Supplementary Information

Deuterium Spin Probes of Backbone Order in Proteins: A ^{2}H NMR Relaxation Study of Deuterated Carbon α Sites

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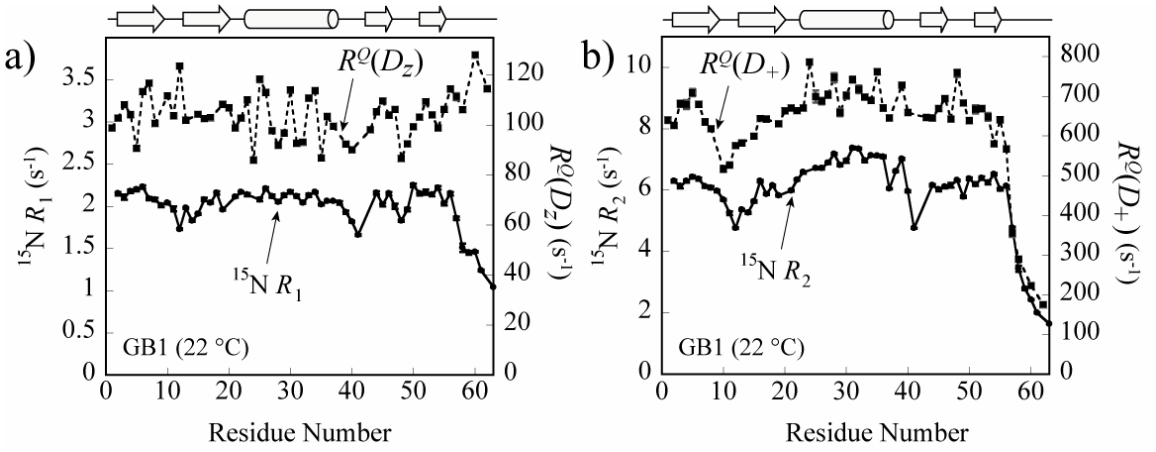

Figure S1. Plots of D^{α} (black rectangles and dashed lines) and ^{15}N (black circles and solid lines) relaxation rates in GB1 (22°C) shown as a function of the protein sequence: **a)** $R^Q(D_Z)$ and ^{15}N R_1 rates; **b)** $R^Q(D_+)$ and ^{15}N R_2 rates. Schematic representation of the secondary structure is shown at the top of the plots: β-strands are depicted with arrows, while α-helices are represented with cylinders.

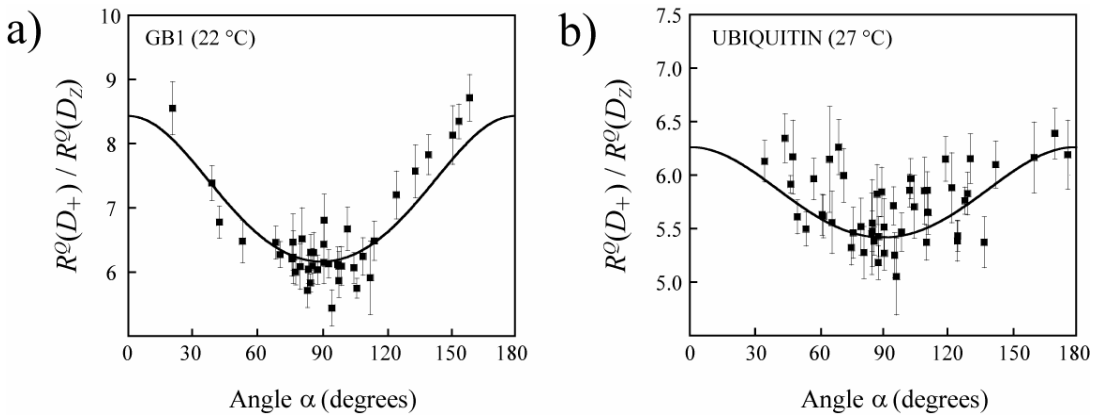

Figure S2. Plots of $R^Q(D_+)/R^Q(D_z)$ as a function of the angle α that each of the C^α-D^α bond vectors subtends with respect to the unique axis of the diffusion tensor, along with the best fits of the data for **a)** GB1 (22°C), and **b)** ubiquitin (27°C). Direction cosines have been extracted from C^α-H^α vector coordinates using protonated crystallographic structures of ubiquitin and GB1 with respective pdb accession codes 1ubq.pdb[1] and 2qmt.pdb[2].

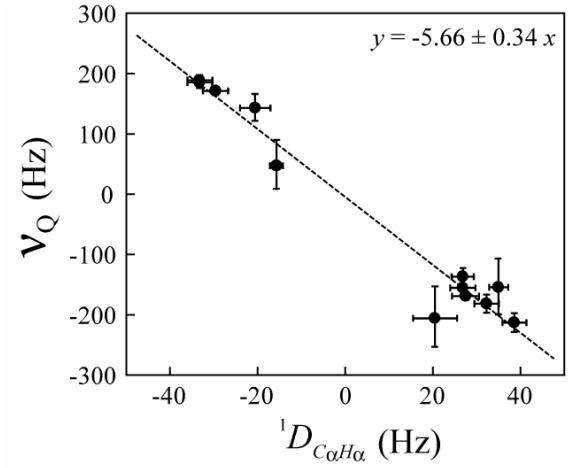

Figure S3. The correlation plot of the ^{2}H quadrupolar splitting, ν_Q (y-axis, Hz), with the $^1D_{C\alpha-H\alpha}$ residual dipolar couplings (x-axis, Hz) in the subset of 11 residues of GB1. The best fit is shown with a dashed line. The values of ν_Q and $^1D_{C\alpha-H\alpha}$ have been assumed to have opposite signs.

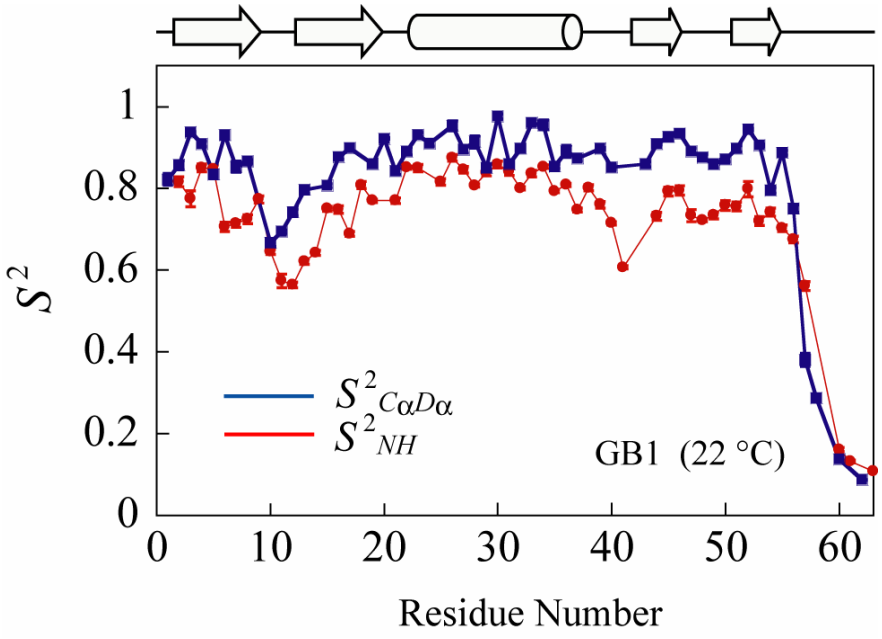

Figure S4. D^{α}-derived $S^2_{C\alpha D\alpha}$ (blue rectangles and lines) and ^{15}N-derived S^2_{NH} (red circles and lines) in GB1 (22°C) plotted as a function of protein sequence. A schematic representation of the secondary structure of GB1 is shown at the top of the plot: β-strands are depicted with arrows, while α-helices are represented with cylinders.

Table S1. $S^2_{C\alpha D\alpha}$ and τ_f Values in Human Ubiquitin Derived from Analysis of D^{α} ^2H Relaxation rates at 10°C, 27°C and 40°C.[a)]

	$S^2_{C\alpha\text{-}D\alpha}$ 10°C	τ_f(ps)[b)] 10°C	$S^2_{C\alpha\text{-}D\alpha}$ 27°C	τ_f(ps)[b)] 27°C	$S^2_{C\alpha\text{-}D\alpha}$ 40°C	τ_f(ps)[b)] 40°C
M1	0.89 (0.01)	-	0.85 (0.01)	-	0.82 (0.01)	-
Q2	0.89 (0.01)	-	0.81 (0.01)	-	0.82 (0.01)	-
I3	-	-	0.89 (0.01)	82.7 (43.1)	0.93 (0.01)	-
F4	0.94 (0.01)	-	0.92 (0.01)	-	0.83 (0.05)	232.3 (51.3)
V5	0.85 (0.01)	-	0.82 (0.02)	136.9 (71.2)	0.83 (0.01)	-
K6	0.87 (0.01)	-	0.84 (0.01)	-	-	
T7	0.89 (0.01)	-	0.86 (0.01)	-	0.77 (0.01)	-
L8	0.73 (0.02)	204.1 (11.7)	-	-	-	-
T9	0.74 (0.01)	69.5 (5.1)	0.78 (0.01)	-	-	-
K11	0.86 (0.01)	-	0.86 (0.01)	-	0.87 (0.02)	-
T12	0.82 (0.01)	-	0.87 (0.02)	-	0.83 (0.01)	-
I13	0.91 (0.02)	154.0 (68.2)	0.86 (0.01)	90.3 (35.7)	0.82 (0.02)	150.4 (30.0)
T14	0.86 (0.01)	-	0.90 (0.02)	-	0.85 (0.01)	-
E16	0.91 (0.01)	-	0.86 (0.02)	95.9 (42.7)	0.84 (0.01)	-
V17	-	-	0.89 (0.01)	-	0.88 (0.01)	-
P19	0.89 (0.01)	-	0.86 (0.01)	-	0.81 (0.01)	-
S20	0.87 (0.01)	51.2 (22.9)	0.85 (0.01)	-	0.82 (0.01)	-
D21	0.92 (0.01)	213.8 (53.4)	0.94 (0.01)	-	-	-
T22	-	-	0.86 (0.01)	112.0 (62.8)	0.92 (0.01)	-
E24	-	-	0.91 (0.01)	-	0.89 (0.01)	-
V26	-	-	0.91 (0.01)	-	0.79 (0.02)	266.8 (31.4)
K27	0.88 (0.03)	-	0.87 (0.02)	-	-	-
A28	0.97 (0.01)	-	0.92 (0.01)	-	0.87 (0.01)	-
K29	0.92 (0.04)	-	0.85 (0.01)	105.1 (53.6)	0.90 (0.01)	-

I30	0.87 (0.02)	-	0.92 (0.01)	-	-	-
Q31	0.93 (0.02)	-	0.90 (0.01)	-	0.89 (0.01)	-
D32	0.92 (0.01)	-	0.89 (0.01)	-	0.87 (0.01)	-
K33	0.90 (0.01)	-	0.86 (0.02)	-	-	-
E34	0.91 (0.01)	-	0.88 (0.01)	-	0.85 (0.01)	-
P38	-	-	0.89 (0.01)	-	0.85 (0.01)	-
D39	0.91 (0.01)	-	0.86 (0.01)	-	0.84 (0.01)	-
Q40	0.94 (0.02)	-	0.90 (0.01)	-	0.88 (0.01)	-
Q41	-	-	0.94 (0.01)	-	-	-
R42	0.89 (0.01)	-	0.92 (0.01)	-	0.89 (0.02)	-
L43	0.87 (0.01)	-	0.94 (0.01)	-	0.82 (0.02)	-
I44	-	-	0.82 (0.01)	-	0.84 (0.01)	-
F45	0.89 (0.01)	128.0 (68.6)	-	-	-	-
A46	0.82 (0.02)	112.7 (26.5)	0.79 (0.01)	-	0.71 (0.03)	137.9 (26.7)
K48	-	-	0.81 (0.01)	-	-	-
Q49	0.90 (0.01)	-	0.86 (0.01)	-	0.84 (0.01)	-
L50	-	-	0.89 (0.01)	-	0.87 (0.01)	-
E51	0.91 (0.01)	-	0.86 (0.01)	-	0.86 (0.01)	-
R54	0.92 (0.01)	-	0.88 (0.01)	-	0.89 (0.01)	-
T55	0.84 (0.01)	-	0.88 (0.01)	-	0.88 (0.01)	-
L56	-	-	-	-	0.94 (0.01)	-
S57	0.92 (0.02)	-	0.92 (0.01)	-	0.91 (0.02)	-
D58	0.97 (0.02)	-	0.92 (0.01)	-	0.91 (0.01)	-
Y59	0.88 (0.02)	-	0.92 (0.01)	-	0.89 (0.01)	-
N60	0.87 (0.02)	-	0.81 (0.01)	-	0.81 (0.01)	-
I61	0.88 (0.01)	-	0.87 (0.01)	-	0.84 (0.01)	-
Q62	-	-	0.81 (0.01)	53.0 (27.8)	0.81 (0.01)	-
K63	0.92 (0.01)	-	0.88 (0.02)	-	0.83 (0.01)	-
E64	0.87 (0.01)	-	0.83 (0.01)	-	0.85 (0.01)	-
S65	0.90 (0.01)	-	0.90 (0.01)	-	0.85 (0.01)	-
T66	0.85 (0.01)	-	0.84 (0.01)	-	0.81 (0.01)	-
L67	0.92 (0.01)	-	0.90 (0.01)	-	0.92 (0.01)	-
H68	0.94 (0.01)	-	0.89 (0.01)	-	0.90 (0.01)	-
L69	0.93 (0.02)	-	0.91 (0.01)	-	0.86 (0.01)	-
V70	0.91 (0.01)	107.7 (16.5)	0.83 (0.01)	204.3 (20.6)	-	-
L71	0.81 (0.02)	208.1 (21.2)	0.77 (0.02)	167.5 (17.7)	-	-
R72	0.62 (0.02)	306.1 (11.7)	0.57 (0.02)	329.9 (35.6)	-	-
L73	0.43 (0.01)	384.6 (6.3)	0.33 (0.01)	339.6 (26.3)	-	-
R74	0.28 (0.01)	319.7 (5.3)	-	-	-	-

[a] Errors in $S^2_{C\alpha D\alpha}$ and τ_f as established from Monte-Carlo simulations[3] are shown in parentheses. [b] τ_f values (in picoseconds) are reported only for the residues that could not be fit to the single-parameter ($S^2_{C\alpha D\alpha}$ only) Lipari-Szabo spectral density function[4,5] with 5% probability of exceeding χ^2.[6]

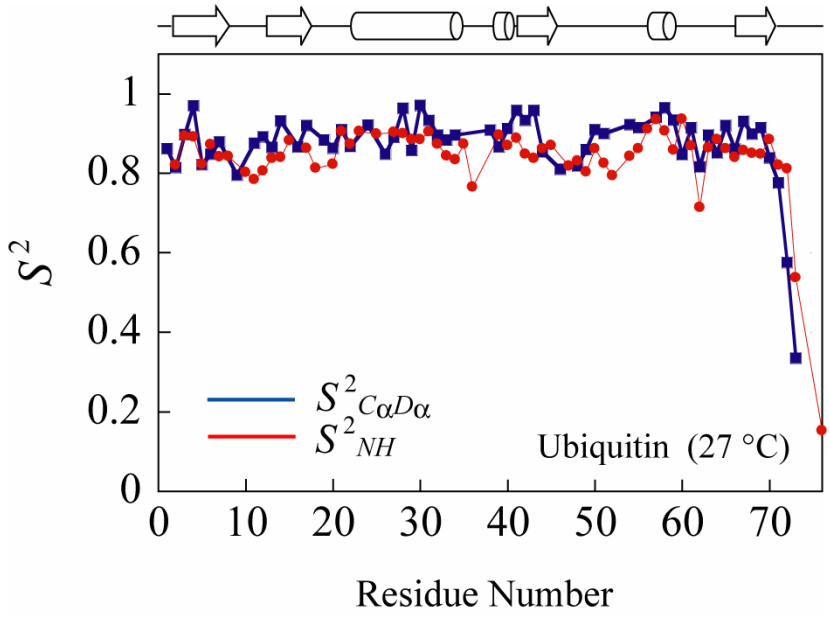

Figure S5. D^{α}-derived $S^2_{C\alpha D\alpha}$ (blue rectangles and lines) and ^{15}N-derived S^2_{NH} (red circles and lines) in ubiquitin (27°C) obtained using common global diffusion tensor parameters ($\tau_{c,eff} = (2D_{||} + 4D_{\perp})^{-1} = 4.05$ ns; $D_{||}/D_{\perp} = 1.20$; $\theta = 7°$; $\phi = -12°$) and $QCC = 171$ kHz, plotted as a function of the protein sequence. A schematic representation of the secondary structure of ubiquitin is shown at the top of the plot: β-strands are depicted with arrows, while α-helices are represented with cylinders.

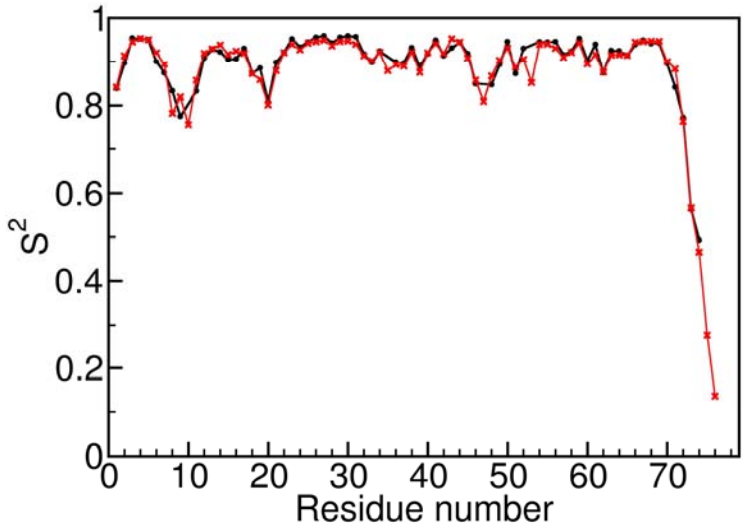

Figure S6. Comparison of $S^2_{C\alpha D\alpha}$ (red, 'x' symbols) with $S^2_{C\alpha C\beta}$ (black, filled circles) order parameters derived from of the 1-μs MD trajectory of ubiquitin (27°C) averaged over a 5-ns time window. See 'Materials and Methods' for the details of MD simulations.

References

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