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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.

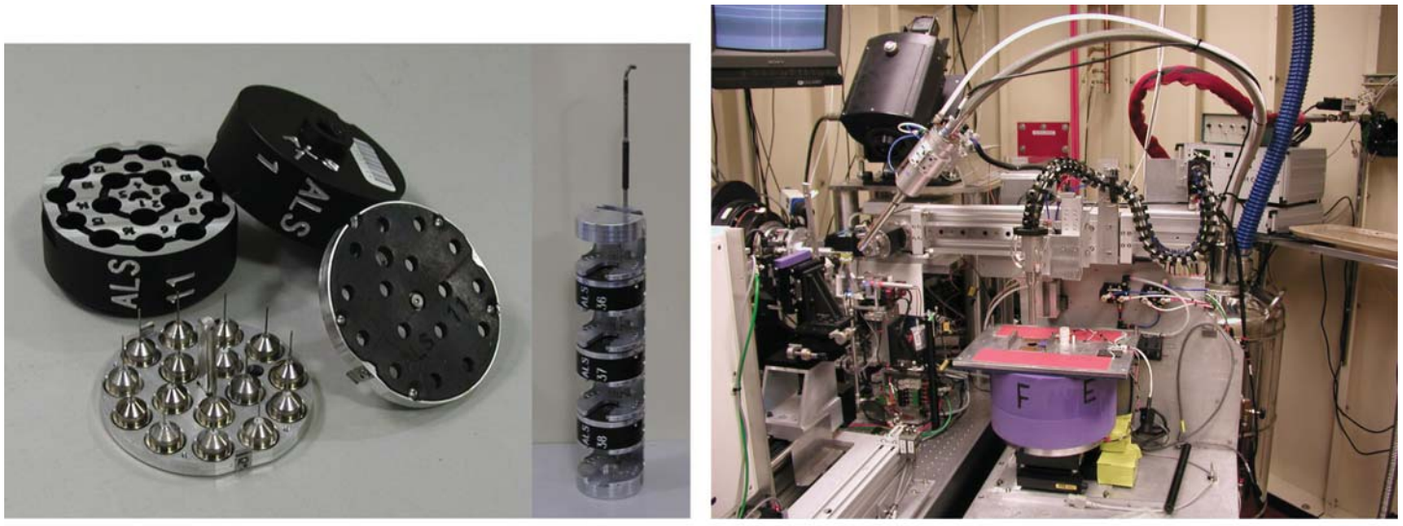
(ii) *It facilitates advanced data-collection techniques.* An experimental station can be fully automated, including integrated data collection and processing whereby the structure-solving software can influence the data-collection process (*e.g.* crystal ranking and data-collection strategy determination). 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



(*a*) (*b*)

Figure 9.2.2.1
(*a*) Hardware for the storage and transport of crystals. Each puck holds up to 16 crystals, and seven can be shipped in a standard cryo-shipper. (*b*) The robotic crystal mounter implemented on Beamline 5.0.2 at the Advanced Light Source.

systems have also been developed (*e.g.* the Rigaku ACTOR and the IRELEC CATS).

There are two basic approaches that have been pursued: (i) the use of commercial multi-axis robots (Muchmore *et al.*, 2000; Cohen *et al.*, 2002; Rigaku ACTOR) and (ii) modular purpose-built systems, such as the one developed at the Lawrence Berkeley Laboratory Advanced Light Source and shown in Fig. 9.2.2.1(*b*) (Snell *et al.*, 2004). All systems must take into consideration that the procedures used to transfer samples from a crystal tray and mount them on the goniometer need to ensure that the crystals be maintained in a frozen frost-free environment and transferred rapidly. For example, the system developed at the Advanced Light Source can be adapted for use with a variety of experimental station configurations. The only significant constraint in the use of this design is that the nitrogen cold stream subsystem must be oriented off-axis from the primary goniometer axis. The first automounter was installed on experimental station BL5.0.3 at the Advanced Light Source, a fixed wavelength station with significant access constraints. This system has been in operation since March 2001. Shortly thereafter, systems were installed on a second monochromatic experimental station, BL5.0.1, and also on a multiwavelength experimental station, BL5.0.2 (Snell *et al.*, 2004). The automounter configuration is essentially identical for all three experimental stations with a high degree of standardization. The sample-mounting robot consists of three main components: the gripper, which holds the sample during transport from the Dewar to the goniometer while keeping it at low temperature and frost-free; the X–Y–θ stage, which performs the actual transport; and the Dewar stage, which supports the samples in a regular array submerged in liquid nitrogen and positions the selected sample for access by the transporter (Fig. 9.2.2.1*b*).

The gripper contains a conically shaped brass collet that can be opened and closed using a small pneumatic actuator (Fig. 9.2.2.2). The collet is precooled to ∼100 K before engaging the sample holder. A sensor inside the collet is used to monitor its temperature. The inner tube is surrounded by a low thermal capacity outer shroud (a very thin stainless steel tube), which provides a sheath flow of warm dry gas to reduce icing and frost formation during exposure to ambient moisture-laden air. During continuous use, ice is periodically removed from the gripper by means of an air-jet heater (*cf.* Fig 9.2.2.1*b*). Additionally, the gripper has a small low-force linear stage (labelled 'small move' in Fig. 9.2.2.2). This stage is used for the final positioning of the gripper on the sample during mounting and dismounting. The sample gripper was originally designed around the standard Hampton metal cap, which is held on the goniometer by a permanent magnet. A modified cap design, which has tighter tolerances and a conical shape rather than a ledge, has been

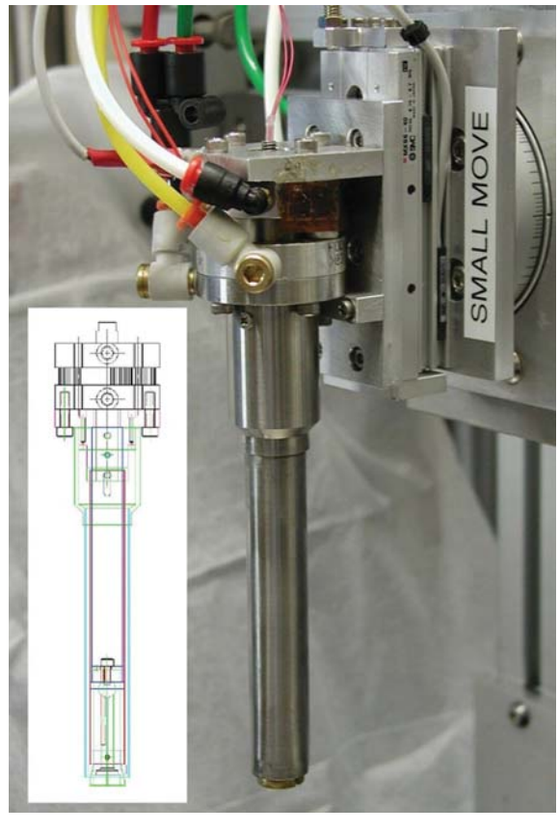


Figure 9.2.2.2
Crystal gripper assembly with low-force actuator (labelled 'small move').

designed to increase automounter reliability (also available from Hampton Research as part No. HR4-779).

The gripper is mounted on a pneumatic X–Y–θ stage, which is used to transport samples between the goniometer and the storage Dewar. A vertical Y stage moves the gripper in and out of the Dewar, a 90° rotational θ stage orients the gripper either horizontally or vertically, and a long horizontal X stage moves the gripper between the Dewar and the goniometer mount points.

When they are not mounted on the goniometer for data collection, the samples are maintained in a small cylindrical Dewar (see Fig. 9.2.2.1*b*). A custom cassette or puck (Fig. 9.2.2.1*a*) facilitates automated handling and bulk transport. Up to seven cassettes (112 samples) can be loaded into the sample Dewar. The Dewar is mounted on an R–θ motorized stage, which is used to position the selected sample for access by the gripper. The Dewar can also be positioned such that the gripper will be inserted into an unoccupied space for a precooling action when boiling of liquid nitrogen might endanger the samples. The Dewar is automatically filled directly from the facility's liquid-nitrogen supply system. During normal operations, an insulated and heated cover reduces icing and evaporation of liquid nitrogen. The gripper reaches into the Dewar through a small heated hole in the cover. The sample cassettes are moved in or out of the Dewar through a small access port in the cover.

The Robosync web site (http://smb.slac.stanford.edu/robosync/), maintained by the SSRL Structural Molecular Biology Group, features a list of biological crystallography beamlines with automated crystal mounting systems.

Important for the efficient and productive use of robotic crystal mounters is their integration into the beamline control system (McPhillips *et al.*, 2002; Snell *et al.*, 2004). The different motions of the automounter can typically be actuated from a software interface, either individually or through scripts which run a sequence of actions. The development of more fully automated capabilities requires the integration of the automounter and beamline control system with the data collection and analysis software. To this end, software has been developed that provides a framework for indexing integration and symmetry determination, along with suggested strategies for data collection (González *et al.*, 2008). When coupled with bright synchrotron beamlines and automounters, the underlying requirements for building fully automated and optimized data collection are mostly in place. The development of intelligent automated control (through *e.g.* artificial-intelligence approaches) should permit fully automated data-collection facilities to collect optimized data from a wide range of structural projects in the most efficient and cost-effective way and with minimal human intervention prior to the scientific analysis of the structures obtained.

9.2.3. Conclusion

In summary, the development of automounters over the past decade has been driven by the demands of greater access to synchrotron resources, the need for high-throughput data collection from projects that require numerous structures (*e.g.* structure-based drug-design and structural genomics programmes) and the demonstrated improvement in data quality for more challenging projects (such as large complexes and membrane proteins) that require the screening of numerous crystals in order to select those with the best diffraction characteristics. A number of different designs have been implemented at synchrotron sources worldwide, to the significant advantage of the structural biology community.

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