International Tables for Crystallography (2006). Vol. F, Chapter 19.4, pp. 438–443.

19.4. Small-angle neutron scattering

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19.4.1. Introduction

Diffuse scatter results when X-ray and neutron beams pass through gases and liquids. It is caused by local inhomogeneities, which all fluids invariably contain, at least transiently, and information about them can be obtained by analysis of the scatter they cause. This diffuse scatter is rotationally symmetric about the direction defined by the incident beam because, on average, gases and liquids are isotropic, and it does depend on scattering angle, 2θ. The intensity of the diffuse X-ray scatter of water, for example, is small at small 2θ, and it reaches a maximum at equivalent Bragg spacings equal to the reciprocal of the average oxygen–oxygen distance. In addition to a 'water ring' at high scattering angles, the diffuse scatter of macromolecular solutions includes a peak at $2\theta = 0$, due to the presence of the macromolecules themselves. If ångstrom-wavelength radiation is used, the central macromolecular peak is entirely contained in the region where $\sin(2\theta) \simeq 2\theta$. This is the region examined in small-angle scattering experiments.

Several properties of macromolecules can be determined by analysing their small-angle solution scattering, among them molecular weight, radius of gyration and maximum linear dimension. Approximate shapes can sometimes be obtained, and if a macromolecule is a complex of different chemical species, information about the distribution of its components may emerge. Hydration and conformational changes are also studied this way.

The molecular properties that can be investigated by small-angle scattering are the same for thermal neutrons and X-rays, but the advantages of neutrons are so great that if the equipment required were not so expensive, not many would do small-angle X-ray scattering (SAXS). They are all manifestations of the differences between the ways in which neutrons and X-ray photons interact with matter. For example, thermal neutrons have very low kinetic energies ($\simeq kT$), and, consequently, the energy they deposit in a sample when they are scattered inelastically is negligible. X-ray photons have large energies, and when they are absorbed or scattered inelastically, damaging amounts of energy are deposited. Thus, samples are 'safer' in neutron beams than they are in X-ray beams.

The cross section for X-ray absorption rises so fast with increasing wavelength that it is impractical to do solution-scattering experiments using X-rays with wavelengths much greater than 1.5 Å. The combination of this and the fact that X-ray beams scatter strongly off the edges of optical-track components makes it difficult to build small-angle X-ray spectrometers that measure diffuse scatter at equivalent reciprocal spacings of 0.001 Å^{-1} or less. The cross section for thermal neutron absorption is small and nearly independent of wavelength over the range 1–10 Å. Furthermore, parasitic neutron scatter is easy to control. Thus, it is comparatively straightforward to build small-angle neutron scattering (SANS) spectrometers that measure diffuse scatter at reciprocal spacings considerably less than 0.001 Å^{-1}.

Even more important to those interested in SANS are the vistas opened up by the huge difference in scattering length that exists between ^{1}H and ^{2}H (henceforth termed H and D), to which we will return below. In brief, the scatter of macromolecular solutions can be significantly altered by replacing some or all of the H atoms with D atoms. This control greatly extends the range of problems that can be addressed by SANS, and the chemical 'cost' is minimal. A perdeuterated molecule is almost identical to its protonated counterpart. The X-ray scattering of substances depends on the number of electrons they contain, and when this number is changed, chemical properties change also.

Relative to SAXS, the sole disadvantage of SANS is a phenomenon called incoherent scatter, which is a comparatively minor aspect of X-ray work. Neutrons are scattered primarily by atomic nuclei, and if a nucleus has spin, its scattering length depends on the orientation of its spin relative to that of each neutron with which it interacts. Since nuclear spins are usually unoriented in SANS samples, this spin-orientation dependence leads to a random atom-to-atom variation in scattering length. Coherent scatter, on which all diffraction effects depend, is determined by average scattering-length values. The scatter due to fluctuations about the average is incoherent, and in the low-angle region incoherent scatter manifests itself as a featureless background that is independent of scattering angle. The cross section for incoherent scattering is very large for H atoms, and since both water and biological macromolecules contain large proportions of H atoms, incoherent scatter is often a dominant source of background.

Some useful general references for small-angle scattering in general and neutron scattering in particular are Bacon (1975), Glatter & Kratky (1982) and Guinier (1955, 1962).

19.4.2. Fundamental relationships

For most purposes, a dilute macromolecular solution can be thought of as a macromolecular gas, and for that reason it is appropriate to apply Debye's theory for gas scatter to macromolecular solutions (Debye, 1915). Debye's master equation can be cast into neutron terms as follows:

$$I(Q) \propto I_0 \sum\sum b_i b_j \sin(Qr_{ij})/(Qr_{ij}), \qquad (19.4.2.1)$$

where $I(Q)$ is the amount of scattered radiation observed at Q, I_0 is the intensity of the incident beam, b_i and b_j are the scattering lengths of the ith and jth atoms in the molecule, r_{ij} is the distance between atoms i and j, and $Q = (4\pi/\lambda)\sin\theta$, λ being the wavelength of the radiation used and θ being half the scattering angle. When applied to molecules in solution, both summations must include not only all the atoms that are covalent components of the molecule in question, but also all the associated solvent atoms, because when a macromolecule dissolves, the inhomogeneity created includes the counterions associated with it, its solvation layer *etc.* Equation (19.4.2.1) holds for a single molecule; if the number of molecules contributing to scattering in some sample is N, the scattering profile measured will be N times the profile due to a single molecule. The inhomogeneities responsible for small-angle scatter have linear dimensions of the order of 10 Å or more and, hence, have volumes that contain large numbers of atoms. In addition, interatomic spacings cannot be resolved using small-angle data. Thus, it is appropriate to discuss small-angle scattering in terms of electron densities for X-rays or scattering-length densities for thermal neutrons. The scattering-length density of a volume, ρ, is given by

$$\rho \equiv \sum b_i/V, \qquad (19.4.2.2)$$

where b_i is the scattering length of the ith atom in volume V and the summation runs over all atoms in the volume.

Recasting the Debye equation in terms of scattering lengths, one obtains

$$I(Q) \propto I_0 \int\int \rho(\mathbf{r}_i)\rho(\mathbf{r}_j)[\sin(Qr_{ij})/(Qr_{ij})]\ \mathrm{d}V_i\ \mathrm{d}V_j,$$

where $\rho(\mathbf{r}_i)$ and $\rho(\mathbf{r}_j)$ are the scattering-length densities in volume elements whose positions are described by vectors $\mathbf{r}_i$ and $\mathbf{r}_j$, $r_{ij} = |\mathbf{r}_i - \mathbf{r}_j|$, and both integrals run over the volume of the entire

macromolecule plus the surrounding perturbed volume. Both this equation and equation (19.4.2.1) implicitly assume that the medium surrounding each molecule is a vacuum, which is not true for the molecules in solution. The effect of solvent on low-angle scattering can be taken care of by subtracting the average scattering length of the solvent, ρ_0, from the scattering-length densities within the molecule. Thus,

$$I(Q) \propto I_0 \int\int [\rho(\mathbf{r}_i) - \rho_0][\rho(\mathbf{r}_j) - \rho_0]_j [\sin(Qr_{ij})/(Qr_{ij})] \, \mathrm{d}V_i \, \mathrm{d}V_j. \tag{19.4.2.3}$$

The quantity $[\rho(\mathbf{r}_i) - \rho_0]$ is a *contrast*, and, as will be shown, contrast manipulation is a major component of SANS experiments.

Equation (19.4.2.3) can be evaluated a second way, because the $(\sin x)/x$ term in the integral depends only on the distances between volume elements, not on their locations in space. Thus, a function $p(r)$ can be defined as follows:

$$p(r) \equiv \int\int [\rho(\mathbf{r}_i) - \rho_0][\rho(\mathbf{r}_i + \mathbf{r}) - \rho_0] \, \mathrm{d}V_i \, \mathrm{d}V\mathbf{r},$$

where the integral in $\mathbf{r}$ runs over all $\mathbf{r}$ such that $|\mathbf{r}| = r$, and the integral in $\mathbf{r}_i$ runs over the entire molecular volume. Written in terms of $p(r)$, equation (19.4.2.3) becomes

$$I(Q) \propto I_0 \int p(r)[\sin(Qr)/(Qr)] \, \mathrm{d}r, \tag{19.4.2.4}$$

where the integral runs from $r = 0$ to $r_{\max}$, the maximum atom-to-atom length within the molecule.

Note that if contrast was constant within a macromolecule, $p(r)$ would be proportional to the distribution of interatomic distances in the molecule, and for that reason $p(r)$ is often called the *length distribution*. Note also that $p(r)$ is simply the molecule's Patterson function, rotationally averaged about its origin. Note, finally, that $p(r)$ is the summation of a large, but finite, number of sharp, discrete interatomic distance peaks, each with its own weight. If the individual interatomic peaks in this 'length spectrum' could be assigned, *i.e.*, if the atoms responsible for each one could be identified, it would be possible to determine the three-dimensional structure of the molecule in question, save for uncertainty about its hand.

Since solution-scattering profiles can be computed by sine transformations of length distributions, it is reasonable to hope that a transformation might exist that enables one to compute length distributions once solution-scattering profiles have been measured. There is (Debye & Bueche, 1949; Debye & Pirenne, 1938):

$$p(r) \propto r \int QI(Q) \sin(Qr) \, \mathrm{d}r. \tag{19.4.2.5}$$

Two practical issues must be addressed when carrying out the operation implied by equation (19.4.2.5) because the integral it contains runs from $Q = 0$ to ∞. Firstly, scattering is never measured at $Q = 0$ due to interference with the direct beam. Secondly, the largest value of Q for which $I(Q)$ is measured is always less than ∞. The absence of data at very small values of Q is easily addressed, because a soundly based method exists for extrapolating the low-angle data to $Q = 0$ (see below). The lack of data at high Q is harder to cope with, but it can be dealt with approximately using Porod's Law (Porod, 1951, 1952) and the impact of its absence on molecular parameters deduced from small-angle data is easy to estimate. In any case, it is important to realize that length distributions represent the sum total of the information that can be extracted from solution-scattering experiments.

The problem of extrapolating small-angle data to $Q = 0$ was solved by Guinier (1939). He demonstrated that, at very small angles,

$$I(Q) \propto I(0) \exp[-(QR_g)^2/3], \tag{19.4.2.6}$$

where R_g is the radius of gyration, and

$$R_g \equiv (\{\int [\rho(\mathbf{r}) - \rho_0]|\mathbf{r}|^2 \, \mathrm{d}V\}/\{\int [\rho(\mathbf{r}) - \rho_0] \, \mathrm{d}V\})^{1/2}. \tag{19.4.2.7}$$

The origin of the vector $\mathbf{r}$ in this equation is the centre of gravity of the macromolecule's scattering-length density distribution, *i.e.*, it is the point where

$$0 = \{\int [\rho(\mathbf{r}) - \rho_0]\mathbf{r} \, \mathrm{d}V\}/\{\int [\rho(\mathbf{r}) - \rho_0] \, \mathrm{d}V\}.$$

It follows from equation (19.4.2.6) that if the lowest-angle data collected are plotted in the form $\ln[I(Q)]$ versus Q^2, a straight line should result, the slope of which is $(R_g^2/3)$ and the intercept of which at $Q = 0$ is $I(0)$. Note that data have to be obtained at scattering angles well inside the region where $I(Q) \sim I(0)/2$ in order for this formula to hold; if the data are thus obtained, a radius of gyration estimate will emerge. The radius of gyration of an object is the root-mean-squared distance between its centre of gravity and the elements of which it is composed.

As might be expected, $I(0)$ and R_g can also be computed from $p(r)$. Consider the magnitude of $I(Q)$ at $Q = 0$. Since the $\sin x/x$ term in equation (19.4.2.4) is 1 at $Q = 0$,

$$I(0) \propto I_0 \int\int [\rho(\mathbf{r}_i) - \rho_0][\rho(\mathbf{r}_j) - \rho_0] \, \mathrm{d}V_i \, \mathrm{d}V_j = \int p(r) \, \mathrm{d}r. \tag{19.4.2.8}$$

Thus, $I(0)$, the forward scatter, is proportional to the integral of the length distribution. It is easy to show that R_g equals $(M/2)^{1/2}$, where M is the second moment of $p(r)$ given by

$$M = [\int r^2 p(r) \, \mathrm{d}r]/[\int p(r) \, \mathrm{d}r]. \tag{19.4.2.9}$$

The average atom-to-atom distance in a molecule, r_{ave}, is easy to compute if $p(r)$ is known from

$$r_{\mathrm{ave}} = [\int r p(r) \, \mathrm{d}r]/[\int p(r) \, \mathrm{d}r]. \tag{19.4.2.10}$$

The reason forward scatter, $I(0)$, is interesting is its dependence on molecular weight. As equation (19.4.2.8) suggests, the forward scatter measured for a sample is proportional to N times the square of the product of the average contrast between a molecule and its solvent and the molecular volume, where N is again the number of molecules contributing to the signal observed. Since average contrasts can be estimated from chemical compositions and partial specific volumes, $I(0)$ measurements can be used to estimate molecular weights. If the $I(0)$ values of solutions of a set of molecules of similar chemical composition are compared, it will be found that $I(0)$ divided by the weight concentration of each sample is proportional to molecular weight.

This procedure for estimating molecular weights can fail. Suppose $\int [\rho(\mathbf{r}_i) - \rho_0) \, \mathrm{d}V = 0$, *i.e.*, the scattering-length density of the solvent is the same as the average scattering-length density of the macromolecule. Then $I(0)$ will be zero, the solution-scattering profile will lack a peak at small angles and no molecular-weight estimate will result. Under these conditions, the macromolecule is said to be 'contrast matched'. It is easy to contrast-match biological macromolecules in the context of SANS experiments, because all biological macromolecules that have not been labelled with ^{2}H have average scattering-length densities between those of H_2O and D_2O (see below).

19.4.3. Contrast variation

19.4.3.1. *Variation of solvent density*

The principle of contrast variation was studied in early work by Bragg & Perutz (1952), who observed that the magnitudes of low-order reflections in X-ray studies of protein crystals were reduced as the salt concentration in the solvent was raised. Following their concept, the effective scattering density of a dissolved particle is

$$\rho(\mathbf{r}) = \rho(\mathbf{r})_{\mathrm{solute}} - \rho(\mathbf{r})_{\mathrm{solvent}} = \rho(\mathbf{r})_{\mathrm{solute}} - \rho_{\mathrm{solvent}},$$

where $\rho_{\rm solvent}$ is the average solvent scattering density. If we take a mean scattering density for the particle, a mean contrast, ρ, is defined:

$$\rho = \rho_{\rm solute} - \rho_{\rm solvent}. \qquad (19.4.3.1)$$

If the solute and solvent have equal densities, they are 'contrast matched', and the scattering from the particle vanishes at zero angle. The particle will nonetheless scatter radiation at larger angles as a consequence of internal density fluctuations, which can be useful in obtaining structural information. In the case of neutron scattering, the solvent density is most often varied by mixing D_2O and H_2O to obtain different percentages of D_2O. This strategy gives a range of solvent densities that includes the densities of most biological molecules. However, biological molecules contain hydrogen atoms that exchange with solvent, so deuteration of the environment alters their scattering density to some extent (see below). In general, all hydrogen atoms not bonded to carbon are potentially exchangeable, but not all of these will actually exchange in a typical experiment.

To describe the variation of the radius of gyration with contrast, Stuhrmann derived the useful relationship (Stuhrmann, 1976; Stuhrmann *et al.*, 1976)

$$R_g^2 = R_c^2 + \alpha/\rho + \beta/\rho^2, \qquad (19.4.3.2)$$

which separates the contributions of the internal structure of the particle (ρ_I) to its radius of gyration from the contributions of the shape (ρ_c). Scattering from the internal structure is independent of contrast; scattering from the shape is contrast dependent. The shape function is defined as having a value of one inside the particle and zero outside. The total scattering density is then

$$\rho(\mathbf{r}) = \rho\rho_c(\mathbf{r}) + \rho_I(\mathbf{r}). \qquad (19.4.3.3)$$

The contrast-independent terms in the Stuhrmann equation are

$$\alpha = (1/V_c)\int \rho_I(\mathbf{r}) r^2 \, {\rm d}^3 r, \qquad (19.4.3.4)$$

$$\beta = (1/V_c^2)\int\int \rho_I(\mathbf{r})\rho_I(\mathbf{r}')\mathbf{r}\mathbf{r}' \, {\rm d}^3 r \, {\rm d}^3 r' \text{ and} \qquad (19.4.3.5)$$

$$R_c^2 = (1/V_c)\int \rho_c(\mathbf{r}) r^2 \, {\rm d}^3 r, \qquad (19.4.3.6)$$

where R_c is the radius of gyration of the shape function and V_c is its volume. The sign and magnitude of α give information on the radial density distribution of scattering density in the particle: if the outer region is higher in density than the inner region, α is positive (as, for example, in lipoproteins); if the inner region is denser, α is negative. The β coefficient represents the displacement of the centre of mass as a function of the contrast and is always positive; in real cases, β is often negligible. The Stuhrmann equation leads to a useful way to represent graphically the radius of gyration data obtained from a series of contrasts: the observed R_g^2 is plotted *versus* $1/\rho$. If β is negligible, the plot is a straight line of slope α, intercepting the $1/\rho$ axis at R_c^2. Thus, R_c is obtained by extrapolation to a point where $\rho = \infty$, and so is often termed the radius of gyration at infinite contrast. This quantity is a representation of the shape of the particle as if it had uniform internal scattering density. In a particle with two discrete regions of density, the radius of gyration for each region can be obtained from such a graph by evaluating R_g^2 where ρ is equal to the density of one region, so that R_g^2 of the non-contrast-matched region is determined. Such measurements can also be made by adjusting the solvent to match the scattering of one region to reveal the scattering of the other.

A parameter that is often useful is the contrast-match point for the particle, which reflects its overall composition including exchange.

$$\rho_M = \sum b_I/V + nd(b_{\rm D} - b_{\rm H})/V, \qquad (19.4.3.7)$$

where ρ_M, the match point, is the solvent scattering length density at which the contrast is zero and n is the number of exchanged hydrogens multiplied by d, the fractional deuteration of the water at the match point. Typically, the match point is obtained by measuring small-angle scattering at a series of D_2O:H_2O ratios, plotting each using a Guinier plot $\{\ln[I(Q) - I(0)]$ *versus* $Q^2\}$ to obtain a value for $I(0)$ by extrapolation, and then plotting $[I(0)/C]^{1/2}$ *versus* $\rho_{\rm solvent}$, where C is the particle concentration. It is often convenient to represent $\rho_{\rm solvent}$ as per cent D_2O. The plot should be a straight line, passing through zero at the contrast-match point. As noted above, the vast majority of biological molecules have contrast-match points at densities between those of H_2O and D_2O. If the particles are compositionally heterogeneous, the observed plot will be a weighted sum of the curves for each of the compositions present and will deviate from a straight line at low contrasts. Thus, the contrast-matching experiment can provide information on both composition and homogeneity.

While contrast variation is most often based on variation of the deuteration level in water, it is also possible to create variation by adding molecules to the solvent. As an example, a study of hydration layers was conducted by adding solute molecules, such as glycerol, at high concentration; the solute molecules alter the solvent scattering length density but do not penetrate the hydration layer (Lehmann & Zaccai, 1984).

19.4.3.2. *Variation of internal contrast*

A second form of contrast variation can be achieved by replacing the hydrogen atoms in biological molecules with deuterium. If, in addition, it is possible to deuterate selected regions of a particle, internal contrasts can be modified to gain information about substructures. In some cases, the opportunity arises because significant biochemical differences are present, as between the RNA and protein portions of a ribosome, the DNA and protein of a nucleosome, or the lipid and protein of a lipoprotein particle. While the intrinsic contrast may be sufficient to provide key information [as, for example, the early finding that DNA is on the outside of nucleosomes (Bradbury *et al.*, 1976; Uberbacher *et al.*, 1982)], it can be accentuated by incorporation of deuterated biochemical precursors or reconstitution from separately labelled components.

Internal contrast can be created in single molecules by differential incorporation of biosynthetic precursors or by chemical synthesis. Differential incorporation was first used to test models of bacteriorhodopsin, using the incorporation of deuterated amino acids supplied to a culture of halophilic archae (Engelman & Zaccai, 1980), and internal labelling was used to document conformations of cholesteryl esters that had been chemically labelled in key positions (see below). Reconstitution from purified components has been used to place deuterated proteins in ribosomes and in other complexes, again with the aim of creating internal contrast to enhance the information obtained in a neutron experiment.

In general, the creation of internal contrast can be viewed as a strategy for enriching the low information content of a solution-scattering experiment by building additional information into the sample. By design, it is known what has been labelled, so the scattering given by contrasting elements provides information about the relationships of the labelled parts to each other or to the particle as a whole. A particularly informative (but difficult) strategy is to use internal contrast to measure distances between locations in a molecule or complex. Such measurements are discussed below.

19.4.3.3. *Relationship of contrasting regions*

Where a particle has two regions of different scattering density, the square of the total observed radius of gyration, R_T^2, can be

obtained as the weighted sum of squares of the two individual radii, R_1^2 and R_2^2, and the square of the distance between the centres of scattering mass of the two regions, d_{12}^2, as

$$R_T^2 = f_1 R_1^2 + f_2 R_2^2 + f_1 f_2 d_{12}^2, \qquad (19.4.3.8)$$

where the f's are the fractions of the total scattering from each of the two regions at the solvent contrast being used in the experiment (Moore *et al.*, 1974). By varying the contrast, a set of differently weighted equations can be obtained, from which the individual radii and the separation can be derived. This method is an alternative to the Stuhrmann analysis described above. An example of the use of this approach, based formally on the parallel axis theorem of mechanics, is found in studies of the ribosome (Moore *et al.*, 1974). An alternative that has proved useful is to combine neutron and X-ray scattering data, since the weighting factors will differ for distinct regions, such as the RNA and protein components of the ribosome (Serdyuk *et al.*, 1979).

19.4.3.4. *The triple isotopic substitution method*

An innovation in the study of subunits in a reconstituted complex was introduced by Serdyuk *et al.* (1994), who devised a difference method to isolate the scattering from a single subunit. The method requires three particles with different deuteration levels in the subunit: one in which the subunit is heavily deuterated (contrast with the complex $= \rho$), one in which the subunit is not deuterated (0), and one in which the subunit is deuterated at an intermediate level ($\rho/2$):

$$I_1(Q) = |C|^2,$$
$$I_2(Q) = |C|^2 + 2(\rho/2)F[CS] + (\rho^2/4)|S|^2 \text{ and}$$
$$I_3(Q) = |C|^2 + 2\rho F[CS] + \rho^2|S|^2,$$

where C is the scattering amplitude of the complex, S is the amplitude of the subunit, and $F[CS]$ is the Fourier transform of the correlation function between the complex and the subunit. Scattering is measured (a) from an equimolar mixture of complexes with heavily deuterated and non-deuterated subunits and (b) from a sample of complexes with subunits with the intermediate level of deuteration. Subtraction of (b) from (a), weighted so that the two curves are equimolar, gives a net curve for the subunit alone (at half the scattering power that would be seen for a solution of the isolated subunits at the same concentration):

$$I_1(Q) + I_3(Q) - 2I_2(Q) = (\rho^2/2)|S|^2. \qquad (19.4.3.9)$$

The difference curve is not influenced by solvent composition, underlying order, concentration or interparticle interference effects. Thus, at the cost of some difficult biochemistry, the small-angle scattering of a subunit belonging to a large assembly can be observed *in situ*. In practice, the mixture is not equimolar, but is adjusted depending on the intermediate level of deuteration, relaxing some of the difficulty of the biochemistry.

19.4.3.5. *Nuclear spin contrast variation*

When atomic nuclei of nonzero spin are placed in a magnetic field, the spins orient. If the temperature is near absolute zero, the orientation results in a polarization that is seen by polarized neutrons, resulting in polarization-dependent scattering. Since polarized neutron sources are available, and since biological materials are rich in hydrogen, Stuhrmann has proposed and tested a measurement based on the following idea (Stuhrmann & Nierhaus, 1996). Consider a complex in which all of the hydrogen has been replaced by deuterium except in one subunit or ligand, and prepare a sample that can be frozen to $T < 0.5$ K, placed in a 2.5 T magnetic field and subjected to dynamic spin polarization. Scattering of polarized neutrons is measured twice, once with the hydrogen spins oriented, and once with the spins selectively depolarized using NMR saturation. The difference contains contributions from the hydrogenated region and a cross term between the region and the rest of the complex. Using a modelling approach, Stuhrmann and his colleagues have deduced a structure that locates transfer RNA molecules on a ribosome from polarized neutron data, revealing the promise of this approach (Stuhrmann & Nierhaus, 1996).

19.4.3.6. *Interpretation of small-angle scattering using models*

There have been many attempts to extract more information from solution-scattering experiments than the radius of gyration and forward scattering, including the distance-measuring strategies discussed below. These attempts are of two kinds: testing models and creating models. Each of these must be cast in the context of the intrinsic information content of a scattering measurement, which can be expressed in terms of the number of independent parameters, n, that can be uniquely extracted from a data set (Moore, 1980).

$$n = Q_{\max} d_{\max}/\pi, \qquad (19.4.3.10)$$

where $Q_{\max}$ is the largest Q at which statistically significant data are measured and $d_{\max}$ is the largest dimension of the particle. A further requirement, normally met in small-angle scattering, is that $Q_{\min} d_{\max}/\pi < 1$.

The information content is a subtle factor in the first class of modelling, where models are tested for agreement with scattering data. Excellent programs have been written for generating predicted scattering curves from atomic coordinates and have been used to explore perturbations between crystal structures and solution organization. A fine example is the work on ATCase by Svergun, Barberato *et al.* (1997); the article also contains references to the programs used.

A more challenging task is to work in the other direction, extracting structural information directly from a scattering curve. Considerable effort has been devoted to work in this area, using approaches based on spherical harmonics, sometimes using sets of spheres to represent structure, and occasionally integrating information from electron microscopy (Svergun, 1994; Svergun, Burkhardt *et al.*, 1997).

19.4.3.7. *Use of forward scattering to measure molecular weights*

The value of the scattering function at zero scattering angle, which is obtained by extrapolation using a Guinier plot, is related to the molecular weight of the particle. In the neutron small-angle scattering case, the incoherent scattering background from hydrogen provides an internal standard. Using the incoherent background as an absolute calibration of the beam intensity, and knowing the concentration and composition of particles, one can obtain good values for the molecular weight, as pointed out by Jacrot & Zaccai (1981) and Zaccai & Jacrot (1983). This approach applies particularly well to proteins, where the average scattering density does not vary much from case to case, and can provide important data on the stoichiometry of oligomeric complexes. The limit in the accuracy of the measurement arises from limitations in knowing the protein concentration.

19.4.4. Distance measurements

19.4.4.1. *Theory and background*

By measuring distances between chemically specified points in a macromolecule or macromolecular complex, useful information may be obtained concerning its structure. In the case of a complex with many subunits, successive inter-subunit distance measurements can be combined by triangulation to generate a three-dimensional map specifying the relative positions of subunits and, perhaps, their individual radii of gyration. The only uncertainty is the handedness if more than three distances are combined. A number of approaches exist for making distance measurements, including NMR, fluorescence and electron-microscope techniques in which labelling is employed. Neutron scattering provides a particularly simple approach to such measurements in a conceptual sense. If we consider two labelled centres in a large macromolecule or complex, the interference of the scattering from the two labelled positions, $I_x(Q)$, can be separated from the scattering of the rest of the complex by measuring the scattering of the unlabelled complex, $I(Q)$, the two singly labelled complexes, $I_1(Q)$ and $I_2(Q)$, and the doubly labelled complex, $I_{12}(Q)$, as they are related by

$$I_x(Q) = [I_{12}(Q) + I(Q)] - [I_1(Q) + I_2(Q)]. \qquad (19.4.4.1)$$

Using the Debye relationship, it can be shown that the cross term will have the approximate form

$$I_x(Q) = 2f_1 f_2 \sin(d_{12}Q)/d_{12}Q, \qquad (19.4.4.2)$$

where the interference intensity results from the correlation between radiation scattered from sites 1 and 2, with scattering strengths f_1 and f_2. It is a damped sinusoidal fringe with a periodicity reciprocally related to the separation between the scattering regions. If the scattering regions are very small compared with the object, there will be nodes at equal intervals in Q; if they are not, $I_x(Q)$ will be a sum of all cross correlations of label positions in the two regions.

19.4.4.2. *Neutron distance measurements*

In the neutron case, the usual application involves deuterium labelling of positions in the two regions, and the cross term becomes

$$I_x(Q) = 2(b_D - b_H)^2 \sum_i \sum_j (\sin d_{ij}Q)/d_{ij}Q, \qquad (19.4.4.3)$$

where the first sum runs over all labelled positions in region 1 and the second over all labelled positions in region 2.

The interference experiment as a technique for studying macromolecules in solution was first proposed in 1947 as an X-ray scattering measurement in which the labels are heavy atoms (Kratky & Worthmann, 1947). It has been discussed several times since and tested (Hoppe, 1972; Vainshtein *et al.*, 1970); however, no biological application has ever been published, since the signals are small (Hoppe, 1972). In 1972, the interference idea was discovered again, but in the context of neutron scattering (Engelman & Moore, 1972). Initially, it was thought that the distance measurement would follow from inspection of the damped sinusoidal term, but it soon became evident that the size and shape of the labelled regions would have an important influence. Using a power series expansion like that originally employed by Guinier, the previous expression becomes (for small Q)

$$I_x(Q) \propto [1 - (1/6)(R_i^2 + R_j^2 + d_{ij}^2)Q^2],$$

where R_i and R_j are the radii of gyration of the subunits *in situ*, and d_{ij} is the separation of their centres of mass. It follows that (Moore *et al.*, 1978; Stöckel *et al.*, 1979)

$$R_g^2 = (1/2)(R_i^2 + R_j^2 + d_{ij}^2). \qquad (19.4.4.4)$$

The second moment of a length distribution, M_{ij}, can be related to a radius of gyration. If $p_{ij}(r)$ is the length distribution of the cross term $I_x(Q)$, then

$$M_{ij} = \int r^2 p_{ij}(r)\, \mathrm{d}r = 2R_g^2.$$

Hence (Moore *et al.*, 1978)

$$M_{ij} = R_i^2 + R_j^2 + d_{ij}^2. \qquad (19.4.4.5)$$

Thus, M_{ij} is the parameter that contains the information in the difference experiment. If the radii of gyration of the labelled regions are small, then the distance is well measured in a single experiment. This would be the case, for example, if the labels were single heavy atoms in an X-ray experiment. However, in most complexes of macromolecules there will be many pairwise protein–protein relationships where the radii and the separation of centres have comparable magnitudes. One approach to extracting distance information is to know the radii of the subunits *in situ*, by estimation from their molecular weights, by measurement of the isolated subunits or from triple isotopic substitution (see above). The first approach is the least desirable but the easiest, and the last approach is correct but laborious.

If a complex has eight or more distinct subunits, the number of possible measurements of M_{ij}, $n(n-1)/2$, is sufficient to solve for the distances and radii, enriching the information obtained from the experiment. The minimum number of measurements required is $4n - 6$, and for large complexes, such as the ribosome, a great excess of possible measurements exists, permitting refinement of the information through redundancy. Moore & Weinstein (1979) have described analytical methods for solving the problem with correct error propagation.

19.4.4.3. *The statistical labelling method*

In many biological assemblies, multiple copies of a given subunit are found. In such cases, statistical labelling methods provide useful information. The basic concept is to produce two samples in which labelled subunits are randomly mixed with unlabelled subunits within the particles, and in which the ratios of labelled to unlabelled subunits is different. The difference in the scattering from the two samples will then contain shape and orientation information for the subunits (Fujiwara & Mendelson, 1996). A simple case is that of a dimer in a larger complex. Random introduction of equimolar labelled (D) and unlabelled (H) subunits will result in a mixture of complexes, where HH:HD:DH:DD = 1:1:1:1. A sample can be prepared with an equimolar mixture of all H and all D so that HH:DD = 1:1. The difference in scattering between these samples will be simply the interference cross term for the dimer, but at half the weight it would have in the case where the subunits were distinctly labelled.

19.4.5. Practical considerations

19.4.5.1. *Feasibility*

For a biological scientist, the first question is whether knowledge of the parameters that can be obtained from neutron scattering is of interest for the biological problem under consideration. If the answer is affirmative, the best course of action is to make contact with individuals who have conducted experiments in the past, as well as with biologists at a neutron-scattering facility. As a preliminary guide, a few general points are pertinent.

For a solution-scattering experiment on an unlabelled sample, typical sample volumes are 0.1–0.2 ml at concentrations of around

10 mg ml^{-1}. For contrast variation, samples dialysed to different D_2O levels will be required, so significant amounts of material need to be at hand. Making a set of measurements will require a few hours at a modern facility.

Longer collection times and significantly greater biochemical efforts will be involved for measurements with labelled material, especially if reconstitution is part of the strategy. Labelled biomolecules are most often produced by growth of organisms in D_2O (Moore & Engelman, 1976; Vanatalu *et al.*, 1993), but strategies using chemical synthesis or providing labelled precursors have also been employed. Preparation of samples with labelled ligands is usually more straightforward than the generation of reconstituted complexes, but still requires tests of homogeneity.

19.4.5.2. *Homogeneity and stability*

For measurements to be made, the samples must be transported to a neutron facility and measured for many hours. This requires stability in the usual biochemical sense, and proper experiments will include assays performed after the measurements. Damage from the neutrons themselves is minimal, but handling and transportation impose greater challenges.

Where mixed samples are employed, stability also involves resistance to exchange of subunits or ligands between complexes in the mixture. This is hard to assess in advance, the first sign of trouble often being an absence of an expected difference signal. Given shipment and measuring times, even a slow exchange is significant. With a consequent qualification of the results, cross-linking may be used to stabilize the complex.

In all scattering measurements, homogeneity of the sample is a great advantage in interpretation. However, some variation is tolerable for many purposes, and the level of purity typically sought for crystallization experiments is not usually required.

19.4.5.3. *Solvent conditions*

Background arises from the incoherent scattering of hydrogen. Consequently, the use of high levels of D_2O in the buffer improves the background, but may not be innocuous, since high levels of D_2O have been seen to induce aggregation. In cases where complexes with labelled subunits are measured, it is advantageous to suppress small-angle background scatter by contrast matching the unlabelled regions *via* solvent adjustment.

19.4.6. Examples

19.4.6.1. *Contrast variation*

An early result that changed ideas about nucleosome organization came from measurements relying on the difference in protein and DNA densities (Baldwin *et al.*, 1975; Bradbury *et al.*, 1976). Information on the organization of serum lipoproteins was also based on intrinsic scattering differences (Stuhrmann *et al.*, 1975; Atkinson & Shipley, 1984). The contrast between RNA and protein was used in early ribosome measurements (Crichton *et al.*, 1977; Moore *et al.*, 1974). Recent examples include detergent binding to membrane proteins (Timmins *et al.*, 1991) and the study of membrane protein–lipid complexes (Jeanteur *et al.*, 1994). Elegant use of contrast variation resulted in a structural explanation of the anti-cooperative binding of tRNA to synthetase, which had resisted study by other methods (Dessen *et al.*, 1978).

19.4.6.2. *Contrast matching*

In some applications, use has been made of contrast matching a large part of a complex to study a component with a contrasting scattering density. Examples include studies of ribosomal proteins *in situ* (Nierhaus *et al.*, 1983; Nowotny *et al.*, 1994), proteins of the DNA-dependant RNA polymerase (Stöckel *et al.*, 1980*a*), studies of muscle proteins (Stone *et al.*, 1998) and a view of a membrane protein in a lipid bilayer (Hunt *et al.*, 1997).

19.4.6.3. *Spin contrast variation*

Some applications of the spin contrast variation method have appeared (Junemann *et al.*, 1998; Nierhaus *et al.*, 1998).

19.4.6.4. *Specific deuteration, combination with X-ray measurements*

Many recent measurements use specific labelling and combine information with data from small-angle X-ray scattering. Studies of ligand binding (Bilgin *et al.*, 1998), hydration layers (Svergun *et al.*, 1998), troponin complexes (Olah *et al.*, 1994) and ribosomes (Svergun *et al.*, 1996) are examples that illustrate the approaches.

19.4.6.5. *Distance measurements and triangulation*

By measuring distances and radii of gyration, models of some large complexes have been created. These include a model of the relative positions of all 21 proteins in the small ribosomal subunit from *E. coli* (Capel *et al.*, 1987), a model of seven proteins from the large subunit (May *et al.*, 1992) and a model of the DNA-dependant RNA polymerase (Stöckel *et al.*, 1980*b*). Intramolecular conformational states of cholesterol esters have been observed using distance measurements on molecules chemically labelled in different regions (Burks & Engelman, 1981).

19.3 (*cont.*)

complex and its implications for signal transduction. *Nature (London)*, **387**, 370–376.
Schmidt, T., Johnson, J. E. & Phillips, W. (1983). *The spherically averaged structures of cowpea mosaic virus components by X-ray solution scattering*. *Virology*, **127**, 65–73.
Schuster, M. & Göbel, H. (1995). *Parallel-beam coupling into channel-cut monochromators using curved graded multilayers*. *J. Phys. D*, **28**, A270–A275.
Seaton, B. A., Head, J. F., Engelman, D. M. & Richards, F. M. (1985). *Calcium-induced increase in the radius of gyration and maximum dimension of calmodulin measured by small-angle X-ray scattering*. *Biochemistry*, **24**, 6740–6743.
Sousa, M. C. & McKay, D. B. (1998). *The hydroxyl of threonine 13 of the bovine 70-kDa heat shock cognate protein is essential for transducing the ATP-induced conformational change*. *Biochemistry*, **37**, 15392–15399.
Srajer, V., Teng, T.-Y., Ursby, T., Pradervand, C., Ren, Z., Adachi, S., Bourgeois, D., Wulff, M. & Moffat, K. (1996). *Photolysis of the carbon monoxide complex of myoglobin: nanosecond time-resolved crystallography* [see *Comments*]. *Science*, **274**, 1726.
Stuhrmann, H. B. (1970). *Interpretation of small-angle scattering functions of dilute solutions and gases. A representation of the structures related to a one-particle-scattering function*. *Acta Cryst.* A**26**, 297–306.
Sunnerhagen, M., Olah, G. A., Stenflo, J., Forsen, S., Drakenberg, T. & Trewhella, J. (1996). *The relative orientation of Gla and EGF domains in coagulation factor X is altered by Ca-2+ binding to the first EGF domain. A combined NMR–small angle X-ray scattering study*. *Biochemistry*, **35**, 11547–11559.
Svergun, D. I. (1992). *Determination of the regularization parameter in indirect-transform methods using perceptual criteria*. *J. Appl. Cryst.* **25**, 495–503.
Svergun, D. I. (1999). *Restoring low resolution structure of biological macromolecules from solution scattering using simulated annealing*. *Biophys. J.* **76**, 2879–2886.
Svergun, D. I., Aldag, I., Sieck, T., Altendorf, K., Koch, M. H. J., Kane, D. J., Kozin, M. B. & Grueber, G. (1998). *A model of the quaternary structure of the Escherichia coli F1 ATPase from X-ray solution scattering and evidence for structural changes in the Delta subunit during ATP hydrolysis*. *Biophys. J.* **75**, 2212–2219.
Svergun, D. I., Barberato, C. & Koch, M. H. J. (1995). *CRYSOL – a program to evaluate X-ray solution scattering of biological macromolecules from atomic coordinates*. *J. Appl. Cryst.* **28**, 768–773.
Svergun, D. I., Barberato, C., Koch, M. H. J., Fetler, L. & Vachette, P. (1997). *Large differences are observed between the crystal and solution quaternary structures of allosteric aspartate transcarbamylase in the R state*. *Proteins Struct. Funct. Genet.* **27**, 110–117.
Svergun, D. I., Koch, M. H. & Serdyuk, I. N. (1994). *Structural model of the 50 S subunit of Escherichia coli ribosomes from solution scattering. I. X-ray synchrotron radiation study*. *J. Mol. Biol.* **240**, 66–77.
Svergun, D. I., Konrad, S., Huss, M., Koch, M. H. J., Wieczorek, H., Altendorf, K., Volkov, V. V. & Grueber, G. (1998). *Quaternary structure of V_1 and F_1 ATPase: significance of structural homologies and diversities*. *Biochemistry*, **37**, 17659–17663.
Svergun, D. I., Richard, S., Koch, M. H. J., Sayers, Z., Kuprin, S. & Zaccai, G. (1998). *Protein hydration in solution: experimental observation by X-ray and neutron scattering*. *Proc. Natl Acad. Sci. USA*, **95**, 2267–2272.
Svergun, D. I., Volkov, V. V., Kozin, M. B. & Stuhrmann, H. B. (1996). *New developments in direct shape determination from small-angle scattering. 2. Uniqueness*. *Acta Cryst.* A**52**, 419–426.
Svergun, D. I., Volkov, V. V., Kozin, M. B., Stuhrmann, H. B., Barberato, C. & Koch, M. H. J. (1997). *Shape determination from solution scattering of biopolymers*. *J. Appl. Cryst.* **30**, 798–802.
Thuman-Commike, P. A., Tsuruta, H., Greene, B., Prevelige, P. E., King, J. & Chiu, W. (1999). *Solution X-ray scattering based estimation of electron cryomicroscopy imaging parameters for reconstruction of virus particles*. *Biophys. J.* **76**, 2249–2261.
Trewhella, J. (1998). *Insights into biomolecular function from small-angle scattering*. *Curr. Opin. Struct. Biol.* **7**, 702–708.
Tsuruta, H., Reddy, V., Wikoff, W. & Johnson, J. (1998). *Imaging RNA and dynamic protein segments with low resolution virus crystallography; experimental design, data processing and implications of electron density maps. J. Mol. Biol.* **284**, 1439–1452.
Tsuruta, H., Vachette, P., Sano, T., Moody, M. F., Amemiya, Y., Wakabayashi, K. & Kihara, H. (1994). *Kinetics of the quaternary structure change of aspartate transcarbamylase triggered by succinate, a competitive inhibitor*. *Biochemistry*, **33**, 10007–10012.
Uversky, V. N., Segel, D. J., Doniach, S. & Fink, A. L. (1998). *Association-induced folding of globular proteins*. *Proc. Natl Acad. Sci. USA*, **95**, 5480–5483.
Wang, J., Harting, J. A. & Flanagan, J. M. (1997). *The structure of ClpP at 2.3 Å resolution suggests a model for ATP-dependent proteolysis*. *Cell*, **91**, 447–456.
Weik, M., Ravelli, R. B. G., Kryger, G., McSweeney, S., Raves, M. L., Harel, M., Gros, P., Silman, I., Kroon, J. & Sussman, J. L. (2000). *Specific chemical and structural damage to proteins produced by synchrotron radiation. Proc. Natl Acad. Sci. USA*, **97**, 623–628.
Wikoff, W., Duda, R., Hendrix, R. & Johnson, J. (1998). *Crystallization and preliminary X-ray analysis of the dsDNA bacteriophage HK97 mature empty capsid*. *Virology*, **243**, 113–118.
Wilbanks, S. M., Chen, L., Tsuruta, H., Hodgson, K. O. & McKay, D. B. (1995). *Solution small-angle X-ray scattering study of the molecular chaperone Hsc70 and its subfragments*. *Biochemistry*, **34**, 12095–12106.
Yamashita, M. M., Almassy, R. J., Janson, C. A., Cascio, D. & Eisenberg, D. (1989). *Refined atomic model of glutamine synthetase at 3.5 Å resolution*. *J. Biol. Chem.* **264**, 17681–17690.
Zheng, Y., Doerschuk, P. C. & Johnson, J. E. (1995). *Determination of three-dimensional low-resolution viral structure from solution X-ray scattering data*. *Biophys. J.* **69**, 619–639.

19.4

Atkinson, D. & Shipley, G. G. (1984). *Structural studies of plasma lipoproteins*. *Basic Life Sci.* **27**, 211–226.
Bacon, G. E. (1975). *Neutron diffraction*. Oxford University Press.
Baldwin, J. P., Boseley, P. G., Bradbury, E. M. & Ibel, K. (1975). *The subunit structure of the eukaryotic chromosome*. *Nature (London)*, **253**, 245–249.
Bilgin, N., Ehrenberg, M., Ebel, C., Zaccai, G., Sayers, Z., Koch, M. H. J., Svergun, D. I., Barberato, C., Volkov, V., Nissen, P. & Nyborg, J. (1998). *Solution structure of the ternary complex between aminoacyl-tRNA, elongation factor Tu, and guanosine triphosphate*. *Biochemistry*, **37**, 8163–8172.
Bradbury, E. M., Baldwin, J. P., Carpenter, B. G., Hjelm, R. P., Hancock, R. & Ibel, K. (1976). *Neutron-scattering studies of chromatin*. *Brookhaven Symp Biol.* **27**, IV97–IV117.
Bragg, W. L. & Perutz, M. F. (1952). *The external form of the haemoglobin molecule. I*. *Acta Cryst.* **5**, 277–283.
Burks, C. & Engelman, D. M. (1981). *Cholesteryl myristate conformation in liquid crystalline mesophases determined by neutron scattering*. *Proc. Natl Acad. Sci. USA*, **78**, 6863–6867.
Capel, M. S., Engelman, D. M., Freeborn, B. R., Kjeldgaard, M., Langer, J. A., Ramakrishnan, V., Schindler, D. G., Schneider, D. K., Schoenborn, B. P. & Sillers, I. Y. (1987). *A complete mapping of the proteins in the small ribosomal subunit of Escherichia coli*. *Science*, **238**, 1403–1406.
Crichton, R., Engelman, D. M., Hass, J., Koch, M. H. J., Moore, P. B., Parfait, R. & Stuhrmann, H. B. (1977). *A contrast variation study of specifically deuterated ribosomal subunits*. *Proc. Natl Acad. Sci. USA*, **74**, 5547–5550.
Debye, P. (1915). *Zerstreuung von Röntgenstrahlen. Ann. Phys. (Leipzig)*, **46**, 809–823.

19.4 (*cont.*)

Debye, P. & Bueche, A. M. (1949). *Scattering by an inhomogeneous solid. J. Appl. Phys.* **20**, 518–525.

Debye, P. & Pirenne, M. H. (1938). *Über die Fourieranalyse von interferometrischen Messungen an freien Molekülen. Ann. Phys. (Leipzig)*, **33**, 617–629.

Dessen, P., Blanquet, S., Zaccai, G. & Jacrot, B. (1978). *Anticooperative binding of initiator transfer RNA Met to methionyl-transfer RNA synthetase from E. coli: neutron scattering studies. J. Mol. Biol.* **126**, 293–313.

Engelman, D. M. & Moore, P. B. (1972). *A new method for the determination of biological quarternary structure by neutron scattering. Proc. Natl Acad. Sci. USA*, **69**, 1997–1999.

Engelman, D. M. & Zaccai, G. (1980). *Bacteriorhodopsin is an inside-out protein. Proc. Natl Acad. Sci. USA*, **77**, 5894–5898.

Fujiwara, S. & Mendelson, R. A. (1996). *In situ shape and distance measurements in neutron scattering and diffraction. Basic Life Sci.* **64**, 385–395.

Glatter, O. & Kratky, O. (1982). *Small angle X-ray scattering*. London: Academic Press.

Guinier, A. (1939). *Diffraction of X-rays of very small angles – application to the study of ultramicroscopic phenomena. Ann. Phys. (Paris)*, **12**, 161–237.

Guinier, A. (1955). *Small angle scattering of X-rays*. New York: John Wiley and Sons.

Guinier, A. (1962). *Small angle X-ray scattering*. In *International tables for X-ray crystallography*, Vol. III. *Physical and chemical tables*, pp. 324–329. Birmingham: Kynoch Press. (Present distributor Kluwer Academic Publishers, Dordrecht).

Hoppe, W. (1972). *New X-ray method for the determination of the quaternary structure of protein complexes. Isr. J. Chem.* **10**, 321–333.

Hunt, J. F., McCrea, P. D., Zaccai, G. & Engelman, D. M. (1997). *Assessment of the aggregation state of integral membrane proteins in reconstituted phospholipid vesicles using small angle neutron scattering. J. Mol. Biol.* **273**, 1004–1019.

Jacrot, B. & Zaccai, G. (1981). *Determination of molecular weight by neutron scattering. Biopolymers*, **20**, 2413–2426.

Jeanteur, D., Pattus, F. & Timmins, P. A. (1994). *Membrane-bound form of the pore-forming domain of colicin A. A neutron scattering study. J. Mol. Biol.* **235**, 898–907.

Junemann, R., Burkhardt, N., Wadzack, J., Schmitt, M., Willumeit, R., Stuhrmann, H. B. & Nierhaus, K. H. (1998). *Small angle scattering in ribosomal structure research: localization of the messenger RNA within ribosomal elongation states. J. Biol. Chem.* **379**, 807–818.

Kratky, O. & Worthmann, W. (1947). *Determination of the configuration of dissolved organic molecules by interferometric measurements with X-rays. Monatsch. Chem.* **76**, 263–281.

Lehmann, M. S. & Zaccai, G. (1984). *Neutron small-angle scattering studies of ribonuclease in mixed aqueous solutions and determination of the preferentially bound water. Biochemistry*, **23**, 1939–1942.

May, R. P., Nowotny, V., Nowotny, P., Voss, H. & Nierhaus, K. H. (1992). *Inter-protein distances within the large subunit from Escherichia coli ribosomes. EMBO J.* **11**, 373–378.

Moore, P. B. (1980). *Small-angle scattering. Information content and error analysis. J. Appl. Cryst.* **13**, 168–175.

Moore, P. B. & Engelman, D. M. (1976). *The production of deuterated E. coli. Brookhaven Symp. Biol.* **27**, V12–V23.

Moore, P. B., Engelman, D. M. & Schoenborn, B. P. (1974). *Asymmetry in the 50S ribosomal subunit of Escherichia coli. Proc. Natl Acad. Sci. USA*, **71**, 172–176.

Moore, P. B., Langer, J. A. & Engelman, D. M. (1978). *The measurement of the locations and radii of gyration of proteins in the 30S ribosomal unit of E. coli by neutron scattering. J. Appl. Cryst.* **11**, 479–482.

Moore, P. B. & Weinstein, E. (1979). *On the estimation of the locations of subunits within macromolecular aggregates from neutron interference data. J. Appl. Cryst.* **12**, 321–326.

Nierhaus, K. H., Lietzke, R., May, R. P., Nowotny, V., Schulze, H., Simpson, K., Wurmbach, P. & Stuhrmann, H. B. (1983). *Shape determinations of ribosomal proteins in situ. Proc. Natl Acad. Sci. USA*, **80**, 2889–2893.

Nierhaus, K. H., Wadzack, J., Burkhardt, N., Junemann, R., Meerwinck, W., Willumeit, R. & Stuhrmann, H. B. (1998). *Structure of the elongating ribosome: arrangement of the two tRNAs before and after translocation. Proc. Natl Acad. Sci. USA*, **95**, 945–950.

Nowotny, P., Ruhl, M., Nowotny, V., May, R. P., Burkhardt, N., Voss, H. & Nierhaus, K. H. (1994). *Direct shape determination of ribosomal proteins in solution and within the ribosome by means of neutron scattering. Biophys. Chem.* **53**, 115–122.

Olah, G. A., Rokop, S. E., Wang, C. L., Blechner, S. L. & Trewhella, J. (1994). *Troponin I encompasses an extended troponin C in the Ca(2+)-bound complex: a small-angle X-ray and neutron scattering study. Biochemistry*, **33**, 8233–8239.

Porod, G. (1951). *The X-ray small-angle scattering of close-packed colloid systems. I. Kolloid-Z.* **124**, 83–144.

Porod, G. (1952). *The X-ray small-angle scattering of close-packed colloid systems. II. Kolloid-Z.* **125**, 51–57.

Serdyuk, I. N., Grenader, A. K. & Zaccai, G. (1979). *Study of the internal structure of E. coli ribosomes by neutron and X-ray scattering. J. Mol. Biol.* **135**, 691–707.

Serdyuk, I. N., Pavlov, M. Yu., Rublevskaya, I. N., Zaccai, G. & Leberman, R. (1994). *Triple isotopic substitution method in small angle scattering. Application to the study of the ternary complex EF-Tu·GTp·aminoacyl tRNA. Biophys. Chem.* **53**, 123–130.

Stöckel, P., May, R., Strell, I., Cejka, Z., Hoppe, W., Heumann, H., Zillig, W. & Crespi, H. (1980*a*). *The core subunit structure in RNA polymerase holoenzyme determined by neutron small-angle scattering. Eur. J. Biochem.* **112**, 411–417.

Stöckel, P., May, R., Strell, I., Cejka, Z., Hoppe, W., Heumann, H., Zillig, W. & Crespi, H. L. (1980*b*). *The subunit positions within RNA polymerase holoenzyme determined by triangulation of centre-to-centre distances. Eur. J. Biochem.* **112**, 419–423.

Stöckel, P., May, R., Strell, I., Cejka, Z., Hoppe, W., Heumann, H., Zillig, W., Crespi, H. L., Katz, J. J. & Ibel, K. (1979). *Determination of intersubunit distances and subunit shape parameters in DNA-dependent RNA polymerase by neutron small-angle scattering. J. Appl. Cryst.* **12**, 176–185.

Stone, D. B., Timmins, P. A., Schneider, D. K., Krylova, I., Ramos, C. H., Reinach, F. C. & Mendelson, R. A. (1998). *The effect of regulatory Ca^{2+} on the in situ structures of troponin C and troponin I: a neutron scattering study. J. Mol. Biol.* **281**, 689–704.

Stuhrmann, H. B. (1976). *Small-angle scattering of proteins in solution. Brookhaven Symp. Biol.* **27**, IV3–IV19.

Stuhrmann, H. B., Haas, J., Ibel, K., Koch, M. H. & Crichton, R. R. (1976). *Low angle neutron scattering of ferritin studied by contrast variation. J. Mol. Biol.* **100**, 399–413.

Stuhrmann, H. B. & Nierhaus, K. H. (1996). *The determination of the in situ structure by nuclear spin contrast variation. Basic Life Sci.* **64**, 397–413.

Stuhrmann, H. B., Tardieu, A., Mateu, L., Sardet, C., Luzzati, V., Aggerbeck, L. & Scanu, A. M. (1975). *Neutron scattering study of human serum low density lipoprotein. Proc. Natl Acad. Sci. USA*, **72**, 2270–2273.

Svergun, D. I. (1994). *Solution scattering from biopolymers: advanced contrast-variation data analysis. Acta Cryst.* A**50**, 391–402.

Svergun, D. I., Barberato, C., Koch, M. H., Fetler, L. & Vachette, P. (1997). *Large differences are observed between the crystal and solution quaternary structures of allosteric aspartate transcarbamylase in the R state. Proteins*, **27**, 110–117.

Svergun, D. I., Burkhardt, N., Pedersen, J. S., Koch, M. H., Volkov, V. V., Kozin, M. B., Meerwink, W., Stuhrmann, H. B., Diedrich, G. & Nierhaus, K. H. (1997). *Solution scattering structural analysis of the 70S Escherichia coli ribosome by contrast variation. II. A model of the ribosome and its RNA at 3.5 nm resolution. J. Mol. Biol.* **271**, 602–618.

Svergun, D. I., Koch, M. H., Pedersen, J. S. & Serdyuk, I. N. (1996). *Structural model of the 50S subunit of E. coli ribosomes from solution scattering. Basic Life Sci.* **64**, 149–174.

19.4 (*cont.*)

Svergun, D. I., Richard, S., Koch, M. H., Sayers, Z., Kuprin, S. & Zaccai, G. (1998). *Protein hydration in solution: experimental observation by X-ray and neutron scattering*. *Proc. Natl Acad. Sci. USA*, **95**, 2267–2272.

Timmins, P. A., Hauk, J., Wacker, T. & Welte, W. (1991). *The influence of heptane-1,2,3-triol on the size and shape of LDAO micelles. Implications for the crystallisation of membrane proteins*. *FEBS Lett.* **280**, 115–120.

Uberbacher, E. C., Mardian, J. K., Rossi, R. M., Olins, D. E. & Bunick, G. J. (1982). *Neutron scattering studies and modeling of high mobility group 14 core nucleosome complex*. *Proc. Natl Acad. Sci. USA*, **79**, 5258–5262.

Vainshtein, B. K., Sosfenov, N. I. & Feigin, L. A. (1970). *X-ray method for determining the gaps between heavy atoms in macromolecules in solution and its use for studying gramicidin derivatives. Dokl. Akad. Nauk SSSR*, **190**, 574–577.

Vanatalu, K., Paalme, T., Vilu, R., Burkhardt, N., Junemann, R., May, R., Ruhl, M., Wadzack, J. & Nierhaus, K. H. (1993). *Large-scale preparation of fully deuterated cell components. Ribosomes from Escherichia coli with high biological activity*. *Eur. J. Biochem.* **216**, 315–321.

Zaccai, G. & Jacrot, B. (1983). *Small angle neutron scattering*. *Annu. Rev. Biophys. Bioeng.* **12**, 139–157.

19.5

Arnott, S. (1980). *Twenty years hard labor as a fiber diffractionist*. *Am. Chem. Soc. Symp. Ser.* **141**, 1–30.

Arnott, S., Chandrasekaran, R., Birdsall, D. L., Leslie, A. G. W. & Ratliff, R. L. (1980). *Left-handed DNA helices*. *Nature (London)*, **283**, 743–745.

Arnott, S., Chandrasekaran, R. & Leslie, A. G. W. (1976). *Structure of the single-stranded polyribonucleotide polycytidylic acid*. *J. Mol. Biol.* **106**, 735–748.

Arnott, S., Chandrasekaran, R., Millane, R. P. & Park, H.-S. (1986). *DNA–RNA hybrid secondary structures*. *J. Mol. Biol.* **188**, 631–640.

Arnott, S., Chandrasekaran, R., Puigjaner, L. C., Walker, J. K., Hall, I. H. & Birdsall, D. L. (1983). *Wrinkled DNA*. *Nucleic Acids Res.* **11**, 1457–1474.

Arnott, S., Dover, S. D. & Wonacott, A. J. (1969). *Least-squares refinement of the crystal and molecular structures of DNA and RNA from X-ray data and standard bond lengths and angles*. *Acta Cryst.* B**25**, 2192–2206.

Arnott, S., Guss, J. M., Hukins, D. W. L., Dea, I. C. M. & Rees, D. A. (1974). *Conformation of keratan sulphate*. *J. Mol. Biol.* **88**, 175–184.

Arnott, S. & Mitra, A. (1984). *X-ray diffraction analysis of glycosaminoglycans*. In *Molecular biophysics of the extracellular matrix*, edited by S. Arnott, D. A. Rees & E. R. Morris, pp. 41–67. New Jersey: Humana Press.

Arnott, S., Scott, W. E., Rees, D. A. & McNab, C. G. A. (1974). *i-Carrageenan: molecular structure and packing of polysaccharide double helices in oriented fibers of divalent cation salts*. *J. Mol. Biol.* **90**, 253–267.

Arnott, S., Wilkins, M. H. F., Fuller, W. & Langridge, R. (1967). *Molecular and crystal structures of double-helical RNA. III. An 11-fold molecular model and comparison of the agreement between the observed and calculated three-dimensional diffraction data for 10- and 11-fold models*. *J. Mol. Biol.* **27**, 535–548.

Arnott, S., Wilkins, M. H. F., Hamilton, L. D. & Langridge, R. (1965). *Fourier synthesis studies of lithium DNA. Part III: Hoogsteen models*. *J. Mol. Biol.* **11**, 391–402.

Arnott, S. & Wonacott, A. J. (1966). *The refinement of the crystal and molecular structures of polymers using X-ray data and stereochemical constraints*. *Polymer*, **7**, 157–166.

Bailey, K., Astbury, W. T. & Rudall, K. M. (1943). *Members of the keratin-myosin group*. *Nature (London)*, **151**, 716–717.

Barrett, A. N., Leigh, J. B., Holmes, K. C., Leberman, R., Sengbusch, P. & Klug, A. (1971). *An electron density map of tobacco mosaic virus at 10 Å resolution*. *Cold Spring Harbor Symp. Quant. Biol.* **36**, 433–448.

Beese, L., Stubbs, G. & Cohen, C. (1987). *Microtubule structure at 18 Å resolution*. *J. Mol. Biol.* **194**, 257–264.

Brünger, A. T., Kuriyan, J. & Karplus, M. (1987). *Crystallographic R-factor refinement by molecular dynamics*. *Science*, **235**, 458–460.

Bryan, R. K., Bansal, M., Folkhard, W., Nave, C. & Marvin, D. A. (1983). *Maximum-entropy calculation of the electron density at 4 Å resolution of Pf1 filamentous bacteriophage*. *Proc. Natl Acad. Sci. USA*, **80**, 4728–4731.

Chandrasekaran, R. (1997). *Molecular architecture of polysaccharide helices in oriented fibers. Adv. Carbohydr. Chem. Biochem.* **52**, 311–439.

Chandrasekaran, R. & Arnott, S. (1989). *The structures of DNA and RNA helices in oriented fibers*. In *Landolt–Börnstein numerical data and functional relationships in science and technology*, Vol. VII/1b, edited by W. Saenger, pp. 31–170. Berlin: Springer-Verlag.

Chandrasekaran, R., Bian, W. & Okuyama, K. (1998). *Three-dimensional structure of guaran*. *Carbohydr. Res.* **312**, 219–224.

Chandrasekaran, R., Giacometti, A. & Arnott, S. (2000*a*). *Structure of poly d(T)·poly d(A)·poly d(T). J. Biomol. Struct. Dynam.* **17**, 1011–1022.

Chandrasekaran, R., Giacometti, A. & Arnott, S. (2000*b*). *Structure of poly (U)·poly (A)·poly (U). J. Biomol. Struct. Dynam.* **17**, 1023–1034.

Chandrasekaran, R., Giacometti, A. & Arnott, S. (2000*c*). *Structure of poly (I)·poly (A)·poly (I). J. Biomol. Struct. Dynam.* **17**, 1035–1045.

Chandrasekaran, R., Puigjaner, L. C., Joyce, K. L. & Arnott, S. (1988). *Cation interactions in gellan: an X-ray study of the potassium salt*. *Carbohydr. Res.* **181**, 23–40.

Chandrasekaran, R. & Radha, A. (1992). *Structure of poly d(A)·poly d(T). J. Biomol. Struct. Dynam.* **10**, 153–168.

Chandrasekaran, R., Radha, A. & Lee, E. J. (1994). *Structural roles of calcium ions and side chains in welan: an X-ray study. Carbohydr. Res.* **252**, 183–207.

Chandrasekaran, R., Radha, A., Lee, E. J. & Zhang, M. (1994). *Molecular architecture of araban, galactoglucan and welan. Carbohydr. Polymers*, **25**, 235–243.

Chandrasekaran, R., Radha, A. & Park, H.-S. (1995). *Sodium ions and water molecules in the structure of poly d(A)·poly d(T). Acta Cryst.* D**51**, 1025–1035.

Chandrasekaran, R., Radha, A. & Park, H.-S. (1997). *Structure of poly d(AI)·poly d(CT) in two different packing arrangements*. *J. Biomol. Struct. Dynam.* **15**, 285–305.

Cochran, W., Crick, F. H. & Vand, V. (1952). *The structure of synthetic polypeptides. I. The transform of atoms in a helix*. *Acta Cryst.* **5**, 581–586.

Cohen, C., Harrison, S. C. & Stephens, R. E. (1971). *X-ray diffraction from microtubules*. *J. Mol. Biol.* **59**, 375–380.

Franklin, R. E. & Klug, A. (1955). *The splitting of layer lines in X-ray fibre diagrams of helical structures: application to tobacco mosaic virus*. *Acta Cryst.* **8**, 777–780.

Fraser, R. D. B., MacRae, T. P., Miller, A. & Rowlands, R. J. (1976). *Digital processing of fibre diffraction patterns*. *J. Appl. Cryst.* **9**, 81–94.

Fraser, R. D. B., MacRae, T. P. & Suzuki, E. (1978). *An improved method for calculating the contribution of solvent to the X-ray diffraction pattern of biological molecules*. *J. Appl. Cryst.* **11**, 693–694.

Gonzalez, A., Nave, C. & Marvin, D. A. (1995). *Pf1 filamentous bacteriophage: refinement of a molecular model by simulated annealing using 3.3 Å resolution X-ray fibre diffraction data*. *Acta Cryst.* D**51**, 792–804.

Gregory, J. & Holmes, K. C. (1965). *Methods of preparing orientated tobacco mosaic virus sols for X-ray diffraction*. *J. Mol. Biol.* **13**, 796–801.

Hamilton, W. C. (1965). *Significance tests on the crystallographic R factor*. *Acta Cryst.* **18**, 502–510.