

MOLECULAR DIMENSIONS MONOGRAPH SERIES®

Controlling nucleation in crystal growth

In screening for crystal growth, once a lead has been found, optimization experiments are performed to slow down or reduce the amount of nucleation. If nucleation competes with crystal growth then microcrystals or spherulites may result. To remove aggregates and micro-particulate matter spin filters offer a convenient way to filter sample solutions. External nucleants can be added in a controlled manner to filtered samples, or use streak seeding.

Containerless crystallization (Chayen N.E. *Protein Engineering* (1996) 9, 10, 927-929) between oils (MD2-05) avoids surface effects which can induce excessive nucleation. This method is employed in the easy to use GelledSurface™ Kit (MD1-12).

Another useful approach is to separate nucleation from crystal growth. Whilst the metastable zone is considered to be an optimum zone for crystal growth it is possible to sustain but not initiate crystal growth. Ideally, crystal nuclei have to be transferred to metastable conditions. Various means of achieving this have been used such as seeding, diluting microbatch drops, and cell constructions that allow reservoir conditions to be changed during crystal growth. A simple method for growing crystals in the metastable zone using the vapour diffusion technique has been described by Saridakis & Chayen (*Protein Science* (2000), 9:755-757), in which the authors obtained better diffracting crystals compared to conventional methods. Coverslips holding hanging drops were first incubated at conditions that normally gave many small crystals, and then transferred, before the appearance of crystals, to reservoirs at concentrations that normally would yield clear drops. The 3D Structure Screen (MD1-13) presents a simple adaptation of this dilution method in a screening kit format.

Traditional approaches to seeding avoid the need for creating conditions for spontaneous nucleation. Therefore seeding can be used to test changes to crystal growth conditions. (Stura E.A. (1999) *Strategy 3: Reverse Screening*. In "Crystallization of Proteins: Techniques, Strategies and Tips. A laboratory manual" (Bergfors T. ed.) International University Line pp113-124.) An interesting approach to seeding comes to light if we reverse this observation and ask – what if spontaneous nucleation conditions are screened as potential seed stocks? In effect this is what is reported by Saridakis. Ideally in this manner drops from "successful wells" containing crystal nuclei will be transferred to metastable conditions for crystal growth.

Although the seed stock in such an experiment cannot be seen, it follows that conditions that ultimately gave rise to crystals must have nucleated. Therefore drops from "nucleation wells" for these conditions could be used as "Quality seed stocks" for different growth conditions in other wells.

