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General formalism for phase combination and phase refinement: a statistical thermodynamics approach in reciprocal space

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The mean-field optimization methodology has been used to recast in a single formalism the problem of phase optimization using an arbitrary energy function in the presence of an experimentally determined phase probability distribution function. It results naturally in the generalization of the notions of figure of merit and centroid phase where the weight of the energy refinement is controlled by an effective temperature in a self-consistent manner. In the limit of high temperature, the formalism reduces of course to the Blow & Crick [*Acta Cryst.* (1959), **12**, 794–802] classical treatment. If a model is available, Sim's [*Acta Cryst.* (1960), **13**, 511–512] weighting scheme for a combined map appears as the first step of a refinement to be conducted until self-consistency is achieved. Assuming that MIR phases exist and that they agree reasonably well with the phases of the model, a first-order expansion gives an estimate of the changes of weights and phases to be performed for the Fourier synthesis. This provides for a new way of doing phase combination that might prove useful in challenging cases of model refinement, *e.g.* in large macromolecular complexes. Thermodynamic considerations have been used to discuss the best determination of weights in phase refinement; they also suggest that a variational expression of maximum likelihood is best suited as a target for refinement because it is the free energy of the system. The formalism readily allows use of solvent flattening, density averaging and the atomicity criterion to refine phases, and automatically assigns a figure of merit to each reflection. Numerical tests of the method are presented in an attempt to resolve the phase-ambiguity problem of protein crystallography in the centrosymmetric $P\bar{1}$ space group using an energy derived from the Sayre equation.

1. Introduction

The aim of this paper is to find a general formalism for phase refinement, where an energy expressed in reciprocal space is being minimized, in the presence of an experimental phase probability distribution function. In the absence of any energy refinement, the classical probabilistic treatment of this problem, as originally proposed by Blow & Crick (1959), is to use figure-of-merit (fom) weighted structure factors and centroid phases to calculate the best map. Then, real-space density-modification techniques are used to improve the phases and facilitate map interpretation and model building. Conversely, if a partial model is available, the Sim weighting scheme is classically applied (Sim, 1959, 1960) before combining this information with that from experiment (Bricogne, 1976).

In the course of the work presented in a recent paper (Delarue, 2000), where Monte Carlo techniques were employed to break phase ambiguity in the $P\bar{1}$ centrosymmetric space group, a formal analogy was drawn between the phase problem and the so-called Ising spin system, a classical model system intensively studied in condensed-matter physics. Here, the analogy is further pursued by employing the technique of mean-field optimization. This method is at the basis of the solution of many statistical thermodynamics problems whenever the combinatorics cannot be explored exhaustively [see *e.g.* Koehl & Delarue (1996) for an application in homology modelling problems]; it is also essential to the theory of neural networks (Hertz *et al.*, 1991).

The formal analogy of some problems in phase determination in crystallography and the Ising system or even the spin-glass problem has been noted earlier (Venkatesan, 1991).

The connection between crystallography and thermodynamics was used with success for small-molecule crystallography (Khachaturyan *et al.*, 1981; Semenovskaya *et al.*, 1985); in this case, the theory was expressed in real space. Here, an entirely new application of mean-field optimization theory derived from statistical mechanics is presented in reciprocal space to refine phases in protein crystallography. Statistical thermodynamics is the natural framework to study this problem because, loosely speaking, the phase distribution probability function contributes to the entropy of the system, which competes with the energy being refined. It results in the generalization of the notion of figure of merit and centroid phase of Blow & Crick (1959) with the weight of the energy being controlled by an effective temperature in a self-consistent manner.

This paper is organized as follows: in the following section, the general theory of mean-field optimization of a system of interacting reflections in any space group is presented, in the presence of biased phase probability distribution functions. Special attention is devoted to the phase-refinement case, with two extreme cases treated in detail: (i) the Sim weighting scheme is recovered and extended to a self-consistent formulation; and (ii) if MIR phase information is available, the best Fourier coefficients for a combined map are rederived: this leads to an entire new view in phase combination. Then, the evaluation of classical thermodynamic functions such as entropy and free energy offers a new interpretation of the maximum-likelihood target function. Finally, the problem of choosing the best temperature is examined in the context of the linear response theory of thermodynamics. Other energy functions to be refined are presented in a general form, all of them derived from real-space constraints, but expressed in reciprocal space, and briefly discussed. The analytical treatment of the minimization of an energy function based on solvent flattening or on the atomicity criterion (for which the formalisms are very similar) is introduced in the main text, while most of the formal derivations on this subject are deferred to Appendices *A* and *B*. The rest of the paper is devoted to numerical tests of the theory.

2. General theory

Suppose that an experimental phase distribution function $P(\varphi_k)$ is available and that one wants to choose, for each reflection k, the phase φ_k that minimizes a global energy function $E(\{\varphi_k\})$ to be specified later. The classical statistical thermodynamics treatment of this problem starts with the evaluation of the partition function Z, which reads:

$$Z = \int \prod_k \mathrm{d}\varphi_k \, P(\varphi_k) \exp[-\beta E(\{\varphi_k\})], \qquad (1)$$

where the integral over each $\mathrm{d}\varphi_k$ is to be performed between 0 and 2π and where $\prod_k \mathrm{d}\varphi_k P(\varphi_k)$ can be seen as the Lebesgue measure of integration of the microcanonical states of phase sets $\{\varphi_k\}$ of energy $E(\{\varphi_k\})$ and Boltzmann weights $\exp[-\beta E(\{\varphi_k\})]$. This partition function Z can be written, with the help of the Dirac delta function δ, as

$$Z = \int \prod_k \mathrm{d}\varphi_k \int \prod_k \mathrm{d}z_k \prod_k \mathrm{d}z_k^* P(\varphi_k)\delta[z_k - \exp(i\varphi_k)]$$
$$\times \, \delta[z_k^* - \exp(-i\varphi_k)] \exp[-\beta E(\{z_k^*, z_k\})]. \qquad (2)$$

In this form, it can be seen that it is now possible to perform the integral over the phase probability distribution, *i.e.* over the $\mathrm{d}\varphi_k$. Indeed, exponentiating the δ function (see for instance Negele & Orland, 1987), using an analogue in complex space of the well known identity

$$\delta(x - x_0) = 1/2\pi \int \mathrm{d}y \exp[iy(x - x_0)] \qquad (3)$$

and omitting the unessential normalization factor $1/2\pi$, equation (2) now reads

$$Z = \int \prod_k \mathrm{d}\varphi_k \int \prod_k \mathrm{d}z_k \prod_k \mathrm{d}\zeta_k \int \prod_k z_k^* \prod_k \mathrm{d}\zeta_k^* P(\varphi_k)$$
$$\times \exp\{i\zeta_k[z_k - \exp(i\varphi_k)]\} \exp\{i\zeta_k^*[z_k^* - \exp(-i\varphi_k)]\}$$
$$\times \exp[-\beta E(\{z_k^*, z_k\})]. \qquad (4)$$

Rearranging the order of integration, one finds

$$Z = \int \prod_k \mathrm{d}z_k \prod_k \mathrm{d}\zeta_k \int \prod_k \mathrm{d}z_k^* \prod_k \mathrm{d}\zeta_k^* \exp\left\{ -\beta E(\{z_k^*, z_k\}) \right.$$
$$+ \sum_k i\zeta_k z_k + i\zeta_k^* z_k^* + \log \int_0^{2\pi} P(\varphi_k) \, \mathrm{d}\varphi_k \exp[-i\zeta_k \exp(i\varphi_k)$$
$$\left. - i\zeta_k^* \exp(-i\varphi_k)] \right\}. \qquad (5)$$

In effect, we have now built a field theory of the phase-refinement problem in crystallography; the ζ_k and ζ_k^* are the conjugate fields of z_k and z_k^*, respectively. Mean-field theory consists in evaluating this integral through the saddle-point approximation, which amounts to looking for the values of the conjugate fields that minimize the exponent in the integral in (5) (see, for instance, Negele & Orland, 1987; Chaikin & Lubensky, 1995). Altogether, this gives four equations by differentiating the exponent in the integral with respect to z_k, z_k^*, $i\zeta_k$ and $i\zeta_k^*$; all these mean-field equations must be satisfied simultaneously:

(i) Derivatives of the exponent in (5) with respect to z_k and z_k^* give

$$i\zeta_k = \beta(\partial E/\partial z_k)_{\mathrm{MF}} \quad \text{and} \quad i\zeta_k^* = \beta(\partial E/\partial z_k^*)_{\mathrm{MF}}, \qquad (6a)$$

which are the local fields felt by reflection k, *to be evaluated at the saddle point MF*.

(ii) Derivatives of the exponent in (5) with respect to $i\zeta_k$ and $i\zeta_k^*$ give

$$z_k^{\mathrm{MF}} = \langle \exp(i\varphi_k) \rangle_{\mathrm{MF}} \quad \text{and} \quad z_k^{*\mathrm{MF}} = \langle \exp(-i\varphi_k) \rangle_{\mathrm{MF}}, \qquad (6b)$$

where we have defined

$$\langle \exp(i\varphi_k)\rangle_{\mathrm{MF}} = \left\{ \int_0^{2\pi} P(\varphi_k)\,\mathrm{d}\varphi_k\,\exp(i\varphi_k)\exp[-i\zeta_k\exp(i\varphi_k)\right.$$
$$\left. - i\zeta_k^*\exp(-i\varphi_k)]\right\}\left\{\int_0^{2\pi} P(\varphi_k)\,\mathrm{d}\varphi_k\right.$$
$$\left. \times\exp[-i\zeta_k\exp(i\varphi_k)-i\zeta_k^*\exp(-i\varphi_k)]\right\}^{-1} \quad (7a)$$

and

$$\langle \exp(-i\varphi_k)\rangle_{\mathrm{MF}} = \langle\exp(i\varphi_k)\rangle_{\mathrm{MF}}^*. \quad (7b)$$

Note that $\langle\exp(i\varphi_k)\rangle_{\mathrm{MF}}$ is no longer of modulus 1 but should be written $\mu_k^{\mathrm{MF}}\exp(i\varphi_k^{\mathrm{MF}})$ instead. μ_k^{MF} plays the role of a generalized figure of merit [see equation (9) below].

Replacing $i\zeta_k$ and $i\zeta_k^*$ by their values using (6a), one finally gets from (6b):

$$z_k^{\mathrm{MF}} = \left\{\int_0^{2\pi} P(\varphi_k)\,\mathrm{d}\varphi_k\,\exp(i\varphi_k)\exp[-\beta(\partial E/\partial z_k)\exp(i\varphi_k)\right.$$
$$\left. - \beta\partial(E/\partial z_k^*)\exp(-i\varphi_k)]\right\}\left\{\int_0^{2\pi} P(\varphi_k)\,\mathrm{d}\varphi_k\right.$$
$$\left. \times\exp[-\beta(\partial E/\partial z_k)\exp(i\varphi_k)-\beta(\partial E/\partial z_k^*)\exp(-i\varphi_k)]\right\}^{-1} $$
$$(8a)$$

and its companion complex-conjugate equation:

$$z_k^{\mathrm{MF}*} = \left\{\int_0^{2\pi} P(\varphi_k)\,\mathrm{d}\varphi_k\,\exp(-i\varphi_k)\exp[-\beta(\partial E/\partial z_k)\exp(i\varphi_k)\right.$$
$$\left. - \beta(\partial E/\partial z_k^*)\exp(-i\varphi_k)]\right\}\left\{\int_0^{2\pi} P(\varphi_k)\,\mathrm{d}\varphi_k\right.$$
$$\left. \times\exp[-\beta(\partial E/\partial z_k)\exp(i\varphi_k)\right.$$
$$\left. - \beta(\partial E/\partial z_k^*)\exp(-i\varphi_k)]\right\}^{-1}. \quad (8b)$$

More generally, equations (8) can be recast into

$$\mu_k^{\mathrm{MF}} = \frac{\int_0^{2\pi} P(\varphi_k)\,\mathrm{d}\varphi_k\,\exp(i\Delta\varphi_k^{\mathrm{MF}})\exp(-\beta Y_k)}{\int_0^{2\pi} P(\varphi_k)\,\mathrm{d}\varphi_k\,\exp(-\beta Y_k)}, \quad (9)$$

where

$$Y_k = (\partial E/\partial z_k)_{\mathrm{MF}}\exp(i\varphi_k) + (\partial E/\partial z_k^*)_{\mathrm{MF}}\exp(-i\varphi_k) \quad (10)$$

is a real number and where we have set $\Delta\varphi_k^{\mathrm{MF}} = \varphi_k - \varphi_k^{\mathrm{MF}}$ for simplicity.

Equations (8) or (9) are self-consistent equations that can be solved numerically iteratively by the Picard method (see below) in reciprocal space, provided that the energy that one wishes to minimize can be expressed in reciprocal space. The route we have taken to derive them is somewhat more complicated but completely equivalent to the simpler and classical variational mean-field treatment (Chaikin & Lubensky, 1995). A formal expression of the variational free energy being minimized by (9) will be provided later in the text.

Basically, what happens is that, in order to perform the phase integration in (2), we have somewhat loosened the constraint on the phases to lie on the unit circle and this results naturally in the weighting of each reflection by a *generalized figure of merit*, which we denote by μ_k^{MF}.

Equations (8) and (9) are the main results of this paper. They can be applied to any energy E to refine and extend phases. It is interesting to check a few points about equation (9).

First, from this equation, the Blow & Crick (1959) formalism is recovered immediately, provided that E depends neither on z_k nor on z_k^* (or $\beta = 0$), *i.e.* there is no phase refinement performed. Indeed, in this case the real part of (9) reads

$$\mu_k^{\mathrm{MF}} = \int_0^{2\pi} P(\varphi_k)\,\mathrm{d}\varphi_k\,\cos\Delta\varphi_k^{\mathrm{MF}}\bigg/\int_0^{2\pi} P(\varphi_k)\,\mathrm{d}\varphi_k$$
$$= \langle\cos\Delta\varphi_k^{\mathrm{BC}}\rangle_{\beta=0}, \quad (11)$$

which is just the so-called Blow & Crick (1959) figure of merit (again, we have set $\Delta\varphi_k^{\mathrm{BC}} = \varphi_k - \varphi_k^{\mathrm{BC}}$). As usual, the imaginary part of (9) helps define the centroid phase φ_k^{BC}.

Second, if the phase distribution is flat [average distribution (AD)], *i.e.* contains no information [$P(\varphi_k) = 1/2\pi$], (9) reads

$$\mu_k^{\mathrm{MF}} = \int_0^{2\pi}\mathrm{d}\Delta\varphi_k\,\cos\Delta\varphi_k^{\mathrm{MF}}\exp(-\beta Y_k)\bigg/\int_0^{2\pi}\mathrm{d}\Delta\varphi_k\,\exp(-\beta Y_k)$$
$$= \langle\cos\Delta\varphi_k^{\mathrm{AD}}\rangle_{P(\varphi_k)=1/2\pi}, \quad (12)$$

which can often be rearranged using modified Bessel functions if Y_k is linear in $\cos\Delta\varphi_k^{\mathrm{MF}}$, as shown in the following section.

3. Application to model refinement

3.1. Phase combination

If a partial model is available, the energy to be minimized may be expressed as $\sum_{\mathbf{r}}[\Delta\rho(\mathbf{r})]^2$ in real space, where $\Delta\rho(\mathbf{r})$ is the difference between the true electron density and that of the model at grid point $\mathbf{r}$. Using Parseval's theorem, this simply amounts to minimizing

$$E = \sum_{\mathbf{k}}|\mathbf{F}_{\mathrm{obs}}(\mathbf{k}) - \mathbf{F}_{\mathrm{calc}}(\mathbf{k})|^2$$
$$= \sum_{\mathbf{k}}[z_k^* F_{\mathrm{obs}}(\mathbf{k}) - \exp(-i\varphi_k^{\mathrm{calc}})F_{\mathrm{calc}}(\mathbf{k})]$$
$$\times [z_k F_{\mathrm{obs}}(\mathbf{k}) - \exp(i\varphi_k^{\mathrm{calc}})F_{\mathrm{calc}}(\mathbf{k})], \quad (13)$$

where it is understood that F_{calc} and F_{obs} have been scaled together and where we have written

$$\mathbf{F}_{\mathrm{calc}}(\mathbf{k}) = F_{\mathrm{calc}}(\mathbf{k})\exp(i\varphi_k^{\mathrm{calc}})$$

and

$$\mathbf{F}_{\mathrm{obs}}(\mathbf{k}) = z_k F_{\mathrm{obs}}(\mathbf{k}) = \mu_k\exp(i\varphi_k)F_{\mathrm{obs}}(\mathbf{k}), \quad (14)$$

anticipating the fact that all structure factors are ultimately to be weighted by a figure of merit.

In this kind of situation where the energy is quadratic (and only in this situation), the mean-field approximation is exact. Indeed, in this case, all the integrals are Gaussian integrals that can be evaluated directly and that lead to the same results

as if evaluated by saddle-point methods (see *e.g.* Mathews & Walker, 1970).

Now, the derivatives $\partial E/\partial z_k$ and $\partial E/\partial z_k^*$ can be calculated explicitly. Indeed, setting

$$\lambda_k = F_{\text{obs}}(\mathbf{k})/F_{\text{calc}}(\mathbf{k}) \tag{15}$$

and

$$X_k = 2F_{\text{obs}}(\mathbf{k})F_{\text{calc}}(\mathbf{k}), \tag{16}$$

one can then write, using the notations $\Delta\varphi_k^{\text{calc}} = \varphi_k - \varphi_k^{\text{calc}}$ and $\Delta\varphi_k^{\text{MF}} = \varphi_k - \varphi_k^{\text{MF}}$:

$$\mu_k^{\text{MF}} = \left\{ \int_0^{2\pi} P(\varphi_k)\,\mathrm{d}\varphi_k \exp(i\Delta\varphi_k^{\text{MF}}) \exp[\beta X_k(\cos\Delta\varphi_k^{\text{calc}} \right.$$
$$\left. - \lambda_k\mu_k^{\text{MF}}\cos\Delta\varphi_k^{\text{MF}})] \right\} \left\{ \int_0^{2\pi} P(\varphi_k)\,\mathrm{d}\varphi_k \exp[\beta X_k(\cos\Delta\varphi_k^{\text{calc}} \right.$$
$$\left. - \lambda_k\mu_k^{\text{MF}}\cos\Delta\varphi_k^{\text{MF}})] \right\}^{-1}. \tag{17}$$

This is equivalent to setting

$$Y_k = -X_k(\cos\Delta\varphi_k^{\text{calc}} - \lambda_k\mu_k^{\text{MF}}\cos\Delta\varphi_k^{\text{MF}}) \tag{18}$$

in (9).

Both real and imaginary parts of (17) will define the centroid mean-field phase φ_k^{MF} and the generalized figure of merit μ_k^{MF} to be used in the Fourier synthesis, through the simple relations

$$\mu_k^{\text{MF}} = \langle\cos\Delta\varphi_k^{\text{MF}}\rangle_\beta$$

and

$$\tan\varphi_k^{\text{MF}} = \langle\sin\varphi_k\rangle_\beta/\langle\cos\varphi_k\rangle_\beta.$$

It is the second term in the sum of the exponent in (17) that makes this equation self-consistent and different from previous treatments of the same problem.

The new mean-field phase φ_k^{MF} is the combined phase and μ_k^{MF} its combined figure of merit. What is appealing in this formulation is that all kinds of information (experimental phase information, current state of the model-building process) are put automatically on the same footing. In other words, phase combination is dealt with in a natural way. Other energy constraints can also be easily taken into account, including physical constraints on the electron-density map (Arnold & Rossmann, 1986, see below), expressed in reciprocal space.

If the phase probability distribution function is known through heavy-atom derivatives, it is always possible to set

$$P(\varphi_k) = \exp(A_k\cos\varphi_k + B_k\sin\varphi_k + C_k\cos 2\varphi_k$$
$$+ D_k\sin 2\varphi_k + K), \tag{19}$$

where K is a scaling factor and A_k, B_k, C_k and D_k are Hendrickson & Lattman coefficients (see *e.g.* Drenth, 1994); it then becomes possible to write (17) in a somewhat more compact analytical form. However, in most cases, it will be more convenient to integrate (17) numerically.

In the following two sections, we will show that (17) reduces to known equations in two limiting cases. We will also show

that, even in these two limiting cases, it is necessary to work with their fully self-consistent expression.

3.2. Self-consistent Sim weighting scheme

Equation (17) can be applied in the situation where there is no experimental *a priori* phase information, but a partial model $F_{\text{part}} = F_{\text{calc}}$ is available; the classical treatment of this situation was first given by Sim (1959, 1960). Indeed, if the phase probability distribution function is flat, $P(\varphi_k)\,\mathrm{d}\varphi_k = 1/2\pi\,\mathrm{d}\varphi_k$ and, if one postulates $\varphi_k^{\text{MF}} = \varphi_k^{\text{calc}}$, the real part of (17) gives

$$\mu_k^{\text{MF}} = \frac{\int_0^{2\pi}\mathrm{d}\Delta\varphi_k\cos\Delta\varphi_k^{\text{MF}}\exp[\beta X_k(1 - \lambda_k\mu_k^{\text{MF}})\cos\Delta\varphi_k^{\text{MF}}]}{\int_0^{2\pi}\mathrm{d}\Delta\varphi_k\exp[\beta X_k(1 - \lambda_k\mu_k^{\text{MF}})\cos\Delta\varphi_k^{\text{MF}}]}$$
$$= I_1(\beta X_k[1 - \lambda_k\mu_k^{\text{MF}}])/I_0(\beta X_k[1 - \lambda_k\mu_k^{\text{MF}}]), \tag{20}$$

where I_1 and I_0 are modified Bessel functions of order 1 and 0, respectively.

In (20), one is naturally led to define an *effective inverse temperature* $\beta_k' = \beta(1 - \lambda_k\mu_k)$, which goes to zero if $\lambda_k\mu_k$ goes to 1; therefore, it has the nice feature of not doing any energy refinement if the figure of merit is already very good, *i.e.* close to 1, meaning that it will not spoil the available experimental phase information (see below).

Equation (20) is the self-consistent equation in μ_k^{MF} that is to be compared with Sim's treatment of the same problem (Sim, 1959, 1960). It is identical to Sim's weighting scheme *at the first cycle of the Picard iteration* (setting for instance $\mu_k^{(0)} = 0$) but may depart from it as the iteration process goes along. Actually, whatever the starting value of μ_k, (20) still resembles Sim's weighting scheme with the understanding that the normalization factor $1/\sum_i f_i^2$ has been absorbed in the inverse temperature β, to follow Sim's original weighting-scheme formulation (Sim, 1959, 1960). This normalization factor appears if one decides to minimize

$$E = \sum_{\mathbf{k}} |\mathbf{F}_{\text{obs}}(\mathbf{k}) - \mathbf{F}_{\text{calc}}(\mathbf{k})|^2 \bigg/ \sum_{\mathbf{k}} |\mathbf{F}_{\text{calc}}(\mathbf{k})|^2 \tag{21}$$

instead of the unnormalized energy defined in (13).

3.3. Best linear Fourier coefficients for the combined MIR map

Equation (17) tells us how to modify both the structure factors (weighting them with a figure of merit) and the phases in order to satisfy the energy-refinement criterion. This is in contrast with what is usually performed in the calculation of a combined map, where a linear combination of $\mu_k^{\text{MF}}F_{\text{obs}}(\mathbf{k})$ and $F_{\text{calc}}(\mathbf{k})$ is used (*e.g.* Read, 1986) as structure factors and where the phases are combined separately (Bricogne, 1976). We will now show that this can actually be seen as a special case of (17) under rather stringent but natural conditions.

To illustrate this point, let us assume that the corrections to Blow & Crick phases are small, *i.e.* that the experimental phases are not too far from the model currently being refined.

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Using the notation

$$\delta\mu_k^{MF} = 1 - \lambda_k\mu_k^{MF} \qquad (22)$$

and assuming $\delta\mu_k^{MF} \ll 1$, which is to be expected if the experimental phases agree reasonably well with the ones derived from the model, *i.e.* $\mu_k^{MF} \sim 1$, (17) now reads

$$\mu_k^{MF} = \frac{\int_0^{2\pi} P(\varphi_k)\,d\varphi_k \exp(i\Delta\varphi_k^{MF})\exp(\beta X_k\delta\mu_k^{MF}\cos\Delta\varphi_k^{MF})}{\int_0^{2\pi} P(\varphi_k)\,d\varphi_k \exp(\beta X_k\delta\mu_k^{MF}\cos\Delta\varphi_k^{MF})}. \qquad (23)$$

Now this can be developed to first order in $\delta\mu_k^{MF}$, leading to

$$\mu_k^{MF} = \mu_k^{BC} + \beta X_k\delta\mu_k^{MF}(\langle\cos^2\Delta\varphi_k^{MF}\rangle - \langle\cos\Delta\varphi_k^{MF}\rangle^2)_{\beta=0}, \qquad (24)$$

where it is the second moment of $\cos(\Delta\varphi_k^{MF})$ that appears in the first-order correction term. Again, the inverse temperature β controls the weight of the energy refinement. We note that, *at the first cycle of Picard refinement*, setting $\mu_k^{(0)} = \mu_k^{BC}$,

$$\mu_k^{MF} = \mu_k^{BC} + \beta X_k\sigma^2(\Delta\varphi_k^{MF})_{\beta=0}(1 - \lambda_k\mu_k^{BC}), \qquad (25)$$

where

$$\sigma^2(\Delta\varphi_k^{MF})_{\beta=0} = (\langle\cos^2\Delta\varphi_k^{MF}\rangle - \langle\cos\Delta\varphi_k^{MF}\rangle^2)_{\beta=0}$$
$$= \sigma^2(\Delta\varphi_k^{BC}) \qquad (26)$$

is the width of the distribution of the cosine of the phase difference with φ_k^{BC}.

We recall that $F_{obs}(\mathbf{k}) = \lambda_k F_{calc}(\mathbf{k})$ and $X_k = 2F_{obs}(\mathbf{k})F_{calc}(\mathbf{k})$ from (15) and (16) and set

$$D_k = 2\beta F_{obs}(\mathbf{k})^2\sigma^2(\Delta\varphi_k^{BC}), \qquad (27)$$

so the coefficients of the Fourier synthesis now read

$$F_{obs}(\mathbf{k})\mu_k^{MF} = \mu_k^{BC}(1 - D_k)F_{obs}(\mathbf{k}) + D_k F_{calc}(\mathbf{k}). \qquad (28)$$

This resembles what is usually performed during the refinement of a model, namely the coefficients of the Fourier synthesis (with φ_k^{calc}) phases are usually taken to be a linear combination of $\mu_k^{BC}F_{obs}(\mathbf{k})$ and $F_{calc}(\mathbf{k})$ (Main, 1979; Read, 1986).

The problem of choosing the right D_k coefficient amounts to choosing the temperature. First of all, it might be advisable to work with normalized structure E factors rather than normal F ones. In this case, the relationship $\langle E^2\rangle = 1$ suggests that the inverse temperature should be chosen to be of order 1. Second, if $\sigma^2(\Delta\varphi_k)_{BC} \ll 1$, the weight D_k of $F_{calc}(\mathbf{k})$ in the Fourier synthesis (28) is small, as it should be. We return to this problem in a following section in more detail (§3.5).

However, whatever the choice of the temperature, even if the first-order expansion is justified, *i.e.* if the model and the experimental phases agree well, (25) is just the first iteration of a process that must be pursued until self-consistency is achieved. In fact, (24) can be solved quite simply and directly for μ_k^{MF}.

In the general case where φ_k^{BC} departs significantly from φ_k^{calc}, the more general formula (17) is the one to be used for phase combination and refinement in its self-consistent version.

3.4. Variational treatment and definition of a new target function: maximum-likelihood revisited

If the partition function Z is known, all the usual thermodynamic quantities can be calculated. For instance, using the convention $k_B = 1$, the energy, free energy and entropy read, respectively:

$$U = -\partial\log Z/\partial\beta \qquad (29)$$
$$\mathcal{F} = -1/\beta\log Z = U - TS \qquad (30)$$
$$S = (U - \mathcal{F})/T. \qquad (31)$$

In particular, the free energy is interesting to compute explicitly since this is the quantity minimized by (9), as we will now show. Setting $\log Z = \log Z^{MF}$, we can write, starting from (5),

$$\mathcal{F} = E^{MF} - \left[\sum_k \mu_k^{MF} Y_k^{MF} + 1/\beta\log\int_0^{2\pi} P(\varphi_k)\,d\varphi_k\exp(-\beta Y_k)\right], \qquad (32)$$

where Y_k is defined as in (10). From this expression, one can immediately notice that the free energy $\mathcal{F}$ bears a strong resemblance to the maximum-likelihood formalism (Bricogne, 1993, 1997; Murshudov *et al.*, 1997).

Indeed, when Y_k is evaluated as in (18) for the model refinement case,

$$Y_k = -X_k(\cos\Delta\varphi_k^{calc} - \lambda_k\mu_k^{MF}\cos\Delta\varphi_k^{MF}),$$

and recalling $X_k = 2F_{obs}(\mathbf{k})F_{calc}(\mathbf{k})$, as defined in (16), we can compute E^{MF} from (13), and replace it in (32) to get

$$\mathcal{F} = \sum_k -\mu_k^{MF\,2}F_{obs}(\mathbf{k})^2 + F_{calc}(\mathbf{k})^2 - 1/\beta\log\left[\int_0^{2\pi} P(\varphi_k)\,d\varphi_k\right.$$
$$\left.\times\exp(-\beta Y_k)\right]. \qquad (33)$$

$\beta\mathcal{F}$ is the maximum likelihood of Murshudov *et al.* (1997), up to an additive constant that defines the origin of the free energy, if one sets $\mu_k^{MF} = 1$ and $\Delta\varphi_k^{MF} = 0$ in the exponential of the integral. This is because it is expressed here in the fully self-consistent formalism [see also the discussion on (17), the self-consistent analogue of the Sim weighting scheme]. The observed structure factors $F_{obs}(\mathbf{k})$ are now weighted by their figure of merit μ_k^{MF} implying that the free energy will decrease if the figure of merit increases as it should. The temperature has the following simple physical interpretation:

$$\beta^{-1} = 2\sigma^2(F_{obs}) + \Sigma_{wc}, \qquad (34)$$

which takes into account the partial nature of the model through Σ_{wc} and the errors in the experimental measurements of F_{obs} (but see also our discussion on the choice of the temperature in the following section).

It is easy to check that one recovers (9) immediately through the condition $\partial\mathcal{F}/\partial\mu_k = 0$. Hence, the best phase and the figure of merit are the ones that minimize the free energy $\mathcal{F}$, which can be seen as a variational form of maximum likelihood.

It is also possible to calculate the free energy in the case of an energy function derived from the Sayre equation (see Appendix A), using equation (62) for Y_k and equation (32) for the free energy.

3.5. Choice of temperature

To ascertain the role of the temperature parameter, we have attempted to see if there is any thermodynamic phase transition if one varies the temperature. To do so, the Jacobian of the exponent in (5) was calculated and minimization of its lowest eigenvalue with respect to the temperature was performed. *It was found that there is no thermodynamic phase transition in the case of an energy given by (17)*. Therefore, there is no preferential choice of the temperature.

However, as already in use in the crystallographic community in different programs such as *X-PLOR* (Brunger *et al.*, 1987), one could be tempted to choose the temperature such that *the weight of the energy refinement is the same as the one coming from the experimental phase probability distribution function*, in some sort of a null hypothesis as to the relative importance of the two terms.

In order to do so, we resort to the theory of linear response in thermodynamics, which states that the response coefficient to a perturbation in the order parameter is

$$\chi = \beta \sigma^2(\Delta\varphi_k^{\mathrm{MF}}). \tag{35}$$

Therefore, if one wants the restoring forces to be equivalent, it is sufficient to set

$$\sigma^2(\Delta\varphi_k^{\mathrm{BC}})_{\beta=0} = \sigma^2(\Delta\varphi_k^{\mathrm{AD}})_{P(\varphi)=1/2\pi} \tag{36}$$

using notations introduced in equations (11), (12) and (26). This defines the temperature ensuring equivalence of information coming from experimental measurements on one side and from energy refinement on the other side.

Another alternative would be to set the specific heat C_v to a maximum as a function of temperature, *i.e.* to set $\partial C_v/\partial\beta = 0$. Using the classical relationship

$$C_v = \beta^2 \partial^2 \log Z/\partial\beta^2 = \beta^2(\langle E^2\rangle - \langle E\rangle^2), \tag{37}$$

which follows from the definition $C_v = \partial U/\partial T$, the condition $\partial C_v/\partial\beta = 0$ can be shown to give the following self-consistent equation:

$$\beta = 2(\langle E^2\rangle - \langle E\rangle^2)/(\langle E^3\rangle - 3\langle E^2\rangle\langle E\rangle + 2\langle E\rangle^3), \tag{38}$$

where all the average quantities on the right-hand side of the equation have to be evaluated at the same temperature as the one specified on the left-hand side of the equation. The temperature at which C_v reaches its maximum is the one at which fluctuations are maximum and where the energy and the entropy compete most effectively.

4. Other energy functions formulated in reciprocal space

4.1. General formalism

Energy functions that express real-space constraints but formulated in reciprocal space can be at least of three kinds, depending on the physical criterion that one wishes the map to satisfy: solvent flattening, the atomicity criterion (Sayre equation) and molecular averaging if there is more than one copy of the molecule in the asymmetric unit. All of these criteria can be expressed in the following general form (Arnold & Rossmann, 1986, and references therein):

$$\mathbf{F}(\mathbf{h})_{\mathrm{modified}} = \sum_{\mathbf{k}} \mathbf{F}_{\mathrm{obs}}(\mathbf{k})\mathbf{A}(\mathbf{h},\mathbf{k}). \tag{39}$$

From this, it is easy to construct an energy to be minimized:

$$E = \sum_{\mathbf{h}} |\mathbf{F}_{\mathrm{obs}}(\mathbf{h}) - \mathbf{F}_{\mathrm{modified}}(\mathbf{h})|^2. \tag{40}$$

For solvent flattening, $\mathbf{A}(\mathbf{h},\mathbf{k})$ takes the form

$$\mathbf{A}(\mathbf{h},\mathbf{k}) = \mathbf{G}(\mathbf{h}-\mathbf{k}), \tag{41}$$

where $\mathbf{G}(\mathbf{h})$ is the Fourier transform of the molecular envelope function.

For the Sayre equation, it is sufficient to set

$$\mathbf{A}(\mathbf{h},\mathbf{k}) = \mathbf{F}(\mathbf{h}-\mathbf{k}). \tag{42}$$

For molecular averaging, one has

$$\mathbf{A}(\mathbf{h},\mathbf{k}) = 1/N \sum_{i=1}^{N} \mathbf{R}_i(\mathbf{h},\mathbf{k})\mathbf{T}_i(\mathbf{h}), \tag{43}$$

where $\mathbf{R}_i$ and $\mathbf{T}_i$ are given by the rotations C_i and translations $\mathbf{d}_i$ needed to superpose the different copies of the molecules present in the asymmetric unit (Rossmann, 1972, and references therein):

$$\mathbf{T}_i(\mathbf{h}) = \exp(-2i\pi\mathbf{h}\cdot\mathbf{d}_i) \tag{44}$$

$$\mathbf{R}_i(\mathbf{h},\mathbf{k}) = 1/U \int_{U_i} \exp\{-2i\pi(\mathbf{k}-\mathbf{h}[C_i])\cdot\mathbf{x}_i\}\,\mathrm{d}\mathbf{x}_i. \tag{45}$$

The molecular averaging techniques developed in real space are extremely powerful, especially in the presence of some initial experimental phase information (Bricogne, 1976). However, they can be used just as well in reciprocal space. The formalisms (9) and (10) developed above indicates how to treat simultaneously the constraint of equal electron density in the different copies of the molecule in the asymmetric unit through equations (39), (40) and (43)–(45) and preliminary and partial phase information in the form of a phase probability distribution function.

It is possible to do the analytical calculation of the best phase and the figure of merits of each reflection in the case of the Sayre energy [equations (39), (40) and (42)], in the presence of external phase information [equation (9)]. The calculation proceeds essentially along the lines detailed above (see Appendix A). The result is then *a set of coupled equations to be solved simultaneously*. The novelty here is that all structure factors entering the equations *are weighted by their respective figure of merit*, which are to be determined self-

consistently. This new treatment may then contribute in this respect to extend the power of direct methods beyond the field of small-molecule crystallography. The phase triplets and quartets also enter the equations, as expected (see Appendix A). If $P(\varphi_k)$ reflects the phase distribution probability function coming from a SIR or a SIRAS experiment, one should recover the analytical results of Hauptman (1982a,b). We have not attempted this, however, as it goes beyond the scope of this work. Instead, we have chosen to do a *numerical* study, where the mean-field equations are solved iteratively. In the following two sections, a few more details are given about the Sayre score and the envelope score and variations thereof, which will be used in the numerical tests presented below.

4.2. Sayre score

The Sayre equation is the reciprocal-space equivalent of a simple relationship in real space, $\rho(\mathbf{r}) \propto \rho^2(\mathbf{r})$, which is valid for sharply peaked electron-density maps (the so-called atomicity condition). The Sayre equation reads (Sayre, 1952)

$$\mathbf{F}(\mathbf{h}) = g(\mathbf{h}) \sum_{\mathbf{k}} \mathbf{F}(\mathbf{k})\mathbf{F}(\mathbf{h} - \mathbf{k}), \qquad (46)$$

where $g(\mathbf{h})$ is a spherically averaged resolution-dependent form factor, which is difficult to estimate analytically at low resolution.

Let us call $\mathbf{F}_S(\mathbf{h})$ the right-hand side of this equation, ignoring the $g(\mathbf{h})$ form factor.

$$\mathbf{F}_S(\mathbf{h}) = \sum_{\mathbf{k}} \mathbf{F}(\mathbf{k})\mathbf{F}(\mathbf{h} - \mathbf{k}), \quad i.e. \ \mathbf{F}(\mathbf{h}) = g(\mathbf{h})\mathbf{F}_S(\mathbf{h}). \quad (47)$$

If the $g(\mathbf{h})$ function is a slowly varying function, these Sayre structure factors $\mathbf{F}_S(\mathbf{h})$ should scale well with the original $\mathbf{F}(\mathbf{h})$ structure factors. Therefore, we ask for a high correlation coefficient between $\mathbf{F}(\mathbf{h})$ and $\mathbf{F}_S(\mathbf{h})$. This has been been shown to work well in a simulated-annealing study of the same problem (Delarue, 2000), using an energy W_{Sayre} such that

$$W_{\text{Sayre}} = 1 - \text{Corr}(\mathbf{F}(\mathbf{h}), \mathbf{F}_S(\mathbf{h})), \qquad (48)$$

where $\text{Corr}(A, B)$ stands for the correlation coefficient between vectors A and B:

$$\text{Corr}(A, B) = (\langle AB \rangle - \langle A \rangle \langle B \rangle)$$
$$\times (\langle A^2 \rangle - \langle A \rangle^2)^{-1/2}(\langle B^2 \rangle - \langle B \rangle^2)^{-1/2}. \quad (49)$$

It turns out that it is not necessary to calculate this correlation coefficient over the entire set of reflections but that a mere subset of the 1000 or so most intense ones are sufficient to obtain a very accurate Sayre score. To speed up the calculation of the energy W_{Sayre}, the list of reflections $\mathbf{k}(\mathbf{h})$ contributing to the summation in (46) is stored once and for all for each reflection $\mathbf{h}$. We have found it easier to work with correlation coefficients rather than with residuals as in (40).

Formally, trying to break down the sign ambiguity in the centrosymmetric space group $P\bar{1}$ is equivalent to trying to break the phase-ambiguity problem in the SIR case in protein crystallography. It may be in order here to mention that there is a long history of using the Sayre equation to tackle this problem, which has recently regained attention (see Weinzierl

et al., 1969; Coulter, 1971; Hendrickson, 1971; Giacovazzo & Siliqi, 1994).

4.3. Envelope score

It is also possible to express in reciprocal space the *a priori* knowledge of the form of the electron density outside the protein, which should be constant and equal to zero. One way to express it is to impose $\rho(\mathbf{r}) = \rho(\mathbf{r})\text{Env}(\mathbf{r})$, where $\text{Env}(\mathbf{r})$ is the characteristic function of the envelope of the molecule; if $\mathbf{G}(\mathbf{k})$ is the Fourier transform of $\text{Env}(\mathbf{r})$, one can define another energy criterion, in reciprocal space:

$$W_{\text{env}} = 1 - \text{Corr}(\mathbf{F}(\mathbf{h}), \mathbf{F}_{\text{env}}(\mathbf{h})) \qquad (50)$$

with the notation

$$\mathbf{F}_{\text{env}}(\mathbf{h}) = \sum_{\mathbf{k}} \mathbf{F}(\mathbf{k})\mathbf{G}(\mathbf{h} - \mathbf{k}) \qquad (51)$$

as defined in (39) and (41).

Hence, one can try to minimize a combination of the two, controlled by the mixing parameter η:

$$W_{\text{tot}} = \eta W_{\text{Sayre}} + (1 - \eta)W_{\text{env}}. \qquad (52)$$

Recent attempts to use the envelope information to break down the phase-ambiguity problem in the SIR case can be found in Gu *et al.* (1997) and Zheng *et al.* (1997).

5. Three different test cases using the Sayre equation

5.1. Mean-field optimization techniques in $P\bar{1}$

For simplicity, most of the numerical tests presented in this work were performed in the centrosymmetric $P\bar{1}$ space group, where phases are constrained to take one of two values, 0 or 180°. Therefore, the integrals in (9) are actually a sum of only two terms, leading to the simple expression

$$m(\mathbf{k}) = -\tanh\{\beta[\partial E/\partial m(\mathbf{k})]\}. \qquad (53)$$

At $T = 0$, $m(\mathbf{k})$ can take only two values: $+1$ or -1 and it is the sign of the reflection $\mathbf{k}$. At intermediate temperatures, it can take any value between -1 and $+1$.

Alternatively, the same method could be used to decide which of the two phases $\varphi_k^{(2)}$ and $\varphi_k^{(1)}$ given by the Harker construct is the true one in the SIR method for each reflection. In this case, the phase $\varphi_{\mathbf{k}}$ of reflection $\mathbf{k}$ would be given by

$$2\varphi_{\mathbf{k}} = (\varphi_{\mathbf{k}}^{(2)} + \varphi_{\mathbf{k}}^{(1)}) + m(\mathbf{k})(\varphi_{\mathbf{k}}^{(2)} - \varphi_{\mathbf{k}}^{(1)}). \qquad (54)$$

It is possible to give a simple physical interpretation to (53) by drawing a formal comparison with the well known Ising spin system, in which now $m(\mathbf{k})$ is the magnetization of a spin $\mathbf{k}$ interacting with an assembly of spins through the following bilinear energy:

$$E = -\sum_{\mathbf{k},\mathbf{k}'} J_{\mathbf{k},\mathbf{k}'} m(\mathbf{k})m(\mathbf{k}'). \qquad (55)$$

In the nearest-neighbour Ising-model system, the sum is restricted to those sites $\mathbf{k}'$ that are close in space to $\mathbf{k}$, a condition that we denote by $\mathbf{k}' \in N(\mathbf{k})$. By convention, each pair interaction is counted only once in the sum. $J_{k,k'}$ is the

coupling constant, which will be assumed here to be site independent, for simplicity, and equal to J ($J > 0$).

Basically, the mean-field formula reads, in the absence of an external field (see for instance Reif, 1965),

$$m(\mathbf{k}) = \tanh\left[\beta J \sum_{\mathbf{k}' \in N(\mathbf{k})} m(\mathbf{k}')\right] = -\tanh(\beta H_{\text{local}}), \quad (56)$$

where $\beta = 1/k_B T$ is the inverse temperature and where $H_{\text{local}} = -J \sum_{\mathbf{k}' \in N(\mathbf{k})} m(\mathbf{k}')$ is the local field, which is different for each spin $\mathbf{k}$ and is sometimes called the mean field felt at position $\mathbf{k}$. One can check that (53) gives (56) directly by calculating explicitly the derivative of the energy E in (55).

Physically, the interpretation of (53) and (56) is straightforward: for a given temperature, a high positive (negative) value of H_{local} forces $m(\mathbf{k})$ towards -1 ($+1$), respectively. A low temperature (high value of β) will increase the contrast between the two different possible values of $m(\mathbf{k})$ and freeze $m(\mathbf{k})$ into either one of them, depending essentially on the sign of H_{local}.

On a practical level, equation (53) was solved numerically, replacing E by W from equation (48) or (52) and evaluating the derivative numerically (Press *et al.*, 1992). An entire update of the spin system (typically 1000 reflections) takes about 4–8 min CPU on a DEC alpha PWS500 workstation, with an EV6 CPU card, depending on whether the energy is defined by (48) or (52).

In order to avoid oscillation problems and ensure convergence, only part of the corrections to the $m(\mathbf{k})$ set of values is taken into account at each step, according to the scheme (the so-called Picard method)

$$m(\mathbf{k})^{(n+1)} = m(\mathbf{k})^{(n)} + \gamma(m(\mathbf{k})^{\text{calc}} - m(\mathbf{k})^{(n)}), \quad (57)$$

where n is the cycle number and $m(\mathbf{k})^{\text{calc}}$ is given by the right-hand side of (53). Typically, a value of 0.10–0.20 was taken for γ and 30–50 cycles were necessary to achieve convergence. We note in passing that (57) is nothing but a (relaxation) Langevin equation at zero noise and friction coefficient $1/\gamma$. This is because (57) can be rewritten, taking into account (33), to give

$$\partial m(\mathbf{k})/\partial n = -\gamma(\partial \mathcal{F}/\partial m(\mathbf{k})).$$

This shows that $\partial \mathcal{F}/\partial n$ is a *negative* number and it therefore guarantees that the free energy of the system will decrease along the iteration process.

5.2. Protein and structure-factor calculations

Working in space group $P\bar{1}$ is far less unnatural than it may seem at first glance; indeed, Berg and co-workers were able a few years ago to crystallize an equal mixture of a protein and its enantiomer in space group $P\bar{1}$ (Berg & Goffeney, 1997); direct methods failed to find the solution of this crystal structure and molecular replacement was used to locate the natural enantiomer in the cell (Zawadzke & Berg, 1993). The protein used by these authors was rubredoxin, a small protein whose unnatural enantiomer was chemically synthesized. The same protein was used here, but with calculated structure factors, calculated using the CCP4 suite of programs (Colla-

borative Computational Project, Number 4, 1994). The Zn^{2+} metal ion was omitted to avoid any strong bias in the Patterson and to satisfy the hypothesis of equal atoms in the unit cell. Sometimes, errors were voluntarily added to simulate the effect of measurement errors. The resolution was limited to 2.5 Å. Calculations were performed with both raw structure factors and normalized E-value structure factors.

The crystallographic coordinates of rubredoxin were taken from the PDB (code 6rxn). The molecule was put in space group $P\bar{1}$ and care was taken that the packing was correct, *i.e.* that no crystallographically equivalent molecule would bump into any other molecule in the cell.

5.3. Generalization of mean-field treatment to P1, with a (discrete) set of four phases

It is possible to simplify equation (9) if the phases are constrained to take only one of the four following values: $0, 90, 180, 270°$, thereby providing a crude sampling of the set of phases in $P1$. This kind of sampling is also often used in small-molecule crystallography and direct methods. In this case, the correlation coefficient in equation (49) has to be calculated in the complex plane, which can be performed by replacing every product AB by AB^*. Then equation (9) simplifies to

$$\mu_k \cos \varphi_k^{\text{MF}} = -\sinh \beta Y_k^{(1)}/(\cosh \beta Y_k^{(1)} + \cosh \beta Y_k^{(2)}) \quad (58a)$$

and

$$\mu_k \sin \varphi_k^{\text{MF}} = -\sinh \beta Y_k^{(2)}/(\cosh \beta Y_k^{(1)} + \cosh \beta Y_k^{(2)}), \quad (58b)$$

where

$$Y_k^{(1)} = (\partial E/\partial z_k)_{\text{MF}} + (\partial E/\partial z_k^*)_{\text{MF}}$$

and

$$Y_k^{(2)} = i(\partial E/\partial z_k)_{\text{MF}} - i(\partial E/\partial z_k^*)_{\text{MF}}$$

are both real numbers. The interpretation is the same as before: if $Y_k^{(1)}$ is large, then $m(\mathbf{k}) = \pm 1$ ($\varphi_k^{\text{MF}} = 0$ or π), whereas, if $Y_k^{(2)}$ is large, then $m(\mathbf{k}) = \pm i$ ($\varphi_k^{\text{MF}} = \pm\pi/2$).

5.4. Overcoming wrongly biased phase probability distribution functions

Suppose phases are restricted to take one of only two values, with probabilities p_{max} and p_{min}, respectively ($p_{\text{max}} = 1 - p_{\text{min}}$). If there is no experimental error, then most likely the right phase will be the one for which the probability is maximum. However, what is the best choice of phases if errors are known to be present? If an energy function E is available, mean-field optimization can be used to discriminate between the two possible phases, even in the presence of a wrongly biased phase distribution function. Indeed, equation (9) reads in this case:

$$m(\mathbf{k}) = -m_{\text{max}}(\mathbf{k}) \tanh[\beta m_{\text{max}}(\mathbf{k}) \partial E/\partial m(\mathbf{k}) + \alpha], \quad (59)$$

where $\alpha = 1/2 \log[(1 - p_{\text{max}})/p_{\text{max}}]$.

This indeed reduces to equation (53) if $p_{\text{max}} = 1/2$. If p_{max} is larger than $1/2$, (59) tends to force $m(\mathbf{k})$ to take the $m_{\text{max}}(\mathbf{k})$

value, as expected, through the α term. If $m_{max}(\mathbf{k})$ is not the true solution, then the term $\partial E/\partial m(\mathbf{k})$ in (59) will tend to balance the α term and reverse the choice of phases.

6. Numerical results

6.1. Resolving phase ambiguity in the centrosymmetric $P\bar{1}$ space group

In order to test the validity of the method, different percentages of the total number of reflections being refined had their phases voluntarily imposed to the right value throughout the optimization process. It was found that 10% of imposed phases is the minimum required to get a definite improvement of the phases (Fig. 1). At 0% of imposed phases, the algorithm does not find the right solution, meaning that the minimization of the energy criterion W_{Sayre} defined in (48) is not sufficient to break down the phase ambiguity.

The temperature at which the simulation was run is unimportant over the range that was tested since it only increases the contrast between the two opposite signs of the phase (Fig. 2); there was no thermodynamic phase transition in any of the simulations that were run. Actually, it is better to freeze the system at the end of the mean-field optimization by lowering the temperature in the last cycle, thereby forcing the system to adopt a value of either $+1$ or -1 for each phase. Adding the envelope information helped somewhat, with an optimum in the respective weights of the Sayre score and the envelope score as defined in (52) of 65 and 35%, respectively (Fig. 3).

Finally, the method was shown to be relatively robust with respect to experimental errors in the measurement of structure-factor moduli (data not shown). At low percentages of imposed phases, the final set of phases at which the system arri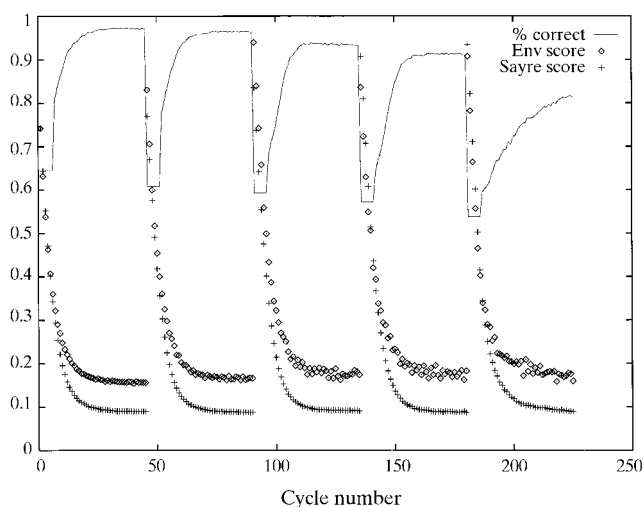ves depends somewhat on the initial configuration. But the general trend is that phase information is recovered as soon as 5–10% of phases are known and imposed (Fig. 4).

6.2. Extension to other space groups

A possible extension of the method to $P1$, or any space group for that matter, was also tried in the course of this work. Here the phases are not constrained to take either of the two values 0 and 180°, but rather a discrete set of only four values, e.g. 0, 90, 180 and 270°, using (58a) and (58b). In this case, (48) has to be redefined to work with complex numbers [basically, each product AB in (49) has to be changed into AB^*]. The Sayre score of the right solution is then only about 0.30, instead of 0.06 in $P\bar{1}$. Therefore, there is insufficient contrast between the right and wrong scores of the Sayre energy defined in (48), when one goes to the complex plane. Further tests were not pursued. Other energy functions would be needed to tackle this problem in space group $P1$ if there is no other phase information available.

6.3. Overcoming errors in biased probability distribution functions

To illustrate further the potentiality of the method, the following test was performed, in which *a priori* probabilities for the two possible phases were made unequal (0.6 *vs* 0.4 in this particular example). However, errors were also deliberately introduced in the process; for instance, 80% of the phases were assigned a probability of 0.6 to their right value and 0.4 to their wrong value, whereas, for the remaining 20%, the probability assignment was reversed. In other words, for those reflections, the phase for which the probability was the highest was the wrong one, *i.e.* $s_{max} = s_{wrong}$ and $s_{min} = s_{right}$. At the beginning of the simulation, phases were assigned according to $s = s_{max}$. Then, phases were refined according

Figure 1
Mean-field optimization of phases at different percentages of imposed phases: from left to right 30, 25, 20, 15, 10% of imposed phases. 45 cycles were performed in each case with $\gamma = 0.1$ using equation (57). The quantity being minimized is the Sayre score W_{Sayre} (crosses), while the envelope score W_{env} (diamonds) and the percentage of correct phases (continuous line) are being monitored but not minimized.
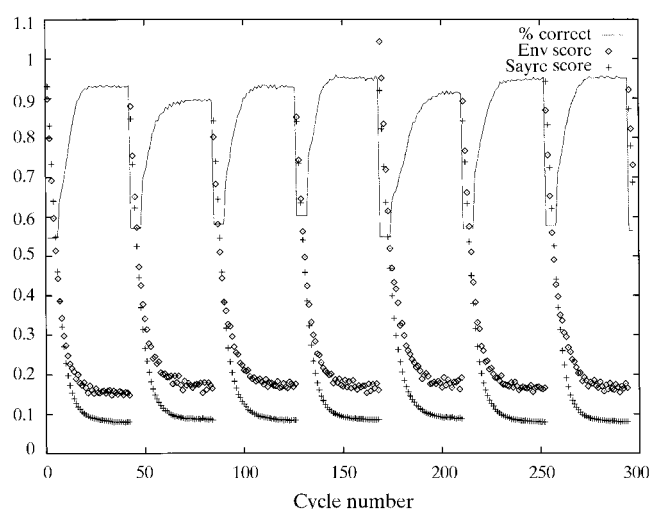

Figure 2
Influence of the temperature on mean-field minimization, at 15% of imposed phases. The simulations were also performed with different starting configurations. Seven different temperatures are presented, covering the thermodynamic phase transition determined by Monte Carlo techniques (Delarue, 2000), *i.e.* from 0.0005 to 0.00001 (left to right).

to (59), at different temperatures. For a given range of temperature, the Sayre energy being minimized allowed us to partially overcome the errors deliberately introduced and a definite phase improvement was observed (Fig. 5).

7. Discussion

There are at least two problems in phase refinement in protein crystallography. One is the definition of an energy function for which the right solution is the absolute minimum, with an energy spectrum such that the 'native' configuration is well detached from all the other (wrong) ones. In the absence of experimental phase information, such a criterion does not exist to our knowledge, meaning that it would be hopeless to *refine* one trial configuration against any existing energy criterion (see Baker *et al.*, 1993). Knowledge of the shape of the molecule can help but is not usually available in real cases. In recent work, the Terwilliger σ_R^2 index (Terwilliger, 1999) proved successful to break the phase ambiguity using multi-start Monte Carlo simulations (Delarue, 2000). One interesting idea would be to impose chain connectivity in the final map, as suggested by Baker *et al.* (1993). Some steps along these lines for low-resolution phasing have been attempted recently (Lunin *et al.*, 1999). Needless to say, mean-field optimization might be used with any kind of new and effective criterion to select good maps *vs* bad ones. All one has to do is to calculate $\partial E/\partial z_k$ (even numerically) and plug it in equation (9).

In the absence of any phase probability bias, the mean-field optimization technique using the Sayre-energy criterion proved successful in the case where at least 5–10% of the phases are known and imposed to their correct value. This seems of limited use at first sight. However, one could argue
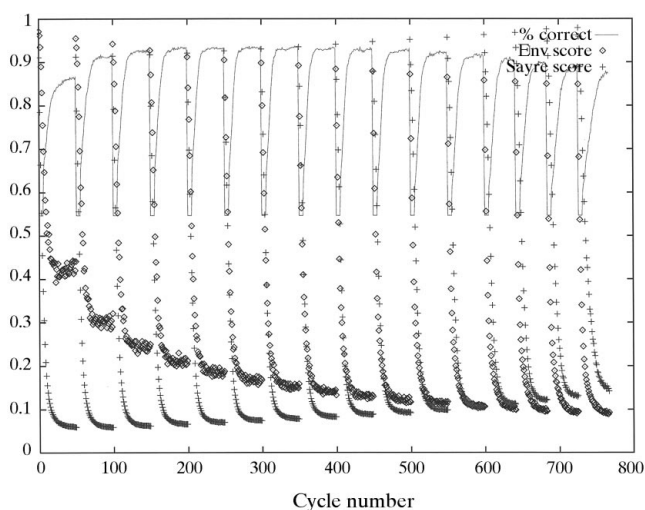
that imposing 10% of the phases is actually easy to do in the SIR method, provided that the space-group system allows for that many centric reflections. Preliminary tests in space group $P2_12_12_1$ did converge to the right solution readily (data not shown). Moreover, the set of imposed phases could come from experimentally determined phases for some reflections (Weckert & Hummer, 1997; Shen, 1998). Test calculations and results of phase refinement using the Sayre equation and a handful of imposed phase reflections have already been reported for protein crystallography (Mo *et al.*, 1996). These methods might become more widely used in the near future.

In addition, mean-field optimization techniques were able to partially overcome phase probability distribution functions plagued with some errors, with a portion of the reflections (up to 30–40%) wrongly biased.

The second difficulty is to treat in the same general formalism both energy refinement and phase probability distribution functions and to ensure that the entire phase space is being explored, avoiding false (local) minima. While energy functions can be expressed in both real and reciprocal spaces, the phase probability distribution functions are obviously expressed in reciprocal space only. Usually, this leads to alternate cycles of map improvement and model building and then further model refinement, in both direct and reciprocal spaces, respectively (Podjarny *et al.*, 1987; Main, 1990). We show here that it is possible to work only in reciprocal space. Indeed, mean-field optimization techniques provide a natural and elegant way to do it, effectively leading to an *analytical* solution giving the best phase and best weight for each reflection. The idea would be then to solve the mean-field self-consistent equations directly by the Picard method for each reflection and, once (almost) perfect phases have been recovered, to use automatic map building procedures (Perrakis *et al.*, 1999) to get an atomic model of the molecule.

Why mean-field optimization? Historically, mean-field theory dates back to P. Weiss a century ago; it was devised to
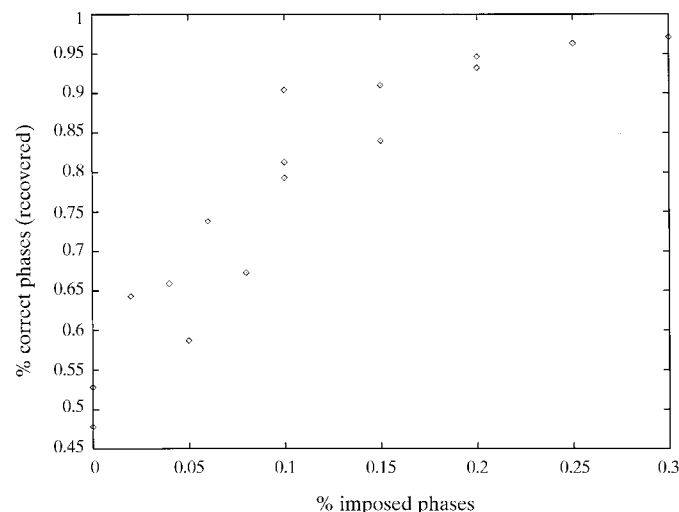

Figure 3
Influence of the η mixing parameter in equation (52) defining the proportions of the envelope and Sayre scores being minimized in mean-field optimization. From left to right, the η value is varied from 5 to 80% in steps of 5%, and the system is submitted to 45 cycles of optimization. The optimum is between 30 and 50%. The percentage of correct phases (continuous line) is presented as well as the envelope score W_{env} (diamonds) and the Sayre score W_{Sayre} (crosses).

Figure 4
Final percentage of correct phases obtained after 40 cycles of mean-field optimization (y axis) for different percentages of imposed phases (x axis). 15 different initial configurations are presented. The mixing parameter η was set to 0.40 and the temperature at 0.0001.

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solve some simple problems in condensed-matter physics, *e.g.* systems of interacting magnetic spins (Reif, 1965). It was successful in many cases presented in classical textbooks (Hertz *et al.*, 1991; Chaikin & Lubensky, 1995). What makes it particularly appealing is that it can be applied to a whole range of problems involving interacting particles, where it represents the simplest possible full *analytical* treatment [see *e.g.* Koehl & Delarue (1996) for an application to molecular homology modelling]. It is, in principle, the method of choice to be tried first, especially if the range of fluctuations of the order parameter, *i.e.* the correlation length, is less than the range of interaction of the particles. Its validity and robustness is especially good if each particle interacts with many neighbours, because in this case the notion of 'mean field' is clearly relevant and effective [see equation (56)]. Indeed, it consists in replacing all pairwise interactions by a 'local field' felt by each spin; this 'local field' is not known at the beginning of the calculation but refined using the self-consistent mean-field equation until convergence is achieved. Its immense advantage is that all the phase space is explored at once.

In the case of phase refinement, mean-field optimization is exact, since the energy being refined is quadratic. It leads to a new view of phase combination which should prove crucial in tough cases of model refinement, especially for very large macromolecular complexes such as ribosomes or eukaryotic RNA polymerases.

8. Conclusions

The formalism presented in this paper allows one to treat simultaneously experimental phase information, as given by a probablity distribution function, and refinement of an energy expressed in reciprocal space. The equations have all the necessary requirements that one might expect from them:
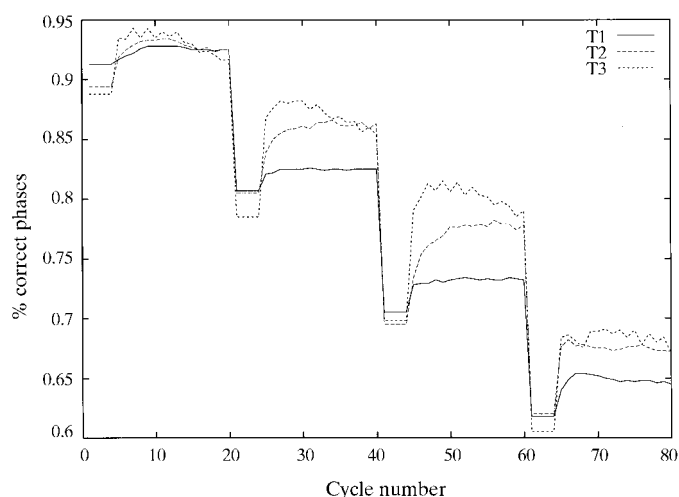

Figure 5
Overcoming wrongly biased probability distributions in $P\bar{1}$, with a proportion of wrongly biased reflections increasing from 10 to 40% by steps of 10% (left to right). 20 cycles of refinement were performed in each case, using equation (57) and $\gamma = 0.25$. Three temperatures have been tried and are presented, namely $T1 = 0.0003$, $T2 = 0.0001$ and $T3 = 0.00003$ and the relative probabilities of the wrong and right phase signs was set at $p_{min} = 0.4$ ($p_{max} = 0.6$) in equation (59).

they reduce to the Blow & Crick (1959) treatment at high temperature, they are similar to the Sim (1959, 1960) weighting scheme for a flat distribution probability function in the presence of a partial model and a first-order expansion of phase refinement in the presence of a reasonably good model gives the expected form for the weighted Fourier synthesis to be performed. However, they go beyond all these particular situations and provide the framework to do phase refinement in general, in the presence of any energy function. Numerical tests have been successfully implemented, aimed primarily at breaking the phase-ambiguity problem in $P\bar{1}$ or in the SIR method of protein crystallography with the help of the Sayre equation.

In addition, we show in Appendix *A* how the mean-field equations naturally lead to the use of figures of merit in direct methods and how to calculate them (*i.e.* using the Sayre equation).

We found the framework of thermodynamics enlightening in at least two instances: first, we could show the formal equivalence of minimizing the free energy of the system and the so-called maximum-likelihood prescription, at least in the case of model refinement. However, we must stress that in this case, as in all the other examples of applications examined in this work, our treatment has an additional term to impose self-consistency. Second, mean-field formalism also allows a fruitful discussion on the weight to be given to the energy refinement, *vs* the experimental phase information, through completely general classical thermodynamics arguments.

APPENDIX *A*
The case of phase refinement using the Sayre equation

Here we present the explicit mean-field equations in the case of the minimization of the Sayre energy W, where normalized structure factors $E_{\mathbf{k}}$ have been used:

$$W = \sum_k \left(z_k E_k - \sum_h z_h z_{k-h} E_h E_{k-h} \right)$$
$$\times \left(z_k^* E_k - \sum_l z_l^* z_{k-l}^* E_l E_{k-l} \right)$$
$$= \sum_k A_k A_k^*. \tag{60}$$

First we need to calculate the two derivatives $\partial W/\partial z_k$ and $\partial W/\partial z_k^*$:

$$\partial W/\partial z_k = E_k A_k^* - 2 \sum_h A_h^* z_{h-k} E_k E_{h-k}$$
$$= z_k^* E_k E_k - 2 E_k \sum_l z_l^* z_{l-k} E_{l-k} E_l$$
$$- E_k \sum_l z_l^* z_{k-l}^* E_{k-l} E_l$$
$$+ 2 \sum_l \sum_h z_l^* z_{h-l}^* z_{h-k} E_{h-k} E_k E_l E_{h-l}$$
$$= z_k^* E_k^2 - 3 E_k \sum_l z_l^* z_{k-l}^* E_{l-k} E_l$$
$$+ 2 \sum_h \sum_l z_l^* z_{h-l}^* z_{h-k} E_k E_l E_{h-l} E_{h-k}. \tag{61}$$

Then, using $z_{l-k} = z_{k-l}^*$, $z_{h-k} = z_{k-h}^*$ and $\partial W / \partial z_k^* = \{\partial W / \partial z_k\}^*$, one can again write (9) as

$$\mu_k^{\mathrm{MF}} = \frac{\int_0^{2\pi} P(\varphi_k) \, d\varphi_k \exp(i\Delta\varphi_k^{\mathrm{MF}}) \exp(-\beta Y_k)}{\int_0^{2\pi} P(\varphi_k) \, d\varphi_k \exp(-\beta Y_k)},$$

with the understanding that

$$Y_k = \mu_k E_k^2 \cos\Delta\varphi_k^{\mathrm{MF}} - 3E_k \sum_l \mu_l \mu_{k-l} E_{k-l} E_l \cos\psi_{k,l}$$
$$+ 2E_k \sum_h \sum_l \mu_l \mu_{h-l} \mu_{k-h} E_l E_{l-h} E_{k-h} \cos\Phi_{h,k,l}, \quad (62)$$

where all the quantities in the exponential are to be evaluated at the mean-field point MF and where we have used the following notations involving the so-called triplet and quartet invariants:

$$\psi_{k,l} = \varphi_l^{\mathrm{MF}} + \varphi_{k-l}^{\mathrm{MF}} - \varphi_k$$

and

$$\Phi_{h,k,l} = \varphi_l^{\mathrm{MF}} + \varphi_{h-l}^{\mathrm{MF}} + \varphi_{k-h}^{\mathrm{MF}} - \varphi_k. \quad (63)$$

This leads to the following free energy being minimized:

$$\mathscr{F} = \sum_k \left\{ \mu_k E_k \sum_l \mu_l \mu_{k-l} E_{k-l} E_l \cos\psi_{k,l}^{\mathrm{MF}} \right.$$
$$- \mu_k E_k \sum_h \sum_l \mu_l \mu_{h-l} \mu_{k-h} E_l E_{l-h} E_{k-h} \cos\Phi_{h,k,l}^{\mathrm{MF}}$$
$$\left. - 1/\beta \log\left[\int_0^{2\pi} P(\varphi_k) \, d\varphi_k \exp(-\beta Y_k) \right] \right\}. \quad (64)$$

In this equation, the sums on the h and l (but not on k) indices could be restricted to the top 1000 or so most intense reflections only. $\psi_{k,l}^{\mathrm{MF}}$ and $\Phi_{h,k,l}^{\mathrm{MF}}$ are evaluated as in (63) but with $\varphi_k = \varphi_k^{\mathrm{MF}}$.

APPENDIX B
The case of phase refinement using solvent flattening

For solvent flattening, the formalism would be very similar [see equations (39)–(42) in the main text]. Here we only quote the results:

$$Y_k = \mu_k E_k^2 \cos\Delta\varphi_k^{\mathrm{MF}} - 2E_k \sum_l \mu_l E_l G_{k-l} \cos(\varphi_k - \varphi_l^{\mathrm{MF}} - \theta_{k-l})$$
$$+ E_k \sum_l \mu_l E_l \Gamma_{k,l} \cos(\varphi_k - \varphi_l^{\mathrm{MF}} - \Theta_{k,l}), \quad (65)$$

where $G_k \exp(i\theta_k)$ is the Fourier transform of the envelope function Env($\mathbf{r}$), and where the quantity

$$\Gamma_{k,l} \exp(i\Theta_{k,l}) = \sum_h G_{h-l} G_{k-h} \exp i(\theta_{k-h} - \theta_{h-l}) \quad (66)$$

can be calculated once and for all before the main calculation. The free energy then simplifies to

$$\mathscr{F} = \sum_k \left\{ \mu_k E_k \sum_l \mu_l E_l G_{k-l} \cos(\varphi_k^{\mathrm{MF}} - \varphi_l^{\mathrm{MF}} - \theta_{k-l}) \right.$$
$$\left. - 1/\beta \log\left[\int_0^{2\pi} P(\varphi_k) \, d\varphi_k \exp(-\beta Y_k) \right] \right\}. \quad (67)$$

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