Supporting Information

Probing Conformational Exchange in Weakly Interacting, Slowly Exchanging Protein Systems via Off-Resonance $R_{1\rho}$ Experiments: Application to Studies of Protein Phase Separation

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Materials and Methods

A Simple Set of Equations Describing $R_{1\rho}$ Relaxation for Two-Site Exchange. Prior to considering the effects of chemical exchange we first focus on the simple case of a single spin whose evolution is described by the Bloch equations [1]

$$\frac{d}{dt}\begin{bmatrix} M_x \\ M_y \\ M_z \end{bmatrix} = \begin{bmatrix} -R_2 & \delta & 0 \\ -\delta & -R_2 & \omega_1 \\ 0 & -\omega_1 & -R_1 \end{bmatrix} \begin{bmatrix} M_x \\ M_y \\ M_z \end{bmatrix} \tag{S1}$$

where the longitudinal and transverse relaxation rates are given by R_1 and R_2, respectively, and a B_1 field of strength ω_1 is applied at an offset of $-\delta(=\omega_{\rm RF}-\omega)$ from the resonance position of the spin. The equilibrium component of z-magnetization has been neglected in Equation S1; the phase cycles in the schemes of Figure 4 or Figure S3 eliminate contributions from it in any event. [2] We transform into a frame where the spin-lock axis makes an angle θ with respect to the z-axis and define this axis to be the z-axis of the new frame. Thus,

$$\hat{R}\begin{bmatrix} M_x \\ M_y \\ M_z \end{bmatrix} = \begin{bmatrix} \cos\theta & 0 & -\sin\theta \\ 0 & 1 & 0 \\ \sin\theta & 0 & \cos\theta \end{bmatrix} \begin{bmatrix} M_x \\ M_y \\ M_z \end{bmatrix} = \begin{bmatrix} \cos\theta M_x - \sin\theta M_z \\ M_y \\ \cos\theta M_z + \sin\theta M_x \end{bmatrix} \tag{S2}$$

where $\sin\theta = \dfrac{\omega_1}{\sqrt{\omega_1^2+\delta^2}}$, $\cos\theta = \dfrac{\delta}{\sqrt{\omega_1^2+\delta^2}}$ and $(\cos\theta M_x - \sin\theta M_z, M_y, \cos\theta M_z + \sin\theta M_x) = (M_{x'}, M_{y'}, M_{z'})$ are the (x,y,z) components of magnetization in the new, tilted frame. Equa-

tion S1 in the rotated basis becomes

$$\frac{d}{dt}\begin{bmatrix} M_{x'} \\ M_{y'} \\ M_{z'} \end{bmatrix} = \hat{R}\begin{bmatrix} -R_2 & \delta & 0 \\ -\delta & -R_2 & \omega_1 \\ 0 & -\omega_1 & -R_1 \end{bmatrix}\hat{R}^{-1}\begin{bmatrix} M_{x'} \\ M_{y'} \\ M_{z'} \end{bmatrix}$$

$$= \begin{bmatrix} -R_2\cos^2\theta - R_1\sin^2\theta & \delta\cos\theta + \omega_1\sin\theta & -(R_2-R_1)\sin\theta\cos\theta \\ -\delta\cos\theta - \omega_1\sin\theta & -R_2 & -\delta\sin\theta + \omega_1\cos\theta \\ -(R_2-R_1)\sin\theta\cos\theta & \delta\sin\theta - \omega_1\cos\theta & -R_2\sin^2\theta - R_1\cos^2\theta \end{bmatrix}\begin{bmatrix} M_{x'} \\ M_{y'} \\ M_{z'} \end{bmatrix}$$

$$= \begin{bmatrix} -R_2\cos^2\theta - R_1\sin^2\theta & \sqrt{\delta^2+\omega_1^2} & -(R_2-R_1)\sin\theta\cos\theta \\ -\sqrt{\delta^2+\omega_1^2} & -R_2 & 0 \\ -(R_2-R_1)\sin\theta\cos\theta & 0 & -R_2\sin^2\theta - R_1\cos^2\theta \end{bmatrix}\begin{bmatrix} M_{x'} \\ M_{y'} \\ M_{z'} \end{bmatrix} \tag{S3}$$

Equation S3 shows that the evolution of the aligned component of interest, $M_{z'}$, is coupled to one of the orthogonal components, $M_{x'}$. However, for large ω_1 the two orthogonal components $M_{x'}$ and $M_{y'}$ interconvert rapidly about the spin-lock axis via the 1,2 and 2,1 elements of the relaxation matrix above. The rapid time evolution of $M_{x'}$ effectively averages cross-relaxation between the $M_{z'}$ and $M_{x'}$ components to 0 so long as long as $\omega_1 \gg R_2$ (or more strictly $\omega_1 \gg |(R_2 - R_1)\sin\theta\cos\theta|$). Thus, cross-relaxation between the $M_{z'}$ and $M_{x'}$ components can be neglected (i.e., the off-diagonal elements $-(R_2 - R_1)\sin\theta\cos\theta$ can be ignored) so that the relaxation of $M_{z'}$ is single exponential with a rate of $R_2\sin^2\theta + R_1\cos^2\theta$. Rapid decay of the orthogonal components due to B_1 inhomogeneity also leads to the same result, although it is of interest that even in the limit of a perfectly homogeneous spin-lock field the magnetization components can become uncoupled.

Next we consider two-site chemical exchange starting from the Bloch–McConnell

equations in the laboratory frame,[3]

$$\frac{d}{dt}\begin{bmatrix} M_x^{\mathrm{G}} \\ M_y^{\mathrm{G}} \\ M_z^{\mathrm{G}} \\ M_x^{\mathrm{E}} \\ M_y^{\mathrm{E}} \\ M_z^{\mathrm{E}} \end{bmatrix} = \begin{bmatrix} -R_{2,\mathrm{G}} - p_{\mathrm{E}}k_{\mathrm{ex}} & \delta_{\mathrm{G}} & 0 & (1-p_{\mathrm{E}})k_{\mathrm{ex}} & 0 & 0 \\ -\delta_{\mathrm{G}} & -R_{2,\mathrm{G}} - p_{\mathrm{E}}k_{\mathrm{ex}} & \omega_1 & 0 & (1-p_{\mathrm{E}})k_{\mathrm{ex}} & 0 \\ 0 & -\omega_1 & -R_1 - p_{\mathrm{E}}k_{\mathrm{ex}} & 0 & 0 & (1-p_{\mathrm{E}})k_{\mathrm{ex}} \\ p_{\mathrm{E}}k_{\mathrm{ex}} & 0 & 0 & -R_{2,\mathrm{E}} - (1-p_{\mathrm{E}})k_{\mathrm{ex}} & \delta_{\mathrm{E}} & 0 \\ 0 & p_{\mathrm{E}}k_{\mathrm{ex}} & 0 & -\delta_{\mathrm{E}} & -R_{2,\mathrm{E}} - (1-p_{\mathrm{E}})k_{\mathrm{ex}} & \omega_1 \\ 0 & 0 & p_{\mathrm{E}}k_{\mathrm{ex}} & 0 & -\omega_1 & -R_1 - (1-p_{\mathrm{E}})k_{\mathrm{ex}} \end{bmatrix} \begin{bmatrix} M_x^{\mathrm{G}} \\ M_y^{\mathrm{G}} \\ M_z^{\mathrm{G}} \\ M_x^{\mathrm{E}} \\ M_y^{\mathrm{E}} \\ M_z^{\mathrm{E}} \end{bmatrix} \tag{S4}$$

where $\delta_i = \omega_i - \omega_{\mathrm{RF}}, i \in \{\mathrm{G},\mathrm{E}\}$. Noting that in the $R_{1\rho}$ experiment magnetization from both ground (G) and excited (E) states are rotated independently onto their effective spin-lock axes, we obtain,

$$\hat{R}\begin{bmatrix} M_x^{\mathrm{G}} \\ M_y^{\mathrm{G}} \\ M_z^{\mathrm{G}} \\ M_x^{\mathrm{E}} \\ M_y^{\mathrm{E}} \\ M_z^{\mathrm{E}} \end{bmatrix} = \begin{bmatrix} \cos\theta_{\mathrm{G}} & 0 & -\sin\theta_{\mathrm{G}} & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ \sin\theta_{\mathrm{G}} & 0 & \cos\theta_{\mathrm{G}} & 0 & 0 & 0 \\ 0 & 0 & 0 & \cos\theta_{\mathrm{E}} & 0 & -\sin\theta_{\mathrm{E}} \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & \sin\theta_{\mathrm{E}} & 0 & \cos\theta_{\mathrm{E}} \end{bmatrix} \begin{bmatrix} M_x^{\mathrm{G}} \\ M_y^{\mathrm{G}} \\ M_z^{\mathrm{G}} \\ M_x^{\mathrm{E}} \\ M_y^{\mathrm{E}} \\ M_z^{\mathrm{E}} \end{bmatrix} = \begin{bmatrix} \cos\theta_{\mathrm{G}} M_x^{\mathrm{G}} - \sin\theta_{\mathrm{G}} M_z^{\mathrm{G}} \\ M_y^{\mathrm{G}} \\ \cos\theta_{\mathrm{G}} M_z^{\mathrm{G}} + \sin\theta_{\mathrm{G}} M_x^{\mathrm{G}} \\ \cos\theta_{\mathrm{E}} M_x^{\mathrm{E}} - \sin\theta_{\mathrm{E}} M_z^{\mathrm{E}} \\ M_y^{\mathrm{E}} \\ \cos\theta_{\mathrm{E}} M_z^{\mathrm{E}} + \sin\theta_{\mathrm{E}} M_x^{\mathrm{E}} \end{bmatrix} = \begin{bmatrix} M_{x'}^{\mathrm{G}} \\ M_{y'}^{\mathrm{G}} \\ M_{z'}^{\mathrm{G}} \\ M_{x'}^{\mathrm{E}} \\ M_{y'}^{\mathrm{E}} \\ M_{z'}^{\mathrm{E}} \end{bmatrix} \tag{S5}$$

and the 6×6 exchange matrix becomes

$$\begin{bmatrix} -R_{2,\mathrm{G}}\cos^2\theta_{\mathrm{G}} - R_1\sin^2\theta_{\mathrm{G}} - p_{\mathrm{E}}k_{\mathrm{ex}} & \sqrt{\delta_{\mathrm{G}}^2+\omega_1^2} & -(R_{2,\mathrm{G}}-R_1)\sin\theta_{\mathrm{G}}\cos\theta_{\mathrm{G}} & (1-p_{\mathrm{E}})k_{\mathrm{ex}}\cos(\theta_{\mathrm{G}}-\theta_{\mathrm{E}}) & 0 & (1-p_{\mathrm{E}})k_{\mathrm{ex}}\sin(\theta_{\mathrm{G}}-\theta_{\mathrm{E}}) \\ -\sqrt{\delta_{\mathrm{G}}^2+\omega_1^2} & -R_{2,\mathrm{G}} - p_{\mathrm{E}}k_{\mathrm{ex}} & 0 & 0 & (1-p_{\mathrm{E}})k_{\mathrm{ex}} & 0 \\ -(R_{2,\mathrm{G}}-R_1)\sin\theta_{\mathrm{G}}\cos\theta_{\mathrm{G}} & 0 & -R_{2,\mathrm{G}}\sin^2\theta_{\mathrm{G}} - R_1\cos^2\theta_{\mathrm{G}} - p_{\mathrm{E}}k_{\mathrm{ex}} & -(1-p_{\mathrm{E}})k_{\mathrm{ex}}\sin(\theta_{\mathrm{G}}-\theta_{\mathrm{E}}) & 0 & (1-p_{\mathrm{E}})k_{\mathrm{ex}}\cos(\theta_{\mathrm{G}}-\theta_{\mathrm{E}}) \\ p_{\mathrm{E}}k_{\mathrm{ex}}\cos(\theta_{\mathrm{G}}-\theta_{\mathrm{E}}) & 0 & -p_{\mathrm{E}}k_{\mathrm{ex}}\sin(\theta_{\mathrm{G}}-\theta_{\mathrm{E}}) & -R_{2,\mathrm{E}}\cos^2\theta_{\mathrm{E}} - R_1\sin^2\theta_{\mathrm{E}} - (1-p_{\mathrm{E}})k_{\mathrm{ex}} & \sqrt{\delta_{\mathrm{E}}^2+\omega_1^2} & -(R_{2,\mathrm{E}}-R_1)\sin\theta_{\mathrm{E}}\cos\theta_{\mathrm{E}} \\ 0 & p_{\mathrm{E}}k_{\mathrm{ex}} & 0 & -\sqrt{\delta_{\mathrm{E}}^2+\omega_1^2} & -R_{2,\mathrm{E}} - (1-p_{\mathrm{E}})k_{\mathrm{ex}} & 0 \\ p_{\mathrm{E}}k_{\mathrm{ex}}\sin(\theta_{\mathrm{G}}-\theta_{\mathrm{E}}) & 0 & p_{\mathrm{E}}k_{\mathrm{ex}}\cos(\theta_{\mathrm{G}}-\theta_{\mathrm{E}}) & -(R_{2,\mathrm{E}}-R_1)\sin\theta_{\mathrm{E}}\cos\theta_{\mathrm{E}} & 0 & -R_{2,\mathrm{E}}\sin^2\theta_{\mathrm{E}} - R_1\cos^2\theta_{\mathrm{E}} - (1-p_{\mathrm{E}})k_{\mathrm{ex}} \end{bmatrix} \tag{S6}$$

As described above in the limit that $\omega_1 \gg R_{2,\mathrm{E}}, R_{2,\mathrm{G}}$ the $-(R_{2,\mathrm{G}} - R_1)\sin\theta_\mathrm{G}\cos\theta_\mathrm{G}$ and $-(R_{2,\mathrm{E}} - R_1)\sin\theta_\mathrm{E}\cos\theta_\mathrm{E}$ off-diagonal elements can be ignored, effectively uncoupling the $M_{z'}$ and $M_{x'}$ terms for states G and E. However, the 1,6 ; 3,4 elements in Equation S6 couple $M^i_{z'}$ with $M^j_{x'}$ ($i = \mathrm{G}, j = \mathrm{E}$ for 3,4; $i = \mathrm{E}, j = \mathrm{G}$ for 1,6). In what follows we focus on the coupling between $M^\mathrm{G}_{z'}$ and $M^\mathrm{E}_{x'}$ from the 3,4 element, but an analogous argument to that presented below holds for the coupling of $M^\mathrm{E}_{z'}$ and $M^\mathrm{G}_{x'}$ from the 1,6 and 6,1 terms as well. The 4,5 and 5,4 elements of Equation S6 lead to a rapid averaging of the $M^\mathrm{E}_{x'}$ and $M^\mathrm{E}_{y'}$ magnetization components about the spin-lock axis for E, as described above, so that E state x' magnetization evolves as

$$M^\mathrm{E}_{x'}(0)\vec{e}^\mathrm{E}_{x'} \rightarrow M^\mathrm{E}_{x'}(0)\{\vec{e}^\mathrm{E}_{x'}\cos(\omega_{\mathrm{eff,E}}t) + \vec{e}^\mathrm{E}_{y'}\sin(\omega_{\mathrm{eff,E}}t)\}, \tag{S7.1}$$

or

$$M^\mathrm{E}_{x'}(0)\begin{bmatrix}\cos\theta_\mathrm{E}\\ 0\\ -\sin\theta_\mathrm{E}\end{bmatrix} \rightarrow M^\mathrm{E}_{x'}(0)\begin{bmatrix}\cos\theta_\mathrm{E}\cos(\omega_{\mathrm{eff,E}}t)\\ \sin(\omega_{\mathrm{eff,E}}t)\\ -\sin\theta_\mathrm{E}\cos(\omega_{\mathrm{eff,E}}t)\end{bmatrix} = \vec{v}_1(t) \tag{S7.2}$$

where $M^\mathrm{E}_{x'}(0)$ is the x' magnetization component at $t = 0$, and $\vec{e}^\mathrm{E}_{j'}$ is a unit vector directed along the j' axis of the spin-lock frame for spin E, $\omega^2_{\mathrm{eff,E}} = \omega_1^2 + \delta^2_\mathrm{E} = \omega_1^2 + (\omega_\mathrm{E} - \omega_\mathrm{RF})^2$ and we have written the evolution with respect to the original, untilted frame in Equation S7.2. In turn, we can write the unit vector along which G state magnetization is locked (z' axis for G) as

$$\begin{bmatrix}\sin\theta_\mathrm{G}\\ 0\\ \cos\theta_\mathrm{G}\end{bmatrix} = \vec{v}_2(t) \tag{S8}$$

The components of $\vec{v}_1(t)$ that are parallel and perpendicular to $\vec{v}_2$ are, in turn, given by

$$M_{x'}^{\mathrm{E}}(0)\sin(\theta_{\mathrm{G}}-\theta_{\mathrm{E}})\cos(\omega_{\mathrm{eff,E}}t)\begin{bmatrix}\sin\theta_{\mathrm{G}}\\0\\\cos\theta_{\mathrm{G}}\end{bmatrix} \tag{S9.1}$$

and

$$M_{x'}^{\mathrm{E}}(0)\begin{bmatrix}\cos(\omega_{\mathrm{eff,E}}t)\cos\theta_{\mathrm{E}}-\sin(\theta_{\mathrm{G}}-\theta_{\mathrm{E}})\cos(\omega_{\mathrm{eff,E}}t)\sin\theta_{\mathrm{G}}\\\sin(\omega_{\mathrm{eff,E}}t)\\-\cos(\omega_{\mathrm{eff,E}}t)\sin\theta_{\mathrm{E}}-\sin(\theta_{\mathrm{G}}-\theta_{\mathrm{E}})\cos(\omega_{\mathrm{eff,E}}t)\cos\theta_{\mathrm{G}}\end{bmatrix} \tag{S9.2}$$

respectively. So long as these are averaged rapidly (by the $\cos(\omega_{\mathrm{eff,E}}t)$ and $\sin(\omega_{\mathrm{eff,E}}t)$ terms) compared to the rate of cross-relaxation between $M_{z'}^{\mathrm{G}}$ and $M_{x'}^{\mathrm{E}}$ (i.e., the 3,4 term), which is fulfilled when $\omega_1 \gg k_{\mathrm{ex}}|\sin(\theta_{\mathrm{G}}-\theta_{\mathrm{E}})|$, the 3,4 coupling term in Equation S6 is ineffective and can be neglected. Thus, in the limit that $\omega_1 \gg R_{2,\mathrm{E}}, R_{2,\mathrm{G}}$ and $\omega_1 \gg k_{\mathrm{ex}}$ the evolution of the spin-locked magnetization components become uncoupled from any of the non spin-locked components such that the exchange matrix can be simplified to a 2×2 form.

Under these conditions, and assuming further that $R_{2,\mathrm{G}} = 0\ \mathrm{s}^{-1}$, $R_1 = 0\ \mathrm{s}^{-1}$ the Bloch–McConnell equations become

$$\frac{d}{dt}\begin{bmatrix}M_{\mathrm{G}}\\M_{\mathrm{E}}\end{bmatrix} = \begin{bmatrix}-p_{\mathrm{E}}k_{\mathrm{ex}} & (1-p_{\mathrm{E}})k_{\mathrm{ex}}\cos\theta_{\mathrm{EG}}\\ p_{\mathrm{E}}k_{\mathrm{ex}}\cos\theta_{\mathrm{EG}} & (1-p_{\mathrm{E}})k_{\mathrm{ex}}-\Delta R_2\sin^2\theta_{\mathrm{E}}\end{bmatrix}\begin{bmatrix}M_{\mathrm{G}}\\M_{\mathrm{E}}\end{bmatrix} \tag{S10}$$

as described in the text, with a solution given by

$$\begin{aligned} M_{\mathrm{G}}(t) &= Ae^{\lambda_{\mathrm{s}}t}+Be^{\lambda_{\mathrm{f}}t}\\ M_{\mathrm{E}}(t) &= Ce^{\lambda_{\mathrm{s}}t}+De^{\lambda_{\mathrm{f}}t}\end{aligned} \tag{S11}$$

where λ_{s} and λ_{f} are the decay rates for the slowly and rapidly relaxing components, respectively. Assuming that the system is at equilibrium at $t = 0$ the coefficients A–D can be

obtained from solving the following initial boundary condition equations

$$\begin{aligned}
M_{\rm G}(0) &= A+B = 1-p_{\rm E} \\
M_{\rm E}(0) &= C+D = p_{\rm E} \\
M'_{\rm G}(0) &= \lambda_{\rm s}A+\lambda_{\rm f}B = -p_{\rm E}k_{\rm ex}(1-p_{\rm E})+(1-p_{\rm E})k_{\rm ex}p_{\rm E}\cos\theta_{\rm EG} = (1-p_{\rm E})p_{\rm E}k_{\rm ex}(\cos\theta_{\rm EG}-1) \\
M'_{\rm E}(0) &= \lambda_{\rm s}C+\lambda_{\rm f}D = p_{\rm E}k_{\rm ex}(1-p_{\rm E})\cos\theta_{\rm EG}-(1-p_{\rm E})k_{\rm ex}p_{\rm E}-p_{\rm E}\Delta R_2\sin^2\theta_{\rm E} \\
&= (1-p_{\rm E})p_{\rm E}k_{\rm ex}(\cos\theta_{\rm EG}-1)-p_{\rm E}\Delta R_2\sin^2\theta_{\rm E}
\end{aligned} \tag{S12}$$

and the coefficients $A-D$ are

$$\begin{aligned}
A &= \frac{\lambda_{\rm f}(1-p_{\rm E})-(1-p_{\rm E})p_{\rm E}k_{\rm ex}(\cos\theta_{\rm EG}-1)}{\lambda_{\rm f}-\lambda_{\rm s}} \\
B &= \frac{-\lambda_{\rm s}(1-p_{\rm E})+(1-p_{\rm E})p_{\rm E}k_{\rm ex}(\cos\theta_{\rm EG}-1)}{\lambda_{\rm f}-\lambda_{\rm s}} \\
C &= \frac{\lambda_{\rm f}p_{\rm E}+p_{\rm E}\Delta R_2\sin^2\theta_{\rm E}-(1-p_{\rm E})p_{\rm E}k_{\rm ex}(\cos\theta_{\rm EG}-1)}{\lambda_{\rm f}-\lambda_{\rm s}} \\
D &= \frac{-\lambda_{\rm s}p_{\rm E}-p_{\rm E}\Delta R_2\sin^2\theta_{\rm E}+(1-p_{\rm E})p_{\rm E}k_{\rm ex}(\cos\theta_{\rm EG}-1)}{\lambda_{\rm f}-\lambda_{\rm s}}
\end{aligned} \tag{S13}$$

with the eigenvalues given by Equation 6 of the text for the case where $p_{\rm E} \ll 1$ or by Equation 4 in general.

Experimental Details. The pulse sequence that we have used for measuring ^{15}N off-resonance $R_{1\rho}$ rates is illustrated in Figure 4 of the main text and follows closely an early version proposed by Akke and Palmer.[4] The experiment is carried out using a constant-time relaxation period (fixed $T_{\rm relax,max}$ value), so that the measured decay rate corresponds to $R_{1\rho}-R_1$ instead of $R_{1\rho}$.[4] In such a scheme the R_2 rates are obtained from the expression $R_2 = \rho/\sin^2\theta_{\rm G}+R_1$, where ρ is the measured decay rate, rather than $R_2 = \rho/\sin^2\theta_{\rm G}-R_1\cot^2\theta_{\rm G}$. In this manner uncertainties in the R_1 rates are not amplified by the $\cot^2\theta_{\rm G}$ factor, although in practice R_1 rates can be easily measured with high accuracy so long as care is taken.[5] Another advantage of the constant-time scheme is that the extent of water

saturation remains constant as a function of T_{relax}, that may be important for systems with fast solvent exchange.[6] Figure S1 compares $R_{2,eff} - R_1$ profiles as a function of ω_1 offset for experiments recorded using a 15 kHz ^{1}H cw decoupling field during $T_{relax,max}$ or a pair of ^{1}H 180° pulses spaced at intervals of $T_{relax,max}/2$, in the absence of chemical exchange. Flat profiles are obtained for the cw case, while small, but noticeable artifacts are generated with the 180° pulses. It is noteworthy that larger artifacts are observed when composite pulse ^{1}H decoupling schemes are used, as described previously.[7] We have validated the methodology by showing that flat $R_{2,eff} - R_1$ profiles are obtained in studies of protein L (for both deuterated and protonated samples), a system for which slow exchange processes are absent (Figure S2).

In order to achieve alignment of ^{15}N magnetization along the appropriate effective field for each spin we have used Adiabatic Half Passage pulses (AHPs) prior to and after each ^{15}N spin-lock period.[8] Although the tanh/tan shape[9] has been employed in our study many other choices of AHP shapes will work equally well so long as the adiabatic condition is fully satisfied.[10] For a B_1 = 2 kHz spin-lock we have used AHPs set to 4 ms and a sweep of 250 kHz. It is noteworthy that the direction of the adiabatic sweep for measuring each offset has been selected such that at the end of the spin-lock period magnetization is returned back to its initial position rather than undergoing a net inversion (i.e., the combined pair of AHPs does not generate a net sweep through the spectrum, but rather the second pulse reverses the effects of the first). Thus, if the spin-lock carrier is placed at a frequency that is upfield of the center of the ^{15}N spectrum for the duration of T_{relax}, the direction of the sweep is from low to high field (low ppm to high ppm) and then reversed to bring the magnetization back to the z-axis, with the opposite scenario for the case where the carrier is placed downfield of the center of the spectrum. This ensures that all amide ^{15}N spins in a protein can be properly spin-locked for the RF power and sweep duration used. It is noteworthy that the pair of AHPs, one before and one after each spin-lock period, are not simply related by a time reversal but also by a phase inversion. The code used to generate

each of the shapes is available from the authors upon request. As discussed below it is necessary to take into account the relaxation that occurs during the AHP pulses so that c_{fast} can be calculated correctly. This is discussed in detail below.

Equations 11 and 12 show that c_{fast} increases as a function of p_{E} and for the system considered here where $p_{\text{E}} = 0.3$ reasonably high quality c_{fast} profiles were obtained experimentally. In many cases, however, p_{E} values will be smaller, complicating extraction of accurate c_{fast} values. For systems where k_{ex} is relatively large (on the order of several hundred per second) so that magnetization is rapidly equilibrated it is possible to perform experiments as indicated in Figure S3A, whereby a series of N spin-lock elements are applied, each separated by a duration that is sufficiently long to ensure proper equilibration. Note that N must be chosen such that $T_{\text{relax}}/N \gg 1/|\lambda_{\text{f}}|$, so that the fast decay component decays completely during each element. It can be shown that for N applications of the spin-lock $c_{\text{fast}}^{N} = 1-(1-c_{\text{fast}}^{1})^{N}$, where c_{fast}^{1} is the c_{fast} value when $N = 1$ (see Figures S3B and S3C).

Correcting T_{relax} Values for Finite Duration of AHPs. Since each AHP has a duration that is typically several ms long (we use 4 ms) it is necessary to take relaxation effects during the pulse into account so that the relative intensities of peaks obtained as a function of T_{relax} (including $T_{\text{relax}} = 0$ where AHPs are not applied) are consistent. This is important for obtaining correct c_{fast} values but will not affect the decay rates measured from a set of non-zero T_{relax} values (corresponding to $|\lambda_{\text{s}}|$). As discussed above, in order to maintain the adiabatic condition over a large range of offsets the direction of the sweep is chosen such that the extent of the spectrum that is 'swept through' is minimized. In what follows we focus on the first AHP during which magnetization rotates from the z-axis to the spin-lock field and calculate the effective correction to T_{relax} based on the magnetization trajectory during the AHP. That is, non-zero values of T_{relax} are corrected slightly to account for the AHP pulse. The correction factor is identical for the first and second AHP.

Assuming that the time dependent angle between the magnetization and the z-axis during the AHP is $\theta(t)$ then the effective magnetization decay is given by

$$\begin{aligned} e^{-\left(R_2\int_0^{\tau_{\mathrm{AHP}}}\sin^2\theta(t)dt+R_1\int_0^{\tau_{\mathrm{AHP}}}\cos^2\theta(t)dt\right)} &= e^{-\left((R_2-R_1)\int_0^{\tau_{\mathrm{AHP}}}\sin^2\theta(t)dt+R_1\int_0^{\tau_{\mathrm{AHP}}}[\cos^2\theta(t)+\sin^2\theta(t)]dt\right)} \\ &= e^{-(R_2-R_1)\int_0^{\tau_{\mathrm{AHP}}}\sin^2\theta(t)dt}e^{-R_1\tau_{\mathrm{AHP}}} \end{aligned} \tag{S14}$$

where τ_{AHP} is the duration of the AHP pulse. The reference plane is recorded with magnetization along the z-axis for the full duration of the constant-time element so that the decay of magnetization for a duration equal to that of the AHP is given by $e^{-R_1\tau_{\mathrm{AHP}}}$. Thus the correction of the intensities of points recorded with $T_{\mathrm{relax}} \neq 0$ (i.e., with the AHP) relative to the $T_{\mathrm{relax}} = 0$ point is given by

$$\frac{e^{-(R_2-R_1)\int_0^{\tau_{\mathrm{AHP}}}\sin^2\theta(t)dt}e^{-R_1\tau_{\mathrm{AHP}}}}{e^{-R_1\tau_{\mathrm{AHP}}}} \tag{S15}$$

The amount that T_{relax} must be corrected, τ_{corr}, is given by the relation

$$\begin{aligned} e^{-(R_{1\rho}-R_1)\tau_{\mathrm{corr}}} &= e^{-(R_2-R_1)\sin^2\theta_{\mathrm{G}}\tau_{\mathrm{corr}}} \\ &= e^{-(R_2-R_1)\int_0^{\tau_{\mathrm{AHP}}}\sin^2\theta(t)dt} \end{aligned} \tag{S16}$$

where θ_{G} is the angle between the spin-lock field and the z-axis for the ground state spin in question. Therefore

$$\tau_{\mathrm{corr}} = \frac{\int_0^{\tau_{\mathrm{AHP}}}\sin^2\theta(t)dt}{\sin^2\theta_{\mathrm{G}}} = \tau_{\mathrm{AHP}}\frac{\int_0^{\tau_{\mathrm{AHP}}}\sin^2\theta(t)dt}{\tau_{\mathrm{AHP}}\sin^2\theta_{\mathrm{G}}} = \tau_{\mathrm{AHP}}\frac{\left\langle\sin^2\theta(t)\right\rangle}{\sin^2\theta_{\mathrm{G}}} = f\times\tau_{\mathrm{AHP}} \tag{S17}$$

It can be shown for B_1 = 2 kHz and a 250 kHz sweep that f is relatively constant over most offsets so long as the majority of the spectrum is not swept through (Figure S4). For the tanh/tan shape in the current work a τ_{corr} value of 0.3×4 ms = 1.2 ms has been used for all ω_1 offsets and all resonances, so that T_{relax} values must be increased by 2.4 ms.

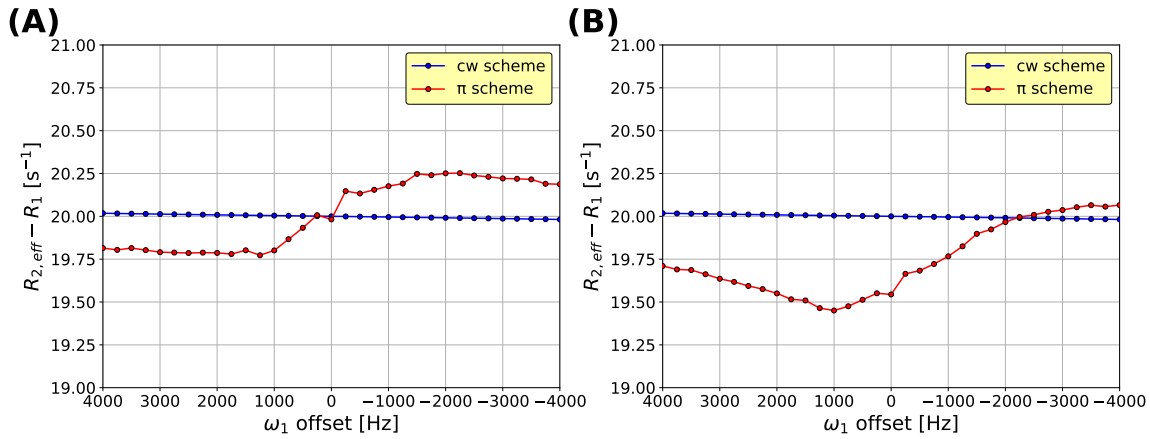

Figure S1. Simulation of $R_{2,\text{eff}} - R_1$ dispersion profiles obtained (i) using ^{1}H cw decoupling (15 kHz) for the duration of the ^{15}N spin-lock element or (ii) by application of a pair of evenly spaced ^{1}H 180° pulses.[11] The simulations are carried out with the following parameters: $p_E = 0$, $R_1 = 0$ s^{-1}, $R_2 = 20$ s^{-1}, η_{xy} = 10 s^{-1}, B_1 field strength is 2000 Hz and B_1 inhomogeneity is not included. Panels (A) and (B) are simulated with a ^{1}H spin flip rate $R_{1H} = 0$ or 10 s^{-1} respectively. In the ideal situation the $R_{2,\text{eff}} - R_1$ profile should be totally flat in the absence of a slow exchange process.

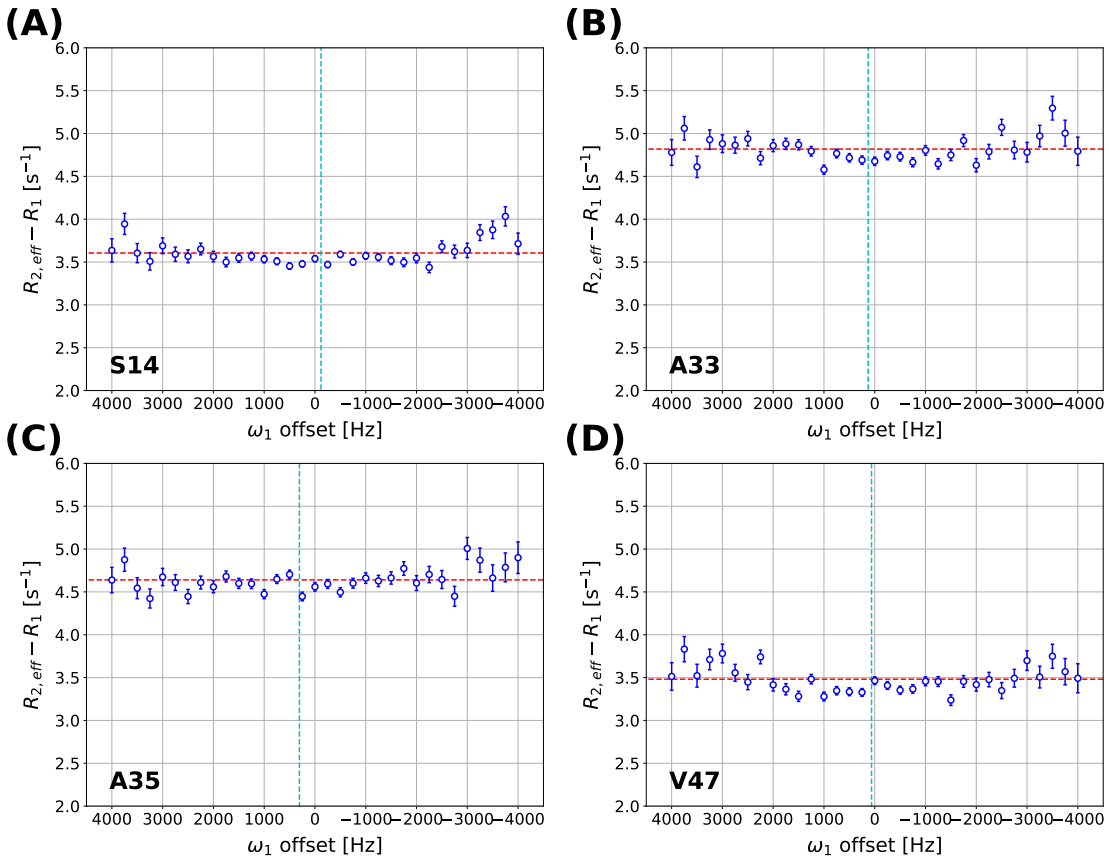

Figure S2. $R_{2,\text{eff}} - R_1$ dispersion profiles from off-resonance ^{15}N $R_{1\rho}$ measurements on a protonated protein L sample, 25 °C. Flat profiles are expected because slow exchange timescale processes have not been observed for this protein. Profiles are shown for four representative residues. *Red dashed lines* indicate the average value of $R_{2,\text{eff}} - R_1$ for each residue, while *cyan dashed lines* indicate the position of ground state chemical shifts. Errors are obtained via a Monte Carlo analysis whereby 1000 datasets are generated.[12]

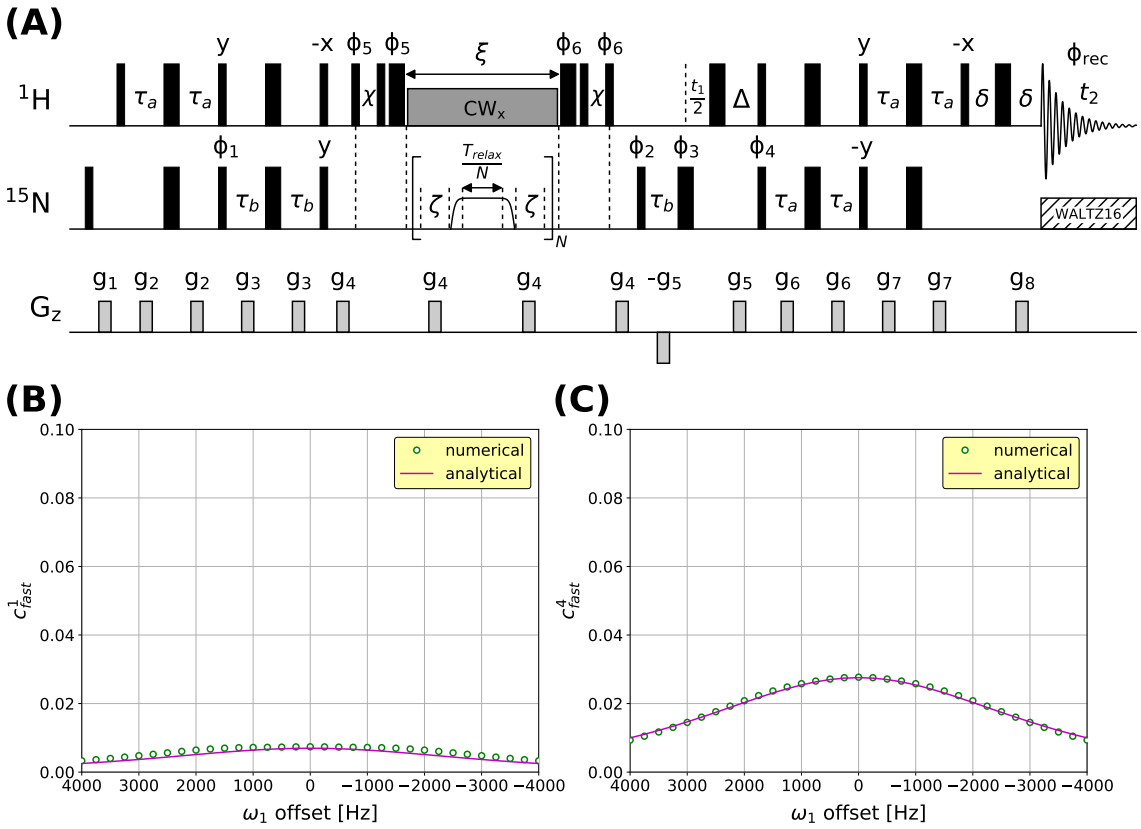

Figure S3. (A) Pulse scheme for measurements on systems with small p_E values and for k_{ex} values such that $T_{relax}/N \gg 1/|\lambda_f|$. The setup is similar to the original scheme in Figure 4, except that the single spin-lock period is divided into N spin-lock periods. $\zeta = (T_{relax,max} - T_{relax})/2N + \tau_{eq}/2$ and $\xi = T_{relax,max} + N\tau_{eq} + 2N\tau_{AHP}$. (B–C) Comparison of c_{fast} values calculated for N = 1 (B) or N = 4 (C) using the pulse scheme of Panel (A). The simulations are carried out with the same set of parameters as for Figures 2 and 3, except that p_E = 0.01. When $p_E \ll 1$ the size of the curve is enhanced roughly N times.

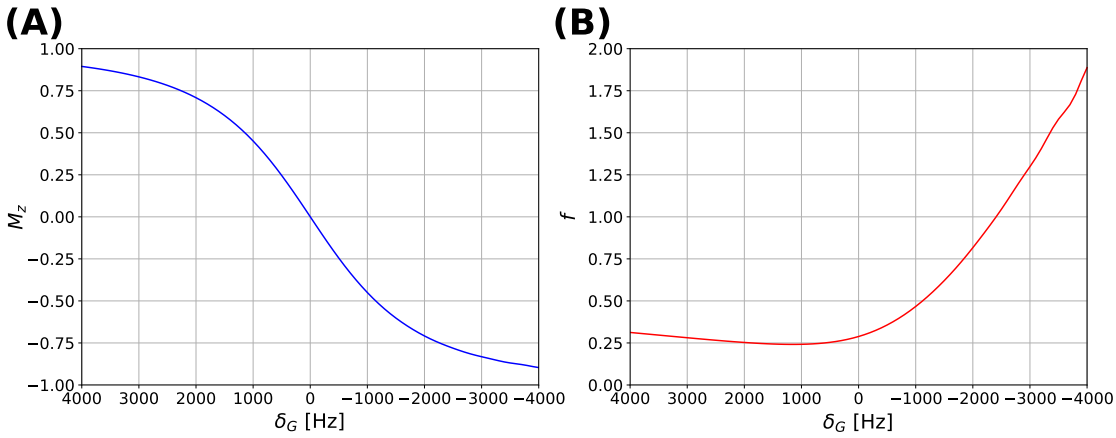

Figure S4. (A) Alignment of magnetization from the application of a single AHP pulse. The simulation is carried out for the first of the two AHP pulses applied in the pulse scheme of Figure 4 or Figure S3A, rotating magnetization from the z-axis to the direction of the effective field. The amplitude and phase of the AHP that is used in the present work is described by the relations: $\omega_1(t) = \omega_1^0 \tanh(10t/\tau_{AHP}), \frac{d\phi(t)}{dt} = \Delta\omega(t) = \omega_{RF} + \Delta\omega^0 \tan[\arctan(50) \times (1 - t/\tau_{AHP})]/50.$[8,9] Simulations are carried out with ω_1^0 = 2 kHz, τ_{AHP} = 4 ms and $\Delta\omega^0$ = 250 kHz. Relaxation effects and slow exchange processes are not included in the simulations. The sweeping direction is from upfield to downfield, with the sweep stopping at the position of the ^{15}N carrier during the ^{15}N spin-lock (ω_{RF}). For the parameters used here there is excellent alignment for resonances within ±4 kHz of ω_{RF}, although for weaker fields the alignment of magnetization that resonates upfield of ω_{RF} is less optimal than that shown here. (B) The factor f that is used for correcting T_{relax} is calculated from Equation S17, and is the same for the second AHP since the magnetization trajectory is simply time reversed from that generated with the first pulse. In (A) and (B) $\delta_G = \omega_G - \omega_{RF}$.

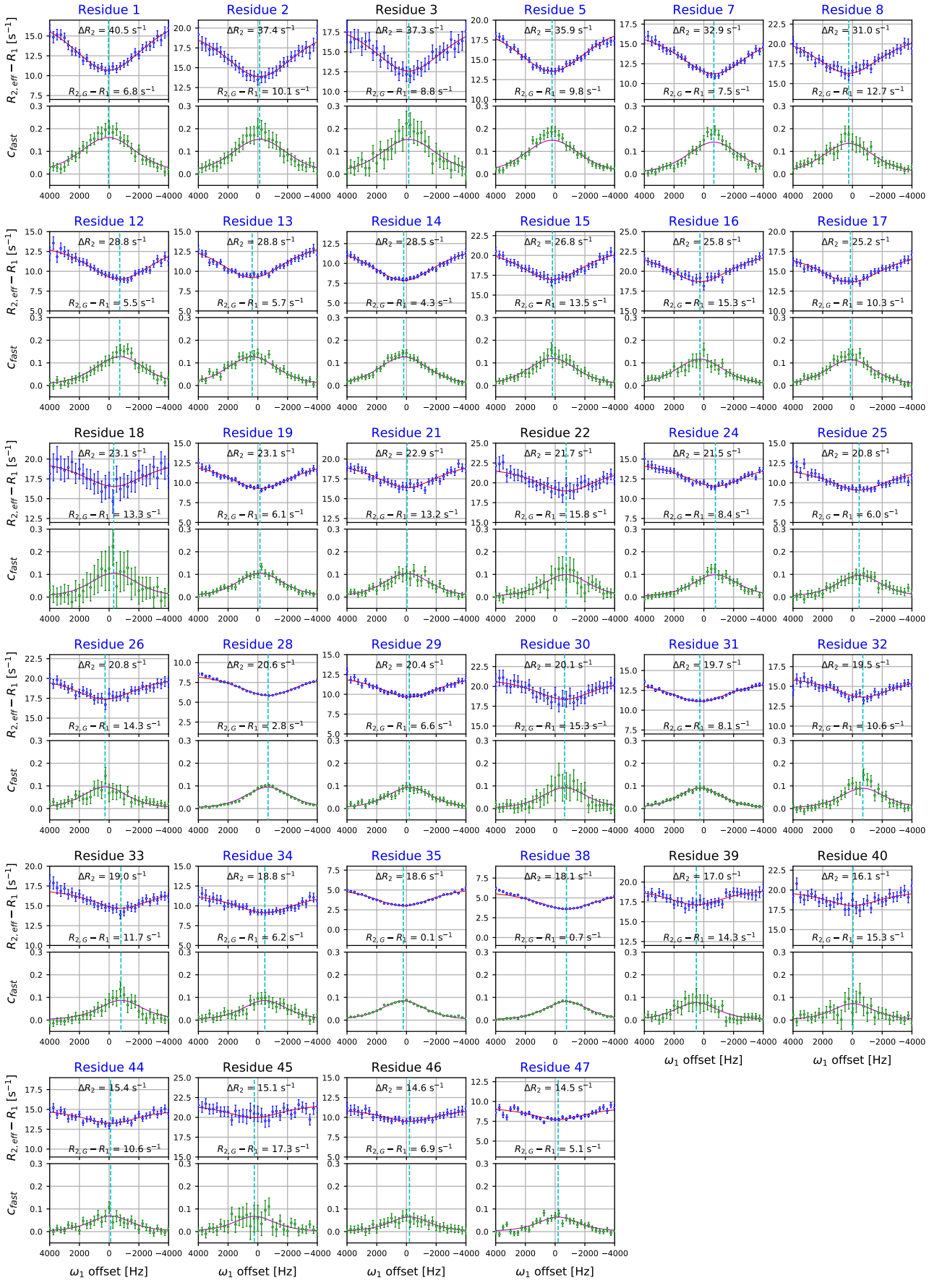
Residue 1
ΔR2 = 40.5 s−1
R2,G − R1 = 6.8 s−1
Residue 2
ΔR2 = 37.4 s−1
R2,G − R1 = 10.1 s−1
Residue 3
ΔR2 = 37.3 s−1
R2,G − R1 = 8.8 s−1
Residue 5
ΔR2 = 35.9 s−1
R2,G − R1 = 9.8 s−1
Residue 7
ΔR2 = 32.9 s−1
R2,G − R1 = 7.5 s−1
Residue 8
ΔR2 = 31.0 s−1
R2,G − R1 = 12.7 s−1
Residue 12
ΔR2 = 28.8 s−1
R2,G − R1 = 5.5 s−1
Residue 13
ΔR2 = 28.8 s−1
R2,G − R1 = 5.7 s−1
Residue 14
ΔR2 = 28.5 s−1
R2,G − R1 = 4.3 s−1
Residue 15
ΔR2 = 26.8 s−1
R2,G − R1 = 13.5 s−1
Residue 16
ΔR2 = 25.8 s−1
R2,G − R1 = 15.3 s−1
Residue 17
ΔR2 = 25.2 s−1
R2,G − R1 = 10.3 s−1
Residue 18
ΔR2 = 23.1 s−1
R2,G − R1 = 13.3 s−1
Residue 19
ΔR2 = 23.1 s−1
R2,G − R1 = 6.1 s−1
Residue 21
ΔR2 = 22.9 s−1
R2,G − R1 = 13.2 s−1
Residue 22
ΔR2 = 21.7 s−1
R2,G − R1 = 15.8 s−1
Residue 24
ΔR2 = 21.5 s−1
R2,G − R1 = 8.4 s−1
Residue 25
ΔR2 = 20.8 s−1
R2,G − R1 = 6.0 s−1
Residue 26
ΔR2 = 20.8 s−1
R2,G − R1 = 14.3 s−1
Residue 28
ΔR2 = 20.6 s−1
R2,G − R1 = 2.8 s−1
Residue 29
ΔR2 = 20.4 s−1
R2,G − R1 = 6.6 s−1
Residue 30
ΔR2 = 20.1 s−1
R2,G − R1 = 15.3 s−1
Residue 31
ΔR2 = 19.7 s−1
R2,G − R1 = 8.1 s−1
Residue 32
ΔR2 = 19.5 s−1
R2,G − R1 = 10.6 s−1
Residue 33
ΔR2 = 19.0 s−1
R2,G − R1 = 11.7 s−1
Residue 34
ΔR2 = 18.8 s−1
R2,G − R1 = 6.2 s−1
Residue 35
ΔR2 = 18.6 s−1
R2,G − R1 = 0.1 s−1
Residue 38
ΔR2 = 18.1 s−1
R2,G − R1 = 0.7 s−1
Residue 39
ΔR2 = 17.0 s−1
R2,G − R1 = 14.3 s−1
Residue 40
ΔR2 = 16.1 s−1
R2,G − R1 = 15.3 s−1
Residue 44
ΔR2 = 15.4 s−1
R2,G − R1 = 10.6 s−1
Residue 45
ΔR2 = 15.1 s−1
R2,G − R1 = 17.3 s−1
Residue 46
ΔR2 = 14.6 s−1
R2,G − R1 = 6.9 s−1
Residue 47
ΔR2 = 14.5 s−1
R2,G − R1 = 5.1 s−1
R2,eff − R1 [s−1]
Cfast
ω1 offset [Hz]

Figure S5 *(previous page).* Experimental ^{15}N $R_{1\rho}$ profiles for Ddx4$_{\text{cond}}$ arranged in order of decreasing ΔR_2. The average χ^2 from all single residue fits is 0.66, while χ^2 from the global fit with a common set of $(p_{\text{E}}, k_{\text{ex}})$ is 0.79. $R_{2,\text{eff}} - R_1$ and c_{fast} profiles are fitted to analytical expressions derived for $\Delta\omega_{\text{GE}} = 0$; the approximation $p_{\text{E}} \ll 1$ is not used due to the large size of p_{E}. The *cyan dashed lines* indicate the ground state chemical shifts. The 26 residues of Ddx4$_{\text{cond}}$ used in the global fit to obtain $(p_{\text{E}}, k_{\text{ex}})$ are indicated in *blue*; these have large ΔR_2 rates and high quality dispersion curves. The best fit $(p_{\text{E}}, k_{\text{ex}})$ values are (29.7%, 17.7 s^{-1}), as discussed in the main text. The positions of the residues in the ^{1}H–^{15}N HSQC spectrum are indicated in Figure S7.

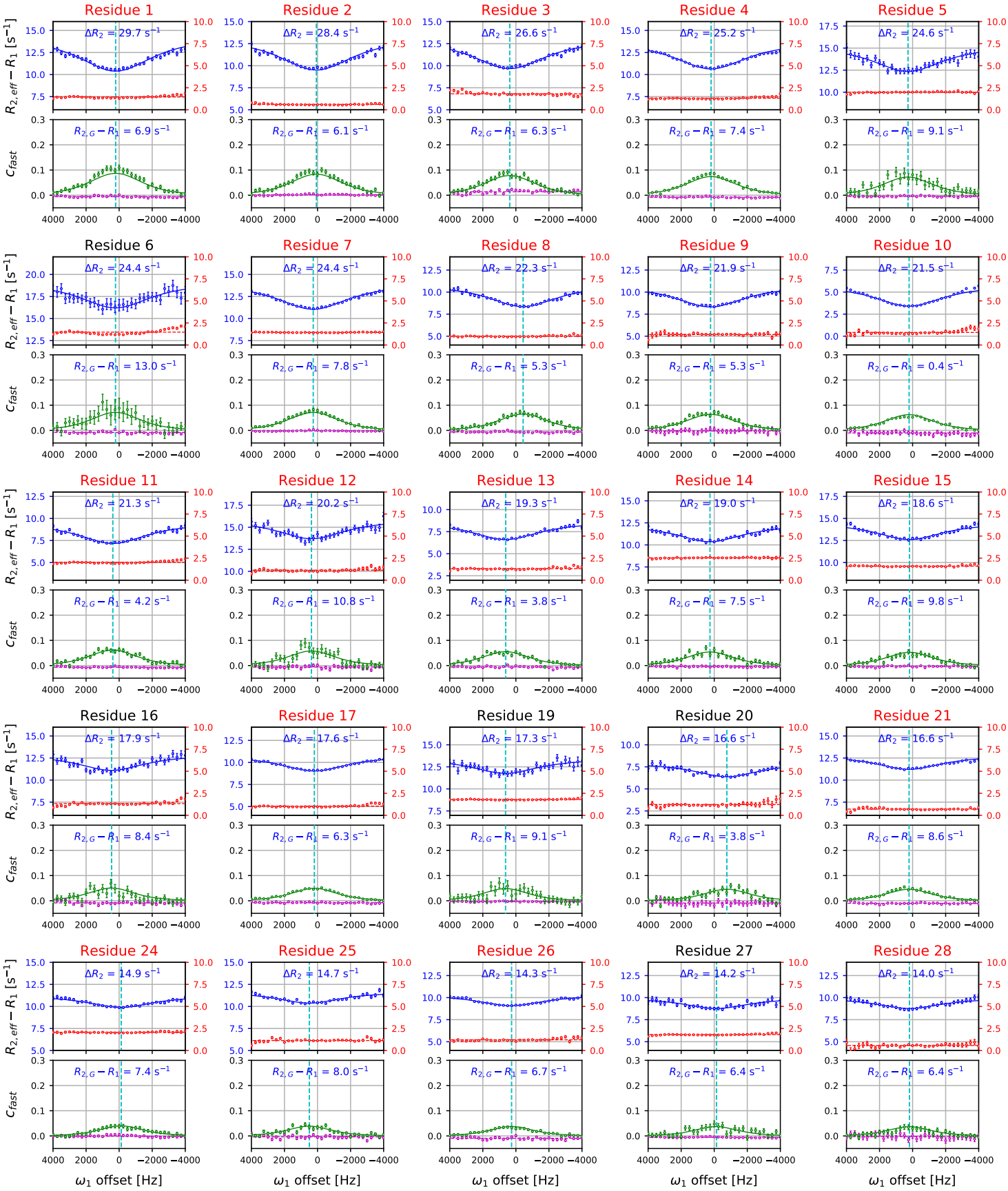
Residue 1
ΔR2 = 29.7 s−1
R2,G − R1 = 6.9 s−1
Residue 2
ΔR2 = 28.4 s−1
R2,G − R1 = 6.1 s−1
Residue 3
ΔR2 = 26.6 s−1
R2,G − R1 = 6.3 s−1
Residue 4
ΔR2 = 25.2 s−1
R2,G − R1 = 7.4 s−1
Residue 5
ΔR2 = 24.6 s−1
R2,G − R1 = 9.1 s−1
Residue 6
ΔR2 = 24.4 s−1
R2,G − R1 = 13.0 s−1
Residue 7
ΔR2 = 24.4 s−1
R2,G − R1 = 7.8 s−1
Residue 8
ΔR2 = 22.3 s−1
R2,G − R1 = 5.3 s−1
Residue 9
ΔR2 = 21.9 s−1
R2,G − R1 = 5.3 s−1
Residue 10
ΔR2 = 21.5 s−1
R2,G − R1 = 0.4 s−1
Residue 11
ΔR2 = 21.3 s−1
R2,G − R1 = 4.2 s−1
Residue 12
ΔR2 = 20.2 s−1
R2,G − R1 = 10.8 s−1
Residue 13
ΔR2 = 19.3 s−1
R2,G − R1 = 3.8 s−1
Residue 14
ΔR2 = 19.0 s−1
R2,G − R1 = 7.5 s−1
Residue 15
ΔR2 = 18.6 s−1
R2,G − R1 = 9.8 s−1
Residue 16
ΔR2 = 17.9 s−1
R2,G − R1 = 8.4 s−1
Residue 17
ΔR2 = 17.6 s−1
R2,G − R1 = 6.3 s−1
Residue 19
ΔR2 = 17.3 s−1
R2,G − R1 = 9.1 s−1
Residue 20
ΔR2 = 16.6 s−1
R2,G − R1 = 3.8 s−1
Residue 21
ΔR2 = 16.6 s−1
R2,G − R1 = 8.6 s−1
Residue 24
ΔR2 = 14.9 s−1
R2,G − R1 = 7.4 s−1
Residue 25
ΔR2 = 14.7 s−1
R2,G − R1 = 8.0 s−1
Residue 26
ΔR2 = 14.3 s−1
R2,G − R1 = 6.7 s−1
Residue 27
ΔR2 = 14.2 s−1
R2,G − R1 = 6.4 s−1
Residue 28
ΔR2 = 14.0 s−1
R2,G − R1 = 6.4 s−1
R2,eff − R1 [s−1]
cfast
ω1 offset [Hz]

Figure S6 ***(previous page).*** As in Figure S5 but for $Ddx4_{14FtoA}$. Residues used to obtain the best fit values (p_E, k_{ex}) = (25.2%, 23.1 s^{-1}) are highlighted in *red* (χ^2 from the global fit is 1.68). $R_{2,eff} - R_1$, c_{fast} profiles in *blue* and *green* correspond to those obtained for the concentrated (370 mg/mL) sample with the corresponding plots for a more dilute (50 mg/mL) sample indicated in *red* and *magenta*. Note the separate *y*-axes for the $R_{2,eff} - R_1$ profiles in each case, with flat dispersion profiles obtained for the dilute protein sample.

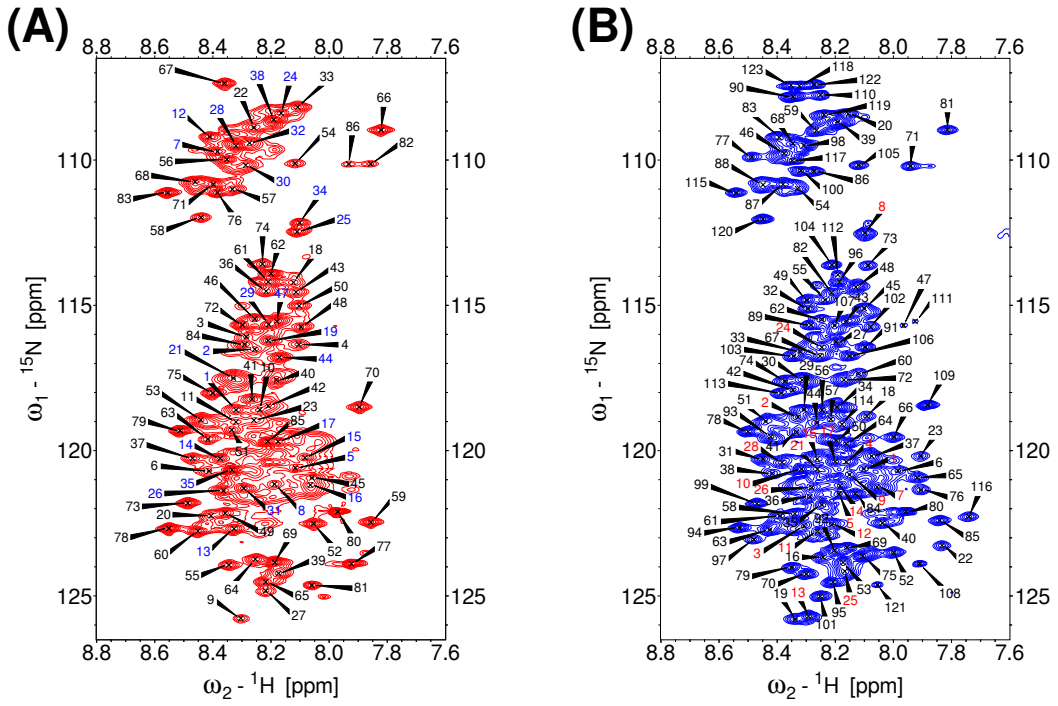

Figure S7. $^{1}H–^{15}N$ HSQC spectra for (A) $Ddx4_{cond}$ and (B) $Ddx4_{14FtoA}$, 800 MHz, 30 °C. Cross peaks are numbered according to the relative sizes of fitted ΔR_2 values, with those residues used to calculate exchange parameters highlighted in *blue* (A) and *red* (B).

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```
/*  N15T1rho_off_res_lek_800_cp

    This pulse sequence will allow one to perform the following experiment:

    15N T1rho, off res determination with enhanced sensitivity PFG 15N selection.

      2D       F1       15N
               F2(acq)  1H (NH)

    REF: Farrow et. al.  Biochemistry  33, 5984-6003 (1994).

    Includes a heat compensation flag so that the net amount of RF into the sample is
independent of the
      spin lock time for the different T2 points of the T2 series; set time_relax_max
to the max time_relax
      value for this

    Note that dps will show the spin lock times negative because run time variables ar
e not updated in dps;
      use hpdisp to verify that things are working

    Modified by LEK on July 22, 2015 from N15T1rho_gd_neh_lek_600

    Use BRF_adiabatic_half_passage.c to generate both rampup and rampdown 4ms pulses
    For a 1 KHz power spin lock the adiabaticity holds for spins 1000 Hz upfield of th
e stop of the swept pulse
      (swept downfield - to higher 15N ppm). For 2 KHz it is 2500 Hz. Use a 100,000 Hz
 sweep.

      BRF_adiabatic_half_passage 4000 1000 100000 0

    To generate a set of R1rho values use a frequency list in Hz values corresponding
to
      the position where the ramp finishes.

    Adiabaticity will ALWAYS be fullfiled for downfield sweeps

    Simulations show that it is best to use a 250 kHz sweep downfield

    Nov 28, 2016 modified by LEK to include a 1H purge that eliminates NzIz before adi
abatic sweep

    Nov 28, 2016 modified by LEK to make 4D such that do all frequencies (F3) , all T2
 points (F2) in a
      single file

    Modified on Mar 10, 2017 to include option to calibrate 15N spin-lock B1 field
      -Dcal_NB1

    Modified on Aug 30, 2017, with several names changed:
      1. p25 -> p15; 2. pl(w)23 -> pl(w)35; 3. cnst23 -> cnst35
    and also changed to 'constant-time scheme' when ip_flg is used
*/

;prosol relations=<triple>

#include <Avance.incl>
#include <Grad.incl>
#include <Delay.incl>

/*************************/
/*    Define phases      */
/*************************/
#define zero ph=0.0
#define one ph=90.0
#define two ph=180.0
#define three ph=270.0

/*************************/
```

```
/*    Define delays       */
/*************************/
define delay hscuba      /* length of 1/2 scuba delay */
  "hscuba=30m"

define delay taua        /* < 1/ 4J(XH)              */
  "taua=d3"

define delay taub        /* ~ 1/ 4J(XH)              */
  "taub=d5"

define delay BigT1
#ifdef cal_NB1
  "BigT1=2.7m"
#else
  "BigT1=d14"
#endif

#ifndef cal_NB1
  define delay time_relax_max
    "time_relax_max=d17"
#endif

define delay time_eq
  "time_eq=d19"

#ifdef ip_flg
  define delay t1_max
#endif

/*************************/
/*    Define pulses      */
/*************************/
define pulse dly_pg1     /* Messerle purge pulse*/
  "dly_pg1=5m"

define pulse dly_pg2     /* Messerle purge pulse*/
  "dly_pg2=dly_pg1/1.62"

define pulse pwh
  "pwh=p1"               /* 1H hard pulse at power level pl1 (tpwr) */

define pulse pwn
  "pwn=p3"               /* PW90 for N pulse at power level pl3 (dhpwr2)  */

#if defined(cp_flg) || !defined(cal_NB1)
  define pulse pw_ml
    "pw_ml=p12"
#endif

#ifdef ip_flg
  define pulse pwh_cpd
    "pwh_cpd=p17"
#endif

#ifdef c_flg
  define pulse pwc_ad
    "pwc_ad=p22"
#endif

#ifdef cp_flg
  define pulse pw_dip
    "pw_dip=p15"
#endif

#ifdef N_sel
  define pulse pwn_sl
    "pwn_sl=p32"
#endif
```

```
#if defined(cp_flg) || !defined(cal_NB1)
  define pulse pwn_ramp
    "pwn_ramp=p33"        /* pwn_ramp is the duration of the ramp up or down, typically
 4 ms */

  define pulse pwn_lk
    "pwn_lk=p34"          /* PW90 for N pulse at power level pl33   */
#endif

/*****************************/
/* Define other parameters */
/*****************************/
#ifndef cal_NB1
  define loopcounter num_sl
    "num_sl=l5"

  define list<delay> time_relax=<$VDLIST>

  define list<frequency> N_offset=<$FQ1LIST>
#endif

"d11=30m"
"in0=inf1/2"              /* t1/2 increment */
"TAU2=0.2u"

#ifdef ip_flg
  define loopcounter ni
    "ni=td1/2"
#endif

/*****************************************************************/
/* f1180: Start t1 at half dwell to get -90/180 phase correction */
/*        in F1 (15N) dim - set zgoptns -Df1180                  */
/*****************************************************************/
#ifdef f1180
  "d0=(in0)/2"
#else
  "d0=0.1u"
#endif

/*******************************************************/
/* Assign cnsts to check validity of parameter range */
/*******************************************************/
#ifdef fsat
  "cnst10=plw10"          /* tsatpwr - set max at 0.00005W */
#endif

#ifdef mess_flg
  "cnst11=plw11"          /* tpwrmess - set max at 2.0W */
#endif

#if defined(cp_flg) || !defined(cal_NB1)
  "cnst12=plw12"
#endif

#ifdef cp_flg
  "plw15=plw12*pow(pw_ml/pw_dip,2)"
  "plw35=plw33*pow(pwn_lk/pw_dip,2)"

  "cnst15=plw15"
  "cnst35=plw35"

  define delay tau_cp
    "tau_cp=(2590.0/90.0)*pw_dip*l1"
#endif

#ifdef ip_flg
  "cnst17=plw17"
```

```
#endif

#ifdef c_flg
  "cnst22=spw22"          /* power level for 13C adiabatic pulse */
#endif

#ifndef cal_NB1
  "cnst31=plw31"          /* dpwr2 - set max at 6W */
#endif

#ifdef N_sel
  "cnst32=spw32"          /* power level for 15N selective (reburp pulse) */
#endif

"cnst33=plw33"            /* power level for 15N spinlock for T1rho */

/*****************************/
/* Initialize loop counters */
/*****************************/
"l2=0"                    /* loop counter to switch Trelax delay time */
"l3=0"                    /* loop counter to switch between IP and AP */

/*******************************************/
/* Set phase alignment and offset for AHPs */
/*******************************************/
"spoal33=1"
"spoal34=0"
"spoal35=1"
"spoal36=0"
"spoff33=0"
"spoff34=0"
"spoff35=0"
"spoff36=0"

;"acqt0=0"
;baseopt_echo

/*******************************/
/* BEGIN ACTUAL PULSE SEQUENCE */
/*******************************/
1 ze
/*****************************************/
/*   Check Validity of Parameter Range   */
/*****************************************/
#ifdef fsat
  if "cnst10 > 0.00005" {
    2u
    print "error: tsatpwr pl10 too large !!!  "
    goto HaltAcqu
  }
#endif

#ifdef mess_flg
  if "cnst11 > 2" {
    2u
    print "error: tpwrmess pl11 too large !!!  "
    goto HaltAcqu
  }
#endif

#ifdef cp_flg
  if "l1%2 != 0" {
    2u
    print "error: l1 should be set as an even number !!!  "
    goto HaltAcqu
  }

  if "cnst15 > 4" {
    2u
```

```
    print "error: pl15 is too high; < 4W !!!  "
    goto HaltAcqu
  }

  if "cnst35 > 60" {
    2u
    print "error: pl35 is too high; < 60W !!!  "
    goto HaltAcqu
  }

  if "tau_cp > 12m" {
    2u
    print "error: dipsi-2 cross polarization duration is too long; < 12ms !!!  "
    goto HaltAcqu
  }
#endif

#ifdef ip_flg
  if "cnst17 > 6" {
    2u
    print "error: pl17 is too high; < 6W !!!  "
    goto HaltAcqu
  }
#endif

#ifdef c_flg
  if "cnst22 > 50" {
    2u
    print "error: power for 13C adiabatic pulse (spw22) is too large !!!  "
    goto HaltAcqu
  }

  if "pwc_ad > 500us" {
    2u
    print "error: pwc_ad is too long"
    goto HaltAcqu
  }
#endif

#ifdef N_sel
  if "cnst32 > 55" {
    2u
    print "error: spw32 power (reburp) is too high !!!  "
    goto HaltAcqu
  }

  if "pwn_sl > 3m" {
    2u
    print "error: pwn_sl 15N reburp (selective) is too long"
    goto HaltAcqu
  }
#endif

#if defined(cp_flg) || !defined(cal_NB1)
  if "cnst12 > 6" {
    2u
    print "error: pl12 is too high; < 6W !!!  "
    goto HaltAcqu
  }

  if "pwn_ramp > 4.01m" {
    2u
    print "error: pwn_ramp is too long; < 4ms"
    goto HaltAcqu
  }

  if "pwn_lk < 125u" {
    2u
    print "error: pwn_lk is too short and spin lock power too high > 125 us"
```

```
    goto HaltAcqu
  }
#endif

#ifndef cal_NB1
  if "time_relax_max > 205m" {
    2u
    print "error: time_relax_max is too long"
    goto HaltAcqu
  }

  if "cnst31 > 8" {
    2u
    print "error: dpwr2 pl31 too large !!!  "
    goto HaltAcqu
  }
#endif

  if "cnst33 > 25" {
    2u
    print "error: pl33 for 15N spin lock too large !!!  "
    goto HaltAcqu
  }

  if "aq > 64m" {
    2u
    print "error: aq is too long"
    goto HaltAcqu
  }

  if "d1 < 2.5s" {
    2u
    print "error: d1 is too short - >= 2.5s"
    goto HaltAcqu
  }

2 d11 do:f3
  10u fq=0:f1
  10u fq=0:f3
  2u pl3:f3              /*  power(dhpwr2) */

/*****************************************/
/* Continue to check real time variables */
/*****************************************/
#ifndef cal_NB1
  "DELTA = time_relax[l2]"
  if "DELTA > 201m" {
    2u
    print "error: time_relax cannot be greater than 200m"
    goto HaltAcqu
  }

  "DELTA = time_relax_max - time_relax[l2]"
  if "DELTA <= 2u" {
    2u
    print "error: time_relax cannot be greater than time_relax_max"
    goto HaltAcqu
  }
#endif

/*************************************************/
/*   Presaturation Period                        */
/*   option for Messrlie purge zgoptn -Dmess_flg   */
/*************************************************/
#ifdef mess_flg
  20u pl11:f1
  (dly_pg1 ph26):f1
  20u
  (dly_pg2 ph27):f1
```

```
  20u
#endif /*mess_flg*/

#ifdef fsat
#if defined(N_comp) && !defined(cal_NB1)
  if "abs(N_offset)*1e6 > 10000" {
    "DELTA2 = time_relax_max"
  }
  else {
    "DELTA2 = time_relax_max - time_relax[l2]"
  }

  10u fq=cnst3:f3
  2u pl33:f3

  DELTA2 cw:f3 ph26
  2u do:f3

  2u pl3:f3
  10u fq=0:f3

  2u pl10:f1

  "DELTA1 = d1 - DELTA2"
  DELTA1 cw:f1 ph26
  2u do:f1

  2u pl1:f1
#else
  2u pl10:f1
  d1 cw:f1 ph26
  2u do:f1
  2u pl1:f1
#endif /*N_comp && !cal_NB1*/
#ifdef fscuba
  hscuba
  (pwh ph26):f1
  (pwh*2 ph27):f1
  (pwh ph26):f1
  hscuba
#endif /*fscuba*/
#else
#if defined(N_comp) && !defined(cal_NB1)
  if "abs(N_offset)*1e6 > 10000" {
    "DELTA2 = time_relax_max"
  }
  else {
    "DELTA2 = time_relax_max - time_relax[l2]"
  }

  10u fq=cnst3:f3
  2u pl33:f3

  DELTA2 cw:f3 ph26
  2u do:f3

  2u pl3:f3
  10u fq=0:f3

  2u pl1:f1

  "DELTA1 = d1 - DELTA2"
  DELTA1
#else
  2u pl1:f1
  d1
#endif /*N_comp && !cal_NB1*/
#endif /*fsat*/
```

```
/*****************************************/
/*  Erase 15N equilibrium magnetization  */
/*****************************************/
  20u UNBLKGRAD
  (pwn ph26):f3

  2u
  p51:gp1
  d16

/*****************************/
/*   Forward H->N transfer   */
/*****************************/
#ifdef cp_flg
  10u fq=cnst1:f1 rpp11 rpp12

  (pwh ph26):f1
  2u pl15:f1 pl35:f3

4 (center (pw_dip*3.556 ph11):f1 (pw_dip*3.556 ph11):f3)
  (center (pw_dip*4.556 ph12):f1 (pw_dip*4.556 ph12):f3)
  (center (pw_dip*3.222 ph11):f1 (pw_dip*3.222 ph11):f3)
  (center (pw_dip*3.167 ph12):f1 (pw_dip*3.167 ph12):f3)
  (center (pw_dip*0.333 ph11):f1 (pw_dip*0.333 ph11):f3)
  (center (pw_dip*2.722 ph12):f1 (pw_dip*2.722 ph12):f3)
  (center (pw_dip*4.167 ph11):f1 (pw_dip*4.167 ph11):f3)
  (center (pw_dip*2.944 ph12):f1 (pw_dip*2.944 ph12 ipp12):f3)
  (center (pw_dip*4.111 ph11):f1 (pw_dip*4.111 ph11 ipp11):f3)
  lo to 4 times l1

  2u pl1:f1 pl3:f3
  (lalign (pwh ph28):f1 (pwn ph1):f3)

  10u fq=0:f1
#else
  (pwh ph26):f1

  2u
  p52:gp2
  d16

#ifdef N_sel
  "DELTA = taua - 2u - p52 - d16 - pwn_sl*0.5"
#else
  "DELTA = taua - 2u - p52 - d16"
#endif
  DELTA

#ifdef N_sel
  (center (pwh*2 ph26):f1 (pwn_sl:sp32 ph26):f3)
#else
  (center (pwh*2 ph26):f1 (pwn*2 ph26):f3)
#endif

  DELTA pl3:f3

  2u
  p52:gp2
  d16

  (pwh ph27):f1
  (pwn ph1):f3

  2u
  p54:gp4
  d16

  "DELTA = taub - 2u - p54 - d16"
  DELTA
```

```
  (center (pwh*2 ph26):f1 (pwn*2 ph26):f3)

  DELTA

  2u
  p54:gp4
  d16

  (pwn ph27):f3
  (pwh ph28):f1
#endif /*cp_flg*/

  2u
  p55:gp5
  d16

  "DELTA = time_eq*0.5"
  DELTA

/************************/
/*   15N R1rho period   */
/************************/
#ifndef cal_NB1
  10u fq=cnst1:f1
  10u fq=N_offset:f3
  2u pl33:f3

  /* Align water magnetization with effective field */
  (pwh ph5):f1
  "DELTA = larger(pw_ml*2.0/PI - pwh*4.0/PI, TAU2)"
  DELTA

  (pwh ph26):f1
  (pwh*2 ph5):f1

  "DELTA = larger(pwh*2.0/PI - 2u, TAU2)"
  DELTA
  /* End of align */

  2u pl12:f1
  (2u cw ph26):f1

6 2u
  "DELTA = (time_relax_max - time_relax[l2])*0.5/num_sl + time_eq*0.5 - 2u"
  DELTA

  if "abs(N_offset)*1e6 > 10000" {
    "DELTA = pwn_ramp + time_relax[l2]/num_sl + 2u + pwn_ramp"
    DELTA
  }
  else {
    if "N_offset > 0" {
      (pwn_ramp:sp35(currentpower) ph26):f3
    }
    else {
      (pwn_ramp:sp33(currentpower) ph26):f3
    }

    "DELTA = time_relax[l2]/num_sl"
    DELTA cpds5:f3 ph26
    2u do:f3

    if "N_offset > 0" {
      (pwn_ramp:sp36(currentpower) ph26):f3
    }
    else {
      (pwn_ramp:sp34(currentpower) ph26):f3
    }
```

```
  }

  "DELTA = (time_relax_max - time_relax[l2])*0.5/num_sl + time_eq*0.5"
  DELTA
  lo to 6 times num_sl

  2u do:f1
  2u pl1:f1

  /* Realign water magnetization along Z */
  "DELTA = larger(pwh*2.0/PI - 2u - 2u, TAU2)"
  DELTA

  (pwh*2 ph6):f1
  (pwh ph26):f1
  "DELTA = larger(pw_ml*2.0/PI - pwh*4.0/PI, TAU2)"
  DELTA

  (pwh ph6):f1
  /* End of realign */

  10u fq=0:f1
  10u fq=0:f3
  2u pl3:f3
#endif /*cal_NB1*/

  "DELTA = time_eq*0.5"
  DELTA

  2u
  p55:gp5
  d16

/*****************************************************************************/
/*  Choose whether to maintain in-phase magnetization during t1 or not  */
/*****************************************************************************/
#ifdef ip_flg
  "t1_max = (ni-1)*in0*2 + in0 + 2u"
  "DELTA = larger(t1_max-d0*2, TAU2)"

  2u pl17:f1
  (pwh_cpd ph27):f1
  (2u cpds1 ph26):f1

  DELTA

  (pwn ph2):f3

#ifdef c_flg
  if "d0 - pwc_ad*0.5 > 2u" {
    "DELTA = d0 - pwc_ad*0.5"
    DELTA
    (pwc_ad:sp22 ph26):f2
    DELTA
  }
  else {
    d0
    d0
  }
#else
  d0
  d0
#endif /*c_flg*/

  2u do:f1
  (pwh_cpd ph29):f1
  2u pl1:f1

  2u
```

```
  p56:gp6*1.0
  d16

  "DELTA = taub - 2u - pwh_cpd - 2u - 2u - p56 - d16"
  DELTA

  (center (pwh*2 ph26):f1 (pwn*2 ph3):f3)

  "DELTA = taub - 2u - p56 - d16"
  DELTA

  2u
  p56:gp6*-1.0
  d16

#else
  (pwn ph2):f3

  "DELTA = taub - 2u - p56 - d16 + 32u + 100u + pwh*2.0"
  DELTA

  2u
  p56:gp6*1.0
  d16

  (pwn*2 ph3):f3

  32u gron0*-1.0
  d0
  100u groff

  (pwh*2 ph26):f1

  2u
  p56:gp6*-1.0
  d16

#ifdef c_flg
  "DELTA = taub - 2u - p56 - d16 - 32u - 100u - pwc_ad"
  DELTA

  (pwc_ad:sp22 ph26):f2
#else
  "DELTA = taub - 2u - p56 - d16 - 32u - 100u"
  DELTA
#endif

  32u gron0*1.0
  d0
  100u groff
#endif /*ip_flg*/

  (center (pwh ph26):f1 (pwn ph4):f3)

  2u
  p57:gp7
  d16

  "DELTA = taua - 2u - p57 - d16"
  DELTA

  (center (pwh*2 ph26):f1 (pwn*2 ph26):f3)

  DELTA

  2u
  p57:gp7
  d16
```

```
  (center (pwh ph27):f1 (pwn ph29):f3)

  2u
  p58:gp8
  d16

  "DELTA = taua - 2u - p58 - d16 - (pwn - pwh)*0.5"
  DELTA

  (center (pwh*2 ph26):f1 (pwn*2 ph26):f3)

  "DELTA = taua - 2u - p58 - d16"
  DELTA

  2u
  p58:gp8
  d16

  (pwh ph28):f1

  BigT1

#ifdef cal_NB1
  if "l3%2 == 0" {
    (center (pwh*2 ph26):f1 (pwn ph26 pwn ph28):f3)
  }
  else {
    (center (pwh*2 ph26):f1 (pwn*2 ph26):f3)
  }
#else
  (pwh*2 ph26):f1
#endif

  2u
  p59:gp9*EA
  d16

  4u BLKGRAD

  "DELTA = BigT1 - 2u - p59 - d16 - 4u - de - 10u - 2u + pwh*2.0/PI"
  DELTA

#ifdef cal_NB1
  10u fq=cnst4:f3
  2u pl33:f3
#else
  10u
  2u pl31:f3
#endif /*cal_NB1*/

/*************************************/
/*   Test how much water left        */
/*   set d13=AQ and zgoptn -Dwater   */
/*   also use small value of rg      */
/*************************************/
#ifdef water
  if "nsdone == 0" {
    d13
    d1
    (pwh*0.1 ph26):f1
  }
  else {
    d13
  }
#endif

/*************************************/
/*   Signal detection and looping    */
/*************************************/
```

```
#ifdef cal_NB1
  if "l3%2 == 0" {
    go=2 ph30 cpds5:f3
  }
  else {
    go=2 ph31 cpds5:f3
  }
#else
  go=2 ph30 cpds3:f3
#endif /*cal_NB1*/

  d11 do:f3 mc #0 to 2
#ifdef cal_NB1
  F3QF(calclc(l3,1))     /* IP or AP */
  F2QF(calclc(l2,0))     /* T2 points */
#else
  F3QF(calclist(N_offset,1))
  F2QF(calclc(l2,1))     /* T2 points */
#endif /*cal_NB1*/
  F1EA(calgrad(EA) & calph(ph4, +180), caldel(d0, +in0) & calph(ph2, +180) & calph(ph3
0, +180) & calph(ph31, +180))

HaltAcqu, 1m
exit

ph1=0 2
ph2=1
ph3=0 0 1 1 2 2 3 3
ph4=0
ph5=1 1 3 3
ph6=3 3 1 1
ph11=1 3 3 1
ph12=3 1 1 3
ph26=0
ph27=1
ph28=2
ph29=3
ph30=0 2 2 0
ph31=1 3 3 1

;d1 : Repetition delay d1
;d3 : taua < 1/4J(NH) 2.38 ms
;d5 : taub = 1/4J(NH) 2.68 ms
;d11 : delay for disk i/o, 30ms
;d14 : BigT1 delay at end for gradient set to 500 u
;d16 : gradient recovery delay, 200us
;d17 : time_relax_max set to MAX of 200 ms only for large offsets of spin lock
;d19 : time_eq
;pl1 : tpwr - power level for pwh
;pl3 : dhpwr2 - power level for 15N pulse pwn
;pl10 : tsatpwr - power level for water presat
;pl11 : tpwrmess - power level for Messerle purge
;pl12 : power level for 1H cw decoupling during 15N SL
;pl15 : power level for 1H dipsi2 cross-polarization
;pl17 : power level for 1H cpd decoupling during t1
;pl22 : d_ad - power level for pwc_ad = p22
;pl31 : dpwr2 - power level for 15N cpd3
;pl33 : power level for 15N spin lock for T1rho
;pl35 : power level for 15N dipsi2 cross-polarization
;spw32 : power level for reburp on 15N (selective)
;p1 : pwh - 1H 90 degree pulse
;p3 : pwn - 15N 90 degree pulse
;p12 : pw_ml -  90 degree pulse for 1H cw-decoupling
;p15 : pw_dip -  90 degree pulse for dipsi-2
;p17 : pwh_cpd -  90 degree pulse for 1H cpd-decoupling
;p22 : pwc_ad - 13C 180o adiabatic pulse
;p32 : pwn_sl for selective pulse on amide N15s
;p33 : pwn_ramp, time for ramp up and ramp down; typically 4 ms at pl33
;p34 : pwn_lk >= 125 us at power of pl33
```

```
;p51 : gradient pulse 51                                    [1000 usec]
;p52 : gradient pulse 52                                    [500 usec]
;p54 : gradient pulse 54                                    [500 usec]
;p55 : gradient pulse 55                                    [1000 usec]
;p56 : gradient pulse 56                                    [625 usec]
;p57 : gradient pulse 57                                    [256 usec]
;p58 : gradient pulse 58                                    [256 usec]
;p59 : gradient pulse 59                                    [256 usec]
;spnam22 : shape for 13C adiabatic pulse
;spnam32 : shape for 15N selective pulse to remove Arg
;spnam33 : shape for ramp up pulse, for N_offset <= 0
;spnam34 : shape for ramp down pulse, for N_offset <= 0
;spnam35 : shape for ramp up pulse, for N_offset > 0
;spnam36 : shape for ramp down pulse, for N_offset > 0
;cpd1 : 1H Waltz-16 decoupling during t1
;pcpd1 : 90 degree pulse for cpd1
;cpdprg1 : waltz16, used for 1H decoupling during t1
;cpd3 : 15N decoupling according to program defined by cpdprg3
;pcpd3 : 1/dmf2 - 90 degree pulse for cpd3
;cpdprg5 : cwp, used for 15N spin-lock
;cnst1 : set to center of NHs - o1 (Hz)
;cnst3 : set to far from o3 for heating at the start. Suggest 500000 (or further)
;cnst4 : set to far from o3 for calibrating J-couplings. Suggest -2000 (B1 = 2kHz)
;cnst12 : power in w for 1H CW during Trelax
;cnst15 : power in w for 1H dipsi-2 cross-polarization
;cnst17 : power in w for 1H Waltz during t1
;cnst35 : power in w for 15N dipsi-2 cross-polarization
;l1 : loop counter for dipsi-2 cycle
;l2 : pointer to Trelax delay time
;l3 : pointer to switch between IP and AP
;l5 : number of 15N spin-lock periods
;inf1 : 1/SW(X) = 2*DW(X)
;in0 : 1/(2*SW(x))=DW(X)
;nd0 : 2
;ns : 2*n
;FnMODE : echo-antiecho

;use gradient ratio:    gp 6 : gp 9
;                         80 : 39.6

;for z-only gradients:
;gpz0: 0.25%
;gpz1: 15%
;gpz2: 25%
;gpz4: 50%
;gpz5: 70%
;gpz6: 80%
;gpz7: 60%
;gpz8: 15%
;gpz9: 39.6%

;use gradient files:
;gpnam1: SMSQ10.32
;gpnam2: SMSQ10.32
;gpnam4: SMSQ10.32
;gpnam5: SMSQ10.32
;gpnam6: SMSQ10.32
;gpnam7: SMSQ10.32
;gpnam8: SMSQ10.32
;gpnam9: SMSQ10.32

;zgoptns: Dfsat, Dfscuba, Df1180, Dfsat, Dmess_flg, Dc_flg, DN_sel, DN_comp, Dip_flg,
Dcp_flg, Dcal_NB1
```