

Supplemental Figures and Tables

Dynamics of GCN4 Facilitate DNA Interaction: A Model-Free Analysis of an Intrinsically Disordered Region

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Figure S1

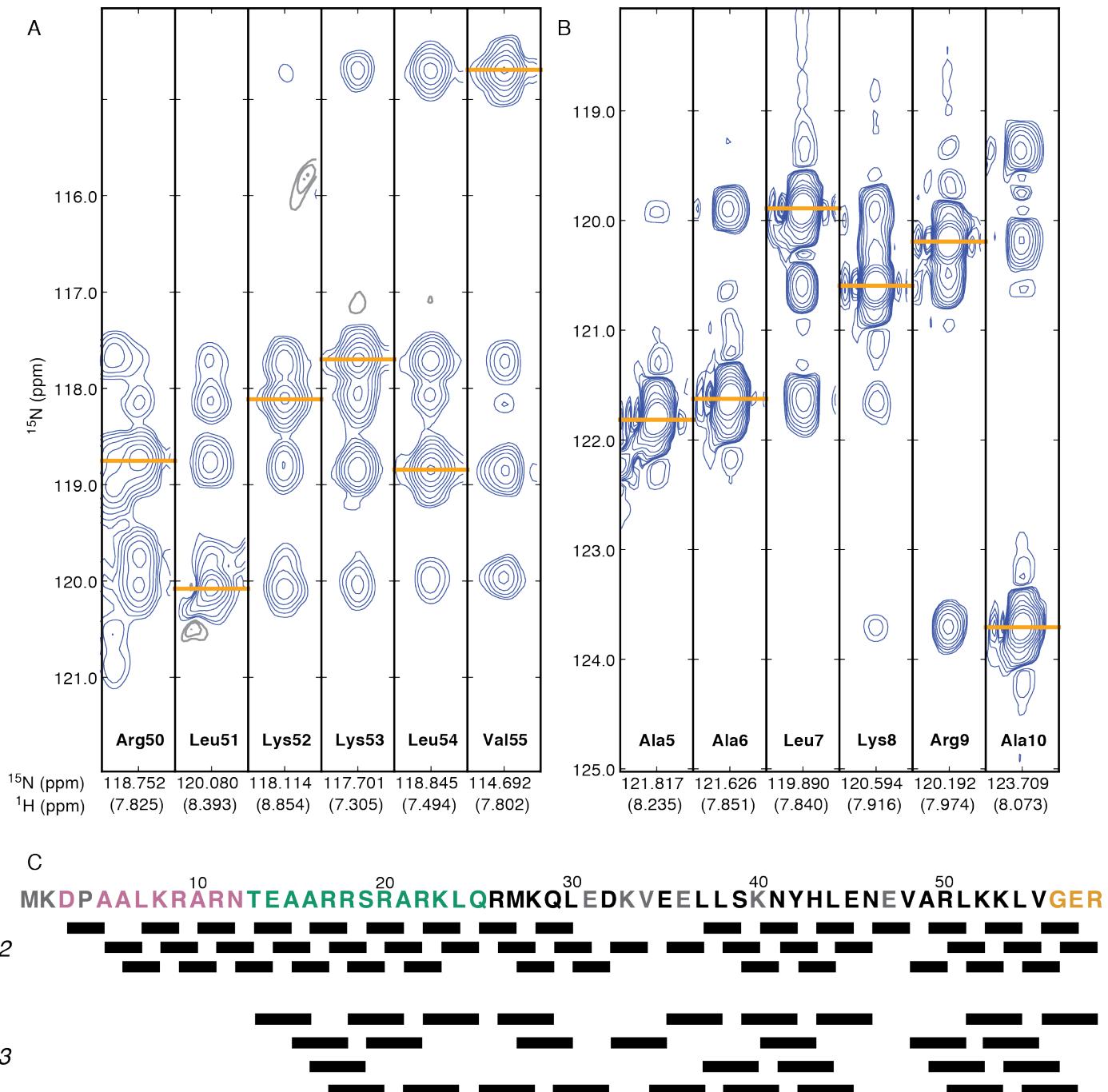


Figure S1. Representative strip plots of U- $[^{15}\text{N}, {^2}\text{H}]$ GCN4 (**A**) coiled-coil region and (**B**) basic region from an ^1H , ^{15}N , ^{15}N HSQC-NOESY-HSQC with 600 ms mixing time. NOE connectivities for i_{-3} to i_{+3} can be observed in panel A, while the connectivities in the disordered region (panel B) tends to only extend from i_{-2} to i_{+2} . Positive and negative contours are shown in blue and black, respectively. (**C**) All connectivities for i_{-3} to i_{+3} are shown. Residues are colored as described in the main text, except for those that were excluded from relaxation analysis and are shown in gray.

Figure S2

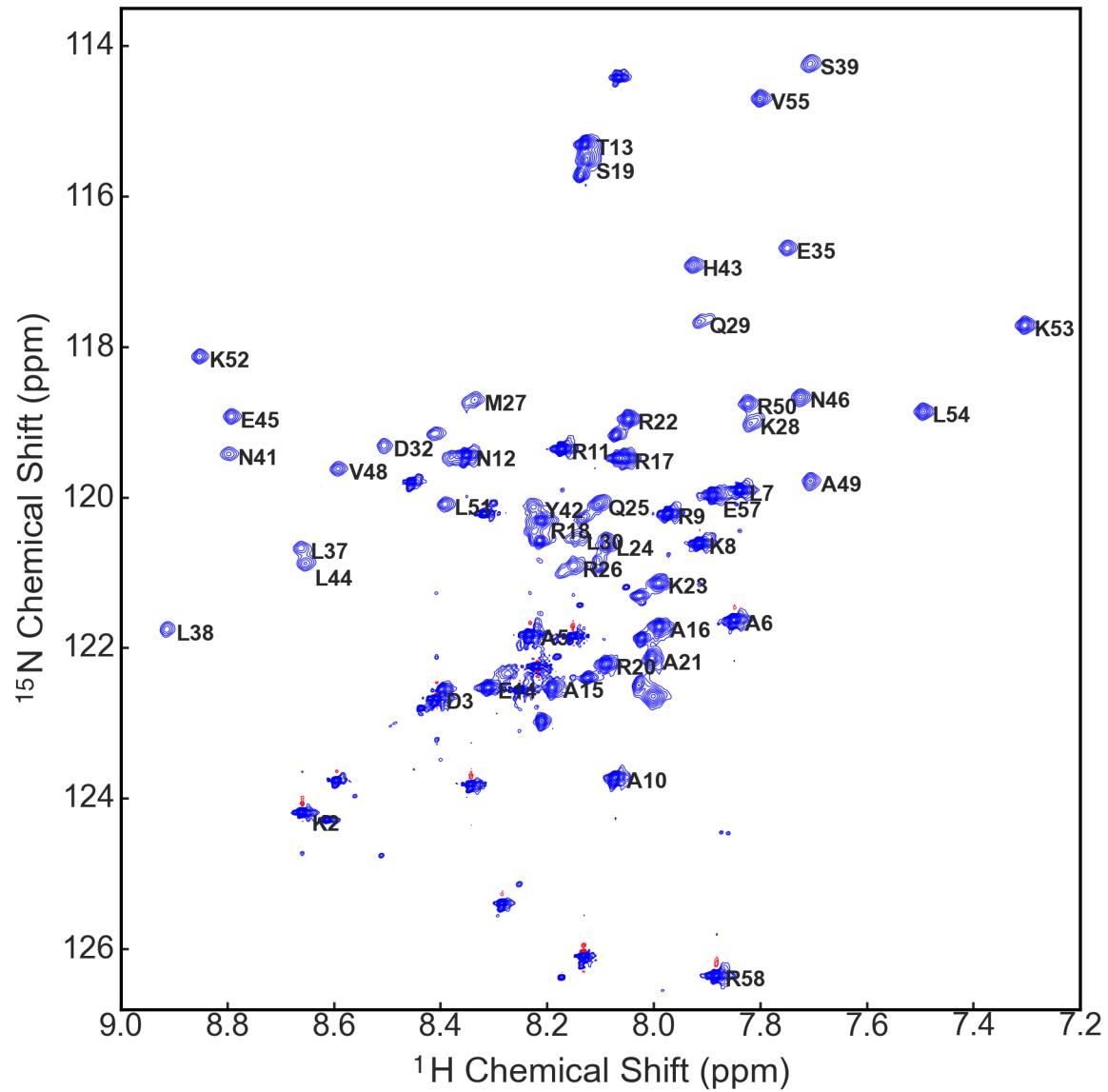


Figure S2. ^1H - ^{15}N HSQC spectrum of U- $[^{15}\text{N}, ^2\text{H}]$ GCN4. Unlabeled peaks correspond to small amounts of proteolysis during expression and purification in the basic region of GCN4.

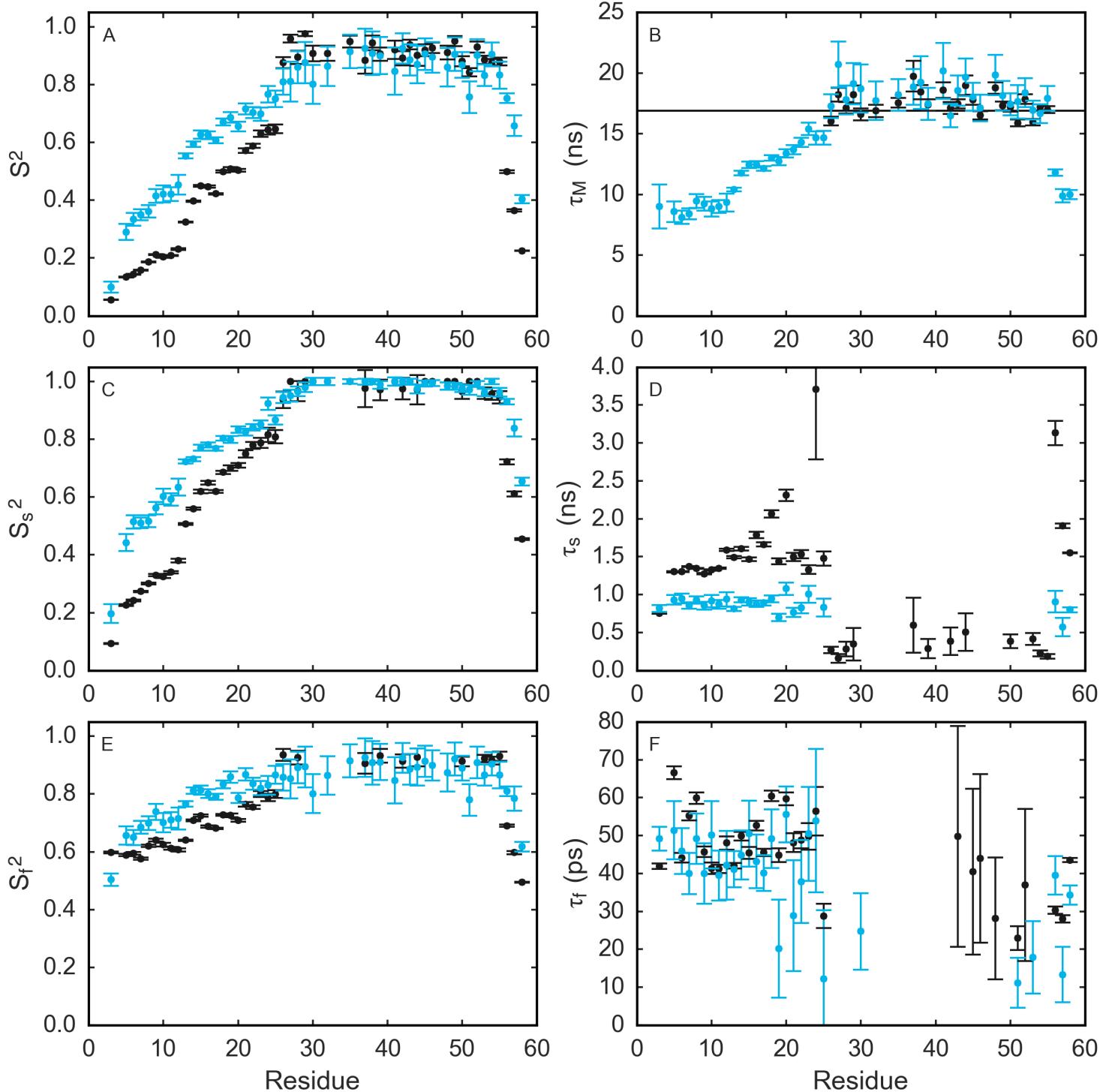
Figure S3

Figure S3. Comparison of Model-free parameters (**A**) S^2 , (**B**) τ_M , (**C**) S_{s^2} , (**D**) τ_s , (**E**) S_{f^2} , and (**F**) τ_f determined from spectral density calculations (blue) and from analysis using the program relax with a fixed τ_M for disordered residues in the basic and C-terminal domains (black). The mean τ_M (16.9 ns) is indicated with a straight, black line.

Table S1. Time points used for measuring R_1 and R_2 relaxation rate constants of GCN4¹

| Experiment | B0 Field (T) | Time Points (s) |
|-------------------------|--------------|--|
| R_1 | 14.1 | 0.02 , 0.06, 0.14 , 0.23, 0.34 , 0.47, 0.69, 0.98 , 1.50 |
| | 16.45 | 0.02 , 0.14, 0.23 , 0.47, 0.98 , 1.50, 1.75 |
| | 18.8 | 0.02 , 0.06, 0.08 , 0.14, 0.23 , 0.47, 0.98 , 1.50, 1.75 |
| | 21.1 | 0.02 , 0.14, 0.23, 0.47 , 0.98 , 1.50 |
| R_2 | 14.1 | 0.004, 0.008 , 0.024 , 0.064 , 0.096, 0.144 , 0.208 |
| | 16.45 | 0.004, 0.008 , 0.024, 0.064 , 0.096, 0.144 , 0.208 |
| | 18.8 | 0.004, 0.008 , 0.024 , 0.064 , 0.096, 0.144 , 0.208 |
| | 21.1 | 0.004, 0.008 , 0.024, 0.064 , 0.096, 0.144 , 0.208 |

¹Time points that were collected in duplicate for error analysis are shown in bold.

Table S2. Amide chemical shifts for U-[¹⁵N, ²H] GCN4

| Residue | | ¹ H (ppm) | ¹⁵ N (ppm) | Residue | | ¹ H (ppm) | ¹⁵ N (ppm) |
|---------|---|-------------------------|--------------------------|---------|---|-------------------------|--------------------------|
| 1 | M | | | 30 | L | 8.14 | 120.5 |
| 2 | K | | | 31 | E | | |
| 3 | D | 8.40 | 122.6 | 32 | D | 8.51 | 119.3 |
| 4 | P | | | 33 | K | | |
| 5 | A | 8.23 | 121.8 | 34 | V | | |
| 6 | A | 7.85 | 121.6 | 35 | E | 7.75 | 116.7 |
| 7 | L | 7.84 | 119.9 | 36 | E | | |
| 8 | K | 7.92 | 120.6 | 37 | L | 8.66 | 120.7 |
| 9 | R | 7.97 | 120.2 | 38 | L | 8.91 | 121.7 |
| 10 | A | 8.07 | 123.7 | 39 | S | 7.71 | 114.2 |
| 11 | R | 8.17 | 119.3 | 40 | K | | |
| 12 | N | 8.35 | 119.4 | 41 | N | 8.80 | 119.4 |
| 13 | T | 8.13 | 115.3 | 42 | Y | 8.22 | 120.1 |
| 14 | E | 8.31 | 122.5 | 43 | H | 7.93 | 116.9 |
| 15 | A | 8.19 | 122.5 | 44 | L | 8.65 | 120.9 |
| 16 | A | 7.99 | 121.7 | 45 | E | 8.79 | 118.9 |
| 17 | R | 8.06 | 119.5 | 46 | N | 7.72 | 118.7 |
| 18 | R | 8.21 | 120.3 | 47 | E | | |
| 19 | S | 8.13 | 115.5 | 48 | V | 8.59 | 119.6 |
| 20 | R | 8.09 | 122.2 | 49 | A | 7.71 | 119.8 |
| 21 | A | 8.05 | 122.2 | 50 | R | 7.82 | 118.8 |
| 22 | R | 8.02 | 120.7 | 51 | L | 8.39 | 120.1 |
| 23 | K | 8.02 | 119.9 | 52 | K | 8.85 | 118.1 |
| 24 | L | 8.09 | 120.6 | 53 | K | 7.30 | 117.7 |
| 25 | Q | 8.10 | 120.1 | 54 | L | 7.49 | 118.9 |
| 26 | R | 8.15 | 120.9 | 55 | V | 7.80 | 114.7 |
| 27 | M | 8.33 | 118.7 | 56 | G | 7.79 | 108.5 |
| 28 | K | 7.82 | 119.0 | 57 | E | 7.89 | 120.0 |
| 29 | Q | 7.92 | 117.7 | 58 | R | 7.88 | 126.3 |

¹Resonance assignments were determined from a ¹H, ¹⁵N, ¹⁵N HSQC-NOESY-HSQC with 600 ms mixing time.

Table S3. Relaxation parameters determined for GCN4 at 300 K¹

| Residue | 14.1 T | | | 16.45 T | | |
|---------|-----------------------------------|-----------------------------------|--------------|-----------------------------------|-----------------------------------|--------------|
| | R ₁ (s ⁻¹) | R ₂ (s ⁻¹) | NOE | R ₁ (s ⁻¹) | R ₂ (s ⁻¹) | NOE |
| 3 | 1.11 ± 0.02 | 3.05 ± 0.38 | -0.76 ± 0.01 | 1.07 ± 0.03 | 1.48 ± 0.11 | -0.41 ± 0.01 |
| 5 | 1.38 ± 0.05 | 4.97 ± 0.84 | -0.14 ± 0.01 | 1.35 ± 0.07 | 5.84 ± 0.31 | 0.06 ± 0.01 |
| 6 | 1.39 ± 0.04 | 5.80 ± 1.15 | -0.01 ± 0.01 | 1.27 ± 0.07 | 6.02 ± 0.33 | 0.13 ± 0.01 |
| 7 | 1.37 ± 0.03 | 6.01 ± 0.97 | -0.05 ± 0.01 | 1.32 ± 0.07 | 5.72 ± 0.29 | 0.10 ± 0.01 |
| 8 | 1.38 ± 0.03 | 6.09 ± 1.07 | -0.03 ± 0.01 | 1.31 ± 0.07 | 8.34 ± 0.37 | 0.11 ± 0.01 |
| 9 | 1.39 ± 0.04 | 6.69 ± 1.23 | 0.02 ± 0.01 | 1.33 ± 0.09 | 7.58 ± 0.38 | 0.17 ± 0.01 |
| 10 | 1.39 ± 0.05 | 7.05 ± 1.56 | 0.06 ± 0.01 | 1.25 ± 0.10 | 7.50 ± 0.52 | 0.18 ± 0.01 |
| 11 | 1.36 ± 0.04 | 7.02 ± 1.30 | 0.06 ± 0.01 | 1.25 ± 0.08 | 7.54 ± 0.40 | 0.19 ± 0.01 |
| 12 | 1.36 ± 0.06 | 7.66 ± 2.04 | 0.14 ± 0.01 | 1.26 ± 0.11 | 7.75 ± 0.61 | 0.32 ± 0.01 |
| 13 | 1.27 ± 0.01 | 9.96 ± 0.29 | 0.18 ± 0.01 | 1.18 ± 0.03 | 10.26 ± 0.24 | 0.26 ± 0.01 |
| 14 | 1.28 ± 0.01 | 10.75 ± 0.33 | 0.26 ± 0.01 | 1.12 ± 0.04 | 12.00 ± 0.32 | 0.42 ± 0.01 |
| 15 | 1.20 ± 0.02 | 12.48 ± 0.54 | 0.27 ± 0.01 | 1.10 ± 0.05 | 12.39 ± 0.47 | 0.26 ± 0.01 |
| 16 | 1.17 ± 0.01 | 12.72 ± 0.55 | 0.27 ± 0.01 | 0.99 ± 0.04 | 14.19 ± 0.50 | 0.36 ± 0.01 |
| 17 | 1.18 ± 0.01 | 11.27 ± 0.33 | 0.27 ± 0.01 | 1.06 ± 0.02 | 12.20 ± 0.27 | 0.35 ± 0.01 |
| 18 | 1.18 ± 0.01 | 13.45 ± 0.53 | 0.34 ± 0.01 | 1.01 ± 0.03 | 14.61 ± 0.47 | 0.43 ± 0.01 |
| 19 | 1.14 ± 0.02 | 13.95 ± 0.87 | 0.28 ± 0.01 | 0.98 ± 0.06 | 14.68 ± 0.73 | 0.36 ± 0.02 |
| 20 | 1.10 ± 0.02 | 13.53 ± 0.67 | 0.35 ± 0.01 | 1.12 ± 0.05 | 12.94 ± 0.46 | 0.29 ± 0.01 |
| 21 | 1.09 ± 0.02 | 14.66 ± 1.09 | 0.34 ± 0.01 | 1.06 ± 0.08 | 15.45 ± 0.94 | 0.42 ± 0.02 |
| 22 | 1.04 ± 0.02 | 15.11 ± 1.08 | 0.35 ± 0.01 | 0.96 ± 0.06 | 16.94 ± 0.87 | 0.38 ± 0.02 |
| 23 | 1.01 ± 0.02 | 16.26 ± 1.27 | 0.36 ± 0.01 | 0.94 ± 0.07 | 19.13 ± 1.30 | 0.43 ± 0.02 |
| 24 | 1.01 ± 0.03 | 16.50 ± 1.96 | 0.52 ± 0.01 | 0.86 ± 0.09 | 18.84 ± 1.77 | 0.53 ± 0.03 |
| 25 | 1.01 ± 0.03 | 15.74 ± 1.85 | 0.49 ± 0.01 | 0.87 ± 0.09 | 17.97 ± 1.63 | 0.52 ± 0.03 |
| 26 | 0.82 ± 0.04 | 19.83 ± 2.78 | 0.58 ± 0.01 | 0.77 ± 0.09 | 22.63 ± 2.48 | 0.40 ± 0.03 |
| 27 | 0.71 ± 0.05 | 23.08 ± 4.40 | 0.68 ± 0.01 | 0.62 ± 0.09 | 28.91 ± 4.47 | 0.67 ± 0.03 |
| 28 | 0.77 ± 0.05 | 21.53 ± 3.67 | 0.69 ± 0.01 | 0.67 ± 0.07 | 24.54 ± 2.52 | 0.74 ± 0.03 |
| 29 | 0.75 ± 0.06 | 23.27 ± 5.89 | 0.72 ± 0.01 | 0.60 ± 0.12 | 27.36 ± 5.67 | 0.77 ± 0.04 |

| | | | | | | |
|-----------|-------------|--------------|--------------|-------------|--------------|-------------|
| 30 | 0.75 ± 0.06 | 19.95 ± 3.30 | 0.80 ± 0.01 | 0.63 ± 0.07 | 23.50 ± 2.22 | 0.72 ± 0.02 |
| 32 | 0.76 ± 0.07 | 22.55 ± 3.82 | 0.78 ± 0.01 | 0.63 ± 0.07 | 25.54 ± 2.90 | 0.83 ± 0.02 |
| 35 | 0.75 ± 0.06 | 23.96 ± 3.43 | 0.78 ± 0.01 | 0.64 ± 0.05 | 26.17 ± 1.99 | 0.80 ± 0.02 |
| 37 | 0.69 ± 0.06 | 25.06 ± 4.19 | 0.74 ± 0.01 | 0.61 ± 0.06 | 29.23 ± 3.15 | 0.78 ± 0.02 |
| 38 | 0.73 ± 0.07 | 24.45 ± 4.07 | 0.86 ± 0.01 | 0.64 ± 0.06 | 26.73 ± 2.61 | 0.75 ± 0.02 |
| 39 | 0.77 ± 0.06 | 22.04 ± 3.40 | 0.71 ± 0.01 | 0.69 ± 0.06 | 24.83 ± 2.30 | 0.71 ± 0.02 |
| 41 | 0.69 ± 0.07 | 27.21 ± 5.27 | 0.80 ± 0.01 | 0.58 ± 0.07 | 27.47 ± 3.12 | 0.85 ± 0.03 |
| 42 | 0.78 ± 0.05 | 20.88 ± 2.58 | 0.73 ± 0.01 | 0.72 ± 0.06 | 22.46 ± 1.72 | 0.75 ± 0.02 |
| 43 | 0.75 ± 0.05 | 23.21 ± 2.98 | 0.77 ± 0.01 | 0.66 ± 0.05 | 25.80 ± 1.79 | 0.74 ± 0.02 |
| 44 | 0.70 ± 0.06 | 24.40 ± 3.37 | 0.72 ± 0.01 | 0.61 ± 0.05 | 28.37 ± 2.67 | 0.77 ± 0.02 |
| 45 | 0.73 ± 0.05 | 25.51 ± 3.26 | 0.72 ± 0.01 | 0.68 ± 0.05 | 26.32 ± 1.90 | 0.77 ± 0.02 |
| 46 | 0.78 ± 0.06 | 22.15 ± 2.76 | 0.75 ± 0.01 | 0.71 ± 0.05 | 24.22 ± 1.69 | 0.75 ± 0.02 |
| 48 | 0.68 ± 0.06 | 23.91 ± 3.37 | 0.76 ± 0.01 | 0.60 ± 0.05 | 26.94 ± 2.38 | 0.71 ± 0.02 |
| 49 | 0.77 ± 0.06 | 23.39 ± 2.95 | 0.75 ± 0.01 | 0.69 ± 0.05 | 25.44 ± 1.96 | 0.73 ± 0.02 |
| 50 | 0.76 ± 0.05 | 22.68 ± 2.33 | 0.69 ± 0.01 | 0.66 ± 0.04 | 24.53 ± 1.52 | 0.74 ± 0.01 |
| 51 | 0.74 ± 0.06 | 21.50 ± 2.04 | 0.74 ± 0.01 | 0.59 ± 0.05 | 22.85 ± 1.55 | 0.76 ± 0.02 |
| 52 | 0.75 ± 0.06 | 23.70 ± 2.73 | 0.74 ± 0.01 | 0.62 ± 0.05 | 25.92 ± 1.71 | 0.78 ± 0.01 |
| 53 | 0.82 ± 0.04 | 20.12 ± 1.67 | 0.68 ± 0.01 | 0.69 ± 0.03 | 22.32 ± 0.97 | 0.70 ± 0.01 |
| 54 | 0.73 ± 0.04 | 22.90 ± 2.12 | 0.65 ± 0.01 | 0.76 ± 0.04 | 24.13 ± 1.36 | 0.65 ± 0.01 |
| 55 | 0.74 ± 0.05 | 21.63 ± 2.08 | 0.63 ± 0.01 | 0.68 ± 0.04 | 22.34 ± 1.23 | 0.61 ± 0.01 |
| 56 | 1.04 ± 0.02 | 13.71 ± 0.52 | 0.54 ± 0.01 | 1.03 ± 0.02 | 14.69 ± 0.29 | 0.56 ± 0.01 |
| 57 | 1.13 ± 0.07 | 10.12 ± 2.17 | 0.36 ± 0.01 | 1.10 ± 0.05 | 11.33 ± 0.44 | 0.42 ± 0.01 |
| 58 | 1.10 ± 0.03 | 6.74 ± 0.53 | -0.01 ± 0.01 | 0.99 ± 0.02 | 6.31 ± 0.10 | 0.08 ± 0.01 |

¹R₁ and R₂ spin relaxation rates at 16.45 T are adjusted for temperature as noted in the text.

Table S3 (continued)

| Residue | 18.8 T | | | | 21.1 T | | | |
|---------|-----------------------------------|-----------------------------------|--------------|--|-----------------------------------|-----------------------------------|--------------|--|
| | R ₁ (s ⁻¹) | R ₂ (s ⁻¹) | NOE | | R ₁ (s ⁻¹) | R ₂ (s ⁻¹) | NOE | |
| 3 | 1.09 ± 0.07 | 3.59 ± 0.04 | -0.18 ± 0.01 | | 1.18 ± 0.01 | 2.16 ± 0.07 | -0.02 ± 0.01 | |
| 5 | 1.24 ± 0.14 | 6.91 ± 0.11 | 0.14 ± 0.01 | | 1.34 ± 0.01 | 5.92 ± 0.04 | 0.29 ± 0.01 | |
| 6 | 1.25 ± 0.16 | 6.20 ± 0.13 | 0.27 ± 0.01 | | 1.28 ± 0.02 | 6.33 ± 0.05 | 0.33 ± 0.01 | |
| 7 | 1.26 ± 0.13 | 7.42 ± 0.12 | 0.20 ± 0.01 | | 1.25 ± 0.01 | 6.70 ± 0.04 | 0.34 ± 0.01 | |
| 8 | 1.27 ± 0.15 | 7.40 ± 0.13 | 0.22 ± 0.01 | | 1.29 ± 0.01 | 7.71 ± 0.05 | 0.35 ± 0.01 | |
| 9 | 1.22 ± 0.17 | 8.12 ± 0.16 | 0.29 ± 0.01 | | 1.24 ± 0.02 | 8.41 ± 0.06 | 0.39 ± 0.01 | |
| 10 | 1.26 ± 0.22 | 8.46 ± 0.22 | 0.32 ± 0.01 | | 1.22 ± 0.02 | 8.09 ± 0.08 | 0.35 ± 0.01 | |
| 11 | 1.24 ± 0.18 | 8.28 ± 0.15 | 0.31 ± 0.01 | | 1.19 ± 0.01 | 8.22 ± 0.06 | 0.39 ± 0.01 | |
| 12 | 1.13 ± 0.22 | 8.97 ± 0.23 | 0.32 ± 0.01 | | 1.18 ± 0.02 | 9.04 ± 0.09 | 0.42 ± 0.01 | |
| 13 | 1.10 ± 0.04 | 11.00 ± 0.08 | 0.35 ± 0.01 | | 1.02 ± 0.01 | 11.71 ± 0.06 | 0.42 ± 0.02 | |
| 14 | 1.04 ± 0.05 | 13.18 ± 0.13 | 0.40 ± 0.01 | | 1.07 ± 0.01 | 14.13 ± 0.09 | 0.48 ± 0.02 | |
| 15 | 1.00 ± 0.07 | 14.92 ± 0.20 | 0.41 ± 0.01 | | 0.98 ± 0.01 | 15.47 ± 0.13 | 0.47 ± 0.02 | |
| 16 | 0.98 ± 0.06 | 14.61 ± 0.22 | 0.36 ± 0.01 | | 0.94 ± 0.01 | 15.41 ± 0.13 | 0.47 ± 0.02 | |
| 17 | 1.07 ± 0.04 | 13.89 ± 0.14 | 0.38 ± 0.01 | | 0.95 ± 0.01 | 14.73 ± 0.09 | 0.49 ± 0.01 | |
| 18 | 0.91 ± 0.05 | 16.16 ± 0.20 | 0.39 ± 0.01 | | 0.94 ± 0.01 | 17.23 ± 0.14 | 0.50 ± 0.02 | |
| 19 | 0.99 ± 0.07 | 16.54 ± 0.33 | 0.39 ± 0.01 | | 0.85 ± 0.01 | 17.03 ± 0.21 | 0.56 ± 0.03 | |
| 20 | 0.96 ± 0.05 | 16.57 ± 0.26 | 0.37 ± 0.01 | | 0.89 ± 0.01 | 17.39 ± 0.18 | 0.49 ± 0.02 | |
| 21 | 0.89 ± 0.09 | 18.14 ± 0.44 | 0.43 ± 0.01 | | 0.82 ± 0.01 | 19.13 ± 0.29 | 0.57 ± 0.03 | |
| 22 | 0.81 ± 0.07 | 18.94 ± 0.39 | 0.41 ± 0.01 | | 0.79 ± 0.01 | 19.39 ± 0.25 | 0.52 ± 0.03 | |
| 23 | 0.83 ± 0.09 | 19.29 ± 0.55 | 0.44 ± 0.01 | | 0.80 ± 0.01 | 20.93 ± 0.40 | 0.48 ± 0.03 | |
| 24 | 0.84 ± 0.16 | 20.88 ± 0.81 | 0.49 ± 0.01 | | 0.74 ± 0.02 | 21.54 ± 0.55 | 0.55 ± 0.05 | |
| 25 | 0.80 ± 0.12 | 20.06 ± 0.79 | 0.57 ± 0.01 | | 0.74 ± 0.02 | 21.32 ± 0.59 | 0.69 ± 0.05 | |
| 26 | 0.68 ± 0.12 | 27.08 ± 1.53 | 0.60 ± 0.01 | | 0.56 ± 0.02 | 26.10 ± 1.01 | 0.68 ± 0.06 | |
| 27 | 0.62 ± 0.14 | 29.90 ± 2.37 | 0.67 ± 0.01 | | 0.51 ± 0.03 | 31.83 ± 2.04 | 0.77 ± 0.06 | |
| 28 | 0.62 ± 0.08 | 27.40 ± 1.94 | 0.70 ± 0.01 | | 0.49 ± 0.02 | 29.19 ± 1.43 | 0.80 ± 0.04 | |

| Residue | 18.8 T | | | 21.1 T | | |
|---------|-----------------------------------|-----------------------------------|-------------|-----------------------------------|-----------------------------------|-------------|
| | R ₁ (s ⁻¹) | R ₂ (s ⁻¹) | NOE | R ₁ (s ⁻¹) | R ₂ (s ⁻¹) | NOE |
| 29 | 0.57 ± 0.14 | 29.82 ± 2.92 | 0.76 ± 0.01 | 0.49 ± 0.03 | 31.86 ± 2.30 | 0.72 ± 0.06 |
| 30 | 0.59 ± 0.09 | 28.01 ± 2.33 | 0.80 ± 0.01 | 0.52 ± 0.03 | 28.66 ± 1.64 | 0.71 ± 0.04 |
| 32 | 0.59 ± 0.08 | 27.60 ± 2.09 | 0.78 ± 0.01 | 0.49 ± 0.03 | 28.80 ± 1.47 | 0.78 ± 0.03 |
| 35 | 0.61 ± 0.05 | 30.14 ± 1.68 | 0.84 ± 0.01 | 0.48 ± 0.02 | 31.46 ± 1.33 | 0.67 ± 0.03 |
| 37 | 0.54 ± 0.07 | 32.60 ± 2.59 | 0.80 ± 0.01 | 0.41 ± 0.03 | 32.06 ± 1.80 | 0.74 ± 0.03 |
| 38 | 0.54 ± 0.08 | 31.31 ± 2.55 | 0.66 ± 0.02 | 0.47 ± 0.03 | 33.63 ± 1.89 | 0.84 ± 0.04 |
| 39 | 0.58 ± 0.06 | 27.78 ± 1.66 | 0.73 ± 0.01 | 0.48 ± 0.02 | 30.13 ± 1.39 | 0.73 ± 0.03 |
| 41 | 0.56 ± 0.10 | 32.03 ± 3.14 | 0.74 ± 0.02 | 0.46 ± 0.03 | 31.65 ± 1.99 | 0.81 ± 0.05 |
| 42 | 0.64 ± 0.07 | 27.32 ± 1.56 | 0.76 ± 0.01 | 0.46 ± 0.02 | 29.93 ± 1.30 | 0.81 ± 0.03 |
| 43 | 0.62 ± 0.05 | 27.94 ± 1.50 | 0.72 ± 0.01 | 0.50 ± 0.02 | 32.34 ± 1.26 | 0.76 ± 0.03 |
| 44 | 0.55 ± 0.07 | 31.77 ± 2.56 | 0.76 ± 0.01 | 0.46 ± 0.03 | 31.86 ± 1.56 | 0.78 ± 0.03 |
| 45 | 0.57 ± 0.06 | 29.53 ± 1.93 | 0.70 ± 0.01 | 0.46 ± 0.02 | 30.57 ± 1.34 | 0.76 ± 0.03 |
| 46 | 0.62 ± 0.05 | 26.86 ± 1.38 | 0.72 ± 0.01 | 0.51 ± 0.02 | 29.44 ± 1.07 | 0.79 ± 0.03 |
| 48 | 0.53 ± 0.06 | 29.54 ± 2.06 | 0.74 ± 0.01 | 0.44 ± 0.02 | 33.06 ± 1.51 | 0.79 ± 0.03 |
| 49 | 0.58 ± 0.05 | 30.17 ± 1.56 | 0.73 ± 0.01 | 0.50 ± 0.02 | 30.91 ± 1.27 | 0.79 ± 0.02 |
| 50 | 0.59 ± 0.04 | 26.73 ± 1.20 | 0.72 ± 0.01 | 0.48 ± 0.01 | 28.73 ± 0.89 | 0.75 ± 0.02 |
| 51 | 0.58 ± 0.05 | 23.31 ± 0.76 | 0.68 ± 0.01 | 0.50 ± 0.02 | 25.49 ± 0.58 | 0.80 ± 0.03 |
| 52 | 0.61 ± 0.06 | 28.16 ± 1.71 | 0.75 ± 0.01 | 0.47 ± 0.02 | 32.43 ± 1.33 | 0.77 ± 0.02 |
| 53 | 0.61 ± 0.03 | 24.76 ± 0.77 | 0.72 ± 0.01 | 0.55 ± 0.01 | 27.28 ± 0.67 | 0.72 ± 0.02 |
| 54 | 0.59 ± 0.04 | 27.07 ± 0.92 | 0.66 ± 0.01 | 0.47 ± 0.01 | 28.20 ± 0.82 | 0.65 ± 0.02 |
| 55 | 0.59 ± 0.04 | 26.59 ± 1.07 | 0.61 ± 0.01 | 0.50 ± 0.02 | 29.02 ± 0.84 | 0.69 ± 0.02 |
| 56 | 0.90 ± 0.02 | 16.14 ± 0.26 | 0.55 ± 0.01 | 0.74 ± 0.01 | 17.18 ± 0.18 | 0.59 ± 0.01 |
| 57 | 0.94 ± 0.07 | 12.25 ± 0.36 | 0.44 ± 0.01 | 0.83 ± 0.02 | 12.85 ± 0.14 | 0.53 ± 0.01 |
| 58 | 0.96 ± 0.03 | 9.37 ± 0.11 | 0.14 ± 0.01 | 0.93 ± 0.01 | 8.46 ± 0.03 | 0.26 ± 0.01 |

Table S4. Model-free parameters for GCN4 determined using the program *relax*¹

| Residue | Model | χ^2 | τ_M (ns) | S^2 | τ_f (ps) | S_f^2 | τ_s (ns) | $S_s^2 (S^2/S_f^2)$ |
|---------|-------|----------|---------------|-------------|---------------|-------------|---------------|---------------------|
| 3 | 6 | 588.33 | | 0.06 ± 0.01 | 41.93 ± 0.70 | 0.60 ± 0.01 | 0.75 ± 0.01 | 0.09 ± 0.01 |
| 5 | 6 | 268.30 | | 0.13 ± 0.01 | 66.59 ± 1.71 | 0.59 ± 0.01 | 1.30 ± 0.01 | 0.23 ± 0.01 |
| 6 | 6 | 30.69 | | 0.14 ± 0.01 | 44.14 ± 1.24 | 0.60 ± 0.01 | 1.30 ± 0.01 | 0.24 ± 0.01 |
| 7 | 6 | 171.52 | | 0.16 ± 0.01 | 55.22 ± 1.24 | 0.58 ± 0.01 | 1.37 ± 0.01 | 0.27 ± 0.01 |
| 8 | 6 | 54.33 | | 0.19 ± 0.01 | 59.86 ± 1.54 | 0.62 ± 0.01 | 1.35 ± 0.01 | 0.30 ± 0.01 |
| 9 | 6 | 30.64 | | 0.21 ± 0.01 | 45.71 ± 1.40 | 0.64 ± 0.01 | 1.28 ± 0.01 | 0.33 ± 0.01 |
| 10 | 6 | 50.12 | | 0.20 ± 0.01 | 41.34 ± 1.48 | 0.62 ± 0.01 | 1.33 ± 0.01 | 0.33 ± 0.01 |
| 11 | 6 | 35.97 | | 0.21 ± 0.01 | 41.21 ± 1.10 | 0.61 ± 0.01 | 1.35 ± 0.01 | 0.34 ± 0.01 |
| 12 | 6 | 122.29 | | 0.23 ± 0.01 | 48.03 ± 1.75 | 0.61 ± 0.01 | 1.59 ± 0.01 | 0.38 ± 0.01 |
| 13 | 6 | 189.80 | | 0.32 ± 0.01 | 41.98 ± 0.73 | 0.64 ± 0.01 | 1.49 ± 0.01 | 0.51 ± 0.01 |
| 14 | 6 | 74.95 | | 0.40 ± 0.01 | 49.84 ± 1.16 | 0.71 ± 0.01 | 1.60 ± 0.02 | 0.56 ± 0.01 |
| 15 | 6 | 68.07 | | 0.45 ± 0.01 | 45.37 ± 1.56 | 0.72 ± 0.01 | 1.47 ± 0.03 | 0.62 ± 0.01 |
| 16 | 6 | 55.95 | | 0.45 ± 0.01 | 52.62 ± 1.25 | 0.69 ± 0.01 | 1.79 ± 0.04 | 0.65 ± 0.01 |
| 17 | 6 | 69.38 | | 0.42 ± 0.01 | 45.51 ± 0.95 | 0.68 ± 0.01 | 1.66 ± 0.03 | 0.62 ± 0.01 |
| 18 | 6 | 59.28 | | 0.50 ± 0.01 | 60.45 ± 1.43 | 0.73 ± 0.01 | 2.06 ± 0.05 | 0.69 ± 0.01 |
| 19 | 6 | 59.24 | | 0.51 ± 0.01 | 44.81 ± 1.84 | 0.73 ± 0.01 | 1.44 ± 0.04 | 0.70 ± 0.01 |
| 20 | 6 | 76.92 | | 0.50 ± 0.01 | 59.67 ± 1.65 | 0.71 ± 0.01 | 2.31 ± 0.07 | 0.71 ± 0.01 |
| 21 | 6 | 30.72 | | 0.57 ± 0.01 | 48.09 ± 2.48 | 0.76 ± 0.01 | 1.50 ± 0.05 | 0.75 ± 0.01 |
| 22 | 6 | 26.61 | | 0.59 ± 0.01 | 48.77 ± 2.17 | 0.76 ± 0.01 | 1.53 ± 0.06 | 0.78 ± 0.01 |
| 23 | 6 | 3.34 | | 0.63 ± 0.01 | 49.78 ± 3.48 | 0.80 ± 0.01 | 1.33 ± 0.06 | 0.79 ± 0.02 |
| 24 | 6 | 6.39 | | 0.64 ± 0.02 | 56.41 ± 6.37 | 0.79 ± 0.01 | 3.71 ± 0.93 | 0.82 ± 0.02 |
| 25 | 6 | 6.77 | | 0.65 ± 0.01 | 28.78 ± 3.17 | 0.80 ± 0.01 | 1.48 ± 0.09 | 0.81 ± 0.02 |
| 26 | 5 | 60.71 | 16.04 ± 0.36 | 0.88 ± 0.02 | | 0.94 ± 0.02 | 0.27 ± 0.04 | 0.94 ± 0.03 |
| 27 | 2 | 9.87 | 18.21 ± 0.56 | 0.96 ± 0.01 | | | 0.16 ± 0.06 | |
| 28 | 5 | 15.94 | 17.13 ± 0.47 | 0.90 ± 0.02 | | 0.93 ± 0.02 | 0.28 ± 0.10 | 0.97 ± 0.03 |
| 29 | 2 | 4.43 | 18.22 ± 0.74 | 0.98 ± 0.01 | | | 0.35 ± 0.21 | |
| 30 | 2 | 66.30 | 16.59 ± 0.49 | 0.91 ± 0.03 | 22.51 ± 28.86 | | | |

| Residue | Model | χ^2 | τ_M (ns) | S^2 | τ_f (ps) | S_f^2 | τ_s (ns) | $S_s^2 (S^2/S_f^2)$ |
|---------|-------|----------|------------------|-----------------|-------------------|-----------------|-----------------|---------------------|
| 32 | 2 | 3.05 | 16.89 \pm 0.50 | 0.91 \pm 0.03 | 19.37 \pm 27.00 | | | |
| 35 | 2 | 48.38 | 17.55 \pm 0.39 | 0.95 \pm 0.02 | 28.02 \pm 45.44 | | | |
| 37 | 5 | 9.60 | 19.74 \pm 1.26 | 0.88 \pm 0.05 | | 0.91 \pm 0.04 | 0.59 \pm 0.36 | 0.98 \pm 0.06 |
| 38 | 2 | 102.63 | 18.43 \pm 0.58 | 0.94 \pm 0.03 | 30.83 \pm 83.09 | | | |
| 39 | 5 | 1.34 | 17.37 \pm 0.48 | 0.91 \pm 0.02 | | 0.93 \pm 0.02 | 0.29 \pm 0.13 | 0.97 \pm 0.04 |
| 41 | 2 | 11.34 | 18.60 \pm 0.65 | 0.92 \pm 0.03 | 21.42 \pm 57.64 | | | |
| 42 | 5 | 9.45 | 17.12 \pm 0.49 | 0.89 \pm 0.03 | | 0.91 \pm 0.02 | 0.39 \pm 0.18 | 0.98 \pm 0.04 |
| 43 | 2 | 15.37 | 17.47 \pm 0.39 | 0.94 \pm 0.02 | 49.78 \pm 29.10 | | | |
| 44 | 5 | 3.10 | 18.98 \pm 0.81 | 0.90 \pm 0.04 | | 0.93 \pm 0.03 | 0.50 \pm 0.25 | 0.97 \pm 0.05 |
| 45 | 2 | 23.27 | 17.82 \pm 0.42 | 0.92 \pm 0.02 | 40.50 \pm 21.86 | | | |
| 46 | 2 | 11.43 | 16.52 \pm 0.33 | 0.93 \pm 0.02 | 43.96 \pm 22.27 | | | |
| 48 | 2 | 7.37 | 18.78 \pm 0.47 | 0.91 \pm 0.02 | 28.12 \pm 16.06 | | | |
| 49 | 2 | 15.65 | 17.30 \pm 0.36 | 0.95 \pm 0.02 | 58.11 \pm 43.61 | | | |
| 50 | 5 | 5.26 | 17.18 \pm 0.35 | 0.88 \pm 0.02 | | 0.91 \pm 0.02 | 0.38 \pm 0.09 | 0.97 \pm 0.03 |
| 51 | 2 | 23.44 | 15.88 \pm 0.28 | 0.84 \pm 0.01 | 22.90 \pm 3.15 | | | |
| 52 | 2 | 8.56 | 17.85 \pm 0.40 | 0.93 \pm 0.02 | 36.95 \pm 20.08 | | | |
| 53 | 5 | 8.18 | 15.95 \pm 0.26 | 0.89 \pm 0.01 | | 0.92 \pm 0.01 | 0.41 \pm 0.08 | 0.96 \pm 0.02 |
| 54 | 5 | 26.87 | 17.19 \pm 0.26 | 0.88 \pm 0.01 | | 0.92 \pm 0.01 | 0.23 \pm 0.04 | 0.96 \pm 0.02 |
| 55 | 5 | 15.15 | 16.99 \pm 0.29 | 0.88 \pm 0.01 | | 0.93 \pm 0.02 | 0.18 \pm 0.03 | 0.95 \pm 0.02 |
| 56 | 6 | 55.91 | | 0.50 \pm 0.01 | 30.34 \pm 0.99 | 0.69 \pm 0.01 | 3.13 \pm 0.16 | 0.72 \pm 0.01 |
| 57 | 6 | 102.05 | | 0.36 \pm 0.01 | 28.05 \pm 0.99 | 0.60 \pm 0.01 | 1.91 \pm 0.03 | 0.61 \pm 0.01 |
| 58 | 6 | 598.91 | | 0.22 \pm 0.01 | 43.53 \pm 0.54 | 0.49 \pm 0.01 | 1.55 \pm 0.01 | 0.45 \pm 0.01 |

¹Relaxation data were analyzed with a fixed τ_M for the basic and disordered C-terminal regions. Input data were from R₁, R₂, and {¹H}-¹⁵N heteronuclear NOE rate constants determined at 14.1, 16.45, 18.8, and 21.1 T.