Supporting Information:

Unraveling the complexity of protein backbone dynamics with combined ¹³C and ¹⁵N solid state NMR relaxation measurements

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1. Spectral densities

1.1 Solution spectral densities

(a) Simple model free (SMF):¹

$$J(\omega) = (1 - S_f^2) \frac{\tau}{1 + (\omega\tau)^2} + S_f^2 \frac{\tau_R^{eff}}{1 + (\omega\tau_R^{eff})^2}, \frac{1}{\tau} = \frac{1}{\tau_f} + \frac{1}{\tau_R^{eff}}$$
(1)

 S_f^2 is the order parameter for internal motion, τ_f is the correlation time for internal motion and τ_R^{eff} is the correlation time for the overall rotational diffusion.

(b) Extended model free (EMF):²

$$J(\omega) = S_{f}^{2} S_{s}^{2} \frac{\tau_{R}^{eff}}{1 + (\omega \tau_{R}^{eff})^{2}} + (1 - S_{f}^{2}) \frac{\tau_{f}^{'}}{1 + (\omega \tau_{f}^{'})^{2}} + S_{f}^{2} (1 - S_{s}^{2}) \frac{\tau_{s}^{'}}{1 + (\omega \tau_{s}^{'})^{2}}, \frac{1}{\tau_{s}^{'}} = \frac{1}{\tau_{s}} + \frac{1}{\tau_{R}^{eff}}, \frac{1}{\tau_{f}^{'}} = \frac{1}{\tau_{f}} + \frac{1}{\tau_{R}^{eff}},$$
(2)

 S_f^2 is the order parameter for fast internal motion, S_s^2 is the order parameter for slow internal motion, τ_f is the correlation time for fast internal motion, τ_s is the correlation time for slow internal motion and τ_R^{eff} is the correlation time for the overall rotational diffusion.

1.2 Solid state spectral densities

(a) Simple model free (SMF) – motion modeled using single time scale (τ_{eff}) and amplitude (S^2) of isotropic motion:³

$$J(\omega) = (1 - S^2) \frac{\tau_{eff}}{1 + (\omega \tau_{eff})^2}$$
(3)

(b) Extended model free (EMF) – motion modeled using fast (τ_f, S_f^2) and slow (τ_s, S_s^2) isotropic motions:⁴

$$J(\omega) = (1 - S_f^2) \frac{\tau_f}{1 + (\omega \tau_f)^2} + S_f^2 (1 - S_s^2) \frac{\tau_s}{1 + (\omega \tau_s)^2}$$
(4)

2. Relaxation rates

2.2 Spin-lattice relaxation

The rank for the spectral density indicated with subscript is retained for record keeping purposes. With the models considered below the subscript can be omitted, as the different rank spectral densities are the same.

(a) Dipolar ¹H-¹⁵N dipolar contribution to ¹⁵N R_1 :

$$R_{1,NH} = \frac{1}{10} \left(\frac{\mu_0}{2\pi} \frac{\gamma_H \gamma_N}{\hbar r_{NH}^3} \right)^2 \left(J_0 \left(\omega_H - \omega_N \right) + 3J_1(\omega_N) + 6J_2(\omega_H + \omega_N) \right)$$
(5)

Assumptions: We consider contribution from the directly bonded proton and the nearby non-directly bonded protons. The effective distance⁵ for non-directly bonded protons contribution was on average estimated to be ~ 1.8 Å.

(b) CSA contribution to ${}^{15}N R_1$:

$$R_{1,NCSA} = \frac{2}{15}\omega_N^2(\sigma_{11}^2 + \sigma_{22}^2 + \sigma_{33}^2 - \sigma_{11}\sigma_{22} - \sigma_{11}\sigma_{33} - \sigma_{22}\sigma_{33})J_1(\omega_N)$$
(6)

S2

¹⁵N CSA: $\sigma_{11} > \sigma_{22} > \sigma_{33}$. The components were parameterized using nitrogen isotropic chemical shift based on linear fits of the CSA components versus isotropic chemical shift for solid-state NMR CSA measurements on crystalline GB1.⁶ The values are given in Table S2.

(c) CSA contribution to 13 C' R_1 .

$$R_{1,C',CSA} = \frac{2}{15}\omega_C^2 \left(\sigma_{11}^2 + \sigma_{22}^2 + \sigma_{22}^2 - \sigma_{11}\sigma_{22} - \sigma_{12}\sigma_{33} - \sigma_{22}\sigma_{33}\right) J_1(\omega_C)$$
(7)

¹³C' CSA: $\sigma_{11} > \sigma_{22} > \sigma_{33}$. The components were parameterized using carbonyl isotropic chemical shift based on linear fits of the CSA components versus isotropic chemical shift for solid-state NMR CSA measurements on crystalline GB1 and are given in Table S2.⁷

(d) Dipolar ¹³C-¹³C contribution to ¹³C' R_1 :

$$R_{1,C'C\alpha} = \frac{1}{10} \left(\frac{\mu_0}{2\pi} \frac{\gamma_C \gamma_C}{\hbar r_{C'C\alpha}^3} \right)^2 \left(J_0 \left(\omega_{C'} - \omega_{C\alpha} \right) + 3J_1(\omega_{C'}) + 6J_2(\omega_{C'} + \omega_{C\alpha}) \right)$$
(8)

Assumptions: $J_0(\omega_{C'} - \omega_{C\alpha})$ was evaluated at a frequency corresponding to 120 ppm for ¹³C.

(e) Dipolar ¹H-¹³C contribution to ¹³C' R_1 :

$$R_{1,C'H} = \frac{1}{10} \left(\frac{\mu_0}{2\pi} \frac{\gamma_H \gamma_C}{\hbar r_{C'H}^3} \right)^2 \left(J_0 \left(\omega_H - \omega_C \right) + 3J_1(\omega_C) + 6J_2(\omega_H + \omega_C) \right)$$
(9)

Assumptions: We considered directly contribution from amide proton and the contribution from non-directly bonded protons. The effective distance⁵ for non-directly bonded protons contribution was on average estimated to be ~ 1.82 Å.

(f) Dipolar ¹³C'-¹⁵N contribution to ¹³C' R_1 :

$$R_{1,C'N} = \frac{1}{10} \left(\frac{\mu_0}{2\pi} \frac{\gamma_C \gamma_N}{\hbar r_{C'N}^3} \right)^2 \left(J_0 \left(\omega_C - \omega_N \right) + 3J_1(\omega_C) + 6J_2(\omega_C + \omega_N) \right)$$
(10)

(g) Dipolar ¹³C α -¹⁵N contribution to ¹⁵N R_1 :

$$R_{1,C\alpha N} = \frac{1}{10} \left(\frac{\mu_0}{2\pi} \frac{\gamma_C \gamma_N}{\hbar r_{C\alpha N}^3} \right)^2 \left(J_0 \left(\omega_C - \omega_N \right) + 3J_1(\omega_N) + 6J_2(\omega_C + \omega_N) \right)$$
(11)

2.3 Spin-lattice relaxation rate in the rotating frame

(a) Dipolar contribution to ¹⁵N R_{10} :

$$R_{1\rho,NH} = \frac{1}{20} \left(\frac{\mu_0}{2\pi} \frac{\gamma_H \gamma_N}{\hbar r_{NH}^3} \right)^2 (4J_0(\omega_1) + 3J_1(\omega_N) + J_0(\omega_H - \omega_N) + 6J_1(\omega_H) + 6J_2(\omega_H + \omega_N))$$
(12)

Assumptions: We also investigated the influence of non-directly bonded protons. The effective distance⁵ for such contributions was on average estimated to be 1.8 Å. The order parameter was assumed to be the same as for NH.

(b) CSA contribution to ${}^{15}N R_{10}$:

$$R_{1\rho,N,CSA} = \frac{1}{45} \left(\sigma_{11}^2 + \sigma_{22}^2 + \sigma_{33}^2 - \sigma_{11}\sigma_{22} - \sigma_{11}\sigma_{33} - \sigma_{22}\sigma_{33} \right) \left(4J_0(\omega_1) + 3J_1(\omega_N) \right)$$
(13)

(c) CSA contribution to 13 C' $R_{1\varrho}$.

$$R_{1\rho,C',CSA} = \frac{1}{45}\omega_C^2 \left(\sigma_{11}^2 + \sigma_{22}^2 + \sigma_{33}^2 - \sigma_{11}\sigma_{22} - \sigma_{11}\sigma_{33} - \sigma_{22}\sigma_{33}\right) \left(4J_0(\omega_1) + 3J_1(\omega_C)\right)$$
(14)

(d) Dipolar ¹³C-¹³C contribution to ¹³C' $R_{1\varrho}$.

$$R_{1\rho,C'C\alpha} = \frac{1}{20} \left(\frac{\mu_0}{2\pi} \frac{\gamma_C \gamma_C}{\hbar r_{C'C\alpha}^3} \right)^2 (4J_0(\omega_1) + J_0(\omega_{C'} - \omega_{C\alpha}) + 9J_1(\omega_C) + 6J_2(2\omega_C))$$
(15)

Assumptions: $J_0(\omega_{C'} - \omega_{C\alpha})$ was evaluated at a frequency corresponding to 120 ppm for ¹³C.

(e) Dipolar ¹H-¹³C contribution to ¹³C' $R_{1\varrho}$.

$$R_{1\rho,C'H} = \frac{1}{20} \left(\frac{\mu_0}{2\pi} \frac{\gamma_C \gamma_H}{\hbar r_{C'H}^3} \right)^2 (4J_0(\omega_1) + 3J_1(\omega_C) + J_0(\omega_H - \omega_{C\alpha}) + 6J_1(\omega_H) + 6J_2(\omega_H + \omega_C))$$
(16)

Assumptions: We also investigated the influence of non-directly bonded protons. The effective distance⁵ for such contributions was on average estimated to be ~ 1.82 Å.

(f) Dipolar ¹³C'-¹⁵N contribution to ¹³C' $R_{1\rho}$:

$$R_{1\rho,C'N} = \frac{1}{20} \left(\frac{\mu_0}{2\pi} \frac{\gamma_C \gamma_N}{\hbar r_{C'N}^3} \right)^2 (4J_0(\omega_1) + 3J_1(\omega_C) + J_0(\omega_C - \omega_N) + 6J_1(\omega_N) + 6J_2(\omega_N + \omega_C))$$
(17)

(g) Dipolar ¹³Ca-¹⁵N contribution to ¹⁵N R_{1p} :

$$R_{1\rho,C\alpha N} = \frac{1}{20} \left(\frac{\mu_0}{2\pi} \frac{\gamma_C \gamma_N}{\hbar r_{C\alpha N}^3} \right)^2 (4J_0(\omega_1) + 3J_1(\omega_N) + J_0(\omega_C - \omega_N) + 6J_1(\omega_C) + 6J_2(\omega_N + \omega_C))$$
(18)

Table S2. Relaxation-active interactions in the peptide plane frame. The ¹⁵N and ¹³C' CSA components were parameterized using linear fits of the CSA components versus isotropic chemical shift for solid-state NMR CSA measurements on crystal-line GB1.^{6,7}

Relaxation active interaction	Geometrical and CSA parameters
¹⁵ N-H	1.02Å
¹⁵ N-CSA	$\sigma_{11}=1.1283\sigma_{iso}+93.77 \text{ (ppm)}^{6}$ $\sigma_{22}=1.0086\sigma_{iso}-42.475 \text{ (ppm)}^{6}$ $\sigma_{33}=0.8631\sigma_{iso}-51.295 \text{ (ppm)}^{6}$
15N-13C'	1.33 Å
15N-13Cα	1.46 Å
$13C'-13C^{\alpha}$	1.525 Å
¹³ C'-H ^N	2.04 Å
¹³ C'-CSA	$\sigma_{11}=0.24\sigma_{iso}+200 \text{ (ppm)}^{7}$ $\sigma_{22}=2.82\sigma_{iso}-305 \text{ (ppm)}^{7}$ $\sigma_{33}=96.5 \text{ (ppm)}^{7}$



Figure S1. Bulk carbonyl ¹³C $R_{1\varrho}$ in [U-¹³C, ¹⁵N]GB1, measured as a function of magic angle spinning (MAS) frequency (with a constant spin-lock amplitude of 17 kHz) at a field of 14.1 T (600 MHz ¹H Larmor frequency). Sample temperature was 27 °C for all experiments, as determined by the chemical shift of water with respect to DSS. Rates were found in 1D by measuring total carbonyl peak integrals at incrementally longer spin-lock pulses. The ¹³C spin-lock fields were calibrated from nutation experiments. ¹³C $R_{1\varrho}$ rates clearly plateau at spinning frequencies greater than ~45 kHz.

Magic angle mis-adjustment

As magic angle spinning plays a crucial role in averaging the interactions contributing to the coherent mechanisms for the magnetization decay, it is important to consider the influence of "mis-setting" the magic angle upon the efficiency of averaging by MAS. We examine the effect of mis-setting the angle of rotation on the measured coherence lifetimes in [1-¹³C]Ala in

Fig. S2, where the measured $R_{1\rho}$ rates for ¹³C' are plotted as a function of the ¹³C' line width measured in a cross-polarization (CP) experiment. At the magic angle (~54.736°) the ¹³C' line width was ~21 Hz, and from here the angle was systematically mis-adjusted up to a setting that yielded a ¹³C' line width of 54 Hz. In the explored range we found that the measured $R_{1\rho}$ changed by less than 2%, suggesting that the $R_{1\rho}$ measurement is relatively forgiving to a slight mis-adjustment of the magic angle.



Figure S2. ¹³C' $R_{1\varrho}$ in [1-¹³C]alanine as a function of deviation of the rotor axis from the magic angle. The horizontal axis depicts ¹³C' line width, itself a function of the rotor angle setting; 21 Hz corresponds to a "well-set" magic angle, while larger line width indicates larger deviation from the magic angle. Measurements were performed at $\omega_{0H}/2\pi = 600$ MHz, $\omega_{I}/2\pi = 600$ kHz and $\omega_{I}/2\pi = 17$ kHz.

Temperature effects

The ¹³C' R_{1p} experiment is relatively robust with respect to sample temperature changes (from r.f.-induced heating) during the spin-lock pulse. We measured the temperature change of the GB1 sample (50 mM salt, pH 5.5) at $\omega_{0H}/2\pi = 600$ MHz upon application of a ¹³C spin-lock pulse prior to acquisition. The temperature was measured based on the chemical shift of water protons (a Bruker au macro for calculating the temperature of the sample based on the chemical shift of water with respect to an internal DSS reference, including effects of pH and salt concentration, can be downloaded from http://www2.warwick.ac.uk/fac/sci/chemistry/research/lewandowski/lewandowskigroup/goodies/). For reference, the sample temperature without any ¹³C irradiation was 26.9 ± 0.5 °C. A total of 50 experiments with 0.3 s of 17 kHz spin-lock irradiation were performed (32 scans per experiment with a recycle delay of 2 s, resulting in a total time for each experiment of \sim 74 s) back to back, for a total of ~62 minutes. The measured sample temperature had increased by 1.5 ± 0.5 °C after a single experiment, but then remained at a constant 28.4 ± 0.5 °C for the remainder of the 62 minute run, showing that equilibrium is reached quickly (a few transients) without the long stabilization time observed for larger rotors at slower spinning frequencies and under the application of high power heteronuclear decoupling. As 0.3 s is at the limit of what must typically be sampled experimentally (we sampled to 0.2 s at 17 kHz for ¹³C, plus a combined 50 ms of 15 kHz slpTPPM decoupling during t_1 and t_2 acquisition periods), internal sample temperatures should not be expected to exceed a temperature 1.5 °C higher than equilibrium at any point during an R_{I_0} experiment. To examine the relationship between temperature increase and spinlock pulse length under typical experimental conditions (60 kHz MAS, 17 kHz spin-lock field strength), further test experiments were conducted with spin-lock pulses ranging from 10 µs to 300.01 ms (see Fig. S3). Naturally, the sample was observed to increase in temperature with increased pulse length, but at a rate of just ~ 0.005 K ms⁻¹ (assuming a linear relationship). The difference in sample temperature between different spin-lock lengths sampled is clearly very small (maximum of 1.5 °C difference between shortest and longest spin-lock pulses employed in an experiment) and for most purposes may be considered negligible. To investigate impact of such temperature changes on the measured ¹³C' R_{10} we have measured this parameter in microcrystalline 100% H₂O [U-²H, ¹³C, ¹⁵N]GB1 where lower spin lock nutation frequencies may be used without introducing large coherent contributions to the rates. For the measurements performed using 8 kHz spin lock nutation frequency (rf induced heating depends quadratically on the B_1 so it should be reduced ~5 times compared to 17 kHz) at 298

K and 303 K, i.e. difference of 5 K, the average site specific ¹³C' R_{1p} differed by 0.7 s⁻¹, which corresponds to ~15% of the rates. This means that at least in GB1 (it may be different for different systems) 1.5 °C difference in sample temperature should not result in rate differences larger than 5%. Currently, this is smaller than the experimental error of the measurement.. Temperature changes between experiments with different spin-lock times may be minimized by employing a "constant heat-ing time" version of experiment, where an additional spin–lock pulse is applied after acquisition in order to keep the overall length of the spin lock pulse constant.



Figure S3. Sample temperature as a function of spin-lock pulse length, as measured by the chemical shift of water protons with respect to internal DSS in a sample of $[U-{}^{13}C, {}^{15}N]GB1$ (50 mM salt concentration, pH 5.5).^{8,9} Experiments were measured at 14.1 T at 60 kHz MAS frequency, with a spin-lock nutation frequency of 17 kHz (corresponding to 2.49 W) and the initial (i.e. in the absence of spin-lock) sample temperature of 26.9 ± 0.5 °C.

These results also illustrate that the technology employed here provides a practical and safe approach for measuring relaxation dispersion for spin-lock frequencies in the range from ~1 kHz to a few tens of kHz (or more if the length of spin-lock is limited to a few tens of milliseconds), which significantly expands the range of time scales accessible with such methodology to a few microseconds, and complementing CPMG in perdeuterated proteins¹⁰. Relaxation dispersion in the solid state could potentially be highly complementary to similar measurements in solution, where currently even with cryo-cooled NMR probe heads the current limit for safe spin-lock field strengths is ~6.4 kHz (corresponding to a minimum detectable time scale of $1/(2\pi^*6.4 \text{ kHz}) \approx 25 \text{ µs})$.¹¹

Polarization transfer

Another potential complication associated with carbonyl $R_{1\varrho}$ experiments is that of polarization transfer between different sites during the spin-lock pulse, namely via isotropic mixing or r.f.-driven spin diffusion mechanisms. In the solution state it has been noted that evolution under homonuclear three-bond scalar couplings can lead to magnetization transfer during spin-locking ("isotropic mixing") between carbonyls of neighboring residues whose resonances are close in chemical shift, leading to inaccurate $R_{1\varrho}$ measurements.¹² To check that neither this nor r.f.-driven spin diffusion would compromise our solidstate experiments, we ran a 2D ¹³C-¹³C experiment (on-resonance with ¹³C') with a "mixing" block (between t_1 and t_2 acquisition) consisting of a 150 ms, 17 kHz spin-lock pulse. After a total of 56 scans (~14.5 hours) no off-diagonal cross-peaks were observed above the noise level (spectrum in Fig. S4) between carbonyls (or between ¹³C' and ¹³C_a), suggesting that neither mechanism is efficient for polarization transfer under the employed conditions. Note, however, that r.f.-driven spin diffusion may become more of a concern for aliphatic carbons.



Figure S4. Spectrum resulting from an experiment to test for the occurrence of isotropic mixing and r.f.-driven spin diffusion during a spin-lock pulse typical of that employed in a ¹³C' $R_{1\varrho}$ experiment. After a 150 ms "mixing" block of 17 kHz ¹³C' irradiation, no ¹³C'-¹³C' cross-peaks are seen above the level of noise (negative contours in black, positive in red-yellow), implying little or no polarization transfer occurs.

Table S3. Summary of measured carbonyl ¹³C and amide ¹⁵N $R_{1\varrho}$ and R_1 rates in [U-¹³C, ¹⁵N]GB1 at fields of 14.1 T and 20.0 T (¹H Larmor frequencies of 600 MHz and 850 MHz respectively). ¹³C and ¹⁵N $R_{1\varrho}$ and R_1 rates were measured with the pulse sequences shown in Figure S10, at a sample temperature of 27 ± 2 °C.

600 MHz					850 MHz				
Residue	$^{13}{\rm C}' R_{1\varrho} (s^{-l})$	$^{13}\text{C}' R_1 (s^{-1})$	¹⁵ N $R_{1\varrho}$ (s ⁻¹)	15 N $R_1 (s^{-1})$	$^{13}\text{C'}R_{1\varrho}(s^{-l})$	$^{13}\text{C}' R_1 (s^{-1})$	¹⁵ N $R_{1\varrho}$ (s ⁻¹)	¹⁵ N R_1 (s ⁻¹)	
1	2.48 ± 0.28	0.11 ± 0.01	-	-	4.50 ± 0.64	0.09 ± 0.01	-	-	
2	0.98 ± 0.27	0.14 ± 0.05	1.59 ± 0.24	0.050 ± 0.008	2.21 ± 0.74	0.13 ± 0.01	1.28 ± 0.23	0.106 ± 0.029	
3	2.41 ± 0.53	0.16 ± 0.02	1.92 ± 0.23	0.039 ± 0.007	4.10 ± 0.72	0.17 ± 0.02	1.34 ± 0.41	0.053 ± 0.021	
4	2.89 ± 0.54	0.22 ± 0.04	1.31 ± 0.12	0.013 ± 0.007	3.81 ± 0.31	0.14 ± 0.01	0.88 ± 0.31	0.009 ± 0.006	
5	0.94 ± 0.32	0.17 ± 0.03	1.72 ± 0.1	0.030 ± 0.006	1.65 ± 0.37	0.24 ± 0.06	1.72 ± 0.38	0.029 ± 0.006	
6	1.59 ± 0.40	0.14 ± 0.04	0.82 ± 0.21	0.023 ± 0.007	2.46 ± 0.45	0.23 ± 0.06	0.73 ± 0.20	0.008 ± 0.013	
7	2.48 ± 0.43	0.30 ± 0.09	0.65 ± 0.23	0.016 ± 0.004	2.94 ± 0.64	0.34 ± 0.08	0.76 ± 0.1	0.023 ± 0.007	
8	2.60 ± 0.28	0.55 ± 0.06	0.97 ± 0.23	0.020 ± 0.003	4.58 ± 0.62	0.39 ± 0.03	0.91 ± 0.24	0.021 ± 0.005	
9	2.82 ± 0.66	0.13 ± 0.02	1.00 ± 0.15	0.031 ± 0.005	6.84 ± 1.52	0.14 ± 0.01	2.03 ± 0.37	0.051 ± 0.012	
10	4.28 ± 0.52	0.18 ± 0.02	1.54 ± 0.49	0.088 ± 0.023	7.09 ± 0.75	0.15 ± 0.01	3.44 ± 0.58	0.058 ± 0.014	
11	5.77 ± 0.43	0.20 ± 0.03	3.86 ± 0.35	0.060 ± 0.004	14.6 ± 2.9	0.22 ± 0.02	8.33 ± 0.96	0.131 ± 0.040	
12	1.50 ± 0.40	0.25 ± 0.06	3.36 ± 0.32	0.048 ± 0.006	3.87 ± 0.76	0.26 ± 0.03	7.58 ± 0.99	0.076 ± 0.033	
13	6.34 ± 0.64	0.24 ± 0.03	1.05 ± 0.18	0.027 ± 0.007	6.30 ± 0.74	0.20 ± 0.01	1.95 ± 0.14	0.026 ± 0.016	
14	2.81 ± 0.66	0.09 ± 0.02	1.26 ± 0.30	0.037 ± 0.004	4.40 ± 0.74	0.09 ± 0.01	2.39 ± 0.34	0.035 ± 0.011	
15	3.26 ± 0.38	0.21 ± 0.03	0.86 ± 0.24	0.031 ± 0.012	2.87 ± 0.44	0.17 ± 0.01	1.52 ± 0.38	0.045 ± 0.008	
16	6.80 ± 0.62	0.16 ± 0.02	1.42 ± 0.29	0.039 ± 0.007	5.66 ± 0.58	0.12 ± 0.01	1.43 ± 0.26	0.031 ± 0.017	
17	6.56 ± 1.26	0.22 ± 0.02	5.94 ± 0.62	0.065 ± 0.016	9.69 ± 0.67	0.15 ± 0.01	4.27 ± 0.39	0.127 ± 0.052	
18	2.35 ± 0.29	0.12 ± 0.01	3.86 ± 0.44	0.059 ± 0.013	3.88 ± 0.75	0.11 ± 0.01	3.00 ± 0.35	0.058 ± 0.011	
19	3.21 ± 0.31	0.14 ± 0.01	0.70 ± 0.15	0.066 ± 0.009	3.51 ± 0.73	0.12 ± 0.01	0.92 ± 0.29	0.064 ± 0.008	
20	3.39 ± 0.55	0.14 ± 0.02	2.22 ± 0.34	0.060 ± 0.006	5.10 ± 0.54	0.07 ± 0.02	2.83 ± 0.34	0.111 ± 0.007	
21	5.21 ± 0.69	0.10 ± 0.02	1.92 ± 0.17	0.043 ± 0.006	5.14 ± 0.63	0.10 ± 0.01	2.99 ± 0.43	0.066 ± 0.019	
22	2.75 ± 0.50	0.09 ± 0.01	2.31 ± 0.38	0.064 ± 0.017	4.28 ± 0.44	0.11 ± 0.01	4.41 ± 0.53	0.106 ± 0.035	
23	1.87 ± 0.23	0.20 ± 0.03	1.78 ± 0.24	0.022 ± 0.005	3.77 ± 0.65	0.19 ± 0.04	2.17 ± 0.44	0.045 ± 0.011	
24	3.89 ± 0.59	0.16 ± 0.03	0.89 ± 0.11	0.031 ± 0.004	3.33 ± 1.20	0.14 ± 0.01	2.04 ± 0.20	0.056 ± 0.017	
25	2.34 ± 0.25	0.16 ± 0.02	3.37 ± 0.37	0.017 ± 0.011	2.73 ± 0.39	0.13 ± 0.01	5.08 ± 1.12	0.009 ± 0.023	
26	2.77 ± 0.33	0.40 ± 0.07	0.91 ± 0.18	0.015 ± 0.008	2.90 ± 0.71	0.26 ± 0.06	1.55 ± 0.22	0.060 ± 0.033	
27	2.01 ± 0.32	0.16 ± 0.05	0.51 ± 0.16	0.026 ± 0.009	3.16 ± 0.75	0.25 ± 0.03	1.18 ± 0.17	0.021 ± 0.009	
28	1.53 ± 0.29	0.38 ± 0.12	1.25 ± 0.23	0.026 ± 0.005	2.34 ± 0.75	0.36 ± 0.10	1.89 ± 0.44	0.024 ± 0.012	
29	1.10 ± 0.23	0.25 ± 0.04	1.60 ± 0.23	0.028 ± 0.004	3.30 ± 0.30	0.13 ± 0.03	1.27 ± 0.32	0.022 ± 0.009	
30	2.61 ± 0.63	0.06 ± 0.01	1.16 ± 0.17	0.018 ± 0.004	3.16 ± 0.60	0.07 ± 0.02	1.47 ± 0.26	0.029 ± 0.012	
31	1.54 ± 0.13	0.20 ± 0.04	0.83 ± 0.17	0.009 ± 0.004	1.10 ± 0.53	0.12 ± 0.02	1.14 ± 0.36	0.026 ± 0.008	
32	1.88 ± 0.35	0.25 ± 0.08	0.89 ± 0.08	0.026 ± 0.003	2.63 ± 0.24	0.18 ± 0.03	1.32 ± 0.17	0.030 ± 0.011	
33	2.01 ± 0.35	0.79 ± 0.21	0.62 ± 0.25	0.005 ± 0.007	2.83 ± 0.44	0.50 ± 0.06	0.78 ± 0.25	0.020 ± 0.015	

		600]	MHz	850 MHz				
Residue	$^{13}\text{C'}R_{1\varrho}(s^{-l})$	$^{13}\text{C'}R_1(s^{-1})$	¹⁵ N $R_{1\varrho}$ (s ⁻¹)	15 N $R_1 (s^{-1})$	$^{13}\text{C}' R_{1\varrho} (s^{-l})$	$^{13}\text{C'}R_1(s^{-1})$	15 N $R_{1\varrho} (s^{-1})$	¹⁵ N R_1 (s ⁻¹)
34	1.30 ± 0.19	0.31 ± 0.09	0.93 ± 0.19	0.008 ± 0.005	1.99 ± 0.37	0.31 ± 0.07	0.83 ± 0.30	0.005 ± 0.02
35	1.65 ± 0.08	0.18 ± 0.03	0.47 ± 0.16	0.022 ± 0.009	2.79 ± 0.15	0.17 ± 0.03	0.96 ± 0.33	0.021 ± 0.014
36	3.82 ± 0.67	0.30 ± 0.06	0.88 ± 0.08	0.021 ± 0.003	3.97 ± 0.42	0.33 ± 0.04	1.5 ± 0.10	0.031 ± 0.005
37	3.30 ± 0.47	0.17 ± 0.02	1.23 ± 0.38	0.027 ± 0.006	3.31 ± 0.42	0.20 ± 0.02	1.01 ± 0.50	0.028 ± 0.010
38	9.85 ± 1.22	0.13 ± 0.02	1.07 ± 0.20	0.029 ± 0.008	8.48 ± 1.09	0.19 ± 0.04	1.67 ± 0.22	0.021 ± 0.008
39	3.12 ± 0.56	0.19 ± 0.03	3.00 ± 0.46	0.050 ± 0.020	5.80 ± 0.85	0.25 ± 0.02	4.62 ± 0.53	0.040 ± 0.017
40	6.14 ± 0.55	0.24 ± 0.04	3.07 ± 0.27	0.101 ± 0.015	6.82 ± 0.76	0.21 ± 0.02	4.26 ± 0.34	0.304 ± 0.058
41	2.74 ± 0.36	0.06 ± 0.02	3.22 ± 0.29	0.204 ± 0.058	2.74 ± 0.70	0.08 ± 0.01	6.04 ± 0.70	0.245 ± 0.039
42	2.85 ± 0.31	0.24 ± 0.07	1.46 ± 0.34	0.087 ± 0.015	2.92 ± 0.46	0.14 ± 0.03	2.42 ± 0.50	0.112 ± 0.013
43	5.70 ± 0.73	0.31 ± 0.06	1.27 ± 0.22	0.027 ± 0.009	4.79 ± 0.64	0.12 ± 0.02	1.68 ± 0.38	0.024 ± 0.016
44	4.00 ± 0.90	0.15 ± 0.02	1.96 ± 0.45	0.019 ± 0.012	4.96 ± 0.48	0.11 ± 0.01	1.84 ± 0.74	0.016 ± 0.010
45	3.32 ± 0.55	0.14 ± 0.01	1.62 ± 0.35	0.012 ± 0.009	3.41 ± 0.39	0.09 ± 0.01	2.02 ± 0.55	0.012 ± 0.014
46	3.20 ± 0.52	0.29 ± 0.05	0.90 ± 0.22	0.041 ± 0.010	3.71 ± 0.93	0.18 ± 0.02	1.18 ± 0.31	0.029 ± 0.009
47	2.83 ± 0.67	0.17 ± 0.03	1.17 ± 0.28	0.033 ± 0.006	4.36 ± 0.34	0.17 ± 0.01	2.06 ± 0.42	0.049 ± 0.021
48	1.83 ± 0.45	0.25 ± 0.03	1.94 ± 0.23	0.030 ± 0.008	3.58 ± 0.48	0.23 ± 0.02	3.01 ± 0.40	0.033 ± 0.013
49	2.56 ± 0.71	0.36 ± 0.12	1.06 ± 0.24	0.043 ± 0.004	2.99 ± 0.64	0.20 ± 0.05	1.72 ± 0.40	0.022 ± 0.009
50	2.06 ± 0.33	0.29 ± 0.08	1.33 ± 0.24	0.039 ± 0.008	2.38 ± 0.77	0.24 ± 0.06	1.70 ± 0.40	0.01 ± 0.014
51	2.15 ± 0.49	0.38 ± 0.06	0.58 ± 0.19	0.012 ± 0.005	2.04 ± 0.66	0.23 ± 0.02	1.26 ± 0.29	0.036 ± 0.016
52	3.13 ± 0.39	0.24 ± 0.07	0.66 ± 0.13	0.009 ± 0.008	3.91 ± 0.52	0.23 ± 0.03	1.68 ± 0.27	0.019 ± 0.011
53	5.29 ± 0.81	0.16 ± 0.05	1.90 ± 0.40	0.003 ± 0.005	7.05 ± 0.99	0.15 ± 0.02	2.88 ± 0.41	0.011 ± 0.007
54	2.14 ± 0.39	0.23 ± 0.08	3.44 ± 0.38	0.005 ± 0.004	3.12 ± 0.38	0.16 ± 0.03	5.05 ± 0.72	0.020 ± 0.009
55	3.75 ± 0.32	0.28 ± 0.01	0.79 ± 0.23	0.001 ± 0.009	5.67 ± 0.70	0.22 ± 0.01	0.85 ± 0.20	0.029 ± 0.011
56	-	-	1.65 ± 0.15	0.065 ± 0.011	-	-	2.40 ± 0.42	0.102 ± 0.018



Figure S5. NCO S³E DCP spectrum measured at $\omega_{0H}/2\pi = 850$ MHz showing resonance assignments. Note that a number of peaks partially overlap and as such the rates extracted from them may be distorted. Assignments are not shown for side-chain cross peaks.



Figure S6. *Top panels*: Ratio of the fast motion contribution to $J_1(\omega_0)$ (with $\tau_f = 80$ ps, $S_f^2 = 0.75$) to that of a slow motion ($S_s^2 = 0.94$, τ_s indicated on the horizontal axis), calculated using SMF (Eq. 1.3) at $\omega_{0H}/2\pi = 600$ and 850 MHz. *Bottom panels*: The result of fitting to a single timescale motion (using SMF: Eq. 1.3 and 1.7-1.18) rates simulated using two timescale motion (using EMF: Eq. 1.4 and 1.7-1.18) with the settings as in the top panels (S_f^2 , S_s^2 and $S_f^2S_s^2$ are indicated by dashed grey lines). We assumed 10% error for the simulated rates in the SMF fit. Note the offset between the order parameter for ¹⁵N and ¹³C' when SMF is used for modeling the data resulting from two timescale motion.



Figure S7. EMF analysis of backbone dynamics in crystalline GB1 based on ¹⁵N R_1 and $R_{1\rho}$ measurements performed at 600 and 850 MHz ¹H Larmor frequency and ¹⁵N dipolar coupling measurements (diamond black line), compared to an analogous analysis with the addition of ¹⁵N R_1 and $R_{1\rho}$ measured at 1 GHz ¹H Larmor frequency (red dashed line) and an analysis where the generalized expressions for $R_{1\rho}$ including the effect of spinning frequency¹³ were used (blue dotted line). In the current case neither of these approaches leads to complete elimination of fitting artifacts.



Figure S8. The effect of r_{NH} and ${}^{15}N \& {}^{13}C'$ CSA on the results of an EMF analysis of backbone dynamics in crystalline GB1. For the fits represented by the red line, $r_{NH} = 1.02$ Å, site specific ${}^{15}N$ CSA from [Wylie, B. J. *et al. Proc. Natl. Acad. Sci.* 2011, 108, 16974–16979.] and site specific ${}^{13}C'$ CSA from ref. [Wylie, B. J. *et al. J. Am. Chem. Soc.* 2007, 129, 5318–9.]

were used. For the fits represented with the black line, $r_{NH} = 1.04$ Å, site specific ¹⁵N CSA from [Wylie, B. J. *et al. Proc. Natl. Acad. Sci.* 2011, 108, 16974–16979.] and site specific ¹³C' CSA from [Wylie, B. J. *et al. J. Am. Chem. Soc.* 2007, 129, 5318– 9.] were used. For the fits represented with the blue line, $r_{NH} = 1.02$ Å, site specific ¹⁵N/¹³C' CSA from [Loth, K.; Pelupessy, P.; Bodenhausen, G. J. Am. Chem. Soc. 2005, 127, 6062–8.] were used. In all cases the EMF analysis was based on ¹⁵N and ¹³C' R_1 and $R_{1\rho}$ performed at 600 and 850 MHz ¹H Larmor frequency with ¹⁵N dipolar coupling measurements used for constraining the overall amplitude of motions (assuming a rigid NH bond length of 1.02 Å). The simulations illustrate that the EMF analysis in the solid state is less sensitive to the choice of bond lengths and CSA compared to in solution.



Figure S9. Comparison of the fast motion parameters from the EMF analysis (see Fig. 5) with various SMF analyses based on relaxation in solution. Reference A is ¹⁴. Reference B is ¹⁵.



Figure S10. Pulse sequences for the site-specific measurement of (a) carbonyl ¹³C $R_{1\varrho}$, (b) amide ¹⁵N $R_{1\varrho}$, (c) carbonyl ¹³C R_1 and (d) amide ¹⁵N R_1 . ¹³C and ¹⁵N frequency offsets are set to the centers of the carbonyl and amide regions respectively. Pulses with a flip angle of $\pi/2$ are indicated with a narrow black rectangle, while π pulses are denoted by a thicker black rectangle. Spin-lock pulses (for (a) and (b)) are indicated in light grey. Indirect and direct acquisition periods are labeled as "t₁"

and "t₂" respectively, while phases are shown as " ϕ ". For all sequences, slpTPPM¹⁶ decoupling is applied on the proton channel during acquisition periods at ¹H amplitude of one quarter of the sample spinning frequency. Site-specific relaxation rates are obtained from curves obtained by monitoring intensity of cross peaks in 2D experiments as a function of relaxation time, τ (length of spin-lock pulse for R_{1q} measurements, delay length for R_1 measurements). No ¹H decoupling is applied during relaxation periods. In each sequence, the rectangle with a dashed outline represents an S³E block, which may be optionally included to improve resolution in the direct dimension by minimizing the effect of one-bond C'-C_a J-couplings.¹⁷ Experiments containing "A" and "B" blocks (which differ in the positioning of the band-selective pulses – see ¹⁷) are run in an interleaved fashion, to be split and recombined when processing. The two different phase cycles associated with these are differentiated by use of square brackets below. The phases of all S³E pulses (including the π pulse on ¹³C during t₁ evolution) are identical, labeled as ϕ_{S3E} . Phase cycling (with S³E):

(a) $\phi_1 = (+y - y), \phi_2 = \phi_3 = \phi_5 = \phi_{S3E} = (+x), \phi_4 = \phi_6 = (+x + x - x - x [A] / -y - y + y + y [B]), \phi_{rec} = (+x - x + x - x).$ (b) $\phi_1 = (+y - y), \phi_2 = \phi_3 = \phi_5 = \phi_6 = \phi_{S3E} = (+x), \phi_4 = (+x + x - x - x [A] / -y - y + y + y [B]), \phi_{rec} = (+x - x + x - x).$ (c) $\phi_1 = (+y - y), \phi_2 = \phi_3 = \phi_5 = \phi_{S3E} = (+x), \phi_4 = (+x + x - x - x [A] / -y - y + y + y [B]), \phi_6 = -\phi_7 = (+y - y [A] / +x - x [B]) \phi_{rec} = (+x - x + x - x).$ (d) $\phi_1 = (+y - y), \phi_2 = \phi_3 = \phi_5 = \phi_{S3E} = (+x), \phi_4 = (+x + x - x - x [A] / -y - y + y + y [B]), \phi_6 = -\phi_7 = (+y - y) \phi_{rec} = (+x - x + x - x).$

Pulse sequences in Bruker (Avance III) format are available to download at: http://www2.warwick.ac.uk/fac/sci/chemistry/research/lewandowski/lewandowskigroup/goodies/

Table S4. Extended model free (EMF) analysis of ¹⁵N & ¹³C' relaxation in crystalline [U-¹³C,¹⁵N]GB1 (data plotted in Fig. 5 in the manuscript). Fitted data include ¹⁵N R_1 and $R_{1\varrho}$ at 600 MHz, 850 MHz, ¹³C' R_1 and $R_{1\varrho}$ at 600 MHz and 850 MHz and NH dipolar order parameters⁶. # indicates peptide plane number following the numberings of the amide nitrogens.

#	S_s^2	error	$ au_{s}$ (ns)	error	S_f^2	error	$ au_{f}$ (ps)	error	χ^2
3	0.995	3.17E-04	428	6	0.790	0.00149	48	4	145.3
4	0.997	4.28E-04	399	42	0.782	3.47E-04	13	4	93.3
5	0.996	2.97E-04	405	18	0.714	3.03E-04	24	3	90.2
6	0.998	0.00576	421	213	0.771	0.00452	18	5	42.0
7	0.999	0.00179	526	182	0.801	0.00146	15	3	27.2
8	0.998	0.0156	386	390	0.832	0.0133	21	5	41.8
9	0.997	3.03E-04	458	20	0.783	3.41E-04	33	4	203.8
10	0.986	0.00278	205	45	0.750	0.00446	82	8	36.0
11	0.990	5.09E-04	445	5	0.719	6.01E-04	45	3	73.7
13	0.997	2.52E-04	420	20	0.813	3.58E-04	33	7	92.2
14	0.994	4.39E-04	429	9	0.755	4.81E-04	34	3	211.5
15	0.997	5.28E-04	400	33	0.773	6.64E-04	39	6	30.2
16	0.996	3.58E-04	401	20	0.817	0.00196	60	8	127.9
17	0.994	0.00587	572	3430	0.776	0.00482	69	11	57.5
18	0.989	0.00163	324	48	0.763	0.00308	65	8	97.2
20	0.995	7.03E-04	563	77	0.664	0.00111	47	3	87.4
21	0.999	0.00202	5200	13800	0.710	0.00146	28	4	24.9
22	0.996	0.00445	669	4500	0.733	0.00345	53	8	17.1
23	0.997	3.96E-04	405	13	0.798	0.00197	65	7	78.8
25	0.993	6.84E-04	431	15	0.766	7.35E-04	38	8	55.4
26	0.997	2.33E-04	452	8	0.783	4.59E-04	37	7	109.7
27	0.997	0.0157	366	242	0.752	0.0122	18	7	78.0
28	0.997	4.03E-04	455	27	0.782	3.39E-04	24	4	46.2
31	0.998	3.30E-04	500	55	0.802	2.67E-04	9	4	22.5
33	0.998	2.43E-04	462	24	0.782	1.92E-04	11	5	55.1
34	0.998	2.81E-04	451	22	0.812	2.30E-04	10	5	102.1
35	0.998	0.00871	415	208	0.781	0.00699	19	7	32.7
37	0.996	5.45E-04	423	38	0.773	4.68E-04	24	5	94.0
38	0.996	4.21E-04	400	35	0.773	4.39E-04	26	5	110.6

	S_s^2	error	$ au_{s}$ (s)	error	S_f^2	error	$ au_{f}$ (s)	error	χ^2
39	0.988	0.0414	299	233	0.780	0.0349	29	11	38.4
40	0.991	0.00114	360	41	0.660	0.00252	100	11	41.9
41	0.860	0.147	31	19400	0.604	0.0953	92	13	31.4
42	0.933	0.0408	24	29700	0.771	0.0329	53	10	12.6
43	0.996	0.00552	376	126	0.783	0.00444	23	8	33.4
44	0.994	7.44E-04	416	38	0.805	6.29E-04	22	9	55.3
45	0.995	4.71E-04	429	17	0.784	4.31E-04	26	6	54.5
46	0.996	6.25E-04	333	46	0.819	0.0031	57	8	107.9
47	0.996	4.40E-04	430	14	0.844	9.14E-04	51	9	85.6
48	0.995	3.48E-04	414	13	0.766	0.00115	48	6	96.5
49	0.996	4.13E-04	412	18	0.724	4.41E-04	30	3	173.4
50	0.951	0.0419	26	175	0.799	0.0342	12	20	21.5
51	0.998	3.45E-04	538	67	0.771	2.69E-04	11	4	36.3
52	0.998	2.55E-04	430	16	0.792	2.38E-04	21	6	144.0
53	0.995	7.57E-04	423	50	0.754	5.77E-04	9	4	46.9
54	0.993	8.85E-04	506	53	0.805	7.20E-04	8	4	21.4
55	0.998	3.73E-04	451	59	0.792	3.16E-04	20	7	39.8

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