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. All data were obtained on $^{1}H,^{15}N$-labeled RNase A.

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.

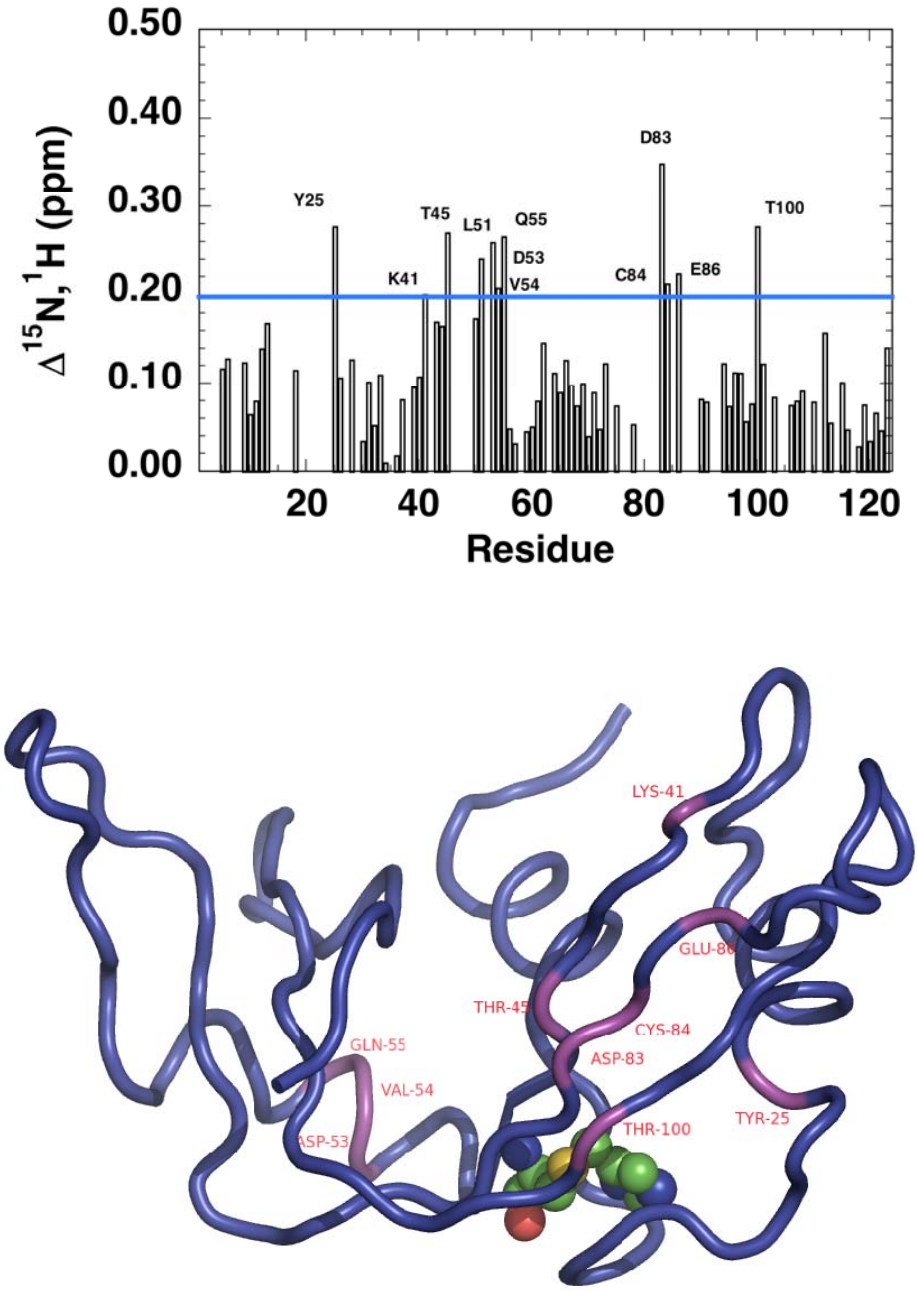

Figure S1. (top) Composite $\Delta^{15}N,^{1}H = \sqrt{\delta_N^2 / 25 + \delta_H^2}$ chemical shift changes for H48C-4MI. The blue line marks 1.5σ above the standard deviation of the mean. Amino acid residues above this line are indicated by their one-letter amino acid code and are (bottom) mapped onto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

Figure S2.

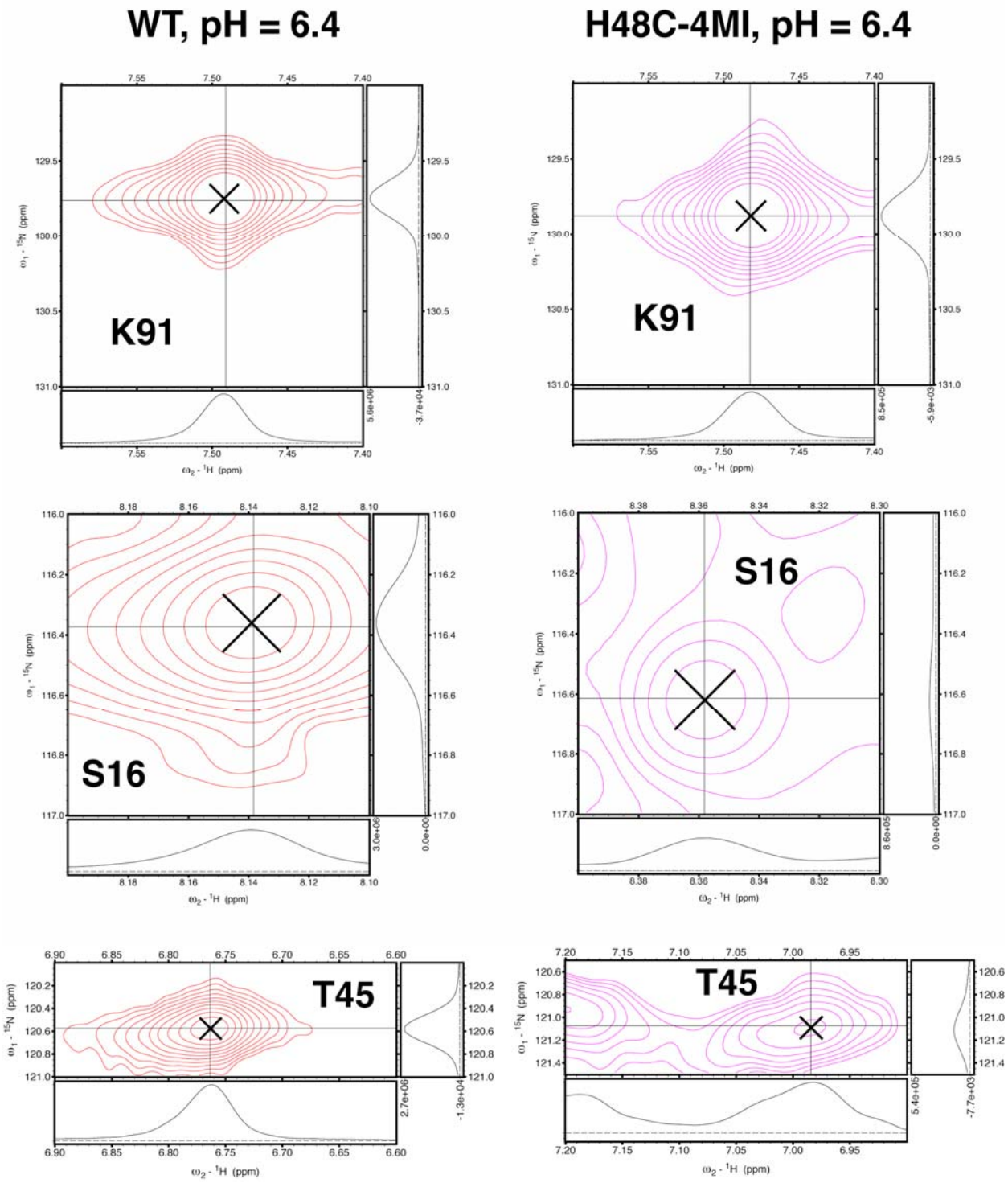

Figure S2. Contour plots for residues in (left) RNase A, pH = 6.4 and (right) H48C-4MI, pH = 6.4. K91 represents a residue in which the peak intensity is not affected in H48C-4MI relative to WT. S16 and T45 are residues in loop1 and beta-strand 1, respectively, in which significant exchange broadening 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.

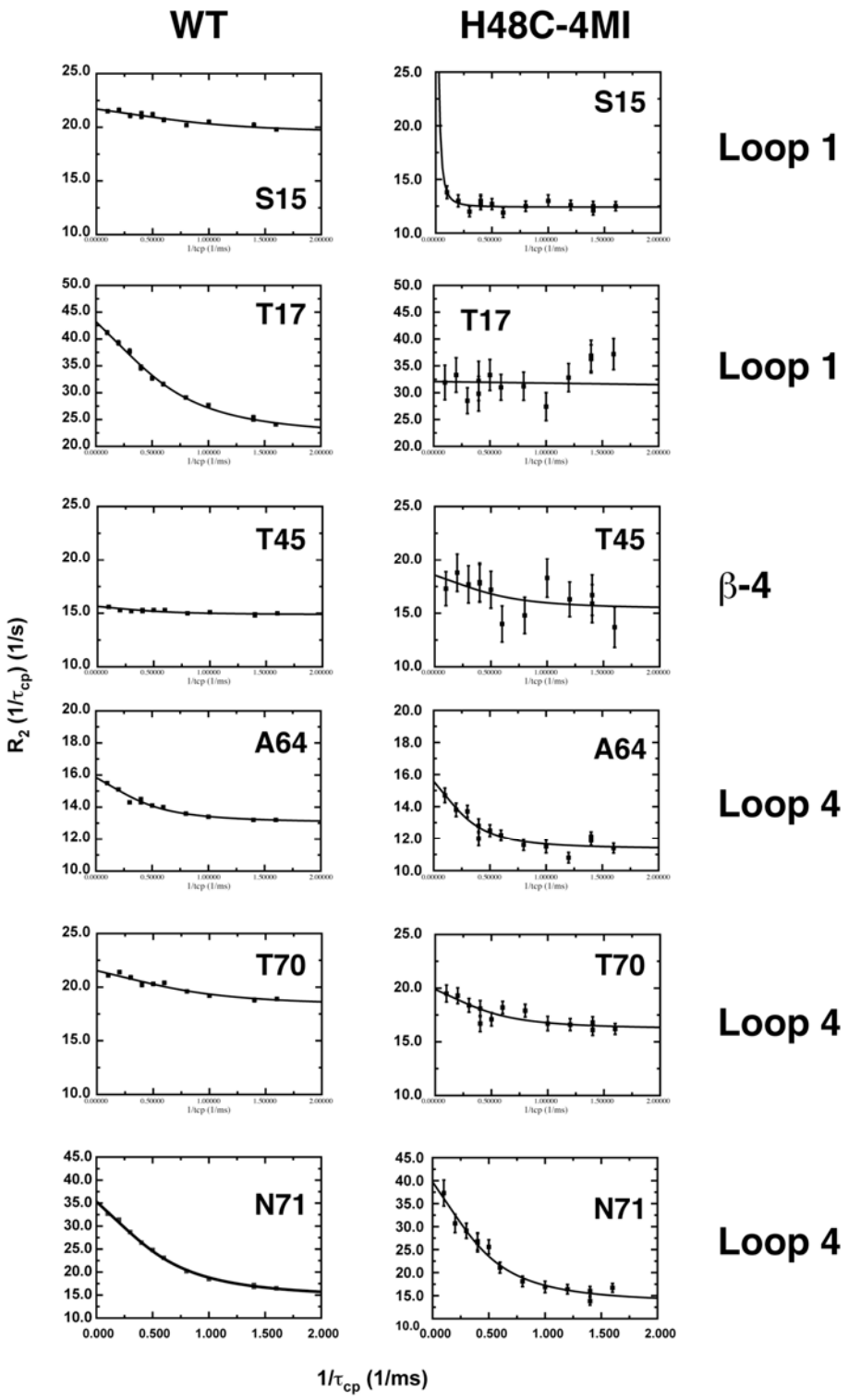

Figure S3. CPMG dispersion data for residues in loop1, β-4, and loop 4 for (left) WT and (right) H48C-4MI at pH = 7.0 and 298K

Figure S4.

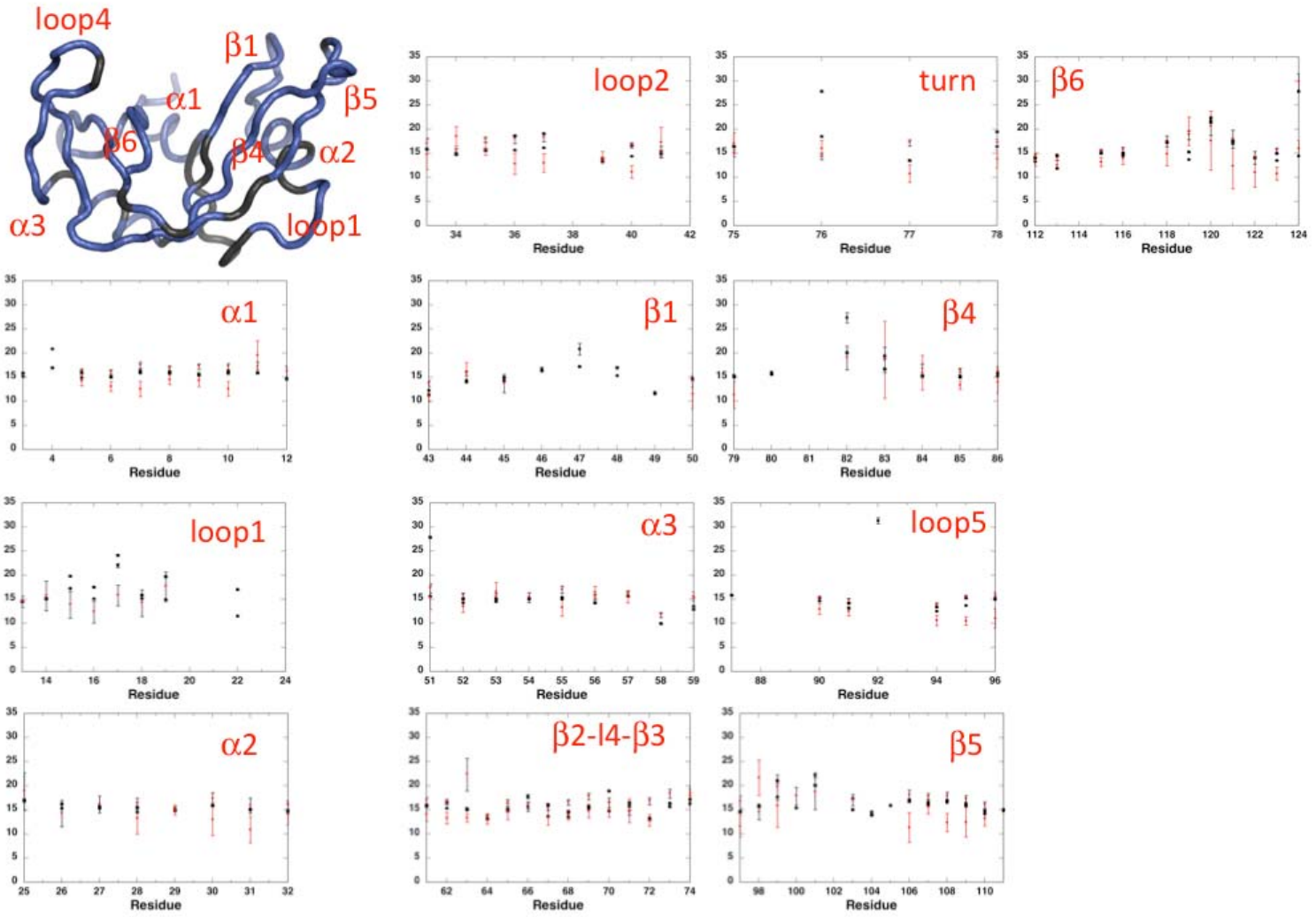
loop4
β1
α1
β5
β6
β4
α2
α3
loop1
loop2
turn
β6
α1
β1
β4
loop1
α3
loop5
α2
β2-l4-β3
β5
Residue

Figure S4. R_2 values grouped by secondary structure element and mapped onto ribbon cartoon of RNase A. Black symbols are WT at pH = 6.4 and red symbols are H48C-4MI at pH =6.4. Black portions of the ribbon indicate residues that are exchange broadened in H48C-4MI. 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. All data were obtained on $^{1}H,^{15}N$-labeled RNase A.

Figure S5.

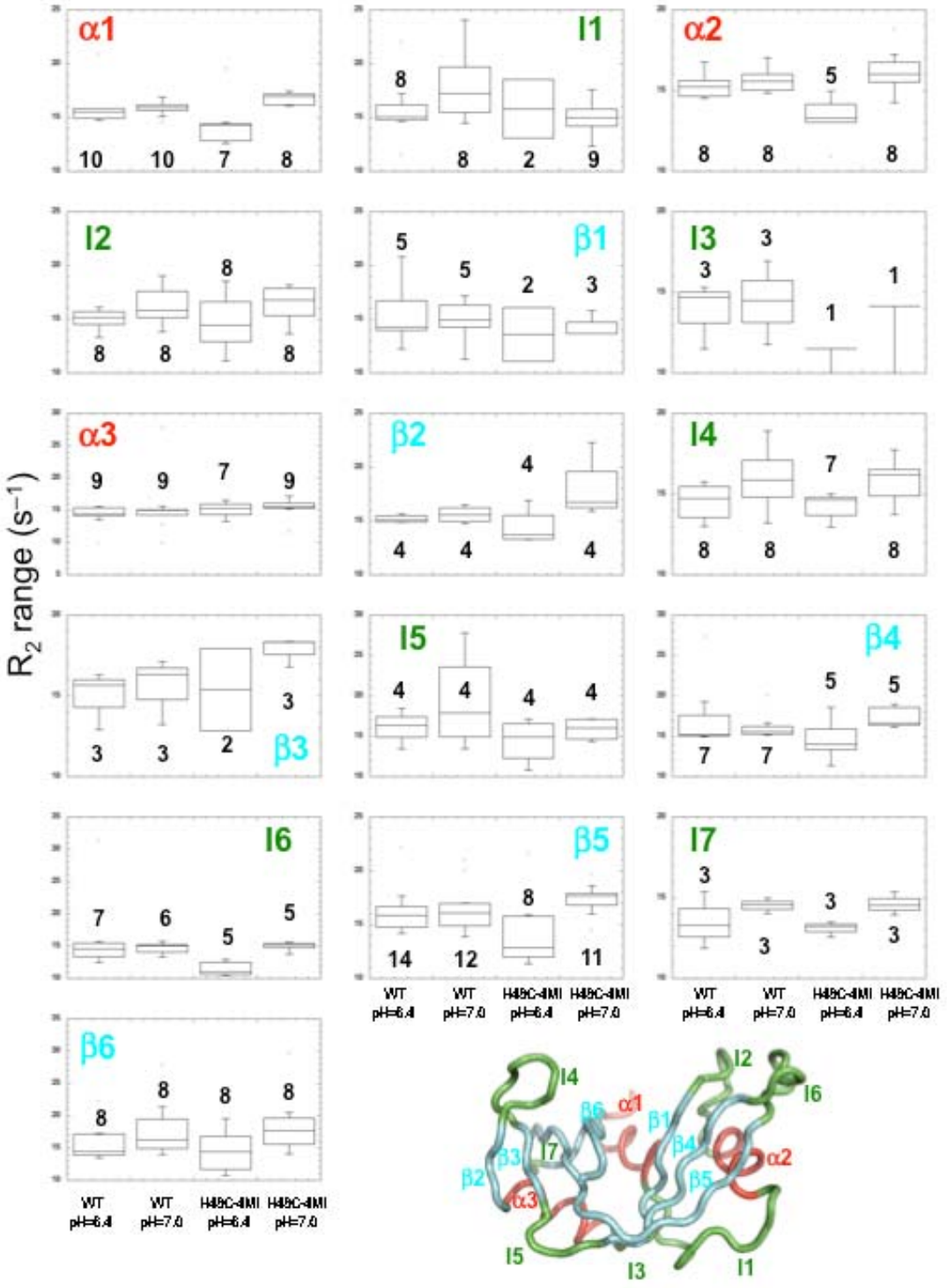

Figure S5. Box plots of R_2 values grouped by secondary structure. The range of measured R_2 values is shown in box plot format for WT and H48C-4MI at pH = 6.4 and 7.0. In the box plots

the center horizontal line represents the median value and the upper and lower parts of the box are represent the 75th and 25th percentiles. The ends of the whiskers are the upper and lower data points and the small circles represent outlier data points. Regions of secondary structure are color-coded cyan (β-strand), red (α-helix), and green (loops and turns). The numbers near each box indicate the number of residues used for each plot. The similarity in R2 values between WT and H48C-4MI is evident in this type of representation. 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. All data were obtained on $^{1}H,^{15}N$-labeled RNase A.

Figure S6.

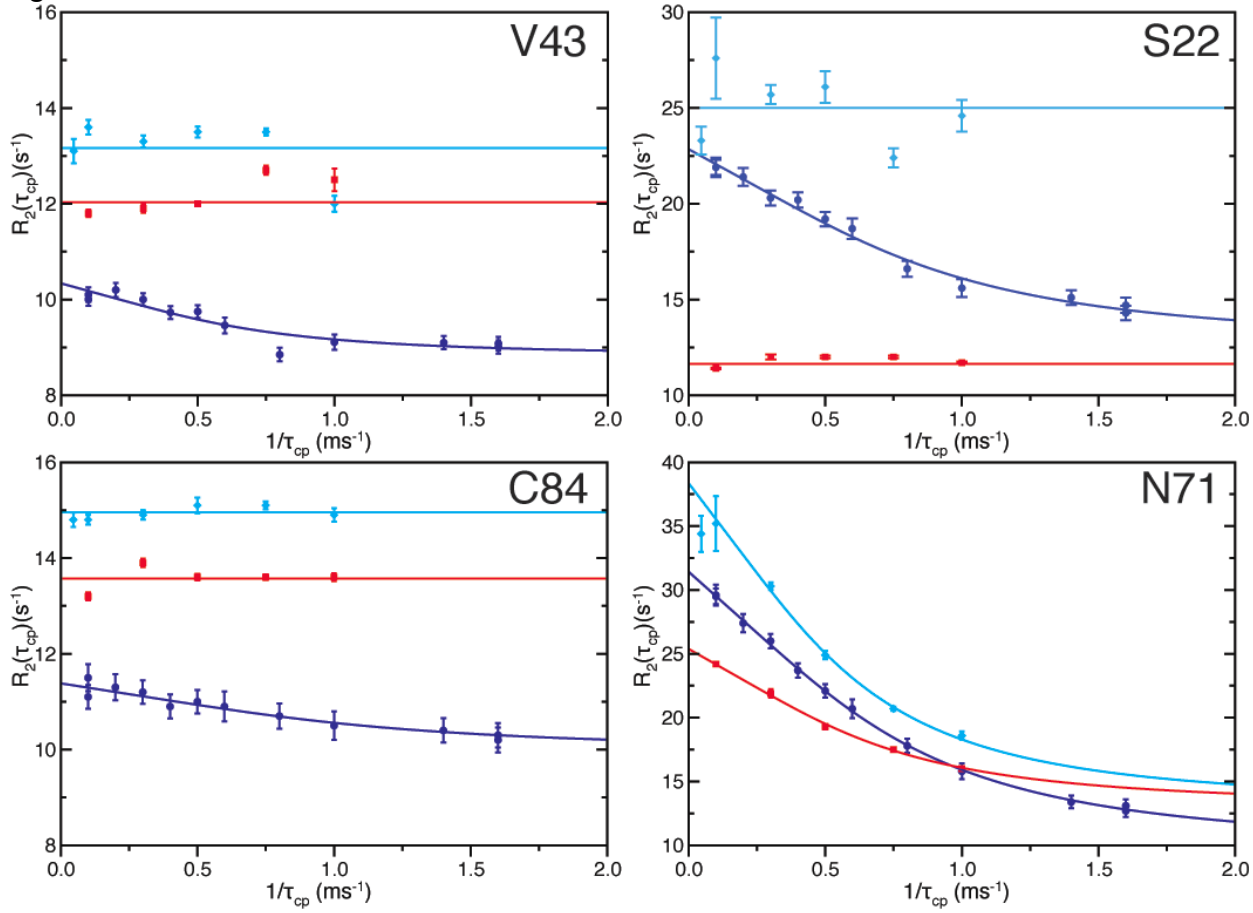

Figure S6. pH dependence of conformational exchange in WT RNase A. pH 7.5 (cyan), pH 4.5 (red), and pH 6.4 (blue). The residues represent β1 (V43), β4 (C84), loop 1 (S22), and loop 4 (N71). The dispersion curves at pH 6.4 were obtained on 98% deuterated ^{15}N enzyme. All other data was obtained for protonated protein.

Figure S7.

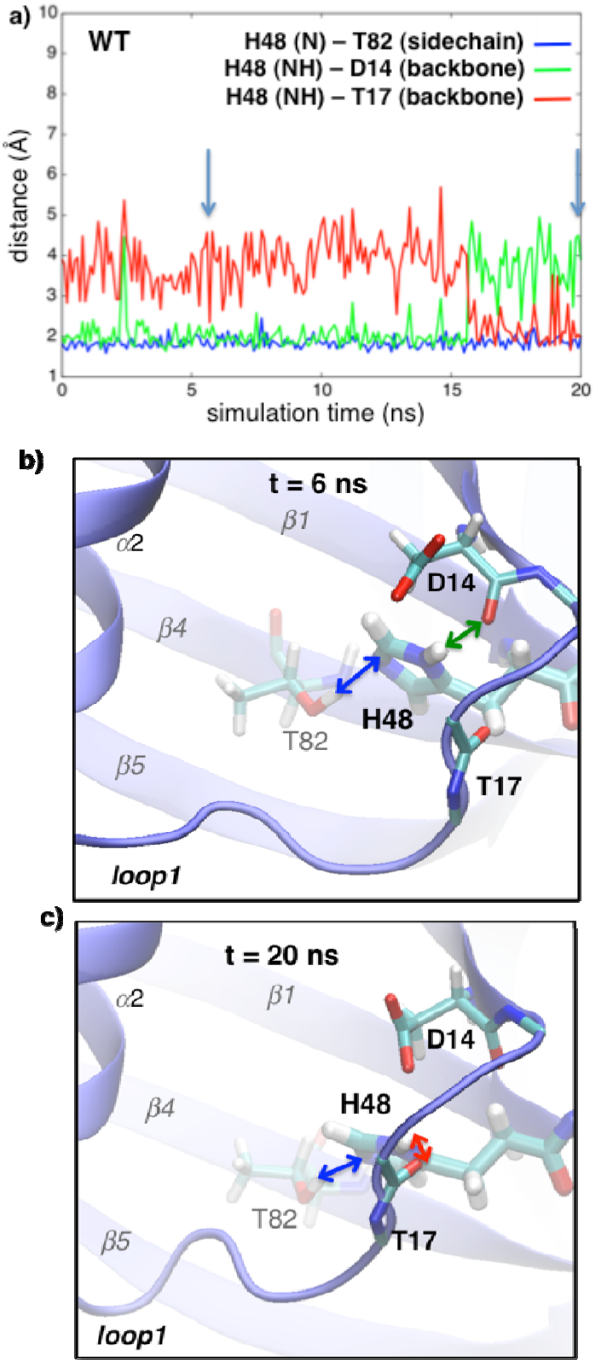

Figure S7. Panel a: Time-dependent bond lengths for hydrogen-bonds of the imidazole ring of H48 with loop 1 (D14 and T17) and β-strand 4 (T82), as observed during a 20 ns MD simulation of WT Ribonuclease A. Panel (b): Snapshot configuration at 6 ns (indicate by arrow in panel (a) showing H-bond interactions between H48 and both T82 and D14 residues. Panel (c): snapshot at 20 ns where H48 forms a H-bond with the backbone carbonyl of T17.

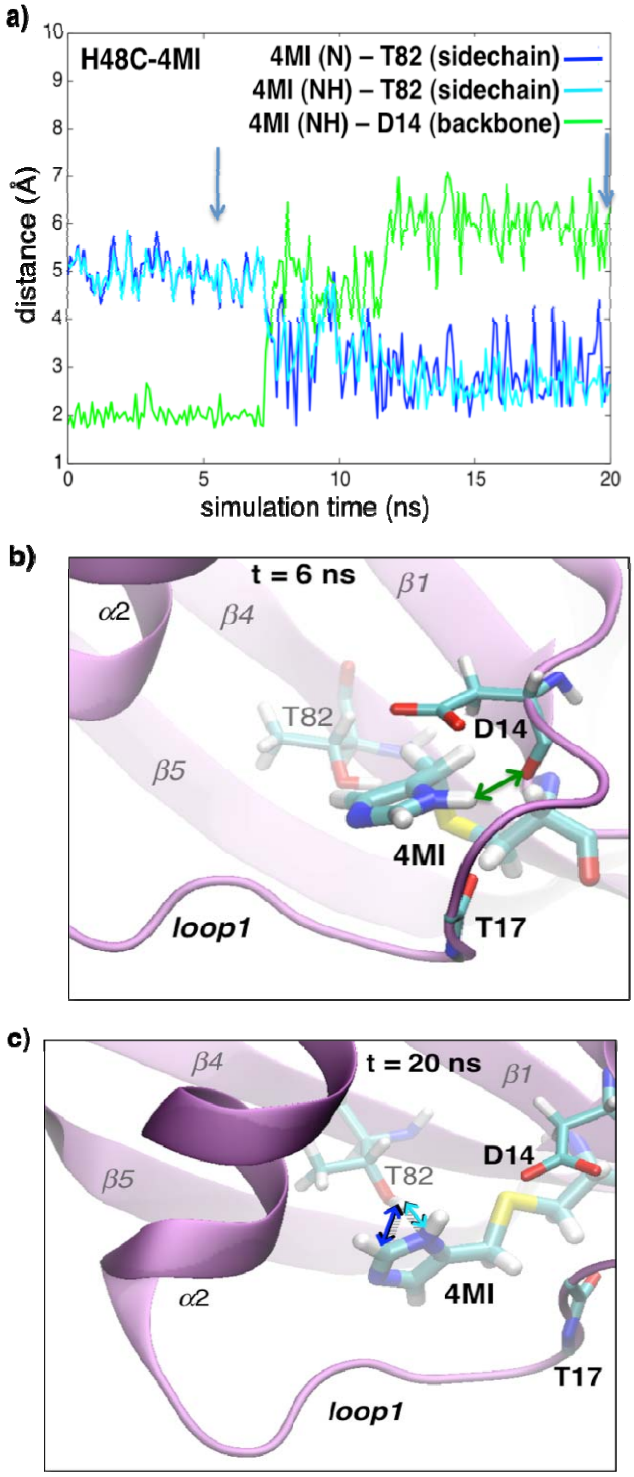

Figure S8. Panel a: Time-dependent bond lengths for hydrogen-bonds of the imidazole ring of 4MI to loop 1 (D14) and β-strand 4 (T82), as observed during a 20 ns MD simulation of H48C-4MI RNase A. Panel (b): Snapshot configuration at 6 ns (indicate by arrow in panel (a)), showing H-bond interactions between 4MI and D14. Panel (c): snapshot at 20 ns, where 4MI forms a H-bond with T82.

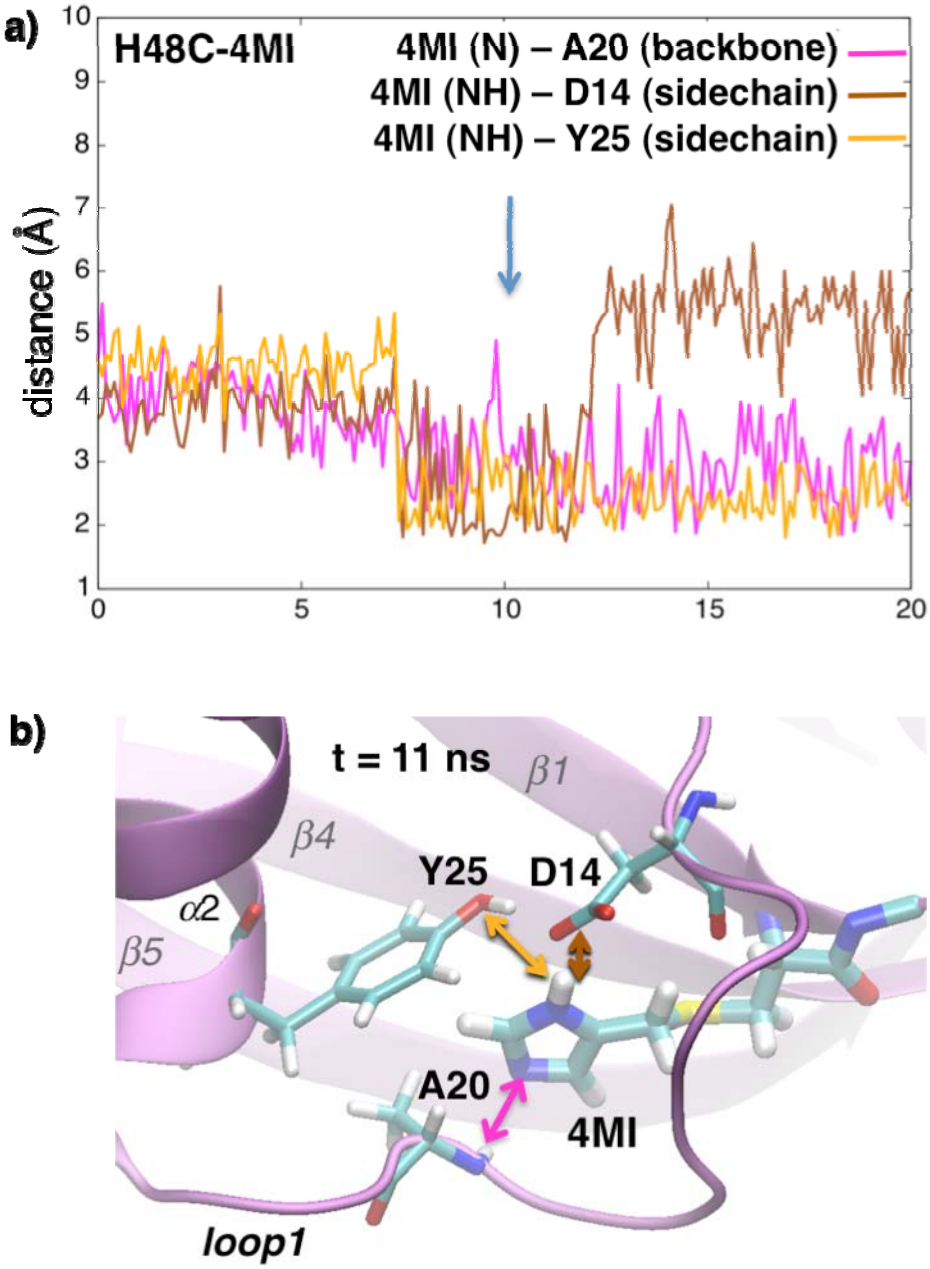

Figure S9. Panel a: Time-dependent bond lengths for hydrogen-bonds of the imidazole ring of 4MI and loop 1 (D14, A20) and α2 (Y25), as observed during a 20 ns MD simulation of H48C-4MI Ribonuclease A. Panel (b): Snapshot configuration at 11 ns (indicate by arrow in panel (a)), showing H-bond interactions between 4MI and D14, Y25 and A20.

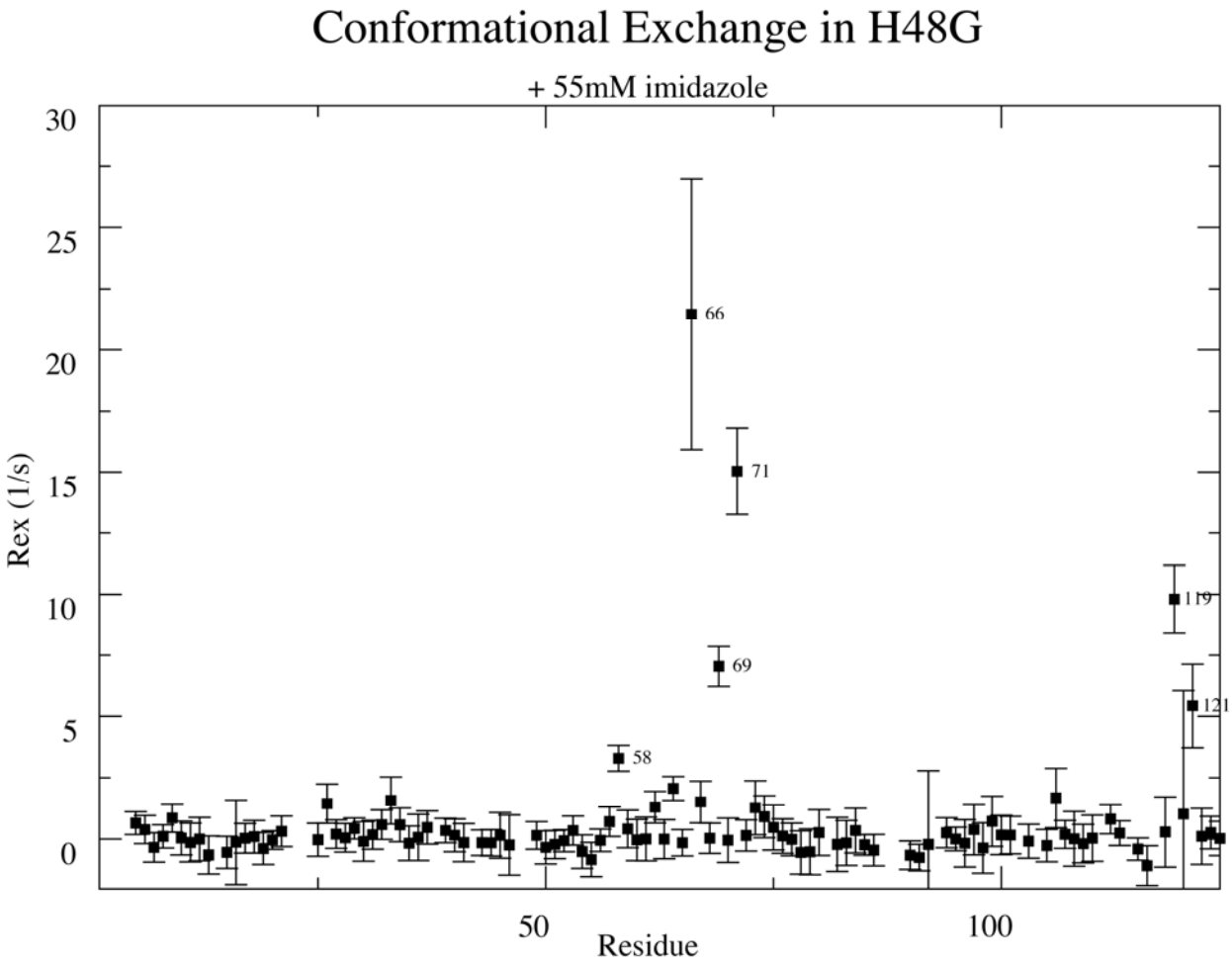

Figure S10. Conformational exchange measurements in H48G + 55 mM imidazole. R_{ex} was measured as the difference in relaxation rates in a CPMG dispersion experiment with τ_{cp} = 0.625 ms and τ_{cp} = 10 ms. Data was obtained at pH = 6.4, T= 298 K, 11.7 T.