Supporting Materials

Table S1. Protonation state of the His residues in the simulation systems of CDK9 and CDK2.

CDK9		CDK2	
His38	HIE	His60	HIE
His75	HIE	His71	HID
His108	HIE	His84	HID
His141	HID	His119	HIE
His147	HIE	His121	HID
His236	HIE	His125	HIE
His312	HIE	His161	HID
		His268	HIE
		His283	HIE
		His295	HIE

Table S2. The results of 5 repeats of SMD simulations.

System	F_{max_1}	F_{max_2}	F_{max_3}	F_{max_4}	F_{max_5}	F_{max}[a]
CDK9/12u	1399	1440	1372	1368	1332	1382±40
CDK2/12u[b]	1092	1142	996	1108	1054	1078±60
CDK2/12u[c]	910	923	909	883	899	904±15
CDK9/4	1258	1277	1224	1228	1356	1268±53
CDK2/4	1025	1019	1123	991	989	1029±55

[a] $F_{max}= \overline{X}$, the uncertainty of them is based on the the 5 repeats of SMD simulations standard deviation ($s=\sqrt{\frac{\sum_{i=1}^{n}(X_i-\overline{X})^2}{n-1}}$)of them (n=5; i=1, 2, 3, 4, 5; $X_i = F_{max\_i}$, $\overline{X} = \frac{1}{n}\sum_{i=1}^{n} X_i$).[b]CDK9/12u system with "inward orientation" of the ligand. [c]CDK9/12u system with "outward orientation" of the ligand.

Table S3. The individual energy components of compound 12u predicted by MM/GBSA.

Residue	CDK9/12u					Residue	CDK2/12u				
	ΔE_{ele}	ΔG_{GB}	ΔE_{vdW}	ΔG_{SA}	ΔG_{bind}		ΔE_{ele}	ΔG_{GB}	ΔE_{vdW}	ΔG_{SA}	ΔG_{bind}
Ile25	-0.16	0.46	-6.20	-0.78	-6.68	Ile10	-0.18	0.70	-5.86	-0.86	-6.20
Gly26	0	0.08	-0.52	-0.02	-0.44	Gly11	0.06	0.04	-1.24	-0.16	-1.30
Gln27	-0.06	0.54	-2.04	-0.36	-1.92	Glu12	-0.44	0.66	-1.04	-0.16	-1.00
Gly28	-0.04	0.12	-0.38	-0.04	-0.36	Gly13	0.10	-0.02	-0.50	-0.10	-0.52
Thr29	0	0.18	-1.40	-0.28	-1.48	Thr14	0.02	0.02	-0.12	0	-0.10
Phe30	0	0.02	-0.08	0	-0.06	Tyr15	-0.02	0.06	-0.16	0	-0.14
Gly31	0.02	0	-0.04	0	-0.02	Gly16	-0.08	0.12	-0.12	0	-0.08
Glu32	-0.1	0.14	-0.18	0	-0.14	Val17	0.04	-0.02	-0.20	0	-0.18
Val33	-0.02	-0.02	-3.06	-0.3	-3.4	Val18	-0.06	0.02	-3.86	-0.46	-4.36
Phe34	-0.06	0.08	-0.50	0	-0.46	Tyr19	-0.02	0.04	-0.44	0	-0.42
Lys35	0.20	0.10	-1.90	-0.12	-1.72	Lys20	0.28	-0.20	-0.44	0	-0.36
Ala46	0.14	-0.04	-2.44	-0.22	-2.58	Ala31	0.12	0	-2.40	-0.20	-2.48
Lys48	-1.76	1.60	-2.36	-0.22	-2.74	Lys33	-1.30	1.28	-2.44	-0.26	-2.70
Val78	-0.08	0.08	-0.08	0	-0.08	Ile63	-0.06	0.06	-0.06	0	-0.06
Val79	-0.12	0.08	-1.32	-0.12	-1.48	Val64	-0.04	0.02	-1.14	-0.10	-1.24
Asn80	0.08	-0.04	-0.24	0	-0.20	Lys65	-0.20	0.22	-0.20	0	-0.16
Phe103	0.10	0.16	-3.38	-0.18	-3.30	Phe80	0.12	0.12	-3.42	-0.18	-3.38
Asp104	-0.56	0.54	-0.68	-0.02	-0.72	Glu81	-0.54	0.50	-0.74	-0.04	-0.82
Phe105	-0.08	0.08	-1.72	-0.02	-1.74	Phe82	-0.28	0.22	-3.26	-0.12	-3.44
Cys106	-1.38	0.92	-2.92	-0.18	-3.56	Leu83	-1.78	1.16	-2.76	-0.16	-3.56
Glu107	-0.58	0.72	-1.48	-0.08	-1.42	His84	-0.08	0.24	-1.30	-0.12	-1.26
His108	0.06	0.04	-1.78	-0.06	-1.72	Gln85	-0.10	0.04	-1.28	-0.12	-1.44
Asp109	-1.02	2.20	-3.82	-0.52	-3.16	Asp86	-0.40	1.62	-2.70	-0.44	-1.94
Leu110	0	0.02	-0.18	0	-0.16	Leu87	0	0.02	-0.12	0	-0.10
Ala111	0.02	-0.02	-0.32	-0.02	-0.32	Lys88	0.08	-0.04	-0.22	0	-0.18
Gly112	0.04	-0.06	-0.16	0	-0.18	Lys89	0.32	-0.22	-0.4	-0.06	-0.36
Ala153	-0.92	0.96	-1.86	-0.42	-2.26	Gln131	-0.30	0.52	-1.92	-0.44	-2.14
Asn154	-0.02	0.14	-0.98	-0.08	-0.94	Asn132	-0.06	0.12	-0.54	-0.02	-0.50
Val155	-0.04	0.02	-0.30	0	-0.3	Leu133	-0.04	0.04	-0.24	0	-0.24
Leu156	-0.04	0.08	-5.52	-0.60	-6.08	Leu134	-0.02	0.04	-4.64	-0.62	-5.26
Ile157	-0.06	0.06	-0.3	0	-0.3	Ile135	-0.04	0.06	-0.20	0	-0.18
Thr158	0	0.02	-0.12	0	-0.08	Asn136	0.02	0	-0.06	0	-0.04
Leu165	0.14	-0.12	-0.18	0	-0.16	Leu143	0.10	-0.10	-0.14	0	-0.12
Ala166	-0.30	0.30	-1.74	-0.14	-1.86	Ala144	-0.3	0.26	-1.08	-0.08	-1.18
Asp167	-1.06	1.40	-2.38	-0.34	-2.40	Asp145	-1.12	1.60	-2.26	-0.44	-2.20

Table S4. The individual energy components of compound 4 predicted by MM/GBSA.

Residue	CDK9/4					Residue	CDK2/4				
	ΔE_{ele}	ΔG_{GB}	ΔE_{vdW}	ΔG_{SA}	ΔG_{bind}		ΔE_{ele}	ΔG_{GB}	ΔE_{vdW}	ΔG_{SA}	ΔG_{bind}
Lys24	-0.66	0.70	-0.18	0	-0.16	Lys9	-0.16	0.26	-0.22	0	-0.14
Ile25	0.18	0.18	-5.18	-0.72	-5.52	Ile10	0.24	0.32	-6.44	-1.02	-6.90
Gly26	-0.04	0.14	-1.54	-0.08	-1.50	Gly11	0.06	0.02	-0.80	-0.12	-0.84
Gln27	0.06	0.06	-0.38	0	-0.26	Glu12	0.08	0	-0.26	-0.02	-0.22
Gly28	0	0	-0.04	0	-0.04	Gly13	0.02	0	-0.06	0	-0.04
Thr29	-0.02	0.08	-0.14	0	-0.10	Thr14	0	0.02	-0.02	0	0
Phe30	-0.14	0.46	-3.64	-0.70	-4.02	Tyr15	0	0	-0.02	0	-0.02
Gly31	-0.10	0.14	-0.28	-0.02	-0.26	Gly16	-0.02	0.04	-0.04	0	-0.02
Glu32	0.36	-0.30	-0.24	0	-0.20	Val17	0	0.02	-0.14	0	-0.12
Val33	0.04	-0.08	-4.44	-0.46	-4.92	Val18	-0.04	0.02	-3.14	-0.44	-3.60
Phe34	0	0.04	-0.42	0	-0.38	Tyr19	0	0.02	-0.44	0	-0.42
Lys35	0.32	-0.26	-0.36	0	-0.28	Lys20	0.94	-0.82	-0.62	-0.02	-0.52
Lys44	0.26	-0.22	-0.08	0	-0.04	Val29	0.02	-0.02	-0.1	0	-0.08
Val45	0.02	0	-0.20	0	-0.18	Val30	0	0.02	-0.24	0	-0.22
Als46	0.08	0	-2.24	-0.20	-2.36	Ala31	0.08	0.02	-2.52	-0.24	-2.66
Leu47	0.02	0.02	-0.56	0	-0.54	Leu32	-0.02	0.04	-0.70	0	-0.68
Lys48	-1.42	1.68	-2.90	-0.34	-2.98	Lys33	-1.48	1.42	-2.46	-0.18	-2.72
Lys49	-0.3	0.34	-0.12	0	-0.08	Lys34	-0.08	0.08	-0.06	0	-0.04
Val78	-0.06	0.06	-0.06	0	-0.06	Ile63	-0.08	0.08	-0.06	0	-0.06
Val79	-0.06	0.04	-1.26	-0.10	-1.38	Val64	-0.02	0	-1.00	-0.06	-1.08
Asn80	0.08	-0.04	-0.20	0	-0.16	Lys65	-0.04	0.08	-0.14	0	-0.12
Leu81	-0.04	0.04	-0.14	0	-0.14	Leu66	-0.02	0.02	-0.12	0	-0.12
Leu101	-0.04	0.04	-0.22	0	-0.22	Leu78	-0.04	0.04	-0.18	0	-0.20
Val102	0.06	-0.06	-0.16	0	-0.14	Val79	0.08	-0.06	-0.14	0	-0.12
Phe103	0.16	0.10	-3.26	-0.18	-3.16	Phe80	0.02	0.16	-3.72	-0.14	-3.66
Asp104	-0.84	0.78	-0.66	-0.02	-0.74	Glu81	-0.86	0.86	-0.88	-0.02	-0.92
Phe105	-0.16	0.18	-3.00	-0.06	-3.04	Phe82	-0.24	0.18	-3.14	-0.18	-3.38
Cys106	-1.86	1.24	-2.50	-0.18	-3.3	Leu83	-1.78	1.38	-3.26	-0.22	-3.88
Glu107	-1.12	1.38	-1.36	-0.14	-1.26	His84	-0.14	0.32	-1.32	-0.14	-1.28
His108	-0.14	0.26	-1.72	-0.08	-1.66	Gln85	-0.14	0.18	-0.86	-0.04	-0.86
Asp109	-2.00	3.32	-4.04	-0.66	-3.36	Asp86	-0.12	1.00	-2.2	-0.34	-1.68
Leu110	0	0.02	-0.20	0	-0.18	Leu87	-0.02	0.04	-0.12	0	-0.10
Ala111	0.1	-0.08	-0.32	-0.02	-0.32	Lys88	-0.18	0.20	-0.28	-0.02	-0.28
Gly112	0.14	-0.14	-0.20	0	-0.18	Lys89	-0.02	0.10	-0.36	-0.06	-0.36
Ala152	-0.08	0.08	-0.14	0	-0.14	Pro130	-0.04	0.04	-0.20	0	-0.20
Ala153	-0.18	0.48	-1.52	-0.34	-1.56	Gln131	-0.32	0.60	-2.84	-0.52	-3.06
Asp167	-0.76	1.20	-2.18	-0.38	-2.14	Asp145	-0.48	1.04	-3.90	-0.54	-3.88

Table S5. The ΔW_{PMF} of 10 cycles of 200 ps US simulations.

	CDK9-12u[a]	**CDK2-12u[b]**	**CDK9-4[a]**	**CDK2-4[a]**
0~200 ps	20.57±0.15[c]	17.81±0.64	21.97±0.18	19.66±0.35
200~400 ps	20.54±0.15	15.22±0.61	18.70±0.14	15.28±0.35
400~600 ps	20.66±0.15	13.03±0.60	17.47±0.16	13.21±0.37
600~800 ps	22.29±0.21	10.43±0.63	17.11±0.20	11.21±0.35
800~1000 ps	20.02±0.24	10.90±0.40	16.15±0.14	12.44±0.42
1000~1200 ps	20.42±0.18	10.77±0.26	16.13±0.16	12.69±0.39
1200~1400 ps	18.72±0.25	10.55±0.37	15.39±0.12	11.34±0.28
1400~1600 ps	18.43±0.12	10.55±0.39	16.71±0.11	12.60±0.17
1600~1800 ps	20.17±0.13	10.00±0.35	15.92±0.14	11.91±0.21
1800~2000 ps	19.34±0.15	9.60±0.25	14.28±0.11	13.40±0.21
average (2 ns)	19.4±1.10[d]	10.1±2.67	15.5±2.12	11.0±2.50

[a]The PMF value was estimated by averaging bins across 18~20 Å of the Reaction Coordinate (CDK9-12u, CDK9-4, and CDK2-4). [b]The PMF value was estimated by averaging bins across 12~16 Å of the Reaction Coordinate (CDK2-12u). [c]The standard deviations ($s=\sqrt{\frac{\sum_{i=1}^{n}(X_i-\bar{X})^2}{n-1}}$) for the 10 cycles of US simulations (0~2000 ps) were estimated from 18~20 Å of the RC for CDK9-12u, CDK9-4, and CDK2-4, and 12~16 Å of the RC for CDK2-12u. [d]The total standard deviations ($s=\sqrt{\frac{\sum_{i=1}^{n}(X_i-\bar{X})^2}{n-1}}$) were estimated from the PMF values of the 10 cycles of US simulations.

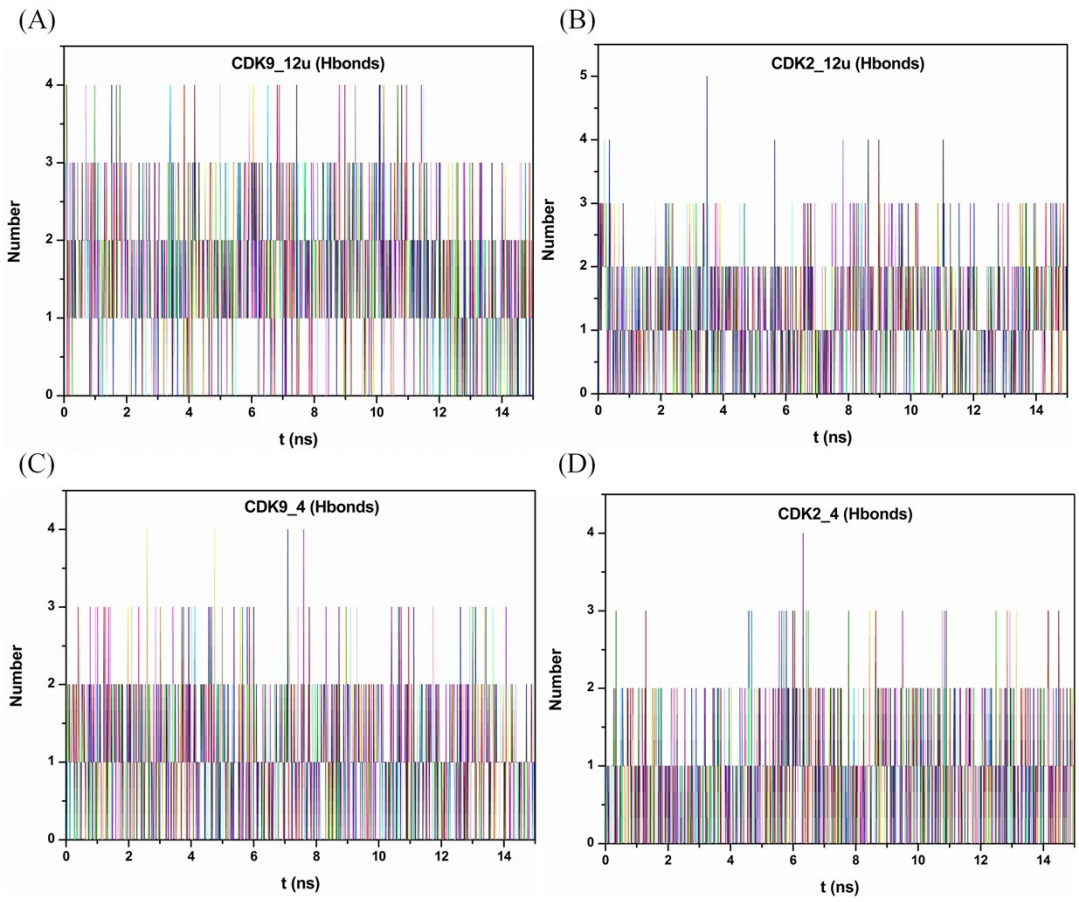

Figure S1. H-bonds interactions of the four systems in the MD simulations.

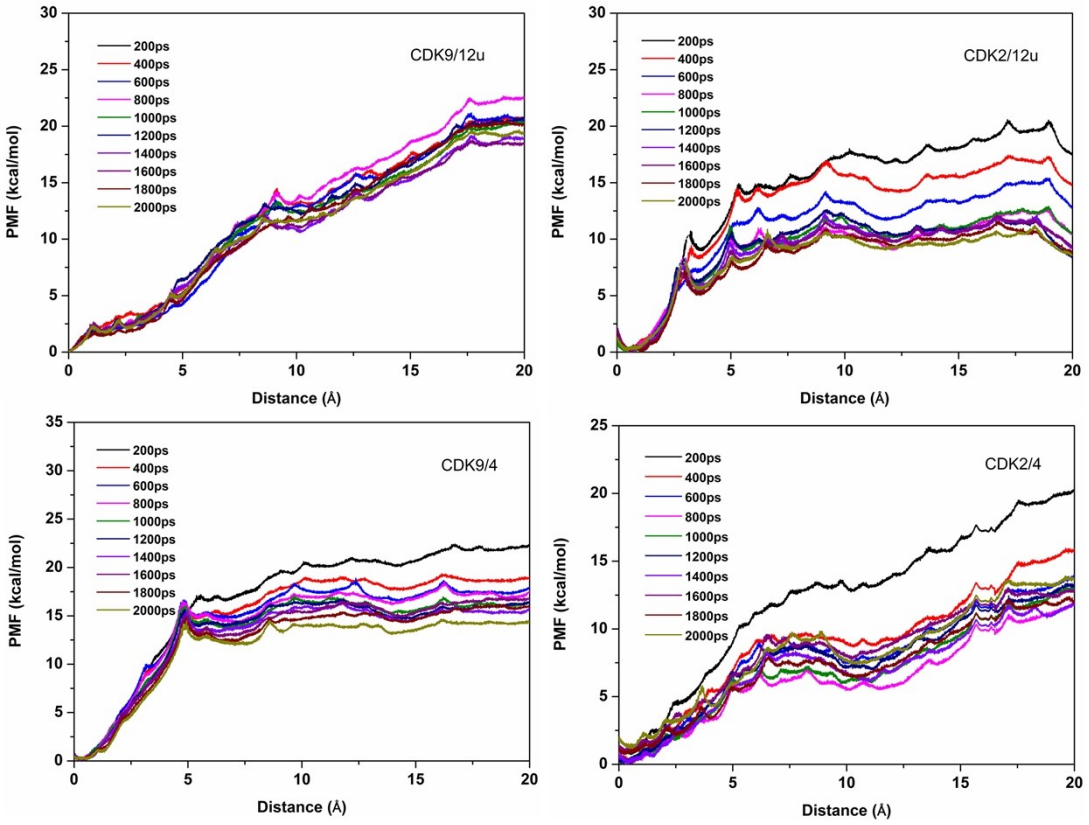

Figure S2. Convergence of PMFs calculated by US, where 2 ns US simulations were performed for each system.

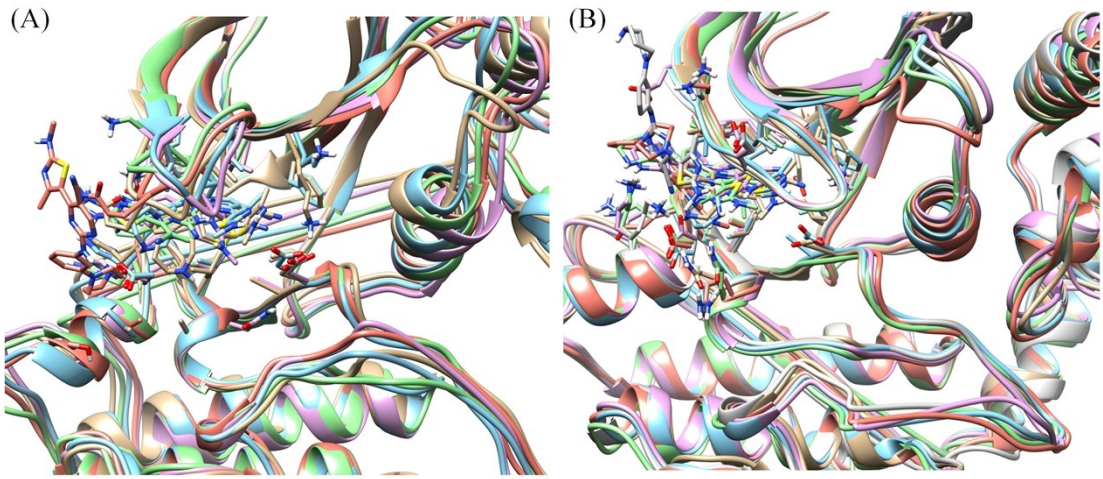

Figure S3. The superimposition of the representative structures extracted from (A) CDK9 and (B) CDK2 US simulations.