

**The use of X-ray crystallography to determine absolute configuration**

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**The use of X-ray crystallography to determine absolute configuration**

Authors:

**H. D. Flack\* and G. Bernardinelli**

Affiliation:

**Laboratoire de Cristallographie, University of Geneva, Switzerland**

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\*Address

H. D. Flack, Laboratoire de Cristallographie, 24 quai Ernest-Ansermet, CH-1211 Genève 4, Switzerland. Phone: +41 2237 96249 Fax: +41 2237 96108 E-mail: Howard.Flack@unige.ch

**Submission for the special project DETERMINATION OF ABSOLUTE CONFIGURATION**

## Abstract

Essential background on the determination of absolute configuration by way of single-crystal X-ray diffraction (XRD) is presented. The use and limitations of an internal chiral reference are described. The physical model underlying the Flack parameter is explained. Absolute structure and absolute configuration are defined and their similarities and differences are highlighted. The necessary conditions on the Flack parameter for satisfactory absolute-structure determination are detailed. The symmetry and purity conditions for absolute-configuration are determined and discussed. The physical basis of resonant scattering is briefly presented and the insights obtained from a complete derivation of a Bijvoet intensity ratio by way of the mean-square Friedel difference are exposed. The requirements on least-squares refinement are emphasized. The topics of right-handed axes, X-ray diffraction intensity measurement, software, crystal-structure evaluation, errors in crystal structures and compatibility of data in their relation to absolute-configuration determination are described. Characterization of the compounds and crystals by the physico-chemical measurement of optical rotation, CD spectra and enantioselective chromatography are presented. Some simple and some complex examples of absolute-configuration determination using combined XRD and CD measurements, using XRD and enantioselective chromatography, and in multiply-twinned crystals clarify the technique. The review concludes with comments on absolute-configuration determination from light-atom structures.

## Introduction

X-ray diffraction (XRD) of single crystals has the capacity to distinguish between the enantiomorphs of a chiral crystal structure and the enantiomers of a chiral molecule. The technique may be applied to compounds of a vast range of chemical composition. Essential chemical information such as the molecular geometry, bond distances and angles, and the packing of the molecules in the crystal are part and parcel of the results of the analysis. However there are limitations. Absolute-configuration determination is a fine detail of crystal-structure determination, which depends on being able to identify small diffraction intensity differences between two crystal-structure models of opposite chirality. With compounds containing only light atoms a significant difference is not guaranteed. The physical reason that these differences are small is described in the section *Resonant scattering and its effect on the diffraction intensities*.

Clearly it makes no sense to claim that an absolute configuration has been determined unless the gross features of the structure and its determination, such as intensity measurements, symmetry, atomic positions, interatomic distances and atomic displacement parameters have been evaluated and shown not to be in error. This review is written for the person who has sufficient knowledge of X-ray crystallography to accomplish this essential step.

Of particular relevance in absolute-configuration determination are the following questions:

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4 - Does the model crystal structure properly represent the crystal structure inside the  
5 crystal(s) that have been measured? Is the crystal structure chiral? Is the model that  
6 of the real crystal structure and not its enantiomorph? Is the compound  
7 enantiomerically pure? Is the assumed space group symmetry neither too low nor too  
8 high?

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10 - Does the model crystal structure properly represent the bulk product from which the  
11 crystal was grown?

12 - Have the bulk and the measured single crystal been sufficiently characterized or  
13 fingerprinted to enable another experimentalist to correctly identify the material  
14 studied?  
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17 In a presentation of the way that absolute-configuration determination is undertaken  
18 by single-crystal X-ray diffraction, the review provides essential background  
19 information and highlights those aspects which are the cause of confusion and error.  
20 Techniques to improve the capacity of single-crystal X-ray diffraction to determine  
21 absolute configuration are reviewed, along with a few examples of other more  
22 complex cases. The all-important topic of characterization of bulk and individual  
23 single crystals is treated and the concluding remarks contain comments on some  
24 current technical limitations. The current review does not go into any detail  
25 concerning the phase diagrams of enantiomeric mixtures as we have recently  
26 contributed detailed information on the absolute-configuration determination from  
27 binary enantiomeric mixtures<sup>1</sup> which are neither enantiomerically pure nor racemates  
28 (scalemates).  
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## 34 **Single-crystal X-ray diffraction techniques using an** 35 **internal chiral reference** 36

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38 The presence in a crystal structure of enantiomerically pure chiral molecules, groups  
39 or chiral centres of known absolute configuration leads directly to the determination  
40 of the absolute configuration of the other constituents of the crystal by making the  
41 image of the atomic arrangement correspond to that of the chiral molecules whose  
42 absolute configuration is known. The chiral molecules (or groups or centres) thus act  
43 as an internal reference. These may be introduced as part of the compound by  
44 chemical reaction or as part of the crystal by co-crystallization using an  
45 enantiomerically pure sample of the reference substance. It is important to stress that  
46 the correctness of absolute-configuration determination using an internal chiral  
47 reference depends crucially on the knowledge of the enantiomeric purity of the  
48 reference material and its indicated absolute configuration. It is not sufficient to  
49 assume that chemical reaction, crystallization or operations of mechanochemistry (*i.e.*  
50 grinding) will necessarily conserve the chirality of the reference material.  
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## 57 **Single-crystal X-ray diffraction techniques exploiting** 58 **resonant scattering** 59 60

## The Flack parameter

The distinction by single-crystal X-ray diffraction of inversion-related models of a non-centrosymmetric crystal structure relies on the phenomenon of resonant scattering (see section *Resonant scattering and its effect on the diffraction intensities*) and is measured by the Flack parameter<sup>2</sup>. The physical model underlying the Flack parameter is that of a crystal twinned by inversion and composed of distinguishable domains, all of these being real phenomena well established in the fields of mineralogy, crystal growth, crystal physics and solid-state physics<sup>3</sup>. The macroscopic crystal is formed of two types of homogeneous and perfectly-oriented domains, the relationship between the two domain types being that of inversion. A simple way of picturing the crystal twinned by inversion is to imagine a racemic conglomerate in which the crystals have stuck together at growth in a perfectly oriented manner giving diffraction patterns that look like those of a single crystal. For a nice illustrative example see<sup>4</sup>. Let  $X$  represent the model crystal structure as given by its cell dimensions, space group and atomic coordinates and  $\bar{X}$  its image inverted through a point. The macroscopic crystal may be represented as  $C = (1 - x) X + x \bar{X}$  for which the Flack parameter  $x$  measures respectively the mole fractions  $(1 - x)$  and  $x$  of the two types of domain  $X$  and  $\bar{X}$ . When  $x = 0$ , there is only one domain in the crystal which is that of the model  $X$ . When  $x = 1$ , there is only one domain in the crystal which is that of the inverted model  $\bar{X}$ . When  $x = 0.3$  both types of domain are present in the crystal in the proportion 70% of  $X$  to 30% of  $\bar{X}$ . The physically meaningful values of  $x$  are  $0 \leq x \leq 1$  but due to statistical fluctuations and systematic errors, experimental values may lie a little outside of this range by a few standard uncertainties. A crystal of an enantiomerically pure compound in the correct absolute configuration has a value of the Flack parameter of zero. In crystallographic jargon one says that the Flack parameter measures the absolute structure of a non-centrosymmetric crystal and from this one may deduce the absolute configuration of the chiral molecules forming the crystal.

## What are absolute structure and absolute configuration?

For convenience, the formal definitions of these quantities are reproduced<sup>5</sup> in the glossary to this review. 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. Note particularly that both the entity under consideration, *viz* crystal structure versus molecule, and the symmetry restrictions, *viz* non-centrosymmetric versus lack of mirror reflection, inversion through a point and rotoinversions, are different. Both terms concern the complete specification of the spatial arrangement of atoms with respect to inversion and require that the sample under investigation be characterized by some other physical measurement.

## Absolute-structure determination

There are conditions under which one may say that the absolute structure of the crystal has been determined satisfactorily<sup>6</sup>. Firstly one wants to know whether the absolute-structure determination is sufficiently precise by looking to see whether the

standard uncertainty  $u$  of the Flack parameter  $x(u)$  is sufficiently small: in general  $u$  should be less than 0.04 but this value may be relaxed to 0.10 for a compound proven by other means to be enantiomerically pure. Secondly the value of the Flack parameter itself should be close to zero within a region of three standard uncertainties *i.e.*  $u < 0.04$  (or  $u < 0.10$  for a chemically proven enantiomeric excess of 100%) and  $|x|/u < 3.0$ . Moreover the crystal and bulk need to be characterized. The above criteria have been established by way of statistical reasoning<sup>6</sup> to ensure that the structure analyst, by an examination of  $x(u)$  alone, does not claim an absolute-structure determination where none is valid. An unfortunate consequence of such a conservative or safe approach is that some borderline but valid absolute-structure determinations are deemed to be unacceptable. Some of the above criteria may be relaxed somewhat by taking account of a broader spectrum of knowledge over a class of compounds in conjunction with other non-diffraction data or accumulated knowledge of a particular instrument but this needs exceedingly careful, individual and expert evaluation.

### Absolute-configuration determination

Once the absolute structure has been determined satisfactorily, it is only then the moment to see whether something can be said about the absolute configuration of its constituent molecules, as not all valid determinations of absolute structure can necessarily lead to the assignment of an absolute configuration. Although the following description of restrictions<sup>7</sup> is self-sufficient, it has to be admitted that more background knowledge on chiral and achiral crystal structures<sup>5</sup> helps in its understanding. The weakest and most easily-applicable restriction is given first and the strongest one is given last. In fact the third restriction is sufficient in itself, the other two not so.

**Space-group restriction:** the simplest restriction is one of space-group symmetry. If the space group contains symmetry operations of the second kind (*i.e.* rotoinversions or roto reflections, glide reflections), it must occur that these operate either intramolecularly, forcing the individual molecules to be achiral, or intermolecularly, forcing an arrangement of pairs of opposite enantiomers. Thus, in the first case, the molecules are achiral and in the second a racemate is present. Consequently it is only in crystals displaying space groups containing exclusively symmetry operations of the first kind (*i.e.* pure or proper rotations, screw rotations) that the determination of absolute configuration is possible (geometric crystal classes: 1, 2, 222, 4, 422, 3, 32, 6, 622, 23 and 432).

**Chiral molecular entity restriction:** to comply with the definition of absolute configuration<sup>5</sup>, one needs to identify a chiral molecular entity and its spatial arrangement in the crystal structure. For example in a couple of alkali tartrate salts<sup>8,9</sup>, the absolute configuration of the tartrate anion (a chiral molecule) was established but correctly no claims to have done so for the sodium or rubidium atoms were made as these are achiral cations and not molecules. The symmetry group of an achiral molecule contains rotoinversion or roto reflection operations and these may or may not be part of the space-group symmetry of the crystal. So one must always examine the spatial arrangement of a candidate molecule for non-crystallographic rotoinversion or roto reflection symmetry operations and if any are found, the molecule is achiral and

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3 its absolute configuration cannot be determined. For example any planar molecule  
4 has mirror symmetry and is achiral whether the mirror plane is part of the space-group  
5 symmetry or not.  
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8 **Solid-state enantiomeric purity restriction:** one needs to verify that all occurrences  
9 of the chiral molecular entity in the crystal structure are the same enantiomer for an  
10 absolute-configuration assignment to be valid. This is of particular concern when the  
11 asymmetric unit contains more than one occurrence of the chiral molecule ( $Z' > 1$ ).  
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14 Some of the above criteria may be relaxed but such studies need exceedingly careful,  
15 individual and expert evaluation as described in the section *Absolute-configuration*  
16 *determination from a bulk racemate by combined CD and XRD*<sup>10</sup>. Bulk samples  
17 which are mixtures of diastereoisomers may give rise to crystals which contain  
18 several of the diastereoisomers either in an ordered or disordered arrangement. The  
19 crystal structure may clearly and distinctly show the configuration of some of the  
20 elements of chirality common to all of the diastereoisomers whereas those elements  
21 which vary amongst the diastereoisomers may be in doubt due to disordered atomic  
22 positions arising from the superposition of more than one diastereoisomer.  
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26 The *space-group restriction* mentioned above implies that absolute configuration may  
27 only be undertaken from chiral crystal structures. The latter are necessarily non-  
28 centrosymmetric but not all non-centrosymmetric crystal structures are chiral.  
29 Achiral non-centrosymmetric crystal structures exist and the absolute structure of  
30 their crystals may be determined. However it is not possible to deduce the absolute  
31 configuration of their constituent molecules for the reasons given above. As we have  
32 explained previously<sup>5</sup> chiral crystal structures are found formed either of  
33 enantiomerically pure (chiral) molecules, or of chiral molecules as a racemate, or of  
34 achiral molecules. Achiral crystal structures, which may be either centrosymmetric or  
35 non-centrosymmetric, are found formed either of chiral molecules as a racemate, or of  
36 achiral molecules. Enantiomerically pure compounds are found to crystallize  
37 exclusively in chiral crystal structures.  
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## 42 Resonant scattering and its effect on the diffraction intensities

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45 Optical systems working with visible light distinguish objects of opposite chirality  
46 without difficulty *i.e.* one can easily see the difference between ones own right and  
47 left hand. The major difference in the diffraction pattern of the right and left hand  
48 occurs in the phases of the inversion-related reflections whereas their amplitudes are  
49 identical in the absence of any resonant effects. Differences in intensities of the latter  
50 occur only if a resonant frequency of the diffracting object is near to that of the  
51 incident electromagnetic radiation. Therein lies the essential difficulty for chirality  
52 distinction using X-rays. As no lens exists for focusing X-rays, one has to rely only  
53 on the intensity of the reflections in the Fraunhofer diffraction pattern. Moreover if  
54 the frequency of the incident X-rays is close to that of some of the atomic electrons  
55 which cause diffraction, it will be only a small proportion of these electrons which are  
56 resonant and the intensity difference between inversion-related reflections (Friedel  
57 opposites in crystallographic jargon) is small. A further complication is that the  
58 resonant frequencies of light atoms occur at long X-ray wavelengths which are very  
59 difficult to access experimentally. It helps to remember that resonant scattering is no  
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more or no less than the response of a forced damped harmonic oscillator of which there are numerous examples in nature. There is nothing anomalous about resonant scattering (apart perhaps from the commonly-used names *anomalous dispersion* and *anomalous scattering*).

An important tool<sup>11</sup> in understanding resonant-scattering effects in X-ray diffraction and of use in the planning of experimentation and the evaluation of results has been provided very recently in an analytical expression for the mean-square Friedel intensity difference for a non-centrosymmetric crystal structure with a centrosymmetric substructure. A related Bijvoet intensity ratio  $\chi$  gives a measure of Friedel differences relative to the average intensity of Friedel opposites. A spreadsheet application available with the publication<sup>11</sup> undertakes the necessary calculations from the elemental composition of the compound for some common X-ray wavelengths. Values of  $10^4 \chi$  called Friedel<sup>11</sup> are calculated both for the case of all atoms arranged non-centrosymmetrically and also allowing for atoms arranged on a centrosymmetric substructure if it is possible to identify these. We now rapidly pass in review some of the principal insights that this work has provided:

- The Bijvoet ratio is largest when all atoms are arranged non-centrosymmetrically and zero when all atoms are arranged centrosymmetrically.
- The Bijvoet ratio is zero when all atoms are of the same chemical element regardless of whether the structure is non-centrosymmetric or centrosymmetric. Such is the case, in the spherical atom approximation, for the chiral crystal structure of elemental Se in the form of a helix<sup>12</sup>.
- The Bijvoet ratio quantifies a contrast and needs both resonant and non-resonant atoms to attain large values.
- Rather surprisingly the presence in an otherwise non-centrosymmetric structure of a centrosymmetric arrangement of resonant atoms of one (heavy) chemical element does not diminish the value of the Bijvoet intensity ratio, an observation which had already been confirmed experimentally<sup>13,14</sup>.
- The analytical form of the Bijvoet ratio shows that there are no classes of Bragg reflections having particularly large or small values. Consequently, in the absence of a model of the crystal structure, no particular reflections nor any specific regions of reciprocal space on average are established as showing large Friedel differences.

Calculation of the Bijvoet ratio at different wavelengths enables an optimal choice of X-ray wavelength to be made prior to experimentation. Further it allows the molecular composition of the crystal to be optimized. Suppose for example that a compound is found to have a Bijvoet ratio that is too small for absolute-configuration determination. One may envisage the synthesis of a suitable derivative or the fabrication of a solvate or co-crystal of the compound having a higher Bijvoet ratio. As has been shown above it is unimportant if the solvent or co-crystal molecule takes an essentially centrosymmetric arrangement in the crystal as this does not tend to diminish the Bijvoet ratio. Moreover we have found<sup>14</sup>, using an approximate form of the Bijvoet ratio<sup>15</sup> and a small set of pseudo-centrosymmetric structures, a relationship between the Bijvoet ratio and the standard uncertainty on the Flack parameter. This relationship allows *a-priori* estimates of the standard uncertainty of the Flack parameter. Work is currently in progress to establish the corresponding relationship between the full Bijvoet ratio and the standard uncertainty on the Flack parameter for a much larger set of non-pseudosymmetric structures. Unfortunately as this review goes to press, we have not yet completed the data analysis to determine the values of



Friedif corresponding to our limiting values of  $u$  of 0.04 and 0.10, and to investigate in more detail the influence of pseudosymmetry.

## Least-squares refinement

Early results<sup>16,17</sup> and subsequent experience from many crystal-structure determinations have shown that the Flack parameter is robust and converges in only a few cycles to its final value during least-squares refinement. However as the Flack parameter is one of many parameters of the physical model of a crystal structure, the values of which are to be found by optimization based on some general criterion, it is essential in the final cycles of optimization that all parameters be varied jointly and simultaneously. If this prescription is not followed, two effects may occur separately or together: (a) the value of the Flack parameter may not correspond to the best value for the optimization criterion and (b) its standard uncertainty may be incorrectly estimated, most frequently underestimated. In the case of least-squares minimization, the final refinement needs to be undertaken by full-matrix least-squares (all parameters varied jointly and simultaneously) and needs to have converged.

Another important aspect of least-squares minimization which needs some words of explanation is that of stabilization and damping<sup>18</sup>. In order to avoid a least-squares refinement becoming unstable and failing to converge, certain numerical techniques, grouped together under the general term *damping*, are often applied automatically or manually. A side effect of these techniques is that stabilized parameters stay close to their starting or target values with standard uncertainties that are systematically underestimated. Biased (wrong) parameter estimates and underestimated standard uncertainties are the result.

## Inverting a model structure

It sometimes happens that a model crystal structure yields a value of the Flack parameter larger than 0.5. In order to represent the majority component in the crystal the model needs to be inverted so the Flack parameter takes a value less than 0.5. In general this inversion is obtained by inversion in the origin by just changing all atomic coordinates  $x,y,z$  into  $-x,-y,-z$  or some point symmetry-equivalent to it. However for the chiral crystal structures which are necessary for absolute-configuration determination, there are some cases where this simple change of coordinates is insufficient or inappropriate. So in the case of a space group belonging to one of the 11 pairs of enantiomorphic space groups ( $P4_1-P4_3$ ;  $P4_122-P4_322$ ;  $P4_12_12-P4_32_12$ ;  $P3_1-P3_2$ ;  $P3_121-P3_221$ ;  $P3_112-P3_212$ ;  $P6_1-P6_5$ ;  $P6_2-P6_4$ ;  $P6_122-P6_522$ ;  $P6_222-P6_422$ ;  $P4_132-P4_332$ ), the space group should also be changed into the other member of the pair. As they occur in enantiomorphic pairs these 22 space groups are the only ones that are correctly described as being chiral<sup>5</sup>. Moreover there are cases where for the standard setting of the space group<sup>19</sup> that the coordinates need to be inverted in some point other than the origin. The coordinates of the appropriate inversion point can be found in the columns *Inversion through a centre at* of the tables of Euclidean normalizers of space groups<sup>20</sup>. For non-standard settings an algorithmic solution to this problem has been provided<sup>16</sup>.

## Characterization of compounds and crystals

The phase diagrams of enantiomeric mixtures can be complicated<sup>21,22</sup> giving rise to solid and liquid phases of different composition. Also kinetic effects play an important role in crystallization. It is for these reasons that for absolute-configuration determination, some characterization or measurement of the enantiomeric purity not only of the bulk but also of the single crystal used for the diffraction studies is recommended. There are three principal methods of characterization:

(OR) The specific rotation of the optical activity in solution. As the measurement of specific rotation is a single-wavelength technique, the presence and effect of impurities can easily go undetected. Moreover OR can not be applied to microgram quantities (*i.e.* a single crystal used for diffraction studies). Also OR can only provide a measure of enantiomeric excess if the specific rotation of the enantiomerically pure compound is sufficiently strong and has been determined previously.

(CD) The visible and near-UV circular dichroism spectrum in solution. The presence and effect of impurities may be readily recognized in a CD spectrum. In favourable circumstances, CD may be applied to the single crystal used for the diffraction measurements taken into solution. For compounds that racemize rapidly in solution, solid-state CD in a KBr disk may be applied to the bulk compound and perhaps even to a powdered single crystal<sup>23,24,25</sup>. One may expect vibrational CD, either IR or Raman, to be used increasingly in the future.

(EC) Enantioselective chromatography. This sensitive technique is applicable to microgram quantities and provides estimates of the enantiomeric excess. It is of course necessary to establish that under the chosen experimental conditions that the two enantiomers are clearly separated. The retention times provide a satisfactory characterization of the two enantiomers.

Regrettably little use is made of differential scanning calorimetry (DSC) by synthetic chemists and structure analysts. Nevertheless the measurement of melting temperatures and enthalpies is a valuable technique for establishing a phase diagram. DSC measurements may be applied to the bulk.

In passing, we also mention that powder diffraction can be useful. The simple expedient of comparing the X-ray powder diffraction pattern of the bulk product with that simulated from the results of a single-crystal study allows the presence in the bulk of polymorphs and crystalline diastereoisomers to be revealed. Clearly although this technique is of no help for detecting racemic conglomerates, it is very helpful for other solid mixtures<sup>26</sup>. As the presence of diastereoisomers has the capability of invalidating the determination of absolute configuration, any method which establishes the number and relative concentration of these isomers should be used to characterize the bulk compound and if possible the single crystal used for the diffraction studies.

## Experimentation and analysis needs impeccable technique

### Right-handed axes

As emphasized and discussed previously<sup>27</sup>, right-handed sets of axes must be used at every stage of an analysis of absolute structure. Of particular danger for the structure analyst are basis transformations performed to bring the unit cell into a standard setting. To maintain right-handed axes, any basis transformation matrix must have a positive determinant. A transformation matrix with a negative determinant will transform a right-handed set of axes into a left-handed set of axes, and conversely. The sign of the determinant can not be spotted simply by counting the number of positive and negative elements in the transformation matrix. The orientation matrix (**UB**) of the crystal on the diffractometer must have a positive determinant. It is standard practice in our laboratory to calibrate every diffractometer after a hardware or software modification with a well-defined reference material of a chiral crystal structure and containing a sufficient amount of resonant scattering. We use enantiomerically pure potassium hydrogen (*2R,3R*) tartrate. With such a test material, structure solution must give a Flack parameter very close to zero for the (*2R,3R*) configuration of the acid tartrate anion.

### X-ray diffraction intensity measurements

It has been established<sup>13,14</sup>, under certain particular conditions which it is not necessary to detail here, that unless intensity measurements of both members,  $hkl$  and  $\bar{h}\bar{k}\bar{l}$  of each pair of Friedel opposites are made and used separately in the least-squares refinement, false values of the Flack parameter may result. It hence seems prudent, whether these particular conditions apply or not, to always measure both members of each Friedel pair. Fortunately with modern-day equipment using area detectors this criterion is easy to achieve and is often the default mode of operation. At the data-reduction stage it is essential for absolute-configuration determination not to average the intensities of Friedel opposites<sup>6</sup>, to transform reflection indices only according to the symmetry operations of the crystal point group<sup>6</sup> and not to use any semi-empirical absorption correction which applies a different correction to the intensities of  $hkl$  and  $\bar{h}\bar{k}\bar{l}$ .

In writing about pairs of Friedel opposites in this review,  $\bar{h}\bar{k}\bar{l}$  should be taken in a general sense to mean  $\bar{h}\bar{k}\bar{l}$  or any Bragg reflection symmetry-equivalent to it under the point group of the crystal. For a non-centrosymmetric crystal  $hkl$  and  $\bar{h}\bar{k}\bar{l}$  are not symmetry-equivalent under the point group of the crystal. Later on in this review the term Friedel coverage is also used. If in the intensity data, for each reflection  $hkl$  the reflection  $\bar{h}\bar{k}\bar{l}$ , or one symmetry-equivalent to it, has been measured then the Friedel coverage is 100%. If for each  $hkl$ ,  $\bar{h}\bar{k}\bar{l}$  or one symmetry-equivalent to it has not been measured then the Friedel coverage is 0%.

## Getting the best out of your software

In the section *Least-squares refinement* above it was pointed out that full-matrix simultaneous refinement of all variables should be used in the final cycles to obtain reliable results. One widely-used least-squares refinement programme, SHELXL93/97<sup>28</sup>, uses by default a sparse-matrix technique<sup>6</sup>, called *hole-in-one*, for the refinement of the Flack parameter. SHELXL may nevertheless be coaxed into doing the appropriate full-matrix calculation<sup>13,29</sup>.

One may make one or two simple checks of any refinement software. The first is to invert the structure and check that a value of the Flack parameter of  $1 - x$  with the same standard uncertainty is obtained. A second check is to undertake the refinement using a different starting value of the Flack parameter which should lead to exactly the same value of  $x(u)$ .

## Crystal-structure evaluation

The results of a crystal-structure determination are transmitted nowadays by means of computer-readable files *viz* a Crystallographic Information Framework<sup>30</sup> (CIF) file which may be used for display, analysis, evaluation and archiving. A great deal may be achieved by the automated evaluation of the information contained in a CIF file concerning the data measurements and the crystal-structure determination. Systems can be designed to make use of a considerable amount of general and specific crystallographic knowledge and know-how in the evaluation of a structure determination, and to alert the structure analyst to ambiguities, contradictions and shortcomings in the information encapsulated in a CIF file. With the aid of these alerts, the data-measurement and structure-refinement procedures may be improved, completed and justified. An essential element is the examination of a graphical representation of the atomic displacement parameters<sup>31</sup>. The most elaborate system currently in operation for the evaluation of crystal-structure determinations is the free-of-charge online checkCIF/PLATON<sup>32,33</sup> operated by the International Union of Crystallography.

## Erroneous crystal structures

One should bear in mind that a structure analysis may be erroneous. An error with which one should be familiar is the one in which the symmetry of a crystal structure has been incorrectly assigned to a non-centrosymmetric space group whereas the crystal structure itself is really centrosymmetric. In an erroneous non-centrosymmetric description of a crystal structure, the conditions for absolute-configuration determination may apparently be achieved which do not of course apply in the true centrosymmetric description<sup>13,14</sup>. The measurement of a whole sphere of reflection intensities (see section *X-ray diffraction intensity measurements*) is a prudent approach to help avoiding falling into this trap. Of equal relevance to absolute-configuration determination are those cases of analysis in which an achiral crystal structure of a racemate (often disordered) with a space group containing rotoinversion operations has erroneously been assigned to a crystal which has in fact a

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chiral crystal structure of an enantiomerically pure compound with a space group containing only rotation and screw rotation operations. This latter case may arise when a bulk racemate crystallizes by spontaneous resolution and the structure analyst force-feeds the structure solution with a racemate. The possibility of undertaking an absolute-configuration is hence lost.

From an analysis of the atomic coordinates and cell parameters, checkCIF/PLATON may provide an alert that a space group of too low symmetry has been chosen. The most common situation is that it may be possible to add a centre of symmetry to the chosen space group. This proposition should be rejected if there is strong evidence to show that the compound is enantiomerically pure in the crystalline state.

Partial-polar ambiguities<sup>34</sup> have the capacity to falsify an absolute-configuration determination. In a crystal-structure solution suffering from a partial-polar ambiguity, some of the atoms are correctly located but the others are images of the real atoms inverted in a point. One may be able to recognize this type of error by a study of interatomic distances and angles.

## Compatibility of chemical and crystallographic data

In our recent study<sup>1</sup> of 135 published crystal structures of metallacycles an appalling 26% had incompatible chemical and crystallographic data. In general, for these incompatible crystal structures, the chemical evidence was adequate to convince us that the bulk products had a composition close to an enantiomeric excess of 100%. The crystal structures were determined as being chiral but with values of the Flack parameter close to 0.5, with a low standard uncertainty, indicative of a crystal twinned by inversion with an overall composition near to that of the racemate, in contradiction to the chemical evidence. We hypothesized that for the incompatible crystal-structure determinations that either the data-reduction software had averaged Friedel opposites or that an empirical absorption correction procedure had tended to eliminate intensity differences between Friedel opposites. Other considerations have also been highlighted<sup>14</sup>.

## Some examples to stretch one's understanding

### Experimental values of the Flack parameter

We owe a debt of gratitude to the referees of this article for suggesting the inclusion of this section giving the interpretation of some typical values of the Flack parameter. We can not stress sufficiently yet again that a necessary step, prior to examining and interpreting the Flack parameter, is to scrutinize the output of a system such as checkCIF/PLATON<sup>32,33,31</sup> to ensure the overall validity of the structure determination. For absolute-configuration determination one pays particular attention to any indications of pseudosymmetry, incorrect space group and insufficient number of intensity measurements of Friedel opposites. Moreover the non-crystallographic

**chemical** evidence concerning the enantiopurity both of the bulk and of the individual single crystal used for the diffraction studies needs to be reviewed.

$x(u) = 0.05(2)$ , space group  $P2_1/c$ : This report is complete rubbish as a crystal structure with this space group is centrosymmetric implying that both absolute structure and the Flack parameter are meaningless. If there are chiral molecules in this structure, they are present as the racemate.

$x(u) = 0.05(2)$ , space group  $Pna2_1$ : Absolute-configuration determination is not possible as this space group contains rotoinversion operations in the form of the glide reflections  $n$  and  $a$ . The crystal structure is non-centrosymmetric and achiral. If there are chiral molecules in this structure, they are present as the racemate. Absolute-structure determination, but not absolute-configuration determination, may have been possible in this case.

$x(u) = 0.05(2)$ , space group  $C2$ , good Friedel coverage, enantiomerically-pure bulk compound: The standard uncertainty on the Flack parameter is less than 0.10 and the Flack parameter itself is within three standard uncertainties of zero. Absolute-structure determination has been achieved. Moreover as the space group contains only pure rotations and screw rotations, the crystal structure is non-centrosymmetric and chiral. The enantiopurity of the bulk compound ensures the enantiopurity of the single crystal used for the diffraction studies and consequently absolute-configuration determination has been achieved. Characterization of the bulk compound by OR, CD or enantioselective chromatography is necessary to make the absolute-configuration determination complete.

$x(u) = 0.05(2)$ , space group  $C2$ , good Friedel coverage, racemic bulk compound: Similar to the case immediately above but the compound has crystallized by spontaneous resolution. For measurements on a series of crystals all refined using the same structure model, about 50% of the crystals would give  $x(u) \approx 0.05(2)$  and the other 50% would give  $x(u) \approx 0.95(2)$ . Absolute configuration is achieved if it is possible to characterize by either CD or enantioselective chromatography the single crystal used for the diffraction study.

$x(u) = -0.1(2)$ : The value 0.2 of the standard uncertainty is larger than either of the limiting values 0.04 or 0.1 and consequently absolute-structure and absolute-configuration determination are not possible from the experimental intensity measurements. The negative value of the Flack parameter is within three standard uncertainties of zero and entirely compatible with the statistical fluctuations inherent in the experimental measurements. Possible remedies to achieve a smaller standard uncertainty on the Flack parameter are more accurate intensity measurements, measurement at a lower temperature, accurate measurement of a selected set of Bragg reflections having the largest model Friedel intensity differences, use of a radiation of different wavelength, synthesis of a derivative containing resonant-scattering atoms and synthesis of a co-crystal or solvate containing resonant scatterers.

$x(u) = 0.00$ : No standard uncertainty on the Flack parameter has been reported and the Flack parameter may not even have been refined. As it is hence impossible to assess the accuracy of the Flack parameter, absolute-structure and absolute-configuration determination have not been achieved.

$x(u) = 0.0(3)$ : This is a situation similar to that of  $x(u) = -0.1(2)$  but with an even greater uncertainty.

$x(u) = 0.81(12)$ : For values of the Flack parameter greater than 0.5, the structure model should be inverted and the refinement restarted.

$x(u) = 0.19(12)$ : When using SHELXL<sup>28</sup> there is the danger that positive values of the Flack parameter noticeably different from zero may not have converged.

One must use the TWIN/BASF commands<sup>13,29</sup> and report the value of BASF1 for the Flack parameter. If this has been done in the present case and there is good Friedel coverage, the crystal is probably twinned by inversion containing 81% of the model and 19% of the inverted model. See the section *Absolute-configuration determination from a bulk racemate by combined CD and XRD*. However  $x(u) = 0.19(12)$  is also rather typical of structure solutions suffering from a partial-polar ambiguity<sup>34</sup>.

$x(u) = 0.49(2)$ , good Friedel coverage, enantiomerically pure bulk compound: The chemical and the crystallographic evidence are contradictory. One needs to examine both very critically. A similar situation pertains when a centrosymmetric crystal structure is obtained for an enantiomerically pure bulk compound. The chemists' curse is the opposite enantiomer as impurity in a binary system for which the melting temperature of the racemic compound is much higher than that of the enantiomerically pure compounds. The crystallographers' curse is bad software implementing inappropriate averaging algorithms or absorption corrections. No absolute-configuration determination is possible. Watch out as well for disordered racemates which may be enantiomerically-pure crystals produced by spontaneous resolution.

$x(u) = 0.042(8)$ , poor Friedel coverage, indications from checkCIF/PLATON that a centre of symmetry needs to be added to the space group, no chemical data indicating that the compound is enantiomerically pure: Due to insufficient intensity data, the refinement of the Flack parameter has probably stuck at its starting value of zero<sup>13,14</sup> for this centrosymmetric structure refined as non-centrosymmetric. The analyst should undertake refinement in the appropriate centrosymmetric space group. No absolute-configuration determination is possible.

$x(u) = 0.042(8)$ , good Friedel coverage, indications from checkCIF/PLATON that a centre of symmetry needs to be added to the space group, strong chemical evidence that the compound is enantiomerically pure: This is a very nice absolute-configuration determination.

$x(u) = 0.49(2)$ , good Friedel coverage, bulk compound is either a racemate or achiral: If there are indications from checkCIF/PLATON that a centre of symmetry needs to be added to the space group, this is most likely the case. If there are no such indications, the crystal is most likely a 50:50 inversion twin. No absolute-configuration determination is possible.

### **Absolute-configuration determination from a bulk racemate by combined CD and XRD**

A chiral chromium complex<sup>35</sup> was synthesized, and crystals were grown from a solution of the racemate. The crystal structure is chiral displaying the space group  $P2_12_12_1$ . One would suspect thus that the crystallization had proceeded by spontaneous resolution giving rise to a racemic conglomerate. Two different crystals were measured by X-ray diffraction and gave values for the Flack parameter<sup>2</sup>  $x$  of 0.36(4) [ $ee$  (*i.e.* enantiomeric excess) = 28(8)%] and 0.90(3) [ $ee = -80(6)\%$ ]. Both crystals are thus twinned by inversion, being in effect oriented agglomerates of enantiomerically pure domains containing molecules of opposite chirality in the manner of hexahelicene<sup>4</sup>. Moreover, the second crystal shows a higher enantiomeric excess than the first but contains a majority of the enantiomer opposite to that present as majority component in the first crystal. The two crystals were put into separate solutions and the CD-spectra of these were measured and normalized to equal crystal

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volume. The CD-spectrum of the solution from crystal 1 is indeed weaker and in form the mirror image of that from crystal 2. The ratio of the enantiomeric excesses from the X-ray diffraction gives a value of -0.35(10) whereas the ratio of the normalized peak heights at 350 nm of the CD spectra is -0.42. The agreement is very good indeed. So long as a CD spectrum of a solution of the crystal used for the diffraction experiment is published with the results of the structure analysis, it will be justifiable to claim that the absolute configuration has been determined. This is very satisfactory considering that one is working from a racemate in solution.

### Absolute-configuration determination relying on enantioselective chromatography

The synthesis of an N-sulphonated aziridine, resulted in an enantiomeric mixture which was found to have an enantiomeric excess of 43% of the (1*R*,3*R*,6*S*) enantiomer<sup>36,37</sup>. The enantiomers were separated by semi-preparative HPLC on Chiracel OD H using hexane/isopropanol 9:1 at 0.5 ml/min giving retention times of 15.3 and 16.3 min. The product from the minority component (retention time 15.3 min) was used to make crystals. Their crystal structure is chiral displaying space group *P2*<sub>1</sub> giving a Flack parameter<sup>2</sup>  $x(u) = -0.03(12)$ . Although the standard uncertainty on  $x$ , 0.12, is very slightly larger than our upper safe limit of 0.10, the conditions of experimentation, experience with other similar compounds, the small value of  $x$  convince us that absolute-structure determination has been achieved. The absolute configuration was determined to be (1*S*,3*S*,6*R*). The retention time and experimental conditions provide a sufficient characterization of the enantiomer in the absolute-configuration determination. In this case, it would not have been possible to use optical activity or CD as these effects are far too weak:  $[\alpha_D] = 0.7^\circ$  for  $ee = 43\%$  and the CD spectrum is flat.

### Determination of absolute configuration from multiply-twinned crystals

A twinned crystal may be viewed as a solid-state agglomerated mixture of rotated and/or inverted copies of the untwinned crystal structure. Each component in this mixture is specified by two attributes.

(i) The volume fraction  $x_i$  of the  $i^{\text{th}}$  component in the macroscopic crystal. This value may be established during structure refinement.

(ii) The isometry relating the orientation of the component to that of the basic one. This twin symmetry operation may be established by arguments of symmetry<sup>38,39,3</sup> and is not unique. It comes from a group *G* of isometries which leave the crystal lattice invariant but not necessarily the crystal structure. The crystal point group *P* is a subgroup of *G*,  $G \supseteq P$ .

As we are dealing here solely with cases in which the crystal structure is chiral, so that *P* is one of the point groups containing only rotations (geometric crystal classes: 1, 2, 222, 4, 422, 3, 32, 6, 622, 23, 432). So long as the criteria given in the sub-section *absolute-configuration determination* are obeyed, it is still possible to proceed to the determination of absolute configuration for the multiply-twinned crystal. Full details



of the group-theoretical analysis with the related restrictions to its application are given in<sup>7</sup> but here it suffices to point out that twin-symmetry operations that have a determinant of +1 are pure rotations and do not change the chirality of the molecules in the crystalline domain upon which they act. On the other hand twin-symmetry operations that have a determinant of -1 are rotoinversions and change the chirality of the molecules. For the purposes of the analysis of absolute configuration, the total amount of rotated-only structure,  $x^+$ , may be deduced by summing the volume fractions corresponding to twin laws of determinant +1,  $x^+ = \sum x_i^+$ , and that of rotated-and-inverted structure,  $x^-$ , may be deduced by summing the volume fractions corresponding to twin laws of determinant -1,  $x^- = \sum x_i^-$ .  $x^-$  is the equivalent of the Flack  $x$  parameter for multiply-twinned crystals possessing a chiral crystal structure. Let us make this clear from an example<sup>40</sup>. The space group is  $P3_1$  which belongs to geometric crystal class 3 and thus the crystal structure is chiral. The structure was refined as a four-component twin:  $k_2 = 0.064(13)$  for matrix 010,100,00-1,  $k_3 = 0.038(17)$  for matrix -100,0-10,00-1 and  $k_4 = 0.329(13)$  for matrix 0-10,-100,001<sup>40</sup>.  $k_1$  may be obtained from the relationship  $k_1 = 1 - k_2 - k_3 - k_4$  to give  $k_1 = 0.569(14)$  for matrix 100,010,001. The twin symmetry operations are of determinant +1 for matrices 1 and 2, and -1 for matrices 3 and 4. In the nomenclature of the current analysis, one has  $x^+_1 (=k_1) = 0.569(14)$ ,  $x^+_2 (=k_2) = 0.064(13)$ ,  $x^-_1 (=k_3) = 0.038(17)$  and  $x^-_2 (=k_4) = 0.329(13)$ , giving  $x^+ (=x^+_1 + x^+_2) = 0.633(17)$  and  $x^- (=x^-_1 + x^-_2) = 0.367(17)$ .  $x^-$  is equivalent to the Flack  $x$  parameter for this multiply-twinned crystal. Its standard uncertainty is low and hence the value of the Flack parameter is significant. The experiment clearly shows that 63% of the crystalline sample contains the structure as determined in space group  $P3_1$  and 37% contains the inverted structure in space group  $P3_2$  making the enantiomeric excess of the crystalline sample 26%. From these measurements, it is clearly not possible to establish the absolute configuration for this compound.

## Concluding remarks

Compounds composed only of light atoms, *i.e.* those having a low value of Friedel<sup>11</sup>, give rise to standard uncertainties on the Flack parameter which are too large for absolute-configuration determination. On the experimental side it may help to measure at a longer wavelength although synchrotron radiation does not seem to provide the easy answer that one might at first imagine<sup>14</sup>. Higher precision intensity measurements on a small set of Bragg reflections selected from the crystal-structure model as having large Friedel differences may be undertaken<sup>41</sup>. In various published<sup>41</sup> and unpublished works, improvement of the uncertainty of the absolute-configuration determination has been attempted by way of alternative statistical procedures to that of least squares. In our view, all the procedures we have examined suffer from the same problem as sparse-matrix least squares by assuming invariance of parameters of the model which should be variable. It is perfectly correct that the average of Friedel opposites<sup>11</sup> calculated from the crystal-structure model is independent of the Flack parameter or the absolute structure. However the corresponding difference of Friedel opposites (of the model) depends both on the Flack parameter **and** all the other atomic parameters of the model. It is the latter dependence which is assumed to be invariant in the procedures we have studied.

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3 Only the procedure of Parsons<sup>42</sup> escapes from this pitfall but needs further  
4 development.  
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## 8 9 Glossary<sup>5</sup>

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12 Absolute configuration<sup>5</sup>: The spatial arrangement of the atoms of a physically  
13 identified chiral molecular entity (or group) and its stereochemical description (*e.g.*,  
14 (*R*) or (*S*), (*P*) or (*M*), D or L, *etc.*)  
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17 Absolute structure<sup>5</sup>: The spatial arrangement of the atoms of a physically identified  
18 non-centrosymmetric crystal and its description by way of unit-cell dimensions, space  
19 group, and representative coordinates of all atoms.  
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22 Flack parameter<sup>5</sup>: The Flack parameter<sup>2</sup> is the molar fraction  $x$  in the defining  
23 equation  $C=(1-x) X + x \bar{X}$ , where  $C$  represents an oriented two-domain-structure  
24 crystal, twinned by inversion, consisting of an oriented domain structure  $X$  and an  
25 oriented inverted domain structure  $\bar{X}$ . In reciprocal space, the Flack parameter<sup>2</sup>  $x$  is  
26 defined by the structure-amplitude equation  $G^2(h, k, l, x)=(1-x) |\mathbf{F}(h k l)|^2 + x$   
27  $|\mathbf{F}(\bar{h} \bar{k} \bar{l})|^2$ . For a multidomain-structure twin of a chiral crystal structure, an  
28 equivalent Flack parameter may be calculated according to the method of Flack and  
29 Bernardinelli<sup>7</sup>.  
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