Application Note: Protein Crystal Seeding with The Crystal Gryphon LCP

Equipped with 3 diverse liquid handling heads, The Crystal Gryphon LCP is a drop setter uniquely suited to set-up protein crystallization trials.

The LCP Arm is a syringe drive that allows for fluid handling independent of solution viscosities typical of protein seed stock stabilization mixtures. The deck eof the Gryphon includes an eight position tube holder for automated serial dilution of seed stock.

Ultimately, seeding experiments are useful methods to optimize the X-ray diffraction of crystals. By finding new stable crystalline growth conditions Microseed Matrix Seeding (Ireton and Stoddard. Acta Crystallogr D Biol Crystallogr. (2004) D60, p.601-5) and improved upon by introducing automation (Darcy et al *Acta Cryst.* (2007). D**63**, 550-554). By providing crystal nuclei to fresh full screen conditions we effectively bypass crystal nucleation events, finding new well chemistries that otherwise might not favor nucleation, but allow for optimal crystalline growth.

Overview of Methods: Protein Crystal Seeding Protocols

1) Initial Seed titration experiment to determine seed density using serial dilution rack.

Serial Dilution Seed stocks.pro

2) Plating 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. Used 25 nl seed stock additions.

Seeded Plate Screen Setup.pro

Case Study: Highlights of a Successful Seeding Experiment

The target: Protein X [Soon to be Released in the PDB]

This particular construct tends to degrade quickly from the C-Terminus, both degradation products present in crystals. No electron density detected for much of the C-terminal.

The following initial full screens were used for the cross seeding trials:

Hampton Research: PEG/ion PEG/Rx Index, Molecular Dimensions: JCSG+, Rigaku: Wizard Classics, Qiagen: PGA, Morpheus MIDAS

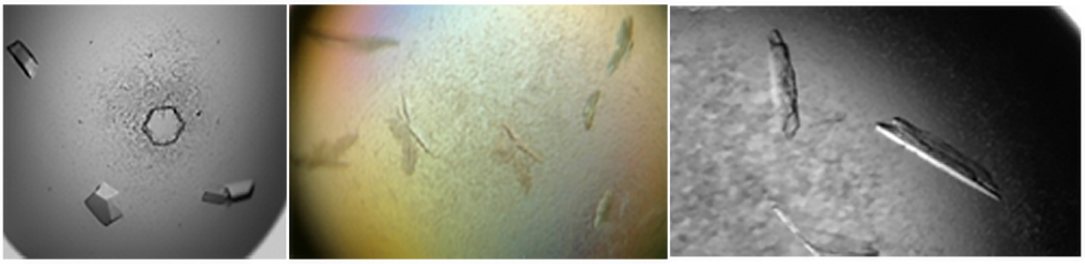

Figure 1: Hits in PGA screen. Needles or Plates, diffracted nominally from to 8-4 Angstroms

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Exhausting conventional methods, many, many standard 2D gridding and Additive screening attempts did not prove to improve X-ray diffraction quality. A very familiar story!

Experimental Work Flow performed on the ARI Crystal Gryphon LCP

8 ul Crystals harvested from several drops in 24 ul stabilization buffer were vortexed with Hampton Seed Beads, diluted 1:10 in SB and then set 1:3(screen):3(protein) in full screen drops

Next the LCP syringe drive of the Crystal Gryphon LCP was loaded with the seed stock mixture.
The same sets of full screens were deployed

General Observations: lots of phase separation and around 10-20 hits per full screen.

Crystal morphology from the resulting seeded cross screens resembled the initial types but appeared to have more regular growth characteristic. There were also other chunky crystals.

Drops chemistries were then further optimized by gridding successfully

Outcomes of the Random Matrix MicroSeeding:

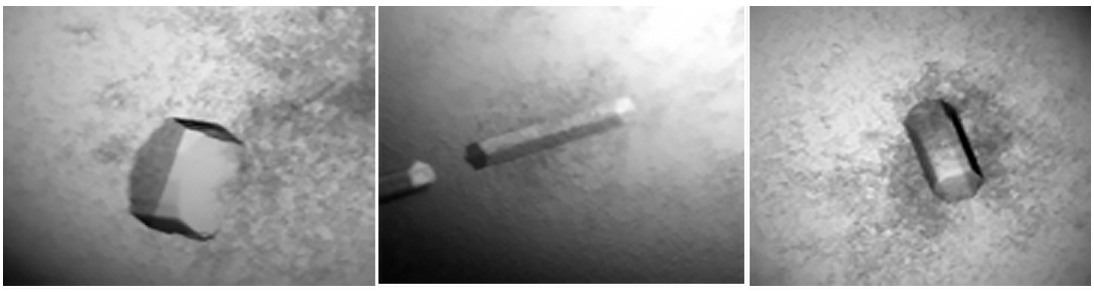

Figure 2: X-ray analysis completed structure. New diffraction limit 2.2 Angstroms

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