Improvement of crystal order and cryo-behaviour: Supply of organic solvent to the naked protein crystal

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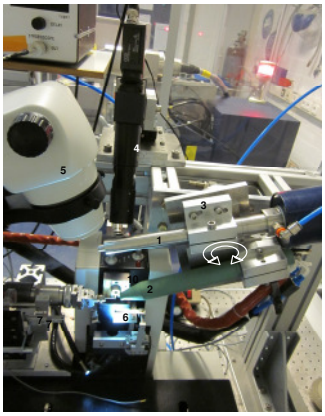
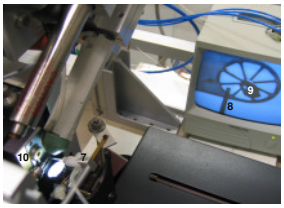
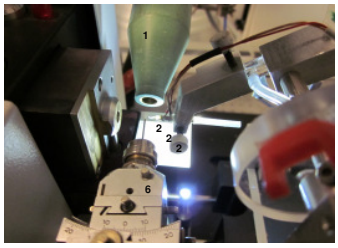
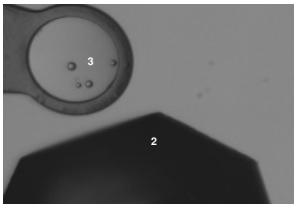


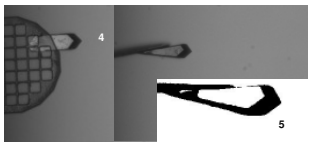


1 Cryo-nozzle
2 Humidifier-nozzle (FMS)
3 Rotational switch (pneumatic)
4 Video System with optics
5 Stereo Microscope
6 Light
7 Sucking device
8 Glass capillary
9 Crystal
10 Xray collimator

CryoSwitch: hardware setup – combined with Xray-camera
Crystal preparation: removal of crystal surrounding liquid in the humidified gas stream by glass capillary

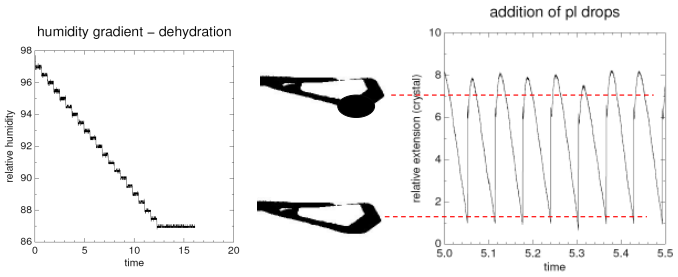






1 Humidifier-nozzle (FMS)
2 PicoDropper
3 Picoliter drops in oil film
4 Naked crystal
5 Binary image of the crystal
6 Goniometer head

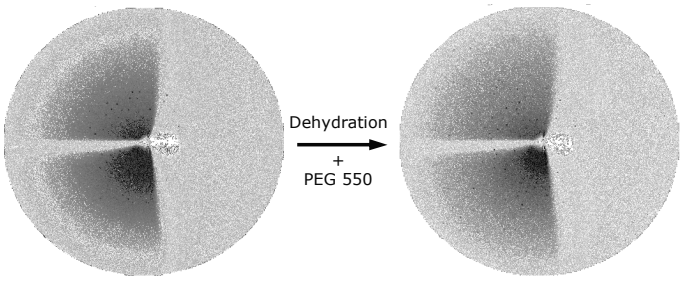
PicoDropper: hardware setup
controlled addition of liquid to the naked protein crystal



Accumulation of organic solvent in the crystal:
Apply of humidity gradient (dehydration)
+ (in parallel)
supply of organic liquid to the naked crystal by pl drops

Dropping is triggered by the crystal extension measurement

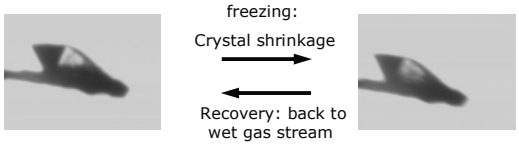
Example 1: Influenza nucleoprotein
Crystallisation condition 15% PEG4000
Problem: diffraction power 5 Å, after freezing 7,5 Å



Trick: Apply of humidity gradient (97% → 87%, 1% steps in 45s)
+
addition of 50% PEG550 to the naked crystal
→ improvement of diffraction power to 3,5 Å
→ Freezing in oil with no loss of crystal quality
→ 2,7 Å at synchrotron

Reference: „Inhibition of influenza virus replication via small molecules that induce the formation of higher-order nucleoprotein oligomers", Gerritz et al, PNAS 2011 (in press)

Example 2: PI3Kδ in complex with p85
Crystallisation condition 16% PEG6000
Problem: - diffraction power prior freezing 3,0 Å, after freezing 7 Å
- any change of the crystallisation solution damages the crystal lattice
- crystal shrinkage induced by the freezing process



Trick: Apply of humidity gradient (97% → 80% 0,15% steps in 15s)
+
addition of 2 M TMAO to the naked crystal
+
cryoSwitch (quick replacement of wet gas stream by the cryo stream)

→ no loss of diffraction power by freezing
→ fully reproducible results
→ 2,7 Å at synchrotron

Reference: „Trimethylamine N-oxide as a versatile cryoprotective agent in macromolecular crystallography", C. Mueller-Dieckmann et al, J. Appl. Cryst. (2011). 44, 433-436

Features:

- **Direct screening of the effect of organic solvent (glycerol, PEG, TMAO, ..) to the crystal**
- **Display of crystal response at every process step by X-rays**
- **Crystal optimization and crystal freezing – separated only by a mouse click**
- **Good reproducibility of experiments**
- **Automation of the processes**
- **General method for crystal freezing**
- **Reversible switch - a second chance for your crystal**
- **Controlled crystal annealing**
- **Naked crystal - easy crystal alignment**
- **Best anomalous signal – no interference with crystal surrounding solution**