search & discovery

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

A free electron laser'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 trace 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 the molecule, when arrayed in a crystal, share the radiation dose.

Unfortunately, many biomolecules don't form crystals (see the story on page XX). 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 techniques circumvent the need for crystals, but all have limitations. Solution nuclear magnetic resonance fails for molecules bigger than the modest limit of 25 kilodaltons; cryo electron miscroscopy can't reveal features smaller than 5 angstroms; 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 beam vaporized the object. But in the few femtoseconds before the object's destruction, enough photons scattered off the object to fill out a diffraction pattern. And, as the team has demonstrated, the diffraction pattern was sharp 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 at the German Electron Synchrotron (DESY) outside Hamburg.

FLASH is a soft x ray laser. As such, it can't resolve structures smaller than a few microns. For its demonstration, Chapman and Hajdu's team used a micron-sized picture drawn on a silicon nitride membrane. But in a few years' time, more intense, harder FELs will come online in Europe, Japan, and the US. The era of single molecule diffraction is approaching.

Finite support constraint

When a beam of coherent photons scatters elastically off an object, the diffracted signal takes on the object's shape in Fourier space. Transforming back to real space to recover the shape is easy in the optical band. A lens suffices. But in the x-ray band, where focusing is more challenging, it's more convenient to transform the diffracted signal mathematically.

To work, the transformation needs both the intensity, which can be detected directly, and the phase of the diffraction pattern, 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 clever solutions.

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.

Sayre's idea was impractical at the time. The detected peaks are so bright

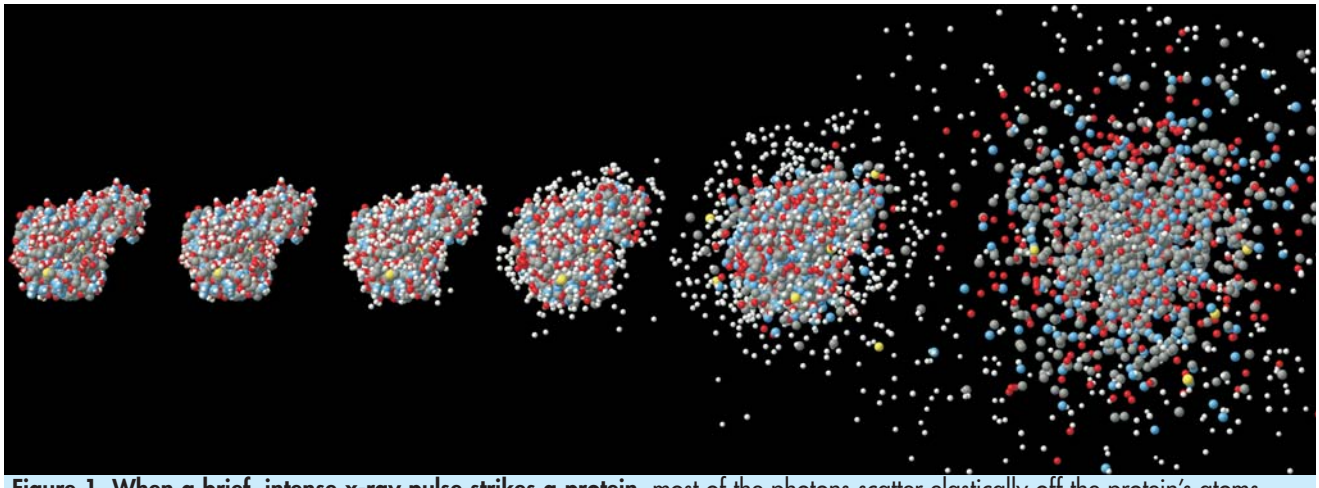

Figure 1. When a brief, intense x-ray pulse strikes a protein, most of the photons scatter elastically off the protein's atoms. The rest ionize the atoms, which leads to a Coulomb explosion. In this simulation, a 2-femtosecond pulse of 12-keV photons encounters lysozyme from the T4 bacteriophage. The 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 XX. (Adapted from ref. 3.)

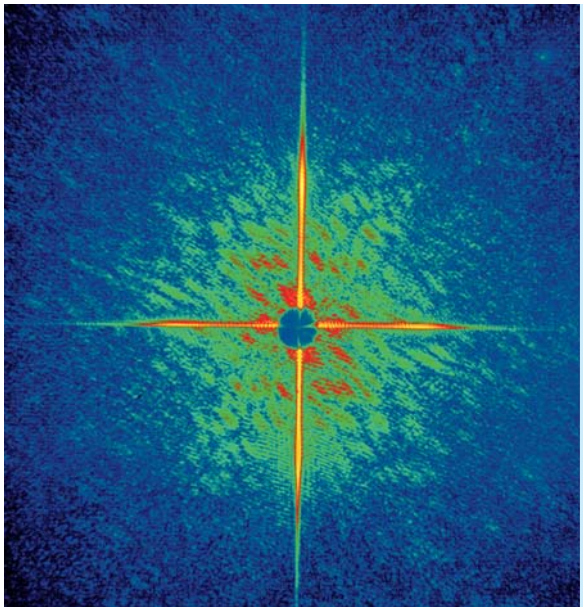

Figure 2. The diffraction pattern from a micron-scale target illuminated by a single, 25-fs pulse of soft x-rays. The prominent cross shape corresponds to the target's rectangular frame, whereas the speckling corresponds to the target's structure. The CCD camera captured the entire pattern and detected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

that they swamp the signal between them. But the diffraction pattern from single object has a much narrower dynamic range. In the 1980s, James Fienup of the University of Rochester adapted Sayre's idea and showed how the real-space image of a single object could be recovered directly from the diffracted signal, provided one made a simple assumption: That the object is finite and isolated.

Fienup applied his method, which he dubbed the finite support constraint algorithm, to resolve the blurry images of spacecraft taken by ground-based telescopes. In a more famous application, he diagnosed the flaw in the *Hubble Space Telescope*'s primary mirror.

In 1998, Sayre and two collaborators from Stony Brook University, Chapman and Jianwei Miao, proposed using Fienup's algorithm 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 the 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.[2] 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 useable 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 destroy its target.

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 hard x-ray FELs turn on in the next few years, they'll be able to deliver 10^{19} photons m^{-2} in femtosecond bursts. Can a protein survive in such a beam 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.[3] In their model, elastically scattered photons promptly leave the molecule and fill out the diffraction pattern. The rest of the photons loose 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.

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.

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 predict that a broad and attainable range of pulse durations, energies, and intensities yields useable 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 field and high frequencies 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 FELs 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 in 25-fs bursts. To image the diffracted photons, the team used a CCD camera. It records every photon, but with a readout time of a few seconds, not a few femotoseconds. The burst of light that accompanies the target's destruction would be detected too, but for a special multilayer mirror designed by Livermore's Saša Bajt. The mirror is tuned to the beam's photons, which, after elastically scattering off the target, are directed by the mirror toward the CCD.

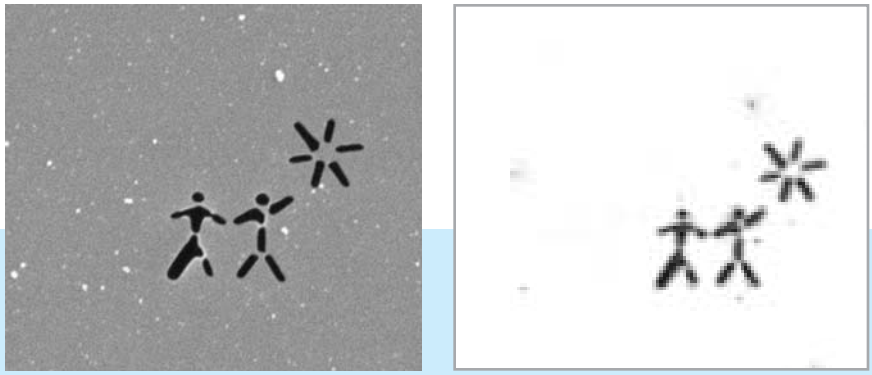

Figure 3. A micron-scale test target was imaged directly by a scanning electron microscope (left) and indirectly by transforming a diffraction pattern (right). Livermore's Sébastien Boutet drew the whimsical image was on a silicon nitride membrane using a focused ion beam. (Adapted from ref. 1.)

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 timescale. The molecules could be wafted across the beam until one of them gets hit.

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References

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2. J. Miao, P. Charalambous, J. Kirz, D. Sayre, *Nature* **400**, 342 (1999).
3. R. Neutze, R. Wouts, D. van der Spool, E. Weckert, J. Hajdu, *Nature* **406**, 752 (2000).