search & discovery

Soft x rays from a free-electron laser resolve a single, micron-sized structure

An FEL's intense beam can destroy a tiny object in femtoseconds, but not before scattered photons escape with the object's structural plan.

If photons are to map an object's structure, their wavelength must be no bigger than the object's finest features. But the shorter the wavelength, the greater the destructive energy each photon packs. Structural biologists get away with using x rays to map proteins and other biomolecules, but only because countless identical copies of a molecule, when arrayed in a crystal, share the radiation dose.

Unfortunately, many biomolecules don't form crystals (see the story on page 23). And some biomolecules, even when they do crystallize, are available in such minute quantities that the diffraction patterns from their paltry crystals are too blurry to yield accurate structures.

Other structure-finding techniques circumvent the need for crystalline samples, but all have limitations. Nuclear magnetic resonance fails for molecules bigger than the modest limit of 25 kilodaltons; cryo-electron microscopy can't reveal features smaller than 4 Å; atomic force microscopy requires immobilizing molecules on a surface, which distorts their shape.

Now, a multi-institute team has used x rays from a free-electron laser to resolve a single object. Damage did occur. Indeed, the FEL's intense, few-femtosecond pulse vaporized the object. But before the object's demise, enough photons scattered off the object to fill out a diffraction pattern. And, as the team has demonstrated, the diffraction pattern was rich enough to faithfully embody the object's structure.[1]

Henry Chapman of Lawrence Livermore National Laboratory in California and Janos Hajdu of Uppsala University in Sweden led the team, which performed the experiment at the FLASH facility of the German Electron Synchrotron (DESY) outside Hamburg.

FLASH is a soft x-ray laser, the first of its kind in the world. As such, it can't resolve features smaller than a few microns. For the demonstration, Chapman, Hajdu, and their coworkers used a micron-sized picture drawn on a silicon nitride membrane. But in a few years' time, harder, more intense FELs will come online in Europe, Japan, and the US.

"It's a very exciting development," says James Fienup of the University of Rochester. "It has the potential for seeing things that have never been seen before."

Seeing stars

When a beam of coherent photons scatters elastically off an object, the diffracted signal embodies the object's spatial frequency spectrum. Each point in the diffraction pattern corresponds to a particular Fourier component. Transforming the pattern to recover a real-space image is easy in the optical band. A lens suffices. But in the x-ray band, where focusing is more challenging, the only currently available option is to transform the diffracted signal mathematically.

To work, the transformation needs both the diffraction pattern's intensity, which can be detected directly, and its phase, which cannot. Inferring the phase is known as the phase problem. Solving it continues to challenge crystallographers, several of whom earned Nobel prizes for their ingenious solutions. (For recent approaches to the phase problem, see the article by Qun Shen, Quan Hao, and Sol Gruner, PHYSICS TODAY, March 2006, page 46.)

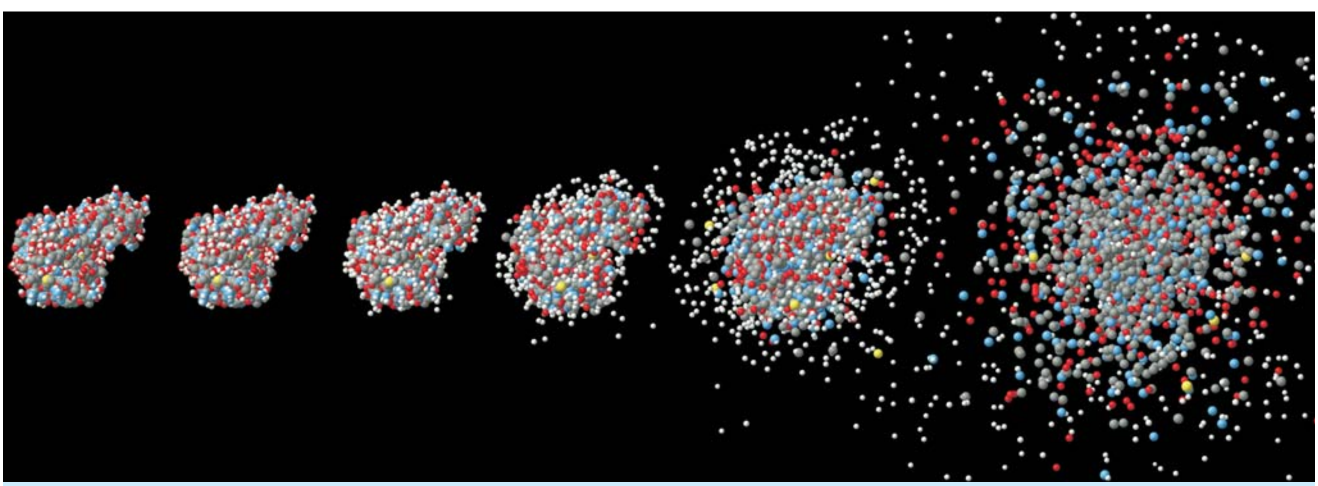

Figure 1. When a brief, intense x-ray pulse strikes a protein, most of the photons scatter elastically off the protein's atoms. The rest ionize the atoms, which leads to a Coulomb explosion. In this simulation, a 2-fs pulse of 12-keV photons encounters lysozyme from the T4 bacteriophage. The atoms are indicated by colors: hydrogen (white), carbon (gray), nitrogen (blue), oxygen (red), sulfur (yellow.) By coincidence, the same protein features in the story on page 23. (Adapted from ref. 3.)

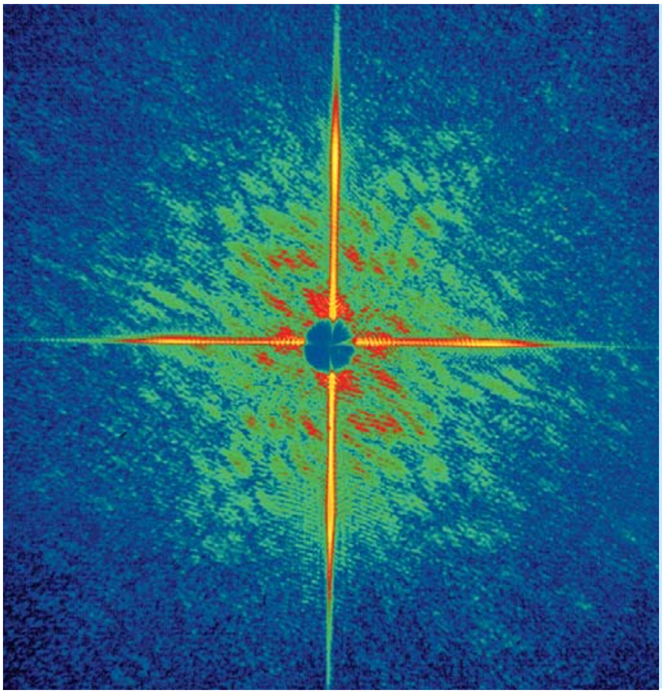

Figure 2. The diffraction pattern from a micron-scale target illuminated by a single, 25-fs pulse of soft x rays. The prominent cross shape corresponds to the target's rectangular frame, whereas the speckling corresponds to the target's structure. The CCD camera captured the entire pattern and detected all the photons. (Adapted from ref. 1.)

A molecule inside a crystal diffracts photons in all directions, but constructive interference from the other, identically arrayed molecules concentrates the signal in a set of Bragg peaks. In 1952 David Sayre realized that the weak signal between the peaks could, in principle, be used to solve the phase problem.[2]

Sayre's idea was impractical at the time. The detected peaks are so bright that they swamp the signal between them. But the diffraction pattern from a single object has a much narrower dynamic range. In the late 1970s Fienup tackled the mathematically equivalent problem of using speckle interferometry to image single stars.

Fienup showed that the real-space image of a single object could be recovered directly, provided one made a simple assumption: That the object is finite and isolated.[3] He applied his method not only to stellar astronomy, but also to resolve the blurry images of spacecraft taken by ground-based telescopes.

In 1998 Sayre and two collaborators from Stony Brook University, Chapman and Jianwei Miao, proposed using Fienup's method to transform the x-ray diffraction patterns from single objects. One year later, the Stony Brook researchers proved the method works. Using 1.7-nm x rays from Brookhaven's National Synchrotron Light Source, they successfully reconstructed the image of a test object, the first six letters of the alphabet drawn on a silicon nitride membrane.[4] Not only were the 1-μm-tall letters resolved, but so were the 30 or so gold particles, 100 nm in diameter, that made up each letter.

Harder photons

To accumulate a usable image, the Stony Brook team bombarded their test object with a trillion photons per square meter for 15 minutes. If administered at the same rate, the hard x rays needed to resolve a protein would be fatal.

But what if the dose were delivered so quickly that the scattered photons left their target before the absorbed photons could destroy it? When SLAC's Linac Coherent Light Source and other FELs turn on in the next few years, they'll be able to deliver the x-ray photons needed to resolve a protein in a few femtoseconds. Can a protein survive such bombardment long enough to produce a structure-yielding pattern?

In 2000, Hajdu and his collaborators simulated case of a protein placed in a hard FEL beam.[5] In their model, elastically scattered photons promptly leave the molecule and fill out the diffraction pattern. The rest of the photons lose energy to the molecule in one of several ways. Some photons knock out inner shell electrons, leading, in the case of light atoms, to the ejection of a second, Auger electron. Some Compton scatter. And some shake off electrons via a recently discovered vibrational mechanism.

Some of the liberated electrons take less than a femtosecond to quit the protein, leaving behind a molecule of positively charged atoms. As figure 1 shows, by about 50 fs, electrostatic repulsion overcomes the atoms' inertia and blows the protein apart in a tiny, light-emitting flash.

X-ray photons interact with the atoms' electrons, not their nuclei. Even before the molecule explodes, absorbed photons perturb the electronic orbitals and alter the paths of any elastically scattered photons that follow. Nevertheless, the simulations predicted that a broad and attainable range of pulse durations, energies, and intensities yields usable diffraction patterns.

Testing

Although the cross sections, lifetimes, and other simulation ingredients are fairly well determined, the simulation itself ran in a regime of high intensity and short wavelength that remains unexplored in the lab. To gauge the feasibility of doing the experiment for real, Chapman, Hajdu, and their team used FLASH, the soft x-ray FEL at DESY. Like the Stony Brook team before them, they imaged a micron-sized picture drawn on a silicon nitride membrane.

FLASH delivers 10^{12} 32-nm photons

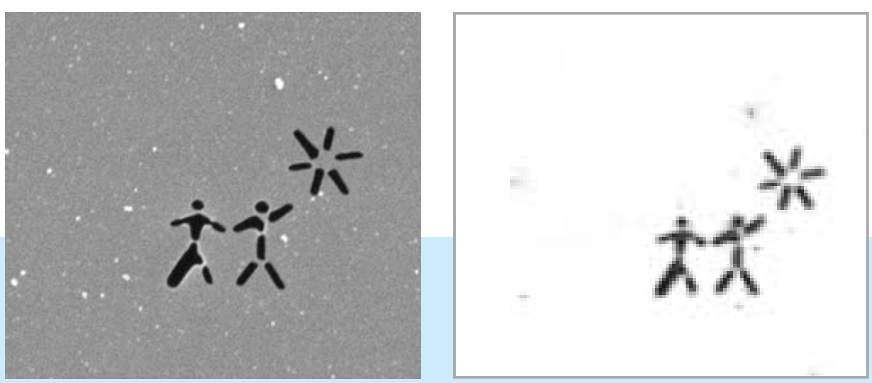

Figure 3. A micron-scale test target was imaged directly by a scanning electron microscope (left) and indirectly by transforming its diffraction pattern (right). Sébastien Boutet of SLAC drew the whimsical image on a silicon nitride membrane using a focused ion beam. (Adapted from ref. 1.)

in 25-fs bursts. To image the diffracted photons, the team used a CCD camera. It recorded every photon, but with a readout time of a few seconds, not a few femtoseconds. The burst of light that accompanies the target's destruction would have been detected too, but was deflected by a multilayer mirror designed by Livermore's Saša Bajt. The mirror is tuned to reflect the beam's photons, which, after elastically scattering off the target, are directed by the mirror toward the CCD. Photons from the explosion don't have the right energy for reflection and miss the CCD. Direct, unscattered photons also miss the CCD; they fly off through a hole in the center of the mirror.

Figure 2 shows the diffraction pattern obtained last year at FLASH, while figure 3 shows both the original test object and its faithful reconstruction. "Without a doubt this is a major milestone," comments Cornell University's Veit Elser. "Up to now the entire enterprise—of using totally destructive imaging events to reconstruct a target—was a fond dream supported by some calculations and simulations."

Graduating from micron-sized membranes to nanometer-sized proteins isn't just a matter of using a harder, brighter beam. Unlike a crystal, a single-protein sample is invisible. But, as Chapman points out, a free, isolated protein would barely move during the experiment's femtosecond time scale. The molecules could be wafted across the beam until one of them gets hit. **Charles Day**

References

1. H. N. Chapman et al., *Nat. Phys.* **2**, 839 (2006).
2. D. Sayre, *Acta Crystallogr.* **5**, 60 (1952).
3. J. R. Fienup, *Opt. Lett.* **3**, 27 (1978).
4. J. Miao, P. Charalambous, J. Kirz, D. Sayre, *Nature* **400**, 342 (1999).
5. R. Neutze, R. Wouts, D. van der Spool, E. Weckert, J. Hajdu, *Nature* **406**, 752 (2000).

High-redshift supernovae indicate that dark energy has been around for 10 billion years

A puzzling dark energy is presumed to be driving the present acceleration of the Hubble expansion. But what was it doing before it became the dominant component of the cosmos?

Since its discovery in 1998, the acceleration of the cosmic Hubble expansion has generally been attributed to some sort of pervasive dark energy that works against the decelerating pull of ordinary gravity. The big question is, What is the nature of that dark energy? Is it simply the unvarying vacuum energy density implied by the cosmological constant Λ that Albert Einstein introduced into general relativity to avoid universal gravitational collapse—and later discarded when the Hubble expansion was discovered? Or is it a more dynamic energy, changing with time as some cosmic scalar field slowly settles into an equilibrium configuration?

A variety of such putative scalar fields have been invoked as so-called quintessence alternatives to Einstein's vacuum energy.[1] One might regard the quintessence scenarios as weak, slow-motion replays of inflation, the primordial scalar-field settling that is thought to have expanded the linear scale of the universe by at least 26 orders of magnitude in its first split second.

The original evidence that the Hubble expansion was speeding up came from the redshifts and luminosities of a few dozen type 1a supernovae, with redshifts $z \equiv \Delta\lambda/\lambda$ up to 0.9, measured by two teams of observers.[2] A type 1a supernova, the thermonuclear explosion of a white dwarf star, serves as an effective standard candle; one can deduce its distance from its apparent brightness and duration. Both teams found that their higher-redshift supernovae were systematically fainter—that is, more distant—than one would expect for a cosmos whose expansion has recently been slowing down, or even coasting. (See the article by Saul Perlmutter in PHYSICS TODAY, April 2003, page 53.)

Looking way back

To explore the nature of the dark energy that now drives the acceleration by overcoming gravitational braking, cosmologists seek to find out how effective it was in much earlier epochs. That means looking for type 1a supernovae at very high redshifts. A supernova observed now with redshift z would have exploded when the linear scale of the cosmos was $1/(1+z)$ of its present size. In cosmology, z often serves as a surrogate for time.

The Higher-Z Supernova Search Team led by Adam Riess of Johns Hopkins University recently reported new data and a new analysis that includes 23 type 1a supernovae with $z > 1$ discovered with the *Hubble Space Telescope* (see figure 1).[3] Earth's atmosphere makes it difficult to find and adequately measure such very distant supernovae with ground-based telescopes. The highest

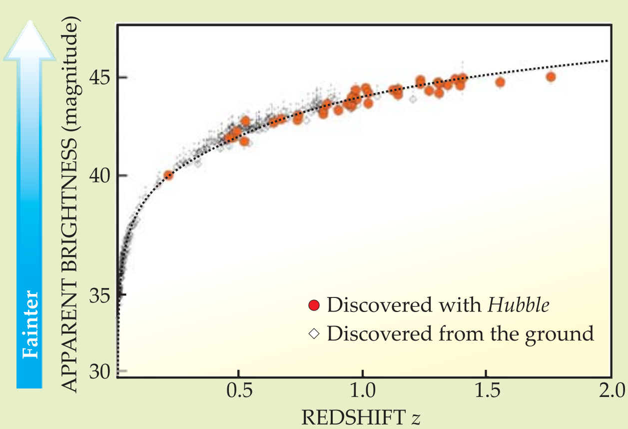

Figure 1. Hubble diagram plotting apparent brightness against redshift for the sample of type 1a supernovae used by the Higher-Z Supernova Search Team to seek evidence, from earlier epochs, of the putative dark energy that now accelerates the cosmic expansion. Particularly useful were the supernovae at redshifts above 1 discovered with the *Hubble Space Telescope*. The curve is a concordance-model fit to the data that assumes the dark energy to be vacuum energy whose density doesn't change as the cosmos expands and now exceeds the mean matter density. (Adapted from ref. 3.)