There and back again: how does a sample's adventure unfold at Diamond?

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This article looks at some of the processes and operations behind sample testing at the UK's national synchrotron facility

There's no such thing as a typical day at Diamond, or a typical sample. Scientists from all over the world use this light source facility for critical experiments that cannot be undertaken anywhere else, enabling them to make huge leaps forward in their research in chemistry, physics and material sciences, life sciences, environmental and Earth science, as well as cultural heritage. On any given day, the intense light focused into Diamond's beamlines could be shining on samples as varied as rechargeable batteries, disease proteins and dinosaur bones.

In life sciences, Macromolecular Crystallography (MX) has for decades been a crucial tool for academic researchers and pharmaceutical companies. MX reveals the shape and arrangement of biological molecules at atomic resolution, providing a highly accurate insight into their function.

This core activity at Diamond, offering seven dedicated beamlines has enabled its dedicated group to move the goalposts of what is feasible for conventional MX and develop techniques and beamlines that can take MX to the next level. Great strides have been made in automation creating high-throughput beamlines that can offer results in minutes. Many experiments, can now also be carried out remotely reducing the need for users to travel and allowing science to continue despite the Covid-19 pandemic.

Transfer block

Sending samples for remote experiments

How does this work? Before anything can take place, researchers need to apply for beamtime; because of high demand for this rare and expensive facility, which is funded by UK taxpayers, Diamond goes to great lengths to ensure the process is fair. So, all potential users must participate in a highly competitive, peer reviewed application process which is open to UK, EU and international scientists. The facility is free at the point of access except for commercial use.

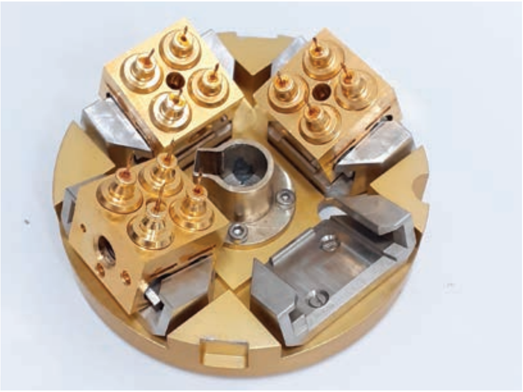
Once beamtime has been granted the researchers need to decide how they will send their samples, the majority of which, for MX investigations, will be protein crystals. This can be a complex process as the samples typically travel deep-frozen in liquid nitrogen which requires highly specialised techniques to achieve good preservation and special shipping systems able to transport the samples safely.

So, a sample's journey starts in the user's home laboratory. Users register each sample they wish to send with Diamond's User Administration System, via which the facility's Safety group checks whether they include any hazardous materials that may require extra safety precautions.

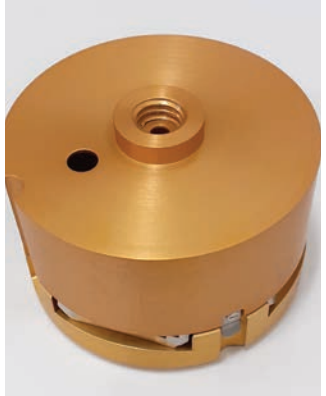
In the user lab, each sample crystal is 'fished' onto the tiny nylon loop at the top of a standardised pin. Each pin is plunged into a Unipuck, which can hold 16 samples, in liquid nitrogen. It's up to the user how many pins they send, but seven Unipucks fit into a standard shipping Dewar, pre-cooled by liquid nitrogen.

Each Diamond user has a login to the ISPyB database, accessed via a web browser. The 'Information System for Protein Crystallography Beamline' is a joint development between Diamond Light Source, the European Synchrotron Radiation Facility, BM14 (e-HTPX) and the EU-funded SPINE project.

The user logs every sample pin to ISPyB, including details about the sample and its location in the Dewar. The user can then simply click on a link within ISPyB to arrange for Diamond's courier to collect the Dewar and deliver it to the Harwell site (and for UK and European academic users, it doesn't cost them a penny).



Block Puck Base



Block Puck Base and Lid

On arrival Dewars are booked into ISPyB by the staff in Goods Handling and collected by Experimental Hall Co-ordinators (EHCs) who, as part of their role, move them into Diamonds storage system. EHCs provide a 24-hour service, ensuring the smooth running of the Synchrotron Building.

Automated sample parking

Diamond's Dewar storage system is like automated parking for samples. It's two storeys high and can hold about 100 Dewars at a time. EHCs or Goods Handling scan each Dewar into the storage system and are responsible for topping up the liquid nitrogen coolant where necessary. While some Dewars may only stay in the storage system for a few hours, they can be kept 'on ice' for weeks if necessary.

The ISPyB database has a section called 'Experiment Kind', which allows users to select the 'recipe' - data collection parameters - for their sample from a predefined list.

For Diamond MX beamlines I03, I04, I04-1, I24, staff check which samples in storage are suitable to be run remotely or automatically in unattended data collection (UDC) mode. They then collect the relevant Dewars from storage, scanning them out. Each puck is then transferred from the shipping Dewar into a special experiment Dewar on the beamline. The beamline Dewar can hold 37 pucks and is designed for robot access. Each puck is carefully scanned into the database to register its position in the experiment Dewar.

At this point, the user is notified that their samples have been loaded onto the beamline for an automatic UDC experiment, or at their scheduled remote visit time they connect to the beamline from anywhere in the world and run the experiment themselves. They can view their experiments progress through ISPyB in real-time if they so choose. A photo is taken when the robot removes the sample from the Dewar, followed by an image of the experiment taking place.

For UDC experiments when the sample is placed in the experimental environment, the beamline uses X-rays to locate and centre the sample automatically before running the experiment with the user specified request. Each diffraction experiment takes two to three minutes, with the robot taking around 15 seconds to swap out the sample for a new one.

Combipuck base with I23 sample holders and combipuck lid

Once an individual experiment is complete, Diamond's computer cluster starts processing the data, which can take between two and thirty minutes, depending on how much data is being processed at the same time. If the user has provided extra data about the nature of the sample, Diamond can do more data processing for them; with good quality data this can often result in automatic structure solution, with results available to access.

The user can access the raw data and the processed data through ISPyB. The raw data is several gigabytes and may not be needed unless the experiment data is later published. The processed data is only megabytes and much easier for users to handle on their local systems.

When the experiment run has finished, the beamline staff unload the samples back into their shipping Dewars and scan them back into the storage system. This notifies the user, who can arrange for their safe return, again through ISPyB.

In-vacuum MX

This procedure for shipping samples to Diamond assumes that the experiment will be carried out in air, which is typical for MX. However, beamline I23 is very different. I23 is currently the only beamline in the world that can run MX experiments in a vacuum.

I23 is designed for long-wavelength MX, which exploits the anomalous scattering properties of elements such as sulphur, phosphorus, potassium, chlorine or calcium. This technique allows researchers to solve the structure of proteins and nucleic acids without the need for additional labelling and identify the locations of these light elements. As X-rays at these wavelengths are strongly absorbed by air, the experiments need to be carried out in a vacuum. This makes it more challenging to keep samples cold and transfer them onto the beamline.

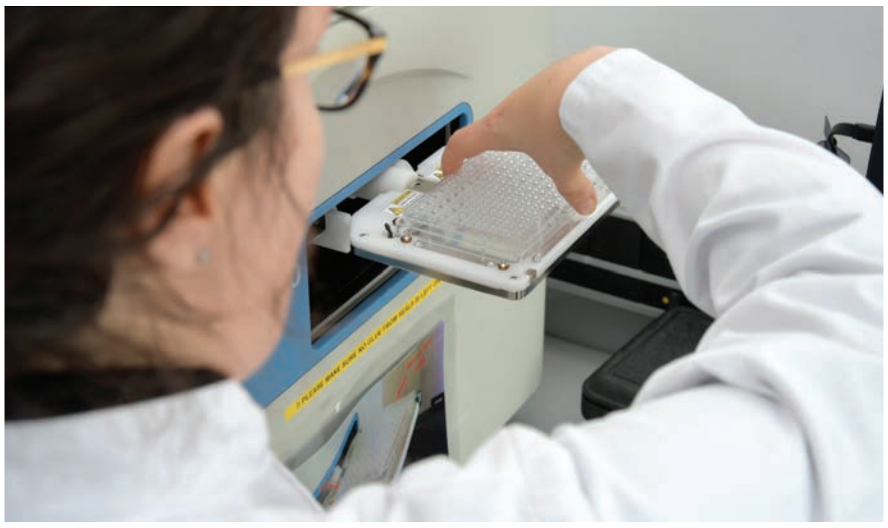
To make life simple for users, the beamline staff on I23 designed a process for sample submission that is as similar to that for standard MX as possible, and uses, apart from the dedicated sample holders which are available on request, only commercially available equipment.

In place of the standard pins, the sample holders for I23 have a thermally conductive copper pin and body, and a strong magnet base. An adaptor is used to make them compatible for shipping, and also means that the samples can be pre-screened on other beamlines. Combipucks are used in place of Unipucks for shipping, and can be loaned out on request.

I23 sample holder with adaptor

123 sample holder

Samples for I23 are logged into ISPyB, shipped to Diamond, and placed into the automated storage area. In the next step the sample holders are removed from the Combipucks and separated from their adaptors. The sample holders are then placed into special transfer blocks, each holding four samples with excellent thermal conductivity and significant thermal mass. These operations are performed in liquid nitrogen, to keep the samples cold. Four transfer blocks are then loaded onto a block puck used to transfer samples to the Cryogenic Transfer System (CTS). Designed and built in-house, the CTS acts as a loading station, shuttle and airlock, taking samples from liquid nitrogen into the vacuum chamber. Once an experiment is complete, the system runs in reverse, returning samples to the user.



96 well plate of crystals being placed in the Echo Acoustic dispenser for dispensing of fragments

96 well plate of crystals in the crystal shifter ready for fishing crystals with a loop mounted on a pin

The pins with the mounted crystals are plunged into liquid nitrogen and placed into pucks

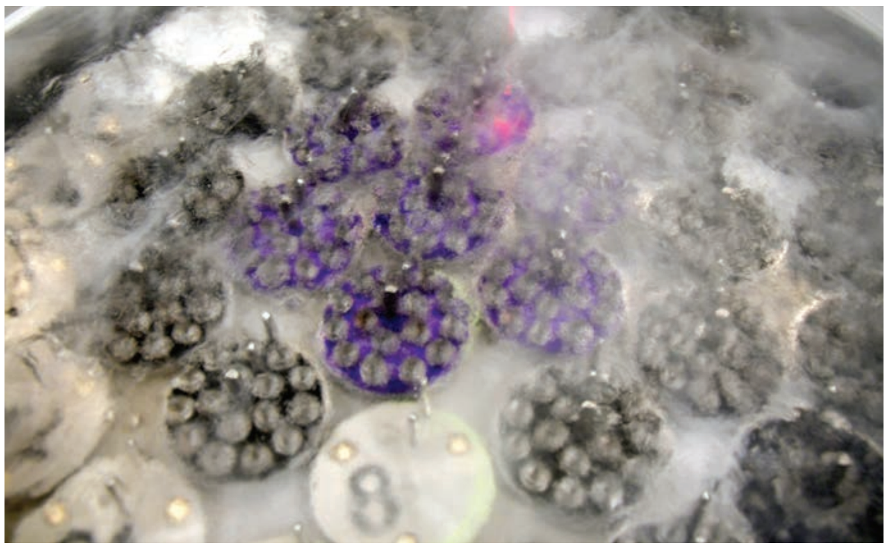
The picks containing 16 mounted pins each are sealed and placed into storage racks in the liquid nitrogen dewar ready to go to the beamline

Fragment screening

When it comes to fragment screening, things are different again.

Most drugs work due to a chemical interaction between the medicine compound and a protein in the body. In fragment screening, researchers test whether tiny fragments of compounds are connecting to their target protein. It's possible to screen fragments much more quickly and easily than larger compounds, and fragment screening has become well-established as a powerful approach to early drug ('lead') discovery.

In partnership with the University of Oxford, Diamond developed the XChem fragment-screening facility which uses a highly streamlined process developed around beamline I04-1 to screen up to 1000 compounds in less than a week. Pre-Covid, users would arrive at Diamond with their samples and use the onsite lab to soak their protein crystals in fragment solutions (Diamond has fragment libraries available, or users can bring their own). Users would then fish their soaked crystals onto pins assisted by a semi-automated Shifter microscope stage, after which they could be left in the capable hands of the beamline staff to be loaded onto the beamline. During the pandemic, the soaking and sample preparation is also being done by the beamline staff.



The samples are loaded into the robot dewar on the beamline, the pins are now attached to the puck lids and pointing upwards ready for the BART robot to load on to the goniometer

Loaded onto pins and into UniPucks at Diamond, samples for fragment screening are not returned to their users - the equipment is cleaned and reused. Although their journey ends at Diamond, the data these samples generate saves hours, months and even years of wasted time.

Every day, hundreds of samples move through Diamond, which can be thought of as a huge machine that uses samples to generate science. More than 14,000 researchers from all academic disciplines and industry use Diamond to conduct experiments, assisted by approximately 700 staff. Each journey is unique, but the destination is the same: increasing our understanding of the world we live in.

References

Search web under 'sample holders and pucks for MX experiments' for manufacturers/suppliers
There are more than one commercial provider of SPINE pins and pucks which are defined standards, see: https://diamond.ac.uk/Instruments/Mx/Common/Common-Manual/Sample-Holders/Pucks-and-pins.html
and for I23 : https://www.diamond.ac.uk/Instruments/Mx/I23/I23/Manual/Sample-Holders.html

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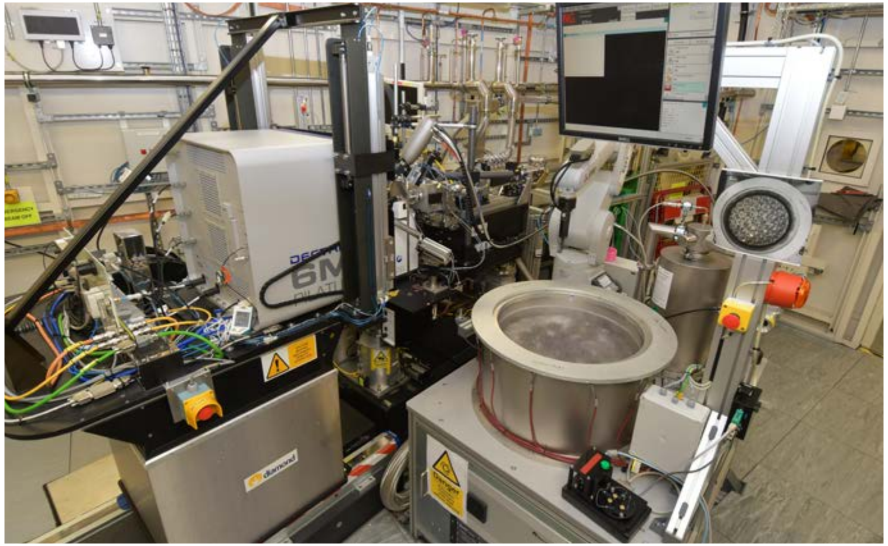

Overview of the IO4-1 hutch