Journal of Biomolecular NMR, 3 (1993) 205–214
ESCOM

J-Bio NMR 104

Computationally efficient gradients for relaxation matrix-based structure refinement including the accommodation of internal motions

Martin J. Dellwo* and Joshua Wand**

Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL 61801, U.S.A.

Received 24 June 1992
Accepted 15 November 1992

Keywords: Rate matrix; Structure refinement; Gradients; Internal motion

SUMMARY

A general method for deriving analytical gradients based on NOESY cross-peak intensities is presented. This method allows for very rapid calculation of exact gradients of cross-peak intensities with respect to parameters directly related to the pairwise dipole–dipole interaction giving rise to the cross peak. In the simplest case, gradients with respect to internuclear separation can be calculated, which allows for the rational modification of distance constraints used in structural refinement. In the general case, any arbitrary level of knowledge of the internal dynamics of the molecule can be introduced, thereby providing a pathway for the experimental determination of motional parameters. The motional characteristics of the internuclear vectors defining dipole–dipole interactions are cast in model-free terms. The form of the gradient circumvents many of the limitations of gradients expressed in terms of Cartesian or dihedral variables. The gradients presented are simple, direct, and exact and require little computational effort to calculate.

INTRODUCTION

Two-dimensional ^{1}H nuclear Overhauser enhancement spectroscopy (NOESY) depends on the dipolar interaction of protons in spatial proximity to one another (Macura and Ernst, 1980). For a simple rigid model, the cross-relaxation rates describing buildup of the NOE between protons are directly proportional to the inverse sixth power of the distance separating pairs of protons, $1/r^6$ (Solomon, 1955). Consequently at short mixing times, the NOESY intensity can be used as a distance measurement by calibration with a known distance (Kumar et al., 1981). For larger molecules, this simple relationship fails significantly at long mixing times because of indirect

*Present address: Eastman Kodak Company, Rochester, NY, U.S.A.
**To whom correspondence should be addressed.

magnetization transfer effects commonly termed spin diffusion (Kumar et al., 1981; Keepers and James, 1984). In addition, internal motions will invalidate the simple rigid rotor approximation, and will lead to calibration errors dependent on the different fluctuations of each internuclear vector.

A complete analysis of the NOESY spectrum, by specification of the rate matrix describing relaxation, accounts rigorously for the effects of spin diffusion and allows a direct analysis of the relationship of cross-peak intensities to a set of internuclear distances (Keepers and James, 1984; Borgias and James, 1988; Mirau, 1988). A rate matrix based on a set of atomic coordinates can be derived by specifying the elements as a function of the internuclear distances. This matrix can then be used to calculate the full matrix of NOESY intensities as a function of the mixing time.

In order to determine a molecular structure with these calculations, one must refine the set of relaxation rates to obtain the best agreement between calculated and observed intensities. Several techniques have been proposed for this (e.g., Borgias and James, 1988; Boelens et al., 1988; Baleja et al., 1992). In many, a penalty function specifying the error in the observed versus calculated intensities is minimized by means of molecular mechanics calculations which change the molecular coordinates while maintaining physically reasonable molecular structures (Borgias and James, 1988; Nilges et al., 1991). At each iteration, a new rate matrix is calculated based on the new coordinates. Such techniques have the advantage of being based directly on the set of distances defined by the atomic coordinates of the structure, but in the absence of parameters describing internal motions, the results are biased by the assumption of rigidity. A modified approach would be to iteratively modify the rate matrix to give the best fit of calculated to observed NOESY data, after which the individual terms of the rate matrix could be interpreted by using various relaxation models (Boelens et al., 1988). This approach suffers by 'decoupling' of the dependence of the rate matrix elements from the underlying physical origin, and could lead to biased results. For instance, the fluctuation of a given internuclear vector gives rise to rate terms in both the diagonal and off-diagonal elements of the rate matrix. These terms are related but not equal. Refinement of the rate matrix without reference to this relationship could lead to inconsistent results.

Recently, several related gradient-based refinement techniques have been proposed which permit one to calculate the dependence of a given cross-peak intensity on a specific proton's molecular coordinates (Nilges et al., 1991; Yip and Case, 1989; Mertz et al., 1992). This has been used in conjunction with molecular mechanics to specify more accurately the changes in molecular coordinates needed to improve agreement between calculated and observed intensities. These techniques, while quite powerful, still suffer from the assumption of internal rigidity and do not account for variations in distances due to internal motions, since the rate matrix is directly coupled to a specific set of coordinates. Moreover, the course of the refinement can depend on a pseudoenergy term, the preferred method for including a distance penalty function into standard molecular mechanics calculations. As pointed out by Yip and Case (1989), the gradient function may be taken with respect to any parameter, not just molecular coordinates. We propose here a gradient-based method which uses a penalty function based on parameters describing the motions of the internuclear vectors. This method would be used to determine realistic bounds on the internuclear distances, in conjunction with either distance-geometry calculations to obtain a family of structures consistent with the observed data, or with a refinement strategy which directly determines model-independent order parameters and correlation times describing the internal motions. More immediately, and perhaps importantly, the form of the gradients allows

any arbitrary level of knowledge of the internal motions of the molecule to be introduced without significant modification.

THEORY

The NOESY spectrum can be calculated directly from the rate matrix describing dipolar relaxation by way of the equation:

$$M(t) = e^{-Rt_{mix}}M(0) \tag{1}$$

where the diagonal matrix M(0) describes the initial intensities for all spins M_i at zero mixing time. It is assumed that the elements M_i have a one-to-one correspondence to individual protons in the molecule of interest. Furthermore, although more complicated expressions for M(0) could occur due to incomplete relaxation or saturation effects (summarized in Dellwo et al., 1992), it is assumed here that $M(0) = k \cdot 1$, where k is a scale factor indicating arbitrary intensity units. These assumptions mean that the rate matrix R is symmetric with real eigenvalues, leading to the result:

$$M(t) = X \cdot e^{-\Lambda t_{mix}} \cdot X^T \tag{2}$$

where Λ is the diagonal matrix of eigenvalues of the rate matrix and X is the matrix whose columns are the corresponding eigenvectors. It should be noted that the order of matrix multiplication is important, and the correct relationship is $R = X \cdot \Lambda \cdot X^T$. Yip and Case (1989) use the notation $R = L^T \cdot \Lambda \cdot L$, but do not otherwise define L. This ambiguity could lead to confusion; we therefore note that $L = X^T$.

The elements of the rate matrix depend on the dipolar relaxation rates describing the interactions of pairs of protons, which in turn depend on the spectral densities describing the motions of the internuclear vectors. For the case of proton–proton relaxation assuming rigid-rotor isotropic tumbling, the rates are given by:

$$\begin{aligned} R_{ij} &= \sigma_{ij} = \frac{\hbar^2\gamma^4}{4r_{ij}^6}(6J(2\omega)-J(0)) \\ R_{ii} &= \rho_i = \sum_{j \neq i} \rho_{ij} \\ \rho_{ij} &= \frac{\hbar^2\gamma^4}{4r_{ij}^6}(J(0)+3J(\omega)+6J(2\omega)) \\ \text{and } J(\omega) &= \frac{2}{5}\left(\frac{\tau_c}{1+\omega^2\tau_c^2}\right) \end{aligned} \tag{3}$$

where r_{ij} is the interproton distance, ω is the proton Larmor frequency in rad/s, γ is the proton gyromagnetic ratio, and τ_c is the correlation time in s/ rad for molecular tumbling. The rate constants are in rad/s. To include variations in interproton distances due to internal motions on a timescale slower than τ_c, the above expressions can be modified by the substitution of an

average $\langle r_{ij}^{-6} \rangle$ for $1/ r_{ij}^{6}$. This derives from consideration of the expression for the autocorrelation function at time zero:

$$C(t) = \left\langle \frac{P_2(\hat{\mu}_{ij}(0) \cdot \hat{\mu}_{ij}(t))}{r_{ij}^3(0) \cdot r_{ij}^3(t)} \right\rangle \tag{4}$$

where at t = 0, this reduces to $\langle r_{ij}^{-6} \rangle$, with the angle brackets denoting the average over all possible conformations. This is the correct expression rather than $\langle 1/ r \rangle^6$ or $\langle 1/ r^3 \rangle^2$ as sometimes appears in the literature.

To account for fast internal motions, the simplest expression for the internal autocorrelation function which contains all the information concerning the internal motions is that proposed by Lipari and Szabo (1982a,b), and leads to the following expressions:

$$\sigma_{ij} = \frac{\hbar^2 \gamma^4}{4} (6J(2\omega) - J(0))$$

$$\rho_{ij} = \frac{\hbar^2 \gamma^4}{4} (J(0) + 3J(\omega) + 6J(2\omega)) \tag{5}$$

$$\text{and } J(\omega) = \frac{2}{5} \left(\tilde{S}_{ij}^2 \frac{\tau_c}{1+\omega^2\tau_c^2} + (\langle r_{ij}^{-6} \rangle - \tilde{S}_{ij}^2) \frac{\tau}{1+\omega^2\tau^2} \right)$$

$$\frac{1}{\tau} = \frac{1}{\tau_c} + \frac{1}{\tau_e(ij)}$$

where $\langle r_{ij}^{-6} \rangle$ has the meaning given above, and $\tilde{S}_{ij}^2$ now is the limiting value of the internal autocorrelation function, $C_I(\infty)$, describing the motion of the vector r_{ij} in a coordinate system rigidly attached to the molecule:

$$\tilde{S}_{ij}^2 = C_I(\infty) = \sum_{m=-2}^{2} \left| \left\langle \frac{C_{2m}(\Omega_{ij})}{r_{ij}^3} \right\rangle \right|^2 \tag{6}$$

Here C_{2m} are second- order spherical harmonics and Ω_{ij} are the polar angles of the vector r_{ij}. The parameter $\tau_e(ij)$ is an effective correlation time for the internal motion, as described by Lipari and Szabo (1982a,b). These parameters, $\tilde{S}_{ij}^2$ and $\tau_e(ij)$, describe the motional behavior of the relevant vector without reference to a specific motional model, and are therefore referred to as model-free parameters. Similar equations describing the relaxation behavior of heteronuclei have been successfully used to analyze the internal motions of peptides and macromolecules, where one can simplify the observed relaxation behavior experimentally and assume a two- spin approximation (Dellwo and Wand, 1989; Kay et al., 1989; Clore et al., 1990; Schneider et al., 1992; Stone et al., 1992). However, determination of model-free parameters for proton– proton relaxation requires the full analysis of the rate matrix describing the coupled behavior of all relaxing protons. Such an analysis can be performed by using a distance- based gradient refinement as described below.

Calculation of the gradient of a penalty function based on NOESY intensities depends on finding the differentiation of $e^{-Rt_{mix}}$ with respect to the parameter of interest. The derivation below follows from the work described by Yip and Case (1989), where the exponential $e^{-Rt_{mix}}$ is expanded and derivatives $\partial/\partial\upsilon$ with respect to some parameter υ can be written:

$$\frac{\partial}{\partial \upsilon}(e^{-Rt_{mix}}) = -t_{mix}\frac{\partial R}{\partial \upsilon} + \frac{t_{mix}^2}{2!}\left(R\frac{\partial R}{\partial \upsilon} + \frac{\partial R}{\partial \upsilon}R\right) - \frac{t_{mix}^3}{3!}\left(R^2\frac{\partial R}{\partial \upsilon} + R\frac{\partial R}{\partial \upsilon}R + \frac{\partial R}{\partial \upsilon}R^2\right) + \ldots \quad (7)$$

The matrix $\partial R/\partial \upsilon$ is a matrix whose elements are $\partial R_{ij}/\partial \upsilon$ and can easily be found from the expressions above for the elements of the rate matrix. After pre- and post-multiplication by $X \cdot X^T$, the derivative can be rewritten as:

$$\frac{\partial}{\partial \upsilon}(e^{-Rt_{mix}}) = -t_{mix}X\left(K - \frac{t_{mix}}{2!}(K\Lambda + \Lambda K) - \frac{t_{mix}^2}{3!}(K\Lambda^2 + \Lambda K\Lambda + \Lambda^2)K + \ldots\right)X^T \quad (8)$$

where $K = X^T \cdot \partial R/\partial \upsilon \cdot X$.

The series above can be summed in closed form since Λ is diagonal, so that the equation can be expressed as:

$$\frac{\partial}{\partial \upsilon}(e^{-Rt_{mix}}) = -t_{mix}X\, J(K, -\Lambda t_{mix})X^T \quad (9)$$

and the matrix function $J(K,-\Lambda t_{mix})$ is defined such that:

$$\begin{aligned} J_{ij}(K,-\Lambda t_{mix}) &= \frac{-K_{ij}}{t_{mix}}\left(\frac{-e^{\lambda_i t_{mix}} - e^{-\lambda_j t_{mix}}}{\lambda_i - \lambda_j}\right) && \lambda_i \neq \lambda_j \\ &= K_{ij}e^{-\lambda_i t_{mix}} && \lambda_i = \lambda_j \end{aligned} \quad (10)$$

As pointed out by Nilges et al. (1991), calculation of the gradient with respect to all molecular coordinates is quite computationally expensive since a different matrix K needs to be computed for every coordinate of interest. In the case of derivatives with respect to atomic coordinates for N protons, there are 3N unique coordinates $\{x,y,z\}_i$, i = 1...N and therefore 3N matrices which can be computed. For a cross peak between two close protons, i and j, the internuclear separation, r_{ij}, can be the main determinant of the NOESY buildup, and will depend on the six coordinates $\{x,y,z\}_i$ and $\{x,y,z\}_j$. The coordinates of just one proton, i, in turn enter into all rate matrix terms involving that proton. The matrix $\partial R/\partial \upsilon$ for $\upsilon = x_i$, y_i, or z_i will have non-zero elements for the row and column i, as well as all diagonal elements. Since for protons $\sigma_{ij} = \sigma_{ji}$ and $\rho_{ij} = \rho_{ji}$, there are 2(N−1) unique derivatives with respect to one coordinate, υ, to be found, corresponding to the (N−1) unique distances, r_{ij}, involving coordinates for proton, i.

If one instead takes the derivative of R with respect to particular distances, there are now N(N−1)/2 unique distances and therefore N(N−1)/2 matrices, $\partial R/\partial \upsilon$, that could be calculated. This rapidly exceeds the number of unique coordinates {x,y,z} for large N. However, since in practice only short-range distances lead to strong cross peaks which would be of primary interest in a structure determination, not all derivatives are of interest. Most likely, a particular observed cross peak, l_{ij}, if not spin-diffusive in nature, will depend strongly on the corresponding distance r_{ij}. This is particularly so with short mixing times. The matrix $\partial R/\partial \upsilon$ where now $\upsilon = r_{ij}$ then has a particularly simple form. All elements will be zero except for $\partial R_{ii}/\partial_{ij}$, $\partial R_{ij}/\partial_{ij}$, $\partial R_{ji}/\partial r_{ij}$ and $\partial R_{jj}/\partial r_{ij}$. The elements $\partial R_{ii}/\partial r_{ij}$ and $\partial R_{jj}/\partial r_{ij}$ will both be equal to $\partial \rho_{ij}/\partial r_{ij}$ since $\rho_{ij} = \rho_{ji}$. Likewise,

$\partial R_{ij}/\partial r_{ij} = \partial \sigma_{ij}/\partial r_{ij} = \partial R_{ji}/\partial r_{ij}$. Thus for a given distance, $\partial R/\partial r_{ij}$ is a symmetrical matrix of four non-zero elements, involving two unique derivatives. This result applies equally for derivatives with respect to $\langle r_{ij}^{-6} \rangle$, $\tilde{S}_{ij}^2$, or $\tau_e(ij)$ since these parameters account for reorientation of the unique vector, r_{ij}. In what follows we use υ_{ij} to refer to any one of the above parameters.

As $\partial R/\partial \upsilon_{ij}$ only contains four non-zero elements, the matrix $K = X^T \cdot \partial R/\partial \upsilon_{ij} \cdot X$ becomes straightforward to compute. The elements K_{ab} for a,b = 1...N can be written as:

$$K_{ab} = (x_{ia}x_{ib} + x_{ja}x_{jb}) \frac{\partial R_{ii}}{\partial R \upsilon_{ij}} + (x_{ia}x_{jb} + x_{ja}x_{ib}) \frac{\partial R_{ij}}{\partial \upsilon_{ij}} \tag{11}$$

where we have taken advantage of the equalities $\partial R_{ii}/\partial \upsilon_{ij} = \partial R_{jj}/\partial \upsilon_{ij}$ and $X_{ka} = X^T{}_{ak}$ for k = i,j. We note that the matrix K is symmetric. The corresponding matrix function, J, is calculated in a straightforward way from K and is also symmetric. Having computed this matrix, the derivative of any cross-peak intensity $I_{uv}(t_{mix})$ with respect to υ_{ij} can be expressed as:

$$\frac{\partial I_{uv}(t_{mix})}{\partial \upsilon_{ij}} = \sum_{a,b}^{N} x_{au}x_{bv}J_{ab} \tag{12}$$

For u = i and v = j, this can be expanded in terms of the eigenvalues and derivative of R to yield:

$$\frac{\partial I_{ij}(t_{mix})}{\partial \upsilon_{ij}} = \sum_{a,b}^{N} \left[(x_{ia}^2 x_{ib}x_{jb} + x_{ia}x_{ja}x_{jb}^2) \frac{\partial R_{ii}}{\partial \upsilon_{ij}} + (x_{ia}^2 x_{jb}^2 + x_{ia}x_{ja}x_{ib}x_{jb}) \frac{\partial R_{ij}}{\partial \upsilon_{ij}} \right] \Gamma_{ab}$$

where

$$\Gamma_{ab} = \frac{e^{-\lambda_a t_{mix}} - e^{-\lambda_b t_{mix}}}{\lambda_a - \lambda_b} \qquad \lambda_a \neq \lambda_b$$
$$= -t_{mix} e^{-\lambda_a t_{mix}} \qquad \lambda_a = \lambda_b \tag{13}$$

This equation is fairly straightforward and is efficient to calculate using a typical workstation configuration. The derivatives of the functions σ_{ij} and ρ_{ij} with respect to υ_{ij} are exceedingly straightforward to determine, and in fact involve terms used to calculate the rate matrix itself. Table 1 contains derivatives of σ_{ij} and ρ_{ij} with respect to the various υ_{ij}. It should be pointed out that, in the context of computing intensity matrices for comparison to observed data, the determination of eigenvalues and eigenvectors of the rate matrix is performed in the process of computing the intensity matrix, and does not represent an additional computational burden when calculating the gradients (Dellwo, M.J., Schneider, D.M. and Wand, A.J., unpublished results).

DISCUSSION

A significant advantage of the gradients derived above is their inherent relationship to the refinement of structural models when using distance constraints. A gradient can be calculated

that most directly relates the observed intensities to the physical interaction that determines them. In addition, the gradients are simpler and more rapid to perform than comparable coordinate-based gradients. Calculation of 600 cross-peak gradients with respect to distance, as given in Eq. 13, in a system of 630 protons, required only 30% additional CPU compared to the calculation of the full matrix of NOE intensities when performed on a Silicon Graphics 4D/35. A similar calculation performed on a Cray Y-MP for three NOESY mixing times required 145 CPU seconds. We emphasize that these calculations are exact and do not make use of any approximations or distance cutoff criteria (Nilges et al., 1991; Yip and Case, 1989; Bonvin et al., 1992; Mertz et al., 1992). Another advantage of the approach described here is that one need not assume a single rigid fixed distance in the refinement procedure. Indeed, the simplicity of the gradients presented here allows for any arbitrary level of knowledge of the internal dynamics of the molecule to be introduced. This is in contrast to gradient methods cast in coordinate or dihedral variables (Nilges et al., 1991; Mertz et al., 1992). Finally, it should be noted that the relatively complex but empirical treatment of internal dynamics presented by Koehl & Lefèvre (1990) reduces to our simpler but exact treatment.

A structure refinement strategy will usually attempt to minimize the difference between experimentally observed NOESY intensities and those calculated on the basis of structural model. This will often involve the specification of an R-factor such as that presented by Bovin et al. (1991), Nilges et al. (1991), or Mertz et al. (1992). If the R-factor is an error function to be minimized,

TABLE I
DERIVATIVES OF RELAXATION RATES[a]

υ_{ij}	r_{ij}	$1/r_{ij}^6$	$\tilde{S}_{ij}^2$	$\langle r_{ij}^{-6}\rangle$	$\tau_e(ij)$
$\frac{\partial\sigma_{ij}}{\partial\upsilon_{ij}}$	$\frac{-6}{r_{ij}^7}F_\sigma(\tau_c)$	$F_\sigma(\tau_c)$	$F_\sigma(\tau_c) - F_\sigma(\tau)$	$F_\sigma(\tau)$	$(\langle r_{ij}^{-6}\rangle - \tilde{S}_{ij}^2)\frac{\partial F_\sigma(\tau)}{\partial\tau_e(ij)}$
$\frac{\partial\rho_{ij}}{\partial\upsilon_{ij}}$	$\frac{-6}{r_{ij}^7}F_\rho(\tau_c)$	$F_\rho(\tau_c)$	$F_\rho(\tau_c) - F_\rho(\tau)$	$F_\rho(\tau)$	$(\langle r_{ij}^{-6}\rangle - \tilde{S}_{ij}^2)\frac{\partial F_\rho(\tau)}{\partial\tau_e(ij)}$

where

$$F_\sigma(t) = \frac{\hbar^2\gamma^4}{10}\left(\frac{6t}{1+4\omega^2t^2} - t\right), \qquad F_\rho(t) = \frac{\hbar^2\gamma^4}{10}\left(t + \frac{3t}{1+\omega^2t^2} + \frac{6t}{1+4\omega^2t^2}\right)$$

and

$$\frac{\partial F_\sigma(t)}{\partial\tau_e(ij)} = \frac{\hbar^2\gamma^4}{10}\left[\frac{6(1-4\omega^2t^2)}{(1+4\omega^2t^2)^2} - 1\right]\frac{\partial t}{\partial\tau_e(ij)}$$

$$\frac{\partial F_\rho(t)}{\partial\tau_e(ij)} = \frac{\hbar^2\gamma^4}{10}\left[1 + \frac{3(1-\omega^2t^2)}{(1+\omega^2t^2)^2} + \frac{6(1-4\omega^2t^2)}{(1+4\omega^2t^2)^2}\right]\frac{\partial t}{\partial\tau_e(ij)}$$

For $t = \tau$,

$$\frac{\partial\tau}{\partial\tau_e(ij)} = \left(\frac{\tau_c}{\tau_c + \tau_e(ij)}\right)^2 = \left(\frac{\tau}{\tau_e(ij)}\right)^2$$

[a] Derivatives with respect to r_{ij} and $1/r_{ij}^6$ result from Eq. 3. Derivatives with respect to $\tilde{S}_{ij}^2$, $\langle r_{ij}^{-6}\rangle$ and $\tau_e(ij)$ result from Eq. 5.

then the gradient of this function with respect to a parameter, υ_{ij}, will require use of Eqs. 12 and 13. Incorporation of the distance-based gradients as described above allows the refinement strategy to be tailored to differing levels of accuracy. The accuracy depends solely on which terms of the gradient are examined, not on prior approximations in the rate matrix or gradient calculations themselves.

For a simple distance-geometry-based analysis, refinement proceeds by specifying a set of distance bounds rather than exact distances. This is fortunate, as the set of distances describing a particular structure are not independent quantities. An arbitrary change in one distance must necessarily change all distances to ensure that the resulting distance matrix can be embedded in E_3 space. By beginning with a set of bounds defined, for example, by the loose bound criteria (Wüthrich, 1986), the gradients presented here provide a means to contract (or expand) the bounds to the widths corresponding to the limits of their inherent accuracy. A gradient which is based on rigid distances, r_{ij}, or the ensemble average, $\langle r_{ij}^{-6} \rangle$, is perhaps best suited to this approach, since actual fixed structures are computed by the distance geometry algorithm. As a first step, specific experimental and calculated cross-peak intensities could be compared, and the distance gradient given by Eq. 13 used to contract specific bounds. In this case, the gradient of the intensity of a cross peak with respect to a change in the distance between the two directly interacting spins is used to change the corresponding bound. At early stages of the refinement, where coarse adjustments of bounds are being undertaken, this is probably sufficient. For a more accurate gradient, the additional terms represented by Eq. 12 need to be summed and would determine the effects of a particular distance on *all* cross peaks. We note here that once the matrix, K, is computed for a given gradient, $\partial R_{ij}/\partial \upsilon_{ij}$, it can be used to find $\partial I_{kl}(t_{mix})\partial \upsilon_{ij}$ for any spins, k or l, of interest without a severe additional computational burden. In either case, the individual gradient terms fully include the effects of spin-diffusion and are completely accurate. In particular, if the goal is to iteratively change a set of bounds rather than fixed distances, the use of Eq. 13 alone is unlikely to impede an iterative refinement, especially at early stages. The decision as to whether a specific bound needs changing will also depend on other factors, such as comparisons made from a family of structures calculated from one set of bounds. For such refinements, not only the gradient must be examined but also the particular distance used in each structure, to determine whether the upper or lower bound, or both, must be raised or lowered. Criteria for changing bounds would involve the magnitude of the gradients found, and where each distance fell within the bounds. When the bounds are sufficiently accurate given the experimental data, then one would expect that the family of structures which satisfies the bounds constraints would encompass the range of distances specified and minimize the R-factor, and that the gradients determined for a given distance, when summed across the family of structures, sum to approximately zero.

A fuller treatment to derive the model-free parameters could be attempted by using an initial structure as a starting point, then refining the matrix R against the penalty function, where R is now a function of the complete set of parameters $\{\tilde{S}_{ij}^2, \langle r_{ij}^{-6} \rangle, \tau_e(ij)\}$ for i,j = 1...N and $i \neq j$. At this level, the refinement is no longer directly dependent upon a particular model structure. The initial structure serves to bootstrap the model-free parameters to rigid rotor where $\tilde{S}_{ij}^2$ equals $\langle r_{ij}^{-6} \rangle$. The refinement would, in principle, involve 3N(N−1)/2 parameters, with each full NOESY spectrum having only N(N−1)/2 independent peaks [or N^2, if non-equilibrium initial intensities are present, Dellwo, M.J., Schneider, D.M. and Wand, A.J., unpublished results]. As many of these cross

peaks will be obscured by spectral overlap or be too weak to measure accurately, any such refinement would require data at multiple mixing times and additionally multiple field strengths, to accurately fit the spectral densities.

As in the above distance-geometry-based strategy, initially only Eq. 13 would be used to direct the refinement of the parameters. However, as described above, at later stages Eq. 12 would need to be included to accurately reflect the effects of indirect magnetization transfer in the complete gradient. Model-free parameters describing long-range interactions will most likely be poorly determined, since cross peaks involving these spins directly will be susceptible to indirect magnetization transfer and will therefore require knowledge of many more interactions in order for the parameters to be extracted with comparable accuracy. Fortunately, internal motions on the NMR time scale in well-packed globular proteins are primarily due to local fluctuations rather than large scale reorganizations (Dellwo and Wand, 1989; Kay et al., 1989; Clore et al., 1990; Schneider et al., 1992; Stone et al., 1992). Thus, for long-range interactions, the apparent order parameters will tend toward unity and the values $\tilde{S}_{ij}^2$ and $\langle r_{ij}^{-6} \rangle$ will tend towards the 'rigid rotor' value $1/r_{ij}^6$. This is easily visualized if one considers that an atom which might fluctuate over a 0.5Å interval will lead to a large variation in the distance r_{ij} between a nearby atom; however, this same fluctuation will have a minimal effect on the distance r_{ij} between a remote atom. Thus the effective approximation that the internuclear vector connecting remote atoms is rigid will be valid and thereby allow a more complete application of Eq. 12. Nonetheless, without a complete set of accurate NOESY intensities, one will not be able to determine all parameters, but only those parameters which directly relate the observed intensities to the underlying physical interaction.

ACKNOWLEDGEMENTS

This work was supported by NIH research grant GM-35940 and a grant (NSF DMB 920002N) from the National Center for Supercomputing Applications (A.J.W.). M.J.D. is the recipient of an NIH predoctoral fellowship (GM-07229) administered by the University of Pennsylvania.

REFERENCES

Baleja, J.D., Moult, J. and Sykes, B.D. (1992) *J. Magn. Reson.*, **87**, 375–384.
Boelens, R., Koning, T.M.G. and Kaptein, R. (1988) *J. Mol. Struct.*, **173**, 299–311.
Bonvin, A.M.J.J., Boelens, R. and Kaptein, R. (1992) *J. Biomol. NMR*, **1**, 305–309.
Borgias, B.A. and James, T.L. (1988) *J. Magn. Reson.*, **79**, 493–512.
Clore, G.M., Driscoll, P.C., Wingfield, P.T. and Gronenborn, A.M. (1990) *Biochemistry*, **29**, 7387–7401.
Dellwo, M.J. and Wand, A.J. (1989) *J. Am. Chem. Soc.*, **111**, 4571–4578.
Kay, L.E., Torchia, D. and Bax, A. (1989) *Biochemistry*, **28**, 8972–8979.
Keepers, J.W. and James, T.L. (1984) *J. Magn. Reson.*, **57**, 404–426.
Koehl, P. and Lefèvre, J-F. (1990) *J. Magn. Reson.*, **86**, 565–583.
Kumar, Anil, Wagner, G., Ernst, R.R. and Wüthrich, K. (1981) *J. Am. Chem. Soc.*, **103**, 3654–3658.
Lipari, G. and Szabo, A. (1982a) *J. Am. Chem. Soc.*, **104**, 4546–4559.
Lipari, G. and Szabo, A. (1982b) *J. Am. Chem. Soc.*, **104**, 4559–4570.
Macura, S. and Ernst, R.R. (1980) *Mol. Phys.*, **41**, 95–117.
Mirau, P. (1988) *J. Magn. Reson.*, **80**, 439–447.
Mertz, J.E., Güntert, P., Wüthrich, K. and Braun, W. (1992) *J. Biomol. NMR.*, **1**, 247–248.
Nilges, M., Habazettl, J., Brünger, A.T. and Holak, T.A. (1991) *J. Mol. Biol.*, **219**, 499–510.

Schneider, D.M., Dellwo, M.J. and Wand, A.J. (1992) *Biochemistry*, **31**, 3645–3652.
Stone, M.J., Fairbrother, W.J., Palmer III, A.G., Reizer, J., Saier, Jr., M.H., and Wright, P.E. (1992) *Biochemistry*, **31**, 4394–4406.
Solomon, I. (1955) *Phys. Rev.*, **99**, 559–565.
Yip, P. and Case, D.A. (1989) *J. Magn. Reson.*, **83**, 643–648.
Wüthrich, K. (1986) *NMR of Proteins and Nucleic Acids*, Wiley, New York.