Chapter 9.2. Robotic crystal loading

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9.2.1. Introduction

The three-dimensional structure of a biological molecule provides fundamental information about how it interacts with other molecules, co-factors and ligands that defines, at a molecular level, how the protein functions in the cellular environment. Protein structure information has also proved to be an invaluable tool in the development of new synthetically derived medicines. The determination of protein structures has significantly accelerated in the past decade, due in large part to advances in protein production (cloning, sequencing, expression and purification), protein crystallography (*e.g.* cryogenic freezing, intense synchrotron X-ray sources, area detectors) and computing. There has been an explosive growth in the use of synchrotron sources for the collection of X-ray diffraction intensities from protein crystals, since synchrotrons can provide bright tunable X-rays which are needed for both high-throughput projects and challenging biomolecular targets such as large complexes and membrane proteins (Wakatsuki & Earnest, 2000).

The convergence of a number of factors in the 1990s and early 2000s motivated the development of robotic crystal mounting – the increase in the number and performance of synchrotron beamlines for biological crystallography, improvements in detector speed, the motorization and computer control of beamlines, advances in computer hardware and software, and an increase in the demand for beam time as structural biologists began to pursue ever more difficult crystallographic projects. Structural genomics efforts and structure-based drug-design programmes benefited significantly, with an increase in the throughput of data collection and analysis for which these systems provided an enabling technology. During the 1990s, the use of cryogenic data collection, instead of mounting in glass capillaries, also allowed synchrotron beamlines to be used more productively, since the bright beams lead very rapidly to the onset of radiation damage unless the crystal is preserved and maintained at low temperatures, typically ∼100–120 K.

9.2.2. Robotic sample loaders

Significant amounts of synchrotron beam time can be lost due to crystal manipulation and misalignment by the beamline user, which becomes more critical with the increase in brightness and stability of the newer synchrotron sources. Crystal-screening and data-collection runs have become significantly shorter. Therefore, more efficient mounting and alignment tools have been developed that reduce the fraction of time spent changing samples, including the implementation of robotic sample loaders (automounters), which can greatly facilitate the throughput and production of crystallography beamlines and other X-ray sources.

There are several benefits to using an automounter system, particularly on bright synchrotron beamlines:

(i) *It facilitates optimum use of synchrotron beam time.* Crystallography experiments are installed in radiation-shielded hutches, which are inaccessible during data collection. Changing the crystal manually requires opening and closing the hutch, initiating the interlocks and performing a hutch search, which typically takes several minutes. The automounter significantly reduces both the sample mounting time and the number of required hutch accesses.

(ii) *It facilitates advanced data-collection techniques.* An experimental station can be fully automated, including integrated data collection and processing whereby the structure-solving software can influence the data-collection process (*e.g.* crystal ranking and data-collection strategy determination). Remote data collection, where the researchers send cryoprotected crystals (maintained near liquid-nitrogen temperatures) to the beamline for robotic mounting and automated (or semi-automated) data collection, can proceed in a flexibly scheduled manner.

(iii) *It facilitates the collection of higher-quality data.* The rapid crystal-interchange mechanism enables the researcher to evaluate a large pool of samples and to select the best crystals from the set.

(iv) *It reduces risk to crystals.* Automated mounting and dismounting of crystals can be done much more reliably than manual handling.

(v) *It facilitates systematic studies of experimental protocols.* Alternative protocols can be performed in a manner and number that would be impractical for humans to perform manually. Furthermore, this can allow for an intelligent system to 'learn' improved methods of data collection and processing.

The major challenge in the design of automation hardware for the mounting and alignment of crystals of biological samples is the necessity to maintain the sample at approximately 100 K, since cryogenic data collection is required at synchrotron beamlines and usually at home sources as well. The process involves the optical screening of several candidate crystals under a microscope, followed by 'freezing' the selected crystal in an amorphous glass formed by the mixing of reservoir and cryoprotectant solutions. In this manner, it is possible to manipulate small or fragile crystals more easily. More importantly, the frozen crystals are far more resistant to X-ray induced radiation damage, a common problem at synchrotron sources. The frozen crystals can then be stored indefinitely in liquid nitrogen and handled with minimum difficulty at cryogenic temperatures. There are established procedures for the manual manipulation and mounting of the crystal while at cryogenic temperatures. At the beamline, the crystals are transferred from the transport container to a small holding Dewar. Next, they are mounted in a secondary sample-transfer tool, and then they are transferred to the X-ray diffractometer. These can be very time-consuming steps when performed manually. Consequently, instrumentation has been developed to minimize the extent of manual manipulations of frozen crystals at the beamline (Snell *et al.*, 2004; Cork *et al.*, 2006) (Fig. 9.2.2.1). Groups at Abbott (Muchmore *et al.*, 2000), at the SSRL (Cohen *et al.*, 2002), at European facilities (Cipriani *et al.*, 2006) and in Japan (Ueno *et al.*, 2006) have similarly produced robotic crystal-mounting systems with a diversity of approaches to achieve the same goal. Commercial

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