International Tables for Crystallography (2006). Vol. F, Section 19.4.3.2, p. 440.

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:

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

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)

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

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

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

The contrast-independent terms in the Stuhrmann equation are

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

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

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

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.

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

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

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