A Vapor Batch Method for Volatile Organics

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Introduction

Combinations of precipitants have often proved more successful in obtaining crystals than the individual precipitant alone. In some cases a few percent of a precipitant such as PEG 400 or an organic solvent have been essential additives for nucleation to occur. In other cases, 10-20% of a co-precipitant has been used, in addition to the primary precipitant, to achieve good quality crystals. A study by Majeed *et al.* (2003) demonstrated that the use of precipitant mixtures significantly enhanced both the probability of crystallization as well as the quality of optimized crystals.

For low temperature data collection, the crystals need to be transferred into an artificial mother liquor containing cryoprotectant before freezing. When crystals are grown in the presence of volatile organic solvents, crystal damage and loss of diffraction quality often result during this transfer procedure. This frequently occurs with the hanging drop vapour diffusion technique where the crystals are exposed to the atmosphere before removal from the drop.

Here, a novel vapor batch method for protein crystallization is described, using a volatile alcohol, isopropanol and PEG 3350 as co-precipitant.

The method uses specially designed vapor batch plates (Douglas Instruments).

Difficulties encountered during crystal handling for the N-terminal domains of N and B tropic MLV capsid proteins were overcome by use of this method, allowing easier manipulation of crystals, improved resolution and decreased mosaicity.

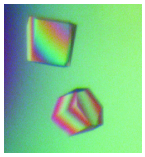
Several other proteins; haemagglutinin, catalase and glucose isomerase, have been crystallized using the vapor batch method in our lab.

References: Enhancing protein crystallization through precipitant synergy. Majeed, S. *et al.*, (2003) Structure, 11 1061-1070.

High resolution structure of a retroviral capsid hexameric amino-terminal domain.

Gulnahar B. Mortuza, Lesley F. Haire, Anthony Stevens, Stephen J. Smerdon, Jonathan P. Stoye & Ian A. Taylor (2004) Nature 431 481-485.

Crystallization of NTD of N-MLV capsid



- Crystals were initially grown in hanging drops from Crystal screen solution no.40; 20% PEG 4000, 20% v/v isopropanol, 0.1M sodium citrate, pH 5.6, (Hampton) 20mg/ml protein in the drop.
- Major problem-harvesting crystals in the presence of isopropanol.
- The crystals disintegrated as soon as the coverslip was opened.

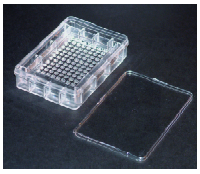
Attempts to overcome the problem

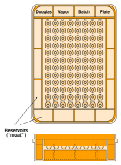
- By using sitting drops,
- Oil over the drops,
- Handling crystals using constant humidity was only partially successful.
- Using microbatch experiments under oil. Crystals were not stable and dissolved after a couple of days.
- Crystals that were X-rayed had high mosaicity and could not be used for structure solution.

Vapor batch crystallization

- Vapor batch trays (Douglas Instruments) designed for both microbatch and vapor diffusion (sitting drop) crystallization.
- The tray has 96 wells in the centre and several reservoirs around the outside – the "moat".
- Water-filled moat preserves microbatch crystals.
- Vapor diffusion experiments can be carried out where up to 96 wells are equilibrated against a single precipitant e.g. ammonium sulphate (AS), NaCl.

Vapor Batch trays (Douglas Instruments)





Procedure

Droplets (2µl) were dispensed under a mixture of silicone/ paraffin oil (Al's oil) using IMPAX 1-5 crystallisation robot.

A 6x4 spreadsheet was set up with xstep software varying: protein, 16-22 mg/ml; PEG 3350, 13-16%; 0.1M sodium citrate pH 5.6.

10% v/v isopropanol was pipetted into the tray's "moat" and the drops equilibrated overnight at 18°C.

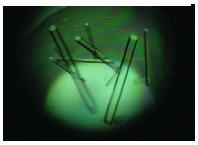
Next day, the 10% v/v isopropanol solution was replaced by 20% v/v isopropanol.

This method was used to grow crystals of NTD N-MLV capsid protein:-

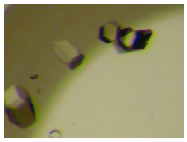
- Crystals appeared after a couple of days.
- Typically they were harvested and frozen after 10 days.
- Crystals were very stable in drops for at least 6 months.
- Diffraction to 1.9Å with low mosaicity.
- Crystals did not grow in the controls without isopropanol in the moat.

NTD N-tropic MLV- capsid protein

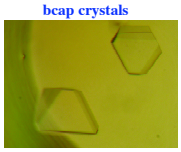
Native crystals



selenomethionine crystals



bcap crystals



An example of a homemade screen for vapor batch with isopropanol.

Salt/Buffer screen

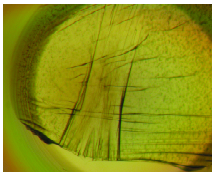
Salt	Citrate pH 6.0	CAC pH 6.5	Imidazole pH 7.0	HEPES pH 7.5	TrisCl pH 8.0	TrisCl pH 8.5
AS	1	2	3	4	5	6
KSCN	7	8	9	10	11	12
MgCl2	13	14	15	16	17	18
NaK tartrate	19	20	21	22	23	24
KCl	25	26	27	28	29	30
AmAc	31	32	33	34	35	36
AmP04	37	38	39	40	41	42
MgS04	43	44	45	46	47	48

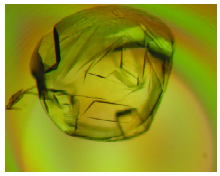
Final concentrations: 0.2M salt, 0.1M buffer when mixed 1:1 with protein.

The moat initially contained 10% v/v isopropanol. This was increased to 20% v/v after a couple of days.

Crystals of glucose isomerase (40mg/ml) were grown in 2 of the 48 conditions. After 1 year, 11 droplets contained protein crystals (checked by needle crush test).

Glucose isomerase crystals





Advantages of vapor batch crystallisation

- Improved crystal stability
- Easier crystal handling.
- Better diffraction from crystals grown under paraffin/silicone oil mixture.

Use of vapor batch trays for screening with volatile solvents.

Low ionic strength PEG screens

e.g. grid screen, varying pH and PEG concentration.

Duplicate trays can be set up +/- isopropanol (or other volatile organic in the moat) to test the effect of the organic solvent on the crystallization.

High salt screens

Isopropanol with AmS04 or P04 grid screen

- set up duplicate trays, +/-10% isopropanol in the moat.

The same principle could be used to test isopropanol (or other volatile additive) with any selected screen dispensed in VB trays.

Acknowledgements

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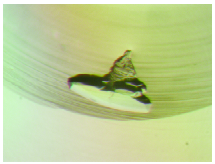
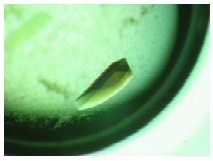
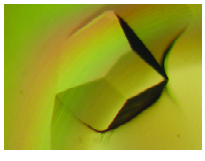
Crystals grown by Vapor Batch with PEG/Isopropanol



haemagglutinin
12% PEG 3350, 50mM HEPES pH 7.5



Catalase
12% PEG 3350, 50mM HEPES pH 6.0



Glucose isomerase:
12% PEG 3350, 50mM HEPES pH 7.5