

AUTOMATION OF THE MACROMOLECULAR CRYSTALLOGRAPHY BEAMLINES AT THE ESRF

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Macromolecular crystallography is now a routine tool in academia and industry alike. Automation is required to maximise throughput on the available beamlines and to cope sensibly with the increasing influx of measurements.

Since the first ESRF beamline for macromolecular crystallography, ID2B, came on-line in September 1994, macromolecular crystallography has developed into a standard tool for scientists in academia and industry alike. It now has a routine role in the drug development cycle and is an intrinsic component of the burgeoning structural genomics field. In order to maintain pace with this explosive growth and augment throughput of generic experiments, such as ligand soaking and <100 kDa structure solution, the beamlines must become automated, reliable and easy to use. Automation will clear the path for the challenging long-term projects requiring many shifts of beam time that will follow structural genomics: the membrane proteins, the functional studies and, perhaps most crucially, the study of complexes of proteins and macromolecules that is central to the understanding of their real-life interactions.

Beamline	Detector	Wavelength range (Å)
ID14-1	165mm MAR CCD	Fixed: 0.93
ID14-2	ADSC Q4 Matrix CCD	Fixed: 0.93
ID14-3	165mm MAR CCD	Fixed: 0.93
ID14-4	ADSC Q4R Matrix CCD	MAD: routinely* 0.94 - 1.15
ID-29	ADSC210 Matrix CCD	MAD: routinely* 0.80 - 2.1

*other wavelengths possible by prior arrangement.

requiring improved efficiency and throughput.

GOAL: GENERIC HIGH THROUGHPUT

Crystallographic data collection represents only a small time slice on the structural genomics pathway, but automation at the beamline is not only concerned with this facet of the process. Beamlines increasingly resemble factory assembly lines, with the goal of automation to streamline the entire structure solution process from

sample mounting, alignment and collection strategy through to data processing, reduction, phasing and even initial 3-D model construction (Figure 1). To accomplish this will require a close collaboration between instrumentationalists and software engineers.

Automation also goes to the heart of the beamline, in the optics hutch. It is of little use automating the experiment if the underlying optical elements are not optimised and reliable as possible. The suite of JSBG beamlines will be made consistent in terms of software and

THE CURRENT BEAMLINES

Five JSBG (Joint Structural Biology Group) beamlines are available for macromolecular crystallography: the ID14 complex with four end-stations and ID29 which, since the end of 2000, has superseded BM14 (now an Anglo-Spanish CRG line). Every six months these beamlines handle over five hundred projects and demand is on the increase,

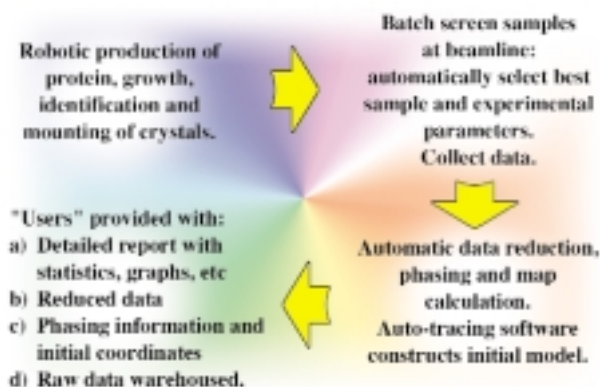
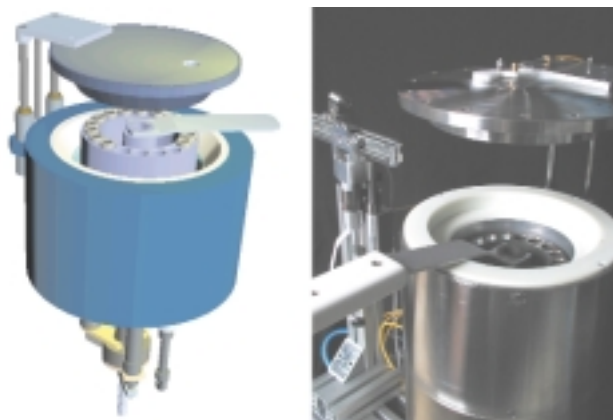


Fig. 1: Target protein to initial 3-D model for generic structure solution.

Fig. 2: (Left) schematic of sample changer cassette and cold box. (Right) the complete changer unit ready to move to a beamline.



hardware, with built-in diagnostic tools to allow rapid alignment or intervention in the event of a breakdown.

Optics hutch and experiment automation together will reduce human error: tired users in the small hours can make unwise decisions, deteriorating their data quality and eventual structural information or indeed the beamline itself.

IN THE PIPELINE: HARDWARE

Plans are already underway to use ID14-3 as a test-bed for beamline automation. New equipment will be installed on the line over the coming months and then rolled out over the other stations once thoroughly tested and proven. The Automation Task Force have designs in hand and the first steps will be taken shortly with particular regard to the beamline optics where automatic alignment tools and instrumentation will be added.

For the experimental hutch, the Joint Instrumentation Group (JIG) of the ESRF and EMBL has developed an automated sample changer. Currently, user groups can take many minutes to retrieve, mount and align a pre-frozen sample, with this process becoming harder as average sample sizes drop from 200 μm several years ago to around 80 μm now. The sample changer is under final bench checks and will soon move to a beamline for on-line testing. The current device, the design of which is extendable, can hold up to twenty samples in Hampton Research cryo-vials (Figure 2) and is simple in conception as well as robust, compact and rapid. With the footprint of a dry-transport dewar and standing lower, it can place or remove a sample in under two seconds on the experiment

goniometer. Once mounted, automatic alignment routines can precisely centre the sample for data collection. Work on this aspect is underway, though pattern recognition of the cryo-cooled crystal in supporting loop will not be simple.

The changer is compatible with a microdiffractometer, also developed by the JIG, and already used for scheduled experiments on the ID13 and ID14 lines much to the delight of users. The microdiffractometer can work with samples down to 5 μm in size and allows remote accurate centring in small beams. A commercialised version is under development.

IN THE PIPELINE: SOFTWARE

With the revolution in data collection speeds, the ergonomics and ease of use of a beamline have become paramount. To this end, the ProDC interface, the user's beamline communication and control centre, is now installed on all beamlines providing a unified interface no matter which detector or equipment is installed. The software takes care of routine data collection tasks, such as safety shutter opening, beam loss checks, auto beam realign and one button edge scans for anomalous diffraction data.

The beamline software also interacts with databases in order to store experiment and beamline parameters. This can allow subsequent automated data processing using stored parameters either on-site or remotely through web-based interfaces, and also the recovery of known beamline configurations in the event of failures. These web interfaces are going through the second generation of development using the ZOPE environment and eventually users can

expect to use the interfaces to query databases with questions such as:

- What did I collect around 28 Feb 2001?
- Can I take a look at the images?
- I'd like to process the data using software X and script Y.
- What are the data processing statistics?
- Can I see the Pattersons?
- Can I trace a 3-D structure?

These questions highlight the advantages and flexibility of a database environment, in comparison to complementary information storage in the headers of image files, where parameters and results can be changed or added at a later date.

THE FUTURE

The beamlines are continuously evolving and projects, such as the automated sample mounting and alignment, are underway to further advance beamline efficiency. The JSBG is aiming for a fully automated beamline to be operational in two to three years' time. In the future, user presence may not be necessary for all measurements with samples routinely sent to beamlines, handled by robots and the processed data (or initial coordinates) sent electronically back to the research groups at home.

WHAT COULD BE DONE?

Assuming one useful structure data set (MAD, SAD, ligand soak, etc.) per hour, an optimised suite of JSBG beamlines could produce 125 structures per day or 25,000 per year. Multiply this by the European synchrotrons and their macromolecular crystallography resources and over 100,000 structures could be produced per year. Even if only one quarter of this total were to be realised, the input of structural information to world databases would be extraordinary. Imagine what could be achieved with that amount of knowledge! ■

Further Information

The JSBG is a joint ESRF and EMBL team of technicians, engineers and scientists operating and maintaining the JSBG beamlines, together with technical developments and in-house research.

(http://www.esrf.fr/exp_facilities/jsbg/jsbg_beamlines.html)