Supplementary Information

Determining Methyl Sidechain Conformations in a CS-ROSETTA Model Using Methyl ^{1}H-^{13}C Residual Dipolar Couplings

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Interpretation of *J*-modulated interferograms of CH_3 methyl groups in the presence of spin relaxation.

The experiment used for the measurement of methyl ^{1}H-^{13}C RDCs is shown in Figure S1, and represents a simplified version of the constant-time HSQC-based scheme of Ottiger et al. (1998), applicable for per-deuterated protein samples with selectively [$^{13}CH_3$]-labeled methyl groups. Accounting for spin relaxation of the lines of the ^{13}C quadruplet during the constant time delay T (Figure S1) rigorously is problematic, as the spin relaxation rates depend on the micro-dynamics parameters of methyl three-fold symmetry axis (the order parameter squared, S^2_{axis}, and the correlation time of fast local motions including methyl rotation, τ_f) that are not known *a-priori*, and vary from one methyl site to another. That is why we have chosen to use an approximate, semi-quantitative approach, and re-cast the modulation of methyl signal intensity in the presence of spin relaxation as,

$$I(t_2) = I_0 \left\{ a \cos(3\pi[{}^1J_{\text{CH}} + D_{\text{CH}}]t_2) \exp(-bR^S_{2,\text{C}}T) + \cos(\pi[{}^1J_{\text{CH}} + D_{\text{CH}}]t_2) \exp(-R^S_{2,\text{C}}T) \right\} \qquad \text{(S1)}$$

where $R^S_{2,\text{C}}$ is the transverse spin relaxation rate of the slow-relaxing (inner) ^{13}C transitions, and the transverse spin relaxation rate of the fast-relaxing (outer) ^{13}C transitions is assumed to be proportional to $R^S_{2,\text{C}}$, $R^F_{2,\text{C}} = b\, R^S_{2,\text{C}}$ (see Eq. (2) of the main text).

In this framework, two effects of relaxation on the *J*-modulation profiles can be distinguished. The first effect relates to deviations of the ratio of ^{13}C outer to inner line intensities (a) from the 'relaxation-free' value of 3 resulting from differential transverse spin relaxation of ^{1}H transitions during the first INEPT of the scheme in Figure S1. Using known expressions for methyl signal intensities in an HSQC experiment in the presence of spin relaxation (Ollerenshaw et al. 2003; Tugarinov et al. 2003; Tugarinov and Kay 2013), it is straightforward to show that at the start of the constant-time period T (*i.e.* for T, t_1, $t_2 = 0$), the value of a is given by,

$$a = \frac{\{\exp(-2R_{2,\mathrm{H}}^{F}\tau_a) + \exp(-2R_{2,\mathrm{H}}^{S}\tau_a)\}(F+S)}{\{\exp(-2R_{2,\mathrm{H}}^{F}\tau_a) - (1/3)\exp(-2R_{2,\mathrm{H}}^{S}\tau_a)\}F + \{\exp(-2R_{2,\mathrm{H}}^{S}\tau_a) - (1/3)\exp(-2R_{2,\mathrm{H}}^{F}\tau_a)\}S} \quad \text{(S2.1)}$$

where

$$F = \exp(-2R_{2,\mathrm{H}}^{F}\tau_a)\int_0^{t_{acq}} \exp(-R_{2,\mathrm{H}}^{F}t)dt = \frac{\exp(-2R_{2,\mathrm{H}}^{F}\tau_a)\left\{1-\exp(-R_{2,\mathrm{H}}^{F}t_{acq})\right\}}{R_{2,\mathrm{H}}^{F}}; \quad \text{(S2.2)}$$

$$S = \exp(-2R_{2,\mathrm{H}}^{S}\tau_a)\int_0^{t_{acq}} \exp(-R_{2,\mathrm{H}}^{S}t)dt = \frac{\exp(-2R_{2,\mathrm{H}}^{S}\tau_a)\left\{1-\exp(-R_{2,\mathrm{H}}^{S}t_{acq})\right\}}{R_{2,\mathrm{H}}^{S}}; \quad \text{(S2.3)}$$

t is the acquisition time in the direct acquisition dimension of the experiment and varies from 0 to t_{acq}; $2\tau_a$ is the duration of the INEPT periods in the scheme of Figure S1, $\sim 1/(2\,{}^1J_{CH})$; and $R_{2,\mathrm{H}}^{S}$ and $R_{2,\mathrm{H}}^{F}$ are transverse spin relaxation rates of the slow- and fast-relaxing methyl ^{1}H transitions, respectively. The plot of a calculated using Eq. (S2) and ^{1}H relaxation rates estimated from the expressions in the references above as a function of S_{axis}^2 for a protein molecule with global rotational correlation time τ_C of 12 ns is shown in Figure S2A.

The second effect of spin relaxation relates to the ratio of 'fast' and 'slow' ^{13}C transverse spin relaxation rates, $b = R_{2,\mathrm{C}}^{F}/R_{2,\mathrm{C}}^{S}$. In the limit where local motions of a methyl group (including methyl rotation) are infinitely fast compared to the global rotational correlation time of the protein ($\tau_f << \tau_C$), and all relaxation mechanisms except ^{13}C-^{1}H dipolar interactions are neglected, $R_{2,\mathrm{C}}^{F} = 9\,R_{2,\mathrm{C}}^{S}$ (Werbelow and Grant, 1977; Kay and Torchia, 1991; Kay and Bull, 1992). However, for methyl groups in a protein molecule of an intermediate size considered here, the effects of local dynamics can be expected to be significant. As a result, the auto- and cross-correlation spectral density functions entering into the expressions for $R_{2,\mathrm{C}}^{F}$ and $R_{2,\mathrm{C}}^{S}$ can no longer be assumed to be equivalent. As an illustration of this effect, we estimated the value of the proportionality factor b as a function of both parameters of methyl dynamics (S_{axis}^2 and τ_f) using full expressions for the spectral density function at all relevant frequencies (Figure S2B). Clearly, the large

range of predicted variability precludes the assignment of any single value to *b*, and the fits to Eq. (S1) have to be performed for a series of (fixed) values of this coefficient.

If contributions to relaxation from dipolar interactions with external ^{1}H spins, methyl ^{13}C chemical shift anisotropy, and fast local dynamics of the methyl three-fold symmetry axis are neglected, relative estimates of the amplitude of methyl motions, described by 'apparent' order parameters squared $S_{\text{axis}}^{2,\text{app}}$, can be obtained from standard relationships (Ollerenshaw et al. 2003; Tugarinov et al. 2003; Tugarinov and Kay 2013), and is given by,

$$S_{\text{axis}}^{2,\text{app}} = 5\left(\frac{4\pi}{\mu_0}\right)^2\left(\frac{r_{\text{HC}}^6}{\gamma_{\text{H}}^2\gamma_{\text{C}}^2\hbar^2\tau_{\text{C}}}\right)R_{2,\text{C}}^F \qquad \text{(S3)}$$

where μ_0 is the vacuum permeability, $\hbar$ is Planck's constant divided by 2π, γ_j is the gyromagnetic ratio of spin *j*, and r_{HC} is the distance between ^{1}H and ^{13}C nuclei in a methyl group (1.135 Å).

By performing multiple fits of experimental *J*-modulation profiles to the expression in Eq. (S1) (Eq. (2) of the main text) with coefficients *a* and *b* set to a wide range of values, we found that the resulting distributions of $S_{\text{axis}}^{2,\text{app}}$, recalculated according to Eq. (S3), are not sensitive to the exact value of *a* as long as *a* falls in the range between 2 and 3 (*a* = 2.7 was used for final analysis), while reasonable distributions of $S_{\text{axis}}^{2,\text{app}}$ are obtained for values of *b* in the range between ~6 to ~8 (*b* = 7 was used to generate the set of $S_{\text{axis}}^{2,\text{app}}$ for final analysis). See Figure S3 for a representative distribution of $S_{\text{axis}}^{2,\text{app}}$ obtained for the ILV methyls of ΔST-DNAJB6b.

Materials and Methods

NMR Sample Preparation. Methyl ^{1}H-^{13}C RDCs were measured using a sample of {U-[^{15}N,^{2}H]; Ileδ1-[$^{13}CH_3$]; Leu,Val-[$^{13}CH_3$,$^{12}CD_3$]}-labeled ΔST-DNAJB6b prepared as described elsewhere (Karamanos et al. 2019) but without removal of the His-tag, aligned in a 4.2 % (v/v) PEG/hexanol mixture in 90% H_2O/10 %D_2O (v/v), or a sample of {U-[^{13}C,^{15}N,^{2}H]; Ileδ1-[$^{13}CH_3$]; Leu,Val-[$^{13}CH_3$,$^{12}CD_3$]}-labeled ΔST-DNAJB6b, 90% H_2O/10% D_2O (v/v), aligned in 12 mg/mL pf1 bacteriophage. Sample conditions were: 200 μM ΔST-DNAJB6b, 20 mM sodium phosphate, pH = 7.0 (uncorrected) and 50 mM NaCl.

NMR Spectroscopy. Measurements of methyl ^{1}H-^{13}C RDCs on ΔST-DNAJB6b were performed using a simplified version of the experiment of Ottiger et al. (1998) (Figure S1). Constant-time periods *T* of 28 ms and 58 ms were employed for the samples aligned in pf1 phage and PEG/hexanol, respectively. *J* evolution t_2 periods of 0, 0.4, 1.6, 2.4, 3.2, 4, 4,8, 5,6, 6,4, 8, 9.6, 11.2, 12.8 and 16 ms were used for the pf1 sample; and t2 periods of 0, 0.4, 0.16, 2.4, 3.2, 4, 4,8, 5,6, 6.4, 8, 9.6, 11.2 and 16 ms were used for the PEG/hexanol sample. Amide ^{1}H-^{15}N RDCs were measured using the ARTSY experiment (Fitzkee and Bax 2010). All spectra were recorded on a 600 MHz, AVANCE HD Bruker spectrometer equipped with a triple *x*,*y*,*z*-gradient cryoprobe. The spectra were processed and analysed with the NMRPipe/NMRDraw (Delaglio et al. 1995) suite of programs and associated software.

Structure Calculations. All structure calculations were performed in Xplor-NIH (Schwieters et al. 2017) using the lowest energy CS-ROSSETA model of ΔST-DNAJB6b (PDB ID: 6U3R, 6U3S) (Karamanos et al. 2019). Simulated annealing calculations were performed with the temperature gradient from 2000 K to 25 K on the JD and CTD domains separately (as they tumble almost independently of each other (Karamanos et al. 2019)) with the coordinates of the backbone fixed, and the side chains given torsional degrees of freedom. Amide ^{1}H-^{15}N RDCs measured on the same sample served to determine the parameters of the alignment tensor for the two domains in each alignment medium. Since only methyl-bearing sidechains are restrained by the RDC data, we used the torsionDB potential of mean force term (Bermejo et al. 2012) to restrain all other sidechains to allowed rotamers. Xplor-NIH scripts for methyl bearing sidechain refinement are available from the authors upon request.

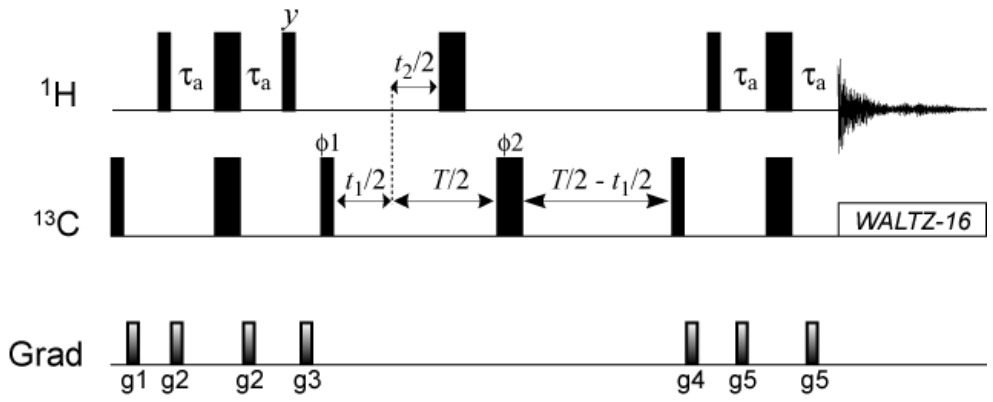

Figure S1. Pulse sequence used for the measurements of methyl ^{1}H-^{13}C RDCs in $^{13}CH_3$ methyl groups of ΔST-DNAJB6b. All narrow and wide rectangular pulses are applied with flip angles of 90° and 180°, respectively, along the *x*-axis unless indicated otherwise. The ^{1}H and ^{13}C carrier frequencies are positioned in the centre of the Ileδ1-Leu-Val methyl region - at 0.5 and 20 ppm, respectively. All ^{1}H and ^{13}C pulses are applied with the highest possible power, while ^{13}C WALTZ-16 decoupling (Shaka et al. 1983) was applied using a 2-kHz field. Delay τ_a = 2.0 ms; constant-time period T is set to $1/J_{CC}$ = 28 ms for uniformly ^{13}C-labeled samples, while, sensitivity permitting, any value of T can be used for protein samples with selectively ^{13}C-labeled methyl groups. The durations and strengths of pulsed-field gradients in units of (ms; G/cm) are: g1 = (1; 30), g2 = (0.5; 20), g3 = (1.4; 30), g4 = (0.7; -20), g5 = (0.3; 25). The phase cycle is: ϕ1 = *x,-x*; ϕ2 = *x,x,-x,-x*; receiver = *x,-x*. Quadrature detection in F_1 is achieved via States-TPPI incrementation of ϕ1.

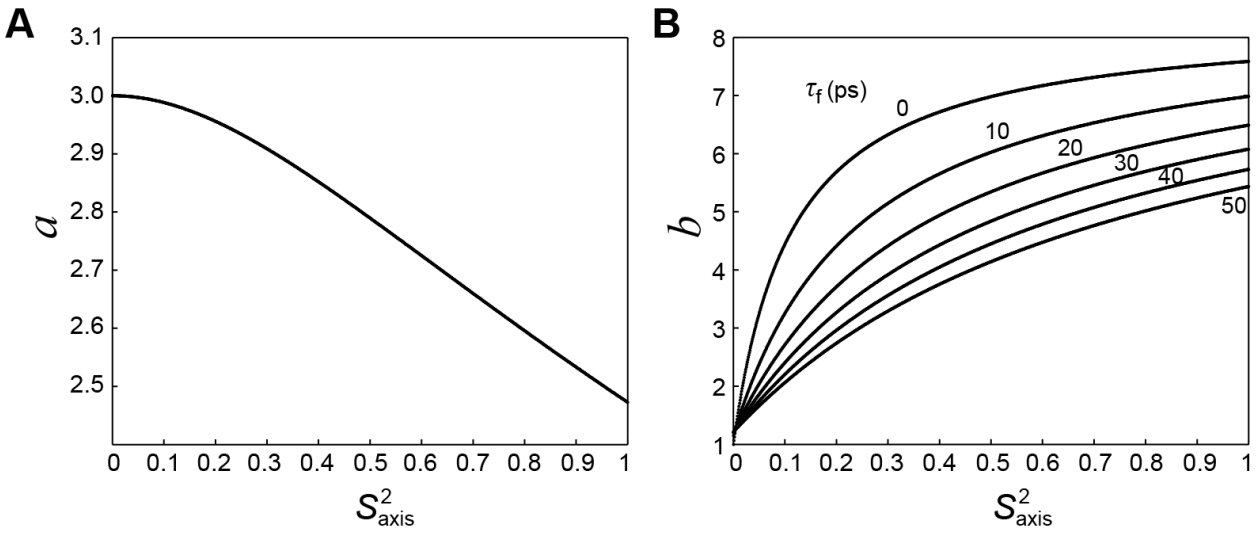

Figure S2. (**A**) Plot of the coefficient *a* as a function of S^2_{axis} calculated using Eqs. (S2) (*a* = 3 in the absence of spin relaxation). Simplified expressions for ^{1}H transverse spin relaxation rates $R^S_{2,H}$ and $R^F_{2,H}$ were used (assuming the equivalence of the auto- and cross-correlated relaxation spectral density functions) that can be found, for example, in Eqs. (3.1) and (3.2) of Tugarinov and Kay (2013). The calculations were performed with the delay τ_a set to 2.0 ms and t_{acq} = 70 ms. (**B**) Plots of the proportionality factor *b* as a function of S^2_{axis} for a series of τ_f values (ps) calculated using full expressions for $R^F_{2,C}$ and $R^S_{2,C}$ (including methyl ^{13}C chemical shift anisotropy, CSA, assumed to be equal to 20 ppm, as well as relaxation due to dipolar interactions with external ^{1}H(^{2}H) spins, placed at 3.5(1.8) Å from methyl proton nuclei). All calculations were performed for a 600 MHz static magnetic field strength and a global rotational correlation time τ_C of 12 ns.

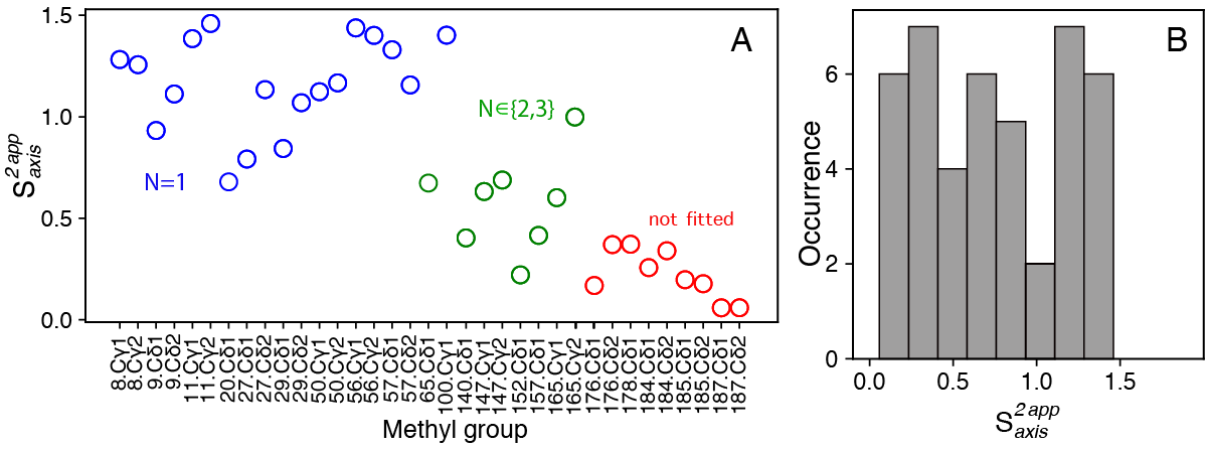

Figure S3. (A) Values of $S^{2,\mathrm{app}}_{\mathrm{axis}}$ obtained for the ILV methyl sites of ΔST-DNAJB6b using Eq. (S3) and the interferogram best-fits using Eq. (S1), with the coefficients '*a*' and '*b*' set to 2.7 and 7, respectively. The methyl sites for which the measured D_{CH} values can be fitted with a single side-chain conformation are shown as blue circles ($N = 1$), the residues whose side-chains were represented as a combination of 2 or 3 rotameric states are shown as green circles ($N \in \{2,3\}$), while flexible residues whose D_{CH} values cannot be fitted to the rotamer-jump model are shown as red circles. (**B**) A histogram of the $S^{2,\mathrm{app}}_{\mathrm{axis}}$ values obtained for the methyls of ΔST-DNAJB6b as indicated in (A).

Supplementary References

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