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Editor's overview

The extraordinary advantages of using neutron scattering and diffraction to study structure and dynamics in molecular biology have been known and applied for more than two decades. But in contrast to X-rays for which high-performance laboratory apparatus is available, there are very few neutron sources in the world with the appropriate characteristics and instrumentation for such experiments and the standard condition set when a neutron experiment is proposed is that 'it *cannot* be done by X-rays'. Neutrons have many properties that are uniquely useful for molecular structure studies in biology using radiation diffraction:

— a choice of wavelength from below 1 Å to more than 10 Å associated with 'soft' energies and negligible absorption in most matter;
— isotope (especially hydrogen–deuterium) effects;
— scattering amplitudes independent of atomic mass;
— a negative coherent amplitude and large incoherent cross-section for ^{1}H.

Furthermore, the energies of neutrons of the appropriate wave-lengths for structural studies correspond to thermal energies for temperatures between a few degrees K to those well in excess of room temperature. In fact, neutrons produced in a reactor or spallation source have their wavelengths 'adjusted' by equilibration at a given temperature; thus 'cold' neutrons have wavelengths of several Ångströms, 'hot' neutrons have wavelengths of a fraction of an Ångström and 'thermal' neutrons have wavelengths close to 1 Å. Because of this wavelength–energy link, neutron inelastic scattering allows the simultaneous study of spatial structure, on the one hand, and dynamics, on the other, at the atomic and molecular levels–motions in proteins, for example, that have been shown to be associated with a biological function.

Very important information can be obtained from high-resolution neutron diffraction experiments in macromolecular crystallography. The claims made in early papers and reviews remain valid: water molecules, protons or H atoms play an essential and often poorly understood role in protein folding and function; neutron diffraction could show us where they are in a structure. Why then are there so few studies in this field, to the extent that neutron scattering is associated much more with solution and inelastic work than with single crystal diffraction? The answer is simply that the flux from the best neutron sources is very much weaker than that of laboratory X-ray generators (the flux comparison with synchrotron radiation is devastating for neutrons) and unreasonably large crystals and long times are required for data collection. Crystal growth is the main stumbling block in high-resolution studies of protein structure and most proteins that have been crystallised so far will just not form crystals of the appropriate size for a neutron study. It is estimated that if neutron crystallography could be done with crystal volumes a factor of 100 smaller than present requirements the field would explode with interesting studies such as, for example, an examination of the charge relay hypothesis in the various families of serine proteases and esterases whose structures have been solved by X-rays. Such a gain is not beyond hope. Although it is out of the question that we should have a higher effective flux from neutron

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sources in the near future, considerable improvements are expected beyond the reactor or spallation source wall, in diffractometer and data collection design and also in the sample itself. The first two papers in this issue address these concerns. Cipriani et al. have developed an image plate detector for neutron diffraction and Gamble et al. present the first atomic resolution study (by X-rays) of a perdeuterated protein to show that it cannot be distinguished from the native (natural abundance) form. This is an important result for future neutron crystallographic studies. The main contribution to background in neutron experiments is the incoherent scattering of hydrogen and a perdeuterated sample could lead to an improvement in signal-to-noise ratio by several orders of magnitude, depending on the resolution.

Recently, a new neutron crystallography approach at low resolution has been developed, suitable for the study of complex structures such as viruses or membrane protein–detergent complexes. Components are separated by contrast variation. Because of the 'low' resolution and long wavelengths used, neutron diffraction is relatively strong and the limitations on minimum crystal size are considerably relaxed compared to atomic resolution studies. Timmins et al. describe a study of detergent organisation in membrane protein crystals and compare it to solution results.

Perdeuterated phycocyanin was used by Bradley et al. to measure the 'liquid' diffraction pattern from an amorphous protein sample as a function of temperature and hydration. They modelled the data quite well by assuming each protein molecule to have itself the structure of a glass with the amino acids playing the role of 'atoms'. It is interesting that such an approach works since it ignores the chemical differences between amino acids. Chemical differences between amino acids were also successfully ignored in the study of protein denaturation by Calmettes et. al in which unfolded yeast phosphoglycerate kinase was modelled as a random polymer coil with excluded volume interactions. Both studies are in step with recent views, resulting from thermodynamic studies of protein folding, on the dominance of packing interactions in a protein interior. The interactions of denatured protein with sodium-dodecyl-sulphate were studied by Ibel et al.

Neutron scattering is particularly well suited to the study of macromolecules in concentrated salt solutions, be they denaturing or stabilising, because of negligible absorption and favourable contrast values. An understanding of the hydration and cosolvent interactions of proteins and nucleic acids in different conditions is essential and in a comprehensive review of decades of data, Eisenberg has derived 'base-line' values for these effects, i.e. water and cosolvent binding to either the stable native or fully unfolded state of the macromolecule in solutions with very high cosolvent concentrations.

Salt solutions are themselves a current and active subject of study and the paper by Bonneté and Zaccai analyses their neutron small angle scattering and cross-sections in usual conditions for the study of proteins. Deliberately broadening the definition of 'Neutrons in Biology' I have included in this issue a number of structural and inelastic neutron scattering studies that are not on biological macromolecules, either because they are informative on the physical chemistry of the environment in which biological interactions take place or because they deal with well-defined model systems. One way to approach an understanding of the crowded environment inside a cell, for example, could be through the study of the structure and dynamics of aqueous gels, as in the papers of Kreuger et al. and Middendorf et al. As already mentioned above, protons play vital roles in biological structure and function; the work of Fillaux et al. addresses fundamental questions on the chemistry of proton dynamics through experiments on polyglycine. These last two papers describe the methods of quasielastic and inelastic neutron scattering at a spallation source. Pebay-Peyroula et al. have examined the location of fluorescent probes in lipid bilayers and describe methods using deuterium labels that could be very powerful in the study of membrane protein insertion, for example.

The atomic resolution structure of a protein is obtained in well-defined crystalline conditions. Solution scattering, on the other hand, provides much more limited structural information but in the environment of our choice. The availability of atomic resolution structures has paved the way for the interpretation of solution data on interactions, for example, by using these structures as a basis. Henderson et al. measured the scattering of yeast phosphoglycerate kinase in the presence of different ligands in

D_2O solution and modelled the results in terms of hydration changes and the known crystal structure of the enzyme.

The challenge of quaternary structure and interaction determination by neutron solution scattering and deuterium labelling is very much of topical interest and the papers of Nowotny et al. and Serdyuk et al. deal with two different powerful approaches that should have a wide applicability. Both are in the field of protein synthesis — on ribosome structure and the interactions of polypeptide elongation factor, respectively.

Finally, inelastic neutron scattering remains irreplaceable for the study of thermal motions in proteins at different levels of time resolution. The review by Smith brings us up to date in this field, not only on the experimental results but also on progress in the theoretical approaches for understanding protein dynamics.

The sixteen papers are not published in the order in which they are discussed above but according to the methodology employed: first, high, medium and low resolution studies, then, solution work and inelastic scattering. Experiments were carried out with high and medium flux reactors and a spallation source in the EU (ILL, LLB, ISIS) and the USA (ORNL, NIST) by a truly international community of scientists.

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