X-ray crystallography and chirality: understanding the limitations

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A D V A N C E D  S E A R C H

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 ahead, for example, the structure of hexachlorocyclohexanes.³ It was not until the 1940s and 1950s that 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.

In 2009, given fair quality crystals, it has become increasingly easy to directly determine the absolute configuration of chiral molecules without the need to form a heavy atom⁴ 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.

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, 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.

² In this context, heavy means an atomic number greater than that of argon.
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 mole-
ucle cannot be superimposed upon an image of itself created by the
action of a mirror, centre of inversion or an improper axis of rota-
tion.\(^6\) 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-centro-
symmetric crystal structures. Absolute configuration is a chemist's
term and refers to chiral molecules.\(^7\) In order to understand how
chiral structures can be used to determine the absolute configuration,

\(^5\) For an explanation of this slightly evasive definition , see La Coupe du Roi below.

\(^6\) In most chemistry laboratories the available wavelengths are 1.5 Å from a source
with a copper target (Cu K\(_\alpha\) radiation) or 0.7 Å (Mo K\(_\alpha\) radiation). Synchrotron
sources have tuneable wavelengths.

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 num-
bers of fundamental building blocks (the unit cells) arranged side
by side in three dimensions. Since each block is the same as its neigh-
bour, this leads to a periodic object which can act as a three dimen-
sional diffraction grating for incident radiation with a wavelength
similar to the dimension of the periodicity. X-rays, with a wave-
length of the order of 1 Å, are a suitable radiation.\(^8\) When a mono-

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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 phenomenon known as the phase problem. Each diffracted beam is proportional to the electron density at the atomic site and is a guide to the atomic number of the atom.

Calculated structure factors, \(|F_o|\), can be computed from this available data. 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.

At this stage, the model consisting of parameters that describe the structure (principally the atomic coordinates) is only approximate. Calculated structure factors, \(|F_o|\), 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 \(\sum|F_o|^2 - |F_c|^2\) 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 refinable 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.

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 angles (from the stalk to the middle and from the bottom of the core to the middle),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\(^1\) created with CRYSTALS\(^2\) and MCE.\(^3\)
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 \((hk l)\) and \((hkl)\) 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 sulphur. The heavier element does not have to be part of the molecule under investigation—a chlorinated solvent of crystallisation would be adequate. Flack and Shmueli proposed 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 recognised that a better strategy would be to include an enantiomeric-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, \(\eta\), applied to the anomalous component of the scattering factor, \(F\). This proved to be very successful for enantiopure crystals, but Flack noted that the parameter only had physical meaning at values of \(\pm 1\). He argued that if a given sample crystal could be regarded as a crystal containing a mole fraction of a given hand equivalent to \(\pm 1\), 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 \(s(x)\) 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 Bernardini 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. 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 \(hkl\), thus the observed data can be written \(|F_o|^2 hkl\) and \(|F_c|^2 hkl\) and the data calculated from the model are written \(|F_o|^2 hkl\) and \(|F_c|^2 hkl\). Thus, for every Friedel pair of reflections the difference, \(Dhkl\), can be calculated \(|F_o|^2 hkl\) – \(|F_c|^2 hkl\), together with its standard uncertainty. A similar term \(|F_o|^2 hkl\) – \(|F_c|^2 hkl\), called \(Dhkl\), can also be computed. This should have the same sign as \(Dhkl\) if the model has the correct hand. Replacing a simple change of sign by a continuously variable parameter \(y\) allows for the possibility of twinning by inversion. The probability of finding a particular value of \(y\) from the observed

![Anomalous Scattering Factors](image)

3. Absolute configuration and absolute structure

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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:

\[ G = \frac{\int y Pu(y) dy}{\int Pu(y) dy} \]

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 (DoF and DFpl) to the single parameter, \( y \). 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 \( K_{\alpha} \) 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 (\( R1 \)) 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, \( \sigma = 0.004 \pm 0.669 \), 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 \( \sigma = 0.018 \pm 0.512 \) 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.

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\textsuperscript{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.\textsuperscript{11}
• 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 $F_w$ versus $F$ 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 $w(F_o^2 - F_c^2)$ or $w(F_o^2 - F_c^2)^{1/2}$ 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 enantiopure 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.

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References


\textsuperscript{11} 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.