

# Selectivity Studies and Free Energy Calculations of AKT Inhibitors

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**Figure S1.** Multiple sequence alignments of three different AKT subtypes. The alignment was generated with the Clustal Omega program. AKT1: 6S9W; AKT2: 3D0E and AKT2\_Comb; AKT3: AAD24196.1. Binding residues unique to different subtypes are highlighted.

**Figure S2.** The Ramachandran plot for the AKT3\_HM model (A) and the AKT3\_AF model (B).

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**Figure S3.** Superposition of AKT1 (red), AKT2 (cyan) and AKT3 (orange). The catalytic domain and the PH domain were highlighted.

**Table S2.** Interacting residues from the CovDock generated poses for the AKT1 (6S9W) models.

**Table S3.** Interacting residues from the CovDock generated poses for the AKT2 (3D0E\_N and 3D0E) models.

**Table S4.** Interacting residues from the CovDock generated poses for the AKT3 model (HM model and AF model).

**Figure S4.** Interacting residues of AKT3 inhibitors (the AF model).

CLUSTAL 2.1 multiple sequence alignment

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6S9W (AKT1)      GSDVAIVKEGWLHKGREYIKTWPRPYFLLKNDGTFIGYKERPDQVDQREAPLNNFSVAQC 60
AAD24196.1      MSDVTIVKEGWVQKRGEYIKNWRPRYFLLKTDGSFIGYKEKPQDQVDLP-YPLNFSVAKC 59
3D0E (AKT2)      ----- 0
AKT2_Comb       MNEVSVIKEGWLHKGREYIKTWPRPYFLLKSDGSFIGYKERPEAPDQTLPLNFSVAEC 60
                  E17 (AKT3)                                N54 (AKT1/2)
                  N53 (AKT3)

6S9W (AKT1)      QLMKTERPRPNTFIIIRCLQWTTVIERTFHVETPEEREETTAIQTVADGLKKQAAAE--M 118
AAD24196.1      QLMKTERPKPNTFIIIRCLQWTTVIERTFHVDTPEEREETTAIQAVADRLQRQEE---E 115
3D0E (AKT2)      ----- 0
AKT2_Comb       QLMKTERPRPNTFVIRCLQWTTVIERTTFHVDSPDEREETWMRAIQMVANSLKQRAPGEDPM 120
                  W80 (AKT1)
                  W79 (AKT3)
                  R86 (AKT2)

6S9W (AKT1)      DFRSGSP--SDNSGAEEMEVSLAKPKHRVTMNEFEYLKLLGKGTFGKVILVKEKATGRYY 176
AAD24196.1      RMNCSPSQIDNIGEEEMDA-STTHHKRKTMNDFDYLLGKGTFGKVILVREKASGKYY 174
3D0E (AKT2)      -----KVTMNDFDYLLGKGTFGKVILVREKATGRYY 33
AKT2_Comb       DYKCGSPSD--SSTTEEMEVAVSKARAKVTMNDFDYLLGKGTFGKVILVREKATGRYY 178
                  :  ***:***:*****:***:***:
                  T162, F163 (AKT2)

6S9W (AKT1)      AMKILKKEVIVAKDEVAHTLTENRVLQNSRHPFLTALKYSFQTHDRLCFVMEYANGGELF 236
AAD24196.1      AMKILKKEVIAKDEVAHTLTESRVLKNTRHPFLTSLKYSFQTKDRLCFVMEYVNGGELF 234
3D0E (AKT2)      AMKILRKEVIAKDEVAHTVTESRVLQNTRHPFLTALKYAFQTHDRLCFVMEYANGGELF 93
AKT2_Comb       AMKILRKEVIAKDEVAHTVTESRVLQNTRHPFLTALKYAFQTHDRLCFVMEYANGGELF 238
                  *****:***:***:*****:***:***:*****:***:***:*****:*****
                  D188, H192, R198 (AKT3)
                  K181                K191 E193 (AKT2)

6S9W (AKT1)      FHLSRERVFSEDRARFYGAEIVSALDYLHSEKNVVYRDLKLENLMLDKDGHIKITDFGLC 296
AAD24196.1      FHLSRERVFSEDRTRFYGAEIVSALDYLHSG-KIVYRDLKLENLMLDKDGHIKITDFGLC 293
3D0E (AKT2)      FHLSRERVFTEERARFYGAEIVSALEYLHSR-DVVYRDIKLENLMLDKDGHIKITDFGLC 152
AKT2_Comb       FHLSRERVFTEERARFYGAEIVSALEYLHSR-DVVYRDIKLENLMLDKDGHIKITDFGLC 297
                  *****:***:***:*****:***:***:*****:*****:*****
                  (AKT1)                Y272, R273, D274                D292 C296
                  (AKT2)                K277, E279, N280                D293
                  (AKT3)                R270, D271

6S9W (AKT1)      KEGIKDGATMKTFCGTPEYLAPEVLEDNDYGRAVDWWGLGVVMYEMMCGRLPFYNQDHEK 356
AAD24196.1      KEGITDAATMKTFCGTPEYLAPEVLEDNDYGRAVDWWGLGVVMYEMMCGRLPFYNQDHEK 353
3D0E (AKT2)      KEGISDGATMKTFCGTPEYLAPEVLEDNDYGRAVDWWGLGVVMYEMMCGRLPFYNQDHER 212
AKT2_Comb       KEGISDGATMKTFCGTPEYLAPEVLEDNDYGRAVDWWGLGVVMYEMMCGRLPFYNQDHER 357
                  *****:***:*****:*****:*****:*****:*****:*****
                  C310 (AKT1), C311 (AKT2)
                  K294, E295, F306, C307 (AKT3)

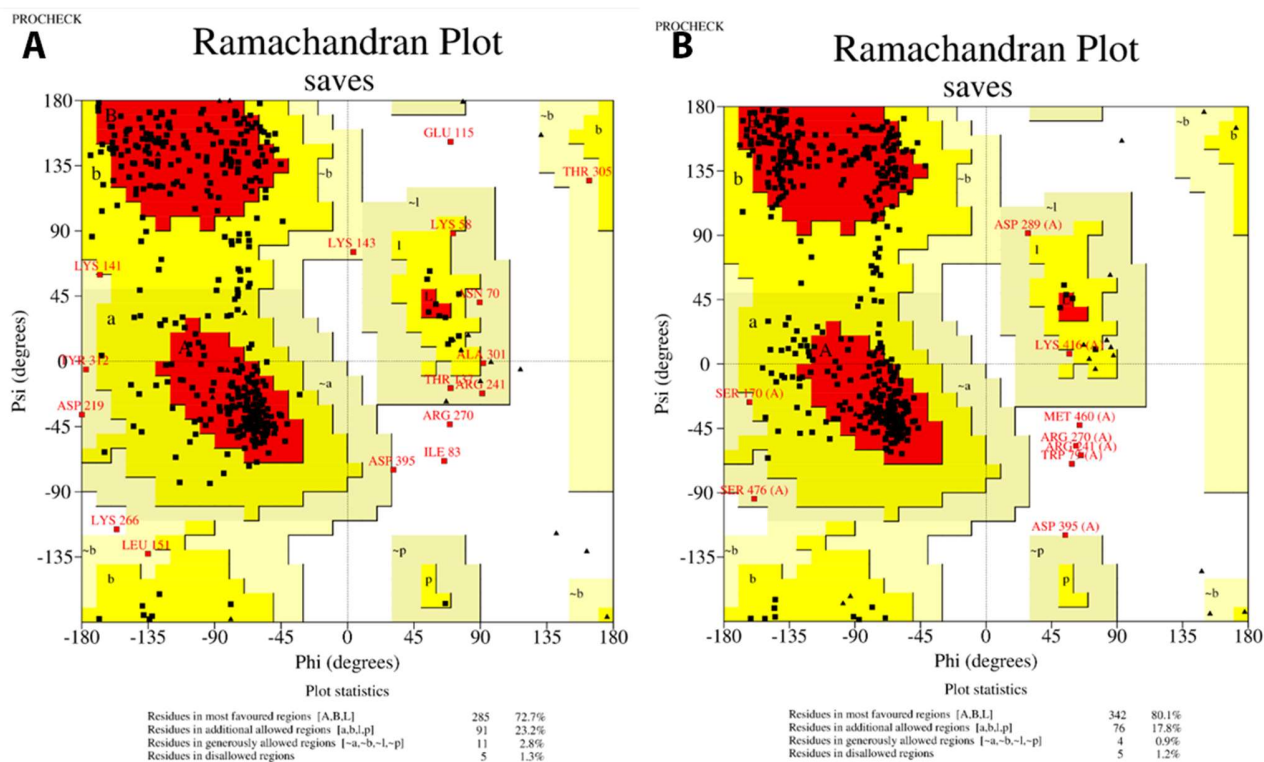
6S9W (AKT1)      LFELILMEEIRFPRTLGPEAKSLLSGLLKDPKQRLGGGSDAKEIMQHRRFFAGIVWQHV 416
AAD24196.1      LFELILMEDIKFPRTLSSDAKSLLSGLLIKDPNKRLGGGPDAKEIMRHSFFSGVNWQDV 413
3D0E (AKT2)      LFELILMEEIRFPRTLSPEAKSLLAGLLKDPKQRLGGGPDAKEVMEHRFFLSINWQDV 272
AKT2_Comb       LFELILMEEIRFPRTLSPEAKSLLAGLLKDPKQRLGGGPDAKEVMEHRFFLSINWQDV 417
                  *****:***:*****:*****:***:***:*****:***:***:***:

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Reported sequence identity between sequences:

1: 6S9W (AKT1)	100.00	82.31	88.74	83.30
2: AAD24196.1	82.31	100.00	83.28	78.79
3: 3D0E (AKT2)	88.74	83.28	100.00	100.00
4: AKT2_Comb	83.30	78.79	100.00	100.00

**Figure S1.** Multiple sequence alignments of three different AKT subtypes. The alignment was generated with the Clustal Omega program. AKT1: 6S9W; AKT2: 3D0E and AKT2\_Comb; AKT3: AAD24196.1. Binding residues unique to different subtypes are highlighted.

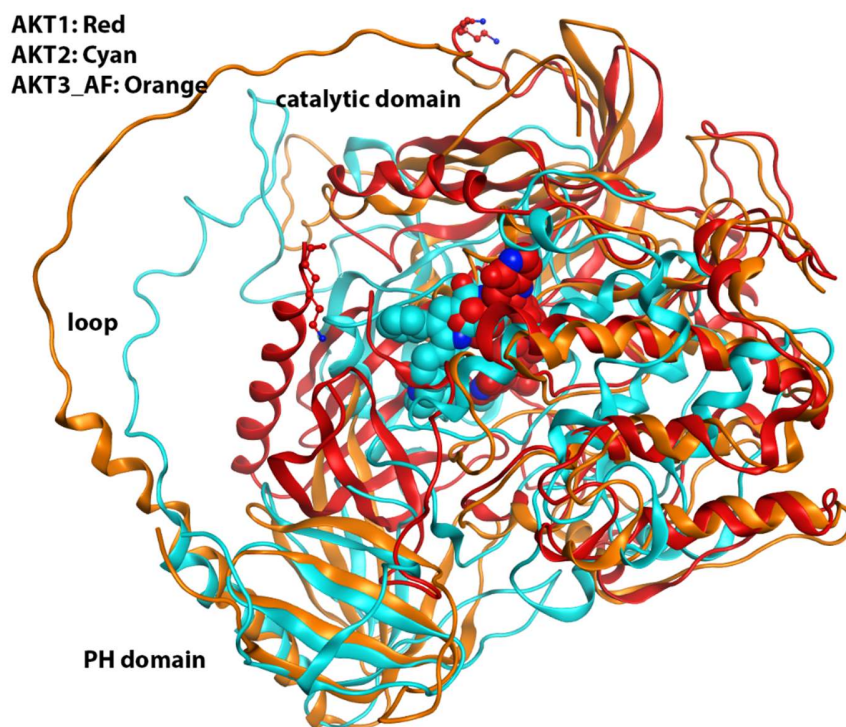


**Figure S2.** The Ramachandran plot for the AKT3\_HM model (A) and the AKT3\_AF model (B).

**Table S1.** Docking CovDock scores (Kcal/mol), the MT-based free energy ( $\Delta G$ , Kcal/mol) and the interacting residues for the AKT1 binding (6S9W and 6S9W\_L models) based on the IC<sub>50</sub> (nM) values of tested compounds.

Compd.	IC <sub>50</sub> (nM)	$\Delta G_{exp}$	6S9W	$\Delta\Delta G$ (6S9W)	6S9W_L	$\Delta\Delta G$ (6S9W_L)	MT1	$\Delta\Delta G$ (MT, 6S9W)
6	1	-12.28	-12.94	0.66	-14.695	2.42	-14.62	2.34
7	38	-10.12	-11.88	1.76	-14.31	4.19	-12.75	2.63
8	490	-8.61	-13.85	5.25	-14.08	5.47	-14.24	5.63
9	381	-8.76	-14.12	5.36	-13.84	5.09	-15.66	6.90
10	39	-10.11	-11.71	1.61	-14.58	4.48	-13.10	3.00
11	1140	-8.11	-12.75	4.64	-14.58	6.48	-14.88	6.77
12	813	-8.31	-11.64	3.33	NA		-16.61	8.30
13	53	-9.92	-12.03	2.10	-15.56	5.64	-11.71	1.79
14	1716	-7.86	-12.76	4.89	-12.11	4.24	-15.00	7.14
15	643	-8.45	-12.49	4.04	-13.88	5.43	-12.34	3.89
16	1281	-8.04	-12.86	4.82	-12.19	4.15	-14.62	6.58
17	98	-9.56	-12.28	2.72	-9.99	0.43	-15.22	5.65
18	527	-8.56	-13.18	4.62	-14.18	5.61	-16.32	7.76

19	604	-8.48	-13.17	4.68	-14.54	6.05	-15.41	6.93
20	9484	-6.85	-12.90	6.05	-12.67	5.82	-15.40	8.55
21	2853	-7.56	-12.00	4.44	-13.27	5.70	-17.15	9.59
22	91	-9.60	-12.65	3.04	-7.65	1.95	-16.64	7.04
23	1797	-7.84	-12.94	5.10	-12.49	4.65	-15.32	7.48
24	22	-10.45	-10.82	0.37	-15.47	5.02	-12.63	2.18
25	1631	-7.89	-11.02	3.13	-9.47	1.57	-13.81	5.91
26	2111	-7.74	-13.07	5.32	-11.81	4.07	-15.81	8.07
27	209	-9.11	-12.11	3.00	-9.13	0.02	-16.11	7.00
28	130	-9.39	-11.22	1.82	-12.18	2.79	-15.62	6.23
29	186	-9.18	-11.71	2.53	-15.91	6.73	-13.39	4.20
30	44	-10.04	-11.90	1.87	-15.30	5.27	-14.52	4.48
31	35	-10.17	-11.44	1.27	-14.56	4.38	-13.24	3.07
32	112	-9.48	-11.66	2.18	-14.38	4.90	-14.34	4.86
33	7087	-7.02	-12.55	5.53	-13.78	6.75	-13.67	6.65
34	4566	-7.28	-12.15	4.87	-13.94	6.66	-16.56	9.27
35	5930	-7.13	-12.92	5.79	-13.30	6.17	-13.98	6.85
mean				3.56		4.56		5.89
stdev				1.66		1.81		2.18



**Figure S3.** Superposition of AKT1 (red), AKT2 (cyan) and AKT3 (orange) and the catalytic domain and the PH domain of the AKT proteins.

**Table S2.** Interacting residues from the CovDock generated poses for the AKT1 (6S9W) models.

Compd.	Interacting residues (6S9W)	Compd.	Interacting residues (6S9W)
6	N54, W80, Y272, D292	21	E17, Q79, W80, N204, E267, Y272, D274
7	N54, W80, Y263, Y272, D274, D292	22	N54, A58, Q59, W80, Y272, D292
8	N54, W80, Y272	23	E17, Q79, W80, Y272, R273, D274
9	N53, N54, W80, Y263, Y272, D292, L295	24	N54, W80, Y272, D292
10	N54, W80, Y272, D292	25	E17, Q79, Y272, R273, D274, E267
11	E17, Q79, W80, Y272, R273, D274, C310	26	N54, W80, Y272, D274, D292
12	E17, Q79, Y272, R273, D274, K297, C310	27	N54, W80, Y272, D292, L295, K297
13	N54, W80, Y272, D292	28	N54, W80, Y272, R273, C310
14	E17, Q79, Y272, R273, D274, C310	29	N54, W80, Y272, D292
15	E17, N54, Q79, Y272, R273, D274	30	N54, W80, Y272, D292
16	E17, Q79, Y263, Y272, R273, D274, C310	31	N54, W80, Y272, D274
17	N54, W80, Y272, D274, D292	32	N54, W80, Y272
18	N54, W80, E267, Y272	33	E17, Q79, Y272, R273, D274
19	N54, W80, Y272, D274, D292	34	N54, W80, Y272, C310
20	E17, A58, W80, R200, E267, Y272, R273, D274, C310	35	N54, W80, T82, Y272, D292

**Table S3.** Interacting residues from the CovDock generated poses for the AKT2 (3D0E\_N and 3D0E) models.

Compd.	Interacting residues (3D0E_N)	Interacting residues (3D0E)
6	F163, K191, E279, D293	F163, K181, E230, A232, E279, D293
7	F163, K277, E279, D293	F163, K181, T197, E279, D293
10	E279	F163, K181, E279, D293
11	F163, E279, K277, D293	F163, E279, D293, K181
12	E279	F163, D293
13	E279	F163, K181, E279, D293
14	F163, K277, D293	F163, E279
15	F163, K191, E193, E279	F163, K181, T197, E279, D293
16	E193, A232, E279	F163, K181, T197, A232, E279, D293
17	R15, K277, N280	F238, E279, F443
18	E193, E279, A232	F163, K181, E279, D293
19	F163, T162, E279, F238	F163, E279, D293
20	F163, E230, E279, D293	F163, E279
21	T162, F238, E279	F238, E279, F443
22	T213, E279	F238, E279, F443
23	F163, E230, E279	E193, K277, L278
24	F163, N280, E279, D293	F163, K181, E279, D293
25	F163, T213, F238, E279, K277	F238, E279, D293
26	F163, T162, E279	F238, E279, T313, F443
27	E279	F238, E279, F443
28	R86, F163, D293	F238, E279, F443
29	E279, E193, T213	F163, K181, A232, E279, D293
30	E279, E193	F163, E279, D293, G312
31	F163, T162, D293	F163, K181, A232, D293
32	F163, T162, E279, D293	F163, K181, T197, E279, D293, F443
33	F163, E193, E230, E279	F163, K181, E279, D293
34	F163, T162, F238	F238, E279, F443
35	F163, K191, E236, E279, D293	F163, E279

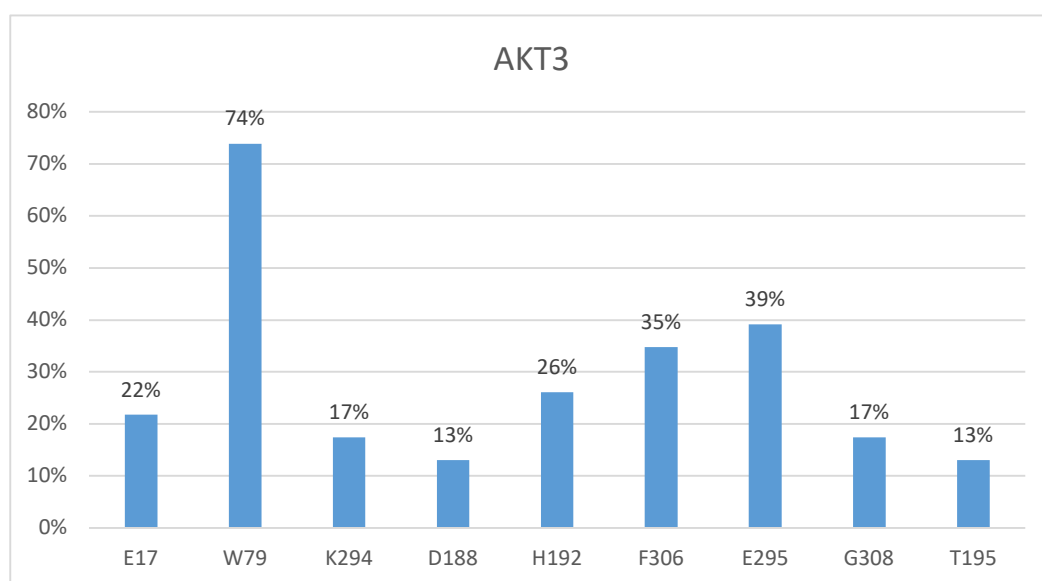
Note: the reduced number of protein-ligand interactions in the AKT2 model was since ligands with >20,000 nM were removed from further analysis.

**Table S4.** Interacting residues from the CovDock generated poses for the AKT3 AF model.

Compd.	Interacting residues (AF)	Compd	Interacting residues (AF)
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<b>6</b>	W79, K294, D188, H192, F306	<b>22</b>	W79, F306
<b>11</b>	E17, W79	<b>23</b>	G308
<b>12</b>	E17, W79, E295	<b>24</b>	W79, E17
<b>13</b>	W79, H192, R198, K294, F306	<b>25</b>	E17, T195, E295
<b>14</b>	W79, T195, R270, F306	<b>26</b>	W79, E295
<b>15</b>	E17	<b>27</b>	W79, T195, R270, F306, G308
<b>16</b>	W79, E295, G308	<b>28</b>	NA
<b>17</b>	W79, F306	<b>30</b>	W79, H192 , R198
<b>18</b>	E295	<b>31</b>	W79, H192, Q470
<b>19</b>	E17, W79, E295	<b>32</b>	W79, H192, E295, K294, D188
<b>20</b>	E295, G308	<b>35</b>	W79, K294, E295, H192, R198, D188, F306
<b>21</b>	W79, F306		

Note: the reduced number of protein-ligand interactions in the AKT3 model was since ligands with >20,000 nM were removed from further analysis.



**Figure S4.** Interacting residues of AKT3 inhibitors (the AF model).