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Detection of Intermolecular Chemical Exchange through Decorrelation of Two-Spin Order

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An initial correlation between two spins is lost when they are separated by intermolecular chemical exchange. This effect, termed "decorrelation by chemical exchange," manifests itself in a decay of the corresponding two-spin modes. It can be used for monitoring intermolecular chemical exchange, as is demonstrated for L-tryptophan where the decay of $^1H^{15}N$ two-spin order provides information on the exchange of indole protons with solvent water. © 1999 Academic Press

Key Words: **intermolecular chemical exchange; decorrelation; multispin order; multiquantum coherence; L-tryptophan; amide proton exchange; HMQC.**

NMR spectroscopy has been instrumental in investigating a variety of chemical exchange effects in liquid phase. Slow forms of exchange can often be studied using isotope exchange methods (*1*). Fast exchange processes can be elucidated on the basis of relaxation data (*2, 3*). Exchange processes falling in an intermediate range (millisecond-to-second) are best investigated by lineshape analysis (*4*), polarization transfer methods (*5*), or multidimensional exchange spectroscopy (*6*).

It was pointed out long ago that manifestations of the chemical exchange in single-quantum spectroscopy are formally similar to those of spin relaxation. Over the past two decades, relaxation studies have been fruitfully extended to multiquantum coherences and multispin orders (*7–9*). By comparison, there have been relatively few chemical exchange studies utilizing multispin modes. The theory of *intra*molecular exchange involving multispin modes has been developed by Szymański (*10*), Gamliel, Luz, and Vega (*11*), Rance (*12*), Wagner *et al.* (*13*) and others. Here we demonstrate how multispin orders can be used to examine *inter*molecular chemical exchange.

Consider a binary system undergoing chemical exchange, $AB + B' \underset{k_2}{\overset{k_1}{\rightleftharpoons}} AB' + B$. According to the Kaplan–Alexander–Binsch formalism (*14, 15*) in the formulation by Kaplan and Fraenkel (*16*) and Muhandiram and McClung (*17*), ensuing changes in the spin density operator ρ_{AB} are described by

$$\frac{d\rho_{AB}}{dt} = -k_1\rho_{AB} + k_1 \mathrm{Tr}_{B'}\{\rho_{AB'} \otimes \rho_B\}, \qquad [1]$$

where $\mathrm{Tr}_{B'}$ denotes the trace over the spin variables of the spin system B′. The direct product notation, $\otimes$, is used here so that Eq. [1] has the same form for spin density operators and the corresponding spin density matrices. Using a basis of traceless product operators $\{A_i\}$ for the spin system A and $\{B_i\}$ for the spin system B, the density operators can be represented as

$$\rho_B = [E^B + \beta \sum_i b_i^B(t)\, B_i]/\mathrm{Tr}_B\{E^B\} \qquad [2a]$$

$$\rho_{AB} = [E^{AB} + \beta \sum_i a_i^{AB}(t)\, A_i + \beta \sum_j b_j^{AB}(t)\, B_j + \beta \sum_{i,j} c_{ij}^{AB}(t)\, A_i B_j]/\mathrm{Tr}_{AB}\{E^{AB}\}. \qquad [2b]$$

Here E^B and E^{AB} are unity operators of the respective spin systems, $\beta = \hbar\omega_0/kT$ is a small constant proportional to the nuclear spin Larmor frequency ω_0, and $a_i(t)$, $b_i(t)$, and $c_{ij}(t)$ are time-dependent coefficients, representing magnetization modes, which are on the order of magnitude of 1. Note that the spin density operators in Eq. [2] are normalized to one in spin space and are not proportional to the concentrations of the respective molecules. This convention is relevant for the form of the exchange superoperator (*18*) and is compatible with Eq. [1].

We are concerned here with the terms A_iB_j that are the products of spin operators A_i and B_j associated with the two subsystems. The evolution of these terms is expressed by the time-dependent coefficients $c_{ij}^{AB}(t)$, which represent multispin modes. Substituting the density operators in the form of Eq. [2] into Eq. [1] and using the property of traceless operators, $\mathrm{Tr}_B\{B_i\} = 0$, we obtain

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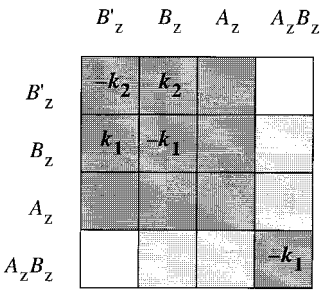

FIG. 1. Exchange matrix (longitudinal manifold) for a weakly coupled two-spin system, A (= ^{15}N), B (= ^{1}H), undergoing intermolecular chemical exchange. Shaded squares correspond to the relaxation matrix elements for the general case involving intra- and intermolecular relaxation (*44*). Light shading indicates cross-correlated cross relaxation.

$$\beta \frac{dc_{ij}^{AB}(t)}{dt} = -k_1\beta c_{ij}^{AB}(t) + k_1\beta^2 a_i^{AB}(t)b_j^{B}(t). \qquad [3]$$

The first term on the right-hand side of Eq. [1] leads to a decay of $c_{ij}^{AB}(t)$, reflecting the loss of observable correlation between spins that are separated by chemical exchange. The second term, representing the production of correlated modes by chemical exchange, is negligible in comparison to the first one since it is proportional to β^2, $\beta \ll 1$ (*19*). We conclude that $c_{ij}^{AB}(t)$ magnetization is not measurably replenished in the process of intermolecular exchange.

According to Eq. [3], any multispin mode which is "broken up" in the process of intermolecular exchange undergoes a monoexponential decay characterized by the respective exchange rate constants. We call this effect "decorrelation by chemical exchange." The monoexponential decay which has been obtained here for the exchange process AB + B′ $\underset{k_2}{\overset{k_1}{\rightleftharpoons}}$ AB′ + B is also encountered in other intermolecular exchange reactions. It is intuitively obvious that multispin orders (or coherences) undergo decorrelation when at least some of the involved spins become separated in the process of intermolecular exchange. In this communication, we concentrate on longitudinal multispin order, such as represented by the term A_zB_z. The analogous exchange-driven decay of multispin coherences, which is not discussed in this paper, is known to have an effect on 1D exchange spectra (*20, 21*).

A more thorough analysis of the exchanging spin system should also account for relaxation effects. Figure 1 illustrates the structure of the exchange and relaxation matrices for the case when A and B bear single spins $\frac{1}{2}$. For the mode corresponding to two-spin order, A_zB_z, the exchange matrix contains only diagonal elements as follows from Eq. [3]. Accordingly, the sum of the exchange matrix elements along the bottom row is nonzero. However, this does not mean that multispin correlations actually disappear as a result of exchange. In fact, they are preserved for a time of the order of T_1, but become unobservable when two spins are separated by chemical exchange and move far apart from each other in the process of translational diffusion.

The structure of the relaxation matrix is shown by a shaded pattern in Fig. 1. Cross-correlated cross-relaxation elements, responsible for the coupling between the two-spin and single-spin modes, are indicated by light shading. In the case when chemical exchange is fast, k_1 is much greater than the cross-correlated cross-relaxation rate constant, and the latter can be treated as a first-order perturbation-theory correction in the eigenbasis of the exchange matrix. This procedure yields a slightly modified effective autorelaxation rate constant for the two-spin order, $T_{1zz}^{\text{eff}\,-1}$. It is also often possible to suppress the effect of cross-correlation terms by specially designed pulse

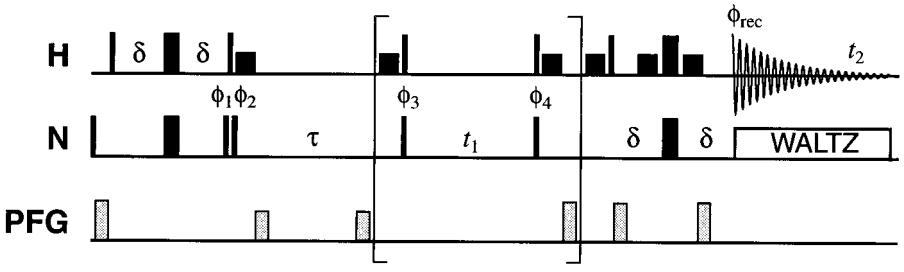

FIG. 2. Pulse sequence for measurements of the N_zH_z decay induced by chemical exchange and spin relaxation. The phase cycle $\phi_1 = (x, x)$; $\phi_2 = (-x, x)$; $\phi_{rec} = (x, -x)$ selects N_zH_z order. The second scan of this phase cycle produces an inverted N_zH_z relaxation profile, whereas H_z and N_z profiles are left in a good approximation unchanged, with both H_z and N_z relaxing from zero toward their equilibrium values. This phase cycle ensures an efficient suppression of cross-correlated cross-relaxation effects so that the obtained decay curves $c_{zz}^{NH}(\tau)$ are monoexponential and $T_{1zz}^{\text{eff}\,-1}$ is close to the two-spin order auto-relaxation rate constant, T_{1zz}^{-1}. The block inserted in square brackets corresponds to the HMQC-type 2D version of the sequence where N_zH_z order is converted into double- and zero-quantum coherences oscillating at the frequencies $\omega_H \pm \omega_N$. Quadrature detection in the 2D version of the sequence is accomplished by incrementing the phase ϕ_4 according to the States–TPPI method (*45*). The complete phase cycle in the 2D version of the experiment is $\phi_1 = 2(x, x)$; $\phi_2 = 2(-x, x)$; $\phi_3 = (x, x, -x, -x)$; $\phi_{rec} = (x, -x, -x, x)$, and in the 1D version $\phi_1 = (x, x, y, y, -x, -x, -y, -y)$; $\phi_2 = (x, -x, y, -y, -x, x, -y, y)$; $\phi_{rec} = 4(x, -x)$. The duration of the delay δ is $1/(4^1J_{NH})$. The duration of the relaxation delay between the scans was set to 1 s (a 10-s delay, used in control experiments, leads to virtually identical values of Γ_{zz}^{NH}). The water suppression scheme includes WATERGATE (*40*) and water flip-back pulses (*41*) with an RF field strength $|\gamma_H B_1|/(2\pi) = 250$ Hz. Pulsed field z gradients (*46*) (15–30 G/cm) are used to purge the unwanted coherences.

sequences, such as the sequence of Fig. 2 (see also Refs. *22*, *23*), so that the residual small effect can be incorporated in $T_{1zz}^{\mathrm{eff}\,-1}$. In both cases, the decay of the two-spin order is approximately monoexponential,

$$c_{zz}^{\mathrm{AB}}(t) \cong c_{zz}^{\mathrm{AB}}(0)\exp(-(k_1 + T_{1zz}^{\mathrm{eff}\,-1})t)$$
$$= c_{zz}^{\mathrm{AB}}(0)\exp(-\Gamma_{zz}^{\mathrm{AB}}t). \qquad [4]$$

Equation [4] constitutes the basis for the proposed method for detection of chemical exchange.

We concentrate on the exchange of labile amide protons in proteins and nucleic acids with solvent water (*24*). A determination of k_1 provides information on the degree of exposure of different residues to the solvent, which is particularly relevant for understanding protein folding (*25*). We test the new approach by studying the exchange of the indole proton in L-tryptophan (inset in Fig. 3). Other labile protons suitable for such measurements at or near ambient conditions are found in lysine, arginine, glutamine, and asparagine side chains (*26*), as well as in the polypeptide backbone (*27*) and in polynucleotides (*28*).

The pulse sequence used for measuring of the N_zH_z decay is shown in Fig. 2. It consists of an initial period of length 2δ for the preparation of the heteronuclear two-spin order N_zH_z. This term is exposed to the influence of chemical exchange (and relaxation) during the subsequent mixing period τ. In the 2D version of the experiment, the remaining part of the N_zH_z mode is converted into heteronuclear zero and double quantum coherences which precess during the evolution time t_1. They are subsequently detected, after a further transfer period of duration 2δ, as ^{1}H single quantum coherence. In the 1D version the t_1 evolution block is omitted.

The use of zero and double quantum coherences in the 2D experiment allows one to separate N_zH_z from other longitudinal orders emerging during the time τ. This could also be achieved with an HSQC-based sequence by using the distinct transformation properties of N_zH_z under the effect of simultaneously applied proton and nitrogen RF pulses. It is also possible to implement an experiment where magnetization originates from a proton other than NH and is later transferred back to this proton for detection (*29*, *30*).

The detection of the exchange becomes feasible in the range $T_{1zz}^{\mathrm{eff}\,-1} \lesssim k_1 \lesssim {}^1J_{\mathrm{NH}}$, where ${}^1J_{\mathrm{NH}}$ is the ^{1}H–^{15}N scalar coupling constant, ~90 Hz. The lower limit of usefulness is imposed by a dominant relaxation term. The upper limit is reached when the build-up of two-spin order during the preparation period is strongly attenuated by fast chemical exchange. The exchange rate constants measured in our experiments fell in the range $0.3\ T_{1zz}^{\mathrm{eff}\,-1} < k_1 < 1.2\ {}^1J_{\mathrm{NH}}$, with $T_{1zz}^{\mathrm{eff}\,-1} \ll {}^1J_{\mathrm{NH}}$.

The pH dependence of the decorrelation decay rate constant $\Gamma_{zz}^{\mathrm{NH}}$ has been measured in aqueous solutions of doubly ^{15}N-labeled L-tryptophan, Fig. 3. Measurements employing several samples with different pH levels are often used to elucidate

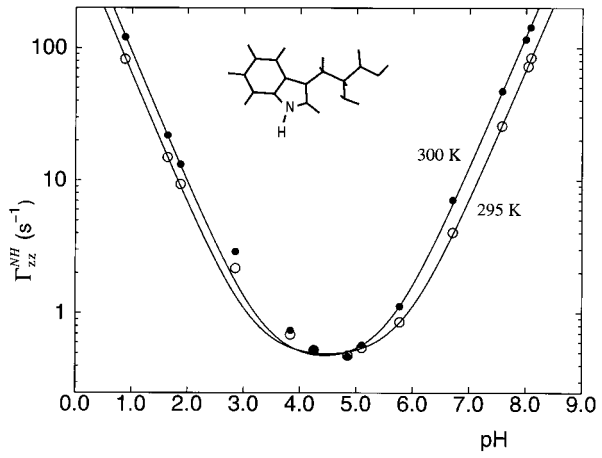

FIG. 3. Apparent decay rate constants $\Gamma_{zz}^{\mathrm{NH}} = k_1 + T_{1zz}^{\mathrm{eff}\,-1}$ for the two-spin order N_zH_z of the indole NH group in L-tryptophan as a function of the pH of an aqueous solution at the temperatures (●) 300 K and (○) 295 K. The samples were prepared with a concentration of 50 mM of tryptophan in 97% H_2O–3% D_2O and buffered with 20 mM phthalate, acetate, phosphate, or borate, depending on the pH range. The curves shown in the plot are obtained by least squares fitting, yielding the values $\log k_{\mathrm{H}} = 2.8$, $\log k_{\mathrm{OH}} = 7.9$, and $\log k_{\mathrm{H}} = 3.0$, $\log k_{\mathrm{OH}} = 8.0$ at 295 K and 300 K, respectively. The latter two values can be compared with the values $\log k_{\mathrm{H}} = 3.0$ and $\log k_{\mathrm{OH}} = 7.8$ from the work by Waelder and Redfield (*33*). The functional dependence used for the fitting of the experimental data does not account for the local pH effects mentioned in the text, possible interactions of the buffer molecules with tryptophan, or a possible variation of $T_{1zz}^{\mathrm{eff}\,-1}$ with pH.

chemical exchange in proteins (*27*, *31*). The measured decorrelation decay times range from 10 ms to 2 s. The strong dependence of $\Gamma_{zz}^{\mathrm{NH}}$ on the pH clearly reveals the presence of exchange since the relaxation term $T_{1zz}^{\mathrm{eff}\,-1}$ is in a good approximation independent of the pH. The measured pH dependence is in good agreement with the results published in the literature (*32–34*) (see caption of Fig. 3). The data were collected at two temperatures in order to test the consistency of results.

Figure 3 also shows the results of the fitting with the function $\Gamma_{zz}^{\mathrm{NH}} = k_{\mathrm{H}}10^{-\mathrm{pH}} + k_{\mathrm{OH}}K_{\mathrm{W}}10^{\mathrm{pH}} + T_{1zz}^{\mathrm{eff}\,-1}$, where k_{H} and k_{OH} are temperature-dependent acid- and base-catalyzed exchange-rate constants that are used, together with $T_{1zz}^{\mathrm{eff}\,-1}$, as fitting parameters, and K_{W} is the temperature-dependent autoionization constant of water ($\log K_{\mathrm{W}} = -13.936$ at 300K). In the logarithmic representation given, the curves are symmetric and have linear asymptotes with the slopes 1 and −1. Near pH 3, the measured rate constants $\Gamma_{zz}^{\mathrm{NH}}$ show a significant deviation from the calculated curves. This deviation has previously been observed by Waelder and Redfield (*33*), who used a saturation transfer experiment to determine k_1 of the indole proton in tryptophan. Based on a fluorescence study, Feitelson (*35*) argued that the deprotonation of the COOH group, which takes place at pH $\gtrsim$ 3, leads to an electric polarization of the neighboring NH_3^+ group, which in turn affects the indole ring. It is possible that this mechanism is responsible for the enhancement of the acid-catalyzed exchange observed at pH $\gtrsim$ 3.

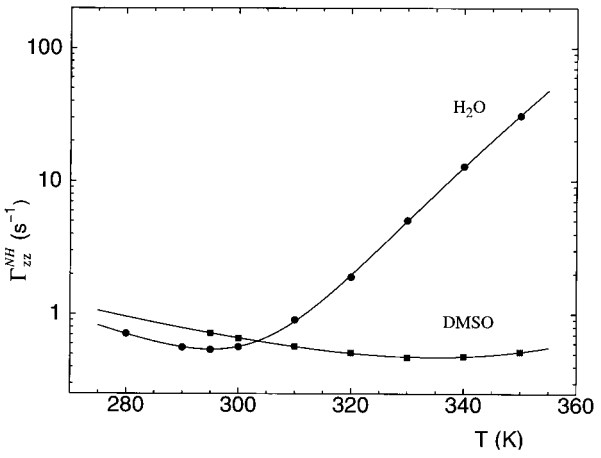

FIG. 4. Apparent decay rate constant $\Gamma_{zz}^{\mathrm{NH}} = k_1 + T_{1zz}^{\mathrm{eff}\,-1}$ for the two-spin order N_zH_z of the indole NH group in L-tryptophan as a function of temperature. The data are plotted for (●) an aqueous solution of 50 mM tryptophan with 20 mM acetate buffer, and (■) a solution of 10 mM tryptophan in deuterated d_6-dimethyl sulfoxide (DMSO) containing a small amount of residual water. The pH of the aqueous sample is 5.07 at 300 K and varies by less than 0.1 pH unit over the temperature range of this experiment. For the aqueous solution, the fitted parameter values are $\log T_{1zz}^{0\,-1} = -4.04$, $E_r = 20.8$ kJ mol^{-1}, $\log k_1^0 = 14.85$, and $E_{ex} = 89.5$ kJ mol^{-1}. The exchange parameter values are typical for NH protons (*47*).

The temperature dependence of $\Gamma_{zz}^{\mathrm{NH}}$, shown in Fig. 4, also puts into evidence the effect of the chemical exchange. The increase of $\Gamma_{zz}^{\mathrm{NH}}$ with temperature is due to the increasing exchange rate constant, k_1, while the decrease reflects the temperature dependence of the relaxation term, $T_{1zz}^{\mathrm{eff}\,-1}$, in the extreme narrowing limit. Strong increase of the decay rate constant $\Gamma_{zz}^{\mathrm{NH}}$ with temperature is observed for aqueous solution of tryptophan (upper curve in Fig. 4). This points toward intermolecular exchange as a main source of the N_zH_z decay. In contrast, $\Gamma_{zz}^{\mathrm{NH}}$ declines with increasing temperature for tryptophan dissolved in aprotic DMSO solvent (lower curve in Fig. 4). In this case, exchange contribution is small and the temperature dependence of $\Gamma_{zz}^{\mathrm{NH}}$ is determined by $T_{1zz}^{\mathrm{eff}\,-1}$ term. The slight rise in $\Gamma_{zz}^{\mathrm{NH}}$, registered in the DMSO sample at higher temperatures, is probably caused by the onset of chemical exchange (the mechanism of exchange in the aprotic solvent is unclear; it can be hypothesized that cations are concentrated in the coordination sphere of tryptophan and that the impurity water is involved). The functional dependence used for fitting the data in Fig. 4, $\Gamma_{zz}^{\mathrm{NH}}(T) = k_1(T) + T_{1zz}^{eff\,-1}(T)$ with $k_1(T) = k_1^0\exp(-E_{ex}/RT)$ and $T_{1zz}^{eff\,-1}(T) = T_{1zz}^{0\,-1}\exp(E_r/RT)$, is based on an Arrhenius temperature dependence of exchange and relaxation correlation times, assuming that the latter fulfills the extreme narrowing conditions. The extracted activation energies and preexponential factors are listed in the figure caption. The temperature dependence of the exchange in the aqueous solution of tryptophan has been also recorded with addition of the alleged exchange-inhibiting agent, *N*-acetyl-D-glucosamine (*36*). We found that the exchange rate constant k_1 dropped by less than 20% in the presence of 0.2 M *N*-acetyl-D-glucosamine.

The functional dependencies described above have been utilized to separate the exchange and relaxation contributions. The fitting of the temperature dependence of the H_2O sample in Fig. 4 leads to k_1 (300 K) = 0.18 s^{-1} and $T_{1zz}^{\mathrm{eff}\,-1}$ (300 K) = 0.38 s^{-1}. Respective contributions determined from fitting the pH dependence, Fig. 3, are 0.15 and 0.41 s^{-1}. The agreement is fair, given the simple character of functional dependencies employed in this analysis.

The fastest and the slowest $\Gamma_{zz}^{\mathrm{NH}}$ rates, corresponding to pH = 8.00 and pH = 4.85 in Fig. 3, respectively, have been remeasured using the 2D version of the sequence shown in Fig. 2. The results agree within a 2% error margin with those obtained from the 1D experiments.

We have also investigated the intermolecular exchange of amide protons in acetamide, which can be considered as a model for the glutamine and asparagine side-chain amides. The results are in agreement with the literature data (*37*). The situation is, however, more involved compared to tryptophan, since the NH_2 group of acetamide displays both inter- and intramolecular exchange. The latter is responsible for a permutation of the two amide protons, cis and trans with respect to the carbonyl oxygen. The presented approach can be extended to treat both exchange pathways in the $\{N_zH_z^{\mathrm{cis}}, N_zH_z^{\mathrm{trans}}\}$ manifold (the results will be reported elsewhere). The decay of three-spin order in this system can be used to measure the sum of the intermolecular exchange rate constants for cis and trans protons.

The approach based on the decorrelation of the multispin modes can be referred to as the Decor method for detection of intermolecular chemical exchange. The Decor method, implemented in this work for two-spin order, offers potential advantages in comparison to other experimental schemes used for exchange measurements. It is particularly convenient that the value of $k_1 + T_{1zz}^{\mathrm{eff}\,-1}$ can be read directly from the decay curve. In comparison, the interpretation of exchange effects in single-quantum experiments requires the consideration at least of a 2×2 exchange matrix, and a full lineshape analysis usually involves large evolution matrices. It is well known that the use of multispin modes has generally the benefit of reducing the dimensionality of the evolution matrices. In the Decor approach, the dimensionality is reduced to 1, thus simplifying the interpretation (this reduction also involves the elimination of cross-correlated cross-relaxation between A_zB_z and other spin orders, such as A_z).

In a good approximation, the proposed method is free of problems concerned with partial saturation of the water signal or radiation damping which are typically encountered in exchange studies of proteins (*27, 38, 39*). In the Decor experiment, the water magnetization itself is not a part of the exchange measurement so that the standard water suppression methods (*40, 41*) can be utilized as in Fig. 2.

The 2D Decor experiment allows one to monitor the exchange of NH protons in proteins with water by recording several 2D spectra for different values of τ. Each spectrum

corresponds to a standard HMQC spectral map where the intensities of the cross-peaks decrease exponentially as a function of time τ. In many cases, the resolution of such spectra is sufficient so that the experiment can be carried out without introducing a third spectral dimension as in heteronuclear-edited proton exchange spectroscopy (*42*).

The proposed experiment measures the sum of k_1 and $T_{1zz}^{\mathrm{eff}\,-1}$, so it is left to the spectroscopist to separate the exchange contribution from the relaxation contribution (unless the latter can be neglected). Their separation based on pH and temperature dependencies was useful in this paper to establish quantitative validity of the Decor approach. In practical exchange studies, it is often possible to neglect the relaxation contribution, in particular when a qualitative verification of exchange process is sufficient. The separation of exchange and auto- or cross-relaxation contributions requires special attention in any exchange measurement on this time scale (*13, 43*).

In conclusion, the use of two-spin order provides a simple and elegant method for the investigation of intermolecular chemical exchange.

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