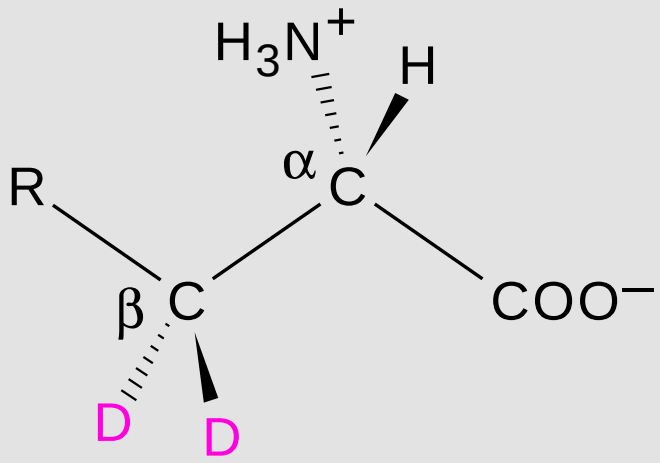
Experimental Structural Biology

Jill Trewhella



A deuteron-labeled amino acid

The key to understanding the functioning of biological macromolecules lies in detailed knowledge of the structures and structural dynamics of the macromolecules themselves and of the assemblages they form. Among the various types of biological macromolecules, the polypeptides and polynucleotides are of particular importance. A polypeptide, or protein, is a linear polymer of amino acids; a polynucleotide, or DNA or RNA molecule, is a linear polymer of either deoxyribonucleotides or ribonucleotides. Proteins are the principal worker molecules of cells, performing essential processes such as catalysis of biochemical reactions, including those involved in extraction of energy from the environment and in synthesis of DNA, of RNA, and of proteins themselves; transport and storage of small molecules and ions; provision of mechanical support and motility; recognition and destruction of foreign substances; generation and transmission of nerve impulses; and regulation of gene expression. DNA molecules are the agents of heredity, containing information that guides not only their own replication but also synthesis of the RNA molecules that in turn guide the synthesis of proteins. Unlike synthetic polymers, polypeptides and polynucleotides assume precise three-dimensional structures, structures that constitute the framework for the chemistry of life. Knowledge of those structures is fundamental to understanding life processes in terms of basic physical and chemical laws of nature.

Before the advances achieved at Los Alamos National Laboratory in experimental structural biology are described, a natural question should be answered. Why is a laboratory whose primary job is the design of nuclear weapons also investigating the structures of biological molecules? The answer lies in the fact that many of the techniques and areas of expertise applicable to research on nuclear weapons are applicable also to research on structural biology. Now gone is the era when it was possible to describe the biological sciences as "soft." Today sophisticated physical, chemical, and analytic techniques are as much a part of biology research as of weapons research, and, equally important, the ability to describe biological processes in terms of basic physics, chemistry, and mathematics is fast increasing.

Prominent among the newer techniques used by structural biologists are laser spectroscopy, neutron scattering, and nuclear-magnetic-resonance spectroscopy. Each involves observation of the results of the interaction of a "probe" with a biological molecule or with its constituent atoms or atomic nuclei. The basis of laser-spectroscopic techniques is the absorption and scattering of laser light (the probe) by molecules; of neutron-scattering techniques, the scattering of neutrons by nuclei; and of NMR spectroscopy, the interaction of magnetic fields with certain nuclei. The utility of each of those techniques to structure determination can often be enhanced by clever strategies for labeling a particular site or region of the molecule of interest with stable isotopes. Such labeling imparts different spectroscopic, neutron-scattering, or magnetic properties to the site or region, and

those differences reflect structural features of the molecule. To date the Laboratory's unique contributions in experimental structural biology have arisen primarily from applications of advanced laser-spectroscopic and neutron-scattering techniques, often in combination with stable-isotope labeling. The future promises a greatly increased emphasis here on the use of high-magnetic-field NMR spectroscopy and stable-isotope labeling in structural studies of biological entities.

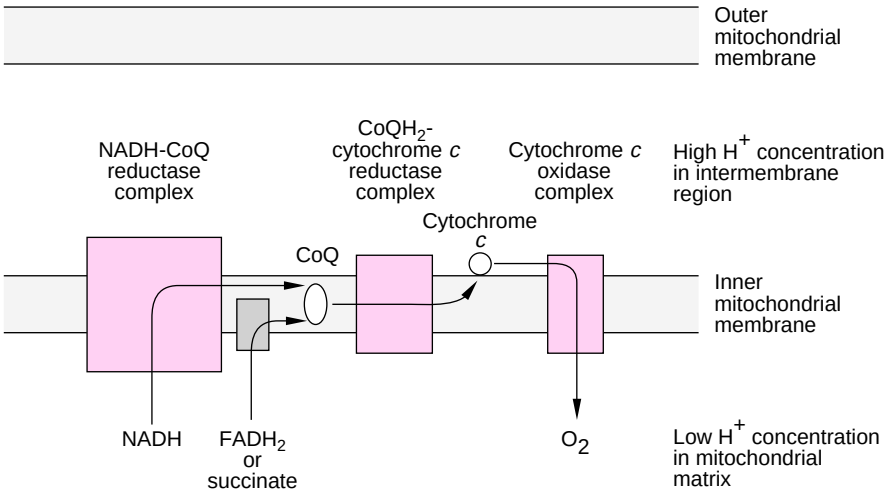


Figure 1. The Respiratory Chain

The process by which living things extract energy from foodstuffs is exceedingly complex and circuitous. The strongly oxidizing molecule O_2 is involved in one portion of the overall "combustion" of foodstuffs by aerobic organisms. That portion, which results in the energy-releasing transfer of electrons from three strongly reducing intermediate products of foodstuff combustion to O_2, is accomplished indirectly. Electrons are transferred to O_2 from NADH, $FADH_2$, and succinate along an ordered sequence of electron carriers that together constitute the respiratory chain. All but two of the electrons carriers are components of four large, catalytically active multiprotein complexes embedded in the inner mitochondrial membrane: the NADH-CoQ reductase complex, the succinate-CoQ reductase complex, the $CoQH_2$-cytochrome *c* reductase complex, and the cytochrome *c* oxidase complex (or simply the cytochrome oxidase complex). Of the remaining two electron carriers one is a component of the protein cytochrome *c* and the other is a component of the organic molecule CoQ. Both cytochrome *c* and CoQ are small, mobile, and catalytically inactive. The individual electron carriers themselves (not shown) include iron-sulfur clusters, copper atoms, and the iron atoms within hemes. The three multiprotein complexes shown in color effect not only electron transfer but also "pumping" of protons (H^+) from the mitochondrial matrix to the region between the outer and inner mitochondrial membranes. The resulting proton gradient, which is augmented by consumption of protons in the reduction of O_2 to H_2O, drives the energy-consuming phosphorylation of ADP to ATP.

Laser Spectroscopy and the Structural Dynamics of Cytochrome Oxidase

Los Alamos National Laboratory is recognized worldwide as a wellspring of innovation in laser science and technology. Our activity in that area concentrated in the past on defense-related programs aimed at separating uranium and plutonium isotopes, observing explosion and shock phenomena, monitoring the products of nuclear reactions, and detecting and tracing uranium, plutonium, and other radioactive species in the environment. More recently, the Laboratory has promoted the use of lasers in many areas unrelated to defense, including laser-spectroscopic studies of the structural dynamics of proteins. Lasers offer spectroscopists light that not only is coherent and intense but also can be generated as pulses of extremely short duration. Such pulses enable resolution of changes in the structure of a functioning protein that occur within times as short as 1 femtosecond (10^{-15} second).

An example of our application of lasers to structural biology focuses on the last of the four large multiprotein complexes along the "respiratory chain" of aerobic organisms (Figure 1). The function of those complexes, which are embedded in the inner membrane of mitochondria, is to extract energy from highly reducing chemical species formed during "combustion" of carbohydrates and fats and to convert that energy into a proton gradient across the inner mitochondrial membrane. The proton gradient then drives the formation of ATP. (Molecules of ATP, adenosine triphosphate, provide the energy required to drive many biochemical reactions, including those that effect muscle contraction, transport of molecules and

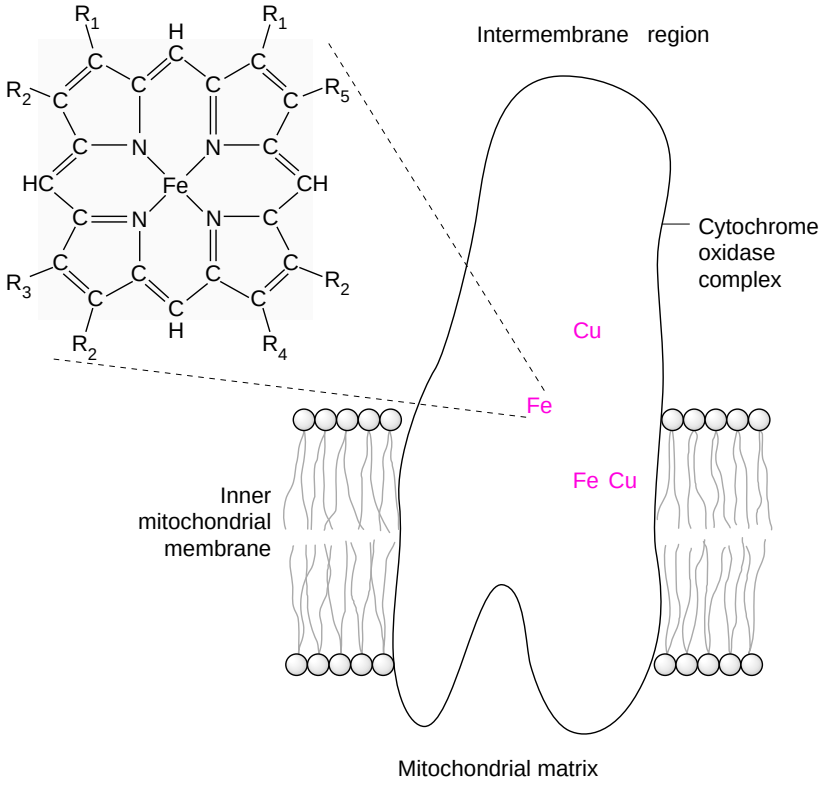


Figure 2. The Cytochrome Oxidase Complex
The molecular weight of the cytochrome oxidase complex (the last and smallest of the four multiprotein complexes in the respiratory chain) is about 200,000 daltons. The general shape of the complex and its situation within the inner mitochrondrial membrane have been determined by electron microscopy. One component of the complex, the enzyme cytochrome oxidase, effects transfer of electrons from cytochrome *c* to O_2 (see Figure 1) and pumping of protons unidirectionally across the inner mitochondrial membrane. Two copper atoms and two iron atoms within cytochrome oxidase play central roles in the electron-transfer reaction. The approximate locations of the metal atoms within cytochrome oxidase have been determined by analysis of its amino-acid sequence. Each copper atom is bound to four specific ligands (not illustrated), and each iron atom occurs as the central atom in one of two identical hemes within the enzyme. Each iron atom lies in the plane formed by the tetrapyrrole ring (screened portion) of the heme; the iron atom can form two additional bonds, one on either side of the plane. When the two hemes of cytochrome oxidase are in place within the cytochrome oxidase complex as depicted in this illustration, their planes are perpendicular to the plane of the page. Hemes occur also in the $CoQH_2$-cytochrome *c* reductase complex of the respiratory chain, in hemoglobin, and in myoglobin. The structures of those hemes differ from that of the hemes in cytochrome oxidase only in the identities of the side chains R_4 and R_5.

ions, and biosynthesis.) The respiratory chain carries out its energy-extraction function by transferring electrons from the highly reducing species to a highly oxidizing species, usually molecular oxygen, O_2. As shown in Figure 1, the electron transfer occurs in a sequence of steps, each brought about by one of the multiprotein complexes. The enzyme within the last multiprotein complex that catalyzes the final electron transfer (from cytochrome *c* to O_2) is called a terminal oxidase. The terminal oxidases found in all plants and animals (and some lower organisms) are heme-copper oxidases, which contain both iron and copper ions. The metal ions work in concert to draw O_2 into the enzyme and activate the O_2 so that it is readily reduced to water. Heme-copper oxidases are estimated to be responsible for about 90 percent of all the biological reduction of O_2 that occurs on Earth.

Cytochrome oxidase, a prominent heme-copper oxidase, is the subject of our studies; Figure 2 shows the locations of its iron and copper sites within the protein complex. Cytochrome oxidase can catalyze the reduction of O_2 at rates approaching 1000 molecules per second. Since a large amount of free energy is released by the reduction of O_2, cytochrome oxidase acts as a powerful biomolecular generator. In fact, 1 mole of the enzyme (about 150 kilograms) can catalyze the production of about 25 million watts of power. The enzyme converts the released energy into a proton gradient not only by catalyzing consumption of protons on the inner side of the inner mitochondrial membrane in the energy-releasing reaction $O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O$ but also by directly "pumping" protons from the

inner to the outer side of the membrane. Obviously elucidating details of the operation of cytochrome oxidase is an important endeavor. Those details can perhaps be applied to practical energy conversion and storage or to developing therapies for respiratory disorders that disrupt normal operation of the enzyme.

By using visible and infrared lasers and the techniques of absorption and Raman spectroscopy, as well as techniques involving polarized light, Laboratory researchers have been able to study the structures and the structural dynamics of the iron and copper sites of cytochrome oxidase. The laser techniques monitor changes in both electronic structure and physical configuration of the enzyme. The processes that have been observed include the binding of O_2, the electron-transfer reactions, and the concomitant responses of the enzyme as a whole and of its metal sites. The dynamics of the processes have been observed at temporal resolutions ranging from picoseconds to thousands of seconds. The data help us understand how the oxidase recognizes and controls the binding of O_2, how it carries out fast electron transfer, how it modifies the dynamics and thermodynamics of electron transfer in response to ambient conditions, and how it performs coupled functions such as proton pumping. Work at the Laboratory over the past several years suggests that binding and dissociation of O_2 at the metal sites and exchange at those sites of O_2 and groups from the oxidase ("ligand shuttling") play key roles in both electron transfer and proton pumping. Shown in Figure 3 are experimentally observed details of the binding of an oxygen molecule to an iron atom in cytochrome oxidase. Other aspects of the operation of cytochrome oxidase are described in the references listed at the end of this article.

Neutron Scattering and the Regulation of Enzymes by Ca^{2+}

From the earliest days the Laboratory's weapons community has needed sources of neutrons to study interactions of neutrons with matter and to measure cross sections for those interactions. The Manuel Lujan, Jr. Neutron Scattering Center (LANSCE) grew out of recognition of the value of neutrons to scientists other than designers of weapons, including condensed-matter scientists and structural biologists. The LANSCE neutron source is pulsed, and therefore the wavelength of any neutron arriving at a detector located at a known distance relative to the source can be determined from the neutron's time of flight from source to detector. That characteristic of a pulsed neutron source has been used to great advantage in development of neutron-scattering instrumentation for studying biological macromolecules ranging in size from 10 to 1000 angstroms (1 angstrom = 10^{-10} meter).

Neutron-scattering techniques can reveal the time-averaged structures of biological macromolecules in solution, in partially ordered configurations (such as those assumed by the macromolecules that are embedded in cellular membranes or by the macromolecules that exist as liquid crystals), and in crystalline form. Because neutrons are electrically neutral, they interact only weakly with the electrons of atoms. However, neutrons do interact strongly with atomic nuclei, and different isotopes of the same element usually have different neutron-scattering lengths. (The neutron-scattering length of a nucleus is directly proportional to the square root of its neutron-scattering cross section.) The difference between the scattering lengths of the two stable isotopes of hydrogen (^1H, the proton, and ^2H, or D, the deuteron) happens to be very large, and, since hydrogen is a major constituent of biological molecules and of water, the fluid of life, that large difference has been exploited to enhance the utility of small-angle neutron scattering to structural biologists.

Small-angle neutron scattering is a technique applied to biological molecules in solution, usually aqueous. Its application to relatively simple molecules, such as single proteins, is fairly straightforward. But its application to complex macromolecules involves invoking some ingenious techniques based on the large difference between the neutron-scattering lengths of the proton and the deuteron. Suppose, as is often the case, that the biological macromolecule of interest consists of two components (an RNA molecule and a protein, say, or two proteins); suppose further that one component, call it A, is of greater interest than the other component, B. Obviously, small-angle neutron-scattering data for A alone would be easier to interpret, but such data must, of course, be obtained without physically separating the two components. That situation can be achieved without too much difficulty if the two components have different chemical compositions (as do an RNA molecule and a protein) and hence different neutron-scattering densities. (The neutron-scattering density of a molecule is

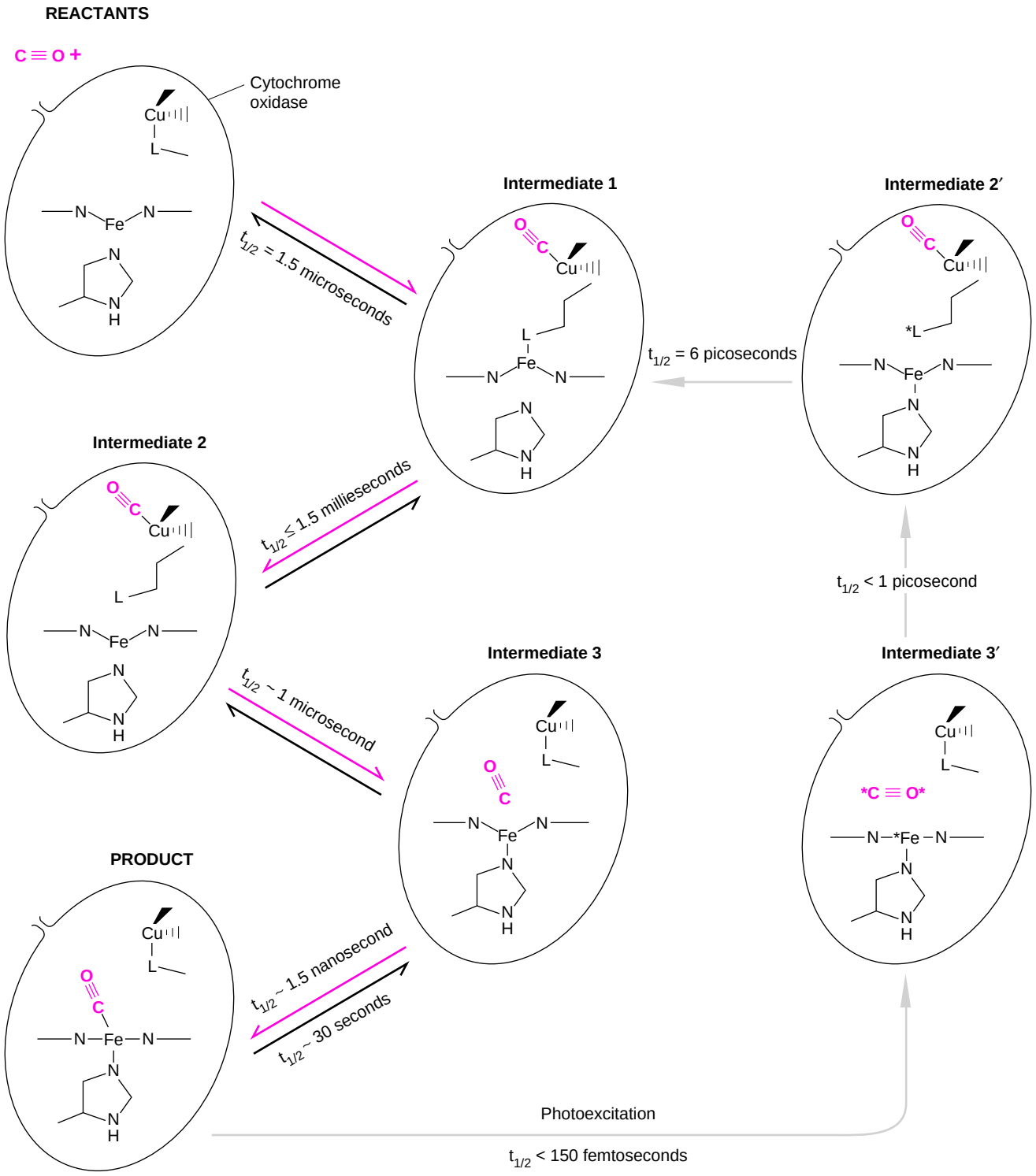


Figure 3. Structural Dynamics of O_2-to-Fe Binding in Cytochrome Oxidase

Before cytochrome oxidase can catalyze the transfer of electrons from cytochrome *c* to O_2 (see Figure 1), the oxygen molecule must bind to the iron atom within a heme of cytochrome oxidase. To study the structural dynamics of the binding separately from the structural dynamics of electron transfer, we used carbon monoxide (CO) as a nonreactive substitute for O_2. We learned that the binding of CO involves the formation of three intermediate species (1, 2, and 3). Intermediate species 1, in which CO is bound to a copper atom, exists only fleetingly (as indicated by the short half-life for the decay of Cu-bound CO to free CO). The resulting low concentration of intermediate 1 hampered observation of the course of the binding reaction under physiological conditions (red amd black arrows), so we artificially increased the concentration of intermediate species 1 by photoexcitation of the product of the binding. The photoexcited product rapidly forms intermediate 1 (gray arrows). Note how the ligand L "shuttles" from Cu to Fe and back again as the different intermediates are formed and how the histidine-imidazole ligand dissociates from and reassociates with the Fe atom. The half-lives listed are those measured or reliably estimated.

equal to the sum of the neutron scattering lengths of the molecule's constituent nuclei divided by the molecule's volume.) Then by manipulating the deuterium-to-hydrogen ratio of the solvent, its neutron-scattering density can be made equal to the neutron-scattering density of B. As a result, B contributes essentially nothing to the neutron-scattering signal, and the neutron-scattering data contain information about only A. In practice, of course, both components of a two-component biological macromolecule are of interest, and structural information for both are obtained separately by matching the solvent's neutron-scattering density first to one component and then to the other.

Suppose now that A and B have very similar neutron-scattering densities (as do two proteins). The technique of solvent matching can still be applied to obtain structural information about only A, but to do so first one component is "deuterated" (at least some of its protons are replaced with deuterons) so that the neutron-densities of the two components are different. Then the neutron-scattering density of the solvent is adjusted to match that of B, again by manipulating the solvent's deuterium-to-hydrogen ratio. (The ability to deuterate biological molecules is a consequence of recombinant DNA technology and exploits the biosynthetic machinery of a bacterium, usually *E. coli*, whose genome has been altered to include a gene for, say, the protein or RNA molecule of interest. Culture of such a bacterium on a medium containing deuterated nutrients leads to production of a deuterated version of the protein or the RNA molecule.)

To extract an even greater amount of structural information about two-component biological macromolecules from small-angle neutron-scattering data, the technique called contrast variation is employed. Contrast variation can be considered a generalization of solvent matching. Implementation of the technique involves collecting a series of data sets, each obtained at a different value of the solvent's neutron-scattering density. The range of solvent neutron-scattering densities spanned by the data sets includes the match point for each component. Like solvent matching, contrast variation is often applied in conjunction with deuteration.

The first neutron-scattering experiments at LANSCE on a biological macromolecule were completed in 1987. The instrument used to carry out those experiments, the Low-Q Diffractometer, was designed specifically for small-angle neutron scattering. The data obtained are generally spherically averaged (because the molecules are generally randomly oriented in solution) and therefore the structural information that can be extracted from the data is inherently of low resolution. In other words, solution experiments provide molecular boundaries and shapes rather than locations of individual atoms or chemical groups. To obtain more detailed information from neutron-scattering experiments, the molecules must be oriented, preferably highly oriented as in a crystal. The regular spacing of the nuclei in a crystal leads to a regular pattern of constructive and destructive interference of the scattered neutrons and hence to a regular pattern, a diffraction pattern, of scattering intensity. Analysis of the pattern provides information about the locations of the nuclei in the crystal. Unfortunately, most biological macromolecules do not occur naturally as crystals, and relatively few are easily crystallized in the laboratory. Solution scattering techniques therefore provide an important window for biomolecular structure determinations.

Since 1987 neutron-scattering experiments have been carried out at LANSCE on a number of biological macromolecules in solution, including the complexes formed by antibodies and the foreign substances they target for elimination; chromatin, the complex of DNA and certain proteins that modulate the accessibility of genetic material to replication and transcription; complexes of DNA with other proteins; and viruses. All the studies utilized contrast variation (and some utilized deuterium labeling) to obtain structural information about individual components of the macromolecules. Perhaps the most significant experiments at LANSCE to date, however, have focused on molecules involved in biochemical regulation.

One of the key issues in biochemistry is the question of how reactions are switched on and off or accelerated and decelerated in different types of cells, at different stages of development of an organism, and in response to external and internal signals. The answer lies in a complex network of chemical messengers, transmitters, and receivers that orchestrate the timing and the rates of reactions in response to physiological stimuli. One of the simplest of the messengers involved in biochemical regulation is the doubly charged ion of calcium, Ca^{2+}. Calcium ions deliver messages to the intended recipients (usually enzymes) indirectly. First the calcium ions bind to one of a family of closely related proteins, the most

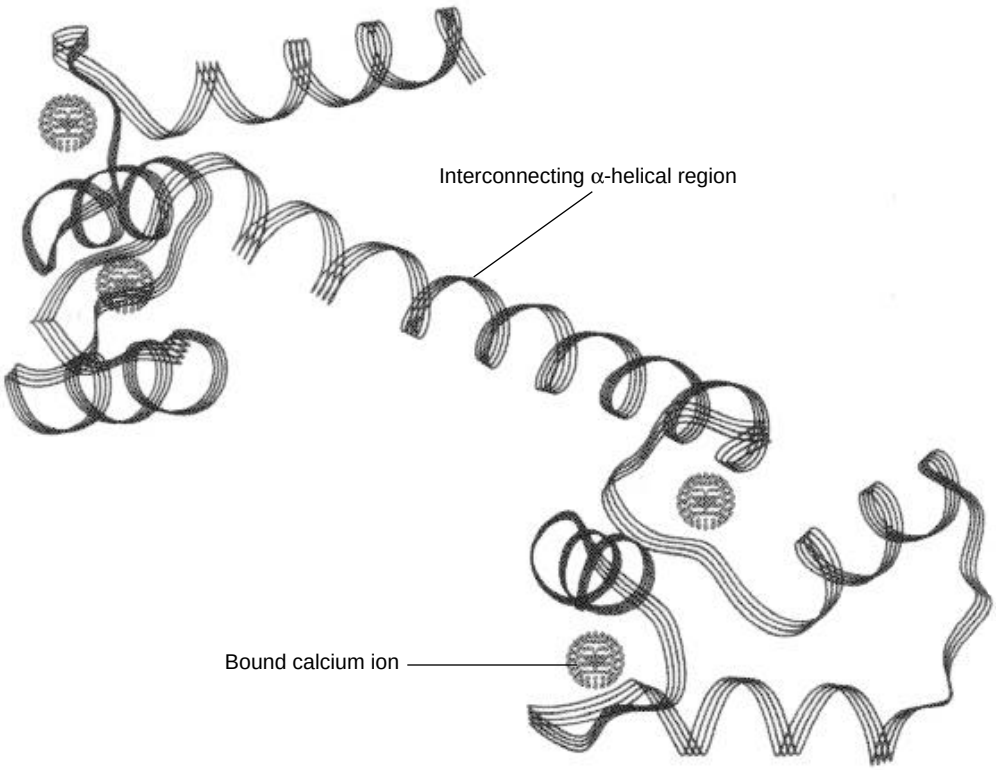


Figure 4. Conformation of Ca^{2+}-Calmodulin
This ribbon diagram depicts the conformation of the polypepetide backbone of calmodulin after Ca^{2+} ions (colored spheres) have bound to the protein. Note the stretch of the protein that exhibits the α-helical conformation and connects the two lobes containing the calcium ions. Structural studies indicate that the interconnecting α-helical region can flex when Ca^{2+}-calmodulin binds to a target enzyme. That flexibility is thought to be responsible for the ability of Ca^{2+}-calmodulin to bind to (and activate) a number of target enzymes, each with a chemically different binding domain.

ubiquitous of which—and the object of our studies—is calmodulin. The resulting Ca^{2+}-calmodulin then binds to and activates one of a number of different target enzymes. The target enzymes of Ca^{2+}-calmodulin are involved in a variety of biological functions, including muscle contraction, neurotransmitter release, and generation of metabolic energy from glycogen. The domains on the target enzymes to which Ca^{2+}-calmodulin binds are as chemically diverse as the enzymes are functionally diverse. In other words, the binding of Ca^{2+}-calmodulin to a target enzyme exhibits not only specificity but also diversity.

Fortunately, Ca^{2+}-calmodulin is one of the biological macromolecules that can be crystallized, and high-resolution x-ray diffraction by others had shown that its structure is unusual, consisting of two football-shaped domains, each containing two Ca^{2+}-binding sites, connected by an extended α helix of about eight turns (Figure 4). The α helix is a common structural motif in proteins, but stretches of α helix in more compact proteins are usually stabilized by intramolecular interactions. No such stabilizing interactions are evident, however, in the case of Ca^{2+}-calmodulin. Neutron-scattering experiments at LANSCE helped unlock the key to understanding the relationship between the unusual structure of Ca^{2+}-calmodulin and its functional diversity and specificity.

We characterized complexes of Ca^{2+}-calmodulin with a number of target enzymes. Two general types of interaction were identified. The more common type involved flexing of the connecting α-helical region of Ca^{2+}-calmodulin and collapse of its two lobes around an α-helical domain in the target enzyme (Figure 5). The flexibility of its interconnecting α-helical region allows Ca^{2+}-calmodulin to collapse around different types of domains with different chemical surfaces and to accommodate different stabilizing chemical interactions. In the other type of interaction we characterized, Ca^{2+}-calmodulin remains extended when it binds to a target enzyme, possibly to facilitate Ca^{2+}-independent interactions. Thus we have shown that the mechanical flexibility of the interconnecting α-helical region of Ca^{2+}-calmodulin is a key to its ability to function as a regulator of many different cellular functions. However, the chemical surface of Ca^{2+}-calmodulin is constant, and the characteristics of that surface must be accommodated in any complex it forms. We know that the binding of Ca^{2+} to calmodulin opens up each of its lobes so that hydrophobic amino-acid side chains are exposed to the solvent. Those exposed hydrophobic side chains are key to the interactions of Ca^{2+}-calmodulin with target enzymes, since the presence of large and specifically spaced hydrophobic side chains is a characteristic feature of

all the binding domains targeted by Ca^{2+}-calmodulin. Thus the functional diversity and the functional specificity of calmodulin are programmed into its structure.

Advances in NMR Spectroscopy

Over the past decade the importance of NMR spectroscopy to structural biologists has increased considerably as a result of the availability of higher magnetic fields and of the means to introduce appropriate isotopes into molecules of interest. The traditional strength of the Laboratory in isotope separation fostered the development here of novel strategies for labeling biological molecules, and those strategies have been widely used by the biophysics community for structural studies. More recently our role as one of the three institutions composing the National High Magnetic Field Laboratory has enabled us to focus advances in both isotope-labeling methodology and high-magnetic-field technology on high-resolution structural studies of biological molecules. (The National High Magnetic Field Laboratory is funded by the National Science Foundation; the other two institutions are Florida State University and the University of Florida. It will make available to NMR spectroscopists magnetic fields as high as 25 teslas; for comparison the magnetic field of the earth is about 0.5×10^{-4} tesla, or 0.5 gauss.)

The past decade has seen the development also of "multidimensional" NMR techniques that, in conjunction with the use of high magnetic fields, provide sufficient resolution to determine, in solution, the locations in three-dimensional space of all the atoms and chemical groups composing a biological molecule. Such detailed structural information was previously obtainable only from x-ray-diffraction and neutron-diffraction studies of crystallized biological molecules. Since 1984, when NMR spectroscopy first yielded a complete three-dimensional structure of a biological molecule in solution (a protein), the technique has yielded similarly detailed structures for about sixty other proteins. Thus, as a structural tool, NMR spectroscopy now rivals x-ray diffraction, which has produced high-resolution structures for about 235 proteins during the approximately 30 years since John Kendrew obtained a high-resolution x-ray structure of myoglobin.

The fundamental basis of NMR spectroscopy is the fact that certain atomic nuclei, including all nuclei with odd mass number, possess

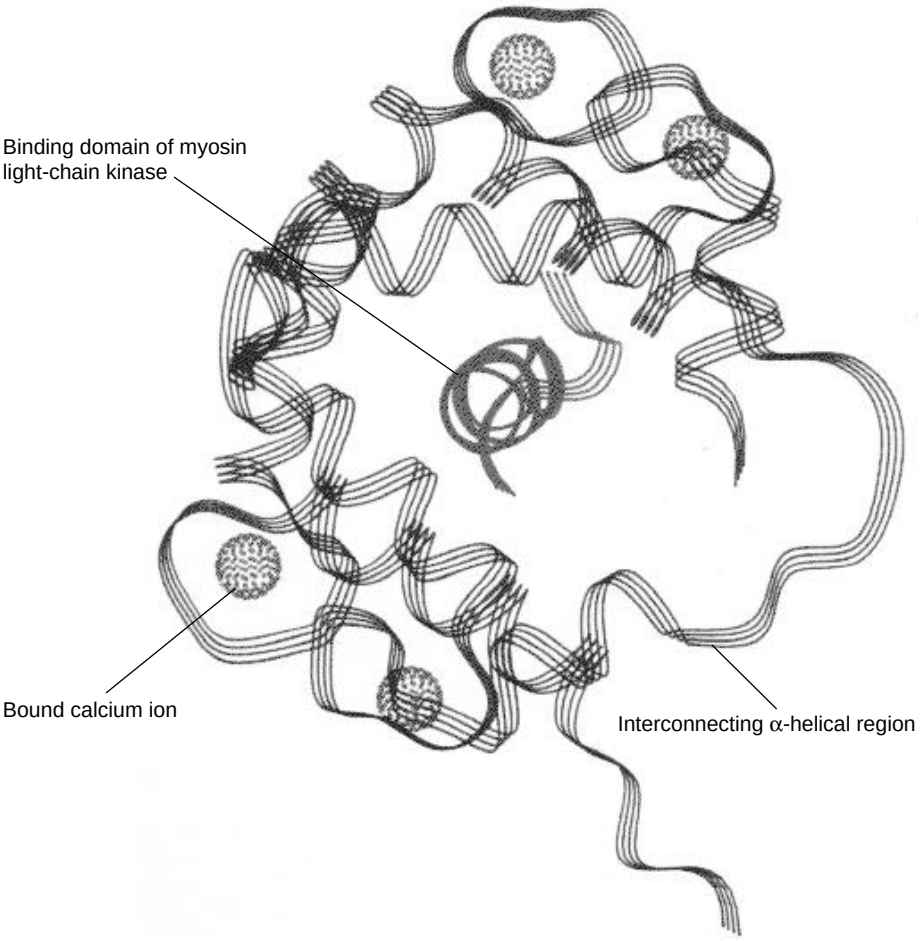


Figure 5. Conformation of Ca^{2+}-Calmodulin Bound to a Target Enzyme

Small-angle neutron-scattering studies of the complex formed by binding of Ca^{2+}-calmodulin to a binding domain within myosin light-chain kinase (one of Ca^{2+}-calmodulin's target enzymes) showed that Ca^{2+}-calmodulin collapses as shown here around the binding domain by virtue of the flexibility of Ca^{2+}-calmodulin's interconnecting α-helical region. NMR-spectroscopic studies of the complex have confirmed this model and provided details of the chemical interactions that stabilize the complex.

nonzero spins and hence nonzero (and characteristic) magnetic moments. Among the stable and biologically relevant nuclei with nonzero magnetic moments are ^{1}H, the naturally abundant isotope of hydrogen; ^{13}C, the naturally rare isotope of carbon; ^{15}N, the naturally rare isotope of nitrogen; ^{17}O, a naturally rare isotope of oxygen; and ^{31}P, the only naturally occurring isotope of phosphorus. (Many radioactive isotopes also possess nonzero magnetic moments, but the use of stable isotopes in NMR spectroscopy avoids the complication of dealing with radioactive materials.)

When a magnetic field is imposed upon a collection of, say, free protons (^{1}H), the alignment of their magnetic moments nearly parallel to the magnetic field produces a net magnetization. Furthermore, the magnetic moments precess (rotate) about an axis parallel to the magnetic field at a frequency, called the Larmor frequency, that is proportional to the product of the proton's magnetic moment and the strength of the magnetic field. (The effect is analogous to the precession of a spinning top about its axis under the influence of gravity.) The frequency of precession is one of the quantities measured in NMR experiments; the measurement techniques will not be detailed here.

When a proton is bound in a molecule, however, the electrons surrounding the proton in effect weaken the magnetic field "felt" by the proton, and as a result the Larmor frequency of the bound proton is slightly different from the Larmor frequency of a free proton. The difference between the two Larmor frequencies is called a chemical shift, and NMR-spectroscopic data are presented as peaks on a chemical-shift axis rather than as absolute frequencies. (The chemical shifts of a nucleus are usually calculated relative to the Larmor frequency not of the free nucleus but of the nucleus in some reference molecule.) If the molecule contains more than one proton (as do most biological molecules) and if all the protons are chemically equivalent (surrounded by identical electronic environments) as they are in, say, methane (CH_4), then the NMR spectrum of the protons in that molecule consists of a single peak at a certain chemical-shift value. On the other hand, if the molecule contains two protons that are not chemically equivalent, as does, say, formic acid (HCOOH), the NMR spectrum of the protons in such a molecule consists of two peaks, each at a different chemical-shift value. As might be expected, the NMR spectrum of the protons in a complicated molecule such as a protein contains numerous closely spaced peaks, some of which are caused by the interaction of the protons with the magnetization created by alignment of their magnetic moments. The peaks differ not only in their locations along the chemical-shift axis but also in their heights and widths. The structural information that can be extracted from those parameters includes proton-proton distances and the angles that specify the relative orientations of the amide planes in a polypeptide.

Currently one of the major drawbacks of NMR spectroscopy is a limitation on the molecular weight of the molecules to which the technique is applicable. The limitation arises from overlaps of the many proton peaks that appear in the NMR spectrum of high-molecular-weight biological molecules. One way to increase the separation of the peaks along the chemical-shift axis is to increase the magnetic-field strength, but the currently available field strengths (about 15 teslas) restrict application of conventional NMR spectroscopy to biological molecules whose molecular weights are less than 15,000 daltons.

Another technique for increasing the separation of the chemical shifts of the protons in a large biological molecule is multidimensional NMR spectroscopy. The sample required for that technique is a collection of molecules that have been synthesized in a manner such that each molecule contains at least one other nuclear species with a nonzero spin. For example, the carbon atoms might contain ^{13}C rather than ^{12}C (the abundant carbon isotope), or the nitrogen atoms might contain ^{15}N rather than ^{14}N (the abundant nitrogen isotope). The magnetization produced by alignment of the magnetic moments of the other nuclear species separates the proton peaks and thus removes many overlaps. Ad Bax and his colleagues at the National Institutes of Health used three-dimensional NMR spectroscopy to obtain a high-resolution solution structure of the complex of Ca^{2+}-calmodulin with the binding domain of one of its target enzymes (myosin light-chain kinase). (That complex, whose molecular weight is 21,000 daltons, is the largest to date for which NMR spectroscopy has provided a detailed solution structure.) The structure deduced by Bax and his colleagues confirmed the model we had extracted from small-angle neutron-scattering data (see preceding section). However, the cost of the sample for the multidimensional NMR experiment was high (between $100,000 and $250,000) primarily because produc-

ing molecules labeled with ^{13}C is expensive but also because the limited sensitivity of current NMR instrumentation requires a large sample. Obviously wider application of multidimensional NMR spectroscopy will require lower sample-production costs. Together with colleagues at the California Institute of Technology we are developing a technique that may decrease ^{13}C-labeling costs by a factor of 15. The technique involves biosynthesis of ^{13}C-labeled molecules by a bacterium that requires only the simplest of nutrients (methanol and ammonia salts). Providing those bacteria with ^{13}C-labeled methanol is much less expensive than providing *E. coli* with labeled versions of the more complex nutrients they require.

As mentioned previously, the Laboratory is also pursuing another advance in NMR spectroscopy through its association with the National High Magnetic Field Laboratory. However, since increasing the magnetic-field strength not only separates but also broadens NMR peaks, the use of very high magnetic fields may obliterate the increased resolution achieved by multidimensional NMR spectroscopy. Therefore realizing the potential of very-high-field NMR spectroscopy may require replacing some of the protons with spin-zero deuterons. To that end we are developing deuteron-labeling strategies based on both biosynthesis and chemical synthesis. Another benefit of the use of high magnetic fields in NMR spectroscopy is an increase in sensitivity, which will allow the use of samples with smaller masses and of dilute solutions, in which molecular aggregation (another source of chemical-shift broadening) is less likely.

Conclusion

Through varied routes, techniques employed in the Laboratory's research on nuclear weapons have found applications in structural biology, an area that constitutes a crucial component of the foundations on which medical research and biotechnology are built. For example, information about the structures and structural dynamics of biological molecules provides understanding of the molecular basis of disease and hence helps in the design of vaccines and therapeutic drugs and of molecular systems for delivering drugs to diseased cells. Thus research on nuclear weapons will affect the quality of human life in ways that were never planned. ■

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Further Reading

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