The SSRL Plate for In-situ Crystallization and Automated Diffraction Data Collection

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BACKGROUND

The aim of this project was to optimize the SSRL In-Situ Crystallization plate (SSRL-ICP), developed by scientists at the Stanford Synchrotron Radiation Lightsource (SSRL) Stanford Linear Accelerator Center (SLAC). The intention was to find the proper conditions to allow protein crystals to be stored at room temperature within the plate for three days or longer. This would allow users to take samples without using potentially degrading cryogenic procedures, as well as allow users to collect crystal refraction data remotely.

Since it is known that protein crystals become dry, and therefore unviable, very quickly when not immersed in crystallization solution or kept in a humid air stream, the focus on this project was to determine the most effective way in which to keep the chambers of the ICP at the correct humidity to maintain viability of the crystals.

METHODS

Initial tests were done using only one type of protein crystal. Lysozyme was chosen because it is both relatively inexpensive to acquire and grows quickly. The crystals are typically uniform in shape and size, allowing researchers to more easily determine the effectiveness of the ICP.

Several well conditions were tested in order to establish the best environment for **humidity**, **stability** of the crystal and solution in the cups, as well as **ease of use**.

Once the proper conditions had been established, testing began with different crystal types. In total, researches tested **lysozyme**, **kemp**, **catalase**, and **thaumatin**.

Crystals were stored in the sealed ICP for several days. Crystals were tested for viability by gathering **X-ray diffraction data** after **24**, **36**, and **48** hours of being sealed within the tray. In addition, a crystal from the same growth batch that had not been sealed within the tray (had been kept in its growing solution) was tested to provide a **control**.

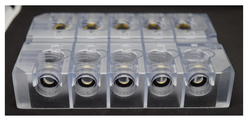

Figure 1: The SSRL-ICP, a 10-well plate intended to store room temperature protein crystals.

Figure 2: The silicon liner for the ICP wells. Referred to as the "well cup".

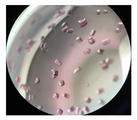

Figure 3: Color-enhanced lysozyme crystals demonstrating uniformity of size and shape of the protein.

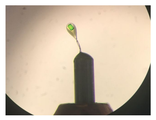

Figure 4: A color-enhanced lysozyme crystal held in a loop within the sealed ICP. The crystal maintains the proper level of humidity within the tray.

DATA

Figure 5: An improperly sealed well caused this lysozyme crystal to become dry in the loop.

Initial tests to determine if the plate could be kept airtight were successful. It was found that an acrylic tape, applied to the top and side opening of the well were sufficient to keep moisture in the well for a significant period of time. The figure to the left shows one of the first failed attempts using only pins to seal the tray.

Further testing revealed that adding **agarose** to the crystallization solution provided both the stability and humidity control to keep the crystals viable for at least 3 days.
An agarose concentration of **0.06% w/v** was found to be ideal, but testing demonstrated that the crystals tolerated a **range** from **0.02-0.08%**

Figure 6: ICP wells filled with a colored agarose solution.

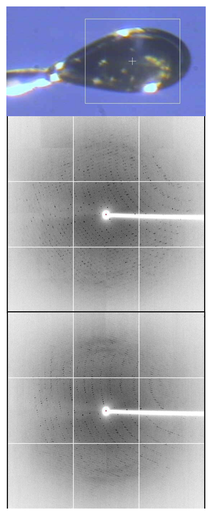

Figure 7: a catalase crystal mounted a loop for X-ray diffraction. The middle image is the diffraction pattern of the control crystal, while the bottom image is a catalase crystal after 36 hours.

X-Ray diffraction was taken of the protein crystals samples kept within the wells throughout the test. Data was gathered 24, 48, and 36 hours after the crystals were initially sealed within the ICP. A crystal from the growing tray was tested as a control for each different test. The image to the right shows a catalase protein crystal. Below are X-ray data from the control and from a crystal which had been stored in the tray for 36 hours. Both diffraction patterns are within normally expected ranges. This pattern was seen for all of the crystals tested.

Lysozyme, thaumatin, and kemp all showed similar results. In fact, none of the tests revealed damage to the crystals or defective X-ray diffraction patterns.

DISCUSSION AND NEXT STEPS

This research was concluded without any major setbacks. All testing parameters were flushed out, and positive outcomes were reached in all aspects.

X-ray data of the protein crystals showed a somewhat surprising trend of positive results.

Next steps for this project are to determine if the SSRL-ICP tray can be used to grow crystals within the tray itself to minimize any manipulation of the often fragile crystals. Additionally, tests should be done to establish whether or not crystals can be shipped within the tray to SLAC for data analysis. This would allow users to send valuable protein crystals for analysis even if the user can not travel to SLAC in person.

Further development and testing of the robotic loading system will be required before users are able to remotely gather data, however the project is progressing quickly.

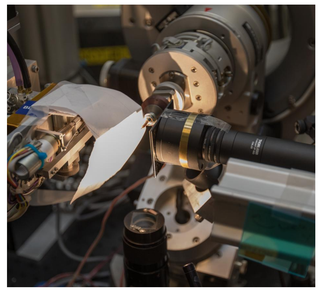

Figure 8 (left): a crystal loaded into a loop in the humid air stream. The crystal is awaiting X-ray diffraction.

Figure 9 (right): The loading tray for the ICPs. This tray will allow users to load crystals for the automatic data collection via a robotic arm. This will reduce handling of fragile crystals.

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