FREE-ELECTRON LASERS

FLASH microscopy

An ultrafast diffractive imaging technique that reconstructs an object's structure from a single short X-ray pulse is an important step towards the superlative spatial and temporal resolution promised by next-generation free-electron lasers.

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The difficulties in determining the structure of macromolecules that cannot be crystallized — which include the overwhelming majority of protein molecules that are responsible for cross-membrane communication — is one of the key problems faced in structural biology today. The development and impending completion of the next generation of X-ray free-electron lasers (XFELs), which generate intense and extremely short bursts of X-rays, promise to help scientists overcome this problem[1], and greatly improve our ability to study and to design complex organic molecules and nanostructured materials. On page 839 of this issue, Chapman and co-workers[2] demonstrate a diffractive imaging technique that could prove vital to realizing such exciting possibilities. In the process, they set a remarkable new record for the time taken to collect a microscopic X-ray image — just 25 femtoseconds.

The diffraction pattern formed by a beam of X-rays incident on a crystal consists of series of discrete Bragg peaks. Crystallographers routinely collect and analyse these patterns to determine the structure of the molecules from which the crystals form. It is not, however, possible to determine the structure from a single pattern. The diffraction pattern cast by a sample is complex, consisting of both amplitude and phase information, but is usually recorded in terms of its intensity, which represents the absolute square of the complex amplitude. Doing so effectively discards the phase information present in the pattern, which is required for the reconstruction. Several effective techniques have been developed over the course of the past 100 years to try to solve this 'phase problem', but all require additional information.

In contrast to the conventionally produced diffraction pattern of a perfect crystal, a non-crystalline object, if illuminated by coherent X-ray radiation, generates a diffuse diffraction pattern characterized not by discrete Bragg peaks but by a continuous landscape of peaks and troughs, and thereby contains more information. Sayre[3] suggested that this additional information should be sufficient to reconstruct the object's structure exactly. In fact, Sayre, Chapman and Miao showed, using model calculations[4], that the mathematical scheme developed by Fienup[5] and others can be used to reconstruct such a pattern. The first experimental demonstration of this was by Miao *et al.*[6], and the technique has been extended by several groups of researchers to a variety of specimens. In all the cases before the work now reported by Chapman *et al.*[2], the patterns were accumulated using a large number of X-ray pulses, most often produced by third-generation synchrotron light sources.

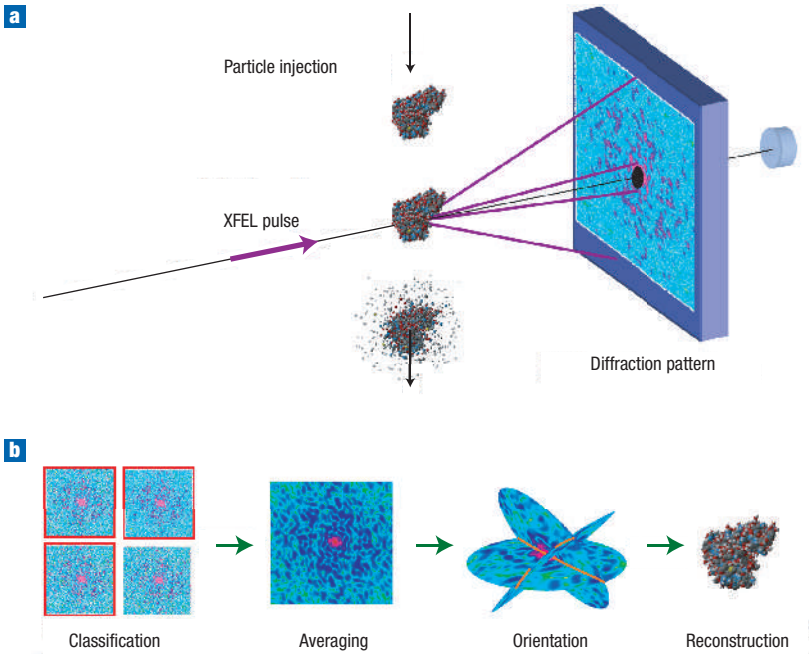

Figure 1 Proposed experiment[8,9] with X-ray free-electron lasers (XFELs) to determine the structure of macromolecules that will not crystallize. **a**, A faint diffraction pattern is captured on the detector before the molecule has a chance to explode. **b**, Millions of such faint patterns are recorded, classified according the orientation of the molecule, averaged, and assembled into a complete three-dimensional data set in reciprocal space. An iterative algorithm is used to obtain the molecular structure from the resulting data set. Reprinted from ref. 8 (copyright (2003) with permission from Elsevier) and from H. Chapman.

To get anywhere close to atomic resolution requires not only short-wavelength radiation, but also a radiation exposure that is many orders of magnitude larger than any single molecule can tolerate. Indeed, conventional macromolecular crystallography is only made possible by the fact that the radiation to which a crystal is exposed is shared among its many molecules. To overcome this, Neutze *et al.*[7] pointed out that with a short-enough exposure it might be possible to record the diffraction pattern of a single molecule before it had a chance to disintegrate. Their calculations, and several more recent ones, suggest that the pulse duration needed to achieve this would be a few femtoseconds, and even then the pattern generated would be too weak to reconstruct. To solve a structure, it will be necessary to expose potentially millions of molecules, sort the patterns according to orientation, combine them into a three-dimensional data set in reciprocal space, and then subject the result to the appropriate reconstruction algorithm[8,9] (see Fig. 1). This scheme works just fine on paper (and in model calculations). Will it work in practice? This is where the next generation of free-electron lasers, and the proof-of-principle demonstrated by Chapman *et al.*[2], step in.

Synchrotrons are capable of producing intense beams of coherent X-rays, but only over relatively long periods of time, of the order of tens of picoseconds. But the next generation of free-electron lasers — which produce X-rays by a process of stimulated radiation from a beam of relativistic electrons — should be able to generate intensities many orders of magnitude greater with pulse lengths of a few tens of femtoseconds, or even less. The work reported by Chapman *et al.*[2] was carried out using the FLASH soft X-ray free-electron laser, which began operation last year at the DESY laboratory in Hamburg. It involved irradiating a pattern, consisting of two micrometre-scale figures etched into a silicon nitride film, with a single 25-fs pulse of 32-nm soft X-rays, the diffraction pattern from which was captured by a high-sensitivity CCD camera. During this pulse, at a power density of 4×10^{13} W cm^{-2}, the sample exploded into a plasma with a temperature of 60,000 K, but not before enough diffraction data were collected to reconstruct its image, making it probably the fastest photograph taken in human history. The success of the reconstruction not only demonstrates that it could be possible to image an object with a single ultrashort X-ray pulse, but also provides independent verification of the spatial and temporal coherence of the output of FLASH, a feature of the source that was expected but by no means guaranteed.

Although the first hard X-ray FEL, the Linac Coherent Light Source at Stanford, will begin operation in just a couple of years, and facilities in Japan and Germany will follow close behind, many hurdles remain before they start to churn out structures at near atomic resolution. Chapman *et al.* demonstrate that within the 25-fs pulse duration, the object does not have a chance to fly apart by more than the limit of resolution, about 60 nm. The object was carefully positioned in the beam, and a single recording was sufficient to create the image. For macromolecules, targeting will be more difficult, the structure will have to be stable to near atomic dimensions during the exposure, and the individual patterns will be weak. Overcoming these hurdles will not be easy, but the potential payoff, especially in terms of the advances it could promote in biology, medicine and materials science, should certainly make the effort worthwhile.

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