Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Protein crystal perfection and its application

John R. Helliwell

Department of Chemistry, University of Manchester, M13 9PL, England, and CCLRC Daresbury Laboratory, Warrington WA4 4AD, England

Correspondence e-mail: john.helliwell@man.ac.uk

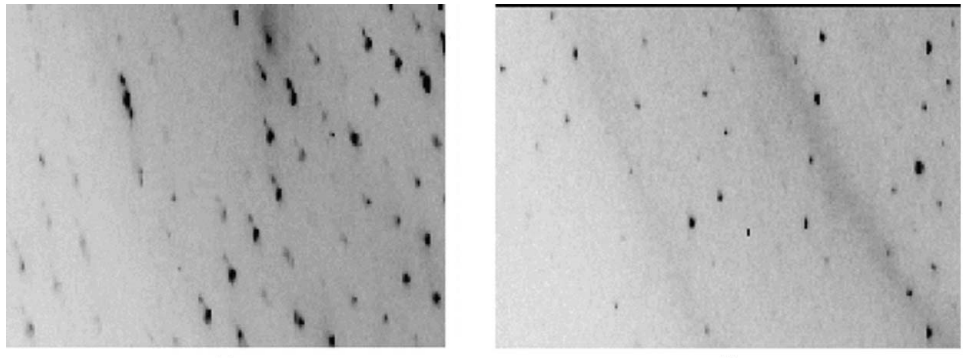
Received 20 September 2004
Accepted 12 January 2005

Knowledge of protein crystal perfection in theory and practice is reviewed. X-ray methods of assessing perfection such as mosaicity, topography and reciprocal-space mapping are described. X-ray diffraction physics applications of protein crystals such as in Laue geometry, the large-angle oscillation technique and forming polychromatic profiles across diffraction spots are covered. Cryo- and room-temperature cases are discussed including in X-ray and neutron protein crystallography. Experience of freezing very large crystals, which are commonly used today in neutron protein crystallography, is highlighted.

1. Introduction

Technology and methods developments have transformed our expectations of structure analysis from crystals. Highlights of the last two decades have included synchrotron radiation, detectors and cryo-crystallography. Thus, smaller crystals can be used, faster phasing and higher resolution can be achieved, as well as larger structures studied (a recent global survey is given in Jiang & Sweet, 2004). Some structures have also been studied by time-resolved Laue techniques including on a sub-nanosecond timescale (Moffat, 1998). In a synergistic development neutron Laue methods, along with very large area neutron sensitive image plates, and new spallation source developments, are enhancing the potential for complete, *i.e* with-H atoms (as deuteriums), structures (Blakeley, Cianci *et al.*, 2004). Neutron studies will also benefit greatly from fully perdeuterated protein production.

The field of biological crystal growth (for a review see Chernov, 1998) has the challenges as ever of crystal size and diffraction quality but now also with throughput issues. Bigger crystals for neutron structural studies are also needed and where they are reasonably perfect. In an interesting twist of this story, freezing of very large crystals of proteins suitable for high resolution neutron data collection is a recent breakthrough (Blakeley, Kalb *et al.*, 2004). Neutron cryo-crystallography benefits the clarity of bound water structure, including the water deuteriums, and also opens up freeze-trap neutron protein structural studies.



(*a*) (*b*)

Figure 1
Textured spots (*a*) can cause difficulties in standard and more exotic data collection regimes *versus* well behaved obviously single spots (*b*). From Helliwell (2002).

2. Experiments where good crystal perfection has been identified as important

The effect of a spoilt spot shape on the diffraction pattern geometric quality is illustrated in Fig. 1. The relevant situations where good perfection is especially valuable include the following (Figs. 2, 3 and 4).

Firstly, where there is a congestion of diffraction spots: (i) building on the protein crystal Laue initiative of Moffat *et al.* (1984) a broader bandpass Laue diffraction pattern ('white beam') approach has been pursued by Helliwell (1984, 1985) (for a recent overview, see Nieh *et al.*, 1999). The Laue approach, in the narrow bandpass exposition, has more recently become relevant for neutron protein data collection at the Institut Laue Langevin with its Laue diffractometer ('LADI'); see Fig. 2. (ii) Large-angle oscillation technique (LOT) diffraction patterns (Weisgerber & Helliwell, 1993); see Figs. 3(*a*) and 3(*b*).

Secondly, within a diffraction spot the angular smearing at any point should be kept small for the case of measuring the polychromatic profile across a diffraction spot (Arndt *et al.*, 1982; Greenhough *et al.*, 1983); see Fig. 4. This requirement is also true for the case of *n*-beam phasing (Weckert & Hummer, 1997).

Thirdly, where diffraction spot intensities are weak such as at high resolution an angularly sharp reflecting range allows the strategy of using fine φ slicing to improve data quality. Since protein crystals have been shown to reach 'silicon-level-quality' (Snell *et al.*, 1995; Fourme *et al.*, 1995; see also Helliwell, 1988) superfine φ-slicing to extend the diffraction resolution limit has been investigated (Borgstahl *et al.*, 2001); see also Fig. 5.

3. Assessing protein crystal perfection

For validating crystal and diffraction quality, *e.g* for publication, various metrics have survived the test of time; diffraction resolution, being paramount, is estimated according to parameters such as $R_{\rm merge}$, average $F/\sigma(F)$ or average $I/\sigma(I)$ and data completeness thus defining the true 'outer shell' of 'observable data'.

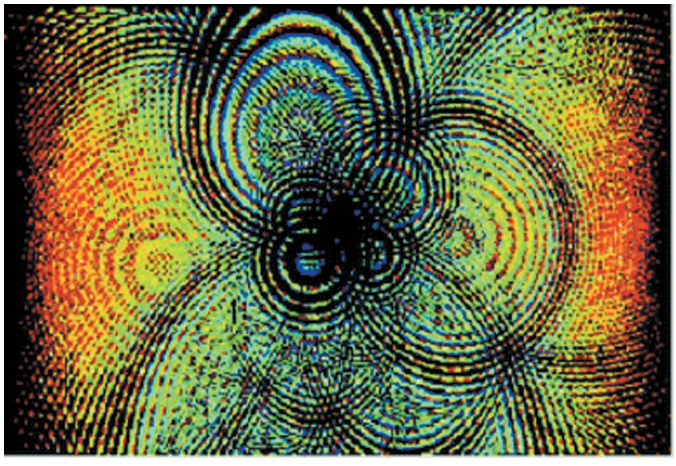


Figure 2
Example of a congested spot pattern. Neutron Laue crystal diffraction pattern simulation from a concanavalin A–glucoside complex. Crystal space group $I2_13$, a = 167.8 Å. The pattern is colour coded for wavelength [red, long wavelength, 3.8 Å through the rainbow colours to blue, short wavelength, 2.8 Å; these are the typical experimental conditions in use at the Institut Laue Langevin Laue Diffractometer (LADI) for biological crystallography]. At the far left and far right of this pattern then is the 'full back-scattering position' neutron reflection data. This is the largest at 50 kDa protein in the asymmeric unit studied so far with neutrons (Gilboa *et al.*, 2001). A colour version of this figure is available in the online edition of the journal.

In addition, however, new methods of characterization of a protein crystal have emerged. These methods have involved mosaicity, topography and reciprocal space mapping measurements (see, for example, Boggon *et al.*, 2000 where each of these methods was used and Fig. 6 for an example). These types of evaluations relate well to the crystal growth conditions and have allowed assessment of new approaches used by crystal growers, such as microgravity, the use of gels and of microbatch growth under oil (see *e.g.* Dong *et al.*, 1999).

The use of topography (see, for example, Fourme *et al.*, 1995; Stojanoff *et al.*, 1996) was introduced as a new technique to be applied to protein crystal perfection studies. The measurement of X-ray

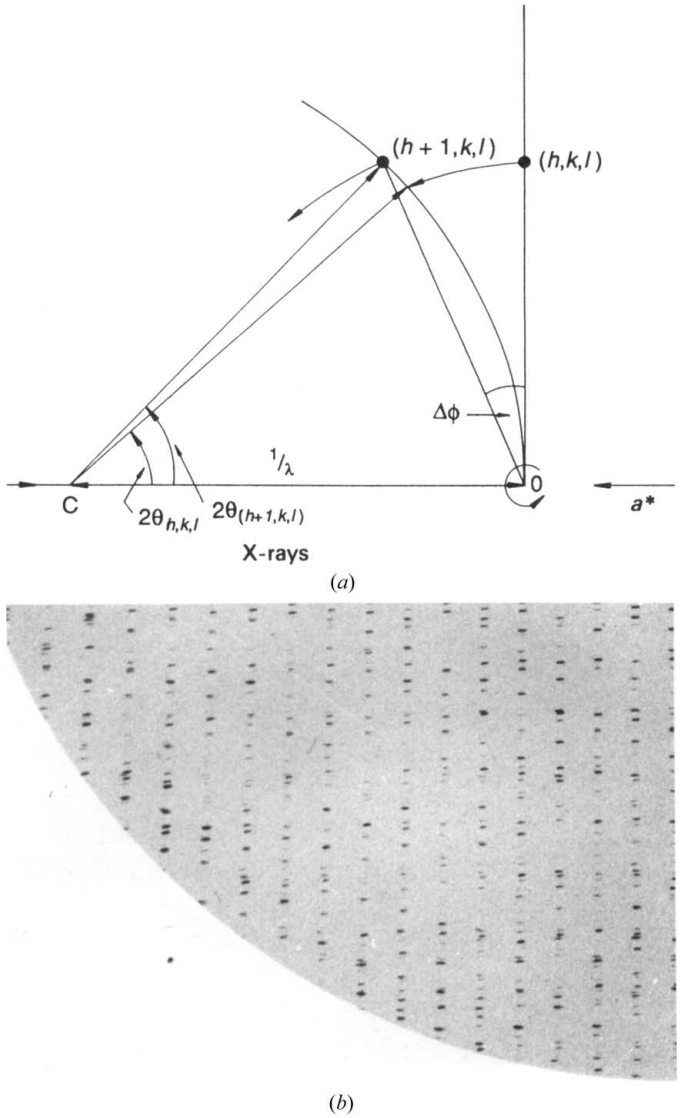


Figure 3
Another example of a congested spot pattern, the case of the large-angle oscillation technique (LOT) diffraction (from Weisgerber & Helliwell, 1993. Reproduced with permission of the Royal Society of Chemistry.). (*a*) The principle behind LOT; the rotation of the crystal beyond the typical $d_{\rm min}$/maximum unit-cell dimension is not in fact limiting as the Bragg angles of these two reciprocal lattice points (h, k, l and h + 1, k, l) are slightly different. (*b*) LOT practice; a 90° rotation X-ray diffraction photograph recorded at SRS station 9.5, wavelength ∼1 Å from concanavalin A saccharide-free crystal form space group $I222$, unit-cell dimensions a = 88, b = 86, c = 62 Å, diffraction resolution at the edge is 5 Å (higher resolution, $d_{\rm min}$ = 3 Å, randomly set crystal diffraction pattern examples are also given in Weisgerber & Helliwell, 1993).

mosaicity was investigated (Helliwell, 1988; Colapietro *et al.*, 1992) as a direct indicator of the physical perfection of the macromolecular crystal. The mosaicity provides a simple measurement of crystal quality independent of many experimental parameters. It has been used to characterize successfully the improvement seen in some microgravity samples *i.e.* a reduction in the reflection mosaic spread providing a corresponding increase in the signal-to-noise ratio of the reflection (Snell *et al.*, 1995; Helliwell *et al.*, 1996; Ng *et al.*, 1997). Mosaicity is the recorded rocking width of the reflection with the instrument contribution deconvoluted out. As such, it requires a beam with geometric and spectral divergence that does not mask the measurements being made. The mosaicity measured from the crystal is a global measure of several effects. Simple rocking-curve measurements cannot separate out the effects arising from strains (*i.e.* *d*-spacing variations) from those arising from classical mosaicity, which involves only rotational misalignments of parts of the crystal. This separation requires that 2θ be determined precisely as well as θ (also termed ω). The technique used for this purpose is the triple-axis spectrometer (Brockhouse, 1955), first used for neutron-scattering studies.

If we wish to reach a microscopic understanding of the crystal defects so that their origin may be identified, diffraction imaging (*i.e.* X-ray diffraction topography) can be a useful tool. It allows the diffracting power of a crystal to be spatially resolved on the micrometre level at an arbitrary sample orientation. Thus, a precise picture of which parts of the sample diffract at which incidence angles can be built up. If the defect density is low enough, it should be possible to determine the nature of an individual defect. A combined mosaicity, topography and reciprocal space mapping set of measurements made on space and earth grown lysozyme crystals as examples are described in Boggon *et al.* (2000).

4. Sharpened mosaicity; the microgravity grown crystal experience

The key concepts that attracted crystal growers, protein or non-protein (*e.g.* semiconductors), to microgravity research is that in essentially weightless conditions density difference fluid flows can be eliminated and sedimentation of the growing crystals is also eliminated (McPherson, 1996). Thus, defects and flaws in the crystals can be reduced, even eliminated. Protein crystals of course differ from semiconductors because the latter crystals are harnessed for their crystalline derived electrical behaviours whereas protein crystals provide the means to work out the structure of the protein in the unit cell of the crystal. Only in exceptional cases are the overall protein crystals the objects of interest. Defects in crystals however can affect not only crystal properties but also the diffraction data from which protein structures are elucidated.

The practical demonstration of convection driven fluid flow was made in a growing lysozyme crystal, earth-based, experiment using Schlieren optics to record direct video images of growing crystals (M. Pusey, peronal communication). This and many other experiments stimulated protein crystal growers to investigate for themselves the microgravity environment. These researchers then often recruited X-ray crystallographers to analyse the perfection of the crystals and in a growing number of cases to analyse the protein structures from those crystals. The field of crystal perfection analysis and structure validation enabled an increasingly exacting set of experimental protocols culminating in analyses of the space *versus* the earth based ground controls carried out in otherwise identical apparatus and physical and chemical conditions and *versus* the best results ever seen on the ground. For the latter however not all the wished for data are routinely available but are nevertheless interesting. It is probably fair to say that the media attention that microgravity research received forced an ever more exacting approach. Clear-cut results did eventually come and in some cases these have served as benchmarks to compare all earth based research results against (see, for example, McPherson, 1996 and references therein; Snell *et al.*, 1995; Ng *et al.*, 2002).

Microgravity research has enriched the field through new concepts, new benchmarks, new methods, new apparatus, new protein crystal structures and better resolved existing protein structures. As knowledge has evolved it has become possible to improve the accuracy of predicting which samples will yield improved results in microgravity and predicting which type of microgravity flight would yield positive results. The method is however of course difficult *i.e.* not having easy access and the dangers of space flight to the astronauts flying on the flights are well known. Overall a principal effect witnessed with microgravity grown crystals has been a sharpened mosaicity. This has increased the peak height of reflections above background. A sharper mosaicity makes sense because it in essence means that the crystal as an ordered array has a longer spatial coherence length. This can be imagined to be readily linked to a more orderly growth environment from a non-convective fluid flow situation in microgravity.

In principle sharpened protein crystal mosaicity should be applicable, with a parallel undulator synchrotron X-ray beam, for ultra-fine-phi-slicing data collection, which will reduce the background contribution to the reflecting range. However radiation damage can quickly take over and broaden the mosaicity. Radiation damage is greatly reduced with freezing of the sample but at the expense of broadened mosaicity. Interest in room temperature

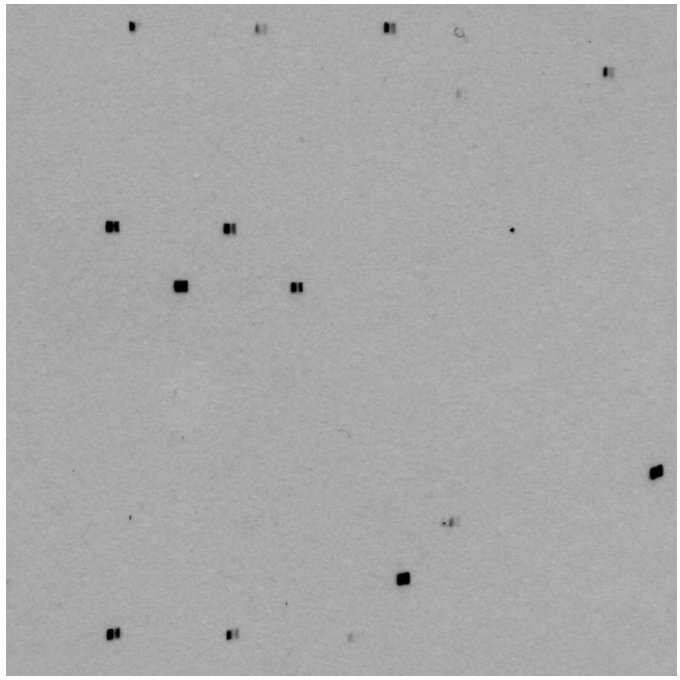


Figure 4
The polychromatic profile across each diffraction spot was achieved here by having a monochromator dispersive setting on SRS station 7.2 and rotating the rhenium-containing test crystal in that essentially monochromatic beam. The bandpass across each spot is ~60 eV and has been set on the rhenium L_{III} absorption edge in this case. The largest absorption at the rhenium XANES peak is seen directly *via* the white lines. Preserving the spectral distribution across each spot rests upon having a non-mosaic block crystal. From Helliwell (2005). Reproduced with permission of Cambridge University Press.

structures, free of any structural changes induced upon freezing (for an example, see Deacon *et al.*, 1997), sustains the interest in sharpened mosaicity, better topographically perfect, crystals. Other factors affect diffraction resolution of course such as the protein molecule overall *B* factor.

5. Application of these concepts of assessing and applying protein crystal perfection to freezing very large crystals for neutron protein crystal structure studies: first experiments

The very big protein crystals needed for neutron protein crystallography offer a rich topic for future crystal perfection assessments, especially checks of the uniformity of diffraction quality across all of the big crystal. Firstly in this section an explanation is given why there is a new excitement afoot in neutron protein crystallography and then a description is given of one recent experiment involving relatively simple quality assessment.

5.1. Why neutrons? the complementarity of X-ray and neutron protein structural studies

Whilst the determination of some H atoms in proteins is now feasible with ultra-high/atomic resolution protein SR X-ray crystallography the mobility of H atoms can abolish their diffraction signal. Since neutron protein crystallography determination of deuteriums at around 2 Å resolution or better matches that at 1.0 Å by SR X-rays, the more mobile H atoms are best determined by the neutron approach. Indeed the bound solvent is a whole category of D atoms which are more efficiently sought by neutron techniques. It is in the more mobile parts of the protein structure, and its bound solvent, that the role of neutron protein crystallography in completing the protein structure protonation details is most obviously needed.

Major investments in neutron sources are occurring and further apparatus developments being made at existing neutron source, which will make an important impact in the study of protein structure and hydration. For example there are enhancement plans for LADI in Grenoble and a new protein deuteration laboratory is recently available there as well; there is a proposed Large Molecule Crystallography (LMX) instrument on ISIS2; recently on-line is the Protein Crystallography System (PCS) at Los Alamos; there is the MANDI, 'Macromolecular Neutron Diffraction Instrument', planned for the new 1.4 MW USA source 'SNS' and similarly an instrument planned at the 1 MW Japanese source, both new MW power level spallation neutron sources that are now under construction. Crystal sizes needed will reduce to ∼0.5 mm on edge.

5.2. Case study: cryo- and room-temperature concanavalin A structures

Since neutrons do not cause radiation damage there has not been a pressing need to cryo-protect crystals. However, there is the advantage to be gained from cryo-crystallography of the reduction of atomic mobility and enhanced nuclear-density definition. Furthermore, collecting data at cryo-temperatures can also reduce background (diffuse scattering), and therefore can aid the signal-to-noise ratio and hence the resolution limit be improved. Cryo-crystallography also opens up the possibility of freeze trapping of intermediates in biological reactions triggered in a protein crystal (Moffat & Henderson, 1995). So, the idea of combining neutron and cryo-crystallography advantages together is of considerable interest.

It seems to have been generally assumed that freezing very large protein crystals is not possible. However, we have recently shown that it is possible to freeze and collect high resolution X-ray (1.65 Å on a rotating anode) and neutron data (2.5 Å on LADI; Fig. 7) from large concanavalin A protein crystals (∼2 and 5 mm^3) as examples (Blakeley, Kalb *et al.*, 2004). These data have allowed a combined 'X + n' protein structure analysis to be undertaken as performed previously with room temperature X-ray and neutron data sets (Habash *et al.*, 2000). Indeed a direct comparison of the structural results between cryo and room temperature allows atomic dynamics to be separated from disorder structural states. These results demonstrate the potential of protein cryo-crystallography with neutrons, thus combining the advantages of the neutron and cryo approaches for studying the structural details of bound water H atoms (as deuteriums) and of protonation states of

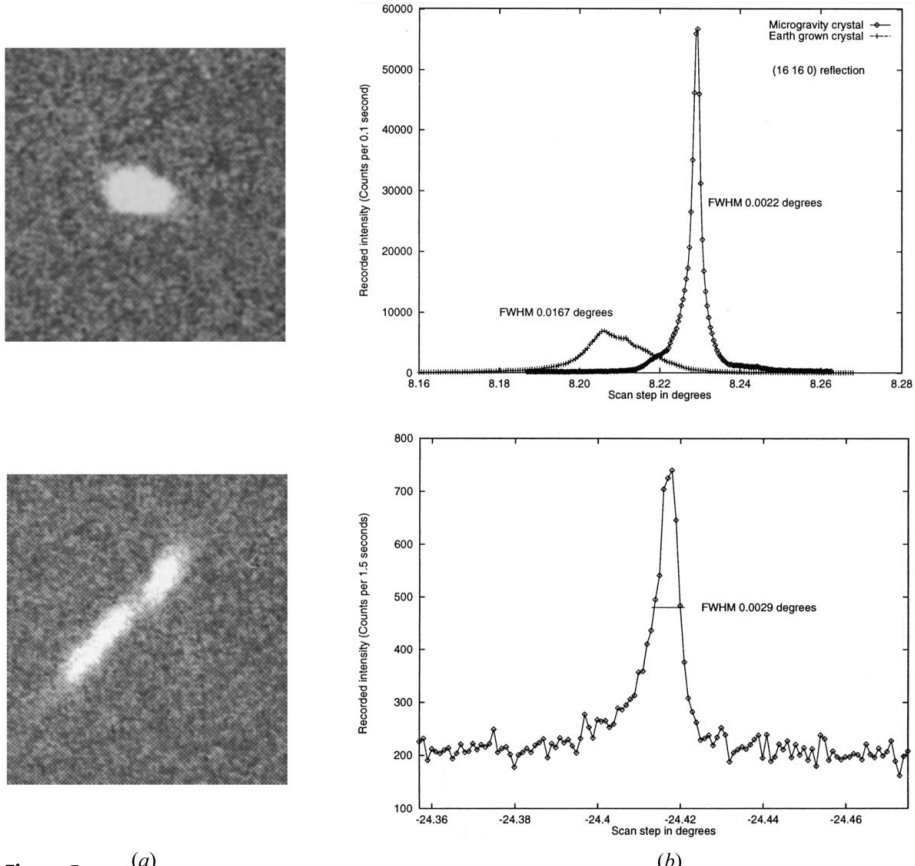
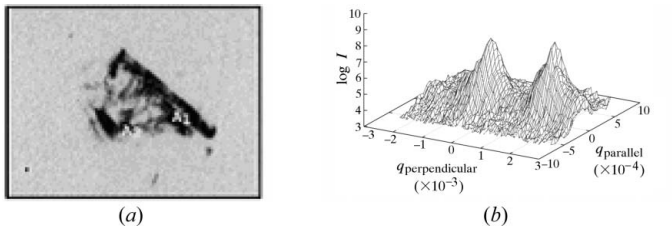


Figure 5
Sharpened mosaicity effect seen for microgravity *versus* ground control lysozyme crystals. (*a*) Lysozyme diffraction spot size and shape studied using ultralong-distance Laue difffraction (2.4 m) at SRS station 9.5 [from Helliwell *et al.* (1996). Copyright (1996) Springer. Reproduced with permission.]. Top, space grown crystal; bottom, earth grown crystal. (*b*) Rocking curve evaluation made at ESRF (from Snell *et al.*, 1995). Top, space *versus* earth grown crystal for an identical reflection; bottom, space grown crystal near to the space crystal diffraction resolution limit, which is beyond the earth crystal resolution limit.

histidines and carboxyl side chains. Most exciting of all, this opens up the possibility of time-resolved neutron freeze-trap biological crystallography. These developments widen considerably the study of the structure, dynamics and function of proteins.

5.3. The assessment of the diffraction quality of a frozen very large concanavalin A crystal

One of the frozen crystals was removed from the neutron LADI diffractometer and transferred in its frozen state onto a laboratory (Cu $K\alpha$) image plate diffraction set up. Using a 200 µm collimator 1° oscillation photos were recorded at different points of the crystal along its length. Fig. 7 shows firstly the neutron LADI pattern from the crystal and secondly the surveyed places of the crystal and associated X-ray diffraction patterns. This shows that the portion of the crystal to have been plunged in first to the liquid nitrogen has the worst diffraction resolution. Three out of five of the diffraction patterns show excellent quality diffraction [1.65 Å resolution, with single, *i.e.* non-split, diffraction spots which is necessary for accurate diffraction data processing but the mosaicity is large at 1°, not ideal for optimizing the reflection intensity signal to noise]. An interpretation of the spatial distribution of diffraction quality along the length of the crystal came from a discussion after my Plenary Lecture from E. Stura who suggested that the leading edge of the crystal in the plunge towards the cryogen would meet higher temperature boil off gas before entering the lowest temperature liquid nitrogen. This is very plausible.



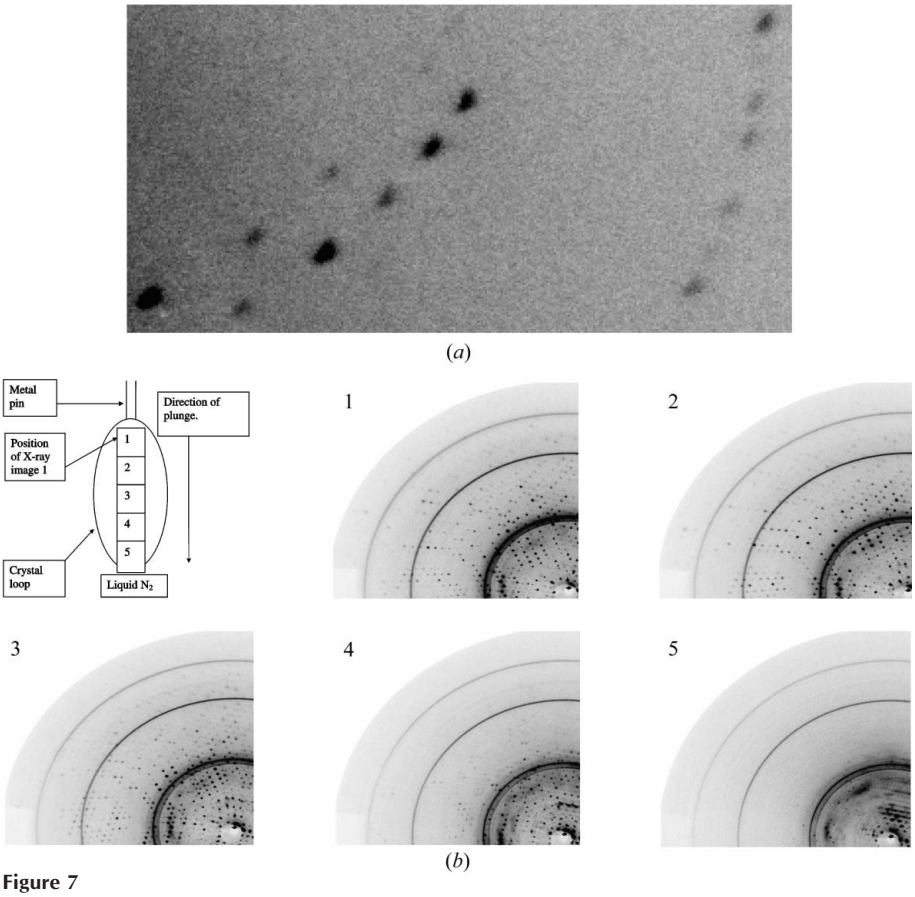
(*a*) (*b*)

Figure 6
Spatial mapping. (*a*) Example topograph of a reflection. (*b*) Example reciprocal space map for a lysozyme crystal reflection (from Boggon *et al.*, 2000)

Clearly the battery of protein crystal perfection X-ray techniques of topography and reciprocal space mapping, besides step scans, diffraction resolution and mosaicity checks, could be made in the future. These would lead to an enhanced understanding of the currently very big (∼1–5 mm^3) crystals world of neutron protein crystallography.

6. Concluding remarks

The limit of protein crystal perfection evaluation has taken one into the territory of the silicon crystal level of quality (Helliwell, 1988). Indeed that fact interested many protein crystal growers since proteins might be regarded as always totally floppy 'balls of fluff' rather than capable of being orderly packable objects over the whole spatial coherence length of a crystal. Arai *et al.* (2004) offered the opinion that crystal quality can be described by a single parameter: the resolution of diffraction. Textured patterns (with split spots in other words), but still diffracting to 'decent resolution', tell us that more parameters are needed to describe one crystal from another than resolution alone (or a relative *B* factor too). Indeed most crystallographers now know that these various parameters are needed to define the various aspects of crystal quality that crop up. However single parameter evaluation as Arai *et al.* (2004) suggests is better suited to the high throughput area of structural genomics.



(*a*) (*b*)

Figure 7
(*a*) Close-up of part of a neutron Laue diffraction pattern from a large cryo-cooled concanavalin A crystal at 15 K (volume of the crystal was ∼5 mm^3) and which illustrates the good spot-shape. (*b*) Step scan across a frozen crystal of concanavalin A, crystal size 2.5 × 1.8 × 1 mm and recorded Cu $K\alpha$ X-ray diffraction patterns (from Blakeley, Kalb *et al.*, 2004, reproduced with permission, copyright, 1993–2004, National Academy of Sciences, USA).

To summarize, the future will then no doubt retain its share of imperfect crystals but they can be better characterized now and what they yield for structural studies by X-ray and neutron crystallography is now also much better than 20 years ago. The techniques developed primarily with synchrotron X-ray sources in mind and with unfrozen crystals now can be brought to bear systematically on frozen crystals and especially on the very big crystals needed for neutron studies. These latter, where a reasonably plentiful supply of crystals can be guaranteed, allow spatial distributions and step scanning approaches to crystal perfection evaluation to be investigated in detail. The X-ray applications requiring perfection, such as preserving either sharp, small sized,

diffraction spots or where spectral profiles are sought within each diffraction spot, will benefit if single domain, radiation protected, single crystals can be produced systematically. The benefiting techniques would be protein crystal profile-phasing and *n*-beam phasing.

Overall, the investigation of protein crystal perfection started as a curiosity driven set of studies. The ever improving SR source machine emittances and protein crystallography instrument resolution functions made it obvious that protein crystals can be of fine perfection (Helliwell, 2005). Calculations had shown that the theoretical limit would be in the silicon quality range of arcseconds (Helliwell, 1988). From these observations it was clear that special apparatus would be required to uncover the experimental details in their full glory and complexity. Crystal perfection assessment has reached a maturity where a good battery of tools and experience is indeed now in place.

I am grateful to former students Dr Susanne Weisgerber, Dr Edward H. Snell and Dr Titus J. Boggon for their contributions to the protein crystal perfection studies between 1991 and 2000, and to Dr Matthew J. Blakeley for the work on cryo neutron protein crystallography between 2001 and 2004. I thank the British Council for support of SW, I thank EPSRC for studentship support of EHS, TJB and MJB; for SW and MJB this included support from the EU and the Institut Laue Langevin, respectively. The research would not have been possible without the following collaborating staff scientists; Dr Marcello Colapietro and Dr Riccardo Spagna at ADONE in Frascati, Dr Edgar Weckert at the ESRF Swiss-Norwegian Beamline, Dr Vivien Stojanoff and Dr Peter Siddons at NSLS, Dr Dean Myles at the Institut Laue Langevin. These facilities are also thanked for SR or neutron beamtime awards. The work performed at SRS involving the polychromatic profiles was performed on SRS station 7.2 by JRH with Dr Steve Rule and Dr Trevor Greenhough, and the ultralong-distance (2.4 m) Laue diffraction perfection evaluation of space and earth grown crystals was undertaken at SRS station 9.5 with Susanne Weisgerber; the SERC Daresbury Laboratory is thanked for SR beamtime and the Swedish Research Council for financial contributions to the SRS 9.5 instrument. The EU Framework Programme provided a twinning research award between JRH and Dr M. Colapietro, which included the work performed at ADONE and the support of SW referred to above.

References

Arai, S., Chatake, T., Suzuki, N., Mizuno, H. & Niimura, N. (2004). *Acta Cryst.* D**60**, 1032–1039.

Arndt, U. W. Greenhough, T. J., Helliwell, J. R., Howard, J. A. K., Rule, S. A. & Thompson, A. W. (1982). *Nature (London)*, **298**, 835–838.

Blakeley, M. P., Cianci, M., Helliwell, J. R. & Rizkallah, P. J. (2004). *Chem. Soc. Rev.* **33**, 548–557.

Blakeley, M. P., Kalb, A. J., Helliwell, J. R. & Myles, D. A. A. (2004). *Proc. Natl Acad. Sci USA*, **101**, 16393–16397.

Boggon, T. J., Helliwell, J. R., Judge, R. A., Olczak, A., Siddons, D. P., Snell, E. H. & Stojanoff, V. (2000). *Acta Cryst.* D**56**, 868–880.

Borgstahl, G. E. O., Vahedi-Faridi, A., Lovelace, J., Bellamy, H. D. & Snell, E. H. (2001). *Acta Cryst.* D**57** 1204–1207.

Brockhouse, B. (1955). *Phys. Rev.* **100**, 601–603.

Chernov, A. (1998). *Acta Cryst.* A**54**, 859–872.

Colapietro, M., Cappuccio, G., Marciante, C., Pifferi, A., Spagna, R. & Helliwell, J. R. (1992). *J. Appl. Cryst.* **25**, 192–194.

Deacon, A. M, Gleichmann, T., Kalb (Gilboa), A. J., Price, H., Raftery, J., Bradbrook, G. M., Yariv, J. & Helliwell, J. R. (1997). *J. Chem. Soc. Faraday Trans.* **93**, 4305–4312.

Dong, J., Boggon, T. J., Chayen, N. E., Raftery, J., Bi, R.-C. & Helliwell, J. R. (1999). *Acta Cryst.* D**55**, 745–752.

Fourme, R., Ducruix, A., Riès-Kautt, M. & Chapelle, B. (1995). *J. Synchrotron Rad.* **2**, 136–142.

Gilboa, A. J. K., Myles, D. A. A., Habash, J., Raftery, J. & Helliwell, J. R. (2001). *J. Appl. Cryst.* **34**, 454–457.

Greenhough, T. J., Helliwell, J. R. & Rule, S. A. (1983). *J. Appl. Cryst.* **16**, 242–250.

Habash, J., Raftery, J., Nuttall, R., Price, H. J., Wilkinson, C., Kalb (Gilboa), A. J. & Helliwell, J. R. (2000). *Acta Cryst.* D**56**, 541–550.

Helliwell, J. R. (1984). *Rep. Prog. Phys.* **47**, 1403–1497.

Helliwell, J. R. (1985). *J. Mol. Struct.* **130**, 63–91.

Helliwell, J. R. (1988). *J. Cryst. Growth*, **90**, 259–272.

Helliwell, J. R. (2002). *J. Synchrotron Rad.* **9**, 1–8.

Helliwell, J. R. (2005). *Macromolecular Crystallography with Synchrotron Radiation.* Cambridge University Press.

Helliwell, J. R., Snell, E. H. & Weisgerber, S. (1996). *Proceedings of the Berlin Conference on Microgravity Research*, edited by L. Ratke, H. Walter & B. Feuerbacher, pp. 155–170. Berlin: Springer-Verlag.

Jiang, J. & Sweet, R. W. (2004). *J. Synchrotron Rad.* **11**, 319–327.

McPherson, A. (1996). *Crystallogr. Rev.* **6**, 157–308.

Moffat, K. (1998). *Acta Cryst.* A**54**, 833–841.

Moffat, K. & Henderson, R. (1995). *Curr. Opin. Struct. Biol.* **5**, 656–663.

Moffat, K., Szebenyi, D. M. & Bilderback, D. H. (1984). *Science*, **223**, 1423–1425.

Ng, J. D., Sauter, C., Lorber, B., Kirkland, N., Arnez, J. & Giegé, R. (2002). *Acta Cryst.* D**58**, 645–652.

Ng, J. D. Lorber, B., Giegé, R., Koszelak, S., Day, J., Greenwood, A. & McPherson, A. (1997). *Acta Cryst.* D**53**, 724–733.

Nieh, Y. P., Raftery, J., Weisgerber, S., Habash, J., Schotte, F., Ursby, T., Wulff, M., Haedener, A., Campbell, J. W., Hao, Q. & Helliwell, J. R. (1999). *J. Synchrotron Rad.* **6**, 995–1006.

Snell, E. H., Weisgerber, S., Helliwell, J. R., Weckert, E., Holzer, K. & Schroer, K. (1995). *Acta Cryst.* D**51**, 1099–1102.

Stojanoff, V., Siddons, D. P., Snell, E. H. & Helliwell, J. R. (1996). *Synchrotron Radiat. News*, **9**, 25–26.

Weckert, E. & Hummer, K. (1997). *Acta Cryst.* A**53**, 108–143.

Weisgerber, S. & Helliwell, J. R. (1993). *J. Chem. Soc. Faraday Trans.* **89**, 2667–2675.