Electronic Supplementary Material (ESI) for Molecular BioSystems.

Supplementary Information

Insight into the key interactions of bromodomain inhibitors based on molecular docking, interaction fingerprint, molecular dynamics and binding free energy calculation

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Supplementary information about the H-bond by waters

H-bonds mediated by crystal waters were reported to be important as well as the H-bonds with residues. Thus, it was necessary to clarify the influence of H-bonds by waters in the interaction. The superimposition of crystal structures showed that crystal waters were clustered in several regions of the active site to form an H-bond network (Figure S3). In particular, the H-bond network at the bottom of the binding cavity seemed to be the most conserved over various crystal structures. The network at least consisted of six crystal waters named as W130, W131, W132, W133, W134 and W135. The W132 was the only water that made direct H-bond with inhibitors in the crystal structures. However, the H-bond between W132 and the inhibitor was at most 43.08% occupancy among all complexes. The H-bond network was unstable as well along the simulation, and the positions of waters were ceaselessly arranged in the complexes with both high active and inactive inhibitors according to the distances between these waters and the H-bond sites on the residues (Figure S4/S5). Especially, some waters slipped away from the active site. It was indicated that the slipping path was the backdoor connected to the bottom of the active site, which was formed by Pro104, Met105 and Asp106. The instability of waters in the binding cavity weakened the importance of the water-mediated H-bond network in the interaction with inhibitors.

Supplementary tables

Table S1. Contribution of vdw energy for each residue (kcal/mol)

PDB code	Crystal ligand	Docked ligand
3P5O	C1 (I-BET762)	
4O74	C2 (BI2536)	
3MXF	C3 ((+)-JQ1)	C7
4E96	C5 (PFI-1)	
4JOS	C6	C8-C9, C11, C13-C15, C17-C20
3ZYU	C10 (I-BET151)	C4
4HXL	C12	
3SVG	C16	

Table S2. Contribution of vdw energy for each residue (kcal/mol)

Compound Number	Residue Number																		
	81	82	83	85	87	88	91	92	93	94	95	97	135	136	139	140	145	146	149
C1	-1.45	-2.34	-0.82	-0.49	-1.08	-0.26	-0.09	-1.81	-0.1	-1.2	-0.04	-0.6	-0.17	-0.88	-1.23	-1.17	-0.77	-3.69	-0.67
C2	-1.78	-2.04	-0.68	-0.95	-1.13	-0.16	-0.55	-3.47	-1.56	-1.61	-0.06	-1.64	-0.24	-0.92	-0.96	-0.62	-0.07	-2	-0.12
C3	-1.38	-2.17	-0.8	-0.29	-1.25	-0.11	-0.08	-2.23	-0.19	-1.33	-0.04	-0.47	-0.22	-0.68	-0.86	-1.14	-0.71	-3.41	-0.61
C4	-1.12	-1.89	-0.63	-0.33	-0.67	-0.08	-0.2	-2.78	-0.33	-1	-0.03	-1.78	-0.42	-0.81	-0.92	-0.35	-0.07	-1.71	-0.09
C5	-1.78	-2.04	-0.68	-0.95	-1.13	-0.16	-0.55	-3.47	-1.56	-1.61	-0.06	-1.64	-0.24	-0.92	-0.96	-0.62	-0.07	-2	-0.12
C6	-1.26	-2.09	-0.92	-0.19	-1.48	-0.14	-0.06	-1.79	-0.03	-0.56	-0.01	-1.08	-0.39	-0.89	-0.58	-0.85	-0.6	-3.05	-0.45
C7	-1.73	-2.34	-1.17	-1.26	-1.31	-0.11	-0.1	-1.88	-0.08	-1.58	-0.08	-1.03	-0.42	-1.07	-1.41	-0.84	-0.05	-1.75	-0.05
C8	-1.41	-1.56	-0.59	-0.17	-1.22	-0.11	-0.09	-1.95	-0.04	-0.64	-0.02	-0.93	-0.31	-0.71	-0.7	-0.64	-0.94	-3.15	-0.64
C9	-1.76	-2.63	-0.91	-0.72	-1.79	-0.13	-0.09	-1.67	-0.06	-0.62	-0.01	-1.21	-0.32	-0.61	-0.09	-0.01	-0.7	-2.98	-0.62
C10	-1.72	-2.3	-0.72	-0.45	-1.51	-0.1	-0.09	-2.29	-0.06	-0.57	-0.02	-0.64	-0.33	-1.01	-0.9	-1.14	-0.64	-3.37	-0.54
C11	-1.52	-2.33	-0.78	-0.17	-0.98	-0.08	-0.07	-1.89	-0.05	-0.61	-0.02	-0.95	-0.25	-0.8	-0.79	-0.78	-0.47	-2.45	-0.47
C12	-2.3	-0.88	-0.14	-0.35	-0.26	-0.03	-2.89	-0.1	-0.77	-0.02	-0.03	-0.04	-0.8	-0.05	0.15	-0.02	-1.5	-0.06	-0.04

C13	-1.07	-1.86	-0.79	-0.85	-1.35	-0.06	-0.03	-1.53	-0.04	-0.53	-0.02	-0.09	-0.28	-0.94	-0.87	-0.79	-0.27	-2.38	-0.35
C14	-1.57	-2.12	-0.76	-0.42	-0.7	-0.07	-0.5	-2	-0.1	-0.41	-0.02	-1.19	-0.22	-0.77	-0.68	-0.59	-0.28	-2	-0.46
C15	-1.33	-2.18	-1.29	-0.32	-0.92	-0.05	-0.02	-0.45	-0.07	-1.12	-0.02	-1.69	-0.95	-1.38	-1.31	-0.13	-1.03	-2.99	-0.79
C16	-1.11	-1.85	-0.69	-0.39	-1.47	-0.13	-0.08	-1.74	-0.04	-0.53	-0.01	-0.65	-0.21	-0.7	-0.65	-1.06	-0.23	-1.82	-0.12
C17	-1.47	-2.34	-0.88	-0.32	-1.27	-0.1	-0.08	-1.76	-0.04	-0.51	-0.01	-0.66	-0.19	-0.83	-0.65	-0.4	-0.65	-3.03	-0.63
C18	-0.49	-2.58	-0.77	-0.89	-1.83	-0.16	-0.05	-1.32	-0.19	-1.12	-0.03	-1.33	-0.3	-0.85	-0.83	-0.4	-0.05	-1.66	-0.09
C19	-0.66	-1.26	-0.61	-0.14	-1.09	-0.08	-0.05	-1.54	-0.04	-0.47	-0.01	-0.66	-0.27	-0.86	-0.67	-0.78	-0.14	-1.97	-0.19
C20	-1.22	-2	-0.76	-0.79	-1.34	-0.08	-0.31	-1.48	-0.04	-0.48	-0.01	-0.77	-0.36	-0.97	-0.74	-0.82	-0.05	-1.41	-0.07

Table S3. Contribution of electrostatic energy for each residue (kcal/mol)

Compound Number	Residue Number																		
	81	82	83	85	87	88	91	92	93	94	95	97	135	136	139	140	145	146	149
C1	-0.58	-0.77	-0.22	0.03	0	-1.52	1.13	0.55	0.05	-0.05	-0.02	-0.82	0.16	-0.22	-0.65	-3.51	1.15	-0.31	-0.28
C2	-0.13	-0.15	0	-0.52	0.09	-0.22	0.15	-1.54	-0.56	0.05	0.04	0.03	0.29	-0.9	-0.43	-2.77	-0.03	0.18	-0.02
C3	-0.45	-0.13	-0.2	0.17	-0.05	-0.94	1.12	-0.15	0.03	-0.02	-0.01	-0.46	0.63	0.59	-0.27	-1.08	1.06	-0.39	-0.18
C4	0.27	-0.24	0.06	-0.2	0.01	0.16	-0.24	-0.48	0.05	-0.06	-0.02	-0.12	0.68	0.23	-0.49	-0.52	-1.26	0.18	0.05
C5	-0.13	-0.15	0	-0.52	0.09	-0.22	0.15	-1.54	-0.56	0.05	0.04	0.03	0.29	-0.9	-0.43	-2.77	-0.03	0.18	-0.02
C6	-0.01	-0.22	-0.15	-0.08	-0.08	-0.05	0.09	-0.07	-0.02	0.04	0.01	-0.44	0.31	0.02	-0.15	-0.58	0.11	-0.07	0
C7	-0.41	-0.39	-0.8	-3.49	0.44	-0.61	-0.06	0.05	-0.02	0.08	0.09	-0.23	0.51	-0.25	-0.66	-2.94	-0.34	0.09	0.04
C8	-0.11	-0.47	-0.03	0.05	-0.03	0.36	-0.53	0.03	-0.01	-0.03	-0.03	-0.15	0.69	0.17	-0.37	-1.48	-1.84	0.03	-0.04
C9	-0.12	-0.46	-0.05	-0.09	-0.06	-0.28	0.39	-0.23	-0.1	0.07	0.05	-0.68	-0.25	-0.09	-0.04	0.02	-0.99	-0.21	-0.15
C10	-0.22	0.72	0.03	-0.27	-0.08	0.19	0.02	-0.11	-0.07	0.06	0.04	-0.69	0.23	-0.22	-0.48	-1.61	-2.31	0.25	-0.21
C11	-0.37	-0.47	-0.12	-0.09	-0.08	0.16	-0.01	0.2	0.06	-0.05	-0.03	0.06	0.32	0.14	-0.38	-1.66	-1.55	0.2	-0.06
C12	-2.03	-0.23	0.03	-0.57	-0.73	0.1	0.6	0.11	-0.11	-0.05	1.62	0.01	-0.48	0.18	-2.79	-0.74	0.08	0.06	-0.13

C13	-0.2	-0.47	-0.16	-0.37	-0.05	0.06	0.11	0.14	0	0	-0.03	0.02	0.36	0.06	-0.42	-1.9	-1.14	0.26	-0.02
C14	-0.16	-0.58	-0.15	-0.27	-0.15	0.6	0.54	0.22	-0.01	-0.04	-0.03	-0.02	0.46	0.39	-0.39	-1.62	-1.04	-0.14	-0.11
C15	-0.56	-0.4	-0.2	-0.28	-0.06	-0.35	0.33	0.14	0.11	-0.08	-0.03	0.27	-1.66	-0.49	0.03	0.09	-0.81	-0.13	-0.18
C16	-0.09	-0.13	-0.06	0.04	-0.07	-0.2	0.4	-0.07	-0.03	0.05	0.04	0.19	-0.02	-0.06	-0.33	-1.93	-1.54	0.42	-0.03
C17	-0.44	-0.52	-0.18	0.09	-0.11	0.1	-0.14	0.16	0.02	-0.03	-0.02	-0.55	-0.05	-0.84	-0.29	-0.26	-5	0.06	-0.12
C18	-0.21	-0.78	-0.09	-0.97	-0.01	-1.16	0.45	-0.23	-0.17	0.02	0.06	-0.17	0.53	0.2	-0.6	-2.18	0.78	-0.05	-0.04
C19	-0.07	-0.43	-0.12	0.1	-0.07	-0.13	0.27	-0.07	0	0.03	0.02	-0.58	0.3	-0.08	-0.43	-1.96	-0.37	0.11	-0.02
C20	0.47	-1.87	-0.26	-0.95	0.11	0.35	-4.86	0.07	-0.08	0.09	0.06	0.04	0.19	-0.29	-0.28	-1.55	-0.59	0.23	0.06

Table S4. Contribution of solvation free energy for each residue (kcal/mol)

Compound Number	Residue Number																		
	81	82	83	85	87	88	91	92	93	94	95	97	135	136	139	140	145	146	149
C1	0.64	1.24	0.11	0.61	-0.06	1.75	-0.92	-0.67	0.08	-0.12	0.03	0.88	-0.16	0.14	0.82	2.87	-0.88	0.1	0.35
C2	0.1	0.75	0.07	1.05	-0.12	0.21	0.51	2	0.67	-0.17	0.01	0.15	-0.28	0.57	0.66	1.88	0.19	-0.17	0.09
C3	0.57	0.6	0.04	0.22	-0.06	0.96	-0.9	0.24	0.16	-0.11	0.02	0.52	-0.43	-0.71	0.37	0.97	-0.86	0.3	0.3
C4	-0.26	0.4	0.05	0.38	-0.1	-0.12	0.52	0.5	0.07	-0.09	0.04	0.22	-0.46	-0.23	0.53	0.44	1.37	-0.42	-0.02
C5	0.1	0.75	0.07	1.05	-0.12	0.21	0.51	2	0.67	-0.17	0.01	0.15	-0.28	0.57	0.66	1.88	0.19	-0.17	0.09
C6	0.28	0.96	0.1	0.24	-0.06	0.12	0.01	-0.1	0.08	-0.06	0	0.89	-0.05	-0.1	0.44	1.39	0.45	-0.15	0.13
C7	0.47	1.07	0.46	3.08	-0.25	0.57	0.29	-0.29	0.15	-0.26	-0.08	0.09	-0.35	0.08	0.78	2.09	0.44	-0.18	0
C8	0.29	0.69	0.09	0.23	-0.1	-0.31	0.72	-0.26	0.08	-0.04	0.04	0.18	-0.5	-0.25	0.4	0.76	3.16	-0.01	0.18
C9	0.27	0.78	0.14	0.65	-0.14	0.28	-0.21	-0.01	0.19	-0.13	-0.04	0.9	0.17	0.36	0.1	0	1.68	-0.17	0.27
C10	0.22	-0.21	0	0.5	-0.09	-0.18	0.18	-0.19	0.15	-0.09	-0.03	0.78	-0.14	0.05	0.67	1.79	3.09	-0.36	0.27
C11	0.54	0.99	0.14	0.37	-0.03	-0.11	0.18	-0.45	0.02	-0.03	0.04	0.02	-0.33	-0.2	0.52	1.27	2.53	-0.32	0.16
C12	2.04	0.29	0.25	0.75	0.75	-0.07	-1.03	-0.02	-0.04	0.07	-1.49	0.03	0.27	-0.04	0.98	0.77	-0.25	-0.04	0.12

C13	0.32	0.72	0.08	1.27	-0.17	-0.06	-0.01	-0.33	0.06	-0.02	0.04	0	-0.29	-0.14	0.54	1.52	1.4	-0.24	0.11
C14	0.34	0.82	0.12	0.81	0.07	-0.58	0.23	-0.35	0.13	0.01	0.04	0.11	-0.41	-0.41	0.49	0.96	1.43	-0.07	0.18
C15	0.71	0.84	0.16	0.62	0	0.36	-0.26	-0.09	-0.06	-0.11	0.05	0.09	0.88	0.76	0.21	-0.01	3.72	-0.08	0.33
C16	0.12	0.3	0.05	0.38	-0.18	0.25	-0.23	-0.25	0.1	-0.09	-0.03	-0.04	0.02	0	0.51	2.02	2.02	-0.52	0.11
C17	0.61	1	0.17	0.3	-0.08	-0.02	0.32	-0.46	0.04	-0.03	0.03	0.67	-0.03	0.66	0.38	0.41	6.6	-0.15	0.26
C18	0.13	1.53	0.21	1.66	-0.16	1.2	-0.33	0.49	0.34	-0.17	-0.03	0.2	-0.38	-0.26	0.63	1.02	-0.61	-0.14	0.09
C19	0.08	0.6	0.1	0.1	-0.13	0.19	-0.17	-0.17	0.06	-0.07	-0.02	0.69	-0.25	-0.06	0.54	1.58	0.5	-0.21	0.08
C20	-0.43	1.95	0.2	0.55	-0.15	-0.27	4.9	-0.31	0.13	-0.11	-0.06	0.05	-0.23	0.24	0.45	1.63	0.69	-0.34	-0.03

Supplementary figures

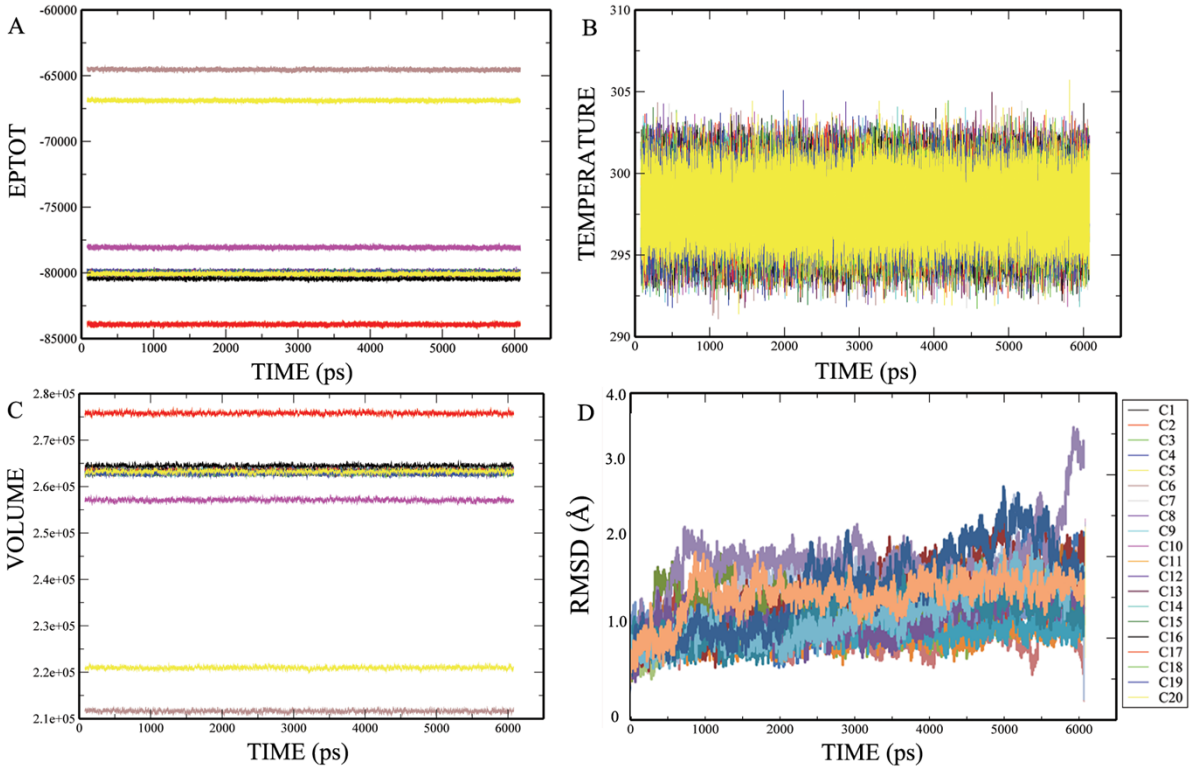

Figure S1. A/B/C/D) Curves representing the fluctuation of potential energy, temperature, volume and RMSD of Cα atoms in amino acids across the molecular dynamic simulation, respectively. The x-axis represents the simulation time. The y-axis represents the potential energy in figure A, temperature in figure B, volume in figure C and the RMSD values of Cα atoms in figure D, respectively. The curves are colored according to the inhibitors.

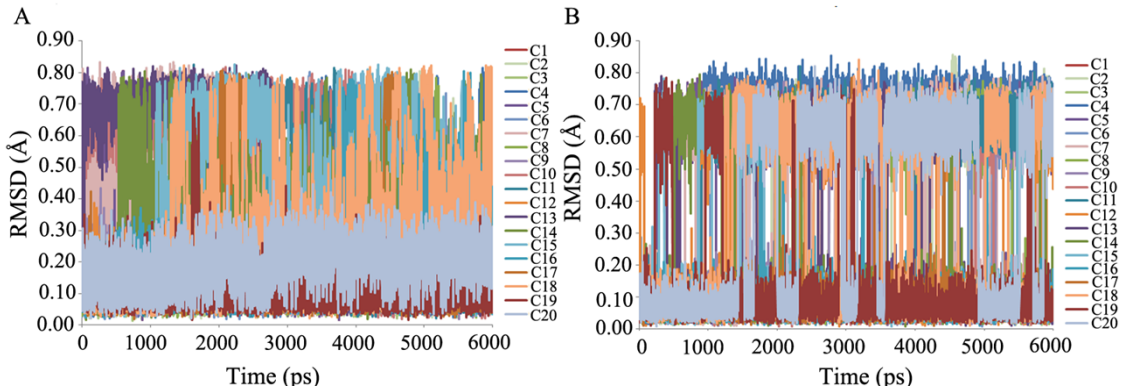

Figure S2. A/B) Curves representing the RMSD values of Gln85 and Ile146 over the whole simulation time, respectively. The x-axis is the simulation time, while the y-axis is the RMSD values. The curves are colored according to the inhibitors.

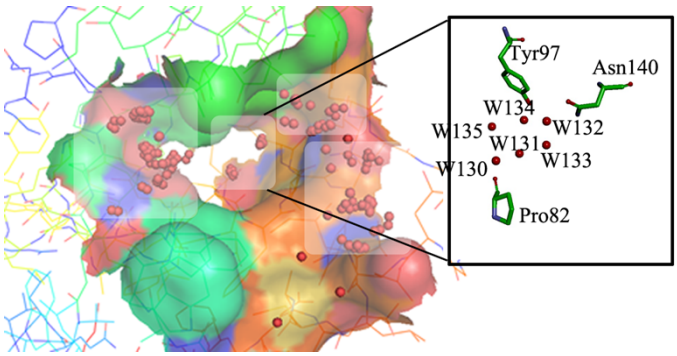

Figure S3. Distribution of crystal waters in the bromodomain by aligning crystal structures. Amino acids are represented using line model. The surface is produced for the residues in the active site of the bromodomain and colored by the types of residues. Waters are shown using ball model and colored red. The water clusters are highlighted using square frames. On the right side, waters (W130-135) are shown in a large scale. The surrounding residues are shown as green stick.

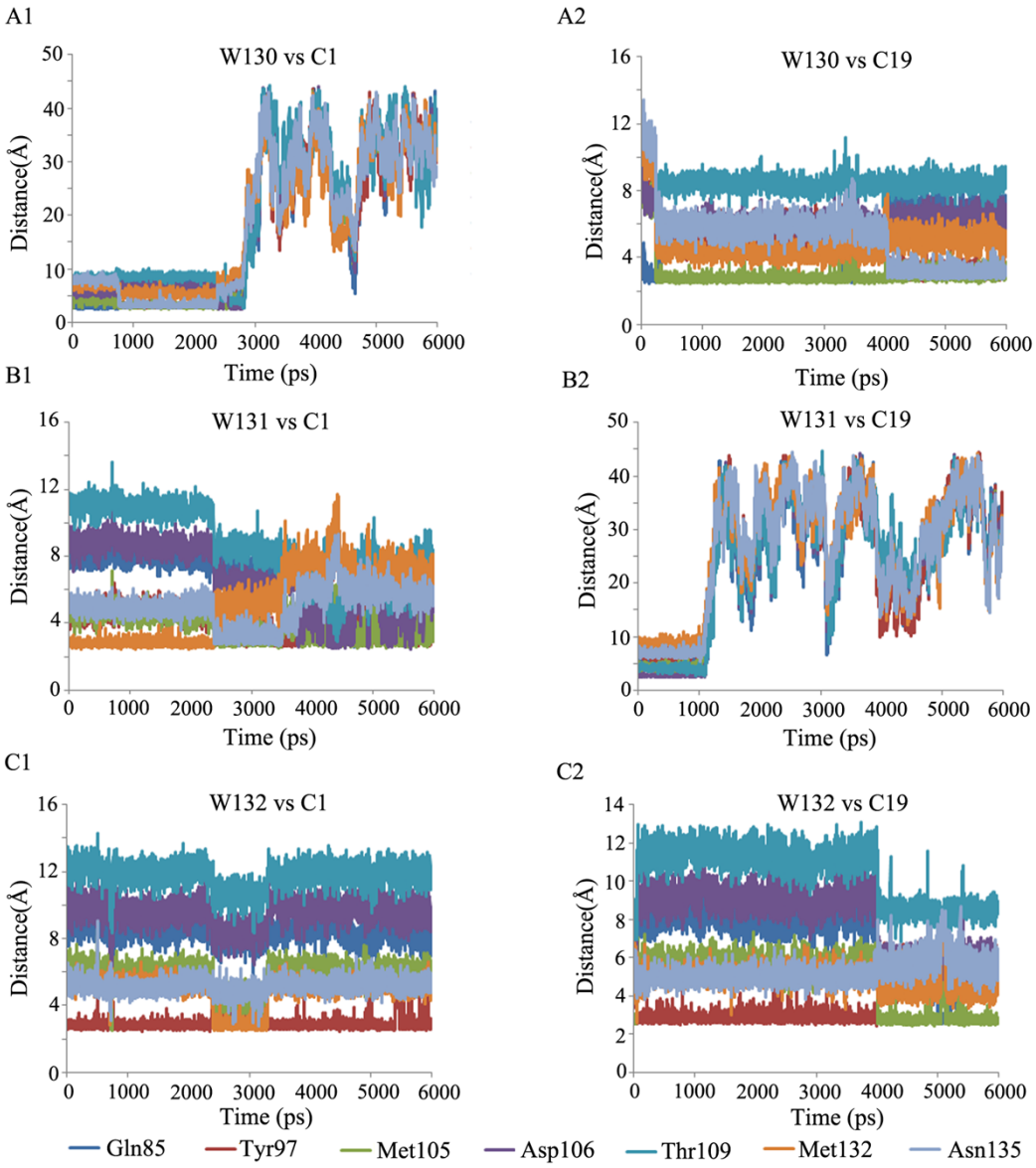

Figure S4. A1/A2) Curves representing the distance variation between the oxygen atoms of W130 and the atoms of the potential residues that can make H-bond with this water in the complexes of C1 (IBET762) and C19, respectively. The x-axis is the simulation time, while the y-axis is the distance values. B1/B2) Curves representing the distance between the oxygen atoms of W131 and the atoms of the potential residues that can make H-bond with this water in the complexes of C1 (IBET762) and C19, respectively. C1/C2) Curves representing the distance between the oxygen atoms of W132 and the atoms of the potential residues that can make H-bond with this water in the complexes of C1 (IBET762) and C19, respectively. The residues are Gln85, Tyr97, Met105, Asp106, Thr109, Met132 and Asn135. All curves are colored by the residues.

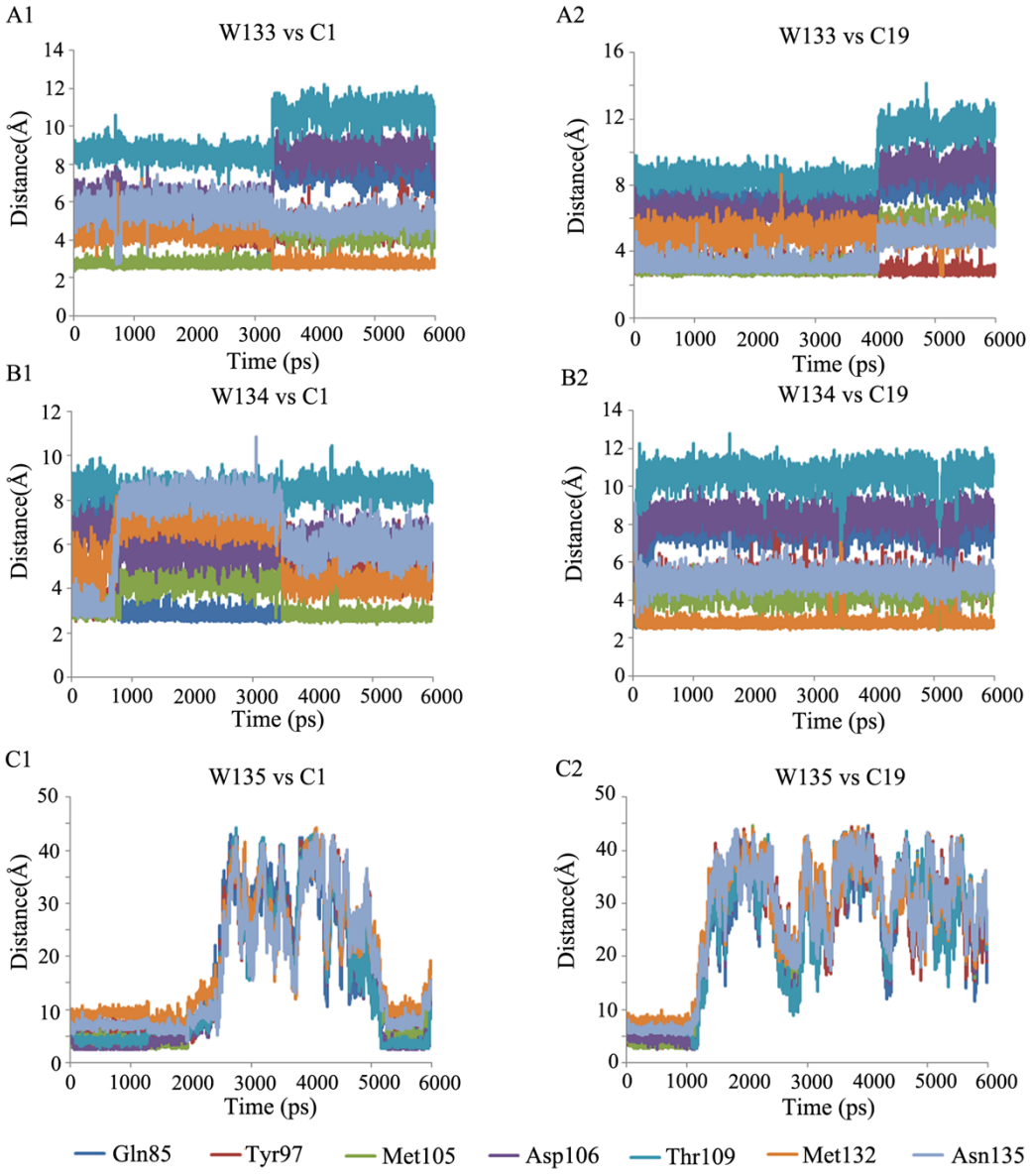

Figure S5. A1/A2) Curves representing the distance variation between the oxygen atoms of W133 and the atoms of the potential residues that can make H-bond with this water in the complexes of C1 (IBET762) and C19, respectively. The x-axis is the simulation time, while the y-axis is the distance values. B1/B2) Curves representing the distance between the oxygen atoms of W134 and the atoms of the potential residues that can make H-bond with this water in the complexes of C1 (IBET762) and C19, respectively. C1/C2) Curves representing the distance between the oxygen atoms of W135 and the atoms of the potential residues that can make H-bond with this water in the complexes of C1 (IBET762) and C19, respectively. The residues are Gln85, Tyr97, Met105, Asp106, Thr109, Met132 and Asn135. All curves are colored by the residues.