

Automatic Phasing of Pure Phase Absorption Two-Dimensional NMR Spectra

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A number of procedures have been reported for the automatic phasing of one-dimensional NMR spectra (1-8) but none for two-dimensional spectra. Unlike the phasing of 1D spectra, optimal manual phasing of pure-phase absorption 2D spectra (9) can be a time consuming procedure. Typically, this is carried out by Fourier transforming the 2D spectrum with phases acquired from inspection of the 1D transforms of the free induction decays of the first few increments, followed by further corrections in both the ω_1 and the ω_2 dimensions. The phase corrections for the latter are usually determined by inspection of a few selected rows and columns. It is therefore clearly desirable to develop a method for the fully automated phase correction of two-dimensional NMR spectra. In this paper we present a simple and efficient solution to this problem.

The spectrum to be phased is a 2D hypercomplex spectrum in which the diagonal of the spectrum lies exactly on the diagonal of the point matrix representing the spectrum. Every point of the hypercomplex matrix consists of four values, RR, RI, IR, and II, which are the real and imaginary parts of the ω_1 and ω_2 dimensions, of which only RR is displayed. Phase correction is carried out by multiplying the original values (RR, . . . , II) by the matrices

$$\begin{bmatrix} c_2 & 0 & 0 & -s_2 \\ 0 & c_2 & -s_2 & 0 \\ 0 & s_2 & c_2 & 0 \\ s_2 & 0 & 0 & c_2 \end{bmatrix} \begin{bmatrix} c_1 & -s_1 & 0 & 0 \\ s_1 & c_1 & 0 & 0 \\ 0 & 0 & c_1 & -s_1 \\ 0 & 0 & s_1 & c_1 \end{bmatrix} \begin{bmatrix} \text{RR} \\ \text{RI} \\ \text{IR} \\ \text{II} \end{bmatrix} = \begin{bmatrix} \text{RR}' \\ \text{RI}' \\ \text{IR}' \\ \text{II}' \end{bmatrix} \quad [1]$$

to generate a new set of values (RR', RI', IR' and II'), where

$$\begin{aligned} s_2 &= \sin \phi_2 & s_1 &= \sin \phi_1 \\ c_2 &= \cos \phi_2 & c_1 &= \cos \phi_1 \end{aligned} \quad [2]$$

and

$$\begin{aligned} \phi_1 &= a_1 + \omega_1(e_1 - a_1)/(N - 1) \\ \phi_2 &= a_2 + \omega_2(e_2 - a_2)/(N - 1). \end{aligned} \quad [3]$$

(N is the number of points in each of the two dimensions.)

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The automatic phasing must find the four parameters (a_1, e_1, a_2, e_2) of the two linear functions in Eq. [3] such that the resulting spectrum is optimally phased. For 2D spectra in which the diagonal is absorptive in-phase (e.g., NOESY, HOHAHA, z-COSY), our approach to the phasing problem involves maximizing the value of the diagonal elements and minimizing the asymmetry of the diagonal peaks. Note that for 2D spectra in which the diagonal is either dispersive (e.g., COSY) or absorptive antiphase (e.g., DQF-COSY, E.COSY), the reverse must be carried out. In the first stage the sum X_1 of all the diagonal elements $RR'(n, n)$ is maximized by varying a_2 and e_2 while keeping $a_1 = e_1 = 0$. The starting values of a_2 and e_2 are set equal to minus the phase angle of the complex number

$$\sum_n RR(n) + i \sum_n RI(n).$$

The resulting values of a_2 and e_2 are then used as starting values for the second stage which minimizes the asymmetry of the diagonal peaks given by

$$X_2 = \sum_n |RR'(n, n+A) - RR'(n, n-A)| + |RR'(n+A, n) - RR'(n-A, n)| \quad [4]$$

taken over all n . Typical values for A are 2, 3, 4, 5, and 6; these give slightly different results and should be chosen according to the apodization function used. The greater the degree of resolution enhancement, the smaller the appropriate value of A .

Optimization for both steps is carried out by a simple program that evaluates the value of X_1 or X_2 at all points differing in the value of only one parameter in the phase angle functions (Eq. [3]) by plus or minus a step value s from the current position; i.e., for the parameters a_2 and e_2 in Stage 1, values of $(a_2 + s, e_2)$, $(a_2 - s, e_2)$, $(a_2, e_2 + s)$, $(a_2, e_2 - s)$ would be examined in turn. The best of these results becomes the new current position if it is better than the value at the current position, otherwise convergence for a given value of s has been achieved. Repeated cycles with decreasing values of s allows one to optimize the phasing parameters to the desired level of accuracy. Typically, three cycles of optimization are sufficient.

An application of the method is shown in Fig. 1 which displays the aromatic (panel A) and high-field portion of the aliphatic (panel B) regions of the 500 MHz proton homonuclear Hartman-Hahn (HOHAHA) spectrum of 6 mM α_1 -purothionin recorded in D_2O . The spectrum was processed on a CONVEX C1-XP. The complete 2D hypercomplex transform (1024×1024 complex points) took about 45 s. The upper panels are unphased. The middle panels are the phase-corrected spectra after 3 cycles of Stage 1 optimization with successive values of 0.2, 0.1, and 0.01 rad for s . The resulting values for the phase parameters, a_2, e_2, a_1 , and e_1 , are 2.80 rad (160°), 2.42 rad (139°), 0 rad, and 0 rad, respectively. This first stage, which takes less than 1 s, results in considerable improvement. It is clear, however, that dispersion elements are still present. The lower panels are the phase-corrected spectra after 2 cycles of Stage 2 minimization with successive values of 0.02 and 0.0066 rad. This yields final values for the phase parameters, a_2, e_2, a_1 , and e_1 , of 3.19 rad (184°), 2.14 rad (123°), 0.32 rad (18.3°), and -0.266 rad (-15.2°), respectively. This stage takes ~ 20 s. As is evident, the spectra in the lower panels are perfectly phased.

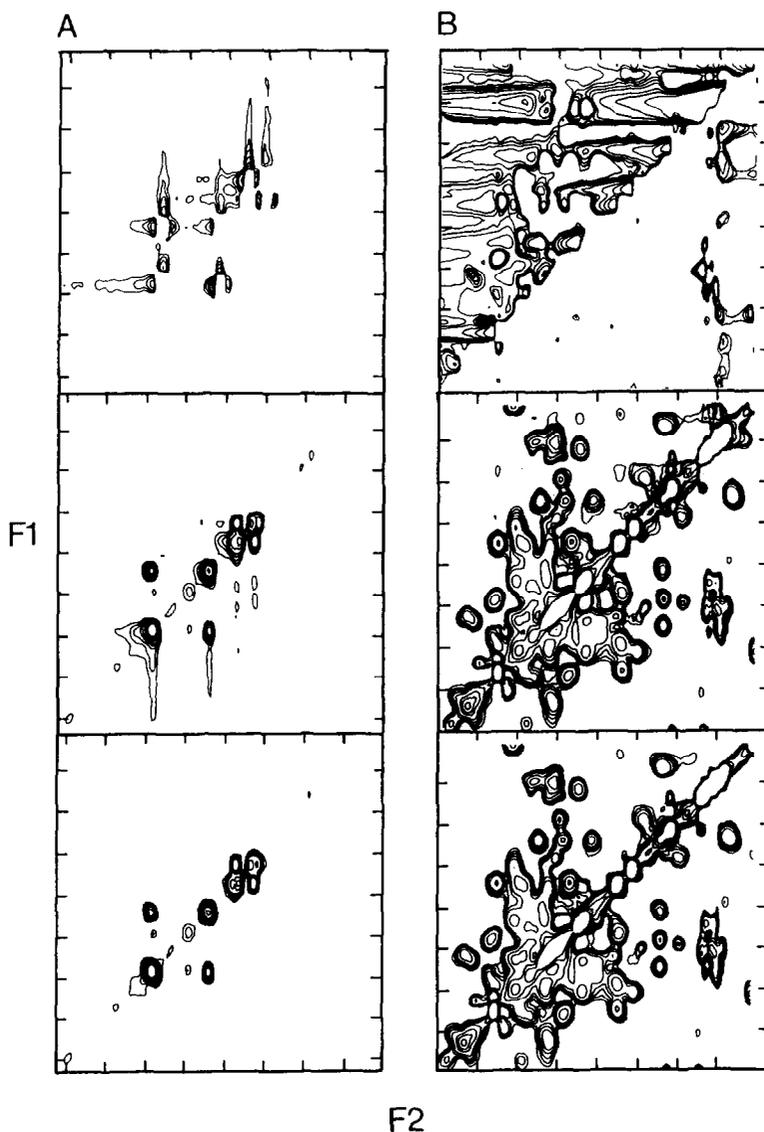


FIG. 1. HOHAHA spectrum of 6 mM α_1 -purothionin recorded in D_2O . The spectrum was acquired with a mixing time of 39 ms using the MLEV17 sequence (10); 512 increments were recorded using the TPPI method (9) with a spectral width of 7042 Hz and zero-filled in the t_1 dimension only to yield after 2D FT a 1024×1024 point hypercomplex matrix with a digital resolution of 6.88 Hz/point. The total measurement time was ~ 6 h. Apodization was carried out using a Lorentzian-to-Gaussian transformation. The value of A in Eq. [4] was 4. The spectra on the left (A) and right (B) hand sides show the aromatic ($\omega_2 = \omega_1 = 8.07$ to 6.31 ppm) and high-field aliphatic ($\omega_2 = \omega_1 = 2.69$ to 0.93 ppm) regions, respectively. The spectrum without phase correction is shown in the top panel, while the middle and lower panels show the spectra after Stages 1 and 2, respectively, of automatic phase correction.

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REFERENCES

1. R. R. ERNST, *J. Magn. Reson.* **1**, 7 (1969).
2. M. M. SIEGEL, *Anal. Chim. Acta* **133**, 103 (1981).
3. S. I. PARKS AND R. B. JOHANNESSEN, *J. Magn. Reson.* **22**, 265 (1976).
4. B. L. NEEF, J. L. ACKERMAN, AND J. S. WAUGH, *J. Magn. Reson.* **25**, 335 (1977).
5. F. G. HERRING AND P. S. PHILPIS, *J. Magn. Reson.* **59**, 489 (1984).
6. C. H. SOTAK, C. L. DUMOULIN, AND M. D. NEWSHAM, *J. Magn. Reson.* **57**, 453 (1984).
7. L. F. GLADDEN AND S. R. ELLIOTT, *J. Magn. Reson.* **68**, 383 (1986).
8. E. C. CRAIG AND A. G. MARSHALL, *J. Magn. Reson.* **76**, 458 (1988).
9. R. R. ERNST, G. BODENHAUSEN, AND A. WOKAUN, "Principles of Nuclear Magnetic Resonance in One and Two Dimensions," Clarendon, Oxford.
10. A. BAX AND D. G. DAVIS, *J. Magn. Reson.* **65**, 355 (1985).