MOLECULAR DIMENSIONS MONOGRAPH SERIES©

Improving crystal quality

Anthony Duff, (School of Molecular and Microbial Biosciences, University of Sydney, Australia), (A.Duff@usyd.edu.au) has carried out a quick survey of peoples' experiences with methods for improving crystallizability and/or resolution of diffraction of proteins (Feb. 2005). Anthony posted a summary of the compiled results to ccp4bb with the following comments, and has kindly agreed for us to reproduce them here.

It appears that cryoannealing is not particularly successful, despite many people trying it. Anthony suggested that fits with it being very easy to do at the point when you intend to throw the crystal away anyway. It is suggested that cryoannealing is just a fairly brutal dehydration method. Dehydration methods seem to be sometimes, but not frequently, successful. Gluteraldehyde was useful in the past in extending the lifetime in the beam of a room temperature crystal. However, some people find that gluteraldehyde can still be very useful even with cryocrystallography. Modification of the protein, whether by methylation of amines, adding BME to Cys or mutating away floppy things, seems to give excellent results with a high probability.

Addition of additives/heavy metals/divalent metals is suggested as a method that often improves crystals.

Most people who responded to the survey recommended nearly everything, even if it had never worked for them. The meaning of "improvement" was left up to the responder to decide. However, Anthony considered it to include the growth of crystals where previously no crystals were obtained, the growth of bigger crystals, the increase of resolution of diffraction of crystals, or the decrease of mosaicity of crystals enabling better data collection.

After these results were posted Marcus D. Collins (Cornell University) mentioned a much more controlled robust protocol for crystal annealing, that is reported to achieve better results than the "take it out of the cold stream and put it back in" method. (Kriminski et al, Acta Cryst **D58** 459-471).

Key to the Survey Results:
Ruins, the method made the crystals/crystallisation worse.
No effect, the method had negligible effect.
Good! an improvement was obtained.
Fantastic, a fantastic improvement was obtained.

Method of crystal improvement	Number of attempts	Best Result	Typical Result	Recommended
Cryoannealing	60	Good!	No effect	No
Cryoannealing	5	No effect	Ruins	No
Cryoannealing	5	Ruins	Ruins	No
Cryoannealing	2	Ruins	Ruins	Yes
Cryoannealing	30	Good!	No effect	Yes
Cryoannealing	5	Ruins	Ruins	Yes
Cryoannealing	5	Ruins	Ruins	Yes
Cryoannealing	5	Ruins	Ruins	No
Cryoannealing	6	Ruins	No effect	Yes
Cryoannealing	30	No effect	Ruins	Yes
Cryoannealing	50	Good!	Good!	Yes
Cryoannealing (1-3 minutes) after dehydrating		No effect		
Cryoannealing (1-3 minutes without previous dehydrating		Good!		
Cryowet annealing	20	Good!	Good!	Yes
Annealby transfer to room temp cryoprotectant	5	No effect	No effect	Yes
Dehydration	1	No effect	No effect	Yes
Dehydration with PEG	22	Good!	Good!	Yes
Dehydration with PEG	3	Good!	Good!	Yes
Dehydration with PEG	5	Ruins	Ruins	Yes
Dehydration with PEG	10	No effect	No effect	Yes
Dehydration with PEG 400	40	Good!	No effect	Yes
Dehydration with PEG 400	40	Good!	No effect	Yes
Dehydrating the crystal by post-growth addition of PEG/Glycerol etc.	3	Good!	No effect	Yes
Dehydration with 1,6-Hexanediol	10	Good!	No effect	Yes
Dehydrating the crystal by post-growth addition of salt	21	Good!	Good!	Yes
Dehydration (by equilibration with the vapour pressure of a different osmolyte) after fishing		FANTASTIC		

Method of crystal improvement	Number of attempts	Best Result	Typical Result	Recommended
the crystal into the loop				
Dehydrating the crystal by waving sample in loop in the air	5	No effect	No effect	Yes
Try finding different cryo-conditions or room temperature diffraction		FANTASTIC		Yes
Annealing with crystals in oil		Ruins	Ruins	
Rehydration with cryo of dried crystals	10	No effect	Ruins	No
Gluteraldehydesoak	15	FANTASTIC	No effect	Yes
Gluteraldehydesoak	30	Good!	Good!	Yes
Gluteraldehydesoak	3	Good!	Good!	Yes
Gluteraldehydesoak	12	Ruins	Ruins	No
Methylating surface lysines	1	FANTASTIC	FANTASTIC	Yes
Methylating surface lysines	1	Ruins	Ruins	Yes
Modification of surface cysteines with BME	8	FANTASTIC	No effect	Yes
Mutating 1-3 surface residues	27	FANTASTIC	Good!	Yes
Mutating surface disordered loops	29	FANTASTIC	FANTASTIC	Yes
Mutating surface residues, especially removing glycosylation sites		FANTASTIC	Good!	Yes
Removing purification tags	60	FANTASTIC	No effect	
Co-crystallization with heavy metals	18	Good!	Good!	Yes
Treatment of existing crystals with heavy metals	10	FANTASTIC	No effect	Yes
Adding additives	20	FANTASTIC	FANTASTIC	Yes
Limited proteolysis of protein for crystalization	20	Good!	No effect	No
Limited proteolysis of protein followed by re-cloning	20	FANTASTIC	No effect	Yes
Optimization of prot:precipitant	20	FANTASTIC	Good!	Yes