Random Microseed Matrix-Screening (rMMS), including LCP seeding

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Introduction

Several new techniques that increase the production of crystal structures have emerged in recent years. Random Microseed Matrix-Screening (rMMS), where seed crystals are added automatically to random crystallization screens, is probably the most important of these [1]. During the eight years since the method was published, theoretical understanding of the method has increased [2 - 4], and several important practical variations of the basic method have gained popularity [5, 6]. We will briefly describe some of these variations, including cross-seeding, and introduce a novel method of making LCP seed stocks by scaling up LCP crystallization conditions [7].

We will also describe a method of generating seed gradients across a plate so that the number of crystals in each LCP bolus can be varied, with a practical example.

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[1] D'Arcy, A., Villard, F, and Marsh, M. "An automated microseed matrix-screening method for protein crystallization." Acta Crystallographica Section D: Biological Crystallography 63.4 (2007): 550-554.

[2] Shaw Stewart, P. D., Kolek, S. A., Briggs, R. A., Chayen, N. E., & Baldock, P. F. (2011). Random microseeding: a theoretical and practical exploration of seed stability and seeding techniques for successful protein crystallization.*Crystal Growth & Design*, *11*(8), 3432-3441.

[3] D'Arcy, A., Bergfors, T., Cowan-Jacob, S. W., & Marsh, M. (2014). Microseed matrix screening for optimization in protein crystallization: what have we learned?. *Acta Crystallographica Section F: Structural Biology Communications*, *70*(9), 1117-1126.

[4] Shaw Stewart, P. D, & Mueller-Dieckmann, J. (2014). Automation in biological crystallization. *Acta Crystallographica Section F: Structural Biology Communications*, *70*(6), 686-696.

[5] Obmolova, G., Malia, T. J., Teplyakov, A., Sweet, R. W., & Gilliland, G. L. (2014). Protein crystallization with microseed matrix screening: application to human germline antibody Fabs. *Structural Biology and Crystallization Communications*, *70*(8).

[6] Abuhammad, A., Lowe, E. D., McDonough, M. A., Shaw Stewart, P. D., Kolek, S. A., Sim, E., & Garman, E. F. (2013). Structure of arylamine N-acetyltransferase from *Mycobacterium tuberculosis* determined by cross-seeding with the homologous protein from *M. marinum*: triumph over adversity. *Acta Crystallographica Section D: Biological Crystallography*, 69(8), 1433-1446.

[7] Kolek SA, Braeuning B, Shaw Stewart PD. A novel microseeding method for the crystallization of membrane proteins in lipidic cubic phase. Acta Crystallographica Section F: Structural Biology Communications. 2016 Apr 1;72(4):307-12.

I'm going to try to convince you that:

Random microseeding (random Microseed Matrix Screening, or rMMS) should be part of your normal workflow.

You should use the method as soon as possible!

Comment: I'm not talking about "classical" microseeding where you add seed crystals to conditions that are similar to your hit.

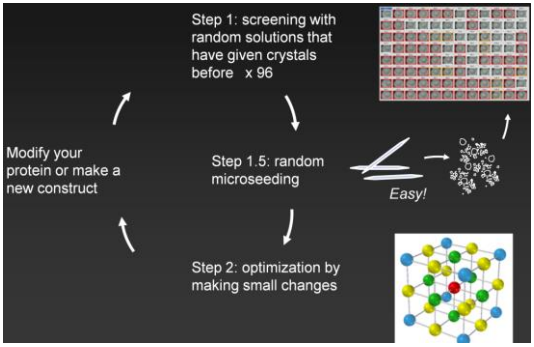
Main Reference:

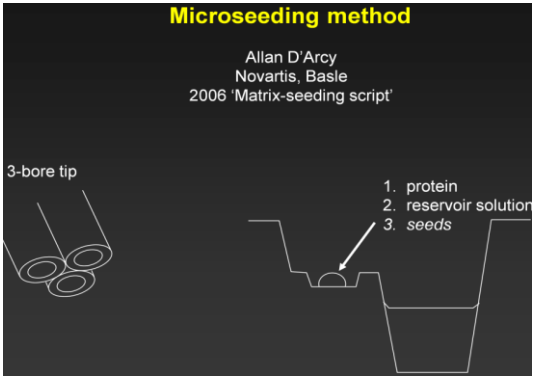
D'Arcy et al. Acta Cryst. (2007). D63:

1. Add seed crystals to a random screen
2. Suspend crushed crystals in the reservoir solution that gave the hits used ("hit solution")
3. Automate!

To get:

(1) more hits
(2) better crystals
(3) the right number of crystals (e.g. for soaking)



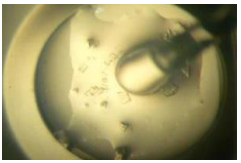


Before we start, comments about robotics:

- Contact dispensing is best for microseeding
- It's very helpful if no seed-stock or protein is wasted
- Optimization:
  - 2-d grid
  - Combinatorial script
  - 7-d multivariate designs

Recommended matrix seeding volumes:

0.3 µl protein
+ 0.2 µl reservoir solution
+ 0.1 µl seed stock
E.g. 3.0 + 2.0 + 1.0 µl for neutron diffraction !

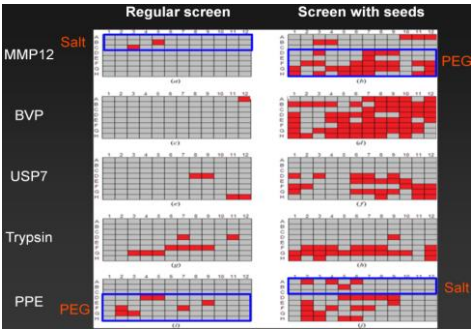


Method of making the seed stock:

See www.douglas.co.uk/mms.htm or sheet
1.Make a seed stock as soon as your crystals stop growing
2. Break crystals with a probe
3. Think about which high-salt solutions are likely to give salt crystals

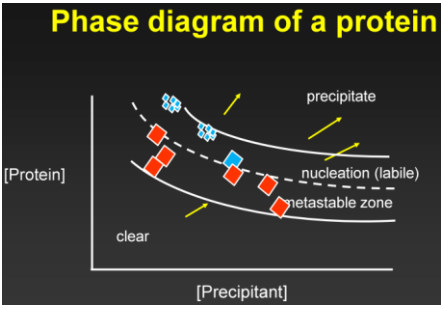
4. Take 50 µl of reservoir solution and add everything from a drop
5. Vortex with a Hampton "Seed Bead"
6. Make a dilution series immediately
7. Freeze

Look after your seeds!



1. Dramatic increase in the number of hits
2. The hits are often in completely different conditions (salt/PEG)

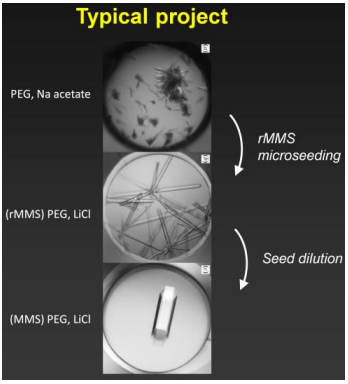
Phase diagram of a protein:



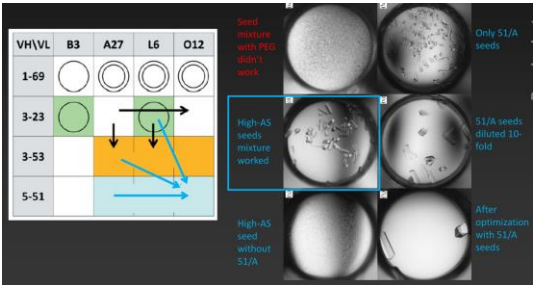
Only the blue conditions would be picked up in a typical screen

The red conditions are picked up in rMMS; these are the best conditions to work with

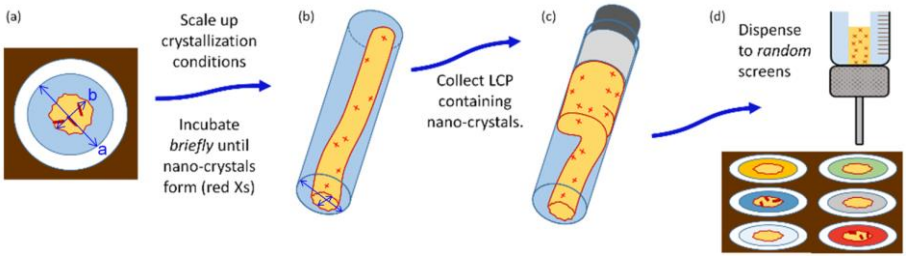
Typical progression of a project:



Cross-seeding, sometimes with mixtures of seed-stocks:

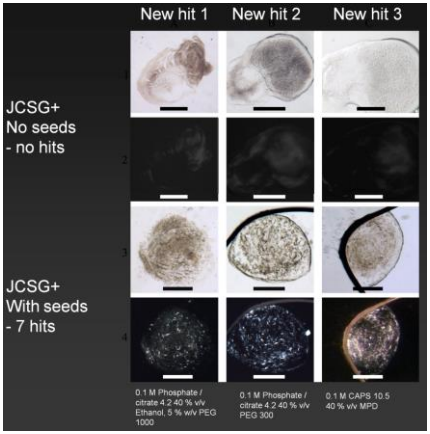


How can we use seeding with LCP crystallization?



The ratio of b/a determines the final conc. of precipitant etc. in the LCPThe absolute size of b determines the timing

New LCP hits picked up:



Special parts required for accurate scaling up: