precision*. Hence, in comparing results from different studies—for example, in comparing two bond lengths, or in trying to decide whether a bond angle is significantly different from that expected on the basis of some theoretical model—it is usually sound not to regard the difference as significant unless it is at least three or more times the s.u. For example, if a bond length is measured to be 1.560 Å with an s.u. of 0.007 Å, it is probably not significantly different from one measured to be 1.542 Å.

There are several known sources of systematic errors in even the more precise crystal structure analyses published to date. Most of these effects are under study in various laboratories and some of the most careful recent studies take them into account. They include:

(1) Scattering factor curves (uncorrected for thermal motion) are normally assumed to be spherically symmetrical, which is clearly not correct for bonded atoms. Extensive studies (both theoretical and experimental) of this asymmetry, which is detectable only in the most precise work, are now under way.
(2) The motions of some molecules in crystals are very complicated, and the usual ellipsoidal approximation for the motion of each atom may be a considerable oversimplification, especially if the motion is appreciable. Furthermore, in some crystals the corre-

lated motions of molecules in different unit cells—so-called "lattice vibrations"—may give rise to appreciable "thermal diffuse scattering" (e.g., streaks extending out from the usual Bragg diffraction peaks). Correction must be made for such effects in the most precise work.

(3) Many errors that can in principle be eliminated—for example, those arising from absorption or instrumental effects—may not have been properly taken into account.

(4) Sometimes the diffracted beam is rediffracted in the crystal when two planes are in a position to "reflect" simultaneously. This can give rise to significant errors in measurements of intensities.

Failure to correct for systematic errors may occur because the errors are regarded as minor and the corrections too complicated to be worthwhile, because an appropriate method of correction is not known, or because the source of error is overlooked. A critical reader will seek to discover what the author has done about known sources of systematic errors. Of course, it takes experience to assess the likely effects of having ignored some of them. Because of the ever-present possibility of systematic errors in even the most careful work, it is usually unwise to regard measured interatomic distances in crystals as more accurate than to the nearest 0.01 Å, although the stated precision may be as low as 0.001 Å. An exception is the now relatively unusual circumstance that the distance involves no parameters at all other than the unit-cell dimensions, for example, the Na^+ to Cl^- distance in NaCl or the C-C distance in diamond, each of which can be measured accurately to better than 0.001 Å at any given temperature. However, even when an interatomic distance is known with high precision and apparent accuracy, it must always be remembered that it represents only the distance between the average positions of the atoms as they vibrate in the crystal. For substances such as rock salt, the root-mean-square amplitude of vibration of the atoms at room temperature is 0.08 Å, and for organic molecules it is larger by a factor of two or three.

Summary

Since there are so many measured reflections (50 to 100 or more per atom in precise structure determinations), the "trial structure" parameters, representing atomic positions and extents of vibration, may be refined to obtain the best possible fit of observed and calculated structure factors.

Difference Fourier methods

Either electron-density or difference electron-density maps may be calculated, the latter being especially useful in the later stages of refinement. A peak in a difference map indicates too little scattering matter in the trial structure, a trough too much. For example, if a hydrogen

atom is left out of a trial structure, a peak will show where the atom must lie in the corrected trial structure. In general a model is adjusted appropriately to give as flat a final difference map as possible; this map should ideally be zero everywhere, but fluctuations will occur as a result of experimental uncertainties or inadequacies of the model used.

Least-squares/maximum likelihood methods

In any crystal structure analysis there are many more observations than parameters to be determined. The best parameters corresponding to some assumed model of the structure are found by minimizing the sum of the squares of the discrepancies between the observed values of $|F|$ (or $|F|^2$) and those calculated for an appropriate trial structure (or a partially refined version of it). Maximum likelihood methods are now increasingly used for structure refinement. These two methods of refinement have only been practicable for three-dimensional data since the advent of high-speed computers.

The correctness of a structure

All the following criteria should be applied:

(1) The agreement of individual structure factor amplitudes with those calculated for the refined model should be consistent with the estimated precision of the experimental measurements of the observations.
(2) A difference map, phased with final parameters for the refined structure, should reveal no fluctuations in electron density greater than those expected on the basis of the estimated precision of the electron density.
(3) Any anomalies in molecular geometry or packing should be scrutinized with great care and regarded with some skepticism.

Structural parameters: Analysis of results

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The results of an X-ray structure analysis are coordinates of the individual, chemically identified atoms in each unit cell, the space group (which gives equivalent positions), and displacement parameters that may be interpreted as indicative of molecular motion and/or disorder. Such data obtained from crystal structure analyses may be incorporated into a CIF or mmCIF (Crystallographic Information File or Macromolecular Crystallographic Information File). These ensure that the results of crystal structure analyses are usefully archived. There are many checks that the crystallographer can make to ensure that the CIF or mmCIF file is correctly informative. For example, the automated validation program PLATON (Spek, 2003) checks that all data reported are up to the standards required for publication by the International Union of Crystallography. It does geometrical calculations on the structure, illustrates the results, finds if any symmetry has been missed, investigates any twinning, and checks if the structure has already been reported. We now review the ways in which these atomic parameters can be used to obtain a three-dimensional vision of the entire crystal structure.

Calculation of molecular geometry

When molecules crystallize in an orthorhombic, tetragonal, or cubic unit cell it is reasonably easy to build a model using the unit-cell dimensions and fractional coordinates, because all the interaxial angles are 90°. However, the situation is more complicated if the unit cell contains oblique axes and it is often simpler to convert the fractional crystal coordinates to orthogonal coordinates before calculating molecular geometry. The equations for doing this for bond lengths, interbond angles, and torsion angles are presented in Appendix 12. If the reader wishes to compute interatomic distances directly, this is also possible if one knows the cell dimensions ($a, b, c, \alpha, \beta, \gamma$), the fractional atomic coordinates (x, y, z for each atom), and the space group. For example, the

square of the distance between two points (x_1, y_1, z_1) and (x_2, y_2, z_2) is

$$\begin{aligned} l^2 &= [(x_1 - x_2)a]^2 + [(y_1 - y_2)b]^2 + [(z_1 - z_2)c]^2 \\ &\quad -[2ab\cos\gamma(x_1 - x_2)(y_1 - y_2)] - [2ac\cos\beta(x_1 - x_2)(z_1 - z_2)] \\ &\quad -[2bc\cos\alpha(y_1 - y_2)(z_1 - z_2)] \\ &= [\Delta x\, a]^2 + [\Delta y\, b]^2 + [\Delta z\, c]^2 - [2ab\cos\gamma\Delta x\ \Delta y] \\ &\quad -[2ac\ \cos\beta\Delta x\ \Delta z] - [2bc\ \cos\alpha\Delta y\ \Delta z] \end{aligned} \tag{12.1}$$

where $\Delta x = x_1 - x_2$, and so forth. This provides an equation for calculating a bond length or other type of interatomic interaction. If the three distances between atoms A, B, and C, where AB = l_1, AC = l_2, BC = l_3, are known, then the angle B–A–C = δ may be calculated with the law of cosines,

$$\cos\delta = \frac{l_1^2 + l_2^2 + l_3^2}{2l_1l_2} \tag{12.2}$$

These two equations [Eqns. (12.1) and (12.2)] are used for most of the preliminary information necessary for analyzing a crystal structure.

Some illustrations of results from some very simple crystal structure studies are shown in Figures 12.1–12.3. For example, sodium chloride, NaCl (Figure 12.1), crystallizes at room temperature in the space group $Fm3m$, a face-centered cubic space group, and the unit-cell dimension is a = 5.6402(2) Å; the 2 in parentheses is a measure of the standard uncertainty in the last place quoted, so that it could be read as a = 5.6402 ± 0.0002 Å. Since this crystal structure involves a sodium ion at the origin ($x = y = z = 0.0$) and a chloride ion at ½, 0, 0, each ion is surrounded by six of the opposite type so that there is no significant buildup of charge (positive or negative) in the crystal. It can be readily calculated that the nearest distance between cations and anions is 2.82 Å. Integration of the experimental electron densities of Na and Cl, assuming that the minimum of electron density between them defines the edge of each atom or ion, shows that they are ions rather than atoms (see Dunitz, 1996). Potassium chloride has a similar structure in a unit cell with a = 6.2931(2) Å and therefore a $K^+ \ldots Cl^-$ distance of 3.15 Å. On the other hand, cesium chloride has a cubic unit cell with a cesium ion at the origin and a chloride ion in the center of the cell at $x = y = z$ = ½ to give a primitive unit cell *(not a body-centered unit cell, because the atoms at the origin and the center of the unit cell are different)*, so that the space group is primitive, $Pm3m$. Since the unit-cell edge is a = 4.120(2) Å, the $Cs^+ \ldots Cl^-$ distance is $4.120 \times (\sqrt{3})/2 = 3.57$ Å. Iron pyrite, FeS_2 (Figure 12.2), also crystallizes in a cubic unit cell, space group $Pa3$, a = 5.4175(5) Å, with an iron atom at the origin and a sulfur atom at x, x, x, where x = 0.39. Iron atoms are shown in black in this figure, with Fe–S distances of 3.05 Å. Sulfur atoms are speckled, and S–S bonds that are about 2.06 Å in length are illustrated in this figure

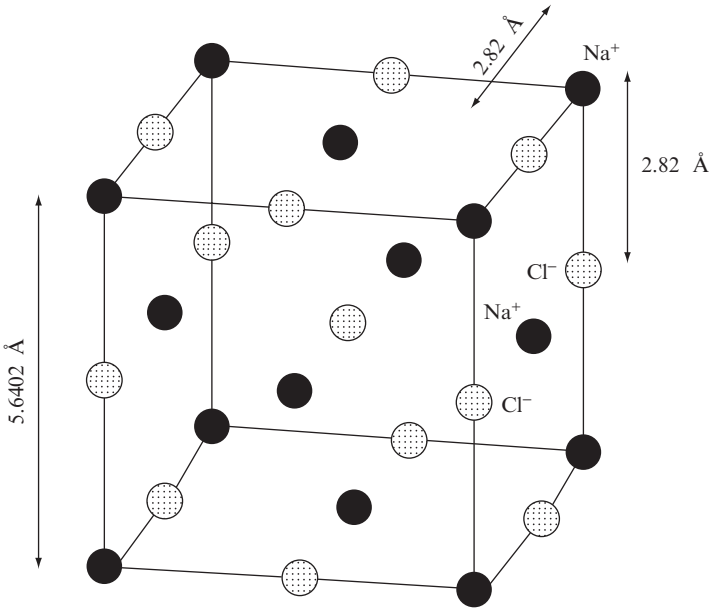

Fig. 12.1 Crystal structure of sodium chloride.
Sodium chloride (Na^+ black, Cl^- stippled circles) (Bragg, 1913).
$4Na^+$ at 0, 0, 0; 0, ½, ½; ½, 0, ½; ½, ½, 0 and $4Cl^-$ at ½, 0, 0; 0, ½, 0; 0, 0, ½; ½, ½, ½.

with black bonds. Diamond, shown in Figure 12.3, crystallizes in a cubic unit cell, a = 3.5597 Å, space group $Fd3m$, with eight carbon atoms per unit cell (Bragg and Bragg, 1913). The crystal structure clearly show the tetrahedral surroundings of each carbon atom and the result is the hardest mineral known. The nearest neighbor to an atom at the origin is the atom at $x = y = z = 1/4$, so that the C–C distance is $3.5597 \times (\sqrt{3})/4 =$ 1.541 Å, the C–C–C bond angle is 109.5°, and the C–C–C–C torsion angles are 60° or 180° depending on which carbon atom is chosen as the fourth (see equations in Appendix 12). Approximate atomic and ionic radii for many common ions in crystals have been derived from data such as these. There is always an element of arbitrariness in assigning radii, and no set is completely consistent, because ions are not "hard spheres," their effective radii varying somewhat with environment. Some typical values, however, are: Na^+, 0.95–1.17 Å; K^+, 1.33–1.49 Å; Cl^-, 1.64–1.81 Å; F^-, 1.16–1.36 Å (Frausto da Silva and Williams, 2001; Brown, 2006). A general analysis of ionic crystals was written by Linus Pauling in 1929, in which he showed how charged groups congregate in a crystal and aim to stay distant from hydrophobic groups (Pauling, 1929).

Of course, much more complicated structures than those illustrated in Figures 12.1–12.3 are now being studied, and the amount of information on bond lengths and the environments of various chemical groupings is escalating. Examples of historical interest include the phthalocyanines (Robertson, 1936), the boron hydrides (Lipscomb,

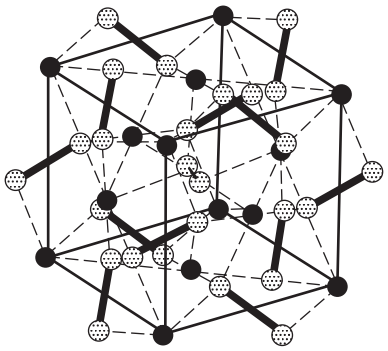

Fig. 12.2 Crystal structure of iron pyrite.
Iron pyrite (fool's gold), FeS_2 (Fe black, S stippled). Space group $Pa\bar{3}$, unit-cell dimensions a = 5.417 Å (Bragg, 1913).
4Fe at 0, 0, 0; 0, ½, ½; ½, 0, ½; ½, ½, 0 (as Na^+ in NaCl); 8S at $\pm(x, x, x; ½ + x, ½ - x, -x; -x, ½ + x, ½ - x; ½ - x, -x, ½ + x$; where x = 0.39).

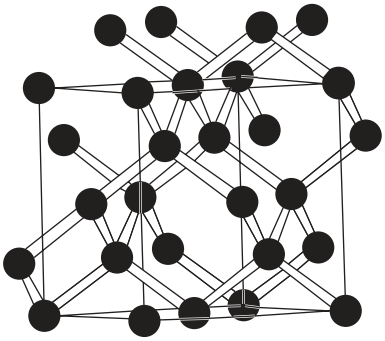

Fig. 12.3 Crystal structure of diamond.

The crystal structure of diamond, showing three-dimensional bonding throughout the crystal (Bragg and Bragg, 1913). This three-dimensional structure accounts for its hardness.

C at 0, 0, 0; 0, $1/2$, $1/2$; $1/2$, 0, $1/2$; $1/2$, $1/2$, 0; $1/4$, $1/4$, $1/4$; $1/4$, $3/4$, $3/4$; $3/4$, $1/4$, $3/4$; $3/4$, $3/4$, $1/4$.

1954), vitamin B_{12} (Hodgkin et al., 1957), myoglobin (Kendrew et al., 1960), hemoglobin (Perutz et al., 1968; Perutz, 1976), lysozyme (Phillips, 1966), and tobacco mosaic virus (Stubbs et al., 1977). Data on the results of X-ray and neutron diffraction studies on crystal structures of small and medium-sized molecules containing at least one carbon atom are available on the Cambridge Structural Database (CSD). This database is maintained by the Cambridge Crystallographic Data Centre in Cambridge, England, founded by Olga Kennard (Allen, 2002). Data files are also available on other types of crystal structures, including inorganic structures (the Inorganic Crystal Structure Database, ICSD) (Bergerhoff and Brown, 1987) and proteins (the RCSB Protein Data Bank) (Bernstein et al., 1977; Berman et al., 2003). A search of the World Wide Web will show the reader that there are many other crystallographic databases available and many computer-based methods of extracting structural information from them.

Molecular conformations

The torsion angles in a molecular structure are frequently of interest (see Appendix 12). These are a measure of the amount of twist about a bond and are defined, for a bonded series of four atoms (A–B–C–D), as the angle of rotation about a bond B–C needed to make the projection of the line B–A coincide with the projection of the line C–D, when viewed along the B–C direction. The positive sense is clockwise for this rotation. Thus the torsion angle is a representation of the structure viewed so that the atom C is completely obscured by atom B, as shown in Figure 12.4. A chain of methylene ($–CH_2–$) groups will generally have a staggered conformation so that torsion angles are 180° for C–C–C–C and 60° for C–C–C–H or H–C–C–H. The torsion angle is actually independent of the direction of view; that is, the A–B–C–D torsion angle equals the D–C–B–A torsion angle. However, for a pair of enantiomers (mirror images) the torsion angles of equivalent sets of

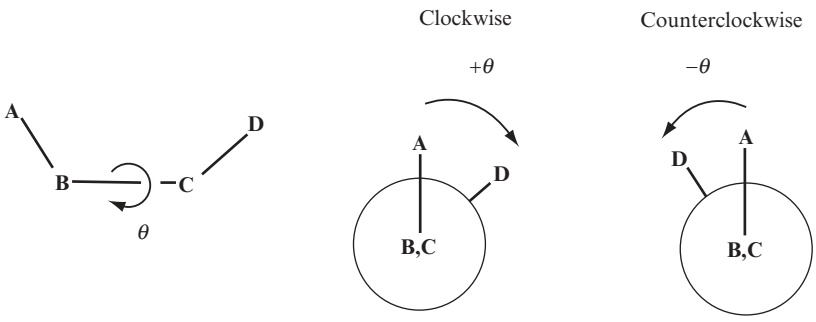

Fig. 12.4 Torsion angles.

Torsion angles measure the amount of twist about a chemical bond. For four bonded atoms A–B–C–D, the torsion angle about the central B–C bond is the extent to which the A–B bond has to be rotated clockwise so that it will eclipse the C–C bond.

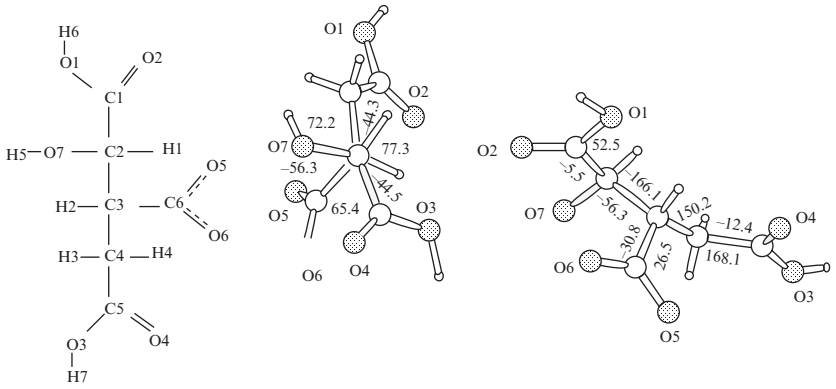

Fig. 12.5 Torsion angles in the isocitrate ion.
The isocitrate ion (see Figure 10.6b), showing some relevant torsion angles.

atoms have opposite signs (Figure 12.4, compare the two diagrams on the right of this Figure). An example of torsion angles in a structure is shown in Figure 12.5. Many studies of molecular structures involve lists of torsion angles because these angles can indicate similarities (or significant variations) in conformation (for example in sugars and in steroids). Another very useful calculation is that of the *least-squares plane* through a group of atoms in a molecule. Such planes can be used points of reference in describing the rest of the molecule, particularly when the shapes of molecules are being compared.

Intermolecular interactions

If one wishes to determine intermolecular distances (that is, distances between atoms in different molecules), then space-group symmetry information aids the calculations. The results are particularly useful for investigating the presence of hydrogen bonds and also for checking whether two molecules are unusually close to each other (an indication either of an unexpected intermolecular interaction or of an incorrect structure). For example, if the compound crystallizes in the space group $P2_12_12_1$, then, by use of Eqn. (12.1) and the information in Figure 7.6, the distance may be calculated, for example, between one atom at x_1, y_1, z_1 and another at $\frac{1}{2} - x_2, 1 - y_2, \frac{1}{2} + z_2$ (where x_1, y_1, z_1 and x_2, y_2, z_2 are the coordinates of two atoms in one molecule). Systematic calculations of distances and angles are now done almost entirely by computer programs, which search for all distances (intramolecular and intermolecular) within a selected range (in Å) around each atom in a chosen molecule. Analysis of intermolecular packing has, in several instances, led to an improved understanding of molecular interactions (see, for example, Bürgi et al., 1973; Kitaigorodsky, 1973; Rosenfield, 1977; Brown, 1988).

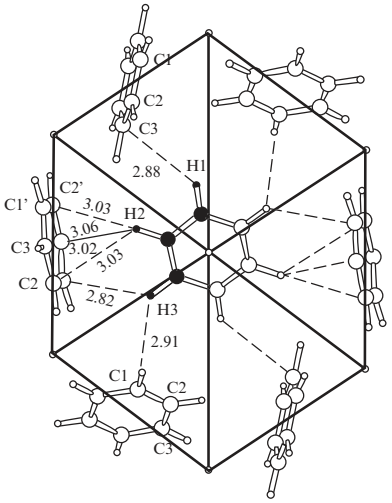

Fig. 12.6 Crystal structure of benzene.

Benzene, space group *Pbca*, $a = 7.44$, $b = 9.55$, $c = 6.92$ Å. Atoms at $\pm\{x, y, z; \frac{1}{2} + x, \frac{1}{2} - y, -z; -x, \frac{1}{2} + y, \frac{1}{2} - z; \frac{1}{2} - x, -y, \frac{1}{2} + z\}$

Atom	*x*	*y*	*z*
C(1)	−0.0569	0.1387	−0.0054
C(2)	−0.1335	0.0460	0.1264
C(3)	−0.0774	−0.0925	0.1295
H(1)	−0.0976	0.2447	−0.0177
H(2)	−0.2409	0.0794	0.2218
H(3)	−0.1371	−0.1631	0.2312

The asymmetric unit is indicated by black atoms (Cox and Smith, 1954; Bacon et al., 1964).

[*] Dunitz (1996) has an extensive discussion of calculations of standard uncertainties of derived quantities, including the need for taking correlations between different parameters into account.

[**] The name "temperature factor" has persisted to denote the constants in the exponential factors in Eqns. (12.3) and (12.4), despite the fact that it has long been recognized that vibrations persist at low temperatures, and that a static disorder may simulate a dynamic one if studies are made only at a single temperature. We use "displacement factor" here in recognition of this problem, that is, that the factor may represent thermal motion and/or disorder of the atom involved (Trueblood et al., 1996).

Benzene, for example, has been studied in the crystalline state at −3° C and by neutron diffraction at −55° C, −135° C, −150°C, and −258°C (because it is a liquid at room temperature) (Cox and Smith, 1954; Bacon et al., 1964; Jeffrey et al., 1987). The last two neutron studies were done on deuterobenzene, C_6D_6. The structure is illustrated in Figure 12.6. The crystals are orthorhombic, space group *Pbca*, with cell dimensions $a = 7.44$, $b = 9.55$, and $c = 6.92$ Å, and with half of a molecule (in black) in the asymmetric unit. Atomic coordinates are listed in the caption to this figure, which shows the molecular packing. The average C–C bond is 1.390 Å and the average C–H bonds are 1.07 Å in length. As shown in the figure, one hydrogen atom of one molecule points toward the π-electron system of the aromatic ring of a neighboring molecule. This kind of C–H. . . π-electron interaction occurs in many crystal structures of aromatic compounds.

Precision

All the quantities listed in a structure analysis (bond lengths, interbond angles, torsion angles, and least-squares planes) have errors that result from experimental errors in the diffraction measurements (see Chapter 4). Furthermore, the atomic scattering model used is not an exact representation of the electron density, merely the sum of ellipsoidal electron densities around each atomic nucleus. Estimates of errors, including those of unit-cell dimensions, may be made from least-squares refinements of the appropriate data, and their values can be used to assess the standard uncertainties in bond lengths, bond angles, and torsion angles. Unsuspected systematic errors may also be present.

As pointed out in Chapter 11, it is always necessary to quote a standard uncertainty with any computed geometrical quantity.[*] The standard uncertainty of a bond length is a function both of the precision in measurement of $|F(hkl)|$ values (expressed in the R value) and of the relative atomic numbers of the various atoms in the structure. For example, the standard uncertainty of a C–C bond in a structure containing only carbon and hydrogen atoms may be 0.002 Å for an R value of 0.05, but can increase to 0.02 Å or more for a structure with $R = 0.05$ that contains a heavy atom.

Atomic and molecular motion and disorder

The extent of atomic motion from vibration and/or disorder of each atom in a structure can also be measured.[**] However, before deriving their values it is important that absorption and other factors that affect the intensity distribution be taken into account; otherwise the parameters will not be a true representation of atomic motion or disorder.

The effect of the vibration of atoms in crystals on the scattering of X rays by these atoms has been discussed in Figure 5.4 and the

accompanying text. The simplest assumption that can be made is that the motion of each atom is the same in all directions; that is, that the motion is isotropic. The decrease of scattering intensity that results from this motion then depends only on the scattering angle and not on the particular orientation of the crystal with respect to the incident X-ray beam. As indicated in Figure 5.4c, such isotropic motion causes an exponential decrease of the effective atomic scattering factors as the scattering angle, 2θ, increases. The scattering factor for an atom at rest, f, is replaced by

$$f e^{-B_{iso}[(\sin^2\theta)/\lambda^2]} \tag{12.3}$$

B_{iso} is related to the average of the square of the amplitude of vibration, $<u^2>$, by $B_{iso} = 8\pi^2 <u^2> \cong 79 <u^2>$. For a typical B value of around 4 $Å^2$ (for an atom in an organic molecule at room temperature), this means that $<u^2>$ is about 0.05 $Å^2$, and the root-mean-square vibration amplitude, $<u^2>^{1/2}$, is then around 0.22 Å. At liquid nitrogen temperatures (near 100 K), B values are typically reduced by a factor of 2 or 3 from those at room temperature, and the root-mean-square amplitude will then be of the order of 0.15 Å. Atomic displacement parameters can be used to establish atomic type if the chemical formula of the compound under study is not known. This was true for the azidopurine that was used to demonstrate resolution in Figure 6.6 (Glusker et al., 1968). When the structure was refined with all atoms as carbon atoms it was found that the atomic displacement factors were lower for the nitrogen atoms, so that the chemical formula was thereby established.

However, it is clear that the approximation of isotropic motion is a poor one for atoms in most crystals, because the environments of these atoms are far from isotropic. The increasing availability of high-speed computers during the last three decades has made it worthwhile to attempt to collect precise intensity data and to analyze these data for relatively subtle effects, such as more complicated patterns of atomic and molecular motion. The next simplest approximation after isotropic motion is to assume that the motion is ellipsoidal—that is, that it can be described by the six parameters of a general ellipsoid rather than the single parameter characteristic of a sphere. These six parameters define the lengths of three mutually perpendicular axes describing the amount of motion in these directions, and the orientation of these ellipsoidal axes relative to the crystal axes. Figure 12.7 illustrates this representation of atomic motion in a portion of the structure of sodium dihydrogen citrate. This diagram was drawn with the computer program ORTEP (Johnson, 1965), which automatically generates stereoscopic images of molecules and represents the molecular motion by ellipsoids. These "thermal ellipsoids," calculated from the atomic displacement factors, show the amount that an atom is displaced in a given direction (indicated by the shape of the ellipsoid, a cigar shape indicating much motion or displacement). The ellipsoid also indicates the direction of maximum motion. The plot of ellipsoids is made at a

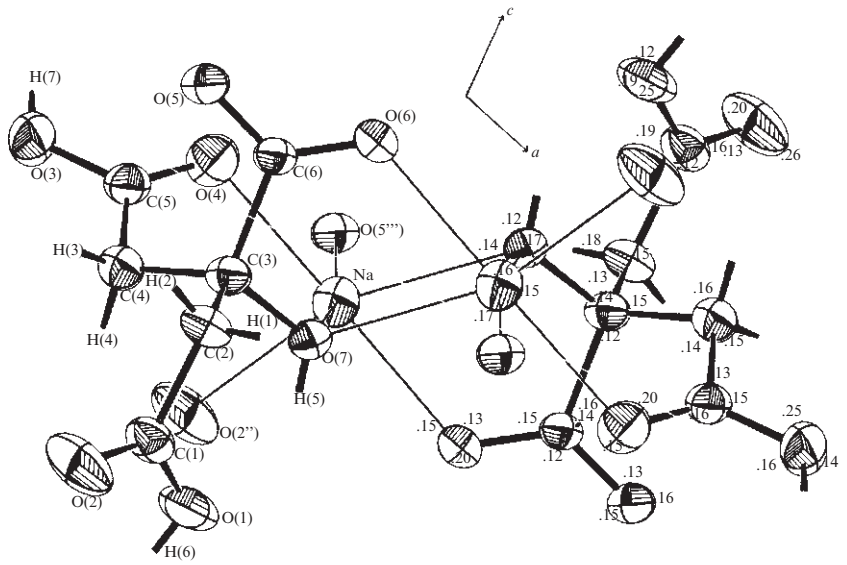

Fig. 12.7 Anisotropic molecular motion.

The anisotropic motion of atoms is usually described by "thermal ellipsoids," as in this example, taken from a study of the structure of sodium dihydrogen citrate and drawn with the program ORTEP (Johnson, 1965). Two complete dihydrogen citrate ions and two sodium ions are shown, grouped around a center of symmetry in the middle of the figure. Two atoms [O(5) and O(2)] of each of two other dihydrogen citrate ions are also shown. In order to simplify the figure, hydrogen atoms are not drawn, but their positions are labeled and the bonds to them are displayed. The thick lines represent covalent bonds; the thin ones denote coordination interactions of oxygen atoms with the sodium ion. The "thermal motion ellipsoids," calculated from the displacement factors, are drawn at 67% of the probability density function for each atom. The three numbers near each of the ellipsoids in the right half of the drawing indicate the root-mean-square displacements (Å) along the three principal axes of that ellipsoid. The anisotropy of the motion is very evident for some of the atoms, especially for those at the ends of the ion; for these peripheral atoms, the motion is always greatest in directions perpendicular to the bonding direction. This result is just what one would expect, and thus is evidence for the reality of this interpretation of the diffraction data. (From Glusker et al. (1965), p. 564, Figure 2.)

selected percentage of the probability density function for the electron density of each atom, that is, the probability of finding an electron in a defined volume of the crystal. It is noteworthy that both the degree of anisotropy and the extent of atomic motion itself vary in different parts of the citrate ion, being greatest for some of the peripheral atoms, such as O(2).

The usual way of taking this kind of ellipsoidal motion into account in the structure factor equations is by means of an anisotropic exponential factor analogous to that in Eqn. (12.3), with six anisotropic vibration parameters, b^{ij} (with superscripts in their labels), as multipliers of the indices for each reflection *hkl* in the exponent, thus:

$$e^{-(b^{11}h^2+b^{22}k^2+b^{33}l^2+b^{12}hk+b^{23}kl+b^{31}hl)} \tag{12.4}$$

Increasingly, anisotropic vibration parameters are reported as components of a symmetric tensor, U, rather than as b values, because the latter are dimensionless and their magnitudes cannot be related to vibration amplitudes without taking the cell dimensions into account.

The relation between the U^{ij} and b^{ij} values is simple:

$$U^{ii} = b^{ii}/2\pi^2 a^{*2}_i, \quad U^{ij} = b^{ij}/4\pi^2 a^*{}_i a^*{}_j \quad (i \neq j) \tag{12.5}$$

The mean square vibration amplitude in any direction, specified by the cosines l of the angles this direction makes with reciprocal axes, is given by

$$\langle u^2 \rangle = U^{11} l_1^2 + U^{22} l_2^2 + U^{33} l_3^2 + 2U^{12} l_1 l_2 + 2U^{23} l_2 l_3 + 2U^{31} l_3 l_1 \tag{12.6}$$

The anisotropic vibration parameters b^{ij} or U^{ij} differ from atom to atom in a structure. The effect of temperature is illustrated in the ellipsoids in Figure 12.8. At the lower temperature, the atoms fill less space.

This ellipsoidal description of atomic motion is a convenient one for computation, unlike more complex models that may be more realistic physically, and it has proved adequate for most structure analyses to date. It is, however, clear that the motions of atoms in crystals may frequently be more complicated; for example, the atoms may move along arcs rather than straight lines, or under the influence of an anharmonic potential function that is steeper on one side of the equilibrium position than on the other. Analysis of such motion requires the best possible data and more complete equations describing the motion (Johnson, 1969). One needs to beware of possible problems; for example, appreciable uncorrected absorption errors in a crystal of irregular shape may be compensated for by spurious anisotropy of motion of some atoms in the structure. However, by suitable choice of radiation and crystal size and shape, such absorption errors can be minimized or corrected for, and the reality of derived anisotropies of atomic motion in many structures has been firmly established.

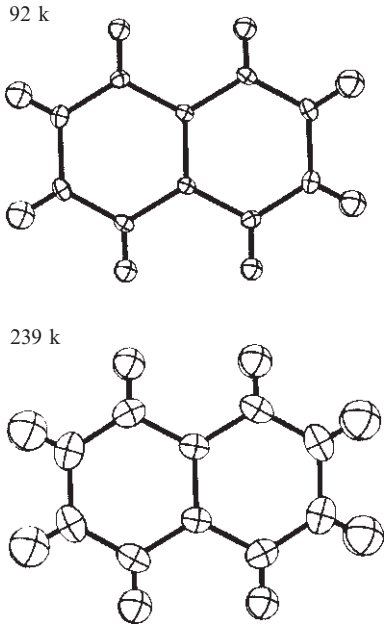

Fig. 12.8 Root-mean-square displacements at two different temperatures.

Two views of napththalene, measured with X rays at 92 K (upper diagram) and 239 K (lower diagram). Note the smaller root-mean-square displacements of the atoms at the lower temperature.

(From Brock and Dunitz (1982). Photograph courtesy C. P. Brock and J. D. Dunitz).

Rigid-body motion

Some molecules may be regarded as nearly rigid bodies, which implies that when they move the relative positions of all atoms (and consequently all interatomic distances) remain constant. The motion may thus be considered to be motion of the molecule as a whole. This is clearly only an approximation, because there are always "internal" vibrations—motion of an atom in the molecule relative to its neighbors—but in many crystals the overall motion of the molecules (or ions) is far greater than the internal vibrations. Analysis of the individual anisotropic thermal parameters of molecules in crystals sometimes reveals striking patterns of molecular motion, which can frequently be correlated with the shape of the molecule and the nature of its surroundings in the crystal. The molecular motion may, in general, be described in terms of three components: a translational motion (vibration along a straight-line path), a librational motion (vibration along an arc), and a combination of translation and libration that may be regarded as vibration along a helical path (Schomaker and Trueblood, 1968; Dunitz et al., 1988). Libration is shown in Figure 12.9.

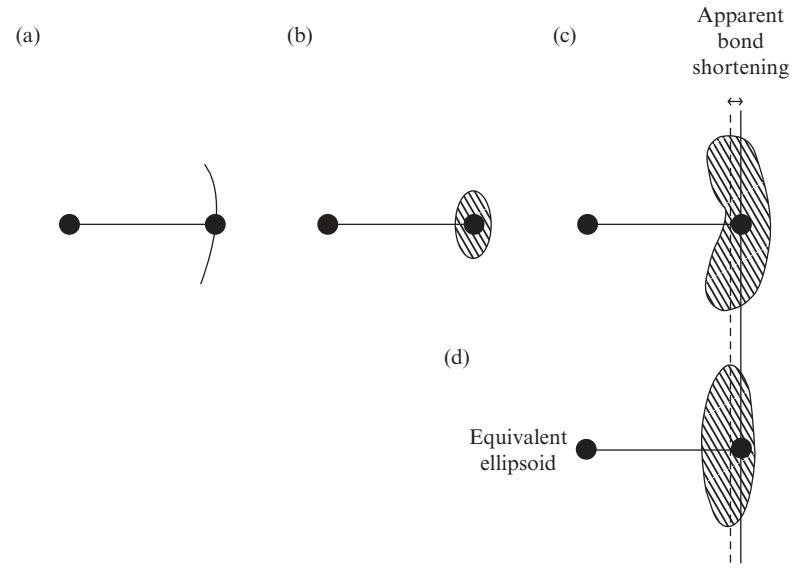

Fig. 12.9 Libration.

Libration causes apparent but not real bond shortening. The movement of the librating atom takes the form of an arc. This is, however, introduced into the structure as an ellipsoid with the result that the bond appears to be shorter, as shown.

Some molecules that are not completely rigid may be composed of segments that are themselves rigid, coupled together in a nonrigid way—for example, molecules such as biphenyl and its derivatives, with appreciable torsional oscillation about the inter-ring bond, or torsional oscillation of the methyl groups in durene (1,2,4,5-tetramethylbenzene). Methods have been developed for analysis of internal torsional motion and similar motions in many molecules, and it has been possible to obtain, from diffraction data, rough estimates of force constants for and barriers to such motions. Since bond-stretching vibrations are small, it was noted by Fred Hirshfeld that a bond length should not change much even if the two atoms composing it are vibrating. This means that the two atoms should move in synchrony along the direction of the bond, but not necessarily in other directions (Hirshfeld, 1976); the anisotropic displacement factors should reflect this condition. This is shown (especially at the higher temperature) in Figure 12.8.

One important consequence of librational motion is that *intra* molecular distances appear to be somewhat foreshortened, especially for distances that are perpendicular to axes about which there is appreciable librational motion. This is shown in Figure 12.9. Approximate corrections to intramolecular distances are not hard to make if the pattern of motion is known, but with molecules that are not rigid, the corrections are not themselves precise, and consequently the corrected distances cannot be. This is an example of a systematic error that can make the accuracy of a derived result considerably poorer than

would be implied by a statistical analysis based on the assumption that only random errors were present. Only wide limits can usually be put on *inter*molecular distances if there is appreciable molecular motion, because the correlation (if any) of the motion of one molecule with that of its neighbors is unknown.

Neutron diffraction

In many ways neutron and X-ray diffraction complement each other, since they involve different phenomena. Neutrons are scattered by nuclei (or any unpaired electrons present, the magnetic moment of the electron interacting with that of the neutron). Although there have been a few studies of the distribution of unpaired electrons (e.g., in certain orbitals of selected transition metal ions), such applications have been rare, and in most crystal diffraction studies with neutrons, all electrons are paired and the scattering of the neutrons is essentially by the nuclei present. X rays, on the other hand, are scattered almost entirely by the electrons in atoms. Hence, if the center of gravity of the electron distribution in an atom does not coincide with the position of the nucleus, atomic positions determined by the two methods will differ. Such differences are particularly noticeable for the positions of hydrogen atoms, unless X-ray data have been collected to an usually high angle corresponding to a $\sin\theta/\lambda$ of near 1.2, nearly twice as great as usual (and thus corresponding to nearly eight times as many data, if all reflections are collected). One disadvantage of neutron diffraction is that larger crystals are needed than for X-ray structure analysis in order to get sufficient diffraction intensity with the neutron flux available from the present reactors. In order to collect data on myoglobin, a crystal with minimum dimensions of 2 mm was needed. One advantage of neutrons is that they do not cause as much radiation damage as do X rays.

The amount of scattering by nuclei does not vary much (or in any regular way) with atomic number. This fact may be used to clear up some ambiguities in an X-ray study. Typical scattering-factor data for X rays and neutrons are listed in Appendix 5. Hydrogen has a negative[†] scattering factor for neutrons (as shown in Figure 12.10 and Appendix 5) while deuterium has a positive one, both quite high, so that these two isotopes may readily be distinguished; as far as X rays are concerned, they are identical (Peterson and Levy, 1952). Neutron diffraction can thus be useful in studying the structures of reaction products that have been labeled with deuterium. It is also possible with neutrons to distinguish atoms with nearly the same atomic number that cannot readily be distinguished with X rays (for example, Fe, Co, and Ni), because their scattering power for neutrons may be very different. Atomic positions for hydrogen or deuterium may be determined as accurately as those for uranium and many other heavy atoms. This is a particularly important advantage of neutron diffraction

[†] If a nucleus has a negative scattering factor, the radiation scattered by that nucleus differs in phase by $180^\circ(\cos 180^\circ = -1)$ from the radiation that would be scattered from a nucleus that has a positive scattering factor and is situated at the same position.

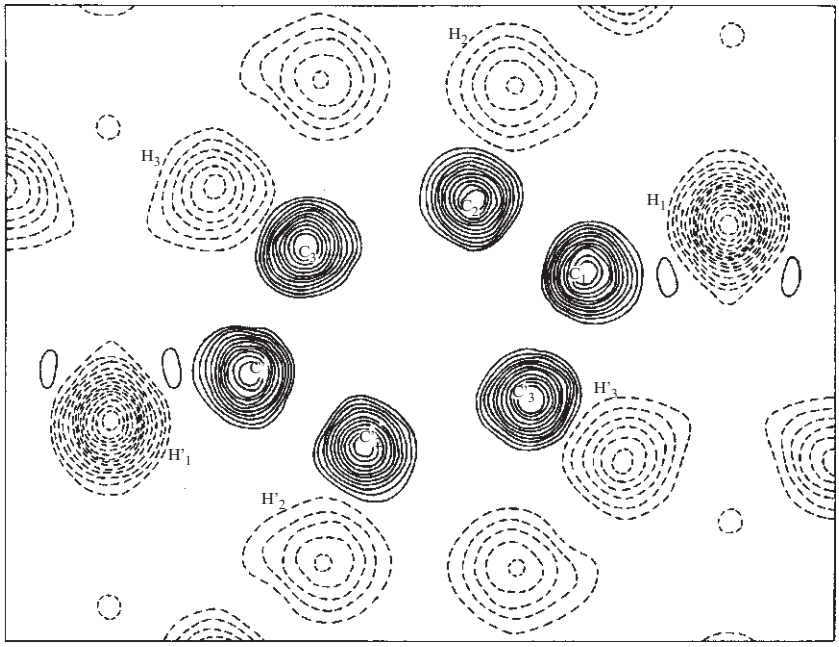

Fig. 12.10 Projection of the neutron scattering density for crystalline benzene.

Positive density (mainly at carbon atom positions) is indicated by full lines; negative density (mainly at hydrogen atom positions) by broken lines. The unlabeled hydrogen atoms are parts of other benzene molecules. The ring plane is not perpendicular to the direction of the projection; thus the ring does not appear as a regular hexagon. The deeper trough at H_1 and H_1' results from the fact that there are two hydrogen atoms superimposed on each other at these positions in this projection.

(Figure courtesy of Dr. G. E. Bacon.)

studies. There may also be anomalous scattering with neutrons, as with X rays. Since nuclei are extremely small relative to the usual neutron wavelengths, which are about 1 Å, the intensity of neutrons scattered from a stationary nucleus would not decrease markedly at high angles, as it would for X rays. Atomic vibrations, even at low temperature, will, however, cause a decrease of intensity at high angles, as with X rays (Figure 5.4).

The combined use of neutron and X-ray diffraction to solve a biochemical problem is illustrated by the analysis of the structure of lithium glycolate (Johnson et al., 1965). Deuterated glycolic acid, HO–CHD–COOH, was prepared biochemically and the structure of the lithium salt determined by X-ray diffraction methods. Since hydrogen and deuterium have the same atomic number they were each located but could not be distinguished by this X-ray method. Crystals of the lithium salt were prepared using lithium hydroxide enriched with the isotope of atomic weight 6. It was then possible to determine the absolute configuration of the lithium salt by neutron diffraction because the scattering amplitude of ^{6}Li is anomalous to neutrons ($0.18 + 0.025i \times 10^{-12}$ cm) and the scattering amplitudes of hydrogen and deuterium (-0.378 and $+0.65 \times 10^{-12}$ cm, respectively) are so

different. This then identified which hydrogen in the molecule was H and which was D and also established the absolute configuration of this glycolate stereoisomer that is acted on by the enzyme lactate dehydrogenase.

Studies of proteins can yield a wealth of structural information because deuterium and hydrogen can be distinguished, and therefore the ionization state of the functional groups in a protein can be found. If the conditions, such as the pH of the crystallization medium, are changed, then the effect of the change on these ionization states will be helpful in understanding how an enzyme accommodates to substrate or inhibitor binding and how hydrogen atoms move throughout the active site. For example, a lysine group may have two or three hydrogen atoms attached to its terminal nitrogen atom; both situations have been seen in neutron studies of the enzyme xylose isomerase (Katz et al., 2006).

Deformation density and difference density studies

The disposition of the electron density in a molecule is of particular interest to chemists since it provides information on what keeps the atoms together in a molecule. The valence-electron scattering of X rays is mainly concentrated in Bragg reflections with low $\sin\theta/\lambda$ values. In order to view the valence-electron density by means of difference electron-density maps, it is necessary to obtain precise and unbiased positional and temperature parameters; this requires high-order data, for which the spherical-atom approximation is more closely valid. When diffraction data are measured to the maximum scattering angles for shorter-wavelength X rays, such as MoKα radiation (λ = 0.7107 Å) or, even better, AgKα radiation (λ = 0.5609 Å), and especially when measurements are made at low temperatures, a large number of experimental data result and the structure perceived in the X-ray experiment—that is, the electron density—is seen at much higher resolution; atoms are therefore located with very high precision.

Some information on the detailed electron distribution in molecules may be obtained by high-resolution X-ray diffraction studies, particularly if the results are combined with neutron diffraction studies. It is possible to look at bonding effects that occur when atoms combine to form molecules. For example, a "deformation density" map may be obtained by calculating the difference electron density between the experimental map and that calculated from the "promolecule" electron density obtained from a model consisting of spherical atoms. This and the other maps described here are affected by the precision of the data used to obtain them and the correctness of the proposed structures. Superpositions that involve computing either an "X − X" map (a difference map using atomic positions from an analysis of only the

high-order X-ray diffraction data) or an "X − N" map (a difference map using atomic positions from a neutron diffraction analysis, and hence atomic nuclear positions) are used to examine the differences between the map from experimental data and that from the promolecule. There are some differences in results from X-ray and neutron studies, and therefore the same displacement parameters (generally from the neutron structure) are used with both the X-ray and neutron atomic coordinates. It has already been pointed out that X-ray diffraction studies give information on the electron density throughout the crystal while neutron diffraction studies give information on atomic nuclei. Therefore the difference between the two maps obtained will contain peaks in positions expected for bonding electrons and for lone pairs of electrons. For several molecules that have been studied (e.g., oxalic acid), quite good agreement exists between the experimental deformation density and a theoretical one, provided the latter model is sufficiently sophisticated [i.e., an extended basis set is used in the theoretical calculation (Pople, 1999)]. For example, the centroid of the electron density of a hydrogen atom is displaced from the nucleus (defined by neutron data) toward the atom it is linked to, as expected for chemical bonding. The future of this area of analysis is bright (Coppens, 1997; Dittrich et al., 2007).

Summary

Molecular geometry

This may be computed from the unit-cell dimensions and symmetry and the values of x, y, and z for each atom that have been derived from electron-density maps or by least-squares methods. Bond lengths, interbond angles, torsion angles, least-squares planes through groups of atoms, and the angles between such planes give much useful chemical information. It is common for crystal structures to be displayed in publications as stereopairs.‡

‡ Such stereodiagrams can be viewed with stereoglasses or the reader can focus on the two images until an image between them begins to form. The reader should allow his/her eyes to relax until the central image becomes three-dimensional. This process requires patience and may take 10 seconds or more.

Atomic and molecular motion and disorder

The fall-off in intensity with increasing scattering angle becomes more pronounced with increasing vibrations of atoms. Atomic vibration itself becomes greater as the temperature of the specimen rises. For spherically symmetrical motion, the reduction in intensity is simply represented by an exponential, $e^{-2B_{iso}[(\sin^2\theta)/\lambda^2]}$. Thermal motion is frequently represented by more sophisticated models, such as an ellipsoid. Atomic disorder can also provide intensity fall-off. With both atomic vibration and disorder the effective size of the atom, which is an average of all such atoms in the crystal, appears to be increased in volume while keeping the same number of electrons within that volume.

Neutron diffraction

Neutrons are scattered by atomic nuclei or by unpaired electrons; X rays are scattered significantly only by the electrons in atoms. Scattering factors for neutrons do not vary systematically with atomic number or atomic weight. Neutron diffraction studies can often clear up ambiguities in X-ray work, and, when the two methods are compared, may give information on the electron distribution that is due to chemical bonding in the molecule. Neutron diffraction is used in protein structural studies, often after an enzyme has been soaked in D_2O in order to insert deuterium in the place of labile hydrogen atoms. The deuterium atoms can be located in the protein electron-density map and therefore it is possible to determine how many (and the percentage of each) H or D atoms are on the oxygen, nitrogen, or sulfur atoms of side chains; this means that neutron crystallography provides a probe of the location of an H or D atom in a hydrogen bond and hence the local pH in a protein (for example, distinguishing $–NH_2$ from $–NH_3^+$).

13 Micro- and noncrystalline materials

The crystalline state is characterized by a high degree of internal order. There are two types of order that we will discuss here. One is *chemical order*, which consists of the connectivity (bond lengths and bond angles) and stoichiometry in organic and many inorganic molecules, or just stoichiometry in minerals, metals, and other such materials. Some degree of chemical ordering exists for any molecule consisting of more than one atom, and the molecular structure of chemically simple gas molecules can be determined by gaseous electron diffraction or by high-resolution infrared spectroscopy. The second type of order to be discussed is *geometrical order*, which is the regular arrangement of entities in space such as in cubes, cylinders, coiled coils, and many other arrangements. For a compound to be crystalline it is necessary for the geometrical order of the individual entities (which must each have the same overall conformation) to extend *indefinitely* (that is, apparently infinitely) in three dimensions such that a three-dimensional repeat unit can be defined from diffraction data. Single crystals of quartz, diamond, silicon, or potassium dihydrogen phosphate can be grown to be as large as six or more inches across. Imagine how many atoms or ions must be identically arranged to create such macroscopic perfection!

Sometimes, however, this geometrical order does not extend very far, and microarrays of molecules or ions, while themselves ordered, are disordered with respect to each other on a macroscopic scale. In such a case the three-dimensional order does not extend far enough to give a sharp diffraction pattern. The crystal quality is then described as "poor" or the crystal is considered to be *microcrystalline*, as in the naturally occurring clay minerals.

On the other hand, in certain solid materials the spatial extent of geometrical order may be less than three-dimensional, and this reduced order gives rise to interesting properties. For example, the geometrical order may exist only in two dimensions; this is the case for mica and graphite, which consist of planar structures with much weaker forces between the layers so that cleavage and slippage are readily observed. In a similar way, certain biological structures such as membranes and

micelles have less than three-dimensional order. Sometimes, however, geometrical order can be increased by external forces. For example, "liquid crystals" can be *temporarily* aligned in three dimensions by externally applied electric or magnetic fields (hence their use in liquid crystal displays in watches, computers, and other instruments). Even less geometrical order is shown by fibers such as silk, hair, and some long-chain polymers that have essentially only one-dimensional order.

Many times there is no evident geometric order beyond the immediate near-neighbor environment of the fundamental building unit. This is characteristic of liquids, glasses, and rubbers, whose spherically symmetrical diffraction patterns indicate that in no direction in space is there geometric order extensive enough to define a period. Such materials are described as *amorphous* and the only regularities seen in the diffraction pattern are those due to recurring bond distances. Thus diffraction patterns from amorphous materials provide information about interatomic distances only when a particular distance stands out from the average of all—usually because it is heavily weighted either by frequent occurrence or by involvement of atoms with scattering factors that are large relative to those of the other atoms present, but occasionally simply because it is unique, with no other distances of comparable magnitude occurring in the sample.

Liquid diffraction

Careful diffraction studies of liquids have provided much valuable structural information on time-averaged interatomic distances; these are spherically symmetrical in space and therefore are generally represented by radial distribution functions, that is, radially averaged electron-density maps. Examples, calculated from the diffraction patterns of water at various temperatures, are shown in Figure 13.1. These show the expected interatomic distances (O–H, O…O, and H…O) and the effects of neighboring molecules, which change as the temperature is raised.

Glass diffraction

Traditional glass, used throughout history to construct containers, windows, and ornaments, is made by fusion of a mixture of lime, silica, and soda and subsequent blowing or pressing of the product into the desired shape. Such glass is, of course, solid at ordinary temperatures. Glass stemware made from it is often referred to as "crystal" in spite of the fact that it is not crystalline. Its diffraction pattern has a halo-like appearance, resembling the diffraction pattern of a liquid; this demonstrates clearly that it is not crystalline and that there is

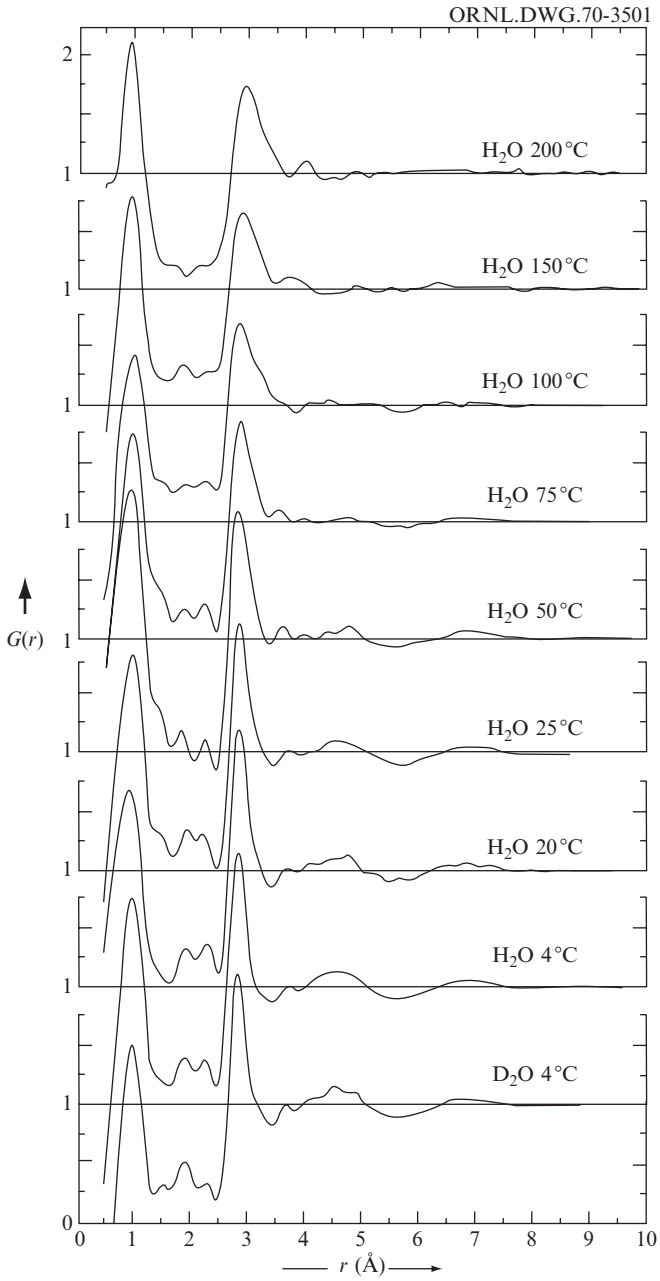

Fig. 13.1 Radial distribution functions.

Radial distribution curves obtained by X-ray diffraction studies on liquid water at temperatures from 4° C to 200° C are shown. Sample pressures were atmospheric up to 100° C; above 100° C, the pressure was equal to the vapor pressure. The vertical coordinate, $G(r)$, for the curves represents a normalized radial distribution function; that is, it gives information on the number of neighbor atoms or molecules at a distance r from an average atom or molecule in the system compared with that expected for a liquid without distinct structure.

no well-defined geometrical order within it. The best model to date of such glass consists of random chains, nets, and three-dimensional arrays of SiO_4 tetrahedra, linked together through oxygen atoms, with appropriately situated cations. Many attempts have been made to fit models with different kinds of short-range order to the observed diffraction patterns and to other quantitative physical and chemical data available on various glasses. This is done in an effort to define more precisely what might be meant by "the structure of glass" (Warren, 1940; Tanaka et al., 1985).

In contrast to the traditional glasses that are the products of fusion and can be "thawed" and reworked without crystallizing, there are now known to be many other glass-forming composition systems and, as a result, there are several ways of generating glasses and other amorphous materials. Each of these gives rise to properties that are useful. For example, amorphous metal films can be made by "splat cooling"—that is, a jet of liquid metal is directed onto a cold surface and therefore is cooled to a solid so rapidly from the melt that it has been deprived of the time required for crystal organization. Another industrial example is provided by the use of a chemical reaction in the gas phase to generate an extremely fluffy amorphous "soot" that may be sintered and compressed to three-dimensional solidity without crystallizing. Optical-waveguide–laser communication technology depends in large measure on the purity, composition control, and perfection of such processes, achievable by starting with pure gases, such as silicon tetrafluoride and oxygen, and reacting them to form a condensed phase of pure silica "soot" where, presumably, the surface is both highly energetic and unique such that particles "join" under pressure without melting (sintering) to form a continuum; such sintering without melting precludes the possibility of any crystallization. A third example is provided by glass-ceramics, which, although noncrystalline as formed, cannot be heated to the softening point because they undergo crystallization from the solid state; this crystallization must be controlled carefully in order to obtain a glass-ceramic with the desired physical properties.

The peak near 1 Å represents the intramolecular O–H interaction and that at 2.9 Å represents hydrogen-bonding interactions between oxygen atoms of neighboring water molecules. A sequence of broad peaks follows, notably those near 4.5 Å and 7 Å, and they may be attributed to preferred distances of separation for second and higher coordination shells. At distances large compared with atomic dimensions, and also with increasing temperature, the values of $G(r)$ merge to unity—that is, to the value for a structureless liquid.

In liquid water the average coordination in the first shell represents about 4.4 molecules (independent of temperature), compared with exactly 4 molecules in ice, in support of the idea that the increase in density when ice melts is due to a small increase in the average coordination number in the first coordination shell. Other details in the distribution curves are compatible with an approximately tetrahedral coordination of molecules, as found in ice.

The curves were kindly provided by Dr. A. H. Narten from Oak Ridge National Laboratory Report 4378, June 1970.

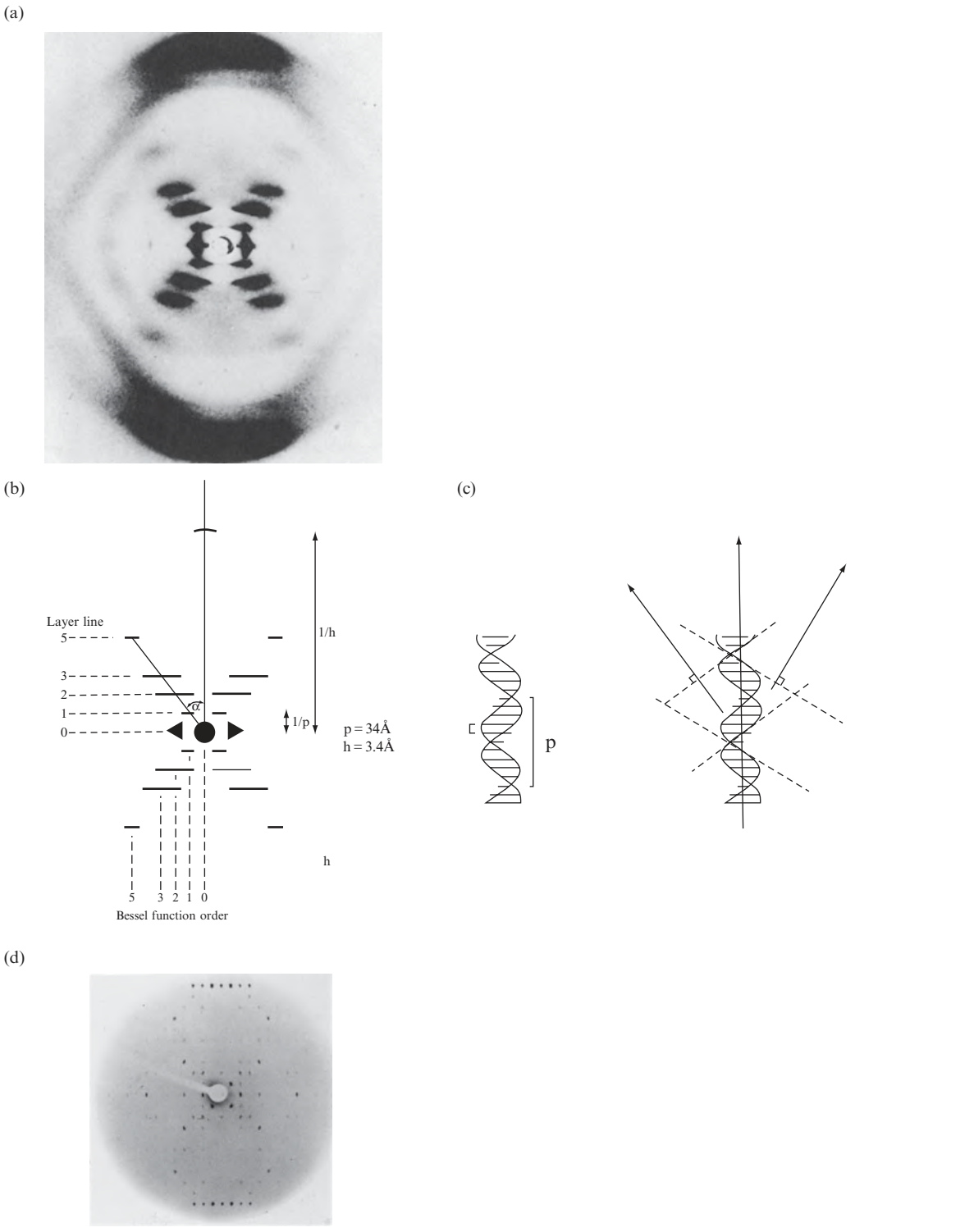

Fig. 13.2 Some diffraction patterns of DNA and polynucleotides.
Diffraction patterns of DNA and of a synthetic polynucleotide. Each diffraction photograph has been taken with the fiber axis vertical.

Fiber diffraction

Fibers have disordered strands aligned within them along the fiber axis (the meridian). If the fiber is rotated about this axis the diffraction pattern does not change much. The diffraction patterns in Figure 3.9 show the effect on the diffraction pattern of partial but incomplete internal order. Figure 3.9d displays quite effectively the result of one-dimensional internal order (characteristic of certain fibers), with elongated streaks instead of spots on the photograph. Many fibers are composed of units with helical structures, with some order along the axis of the helix, but often little order in the packing of adjacent helical units. DNA, certain fibrous proteins, and many other natural and synthetic materials have such structures. An X-ray photograph of DNA is shown in Figure 13.2a; note that the fiber axis is vertical in Figure 13.2, but horizontal in Figure 3.9d.

The coordinates of the atoms in a helical structure are best described by cylindrical polar coordinates, and the scattering factor of a cylindrical system is most appropriately represented in terms of Bessel functions. A zeroth-order Bessel function is high near the origin and then dies away like a ripple in a pond, while higher-order Bessel functions are zero at the origin and then rise to a peak at a distance proportional to their order and then die away, again like a ripple. These Bessel functions are used in calculating the Fourier transform of a helix, which describes the scattering pattern of the helix. The "cross" that is so striking in Figure 13.2a is characteristic of helical diffraction patterns. The diffraction pattern is analyzed in Figure 13.2b and its relationship to DNA structure is shown in Figure 13.2c. Because the helix is periodic along the axial direction, layer lines are formed. Two chief pieces of information may be derived from such a photograph as that in Figure 13.2a. These are the distance between "equivalent" units of the helical structure

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(a) B-DNA, the diffraction of which is illustrated, is a form of DNA in which the individual molecules are packed together less regularly. This fibrous noncrystalline form is that for which Watson and Crick first proposed their famous DNA helical structure. The fibers are randomly oriented around the fiber axes, and a helical diffraction pattern with a characteristic cross is obtained. Remember that short spacings in reciprocal space (the diffraction photograph) represent large spacings in real space. The peaks at the top and bottom of the photograph represent the stacked DNA bases, 3.5 Å apart. The "cross" represents spacings between the turns of the helix. (Photograph courtesy of Dr. R. Langridge.) (Langridge et al., 1957.)

(b) Analysis of the diffraction pattern of DNA shown in Figure 13.2a.

(c) DNA structure showing the stacked bases and the phosphodiester backbone. Periodicities in the structures of both of these are seen in the diffraction photograph.

(d) Precession photograph of a crystalline decameric polynucleotide CGAQTCGATCGn (Grzeskowiak et al., 1991). This photograph is a sampling of the fiber diffraction pattern in (a). Therefore it is clear which is the direction of stacked bases (vertical). (Photograph courtesy of Dr. Richard E. Dickerson.)

(for example, the base pairs in DNA) and the distance along the helix needed for one complete turn. From these two data the pitch of the helix can be deduced (see Watson and Crick, 1953; Franklin and Gosling, 1953; Wilson, 1966; Holmes and Blow, 1965; Squire, 2000).

The diffraction pattern of a crystalline dodecameric fragment of DNA is shown in Figure 13.2d (Dickerson et al., 1985; Grzeskowiak et al., 1991). Note that Figure 13.2d represents a sampling of the diffraction pattern in Figure 13.2a, so that one immediately knows the orientation of the molecules in the crystal (for example, the fiber direction). High-resolution studies of polynucleotides have provided much information on nucleic acid structure and function.

Small-angle scattering

Structural features that are large compared with the wavelength of the radiation being used cause significant scattering only at small angles (Figures 3.1 and 5.4). "Small-angle scattering" at angles 2θ no larger than a few degrees is thus used to measure long-range structure. For example, for a biological macromolecule it may be used to measure the radius of gyration and to study the hydration of the macromolecule. It has been widely applied to the study of liquids, polymers, liquid crystals, and biological membranes. The radiation used may be X rays (small-angle X-ray scattering, SAXS) or neutrons (small-angle neutron scattering, SANS). The method is very useful because it can provide information on partially or totally disordered systems. Therefore particles can be studied under physiological conditions (Guinier and Fournet, 1955; Brumberger, 1994; Koch et al., 2003; Kasai and Kakudo, 2005).

Powder diffraction

The diffraction pattern of a powder (packed in a capillary tube) may be considered that of a single crystal but with the pattern of the crystal in all possible orientations (as are the crystallites in the capillary tube). Powder diffraction is an extremely powerful tool for the identification of crystalline phases and for the qualitative and quantitative analyses of mixtures (Cullity, 1978). It is used for analysis of unit-cell parameters as a function of temperature and pressure and to determine phase diagrams (diagrams showing the stable phases present as a function of temperature, pressure, and composition). A very useful compilation

of common powder diffraction patterns, the Powder Diffraction File (PDF), is maintained by the International Centre for Diffraction Data (ICDD). This file contains d-spacings (related to angle of diffraction) and relative intensities of observable diffraction peaks. A comparison of a powder diffraction pattern obtained experimentally with the highest diffracted intensities of some powder diffraction patterns in the file, a search that can be done by computer, will often reveal the chemical composition of a powder. Thus, the method is of great importance industrially and forensically. For example, the composition of particles in an industrial smokestack may be determined by analysis of the diffraction pattern. Other useful information can also come from powder diffraction studies. For example, an analysis of profile broadening (Figure 13.3) can lead to an estimate of average crystallite sizes in the specimen.

Powder methods may even be used for simple structural studies. There are now sophisticated methods, originally introduced by Hugo Rietveld in 1967, for the adjustment of parameters to give the best fit with an experimental powder diffraction pattern (Rietveld, 1969; Young, 1993; Jenkins and Snyder, 1996). The technique is now used for the structure determination of simple structures and can provide precise unit-cell dimensions, atomic coordinates, and temperature factors in the same way that crystal diffraction studies do. The Rietveld method is, of course, of great value when suitably large crystals cannot be grown. It uses a least-squares approach to obtain agreement between a theoretical line profile and the measured diffraction profile. The introduction of this technique was a significant step forward in the diffraction analysis of powder samples as, unlike other techniques at that time, it was able to deal reliably with strongly overlapping reflections. Larger and larger structures are now being tackled.

Summary

Studies of structures that are not fully crystalline

The diffraction patterns of liquids and glasses are spherically symmetrical and only radial information can be obtained. However, from substances exhibiting partial order, more information may be derived. For example, for a helical structure, the pitch of the helix and the repeat distance along it can be deduced.

Powder diffraction

The diffraction pattern of a powder also gives only radial information, since the powder contains crystallites in all possible orientations.

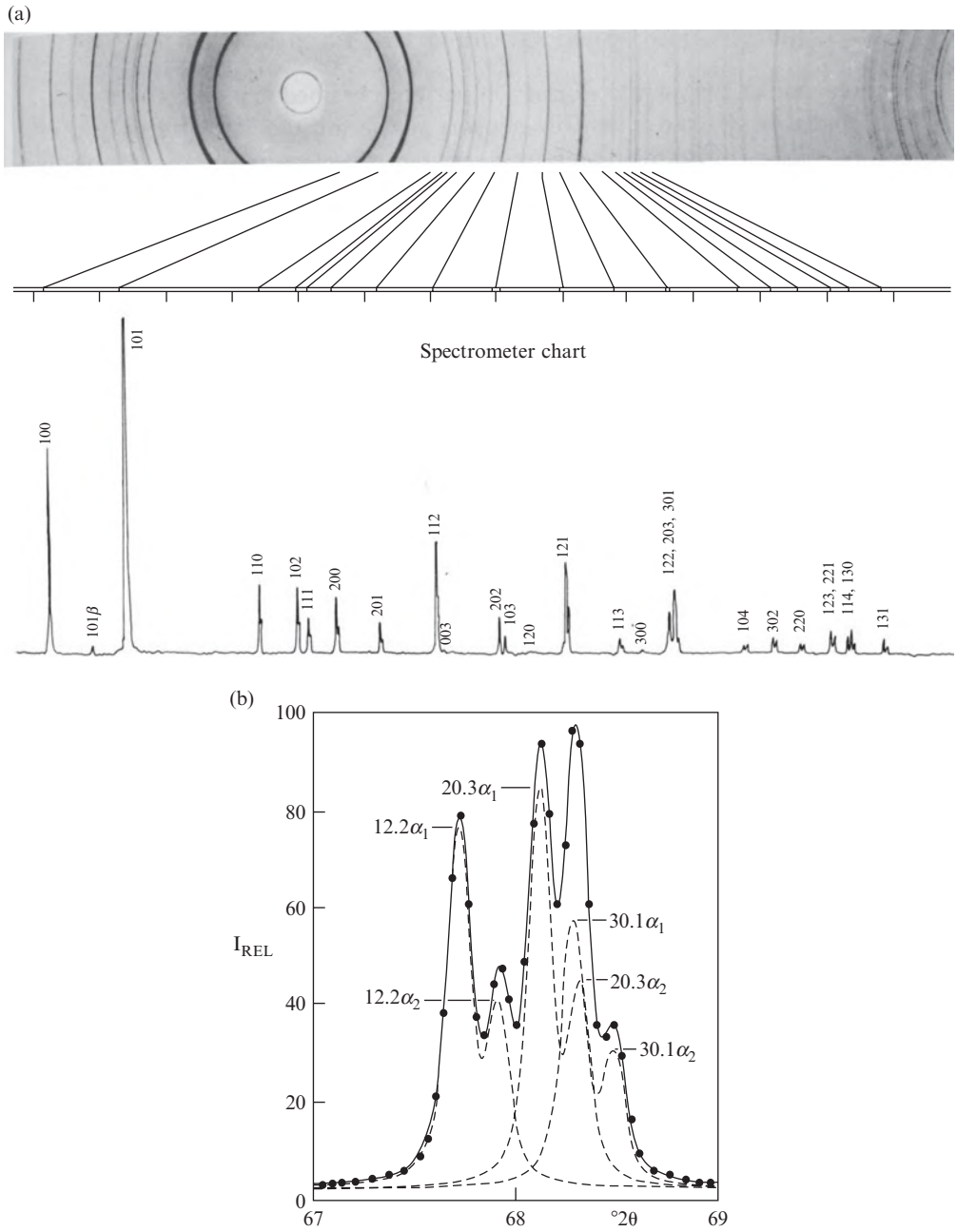

Fig. 13.3 Powder diffraction.

(a) Comparison of an 11.46 cm diameter powder camera film (upper photograph) with a scanned diffractometer pattern of quartz (with copper Kα radiation).

(b) Profile fitting of a portion of the diffraction pattern of quartz. The dots are experimental points from step-scanning and the dashed lines are the individual results for each reflection. The sum is represented by a solid line. In this figure the peak identifications "12.2," "20.3," and "30.1" represent, respectively, the 122, 203, and 301 Bragg reflections for this crystal. Note the separation of the α_1 and α_2 wavelengths of the radiation (wavelengths 1.5405 Å and 1.5443 Å, respectively).

(Photographs and diagram courtesy of Dr. William Parrish.)

Powder diffraction is used for the identification of crystalline phases and for the qualitative and quantitative analysis of mixtures. When suitable crystals are not available, the Rietveld method has made evident the power of powder diffraction to determine three-dimensional crystal structures that otherwise could not have been studied.

14 Outline of a crystal structure determination

Small-molecule crystals

The stages in a crystal structure analysis by diffraction methods are summarized in Figure 14.1 for a substance with fewer than about 1000 atoms. The principal steps are:

(1) First it is necessary to obtain or grow suitable single crystals; this is sometimes a tedious and difficult process. The ideal crystal for X-ray diffraction studies is 0.2–0.3 mm in diameter. Somewhat larger specimens are generally needed for neutron diffraction work. Various solvents, and perhaps several different derivatives of the compound under study, may have to be tried before suitable specimens are obtained.
(2) Next it is necessary to check the crystal quality. This is usually done by finding out if the crystal diffracts X rays (or neutrons) and how well it does this.
(3) If the crystal is considered suitable for investigation, its unit-cell dimensions are determined. This can usually be done in 20 minutes, barring complications. The unit-cell dimensions are obtained by measurements of the locations of the diffracted beams (the reciprocal lattice) on the detecting device, these spacings being reciprocally related to the dimensions of the crystal lattice. The space group is deduced from the symmetry of, and the systematic absences in, the diffraction pattern.
(4) The density of the crystal may be measured if the crystals are not sensitive to air, moisture, or temperature and can survive the process. Otherwise an estimated value (about 1.3 g cm^{-3} if no heavy atoms are present) can be used. This will give the formula weight of the contents of the unit cell. From this it can be determined if the crystal contains the compound chosen for study, and how much solvent of crystallization is present.
(5) At this point it is necessary to decide whether or not to proceed with a complete structure determination. The main question is, of course, whether the unit-cell contents are those expected. One must try to weigh properly the relevant factors, among which are:

(i) Quite obviously, the intrinsic interest of the structure.

(ii) Whether the diffraction pattern gives evidence of twinning, disorder, or other difficulties that will make the analysis, even if possible, at best of limited value. This will depend in part on the type of information sought.

If the answer to (ii) is unfavorable, another crystal specimen or polymorph (with a different crystalline form) may be sought. However, under happy circumstances, one can proceed.

(6) Once a decision has been made to proceed, the next stage is to record, usually with a diffractometer equipped with an area detector (e.g., CCD or imaging plate), the locations and intensities of the accessible diffraction maxima. The intensities must then be appropriately correlated, averaged, and multiplied by various geometrical factors to convert them to relative values of $|F|$. For a typical molecular structure, there may be between 10^3 and 10^4 unique diffraction maxima to be measured, or even more with a very large molecule. The normal time involved in the collection and estimation of these intensity data is from a few hours to several days, the exact amount depending on the equipment available and the experience and other concurrent obligations of the experimenter. The data processing is done with a computer as are all subsequent steps, appreciably reducing the necessary time involved in the analysis.

(7) Next it is necessary to attempt to get a "trial structure" or approximate relative phases. Generally, direct methods and Patterson methods are carried out with a computer-based "black box," indicated by shading in the flow chart. The excellent software now available will make most of the necessary structure solution decisions that the user requires. However, if problems arise, an understanding of the entire process will be necessary (hence this book). If all goes well, the normal procedure is to try some of the direct-methods programs, or to calculate a three-dimensional Patterson map with the aim of finding any heavy atom(s), or some recognizable portion of the molecule that may be present. Meanwhile, measurement of diffraction data on other related compounds whose crystal structures may prove easier to solve (if this one is unusually stubborn) should be considered; every laboratory has its collection of unsolved structures, some of which yield to new and improved methods or brighter minds that come along, and a few of which persist indomitably against all challengers.

(8) Hydrogen atoms, which are weak diffractors of X rays, are often visible in a difference electron-density map. Alternatively, their positions can often be calculated. Refinement (usually by a least-squares method) may then be carried out. One way to ensure that hydrogen atoms are correctly placed is to do a neutron diffraction study on a deuterated specimen.

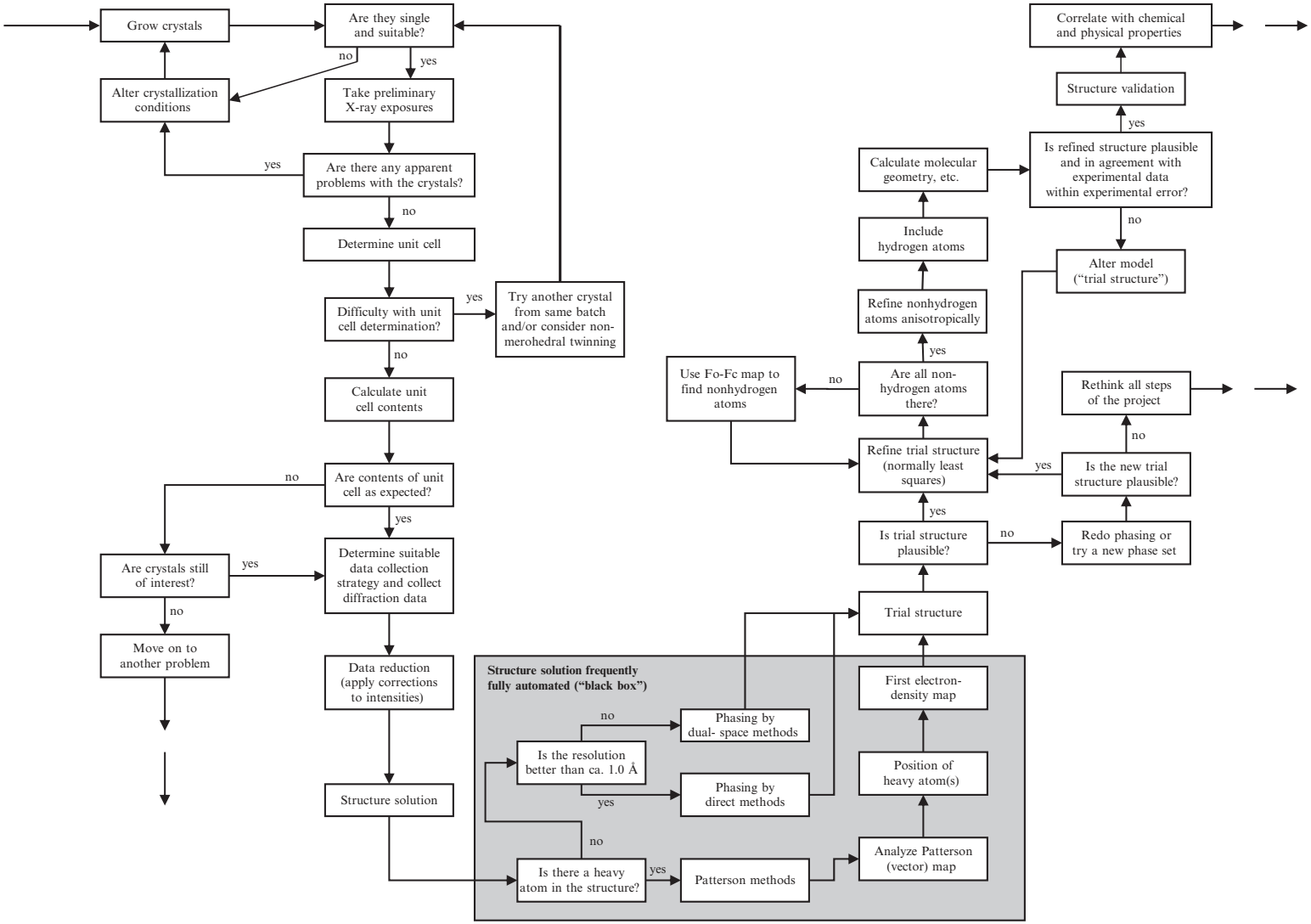

Fig. 14.1 The course of a structure determination by single-crystal X-ray diffraction.
Flow diagram for determination of small structures (10^2 or fewer atoms per asymmetric unit).
(We are grateful to Dr. Peter Müller for help in the preparation of this diagram.)

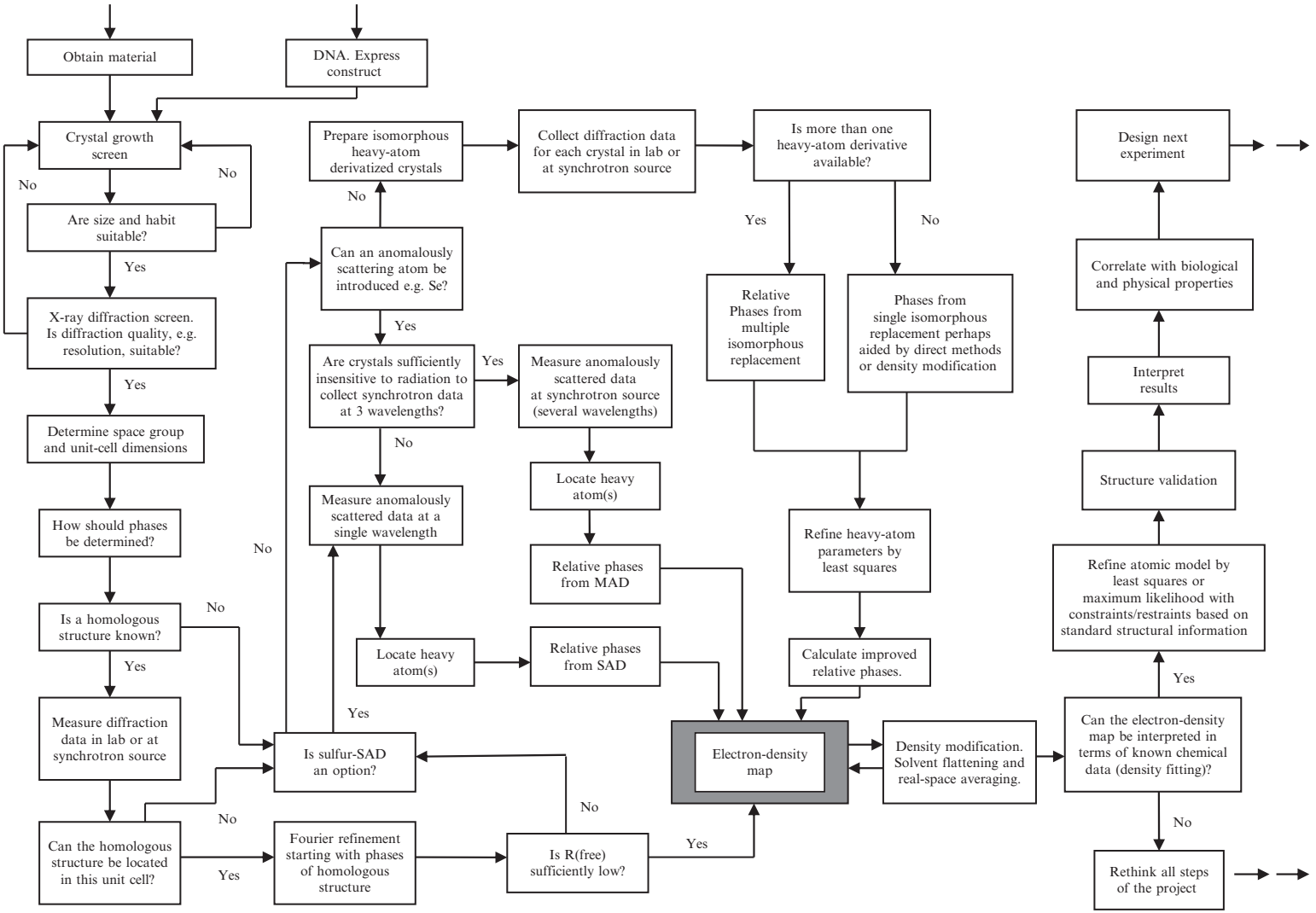

Fig. 14.2 The course of a structure determination by single-crystal X-ray diffraction.
Flow diagram for determination of macromolecular structures (10^3 or more atoms per asymmetric unit).
(We are grateful to Drs. David Eisenberg and Peter Müller for help in the preparation of this diagram.)

(9) A satisfactory trial structure is one that is chemically plausible and for which there is good agreement between observed and calculated structure factors. It must then be refined, as discussed earlier. The resulting structure should have an R index [Eqn. (6.9)] consistent with the precision of the data that were collected, and should meet the criteria discussed earlier under the heading "The correctness of a structure" in Chapter 11 (see Müller, 2009).
(10) When the refinement is complete, the molecular geometry can be calculated and analyzed.
(11) One by-product of a complete and successful structure analysis of an optically active material can be a determination of its absolute configuration, provided that it contains an atom that absorbs sufficiently the X rays being used. This technique has been applied to many organic natural products and was discussed and illustrated in Chapter 10.

Macromolecular crystals

When a macromolecule is crystallized, somewhat different techniques are used to determine its structure (Figure 14.2). The principal steps are:

(1) The material is obtained either by extraction from a biological or chemical specimen, or, if it is a protein, by cloning its gene into a high-expression system. The material so produced needs to have been carefully purified; mass spectrometry and electrophoretic techniques help here. Suitable single crystals are then (hopefully) grown by vapor diffusion of solvent or related methods (Chapter 2 and Figure 2.1). If a suitable crystal is obtained, it is mounted, ready for diffraction studies.
(2) The unit-cell dimensions, space group, and density are determined. These will indicate if the analysis is feasible or not. Sometimes a subunit of an enzyme or other large macromolecule is the asymmetric unit. This should make the structure analysis feasible. On the other hand, it sometimes happens that several molecules comprise the asymmetric unit. This is not always unfortunate, because the resulting additional symmetry in the Patterson function may provide valuable help in solving the structure.
(3) Then it is necessary to assess the degree of order in the crystal under study. This is determined by the measurable Bragg reflections at the highest $\sin\theta/\lambda$ values (which indicate the expected resolution of the measured structure). It must then be decided whether the ultimate resolution will be sufficient to provide information about the detailed structure. If the resolution is

poor, one must try to grow better crystals or look for another source of the biological macromolecule (e.g., a different animal or bacterium).

(4) The next question is whether there is a homologous structure already reported in the crystallographic literature. The structure being sought (the homologous structure) probably has approximately the same amino acid sequence and similar enzymatic activity to the protein investigated (the protein under study). To find out if there is such a homologous structure in the crystallographic literature, it is necessary to search the Protein Data Bank; this is available on the World Wide Web. If such a homologous protein can be found, it is assumed that the foldings of both proteins (the homologous protein and the protein under study) are similar. Therefore diffraction data for the protein under study are measured. An attempt is then made, usually by Patterson methods, to determine the location of the homologous protein molecule in the unit cell of the protein crystal under study. If this works out, the phases for the crystal under study can be calculated and refined and an electron-density map produced.

(5) If no homologous structure is available, there might be an opportunity for sulfur-SAD phasing if sulfur is present in the molecule. This method is currently used frequently and it does not require any heavy metals or homologous structures, only good data to 2.5 Å resolution. Single-wavelength anomalously scattered X-ray data plus direct methods (to locate the sulfur atoms) will give phases for an electron-density map.

(6) In the absence of sulfur or a strong anomalous scatterer, it will be necessary to make conventional heavy-atom derivatives, measure the diffraction data for the native crystal and each of its heavy-atom derivatives that have been successfully crystallized, and then determine the phases by isomorphous replacement. For some proteins, side chains containing heavy atoms, such as selenium, iodine, or bromine, may be genetically engineered into them. The best heavy atoms are those that scatter anomalously with X rays from either a laboratory X-ray tube or a synchrotron source (with the possibility of X-ray wavelength tuning to required values). The heavy-atom parameters are then refined by least-squares methods. Improved phases are then derived, and an electron-density map is computed.

(7) If an atom with a strong anomalous signal can be introduced into the crystal, the measurement of anomalous data is probably the best way to go (that is, by MAD or SAD phasing). If anomalous data [i.e., $I(hkl)$ and $I(\overline{hkl})$] are an option it is necessary to determine if the crystal will survive many data collections, since X rays damage protein crystals. The single-wavelength anomalous

dispersion (SAD) method (mentioned above for sulfur-containing proteins) is used if the crystals are fragile or if it is more convenient to study them in the investigator's laboratory with standard X-ray tubes. Sturdier crystals can be studied by the multiwavelength anomalous dispersion (MAD) method, in which several data sets at different wavelengths near and far from the absorption edge of the anomalously scattering atom are measured. Selenomethione is often introduced in place of methionine in proteins and acts as the anomalous scatterer. The advantage of MAD phasing is that only one crystal is needed, but it is generally necessary to go to a synchrotron source to obtain the required X-ray wavelengths.

(8) In each of these methods, the result is an electron-density map. This is probably a good place to stress that this map does not constitute "data," and to remind the reader that the primary experimental data are the Bragg reflections. The map is totally dependent on the phases that have been input into the calculation. These phases may be improved by density modification, which includes solvent flattening for crystal structures with large areas occupied by solvent and real-space averaging for structures with noncrystallographic symmetry.

(9) If a protein crystal structure is under study, it is usual first to "trace the chain" of the polypeptide backbone. The determination of side-chain coordinates for the protein follows from a knowledge of the amino acid sequence of the protein and the fitting of a model of each amino acid to the electron density on a computer screen. Without sequence information, the analysis of the electron-density map is difficult unless phasing is good to atomic resolution (as is the case with increasingly many investigations). If the macromolecule under study is a nucleic acid, the phosphate groups and the bases are sought from the electron-density map as a preliminary to phasing the electron-density map.

(10) For an enzyme, the question of the location of the active site of the catalytic process then arises. This may often be found by soaking into native crystals either inhibitors, poor substrates (if the substrate is too good, reaction may readily occur), or cofactors. Then diffraction data are measured and a difference electron-density map is calculated using phases from both the native protein and the liganded complex. In this way the site of attachment of a substrate may be evident, suggesting that this is the active site of the enzyme. At this stage, neutron diffraction studies on deuterated proteins and/or their ligands can yield powerful information on the protonation state of each functional group under the particular experimental conditions at which the crystals formed. Therefore a combination of X-ray and neutron diffraction investigations is encouraged.

Concluding remarks

We have attempted to present enough about the details of structure determination so that an attentive reader can appreciate how the method works. As mentioned earlier, a glossary and list of references (including a short bibliography) have been included so that those interested may delve further into the subject. Do not forget to use search engines in the World Wide Web, as there are many useful articles and reprints available for study. We will now summarize by answering our initial questions.

Why use crystals and not liquids or gases?

A crystal has a precise internal order and gives a diffraction pattern that can be analyzed in terms of the shape and contents of one repeating unit, the unit cell. This internal order is lacking in liquids and gases and for these only radial information may be derived. Such information may be of use in distinguishing between possible structures, but, for detailed results in terms of molecular structure and intermolecular interactions, the analysis of crystals (or powders) is necessary.

Why use X rays or neutrons and not other radiation?

These radiations are scattered by the components of atoms and have wavelengths that are of the same order of magnitude as the distances between atoms in a crystal (approximately 10^{-10} m). Hence they lead to diffraction effects on a scale convenient for observation and measurement.

What experimental measurements are needed?

The unit-cell dimensions and the density of the crystal, and the indices and intensities of all observable Bragg reflections.

What are the stages in a typical structure determination?

These stages have been described above in detail for both small molecules and macromolecules, and further information may be obtained from the World Wide Web. The stages involve the preparation of a

crystal, the indexing and measurement of intensities in the diffraction pattern, the determination of a "trial structure," and the refinement of this structure.

Why is the process of structure analysis often lengthy and complex?

Because 50 to 100 distinct intensity measurements are needed per atom in the asymmetric unit for a resolution of 0.75 Å, because the determination of a trial structure may be difficult, because the refinement requires much computation, and because in the end so much structural information is obtained that analysis of it takes time. Many structures are readily or even automatically solved, while others, tackled by the same competent crystallographer, may take months or years to solve. It is hard for the noncrystallographer, who may have been led to believe that the determination of structure is now almost automatic, to comprehend this "never-never land" in which crystallographers occasionally find themselves while trying to arrive at a trial structure for certain crystals.

Why is it necessary to "refine" the approximate structure that is first obtained?

Because the initially estimated phases may give a poor image of the scattering matter. Since the least-squares equations are not linear, many cycles of refinement are usually necessary. By refinement, one can tell whether the approximate structure is correct and obtain the best possible atomic positions consistent with the experimental data and the assumed structural model.

How can one assess the reliability of a structure analysis?

By checking the standard uncertainties of the derived results, by considering measures of the agreement of the values of the observed $|F_o|$ with the values of the calculated $|F_c|$, by the absence of any unexplained peaks in a final difference map, and by the chemical reasonableness of the resulting structure.

We hope we have made it possible for you to read accounts of X-ray structure analyses with some appreciation of the scope and the limitations of the work described. Perhaps you are even interested enough to want to try the techniques yourself. If so, trust that this introduction serves as a useful background and reference. But also

we hope that you realize that there is more to the crystallographer's discipline than just diffraction methods. When the crystal structure is known, it is a first step in the interpretation of physical properties, chemical reactivity, or biological function in terms of the three-dimensional structures and conformations of the component molecules or ions.