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Supporting Information for 'BRD4: Quantum mechanical protein-ligand binding free energies using the full-protein DFT-based QM-PBSA method'

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1 Input and output files

QM-PBSA input and output files are provided at the University of Southampton Institutional Repository (https://doi.org/10.5258/SOTON/D2384). Additionally, the outputs of the MM-PBSA, MM-GBSA and normal mode calculations are provided.

2 Ligand Set

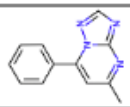
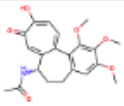
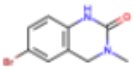
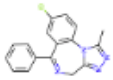
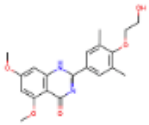
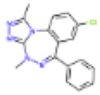
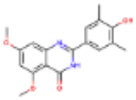
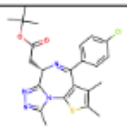
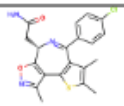
Ligand Number	Ligand Structure	G_bind (kcal/mol)	Source
1		Non-Binder	[1]
2		Weak-Binder	[1]
3		-5.95	[2]
4		-6.36	[3]
5		-7.40	[4]
6		-7.84	[5]
7		-8.16	[4]
8		-8.99	[5]
9		-9.64	[6]
10		-10.41	[7]

Figure 1: Benchmarking ligand set for BRD4(1) with ligand structure, experimental binding energies and original publications [1–7] as compiled by Mobley et al.[8]

3 Equilibration Protocol

- Minimization 1: 1000 steps conjugate gradient and 1000 steps steepest descent, nonbonded cut off is 8 Å, restraint of 1000 $\text{kcal} \cdot \text{mol}^{-1} \cdot \text{Å}^{-2}$ on all heavy atoms.
- Minimization 2: 1000 steps conjugate gradient and 1000 steps steepest descent, nonbonded cut off is 8 Å, restraint of 1000 $\text{kcal} \cdot \text{mol}^{-1} \cdot \text{Å}^{-2}$ on complex heavy atoms.
- Heating: 100 ps of heating of the solvent with NVT ensemble from 100 K to 300. Heavy atoms of the complex restraint with 1000 $\text{kcal} \cdot \text{mol}^{-1} \cdot \text{Å}^{-2}$.
- Density: Adjust the size of the box via 500 ps of NPT and heavy atoms of the complex restraint with 1000 $\text{kcal} \cdot \text{mol}^{-1} \cdot \text{Å}^{-2}$.
- Cooling: 100 ps of cooling of the solvent with NVT ensemble from 300 K to 100. Heavy atoms of the complex restraint with 1000 $\text{kcal} \cdot \text{mol}^{-1} \cdot \text{Å}^{-2}$.
- Minimization: 1000 steps conjugate gradient and 1000 steps steepest descent, nonbonded cut off is 8 A, for each decreasing constraint of 1000, 500, 100, 50, 20, 10, 5, 2, 0 $\text{kcal} \cdot \text{mol}^{-1} \cdot \text{Å}^{-2}$.
- Heating: 100 ps of heating of the system with NVT ensemble from 100 K to 300 K with no constraints.
- Equilibration: 100 ps simulation at 300 K in NPT ensemble with no constraints.

4 Absolute binding free energies

	Energies (kcal/mol)				
Ligand	Gas-phase	G_bind	G_bind(IE)	G_bind(Nmode)	G_bind Experiment
1	-36.1	-34.3	-29.4	-19.4	0
2	-45.2	-37.3	-27.0	-20.9	0
3	-45.8	-44.1	-37.6	-26.2	-5.95
4	-32.6	-26.2	-15.0	-10.7	-6.36
5	-44.5	-40.4	-33.8	-23.3	-7.40
6	-55.3	-42.7	-36.2	-23.8	-7.84
7	-48.4	-45.2	-40.0	-26.7	-8.16
8	-42.9	-42.5	-34.7	-24.8	-8.99
9	-47.2	-46.4	-40.2	-28.4	-9.64
10	-47.7	-46.9	-38.5	-27.8	-10.4
Mean without Lig4	-45.9	-42.2	-35.3	-24.6	-6.5
Lig4 - Mean	13.3	16.0	20.3	13.8	0.1
Lig4 - Lig 1	3.49	8.09	14.47	8.64	-6.36

Table 1: QM-PBSA absolute gas phase energies and binding energies without entropy and with interaction entropy and normal mode correction terms. Mean energies calculated without ligand 4. Difference between ligand 4 energies and mean without ligand 4 and difference between ligand 4 and ligand 1, the non binder, shown at the bottom of the Table. The non-binder (ligand 1) and weak binder (ligand 2) are allocated experimental binding energies of 0 kcal/mol.

5 RMSDtr and Spearman's r_s with BASE and Bootstrapped Errors

Snapshots	Method	Entropy	RMSDtr (kcal/mol)	BASE	Boostrapped SE
50	QM-PBSA	No Entropy	1.77	0.19	0.15
		Normal Mode	1.60	0.23	0.26
		Interaction Entropy	2.30	0.21	0.20
	MM-PBSA	No Entropy	2.99	0.18	0.18
		Normal Mode	3.05	0.24	0.25
		Interaction Entropy	3.84	0.45	0.41
	MM-GBSA	No Entropy	2.66	0.20	0.15
		Normal Mode	2.17	0.47	0.27
		Interaction Entropy	3.22	0.21	0.29
4500	MM-PBSA	No Entropy	2.89	0.12	0.18
		Normal Mode	NA	NA	NA
		Interaction Entropy	6.18	0.71	1.15
	MM-GBSA	No Entropy	2.07	0.05	0.13
		Normal Mode	NA	NA	Na
		Interaction Entropy	5.54	0.73	1.10

Table 2: Root mean squared deviation of calculated binding energies shifted by the mean signed error against the experimental binding energies of 7 binders in BRD4 in kcal/mol using QM-PBSA, MM-PBSA, and MM-GBSA without entropy and with normal mode and interaction entropy term. RMSDtr values for MM are shown over 50 and over the complete set of 4500 snapshots. Block average standard errors and bootstrapped standard errors provide an estimate of the uncertainty in the RMSDtr metrics. Normal mode entropies only calculated for 50 snapshots.

Snapshots	Method	Entropy	Spearman's r	BASE	Boostrapped SE
50	QM-PBSA	No Entropy	0.83	0.03	0.02
		Normal Mode	0.85	0.08	0.06
		Interaction Entropy	0.76	0.06	0.08
	MM-PBSA	No Entropy	0.01	0.07	0.04
		Normal Mode	-0.30	0.15	0.19
		Interaction Entropy	-0.13	0.09	0.11
	MM-GBSA	No Entropy	0.79	0.02	0.05
		Normal Mode	0.53	0.06	0.09
		Interaction Entropy	0.48	0.06	0.11
4500	MM-PBSA	No Entropy	-0.03	0.04	0.04
		Normal Mode	NA	NA	NA
		Interaction Entropy	0.03	0.12	0.21
	MM-GBSA	No Entropy	0.78	0.03	0.04
		Normal Mode	NA	NA	NA
		Interaction Entropy	0.53	0.09	0.14

Table 3: Spearman's rank order coefficient, r, of calculated binding energies against the experimental binding energies of 9 ligands (binders and non-binders) in BRD4 using QM-PBSA, MM-PBSA, and MM-GBSA without entropy and with normal mode and interaction entropy term. r values for MM are shown over 50 and over the complete set of 4500 snapshots. Block average standard errors and bootstrapped standard errors provide an estimate of the uncertainty in the metrics. Normal mode entropies only calculated for 50 snapshots.

6 Interaction Entropy

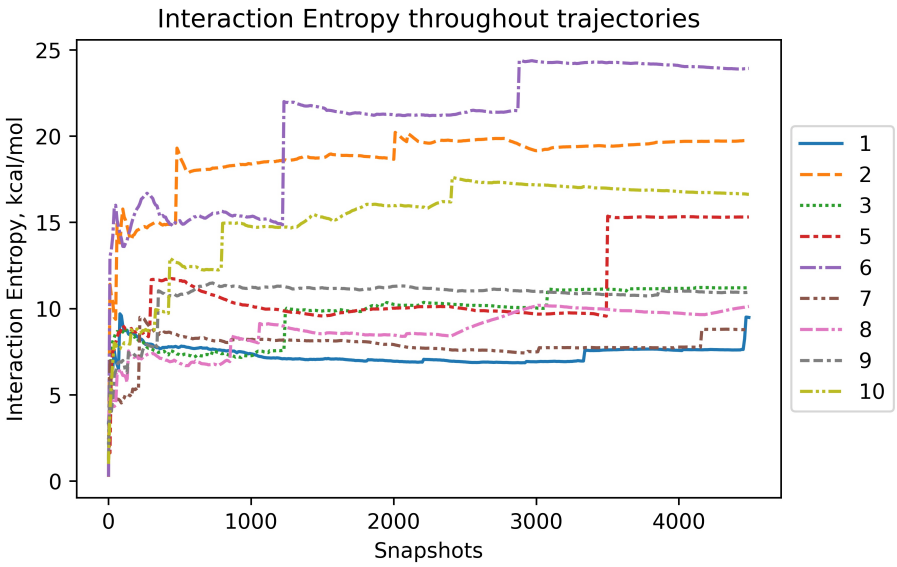


Figure 2: Interaction entropy correction term in kcal/mol for MM-PBSA as increasing numbers of snapshots are included for each ligand binding to BRD4.

The inclusion of an IE correction term increases RMSDtr and worsens rank ordering in all cases. The negative impact of the IE term is especilly pronounced when all 4500 snapshots are included in the MM-PBSA and MM-GBSA calculations. Overall, the IE correction also increases the BASE and bootstrapped SE significantly.Figure 2 shows the calculated IE correction for MM-PBSA as increasing numbers of snapshots are included, up to 4500. The sharp increases in IE indicate that individual snapshots with peak interaction energies are dominating the IE results. We also observe that the IE tends to increase as more snapshots are included for almost all ligands. This observation is in line with the findings of Ekberg et al. [9] who showed that IE increases with the number of snapshots sampled and that the exponential average is poorly conditioned. They suggest that when the standard deviation of the interaction energy is larger than 3.5 kcal/mol, the interaction entropy method becomes almost impossible to converge. In our test set the standard error of the interaction energy is 4.8 kcal/mol when averaged across the ligand set. Thus, according to Ekberg et al. the IE method is not applicable. Similarly, Menzer et al. [10] observed that the IE term is dominated by rare events. Also in 2018, Kohut et al. [11] showed the IE depends strongly on the highest peak interaction energies and does not significantly change after a peak energy has distorted the IE term. This behaviour is mirrored in our results in Figure 2. Kohut et al. concluded that especially when conformational changes occur, the IE method is not applicable.

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