Supporting Information

Ligand Gaussian accelerated molecular dynamics (LiGaMD): Characterization of ligand binding thermodynamics and kinetics

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Table S1 Summary of host-guest binding thermodynamics and kinetics obtained from (A) experimental data and (B) cMD simulations from Ref. [1]. Results are obtained for the binding of aspirin and 1-butanol guests to the β-cyclodextrin (CD) host. In simulations, the CD host has been modeled with both the GAFF and q4MD force fields. ΔG is the ligand binding free energy. The ΔG_{comp1} and ΔG_{comp2} are ligand binding free energies calculated using two different algorithms as adapted from Ref. [1]. k_{on} and k_{off} are the kinetic dissociation and binding rate constants, respectively, with N_D and N_B being the number of host-guest dissociation and binding events collected from individual simulations.

Host	Ligand	ΔG (kcal/mol)	k_{on} ($\times 10^8$ M·s^{-1})	k_{off} ($\times 10^6$ s^{-1})
CD	Aspirin	-3.74 ± 0.00	7.2 ± 0.04	1.3 ± 0.03
	1-Butanol	-1.67 ± 0.19	2.8 ± 0.8	38 ± 6

(A) Experimental data

Host	Ligand	cMD	N_D	N_B	ΔG_{comp1} (kcal/mol)	ΔG_{comp2} (kcal/mol)	k_{on} ($\times 10^8$ M·s^{-1})	k_{off} ($\times 10^6$ s^{-1})
CD: GAFF	Aspirin	9500 ns	133	133	-3.84 ± 0.35	-2.27 ± 0.06	11 ± 0.1	24 ± 3
	1-Butanol	6500 ns	42	42	1.01 ± 0.59	-0.41 ± 0.10	2.2 ± 0.1	110 ± 20
CD: q4MD	Aspirin	6000 ns	17	18	-6.33 ± 0.37	-4.11 ± 0.05	32 ± 3	3.1 ± 0.9
	1-Butanol	5000 ns	89	89	-1.33 ± 0.34	-2.27 ± 0.02	15 ± 0.3	33 ± 0.7

(B) cMD simulations.

Table S2 The guest binding and unbinding time periods (τ_B and τ_U) recorded from LiGaMD simulations of host-guest binding systems.

Host	Ligand	GaMD (300 ns x 3)	τ_B (ns)	τ_U (ns)
CD: GAFF	Aspirin	LiGaMD	32.0228, 8.5966, 3.1112, 39.9596, 21.2604	9.9081, 194.0138, 86.3999
		LiGaMD_Dual	8.2607, 0.796, 7.9322, 0.5776, 4.5163, 1.758, 5.9326	201.0795, 13.8457, 24.4303, 239.0854, 8.3765
CD: q4MD	Aspirin	LiGaMD	31.9081, 61.633, 43.8801, 8.2827, 16.9167, 20.3661, 14.1932, 19.3123, 27.4969	1.0855, 2.1165, 86.4148, 35.6304, 17.3932, 115.365, 89.763
		LiGaMD_Dual	3.907, 30.0912, 36.3697, 1.4833, 10.1038, 1.7181, 12.8671, 56.2154, 3.9519, 8.4395, 8.9309, 4.4481, 4.2826, 26.9633, 38.3872, 20.3627, 9.6437, 44.3214	8.4001, 29.2999, 6.2442, 42.1422, 12.1334, 39.4287, 54.0979, 62.0065, 15.8736, 45.7133, 6.6287, 29.8733, 13.1733, 60.1833, 65.7046, 9.4815
	1-Butanol	LiGaMD	5.4329, 2.5518, 11.281, 3.0967, 6.9, 2.606, 0.7952, 3.3571, 1.0371, 1.1861, 5.05, 0.9876, 1.275, 0.7967, 0.4231, 1.0814, 1.0247, 0.9549, 0.7596, 4.4845	5.1734, 72.0719, 51.3295, 15.962, 56.6445, 0.9302, 16.088, 12.9821, 19.5307, 84.7936, 62.3141, 27.7493, 39.8894, 61.5153, 32.1647, 24.3296, 24.2369, 38.1307, 27.2135, 79.995
		LiGaMD_Dual	1.4312, 2.3294, 4.6188, 1.5447, 1.2259, 2.1735, 1.6123, 1.8385, 0.8007, 5.3053, 0.6236, 0.9946, 0.924, 0.3333, 4.6931, 1.8042, 0.8413, 2.2369, 1.7726, 2.692, 4.133, 1.7794, 8.4176, 2.262, 1.5251, 6.0076, 1.262, 1.5508, 1.1358, 1.6418	1.8388, 7.8776, 20.9217, 79.5963, 41.6825, 50.1632, 22.8479, 1.5881, 11.4997, 18.477, 65.5491, 16.961, 47.0128, 8.5518, 2.548, 2.8552, 5.14, 16.607, 5.6482, 41.906, 35.6489, 4.0918, 18.9406, 20.069, 14.6097, 8.0379, 85.2561, 4.8654, 24.6768, 45.8237

Table S3 Energy barriers of host-guest dissociation ("off") and binding ("on") calculated from the reweighed ($\Delta \boldsymbol{F}$) and modified (no reweighting, $\Delta \boldsymbol{F^*}$) free energy profiles, curvatures of the reweighed (w) and modified (w^*) free energy profiles near the guest Bound ("B"), Barrier ("Br") and Unbound ("U") states, and the ratio of apparent diffusion coefficients calculated from the LiGaMD and LiGaMD_Dual simulations without reweighting (modified, D^*) and with reweighting (D). Results are listed for the following systems: (A) aspirin binding to CD with the GAFF force field, (B) aspirin binding to CD with the q4MD force field and (C) 1-butanol binding to CD with the q4MD force field.

Sim	$\Delta \boldsymbol{F}$ **(kcal/mol)**		$\Delta \boldsymbol{F^*}$ **(kcal/mol)**		***w***			***w****			***D**/D***	
	Off	**On**	**Off**	**On**	**B**	**Br**	**U**	**B**	**Br**	**U**	**Off**	**On**
LiGaMD	3.09 ± 0.37	0.61 ± 0.37	1.49 ± 0.35	1.87 ± 0.28	0.38 ± 0.21	0.08 ± 0.04	0.11 ± 0.01	0.32 ± 0.08	0.07 ± 0.08	0.15 ± 0.01	1.14	1.53
LiGaMD_Dual	2.06 ± 0.28	0.40 ± 0.34	0.59 ± 0.37	1.83 ± 0.28	0.49 ± 0.08	0.07 ± 0.05	0.12 ± 0.03	0.35 ± 0.03	0.05 ± 0.15	0.13 ± 0.01	1.23	0.85

(A) CD (GAFF) – Aspirin

Sim	$\Delta \boldsymbol{F}$ **(kcal/mol)**		$\Delta \boldsymbol{F^*}$ **(kcal/mol)**		***w***			***w****			***D**/D***	
	Off	**On**	**Off**	**On**	**B**	**Br**	**U**	**B**	**Br**	**U**	**Off**	**On**
LiGaMD	4.00 ± 0.34	0.97 ± 0.35	2.18 ± 0.28	1.95 ± 0.37	0.41 ± 0.08	0.09 ± 0.08	0.13 ± 0.03	0.32 ± 0.05	0.08 ± 0.02	0.13 ± 0.02	0.84	0.12
LiGaMD_Dual	4.46 ± 0.22	0.67 ± 0.39	2.25 ± 0.23	1.67 ± 0.24	0.46 ± 0.03	0.10 ± 0.12	0.18 ± 0.02	0.33 ± 0.02	0.08 ± 0.12	0.15 ± 0.00	0.92	1.80

(B) CD (q4MD) – Aspirin

Sim	$\Delta \boldsymbol{F}$ **(kcal/mol)**		$\Delta \boldsymbol{F^*}$ **(kcal/mol)**		***w***			***w****			***D**/D***	
	Off	**On**	**Off**	**On**	**B**	**Br**	**U**	**B**	**Br**	**U**	**Off**	**On**
LiGaMD	2.65 ± 0.34	1.59 ± 0.42	1.37 ± 0.30	2.09 ± 0.19	2.23 ± 0.02	0.07 ± 0.05	0.12 ± 0.00	2.42 ± 0.02	0.06 ± 0.03	0.14 ± 0.01	0.94	1.28
LiGaMD_Dual	2.83 ± 0.24	1.35 ± 0.25	1.82 ± 0.16	2.21 ± 0.11	2.24 ± 0.00	0.07 ± 0.07	0.03 ± 0.00	2.82 ± 0.03	0.06 ± 0.01	0.12 ± 0.04	0.98	1.11

(C) CD (q4MD) – 1-Butanol

Table S4 The ligand binding and unbinding time periods (τ_B and τ_U) recorded from LiGaMD_Dual simulations of the trypsin-benzamidine binding system.

System	ID	τ_B (ns)	τ_U (ns)
Trypsin - BEN	Sim1	14.90, 8.20, 28.90	165.90, 77.00, 93.40
	Sim2	26.10, 14.00, 27.00, 18.80, 124.10	11.50, 270.70, 70.20, 415.70
	Sim3	47.39, 31.59, 12.63, 18.29, 49.09, 19.84, 41.00, 15.00, 85.13, 17.19, 60.59	145.08, 17.21, 18.71, 28.50, 38.37, 18.52, 32.75, 205.65, 13.37, 32.84
	Sim4	32.10, 70.00, 38.70, 30.30	210.40, 9.40, 21.70, 496.90
	Sim5	7.00, 46.70, 31.30, 39.60	279.80, 179.30, 265.80

Table S5 Energy barriers of trypsin-benzamidine dissociation ("off") and binding ("on") calculated from the reweighed ($\Delta \boldsymbol{F}$) and modified (no reweighting, $\Delta \boldsymbol{F^*}$) free energy profiles, curvatures of the reweighed (w) and modified (w^*) free energy profiles near the guest Bound ("B"), Barrier ("Br") and Unbound ("U") states, and the ratio of apparent diffusion coefficients calculated from the LiGaMD_Dual simulations without reweighting (modified, D^*) and with reweighting (D).

Sim	$\Delta \boldsymbol{F}$ **(kcal/mol)**		$\Delta \boldsymbol{F^*}$ **(kcal/mol)**		***w***			***w****			***D*/D***	
	Off	**On**	**Off**	**On**	**B**	**Br**	**U**	**B**	**Br**	**U**	**Off**	**On**
LiGaMD_Dual	12.17 ± 1.54	3.04 ± 2.04	1.37 ± 0.56	2.40 ± 0.41	2.39 ± 0.21	0.12 ± 0.16	0.06 ± 0.01	0.99 ± 0.05	0.04 ± 0.05	0.06 ± 0.02	1.06	15.07

Figure S1 Time courses of host-guest distances calculated from (A) LiGaMD and (B) LiGaMD_Dual simulations of CD using the GAFF force field with aspirin, (C) LiGaMD and (D) LiGaMD_Dual simulations of CD using the GAFF force field with 1-butanol, (E) LiGaMD and (F) LiGaMD_Dual simulations of CD using the q4MD force field with aspirin, (G) LiGaMD and (H) LiGaMD_Dual simulations of CD using the q4MD force field with 1-butanol.

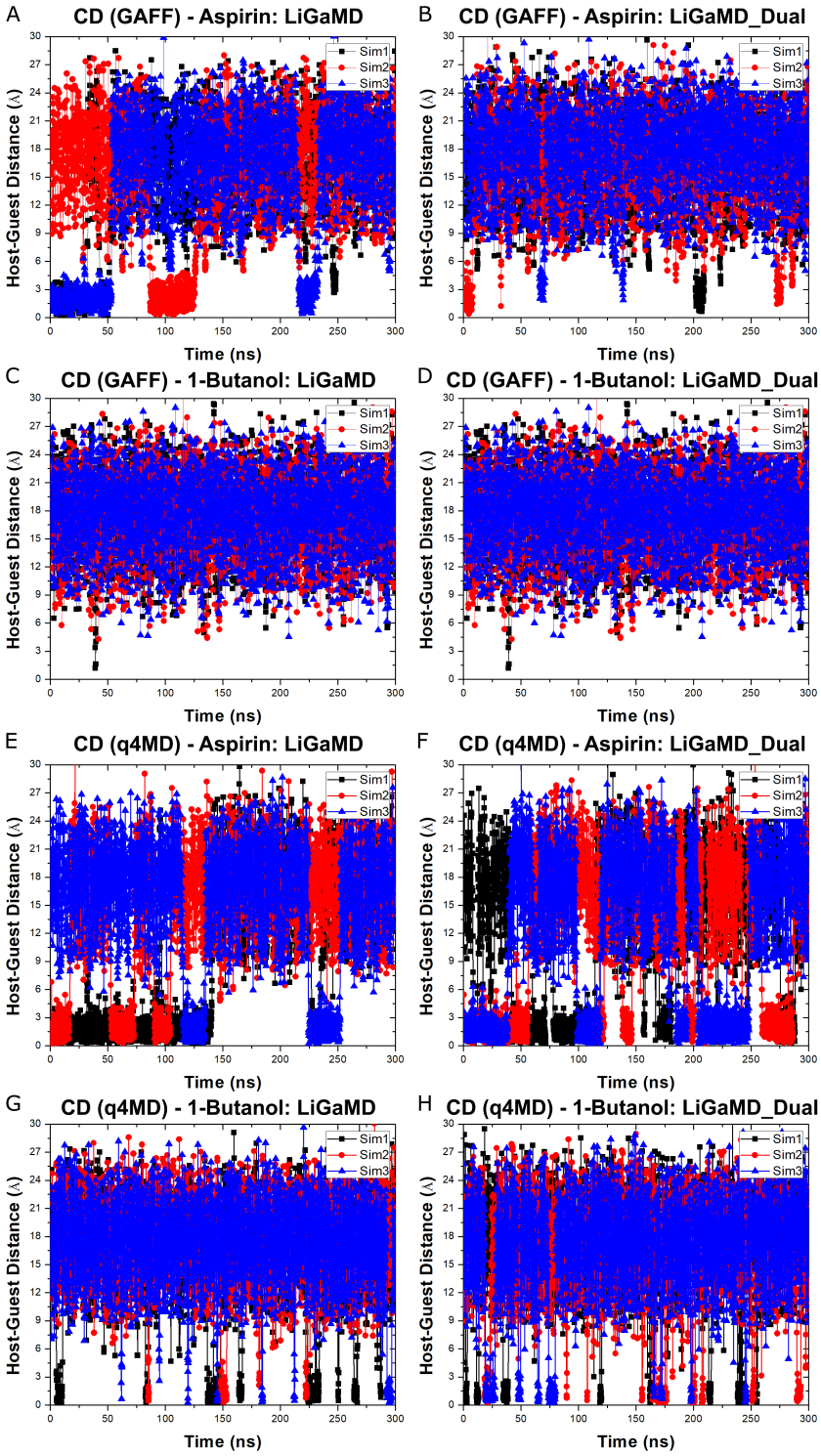


Figure S2 Reweighted and modified PMF profiles of guest 1-butanol binding to the CD host modeled with the GAFF force field: (A) LiGaMD and (B) LiGaMD_Dual simulations.

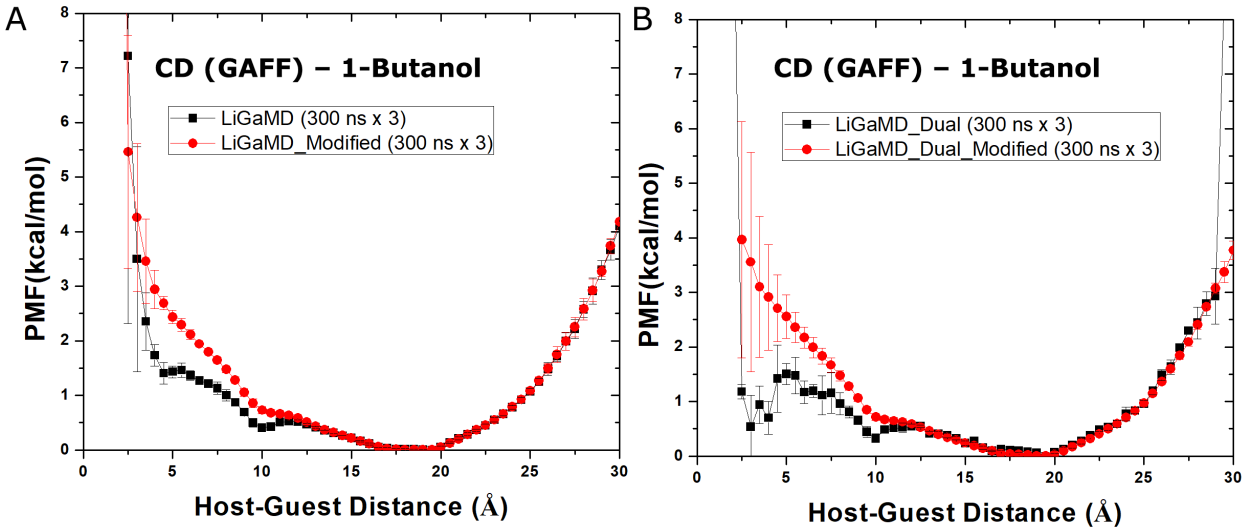


Figure S3 Time courses of the benzamidine ligand RMSD relative to the X-ray conformation obtained from LiGaMD_Dual equilibration simulations of trypsin, where the input parameter σ_{0P} was increased from 1.0 to 6.0 with the threshold energy set to upper bound for applying boost potential to the ligand non-bounded potential energy.

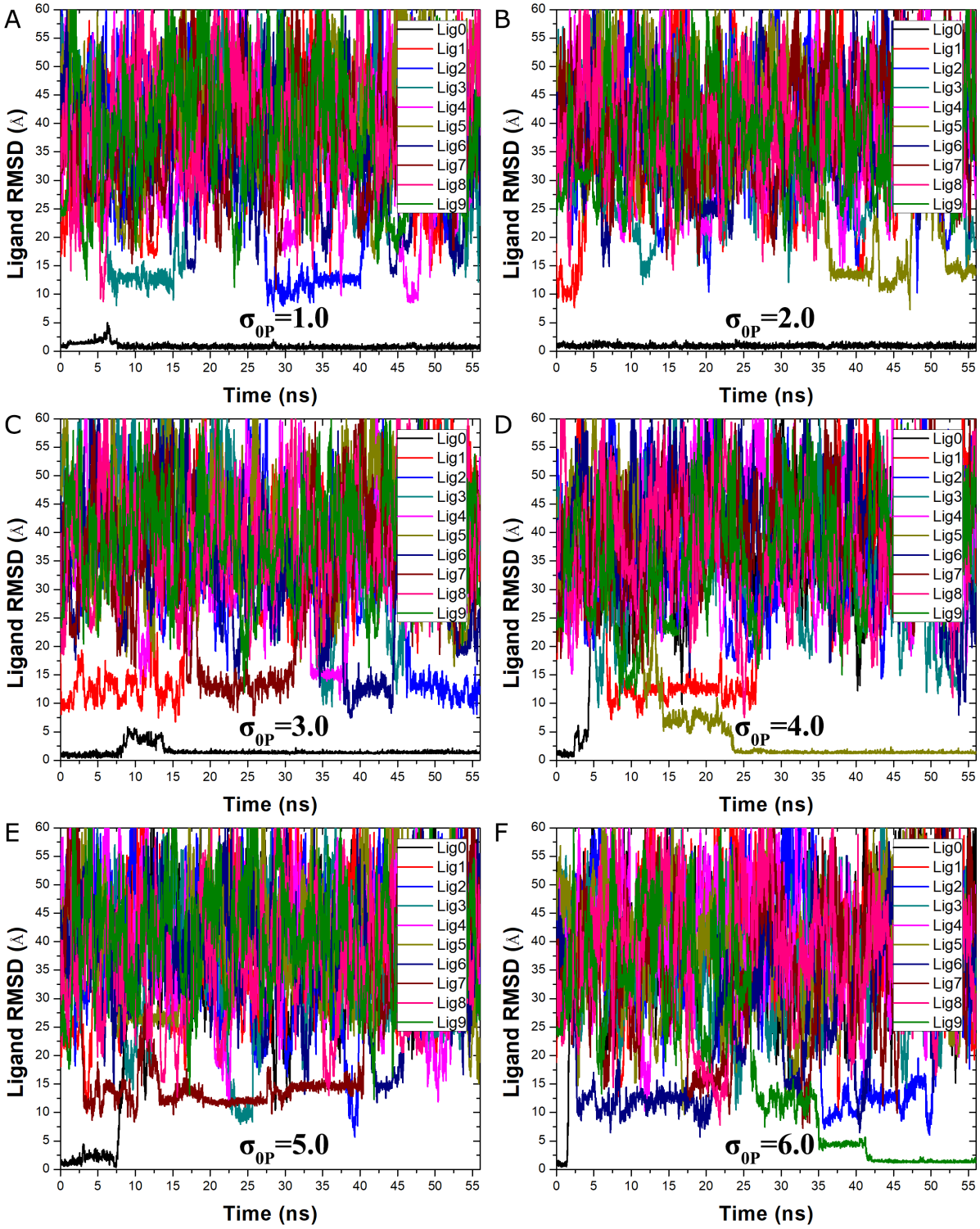


Figure S4 RMSD of the benzamidine (BEN) ligand relative to the X-ray crystal conformation calculated from LiGaMD_Dual simulations as listed in **Table 4**: (A) Sim1, (B) Sim2, (C) Sim3, (D) Sim4 and (E) Sim5.

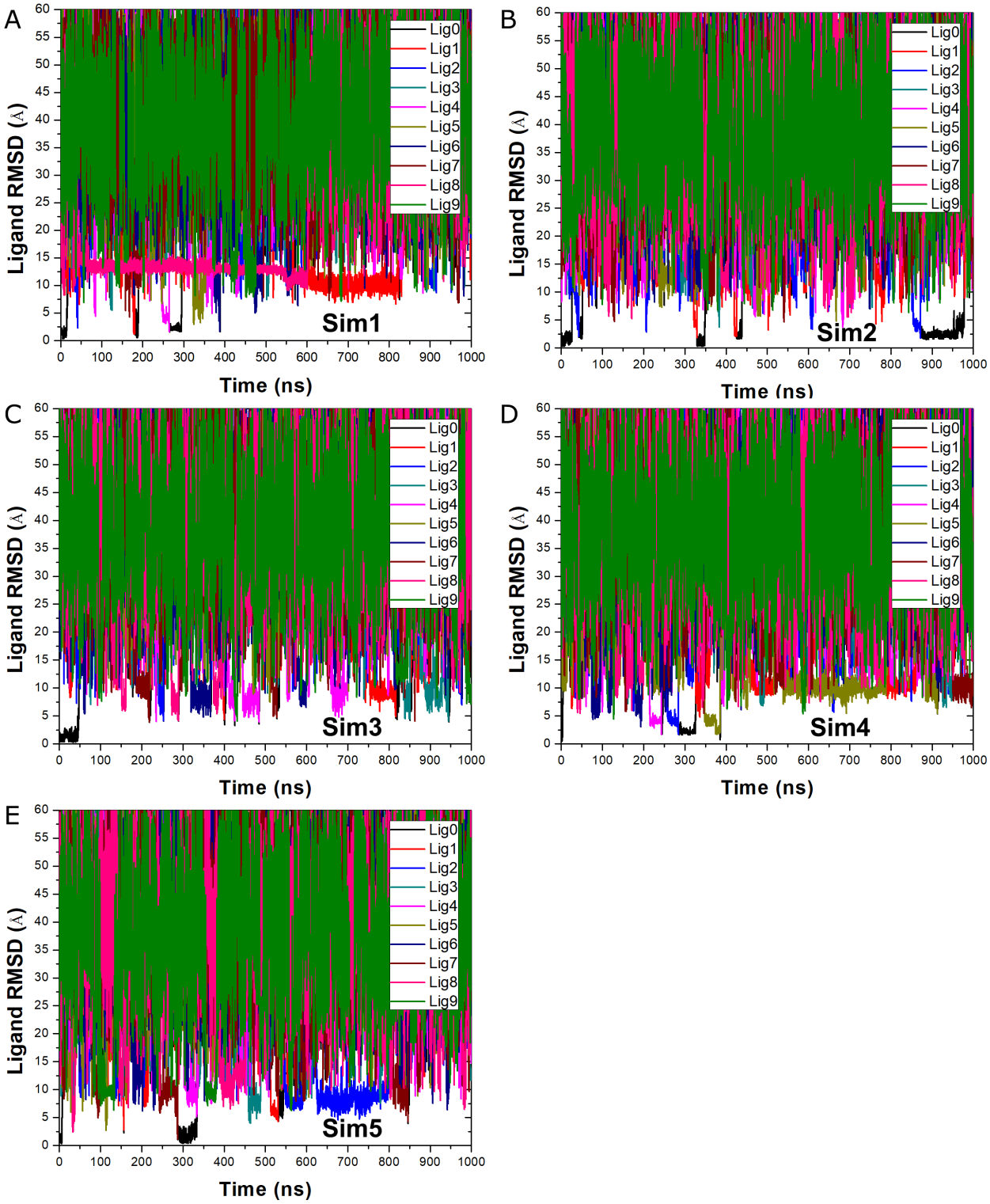


Figure S5 Distances between the N atom in benzamidine and CG atom of Asp189 in trypsin calculated from five LiGaMD_Dual simulations as listed in **Table 4**: (A) Sim1, (B) Sim2, (C) Sim3, (D) Sim4 and (E) Sim5. Distance plots of Sim2 are provided in **Figure 5A**.

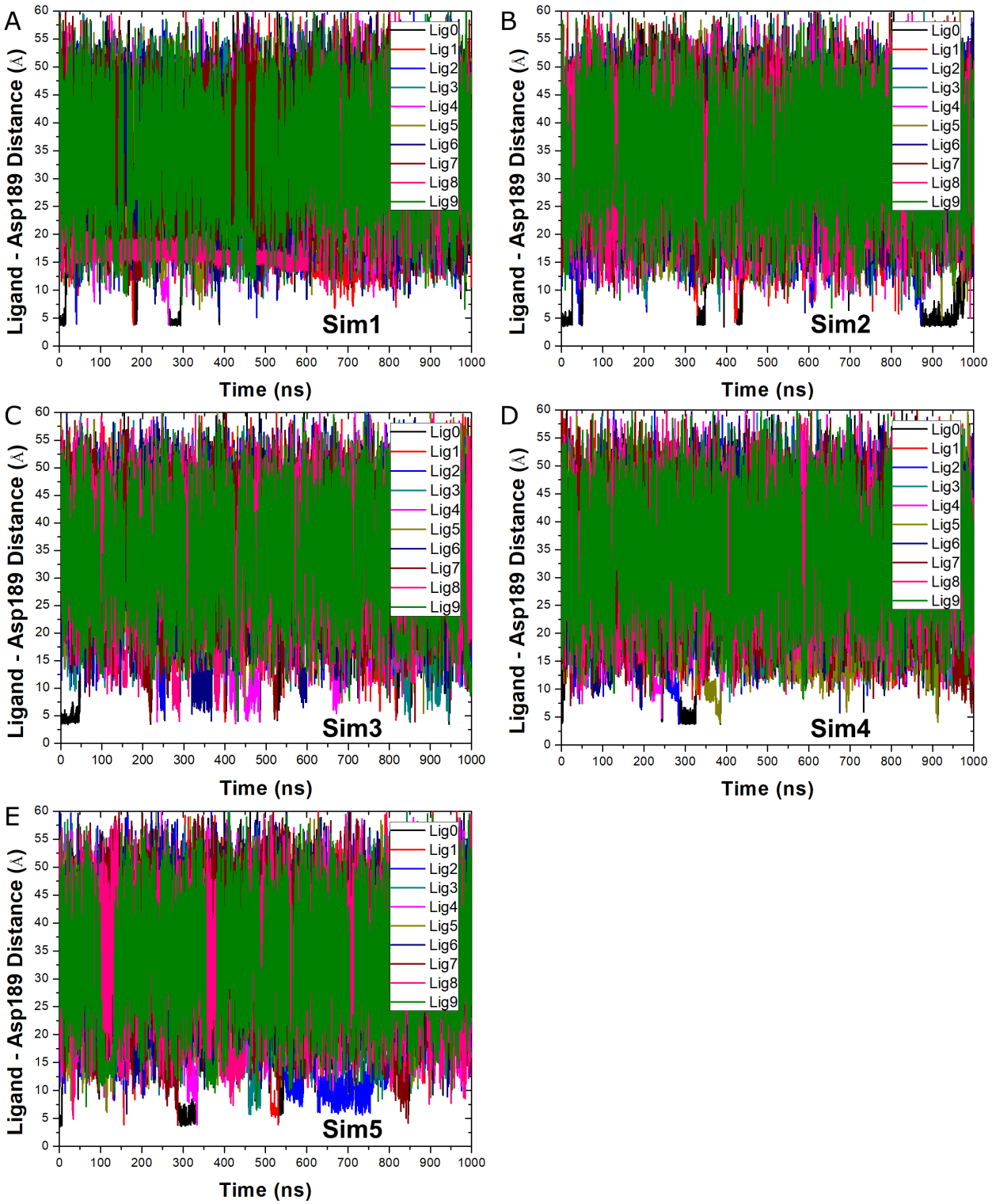


Figure S6 Reweighted 2D PMF profiles of the BEN:N – Asp189:CG and Trp215:NE – Asp189:CG atom distances calculated from five individual 1000 ns LiGaMD_Dual simulations of the benzamidine inhibitor binding to trypsin: (A) Sim1, (B) Sim2, (C) Sim3, (D) Sim4 and (E) Sim5.

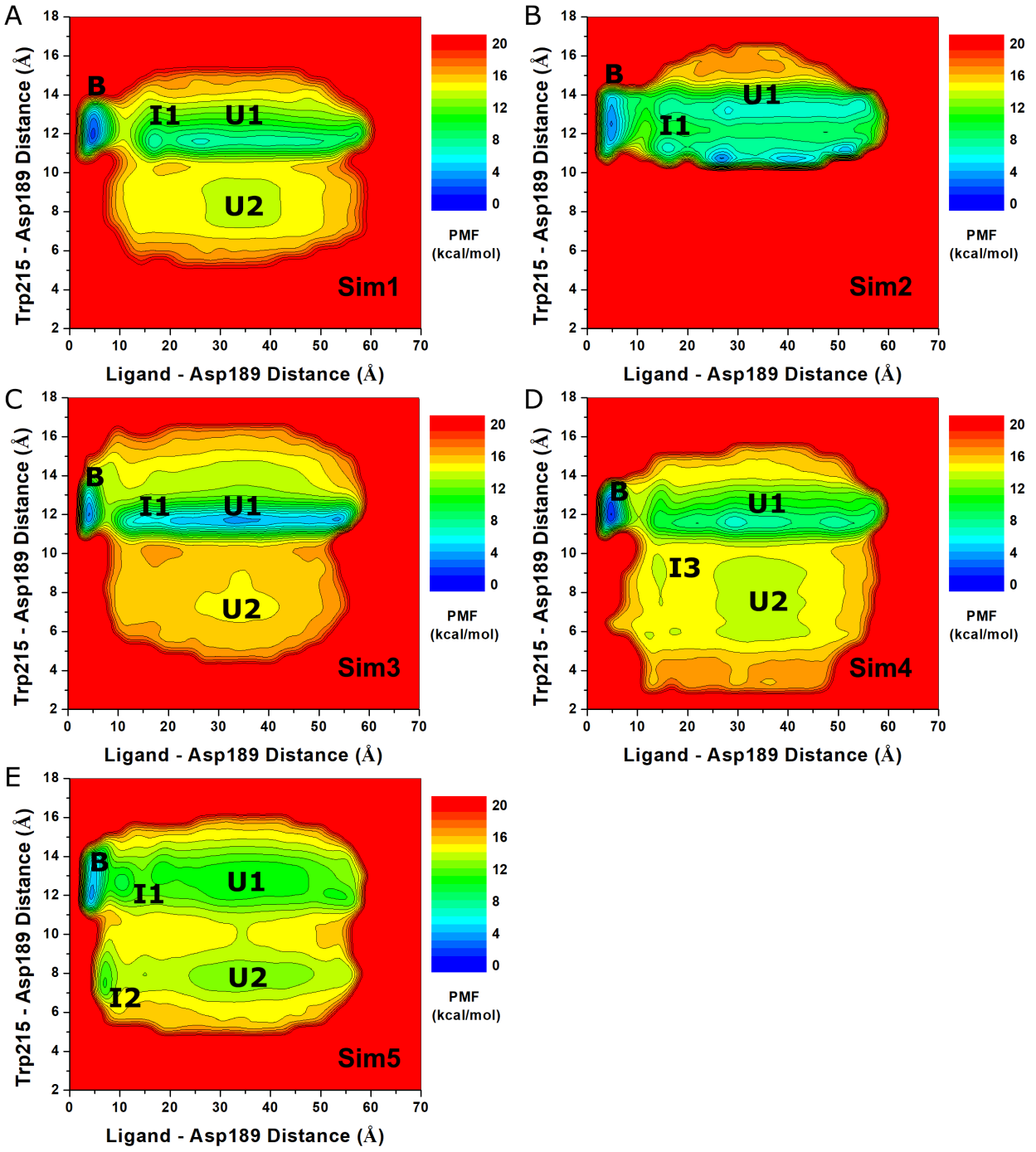
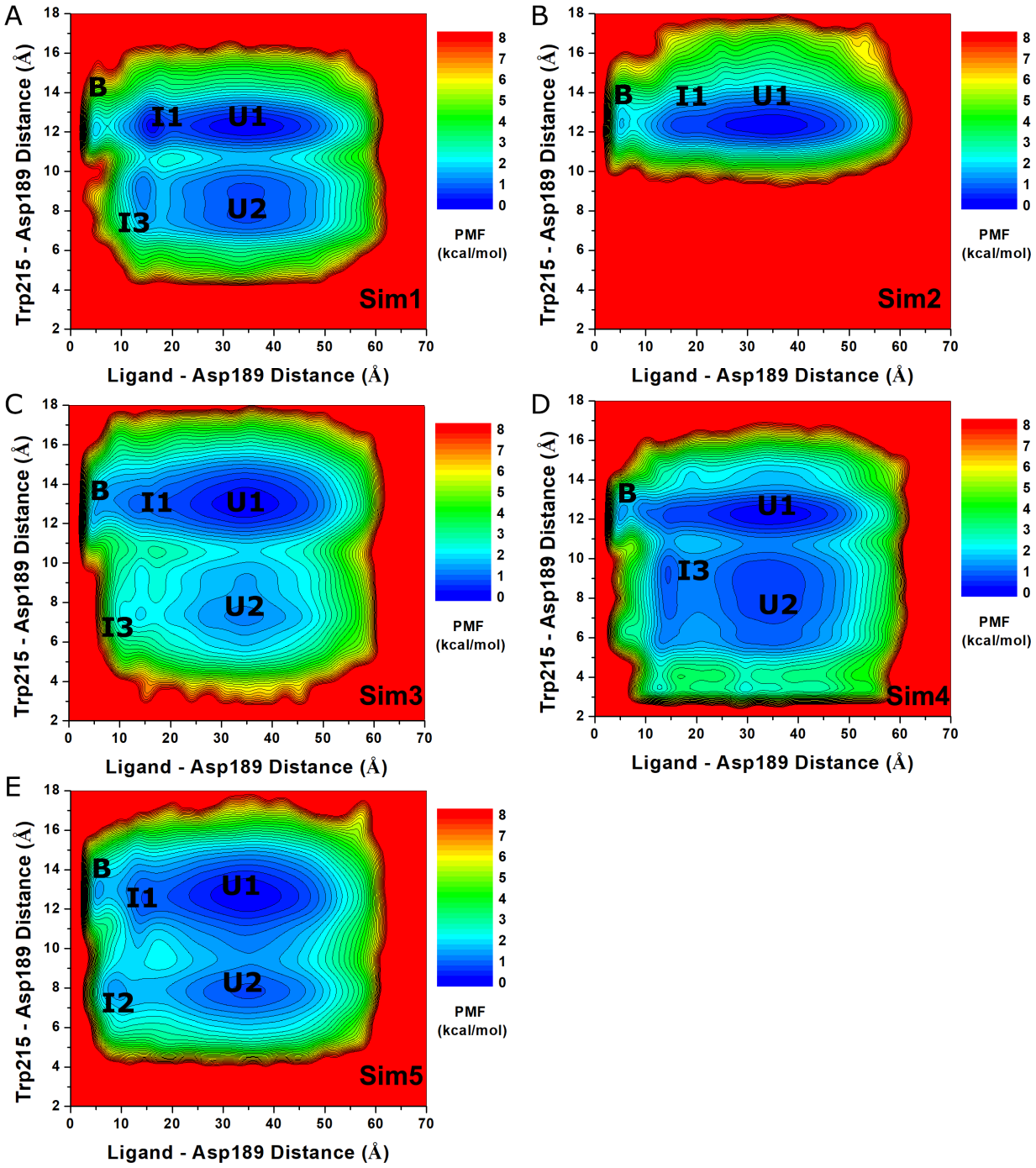


Figure S7 Modified 2D PMF profiles of the BEN:N – Asp189:CG and Trp215:NE – Asp189:CG atom distances calculated from five individual 1000 ns LiGaMD_Dual simulations of the benzamidine inhibitor binding to trypsin: (A) Sim1, (B) Sim2, (C) Sim3, (D) Sim4 and (E) Sim5.



References:

1. Tang, Z. Y.; Chang, C. E. A., Binding Thermodynamics and Kinetics Calculations Using Chemical Host and Guest: A Comprehensive Picture of Molecular Recognition. *J Chem Theory Comput* **2018,** *14* (1), 303-318.