Supporting Information of "Cosolute Paramagnetic Relaxation Enhancements Detect Transient Conformations of Human Uracil DNA Glycosylase (hUNG)" by Yan Sun, Joshua I. Friedman and James T. Stivers

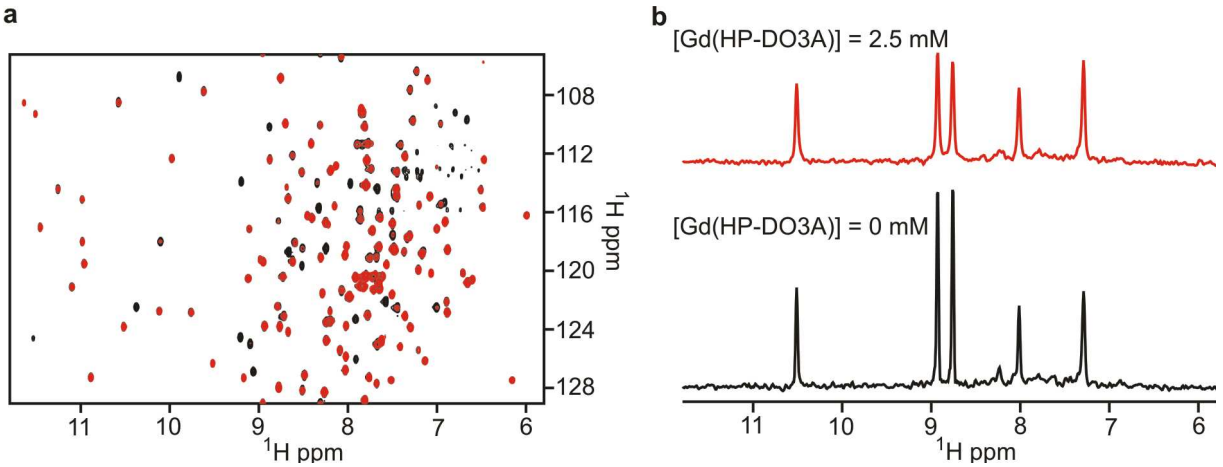

Figure S1. The ^{1}H-^{15}N TROSY spectra of DNA-bound hUNG and the effect of the Gd(HP-DO3A) paramagnetic cosolute. (a) The spectra were obtained with isotropically ^{2}H, ^{15}N-enriched hUNG-DNA complex samples with no Gd(HP-DO3A) (black) or [Gd(HP-DO3A)] = 2.5 mM (red). (b) Representative 1D slices (f_1 = 123.8 ppm) of the spectra in (a).

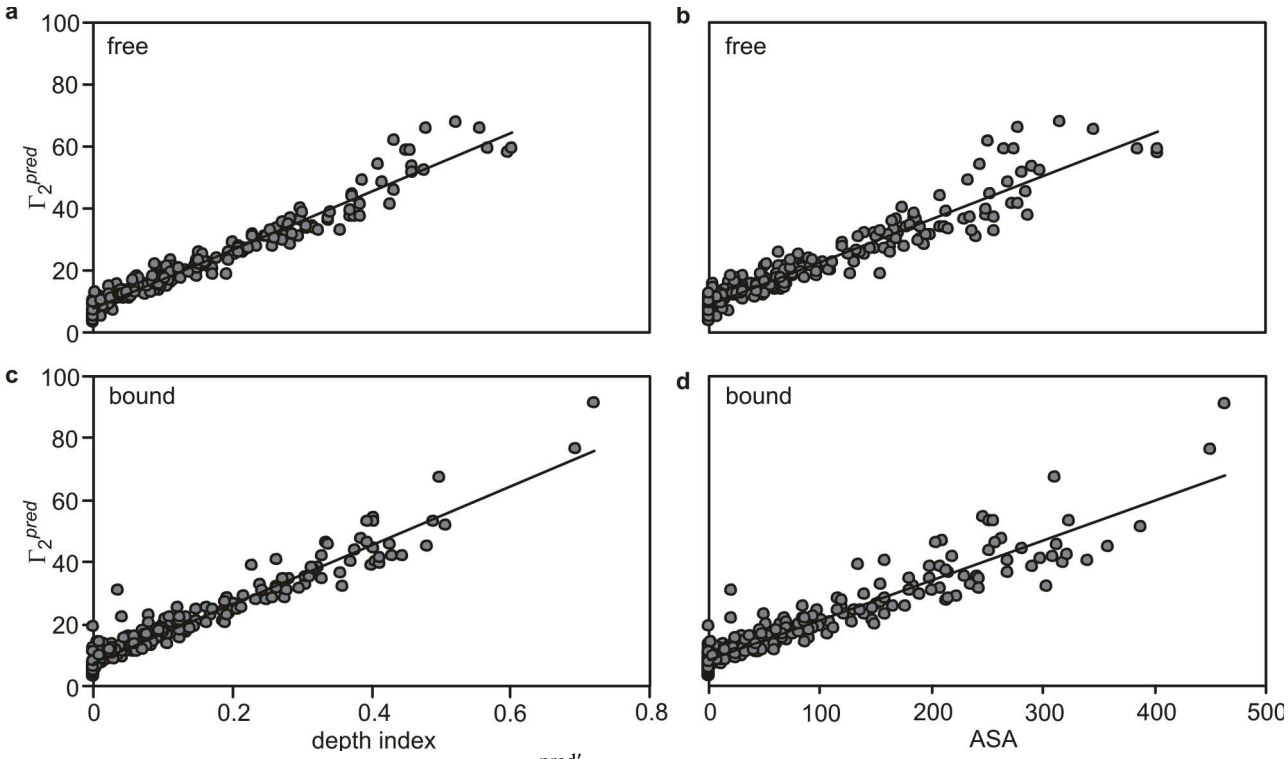

Figure S2. The predicted PRE values ($\Gamma_2^{pred'}$) as a function of calculated depth index for (a) free and (c) DNA-bound hUNG, and the predicted PRE values ($\Gamma_2^{pred'}$) as a function of accessible surface areas (ASA) for both the (b) free hUNG and (d) its complex with non-target DNA. The radius for the sample sphere centered on each amide was 10 and 9 Å in the calculations for the depth index and ASA, respectively.

Table S1. Summary of experimentally measured and calculated parameters for hUNG backbone amides.

Res.	amino acid	$\Gamma_2^{Meas'}$ (s^{-1} mM^{-1}) [a]				$\Gamma_2^{Pred'}$ ($\times 10^{-4}$) [e]		$\Delta\delta$ [f] (ppm)	NMA [g] (arb. u.)	k_{ex} [h] (s^{-1})	depth index [i]		ASA [j]	
		free	error	DNA-bound	error	free	DNA-bound				free	DNA-bound	free	DNA-bound
84	Phe	57.62	2.94	59.44	5.85	61.95	32.51	0.011	0.211	N/A	0.434	0.306	251.5	234.9
85	Phe	29.06	2.17	27.62	0.53	28.83	18.82	0.015	0.214	N/A	0.202	0.092	119.6	62.4
86	Gly	28.92	2.54	32.05	4.36	15.13	11.18	0.004	0.24	N/A	0.079	0.023	46.1	16.6
87	Glu	70	0	70	0	54.09	38.86	0.005	0.317	N/A	0.41	0.227	243.5	134.6
88	Ser	n. a. [b]	n. a.	n. a.	n. a.	36.86	29.20	n. a.	0.348	N/A	0.297	0.215	184.0	139.3
89	Trp	29.11	1.28	27.41	5.27	15.77	14.48	0.006	0.338	N/A	0.059	0.049	34.0	34.2
90	Lys	19.52	1.19	15.16	1.27	15.44	13.38	0.004	0.302	N/A	0.033	0.016	4.4	1.6
91	Lys	17.53	1.04	14.7	0.8	24.28	24.46	0.009	0.346	N/A	0.152	0.148	80.2	96.7
92	His	12.49	1.2	17.58	3.05	20.74	18.91	0.008	0.371	N/A	0.124	0.103	76.9	79.9
93	Leu	8.66	0.31	12.8	1.18	17.60	20.36	0.006	0.316	N/A	0.091	0.142	50.6	108.3
94	Ser	11.46	1	13.74	0.72	30.32	25.36	0.007	0.293	N/A	0.27	0.207	189.8	165.4
95	Gly	13.58	0.41	13.86	0.49	53.76	43.52	0.009	0.403	N/A	0.459	0.377	290.2	252.5
96	Glu	7.79	0.31	11.57	1.17	27.26	26.39	0.006	0.38	N/A	0.215	0.2	148.9	150.4
97	Phe	7.86	0.62	9.23	0.43	25.55	28.62	0.005	0.357	N/A	0.214	0.277	134.0	222.8
98	Gly	11.83	0.89	10.96	0.86	51.45	52.91	0.008	0.563	N/A	0.458	0.49	281.6	323.7
99	Lys	7.37	0.4	10.15	0.98	36.39	44.38	0.007	0.475	N/A	0.337	0.403	229.2	281.9
100	Pro	— [c]	—	—	—	—	—	—	0.376	—	0.596	0.507	404.0	387.5
101	Tyr	14.03	0.23	14.34	0.39	58.95	47.32	0.004	0.282	N/A	0.451	0.384	264.4	262.6
102	Phe	4.99	0.54	15.12	1.39	23.40	23.01	0.005	0.316	N/A	0.111	0.08	76.9	49.2
103	Ile	6.81	1.03	9.35	0.65	25.64	28.03	0.004	0.529	N/A	0.153	0.193	62.5	114.9
104	Lys	7.58	0.37	8.55	0.47	31.78	31.86	0.02	0.709	N/A	0.276	0.264	170.0	184.2
105	Leu	4.76	0.01	7.31	0.74	21.16	19.77	0	0.68	N/A	0.116	0.1	66.9	64.7
106	Met	n. o. [d]	n. o	n. o	n. o	19.01	19.70	n. o	0.765	N/A	0.108	0.127	65.8	94.8
107	Gly	12.18	0.93	12.08	0.97	36.43	36.45	0.007	1.155	N/A	0.297	0.315	185.5	214.9
108	Phe	10.64	0.49	13.16	0.82	31.83	32.62	0.005	1.376	N/A	0.23	0.239	140.4	154.7
109	Val	n. o	n. o	n. o	n. o	16.22	15.56	n. o	1.214	N/A	0.069	0.068	30.8	40.5
110	Ala	n. o	n. o	n. o	n. o	24.94	26.64	n. o	1.527	N/A	0.196	0.2	140.4	154.1
111	Glu	13.7	0.33	14.33	0.36	31.57	30.99	0.008	2.042	N/A	0.278	0.263	198.9	207.2
112	Glu	n. o	n. o	n. o	n. o	21.56	20.08	n. o	1.86	N/A	0.091	0.079	50.5	52.4
113	Arg	13.71	0.26	14.19	0.73	21.30	21.25	0.012	1.69	N/A	0.107	0.103	62.5	68.1
114	Lys	n. o	n. o	n. o	n. o	33.88	34.73	n. o	2.283	N/A	0.279	0.281	176.8	198.8
115	His	n. o	n. o	n. o	n. o	37.04	35.30	n. o	2.532	N/A	0.339	0.305	234.2	240.5
116	Tyr	60.1	7	55.59	0.7	33.74	35.01	0.007	2.096	N/A	0.308	0.311	207.5	229.4
117	Thr	70	0.05	70	0	48.63	45.89	0.007	1.532	N/A	0.417	0.395	268.5	256.5
118	Val	28.97	1.81	40.97	5.14	27.81	24.08	0.013	0.923	N/A	0.208	0.188	120.7	130.8
119	Tyr	n. o	n. o	n. o	n. o	9.74	8.58	n. o	0.5	N/A	0.008	0.002	1.1	0.0
120	Pro	—	—	—	—	—	—	—	0.176	N/A	0.028	0.037	9.9	26.0
121	Pro	—	—	—	—	—	—	—	0.254	N/A	0.256	0.235	148.2	158.1
122	Pro	—	—	—	—	—	—	—	0.496	N/A	0.148	0.187	109.8	146.2
123	His	n. o	n. o	n. o	n. o	37.65	38.77	n. o	0.487	N/A	0.37	0.399	249.8	289.6
124	Gln	12.73	0.65	14.26	0.49	16.63	17.42	0.007	0.317	N/A	0.085	0.134	17.6	79.7
125	Val	8.05	0.72	7.55	0.63	10.06	10.15	0.006	0.166	N/A	0.003	0.002	1.1	0.0
126	Phe	7.33	0.1	8.48	0.55	8.94	8.57	0.011	0.205	N/A	0.001	0.005	0.0	0.0
127	Thr	7.15	0.54	7.81	0.6	7.44	7.26	0.006	0.302	N/A	0.007	0.01	1.1	3.2
128	Trp	4.17	0.1	5.88	0.34	5.96	5.48	0.006	0.338	N/A	0	0	0.0	0.0
129	Thr	29.93	1.8	52.14	6.56	7.53	6.77	0	0.347	N/A	0.002	0.004	0.0	0.0
130	Gln	19.92	1.39	25.79	3.68	12.00	9.48	0.002	0.283	N/A	0.074	0.04	48.3	29.7
131	Met	27.9	0.63	27.85	2.82	13.59	13.23	0.003	0.351	N/A	0.096	0.084	63.7	60.8

Res.	amino acid	$\Gamma_2^{Meas'}$ (s^{-1} mM^{-1})				$\Gamma_2^{Pred'}$ ($\times 10^{-4}$)		$\Delta\delta$ (ppm)	NMA (arb. u.)	k_{ex} (s^{-1})	depth index		ASA	
		free	error	DNA-bound	error	free	DNA-bound				free	DNA-bound	free	DNA-bound
132	Cys	28.52	1.6	28.87	1.8	16.01	14.81	0.004	0.296	N/A	0.112	0.105	70.3	69.5
133	Asp	30.94	4.19	61.33	9.06	40.32	54.26	0.006	0.319	N/A	0.297	0.402	174.5	245.5
134	Ile	15.87	1.53	28.77	1.83	16.33	16.48	0.01	0.387	N/A	0.088	0.118	51.6	107.3
135	Lys	15.24	1.04	27.29	1.75	21.16	23.35	0.007	0.389	N/A	0.156	0.195	83.1	140.2
136	Asp	16.6	2.18	26.17	2.12	16.75	20.15	0.006	0.397	N/A	0.115	0.19	51.4	148.8
137	Val	12.35	0.56	12.95	1.28	11.27	12.42	0.005	0.408	N/A	0.025	0.045	13.8	32.1
138	Lys	5.33	0.09	6.16	0.43	6.68	7.64	0.006	0.479	N/A	0.001	0.007	0.0	2.6
139	Val	n. o	n. o	n. o	n. o	5.72	5.94	n. o	0.361	N/A	0	0	0.0	0.0
140	Val	n. o	n. o	n. o	n. o	4.49	4.35	n. o	0.369	N/A	0	0	0.0	0.0
141	Ile	n. o	n. o	n. o	n. o	3.57	3.61	n. o	0.415	N/A	0	0	0.0	0.0
142	Leu	n. o	n. o	n. o	n. o	4.58	3.67	n. o	0.588	N/A	0	0	0.0	0.0
143	Gly	11.33	0.33	9.26	0.45	4.05	3.22	0.023	0.565	N/A	0	0	0.0	0.0
144	Gln	56.8	1.83	50.24	9.78	7.02	3.30	0.024	0.74	900	0.028	0	17.5	0.0
145	Asp	70	2.82	54.44	2.65	11.05	3.47	0.013	0.666	900	0.053	0	28.7	0.0
146	Pro	—	—	—	—	—	—	—	0.6	N/A	0.011	0	5.5	0.0
147	Tyr	9.58	0.2	11.77	0.83	7.47	4.29	0.023	0.608	N/A	0.017	0	13.2	0.0
148	His	26.36	1.77	17.77	3.55	18.32	7.02	0.015	0.889	900	0.148	0.003	96.6	0.0
149	Gly	15.23	1.03	15.94	2.38	20.65	10.70	0.024	0.86	N/A	0.169	0.029	107.5	21.8
150	Pro	—	—	—	—	—	—	—	0.607	N/A	0.314	0.329	211.6	242.3
151	Asn	13.97	0.16	13.89	0.42	27.97	41.61	0.002	0.394	N/A	0.257	0.33	175.7	219.3
152	Gln	8.40	0.66	9.09	0.43	17.20	16.01	0.008	0.62	N/A	0.06	0.095	25.3	66.8
153	Ala	6.13	0.18	6.50	0.45	9.50	8.24	0.012	0.572	900	0	0.003	0.0	0.0
154	His	4.58	0.32	5.41	1.45	6.41	6.24	0.017	0.535	N/A	0	0	0.0	0.0
155	Gly	6.64	0.24	12.15	1.81	6.00	5.41	0.021	0.431	N/A	0	0	0.0	0.0
156	Leu	6.36	0.91	5.10	0.99	5.24	5.50	0.011	0.611	900	0	0.002	0.0	0.0
157	Cys	4.17	0.19	3.54	0.4	3.76	3.41	0.005	0.611	N/A	0	0	0.0	0.0
158	Phe	12.64	1.23	11.74	0.96	5.05	3.48	0.009	0.807	N/A	0.011	0	6.6	0.0
159	Ser	n. o	n. o	n. o	n. o	4.65	4.24	n. o	0.808	N/A	0	0	0.0	0.0
160	Val	3.02	0.51	5.92	1.11	7.91	6.95	0	0.884	N/A	0	0	0.0	0.0
161	Gln	n. o	n. o	n. o	n. o	12.99	13.6	n. o	0.989	N/A	0.029	0.028	9.8	12.1
162	Arg	13.64	0.35	14.5	0.56	33.56	29.49	0.009	1.21	N/A	0.265	0.251	166.6	189.6
163	Pro	—	—	—	—	—	—	—	1.497	N/A	0.568	0.721	384.8	462.4
164	Val	8.49	0.29	9.57	0.55	28.14	40.15	0.006	1.362	N/A	0.285	0.407	194.0	339.6
165	Pro	—	—	—	—	—	—	—	1.335	N/A	0.385	0.371	271.6	267.7
166	Pro	—	—	—	—	—	—	—	1.079	N/A	0.043	0.036	15.8	20.6
167	Pro	—	—	—	—	—	—	—	1.055	N/A	0.34	0.113	213.6	70.7
168	Pro	—	—	—	—	—	—	—	1.255	N/A	0.373	0.051	253.1	29.5
169	Ser	26.96	0.99	26.39	1.73	20.43	5.97	0.015	1.176	900	0.173	0.011	107.2	11.5
170	Leu	9.14	0.4	12.89	1.12	10.98	5.72	0.012	0.91	N/A	0.035	0.007	14.3	0.8
171	Glu	8.86	0.26	8.51	0.28	15.52	8.98	0.015	0.774	N/A	0.069	0.028	38.4	16.8
172	Asn	12.18	1.07	8.97	0.55	13.63	8.12	0.019	0.928	N/A	0.043	0.01	18.6	5.3
173	Ile	n. o	n. o	n. o	n. o	8.77	7.18	n. o	0.717	N/A	0	0	0.0	0.0
174	Tyr	n. o	n. o	n. o	n. o	9.50	8.16	n. o	0.507	N/A	0.003	0.005	0.0	1.0
175	Lys	n. o	n. o	n. o	n. o	15.78	14.70	n. o	0.645	N/A	0.076	0.045	43.9	32.8
176	Glu	n. o	n. o	n. o	n. o	13.57	11.95	n. o	0.828	N/A	0.039	0.022	14.3	9.5
177	Leu	n. o	n. o	n. o	n. o	10.82	9.73	n. o	0.646	N/A	0.004	0.002	0.0	0.0
178	Ser	14.66	0.94	25.37	2.84	20.68	17.40	0	0.652	N/A	0.121	0.097	83.5	70.4
179	Thr	8.95	0.35	9.68	0.24	30.85	26.17	0.001	0.96	N/A	0.295	0.248	240.5	212.9
180	Asp	9.70	0.66	13.05	1.25	22.06	19.34	0.008	1.002	N/A	0.161	0.127	100.9	86.0
181	Ile	6.79	0.19	8.55	0.38	17.63	15.09	0.005	0.897	N/A	0.086	0.048	31.1	23.2

Res.	amino acid	$\Gamma_2^{Meas'}$ (s^{-1} mM^{-1})				$\Gamma_2^{Pred'}$ ($\times 10^{-4}$)		$\Delta\delta$ (ppm)	NMA (arb. u.)	k_{ex} (s^{-1})	depth index		ASA	
		free	error	DNA-bound	error	free	DNA-bound				free	DNA-bound	free	DNA-bound
182	Glu	14.2	0.34	15.35	1.41	43.99	40.50	0.008	1.11	N/A	0.372	0.264	208.6	157.2
183	Asp	14.52	0.52	15.24	1.27	37.36	39.49	0.011	0.999	N/A	0.376	0.411	257.0	318.6
184	Phe	9.87	0.64	9.65	0.29	31.21	25.44	0.007	0.704	N/A	0.247	0.164	161.3	119.7
185	Val	13.46	0.5	4.36	0.38	27.79	31.00	0.014	0.39	N/A	0.236	0.295	163.6	241.8
186	His	13.42	0.48	14.13	0.3	67.67	66.10	0.007	0.11	N/A	0.522	0.498	315.5	311.4
187	Pro	—	—	—	—	—	—	—	0.4	N/A	0.479	0.322	277.6	208.5
188	Gly	12.93	1.17	25.59	3.1	39.61	40.48	0.002	0.737	N/A	0.371	0.411	248.8	298.0
189	His	11.26	0.52	9.52	0.34	26.89	28.05	0.008	0.872	N/A	0.21	0.257	158.2	214.4
190	Gly	5.83	0.23	8.62	0.63	14.55	15.95	0.009	0.903	N/A	0.023	0.058	4.4	37.3
191	Asp	6.79	0.5	8.14	0.65	12.34	13.59	0.009	0.85	N/A	0.024	0.014	9.9	0.0
192	Leu	13.23	0.99	15.83	1.91	7.85	7.88	0	0.668	N/A	0.007	0.004	0.0	0.0
193	Ser	12.68	1.2	14.78	0.96	13.65	12.14	0	0.689	N/A	0.088	0.054	59.3	38.7
194	Gly	15.42	1.25	15.18	1.47	19.23	16.47	0.006	0.675	N/A	0.13	0.107	78.9	75.2
195	Trp	7.27	0.16	10.52	0.37	9.84	8.92	0.005	0.528	N/A	0.027	0.006	12.1	0.8
196	Ala	n. o	n. o	n. o	n. o	8.11	7.29	n. o	0.487	N/A	0.008	0	0.0	0.0
197	Lys	13.01	0.88	13.07	0.9	15.09	13.72	0.011	0.533	N/A	0.107	0.077	68.0	57.3
198	Gln	13.54	0.43	13.36	0.82	14.20	15.37	0.003	0.6	N/A	0.082	0.092	57.2	66.0
199	Gly	13.33	0.59	13.6	0.98	10.69	11.03	0.006	0.54	N/A	0.044	0.055	29.7	42.6
200	Val	n. o	n. o	n. o	n. o	6.73	7.09	n. o	0.429	N/A	0.001	0.004	0.0	0.0
201	Leu	n. o	n. o	n. o	n. o	4.26	4.56	n. o	0.437	N/A	0	0	0.0	0.0
202	Leu	3.40	0.25	2.85	0.3	3.99	3.94	0.012	0.532	N/A	0	0	0.0	0.0
203	Leu	n. o	n. o	n. o	n. o	3.52	3.13	n. o	0.523	N/A	0	0	0.0	0.0
204	Asn	5.42	0.42	2.85	0.7	3.79	3.23	0.005	0.518	N/A	0	0	0.0	0.0
205	Ala	13.48	0.75	11.6	0.9	5.28	3.55	0.011	0.5	N/A	0	0	0.0	0.0
206	Val	n. o	n. o	n. o	n. o	5.50	4.35	n. o	0.443	N/A	0.001	0	0.0	0.0
207	Leu	n. a.	n. a.	n. a.	n. a.	5.51	4.99	n. a.	0.27	N/A	0	0	0.0	0.0
208	Thr	n. o	n. o	n. o	n. o	5.75	5.14	n. o	0.303	N/A	0	0	0.0	0.0
209	Val	n. o	n. o	n. o	n. o	7.26	6.31	n. o	0.579	N/A	0	0	0.0	0.0
210	Arg	12.27	1.57	11.67	2.41	11.87	7.33	0.003	1.114	N/A	0.008	0.004	0.0	1.1
211	Ala	14.5	0.47	14.73	0.8	18.27	16.77	0.012	1.124	N/A	0.062	0.072	31.3	46.0
212	His	14.61	0.56	22.3	1.09	24.03	14.79	0.016	1.248	N/A	0.178	0.083	90.2	49.5
213	Gln	14.87	0.83	20.18	1.69	25.61	12.67	0.011	1.551	N/A	0.195	0.075	135.9	59.7
214	Ala	17.77	3.12	30.59	3.51	65.66	10.05	0.017	1.551	N/A	0.556	0.042	346.4	20.5
215	Asn	32.34	4.87	29.56	1.79	18.62	4.78	0.002	1.326	N/A	0.173	0.006	126.9	6.6
216	Ser	14.7	0.66	25.75	3.01	22.21	6.48	0.009	1.418	N/A	0.158	0.01	97.6	4.7
217	His	13.57	0.41	13.73	0.73	13.68	6.16	0.015	1.197	N/A	0.048	0.005	27.5	3.2
218	Lys	15.19	0.95	15.04	1.07	18.34	7.08	0.016	0.978	N/A	0.1	0.006	61.9	2.1
219	Glu	14.01	0.24	24.99	3.21	18.65	12.30	0.019	0.979	N/A	0.117	0.08	60.2	53.4
220	Arg	13.64	0.34	14.69	2.15	18.15	14.68	0.005	0.89	N/A	0.11	0.1	75.6	80.0
221	Gly	13.53	0.56	13.9	1.77	21.17	19.59	0.004	0.591	N/A	0.146	0.147	92.2	101.8
222	Trp	9.45	0.83	12.1	1.67	10.64	8.96	8E-04	0.269	N/A	0.022	0.035	9.9	24.4
223	Glu	12.48	0.8	12.86	1.22	12.28	10.18	0.007	0.352	N/A	0.042	0.047	19.7	33.6
224	Gln	6.04	0.49	7.22	0.95	18.64	18.82	0.011	0.356	N/A	0.192	0.146	154.8	98.9
225	Phe	n. o	n. o	n. o	n. o	11.90	11.09	n. o	0.248	N/A	0.039	0.04	13.2	22.4
226	Thr	n. o	n. o	n. o	n. o	7.95	7.36	n. o	0.389	N/A	0.006	0.006	0.0	0.0
227	Asp	8.79	0.42	9.33	0.5	12.86	10.91	0.004	0.485	N/A	0.082	0.06	50.5	43.9
228	Ala	n. o	n. o	n. o	n. o	16.62	13.97	n. o	0.471	N/A	0.109	0.079	66.9	49.8
229	Val	n. o	n. o	n. o	n. o	9.34	8.31	n. o	0.461	N/A	0.023	0.014	10.9	8.4
230	Val	n. o	n. o	n. o	n. o	8.20	8.18	n. o	0.541	N/A	0.015	0.015	2.2	10.5
231	Ser	8.55	0.14	9.09	0.54	14.61	14.02	0.006	0.591	N/A	0.097	0.086	68.0	59.2

Res.	amino acid	$\Gamma_2^{Meas'}$ (s^{-1} mM^{-1})				$\Gamma_2^{Pred'}$ ($\times 10^{-4}$)		$\Delta\delta$ (ppm)	NMA (arb. u.)	k_{ex} (s^{-1})	depth index		ASA	
		free	error	DNA-bound	error	free	DNA-bound				free	DNA-bound	free	DNA-bound
232	Trp	10.05	0.75	8.98	0.74	15.24	12.86	0.002	0.543	N/A	0.088	0.061	54.9	53.1
233	Leu	n. o	n. o	n. o	n. o	9.41	9.10	n. o	0.532	N/A	0.006	0.002	1.1	0.0
234	Asn	n. o	n. o	n. o	n. o	10.87	11.07	n. o	0.543	N/A	0.008	0.008	0.0	0.5
235	Gln	12.6	0.96	13.6	0.55	19.07	20.08	0.002	0.63	N/A	0.112	0.121	46.1	80.4
236	Asn	n. o	n. o	n. o	n. o	20.47	22.79	n. o	0.637	N/A	0.15	0.169	77.2	121.7
237	Ser	11.66	0.75	13.67	0.31	19.77	24.85	0.006	0.554	N/A	0.124	0.202	83.0	147.5
238	Asn	14.31	0.27	15.7	1.53	30.78	46.51	0.008	0.495	N/A	0.249	0.334	151.1	209.9
239	Gly	59.99	5.79	58.32	3.95	22.35	30.70	0.003	0.336	N/A	0.163	0.278	103.0	199.7
240	Leu	11.93	0.33	14.05	0.29	11.23	12.69	0.002	0.296	N/A	0.027	0.039	6.5	19.2
241	Val	n. o	n. o	n. o	n. o	6.94	7.28	n. o	0.181	N/A	0	0	0.0	0.0
242	Phe	n. o	n. o	n. o	n. o	7.59	7.78	n. o	0.171	N/A	0.004	0.004	0.0	0.5
243	Leu	n. o	n. o	n. o	n. o	5.05	4.84	n. o	0.23	N/A	0	0	0.0	0.0
244	Leu	n. o	n. o	n. o	n. o	8.34	8.24	n. o	0.297	N/A	0.003	0.01	0.0	0.0
245	Trp	n. o	n. o	n. o	n. o	6.78	5.05	n. o	0.54	900	0	0	0.0	0.0
246	Gly	70	6.72	54.69	2.22	14.90	7.94	0.064	0.805	900	0.078	0.021	42.5	14.5
247	Ser	n. a.	n. a.	n. a.	n. a.	36.46	11.60	n. a.	0.728	N/A	0.285	0.061	168.9	47.3
248	Tyr	n. a.	n. a.	n. a.	n. a.	26.48	8.92	n. a.	0.649	N/A	0.218	0.031	133.5	24.2
249	Ala	70	0	59.57	6.07	11.96	6.13	0.018	0.585	900	0.037	0.007	21.3	0.0
250	Gln	31.07	4.24	30.26	2.49	14.87	10.78	0.049	0.569	N/A	0.067	0.063	30.7	41.0
251	Lys	n. o	n. o	n. o	n. o	24.84	15.60	n. o	0.638	N/A	0.205	0.125	126.1	90.2
252	Lys	20.89	0.98	14.73	1.06	18.86	12.89	0.006	0.599	N/A	0.125	0.076	75.7	58.9
253	Gly	13.38	0.54	13.25	0.76	14.18	17.88	0.016	0.54	N/A	0.06	0.131	28.6	90.0
254	Ser	15.27	1.07	24.8	1.7	29.64	41.78	0.01	0.613	N/A	0.273	0.444	188.8	308.5
255	Ala	14.88	0.76	26.02	2.36	32.64	31.72	0.016	0.65	N/A	0.355	0.358	256.9	303.0
256	Ile	11.99	0.35	13.68	0.67	22.91	22.86	0.003	0.569	N/A	0.195	0.192	131.6	157.5
257	Asp	13.63	0.44	14.28	0.55	22.26	24.45	0.007	0.509	N/A	0.152	0.172	97.6	116.5
258	Arg	15.51	1.28	26.79	2.64	58.93	53.05	0.004	0.375	N/A	0.456	0.402	273.3	251.7
259	Lys	28.36	1.38	28.53	3.63	52.08	42.11	0.006	0.333	N/A	0.477	0.431	297.4	322.5
260	Arg	n. o	n. o	n. o	n. o	31.42	45.57	n. o	0.354	N/A	0.286	0.428	194.5	313.3
261	His	26.94	1.21	27.8	1.13	20.20	22.25	0.007	0.292	N/A	0.132	0.134	67.6	93.3
262	His	12.06	0.53	14.3	0.39	12.43	11.90	0.015	0.109	N/A	0.048	0.034	21.7	18.4
263	Val	14.11	0.24	14.85	0.82	31.08	27.42	0.009	0.156	N/A	0.228	0.189	135.0	119.7
264	Leu	n. o	n. o	n. o	n. o	11.42	11.78	n. o	0.219	N/A	0.054	0.07	40.6	59.7
265	Gln	15.3	1.21	25.14	1.84	38.53	45.72	0.015	0.421	N/A	0.302	0.337	185.4	203.6
266	Thr	15.11	0.99	15.06	1.01	17.38	13.52	0.032	0.713	N/A	0.126	0.108	85.6	86.8
267	Ala	15.86	1.81	14.89	1.43	26.85	23.70	0.031	1.127	N/A	0.223	0.206	144.9	135.8
268	His	70	0	58.06	3.5	17.48	7.30	0.029	1.276	N/A	0.097	0.011	60.3	8.1
269	Pro	—	—	—	—	—	—	—	1.161	N/A	0.001	0	0.0	0.0
270	Ser	70	0	55.44	0.95	13.06	4.34	0.076	1.563	N/A	0.06	0	42.8	0.0
271	Pro	—	—	—	—	—	—	—	1.971	N/A	0.273	0	165.4	0.0
272	Leu	59.89	9.03	51.18	8.2	41.37	6.27	0.098	2.537	900	0.426	0	277.9	0.0
273	Ser	26.69	1.32	27.19	1.26	25.22	6.32	0.07	2.363	N/A	0.159	0	81.4	0.0
274	Val	54.33	2.36	53.75	3.83	22.26	6.77	0.016	1.999	900	0.09	0	51.6	0.0
275	Tyr	27.65	0.52	28.35	1.48	32.70	12.07	0.065	2.149	N/A	0.255	0.037	154.1	20.3
276	Arg	28.42	1.35	27.8	2.14	33.29	16.37	0.014	2.289	N/A	0.317	0.122	215.9	84.9
277	Gly	25.99	1.91	26.52	1.68	45.55	24.72	0.06	1.963	N/A	0.434	0.242	284.2	179.4
278	Phe	14.78	0.71	16.19	2.2	22.55	15.68	0.013	1.458	N/A	0.16	0.123	112.0	92.9
279	Phe	13.65	0.35	13.75	0.75	23.10	15.95	0.037	1.449	N/A	0.149	0.111	93.2	83.9
280	Gly	13.07	0.75	13.87	0.56	34.75	31.06	0.025	1.438	N/A	0.282	0.273	181.1	179.6
281	Cys	12.94	0.84	13.55	0.67	25.49	24.01	0.011	1.263	N/A	0.21	0.214	162.3	175.9

Res.	amino acid	$\Gamma_2^{Meas'}$ (s^{-1} mM^{-1})				$\Gamma_2^{Pred'}$ ($\times 10^{-4}$)		$\Delta\delta$ (ppm)	NMA (arb. u.)	k_{ex} (s^{-1})	depth index		ASA	
		free	error	DNA-bound	error	free	DNA-bound				free	DNA-bound	free	DNA-bound
282	Arg	9.27	0.52	12.55	1.22	19.21	18.10	0.006	1.013	N/A	0.137	0.138	96.5	112.8
283	His	3.36	0.64	2.81	0.42	11.37	11.07	0.015	0.757	N/A	0.007	0.025	0.0	15.8
284	Phe	4.65	0.27	5.83	0.53	9.43	7.79	0.006	0.572	N/A	0	0	0.0	0.0
285	Ser	7.50	0.29	8.39	0.39	13.67	12.25	0.001	0.737	N/A	0.04	0.028	27.4	21.6
286	Lys	12.65	0.46	13.58	0.61	16.26	15.03	0.011	0.768	N/A	0.085	0.044	50.6	23.9
287	Thr	n. o	n. o	n. o	n. o	10.37	9.76	n. o	0.595	N/A	0.012	0.008	0.0	3.7
288	Asn	6.12	0.34	5.87	0.07	12.29	10.44	0.003	0.638	N/A	0.039	0.028	28.5	24.2
289	Glu	12.36	0.82	13.37	0.73	20.83	20.75	0.004	0.861	N/A	0.122	0.125	73.6	86.5
290	Leu	n. o	n. o	n. o	n. o	17.61	16.75	n. o	0.735	N/A	0.067	0.073	23.0	48.2
291	Leu	n. o	n. o	n. o	n. o	12.86	14.26	n. o	0.651	N/A	0.002	0.01	0.0	1.3
292	Gln	8.64	0.51	10.82	1.28	23.07	22.11	0.005	0.919	N/A	0.153	0.125	73.0	86.3
293	Lys	13.22	0.64	14.84	0.91	34.22	37.48	0.005	1.032	N/A	0.306	0.318	190.7	212.8
294	Ser	14.24	0.24	15.01	0.99	32.97	36.49	0.007	0.908	N/A	0.324	0.355	236.9	268.3
295	Gly	14.18	0.36	19.62	1.99	59.08	76.45	0.006	1.078	N/A	0.602	0.694	403.8	450.8
296	Lys	13.46	0.43	14.16	0.25	37.64	45.04	0.004	0.98	N/A	0.383	0.479	287.3	359.2
297	Lys	28.9	1.57	29.36	2.69	48.92	53.30	0.007	0.989	N/A	0.387	0.395	232.7	256.4
298	Pro	—	—	—	—	—	—	—	0.864	N/A	0.094	0.078	67.7	66.3
299	Ile	5.44	0.48	6.22	0.16	12.48	11.59	0.005	0.582	N/A	0.043	0.023	25.3	14.2
300	Asp	7.76	0.12	9.49	0.21	17.44	17.75	0.007	0.685	N/A	0.104	0.096	63.8	60.0
301	Trp	6.81	0.06	6.93	0.39	13.16	13.52	0.01	0.501	N/A	0.05	0.058	30.5	41.6
302	Lys	8.07	0.58	9.15	0.78	16.46	17.88	0.006	0.478	N/A	0.056	0.124	23.2	89.2
303	Glu	9.46	0.57	12.55	1.49	18.20	20.40	0.006	0.594	N/A	0.085	0.164	19.7	128.4
304	Leu	23.83	1.43	20.95	2.31	30.40	38.47	0.005	0.61	N/A	0.262	0.315	167.8	198.6

[a] The PRE values were measured using samples with $[Gd^{3+}]$ = 1.0, 2.5, 5.0, 10.0 mM. The linear slopes of the PRE values at different Gd^{3+} concentrations were obtained as the molar relaxivity (Γ_2^{Meas}). Errors in the relaxation rates were propogated from the estimated errors in the peak intensities by measuring corresponding variances in the duplicated spectra acquired.

[b] n. a. ≡ no assignment.

[c] No amide protons because they are proline residues.

[d] n. o. ≡ not observable. For ^2H labeled hUNG, these residues exchange with water extremely slowly, so they show either no or very weak signals in the ^1H-^{15}N HSQC spectra.

[e] The predicted PREs (Γ_2^{Pred}) were calculated using the grid-based approach (1, 2) and the atom coordinates obtained from the crystal structures of the free enzyme (1AKZ) and its non-specific DNA complex (2OXM).

[f] Heteronuclear (^1H, ^{15}N) weighted chemical shift perturbations.

[g] Amide nitrogen displacement using the lowest frequency normal mode analysis for the free hUNG (pdb code 1AKZ).

[h] Exchange constants that describes dynamic exchange between two states. The exchange rates were evaluated using NMR relaxation experiments previsouly (3)

[i,j] Depth index and accessible surface area (ASA) calculated using SADIC program and Gd^{3+} probe size in the form of ProHance (3.5 Å)(1, 4) .

Table S2. Summary of structural and dynamic properties of residues in the protected sites

Res.	Amino acid	polarity	Structure feature [a]	Conservation score [b]	Depth Index [c]		$\Gamma_2^{Meas'}/\Gamma_2^{Pred'}$ [d]	
					free	DNA-bound	Free	DNA-bound
95	Gly	nonpolar	h	2.431	0.459	0.377	0.25	0.31
96	Glu	negative	h	-0.78	0.215	0.2	0.29	0.43
97	Phe	nonpolar	h	-0.357	0.214	0.277	0.30	0.32
98	Gly	nonpolar	h	2.428	0.458	0.49	0.23	0.20
99	Lys	positive	c	-0.025	0.337	0.403	0.20	0.22
100	Pro	nonpolar	h	0.838	0.596	0.507	N/A	N/A
101	Tyr	polar	h	-0.862	0.451	0.384	0.23	0.30
102	Phe	nonpolar	h	-0.319	0.111	0.08	0.21	0.65
103	Ile	nonpolar	h	2.401	0.153	0.193	0.26	0.33
104	Lys	psotive	h	2.433	0.276	0.264	0.23	0.26
105	Leu	nonpolar	h	-0.516	0.116	0.1	0.22	0.36
106	Met	nonpolar	h	0.62	0.108	0.127	N/A	N/A
107	Gly	nonpolar	h	2.434	0.297	0.315	0.33	0.33
108	Phe	nonpolar	h	0.699	0.23	0.239	0.33	0.40
182	Glu	negative	c	2.323	0.372	0.264	0.32	0.37
183	Asp	negative	c	-0.148	0.376	0.411	0.38	0.38
184	Phe	nonpolar	c	0.366	0.247	0.164	0.31	0.37
185	Val	nonpolar	c	2.434	0.236	0.295	0.48	0.14
186	His	polar	c	0.38	0.522	0.498	0.2	0.21
187	Pro	nonpolar	c	-0.881	0.479	0.322	N/A	N/A
188	Gly	nonpolar	c	2.409	0.371	0.411	0.32	0.63
292	Gln	polar	h	2.434	0.153	0.125	0.37	0.48
293	Lys	positive	h	2.433	0.306	0.318	0.38	0.39
294	Ser	polar	h	0.892	0.324	0.355	0.43	0.41
295	Gly	nonpolar	c	-0.428	0.602	0.694	0.24	0.25
296	Lys	positive	c	2.227	0.383	0.479	0.35	0.31
297	Lys	positive	c	2.434	0.387	0.395	0.58	0.54

[a] h ≡ helical; c ≡ coil

[b] Lower conservation score indicates the functionally important amino acids on the protein surface which have slow evolution rates and was calculated through the web server http://consurf.tau.ac.il/ based on the phylogenetic relations between its close sequence homologues.

[c] Depth index calculated using SADIC program and Gd^{3+} probe size in the form of ProHance (3.5 Å).

[d] The ratios between the measured and predicted PRE values across the protein sequence for free hUNG and its complex with DNA.

Table S3. Summary of structural and dynamic properties of residues in the hyper-reactive sites

Res.	Amino acid	polarity	Structure Feature [a]	Conservation score [b]	Depth index [c]		$\Gamma_2^{Meas}/\Gamma_2^{Pred}$ [d]	
					free	DNA-bound	free	DNA-bound
129	Thr	polar	h	-0.385	0.002	0.004	3.97	7.70
130	Gln	polar	h	0.596	0.074	0.04	1.66	2.72
131	Met	nonpolar	h	2.374	0.096	0.084	2.05	2.11
132	Cys	polar	c	-0.765	0.112	0.105	1.78	1.95
144	Gln	polar	c	-0.952	0.028	0	8.09	15.23
145	Asp	negative	c	-0.952	0.053	0	6.34	15.68
169	Ser	polar	h	-0.955	0.173	0.011	1.32	4.42
170	Leu	nonpolar	h	-0.945	0.035	0.007	0.83	2.25
214	Ala	nonpolar	c	-0.715	0.556	0.042	0.27	3.05
215	Asn	polar	c	-0.578	0.173	0.006	1.74	6.18
216	Ser	polar	c	-0.937	0.158	0.01	0.66	3.97
246	Gly	nonpolar	c	-0.945	0.078	0.021	4. 7	6.89
247	Ser	polar	h	0.04	0.285	0.061	N/A	N/A
248	Tyr	polar	h	-0.382	0.218	0.031	N/A	N/A
249	Ala	nonplar	h	-0.954	0.037	0.007	5.85	9.71
250	Gln	polar	h	-0.412	0.067	0.063	2.09	2.81
268	His	polar	c	-0.953	0.097	0.011	4.00	7.95
269	Pro	nonpolar	c	-0.947	0.001	0	N/A	N/A
270	Ser	polar	c	-0.955	0.06	0	5.36	12.76
271	Pro	nonpolar	c	-0.913	0.273	0	N/A	N/A
272	Leu	nonpolar	c	-0.682	0.426	0	1.45	8.16
273	Ser	polar	c	-0.921	0.159	0	1.09	4.30
274	Val	nonpolar	c	-0.712	0.09	0	2.44	7.94

[a] h ≡ helical; c ≡ coil; s ≡ strand

[b] The lower conservation score indicates the functionally important amino acids on the protein surface which have slow evolution rates and was calculated through the web server http://consurf.tau.ac.il/ based on the phylogenetic relations between its close sequence homologues.

[c] Depth index calculated using SADIC program and Gd^{3+} probe size in the form of ProHance (3.5 Å).

[d] The ratios between the measured and predicted PRE values across the protein sequence for free hUNG and its complex with DNA.

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