Electronic Supplementary Material (ESI) for Chemical Science.

A combination of solid-state NMR and MD simulations reveals the binding mode of a rhomboid protease inhibitor

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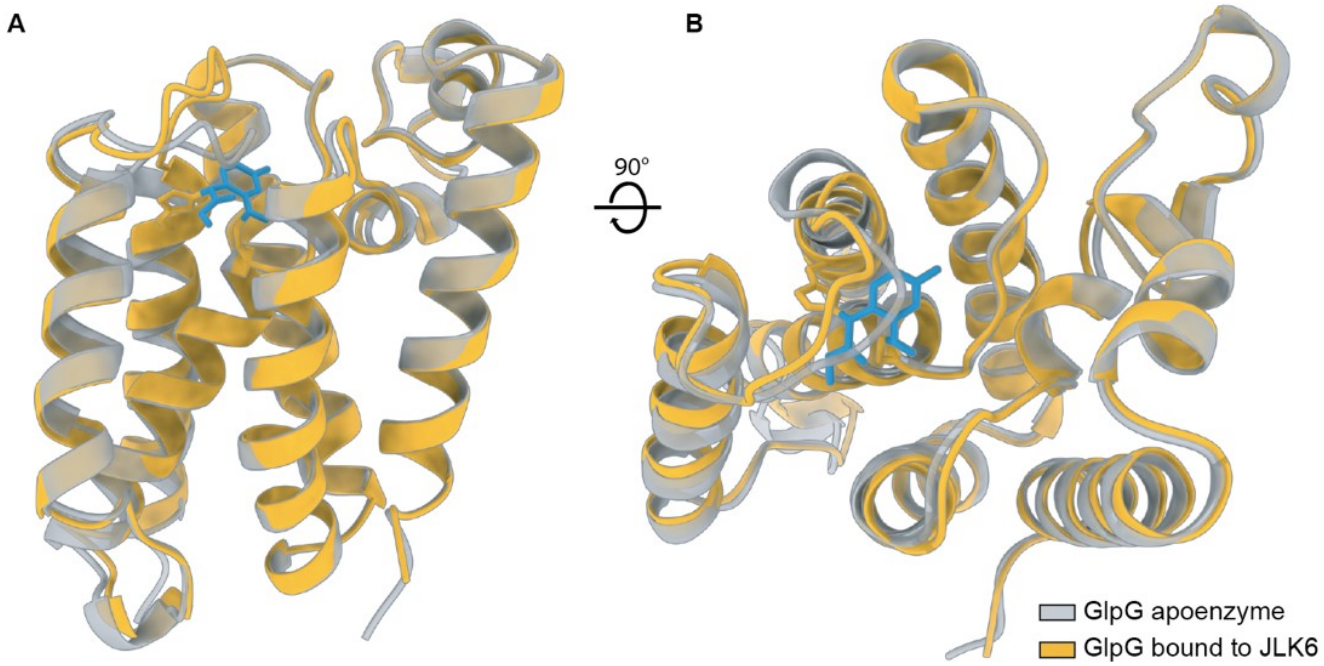

Figure S1. 3D Structures of GlpG with (yellow, PDB ID: 2XOW) and without (grey, PDB ID: 2IC8) JLK6 inhibitor bound. **A.** Side view of GlpG with and without JLK6 inhibitor bound. **B.** Top view of GlpG with and without JLK6 inhibitor bound. Adapted from Vinothkumar et al. [1].

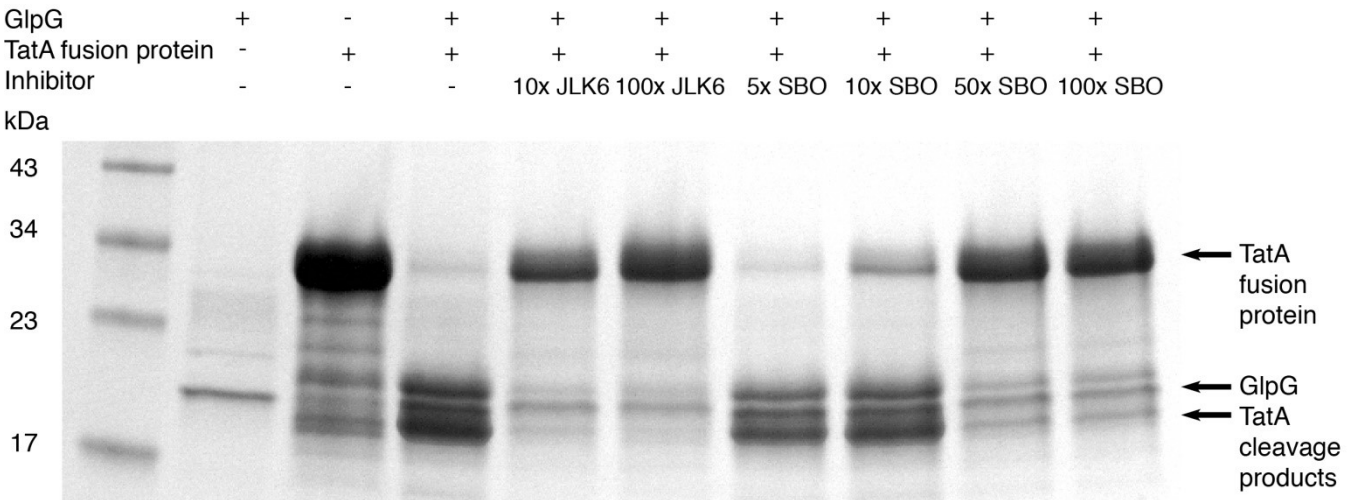

Figure S2. SDS-PAGE of GlpGΔN digesting TatA fusion protein (6His-SUMO-TatA-FLAG) in liposomes without and with inhibitors at different concentrations. Cleavage products are SUMO-TatA_N and TatA-FLAGΔN. 10x JLK6 and 50x SBO fully inhibit the substrate cleavage through GlpG.

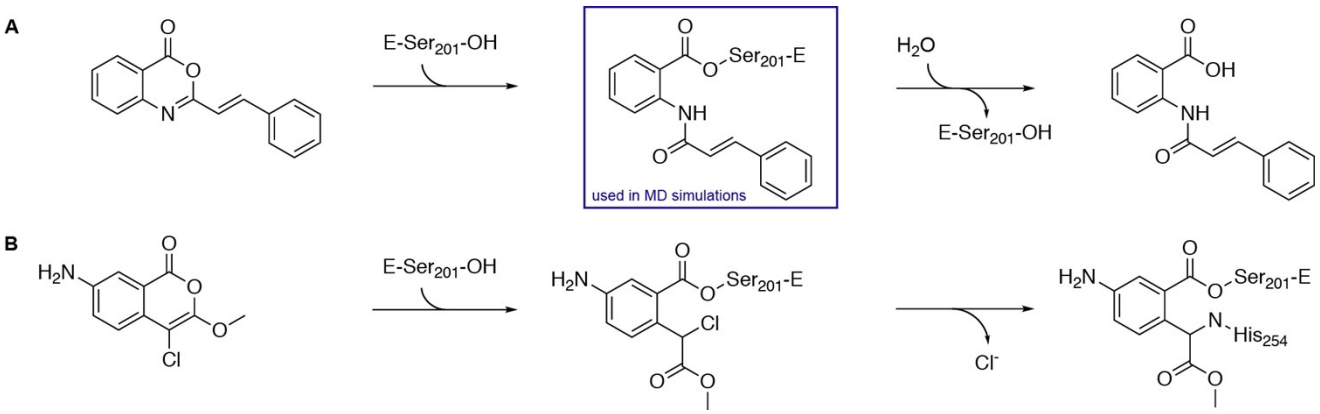

Figure S3. Reaction of 2-styryl-4*H*-3,1-benzoxazin-4-one (SBO, top) and 7-amino-4-chloro-3-methoxy-1*H*-2-benzopyran (JLK6, bottom). JLK6 blocks the enzyme irreversibly and SBO blocks it in a reversible manner[1–3]. **A.** The reaction of S201 with SBO results in ring opening and formation of an acyl intermediate followed by reactivation upon hydrolysis. The SBO intermediate bound to GlpG used in our MD simulations is highlighted by the blue box. **B.** The nucleophilic attack of S201 on JLK6 results in ring opening and formation of an acyl intermediate. The subsequent reaction with H254 leads to a double covalently bonded end product with the enzyme.

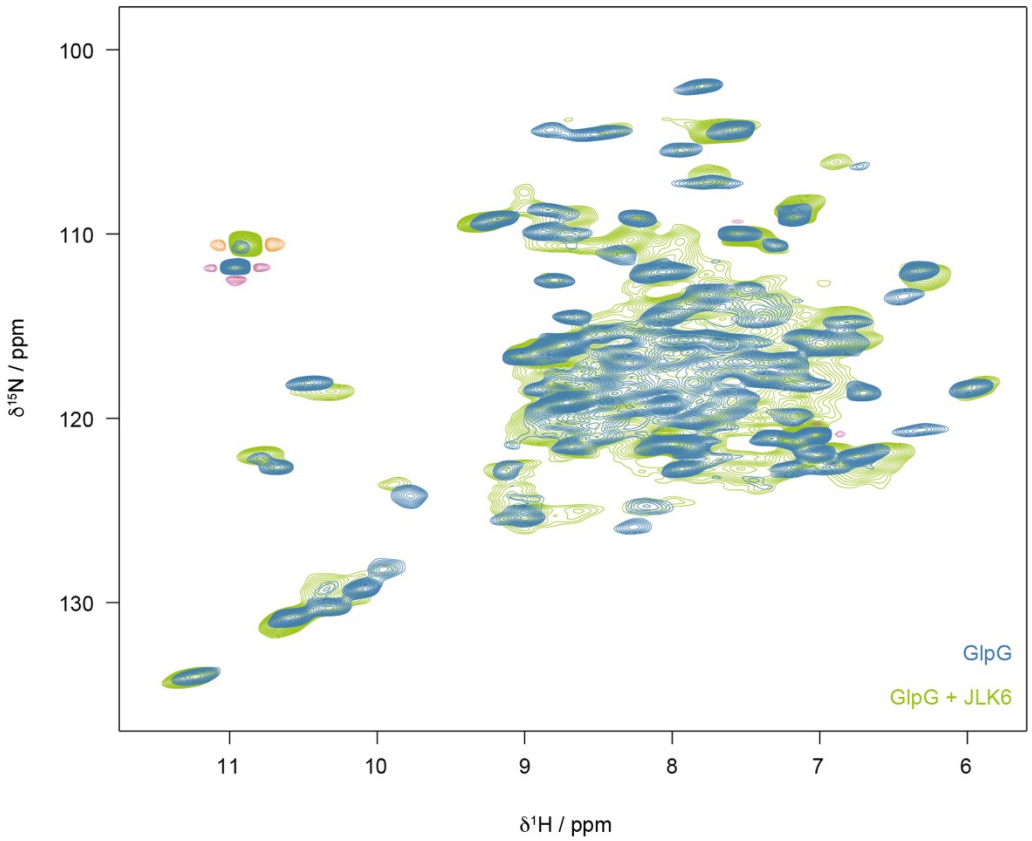

Figure S4. 2D ^{1}H-^{15}N correlation spectra of GlpG (blue) and GlpG bound to JLK6 (green). The spectra were recorded on a 600 MHz spectrometer using a 1.9 mm probe, operating at an MAS rate of 40 kHz, and at a sample temperature of 22 °C.

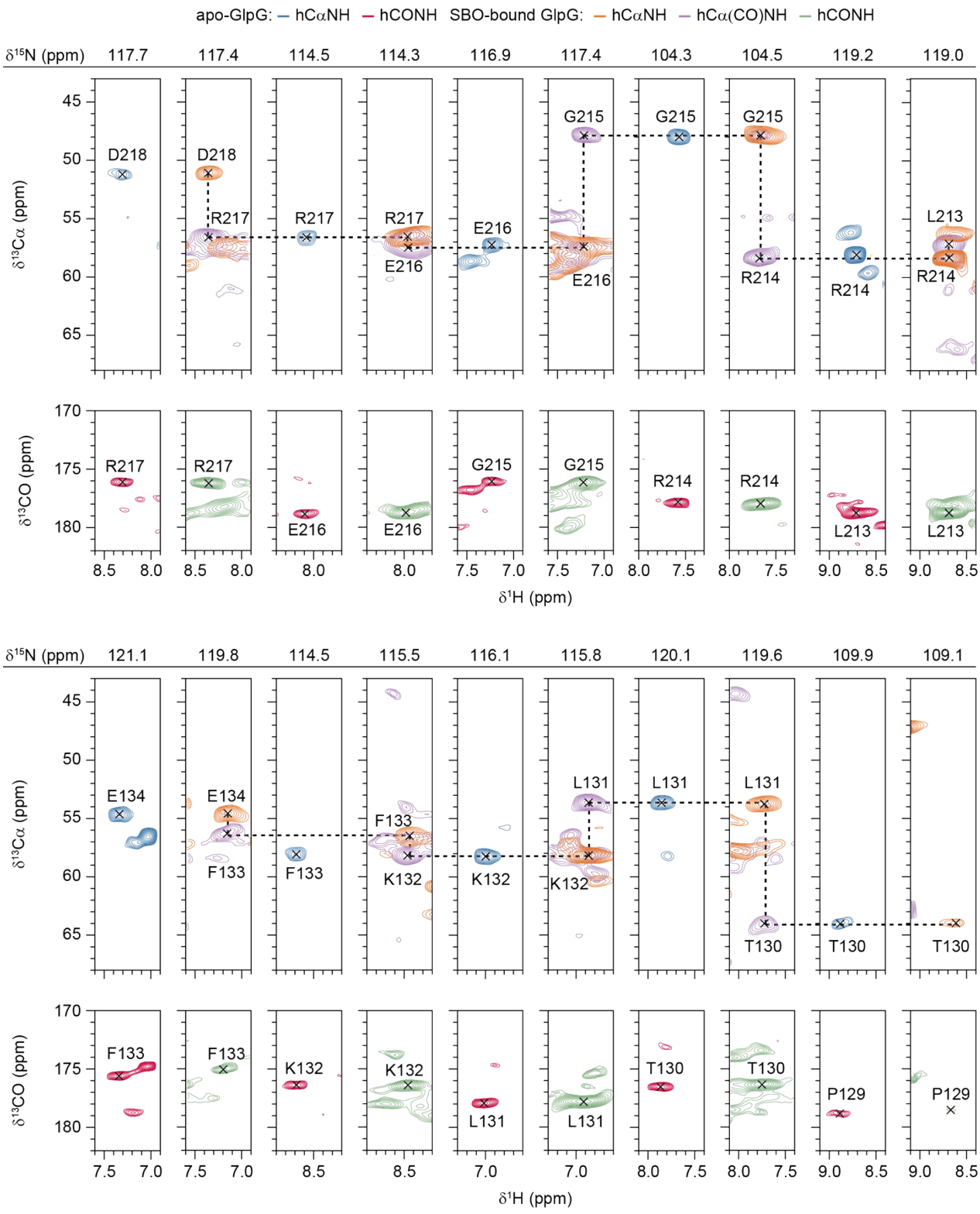

Figure S5. Assignment transfer from apo-GlpG to SBO-bound GlpG. The inhibitor-bound samples were assigned, based on the assignments of apo-GlpG, using 3D hCαNH, hCα(CO)NH and hCONH spectra. The top panel shows the assignment procedure for residues D218 to L213 and the bottom panel for residues E134 to P129. First the H, N and Cα chemical shifts for residue *i* were identified by comparing the hCαNH spectra between apo-GlpG (blue) and SBO-bound GlpG (orange). The CO chemical shift for residue *i-1* was identified by comparing the hCONH spectra (red for apo-GlpG and green for SBO-bound GlpG). The Cα chemical shift for residue *i-1* was identified in the hCα(CO)NH spectrum of SBO-bound GlpG (purple). The H and N chemical shifts for residue *i-1* were identified in the hCαNH spectrum of SBO-bound GlpG (orange), again comparing with the hCαNH spectrum of apo-GlpG (blue). A backbone walk could then be performed using the hCαNH and hCα(CO)NH spectra (indicated by a dashed line), aided by comparisons of the hCONH spectra. The labelled peaks in the spectra represent the assigned ^{13}C chemical shifts. In the HCαNH spectra a labelled peak represents the H, N and Cα chemical shifts of residue *i*, in the hCα(CO)NH spectrum a labelled peak represents the H and N chemical shifts of residue *i* and the Cα chemical shift of residue *i-1*, and in the hCONH spectra a labelled peak represents the H and N chemical shifts of residue *i* and the CO chemical shift of residue *i-1*.

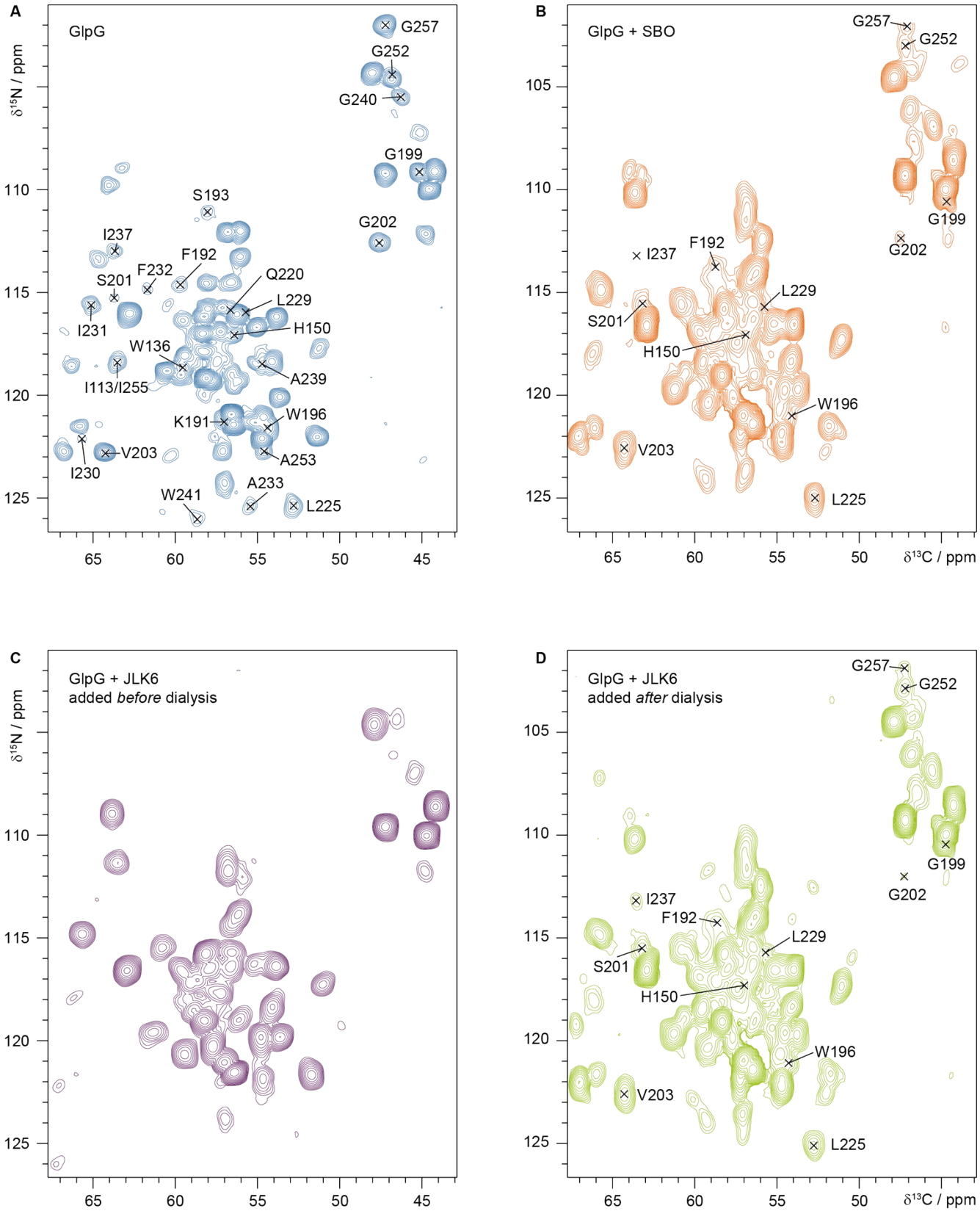

Figure S6. 2D NCα projections from 3D hCαNH spectra of GlpG alone (**A**), with SBO (**B**), with JLK6 added before dialysis (**C**) and JLK6 added after dialysis (**D**). Disappearing and "re-appearing" residues are indicated in the spectra. The spectra were recorded on a 600 MHz spectrometer using a 1.9 mm probe, operating at an MAS rate of 40 kHz, and at a sample temperature of 22 °C.

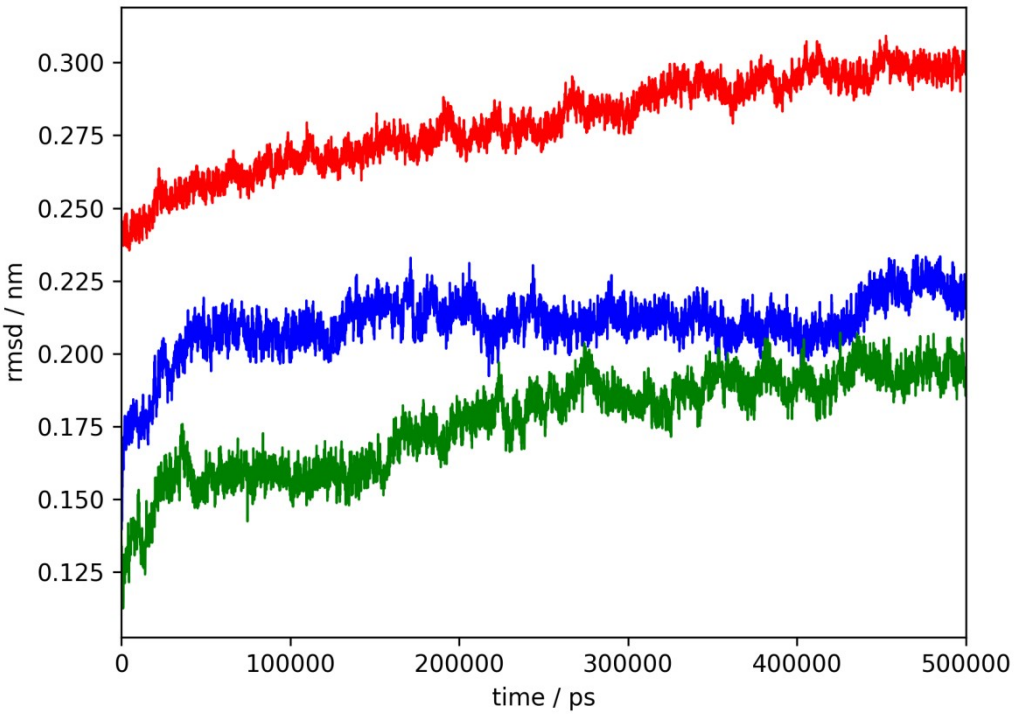

Figure S7. Evolution of the averaged RMSD of the protein backbone starting from *poseA* (blue), *poseB* (green), and apo-GlpG (red).

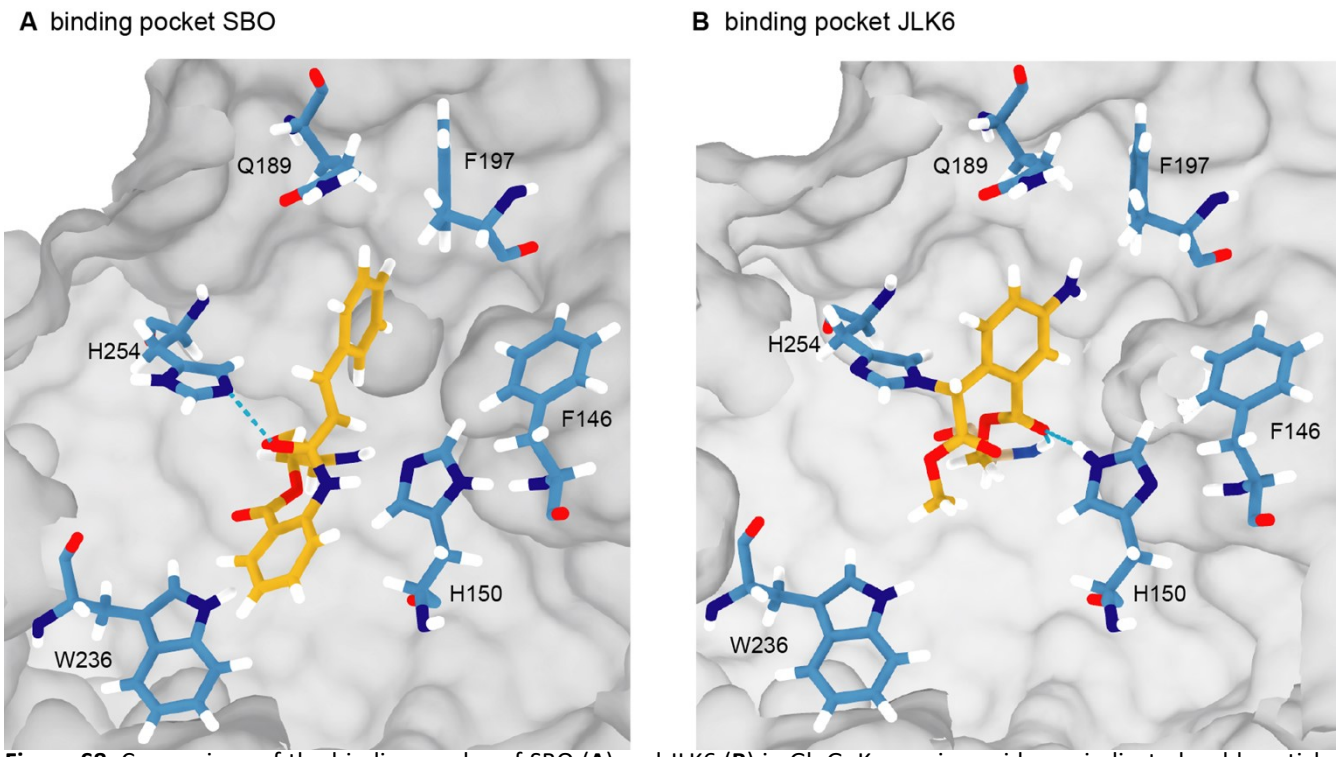

Figure S8. Comparison of the binding modes of SBO (**A**) and JLK6 (**B**) in GlpG. Key amino acids are indicated as blue sticks, inhibitors are shown in yellow and light blue dashed lines indicate hydrogen bonds.

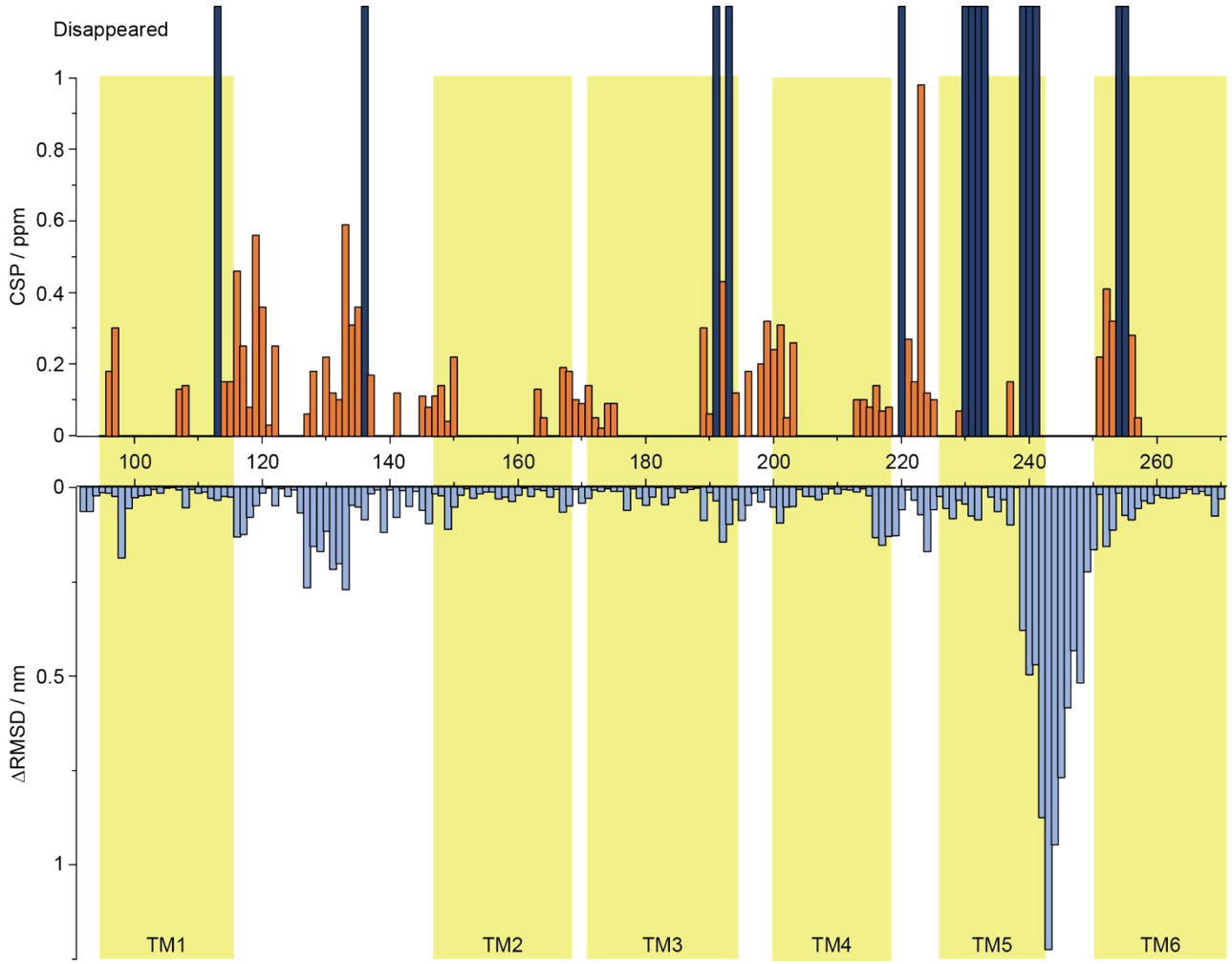

Figure S9. Comparison between CSPs based on solid-state NMR data and RMSD values based on MD simulations. The CSPs between apo-GlpG and SBO-bound GlpG (top) are shown as orange bars, dark blue bars represent residues that disappear upon binding of SBO. RMSD differences (ΔRMSD) calculated between simulations of apo-GlpG and SBO-bound GlpG (bottom) are shown as light blue bars. TM helices are indicated with yellow shading.

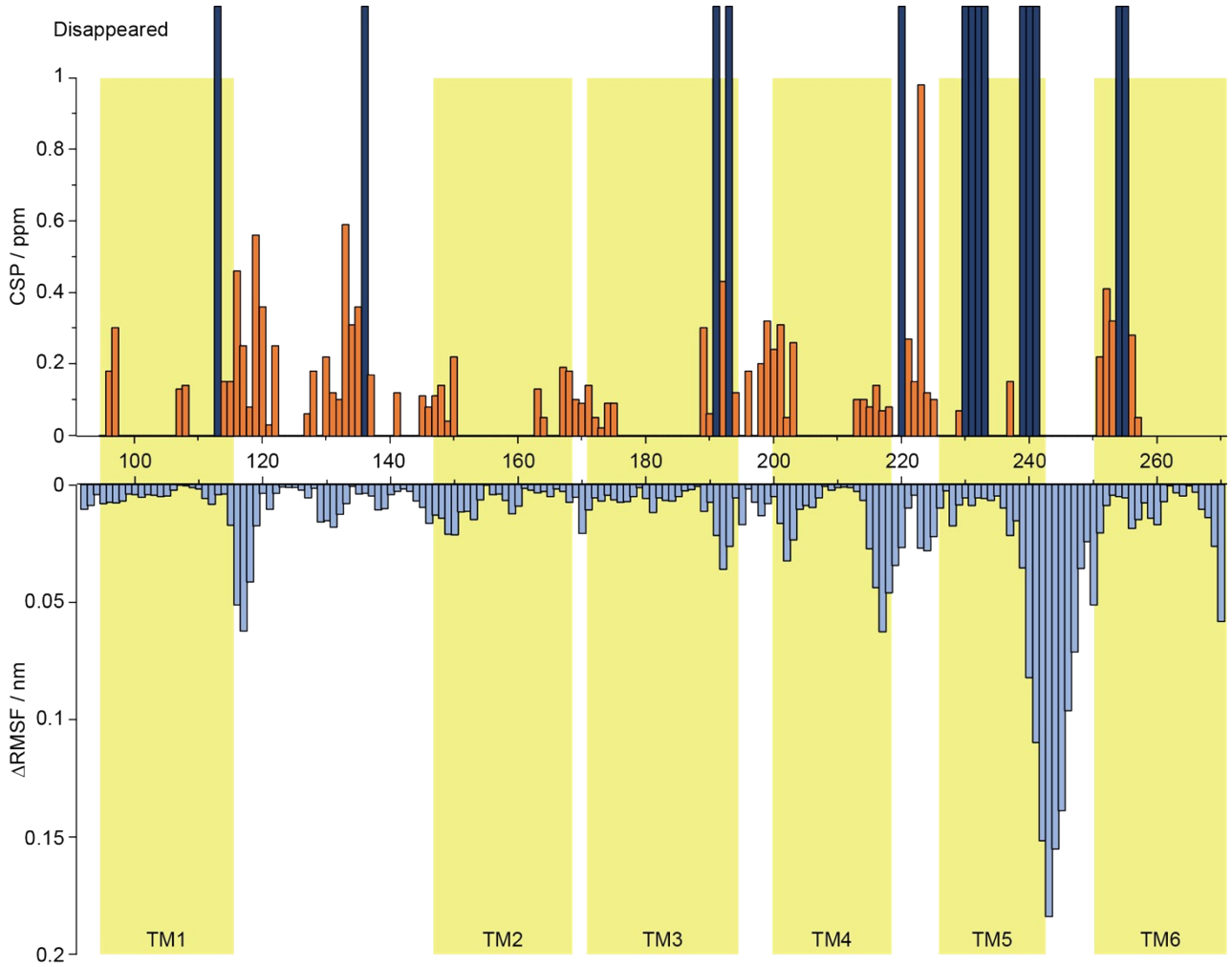

Figure S10. Comparison between CSPs based on solid-state NMR data and RMSF values based on MD simulations. The CSPs between apo-GlpG and SBO-bound GlpG (top) are shown as orange bars, dark blue bars represent residues that disappear upon binding of SBO. RMSF differences (ΔRMSF) calculated between simulations of apo-GlpG and SBO-bound GlpG (bottom) are shown as light blue bars. TM helices are indicated with yellow shading.

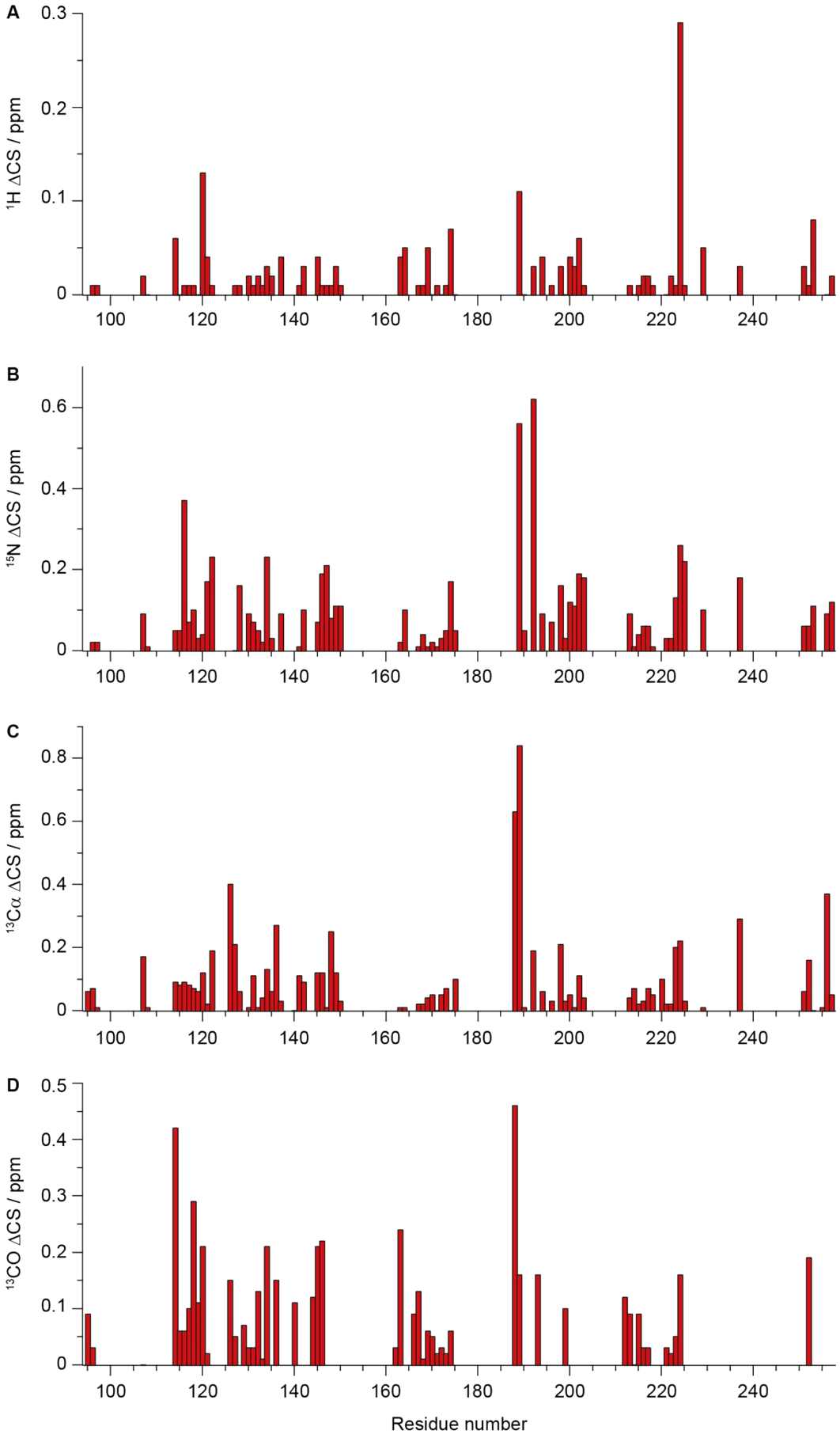

Figure S11. Chemical shift comparison between JLK6 and SBO bound GlpG. The absolute chemical shift differences are plotted as a function of residue number for ^{1}H in **A**, ^{15}N in **B**, ^{13}Ca in **C** and ^{13}CO in **D**. The plots are based on the chemical shift assignments listed in tables S2 and S3.

Tables

Table S1. Chemical shift assignments (ppm) for apo-GlpG based on previous assignments[4]. Only residues that could be identified in 3D hCaNH and hCONH spectra are included.

Residue		H	N	C	CA	Residue		H	N	C	CA
95	Pro	-	-	179.1	65.37	188	Val	-	-	176.82	66.19
96	Val	10.68	122.7	179.83	66.85	189	Gln	8.03	119.28	178.22	57.82
97	Thr	8.39	118.84	-	60.49	190	Gln	7.66	116.02	178.75	56.21
106	Val	-	-	177.46	-	191	Lys	7.18	121.22	177.95	56.95
107	Val	8.23	118.53	178.24	66.27	192	Phe	7.41	114.6	178.29	59.74
108	Phe	8.81	119.34	-	56.22	193	Ser	8.35	111.08	175.31	58.02
112	Gln	-	-	177.78	56.47	194	Gly	8.15	112.11	-	44.75
113	Ile	7.84	118.04	177.6	63.46	195	Pro	-	-	174.48	64.44
114	Leu	9.03	116.67	178.78	55.03	196	Trp	7.87	121.56	-	54.37
115	Gly	7.18	109.11	173.11	44.2	197	Phe	-	-	172.78	55.62
116	Asp	7.88	118.17	178.65	57.39	198	Gly	8.53	104.62	173.36	46.82
117	Gln	8.31	116.33	177.26	59.5	199	Gly	8.25	109.12	-	45.16
118	Glu	8.24	118.35	176.96	58.98	200	Leu	8,744	119.22	178.59	54.56
119	Val	6.41	113.36	178.67	64.58	201	Ser	8.46	115.41	176.54	63.98
120	Met	8.57	119	178.06	59.61	202	Gly	8.8	112.55	174.97	47.57
121	Leu	7.91	115.79	180.5	58	203	Val	7.93	122.77	-	64.28
122	Trp	7.39	117.85	-	58.29	212	Trp	-	-	178.34	61.19
126	Pro	-	-	177.72	64.68	213	Leu	8.23	117.03	178.76	57.22
127	Phe	5.97	118.35	173.25	54.11	214	Arg	8.72	119.16	177.84	58.06
128	Asp	7.02	122.01	-	51.36	215	Gly	7.57	104.32	176.06	47.99
129	Pro	-	-	178.81	64.74	216	Glu	7.24	116.89	178.83	57.24
130	Thr	8.89	109.85	176.52	64.02	217	Arg	8.08	114.5	176.12	56.55
131	Leu	7.86	120.08	177.88	53.66	218	Asp	8.31	117.68	-	51.22
132	Lys	7	116.14	176.38	58.24	219	Pro	-	-	178.6	64.39
133	Phe	8.68	114.54	175.59	58.11	220	Gln	7.95	115.85	176.07	56.69
134	Glu	7.34	121.09	176.76	54.62	221	Ser	7.72	115.6	176.04	60.41
135	Phe	7.46	116.97	174.86	58.65	222	Gly	7.76	107.22	173.22	45.12
136	Trp	6.71	118.63	176.93	59.58	223	Ile	7.78	119.69	173.38	58.08
137	Arg	7.18	122.69	-	57.1	224	Tyr	7.6	119.06	173.11	56.51
140	Thr	-	-	174.78	65.61	225	Leu	9	125.4	-	52.79
141	His	7.03	120.93	-	56.58	228	Gly	-	-	174.7	45.13
144	Met	-	-	171.59	-	229	Leu	6.81	115.99	179.5	55.73
145	His	6.33	111.99	175.32	56.01	230	Ile	7.92	121.87	177.43	65.68
146	Phe	10.46	118.07	175.31	59.37	231	Ile	7.1	115.61	176.82	65.1
147	Ser	8.03	112.05	174.02	56.8	232	Phe	6.79	114.78	177.6	61.6
148	Leu	9.78	124.2	177.72	56.97	233	Ala	9.21	125.52	-	55.46
149	Met	8.77	115.87	178.67	58.05	236	Trp	-	-	177.21	57.58
150	His	7.79	117.06	-	56.44	237	Ile	7.48	112.97	-	63.67
162	Gly	-	-	175.5	46.3	238	Val	-	-	177.69	66.86
163	Gly	6.71	106.33	176.34	46.62	239	Ala	8.7	118.44	180.36	54.67
164	Ala	6.75	122.07	-	54.72	240	Gly	7.94	105.46	177.22	46.27
166	Glu	-	-	179.29	58.66	241	Trp	8.26	125.94	-	58.62
167	Lys	8.32	116.99	178.28	58.28	250	Ala	-	-	177.84	50.53
168	Arg	7.76	113.27	177.44	56.04	251	Asn	8.5	121.03	178.36	55.41
169	Leu	8.8	116.19	177.76	53.81	252	Gly	8.82	104.4	174.44	46.82
170	Gly	7.57	109.96	173.84	44.52	253	Ala	6.96	122.68	179.79	54.57
171	Ser	8.71	116.03	175.91	62.77	254	His	7.07	118.16	177.2	56.98
172	Gly	9.19	109.19	174.83	47.22	255	Ile	8.33	118.4	176.98	63.47
173	Lys	8.02	121.43	177.77	56.43	256	Ala	7.92	121.29	178.74	55.15
174	Leu	6.99	115.77	180.01	57.07	257	Gly	7.8	101.99	-	47.25
175	Ile	8.69	121.52	-	65.82						

Table S2. Chemical shift assignments (ppm) for GlpG with JLK6.

Residue		H	N	C	CA	Residue		H	N	C	CA
95	Pro	-	-	178.93	65.25	168	Arg	7.83	113.96	177.36	56.27
96	Val	10.77	122.03	179.56	67.04	169	Leu	8.92	116.52	177.64	54
97	Thr	8.28	119.67	-	61.25	170	Gly	7.5	109.98	173.98	44.71
106	Val	-	-	177.49	-	171	Ser	8.72	116.6	176	62.97
107	Val	8.12	118.14	177.97	66.37	172	Gly	9.25	109.3	174.9	47.24
108	Phe	8.65	119	-	56.47	173	Lys	8	121.35	177.87	56.4
113	Ile	-	-	177.11	63.14	174	Leu	7.12	116.11	180.1	57.32
114	Leu	8.86	116.5	178.08	55.12	175	Ile	8.65	121.62	-	65.98
115	Gly	7.12	108.49	174.01	44.21	188	Val	-	-	176.49	65.66
116	Asp	8.04	119.84	178.67	57.71	189	Gln	7.73	118.59	178.06	58.38
117	Gln	8.62	116.93	177.39	59.05	190	Gln	7.64	116.17	-	56.37
118	Glu	8.13	118.31	177.8	58.76	192[a]	Phe	7.35	114.25	-	58.57
119	Val	6.86	114.85	178.72	65.82	193	Ser	-	-	175.07	-
120	Met	8.88	120.43	177.66	59.33	194	Gly	8.02	111.8	-	44.89
121	Leu	7.9	115.88	179.43	58.04	196[a]	Trp	7.68	120.99	-	54.37
122	Trp	7.37	117.15	-	58.58	198	Gly	8.36	104.27	-	46.74
126	Pro	-	-	177.52	64.59	199[a]	Gly	8.26	110.25	177.51	44.82
127	Phe	5.91	118.34	173.28	54.06	200[a]	Leu	8.92	120.4	-	54.65
128	Asp	7.08	121.63	-	51.74	201[a]	Ser	8.19	115.53	-	63.21
129	Pro	-	-	178.56	64.73	202[a]	Gly	8.89	112.17	-	47.44
130	Thr	8.67	109.15	176.32	63.97	203[a]	Val	7.51	122.68	-	64.34
131	Leu	7.73	119.69	177.83	53.82	212	Trp	-	-	178.23	60.77
132	Lys	6.9	115.84	176.51	58.2	213	Leu	8.2	117.53	178.67	57.28
133	Phe	8.43	115.5	175.08	56.47	214	Arg	8.7	119.04	177.93	58.29
134	Glu	7.19	119.53	177.26	54.54	215	Gly	7.68	104.46	176.22	47.9
135	Phe	7.55	116.89	-	59.83	216	Glu	7.24	117.49	178.74	57.43
136	Trp	-	-	176.61	59.98	217	Arg	8	114.38	176.15	56.53
137	Arg	7.02	121.9	-	57.25	218	Asp	8.36	117.37	-	51.06
140	Thr	-	-	175.19	65.68	220	Gln	-	-	-	57.3
141	His	7.12	120.93	-	57.04	221	Ser	8.14	115.55	176.08	60.82
142	Ala	6.66	122.53	-	52	222	Gly	7.7	106.9	173.28	45.5
144	Met	-	-	171.7	-	223	Ile	7.39	115.42	173.35	58.58
145	His	6.29	112.44	175.09	55.98	224	Tyr	7.38	118.36	173.25	56.51
146	Phe	10.35	118.49	175.02	59.6	225[a]	Leu	8.95	125.21	-	52.7
147	Ser	8.05	111.74	-	56.87	229[a]	Leu	6.7	115.83	-	55.82
148	Leu	9.82	123.53	-	57.1	237[a]	Ile	7.47	113.17	-	63.56
149	Met	8.74	115.9	-	58.17	251	Asn	8.49	120.58	-	54.88
150[a]	His	7.59	117.37	-	57.03	252[a]	Gly	8.53	102.94	176.11	47.19
162	Gly	-	-	175.48	-	253	Ala	6.6	121.94	-	54.74
163	Gly	6.91	106.08	176.14	46.81	255	Ile	-	-	-	62.95
164	Ala	6.78	122.2	-	54.71	256	Ala	7.95	121.49	-	56.4
166	Glu	-	-	179.11	-	257[a]	Gly	7.77	101.92	-	47.18
167	Lys	8.22	116.41	178.23	57.88						

[a] Amide protons only back-exchanged when JLK6 was added after dialysis.

Table S3. Chemical shift assignments (ppm) for GlpG with SBO.

Residue		H	N	C	CA	Residue		H	N	C	CA
95	Pro	-	-	178.84	65.19	171	Ser	8.71	116.59	175.98	62.97
96	Val	10.76	122.01	179.53	67.11	172	Gly	9.25	109.33	174.87	47.19
97	Thr	8.29	119.65	-	61.24	173	Lys	8.01	121.4	177.89	56.47
107	Val	8.1	118.05	177.97	66.2	174	Leu	7.05	115.94	180.04	57.32
108	Phe	8.65	118.99	-	56.46	175	Ile	8.65	121.57	-	66.08
114	Leu	8.8	116.55	178.5	55.21	188	Val	-	-	176.95	65.03
115	Gly	7.12	108.54	174.07	44.29	189	Gln	7.84	118.03	178.22	57.54
116	Asp	8.05	120.21	178.73	57.62	190	Gln	7.64	116.12	-	56.38
117	Gln	8.63	116.86	177.29	59.13	191	Lys			177.83	
118	Glu	8.14	118.41	177.51	58.83	192	Phe	7.38	114.87	178.23	58.38
119	Val	6.86	114.88	178.61	65.76	193	Ser	8.39	110.67	175.23	-
120	Met	8.75	120.47	177.87	59.21	194	Gly	7.98	111.89	-	44.83
121	Leu	7.86	115.71	179.41	58.02	195	Pro	-	-	174.35	-
122	Trp	7.36	116.92	-	58.77	196	Trp	7.67	120.92	-	54.4
126	Pro	-	-	177.37	64.99	197	Phe	-	-	172.73	55.88
127	Phe	5.92	118.34	173.33	54.27	198	Gly	8.33	104.11	173.54	46.53
128	Asp	7.07	121.47	-	51.8	199	Gly	8.26	110.28	177.61	44.85
129	Pro	-	-	178.63	-	200	Leu	8.88	120.28	-	54.6
130	Thr	8.65	109.06	176.35	63.96	201	Ser	8.16	115.64	176.48	63.2
131	Leu	7.74	119.62	177.8	53.71	202	Gly	8.83	112.36	175.06	47.55
132	Lys	6.88	115.79	176.38	58.21	203	Val	7.5	122.5	-	64.3
133	Phe	8.44	115.52	175.09	56.43	212	Trp	-	-	178.35	-
134	Glu	7.16	119.76	177.05	54.67	213	Leu	8.19	117.44	178.76	57.32
135	Phe	7.53	116.86	-	59.77	214	Arg	8.7	119.03	177.93	58.36
136	Trp	-	-	176.76	59.71	215	Gly	7.67	104.5	176.13	47.88
137	Arg	7.06	121.99	-	57.22	216	Glu	7.22	117.43	178.77	57.46
140	Thr	-	-	175.3	65.68	217	Arg	7.98	114.32	176.18	56.6
141	His	7.11	120.94	177.38	56.93	218	Asp	8.37	117.36	-	51.11
142	Ala	6.63	122.63	-	51.91	220	Gln	-	-	-	57.2
144	Met	-	-	171.58	55.58	221	Ser	8.14	115.58	176.05	60.8
145	His	6.25	112.37	175.3	55.86	222	Gly	7.72	106.93	173.3	45.52
146	Phe	10.36	118.3	175.24	59.48	223	Ile	7.4	115.29	173.4	58.38
147	Ser	8.04	111.53	173.95	56.86	224	Tyr	7.67	118.62	173.09	56.73
148	Leu	9.83	123.61	177.92	56.85	225	Leu	8.94	124.99	-	52.73
149	Met	8.71	115.79	178.27	58.05	228	Gly	-	-	175.42	-
150	His	7.58	117.26	-	57	229	Leu	6.75	115.73	-	55.83
162	Gly	-	-	175.51	-	236	Trp	-	-	177.27	58.57
163	Gly	6.87	106.1	176.38	46.82	237	Ile	7.5	113.35		63.27
164	Ala	6.73	122.3	-	54.72	251	Asn	8.46	120.52	179.18	54.82
166	Glu	-	-	179.2	58.88	252	Gly	8.52	103	175.92	47.03
167	Lys	8.21	116.4	178.36	57.86	253	Ala	6.52	121.83	-	54.74
168	Arg	7.84	114	177.35	56.25	255	Ile	-	-	177.06	62.94
169	Leu	8.87	116.53	177.7	53.96	256	Ala	7.95	121.4	178.79	56.03
170	Gly	7.5	110	174.03	44.76	257	Gly	7.75	102.04	-	47.13

Table S4. Comparison between assigned residues for apo-GlpG and GlpG with SBO or JLK6. CSPs (in ppm) represent chemical shift perturbations for HNCA, calculated using equation (1) from the main text.

Residue		SBO	JLK6	Residue		SBO	JLK6
96	Val	0.18	0.17	189	Gln	0.30	0.29
97	Thr	0.30	0.31	190	Gln	0.06	0.06
107	Val	0.13	0.11	191	Lys	gone	gone
108	Phe	0.14	0.14	192	Phe	0.43	0.38
113	Ile	gone	gone	193	Ser	gone	gone
114	Leu	0.15	0.11	194	Gly	0.12	0.12
115	Gly	0.15	0.16	196	Trp	0.18	0.16
116	Asn	0.46	0.39	198	Gly	0.20	0.14
117	Gln	0.25	0.26	199	Gly	0.32	0.31
118	Glu	0.08	0.09	200	Leu	0.24	0.28
119	Val	0.56	0.57	201	Ser	0.31	0.29
120	Met	0.36	0.37	202	Gly	0.05	0.12
121	Leu	0.03	0.02	203	Val	0.26	0.24
122	Trp	0.25	0.18	213	Leu	0.10	0.11
127	Phe	0.06	0.04	214	Arg	0.10	0.08
128	Asp	0.18	0.15	215	Gly	0.08	0.08
130	Thr	0.22	0.20	216	Glu	0.14	0.14
131	Leu	0.12	0.12	217	Arg	0.07	0.05
132	Lys	0.10	0.09	218	Asp	0.08	0.09
133	Phe	0.59	0.58	220	Gln	gone	gone
134	Glu	0.31	0.35	221	Ser	0.27	0.28
135	Phe	0.36	0.38	222	Gly	0.15	0.15
136	Trp	gone	gone	223	Ile	0.98	0.96
137	Arg	0.17	0.20	224	Tyr	0.12	0.20
141	His	0.12	0.15	225	Leu	0.10	0.06
145	His	0.11	0.10	229	Leu	0.07	0.08
146	Phe	0.08	0.13	230	Ile	gone	gone
147	Ser	0.11	0.07	231	Ile	gone	gone
148	Leu	0.14	0.15	232	Phe	gone	gone
149	Met	0.04	0.04	233	Ala	gone	gone
150	His	0.22	0.23	237	Ile	0.15	0.06
163	Gly	0.13	0.15	239	Ala	gone	gone
164	Ala	0.05	0.03	240	Gly	gone	gone
167	Lys	0.19	0.19	241	Trp	gone	gone
168	Arg	0.18	0.17	251	Asn	0.22	0.19
169	Leu	0.10	0.12	252	Gly	0.41	0.43
170	Gly	0.09	0.07	253	Ala	0.32	0.27
171	Ser	0.14	0.14	254	His	gone	gone
172	Gly	0.05	0.05	255	Ile	gone	gone
173	Lys	0.02	0.02	256	Ala	0.28	0.40
174	Leu	0.09	0.13	257	Gly	0.05	0.03
175	Ile	0.09	0.06				

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