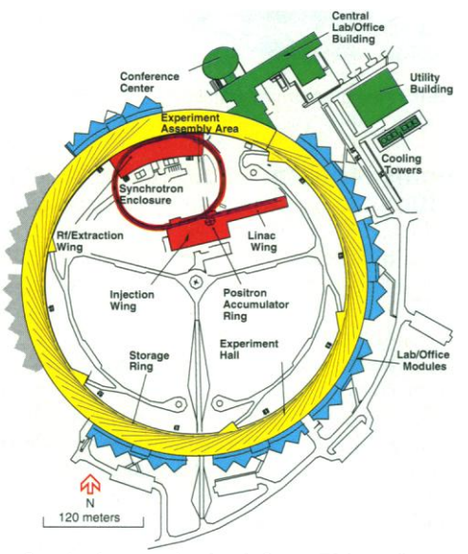
Switching On a Brilliant Light

Soon to be the world's brightest source of hard x-rays, the Advanced Photon Source is a beacon for crystallographers, solid-state physicists, and materials scientists

ARGONNE, ILLINOIS—Construction crews are still swarming around the 350-meter-wide donut that houses the Advanced Photon Source (APS) here at Argonne National Laboratory. But inside, the scientific action has already begun. Last Sunday the $811 million machine, which is funded by the Department of Energy, entered a final round of tests as charged particles were injected into the APS's main ring. Whirling around it at nearly the speed of light, they threw off bursts of x-rays like sparks from a pinwheel. This is what those who work on the machine call "first light," and it offers a glimmer of what is to come when the APS powers up for its first experiments within the next year.

At that point the "brilliance" of the radiation—a combined measure of intensity and factors such as a beam's tightness and coherence—will be thousands of times greater than previous-generation machines could muster at hard x-ray wavelengths, less than an angstrom or so. Just as important to the consortia of basic researchers and industry scientists who will be working at dozens of beamlines around the machine (see box on p. 1905), the machine's design will make it easy for each group to tailor the brilliant light independently to fit its own needs. With the start-up of the APS, the United States has drawn even with Europe, whose European Synchrotron Radiation Facility (ESRF) began operation last year in Grenoble, France, at slightly lower particle energies—6 billion electron volts (GeV) compared to the APS's 7 GeV. And it has opened up a lead over Japan, whose 8-GeV SPring-8 synchrotron is not scheduled for completion until 1998.

Many researchers predict that this trio of x-ray searchlights will have its most dramatic impact on protein crystallography, the effort to work out molecular structures by diffracting x-rays through protein crystals (*Science*, 3 February, p. 620). The brilliance of the APS and its rivals means "the difference between high-resolution details being measurable and losing them," says Ada Yonath, a biologist at the Weizmann Institute in Rehovot, Israel, who plans to enlist the APS in her effort to solve the structure of the cell's protein-making factory, the ribosome. Other researchers are enamored of the APS's ability to deliver a series of brief x-ray blasts so brilliant that they should be able to record movies of atomic or molecular rearrangements in polymers, semiconductors, and liquids as they happen—phenomena visible in the past only as blurs or as snapshots.



SOURCE: ARGONNE NAT'L LABORATORY

Sparks from a pinwheel. Some 70 beamlines emanate from the APS's main ring. Inside it is hardware that generates positrons, which circulate in the ring and produce x-rays.

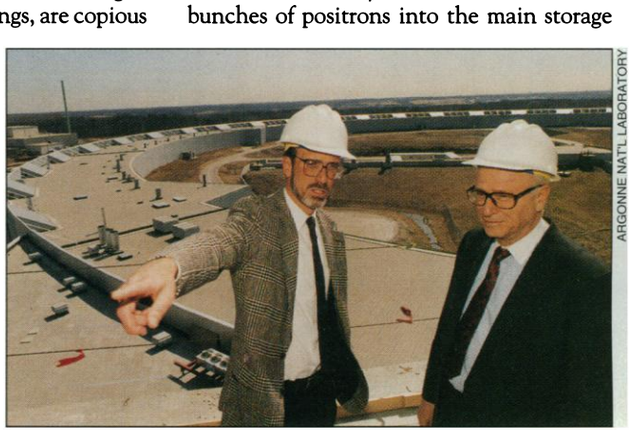
The spark that gives life to all this research is an effect called synchrotron radiation—the narrow cone of illumination, like the headlight on a freight train, given off by charged particles moving at nearly the speed of light when they are forced to change direction. The higher the energy of the particles, the narrower the cone angle and the shorter the average wavelength in the bundle of radiation. Synchrotron accelerators, which use radio-frequency cavities to propel bunches of charged particles around evacuated rings, are copious generators of this light. Because synchrotron radiation saps the particles' energy, early accelerator builders regarded it as an annoyance. But researchers in other fields, such as biology, found it was spectacularly useful. They flocked to the physics facilities, where they "piggybacked on the physicists, who were the rulers of the synchrotron," jokes Jonathan Greer, a senior research fellow in pharmaceutical discovery at Abbott Laboratories near Chicago.

By the 1980s, synchrotron radiation devotees commanded machines of their own, culminating with the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory. But the radiation thrown off as these machines whipped particles through "bending" magnetic fields had drawbacks. The beams from bending magnets tended to fan out, which reduced their intensity, and they consisted of many different wavelengths, lowering the usable intensity still further for researchers who needed to single out particular wavelengths. What's more, even at the energy of the NSLS, 2.5 GeV, the particles couldn't generate the very hard x-rays that can probe materials and molecules at high resolution.

A new generation

The APS and its European and Japanese competitors vault over those drawbacks by incorporating new magnetic devices that can generate narrow, coherent beams, concentrated in just a few wavelengths, and by accelerating the particles to higher energies than ever before. At the APS, those particles are positrons, the antimatter counterparts of electrons. They begin their journey in a thicket of hardware that nestles inside the main ring. There electrons, emitted from a hot filament and accelerated, smash into a tungsten sheet to produce the positrons. In quick succession, the positrons pass through a linear accelerator, a small "accumulator" ring, and a booster synchrotron, which kicks their energy all the way up to 7 GeV.

The booster synchrotron then shoots bunches of positrons into the main storage



ARGONNE NAT'L LABORATORY

X-ray vision. APS Director David Moncton *(left)* and Alan Schriesheim, director of Argonne National Laboratory, overlook the main ring, which measures more than a kilometer around.

Teaming Up to Catch Some Rays

The Advanced Photon Source (APS) will serve up hard x-rays of unmatched quality and brilliance, but that's where its job will end, for the most part—and that's also where human factors, with all their potential for controversy, begin. Once the photons stream out of the synchrotron ring's beryllium windows and into the experiment hall that encircles the ring, it will be up to the users of the facility to put them to good use. The APS will provide design advice and offer some ready-made x-ray optics for purchase. But the users themselves will have to fund, build, and run the beamlines and detectors that will turn the radiation into data on everything from protein structures to polymer processing (see main text).

This arrangement is spurring the creation of eclectic research consortia known as Collaborative Access Teams, or CATs, that include private companies, national laboratories, university research groups, and federal agencies. "The level of collaboration is really new," says APS Director David Moncton—and so are some of the accompanying dilemmas.

At earlier synchrotrons, such as Brookhaven National Laboratory's National Synchrotron Light Source, a single large company could afford to control an entire beamline. But setting up a beamline to harness the far brighter x-rays at the APS takes a $5 million "entry-level" investment for basic optics, detectors, computers, and other apparatus, well beyond the means of any single research group. The result, says Moncton, is some "interesting bedfellows."

One CAT, for example, consists of 12 major pharmaceutical companies; although they compete in the marketplace, they are sharing beamlines and equipment aimed at deciphering the structure of biological molecules that could make good drug targets. Such arrangements—and so far there are 14 others—complicate the management of the project, resulting in an unrelenting schedule of meetings and progress reports. "It's hypermanaged," says Edwin Westbrook, a researcher at Argonne National Laboratory who leads a CAT aimed at studies of structural biology.

What's more, proprietary and basic research don't always mix easily. Some industry researchers are upset over plans to charge supplemental user fees of $5000 a day to companies when they are doing proprietary, nonpublishable research. "We've paid so much money already to be there," argues Randolph Barton Jr. of E. I. du Pont de Nemours and Co., which has formed a CAT with Northwestern University and Dow Chemical Co. to study surfaces and polymers. "We'd like to see [the rule] changed." Some industry officials, however, say they welcome the fees because they help establish clear title to proprietary research.

Martha Krebs, director of DOE's office of energy research, which will fund the APS's $95 million in annual operating costs through its office of basic energy sciences, declined to comment on the user fees controversy. But in spite of the rough spots, she insists that the APS is "a great example of teamwork." The diversity of users, Krebs says, will help it produce "good science that has impact on our lives."

–J.G.

ring "like peas from a high-tech pea shooter," says APS Director David Moncton. Within the ring—actually an 80-sided polygon, with alternating long and short sides—each bunch circulates for hours before decaying, its lifetime enhanced because the particles are positrons rather than the electrons that are the staple of most other synchrotrons. Positrons, being positively charged, repel the sparse gas ions left behind in the ring's high vacuum, pushing them out of the way and suppressing the collisions that could disrupt the beam.

At the vertices, the positron bunches whip through bending magnets and emit a higher energy version of the broadband x-rays available at earlier machines. But in 35 of the straight sections, the positrons speed through magnetic devices of another sort, known as wigglers and undulators. Instead of swinging the positrons through wide turns, explains Gopal Shenoy, experimental facilities director at APS, undulators force the particles through a gauntlet of magnets, where they jiggle back and forth in a tight, sinusoidal path roughly 20 microns across. At each jiggle, the particles fire x-rays down the tube.

These x-ray pulses add up to form a beam that, because of the particles' high energy, spreads out by less than 1 part in 10,000. Most of its energy is concentrated in a fundamental wavelength and its harmonics—wavelengths that add up coherently, crest to crest and trough to trough, from one bend to another. What's more, these wavelengths can be tuned, either by filtering out specific harmonics or by mechanically adjusting the undulator. By moving its magnetic "teeth" closer together, experimenters can force the particles to make wider excursions from side to side, slowing their progress through the undulator. The effect is to lengthen the wavelengths of the output radiation.

The resulting x-ray beams not only far outshine the light from earlier generations of synchrotrons but, because of the extra GeV of particle energy, should also be tunable over a wider range than even the radiation from the ESRF. Yves Petroff, ESRF's director, isn't so sure, but whatever the relative merits of the two machines, researchers planning to work at the APS are impressed with its potential. "It's because APS was designed for undulators that the machine is so wonderful," says Edwin Westbrook, director of Argonne's Structural Biology Center.

Flashes of insight

Yonath of the Weizmann Institute couldn't agree more. The only way to begin understanding how messenger RNA gets translated into peptide chains in a cell, says Yonath, is to solve the molecular structure of the ribosome, the assemblage of proteins and RNA that performs the task. But that is easier said than done. Like other crystallographers, Yonath begins by trying to crystallize her target molecules. When x-rays are diffracted through a crystal, the multiple copies of the molecule or protein complex intensify the diffraction pattern, an array of dots whose intensities and phases can be mathematically decoded to reveal the molecule's structure.

Unfortunately, large biological molecules like those in the ribosome tend to produce small crystals that diffract weakly. And because the quality of the crystals is uneven, the standard approach to determining the phases of the diffracted x-rays—tagging two or more crystals with different heavy atoms—doesn't work well. Yonath hopes to do better with newer methods that extract enough information from a single crystal by illuminating it with several different x-ray wavelengths. Those methods require a source that is both brilliant and tunable—and Yonath, like researchers planning to apply them to other molecules, hopes the APS's undulator radiation will answer the need.

Not all problems in biology, however, can be solved with still shots of molecular structures. In other cases the important processes happen as the molecules fidget, move, and recombine. And because the undulator radiation will be available in short bursts, each generated when a single bunch of positrons rattles through the undulator, some of these proteins should be visible in motion. Like the flashes of a strobe light, the x-ray bursts should be able to capture a molecule's changing shape moment by moment.

The x-ray movie business also beckons to materials scientists. Researchers from E. I.

du Pont de Nemours and Co., for example, plan to set up a small nylon spinning and treatment operation in front of an APS beamline. By lighting up the process with bursts of x-rays, they hope to learn how nylon forms intermingled regions of crystalline and amorphous phases as it is spun—a structure that "has a huge influence on really important properties of the fiber," such as how it takes up dye, says DuPont researcher Randolph Barton Jr.

The narrow range of wavelengths in these bursts—their coherence—should be a boon to biologists and materials scientists who study molecular dynamics with so-called "speckle" measurements. In this technique, researchers train x-ray pulses on a sample and monitor the reflections. Because the x-rays are coherent, the reflections from an irregular sample should interfere with each other: constructively, to produce bright speckles, and destructively, to produce dark regions. Like the dapples on the ceiling of an indoor pool, these speckles contain information about the size and motion of "ripples" in the sample—say, the conformational oscillations of a protein in solution or the topographical fluctuation of a semiconductor's surface as the temperature changes.

Physicists and chemists in other fields also expect their samples to light up with new results, from studies of microscopic magnetic structures, say, or microchemical assays of trace elements. But Arthur Bienenstock, director of the Stanford Synchrotron Radiation Laboratory, cautions against trying to predict everything the APS's bright light will reveal. "All our experience indicates that once you provide a significant [improvement], there will be new science that you just didn't anticipate at all. Creative people will find new uses of the radiation."

–James Glanz

James Glanz is a science writer in Chicago.

IMMUNOLOGY

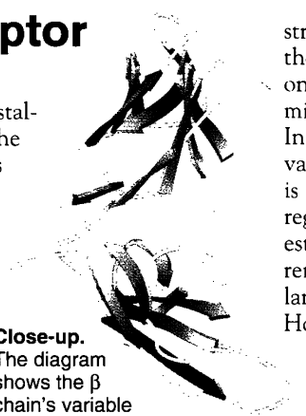
Taking a First Look at a T Cell Receptor

It isn't hard to understand why immunologists place such a high value on understanding the T cell receptor (TCR). Located on the surfaces of the immune system's T cells, this protein complex allows those crucial cells to recognize foreign proteins and initiate immune responses, including the responses needed to fight off pathogenic viruses. But even though researchers cloned the genes for the proteins that make up the T cell receptor in 1983, it has been frustratingly difficult to get a direct look at their three-dimensional structures—until now.

On page 1984, Graham Bentley and Ginette Boulot of the Pasteur Institute in Paris, Klaus Karjalainen at the Basel Institute for Immunology in Switzerland, and Roy Mariuzza at the University of Maryland present the first x-ray crystallographic structure of the β chain, which, along with the α chain, is one of the two proteins that form the site where the TCR recognizes antigens, or pieces of protein. "This is the first breakthrough in hard data on the TCR [structure]," says immunologist John Kappler of the National Jewish Center for Immunology and Respiratory Medicine in Denver.

Kappler cautions, however, that Bentley, Mariuzza, and their colleagues have solved only part of the receptor structure. The β chain, he points out, may look different when found in its normal tight association with the α chain. Still, other researchers will now be able to use the group's results to achieve the next step more quickly: solving the structure of the whole receptor, a feat that will help immunologists understand just how the TCR initiates immune responses. "Finally this problem is yielding to technology," says immunologist Ron Schwartz of the National Institute of Allergy and Infectious Diseases.

Indeed, before the collaborators could determine the β chain structure, they had to apply a technological fix to a problem that has long hindered efforts to obtain crystals of TCR proteins adequate for crystallographic analysis. A few of the amino acids in the proteins carry long chains of sugars, which hinder their ability to form crystals. To get around this problem, Karjalainen used the technique of site-directed mutagenesis to change some amino acids in the β chain into others to which carbohydrates can't be added—and struck lucky with a modified protein that yielded good crystals. This enabled the researchers to obtain the protein structure at a resolution of 1.7 angstroms, good enough to see the positions of all the atoms.



SOURCE: BENTLEY *ET AL.*

Close-up. The diagram shows the β chain's variable *(upper portion)* and constant regions.

The structure confirmed one thing immunologists had already suspected on the basis of the amino acid sequences of the T cell receptor proteins: The β chain's three-dimensional structure resembles that of the immunoglobulins, the proteins that form antibodies and which also bind specific antigens. Both the immunoglobulin and T cell receptor proteins have variable regions, which vary from one molecule to another and form the antigen binding site, plus constant regions, which are the same in all proteins of the same type. Earlier structural studies of the immunoglobulins showed that the sequences that make up each of these regions fold in a characteristic way: The strands weave back and forth, forming a pleated sheet that folds into a sandwichlike structure. The β chain also has this characteristic "immunoglobulin fold." And again as in the immunoglobulins, the amino acids that vary the most—and are thus likely to be involved in antigen binding—form small loops at the edge of the variable domain.

The TCR β chain structure does show some deviations from the immunoglobulin structure, however, and some of these differences may shed light on how the T cell receptor transmits signals to the cell's interior. In the β chain, for example, the variable domain of the molecule is much closer to the constant region. "I'm particularly interested in that elbow ... whether it remains that rigid," says molecular biologist Mark Davis of the Howard Hughes Medical Institute at Stanford University, whose group was among the first to clone a TCR gene. Both Davis and the authors speculate that this rigidity might be involved in telling the T cell that its receptor has bound an antigen.

How the hinge functions, however, is not at all clear. And that's only one of several questions remaining about T cell receptor structure and function. In particular, immunologists want to see the whole structure—β and α chains together—to confirm that the current structure is maintained in the dimer. As Kappler cautions, "Some of the unusual features might disappear when the α chain is there."

But the current work should help in snaring the entire structure. Crystallographer Pamela Bjorkman of the California Institute of Technology, who is also working on the T cell receptor structure, predicts that continuing the team's strategy of reducing carbohydrate binding by T cell receptor proteins "should allow the growth of well-ordered crystals" of the complete two-protein molecule. What's more, Kappler says, obtaining the three-dimensional structure of the β chain should help in solving the structure of the dimer, because knowing half the structure should make it easier to get the rest. And then, at last, immunologists should get a complete view of how the TCR works.

–Claire O'Brien

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