WHITMAN COLLEGE

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Gentle equilibration of macromolecular crystals to new solutions with minimal handling.

Background

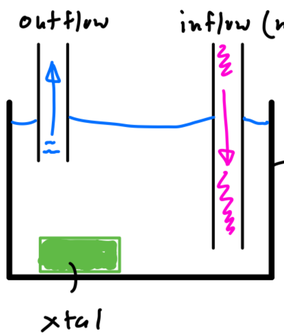
Macromolecular crystals are easily damaged during transfer to new solutions due to handling and/or osmotic effects. Here we explore two methods to gently equilibrate crystals to new solutions with minimal handling.

Approach

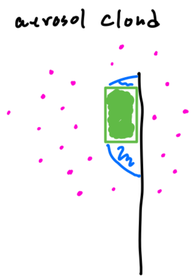
old solution

new solution

Liquid Based

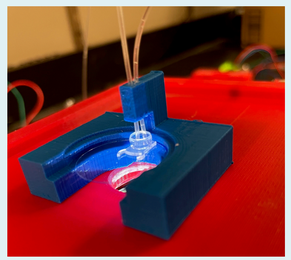


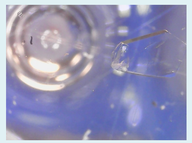
Aerosol Based



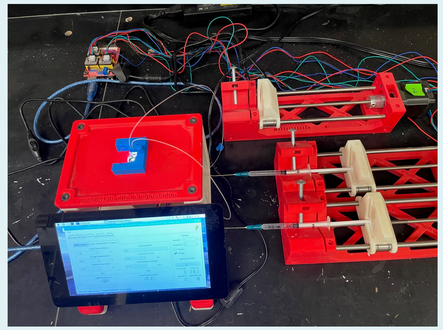
Results

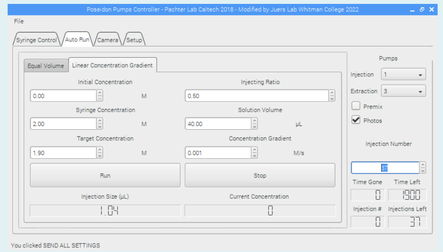
Liquid Based



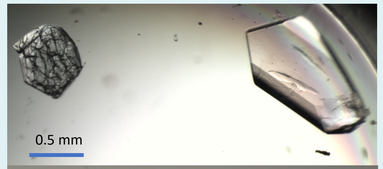


Top: 40 µL pot (pcr tube cap) with inflow and outflow tubes. Bottom: view from below showing crystal on right.



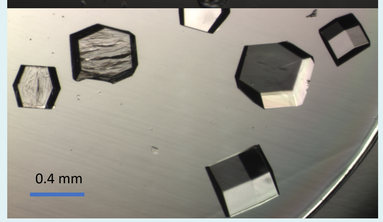


Top: system showing open-source syringe pumping system[1] with enhancements for crystal equilibration. Bottom: auto gradient view of control software



Alpha lactalbumin
0 -> 25 % glycerol
Direct (left) vs 50 min linear gradient

Thermolysin
~2 M AmSO4 -> 0 M Am SO4
Direct (left) vs 15 min linear gradient



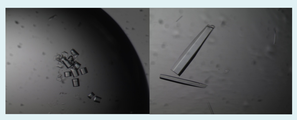
Lysozyme
8 % NaCl -> 3% NaCl
Direct (left 2 xtals vs 40 min linear gradient

Aerosol Based

Batch/Closed System Method

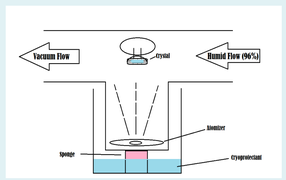
Draw vape-pen generated aerosol from syringe

Eject aerosol into cryovial with loaded sample and incubate.

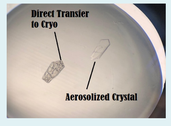


Thermolysin
~2 M AmSO4 -> 0 M Am SO4
Direct (left) vs ~ 10 min incubation in vial

Process/Open System Method



Cryoprotectant is directed towards a loop mounted crystals sitting in a cryovial with a nebulizer plate.



Alpha lactalbumin
0 -> 25 % glycerol
Direct (left) vs ~5 min incubation in vial

Summary

The liquid based approach has proven to be robust and reliable.

The aerosol based approach has been shown to work in principle. The many process variables make it less reliable. However it has potential to be a more rapid method, and further development is underway.

References/Acknowledgments

1. **Sina Booeshaghi, Eduardo da Veiga Beltrame, Dylan Bannon, Jase Gehring and Lior Pachter, Principles of open source bioinstrumentation applied to the poseidon syringe pump system, Scientific Reports 9, Article number: 12385 (2019)**
2. This work was supported in part by a grant from the National Institutes of Health (GM0900248).