Molecular Dimensions

Soluble Proteins

3D Structure Screen MD1-13

3D Structure Screen - Improve crystal quality by separating nucleation and growth. Formulated for growing protein crystals in the metastable zone

A targeted sparse matrix kit of 48 × 10 mL conditions.

Features of 3D Structure Screen:

- Separate nucleation from crystal growth.
- A simple method for growing crystals in the metastable zone.
- Contains 48 conditions covering a range of pH, PEGs and salt additives.
- Obtain much larger and better diffracting crystals compared to conventional methods.

Introduction

This is a method to get nucleation "going" and then "back off" before the nucleation becomes excessive. It can be used for optimisation when small useless crystals are formed, and improvement cannot be obtained neither by fine-tuning the concentrations of the protein and precipitating agents nor by addition of additives. In practice, cover-slips holding the drops are incubated for some time over reservoir solutions that normally give many small crystals and after given times the cover-slips are transferred over reservoirs with lower precipitant concentrations that would normally yield clear drops. This method can also be used for screening.

Background

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 (Figure 1). Various means of achieving this have been used such as seeding, diluting microbatch drops, and well constructions that allow reservoir conditions to be changed during crystal growth.

The 3D Structure Screen presents a simple adaptation of the dilution method to the hanging drop vapour diffusion technique in a screening kit format. In this kit, the crystal growth trial is performed as a two-step method. Firstly, conditions are set-up for nucleation and then the drops on their coverslips are transferred to wells with a lower concentration of precipitant for crystal growth (Figure 2).

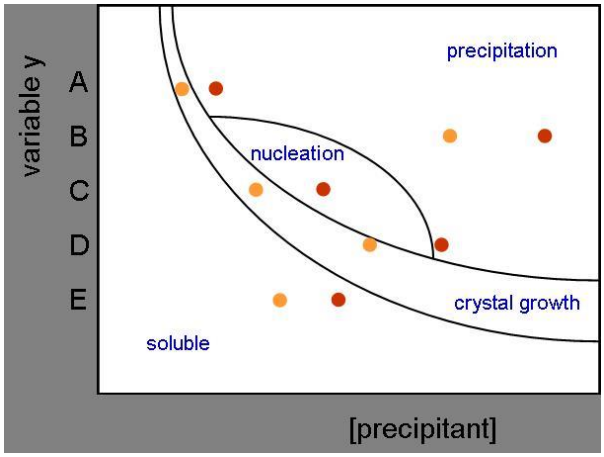

Figure 1. A schematic of a phase diagram showing position of initial (black dots) crystallization conditions and new positions (grey dots) with 'backed-off' conditions.

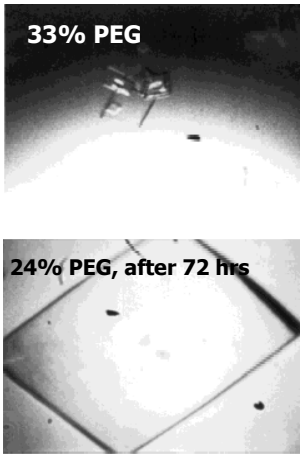

Figure 2. Successful crystallization after 'backing-off' procedure.

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Method of use #1

Set up a 24-well crystal growth plate in the normal manner using solutions 1-1, 2-1, 3-1, etc…24-1. Incubate protein drops (often 1µl plus 1µl of reservoir solution) as hanging drops on coverslips for 24 hours. Then transfer the coverslips to a second plate prepared with solutions 1-2, 2-2, 3-2, etc. …24-2. Examine this plate daily for crystal growth in the normal manner.

Method of use #2

Set-up the full kit as a 48 reagent screen. Examine the drops after 24 hours. For wells where precipitate has formed with the high concentration precipitant (set 1) and nothing can be seen in the corresponding drop from set 2, repeat the trial with the appropriate set 1 reagents and transfer to a corresponding set 2 reagent well after 6 hours.

Examine the original trays after 48 hours and where precipitate has formed in set 1 but nothing in set 2 repeat the trial with these conditions from set 1 and do a transfer to the set 2 reagent at 12 hours.

Method of use #3 - Quality seeding

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[2]. 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 Saradakis, E. & Chayen, N. Although a seed stock in the first part of the experiment (nucleation) 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 other different growth conditions. In this third method of use "seeds" from nucleation drops may be added to precipitant drops for the crystal growth conditions to seed crystal growth.

Formulation Notes

3D Structure Screen reagents are formulated using ultrapure water (>18.0 MΩ) and are sterile-filtered using 0.22 µm filters. No preservatives are added.

Molecular Dimensions will be happy to discuss the precise formulation of individual reagents.

Individual reagents and stock solutions for optimisation are available from Molecular Dimensions.

Enquiries regarding 3D Structure Screen formulation, interpretation of results or optimisation strategies are welcome. Please e-mail, fax or phone your query to Molecular Dimensions.

References

1. Saradakis & Chayen (Protein Science (2000), 9:755-757).
2. Chayen & Saradakis (2002) Acta Cryst. D58, 921.
3. 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

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3D Structure Screen Conditions 1 -48 MD1-13

Screen #	Conc.	Salt	Conc.	Buffer	pH	Conc.	Precipitant1
1-1	0.02 M	Calcium chloride dihydrate	0.1 M	Sodium acetate	4.6	30 % v/v	MPD
1-2	0.2 M	Ammonium acetate	0.1 M	Sodium acetate	4.6	30 % w/v	PEG 4000
1-3	0.2 M	Ammonium sulfate	0.1 M	Sodium acetate	4.6	25 % w/v	PEG 4000
1-4	2.0 M	Sodium formate	0.1 M	Sodium acetate	4.6		
1-5	1.0 M	Ammonium phosphate monobasic	0.1 M	Sodium citrate	5.6		
1-6	0.2 M	Magnesium acetate tetrahydrate	0.1 M	Sodium cacodylate	6.5	20 % w/v	PEG 8000
1-7	0.2 M	Magnesium acetate tetrahydrate	0.1 M	Sodium cacodylate	6.5	30 % v/v	MPD
1-8	0.2 M	Sodium acetate trihydrate	0.1 M	Sodium cacodylate	6.5	30 % w/v	PEG 8000
1-9	0.2 M	Zinc acetate dihydrate	0.1 M	Sodium cacodylate	6.5	18 % w/v	PEG 8000
1-10	0.2 M	Calcium chloride dihydrate	0.1 M	Sodium HEPES	7.5	28 % v/v	PEG 400
1-11	1.6 M	Potassium/sodium phosphate	0.1 M	Sodium HEPES	7.5		
1-12	1.5 M	Lithium sulfate	0.1 M	Sodium HEPES	7.5		
1-13	1.4 M	Sodium citrate tribasic dihydrate	0.1 M	Sodium HEPES	7.5		
1-14	2.0 M	Ammonium sulfate	0.1 M	Sodium HEPES	7.5	2 % v/v	PEG 400
1-15			0.1 M	Sodium HEPES	7.5	20 % w/v	PEG 4000
						10 % v/v	2-Propanol
1-16	2.0 M	Ammonium sulfate	0.1 M	Tris	8.5		
1-17	0.2 M	Magnesium chloride hexahydrate	0.1 M	Tris	8.5	30 % w/v	PEG 4000
1-18	0.2 M	Sodium citrate tribasic dihydrate	0.1 M	Tris	8.5	30 % v/v	PEG 400
1-19	0.2 M	Lithium sulfate	0.1 M	Tris	8.5	30 % w/v	PEG 4000
1-20			0.1 M	Tris	8.5	8 % w/v	PEG 8000
1-21	0.2 M	Ammonium sulfate				30 % w/v	PEG 8000
1-22	4.0 M	Sodium formate					
1-23	0.05 M	Potassium phosphate monobasic				20 % w/v	PEG 8000
1-24						30 % w/v	PEG 1500
2-1	0.02 M	Calcium chloride dihydrate	0.1 M	Sodium acetate	4.6	21 % v/v	MPD
2-2	0.2 M	Ammonium acetate	0.1 M	Sodium acetate	4.6	21 % w/v	PEG 4000
2-3	0.2 M	Ammonium sulfate	0.1 M	Sodium acetate	4.6	17.5 % w/v	PEG 4000
2-4	1.4 M	Sodium formate	0.1 M	Sodium acetate	4.6		
2-5	0.7 M	Ammonium phosphate monobasic	0.1 M	Sodium citrate	5.6		
2-6	0.2 M	Magnesium acetate tetrahydrate	0.1 M	Sodium cacodylate	6.5	14 % w/v	PEG 8000
2-7	0.2 M	Magnesium acetate tetrahydrate	0.1 M	Sodium cacodylate	6.5	21 % v/v	MPD
2-8	0.2 M	Sodium acetate trihydrate	0.1 M	Sodium cacodylate	6.5	21 % w/v	PEG 8000
2-9	0.2 M	Zinc acetate dihydrate	0.1 M	Sodium cacodylate	6.5	12.6 % w/v	PEG 8000
2-10	0.2 M	Calcium chloride dihydrate	0.1 M	Sodium HEPES	7.5	19.6 % v/v	PEG 400
2-11	1.12 M	Potassium/sodium phosphate	0.1 M	Sodium HEPES	7.5		
2-12	1.05 M	Lithium sulfate	0.1 M	Sodium HEPES	7.5		
2-13	0.98 M	Sodium citrate tribasic dihydrate	0.1 M	Sodium HEPES	7.5		
2-14	2.0 M	Ammonium sulfate	0.1 M	Sodium HEPES	7.5	1.4 % v/v	PEG 400
2-15			0.1 M	Sodium HEPES	7.5	14 % w/v	PEG 4000
						7 % v/v	2-Propanol
2-16	1.4 M	Ammonium sulfate	0.1 M	Tris	8.5		
2-17	0.2 M	Magnesium chloride hexahydrate	0.1 M	Tris	8.5	21 % w/v	PEG 4000
2-18	0.2 M	Sodium citrate tribasic dihydrate	0.1 M	Tris	8.5	21 % v/v	PEG 400
2-19	0.2 M	Lithium sulfate	0.1 M	Tris	8.5	21 % w/v	PEG 4000
2-20			0.1 M	Tris	8.5	5.6 % w/v	PEG 8000
2-21	0.2 M	Ammonium sulfate				21 % w/v	PEG 8000
2-22	2.8 M	Sodium formate					
2-23	0.05 M	Potassium phosphate monobasic				14 % w/v	PEG 8000
2-24						21 % w/v	PEG 1500

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Abbreviations:
Sodium HEPES; 2-(4-(2-Hydroxyethyl)-1-piperazinyl)ethanesulfonic Acid Sodium Salt, **MPD;** 2,4-methyl pentanediol, **PEG**; Polyethylene glycol, **Tris**; 2-Amino-2-(hydroxymethyl)propane-1,3-diol,

Manufacturer's safety data sheets are available from our website or by scanning the QR code here:

Re-Ordering details:

Catalogue Description	Pack size	Catalogue Code
3D Structure Screen	48 x 10 mL	MD1-13
Heavy + Light (3D Structure Screen) Twin Pack HT-96	48 x 1 mL (x 2)	MD1-35
Eco Screens		
3D Structure Eco Screen	48 x 10 mL	MD1-13-ECO
Heavy + Light (3D Structure Screen) Twin Pack HT-96 Eco Screen	48 x 1 mL (x 2)	MD1-35-ECO
Single Reagents		
3D Structure Screen single reagents	100 mL	MDSR-13-tube number
Heavy + Light (3D Structure Screen) Twin Pack HT-96 single reagent	100 mL	MDSR-35-well number

For 3D Structure Screen stock solutions please visit the Optimization section on our website.