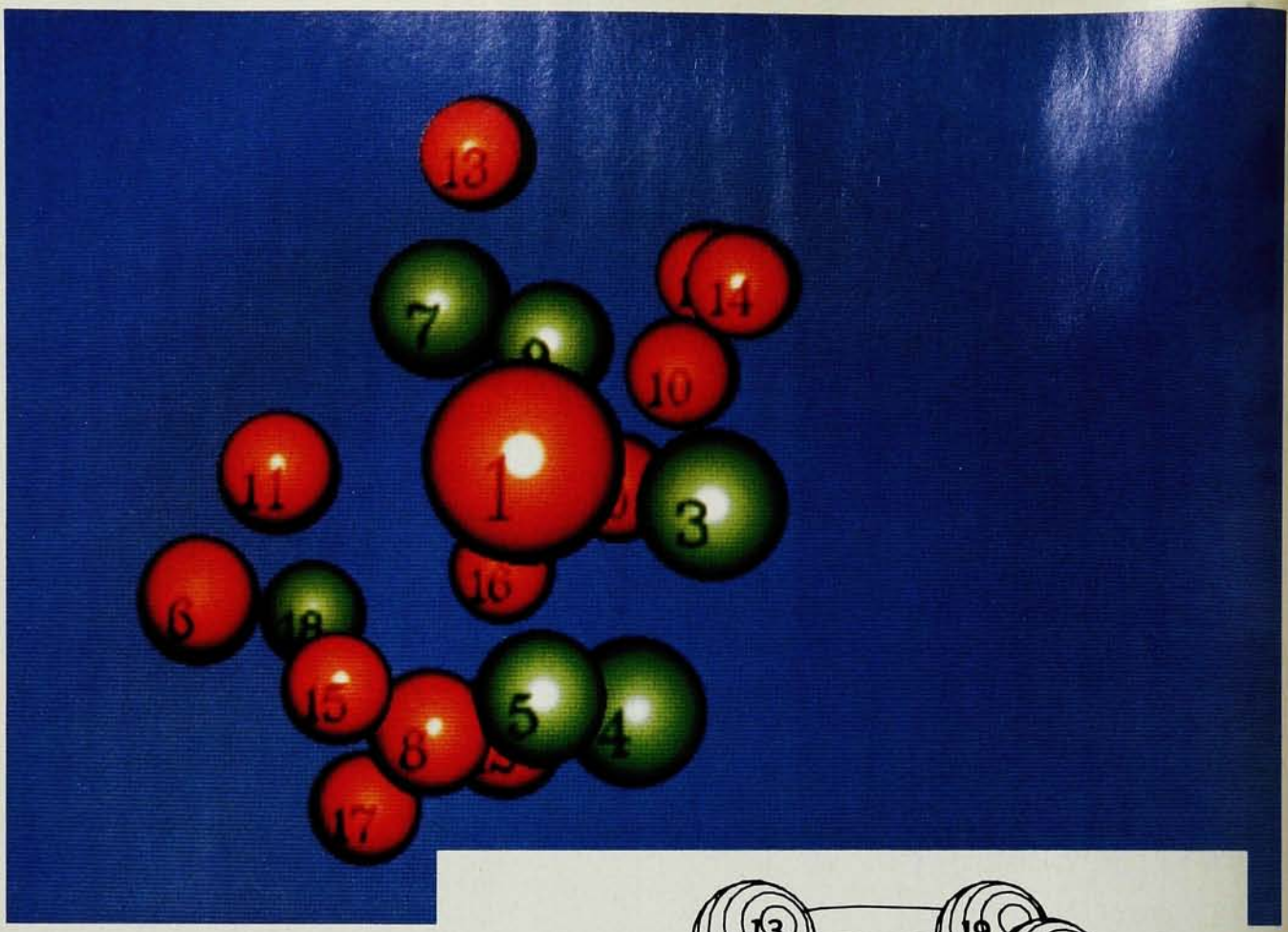

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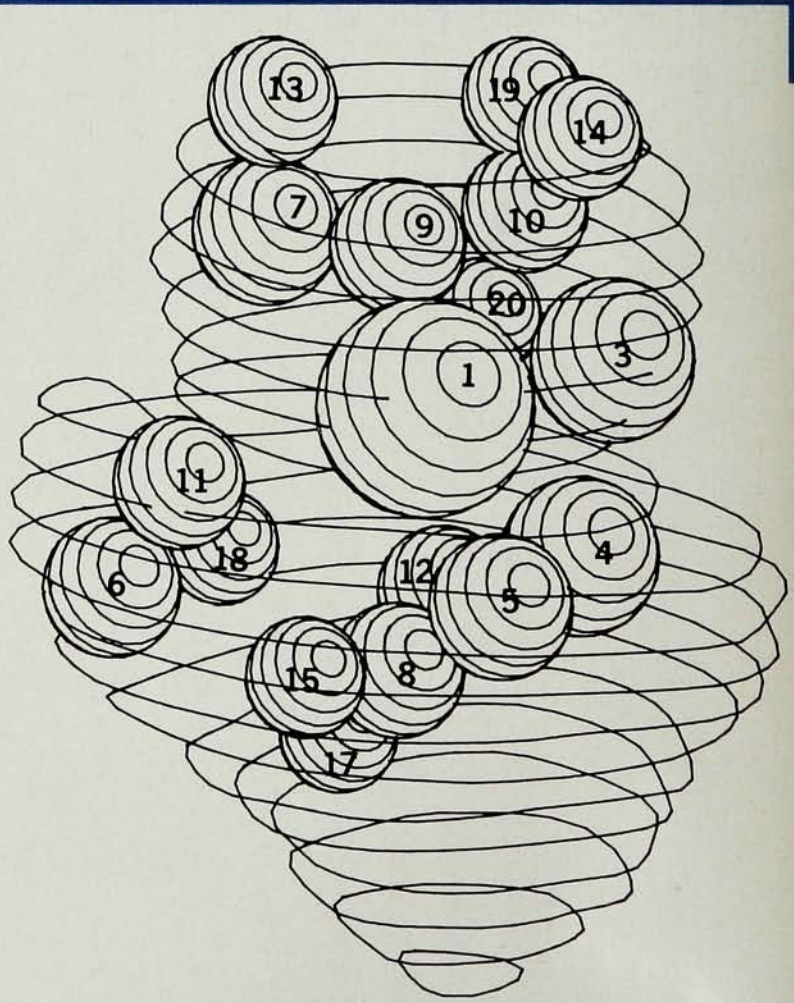

b

Three-dimensional representation of positions of proteins in the 30S ribosomal subunit of the bacteria *Escherichia coli*. In **a** the proteins are shown as spheres. The height of the structure is 170 Å. The RNA matrix is not shown. The figure on the right (**b**) is obtained by the superposition of the electron-microscopic image of the RNA matrix with a protein map similar to the one shown in **a**. The computergraph is based on data from reference 22. (Courtesy of Morten Kjeldgaard) Figure 1

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Applications of neutron scattering to biology

Investigation of the structure of biological macromolecules, until recently possible only with electrons and x rays, has been significantly enhanced by the use of high-flux neutron beams.

Peter B. Moore

During the past 20 years neutron scattering has developed into an important tool for the determination of the structure of biologically important compounds. Traditionally these kinds of problems have been solved by x-ray crystallography or electron microscopy, both of which require far cheaper and simpler instrumentation. The justification for the use of neutrons in biological research, which might seem at first an outrageous extravagance, is contained in a single word: "hydrogen." The scattering of neutrons from hydrogen (and deuterium) is far larger compared to other atoms than is the comparative scattering of x rays and electrons; neutrons, therefore, are a much better probe for these light atoms.

Biological neutron experiments fall into two categories: At high resolution, neutrons are used to determine the placement of hydrogen atoms in biological molecules, and at lower resolution these experiments exploit the difference in sensitivity of neutron radiation to hydrogen and to deuterium to determine the location of molecular components in large structures. One of the more interesting results obtained with low-resolution experiments so far, the location of protein spheres in a section of chromosome, is shown in figure 1.

Biology and molecular structure

The properties of organisms that identify them as living, such as motility and reproduction, are to a large extent manifestations of chemical events occurring within them. A century of biochemical study has shown that these chemical events, in turn, reflect the properties of the macromolecular components an organism contains: its proteins, nucleic acids, lipids and polysaccharides. The intimate involvement of macromolecular materials in life processes has made these polymeric substances the object of intensive study for many decades.

In many respects the interest of biophysical chemists studying the properties of biopolymers are similar to those of the polymer chemists examining synthetic polymers, and their experimental strategies are closely related. However, one should bear in mind that while synthetic polymer preparations are commonly mixtures of molecular species differing in molecular weight, sequence, and configuration, and thus appropriately characterized statistically, biological macromolecules tend to be highly nonstatistical. A preparation of a given protein is a population of molecules of identical covalent structure with identical three-dimensional folded structures within the limits imposed by thermal vibrations. Indeed it is the three-dimensional structure of these macromolecules that endows them with their biologically important properties. The challenge is to understand what these three-dimensional structures are and how the physiological properties of these molecules arise from them.

From time to time, investigations of this kind succeed, and when they do the impact can be substantial. A most striking case in point was the discovery of the structure of DNA in 1953 by James Watson and Francis Crick. The structure of DNA immediately suggested how the molecule worked as a storage device for genetic information and how it is replicated—its two key functions. Molecular biologists have productively explored the implications of these findings for the past 30 years. The end is still not in sight.

Because molecular structure is of extreme importance in biology, any new technique for investigating structures will be eagerly embraced by biologists. In the late 1960s when the current generation of high-flux beam reactors was commissioned or built, some biophysical chemists were granted access to beam time. They hoped that neutron beams would yield novel information about the structure and properties of biological macromolecules, and that the new reactors would produce thermal fluxes that were high enough to make experiments with these weakly scattering biological materials rewarding. One of the most valuable instruments to biologists is the D11 small-angle neutron spectrometer at the high-flux reactor of the Institut Laue–Langevin in Grenoble, France (see figure 2).

Neutrons and biological structure

Neutron beams of useful flux obtained from typical thermal neutron sources have wavelengths ranging from about 1 Å to 10 Å. Using radiation in this wavelength range, one can examine the structures of objects having linear dimensions as large as 500 Å, the size of some viruses, and macromolecular aggregates. Provided the material of interest crystallizes appropriately, the resolution available in a neutron study can lead to a description of its structure at the atomic level.

Both in terms of the resolution achievable and the size range of objects accessible, there is a strong overlap between the capability of x rays and of neutrons. Moreover, electron microscopy is available for studying the structure of the larger objects.

Hydrogen. The end product of an x-ray crystallographer's work is a description of a molecule in terms of an

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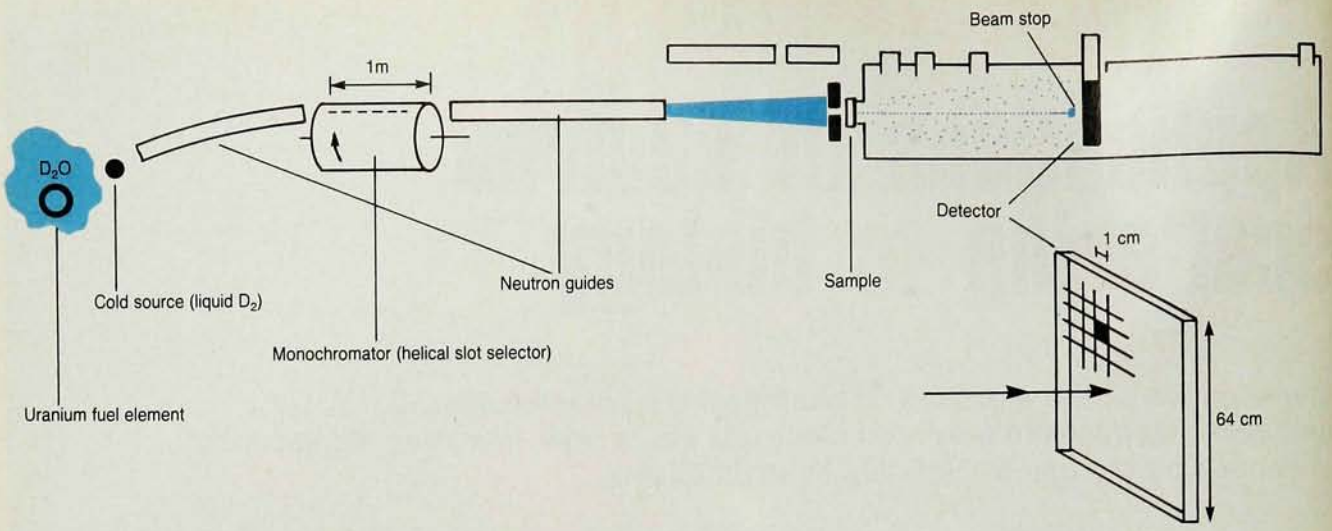


Small-angle neutron spectrometer at the high-flux reactor of the Institut Laue–Langevin in Grenoble. For a description of the instrument see the article by Roger Pynn and Brian Fender on page 46 . Figure 2

electron-density map. Hydrogen, having only a single electron, is never conspicuous in such maps. Even in a well-determined structure of a small molecule, the placement of hydrogens is subject to substantial error. Unfortunately, the electron-density maps obtained in x-ray crystallographic studies of biological macromolecules seldom yield the resolution or the favorable ratio of data characteristic of electron-density maps for small molecules. In the relatively low-quality electron-density maps that one obtains for macromolecules, hydrogen simply disappears.

Table 1 compares the scattering lengths of atoms of biological significance for both x rays and neutrons. Scattering lengths for x rays are proportional to atomic number, making C, N and O far more conspicuous than H in x-ray diffraction; in the case of neutrons, however, the magnitude of the scattering length of H^1 is within a factor two of other atoms. The negative sign of its scattering length indicates that the neutron wave produced by scattering from H^1 is 180 degrees out of phase with respect to the wave produced when a carbon atom, for example, scatters a neutron from the same location in space. Thus in a neutron-scattering density map of a molecule, H^1 will appear as a negative hole while other atoms are represented as positive peaks. Negative or not, however, H^1 will be detected more easily with neutrons than with x rays, and protons are visible in the (relatively) low-quality maps produced when macromolecular crystals are solved by neutron techniques.

A second point that emerges from table 1 is the striking difference in scattering length between hydrogen and deuterium. The former is negative while the latter is positive. If hydrogen is substituted by deuterium, then at high resolution all the H^1 holes in the scattering-length map will be replaced by positive H^2 peaks. One should bear in mind that most biological materials contain more hydrogen atoms than all other atomic species put together. Thus one can achieve marked alterations in the total scattering length of a biomolecule with H^2 labeling, a fact of considerable significance in low-resolution experiments. Only modest alterations in the chemical properties of the substances are caused by H^2 labeling. Indeed, there is no other combination of labeling method and radiation choice that achieves so much at so little cost to the integrity of the material under study. I will show what use one can make of this fact later in this article.

High-resolution studies

A major objective of those who started biological work with neutrons in the late 1960s was the determination of the crystal structures of proteins at a resolution as high as possible. A number of issues stimulated this interest. The first concerned hydrogen bonds, which in part are responsible for the stabilization of the three-dimensional structure of proteins. (For an overview of protein structure, see reference 1.) It is, in fact, typical for protein structures derived from x-ray scattering not to exhibit the hydrogen bonds directly because the shared hydrogen atoms are invisible in the maps. The existence of these bonds must therefore be inferred from the proximity of appropriate donor and acceptor groups. In a neutron study, these interactions are directly observable. The ability to visualize hydrogen bonds directly was also important to those interested in the way enzymes work, as opposed to structure as such, because the catalytic configurations of these molecules commonly involve complex hydrogen-bonded networks, and alterations in proton chemistry often accompany enzyme action.

A second issue, closely related to the first, concerns solvent structure. It has been known for a long time that the water that immediately surrounds a macromolecule in solution has properties that distinguish it from bulk solvent. Because solvation is believed to be an important influence in the folding of macromolecules, one wants to see how water is structured around the surfaces of biological macromolecules in detail. In an x-ray structure, such water is observable because the oxygen atoms are visible. The problem is that these solvent structures are seldom perfectly ordered, and many of the H_2O sites are not occupied all the time, weakening the contribution these sites make to the map. At some point in the analysis there comes a stage where it is difficult to distinguish a water site that is partially occupied from noise in the map. In a neutron map all three atoms in H_2O can be seen and the distinctive shape of the molecule helps to distinguish it from noise.

A third reason for solving protein structures at high resolution was discovered by accident, I suspect. Biological molecules contain a large number of hydrogen atoms and most of the molecules prefer to exist in aqueous media, which are also rich in hydrogen. The presence of so much hydrogen brings with it a burden. In addition to having a reasonably large coherent scattering length, hydrogen also has an exceptionally large incoherent cross section.

Incoherent hydrogen scattering contributes a large background to any scattering or diffraction measurement done on samples containing it. In addition, its presence effectively limits the permissible thickness of hydrogenated scattering specimens to a few millimeters. A simple expedient that alleviates both problems is to replace H_2O in the solvent with D_2O.

An interesting process begins as soon as the solvent is changed to D_2O. Many of the protons in biological molecules are chemically labile, and these begin exchanging with deuterium in the water. What is observed, however, is that not all groups chemically capable of exchange succeed in doing so; some are protected from exchange by their position in the molecule.

The pattern of protection is far more interesting than that expected from simple steric hindrance considerations based on the molecular structure.[3] Some groups, apparently well protected from solvent, do exchange; others do not. Thus the exchange pattern gives evidence about the dynamic properties of the molecule because flexibility is clearly required to permit exchange at interior groups. Neutron data collected in D_2O readily distinguish exchanged from unexchanged sites and thus give valuable information on the long-time limit of the exchange process.

There are a number of other techniques that are used to obtain information on these processes. Tritium exchange experiments, for example, have been done for years.[4] The degree of replacement of H^1 by H^3 atoms is measured by changes in radioactivity. While tritium exchange can give good kinetic data, it is hard to discover which groups are exchanging by this method[5] because the information sought tends to be lost during analysis. Nuclear magnetic resonance can also be used for studies of this kind. For smallish macromolecules with molecular weights of less than 10 000, where assignments can be made, nmr can identify the slowly exchanging groups and measure their exchange rates.[6] The neutron method is not limited to small molecules, unlike the nmr technique, but at present it can give no kinetic data.

The fourth and final motivation came from the recognition that one might be able to distinguish O and N in a neutron map (see table 1). There are a number of residues in protein, such as asparagine, which terminate in amides. In an x-ray map it is hard to distinguish the carbonyl side of an amide group from its amine side. However, in a comparable neutron map, the difference between N and O is larger than in the x-ray counterpart, and the attached hydrogens are also visible, so one can readily place the groups correctly in space.

Neutron protein crystallography

Because of the low flux provided by most neutron sources, it is not easy to collect neutron data from protein crystals. Thus the only proteins that could be studied at first were those that could form very large crystals and give high data-collection rates. The structure of myoglobin was determined using a crystal of 24 mm^3 in volume, which is an exceptional size for a protein crystal.[7] In the past decade, substantial progress has been made in the instruments and techniques used for collecting neutron-scattering data, reducing the need for unusually large crystals and broadening the range of approachable problems. A crystal 1 mm^3 in

Glossary

Chromosome: Any of a number (specific for each species) of threadlike structures found in the cell nucleus. Chromosomes are microscopically visible during cell division and consist of complexes of densely coiled DNA molecules and proteins.

Deoxyribonucleic acid (DNA): A macromolecule that is a linear polymer of deoxyribonucleotides. These molecules function as the storage device for genetic information, encoded in the sequence of nucleotides in the polymer.

Deuteration: The replacement of an H^1 atom by an H^2 atom in a molecule.

Lipid: One of a diverse class of biological molecules of modest molecular weight (about 500) that are sparingly soluble in water. Many molecules of this class will aggregate in aqueous media to form two-dimensional sheets, which are the basis for the membranous structures in living organisms.

Myoglobin: A protein abundant in muscle that serves as an oxygen storage medium. This protein was the first to have its structure determined crystallographically.

Nucleic acid: DNA or RNA molecules, especially those of biological origin.

Polysaccharide: A molecule that is either a linear or branched polymer of sugars. Some polysaccharides serve as energy stores—for example, glycogen and starch; others have structural functions—for example, cellulose.

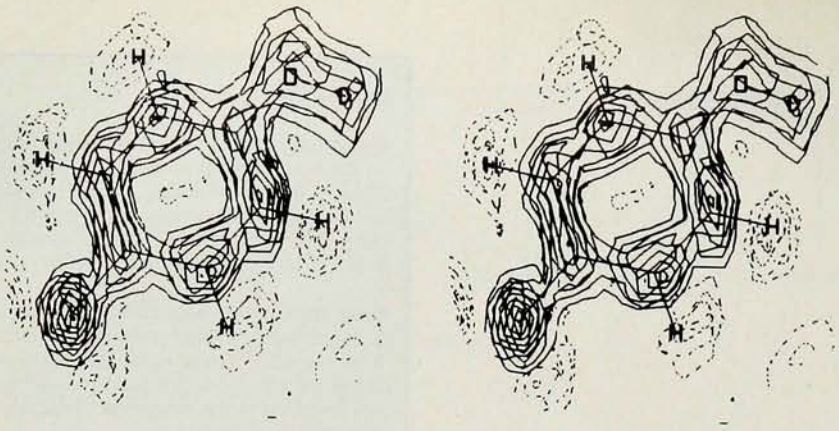
Protein: A molecule that is a linear copolymer of amino acids, especially one of biological origin. Proteins are the main macromolecular constituents of living matter, accounting for roughly half the dry weight of the average organism. Many proteins are enzymes; that is, they function as catalysts.

Ribonucleic acid (RNA): A macromolecule that is a linear polymer of ribonucleotides. These molecules are chemically similar to DNA and function primarily in the processes that lead to gene expression.

RNA polymerase: A protein enzyme found in all organisms that catalyzes the synthesis of RNA copies of DNA molecules.

Ribosome: A complex of protein and RNA of high molecular weight (about 3×10^6 daltons) that catalyzes the polymerization of amino acids into protein, a process directed ultimately by the nucleotide sequences in DNA.

Transfer RNA: (tRNA) a class of RNA molecules with molecular weights near 25 000 that all have similar three-dimensional structures and serve as carriers of amino acids in protein synthesis.



Stereoscopic view of the tyrosine residue of the plant protein crambin obtained by high-resolution neutron crystallography. The depth effect becomes visible when viewed with a stereoscope. Reprinted from M. M. Teeter, A. A. Kossiakoff, in *Neutrons in Biology*, B. Schoenborn, ed., Plenum, New York, (1983), p. 335. Figure 3

volume will yield useful data in an acceptable length of time today. (An interesting feature of neutron crystallography is that neutrons, unlike x rays, do not damage crystals: Their energies are only on the order of kT, much too small to cause ionization. Crystals last "forever" in a neutron beam.)

About six proteins have been studied[8] by neutron crystallography at Brookhaven, the National Bureau of Standards and the Institut Laue–Langevin. What emerges from the data published so far is that the promises and hopes that led to the development of this field are going to be fulfilled. But we have found that the exchange properties and solvent structures of the proteins examined so far are so diverse that it is hard to deduce any general principles, and we need to see more examples. It is also clear that it would be interesting to attempt to measure exchange kinetics. Perhaps cryogenic methods to slow exchange processes and higher-flux sources to speed data collection will allow us to get some data of this kind.

Figure 3 shows a stereoscopic view of a small portion of the neutron-scattering map deduced for the plant protein crambin. This molecule crystallizes in a remarkably well-ordered lattice and gives diffraction patterns that provide exceptional resolution. The figure shows a tyrosine residue at 1.4 Å resolution. The protein was soaked in a mixture of D_2O and deuterated ethanol for one week prior to data collection. The aromatic-ring hydrogens are clearly delineated as negative intensity features; the hydroxyl proton is clearly positive because it contains H^2. (In this case the exchange pattern is the one anticipated on chemical grounds.)

It should be noted that no one has developed methods for solving macromolecular structures using neutron data alone. The only protein crystals studied by neutrons have already been solved using x rays; the neutron structure is obtained by refinement. Neutron analysis is, in fact, a method to obtain supplementary information on a protein crystal structure that has been solved by x-ray methods. A few months of data collection on a neutron spectrometer followed by some computation is cheap compared to the investment necessary to get an x-ray protein structure in the first place, and the information gained is substantial.

In the future there undoubtedly will be a steady flow of biochemists motivated by issues such as the ones described above, bringing protein crystals to reactors. Perhaps they will soon start bringing crystals of nucleic acids as well. Nucleic acids are densely charged polyelectrolytes and the surrounding solvent region may be quite interesting.

Low-resolution studies

In my mind there is little doubt that the results obtained using neutrons crystallographically will be, in the long run, the most significant contribution of neutron research to biology. Nevertheless, in the literature of the past 15 years there have been ten or more papers describing the use of neutrons to study structures at much lower resolution for every paper involving high-resolution crystallography. All of these experiments exploit the difference in scattering lengths between H^1 and H^2 in one way or another.

In a low-resolution experiment, by definition, individual atoms are not visualized. It is not the scattering lengths of individual atoms that are interesting, but the bulk, average scattering properties, which can be conveniently characterized in terms of scattering-length densities. A few scattering-length densities for substances of biological interest are given in table 2. Water has a scattering-length density close to zero; its two hydrogen atoms ($b = -0.374\times10^{-12}$ cm) just about cancel out the positive contribution ($b = +0.667\times10^{-12}$ cm) due to its oxygen. The scattering-length density of D_2O, on the other hand, is strongly positive because the negative contributions of the hydrogen atoms have been replaced with positive scattering lengths due to deuterium. The differences in scattering-length density for different biological materials reflect the relative abundances of hydrogen in these substances. In every case, perdeuteration produces striking increases in scattering-length density.

Many biological objects are composites consisting of two different chemical species and hence vary significantly in scattering-length density within their volumes. One can obtain low-resolution information on the disposition of the two materials (for example, radius of gyration, shape, separation) in such a structure by using what is called "contrast variation."[9] In effect, by adding D_2O to the water in which the structure is dissolved, one can arrange the difference in scattering-length density (the contrast) between one of the components and the solvent to be zero. In this solvent the unmatched component will dominate the scattering, and one can obtain information about its size, shape and so on. Changing the contrast to match the second component will yield comparable information about the first.

Because contrast-variation experiments are easy to implement biochemically and because data are collected in a straightforward manner, many biologists have used this technique. Among the successes achieved by this method one would have to count the studies done on nucleosomes.[10,11] Nucleosomes, which consist of DNA and protein, are the unit of structure in chromosomes. An example of results obtained in such a study is given in figure

4, which shows low-resolution scattering-density maps of crystals of nucleosome particles in solvents with varying D_2O content. These and other studies done on nucleosomes in solution have led to a low-resolution model for this structure, which has now been confirmed[12] crystallographically at much higher resolution.

Viruses have also been investigated with the contrast-variation method. Viruses crystallize and the structures of some of them are now known at high resolution.[13] A shortcoming of the structures in hand is that only the viral protein coat is crystallizing. The nucleic acid contained in the coat is not ordered with respect to it, and so while the crystal structures are very informative about the protein coat, they tell us next to nothing about the placement of the nucleic acid within it. Contrast variation has been used[14] to obtain low-resolution information on this point. Contrast variation has also been helpful in the analysis of the structures of lipid bilayers containing protein.

An application of contrast variation which is particularly intriguing has been explored by Guiseppe Zaccai and Bernard Jacrot at the Institut Laue–Langevin. Using contrast-variation studies on transfer RNA, they have obtained[15] strong evidence for the existence of an electrostricted layer of water around these molecules.

Deuteration experiments

In many respects the most elegant low-resolution neutron experiments are those involving selective deuteration of the object under investigation. Localized deuteration experiments are low-resolution analogues of the isomorphous replacement experiments that are used to derive macromolecular crystal data. The result of the analysis of isomorphous replacement data in an x-ray experiment is the determination of the placement of a heavy atom in a crystal. In the low-resolution neutron case, the atoms whose positions are determined are the deuterium atoms used as labels.

Table 1. Scattering lengths

Element	neutrons $b\times10^{12}$ cm	x rays $b\times10^{12}$ cm
H^1	−0.374	0.28
H^2	+0.667	0.28
C	+0.665	1.69
N	+0.94	1.97
O	+0.58	2.25
P	+0.51	4.23
S	+0.28	4.50

X-ray scattering lengths are given for a scattering angle of zero.
Data taken from reference 2.

A number of incisive experiments have been done in this way. Among them one would certainly include the series of experiments done[16] to position lipid molecules in membrane bilayers. In this case bilayers were formed from lipids chemically labeled with H^2 at specific positions. The perturbation in bilayer diffraction caused by the incorporated deuterium revealed the locations of the deuterated positions of the lipid molecules in the structure.

Several macromolecular aggregates have been or are being now investigated by specific deuteration methods to determine the position of macromolecular subunits within these structures. A full study has been completed[17] on the enzyme RNA polymerase, a five subunit structure from *Escherichia coli*. The 30S ribosomal subunit from *E. coli*, containing 22 macromolecular components, is being studied by similar methods; 19 of its components have been located.[18] The 50S subunit of the ribosome is being examined[19] in a set of experiments presently getting underway at the Institut Laue–Langevin.

Table 2. Scattering-length densities

Substance	H^1 substituted	H^2 substituted
H_2O	−0.55	+6.36
Protein	+3.11	+8.54
Nucleic acid	+4.44	+7.44
Carbohydrate	+4.27	+8.07
Fatty acid	−0.01	+6.89

Scattering-length densities are given in units of 10^{-14} cm/Å^3, or 10^{10} cm^{-2}. These average values are obtained by summing the scattering lengths of all atoms in a molecule and dividing the result by the molecular volume. For H^2 substituted materials it is assumed that all exchangable hydrogen sites are occupied by H^2.

Figure 1a is a view of the protein map of the 30S ribosomal subunit from the bacterium *E. coli*, displaying the position of the currently known 19 proteins. Ribosomes occur in all organisms and are responsible for protein synthesis; the ribosome from *E. coli*, part of which is shown here, is typical. Proteins are represented as spheres for convenience. The volumes of the spheres represent the volume occupied by each protein to the scale of the figure. The results of 79 independent scattering experiments are combined in this map. The fact that the proteins are apparently "floating" in space is an illusion created by the fact that the RNA portion of the ribosome to which the proteins attach is not shown.

The appearance of the RNA matrix of the 30S ribosomal subunit in the electron microscope is well known. It is possible to orient the neutron map of protein positions within the shape obtained with an electron microscope because the sites where the proteins come to the surface of the structure have been located electronmicroscopically—using antibodies raised against specific proteins as staining agents. Figure 1b shows such a superposition of neutron-derived protein locations into the microscopic image. The electron-microscopic data used[22] in this case were obtained by George Stoeffler and Marina Stoeffler-Meilicke.

The production of appropriately labeled samples for experiments of this kind places heavy demands on biochemical technology. In terms of the labor involved, a specific deuteration experiment typically consists of 95% sample preparation and 5% data collection and analysis. The number of structures sufficiently well known to permit the production of suitable amounts of appropriately labeled material is still small. There is a "learning curve" connected with the preparation and manipulation of any biochemical system, however, so it is reasonable to anticipate that more and more structurally interesting systems will arrive at the stage where specific-deuteration experiments can give interesting information and where the technical means of doing the work are in hand.

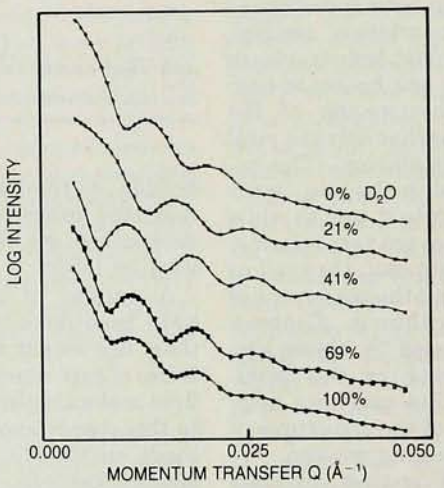
While one can think of counterparts to many of these experiments using electron microscopy or x-ray scattering, and although some have been attempted, none work as well as their neutron cousins. These are experiments where the peculiar properties of neutron radiation happen to fit the biological problem particularly well.

Inelastic scattering

In the past few years some work has been done[20,21] on inelastic scattering in biological polymers. However, very little experience has been gained so far: One can obtain data only slowly from biological materials and the competing demands for time on the few existing instruments suited for this work have limited what the biologists could do.

What is clear from the data is that proteins have inelastic modes corresponding to whole-molecule vibrations and that these low-frequency modes

Contrast-variation experiments



A demonstration of the influence of solvent scattering-length density on solution scattering curves may be found in the figure above. The data obtained by Stephen Cusack and his colleagues at the Institut Laue–Langevin are plotted as the logarithm of scattered neutron intensity versus scattering angle, represented here by the momentum transfer Q, given by $4\pi\sin\theta/\lambda$, where θ is half the scattering angle. Several scattering profiles are shown. They are collected on the same material in solvents varying in D_2O content. (The curves are shifted in the vertical direction for convenience.)

The material examined is a derivative of influenza virus, which is a complex aggregate of protein, RNA, lipid and carbohydrate. Because the virus is nearly spherical, its scattering shows secondary maxima. These maxima shift in position as the D_2O concentration in the solvent is altered, indicating that the average radius of the net scattering power in the particle is changing with contrast. The slopes of the scattering curves as they approach $Q = 0$ also depend strongly on solvent composition, echoing the same message as the positions of the secondary maxima: the scattering-length density of the virus is not uniformly distributed in the radial direction. Systematic analysis of such constant series enables one to deduce what the radial distribution of scattering-length density is within the virus. Since these variations in density reflect differences in the scattering-length densities of the chemical constituents of the virus, knowledge of the radial distribution of scattering-length density gives valuable information about the way these constituents are arranged in space.

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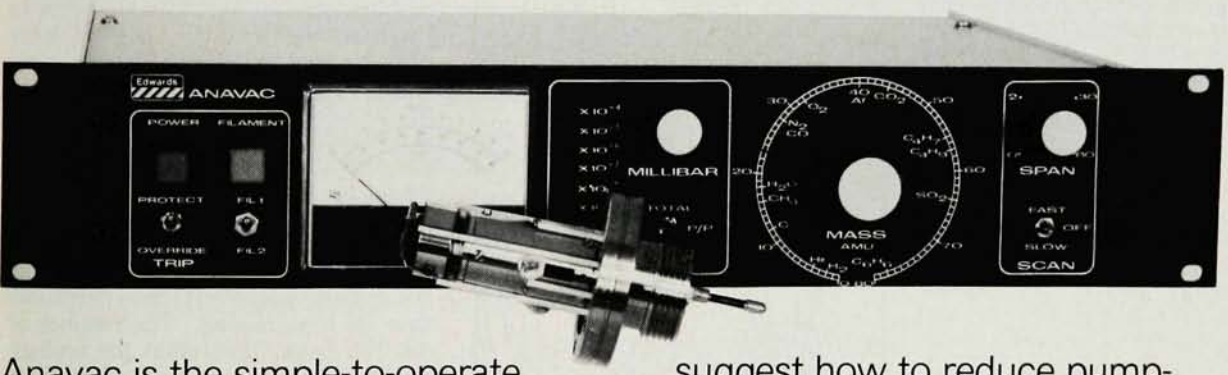

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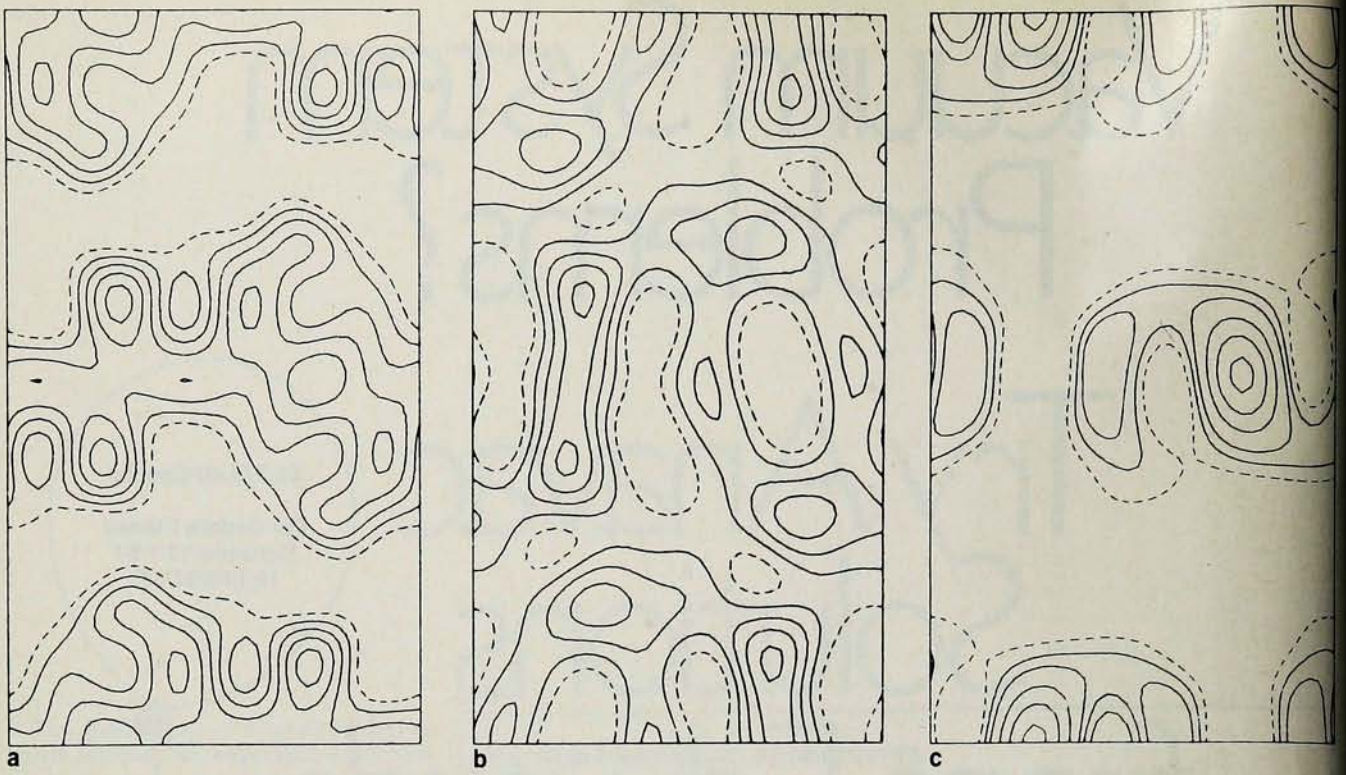

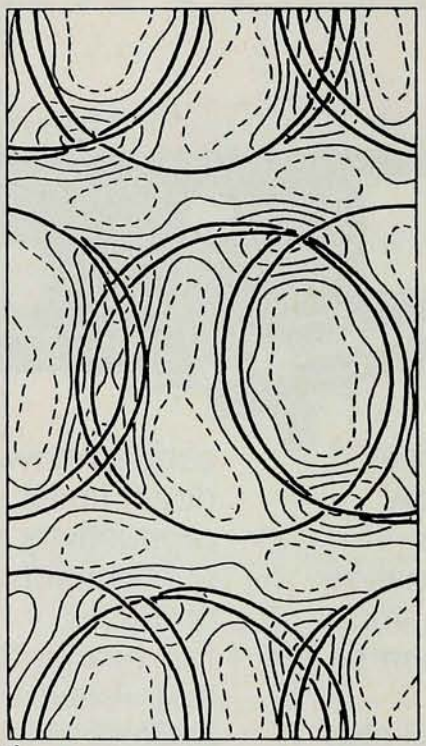

Low-resolution neutron maps of crystals of nucleosome particles in solvents varying in D_2O content. The resolution is 25 Å. In **a** the solvent is 100% D_2O, both the DNA and the protein are visible. In **b** the solvent contains 39% D_2O and matches the scattering-length density of protein, rendering the DNA visible. In **c** the solvent contains 65% D_2O and matches the scattering-length density of DNA, making the protein visible. In **d** the trajectories of the DNA coils around the nucleosomes are shown, the density lines represent calculated values. Reprinted from G. A. Bentley, J. T. Finch, A. Lewit-Bentley, *J. Mol. Biol.* **145**, 771 (1981). Figure 4

respond in interesting ways to biologically significant alterations in molecular state, such as substrate binding. It is not yet clear how this field will develop; the spectra are seriously overlapped and assignment of spectral characteristics to molecular processes is very difficult. There is, however, a connection between these measurements and the theoretical area of molecular dynamics. In the past decade biochemists have made efforts to simulate the dynamic properties of proteins and nucleic acids computationally. These calculations predict in effect the inelastic spectra biological macromolecules should have. A comparison of data with theory in this area would be extremely helpful.

In evaluating the progress made in the application of neutron techniques to biology, one should bear in mind that the resources devoted to the effort have thus far been modest. The number of neutron beam lines used for biology worldwide has never been more than about six, and most lines used by biologists have been shared with scientists from other disciplines. The results obtained in return for this investment have been gratifying.

In the future it is reasonable to anticipate continued crystallographic work on biological macromolecules and further studies using the low-resolution methods already established. Given the history of the past decade, there is every reason to hope that new and exciting approaches will develop.

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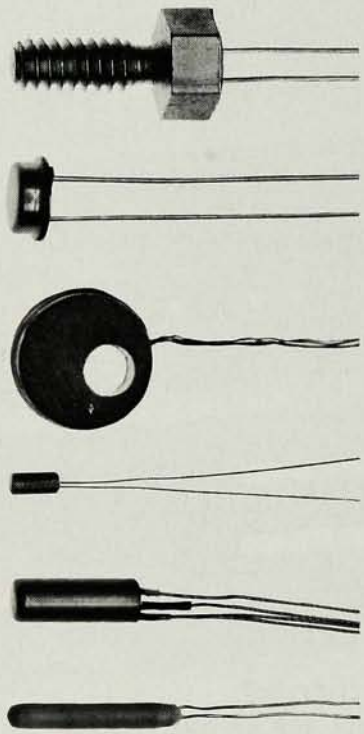

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