Supporting Information

ja-2009-039373

Importance of Ligand Reorganization Free Energy in Protein-Ligand Binding-Affinity Prediction

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Table S1. List of crystal structures used in Figure 2.

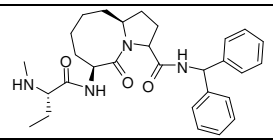
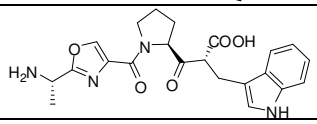
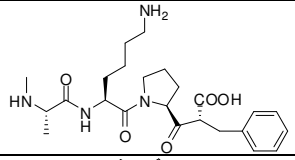
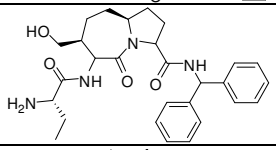
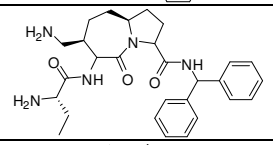
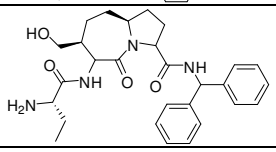
PDB ID	Reference	Ligand in the binding site	Resolution (Å)
1G73	[1]	Ala-Val-Pro-Ile	2.00
1NW9	[2]	Ala-Thr-Pro-Phe	2.40
2JK7	[3]		2.82
2OPY	[4]		2.80
2OPZ	[4]	Ala-Val-Pro-Phe	3.00
2VSL	[5]		2.10
3CLX	[6]		2.70
3CM2	[6]		2.50
3CM7	[6]		3.10

Table S2. Binding affinity estimates based on different protein-ligand conformation selection criteria and scoring functions. The unit for RT ln(K_i) is kcal/mol. Scores from X-Score is kcal/mol. Scores from Drugscore and M-Score are dimensionless in which more negative values correspond to higher potencies. SD stands for standard deviation.

Pose selection		**Dock**			**Lowest Score**			**Ensemble (1ns)**					
Compound	RT ln (Ki)	X-Score	Drugscore	M-Score	X-Score	Drugscore	M-Score	X-Score	SD	Drugscore	SD	M-Score	SD
1	-8.92	-7.27	-416688.00	-151.88	-8.45	-497309.00	-207.40	-7.90	0.27	-455056.51	23772.16	-177.34	16.08
2	-7.30	-7.48	-426793.00	-182.80	-8.43	-467966.00	-215.88	-7.81	0.36	-426141.29	20164.95	-184.80	16.42
3	-6.65	-7.37	-428925.00	-145.84	-8.23	-441610.00	-196.39	-7.69	0.29	-409691.95	19760.11	-155.89	16.89
4	-7.66	-7.23	-440399.00	-168.85	-8.31	-466304.00	-191.47	-7.63	0.33	-424092.44	21224.96	-161.30	12.77
5	-7.31	-7.45	-393940.00	-138.50	-8.15	-442246.00	-186.98	-7.47	0.28	-405678.93	21477.72	-148.31	16.68
6	-8.04	-7.37	-452325.00	-145.67	-8.63	-491495.00	-194.73	-8.14	0.29	-450089.66	23226.78	-157.81	18.20
7	-9.08	-7.33	-459131.00	-143.83	-8.38	-479915.00	-173.67	-7.70	0.30	-434931.29	22488.78	-142.22	16.63
8	-9.20	-7.43	-442239.00	-158.77	-8.52	-474017.00	-181.25	-7.86	0.30	-434758.66	21671.54	-150.76	13.34
9	-7.24	-6.52	-419361.00	-185.30	-7.63	-388564.00	-213.12	-6.99	0.28	-357985.59	23893.30	-182.68	14.07
10	-9.31	-7.88	-477814.00	-156.64	-8.24	-498394.00	-199.37	-7.82	0.26	-450065.76	21364.42	-177.84	13.37
11	-10.40	-8.01	-459274.00	-144.72	-8.61	-542015.00	-222.30	-7.85	0.33	-460683.83	31151.62	-187.94	18.48
12	-10.30	-7.24	-486725.00	-180.44	-8.42	-514874.00	-220.60	-7.97	0.26	-479616.29	20458.77	-182.53	16.61
13	-8.08	-6.89	-471099.00	-177.63	-8.40	-505112.00	-207.54	-6.74	0.57	-373538.44	47114.53	-178.12	14.54
14	-5.69	-7.18	-444296.00	-163.31	-8.19	-483143.00	-203.77	-7.65	0.28	-427269.12	27380.52	-177.00	12.70
15	-9.68	-7.39	-473666.00	-169.74	-8.70	-513764.00	-217.77	-8.16	0.25	-467610.44	23495.53	-186.35	17.79
16	-7.34	-7.46	-439710.00	-135.57	-8.67	-533195.00	-221.49	-8.25	0.24	-485782.98	22524.76	-179.39	22.18
17	-5.82	-7.31	-468412.00	-140.24	-8.91	-521847.00	-193.38	-8.35	0.29	-481086.10	19303.20	-163.86	14.39
18	-8.81	-6.25	-354290.00	-177.12	-8.66	-532402.00	-241.75	-8.00	0.25	-475203.95	24669.26	-207.10	16.55
19	-9.31	-8.31	-405320.00	-147.81	-8.75	-499577.00	-204.70	-8.11	0.26	-456651.73	18873.14	-176.86	13.63
20	-10.35	-8.25	-491799.00	-209.36	-8.77	-570451.00	-238.78	-8.17	0.31	-494060.22	29602.31	-209.42	16.76
21	-10.50	-7.37	-492148.00	-176.17	-9.36	-591848.00	-242.99	-8.28	0.47	-496076.41	43229.06	-200.71	20.79
22	-11.47	-7.87	-503021.00	-207.21	-9.20	-540905.00	-273.17	-8.26	0.32	-493203.85	27584.53	-213.81	24.71
23	-9.79	-7.71	-481055.00	-168.03	-8.68	-575214.00	-245.04	-8.21	0.26	-504054.80	26403.78	-210.45	20.26
24	-9.11	-7.95	-540218.00	-205.93	-8.81	-514034.00	-232.71	-8.13	0.30	-459025.90	24405.49	-197.86	14.79
25	-10.72	-7.74	-474838.00	-201.93	-8.72	-501082.00	-224.43	-8.19	0.25	-457798.22	23781.09	-193.82	17.28
26	-11.28	-7.54	-514372.00	-240.79	-8.90	-531202.00	-258.72	-8.28	0.24	-492984.90	23880.09	-224.51	17.35
27	-11.02	-7.50	-496308.00	-227.32	-8.99	-570804.00	-261.20	-8.26	0.33	-508496.98	32550.38	-215.98	20.50
28	-10.26	-7.41	-503721.00	-201.51	-8.76	-543175.00	-263.72	-8.31	0.22	-498580.68	19282.05	-225.62	14.24
29	-8.41	-7.54	-519222.00	-203.63	-8.54	-526968.00	-245.23	-8.08	0.24	-480823.46	22154.50	-204.20	21.44
30	-9.20	-7.58	-482779.00	-220.36	-9.09	-548060.00	-269.99	-8.36	0.38	-483624.95	34965.43	-230.00	21.25
31	-8.51	-7.52	-431416.00	-148.71	-8.49	-480940.00	-191.07	-7.77	0.29	-440792.05	14864.24	-160.06	15.20

Table S3. Binding affinity calculations based on the MM-GBSA method. Numbers in parentheses are standard deviations. The standard deviation of ΔG is calculated as the squared root of the sum of squares of standard deviations of ΔH and TΔS.

Compound	RT ln (Ki)	ΔG (elec)	ΔG (vdw)	ΔH	TΔS	ΔG	ΔG + $\delta\Delta G_{re}$
1	-8.92	-190.29(11.87)	-35.13(2.34)	-41.83(3.45)	-20.47(6.52)	-21.36 (7.38)	-19.07
2	-7.30	-198.25(13.37)	-33.53(3.86)	-40.27(4.33)	-22.15(3.02)	-18.12(5.28)	-15.30
3	-6.65	-189.07(15.22)	-32.99(2.65)	-39.05(3.26)	-21.19(3.10)	-17.86(4.50)	-13.44
4	-7.66	-183.74(10.89)	-35.08(3.42)	-40.35(3.30)	-23.62(3.87)	-16.73(5.09)	-15.10
5	-7.31	-192.86(13.55)	-32.27(3.21)	-38.14(3.39)	-21.83(3.34)	-16.31(4.76)	-14.87
6	-8.04	-168.79(12.59)	-37.08(2.93)	-42.12(2.92)	-20.69(3.96)	-21.43(4.92)	-19.82
7	-9.08	-203.59(12.86)	-34.90(3.32)	-41.74(2.43)	-20.60(4.32)	-21.14(4.96)	-19.57
8	-9.20	-193.53(10.20)	-35.89(3.09)	-42.57(3.18)	-23.03(5.44)	-19.54(6.30)	-17.68
9	-7.24	-208.43(17.96)	-29.61(3.51)	-34.59(3.16)	-21.56(5.16)	-13.03(6.05)	-14.88
10	-9.31	-175.92(10.68)	-33.70(3.19)	-39.80(3.09)	-23.49(2.87)	-18.58(4.22)	-17.44
11	-10.40	-169.61(15.74)	-36.89(3.76)	-41.14(3.62)	-23.01(2.57)	-21.07(4.44)	-20.91
12	-10.30	-186.03(13.84)	-40.95(3.24)	-46.60(2.76)	-23.19(3.58)	-23.41(4.52)	-22.29
13	-8.08	-197.50(10.29)	-29.43(4.61)	-36.47(5.03)	-20.07(4.14)	-16.4(6.52)	-16.10
14	-5.69	-186.58(13.63)	-32.93(3.08)	-38.29(3.75)	-23.14(6.10)	-15.15(5.59)	-13.22
15	-9.68	-201.62(11.93)	-38.25(2.56)	-46.51(3.43)	-25.61(2.79)	-20.9(4.42)	-20.86
16	-7.34	-173.76(12.98)	-40.43(3.24)	-45.84(4.33)	-25.00(3.18)	-20.84(5.37)	-19.17
17	-5.82	-195.79(13.67)	-41.24(3.37)	-45.71(4.37)	-25.88(5.02)	-19.83(6.66)	-16.63
18	-8.81	-177.37(9.96)	-39.98(2.86)	-44.34(3.01)	-23.26(2.60)	-21.08(3.98)	-16.49
19	-9.31	-204.44(13.08)	-35.42(3.11)	-43.77(3.08)	-21.30(4.66)	-22.47(5.59)	-19.31
20	-10.35	-195.38(10.27)	-38.68(3.57)	-45.78(2.89)	-19.46(3.09)	-22.71(4.23)	-22.11
21	-10.50	-284.60(25.43)	-40.69(3.35)	-44.58(3.99)	-26.26(7.10)	-18.32(8.15)	-18.38
22	-11.47	-184.86(17.09)	-39.92(3.47)	-46.39(3.00)	-24.41(3.08)	-22.62(4.30)	-19.13
23	-9.79	-187.15(12.74)	-42.42(2.34)	-47.27(3.34)	-21.99(5.54)	-25.28(6.47)	-20.04
24	-9.11	-200.61(12.91)	-37.47(2.97)	-42.39(3.02)	-20.85(3.01)	-21.54(4.26)	-18.07
25	-10.72	-188.07(14.59)	-37.12(2.68)	-43.89(2.87)	-22.53(3.73)	-21.36(4.71)	-19.59
26	-11.28	-181.18(14.33)	-41.33(3.40)	-46.20(3.67)	-24.09(3.58)	-22.11(5.13)	-23.03
27	-11.02	-173.25(15.64)	-42.32(3.22)	-50.52(3.51)	-24.81(5.07)	-25.71(6.17)	-23.78
28	-10.26	-193.51(9.83)	-40.91(2.56)	-49.18(3.48)	-22.08(4.42)	-27.1(5.63)	-19.81
29	-8.41	-179.37(11.99)	38.92(2.90)	-44.87(3.30)	-19.77(3.00)	-25.1(4.46)	-17.25
30	-9.20	-191.77(12.33)	-39.81(3.83)	-44.76(4.26)	-22.76(4.33)	-22.49(6.08)	-19.15
31	-8.51	-201.11(9.55)	-37.06(2.35)	-44.76(2.67)	-23.10(4.14)	-21.66(4.93)	-18.73
32	-8.34	-184.12(12.31)	-40.88(3.26)	-45.24(3.85)	-24.85(6.38)	-20.84(7.45)	-19.14
33	-7.68	-182.9(10.95)	-40.89(3.02)	-45.71(2.88)	-23.40(2.85)	-22.31 (4.05)	-17.40
34	-8.96	-182.96(12.09)	-42.22(2.44)	-44.23(3.20)	-27.26(2.56)	-16.97(4.10)	-18.29
35	-7.54	-181.39(9.88)	-40.45(3.26)	-45.85(3.11)	-22.80(3.50)	-23.05(4.68)	-14.08
36	-8.52	-195.62(11.50)	-40.73(3.72)	-45.10(3.42)	-24.86(2.83)	-20.24(4.44)	-15.21
37	-9.35	-176.64(9.81)	-44.67(2.88)	-49.56(3.61)	-25.06(3.85)	-24.5(5.28)	-17.22
38	-9.49	-177.22(9.85)	-45.48(3.34)	-50.97(3.22)	-22.81(5.39)	-28.16(6.28)	-21.13
39	-8.08	-198.00(11.60)	-42.93(2.92)	-49.45(3.28)	-24.01(3.32)	-25.44(4.67)	-17.80
40	-9.11	-180.67(11.33)	-42.85(3.35)	-50.24(2.96)	-23.96(4.26)	-26.28(5.19)	-20.32
41	-9.17	-241.85(12.82)	-38.01(3.71)	-44.79(3.64)	-19.96(5.47)	-24.83(6.57)	-20.92

Table S4. Ligand reorganization free energy upon binding ($\delta\Delta G_{re}$) calculated using trajectories from the 1 ns protein-ligand complex simulations. The protein-free ligand simulations were performed for 10 ns. The number of rotable bonds is calculated by a utility program in X-Score.

Compound	RT ln(Ki)	$\delta\Delta G_{re}$	$\delta\Delta H_{re}$	$\delta\Delta T\Delta S_{re}$	# of rotable bonds
1	-8.92	2.29	3.62	1.33	7
2	-7.30	2.82	4.21	1.39	5
3	-6.65	4.42	5.12	0.70	7
4	-7.66	1.63	2.97	1.34	9
5	-7.31	1.44	2.15	0.71	7
6	-8.04	1.61	2.52	0.91	7
7	-9.08	1.57	2.44	0.87	7
8	-9.20	1.86	3.36	1.50	7
9	-7.24	-1.85	-1.99	-0.14	5
10	-9.31	1.14	2.51	1.37	8
11	-10.40	0.16	1.69	1.53	8
12	-10.30	1.12	1.40	0.28	9
13	-8.08	0.30	-0.03	-0.33	8
14	-5.69	1.93	3.13	1.20	8
15	-9.68	0.03	1.35	1.31	8
16	-7.34	1.67	2.76	1.09	8
17	-5.82	3.20	4.06	0.86	9
18	-8.81	4.59	5.24	0.65	7
19	-9.31	3.16	3.55	0.39	5
20	-10.35	0.60	1.07	0.47	7
21	-10.50	-0.08	-0.11	-0.05	7
22	-11.47	3.08	4.26	0.77	7
23	-9.79	5.24	5.83	0.59	8
24	-9.11	3.47	3.47	0.00	5
25	-10.72	1.77	3.37	1.60	5
26	-11.28	-0.92	0.15	1.07	7
27	-11.02	1.93	2.81	0.88	9
28	-10.26	7.29	8.30	1.01	7
29	-8.41	7.85	8.44	0.59	7
30	-9.20	3.34	4.73	1.39	5
31	-8.51	2.93	4.34	1.41	9

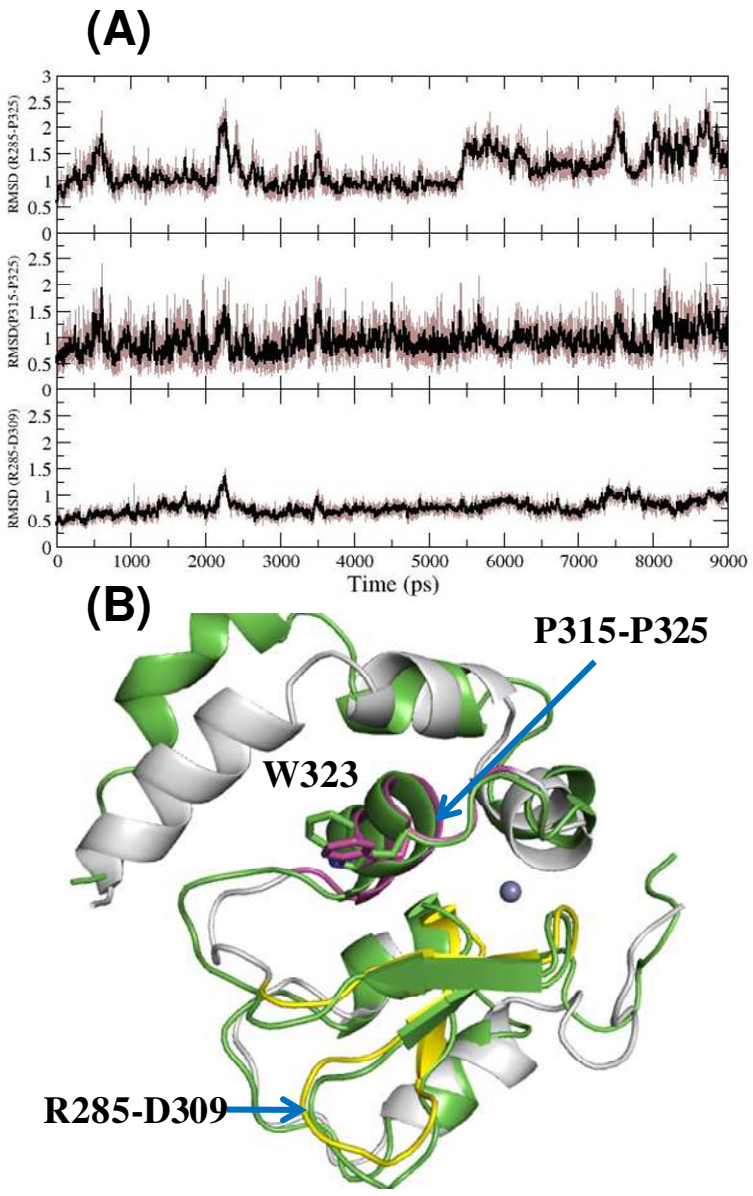
Table S5. Average radius of gyration (Rg) of the ligands in protein-bound and protein-free (10ns) conformations. SD is standard deviation.

Compound #	Rg(bound)	STD	Rg(free)	STD	ΔRg(bound-free)
1	4.60	0.07	4.08	0.24	0.52
2	4.56	0.10	3.94	0.19	0.62
3	4.20	0.05	3.92	0.12	0.28
4	4.50	0.06	4.04	0.15	0.46
5	4.16	0.05	3.89	0.12	0.27
6	4.68	0.08	4.22	0.19	0.46
7	4.34	0.11	3.99	0.16	0.35
8	4.56	0.08	4.04	0.16	0.52
9	4.04	0.06	4.08	0.11	-0.05
10	4.73	0.14	4.18	0.28	0.56
11	5.04	0.61	4.38	0.11	0.66
12	4.89	0.06	4.48	0.12	0.41
13	4.50	0.27	4.45	0.29	0.05
14	4.66	0.13	4.22	0.36	0.45
15	4.64	0.07	4.16	0.17	0.49
16	4.60	0.08	4.24	0.18	0.36
17	4.48	0.21	4.23	0.23	0.25
18	4.86	0.06	4.25	0.13	0.60
19	4.56	0.08	4.03	0.18	0.54
20	4.95	0.06	4.48	0.17	0.47
21	4.89	0.06	4.90	0.12	0.00
22	5.07	0.07	4.81	0.14	0.26
23	4.98	0.05	4.57	0.17	0.41
24	4.47	0.06	4.22	0.19	0.25
25	4.84	0.05	4.28	0.16	0.56
26	5.05	0.05	4.68	0.23	0.37
27	4.95	0.09	4.70	0.19	0.25
28	5.01	0.07	4.41	0.11	0.60
29	4.89	0.05	4.36	0.08	0.53
30	4.93	0.06	4.25	0.11	0.68
31	4.51	0.06	4.02	0.09	0.49
Average	4.68	0.10	4.22	0.19	0.41

Table S6. Intercepts and slopes of the linear regression in the leave-one-out analyses. The parameters were calculated using R^7 based on 30 data points.

	ΔG		$\Delta G+\delta\Delta G_{re}$	
Compound #	**intercept**	**slope**	**intercept**	**slope**
1	-9.4508	1.2462	-5.8410	1.4073
2	-9.5924	1.2349	-6.0756	1.3867
3	-9.4430	1.2495	-6.4957	1.3436
4	-9.9407	1.2029	-6.1753	1.3781
5	-10.0516	1.1906	-6.1865	1.3760
6	-9.1871	1.2713	-5.4671	1.4416
7	-9.4725	1.2453	-5.8508	1.4051
8	-9.4745	1.2516	-5.8613	1.4117
9	-10.9140	1.1065	-6.1712	1.3773
10	-9.4367	1.2600	-5.8388	1.4157
11	-8.8347	1.3333	-5.9303	1.3981
12	-9.6402	1.2236	-6.1502	1.3676
13	-9.9321	1.2070	-6.0274	1.3931
14	-10.2337	1.1676	-6.2105	1.3712
15	-9.4359	1.2531	-5.9523	1.3921
16	-8.9275	1.2991	-5.1250	1.4784
17	-7.9061	1.4086	-4.5578	1.5422
18	-9.4468	1.2472	-5.9495	1.4042
19	-9.4997	1.2384	-5.8690	1.4053
20	-9.5294	1.2390	-6.1248	1.3714
21	-8.7283	1.3464	-5.4646	1.4606
22	-9.1019	1.2929	-4.9253	1.5241
23	-9.7625	1.2005	-5.8951	1.4021
24	-9.4703	1.2442	-5.8694	1.4088
25	-9.1697	1.2863	-5.5803	1.4442
26	-9.0575	1.2989	-6.2449	1.3592
27	-10.1116	1.1651	-6.4747	1.3291
28	-10.1683	1.1501	-5.7933	1.4169
29	-8.9590	1.2849	-5.9086	1.4038
30	-9.4802	1.2400	-5.8641	1.4058
31	-9.3388	1.2557	-5.7853	1.4126

Figure S1. (A) Backbone RMSD of XIAP BIR3 with a reference to XIAP BIR3 in the crystal structure between Smac and XIAP BIR3 from a 9 ns simulation. The alignments are based on residues from R285 to D309 where the Smac peptide binds, P315 to P325 and R285 to D309 respectively. The black lines are a 20-point running average. (B) Alignment of XIAP BIR3 between Smac-bound crystal structure (grey color) and the final conformation (green color) from the 9 ns simulation.



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