MS49-O4 Microseed matrix-screening (rMMS): introduction, theory, practice and a new technique for membrane protein crystallization in LCP

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Random Microseed Matrix-Screening (rMMS), where seed crystals are added automatically to random crystallization screens, is a significant recent breakthrough in protein crystallization [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 emerged [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. 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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MS49-O5 The Highs And Lows Of Extreme Conditions Crystallisation

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Classical crystallisation of compounds commonly occurs through the evaporation of a solvent from a solution of dissolved material. This solution is allowed to become supersaturated, enabling nucleation and crystal formation/growth to occur. Although this approach is highly successful there are situations where alternative methods need to be employed. One example is observed when the desired product exists in the liquid phase under standard conditions; evaporation in this case then only leads to the loss of material, and not a crystalline material suitable for diffraction studies.

It is well understood that perturbing a material from nominally ambient conditions, via the application of pressure or reduced temperatures, can lead to a change in phase. If the perturbations are suitably controlled they can lead to the spontaneous crystallisation of samples that are liquid at room temperature and pressure. The large degree of flexibility in thermodynamic variables and the way in which they are applied in this method affords many, subtly different, routes to the solid state. This in turn opens up a pathway to a polymorphic playground of possibilities.

The techniques of in-situ crystallisation via high pressure and low temperature will be explained and highlighted with example case studies. This ‘how to …’ will demonstrate the practicalities, optimisations and tricks that will enable users to enhance their success rates with these techniques.

Keywords: crystallisation, extreme conditions, high pressure, low temperature