Application Note SC-XRD 513

D8 VENTURE with ISX Stage

- Automated *in situ* Crystal Screening

Introduction

Despite many recent advancements in data collection and processing, it remains a major bottleneck in protein crystallography workflows to obtain diffraction-quality crystals to begin with. It is common practice to test crystals for diffraction quality only after harvesting, cryo-protecting, and cooling them—all of which may harm the crystal's diffraction quality. Not only is low-temperature crystal screening time-consuming and laborious, it does not allow the researcher to directly ascertain the impact of the these manipulations on the sample's diffraction limits.

At synchrotrons a few years ago, a new method emerged that allows researchers to study crystals' diffraction properties directly in their growth environment—a fast, convenient technique called in situ crystallography. It provides a basis for assessment of potential crystal damage caused by physical manipulation, non-optimal cryo-protection, or dehydration by setting the benchmarks to optimize the strategies for crystal transfer and protection.

Figure 1: ISX stage mounted on a D8 VENTURE with KAPPA.

The ISX stage for D8 VENTURE (Figure 1) is the ideal in-house solution for automated screening of crystals directly in crystallization plates:

- Easy stage mounting and unmounting, with typical conversion time under five minutes
- Software-driven, single-click well selection for rapid optical screening of the entire plate
- Multiple object selection and queuing for automated diffraction screening
- Rapid unit cell characterization to confirm crystal identity and streamline subsequent data collection

Optical and X-ray Screening of Glucose Isomerase Crystallization Trials

The ISX stage was mounted onto the D8 VENTURE's KAPPA goniometer. The system features an IμS 3.0 X-ray source with HELIOS MX optics and a PHOTON II CPAD detector.

Glucose isomerase was crystallized under a variety of conditions in a single MiTeGen in situ-1 plate. The crystallization tray was inserted into the ISX stage at room temperature (with no further manual intervention, minimizing potential crystal disturbance).

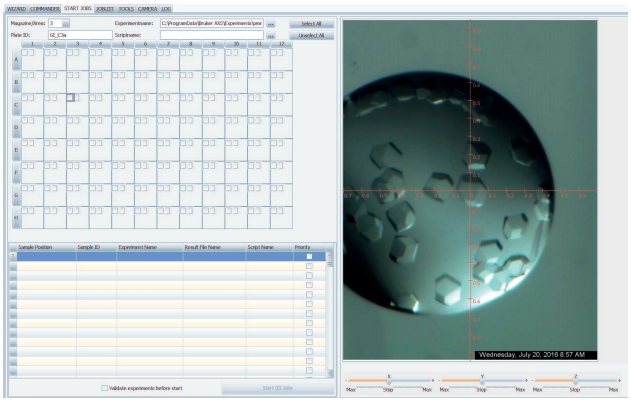


Figure 2: ISX stage control software with plate navigation area, image viewer, and job queue.

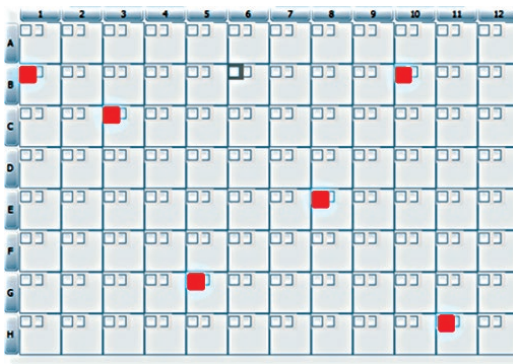


Figure 3: The ISX stage is capable of unattended screening of all wells. Wells forwarded for diffraction screening are shown in red.

Process	Samples analyzed	Time
Optical examination	96 drops	20 min
Diffraction screening	6 drops, 28 objects	30 min
Crystal characterization	1 crystal	10 min
Total		60 min

Table 1: A one-hour experiment: ISX screening and lattice parameter determination.

Parameter	Value
Bravais lattice type	I-centered orthorhombic
a, b, c (Å)	94.05, 99.50, 103.13
α, β, γ (°)	90.0, 90.0, 90.0
Mosaicity (°)	0.42
Resolution limit (Å)	1.80

Table 2: Orthorhombic glucose isomerase crystal parameters from drop G5.

The entire plate was accessed without the need to rotate (Figure 2). All 96 drops were visually inspected by selecting the well of interest with a mouse click in the ISX stage control software's plate navigation area (Figure 3).

Optical inspection of the entire plate identified six drops containing crystalline material (Table 1). Objects of interest within these drops were added to a job queue for automated X-ray analysis by clicking on the objects in the viewing area. Upon starting the job, the diffraction quality of each selected object was measured by collecting a single 60 second, 0.5° frame.

A total of 28 objects in six different drops were screened for X-ray diffraction in 30 minutes. Representative results from each drop are shown in Table 3.

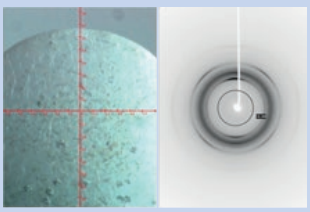
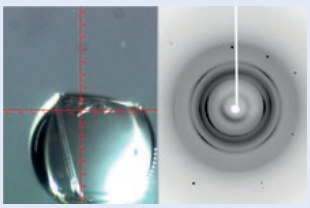
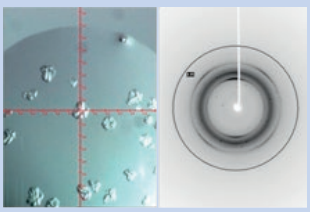
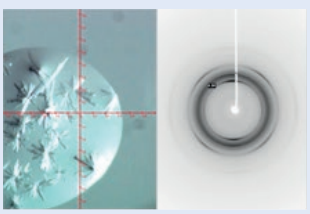
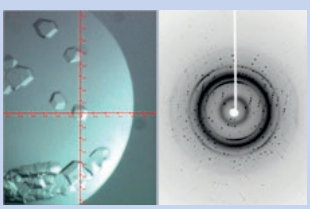
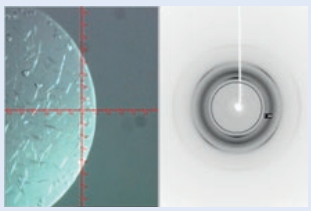
Drop	Optical/diffraction image	Optical interpretation	Diffraction result
B1		Shower of microcrystals	Protein powder diffraction to 10 Å
B10		Several large crystals with good morphology	Several intense, widely-spaced reflections indicate salt crystals
C3		A number of 30 µm crystals, poor morphology	Diffraction from a single protein lattice to 5 Å
E8		Clusters of crystalline needles, 100 µm in length	Diffraction from a single protein lattice to 5 Å
G5		Several 100 µm single crystals, with good morphology	Single protein lattice diffracting to 1.5 Å
H11		Shower of small crystals, 2-4 µm in length	Diffraction from several crystals showed protein powder diffraction to 7 Å

Table 3: Representative results from six drops with 28 objects examined in 30 minutes.

Fast crystal characterization

A crystal from drop G5 was selected for characterization. A total of 5° of data was collected in 10 minutes using 60 second exposures of 0.5° to provide the crystal parameters described in Table 2. The lattice parameters are consistent with those of published orthorhombic-I glucose isomerase data (Figure 4).

Conclusions

The D8 VENTURE, equipped with the IXS stage for in situ crystallography, offers an extremely convenient method to quickly ascertain the success of crystallization trials. The system can routinely provide further insight into the crystallization experiment, enabling the successful identification of leads and their optimization. As a result, the number of unsuccessful attempts harvesting crystals will decline, valuable labor time will be used more efficiently, and last but not least, the number of proteins successfully crystallized will significantly increase.

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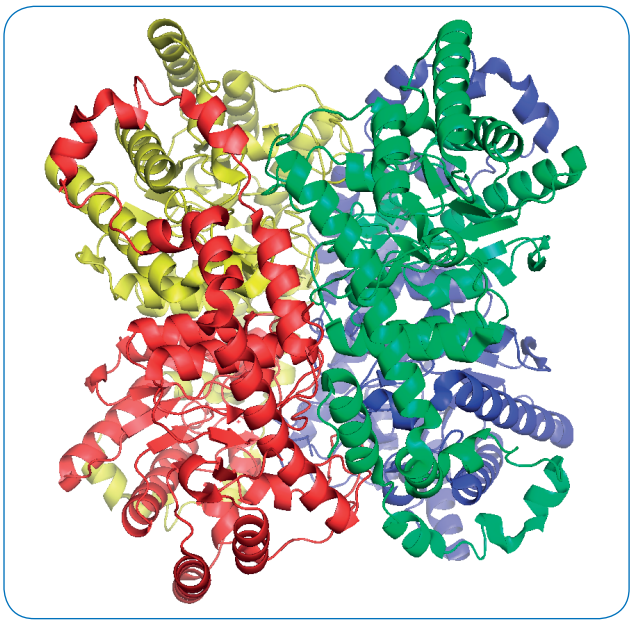

Figure 4: Biological assembly of glucose isomerase (1MNZ from PDB) created with PyMOL.

Automated crystallization screening

- Identify crystalline material
- Discriminate salt from protein
- Quickly identify optimal crystallization conditions
- Provide comparative diffraction-quality data for optimization of cryo-protection

Convenient crystal characterization

- Determine unit cell parameters and crystal symmetry
- Identify alternative crystal forms arising from heavy-atom soaks or ligand binding

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