Biologists and other researchers are lining up at synchrotrons to probe materials and molecules with hard x-rays, and demand is growing fast; new magnet technologies may soon relieve the crush

Wiggling and Undulating Out Of an X-ray Shortage

These are heady days at the world's most powerful x-ray sources. Materials scientists, chemists, and biologists are flocking to the stadium-sized machines for front-row views of the structure and chemical behavior of materials and molecules. But like fans at a World Series game, they have to fight the crowds. And there is a waiting list for tickets, especially for the best seats—those at a handful of synchrotrons that produce highly energetic, or hard, x-ray beams, which are ideal for figuring out the structure of complex proteins all the way down to the atomic level. "There's been an explosion of interest in the hard x-ray range," says Herman Winick, a synchrotron physics expert at the Stanford Synchrotron Radiation Laboratory (SSRL) in Menlo Park, California.

MAGNET UPGRADES

Facility	Energy (GeV)	Scheduled Completion
SSRL/California	3.0	2002
ALS/California	1.9	2001
CAMD/Louisiana	1.6–1.8	2001
BESSY-1/Germany*	1.0	Awaiting approval

NEW SYNCHROTRONS

Host Country	Energy (GeV)	Scheduled Completion
China	2.5	2002–2003
Canada	2.9	2003
United Kingdom	3.0	2004, if approved
Spain	2.5	Awaiting approval
Switzerland	2.4	2001

* To be moved to Middle East.

Among the triggers for this explosion have been spectacular demonstrations of the power of synchrotron beams to determine the precise shapes of important molecules, providing insights into how they work and clues to the design of drugs to interact with them. The latest, published just this week, is the long-sought structure of the ribosome, the cell's protein factory (see sidebar on p. 1343). And the demand for such information is growing fast as labs around the world identify more and more of the intricate trysts that take place between proteins as they convey biochemical signals between and within cells. Once researchers figure out which proteins interact in these signaling pathways, they are eager to know the intimate details of how they fit together. Adding to this information demand are the vast numbers of new proteins being discovered through genome sequencing efforts around the world—a deluge that has prompted the National Institute of General Medical Sciences (NIGMS) to launch a $3 million a year program to automate crystallography for structural genomics (see sidebar on p. 1345). As a result, says NIGMS director Marvin Cassman, waiting lists for structural biology beamlines around the world are growing. Indeed, demand outstrips supply by a factor of 4 for hard x-ray "beamtime" at one busy x-ray port at SSRL.

Help is on the way, however. The number of hard x-ray beamlines devoted to structural biology, protein crystallography, and environmental science is set to increase dramatically in the next few years. One reason: New techniques for making x-rays are enabling smaller, cheaper machines to create x-ray beams nearly as powerful as those produced by top-of-the-line, $1 billion behemoths like the Advanced Photon Source (APS) in Argonne, Illinois. These advances are bringing powerful new synchrotrons within reach of countries like China and Canada, as well as prompting biomedical research funders—such as the U.S. National Institutes of Health (NIH) and Britain's Wellcome Trust—to collectively pump hundreds of millions of dollars into synchrotron construction. "There really is a pretty big surge in new capabilities that is coming online," says Columbia University crystallographer Wayne Hendrickson.

Slow start

Biologists and other researchers now lining up for access to beamlines have come a long way from the early days of synchrotron radiation studies, when they lived off the wastes produced by particle physics experiments. For accelerator physicists, the x-rays generated by particles that speed around their giant atom-smashers are a drag, literally. As charged particles zip around an accelerator ring, guided by massive magnets, they shed x-ray photons like baggage flying out of a convertible screeching around a tight corner. The departing x-rays rob the particles of energy, making it harder to accelerate them to higher speeds.

But the particle physicists' losses turned out to be other researchers' gains. Engineers figured out a way to corral the unwanted x-rays and focus them down into a hair-thin beam that could be trained on samples ranging from crystallized proteins to minerals squeezed in a diamond anvil to mimic pressures at Earth's core. Early experiments with these penetrating probes were anything but straightforward, however. To carry out the first synchrotron-based protein crystallography experiments in 1975, for example, Keith Hodgson and his SSRL colleagues essentially had to build their own makeshift beamline to collect their data. "It was a very difficult way of discovery," says Hodgson. But it was worth the effort: The 1000-fold increase in x-rays compared with the small lab-based sources then available sped up experiments dramatically, and the results quickly dispelled fears that the intense x-ray beams would destroy delicate samples.

Synchrotrons were no panacea, however. The finicky accelerator rings in the early machines often malfunctioned, going off-line for weeks at a time—a major inconvenience for researchers visiting a facility for a tightly scheduled block of time. Protein crystallographers, one of the largest groups of users today, also faced the challenge of transporting fragile protein crystals, painstakingly grown in the lab, to the facility. As a result, says crystallographer Janet Smith of Purdue University in West Lafayette, Indiana, the chance of an experiment working out in the early days was no better than 50–50. Indeed, for most crystallographers, it was less hassle

Upgrade Brings Hope to Berkeley's Advanced Light Source

Recent advancements in synchrotron technology (see main text) have come to the rescue of the Advanced Light Source (ALS), a "soft" or low-energy x-ray synchrotron at Lawrence Berkeley National Laboratory in California. Two years ago, ALS researchers were stunned by a harsh review from a scientific advisory panel assembled by the U.S. Department of Energy (DOE), which oversees the facility. But last month, another panel concluded that the ALS is steering a much-improved course, thanks in part to upgrades that enable it to generate hard x-rays for protein crystallography and other uses.

Completed in 1993 at a cost of $100 million, the ALS became one of the world's premier sources for soft, or long-wavelength, x-rays, which it generates from a beam of electrons accelerated to 1.9 giga electron volts. That makes the facility a prime spot for studies such as tracking the electric and magnetic behavior of superconductors or mapping the chemical identities of a wide variety of elements in a sample. But in 1997, an influential DOE advisory panel headed by Massachusetts Institute of Technology physicist Robert Birgeneau concluded that demand for synchrotron beamtime was growing fastest for hard x-rays, the short-wavelength, high-energy radiation that x-ray crystallographers need for probing molecular structures. If money was tight, the panel concluded, DOE should spend it first on upgrades of hard x-ray machines such as the Stanford Synchrotron Radiation Laboratory and the National Synchrotron Light Source at Brookhaven National Laboratory in Upton, New York—even before paying the regular operating budget at ALS (*Science*, 17 October 1997, p. 377). The recommendation left many ALS researchers concerned about the future.

Turnaround. A harsh review 2 years ago raised doubts about ALS's future. Now, the reviews are enthusiastic.

Now, however, the ALS is getting a new lease on life. Two years ago, it opened its first x-ray crystallography beamline, which benefits from a new superconducting magnet, called a wiggler, that can wring hard x-rays from the ALS's moderate-energy electrons. And more hard x-rays are on the way. A set of three new "superbend" magnets, capable of turning out hard x-rays, is being installed at a cost of only about $4 million. When completed in 2002, the new magnets could supply hard x-rays to another 12 protein crystallography beamlines, bringing the total to 13—about the same number as planned for the nation's premier hard x-ray source, the Advanced Photon Source in Argonne, Illinois. "Nobody anticipated the Berkeley ring would be much good for this kind of science," says Janet Smith, an x-ray crystallographer at Purdue University in West Lafayette, Indiana. "But now the ALS is flourishing."

The makeover caught the eye of ALS reviewers, who were asked to assess the facility's progress by the University of California, which runs the lab for DOE. The review concluded that the shift toward hard x-rays, in addition to changes in management and efforts to be more responsive to users, ranked as "excellent/outstanding." The glowing review isn't expected to have any immediate impact on ALS's budget, says Tom Russell, a polymer physicist at the University of Massachusetts, Amherst, who served on both the current review team as well as the Birgeneau panel. However, he adds, "it can't hurt." **–R.F.S.**

to keep using the plodding but trusty x-ray tube in the lab.

Most of those teething problems have now been solved. New beamlines and accelerator rings "are pretty much bullet proof," rarely going off-line during scheduled operation, says Smith. And researchers now stabilize their crystals by freezing them with liquid nitrogen, making them easier to transport and more resistant to radiation damage from the even stronger x-ray beams at today's sources. That has increased the chance of a successful experiment to well over 90%, she says. And increased computer power and the ability to select the precise wavelength of x-rays has made it possible to solve novel protein structures in just days or weeks, rather than the months to years it took a few decades ago.

As a result, "everybody wants to go to synchrotrons now," says Smith. The crowds include not just the traditional users but some new groups as well. "At one time, determining a protein structure was the province of x-ray crystallographers," notes Cassman. "That's not totally true anymore. Cell biologists who find a protein want to know the structure [themselves]."

Souped-up wigglers

A few years ago, prospects for meeting this surging demand were grim—and expensive. Although bio-centered beamlines were—and still are—being added at the large hard x-ray facilities, space was going fast. New hard x-ray machines were running about $1 billion apiece, and few were likely to be funded. X-rays from the relatively inexpensive soft x-ray machines didn't have enough juice to penetrate protein crystals. But recent improvements in the technology of the magnets at the core of the particle accelerators have dramatically changed the picture by allowing low-energy accelerators to create the abundant high-energy hard x-rays prized by biology researchers.

One source of x-rays from a synchrotron is the machine's particle-steering "bending" magnets. But other pieces of equipment called undulators and wigglers, essentially strings of magnets with alternating polarity, dramatically increase the x-ray output. As the charged particles fly through the devices, the alternating magnetic field causes them to wiggle back and forth like a slalom skier whipping through a series of tight gates. In an undulator, for example, at each turn, the particle emits a broad spectrum of x-rays whose wavelengths are linked to the regular interval in the array of magnets. Some of the x-ray photons will have the same wavelength as the distance between magnets; others are "overtones" of these wavelengths. The phenomenon is a bit like playing a note on a violin, which can sound not only the note itself but also harmonic overtones.

These shorter wavelength, or higher energy, x-ray overtones are key to generating abundant hard x-ray beams prized for experiments. The higher the harmonic, the more energetic are the x-rays. But slight defects in the magnetic structures of undulators long made it impossible to generate x-rays beyond the third and fifth harmonics. So engineers had to rely on another strategy to get those third and fifth harmonics into the hard x-ray range: raw power. They cranked up the speed of the particles whipping around the synchrotron racetrack so that they have more energy to shed when they pass through

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the alternating magnets. The APS, for example, hurls electrons around the ring at 7 giga electron volts (GeV).

In the early 1990s, Pascal Elleaume and his colleagues at the European Synchrotron Radiation Facility in Grenoble, France, cracked the harmonic barrier. They showed that a standard magnet-tweaking practice called shimming—essentially precisely shaping a magnet's field by adding tiny bits of magnetic material to it—could greatly improve an undulator's performance. "[That] made it possible to reach much higher harmonics, thus opening the way for lower energy rings to reach hard x-rays with high brightness," says SSRL's Winick. Instead of needing a billion-dollar, 6- to 8-GeV ring, researchers could now think about generating the high fluxes of hard x-rays with intermediate-sized rings that have energies of about 2.5 to 3 GeV and cost $100 million to $200 million. A related set of advances is also paving the way for even smaller, 1-GeV machines to produce hard x-rays. The key is the use of wigglers that harbor high-field superconducting magnets to bend the particle beam more tightly, which causes the charged particles to emit more energetic x-rays.

New intermediate-energy synchrotrons are currently on the drawing board in Canada, the United Kingdom, Switzerland, and China. Some of these machines have been in the planning stages for nearly a decade and have recently been revised to take advantage of the new magnet technology. Five years ago, for example, Canada decided to build a 1.5-GeV soft x-ray machine, but the plans were revamped in 1997 when it became clear that a machine with only a little more power could provide hard x-rays. Canada is now planning a 2.9-GeV facility, which is expected to open in 2003. Planned synchrotrons in the United Kingdom, China, and Spain also underwent recent redesigns to take advantage of the new hardware. "It makes sense to build accelerators that will not break the bank," says Joan Bordas, who is heading Spain's effort to build its first synchrotron, the Synchrotron Light Source, which has yet to secure its final funding.

Numerous existing facilities are also planning upgrades to take advantage of the new insertion devices. Last month, for example, NIH announced plans to provide an initial $14 million toward a $53 million upgrade of the SSRL facility, which will use the new magnet technology to become a high-flux "third generation source" (*Science*, 30 July, p. 650). Similarly, the CAMD facility in Baton Rouge, Louisiana, and Germany's BESSY-1 ring, which is being donated to the Middle East (*Science*, 25 June, p. 2077), are planning upgrades with superconducting magnets to generate hard x-rays. But the Advanced Light Source at Lawrence Berkeley National Laboratory in California probably stands to gain the most from the new technologies. The facility will soon be retrofitted with new superconducting bending magnets, and its fortunes appear to be turning around after a recent poor scientific review (see sidebar on p. 1344).

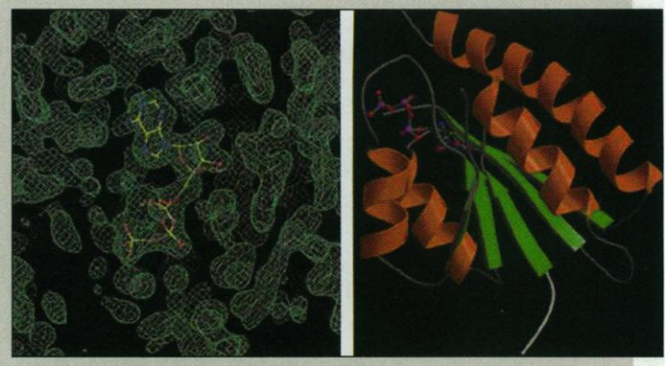
As many experts see it, these develop-

The Automated Approach to Protein Structure

If Thomas Earnest and a handful of like-minded colleagues have their way, robots may someday put human crystallographers out of work. Earnest, who heads a new crystallography beamline at the Advanced Light Source in Berkeley, California, is working with a handful of other groups to automate every stage of determining protein structure—from generating the proteins to crystallizing them, blasting them with x-rays, and analyzing the data. "We're really trying to minimize the amount of human intervention necessary," says Earnest.

It's easy to see why. Genome projects around the world are now churning out the DNA sequences that code for proteins by the tens of thousands every year. The Human Genome Project alone is set to complete the sequencing of the 80,000 to 100,000 human genes by the end of 2001, and researchers have no idea what most of their proteins do. They will be looking for clues from the molecules' three-dimensional structures.

Getting those structures will be a huge challenge with today's techniques. Researchers must first coax bacteria to overexpress the protein they are interested in, isolate the protein, find the right conditions that cause it to crystallize, and purify the crystals. Next comes the trip to a synchrotron, where the crystals are bombarded with x-rays. If they actually diffract the x-rays in a manner that provides useful information, the data must be collected and analyzed. From start to finish, the whole process can take months or years to determine a single structure; amassing the current tally of some 10,500 structures has taken decades. "You just can't solve enough structures with the normal techniques," says Earnest. "At current rates, it would take decades to get this accomplished."



From form to function. Structure of protein from *Methanococcus jannaschii* helped pin down its role as a molecular switch for chemical reactions.

That's why a variety of labs are looking to robots for help. Earnest, Ray Stevens—a molecular biologist with a joint appointment at the Novartis Institute for Functional Genomics (NIFG) and The Scripps Research Institute in La Jolla, California—and their colleagues are working on a robotic system for loading crystals, checking to see if they diffract, and collecting data that they hope will dramatically boost the pace of work at synchrotron beamlines. Whereas today's top-of-the-line crystallography beamlines can collect complete data sets on two crystals per day, "we should be able to do 10 to 15 data sets, if not more," says Earnest. NIFG researchers are also developing similar robotic systems for the front end of the effort: overexpressing, isolating, and crystallizing proteins.

Meanwhile, on the software side, Peter Kuhn and his colleagues at Stanford, as well as Robert Sweet's team at Brookhaven National Laboratory in Upton, New York, are testing programs to allow users to run the beamline machines from work stations at their home institutions. And Tom Terwilliger of Los Alamos National Lab in New Mexico and his colleagues recently unveiled a new software package called Solve, which quickly converts raw diffraction data into 3D protein structures.

Help is also starting to roll in from funding agencies. This summer the National Institute of General Medical Sciences launched a new $3 million a year program to fund up to six high-throughput crystallography pilot centers to test some of the new technology. In time, researchers should be able to stay at their home institutions and simply send their samples to a synchrotron via overnight mail for processing. Such a strategy won't work with the most complex proteins, which will need extra experimental care. However, says Earnest, "most proteins are amenable to a FedEx approach." **–R.F.S.**

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ments have virtually eliminated the need for additional APS-sized and -priced machines. Indeed, "it's unlikely another one will be built in the near future, if ever," says SSRL's Hodgson. But Hodgson and other synchrotron experts are quick to add that the new technology in no way makes the behemoth machines obsolete. Even with the new technology, the smaller sources cannot produce beams as bright or as tightly focused as those of their big siblings. Winick and others predict that most of the growth in crystallography will take place at the smaller machines, freeing up beamtime on the big synchrotrons for experiments that take full advantage of their capabilities, such as making x-ray movies of proteins in motion. "The bread-and-butter stuff can be done without having to go to the very large machines," says Bordas.

It will take several years before many of the upgrades and new facilities are completed. But if the new generation of magnet technology lives up to its billing, other smaller, cheaper synchrotrons will likely follow. For biologists, that will provide a welcome relief from today's crowds. **–ROBERT F. SERVICE**

CHEMISTRY

Brazil Lobbies for First Nobel

Brazilian scientists hope to convince Swedish colleagues that one of their patriarchs of biodiversity, Otto Richard Gottlieb, deserves a Nobel Prize

RIO DE JANEIRO—Brazil's science community has begun an unusually visible campaign to promote the Nobel Prize candidacy of Otto Richard Gottlieb, a revered figure in that country, for his contributions to the study of biodiversity and plant chemistry. But the effort is a long shot. In addition to the difficulty of touting someone from the developing world working in a relatively obscure field, Gottlieb's advocates are up against the secretive world of the Nobel selection process.

The Royal Swedish Academy of Sciences, which awards the chemistry prize, does not accept unsolicited nominations and does not discuss who is under consideration. But for 3 years running, its chemistry committee, as part of its normal outreach efforts, has invited the Brazilian Chemistry Association to submit the names of worthy candidates. The association has used the opportunity to promote the 79-year-old Gottlieb, born in Czechoslovakia of a Brazilian mother, who moved to Brazil on the eve of World War II.

Prize fighter. Brazil makes a push for chemist Otto Gottlieb.

Gottlieb's candidacy has attracted extensive media attention here, thanks to the efforts of Peter Rudolf Seidl, a chemist at the Federal University of Rio de Janeiro. Earlier this year, Seidl began making the rounds of government offices and scientific societies to build support for Gottlieb's candidacy, which by then had been endorsed by the Brazilian Chemistry Society. With public pressure building, last month the Brazilian Academy of Sciences took the unusual step of throwing its weight behind the effort, although academy officials are still debating how best to communicate that support to their Swedish counterparts, which award Nobel Prizes in physics, economics, and literature, too.

Trained in Brazil, Gottlieb spent decades in the Amazonian rainforests, helping to create a taxonomy system that classifies plants not by their physical characteristics but by the chemicals they produce. Colleagues say he made important contributions to the field of natural products at a time when Brazil was virgin territory to most of the world's chemists. "He played a big role in developing what is now called biodiversity," says Norman Lewis, director of the Institute of Biological Chemistry at Washington State University in Pullman and regional editor for *Phytochemistry*, which is planning a special issue next year in Gottlieb's honor. "He recognized that plants are factories that produce a variety of chemical compounds, depending on their environments."

Before Gottlieb began his work, Seidl says, studying biodiversity was like trying to repair a Swiss watch without understanding how its sophisticated mechanism works: "He provided the theoretical base, quantifying and bringing coherence to the study of this area." Chemistry Nobelist Roald Hoffmann of Cornell University agrees that Gottlieb has made major contributions to several fields. "He is the premier Brazilian organic chemist and one of the world's outstanding phytochemists and biogeochemists as well," says Hoffmann. "His work deserves the highest honors of our profession, including the Nobel Prize."

Gottlieb retired from the University of São Paulo in 1990. But he has transformed the living room of his modest apartment in the Copacabana neighborhood here into a minilaboratory that serves as home base for a dozen researchers, graduate students, librarians, and a secretary working on one project or another. Despite the recent outpouring of media attention to the academy's efforts, Gottlieb maintains a deep humility about his status in the scientific community. "I think there are people who are more clever and capable than I am," he says. "I am a product of this nation, and by this nation I have been more than adequately compensated."

The uphill campaign to capture Brazil's first Nobel Prize has the strong backing of the country's scientific establishment, members of which are speaking out on his behalf. "The nomination of Professor Gottlieb for the Nobel Prize would do his work justice and would be a great distinction for Brazil," says José Israel Vargas, special assistant to the Brazilian president. A former minister for science and technology, Vargas is currently president of the Third World Academy of Sciences (TWAS), which awarded Gottlieb its chemistry prize in 1991. "TWAS supports the nomination of Dr. Gottlieb for the Nobel Prize. And so do I," says its executive director, Mohamed H. A. Hassan.

Gottlieb is also well known for building the country's scientific infrastructure, having seeded the faculties of many of Brazil's leading universities and research institutes with more than 100 of his students. "If there was a popular vote in Brazil for the Nobel, he'd be near the top of the list," says Lewis, who adds that Gottlieb is still going strong thanks to an "insatiable curiosity."

It is not clear that any of this advocacy will make any difference, however. "I am sorry to inform you that most of your questions cannot be answered due to our secrecy rules," says Lennart Eberson, chair of the chemistry committee and a professor at the University of Lund in Sweden, in response to an e-mail query from *Science*. "The only thing I can say is that we every year ask a large number of individuals to nominate candidates for the Nobel Prize. Many nominations are received, and each candidate is evaluated." **–CASSIO LEITE VIEIRA**

Cassio Vieira is a free-lance writer in Rio de Janeiro.

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