

Methods.

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Dynamics Fitting

The various models were fit to the relaxation data using an in-house fitting model implemented using the C programming language. This model can be found at <http://www.website.com>. Expressions for the rates and spectral densities for the models can be found below. Minimization was performed using the Nelder-Mead algorithm¹, using N repeats with random starting points. Table 1 shows the variation in the random starting points for each model, along with the bounds used in the fitting to ensure physical solutions were found¹.

Table 1: Parameters used in the dynamic model fitting. SMF refers to Simple Model Free, EMF refers to Extended Model Free, GAF refers to Gaussian Axial Fluctuation Model, 3D and 6D refer to the dimensionality of the axial fluctuations. A prefix of T indicates temperature dependence. * indicates these are bound via the non-temperature dependent forms.

Parameter	Description	Units	Models	Start Range	Bound Range
τ	Correlation time	s	SMF	$0 - 10^{-8}$	≥ 0
τ_0	Correlation preexponential	s	SMFT	$0 - 10^{-15}$	≥ 0

¹(see chisq.c lines 34-211, main.c 135-204. I've omitted orientation variation for now.). crosen.c includes Nelder-Mead method.

Parameter	Description	Units	Models	Start Range	Bound Range
τ^s	Slow motion correlation time	s	EMF/GAF	$0 - 10^{-8}$	$10^{-5} \geq \tau \geq 0$
τ^f	Fast motion correlation time	s	EMF/GAF	$0 - 10^{-11}$	$10^{-5} \geq \tau \geq 0$
τ_0^s	Slow preex- ponential factor	s	EMFT/GAFT	$0 - 10^{-15}$	*
τ_0^f	Fast preexpo- nential factor	s	EMFT/GAFT	$0 - 10^{-20}$	*
S^2	Order parameter	-	SMF	$0.5 - 1.0$	$1 \geq S^2 \geq 0$
S_s^2	Slow order parameter	-	EMF/EMFT	$S_{NH}^2 - 1.0$	$1 \geq S^2 \geq 0$
S_f^2	Fast order parameter	-	EMF/EMFT/3D- GAF/3D- GAFT	$S_{NH}^2 - 1.0$	$1 \geq S^2 \geq 0$
$\sigma_{\alpha/\beta/\gamma}^s$	Angular deflections for slow motions	radians	3D/6D- GAF/GAFT	$0.00 - 0.25$	$0.52 \geq \sigma \geq 0.00$
$\sigma_{\alpha/\beta/\gamma}^f$	Angular deflections for fast motions	radians	6D- GAF/GAFT	$0.00 - 0.25$	$0.52 \geq \sigma \geq 0.00$

Parameter	Description	Units	Models	Start Range	Bound Range
Ea	Correlation time activation energy	J mol ⁻¹	SMFT	0 – 60000	*
Ea ^s	Slow correlation time activation energy	J mol ⁻¹	EMFT/GAFT	0 – 60000	*
Ea ^f	Fast correlation time activation energy	J mol ⁻¹	EMFT/GAFT	0 – 60000	*

14 The best fit parameters were found for all models by minimizing the χ^2 target function:

$$\chi^2 = \sum_i W_i \frac{(X_{i,exp} - X_{i,calc})^2}{\sigma_{i,exp}^2}$$

15 where X_i are relaxation rates and dipolar order parameter measurements and σ_i appropriate
16 experimental errors². W_i is a weighting which is taken to be 1 for all relaxation measurements and
17 100 for dipolar order parameters to reflect the increased model reliance on these order parameters.²
18 The rigid limit NH distance was assumed to be N Ang.

19 Errors were estimated using Monte Carlo error analysis using N iterations. Relaxation rates were
20 back-calculated from the model fit parameters, and random noise within experimental error was
21 added using the Box-Muller method. The model was then fit to these new relaxation rates. This

²See chisq.c lines 246-248 for relaxation data fitting, and lines 254-288 for order parameter fitting

22 procedure was repeated N times per residue, and the error is given as twice the standard deviation
 23 of all of these repeats³.

24 Simple Model Free

25 The Simple Model Free model was implemented as outlined in Lipari *et al.* 1982³. In brief, the
 26 spectral density is taken as⁴:

$$J(\omega \text{ (rad s}^{-1}\text{)}) = \frac{(1 - S^2) \tau}{(1 + (\omega \tau)^2)}$$

27 Where ω is the frequency of interest, and the other parameters are as defined in Table 1. The
 28 contribution to the R_1 relaxation rate from a dipolar interaction was obtained using⁴⁵:

$$R_{1, \text{dipolar}}(\Omega, \omega) = \frac{1}{10} \omega_D^2 (J(\omega - \Omega) + 3 \times J(\omega) + 6 \times J(\omega + \Omega))$$

29 Where Ω is the Larmor frequency for the nuclei being observed, ω is the Larmor frequency for the
 30 dipolar coupled nuclei, and the dipolar coupling is given by ω_D . The CSA contribution is⁶:

$$R_{1, \text{CSA}}(\Omega) = \frac{2}{15} (\delta_{11}^2 + \delta_{22}^2 + \delta_{33}^2 - \delta_{11}\delta_{22} - \delta_{11}\delta_{33} - \delta_{22}\delta_{33}) \Omega^2 \times J(\Omega)$$

31 In which δ_{11}^2 are components of the CSA, parameterized using the isotropic chemical shift of the
 32 given nuclei. Defining J_k as follows (where ω_r and ω_1 are the spinning frequency and spin lock
 33 frequency in Hz, respectively)⁷.

³Errors.c lines 26-35 for the Box-Muller method, 48-60 for standard deviation calculation. Errors calculated in calc_errors(). Back calculation of rates is line 131, adding error is done 132. Simplex performed 141. Statistics and output done main.c lines 457-460.

⁴Smf.c, lines 15-18

⁵Smf.c, SMF_Dipolar_R1 33-40

⁶Smf.c eg 123-125

⁷This is J0sum, eg 177-181 in smf.c and 203-207 in emf.c

$$J_k(\omega_r, \omega_1) = \frac{2}{3}J(2\pi(\omega_1 - 2\omega_r) + \frac{2}{3}J(2\pi(\omega_1 + 2\omega_r) + \frac{4}{3}J(2\pi(\omega_1 - \omega_r) + \frac{4}{3}J(2\pi(\omega_1 + \omega_r)$$

³⁴ The dipolar contribution to the $R_{1\rho}$ rate is taken as⁸;

$$R_{1\rho, \text{ dipolar}}(\Omega, \omega) = \frac{1}{20}\omega_D^2(J_k + 3 \times J(\Omega) + J(\omega - \Omega) + 6 \times J(\omega) + 6 \times J(\omega + \Omega))$$

³⁵ And correspondingly⁹

$$R_{1\rho, \text{ CSA}}(\Omega) = \frac{1}{45}(\delta_{11}^2 + \delta_{22}^2 + \delta_{33}^2 - \delta_{11}\delta_{22} - \delta_{11}\delta_{33} - \delta_{22}\delta_{33})\Omega^2 \times (J_k + 3 \times J(\Omega))$$

³⁶ For ^{15}N , contributions from N-H, N-Hr, N-C and N-Ca dipolar interactions were considered. For
³⁷ ^{13}C , C-H, C-Hr, C-N and C-C dipolar interactions were considered. The effective distances used
³⁸ for calculation of dipolar couplings are given in Table 2.

Bond	Length / Å
N-H	1.02 ⁵
N-H (rest)	1.80 ⁵
N-C	1.33 ⁵
N-C α	1.46 ⁵
C-H	2.04 ⁶
C-H (rest)	1.82 ⁵
C-N	1.33 ⁵
C-C	1.53 ⁶

⁸Smf.c 58-65, emf.c 81-88.

⁹Smf.c 205-207 and emf.c 231-233.

39 Extended Model Free

40 The Extended Model Free spectral density was taken as:⁷¹⁰:

$$J(\omega \text{ (rad s}^{-1}\text{)}) = \frac{(1 - S_{\text{fast}}^2) \tau_{\text{fast}}}{(1 + (\omega \tau_{\text{fast}})^2)} + \frac{S_{\text{fast}}^2 (1 - S_{\text{slow}}^2) \tau_{\text{slow}}}{(1 + (\omega \tau_{\text{slow}})^2)}$$

41 The form of the relaxation rates was as in Simple Model Free.

42 Gaussian Axial Fluctuations

43 The GAF spectral density was taken as¹¹

$$J(\omega) = \frac{(1 - S_{\text{fast}}^2) \tau_{\text{fast}}}{(1 + (\omega \tau_{\text{fast}})^2)} + \frac{1}{P_2(\cos(\theta_u))} \frac{S_{\text{fast}}^2 (P_2(\cos(\theta_u)) - S_{\text{slow}}^2) \tau_{\text{slow}}}{(1 + (\omega \tau_{\text{slow}})^2)}$$

44 Where $P_2(\cos(\theta_u))$ equals 1 for autocorrelated motions and -1/2 for cross correlated motions.

45 The GAF order parameters were obtained as by Lienin et al, 1998⁸ via¹²

$$S_u^2 = \frac{4\pi}{5} \sum_{l, k, k', m, m'=-2}^2 (-i)^{k-k'} \exp \left[-\frac{\sigma_\alpha^2 (k^2 + k'^2)}{2} - \sigma_\beta^2 l^2 - \frac{\sigma_\gamma^2 (m^2 + m'^2)}{2} \right] \times d_{kl}^{(2)} \left(\frac{\pi}{2} \right) d_{k'l}^{(2)} \left(\frac{\pi}{2} \right) d_{mk}^{(2)}$$

46 In which $d_{mm'}^{(2)}, (\frac{\pi}{2})$ are reduced Wigner matrix elements evaluated at $\frac{\pi}{2}$, $Y_{2m}(\theta(t), \phi(t))$ are second
 47 order spherical harmonics, and $\mathbf{e}_\alpha^{\text{pp}}, \mathbf{e}_\beta^{\text{pp}}, \mathbf{e}_\gamma^{\text{pp}}$ are principal axes rigidly attached to the peptide plane.
 48 The deflection angles, $\sigma_\alpha, \sigma_\beta, \sigma_\gamma$ refer to Gaussian rotations about these axes. Here, we have taken
 49 the same definition of these principal axes as in Lienin et al, 1998.⁸ Relaxation rates were obtained.
 50 Dipolar contributions to the relaxation rates were taken as in EMF, while CSA contributions were
 51 calculated as:⁹¹³:

¹⁰Emf.c 14-24.

¹¹The case for autocorrelated is just J0_EMF, emf.c 14-24. The cross correlate is emf.c 30-40.

¹²Gaf.c 103-177. Note I've messed it around a bit. The individual terms of the exponential are calculated once per loop, and I've considered that the Y2m components have no real components and so if we're selecting for real order parameters we can ignore any cases where $(-i)^{\wedge(k-k')}$ is imaginary.

¹³Gaf.c, 248-256, 416-422.

$$R_{1, \text{CSA}}^X(\Omega) = \frac{1}{15} \times (\delta_{xx} - \delta_{zz})^2 \times \Omega^2 \times J(\Omega)$$

$$R_{1, \text{CSA}}^Y(\Omega) = \frac{1}{15} \times (\delta_{yy} - \delta_{zz})^2 \times \Omega^2 \times J(\Omega)$$

$$R_{1, \text{CSA}}^{XY}(\Omega) = \frac{1}{15} \times (\delta_{xx} - \delta_{zz})(\delta_{yy} - \delta_{zz}) \times \Omega^2 \times J(\Omega)$$

$$R_{1, \text{CSA}}(\Omega) = R_{1, \text{CSA}}^X(\Omega) + R_{1, \text{CSA}}^Y(\Omega) + 2 \times R_{1, \text{CSA}}^{XY}(\Omega)$$

52 For $R_{1\rho}$, CSA contributions to the relaxation rate were taken as¹⁴:

$$R_{1\rho, \text{CSA}}^X(\Omega) = \frac{1}{15} \times (\delta_{xx} - \delta_{zz})^2 \times \Omega^2 \times (J_k + 3 \times J(\Omega))$$

$$R_{1\rho, \text{CSA}}^Y(\Omega) = \frac{1}{15} \times (\delta_{yy} - \delta_{zz})^2 \times \Omega^2 \times (J_k + 3 \times J(\Omega))$$

$$R_{1\rho, \text{CSA}}^{XY}(\Omega) = \frac{1}{15} \times (\delta_{xx} - \delta_{zz})(\delta_{yy} - \delta_{zz}) \times \Omega^2 \times (J_k + 3 \times J(\Omega))$$

$$R_{1\rho, \text{CSA}}(\Omega) = R_{1\rho, \text{CSA}}^X(\Omega) + R_{1\rho, \text{CSA}}^Y(\Omega) + 2 \times R_{1\rho, \text{CSA}}^{XY}(\Omega)$$

53 Temperature Dependence

54 For the temperature dependent models we assumed that the timescales of motion would be time
 55 dependent according to the Arrhenius equation¹⁵:

¹⁴Gaf.c, 36-60 implements each summation, then 333-336 and 501-503.

¹⁵eg chisq.c 46, 82-83, 128-129, 172-173.

$$\tau(T) = \tau^0 \exp\left(\frac{E_a}{RT}\right)$$

56 Fitted Correlation Functions

57 Using the parameters obtained from the dynamics model fitting described above we determined the
 58 form of the correlation functions for these motions. For Simple Model Free analysis, the correlation
 59 functions were plotted according to:³¹⁶:

$$C(t) = S^2 + (1 - S^2) \exp\left(\frac{-t}{\tau}\right)$$

60 For Extended Model Free, the following form was used:⁷¹⁷:

$$C(t) = S^2 + (1 - S_f^2) \exp\left(\frac{-t}{\tau_f}\right) + S_f^2 (1 - S_s^2) \exp\left(\frac{-t}{\tau_s}\right)$$

61 AMBER MD

62 A molecular dynamics trajectory for GB1 in a supercell was computed using AMBER MD¹⁰. The
 63 coordinates of the X-ray structure of GB1 (PDB:2gi9) were taken as a starting conformation. A
 64 unit cell containing 4 GB1 molecules was created using the AMBER MD *UnitCell* utility, which was
 65 propagated by *PropPDB* to produce a 3x3x3 supercell containing 108 GB1 proteins. To this, 108
 66 PO₄³⁻ counter ions were added using *AddToBox*, along with 108 MPD and 648 IPA cocrystallising
 67 ligands. Finally, 12852 water molecules were added to the box. This was then charge balanced
 68 by addition of sodium ions, to give an overall box size of 75.591 Å x 107.152 Å x 150.822 Å. The
 69 ff14SB¹¹ forcefield was used for the GB1 proteins, while TIP3P was used for water and GAFF for
 70 the cocrystals.

¹⁶Correlation.c, 171-172

¹⁷Correlation.c, 134-136

71 The system was heated to 300 K and allowed to equilibrate for 25 ps with a timestep of 0.5 fs. The
 72 system was then simulated for a full 1 μ s run with a 2 fs timestep and a cut off of 11 Å for non-
 73 bonded interactions. The temperature was maintained at 300 K using Langevin thermostat, and
 74 the SHAKE algorithm¹² was applied to all bond lengths involving a hydrogen atom. Anisotropic
 75 pressure scaling was used with periodic boundary conditions.

76 Correlation functions for NH, CH and CN bonds were extracted from the final trajectories for
 77 each of the 108 GB1 molecules simulated in the following manner. *Pytraj*¹³ was used align the
 78 supercells to minimize the effects of overall tumbling. Atomic coordinates were then extracted, and
 79 for each time step the orientation of the relevant atomic bond vector was obtained. The correlation
 80 function was then calculated using⁹

$$C_{\mu\nu}^{\text{int}}(k\Delta t) = \frac{1}{(N-k)} \sum_{i=1}^{N-k} \frac{3(\mathbf{e}_{\mu,i+k} \cdot \mathbf{e}_{\nu,i})^2 - 1}{2}$$

81 Bibliography

- 82 1. Nelder, J. A. & Mead, R. A Simplex Method for Function Minimization. *The Computer Journal*
 83 **7**, 308–313 (1965).
- 84 2. Lamley, J. M. *et al.* Unraveling the complexity of protein backbone dynamics with combined
 85 ¹³C and ¹⁵N solid-state NMR relaxation measurements. *Physical Chemistry Chemical Physics*
 86 **17**, 21997–22008 (2015).
- 87 3. Lipari, G. & Szabo, A. Model-free approach to the interpretation of nuclear magnetic resonance
 88 relaxation in macromolecules. 1. Theory and range of validity. *Journal of the American Chemical*
 89 *Society* **104**, 4546–4559 (1982).
- 90 4. Kurbanov, R., Zinkevich, T. & Krushelnitsky, A. The nuclear magnetic resonance relaxation
 91 data analysis in solids: General R₁ / R_{1ρ} equations and the model-free approach. *The Journal*
 92 *of Chemical Physics* **135**, 184104 (2011).
- 93 5. Ferrage, F., Pelulessy, P., Cowburn, D. & Bodenhausen, G. Protein Backbone Dynamics

- 94 through $^{13}\text{C}'$ – ^{13}C Cross-Relaxation in NMR Spectroscopy. *J. Am. Chem. Soc.* **128**, 11072–
95 11078 (2006).
- 96 6. Engh, R. A. & Huber, R. {Accurate bond and angle parameters for X-ray protein structure
97 refinement}. *Acta Crystallographica Section A* **47**, 392–400 (1991).
- 98 7. Clore, G. M. *et al.* Deviations from the simple two-parameter model-free approach to the
99 interpretation of nitrogen-15 nuclear magnetic relaxation of proteins. *Journal of the American*
100 *Chemical Society* **112**, 4989–4991 (1990).
- 101 8. Lienin, S. F., Bremi, T., Brutscher, B., Brüschweiler, R. & Ernst, R. R. Anisotropic Intramolec-
102 ular Backbone Dynamics of Ubiquitin Characterized by NMR Relaxation and MD Computer
103 Simulation. *Journal of the American Chemical Society* **120**, 9870–9879 (1998).
- 104 9. Bremi, T. & Brüschweiler, R. Locally Anisotropic Internal Polypeptide Backbone Dynamics by
105 NMR Relaxation. *Journal of the American Chemical Society* **119**, 6672–6673 (1997).
- 106 10. Case, D. A. *et al.* The Amber biomolecular simulation programs. *Journal of Computational*
107 *Chemistry* **26**, 1668–1688 (2005).
- 108 11. Maier, J. A. *et al.* ff14SB: Improving the Accuracy of Protein Side Chain and Backbone
109 Parameters from ff99SB. *J. Chem. Theory Comput.* **11**, 3696–3713 (2015).
- 110 12. Ryckaert, J.-P., Ciccotti, G. & Berendsen, H. J. C. Numerical integration of the cartesian
111 equations of motion of a system with constraints: molecular dynamics of n-alkanes. *Journal of*
112 *Computational Physics* **23**, 327–341 (1977).
- 113 13. Nguyen, H., Roe, D. R., Swails, J. & Case, D. A. PYTRAJ: Interactive data analysis for
114 molecular dynamics simulations. *New Brunswick, NJ: Rutgers University* (2016).