Expected results:
By loosening the cap you are allowing evaporation for a limited time. What you are effectively doing is inducing nucleation which you then arrest when re-sealing. This will drive the system into supersaturation and hopefully give you a hit which you can then proceed to optimise using the technique of your choice.

The method can also be used for optimization as described in Govada, L. and Chayen, N.E. (2009) *Crystal Growth & Design* 9, 1729-1732.

INTRODUCING NUCLEANTS INTO CRYSTALLIZATION TRIALS

Chayen N.E., Saridakis E., Sear, R.P. (2006) *PNAS* 103, 597-601
Saridakis, E. and Chayen, N.E. (2009) *Trends in Biotechnology* 27, 99-106
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Saridakis, E. and Chayen. N.E (2013) *Trends in Biotechnology* 31, 515-520.
Khurshid *et al.* (2014) *Nature Protocols* 9 (2014)Pages: 1621–1633.
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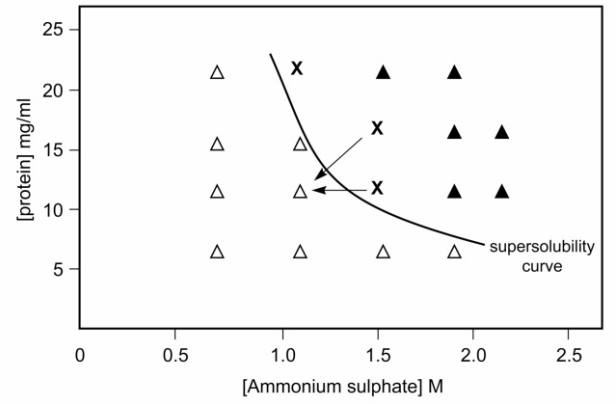
Govada *et al.* (2016) *Scientific Reports - Nature* 6:20053 DOI: 10.1038/srep20053
http://www.imperialinnovations.co.uk/CRMIP

First, determine the metastable zone as follows:
1. Note the conditions in a 'hit' of a screen that have yielded poor crystals or a crystalline precipitate.
2. Dispense (manually or by a robot) 10-24 trials using a crystallization method of your choice, varying the protein and precipitant concentrations in steps (as instructed in point 3 below) on a matrix grid.
3. The concentrations of protein and precipitating agents should be above and below the concentrations that gave the conditions of the hit. Alternatively the pH, temperature, or another parameter to which solubility is sensitive can be varied.
4. Plot the results and you will obtain the supersolubilty curve. e.g. diagram below.:For example, if a screen produced 'hits' at conditions of 10-22 mg/ml of protein and 1-1.5 M Ammonium sulphate, the experiments are set at concentrations ranging from 5-25 mg/ml protein versus 0.5-2.2 M ammonium sulphate, thus covering a range of conditions above and below the 'hits. The area just below the curve is the metastable zone. **X** represent conditions obtained from screening 'hits', giving low quality crystals or crystalline precipitate. Δ represent clear drops; ▲ represent precipitate.



Insert your nucleant at conditions just below the curve (where the heads of the arrows are pointing) using fine tweezers and let the trial incubate as you would normally do.

Nucleant are used mostly for optimization but can also be used for screening. When nucleants are placed in supersaturated conditions, crystals will usually appear faster.

References on crystallization methods in microbatch and vapour diffusion (and some in microgravity)

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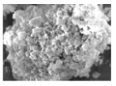


Molecular Dimensions

moleculardimensions.com

Naomi's Nucleant MD2-07

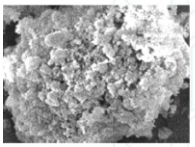
Towards a 'universal' nucleant for protein crystallization,
Developed and patented at Imperial College London, the most effective nucleant of any material tested.

Method of use:

- Simply add a single grain* to a crystallization drop. *Easy to place with fine tweezers or a whisker.*
- Use in screening or optimization to nucleate supersaturated conditions.
- Use in optimization where excessive nucleation occurs (i.e. lots of tiny crystals). *Back off the precipitant concentration to the metastable zone and then use a grain to nucleate.*
- Negates twinning.
- Protein crystals are easily detached from the nucleant using a whisker or a cryo loop.

Introduction

This material (bio-glass) (CaO-P_2O_5-SiO_2) has a highly porous surface with cavities of similar sizes to proteins. It is hypothesised that the cavities entrap protein molecules, thereby encouraging nucleation and crystal formation.

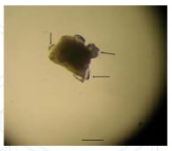


Scanning electron micrograph showing the highly porous nature of this material.

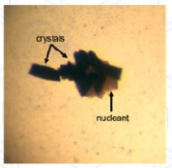
To date Naomi's Nucleant has facilitated the crystallization of 14 proteins, the highest number reported for any single nucleant. Many of these proteins have proven difficult to crystallize and some of these, including membrane proteins, have only been crystallized in the presence of Naomi's Nucleant.

In addition to test proteins those proteins that can be named are: multi drug resistance protein (a membrane protein), modified cyclodextrine, oxyntomodulin, myosin binding protein C, lobster shell α-crustacyanin, c-phycocyanin, α-actinin actin binding protein. Several other proteins have also been crystallized but cannot be named at this time.

Often the crystals obtained were of increased diffracting quality compared to those resulting from standard techniques. For example myosin binding protein C diffracted to 1.6Å compared to 3Å.



Crystals (arrowed) of β-lactamase grown on a grain of Naomi's Nucleant by Rosalida leone at imperial College, London.



Crystals of lobster shell α-crustacyanin grown on a grain of Naomi's Nucleant.

Contact Us

Molecular Dimensions Ltd. would be very grateful if investigators were prepared to provide feedback on their own experiences with Naomi's Nucleant. Crystallization reports or pictures can be e-mailed to enquiries@moleculardimensions.com

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*** Please note:** There is a wide variety of grain sizes in each vial. Every grain is useable as a nucleant – even the very small ones (which are still much larger than a protein molecule!)

Ordering information

Naomi's Nucleants	1 vial	MD2-07
(approx 3mg)	(approx. 300 grains)	
Fine tweezers	1	MD9-25

Molecularly Imprinted polymers

http://www.imperialinnovations.co.uk/CRMIP

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