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.

references