X-RAY CRYSTALLOGRAPHY

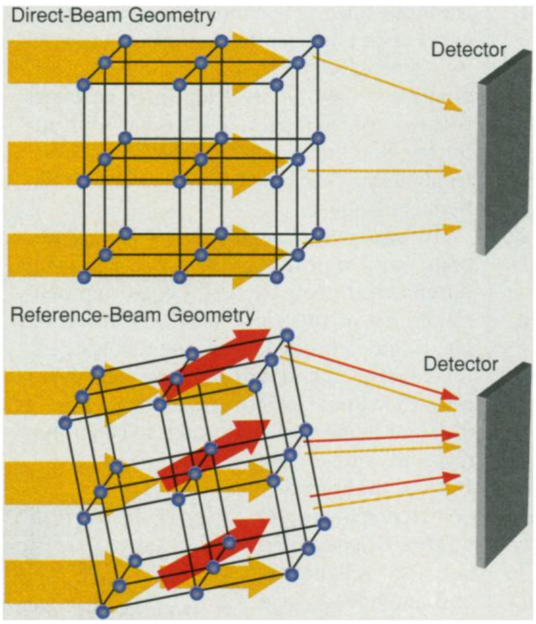
Beam Splitter Teases Out Phase Secrets

X-ray crystallography, the workhorse of structural biology, may soon be hefting a lighter load. The technique is widely used to produce three-dimensional maps of the jumble of atoms that make up proteins and other molecules. Although the technique is enormously powerful—these days it can decipher the precise structure of molecules containing tens of thousands of atoms—it is also immensely laborious. Mapping out a large molecule often requires researchers to add heavy metals to some crystals of the molecule, scatter x-rays off each crystal in many different orientations, and then compare all of the resulting diffraction patterns. The process can take years of work. But now a Cornell University researcher has come up with a new technique that should considerably speed up the process.

Qun Shen, an x-ray physicist at the Cornell High Energy Synchrotron Source in Ithaca, New York, reports in the 13 April *Physical Review Letters (PRL)* that he has developed a new technique that may do away with the need to make heavy-metal versions of the crystal. Shen uses the crystal itself to split the x-ray beam in two, which results in two beams passing through the crystal at slightly different angles. This produces diffraction patterns that provide the data needed to calculate the molecule's structure.

"There is a lot of potential here," says Michael Rossman, an x-ray crystallographer at Purdue University in West Lafayette, Indiana. He adds that the new technique still has a few bugs to work out before it can be widely adopted. Most important, it requires painstaking work to align the crystal in the first place. But if that procedure can be automated, Rossman and others say the technique could substantially speed the work of protein mappers.

The process of diffraction is the key to x-ray crystallography: X-rays ricochet in slightly different directions from different types of atoms depending on the number of electrons they carry. In a conventional setup, x-rays scatter off atoms in the crystal and hit a detector in a characteristic pattern of dots arrayed in concentric ellipses. This diffraction pattern is the result of interference between ricocheting x-ray waves. Where the peaks and troughs of separate waves rise and fall in unison, they add together, producing a bright spot on the detector. Where the peaks and troughs are out of synch, they cancel each other out, leaving the detector blank. The intensity of the spots and the space between them reveal essential information about the distances between atoms in the crystal. But to know exactly where each atom sits relative to its neighbors, researchers must find out the relative positions of the scattered x-rays, known as their phase, which is not recorded by the detectors.



Split vision. Tilting the crystal *(bottom)* splits the x-ray beam and generates diffraction patterns containing phase information.

ILLUSTRATION: P. RIEHN SOURCE: Q. SHEN

One of the most common ways researchers get this information is by making a version of the protein crystals with heavy-metal atoms inserted in the core of each protein. The large number of electrons around these metals deflect x-rays moving through the crystal in a different manner. And by comparing patterns from crystals with and without the metal, researchers can triangulate the position of the metal atom and then work out the positions of other atoms in the structure relative to that point.

Shen's setup extracts the phase information in a very different way. First, he aligns the crystal precisely so that initially the x-ray beam hits the crystal planes at a very narrow angle. Each plane acts like a mirror, reflecting a large portion of the x-rays in one direction, and so producing two beams passing through the crystal at different angles. Both beams are diffracted by the atoms of the crystal, and as they emerge they interfere with each other, creating a diffraction pattern. The key is that this pattern is now sensitive to phase: Slightly changing the tilt of the crystal changes the angle—and hence phase—of the reflected second beam, which in turn alters the intensity of each spot in the final diffraction pattern. "By looking at the interference between the two beams, we can get the relative phase information of the diffracted waves," says Shen. That information can then be plugged into a software program to solve the protein's structure.

In the *PRL* paper, Shen reports using this technique to get the phase information needed to solve the structure of semiconductor materials, which are simple to interpret because they have only a small number of atoms in each unit cell. But he and his Cornell colleagues are now trying the technique on a crystallized protein known as lysozyme. If the experiment succeeds, it could help molecular cartographers draw their maps with ease and speed.

–Robert F. Service

PALEOCLIMATOLOGY

A Dusty Ice Age Trigger Looks Too Weak

A study of interplanetary dust has dealt a setback to an upstart proposal about what drives the ice ages. Most climate researchers believe that a slow redistribution of the sun's heat across the globe, driven by cyclic variations in the shape of Earth's orbit, drives the advance and retreat of ice sheets every 100,000 years. But physicist Richard Muller of the University of California, Berkeley, has argued that another kind of orbital cycle, the changing tilt of the orbital plane, could dip Earth in and out of a cloud of interplanetary dust that could somehow alter climate (*Science*, 11 July 1997, p. 183).

Now, on page 874 of this issue, two planetary scientists report calculations suggesting that dust should indeed fall to Earth in a 100,000-year cycle that matches the ice ages. What drives the cycle, however, is the changing ellipticity of Earth's orbit—the orbital change commonly thought to drive the ice ages—rather than Muller's tilting. And the relatively tiny amount of dust delivered to Earth varies by only a factor of 2 to 3. That's hardly enough to trigger anything so dramatic as an ice age, says geochemist Kenneth Farley of the California Institute of Technology in Pasadena: "It's awfully small to do much of anything."

The tenuous dust cloud that envelops the inner planets is visible to the naked eye on a clear night, but to calculate how much dust may fall to Earth, the orbital behavior of individual dust particles must be known. So Stephen Kortenkamp of the Carnegie Institution of Washington's Department