Random Microseed Matrix Screening (rMMS): A new technique where the method is applied to membrane proteins in Lipidic Cubic Phase

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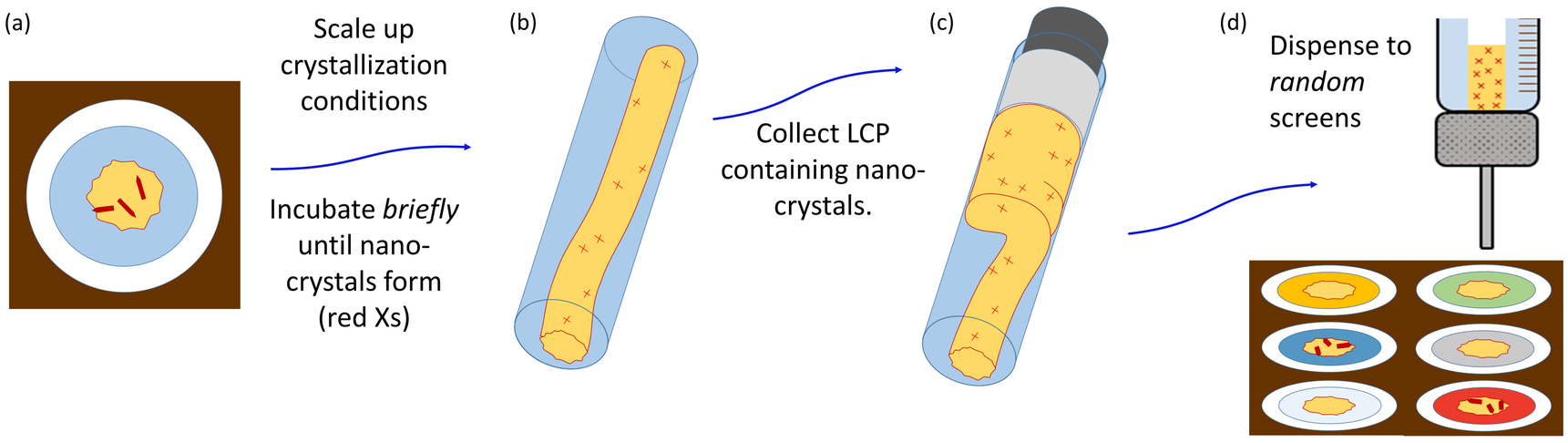
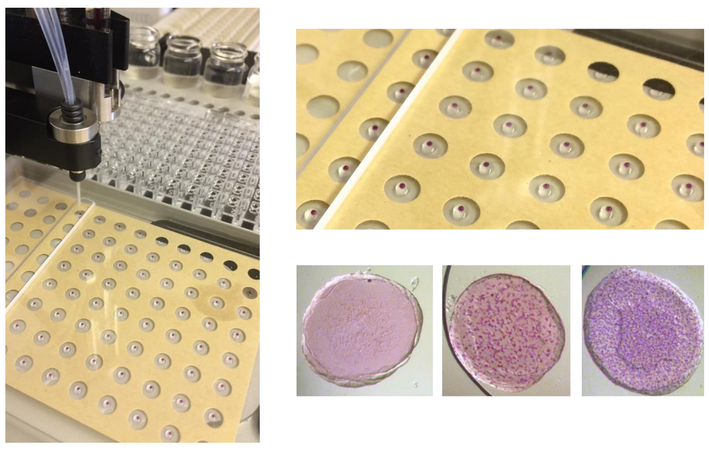
Introduction

- Inspired by the success of random Microseed Matrix Screening (rMMS), we have adapted rMMS to the crystallization of membrane proteins in LCP.
- LCP seed stock is made by scaling up LCP crystallization conditions without changing critical parameters.
- Seed crystals are grown directly in LCP, and (as with conventional rMMS) seeding is combined with an additive experiment.
- We used the method with the bacterial integral membrane protein OmpF: without microseeding, one new hit was found, but with LCP-rMMS eight new hits were found.
- We also demonstrate a method of generating seed gradients, which allows the number of crystals to be varied.

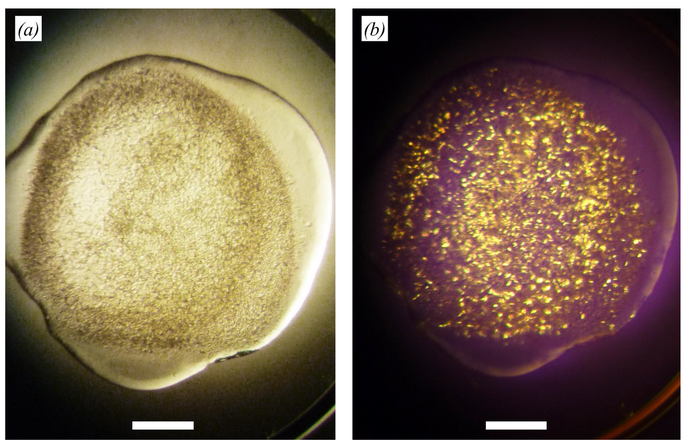
Preparing the seed stock



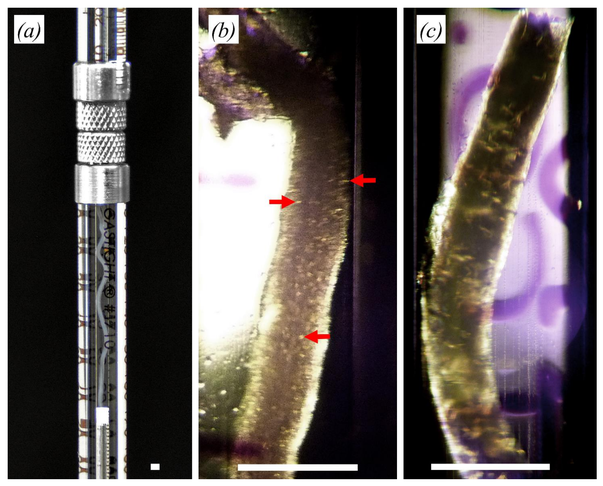
1. Identify Initial Hit



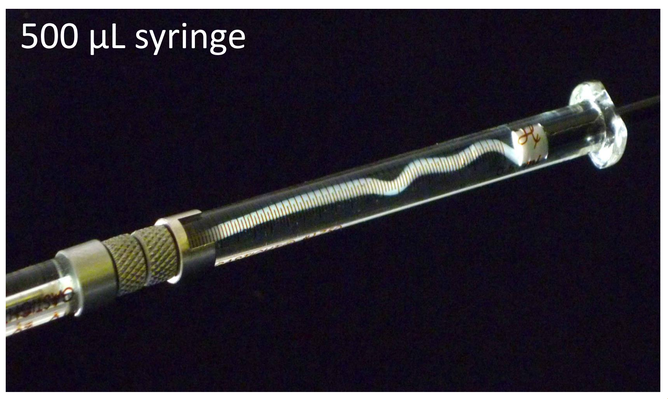
- Screening experiments were dispensed to glass sandwich plates.
- We identified wells with high nucleation to make the seed stock.
- We used a published crystallization condition: 1.25 M K thiocyanate, 2.1 M Li nitrate and 0.1 M Na acetate pH 4.6. (Efremov & Sazanov, 2012)



2. Make the LCP Seed Stock



- Inject LCP into a syringe containing the crystallization cocktail.
- When the first sign of nucleation is observed, collect the LCP seed stock mixture and dispense straight away.
- It is important to collect the LCP seed stock before the crystals grow too much to ensure there is un crystallized protein in the LCP seed stock mixture.
- Using a 250µL or 500µL syringe allows more seed stock to be produced. It also improves reproducibility by better re-creating the crystallization environment and dimensions important to crystallization of a sandwich plate.

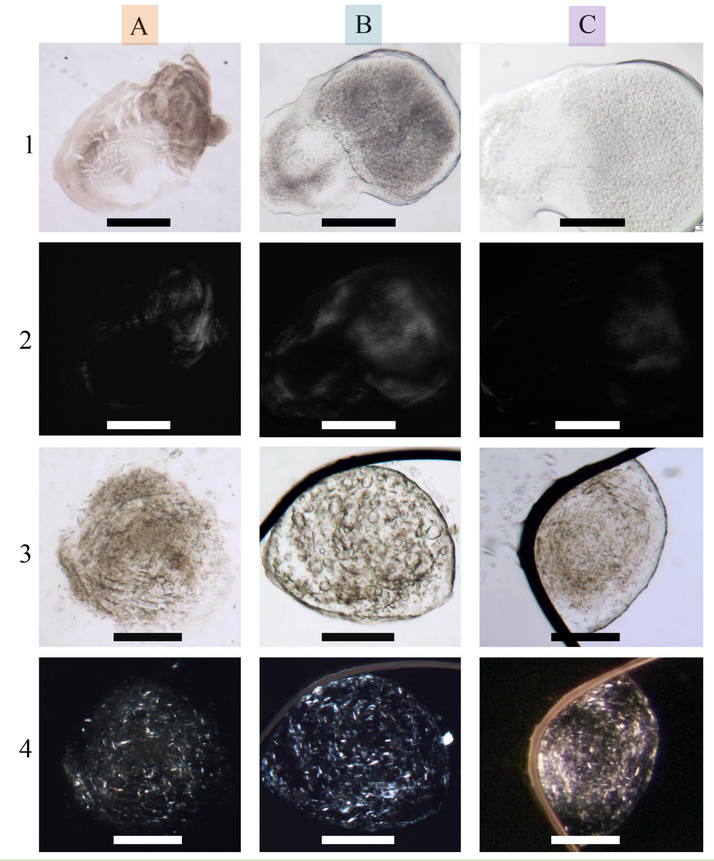


3. Dispense LCP rMMS Experiment

- Dispense the LCP seed stock to new screen.
- We found new hit conditions using the LCP-rMMS method.
- The main precipitant of the new hits were in all cases organic (PEG, ethanol or MPD), whereas the seed crystals were high-salt conditions.

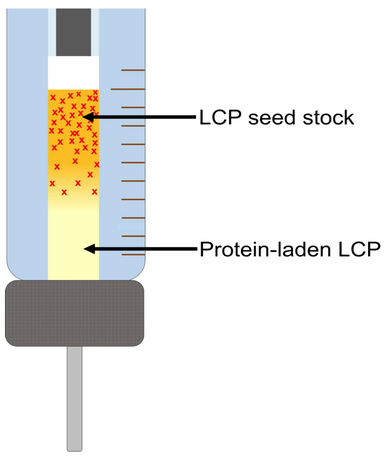
Summary of new hits obtained in 120 conditions using LCP-rMMS.

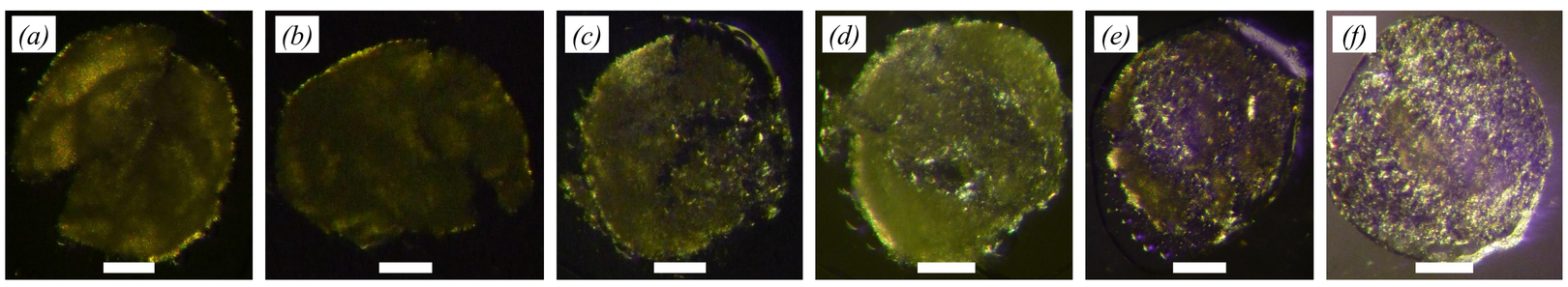
			Without seeding		With seeding	
Crystallization screen	Condition No.	Chemical composition	Crystals?	Crystal size (µm)	Crystals?	Crystal size (µm)
JCSG+	17	40% MPD, 5% PEG 8K, 0.1 *M* sodium cacodylate pH 6.5	–		Yes	20–30
JCSG+	18	40% ethanol, 5% PEG 1K, 0.1 *M* phosphate–citrate pH 4.2	–		Yes	20–30
JCSG+	22	50% PEG 200, 0.2 *M* $MgCl_2$, 0.1 *M* sodium cacodylate pH 6.5	–		Yes	5–10
JCSG+	30	40% PEG 300, 0.1 *M* phosphate–citrate pH 4.2	–		Yes	10–20
JCSG+	43	40% PEG 400, 0.2 *M* lithium sulfate, 0.1 *M* Tris–HCl pH 8.5	–		Yes	10–20
JCSG+	53	40% MPD, 0.1 *M* CAPS pH 10.5	–		Yes	20–30
JCSG+	64	20% Jeffamine M-600 pH 7.0, 0.1 *M* Na HEPES pH 7.5	Yes	10–20	–	
JCSG+	66	10% MPD, 0.1 *M* Na bicine pH 9.0			Yes	10–20
Membrane 1	5	48% PEG 400, 0.2 *M* $CaCl_2$, 0.1 *M* HEPES pH 7.5	–		Yes	10–20



Preparation of LCP Gradient

- The ability to control the number of crystals per drop by diluting the seed stock is an important advantage of the rMMS method as applied to soluble proteins.
- An LCP seed stock gradient can be produced by mixing LCP seed stock and LCP without seeds together into a single syringe .
- The mixture was dispensed into a sandwich plate where all wells contained an aqueous solution that had previously given crystals with the LCP seed stock.
- A gradient effect was observed, however the length of the gradient was comparatively short, resulting in wells with clusters of crystals within clear LCP.





Key Points

- We conclude that an LCP seed stock can easily be produced within gas-tight syringes by following a simple protocol.
- This protocol allows LCP crystallization conditions to be scaled up very easily and reliably because the physical dimensions that are critical for crystallization are maintained.
- We tested the method with the membrane protein OmpF from E. coli. The method worked well, increasing the number of hits from one to nine and identifying several new precipitants that supported crystallization.
- It is possible to dispense a LCP seed stock gradient, however we would have preferred to see individual crystals that were well separated from other crystals to encourage the growth of large crystals.

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