

Macro-seeding – a key to high resolution complex structures

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Summary:

Seeding is a simple and powerful method of obtaining high-quality crystals in protein crystallization. In this technique, single crystals are transferred into new drops where the conditions may be different from where the seed was obtained.

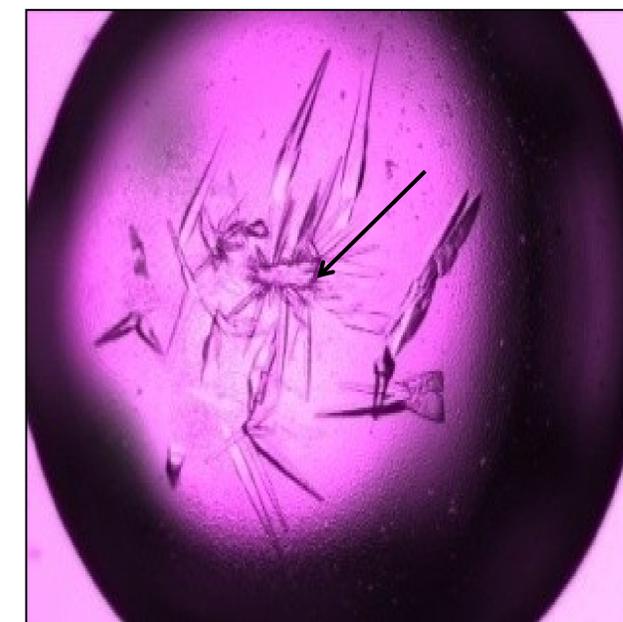
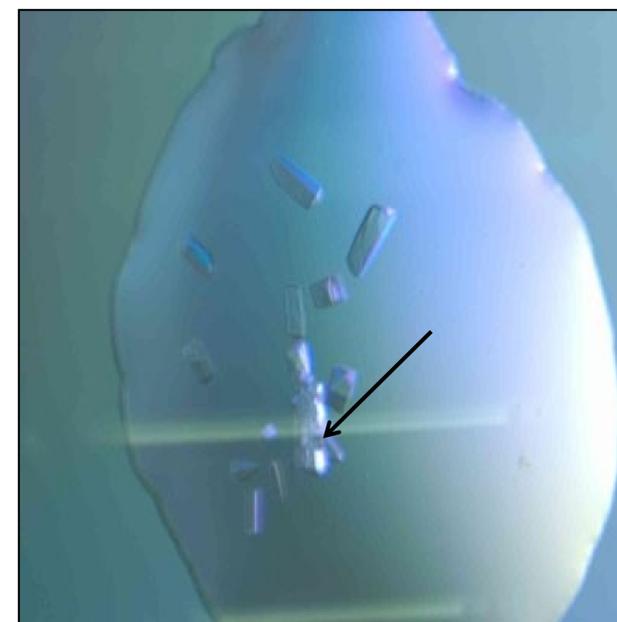
In this poster, I present a successful application of macro-seeding that enabled improved success rate of co-crystallization and improvement in resolution for a set of diverse compounds. Using the improved protocol, a large number of complex structures could be determined and successfully used to guide LG and LO chemistry.

Before macro-seeding protocol in place:

- Crystallization protocol available (two 96-condition optimization screens), but require a number of iterations depending on the compounds
 - 96 to 240 drops needed to be set up for each compound
 - 100µl protein used for each compound
- Four different crystal forms identified under approximately the same conditions
- Resolution was around 2.5Å
- Crystals appeared 7 to 10 days after set up. Typically 2-3 months from request to deliver the structure

The crystallization protocol (macro-seeding):

- Add 0.2µl compound (stock 100mM) to 10µl protein (around 8mg/ml), incubate on ice and spin down if heavy precipitation
- Seeding: 1. wash single crystal (e.g. used for data collection of other compound) in well solution several times
 - 2. add a crystal to a fresh, unequilibrated drop that contains a new compound
- Crystals appear the day after set up (crystals show up immediately for some complexes) and continue to grow a few more days. Typically many single crystals grow from the edges of the macro seed



Crystals after macro-seeding for different compounds. Seed crystals indicated by arrows

After macro-seeding protocol in place:

- A very simple macro-seeding protocol worked for several different compound series
 - 2 drops needed to be set up for each compound
 - 10µl protein used for each compound
- Reproducible high symmetry (space group P4₂) crystal form
- Resolution was higher than 2Å
- Crystals appeared in 2 days and from request to deliver the structure between 2-4 weeks

(Twenty-six different compounds were tested and solved)

