Slow Methyl Axes Motions in Perdeuterated Villin Headpiece Subdomain Probed by Cross-Correlated NMR Relaxation Measurements

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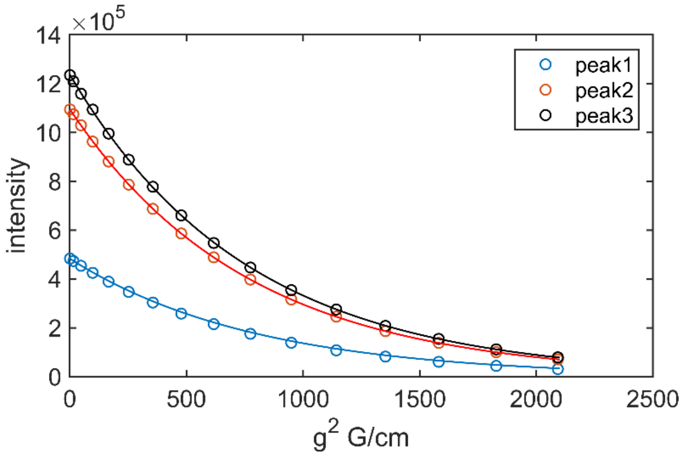
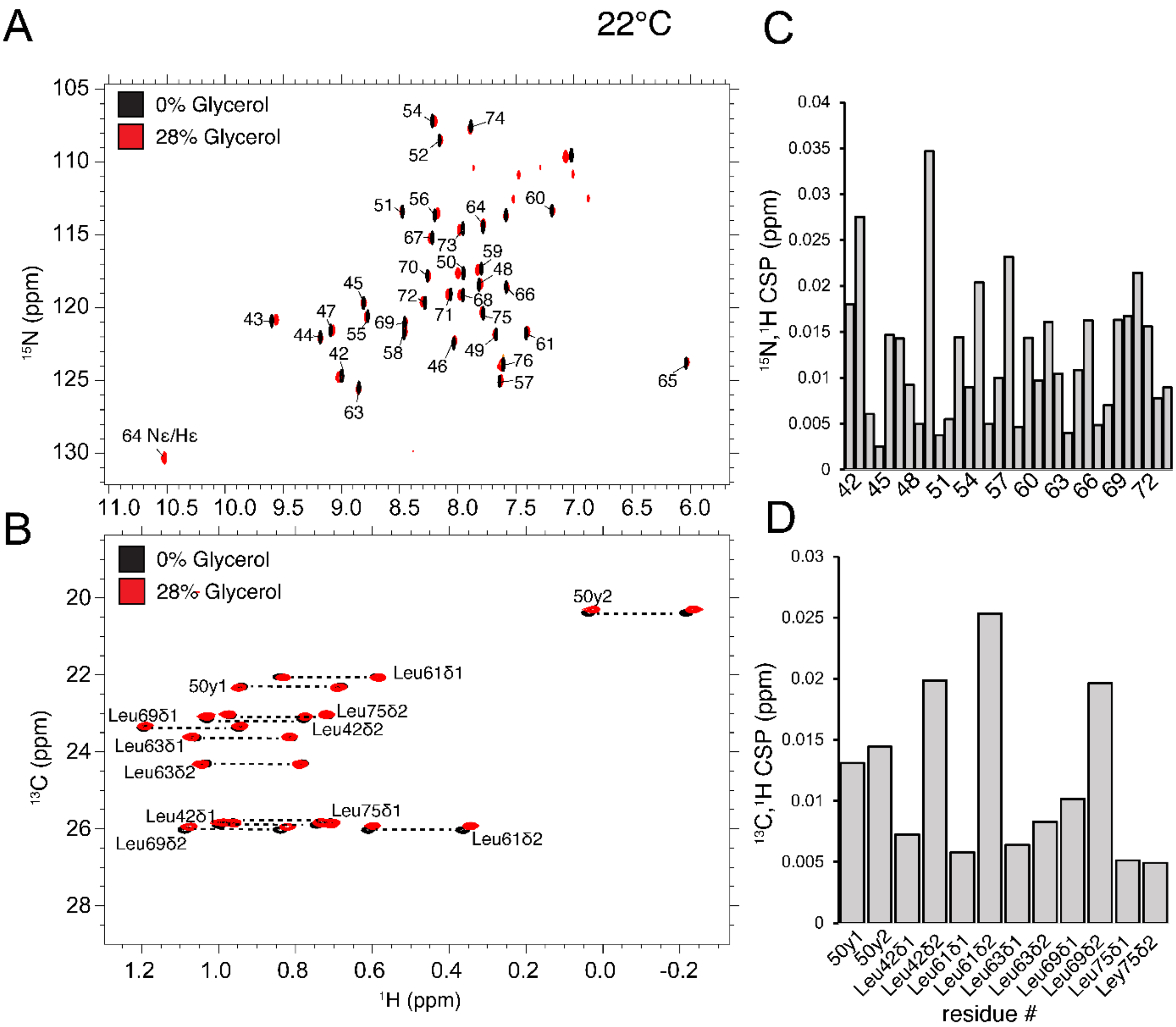
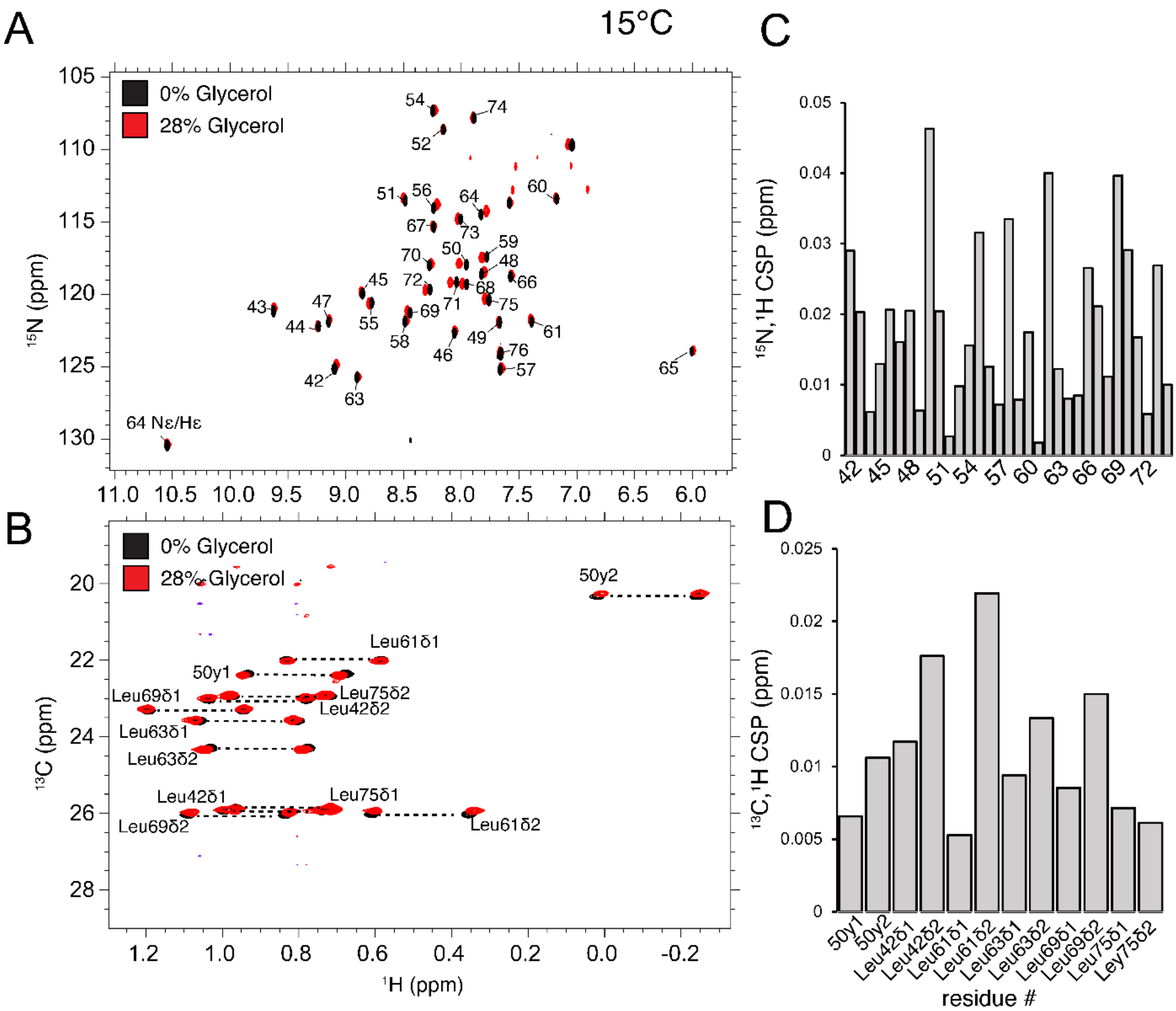
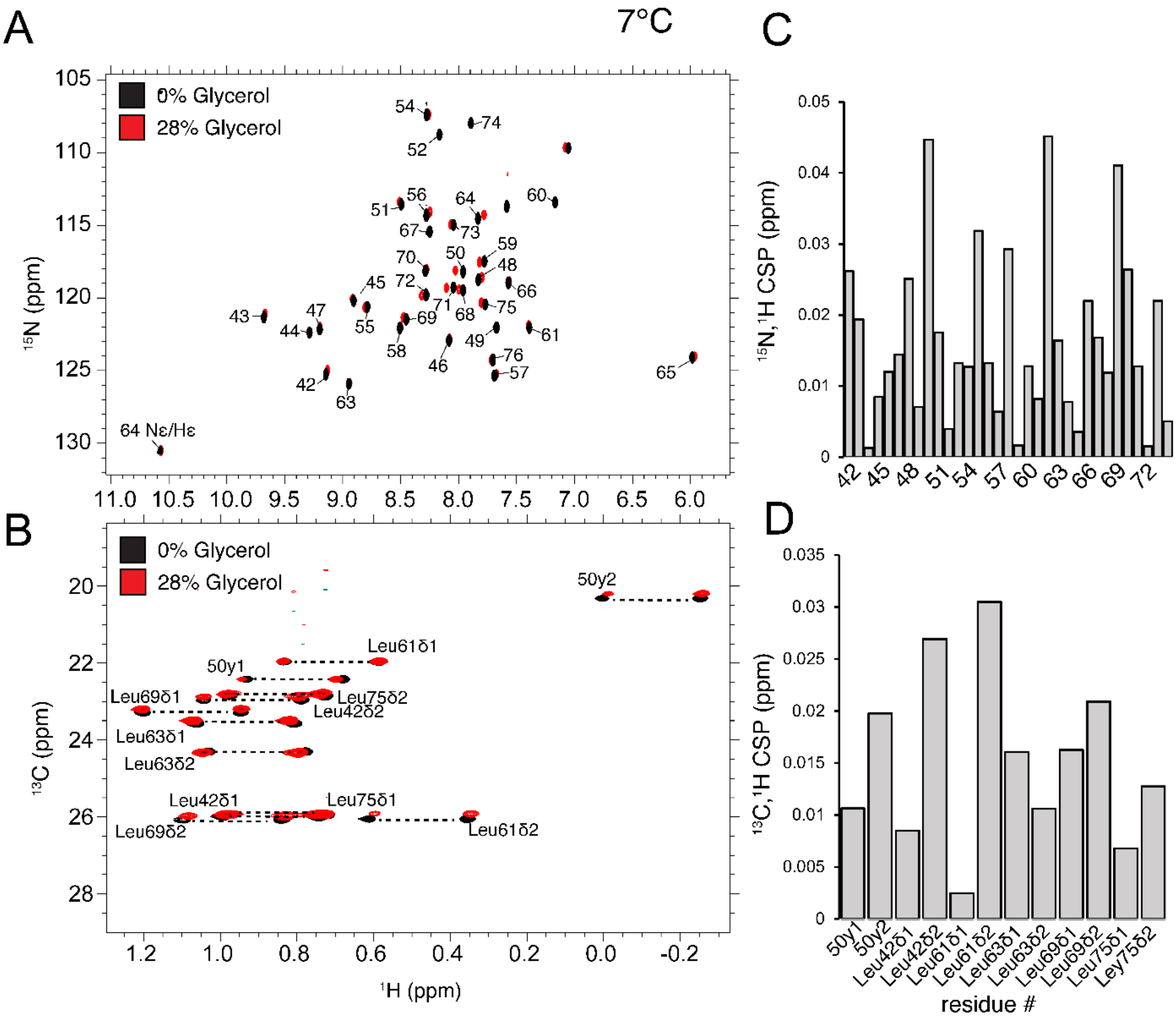


Figure S1. Sample DOSY decay curves (perdeuterated/LV protonated sample at 15 ºC) for the residual proton peaks of glycerol-d8 (numbered from downfield to upfield positions). Intensity is given in arbitrary units versus gradient strength. The lines represent exponential fits to the data.


A
22°C
0% Glycerol
28% Glycerol
^{15}N (ppm)
64 Nε/Hε
B
0% Glycerol
28% Glycerol
^{13}C (ppm)
^{1}H (ppm)
50γ2
Leu61δ1
50γ1
Leu69δ1
Leu75δ2
Leu42δ2
Leu63δ1
Leu63δ2
Leu42δ1
Leu75δ1
Leu69δ2
Leu61δ2
C
^{15}N,^{1}H CSP (ppm)
D
^{13}C,^{1}H CSP (ppm)
residue #


A
15°C
0% Glycerol
28% Glycerol
^{15}N (ppm)
B
^{13}C (ppm)
^{1}H (ppm)
C
^{15}N,^{1}H CSP (ppm)
D
^{13}C,^{1}H CSP (ppm)
residue #


A
7°C
C
0% Glycerol
28% Glycerol
^{15}N (ppm)
64 Nε/Hε
B
0% Glycerol
28% Glycerol
^{13}C (ppm)
50γ2
50γ1
Leu61δ1
Leu69δ1
Leu75δ2
Leu42δ2
Leu63δ1
Leu63δ2
Leu42δ1
Leu75δ1
Leu69δ2
Leu61δ2
^{1}H (ppm)
^{15}N,^{1}H CSP (ppm)
D
^{13}C, ^{1}H CSP (ppm)
50γ1
50γ2
Leu42δ1
Leu42δ2
Leu61δ1
Leu61δ2
Leu63δ1
Leu63δ2
Leu69δ1
Leu69δ2
Leu75δ1
Ley75δ2
residue #

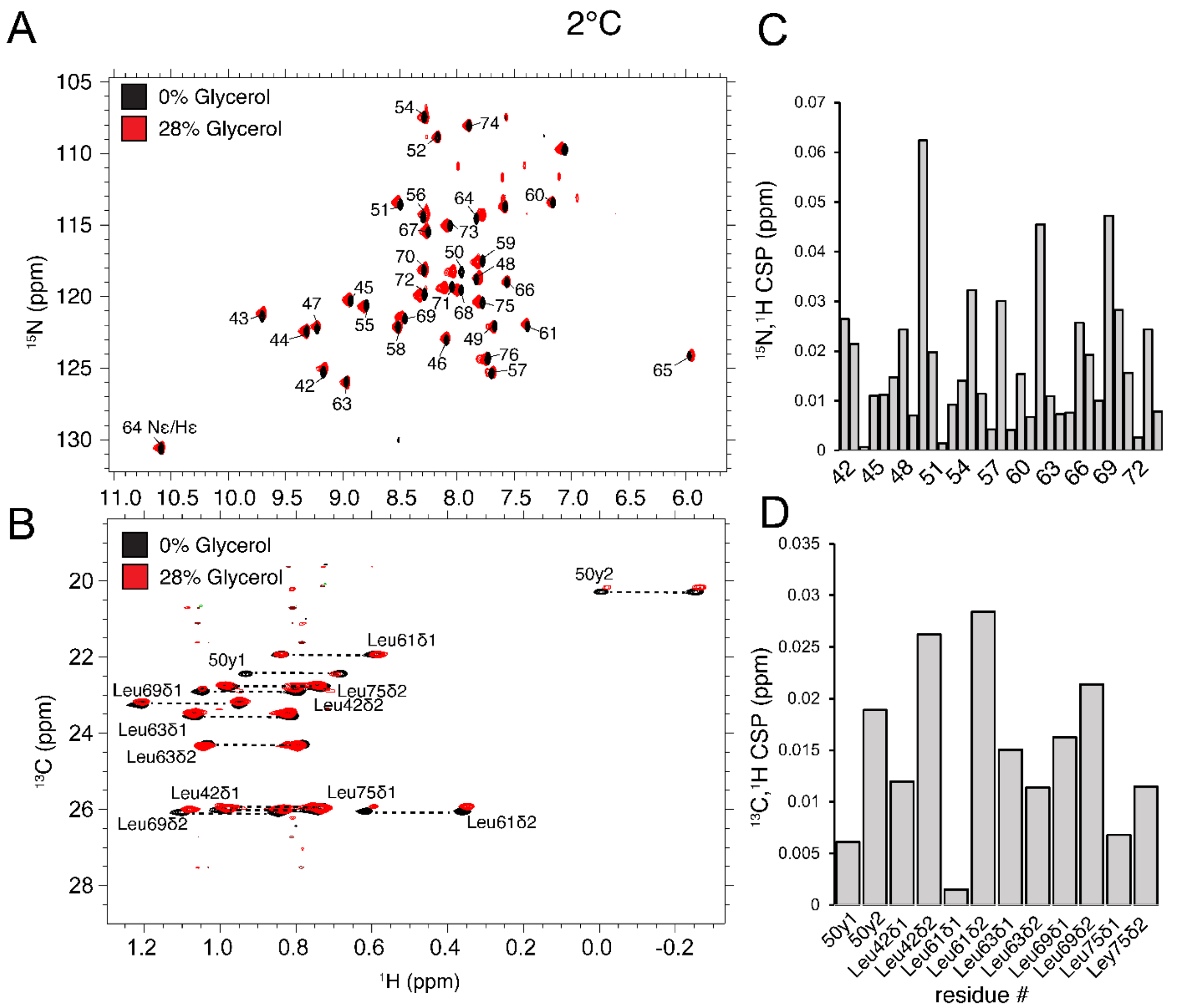


Figure S2. Additional backbone (A) and methyl (B) correlation spectra and corresponding differences in CSPs (C, D) in the presence and absence of glycerol, equivalent to Figure 4 of the main text for four additional temperatures, listed directly above the panels.

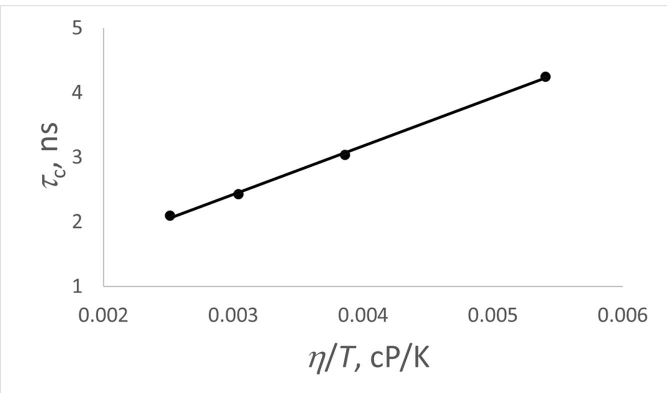


Figure S3. τ_c versus η/T for different glycerol content in the 0 to 28% *w/w* range at 32 ºC for the homogeneously ^{15}N-labeled and perdeuterated HP36 protein. The line represents a linear fit to the data.

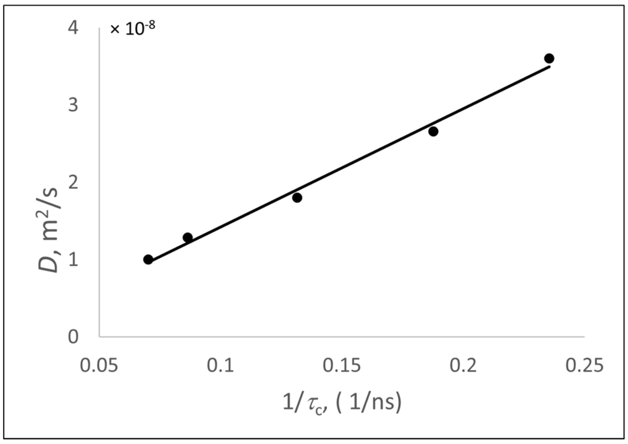


Figure S4. Inverse tumbling time τ_c versus diffusion coefficient D from DOSY measurements for the for the homogeneously ^{15}N-labeled and perdeuterated HP36 protein at 28 % *w/w* content obtained in 2 to 32 ºC temperature range. The line represents linear fit to the data.

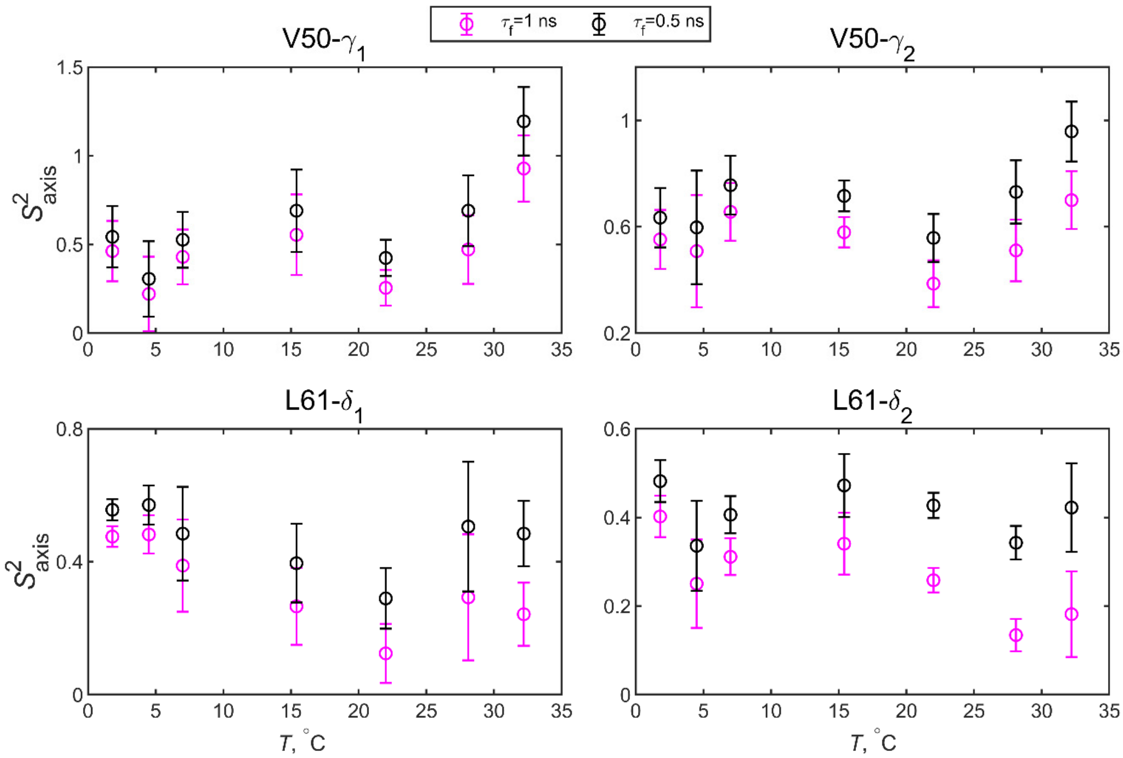


Figure S5. Methyl axis order parameters S^2_{axis} versus temperature for perdeuterated/LV protonated HP36 protein for V50 and L61 sites, obtained from the H_i-H_j/H_i-C dipole-dipole cross-correlated relaxation rates and fitted according to the spectral density of Eq. (6) of the main text, while fixing τ_f at either 1 ns (magenta) or 0.5 ns (black).

Table S1. Diffusion coefficients D from DOSY measurements at 15, 22, and 32 ºC for the perdeuterated LV protein and ^{15}N-labeled proteins in the presence of 28% *w/w* glycerol. The analysis was performed for the three residual proton peaks of glycerol-d8 (see Figure S1) and the reported value represents the average between the three peaks. The average ratio of diffusion coefficients between the two samples for the three temperatures is 1.0, confirming very similar glycerol concentrations.

T, ºC	15		22		32	
	D	error	*D*	error	*D*	error
D, LV protein (m^2/s)	$1.82 \cdot 10^{-8}$	$3.1 \cdot 10^{-11}$	$2.59 \cdot 10^{-8}$	$1.4 \cdot 10^{-10}$	$3.59 \cdot 10^{-8}$	$1.1 \cdot 10^{-10}$
D, ^{15}N-labeled protein (m^2/s)	$1.80 \cdot 10^{-8}$	$4.5 \cdot 10^{-11}$	$2.66 \cdot 10^{-8}$	$7.1 \cdot 10^{-11}$	$3.60 \cdot 10^{-8}$	$1.2 \cdot 10^{-10}$
RATIO (LV to ^{15}N-labeled)	1.0		0.97		1.0	

Table S2. ^{15}N R_1 and $R_{1\rho}$ values in the absence of glycerol and at 28% glycerol-d8 content, measured for the homogeneously ^{15}N-labeled and perdeuterated HP36 protein.

***R*₁ and *R*₁ρ at 32°C and 0% glycerol.**

Residue #	R_1 (s^{-1})	$R_{1\rho}$ (s^{-1})
42	1.738 ± 0.004	2.687 ± 0.027
43	2.197 ± 0.01	3.086 ± 0.009
44	2.359 ± 0.018	3.266 ± 0.014
45	2.371 ± 0.012	3.308 ± 0.012
46	2.381 ± 0.007	3.255 ± 0.012
47	2.464 ± 0.005	3.471 ± 0.01
48	2.443 ± 0.014	3.516 ± 0.012
49	2.418 ± 0.007	3.405 ± 0.012
50	2.303 ± 0.008	3.183 ± 0.012
51	2.431 ± 0.013	3.585 ± 0.011
52	2.481 ± 0.014	3.44 ± 0.012
54	2.332 ± 0.024	3.251 ± 0.01
55	2.473 ± 0.017	3.564 ± 0.014
56	2.478 ± 0.001	3.543 ± 0.013
57	2.515 ± 0.004	3.594 ± 0.013
58	2.549 ± 0.01	3.734 ± 0.016
59	2.547 ± 0.003	3.752 ± 0.013
60	2.412 ± 0.017	3.392 ± 0.014
61	2.482 ± 0.019	3.506 ± 0.013
63	2.386 ± 0.004	3.306 ± 0.012
64	2.326 ± 0.003	3.414 ± 0.013
65	2.456 ± 0.019	3.405 ± 0.008
66	2.409 ± 0.012	3.408 ± 0.01
67	2.449 ± 0.009	3.496 ± 0.014
68	2.414 ± 0.014	3.401 ± 0.014
69	2.496 ± 0.011	3.432 ± 0.011

70	2.423 ± 0.011	3.411 ± 0.012
71	2.452 ± 0.011	3.485 ± 0.012
72	2.359 ± 0.022	3.313 ± 0.012
73	2.424 ± 0.014	3.44 ± 0.014
74	2.291 ± 0.009	3.147 ± 0.011
76	1.554 ± 0.039	1.99 ± 0.005

R_1 and $R_{1\rho}$ at 32°C and 28% glycerol.

Residue #	R_1 (s^{-1})	$R_{1\rho}$ (s^{-1})
42	1.929 ± 0.019	4.333 ± 0.041
43	2.363 ± 0.007	5.648 ± 0.244
44	2.523 ± 0.012	5.76 ± 0.117
45	2.473 ± 0.011	5.487 ± 0.109
46	2.545 ± 0.009	5.342 ± 0.016
47	2.575 ± 0.009	5.71 ± 0.049
48	2.515 ± 0.011	5.778 ± 0.047
49	2.538 ± 0.013	6.037 ± 0.154
50	2.445 ± 0.009	5.295 ± 0.016
51	2.497 ± 0.009	5.697 ± 0.032
52	2.512 ± 0.01	5.549 ± 0.022
54	2.467 ± 0.009	5.524 ± 0.203
55	2.444 ± 0.008	5.878 ± 0.16
56	2.507 ± 0.006	5.778 ± 0.026
57	2.528 ± 0.01	5.933 ± 0.047
58	2.516 ± 0.023	6.396 ± 0.108
59	2.512 ± 0.009	6.126 ± 0.166
60	2.464 ± 0.009	5.568 ± 0.117
61	2.463 ± 0.011	6.313 ± 0.179
63	2.551 ± 0.011	5.382 ± 0.136
64	2.444 ± 0.008	5.453 ± 0.1
65	2.581 ± 0.017	5.628 ± 0.034
66	2.506 ± 0.011	5.609 ± 0.132
67	2.531 ± 0.011	5.762 ± 0.083
68	2.509 ± 0.01	6.133 ± 0.09
69	2.587 ± 0.009	5.838 ± 0.114
70	2.536 ± 0.011	5.705 ± 0.041
71	2.52 ± 0.037	5.896 ± 0.059
72	2.506 ± 0.008	5.801 ± 0.129
73	2.521 ± 0.008	5.939 ± 0.129
74	2.354 ± 0.009	5.103 ± 0.024
76	1.855 ± 0.004	3.136 ± 0.009

R_1 and $R_{1\rho}$ values measured at 22°C and 0% glycerol.

Residue #	R_1 (s^{-1})	$R_{1\rho}$ (s^{-1})
42	1.816 ± 0.014	2.804 ± 0.01
43	2.305 ± 0.061	3.452 ± 0.208
44	2.429 ± 0.079	3.598 ± 0.197
45	2.429 ± 0.063	4.135 ± 0.227

46	2.471 ± 0.094	3.616 ± 0.221
47	2.533 ± 0.087	4.292 ± 0.095
48	2.537 ± 0.084	4.352 ± 0.141
49	2.534 ± 0.09	4.224 ± 0.219
50	2.398 ± 0.081	3.915 ± 0.237
51	2.52 ± 0.111	3.892 ± 0.188
52	2.595 ± 0.165	4.018 ± 0.225
54	2.412 ± 0.05	4.214 ± 0.201
55	2.571 ± 0.047	4.593 ± 0.162
56	2.522 ± 0.154	4.348 ± 0.197
57	2.601 ± 0.174	4.467 ± 0.145
58	2.637 ± 0.089	4.13 ± 0.138
59	2.623 ± 0.239	4.545 ± 0.221
60	2.465 ± 0.07	4.343 ± 0.158
61	2.54 ± 0.093	4.457 ± 0.051
63	2.418 ± 0.044	4.11 ± 0.157
64	2.421 ± 0.087	4.26 ± 0.176
65	2.632 ± 0.071	3.772 ± 0.225
66	2.529 ± 0.171	4.287 ± 0.184
67	2.527 ± 0.131	4.376 ± 0.191
68	2.486 ± 0.066	4.342 ± 0.247
69	2.551 ± 0.088	4.325 ± 0.218
70	2.504 ± 0.112	4.263 ± 0.156
71	2.512 ± 0.051	3.869 ± 0.273
72	2.436 ± 0.064	4.178 ± 0.215
73	2.558 ± 0.049	4.468 ± 0.253
74	2.372 ± 0.113	3.497 ± 0.221
76	1.661 ± 0.016	2.169 ± 0.005

R_1 and $R_{1\rho}$ values measured at 22°C and 28% glycerol.

Residue #	R_1 (s^{-1})	$R_{1\rho}$ (s^{-1})
42	1.9 ± 0.009	4.939 ± 0.021
43	2.224 ± 0.006	6.777 ± 0.18
44	2.319 ± 0.007	7.016 ± 0.202
45	2.299 ± 0.008	6.547 ± 0.177
46	2.397 ± 0.01	6.498 ± 0.029
47	2.389 ± 0.009	6.991 ± 0.089
48	2.346 ± 0.011	6.911 ± 0.083
49	2.369 ± 0.01	6.892 ± 0.207
50	2.29 ± 0.01	6.332 ± 0.025
51	2.33 ± 0.01	6.799 ± 0.035
52	2.313 ± 0.014	6.6 ± 0.037
54	2.301 ± 0.008	6.588 ± 0.14
55	2.233 ± 0.007	7.517 ± 0.144
56	2.259 ± 0.011	6.885 ± 0.074
57	2.301 ± 0.008	7.032 ± 0.111
58	2.315 ± 0.01	7.574 ± 0.165
59	2.312 ± 0.01	7.379 ± 0.057

60	2.279 ± 0.01	6.698 ± 0.078
61	2.276 ± 0.007	7.321 ± 0.205
63	2.345 ± 0.006	6.93 ± 0.252
64	2.309 ± 0.009	6.592 ± 0.105
65	2.414 ± 0.021	6.669 ± 0.031
66	2.342 ± 0.01	6.817 ± 0.128
67	2.329 ± 0.008	6.883 ± 0.098
68	2.347 ± 0.008	6.739 ± 0.087
69	2.413 ± 0.013	6.836 ± 0.163
70	2.351 ± 0.011	6.916 ± 0.051
71	2.347 ± 0.007	7.02 ± 0.091
72	2.343 ± 0.008	7 ± 0.206
73	2.327 ± 0.009	7.011 ± 0.189
74	2.206 ± 0.011	6.403 ± 0.124
76	1.899 ± 0.005	3.79 ± 0.008

R_1 and $R_{1\rho}$ values at 15°C and 0% glycerol.

Residue #	R_1 (s^{-1})	$R_{1\rho}$ (s^{-1})
42	1.939 ± 0.004	3.744 ± 0.063
43	2.426 ± 0.024	4.599 ± 0.154
44	2.519 ± 0.065	4.763 ± 0.175
45	2.547 ± 0.056	4.901 ± 0.14
46	2.602 ± 0.146	4.83 ± 0.024
47	2.648 ± 0.007	5.051 ± 0.098
48	2.632 ± 0.064	5.14 ± 0.104
49	2.619 ± 0.094	5.363 ± 0.184
50	2.506 ± 0.007	4.76 ± 0.048
51	2.593 ± 0.043	5.043 ± 0.109
52	2.632 ± 0.133	4.827 ± 0.059
54	2.544 ± 0.017	5.035 ± 0.213
55	2.545 ± 0.01	5.413 ± 0.159
56	2.565 ± 0.061	5.356 ± 0.117
57	2.625 ± 0.159	5.114 ± 0.089
58	2.642 ± 0.068	5.566 ± 0.125
59	2.652 ± 0.137	5.455 ± 0.046
60	2.563 ± 0.01	4.967 ± 0.049
61	2.589 ± 0.006	5.459 ± 0.146
63	2.532 ± 0.008	4.689 ± 0.134
64	2.528 ± 0.068	4.975 ± 0.069
65	2.663 ± 0.009	4.696 ± 0.098
66	2.619 ± 0.092	5.233 ± 0.089
67	2.62 ± 0.006	5.219 ± 0.09
68	2.59 ± 0.05	4.983 ± 0.089
69	2.65 ± 0.074	5.248 ± 0.096
70	2.605 ± 0.052	5.128 ± 0.054
71	2.603 ± 0.078	5.003 ± 0.047
72	2.577 ± 0.006	4.874 ± 0.11
73	2.583 ± 0.074	5.036 ± 0.094
74	2.462 ± 0.005	4.671 ± 0.104

76	1.882 ± 0.037	3.067 ± 0.244

R_1 and $R_{1\rho}$ values measured at 15°C and 28% glycerol.

Residue #	R_1 (s^{-1})	$R_{1\rho}$ (s^{-1})
42	1.645 ± 0.007	6.513 ± 0.088
43	1.832 ± 0.007	8.371 ± 0.13
44	1.837 ± 0.007	8.669 ± 0.091
45	1.838 ± 0.007	8.771 ± 0.145
46	1.95 ± 0.009	8.618 ± 0.225
47	1.904 ± 0.008	8.543 ± 0.359
48	1.846 ± 0.006	9.341 ± 0.236
49	1.9 ± 0.009	8.927 ± 0.199
50	1.855 ± 0.012	8.542 ± 0.246
51	1.828 ± 0.008	9.568 ± 0.415
52	1.847 ± 0.009	8.825 ± 0.48
54	1.868 ± 0.006	8.075 ± 0.061
55	1.738 ± 0.008	9.609 ± 0.049
56	1.762 ± 0.01	9.274 ± 0.463
57	1.815 ± 0.006	9.309 ± 0.465
58	1.778 ± 0.006	9.349 ± 0.358
59	1.787 ± 0.007	9.263 ± 0.341
60	1.798 ± 0.008	8.565 ± 0.287
61	1.801 ± 0.009	9.459 ± 0.158
63	1.852 ± 0.006	8.691 ± 0.122
64	1.845 ± 0.01	8.783 ± 0.244
65	1.965 ± 0.019	9.822 ± 0.359
66	1.872 ± 0.007	9.215 ± 0.454
67	1.862 ± 0.009	9.263 ± 0.373
68	1.885 ± 0.007	8.63 ± 0.405
69	1.922 ± 0.007	8.365 ± 0.371
70	1.886 ± 0.008	9.149 ± 0.276
71	1.879 ± 0.01	8.913 ± 0.355
72	1.909 ± 0.007	8.534 ± 0.301
73	1.848 ± 0.009	9.129 ± 0.091
74	1.771 ± 0.011	8.282 ± 0.239
76	1.812 ± 0.005	5.275 ± 0.37

R_1 and $R_{1\rho}$ values measured at 7°C and 0% glycerol.

Residue #	R_1 (s^{-1})	$R_{1\rho}$ (s^{-1})
42	1.904 ± 0.034	5.065 ± 0.175
43	2.316 ± 0.038	6.425 ± 0.162
44	2.372 ± 0.025	5.969 ± 0.179
45	2.393 ± 0.029	6.258 ± 0.134
46	2.503 ± 0.08	6.006 ± 0.051
47	2.503 ± 0.007	6.482 ± 0.141
48	2.445 ± 0.053	6.893 ± 0.163
49	2.503 ± 0.024	6.507 ± 0.236
50	2.399 ± 0.005	5.947 ± 0.093
51	2.443 ± 0.055	6.285 ± 0.084

52	2.457 ± 0.008	6.091 ± 0.016
54	2.402 ± 0.031	5.736 ± 0.213
55	2.334 ± 0.043	6.852 ± 0.178
56	2.358 ± 0.009	6.643 ± 0.114
57	2.446 ± 0.054	6.374 ± 0.083
58	2.437 ± 0.006	7.027 ± 0.122
59	2.442 ± 0.033	6.695 ± 0.172
60	2.405 ± 0.034	6.157 ± 0.111
61	2.401 ± 0.008	6.542 ± 0.179
63	2.377 ± 0.033	5.804 ± 0.2
64	2.418 ± 0.085	6.519 ± 0.137
65	2.524 ± 0.009	6.283 ± 0.053
66	2.471 ± 0.056	6.333 ± 0.128
67	2.46 ± 0.08	6.458 ± 0.057
68	2.461 ± 0.008	6.503 ± 0.12
69	2.49 ± 0.01	6.305 ± 0.16
70	2.467 ± 0.005	6.35 ± 0.095
71	2.458 ± 0.006	6.359 ± 0.088
72	2.447 ± 0.04	6.442 ± 0.125
73	2.415 ± 0.009	6.632 ± 0.163
74	2.331 ± 0.005	6.088 ± 0.133
76	1.989 ± 0.08	3.897 ± 0.011

R_1 and $R_{1\rho}$ values measured at 7°C and 28% glycerol.

Residue #	R_1 (s^{-1})	$R_{1\rho}$ (s^{-1})
42	1.381 ± 0.007	9.469 ± 0.147
43	1.413 ± 0.008	12.178 ± 0.478
44	1.384 ± 0.008	11.482 ± 0.843
45	1.386 ± 0.007	12.058 ± 1.037
46	1.474 ± 0.01	12.876 ± 0.284
47	1.435 ± 0.01	14.264 ± 0.467
48	1.383 ± 0.009	15.116 ± 0.513
49	1.442 ± 0.008	12.027 ± 0.973
50	1.393 ± 0.01	12.839 ± 0.406
51	1.367 ± 0.009	12.855 ± 0.333
52	1.374 ± 0.009	12.626 ± 0.107
54	1.405 ± 0.007	11.288 ± 0.362
55	1.296 ± 0.006	12.474 ± 0.818
56	1.305 ± 0.009	14.533 ± 0.536
57	1.352 ± 0.009	13.519 ± 0.395
58	1.334 ± 0.008	13.672 ± 0.153
59	1.319 ± 0.01	15.45 ± 0.421
60	1.329 ± 0.01	13.744 ± 0.315
61	1.344 ± 0.009	14.844 ± 0.473
63	1.39 ± 0.006	12.138 ± 0.334
64	1.394 ± 0.007	13.656 ± 0.462
65	1.476 ± 0.018	14.09 ± 0.478
66	1.404 ± 0.011	13.031 ± 0.28
67	1.387 ± 0.008	14.268 ± 0.41

68	1.405 ± 0.01	14.427 ± 0.348
69	1.403 ± 0.016	13.41 ± 0.159
70	1.412 ± 0.012	13.243 ± 0.332
71	1.411 ± 0.01	14.344 ± 0.354
72	1.45 ± 0.011	14.041 ± 0.459
73	1.379 ± 0.01	14.799 ± 0.679
74	1.346 ± 0.01	13.03 ± 0.402
76	1.616 ± 0.005	8.291 ± 0.14

R_1 and $R_{1\rho}$ values measured at 2°C and 0% glycerol.

Residue #	R_1 (s^{-1})	$R_{1\rho}$ (s^{-1})
42	1.813 ± 0.003	5.781 ± 0.23
43	2.153 ± 0.021	7.364 ± 0.163
44	2.204 ± 0.016	7.429 ± 0.151
45	2.22 ± 0.006	7.475 ± 0.153
46	2.327 ± 0.007	6.939 ± 0.052
47	2.302 ± 0.005	7.437 ± 0.055
48	2.258 ± 0.005	7.876 ± 0.171
49	2.318 ± 0.046	7.729 ± 0.131
50	2.215 ± 0.006	6.932 ± 0.048
51	2.228 ± 0.006	7.168 ± 0.133
52	2.247 ± 0.007	7.104 ± 0.034
54	2.23 ± 0.025	6.475 ± 0.201
55	2.139 ± 0.026	8.049 ± 0.225
56	2.16 ± 0.051	7.376 ± 0.077
57	2.231 ± 0.011	7.628 ± 0.048
58	2.206 ± 0.063	7.926 ± 0.128
59	2.214 ± 0.052	8.201 ± 0.189
60	2.19 ± 0.005	7.102 ± 0.093
61	2.208 ± 0.054	7.776 ± 0.162
63	2.201 ± 0.032	7.281 ± 0.121
64	2.227 ± 0.004	7.331 ± 0.07
65	2.323 ± 0.01	7.286 ± 0.036
66	2.277 ± 0.006	7.285 ± 0.042
67	2.253 ± 0.007	7.489 ± 0.132
68	2.271 ± 0.073	7.494 ± 0.139
69	2.318 ± 0.051	7.467 ± 0.083
70	2.271 ± 0.007	7.341 ± 0.029
71	2.254 ± 0.007	7.277 ± 0.09
72	2.285 ± 0.006	7.272 ± 0.102
73	2.235 ± 0.006	7.587 ± 0.147
74	2.153 ± 0.006	6.879 ± 0.073
76	1.93 ± 0.087	4.305 ± 0.232

R_1 and $R_{1\rho}$ values measured at 2°C and 28% glycerol.

Residue #	R_1 (s^{-1})	$R_{1\rho}$ (s^{-1})
42	1.238 ± 0.008	12.689 ± 0.041
43	1.162 ± 0.009	14.999 ± 0.075
44	1.13 ± 0.008	15.889 ± 0.062

45	1.133 ± 0.012	16.225 ± 0.081
46	1.2 ± 0.011	15.656 ± 0.115
47	1.165 ± 0.01	16.837 ± 0.099
48	1.126 ± 0.008	17.051 ± 0.109
49	1.176 ± 0.012	16.144 ± 0.081
50	1.127 ± 0.009	15.722 ± 0.088
51	1.108 ± 0.009	16.309 ± 0.137
52	1.118 ± 0.011	15.934 ± 0.176
54	1.141 ± 0.01	13.984 ± 0.064
55	1.051 ± 0.005	17.007 ± 0.08
56	1.055 ± 0.008	18.777 ± 0.153
57	1.093 ± 0.011	17.336 ± 0.123
58	1.073 ± 0.011	7.245 ± 0.156
59	1.071 ± 0.009	19.436 ± 0.129
60	1.071 ± 0.012	16.166 ± 0.125
61	1.083 ± 0.011	17.23 ± 0.138
63	1.121 ± 0.006	15.166 ± 0.063
64	1.142 ± 0.011	15.883 ± 0.085
65	1.192 ± 0.017	16.322 ± 0.097
66	1.138 ± 0.015	16.636 ± 0.096
67	1.124 ± 0.01	16.921 ± 0.125
68	1.127 ± 0.012	18.572 ± 0.113
69	1.167 ± 0.01	6.902 ± 0.207
70	1.14 ± 0.007	16.826 ± 0.13
71	1.136 ± 0.013	17.061 ± 0.137
72	1.186 ± 0.009	16.276 ± 0.112
73	1.107 ± 0.007	16.541 ± 0.116
74	1.112 ± 0.01	15.122 ± 0.098
76	1.458 ± 0.006	10.808 ± 0.03

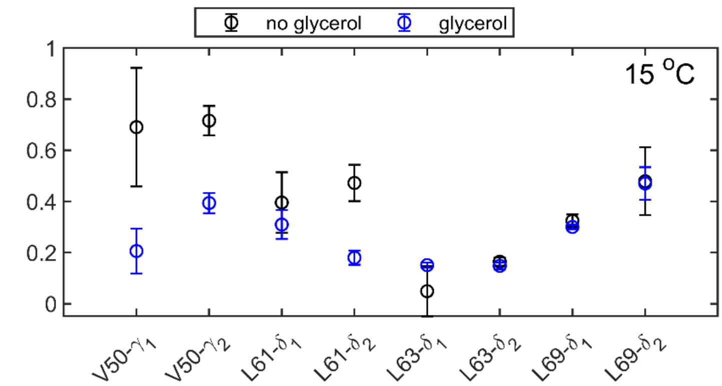


Figure S6. Comparison of methyl axis order parameters S^2_{axis} in the perdeuterated/LV protonated sample at 15 ºC ºC in absence of glycerol-d8 (black circles) and in the presence of 28% *w/w* glycerol (blue circles) versus site identity. The fits were done according to Eq. (4) with τ_f fixed at 500 ps. L75-δ_2 and L42-δ_2 sites are not included due to spectral overlap in the presence of glycerol at this temperature. In similarity to the results at 22ºC, differences are observed for the V50 and L61 residues, for which the better choice of spectral densities is Eq. (6) representing a direct coupling between overall and internal motions.