Supporting Information

Computational Analysis of Binding Free Energies, Hotspots and Binding Mechanism in Bcl-2/Bcl-xL Binding to Bad/Bax

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Tables

Table S1. Components of the binding free energy and corresponding standard error of the mean calculated by various methods. (in unit of kcal/mol)

System	PBSA				$-T\Delta S$		AS-IE					ΔG_{exp}
	ΔE_{ele}	ΔE_{vdw}	ΔG_{pb}	ΔG_{np}	Nmode	IE	ΔE_{ele}	ΔE_{vdw}	ΔG_{pb}	ΔG_{np}	IE	
Bcl-xL/Bad	-363.4±3.2	-132.6±0.5	399.1±2.9	-16.0±0.0	55.4±2.5	82.9±0.0	26.7±0.4	53.5±0.5	-29.3±0.4	2.6±0.0	-35.3±0.0	-12.6
Bcl-xL/Bax	-228.5±2.6	-111.3±0.5	256.4±2.3	-13.0±0.0	49.3±1.1	71.9±0.0	38.1±0.4	47.4±0.4	-37.2±0.5	1.7±0.0	-40.4±0.0	-9.2
Bcl-2/Bad	-295.2±1.9	-104.6±0.4	333.7±1.6	-14.1±0.0	47.9±1.1	50.1±0.0	25.1±0.3	48.4±0.4	-31.7±0.3	3.3±0.0	-30.4±0.0	-10.2~-12.6
Bcl-2/Bax	-252.7±3.3	-132.4±0.6	276.2±2.9	-14.6±0.0	56.8±0.7	96.6±0.0	34.6±0.4	59.2±0.5	-31.1±0.3	1.8±0.0	-52.5±0.0	-10.2

Table S2. Residue-specific binding free energies within 5 Å around the binding interface between Bcl-xL and Bad/Bax from AS-IE calculations. (in unit of kcal/mol)

Residues	ΔE_{ele}		ΔE_{vdW}		ΔG_{pb}		ΔG_{np}		$-T\Delta S$		$\Delta\Delta G_{bind}^{x\to a}$	
	Bcl-xL / Bad	*Bcl-xL / Bax*	*Bcl-xL / Bad*	*Bcl-xL / Bax*	*Bcl-xL / Bad*	*Bcl-xL / Bax*	*Bcl-xL / Bad*	*Bcl-xL / Bax*	*Bcl-xL / Bad*	*Bcl-xL / Bax*	*Bcl-xL / Bad*	*Bcl-xL / Bax*
Phe101	-0.51	0.12	6.09	4.09	-2.37	-2.71	0.02	-0.06	-1.03	-2.22	2.20	-0.79
Arg104	10.90	5.36	2.77	3.29	-6.86	-4.79	0.09	0.52	-4.33	-3.21	2.56	1.18
Tyr105	5.59	-0.58	5.63	5.62	-5.35	-0.82	0.40	0.46	-4.38	-1.43	1.89	3.25
Phe109	0.48	0.35	1.86	1.07	-0.57	-1.42	-0.07	-0.01	-0.16	-0.05	1.55	-0.06
Leu112	0.15	0.61	2.31	1.71	-0.99	-1.26	0.05	0.08	-0.52	-0.69	1.01	0.44
Gln115	1.38	5.92	1.03	3.50	-1.55	-6.03	0.11	0.27	-1.24	-1.13	-0.28	2.52
Leu116	0.03	0.58	3.92	2.34	0.26	0.45	0.00	-0.12	-1.15	-1.79	3.07	1.45
Gln125	-0.86	0.17	0.65	0.28	0.70	-0.29	0.01	0.02	-0.56	-2.00	-0.06	-1.82
Gln129	1.89	7.77	2.80	0.83	-2.99	-6.77	0.27	0.15	-1.10	-6.13	0.87	-4.15
Val130	0.39	0.18	1.44	3.85	-0.40	1.06	0.00	-0.05	-0.67	-0.55	0.76	4.49
Leu134	0.90	1.17	4.05	3.43	-1.49	-2.06	-0.06	-0.09	-0.56	-1.34	2.83	1.11
Arg136	-10.86	-4.54	3.13	0.23	8.67	3.89	0.32	0.00	-0.84	-1.44	0.42	-1.86
Asp137	6.91	5.55	0.51	-0.75	-5.90	-3.21	0.04	0.04	0.39	-4.34	1.95	-2.71
Asn140	2.83	3.19	1.04	1.39	-3.05	-1.05	0.24	0.02	-2.48	-2.53	-1.42	1.02
Arg143	4.75	6.29	1.89	3.29	-2.19	-3.30	0.40	0.16	-5.88	-5.40	-1.03	1.06
Leu198	0.19	0.55	0.83	3.31	-1.43	-1.49	0.13	0.23	-0.16	-0.65	-0.44	1.94
Tyr199	-0.11	-0.62	5.61	4.00	-1.13	-0.62	0.31	-0.01	-1.10	-1.76	3.59	0.99

Table S3. Residue-specific binding free energies within 5 Å around the binding interface between Bcl-2 and Bad/Bax from AS-IE calculations. (in unit of kcal/mol)

	ΔE_{ele}		ΔE_{vdW}		ΔG_{pb}		ΔG_{np}		$-T\Delta S$		$\Delta\Delta G_{bind}^{x\to a}$	
Residues	***Bcl-2 / Bad***	***Bcl-2 / Bax***	***Bcl-2 / Bad***	***Bcl-2 / Bax***	***Bcl-2 / Bad***	***Bcl-2 / Bax***	***Bcl-2 / Bad***	***Bcl-2 / Bax***	***Bcl-2 / Bad***	***Bcl-2 / Bax***	***Bcl-2 / Bad***	***Bcl-2 / Bax***
Phe104	0.41	0.20	3.71	5.01	-2.66	-1.72	0.13	-0.11	-0.63	-0.88	0.96	2.50
Arg107	3.12	8.78	3.04	5.55	-2.33	-6.64	0.39	0.42	-2.25	-3.91	1.96	4.20
Tyr108	1.46	-0.42	2.80	5.38	-1.47	-0.11	-0.03	-0.04	-1.77	-1.66	1.00	3.14
Arg110	4.89	7.98	-0.29	-2.08	-3.43	-4.88	0.41	0.04	-3.28	-7.59	-1.70	-6.52
Phe112	0.65	0.12	4.55	5.78	-3.47	-3.04	0.60	0.44	-1.32	-2.18	1.01	1.12
Met115	0.04	0.64	1.28	1.75	-1.40	-0.98	-0.04	-0.08	-0.83	-0.53	-0.95	0.80
Gln118	1.25	-2.38	4.57	5.36	-2.39	0.36	0.50	0.17	-2.36	-1.14	1.57	2.37
Leu119	0.41	0.38	1.11	0.75	-1.99	-0.22	0.09	-0.06	-0.02	-0.60	-0.40	0.25
Thr132	0.62	-	2.12	-	-1.03	-	0.10	-	-0.33	-	1.48	-
Val133	0.11	-0.26	1.95	2.31	0.22	0.53	0.00	0.05	-1.89	-1.26	0.38	1.36
Glu136	15.67	11.99	2.03	-1.30	-12.19	-9.07	0.10	0.27	-4.84	-7.10	0.78	-5.22
Leu137	0.45	0.52	4.04	3.31	-0.97	-0.92	-0.02	-0.10	-0.80	-1.19	2.70	1.62
Arg139	-10.06	0.57	0.86	1.54	8.69	0.45	0.03	0.37	-0.30	-6.24	-0.79	-3.32
Asp140	9.79	2.44	-1.18	-0.71	-7.24	-0.59	0.07	0.02	-4.59	-4.79	-3.15	-3.63
Asn143	4.53	4.79	1.97	1.56	-2.56	-2.71	0.10	0.05	-0.75	-2.78	3.29	0.92
Arg146	-3.46	5.47	3.67	6.28	3.33	-3.50	0.13	0.10	-1.17	-3.58	2.51	4.77
Leu201	0.01	1.19	1.57	4.20	-0.96	-2.45	0.26	0.21	-0.54	-0.74	0.34	2.41
Tyr202	0.46	0.29	1.59	2.69	-0.72	-1.18	0.10	0.02	-0.34	-0.52	1.08	1.30
Pro204	0.09	0.54	0.14	2.72	-0.10	0.61	0.00	0.04	-0.01	-1.06	0.12	2.84

Figures

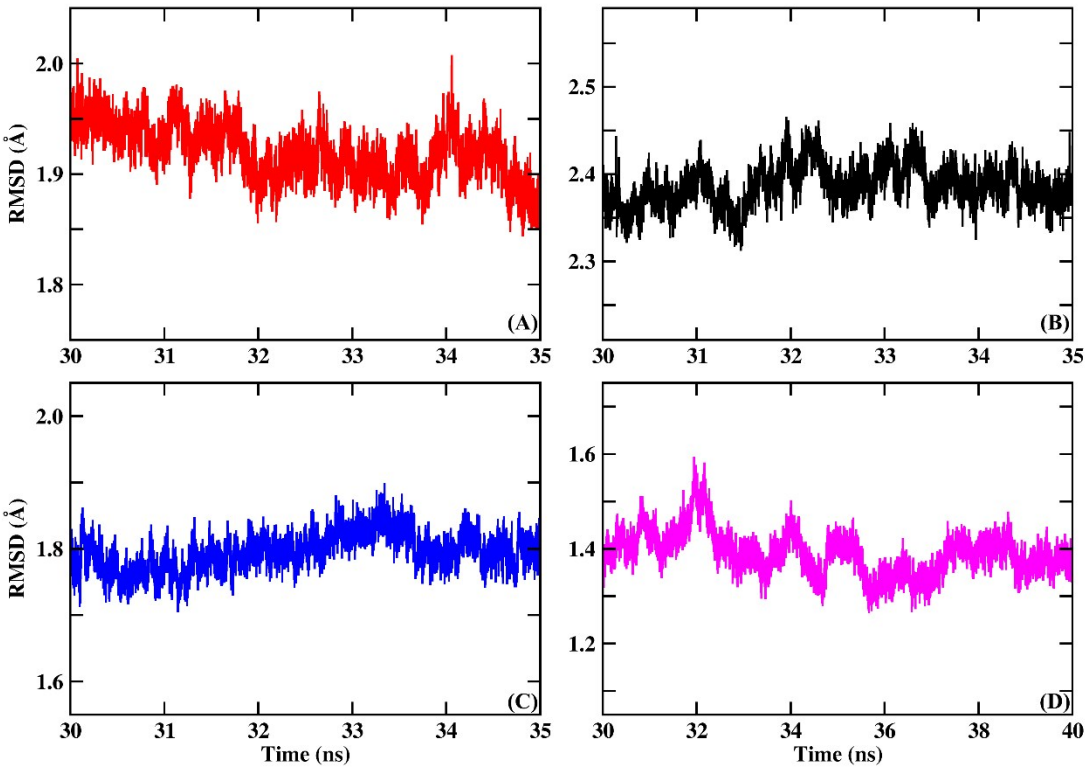

Figure S1. The root-mean-square deviation (RMSD) of all atoms relative to the corresponding native structure for Bcl-xL/Bad (A), Bcl-xL/Bax (B), Bcl-2/Bad (C), and Bcl-2/Bax (D), respectively.

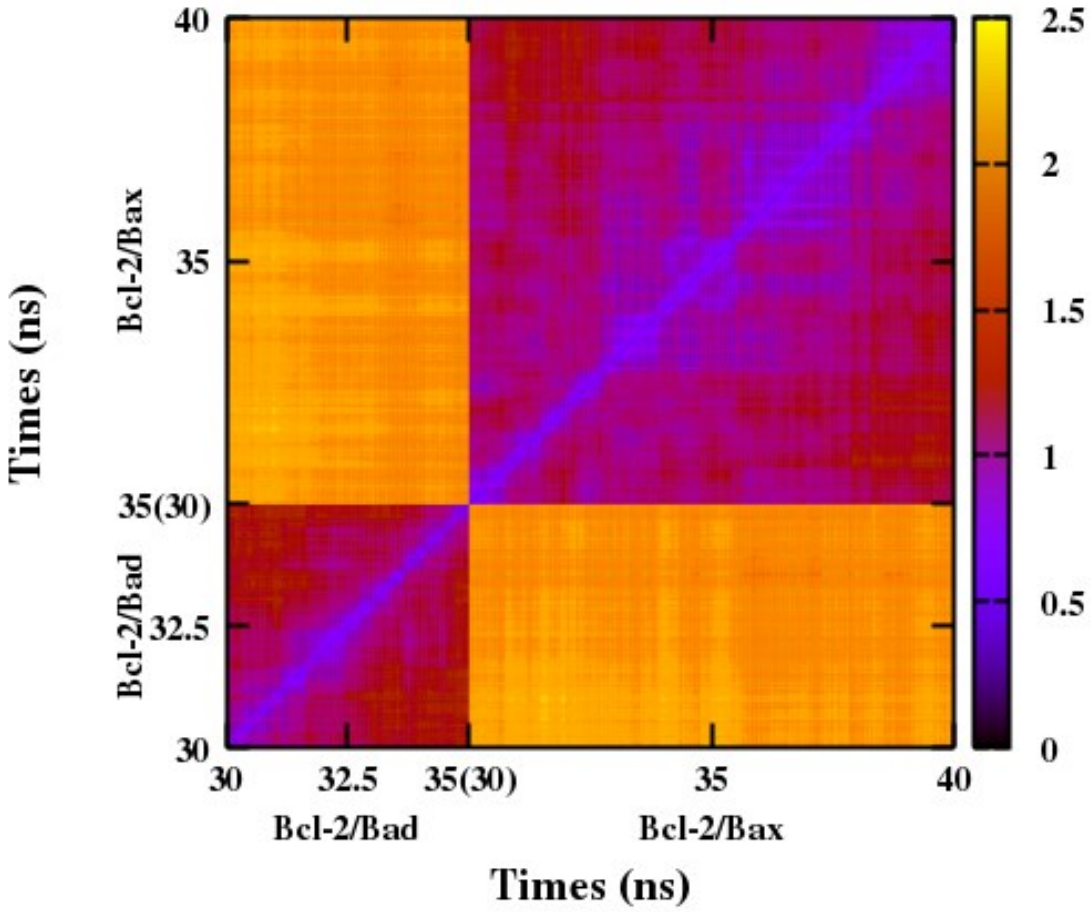

Figure S2. Cross-comparison of 2D-RMSD of Bcl-2 protein from Bcl-2/Bad and Bcl-2/Bax systems in all trajectories with units of Å.

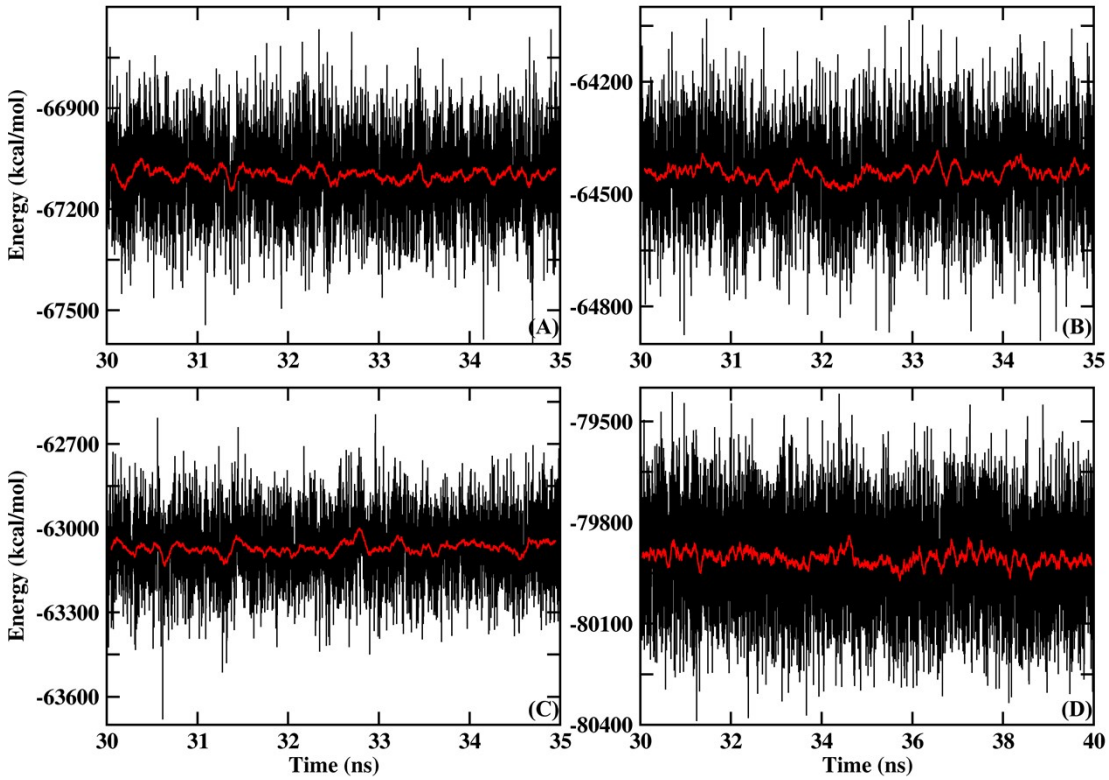

Figure S3. The potential energies of Bcl-xL/Bad (A), Bcl-xL/Bax (B), Bcl-2/Bad (C), and Bcl-2/Bax (D) systems as a function of time observed during MD simulation. The red solid line represents a 100 ps running average.

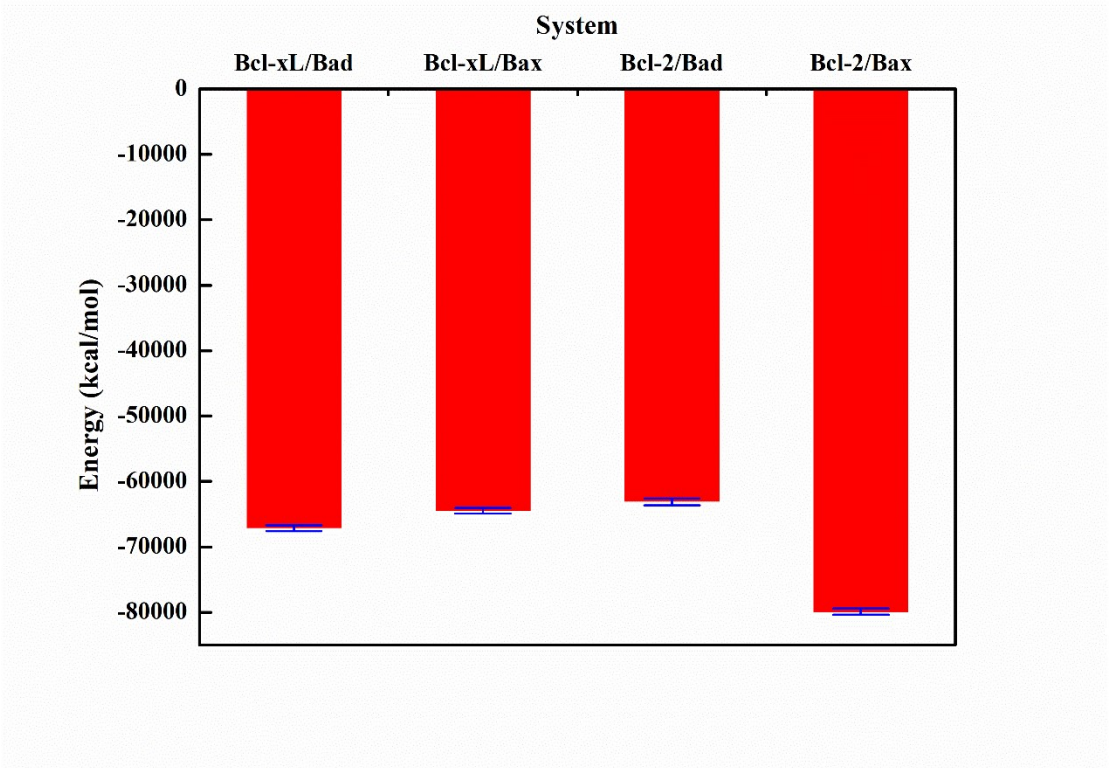

Figure S4. The red histograms denote the average value of potential energies of Bcl-xL/Bad, Bcl-xL/Bax, Bcl-2/Bad, and Bcl-2/Bax systems, with blue lines denote error bar.