Solid-state NMR provides evidence for small-amplitude slow domain motions in a multi-spanning transmembrane α-helical protein

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Supporting Information
1. Materials and Methods

![Figure S1](image)

**Figure S1.** Dipolar recoupling and $^{15}$N relaxation measurement pulse sequences used in this study. Hollow and filled bars represent 90° and 180° pulses, respectively. (A1) $^{15}$N detected and (A2) $^{13}$Cα detected TMREV recoupling was used to probe $^{15}$N-$^1$H dipolar couplings. TMREV pulse train was applied for increasing duration during a constant-time interval of length 12$t_r$ (m=6). Four MREV$^{12,3}$ blocks were applied per rotor period $t_r$ (n=4), with the overall phase of each block incremented by 90° (TMREV-4). TPPM decoupling was used for the remainder of the 12$t_r$ period. Phase tables were as follows: $f_1=y-x, f_2=x, f_3=x, f_4=x x y y -x -x -y -y, f_{rec}=x x x y x y y y -x y -y -y$. (B) For $^{15}$N R$_1$ measurements 2D NCA and afterglow NCO spectra were acquired as a function of recovery time $\tau$ of 1s, 5s, 10s, 20s and 30s. The following phases were used: $f_1=x x x x -x, f_2=x x x x -x -x x -x -x, f_3=x x x x x x x x x x, f_4=x x x x x, f_5=8(x) 8(-x), f_6=8(x) 8(-x), f_7=x, f_8=-x, f_{rec}=x x x x -x x x x -x x x x x x x x x x$. (C) For R$_{1p}$ measurements 2D NACAB correlation spectra were recorded as a function of a spinlock time $\tau$. The phases were as follows: $f_1=y -y, f_2=x, f_3=x, f_4=x x y y -x -y -y -y, f_{rec}=x x y y -y -x -y y y$. The phase of all other pulses including the spinlock pulse was x.
Figure S2. A comparison of $^{15}$N spectra of ASR collected on a 600 MHz spectrometer at a spinning rate of 8 kHz, and at 7 °C and 30 °C. Spinning sideband intensities remain unchanged and match the theoretical simulation of a typical $^{15}$N chemical shift anisotropy (CSA) tensor, overall indicating that the protein does not undergo rotational diffusion. Simulation was performed using the program SIMPSON$^6$ with the CSA parameters of $\delta_{\text{CSA}} = -109$ ppm, $\eta = 0.2.$

Figure S3. Amino acid sequence and the topological model of ASR. Helices are shown as rectangles and are labelled A through G. Assigned residues at 7 °C are shown as blue and green circles; assigned residues at 30 °C are shown as green circles. The cytoplasmic side is on top.
2. Chemical shift perturbations and cross peak intensities at 30 °C.

Figure S4. (A) A comparison of cross peak intensities in the CANCO experiments recorded at 7 °C and 30 °C. (B) Chemical Shift Index $\delta_{C\alpha} - \delta_{C\beta}$ at 30 °C. (C) $^{15}$N amide and (D) $^{13}$C$\alpha$ chemical shift difference between 30 °C and 7 °C.
Figure S5. Bulk $R_{1p}$ measurements at a spinning frequency of 14.3 kHz, at 7 °C and 30 °C for (A) $^1$H, (B) $^{13}$C and (C) $^{15}$N.

3. Estimation of the residual coherent contributions to the $^{15}$N $R_{1p}$ relaxation rates at fast MAS rates.

Figure S6. Experimental $^{15}$N $R_{1p}$ trajectories measured in N-acetyl-[U-$^{13}$C, $^{15}$N-labeled]-Val-Leu peptide at a spinning frequency of 50 kHz and at the magnetic field strength of 800 MHz.

$R_{1p}$ relaxation rates measured in fully protonated samples may include coherent contributions from anisotropic interactions which are incompletely averaged by the fast (50-55
kHz) magic angle spinning. To estimate an order of magnitude of the residual coherent effects, we measured $^{15}$N $R_{1\rho}$ relaxation rates in a model dipeptide N-Ac-[$^{13}$C, $^{15}$N-labeled]-Val-Leu (NAVL), under typical experimental conditions which were used to measure relaxation rates in ASR at 7 °C ($\omega_r/2\pi=50$ kHz, $^{15}$N lock RF field $\omega_1/2\pi=12$ kHz). Previous multiple solid-state NMR measurements (e.g., internuclear distance measurements) on NAVL indicate rigid peptide in which intermediate time scale motions are largely suppressed. Rotations of the methyl groups likely present in the peptide at the experimental temperatures are expected to occur on a fast time scale and would have minimal contributions to the transverse relaxation. Thus, we expect that $R_{1\rho}$ rates to be governed by the residual anisotropic interactions. $R_{1\rho}$ rates in NAVL were determined to be $\sim 0.23\pm 0.09$ s$^{-1}$ and $0.42\pm 0.13$ s$^{-1}$ (Figure S6) for valine and leucine, respectively, with both values being less than the lowest $R_{1\rho}$ rate of 0.60 s$^{-1}$ measured in ASR for protonated amide nitrogen atoms.

4. Bulk order parameter measurements at 30 °C.

As explained in the main text, ASR samples were found to be unstable at 30 °C on the time scale of a full 3D DIPSHIFT experiment. To probe the amplitudes of motions, we performed the bulk order parameter measurements using $^{15}$N- and $^{13}$C-detected DIPSHIFT experiments with TMREV-4 recoupling (Figures S1A1, A2). $^{15}$N and 1D NCA spectra are shown in Figure S7A, C, and representative dipolar dephasing curves are in Figure S7B, D. The observed small changes in the extracted dipolar order parameters are comparable to the small change in the order parameter of $^{15}$N-labeled crystalline sample of N-Ac-Valine (Table S1). Thus, small differences in the dipolar coupling at 7 °C and 30 °C are representative of the systematic effects from the equipment rather than from changes in the amplitudes of motions in ASR.
Figure S7. TMREV-4 measurements of the $^1$H-$^{15}$N bulk order parameter in the 1,3-ASR sample at 7 °C and 30 °C. (A) 1D NCA spectrum with some amino acid type-specific assignments indicated in the figure. (B) Representative TMREV $^1$H-$^{15}$N dipolar dephasing from the bulk peak in the $^{13}$C detected spectrum at 7 °C and 30 °C. (C) Nitrogen spectrum with amino acid assignment assignments. (D) Representative nitrogen-detected TMREV $^1$H-$^{15}$N dipolar dephasing curves of the Thr/Ser/Gly peak at 7 °C and 30 °C.

Table S1. Order parameters measured from TMREV experiments at 7 °C and 30 °C

| Sample      | Peak labeling               | $S_{NH}^2$ at 7 °C     | $S_{NH}^2$ at 30 °C |
|-------------|------------------------------|------------------------|---------------------|
| 1,3-ASR     | 13C-detected (Fig. S6C)     | Bulk no prolines       | 0.843±0.007         | 0.788±0.009         |
|             |                               | Thr                    | 0.830±0.040         | 0.890±0.060         |
|             |                               | Thr/Ser/Gly            | 0.823±0.021         | 0.779±0.025         |
| NAV         |                               |                        | 0.883±0.015         | 0.869±0.019         |
5. Site-specific relaxation rates and order parameter measurements

**Figure S8.** (A-B) Representative NCA spectra collected on a 1,3-ASR sample and at 30 °C. (A) represents a starting point of the $R_1$ relaxation trajectory (0 s delay); (B) corresponds to the 30 s delay. (C-D) NCACB spectra collected at 30 °C, and representing two points of the $R_{1\rho}$ relaxation trajectories with the delay times of 0s (C) and 300ms (D). Negative peaks resulting from the double-quantum DREAM mixing are shown in red.

**Table S2.** Relaxation rates and order parameters measured at 7 °C and 30 °C

| Residue | $R_1$ @7°C, 600MHz (s$^{-1}$) | $R_1$ @7°C, 800MHz (s$^{-1}$) | $R_{1\rho}$ @7°C, 800MHz (s$^{-1}$) | $S_{\rho H}$ @7°C, 600MHz (s$^{-1}$) | $R_1$ @30°C, 800MHz (s$^{-1}$) | $R_{1\rho}$ @30°C, 800MHz (s$^{-1}$) |
|---------|-------------------------------|-----------------------------|--------------------------------|----------------------------------|-----------------------------|----------------------------------|
| L7      | 0.0170 ± 0.0008                | 0.0175 ± 0.0010             | 3.8 ± 0.6                     | 0.880 ± 0.013                    | 0.0155 ± 0.0011             | 4.3 ± 0.6                        |
| W9      |                               |                             |                                |                                 | 5.0 ± 1.6                   |                                  |
| I10     |                               |                             |                                |                                 |                             | 1.7 ± 0.8                       |
| V12     |                               |                             |                                | 0.925 ± 0.033                    | 0.0019 ± 0.0019             | 1.4 ± 0.4                       |
| A13     | 0.0250 ± 0.0100                | 0.0105 ± 0.0013             | 2.0 ± 0.9                     | 0.908 ± 0.021                    | 0.0030 ± 0.0016             | 2.3 ± 0.5                       |
| G14     | 0.0101 ± 0.0019                | 0.0040 ± 0.0030             |                                | 0.803 ± 0.016                    | 0.0060 ± 0.0030             |                                  |
| M15     |                               |                             | 0.5 ± 0.7                     | 0.870 ± 0.022                    | 0.0125 ± 0.0019             | 1.9 ± 0.7                       |
|   |   |   |   |   |
|---|---|---|---|---|
| G18 | 0.0050 ± 0.0014 | 0.0040 ± 0.0040 | 2.4 ± 1.0 | 0.837 ± 0.029 |
| A19 | 0.0120 ± 0.0006 | 0.0110 ± 0.0010 | 0.859 ± 0.019 | 0.0065 ± 0.0019 |
| L20 | 0.0195 ± 0.0017 | 0.0100 ± 0.0020 | 0.867 ± 0.013 | 0.0105 ± 0.0010 |
| F22 | 0.0130 ± 0.0020 | 0.869 ± 0.026 | 0.0270 ± 0.010 |
| S24 | 0.0185 ± 0.0016 | 0.0275 ± 0.0020 | 0.0250 ± 0.003 |
| L25 | 0.0400 ± 0.0070 | 0.0410 ± 0.0070 | 0.0275 ± 0.0019 |
| N28 | 0.0190 ± 0.0040 | 0.0450 ± 0.0110 | 0.0580 ± 0.011 |
| P29 | 0.0200 ± 0.0040 | 0.0220 ± 0.0020 | 0.0200 ± 0.004 |
| V32 | 0.0200 ± 0.0040 | 0.0370 ± 0.0090 | 0.0280 ± 0.007 |
| P33 | 0.0460 ± 0.0050 | 0.0340 ± 0.0050 | 11.0 ± 2.0 | 0.774 ± 0.037 |
| E36 | 0.0120 ± 0.0020 | 0.0100 ± 0.0050 | 0.0420 ± 0.004 |
| M41 | 0.0006 ± 0.0009 | 0.0012 ± 0.0018 | 0.0030 ± 0.004 |
| I43 | 0.0060 ± 0.0009 | 0.0055 ± 0.0011 | 0.0008 ± 0.0012 |
| S47 | 0.0060 ± 0.0012 | 0.0030 ± 0.0020 | 1.1 ± 0.4 |
| G48 | 0.0070 ± 0.0013 | 0.869 ± 0.015 | 1.1 ± 0.5 |
| A50 | 0.0065 ± 0.0008 | 0.0060 ± 0.0010 | 0.0006 ± 0.0009 |
| Y51 | 0.0065 ± 0.0019 | 0.0050 ± 0.0020 | 0.799 ± 0.011 |
| M52 | 0.19 ± 0.5 | 0.878 ± 0.026 | 0.0065 ± 0.0018 |
| A53 | 0.0110 ± 0.0020 | 0.0150 ± 0.0020 | 1.2 ± 0.6 |
| M54 | 0.0150 ± 0.0012 | 0.0135 ± 0.0016 | 3.0 ± 0.7 |
| A55 | 0.0170 ± 0.0030 | 0.0060 ± 0.0040 | 0.0007 ± 0.0020 |
| I56 | 0.0220 ± 0.0020 | 0.0250 ± 0.0030 | 0.0180 ± 0.0070 |
| G59 | 0.0320 ± 0.0050 | 0.0140 ± 0.0090 | 5.4 ± 1.9 |
| K60 | 0.0320 ± 0.0040 | 0.0660 ± 0.0050 | 19.0 ± 14.0 |
| E62 | 0.0370 ± 0.0030 | 0.0510 ± 0.0090 | 1.0 ± 0.7 |
| A63 | 0.0890 ± 0.0070 | 0.0670 ± 0.0070 | 0.806 ± 0.02 |
| A64 | 0.0610 ± 0.0060 | 0.0430 ± 0.0060 | 0.0740 ± 0.0320 |
| H69 | 0.0650 ± 0.0040 | 0.0660 ± 0.0050 | 16.0 ± 4.0 |
| H71 | 0.0890 ± 0.0070 | 0.0762 ± 0.0028 | 0.0420 ± 0.0100 |
| A71 | 0.0330 ± 0.0040 | 0.0270 ± 0.0070 | 9.0 ± 3.0 |
| G65 | 0.0330 ± 0.0030 | 0.0510 ± 0.0090 | 0.0850 ± 0.0260 |
| Q66 | 0.0200 ± 0.0060 | 0.0860 ± 0.027 | 0.0630 ± 0.0120 |
| I67 | 0.0330 ± 0.0030 | 0.0290 ± 0.0030 | 0.0740 ± 0.0320 |
| A68 | 0.0070 ± 0.0020 | 0.0100 ± 0.0040 | 6.1 ± 1.6 |
| H69 | 0.0110 ± 0.0012 | 0.0100 ± 0.0015 | 0.878 ± 0.03 |
| A71 | 0.0061 ± 0.0018 | 0.0050 ± 0.0020 | 0.0340 ± 0.0080 |
| R72 | 0.0075 ± 0.0011 | 0.0045 ± 0.0014 | 7.0 ± 2.0 |
| Y73 | 0.0180 ± 0.0015 | 0.0105 ± 0.0017 | 0.817 ± 0.045 |
| D75 | 0.0070 ± 0.0030 | 0.091 ± 0.0032 | 0.0110 ± 0.0050 |
| W76 | 0.0200 ± 0.0040 | 0.0931 ± 0.0033 | 1.0 ± 0.4 |
| M77 | 0.0030 ± 0.0015 | 0.0095 ± 0.0016 | 0.914 ± 0.0018 |
| V78 | 0.0040 ± 0.0016 | 0.0070 ± 0.0030 | 0.9 ± 0.5 |
| T79 | 0.0070 ± 0.0030 | 1.7 ± 0.5 | 0.821 ± 0.014 |
| T80 | 0.0065 ± 0.0013 | 0.0010 ± 0.0020 | 0.9 ± 0.5 |
| P81 | 0.0009 ± 0.0013 | 0.0010 ± 0.0020 | 0.0008 ± 0.0011 |
|   | 0.3 ± 0.5 | 0.046 ± 0.014 | 1.3 ± 0.4 |
|   | 0.0070 ± 0.0020 | 0.941 ± 0.041 | 0.8 ± 0.4 |
|   | 0.0009 ± 0.0013 | 0.0065 ± 0.0020 | 0.5 ± 0.6 |
|   |   |   |   |   |   |
|---|---|---|---|---|---|
| L82 | 0.0080 ± 0.0007 | 0.0050 ± 0.0010 | 0.912 ± 0.015 | 0.0075 ± 0.0009 | 2.0 ± 0.4 |
| L84 | 0.0115 ± 0.0011 | 0.0120 ± 0.0050 | 0.0050 ± 0.0010 | 1.1 ± 0.7 |
| L85 | 0.0050 ± 0.0008 | 0.0190 ± 0.0030 | 0.0050 ± 0.0010 | 0.9 ± 0.4 |
| S86 | 0.0050 ± 0.0008 | 0.0050 ± 0.0010 | 0.0050 ± 0.0010 | 0.9 ± 0.4 |
| S88 | 0.912 ± 0.015 | 0.0075 ± 0.0009 | 0.0075 ± 0.0009 | 2.7 ± 0.6 |
| T90 | 0.0120 ± 0.0050 | 0.0120 ± 0.0050 | 0.0120 ± 0.0050 | 3.2 ± 1.0 |
| A91 | 3.1 ± 1.1 | 0.9 ± 0.4 | 0.9 ± 0.4 | 3.6 ± 1.2 |
| M92 | 0.0190 ± 0.0030 | 0.0190 ± 0.0030 | 0.0190 ± 0.0030 | 3.1 ± 0.8 |
| F94 | 0.0050 ± 0.0008 | 0.0050 ± 0.0008 | 0.0050 ± 0.0008 | 1.4 ± 0.6 |
| I95 | 0.0160 ± 0.0020 | 0.0160 ± 0.0020 | 0.0160 ± 0.0020 | 3.7 ± 1.1 |
| K96 | 0.0360 ± 0.0100 | 0.0360 ± 0.0100 | 0.0360 ± 0.0100 | 4.3 ± 1.1 |
| T100 | 0.0230 ± 0.0060 | 0.0070 ± 0.0070 | 0.0070 ± 0.0070 | 7.0 ± 2.0 |
| I102 | 0.0125 ± 0.0017 | 0.0090 ± 0.0030 | 0.0090 ± 0.0030 | 0.8 ± 0.7 |
| G103 | 2.1 ± 0.9 | 0.9 ± 0.4 | 0.9 ± 0.4 | 3.6 ± 1.2 |
| F104 | 0.0120 ± 0.0080 | 0.0120 ± 0.0080 | 0.0120 ± 0.0080 | 1.8 ± 0.9 |
| S107 | 0.0080 ± 0.0017 | 0.0022 ± 0.0019 | 0.0022 ± 0.0019 | 0.9 ± 0.4 |
| T108 | 1.3 ± 0.5 | 0.885 ± 0.015 | 0.885 ± 0.015 | 3.2 ± 1.0 |
| Q109 | 0.878 ± 0.021 | 0.0140 ± 0.0020 | 0.0140 ± 0.0020 | 0.9 ± 0.4 |
| I110 | 0.0070 ± 0.0013 | 0.060 ± 0.0030 | 0.060 ± 0.0030 | 0.9 ± 0.4 |
| V111 | 0.0050 ± 0.0007 | 0.085 ± 0.0018 | 0.085 ± 0.0018 | 0.9 ± 0.4 |
| V112 | 0.0050 ± 0.0007 | 0.0060 ± 0.0016 | 0.0060 ± 0.0016 | 0.9 ± 0.4 |
| T114 | 0.0070 ± 0.0018 | 1.7 ± 0.8 | 1.7 ± 0.8 | 0.9 ± 0.4 |
| S115 | 0.0080 ± 0.0007 | 0.0095 ± 0.0010 | 0.0095 ± 0.0010 | 0.9 ± 0.4 |
| G116 | 0.865 ± 0.013 | 0.865 ± 0.013 | 0.865 ± 0.013 | 0.9 ± 0.4 |
| L117 | 0.0080 ± 0.0007 | 0.0095 ± 0.0010 | 0.0095 ± 0.0010 | 0.9 ± 0.4 |
| I118 | 0.0180 ± 0.0007 | 1.9 ± 0.5 | 1.9 ± 0.5 | 0.9 ± 0.4 |
| A119 | 0.0070 ± 0.0030 | 0.0130 ± 0.0030 | 0.0130 ± 0.0030 | 0.9 ± 0.4 |
| D120 | 0.0070 ± 0.0007 | 0.0130 ± 0.0030 | 0.0130 ± 0.0030 | 0.9 ± 0.4 |
| L121 | 0.891 ± 0.030 | 0.891 ± 0.030 | 0.891 ± 0.030 | 0.9 ± 0.4 |
| S122 | 0.0080 ± 0.0007 | 0.0095 ± 0.0010 | 0.0095 ± 0.0010 | 0.9 ± 0.4 |
| E123 | 0.0080 ± 0.0007 | 0.0130 ± 0.0030 | 0.0130 ± 0.0030 | 0.9 ± 0.4 |
| R124 | 0.0080 ± 0.0007 | 0.0130 ± 0.0030 | 0.0130 ± 0.0030 | 0.9 ± 0.4 |
| D125 | 0.0080 ± 0.0007 | 0.0130 ± 0.0030 | 0.0130 ± 0.0030 | 0.9 ± 0.4 |
| W126 | 0.0210 ± 0.0030 | 0.891 ± 0.059 | 0.891 ± 0.059 | 0.9 ± 0.4 |
| L130 | 0.0250 ± 0.0020 | 0.0310 ± 0.0060 | 0.0310 ± 0.0060 | 0.9 ± 0.4 |
| W131 | 0.0100 ± 0.0014 | 0.0110 ± 0.0020 | 0.0110 ± 0.0020 | 0.9 ± 0.4 |
| Y132 | 0.835 ± 0.031 | 0.0230 ± 0.0050 | 0.0230 ± 0.0050 | 0.9 ± 0.4 |
| C134 | 0.843 ± 0.018 | 0.0106 ± 0.0012 | 0.0106 ± 0.0012 | 0.9 ± 0.4 |
| G135 | 0.878 ± 0.017 | 2.2 ± 0.6 | 2.2 ± 0.6 | 0.9 ± 0.4 |
| V136 | 0.895 ± 0.021 | 5.2 ± 1.0 | 5.2 ± 1.0 | 0.9 ± 0.4 |
| C137 | 0.814 ± 0.016 | 3.6 ± 0.9 | 3.6 ± 0.9 | 0.9 ± 0.4 |
| A138 | 0.8 ± 0.6 | 6.2 ± 1.8 | 6.2 ± 1.8 | 0.9 ± 0.4 |
| I142 | 0.814 ± 0.016 | 3.6 ± 0.9 | 3.6 ± 0.9 | 0.9 ± 0.4 |
|   |   |   |
|---|---|---|
| W144 | 0.0080 ± 0.0030 | 0.050 ± 0.0050 | 0.0075 ± 0.0016 |
| G145 | 0.0100 ± 0.0017 | 0.030 ± 0.0030 | 0.0260 ± 0.0100 |
| I146 | 0.0210 ± 0.0020 | 0.0280 ± 0.0040 | 0.0120 ± 0.0030 |
| N148 | 0.0020 ± 0.0020 | 0.0120 ± 0.0040 | 0.0200 ± 0.0050 |
| P149 | 0.0150 ± 0.0050 | 0.0070 ± 0.0010 | 0.0080 ± 0.0030 |
| K153 | 0.0640 ± 0.0004 | 0.0190 ± 0.0080 | 0.0033 ± 0.0009 |
| S158 | 0.0390 ± 0.0020 | 0.0320 ± 0.0030 | 0.0470 ± 0.0080 |
| S159 | 0.0120 ± 0.0030 | 0.0340 ± 0.0050 | 0.0120 ± 0.0040 |
| L161 | 0.0080 ± 0.0017 | 0.0070 ± 0.0030 | 0.0240 ± 0.0050 |
| K167 | 0.0120 ± 0.0020 | 0.0120 ± 0.0040 | 0.0200 ± 0.0110 |
| L168 | 0.0125 ± 0.0007 | 0.0115 ± 0.0009 | 0.0070 ± 0.0030 |
| V169 | 0.0090 ± 0.0020 | 0.0120 ± 0.0040 | 3.5 ± 1.5 |
| T170 | 0.0120 ± 0.0020 | 0.0050 ± 0.0020 | 0.0260 ± 0.0100 |
| Y171 | 0.0020 ± 0.0020 | 0.0070 ± 0.0040 | 0.0033 ± 0.0009 |
| F172 | 0.0100 ± 0.0030 | 0.040 ± 0.0030 | 0.0120 ± 0.0030 |
| I173 | 0.0080 ± 0.0020 | 0.0070 ± 0.0040 | 0.0030 ± 0.0040 |
| I177 | 0.0099 ± 0.0016 | 0.0120 ± 0.0040 | 0.0120 ± 0.0030 |
| G178 | 0.0120 ± 0.0020 | 0.0100 ± 0.0020 | 0.0030 ± 0.0040 |
| Y179 | 0.0120 ± 0.0020 | 0.0120 ± 0.0040 | 0.0050 ± 0.0040 |
| P180 | 0.0088 ± 0.0012 | 0.0200 ± 0.0040 | 3.5 ± 1.5 |
| I181 | 0.0090 ± 0.0011 | 0.0110 ± 0.0030 | 0.0260 ± 0.0100 |
| V182 | 0.0120 ± 0.0020 | 0.0050 ± 0.0020 | 1.6 ± 1.0 |
| I184 | 0.0080 ± 0.0020 | 0.0070 ± 0.0040 | 0.0030 ± 0.0040 |
| I185 | 0.0099 ± 0.0016 | 0.0120 ± 0.0040 | 0.0030 ± 0.0040 |
| G186 | 0.0190 ± 0.0060 | 0.0170 ± 0.0080 | 0.0060 ± 0.0030 |
| G187 | 0.0090 ± 0.0020 | 0.0120 ± 0.0040 | 0.0120 ± 0.0030 |
| P188 | 0.0088 ± 0.0012 | 0.0200 ± 0.0040 | 3.5 ± 1.5 |
| I189 | 0.0020 ± 0.0030 | 0.0160 ± 0.0050 | 0.0120 ± 0.0030 |
| G190 | 0.0120 ± 0.0020 | 0.0120 ± 0.0040 | 0.0120 ± 0.0030 |
| F190 | 0.0090 ± 0.0020 | 0.0120 ± 0.0040 | 0.0120 ± 0.0030 |
| G191 | 0.0080 ± 0.0020 | 0.0120 ± 0.0040 | 0.0120 ± 0.0030 |
| I193 | 0.0360 ± 0.0060 | 0.0370 ± 0.0080 | 0.0350 ± 0.0070 |
| N194 | 0.0560 ± 0.0080 | 0.0310 ± 0.0090 | 0.0420 ± 0.0070 |
| Q195 | 0.0040 ± 0.0060 | 0.0120 ± 0.0050 | 0.0180 ± 0.0040 |
| D198 | 0.0175 ± 0.0012 | 0.0140 ± 0.0016 | 0.0160 ± 0.0030 |
| F202 | 0.0190 ± 0.0040 | 0.0160 ± 0.0050 | 0.0210 ± 0.0050 |
| L204 | 0.0160 ± 0.0008 | 0.0160 ± 0.0011 | 0.0135 ± 0.0007 |
| L205 | 0.0110 ± 0.0011 | 0.0100 ± 0.0016 | 0.0120 ± 0.0010 |
| P206 | 0.0060 ± 0.0040 | 0.0230 ± 0.0120 | 0.0030 ± 0.0030 |
| F207 | 0.0030 ± 0.0009 | 0.0080 ± 0.0009 | 0.0030 ± 0.0030 |
Figure S9. Backbone $^{15}$N $R_1$ (A) and $R_{1p}$ (B) relaxation rates measured on a 800 MHz spectrometer at 7 °C (red squares) and 30 °C (black circles). This is the same as Figure 4 except only residues in the center and extracellular sides are shown. Relaxation rates of residues in the interhelical loops and on the cytoplasmic sides of TM helices (shown in grey) have been removed from clarity. Figure 4 represents the full version of this figure.

Table S3. Average $R_1$ relaxation rates for non-proline residues of helices and loops at fields of 600 MHz and 800 MHz and temperatures of 7 °C and 30 °C.

|          | $R_1$ @7°C, 600 MHz (s$^{-1}$) | $R_1$ @7°C, 800 MHz (s$^{-1}$) | $R_1$ @30°C, 800 MHz (s$^{-1}$) |
|----------|-------------------------------|-------------------------------|-------------------------------|
| Helix A  | 0.015±0.002                   | 0.012±0.001                   | 0.014±0.001                   |
| Loop AB  | 0.040±0.007                   | 0.031±0.004                   | 0.058±0.001                   |
| Helix B  | 0.014±0.001                   | 0.011±0.001                   | 0.012±0.001                   |
| Loop BC  | 0.039±0.001                   | 0.034±0.002                   | 0.045±0.004                   |
| Helix C  | 0.009±0.001                   | 0.008±0.001                   | 0.007±0.001                   |
Table S4. Average $R_{1p}$ relaxation rates for non-proline residues in the seven helices and loops at 800 MHz magnetic field and temperatures of 7 °C and 30 °C.

| Region    | $R_{1p}$ @7°C, (s$^{-1}$) | $R_{1p}$ @30°C, (s$^{-1}$) |
|-----------|--------------------------|----------------------------|
| Helix A   | 2.2±0.4                  | 2.8±0.4                    |
| Loop AB   | N/A*                     | N/A*                       |
| Helix B   | 4.1±0.5                  | 2.0±0.3                    |
| Loop BC   | 13.6±1.3                 | 9.0±3.0                    |
| Helix C   | 1.5±0.2                  | 1.45±0.15                  |
| Loop CD   | 2.0±0.7                  | 3.6±1.2**                  |
| Helix D   | 2.5±0.3                  | 2.9±0.4                    |
| Loop DE   | 5.2±1.0**                | 4.2±1.1**                  |
| Helix E   | 4.1±0.8                  | 3.7±0.5                    |
| Loop EF   | 10.0±3.0**               | 9.0±2.0**                  |
| Helix F   | 2.6±0.5                  | 2.6±0.4                    |
| Loop FG   | 16.0±4.0                 | 4.4±0.9                    |
| Helix G   | 7.0±1.5                  | 2.6±0.5                    |

Notes: *No residues detected in this region. **Single residue detected in this region.
6. Simple Model Free Fit

For residues with at least 5 out of 6 experimentally measured $^1$H dipolar $S_{NNH}^2$, $R_1$ at 600MHz and 7 °C, $R_1$ at 800MHz and 7 °C, $R_1$ at 800MHz and 30 °C, and $R_{1p}$ at 800 MHz and 30°C the best fit single motional order parameter $S_{eff}^2$, timescale $\tau_{c,eff}$ and activation energy $E_a$ were determined by minimizing the following $\chi^2$ equation.

$$\chi^2 = \left(\frac{s_{NNH,exp} - s_{eff}^2}{\sigma_{s_{NNH}^2}}\right)^2 + \left(\frac{(R_{1,600,exp}-R_{1,600,bc})^2}{\sigma_{R_{1,600}^2}}\right)^2 + \left(\frac{(R_{1,800,exp}-R_{1,800,bc})^2}{\sigma_{R_{1,800}^2}}\right)^2 + \left(\frac{(R_{1p,7C,exp}-R_{1p,7C,bc})^2}{\sigma_{R_{1p,7C}^2}}\right)^2 + \left(\frac{(R_{1,30C,exp}-R_{1,30C,bc})^2}{\sigma_{R_{1,30C}^2}}\right)^2 + \left(\frac{(R_{1p,30C,exp}-R_{1p,30C,bc})^2}{\sigma_{R_{1p,30C}^2}}\right)^2$$  \hspace{1cm} (S1)

Where $\sigma$ denotes the experimental uncertainty in the measured values and the subscripts $exp$ and $bc$ denote the experimentally measured and back calculated values, respectively. Back calculated relaxation rates were determined using Eqs. 1-7 from the main text. The reduced $\chi^2$ and the degrees of freedom $(d.f.)$ are defined as

$$\chi^2_{\text{reduced}} = \frac{\chi^2}{d.f.}$$  \hspace{1cm} (S2)

$$d.f. = \text{number of experimental values} - \text{number of fit values} - 1$$

We also calculate the Akaike’s Information Criterion (AIC),$^{14}$ Bayesian Information Criterion (BIC)$^{15}$ and corrected Akaike’s Information Criterion (AICc)$^{16}$ which are defined with respect to the number of fit parameters $k$ and the number of experimental data points included in the fit $n$. These values are calculated for each residue (see Table S5) and to allow comparison to EMF they are calculated for each molecular fragment (see Table S7).

$$AIC = \chi^2 + 2k$$  \hspace{1cm} (S3)

$$BIC = \chi^2 + k \cdot \ln (n)$$  \hspace{1cm} (S4)

$$AICc = AIC + \frac{2k(k+1)}{(n-k-1)}$$  \hspace{1cm} (S5)

Uncertainties in the best fit parameters $S_{eff}^2$, $\tau_{c,eff}$ and $E_a$ were determined by a Monte Carlo procedure. First, the best fit parameters were used to back calculate all experimental data.
Gaussian distributed noise was then added to each of the back-calculated values for 5000 times, and the $\chi^2$ minimization was done for each iteration to determine the distribution of the fit parameters.

Table S5. Summary of best fit SMF results

| Residue Number | $S_{eff}^2$ | $\tau_{c,eff}$ | $E_a (kJ/mol)$ | reduced $\chi^2$ | d.f. | AIC | BIC | AICc |
|----------------|------------|----------------|----------------|-----------------|-----|-----|-----|-----|
| L7             | 0.933      | 44ps           | 4.1            | 44.5            | 2   | 95.1| 94.5| 107.1|
| A13            | 0.965      | 51ps           | 36.5           | 13.8            | 2   | 33.5| 32.9| 45.5|
| A19            | 0.912      | 11ps           | 0.0            | 5.9             | 1   | 11.9| 10.7| 35.9|
| E36            | 0.853      | 45ns           | 7.1            | 1.1             | 1   | 7.1 | 5.9 | 31.1|
| S47            | 0.947      | 19ps           | 55.0           | 5.9             | 2   | 17.8| 17.1| 29.8|
| M52            | 0.931      | 15ps           | 0.0            | 12.5            | 2   | 31.0| 30.4| 43.0|
| M54            | 0.955      | 49ps           | 17.8           | 24.1            | 2   | 54.2| 53.5| 66.2|
| A55            | 0.937      | 35ps           | 16.4           | 14.1            | 2   | 34.3| 33.7| 46.3|
| I56            | 0.940      | 58ns           | 22.2           | 4.6             | 1   | 10.6| 9.5 | 34.6|
| G59            | 0.868      | 58ns           | 7.0            | 5               | 1   | 11.0| 9.8 | 35.0|
| K60            | 0.921      | 47ns           | 2.9            | 6.2             | 2   | 18.4| 17.8| 30.4|
| E62            | 0.807      | 47ns           | 8.4            | 3.4             | 1   | 9.4 | 8.2 | 33.4|
| A63            | 0.825      | 36ns           | 6.5            | 16.7            | 1   | 22.7| 21.6| 46.7|
| A64            | 0.797      | 34ns           | 0.0            | 3.1             | 2   | 12.1| 11.5| 24.1|
| G65            | 0.791      | 91ns           | 35.7           | 0.5             | 2   | 7.0 | 6.4 | 19.0|
| Q66            | 0.865      | 52ns           | 25.0           | 9.8             | 1   | 15.8| 14.6| 39.8|
| A68            | 0.905      | 40ns           | 5.8            | 2.9             | 2   | 11.7| 11.1| 23.7|
| H69            | 0.947      | 89ns           | 8.0            | 2.7             | 2   | 11.3| 10.7| 23.3|
| A71            | 0.960      | 45ps           | 13.1           | 11.2            | 2   | 28.4| 27.8| 40.4|
| R72            | 0.960      | 23ps           | 4.1            | 8.3             | 2   | 22.7| 22.0| 34.7|
| Y73            | 0.956      | 25ps           | 56.0           | 10.6            | 2   | 27.2| 26.6| 39.2|
| V78            | 0.977      | 46ps           | 42.4           | 7.2             | 2   | 20.4| 19.7| 32.4|
| T79            | 0.871      | 6ps            | 52.4           | 6.6             | 2   | 19.2| 18.6| 31.2|
| T80            | 0.982      | 72ps           | 4.5            | 7.2             | 2   | 20.3| 19.7| 32.3|
| A91            | 0.948      | 40ns           | 10.8           | 1.5             | 2   | 9.1 | 8.4 | 21.1|
| I102           | 0.936      | 34ps           | 0.0            | 11.4            | 2   | 28.9| 28.3| 40.9|
| S107           | 0.960      | 20ps           | 0.0            | 13.3            | 2   | 32.6| 32   | 44.6|
| V112           | 0.946      | 27ps           | 8.5            | 5.1             | 2   | 16.2| 15.5| 28.2|
| L117           | 0.917      | 18ps           | 3.0            | 15.4            | 1   | 21.4| 20.2| 45.4|
| D120           | 0.980      | 66ps           | 0.0            | 7.5             | 2   | 21.0| 20.3| 33.0|
| L121           | 0.931      | 32ps           | 0.0            | 23.9            | 2   | 53.7| 53.1| 65.7|
| I146           | 0.952      | 70ns           | 15.7           | 8.6             | 1   | 14.6| 13.4| 38.6|
| N148           | 0.903      | 61ns           | 3.8            | 5.7             | 2   | 17.5| 16.8| 29.5|
| Residue Number | $S_{eff}^2$ range | $\tau_{c,eff}$ range | $E_a$ (kJ/mol) |
|----------------|-------------------|----------------------|----------------|
| L7             | 0.906 - 0.966     | 31 - 98 ps           | 5±2            |
| A13            | 0.924 - 0.986     | 20 - 176 ps          | 40±21          |
| A19            | 0.974 - 0.847     | 6 - 21 ps            | <16            |
| E36            | 0.761 - 0.912     | 29 - 87 ps           | <5             |
| S47            | 0.914 - 0.985     | 11 - 67 ps           | 65±35          |
| M52            | 0.876 - 0.980     | 7 - 69 ps            | <13            |
| M54            | 0.894 - 0.986     | 19 - 245 ps          | 20±11          |
| A55            | 0.903 - 0.980     | 22 - 124 ps          | 19±12          |
| I56            | 0.815 - 0.983     | 13 - 186 ps,         | <1             |
|                | 0.919 - 0.975     | 27 - 98 ns           | 28±8           |
| G59            | 0.851 - 0.903     | 51 - 87 ns           | 13±8           |
| K60            | 0.915 - 0.939     | 45 - 63 ns           | 8±6            |
| E62            | 0.748 - 0.865     | 34 - 69 ps           | <3             |
| A63            | 0.774 - 0.869     | 43 - 81 ps           | <8             |
| A64            | 0.739 - 0.847     | 47 - 91 ps           | 8±5            |
|                | 0.763 - 0.819     | 32 - 45 ns           | 3±3            |
| G65            | 0.766 - 0.840     | 85 - 138 ns          | 41±11          |
| Q66            | 0.860 - 0.903     | 51 - 76 ns           | 34±6           |
| A68            | 0.837 - 0.981     | 29 - 659 ps          | <14            |
|                | 0.889 - 0.921     | 38 - 53 ns           | 10±7           |
| H69            | 0.936 - 0.970     | 71 - 162 ns          | 12±11          |
| A71            | 0.925 - 0.986     | 24 - 180 ps          | 15±10          |
| R72            | 0.893 - 0.987     | 8 - 102 ps           | <18            |
| Y73            | 0.919 - 0.987     | 13 - 97 ps           | 66±35          |
| V78            | 0.914 - 0.988     | 11 - 112 ps          | 54±37          |
| Molecular Fragment | Degrees of freedom | Reduced $\chi^2$ | AIC | BIC | AICc |
|--------------------|--------------------|------------------|-----|-----|------|
| Helix A (L7-R27)   | 5                  | 23.3             | 116.6 | 128.6 | 131.5 |
| Helix B (Q34-I56)  | 11                 | 10.3             | 113.2 | 137.2 | 151.3 |
| BC loop (K60-A68)  | 11                 | 2.3              | 25.2  | 49.2  | 63.4  |
| Helix C (Y70-A91)  | 20                 | 5.3              | 105.2 | 147.2 | 183.7 |
| Helix D (T100-L121)| 14                 | 8.7              | 122.4 | 152.4 | 173.4 |
| Helix E (D125-N148)| 2                  | 5.7              | 11.5  | 17.5  | 16.8  |
| Helix F (S159-I184)| 11                 | 4.3              | 47.8  | 71.8  | 85.9  |
| FG loop (I185-N194)| 5                  | 1.3              | 6.3   | 18.3  | 21.2  |
| Helix G (Q195-L221)| 2                  | 0.8              | 1.7   | 7.7   | 7     |

Table S7. Summary of SMF results by molecular fragment
Figure S10. Representative histograms of the populations of 5000 Monte Carlo SMF fits for residues G14, A19, A68, H69, I185 and G189. Histograms of the correlation time $\tau_{c,\text{eff}}$ are shown in the left column, histograms of order parameter $S^2_{\text{eff}}$ are in the center column, and histograms of the activation energy $E_a$ are shown in the right column.
Figure S11. Comparison of experimental and SMF best fit (A) $R_1$ and (B) $R_{1p}$ relaxation rates at 30 °C. The best fit solution is shown as black circles, and the second best fit result is shown in grey.

Figure S12. Reduced $\chi^2$ for the SMF fit as a function of residue number.
7. Modified Extended Model Free Fit

In this modified EMF fit the spectral density function $J(\omega)$ is modelled assuming the presence of fast and slow motions where the fast motional parameters $S_{f,loc}^2$, $\tau_{f,loc}$, and $E_{a,loc}$ were defined locally for each residue and the slow motional parameters $S_{s,loc}^2$, $\tau_{s,loc}$ and $E_{a,col}$ were defined globally for all residues within the given molecular fragment (7 helices, BC and FG loops). The modified EMF approach was used for residues with all six experimental measurements available (Table 1, main text): $^{15}$N-$^1$H dipolar order parameters $S_{NH}^2$, $R_1$ at 600 MHz and 7 °C, $R_1$ at 800 MHz and 7 °C, $R_{1p}$ at 800 MHz and 7 °C, $R_1$ at 800 MHz and 30 °C, and $R_{1p}$ at 800 MHz and 30 °C. The best fit parameters were determined by minimizing $\chi^2$ for each of the nine fragments by using a grid search followed by steepest decent procedure:

$$
\chi^2 = \sum_{i=1}^{N_1} \left[ \frac{(S_{NH,exp}^2 - S_{f,loc,i}^2 S_{s,loc,i}^2)^2}{\sigma_{S_{NH,i}}^2} + \frac{(R_{1,600,exp} - R_{1,600,bcl})^2}{\sigma_{R_{1,600,i}}^2} + \frac{(R_{1,800,exp} - R_{1,800,bcl})^2}{\sigma_{R_{1,800,i}}^2} + \frac{(R_{1,30c,exp} - R_{1,30c,bcl})^2}{\sigma_{R_{1,30c,i}}^2} + \frac{(R_{1,p,7c,exp} - R_{1,p,7c,bcl})^2}{\sigma_{R_{1,p,7c,i}}^2} + \frac{(R_{1,p,30c,exp} - R_{1,p,30c,bcl})^2}{\sigma_{R_{1,p,30c,i}}^2} \right],
$$

(S6)

where relaxation rates are defined by Eqs 1-6 and 9 from the main text. The summation extends over all residues with all 6 experimentally measured values available within a molecular fragment ($N_1$). The reduced $\chi^2$ and the degrees of freedom ($d.f.$) are defined as

$$
\chi^2_{reduced} = \frac{\chi^2}{d.f.},
$$

(S7)

d.f. = number of experimental values – number of fit values – 1

$$
= (6 \cdot N_1) - (3 \cdot N_1 + 3) - 1 = 3 \cdot N_1 - 4.
$$

(S8)

Uncertainties in the best fit parameters $S_{f,loc}^2$, $S_{s,loc}^2$, $\tau_{f,loc}$, $\tau_{s,loc}$, $E_{a,loc}$ and $E_{a,col}$ were determined by the Monte Carlo procedure. First, the best fit parameters were used to back calculate experimental data. Gaussian distributed noise was then added to each of the back-calculated values for 1000 iterations, and the $\chi^2$ minimization was done for each iteration to determine the distribution of the fit parameters.
### Table S8. Summary of EMF results by molecular fragment

| Molecular Fragment | Degrees of freedom | Reduced $\chi^2$ | AIC | BIC | AICc |
|--------------------|--------------------|------------------|-----|-----|------|
| Helix A (L7-R27)   | 2                  | 6.3              | 30.5| 35.0| 120.5|
| Helix B (Q34-I56)  | 8                  | 3.6              | 59.0| 76.6| 119.0|
| BC loop (K60-A68)  | 8                  | 4.0              | 61.9| 79.6| 121.9|
| Helix C (Y70-A91)  | 17                 | 2.2              | 85.5| 127.2| 156.0|
| Helix D (T100-L121)| 11                 | 3.6              | 76.0| 101.3| 138.2|
| Helix E (D125-N148)| 0                  | n/a              | n/a | n/a | n/a  |
| Helix F (S159-I184)| 8                  | 1.7              | 43.4| 61.1| 103.5|
| FG loop (I185-N194)| 2                  | 3.3              | 24.8| 29.1| 114.8|
| Helix G (Q195-L221)| 0                  | n/a              | n/a | n/a | n/a  |

### Table S9. Summary of Best Fit EMF results by residue.

| Residue Number | $S^2_{s,\text{col}}$ | $\tau_{s,\text{col}}$ (ns) | $S^2_{f,\text{loc}}$ | $\tau_{f,\text{loc}}$ (ps) | $E_{a,\text{col}}$ (kJ mol$^{-1}$) | $E_{a,\text{loc}}$ (kJ mol$^{-1}$) |
|----------------|----------------------|-----------------------------|----------------------|-----------------------------|----------------------------------|----------------------------------|
| L7             | 0.980                | 105                         | 0.952                | 55                          | 2                                | 4                                |
| A13            | 0.980                | 105                         | 0.973                | 56                          | 2                                | 43                               |
| S47            | 0.990                | 123                         | 0.951                | 17                          | 4                                | 49                               |
| M52            | 0.990                | 123                         | 0.951                | 18                          | 4                                | 0                                |
| M54            | 0.990                | 123                         | 0.970                | 67                          | 4                                | 20                               |
| A55            | 0.990                | 123                         | 0.949                | 40                          | 4                                | 16                               |
| K60            | 0.953                | 90                          | 0.931                | 40                          | 0                                | 8                                |
| A64            | 0.953                | 90                          | 0.868                | 105                         | 0                                | 23                               |
| G65            | 0.953                | 90                          | 0.838                | 28                          | 0                                | 0                                |
| A68            | 0.953                | 90                          | 0.970                | 190                         | 0                                | 0                                |
| A71            | 0.996                | 231                         | 0.964                | 50                          | 8                                | 15                               |
| R72            | 0.996                | 231                         | 0.970                | 30                          | 8                                | 4                                |
| Y73            | 0.996                | 231                         | 0.959                | 26                          | 8                                | 45                               |
| V78            | 0.996                | 231                         | 0.969                | 31                          | 8                                | 36                               |
| T79            | 0.996                | 231                         | 0.874                | 6                           | 8                                | 43                               |
| T80            | 0.996                | 231                         | 0.990                | 146                         | 8                                | 4                                |
| A91            | 0.996                | 231                         | 0.989                | 543                         | 8                                | 0                                |
| I102           | 0.978                | 77                          | 0.970                | 51                          | 14                               | 0                                |
| S107           | 0.978                | 77                          | 0.990                | 14                          | 14                               | 0                                |
| V112           | 0.978                | 77                          | 0.968                | 31                          | 14                               | 28                               |
| Residue Number | $S^2_{s,col}$ | $\tau_{s,col}$ (ns) | $S^2_{f,loc}$ | $\tau_{f,loc}$ (ps) | $E_{a,col} (kJ/mol)$ | $E_{a,loc} (kJ/mol)$ |
|----------------|---------------|---------------------|---------------|---------------------|----------------------|---------------------|
| L7             | 0.970 - 0.992 | 66 - 231            | 0.920 - 0.974 | 34 - 115            | <7                   | 4 ± 3               |
| A13            | 0.970 - 0.992 | 66 - 231            | 0.930 - 0.990 | 19 - 209            | <7                   | 37 ± 10             |
| S47            | 0.982 - 0.996 | 66 - 270            | 0.912 - 0.984 | 7 - 51              | 5 ± 5                | 38 ± 13             |
| M52            | 0.982 - 0.996 | 66 - 270            | 0.893 - 0.990 | 6 - 67              | 5 ± 5                | <15                 |
| M54            | 0.982 - 0.996 | 66 - 270            | 0.896 - 0.990 | 18 - 250            | 5 ± 5                | 21 ± 11             |
| A55            | 0.982 - 0.996 | 66 - 270            | 0.912 - 0.977 | 23 - 96             | 5 ± 5                | 18 ± 10             |
| K60            | 0.943 - 0.964 | 66 - 144            | 0.893 - 0.968 | 24 - 86             | <9                   | 13 ± 13             |
| A64            | 0.943 - 0.964 | 66 - 144            | 0.803 - 0.927 | 64 - 287            | <9                   | 26 ± 9              |
| G65            | 0.943 - 0.964 | 66 - 144            | 0.784 - 0.894 | 18 - 48             | <9                   | <27                 |
| A68            | 0.943 - 0.964 | 66 - 144            | 0.906 - 0.989 | 46 - 707            | <9                   | <19                 |
| A71            | 0.990 - 0.996 | 90 - 316            | 0.930 - 0.989 | 23 - 186            | 9 ± 6                | 16 ± 10             |
| R72            | 0.990 - 0.996 | 90 - 316            | 0.895 - 0.991 | 8 - 113             | 9 ± 6                | 7 ± 10              |
| Y73            | 0.990 - 0.996 | 90 - 316            | 0.925 - 0.990 | 11 - 95             | 9 ± 6                | 39 ± 12             |
| V78            | 0.990 - 0.996 | 90 - 316            | 0.908 - 0.991 | 8 - 121             | 9 ± 6                | 30 ± 14             |
| T79            | 0.990 - 0.996 | 90 - 316            | 0.842 - 0.905 | 2 - 10              | 9 ± 6                | 39 ± 19             |
| T80            | 0.990 - 0.996 | 90 - 316            | 0.936 - 0.991 | 18 - 247            | 9 ± 6                | <18                 |
| A91            | 0.990 - 0.996 | 90 - 316            | 0.934 - 0.991 | 42 - 918            | 9 ± 6                | <13                 |
| I102           | 0.974 - 0.990 | 66 - 169            | 0.893 - 0.990 | 14 - 196            | 17 ± 7               | <10                 |
| S107           | 0.974 - 0.990 | 66 - 169            | 0.931 - 0.992 | 3 - 52              | 17 ± 7               | <33                 |
| V112           | 0.974 - 0.990 | 66 - 169            | 0.900 - 0.991 | 8 - 129             | 17 ± 7               | 24 ± 16             |
| D120           | 0.974 - 0.990 | 66 - 169            | 0.857 - 0.990 | 2 - 42              | 17 ± 7               | <26                 |
| L121           | 0.974 - 0.990 | 66 - 169            | 0.914 - 0.990 | 19 - 193            | 17 ± 7               | <8                  |
| T170           | 0.988 - 0.996 | 169 - 593           | 0.802 - 0.971 | 6 - 52              | 14 ± 9               | <17                 |
| Y171           | 0.988 - 0.996 | 169 - 593           | 0.940 - 0.990 | 26 - 321            | 14 ± 9               | 28 ± 13             |
| T173           | 0.988 - 0.996 | 169 - 593           | 0.906 - 0.991 | 16 - 321            | 14 ± 9               | 18 ± 12             |
| G178           | 0.988 - 0.996 | 169 - 593           | 0.748 - 0.988 | 4 - 70              | 14 ± 9               | 26 ± 17             |
| I185           | 0.931 - 0.953 | 90 - 231            | 0.874 - 0.991 | 8 - 147             | 40 ± 9               | <19                 |
| G189           | 0.931 - 0.953 | 90 - 231            | 0.894 - 0.988 | 19 - 189            | 40 ± 9               | 30 ± 15             |

Table S10. Summary of Monte Carlo EMF results by residue.
All intervals are given at a 95% confidence level.
Figure S13. Representative histograms of the population of all 6 fit parameters ($S^2_{s,col}$, $S^2_{f,loc}$, $\tau_{s,col}$, $\tau_{f,loc}$, $E_{a,col}$ and $E_{a,loc}$) from 1000 Monte Carlo EMF fits for residues I102 and I185.
Figure S14. Comparison of experimental and EMF back-calculated (A) $R_1$ and (B) $R_{1p}$ relaxation rates at 30 °C.

Table S11. Summary of MF-3DGAF results by molecular fragment*

| Molecular Fragment | Data points | Fit parameters | $\chi^2$ | AIC  | BIC  | AICc |
|--------------------|-------------|----------------|---------|------|------|------|
| Helix A (L7-R27)   | 13          | 13             | 3.7     | 29.7 | 37.0 | n/a  |
| Helix B (Q34-I56)  | 27          | 19             | 17.3    | 55.3 | 80.0 | 163.9|
| BC loop (K60-A68)  | 28          | 19             | 17.5    | 55.5 | 80.8 | 150.5|
| Helix C (Y70-A91)  | 45          | 28             | 40.0    | 96.0 | 146.6| 197.5|
| Helix D (T100-L121)| 45          | 22             | 20.94   | 20.9 | 95.8 | 165.5|
| Helix E (D125-N148)| n/a         | n/a            | n/a     | n/a  | n/a  | n/a  |
| Helix F (S159-I184)| 25          | 19             | 10.4    | 48.4 | 71.5 | 200.4|
| FG loop (I185-N194)| 13          | 13             | 4.3     | 30.3 | 37.6 | n/a  |
| Helix G (Q195-L221)| n/a         | n/a            | n/a     | n/a  | n/a  | n/a  |
Table S12. Summary of MF-3DGAF by residue.

| Residue Number | Residue | $\sigma_{\alpha}(\degree)$ | $\sigma_{\beta}(\degree)$ | $\sigma_{\gamma}(\degree)$ | $\Delta\phi(\degree)$ | $\Delta\theta(\degree)$ | $\tau_{\alpha,280K}$ (ns) | $S_{F,loc}^2$ | $\tau_{\gamma,280K}$ (ps) | $E_{a,cot}(kJ/mol)$ | $E_{a,loc}(kJ/mol)$ |
|----------------|---------|----------------------------|---------------------------|---------------------------|---------------------|---------------------|--------------------------|----------------|--------------------------|------------------|------------------|
| L7             | L       | 0.0                        | 2.3                       | 4.4                       | -55.1               | -0.1                | 148                      | 0.95           | 54                       | 0                | 0                |
| A13            | A       | 0.0                        | 2.3                       | 4.4                       | -55.1               | -0.1                | 148                      | 0.97           | 58                       | 0                | 0.8              |
| S47            | S       | 0.7                        | 0.0                       | 18.6                      | 55.0                | -15.6               | 192                      | 0.95           | 18.6                     | 2.6              | 2.8              |
| M52            | M       | 0.7                        | 0.0                       | 18.6                      | 55.0                | -15.6               | 192                      | 0.93           | 13.4                     | 2.6              | 8.4              |
| M54            | M       | 0.7                        | 0.0                       | 18.6                      | 55.0                | -15.6               | 192                      | 0.99           | 765                      | 2.6              | 0                |
| A55            | A       | 0.7                        | 0.0                       | 18.6                      | 55.0                | -15.6               | 192                      | 0.95           | 40.3                     | 2.6              | 0                |
| K60            | K       | 0.0                        | 5.2                       | 11.7                      | -30.4               | 36.2                | 73                       | 0.94           | 43.7                     | 6.2              | 0                |
| A64            | A       | 0.0                        | 5.2                       | 11.7                      | -30.4               | 36.2                | 73                       | 0.93           | 179                      | 6.2              | 9.8              |
| G65            | G       | 0.0                        | 5.2                       | 11.7                      | -30.4               | 36.2                | 73                       | 0.84           | 24.3                     | 6.2              | 7.5              |
| A68            | A       | 0.0                        | 5.2                       | 11.7                      | -30.4               | 36.2                | 73                       | 0.99           | 1000                     | 6.2              | 5.4              |
| A71            | A       | 4.3                        | 0.0                       | 0.0                       | -70.7               | 6.5                 | 65                       | 0.97           | 51.4                     | 11.8             | 6.1              |
| R72            | R       | 4.3                        | 0.0                       | 0.0                       | -70.7               | 6.5                 | 65                       | 0.96           | 11.5                     | 11.8             | 18.6             |
| Y73            | Y       | 4.3                        | 0.0                       | 0.0                       | -70.7               | 6.5                 | 65                       | 0.97           | 19.2                     | 11.8             | 37.2             |
| V78            | V       | 4.3                        | 0.0                       | 0.0                       | -70.7               | 6.5                 | 65                       | 0.97           | 36.5                     | 11.8             | 0                |
| T79            | T       | 4.3                        | 0.0                       | 0.0                       | -70.7               | 6.5                 | 65                       | 0.88           | 4.3                      | 11.8             | 0                |
| T80            | T       | 4.3                        | 0.0                       | 0.0                       | -70.7               | 6.5                 | 65                       | 1.00           | 787                      | 11.8             | 12.6             |
| A91            | A       | 4.3                        | 0.0                       | 0.0                       | -70.7               | 6.5                 | 65                       | 0.98           | 25.5                     | 11.8             | 20.1             |
| I102           | I       | 0.0                        | 5.8                       | 0.0                       | -74.7               | 54.3                | 133                      | 0.99           | 210                      | 6.0              | 13.0             |
| S107           | S       | 0.0                        | 5.8                       | 0.0                       | -74.7               | 54.3                | 133                      | 1.00           | 773                      | 6.0              | 0                |
| V112           | V       | 0.0                        | 5.8                       | 0.0                       | -74.7               | 54.3                | 133                      | 0.95           | 27.4                     | 6.0              | 1.7              |
| D120           | D       | 0.0                        | 5.8                       | 0.0                       | -74.7               | 54.3                | 133                      | 0.94           | 15.8                     | 6.0              | 48.2             |
| L121           | L       | 0.0                        | 5.8                       | 0.0                       | -74.7               | 54.3                | 133                      | 0.99           | 179                      | 6.0              | 11.5             |
| T170           | T       | 1.6                        | 0.0                       | 12.0                      | 62.5                | -17.1               | 482                      | 0.90           | 16.4                     | 11.4             | 0                |
| Y171           | Y       | 1.6                        | 0.0                       | 12.0                      | 62.5                | -17.1               | 482                      | 0.99           | 237                      | 11.4             | 25.3             |
| T173           | T       | 1.6                        | 0.0                       | 12.0                      | 62.5                | -17.1               | 482                      | 1.00           | 888                      | 11.4             | 0                |
| G178           | G       | 1.6                        | 0.0                       | 12.0                      | 62.5                | -17.1               | 482                      | 0.91           | 13.8                     | 11.4             | 4.6              |
| I185           | I       | 18.6                       | 0.0                       | 0.0                       | -62.6               | -7.8                | 131                      | 0.93           | 19.2                     | 23.0             | 0                |
| G189           | G       | 18.6                       | 0.0                       | 0.0                       | -62.6               | -7.8                | 131                      | 0.97           | 72.3                     | 23.0             | 23.5             |
Figure S15. Movie of a mode 7 from Normal Mode Analysis of ASR trimer performed using Nomad-Ref webserver."}

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