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).

references