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Mini-Review

X-ray crystallography and its impact on understanding bacterial cell wall remodeling processes

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ABSTRACT

The molecular structure of matter defines its properties and function. This is especially true for biological macromolecules such as proteins, which participate in virtually all biochemical processes. A three dimensional structural model of a protein is thus essential for the detailed understanding of its physiological function and the characterization of essential properties such as ligand binding and reaction mechanism. X-ray crystallography is a well-established technique that has been used for many years, but it is still by far the most widely used method for structure determination. A particular strength of this technique is the elucidation of atomic details of molecular interactions, thus providing an invaluable tool for a multitude of scientific projects ranging from the structural classification of macromolecules over the validation of enzymatic mechanisms or the understanding of host–pathogen interactions to structure-guided drug design. In the first part of this review, we describe essential methodological and practical aspects of X-ray crystallography. We provide some pointers that should allow researchers without a background in structural biology to assess the overall quality and reliability of a crystal structure. To highlight its potential, we then survey the impact X-ray crystallography has had on advancing an understanding of a class of enzymes that modify the bacterial cell wall. A substantial number of different bacterial amidase structures have been solved, mostly by X-ray crystallography. Comparison of these structures highlights conserved as well as divergent features. In combination with functional analyses, structural information on these enzymes has therefore proven to be a valuable template not only for understanding their mechanism of catalysis, but also for targeted interference with substrate binding.

X-ray crystallography: principles, possibilities and challenges

Overview

The determination of the crystal structure of diamonds by William Henry Bragg and his son William Lawrence Bragg in 1913 can be viewed as the birth of X-ray crystallography. Thus, this method has only recently turned one hundred years old, and can now look back on a century in which it has often enriched and sometimes revolutionized scientific research. To mark the occasion, the year 2014 has been announced as the "International Year of Crystallography" (iycr2014, 2014) and the journals *Nature* and *Science* have dedicated special issues to review the historical milestones of X-ray crystallography, its achievements, developments, and future prospects (NATURE, 2014; SCIENCE, 2014). The increasing level of automation in the crystallographic pipeline over these last 100 years has led to a tremendous and constantly growing number of structures deposited in the PDB today, the PDB features over 100.000 structures. In combination with the rapid technical evolution of X-ray crystallographic techniques in areas such as membrane protein structure determination or room-temperature structure determination at synchrotrons, as well as the possibilities offered by free electron laser X-ray (XFEL) sources (Garman, 2014), this promises that macromolecular crystallography will continue to have considerable impact in a broad range of scientific research fields in the future.

X-ray crystallography is not a direct imaging technique that focuses visible light scattered from objects through refractive lenses to create a magnified image of the object. Rather, it exploits the fact that X-rays with wavelengths between 0.05 and 5.0 nm are scattered by the electron shells of atoms and thus provide the

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possibility to obtain structural information of molecules at near-atomic resolution. However, due to the refractive index of X-rays in different materials, which is essentially equal and close to unity, it is not possible to obtain direct atomic resolution images of a single protein molecule (or other macromolecule) through simple focusing of scattered X-rays (Rupp, 2010; Sumner, 2014). Nevertheless, diffraction images can be obtained from protein crystals in X-ray diffraction experiments. These images carry information about the content of the crystal's unit cell (the protein of interest), but this information is encoded in intensity distributions of reflections in "reciprocal space" and thus not easily accessible. With the help of Fourier transformations, this information can be "translated" back into molecular "real" space, giving rise to an image of the crystallized molecule. The Fourier transformation is a straightforward mathematical operation that requires two terms as Fourier coefficients: (i) the structure factor amplitudes, which can be obtained from the recorded diffraction spot intensities, and (ii) the relative phase angle corresponding to each observed diffraction spot (Rupp, 2010). Since these phase angles are not directly accessible by experimental methods, they must be obtained in additional so-called phasing approaches, which can involve further experiments or molecular replacement calculations with the help of the phases from related known structures. This is generally known as the "phase problem" in crystallography, and it is one reason X-ray crystallographic structure determinations can remain challenging even today. Once initial phases are determined, a first electron density map can be calculated, and this map provides the basis for molecular model building and structural refinement (Fig. 1).

Experimental approach and challenges

An X-ray crystallographic structure determination requires one or typically several crystals of the molecule of interest. In 1937 James Sumner demonstrated that proteins can be crystallized and must therefore have a regular, ordered structure (Sumner, 1937). Despite efforts to standardize and automate this process, growing crystals remains a major bottleneck in crystallography. Crystal formation requires sufficient amounts of highly pure protein, which is usually obtained using recombinant expression systems in bacteria, yeast, insect cells, or mammalian cell lines. Using these approaches, even challenging proteins carrying post-translational modifications or membrane proteins can often be expressed.

Although the parameters governing the process of protein crystallization are now better understood, it is not yet possible to predict the conditions under which a particular protein will crystallize (Garman, 2014), and thus crystallization conditions have to be screened empirically. Moreover, since diffraction power can vary tremendously from crystal to crystal, several additional rounds of fine-screening and crystal optimization are often necessary to obtain diffraction-quality crystals. Even with the use of robotic platforms that can routinely dispense low-volume drops (as low as 50 nL protein + 50 nL of precipitant solution), this screening typically still requires milligram amounts of pure, homogeneous protein solutions that are not always easy to obtain.

Wavelength (energy) and brilliance (flux) of the X-ray beam itself are also important factors influencing diffraction power and data quality. In some cases, such as in experimental phasing strategies with the help of anomalous scatterers, it is even necessary to record several data sets (see below) at different wavelengths. The evolution of storage ring sources to the currently available third-generation synchrotron sources with tunable wavelengths in conjunction with fast and accurate X-ray detectors has greatly facilitated the performance and efficiency of X-ray crystallography in the last decades. Nowadays, even weakly diffracting or smaller crystals can be used for structure determination (Garman, 2014).

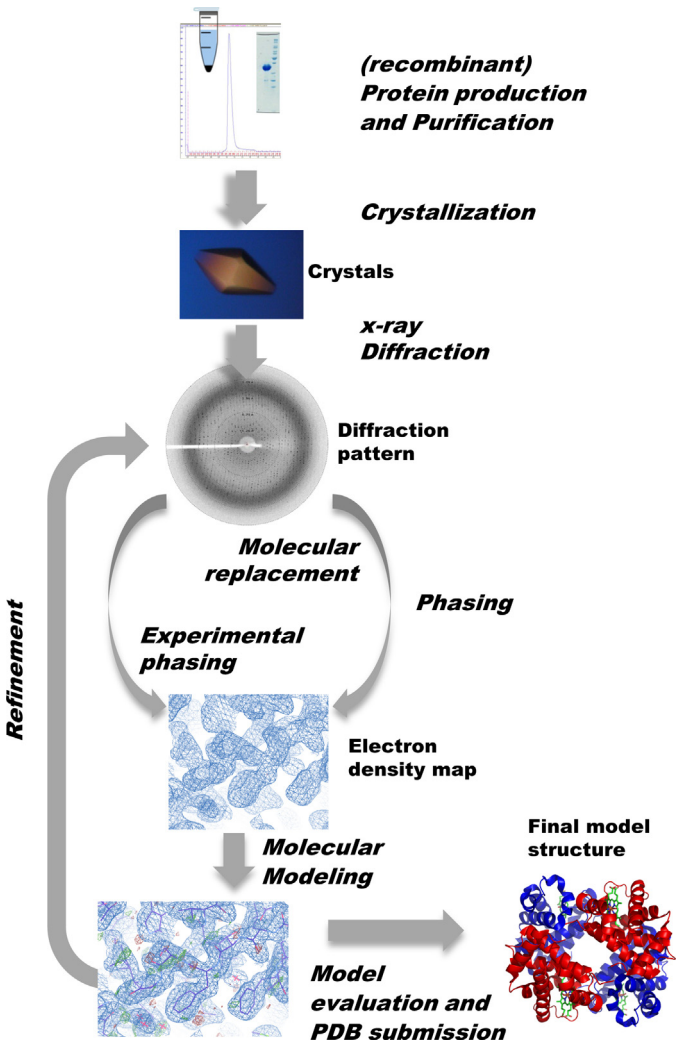

Fig. 1. X-ray crystallographic workflow. Schematic diagram showing the workflow for macromolecular structure determination by X-ray crystallography.

Once X-ray data from a crystal are available, the intensities of the reflections need to be extracted from the data images and processed further. Here, all spots recorded on the diffraction images are indexed according to the crystal's space group, and their intensities are subsequently integrated. The processed data (the "data set") then forms the basis for phase determination. A number of streamlined program packages are available nowadays that can overcome many difficulties in data interpretation and phasing with limited user adjustment. In some cases, crystallographic software packages are even capable of solving structures without human intervention. However, since data processing and phasing have a major impact on the resulting structural information, while leaving room for dramatic misinterpretations at several stages, it is still essential to assess their outputs for biochemical plausibility. Moreover, the automated approaches typically fail when challenging macromolecules, such as large, poorly diffracting complexes or complicated crystal packing arrangements are analyzed. Therefore, the input of human intelligence and experience is still essential.

Evaluating crystal structures

A typical scientist does perhaps not need to understand the intricate details and challenges of molecular structure determination, but he or she must be able to critically evaluate a structure