Isolation of Selected Exchange Processes in Nuclear Magnetic Resonance

Abstract: We have shown that it is possible to inhibit the transfer of magnetization in a system with several exchanging sites in dynamic equilibrium, as in a mixture of *cis*- and *trans*-$ZrCl_4L_2$ with excess free ligand L (L = $(CH_3O)_3PO$). The forward and backward reaction rates involving two selected sites can be studied while the effect of competing exchange processes is "quenched". This can be achieved either by selective inversion of the magnetization of the two chosen sites in the course of the reaction interval, or alternatively by inversion of the magnetization of all other sites in the exchange network. The rate of exchange from the *free* to the *cis* site was determined to be $k_{cis \leftarrow free} = 0.018\ s^{-1}$. In the usual methods, this process would tend to be overshadowed by the almost two hundred times faster competing exchange process from the *cis* to the *trans* site ($k_{trans \leftarrow cis} = 3.32\ s^{-1}$).

Keywords
exchange processes · kinetics · NMR spectroscopy · zirconium complexes

One of the most fascinating aspects of magnetic resonance is the possibility of determining rates of chemical reactions in systems in dynamic equilibrium, where the turnover due to forward and back reactions precisely cancels, so that there is no net transfer of material.[1] In most forms of spectroscopy, there is little or no evidence that any dynamic processes are taking place, since the concentrations remain time-independent, but magnetic resonance allows one to observe the transfer of magnetization rather than the transfer of material, and it is sufficient that the chemical shifts of the nuclei be affected in the course of the chemical reaction to make the exchange apparent. This effect can be exploited in line-shape studies[2, 3] and in experiments where the longitudinal magnetization of a chosen site is perturbed by selective saturation or inversion.[4–7] Exchange processes lead to a redistribution of the perturbed magnetization. This effect can be visualized very effectively by two-dimensional exchange spectroscopy (EXSY),[4, 5] which is essentially equivalent to nuclear Overhauser effect spectroscopy (NOESY).[6, 7] In this paper, we show that it is possible to modify the behavior of the magnetization in exchanging systems in such a way that they behave as *if all exchange rates were quenched except for those leading to the interconversion of a selected pair of sites*. This can be achieved by selectively inverting suitably chosen magnetization components in the course of the mixing time τ_m. The chemical exchange processes proceed unhindered, and the dynamic equilibrium between the chemical reactions is not perturbed. It is only the magnetization that behaves as if the rates were modified. This approach is related to the suppression of spin diffusion by quenching undesirable indirect external trouble in nuclear Overhauser effect spectroscopy (QUIET-NOESY),[8] and we therefore refer to the experiment described here as QUIET-EXSY.

Consider a system in chemical exchange, where we wish to focus attention on the conversion of magnetization from a "source" site A to a "target" site X. There may be one or several "clandestine" sites K, K′, . . ., so that the magnetization transfer due to the direct conversion A → X may be contaminated by two-step processes such as A → K → X, etc. To resolve this entangled situation, several approaches are possible, in analogy to Overhauser studies. The first consists in taking into account all pathways in a full matrix analysis[9, 10] requiring the consideration of the whole network (chemical exchange and cross-relaxation) and the determination of all the information at once. In a less demanding approach, the rates can be determined one after the other by using the initial rate approximation,[11, 12] which requires the acquisition of a series of one- or two-dimensional spectra (NOESY or EXSY) with mixing times that are short on the timescale of the fastest dynamic process. A third approach relies on the cancellation of unwanted effects by manipulating the magnetization.[8, 13–23] Earlier studies have focused on the selective saturation of unwanted sites, which requires knowledge of their resonance frequencies.[13–18] The experiment proposed here also relies on the cancellation of unwanted effects, but only the resonance frequencies of the sites of interest must be known and no further assumptions need to be made concerning the number and frequencies of the clandestine sites. Misleading contributions due to two-step processes such as A → K → X can be removed by selective inversion of longitudinal magnetization in the middle of the mixing time τ_m. This approach does not require any continuous irradiation, thus eliminating uncertainties about the efficiency of saturation, and heating of the sample due to protracted irradiation can be avoided.

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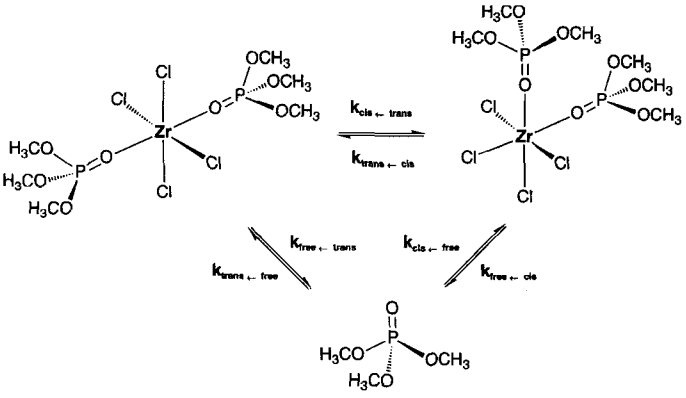
Results

To illustrate our method, we have chosen a three-site exchange network consisting of *trans*-$ZrCl_4L_2$, *cis*-$ZrCl_4L_2$, and an excess of the *free* ligand L (L = $(CH_3O)_3PO$, Scheme 1). This equilibrium has been studied by Merbach and co-workers.[24, 25]



Scheme 1. Exchange equilibria of *trans*-$ZrCl_4L_2$, *cis*-$ZrCl_4L_2$, and the *free* ligand $(CH_3O)_3PO$.

The proton-decoupled ^{31}P spectrum of our sample (Fig. 1) consists of three singlets, which correspond to the phosphate resonances of the *trans*, *cis*, and *free* ligands, respectively. We may invert the *trans* resonance (source site A) selectively and apply a nonselective 90° monitoring pulse after a mixing time τ_m to excite transverse magnetization and observe the entire spectrum. In the difference spectrum obtained by subtracting the signals from an ordinary spectrum, the peak intensities decay asymptotically to zero for large τ_m. The sequence shown in Figure 2a allows the intensity of EXSY cross-peaks to be measured in a selective one-dimensional fashion. As shown in Figure 3a–c obtained with this pulse sequence, one can readily observe the migration of magnetization from the source site A (*trans*) to the target site X (*cis*), but also to the clandestine site K (*free*). In the proposed modification of this experiment (the QUIET-EXSY experiment shown in Fig. 2b), the longitudinal magnetization of the clandestine site K (*free*) is selectively inverted in the middle of the mixing time. In Figure 3d–f, this is indicated in shorthand notation by using an overbar (e.g., $\overline{free}$) to symbolize the change of sign of the longitudinal magnetization. The same result could be achieved by a doubly-selective inversion of both the source and the target site, that is, $\overline{trans}$ and $\overline{cis}$. This approach would also be effective if the resonance frequency of the clandestine *free* site were unknown, or indeed if there were an unknown number of invisible clandestine sites. In both cases, the transfer of magnetization from A (*trans*) to K (*free*) changes sign during the second half of τ_m, so that the net transfer is very small at the end of the mixing period. The magnetization thus behaves, to first order, as if there were no reaction from A (*trans*) to K (*free*), nor, for that matter, from K (*free*) to X (*cis*). The time-dependence of the signals reflects a simplified effective kinetic matrix, where only the interconversion of the A and X sites, that is, of the *trans* and *cis* isomers, seems to occur, as illustrated in Figure 3d–f. The remaining effective exchange matrix only has dimensions 2 × 2. One might say loosely that the conversion to and from the clandestine K site (i.e., to and from the *free* ligand) has been "decoupled" by selective inversion. A different effect, shown in Figure 3g–i, can be achieved if the roles of the target and clandestine sites are swapped, that is, if the target X is identified with the *free* site and the clandestine site K with the *cis* resonance. In this case, only the exchange between the *trans* and *free* sites should be observed, since the *cis* site should be "decoupled". In spite of the inversion of the *cis* resonance ($\overline{cis}$, Fig. 3g–i), or equivalently the simultaneous inversion of both the $\overline{trans}$ and $\overline{free}$, the conversion of the magnetization from *trans* to *cis* (Fig. 3h) is not completely suppressed because of second-order effects.

Fig. 1. Proton-decoupled ^{31}P NMR spectrum of a sample of *trans*-$ZrCl_4L_2$, *cis*-$ZrCl_4L_2$, and *free* ligand $(CH_3O)_3PO$ in $CDCl_3$.

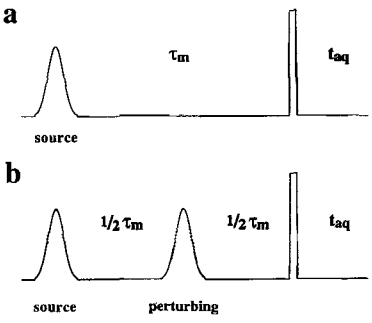
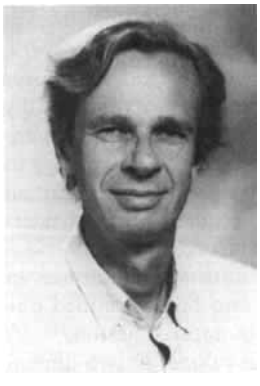


Fig. 2. Pulse sequences for two experiments: a) selective inversion experiment (selective EXSY) and b) quenching undesirable indirect external trouble in exchange spectroscopy (QUIET-EXSY).



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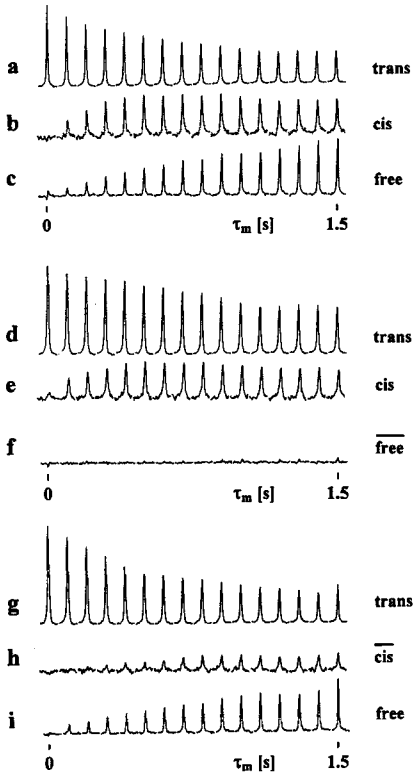

Fig. 3. Time-dependence of the ^{31}P resonances of the mixture of Scheme 1: a–c) difference spectra obtained with the sequence of Figure 2a by initially inverting the *trans* resonance (source site A); d–f) QUIET-EXSY experiments obtained with the pulse sequence of Figure 2b by applying an additional selective inversion pulse to the *free* resonance (clandestine site K, inversion symbolized by overbar) in the middle of the τ_m interval; and g–i) analogous QUIET-EXSY experiments, but swapping the roles of the sites K and X by using a selective inversion of the *cis* resonance ($\overline{cis}$, now clandestine site K) in the middle of the τ_m interval.

The isolation of a reaction pathway can be understood with the help of numerical simulations of the behavior of magnetization in the presence of chemical exchange as described by a set of coupled differential Equations (1),[26] where $\Delta\vec{M}(t)$ describes

$$\frac{d\Delta\vec{M}(\tau_m)}{dt} = \boldsymbol{L}\Delta\vec{M}(\tau_m) \quad (1)$$

the deviation of longitudinal magnetization from thermal equilibrium, and $\boldsymbol{L}$ (= $\boldsymbol{K}$–$\boldsymbol{R}$) is a matrix describing all incoherent processes taking place during the mixing time τ_m, which is given by the difference of the kinetic matrix $\boldsymbol{K}$ describing exchange and the relaxation matrix $\boldsymbol{R}$.[26] The set of differential equations has the formal solution shown in Equation (2), which allows

$$\Delta\vec{M}(\tau_m) = \exp\{\boldsymbol{L}\tau_m\}\Delta\vec{M}(0) \quad (2)$$

one to simulate the evolution of magnetization during the two halves of the mixing time of the QUIET-EXSY sequence of Figure 2b and *during* the selective pulses (Fig. 4). These simulations were carried out for a three-site system [Eq. (3)] with rates

$$\boldsymbol{L} = \begin{pmatrix} L_{AA} & L_{AK} & L_{AX} \\ L_{KA} & L_{KK} & L_{KX} \\ L_{XA} & L_{XK} & L_{XX} \end{pmatrix} = \begin{pmatrix} -R_1^A - k_{AA} & k_{AK} & k_{AX} \\ k_{KA} & -R_1^K - k_{KK} & k_{KX} \\ k_{XA} & k_{XK} & -R_1^X - k_{XX} \end{pmatrix} = \begin{pmatrix} -6.5 & 5.0 & 0.5 \\ 5.0 & -7.0 & 1.0 \\ 0.5 & 1.0 & -2.5 \end{pmatrix} \quad (3)$$

chosen for the sake of illustration; k_{ii} is given by Equation (4), where k_{ij}, also denoted $k_{i\leftarrow j}$, expresses the first-order rate constant of the reaction j → i. The relaxation matrix $\boldsymbol{R}$ was assumed to have $R_{ii} = 1\ s^{-1}$ and $R_{ij} = 0\ s^{-1}$. Figure 4 shows the time

$$k_{ii} = \sum_{j \neq i} k_{ji} \quad (4)$$

evolution of the longitudinal magnetization components in the course of three different experiments, namely, selective EXSY, QUIET-EXSY, and EXSY with continuous low-power irradiation of the clandestine site K throughout τ_m as proposed by Hoffman and Forsén.[13–15] These curves show that the selective EXSY experiment is strongly influenced by the clandestine site K. This makes the direct determination of the exchange rates difficult, since the indirect migration of magnetization through the perturbing site K is significant. On the other hand, the simulations of QUIET-EXSY including the effects of relaxation during the selective pulses (using 180° Gaussian pulses of 21 ms duration) are very close to the ideal case of complete saturation. For the magnetization of the target site X in the three-site system considered, low-power irradiation of the perturbing site K leads to identical results as perfect saturation, which was simulated by setting the longitudinal magnetization of site K to zero at all times. The oscillations apparent in Figure 4c indicate that the two experiments are, however, not completely equivalent. Moreover, from an experimental point of view, saturation is hard to achieve, it is difficult to assess its efficiency, and it is not easy to saturate perfectly n–2 sites simultaneously when n is larger than three. On the other hand, the selective inversions proposed in this work are straightforward to implement and allow a two-site subsystem to be isolated from an arbitrary n-site system.

Further manipulations might be needed if the exchange rates are fast on the timescale over which the mixing interval is sampled. In these cases, the "decoupling" effect achieved by a single

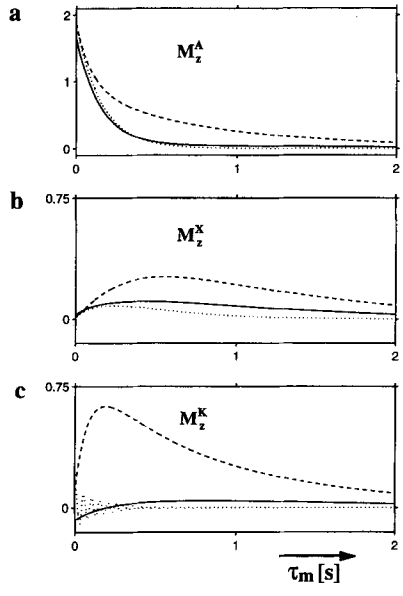

Fig. 4. Numerical simulations of the time-evolution of longitudinal magnetization components of a three-site system described by Equation (3) in the course of three different experiments: selective EXSY (- - -), QUIET-EXSY (—), and EXSY (····) with continuous saturation of the clandestine site K during τ_m. a) Decay of the magnetization of the source site A. b) Build-up curve for the target site X. c) Build-up curve for the clandestine site K. Note the transient oscillations for site K due to the nutation of the magnetization driven by the saturating field, which are damped by T_2^K assumed to be equal to k_{KK}.

inversion pulse can be insufficient, because it is only effective to first order, as shown for example in Figure 3h. In such cases, several consecutive inversion pulses can be used, for example at $^1/_4\tau_m$ and $^3/_4\tau_m$, or more generally at $[(2k + 1)/2n]\tau_m$ with k and n integer, in analogy to QUIET-NOESY and related experiments.[8, 19, 20] If the number of selective inversion pulses inserted in the mixing period increases, the net effect tends towards what can be obtained by continuous saturation. This is confirmed by the numerical simulations of Figure 5. The curves show that, with two inversions instead of one, the results are much closer to saturation.

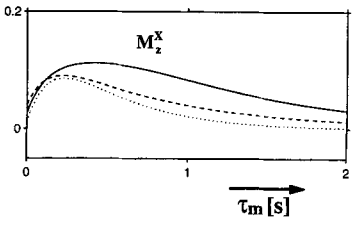

Fig. 5. Numerical simulation for the time evolution of the longitudinal magnetization component of the target site X in the three-site system described by Equation (3). The curves represent QUIET-EXSY (—) with a 21 ms 180° Gaussian inversion pulse at $^1/_2\tau_m$ as in Figure 4b, QUIET-EXSY with two 21 ms 180° Gaussian inversion pulses at $^1/_4\tau_m$ and $^3/_4\tau_m$ (- - -), and EXSY with continuous saturation of the clandestine site K throughout τ_m (····).

In the $ZrCl_4L_2$ system, further insight can be obtained by initially inverting the magnetization of various "source" spins other than those of the *trans* resonance. This would amount to comparing cross-sections taken from two-dimensional exchange matrices at different frequencies in the ω_1 domain. All curves shown in Figure 3 can be fitted together in order to retrieve the exchange rates, as shown in Figure 6.

These fits were obtained as described in the Experimental Section below. The pseudo first order rates determined at 250 K can be presented in the form of Equation (3) [Eq. (5)], where the errors have been estimated as discussed below.

$$L = \begin{pmatrix} L_{trans,\,trans} & L_{trans\leftarrow cis} & L_{trans\leftarrow free} \\ L_{cis\leftarrow trans} & L_{cis,\,cis} & L_{cis\leftarrow free} \\ L_{free\leftarrow trans} & L_{free\leftarrow cis} & L_{free,\,free} \end{pmatrix} = \begin{pmatrix} -1.47\pm0.02 & 3.32\pm0.20 & 0.138\pm0.006 \\ 0.71\pm0.20 & -3.63\pm0.15 & 0.018\pm0.005 \\ 0.431\pm0.006 & 0.270\pm0.005 & -0.220\pm0.006 \end{pmatrix} \quad (5)$$

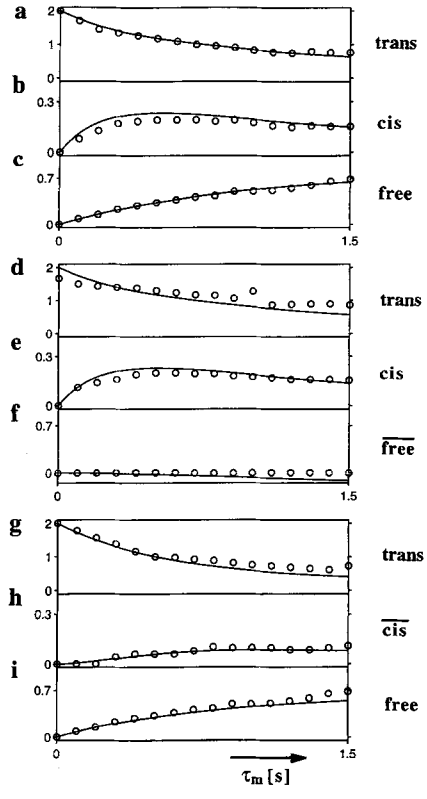

Fig. 6. Simulated time evolution of the three sites in $ZrCl_4L_2$ corresponding to the matrix of Equation (5). The experimental data points are represented by circles.

Discussion

Exchange matrices, in contrast to the relaxation matrices which describe cross-relaxation, are generally asymmetrical, as the equilibrium populations are unequal. Furthermore, the "kinetic window" of possible exchange rates is far wider than the typical range of cross-relaxation rates. One of the chief motivations in developing our technique was the wish to measure slow exchange rates that tend to be overshadowed by faster competing processes. Selective experiments should allow exchange rates to be determined with improved accuracy.

The idea of continuous saturation has been proposed in the original work of Hoffman and Forsén[13–15] and biophysical implications have been discussed by Spencer et al.[27] Proper saturation (as opposed to inhomogeneous scrambling of the magnetization) is, however, difficult to achieve, since it can only be obtained on a timescale on the order of T_2. Thus, the use of selective inversion pulses as in QUIET-EXSY allows faster exchange processes to be investigated, which are not be accessible to traditional methods involving saturation.

In systems with more than three sites, it is generally not possible to "decouple" all undesirable sites with a simple monochromatic inversion pulse, unless they happen to be degenerate. With QUIET-EXSY experiments, one has the option of simultaneously inverting all sites K, K', ... that should be excluded, or simultaneously inverting the two sites A and X that one wishes to investigate. For the rates of Equation (3), numerical simulations show that the build-up plots are virtually indistinguishable, even when exchange and relaxation during the pulses are taken into account. Doubly selective inversion can easily be achieved by using cosine-modulated pulses.[8, 28] The inversion of the two sites A and X makes it possible to suppress competing processes regardless of the number of clandestine sites. This stands in contrast to earlier methods for the suppression of unwanted processes.[13–17, 29]

Conclusions

The QUIET-EXSY method presented in this paper allows one to focus on a particular reaction pathway of interest, without needing to investigate the whole exchange network. This opens possibilities for the determination of exchange rates in systems where some of the perturbing sites may not be known. Because the new methods involve selective inversion pulses, they are more versatile than traditional saturation methods, and the outcome of the experiments is less prone to artefacts due to sample heating or incomplete saturation.

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Experimental Section

The sample was prepared as described by Merbach and co-workers [24,25] by mixing appropriate quantities of $ZrCl_4$ (0.12 mol) and trimethyl phosphate $(CH_3O)_3PO$ (0.54 mol) in $CDCl_3$ under a dry nitrogen atmosphere. The proton-decoupled ^{31}P NMR spectrum of the sample measured at 250 K, 121.5 MHz, and atmospheric pressure with a Bruker MSL 300 spectrometer (Fig. 1) consists of three singlets at relative offsets of 0, 83, and 791 Hz, which correspond to the phosphate groups of *trans*-$ZrCl_4L_2$, *cis*-$ZrCl_4L_2$, and the *free* ligand $(CH_3O)_3PO$, respectively, with relative integrals of 4.7: 1: 14.8.

The initial selective inversion (Fig. 2) was achieved by a Gaussian pulse truncated at 2.5% with a 180° nutation angle and 21 ms duration. The inversion profile of a Gaussian pulse is very sharp, but suitable for singlets. If multiplets with a finite width must be inverted, it is advisable to use shaped pulses with a "top hat" profile such as Q^3 or I-BURP-2 pulses [30,31]. However, for a given selectivity, more sophisticated shaped pulses tend to have longer durations than Gaussian pulses and are therefore less suitable for studying fast exchange processes. In this example, the 180° Gaussian pulse turned out to be useful to invert separately the *trans* and the *cis* sites which are only 83 Hz apart at a static field of 7 T. In QUIET-EXSY experiments (Fig. 2b), the longitudinal magnetization of the clandestine site K was selectively inverted in the middle of τ_m. For a three-site system, it is immaterial whether the selective inversion is applied to the clandestine site or simultaneously to the source and target sites. In the more general case of a system with n sites, it is preferable in practice to invert both the source and the target sites. This can be achieved by modulating the shape of the selective inversion, say a 180° Gaussian pulse; by a cosine function with a frequency equal to half the chemical shift difference, provided the carrier is set midway between the two shifts [28]. Although this approach requires the use of a different frequency modulation scheme for every pair of sites, it has the advantage that it does not presuppose knowledge of the chemical shifts of the clandestine sites. In Figure 2, the rectangular pulse represents a nonselective 90° pulse. Both sequences are combined with proton decoupling, and difference spectroscopy is applied by selectively inverting the source site A in odd transients only and subtracting signals obtained without initial selective inversion in even transients.

The spectra of Figure 3 were obtained with the pulse sequences of Figure 2 with an initial 180° Gaussian of 21 ms duration. The three resonances were observed after a nonselective 90° pulse of 22 μs. Only three narrow windows (20 Hz wide) are shown for each τ_m value. The mixing time τ_m was incremented from 0 to 1.5 s. For all three series of spectra, the *trans* resonance was used as a source site. In the QUIET-EXSY experiments (pulse sequence of Fig. 2b) of Figure 3d–i, an additional selective inversion pulse was applied to the clandestine site K, that is, at the *free* resonance in the middle of the τ_m interval (inversion symbolized by overbar on $\overline{free}$). The conversion of magnetization from the source site A to clandestine site K is completely inhibited, although the chemical reaction from A to X proceeds unhindered. For clarity, the vertical scales of the plots were amplified with ratios 1:5:2 in a–c and 1:3:1.2 in d–f and g–i.

The simulations of Figures 4–6 were obtained with routines written with the MATLAB program [32]. Numerical simulations in Figure 4 show the evolution with time of longitudinal magnetization components of a three-site system described by Equation (3) in the course of three different experiments: 1) selective EXSY with the pulse sequence of Figure 2a simulated with an initial 21 ms 180° Gaussian inversion pulse, which gives the same τ_m-dependence as a series of two-dimensional EXSY spectra; 2) QUIET-EXSY with the sequence of Figure 2b with an initial 21 ms 180° Gaussian inversion pulse and a second 21 ms 180° Gaussian inversion pulse at $^1/_2\tau_m$; and 3) EXSY with continuous saturation of the clandestine site K during τ_m. The simulations take into account relaxation and exchange effects during selective pulses. For Figures 4 and 5, the chemical shifts were assumed to be 300 Hz apart from each other, no scalar couplings were considered, and transverse relaxation and exchange occuring during the pulses were taken into account assuming $T_2^i = 1/k_{ii}$ defined in Equation (4).

The fittings of Figure 6 were obtained with a nonlinear SIMPLEX algorithm applied to Equation (2) in which the matrix exponentials were calculated via eigenvalues and eigenvectors. The matrix $\boldsymbol{R}$, or a submatrix thereof, was fitted to the experimental data. Independent fits were obtained for the same experimental data by using the SIMPLEX algorithm from the MINUIT package [33], yielding nearly identical results, but with the error estimates given in Equation (4).

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