# α-Helices as Alignment Reporters in Residual Dipolar Coupling Analysis of Proteins

Yang Shen, Marshall J. Smith, John M. Louis and Ad Bax

Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-0520, U.S.A.

# SUPPORTING INFORMATION

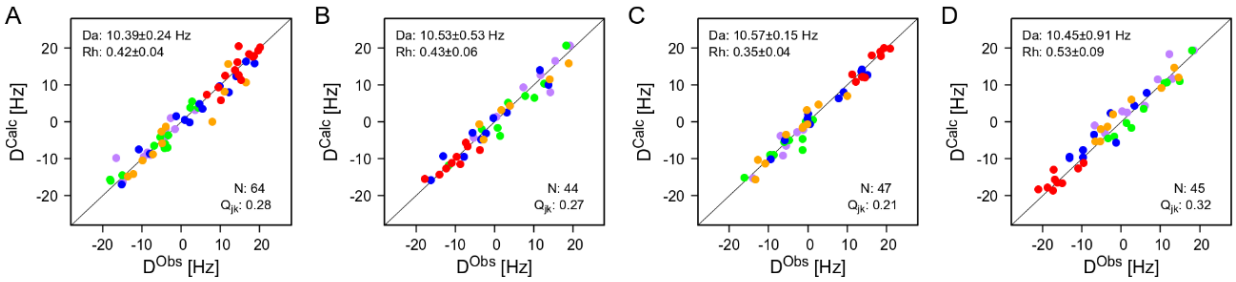


**Figure S1.** RDC analysis of α-helices in $Ca^{2+}$-calmodulin's N-terminal domain. Fit of the normalized $^1D_{NH}$ (●), $^1D_{H\alpha C\alpha}$ (●), $^1D_{C'N}$ (●), $^1D_{C\alpha C'}$ (●), and $^2D_{H\alpha C'}$ (●) RDCs, reported by Chou et al.(Chou *et al.* 2001) against values predicted by SVD fits to helices taken from the 1-Å X-ray structure (PDB entry 1EXR) (Wilson and Brunger 2000). (A) helix 1 (E6-F19); (B) helix 2 (T29 to S38); (C) helix 3 (E45 to E54); and (D) helix 4 (F65-R74).

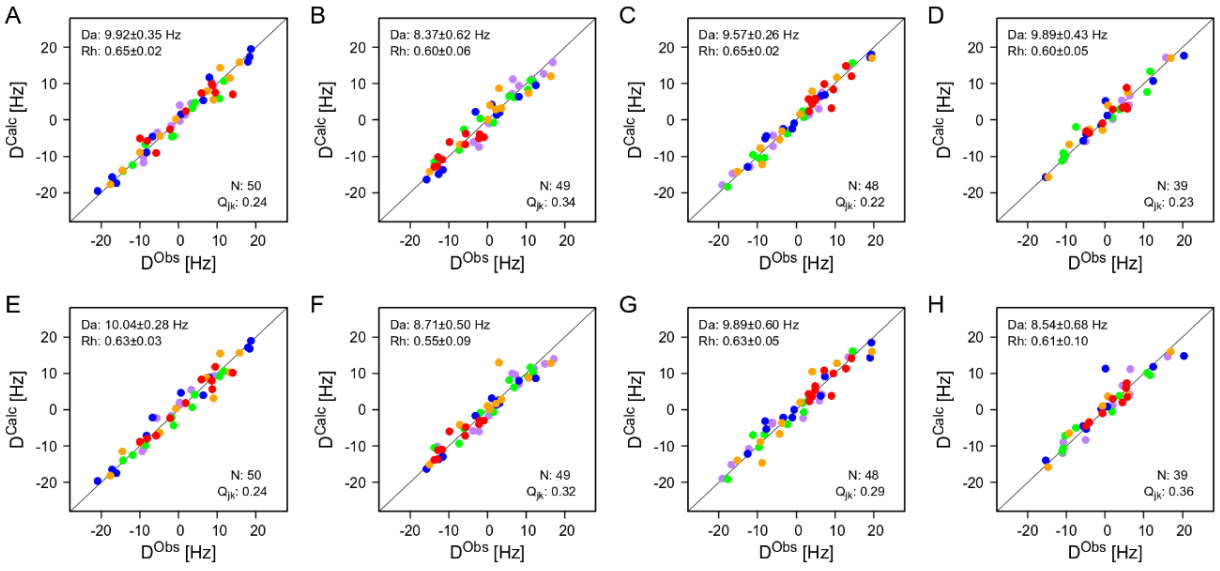


**Figure S2.** RDC analysis of α-helices in $Ca^{2+}$-calmodulin's C-terminal domain. Fit of the normalized $^1D_{NH}$ (●), $^1D_{H\alpha C\alpha}$ (●), $^1D_{C'N}$ (●), $^1D_{C\alpha C'}$ (●), and $^2D_{H\alpha C'}$ (●) RDCs, reported by Chou et al.(Chou *et al.* 2001) against values predicted by an SVD fit to the 1-Å X-ray structure (PDB entry 1EXR) (Wilson and Brunger 2000). (A-D) Individual SVD fits of normalized experimental RDCs in the four helices to the coordinates of the 1-Å X-ray structure (PDB entry 1EXR) (A-D) or to ideal helix (E-H). (A,E) helix 5 (E82-F92); (B,F) helix 6 (A102 to N111); (C,G) helix 7 (D118 to E127); and (D,H) helix 8 (Y138-M145).

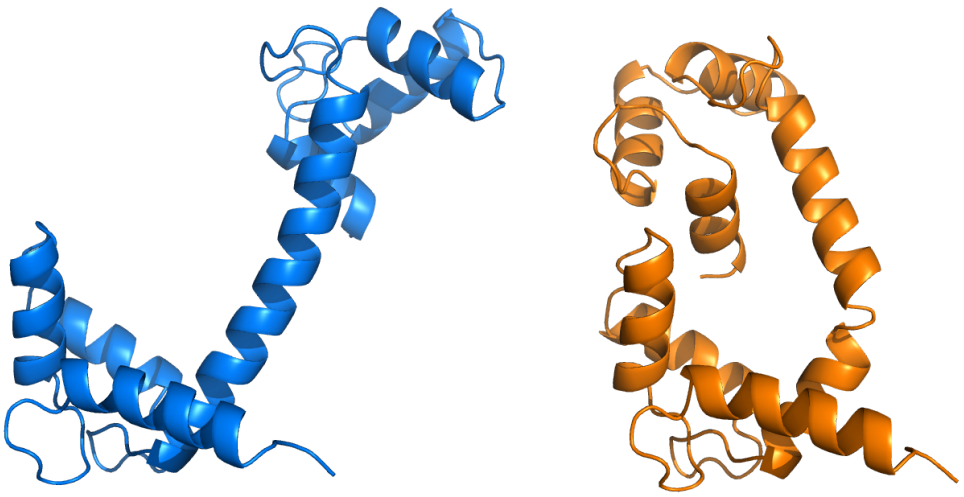

**Figure S3.** Ribbon diagram of the X-ray structure of $Ca^{2+}$-calmodulin (1EXR) before (left) and after (right) altering its C-terminal domain orientation such that the principal axes of the N- and C-terminal alignment tensors become parallel to one another.

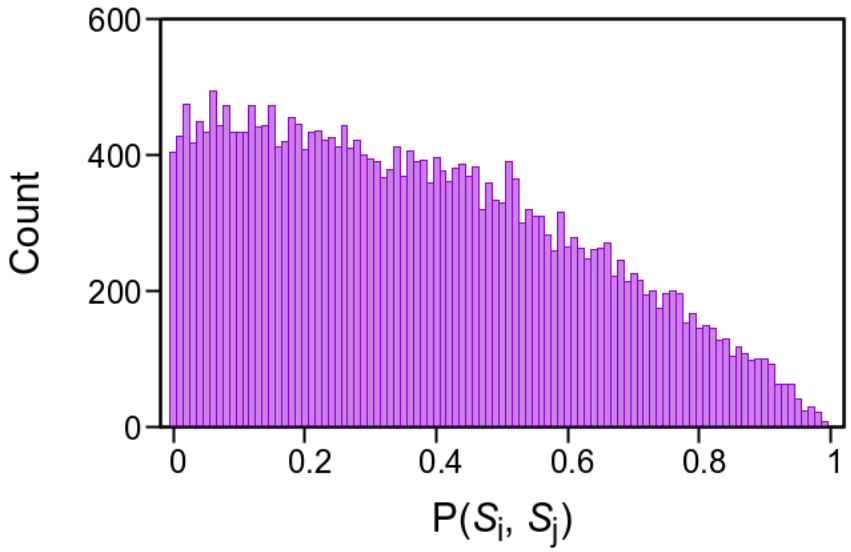


**Figure S4.** Histogram of normalized scalar products $P(S_i, S_j)$ calculated for 30,000 randomly obtained alignment tensor pairs.

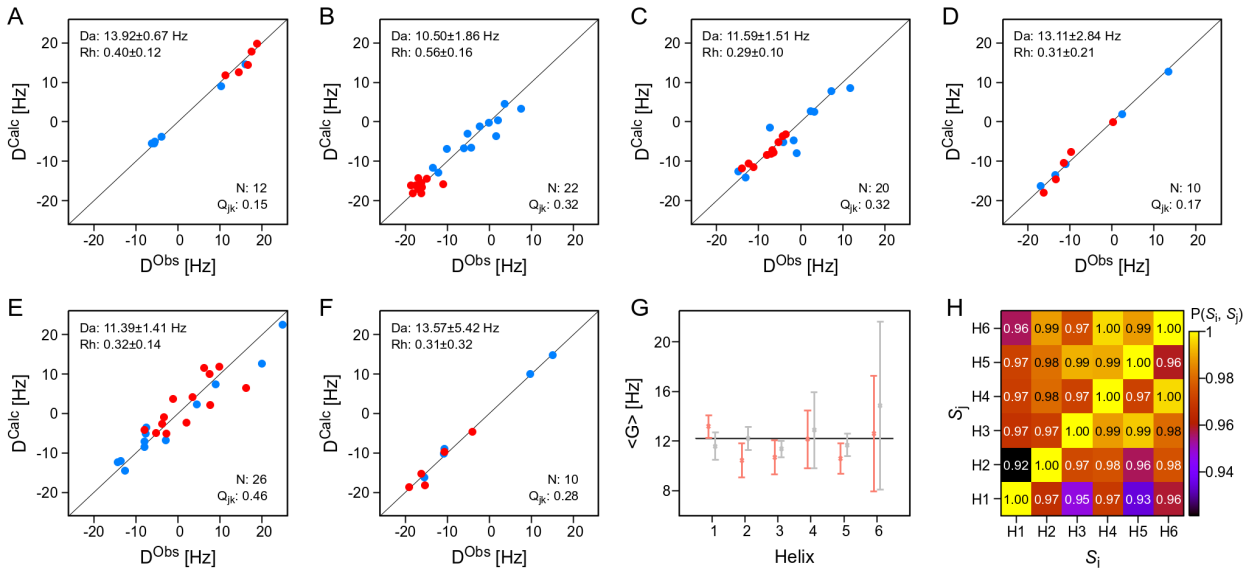


**Figure S5.** RDC analysis of α-helices in dimeric SARS-CoV-2 MPro. Fits of the normalized $^{1}D_{NH}$ (●), $^{2}D_{HC'}$ (●) RDCs to the X-ray coordinates of homodimeric MPro (PDB entry 7K3T) are shown for the following helices: (A) Y54-I59; (B) T201-I213; (C)L227-T237; (D) D245-L250; (E) V261-Q273; and (F) F294-Q299. The generalized sampling parameter for these RDCs, Ξ, is 0.33. (G) Generalized alignment strengths, *G*, obtained for the six helices when using 7K3T (grey) or idealized helices (orange) as reference structures; the reference G value of 12.20 obtained for all six helices (H1 to H6) is plotted as the horizontal line. (H) Normalized scalar products P($S_i$, $S_j$) [i,j="H1" to "H6"] for the six alignment tensors *S* obtained when fitting the helical RDCs to the coordinates of the homodimeric X-ray structure (7K3T) (upper-left half), and to ideal helices that were best-fit superimposed on 7K3T (lower-right half).

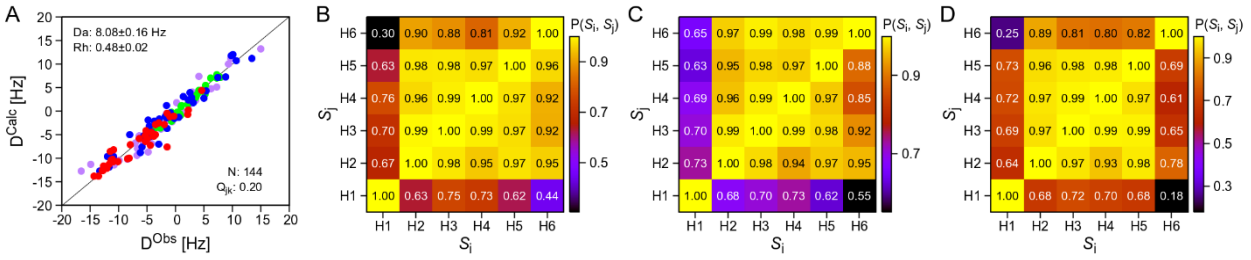


**Figure S6.** (A) Fits of the normalized $^{1}D_{NH}$ (●), $^{1}D_{C'N}$ (●), $^{1}D_{C\alpha C'}$ (●), and $^{2}D_{HNC'}$ (●) RDCs measured in the monomeric state to the X-ray coordinates of homodimeric MPro (PDB entry 7K3T) are shown for RDCs measured for the five helices in the C-terminal domain. (B-D) Normalized scalar products P($S_i$, $S_j$) [i = "H1" to "H6"] for the six alignment tensors *S* obtained when fitting the RDCs to the actual X-ray coordinates of the helices in the monomeric X-ray structures (upper-left half) of (B) 2QCY, (C) 2PWX and (D) 3F9E, or to the best-fit superimposed individual idealized helices (lower-right half).

**Table S1**. Geometry parameters of the ideal helix used in this work.

| | |
|---|---|
| ϕ | -62.8° |
| ψ | 41.6° |
| ω | 179.0° |
| H-N bond length[a] | 0.99 Å |
| In-plane angle $H_i$-$N_i$-$C^{\alpha}_i$ | 116.5º |
| Out-of-plane ($C'_{i-1}$-$N_i$-$C^{\alpha}_i$) orientation | 1.0° |

[*] All other structural parameters follow the AMBER94 library (Cornell *et al.* 1995) used by MOLMOL (Koradi *et al.* 1996).

[a] This bond length provides optimal RDC fits for $^2D_{HNC'}$ (Robertson *et al.* 2021) and is not used for calculating the $^1$H-$^{15}$N dipolar interaction constant that is librationally averaged.(Yao *et al.* 2008)

**Table S2.** Experimental $D_{NH}$, $D_{C'H}$, $D_{C'C}$, $D_{NC'}$, RDC values for the alpha helices of monomeric MPro$^{10–306,H41Q}$.

| Residue Number | Residue Name | Atom | Residue Number | Residue Name | Atom | D (Hz) | Error[a] (Hz) |
|---|---|---|---|---|---|---|---|
| 54 | Tyr | N | 54 | Tyr | HN | -3.90 | 0.15 |
| 55 | Glu | N | 55 | Glu | HN | 3.77 | 0.20 |
| 56 | Asp | N | 56 | Asp | HN | 3.50 | 0.23 |
| 57 | Leu | N | 57 | Leu | HN | -3.13 | 0.32 |
| 59 | Ile | N | 59 | Ile | HN | 5.11 | 0.16 |
| 201 | Thr | N | 201 | Thr | HN | -3.81 | 0.37 |
| 202 | Val | N | 202 | Val | HN | -5.27 | 0.22 |
| 203 | Asn | N | 203 | Asn | HN | -11.10 | 0.32 |
| 204 | Val | N | 204 | Val | HN | -4.25 | 0.58 |
| 227 | Leu | N | 227 | Leu | HN | -11.37 | 0.13 |
| 229 | Asp | N | 229 | Asp | HN | -14.38 | 0.20 |

| Residue Number | Residue Name | Atom | Residue Number | Residue Name | Atom | D (Hz) | Error[a] (Hz) |
|---|---|---|---|---|---|---|---|
| 230 | Phe | N | 230 | Phe | HN | -11.72 | 0.18 |
| 231 | Asn | N | 231 | Asn | HN | -10.94 | 0.18 |
| 232 | Leu | N | 232 | Leu | HN | -11.32 | 0.22 |
| 233 | Val | N | 233 | Val | HN | -13.58 | 0.38 |
| 234 | Ala | N | 234 | Ala | HN | -11.29 | 0.37 |
| 235 | Met | N | 235 | Met | HN | -4.95 | 0.25 |
| 236 | Lys | N | 236 | Lys | HN | -11.47 | 0.33 |
| 237 | Tyr | N | 237 | Tyr | HN | -12.86 | 0.40 |
| 245 | Asp | N | 245 | Asp | HN | -1.49 | 0.13 |
| 246 | His | N | 246 | His | HN | 2.29 | 0.16 |
| 247 | Val | N | 247 | Val | HN | -1.03 | 0.18 |
| 248 | Asp | N | 248 | Asp | HN | -5.30 | 0.18 |
| 249 | Ile | N | 249 | Ile | HN | -6.43 | 0.28 |
| 250 | Leu | N | 250 | Leu | HN | 4.65 | 0.40 |
| 261 | Val | N | 261 | Val | HN | -3.53 | 0.11 |
| 262 | Leu | N | 262 | Leu | HN | -10.10 | 0.18 |
| 263 | Asp | N | 263 | Asp | HN | 2.20 | 0.20 |
| 264 | Met | N | 264 | Met | HN | -2.94 | 0.45 |
| 266 | Ala | N | 266 | Ala | HN | -12.72 | 0.26 |
| 267 | Ser | N | 267 | Ser | HN | -0.33 | 0.10 |
| 269 | Lys | N | 269 | Lys | HN | -12.12 | 0.30 |
| 270 | Glu | N | 270 | Glu | HN | -8.50 | 0.17 |
| 271 | Leu | N | 271 | Leu | HN | -4.42 | 0.48 |
| 273 | Gln | N | 273 | Gln | HN | -12.75 | 5.17 |
| 294 | Phe | N | 294 | Phe | HN | -10.91 | 0.16 |
| 296 | Val | N | 296 | Val | HN | -1.48 | 0.19 |
| 297 | Val | N | 297 | Val | HN | -5.17 | 0.20 |
| 298 | Arg | N | 298 | Arg | HN | -8.00 | 0.14 |
| 299 | Gln | N | 299 | Gln | HN | -1.99 | 0.12 |
| 54 | Tyr | N | 53 | Asn | C | -0.40 | 0.02 |
| 55 | Glu | N | 54 | Tyr | C | -0.34 | 0.03 |
| 56 | Asp | N | 55 | Glu | C | 1.00 | 0.03 |
| 57 | Leu | N | 56 | Asp | C | -0.58 | 0.04 |
| 201 | Thr | N | 200 | Ile | C | 1.30 | 0.05 |
| 202 | Val | N | 201 | Thr | C | 0.63 | 0.02 |
| 203 | Asn | N | 202 | Val | C | -0.84 | 0.04 |
| 204 | Val | N | 203 | Asn | C | 1.30 | 0.08 |
| 227 | Leu | N | 226 | Thr | C | 0.95 | 0.02 |

| Residue Number | Residue Name | Atom | Residue Number | Residue Name | Atom | D (Hz) | Error[a] (Hz) |
|---|---|---|---|---|---|---|---|
| 228 | Asn | N | 227 | Leu | C | 0.89 | 0.01 |
| 229 | Asp | N | 228 | Asn | C | 0.01 | 0.02 |
| 230 | Phe | N | 229 | Asp | C | 0.90 | 0.02 |
| 231 | Asn | N | 230 | Phe | C | 0.07 | 0.02 |
| 232 | Leu | N | 231 | Asn | C | 1.93 | 0.03 |
| 233 | Val | N | 232 | Leu | C | -0.66 | 0.04 |
| 234 | Ala | N | 233 | Val | C | 0.77 | 0.04 |
| 235 | Met | N | 234 | Ala | C | 0.26 | 0.03 |
| 236 | Lys | N | 235 | Met | C | 1.32 | 0.04 |
| 237 | Tyr | N | 236 | Lys | C | 0.19 | 0.04 |
| 246 | His | N | 245 | Asp | C | -0.14 | 0.02 |
| 247 | Val | N | 246 | His | C | 1.03 | 0.02 |
| 248 | Asp | N | 247 | Val | C | 0.53 | 0.02 |
| 249 | Ile | N | 248 | Asp | C | -1.27 | 0.04 |
| 250 | Leu | N | 249 | Ile | C | 1.95 | 0.06 |
| 261 | Val | N | 260 | Ala | C | 0.27 | 0.01 |
| 262 | Leu | N | 261 | Val | C | 0.87 | 0.02 |
| 263 | Asp | N | 262 | Leu | C | -1.01 | 0.03 |
| 264 | Met | N | 263 | Asp | C | 1.82 | 0.06 |
| 266 | Ala | N | 265 | Cys | C | -0.17 | 0.03 |
| 269 | Lys | N | 268 | Leu | C | 0.71 | 0.03 |
| 270 | Glu | N | 269 | Lys | C | -0.66 | 0.02 |
| 271 | Leu | N | 270 | Glu | C | 1.55 | 0.07 |
| 273 | Gln | N | 272 | Leu | C | 0.76 | 0.02 |
| 294 | Phe | N | 293 | Pro | C | 1.04 | 0.02 |
| 296 | Val | N | 295 | Asp | C | 1.83 | 0.03 |
| 297 | Val | N | 296 | Val | C | -0.17 | 0.02 |
| 299 | Gln | N | 298 | Arg | C | 0.21 | 0.02 |
| 53 | Asn | C | 53 | Asn | CA | 1.97 | 0.09 |
| 54 | Tyr | C | 54 | Tyr | CA | 0.61 | 0.10 |
| 55 | Glu | C | 55 | Glu | CA | -1.65 | 0.11 |
| 56 | Asp | C | 56 | Asp | CA | -0.33 | 0.14 |
| 200 | Ile | C | 200 | Ile | CA | -1.26 | 0.22 |
| 201 | Thr | C | 201 | Thr | CA | -1.19 | 0.13 |
| 202 | Val | C | 202 | Val | CA | 0.99 | 0.15 |
| 203 | Asn | C | 203 | Asn | CA | 1.08 | 0.20 |
| 226 | Thr | C | 226 | Thr | CA | 0.26 | 0.05 |
| 228 | Asn | C | 228 | Asn | CA | -0.32 | 0.07 |

| Residue Number | Residue Name | Atom | Residue Number | Residue Name | Atom | D (Hz) | Error[a] (Hz) |
|---|---|---|---|---|---|---|---|
| 229 | Asp | C | 229 | Asp | CA | 0.62 | 0.08 |
| 230 | Phe | C | 230 | Phe | CA | -1.25 | 0.07 |
| 231 | Asn | C | 231 | Asn | CA | -0.97 | 0.08 |
| 232 | Leu | C | 232 | Leu | CA | -0.85 | 0.12 |
| 233 | Val | C | 233 | Val | CA | 2.03 | 0.12 |
| 234 | Ala | C | 234 | Ala | CA | -2.34 | 0.08 |
| 235 | Met | C | 235 | Met | CA | -1.13 | 0.10 |
| 236 | Lys | C | 236 | Lys | CA | 0.51 | 0.12 |
| 244 | Gln | C | 244 | Gln | CA | 2.21 | 0.04 |
| 245 | Asp | C | 245 | Asp | CA | -0.76 | 0.08 |
| 246 | His | C | 246 | His | CA | -0.59 | 0.07 |
| 247 | Val | C | 247 | Val | CA | -0.18 | 0.07 |
| 248 | Asp | C | 248 | Asp | CA | 1.76 | 0.14 |
| 249 | Ile | C | 249 | Ile | CA | -0.31 | 0.16 |
| 260 | Ala | C | 260 | Ala | CA | -2.81 | 0.05 |
| 261 | Val | C | 261 | Val | CA | 1.49 | 0.07 |
| 262 | Leu | C | 262 | Leu | CA | 0.57 | 0.09 |
| 263 | Asp | C | 263 | Asp | CA | -0.86 | 0.18 |
| 264 | Met | C | 264 | Met | CA | -2.19 | 0.15 |
| 265 | Cys | C | 265 | Cys | CA | 2.69 | 0.09 |
| 268 | Leu | C | 268 | Leu | CA | 0.28 | 0.08 |
| 269 | Lys | C | 269 | Lys | CA | 0.45 | 0.09 |
| 270 | Glu | C | 270 | Glu | CA | -0.30 | 0.11 |
| 272 | Leu | C | 272 | Leu | CA | 2.14 | 0.08 |
| 293 | Pro | C | 293 | Pro | CA | 1.76 | 0.09 |
| 294 | Phe | C | 294 | Phe | CA | 0.13 | 0.08 |
| 295 | Asp | C | 295 | Asp | CA | -0.57 | 0.07 |
| 298 | Arg | C | 298 | Arg | CA | -0.61 | 0.04 |
| 53 | Asn | C | 54 | Tyr | HN | -2.60 | 0.17 |
| 54 | Tyr | C | 55 | Glu | HN | -0.25 | 0.22 |
| 55 | Glu | C | 56 | Asp | HN | 3.61 | 0.24 |
| 56 | Asp | C | 57 | Leu | HN | -1.25 | 0.33 |
| 58 | Leu | C | 59 | Ile | HN | 2.74 | 0.14 |
| 200 | Ile | C | 201 | Thr | HN | 2.09 | 0.45 |
| 201 | Thr | C | 202 | Val | HN | 1.19 | 0.26 |
| 202 | Val | C | 203 | Asn | HN | -4.99 | 0.38 |
| 203 | Asn | C | 204 | Val | HN | 0.77 | 0.36 |
| 226 | Thr | C | 227 | Leu | HN | -0.51 | 0.09 |

| Residue Number | Residue Name | Atom | Residue Number | Residue Name | Atom | D (Hz) | Error[a] (Hz) |
|---|---|---|---|---|---|---|---|
| 227 | Leu | C | 228 | Asn | HN | 0.42 | 0.11 |
| 228 | Asn | C | 229 | Asp | HN | -1.61 | 0.12 |
| 229 | Asp | C | 230 | Phe | HN | -1.07 | 0.13 |
| 230 | Phe | C | 231 | Asn | HN | -1.26 | 0.13 |
| 231 | Asn | C | 232 | Leu | HN | 2.42 | 0.14 |
| 232 | Leu | C | 233 | Val | HN | -3.32 | 0.32 |
| 233 | Val | C | 234 | Ala | HN | -2.13 | 0.27 |
| 234 | Ala | C | 235 | Met | HN | 0.05 | 0.16 |
| 235 | Met | C | 236 | Lys | HN | 1.72 | 0.22 |
| 236 | Lys | C | 237 | Tyr | HN | -2.28 | 0.23 |
| 244 | Gln | C | 245 | Asp | HN | -1.90 | 0.13 |
| 245 | Asp | C | 246 | His | HN | -0.35 | 0.14 |
| 246 | His | C | 247 | Val | HN | 1.37 | 0.13 |
| 247 | Val | C | 248 | Asp | HN | -0.46 | 0.14 |
| 248 | Asp | C | 249 | Ile | HN | -5.54 | 0.33 |
| 249 | Ile | C | 250 | Leu | HN | 5.00 | 0.39 |
| 260 | Ala | C | 261 | Val | HN | 1.01 | 0.08 |
| 261 | Val | C | 262 | Leu | HN | -0.85 | 0.14 |
| 262 | Leu | C | 263 | Asp | HN | -2.37 | 0.18 |
| 263 | Asp | C | 264 | Met | HN | 3.25 | 0.27 |
| 264 | Met | C | 265 | Cys | HN | -1.51 | 0.30 |
| 265 | Cys | C | 266 | Ala | HN | -2.80 | 0.19 |
| 266 | Ala | C | 267 | Ser | HN | 0.98 | 0.20 |
| 268 | Leu | C | 269 | Lys | HN | -1.41 | 0.17 |
| 269 | Lys | C | 270 | Glu | HN | -3.60 | 0.20 |
| 270 | Glu | C | 271 | Leu | HN | 2.65 | 0.20 |
| 272 | Leu | C | 273 | Gln | HN | -1.40 | 0.16 |
| 293 | Pro | C | 294 | Phe | HN | -0.57 | 0.16 |
| 294 | Phe | C | 295 | Asp | HN | -3.82 | 0.17 |
| 295 | Asp | C | 296 | Val | HN | 3.11 | 0.15 |
| 296 | Val | C | 297 | Val | HN | -0.54 | 0.30 |
| 297 | Val | C | 298 | Arg | HN | -2.22 | 0.13 |
| 298 | Arg | C | 299 | Gln | HN | -0.18 | 0.09 |

[a] The error only accounts for the statistical uncertainty based on the signal to noise ratio in the reference and attenuated spectra (Fitzkee and Bax 2010) and does not include systematic errors that can result from baseline undulations or other factors that can impact peak intensities.

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