Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information

Deciphering the Mechanisms of the Selective Inhibition for the tandem BD1/BD2 in BET-Bromodomains family

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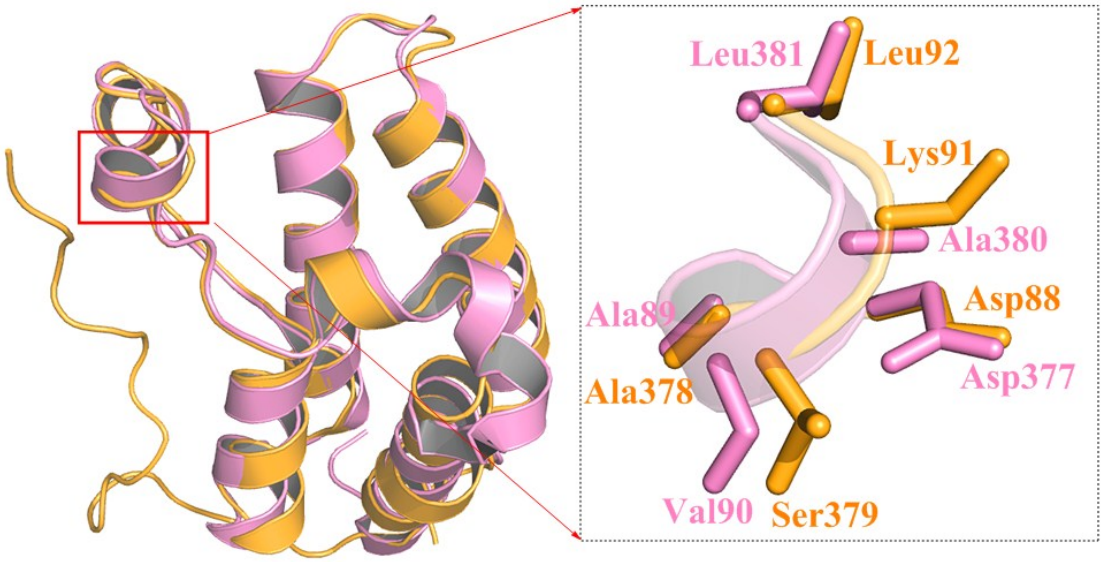

Fig. S1 Crystal structure alignment of BRD4-BD1 and BRD2-BD2. The key part of the ZA-loop is highlighted (right). Residues in BD1 are highlighted in bright-orange and in BD2 are marked in pink.

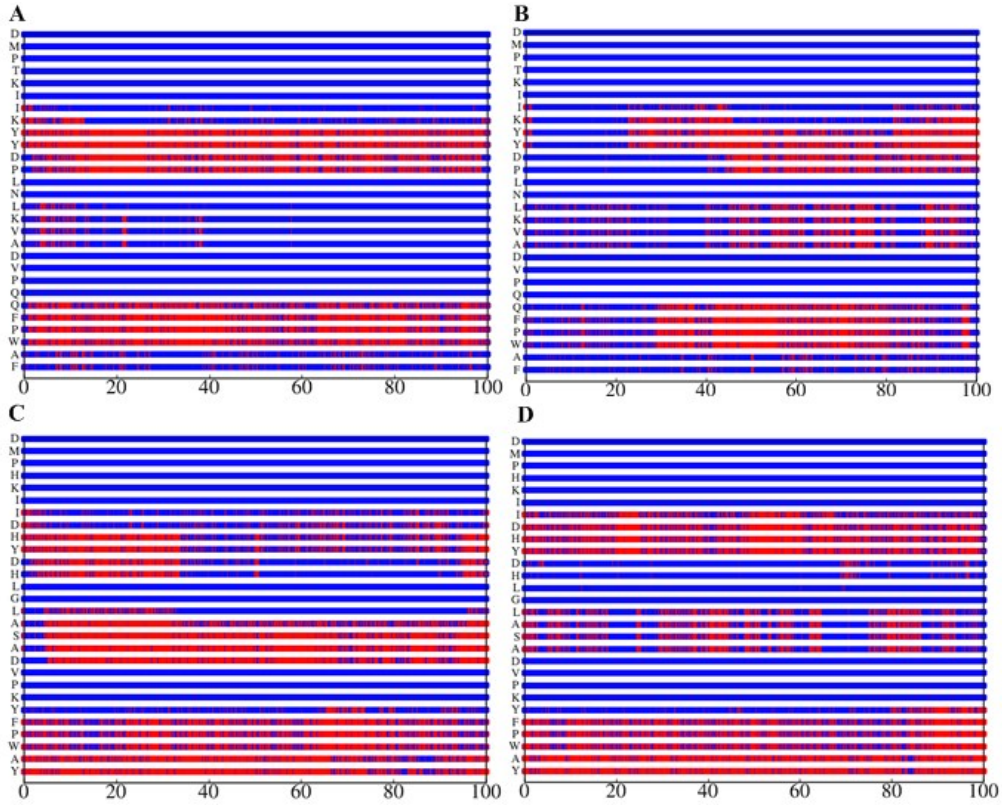

Fig. S2 The secondary structure evolutions of the whole ZA-loop. Helix is marked in red and loop in blue. (A) For apo-BRD4-BD1 simulation system. (B) For RVX-208 bounded BRD4-BD1 complex. (C) For apo-BRD2-BD2 simulation system. (D) For RVX-208 bounded BRD2-BD2 complex.

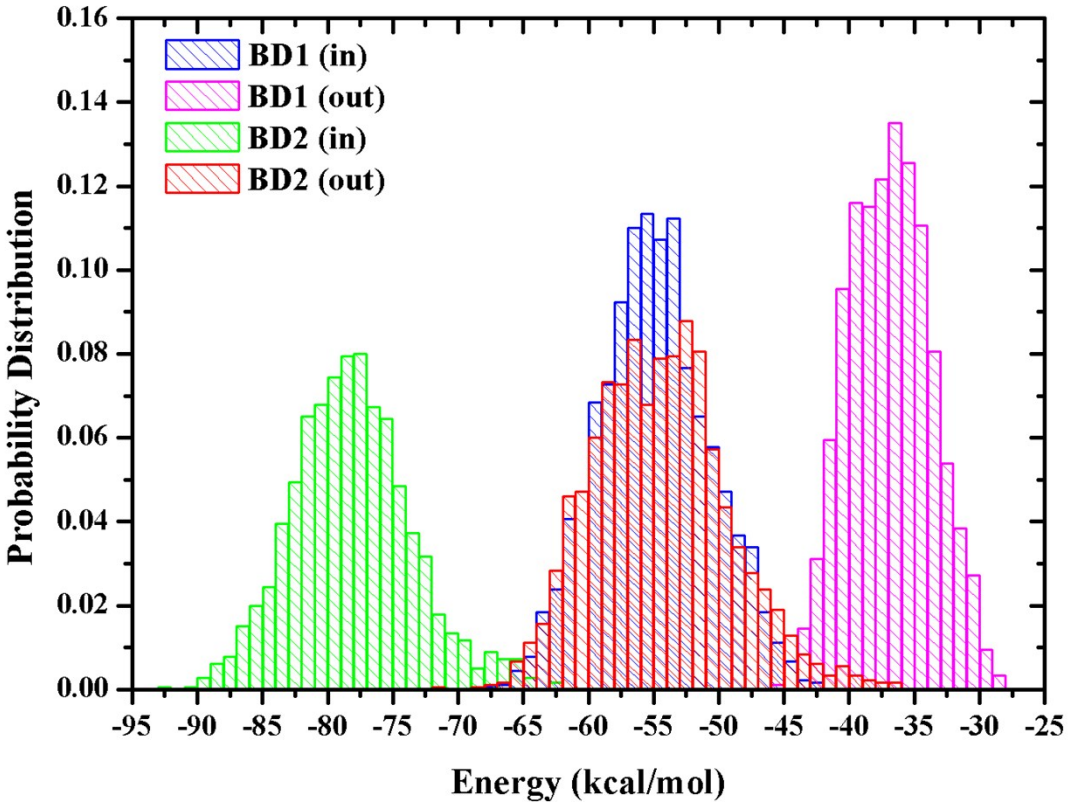

Fig. S3 The calculated Electrostatics and Van der Waals interaction energies between the QM subsystem (i.e., RVX-208) and its protein environment.

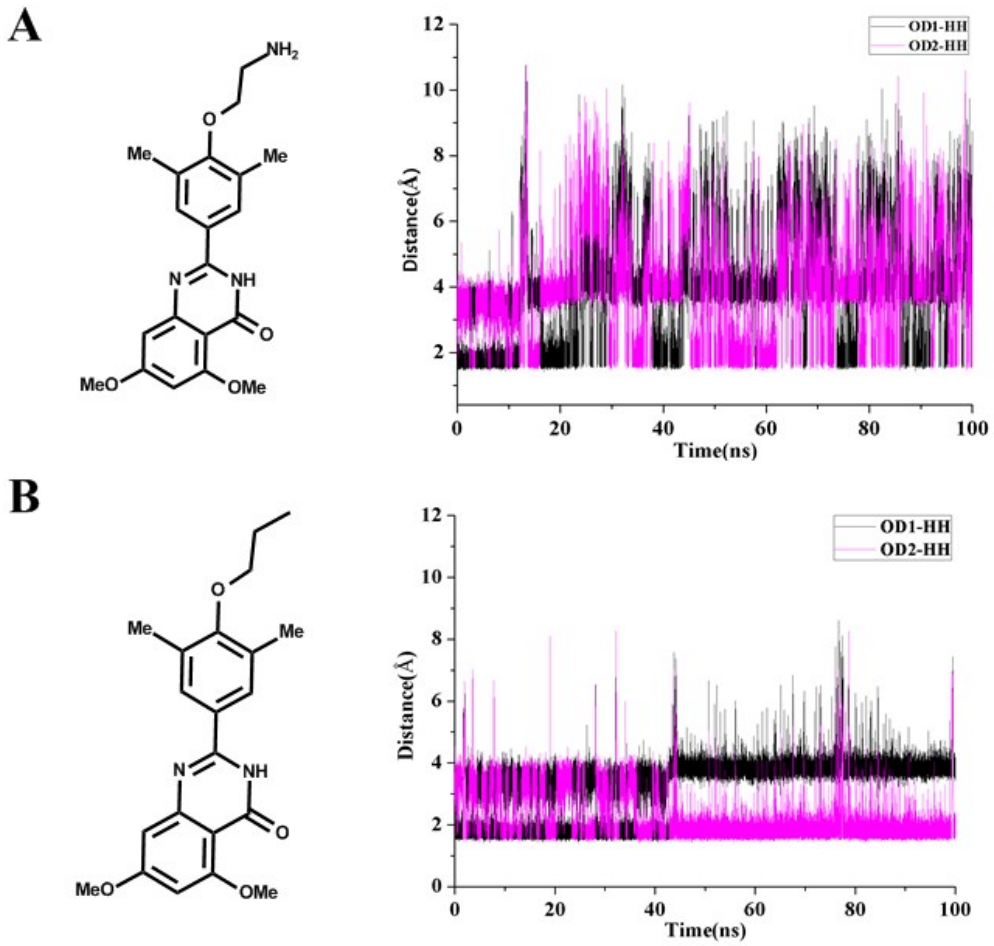

Fig. S4 The two designed small molecules and the internal H-bond distance change along the MD trajectory in BRD4-BD1.

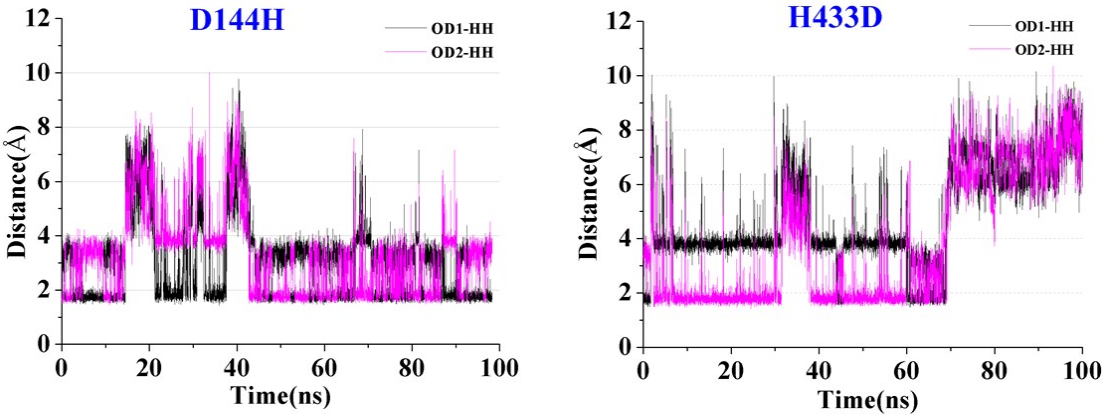

Fig. S5 The internal H-bond variation in D144H BD1 and H433D BD2 models.

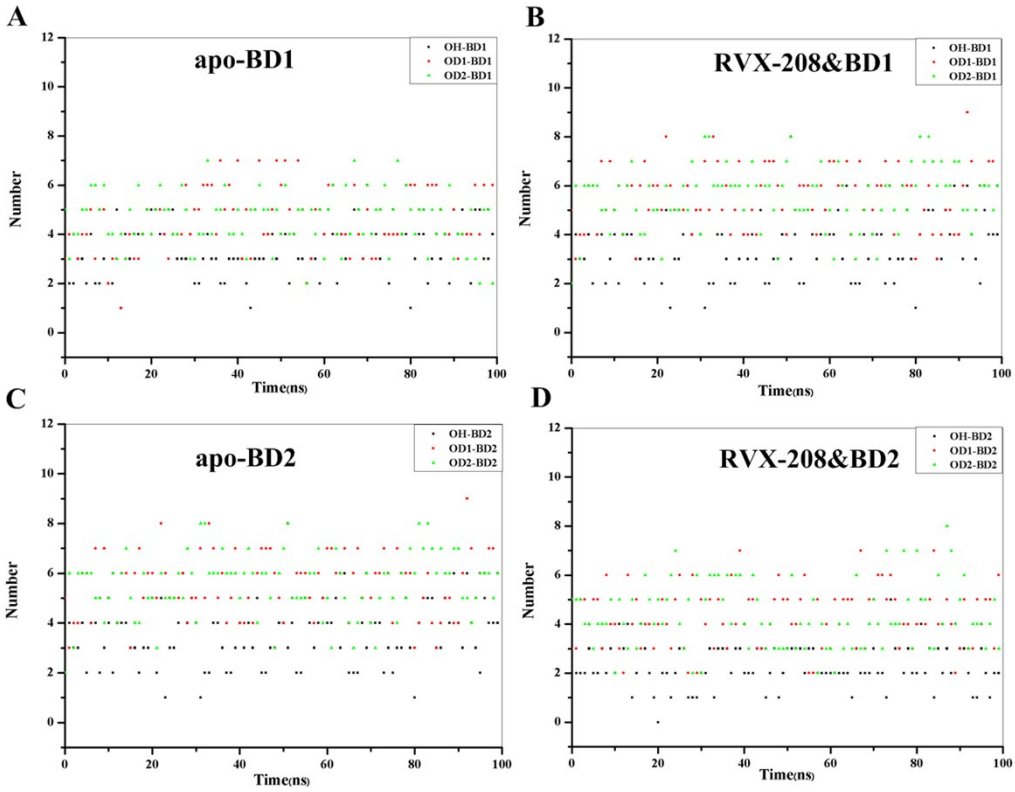

Fig. S6 The number of water molecules around 3.5 Å from the selected atoms of Asp96 and Tyr139 in apo-/holo- BD1/BD2. The OH refers to the hydroxyl oxygen of Tyr139. The OD1 and OD2 refer to the two carboxyl oxygens of Asp96, respectively. As shown in Figures, no matter in apo- or holo- BD1/BD2, there are about 4~5 water molecules around the conserved D-Y dyad (Asp96 and Tyr139) in most case. It indicates that the existence of the solvent water would not destroy the intrinsic D-Y hydrogen bond.

Table S1 Energy contribution analysis (kcal/mol) of the key residues in RVX-208 bounded BRD4-BD1/BRD2-BD2 complexes via QM/MM calculation. The energy contribution of each residue included the Van der Waals and electrostatic interaction. The residues with the notable contribution on stabilization energy were marked in bold.

Models		Key Residues							
		P82/371	L92/381	L94/383	Y97/386	C136/425	Y139/428	D144/H433	I146/V435
BD1	In	**-5.72**	**-4.93**	-1.31	-1.80	**-10.94**	**-8.00**	2.23	**-5.93**
	Out	**-4.57**	-1.42	**-2.40**	-2.19	**-7.41**	**-3.68**	0.79	**-4.00**
BD2	In	**-8.62**	-2.27	-5.62	-4.79	**-13.18**	**-5.91**	**-14.16**	**-8.05**
	Out	-3.24	-0.96	-1.77	**-4.00**	**-8.24**	**-6.51**	**-10.60**	**-4.51**

Table S2 The calculated binding free energies of RVX-208 in wild and mutant models from MMPB/GBSA calculations.

Free energy analysis (kcal/mol) for binding of compound RVX-208						
Models	$\triangle E_{ele}$	$\triangle E_{vdw}$	$\triangle$Gsol	-T$\triangle$S	$\triangle G_{binding}$(PB)	$\triangle G_{binding}$(GB)
BRD2-BD2 with RVX-208	-6.07	-41.32	9.42	-18.29	-15.4±3.32	-19.68±2.66
H433D	-9.66	-33.64	14.29	-17.35	-7.88±3.30	-11.66±2.79
BRD4-BD1 with RVX-208	-30.51	-42.68	39.35	17.94	-8.38±3.40	-15.90±2.50
D144H	3.63	-46.18	5.93	-18.21	-15.27±4.56	-18.42±4.99