STRUCTURAL BIOLOGY

# Solving XFEL's image problem

**Improved XFEL crystallography data processing methods enable structure determination from limited samples.**

The use of X-ray free-electron lasers (XFELs) for protein structure determination permits the analysis of crystals that are too small or poorly diffracting for synchrotron radiation. Ultrafast XFEL pulses also make it possible to visualize molecular structures before the occurrence of radiation damage. However, a major drawback of XFELs is the need to obtain tens to hundreds of thousands of images to solve a high-resolution structure.

Two teams—led by David Stuart at the University of Oxford (Ginn *et al.*, 2015), and William Weis and Axel Brunger at Stanford University (Uervirojnangkoorn *et al.*, 2015)—now report improved data processing methods that promise to increase the efficiency of structure determination using XFELs.

"In early experiments, they were using literally tens or hundreds milligrams of proteins, which can be an absolute nonstarter for some problems," says Weis. Because the still images of a microcrystal collected using an XFEL contain less information than the diffraction data recorded by rotating a single large crystal during conventional crystallography, greater numbers of images are needed. "That was really the goal of our work: to improve the accuracy of the data and to need much fewer data to solve structures," Weis notes.

In order to solve this problem, the team led by Brunger and Weis adapted classical post-refinement methods to the specific properties of XFEL diffraction data. "With XFEL you get only a partial intensity and you have to figure out what the full intensity equivalent would have been. That's the heart of the problem," says Weis.

Using their new model, and a setup where the crystals are held in place with a goniometer, Brunger and Weis's team determined the structures of two proteins—hydrogenase and myoglobin—using a small number of crystals and as few as 100 images. With a more standard XFEL injector setup where small crystals are streamed through the beam, they also determined the structure of thermolysin to a resolution of 2.1–2.6 angstroms using only 2,000 diffraction images. "We're now pretty convinced that you don't need as much data as people have been collecting," concludes Weis.

Stuart had been thinking along similar lines, hoping to find a better way to analyze diffraction data than the current standard Monte Carlo approach. "Our guess was that if you could get a more accurate orientation matrix, you could be more precise in predicting whether a reflection was present or not," he says. Whereas Weis and Brunger took a global approach using all collected images during refinement, the method developed by Stuart's team considers one image at a time.

With their own algorithm to model partial reflections to their full intensity—developed by graduate student Helen Ginn—Stuart's team succeeded in determining a room-temperature structure of CPV17, the smallest cytoplasmic polyhedrosis virus polyhedra yet characterized. Fewer than 6,000 CPV17 crystals, each barely 1 micrometer in size, were sufficient to obtain the 1.75-angstrom-resolution XFEL structure, thereby demonstrating the power of the approach for addressing a difficult structure that could not be solved using synchrotron radiation.

"It was a difficult molecular replacement solution because we didn't have a good starting model … and so we really needed very good high-resolution data to allow us to be able to refine the structure and get useful phases," says Stuart. Regarding the amount of data needed, Ginn adds, "We could certainly manage with quite a lot less," as she found that completeness of the map approached 100% with about 2,000 images.

Considering the current severely limited access to XFEL beam time, the new data processing approaches developed by the two teams should allow a greater number of experiments to be carried within a given time window. Stuart predicts that "this will have a major impact in the efficiency of use of the [XFEL] machines."

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RESEARCH PAPERS

Ginn, H.M. *et al.* Structure of CPV17 polyhedrin determined by the improved analysis of serial femtosecond crystallographic data. *Nat. Commun.* **6**, 6435 (2015).

Uervirojnangkoorn, M. *et al.* Enabling X-ray free electron laser crystallography for challenging biological systems from a limited number of crystals. *eLife* doi:10.7554/eLife.05421 (17 March 2015).