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Effects of *J* couplings and unobservable minor states on kinetics parameters extracted from CEST data

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ABSTRACT

Chemical exchange saturation transfer (CEST) experiments have emerged as a powerful tool for characterizing dynamics and sparse populated conformers of protein in slow exchanging systems. We show that *J* couplings and 'invisible' minor states can cause systematic errors in kinetics parameters and chemical shifts extracted from CEST data. For weakly coupled spin systems, the *J* coupling effect can be removed using an approximation method. This method is warranted through detailed theoretical derivation, supported by results from simulations and experiments on an acyl carrier protein domain. Simulations demonstrate that the effect of 'invisible' minor states on the extracted kinetics parameters depends on the chemical shifts, populations, exchange rates of the 'invisible' states to the observed major or minor state and exchange models. Moreover, the extracted chemical shifts of the observed minor state can also be influenced by the "invisible" minor states. The presence of an off-pathway folding intermediate in the acyl carrier protein domain explains why the exchange rates obtained with a two-state model from individual residues that displayed only two obvious CEST dips varied significantly and the extracted exchange rates for ^{15}N and ^{13}CO spins located in the same peptide bond could be very different. The approximation method described here simplifies CEST data analysis in many situations where the coupling effect cannot be ignored and decoupling techniques are not desirable. In addition, this study also raises alerts for 'invisible' minor states which can cause errors in not only kinetics parameters but also chemical shifts of the observed minor state.

1. Introduction

Protein dynamics has been an increasingly interesting area with growing awareness that protein stays in more than one conformations [1–9]. Large biomolecules such as protein which usually present in multiple forms are themselves exchanging systems, in which there exist dynamic equilibriums between differently populated forms of the molecules. Traditional biophysical methods have been often successful in the study of dominant (most populated) structures of large biomolecules, however, are limited in quantitatively studying kinetics and thermodynamics of the exchange processes, where exist sparsely populated and transiently formed conformations that are often invisible in most imaging techniques. The situation has been improving over the past half century with the development of new biophysical methods, including new nuclear magnetic resonance (NMR) techniques. Experimental methods for quantifying chemical exchange by NMR mainly includes $R_{1\rho}$ relaxation dispersion [8,10], Carr Purcell Meiboom Gill (CPMG) relaxation dispersion [6,7,11], longitudinal magnetization exchange, and line shape analysis [12,13]. CPMG relaxation dispersion experiments, developed in the past half of a century, are a powerful technique for intermediate exchange processes (approximately 200–2000 s^{-1}) [6]. Just recently, researchers have demonstrated the extension of the CPMG-based method to the study of fast exchange processes (as high as 5000–6000 s^{-1}) [7]. For slow exchange processes (less than 200 s^{-1}) where CPMG methods become impotent to extract the chemical shifts of transient and small populated conformations, the chemical exchange saturation transfer (CEST) NMR technique [4] now emerges as a powerful tool to fulfill such a gap.

To determine kinetic parameters of exchange processes, it is indispensable to fit experimental data to an exchange model based on a set of n coupled first order linear differential equations (i.e., Bloch–McConnell equations). In the analysis of CEST data, the number of coupled homogenous differential equations (n) is equal to $2 \times 4^m - 1$, where m is the number of nuclear spins that are weakly coupled in an m-spin system [14,15]. For a ^{15}N-labeled sample each ^{15}N spin can be treated as a 1-spin system under ^{1}H-decoupling, while for a uniformly ^{13}C, ^{15}N-labeled sample each

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^{15}N spin is coupled to three ^{13}C spins and has to be treated as a 4-spin system in the ^{15}N CEST data analysis. Extraction of kinetics parameters from the CEST data is often time-consuming. For example, it can take about four days of computation time on a personal computer to obtain global kinetics parameters and their errors (obtained by 100 repetitions) by fitting ^{15}N CEST data from 29 residues recorded on a ^{15}N-labeled sample to a two-state exchange model. Note that n = 7, 31, 127 and 511 for 1-, 2-, 3- and 4-spin systems, respectively. Therefore, it seems formidably difficult to analyze the CEST data for spins in 3- and 4-spin systems though it is not impossible without any simplification. Recently, it has been proposed to treat each line of a multiplet that arises from scalar couplings as an isolated 1-spin system [16,17]. This approximation method simplifies data analysis, but the goodness of the method has not been fully explored through simulation.

Most of protein kinetics studies to date were conducted based on the assumption of a two-state exchange model, although protein dynamics in most cases are expected, empirically speaking, to be more complex. The two-state model has been the most commonly used mainly because of its simplicity and the belief that in some cases, a multi-state exchange can be simplified as a two-state process with an ‘effective’ exchange rate and population. But multi-state dynamics study has not been an untouched region. For instance, CPMG studies on mutants of Fyn SH3 domains have detected low-populated folding intermediates together with unfolded and fully folded states [18], our lab has recently showed direct evidence of a third state undergoing slow exchange with a major state or minor state using CEST experiments on an acyl carrier protein domain of CalE8 (meACP) [5]. Multi-state exchanges, however, are challenging to be accurately characterized. This is true even for slow chemical exchanges monitored by CEST experiments which are potent for characterizing such processes. Thus the two-state model is still preferable when no direct evidence of a third state appears in experiments. However, previous studies have indicated that using a two-state model to model a multi-state exchange process may cause deviation in extracted chemical shifts of the minor state [16], populations, exchange rates and other parameters [19]. Such effects on the extracted kinetics have not been well noted and evaluated.

Here we provide the theoretical basis for the approximation method used to analyze CEST data of spins in a weakly coupled multiple-spin system. The goodness of the method was evaluated by comparing the results obtained computationally on a 2-spin system with and without ‘J coupling consideration’. The approximation method can be extended to any weakly coupled multiple-spin systems. We also applied the method to analyze the experimental ^{15}N and ^{13}CO CEST data recorded on a ^{13}C, ^{15}N-labeled meACP sample. We simulated how the existence of an ‘invisible’ second minor state affects extracted kinetic parameters with a two-state model and evaluated reliability of the extracted results of meACP.

2. Theoretical basis

For a single nuclear spin system, the time evolution of density operators is described by [20]:

$$\frac{\mathrm{d}}{\mathrm{dt}}\begin{bmatrix} E/2 \\ I^+ \\ I^- \\ I_z \end{bmatrix} = -\begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & R_2 - i\Omega & 0 & i\omega_1^+ \\ 0 & 0 & R_2 + i\Omega & -i\omega_1^- \\ -2R_1M_{eq} & i\omega_1^-/2 & -i\omega_1^+/2 & R_1 \end{bmatrix}\begin{bmatrix} E/2 \\ I^+ \\ I^- \\ I_z \end{bmatrix} \tag{1}$$

where E is the unit operator, $I^+ = I_x + iI_y$ and $I^- = I_x - iI_y$, I_x, I_y and I_z are the x, y, and z components of angular momentum operator I, R_1 and R_2 are the longitudinal and transverse relaxation rates, M_{eq} is the equilibrium magnetization, Ω is the difference of the resonant frequency of spin I and the frequency of the radiofrequency (RF) field applied to the system and Ω is called offset hereafter, $\omega_1^+ = \gamma B_1 e^{+i\phi}, \omega_1^- = \gamma B_1 e^{-i\phi}$, B_1 and ϕ are the strength and phase of the applied RF field, respectively, and γ is the gyromagnetic ratio of the spin. In the CEST experiment [4] or R_1 measurement [21], one often aligns alternatively the magnetization along the +z axis and $-z$ axis just before the CEST period or relaxation delay (or at time zero) and inverts the receiver phase every the other scan. In this way, the observed values for $[E/2\, I^+ I^- I_z]$ are $[0\,0\,0\,c]$ at time zero, where c is a constant. When $E(0) = 0$, Eq. (1) is reduced into

$$\frac{\mathrm{d}}{\mathrm{dt}}\begin{bmatrix} I^+ \\ I^- \\ I_z \end{bmatrix} = -\begin{bmatrix} R_2 - i\Omega & 0 & i\omega_1^+ \\ 0 & R_2 + i\Omega & -i\omega_1^- \\ i\omega_1^-/2 & -i\omega_1^+/2 & R_1 \end{bmatrix}\begin{bmatrix} I^+ \\ I^- \\ I_z \end{bmatrix} \tag{2}$$

For a weakly coupled two-spin system (I and S), a complete base set can be formed by the following 16 operators: $\left[\frac{E}{2}\ I^+S^\alpha\ I^-S^\alpha\ I_zS^\alpha\ I^+S^\beta\ I^-S^\beta\ I_zS^\beta\ I^\alpha S^+\ I^\alpha S^-\ I^\beta S^+\ I^\beta S^-\ S_z\ I^+S^-\ I^-S^+\ I^+S^+\ I^-S^-\right]$, where $I^\alpha = E/2 + I_z$, $I^\beta = E/2 - I_z$, $S^\alpha = E/2 + S_z$ and $S^\beta = E/2 - S_z$. Due to the J coupling between spins I and S, spin I gives rise to two lines (peaks). The magnetizations for the down-field line correspond to $[I^+S^\alpha\ I^-S^\alpha\ I_z\ S^\alpha]$, while those for the up-field line corresponds to $[I^+S^\beta\ I^-S^\beta\ I_zS^\beta]$. Under the initial condition of $E(0) = 0$, the evolution of the operators is given by

$$\frac{\mathrm{d}}{\mathrm{dt}}\boldsymbol{M} = -\boldsymbol{R}\cdot\boldsymbol{M} \tag{3}$$

where $\boldsymbol{M}$ is a 15 × 1 column vector,

$$\boldsymbol{M} = [I^+S^\alpha\ I^-S^\alpha\ I_zS^\alpha\ I^+S^\beta\ I^-S^\beta\ I_zS^\beta\ I^\alpha S^+\ I^\alpha S^-\ I^\beta S^+\ I^\beta S^-\ S_z\ I^+S^-\ I^-S^+\ I^+S^+\ I^-S^-]^t, \tag{4}$$

“t” is the transpose operator, $\boldsymbol{R}$ is a matrix derived from the matrix [14,15,22] based on Cartesian spin operators.

$$\boldsymbol{R} = \begin{bmatrix}
D_1 & 0 & i\omega_{1I}^+ & -\frac{R_{1S}}{2} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -\frac{i\omega_{1S}^+}{2} & 0 & \frac{i\omega_{1S}^-}{2} & 0 \\
0 & D_2 & -i\omega_{1I}^- & 0 & -\frac{R_{1S}}{2} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \frac{i\omega_{1S}^-}{2} & 0 & -\frac{i\omega_{1S}^+}{2} \\
\frac{i\omega_{1I}^-}{2} & -\frac{i\omega_{1I}^+}{2} & D_3 & 0 & 0 & -\frac{R_{1S}}{2} & \frac{i\omega_{1S}^-}{4} & -\frac{i\omega_{1S}^+}{4} & -\frac{i\omega_{1S}^-}{4} & \frac{i\omega_{1S}^+}{4} & \frac{(\sigma+\delta_S)}{2} & 0 & 0 & 0 & 0 \\
-\frac{R_{1S}}{2} & 0 & 0 & D_4 & 0 & i\omega_{1I}^+ & 0 & 0 & 0 & 0 & 0 & \frac{i\omega_{1S}^+}{2} & 0 & -\frac{i\omega_{1S}^-}{2} & 0 \\
0 & -\frac{R_{1S}}{2} & 0 & 0 & D_5 & -i\omega_{1I}^- & 0 & 0 & 0 & 0 & 0 & 0 & -\frac{i\omega_{1S}^-}{2} & 0 & \frac{i\omega_{1S}^+}{2} \\
0 & 0 & -\frac{R_{1S}}{2} & \frac{i\omega_{1I}^-}{2} & -\frac{i\omega_{1I}^+}{2} & D_6 & -\frac{i\omega_{1S}^-}{4} & \frac{i\omega_{1S}^+}{4} & \frac{i\omega_{1S}^-}{4} & -\frac{i\omega_{1S}^+}{4} & \frac{(\sigma-\delta_S)}{2} & 0 & 0 & 0 & 0 \\
0 & 0 & \frac{i\omega_{1S}^+}{2} & 0 & 0 & -\frac{i\omega_{1S}^+}{2} & D_7 & 0 & -\frac{R_{1I}}{2} & 0 & \frac{i\omega_{1S}^+}{2} & 0 & -\frac{i\omega_{1I}^+}{2} & \frac{i\omega_{1I}^-}{2} & 0 \\
0 & 0 & -\frac{i\omega_{1S}^-}{2} & 0 & 0 & \frac{i\omega_{1S}^-}{2} & 0 & D_8 & 0 & -\frac{R_{1I}}{2} & -\frac{i\omega_{1S}^-}{2} & \frac{i\omega_{1I}^-}{2} & 0 & 0 & -\frac{i\omega_{1I}^+}{2} \\
0 & 0 & -\frac{i\omega_{1S}^+}{2} & 0 & 0 & \frac{i\omega_{1S}^+}{2} & -\frac{R_{1I}}{2} & 0 & D_9 & 0 & \frac{i\omega_{1S}^+}{2} & 0 & \frac{i\omega_{1I}^+}{2} & -\frac{i\omega_{1I}^-}{2} & 0 \\
0 & 0 & \frac{i\omega_{1S}^-}{2} & 0 & 0 & -\frac{i\omega_{1S}^-}{2} & 0 & -\frac{R_{1I}}{2} & 0 & D_{10} & -\frac{i\omega_{1S}^-}{2} & -\frac{i\omega_{1I}^-}{2} & 0 & 0 & \frac{i\omega_{1I}^+}{2} \\
0 & 0 & \sigma + \delta_S & 0 & 0 & \sigma - \delta_S & \frac{i\omega_{1S}^-}{2} & -\frac{i\omega_{1S}^+}{2} & \frac{i\omega_{1S}^-}{2} & -\frac{i\omega_{1S}^+}{2} & D_{11} & 0 & 0 & 0 & 0 \\
-\frac{i\omega_{1S}^-}{2} & 0 & 0 & \frac{i\omega_{1S}^-}{2} & 0 & 0 & 0 & \frac{i\omega_{1I}^+}{2} & 0 & -\frac{i\omega_{1I}^+}{2} & 0 & D_{12} & 0 & 0 & 0 \\
0 & \frac{i\omega_{1S}^+}{2} & 0 & 0 & -\frac{i\omega_{1S}^+}{2} & 0 & -\frac{i\omega_{1I}^-}{2} & 0 & \frac{i\omega_{1I}^-}{2} & 0 & 0 & 0 & D_{13} & 0 & 0 \\
\frac{i\omega_{1S}^+}{2} & 0 & 0 & -\frac{i\omega_{1S}^+}{2} & 0 & 0 & \frac{i\omega_{1I}^+}{2} & 0 & -\frac{i\omega_{1I}^+}{2} & 0 & 0 & 0 & 0 & D_{14} & 0 \\
0 & -\frac{i\omega_{1S}^-}{2} & 0 & 0 & \frac{i\omega_{1S}^-}{2} & 0 & 0 & -\frac{i\omega_{1I}^-}{2} & 0 & \frac{i\omega_{1I}^-}{2} & 0 & 0 & 0 & 0 & D_{15}
\end{bmatrix} \tag{5}$$

In Eq. (5), $\omega_{1I}(\omega_{1S})$ is the RF field (in rad/s) applied to spin I (S); R_{1I} (R_{1S}) is the longitudinal relaxation rate of spin I (S); σ is the cross-relaxation rate between I_z and S_z; δ_s and η_s are the longitudinal and transverse cross-correlated relaxation rates attributed to the interaction between dipole I–S and chemical shift anisotropy (CSA) of spin S. Under the following approximations:

$$\begin{aligned} R_I^a &\approx R_{2I} + R_{1S} \\ R_S^a &\approx R_{2S} + R_{1I} \\ R_{IS} &\approx R_{1I} + R_{1S} \end{aligned} \tag{6}$$

where R_I^a (R_S^a) is the relaxation rate for I^+S_z (I_zS^+) or I^-S_z (I_zS^-), R_{2I} (R_{2S}) is the transverse relaxation of spin I (S), R_{IS} is the relaxation rate for I_zS_z, and the diagonal elements are given by