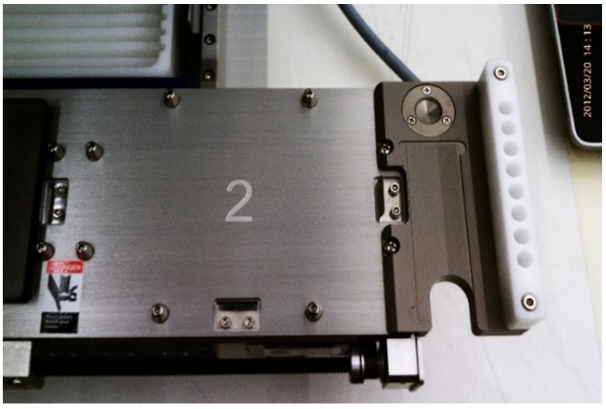
APPLICATION NOTE: Protein Crystal Seeding with The Crystal Gryphon LCP

Equipped with 3 diverse liquid handling heads The Crystal Gryphon LCP is a leader in the setup of protein crystallization trials. This machine is uniquely suited to set-up protein crystal seeding operations. Here we show an example of how to perform complex protein seeding experiments, which truly demonstrate the unique experimental capability of the instrument.



The LCP Arm is a syringe drive that allows low volume fluid handling independent of high viscosity solutions typical of protein seed stock stabilization mixtures.

Key Attributes to Perform Protein Crystal Seeding:

- 3 fluid handling heads are unique in the industry
- Durable, reliable and compact
- Fast and accurate
- 1nl to 1 ml range
- Contact and non-contact low volume dispensing
- LCP Deck extension used for serial dilutions of seed stocks

Crystal cross seeding experiments provide another route to protein crystal optimization:

Ultimately, crystal seeding experiments are useful to find new conditions that increase the nominal diffraction of crystals by finding new stable crystalline growth conditions. By providing crystal nuclei to the screen conditions we can effectively bypass the crystal nucleation event, finding new well chemistries that otherwise would not favor nucleation, but allow for larger and more optimal crystalline growth.

Collaborative seeding experiments with various laboratories:

Crystal seeding experiments were successfully conducted between ARI and several laboratories, although not all protein systems are amenable to these approaches. As always, it can often be the protein construct and not the crystallization chemistry that is most responsible for crystal integrity. However, several experiments were conducted at ARI to show that the mechanism of the machine is effective, and in all cases crystals were redetected in the new screens that were cross seeded. Some of the scientists and laboratories we have worked with include:

Sara Codding, former graduate student in Andrew Karplus' lab at Oregon State University

Ping Yuan former Research Associate and Xiaolin Wen, Research Associates in the laboratory of Theodore Jardetsky at Stanford University

Geoff Feld, Postdoctoral fellow in the laboratory of Brent Segelke at Lawrence Livermore National Laboratory.

OVERVIEW:

- Crystal cross seeding is a complex experiment which requires the additions of three simultaneous additions of reagent classes: screen mixtures, new protein stock solution, and protein crystal seeds in seed stabilization solutions.

- Protein crystal seeds are added using the LCP syringe drive and the non-contact ARI Nano dispenses protein stock solutions. This is followed by screen reagent additions with the 96-Way Head.

- Cross seeding experiments are often tried in parallel with grid optimization methods, but are sometimes the best or only way to proceed if the reagents of grid optimization are insufficient to improve diffraction quality.

REPRESENTATIVE STUDY:

In all cases the same experimental series and methods were conducted in order to cross matrix seed an initial protein crystalline hit chemistry into new full screen chemistries. We will summarize the outcome from the most recent set of experiments with Geoff Feld on FTN0149, a protein originating from the organism, *Francisella novicida U112*.

Background: The original FTN0149 crystal diffracted well to 2.0 angstroms resolution. Crystals were shown to be rod-like spurs (with feathering), producing interference from multiple lattice reflections causing overlaps in the X-ray diffraction experiment. We conducted the cross seeding experiment to see if we could alleviate this poor crystal morphology and grow single crystal forms that could diffract well and remove the multiple crystal problem for this system. This would inevitably increase the completeness of the data, especially at higher resolution angles were reflection overlapping is more likely to occur.

Well solution of initial FTN0149 crystal seeds: 20% PEG4000, 0.1 M Na Acetate pH 4.5

Protein buffer: 9.4 mg/mL FTN0149 Se-Met in 1 mM Zn Acetate, 0.01 M HEPES pH 7.5, 0.05 M NaCl

METHODS:

Protein Crystal Seeding Using the ARI Nano and the LCP arm.

1) Initial Seed titration experiment to determine seed density.

Serial Dilution Seed stocks.pro

2) Plating of initial seed titration in native crystal growth condition

Seed Stock Titration Plate Setup.pro

3) Use protein seeds of initial crystal form and add into new full screens

Seeded Plate Screen Setup.pro

Evaluation of crystal seeding experiments:

Optimization of seeded crystal hits by grid optimization, etc. to develop new optimized conditions, which will be followed by X-ray analysis to determine diffraction quality.

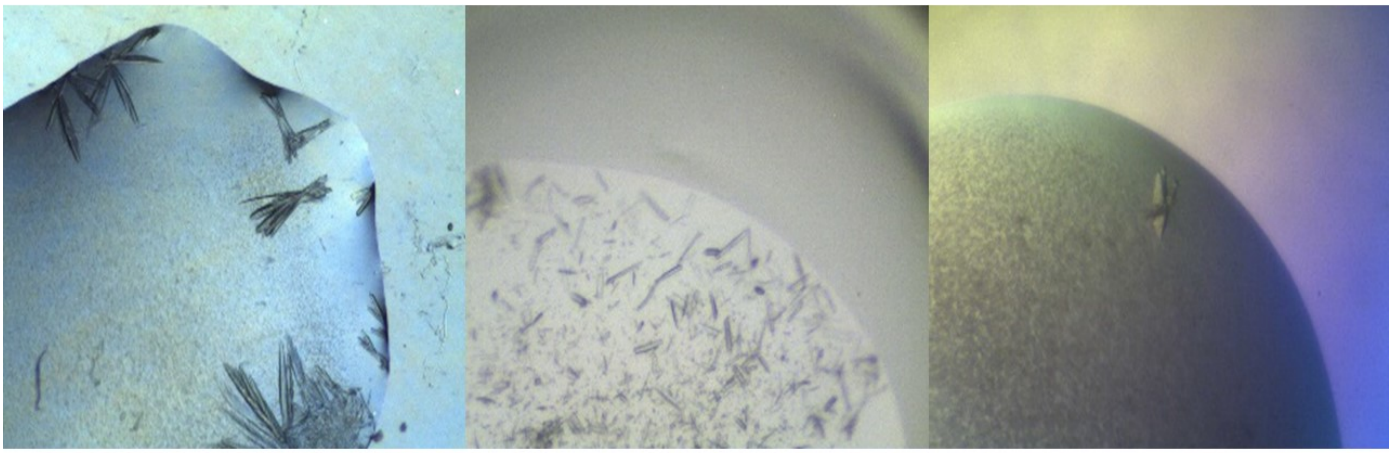
An optimized grid was designed and created on the ARI Scorpion around one of the new seeded hit conditions, Hampton Research PEGRxHT H11.

Well solution of PEGRxHT H11: 0.2M $MgCl_2$ hexahydrate, 0.1 M Na Citrate tribasic dihydrate pH 5.0, 10% PEG 20K

The grid conditions were a shift of pH 5.0 to pH 6.4 along the y axis using Na Citrate buffer and a shift of PEG 20K concentrations from 6% to 16% along the X axis of the tray.

The scorpion also set up the 1ul + 1ul drops after a 0.5 ul seed bolus was delivered to the drop platform of the 24 well INTELLIPLATE using the Gryphon's LCP arm.

Outcome of the 3 stages of this experiment, pictures taken on ARI Cryscam:



Picture 1: The initial crystal hit was grown by grid optimization. Note the sharded feathery bundled appearance of the crystal morphology. That is what we are trying to correct with this seeding experiment.

Picture 2: is one of the initial hits we detected after cross seeding the crystals into four full screens. Note that although the crystals are small, they are regular and single and not in bundles. This hit was grid optimized by the Scorpion.

Picture 3: shows a hit from a seeding experiment conducted on a 24 Way grid optimization run produced on the ARI Scorpion and seeded on the Crystal Gryphon LCP. Note: the chunky single crystal detected during this experiment seems to have a dramatic change in the morphology of the crystal. The initial feathering morphology appears to be alleviated.

CONCLUSIONS:

The crystals initially grew as single crystals that appear to have a slightly different morphology than the original crystals, namely the feathering and multiple lattices apparent on the original crystal have greatly been reduced. The crystals also appear to be thicker better formed. They also do not polarize light to the same extent as the original crystal (perhaps now a P4 spacegroup v. the original P321 spacegroup).

The other exciting outcome so far was a possible shift upwards in the pH in the new condition found above the original pH of 4.5. The crystals are growing in a new condition buffered at a pH value of 5.0 – 6.4. pH shifts are often observed to have effects on the morphology of crystals.

DISCUSSION:

The Crystal Gryphon LCP is a unique fluid handling instrument providing a powerful yet economical tool set to address your laboratory's needs. This device sets up conventional protein crystallization trials, is optimized as an LCP dispenser, has a useful full range of general fluid handling capability, and yet is a precise seeding tool as demonstrated by the experiments reported here. Taken together there has never been anything that has close to the full range of capability that the Crystal Gryphon LCP offers to the research laboratory.

The ability to successfully handle the variety of solution properties demonstrated for crystal seeding operations is essential for a fluid handling device to succeed at this experiment. The Crystal Gryphon LCP has an unmatched capability for this operation's requirement.

No other robotic device is available offering the capability and capacity of a Crystal Gryphon LCP:

- Speedy Set ups allow for smaller volumes, especially for seeding operations
- Accuracy and precision at low volumes independent of fluid class
- Versatile small footprint providing a variety of methods development options
- Experimental outcomes year after year from a proven, fully supported durable device