Crystal Dehydration

A novel device for hydration control of macromolecular crystals is available on ESRF beamlines.

The diffraction properties of crystals can often be improved by controlled dehydration. The EMBL and ESRF have developed, and now operate as standard, a novel device for controlling crystal hydration while mounted on a standard macromolecular crystallography beamline. This allows the fine-tuning of the dehydration protocol and enhances the possibility to fully characterise a given system, thus increasing the chances of finding a suitable dehydration protocol. In addition, the ease and simplicity of its use makes these experiments feasible within a reasonable time.

Among the different effects that may be observed during dehydration are space group changes, unit cell shrinkage, mosaic spread changes, spot profile improvement and an increase in diffraction resolution limits.

The device delivers an air stream of precise relative humidity that can be used to alter the amount of water in macromolecular crystals and is rapidly installed. Samples are mounted on mesh loops, and the progress of dehydration can be monitored both optically and by the acquisition of diffraction images. Once the optimal hydra-tion level is obtained, cryo-cooling is easy to achieve by hand or by using a sample changer.

Practical Details

What do I need ?

Several unfrozen crystals from approximately the same crystallisation condition which you do not mind sacrificing

- Mother liquor solutions
- Mesh loops mounted on SPINE bases
- ESRF pucks filled with empty SPINE standard vials

How long does it take ?

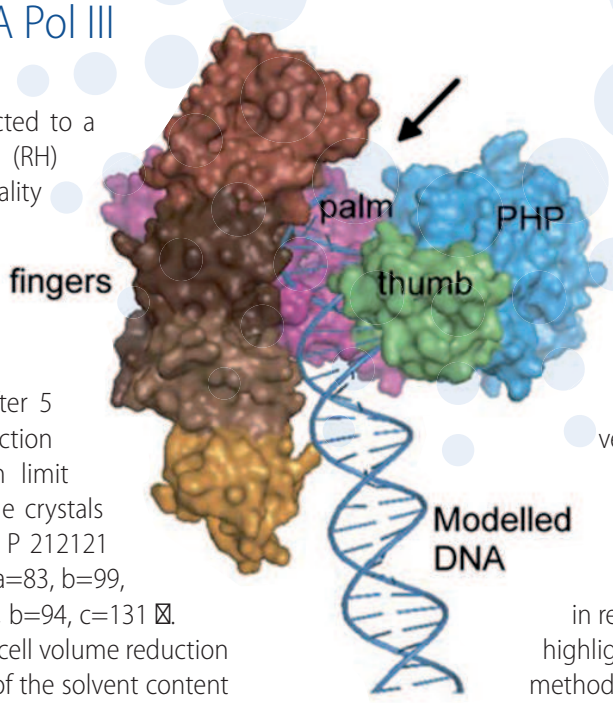
- A quick test can be made to see if your crystal system is susceptible to changes in dehydration within a few hours.
- Dehydration protocols vary considerably between different crystal systems so refining a dehydration protocol can be time-consuming work and requires several crystals. It usually takes 24 hours to explore and refine a dehydration protocol.
- Once a protocol is found, crystals can be conditioned rapidly and stored for data collection on a different beamline.

Will it work ?

- In 10-20% of cases, dehydration results in an increase in the observed diffraction limit.
- If your crystal has a high solvent content and/or low symmetry, dehydration may improve it.
- If you have observed variability in cell dimensions after cryo-cooling, this may indicate that your system can be easily changed by dehydration.

CASE STUDY

Escherichia coli DNA Pol III



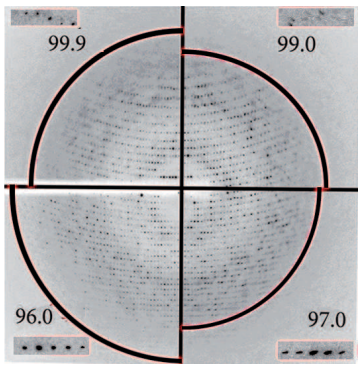
When these crystals are subjected to a descending Relative Humidity (RH) gradient, a decrease in the quality of the diffraction is observed at around 96% RH, such that it is not possible to index the diffraction pattern until the RH reaches 89%. Here, the diffraction starts to recover. After 5 to 10 minutes at 88% RH, diffraction is restored and the resolution limit increases by 1Å to ca 2.8 Å. The crystals undergo a transition; the initial P 212121 unit cell with dimensions of ca a=83, b=99, c=144 Å, is reduced to ca a=83, b=94, c=131 Å. This corresponds to a 14% total cell volume reduction and an approximate reduction of the solvent content from 57% (v/v) to 50%.

These crystals have been previously reported to increase their diffraction limits when dehydrated using the FMS (Lamers et al., 2006, Sanchez-Weatherby et al. 2009). Despite the fact that the two devices work in a very different manner, these crystals undergo the same transition. The changes happen at almost identical RH values and the increase in resolution is comparable. This highlights the robust nature of the method and its reproducibility.

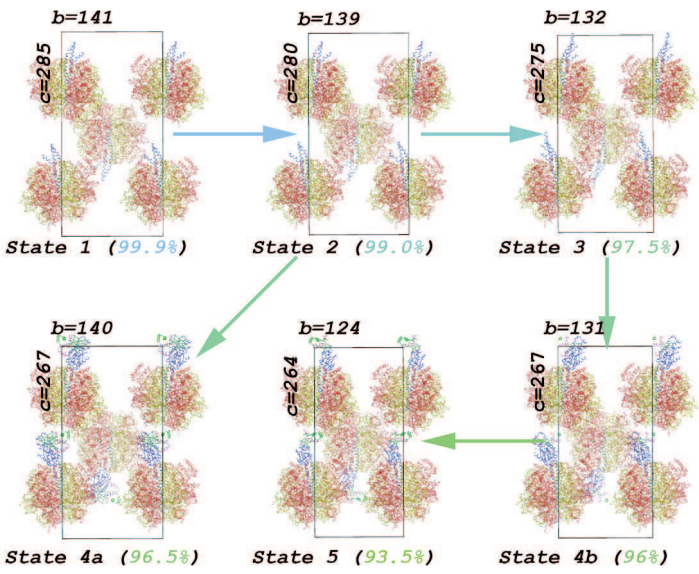
CASE STUDY

Bovine F_1-ATPase

Crystals of bovine F1-ATPase demonstrate more complex behaviour when subjected to controlled dehydration. These crystals have previously been characterised when dehydrated using the FMS (Bowler et al., 2006b, a, 2007); however, the ease and simplicity of the new device, coupled with the much greater time resolution that a synchrotron beamline offers, has allowed a much more detailed study of the changes undergone by these crystals upon dehydration. This system has furthered our understanding of the process of crystal dehydration and has helped in defining the general principles involved.



Changes in X-ray diffraction of F_1-ATPase crystals during dehydration. Each quadrant shows the different resolution limits of each dehydration state: 3 Å, 3.8 Å, 4 Å and 2.5 Å . The inserts show a magnified view of the same area on the detector, demonstrating the improvement in Bragg peak profile after dehydration.



The structures of the different transition states of F_1-ATPase. Crystal packing of F_1-ATPase crystals at each dehydration state. The different unit cells are shown viewed along the a axis. The asymmetric unit and symmetry related particles are shown as Cα traces coloured by subunit (α- red, β- yellow, γ- blue, δ- magenta and ε- green). Arrows indicate the different paths that crystals can follow.