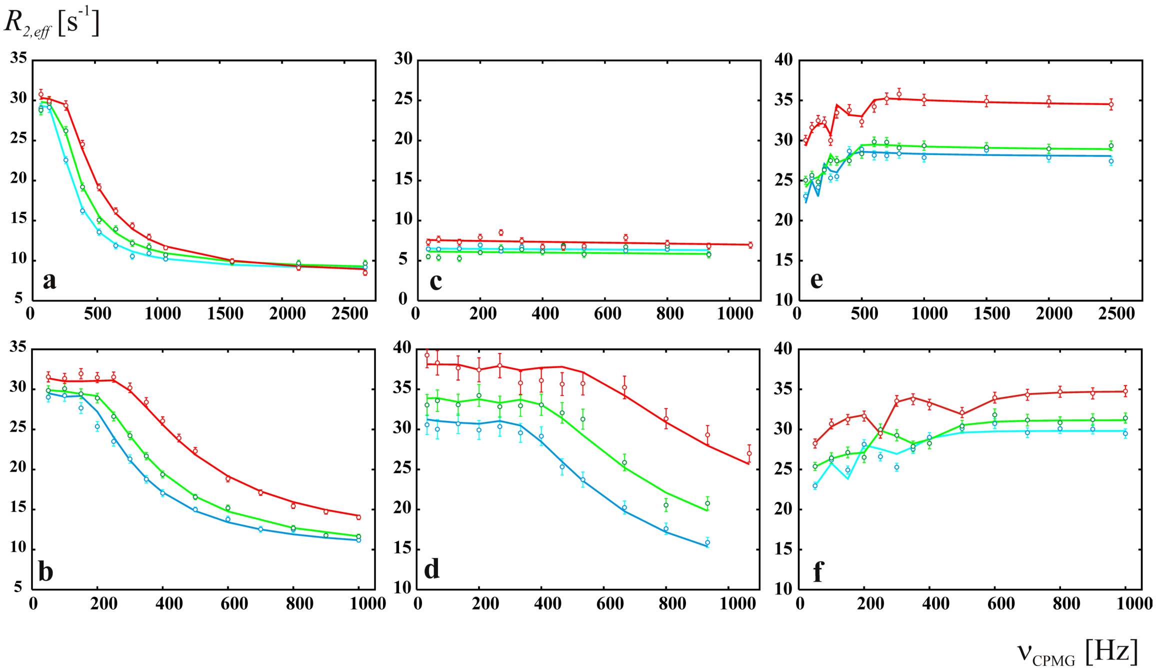
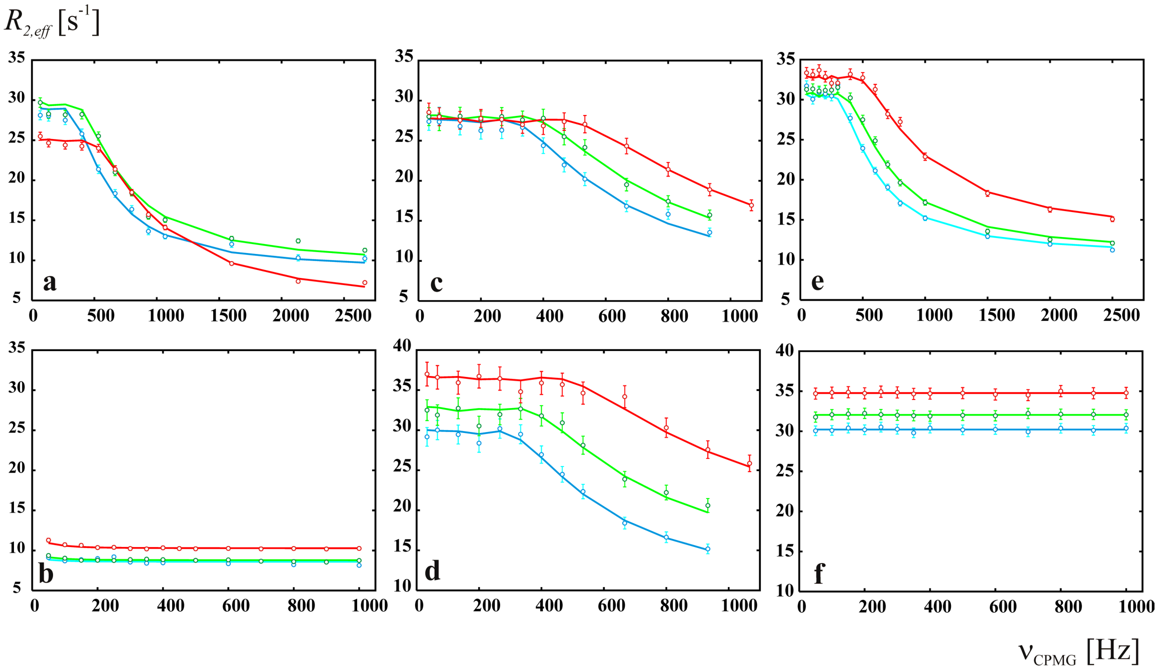
Supporting Information.

Below are three figures (as described in the legend to Figure 1) showing the six dispersion profiles for Glu11, Lys22 and Val 58.

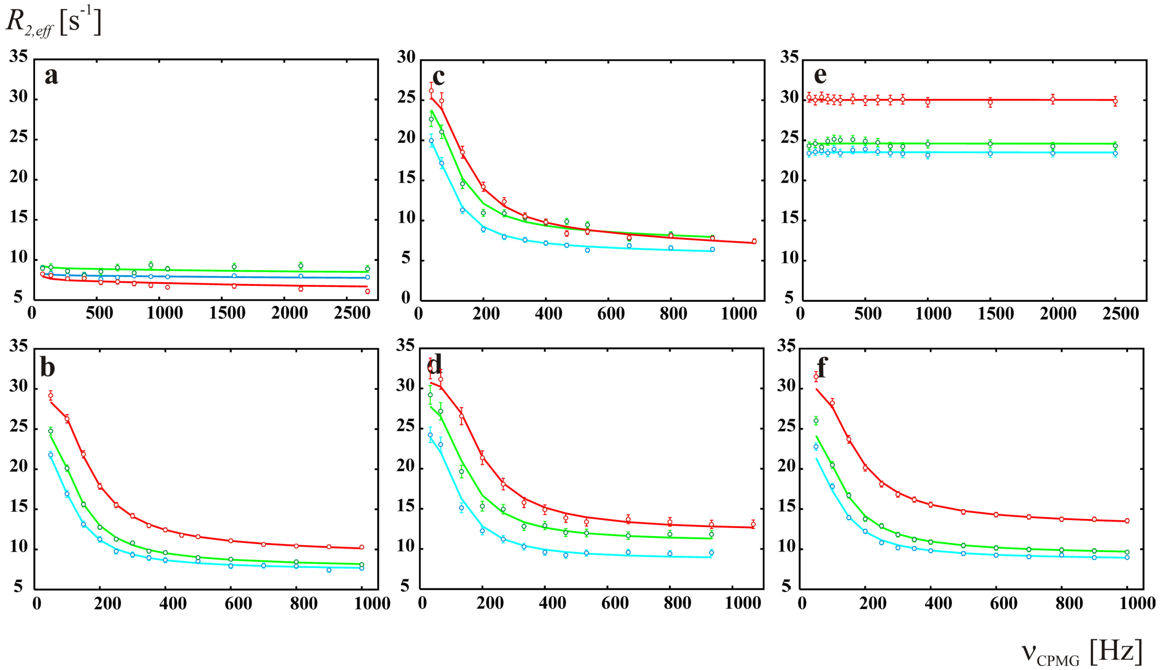
E 11



K 22



V 58



$R_{2,eff}$ [s^{-1}]
a
b
c
d
e
f
35
30
25
20
15
10
5
0
0
500
1000
1500
2000
2500
200
400
600
800
1000
ν_{CPMG} [Hz]

Theoretical Framework for 2- and 3-site chemical exchange.

Below we present the equations necessary to calculate dispersion profiles generated from the 6 experiments described in the text. Although much of the material can be found in several previous papers by our laboratory in connection with 2-site chemical exchange[1-3], what follows below is a consolidation of this work as well as a generalization to the case of 3-site exchange.

Theoretical $R_{2,eff}$ rates for ^{1}H SQ, ^{15}N SQ, ^{1}H-^{15}N DQ, ^{1}H-^{15}N ZQ, ^{1}H MQ and ^{15}N MQ experiments in the case of (i) 2-site exchange between folded state F and low populated unfolded state U ($F \leftrightarrow U$) and (ii) 3-site exchange between states F, I (intermediate) and U ($F \leftrightarrow I \leftrightarrow U$) are calculated as:

$$R_{2,eff} = -\frac{1}{T}\ln\frac{M_F(T)}{M_F(0)} \qquad \text{(S1)}$$

where $M_F(0)$ is the initial magnetization of state F and $M_F(T)$ is magnetization of state F after relaxation period T. In the case of 2-site exchange $M_F(t)$ is a component of the vector $\mathbf{M}(t) = (M_F(t), M_U(t))^T$, while for a 3-site process $M_F(t)$ is an element of $\mathbf{M}(t) = (M_F(t), M_I(t), M_U(t))^T$. Magnetization $M_\pm$ ($M = M_+ + M_-$) that evolves during the period T is of the form $H_\pm$ in ^{1}H SQ, $N_\pm$ in ^{15}N SQ, $H_\pm N_\pm$ in ^{1}H-^{15}N DQ, $H_\mp N_\pm$ in ^{1}H-^{15}N ZQ and $H_+N_+ + H_-N_- + H_-N_+ + H_+N_-$ in ^{1}H MQ and ^{15}N MQ experiments.

The evolution of magnetization in any of the ^{1}H SQ, ^{15}N SQ, ^{1}H-^{15}N DQ and ^{1}H-^{15}N ZQ dispersion experiments is described by a similar set of equations, S2-S4. The application of a $(\delta\text{-}180^\circ\text{-}\delta)_{2n}$ sequence of length $T = 4n\delta$, comprised of ^{1}H 180° refocusing pulses (^{1}H SQ), ^{15}N 180° pulses (^{15}N SQ) or simultaneous ^{1}H and ^{15}N 180° pulses (^{1}H-^{15}N DQ, ^{1}H-^{15}N ZQ experiments), produces $\mathbf{M}(T)$ given by:

$$\mathbf{M}_\pm(T) = \left(\mathbf{A}_\pm\mathbf{A}_\mp\mathbf{A}_\mp\mathbf{A}_\pm\right)^n \mathbf{M}_\pm(0)\,, \qquad \text{(S2)}$$

where $\mathbf{M}(0)$ is proportional to $(p_F, p_U)^T$ or $(p_F, p_I, p_U)^T$ in the case of 2- or 3-site exchange, respectively, and p_F, p_I and p_U are populations of states F, I and U. In the case of 2-site exchange the evolution matrix $\mathbf{A}$ is given by:

$$\mathbf{A}_{\pm} = \exp\left(\begin{bmatrix} -R_{2F}-k_{FU} & k_{UF} \\ k_{FU} & -R_{2U}-k_{UF}\pm i\Delta\omega_{FU} \end{bmatrix}\cdot\delta\right), \tag{S3}$$

where $i=\sqrt{-1}$, $\Delta\omega_{kl}$ is the frequency difference between states k and l (radians/s) given by $\Delta\omega_{kl} = \Delta\omega_{H,kl} = \omega_H\Delta\varpi_{H,kl}$ (^{1}H SQ), $\Delta\omega_{kl} = \Delta\omega_{N,,kl} = \omega_N\Delta\varpi_{N,kl}$ (^{15}N SQ), $\Delta\omega_{kl} = \Delta\omega_{H,kl}+\Delta\omega_{N,kl} = \omega_H\Delta\varpi_{H,kl}+\omega_N\Delta\varpi_{N,kl}$ (^{1}H-^{15}N DQ) or $\Delta\omega_{kl} = \Delta\omega_{H,kl}-\Delta\omega_{N,kl} = \omega_H\Delta\varpi_{H,kl}-\omega_N\Delta\varpi_{N,kl}$ (^{1}H-^{15}N ZQ experiments), $\Delta\varpi_{H,kl}$ and $\Delta\varpi_{N,kl}$ are ^{1}H and ^{15}N chemical shift differences (ppm) between states k and l, ω_H and ω_N are Larmour frequencies of ^{1}H and ^{15}N nuclei ($\omega=-\gamma B_o$), k_{kl} is the rate constant for the transition from state k to l, and $R_{2,k}$ is the (effective) intrinsic transverse relaxation rate in state k for the coherence of interest (here we assume that $R_{2,k}$ are the same for all exchanging states). In the case of 3-site exchange the evolution matrix **A** is given by:

$$\mathbf{A}_{\pm} = \exp\left(\begin{bmatrix} -R_{2F}-k_{FI} & k_{IF} & 0 \\ k_{FI} & -R_{2I}-k_{IF}-k_{IU}\pm i\Delta\omega_{FI} & k_{UI} \\ 0 & k_{IU} & -R_{2U}-k_{UI}\pm i\Delta\omega_{FU} \end{bmatrix}\cdot\delta\right). \tag{S4}$$

Note that direct transitions between states F and U are not allowed in the present model of 3-site exchange ($F\leftrightarrow I\leftrightarrow U$) (*i.e.*, $k_{FU}=k_{UF}=0$). For minimization purposes we recast evolution matrices **A** in terms of the exchange rate constants $k_{ex,kl} = k_{kl}+k_{lk}$ and populations of the exchanging states p_k ($\sum_k p_k = 1$), related to the rate constants k_{kl} through the conditions of microscopic reversibility (*i.e.*, detailed balance) $p_k k_{kl} = p_l k_{lk}$.

The theoretical description of ^{1}H and ^{15}N MQ experiments is slightly different from that of the corresponding ^{1}H SQ, ^{15}N SQ, ^{1}H-^{15}N DQ and ^{1}H-^{15}N ZQ schemes. Here the magnetization of interest at the beginning of the relaxation period T is given by a 1:1 combination of double- and zero-quantum components: $\mathbf{M}_+(0)+\mathbf{M}_-(0) = \mathbf{DQ}_+(0)+\mathbf{ZQ}_+(0)+\mathbf{DQ}_-(0)+\mathbf{ZQ}_-(0)$, where $\mathbf{DQ}_\pm(0)$ and $\mathbf{ZQ}_\pm(0)$ correspond to $H_\pm N_\pm$ and $H_{\mathrm{m}}N_\pm$ operators, respectively ($\mathbf{M}(0)$ is proportional to $(p_F, p_U)^T$ and $(p_F, p_I, p_U)^T$ for 2- and 3-site exchange, as before). Let us focus on the ^{15}N MQ experiment, recognizing that the equations pertaining to the ^{1}H MQ sequence can be simply obtained by switching the indices H and N). Application of the sequence, $(\delta\text{-}180_N\text{-}\delta)_n\text{-}180_H\text{-}(\delta\text{-}180_N\text{-}\delta)_n$, with length $T = 4n\delta$, produces $\mathbf{M}(T) = (\mathbf{DQ}(T)+\mathbf{ZQ}(T))/2$ where:

$$\mathbf{DQ}_{\pm}(T) = \mathbf{A}_{\pm}\mathbf{B}_{\pm}\mathbf{ZQ}_{\pm}(0)\,, \tag{S5}$$

$$\mathbf{ZQ}_{\pm}(T) = \mathbf{B}_{\pm}\mathbf{A}_{\pm}\mathbf{DQ}_{\pm}(0)\,,$$

$\mathbf{A}_{\pm} = (\mathbf{D}_{\pm}\mathbf{Z}_{\mp}\mathbf{Z}_{\mp}\mathbf{D}_{\pm})^{n/2}$ and $\mathbf{B}_{\pm} = (\mathbf{Z}_{\pm}\mathbf{D}_{\mp}\mathbf{D}_{\mp}\mathbf{Z}_{\pm})^{n/2}$. In the case of 2-site exchange, the matrices for $\mathbf{D}_{\pm}$ and $\mathbf{Z}_{\pm}$ are given by:

$$\mathbf{D}_{\pm} = \exp\left(\begin{bmatrix} -R_{2F} - k_{FU} & k_{UF} \\ k_{FU} & \pm i\Delta\omega_{H,FU} \pm i\Delta\omega_{N,FU} - R_{2U} - k_{UF} \end{bmatrix} \cdot \delta\right) \tag{S6}$$

$$\mathbf{Z}_{\pm} = \exp\left(\begin{bmatrix} -R_{2F} - k_{FU} & k_{UF} \\ k_{FU} & \mp i\Delta\omega_{H,FU} \pm i\Delta\omega_{N,FU} - R_{2U} - k_{UF} \end{bmatrix} \cdot \delta\right).$$

While for 3-site exchange they become:

$$\mathbf{D}_{\pm} = \exp\left(\begin{bmatrix} -R_{2F} - k_{FI} & k_{IF} & 0 \\ k_{FI} & \pm i\Delta\omega_{H,FI} \pm i\Delta\omega_{N,FI} - R_{2I} - k_{IF} - k_{IU} & k_{UI} \\ 0 & k_{IU} & \pm i\Delta\omega_{H,FU} \pm i\Delta\omega_{N,FU} - R_{2U} - k_{UI} \end{bmatrix} \cdot \delta\right) \tag{S7}$$

$$\mathbf{Z}_{\pm} = \exp\left(\begin{bmatrix} -R_{2F} - k_{FI} & k_{IF} & 0 \\ k_{FI} & \mp i\Delta\omega_{H,FI} \pm i\Delta\omega_{N,FI} - R_{2I} - k_{IF} - k_{IU} & k_{UI} \\ 0 & k_{IU} & \mp i\Delta\omega_{H,FU} \pm i\Delta\omega_{N,FU} - R_{2U} - k_{UI} \end{bmatrix} \cdot \delta\right)$$

Equations S1-S7 allow the calculation of the theoretical $R_{2,eff}$ values for the 6 dispersion experiments presented in the paper as a function of the pulse repetition frequency in CPMG-type refocusing sequences, $\nu_{CPMG} = 1/(4\delta)$, and the parameters of the 2- and 3-site exchange models.

References

(1) Korzhnev, D. M.; Kloiber, K.; Kay, L. E. *J Am Chem Soc* **2004**, *126*, 7320-7329.

(2) Korzhnev, D. M.; Kloiber, K.; Kanelis, V.; Tugarinov, V.; Kay, L. E. *J Am Chem Soc* **2004**, *126*, 3964-3973.

(3) Korzhnev, D. M.; Salvatella, X.; Vendruscolo, M.; Di Nardo, A. A.; Davidson, A. R.; Dobson, C. M.; Kay, L. E. *Nature* **2004**, *430*, 586-590.