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Solution of the phase problem at non-atomic resolution by the phantom derivative method

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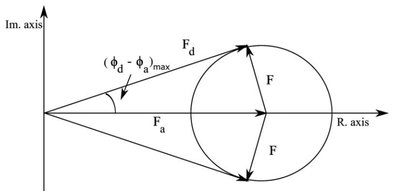
For a given unknown crystal structure (the *target*), n random structures, arbitrarily designed without any care for their chemical consistency and usually uncorrelated with the target, are sheltered in the same unit cell as the target structure and submitted to the same space-group symmetry. (These are called ancil structures.) The composite structures, whose electron densities are the sum of the target and of the ancil electron densities, are denoted *derivatives*. No observed diffraction amplitudes are available for them: in order to emphasize their unreal nature, the term *phantom* is added. The paper describes the theoretical basis by which the phantom derivative method may be used to phase the target structure. It may be guessed that 100–300 ancil structures may be sufficient for phasing a target structure, so that the phasing technique may be denoted as the multiple phantom derivative method. Ancil phases and amplitudes may be initially combined with observed target magnitudes to estimate amplitudes and phases of the corresponding phantom derivative. From them suitable algorithms allow one to obtain poor target phase estimates, which are often improved by combining the indications arising from each derivative. Probabilistic criteria are described to recognize the most reliable target phase estimates. The method is cyclic: the target phase estimates just obtained are used to improve amplitudes and phases of each derivative, which, in their turn, are employed to provide better target phase estimates. The method is a fully *ab initio* method, because it needs only the experimental data of the target structure. The term derivative is maintained with reference to SIR–MIR (single isomorphous replacement–multiple isomorphous replacement) techniques, even if its meaning is different: therefore the reader should think of the phantom derivative method more as a new method than as a variant of SIR–MIR techniques. The differences are much greater than the analogies. The paper also describes how phantom derivatives may be used for improving structure models obtained *via* other *ab initio* or non-*ab initio* techniques. The method is expected to be insensitive to the structural complexity of the target and to the target experimental data resolution, provided it is better than 4–6 Å.

1. Introduction

The isomorphous replacement method was first applied by W. H. Bragg to solve NaCl and KCl structures. Updated by Robertson & Woodward (1937), the technique found a definitive formulation in the papers by Green *et al.* (1954) and by Bragg & Perutz (1954). The method may be summarized as follows: (i) the diffraction data of the structure one wants to solve (the *target structure*) are collected; (ii) a new compound (the *derivative*) is crystallized, in which one or more heavy atoms are incorporated into the target structure; (iii) the target and the derivative diffraction data are simultaneously used to solve the target structure.

According to the theoretical basis of the method, the derivative has to be isomorphous to the target: that is, the incorporation of a few heavy atoms should not seriously



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disturb the target structure. If this condition is heavily violated, the isomorphism is destroyed and the method is useless.

The above case is reported as single isomorphous replacement (SIR). More derivatives of the same target structure may be experimentally prepared: if isomorphous with it, they will provide useful diffraction data, the simultaneous use of which may lead to a more straightforward solution of the target structure. This case is denoted as the multiple isomorphous replacement (MIR) case.

SIR and MIR techniques are usually reported in the literature as non-*ab initio* phasing methods, because they need additional measurements, supplementary to those provided by the target structure. Their popularity has decreased over time: indeed protein crystallographers today prefer other non-*ab initio* techniques like molecular replacement (MR; Rossmann & Blow, 1962) and anomalous dispersion techniques (SAD, single anomalous dispersion, and MAD, multiple anomalous dispersion). The main reasons are as follows: (i) SIR and MIR are demanding in terms of isomorphism; furthermore, supplementary (with respect to the target) diffraction data are needed. (ii) MR is often preferred because model molecules similar to that present in the target structure are increasingly available and because supplementary diffraction data are no longer needed. (iii) SAD–MAD are mainly used when good MR models are not available. Anomalous dispersion data simulate nearly perfect isomorphism and are easily collected at synchrotrons.

Let us focus our attention on SIR–MIR methods. If n derivatives have been submitted to a diffraction experiment, then the set $\{|F|\}$ (diffraction amplitudes of the target structure) and the n sets $\{|F_d\,(j)|\}$, $j = 1, \ldots\, n$ (diffraction amplitudes of the n derivatives) are available: their prior knowledge is the key for solving the target structure. In particular, the heavy-atom substructures are first solved and, then, by exploiting this supplementary information, the full target structure may be determined. It is also implicit in the method that the scattering power of the heavy-atom substructure is small with respect to the scattering power of the target structure (otherwise isomorphism is destroyed).

The main question faced in this paper, related to the above summarized method of the isomorphous derivatives, is the following. May the prior knowledge of a crystal structure (called from now on an *ancil structure*, from the Latin *ancilla*), with the same unit-cell parameters and the same space group as the target structure, be used to solve the target structure even if it is completely uncorrelated with the target? If the answer to the above question is yes, one is not obliged to pick up, from the crystallographic archives, structures satisfying some required characteristics: any artificial structure may be chosen as ancil. We will show in this paper that: (i) it is not necessary that the ancil structures are real structures; much easier is to use one or more simulated, non-realistic structures, properly designed to satisfy specific characteristics. (ii) The prior knowledge of more ancil structures may be used to solve the target structure or to extend and refine phase estimates obtained by any other phasing approach.

The method will be described in detail in the next sections; here we summarize the guidelines and the notation.

Let $\rho(\mathbf{r})$ be the electron density of the target structure, $|F|$ and φ the corresponding amplitudes and phases. The $|F|$'s are known from a diffraction experiment, the φ values are unknown.

For any arbitrarily designed ancil structure, let $\rho_{\rm a}(\mathbf{r})$ be its electron density; then both $|F_{\rm a}|$ and $\varphi_{\rm a}$, the corresponding amplitudes and phases, are *a priori* known.

$\rho_{\rm d}(\mathbf{r})$ will be the electron density of the composite structure, here still called derivative, defined by the equation

$$\rho_{\rm d}(\mathbf{r}) = \rho(\mathbf{r}) + \rho_{\rm a}(\mathbf{r}). \tag{1}$$

It incorporates, in the same unit cell as the target, both the atoms of the target and the atoms of the ancil structure. It is supposed that the two structural components are not modified when they are part of the derivative. $|F_{\rm d}|$ and $\varphi_{\rm d}$ will denote amplitude and phase of the derivative structure factor, both unknown when phasing attempts start.

The derivative, as well as the ancil structure, is not a real structure; therefore, its diffraction amplitudes cannot be experimentally measured. Many ancil structures may be created and, correspondingly, many derivatives may be obtained, all devoid of experimental data. The phasing method described in this paper will use the experimental diffraction amplitudes of the target structure $|F|$, and the calculated diffraction amplitudes and phases of the ancil structures $|F_{\rm a}|$ and $\varphi_{\rm a}$, to progressively provide better and better estimates of the amplitudes and phases of the derivative structures. This information may be used to phase the target structure.

Since the derivatives are not real structures, the method described here will be called the phantom derivative method (PhD will be its acronym): we will also distinguish between the single phantom derivative (SPhD), only useful for describing the main characteristics of the method, and the multiple phantom derivative (MPhD) techniques, the suggested practical approach. PhD is here described as a fully *ab initio* phasing approach because it only needs the diffraction amplitudes of the target structure, but it may also be used for non-*ab initio* purposes.

2. About the ancil structures

We stated in §1 that an ancil structure may be any artificial structure with the same unit cell and the same space group as the target structure. The second condition is not strictly necessary: indeed several degrees of freedom exist, each one presenting specific consequences. For example, the ancil space group may be completely different from that of the target; in this case the derivative space group is usually different from the target and ancil space groups. That may generate practical difficulties in the management of the reflection symmetry. Also, a supergroup of the target group may be chosen for the ancil, belonging to the same crystal system as the target (*e.g.* the ancil symmetry may be $P2/m$ when the target space group is $P2$). In this case the derivative and target space groups coincide, but both are different from the ancil space group.

In general we will adopt the choice that target, ancil and derivative space groups coincide. Since our phasing method does not require that $\rho_a(\mathbf{r})$ and, therefore, $\rho_d(\mathbf{r})$ are chemically sound, we will suppose that the atomic positions of each ancil structure are randomly generated (or equivalently, the ancil reflection phases are randomly fixed): thus the ancil structures are completely independent of each other. However, ancil structures related to the target may also be chosen (see Appendix *E* for an example). Some practical convenience criteria should guide the choice of the ancil structures and they are described below.

The Fourier transform of equation (1) gives

$$F_d = F + F_a. \qquad (2)$$

At the beginning of the phasing process, the values of $|F_d|$, φ_d and φ are unknown, while $|F|$, $|F_a|$ and φ_a are known. Since $\rho_a(\mathbf{r})$ is an arbitrary structure, the phases φ_a can be supposed to be uncorrelated with the φ's. It is therefore impossible to estimate the derivative amplitudes and phases by applying equation (2). Some exceptions however may be found: one occurs when the condition

$$|F_a| > S_p|F| \qquad (3)$$

is satisfied, where S_p is a number sufficiently larger than unity. In this case, in equation (2), $|F|$ may be neglected with respect to $|F_a|$ and

$$|F_d| \simeq |F_a|, \quad \varphi_d \simeq \varphi_a. \qquad (4)$$

We will denote by {P} the subset of reflections satisfying the conditions (3); the letter P stands for directly 'phasable' and suggests that derivative phases and amplitudes of reflections satisfying (3) may be approximately known.

The other exception occurs when the condition

$$|F| > S_u|F_a| \qquad (5)$$

is satisfied, where S_u is a number sufficiently larger than unity. In this case, in equation (2), $|F_a|$ may be neglected with respect to $|F|$ and

$$|F_d| \simeq |F|, \quad \varphi_d \simeq \varphi. \qquad (6)$$

We will denote by {U} the subset of reflections satisfying the condition (5); the letter U suggests that, at an initial step of the PhD procedure, the derivative amplitudes of the {U} reflections may be approximately estimated *via* (6), but the corresponding phases are in practice 'undetermined' because the φ's are unknown at this stage.

The set of reflections not belonging to {P} or to {U} will be denoted by {I}; their phases are in general weakly correlated with the phases φ or φ_a. Equations (4) and (6) state that derivative amplitudes and phases of the {P} reflections may be carefully estimated if S_p is sufficiently large, while only the amplitudes of the derivative reflections belonging to {U} may be evaluated. Furthermore, no careful estimate is immediately possible for the derivative phases of reflections belonging to {I} and {U}. A way of determining them may be: first estimate derivative amplitudes and phases of the reflections belonging to {P}, and after extend the phase information to {I} and {U} reflections. No matter which approach one may use for phase extension, a necessary condition for the success of such a procedure is that the number of reflections belonging to {P} is sufficiently large. If the condition is satisfied and the procedure succeeds, the gain of information obtained after the extension process will be proportional to the number of phased reflections belonging to {I} and {U}.

Unfortunately the conditions (3) and (5), defining {P} and {U}, are in opposition. Indeed {P} will contain a large number of reflections if the scattering power of the ancil structure is chosen to be much larger than the scattering power of the target. This choice, however, will correspondingly reduce the number of reflections belonging to {U}: in this case the derivative is dominated by the ancil, and will hardly provide useful information on the target. On the contrary, choosing a scattering power of the ancil structure that is much lower than that of the target will make it more difficult to establish good starting estimates for the derivative. Thus, a sensible choice, assumed throughout this paper, may be to fix the scattering power of the ancil structure nearly equal to that of the target (even if in principle it may be freely chosen). Accordingly

$$\sum_{j=1}^{N_a} Z_j^2 = \sum_{j=1}^{N} Z_j^2$$

will hold, where N_a and N are the number of atoms in the unit cells of the ancil and of the target structure, respectively, and Z_j is the atomic number of the jth atom. To better accomplish the purpose, target and ancil unit cells may contain the same atomic species and the same number of atoms per atomic species. In this case $N_a = N$, and it is then possible to associate to the ancil atoms the average thermal factor of the target structure as estimated *via* the Wilson plot. In this case

$$\sum_{j=1}^{N_a} f_j^2 \simeq \sum_{j=1}^{N} f_j^2$$

will hold for any reflection, no matter the Bragg angle.

It may however be stressed that a different choice may be made: all the scattering power of the ancil structure may be concentrated into a few heavy atoms. No theoretical reason thwarts this practice, and at the moment it may not be stated which choice may be more effective.

In accordance with the above assumptions, the following relation arises:

$$R = |E| = |F|/{\textstyle\sum}^{1/2}, \; R_a = |E_a| = |F_a|/{\textstyle\sum}^{1/2},$$
$$R_d = |E_d| = |F_d|/(2\textstyle\sum)^{1/2},$$

where R, R_a and R_d are the normalized structure-factor moduli of target, ancil and derivative, respectively. Furthermore $\sum = \varepsilon(\sum_{j=1}^{N} f_j^2)^{1/2}$, where ε is the Wilson parameter, space-group and reflection dependent, necessary for the correct structure-factor normalization.

It is useful to notice that, under the above assumptions, if a pair $|F_a|$ and $|F|$ satisfies the relation (3) or (5), the corresponding normalized amplitudes will satisfy the relations $R_a >$

S_pR or $R > S_uR_a$, respectively. In other words, a reflection is associated to the subset {P}, {I} or {U} no matter if R is used instead of $|F|$.

We use now the Wilson statistics to check if the amount of phase information associated to the {P} reflections, as defined by a given S_p value, may be sufficient for a successful phase extension to {I} and {U} reflections.

In Appendix *A* we have calculated, for the centric and for the acentric cases, and in agreement with Wilson distributions of the amplitudes, the percentage of reflections for which $R_a \geq S_pR$. For acentric reflections we obtained

$$\mathrm{PERC}_1(S_p) = \frac{1}{S_p^2 + 1} \tag{7}$$

and for centric ones

$$\mathrm{PERC}_{\bar{1}}(S_p) = \frac{2}{\pi}\arctan(1/S_p). \tag{8}$$

The curves (7) and (8) are shown in Fig. 1. In both cases PERC = 1 when $S_p = 0$ (in this case $R_a \geq 0$ for all the reflections), and PERC = 0.5 when S_p = 1 [as expected for two uncorrelated Wilson distributions $P(R_a)$ and $P(R)$, $R_a > R$ should hold for half the number of reflections]. Furthermore

$$\begin{aligned} &\mathrm{PERC}_1(S_p) > \mathrm{PERC}_{\bar{1}}(S_p) \text{ for } S_p < 1,\\ &\mathrm{PERC}_1(S_p) < \mathrm{PERC}_{\bar{1}}(S_p) \text{ for } S_p > 1. \end{aligned}$$

In detail, if $S_p = 3$, then PERC_1 is expected to be close to 0.1 and $\mathrm{PERC}_{\bar{1}}$ to 0.2. In practice, it may be expected that, correspondingly, {P} would approximately contain 0.1 or 0.2 of the total number of the observed reflections. If we set $S_p = 2$, then PERC_1 is about 0.2 and $\mathrm{PERC}_{\bar{1}}$ is about 0.3. Equations similar to (7) and (8) hold also for {U} reflections. Indeed

$$\mathrm{PERC}_1(S_u) = \frac{1}{S_u^2 + 1} \tag{9}$$

and

$$\mathrm{PERC}_{\bar{1}}(S_u) = \frac{2}{\pi}\arctan(1/S_u) \tag{10}$$

will be the percentage of reflections for which $|F| > S_u|F_a|$ for the acentric and for the centric case, respectively. S_p and S_u may in principle be different from each other. Increasing or decreasing S_p will enlarge or diminish the number of derivative reflections with phases approximately known; increasing or decreasing S_u will increase or diminish the derivative reflections to phase.

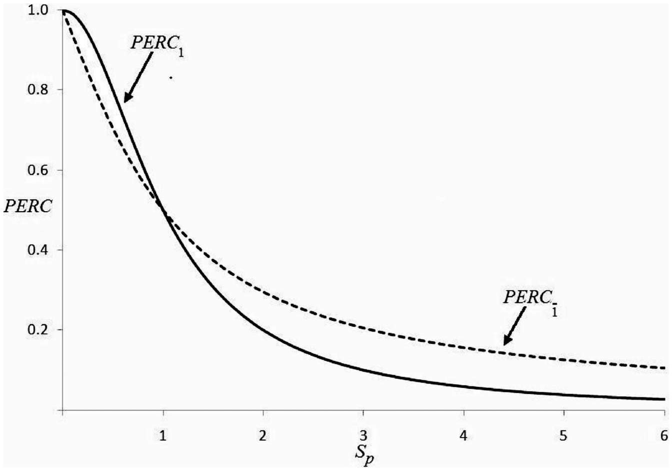

Figure 1
For a pair of uncorrelated target and ancil structures PERC is the percentage of reflections for which $R_a \geq S_pR$, where R_a and R are the normalized structure-factor amplitudes of the two structures. S_p is allowed to vary from 0 to infinite: this last value may be attained when R = 0. In the figure the curve is reported only for S_p in the interval (0, 6). PERC_1 and $\mathrm{PERC}_{\bar{1}}$ are the percentages for acentric and centric structures, respectively.

Equations (7) and (8) show that the number of reflections with phase $\varphi_d \simeq \varphi_a$ (and therefore belonging to the set {P}) may be sufficiently large for a successful phase extension process if $S_p \simeq 2$.

A short additional notation may be useful. In SIR–MIR techniques the isomorphism between native and derivative was a basic condition for the success of the phasing procedure. Isomorphism implies that $\rho_d(\mathbf{r}) \simeq \rho(\mathbf{r})$ for most of the $\mathbf{r}$ points; such a condition may be violated only in small regions of the unit cell, as an effect of the added heavy atoms, whose number has to be very small with respect to the number of native protein atoms. As a necessary effect in reciprocal space, the derivative phase is expected to be close to the native protein phase for most of the reflections. In PhD the traditional concept of isomorphism is absolutely superfluous. Indeed, the ancil structure has the same scattering power as the target structure, and therefore the condition $\rho_d(\mathbf{r}) \simeq \rho(\mathbf{r})$ is severely violated and, for a large subset of reflections, the derivative phases are no longer expected to be close to the target phases. That is the reason why the adjective isomorphous is not associated with phantom derivative.

3. Estimating amplitudes and phases for a phantom derivative

We stated in §2 that good estimates of the derivative phases may be obtained for reflections belonging to the set {P}, provided S_p is sufficiently large. The number of such reflections has been estimated in §2 for each S_p value: we need now to evaluate the quality of the corresponding phases. A probabilistic theory assessing how probable is the relation $\varphi_d \simeq \varphi_a$ is described in Appendix *B* [see equations (86) and (87)]. The theory clearly indicates that the relation $\varphi_d \simeq \varphi_a$ is supported for any type of reflection (no matter whether of type {P}, {I} or {U}); the indication is stronger if R_a is sufficiently large. In this section we want to describe a simple algebraic theory allowing a simple management of the practical aspects.

We want to calculate the average phase error

$$\langle|\varphi_d - \varphi_a|\rangle$$

between the unknown derivative phase φ_d and the assigned φ_a value (φ_a perfectly represents the ancil phase, and is known without error). In order to do that we consider the relationship

$$|F_{\rm d}|\exp(i\varphi_{\rm d}) = |F_{\rm a}|\exp(i\varphi_{\rm a}) + |F|\exp(i\varphi). \qquad (11)$$

The maximum value of $|\varphi_{\rm d} - \varphi_{\rm a}|$ occurs (see Fig. 2) when $F_{\rm d}$ is tangent to the circle with radius $|F|$ and centred at the tip of the vector $F_{\rm a}$. Then

$$|\sin(\varphi_{\rm d} - \varphi_{\rm a})|_{\max} = \frac{|F|}{|F_{\rm a}|} \qquad (12)$$

from which

$$|\varphi_{\rm d} - \varphi_{\rm a}|_{\max} = \arcsin\frac{1}{S}$$

with $S = |F_{\rm a}|/|F|$.

It is worthwhile noticing that if $|F_{\rm a}| > |F|$ $|\varphi_{\rm d} - \varphi_{\rm a}|_{\max}$ may never overcome 90°.

Fig. 3 shows the trend of $|\varphi_{\rm d} - \varphi_{\rm a}|_{\max}$ *versus* S: $|\varphi_{\rm d} - \varphi_{\rm a}|_{\max}$ strongly decreases when S increases. For $S = 2$ the allowed maximum deviation of $\varphi_{\rm d}$ from $\varphi_{\rm a}$ is about 30°, for $S = 3$ the value is 20°. Accordingly, if we associate (in the first steps of the phasing procedure) the $\varphi_{\rm a}$ value to $\varphi_{\rm d}$, 30° and 20° are the maximum phase errors we can commit, respectively.

Let us now collectively consider the reflections belonging to {P}, when we choose $S_p = 2$ (in this case we are dealing with reflections for which $R_{\rm a} > 2R$). For those reflections for which exactly $S = S_p$ (and therefore $R_{\rm a} = 2R$) it will be $|\varphi_{\rm d} - \varphi_{\rm a}|_{\max} = 30°$; for the rest of the reflections (belonging to {P} but with $S > 2$) it will be $|\varphi_{\rm d} - \varphi_{\rm a}|_{\max} < 30°$. The success of a phase extension procedure (that trying to phase the reflections belonging to {I} and {U} given the amplitudes and the phases of the reflection belonging to {P}), more than by the maximum phase error, is decided by the average phase error $\langle|\varphi_{\rm d} - \varphi_{\rm a}|\rangle$ calculated over all the {P} reflections. Certainly $\langle|\varphi_{\rm d} - \varphi_{\rm a}|\rangle$, for a given S_p, is significantly smaller than $|\varphi_{\rm d} - \varphi_{\rm a}|_{\max}$ calculated at the chosen S_p value. Our first conclusion is therefore that the derivative phases for the reflections belonging to the subset {P} are certainly carefully estimated provided $S_p > 2$. Correspondingly, the percentage of derivative reflections with carefully estimated phase values is PERC(2) $\simeq$ 0.3 or 0.2 according to whether the space group is centric or acentric.

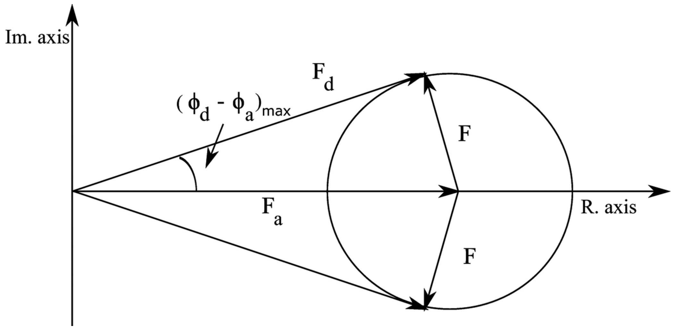

Figure 2
Amplitudes and phase of $F_{\rm a}$ are both *a priori* known. In the figure, for simplicity, we choose $\varphi_{\rm a} = 0$ but it may be whichever (the figure may be rotated if one wants to choose a different $\varphi_{\rm a}$ value). F, the structure factor of the target structure, has known amplitude and unknown phase. It starts at the tip of the vector $F_{\rm a}$ and may end at any point of the circle with radius $|F|$. $F_{\rm d}$ is the vectorial sum of F and $F_{\rm a}$. In the figure F is oriented so as to correspond to the maximum allowed values of $|\varphi_{\rm d} - \varphi_{\rm a}|$.

Let us now consider the size of the errors involved in the approximation $|F_{\rm d}| \simeq |F_{\rm a}|$ as stated by the condition (3) for the subset {P}. From the general relationship

$$|F_{\rm d}|^2 = |F_{\rm a}|^2 + |F|^2 + 2|F_{\rm a}F|\cos(\varphi_{\rm a} - \varphi) \qquad (13)$$

the largest and the smallest allowed values of $|F_{\rm d}|$, say $|F_{\rm d}|_{\max}$ and $|F_{\rm d}|_{\min}$, respectively, are $|F_{\rm d}|_{\max} = |F_{\rm a}| + |F|$ and $|F_{\rm d}|_{\min} = |F_{\rm a}| - |F|$, the first obtained when $\varphi = \varphi_{\rm a}$ and the second when $\varphi = \varphi_{\rm a} + \pi$ (see Fig. 4*a*). Then the maximum relative error on $|F_{\rm d}|$ will be

$$\left(\frac{|\Delta|F_{\rm d}||}{|F_{\rm d}|}\right)_{\max} = \frac{|F_{\rm d}|_{\max} - |F_{\rm d}|_{\min}}{|F_{\rm d}|_{\min}} = \frac{2|F|}{|F_{\rm a}| - |F|}. \qquad (14)$$

First, we notice that the maximum relative error may be very large when $|F_{\rm a}| \simeq |F|$. Second, if we assume $S = |F_{\rm a}|/|F| = 2, 3$ or 4, the maximum relative error may reach 2, 1 or 2/3, respectively, quite large indeed. Luckily this situation will occur only when $|F_{\rm d}|$ is calculated under the following assumption: $\varphi_{\rm a}$ is estimated close to φ but the real relation is $\varphi_{\rm a} \simeq \varphi + \pi$, a condition which, for an acentric structure, statistically happens in a relatively small percentage of cases (φ and $\varphi_{\rm a}$ are uncorrelated indeed). For example, let us consider the reverse case, which also implies a π error in the relation $\varphi_{\rm a} \simeq \varphi$: it occurs when $|F_{\rm d}|$ has been calculated on the assumption $\varphi_{\rm a} \simeq \varphi + \pi$ but the real relation is $\varphi_{\rm a} \simeq \varphi$. Then the maximum relative error for $S = 2, 3, 4$ is

$$\frac{2|F|}{|F_{\rm a}| + |F|} = \frac{2}{3}, \frac{1}{2}, \frac{2}{5},$$

respectively. In practice the relative errors on the $|F_{\rm d}|$ estimates will be significantly smaller than their maxima because the ancil structure is uncorrelated with the target structure, and therefore there is no reason for systematically assuming, in an acentric structure, that $\varphi_{\rm a} \simeq \varphi$ or $\varphi_{\rm a} \simeq \varphi + \pi$.

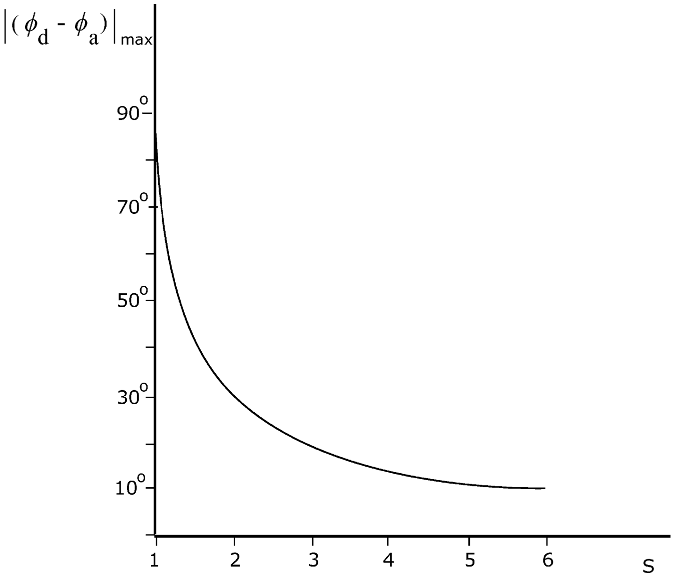

Figure 3
$|\varphi_{\rm d} - \varphi_{\rm a}|_{\max}$ is the maximum phase error one can commit when the assumption $\varphi_{\rm d} \simeq \varphi_{\rm a}$ is accepted for the subset {P}. $|\varphi_{\rm d} - \varphi_{\rm a}|_{\max}$ is drawn *versus* $S = |F_{\rm a}|/|F|$.

A general situation is described in Fig. 4(*a*), where the following conditions were assumed:

$$|F_a| > |F|, \ \ |\varphi_d - \varphi_a| \neq |\varphi_d - \varphi_a|_{\max} \text{ and } (\varphi_d - \varphi_a) \neq 0, \pi.$$

Then, for the chosen value of $(\varphi_d - \varphi_a)$, there are two possible values of $|F_d|$, say $|F_{d1}|$ and $|F_{d2}|$, corresponding to the vectors OA_1 and OA_2, respectively (from now on, by definition, $|F_{d2}| > |F_{d1}|$). Correspondingly, two possible F vectors are allowed, denoted by F_1 and F_2 in the figure.

In accordance with the above observations, the amplitudes $|F_a|$ of the {P} reflections will simulate well the amplitudes $|F_d|$ only if S_p is very large. But choosing very large values of S_p will diminish the number of reflections belonging to {P}, and therefore will reduce the amount of information available for the subsequent steps of phase extension.

In conclusion, the error on the amplitudes is more critical, for the success of the phase-extension process, than the error on the phases (against the common belief that phases are more important than amplitudes). This statement may be more easily accepted if one thinks that the information on the interatomic distances is deposited in the diffraction amplitudes which entirely define the crystal structure (see Giacovazzo, 2014, where the *basic postulate of structural crystallography* has been established. According to it, only one chemically sound structure exists that is compatible with the experimental diffraction data and therefore with the interatomic distances).

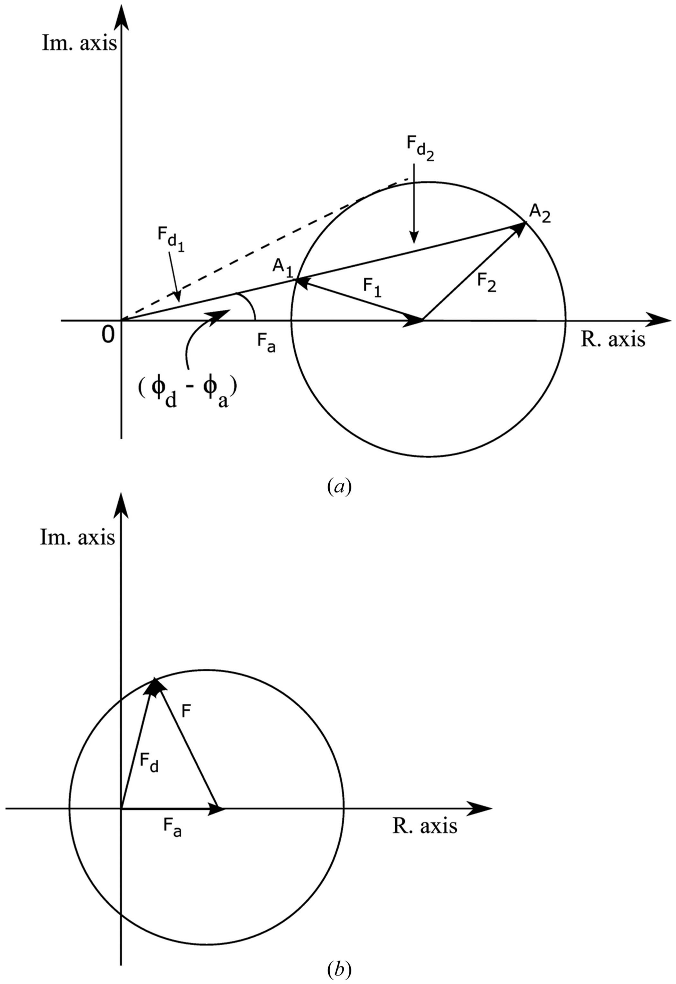

Figure 4

For drawing simplicity, $\varphi_a = 0$ has been assumed. (*a*) Case $|F_a|/|F| > 1$. For a given $(\varphi_d - \varphi_a)$, $|F_{d1}| = OA_1$ and $|F_{d2}| = OA_2$ are both allowed as derivative amplitudes. Correspondingly F_1 and F_2 are both allowed as target structure factors. (*b*) Case $|F_a|/|F| < 1$. For a given $(\varphi_d - \varphi_a)$ only one $|F_d|$ and only one target structure factor are admitted.

There are two different ways to minimize the error on the amplitudes. The first exploits the hypothesis that the ancil and the target structures are uncorrelated; then $\langle\cos(\varphi_a - \varphi)\rangle$ is expected to vanish and the best amplitude estimate, say $|F_{\rm dest}|$, is readily obtained from equation (13):

$$|F_{\rm dest}|^2 = |F_a|^2 + |F|^2. \qquad (15)$$

Under the assumptions made in §2 the relation (15), in terms of normalized structure factors, is equivalent to

$$R^2_{\rm dest} = \frac{1}{2}(R^2_a + R^2).$$

Equation (15) is a probabilistic relation only valid for acentric reflections. For centric ones F_a and F are collinear: therefore, in the absence of supplementary phase information, if $|F| > |F_a|$ then $|F_d|$ may take two values, $|F| + |F_a|$ or $|F| - |F_a|$, the mean of which is $|F|$. If $|F_a| > |F|$ then the allowed $|F_d|$ values are $|F_a| + |F|$ or $|F_a| - |F|$, the average of which is $|F_a|$. Accordingly, the best estimate of $|F_d|$ in the absence of phase information is $|F|$ if $|F| > |F_a|$, is $|F_a|$ when $|F_a| > |F|$. In terms of normalized amplitudes this statement is equivalent to: the best estimate of R_d in the absence of phase information is $R/2^{1/2}$ if $R > R_a$, is $R_a/2^{1/2}$ when $R_a > R$.

Other ways of minimizing the errors on the derivative amplitudes and of correcting the estimates provided by equation (15) are described in §4: since they require some information on φ_d, they are not useful in the very first PhD step.

So far we have focused our attention on the reflections belonging to {P}. The relation $\varphi_d \simeq \varphi_a$ may also be applied for estimating the derivative phases of the reflections belonging to {I} [see distribution (86) for the probabilistic treatment]. In Fig. 4(*b*) we show a case for which $|F| > |F_a|$: now for a given value of φ_d there is only one feasible value of φ. The value of φ_d is weakly correlated with φ_a and therefore the relation $\varphi_d \simeq \varphi_a$ is weaker than for the set {P}.

The derivative phases of the reflections {U} are even less correlated with the corresponding φ_a values: indeed they are strongly correlated with the unknown φ values. The {U} amplitudes, however, can be estimated with larger accuracy if $S_u = |F|/|F_a|$ is sufficiently large (in this case $|F_d|$ is close to the measured $|F|$ value).

4. About the phasing potential and limits of the single phantom derivative

Let us suppose that the procedure outlined in §§2 and 3 has been applied. The new scenario, from the point of view of the gained information, is the following:

(i) The observed amplitudes $|F|$ of the target structure and the calculated amplitudes and phases ($|F_{\rm a}|$ and $\varphi_{\rm a}$) of the ancil structure are known *a priori*.

(ii) If S_p has been chosen sufficiently large, good estimates of the derivative phases $\varphi_{\rm d}$ (*i.e.* $\varphi_{\rm d} \simeq \varphi_{\rm a}$) for reflections belonging to the set {P} should have been obtained, together with rough estimates of their amplitudes as provided by equation (15) or by the related formula for centric reflections.

(iii) Rough estimates of derivative phases and amplitudes are available for the reflections belonging to the set {I}.

(iv) The derivative phases of the reflections belonging to {U} are hardly estimated, but their amplitudes may be obtained *via* equation (6) if S_u is sufficiently large.

Owing to point (ii) it is possible, at least for reflections belonging to the subset {P}, to approximate the derivative structure factors by using the amplitudes (15) as derivative amplitudes, and values $\varphi_{\rm a}$ as derivative phases. They may constitute the seed of a phasing process based on EDM (electron-density modification techniques; Cowtan, 1999; Abrahams, 1997; Abrahams & Leslie, 1996; Refaat & Woolfson, 1993; Giacovazzo & Siliqi, 1997) procedures for phase extension and refinement, which might lead to better estimates of $\rho_{\rm d}(\mathbf{r})$: by its Fourier inversion, $|F_{\rm dinv}|$ and $\varphi_{\rm dinv}$ may be obtained, which, under suitable conditions, may be considered as new supplementary estimates of $|F_{\rm d}|$ and $\varphi_{\rm d}$, the true derivative amplitudes and phases.

In EDM techniques a weight is associated to each reflection, which is expected to be correlated with the reliability of the corresponding model phase. In early times the weight was usually calculated in accordance with Sim (1959), and essentially coincides with the product $2RR_{\rm calc}$, where R and $R_{\rm calc}$ are the normalized amplitudes of the target and of the model, respectively. Sim probabilistic treatment is based on the assumption that the model is part of the target structure, and that the atomic positions of the model coincide with the atomic positions of the target. In more recent years this too strict assumption has been abandoned (Srinivasan & Ramachandran, 1965; Read, 1986; Carrozzini, Cascarano, Giacovazzo & Mazzone, 2013) and the weight has been modified into $I_1(X)/I_0(X)$, where I_1 and I_0 are modified Bessel functions of order 1 and 0, respectively,

$$X = 2\sigma_A R R_p/(1-\sigma_A^2).$$

σ_A is a parameter taking into account the correlation factor between model and target structure: it is expected to be close to 0 when they are uncorrelated, and close to 1 when they are strongly correlated.

Since the derivative amplitudes are not available, the above σ_A weighting scheme cannot be applied to the derivative structures, at least in the first PhD step. It should however be possible to use amplitudes (15) as substitutes of the unavailable observed derivative amplitudes: then the {P} reflections (eventually integrated by {I} reflections) may be used for the calculation of the first derivative electron density, by associating to them the phases $\varphi_{\rm d} = \varphi_{\rm a}$. A few EDM cycles may then be applied for extending and refining derivative phases, by employing the weight $I_1(X)/I_0(X)$. This time

$$X = 2\sigma_A R_{\rm dinv} R_{\rm dest}/(1-\sigma_A^2)$$

may be used, where $R_{\rm dest}$ is given by (15), and $R_{\rm dinv}$ is obtained by Fourier inversion of the current electron-density map. Now σ_A expresses the correlation between the calculated amplitudes (15) and the current derivative model amplitudes. Since amplitudes (15) are only rough approximations of the true derivative amplitudes, in accordance with the considerations described in §3, the EDM procedure may diverge and therefore should be stopped after a few cycles.

Let us suppose for a moment that $\varphi_{\rm dinv}$ is a better estimate of $\varphi_{\rm d}$ than $\varphi_{\rm a}$. Then its value may be used for a more accurate estimate of $|F_{\rm d}|$. Indeed for each reflection the triangle defined by $F_{\rm a}$, F and $F_{\rm d}$ may be exploited, from which, by squaring right and left sides of equation (11), the Carnot quadratic relation

$$|F_{\rm d}^2| - 2|F_{\rm d}||F_{\rm a}|\cos(\varphi_{\rm d}-\varphi_{\rm a}) - (|F|^2 - |F_{\rm a}^2|) = 0$$

may be obtained. Its allowed roots are given by

$$|F_{\rm d}| = |F_{\rm a}|\cos(\varphi_{\rm d}-\varphi_{\rm a}) \pm [|F|^2 - |F_{\rm a}|^2\sin^2(\varphi_{\rm d}-\varphi_{\rm a})]^{1/2}. \qquad (16)$$

Since it has been supposed that $\varphi_{\rm dinv}$ is a better approximation of $\varphi_{\rm d}$, then it may be introduced at the right-hand side of equation (16) to obtain a new estimate of $|F_{\rm d}|$: in practice, the relation (15) may be abandoned in favour of the following estimate:

$$|F_{\rm dest}| \simeq |F_{\rm a}|\cos(\varphi_{\rm dinv}-\varphi_{\rm a}) \pm [|F|^2 - |F_{\rm a}|^2\sin^2(\varphi_{\rm dinv}-\varphi_{\rm a})]^{1/2}. \qquad (17)$$

For reflections with $S < 1$ (they belong to the sets {I} or {U}) equation (17) will provide only one real and positive root, corresponding to the plus sign, which may be assumed to be the new $|F_{\rm d}|$ estimate (see Fig. 4*b*).

For reflections for which $S > 1$ two real roots may be obtained for $|F_{\rm d}|$ (see Fig. 4*a*), say $|F_{\rm d1}|$ and $|F_{\rm d2}|$, provided

$$|F|^2 - |F_{\rm a}|^2\sin^2(\varphi_{\rm dinv}-\varphi_{\rm a}) > 0,$$

or equivalently, provided

$$|\sin(\varphi_{\rm dinv}-\varphi_{\rm a})| < |F|/|F_{\rm a}| = S. \qquad (18)$$

We notice that equation (18) establishes a limit for $\varphi_{\rm dinv}$ (and also for $\varphi_{\rm d}$, even if it is unknown at this stage of the phasing process) which cannot be overcome during EDM refinement (see the broken line in Fig. 4*a*). If the EDM refinement changes $\varphi_{\rm dinv}$ in such a way that equation (18) is violated, then $\varphi_{\rm dinv}$ has to be reset to satisfy the limit. It should be noticed that the inequality (18) has an algebraic nature, not subjected to probabilistic effects: indeed $|F|$ and $|F_{\rm a}|$ are well known, the first from the experiment, the second from the generated ancil structure.

Equation (18) is a useful restraint on the admitted phases, which thwarts EDM procedures to gradually lose the original phase information stored in the {P} reflections. For such reflections $\varphi_{\rm a}$ and $\varphi_{\rm d}$ are very close, and thus the passage from ancil to derivative values is not an important task. The situation is quite different for {U} reflections, for which φ and $\varphi_{\rm d}$ are very close to each other, but both are practically uncorrelated with φ_a. Driving their phases from φ_a to φ_d is quite a difficult job: only when that is attained will passing from φ_d to φ become easy. Obtaining the derivative phases of the {I} reflections starting from the φ_a values of the {P} reflections shows an intermediate degree of difficulty. Since {I} and {U} subsets contain the large majority of the reflections, it is clear that the use of the restraints (18) cannot guarantee the correct migration of the phases from the starting φ_a to the true derivative values.

Indeed, why should EDM procedures be able to improve derivative phase estimates? Certainly the average small starting phase error of the {P} reflections might facilitate the process. On the other hand, the incertitude on the derivative amplitudes will make EDM procedures less efficient than in the usual applications, where the amplitudes are experimentally known. In fact, in this PhD step we are replacing the true derivative diffraction amplitudes by the statistical estimates (15): since the experimental amplitudes contain information on the interatomic vectors, and these last ones define the structure, using (15) violates a condition for successful phasing. EDM should only succeed if an increasing number of reflections belonging to {I} and {U} start to be well phased: then the contribution of the target electron density to the derivative density should progressively increase and should drive the current derivative phase estimates closer and closer to the true values.

Unfortunately, such hope is not well founded: the lack of experimental diffraction amplitudes for each derivative hinders such a virtuous phasing process, and the EDM procedure very likely will confirm a distorted ancil structure rather than provide a useful derivative model.

From the above considerations it may be concluded that SPhD is probably unable to provide, *via* EDM techniques, accurate derivative amplitudes and phases just starting from an initial electron-density map based on amplitudes (15) and phases φ_a. As a consequence, it will not be possible to deduce, from the current derivative model, accurate φ estimates *via* the probabilistic formulas which will be described in §7 and Appendix *B*.

There is a supplementary mathematical reason making the φ estimation more difficult: indeed even small errors on φ_d may transform into large errors on φ. In order to more clearly describe this effect, let us consider, as a first example, the reflections for which $S > 1$. From Fig. 4(*a*) it is easy to derive the relation [demonstrated in Appendix *C*: see equation (89)]

$$|F|\sin(\varphi - \varphi_a) = |F_d|\sin(\varphi_d - \varphi_a) \qquad (19)$$

from which, using equation (16) with $|F_a| = S|F|$, the geometrical relation

$$\begin{aligned}\sin(\varphi - \varphi_a) = \sin(\varphi_d - \varphi_a)\{&S\cos(\varphi_d - \varphi_a)\\ &\mp [1 - S^2\sin^2(\varphi_d - \varphi_a)]^{1/2}\} \qquad (20)\end{aligned}$$

is derived. The plus sign in equation (20) corresponds to the F_{d2} choice, the minus sign to the F_{d1} selection. It is important to notice that equation (19) is satisfied [and therefore $\sin(\varphi - \varphi_a)$ and $\sin(\varphi_d - \varphi_a)$ have the same sign] whichever of F_{d1} or F_{d2} is chosen, and whatever the φ_a value is.

Equation (20) may be used to describe how wrong $(\varphi_d - \varphi_a)$ estimates, or wrong choices between F_{d1} and F_{d2}, transform into wrong $(\varphi - \varphi_a)$ estimates. In order to illustrate such an effect when a wrong choice between F_{d1} and F_{d2} is made, we show in Fig. 5 the case $S \simeq 1.48$, $\varphi_a = 0$ and $\varphi_d = 16°$: we first assume that φ_d is precisely estimated. A wrong choice between F_{d1} and F_{d2} may imply a big error on the φ estimate. Indeed, according to equation (20), if we chose F_{d2} as representative of F_d, then φ is estimated to be close to $\varphi_2 \simeq 40°$, if we chose F_{d1} then φ is expected to be close to $\varphi_1 \simeq 172°$. The corresponding error attains π if $(\varphi_d - \varphi_a)$ is close to zero and we wrongly choose F_d, reduces to zero when $(\varphi_d - \varphi_a)$ is close to the limit value (in this case F_d is tangent to the F_a circle, and F_{d1} and F_{d2} coincide). In Fig. 5 φ_2 and φ_1 values corresponding to other selected φ_d angles are reported, to underline the phase ambiguity related to the choice between F_{d1} and F_{d2}.

Let us now again consider the case in which $S \simeq 1.48$, $\varphi_a = 0$, $\varphi_d = 16°$, but now we suppose that φ_d is estimated with uncertainty lying in the range (0°, 30°). If φ_d is estimated to be 0° (and therefore with an error of 16°), then also φ_2 is estimated to be 0°, with an error of 40°. If φ_d is estimated to be 35° (and therefore with an error of 19°), then φ_2 is estimated to be 87°, with an error of about 47°. It is therefore also seen that modest errors on φ_d have magnified consequences on the φ_2 estimates. For the above two φ_d errors φ_1 takes the values 180° and 157°, respectively (instead of the correct value 172°). It may be concluded that φ_1's are less sensible to errors on φ_d than φ_2's.

We come now to analyse a further drawback frequently met in SPhD, particularly critical at the beginning of the phasing process, when φ_a's are the best phases to associate to the derivative. As we stated before, the reflections with $S > 1$ have strong constraints on the allowed values of $|\sin(\varphi_d - \varphi_a)|$ [see equation (18)], while no constraint may be applied to reflec-

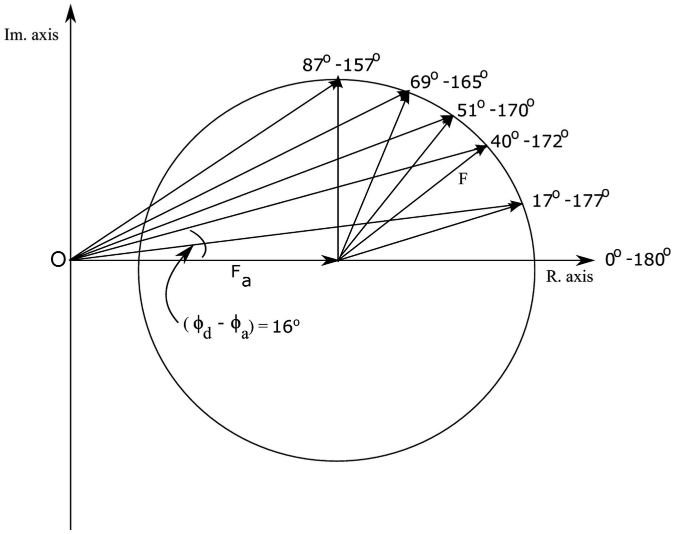

Figure 5
Case $S = F/F_a \simeq 1.48$. The F_d vectors start from O, with some selected φ_d angles. Values of of φ_1 and φ_2 for some selected φ_d values are reported.

tions with $S < 1$. In both the cases, for a given reflection (*hkl*), it is not rare that EDM procedures may suggest a positive sign of $\sin(\varphi_d - \varphi_a)$ while the correct sign is negative, or *vice versa*. Such behaviour is also usual in any standard *ab initio* phasing procedure, when target amplitudes are experimentally available and EDM procedures are applied to improve the target model: some phases are driven in the correct direction, some others are worsened. This behaviour is emphasized in PhD where EDM techniques are applied to extend and improve derivative phases in the absence of observed amplitudes.

With reference to Fig. 5 let us suppose that the EDM process suggests $\varphi_d = -16°$ instead of the correct value $\varphi_d = +16°$. In this case the allowed φ estimate should be $\varphi_2 \simeq -43°$ and $\varphi_1 \simeq 188°$. Thus, the error $|\Delta\varphi_d| = 32°$ may produce a much larger error on φ_2, in our case equal to $|\Delta\varphi_2| = 80°$ and an error on φ_1 equal to $|\Delta\varphi_1| = 16°$: owing to projective laws $|\Delta\varphi_2|$ will always be larger than $|\Delta\varphi_1|$. It may be concluded that a wrong estimate of the $\sin(\varphi_d - \varphi_a)$ sign, as frequently obtained at the end of an EDM procedure, may generate large errors on the target phase estimates.

The case $S < 1$ may be treated along the same lines, by remembering that the ambiguity between F_{d1} and F_{d2} does not exist anymore. In practice (see Fig. 4*b*) the error on φ_d will be transferred to φ, and this will be particularly magnified when φ_d is close to φ_a.

The problem of the wrong $\sin(\varphi_d - \varphi_a)$ estimates is extensively treated in Appendix *D*, where an algorithm is also described to reduce such critical errors.

5. The origin and the enantiomorph problem in the PhD method

When SIR techniques are used to solve a protein structure, isomorphous differences are employed to define the heavy-atom substructure: then the so-gained information is used in a second step for phasing the protein. MIR are often preferred to SIR techniques in order to overcome the enantiomorph ambiguity, to reduce the noise and to emphasize the signal.

Let us consider for simplicity the two isomorphous derivative case (as usual, when dealing with SIR–MIR methods, the subscripts P, H and d will denote native, heavy-atom and derivative structures). Since the heavy-atom binding sites in the second derivative usually do not coincide with those of the first one, then $F_H(1) \neq F_H(2)$ and $F_d(1) \neq F_d(2)$. Then the system of equations

$$\varphi_P = \varphi_H(1) + \cos^{-1}\{[|F_d(1)|^2 - |F_P|^2 - |F_H(1)|^2]/2|F_P F_H(1)|\} \qquad (21a)$$

$$\varphi_P = \varphi_H(2) + \cos^{-1}\{[|F_d(2)|^2 - |F_P|^2 - |F_H(2)|^2]/2|F_P F_H(2)|\} \qquad (21b)$$

may be applied to phase the native structure. The two equations, however, may be used only if the heavy-atom positions are referred to the same origin. If the first derivative is considered the best one, the difference Fourier synthesis with coefficients

$$[|F_d(2)| - |F_P|]\exp(i\varphi_P)$$

is calculated, where the phase angles φ_P are defined according to the first derivative: the synthesis directly provides the heavy-atom positions of the second derivative, referred to the same origin as the first one. In this way the two derivatives also define the same enantiomorph for the native protein. Finally, fixing the correct enantiomorph for the native protein is only a problem of choosing between two alternatives. The method is easily extended to the case in which more derivative data are collected.

In the MPhD approach the origin problem also exists, but with different characteristics: indeed all the atomic positions of the ancil structures are created *via* random numbers between 0 and 1, and therefore are related to the same origin. Since the phases of each ancil structure are known, the situation is similar to that available in the second step of the MIR techniques, when all the heavy-atom substructures have been determined with respect to the same origin. Now the arbitrary generation of the ancil structures unequivocally fixes the correct enantiomorph for each ancil. Therefore, in MPhD an arbitrarily large number of ancil structures, perfectly known, plays the same role as the few solved (and related to the same origin) heavy-atom substructures in the MIR case. However, this time, equations like (21*a*) and (21*b*) cannot be applied because the derivative amplitudes are not measured, and this specific characteristic creates in PhD a different type of origin and enantiomorph ambiguity.

In SIR–MIR the derivative is physically created by adding heavy atoms to the native protein: this condition is reflected in reciprocal space by the relation

$$|F_d|^2 = |F_H|^2 + |F_P|^2 + 2|F_H F_P|\cos(\varphi_H - \varphi_P), \qquad (22)$$

where φ_H and φ_P refer to the same origin. $|F_d|$'s, as calculated by equation (22), and observed derivative amplitudes will fit quite well provided target and heavy-atom model substructures are referred to the same origin. In SIR–MIR techniques the identification of the correct solution relies on suitable figures of merit (*e.g.* on Cullis and Krout *R* factors and/or on the so-called *phasing power* criterion) based on the fitting between observed and calculated derivative amplitudes. Also the most popular refinement scheme, the so-called *phase refinement* (Dickerson *et al.*, 1961; Terwilliger & Eisenberg, 1983), is based on the same fitting.

If $|F_d|$ is not measured, like in PhD, in the first step of the phasing process we are obliged to replace the relation

$$|F_d|^2 = |F_a|^2 + |F|^2 + 2|F_a F|\cos(\varphi_a - \varphi) \qquad (22')$$

by (15). The identification of suitable figures of merit, as well as the success of the phase refinement process, is then much more difficult.

If the last term on the right-hand side of equation (22′) is cancelled [as the application of equation (15) requires], what does that imply in direct space? The phase information is cancelled and therefore we are using as coefficients of the first derivative electron-density map a quantity which does not depend on $\cos(\varphi_a - \varphi)$, and therefore is independent of the possible origin shift between ancil and target structure. Using relation (15) as a substitute of the calculated derivative amplitude will then leave undetermined the ancil origin among the *allowed* ones (Hauptman & Karle, 1956; Giacovazzo, 1974). For example, in $P\overline{1}$ the derivative amplitude (15) is compatible with ancil structures shifted by

$$\begin{array}{cccc} (0,0,0), & (0,0,1/2), & (0,1/2,0), & (1/2,0,0), \\ (1/2,1/2,1/2), & (1/2,1/2,0), & (1/2,0,1/2), & (0,1/2,1/2). \end{array}$$

In $P2$ the derivative amplitudes (15) will be compatible with ancil structures shifted along any of the twofold axes. In conclusion, the amplitude (15) is not specific to a single derivative [being devoid of the phase component present in equation (22′)], but represents the average amplitude of the family of derivatives which may be created by shifting the ancil origin by allowed origin translations.

An additional problem of enantiomorphism is present in the PhD first step. Let us consider two derivative electron densities, the first corresponding to $\rho_{\rm d}(\mathbf{r}) = \rho_{\rm a}(\mathbf{r}) + \rho(\mathbf{r})$, the second corresponding to $\tilde{\rho}_{\rm d}(\mathbf{r}) = \rho_{\rm a}(\mathbf{r}) + \rho(-\mathbf{r})$: here the enantiomorph of the target structure has been used for obtaining $\tilde{\rho}_{\rm d}(\mathbf{r})$. The two derivatives are substantially different: in the first case the derivative structure factor is $F_{\rm a}(\mathbf{h}) + F(\mathbf{h})$, in the second case it is $F_{\rm a}(\mathbf{h}) + F(-\mathbf{h})$. In Fig. 6 $\varphi_{\rm d}$ and $\tilde{\varphi}_{\rm d}$ denote the corresponding phases: it is immediately seen that, in general, $\tilde{\varphi}_{\rm d} \neq \varphi_{\rm d}$. Furthermore, the Fourier amplitudes for the first derivative should be the root square of

$$|F_{\rm d}|^2 = |F_{\rm a}|^2 + |F|^2 + 2|F_{\rm a}F|\cos(\varphi_{\rm a} - \varphi)$$

while those of the second derivative should be

$$|\tilde{F}_{\rm d}|^2 = |F_{\rm a}|^2 + |F|^2 + 2|F_{\rm a}F|\cos(\varphi_{\rm a} + \varphi).$$

$F_{\rm d}(\mathbf{h})$ and $\tilde{F}_{\rm d}(\mathbf{h})$ have therefore different amplitudes. If the $|F_{\rm d}|$'s or the $|\tilde{F}_{\rm d}|$'s were measured, the transition from $\varphi_{\rm a}$ to the derivative phases should be relatively easy, owing to the structural information contained in the measured amplitudes. If in the PhD first phasing steps the approximation (15) is used, then the same amplitude $(|F_{\rm a}|^2 + |F|^2)^{1/2}$ should equally correspond to both the derivatives. In other words, the estimated derivative amplitudes (15) equally represent both the derivative $\rho_{\rm a}(\mathbf{r}) + \rho(\mathbf{r})$ and the derivative $\rho_{\rm a}(\mathbf{r}) + \rho(-\mathbf{r})$. It is then not surprising that, in the first step of the phasing process, EDM procedures may drive some derivative phases from $\varphi_{\rm a}$ towards $\varphi_{\rm d}$ or to $\tilde{\varphi}_{\rm d}$.

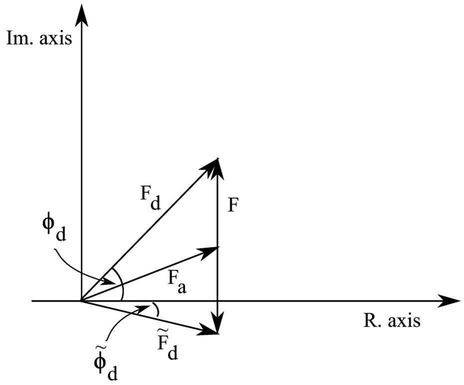

Figure 6
$F_{\rm a}$, F and $F_{\rm d}$ are structure factors of the ancil, target and derivative structure, respectively. $\tilde{F}_{\rm d}$ is the derivative structure factor corresponding to the enantiomorph of the target structure. In general $\tilde{F}_{\rm d} \neq F_{\rm d}$, unless $\varphi_{\rm a} = 0, \pi$.

Luckily in MPhD the ancil phases are known with extreme accuracy. Thus the above ambiguities on the origin and enantiomorph, generated by the unsatisfactory amplitudes used in the first steps of the phasing procedure, are reduced as soon as it becomes possible to apply relationships which estimate the derivative amplitudes as a function of $\varphi_{\rm d}$ and $\varphi_{\rm a}$. Indeed, it is then possible to replace the approximation (15) by the better amplitude estimates given by the relation (17), which now depends on the phase difference $(\varphi_{\rm dinv} - \varphi_{\rm a})$. If $\varphi_{\rm dinv}$ is a better approximation of the derivative phase then also the amplitude $|F_{\rm d}|$ will be a more precise estimate of the true derivative amplitude, now sensitive to the origin and to the enantiomorph.

Even if in MPhD the derivative amplitudes are not measured, strong constraints may be found among the various derivatives, which may make it easier for the derivative phase estimates to converge to the same enantiomorph and then the crystal structure solution. In order to make an example, let us consider a couple of derivatives, the jth and the kth. From relation (11)

$$\begin{aligned} |F|\exp(i\varphi) &= |F_{\rm d}(k)|\exp[i\varphi_{\rm d}(k)] - |F_{\rm a}(k)|\exp[i\varphi_{\rm a}(k)] \\ &= |F_{\rm d}(j)|\exp[i\varphi_{\rm d}(j)] - |F_{\rm a}(j)|\exp[i\varphi_{\rm a}(j)] \end{aligned}$$

is easily derived, from which

$$\begin{aligned} |F_{\rm d}(j)|\sin\varphi_{\rm d}(j) = {}& |F_{\rm d}(k)|\sin\varphi_{\rm d}(k) + |F_{\rm a}(j)|\sin\varphi_{\rm a}(j) \\ & - |F_{\rm a}(k)|\sin\varphi_{\rm a}(k) \end{aligned}$$

and

$$\begin{aligned} |F_{\rm d}(j)|\cos\varphi_{\rm d}(j) = {}& |F_{\rm d}(k)|\cos\varphi_{\rm d}(k) + |F_{\rm a}(j)|\cos\varphi_{\rm a}(j) \\ & - |F_{\rm a}(k)|\cos\varphi_{\rm a}(k) \end{aligned}$$

are obtained. Then

$$\begin{aligned} \tan\varphi_{\rm d}(j) &= \frac{|F_{\rm d}(k)|\sin\varphi_{\rm d}(k) + |F_{\rm a}(j)|\sin\varphi_{\rm a}(j) - |F_{\rm a}(k)|\sin\varphi_{\rm a}(k)}{|F_{\rm d}(k)|\cos\varphi_{\rm d}(k) + |F_{\rm a}(j)|\cos\varphi_{\rm a}(j) - |F_{\rm a}(k)|\cos\varphi_{\rm a}(k)} \\ &= \frac{T}{B} \end{aligned} \tag{23}$$

and

$$|F_{\rm d}(j)| = (T^2 + B^2)^{1/2}.$$

It is easy to see that if $F_{\rm d}(k)$ has been defined in modulus and phase (eventually by choosing between $F_{\rm d1}$ and $F_{\rm d2}$ if the reflection belongs to {P}), then $F_{\rm d}(j)$ is unequivocally fixed in modulus and phase, given the prior knowledge of $\varphi_{\rm a}(j)$. The conclusion is not strange, even if the ancil structures k and j are uncorrelated. Indeed, fixing $F_{\rm d}(k)$ is sufficient to define F in modulus and phase *via* equation (11): on its turn, once F has been fixed, $F_{\rm d}(j)$ may be estimated *via* the same equation (11).

In order to give a simple description of the constraints we consider n centric ancil structures, crystallizing in a space group which is a centric supergroup of the target space group (*e.g.* in $P2/m$ if the target group is $P2$). In Fig. 7, for the same reflection (*hkl*), we show the diagrams for three derivatives: $|F_a| > |F|$ for the first two derivatives, $|F_a| < |F|$ for the third one. In all the three cases φ_a may only take values 0 or π, while both F_d and F will show the symmetry of the target space group: we will assume that in the figures φ_d, φ_a and φ take their true values. Since F does not change amplitude and orientation by changing derivative, in all the three diagrams $|F_a|$ may vary, but the three circles have the same radius $|F|$, and the vector F maintains the same orientation. Accordingly, if in Fig. 7(*a*) the orientation of F corresponds to F_2, in the second diagram it must correspond to F_1: this implies a strong constraint on the choice between F_1 and F_2 when two or more derivatives are used.

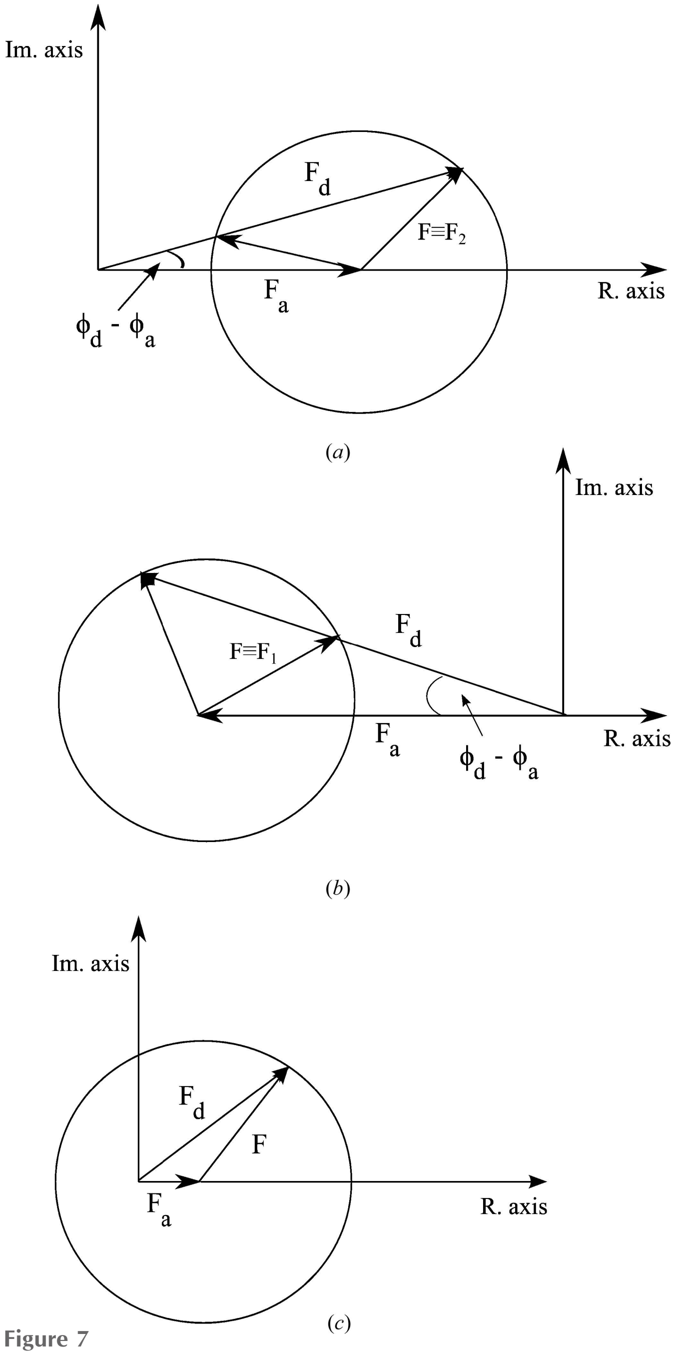

Figure 7
(*a*), (*b*) The case $|F_a| > |F|$ is illustrated for $\varphi_a = 0$ and $\varphi_a = \pi$, respectively. (*c*) The case $|F_a| < |F|$ is illustrated when $\varphi_a = 0°$. In all the figures the letter F indicates the true target structure factor.

In the three Figs. 7(*a*), 7(*b*), 7(*c*), for the chosen reflection (*hkl*), the condition characterizing the correct enantiomorph is the positive value of $\sin\varphi_d$ (see Appendix *C*) but it may be easily understood that for another reflection $\sin\varphi_d < 0$ may characterize the correct enantiomorph for the three derivatives. In more explicit words, if for any chosen (*hkl*) reflection the three EDM procedures, independently applied to the three derivatives, end with three phases φ_d for which $\sin\varphi_d > 0$, then the derivatives coherently define a phase corresponding to the correct enantiomorph or coherently indicate a phase corresponding to the wrong enantiomorph: one is not allowed to decide which of the two alternatives occurs because φ is unknown. If the three EDM processes end with phases φ_d for which the values of $\sin\varphi_d$ do not have the same sign, then it may be concluded that they provide contrasting indications for the enantiomorph. $\sin\varphi_d$ is therefore a coherence criterion not a tool for recognizing if the phases correspond to the correct enantiomorph.

The above considerations, suitably modified, may be extended to the case in which the ancil structures show the same symmetry as the target structures. In Appendix *C* we found a practical criterion for deciding if two derivatives coherently define phase values corresponding to the same (correct or wrong) enantiomorph for a given reflection (*hkl*): if $\mathrm{EN} = \sin(\varphi_d - \varphi_a)\sin\varphi_a$ has the same sign for both the derivatives, it is assumed that the phases are coherently referred to the same enantiomorph. In order to check and improve the coherence of the phase indications provided by different derivatives a statistical algorithm has been described in Appendix *D*.

6. About the role of $R_{\rm dest}$ and $R_{\rm dinv}$ in the PhD phasing procedure

Let us consider the following conditional distribution function, obtained by Giacovazzo & Siliqi (2002) for the SIR case:

$$P(\varphi_{\rm P}|R_{\rm P}, R_{\rm d}, R_{\rm H}, \varphi_{\rm H}) \simeq [2\pi I_0(G)]^{-1}\exp[G\cos(\varphi_{\rm P} - \varphi_{\rm H})], \tag{24}$$

where $\varphi_{\rm P}$ is the phase of the native protein, and

$$G = \frac{2(|F_{\rm d}| - |F_{\rm P}|)|F_{\rm H}|}{|\mu_{\rm sir}|^2} = \frac{2(R_{\rm d} - R_{\rm P})R_{\rm H}}{\sigma^2_{\rm sir}} = \frac{2(\Delta_{\rm iso})_{\rm norm}R_{\rm H}}{\sigma^2_{\rm sir}}. \tag{25}$$

In accordance with the standard notation for SIR–MIR techniques, the subscripts P, H and d denote native, heavy-atom and derivative structure, respectively. $R_{\rm P}$ and $R_{\rm d}$ are observed amplitudes both normalized with respect to the native protein scattering power [*i.e.* $R_{\rm P} = |F_{\rm P}|/(\sum_{\rm P})^{1/2}$ and $R_{\rm d} = |F_{\rm d}|/(\sum_{\rm P})^{1/2}$, where $\sum_{\rm P} = \sum_{j=1}^{N_{\rm P}} f_j^2$, and $N_{\rm P}$ is the number of atoms in the native protein unit cell]. $R_{\rm H}$ and $\varphi_{\rm H}$ are calculated amplitude and phase of the heavy-atom substructure, respectively, with the amplitude normalized with respect to the native protein scattering power [*i.e.* $R_{\rm H} = |F_{\rm H}|/(\sum_{\rm P})^{1/2}$].

$(\Delta_{\rm iso})_{\rm norm}$ is the normalized version of the classical $\Delta_{\rm iso} = (|F_{\rm d}| - |F_{\rm P}|)$ coefficient. $\sigma^2_{\rm sir}$ is the normalized variance connected to the global error on the derivative, defined by $\sigma^2_{\rm sir} = \langle |\mu_{\rm sir}|^2 \rangle / \sum_{\rm P}$, where $|\mu_{\rm sir}| \exp(i\varsigma_{\rm sir})$ represents the cumulative error, the components of which are a lack of isomorphism, errors in measurements and errors in the heavy-atom substructure.

The reliability of the $\varphi_{\rm P}$ estimate is ruled by the concentration parameter G: if $R_{\rm d} > R_{\rm P}$ then $G > 0$ and $\varphi_{\rm P}$ is expected to be close to $\varphi_{\rm H}$; if $R_{\rm d} < R_{\rm P}$ then $G < 0$ and $\varphi_{\rm P}$ is expected to be close to $\varphi_{\rm H} + \pi$. In both cases the phase indication is reinforced if $R_{\rm H}$ is large. According to the above definitions the following relations are expected: $\langle R^2_{\rm P} \rangle = 1$, $\langle R^2_{\rm d} \rangle > 1$ but very close to unity, $\langle R^2_{\rm H} \rangle$ much closer to 0 than to unity: that is due to the small ratio $(\sum_{\rm H})/(\sum_{\rm P})$. The large reliability of the estimate (24) relies therefore on the small value of $\sigma^2_{\rm sir}$, which is much smaller than unity for usual isomorphous derivatives, where it is expected to be in the range 0.05–0.15 (indeed $|\mu_{\rm sir}|^2$ is usually a small fraction of $\sum_{\rm P}$).

A detail which will have some relevance in the next considerations is the following: since $\langle |F_{\rm d}| \rangle > \langle |F_{\rm P}| \rangle$ by definition, the number of reflections for which the relation $\varphi_{\rm P} \simeq \varphi_{\rm H}$ is expected to hold is a bit larger than the number for which $\varphi_{\rm P} \simeq \varphi_{\rm H} + \pi$.

The main question of this section is: how may it be possible to exploit relations similar to (24) in a PhD procedure, where $R_{\rm d}$ is not measurable? In the starting PhD step the substitute of $R_{\rm d}$, say its statistical estimate $R_{\rm dest}$ given by equation (15), relies on a basic assumption: that φ is uniformly distributed on the trigonometric circle, and that it is statistically independent of $\varphi_{\rm a}$. That is in perfect agreement with the random generation of the ancil structures. On the basis of the above considerations one might conclude that the prior knowledge of $\varphi_{\rm a}$ cannot provide any information on φ. The following remarks suggest a possible different conclusion:

(i) While in SIR–MIR, for a given reflection (*hkl*), the difference between the amplitudes $|F_{\rm d}|$ and $|F|$ is expected to be small (indeed isomorphism and good experimental data are basic conditions for SIR–MIR success), in PhD the average difference between $|F_{\rm d}|$ and $|F|$ is much larger (because of the large scattering power of the ancil structure). Also statistically large is the difference between $|F_{\rm d}|$ and $|F_{\rm a}|$. These conditions permit that the error with which $|F_{\rm dest}|$ approximates $|F_{\rm d}|$ is, in favourable conditions, not too critical for phasing.

(ii) In some conditions $R_{\rm dest}$, as provided by equation (15), may be a good approximation of $R_{\rm d}$. Let us consider three asymptotic examples.

If $F = F_{\rm a}$ then $|F_{\rm dest}| = 2^{1/2}|F_{\rm a}| = 2^{1/2}|F|$, from which $R = R_{\rm a}$ and $R_{\rm dest} = R = R_{\rm a}$. In this case the maximum and minimum values of $R_{\rm d}$ are $2^{1/2}R$ and 0, respectively. This is the situation in which the relation $R_{\rm dest} \simeq R_{\rm d}$ may be severely violated. Owing to the random distribution of φ around $\varphi_{\rm a}$, $R_{\rm d}$ may take any value in the interval $(0,\ 2^{1/2}R)$ with equal probability. Such an interval is too large to allow $R_{\rm dest}$ to be a good approximation of $R_{\rm d}$.

If $R = SR_{\rm a}$ with S sufficiently larger than unity, then $R_{\rm dest} = R_{\rm a}[(1+S^2)/2]^{1/2}$ and the maximum and minimum values of $R_{\rm d}$ are $R_{\rm a}[(S+1)/2^{1/2}]$ and $R_{\rm a}[(S-1)/2^{1/2}]$, respectively. Again, owing to the random distribution of φ around $\varphi_{\rm a}$, $R_{\rm d}$ may take any value in the interval $\{R_{\rm a}[(S+1)/2^{1/2}], R_{\rm a}[(S-1)/2^{1/2}]\}$ with the same probability, but this time the amplitude of the allowed interval, say $2^{1/2}R_{\rm a}$, is small if $R_{\rm a}$ is small. In conclusion, for reflections for which $R \gg R_{\rm a}$, $R_{\rm dest} = R_{\rm a}[(1+S^2)/2]^{1/2} = [(R^2_{\rm a} + R^2)/2]^{1/2}$ is a useful approximation of $R_{\rm d}$.

A similar situation occurs when $R_{\rm a} = SR$ with S sufficiently larger than unity. Then $R_{\rm dest} = R[(1+S^2)/2]^{1/2}$ with $R[(S+1)/2^{1/2}]$ and $R[(S-1)/2^{1/2}]$ as maximum and minimum values, respectively. Since the interval $\{R[(S+1)/2^{1/2}], R[(S-1)/2^{1/2}]\}$ is small if R is small, $R_{\rm dest} = [(R^2_{\rm a} + R^2)/2]^{1/2}$ may be a useful approximation of $R_{\rm d}$ for reflections for which $R_{\rm a} \gg R$.

(iii) In the initial PhD step one is obliged to assume that φ and $\varphi_{\rm a}$ are uncorrelated: then from (13) relationship (15) arises. If some information is gained on $\varphi_{\rm d}$ then $R_{\rm dest}$ may be obtained from equation (17): if $\varphi_{\rm d}$ is sufficiently accurate, then also $R_{\rm dest}$ will gain in accuracy, particularly when $R > R_{\rm a}$.

The above considerations allow, in favourable situations, the use of $R_{\rm dest}$ as representative of $R_{\rm d}$ in probabilistic formulas estimating φ or $\varphi_{\rm d}$, and therefore open the door for crystal structure solution. Furthermore, the same considerations suggest that the variance, connected to the current $R_{\rm dest}$ estimate and therefore variable with the PhD step, has to be taken into account when probabilistic formulas, described in §7, are applied.

In sections §§8–10 the reader will see that EDM modification techniques play a central role in the PhD approach. By inversion of the last electron-density map the parameters $\varphi_{\rm dinv}$ and $R_{\rm dinv}$ are provided: $\varphi_{\rm dinv}$ may be considered the current best estimate of $\varphi_{\rm d}$, $R_{\rm dinv}$ may be employed as a supplementary parameter for supporting $\varphi_{\rm dinv}$. For example, if the EDM cycles drive the derivative phase to $\varphi_{\rm dinv}$, a large value of $R_{\rm dinv}$ may be considered a support to the relation $\varphi_{\rm d} \simeq \varphi_{\rm dinv}$; if $R_{\rm dinv}$ is too small the phase indication cannot be considered sufficiently reliable. Both $R_{\rm dinv}$ and $\varphi_{\rm dinv}$ may work together with $R_{\rm dest}$ to select the reflections for which non-vanishing phase estimates may be obtained.

7. Reciprocal-space tools for estimating derivative and target phases

In §6 we recalled the basic probabilistic formula suggested by Giacovazzo & Siliqi (2002) for estimating, by SIR techniques, the native protein phase $\varphi_{\rm P}$ given $R_{\rm P}$, $R_{\rm d}$, $R_{\rm H}$ and $\varphi_{\rm H}$. Such a formula cannot be applied as it is to PhD. To find the most suitable phasing tools we should consider the distributions derived in Appendix *B*, and investigate their practical usefulness for the PhD approach. We will divide this section into five subsections, according to the main five phase relationships suggested in Appendix *B*.

Every conditional phase distribution will show a variance term, say $\sigma^2_{\rm PhD}$, which depends on the specific probabilistic formula, on the specific reflection, and on the PhD step in which the phase is estimated. The considerations described in Appendix *B* suggest that $\sigma^2_{\rm PhD}$ may attain unity and more in the first steps of a PhD procedure, and that will severely deplete the reliability of the estimates. In more advanced PhD steps the variance is expected to diminish, and therefore the accuracy of the phase estimates will improve.

7.1. The *G* reliability parameter

Equations (24) and (25) may be adapted to PhD by letting $F_{\rm a}$ and F play the role of $F_{\rm H}$ and $F_{\rm P}$, respectively. Furthermore $\sigma^2_{\rm sir}$ has to be numerically reconsidered because the conditions under which it was estimated for SIR–MIR do not hold in PhD. The calculations reported in Appendix *B* indicate that, under the hypothesis $\varphi_{\rm d} \simeq \varphi$, equations (24) and (25) should be substituted by

$$P(\varphi|R, R_{\rm dest}, R_{\rm a}, \varphi_{\rm a}) \simeq [2\pi I_0(G)]^{-1} \exp[G\cos(\varphi - \varphi_{\rm a})] \quad (26)$$

and

$$G = \frac{(|F_{\rm dest}| - |F|)|F_{\rm a}|}{\langle|\mu_{\rm PhD}|^2\rangle} = \frac{(2^{1/2}R_{\rm dest} - R)R_{\rm a}}{\sigma^2_{\rm PhD}}, \quad (27)$$

respectively. Distribution (26) suggests that if $R_{\rm dest} > R/2^{1/2}$ then φ is expected to be close to $\varphi_{\rm a}$; if $R_{\rm dest} < R/2^{1/2}$ then φ is expected to be close to $\varphi_{\rm a} + \pi$. The phase indication is stronger when $R_{\rm a}$ is sufficiently large.

Owing to the basic hypothesis $\varphi_{\rm d} \simeq \varphi_{\rm a}$ the distribution

$$P(\varphi|R, R_{\rm dest}, R_{\rm a}, \varphi_{\rm d}) \simeq [2\pi I_0(G)]^{-1} \exp[G\cos(\varphi - \varphi_{\rm d})] \quad (26')$$

is equally supported, sharing with (26) the same reliability *G* factor. Accordingly, if $R_{\rm dest} > R/2^{1/2}$, then φ is expected to be close to $\varphi_{\rm d}$; if $R_{\rm dest} < R/2^{1/2}$ then φ is expected to be close to $\varphi_{\rm d} + \pi$. Again, the phase indication is stronger when $R_{\rm a}$ is sufficiently large.

Equation (26) is more appealing than (26′) if one considers the fact that $\varphi_{\rm a}$ is precisely known by hypothesis, while $\varphi_{\rm d}$ is only estimated. On the other hand, φ is always closer to $\varphi_{\rm d}$ than to $\varphi_{\rm a}$ by geometrical reasons (essentially because $F = F_{\rm d} - F_{\rm a}$; see Figs. 2 or 4). Thus which of (26) or (26′) should be preferred depends on the accuracy with which $\varphi_{\rm d}$ is estimated: the accuracy varies with the specific reflection and with the phasing step.

The efficiency of distribution (26) depends on the formula by which $R_{\rm dest}$ is calculated and therefore on $\sigma^2_{\rm PhD}$ (see Appendix *B*): improving $R_{\rm dest}$ diminishes $\sigma^2_{\rm PhD}$ and therefore improves the φ estimate. Let us now guess about the types of reflections to which (26) or (26′) may be usefully applied. The condition $\varphi_{\rm d} \simeq \varphi$ systematically occurs when $R \gg R_{\rm a}$: in this case (see §6) $R_{\rm d} \simeq R/2^{1/2}$ and (26) and (26′) do not provide reliable phase indications for φ.

In the initial PhD step, where $R_{\rm dest}$ is estimated *via* equation (15), the probability that the condition $2^{1/2}R_{\rm d} > R$ is satisfied is larger if $R_{\rm a} > R$ (see §6): the subcase $R_{\rm a} \gg R$ should however not be useful because then the relation $\varphi_{\rm d} \simeq \varphi$ is no longer obeyed. Thus (26) and (26′) may be usefully applied to reflections for which $R_{\rm a} > R$ but $R_{\rm a}/R$ is not too big, otherwise the basic hypothesis $\varphi \simeq \varphi_{\rm d}$ is no longer valid.

If $R > R_{\rm a}$, the probability of the relation $\varphi \simeq \varphi_{\rm d}$ is higher than in the preceding cases, but the phase indications ($\varphi \simeq \varphi_{\rm a}$ or $\varphi \simeq \varphi_{\rm a} + \pi$) will have small reliability because $R_{\rm d} \simeq R/2^{1/2}$ (see §6). Then *G* is expected to be small because, on average, both the differences $|2^{1/2}R_{\rm d} - R|$ and $R_{\rm a}$ will be small.

7.2. The S_I reliability parameter

The distribution (26) relies on a basic assumption, say $\varphi \simeq \varphi_{\rm d}$. It has been underlined that this condition is often not fulfilled in the PhD approach, because we assume that target and ancil structures have the same scattering power. If one assumes that target and derivative phases are not correlated, equations (74)–(76) may be obtained and fully transferred in a useful form as follows:

$$P(\varphi|R, R_{\rm dest}, R_{\rm a}, \varphi_{\rm a}) \simeq [2\pi I_0(S_I)]^{-1} \exp[S_I\cos(\varphi - \varphi_{\rm a})], \quad (28)$$

where

$$S_I = L - \frac{RR_{\rm a}}{\sigma^2_{\rm PhD}} \quad (29)$$

and *L* is defined by

$$D_1(L) = D_1\left(\frac{2^{1/2}R_{\rm dest}R}{\sigma^2_{\rm PhD}}\right) D_1\left(\frac{2^{1/2}R_{\rm dest}R_{\rm a}}{\sigma^2_{\rm PhD}}\right). \quad (30a)$$

Equation (28) is more general than (26), and suggests that, if $S_I > 0$, then the relation $\varphi \simeq \varphi_{\rm a}$ is supported, if $S_I < 0$, then φ is expected to be close to $\varphi_{\rm a} + \pi$. As should be expected, (28) and (26) do not coincide. Indeed if (26) is used, only the difference $(2^{1/2}R_{\rm dest} - R)$ establishes whether the most probable value of φ is $\varphi_{\rm a}$ or $\varphi_{\rm a} + \pi$. According to (28) the phase indication depends on a more intricate relation between R, $R_{\rm a}$ and $R_{\rm dest}$: at odds with equation (26), equation (28) is symmetric with respect to R and $R_{\rm a}$, and is highly sensitive to the $\sigma^2_{\rm PhD}$ value, in the sense that, in some cases, for given values of R, $R_{\rm a}$ and $R_{\rm dest}$, distribution (28) may estimate φ close to $\varphi_{\rm a}$ or to $\varphi_{\rm a} + \pi$ according to the chosen $\sigma^2_{\rm PhD}$ value. That is mainly due to the non-linear trend of the modified Bessel functions.

A simple example showing the difference between (26) and (28) is shown below. Let us suppose that both R and $R_{\rm a}$ are small, so that $R_{\rm dest}$ is sufficiently small to make both the terms $D_1[(2^{1/2}R_{\rm dest}R)/\sigma^2_{\rm PhD}]$ and $D_1[(2^{1/2}R_{\rm dest}R_{\rm a})/\sigma^2_{\rm PhD}]$ small. Then the approximation $D_1(x) \simeq x/2$ may be used, from which $L \simeq [(RR_{\rm a}R^2_{\rm dest})/\sigma^4_{\rm PhD}]$ and

$$S_I = \frac{RR_{\rm a}}{\sigma^2_{\rm PhD}}\left(\frac{R^2_{\rm dest}}{\sigma^2_{\rm PhD}} - 1\right). \quad (30b)$$

While in (26) the positivity of the reliability parameter *G* only depends on the difference $(2^{1/2}R_{\rm dest} - R)$, the positivity of S_I when both R and $R_{\rm a}$ are small depends on the difference $(R^2_{\rm dest}/\sigma^2_{\rm PhD} - 1)$.

Let us now guess about the type of reflections to which (28) may be usefully applied. If both R and $R_{\rm a}$ are sufficiently small the definition of S_I given in (30*b*) is valid: S_I is expected to be negative, the relation $\varphi \simeq \varphi_a + \pi$ is suggested but its reliability is small.

If both R and R_a are sufficiently large then S_I is expected to be negative. Let us suppose in addition that R and R_a have comparable amplitudes: then $R_{\rm dest}$ is smaller than R and R_a and S_I is expected to be negative with larger reliability. Accordingly, the relation $\varphi \simeq \varphi_a + \pi$ is expected to hold for reflections for which R and R_a are large and with similar amplitudes. In this case however φ and φ_a are correlated, against the founding hypothesis. That weakens the predictability of the concentration parameter (29).

If R is large and R_a is small then $R_{\rm dest} \simeq R_d \simeq R$. In this case the two components of S_I on the right-hand side of (29) have comparable size and consequently S_I is expected to be small. For such reflections, as well as for the reflection for which R is small and R_a is large [R and R_a play a symmetric role in (29)], weak phase indications are obtained.

7.3. The S_U reliability parameter

In Appendix *B* a third conditional phase relationship has been derived [see equation (80)] allowing one to obtain target phase estimates when the information on φ_a does not contribute to the φ determination. It may be written down in a form useful for a practical PhD procedure as follows:

$$P(\varphi|R, R_{\rm dest}, R_a, \varphi_{\rm dest}) \simeq [2\pi I_0(S_U)]^{-1} \exp[S_U \cos(\varphi - \varphi_{\rm dest})], \tag{31}$$

where

$$S_U = \frac{2^{1/2} R R_{\rm dest}}{\sigma^2_{\rm PhD}} - L_U \tag{32a}$$

and

$$D_1(L_U) = D_1\left(\frac{2^{1/2} R_a R_{\rm dest}}{\sigma^2_{\rm PhD}}\right) D_1\left(\frac{R_a R}{\sigma^2_{\rm PhD}}\right). \tag{33}$$

$\varphi_{\rm dest}$ may coincide with $\varphi_{\rm dinv}$ as obtained by Fourier inversion at the end of some EDM cycles or by any other better estimate (see §§8–10).

The distribution (31) suggests that $\varphi \simeq \varphi_{\rm dest}$ if S_U is positive and sufficiently large: that occurs when R and $R_{\rm dest}$ are sufficiently large and R_a is sufficiently small (we are therefore dealing with {U} reflections).

If R and $R_{\rm dest}$ are small then the two components of S_U on the right-hand side of (32*a*) are in conflict and the reliability of the phase indication is vanishing. Also the case in which $R_a > R$ cannot be safely treated by equation (31) because φ_a is statistically correlated with φ, and therefore φ contributes to fix its value against the hypothesis.

Let us now guess about the type of reflections to which (31) may be usefully applied.

If $R \gg R_a$ then the φ_a value does not influence the φ estimate. In this case $R_{\rm dest} \simeq R_d \simeq R$ (see §6) and S_U is strongly positive. The relation $\varphi \simeq \varphi_d$ is then expected to hold even if with a relatively small accuracy.

The subcase $R_a \simeq 0$ deserves a special mention because R may freely vary. The approximation $D_1(x) \simeq x/2$ is perfectly valid and (32*a*) reduces to

$$S_U = \frac{2^{1/2} R R_{\rm dest}}{\sigma^2_{\rm PhD}}\left(1 - \frac{R_a^2}{\sigma^2_{\rm PhD}}\right), \tag{32b}$$

which suggests that $\varphi \simeq \varphi_{\rm dest}$ is highly reliable when R is sufficiently large.

The main problem for distribution (31) does not depend on how $R_{\rm dest}$ approximates R_d (the approximation is quite good when $R \gg R_a$) but on how $\varphi_{\rm dest}$ approximates φ_d. If the approximation is good then the φ estimate is certainly accurate.

Distribution (31) has a dynamic character: any accuracy gain in the $\varphi_{\rm dest}$ estimate is immediately transferred in a larger accuracy of the φ estimate.

The less extreme case in which $R > R_a$ but R/R_a is not too large can also be treated by distribution (31), but now $\sigma^2_{\rm PhD}$ is larger and simultaneously the founding hypothesis (according to which φ_a does not contribute to define φ) is less well satisfied. Better estimates of $\varphi_{\rm dest}$ contribute to make this case more useful.

7.4. The S_d reliability parameter

In Appendix *B* the conditional distribution $P(\varphi_d|R, R_d, R_a, \varphi_a)$ was obtained [see equations (83)–(87)]. In order to make it useful for PhD, we replace R_d by $R_{\rm dest}$ and we obtain

$$P(\varphi_d|R, R_{\rm dest}, R_a, \varphi_a) \simeq [2\pi I_0(S_d)]^{-1} \exp[S_d \cos(\varphi_d - \varphi_a)], \tag{34}$$

where

$$S_d = \frac{2^{1/2} R_{\rm dest} R_a}{\sigma^2_{\rm PhD}} + L_d \tag{35a}$$

and L is defined by

$$D_1(L_d) = D_1\left(\frac{2^{1/2} R R_{\rm dest}}{\sigma^2_{\rm PhD}}\right) D_1\left(\frac{2 R R_a}{\sigma^2_{\rm PhD}}\right). \tag{36}$$

According to distribution (34) S_d is always positive no matter the values of R, R_a and $R_{\rm dest}$, and therefore the relation $\varphi_d \simeq \varphi_a$ is always expected. That may also be deduced by using in equation (36) the relation $D_1(x) \simeq x/2$, from which

$$S_d = \frac{R_{\rm dest} R_a}{\sigma^2_{\rm PhD}}\left(2^{1/2} + \frac{R^2}{\sigma^2_{\rm PhD}}\right). \tag{35b}$$

In accordance with the above indications we assume $\varphi_d \simeq \varphi_a$ at the beginning of the phasing procedure, when the phases of the reflections belonging to the set {P} may be used to calculate the first derivative electron-density map (see §8).

It may also be noticed that while R, $R_{\rm dest}$ and R_a are all present in equation (36), only R_a and $R_{\rm dest}$ appear in the first term of the S_d expression. Thus high values of R_a reinforce the relation $\varphi_d \simeq \varphi_a$, small values of R_a make the expectation weaker. This behaviour gives a probabilistic basis to the algebraic considerations described in §2, where we divided the observed reflections into the subsets {P}, {I} and {U} according to the ratio $|F_{\rm a}|/|F|$.

7.5. Phasing in more advanced steps of the PhD phasing process

Let us suppose that, at a given step of the PhD procedure, $|F_{\rm dest}|$ and $\varphi_{\rm dest}$ are sufficiently accurate estimates of $|F_{\rm d}|$ and $\varphi_{\rm d}$, respectively (we will see in the next sections that this result cannot be attained without extensive use of MPhD). Then, from equation (11) the following relations arise:

$$|F|\cos\varphi \simeq |F_{\rm dest}|\cos\varphi_{\rm dest} - |F_{\rm a}|\cos\varphi_{\rm a}$$

and

$$|F|\sin\varphi \simeq |F_{\rm dest}|\sin\varphi_{\rm dest} - |F_{\rm a}|\sin\varphi_{\rm a},$$

from which

$$\tan\varphi \simeq \frac{|F_{\rm dest}|\sin\varphi_{\rm dest} - |F_{\rm a}|\sin\varphi_{\rm a}}{|F_{\rm dest}|\cos\varphi_{\rm dest} - |F_{\rm a}|\cos\varphi_{a}}. \qquad (37)$$

Equation (37) is not suitable for the initial PhD step because it is based on the simultaneous accurate estimate of $|F_{\rm dest}|$ and of $\varphi_{\rm dest}$: it may be very useful, however, in the late PhD steps for refining the current phase estimates.

8. PhD real-space tools

In §4 we analysed the expected behaviour of a standard EDM procedure, when involved in the improvement of an initial derivative electron-density map calculated by using amplitudes (15) and phases $\varphi_{\rm a}$ of the {P} reflections: *i.e.* by using the Fourier coefficients

$$(|F|^2 + |F_{\rm a}|^2)^{1/2}\exp(i\varphi_{\rm a}). \qquad (38)$$

The relation $\varphi_{\rm d} \simeq \varphi_{\rm a}$ is well obeyed for {P} reflections; also the amplitude $(|F|^2 + |F_{\rm a}|^2)^{1/2}$ is not too rough an approximation of the true $|F_{\rm d}|$ values. Indeed for most of the {P} reflections it is $|F_{\rm d}| \simeq |F_{\rm a}|$ because of the weak contribution of the $|F|$'s to the coefficients (38). However, if coefficients (38) are used, negligible information on the target structure is transferred to the EDM procedure, which therefore is expected to be unable to drive the ancil towards the derivative phases. The procedure is indeed dominated by the ancil and EDM cycles are expected to provide a distorted ancil structure rather than a useful derivative model.

In the first MPhD steps a hybrid electron density may be alternatively calculated: for each jth derivative the phases $\varphi_{\rm a}(j)$ of the reflections for which $|F_{\rm a}(j)| > K|F|$ with K between 1 and 2 may be used, but now target amplitudes are associated to them. In other words, Fourier coefficients

$$|F|\exp[i\varphi_{\rm a}(j)] \qquad (39)$$

are employed, in order to respect the hybrid nature of the derivative. The coefficients (39) are now no longer completely dominated by the jth ancil: indeed, the amplitudes $|F|$ are correlated with the interatomic vector distribution of the target structure, and may be more suitable for driving the ancil phases closer to the target, and therefore closer to the derivative phases.

It should be clear that we do not expect EDM procedures, applied to initial density maps calculated *via* Fourier coefficients (39), to completely reveal the true derivative or the target electron-density map. Indeed, even if $\varphi_{\rm a}(j)$ is a good approximation of $\varphi_{\rm d}(j)$ for any j (see Fig. 3), the number of {P} reflections is a small percentage of the number of observed reflections (see Fig. 1); furthermore, by definition, the {P} set is characterized by a large percentage of small $|F|$ amplitudes. The final electron-density map, based on such a hybrid EDM procedure, is however expected to be capable of estimating $\varphi_{\rm d}(j)$ phases with an accuracy better than that of standard EDM procedures. This is certainly an important advantage for some probabilistic formulas described in §7, which estimate φ from the current $\varphi_{\rm d}(j)$ values.

There is an important restraint which may be used when the hybrid or the usual EDM procedure is applied [see equation (18)]. In every EDM cyle and for each jth derivative the inequality

$$|\sin[\varphi_{\rm d}(j) - \varphi_{\rm a}(j)]| < \frac{|F|}{|F_{\rm a}(j)|}$$

should be respected by any reflection with $|F_{\rm a}(j)| > |F|$. That avoids too large deviations of the derivative from the ancil phases.

Let us now suppose that, for each derivative, sufficiently good $\varphi_{\rm d}(j)$ estimates become available (no matter the source of information): then derivative electron-density maps may be calculated *via* Fourier coefficients

$$|F_{\rm dest}(j)|\exp[i\varphi_{\rm d}(j)],$$

where $|F_{\rm dest}(j)|$ may be obtained by equation (15), or by equation (16) if $\varphi_{\rm d}(j)$ is known with sufficient accuracy, or also it may be replaced by $|F|$ as suggested by equation (39). The corresponding density map is denoted by $\rho_{\rm dest}(j)$. It may be approximately considered as the sum of the well known ancil structure $\rho_{\rm a}(j)$ with $\rho_{\rm est}(j)$, a distorted model electron density of the target structure contained in the jth derivative density map but still not recognizable:

$$\rho_{\rm dest}(j) = \rho_{\rm est}(j) + \rho_{\rm a}(j).$$

Let us now consider the sum

$$\rho_{\rm dsum} = \sum_{j=1}^{n}\rho_{\rm dest}(j) = \sum_{j=1}^{n}\rho_{\rm est}(j) + \sum_{j=1}^{n}\rho_{\rm a}(j). \qquad (40a)$$

The $\rho_{\rm a}(j)$ maps are randomly created and therefore their sum in equation (40*a*) is not expected to provide serious overlapping of the ancil electron densities: it will be more a background of the $\rho_{\rm dsum}$ map rather than a useful signal. On the contrary, if each $\rho_{\rm dest}(j)$ map contains some structural characteristics of the real target map, the electron density corresponding to a given target structural fragment may be present in more derivatives, the density of another fragment may survive in other derivative maps *etc.* It is therefore expected that $\rho_{\rm dsum}$ may emphasize the real target features, while the sum of the ancil structural features will generate the background of the $\rho_{\rm dsum}$ map. In a statistical sense, the contrast (target density)/(background density) will increase with n: since n may be (at least in *ab initio* approaches) in the interval 100–300, $\rho_{\rm dsum}$ may provide a target model much better than that hidden in any single $\rho_{\rm est}(j)$'s.

Equation (40*a*) may also be rewritten as

$$\sum_{j=1}^{n} \rho_{\rm est}(j) = \sum_{j=1}^{n} \rho_{\rm dest}(j) - \sum_{j=1}^{n} \rho_{\rm a}(j) = \sum_{j=1}^{n} [\rho_{\rm dest}(j) - \rho_{\rm a}(j)]. \quad (40b)$$

According to equation (40*b*) each difference Fourier synthesis provides a model of the target electron density: summing the n models, as suggested by equation (40*b*), would emphasize the real structural features of the target and therefore increase the accuracy of the resulting target model.

Equations (40*a*) and (40*b*) are equivalent from a mathematical point of view but probably not in practice. Indeed, according to equation (40*a*), the model is estimated *via* the sum of electron densities which are supposed to be positive; furthermore, the efficiency of $\rho_{\rm dsum}$ relies on the necessary correlation between the derivative and the target electron density. According to equation (40*b*) the target density is estimated *via* n difference electron densities which are supposed to be negative in wide regions of the unit cell. Refining by EDM techniques the two sum maps requires different types of density modification and therefore the two procedures may end with different accuracies.

To better understand the usefulness of the relations (40*a*) and (40*b*), let us consider the Fourier transform of equation (40*b*):

$$\sum_{j=1}^{n} F_{\rm est}(j) = \sum_{j=1}^{n} F_{\rm dest}(j) - \sum_{j=1}^{n} F_{\rm a}(j),$$

where $F_{\rm dest}(j)$ is the current derivative structure-factor estimate available at a given PhD step. If $F_{\rm est}(j) = F_{\rm dest}(j) - F_{\rm a}(j)$ is the target structure-factor estimate arising from the jth derivative, then

$$\langle F \rangle = \frac{1}{n} \sum_{j=1}^{n} F_{\rm est}(j) = \frac{1}{n} \left[\sum_{j=1}^{n} F_{\rm dest}(j) - \sum_{j=1}^{n} F_{\rm a}(j) \right].$$

$\langle F \rangle$ is the expected value of the target structure factor for a given (hkl) reflection obtained by combining the corresponding n target structure-factor estimates. Owing to the random nature of the ancil structures, if n is sufficiently large, the vectors $F_{\rm a}(j)$ will be uniformly distributed on the trigonometric circle, and their sum is expected to be close to zero. Thus the sum of the n vectors $F_{\rm dest}(j)$ will constitute an estimate of the target structure factor: the reliability of the estimate will increase with n and with the correlation between derivative and target structure.

The above procedure does not have a counterpart in the MIR case.

A final remark is necessary. The use of the function $\rho_{\rm sum}$ is expected to fail if $\rho_{\rm est}(j), j = 1, \ldots, n$, are estimates of ρ for half the derivatives, and estimates of its enantiomorph structure $\overline{\rho}$ for the second half of the derivatives. The question therefore may be more clearly expressed as follows: if the hybrid Fourier synthesis with coefficients (39) is used to approximate the jth derivative electron density, why should the EDM procedure bring the derivative map towards ρ instead of towards $\overline{\rho}$?

In order to suggest a possible solution of the problem, let us consider the Fourier coefficients of the hybrid synthesis. The amplitudes $|F|$ have the same values for the two enantiomorph structures and, therefore, if singly considered, they cannot influence which of the two enantiomorphs will be preferred by the EDM procedure.

Let us now consider the phase component $\varphi_{\rm a}(j)$. A reasonable assumption, suggested by the random creation of the ancil structures, is that for a given jth ancil structure, $\varphi_{\rm a}(j)$ is uniformly distributed around the corresponding φ value, so that the expected value of $|\varphi_{\rm a}(j) - \varphi|$ is $\pi/2$. Obviously $\varphi_{\rm a}(j)$ is expected to be uniformly distributed also around $-\varphi$, so that $\langle|\varphi_{\rm a}(j) + \varphi|\rangle \simeq \pi/2$. Thus, according to the above hypotheses, if only the $\varphi_{\rm a}(j)$'s are considered, there is no theoretical reason for assessing that the EDM procedure would drive the derivative phases towards ρ rather than towards $\bar{\rho}$.

Let us now consider all together $\varphi_{\rm a}(j)$, R and $R_{\rm dest}(j)$, and let us assume that a phase relationship is available establishing that $\varphi \simeq \varphi_{\rm a}(j)$. If $\varphi_{\rm a}(j) = 50^\circ$, the phase relationship will indicate $\varphi \simeq 50^\circ$ while $\varphi \simeq -50^\circ$ is not suggested. The use of more derivatives is expected to reinforce the correct enantiomorph characterization of the target structure, provided the phase relationships have sufficient reliability. In conclusion, if the phase estimates are a bit better than random, the correct enantiomorph ρ will be slightly preferred by the EDM procedure, so that more than half of the n derivatives will suggest φ values closer to the correct than to the false derivative.

9. Phasing Step 1: first target phase estimates and first attempts for overcoming the enantiomorph problem

Let us suppose that n different ancil structures were created with electron density $\rho_{ai}(\mathbf{r})$, $i = 1, \ldots, n$: $|F_{\rm a}(i)|$ and $\varphi_{\rm a}(i)$ are the corresponding calculated amplitudes and phases for a given structure factor. $|F_{\rm d}(i)|$ and $\varphi_{\rm d}(i)$ will be the corresponding true amplitudes and phases for the ith derivative structure factor. The new scenario, after the analysis described in §§4–8, is the following:

(*a*) For the same target structure ($|F|$ and φ will again denote amplitude and phase of the target reflection), n different sets $\{\rm P\}_i$, $\{\rm U\}_i$ and $\{\rm I\}_i$ are available, defined by the following conditions:

Set $\{\rm P\}_i$: $|F_{\rm a}(i)| > S_p|F|$;
Set $\{\rm U\}_i$: $|F| > S_u|F_{\rm a}(i)|$;
Set $\{\rm I\}_i$: includes all the reflections not belonging to $\{\rm P\}_i$ or $\{\rm U\}_i$.

S_p and the S_u values may also be different for different ancils: we will assume for simplicity that S_p and S_u are the same for all the ancils.

(*b*) Since the n ancil structures are supposed to be uncorrelated, the sets of reflections satisfying $|F_{\rm a}(i)| > S_p|F|$ or $|F| > S_u|F_{\rm a}(i)|$ for the ith ancil structure usually do not coincide with the sets of reflections for which $|F_{\rm a}(j)| > S_p|F|$ or $|F| > S_u|F_{\rm a}(j)|$ when $i \neq j$. Thus a given reflection (*hkl*) may belong to $\{{\rm P}\}_i$ for the *i*th derivative, to $\{{\rm U}\}_j$ for the *j*th derivative and to $\{{\rm I}\}_k$ for the *k*th derivative.

(*c*) The derivative phases for reflections for which $|F_{\rm a}(i)| > |F|$ are confined in a range around $\varphi_{{\rm a}i}$, the restraint being very sharp for high values of *S*. The difficult choice between $F_{\rm d1}$ and $F_{\rm d2}$ is a strong source of error for the φ estimate: even if the choice is correctly made, a small error on $\varphi_{\rm d}(i)$ may be responsible for large errors on φ [see §4, equation (20) and its consequences].

Furthermore, EDM procedures applied to derivative data often suggest positive $\sin(\varphi_{\rm d} - \varphi_{\rm a})$ estimates, while the correct values are negative, or *vice versa*. For each (*hkl*) this is the problem of the enantiomorph identification of $\varphi_{\rm d}(i)$ with respect to $\varphi_{\rm a}(i)$ for each *i*th derivative.

In order to break down the enantiomorph ambiguity and complete the full target phasing pathway, three conditions are necessary:

(i) The prior phase information available for each *j*th ancil structure should be safely transferred into derivative phase and amplitude estimates of the corresponding *j*th derivative, $\{{\rm I}\}_j$ and $\{{\rm U}\}_j$ reflections included.

(ii) All the derivative enantiomorphs should progressively converge to the same enantiomorph. As we observed, that requires a preliminary step: the percentage of reflections with derivative phase compatible with one enantiomorph during the EDM process should progressively become larger during the EDM process. That implies the use of derivative phase and amplitudes progressively better than the $\varphi_{\rm a}(j)$'s and amplitudes (15), respectively.

(iii) The gained information on derivative amplitudes and phases is progressively transferred into better φ estimates.

Only if the above conditions are satisfied may the combined use of phantom derivatives lead to the crystal structure solution of the target structure. We will show in this and in the next section how the above conditions may be satisfied. In the next paragraph the very first step of the phasing process is described.

In accordance with the distribution (34) the approximation $\varphi_{\rm d}(j) \simeq \varphi_{\rm a}(j)$ is initially accepted for any *j*th derivative and for any (*hkl*) reflection: thus distributions (26) or (28) may be immediately used for the first target phase estimates.. As described in §7 such an approximation is well satisfied for the reflections belonging to the subset {P}, while it is too rough for {I} or {U} reflections. Then the mixed Fourier synthesis with coefficients defined by equation (39) may be calculated for each *j*th derivative: phase refinement and extension may be performed by EDM cycles. The Fourier inversion of the last derivative electron-density map is expected to provide estimates of the derivative phases [*i.e.* $\varphi_{\rm dest}(j) = \varphi_{\rm dinv}(j)$] better than the initial ones [*i.e.* $\varphi_{\rm dest}(j) = \varphi_{\rm a}(j)$] at least for {I} and {U} reflections.

During each EDM cycle the important restraint (12) is applied to all the reflections with $|F_{\rm a}(j)| > |F|$: if in some EDM cycles $|\sin(\varphi_{\rm dinv}(j) - \varphi_{\rm a}(j))| > |F|/|F_{\rm a}(j)|$ then $\varphi_{\rm dinv}(j)$ is reset so as to satisfy equation (12).

It is now possible, for each (*hkl*) reflection and from each *j*th derivative, to obtain a φ estimate *via* equations (26), (28) or (31), by exploiting the corresponding observed amplitude *R*, the calculated amplitude $R_{\rm a}(j)$, the $R_{\rm dest}(j)$ value calculated *via* equation (15), and the calculated phase angle $\varphi_{\rm dinv}(j)$ obtained at the end of the EDM cycles. Accordingly, a large number of φ estimates may be available for the same φ_{hkl}.

The reader, however, should not conclude that $3n$ φ estimates will be available for each (*hkl*): indeed for the *j*th derivative the chosen (*hkl*) reflection may belong to the subset {P} and therefore may be usefully estimated by equation (26), but not *via* the distribution (31), which is more suitable for estimating the target phase of the {U} reflections. It may also occur that φ_{hkl} cannot be estimated by any of the distributions (26), (28) or (31) with sufficient probability to be included in next calculations.

In spite of the above limitations one might think that this approach overdetermines φ if *n* is sufficiently large: unfortunately the situation is different. Indeed, as described before, the $R_{\rm d}(j)$ are not experimentally available for any *j*, $\sigma^2_{\rm PhD}(j)$ is correspondingly large and therefore all the estimates are expected to be only a little better than random. The estimates may also show a large variability; for some ancil structures the average phase error may be significantly smaller than 90°, and for others significantly larger. Indeed the enantiomorph problem described in §§5 and 6 is a severe obstacle to an accurate target phasing: some derivatives may converge towards $\rho(\mathbf{r})$ and some others towards $\rho(-\mathbf{r})$.

An acceptable φ phasing may only be obtained by combining all the available different estimates: luckily target phase indications originating from different derivatives may be considered statistically independent, because the ancil structures are randomly created. In order to accomplish this task, let us first consider the problem from the MIR point of view. In order to combine the phase indications arising from *n* different (known) heavy-atom substructures and from the measured derivative amplitudes, Giacovazzo & Siliqi (2002) derived the conditional probability distribution

$$P\big(\varphi_{\rm P}|R_{\rm P}, \big\{R_{\rm d}(j), R_{\rm H}(j), \varphi_{\rm H}(j)\big\}\big) \simeq [2\pi I_0(\alpha_{\rm P})]^{-1}\exp[\alpha_{\rm P}\cos(\varphi_{\rm P} - \theta_{\rm P})], \qquad (41)$$

where

$$\tan\theta_{\rm P} = \frac{\sum_{j=1}^{n} G_{\rm P}(j)\sin\varphi_{\rm H}(j)}{\sum_{j=1}^{n} G_{\rm P}(j)\cos\varphi_{\rm H}(j)} = \frac{T_{\rm P}}{B_{\rm P}},$$

$$G_{\rm P}(j) = \frac{2[R_{\rm d}(j) - R_{\rm P}]R_{\rm H}(j)}{\sigma^2_{\rm sir}(j)}$$

and

$$\alpha_{\rm P} = (T_{\rm P}^2 + B_{\rm P}^2)^{1/2}.$$

The necessary conditions for the validity of equation (41) are (see §7): (i) $\varphi_{\rm P} \simeq \varphi_{\rm d}(j), j = 1, \ldots, n$; and (ii) $R_{\rm d}(j), j = 1, \ldots, n$ experimentally known.

Equation (41) cannot be adapted to MPhD without significant limitations. Indeed the condition (i) is only valid for reflections belonging to the $\{\mathrm{U}\}_j$ subsets. Condition (ii) is not verified (R_{d}'s may only be estimated): accordingly $\sigma^2_{\mathrm{PhD}}(j)$ should replace $\sigma^2_{\mathrm{sir}}(j)$. For PhD equation (41) transforms into

$$P\big(\varphi|R, \{R_{\mathrm{dest}}(j), R_{\mathrm{a}}(j), \varphi_{\mathrm{a}}(j)\}\big) \simeq [2\pi I_0(\alpha)]^{-1}\exp[\alpha\cos(\varphi-\theta_{\mathrm{init}})] \tag{42}$$

where

$$\tan\theta_{\mathrm{init}} = \frac{\sum_{j=1}^{n} G(j)\sin\varphi_{\mathrm{a}}(j)}{\sum_{j=1}^{n} G(j)\cos\varphi_{\mathrm{a}}(j)} = \frac{T_G}{B_G}, \tag{43a}$$

$$G_j = \frac{[2^{1/2}R_{\mathrm{dest}}(j)-R]R_{\mathrm{a}}(j)}{\sigma^2_{\mathrm{PhD}}(j)}$$

and

$$\alpha_G = (T_G^2+B_G^2)^{1/2}. \tag{44}$$

θ_{init} is the most probable value for φ and α_G is its reliability parameter. At a first approximation it may be assumed that $\sigma^2_{\mathrm{PhD}}(j)$ does not vary with j, because all the ancils are randomly generated. However, in accordance with §6 σ^2_{PhD} should vary with (*hkl*).

Since the distribution (42) is based on the assumption that $\varphi_{\mathrm{d}}(j) \simeq \varphi$, equation (43*a*) may also be replaced by

$$\tan\theta_{\mathrm{init}} = \frac{\sum_{j=1}^{n} G(j)\sin\varphi_{\mathrm{dest}}(j)}{\sum_{j=1}^{n} G(j)\cos\varphi_{\mathrm{dest}}(j)} = \frac{T_G}{B_G}. \tag{43b}$$

Owing to the fact that the condition $\varphi_{\mathrm{dinv}}(j) \simeq \varphi_{\mathrm{a}}(j)$ is not obeyed for reflections $\{\mathrm{I}\}_j$ or $\{\mathrm{U}\}_j$, equations (42)–(44) cannot be applied to them. To derive probability distributions valid for such a type of reflection distribution (28) should be invoked and suitably modified to fit MPhD conditions. We obtain

$$\tan\theta_{\mathrm{init}} = \frac{\sum_{j=1}^{n} S(j)\sin\varphi_{\mathrm{a}}(j)}{\sum_{j=1}^{n} S(j)\cos\varphi_{\mathrm{a}}(j)} = \frac{T_S}{B_S}, \tag{45}$$

where each $S(j)$ is defined by equation (39). Again

$$\alpha_L = (T_S^2+B_S^2)^{1/2} \tag{46}$$

is the reliability factor of the phase estimate.

The third conditional distribution for estimating target phases is given by equation (31): it may be invoked when it is supposed that the phase φ_{a} does not contribute to the estimate of φ. Then

$$\tan\theta_{\mathrm{init}} = \frac{\sum_{j=1}^{n} S_U(j)\sin\varphi_{\mathrm{dest}}(j)}{\sum_{j=1}^{n} S_U(j)\cos\varphi_{\mathrm{dest}}(j)} = \frac{T_U}{B_U}, \tag{47}$$

where each $S_U(j)$ is given by equation (32a) and

$$\alpha_U = (T_U^2+B_U^2)^{1/2}$$

is the reliability parameter of the target phase estimate.

In practical applications the estimates θ_{init} are not separately obtained *via* equations (43a) or (45) or (47). Indeed, as stated before, the same reflection (*hkl*) may belong to the subset $\{\mathrm{P}\}_i$ for the *i*th derivative, to $\{\mathrm{U}\}_j$ for the *j*th derivative and to $\{\mathrm{I}\}_k$ for the *k*th derivative: accordingly, sometimes φ_{hkl} may be estimated *via* the *G* parameter, other times *via* the *S* or the S_U parameters. Therefore a more realistic mathematical representation of the formula estimating φ is given by

$$\tan\theta_{\mathrm{init}} = \frac{\sum_{j=1}^{m} \Gamma(j)\sin\varphi_{\gamma}(j)}{\sum_{j=1}^{m} \Gamma(j)\cos\varphi_{\gamma}(j)} = \frac{T_\Gamma}{B_\Gamma} \tag{48}$$

with

$$\alpha_\Gamma = (T_\Gamma^2+B_\Gamma^2)^{1/2}$$

as the corresponding reliability parameter.

$\Gamma(j)$ will coincide with $G(j)$, $S(j)$ or $S_U(j)$ according to which among equations (43a), (45) or (47) has been used for deriving the φ estimate from the *j*th derivative. $\varphi_\gamma(j)$ represents $\varphi_{\mathrm{a}}(j)$ if equations (43a) or (45) are used, represents $\varphi_{\mathrm{dest}}(j)$ if equation (47) is employed. *m* is the total number of phase relationships really used in practice for estimating the given target phase.

It may now be expected that, by application of equation (48), a sufficient number of target reflections may be estimated with accuracy better than random. The target electron density should then be computed and submitted to EDM procedures. However, phase refinement and extension are expected to succeed only if the average target phase error is sufficiently small, but at this stage the condition may be verified only for small structures. Further steps are therefore necessary to allow PhD to succeed.

10. Second and subsequent PhD phasing steps

At the end of Step 1 the phase estimates θ_{init}, obtained *via* equations (42)–(48), are available for a given subset of target reflections: θ_{init} is expected to estimate the target phase with an accuracy better than random. From them it is easy to obtain, for any reflection (*hkl*), new derivative amplitude and phase estimates *via* the relation

$$|F_{\mathrm{ds}}(j)|\exp[i\varphi_{\mathrm{ds}}(j)] = |F_{\mathrm{a}}(j)|\exp[i\varphi_{\mathrm{a}}(j)] + |F|\exp(i\theta_{\mathrm{init}}),\quad j=1,\ldots,n, \tag{49}$$

from which

$$|F_{\mathrm{ds}}(j)|^2 = |F_{\mathrm{a}}(j)|^2 + |F|^2 + 2|FF_{\mathrm{a}}(j)|\cos[\theta_{\mathrm{init}}-\varphi_{\mathrm{a}}(j)] \tag{50}$$

and

$$\tan\varphi_{\mathrm{ds}}(j) = \frac{|F_{\mathrm{a}}(j)|\sin\varphi_{\mathrm{a}}(j) + |F|\sin\theta_{\mathrm{init}}}{|F_{\mathrm{a}}(j)|\cos\varphi_{\mathrm{a}}(j) + |F|\cos\theta_{\mathrm{init}}} \tag{51}$$

are derived. Since $\varphi_{\mathrm{a}}(j)$ for any *j* value is known without ambiguity, and since the amplitudes $|F_{\mathrm{a}}(j)|$ and $|F|$ on the right-hand side of equation (51) are precisely known, the accuracy of the phase relationship $\varphi_{\mathrm{ds}}(j) \simeq \varphi_{\mathrm{d}}(j)$ will only depend on the θ_{init} reliability. We can therefore associate to all the new estimates $\varphi_{\mathrm{ds}}(j), j=1,\ldots,n$, the same weight $D_1(\alpha_\Gamma)$ calculated for θ_{init}. Since a reasonable weight is now available for each $\varphi_{\mathrm{ds}}(j)$, only the most reliable derivative phase estimates may be used for the next steps of the phasing process. It is worthwhile noticing that, if by chance or by science θ_{init} is an accurate estimate of the target phase, then both $|F_{\mathrm{ds}}(j)|$ and $\varphi_{\mathrm{ds}}(j)$ are accurately estimated.

Obviously, in accordance with relation (18), for reflections for which $R_{\rm a}(j)/R > 1$ the inequality

$$|\sin[\varphi_{\rm ds}(j) - \varphi_{\rm a}(j)]| \le R/R_{\rm a}(j)$$

should be satisfied. If $\varphi_{\rm ds}(j)$ does not satisfy such a constraint, it should be reset to a value satisfying the inequality. That also involves a corresponding back-correction on the $\theta_{\rm init}$ estimate.

The real advantages of equation (51) with respect to the estimates available at the beginning of Step 1 are the following. The relation $\varphi_{\rm d}(j) \simeq \varphi_{\rm ds}(j)$ is expected to be more reliable than the relation $\varphi_{\rm d}(j) \simeq \varphi_{\rm a}(j)$ available at the beginning of the PhD phasing procedure. This expectation is based on the following observation. Let us consider $F_{\rm d}(j)$ and $F_{\rm a}(j)$ as vectors with fixed amplitudes and phases, and let us assume that $\varphi_{\rm a}(j)$ is known while $\varphi_{\rm d}(j)$ remains unknown. Then φ will be randomly distributed on the trigonometric circle and the application of equation (51) cannot provide phase estimates better than $\varphi_{\rm ds}(j) \simeq \varphi_{\rm a}(j)$. If some information on φ is available, such as that provided by equations (42)–(48), the random nature of φ is reduced and equation (51) is expected to provide the more accurate prediction $\varphi_{\rm d}(j) \simeq \varphi_{\rm ds}(j)$.

Furthermore, while at the beginning of Step 1 for each jth derivative, only derivative phase estimates for the reflections $\{{\rm P}\}_j$ were available through the relation $\varphi_{\rm dest}(j) = \varphi_{\rm a}(j)$, now equation (51) is also extended to reflections $\{{\rm I}\}_j$ and $\{{\rm U}\}_j$. However, for reflections with large ratios $R_{\rm a}(j)/R$, the original relation $\varphi_{\rm d}(j) \simeq \varphi_{\rm a}(j)$ may be restated owing to its large accuracy. Finally, it may be concluded that, according to the probabilistic approach so far described, estimates $|F_{\rm ds}(j)|$ and $\varphi_{\rm ds}(j)$ of the derivative amplitudes and phases are available which are more useful than those obtained when Step 1 started.

As soon as $|F_{\rm ds}(j)|$ and $\varphi_{\rm ds}(j)$ are obtained for each derivative, the new phases may be used to start an EDM procedure, where for each jth derivative, the hybrid Fourier synthesis with coefficients (see §8)

$$|F|\exp[i\varphi_{\rm ds}(j)]$$

may be used. Unlike in Step 1, where only {P} reflections were used for calculating the first derivative density map, in this second step an enlarged set of phased reflections is available belonging to the subsets {P}, {I} and {U}, so making the full EDM procedure more effective. At the end of the EDM cycles, each cycle performed under the restraint (18), $\rho_{\rm dest}(j)$ is available for each j.

A last point deserves to be clarified. In this Step 2, where the $\theta_{\rm init}$ estimates of the target phases are available, all the derivative phase estimates corresponding to the same (hkl) reflection are consistent with the same enantiomorph choice because they are constructed *via* equations (49)–(51), that is by using the same $\theta_{\rm init}$ value. Therefore, for a given reflection (hkl) the choice may be correct for all the derivatives or wrong for all of them. The increased derivative phase accuracy gained *via* equations (49)–(51) will make the correct enantiomorph definition easier.

Let us now assume that the procedure just described has been applied to all the n derivatives: then n final electron-density maps $\rho_{\rm dest}(j), j = 1, \ldots, n$, are accessible, which may be summed to obtain the map $\rho_{\rm dsum}$ defined by equation (40*a*). As described in §7, $\rho_{\rm dsum}$ should emphasize the target features present in the various $\rho_{\rm dest}(j)$ and, simultaneously, the various ancil structural features present in them are expected to be confined to the background. The Fourier inversion of $\rho_{\rm dsum}$ will produce new φ estimates, to which equations (49)–(51) may be applied for obtaining the corresponding $|F_{\rm ds}(j)|$ and $\varphi_{\rm ds}(j)$ estimates. The four-step procedure

$$\begin{aligned}[|F_{\rm ds}(j)|, \varphi_{\rm ds}(j)] &\to \sum_{j=1}^{n} \rho_{\rm dest}(j) \to \rho_{\rm dsum} \to \{\varphi\} \\ &\to [|F_{\rm ds}(j)|, \varphi_{\rm ds}(j)] \qquad (52)\end{aligned}$$

may become cyclic. At the end, $|F_{\rm dinv}(j)|$ and $\varphi_{\rm dinv}(j)$ values will be produced which are expected to be improved approximations of $|F_{\rm d}(j)|$ and $\varphi_{\rm d}(j)$.

An analogous cyclic procedure may be applied if, instead of calculating $\rho_{\rm dsum}$, use is made of the sum of the difference electron densities (40*b*). Such a sum will provide, by Fourier inversion, better φ estimates which, on their turn, will be used to generate better derivative structure-factor estimates.

$\varphi_{\rm dinv}(j)$ should be the best current estimate of the jth derivative phase. $|F_{\rm dinv}(j)|$ may or may not be the best estimate of $|F_{\rm d}(j)|$, depending on the quality of the current jth derivative model. Indeed, when good estimates of $\varphi_{\rm dinv}(j)$ are available, better estimates of $|F_{\rm d}(j)|$ may be obtained *via* equation (17). We will again denote by $|F_{\rm dest}(j)|$ the best approximation of $|F_{\rm d}(j)|$ and we will describe how the $|F_{\rm dest}(j)|$'s and the $\varphi_{\rm dinv}(j)$'s may be used for a new φ estimate. That can essentially be done in two different ways.

If $R_{\rm dest}(j)$ and $\varphi_{\rm dinv}(j)$ values are supposed to be still inaccurate estimates of the true derivative amplitude and phase, then equations (43a), (45) or (47) may be applied. The only difference with Step 1 is that now the new $R_{\rm dest}(j)$ and $\varphi_{\rm dinv}(j)$ are expected to be closer to the true derivative amplitudes and phases, and therefore they are potentially able to improve the $\theta_{\rm init}$ estimates obtained at the end of Step 1.

If the $R_{\rm dest}(j)$ and $\varphi_{\rm dinv}(j)$ are assumed to be reliable approximations of $R_{\rm d}(j)$ and $\varphi_{\rm d}(j)$, then the distribution (see Appendix *B*)

$$\begin{aligned}P[\varphi|R, R_{\rm dest}(j), R_{\rm a}(j), \varphi_{\rm a}(j), \varphi_{\rm dinv}(j)] \\ \simeq [2\pi I_0(X)]^{-1}\exp\{X\cos[\varphi - \xi(j)]\} \qquad (53)\end{aligned}$$

may be used, for which the most probable value of φ is $\xi(j)$, given by

$$\tan\xi(j) = \frac{R_{\rm dest}(j)\sin\varphi_{\rm dinv}(j) - R_{\rm a}(j)\sin\varphi_{\rm a}(j)}{R_{\rm dest}(j)\cos\varphi_{\rm dinv}(j) - R_{\rm a}(j)\cos\varphi_{\rm a}(j)}. \qquad (54)$$

The parameter

$$\begin{aligned}X(j) = 2R\{R^2_{\rm dest}(j) + R^2_{\rm a}(j) + 2R_{\rm dest}(j)R_{\rm a}(j) \\ \times\cos[\varphi_{\rm dinv}(j) - \varphi_{\rm a}(j)]\}^{1/2} \qquad (55)\end{aligned}$$

is its reliability factor. The relations (53)–(55) fully exploit the prior available information and are expected to provide more sensitive φ estimates.

Luckily, in the MPhD approach the indications arising from a large number of derivatives may be combined: we obtain as target phase estimate

$$\tan \xi_{\rm ov} = \frac{\sum_{j=1}^{n} R_{\rm dest}(j) \sin \varphi_{\rm dinv}(j) - R_{\rm a}(j) \sin \varphi_{\rm a}(j)}{\sum_{j=1}^{n} R_{\rm dest}(j) \cos \varphi_{\rm dinv}(j) - R_{\rm a}(j) \cos \varphi_{\rm a}(j)} = \frac{T_{\rm ov}}{B_{\rm ov}}, \qquad (56)$$

with reliability factor equal to

$$\alpha_{\rm ov} = (T_{\rm ov}^2 + B_{\rm ov}^2)^{1/2}.$$

The generalization of Step 2 into Step i with $i > 2$ is now obvious.

11. The multiple phantom derivative method as non-*ab initio* technique

Ab initio phasing methods, even designed to drive random phases towards the correct ones, are often useful for improving the phases obtained by other methods, no matter if *ab initio* or non-*ab initio*. For example, this is the case of the *VLD* (*Vive la Difference*) approach (Burla, Giacovazzo & Polidori, 2011; Burla, Carrozzini *et al.*, 2011) which may be employed, together with EDM techniques, to extend and refine phases obtained by MR methods (Carrozzini, Cascarano, Comunale *et al.*, 2013).

Let us suppose that a phasing process (*e.g.* MR, SIR–MIR, SAD–MAD or any *ab initio* technique) has been undertaken for phasing a given target structure, and that the method provides a model structure, with electron density $\rho_{\rm M}(\mathbf{r})$, from which it is difficult to recover the target structure. Such difficulties mainly arise from:

(i) A too rough model. In MR that usually occurs when the sequence identity between target and model molecule is too low, in SIR–MIR when there is a severe lack of isomorphism between derivatives and native protein, in SAD–MAD when the ratio *anomalous signal/noise* is too small.

(ii) Too low resolution of the data phased *via* MR, SIR–MIR or SAD–MAD. Then the phase extension to the higher native protein resolution may be difficult.

(iii) If an *ab initio* approach is used, it is not rare, for structure sizes overcoming 250 non-H atoms in the asymmetric unit, that a rough model is obtained which cannot be refined by standard techniques.

In all the above cases PhD is potentially able to add information supplementary to that contained in the experimental data. It is that provided by the calculated data of tens or hundreds of ancil and derivative structures. All the additional data may be computed up to the native data resolution, and therefore the limitations (ii) are overcome.

Let us now consider how PhD, working in reciprocal space, may help when a too rough model is available [points (i) and (iii)]. Let $|F|$ and φ be modulus and phase of the target reflection (hkl), $|F_{\rm M}|$ and $\varphi_{\rm M}$ be modulus and phase of the best model electron density $\rho_{\rm M}(\mathbf{r})$, no matter if derived from a molecular model or by inversion of an electron-density map. Then n ancil structures are created with the same characteristics described in the preceding sections: from them the values of structure factors $F_{\rm dM}(j)$ may be obtained, where

$$F_{\rm dM}(j) = F_{\rm M} + F_{\rm a}(j), \;\; j = 1, \ldots, n. \qquad (57)$$

From equation (57) the following phase and amplitudes arise:

$$\tan \varphi_{\rm dM}(j) = \frac{|F_{\rm M}| \sin \varphi_{\rm M} + |F_{\rm a}(j)| \sin \varphi_{\rm a}(j)}{|F_{\rm M}| \cos \varphi_{\rm M} + |F_{\rm a}(j)| \cos \varphi_{\rm a}(j)} = \frac{T_{\rm M}}{B_{\rm M}} \qquad (58)$$

and

$$\begin{aligned} |F_{\rm dM}(j)|^2 &= T_{\rm M}^2 + B_{\rm M}^2 \\ &= |F_{\rm M}|^2 + |F_{\rm a}(j)|^2 + 2|F_{\rm M}||F_{\rm a}(j)| \cos[\varphi_{\rm M} - \varphi_{\rm a}(j)]. \end{aligned}$$

$F_{\rm dM}(j)$ is the estimated structure factor corresponding to the jth derivative electron density $\rho_{\rm M}(\mathbf{r}) + \rho_{{\rm a}j}(\mathbf{r})$.

When a model is available there are three strong advantages with respect to the *ab initio* case:

(i) The problem of fixing the correct enantiomorph of the jth derivative phases with respect to the $\varphi_{\rm a}(j)$'s is not usually present (unless the model is centric while the target is acentric).

(ii) The amplitudes $|F_{\rm dM}(j)|$ are better estimates of the true derivative amplitudes than the amplitudes (15), which are the obliged choice when no model is available. That makes PhD convergence faster.

(iii) While in *ab initio* PhD applications $\varphi_{\rm a}(j)$'s are the best phase estimates for the jth derivative (and therefore are sufficiently accurate only for {P} reflections), now better estimates [say $\varphi_{\rm dM}(j)$] are available for all the measured target reflections. Thus Step 1 described in §9 may be skipped: indeed $|F_{\rm dM}(j)|$ and $\varphi_{\rm dM}(j)$ play for non-*ab initio* phasing attempts the same role played by $|F_{{\rm d}n}(j)|$ and $\varphi_{{\rm d}n}(j)$ in the *ab initio* phasing procedures [see equations (49)–(51)]. Accordingly, non-*ab initio* phasing may follow the approach described in §10.

In addition, direct-space methods like those described in §8 may also be used. If a model structure $\rho_{\rm M}$ is available from another phasing technique, then n derivative model electron densities

$$\rho_{\rm dM}(j) = \rho_{\rm M} + \rho_{\rm a}(j), \;\; j = 1, \ldots, n,$$

may be calculated, which may after be submitted to EDM procedures. As described in §8, their sum function may be used for revealing details of the target structure not available in the original model.

12. Conclusions

In order to make conclusive remarks about the nature of the PhD approach, we first summarize similarities and differences with the classical SIR–MIR techniques.

The derivative structures are obtained *via* soaking or co-crystallization methods, often requiring patience and determination.

Diffraction data are collected both for the target and for the derivative structures.

The attachment of the heavy atoms should not be very extensive: the number of binding sites should usually be small, so as to generate heavy-atom substructures with scattering powers much smaller than that of the target, so supporting the phase relation $\varphi \simeq \varphi_{\rm d}$.

The isomorphism between the native and the derivative structures must be sufficiently good. Usually a first control on the isomorphism is made by checking if the unit cells of the target and of the derivatives differ by more than 1.5–2%. A second control is made by checking if the diffraction data differ by more than 15–25%.

The above features locate SIR–MIR among non-*ab initio* phasing methods. It is the redundancy of the experimental information which permits the crystal structure solution, even at resolution worse than 4–6 Å.

Let us now consider PhD. In this method the target plays the role of the native protein and the ancil structures that of the heavy-atom substructures. We notice:

While in SIR–MIR the heavy-atom substructures are unknown and their solution is the first step of the phasing procedure, in the PhD technique the ancil structures are freely generated, may be constituted by heavy and/or light atoms according to the fancy of the practitioner, are usually randomly constructed, and their scattering power may be any.

Practical aspects suggest that an ancil structure with scattering power similar to that of the target may be more useful. Accordingly, the classical crystallographic concept of isomorphism between derivative and target structure has no role in PhD.

Ancils constituted by heavy atoms are not necessary. It has still to be checked if ancil structures with a few heavy atoms with overall scattering power similar to that of the target may be more or less useful than ancil structures with the same target chemical composition. Furthermore, ancils constituted by light atoms may be used even if the target structure contains heavy atoms.

Owing to the artificial nature of the ancil structure, symmetry and unit cell may be exactly the same for the target and for the phantom derivative. Thus the minimum interatomic distance (*e.g.* 1.36 Å), which has often been considered one of the parameters necessary for crystal structure solution, is severely violated by the phantom derivative structures. Cascarano *et al.* (1992) observed that the deviations of the Wilson plot from a straight line are connected to the interatomic distances, and that the average $\langle |F|^2 \rangle$, calculated at a given Bragg angle, shows maxima which depend on the smallest interatomic distances: *i.e.* such distances may be calculated by inverse Fourier transform of the Wilson plot. The conditions for guaranteeing a minimum interatomic distance are not present in a phantom derivative. Indeed, in the same unit cell of the target structure each derivative hosts twice the number of atoms and, according to the random nature of the ancil structures, it may frequently occur that in the phantom derivative two atoms are nearly overlapping. Obviously, an ancil structure may be created by hindering two ancil atoms from being closer than a minimum distance, but it is impossible to avoid ancil atoms overlapping or being too close to the target atoms.

$|F_{\rm d}|$, the structure-factor amplitude of the derivative electron density $\rho_{\rm d}(\mathbf{r}) = \rho(\mathbf{r}) + \rho_{\rm a}(\mathbf{r})$, is not experimentally available, it may only be estimated: thus the redundancy of the SIR–MIR experimental information has been lost. On the other hand, the average differences between phantom derivative amplitudes and target diffraction amplitudes are much larger than in the SIR–MIR methods.

The enantiomorphism problem is present both in SIR–MIR and in PhD, but its nature and the way in which it has to be solved are different.

A large number of phantom derivatives may be used (there is only a computing limit) in MPhD, to compensate, with respect to MIR techniques, for the absence of observed derivative amplitudes.

The phantom derivative technique needs only the target diffraction amplitudes and therefore is an *ab initio* approach.

All the above differences suggest that PhD should be considered an *ex-novo ab initio* phasing method rather than an evolution of the SIR–MIR techniques.

So far PhD has been described as a unique solution approach: *e.g.* from the available prior information on $|F|$, $|F_{\rm a}|$, $\varphi_{\rm a}$, only one solution, wrong or correct, is provided. In practice, such representation is just a simple way to illustrate the method: the number of obstacles in the way of succeeding is extremely large and multi-solution techniques should be more useful. Once the n ancil structures have been selected, the phase estimates provided by the ancil and derivative models may be combined in different ways: different combinations will correspond to different phasing trials. For brevity, this topic, of more practical character, is not discussed here, where it seems more useful to add some supplementary observations on the founding PhD conjecture.

It is a common belief that the knowledge of one or more structures, completely uncorrelated with the target structure, cannot provide any valuable information on the target structure. The phasing procedure described above rejects such a belief. The reader is referred to two other cases where some additional information apparently arises from nothing. The first is the *free lunch* approach (Caliandro *et al.*, 2005*a*,*b*). Non-measured amplitudes and their corresponding phases are extrapolated from a model structure and used to improve it. The model may be well correlated (*e.g.* when obtained from successful *ab initio* or non-*ab initio* phasing approaches), or completely uncorrelated with the target structure (in the case where the model is randomly generated in an *ab initio* approach or obtained at a certain stage of an unsatisfactory phasing process). In the first case the extrapolated amplitudes and phases aim at improving the data resolution and therefore the quality of the electron-density maps; in the second case they may improve the efficiency of the phasing process and make the difference between success and failure [see, for example, the use of 'free lunch' made by the program *SIR2011* (Burla, Caliandro *et al.*, 2012) or by *ACORN* (Yao *et al.*, 2006) or by Usón *et al.* (2007)].

The second example concerns *VLD*. Let

$$\rho(\mathbf{r}),\ \rho_{\rm p}(\mathbf{r})\ \text{and}\ \rho_{\rm q}(\mathbf{r})$$

be target, model and true difference electron density, respectively [by definition

$$\rho_{\rm q}(\mathbf{r}) = \rho(\mathbf{r}) - \rho_{\rm p}(\mathbf{r}),$$

no matter the quality of the model electron density]. It is usual to estimate the true difference electron density by the Fourier transform calculated *via* the coefficients (Read, 1986)

$$m_{\mathbf{h}}F_{\mathbf{h}} - D_{\mathbf{h}}F_{\rm p\mathbf{h}},$$

where F and $F_{\rm p}$ are the structure factors of the target and of the model structure, respectively, m and D are statistical parameters related to the quality of the model.

$\rho_{\rm q}(\mathbf{r})$ is well estimated by the Read formula only when the quality of the model is sufficiently high; the formula is useless for low-quality models. That agrees well with the common belief that, when the model is uncorrelated with the target, the difference Fourier synthesis should not provide any information on the target structure.

More recently (Burla *et al.*, 2010) a new difference Fourier synthesis has been proposed which is able to provide information on the target structure also when the model is uncorrelated with the target: the new formula coincides with the Read formula only when the correlation is sufficiently high. Owing to such a property, the new formula has been the main tool for the *VLD ab initio* phasing approach.

The PhD method proposed here is therefore the most radical example of exploiting model structures uncorrelated with the target. For the first time, artificial, freely chosen structures (the *ancils*), no matter if chemically sound, with or without any control on allowed interatomic distances and angles, sheltered in the same unit cell in which the unknown real structure (*the target*) crystallizes, are used to obtain simulated derivatives which in their turn are employed to phase the target. As for the ancil, the derivative structures are also not real structures, and therefore they are devoid of experimental data. The phasing algorithms described in this paper suggest mathematical tools for phasing the target crystal structure from ancil structures completely uncorrelated with the target.

But, what is the information source allowing the phasing process to succeed? It may be identified in the positivity of the electron density, which is exploited both by EDM cycles, repeatedly performed in the PhD approach, and by the PhD probabilistic formulas used for phasing target reflections.

Each jth derivative electron density satisfies the positivity criterion: indeed the derivative is the sum of two component structures, both positively defined, one perfectly known (the ancil) and the other unknown (the target). Ancil phases and amplitudes obtained by combining ancil and target amplitudes are initially used in EDM cycles to improve, by application of the positivity criterion, the jth derivative phases and amplitudes. The ambiguous nature of the derivative, however, is a strong obstacle to the success: indeed the natural evolution of each starting derivative model towards the correct one is usually difficult because there are weak reasons in favour and strong against. The main reason against is that EDM techniques have a very low efficiency owing to the fact that a basic ingredient, the measured derivative amplitudes, is not available. The difficulty may be overcome when MPhD techniques rather than SPhD are used, but a large number of ancil structures are needed for success.

The estimate of the target phases may also be provided by the probabilistic relationships exploiting the prior knowledge of the ancil amplitudes and phases, and of the target observed amplitudes. Such relationships are again based on the positivity of the electron density (that corresponds in reciprocal space to the positivity of the scattering factors) and are the PhD counterpart of the relationships designed for SIR–MIR techniques when the heavy-atom substructures are already known. The efficiency of SIR–MIR probabilistic relationships is still high at resolution far from atomic (as a rule of thumb, they may also work at 4–6 Å resolution). If the weaker nature of the PhD phase relationships (generated by ignoring the derivative amplitudes) is fully compensated by the large number of phantom derivatives, it may be expected that PhD should work up to the resolution limit at which both EDM and SIR–MIR work. Since both provide useful information up to 4–6 Å, PhD is then expected to be an *ab inito* phasing method which, for the first time in crystallographic history, may succeed at 4–6 Å resolution, without any upper limit for the structural complexity. If the phase information provided by derivative amplitudes to SIR–MIR techniques is only partially compensated by the large number of derivatives, then PhD limits will be more strict in terms of structure complexity and data resolution. It is impossible at the moment to foresee which of the two cases will occur. In the first case PhD would represent a strong discontinuity with the current phasing methods working at non-atomic resolution. They essentially are:

(i) Patterson techniques (Caliandro *et al.*, 2014): they are occasionally able to solve structures up to 2 Å resolution provided heavy atoms are in the unit cell (a very severe limit).

(ii) Direct methods based on a recently published probabilistic formula (Burla, Carrozzini *et al.*, 2012) estimating triplet invariants from a model electron-density map which is progressively created during the phasing process (Burla *et al.*, 2015*a*). Also this approach is occasionally able to solve crystal structures up to 2 Å resolution provided heavy atoms are in the unit cell.

(iii) Arcimboldo approach (Rodríguez *et al.*, 2012). It uses different types of prior information to locate in the unit cell small molecular fragments supposed to be present in the target structure. It works also at about 2 Å but does not need the presence of heavy atoms.

The number of ancil structures, and therefore of phantom derivatives, necessary for solving a macromolecular structure may at the moment only be guessed: as a rule of thumb, it is estimated at between 100 and 300 for *ab initio* purposes, some tens for non-*ab initio*, numbers which are expected to be independent of the structural complexity and of the experimental target data resolution.

The PhD approach described in this paper is essentially speculative. However, we anticipate that PhD will be applied, in its non-*ab initio* version, to extend phases and to refine electron-density maps obtained by MR (Burla *et al.*, 2015*b*). A set of test structures was selected with average phase error larger than 50°, and PhD was applied to them to improve the quality of the model. PhD efficiency was compared with that of other current techniques: the results showed the superior capacity of PhD in refining phases. Such application is the first confirmation of the founding conjecture of this paper: random structures (say ancils) may be used to make the crystal structure solution of a target structure completely uncorrelated with the ancils easier.

APPENDIX *A*

Let us assume that the electron densities of the target and of the ancil structures are uncorrelated with each other. We are interested in calculating the expected percentage of reflections for which $R_{\rm a} \geq S_p\, R$, where $R_{\rm a}$ is the normalized amplitude of the ancil structure and R is the normalized diffraction amplitude of the target structure. We will assume that both of them obey the Wilson distribution $P(R)$. Then the required percentage coincides with

$$\mathrm{PERC}(S_p) = \int_0^\infty P(R) \int_{S_pR}^\infty P(R_{\rm a})\,{\rm d}R\,{\rm d}R_{\rm a}. \qquad (59)$$

For the acentric case the right-hand side of equation (59) is equal to

$$4\int_0^\infty R\exp(-R^2)\,{\rm d}R\left[\int_{S_pR}^\infty R_{\rm a}\exp(-R_{\rm a}^2)\,{\rm d}R_{\rm a}\right]{\rm d}R$$
$$= 2\int_0^\infty R\exp(-R^2)\int_{(S_pR)^2}^\infty \exp(-y)\,{\rm d}y,$$

where $y = R_{\rm a}^2$. Since

$$\int_u^\infty x^{\nu-1}\exp(-\mu x)\,{\rm d}x = \mu^{-\nu}\Gamma(\nu, \mu u),$$

where Γ is the incomplete gamma function, and since

$$\Gamma(1+\nu, u) = \nu!\exp(-u)\sum_{m=0}^{\nu}\frac{u^m}{m!}\ [\nu = 0, 1, \ldots],$$

$\mathrm{PERC}(S_p)$ reduces to

$$2\int_0^\infty R\exp[-(S_p^2+1)R^2]\,{\rm d}R.$$

Owing to the relation

$$\int_0^\infty x^{2n+1}\exp(-px^2)\,{\rm d}x = \frac{n!}{2p^{n+1}}$$

we obtain

$$\mathrm{PERC}(S_p) = \frac{1}{S_p^2+1}. \qquad (60)$$

For the centric case equation (59) becomes

$$\mathrm{PERC}_{\bar 1}(S_p) = \frac{2}{\pi}\int_0^\infty \exp\left(-\frac{R^2}{2}\right)\left[\int_{S_pR}^\infty \exp\left(-\frac{R_{\rm a}^2}{2}\right){\rm d}R_{\rm a}\right]{\rm d}R. \qquad (61)$$

Equation (61), after the change of variable $x = R_{\rm a}/2^{1/2}$, becomes

$$\mathrm{PERC}_{\bar 1}(S_p) = \left(\frac{2}{\pi}\right)^{1/2}\int_0^\infty \exp\left(-\frac{R^2}{2}\right)\left[1-\Phi\left(\frac{S_pR}{2^{1/2}}\right)\right]{\rm d}R, \qquad (62)$$

where Φ is the error function defined by

$$\Phi(x) = \frac{2}{\pi^{1/2}}\int_0^x \exp(-t^2)\,{\rm d}t.$$

Submitting equation (62) to the change of variable $z = S_pR/2^{1/2}$ leads to

$$\frac{2}{S_p(\pi^{1/2})}\int_0^\infty \exp\left(-\frac{z^2}{S_p^2}\right)[1-\Phi(z)]\,{\rm d}z.$$

Owing to the integral

$$\int_0^\infty \exp(-\mu^2x^2)[1-\Phi(x)]\,{\rm d}x = \frac{\arctan\mu}{\mu(\pi^{1/2})}$$

we obtain

$$\mathrm{PERC}_{\bar 1}(S_p) = \frac{2}{\pi}\arctan(1/S_p). \qquad (63)$$

We are also interested in estimating the percentage of reflections for which $R > S_uR_{\rm a}$ (they constitute the set {U}). The corresponding formulas will coincide with equations (60) and (63) but S_u will replace S_p.

APPENDIX *B*

This Appendix aims to provide the mathematical support to the probabilistic formulas which may be used in the PhD phasing approach. We will first recall some basic results obtained for SIR–MIR techniques *via* the method of joint probability distribution functions, and then we will modify them to fit the PhD scenario.

Let $E_{\rm P}, E_{\rm d}, E_{\rm H}$ be the normalized structure factors (for more detailed definitions see §2) of the target, derivative and heavy-atom structure, respectively, for the classical SIR case. The most general distribution for treating the SIR case (Giacovazzo *et al.*, 2004) is

$$
\begin{aligned}
&P(R_{\mathrm{P}}, R_{\mathrm{d}}, R_{\mathrm{H}}, \varphi_{\mathrm{P}}, \varphi_{\mathrm{d}}, \varphi_{\mathrm{H}}) \\
&\quad \simeq Z R_{\mathrm{P}} R_{\mathrm{d}} R_{\mathrm{H}} \exp\{-[\lambda_{11} R_{\mathrm{H}}^2 + \lambda_{22} R_{\mathrm{P}}^2 + \lambda_{33} R_{\mathrm{d}}^2 \\
&\qquad + 2\lambda_{12} R_{\mathrm{H}} R_{\mathrm{P}} \cos(\varphi_{\mathrm{H}} - \varphi_{\mathrm{P}}) + 2\lambda_{13} R_{\mathrm{H}} R_{\mathrm{d}} \cos(\varphi_{\mathrm{d}} - \varphi_{\mathrm{H}}) \\
&\qquad\quad + 2\lambda_{23} R_{\mathrm{P}} R_{\mathrm{d}} \cos(\varphi_{\mathrm{d}} - \varphi_{\mathrm{P}})]\}, \qquad (64)
\end{aligned}
$$

where Z is a scaling parameter and $\lambda_{ij}, i, j = 1, \ldots, 3$ are suitable coefficients. For the sake of simplicity we do not recall the definitions of all the λ_{ij}'s, but only those which are useful for our purposes:

$$\lambda_{12} = \left(\sum_{\mathrm{P}} \sum_{\mathrm{H}}\right)^{1/2} / \langle|\mu|^2_{\mathrm{sir}}\rangle, \qquad (65a)$$

$$\lambda_{13} = -\left(\sum_{\mathrm{d}} \sum_{\mathrm{H}}\right)^{1/2} / \langle|\mu|^2_{\mathrm{sir}}\rangle, \qquad (65b)$$

$$\lambda_{23} = -\left(\sum_{\mathrm{P}} \sum_{\mathrm{d}}\right)^{1/2} / \langle|\mu|^2_{\mathrm{sir}}\rangle. \qquad (65c)$$

From equation (64) several marginal and/or conditional distributions may be derived. For example, $P(R_{\mathrm{P}}|R_{\mathrm{d}}, R_{\mathrm{H}})$ was used by Giacovazzo (2014) for estimating the heavy-atom substructure amplitudes given the values of R_{d} and R_{H}. $P(\varphi_{\mathrm{P}}|R_{\mathrm{P}}, R_{\mathrm{d}}, \varphi_{\mathrm{H}})$ was used to estimate the native protein phase when the heavy-atom substructure was already solved (Giacovazzo & Siliqi, 2002). Owing to the constraint $F_{\mathrm{d}} = F_{\mathrm{P}} + F_{\mathrm{H}}$ all the above distributions were useful only because errors were associated to the observations.

In PhD derivative and target phases are more weakly correlated than in SIR; therefore the relation $\varphi_{\mathrm{P}} \simeq \varphi_{\mathrm{d}}$ cannot always be transferred in PhD notation as $\varphi \simeq \varphi_{\mathrm{d}}$. The relation $\varphi_{\mathrm{P}} \simeq \varphi_{\mathrm{d}}$ reduces the number of variables in SIR and, consequently, the number of conditional distributions necessary to treat it. In PhD techniques more marginal and /or conditional distributions are therefore necessary to take into account the specific nature of the method. Furthermore, as we will see in this Appendix, the reliability of the phase indications arising from them is much smaller than in SIR.

Luckily, the mathematical expressions of such distributions clearly suggest the subsets of reflections to which the different PhD conditional distributions should be applied with expected non-vanishing accuracy.

Let us first recall that, in a paper dedicated to the study of SIR–MIR techniques (Giacovazzo & Siliqi, 2002), the following conditional joint probability distribution was obtained:

$$
\begin{aligned}
&P(R_{\mathrm{P}}, R_{\mathrm{d}}, \varphi_{\mathrm{P}}, \varphi_{\mathrm{d}}|R_{\mathrm{H}}, \varphi_{\mathrm{H}}) \\
&\quad \simeq \pi^{-2}\left(\frac{R_{\mathrm{P}} R_{\mathrm{d}}}{\sigma^2}\right) \exp\left\{-\frac{1}{\sigma^2}[R_{\mathrm{d}}^2 + R_{\mathrm{H}}^2 - 2R_{\mathrm{d}} R_{\mathrm{H}} \cos(\varphi_{\mathrm{d}} - \varphi_{\mathrm{H}}) \right. \\
&\qquad + (1+\sigma^2) R_{\mathrm{P}}^2 - 2R_{\mathrm{d}} R_{\mathrm{P}} \cos(\varphi_{\mathrm{d}} - \varphi_{\mathrm{P}}) \\
&\qquad\quad \left. + 2R_{\mathrm{P}} R_{\mathrm{H}} \cos(\varphi_{\mathrm{P}} - \varphi_{\mathrm{H}})]\right\}. \qquad (66)
\end{aligned}
$$

Since in PhD R_{a}, φ_{a} are known by hypothesis, equation (66) is a useful starting point for deriving analogous conditional distributions valid in PhD. From such an adapted conditional distribution it will be possible to derive initial estimates of φ and φ_{d} given the available prior information.

The following considerations are necessary for adapting equation (66) to PhD conditions:

(i) Amplitudes and phases of the ancil structure are known without error, but R_{d}'s are not measured. They may be only estimated: *e.g.* the estimates may be obtained by Fourier inversion during an EDM procedure, or, for special subsets of reflections, by algebraic considerations (see §6). The estimates may be largely inaccurate, particularly in the first stages of the phasing process. As a consequence, it may be expected that the PhD substitute of σ^2_{sir} (let us call it σ^2_{PhD}) should vary according to the correlation between the current and the target model.

(ii) According to point (i), in PhD, where the traditional isomorphism concept is no longer valid, $|\mu_{\mathrm{sir}}|^2$ has to be replaced by $|\mu_{\mathrm{PhD}}|^2$: obviously its larger size mainly depends on the fact that $|F_{\mathrm{d}}|$ is not measured. Let us first consider the initial PhD step, where R_{dest} and the true R_{d} are very weakly correlated. In this case R_{dest} and R_{d} may be considered as variables independently distributed according to the Wilson statistics. Then, for the type of ancil structures chosen in this paper (*i.e.* the scattering power of the ancil equal to the scattering power of the target), σ^2_{PhD} may be estimated as follows:

$$\sigma^2_{\mathrm{PhD}} \simeq \langle(R_{\mathrm{dest}} - R_{\mathrm{d}})^2\rangle = 2 - 2(\langle R_{\mathrm{dest}}\rangle\langle R_{\mathrm{d}}\rangle) \simeq 0.43$$

for an acentric crystal and

$$\sigma^2_{\mathrm{PhD}} \simeq 2 - 2(\langle R_{\mathrm{dest}}\rangle\langle R_{\mathrm{d}}\rangle) \simeq 0.73$$

for a centric crystal, against the range (0.05–0.15) usually covered by σ^2_{sir}. In real cases, where R_{dest} is obtained by Fourier inversion of a modified electron-density map, it is not expected to be distributed according to Wilson statistics; therefore the variance may be larger than that estimated *via* the above equations, at least in the first PhD steps.

There is a second reason responsible for an additional increase of σ^2_{PhD}. In the conclusions (see §12) it is recalled that the interatomic distances define the oscillations of the Wilson plot, and that, *vice versa*, the smallest interatomic distances may be estimated by inverse Fourier transform of the Wilson plot (Cascarano *et al.*, 1992). In real structures it is the concept of minimal interatomic distance which leads to Wilson plots typical of real crystal structures. In agreement with the above statement, Morris *et al.* (2004) showed that Wilson plots of proteins are very similar to each other in spite of the wide spread of secondary structure characteristics.

In a phantom derivative the concept of minimum interatomic distance has no role, so that the Wilson plot of a phantom derivative, even if it was obtained by using the true derivative amplitudes (instead of the amplitudes calculated by Fourier inversion), is not expected to fit the Wilson plots of real structures. Accordingly, the amplitude normalization procedure of the phantom derivatives cannot be based on the same principles used for real structures, and will lead to a larger spread between true and calculated normalized derivative amplitudes. Owing to all the above considerations it may be supposed that $\sigma^2_{\rm PhD}$ may also attain 1 in the first PhD step. Such high values weaken the reliability of the probabilistic estimates provided by equations (66), (26), (28) or (31).

(iii) In accordance with points (i) and (ii) equation (66) may be adapted to PhD by first applying the transformation $\sum_{\rm P} \rightarrow \sum$ and $\sum_{\rm H} \rightarrow \sum_{\rm a}$, and after by replacing $\langle|\mu|^2_{\rm sir}\rangle$ by $\langle|\mu|^2_{\rm PhD}\rangle$. In accordance with the hypothesis $\sum = \sum_{\rm a} = 0.5\sum_{\rm d}$ equations (65*a*), (65*b*), (65*c*) transform into

$$\lambda_{12} = \sum/\langle|\mu|^2_{\rm sir}\rangle = 1/\sigma^2_{\rm PhD}, \tag{67a}$$

$$\lambda_{13} = -2^{1/2}\sum/\langle|\mu|^2_{\rm PhD}\rangle = -2^{1/2}/\sigma^2_{\rm PhD}, \tag{67b}$$

$$\lambda_{23} = -2^{1/2}\sum/\langle|\mu|^2_{\rm PhD}\rangle = -2^{1/2}/\sigma^2_{\rm PhD}. \tag{67c}$$

Now we can rewrite the required conditional distribution valid for PhD as follows:

$$\begin{aligned} &P(R, R_{\rm d}, \varphi, \varphi_{\rm d}|R_{\rm a}, \varphi_{\rm a}) \\ &\quad \simeq C\exp\bigg\{-\frac{1}{\sigma^2_{\rm PhD}}[RR_{\rm a}\cos(\varphi-\varphi_{\rm a}) - 2^{1/2}R_{\rm d}R_{\rm a}\cos(\varphi_{\rm d}-\varphi_{\rm a}) \\ &\qquad - 2^{1/2}R_{\rm d}R\cos(\varphi_{\rm d}-\varphi)]\bigg\}. \end{aligned} \tag{68}$$

For the sake of simplicity, we do not report in equation (68) the explicit expressions of all the terms which do not depend on the phases, because they are not useful for our purposes: we represent them by *C*.

If the parameters $R, R_{\rm d}, R_{\rm a}, \varphi_{\rm a}, \varphi_{\rm d}$ are estimated with high accuracy then the conditional distribution

$$P(\varphi|R, R_{\rm d}, R_{\rm a}, \varphi_{\rm a}, \varphi_{\rm d}) \simeq [2\pi I_0(X)]^{-1}\exp[X\cos(\varphi-\xi)] \tag{69}$$

is obtained, where the most probable value of φ is ξ, given by

$$\tan\xi = \frac{2^{1/2}R_{\rm d}\sin\varphi_{\rm d} - R_{\rm a}\sin\varphi_{\rm a}}{2^{1/2}R_{\rm d}\cos\varphi_{\rm d} - R_{\rm a}\cos\varphi_{\rm a}}, \tag{70}$$

and

$$X = \frac{R}{\sigma^2_{\rm PhD}}[2R^2_{\rm d} + R^2_{\rm a} - 2(2^{1/2})R_{\rm d}R_{\rm a}\cos(\varphi_{\rm d}-\varphi_{\rm a})]^{1/2} \tag{71}$$

is its reliability factor. $D_1(X)$ may be the weight to associate to the estimate ξ.

Let us now suppose that $\varphi_{\rm d}$ is unknown while $R, R_{\rm d}, R_{\rm a}, \varphi_{\rm a}$ are known with high accuracy. Then from equation (68) the marginal distribution

$$\begin{aligned} &P(R, R_{\rm d}, \varphi|R_{\rm a}, \varphi_{\rm a}) \\ &\quad \simeq C\exp\bigg\{-\frac{1}{\sigma^2_{\rm PhD}}[RR_{\rm a}\cos(\varphi-\varphi_{\rm a})]\bigg\}I_0\left(\frac{2^{1/2}R_{\rm d}R_{\rm dcalc}}{\sigma^2_{\rm PhD}}\right) \end{aligned} \tag{72}$$

is obtained, where

$$R_{\rm dcalc} = [R^2 + R^2_{\rm a} + 2RR_{\rm a}\cos(\varphi-\varphi_{\rm a})]^{1/2}.$$

Equation (72) is able to provide, *via* numerical methods, the most probable value of φ, but does not immediately suggest any immediate practical use: for this purpose it is necessary to derive the conditional distribution $P(\varphi|R, R_{\rm d}, R_{\rm a}, \varphi_{\rm a})$. Such a distribution may be obtained by using the same assumption (say $\varphi_{\rm P} \simeq \varphi_{\rm d}$) employed by Giacovazzo & Siliqi for deriving in the SIR case the distribution (24) from $P(R_{\rm P}, R_{\rm d}, \varphi_{\rm P}, \varphi_{\rm d}|R_{\rm H}, \varphi_{\rm H})$. By adapting such a distribution to PhD and by assuming $\varphi \simeq \varphi_{\rm d}$ equations (26) and (27) are obtained. This is the first probabilistic result which may be used in the phasing procedure.

The assumption $\varphi_{\rm P} \simeq \varphi_{\rm d}$ in SIR–MIR is fully justified by the fact that the scattering power of the heavy-atom substructure is usually negligible with respect to the protein scattering power. The same assumption does not hold for the PhD approach (say, φ is no more obliged to be very close to $\varphi_{\rm d}$) because $\rho_{\rm a}(\mathbf{r})$, the PhD substitute of $\rho_{\rm H}(\mathbf{r})$, may have the same or a larger scattering power than that of the target structure: therefore φ and $\varphi_{\rm d}$ are more weakly correlated.

In order to relax the assumption $\varphi \simeq \varphi_{\rm d}$ we introduce into equation (72) the following approximation (Giacovazzo, 1979):

$$\begin{aligned} &I_0[L^2_1 + L^2_2 + 2L_1L_2\cos(\varphi_1-\varphi_2)] \\ &\quad \simeq \frac{I_0(L_1)I_0(L_2)}{I_0(L)}\exp[L\cos(\varphi_1-\varphi_2)], \end{aligned} \tag{73}$$

where L is defined by the relation

$$D_1(L) = D_1(L_1)D_1(L_2).$$

We obtain

$$P(\varphi|R, R_{\rm d}, R_{\rm a}, \varphi_{\rm a}) \simeq [2\pi I_0(S_I)]^{-1}\exp[S_I\cos(\varphi-\varphi_{\rm a})], \tag{74}$$

where

$$S_I = L - \frac{RR_{\rm a}}{\sigma^2_{\rm PhD}} \tag{75}$$

and L is defined by

$$D_1(L) = D_1\left(\frac{2^{1/2}R_{\rm d}R}{\sigma^2_{\rm PhD}}\right)D_1\left(\frac{2^{1/2}R_{\rm d}R_{\rm a}}{\sigma^2_{\rm PhD}}\right). \tag{76}$$

Equations (73)–(76) constitute the second tool for estimating the target phases.

There is a third way of estimating target phases. Let us suppose that $\varphi_{\rm d}$ and φ are weakly correlated and that we want to estimate φ from equation (68) when $\varphi_{\rm a}$ is integrated over all possible values. In this case the $\varphi_{\rm a}$ value should not influence the value of φ so much, as occurs when $R_{\rm a} \ll R$. The integration leads to

$$\begin{aligned} &P(R, R_{\rm d}, R_{\rm a}, \varphi, \varphi_{\rm d}) \\ &\quad \simeq C\exp\left[\frac{2^{1/2}}{\sigma^2_{\rm PhD}}RR_{\rm d}\cos(\varphi_{\rm d}-\varphi)\right]I_0\left(\frac{R_{\rm a}R_{\rm acalc}}{\sigma^2_{\rm PhD}}\right), \end{aligned} \tag{77}$$

where

$$R_{\rm acalc} = [2R^2_{\rm d} + R^2 - 2(2^{1/2})RR_{\rm d}\cos(\varphi_{\rm d}-\varphi)]^{1/2}.$$

In order to derive the conditional distribution of φ we expand the modified Bessel function in two ways:

(i) According to $I_0(x) \simeq \exp(x^2/4)$ when x is sufficiently small. Then

$$P(R, R_{\rm d}, R_{\rm a}, \varphi|\varphi_{\rm d}) \simeq [2\pi I_0(G_U)]^{-1}\exp[G_U\cos(\varphi-\varphi_{\rm d})], \tag{78}$$

where

$$G_U = \frac{2^{1/2}RR_{\rm d}}{\sigma^2_{\rm PhD}}\left(1-\frac{R^2_{\rm a}}{2\sigma^2_{\rm PhD}}\right). \quad (79)$$

The concentration parameter G_U is not very useful because the truncated expansion of the modified Bessel function holds only if R and $R_{\rm d}$ are both sufficiently small. Since $R_{\rm a}$ is also small by hypothesis, (79) cannot provide sufficiently reliable phase estimates.

(ii) According to equation (73), we then obtain

$$P(\varphi|R, R_{\rm d}, R_{\rm a}, \varphi_{\rm d}) \simeq [2\pi I_0(S_U)]^{-1}\exp[S_U\cos(\varphi-\varphi_{\rm d})], \quad (80)$$

where

$$S_U = \frac{2^{1/2}RR_{\rm d}}{\sigma^2_{\rm PhD}} - L_U \quad (81)$$

and

$$D_1(L_U) = D_1\left(\frac{2^{1/2}R_{\rm d}R_{\rm a}}{\sigma^2_{\rm PhD}}\right)D_1\left(\frac{RR_{\rm a}}{\sigma^2_{\rm PhD}}\right). \quad (82)$$

If R and $R_{\rm d}$ are sufficiently large and $R_{\rm a}$ is sufficiently small then S_U is positive: in this case φ is expected to be close to $\varphi_{\rm d}$ with reliability factor given by S_U. Distribution (80) cannot provide reliable estimates of the type $\varphi \simeq \varphi_{\rm d} + \pi$: indeed that would require that R and $R_{\rm d}$ are small and $R_{\rm a}$ sufficiently large. This last condition is in conflict with the hypothesis that $R_{\rm a}$ is small.

While in SIR–MIR techniques the conditional distribution of $\varphi_{\rm d}$ is never considered because it is very similar to that of the native protein (indeed the scattering powers of the heavy-atom substructure are negligible with respect to that of the protein), in PhD it should be explored because target and ancil structures may have comparable scattering powers. Let us first assume that the condition $\varphi \simeq \varphi_{\rm d}$ is satisfied (practically, we are then studying the case of {U} reflections). Then

$$P(\varphi_{\rm d}|R, R_{\rm d}, R_{\rm a}, \varphi_{\rm a}) \simeq [2\pi I_0(G_{\rm d})]^{-1}\exp[G_{\rm d}\cos(\varphi-\varphi_{\rm a})] \quad (83)$$

is obtained, where

$$G_{\rm d} = \frac{(2^{1/2}R_{\rm d}-R)R_{\rm a}}{\sigma^2_{\rm PhD}}. \quad (84)$$

Owing to the assumption $\varphi \simeq \varphi_{\rm d}$ the concentration parameter $G_{\rm d}$ coincides with the parameter G given by equation (27): accordingly, φ and $\varphi_{\rm d}$ are equally distributed.

Let us now estimate $\varphi_{\rm d}$ given R, $R_{\rm d}$, $R_{\rm a}$, $\varphi_{\rm a}$, whatever the φ value (that is more appropriate for {I} or {P} reflections). To do that we reconsider the distribution (68) and we derive the marginal probability distribution

$$\begin{aligned} &P(R, R_{\rm d}, \varphi_{\rm d}|R_{\rm a}, \varphi_{\rm a}) \\ &\simeq C\exp\left\{\frac{2^{1/2}}{\sigma^2_{\rm PhD}}[R_{\rm d}R_{\rm a}\cos(\varphi_{\rm d}-\varphi_{\rm a})]\right\}I_0\left(\frac{RR_{\rm calc}}{\sigma^2_{\rm PhD}}\right), \quad (85)\end{aligned}$$

where

$$R^2_{\rm calc} = 2R^2_{\rm d} + R^2_a - 2(2^{1/2})R_{\rm d}R_{\rm a}\cos(\varphi_{\rm d}-\varphi_{\rm a}).$$

If approximation (73) is introduced into equation (85) the conditional distribution (86) is obtained:

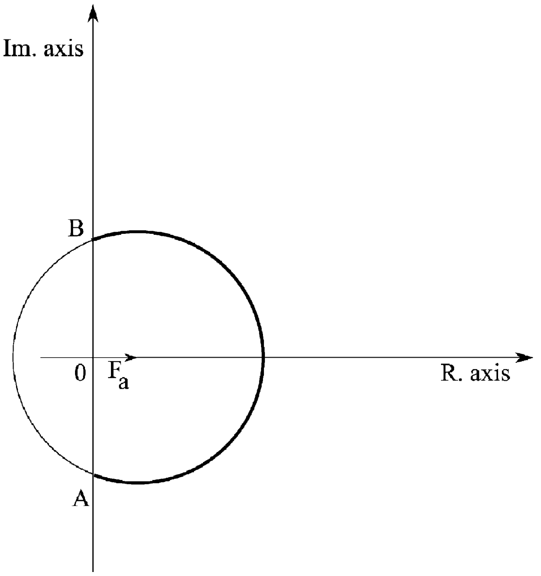

Figure 8
Case $F_{\rm a} \ll F$. The probability that $\cos(\varphi_{\rm d}-\varphi_{\rm a}) > 0$ is proportional to the ratio 'length of the arc AB in bold/length of the circle'.

$$P(\varphi_{\rm d}|R, R_{\rm d}, R_{\rm a}, \varphi_{\rm a}) \simeq [2\pi I_0(S_{\rm d})]^{-1}\exp[S_{\rm d}\cos(\varphi_{\rm d}-\varphi_{\rm a})], \quad (86)$$

where $S_{\rm d} = (2^{1/2}R_{\rm d}R_{\rm a})/(\sigma^2_{\rm PhD}) + L_{\rm d}$ and $L_{\rm d}$ is now defined by

$$D_1(L_{\rm d}) = D_1\left(\frac{2^{1/2}R_{\rm d}R}{\sigma^2_{\rm PhD}}\right)D_1\left(\frac{R_{\rm a}R}{\sigma^2_{\rm PhD}}\right). \quad (87)$$

Let us now compare distributions (84) and (87). They do not coincide: indeed according to equation (87) $\varphi_{\rm d} \simeq \varphi_{\rm a}$ if $R_{\rm d} > R/2^{1/2}$, $\varphi_{\rm d} \simeq \varphi_{\rm a} + \pi$ if $R_{\rm d} < R/2^{1/2}$. According to equation (87) $\varphi_{\rm d} \simeq \varphi_{\rm a}$ is always supported, no matter the values of $R_{\rm d}$ and R.

It is useful to notice that all the Figs. 2–7 support the indications of equation (87). A more explicit example showing that the expectation $\varphi_{\rm d} \simeq \varphi_{\rm a}$ is always more probable than $\varphi_{\rm d} \simeq \varphi_{\rm a} + \pi$ is illustrated in Fig. 8, for the most unfavourable case $|F| \gg |F_{\rm a}|$. In Fig. 8 we assume $\varphi_{\rm a} = 0$, but any different choice may be made without any change in the conclusions. Let us suppose that φ and $\varphi_{\rm a}$ are independent variables: they are therefore uncorrelated and φ spans the circle in a uniform way [a reasonable assumption, owing to the fact that $\rho_{\rm a}(\mathbf{r})$ is randomly fixed]. The probabilities that $\varphi_{\rm d} \simeq \varphi_{\rm a}$ or $\varphi_{\rm d} \simeq \varphi_{\rm a} + \pi$ are then proportional to the lengths of the two arcs AB, one at the right and the other at the left of the imaginary axis, respectively. Accordingly $\varphi_{\rm d} \simeq \varphi_{\rm a}$ is always the most probable phase relationship, unless $|F_{\rm a}| = 0$: only in this case is $\varphi_{\rm d}$ uniformly distributed on the trigonometric circle.

APPENDIX *C*

We stated in the main text that, when no model is available for the target structure, it is difficult, at an early step of an EDM procedure and for a given reflection (*hkl*), to decide if for the *j*th phantom derivative the sign of $\sin[\varphi_{\rm dinv}(j) - \varphi_{\rm a}(j)]$ coincides with the true sign of $\sin[\varphi_{\rm d}(j) - \varphi_{\rm a}(j)]$ or with its opposite. We also suggested in §6 that, under suitable conditions, it is possible to establish whether two different derivatives coherently suggest the same (no matter if wrong or correct) enantiomorph for a given (*hkl*) reflection. To deal with this question we observe that if $\overline{F}_{aj}$ is the complex conjugate of F_{aj}, then

$$\begin{aligned} F_{\rm d}(j)\cdot \bar{F}_{\rm a}(j) &= [F_{\rm a}(j)+F]\cdot \bar{F}_{\rm a}(j) \\ &= |F_{\rm a}(j)|^2 + |F_{\rm a}(j)F|\exp\{i[\varphi-\varphi_{\rm a}(j)]\} \end{aligned}$$

from which

$$|F_{\rm d}(j)F_{\rm a}(j)|\cos[\varphi_{\rm d}(j)-\varphi_{\rm a}(j)] = |F_{\rm a}(j)|^2 + |F_{\rm a}(j)F|\cos[\varphi-\varphi_{\rm a}(j)] \qquad (88)$$

and

$$|F_{\rm d}(j)F_{\rm a}(j)|\sin[(\varphi_{\rm d}(j)-\varphi_{\rm a}(j)] = |F_{\rm a}(j)F|\sin[\varphi-\varphi_{\rm a}(j)]. \qquad (89)$$

From (89) we obtain

$$\sin[\varphi_{\rm d}(j)-\varphi_{\rm a}(j)]\times\sin[\varphi-\varphi_{\rm a}(j)]>0. \qquad (90)$$

For a given reflection (*hkl*) relationship (90) states that, if the correct enantiomorph is obtained, the two sines must have the same sign. Unfortunately the value of φ is unknown and therefore (90) cannot be applied in practice, unless the space group of the ancil structure is a centric supergroup of the acentric space group of the target. In this case (90) reduces to

$$\sin[\varphi_{\rm d}(j)-\varphi_{\rm a}(j)]\sin\varphi\times\cos\varphi_{\rm a}(j)>0.$$

For a given (*hkl*) the sign of $\sin\varphi$ is an unknown but fixed parameter for each derivative. Therefore in an EDM procedure all the derivatives will coherently define the same enantiomorph (wrong or correct) if

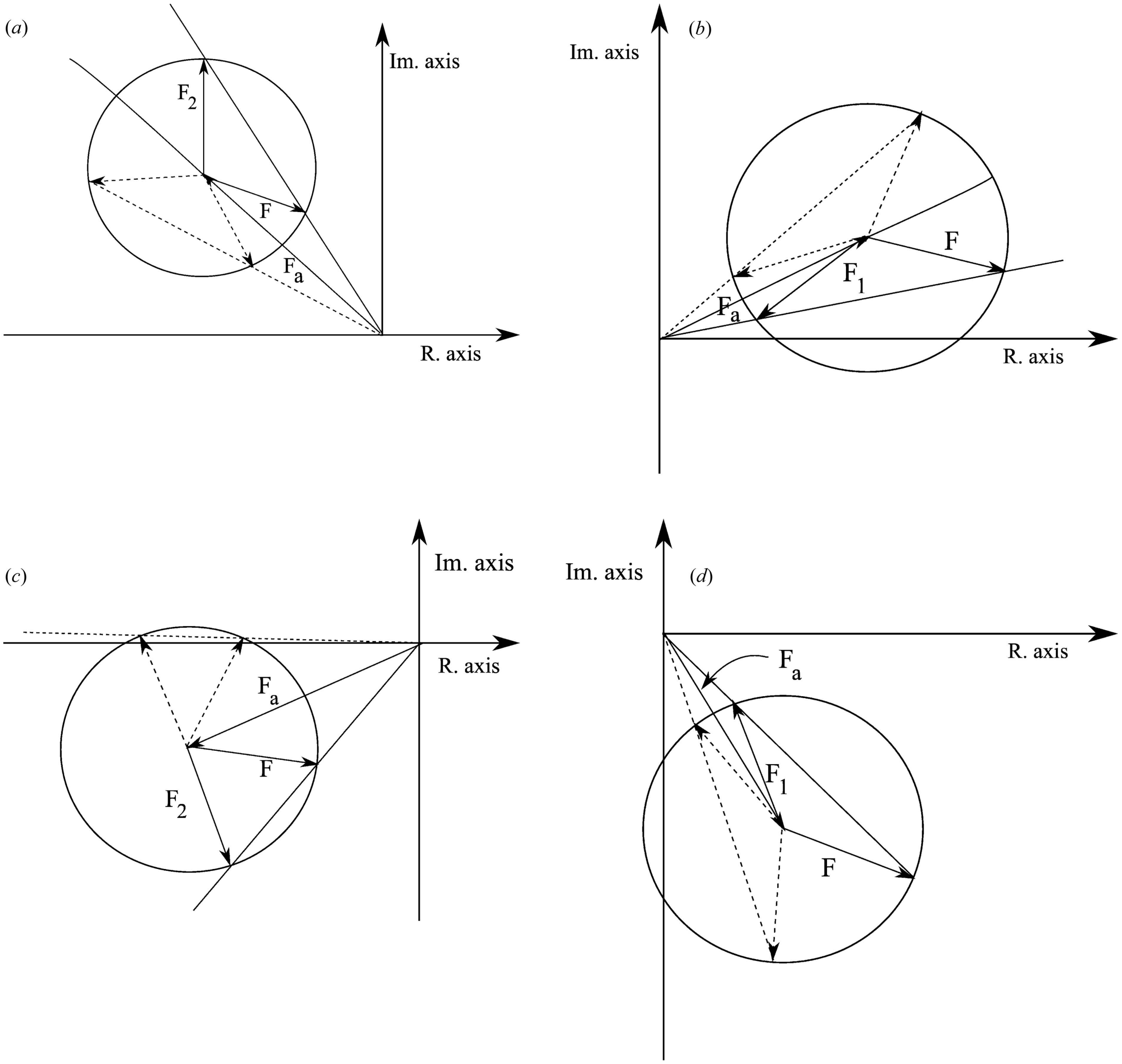

Figure 9
Diagrams for four derivatives with $|F_{\rm a}\,(j)\,|>|F|$ are illustrated in (*a*)–(*d*). The correct enantiomorphs are indicated by unbroken lines, the wrong ones by broken lines. In each case, given the correct enantiomorph, one has to choose between $F_{\rm d1}$ and $F_{\rm d2}$, and therefore between F_1 and F_2. The correct target structure factor in all the four diagrams is indicated by F. In all the four cases the correct enantiomorph corresponds to the positive value of $\mathrm{EN}=\sin(\varphi_{\rm d}-\varphi_{\rm a})\sin\varphi_{\rm a}$.

$$\mathrm{EN}_C = \sin[\varphi_{\rm dinv}(j) - \varphi_{\rm a}(j)] \times \cos\varphi_{\rm a}(j)$$
$$= \sin\varphi_{\rm dinv}(j)\cos^2\varphi_{\rm a}(j) = \sin\varphi_{\rm dinv}(j)$$

has the same sign for each derivative.

In order to find a criterion valid for the case in which the ancil electron density satisfies the same space group of the target structure, let us consider, for a fixed reflection (hkl), the four diagrams in Fig. 9. Each diagram corresponds to a different derivative. We assumed for all the four cases that $|F_{\rm a}(j)| > |F|$, but the conclusions will also be valid if for some or for all the derivatives $|F_{\rm a}(j)| < |F|$. In Fig. 9(a) we will suppose that the correct enantiomorph is indicated by unbroken lines, while the broken lines correspond to the wrong one. The same notation is adopted in Figs. 9(b)–9(d). By hypothesis the condition characterizing the correct target enantiomorph for all the four depicted cases is the positive value of

$$\mathrm{EN} = \sin(\varphi_{\rm d} - \varphi_{\rm a})\sin\varphi_{\rm a},$$

the wrong one by a negative value of EN. We then assume EN as a coherence criterion: for a given reflection (hkl) the correct enantiomorph is coherently defined by all the derivatives if EN has the same sign for all of them. As for centric ancil structures, in practical applications we cannot guess whether EN > 0 defines for a given (hkl) reflection the correct or the wrong enantiomorph, but the criterion may help us to decide if the various derivatives provide for $\varphi_{\rm d}$ the same or different enantiomorphs.

APPENDIX *D*

In the MIR case the enantiomorph problem is automatically solved by the combined use of more derivatives; in the MPhD approach the solution of the problem is more difficult, particularly in the initial step of the phasing process where we are obliged to assume $\varphi_{\rm d} = \varphi_{\rm a}$ and amplitudes given by (15). In the absence of observed derivative amplitudes the use of equation (15) in EDM procedures may drive the initial derivative model towards $\rho_{\rm d}(\mathbf{r}) = \rho_{\rm a}(\mathbf{r}) + \rho(\mathbf{r})$, or towards $\tilde{\rho}_{\rm d}(\mathbf{r}) = \rho_{\rm a}(\mathbf{r}) + \rho(-\mathbf{r})$ (see §6). Similar problems may be encountered when EDM techniques are applied by using hybrid Fourier syntheses (see §8). In this Appendix we treat the following problem: in the initial MPhD steps it may occur that, for the same reflection (hkl), for one derivative the EDM procedure suggests $\sin[\varphi_{\rm dinv}(j) - \varphi_{\rm d}(j)]$ values with sign opposite to that necessary for making the derivative phase closer to the true one, and for another derivative the sign may be the same. An algorithm for checking how coherent are the derivatives in suggesting the same sign for $\sin[\varphi_{\rm dinv}(j) - \varphi_{\rm d}(j)]$, and for eventually improving their coherence, is here described.

For simplicity, let us first consider a two derivative case, under the assumption that, for a target structure with NREF observed reflections, EDM techniques are able to estimate the correct enantiomorph with a frequency $g > 0.5$ for each of the two derivative structures. For the moment we will consider g equal for all the reflections and for all the derivatives: in reality, it depends on the specific reflection and on the specific jth derivative, so that g should be replaced by $g_j(hkl)$ to make more evident the dependence. This generalization is not included here: presently the approximation g = const. will be assumed. In the first steps of the MPhD approach g is not expected to be larger than 0.55–0.60. Consider first Figs. 7(a) and 7(b), obtained when the ancil structure symmetry complies with a centric supergroup of the target group. Three alternatives may occur at a given EDM cycle for the selected (hkl) reflection:

(i) The correct enantiomorph is simultaneously (and independently) defined in both the figures. The occurrence of this event will be proportional to g^2.

(ii) The false enantiomorph is simultaneously suggested in both the figures. The occurrence of this event is proportional to $(1 - g)^2$.

(iii) Two opposite enantiomorphs are suggested: it may occur two times, the first when the correct enantiomorph is found in Fig. 7(a), and the wrong one is found in Fig. 7(b), the second time when the indications are reversed. The overall frequency is $2g \times (1 - g)$.

The cases (i) and (ii) may be separated from the cases (iii) if it is possible to define a criterion, say EN_C (see Appendix C), which is positive for coherent enantiomorph indications, and negative for contradictory enantiomorph indications. In the case illustrated in Fig. 7 $\mathrm{EN}_C = \sin\varphi_{\rm d}$. However, in practical applications it is impossible to distinguish between (i) and (ii): thus the reflections belonging to (i) or (ii) may be regrouped in a *Group* to which $\mathrm{NREF}[g^2 + (1 - g)^2]$ reflections are expected to belong. For $\mathrm{NREF} \times g^2$ of them the correct enantiomorph is probably chosen, for $\mathrm{NREF} \times (1 - g)^2$ the wrong enantiomorph is probably indicated.

As a numerical example, let us suppose that NREF = 30 000 and $g = 0.6$. In accordance with definitions, it may be expected that for each derivative the EDM procedures will indicate the correct enantiomorph for about $\mathrm{NREF} \times g$ = 18 000 reflections. A coherent enantiomorph will be indicated for $\mathrm{NREF}[g^2 + (1 - g)^2]$ = 15 600 reflections, and for $\mathrm{NREF} \times g^2$ = 10 800 of them the correct enantiomorph is expected to be suggested. The frequency of the correct enantiomorph over the 15 600 selected reflections is $g^2/[g^2 + (1 - g)^2] = 0.69$, instead of the starting 0.60 frequency. The gain of information is however compensated by the loss of information on the remaining 14 400 reflections, for which the probability of finding the correct enantiomorph is 0.50.

We can now extend the above observations to the general case of n derivatives. We will indicate by c the case in which a derivative provides the correct enantiomorph and by w the opposite case. We will distinguish between:

Case 0: denoted as c^n. All the n derivatives suggest the correct enantiomorph (we have here zero contrasting indications: that explains the notation). The number of reflections falling in this case is expected to be $\mathrm{NREF} \times g^n$.

Case 1: denoted $c^{n-1}w$. All the derivatives except one suggest a coherent enantiomorph. This case includes all the permutations $wcc\ldots c, cwc\ldots c, ccc\ldots w$. Their number is equal to n, which is the number of permutations of n elements, one of which is repeated one time and the second repeated $(n - 1)$ times. We regroup all the permutations in a unique case. The reflections belonging to it are expected to be NREF $\times\, n \times g^{n-1}(1-g)$.

Case 2: denoted $c^{n-2}w^2$. All the derivatives except two suggest a coherent enantiomorph. This case includes all the permutations *wwcc . . . c*, *wcwc . . . c etc.* Their number is equal to $n!/[2!(n-2)!]$, which is the number of permutations of n elements, one of which is repeated two times and the second repeated $(n-2)$ times. The number of reflections falling in this *Case* is NREF$\{n!/[2!(n-2)!]\}g^{n-2}(1-g)^2$.

Case n − 2: denoted as c^2w^{n-2}. The number of reflections belonging to it is NREF$\{n!/[2!(n-2)!]\}g^2(1-g)^{n-2}$.

Case n − 1: denoted as cw^{n-1}. The number of reflections belonging to it is NREF $\times\, n \times g(1-g)^{n-1}$.

Case n: denoted as w^n. The number of reflections belonging to it is NREF$(1-g)^n$.

The number of reflections belonging to the *Case k* is therefore

$$\mathrm{NREF}\frac{n!}{k!(n-k)!}g^k(1-g)^{n-k}, \qquad (91)$$

say, the number of reflections multiplied by the binomial distribution for n Bernoulli trials. Analogously, the number of reflections belonging to the *Case n − k* will be

$$\mathrm{NREF}\frac{n!}{k!(n-k)!}g^{n-k}(1-g)^k. \qquad (92)$$

As stated before, we are unable to experimentally distinguish the *Case k* from the *Case n − k*. Thus we will regroup them together in the *Group k*, whose occurrence frequency is

$$\mathrm{OCC}(k) = \frac{n!}{k!(n-k)!}t[g^{n-k}(1-g)^k + g^k(1-g)^{n-k}], \qquad (93)$$

where k goes from 0 to $n/2$ if n is even, from 0 to $(n-1)/2$ if n is odd. $t = 1$ when $k \neq (n-k)$ and $t = 1/2$ when $k = (n-k)$. The number of reflections belonging to the *Group k* is

$$\mathrm{REF}(k) = \mathrm{NREF} \times \mathrm{OCC}(k). \qquad (94)$$

The frequency of finding the correct enantiomorph among the reflections belonging to the *Group k* is expected to be the ratio:

$$\begin{aligned}\mathrm{ENANT}(k)\\ &= \frac{[(n-k)g^{n-k}(1-g)^k + kg^k(1-g)^{n-k}]}{[(n-k)g^k(1-g)^{n-k} + kg^{n-k}(1-g)^k] + [kg^k(1-g)^{n-k} + (n-k)g^{n-k}(1-g)^k]}\\ &= \frac{[(n-k)g^{n-k}(1-g)^k + kg^k(1-g)^{n-k}]}{n[g^k(1-g)^{n-k} + g^{n-k}(1-g)^k]}. \qquad (95)\end{aligned}$$

At numerator and denominator the coefficients k and $n-k$ correspond to the number of c symbols in each permutation.

Let us now consider, as a numerical example, the three derivative cases illustrated in Fig. 7. The possible *Cases* are four, defined as: *Case 0*, permutations of type *ccc*; *Case 1*, permutations of type *ccw, cwc, wcc*; *Case 2*, permutations of type *wwc*, *wcw*, *cww*; *Case 3*, permutations of type *www*.

The *Cases 0* and *3* may be regrouped in the *Group 0* because, for each reflection belonging to it, $\mathrm{EN}_C > 0$ or $\mathrm{EN}_C < 0$ for all the three derivatives. The corresponding expected number of reflections is

$$\mathrm{REF}(0) = \mathrm{NREF} \times [g^3 + (1-g)^3] = 8400;$$

6480 are expected to have the correct enantiomorph, 1920 reflections are expected to have the wrong one. For the subset of the 8400 reflections selected above the probability of finding the correct enantiomorph is 0.77, much larger than g.

The *Cases 1* and *2* are regrouped in the *Group 1*. The corresponding expected number of reflections is

$$\begin{aligned}\mathrm{REF}(1) &= \mathrm{NREF} \times 3[g^2(1-g) + g(1-g)^2] = 21\,600,\\ \mathrm{ENANT}(1) &= [2g^2(1-g) + g(1-g)^2]/[3g^2(1-g) + 3g(1-g)^2]\\ &= 0.522.\end{aligned}$$

Again, the higher probability of defining the correct enantiomorph for the *Group 0* has its counterpart in the worst (less than 0.6) probability for the *Group 1*.

How to exploit the above theoretical considerations in order to improve the coherence and the correctness of the enantiomorph indications, as available at a given step of an EDM procedure? Let us consider, for an n derivative case, the *Group j* (with $j \neq 0$): for a certain number of reflections the correct enantiomorph is coherently defined by $n-j$ derivatives in opposition to the enantiomorph suggested by j derivatives. If $n-j$ is sufficiently larger than j, then the $n-j$ enantiomorph indications will have a large probability of being true. It is then advisable to replace the original enantiomorph defined for the j derivatives by the enantiomorph suggested by the $n-j$ derivatives.

APPENDIX *E*

The complete freedom in choosing the ancil structures allows PhD to profit from special relationships between two ancil structures, because they may generate additional constraints. For example, two ancils might have some atomic positions in common while the rest of the two structures is randomly fixed. This condition may help to fix the enantiomorph during PhD phasing.

Let us briefly consider the case in which $\rho_{\rm a}(\mathbf{r})$ is the electron density of the first ancil and $\rho_{\rm a}(-\mathbf{r})$ is the density of the second ancil. Then

$$|F_{\rm d}(1)|\exp[i\varphi_{\rm d}(1)] = |F|\exp(i\varphi) + |F_{\rm a}(1)|\exp[i\varphi_{\rm a}(1)] \qquad (96)$$

and

$$|F_{\rm d}(2)|\exp[i\varphi_{\rm d}(2)] = |F|\exp(i\varphi) + |F_{\rm a}(1)|\exp[-i\varphi_{\rm a}(1)], \qquad (97)$$

where $|F_{\rm d}(1)|$, $\varphi_{\rm d}(1)$ are, respectively, amplitude and phase corresponding to the first derivative and $|F_{\rm d}(2)|$, $\varphi_{\rm d}(2)$ are, respectively, amplitude and phase corresponding to the second derivative. From equations (96) and (97) we obtain

$$|F_{\rm d}(1)|^2 = |F_{\rm a}(1)|^2 + |F|^2 + 2|F_{\rm a}(1)F|\cos[\varphi_{\rm a}(1) - \varphi]$$

and

$$|F_{\rm d}(2)|^2 = |F_{\rm a}(1)|^2 + |F|^2 + 2|F_{\rm a}(1)F|\cos[\varphi_{\rm a}(1) + \varphi],$$

from which

$$|F_{\rm d}(1)|^2 - |F_{\rm d}(2)|^2 = 4|F_{\rm a}(1)F|\sin\varphi_{\rm a}(1)\sin\varphi.$$

The two derivatives will have the same amplitude only if $\varphi = 0, \pi$. Four additional relationships arise:

$$\begin{aligned}|F_{\rm d}(1)|\cos\varphi_{\rm d}(1) + |F_{\rm d}(2)|\cos\varphi_{\rm d}(2)&\\ = 2|F|\cos\varphi + 2|F_{\rm a}(1)|\cos\varphi_{\rm a}(1),&\end{aligned} \tag{98}$$

$$|F_{\rm d}(1)|\cos\varphi_{\rm d}(1) - |F_{\rm d}(2)|\cos\varphi_{\rm d}(2) = 0, \tag{99}$$

$$|F_{\rm d}(1)|\sin\varphi_{\rm d}(1) + |F_{\rm d}(2)|\sin\varphi_{\rm d}(2) = 2|F|\sin\varphi, \tag{100}$$

$$|F_{\rm d}(1)|\sin\varphi_{\rm d}(1) - |F_{\rm d}(2)|\sin\varphi_{\rm d}(2) = 2|F_{\rm a}(1)|\sin\varphi_{\rm a}(1). \tag{101}$$

Their main advantages are the following:

Equation (99) establishes a restraint on the values of $|F_{\rm d}(2)|$ and $\varphi_{\rm d}(2)$ when $|F_{\rm d}(1)|$ and $\varphi_{\rm d}(1)$ have been estimated. In particular $\cos[\varphi_{\rm d}(2)]$ and $\cos[\varphi_{\rm d}(1)]$ must have the same sign. That is, if the two derivative phases, once subjected to EDM techniques, independently migrate so that the two cosines have opposite signs, then the EDM indication violates the expected relationship.

When $\varphi_{\rm a}$ is occasionally close to 0 or π, $\sin\varphi_{\rm d}(1)$ and $\sin\varphi_{\rm d}(2)$ must have the same sign [see equation (101)]. Again, the expectation is violated if an EDM procedure ends with opposite signs for the two sines. It may also be noticed that if $\sin\varphi_{\rm d}(1)$ and $\sin\varphi_{\rm d}(2)$ have the same sign, that is also the sign of $\sin\varphi$, according to equation (100).

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