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Full matrix analysis of cross-relaxation fails in fractionally deuterated molecules

Zsolt Zolnai*, Nenad Juranić & Slobodan Macura**
Department of Biochemistry and Molecular Biology, Mayo Graduate School, Mayo Clinic and Mayo Foundation, Rochester, MN 55905, U.S.A.

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Abstract

We have analyzed cross-relaxation in fractionally deuterated molecules and showed that the full matrix analysis fails except when the dilution is extreme. This is because the isotopic dilution alters the matrix exponential relationship between the observed spectrum and the cross-relaxation rate constants sought. Consequently, an average of the spectra of various isotopomers differs from the matrix exponential of an average relaxation matrix. We have derived a series expansion that allows the determination of the cross-relaxation rate constants in arbitrarily deuterated molecules.

The technique of isotope dilution of the proton spin reservoir is a useful tool for improving the spectra of macromolecules (LeMaster, 1989; Venters et al., 1995; Grzesiek et al., 1995; Nietlispach et al., 1996). The improvement stems from the decrease of the longitudinal and transverse relaxation rates. This implies that the autorelaxation rates of equivalent protons change with the degree of isotope exchange. Hence, in a partially deuterated molecule, considerable variability in autorelaxation rates may exist from site to site. Thus, the cross-peak volumes alone are not a good gauge of cross-relaxation rates. Another important consequence of isotope dilution is that the cross and diagonal peaks are reduced to a different extent by random fractional isotope exchange. Although all the protons from a given site contribute to the diagonal peak, the contribution to the cross-peak comes only from pairs of protons that participate in cross-relaxation (Wagner, 1980). This is analogous to the signal intensity in a ^{13}C–^{13}C INADEQUATE experiment (Bax et al., 1981) where only 0.012% (1.1% of the 1.1% present ^{13}C) of the nuclei contribute to the signal, because 0.012% is the concentration of ^{13}C–^{13}C spin pairs. In homonuclear cross-relaxation, in addition to the two-spin interactions, one needs to account for multispin effects. Here, we present a quantitative analysis of a random or selective partially deuterated cross-relaxation system.

The two-dimensional (2D) exchange spectrum, experimentally obtained at a mixing time τ_m is represented by a matrix of peak volumes $\boldsymbol{A}(\tau_m)$, which is related to the dynamic matrix $\boldsymbol{L}$ by (Juranić et al., 1997)

$$\boldsymbol{A}(\tau_m) = e^{L\tau_m}\boldsymbol{N}a_0. \tag{1}$$

In the absence of chemical exchange, Equation 1 becomes

$$\boldsymbol{A}(\tau_m) = e^{-R\tau_m}\boldsymbol{N}a_0, \tag{2}$$

where $\boldsymbol{R}$ is the relaxation matrix, a_0 the volume of a single spin, and $\boldsymbol{N}$ the diagonal matrix of spin populations, $\boldsymbol{N} = \mathrm{diag}(n_i, \ldots, n_M)$; n_j is the number of spins at spin site i.

The normalized relaxation matrix $\boldsymbol{R}^0$, $\boldsymbol{R}^0 = \boldsymbol{N}^{-1}\boldsymbol{R}$, can be obtained by full matrix analysis (FMA), solving Equation 2:

$$\boldsymbol{R}^0 = -\frac{1}{\tau_m}\boldsymbol{N}^{-1}\ln\left(\boldsymbol{A}(\tau_m)(\boldsymbol{N}a_0)^{-1}\right). \tag{3}$$

*On leave from the Mathematical Institute, Knez Mihailova 35, 11000 Beograd, Yugoslavia.
**To whom correspondence should be addressed.

The Taylor series expansion of Equation 2 is

$$\frac{\boldsymbol{A}_{ij}(\tau_m)}{n_i n_j a_0} = \frac{\delta_{ij}}{n_i} - \tau_m \boldsymbol{R}^0_{ij} + \frac{\tau_m^2}{2}\sum_k n_k \boldsymbol{R}^0_{ik}\boldsymbol{R}^0_{kj} - \frac{\tau_m^3}{6}\sum_{k,l} n_k n_l \boldsymbol{R}^0_{ik}\boldsymbol{R}^0_{kl}\boldsymbol{R}^0_{lj} + \ldots. \quad (4)$$

The diagonal elements $\boldsymbol{R}^0_{ii}$ of the dynamic matrix depend on all the rates by which the particular site loses magnetization,

$$\boldsymbol{R}^0_{ii} = \boldsymbol{R}^{ex}_{ii} + (n_i - 1)\boldsymbol{R}^{\text{auto}}_{ii} + \sum_{j\neq i} c_{ij}\boldsymbol{R}^0_{ij}, \quad (5)$$

where $\boldsymbol{R}^0_{ij}$ represents the magnetization exchange with other sites, $\boldsymbol{R}^{\text{auto}}_{it}$ the losses due to autorelaxation, and $\boldsymbol{R}^{ex}_{ii}$ the losses due to external sources of relaxation. The coefficients c_{ii} reflect that the cross-relaxation is associated with overall relaxation ($\mid c_{ij} \mid > 1$) and that the cross-relaxation rates in a spin-diffusion regime are negative:

$$c_{ij} = \begin{cases} > 1, \boldsymbol{R}_{ij} > 0 \text{ (rotating frame or small molecules)} \\ \leq -1, \boldsymbol{R}_{ij} < 0 \text{ (large molecules)}. \end{cases} \quad (6)$$

To simplify the formulas, assume that the system is in the spin diffusion limit ($c_{ij} = -1$). Eliminating the dependence among the variables in Equation 4 by using Equation 5, one can obtain its canonical form:

$$\frac{\boldsymbol{A}_{ij}(\tau_m)}{n_i n_j a_0} = \begin{cases} -\tau_m \boldsymbol{R}^0_{ij} + \frac{\tau_m^2}{2}\left(\sum_{k\neq i,j} n_k \boldsymbol{R}^0_{ik}\boldsymbol{R}^0_{kj} - \sum_k n_i \boldsymbol{R}^0_{ij}\boldsymbol{R}^0_{ik} - \sum_k n_j \boldsymbol{R}^0_{ij}\boldsymbol{R}^0_{jk}\right) + \ldots, i \neq j \\ \frac{1}{n_i} + \tau_m \sum_k \boldsymbol{R}^0_{ik} + \frac{\tau_m^2}{2}\left(\sum_{k\neq i} n_k (\boldsymbol{R}^0_{ik})^2 + \sum_{k,l} n_i \boldsymbol{R}^0_{ik}\boldsymbol{R}^0_{il}\right) + \ldots, i = j. \end{cases} \quad (7)$$

Also, for the sake of clarity, in Equation 7 we put $R^0_{ii} = -R^{ex}_{ii} - (n_i - 1)R^{\text{auto}}_{ii}$. Note that in all the above equations it is assumed that the spin sites are fully protonated.

In an isotope-diluted system, site i contains protons with probability p_i. Then, for a fully protonated site, $p_i = 1$, and for a fully deuterated site, $p_i = 0$. For a multistep magnetization transfer between spin sites i and j mediated by sites $k, l, ..., m$, all the involved sites must contain protons. Let $p_{ikl\ldots mj}$ be the probability that each of the sites $i, k, l, ..., m$, and j contains a proton. The probability, of the compound event $ikl\ldots mj$ is (Kolmogorov, 1982)

$$p_{ikl\ldots mj} = p_i\, p_{k|i}\, p_{l|ik} \ldots p_{j|ikl\ldots m}, \quad (8)$$

where $p_{j|ikl\ldots m}$ denotes the conditional probability that site j contains a proton if sites $i, k, l, \ldots, m$ also contain protons ($i \neq k \neq l \neq \ldots \neq m \neq j$). For uncorrelated isotope exchange

$$p_{ikl\ldots mj} = p_i\, p_k\, p_l \ldots p_m\, p_j, \quad (9)$$

and, for fully correlated exchange

$$p_{ikl\ldots mj} = p_i = p_k = p_l = \ldots = p_m = p_j. \quad (10)$$

The experimentally obtained 2D cross-relaxation spectrum of an isotopically diluted system is the ensemble average of 2D cross-relaxation spectra of isotopomers of partially deuterated molecules,

$$\boldsymbol{A}^D(\tau_m) = \langle \boldsymbol{A}(\tau_m)\rangle = \left\langle e^{-R^k\tau_m}\boldsymbol{N}a_0\right\rangle = \left\langle e^{-R^k\tau_m}\right\rangle \boldsymbol{N}a_0. \quad (11)$$

$\boldsymbol{R}^k$ is the relaxation matrix that corresponds to an isotopomer with particular proton configuration, and $<>$ denotes the ensemble average. Equation 11 cannot be written in the form of Equation 1, because the ensemble averaging is taken over the spectra and not over the relaxation matrices, hence

$$\langle \boldsymbol{A}(\tau_m)\rangle \neq e^{-<R^k>\tau_m}\boldsymbol{N}a_0. \quad (12)$$

Therefore, in partially deuterated systems, the values of R^0_{ij} cannot be determined by full matrix analysis (Equation 3). However, Equation 11 can be expanded into a Taylor series, and a formula similar to Equation 7 can be obtained.

For an M-spin system, the number of different isotopomers is 2^M (each spin site can contain a proton or a deuteron). The isotopomers have the same geometry but different number and distribution of protons. If the

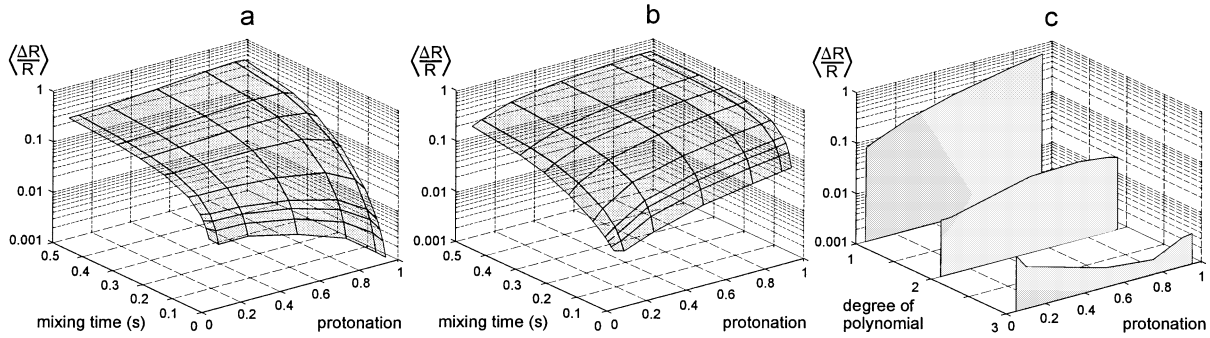

Figure 1. Relative errors in cross-relaxation rates for different degrees of protonation (5%, 10%, 25%, 50%, 75%, 90% and 95%) in cyclo-(L-Pro-Gly) dipeptide. Only a representative subset of 11 cross-relaxation rates that can be measured accurately (Zolnai et al., 1997) is shown. The longest corresponding distance is 4.14 Å. The mixing times used for calculations are: 0.00006, 0.01, 0.02, 0.03, 0.04, 0.08, 0.16, 0.24, 0.32, and 0.48 s. (a) Normalized FMA, Equation 15. (b) Like (a), but with Equation 16. (c) Relative errors in cross-relaxation rates determined by fitting Equation 17 as a function of the degree of protonation. For all degrees of fit, the normalized data obtained at the first six mixing times were used, except for geminals, when only four mixing times were used. All errors are the average values of the 11 relative errors and are plotted on a 10-base logarithmic scale.

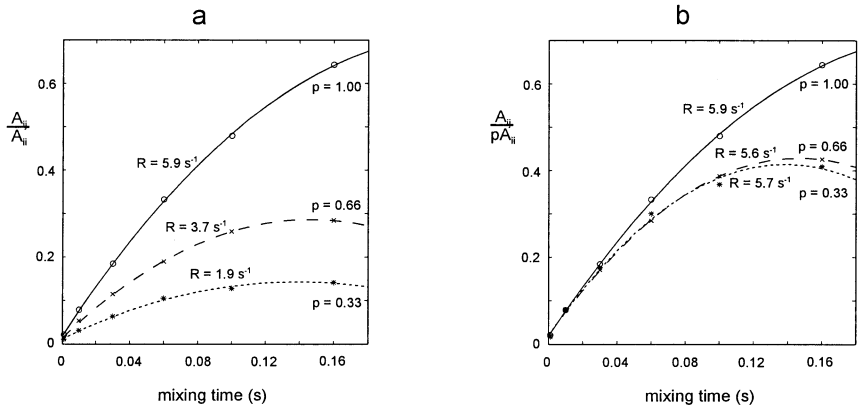

Figure 2. The effect of isotope dilution on cross-relaxation buildup rate of the glutamine sidechain amide protons. The apparent initial buildup rate of the normalized cross-peak volumes (Equation 4) decrease with the degree of deuteration (a). A normalization according to Equation 17 produces the same initial buildup rate in all cases (b). The smooth curves are second order polynomials fitted to experimental data.

sites i and j are protonated, the cross-relaxation rates R_{ij}^k corresponding to different isotopomers will be the same irrespective of the protonation of the other sites. If either site is deuterated, R_{ij}^k is equal to zero. Hence, the terms in the series expansion of isotopomer's volume matrix will be either the same as in Equation 7, or zero. Therefore, the series expansion of Equation 11 can be obtained by introducing the probabilities p_i into Equation 7. For example, for $i \neq j$ the linear term becomes $p_{ij} R_{ij}^0$, because R_{ij}^0 figures in the ensemble average only when both of the sites i and j are protonated, and the probability of that event is p_{ij}. Similarly, in the quadratic term, a product $R_{ik}^0 R_{kj}^0$ is nonzero only when sites i, j, and k contain protons, and that happens with probability p_{ijk}. Accordingly, for an isotope-diluted sample, Equation 7 becomes

$$\frac{\boldsymbol{A}_{ij}^D(\tau_m)}{n_i n_j a_0} = \begin{cases} -\tau_m p_{[ij]} \boldsymbol{R}_{ij}^0 \\ \quad + \frac{\tau_m^2}{2} \left(\sum_{k \neq i,j} n_k p_{[ijk]} \boldsymbol{R}_{ik}^0 \boldsymbol{R}_{kj}^0 \right. \\ \quad - \sum_k n_i p_{[ijk]} \boldsymbol{R}_{ij}^0 \boldsymbol{R}_{ik}^0 \\ \quad \left. - \sum_k n_j p_{[ijk]} \boldsymbol{R}_{ij}^0 \boldsymbol{R}_{jk}^0 \right) \\ \quad + \ldots, i \neq j \\ \frac{p_i}{n_i} + \tau_m \sum_k p_{[ik]} \boldsymbol{R}_{ik}^0 \\ \quad + \frac{\tau_m^2}{2} \left(\sum_{k \neq i} n_k p_{[ik]} (\boldsymbol{R}_{ik}^0)^2 \right. \\ \quad \left. + \sum_{k,l} n_i p_{[ik]} \boldsymbol{R}_{ik}^0 \boldsymbol{R}_{il}^0 \right) \\ \quad + \ldots, i = j. \end{cases} \tag{13}$$

where $[ikj]$ denotes the nonrepetitive indices in the set generated by i, j, and k (e.g., $[123] = 123$, $[232] = 23$, $[111] = 1$) and the probabilities p are defined as in Equations 8–10. Again, in the final equation, we put $R^0_{ii} = -R^{ex}_{ii} - (n_i - 1)R^{\text{auto}}_{ii}$.

In general, Equation 13 is different from the exponential form, which would allow the use of full matrix analysis. However in certain cases it can be approximated by an exponential expression. For example, suppose that the isotope exchange is uncorrelated, and for every i, $p_i = p$. Then, if the mixing time τ_m converges toward zero, or if $p \to 1$, Equation 13 approaches

$$\boldsymbol{A}^D(\tau_m) = pe^{-pR\tau_m}\boldsymbol{N}a_0. \quad (14)$$

Hence, the dynamic matrix can be retrieved using an appropriately modified FMA, namely,

$$\boldsymbol{R}^0 = -\frac{1}{p\tau_m}\boldsymbol{N}^{-1}\ln\left(\frac{1}{p}\boldsymbol{A}^D(\tau_m)(\boldsymbol{N}a_0)^{-1}\right). \quad (15)$$

This expression is of limited value. For arbitrary p, it is valid for short mixing times only (as a linear approximation); for longer mixing times it is valid only for low degrees of dilution. However, this is the best approximation, and here we use it just to demonstrate the problems of FMA in isotope diluted-systems.

With the increase of isotope dilution ($p \to 0$), spin diffusion is quenched and Equation 13 transforms into the equation of the isolated spin pair, where

$$\boldsymbol{R}^0_{ij} = \frac{\ln\left(1 - \frac{2}{p^2}\frac{\boldsymbol{A}^D_{ij}(\tau_m)}{a_0}\right)}{-2\tau_m}. \quad (16)$$

The FMA-type formulas are of limited value because they cannot be used for practically important isotope-dilution ranges. Here, we derive a formula that can be applied for arbitrary isotope dilution. A series expansion that is independent of both external and autorelaxation rates up to the second order can be obtained using Equation 13:

$$\frac{1}{2}\frac{\boldsymbol{A}^D_{ij}(\tau_m)}{p_{ij}}\left(\frac{p_j}{n_i\boldsymbol{A}^D_{jj}(\tau_m)} + \frac{p_i}{n_j\boldsymbol{A}^D_{ii}(\tau_m)}\right) = -\tau_m\boldsymbol{R}^0_{ij} + \frac{\tau_m^2}{2}\sum_{k\neq i,j} n_k\frac{p_{ikj}}{p_{ij}}\boldsymbol{R}^0_{ik}\boldsymbol{R}^0_{kj} + \ldots. \quad (17)$$

Its main virtue is that it takes into account all the relevant variables: the spin populations, the degree of site protonation, and the correlation of isotope exchange among the sites. Equation 17 is an infinite series; however, in practice, as for fully protonated systems, it is used mostly up to the second order.

To assess the extent of errors in the determination of cross-relaxation rates caused by different models, we have applied the derived formulas (Equations 15, 16, and 17) to the cross-relaxation spectra generated by the Monte Carlo simulation. We have chosen cyclo-(L-Pro-Gly), a 10-spin system that at low temperatures exhibits cross-relaxation properties like a small protein (Juranić et al., 1997). We first generated a set of structures randomly deuterated with the desired probabilities (desired degree of isotope exchange). All the structures had the same geometry, and the only difference among them was in the distribution of remaining protons. The number of samples for the Monte Carlo simulation was determined so that on average we have 100 samples per isotopomer at the given degree of protonation. For each isotopomer we generated a relaxation matrix $\boldsymbol{R}^k$ assuming $\omega_0/2\pi = 500$ MHz, $\tau_c = 3.8$ ns, and $R_{ex} = 0$. Taking the matrix exponential of each $\boldsymbol{R}^k$, we obtained the NOESY spectra of individual isotopomers, whose ensemble average yielded the resulting NOESY spectrum, according to Equation 11. Figures 1a,b show the 10-base logarithm of the relative errors in cross-relaxation rates obtained by normalized FMA (Equations 15 and 16), and Figure 1c shows the 10-base logarithm of the relative errors of linear, quadratic, and cubic fitting of Equation 17. Shown are the averages of the relative errors of 11 cross-relaxation rates from cyclo-(L-Pro-Gly) that can be measured accurately (Zolnai et al., 1997). The errors represent only the error of the model used. As expected, the error of Equation 15 (Figure 1a) decreases with increase of protonation and with the decrease of the mixing time. In contrast, Equation 16 (Figure 1b) shows the minimal errors at low degrees of protonation and again at shorter mixing times.

The most important property of the fitting of series expansion, Equation 17, is that the errors are independent of the degree of protonation. The errors rapidly decrease with the increase of the polynomial degree and, in principle, could be made arbitrarily small. In practice, due to the random errors, a quadratic fitting, for which the model error is around 10%, may be sufficient. Besides exhibiting smaller errors than either FMA formula, the most important advantage of Equation 17 is that it can be used across the full range of deuteration degree and with arbitrary isotope exchange correlations among different proton/deuteron

sites. This is particularly useful for an emerging class of selective isotope labeling experiments, in which the intermolecular contacts are deduced from the cross-relaxation rates of remaining protons (Walters et al., 1997). Because the labeling is not absolutely selective, during the calculation of cross-relaxation rates the variable degree of isotope exchange must be taken into account, and this is accomplished by Equation 17.

For an experimental demonstration of the effect of random deuteration, we examined the cross-relaxation between protons of the glutamine side chain amide group. These protons are 1.75 Å apart in a rigid geometry, they are spectroscopically well resolved, and they readily exchange with water. Three 10 mM samples with different degree of deuteration (100%, 66%, 33% H_2O/D_2O) were prepared by dissolving a commercial glutamine (Aldrich) in corresponding mixtures of H_2O/D_2O, followed by 6 h of equilibration; then the samples were mixed with DMSO-d_6 in volume ration 1:3. For NMR measurements, the samples were cooled at $-40\,^{\circ}C$, to bring the cross-relaxation into spin-diffusion regime and to diminish the chemical exchange. The degree of deuteration was determined by comparison of amide proton resonances with the H^{α} resonance, and was found to be the same as the H_2O/D_2O ratio. To construct the build-up curves, the 2D NOESY spectra were recorded at mixing times of 0, 30, 60, 100 and 160 ms on a Bruker AMX-300 spectrometer.

Figure 2 shows the build-up curves obtained by fitting the experimental data to Equations 14 and 17. As predicted, the uncorrected cross-relaxation rates depend on the degree of deuteration, Figure 2a, whereas the build-up curves calculated by Equation 17 yield the same cross-relaxation rate within the error limits of the model, irrespective of the degree of deuteration.

In conclusion, we have shown that in random partially deuterated systems the use of full matrix analysis is inappropriate except for extreme proton or deuteron concentrations. We have derived a series expansion for cross-relaxation spectra of isotope-diluted molecules, and we have shown that the polynomial (in practice quadratic) buildup analysis is the method of choice for calculating the cross-relaxation rates in deuterated molecules. It should be obvious that the chemical exchange rates are unaffected by isotope dilution, except for weaker sensitivity due to the reduced concentration of observed spins. This different sensitivity of the chemical exchange and cross-relaxation to isotope dilution could be used to separate the two processes when both take place between the same sites.

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