Supporting Information

Evaluating the influence of initial magnetization conditions on extracted exchange parameters in NMR relaxation experiments: applications to CPMG and CEST

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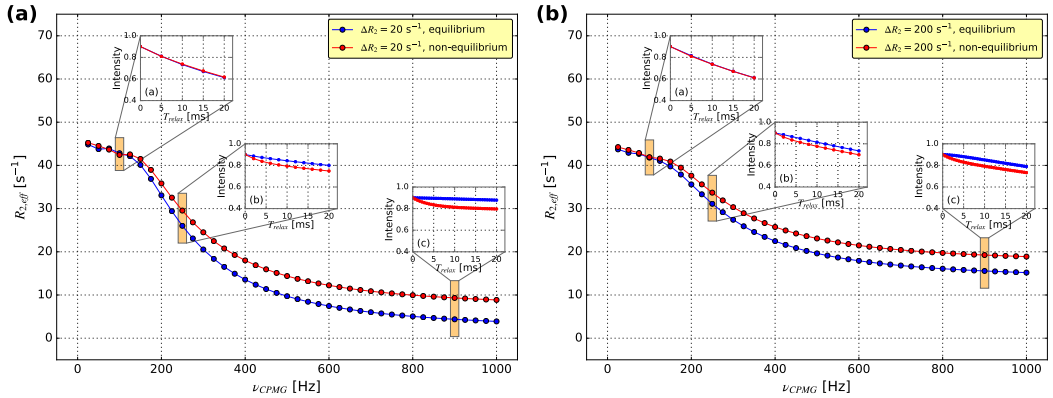

Fig. S1 Simulated ^{15}N CPMG relaxation dispersion profiles (500 MHz) for a single residue undergoing two-site chemical exchange using the following parameters: $T_{relax} = 20$ ms, $p_E = 0.10$, $k_{ex} = 500$ s^{-1}, $\Delta\varpi_{GE} = 5$ ppm, $R_{2,G} = 0$ s^{-1} and $R_{2,E} = 20$ s^{-1} (panel **a**) or $R_{2,E} = 200$ s^{-1} (panel **b**).

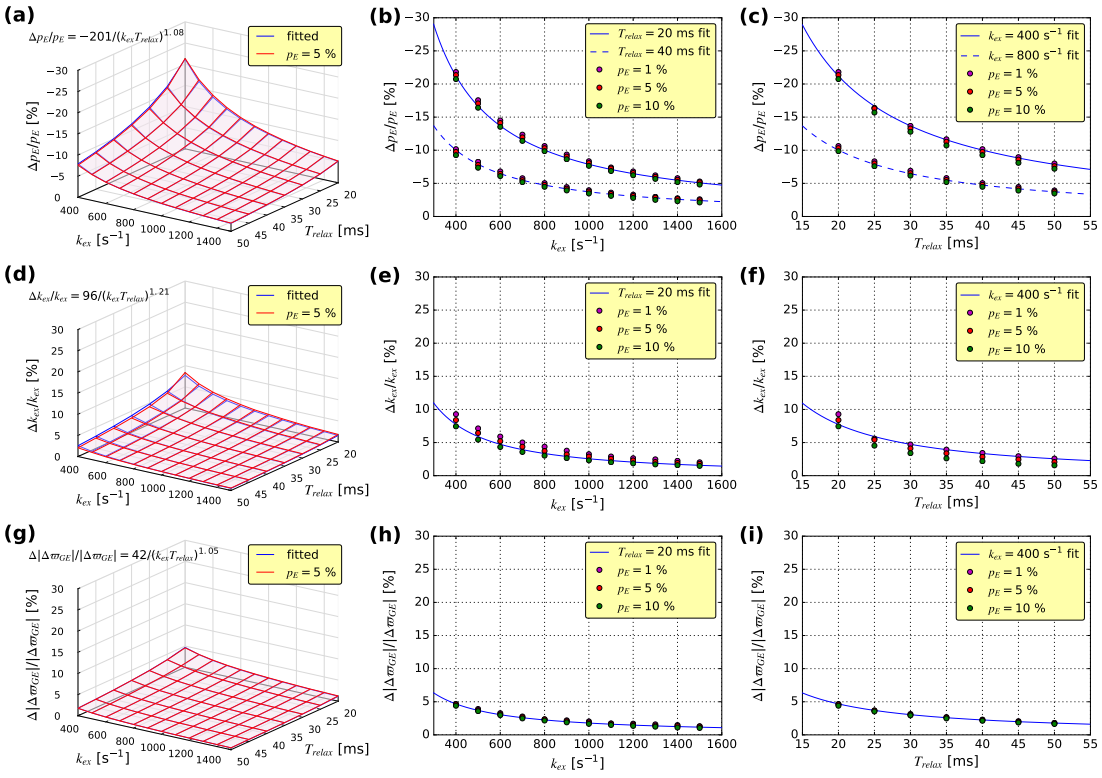

Fig. S2 Systematic errors in exchange parameters from fitting ^{15}N CPMG dispersion profiles generated using 'non-equilibrium' initial conditions with a model that assumed 'equilibrium' boundary conditions, as in Figure 2 (main text), but with data generated with $R_{2,G} = 0$ s^{-1}, $R_{2,E} = 20$ s^{-1}.

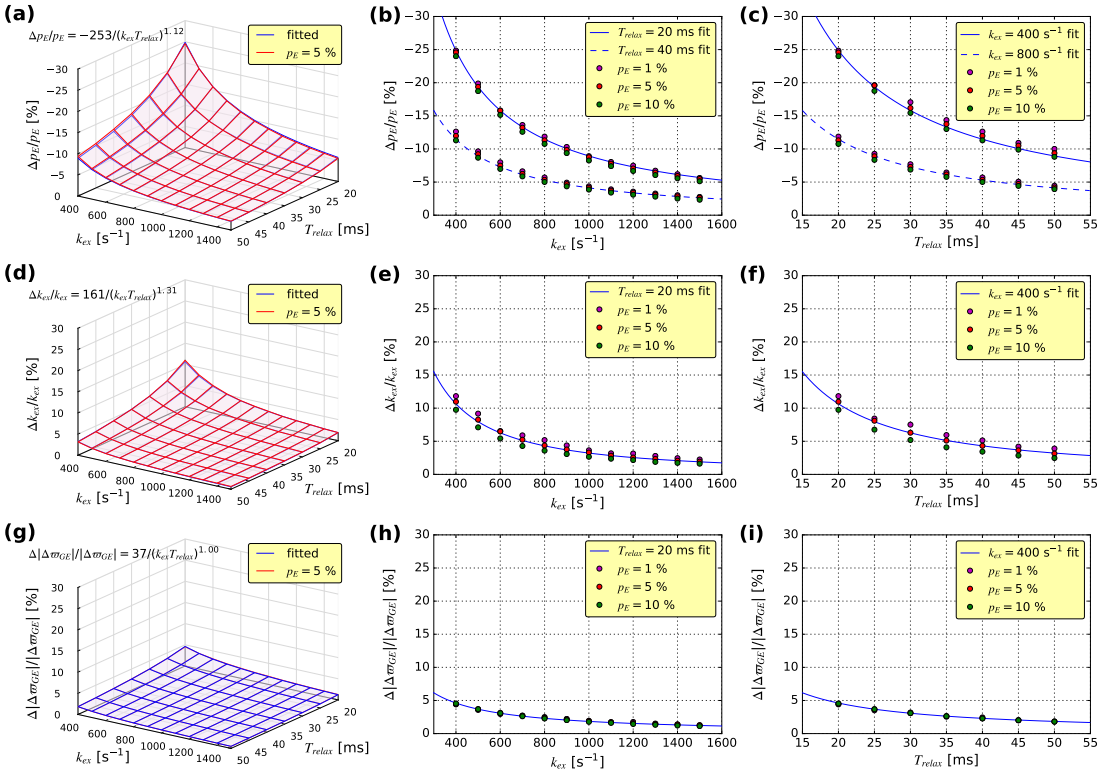

Fig. S3 As in Figure S2 but with $R_{2,G} = 0$ s^{-1}, $R_{2,E} = 200$ s^{-1}.

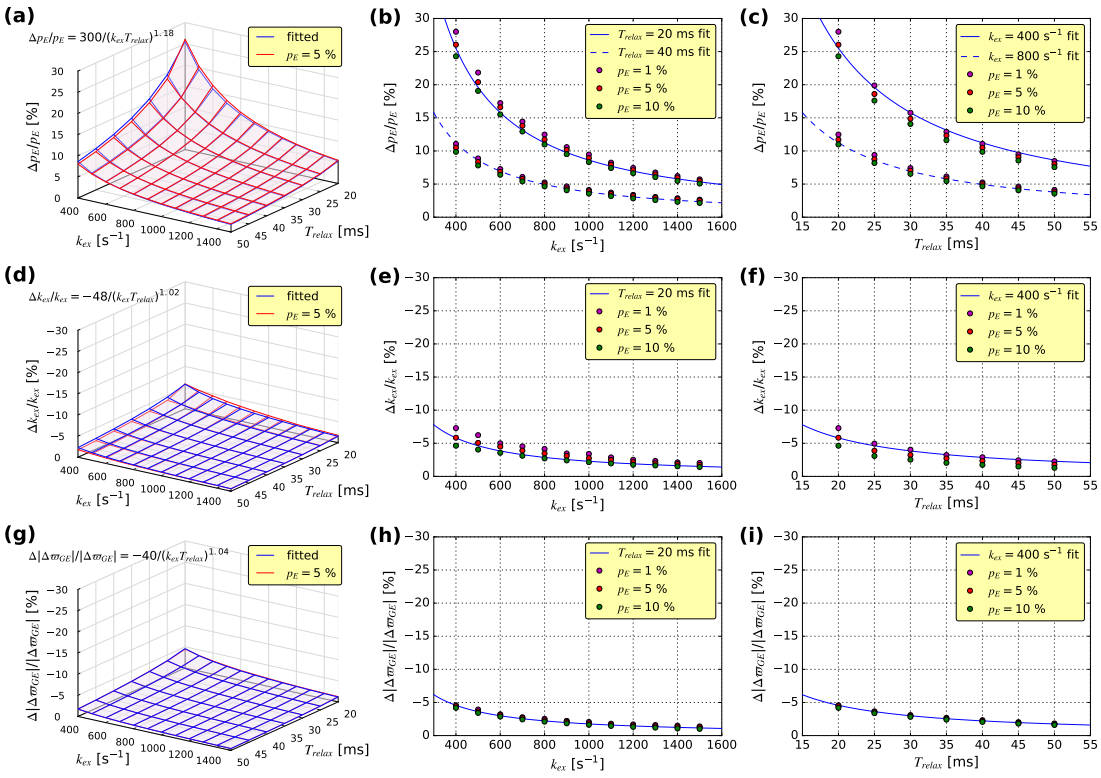

Fig. S4 As in Figure 2 of the main text but where CPMG data sets simulated with 'equilibrium' initial boundary conditions were fit with a model that assumes 'non-equilibrium' conditions. $\Delta R_2 = 0$ s^{-1}.

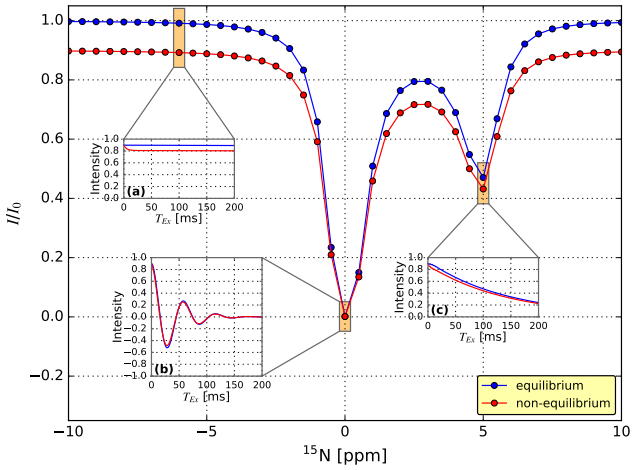

Fig. S5 ^{15}N CEST profiles (600 MHz) for single residue undergoing two-site chemical exchange simulated with the following parameters: $T_{Ex} = 200$ ms, $p_E = 0.10$, $k_{ex} = 200$ s^{-1}, $\Delta\varpi_{GE} = 5$ ppm, $B_1 = 15$ Hz, $R_1 = 0$ s^{-1}, $R_2 = 10$ s^{-1}. Note small differences in intensities at the position of the minor dip when different initial boundary conditions are used (inset **c**).

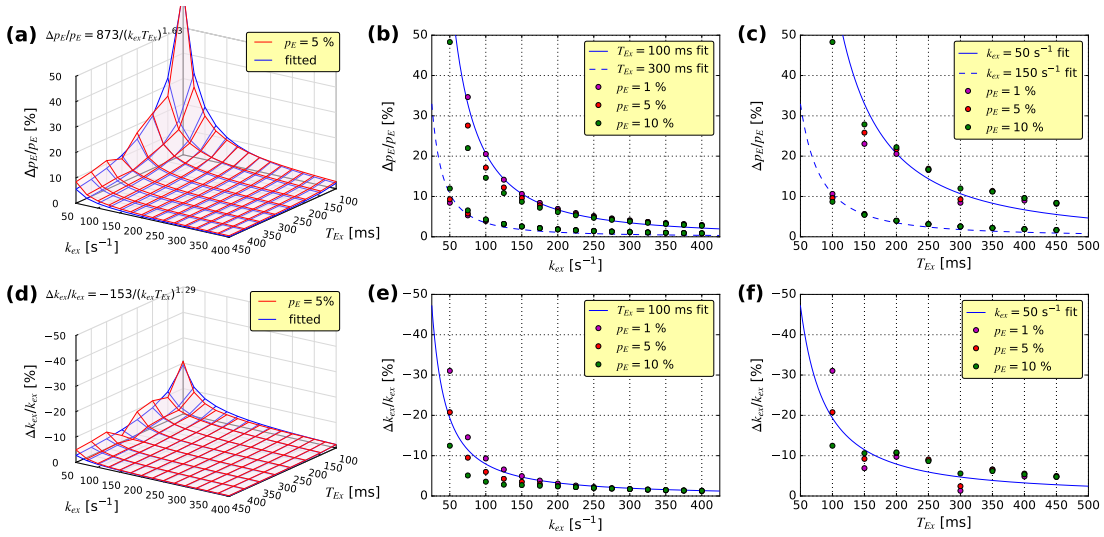

Fig. S6 As in Figure 4 of the text but where CEST profiles simulated with 'equilibrium' initial conditions were fit to a model that assumed 'non-equilibrium' boundary conditions.

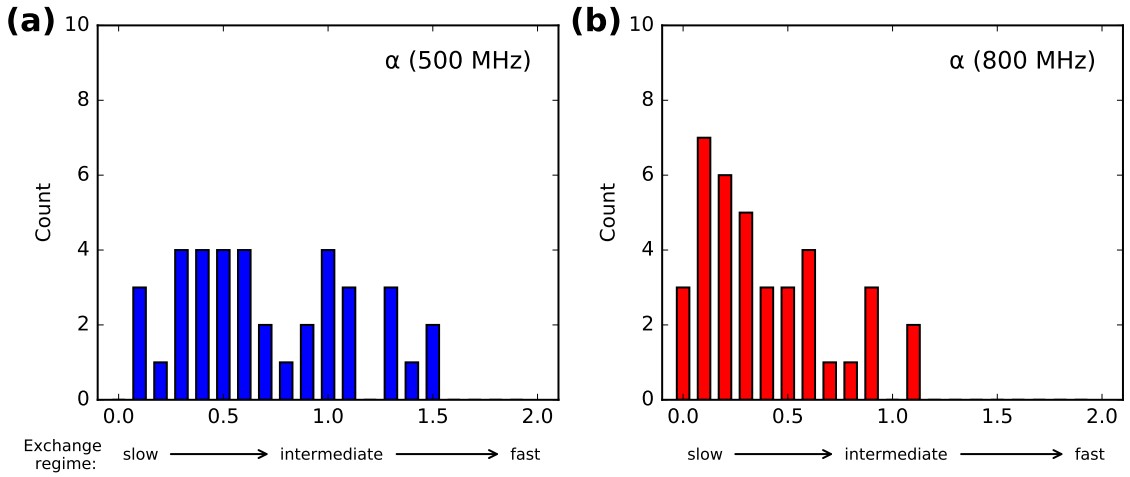

Fig. S7 Histogram of α values (Millet et al. 2000) as calculated for residues in L24A FF used in analyses described in the text. It is clear that a range of α values are represented by the experimental data.

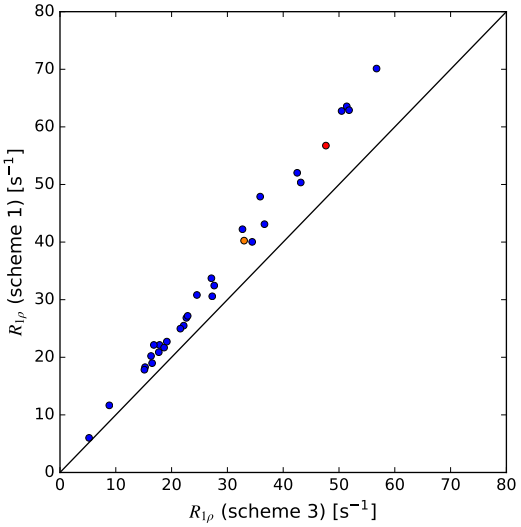

Fig. S8 Linear correlation plot of amide ^{1}H $R_{1\rho}$ rates from the intrinsically disordered 4E-BP2 protein, 600 MHz, 30 °C, measured using schemes 1 (y-axis) and 3 (x-axis) (see Figure 6). In total 31 non-overlapping residues were included in the analysis. The correlation coefficient between the two data sets is $r^2 = 0.996$ and the mean value of $R_{1\rho}$ obtained from scheme 1 is 6 s^{-1} larger than from scheme 3 due to ^{1}H–^{1}H cross-relaxation effects. The ratio of ^{1}H $R_{1\rho}$ rates is much lower than the predicted value of 1.8 due to significant contributions from solvent exchange. Points corresponding to G6 and R20 that are highlighted in Figure 6 of the main text are colored in red and orange, respectively.

Table S1 The pre-factor A and the exponential factor c, for the equation $A/(k_{ex}T_{relax})^c$ describing the relative errors in fitting parameters when CPMG profiles generated with 'equilibrium' boundary conditions are fit using non-equilibrium initial values of magnetization.

	$\Delta R_2 = 0\ s^{-1}$		$\Delta R_2 = 20\ s^{-1}$		$\Delta R_2 = 200\ s^{-1}$	
	A	c	A	c	A	c
p_E	300	1.18	312	1.19	498	1.30
k_{ex}	−48	1.02	−53	1.04	−113	1.21
$\Delta\varpi_{GE}$	−40	1.04	−40	1.04	−39	1.02

Table S2 The pre-factor A and the exponential factor c, for the equation $A/(k_{ex}T_{Ex})^c$ describing the relative errors in fitting parameters when CEST profiles generated with 'equilibrium' boundary conditions are fit using non-equilibrium initial values of magnetization. The ground and excited state spins are assumed to have the same R_1 rate, indicated in the top horizontal line.

	$R_1 = 0\ s^{-1}$		$R_1 = 2\ s^{-1}$		$R_1 = 4\ s^{-1}$	
	A	c	A	c	A	c
p_E	526	1.44	873	1.63	2883	2.13
k_{ex}	−97	1.13	−153	1.29	−340	1.59

References

Millet O, Loria JP, Kroenke CD, Pons M, Palmer AG (2000) The static magnetic field dependence of chemical exchange linebroadening defines the NMR chemcial shift time scale. J Am Chem Soc 122:2867-2877