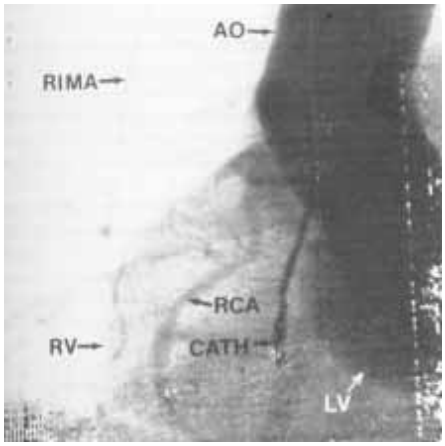
Biological Applications of SYNCHROTRON RADIATION

by ROBERT A. SCOTT

Intense X-rays from synchrotrons are used to determine biological structure at every level of spatial resolution.

ANYONE LOOKING for the strategic importance of high-energy physics research to the nation need look no further than the applications spawned by the harnessing of synchrotron radiation as a spinoff from particle storage rings. Synchrotron radiation (SR) light sources have given rise to technological innovations in a number of fields including chemistry, materials research, geology, and biology.



An example of a coronary angiogram recorded at the Stanford Synchrotron Radiation Laboratory in a 64 year old man. Visible are the aorta (AO), the right internal mammary artery (RIMA), the right coronary artery (RCA), and the left ventricle (LV). This image was recorded after the contrast agent had cleared the right heart so the right ventricle (RV) is virtually transparent [from Nucl. Instr. Meth. A266, *252–259 (1988)].*

It is ironic that the intense ionizing X-rays provided by SR that can do so much damage to biological specimens (e.g., human tissue) have contributed to a revolution in our methods of investigation of a number of biological systems and processes. To date, this revolution can be described in terms of improvements in our ability to determine the structure of biological materials at every level of spatial resolution, from macroscopic imaging to atomic-level structural detail. As the next generation of SR sources turn on during the next few years, a second revolution will occur in the enhanced temporal resolution of these techniques; tantalizing glimpses of the power of these new time-resolved methods are already available today. In this article, I review these SR-based techniques of structural biology and give examples of how they have been applied to problems of biomedical and biotechnological importance.

Most of the studies discussed here use photons in the X-ray region of the synchrotron radiation spectrum. The major advantage that X-rays have over photons in other regions of the electromagnetic spectrum is that they possess wavelengths that are on the order of molecular dimensions [in the Ångstrom (Å), 10^{-10} m, range]. Thus it is a special property of X-rays that their interaction with matter is directly sensitive to structure at the molecular level. Most of the biological applications dealing with this level of structural detail take advantage of this property. However, X-rays can also be used in spectroscopy, that is, measurement of the wavelength-dependent absorption or scattering of X-rays by matter. In spectroscopy-based applications, the X-rays are used to excite electrons within atoms and molecules, and these excitations are wavelength dependent. If a biological specimen absorbs strongly X-rays of one wavelength, but does not absorb X-rays of another wavelength, then comparing the two-dimensional absorption patterns of a specimen at the two different wavelengths gives rise to *contrast* which can be used to image that specimen. This allows access to structure at a macroscopic (millimeter, 10^{-3} m) or microscopic (submicrometer, 10^{-6} m) level. Let's start by looking at these latter imaging techniques and then work our way down to the atomic level.

X-RAY IMAGING TECHNIQUES

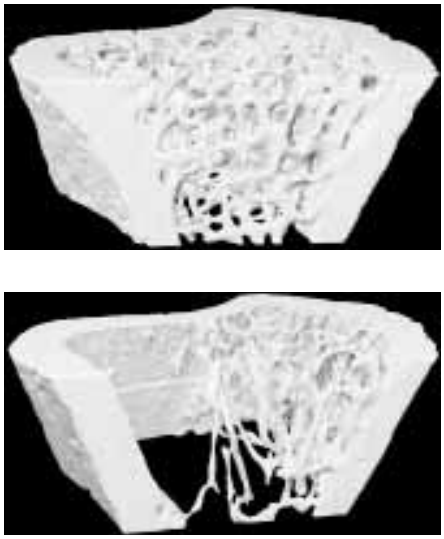
Coronary Angiography

A premiere example of the application of synchrotron radiation to the medical field is the development of non-invasive coronary angiography [see "Imaging the Heart Using Synchrotron Radiation," by George A. Brown, Fall/Winter 1993 *Beam Line* page 22]. Most patients today being tested for arterial restriction or blockage are treated by invasive angiography, which involves catheter-based arterial injection of contrast agent, an inherently dangerous procedure. SR-based coronary angiography takes advantage of the tunability of SR by using a contrast agent containing iodine and measuring images above and below the iodine K absorption edge at 33.16 keV. (K edges are sharp discontinuities in the X-ray absorption coefficient at the energy required to photodissociate an electron bound

in the 1*s* orbital of atoms of a given element. In this case, iodine-containing materials are nearly transparent to X-rays with energies below 33.16 keV, but nearly opaque to X-rays with energies above 33.16 keV.) This yields high-contrast images after subtraction of the image obtained with X-rays below the iodine edge from the image obtained above the iodine edge. The high contrast allows the contrast agent to be delivered intravenously rather than by arterial injection, eliminating much of the risk in the procedure. The standard procedure uses a broad SR beam that is intercepted by a horizontal linear position-sensitive detector behind the patient. The patient is then scanned vertically so that the SR beam passes through all sections of the heart. A fast shutter is used to control the irradiation time at each vertical position. This results in line-scan images much like the images seen on a television. Digital subtraction and image processing yield high-quality two-dimensional coronary images. This field has advanced from the inception of the idea and first experiments at Stanford Synchrotron Radiation Laboratory (SSRL) in 1979 to the establishment of a clinical facility at the National Synchrotron Light Source at Brookhaven called the Synchrotron Medical Research Facility. Recent advances in SR-based angiography include attempts to develop it into a real-time imaging procedure and the extension from two- to three-dimensional imaging. In the latter development, two SR beams strike the patient at slightly different angles, generating stereo-pair images that can be viewed as three-dimensional objects.

X-ray Computed Tomography

True three-dimensional medical imaging is also being pursued in projects at several SR sources to develop X-ray computed tomography. Eventually, this technique is expected to yield higher resolution images than the conventional positron emission tomography (PET). A monochromatic horizontal X-ray fan beam is used to give a projection image of a subject that is rotated about a vertical axis, and a slice of the three-dimensional image is reconstructed. Stepping the subject vertically allows for other slices to be acquired, then stacked into a three-dimensional image. Use of X-rays of various wavelengths and digital subtraction allows imaging of the distribution of materials containing different elements, a feat that is impossible with PET. An example of *in vivo* imaging of bone structure is shown in the figure. This experiment was performed on live anesthetized rats that had been ovariectomized to investigate a correlation between estrogen depletion and bone density that may be responsible for the high level of osteoporosis in older women.



Effect of estrogen depletion on bone volume. The top image shows a slice of the proximal tibia of a 6-month-old rat, and the bottom image shows the same slice 5 weeks after ovariectomy. An overall 60% loss in bone volume has resulted from the estrogen depletion induced by ovariectomy, and disconnected bone fragments are visible. These studies are designed to detail the effects of estrogen depletion in osteoporosis in older women. [For more information on the X-ray tomographic microscope used for this study, see Annu. Rev. Mater. Sci. 22, 121-152 (1992)].

Soft X-ray Microscopy

Both angiography and tomography can image structures at about 10^{-3}–10^{-5} m resolution. For resolution in the 10^{-6}–10^{-8} m range, X-ray microscopy has been shown to be the method of choice. To reach this resolution, the SR X-ray beam must be focused, and the technology for doing this is available only for soft X-rays (in the 100-eV range). Soft X-ray microscopy has a number of

Mapping of protein and DNA in bull sperm, using the Stony Brook scanning transmission X-ray microscope (STXM) at the National Synchrotron Light Source. The upper set of images is for a sperm with tail missing, but at higher magnification (scale bar equals two microns). Images were collected at six wavelengths near the carbon K absorption edge, where protein and DNA spectra differ. Images on the left are at one of these six wavelengths. Analysis of all six leads to the protein and DNA maps shown in center and right. (Work of Xiaodong Zhang. Specimen courtesy of Dr. Rod Balhorn, Livermore.)

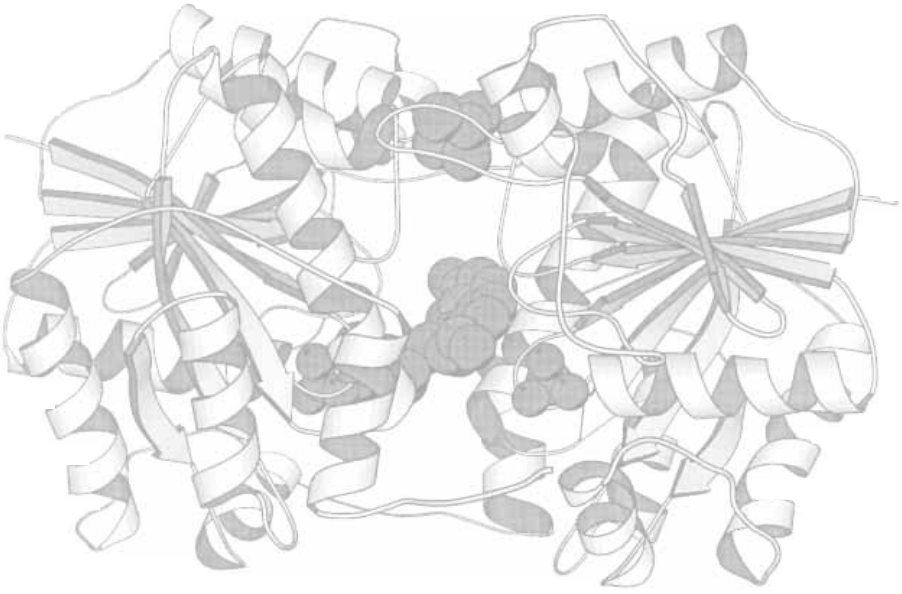
advantages over other recently developed microscopy techniques (such as scanning tunneling microscopy, atomic force microscopy, scanning electron microscopy), the most important one being the ability to image through thick samples (the other techniques "see" only surfaces of objects). This advantage is most important for biological applications such as imaging live cells in a wet environment.

Another advantage of SR-based soft X-ray microscopy is the ability to tune the energy of the radiation to provide contrast dependent on the chemical makeup of different regions of the sample (chemical contrast). For example, the different chemical makeup of nucleic acids (DNA and RNA) compared to proteins allows for microscopic imaging of the ultrastructure and composition of chromosomes. Even regions of the sample that have similar elemental composition but different chemical characteristics (that is, the atoms occur in different molecules with different bonding patterns) can be distinguished using the microscope as a "microprobe." In this usage, the X-ray beam is focused on a particular point of the sample, and the X-ray absorption spectrum is scanned to identify the chemical makeup at that point. This can be done at a number of points in the sample that appear with different contrast in the microscopic image, characterizing the spatial distribution of materials in the sample.

To move from structure at a biological or anatomical level to structure at a biochemical level, we have to change our focus from imaging to spectroscopy. Now we can take advantage of the short wavelength of X-ray photons to yield molecular structure.

X-RAY SPECTROSCOPY

On a biochemical level, scientists are interested in the structures of biomolecules (proteins, nucleic acids, lipids) and the ultrastructure of various assemblies of these molecules, such as chromosomes, ribosomes, membranes, etc. The availability of a tunable source of X-rays like SR has made a large impact on these studies as well. Just as with the imaging applications already discussed, this tunability allows development of contrast to focus attention on the macromolecule of interest or a portion of it and allow one to distinguish it from its environment. Also, as we narrow down our attention to structure at nanometer or Å (10^{-9}–10^{-10} m) resolution, it is the characteristic sensitivity of X-rays with wavelengths that match this length scale to molecular structure that dominates the information attained.

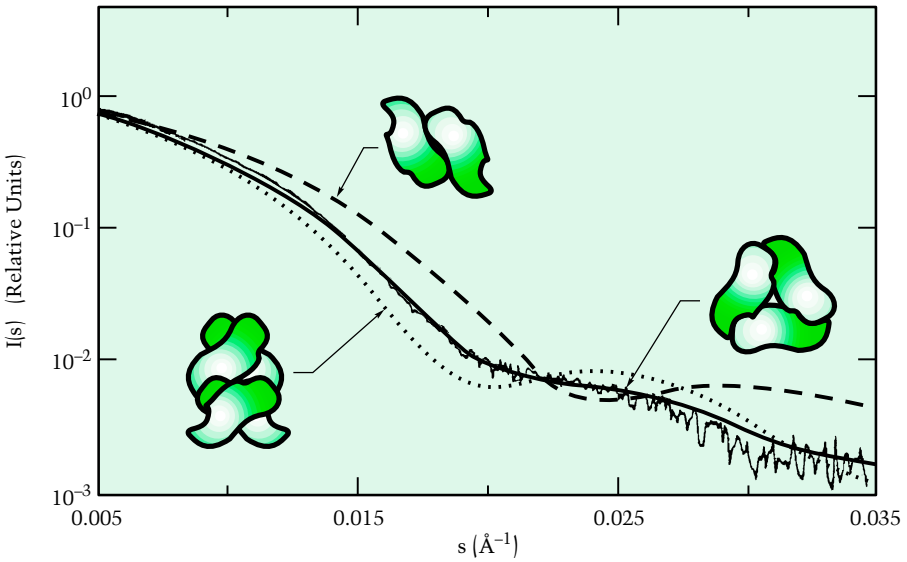


The structure of the Fe protein from the enzyme nitrogenase, solved by X-ray diffraction. The two subunits are joined at the top by the [4Fe-4S] cluster, and the MgATP is expected to bind between the subunits near the bottom. The larger radius of gyration observed for the Fe protein in the absence of MgATP (see text) could arise from separation of the subunits at the bottom. (Figure provided by D. C. Rees, California Institute of Technology.)

X-ray Scattering

One method of obtaining information about biomolecules at molecular resolution is X-ray scattering. In these experiments, X-rays incident upon a sample are scattered from nanometer-sized structures (particles, molecules) in a way that is sensitive to the size and shape of these structures. Placement of an X-ray detector directly behind the sample would measure the transmission of X-rays directly through the sample, but moving the detector away from this position by small angles allows detection of the angle-dependent intensity of scattered X-rays; it is this angle dependence that contains the structural information, leading to techniques referred to as small-angle X-ray scattering (SAXS) and wide-angle X-ray scattering (WAXS). Sensitivity to larger structures increases as the angle decreases, and SAXS has had the largest impact on structure determination of biomolecules (typical protein molecules have length dimensions in the 20–200 Å range).

A globular protein molecule tumbling in solution can be represented as a spherically symmetric structure the size of which can be defined by one parameter, the radius of gyration *(R_g)*. SAXS can be used to measure R_g values for proteins in solution, and several applications of the technique have used this ability to look at changes in the conformation of proteins after some treatment. A recent example of this type of experiment was carried out at SSRL on the iron protein of the enzyme nitrogenase. This enzyme is responsible for nitrogen fixation (reduction of N_2 in the atmosphere to NH_3 that can be assimilated by plants), which occurs in soil bacteria and the root nodules of leguminous plants. The obvious importance of this process to agriculture makes the understanding of nitrogenase function a worthwhile pursuit. Two proteins are involved in the nitrogenase enzyme complex: the "iron (Fe) protein" that contains a cluster of four iron atoms and the "molybdenum-iron (MoFe) protein" that catalyzes the actual reduction of N_2 through the action of an iron- and molybdenum-containing cofactor (FeMoco). The function of nitrogenase requires reducing equivalents (electrons) to be donated from the Fe protein to the MoFe protein, and this electron transfer requires the binding of another cofactor (the magnesium salt of adenosine triphosphate MgATP) to the Fe protein. How the binding of MgATP to the Fe protein affects its interaction with the MoFe protein was the subject of a SAXS study in which a mutated form of the Fe protein that is unable to perform this electron transfer was compared to the natural (wild-type) form. It was found that the wild-type Fe protein had a R_g of 27.2±0.4 Å in the absence of MgATP, whereas addition of MgATP reduced

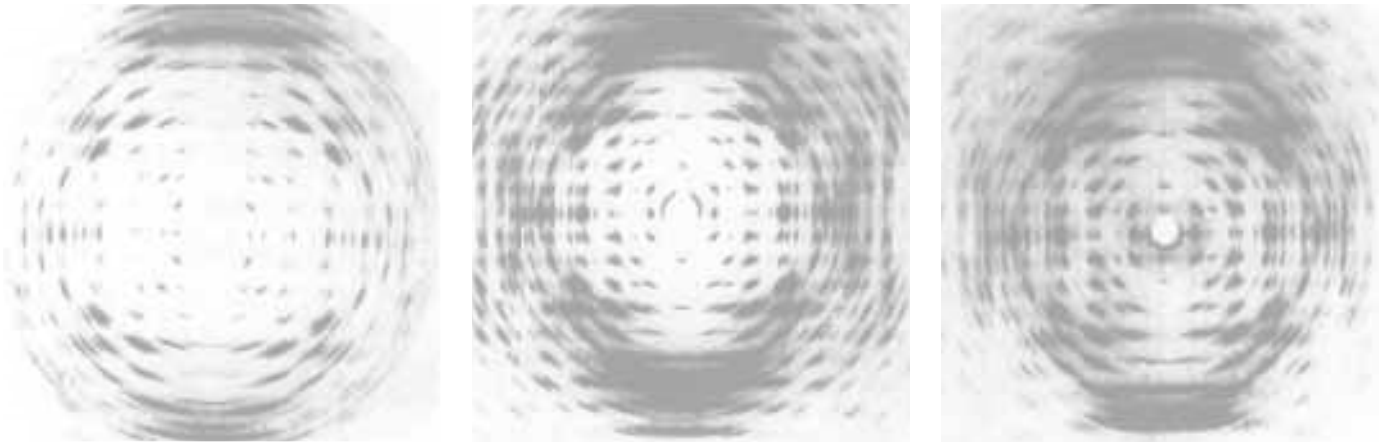


Solution small-angle X-ray scattering profile for the enzyme nitrite reductase from Achromobacter xylosoxidans. The dashed and solid lines represent the simulated data for a set of possible aggregation states (dimer, trimer, tetramer) of this enzyme. These data indicate predominantly the presence of trimers in solution. [J. G. Grossmann et al., Biochemistry 32, 7360-7366 (1993)]

this to 24.6±0.4 Å. By contrast, the mutated form of Fe protein had a R_g of 27.2±0.4 Å, regardless of the presence of MgATP. It appears therefore that a necessary prerequisite for Fe protein interaction with MoFe protein is a MgATP-induced compression of the Fe protein structure. The atomic-resolution structure of the Fe protein suggests that a plausible structural change is the "folding" of the two lobes (subunits) of the molecule around the suspected MgATP binding site.

This experiment points to the potential contribution of SAXS to the study of protein folding in general. One of the major pursuits of protein biochemists is the search for an understanding of how the sequence of amino acid residues in a protein dictates the folding of that protein into a homogeneous globular structure. Such an understanding would revolutionize protein engineering, i.e., the ability to design genetically protein-based enzymes to carry out biotechnologically important reactions. Experiments that can give information about the structure of the unfolded state or intermediate partially folded states of proteins and the kinetics of folding will enhance this understanding. Time-dependent SAXS is a technique that is just beginning to be used for this purpose. In this technique, a stopped-flow apparatus is used to mix rapidly a solution of a protein under conditions favoring the unfolded state with another solution designed to trigger protein folding; SAXS data are collected as a function of time following mixing. Application of this technique to myoglobin (the 0_2-storage heme protein present in vertebrate muscle tissue) showed a rapid conversion (with a half-life of ≈ 1 second) from an unfolded state with R = 32±3 Å to a final state with R_g = 20±2 Å, which is very close to the native state (R_g = 18±1 Å).

Often the functionally viable form of an enzyme is a collection of more than one copy of the protein molecule. These clusters sometimes contain two (a dimer), three (a trimer), or four (a tetramer) protein molecules and are referred to generically as the aggregation state of the enzyme. Newer methods for analysis of SAXS data based on construction of three-dimensional models for the macromolecules followed by simulation of the expected SAXS pattern can lead to determination of the shape and aggregation state of biological macromolecules. One example involves the use of SAXS to determine the solution aggregation state of a copper-containing enzyme nitrite reductase, which is important in denitrification. Early molecular weight determinations suggested a dimeric enzyme in solution, but the crystallographic analysis (see figure) of the structure detected a trimer. With the shape of the protein molecule from the crystal structure, computational models of dimers, trimers, and tetramers were tested by simulating the expected SAXS profiles and comparing them with the observed SAXS data.



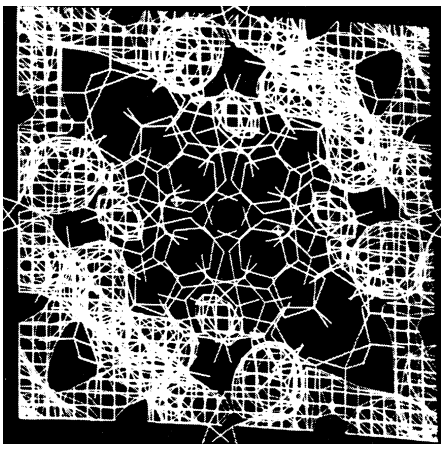
It was clearly demonstrated that only a trimeric arrangement of protein molecules could accurately simulate the observed SAXS data, convincing proof for the existence of trimers in solution as well as in crystals.

Membrane X-ray Diffraction

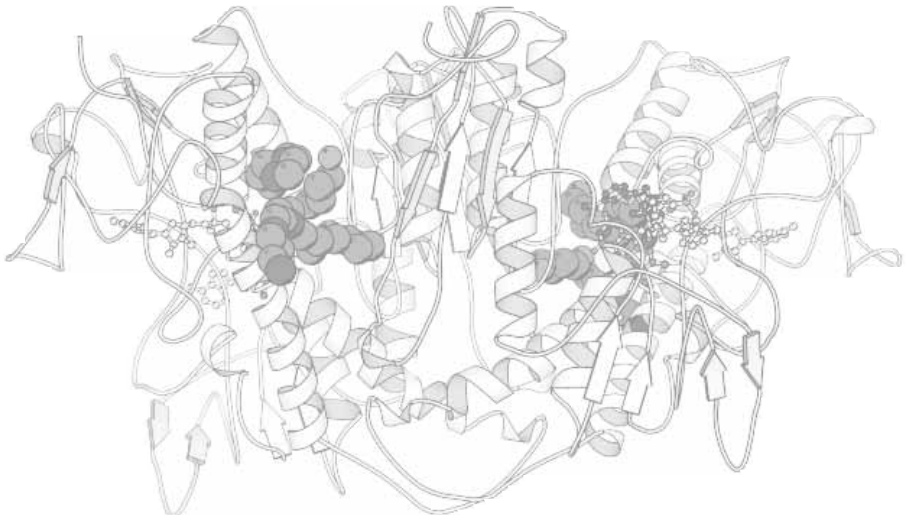
Another area of biochemical research that is impacted by X-ray scattering experiments is the study of phase transitions in lipid membrane bilayers. The goal behind these studies is to elucidate the nature of localized structural changes that must occur in biological membranes to allow various transmembrane transport functions to occur. Scientists are studying model bilayer systems that have more easily interpretable simple phase transitions by using SR X-rays. In these studies, regular two-dimensional membranes are constructed from component lipid molecules, resulting in well-defined "diffraction" peaks as opposed to the smoothly varying angle-dependent scattered intensity observed in SAXS. Different lipid arrangements (phases) give rise to different sets of diffraction peaks which can be followed as a function of temperature or time following a temperature jump.

Fiber X-ray Diffraction

One-dimensional long-range orientation of biomolecules (into fibers) can also give rise to X-ray diffraction, and this is the basis for a large number of experiments designed to understand the molecular details of muscle contraction and the structure of fibrous proteins (e.g., the connective tissue collagen) and nucleic acids (polynucleotides). Muscle contraction involves the sliding of actin molecules in thin filaments past the thick filaments that are composed of molecules of myosin. The structures of the relaxed, tensioned, and contracted states of muscle fiber bundles or even single fibers can be investigated by X-ray fiber diffraction techniques. Control of this contraction by Ca^{2+} and ATP has also been investigated, the latter in a time-resolved experiment in which ATP generated instantaneously by a light-induced chemical reaction induces contraction which is then followed with sub-millisecond time resolution. Fiber diffraction has also been employed in structural investigations of double-helical DNA molecules. In particular, transformation of a DNA fiber from one conformation (D form) to another (B form) can be induced by humidity changes and has been followed by monitoring characteristic changes in the fiber diffraction pattern. In another experiment, the location of cations (Rb^+, K^+, Li^+) in the double helix was deduced by interpreting changes in the fiber diffraction pattern. The interpretation took advantage of the much better X-ray scattering from Rb^+ compared to Li^+. A better understanding of



Fiber diffraction patterns obtained at the SRS in Daresbury on fibers of the D form of DNA The alkali metal cation neutralizing the DNA phosphate backbone has been isomorphously replaced with rubidium (top left), potassium (top center), and lithium (top right), altering the diffraction pattern. Comparison of the rubidium to the lithium diffraction patterns allows a calculation of a Fourier difference map that locates the positions of the alkali metal cations (lower frame; the "wire-mesh blobs" indicate extra electron density in the rubidium compared to the lithium structure). [From W. Fuller, V. T. Forsyth, and A. Mahendrasingam in Synchrotron Radiation and Biophysics *(S. S. Hasnain, Ed.), Ellis Horwood: Chichester, 1990, pp. 201-222.]*



Crystallographically determined structure of the enzyme trypanothione reductase, which is the target for drug design in the control of sleeping sickness trypanosome parasites (see text). The two areas of dark spheres on the left (and behind the right) of the molecule are the substrate analogs that occupy the active site of this enzyme. [See Eur. J. Biochem. 213, *67–75 (1993). Figure generated with MolScript, P. Kraulis,* J. Appl. Crystallogr., 24, *946–950 (1991)]*

DNA conformations and interaction with physiological cations (K^+, Mg^{2+}, Ca^{2+}) should eventually allow the definition of DNA conformational changes that influence regulation of transcription and translation (the synthesis of proteins from genetic material).

Single-Crystal X-ray Diffraction

These X-ray scattering and fiber diffraction techniques give information about the overall size and shape of biological macromolecules, but when it is feasible to grow single crystals of these macromolecules, X-ray diffraction can be used to obtain a near-atomic resolution (in the few Å range) structure (a crystal structure) that provides much more information. Knowledge of the structure of an enzyme at this level allows researchers to identify the chemical characteristics of the protein residues in the "active site" of the enzyme and helps them predict how chemical transformations are catalyzed by the enzyme. It may also allow the design of specific potent inhibitors of the enzyme's activity; for example, inhibition of enzymes that are crucial for the growth or infectiousness of pathogens (viruses, bacteria) might arrest the development of the disease that they cause. This rational (structure-based) drug design is a major component of the research efforts of many pharmaceutical companies. A crystal structure may also be the first step in a process known as structure-based protein engineering. Geneticists' ability to alter the gene that codes for a particular enzyme allows for engineering enzymes to be stable at higher temperatures or to carry out new chemical reactions, both of intense biotechnological interest. Knowing the structure of an enzyme allows the researchers to focus on the important residues to alter in these engineering attempts.

Single crystals consist of a repeating three-dimensional lattice of molecules, and this long-range order allows the diffraction of X-rays at discrete angles, generating a diffraction pattern that can be Fourier transformed into a map of the electron density of the molecules in the crystal. The atoms and bonds of the molecule then must be fitted into this electron density map in a process known as refinement. With considerable effort, which traditionally involves binding heavy metals to the macromolecule to act as landmarks in the electron density map, this process yields the atomic-level structure (the position of each atom) of the macromolecule. Although this process can be accomplished with laboratory X-ray sources, SR has made an impact in a number of ways. First, the intensity of the SR-based X-ray source allows data to be collected much more quickly, before the single crystals have time to degrade because of X-ray induced damage. The intensity and collimation of the SR X-rays also allow diffraction data to be collected and crystal structures to be solved for smaller crystals than required for laboratory-based X-ray sources. Most importantly, the ability to change the wavelength (the tunability) of the SR X-ray beam has had a major impact on macromolecular X-ray crystallography. It has allowed the development of a technique known as MAD (multiple-wavelength anomalous dispersion) that uses diffraction data collected at wavelengths near the X-ray absorption edge of one or more heavy atoms in the macromolecule to facilitate the experimenter's ability to "phase" the diffraction data (part of the transformation to the electron density map) without having to bind exogenous heavy atoms. A general approach has been recently developed by Wayne A. Hendrickson and coworkers at Columbia University for recombinant proteins (proteins which are made by incorporating their cloned genes into another host for production) in which methionine amino acid residues (which contain a sulfur atom in their natural form)

are replaced by selenomethionines (which contain a selenium atom in place of the sulfur), and X-rays just below and above the Se K X-ray absorption edge are used in the MAD technique.

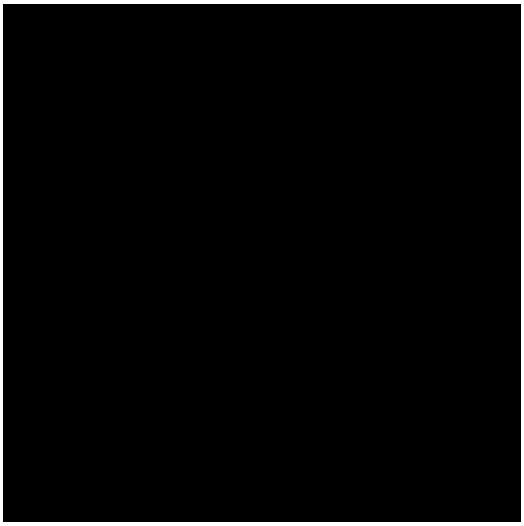
As an example of a project involving rational drug design, SR was used to solve the crystal structure of the enzyme trypanothione reductase, an enzyme that is critical for the metabolism of the trypanosome, a protozoan parasite that causes sleeping sickness. Since humans and domestic animals that are infected by this parasite do not have this enzyme, it is a good target for the selective action of a designed drug. The first step in this project was to solve the crystal structure of the enzyme (requiring SR owing to the small size of the crystals obtainable) in the presence of one of the natural substrates. The substrate serves as an accurate indicator of the active site of the enzyme, which will then be the target for designing inhibitors that will bind tightly to the active site and block substrate access. Without the products of this enzymatic reaction, the trypanosome will die, and the designed inhibitor may become the next-generation drug for treating trypanosomal infection.

Laue X-ray Diffraction

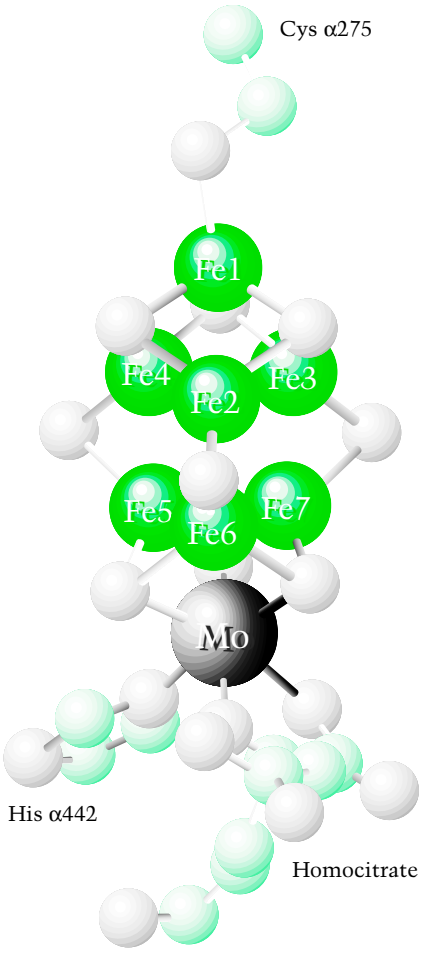
Although the static structures resulting from standard X-ray diffraction studies hold significant information about enzyme active sites, these structures cannot yield the most important information regarding the dynamics of the enzymatic reaction itself. The future holds considerable promise about the ability to perform "kinetic crystallography" in which diffraction patterns are collected within milliseconds as snapshots of the entire enzyme structure during the course of the enzymatic reaction. Referred to as Laue diffraction, the most promising technique involves the use of polychromatic rather than monochromatic SR, so that a large number of lattice planes in the sample give rise to diffraction spots, generating a relatively complete set of diffraction data in a single shot. With fast photochemical methods of releasing caged substrates or cofactors contained within the crystal in milliseconds, the Laue method could allow scientists to track the course of an enzymatic reaction using structures at atomic resolution.

X-ray Absorption Spectroscopy

Although over 100 new macromolecular crystal structures are appearing each year, not every enzyme is amenable to this level of structural characterization; the most common difficulty is the inability to grow suitable crystals of your favorite enzyme. For metalloenzymes, one need not despair since the availability of SR has also driven the revival of the technique of X-ray absorption spectroscopy (XAS) that can give some structural information about metal sites in enzymes for non-crystalline samples (e.g., in frozen solution). In most metalloenzymes, this is very useful since the metal is usually part of the active site, the "business end" of the macromolecule. The structural information from this technique comes from analysis of the EXAFS (extended X-ray absorption fine



White radiation Laue diffraction pattern from a single crystal of the protein isocitrate dehydrogenase (IDH) from E. coli (IDH is an enzyme that catalyzes the conversion of isocitrate to α-ketoglutarate and CO_2) recorded on SSRL beam line 10-2. The X-ray brightness of BL 10-2 is sufficient for diffraction patterns of this type, containing many thousands of usable diffraction peaks, to be recorded in a few tens of milliseconds on photographic X-ray film, and in only a few milliseconds on imaging plates. (Imaging plates are becoming the detection method of choice for SR X-ray diffraction owing to their large format, high resolution, high dynamic range, sensitivity, and reusability.) This provides the possibility of time-resolved studies in protein crystallography. The photograph was taken by Barry L. Stoddard of the Fred Hutchinson Cancer Research Center, Seattle, and R. Paul Phizackerley and S. Michael Soltis of SSRL/SLAC.



The structure of the iron-molybdenum cofactor (FeMoco) of the enzyme nitrogenase solved by X-ray diffraction. This is the cluster at which N_2 reduction is believed to take place. X-ray absorption spectroscopy successfully defined the Mo-Fe, Mo-S, Mo-O, Fe-S, and Fe-Fe distances before the structure was solved by X-ray diffraction, but was unable to predict the exact arrangement of the atoms in three-dimensional space. (Figure provided by D. C. Rees, California Institute of Technology.)

structure) which consists of energy-dependent oscillations in the absorption by the metal of X-rays with energies just above the metal's K absorption edge. In essence, the photoelectron produced by metal absorption of an X-ray photon scatters from nearby electron density, probing the presence of other atoms in the vicinity of the metal. What results is a "radial map" of the metal environment with information about distances and types of atoms in the neighborhood. This technique can define the protein residues that bind the metal atom, define the number of residues bound, and detect the binding of substrates or inhibitors to the metal. This sort of information is very useful in the absence of a three-dimensional structure of all atomic positions in helping define the active site of the metalloenzyme.

As an example, consider the nitrogenase enzyme mentioned earlier. The MoFe protein of this enzyme complex contains a FeMo cofactor where the substrate N_2 reacts. The structure of this metal cluster was probed by EXAFS years before the crystal structure became available. Although the EXAFS data did not give a complete picture of the structure of this cluster, all the essential metrical details from the Mo EXAFS analysis were correct. The recent solution of the crystal structure of the MoFe protein has opened up the possibility of understanding the chemical mechanism of nitrogen fixation, the first step toward engineering this ability into crops that could then produce their own fertilizer.

SR can also be used to probe the electronic structure of metals in biological molecules. In XAS, close examination of the K X-ray absorption edge region can reveal peaks and shoulders just before the edge arising from electronic excitation from the 1*s* orbital to valence orbitals. Analysis of this region of the spectrum (sometimes referred to as XANES, X-ray absorption near-edge structure) can yield information about the electronic structure and symmetry of the metal site. Certain symmetries are correlated with metal-site reactivity and so can aid in structural and functional characterization of the metalloenzyme.

As for many of the other SR-based techniques discussed, time-resolved XAS holds promise for being able to watch metalloenzyme active sites in action. Photochemical release of substrate or photolysis of metal-bound inhibitor might be used as a trigger to initiate reactions that can then be followed by collection of XAS data on a sub-millisecond timescale. The SR intensity and detector capability required for such experiments will become available during the development of the next-generation SR sources.

As one can see from this summary, synchrotron radiation has had a tremendous impact on the field of structural biology. In addition to being able to collect X-ray based data more efficiently using existing techniques, new techniques have been developed that were not possible without SR. As we move into the next millenium, look for another revolution in the development of temporal resolution that will allow SR-based techniques to examine not only the *structure* but also the *function* of biological systems.