

Two-dimensional exchange spectroscopy revisited: Accounting for the number of participating spins*

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The magnetization exchange between two groups of equivalent spins each having different populations and overall relaxation rates has been analyzed. The results suggest that either the spin population difference or the overall relaxation rate difference in an exchange spectrum can produce cross-peaks with volumes larger than that of the corresponding diagonal line. This is important for interpretation of the magnetization exchange between water and macromolecular protons where both the population differences and auto-relaxation rate differences can be very large. Theoretical predictions of peak volume evolution were experimentally verified in the intermolecular magnetization exchange between the water and labile amide proton in a model system *N*-acetylglycine/water.

Keywords: NMR, exchange spectroscopy, chemical exchange, hydrogen exchange.

INTRODUCTION

In the twenty years since its inception,^{1,2} two-dimensional exchange spectroscopy has matured into a well-established method for the study of incoherent magnetization transfer.³ The principal sources of incoherent magnetization transfer are chemical exchange^{1,4} and cross-relaxation.^{1,5} Although the two processes are physically quite different, their experimental manifestations are almost indistinguishable and the phenomenological description of both is similar. Thus, the magnetization exchange in an *N*-spin system is described formally over the dynamic matrix L whose off-diagonal elements are the magnetization exchange rate constants L_{ij} , and the population matrix $N_p = \{\text{diag } n_i\}$ whose elements are equilibrium populations n_i :

$$A(t_m) = \exp(+Lt_m) NA_0. \quad (1)$$

$A(t_m)$ is a matrix containing peak volumes of the exchange spectrum recorded with a mixing time of t_m , and A_0 is the peak volume of a single spin. If an exchange

* Dedicated to Professor Slobodan Ribnikar on the occasion of his 70th birthday.

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experiment is recorded so that initially each spin site is in thermal equilibrium, the matrix A is symmetric, $A_{ij} = A_{ji}$. The dynamic matrix, L , is not necessarily symmetric, $L_{ij} \neq L_{ji}$, but is quasi-symmetric.^{6,7}

$$n_i L_{ji} = n_j L_{ij} \quad (2)$$

a condition imposed by the principles of detailed balance and micro reversibility.⁸

By rearranging Eq. (2) a normalized magnetization exchange rate constant

$$L_{ij}^0 = L_{ji}^0 = \frac{L_{ij}}{n_i} = \frac{L_{ji}}{n_j} \quad (3)$$

which represents a fraction of the unit spin magnetization exchanged in the unit time interval can be obtained. The actual magnetization exchange rate constants can then be expressed over the site populations n_i and the normalized magnetization exchange rate constants L_{ij}^0 as

$$\begin{aligned} L_{ij} &= n_i L_{ij}^0 \\ L_{ji} &= n_j L_{ij}^0 \end{aligned} \quad (4)$$

The normalized elements of the dynamic matrix are linear combinations of their respective chemical exchange rate constant k_{ij}^0 and cross-relaxation rate constant σ_{ij}^0 :

$$L_{ij}^0 = k_{ij}^0 - \sigma_{ij}^0. \quad (5)$$

Thus, to calculate the normalized magnetization exchange rate constant L_{ij}^0 (which is the only parameter that can be interpreted in terms of either chemical exchange rate constants or interproton distances or molecular mobility) a knowledge of the populations of individual spin sites is essential.

Whereas Eq. (1) can be solved numerically for an arbitrary N -spin system, an explicit solution is possible only for special cases involving two-,^{1,5} three-,⁷ and four-spins.^{7,9,10} Here we present an explicit solution for a system of two groups of equivalent spins with unequal populations and different overall relaxation rates. The importance of this system stems from the recent interest in the magnetization exchange between the water protons and (labile) protons of various macromolecules.^{11,12} In particular, this work was triggered by the dilution enhanced exchange spectroscopy (DEEXY)¹³ experiment in the protein/water system where cross-peaks much larger than the diagonal-peaks were observed.¹⁴

THEORY

A. Basic equations. We consider a group of spins A and B with populations n_A and n_B , having overall relaxation rates R_A and R_B respectively, and a normalized magnetization exchange rate constant L_0 ($L_0 = L_{AB}^0 = L_{BA}^0$). By using Eq.(4) to express the magnetization exchange rate constants, L_{AB} , L_{BA} over the normalized

rate constant, L_0 , the spectral, A , dynamic, L , and population. N_p , matrices are

$$\mathbf{A}(t_m) = \begin{pmatrix} a_{AA}(t_m) & a_{AB}(t_m) \\ a_{BA}(t_m) & a_{BB}(t_m) \end{pmatrix}; \mathbf{L} = \begin{pmatrix} -R_A - n_B L_0 & n_A L_0 \\ n_B L_0 & -R_B - n_A L_0 \end{pmatrix}; \mathbf{N}_p = \begin{pmatrix} n_A & 0 \\ 0 & n_B \end{pmatrix} \quad (6)$$

It is important to note the convention used for the matrix representation of dynamic systems. The first index designates the final and the second, the initial state, *i.e.*, L_{AB} denotes the rate constant for the magnetization transfer from group B to group A.⁸ For brevity we define the mismatch parameter d , and use, as before,⁵ the cross-relaxation rate constant R_C , and the leakage relaxation rate constants R_L :

$$d = (R_A - R_B) - (n_A - n_B)L_0 \quad (7)$$

$$R_C = \sqrt{d^2 + 4n_A n_B L_0^2} \quad (8)$$

$$R_L = \frac{1}{2} [(R_A + R_B) + (n_A + n_B)L_0] - \frac{1}{2}R_C. \quad (9)$$

Then, the diagonal a_{AA} , a_{BB} , and the cross-peak volumes a_{AB} , a_{BA} in the exchange spectrum recorded at the mixing time t_m are:

$$a_{AA}(t_m) = \frac{1}{2} n_A A_0 \exp(-R_L t_m) \left[\left(1 - \frac{d}{R_C}\right) + \left(1 + \frac{d}{R_C}\right) \exp(-R_C t_m) \right] \quad (10)$$

$$a_{BB}(t_m) = \frac{1}{2} n_B A_0 \exp(-R_L t_m) \left[\left(1 + \frac{d}{R_C}\right) + \left(1 - \frac{d}{R_C}\right) \exp(-R_C t_m) \right] \quad (11)$$

$$a_{AB}(t_m) = a_{BA}(t_m) = \frac{n_A n_B L_0}{R_C} A_0 \exp(-R_L t_m) [1 - \exp(-R_C t_m)] \quad (12)$$

For $R_L > 0$ the diagonal-peak volumes decrease steadily toward zero while the cross-peak volume decreases only after reaching a maximum at t_{\max} ⁵

$$t_{\max} = \frac{1}{R_C} \ln \left(\frac{R_L + R_C}{R_L} \right) \quad (13)$$

Equations(10)–(12) suggest that the cross-peak volume can be larger than the volume of the smaller diagonal, Fig. 1. The volumes a_{AB} and a_{AA} become equal at

$$t_x^A = \frac{1}{R_C} \ln \frac{2n_B L_0 + d + R_C}{2n_B L_0 + d - R_C}. \quad (14)$$

Similar expressions for volumes a_{BB} and a_{AB} can be obtained from Eq. (14) by permuting the indices A and B respectively. (Index permutation also implies the sign change in d , *cf.* Eq.(7)). By the principle of detailed balance (obviously), cross-peak volume can exceed the volume of only one (smaller) diagonal at a time.

It is irrelevant whether the diagonal line is smaller because of the smaller initial magnetization of the higher overall relaxation rate. In the special case, when

$$\frac{R_A}{L_0} - 2n_A = \frac{R_B}{L_0} - 2n_B. \quad (15)$$

the differences in the spin site populations and the autorelaxation rates compensate and the volume crossing of cross-peak and diagonal-peak does not occur. Then, the cross-peak and both diagonal-peak volumes asymptotically approach each other in the course of mixing time.

B. Cross-peak volume normalization. A convenient method to determine the normalized magnetization exchange rate L_0 is to analyze the initial build-up rates of the cross-peak volume,⁵ or to analyze the build-up rates of normalized cross-peak volumes^{6,15}

$$\frac{a_{AB}(t_m)}{n_B a_{AA}(t_m)} \approx L_0 t_m + \frac{1}{2} L_0 [(R_A - R_B) - L_0(n_A - n_B)] t_m^2 + O[t_m^3]. \quad (16)$$

It is also useful to normalize the cross-peak volume by the population weighted arithmetic average of the diagonals, a^{wa} :

$$a^{wa}(t_m) = \frac{n_B a_{AA}(t_m) + n_A a_{BB}(t_m)}{2}, \quad (17)$$

because the normalized volume intensity is a linear function of mixing time up to the third order

$$\frac{2a_{AB}}{n_B a_{AA} + n_A a_{BB}} = \frac{2L_0}{R_C} \tanh\left(\frac{R_C t_m}{2}\right) \approx L_0 t_m + \frac{1}{24} (L_0 R_C^2 - 12n_A n_B L_0^3) t_m^3 + O[t_m^4]. \quad (18)$$

When one diagonal, a_{BB} , is not known, Eq. (17) can be expressed over the other parameters as

$$a_{AA}^{wa}(t_m) = \frac{n_A n_B A_0}{R_C + d} \left[d \exp(-R_L t_m) + \frac{R_C a_{AA}(t_m)}{n_A A_0} \right]. \quad (19)$$

In either case

$$\frac{a_{AB}(t_m)}{a_{AA}^{wa}(t_m)} = \frac{2L_0}{R_C} \tanh\left(\frac{R_C t_m}{2}\right) \approx L_0 t_m + O[t_m^3]. \quad (20)$$

C. Special cases. With added constraints, Eqs. (10)–(12) reduce to simpler, already known, forms.

1. $R_A = R_B = R_0$. Such a system has been analyzed before, with $R_0 = 0$.⁷ Expressions for cross- and leakage relaxation rates and peak volumes are greatly simplified in comparison to Eqs. (8)–(12):

$$(R_C)_R = (n_A + n_B)L_0 \quad (21)$$

$$(R_L)_R = \frac{1}{2}(R_A + R_B) = R_0 \quad (22)$$

$$(a_{AA})_R = \frac{n_A n_B}{n_A + n_B} A_0 \exp(-R_0 t_m) \left\{ \frac{n_A}{n_B} + \exp[-(n_A + n_B)L_0 t_m] \right\} \quad (23)$$

$$(a_{BB})_R = \frac{n_A n_B}{n_A + n_B} A_0 \exp(-R_0 t_m) \left\{ \frac{n_B}{n_A} + \exp[-(n_A + n_B)L_0 t_m] \right\} \quad (24)$$

$$(a_{AB})_R = \frac{n_A n_B}{n_A + n_B} A_0 \exp(-R_0 t_m) \{1 - \exp[-(n_A + n_B)L_0 t_m]\} \quad (25)$$

$$(a_{AA}^{wA})_R = \frac{n_A n_B}{2} A_0 \exp(-R_0 t_m) \{1 + \exp[-(n_A + n_B)L_0 t_m]\} \quad (26)$$

$$\left(\frac{a_{AB}}{a_{AA}^{wA}} \right)_R = \frac{2}{n_A + n_B} \tanh \left[\frac{(n_A + n_B)L_0 t_m}{2} \right] \approx L_0 t_m + O[t_m^3] \quad (27)$$

$$(t_x^A) = \frac{1}{(n_A + n_B)L_0} \ln \frac{2n_B}{n_B - n_A} \quad (28)$$

The subscript R in Eqs. (21)–(28) indicates that the expressions apply for the special case of equal overall relaxation rates. Because the mixing time at which the diagonal- and cross-peak volumes become equal depends only on the number of spins, this case is potentially useful for determining the number of spins in the intermolecular magnetization exchange.

2. $n_A = n_B = n_0$. The equality of the spin populations in the two sites reduces Eqs. (8)–(12) to Eqs. (22), (23) of our previous work.⁵ The previous analysis and conclusions are strictly valid only when the numbers of spins in the two groups are equal. However, if the difference ($n_A - n_B$) is small the original expressions may hold for mixing times up to $t_m L_0 \approx 1$. When the site populations are equal, the cross-peak volume a_{AB} may exceed the diagonal volume a_{AA} when $\Delta R = R_A - R_B > 0$ and volume a_{BB} when $\Delta R < 0$. For the special case when $R_B = 0$, the cross- and diagonal-peak volumes become equal when the cross-peak reaches a maximum

$$(t_x^A)_n = (t_{\max})_n \quad (29)$$

The index n points to a special condition, $n_A = n_B$. An increase in the relaxation rate of spin site B, $R_B > 0$, leads to the earlier appearance of a cross-peak maximum ensuring that in general

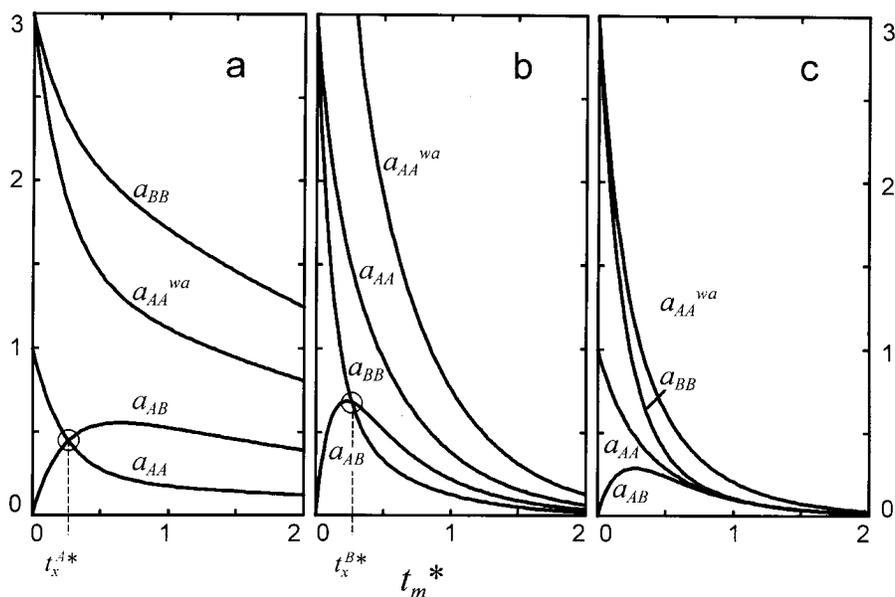


Fig. 1. Dependence of peak volumes, a_{AA} , a_{BB} , and a_{AB} ($= a_{BA}$) in an $n_A n_B$ spin system on the reduced mixing time, $t_m^* = t_m L_0$: a) $n_A = 1$; $n_B = 3$; $R_A^* = R_A/L_0 = 0.5$; $R_B^* = R_B/L_0 = 0.5$; b) $n_A = n_B = 3$; $R_A^* = 0.5$; $R_B^* = 4$; c) $n_A = 1$; $n_B = 3$; R_A^*, R_B^* , satisfy Eq. (15); $R_A^* = 0$; $R_B^* = 4$. The smaller diagonal volume (the site having a smaller population or a faster overall relaxation) becomes equal to the volume of the cross-peak at the mixing time t_x^* ($t_x^* = t_x L_0$). The volume crossing does not occur only in the special case (c). A corrected diagonal a_{AA}^{wa} is the weighted average of the diagonals a_{AA} and a_{BB} , Eq. (17). It is useful for initial build-up rate analysis because it produces normalized cross-peak linear up to the third order in t_m , Eq. (20).

$$(t_x^*)_n \geq (t_{\max})_n \quad (30)$$

Thus, for $n_A = n_B$, cross- and diagonal-peak volumes may become equal only past the mixing time at which the cross-peak reaches a maximum value.

3. $(R_A - R_B) = 2(n_A - n_B)L_0$. This condition, also expressed by Eq. (15), ensures that the cross-peak volume is always smaller than the volume of diagonals. Thus, with increasing mixing time the diagonal- and the cross-peak volumes asymptotically approach each in a manner analogous to an isolated spin pair

$$\lim_{t_m \rightarrow \infty} \left(\frac{a_{AB}}{a_{AA}} \right)_S = \lim_{t_m \rightarrow \infty} \left(\frac{a_{AB}}{a_{BB}} \right)_S = 1$$

The subscript S denotes that these expressions are only valid for the special condition defined by Eq. (15). Interestingly, expressions for the cross- and leakage relaxation rates are the same as in case (1.), Eqs. (21), (22)

$$(R_C)_S = (R_C)_R; \quad (R_L)_S = (R_L)_R.$$

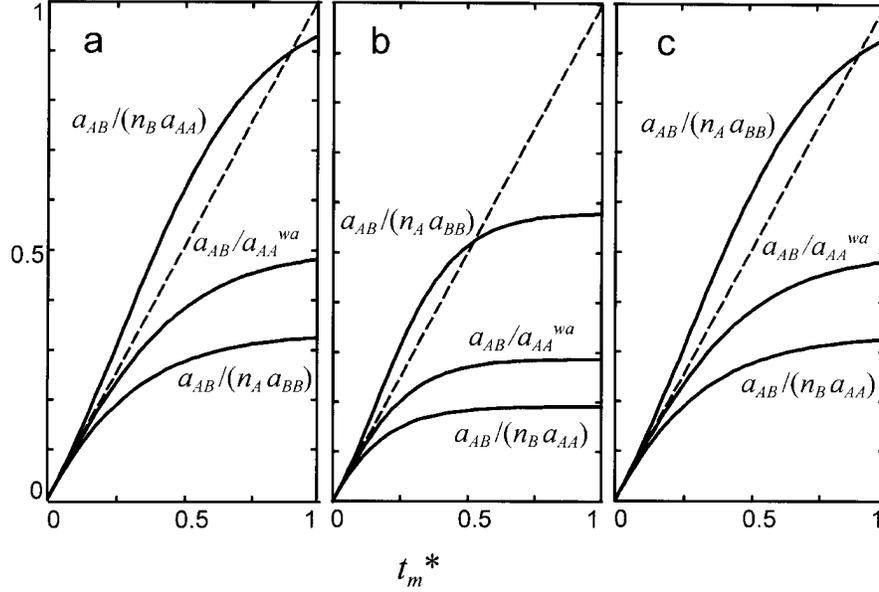


Fig. 2. Dependence of normalized peak volumes in a $n_A n_B$ spin system on the reduced mixing time, $t_m^* = t_m L_0$: a) $n_A = 1$; $n_B = 3$; $R_A^* = R_A/L_0 = 0.5$; $R_B^* = R_B/L_0 = 0.5$; b) $n_A = n_B = 3$; $R_A^* = 0.5$; $R_B^* = 4$; c) $n_A = 1$; $n_B = 3$; R_A^* , R_B^* , satisfy Eq. (15): $R_A^* = 0$; $R_B^* = 4$. Normalization by a_{AA}^{wa} gives build-up lines that closely follow a straight $L_0 t_m$ (dashed) line. Systems a) and c) have the same build-up curves (except that the two diagonals are permuted) although the evolution of their peak volumes (Figs. 1a and 1c) is quite different.

Similarly, expressions for the cross-peak volumes are the same

$$(a_{AB})_S = (a_{AB})_R$$

while the expressions for the diagonal-peaks are symmetrically proportional:

$$n_B(a_{AA})_S = n_A(a_{BB})_R.$$

Such symmetry implies equality of the weighted diagonal volume sums

$$n_B(a_{AA})_S + n_A(a_{BB})_S = n_B(a_{AA})_R + n_A(a_{BB})_R$$

which subsequently yields

$$\left(\frac{a_{AB}}{a^{wa}} \right)_S = \left(\frac{a_{AB}}{a^{va}} \right)_R$$

The last equation shows that the normalized cross-peak in cases 1. and 3. are identical even if the individual diagonal lines have different time evolutions.

4. $n_A = n_B = n_0$ and $R_A = R_B = R_0$. This case may look trivial because it reduces the problem to the spin pair interaction. However, to extract a genuine magnetization exchange rate constant L_0 , it is essential to know the site populations n_0 . Because

of the equality of peak volumes it is impossible to discern system $A_n B_n$ from $n(AB)$. Thus, in order to extract a normalized magnetization exchange rate constant, n_0 must be deduced independently.

Figure 1 shows the time evolution of peak volumes for the first three cases. The most notable is that in the absence of data for the larger diagonal (for example if the larger diagonal overlaps with a strong solvent signal) it is impossible to tell whether the peak volume crossing is caused by different spin populations or different overall relaxation rates. In practice, both can contribute and only detailed analysis (vide infra) can reveal their individual contributions.

Figure 2 shows the build-up curves for the same three cases. Although derived with different parameters, Figures 2a and 2c are almost identical, the only difference being that indices AA and BB are permuted. This clearly indicates that a thorough analysis of the peak volume time evolution is needed for a complete understanding of the system.

THE MODEL SYSTEM: *N*-ACETYL GLYCINE/WATER

For quantitative verification of the derived equations we have used a model system, *N*-acetyl glycine, in which a single amide proton may exchange with water protons. The chemical exchange rate can be controlled by altering the temperature, pH, concentration or solvent composition.

EXPERIMENTAL

A. Sample preparation

N-acetyl(-d₆)-glycine(-2-¹³C-¹⁵N) was prepared by condensation of acetic anhydride-d₆ (Cambridge Isotopes Laboratories, Inc., Andover, MA, USA) with glycine-2-¹³C-¹⁵N(CIL) according to published procedures.¹⁶ An NMR sample was made by dissolving 10 mg of *N*-acetyl-glycine in 1 ml of water with desired D/H = 15. The unbuffered sample had pH* = 2.4 (uncorrected reading for isotope effects).

B. NMR experiment

To eliminate possible problems associated with radiation damping, a series of one-dimensional (1D) difference experiments were recorded instead of the standard 2D exchange experiments. The equivalence of 1D experiments (used in this work), and 2D experiments, which were analyzed theoretically, is proved in the Appendix.

1D difference measurements were conducted at 45 °C on a Bruker 600 MHz Avance spectrometer using the pulse sequence diagramed in Fig. 3. Experimental details of the sequence are given in the figure caption. The pulse sequence was designed after the WNOESY sequence¹² with improved sensitivity¹⁷ and a modification to ensure complete removal of water magnetization radiation dumping. Specifically, water inversion by soft pulses was avoided, and during J-coupling evolution periods water magnetization was dephased and rephased by weak gradients. Data were collected in an interleave mode storing "d+cp" and "d-cp" experiments separately ("d" represents amide diagonal volume data and "cp" amide/water cross-peak volume data). As is shown in the Appendix, three scans are adequate to recover the cross- and diagonal-peaks. However, to follow the phase rotations of a 2D NOESY¹⁸ experiment exactly, one additional scan (scan 0) (I-III described in Appendix) is recorded. To illustrate the relationship between the cross- and the diagonal-peaks a 2D exchange spectrum¹ with $t_m = 4$ s was recorded.

C. Data analysis

Experimental data were transformed into pure diagonal and pure cross-peak volume data by summing or subtracting "d+cp" and "d-cp" data, respectively. The mixing time dependence of the

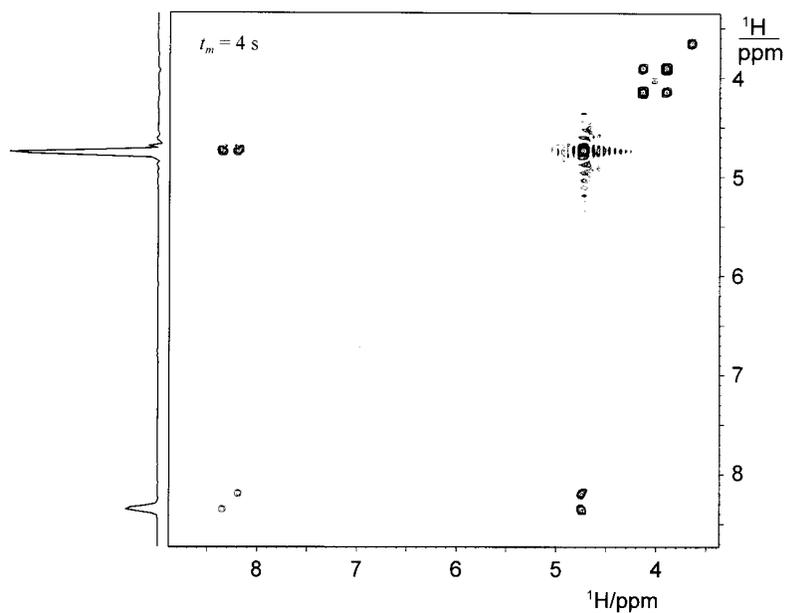


Fig. 4. Contour plot of a 2D exchange spectrum of ^{15}N enriched *N*-acetyl-glycine in water with a D/H ratio in water 15/1. The amide resonance at 8.3 ppm is split into a doublet ($^1J_{\text{NH}} \approx 90$ Hz) due to heteronuclear coupling to ^{15}N . A cross section along the amide resonance at 8.3 ppm clearly shows that the cross-peak is much larger than corresponding diagonal.

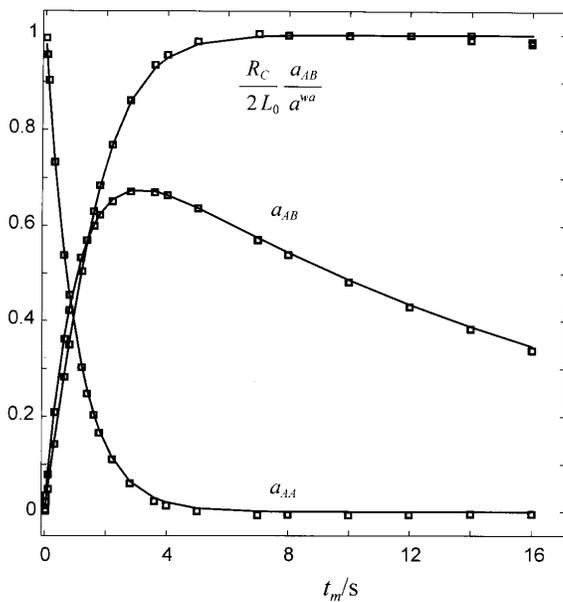


Fig. 5. Time evolution of peak volumes in chemical exchange between the *N*-acetyl-glycine amide protons and water protons. Squares represent experimental points and full lines the best fit according to Eqs. (10), (12), (18) and (19).

diagonal- and cross-peak volumes were fitted by a grid search according to Eqs. (10), (12), (19) and (20). The error limits for each parameter were found by individually varying the parameter value until the standard deviation of the best fit is doubled.

RESULTS

Figure 4 shows a 2D exchange spectrum at 4 s mixing time which clearly indicates that the cross-peak with water is much larger than the amide diagonal-peak. A representative data on time evolution of the cross-diagonal and normalized cross-peak volumes is shown in Fig. 5. Individual points represent experimental data and continuous lines the theoretical curves whose parameters were optimized by the grid search. For the sample with $D/H = 15$ and stoichiometric ratio $n_B/n_A = 550$, the best fit values obtained are $n_B = 570 \pm 400$, $n_B L_0 = 0.76 \pm 0.03 \text{ s}^{-1}$, $R_A = 0.19 \pm 0.02 \text{ s}^{-1}$, $R_B = 0.056 \pm 0.002 \text{ s}^{-1}$.

DISCUSSION AND CONCLUSIONS

Like other methods, 2D exchange spectroscopy studies dynamic systems by inserting a suitable label and monitoring its redistribution among the system components in time. The labels are resonance frequencies of the respective spin sites and are introduced as signal modulation by the frequencies during the evolution time. At the end of the evolution time, the magnetization components are left unperturbed for a brief period, the mixing time, during which magnetization exchange take place. After mixing, the magnetization redistribution is detected during a subsequent acquisition period.

The principal advantages of 2D exchange spectroscopy in multi site exchange are its inherent ability to label and monitor redistribution of all components simultaneously and that the label (nuclear magnetization) does not perturb the original dynamic equilibrium. A disadvantage is nuclear spins relaxation during the exchange. If all spin sites have similar relaxation rates then the relaxation leads merely to the sensitivity losses. However, a difference in relaxation rates among spin sites can be an additional cause of magnetization redistribution. For example, in the special case when $n_A = n_B$ and $R_B = 0$, the times of peak volume crossing and cross-peak maximum coincide, Eq. (29). Magnetization from site A migrates to site B until the original A magnetization becomes equally distributed among the two sites. At that moment the cross-peak reaches its maximum value and the diagonal- and cross-peak volumes become equal. Additionally, the exchange process that always occurs, irrespective of the magnetization distribution, does not lead to net magnetization transfer. However, relaxation continues to diminish magnetization at the site A. Since it is assumed that the site B by itself does not relax, the only way for its magnetization to "leak out" is through relaxation of site A after magnetization back-transfer, ($B \rightarrow A$). The overall effect is that after the maximum, the cross-peak volume a_{AB} becomes larger than the faster relaxing diagonal a_{AA} , even if $n_A = n_B$. The same process takes place when both sites relax with different rates, $R_A \neq R_B$, but the situation becomes complicated by the additional relaxation of site B. In special cases when the populations of both sites are equal, ($n_A = n_B$, $d = R_A - R_B$), the cross-peak

volume may become equal to the diagonal-peak volume of the faster relaxing site only at times greater than the t_{\max} , Eq. (30). This can be observed practically only in experiments with high signal to noise ratio since the difference in the peak volumes can be masked by the loss of signal due to the relaxation. Equation (13) evaluates the exact point at which $a_{AA} = a_{AB}$ for any combination of relaxation rates.

An important parameter to be analyzed is the influence of site populations because their difference can lead to more pronounced peak volume differences (e.g., $a_{AB} > a_{AA}$) even at very short mixing times. When $n_A \neq n_B$ the principal source for the cross-peak volume overshoot is the law of mixing. If the populations of sites A and B are n_A and n_B , then their respective mole fractions are $n_A/(n_A + n_B)$ and $n_B/(n_A + n_B)$. Neglecting all relaxation processes, the original magnetization of site A, $n_A I_0$, will be distributed, after sufficient time, between the sites A and B according to their respective fractions: $I_0 n_A^2/(n_A + n_B)$ remains at A, and $I_0 n_A n_B/(n_A + n_B)$ migrates to B. If $n_A < n_B$ then the fraction of magnetization transferred from A to B may be much larger than the fraction that remains at A. Indeed, in the limit $R_C t_m \rightarrow \infty$, neglecting leakage relaxation, $a_{AA} \propto n_A^2/(n_A + n_B)$ and $a_{AB} \propto n_B/(n_A + n_B)$. Thus, a site with smaller population becomes quickly depleted merely by the law of mixing and the respective diagonal-peak volume becomes unusually low, $a_{AB} > a_{AA}$.

The equations derived herein are quantitatively verified in a system composed of *N*-acetyl-glycine/water chemical exchange between the glycine amide and water protons can readily be observed. A grid search fit of the experimental cross- and diagonal (glycine only) peaks according to Eqs. (10), (12) (19) and (20), was performed. The water diagonal, a_{BB} , was not recorded (and thus not used in the fitting procedure) for two reasons. First, to ensure accurate quantitation of the amide signal, the water signal was filtered out during the acquisition. Second, even if recorded (it would be easy in experiments without filtering) the water diagonal should be used with caution since not necessarily all the water molecules may participate in the exchange process. Rather than explicitly using the number of participating water molecules, the number is obtained from the fit of the cross-peak and amide diagonal data. Figure 5 illustrates the thorough agreement between the experiment and the theory. The derived value for the number of participating water molecules, $n_B = 570 \pm 400$, matches the stoichiometric concentration, $n = 550$, within the error of the method. Such a large error is caused by the low sensitivity of the magnetization transfer to the number of participating spins when the ratio $n_B/n_A > 20$. A low sensitivity to the number of participating spins for large n_B/n_A ratios is an intrinsic weakness of the method.

The agreement between the experimental points and theoretical curves has several important practical implications. The derived expressions accurately predict the crossing of the diagonal- and cross-peaks, and for the two groups of equivalent spins, the expressions are exact in the whole range of mixing times. The equations also explain a difference between chemical exchange and cross-relaxation regarding the peak volume

crossing. In chemical exchange, the magnetization exchange rate constant k , is unrelated to overall relaxation rates and the volume crossing caused either by the population difference of the overall relaxation rate difference can be easily observed. In contrast, the cross-relaxation rate constant σ , in small molecules is related to the overall relaxation rate,¹⁹ ($R_A = 2 n_A L_0 + R_{ex}$) in a manner similar to Eq. (12). Thus, for similar external relaxation rates the cross- and diagonal-peak volumes may become equal only at very long mixing times. In small molecules the peak volume crossing is seldom observed because cross-relaxation is rarely monitored at such a long mixing times.

The derived equations presented are particularly important for the study of chemical exchange between labile solute protons and solvent protons where the number of participating protons does not necessarily need to match the stoichiometric ratio in the mixture. Thus, the equations can provide insight into the elementary relationship among the exchanging partners which is not readily available by other methods.

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ИЗВОД

ДВОДИМЕНЗИОНАЛНА СПЕКТРОСКОПИЈА ИЗМЕНЕ: ИСПИТИВАЊЕ УТИЦАЈА БРОЈА СПИНОВА

НЕНАД ЈУРАНИЋ, ЖОЛТ ЗОЛНАИ И СЛОБОДАН МАЦУРА

Одсек за биохемију и молекуларску биологију, Мејо клиника, Рочестер, Минесота, САД

Испитивали смо размену магнетизације између две групе еквивалентних спинова које имају различите концентрације и различите брзине укупне релаксације и показали да разлика у концентрацијама или у брзинама укупне релаксације, у дводимензионалном спектру, може да доведе до појаве недијагоналних спектралних линија са запремином већом од запредине њима припадајућих дијагоналних линија. Ово је посебно важно за тумачење размене магнетизације између молекула воде и изменљивих протона макромолекула. Теоријска предвиђања су експериментално потврђена у хемијској измени између протона воде и амидног протона у моделном систему *N*-ацетил-глицин/вода.

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APPENDIX

*Equivalence of 1D difference and 2D exchange experiments*A dynamic matrix L

$$L = \begin{pmatrix} -\sum_{i \neq 1} L_{i1} - R_1 & L_{12} & \dots & L_{1N} \\ L_{21} & -\sum_{i \neq 2} L_{i2} - R_2 & \dots & L_{2N} \\ \vdots & \vdots & \ddots & \vdots \\ L_{N1} & L_{N2} & \dots & -\sum_{i \neq N} L_{iN} - R_N \end{pmatrix} \quad (\text{A-1})$$

completely describes the magnetization exchange in a system consisting of N groups of equivalent spins. Matrix elements L_{ij} represent magnetization exchange rate constants for the magnetization transfer from group j to group i , and R_i all relaxation rates by which the magnetization from group i is lost into the environment. An exchange experiment recorded with a mixing time t_m consists of cross- and diagonal-peaks whose volumes $a_{ij}(t_m)$ and $a_{ii}(t_m)$ form a spectral matrix $A(t_m)$

$$A(t_m) = \begin{pmatrix} a_{11}(t_m) & a_{12}(t_m) & \dots & a_{1N}(t_m) \\ a_{21}(t_m) & a_{22}(t_m) & \dots & a_{2N}(t_m) \\ \vdots & \vdots & \ddots & \vdots \\ a_{N1}(t_m) & a_{N2}(t_m) & \dots & a_{NN}(t_m) \end{pmatrix} \quad (\text{A-2})$$

The dynamic matrix and the spectral matrix are related by

$$\mathbf{A}(t_m) = \exp(\mathbf{L} t_m) \mathbf{N}_p \mathbf{A}_0 \quad (\text{A-3})$$

where A is a peak volume of a single spin and N_p the population matrix

$$\mathbf{N}_p = \begin{vmatrix} n_1 & & 0 \\ & n_2 & \\ 0 & & n_N \end{vmatrix} \quad (\text{A-4})$$

Alternatively, the dynamic matrix can be deduced from a series of 1D difference spectra. In a system with N groups of equivalent spins the deviation of the total magnetization from equilibrium, m , can be represented as an N dimensional column vector, $\mathbf{m}(t_m)$, of individual magnetization components $m_i(t_m)$, ($m_i(t_m) = I_i(t) - I_i(0)$; $I_i(t)$ actual, $I_i(0)$ equilibrium magnetization)

$$\mathbf{m}(t_m) = \begin{vmatrix} m_1(t_m) \\ m_2(t_m) \\ \vdots \\ m_N(t_m) \end{vmatrix} \quad (\text{A-5})$$

The time evolution of the vector $\mathbf{m}(t_m)$ can be described by the system of differential equations

$$\dot{\mathbf{m}}(t_m) = \mathbf{L} \mathbf{m}(t_m) \quad (\text{A-6})$$

where \mathbf{L} is the same dynamic matrix and $\mathbf{m}(t_m)$ a column vector of the time derivatives of the individual magnetization components:

$$\dot{\mathbf{m}}(t_m) = \begin{vmatrix} \dot{m}_1(t_m) \\ \dot{m}_2(t_m) \\ \vdots \\ \dot{m}_N(t_m) \end{vmatrix} \quad (\text{A-7})$$

The general solution

$$\mathbf{m}(t_m) = \exp(\mathbf{L} t_m) \mathbf{m}_0 \quad (\text{A-8})$$

depends on the initial conditions, $\mathbf{m}_0 = \mathbf{m}(0)$

$$\mathbf{m}_0 = \begin{vmatrix} m_{0,1} \\ m_{0,2} \\ \vdots \\ m_{0,N} \end{vmatrix} \quad (\text{A-9})$$

Equations (A-3) and (A-8) have a common exponential term. This helps to establish a relation between the magnetization vector \mathbf{m} and the spectral matrix A

$$\mathbf{m}(t_m) = \frac{1}{A_0} \mathbf{A}(t_m) \mathbf{N}_p^{-1} \mathbf{m}_0 \quad (\text{A-10})$$

or in a scalar form

$$m_i(t_m) = \frac{1}{A_0} \sum_j \frac{a_{ij}(t_m)}{n_j} m_{0,j} \quad (\text{A-11})$$

Thus, the magnetization vector component is a sum of the spectral matrix elements taken along

the matrix row, weighted by the corresponding spin populations. Individual matrix elements, a_{ij} , can be obtained from N experiments recorded with linearly independent initial conditions. The simplest way to get a set of linearly independent initial conditions is to perturb one spectral line at a time. If the perturbation is performed in each experiment with the same tip angle, then the initial non-equilibrium state of each component is a constant fraction, c , of the initial magnetization, $n_j I_0$

$$\mathbf{m}^j_0 = c n_j A_0 \mathbf{e}_j = c n_j I_0 \begin{pmatrix} 0_1 \\ \vdots \\ 0_{j-1} \\ 1_j \\ 0_{j+1} \\ \vdots \\ 0_N \end{pmatrix} \quad (\text{A-12})$$

(Note that the peak volume of a single spin is the same as the equilibrium magnetization of a single spin, $A_0 = I_0$).

For each of the N experiments with initial conditions $\mathbf{m}_0^1, \mathbf{m}_0^2, \dots, \mathbf{m}_0^N$ exists a system of differential equations

$$\mathbf{m}^j = \mathbf{m}(t_m) = \exp(\mathbf{L} t_m) \mathbf{m}_0^j \quad (\text{A-13})$$

Then, the solution of N such systems of N differential equations can be grouped into a matrix equation of size $N \times N$

$$\mathbf{M} = \exp(\mathbf{L} t_m) \mathbf{M}_0 \quad (\text{A-14})$$

where

$$\mathbf{M} = (\mathbf{m}^1, \mathbf{m}^2, \dots, \mathbf{m}^N) = \begin{pmatrix} m_1^1 & m_1^2 & \dots & m_1^N \\ m_2^1 & m_2^2 & \dots & m_2^N \\ \vdots & \vdots & & \vdots \\ m_N^1 & m_N^2 & \dots & m_N^N \end{pmatrix} \quad (\text{A-15})$$

and

$$\mathbf{M}_0 = (\mathbf{m}_0^1, \mathbf{m}_0^2, \dots, \mathbf{m}_0^N) = \begin{pmatrix} m_{0,1}^1 & m_{0,1}^2 & \dots & m_{0,1}^N \\ m_{0,2}^1 & m_{0,2}^2 & \dots & m_{0,2}^N \\ \vdots & \vdots & & \vdots \\ m_{0,N}^1 & m_{0,N}^2 & \dots & m_{0,N}^N \end{pmatrix} \quad (\text{A-16})$$

If in every experiment only one line is perturbed according the Eq. (A-12), then

$$\mathbf{M}_0 = c \mathbf{I}_0 \mathbf{N}_p \quad (\text{A-17})$$

and

$$\mathbf{M} = c \exp(\mathbf{L} t_m) \mathbf{N}_p \mathbf{I}_0 \quad (\text{A-18})$$

Comparing Eqs. (A-3) and (A-18) we find

$$\mathbf{M} = c \mathbf{A} \quad (\text{A-19})$$

i.e., 2D exchange spectrum is equivalent to a series of N difference 1D experiments in which one spectral line is perturbed at a time according to Eq. (A-12). Because the reference spectrum (1D

spectrum without perturbation) is the same for all non equilibrium spectra, the total number of 1D experiments needed to recover the dynamic matrix is $N+1$. The reference spectrum is not needed for 1D exchange experiments recorded in the rotating frame because then the equilibrium magnetization is zero. Thus in the rotating frame N 1D experiments are sufficient.

Now it is easy to derive relations for a system with two groups of equivalent spins. If the two groups A and B have populations n_A and n_B , and initial non equilibrium states m_A^0, m_B^0 , then from Eq. (A-11)

$$\begin{aligned} m_A &= \frac{m_A^0}{n_A I_0} a_{AA} + \frac{m_B^0}{n_B I_0} a_{AB} \\ m_B &= \frac{m_B^0}{n_B I_0} a_{BB} + \frac{m_A^0}{n_A I_0} a_{AB} \end{aligned} \quad (\text{A-20})$$

where a is a peak volume in a 2D exchange spectrum and I_0 equilibrium magnetization of a single spin. For $N = 2$ a total of three 1D experiments are needed to obtain all elements (peak volumes) of spectral matrix:

Experiment number	Initial spin orientation	m_A^0	m_B^0	$m_A(t_m)$	$m_B(t_m)$
I	A \uparrow B \uparrow	0	0	0	0
II	A \uparrow B \downarrow	0	$-2n_B M_0$	$-2 a_{AB}$	$-2 a_{BB}$
III	A \downarrow B \uparrow	$-2n_A M_0$	0	$-2 a_{AA}$	$-2 a_{AB}$

Then

$$\begin{aligned} m_A^I - m_A^{III} &= 2a_{AA} & m_B^I - m_B^{III} &= 2a_{AB} \\ m_A^I - m_A^{II} &= 2a_{AB} & m_B^I - m_B^{II} &= 2a_{BB} \end{aligned} \quad (\text{A-21})$$

For rotating frame exchange, with the same initial conditions, only experiments II and III are needed. Then, Eqs. (A-21) apply with $m^I = 0$ and factor one (instead of 2).

With current hardware it is easy to perform selective spin inversion, a selective inversion experiment may be advantageous compared to the 2D exchange experiment in a system with two groups of spins. Provided excessive signal averaging is not needed, the selective inversion experiment is much faster than the 2D exchange experiment. For slowly relaxing groups, like protons in isotope diluted water, the perturbed magnetization can be realigned along the z -axis after the mixing period which enables much shorter repetition times. Finally, selective inversion with shaped pulses could be achieved within the order of tens of a millisecond compared to evolution time in 2D experiment that can be as long as hundreds of milliseconds. Thus, provided the resonances of the groups A and B are well separated, in a two group of equivalent spins, 1D difference spectroscopy is more efficient than 2D exchange spectroscopy.