STRUCTURAL BIOLOGY

Diffraction before destruction

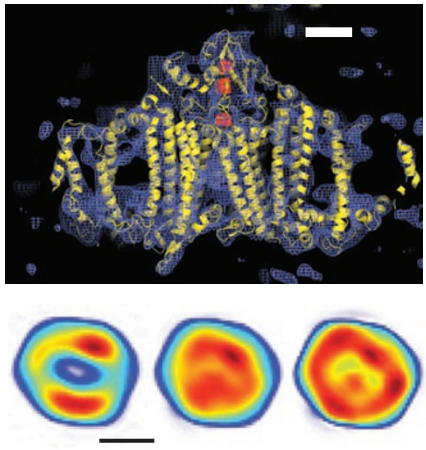
Two recent reports demonstrate the first applications of X-ray free-electron lasers to look at biological structures.

For decades, crystallography has been the best technology for determining the three-dimensional structure of a biological molecule. But although structure determination has become fairly routine for small, well-behaved proteins, many of the most interesting structures, from membrane proteins to large macromolecular assemblies to viruses, remain intractable to the process. The major bottleneck lies in the difficulty in generating large, high-quality crystals—a necessary experimental step that can range from merely challenging to pretty much impossible.

With the construction of ultrabright, ultrafast hard X-ray free-electron lasers (XFELs), crystallographers may be able avoid the headaches associated with trying to grow good crystals. A large collaborative group of researchers now shows that these powerful instruments allow X-ray diffraction analysis of nanocrystals of a large protein complex (Chapman *et al.*, 2011) and of an uncrystallizable virus (Seibert *et al.*, 2011).

Many protein structures nowadays are solved using data collected at synchrotron X-ray beamlines. In theory, synchrotron radiation should allow high-quality diffraction data to be obtained with smaller crystals, but in practice, the required higher X-ray dose results in extensive damage before the signal can even be recorded. XFELs are a billion times brighter than the best synchrotron beamlines, producing beams that are even more damaging to crystals. However, as the X-rays from these sources are applied in extremely short pulses, theoretically, diffraction data could be collected before the radiation damage to the crystal has time to occur. Janos Hajdu of Uppsala University first reported this idea in 2000.

Hajdu, Henry Chapman of the Center for Free-Electron Laser Science at Deutsches Elektronen Synchrotron (DESY) and the University of Hamburg, and their colleagues had to wait to test the theory, while the instrument allowing ultrashort X-ray pulses was being constructed. The Linac Coherence Light Source (LCLS) at SLAC National Accelerator Laboratory in Menlo Park, California, USA is the world's first hard XFEL; the laser has only been up and running since April 2009. After being granted beam time at the LCLS, the researchers, led by Chapman, tested their hypothesis that ultrashort X-ray pulses could 'outrun' crystal damage (Chapman *et al.*, 2011).



XFEL-based diffraction. Reconstruction of photosystem I from 70-femtosecond X-ray diffraction on the LCLS (top; scale bar, 20 Ångstroms). Single-shot reconstructed images of mimiviruses (bottom; scale bar, 200 nanometers). Reprinted from *Nature*.

Researchers in Petra Fromme's laboratory at Arizona State University generated nanocrystals of the very large membrane protein complex, photosystem I. Unlike growing large crystals, explains Chapman, "it might be possible that nanocrystals can be made quite readily, by driving your protein solution into supersaturation and precipitating out nanocrystals really quickly." They used a novel liquid jet developed in John Spence's lab, also of Arizona State University, to flow a stream of nanocrystals over the X-ray beam. Using rapid-readout X-ray detectors developed at the Max Planck Semiconductor Laboratory, it was possible to record millions of diffraction patterns from individual nanocrystals. With the XFEL diffraction data in hand, the methods for solving the three-dimensional structure are basically the same as what a crystallographer with synchrotron diffraction data would use. The researchers finally reconstructed the photosystem I structure at 8.5-Ångstrom resolution.

In a companion project led by Hajdu, the researchers used the LCLS to obtain high-quality, single-shot diffraction data of the mimivirus particle (Seibert *et al.*, 2011). Mimivirus, so-called because it mimics a microbe, is among the largest known viruses. Its size prevents it from a full three-dimensional reconstruction by cryo-electron microscopy, and the fibers coating the capsid prevent it from being crystallized. The rapid X-ray pulses allowed them to collect quality diffraction data on a stream of aerosolized mimivirus particles sprayed into the beam path, before they were destroyed. The reconstructed mimivirus images at 32 nanometer resolution agreed with previous cryo-electron microscopy findings.

"The biggest challenge," notes Chapman, "was working with these very intense X-ray pulses that melt everything in their path. We had to be very careful that we didn't burn holes in the detector or anything else."

These two reports are necessarily proof-of-principle studies. XFEL instrumentation will need further development to generate the shorter wavelengths, shorter pulses and higher brightness necessary to achieve the high resolution that biologists want. But these investigations give us a taste of the many exciting applications to come, including time-resolved studies of molecular dynamics. And of course, everyone is excited about possible single-molecule structure determination using XFELs. Says Chapman, "every time we do experiments, we are getting closer."

Allison Doerr

RESEARCH PAPERS

Chapman, H.N. *et al.* Femtosecond X-ray protein nanocrystallography. *Nature* **470**, 73–77 (2011).

Seibert, M.M. *et al.* Single mimivirus particles intercepted and imaged with an X-ray laser. *Nature* **470**, 78–81 (2011).