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Sources of inspiration

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Abstract

Synchrotrons have long been the preferred X-ray sources for crystallography, but competition has arrived with the advent of X-ray free-electron lasers. A synchrotron expert and an advocate of free-electron lasers discuss the prospects of the respective source types for applications in structural biology.

Sophisticated synchrotrons

SEAN MCSWEENEY

During the 20 years that biological structures have been solved using modern synchrotron sources, the hundreds of thousands of experiments performed have revolutionized the process of determining macromolecular structures. These high-intensity, well-collimated X-ray beams continually drive biologists to try new approaches, pushing our capabilities to reveal ever-larger molecular complexes at atomic resolution. The usefulness of these X-ray beams has also driven a steady rise in the number of crystallographic instruments at synchrotron facilities. Structural biology has thus increasingly been used as a major tool for generating fundamental biological knowledge — much of which has benefited society by aiding the discovery of new drugs.

When third-generation synchrotrons (also known as undulator-based storage rings) began operating in 1994, only two or three crystal structures were being deposited each week in the Protein Data Bank, an international repository for protein structures. Since then, the number of beamlines — the specialist instrumentation that enables light from synchrotrons to be used in experiments — has risen considerably, mostly at third-generation sources. The number of structural biologists has increased in parallel with the ease with which X-ray diffraction revealed natural structures. At present, around 8,000 distinct structures are deposited each year, approximately one per hour. This demonstrates how continual innovation by synchrotron-facility scientists and users has made the existing sources incredibly productive. The list of their achievements is encyclopaedic, and includes the development of: automated sample handling, advanced detectors, improved software, new crystallographic methods and stable X-ray optics that produce microscopic X-ray beams.

These micro-focused X-ray beams may prove to be crucial to structural biologists in the future^{1,2}. Five Nobel prizes have been awarded for work that depended on synchrotron X-ray studies. The most recent of these — the Nobel Prize in Chemistry 2012, which was awarded, in part, for the determination of the structures of G-protein-coupled receptors³ — required the sort of micro-beam that became available only recently. Such beams allowed the delivery of a high flux of X-rays in the tiny volume that was needed to collect crystallographic data from the fragile protein crystals involved.

Further beamline developments will continue until it is possible to truly tune experiments, controlling beam size, shape, flux and wavelength, thereby enabling optimal extraction of information from crystal samples. Storage-ring developments will also continue: the fourth generation of synchrotrons is currently under construction, and will eventually produce flux densities a thousand to a million times higher than those of current state-of-the-art instruments, allowing new experimental approaches and scientific discoveries.

Impressive results from free-electron lasers (FELs) have made some people wonder whether conventional storage-ring sources will continue to have a major role in driving structural biology. I contend that both tools are developing synergistically, and that we are still far from being able to realize the full potential of storage-ring sources in particular. In the next decade, scientists will benefit from synchrotrons even more than they do now, as a result of innovations that are spurred, in part, by FELs. For example, a recent study⁴ reports how intense, micro-focused X-ray beams from a synchrotron, combined with data-analysis techniques previously developed for FEL experiments, have enabled structures to be determined from micrometre-scale crystals (Fig. 1). It is fair to say that the future is bright for synchrotrons in structural biology.

Leading-edge lasers

PETRA FROMME

Free-electron lasers⁵ have opened up a new era in structural biology⁶, for several reasons. For starters, FELs allow structures to be determined from nanometre-scale crystals that contain only a few hundred molecules. These nanocrystals are easier to grow and have fewer defects than the macroscopic crystals used for conventional crystallography.

This is especially helpful for proteins that are difficult to crystallize, such as large complexes and proteins embedded in membranes. Recently, a structure was determined with a FEL using nanocrystals prepared by overexpressing a protein in insect cells⁷ (Fig. 1). This method of preparation seems to be applicable to many proteins, and could save years that would otherwise be spent crystallizing proteins using conventional methods.

FELs also overcome one of the main obstacles in crystallography: that proteins are often damaged by conventional X-ray sources. X-ray pulses from FELs are extremely intense and so completely destroy molecules and crystals. But because the pulses have only femtosecond duration (1 femtosecond is 10^{-15} seconds), diffraction patterns can be detected before the molecules are destroyed⁸. This overcomes the size limit for crystals, as noted earlier. It also allows damage-free structures to be determined from radiation-sensitive crystals. This is

especially important for proteins that contain metal centres, which tend to undergo X-ray-induced chemical reduction.

Biomolecules are dynamic, but most crystal structures provide only a static picture of such molecules in one state. By contrast, time-resolved femtosecond crystallography using FELs allows researchers to make ‘molecular movies’ — a series of snapshots — of biomolecules in action. For proteins whose reactions can be triggered by light, X-ray pulses fired at different times after a light trigger enable the structures of different reaction intermediates to be obtained⁹.

Not all protein reactions are light driven, however. Methods are therefore being developed in which rapid mixing of protein nanocrystals with a solution of the protein’s substrate triggers a reaction; X-ray pulses are then fired at the sample at different time intervals after mixing. This should enable all the steps of drug transport through a receptor to be visualized, for example.

The current main limitation of structural biology research with FELs is access to beam time at the two sources in the United States and Japan. But, with the opening of the European FEL and the Swiss FEL in 2015 or 2016, available beam time will increase significantly. Furthermore, the European FEL will allow up to 10,000 images to be collected per second, so that a full data set can be acquired in 5 minutes, rather than the 3 hours required at present.

It is the dream of structural biologists to determine atomic structures from the X-ray diffraction of single molecules, but this is not yet within our grasp. To reach this goal major challenges have to be met: the flux of X-ray photons from FELs must be increased by at least 1,000-fold to detect the weak diffraction of individual biomolecules at atomic resolution. In addition, the duration of pulses may have to be shortened to less than a femtosecond, to allow for diffraction before destruction of single molecules.

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Figure 1. Protein structures from micrometre-sized crystals

The cathepsin B protein of the parasitic microbe *Trypanosoma brucei* is a potential target for drugs to combat sleeping sickness. The crystal structure of the protein (grey) in complex with an inactivating peptide (multicoloured) was first determined⁷ from micrometre-sized crystals grown *in vivo*, using X-rays from a free-electron laser (FEL). The structure has since been validated⁴ on a synchrotron using a method inspired by techniques developed for FELs.