Supporting information for ‘Reengineering rate-limiting, millisecond enzyme motions by introduction of a unnatural amino acid’ by Watt, Rivalta, Whittier, Batista, and Loria

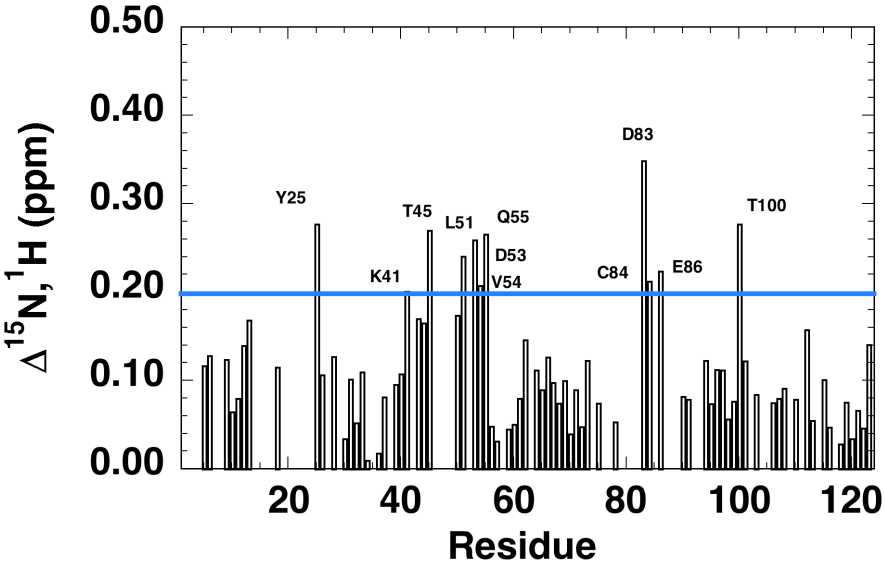
Table S1. Data for both sets was acquired at 298 K and 14.1T. R_2 values were determined in a relaxation compensated CPMG with τ_{cp} = 0.3125 ms. Red residues indicate proline residues, blank spaces are unassigned or overlapped, and EB means exchange broadened.

Residue	WT pH = 6.4	error	WT pH = 7.0	error	H48C-4MI pH = 6.4	error	H48C-4MI pH = 7.0	error
3	15.14	0.11	15.85	0.06				
4	20.88	0.17	16.93	0.07				
5	14.90	0.10	16.06	0.05	14.31	1.12	16.25	0.51
6	14.95	0.09	15.13	0.05	13.11	0.99	16.11	0.46
7	15.87	0.09	16.28	0.04	12.60	1.58	17.48	0.67
8	15.76	0.11	16.13	0.06	14.57	1.04	16.87	0.53
9	15.36	0.11	15.70	0.04	14.38	1.28	17.23	0.55
10	15.80	0.12	16.22	0.06	12.64	1.51	17.22	0.64
11	15.87	0.15	15.88	0.07	19.60	3.00	17.14	1.03
12	14.79	0.15	14.60	0.07	EB		16.13	1.18
13	14.67	0.13	14.51	0.07	EB		14.53	1.21
14	15.04	0.15	15.19	0.07	EB		15.73	3.08
15	17.27	0.25	19.77	0.12	EB		13.85	2.74
16	15.11	0.15	17.50	0.08	EB		12.35	2.26
17	22.04	0.42	24.10	0.17	EB		15.81	2.15
18	15.14	0.11	15.85	0.06	EB		14.22	2.74
19	14.95	0.11	19.67	0.09	EB		17.62	3.08
20	EB		EB		13.11	0.99	15.01	1.66
21	EB		EB		18.56	2.02	16.10	2.84
22	11.52	0.17	17.01	0.18				
25	16.78	0.12	17.06	0.07	EB		18.81	3.93
26	15.34	0.18	16.21	0.08	EB		14.27	2.74
27	15.31	0.14	15.70	0.04	EB		16.16	1.73
28	14.54	0.11	15.51	0.06	13.32	3.25	16.28	1.18
29	14.80	0.08	15.05	0.03	14.99	1.03	15.21	0.43
30	15.95	0.16	15.86	0.08	13.05	3.38	17.26	1.36
31	15.18	0.13	15.05	0.07	10.91	2.72	15.88	1.61
32	14.55	0.12	14.85	0.06	14.18	2.31	15.92	1.03
33	15.80	0.16	15.85	0.08	14.80	3.15	16.94	1.22
34	14.70	0.12	14.98	0.06	18.56	2.02	15.47	0.68
35	15.53	0.11	15.85	0.06	15.92	1.32	17.86	0.55
36	15.69	0.16	18.63	0.09	12.87	2.22	17.96	0.96
37	16.15	0.11	19.08	0.08	13.05	1.93	18.22	0.79
39	13.34	0.09	13.87	0.05	14.17	1.26	13.68	0.46
40	14.40	0.11	16.63	0.07	11.17	1.31	16.69	0.57
41	14.85	0.13	15.39	0.07	17.38	3.07	15.27	1.20
42								
43	12.27	0.10	11.31	0.05	11.13	1.18	13.70	0.60
44	14.01	0.12	14.28	0.06	EB		15.84	0.51
45	14.30	0.17	14.98	0.08	EB		13.70	1.89

46	16.75	0.34	16.34	0.12	EB		EB	
47	20.85	1.16	17.21	0.20	EB		EB	
48	15.34	0.14	16.96	0.22	EB		EB	
49	11.53	0.11	11.80	0.05	EB		EB	
50	14.72	0.13	14.51	0.06	11.53	3.08	14.16	1.16
51	15.64	0.11	27.85	0.17	15.36	2.48	17.27	0.94
52	14.29	0.08	15.13	0.05	13.58	1.26	15.84	0.51
53	14.51	0.10	15.13	0.04	16.62	1.93	15.58	0.67
54	15.19	0.11	15.07	0.06	EB		15.39	1.02
55	15.44	0.13	15.06	0.07	13.35	1.83	16.98	0.78
56	14.18	0.11	14.32	0.05	16.48	1.24	15.50	0.56
57	15.59	0.11	15.69	0.06	15.54	1.27	16.15	0.55
58	9.89	0.11	9.96	0.05	EB		11.76	0.53
59	13.55	0.13	12.89	0.06	15.21	1.39	15.25	0.56
60	14.91	0.16	14.77	0.08	16.95	1.63	15.93	0.70
61	15.71	0.15	15.96	0.08	14.17	1.55	16.93	0.69
62	15.34	0.14	16.56	0.07	13.32	1.21	16.65	0.59
63	14.98	0.09	15.25	0.05	13.38	0.86	22.33	3.38
64	13.02	0.11	13.23	0.05	12.97	0.90	13.76	0.45
65	14.73	0.16	15.14	0.08	14.67	1.71	16.47	0.73
66	15.67	0.97	17.76	0.42	EB		EB	
67	13.66	0.16	16.08	0.09	13.42	1.55	15.95	0.75
68	13.49	0.12	14.55	0.06	13.99	1.05	15.37	0.47
69	15.31	0.14	15.70	0.04	14.83	1.39	16.56	0.59
70	14.77	0.16	18.94	0.10	15.04	1.51	17.76	0.69
71	15.78	0.27	16.47	0.12	14.85	2.48	16.53	1.00
72	12.94	0.14	13.25	0.07	12.87	1.17	14.46	0.54
73	15.70	0.20	16.36	0.07	EB		16.82	0.75
74	16.34	0.22	17.15	0.10	17.96	2.05	18.38	0.92
75	16.39	0.18	16.46	0.09	17.17	2.18	18.35	0.93
76	18.51	0.16	27.85	0.17	16.05	1.65	15.00	0.72
77	13.49	0.11	13.51	0.06	10.83	1.81	14.39	0.67
78	16.39	0.12	19.42	0.08	13.85	1.89	17.19	0.68
79	15.03	0.15	15.30	0.07	11.40	2.84	17.14	0.86
80	16.01	0.18	15.62	0.09	22.38	27.95	EB	
82	27.36	1.07	20.09	0.18	EB		19.01	2.44
83	19.33	0.43	16.71	0.12	EB		18.62	2.62
84	15.23	0.15	15.19	0.08	15.96	3.59	16.68	1.11
85	14.98	0.09	15.25	0.05	13.38	0.86	16.42	0.43
86	15.21	0.14	15.82	0.07	14.05	2.37	16.18	1.06
87	15.79	0.11	15.83	0.06				
90	14.62	0.09	15.28	0.05	13.00	1.10	15.23	0.44
91	13.16	0.08	14.14	0.05	12.56	1.00	14.80	0.41
92	31.33	0.63	55.45	8.41	EB		EB	
93								
94	12.53	0.09	13.38	0.05	10.64	1.07	13.82	0.46
95	13.71	0.07	15.25	0.04	10.55	0.88	15.49	0.41
96	15.26	0.14	15.03	0.07	11.09	2.02	15.65	0.82
97	14.73	0.15	14.54	0.07	11.64	2.28	16.65	1.31

98	15.65	0.16	15.95	0.08	21.71	3.67	14.46	1.49
99	17.70	0.16	21.04	0.11	15.96	4.52	19.69	2.62
100	15.40	0.15	EB		EB		17.85	1.86
101	22.23	0.50	20.10	0.14	EB		18.66	3.54
103	17.36	0.17	15.05	0.03	EB		17.05	1.18
104	14.57	0.18	13.91	0.08				
105	15.94	0.20			EB		EB	
106	16.73	0.19	17.04	0.09	11.40	3.08	18.04	1.10
107	15.92	0.16	16.46	0.09	15.70	1.49	17.71	0.70
108	16.62	0.21	16.95	0.10	12.41	1.87	17.80	0.87
109	15.87	0.09	16.28	0.04	12.51	3.08	17.14	0.86
110	14.21	0.16	14.80	0.08	13.29	1.55	16.03	0.68
111	14.80	0.08	15.05	0.03	EB		EB	
112	13.31	0.13	14.05	0.06	13.52	1.13	14.55	0.52
113	11.90	0.08	14.58	0.05	12.59	0.90	13.95	0.40
114								
115	15.40	0.10	15.00	0.06	13.24	0.94	15.39	0.48
116	14.53	0.13	15.00	0.06	14.02	1.30	15.67	0.60
117								
118	17.32	0.24	17.38	0.11	14.91	2.48	17.53	1.12
119	13.71	0.07	15.25	0.04	19.61	2.95	18.81	0.96
120	22.30	0.34	21.44	0.15	17.66	6.14	20.56	1.81
121	17.13	0.29	17.59	0.14	12.41	4.75	17.96	1.86
122	14.17	0.22	14.04	0.11	11.09	3.08	14.10	1.32
123	13.50	0.11	14.95	0.06	10.81	1.33	15.57	0.55
124	14.44	0.13	27.85	0.17	16.05	1.65	29.74	1.76

Figure S1.



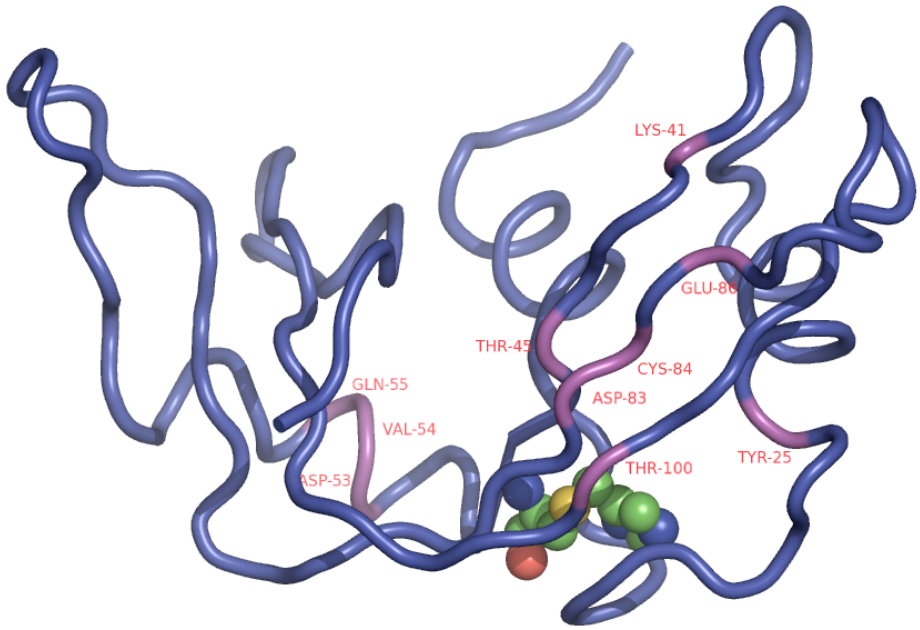


Figure S1. (top) Composite $\Delta^{15}N,^{1}H = \sqrt{\delta_N^2 / 25 + \delta_H^2}$ chemical shift changes for H48C-4MI. The blue line marks 1.5σ above the standard deviation of the mean. Amino acid residues above this line are indicated by their one-letter amino acid code and are (bottom) mapped onto the RNase A ribbon structure in magenta. Unassigned loop 1 residues are shown in gray.

Figure S2.

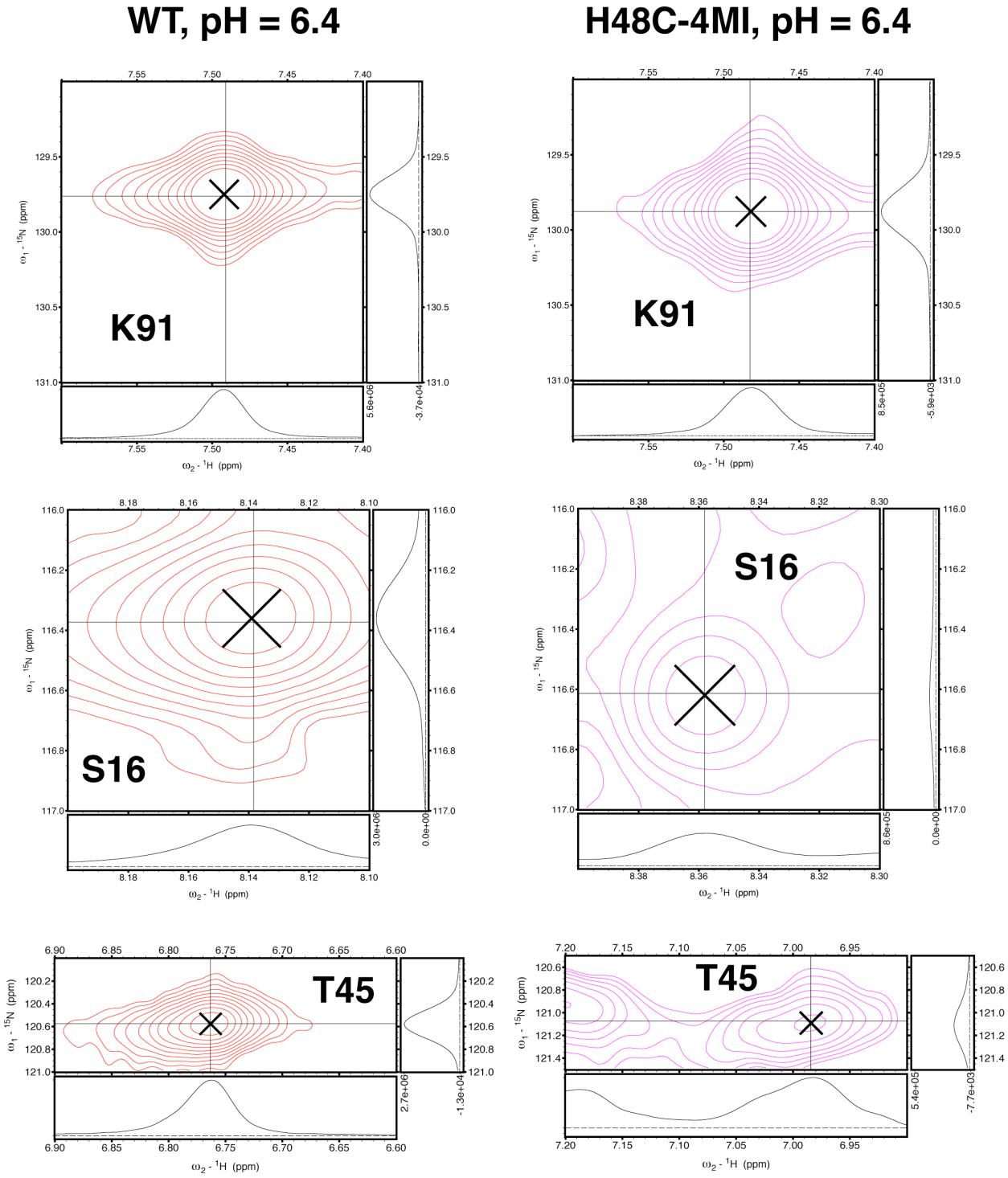


Figure S2. Contour plots for residues in (left) RNase A, pH = 6.4 and (right) H48C-4MI, pH = 6.4. K91 represents a residue in which the peak intensity is not affected in H48C-4MI relative to WT. S16 and T45 are residues in loop1 and beta-strand 1, respectively, in which significant exchange broadening occurs in the H48C-4MI enzyme. 1D slices with intensity ranges show the significant reduction in S/N for H48C-4MI residues. Data was identically acquired at 14.1 T and 298 K.

Figure S3.

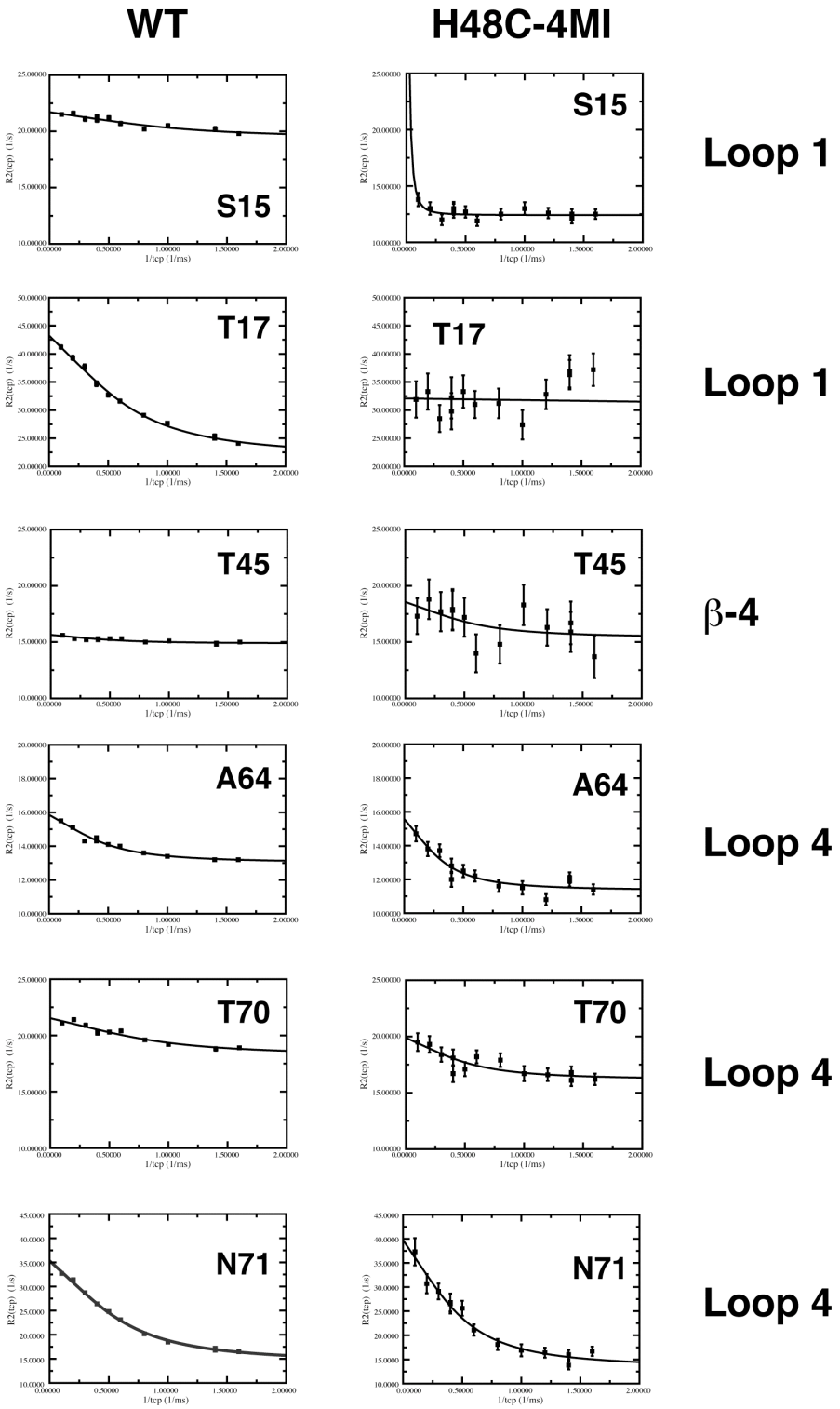


Figure S2. CPMG dispersion data for residues in loop1, b-4, and loop 4 for (left) WT and (right) H48C-4MI at pH = 7.0 and 298K.