otic; at the smallest scales they consist of individual particles. Somewhere in between, liquid molecules interact fleetingly with their neighbors, arranging themselves into structures that are gone again in a flash. For brief moments, the water may even appear to be a solid. These transitory get-togethers by molecules are called collective excitations, and they may affect everyday properties of a liquid, such as chemical reactions, thermal properties, and the way sound waves propagate.

Collective excitations are also thought to be responsible for the phenomenon of "fast sound" in water. Sound usually travels at about 1400 meters per second in water, but about 4000 meters per second in ice. Computer simulations predict that collective excitations in water allow a second, faster form of sound to travel at 3200 meters per second. Paris-based physicist José Teixeira and his colleagues first glimpsed fast sound in 1985 using neutron scattering, although further neutron studies a decade later cast doubt on their findings.

However, Sette and colleagues from ESRF and from the University of L'Aquila in Italy confirmed the effect by setting off fast sound waves in water with inelastically scattered x-rays. "We were able to show that you can have in a liquid a transition to properties characteristic of the solid when you consider time and length scales which are short and small [enough]," says Sette, who reported the result in the 1 July 1996 issue of *Physical Review Letters*.

Solid but disordered materials such as glass are also coming under the gaze of inelastic x-ray scattering. Glass is remarkably good at absorbing heat, for example, and Sette led scattering experiments at ESRF showing that some of this ability "must come from high-frequency acoustic waves." He notes that he and his colleagues "were able to observe and to measure and to characterize [these waves] in a glass, and this was not possible before."

Despite their newfound abilities, x-rays are not about to replace neutrons as a tool for studying matter. Laundy describes the two as complementary, a sentiment echoed by Sette. When it is possible to use them, "neutrons are by far ... the best technique to look at the dynamics," he says. And even though x-rays can probe magnetism in ways neutrons cannot in many situations, "neutrons are still the probe of choice to determine a magnetic structure," says Vettier, because their magnetic scattering is so much stronger. But researchers at the new synchrotron sources are learning fast, and x-rays may not remain the underdog for long.

–Andrew Watson

Andrew Watson is a writer in Norwich, U.K.

BIOLOGY

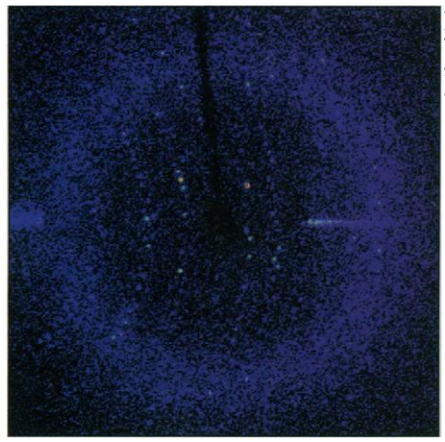
Brightness Speeds Search for Structures Great and Small

To Eva Pebay-Peyroula and other x-ray crystallographers, bacteriorhodopsin has brought 20 years of frustration. Beginning in the mid-1970s, researchers managed to coax this large protein (which helps convert sunlight to chemical energy in bacteria) into crystals, the starting point for x-ray experiments that they hoped would determine the protein's three-dimensional (3D) atomic structure and reveal how it does its job. But while other experiments have unraveled a good deal of the molecule's structure, the x-ray studies never panned out. The crystals were either too small or too disorderly to be useful, making the atomic pictures come out fuzzy at best.

Last winter, however, Pebay-Peyroula, a crystallographer at the University of Grenoble in France, finally triumphed over the elusive protein. Together with crystal growers Ehud Landau and Jurg Rosenbusch of the University of Basel in Switzerland, Pebay-Peyroula took a newly grown batch of pinhead-sized crystals—no bigger than the ones that had failed in earlier studies—to a new ultrafine x-ray beam at the European Synchrotron Radiation Facility (ESRF) in Grenoble. They walked away with the first high-resolution x-ray picture of the molecule, the details of which she presented at last month's European Biophysics Congress in Orleans, France. The new structure not only reveals new aspects of how the water molecules at the core of bacteriorhodopsin help pump protons across cell membranes to help generate chemical energy; it also highlights the new frontier of molecular biology being made possible at the latest generation of synchrotrons. "It's a really big success that could only have been done on this beamline," says Stephen Cusack, a crystallographer at the European Molecular Biology Laboratory's facility in Grenoble.

ESRF, which has been up and running since 1994, is one of three so-called "third-generation" synchrotron sources that turn out highly energetic, or hard, x-rays; the other two are just starting operations in the United States and Japan. The main advantage of these stadium-sized machines, which generate radiation by accelerating charged particles to high energies and sending them along tightly curving paths, is that "their beams are fantastically bright compared with other sources," says Wayne Hendrickson, a biochemist at Columbia University in New York City. They are at least 100 times as bright, in fact, thanks to the high energies of the particles, as well as the addition of specialized instruments designed to enhance and focus the beams. That puts them "head and shoulders above other machines," says Edwin Westbrook, a crystallographer who heads a structural biology collaboration at the Advanced Photon Source (APS), the United States' third-generation synchrotron, which officially opened for business in May 1996.

That brightness, according to Westbrook, Hendrickson, and others, will allow researchers to study biomolecules as never before. For the first time, the ultrasmall crystals of proteins such as bacteriorhodopsin are yielding enough data for researchers to determine their structures. The intense beams are lighting up the atomic landscapes of protein complexes, such as viruses, that are too large to be studied with fainter beams. They are speeding discoveries by turning out, in just seconds, the amount of data that previous machines required minutes or hours to amass. That speed is also helping researchers make high-



A. HOWARD/IIT

Connect the dots. Spots produced by a beam of x-rays diffracted from a protein crystal.

speed movies of proteins as they undergo shape changes (*Science*, 27 June, p. 1986). Moreover, because the beams are so bright, they can reveal details hidden in partially ordered samples, such as the molecular events responsible for muscle contractions.

Starting small. While synchrotrons got their start as scientific toys for physicists, chemists, and materials researchers, today biologists make up the fastest growing set of users, up from 5% just 10 years ago to 30% today. The reason: The machines' hair-thin x-ray beams are ideal for determining the 3D structure of proteins. While such structures can be deter-

mined with a number of techniques, the most popular is a method known as diffraction, in which an x-ray beam ricochets off innumerable copies of a protein lined up in a crystal. By analyzing the pattern of deflected x-rays recorded by a detector, researchers can reconstruct a precise 3D map of a protein's constituent atoms.

At least, that's the theory. Reaching this goal is fraught with difficulties, including the fact that x-rays interact only weakly with the types of atoms in biological crystals. This means that only a tiny fraction of the x-rays are actually deflected from their flight path and provide information about the molecule's structure. Researchers have long tried to get around this by growing relatively large crystals—about the size of a sesame seed—with an enormous number of copies of the protein, in hopes that the added protein copies would deflect a greater percentage of a beam's x-rays. "But there are many cases where you cannot grow large crystals," says Christian Riekel, a microfocus beamline expert at the ESRF. Many proteins have an irregular shape, preventing them from packing together easily, and some can fold up in more than one 3D shape, which again prevents a regular assembly.

Bacteriorhodopsin is one such case. Other similar proteins that span cell membranes are just as finicky. While researchers have solved the structure of more than 2000 nonmembrane proteins, they have only managed about 10 or so membrane proteins. Yet the structures of membrane proteins are eagerly sought, as they include receptors, ion channels, and other vital cell components. With the new beams, such as the ESRF's microfocus beamline (which can focus 200 billion photons a second on a spot just 10 micrometers in diameter) researchers are hopeful that the trickle of membrane protein structures will soon swell to a stream.

The reason for the optimism is that the increased x-ray intensity allows tiny crystals with fewer copies of a given protein to produce a meaningful diffraction pattern. "Smaller crystals suffice," says Cusack. "That's a good thing, because it means that the structure of more proteins can be solved." Ada Yonath, a crystallographer with a joint appointment at Israel's Weizmann Institute for Science and the Max Planck Research Unit for Ribosomal Structure in Hamburg, Germany, agrees. "In 1972, we got microcrystals of an antibody that we were interested in," says Yonath. "But at the time, we just threw them away, whereas today we'd be able to use them." Adds Tom Irving, a synchrotron expert at the Illinois Institute of Technology (IIT) in Chicago, "In the old days, you studied what you could study. Today you study what you want to study."

But the new tightly focused beams are not without their problems: With increased intensity comes radiation damage. Beams at second-generation sources, such as the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory in Upton, New York, can typically focus their beams down to a spot size of 100 micrometers across. Microfocus beams at the APS and ESRF, meanwhile, regularly pack an even greater flux of photons into an even smaller spot, about 30 micrometers and below. As a result, "you get intense radiation damage because you're putting so many photons in such a small area," says Cusack. "It rapidly damages crystals."

C. LIMA AND E. WESTBROOK/COLUMBIA UNIV.

Going MAD. Selenium atoms (white cages) in this fhit protein alter the diffraction of incoming x-rays, making it easier to determine the protein's 3D structure.

In addition to damaging the proteins directly, the high-energy photons can break bonds in the solvent surrounding them, creating highly reactive "free radicals" that eat away at bonds in the protein. Over the past few years, researchers have taken to chilling their crystals to about 100 kelvin with liquid nitrogen in an effort to halt the diffusion of the free radicals, but this is less effective under the more intense beams of ESRF and APS. Westbrook and others have shown in preliminary experiments that cooling the samples to even lower temperatures with liquid helium seems to improve matters somewhat. But "the jury is still out" about whether the improvement will be enough, says Cusack.

The big picture. Another area benefiting from the new high-power x-ray beams is the attempt to determine the atomic structure of heavyweight proteins and large complexes of proteins, DNA, and RNA, such as viruses and cellular organelles. While x-ray diffraction has long worked wonders for solving the structures of small proteins, measuring 75 or so angstroms in diameter, researchers have had a much harder time using it to map the atomic landscape of large structures, such as viruses, in which the smallest repeating unit in the crystal, known as the unit cell, can measure 1000 angstroms across or more.

The huge number of atoms in viruses and other large structures is one problem. Because of it, these structures create more diffraction spots on the detectors, each one formed by x-rays glancing off a different plane of atoms in the crystal. If too many spots are created, they begin to overlap, making it hard for researchers to separate them out. A second problem has been intensity. Because the structures are larger, a crystal of a given size will contain fewer copies of the unit cell. And unless a larger number of photons are blasted at the crystal, the spots will be dimmer, making them harder to distinguish from background noise.

In the new generation of synchrotrons, better x-ray detectors have greatly eased the first problem, while the higher brilliance beams dramatically reduced the second. "If you have more [x-ray] intensity, each spot will be stronger, and it makes it possible to go to larger assemblies," says Michael Rossmann, a crystallographer at Purdue University who in 1985 solved the first crystal structure of a virus—one far smaller than the viruses being mapped today.

While work on these macro structures has been progressing at second-generation sources for years, third-generation sources are only now beginning to weigh in. And the results, says Hendrickson, are "spectacular." At last month's Protein Society meeting in Boston, for example, a British team led by Oxford University molecular biophysicist David Stuart and Peter Mertens, a virologist at the Institute of Animal Health at Pirbright, reported using ESRF to help them map the atomic pattern of the bluetongue virus, which infects sheep and cattle. This structure, the largest ever solved, is made up of about 1000 proteins and has a unit cell that measures about 1100 angstroms on a side and 1600 angstroms high. According to Stuart, "it was a bit of a stretch even at a third-generation source."

But Stuart adds that the bluetongue virus is not likely to remain at the top of the heap forever. "As time goes on, the things that are of biological interest will be different and have larger and larger structures and be made up of complexes of proteins," says Stuart. "Having access to these machines makes looking at these complexes possible."

Tim Richmond and his colleagues at the Swiss Federal Institute of Technology (ETH) in Zurich have been hard at work at another heavyweight project. They also recently used ESRF to collect diffraction data on the nucleosome, a protein-DNA complex that is the fundamental repeating unit in chromosomes, and they too expect to publish a high-resolution structure soon. Yonath and her colleagues are also completing work on a new high-resolution image of the ribosome, the cellular machine responsible for building proteins.

Proteins go MAD. In addition to solving larger structures and probing smaller crystals, researchers at third-generation sources expect to solve structures more rapidly as well. Not only does the higher flux of photons from the machines speed data collection, but the improved ability of the new machines to tune the wavelength of their x-rays allows researchers to expand their use of a new high-speed technique for solving protein structures: multiwavelength anomalous diffraction. MAD, as it's called, cuts down on the number of crystals that researchers must image to piece together an atomic-scale map.

The technique cuts to the heart of modern crystallography's greatest challenge—the fact that conventional lenses cannot focus x-rays. Robert Stroud, a crystallographer at the University of California, San Francisco, explains that a diffraction pattern is somewhat like the blurry pattern that hits a screen if light is projected through a slide without then being focused by a lens. Without a lens to reveal the image, researchers must use mathematical techniques to reconstruct the pattern of atoms that gave rise to the diffraction pattern.

That requires knowing two properties of the x-rays. The first is the intensity of the diffraction spots; the second is the relative position of the wave forms themselves, known as their phase. By plugging these numbers into an equation, researchers can work out the 3D atomic pattern of atoms in the crystal.

But while the intensity of the spots can be measured simply by counting the number of photons that hit the detector at each position, the phases of the scattered waves cannot be determined by looking at a single diffraction image. To find out the phase of the x-rays, researchers normally take multiple images of at least two crystals of the protein, one of which typically has a metal atom inserted into the protein's structure so that it produces subtly different diffraction patterns. Then, by comparing the two sets of patterns, the researchers can determine the precise position of the metal atom, much as surveyors can triangulate their own position by knowing the direction and distance to two separate points. This point of reference then helps the crystallographers infer the relative phases of the waves scattering from other atoms in the protein.

This technique of analyzing multiple crystals of the same protein has a long track record of success, but in the late 1980s, Hendrickson and his colleagues at Columbia showed that they could achieve the same result with just a single metal-containing protein. They did so by taking multiple diffraction patterns with x-rays of slightly different wavelengths. The metal atom scatters photons differently if their frequency is tuned slightly above or below a characteristic "resonant" value, yielding the multiple diffraction images needed to determine the critical phase information. The technique has since swept through the crystallography community, because it means that researchers now need only crystallize one form of their protein.

Results from initial MAD studies at third-generation machines are beginning to come in. Christopher Lima, a postdoctoral in Hendrickson's lab, and colleagues at Columbia and at Argonne National Laboratory in Argonne, Illinois, report in the 15 June issue of *Structure* that they used MAD to determine the atomic map of a putative tumor-suppressor protein, which is thought to be disrupted in lung cancer. This result—the first biological structure announced from the APS—will undoubtedly be the first of many. Because third-generation sources can tune their x-rays over a wider range of wavelengths and because their high power increases the characteristic scattering from the metal atom, use of the technique "is likely to grow exponentially," says Yonath.

D. STUART/OXFORD UNIV.

Heavyweight champ. With the help of ESRF, British researchers solved the structure of the bluetongue virus.

Looking beyond crystals. While solving protein structures is likely to be a mainstay for the new beamlines, researchers expect the machines to help shed light on other long-intractable biological questions as well. At the APS, for example, teams are gearing up to use the intense x-ray beams to explore everything from how muscles twitch to how plants absorb nutrients from the soil.

Like protein crystallography studies, most of these projects had their start at earlier synchrotrons, and researchers are hoping that the new high-brilliance beams will help them improve their data. For the past several years, for example, University of Chicago geophysicist Stephen Sutton and his colleagues have been using Brookhaven's second-generation NSLS beams in the hope of determining just how a fungus manages to cause "take-all" disease, which kills up to 20% of all wheat crops.

Researchers have long known that the key effect of the disease is to make the roots of wheat plants unable to absorb manganese, an essential nutrient—the lack of which in turn makes the plants more vulnerable to the fungus. One possibility is that in the soil surrounding the roots, the fungus alters the electronic state of the metal atoms from manganese 2, a variety easily absorbed by the plant, to manganese 4, a form that the plant cannot absorb. As Sutton explains, "this makes the manganese unavailable to the plant, so it becomes manganese deficient and can't protect itself against the invading fungus."

Because the different electronic states of the metal absorb x-rays at slightly different wavelengths, Sutton and his colleagues had attempted to use the NSLS to map the distribution of manganese 2 and 4 in wheat roots and surrounding soil. However, he says, "the resolution [from the NSLS] hasn't been sufficient to look at the details of this reaction." They are hoping that the APS's tighter focus and higher photon flux will provide them the boost they need. In a related set of experiments at the University of Georgia, environmental geochemist Paul Bertsch and his colleagues are mapping the way certain plants take up plutonium, uranium, and chromium from the soil, in an effort to learn how these plants manage to concentrate the metals without succumbing to their toxic effects.

Slightly farther afield, IIT's Irving plans to train the APS beam on muscle fibers in hopes of witnessing the molecular events of a twitch close up. With each muscle contraction, Irving explains, interleaving assemblies of protein filaments known as actin and myosin exert force on one another to shorten the overall length of the muscle. As early as 1971, Gerold Rosenbaum, Kenneth Holmes and their colleagues attempted to witness this supramolecular dance using x-ray scattering. But the effort was hampered, in part because earlier synchrotrons delivered too few photons to provide good resolution. Now Irving believes they are finally on the verge of a breakthrough.

It is a sentiment that many of his synchrotron colleagues around the world share. "With the third-generation sources," says Irving, "we finally have the flux to do what we wanted to do in the first place."

–Robert F. Service