

Mapping the Hydrogen Bond Networks in the Catalytic Subunit of Protein Kinase A using H/D Fractionation Factors

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SUPPLEMENTARY INFORMATION

Figures S1-S10

Table S1

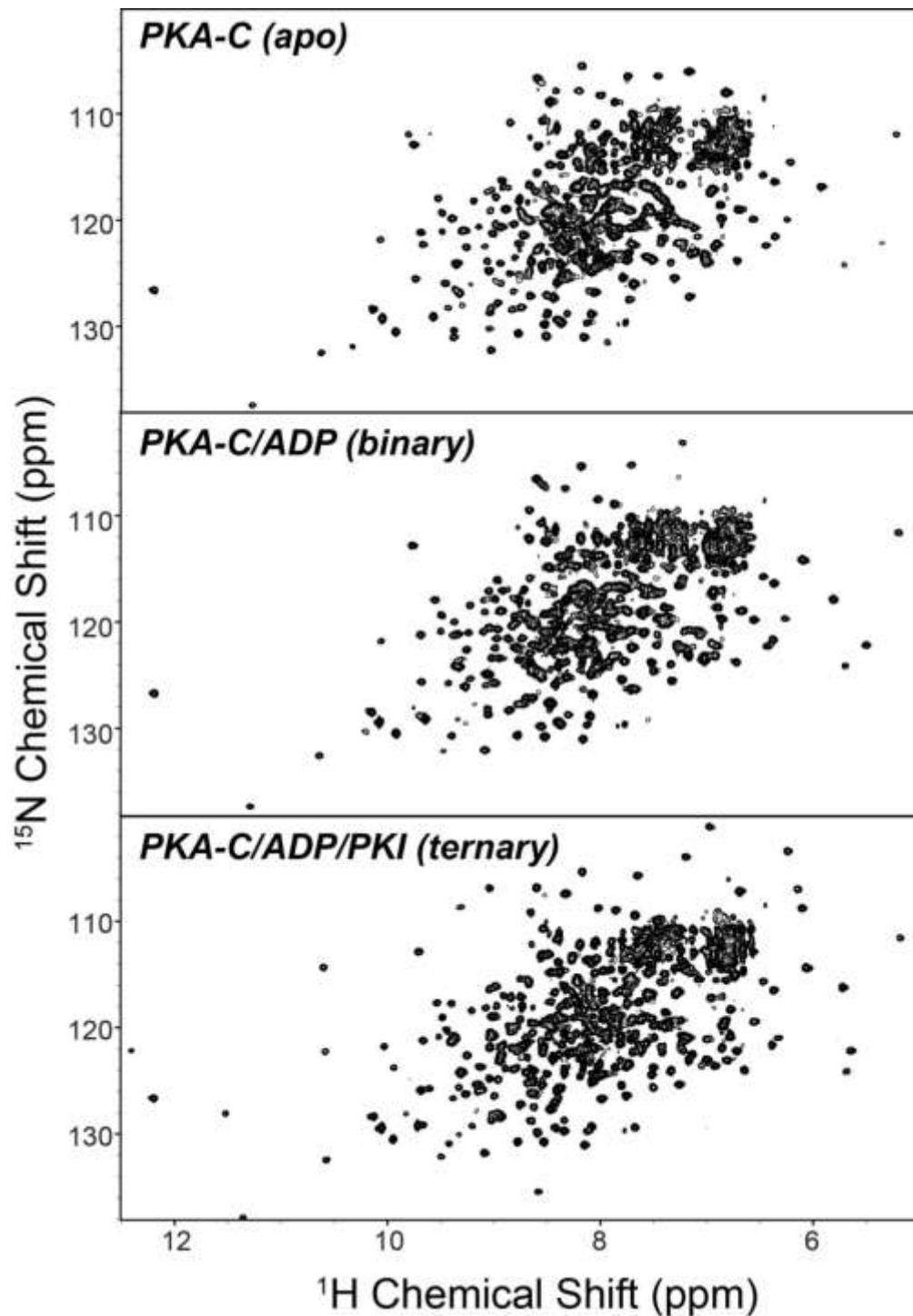


Figure S1. $[^1\text{H}, ^{15}\text{N}]$ -TROSY-HSQC spectra of the three major conformational states of PKA-C used in this study.

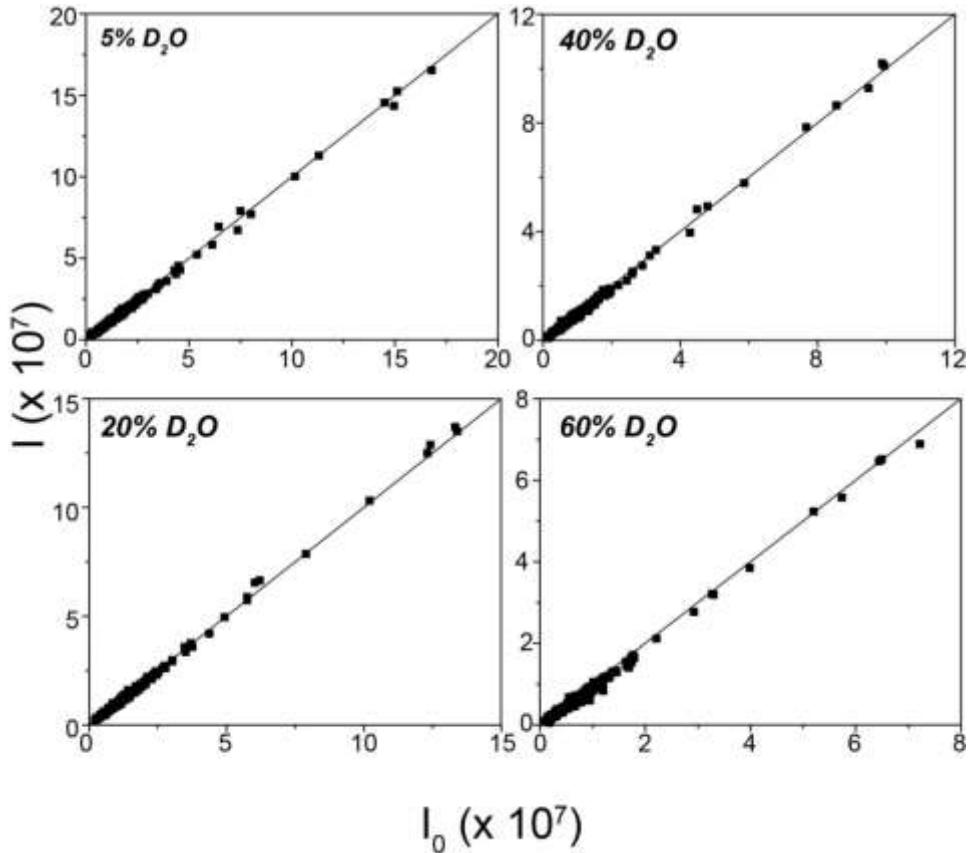


Figure S2. Plots showing the correlation of the peak intensities used in the analysis of the fractionation factors of the amide resonances of the ternary form of PKA-C at the day of actual measurement (I_0) and 7 days after (I). The linear correlation suggests that the 24-hour incubation time is enough for sufficient isotope exchange.

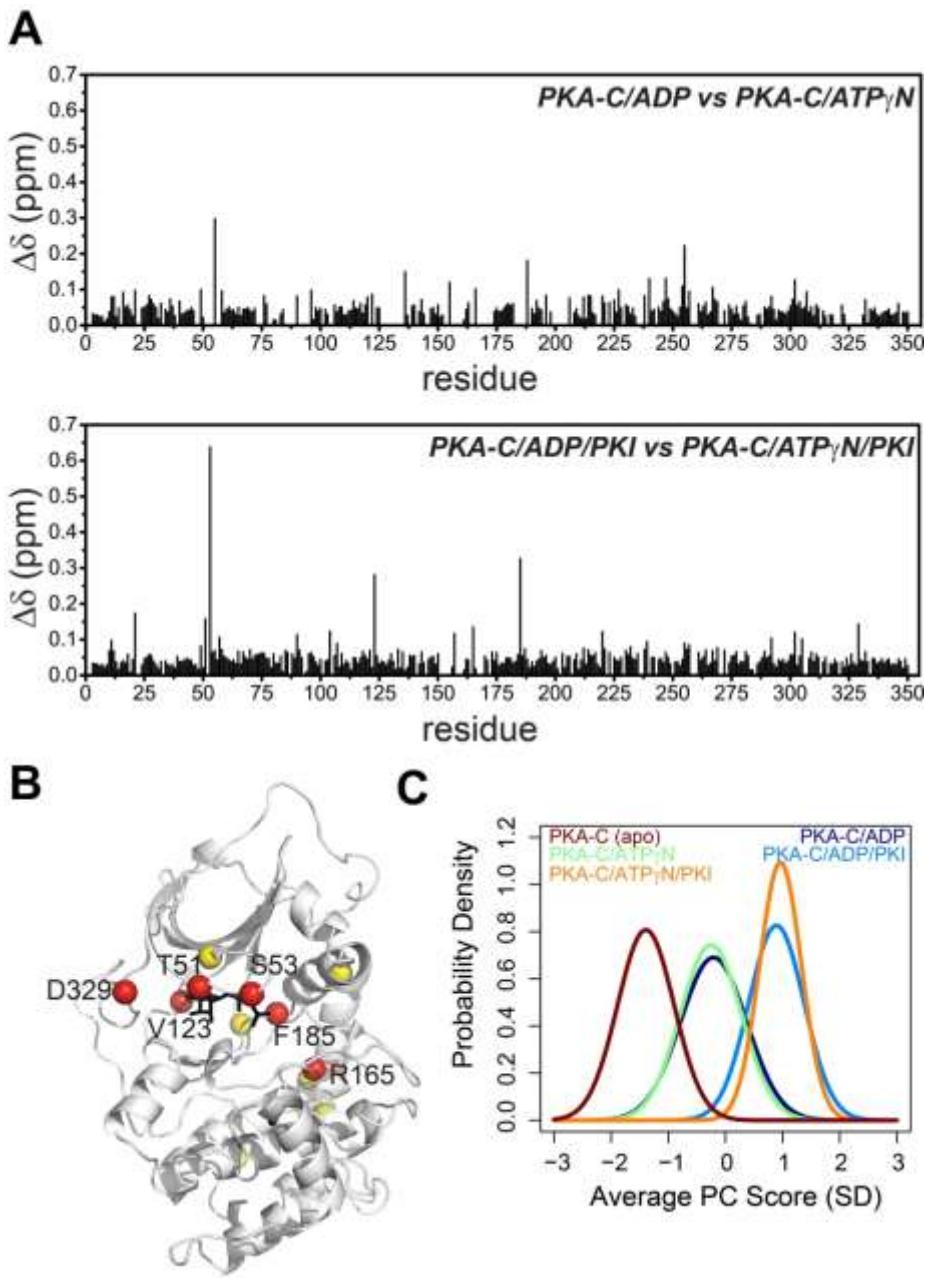


Figure S3. **A.** Plots of the chemical shift changes upon addition of ADP and ATP γ N (top) and ADP/PKI₅₋₂₄ and ATP γ N/PKI₅₋₂₄ (bottom). **B.** Most significant chemical shift differences mapped onto the structure of PKA-C, PDB: 1ATP. Red spheres indicate residues with $\Delta\delta$ greater than one standard deviation from the mean (i.e. $\Delta\delta > 0.12$); yellow spheres are residues with $\Delta\delta > 0.10$. **C.** CONCISE analysis⁴² of the amide chemical shifts showing that the average conformations of the binary and ternary complexes using ADP as nucleotide are similar to their ATP γ N counterparts.

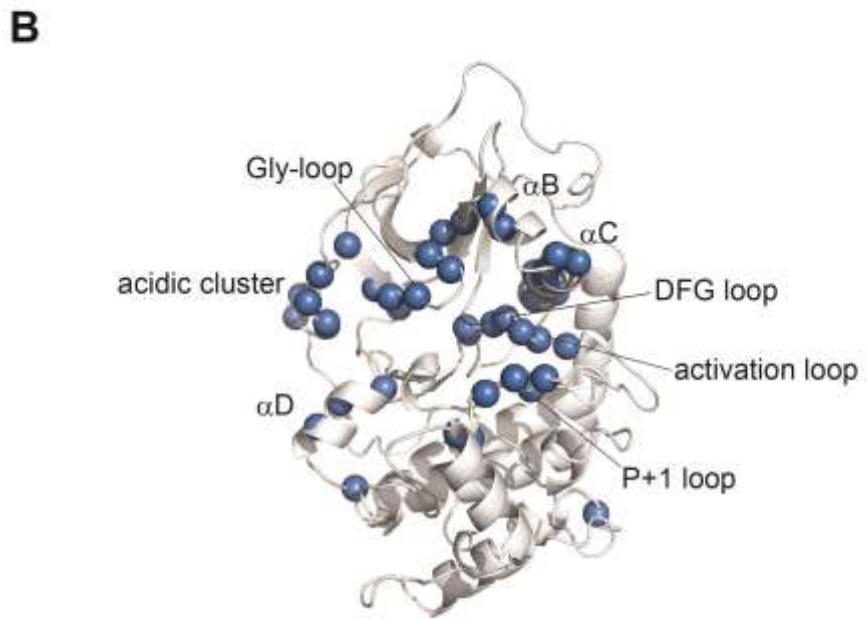
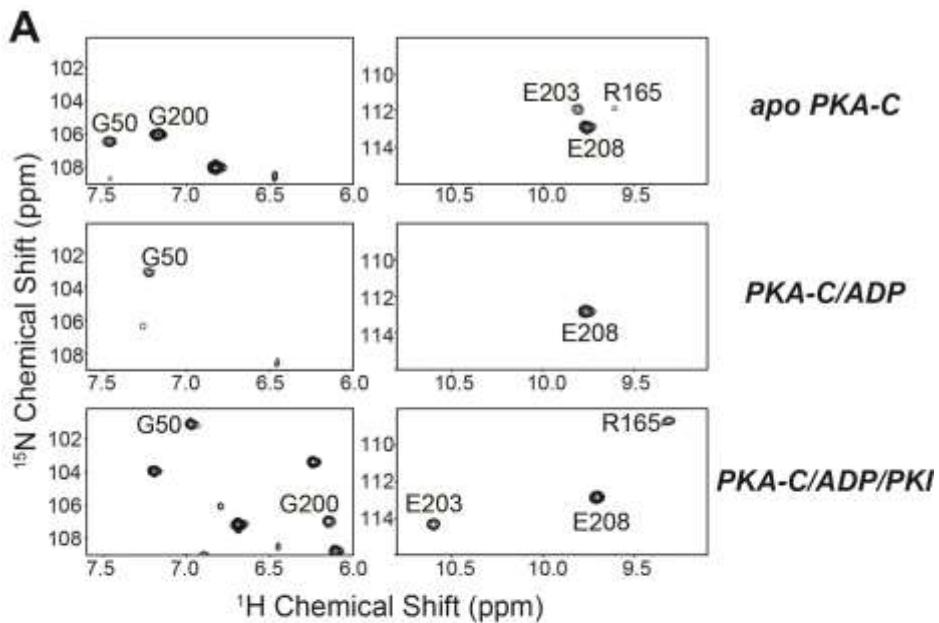


Figure S4. Residues that are exchange-broadened in the spectrum of PKA-C/ADP. (A) Portions of $[^1\text{H}, ^{15}\text{N}]$ -TROSY-HSQC spectra of the three states, showing the exchange broadening in the binary form. (B) These residues include Gly-rich loop, B-, C-, and D-helices, DFG loop, activation loop, P+1 loop and the acidic cluster in C-terminal tail. Gly50 and Gly55 are parts of the Gly-rich loop, Arg165 is part of the catalytic loop, Gly200 and Asp203 are parts of the P+1 loop.

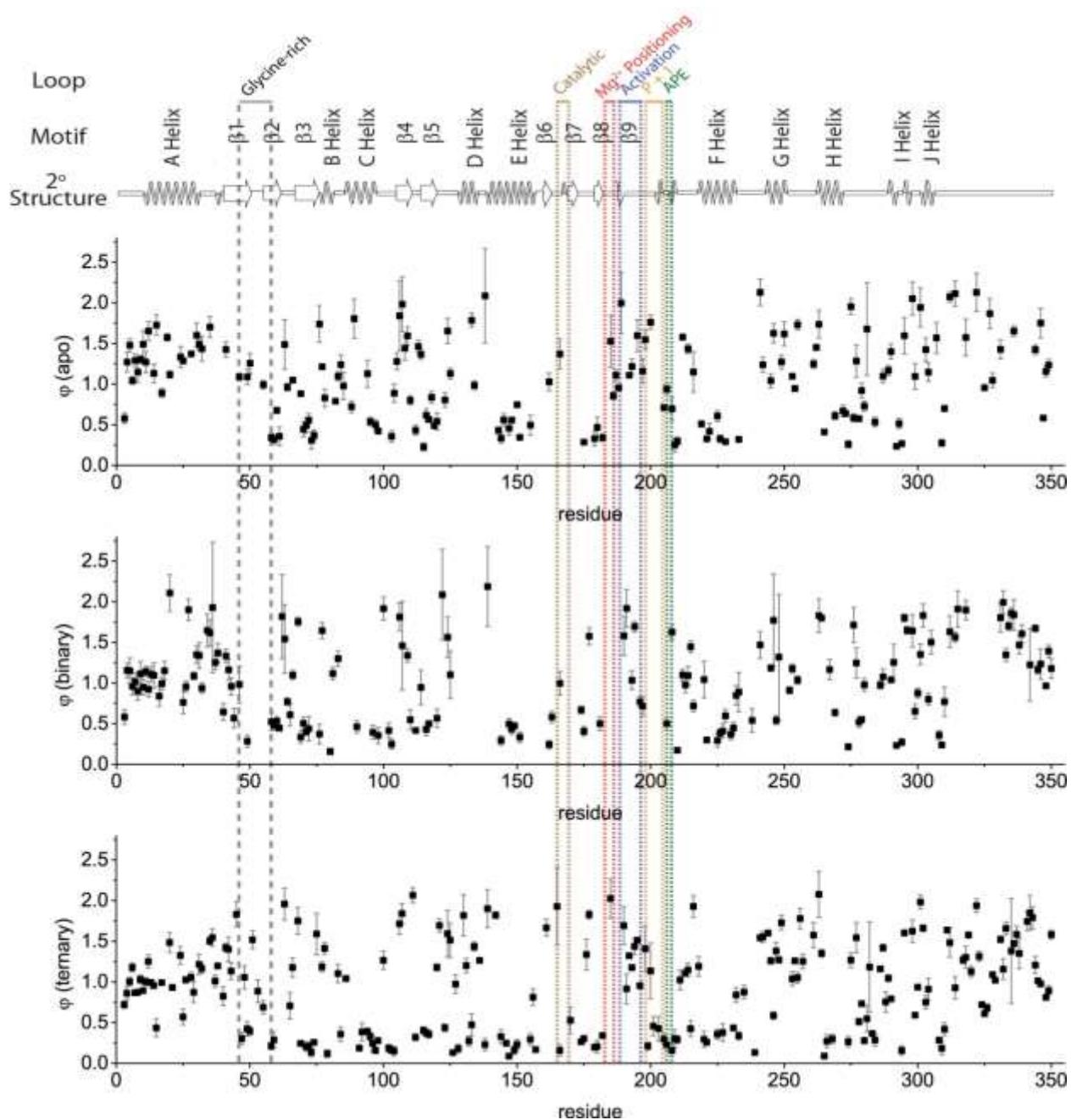


Figure S5. Fractionation factors of the amide resonances of PKA-C in three different states: apo, binary (ADP-bound), and ternary (ADP- and PKI-bound) are plotted versus residue number.

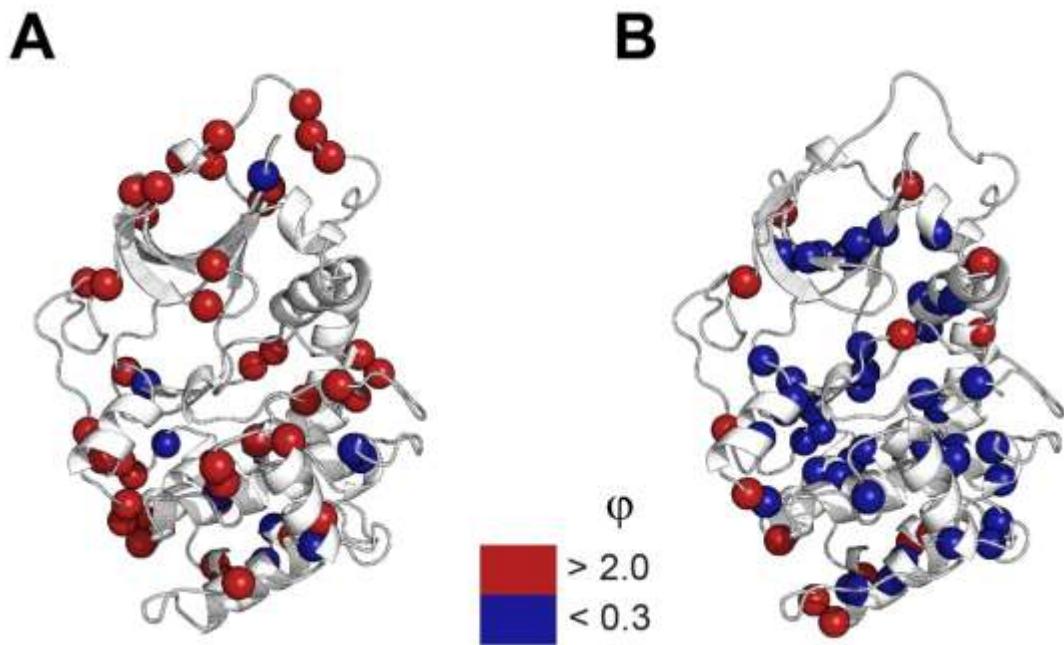


Figure S6. Mapping of the residues with fractionation factors greater than 2.0 and less than 0.3 on the X-ray structure (1ATP). (A) Apo form, (B) ternary form. The H-bond strengths of several residues in the apo form are significantly weaker than the corresponding ternary complex. Also, residues with $\phi > 2.0$ are mostly situated on the solvent-exposed, unstructured region of the kinase; while those with $\phi < 0.3$ are buried within the enzyme.

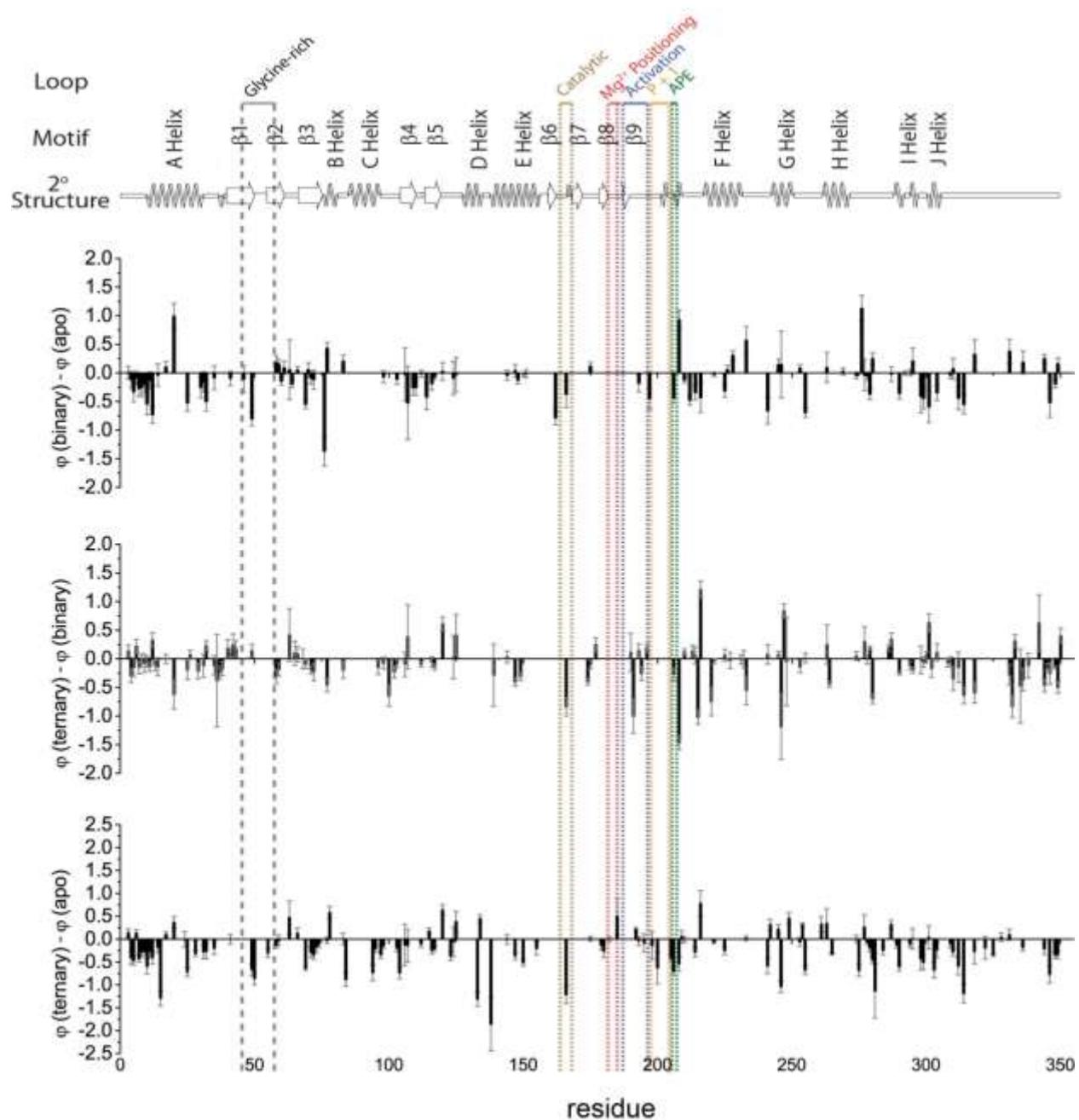


Figure S7. Fractionation factors differences for the amide resonances of PKA-C among the different states.

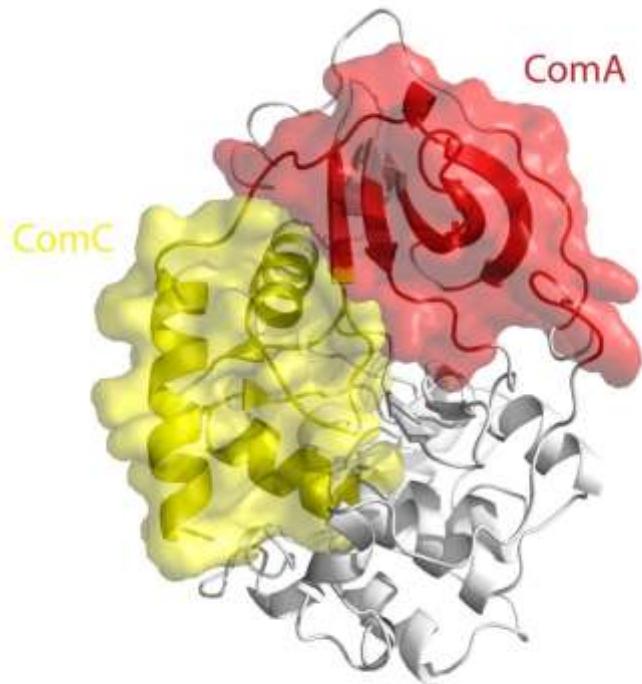


Figure S8. Regions showing the A and C communities, red and yellow, respectively. Illustration adapted from McClendon et al.⁶

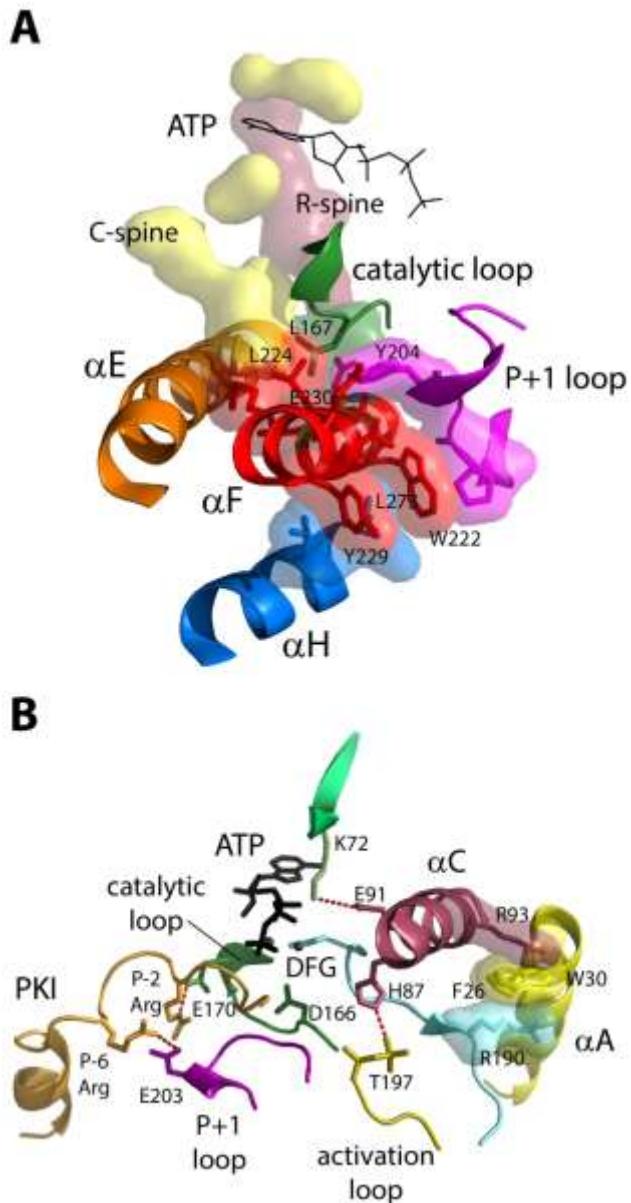


Figure S9. Signal integration motifs in PKA-C. (A) The F-helix (red) is a central scaffold where the catalytic and regulatory spines are tethered. It also forms hydrophobic packing with the H-helix (blue) and catalytic loop (green). It also forms a salt bridge and hydrophobic packing with the P+1 loop (magenta). (B) The C-helix (dark pink) integrates the important catalytic elements in the active site with the small lobe and A-helix (yellow).

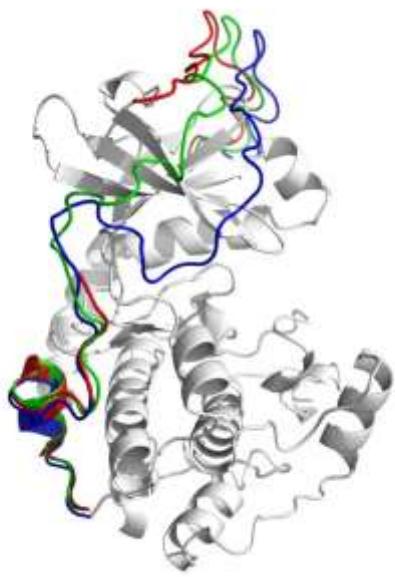


Figure S10. The orientations of the C-terminal tail in three different conformations of PKA as observed in the crystal structures: PDB ID: 1J3H (apo, red), 4NTT (binary, green), 1ATP (ternary, blue).

Table S1. Equilibrium H/D Fractionation Factors of the Residues in Three Major States of PKA-C

Residue	Apo	Binary (PKA/ADP)	Ternary (PKA/ADP/PKI)
3	0.58 ± 0.07	0.58 + 0.09	0.72 + 0.04
4	1.27 ± 0.12	1.16 + 0.09	0.86 + 0.08
5	1.48 ± 0.08	1.15 + 0.16	1.00 + 0.05
6	1.04 ± 0.05	0.96 + 0.11	1.18 + 0.06
7	1.29 ± 0.08	1.02 + 0.11	0.87 + 0.01
8	1.15 ± 0.12	0.90 + 0.11	0.87 + 0.03
9	1.30 ± 0.05	1.11 + 0.15	1.03 + 0.05
10	1.49 ± 0.16	0.95 + 0.09	0.89 + 0.02
11	1.27 ± 0.13	1.14 + 0.06	1.00 + 0.05
12	1.65 ± 0.12	0.92 + 0.10	1.25 + 0.08
13	-	1.11 + 0.09	0.99 + 0.04
14	1.13 ± 0.12	1.10 + 0.16	0.95 + 0.02
15	1.73 ± 0.13	-	0.44 + 0.11
16	-	0.84 + 0.12	-
17	0.89 ± 0.05	0.99 + 0.08	0.99 + 0.01
18	-	1.15 + 0.11	-
19	1.57 ± 0.04	-	-
20	1.12 ± 0.04	2.11 + 0.22	1.49 + 0.12
21	-	-	0.93 + 0.02
24	1.33 ± 0.13	-	1.33 + 0.11
25	1.29 ± 0.03	0.76 + 0.14	0.57 + 0.10
26	-	0.96 + 0.07	1.03 + 0.04
27	-	1.90 + 0.13	ND
28	1.37 ± 0.02	-	1.06 + 0.06
29	-	1.09 + 0.05	0.87 + 0.13
30	1.60 ± 0.15	1.35 + 0.11	-
31	1.49 ± 0.07	1.34 + 0.16	1.22 + 0.12
32	1.44 ± 0.15	0.94 + 0.06	1.17 + 0.05
34	-	1.65 + 0.20	-
35	1.70 ± 0.13	1.62 + 0.16	1.50 + 0.04
36	-	1.93 + 0.80	1.55 + 0.11
37	ND	1.25 + 0.09	1.01 + 0.07
38	-	1.37 + 0.12	1.20 + 0.03
40	ND	0.64 + 0.10	0.82 + 0.12

41	1.43 ± 0.09	$1.33 + 0.08$	$1.42 + 0.05$
42	<i>ND</i>	$1.16 + 0.12$	$1.40 + 0.15$
43	-	$0.96 + 0.11$	$1.13 + 0.10$
44	-	$0.57 + 0.12$	-
45	<i>ND</i>	-	$1.83 + 0.15$
46	1.09 ± 0.05	$0.98 + 0.23$	-
47	-	-	$0.30 + 0.08$
48	-	-	$1.06 + 0.14$
49	1.09 ± 0.10	$0.28 + 0.08$	$0.42 + 0.09$
50	1.26 ± 0.12	<i>ND</i>	$0.40 + 0.05$
51		-	$1.52 + 0.11$
52	<i>ND</i>	-	-
53	-	-	$0.89 + 0.14$
55	0.99 ± 0.05	-	$0.69 + 0.08$
56	<i>ND</i>	-	-
58	0.34 ± 0.05	$0.52 + 0.11$	$0.21 + 0.05$
59	0.32 ± 0.05	$0.47 + 0.06$	$0.29 + 0.10$
60	0.68 ± 0.04	$0.53 + 0.06$	-
61	0.36 ± 0.11	$0.45 + 0.01$	-
62	<i>ND</i>	$1.82 + 0.52$	<i>ND</i>
63	1.49 ± 0.31	$1.54 + 0.42$	$1.96 + 0.19$
64	0.96 ± 0.05	$0.77 + 0.05$	-
65	-	$0.61 + 0.14$	$0.71 + 0.16$
66	1.05 ± 0.02	$1.10 + 0.06$	$1.18 + 0.12$
68	-	$1.75 + 0.05$	$1.75 + 0.16$
69	0.88 ± 0.02	$0.33 + 0.07$	$0.24 + 0.03$
70	0.45 ± 0.09	$0.50 + 0.08$	<i>ND</i>
71	0.49 ± 0.10	$0.40 + 0.04$	$0.21 + 0.06$
72	0.56 ± 0.08	$0.44 + 0.15$	$0.21 + 0.05$
73	0.31 ± 0.11	-	<i>ND</i>
74	0.37 ± 0.07	-	$0.26 + 0.02$
75	-	-	$1.59 + 0.26$
76	1.74 ± 0.23	$0.37 + 0.12$	-
77	1.21 ± 0.03	$1.65 + 0.10$	$1.19 + 0.06$
78	0.83 ± 0.11	-	$1.41 + 0.07$
79	-	-	<i>ND</i>
80	-	$0.16 + 0.04$	-

81	-	1.12 + 0.08	-
82	0.79 ± 0.01	-	-
83	1.10 ± 0.05	$1.30 + 0.09$	$1.10 + 0.11$
84	1.24 ± 0.12	-	$0.36 + 0.09$
85	0.98 ± 0.17	-	ND
86	-	-	$1.04 + 0.02$
88	0.72 ± 0.07	-	-
89	1.81 ± 0.24	-	-
90	-	$0.46 + 0.08$	-
91	-	-	$0.19 + 0.04$
92	-	-	$0.38 + 0.11$
94	1.13 ± 0.17	-	$0.40 + 0.03$
95	0.54 ± 0.05	-	$0.33 + 0.05$
96	-	$0.39 + 0.08$	$0.24 + 0.09$
97	0.50 ± 0.07	-	$0.16 + 0.04$
98	0.42 ± 0.04	$0.36 + 0.10$	$0.28 + 0.03$
99	ND	-	-
100	ND	$1.92 + 0.14$	$1.27 + 0.11$
102	-	$0.42 + 0.09$	$0.19 + 0.05$
103	0.36 ± 0.07	$0.25 + 0.06$	$0.16 + 0.04$
104	0.89 ± 0.11	-	$0.15 + 0.06$
105	1.28 ± 0.10	-	-
106	1.84 ± 0.43	$1.81 + 0.17$	$1.72 + 0.13$
107	1.98 ± 0.34	$1.46 + 0.55$	$1.84 + 0.12$
108	1.44 ± 0.15	ND	ND
109	1.59 ± 0.11	$1.34 + 0.08$	-
110	0.80 ± 0.06	$0.55 + 0.12$	ND
111	ND	ND	$2.07 + 0.09$
112	0.43 ± 0.06	$0.42 + 0.02$	$0.32 + 0.04$
113	1.46 ± 0.08	ND	-
114	1.37 ± 0.06	$0.95 + 0.21$	-
115	0.23 ± 0.05	ND	$0.41 + 0.04$
116	0.62 ± 0.08	$0.43 + 0.08$	$0.37 + 0.04$
117	0.56 ± 0.03	$0.50 + 0.02$	$0.36 + 0.05$
118	0.84 ± 0.07	-	-
119	0.49 ± 0.03	-	-
120	0.54 ± 0.11	$0.57 + 0.11$	$1.18 + 0.04$

121	-	-	1.70 + 0.08
122	-	2.09 + 0.56	-
123	0.81 ± 0.09	-	0.44 + 0.05
124	1.65 ± 0.15	1.56 + 0.25	1.59 + 0.29
125	1.13 ± 0.07	1.10 + 0.29	1.51 + 0.21
126	-	-	ND
127	-	-	0.97 + 0.11
128	-	-	0.18 + 0.05
129	-	-	ND
130	-	-	1.82 + 0.25
131	-	-	1.20 + 0.09
132	-	-	0.27 + 0.06
133	1.79 ± 0.09	-	0.47 + 0.13
134	0.99 ± 0.06	-	1.44 + 0.06
136	-	ND	1.27 + 0.03
137	-	ND	ND
138	2.09 ± 0.58	-	0.23 + 0.07
139	ND	2.19 + 0.49	1.90 + 0.23
140	ND	ND	ND
142	ND	ND	1.82 + 0.04
143	0.43 ± 0.04	ND	-
144	0.33 ± 0.06	0.29 + 0.06	0.33 + 0.08
145	0.56 ± 0.08	-	-
146	-	-	0.25 + 0.03
147	0.46 ± 0.08	0.50 + 0.07	ND
148	0.56 ± 0.02	0.43 + 0.07	-
149	ND	0.47 + 0.04	0.16 + 0.06
150	0.75 ± 0.03	ND	0.23 + 0.07
151	0.35 ± 0.03	0.33 + 0.07	-
155	0.50 ± 0.12	ND	0.29 + 0.07
156	-	-	0.81 + 0.10
157	-	-	0.17 + 0.03
160	ND	-	-
161	ND	-	1.67 + 0.10
162	1.03 ± 0.11	0.24 + 0.05	-
163	-	0.58 + 0.07	-
165	ND	-	1.93 + 0.48

166	1.37 ± 0.18	$1.00 + 0.14$	$0.16 + 0.07$
170	-	-	$0.53 + 0.16$
174	-	$0.67 + 0.05$	$0.26 + 0.03$
175	0.29 ± 0.03	$0.41 + 0.06$	$0.30 + 0.04$
176	ND	ND	$1.34 + 0.19$
177	-	$1.58 + 0.10$	$1.83 + 0.06$
178	ND	ND	-
179	0.33 ± 0.09	ND	$0.20 + 0.03$
180	0.47 ± 0.12	ND	$0.21 + 0.08$
181	-	$0.50 + 0.09$	-
182	0.34 ± 0.05	-	$0.34 + 0.02$
184	-	-	ND
185	1.53 ± 0.32	-	$2.03 + 0.24$
186	0.86 ± 0.05	-	ND
187	1.11 ± 0.07	-	ND
188	0.96 ± 0.02	ND	ND
189	2.00 ± 0.38	-	-
190	ND	$1.58 + 0.24$	$1.69 + 0.24$
191	ND	$1.92 + 0.23$	$0.92 + 0.18$
192	1.11 ± 0.03	-	$1.32 + 0.03$
193	1.22 ± 0.09	$1.03 + 0.11$	$1.18 + 0.04$
194	-	$1.69 + 0.07$	$1.44 + 0.10$
195	1.60 ± 0.20	-	$1.51 + 0.03$
196	-	$0.77 + 0.06$	$0.95 + 0.03$
197	1.16 ± 0.15	$0.71 + 0.11$	-
198	1.55 ± 0.11	ND	$1.41 + 0.28$
199	ND	-	$0.21 + 0.05$
200	1.76 ± 0.09	-	$1.14 + 0.34$
201	ND	-	$0.46 + 0.12$
203	ND	-	$0.43 + 0.15$
205	0.71 ± 0.01	-	$0.30 + 0.07$
206	0.94 ± 0.05	$0.50 + 0.08$	$0.23 + 0.07$
208	0.70 ± 0.14	$1.63 + 0.09$	$0.16 + 0.07$
209	0.25 ± 0.08	-	$0.30 + 0.11$
210	0.30 ± 0.05	ND	$0.29 + 0.05$
211	-	-	$1.02 + 0.12$
212	1.58 ± 0.03	$1.10 + 0.07$	-

213	-	0.98 + 0.09	1.11 + 0.04
214	1.43 ± 0.06	1.09 + 0.12	1.15 + 0.08
215	-	1.45 + 0.07	0.42 + 0.10
216	1.15 ± 0.25	0.72 + 0.07	1.93 + 0.13
218	-	-	1.19 + 0.12
219	0.51 ± 0.04	-	-
220	-	1.04 + 0.23	0.29 + 0.10
221	0.33 ± 0.02	0.30 + 0.03	0.26 + 0.03
222	0.42 ± 0.10	-	-
225	0.61 ± 0.07	0.29 + 0.08	0.36 + 0.06
226	0.33 ± 0.05	0.39 + 0.06	-
227	-	0.41 + 0.08	0.38 + 0.11
228	0.29 ± 0.03	0.60 + 0.08	-
230	-	0.37 + 0.07	-
231	-	0.45 + 0.07	0.44 + 0.03
232	-	0.85 + 0.12	0.84 + 0.09
233	0.32 ± 0.01	0.89 + 0.24	0.34 + 0.05
235	-	-	0.88 + 0.08
238	-	0.54 + 0.14	-
239	-	-	ND
240	ND	ND	ND
241	2.13 ± 0.16	1.47 + 0.16	1.54 + 0.04
242	1.24 ± 0.09	ND	1.55 + 0.07
244	ND	ND	1.60 + 0.03
245	1.04 ± 0.09	1.19 + 0.04	1.26 + 0.04
246	1.63 ± 0.12	1.77 + 0.57	0.59 + 0.05
247	-	0.54 + 0.05	1.38 + 0.11
248	-	1.32 + 0.77	1.27 + 0.04
249	1.27 ± 0.08	-	1.73 + 0.09
250	1.62 ± 0.15	-	-
251	ND	-	-
252	-	0.91 + 0.04	-
253	1.10 ± 0.03	1.18 + 0.06	1.04 + 0.09
254	0.95 ± 0.02	ND	1.26 + 0.03
255	1.73 ± 0.07	1.04 + 0.05	1.05 + 0.07
256	ND	ND	1.78 + 0.13
257	-	-	1.25 + 0.09

261	1.25 ± 0.05	ND	1.58 + 0.15
262	1.45 ± 0.03	ND	ND
263	1.74 ± 0.17	1.83 + 0.20	2.08 + 0.28
264	-	1.80 + 0.02	1.35 + 0.05
265	0.41 ± 0.01	ND	ND
266	-		0.27 + 0.08
267	-	1.17 + 0.12	0.30 + 0.06
268	-	-	-
269	0.61 ± 0.05	0.64 + 0.04	-
272	0.67 ± 0.07	-	-
273	0.64 ± 0.03	-	-
274	0.26 ± 0.05	0.22 + 0.04	0.26 + 0.07
275	1.95 ± 0.10	ND	1.27 + 0.09
276	0.58 ± 0.05	1.71 + 0.22	-
277	1.28 ± 0.20	1.25 + 0.19	1.55 + 0.18
278	0.58 ± 0.03	0.52 + 0.07	0.51 + 0.04
279	0.92 ± 0.09	0.55 + 0.03	0.73 + 0.02
280	0.73 ± 0.06	0.98 + 0.08	0.28 + 0.04
281	1.68 ± 0.57	ND	0.55 + 0.16
282	-	-	1.18 + 0.55
283	-	-	0.37 + 0.03
284	0.53 ± 0.06	-	0.29 + 0.07
286	-	0.98 + 0.05	1.16 + 0.02
287	1.10 ± 0.08	1.08 + 0.10	1.42 + 0.04
288	-	-	0.75 + 0.13
289	1.17 ± 0.06	-	1.05 + 0.04
290	1.40 ± 0.09	1.04 + 0.03	0.79 + 0.02
291	ND	1.25 + 0.22	ND
292	0.24 ± 0.03	0.23 + 0.02	-
293	0.51 ± 0.07	-	ND
294	0.27 ± 0.03	0.27 + 0.04	0.16 + 0.06
295	1.60 ± 0.22	1.80 + 0.05	1.60 + 0.01
296	ND	1.65 + 0.05	ND
297	ND	ND	ND
298	2.05 ± 0.21	1.64 + 0.20	1.63 + 0.14
299	1.10 ± 0.16	0.65 + 0.09	0.59 + 0.01
300	-	0.88 + 0.06	0.94 + 0.02

301	1.94 ± 0.24	$1.35 + 0.13$	$1.98 + 0.09$
302	<i>ND</i>	$1.83 + 0.14$	$1.66 + 0.04$
303	1.42 ± 0.15	-	$0.75 + 0.08$
304	1.15 ± 0.11	$0.80 + 0.07$	$0.91 + 0.13$
305	-	$1.50 + 0.14$	-
307	1.57 ± 0.19	<i>ND</i>	-
308	-	$0.36 + 0.06$	$0.28 + 0.04$
309	0.28 ± 0.04	$0.24 + 0.03$	$0.18 + 0.08$
310	0.70 ± 0.03	$0.77 + 0.18$	$0.42 + 0.08$
311	<i>ND</i>	<i>ND</i>	$1.64 + 0.01$
312	2.07 ± 0.06	$1.63 + 0.19$	$1.48 + 0.18$
314	2.11 ± 0.15	$1.56 + 0.06$	$0.93 + 0.13$
315	<i>ND</i>	$1.91 + 0.22$	<i>ND</i>
317	-	<i>ND</i>	$1.27 + 0.05$
318	1.57 ± 0.23	$1.90 + 0.12$	$1.30 + 0.13$
319	-	-	$1.58 + 0.04$
320	-	-	$1.13 + 0.07$
322	2.13 ± 0.24	<i>ND</i>	$1.94 + 0.08$
323	-	-	$1.31 + 0.06$
324	-	-	$0.72 + 0.02$
325	0.96 ± 0.02	-	$0.61 + 0.02$
326	-	-	$0.68 + 0.05$
327	1.87 ± 0.18	-	<i>ND</i>
328	1.05 ± 0.09	-	$1.09 + 0.01$
329	-	-	$1.02 + 0.02$
330	<i>ND</i>	-	<i>ND</i>
331	1.43 ± 0.12	$1.80 + 0.18$	$1.52 + 0.04$
332	-	$1.99 + 0.14$	$1.16 + 0.13$
333	-	$1.35 + 0.08$	$1.66 + 0.08$
334	<i>ND</i>	$1.70 + 0.06$	-
335	-	$1.86 + 0.07$	$1.38 + 0.64$
336	1.65 ± 0.07	$1.84 + 0.19$	$1.47 + 0.04$
337	<i>ND</i>	-	$1.59 + 0.10$
338	-	$1.47 + 0.11$	$1.35 + 0.19$
339	-	$1.61 + 0.11$	-
341	<i>ND</i>	<i>ND</i>	$1.74 + 0.11$
342	<i>ND</i>	$1.22 + 0.45$	$1.85 + 0.21$

343	<i>ND</i>	<i>ND</i>	$1.79 + 0.14$
344	1.42 ± 0.06	$1.67 + 0.04$	$1.21 + 0.10$
345	-	$1.16 + 0.05$	$1.02 + 0.02$
346	1.75 ± 0.18	$1.23 + 0.19$	$0.98 + 0.04$
347	0.58 ± 0.03	-	<i>ND</i>
348	1.16 ± 0.07	$0.97 + 0.02$	$0.81 + 0.02$
349	1.23 ± 0.07	$1.39 + 0.08$	$0.89 + 0.06$
350	-	$1.18 + 0.12$	$1.58 + 0.05$

*ND = not determined, either due to extreme values of fractionation factors (<0.20 or > 2.00, italicized) or poor correlation coefficient ($R^2 < 0.70$, non-italicized). Dashed entries mean that either there is no assignment for that residue or the peak is overlapped.