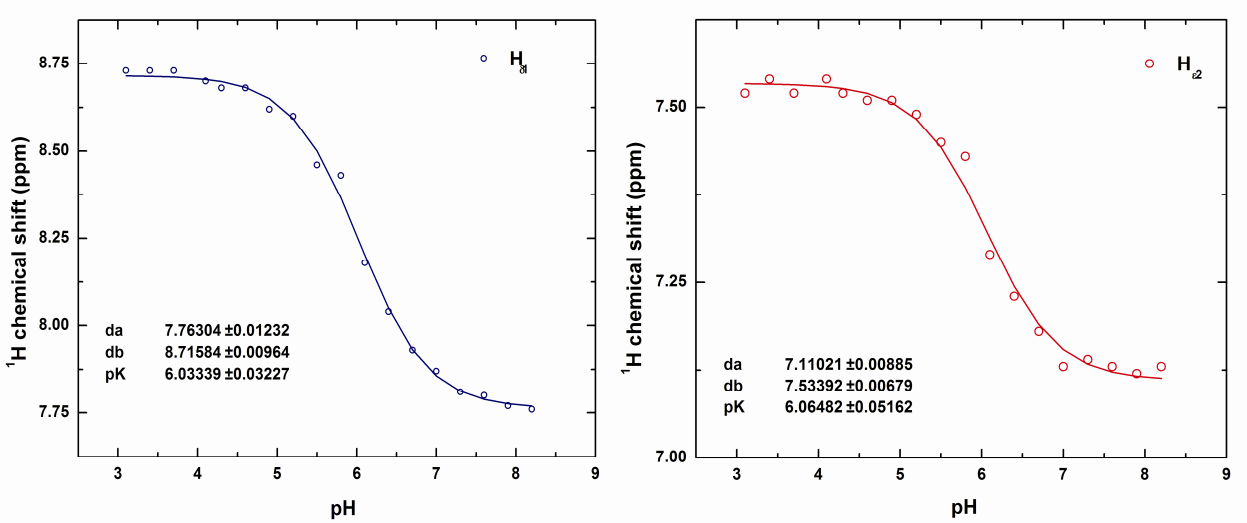
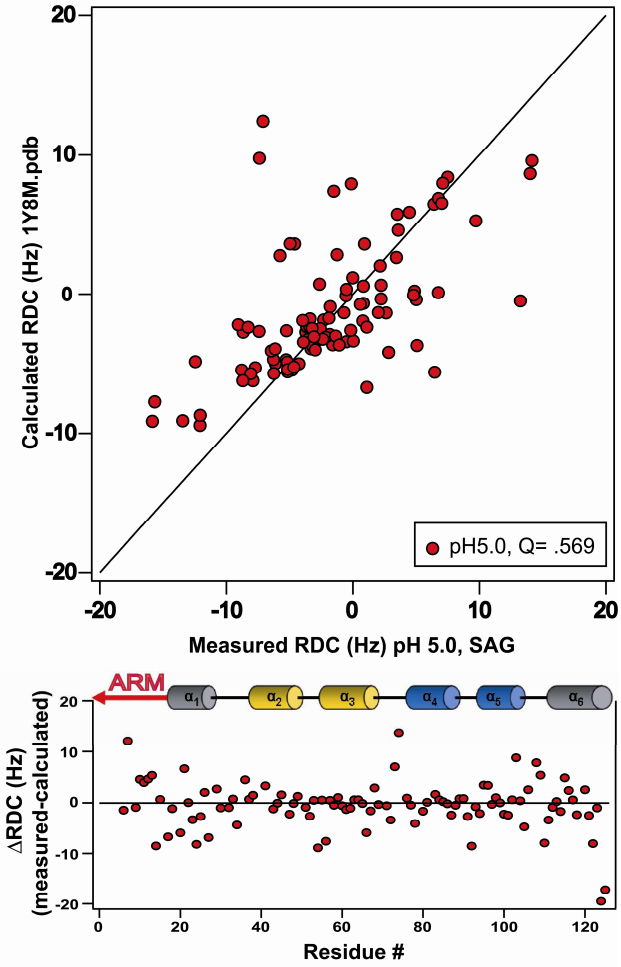
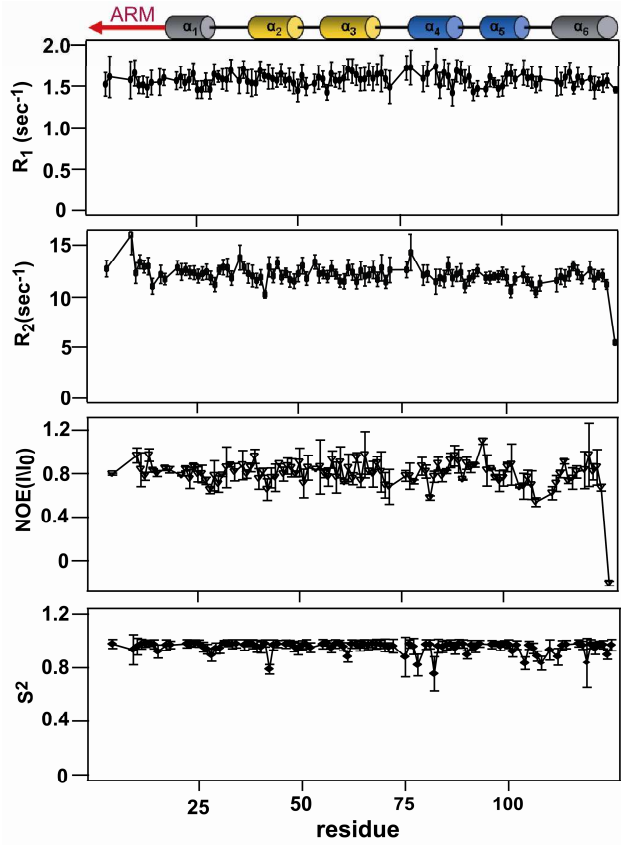


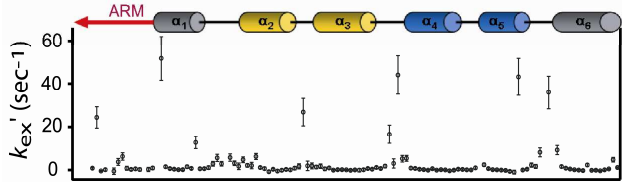
Supplemental Figure 1. pK_a estimation of His106 of yFis1ΔTM. Given that the pI of yFis1ΔTM is estimated to be around 7.7, we reasoned that the chemical shift changes between pH 7.4 and 5.0 might arise from the sole His residue, His106. To measure this pKa value, we collected a series of 1D Long-range HMQC experiments at 500 MHz on a 0.5 mM sample of ^{15}N-labelled yFis1ΔTM, without buffer, 100 mM NaCl as a function of pH. The data were fit to the $\delta = (\delta_{his-} \times 10^{-pH} + \delta_{hisH} \times 10^{-pKa}) / (10^{-pKa} + 10^{-pH})$ to estimate the value of pK_a = 6.04 ± 0.06 for the sole histidine in this protein, His106.



Supplemental Figure 2. Residual dipolar coupling analysis of Fis1ΔTM at 14.2 T, 25 °C, pH 5.0 We first determined an alignment tensor using *FitAlign* for a subset of our RDC data that corresponding to those residues that showed no pH dependence to their chemical shifts and lie in the core of the structure (helices 2-5). From this alignment tensor, we used the program *euler_pdb* to rotate the 1Y8M.pdb coordinates into this frame, and then calculated the predicted RDC values from this aligned structure using the program *calc_rdip2*. Differences between the structure and RDC values measured at pH 5.0 appear to be smaller and more random than those seen at pH 7.4 (Fig 3). We thank Prof. Joel Tolman for providing software and guidance for this analysis.

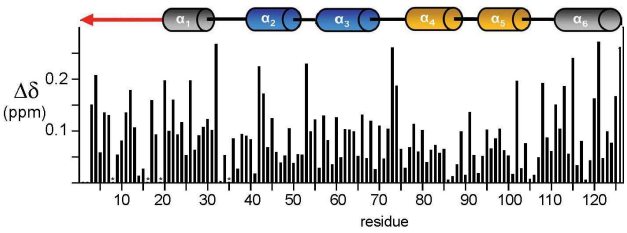


Supplemental Figure 3. NMR spin relaxation data of Fis1ΔTM at 14.2 T, 25 °C, pH 7.4 as a function of residue number. The spin-lattice relaxation rate constant R_1 (top panel), the spin-spin relaxation rate constant R_2 (second panel), the steady-state heteronuclear NOE (third panel) were analyzed using the FastModelfree approach (49, 50, 59, 83) to give a squared generalized order parameter S^2 (bottom panel). From this analysis the rotational correlation time of Fis1ΔTM is τ_m = 8.25 ± 0.32 ns.

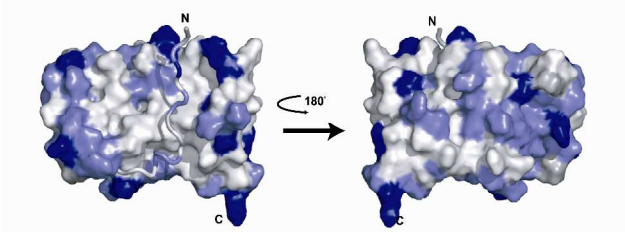


Supplemental Figure 4. NMR saturation transfer data of Fis1ΔTM at 14.2 T, 25 °C, pH 7.4 as a function of residue number. The pH-corrected rate constants for exchange of the amide backbone with solvent protons, k'_{ex}, are derived from identical experiments recorded for an ^{15}N-labeled sample of Fis1ΔTM at pH 7.4.

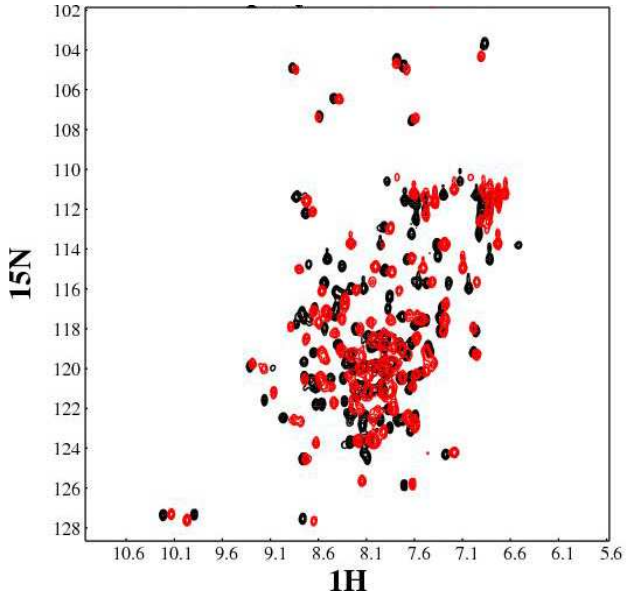
a)



b)



Supplemental Figure 5. NMR chemical shifts differ at 40 °C from 25 °C at pH 7.4 for a subset of residues. (a) The observed change in chemical shift, Δδ, between 25 °C and 40 °C is plotted as a function of residue number where $\Delta\delta = [(^{1H}\delta_{25C} - {}^{1H}\delta_{40C})^2 + \{¼ (^{15N}\delta_{25C} - {}^{15N}\delta_{40C})\}^2]^{½}$. (b) mapping of the observed chemical shift changes onto a surface representation of the pH 5.5 structure of Fis1ΔTM (1y8m.pdb (29)). This figure was made with the software PYMOL (81) using a color gradient (82) to display average chemical shift differences from 0 to 0.27 ppm (white to dark blue, respectively). The residues for which no chemical shift data is available are colored light gray. The chemical shift differences ranged from 0 to 0.27 ppm with an average value of 0.094 ± 0.063 ppm. Only 24% of the residues displayed a temperature-induced difference greater than two standard deviations (> 0.125ppm), which can arise from conformational changes as well as differences in hydrogen bond lengths.



Supplemental Figure 6. I85C-Fis1ΔTM remains well-folded at 40 ºC. ^{1}H-^{15}N HSQC spectra of a 0.32 mM sample of I85C-Fis1ΔTM at pH 7.4, 25 ºC (black) and 40 ºC (red). NMR experiments were performed at 14.2 T on a Bruker Avance600 spectrometer outfitted with a TXI cryoprobe at 298 K. Two-dimensional ^{1}H–^{15}N heteronuclear single quantum correlation (HSQC) experiments were collected using WATERGATE for solvent suppression (39). Uniformly ^{15}N-labeled Fis1ΔTM samples were prepared by extensive buffer exchange into buffer containing 100mM phosphate pH 7.4, 50 mM NaCl, 5 mM DTT and 10% $^{2}H_2O$. HSQC spectra were collected at 20 scans per increment, 1278 (t_2) x 160 (t_1) complex points with acquisition times of 64 ms (^{1}H) and 48 ms (^{15}N). The carrier frequencies were centered on the chemical shift of water in ^{1}H and in the center of the amide region at 115.5 ppm in ^{15}N. NMR data processing was carried out using NMRPipe (40) and subsequently analyzed with NMRView (41, 42).

Supplemental Table 1. RDC values of yFis1ΔTM collected at pH 5.0 and 7.4 at 25 °C.

res_5	rdc 5	stdev 5	res_74	rdc 74	stdev 74	ΔRDC	stdev Δ
6	4.458477	0.762255	6	-0.40486	0.264667	4.863335	0.806896
7	6.459048	0.581251	7	5.944305	2.038269	0.514743	2.119527
10	14.15423	1.593188	10	13.4166	5.287409	0.737626	5.522222
11	2.625907	0.30688	11	3.346429	1.572962	-0.72052	1.602618
12	4.86765	0.569649	12	3.050659	1.189363	1.816991	1.318744
13	14.01414	0.928236	13	11.58219	1.971645	2.431949	2.179221
14	-5.76902	2.376327	14	-3.65255	2.290168	-2.11647	3.300273
15	-4.80776	0.565979	15	-7.60573	0.686266	2.79797	0.889546
17	-15.8429	2.728571	17	-18.1477	1.56268	2.304847	3.14437
18	-6.12548	0.36114	18	-6.01307	0.680523	-0.1124	0.770411
21	6.75415	1.127891	21	7.903476	1.873407	-1.14933	2.186731
22	-2.7914	1.509526	22	-4.49284	3.806867	1.70144	4.09523
24	-4.60554	0.230676	24	-6.75296	0.432012	2.147418	0.489741
25	0.909263	1.250617	25	1.181406	0.930086	-0.27214	1.558558
26	-1.60133	1.368395	26	-7.85388	1.029515	6.252546	1.712427
27	-9.0515	0.882762	27	-13.4495	1.279936	4.397961	1.554833
29	0.786148	0.46986	29	-0.99564	0.325194	1.781784	0.571419
30	-3.71979	1.909712	30	-8.37802	1.03212	4.658227	2.170776
32	7.484953	1.183106	32	10.62687	1.132956	-3.14192	1.638088
33	-4.27193	0.202984	33	-7.46652	2.32605	3.194585	2.33489
34	-13.4474	0.476773	34	-18.2014	0.764299	4.754004	0.900814
36	9.714617	0.40131	36	10.80716	0.472383	-1.09255	0.619835
37	-2.34542	0.635892	37	-2.90049	0.551782	0.555073	0.841916
38	0.822983	0.79899	38	0.732426	0.600828	0.090557	0.99969
41	2.00778	1.104364	41	2.502481	0.422791	-0.4947	1.182528
43	-0.01578	1.531013	43	0.721928	1.19504	-0.73771	1.942195
44	6.404148	0.690686	44	8.187683	0.671395	-1.78354	0.963233
45	2.220012	1.872339	45	5.32827	1.366053	-3.10826	2.317705
47	3.503798	0.784064	47	-8.13991	1.095703	11.64371	1.347338
48	6.765197	0.520685	48	8.658724	0.577502	-1.89353	0.777574
51	7.09959	0.635216	51	11.18053	1.326583	-4.08094	1.470824
54	-1.5145	2.901927	54	6.269439	1.861067	-7.78394	3.447427
55	-2.5026	0.440996	55	-3.46252	0.779661	0.959917	0.895739
56	-12.4499	1.764849	56	-10.233	0.855032	-2.21694	1.961064
57	-3.02675	0.601186	57	-4.56618	0.955584	1.539426	1.128966
58	-2.31104	1.107667	58	-14.958	1.563706	12.64694	1.916273
60	-6.25068	0.562976	60	-10.3457	0.609416	4.095031	0.829657
61	-3.65611	0.603265	61	-6.55136	0.301814	2.895256	0.674552
62	-3.40231	1.239078	62	-3.56765	1.858483	0.165336	2.233668
63	-2.44051	0.39847	63	-6.28876	0.963329	3.84825	1.042488
64	-3.30897	2.11171	64	-8.7494	1.785947	5.440431	2.76567
65	-2.57741	3.562939	65	-5.77269	0.650307	3.19528	3.6218
66	-8.28902	2.902417	66	-4.21594	1.069198	-4.07308	3.09309
67	-6.27564	0.837893	67	-7.56282	1.011062	1.287179	1.313131
68	-0.50074	2.503956	68	-8.25356	0.473855	7.752822	2.548399
69	-3.57233	1.028406	69	-4.56633	1.364208	0.993996	1.708415
71	-5.27621	0.695455	71	-9.77836	0.397686	4.502145	0.801132
72	-2.63291	4.208909	72	1.17532	0.9256	-3.80823	4.309484
76	-2.15295	1.289336	76	-7.91534	1.237288	5.762389	1.786972

77	-0.54694	1.630773		77	0.722232	1.548615	-1.26917	2.248917
78	-1.24999	1.181898		78	-1.06167	1.020151	-0.18832	1.561279
80	-3.39564	1.122623		80	-5.90794	1.966983	2.512307	2.264797
81	2.154125	1.150178		81	4.480214	2.277488	-2.32609	2.551443
83	-1.36894	1.189538		83	-5.43006	2.856647	4.061112	3.09442
84	-3.16337	1.408634		84	-6.52961	0.704916	3.366233	1.575168
85	0.829522	0.843101		85	1.613346	1.30536	-0.78382	1.553957
86	-1.8958	0.728788		86	-1.61	0.650911	-0.28581	0.977147
88	-3.911	0.721962		88	-4.52768	1.444976	0.61668	1.615297
89	3.438684	0.90158		89	4.842775	0.492349	-1.40409	1.027256
90	-2.38103	1.27184		90	-4.89861	1.555946	2.517582	2.009614
91	-12.0851	0.399388		91	-12.5279	0.817818	0.442807	0.91013
92	-4.95759	1.518606		92	-0.28116	2.108543	-4.67643	2.598484
93	-3.22347	0.554462		93	-3.61405	0.570734	0.39058	0.795717
94	-3.97184	0.258556		94	-3.59306	5.462112	-0.37879	5.468228
95	1.103178	1.410363		95	-4.79744	0.973521	5.900614	1.713729
96	0.034833	3.50119		96	-4.68361	1.171956	4.718439	3.692128
97	-5.18218	0.789589		97	-5.86945	0.068374	0.687269	0.792544
98	-2.98443	0.35776		98	-4.0323	0.77165	1.04787	0.85055
99	-3.08333	0.927677		99	-6.89536	0.690337	3.812034	1.156352
100	-6.14553	1.591145		100	-4.52616	1.076837	-1.61937	1.921281
101	-7.7208	0.833909		101	-8.32553	0.62511	0.604727	1.042193
102	-0.72008	1.253782		102	-1.44355	0.404509	0.723475	1.317421
103	5.069381	1.428545		103	-4.94973	0.735823	10.01911	1.606915
104	-5.17079	0.784464		104	-7.00065	0.404938	1.829863	0.882813
105	-7.41113	2.200693		105	-4.82406	0.450741	-2.58707	2.246379
106	2.2195	0.382034		106	-1.76153	0.929566	3.981035	1.005009
108	1.101512	2.172275		108	-9.19397	0.781145	10.29548	2.308455
110	-15.6611	1.043727		110	-14.6549	1.586668	-1.00623	1.899179
112	-0.51344	0.498329		112	0.245562	1.527971	-0.75901	1.607179
113	-5.16014	1.201291		113	-7.38938	2.053296	2.229233	2.378891
114	-7.90279	0.463832		114	-6.93294	0.650915	-0.96985	0.799269
115	4.793329	3.042462		115	1.446443	2.101695	3.346887	3.697796
116	-0.17343	1.156065		116	1.890554	2.351499	-2.06398	2.620311
117	-4.66229	0.795135		117	-5.92392	0.635882	1.261627	1.018128
118	-8.06972	1.457864		118	-7.84733	0.448496	-0.22238	1.525292
120	-1.09119	1.120302		120	-4.59721	0.273077	3.506021	1.153103
121	-8.70873	1.348314		121	-7.99385	1.522983	-0.71487	2.034067
122	-0.12624	1.203558		122	0.044731	1.044636	-0.17097	1.59368
123	3.586862	5.000746		123	4.6885	0.478755	-1.10164	5.023611
124	-7.10305	0.876055		124	-4.99279	0.624498	-2.11026	1.075858
125	-7.39253	0.712506		125	-3.95455	1.83718	-3.43797	1.970507