

## Macromolecular Crystal Quality

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*Author address:*

Edward H. Snell, NASA Laboratory for Structural Biology, Code SD46, NASA, MSFC, Huntsville AL 35812.

Gloria E. O. Borgstahl, University of Toledo, Department of Chemistry, Wolfe Hall Room 3214, 2801 West Bancroft Street, Toledo, Ohio 43606-3390.

Henry D. Bellamy, Stanford Synchrotron Radiation Laboratory, 2575 Sand Hill Rd. MS 69, Menlo Park, CA 94025\*

*Proofs should be sent to Dr. Edward Snell*

*Phone: (256) 544-5570*

*Fax: (256) 544-9305*

*Email: eddie.snell@msfc.nasa.gov*

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\* Current Address:

Center for Advanced Microstructures and Devices  
6980 Jefferson Hwy.  
Baton Rouge LA 70806.

# Macromolecular Crystal Quality

By Edward H. Snell, Henry D. Bellamy and Gloria E. O. Borgstahl

“That which is striking and beautiful is not always good, but that which is good is always beautiful.”

Ninon De L'Enclos

## ***Introduction***

There are many ways of judging a good crystal. Which we use depends on the qualities we seek. For gemstones size, clarity and impurity levels (color) are paramount. For the semiconductor industry purity is probably the most important quality. For the structural crystallographer the primary desideratum is the somewhat more subtle concept of internal order. In this chapter we discuss the effect of internal order (or the lack of it) on the crystal's diffraction properties.

The internal order of the crystal can be characterized by a correlation length *i.e.* the distance over which the atoms in two unit cells are “accurately” related by the crystal symmetry operators. For random disorder between adjacent unit cells the greater the distance the less accurate the correlation. Therefore the correlation length depends in part on the accuracy of the correlation required which in turn depends on the resolution of the diffraction. An atom will only contribute to the intensity of a diffraction spot if its disorder relative to symmetry-related atoms is small compared to the resolution of the spot. Hence, for random disorder, as resolution increases the effective correlation length

decreases and the number of unit cells contributing coherently to the diffraction decreases. Random disorder is a major contributor to the reduction in diffracted intensity with increasing resolution. (In fact this is why the “Temperature Factor” has been renamed the “Atomic Displacement Factor”.) Disorder can be described as long-range or short-range. In general long-range disorder in the crystal gives rise to localized effects in reciprocal space and *vice versa*<sup>1, 2</sup>. For example crystal mosaicity which is a large-scale property in real space causes the localized effect of broadened spots in reciprocal space whereas the effect of random disorder between adjacent unit cells is a global, resolution-dependant reduction in diffracted intensity in reciprocal space. Thus careful measurements of the diffraction from macromolecular crystals can reveal the degree and nature of their disorder. Since macromolecular crystals are, by the standard of small molecule crystals, not very good crystals they offer a fruitful field for the study of disorder. It is our hope that a better understanding of the nature and causes of disorder in macromolecular crystals can lead to the production of better crystals.

### ***Crystal mosaicity and domain structure***

The crystal properties that are amenable to investigation by reflection analysis are mosaicity and domain structure. Mosaicity by profile analysis and domain structure by topography and reciprocal space mapping. The mosaic model of crystals was proposed by Darwin<sup>3</sup> and approximates the crystal to an array of perfectly ordered volumes (domains) slightly misaligned with respect to each other. (The boundaries between these domains are ignored and no model for them is proposed.) We use this model as a first approximation to the real crystal as topographic evidence has revealed these domains<sup>1</sup>

and reasonably accurate calculations can be made from the model. In addition to having small random misalignments the domains can be of varying volume and the unit cells in the crystal can vary (generally due to impurities). Each of these phenomena has a distinct effect on the crystal<sup>1, 2</sup>. Figure 1 shows crystals as being made up of distinct domains according to the Darwin model and illustrates how physical effects described by the mosaic model can be manifested in reciprocal space mapping (center) and reflection profile (rocking width) measurements (right side). The vectors  $q_{\text{parallel}}$  and  $q_{\text{perpendicular}}$  in Figure 1 center are parallel and perpendicular to the scattering vector and are coincident with  $\omega/2\theta$  and  $\omega$  respectively<sup>4, 5</sup> (Figure 2). In the case shown in figure 1(a) all the domains are well aligned so their contributions to the reciprocal lattice point overlap. Misalignment of the domains, figure 1(b) broadens the reciprocal lattice point along  $q_{\text{perpendicular}}$  but causes no broadening in the along  $q_{\text{parallel}}$ . Figure 1(c) shows small, well-aligned domains, the lattice point is broadened in the  $q_{\text{parallel}}$  direction. If the volume of the domains becomes very small the reflections will become broadened from Fourier truncation effects. A single domain is shown in Figure 1(d) has lattice parameter variation that also broadens the reciprocal lattice point in the  $q_{\text{parallel}}$  direction. The lattice variation between the unit cells causes a reflection to have slightly different  $2\theta$  values resulting in broadened reflections. Volume effects and lattice parameter variation can only be distinguished by making measurements at multiple resolutions. Volume effects are resolution independent, whereas lattice effects are resolution dependent. In a realistic case, Figure 1(e), point, line, and plane defects, volume and misalignment all contribute to broaden the reciprocal lattice point in both dimensions. All of the effects can be anisotropic. The analysis of individual reflections can provide a measure of the long-

range order within the crystal. And by making measurements in multiple regions of reciprocal space crystal anisotropy can be investigated. Reflection analysis does not provide information about disordered loops and side chains, thermal vibrations and other kinds of short-range disorder.

## ***Experimental methods***

Crystal volume and physical appearance under the microscope give a qualitative description of crystal quality at best. The diffraction quality of a crystal is determined by features too small to be observed at optical wavelengths. Detailed analysis in reciprocal space provides a quantitative measure. X-ray diffraction analysis techniques can be categorized into volume integrating, imaging and three-dimensional profiling techniques<sup>6</sup>. A unifying requirement in all three methods is that the properties of the incident X-ray beam should not mask the diffraction properties being measured. The relevant properties are vertical and horizontal divergence, bandwidth and spectral dispersion.

In the traditional Ewald sphere construction the sphere is a differentially thin shell. This corresponds to a perfectly monochromatic beam with no angular divergence. A beam with non-zero beam divergence and finite bandwidth can be modeled by Ewald spheres with finite shell thickness (figure 2, inset)<sup>7, 8</sup>. A perfect crystal would have extremely small, almost infinitesimal, reciprocal lattice points. However the mosaicity of a real crystal broadens the reciprocal lattice points into finite volumes. If the reciprocal lattice point can be totally encompassed in the thickness of the shell of the Ewald sphere then the effect of the crystal quality on the reflection parameters will be masked and in

effect only the beam parameters will be measured. When investigating crystal quality the probe, the X-ray beam, has to be carefully configured to prevent this. Typically in ordinary data collection the beam is focused to increase flux on the crystal. At synchrotron beamlines the bandwidth is not as narrow as it could be for the same reason.

An alternative approach is the Laue method which uses polychromatic (“white”) incident radiation to illuminate a stationary crystal<sup>9</sup>. The method is extremely sensitive to the mosaicity of crystals and simultaneously records a large number of reflections. Like the monochromatic method the Laue experiments require a highly parallel incident beam.

## **The incident X-ray beam – diffraction geometry**

The contribution of the vertical and horizontal crossfire angles at the sample,  $\gamma_v$  and  $\gamma_h$  respectively, and the bandwidth,  $\delta\lambda/\lambda$ , can be modeled in the Ewald construction (Figure 2). The beam divergence can be modeled by replacing the sphere with the locus of spheres resulting from a rotation of the nominal sphere around the origin of the reciprocal lattice,  $O$ , through  $\gamma_v$  and  $\gamma_h$ . The effect of finite bandwidth is modeled by two limiting spheres<sup>10</sup> with radii  $1/(\lambda-\Delta\lambda/2)$  and  $1/(\lambda+\Delta\lambda/2)$  that intersect at the origin (Figure 2). An additional effect is that as the crystal is rotated the reflections pass through the Ewald sphere with trajectories at differing angles of incidence to the surface of the sphere. This, of course, is the Lorentz effect and causes the angular width of the reflection to be increased independently of the quality of the crystal or the characteristics of the incident beam. For quantitative data processing, we must leave Ewald’s sphere behind and approach the problem analytically rather than geometrically. In the case of a horizontal rotation axis, the angular width for a reflection is given by<sup>11, 12</sup>;

$$|\phi_R| = \sqrt{L^2 \zeta^2 \gamma_h^2 + \gamma_v^2} + \frac{L}{d} \cos \theta_{hkl} \left[ \eta + \left( \frac{\delta \lambda}{\lambda} \right) \tan \theta_{hkl} \right] \quad (1)$$

Here,  $\phi_R$  is the measured reflection width,  $\zeta$  is the position of the corresponding reciprocal lattice point projected onto the rotation axis,  $d$  is the resolution ( $d = \lambda / 2 \sin \theta_{hkl}$ ),  $\eta$  is the mosaic spread and  $L$  is the correction for the Lorentz effect. If  $h$  and  $v$  are the horizontal (along the rotation axis) and vertical distance of the observed reflection from the direct beam position then  $\zeta^2$  is given by;

$$\zeta^2 = \left( \frac{h^2}{h^2 + v^2} \right) \sin^2(2\theta_{hkl}) \quad (2)$$

The Lorentz correction is given by;

$$L = \frac{1}{\sqrt{\sin^2(2\theta_{hkl}) - \zeta^2}} \quad (3)$$

The reflection angle  $2\theta_{hkl}$ , is given by;

$$2\theta_{hkl} = \tan^{-1} \left( \frac{\sqrt{h^2 + v^2}}{XTD} \right) \quad (4)$$

where  $XTD$  is the crystal to detector distance.

It can be seen that  $\gamma_v$  broadens the reflections universally over the detector whereas the effect of  $\gamma_h$  on the reflection width depends on the position of the reflection on the detector and is maximum along the horizontal. The Lorentz effect is always maximal along the rotation axis which in this case is horizontal. The wavelength

dispersion term has its largest effect on high-resolution reflections. In Eq.1 the correlated dispersion is ignored. Correlated dispersion is the variation of the wavelength across the beam and is negligible with the x-ray optics suitable for reflection analysis. Accurate structural and crystal quality data collection has to overcome or correct for these contributions to the reflection profile in the integration process.

In the Laue case the mosaicity,  $\eta$ , is derived from the radial extension,  $\Delta_{radial}$ , of the reflections;

$$\Delta_{radial} = 2\eta \frac{XTD}{\cos^2 2\theta} \quad (5)$$

This assumes an incident beam of zero divergence and the relationship becomes less trivial if that criterion is not met. A large crystal to film distance (2.4 m was used in<sup>9</sup>) and a fine pixel size detector, e.g. X-ray film, are required to make accurate measurements of  $\Delta_{radial}$ .

## **The incident X-ray beam – practical considerations**

The ideal use of synchrotron radiation is in the unfocused case with a low bandpass monochromator. The method of Multiple Anomalous Dispersion (MAD) also requires a highly monochromatic beam and these beamlines, operated in unfocused modes, are ideal for investigating crystal quality. MAD beamlines use monochromators with  $\delta\lambda/\lambda$  values on the order of  $10^{-4}$ . Typical beamlines in normal operation *i.e.* with a focusing mirror, have vertical divergences, on the order of  $10^{-3}$  radians and horizontal



divergences of several times that. The reflections will be broadened significantly (Eq. 1) and the crystal properties completely masked.

An example of what can be achieved in terms of beam properties is provided by experiments performed at Stanford Synchrotron Radiation Laboratory (SSRL) beamline 1-5 (Figure 3(a)). At the expense of X-ray intensity, the focusing mirror was dropped out of the direct beam path in order to achieve values of 19.5 and 48  $\mu$ radians at the full width at half maximum (FWHM) for  $\gamma_v$  and  $\gamma_h$ , respectively. The bandwidth from the double crystal Si(111) monochromator is  $2.43 \times 10^{-4}$  and the correlated dispersion of the beam at the sample position is calculated to be  $2.50 \times 10^{-4}$  Å/mm (at 1.000 Å) in the vertical direction with no horizontal dispersion. The contribution of the instrument to the reflection profiles measured is a broadening of 0.0016° minimally. The broadening is least along the equatorial plane, *i.e.* perpendicular to the horizontal rotation axis. Beamline 1-5 is a bending magnet beamline, an unfocused beam from an undulator source would be more intense with even less divergence. Typical laboratory sources with focusing mirrors or graphite monochromators are not suitable instruments to study macromolecular crystal quality due to high beam divergence. The home source can be configured for crystal quality measurements but only at the expense of X-ray intensity. For example, a Bartels type<sup>13</sup> monochromator can be used to condition the beam (Figure 3a). This type of monochromator can achieve a geometric divergence of 52  $\mu$ radians and a spectral divergence of  $1.5 \times 10^{-4}$  using the Ge(220) reflection. Other optical systems, *e.g.* parabolic graded mirrors can achieve reductions in the divergence characteristics<sup>14</sup> while increasing the available flux but do not approach that available from the synchrotron.

Because of the inherently low intensity of the highly collimated and monochromatic X-rays from laboratory sources and the weak scattering of macromolecular crystals these sources are best used for the detailed study of reflections identified as containing useful information from previous synchrotron based analysis. In this way the synchrotron and the laboratory X-ray source can be used in a complementary fashion.

The methods used for crystal quality measurements are reflection profiling, topography and reciprocal space mapping. They have in common the requirement that the X-ray beam illuminates a reciprocal space volume smaller than that of the reciprocal lattice points being measured. The experimental setup for each is illustrated in Figure 3. For reflection profiling (termed mosaicity analysis when the effects other than the crystal are deconvoluted out) the instrumental setup is identical to standard modern structural data collection with the exception that an unfocused beam is used and the rotation angle between successive images is very small, typically on the order of the instrument resolution function (a step of  $0.001^\circ$  was used for the SSRL beamline 1-5 configuration described above)<sup>15</sup>. The Bartels monochromator consisting of two channel-cut crystals each having its own (n,-n) double reflection geometry is also illustrated, figure 3(a). The first crystal produces a beam with a relatively large bandwidth but with a high correlation between the wavelength and beam direction. The second crystal is set such that the beam from the first crystal will strike it in dispersive geometry such that only a certain combination of wavelength and direction is passed out of the monochromator. Finally the fourth reflection in the second crystal returns the now spectrally and geometrically collimated beam to its original direction. For topography, Figure 3(b) the area or point

detector is replaced with a fine grain film or a nuclear emulsion plate. Topography and reflection profiling can be accomplished using similar experimental setups. If the detector used for reflection profiling has sufficiently high spatial resolution the topographs can be recorded simultaneously. Reciprocal space mapping is shown in Figure 3(c). The analyzer crystal is made of the same material as the monochromator crystal(s).

## ***Measuring the Quality of a Crystal***

### **Mosaicity**

We see from Eq. 1 that the width of a reflection profile,  $\phi_R$ , is a function of the beam parameters, experimental geometry and mosaicity. The angular extent of the reflection profile is termed the rocking width, generally evaluated as the FWHM of the rocking curve. The mosaicity is the contribution of the crystal to the measured rocking width. Thus mosaicity is the angular width of the reflection profile deconvoluted from beam, spectral and Lorentz effects (Eq. 1). Mosaicity analysis measures the rocking width and deconvolutes the mosaicity from the other factors in the measured rocking width.

Shaikovitch and Kam<sup>16</sup> published one of the first studies on the use of reflection profiling as an indicator of macromolecular crystal perfection. Subsequently Helliwell and coworkers made use of the synchrotron radiation properties described previously to minimize the geometric and spectral contributions of the X-ray source to the experimental data<sup>15, 17</sup>. The first measurements of mosaicity were made by recording

reflections individually with a scintillation counter mounted in the equatorial (vertical) plane and by rotating the crystal about a horizontal axis<sup>9, 15, 17-19</sup>. This experimental setup minimized the Lorentz effect and eliminated the contribution from the horizontal divergence of the synchrotron beam, (Eq. 1). Mosaicity analysis of chicken egg white lysozyme, apocrustacyanin-C<sub>1</sub>, and thaumatin crystals established a physical basis for the improvements seen in these microgravity-grown samples. The reduction in the mosaic spread in the microgravity-grown crystals produced a corresponding increase in the signal-to-noise ratio of the reflection. The minimum mosaicities recorded were 0.005° for lysozyme, 0.030° for apocrustacyanin C<sub>1</sub>, 0.018° for thaumatin<sup>9, 19, 20</sup>.

Earlier methods<sup>1, 9, 15, 18</sup> looked at a few, low-resolution reflections recorded one at a time. The results although intriguing were not statistically robust due to the paucity of data. We therefore developed a method using an area detector<sup>12</sup> as did Ferrer & Roth<sup>21</sup>. Our method combined superfine  $\phi$  slicing data collection, unfocused monochromatic synchrotron radiation with a charge coupled device (CCD) area detector in order to collect, index and analyze hundreds of reflections in a short time<sup>12, 22</sup>. The crystal mosaicity,  $\eta$ , can be deconvoluted from the measured reflection width  $\phi_R$ , by rearranging Eq.1 above to<sup>12, 22</sup>,

$$\eta = \frac{|\phi_R| - \sqrt{L^2 \zeta^2 \gamma_h^2 + \gamma_v^2}}{\frac{L}{d} \cos \theta_{hkl}} - \left( \frac{\delta \lambda}{\lambda} \right) \tan \theta_{hkl} \quad (6)$$

This method was first applied<sup>9</sup> to crystals of *E. coli* manganese superoxide dismutase (MnSOD)<sup>23</sup>. In one degree of data, the mosaicities of 260 reflections were measured. The mosaicity averaged 0.010° (s.d. 0.004°), measured as the FWHM, and ranged from 0.001° to 0.019°. Each reflection could be fitted with two Gaussian curves indicating that the crystal was composed of at least two mosaic domains. Indexing the reflections proved critical and allowed the anisotropic mosaicity to be related to the crystal packing based on the work of Ferrer and Roth<sup>21</sup>. Another study on lysozyme<sup>24</sup> developed a general expression;

$$\eta_{hkl}^{calc} = \frac{\eta_{abc} \left( \frac{(ah)^2 + (bk)^2 + (cl)^2}{a^2 + b^2 + c^2} \right) + \eta_{def} \left( \frac{(dh)^2 + (ek)^2 + (fl)^2}{d^2 + e^2 + f^2} \right) + \eta_{mno} \left( \frac{(mh)^2 + (nk)^2 + (ol)^2}{m^2 + n^2 + o^2} \right)}{h^2 + k^2 + l^2} + \eta_{const} \quad (7)$$

where  $(a,b,c)$ ,  $(d,e,f)$  and  $(m,n,o)$  are real space vectors in the crystal lattice coordinate system,  $h$ ,  $k$  and  $l$  are the reflection indices and  $\eta_{const}$  the isotropic component of the mosaicity. Lysozyme proved to be isotropic in terms of mosaicity but this equation allows anisotropic mosaicity to be probed in terms of any defined direction *e.g.* one related to the lattice or to the surface morphology.

Evaluating a statistically valid sample of indexed reflections becomes very important in comparing the quality of multiple crystals, for example, crystals grown by different methods, crystals of different morphologies, or for comparing crystal manipulations such as cryocooling protocols. As an example we describe a comparison of insulin crystals grown on earth with those grown in microgravity<sup>25</sup>. Using superfine  $\phi$  sliced data between 447 and 502 reflections were profiled for each of six microgravity grown insulin crystals. Between 14 to 174 reflections were profiled for equivalently

accumulated data from six earth crystals (the earth crystals were much weaker diffractors so it was not possible to collect as many reflections from them). The crystals were not cryocooled. The best microgravity crystals had an average  $\eta$  of  $0.002^\circ$  with a standard deviation of only  $0.001^\circ$  - near the limit of resolution of the instrument configuration used. Two of the earth crystals had fairly low mosaicity with average  $\eta$  values of  $0.013^\circ$  (*s.d.*  $0.004^\circ$ ) and  $0.017^\circ$  (*s.d.*  $0.005^\circ$ ), respectively, yet these  $\eta$  values were 6.5 and 8.5 times higher than the best microgravity crystals and both crystals were relatively poor diffractors. For any given earth crystal, the  $\eta$  values for individual reflections varied over a surprisingly large range, with standard deviations of  $0.004$  to  $0.024^\circ$ . The spread in  $\eta$  for microgravity crystals was 4-5 fold narrower with standard deviations ranging from  $0.001$  to  $0.005^\circ$ . In a few cases the best earth  $\eta$  values overlap the worst microgravity values. This illustrates the importance of collecting a statistically significant number of reflections from each sample as an unlucky selection of a few reflections could lead to an erroneous conclusion. A non-parametric, distribution free, Mann-Whitney rank sum test confirms that the microgravity and the earth data are statistically different from each other at the 99% confidence interval. It is important, not only to collect a statistically significant number of reflections but also to collect data from multiple samples in this case 6 crystals of each kind. The microgravity crystals were on average 34 times larger, had 7 times lower mosaicity, had 54 times higher reflection peak heights and diffracted to significantly higher resolution than their earth grown counterparts. Figure 4 shows an example of a reflection profile for one of the earth-grown crystals decomposed into three Gaussians. Figure 5 illustrates the effect of reduced mosaicity on the quality of the data obtained from examples of the insulin crystals in the study described. Crystals with

reduced mosaicity produced data with a higher signal to noise ratio. The mosaicity of a crystal is not directly related to diffraction resolution, but crystals of lower mosaicity produce a higher peak intensity that may be detectable at higher resolution.

During structural data collection the correct  $\phi$  step can take advantage of a reduced mosaicity to maximize the signal to noise thereby improving the useful resolution in the data<sup>26</sup>. Reduced mosaicity increases the number of fully recorded reflections per image, and reduces spatial overlap<sup>27</sup>. Fine-sliced images using oscillation methods can be used to take advantage of low mosaicity but the method does present difficulties. The data may suffer from increased detector readout noise and the shorter, narrower images place more stringent requirements on the hardware for shutter timing and goniometer control<sup>26</sup>. The time lost during detector readout is also increased. In studying mosaicity superfine  $\phi$  slicing provides the necessary detail. For structural data collection where the beam is not as parallel and possibly not as monochromatic there is little or nothing to be gained with oscillations less than one half of the greater of the beam contribution or the crystal mosaicity. It is, of course, important to understand the characteristics of the beamline before starting and to process the data as it is collected in order to maximize the quality of data that can be collected.

## Topography

X-ray topography is an imaging technique based on the reflection of X-rays by a set of planes in the lattice where irregularities cause locally changing diffracted intensities (contrast) in topographic images of individual reflections<sup>1</sup>. Topographs are a measure of the scattering power of a crystal as a function of position across the diffracted

X-ray beam. Essentially it is an image of the diffracting parts of the crystal at a particular, stationary, orientation. In most cases it is not the defect but the lattice surrounding the defect that produces the contrast. Intensity variations are related to the type and volume distribution of defects. Three causes of contrast are, orientation variations due to domain misalignment, extinction due to a high strain gradient and dynamical scattering effects, small for weakly scattering macromolecules. A high quality region of the crystal will have a uniform dark or light area in the topograph. The maximum spatial resolution obtainable in a topograph is about 2-3  $\mu\text{m}$  with photographic film and 1  $\mu\text{m}$  with nuclear emulsion plates.

Topography on macromolecular crystals was suggested by Shaikevitch & Kam<sup>16</sup>. Stojanoff & Siddons<sup>28</sup> used the white Laue beam to study lysozyme crystals. Highly strained regions, high densities of defects and also quite perfect regions were seen. The topographs were surprisingly detailed. Fourme *et al.*<sup>18</sup> used reflection profiles to take topographs at different Bragg angles of multiple peaks seen in the same reflection, again from lysozyme. They discovered separate regions or domains of the crystal contributing to each peak of the total reflection.

Topography has been used as an effective technique to study the effect of solution variations during crystal growth<sup>29</sup>. Topography of lysozyme crystals subjected to deliberate variation of temperature, pH or mother liquor concentrations during their growth revealed several general effects. In crystals subjected to a pH change the scattered intensity from the boundary layer just outside the pre-change region differs strongly from both earlier and subsequent regions. The lattice growing during the change



is more disordered than that before and that shortly after. It seems that crystal perfection recovers in subsequent lattice growth. A similar effect is seen for concentration changes of both the protein and salt. Temperature change causes a difference in the mosaicity or lattice dimensions. Lysozyme is relatively insensitive to changes in growth conditions compared to most macromolecules so the changes employed were large. Temperature was changed from 295 to 288 K, pH from 4 to 5 and in combination protein concentration reduced from 65 to 11 mg ml<sup>-1</sup> while salt was increased from 0.45 to 1.2 M. The authors studied the effects of protein concentration by transferring growing crystals from a 27 to a 41 mg ml<sup>-1</sup> protein concentration solution. A factor of 3 increase in the growth rate did not produce substantial features in the resulting topographs. This suggests that in the growth process, a change of protein concentration in the drop does not affect the quality of the resulting crystal. This is an important result as concentration is constantly changing as the crystal grows. The crystal growth experiment is carried out in a dynamically changing solution environment. By application of reflection profiling in the same experiment it was concluded that the contrast variation seen in the topograph is primarily due to lattice mosaicity, figure 1(b). Topographs acquired at successive angles within the reflection profile will map out the contribution of the crystal to each point of that profile. Figure 6 illustrates topographs from two high quality lysozyme crystals. In 6(a) and 6(b) the crystal clearly consists of two major domains whereas the crystal illustrated in 6(c) and 6(d) consists of several domains separated by boundary areas<sup>1</sup>.

With an undulator source the geometric divergence can be very small and the spatial resolution in the topograph high. The different growth sectors within the crystal can be imaged, and more remarkably, fringes at the boundaries of those growth sectors<sup>4</sup>.

Topography provides a strong but qualitative method suited to the study of crystal growth and other practical applications such as the study of cryoprotectant effects on cooling.

## **Reciprocal Space Mapping**

Although the term reciprocal space mapping can be used to describe all methods of diffraction data collection<sup>30</sup> we use it in a more limited sense to describe examining a volume of reciprocal space in two or three dimensions. Reflection profiling and topography image the reciprocal lattice over a relatively large volume. Much information about the shape of the reciprocal lattice point is lost. Reciprocal space mapping provides the shape information lost from the other techniques. The effects shown in Figure 1 contribute to the measured mosaicity. Reciprocal space mapping allows us to understand mosaicity in terms of the components shown that contribute to it.

Reciprocal mapping is achieved by sampling the reflection profile using an analyzer crystal in the path of the diffracted beam, figure 3(c). The reciprocal space map is recorded by mapping in the analyzer crystal and detector axis. The direction of these scans is illustrated in Figure 2 where  $\omega$  translates to  $q_{\text{parallel}}$  and  $\omega/2\theta$  to  $q_{\text{perpendicular}}$  of Figure 1. Reciprocal space mapping is a high fidelity, time consuming technique at the expense of the number of reflections that can be studied. It is effectively used in combination with area detector mosaicity studies where reflections of interest are identified for later study in detail.

Reciprocal space mapping of macromolecular crystals was first performed in the laboratory<sup>31</sup> using a Bartels monochromator system. Lysozyme was extremely weakly scattering but produced very sharp profiles<sup>30</sup>. Later experiments with synchrotron radiation produced similar results, Figure 7<sup>1</sup>. By recording maps at multiple  $\chi$  positions a three-dimensional profile of the reciprocal lattice can be built up<sup>30</sup>. Lysozyme crystals were found to present a complex analysis problem as reciprocal space mapping data reveals that they appear to lie at the convergence of the kinematical (ideally imperfect crystal model) and dynamical (ideally perfect crystal model) treatments of diffraction<sup>14</sup>. Kinematical diffraction ignores the interaction of wave fields within the crystal and is valid for a crystal that is small compared to the extinction distance  $\varepsilon$  defined by <sup>32</sup>;

$$\varepsilon = \frac{V_c}{r_0 C |F_h| \lambda} \quad (8)$$

where  $C$  is the polarization factor,  $V_c$  the volume of the unit cell,  $r_0$  the classical electron radius,  $|F_h|$  the amplitude of the structure factor and  $\lambda$  the wavelength. Dynamical theory allows the coupling of the wave fields within the crystal and accounts for extinction effects. For X-ray wavelengths and macromolecular crystals the extinction distance can be on the order of a mm<sup>14, 17, 18</sup>. The mosaicity can be predicted from both kinematical and dynamical theory. The values predicted from both theories turn out to be similar<sup>14</sup>. Dynamical theory can have an important impact for structural crystallography on the accuracy of the integrated intensities especially of the lower resolution more intense reflections. Polykarpov & Sawyer<sup>33</sup> derived an extinction correction to take account of dynamical properties in macromolecular crystals. They found that in the case of alcohol dehydrogenase the correction may be as much as 15% for the strongest, low

resolution reflections and that as many as 20% of all the reflections at a resolution lower than 3.4 Å had to be corrected by more than 2% compared to kinematical diffraction data.

The considerable length of time required for reciprocal space mapping makes radiation damage a concern. Fortunately when unfocused, highly monochromatic radiation is used samples receive far lower doses than for an equivalent time of structural data collection. Radiation damage is both time and dose related but Voltz & Matyi<sup>34</sup> report a case of 5 days of continuous radiation not affecting data from a lysozyme sample on a well conditioned laboratory beam.

Reciprocal space mapping reveals information that cannot be seen through mosaicity or topography. The technique has been used with great success in the semiconductor industry due to a comprehensive practical and theoretical understanding of the sample material<sup>30</sup>. Macromolecular crystals are far more complex systems and theory has yet to catch up with experiment in understanding just how much information reciprocal space mapping can reveal in the macromolecular world. It is one of the developing areas in crystal quality analysis.

### ***The complete picture***

Mosaicity, topography and reciprocal space mapping are all techniques to probe the physical characteristics of the crystals by their interaction with X-rays. The techniques described are complementary. For example Boggon *et al.*<sup>1</sup> combined the three techniques with synchrotron radiation in the study of microgravity and ground grown crystals. Only a small number of samples were used but microgravity crystals showed a reduced mosaicity. Reciprocal space maps saw no change in stress and topography

showed that the majority of the crystal was contributing to the peak of the reflection at the appropriate Bragg angle in the microgravity case. Each technique provided unique information and each technique also provided complementary information.

In terms of structural crystallography, *i.e.* solving and understanding the structure of a macromolecule of interest, having a high quality crystal is clearly desirable. The techniques described here are not part of routine data collection. Of the techniques described mosaicity measurement is of the most immediate use. The quality of the data can be optimized by matching the oscillation range to the mosaicity. The background in an oscillation image builds up throughout the oscillation range but the reflection is only recorded over a finite angle. In the future “ideal” data collection may be possible by continuous rotation with real time detector readout offering effectively infinitely fine slicing.

Mosaicity, topography and reciprocal space mapping are diagnostic techniques that allow us to ask questions about the practical effects of the crystal growth process and the data collection practices in order to optimize them. They offer quantitative data about crystal growth methods, biochemical properties and practical matters such as cryocooling protocols, cryogens and crystal handling for automated studies. The resolution of the structural data and corresponding electron density maps provide us with an indication of the short-range quality. The techniques described here give us a measure of long-range order. Many of the crystal quality techniques have been developed with lysozyme, the future will see them being applied to more real life cases. Eventually crystal growth, now an empirical process of rational trial and error guided by past experience, may be understood in far greater detail with information from reflection analysis. A surprise has

been just how ordered macromolecular crystals can be. This offers potential in new phasing methods such as multiple beam diffraction<sup>35</sup> and the exploitation of the coherent radiation opportunities available at third generation synchrotron sources.

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### ***Figures.***

Figure 1. Schematic diagram illustrating the influence of various physical properties of the crystal (left) on the reciprocal lattice point volume (shown in two-dimensions center) and the recorded reflection profile width (right). In (a) the crystal has a mosaic domain structure but the domains are well aligned. In (b) the domains are misaligned with respect to each other. This can be an anisotropic effect. Sharp reflections from each domain are distributed smearing out the overall profile. Well aligned domains are shown in (c) with a reduced volume. This can be anisotropic but is resolution independent. Fourier truncation effects cause smearing out of the reflections from each domain when compared to larger domains. An enlargement of a single domain is shown in (d) with lattice variations and the reciprocal space map from a number of those domains illustrated. The effect can be anisotropic and is resolution dependent. Finally (e) shows a

realistic case where a number of effects contribute. The effect of imperfections in the crystal are to smear the reflection intensity out and reduce the overall peak intensity.

Figure 2. Graphical illustration of reciprocal lattice broadening from crystal mosaicity, and the Ewald sphere illuminated volume broadening from the geometric and spectral aspects of the X-ray source. The hexagon has been enlarged below the Ewald sphere construction for ease of viewing. Shown are the  $\omega$  and  $\omega/2\theta$  scans used in reciprocal space mapping. Dimensions have been exaggerated. Adapted from<sup>8,10</sup>.

Figure 3. Schematic diagram of the experimental setup to perform (a) reflection profiling (to obtain mosaicity) at the synchrotron using a double crystal monochromator and in the laboratory with a Bartels monochromator, (b) topography using film/nuclear emulsion plates and (c) reciprocal space mapping showing the addition of an analyzer crystal.

Figure 4. Profile of the (5, -16, 3) reflection from insulin<sup>25</sup>. This reflection was collected at a  $2\theta$  angle of  $222.9^\circ$  and was accurately fitted by the sum of three Gaussians. The measured FWHM,  $\phi_R$ , was  $0.036^\circ$  with a mosaicity,  $\eta$ , after suitable deconvolution of  $0.010^\circ$ .

Figure 5. Crystal quality comparison of insulin crystals used in a microgravity versus ground-growth study. Mosaicity and background-subtracted intensity are plotted against resolution. The data were cut off at the detector edge. Maximum intensity normalized to a 2s exposure is plotted on a log scale. Further details can be found in<sup>18</sup>.

Figure 6. Topographs taken from two high quality lysozyme crystals<sup>1</sup>. Each topograph is a greatly magnified image of a reflection. In (a) and (b) the crystal is 1.1 mm by 0.9 mm in projection and defined regions are seen at the different reflections of (a) and (b). Some scattering is also seen on the crystal edges, probably due to mounting. In (c) and (d) the crystal is 1.5 mm by 1.1 mm in projection. In this case an array of domains is seen separated by a boundary layer. The different reflections (c) and (d) illustrate a region in the lower right of the crystal coming into the Bragg diffracting condition at the current  $\phi$  orientation. The properties of the monochromatic beam are well illustrated in this case showing the clearly defined shape of the crystal rather than any collimation or divergence properties.

Figure 7. Example of a reciprocal space map of reflection (13 1 8) from a lysozyme crystal of 0.7 x 0.7 x 0.4 in dimension<sup>2</sup>. The mosaicity for this sample was 0.002° with  $q_{\text{parallel}}$  of  $1.0 \times 10^{-4}$  and  $q_{\text{perpendicular}}$  of  $0.9 \times 10^{-4}$  at full width at half height maximum. The units of  $q$  are  $2\pi/\lambda$  with  $\lambda$  being 1.0 Å in this case. Further details can be found in<sup>1</sup>.

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