

Biophysical Journal, Volume 120

Supplemental information

**Multiscale computational study of ligand binding pathways: Case of
p38 MAP kinase and its inhibitors**

Yu-ming M. Huang

No.	DFG conf.	inhibitor	# of inhibitor diffused	threshold energy	adaptive GaMD	simulation length (ns)	com-RMSD (Å)	ha-RMSD (Å)
1	in	SB2	1	lower	no	2000	>10	>10
2	in	SB2	1	lower	no	1947	>10	>10
3	in	SB2	1	lower	no	1918	>10	>10
4	in	SB2	1	lower	no	1748	>10	>10
5	in	SB2	1	lower	no	1888	>10	>10
6	in	SB2	1	lower	no	1977	>10	>10
7	in	SB2	1	lower	no	1946	>10	>10
8	in	SB2	1	lower	no	1892	>10	>10
9	in	SB2	1	lower	no	1999	>10	>10
10	in	SB2	1	lower	no	1985	>10	>10
11	in	SB2	8	lower	no	1931	>10	>10
12	in	SB2	8	lower	no	1927	>10	>10
13	in	SB2	8	lower	no	1927	>10	>10
14	in	SB2	8	lower	no	1865	>10	>10
15	in	SB2	8	lower	no	1810	>10	>10
16	in	SB2	8	lower	no	1864	>10	>10
17	in	SB2	8	lower	no	1906	>10	>10
18	in	SB2	8	lower	no	1853	>10	>10
19	in	SB2	8	lower	no	1759	>10	>10
20	in	SB2	8	lower	no	1947	>10	>10
21	in	SB2	1	lower	yes	500	>10	>10
22	in	SB2	1	lower	yes	500	>10	>10
23	in	SB2	1	lower	yes	500	>10	>10
24	in	SB2	1	lower	yes	500	>10	>10
25	in	SB2	1	lower	yes	500	>10	>10
26	in	SB2	1	lower	yes	400	>10	>10
27	in	SB2	1	lower	yes	373	>10	>10
28	in	SB2	1	lower	yes	400	>10	>10
29	in	SB2	1	lower	yes	400	>10	>10
30	in	SB2	1	upper	yes	500	>10	>10
31	in	SB2	1	upper	yes	500	0.144	1.655
32	in	SB2	1	upper	yes	500	3.149	6.449
33	in	SB2	1	upper	yes	500	>10	>10
34	in	SB2	1	upper	yes	500	>10	>10
35	in	SB2	1	upper	yes	500	>10	>10
36	in	SB2	1	upper	yes	500	>10	>10
37	in	SB2	1	upper	yes	1400	0.126	1.236
38	in	SB2	1	upper	yes	1400	0.364	1.614
39	in	SB2	1	upper	yes	500	>10	>10
40	in	SB2	1	upper	yes	1410	1.770	5.376
41	in	SB2	1	upper	yes	500	>10	>10
42	in	SB2	1	upper	yes	500	>10	>10

43	in	SB2	1	upper	yes	300	>10	>10
44	in	SB2	1	upper	yes	500	>10	>10
45	in	SB2	8	upper	yes	500	>10	>10
46	in	SB2	8	upper	yes	500	>10	>10
47	in	SB2	8	upper	yes	500	1.809	2.330
48	in	SB2	8	upper	yes	500	>10	>10
49	in	SB2	8	upper	yes	500	>10	>10
50	in	SB2	8	upper	yes	500	>10	>10
51	in	SB2	8	upper	yes	700	1.784	4.308
52	in	SB2	8	upper	yes	1468	0.367	1.553
53	in	SB2	8	upper	yes	612	>10	>10
54	in	SB2	8	upper	yes	707	>10	>10
55	in	SK8	1	lower	yes	500	>10	>10
56	in	SK8	1	lower	yes	500	>10	>10
57	in	SK8	1	lower	yes	500	>10	>10
58	in	SK8	1	lower	yes	500	>10	>10
59	in	SK8	1	lower	yes	500	>10	>10
60	in	SK8	8	lower	no	1901	>10	>10
61	in	SK8	8	lower	no	1920	>10	>10
62	in	SK8	8	lower	no	1863	>10	>10
63	in	SK8	8	lower	no	1748	0.013	0.386
64	in	SK8	8	lower	no	2000	>10	>10
65	in	SK8	8	lower	no	2000	>10	>10
66	in	SK8	8	lower	no	1900	>10	>10
67	in	SK8	8	lower	no	1954	3.437	5.474
68	in	SK8	8	lower	no	18078	>10	>10
69	in	SK8	8	lower	no	1902	>10	>10
70	in	SK8	1	upper	yes	400	>10	>10
71	in	SK8	1	upper	yes	500	>10	>10
72	in	SK8	1	upper	yes	500	>10	>10
73	in	SK8	1	upper	yes	500	>10	>10
74	in	SK8	1	upper	yes	500	>10	>10
75	in	SK8	1	upper	yes	500	>10	>10
76	in	SK8	1	upper	yes	500	>10	>10
77	in	SK8	1	upper	yes	500	>10	>10
78	in	SK8	1	upper	yes	500	>10	>10
79	in	SK8	1	upper	yes	500	>10	>10
80	in	SK8	1	upper	yes	500	>10	>10
81	in	SK8	1	upper	yes	500	>10	>10
82	in	SK8	1	upper	yes	500	>10	>10
83	in	SK8	1	upper	yes	900	>10	>10
84	in	SK8	1	upper	yes	500	>10	>10
85	in	SK8	8	upper	yes	400	>10	>10
86	in	SK8	8	upper	yes	500	>10	>10
87	in	SK8	8	upper	yes	900	0.015	0.318

88	in	SK8	8	upper	yes	500	>10	>10
89	in	SK8	8	upper	yes	500	>10	>10
90	in	SK8	8	upper	yes	900	0.089	1.131
91	in	SK8	8	upper	yes	807	>10	>10
92	in	SK8	8	upper	yes	856	0.074	1.137
93	in	SK8	8	upper	yes	900	0.016	0.345
94	in	SK8	8	upper	yes	404	>10	>10
95	in	PUA	1	lower	yes	500	>10	>10
96	in	PUA	1	lower	yes	500	>10	>10
97	in	PUA	1	lower	yes	500	>10	>10
98	in	PUA	1	lower	yes	500	>10	>10
99	in	PUA	1	lower	yes	500	>10	>10
100	in	PUA	1	upper	yes	500	>10	>10
101	in	PUA	1	upper	yes	500	>10	>10
102	in	PUA	1	upper	yes	500	>10	>10
103	in	PUA	1	upper	yes	500	>10	>10
104	in	PUA	1	upper	yes	500	>10	>10
105	in	PUA	8	upper	yes	500	>10	>10
106	in	PUA	8	upper	yes	500	>10	>10
107	in	PUA	8	upper	yes	500	>10	>10
108	in	PUA	8	upper	yes	500	>10	>10
109	in	PUA	8	upper	yes	500	>10	>10
110	out	SB2	1	lower	yes	500	>10	>10
111	out	SB2	1	lower	yes	500	>10	>10
112	out	SB2	1	lower	yes	500	>10	>10
113	out	SB2	1	lower	yes	500	>10	>10
114	out	SB2	1	lower	yes	500	>10	>10
115	out	SB2	8	lower	no	1334	>10	>10
116	out	SB2	8	lower	no	1887	>10	>10
117	out	SB2	8	lower	no	1910	>10	>10
118	out	SB2	8	lower	no	1859	>10	>10
119	out	SB2	8	lower	no	1996	>10	>10
120	out	SB2	8	lower	no	1826	>10	>10
121	out	SB2	8	lower	no	1685	>10	>10
122	out	SB2	8	lower	no	1855	>10	>10
123	out	SB2	8	lower	no	1954	>10	>10
124	out	SB2	8	lower	no	2000	>10	>10
125	out	SB2	1	upper	yes	500	>10	>10
126	out	SB2	1	upper	yes	500	>10	>10
127	out	SB2	1	upper	yes	426	>10	>10
128	out	SB2	1	upper	yes	500	>10	>10
129	out	SB2	1	upper	yes	3000	>10	>10
130	out	SB2	1	upper	yes	500	>10	>10
131	out	SB2	1	upper	yes	416	>10	>10
132	out	SB2	1	upper	yes	500	>10	>10

133	out	SB2	1	upper	yes	856	>10	>10
134	out	SB2	1	upper	yes	500	>10	>10
135	out	SB2	1	upper	yes	2000	>10	>10
136	out	SB2	1	upper	yes	2000	>10	>10
137	out	SB2	1	upper	yes	2000	>10	>10
138	out	SB2	1	upper	yes	1200	0.008	1.131
139	out	SB2	1	upper	yes	2000	>10	>10
140	out	SB2	1	upper	yes	2000	>10	>10
141	out	SB2	1	upper	yes	2000	>10	>10
142	out	SB2	1	upper	yes	2000	>10	>10
143	out	SB2	1	upper	yes	2000	>10	>10
144	out	SB2	1	upper	yes	2000	>10	>10
145	out	SB2	8	upper	yes	500	1.079	6.109
146	out	SB2	8	upper	yes	500	>10	>10
147	out	SB2	8	upper	yes	500	0.952	3.712
148	out	SB2	8	upper	yes	500	>10	>10
149	out	SB2	8	upper	yes	500	>10	>10
150	out	SB2	8	upper	yes	500	>10	>10
151	out	SB2	8	upper	yes	1929	2.417	4.791
152	out	SB2	8	upper	yes	500	>10	>10
153	out	SB2	8	upper	yes	500	>10	>10
154	out	SB2	8	upper	yes	2000	1.202	1.842
155	out	SB2	8	upper	yes	1500	0.008	0.748
156	out	SB2	8	upper	yes	1500	0.008	0.822
157	out	SB2	8	upper	yes	4000	>10	>10
158	out	SB2	8	upper	yes	1500	0.095	0.777
159	out	SB2	8	upper	yes	2000	>10	>10
160	out	SB2	8	upper	yes	2000	>10	>10
161	out	SB2	8	upper	yes	1400	0.007	0.577
162	out	SB2	8	upper	yes	1400	0.021	1.127
163	out	SB2	8	upper	yes	2000	>10	>10
164	out	SB2	8	upper	yes	1300	0.004	1.228
165	out	SK8	1	lower	yes	900	>10	>10
166	out	SK8	1	lower	yes	900	>10	>10
167	out	SK8	1	lower	yes	500	>10	>10
168	out	SK8	1	lower	yes	500	>10	>10
169	out	SK8	1	lower	yes	900	>10	>10
170	out	SK8	1	upper	yes	500	>10	>10
171	out	SK8	1	upper	yes	500	>10	>10
172	out	SK8	1	upper	yes	500	>10	>10
173	out	SK8	1	upper	yes	500	>10	>10
174	out	SK8	1	upper	yes	500	>10	>10
175	out	SK8	8	upper	yes	500	>10	>10
176	out	SK8	8	upper	yes	500	>10	>10
177	out	SK8	8	upper	yes	500	>10	>10

178	out	SK8	8	upper	yes	500	>10	>10
179	out	SK8	8	upper	yes	500	>10	>10
180	out	DU	4	lower	no	1907	>10	>10
181	out	DU	4	lower	no	1960	>10	>10
182	out	DU	4	lower	no	1931	>10	>10
183	out	DU	4	lower	no	2086	5.145	6.815
184	out	DU	4	lower	no	1975	>10	>10
185	out	DU	4	lower	no	1985	>10	>10
186	out	DU	4	lower	no	2000	>10	>10
187	out	DU	4	lower	no	1486	>10	>10
188	out	DU	4	lower	no	1903	>10	>10
189	out	DU	4	lower	no	1949	>10	>10
190	out	DU	1	upper	yes	500	>10	>10
191	out	DU	1	upper	yes	500	>10	>10
192	out	DU	1	upper	yes	1500	>10	>10
193	out	DU	1	upper	yes	500	>10	>10
194	out	DU	1	upper	yes	500	>10	>10
195	out	DU	1	upper	yes	900	>10	>10
196	out	DU	1	upper	yes	500	>10	>10
197	out	DU	1	upper	yes	500	>10	>10
198	out	DU	1	upper	yes	500	>10	>10
199	out	DU	1	upper	yes	500	>10	>10
200	out	DU	4	upper	yes	500	>10	>10
201	out	DU	4	upper	yes	900	>10	>10
202	out	DU	4	upper	yes	900	>10	>10
203	out	DU	4	upper	yes	900	>10	>10
204	out	DU	4	upper	yes	500	>10	>10
205	out	DU	4	upper	yes	900	>10	>10
206	out	DU	4	upper	yes	1953	4.159	6.468
207	out	DU	4	upper	yes	500	>10	>10
208	out	DU	4	upper	yes	500	>10	>10
209	out	DU	4	upper	yes	500	>10	>10
210	out	DU	4	upper	yes	2000	>10	>10
211	out	DU	4	upper	yes	3000	0.008	1.039
212	out	DU	4	upper	yes	1949	3.249	6.056
213	out	DU	4	upper	yes	2000	>10	>10
214	out	DU	4	upper	yes	2000	>10	>10
215	out	DU	4	upper	yes	2000	>10	>10
216	out	DU	4	upper	yes	1991	7.039	8.274
217	out	DU	4	upper	yes	2000	>10	>10
218	out	DU	4	upper	yes	1961	9.664	10.603
219	out	DU	4	upper	yes	2000	3.910	6.601
220	out	PUA	1	lower	no	2000	>10	>10
221	out	PUA	1	lower	no	1902	>10	>10
222	out	PUA	1	lower	no	1978	>10	>10

223	out	PUA	1	lower	no	1902	>10	>10
224	out	PUA	1	lower	no	1902	>10	>10
225	out	PUA	1	lower	no	1600	>10	>10
226	out	PUA	1	lower	no	1886	>10	>10
227	out	PUA	1	lower	no	2000	>10	>10
228	out	PUA	1	lower	no	2000	>10	>10
229	out	PUA	1	lower	no	1838	>10	>10
230	out	PUA	1	lower	yes	500	>10	>10
231	out	PUA	1	lower	yes	500	>10	>10
232	out	PUA	1	lower	yes	500	>10	>10
233	out	PUA	1	lower	yes	500	>10	>10
234	out	PUA	1	lower	yes	500	>10	>10
235	out	PUA	1	upper	yes	500	>10	>10
236	out	PUA	1	upper	yes	500	>10	>10
237	out	PUA	1	upper	yes	500	>10	>10
238	out	PUA	1	upper	yes	500	>10	>10
239	out	PUA	1	upper	yes	800	2.815	5.730
240	out	PUA	1	upper	yes	13600	0.009	1.213
241	out	PUA	1	upper	yes	500	>10	>10
242	out	PUA	1	upper	yes	900	>10	>10
243	out	PUA	1	upper	yes	1455	>10	>10
244	out	PUA	1	upper	yes	500	>10	>10
245	out	PUA	1	upper	yes	500	>10	>10
246	out	PUA	1	upper	yes	333	>10	>10
247	out	PUA	1	upper	yes	425	>10	>10
248	out	PUA	1	upper	yes	1400	>10	>10
249	out	PUA	1	upper	yes	500	>10	>10
250	out	PUA	1	upper	yes	2000	3.553	6.947
251	out	PUA	1	upper	yes	2000	>10	>10
252	out	PUA	1	upper	yes	2000	>10	>10
253	out	PUA	1	upper	yes	2000	>10	>10
254	out	PUA	1	upper	yes	2000	>10	>10
255	out	PUA	1	upper	yes	2000	>10	>10
256	out	PUA	1	upper	yes	10900	1.117	5.389
257	out	PUA	1	upper	yes	2000	>10	>10
258	out	PUA	1	upper	yes	2000	>10	>10
259	out	PUA	1	upper	yes	2000	>10	>10
260	out	PUA	8	upper	yes	500	>10	>10
261	out	PUA	8	upper	yes	500	>10	>10
262	out	PUA	8	upper	yes	500	>10	>10
263	out	PUA	8	upper	yes	500	>10	>10
264	out	PUA	8	upper	yes	500	>10	>10
265	out	PUA	8	upper	yes	1964	4.065	6.437
266	out	PUA	8	upper	yes	1500	>10	>10
267	out	PUA	8	upper	yes	407	>10	>10

268	out	PUA	8	upper	yes	1827	>10	>10
269	out	PUA	8	upper	yes	403	>10	>10

Table S1: List of GaMD simulations. Four inhibitors, SB2, SK8, DU, and PUA, were diffused to both DFG-in and DFG-out p38. We placed one, four, or eight inhibitors in a water box to obtain the inhibitor association pathways with a single protein. The threshold energy level was set to either lower or upper bound. The adaptive GaMD indicates that the boost potential was updated until the end of simulations, while the boost energy was only updated in the first 100 ns and remained at a certain number in the rest of simulations, namely non-adaptive GaMD. Also, we computed two types of RMSD values, com-RMSD and ha-RMSD. Red highlights the simulations that the inhibitors reached the active site with both low com-RMSD and ha-RMSD. Blue highlights the simulations showing low com-RMSD but high ha-RMSD, suggesting the inhibitors were located in the active site, yet the key protein-ligand interactions were not completely formed.

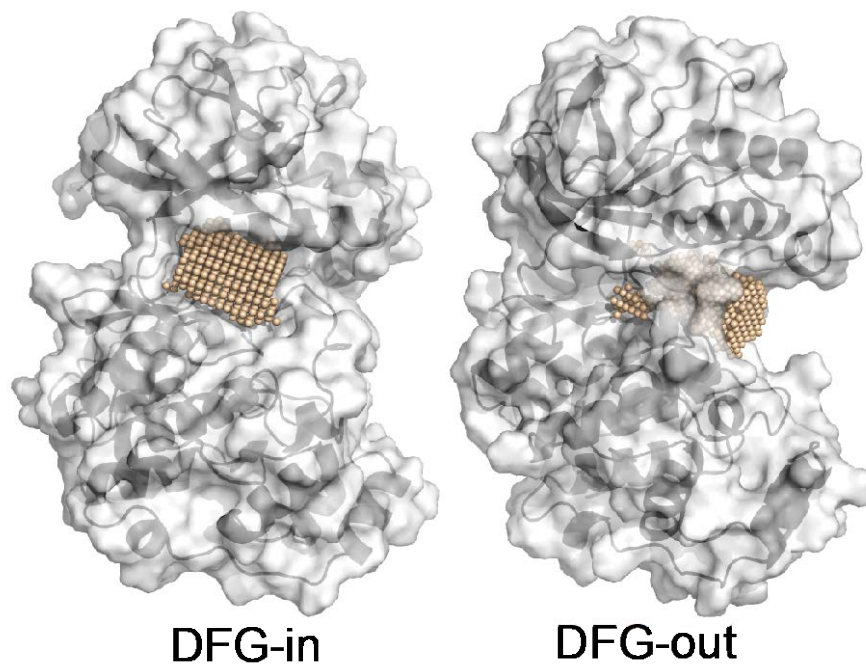


Figure S1: Comparison of the binding channel of two apo p38 crystal structures.

The beads filled in the channel were used to calculate the volume.

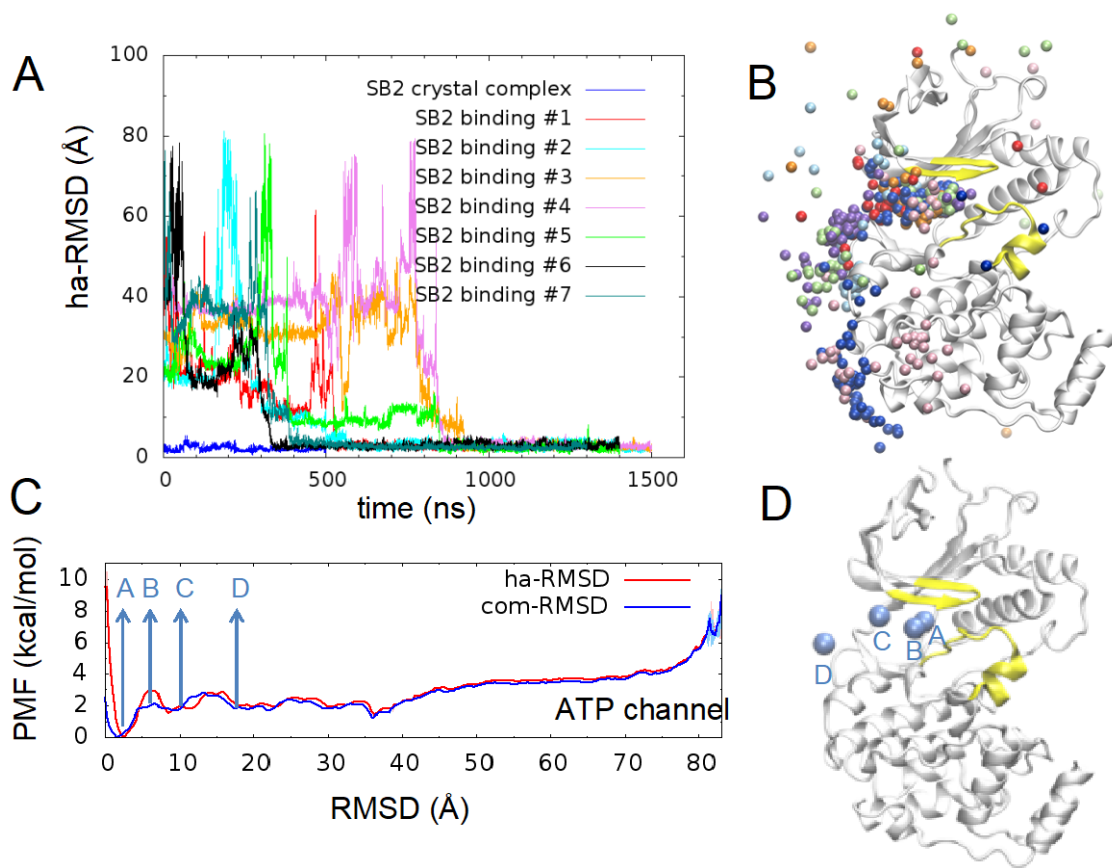


Figure S2: Association of SB2 to DFG-out p38. (A) com-RMSD and ha-RMSD plot of seven binding trajectories. (B) Association of the successful binding paths labeled by seven different colored beads. Yellow indicates the p38 Gly-rich and activation loop. (C) 1D PMF plot according to the ATP channel. The letters represent each low-energy state. (D) Blue beads indicate the SB2 positions at the low-energy states along the ATP channel.

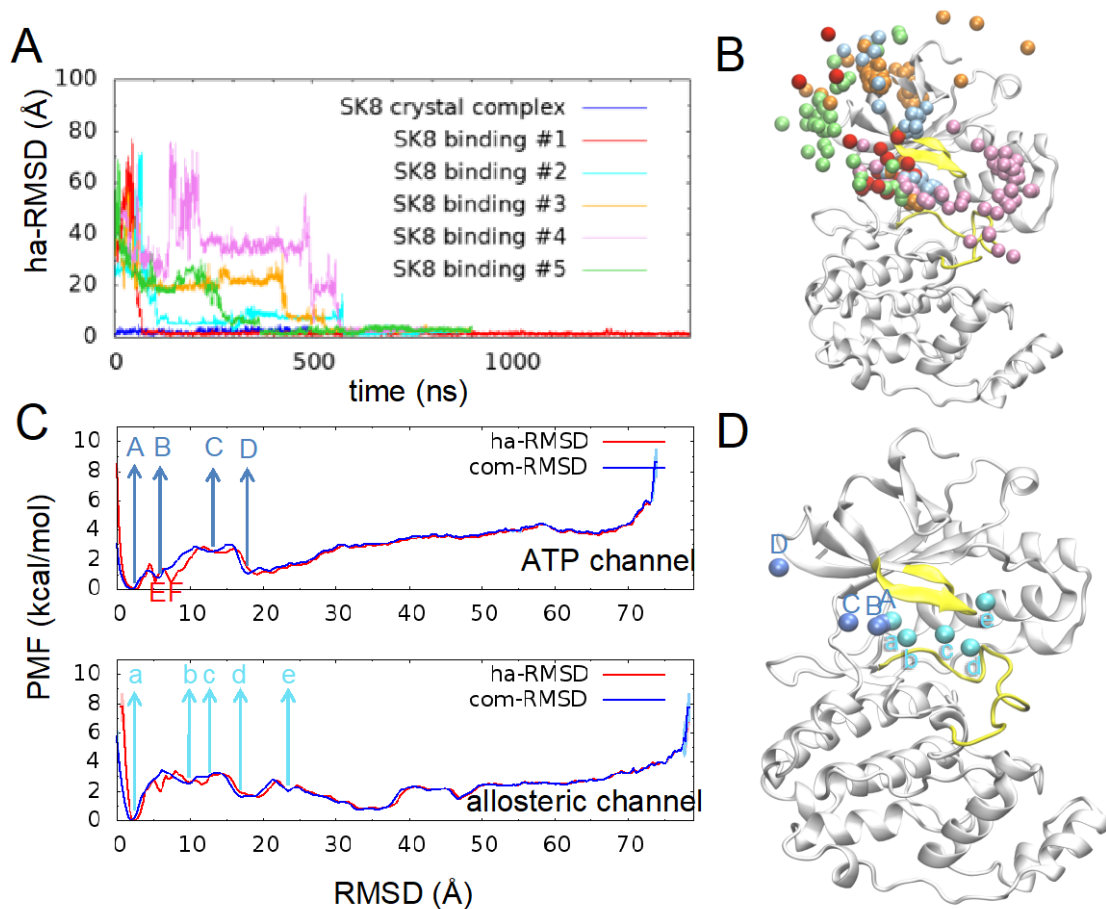


Figure S3: Association of SK8 to DFG-in p38. (A) com-RMSD and ha-RMSD plot of five binding trajectories. (B) Association of the successful binding paths labeled by five different colored beads. Yellow indicates the p38 Gly-rich and activation loop. (C) The 1D PMF plot along the ATP and allosteric channel. The letters label each low-energy state. (D) Blue and cyan beads indicate the SK8 positions at the low-energy states along the ATP and allosteric channel, respectively.

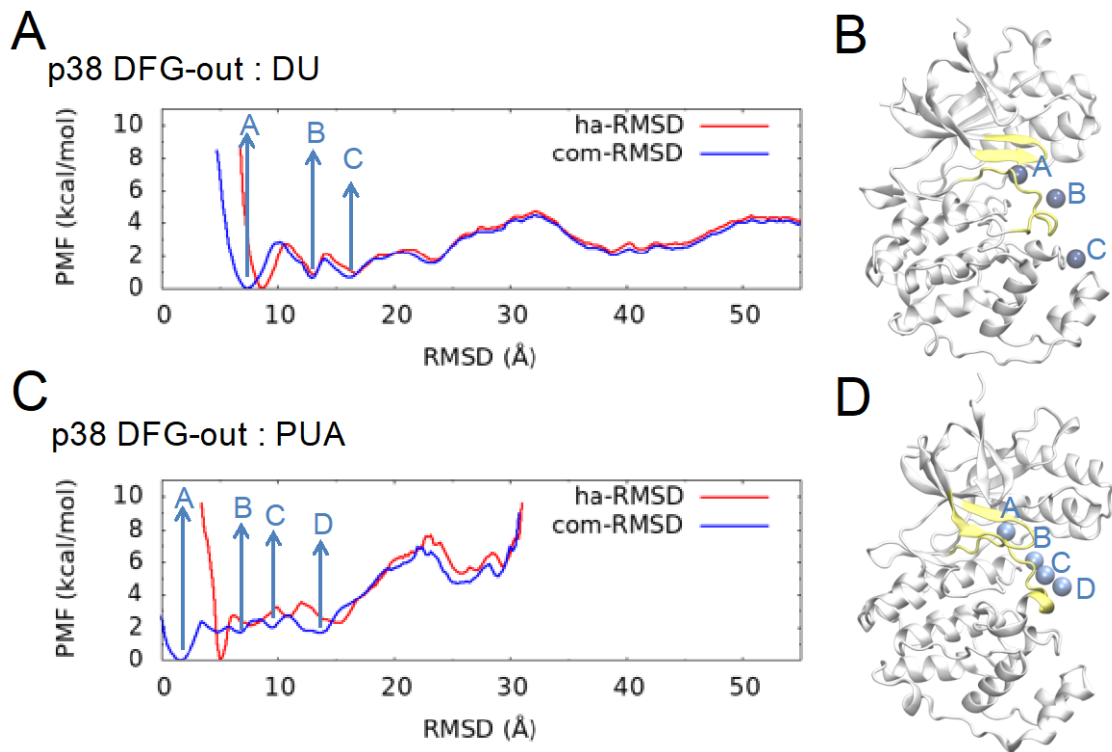


Figure S4: Association of DU and PUA to DFG-in p38. (A) com-RMSD and ha-RMSD plot of the DU binding trajectory. (B) Blue beads indicate the DU positions at the low-energy states along the allosteric channel. Yellow indicates the p38 Gly-rich and activation loop. (C) com-RMSD and ha-RMSD plot of the PUA binding trajectory. (D) Blue beads indicate the PUA positions at the low-energy states along the allosteric channel.

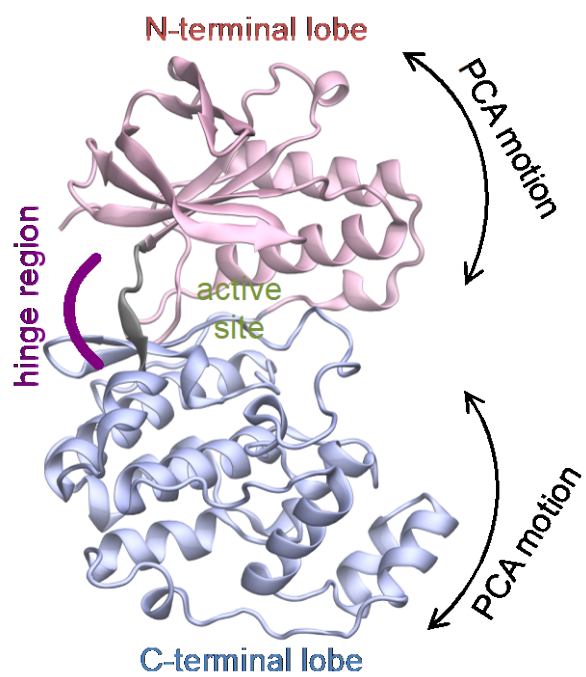


Figure S5: The correlation motions of the apo p38 protein calculated by PCA.

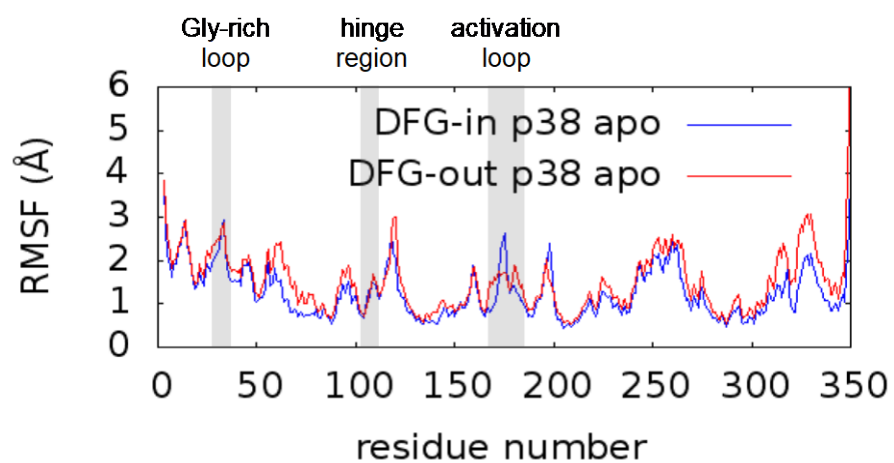


Figure S6: The RMSF calculations of the apo p38 protein.

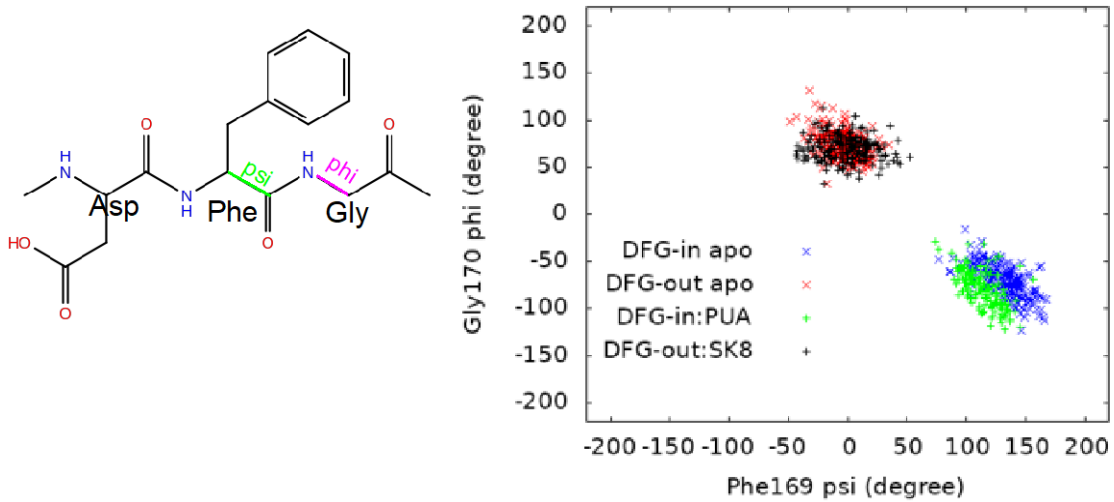


Figure S7: The dihedral angles of DFG motifs. We computed Phe169 psi angles and Gly170 phi angles using the trajectories of apo DFG-in p38, apo DFG-out p38, DFG-in:PUA association, and DFG-out:SK8 association simulations.