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The phase problem in neutron crystallography

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The straightforward solution of the crystal structure of cyclosporin ($C_{62}H_{111}N_{11}O_{12}\cdot H_2O$) by a modified *Shake-and-Bake* procedure, using experimental neutron diffraction data alone, shows that the positivity of the density function is not a necessary prerequisite for solving the phase problem. The initial applications suggest the intriguing possibility that positivity may actually be a hindrance.

1. Introduction

It is now 54 years since the birth of direct methods with the paper of Harker & Kasper (1948) who made implicit use of the positivity of the electron-density function to deduce, for the first time, inequality relationships among the structure factors. Shortly thereafter, Karle & Hauptman (1950) stressed the positivity property of the electron density as the source of the inequality relationships and derived all inequalities based on this property. Presumably because of this background, it came to be generally believed for some 20 years after the birth of direct methods that the validity of this approach rested on the twin pillars of positivity and atomicity. It was not until 1969 (Sikka, 1969) that this view was finally challenged. With the solution of the structure of melampodin, $C_{21}H_{24}O_9$ (Bernal & Watkins, 1972) directly from neutron diffraction data and using standard direct-methods techniques, it became clear that positivity was not an essential prerequisite, at least for small structures (fewer than 60 atoms) and when the amount of scattering from the H atoms is less than 30%. Subsequent work confirmed this initial conclusion (Cascarano *et al.*, 1992; Verbist *et al.*, 1992; Altomare *et al.*, 1994; Giacovazzo, 1998; Wilson, 1999).

In the meantime, Hauptman (1976) had laid the theoretical foundation for the extension of direct methods to neutron diffraction where, owing to the presence of the H atoms (among others), neutron scattering factors could be negative; hence density functions can, in this case, take on negative as well as positive values. Combining this theoretical development with the traditional *Shake-and-Bake* algorithm for phase determination yields a modified *Shake-and-Bake* procedure, here called *Neutron Shake-and-Bake*, which, as described here, significantly increases the power of direct methods in applications when neutron diffraction data alone are available. In fact, the resulting increase in the power of direct methods is such that it now appears, on the basis of the evidence presented here, that positivity may be, at best, irrelevant, at worst, an impediment.

2. Summary

The probabilistic theory of structure invariants, when neutron diffraction data alone are available, yields, as in the X-ray case, the formulation of the phase problem as a problem in constrained global minimization (the minimal principle). The latter, in turn, leads (again as in the X-ray case) to a modified *Shake-and-Bake* algorithm (*Neutron Shake-and-Bake*) that explicitly exploits the property of the neutron density function, which, owing to the presence of H atoms ($_1H^1$), can take negative as well as positive values. Successful applications, when only experimental neutron data are available, confirm the validity of this theoretical analysis.

3. Normalized structure factor $E_{\mathbf{H}}$ and neutron density function $\rho(\mathbf{r})$

For each fixed reciprocal-lattice vector, $\mathbf{H}$, the normalized structure factor $E_{\mathbf{H}}$ is defined by

$$E_{\mathbf{H}} = |E_{\mathbf{H}}| \exp(i\varphi_{\mathbf{H}}) = (1/\sigma_2^{1/2}) \sum_{j=1}^{N} f_j \exp(2\pi i \mathbf{H}\cdot\mathbf{r}_j), \quad (1)$$

where

$$\sigma_n = \sum_{j=1}^{N} f_j^n, \quad (2)$$

f_j is the neutron scattering factor of the atom labeled j and N is the total number of atoms in the unit cell. The corresponding neutron density function is then

$$\rho(\mathbf{r}) = \langle E_{\mathbf{H}} \exp(-2\pi i \mathbf{H}\cdot\mathbf{r})\rangle_{\mathbf{H}} \quad (3)$$

$$\rho(\mathbf{r}) = (1/\sigma_2^{1/2}) \left\langle \sum_{j=1}^{N} f_j \exp 2\pi i \mathbf{H}(\mathbf{r}_j - \mathbf{r}) \right\rangle_{\mathbf{H}} \quad (4)$$

$$\rho(\mathbf{r}) = 0 \qquad \text{if } \mathbf{r} \neq \mathbf{r}_j \quad (5)$$

$$\rho(\mathbf{r}) = (1/\sigma_2^{1/2}) f_j \quad \text{if } \mathbf{r} = \mathbf{r}_j \quad (6)$$

$$\rho(\mathbf{r}) \lesssim 0 \quad (7)$$

according as **r** is the position vector of an H atom or (usually) of a non-H atom, respectively. Let $N = N_p$ (positive) + N_n (negative) atoms.

4. Identities amongst the phases $\varphi_{\mathbf{H}}$

Because there are many more observable magnitudes $|E_{\mathbf{H}}|$ than there are atoms N in the unit cell, and because (1) is a finite sum, it follows that a large number of relationships among the normalized structure factors $E_{\mathbf{H}}$ exist. If magnitudes $|E|$ are assumed to be known, it follows also that there exist many identities among the phases alone, dependent of course on the presumed known magnitudes $|E|$, which must of necessity be satisfied.

5. The conditional probability distribution of the triplet $\Phi_{\mathbf{HK}}$

For each pair of reciprocal-lattice vectors **H**, **K**, define the triplet phase invariant $\Phi_{\mathbf{HK}}$ by

$$\Phi_{\mathbf{HK}} = \varphi_{\mathbf{H}} + \varphi_{\mathbf{K}} + \varphi_{-\mathbf{H}-\mathbf{K}} \tag{8}$$

and parameter $A_{\mathbf{HK}}$ by

$$A_{\mathbf{HK}} = (2\sigma_3/\sigma_2^{3/2})|E_H E_K E_{H+K}|, \tag{9}$$

where σ_n is given by (2).

It should be stressed at this point that, since σ_3 is the sum of the cubes of the neutron scattering factors f_j and since some of these may now be negative, it follows that A values [equation (9)] may be significantly smaller in the case of neutron diffraction than in the case of X-ray diffraction when all the atomic scattering factors are positive.

Refer to Hauptman (1976) for the conditional probability distribution, $P(\Phi|A_{\mathbf{HK}})$, valid for both neutrons and X-rays, of the triplet $\Phi_{\mathbf{HK}}$, given $A_{\mathbf{HK}}$, and the conditional expectation value, $\varepsilon(\cos\Phi_{\mathbf{HK}}|A_{\mathbf{HK}})$, of the cosine of $\Phi_{\mathbf{HK}}$, given $A_{\mathbf{HK}}$:

$$P(\Phi|A_{\mathbf{HK}}) = [1/2\pi I_0(A_{\mathbf{HK}})]\exp(A_{\mathbf{HK}}\cos\Phi) \tag{10}$$

$$\varepsilon(\cos\Phi_{\mathbf{HK}}|A_{\mathbf{HK}}) \approx I_1(A_{\mathbf{HK}})/I_0(A_{\mathbf{HK}}), \tag{11}$$

where Φ represents the triplet $\Phi_{\mathbf{HK}}$ and I_0 and I_1 are modified Bessel functions. The distribution (10) implies that the conditional variance of $\cos\Phi_{\mathbf{HK}}$, given $A_{\mathbf{HK}}$, is inversely correlated with $A_{\mathbf{HK}}$. Hence the larger the magnitude of $A_{\mathbf{HK}}$ the better is the estimate

$$\cos\Phi_{\mathbf{HK}} \approx I_1(A_{\mathbf{HK}})/I_0(A_{\mathbf{HK}}). \tag{12}$$

Since, as already stressed, $A_{\mathbf{HK}}$ values tend to be smaller for neutrons than for X-rays, the estimate (12) for $\cos\Phi_{\mathbf{HK}}$ is less reliable for neutrons than for X-rays. One might therefore anticipate that the direct-methods approach would be less robust for neutrons than it is for X-rays. That precisely the opposite is the case, as shown in the sequel, is, therefore, not only unexpected, but an important consequence of our analysis

6. The phase problem is a problem in constrained global minimization

In analogy with the X-ray diffraction case and referring to (12), one defines the minimal function $m(\Phi)$ for neutrons, a function of the phases, by means of

$$m(\Phi) = \sum_{\mathbf{H},\mathbf{K}} A_{\mathbf{HK}}\left\{\cos\Phi_{\mathbf{HK}} - [I_1(A_{\mathbf{HK}})/I_0(A_{\mathbf{HK}})]\right\}^2 \Big/ \sum_{\mathbf{H},\mathbf{K}} A_{\mathbf{HK}} \tag{13}$$

and formulates the minimal principle: the constrained global minimum of $m(\Phi)$ yields the true values of the phases for any choice of origin and enantiomorph. It should be noted that, although $m(\Phi)$ is defined explicitly as a function of the triplet phase invariants $\Phi_{\mathbf{HK}}$, it becomes, *via* (8), an implicit function of the individual phases.

It is important to stress the role of the constraints (§4), which consist of the identities that the phases must of necessity satisfy. The unconstrained global minimum of $m(\Phi)$, in contrast, does not yield the answer we seek.

7. *Neutron Shake-and-Bake*

It is one thing to formulate the phase problem as a problem in constrained global minimization; it is quite another to find this minimum. Traditional *Shake-and-Bake*, modified to accommodate neutron scattering, serves as a guide.

1. For an N-atom structural determination, select the largest (~$5N$) $|E|$ values, thus identifying the phases whose values we seek. Using these $|E|$s, identify the triplets and calculate their A values. Select those triplets having the largest A values (~$50N$ triplets). These are the triplets to be used in the phase-determination procedure.

2. Choose the initial structural coordinates at random, but assign values of $\rho(\mathbf{r})$ at atomic position vectors in accordance with the known molecular chemistry [(4)–(7)].

3. Calculate the initial values of the phases *via* (1) and the associated value of $m(\Phi)$ *via* (13).

4. Take a particular $\varphi_{\mathbf{H}}$; adjust its value, *via* a parameter shift technique, to reduce the initial value of $m(\Phi)$; then retain the new value and proceed to do this for all $5N$ phases. Do two iterations through the set of phases.

5. Calculate a new neutron-density function $\rho(\mathbf{r})$, *via* (3), using these new refined phases and their observed magnitudes $|E|$.

6. Impose constraints *via* a density-modification protocol: rank the peak values of the new density function from the N_p largest positive values to the N_n most negative, thus associating with each peak (or putative atomic position) a unique atom. Then re-assign the corresponding density values in accordance with the known chemistry and neutron scattering factors *via* (4) or (6). The density function is set equal to zero at all other (non-atomic) position vectors.

7. End of cycle. Do a specified number (~N) of cycles. Note the final value of the minimal function $m(\Phi)$.

8. Do a specified number of trials and make a histogram of the final $m(\Phi)$ values. Is this histogram bimodal? If so, the

smallest values of $m(\Phi)$ identify the phase sets that yield the solution of the crystal structure we seek.

8. Experimental data

Neutron diffraction data for the crystal structure of the native $_1H^1$ isotope of cyclosporin A (Knott *et al.*, 1990) had been deposited with the structural report: $C_{62}H_{111}N_{11}O_{12} \cdot H_2O$, $P2_12_12_1$, $a = 12.67$, $b = 15.68$, $c = 36.30$ Å, λ(neutron) = 1.184 Å, $Z = 4$. These 4121 data were 88% complete at a limiting resolution of 0.916 Å. A hard copy of the data was obtained from the Managing Editor of the journal and manually transcribed into computer-readable format. The F_{obs} data were processed to provide normalized $|E|$ values appropriate for this neutron data set (Blessing *et al.*, 1998). The neutron scattering factors used were $f_N = 9.36$, $f_C = 6.6$, $f_O = 5.8$ and $f_H = -3.7$. Note that, since f_H is now negative, the values of σ_3 and $\sigma_3/\sigma_2^{3/2}$, as already stressed, will be less than the corresponding values for X-rays. Indeed, as anticipated, the average A value of the triplets used in the determination of this structure was much less than the corresponding X-ray value for structures of comparable complexity. In fact, among the 9603 triplets generated from the largest 900 $|E|$ values used to determine this 199 atom structure (86 non-H atoms, 113 H atoms), only 2 triplets had A values greater than 1.5. For comparison, in the X-ray structure determination of gramicidin (Langs, 1988), an exceedingly difficult 300+ atom $P2_12_12_1$ structure, there were 154 triplets that had A values in excess of 1.5.

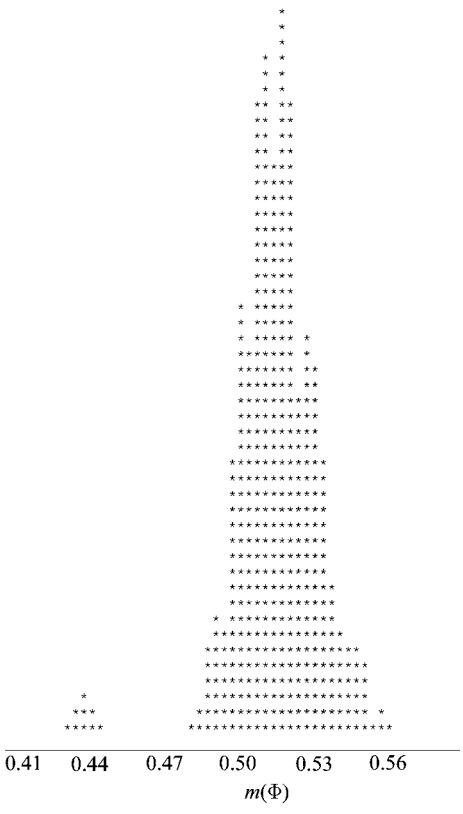

Figure 1
Histogram of the 1000 direct-methods trials computed for the $_1H^1$ neutron diffraction data set measured for cyclosporin A. The small cluster of points representing solutions (~2%) are centered on an $m(\Phi)$ value of 0.43, which is to the left of the large peak that identifies the non-solutions. Note the bimodal character of the histogram, which clearly separates the solutions from non-solutions.

9. The application

As described in §7, the traditional (X-ray) *Shake-and-Bake* procedure (DeTitta *et al.*, 1994; Weeks *et al.*, 1994; Weeks & Miller, 1999) was modified to accept both positive and negative peak positions interpolated from E maps and incorporated into the structure-factor calculations. 1000 randomly seeded trials were first tested, where each trial was subjected to 200 cycles of phase refinement. The phases for the 900 selected $|E|$ values were each in turn permuted by a 90° parameter-shift scheme to determine the constrained global minimum of $m(\Phi)$, and then assigned those values.

The phasing trials showed that the $_1H^1$ neutron structure is readily solved with *Neutron Shake-and-Bake* (see histogram, Fig. 1) with a success rate of about 2%. The initial r.m.s. phase error of these solutions was ~45° for the 900 data used. Maps of these solutions, however, were not as good in displaying the details of the negative density positions of H atoms (contoured in red in Fig. 2) as well as the non-H atoms which appear as positive blue density. Although many of the 113 H atoms were located in convincing negative density, there were many spurious negative peaks that occurred in the middle of bonds between the non-H atoms of the structure, and are most probably artifacts of the series termination of the E maps. Since true H-atom positions are expected to have a single bonding contact of ~1.0 Å to the non-H-atom skeleton of the molecule, the list of tentative H-atom sites from the E map was edited to exclude peaks that have more than one such contact to the rest of the molecule. In this manner, the spurious, mid-bond peaks were excluded from the structure-factor calcula-

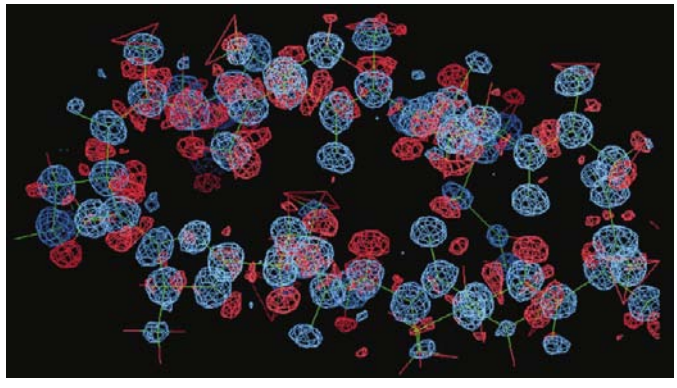

Figure 2
E map of the initial direct-methods solution. The C, N, O skeleton of the molecule is displayed in positive blue density >3$\sigma(\rho)$, while the potential hydrogen sites are displayed in negative red density <−4$\sigma(\rho)$. Although many of the H-atom positions are located in negative density, there exist many strong negative features in the middle of the bonds of the C, N, O substructure, probably due to series termination of the E map. The phase error of this map is ~45° for the 900 E values.

tions, resulting in a map of greatly improved quality (Fig. 3). The r.m.s. phase error was correspondingly reduced from the initial value of ~45 to ~37°. However, only about a third of the expected 113 H-atom sites were found in negative density that is less than $-3\sigma(\rho)$, at which point most of the spurious negative-density features were not visible. This was to be expected since the temperature factors of the H atoms from the structure refinement (Knott *et al.*, 1990) are about 6 Å^2 units higher than the atoms to which they are bonded. When this E map is used to extend and refine phases to the full set of 4121 F_{obs} data (Langs *et al.*, 2000), the r.m.s. phase error was further reduced to ~15°. The resultant F map (Fig. 4) was virtually indistinguishable from that of the final least-squares refined structure where 20% of the H atoms still fail to register in density below the $-3\sigma(\rho)$ level.

10. Positivity: help or hindrance?

Now that positivity is known not to be required for the solution of the phase problem even for moderately complex structures, it is natural to ask whether it helps or hinders. In order to answer this question, we calculated an error-free neutron data set for the deuterium (D = $_1H^2$, $f_D = 6.67$) isotope of the refined cyclosporin structure, where all neutron scattering factors are now positive, as well as an error-free $_1H^1$ data set. Once again, for both structures, diffraction data were calculated for only those 4121 reflections recorded in the experimental set. For each structure, the largest 900 $|E|$ values and ~9000 triples were employed. Once again 1000 random trials were iterated for 200 refinement cycles. As expected, the success rate for the $_1H^1$ structure was somewhat higher (~4%) than before (~2%), when experimental data had been used. The r.m.s. phase error was ~31° as compared to the ~37° value obtained employing the experimental data.

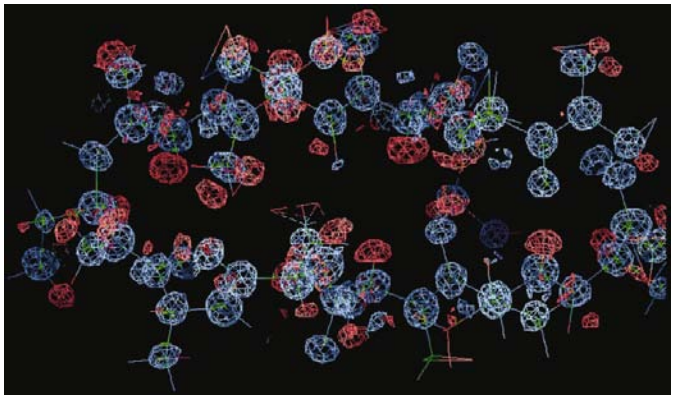

Figure 3
E map of a solution produced by the direct-methods procedure after the structure-factor loop of the program had been modified to exclude potential H atoms that form more than one bonding contact to the tentative C, N, O skeleton of the molecule. Map contour levels are the same as for Fig. 2. Note that the spurious mid-bond peaks of negative density have been largely eliminated. The map phase error has been reduced from ~45 to ~37° for the 900 E values involved.

Quite unexpectedly, however, the companion analysis of the deuterated, error-free data set failed to produce any solutions in 1000 trials (however, see §12 below). We therefore conclude that positivity is not only not required for the solution of the phase problem, it appears to be an impediment.

11. Discussion

One naturally asks why it appears to be easier to solve the phase problem with neutrons than with X-rays. In particular, why was this 199 atom neutron structure so readily solved by direct methods (*Neutron Shake-and-Bake*)? In X-ray direct-methods applications, the number of E values must exceed the number of non-H atoms by a factor of 8 or more in order for the E maps to have sufficient detail to reveal those atomic sites. H atoms may be ignored in this estimate owing to their low X-ray scattering power. This neutron application used only 900 $|E|$ values (approximately 4.5 × 199) to display 199 atoms of comparable scattering power. But half of the atoms are expected to fall in positive density and the remainder in negative density, and there is no competition for all 199 atoms to fit in the limited positive volume features of the map. Under these circumstances, one does not require as many reflections to resolve the atoms from one another. Also, the H atoms in negative density are more cleanly resolved from their bonded partners in positive density than would be the case for the deuterium structure, where both fall in positive density and would be difficult to resolve at greater than 1 Å resolution. Furthermore, the closest atomic contacts in the negative density are about 1.8 Å for vicinal sites of H atoms bonded to the same C or N atom, which exceeds the 1.5 Å resolution limit for atoms in the positive density. Although the triplet A values of this $_1H^1$ neutron application are quite small relative to those for X-ray determinations, the relative ease with which solutions are obtained shows that the ability to exploit negative map features facilitates the phase-determination process. Thus, not only is positivity not a prerequisite for success in direct-methods applications, it now appears that the existence

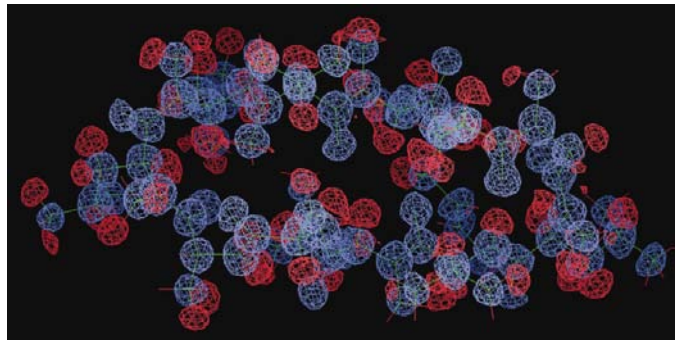

Figure 4
F map produced by extending the phases from the 900 $|E|$ values by peaklist optimization. This map is contoured at $\pm 3\sigma(\rho)$; the phase error of the 4121 terms is ~15°. Although all 113 H atoms reside in negative density, still about 20% of these sites fail to display in negative density at less than the $-3\sigma(\rho)$ contour that is shown.

Table 1
Application of *NSnB* and *RANTAN* to $C_{22}H_{32}O_6$ (neutron diffraction data) at five resolutions.

	NSnB		*RANTAN*	
Resolution (Å)	Success rate (%)	Initial r.m.s. phase error (°)	Success rate (%)	Initial r.m.s. phase error (°)
0.80	5.0	11	2.2	31
1.00	6.0	20	0	–
1.05	6.0	28	0	–
1.10	2.2	36	0	–
1.15	0.03†	54	0	–

† Three solutions in 10000 trials.

of both negative and positive regions of the density function actually facilitates the process of phase determination as compared to their related $_1H^2$ structures.

12. *Neutron Shake-and-Bake* does solve deuterated cyclosporin after all, albeit at a greatly reduced success rate

Neutron Shake-and-Bake can solve the deuterated cyclosporin structure provided that the number of refinement cycles per trial is increased from (the default) 200 to 400 cycles. In this case, 3 of the 1000 trials do converge to solution with a (reduced) success rate of 0.3% and a (relatively large) r.m.s. phase error of ~50°. With the same increase from 200 to 400 cycles per trial, the success rate for the native hydrogen structure increases from 4 to ~6%. Thus, as one goes from the deuterated to the native cyclosporin, the success rate increases from 0.3 to 6%. Hence, once again, positivity proves to be a hindrance, definitely not a help.

13. *Neutron Shake-and-Bake versus* traditional direct methods

1. *Neutron Shake-and-Bake (NSnB)*. *NSnB* and traditional direct methods (*e.g. RANTAN*; Yao, 1981) were applied to error-free neutron diffraction data for the small molecule 9α-methoxycortisol, $C_{22}H_{32}O_6$ (Weeks *et al.*, 1976) using default options for each of the five resolutions shown in Table 1. The results, summarized in Table 1, clearly show the superior performance of *NSnB*, which finds solutions at every resolution whereas *RANTAN* succeeds at only the highest resolution. In all cases (except the *NSnB* application to $C_{22}H_{32}O_6$ at 1.15 Å, when 10000 trials were run), 8192 trials were carried out at each resolution in order to obtain reliable statistics.

2. *RANTAN*. Finally, *RANTAN* was applied to the experimental neutron diffraction data set for cyclosporin A described in §9. After 16384 trials, no solutions were obtained. Thus, while traditional direct methods will determine small structures (fewer than 60 atoms) with neutrons, they appear to be inadequate when challenged by a structure as large as cyclosporin A (199 atoms). As already stressed (§9), *Neutron Shake-and-Bake*, on the other hand, readily solves the cyclosporin structure (success rate of 4% with error-free data, 2% with experimental data).

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