Reduced spectral density mapping for proteins: Validity for studies of ^{13}C relaxation

R. Andrew Atkinson* & Jean-François Lefèvre
UPR 9003 du CNRS, Ecole Supérieure de Biotechnologie de Strasbourg, Bld. Sébastien Brant, F-67400 Illkirch, France

Abstract

Spectral density mapping provides direct access to protein dynamics with no assumptions as to the nature of the molecule or its dynamic behaviour. Reduced spectral density mapping characterises a protein's motions at a lower experimental burden, assuming that the spectral density function J(ω) is flat around ω_H. This introduces little error for ^{15}N relaxation data but is less valid for ^{13}C studies, perturbing J(ω_C) considerably to an extent that depends on the nature of the molecule's motions. We propose the fitting of spectral density at high frequencies to a single Lorentzian and show that the true values of the spectral density lie between those determined by the two approximations.

The measurement of ^{15}N and ^{13}C relaxation rates enables the detailed study of protein dynamics (Palmer, 1997). At present, these data are commonly analysed using the so-called 'model-free' approach, described by Lipari and Szabo (1982a,b), in which the equations for the relaxation rates (Abragam, 1961) are fitted using three parameters, namely the order parameter, S^2, an overall correlation time, τ_c, and a correlation time for internal motion, τ_i. A fourth parameter, R_{ex}, an exchange rate term, may be added to the transverse relaxation rate, if required for a satisfactory fit. The drawbacks of this approach are becoming apparent, in particular the loss of information on nanosecond time-scale motions (Korzhnev et al., 1997) and the difficulty in interpreting data from anisotropic molecules (Luginbühl et al., 1997).

Spectral density mapping has been proposed as a more straightforward manner in which to analyse relaxation data (Peng and Wagner, 1992a,b, 1995; Farrow et al., 1995a; Ishima and Nagayama, 1995a; Dayie et al., 1996; Lefèvre et al., 1996), requiring no assumptions and allowing a simple reading of the molecule's dynamic behaviour. Indeed, on passing from

relaxation rates to spectral density values, no assumptions as to the molecule's shape or motions are made, while the information we require on the dynamics of the system are readily apparent. Models are, of course, then necessary to extract quantitative information, but not to interpret the data in terms of flexibility, conformational exchange and anisotropy, or to compare the behaviour of related systems. Although simple to implement and interpret, the approach has not been widely used to date (Farrow et al., 1995b; Ishima and Nagayama, 1995b; Peng and Wagner, 1995; Lefèvre et al., 1996; Mer et al., 1996; van Heijenoort et al., 1998). More regrettably perhaps, the minimum set of three relaxation rates is not always measured, rendering impossible the subsequent re-analysis of relaxation data by spectral density mapping.

Spectral density mapping simply involves solving Abragam's equations for heteronuclear relaxation rates (Abragam, 1961), expressed in terms of the spectral density. In the full approach (Peng and Wagner, 1992a,b, 1995), a set of six independent relaxation rate measurements ($R_X(X_z)$, $R_X(X_x)$, $R_X(H_z \rightarrow X_z)$, $R_X(2X_zH_z)$, $R_X(2X_xH_z)$, $R_H(H_z)$) is required to determine the spectral density, $J(\omega)$, at the five frequencies appearing in the equations (0, ω_X, $\omega_H - \omega_X$,
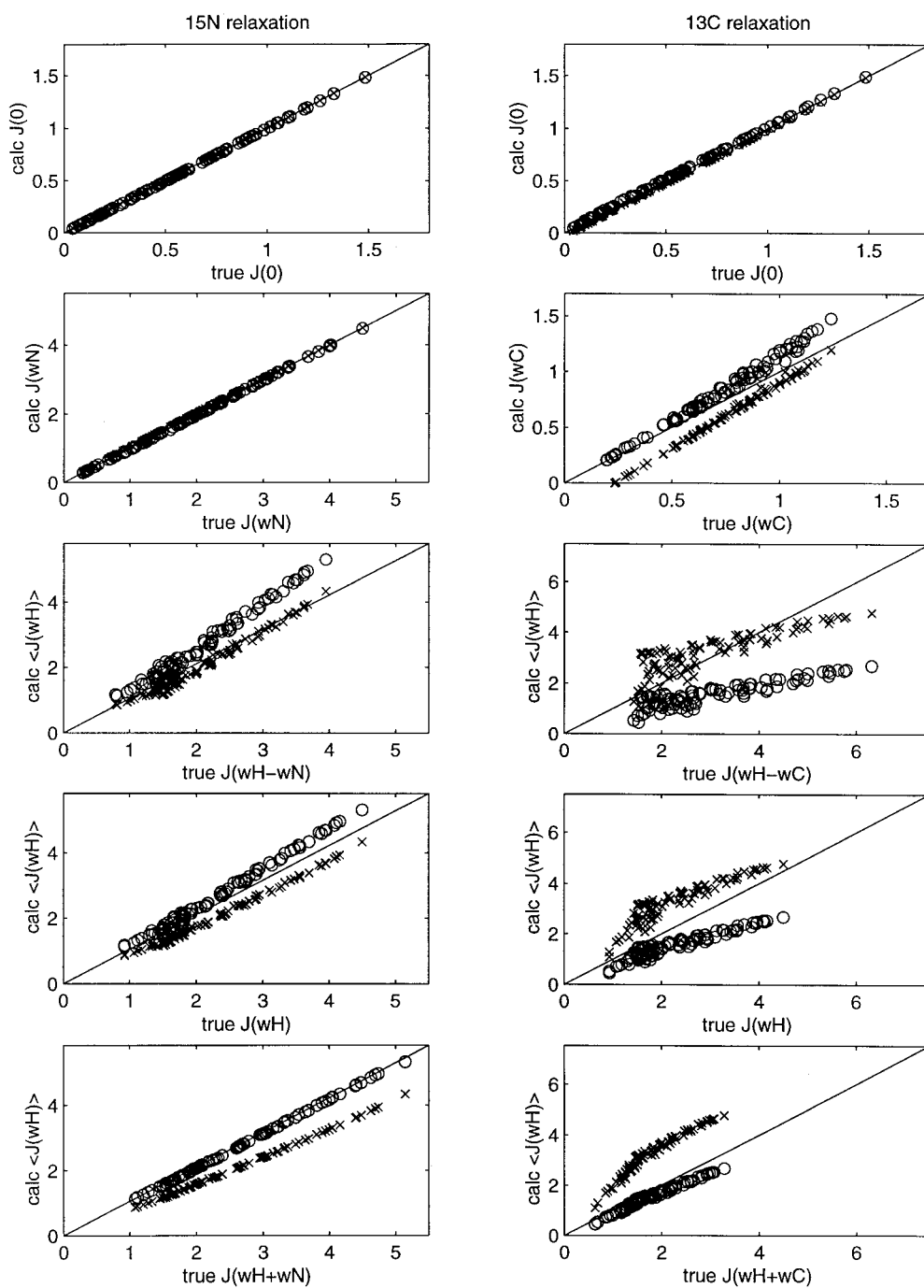
*To whom correspondence should be addressed.

Figure 1. Calculated spectral density values (in ns.rad^{-1}) at frequencies 0, ω_X, $\omega_H - \omega_X$, ω_H, $\omega_H + \omega_X$ plotted against the true values for X–^{1}H vectors relaxed by various contributions of three motions with correlation times of 4 ns, 0.4 ns and 0.04 ns. The values of the spectral density calculated assuming $J(\omega)$ to be flat at ω_H (o) and using a single Lorentzian to fit the high frequency region (x) are shown.

ω_H, $\omega_H + \omega_X$), and the dipolar term for the relaxation of the amide proton by other protons, $\rho_{HH'}$. The equations may be expressed most simply in matrix form:

$$\begin{pmatrix} R_X(X_z) \\ R_X(X_x) \\ R_X(H_z \rightarrow X_z) \\ R_X(2X_zH_z) \\ R_X(2X_xH_z) \\ R_H(H_z) \end{pmatrix} =$$

$$\begin{pmatrix} 0 & E & A & 0 & 6A & 0 \\ 2E/3 & E/2 & A/2 & 3A & 3A & 0 \\ 0 & 0 & -A & 0 & 6A & 0 \\ 0 & E & 0 & 3A & 0 & 1 \\ 2E/3 & E/2 & A/2 & 0 & 3A & 1 \\ 0 & 0 & A & 3A & 6A & 1 \end{pmatrix} \begin{pmatrix} J(0) \\ J(\omega_X) \\ J(\omega_H - \omega_X) \\ J(\omega_H) \\ J(\omega_H + \omega_X) \\ \rho_{HH'} \end{pmatrix}$$

or

$$\mathbf{R} = \mathbf{C} \times \mathbf{J} \tag{1}$$

where

$$A = \left(\frac{\mu_0}{4\pi}\right)^2 \cdot \frac{\gamma_H^2 \gamma_X^2 h^2}{4r_{HX}^6}$$

$$B = \frac{\Delta_X^2 \omega_X^2}{3}$$

$$E = 3A + B$$

where μ_0 is the permeability of the vacuum, γ_H and γ_X are the gyromagnetic ratios of the ^{1}H and X nuclei respectively, r_{XH} is the internuclear X–^{1}H bond distance and Δ_X is the chemical shift anisotropy of X.

The values of the spectral density, $J(\omega)$, may thus be obtained by solving the equations, or by inverting the matrix $\mathbf{C}$ and applying

$$\mathbf{J} = \mathbf{C}^{-1} \times \mathbf{R} \tag{2}$$

A more practical approach in experimental terms, referred to as 'reduced' spectral density mapping (Farrow et al., 1995a; Ishima and Nagayama, 1995a; Peng and Wagner, 1995; Lefèvre et al., 1996), requires only three relaxation rates, $R_X(X_z)$, $R_X(X_x)$, and $R_X(H_z \rightarrow X_z)$, corresponding to the measurement of T_1, T_2 and the heteronuclear NOE, respectively. It is assumed that the spectral density function is flat

around ω_H, allowing the equations for the relaxation rates to be simplified by setting $J(\omega_H - \omega_X) = J(\omega_H) = J(\omega_H + \omega_X)$. The equations expressed in matrix form are then

$$\begin{pmatrix} R_X(X_z) \\ R_X(X_x) \\ R_X(H_z \rightarrow X_z) \end{pmatrix} =$$

$$\begin{pmatrix} 0 & E & 7A \\ 2E/3 & E/2 & 13A/2 \\ 0 & 0 & 5A \end{pmatrix} \begin{pmatrix} J(0) \\ J(\omega_X) \\ \langle J(\omega_H) \rangle \end{pmatrix} \tag{3}$$

For ^{15}N data, Farrow et al. (1995a) have proposed that $J(0.87\omega_H)$ may be determined from $R_N(N_z)$ and $R_N(H_Z \rightarrow N_z)$ alone, from which $J(0.921\omega_H)$ and $J(0.955\omega_H)$ may be estimated by a number of methods and used to calculate $J(0)$ and $J(\omega_N)$. Ishima and Nagayama (1995a), on the other hand, have shown that the term $\langle J(\omega_H) \rangle$ in Equation 3 may be replaced by $J(\omega_H + \omega_N)$. We show here, through model calculations, that, for ^{15}N relaxation data, this latter simplification is indeed a good approximation to the true spectral density. For ^{13}C relaxation data, however, the approximation is less valid. We show that the extent of the discrepancy between true and determined values of the spectral density depends on the nature of the molecule's motions.

We set up model spectral density functions, for ^{15}N–^{1}H and ^{13}C–^{1}H vectors. The case presented here involves three uncorrelated motions with correlation times (τ_α, τ_β, τ_γ) of 4 ns, 0.4 ns and 0.04 ns. The weighting, a_α, of the motion with the longest correlation time, τ_α, was set to 1 while a_β and a_γ were selected at random between 0 and 1, and the spectral density calculated at frequencies 0 ω_X, $\omega_H - \omega_X$, ω_H and $\omega_H + \omega_X$, for a ^{1}H frequency of 600 MHz, using

$$J(\omega) = \frac{2}{5} \cdot \sum_{i=1,4} A_i \cdot \frac{\tau_i}{1 + \omega^2 \tau_i^2} \tag{4}$$

where

$$A_1 = (1 - a_\beta) \cdot (1 - a_\gamma)$$
$$A_2 = a_\beta \cdot (1 - a_\gamma)$$
$$A_3 = (1 - a_\beta) \cdot a_\gamma$$
$$A_4 = a_\beta \cdot a_\gamma$$

and

$$\frac{1}{\tau_1} = \frac{1}{\tau_\alpha}$$

$$\frac{1}{\tau_2} = \frac{1}{\tau_\alpha} + \frac{1}{\tau_\beta}$$

$$\frac{1}{\tau_3} = \frac{1}{\tau_\alpha} + \frac{1}{\tau_\gamma}$$

$$\frac{1}{\tau_4} = \frac{1}{\tau_\alpha} + \frac{1}{\tau_\beta} + \frac{1}{\tau_\gamma}$$

from which model sets of relaxation rates, $R_X(X_z)$, $R_X(X_x)$ and $R_X(H_z \rightarrow X_z)$, could be recalculated, using the full expressions given in Equation 1.

The matrix approach was applied as described above (Equation 3). A second approach, involving the fitting of the spectral density function around ω_H by a single Lorentzian, characterised by a correlation time, τ_H, was also used to calculate the spectral density from the same sets of relaxation rates. Implementation of this modification requires a fitting procedure. Here, we applied a simulated annealing algorithm, as described by Kirkpatrick et al. (1983) and implemented by Goffe et al. (1994), although any other minimisation protocol might be used. The relaxation rates are fitted using three parameters, $J(0)$, $J(\omega_X)$ and τ_H. The values of $J(\omega_H - \omega_X)$, $J(\omega_H)$ and $J(\omega_H + \omega_X)$ are recalculated using the trial value of τ_H using

$$J(\omega) = \frac{2}{5} \cdot \frac{\tau_H}{1 + \omega^2 \tau_H^2} \tag{5}$$

and the trial values of the relaxation rates, $R_X(X_z)$, $R_X(X_x)$ and $R_X(H_z \rightarrow X_z)$, are calculated as in Equation 1.

The performance of the fitting routine may be assessed by also solving the equations (Equation 3) used in the matrix approach described above – the values of $J(\omega)$ were found to match those obtained by the matrix method. In addition, the error term may be used to assess variation in the quality of the fit between residues.

The true and calculated spectral density values at each of the five frequencies, $(0, \omega_X, \omega_H - \omega_X, \omega_H, \omega_H + \omega_X)$ are plotted in Figure 1, for both ^{15}N–^1H and ^{13}C–^1H vectors. For ^{15}N relaxation, the spectral density at 0 and ω_N are faithfully recalculated by both methods. The spectral density $\langle J(\omega_H) \rangle$, recalculated assuming $J(\omega)$ to be flat at ω_H, corresponds closely to $J(\omega_H + \omega_N)$, in accord with Ishima and Nagayama (1995a), while that using a Lorentzian fit lies closer to $J(\omega_H - \omega_N)$.
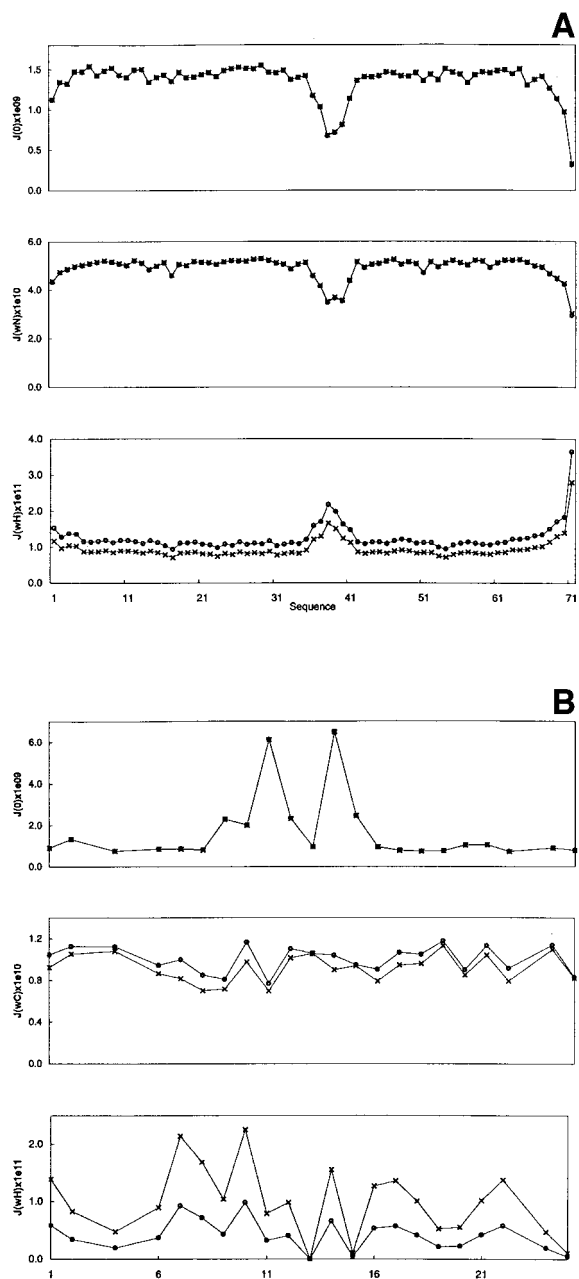

Figure 2. Values of the spectral density (in ns.rad^{-1}) for backbone X–^1H vectors against sequence, obtained assuming $J(\omega)$ to be flat at ω_H (o) and using a single Lorentzian to fit between $(\omega_H - \omega_X)$ and $(\omega_H + \omega_X)$ (x). (A) Data for ^{15}N–^1H vectors in calcium-loaded calbindin D$_{9k}$, measured at 500 MHz ^1H frequency and at 35 °C. (B) Data for ^{13}C–^1H vectors in ω-conotoxin MVIIA, measured at natural abundance, at 600 MHz ^1H frequency and 10 °C.

For ^{13}C relaxation data, however, the situation is rather different, due to the higher frequency of the ^{13}C nucleus compared to ^{15}N: ω_C is larger and closer to ω_H and the approximation that $J(\omega)$ is flat between $J(\omega_H - \omega_X)$ and $J(\omega_H + \omega_X)$ introduces more error than for ^{15}N (at 600 MHz ^{1}H frequency, $2\omega_N = 122$ MHz, while $2\omega_C = 302$ MHz). Both methods yield $J(0)$ well, but $J(\omega_C)$ values are perturbed to a far greater extent than were $J(\omega_N)$ values, above (Figure 1). Again, the assumption that $J(\omega)$ is flat at ω_H gives a value of $\langle J(\omega_H) \rangle$ closest to $J(\omega_H + \omega_C)$, but the divergence is greater than for ^{15}N. The values obtained assuming a single Lorentzian do not correlate well with the spectral density at any single frequency around $J(\omega_H)$. In all cases, the true values of $J(\omega_H)$ and $J(\omega_H + \omega_C)$ lie between those calculated by the two methods.

The failure to yield accurate values of $J(\omega_C)$ is perhaps the most worrying feature of the results. As for $J(\omega_H)$ above, the assumption that $J(\omega)$ is flat yields reasonable values for $J(\omega_C)$ only in certain cases. When these values diverge the most from the true values of $J(\omega_C)$, the use of a single Lorentzian yields better values. Again, the true $J(\omega_C)$ always lies between the two values.

The consequences of these observations on the interpretation of experimental relaxation data are illustrated in Figure 2. Spectral density values calculated using the two methods are plotted against sequence number for ^{15}N–^{1}H vectors in the calcium-loaded form of calbindin, measured at 500 MHz ^{1}H frequency and 35 °C (Kördel et al., 1992), and for the ^{13}C–^{1}H vectors in the 25-residue ω-conotoxin MVIIA, measured at natural abundance at 600 MHz ^{1}H frequency and 10 °C (unpublished results). As noted above, no modification to the matrix method is required for ^{15}N data and the spectral density at high frequency may be most accurately referred to as $J(\omega_H + \omega_N)$, in accord with Ishima and Nagayama (1995a). For the ^{13}C data, however, $J(\omega_C)$ and $\langle J(\omega_H) \rangle$ cannot be reliably determined but lie between the two values plotted. A linear fit to the plot of $J(0)$ vs. $J(\omega_X)$ may be used to extract correlation times from spectral density data (Lefèvre et al., 1996): use of the two sets of spectral densities independently yields overall correlation times, τ_c, for ω-conotoxin MVIIA of 3.75 ns and 3.92 ns, assuming $J(\omega_H)$ to be flat at ω_H or fitting with a Lorentzian, respectively. However, since the contributions of different motions to the relaxation of individual ^{13}C–^{1}H vectors may vary across the sequence, it may not be appropriate to fit the individual

data sets. A linear fit to the combined sets of spectral densities gives a correlation time of 3.82 ns. This behaviour can be rationalised by analysing the values of the spectral densities and the slopes of the curves corresponding to the various components. The slope of the Lorentzian is given by

$$\frac{dJ(\omega)}{d\omega} = -\frac{4}{5} \cdot \frac{\tau^3 \omega}{(1 + \omega^2 \tau^2)^2} \qquad (6)$$

Values of $J(\omega_H)$ for correlation times of 4, 0.4 and 0.04 ns are 0.70×10^{-11}, 4.89×10^{-11}, and 1.56×10^{-11} ns.rad^{-1} respectively while the slopes are -3.70×10^{-21}, -18.0×10^{-21} and -0.19×10^{-21} ns.rad^{-2} at ω_H. The motion with a correlation time of 0.4 ns is the largest in magnitude and has the steepest slope. Thus, when this motion contributes significantly to the spectral density, a fit with a single Lorentzian performs better in determining $J(\omega_C)$ and $J(\omega_H)$. Conversely, when this motion contributes little, the fit with a single Lorentzian performs poorly and the assumption that $J(\omega)$ is flat gives a better estimation of $J(\omega_C)$ and $J(\omega_H)$. Since we cannot know the relative contributions of different motions a priori, use of both methods is necessary.

Acknowledgements

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