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X-ray crystallography and chirality: understanding the limitations

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A R T I C L E   I N F O

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This paper is dedicated to Professor George Fleet, on the occasion of his 65th birthday

A B S T R A C T

Advances in hardware and software have made X-ray crystallography even more attractive as the first-option method for structure analysis. For most organic materials containing up to 100 non-hydrogen atoms, getting from the initial visual examination of the sample to producing publication-ready tables and pictures should usually be achievable in a single morning. Improvements in hardware have also increased reliability of the determination of absolute configuration. A recently published new algorithm may extend the range of applicability of the method.

Contents

1. Introduction

Ever since Pasteur, in 1848,[1] had the good fortune to be working with sodium ammonium tartrate, and to have paid sufficient attention in detail to see that crystals of the material were of two hands, there has been a strong link between crystallography and chirality.[2] What is remarkable is that at that time very little was known about molecular structure, and nothing about the shape of molecules. ‘Seeing’ molecules with X-rays was more than half a century into the future, for example, the structure of hexachlorocyclohexane by Hendricks and Bilicke.[3]

In 1994 Hope[4] wrote ‘*Unfortunately, the chemical community in general is not enjoying the full benefits of [the advances in crystallographic hardware and software]. . . . The result is that X-ray crystallography in many laboratories is still regarded as a time-consuming, expensive method of last resort*’.

In 2009, given fair quality crystals, X-ray structure analysis can generally yield the total connectivity and relative configuration of all non-hydrogen atoms in small (up to 100 atoms) organic molecules in less than 1–3 h. Given slightly better quality crystals, it can also reveal most of the hydrogen atoms. Advances in computers, modern CCD diffractometers and the routine availability of cryo-coolers working at 100–150 K, mean that hardware is no longer a serious issue. The major bottle-neck for X-ray analysis is the production of suitable crystals—an art once practiced by most synthetic chemists, but sadly almost lost since the advent of chromatographic methods for sample purification.

Given good quality crystals, it has become increasingly easy to directly determine the absolute configuration of chiral molecules without the need to form a heavy atom[†] derivative or complex. In order to understand the outcome of this kind of analysis, it is important to have a clear understanding of the nature of the sample—in particular whether or not the bulk sample is enantiomerically pure. For enantiopure bulk samples, it can be assumed that the structure revealed by the X-ray analysis of one single crystal is the same as that of the rest of the sample, and the analysis simply has to distinguish between one hand and its opposite.

If it cannot be guaranteed that the bulk sample is enantiopure, the interpretation of the crystallographic results becomes much more complicated. X-ray crystallographic analysis works with a single crystal which weighs perhaps no more than 1 μg, selected from the batch of crystals sent for analysis, and chosen for its good physical appearance and quality of diffraction. It may not be typical

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[†] In this context, heavy means an atomic number greater than that of argon.

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Table 1
Division of the 32 crystal classes according to property

	Crystal characteristics		Flack classification[a,8]	Crystal class
1	Centrosymmetric	Achiral	CA	$\bar{1}$, $2/m$, mmm, $4/m$, $4/mmm$, $\bar{3}$, $\bar{3}m$, $6/m$, $6/mmm$, $m\bar{3}$, $m\bar{3}m$
2	Non-centrosymmetric	Achiral	NA	m, $mm2$, $\bar{4}$, $4mm$, $\bar{4}2m$, $3m$, $\bar{6}$, $6mm$, $\bar{6}2m$, $\bar{4}3m$
3	Non-centrosymmetric	Chiral	NC	1, 2, 222, 4, 422, 3, 32, 6, 622, 23, 432

[a] The Flack Classification CA corresponds to Centrosymmetric Achiral etc.

Table 2
Relationships between molecular properties, nature of the solution and nature of possible crystals

Solution	Chiral molecule								Achiral Molecule		
	Enantiopure chiral		Enantiomeric mixture*						Homogeneous		
Molecular composition of the single crystal	Enantiopure chiral		Enantiopure chiral	Enantiomeric mixture					—	—	—
Crystal structure	Achiral	Non-centro-symmetric, chiral	Conglomerate (collection of resolved crystals)	Inversion twinned	Non-centrosymmetric achiral	Racemic		Disordered solid solution	Non-centro-symmetric chiral	Non-centro-symmetric achiral	Centro-symmetric achiral
Flack classification		NC	NC	NC	NA	NA	CA		NC	NA	CA
Typical space group	Not known in nature	$P2_12_12_1$	$P6_1$ and $P6_5$	$P2_1$(twinned)	Pc	$P2_1$	$P2_1/c$	any	$P3_121$	Pn	$P2_1/c$

[*] A pair of enantiomeric molecules can associate to form the building block of an achiral crystal structure. The local symmetry of the building block does not coincide with any crystallographic symmetry.

of the whole batch, so some other technique, such as circular dichroism (CD) or enantioselective chromatography must be used to verify (after the X-ray data have been measured) that the chosen single crystal has the same characteristics as the bulk sample. Note that all physical measurements have limits on both precision and accuracy so that it may be difficult to say with absolute confidence that a given bulk sample is enantiopure. Jacques, Collet and Wilen[5] discuss all of these issues in great detail.

A molecule can be chiral (have a 'hand') or achiral. A chiral molecule cannot be superimposed upon an image of itself created by the action of a mirror, centre of inversion or an improper axis of rotation.[6] Conversely, the symmetry group of an achiral molecule will contain a mirror, centre of inversion or an improper axis of rotation, and so can be superimposed upon an image of itself created by the action of a mirror, centre of inversion or an improper axis of rotation.

Flack says, '*Absolute structure leads to absolute configuration. Absolute structure is a crystallographer's term and applies to non-centrosymmetric crystal structures. Absolute configuration is a chemist's term and refers to chiral molecules*'.[7] In order to understand how crystal structures can be used to determine the absolute configuration, it is necessary to understand that (for this purpose) there are three types of space group, that is, symmetry operators controlling the arrangements of motifs within the unit cell (see Table 1).

Crystals of type 1 are centrosymmetric and hence achiral, meaning every motif is accompanied by its inverse. Crystals of type 2 are non-centrosymmetric, but are still achiral. Here the crystal has a sense of 'top' and 'bottom', but within the unit cell each motif is accompanied by its inverse. It is only crystals of type 3 which are experimentally observed to be built without motifs of opposing hands.[‡] The International (Hermann-Maugin) space group symbol for a material in class 1 or 2 will include either a minus sign (bar) or a letter after the capital letter representing the lattice type.

A collection of chiral molecules all of the same hand (i.e., enantiomerically pure) normally assemble themselves into a chiral, non-centrosymmetric, crystal (e.g., sucrose, in $P2_1$). A collection of achiral molecules normally assemble themselves into a centrosymmetric achiral crystal (e.g., benzene, in $Pbca$). However, it is also possible for achiral molecules to assemble themselves into a non-centrosymmetric chiral crystal. For example, achiral silica, SiO_4 tetrahedra, can form left- or right-handed threefold helices giving rise to d-quartz in $P3_221$ or l-quartz, in $P3_121$. In this case, the crystallographic motif is the spiral, not an isolated SiO_4 moiety.

A solution containing chiral molecules of both hands can crystallise in four ways. They can form achiral centrosymmetric crystals containing equal numbers of molecules of each hand; they can spontaneously resolve to form a conglomerate consisting of a mechanical mixture of crystals, each crystal uniquely containing molecules of one hand or the other, or they can crystallise as a twin by inversion. A twin by inversion will be in a non-centrosymmetric space group, but actually contains zones in which the crystal structure has one orientation juxtaposed against zones with the inverse orientation. The zones may be sufficiently large to be visible with a microscope, or may be undetectable optically. If the total weights of the zones of each hand are roughly the same, a solution made from the crystal will show no optical rotation. There is also the possibility that a given crystal is a solid state mixture of the two enantiomers. The chart above (Table 2) summarises these options, which may help to emphasise both that a *crystal structure* may be chiral or achiral, and the molecules from which it is made may be chiral or achiral. The use of homochiral molecules to form an achiral motif that packs to yield achiral crystals is not seen in Nature. Flack[8] discusses why this may be so, even though non-crystallographic models can be devised (e.g., *la Coupe du Roi*,[9] Fig. 1, in which an achiral spherical fruit can be cut into two identical chiral moieties).

2. Understanding crystal structure analysis

A good introduction to modern crystallography can be found in Clegg's Crystal Structure Determination.[10] Briefly, the process works as follows: the macroscopic crystal is composed of very large numbers of fundamental building blocks (the unit cells) arranged side by side in three dimensions. Since each block is the same as its neighbour, this leads to a periodic object which can act as a three dimensional diffraction grating for incident radiation with a wavelength similar to the dimension of the periodicity. X-rays, with a wavelength of the order of 1 Å, are a suitable radiation.[§] When a mono-

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[‡] For an explanation of this slightly evasive definition , see La Coupe du Roi below.

[§] In most chemistry laboratories the available wavelengths are 1.5 Å from a source with a copper target (Cu Kα radiation) or 0.7 Å (Mo Kα radiation). Synchrotron sources have tuneable wavelengths.

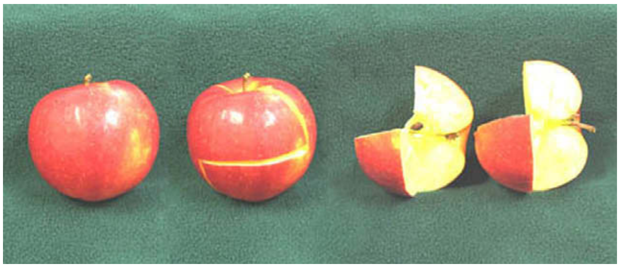

Figure 1. La Coupe du Roi involves four cuts: two half-hemispherical cuts at right angles (from the stalk to the middle and from the bottom of the core to the middle), followed by cuts joining the ends of the first two, penetrating to the middle, approximately in the shape of a triangle. The resulting pieces are chiral, with the same hand, but can be re-assembled to give an achiral apple.

chromatic beam falls on a crystal, diffracted beams emerge which can be recorded on a suitable detector. The *location* of these beams on the detector depends only on the size and orientation of the unit cell, but not on the contents of the unit cell. Each diffracted beam is allocated three indices (h,k,l) which express the relationship between the beam and the unit cell, and act as labels. The *intensity* of each beam is a function (Fourier transform) of the electron density throughout the entire unit cell. This means that *every* atom in the unit cell contributes to *each* diffracted beam. Since the physical phenomenon is a diffraction effect, each emergent beam has both an intensity and a phase angle. The intensity is easily measured with some type of photon detector, but for practical purposes the phase is not measurable, leading to the well-known *Crystallographic Phase Problem*. Developments in the period 1950–1980 lead to statistical methods for reliably estimating the phases, so that the problem has largely disappeared for small organic molecules. The measured intensities are converted to the *observed structure factors,* $|F_o|$, by applying instrument-dependant corrections. With the structure amplitude and the phase angle of each diffracted beam known, a reverse Fourier transform using *all* the available data will reveal the electron density at *any* point in the unit cell. This is a fundamental difference between X-ray crystal structure analysis and most other spectroscopic methods. It is not possible to exclusively associate some diffracted beams with some atomic feature—the whole crystal structure contributes to each diffracted beam, and all possible beams must be included in the calculation in order to reveal any atomic feature. The calculated electron density throughout the unit cell is generally scanned for local maxima, and these are associated with atoms (Fig. 2). The magnitude of the electron density at the atomic site is a guide to the atomic number of the atom.

At this stage, the model consisting of parameters that describe the structure (principally the atomic coordinates) is only approximate. *Calculated structure factors*, $|F_c|$, can be computed from this model and the model sequentially adjusted to get a best fit between the observed $|F_o|$ and calculated $|F_c|$ values. *All* the model parameters are simultaneously adjusted to give the best fit for *all* the data, usually by the method of least squares. The quality of the fit is reflected in $\sum(|F_o|^2 - |F_c|^2)^2$ summed over all the data, which clearly gets smaller as the calculated structure factors better match the observed data. It is important to realise that the consequences of any disagreements between individual $|F_o|$ and $|F_c|$ are distributed over all the refineable parameters. As the model is improved, additional fine detail is included and finally, if the structure is in a non-centrosymmetric space group, the absolute structure is assigned.

In practice, this over-view of the methodology follows quite closely the actual procedure for the majority of analyses, although somewhere between 5% and 15% of analyses may prove to be troublesome. The complexity of the crystallography is only loosely correlated with the complexity of the chemistry, so that sometimes quite trivial materials raise complex crystallographic problems, making it difficult to predict with any certainty how long an analysis will take.

Even if good crystals exist, experimental issues can occasionally degrade the quality of the analysis, the most common problem being *disorder*. Of the 11199 structures deposited in the Cambridge Crystallographic Data Base in 2007, 25% were marked as having some form of disorder. X-ray crystallography not only locates the average positions of each atom in the unit cell, but it also determines the mean square displacements from the average positions. In diagrams, these displacements are generally represented by an ellipsoid which maps out the possible places an atom may occupy, often plotted at a 50% probability level. This uncertainty about an atomic position occurs because the experiment takes a long time, when compared with atomic or molecular vibrations, so that the result is a time average. In addition, the sample contains many millions of unit cells all of which may not be quite identical, so that the resulting structure is also a space average. This may become evident as atoms or groups of atoms appear to occupy alternative positions, for example as a result of ring-flipping. Together, these deviations from ideality are called disorder and their influence on the analysis depends upon the severity of the disorder, and which atoms are involved.

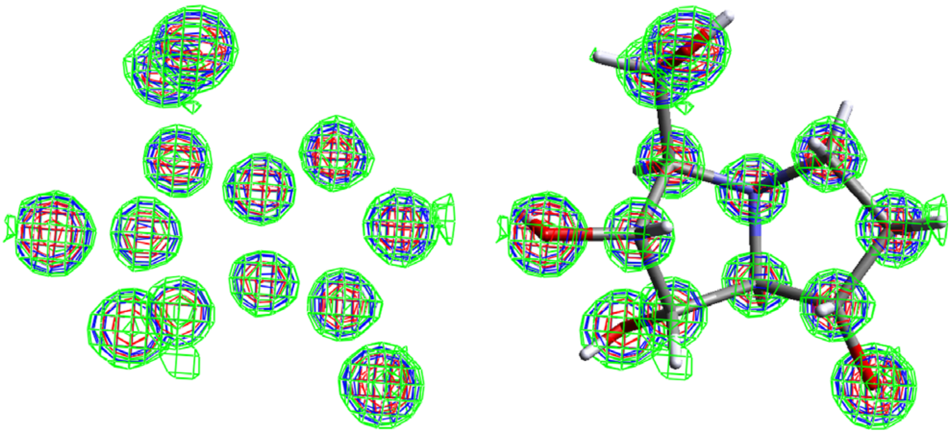

Figure 2. The electron density (left) is what X-rays 'see'. Atoms (right) are inserted into regions of high density, giving the traditional representation of a crystal structure. Images of epialexine[11] created with CRYSTALS[12] and MCE.[13]

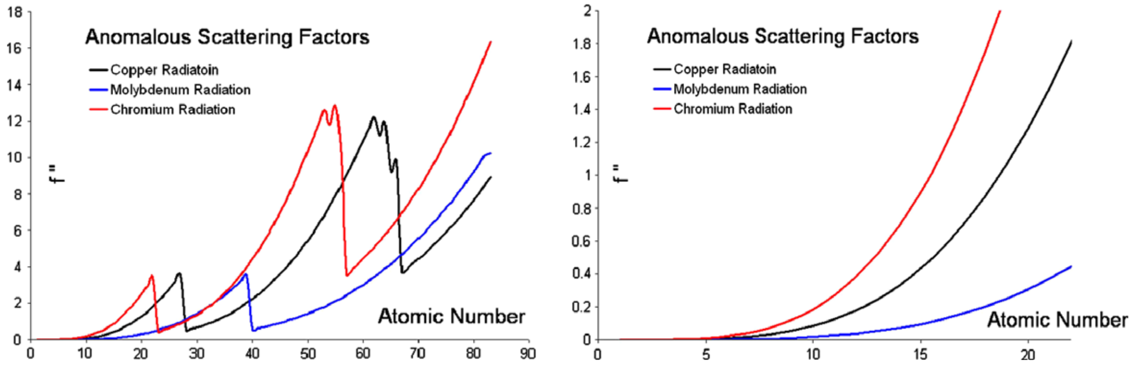

Figure 3. Anomalous scattering factors for all atoms (left) and for light atoms (right). The largest values for the anomalous scattering occur just before an absorption edge (left), a property which can be exploited using the variable wavelength radiations available at synchrotron facilities. For atoms with atomic number less than 20 (right) the anomalous scattering with laboratory sources is lowest for molybdenum Kα radiation.

3. Absolute configuration and absolute structure

Since crystal structure determination obtains the relative positions in space, it is always possible to obtain the relative configuration of one stereogenic centre with respect to another, known centre.[¶] Thus, one way to determine the absolute configuration is to introduce a reference centre. This can either be part of the molecule in question, or, if the material under investigation is an acid or base, it can be co-crystallised with a complex ion of known absolute configuration and the configuration of the new material inferred from the configuration of the known moiety.

If this route is not available, then the absolute structure of a single crystal may be determined. Pairs of reflections (hkl and $\bar{h}\bar{k}\bar{l}$) known as *Friedel* or *Bijvoet Pairs* can be examined. Friedel pairs have exactly the same intensity for centrosymmetric structures, but in non-centrosymmetric cases an effect known as *anomalous* (or *resonant*) *scattering* leads to small differences in the measured intensities. It is these anomalous (or Friedel or Bijvoet) differences which carry information about the absolute structure. The magnitude of the differences depends upon the sample and the experiment. In general, the anomalous differences increase as a function of atomic number, thus it was frequently said 'you need a bromine to determine the absolute configuration'. However, the anomalous differences also depend on the wavelength of X-rays used, generally increasing with the wavelength (Fig. 3). For light atom structures (those containing only C, H, N or O) the anomalous scattering with molybdenum radiation is very small. If the absolute structure is required, the analyst would prefer to use copper radiation, or if that is unavailable, they may ask the chemist to form crystals containing a heavier element, such as chlorine or sulfur. The heavier element does *not* have to be part of the molecule under investigation—a chlorinated solvent of crystallisation would be adequate.[7] Flack and Shmueli[14] propose a way of estimating in advance the likely robustness of an absolute structure determination from the chemical composition of the material and the wavelength of the radiation to be used.

If data can be obtained with discernable anomalous differences, there are broadly two ways for attempting to assign the absolute structure. The traditional method performed refinements of a structure and its inverse, and then tried to determine which model best agreed with the observed structure factors. Since the anomalous differences are small for organic materials, it was recommended to carefully re-measure those reflections with the largest anomalous differences. However, Rogers[15] recognised that a better strategy would be to include an enantio-sensitive parameter directly into the refinement so that its interaction with the other parameters (statistical correlation) could be accounted for. His parameter was a multiplier, η, applied to the anomalous component of the scattering factor, f''. This proved to be very successful for enantiopure crystals, but Flack[16] noted that the parameter only had physical meaning at values of ±1. He argued that if a given sample crystal could be regarded as a crystal containing a mole fraction of a given hand equivalent to $1 - x$, and x mole fraction of the other, the value of x had physical meaning throughout the range 0–1, and successfully accounted for twinning by inversion. Experience has shown that refinement of Flack's parameter is robust against other deficiencies in the analysis, presumably because discrepancies in the residual, $\sum w(|F_o|^2 - |F_c|^2)^2$, due to these deficiencies cannot be replicated by adjustment of x. Since x is a parameter in the refinement, its standard uncertainty $u(x)$ can also be determined. This is very important, since it provides a measure of the confidence one can have in the assignment of the absolute structure. Flack and Bernardinelli[17] give confidence intervals for the assignment of the absolute structure of materials of new samples. For a sample of unknown enantiopurity, the value of $u(x)$ must be less than about 0.04 for x to be a reliable assignment of the chirality of the major (and possibly only) component of the crystal. Thus, if the refinement yields $x = 0.10(4)$, this can be taken as a strong indication that the bulk of the crystal is of the stated configuration, but there is also the possibility that there is about 10% of the opposite configuration. If the material is known to be enantiopure, then the condition on $u(x)$ can be relaxed to 0.10 and a value of x in the range 0.3 to -0.3 would be considered as confirming the stated configuration. In the case of crystals twinned by inversion, there is no special reason in the general case why the ratio should be 50:50 (twinning by inversion, often referred to as racemic twinning).

Increasingly, there has been a suspicion that these conditions are over-cautious. Hooft et al.[18] have returned to analysing the difference between the observed data and those computed from an enantiopure model of arbitrary hand (i.e., with a Flack x defined to be exactly zero). The Friedel pairs are generally referred to using the descriptors hkl and $\bar{h}\bar{k}\bar{l}$, thus the observed data can be written $|F_o|^2hkl$ and $|F_o|^2\bar{h}\bar{k}\bar{l}$ and the data calculated from the model are written $|F_c|^2hkl$ and $|F_c|^2\bar{h}\bar{k}\bar{l}$. Thus, for every Friedel pair of reflections the difference, D$o$hkl, can be calculated $|F_o|^2hkl - |F_o|^2\bar{h}\bar{k}\bar{l}$, together with its standard uncertainly. A similar term $(|F_c|^2hkl - |F_c|^2\bar{h}\bar{k}\bar{l})$, called D$c$hkl, can also be computed. This should have the same sign as D$o$hkl if the model has the correct hand. Replacing a simple change of sign by a continuously variable parameter (γ) allows for the possibility of twinning by inversion. The probability of finding a particular value of γ from the observed

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[¶] For a molecule with two stereogenic centres, crystals of the enantiopure (RR)- and (SS)-isomers are equivalent except for a change of hand. The (RS)-isomer will have different crystallographic properties (unit cell etc) and will be quite distinct.

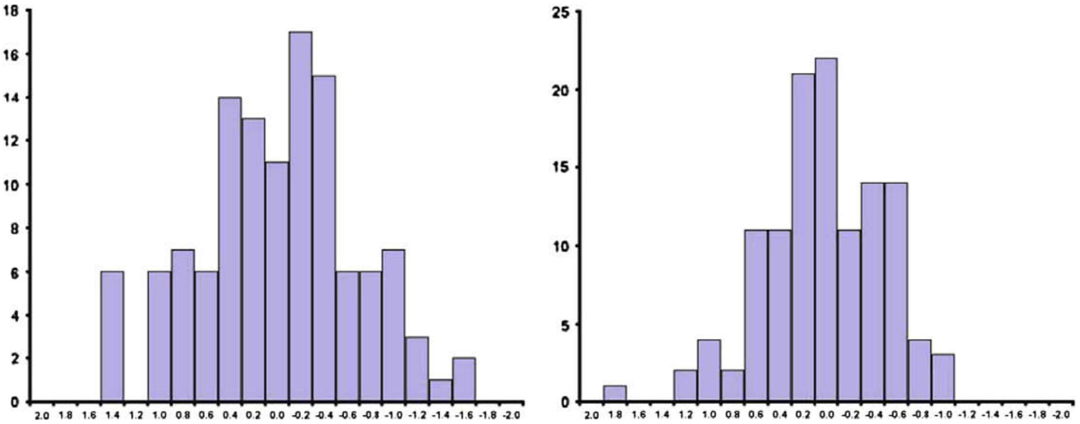

Figure 4. Frequency distributions for the Flack *x* parameter (left) and the Hooft *y* parameter (right) for 120 compounds of known absolute configuration. The information comes from routine structure determinations not specifically intended to be used for absolute structure determinations.

data can be computed, providing that the data are drawn from a reasonably well-behaved distribution, such as a Gaussian, the distributions can be compared. This can formally be written as:

$$G = \frac{\int \gamma Pu(\gamma)d\gamma}{\int Pu(\gamma)d\gamma}$$

From *G*, a parameter can be derived, called '*y*', which lies in the interval 0–1 and behaves something like the Flack *x* parameter. Since the calculation is working with distributions, there is the possibility of directly estimating the probability, P(2), for an enantiopure material that the assigned hand is correct. For a material of unknown enantiopurity, the probabilities of three outcomes, P(3), (correct assignment, 50:50 twin, incorrect assignment) can be computed. Hooft et al.[18] remark, 'These probabilities can be surprisingly decisive, even when the resonant scattering signal is very weak'. The Hooft *y* is computed statically from a finalised refinement, in a completely different way from the Flack *x*, so that while the behaviour of these two estimators can be expected to be largely similar, they can differ in detail, and in their response to unidentified errors in the data or short comings in the model. At the end of a refinement without the Flack parameter, the Hooft analysis assigns all the trends in the observed and computed Friedel differences (Do*hkl* and Dc*hkl*) to the single parameter, γ. In the results published to date, the major difference from the Flack *x* appears to be the reduced standard uncertainty in *y*, seemingly giving it greater enantiomer distinguishing power.

In order to get a snap-shot of the performance of the Flack and Hooft parameters, 120 recent data sets measured from light atom materials known to be enantiopure were examined. The X-ray data were measured using Mo Kα radiation on a Nonius KCCD area detector diffractometer. Routine data collection strategies were employed without the intention of using the results for absolute structure determination (COLLECT),[19] with conventional data processing (DENZO and SCALEPACK)[20] and usual structure refinement methods (CRYSTALS).[12] The conventional *R*-factors (*R*1) measured for all observed data lie in the range 2.34–11.48%, with only 16 of the 120 data sets giving R1 greater than 7%. Figure 4 is a histogram of the frequency distribution of the Flack and Hooft parameters. It broadly supports the suspicion that even without taking special care to measure all Friedel pairs, the Flack parameter tends to a value of less than 0.5. The observed mean and sample standard deviation, $\langle x \rangle = 0.004 \pm 0.669$, suggest that based on routine measurements of the X-ray data, the Flack parameter can be indicative. The corresponding plot for the Hooft parameter with a mean and sample standard deviation $\langle y \rangle = 0.018 \pm 0.512$ is substantially the same as the Flack distribution. The means and sample deviations computed with weights of $1/\sigma^2$ are not very different from the unweighted values. If definitive assignments are required, the experiment must be more carefully designed. Figure 5 shows the distribution of P(2), the probability that the structure is correct for an enantiopure sample, and P(3), the probability for an enantiopure sample allowing the additional possibility that the crystal is racemically twinned for the 120 samples. The observed distribution of P(2) (which should be 100% in the first bin for these samples) is reassuring whereas the distribution of P(3) correct probabilities is far from reassuring. It seems that the quality of modern data is such that the absolute structure determinations of enantiopure light atom structures using Mo radiation could be usefully indicative when driving a research programme, even if not entirely suitable for patent depositions.

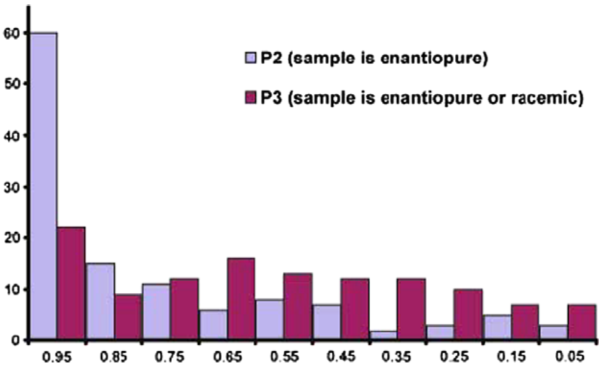

Figure 5. Normalised frequency distribution for the calculated probabilities from 120 samples of known enantiopurity. The probability (P2) that the structures were correctly computed and the probability (P3) which also allows the possibility that the crystals were racemically twinned are shown. If the sample is not known to be enantiopure, the probability of a clear-cut outcome is much reduced.

4. Practicalities

If it is of crucial importance to reliably assign the absolute configuration to a novel compound, then it is important to discuss the strategy with the crystallographer before the X-ray work is started. A check-list of topics to be considered includes:

- The enantiomeric excess (ee) of the bulk sample should be determined before any X-ray work is begun.

- Flack and Shmueli[14] give an expression for estimating the mean-square Friedel difference from a material given its chemical composition and radiation wavelength. From this, it is possible to estimate the likely standard uncertainty of the Flack parameter from a carefully performed experiment.
- If the material does not contain any atoms with strongly resonant scatterers with the available radiation, is it permitted/possible to form either a derivative or solvate containing a heavier atom?
- If the material is an acid or a base, can a crystalline salt be formed with a counter-ion of known absolute configuration?
- An excellent quality crystal should be selected and care taken to minimise systematic errors in the data (such as absorption).

After the structure has been determined, the following points should also be verified:

- The 'checkCIF' website does not generate any serious Alerts.[||]
- The X-ray data collection should have close to 100% coverage of the Friedel pairs within the scattering limit of the material.
- The refined model should give a substantially flat difference electron density plot.
- The weighted and unweighted '*R* factors' are adequately small.
- An F_o versus F_c plot should show no outliers or systematic deviation from a straight line with unit gradient.
- The refinement weights should be carefully assessed to ensure that the weighted residual $w(F_o - F_c)^2$ or $w'(F_o^2 - F_c^2)^2$ shows no trend as a function of either resolution or magnitude of F_c.
- The Normal Probability Plot of the weighted residual is essentially linear, passing through the origin and with unit gradient.
- The standard uncertainty of the Flack parameter is adequately small.
- The value of the Flack parameter is close to zero.

5. Conclusion

Given high quality samples and appropriate hardware and software, X-ray crystal structure determination offers the possibility of the ab initio determination of the absolute configuration of organic materials. As with all physical measurements, there are uncertainties about the outcome. With care, these uncertainties can be reduced, and in any case the uncertainty can be quantified. The standard uncertainty in the Flack parameter is the key to its interpretation. A value of 0.10 ± 0.04 could mean that there is a 99% probability that the material is more than 80% enantiopure. A value of 0.00 ± 0.04 could mean that there is a 99% probability that the material is more than 90% enantiopure. The distribution of P(2) for the enatiopure materials surveyed in this work suggests that the Flack criteria are conservative, and that with care, for some purposes a more liberal interpretation could be made.

Acknowledgements

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[||] CheckCIF is a web-based utility run by the International Union of Crystallography for performing machine-validation of crystal structures. http://journals.iucr.org/services/cif/checking/checkform.html.