Late-stage Crystal Failures and the Protein Crystal Harvesting Bottleneck

JCSG Joint Center for Structural Genomics
Developing HT methods for Gene to Structure and Function

PSI:BIOLOGY

Square One Systems Design

Marc C. Deller[a], Jace Walsh[b], Robert Viola[b], Marc A. Elsliger[a], Ian A. Wilson[a], Bernhard Rupp[c]

[a] Joint Center for Structural Genomics, The Scripps Research Institute, La Jolla, CA 92037, USA, [b] Square One Systems Design, Jackson, WY 83002, USA, [c] k.-k. Hofkristallamt, Vista, CA 92084. E-mail: mdelller@scripps.edu or hofkristallamt@gmail.com, http://www.ruppweb.org/robot/. **Work sponsored by NIH STTR Phase II Grant No. 2 R42 GM073278-02A1 and JCSG funded by Grant U54 GM094586.**

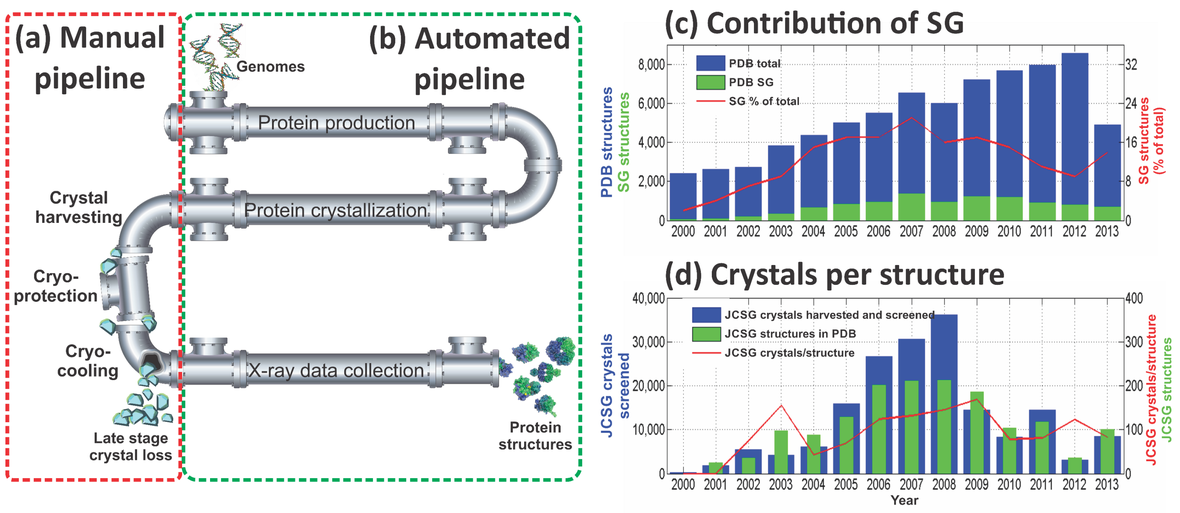
High-throughput protein crystallography and structural genomics (HTPX/SG) have revolutionized structural biology. Approximately 10% of all protein structures deposited in the Protein Data Bank (PDB) are a result of HTPX/SG efforts [1]. To enable the significant increase in output, most HTPX/SG centers have embraced automation and robotics at all stages of the structure determination process. However, a process bottleneck remains at the crystal harvesting stage, which is still largely carried out by hand [1]. Automation of crystal harvesting offers several advantages over traditional manual methods: (a) harvesting of microcrystals too small for reliable harvesting by hand, (b) reduced late-stage failures due to non-optimal crystal handling and cryocooling and (c) increased throughput. In collaboration with Square One Systems Design, the Universal Micromanipulation Robot (UMR) is being developed and tested at the Joint Center for Structural Genomics (JCSG) to embrace the potential benefits of automation, particularly in the areas of microcrystal harvesting and improved cryocooling [1,2,3]. The UMR system has recently been modified to enable harvesting of microcrystals into SSRL sample mounting grids. High-density crystal harvesting will be essential to enable the use of high-brilliance X-ray sources from fourth-generation synchrotrons, free-electron lasers (FEL) and microfocus beamlines. Additionally, the UMR system has been modified to enable high-speed cryocooling (hyperquenching)[4,5]. Such a system offers the potential to relieve the harvesting bottleneck and reduce late-stage crystal failures.

1) Deller, M. C. and Rupp, B. (2014), Acta Cryst., F70: 133–155.
2) Viola, R., Carman, P., Walsh, J., Frankel D., Rupp, B. (2007). J. Struct. Funct. Genomics. 8, 145-152.
3) Viola, R., Carman, P., Walsh, J., Miller, E., Benning, M., Frankel, D., McPherson, A., Cudney, B., Rupp, B. (2007). J. Appl. Cryst. 40, 539-545.
4) Warkentin, M., Berejnov, V., Husseini, N.S., Thorne, E. (2006). J. Appl. Cryst. 39, 805-811.
5) Warkentin, M., Thorne, R.E. (2007). J. Struct. Funct. Genomics. 8, 141-144.

The **Universal Micromanipulation Robot** (UMR) is designed to perform the **full range of tasks necessary for protein crystal harvesting**. The platform is currently being optimized to enable **hyperquenching for HTPX/SG** at the JCSG:

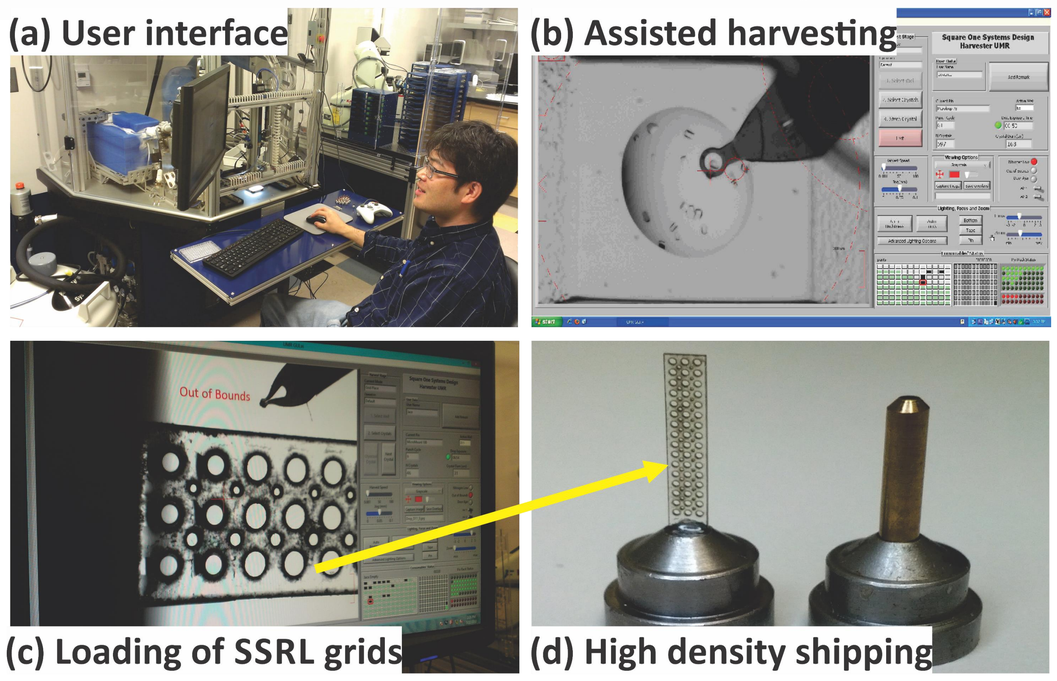
- **Protein crystals are cooled to 77K or 100K** to reduce radiation damage during data collection.
- **Current cooling procedures often result in hexagonal ice formation** which can reduce resolution and increase mosaicity.
- **Cryoprotectant is required to prevent hexagonal ice formation** within the crystal. This is both time-consuming, potentially damaging to the crystal and creates a process bottleneck. Additionally, late-stage crystal losses due to failed harvesting and poor cryoprotection techniques can occur (see **Panel 1**).
- **Automation of crystal harvesting processes streamlines pipeline** and increases throughput, reduces late-stage crystal failures and embraces new technologies such as FEL sources that require high-density crystal shipping (see **Panel 2**).
- **UMR couples drip cryo cryoprotection and hyperquenching** in order to limit late-stage crystal loss. Furthermore, analysis of the quench speeds and temperature profiles will provide a framework for optimal cryoprotection and cooling methods (see **Panel 3**).
- **Current methodologies involve cooling protein crystals relatively slowly** (~1000 K/s) which allows time for damaging hexagonal ice formation. High rates of cooling (10,000-20,000 K/s) are predicted to be sufficient for the hyperquenching of protein crystals (see **Panel 4**).

Panel 1 – Manual crystal harvesting presents a process bottleneck



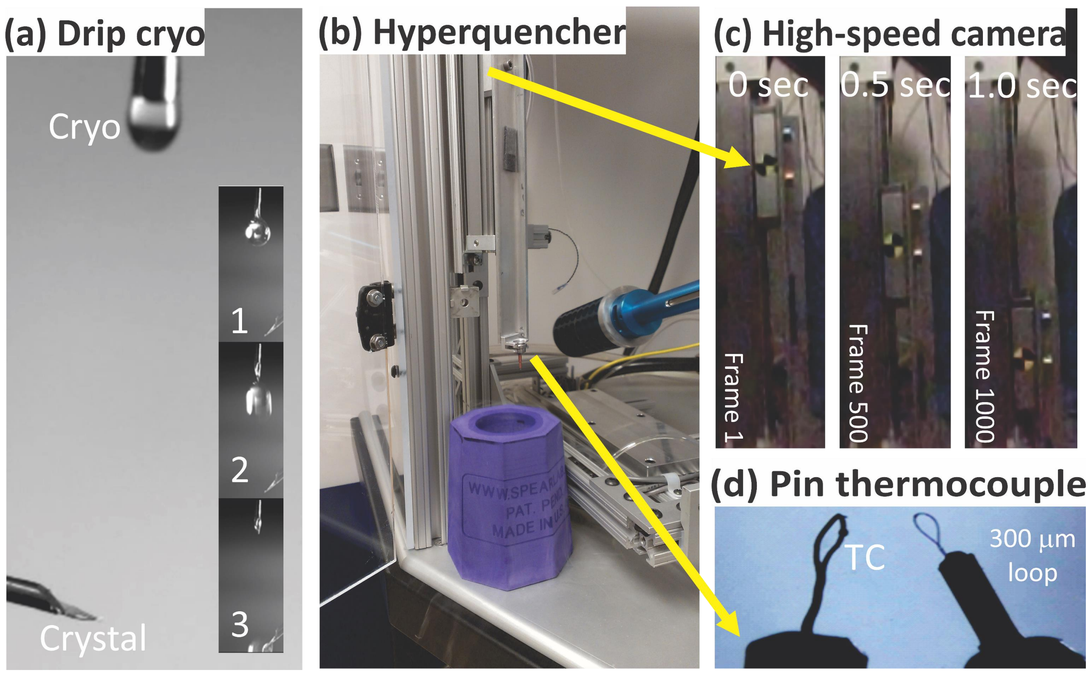
(a) Crystal harvesting remains one of the few manual processes in a typical HTPX/SG pipeline. This creates a process bottleneck and a potential for late-stage crystal failures. (b) A typical HTPX/SG automation pipeline. (c) ~10% contribution of SG structures to PDB. (d) ~100 crystals/per structure at the JCSG.

Panel 2 – High-throughput harvesting into SSRL grids



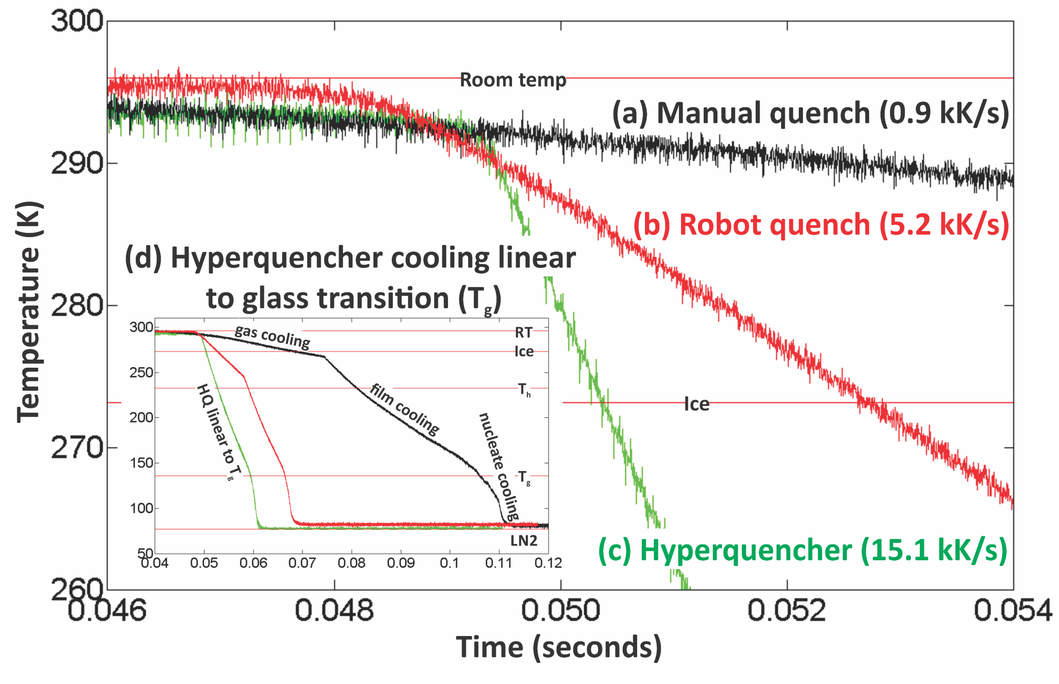
(a)→(d) Workflow of robot-assisted harvesting into SSRL sample mounting grids. UMR represents an opportunity for high-density shipping to FEL sources.

Panel 3 – Combination drip cryoprotection and hyperquenching



(a) Automated drip cryo mechanism for harvested crystals provides insulation from film boiling regime of liquid nitrogen (PFO oil). (b) Directly coupled pneumatic hyperquencher cools crystals at high-speed (5 m/s) in nucleate boiling regime of liquid nitrogen. (c) High-speed camera (1000 frames/s) allows accurate calculation of hyperquencher speeds. (d) High-rate data acquisition thermocouple allows for precise monitoring of cooling rates.

Panel 4 – Hyperquenching and increased rates of cooling



(a) Slow cooling rate of manual cryocooling in liquid nitrogen provides opportunity for damaging hexagonal ice formation. (b) intermediate cooling rate of robot-assisted cooling in liquid nitrogen. (c) High-speed pneumatic cryocooling, via the hyperquencher, increases the probability of amorphous ice formation (vitreous/glassy/unstructured ice) which is less damaging to the protein crystal. (d) Overall temperature profile of hyperquencher (green) in comparison to a traditional manual (black) and robot-assisted (red) quench.