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Inducing phase changes in crystals of macromolecules: Status and perspectives for controlled crystal dehydration

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ABSTRACT

The increase in the number of large multi-component complexes and membrane protein crystal structures determined over the last few years can be ascribed to a number of factors such as better protein expression and purification systems, the emergence of high-throughput crystallization techniques and the advent of 3rd generation synchrotron sources. However, many systems tend to produce crystals that can be extremely heterogeneous in their diffraction properties. This prevents, in many cases, the collection of diffraction data of sufficient quality to yield useful biological or phase information. Techniques that can increase the diffraction quality of macromolecular crystals can therefore be essential in the successful conclusion of these challenging projects. No technique is universal but encouraging results have been recently achieved by carrying out the controlled dehydration of crystals of biological macromolecules. A new device that delivers a stream of air with a precisely controlled relative humidity to the complicated sample environment found at modern synchrotron beamlines has been conceived at the EMBL Grenoble and developed by the EMBL and the ESRF as part of the SPINE2 complexes project, a European Commission funded protein structure initiative. The device, the HC1b, has been available for three years at the ESRF macromolecular crystallography beamlines and many systems have benefitted from on-line controlled dehydration. Here we describe a standard dehydration experiment, highlight some successful cases and discuss the different possible uses of the device.

1. Introduction

Phase changes in crystalline samples can be effected by a number of means. High pressure can be used to induce phase changes that alter lattice packing or physically change the positions of the molecules within the lattice, see for example (Boldyreva et al., 2006; Fourme et al., 2002). In less robust systems, such as crystals of biological macromolecules, changes in the relative humidity (RH) of the air surrounding the sample, or in the molar fraction of water in the mother liquor that surrounds them, can also induce phase changes. However, in the case of biological macromolecules, the effects are greatest on the order and arrangement of the lattice, with the effect on the molecules themselves being limited (Bowler et al., 2006a; Kachalova et al., 1991; Morozova and Morozov, 1982; Sanchez-Weatherby et al., 2009). Given the large solvent content (50–80%) and tendency to disorder of protein crystals, methods that can reduce these effects are of great interest. From the earliest days of macromolecular crystallography (MX) the effects and potential benefits of crystal dehydration have been recognised (Berthou et al., 1972; Einstein and Low, 1962; Huxley and Kendrew, 1953; Perutz, 1946). However, the lack of a method to accurately control and change the RH of the gas surrounding a crystal and, crucially, characterise changes in diffraction quality during dehydration, has limited the use of this technique to a few cases, with improvement in diffraction often discovered by chance (Abergel, 2004; Adachi et al., 2009; Cramer et al., 2000; Esnouf et al., 1998; Fratini et al., 1982; Gupta et al., 2010; Heras et al., 2003; Kuo et al., 2003; Nakamura et al., 2007; Sam et al., 2006; Vijayalakshmi et al., 2008; Yap et al., 2007). Many techniques have been

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developed to dehydrate crystals (Amunts et al., 2007; Heras and Martin, 2005; Newman, 2006) but as these often involve the transfer of crystals between solutions, they can prove difficult to reproduce. In order to dehydrate crystals reproducibly, a number of dedicated devices have been designed that allow control of the RH surrounding a crystal (Einstein, 1961; Huxley and Kendrew, 1953; Pickford et al., 1993; Sjogren et al., 2002). One of the most successful has been the Free Mounting System (FMS) as it allows the dehydration of crystals while monitoring its effects using X-ray diffraction (Kiefersauer et al., 1996, 2000). This device demonstrated the effectiveness and usefulness of a well controlled dehydration device on the diffraction properties of crystals (Bowler et al., 2006a,b, 2007; Dobbek et al., 1999; Engel et al., 2003; Henrich et al., 2003; Koch et al., 2004; Kyrieleis et al., 2005). However, the design of the FMS was not very compatible with the crowded experimental environment found at a modern synchrotron beamline. This stimulated, within SPINE2, the design of a new device that would allow dehydration experiments to be performed at a synchrotron beamline, with minimal perturbation to the experimental configuration, allowing use of standard equipment such as a sample changer. The result is a humidity control device (HC1b) (Sanchez-Weatherby et al., 2009) which can deliver a precisely controlled stream of humidified air to the sample position with no interference to the standard experimental constellation. The device has been in regular use at the ESRF for three years and can now be requested as standard equipment for all experiments. Here we describe the experience gained following regular use of the HC1b at a synchrotron beamline, some results and some new applications of the device.

2. Materials and methods

The device has been designed to be ergonomic, easy to install and compatible with a standard MX beamline environment. Based on the nozzle of a standard cryostream, the device can be quickly and easily installed as the beamline is already equipped with the appropriate mounting (Fig. 1A and B). The components and operation of the device have been fully described (Sanchez-Weatherby et al., 2009) and will not be considered here. Instead, the experimental method and some results will be presented. No two samples react in the same way to dehydration but, in the cases where a resolution increase is observed, these are typically in the range of ~6 Å improving to ~4 Å or ~3 Å improving to ~2 Å. A typical dehydration experiment is composed of four steps that are outlined below.

2.1. Determination of the mother liquor equilibrium relative humidity

At the start of a dehydration experiment, the equilibrium RH of the mother liquor of the crystal being studied must be determined. If the value is too high the crystal will dissolve and if too low changes in the crystal may be effected too quickly without an indication of initial diffraction quality. The equilibrium point is found experimentally by placing a drop of the mother liquor in a loop

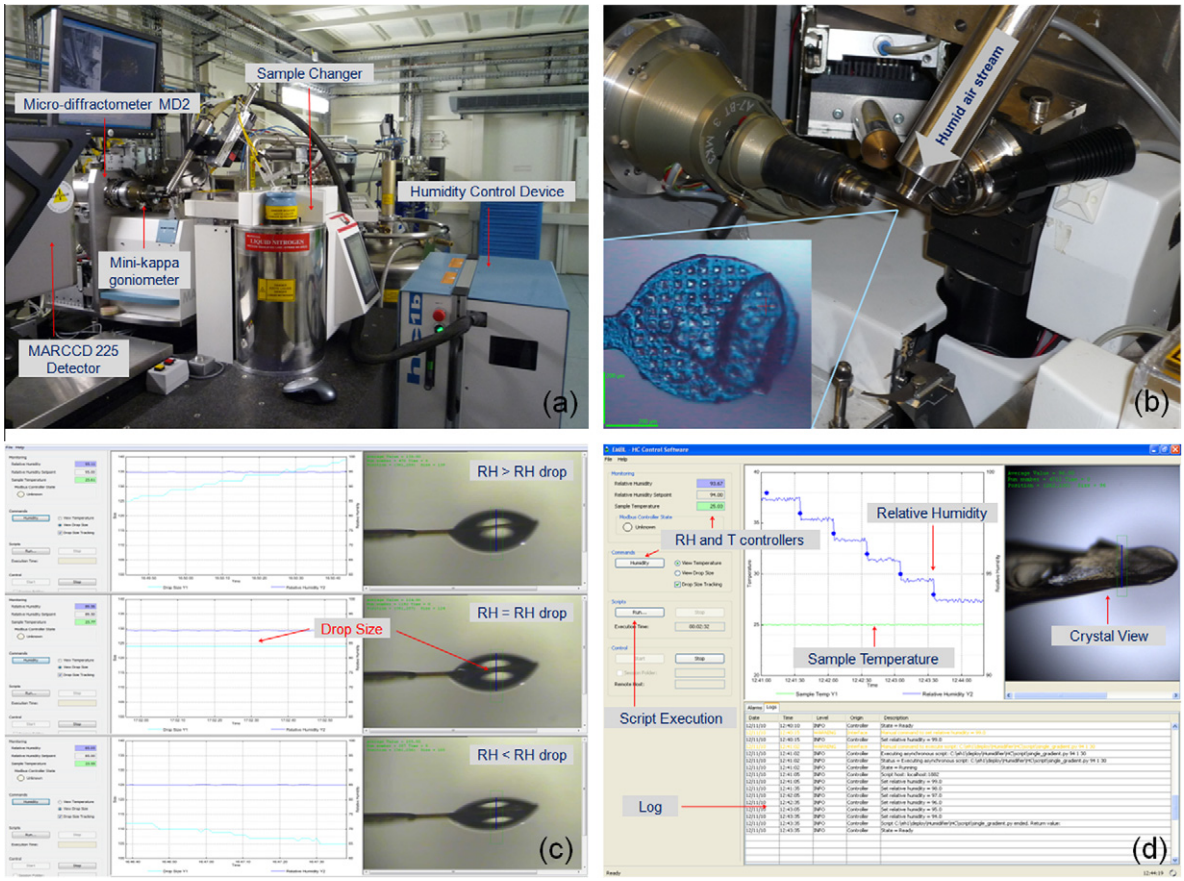

Fig. 1. (a) The humidity control device HC1 installed in the BM14 experimental hutch (ESRF). (b) Detail of the sample environment. The crystals are mounted in mesh loops and are kept under the humid air stream during the dehydration process. (c) Automatic drop area recognition and tracking of the drop size for the determination of the initial relative humidity value. The drop size is plotted against time (cyan) and the RH value is adjusted until the drop reaches the equilibrium with the humid air stream. (d) The Java/Python HC1 control software showing all the functions available: relative humidity and temperature controllers, script execution, time plot graph of humidity, sample temperature and/or drop size and crystal image.

at a chosen starting RH and monitoring the size of a defined region of the drop using specific image processing software (Fig. 1C). An increasing size indicates that the RH is too high, a decreasing size indicates the RH is too low. Once the point of RH equilibrium has been determined this is used as the starting point for all further experiments with a particular system. While the RH equilibrium for certain salt solutions is well documented (Rockland, 1960) no such information exists for commonly used crystallization buffers. In order to define an approximate value for the starting point of experiments, the HC1b was used to measure the relative humidity of binary solutions of the most commonly used cryoprotective agents and precipitants with deionized water. Solutions were made gravimetrically at 50.0% w/w (sucrose, glucose, xylitol, glycerol, and the PEGs 8000, 4000, 2000, 1000, 600, 400 and 200), and for ethylene glycol a range of concentrations was used (80.0%, 60.0%, 50.0%, 40.0%, 30.0%, 20.0%, 15.0%, 10.0% and 5.0% w/w), see Fig. 2. Concentrations may be converted from *w/w* to *w/v* using: $(w/v) = (w/w) \times \rho$, where ρ is the density of the liquid (Alcorn and Juers, 2010). This yields, for EG, PEG200-PEG8000, glycerol, xylitol, glucose and sucrose: 50% (w/w) = 53%, 54%, 57%, 59%, 61%, 62% (w/v), respectively. A round LithoLoop (Molecular Dimensions, Suffolk, UK) was mounted on the HC1b and a drop of the solution of interest was placed on the loop with a pipette. The humidity output by the HC1b was adjusted until the drop diameter was stable. This was repeated a few times until the drop diameter was stable upon initial placement on the loop. The samples were tested at ambient temperature ranging between 21.5 and 22.0 °C. It can be seen that for ethylene glycol the relative humidity scales linearly with the molar fraction of water and that decreasing the molecular weight of PEGs also decreases the equilibrium RH (Fig. 2). This allows a pre-selection of starting points as an estimate can be made from the precipitant concentration. For example, 50% (w/w) glycerol has a molar fraction of water of 0.84 and an equilibrium RH of 85%; mid range PEGs vary between 0.95 and 0.97 mol fraction of water and are between 93% and 95% RH (Fig. 2). This greatly facilitates the initial stages of an experiment.

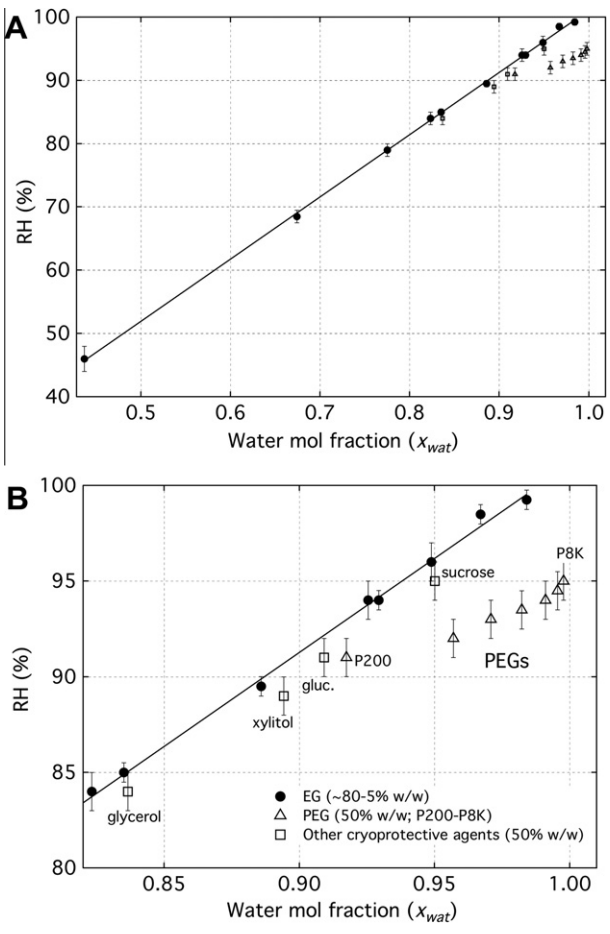

Fig. 2. A. Plot of relative humidity (RH) vs. mol fraction of water ($x_{wat} = \frac{\#\text{moles water}}{\#\text{moles water} + \text{moles cryoprotective agent}}$) for binary solutions of ethylene glycol (filled circles), cryoprotective agents (squares) and precipitants (diamonds) with water. The line is a linear fit to the ethylene glycol data points. B. Detail of the higher x_{wat} values showing the precipitants and cryoprotective agents. EG = ethylene glycol. PEG = polyethylene glycol.

2.2. Inducing a phase change

Once the equilibrium RH has been found, a crystal can be mounted in the air stream. Changes in the RH surrounding a crystal are transmitted through the lattice more efficiently when crystals are mounted free from surrounding mother liquor. We have found that mesh loops are the best support for crystals as the contact area between bare crystals and the mount is much greater, thereby preventing crystal slippage or loss. After mounting, excess mother liquor is removed with a wick and an initial diffraction image taken to evaluate the diffraction qualities of the crystal and determine the initial lattice parameters. These can then be compared to results from previous experiments and used to determine the reference point for comparison during a dehydration experiment. Experience has demonstrated that a cautious approach should be used when starting dehydration as transformations can be induced with only very small changes in the RH. A reduction in the RH that is too fast may lead to beneficial transitions being missed. Therefore, a slow reduction in the RH (usually in steps of 0.5%) with an equilibration period of 5 min (or longer if crystals are large) is advocated. This should be continued until a change is observed. The changes most commonly observed at this stage are transformations in point group; unit cell parameters; mosaic spread; an increase in the overall intensity (particularly the $\langle I/\sigma I \rangle$ in the high resolution bins) and profile changes in the Bragg peaks. The diffraction limit may increase or diffraction may disappear completely. As the experiment is conducted at room temperature, the effects of radiation damage must be accounted for. In general, the exposure time is limited to the minimum required that yields sufficient data for characterisation of the diffraction pattern. In the case of lengthy dehydration, where the effects of radiation damage are apparent before a change has been induced, a new crystal must be mounted. The crystal can then be subjected to dehydration to the same RH as the previous crystal but with only a single exposure, upon mounting, to compare crystal quality. At this stage important information has been gained – whether dehydration has an effect on the sample and at what RH it occurs.

2.3. Optimising the phase change

Once a change has been observed the protocol must be optimised. In many cases, a transition is observed during step 2 of the experiment but an improvement in diffraction properties is only achieved by altering the dehydration protocol. The rearrangements that occur between symmetry related molecules are often complex and vary enormously depending on the crystal system. Therefore, it is crucial that a number of different protocols are tried in order to induce these changes in a manner that leads to the most ordered configuration. The variables include: the RH step size; equilibration time; annealing (cycling repeatedly between different RH values); number of steps and total time for the protocol. These variables have to be investigated thoroughly as the optimum rearrangement of symmetry-related molecules will sometimes only occur with very specific speed and timing of dehydration.

2.4. Cryocooling

While the HC1b can be used for room temperature data collection it is usually necessary to cryocool crystals in order to collect a data set of the best quality with reduced radiation damage. As the crystals are mounted in the absence of mother liquor, they can usually be cryo-cooled directly without the need for a cryoprotectant. This is typically performed using a sample changer (Cipriani et al., 2006; Jacquamet et al., 2009; Ohana et al., 2004; Snell et al., 2004) as conditioned crystals can be stored and easily transferred for subsequent data collection. With sample changers using wet mounting protocols the crystal is simply plunged into the liquid nitrogen contained in the vial. With dry mounting protocols unmounting is performed with the end effector warm and then the sample plunged into liquid nitrogen. In the absence of a sample changer, samples can be cryocooled by unmounting by hand and plunging into liquid nitrogen using cryo-tongs. In a few cases, crystals cannot be directly cryocooled and a cryoprotectant must be added. This is achieved by adding a small drop of oil or cryoprotectant solution to the crystal just before unmounting with a sample changer. The question of why crystals usually do not require added cryoprotectant, and curiously, why some do, is still open. The fact that the crystals are free from surrounding mother liquor probably accounts for part of their capacity to be cryocooled without added penetrative cryoprotectants. The hydrogen bonding networks of the remaining water molecules may inhibit the nucleation of ice crystals within the solvent channels. This has been demonstrated for slow cooling of protein crystals (Warkentin and Thorne, 2009).

2.5. Methods for examples

Cryoprotective agents were purchased from Acros Organics (Geel, Belgium), with the exceptions of glucose, xylitol, glycerol (Sigma–Aldrich, Lyon, France), PEG 2000 (Alpha Aesar, Schiltigheim, France) and PEG 400 (Labosi, Elancourt, France). Crystals of pea (*Pisum sativum* var. *alaska*) chloroplast photosystem I (PSI) were kindly provided by Prof. Nathan Nelson (Department of Molecular Biology and Biochemistry, Tel Aviv University, Israel). Crystals of human phosphoglycerate kinase (hsPGK) were grown as previously described (Cliff et al., 2010; Zerrad et al., 2011). Crystals of *L. lactis* β-phosphoglucomutase (βPGM) were grown as described (Baxter et al., 2010). Amyloid fibres were prepared as described (Tiggelaar et al., 2011).

3. Results and discussion

The HC1b has been in service for three years at the ESRF and is now also available at other synchrotrons (DLS, UK; MAX-Lab, Sweden and CLS, Canada). Many samples have been found that benefit from controlled dehydration with one recently published example (Kadlec et al., 2011). However, as many are not yet published and remain confidential we will outline four successful cases that highlight the effects seen from dehydration.

3.1. Improvement in the diffraction limit of crystals of plant photosystem I

An example of the type of crystals of a large macromolecular complex for which diffraction limits dramatically improve as a result of controlled dehydration with the HC1b is pea chloroplast photosystem I (PSI). Plant photosystem I is the most common multisubunit membrane protein complex on Earth, which together with photosystem II harnesses the energy from sunlight and converts it to chemical energy. Undehydrated crystals of this large (530 kDa) membrane protein complex usually diffract poorly ($d_{min} \approx 6$ Å). When conditioned with the HC1b, these crystals undergo a transition between their RH humidity of 99% and 97%. A significant improvement in diffraction limit from *ca* 6 Å to 4 Å is seen (measured by the resolution bin at which $\langle I/\sigma I \rangle \geqslant 2$), accompanied by visual improvements in diffraction quality (Fig. 3). While the space group remains monoclinic there is a reduction in the lengths of all axes of the unit cell and an increase in the β angle of 5° (Table 1). The crystals were extremely sensitive to the dehydration protocol. A reduction of RH below 97% destroyed the order in the lattice and a single step reduction in RH from 99% to 97% was required to induce the phase change that produced the improved diffraction limit. Increasing PEG concentrations was already known to improve the diffraction properties of crystals of PSI dramatically (Amunts et al., 2007), using this protocol d_{min} is increased to 3.85 Å and in comparison to the results obtained here it is clear that further transitions can be achieved, further reducing the unit cell volume and increasing the β angle and the diffraction limit using this method of dehydration (Table 1). However, using the HC1b has advantages over PEG dehydration, in particular the ability to consistently reproduce improved diffraction with a controlled dehydration protocol.

3.2. Conditioning and cryocooling of crystals of human phosphoglycerate kinase

While controlled dehydration often leads to improvements in the diffraction limit its use can also have other advantages. Here, the benefit of avoiding using cryoprotective agents in human phosphoglycerate kinase crystals (hPGK) is described. PGK catalyses the transfer of a phosphoryl group from 1,3-bisphosphoglycerate (BPG) to ADP in the first ATP-generating step of glycolysis. Crystals of the open conformation of hPGK were grown by vapour diffusion in a buffer containing 26% PEG 4000 (w/v) and displayed excellent external morphology. However, the addition of all cryoprotective agents tried resulted in a complete disintegration of the crystals. Crystals mounted and cryocooled directly from the mother liquor also resulted in poor quality diffraction with ice rings (Figure 4A) and a low resolution limit ($d_{min} \approx 3.0$ Å) that could not be indexed. Controlled dehydration was then attempted as a method to cryocool the crystals. The equilibrium RH of the mother liquor was measured at 98.5% RH and crystals were mounted using micromeshes (MiTeGen, Ithica, NY, USA). Dehydration below 97.5% resulted in a loss of diffraction; however, the removal of mother liquor and an equilibration of 10 min at 97.5% RH allowed the crystals to be cryocooled without the need for cryoprotectant. After conditioning with the HC1b the crystals diffracted to 1.8 Å with no ice rings and excellent data processing statistics (Table 2 and Fig. 4B). The small shift, 1% in RH, illustrates the level of sensitivity of some protein crystals to changes in relative humidity and the accuracy that can be achieved with the HC1b. The ability to remove the mother liquor surrounding a crystal in a stable and controlled manner allowed data collection from a previously intractable system.

3.3. Room temperature data collection

The device also provides the means for routine room temperature data collection. Here, the isomerase β-phosphoglucomutase (βPGM) from *L. lactis* was investigated. As a model system for the study of phosphoryl transfer, complexes of the protein with metal fluorides have been studied by X-ray crystallography in combination with ^{19}F-NMR (Baxter et al., 2009, 2010). However, the question arose as to the similarity between observed fluorine resonances measured at ambient temperatures and the atomic positions determined by X-ray crystallography at 100 K. To this end, a room temperature data set was collected using the HC1b on beamline ID14-1. Crystals of the βPGM glucose-6-phosphate

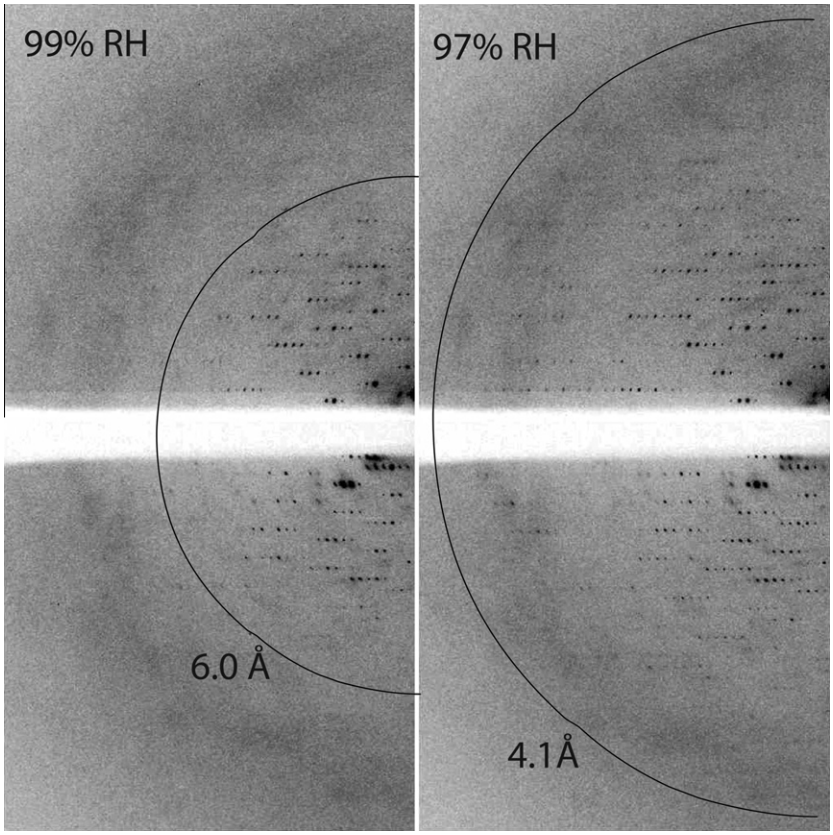

Fig. 3. Changes in the X-ray diffraction limit of crystals of plant photosystem I. After decreasing the RH to 97% in a single step the diffraction limit of the crystals increases from 6 to 4 Å accompanied by an increase in the β angle of 5° and a 7% reduction in the unit cell volume. Resolution limits were defined by the highest resolution bin at which $\langle I/\sigma I\rangle \geqslant 2$.

Table 1
Changes induced in crystals of pea chloroplast photosystem I during dehydration.

RH (%)	Space group	*a* (Å)	*b* (Å)	*c* (Å)	*β* (°)	Mosaic spread (°)	$\langle I/\sigma I\rangle = 2$ (Å)
99	$P2_1$	131	199	141	86.5	0.6	5.8
97	$P2_1$	128	194	137	91.8	0.4	4.1
PEG dehydrated[a]	$P2_1$	120	189	128	95.2	–	3.85[b]

[a] Data from (Amunts et al., 2007).
[b] From a cryocooled crystal on the ESRF microfocus beamline ID23-2.

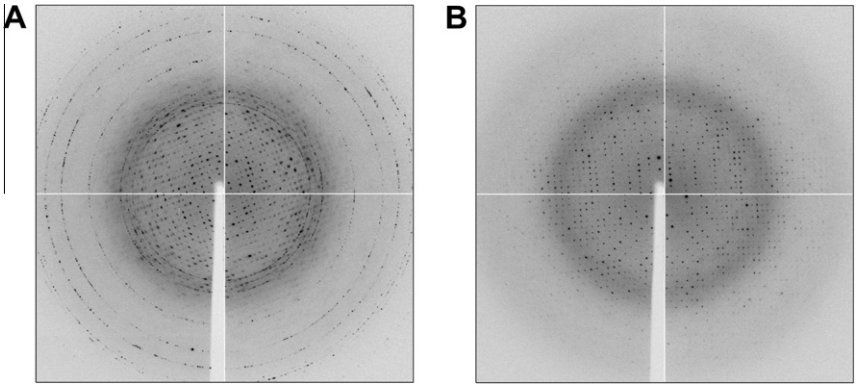

Fig. 4. Cryocooled crystals of human phosphoglycerate kinase. A. Crystals of human phosphoglycerate kinase cryocooled directly from the mother liquor. Diffraction quality is poor, ice rings are present and the resolution is low (d_{min} = 3.0 Å) no indexing solution was found. B. Crystals mounted at 98.5% RH and equilibrated at 97.5% for 10 min, then cryocooled. Diffraction is of excellent quality and no crystalline ice is present. A data set was collected to 1.77 Å.

MgF_3^- complex were mounted directly from the mother liquor on micromesh loops at an RH of 97%, the equilibrium RH determined for the crystallisation conditions (28% PEG 4000 (w/v) and 250 mM sodium acetate). As no dehydration was foreseen in the experi-

Table 2
Data processing and refinement statistics for conditioned crystals of hsPGK and room temperature data collection on a crystal of βPGM.

Structure	hsPGK 3PG	Room temperature β-PGM.G6P.MgF_3^-
Space group	$P2_12_12$	$P2_12_12_1$
Wavelength (Å)	0.933	0.933
Unit cell dimensions (Å) a, b, c	61.4, 72.8, 93.4	37.9, 54.6, 105.7
Resolution range (Å)	20–1.77	20–1.47
Number of unique reflections	41,038	39,199
Multiplicity[a]	3.5 (3.4)	2.3 (2.2)
Completeness[a] (%)	98.7 (97.8)	95.1 (90.3)
R_{merge}[a,b]	0.08 (0.53)	0.05 (0.20)
$I/\sigma(I)$[a]	11.3 (2.2)	12.1 (4.9)
Wilson B factor (Å^2)	20.4	12.5

[a] Statistics for the highest resolution bin (1.86–1.77 Å for hsPGK and 1.55–1.47 Å for βPGM) are shown in parenthesis.
[b] $R_{merge} = \sum_h\sum_i|I(h)-I(h)_i|/\sum_h\sum_i I(h)_i$, where $I(h)$ is the mean weighted intensity after rejection of outliers.

ment the mother liquor was not removed and remained surrounding the crystal. To alleviate the problems of radiation damage, a helical data collection protocol was used (Flot et al., 2010). This allowed the continuous exposure of fresh crystal volumes during data collection. In this manner a complete data set was collected to $d_{min} \approx 1.47$ Å with excellent data processing and refinement statistics (Table 2). After refinement it was ascertained that a minor conformation of the metal fluoride that had been observed in NMR experiments at 294 K could be observed in the crystals at the same temperature, the structure is currently being refined. The HC1b allowed the rapid and simple collection of room temperature data with minimal manipulation of the crystals. As the crystal is mounted using the conventional ESRF experimental environment this also allows the use of advanced data collection protocols, such as helical data collections or mini-kappa goniometer strategies that some other room temperature mounting systems can restrict.

3.4. Hydration of amyloid fibres

The HC1b also offers major opportunities for the study of fibrous systems. Many biological fibres are highly water sensitive and careful variation of the humidity of the sample environment can be used not only to optimise the quality of diffraction data but also to carry out detailed studies of water-driven structural transitions. Examples of such systems include nucleic acids, polysaccharides, and amyloid fibres. The association between neighbouring filaments and the nature of the involvement of water in forming these filaments are of central interest for an understanding of the various models that have been proposed for amyloid. Recently, structural studies on synthetic amyloid fibres have been carried out using the HC1b system. This work, which was carried out in parallel with high-angle neutron fibre diffraction studies (Tiggelaar et al., 2011), has resulted in unique observations of a structural transition. The high precision of the HC1b system has allowed an exceptional degree of control in characterising a reproducible and reversible structural change that occurs at very high levels of hydration (Fig. 5). The deployment of this type of humidity control system is likely to be of major importance in enhancing the quality of both X-ray and neutron fibre diffraction analyses.

4. Conclusions

Techniques that can improve the diffraction properties of crystals are often an essential component in the tool kit available to Structural Biologists. The improvements in diffraction limit, space group changes and general lattice order that can result from the dehydration of crystals can make the difference between gaining relevant biological or phase information and an abandoned project. However, crystal dehydration has, so far, mainly been applied to apparently intractable cases where many other techniques have been used, but failed to improve diffraction quality. It is hoped that as the HC1b becomes more widely used the benefits of controlled dehydration will be seen for more standard systems where small

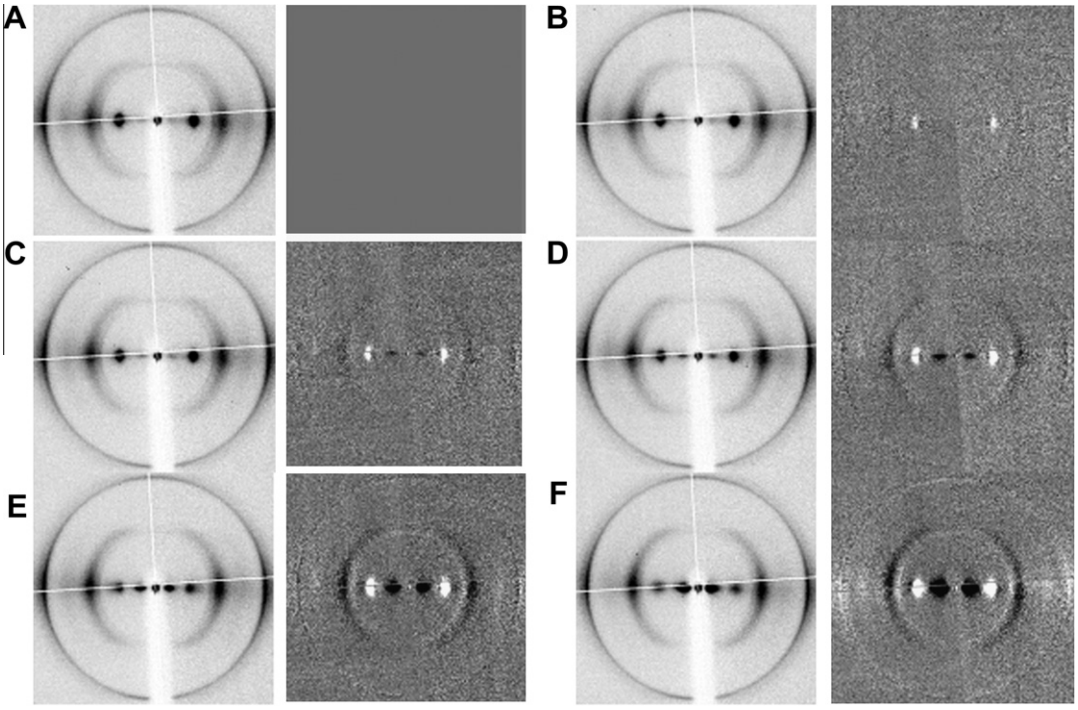

Fig. 5. Selected X-ray diffraction patterns from a series recorded from an amyloid sample. The HC1b was used to adjust the relative humidity of the sample environment over a range from 75% to 100% (A–F in steps of 5%). Adjacent to each image is a difference pattern obtained by subtracting the first image in the sequence from subsequent images – thus allowing a visual indication of positional and intensity changes.

improvements in diffraction limit or phasing power will prove useful. At the ESRF, the HC1b will soon have an interface within the beamline control GUI MXCuBE (Gabadinho et al., 2010). The integration of the device into this automated environment will not only provide a single interface for data collection and dehydration but will also allow the use of on-line data analysis using EDNA/BEST (Incardona et al., 2009; Popov and Bourenkov, 2003) to automatically characterise phase changes and predict the lifetime of a crystal in the beam. A dehydration pipeline will be implemented that will allow the use of a number of standard dehydration protocols and the visualisation of results in the beamline LIMS system ISPyB (Beteva et al., 2006). Experiments will also be enhanced by the use of mesh scans (Bowler et al., 2010) and helical scans (Flot et al., 2010) to alleviate the effects of radiation damage.

While the HC1b has clearly shown its advantages in MX it also has applications in other fields. The device has been used in the collection of protein powder diffraction data. In this case the HC1b enabled the 'wet mounting' of the sample on a micromesh allowing data collection from a very small amount of sample (Y. Watier (ESRF), personal communication). Neutron crystallography has also benefitted from the device. As radiation damage is not a factor in a neutron beam, a fast and simple room temperature mounting system is an advantage. This is coupled with the ability to exchange water for deuterium on-line, in order to change the contrast, thereby avoiding the need to interrupt data collection by exchanging crystals and performing lengthy soaking experiments.

As more examples are examined and more devices commissioned at other synchrotrons (CLS, DLS and MAX-Lab) it is hoped that general rules on dehydration protocols can be developed. A trilateral collaboration was initiated in 2009 between the EMBL-Grenoble/ESRF, Diamond and Max-Lab with the objective of creating a database of dehydration experiments and associated tools to assist HC1b users in planning new experiments and to look for trends in the susceptibility of crystals to dehydration.

Acknowledgments

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References

Abergel, C., 2004. Spectacular improvement of X-ray diffraction through fast desiccation of protein crystals. Acta Cryst. D 60, 1413–1416.

Adachi, H., Umena, Y., Enami, I., Henmi, T., Kamiya, N., Shen, J.R., 2009. Towards structural elucidation of eukaryotic photosystem II: Purification, crystallization and preliminary X-ray diffraction analysis of photosystem II from a red alga. BBA-Bioenergetics 1787, 121–128.

Alcorn, T., Juers, D.H., 2010. Progress in rational methods of cryoprotection in macromolecular crystallography. Acta Cryst. D 66, 366–373.

Amunts, A., Drory, O., Nelson, N., 2007. The structure of a plant photosystem I supercomplex at 3.4 A resolution. Nature 447, 58–63.

Baxter, N.J., Hounslow, A.M., Bowler, M.W., Williams, N.H., Blackburn, G.M., Waltho, J.P., 2009. MgF_3^- and alpha-galactose 1-phosphate in the active site of beta-phosphoglucomutase form a transition state analogue of phosphoryl transfer. J. Am. Chem. Soc. 131, 16334–16335.

Baxter, N.J., Bowler, M.W., Alizadeh, T., Cliff, M.J., Hounslow, A.M., Wu, B., Berkowitz, D.B., Williams, N.H., Blackburn, G.M., Waltho, J.P., 2010. Atomic details of near-transition state conformers for enzyme phosphoryl transfer revealed by MgF_3^- rather than by phosphoranes. Proc Natl Acad Sci USA 107, 4555–4560.

Berthou, J., Cesbron, F., Laurent, A., 1972. New experimental method for direct determination of water-content of protein crystals. J. Mol. Biol. 71, 809.

Beteva, A., Cipriani, F., Cusack, S., Delageniere, S., Gabadinho, J., Gordon, E.J., Guijarro, M., Hall, D.R., Larsen, S., Launer, L., Lavault, C.B., Leonard, G.A., Mairs, T., McCarthy, A., McCarthy, J., Meyer, J., Mitchell, E., Monaco, S., Nurizzo, D., Pernot, P., Pieritz, R., Ravelli, R.G., Rey, V., Shepard, W., Spruce, D., Stuart, D.I., Svensson, O., Theveneau, P., Thibault, X., Turkenburg, J., Walsh, M., McSweeney, S.M., 2006. High-throughput sample handling and data collection at synchrotrons: embedding the ESRF into the high-throughput gene-to-structure pipeline. Acta Cryst. D 62, 1162–1169.

Boldyreva, E.V., Sowa, H., Seryotkin, Y.V., Drebushchak, T.N., Ahsbahs, H., Chernyshev, V., Dmitriev, V., 2006. Pressure-induced phase transitions in crystalline L-serine studied by single-crystal and high-resolution powder X-ray diffraction. Chem. Phys. Lett. 429, 474–478.

Bowler, M.W., Montgomery, M.G., Leslie, A.G., Walker, J.E., 2006a. Reproducible improvements in order and diffraction limit of crystals of bovine mitochondrial F_1-ATPase by controlled dehydration. Acta Cryst. D 62, 991–995.

Bowler, M.W., Montgomery, M.G., Leslie, A.G., Walker, J.E., 2006b. How azide inhibits ATP hydrolysis by the F-ATPases. Proc. Natl. Acad. Sci. USA 103, 8646–8649.

Bowler, M.W., Montgomery, M.G., Leslie, A.G., Walker, J.E., 2007. Ground state structure of F_1-ATPase from bovine heart mitochondria at 1.9 Å resolution. J. Biol. Chem. 282, 14238–14242.

Bowler, M.W., Guijarro, M., Petitdemange, S., Baker, I., Svensson, O., Burghammer, M., Mueller-Dieckmann, C., Gordon, E.J., Flot, D., McSweeney, S.M., Leonard, G.A., 2010. Diffraction cartography: applying microbeams to macromolecular crystallography sample evaluation and data collection. Acta Cryst. D 66, 855–864.

Cipriani, F., Felisaz, F., Launer, L., Aksoy, J.S., Caserotto, H., Cusack, S., Dallery, M., di-Chiaro, F., Guijarro, M., Huet, J., Larsen, S., Lentini, M., McCarthy, S., McSweeney, R., Ravelli, M., Renier, M., Taffut, C., Thompson, A., Leonard, G.A., Walsh, M.A., 2006. Automation of sample mounting for macromolecular crystallography. Acta Cryst. D 62, 1251–1259.

Cliff, M.J., Bowler, M.W., Varga, A., Marston, J.P., Szabo, J., Hounslow, A.M., Baxter, N.J., Blackburn, G.M., Vas, M., Waltho, J.P., 2010. Transition state analogue structures of human phosphoglycerate kinase establish the importance of charge balance in catalysis. J. Am. Chem. Soc. 132, 6507–6516.

Cramer, P., Bushnell, D.A., Fu, J.H., Gnatt, A.L., Maier-Davis, B., Thompson, N.E., Burgess, R.R., Edwards, A.M., David, P.R., Kornberg, R.D., 2000. Architecture of RNA polymerase II and implications for the transcription mechanism. Science 288, 640–649.

Dobbek, H., Gremer, L., Meyer, O., Huber, R., 1999. Crystal structure and mechanism of CO dehydrogenase, a molybdo iron-sulfur flavoprotein containing S-selanylcysteine. Proc. Natl. Acad. Sci. USA 96, 8884–8889.

Einstein, J.R., 1961. Humidity control device for buerger precession camera. J Sci. Instr. 38, 449.

Einstein, J.R., Low, B.W., 1962. Insulin – Some shrinkage stages of sulfate and citrate crystals. Acta Cryst. 15, 32.

Engel, M., Hoffmann, T., Wagner, L., Wermann, M., Heiser, U., Kiefersauer, R., Huber, R., Bode, W., Demuth, H.U., Brandstetter, H., 2003. The crystal structure of dipeptidyl peptidase IV(CD26) reveals its functional regulation and enzymatic mechanism. Proc. Natl. Acad. Sci. USA 100, 5063–5068.

Esnouf, R.M., Ren, J., Garman, E.F., Somers, D.O., Ross, C.K., Jones, E.Y., Stammers, D.K., Stuart, D.I., 1998. Continuous and discontinuous changes in the unit cell of HIV-1 reverse transcriptase crystals on dehydration. Acta Cryst. D 54, 938–953.

Flot, D., Mairs, T., Giraud, T., Guijarro, M., Lesourd, M., Rey, V., van Brussel, D., Morawe, C., Borel, C., Hignette, O., Chavanne, J., Nurizzo, D., McSweeney, S., Mitchell, E., 2010. The ID23–2 structural biology microfocus beamline at the ESRF. J. Synch. Rad. 17, 107–118.

Fourme, R., Ascone, I., Kahn, R., Mezouar, M., Bouvier, P., Girard, E., Lin, T.W., Johnson, J.E., 2002. Opening the high-pressure domain beyond 2 kbar to protein and virus crystallography – Technical advance. Structure 10, 1409–1414.

Fratini, A.V., Kopka, M.L., Drew, H.R., Dickerson, R.E., 1982. Reversible Bending and Helix Geometry in a B-DNA Dodecamer – Cgcgaattbrcgcg. J.Biol. Chem. 257, 4686–4707.

Gabadinho, J., Beteva, A., Guijarro, M., Rey-Bakaikoa, V., Spruce, D., Bowler, M.W., Brockhauser, S., Flot, D., Gordon, E.J., Hall, D.R., Lavault, B., McCarthy, A.A., McCarthy, J., Mitchell, E., Monaco, S., Mueller-Dieckmann, C., Nurizzo, D., Ravelli, R.B.G., Thibault, X., Walsh, M.A., Leonard, G.A., McSweeney, S.M., 2010. MxCuBE: a synchrotron beamline control environment customized for rnacromolecular crystallography experiments. J. Sync. Rad. 17, 700–707.

Gupta, V., Gupta, R.K., Khare, G., Salunke, D.M., Surolia, A., Tyagi, A.K., 2010. Structural ordering of disordered ligand-binding loops of biotin protein ligase into active conformations as a consequence of dehydration. Plos One 5.

Henrich, S., Cameron, A., Bourenkov, G.P., Kiefersauer, R., Huber, R., Lindberg, I., Bode, W., Than, M.E., 2003. The crystal structure of the proprotein processing proteinase furin explains its stringent specificity (vol 10, p. 520, 2003). Nat. Struct. Biol. 10, 669.

Heras, B., Martin, J.L., 2005. Post-crystallization treatments for improving diffraction quality of protein crystals. Acta Cryst. D 61, 1173–1180.

Heras, B., Edeling, M.A., Byriel, K.A., Jones, A., Raina, S., Martin, J.L., 2003. Dehydration converts DsbG crystal diffraction from low to high resolution. Structure 11, 139–145.

Huxley, H.E., Kendrew, J.C., 1953. Discontinuous lattice changes in haemoglobin crystals. Acta Cryst. 6, 76–80.

Incardona, M.F., Bourenkov, G.P., Levik, K., Pieritz, R.A., Popov, A.N., Svensson, O., 2009. EDNA: a framework for plugin-based applications applied to X-ray experiment online data analysis. J. Synch. Rad. 16, 872–879.

Jacquamet, L., Joly, J., Bertoni, A., Charrault, P., Pirocchi, M., Vernede, X., Bouis, F., Borel, F., Perin, J.P., Denis, T., Rechatin, J.L., Ferrer, J.L., 2009. Upgrade of the CATS sample changer on FIP-BM30A at the ESRF: towards a commercialized standard. J. Synch. Rad. 16, 14–21.

Kachalova, G.S., Morozov, V.N., Morozova, T.Y., Myachin, E.T., Vagin, A.A., Strokopytov, B.V., Nekrasov, Y.V., 1991. Comparison of structures of dry and wet hen egg-white lysozyme molecule at 1.8a resolution. FEBS Lett. 284, 91–94.

Kadlec, J., Hallacli, E., Lipp, M., Holz, H., Sanchez-Weatherby, J., Cusack, S., Akhtar, A., 2011. The molecular basis for the recruitment of MOF and MSL3 into the dosage compensation complex by MSL1. Nat. Struct. Mol. Biol. 18, 142–149.

Kiefersauer, R., Stetefeld, J., GomisRuth, F.X., Romao, M.J., Lottspeich, F., Huber, R., 1996. Protein-crystal density by volume measurement and amino-acid analysis. J. Appl. Cryst. 29, 311–317.

Kiefersauer, R., Than, M.E., Dobbek, H., Gremer, L., Melero, M., Strobl, S., Dias, J.M., Soulimane, T., Huber, R., 2000. A novel free-mounting system for protein crystals: transformation and improvement of diffraction power by accurately controlled humidity changes. J. Appl. Cryst. 33, 1223–1230.

Koch, M., Breithaupt, C., Kiefersauer, R., Freigang, J., Huber, R., Messerschmidt, A., 2004. Crystal structure of protoporphyrinogen IX oxidase: a key enzyme in haem and chlorophyll biosynthesis. EMBO J. 23, 1720–1728.

Kuo, A., Bowler, M.W., Zimmer, J., Antcliff, J.F., Doyle, D.A., 2003. Increasing the diffraction limit and internal order of a membrane protein crystal by dehydration. J. Struct. Biol. 141, 97–102.

Kyrieleis, O.J.P., Goettig, P., Kiefersauer, R., Huber, R., Brandstetter, H., 2005. Crystal structures of the tricorn interacting factor F3 from *Thermoplasma acidophilum*, a zinc aminopeptidase in three different conformations. J. Mol. Biol. 349, 787–800.

Morozova, T.Y., Morozov, V.N., 1982. Viscoelasticity of protein crystal as a probe of the mechanical-properties of a protein molecule – hen egg-white lysozyme. J. Mol. Biol. 157, 173–179.

Nakamura, A., Wada, C., Miki, K., 2007. Expression and purification of F-plasmid RepE and preliminary X-ray crystallographic study of its complex with operator DNA. Acta Cryst. F 63, 346–349.

Newman, J., 2006. A review of techniques for maximizing diffraction from a protein crystal *in stilla*. Acta Cryst. D 62, 27–31.

Ohana, J., Jacquamet, L., Joly, J., Bertoni, A., Taunier, P., Michel, L., Charrault, P., Pirocchi, M., Carpentier, P., Borel, F., Kahn, R., Ferrer, J.L., 2004. CATS: a cryogenic automated transfer system installed on the beamline FIP at ESRF. J. Appl. Cryst. 37, 72–77.

Perutz, M.F., 1946. The composition and swelling properties of haemoglobin crystals. Trans. Faraday Soc. B42, 187–195.

Pickford, M.G., Garman, E.F., Jones, E.Y., Stuart, D.I., 1993. A design of crystal mounting cell that allows the controlled variation of humidity at the protein crystal during X-ray diffraction. J. Appl. Cryst. 26, 465–466.

Popov, A.N., Bourenkov, G.P., 2003. Choice of data-collection parameters based on statistic modelling. Acta Cryst. D 59, 1145–1153.

Rockland, L.B., 1960. Saturated salt solutions for static control of relative humidity between 5 and 40 °C. Anal. Chem. 32, 1375–1376.

Sam, M.D., Abbani, M.A., Cascio, D., Johnson, R.C., Clubb, R.T., 2006. Crystallization, dehydration and preliminary X-ray analysis of excisionase (Xis) proteins cooperatively bound to DNA. Acta Cryst. F 62, 825–828.

Sanchez-Weatherby, J., Bowler, M.W., Huet, J., Gobbo, A., Felisaz, F., Lavault, B., Moya, R., Kadlec, J., Ravelli, R.B.G., Cipriani, F., 2009. Improving diffraction by humidity control: a novel device compatible with X-ray beamlines. Acta Cryst. D 65, 1237–1246.

Sjogren, T., Carlsson, G., Larsson, G., Hajdu, A., Andersson, C., Pettersson, H., Hajdu, J., 2002. Protein crystallography in a vapour stream: data collection, reaction initiation and intermediate trapping in naked hydrated protein crystals. J. Appl. Cryst. 35, 113–116.

Snell, G., Cork, C., Nordmeyer, R., Cornell, E., Meigs, G., Yegian, D., Jaklevic, J., Jin, J., Stevens, R.C., Earnest, T., 2004. Automated sample mounting and alignment system for biological crystallography at a synchrotron source. Structure 12, 537–545.

Tiggelaar, S.M., Mossou, E., Callow, P., Callow, S., Teixeira, S.C.M., Mitchell, E.P., Mitraki, A., Forsyth, V.T., 2011. Neutron fibre diffraction studies of amyloid using H2O/D2O isotopic replacement. Acta Cryst. F67, 332–335.

Vijayalakshmi, L., Krishna, R., Sankaranarayanan, R., Vijayan, M., 2008. An asymmetric dimer of beta-lactoglobulin in a low humidity crystal form – Structural changes that accompany partial dehydration and protein action. Proteins-Structure Function and Bioinformatics 71, 241–249.

Warkentin, M., Thorne, R.E., 2009. Slow cooling of protein crystals. J Appl. Cryst. 42, 944–952.

Yap, T.L., Chen, Y.L., Xu, T., Wen, D.Y., Vasudevan, S.G., Lescar, J., 2007. A multi-step strategy to obtain crystals of the dengue virus RNA-dependent RNA polymerase that diffract to high resolution. Acta Cryst. F 63, 78–83.

Zerrad, L., A. Merli, G.F. Schröder, A. Varga, E. Graczer, P. Pernot, A. Round, M. Vas, and M.W. Bowler, 2011. A spring loaded release mechanism regulates domain movement and catalysis in phosphoglycerate kinase. J. Biol. Chem. doi:10.1074/jbc.M110.206813.