

Supporting Information

Alchemical Approach Performance in Calculating the Ligand-Binding Free Energy

Son Tung Ngo,^{ab*} Quynh Mai Thai,^{ab} Trung Hai Nguyen,^{ab} Nguyen Ngoc Tuan,^c T. Ngoc Han
Pham,^d Huong T. T. Phung,^d and Duong Tuan Quang^{e*}

^a*Laboratory of Biophysics, Institute for Advanced Study in Technology, Ton Duc Thang University, Ho Chi Minh City, Vietnam*

^b*Faculty of Pharmacy, Ton Duc Thang University, Ho Chi Minh City, Vietnam*

^c*Faculty of Applied Sciences, Ton Duc Thang University, Ho Chi Minh City, Vietnam*

^d*NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam*

^e*Department of Chemistry, Hue University, Thua Thien Hue Province, Hue City, Vietnam*

*Email: ngosontung@tdtu.edu.vn and dtquang@hueuni.edu.vn

Sequence comparison between *Homo sapiens* and *Rattus norvegicus* of Glutamate Receptor, Ionotropic Kainate 1

Pairwise Alignment

Sequence 1: 2ZNS (*Homo sapiens*)

Sequence 2: 4DLD (*Rattus norvegicus*)

Sequence ends allowed to slide over each other

Alignment score: 248

Identities: 0.9766537

Similarities: 0.9766537

Similarity Matrix: MATCH

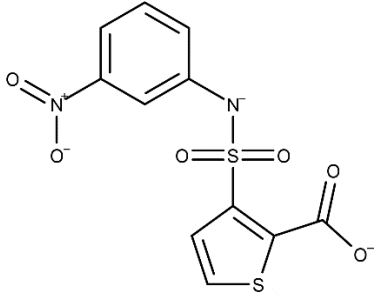
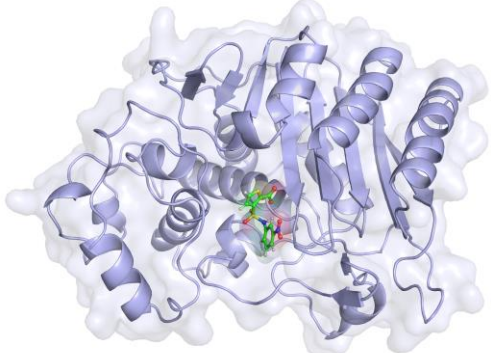
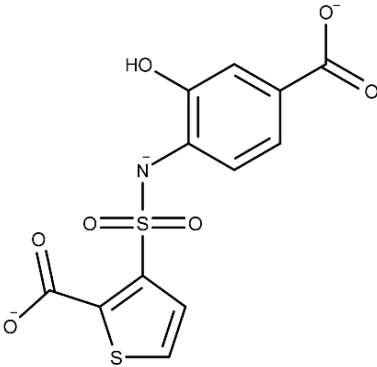
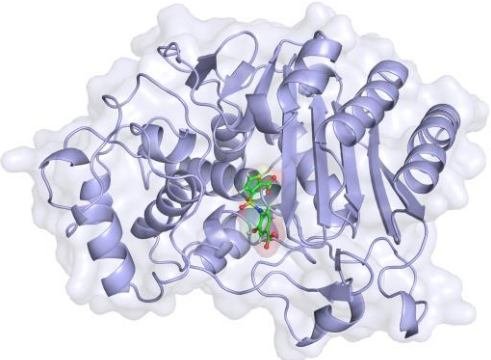
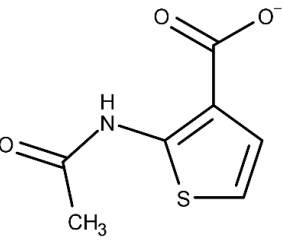
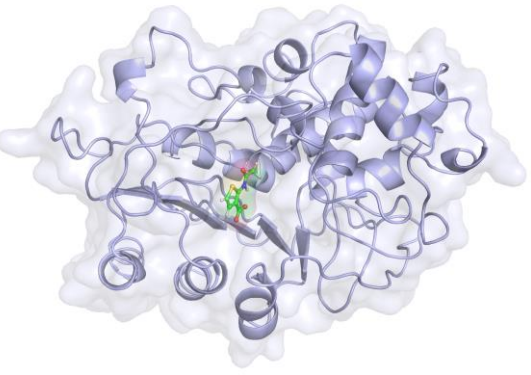
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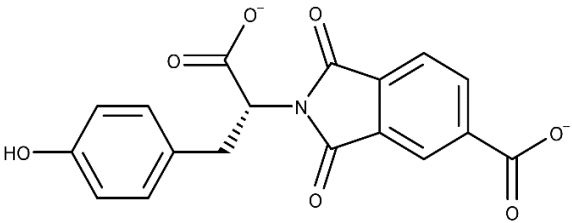
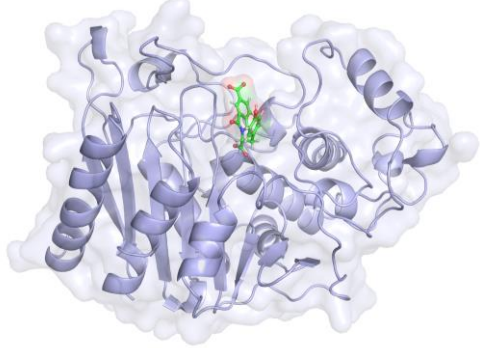
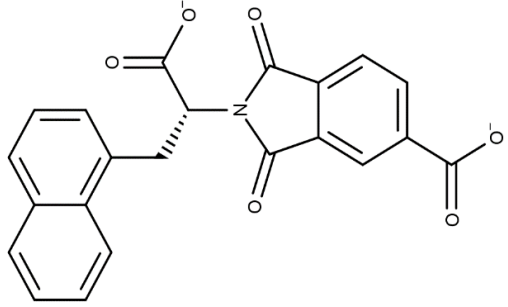
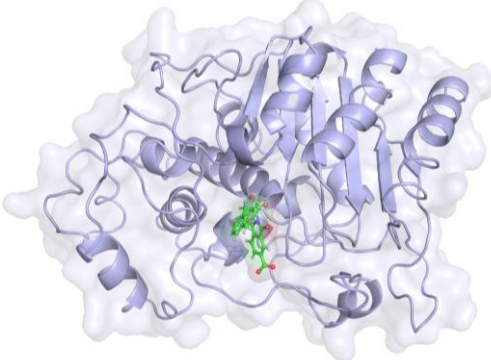
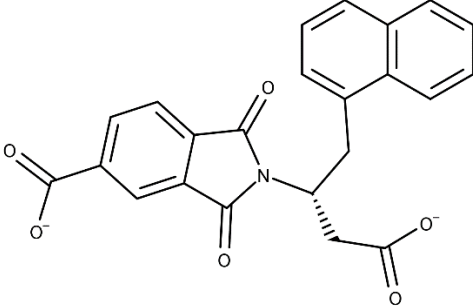
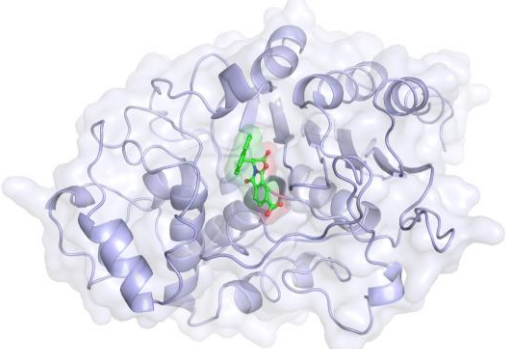
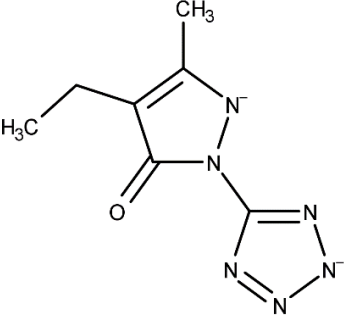
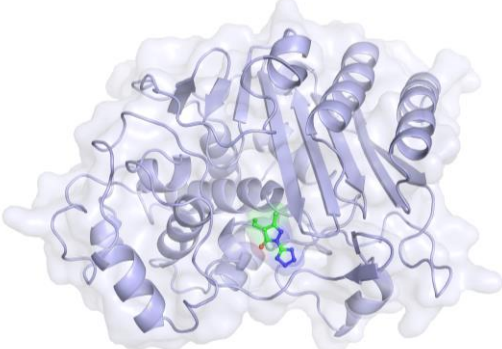
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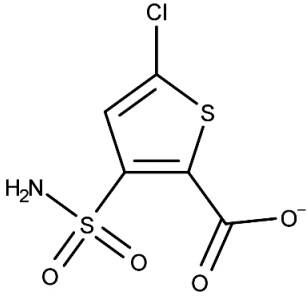
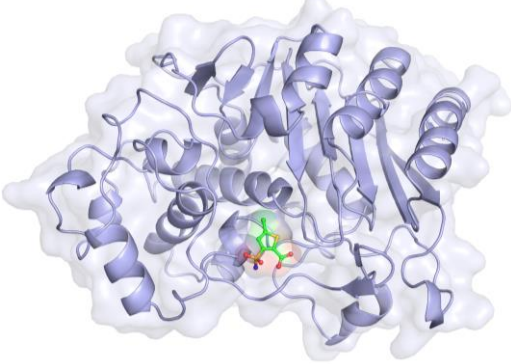
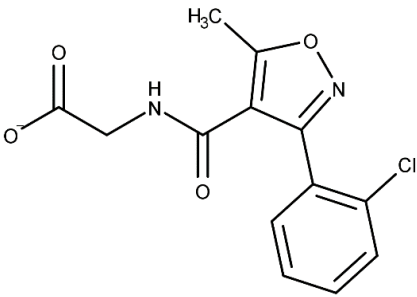
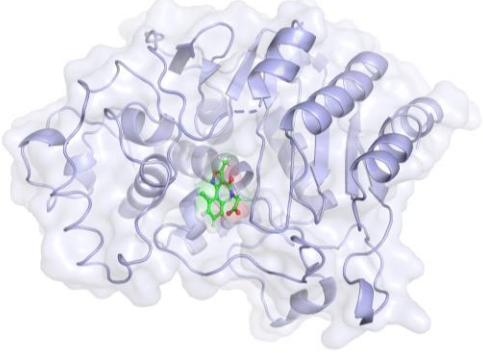
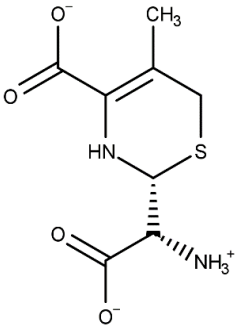
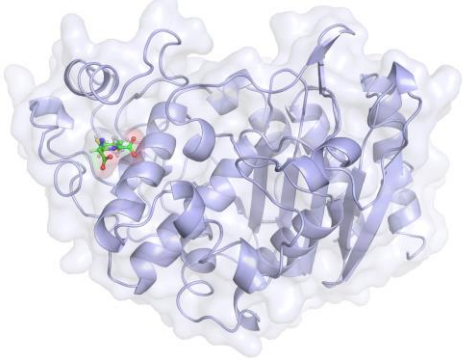
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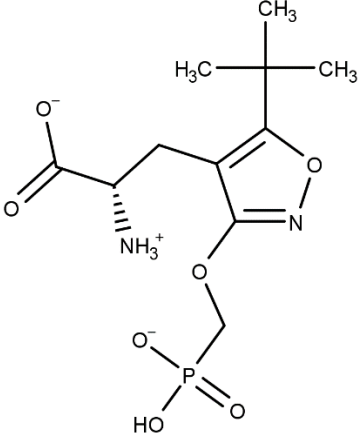
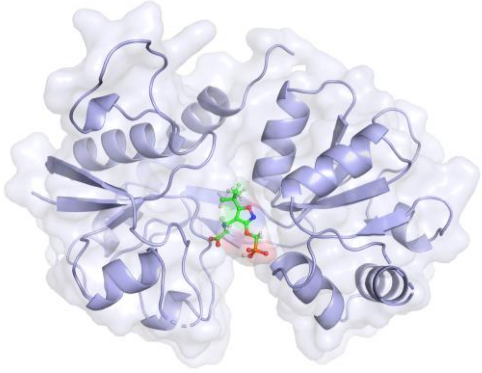
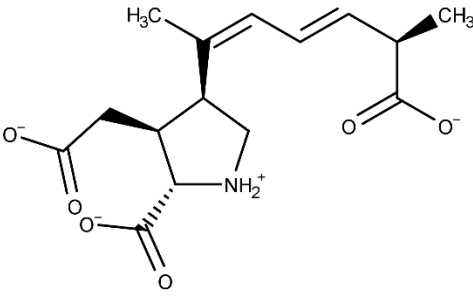
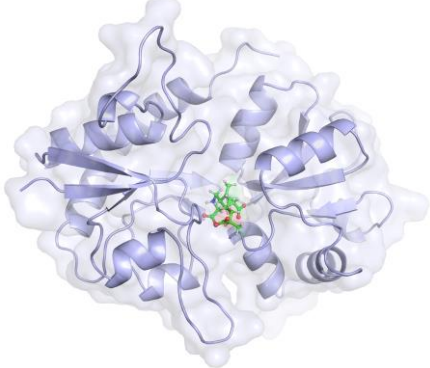
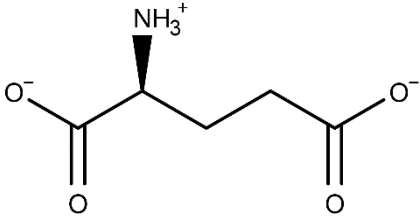
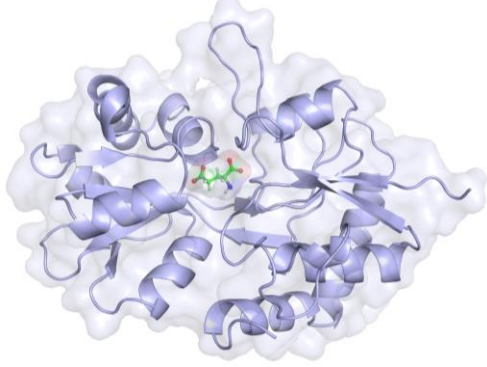
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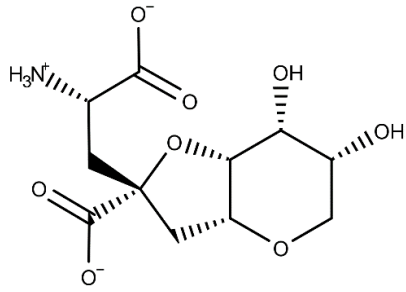
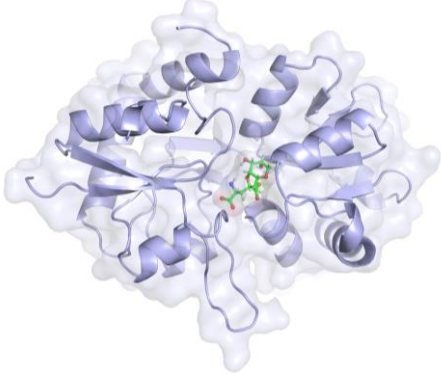
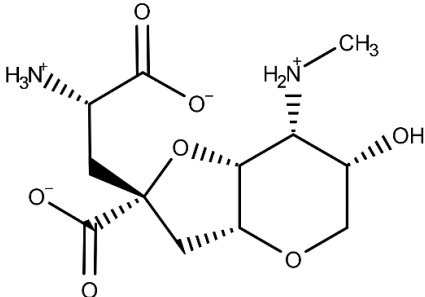
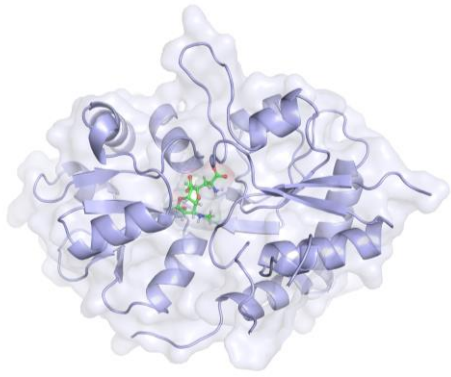
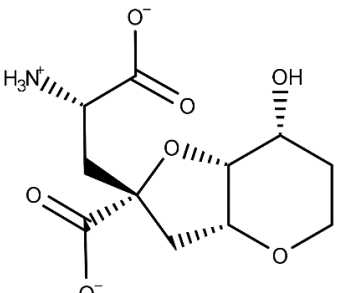
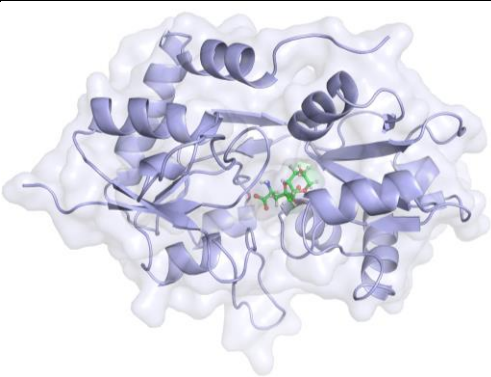
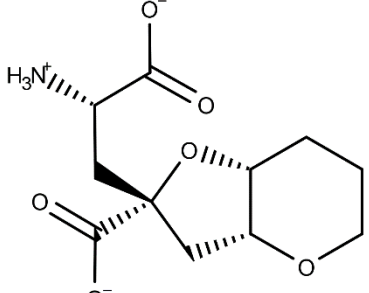
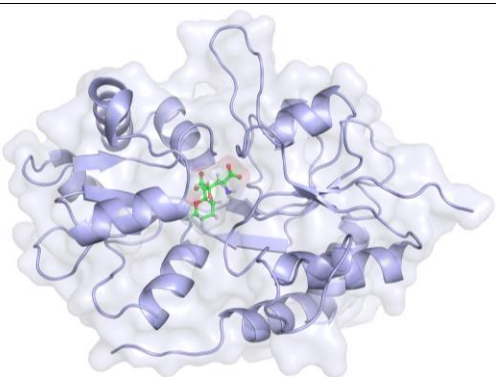
Table S1. 2D and 3D structure for all ligands of AmpC, GluK1, Hsp90 and SARS-CoV-2 Mpro systems

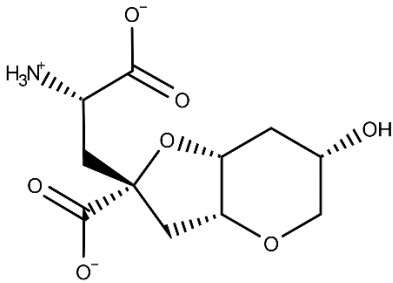
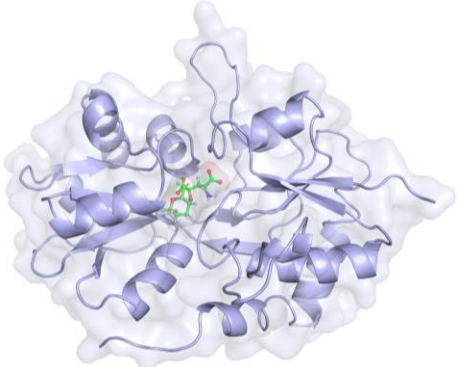
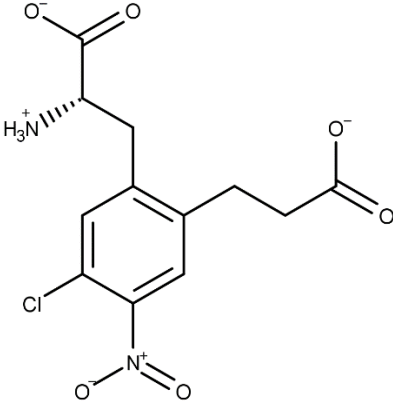
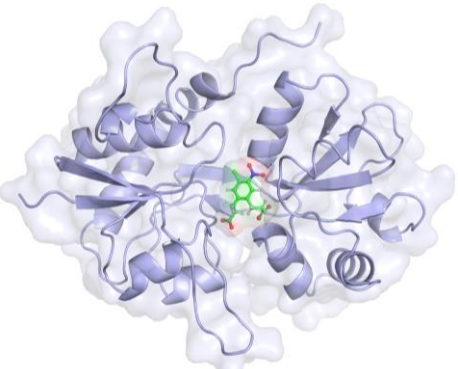
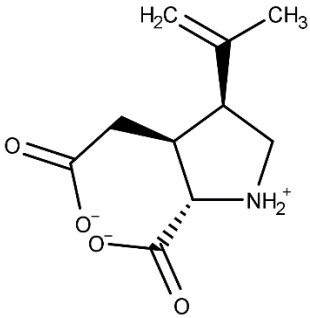
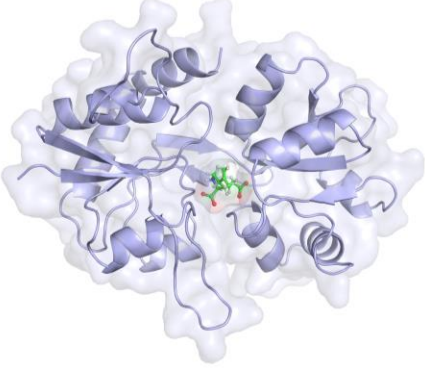
N ^o	PDB ID	2D Structure	3D Structure
1	1XGI	 <p>The 2D chemical structure of ligand 1XGI consists of a central sulfonamide group (-SO₂-NH-) connecting a 4-nitrophenyl ring and a 2-carboxylthiophen-5-yl ring.</p>	 <p>The 3D structure shows the ligand 1XGI (stick representation) bound within the active site of a protein, which is shown as a blue ribbon structure with a white surface representation.</p>
2	1XGJ	 <p>The 2D chemical structure of ligand 1XGJ features a central sulfonamide group (-SO₂-NH-) connecting a 4-hydroxy-3-carboxylphenyl ring and a 2-carboxylthiophen-5-yl ring.</p>	 <p>The 3D structure shows the ligand 1XGJ (stick representation) bound within the active site of a protein, which is shown as a blue ribbon structure with a white surface representation.</p>
3	2HDU	 <p>The 2D chemical structure of ligand 2HDU consists of a central thiophene ring substituted with a methylacetamido group (-NH-CO-CH₃) and a carboxylate group (-COO⁻).</p>	 <p>The 3D structure shows the ligand 2HDU (stick representation) bound within the active site of a protein, which is shown as a blue ribbon structure with a white surface representation.</p>

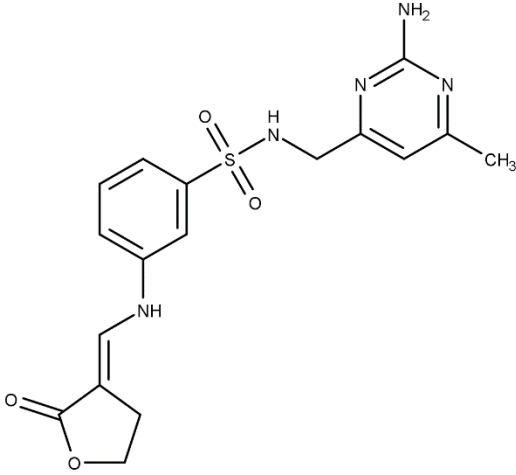
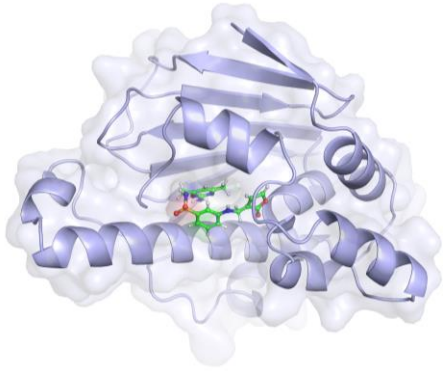
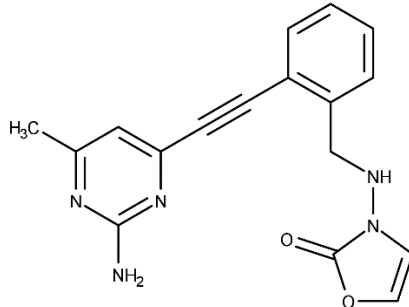
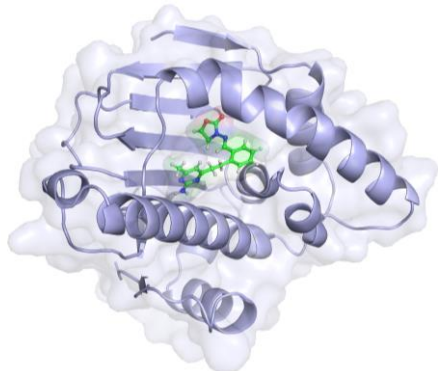
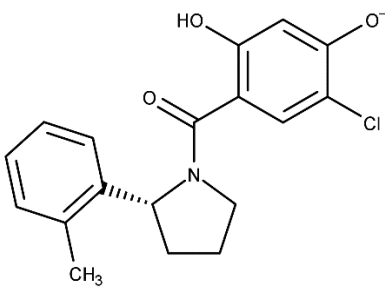
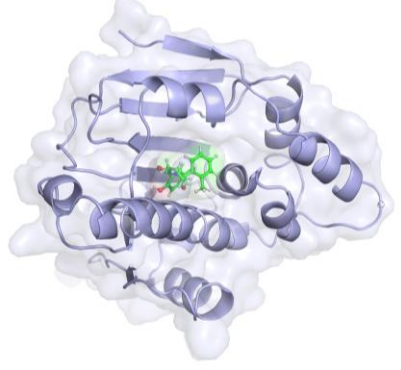
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6	2R9X		
7	3GR2		

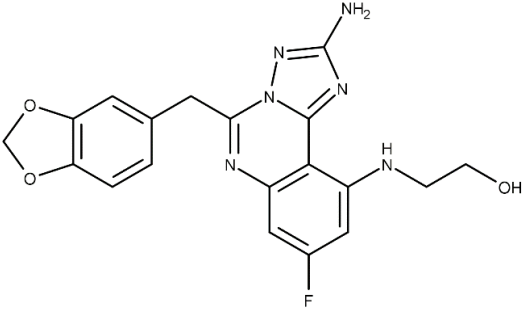
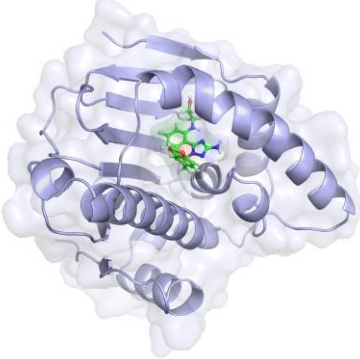
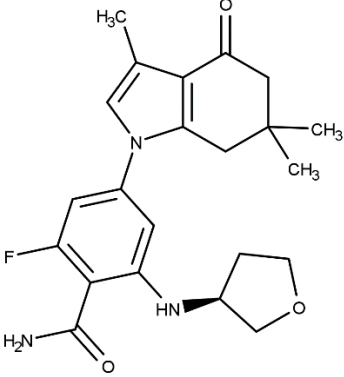
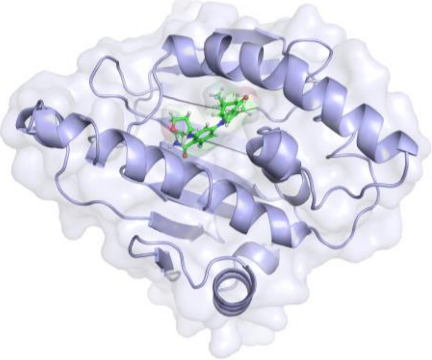
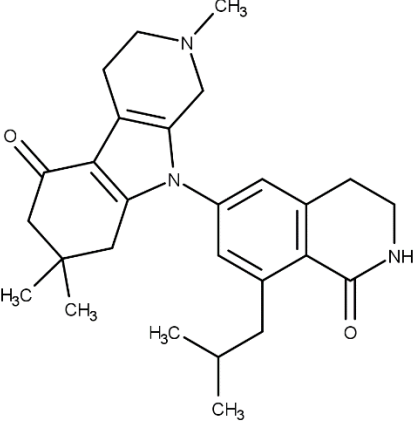
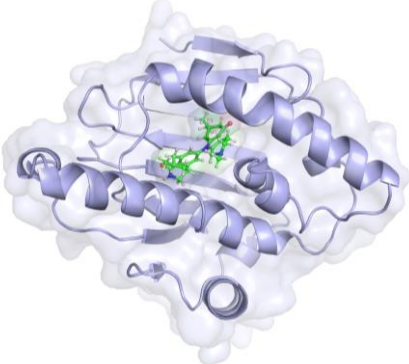
8	4KZ3	 <p>Chemical structure of 5-chloro-L-methionine, a sulfur-containing amino acid. It features a thiazolidine ring with a chlorine atom at the 5-position, a methyl group at the 2-position, and a carboxylate group at the 4-position.</p>	 <p>3D ribbon diagram of a protein structure (PDB ID: 4KZ3) showing a ligand (5-chloro-L-methionine) bound in the active site. The protein is shown in blue ribbon, and the ligand is shown in green sticks.</p>
9	4KZ5	 <p>Chemical structure of a substituted benzimidazole derivative. It features a benzimidazole ring system with a methyl group at the 2-position, a chlorine atom at the 6-position, and a carboxamide group at the 4-position.</p>	 <p>3D ribbon diagram of a protein structure (PDB ID: 4KZ5) showing a ligand (substituted benzimidazole) bound in the active site. The protein is shown in blue ribbon, and the ligand is shown in green sticks.</p>
10	4OKP	 <p>Chemical structure of a substituted thiazolidine derivative. It features a thiazolidine ring system with a methyl group at the 4-position, a carboxylate group at the 2-position, and a protonated amine group at the 3-position.</p>	 <p>3D ribbon diagram of a protein structure (PDB ID: 4OKP) showing a ligand (substituted thiazolidine) bound in the active site. The protein is shown in blue ribbon, and the ligand is shown in green sticks.</p>

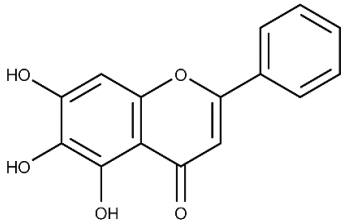
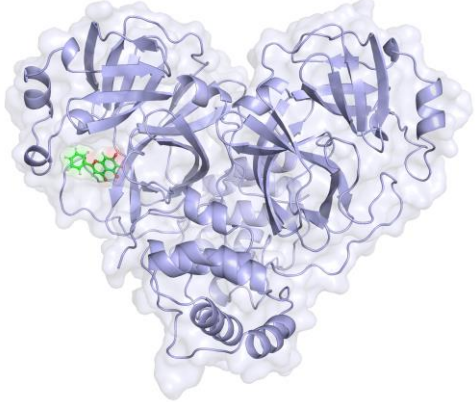
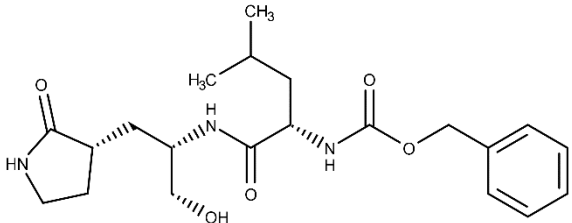
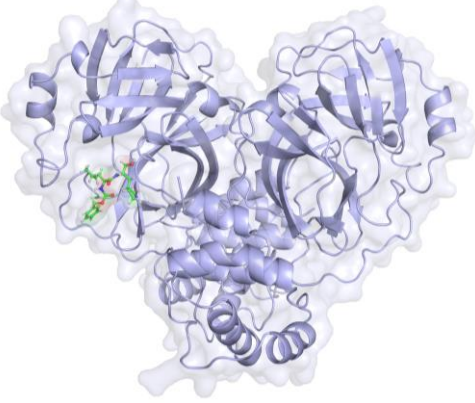
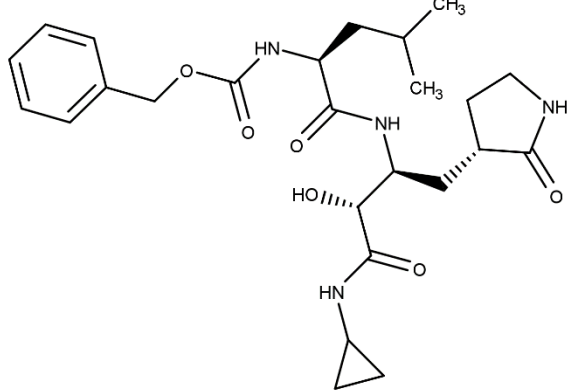
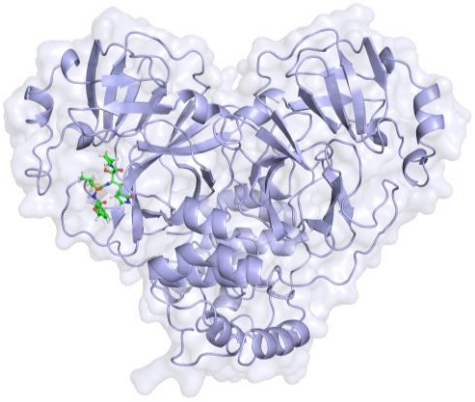
11	1VSO	 <p>The chemical structure of 1VSO is a complex molecule. It features a central five-membered ring containing an oxygen atom and a nitrogen atom. Attached to this ring are a methyl group (CH₃), a tert-butyl group (C(CH₃)₃), a carboxylate group (COO⁻), and a phosphate group (PO₃⁻). A side chain containing an ammonium group (NH₃⁺) is also present.</p>	 <p>A 3D ribbon diagram of the protein structure 1VSO, shown in blue. The protein is surrounded by a white surface representation. The ligand 1VSO is shown as a stick model in the center, with atoms colored by element: carbon (green), oxygen (red), nitrogen (blue), and phosphorus (orange).</p>
12	2PBW	 <p>The chemical structure of 2PBW is a complex molecule. It features a central five-membered ring containing an oxygen atom and a nitrogen atom. Attached to this ring are a methyl group (H₃C), a carboxylate group (COO⁻), and a side chain containing a methyl group (CH₃) and a carboxylate group (COO⁻). The side chain also includes a double bond and a methyl group (CH₃).</p>	 <p>A 3D ribbon diagram of the protein structure 2PBW, shown in blue. The protein is surrounded by a white surface representation. The ligand 2PBW is shown as a stick model in the center, with atoms colored by element: carbon (green), oxygen (red), and nitrogen (blue).</p>
13	2ZNS	 <p>The chemical structure of 2ZNS is a simple molecule. It features a central five-membered ring containing an oxygen atom and a nitrogen atom. Attached to this ring are a methyl group (CH₃), a carboxylate group (COO⁻), and a side chain containing a methyl group (CH₃) and a carboxylate group (COO⁻). The side chain also includes a double bond and a methyl group (CH₃).</p>	 <p>A 3D ribbon diagram of the protein structure 2ZNS, shown in blue. The protein is surrounded by a white surface representation. The ligand 2ZNS is shown as a stick model in the center, with atoms colored by element: carbon (green), oxygen (red), and nitrogen (blue).</p>

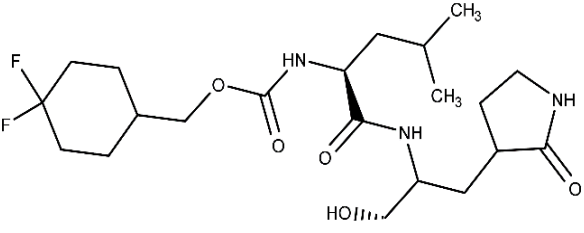
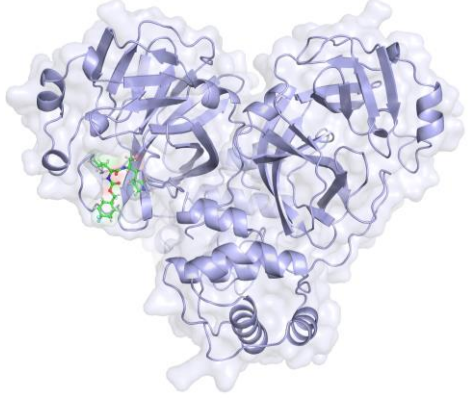
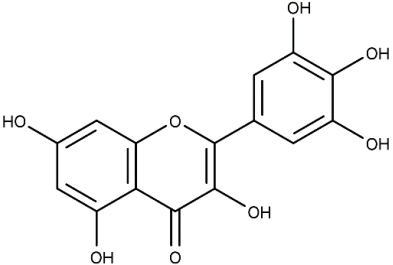
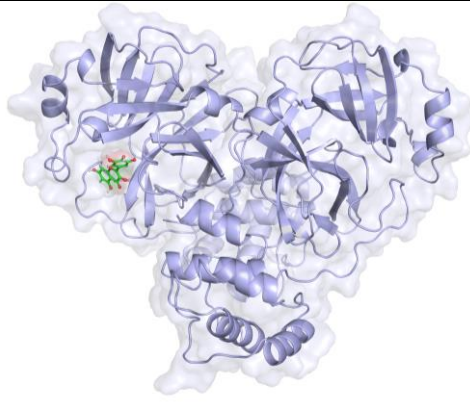
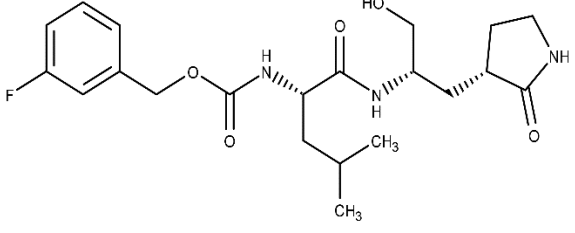
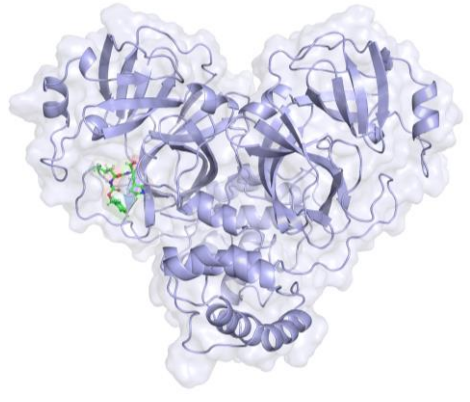
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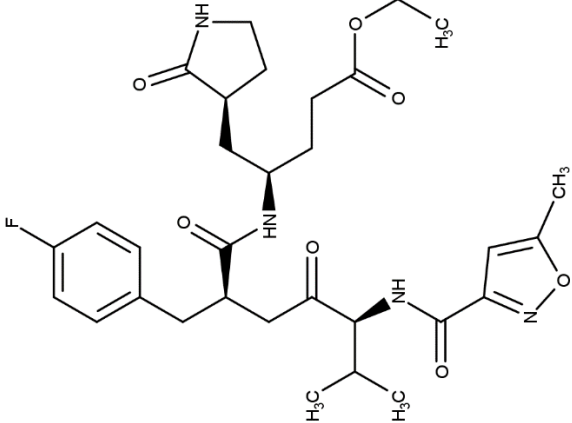
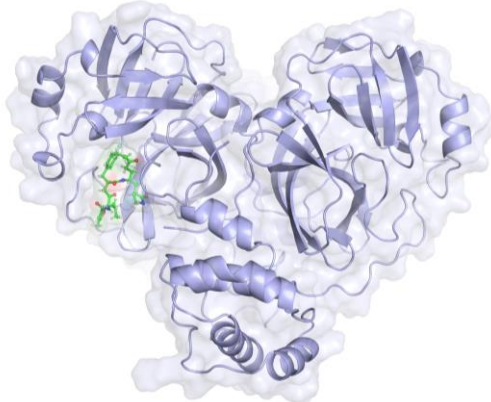
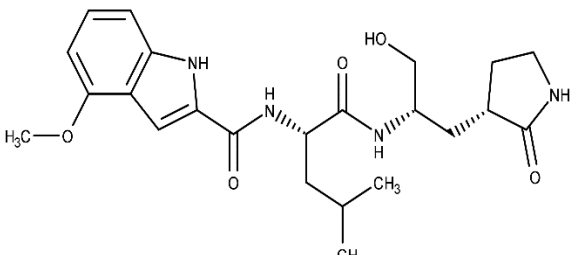
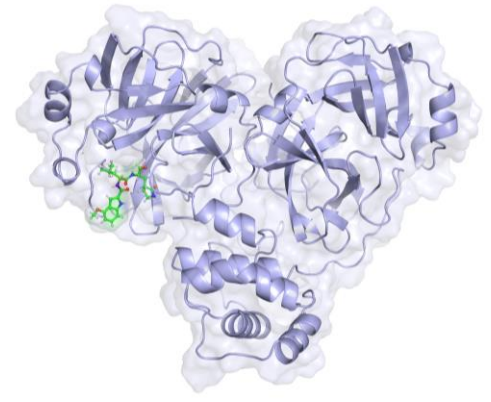
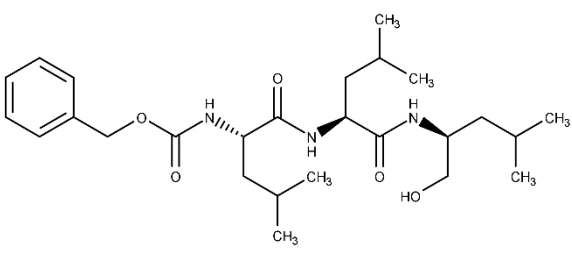
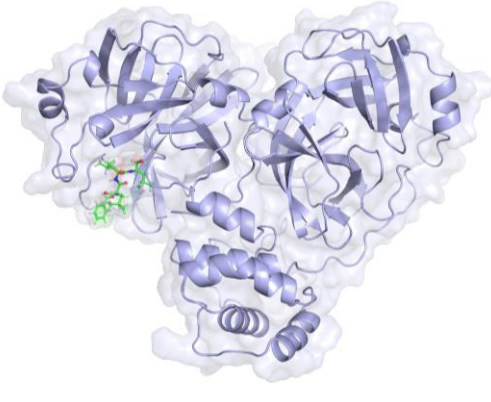
18	3FVK	 <p>Chemical structure of 3FVK, a cyclic peptide derivative. It features a central five-membered ring with an oxygen atom. Attached to this ring are a methylammonium group (H_3N^+), a carboxylate group (COO^-), a hydroxyl group (OH), and a side chain containing another carboxylate group (COO^-).</p>	 <p>3D ribbon diagram of a protein structure (blue) with the ligand 3FVK (green sticks) bound in the active site. The protein surface is shown in white.</p>
19	4DLD	 <p>Chemical structure of 4DLD, a linear molecule. It consists of a central benzene ring substituted with a chlorine atom (Cl), a nitro group (NO_2), and a side chain containing a methylammonium group (H_3N^+), a carboxylate group (COO^-), and a propyl chain ending in another carboxylate group (COO^-).</p>	 <p>3D ribbon diagram of a protein structure (blue) with the ligand 4DLD (green sticks) bound in the active site. The protein surface is shown in white.</p>
20	4E0X	 <p>Chemical structure of 4E0X, a cyclic peptide derivative. It features a central five-membered ring with an oxygen atom and a methylammonium group (NH_2^+). Attached to this ring are a methyl group (CH_3), a vinyl group ($\text{H}_2\text{C}=\text{CH}$), and a side chain containing a carboxylate group (COO^-).</p>	 <p>3D ribbon diagram of a protein structure (blue) with the ligand 4E0X (green sticks) bound in the active site. The protein surface is shown in white.</p>

21	2QG0	 <p>Chemical structure of ligand 2QG0: A 2,4-diaminopyrimidin-5-ylmethylsulfonamide derivative. The pyrimidine ring has an amino group (NH₂) at position 2 and a methyl group (CH₃) at position 4. The 5-position is substituted with a methylene group (-CH₂-) which is further substituted with a sulfonamide group (-SO₂NH-). The nitrogen of the sulfonamide group is attached to a para-substituted benzene ring. This benzene ring is also substituted at the other para position with an imine group (-NH-CH=) which is attached to a furan-2(5H)-one ring.</p>	 <p>3D ribbon and surface representation of protein 2QG0. The protein backbone is shown as a blue ribbon, and the surface is shown as a white mesh. The ligand is shown as a stick model with green carbon atoms, red oxygen atoms, and blue nitrogen atoms, bound within the protein's binding pocket.</p>
22	2QG2	 <p>Chemical structure of ligand 2QG2: A 2-amino-5-methylpyrimidin-4-ylmethylsulfonamide derivative. The pyrimidine ring has an amino group (NH₂) at position 2 and a methyl group (H₃C) at position 5. The 4-position is substituted with a methylene group (-CH₂-) which is further substituted with a sulfonamide group (-SO₂NH-). The nitrogen of the sulfonamide group is attached to a benzene ring. This benzene ring is also substituted at the para position with an imine group (-NH-CH=) which is attached to a furan-2(5H)-one ring.</p>	 <p>3D ribbon and surface representation of protein 2QG2. The protein backbone is shown as a blue ribbon, and the surface is shown as a white mesh. The ligand is shown as a stick model with green carbon atoms, red oxygen atoms, and blue nitrogen atoms, bound within the protein's binding pocket.</p>
23	3K97	 <p>Chemical structure of ligand 3K97: A 2-(2-methylphenyl)pyrrolidine-1-carboxamide derivative. The pyrrolidine ring is substituted at the 2-position with a methyl group (CH₃) and at the 1-position with a carbonyl group (-C(=O)-). The nitrogen of the carbonyl group is attached to a benzene ring. This benzene ring is also substituted at the para position with a hydroxyl group (-OH) and at the other para position with a chlorine atom (-Cl). The oxygen of the hydroxyl group is shown as O⁻.</p>	 <p>3D ribbon and surface representation of protein 3K97. The protein backbone is shown as a blue ribbon, and the surface is shown as a white mesh. The ligand is shown as a stick model with green carbon atoms, red oxygen atoms, and blue nitrogen atoms, bound within the protein's binding pocket.</p>

28	4CWT		
29	4NH8		
30	4O07		

31	6M2N		
32	6WTT		
33	6XBG		

34	6XMK	 <p>Chemical structure of ligand 6XMK, featuring a piperidine ring substituted with two fluorine atoms, connected via a methylene group to a carbonyl group. This carbonyl is further linked to a complex chain containing a secondary amine, a methyl group, a hydroxyl group, and a pyrrolidine ring.</p>	 <p>3D ribbon diagram of the protein structure (blue) with the ligand 6XMK (green sticks) bound in the active site, overlaid on a light blue surface representation.</p>
35	7B3E	 <p>Chemical structure of ligand 7B3E, a flavone derivative with multiple hydroxyl groups on the A and C rings.</p>	 <p>3D ribbon diagram of the protein structure (blue) with the ligand 7B3E (green sticks) bound in the active site, overlaid on a light blue surface representation.</p>
36	7LCR	 <p>Chemical structure of ligand 7LCR, featuring a piperidine ring substituted with two methyl groups and a hydroxyl group, connected via a carbonyl group to a benzene ring with a fluorine substituent. It also includes a pyrrolidine ring.</p>	 <p>3D ribbon diagram of the protein structure (blue) with the ligand 7LCR (green sticks) bound in the active site, overlaid on a light blue surface representation.</p>

37	7L8I	 <p>Chemical structure of ligand 7L8I, featuring a central chain with a 4-fluorophenyl group, a pyrrolidine ring, a propanoic acid ethyl ester, a methyl group, and a 5-methylisoxazole-3-carboxamide group.</p>	 <p>3D ribbon diagram of protein 7L8I (blue) with the ligand (green sticks) bound in the active site, overlaid on a white surface representation of the protein.</p>
38	7LDL	 <p>Chemical structure of ligand 7LDL, featuring a central chain with a 5-methoxy-1H-indole-3-carboxamide group, a piperidine ring with two methyl groups, a hydroxymethyl group, and a pyrrolidine ring.</p>	 <p>3D ribbon diagram of protein 7LDL (blue) with the ligand (green sticks) bound in the active site, overlaid on a white surface representation of the protein.</p>
39	7NG3	 <p>Chemical structure of ligand 7NG3, featuring a central chain with a benzyl carbamate group, a piperidine ring with two methyl groups, a hydroxymethyl group, and a 2-methylbutyl group.</p>	 <p>3D ribbon diagram of protein 7NG3 (blue) with the ligand (green sticks) bound in the active site, overlaid on a white surface representation of the protein.</p>

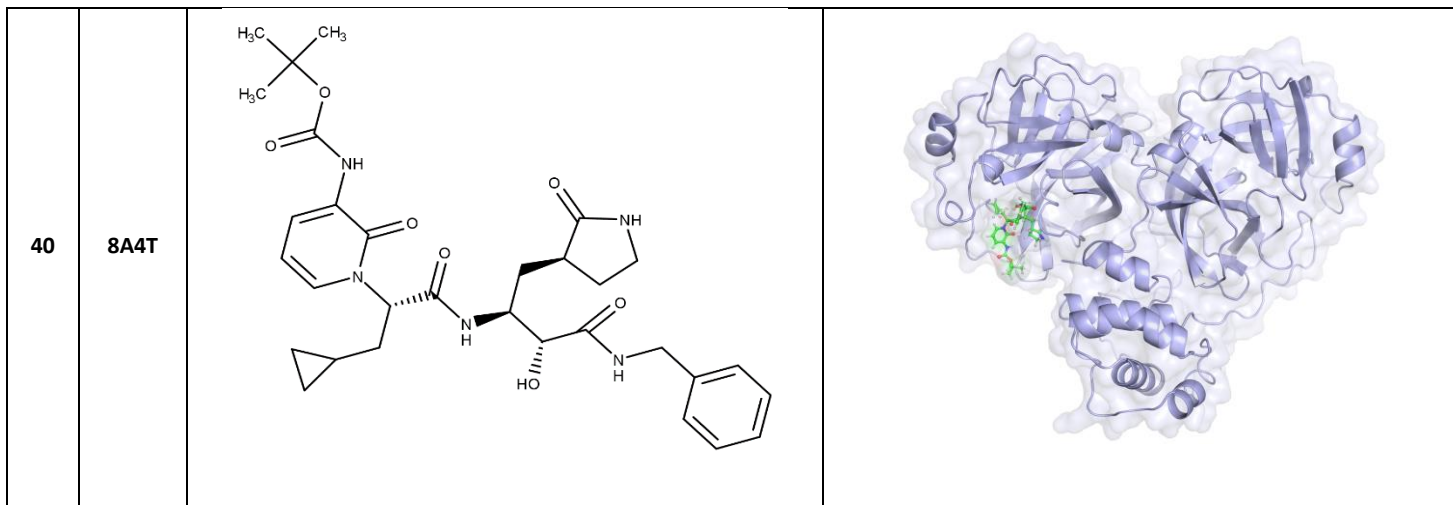
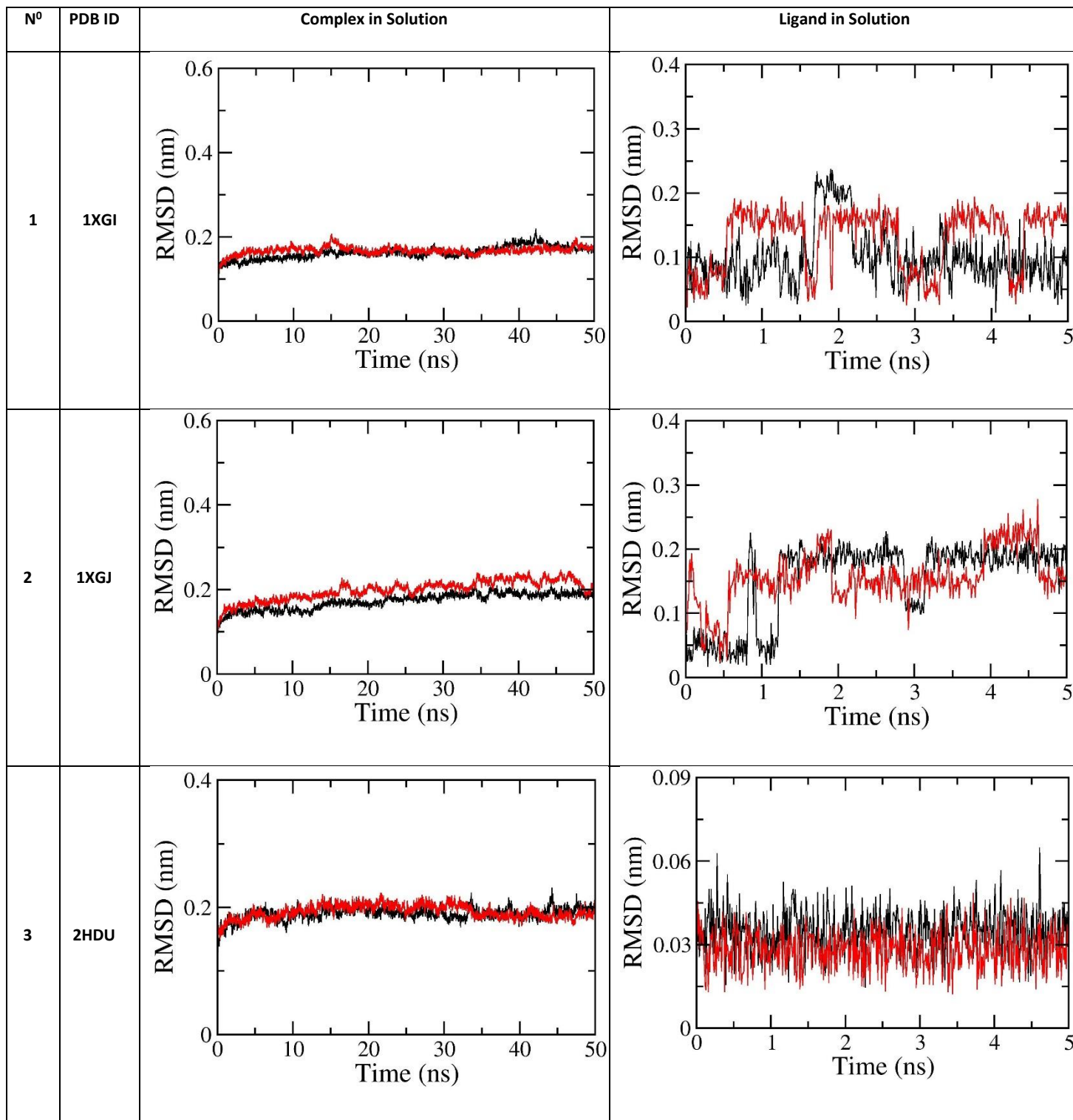
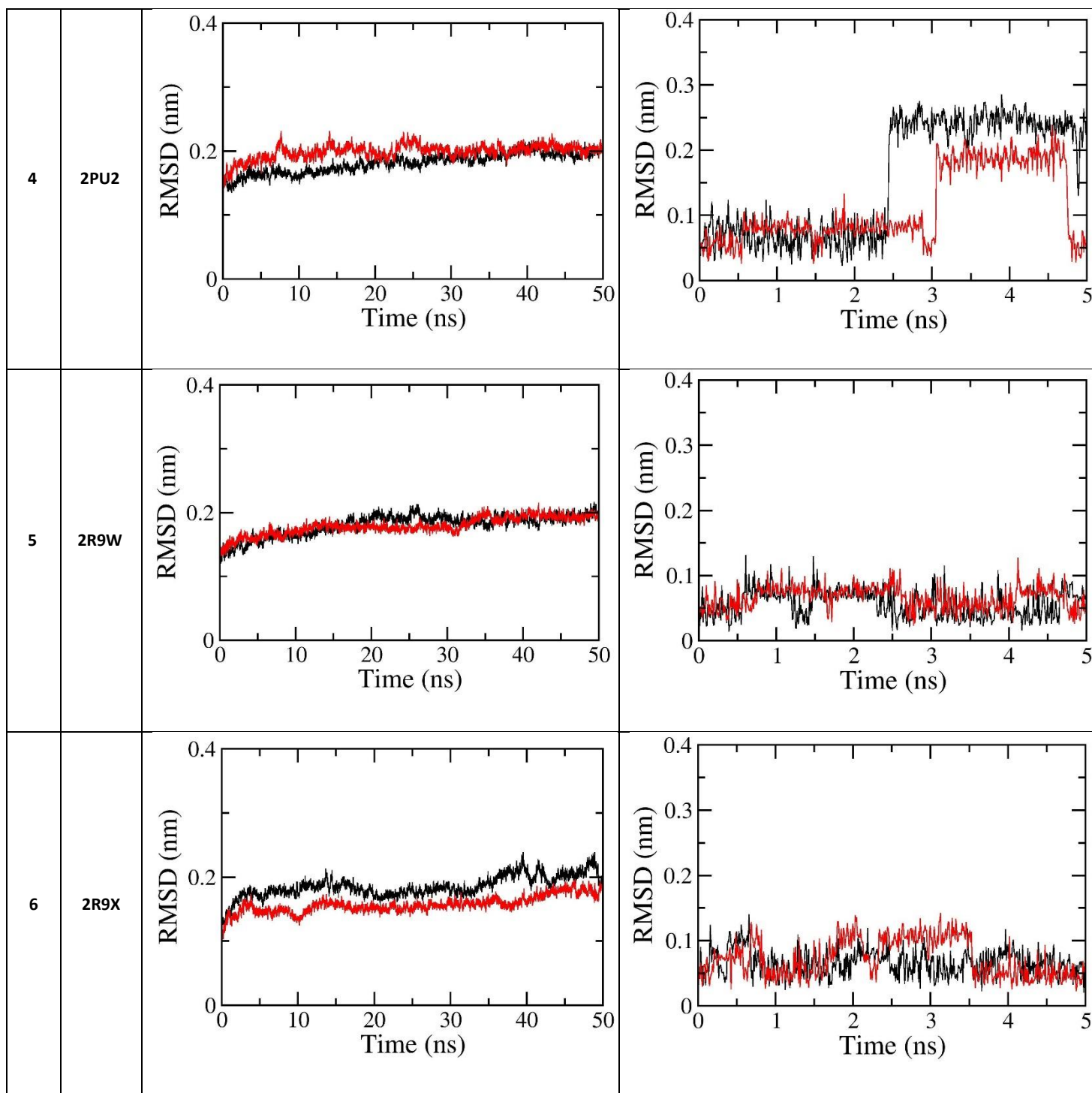
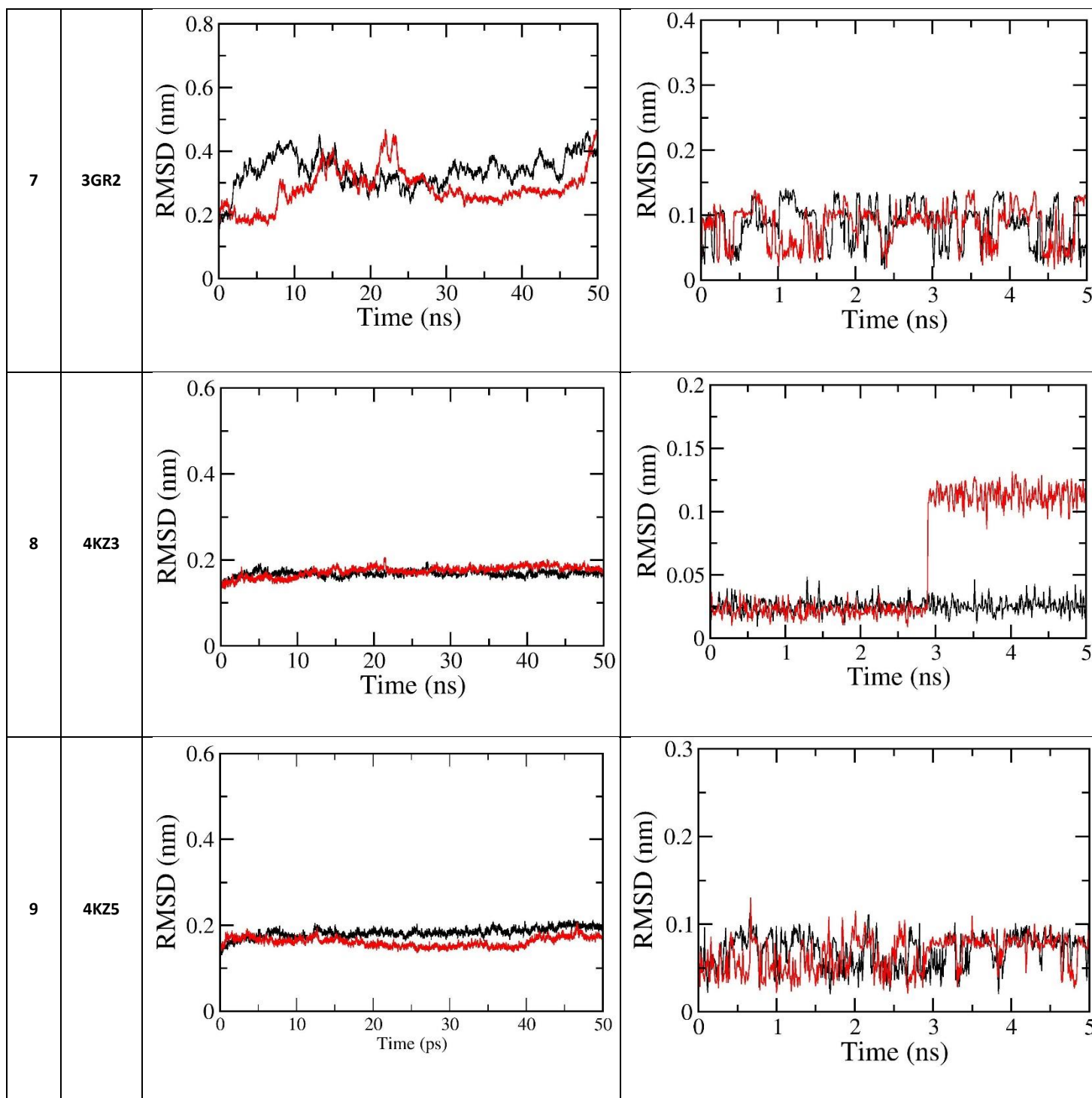


Table S2. All-atom RMSD of AmpC + ligand and ligand in solution systems.







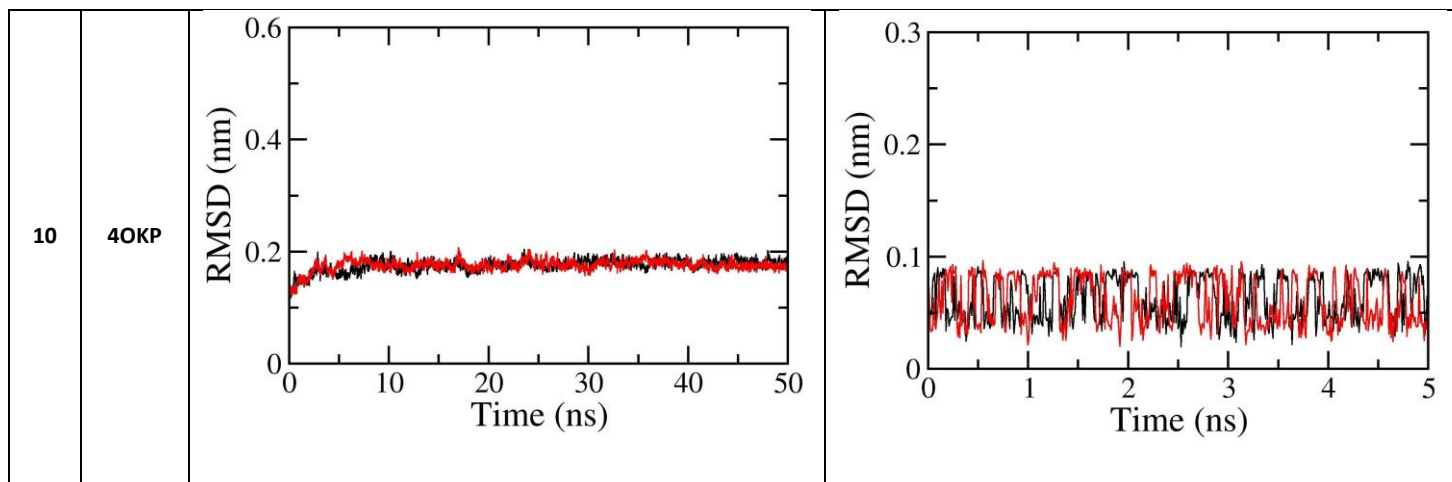
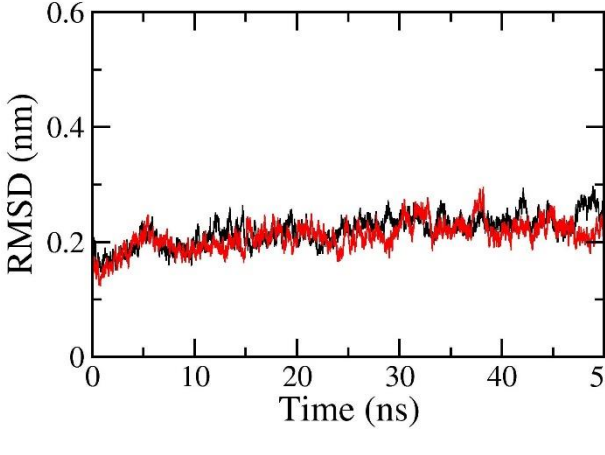
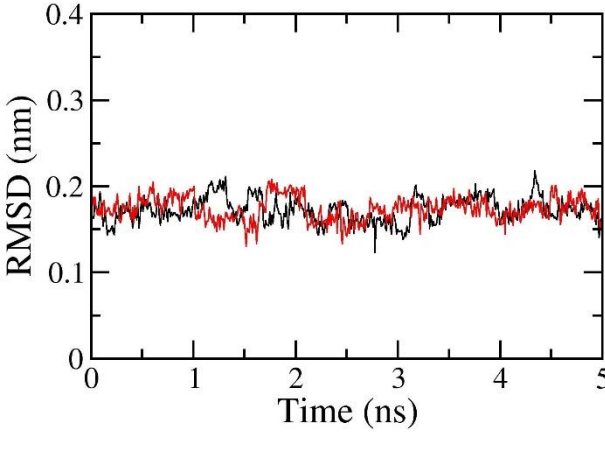
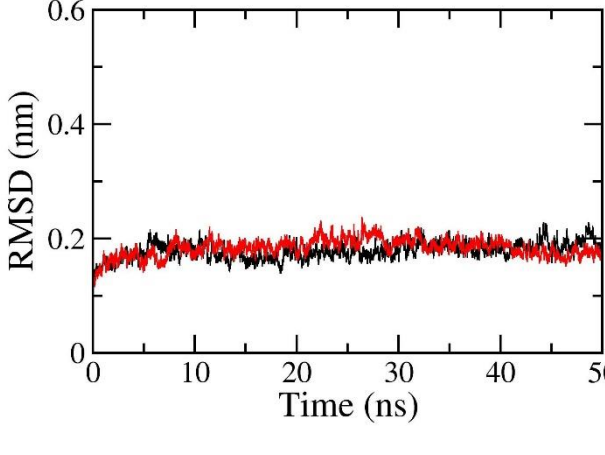
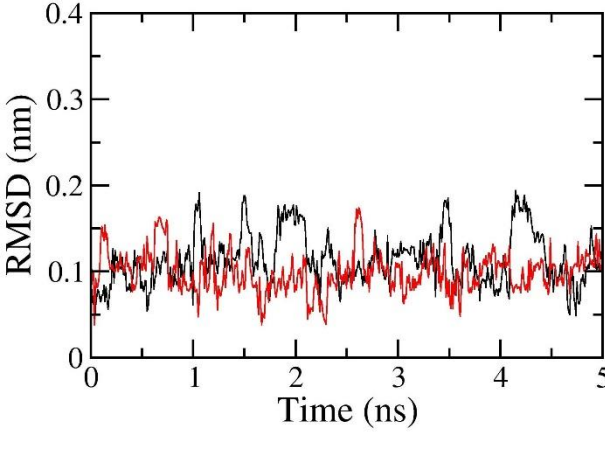
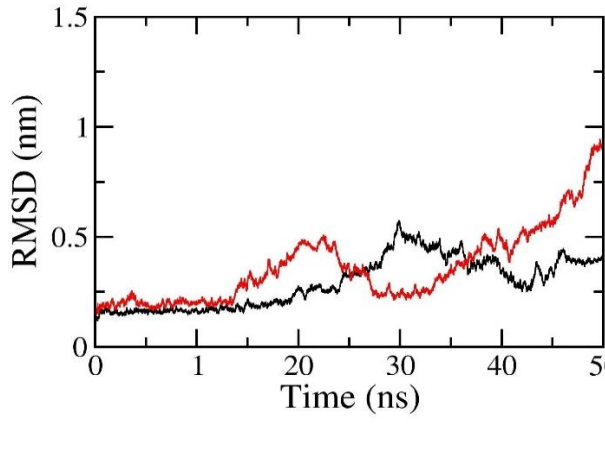
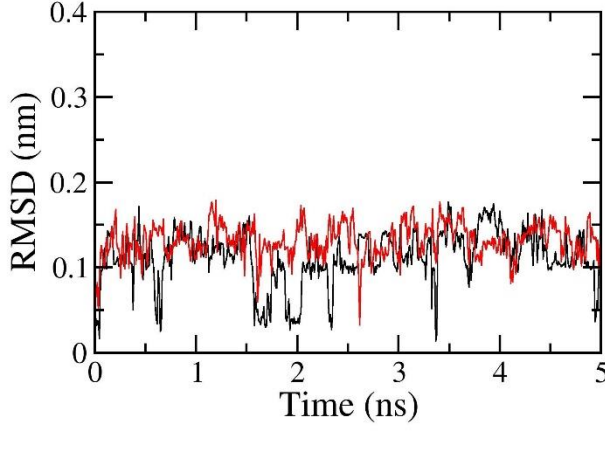
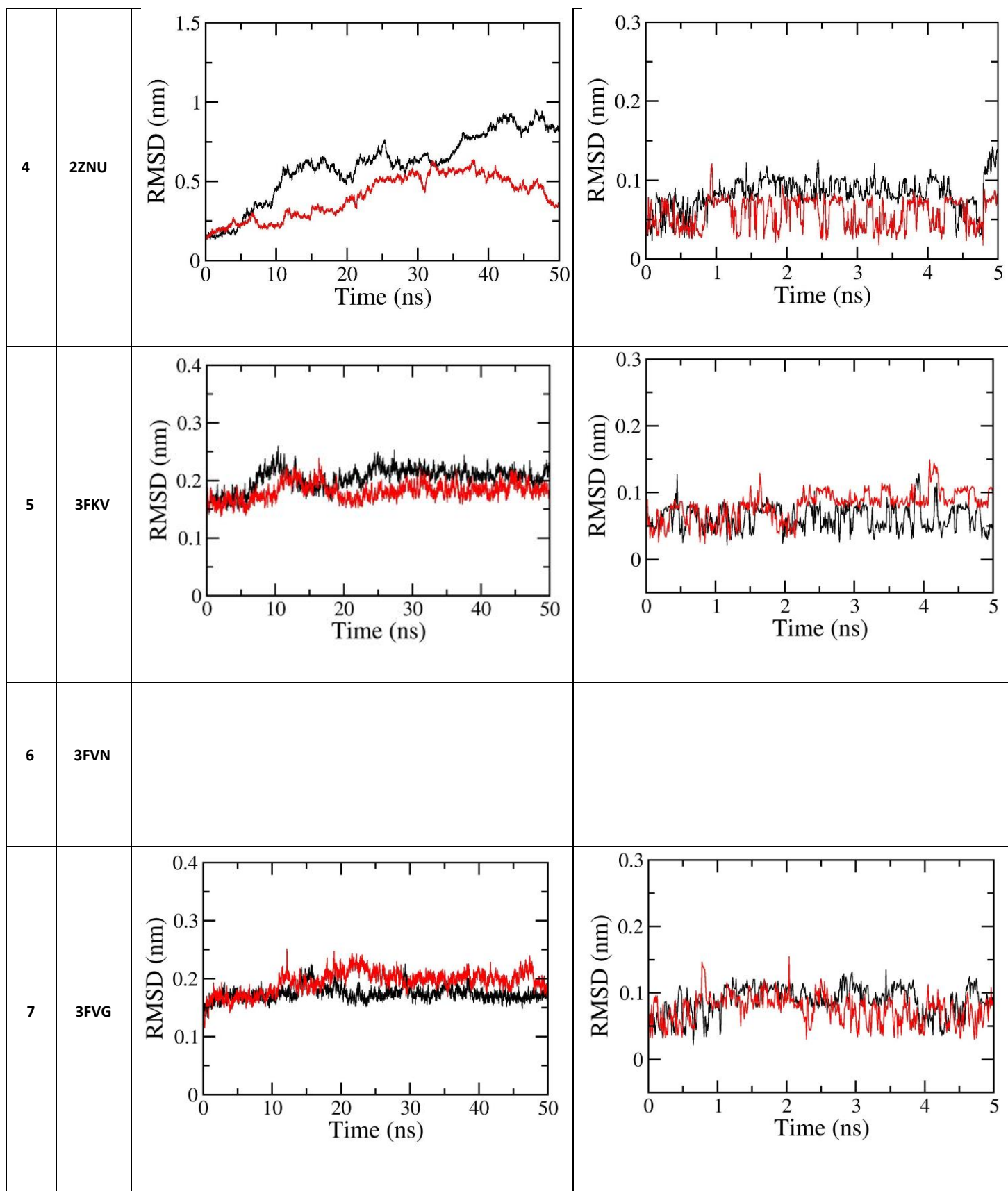


Table S3. All-atom RMSD of GluK1 + ligand and ligand in solution systems.

N ^o	PDB ID	Complex in Solution	Ligand in Solution
1	1VSO	 <p>RMSD (nm) vs Time (ns) for 1VSO complex in solution. The y-axis ranges from 0 to 0.6 nm, and the x-axis ranges from 0 to 50 ns. Two lines (black and red) fluctuate between approximately 0.15 and 0.3 nm.</p>	 <p>RMSD (nm) vs Time (ns) for 1VSO ligand in solution. The y-axis ranges from 0 to 0.4 nm, and the x-axis ranges from 0 to 5 ns. Two lines (black and red) fluctuate between approximately 0.1 and 0.2 nm.</p>
2	2PBW	 <p>RMSD (nm) vs Time (ns) for 2PBW complex in solution. The y-axis ranges from 0 to 0.6 nm, and the x-axis ranges from 0 to 50 ns. Two lines (black and red) fluctuate between approximately 0.1 and 0.2 nm.</p>	 <p>RMSD (nm) vs Time (ns) for 2PBW ligand in solution. The y-axis ranges from 0 to 0.4 nm, and the x-axis ranges from 0 to 5 ns. Two lines (black and red) fluctuate between approximately 0.05 and 0.2 nm.</p>
3	2ZNS	 <p>RMSD (nm) vs Time (ns) for 2ZNS complex in solution. The y-axis ranges from 0 to 1.5 nm, and the x-axis ranges from 0 to 50 ns. Two lines (black and red) show a significant increase from approximately 0.2 nm at 0 ns to 1.0 nm at 50 ns.</p>	 <p>RMSD (nm) vs Time (ns) for 2ZNS ligand in solution. The y-axis ranges from 0 to 0.4 nm, and the x-axis ranges from 0 to 5 ns. Two lines (black and red) fluctuate between approximately 0.05 and 0.15 nm.</p>



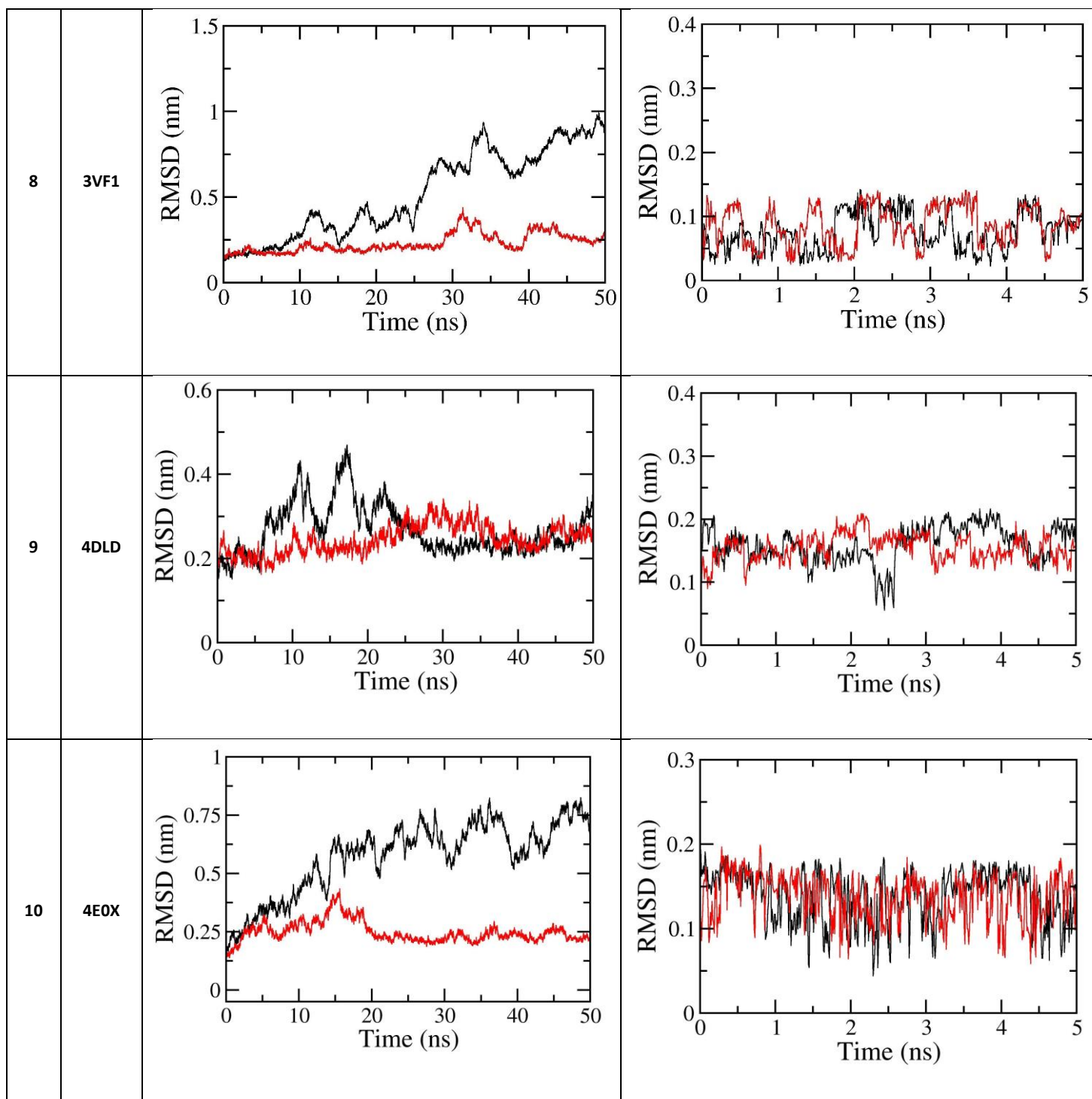
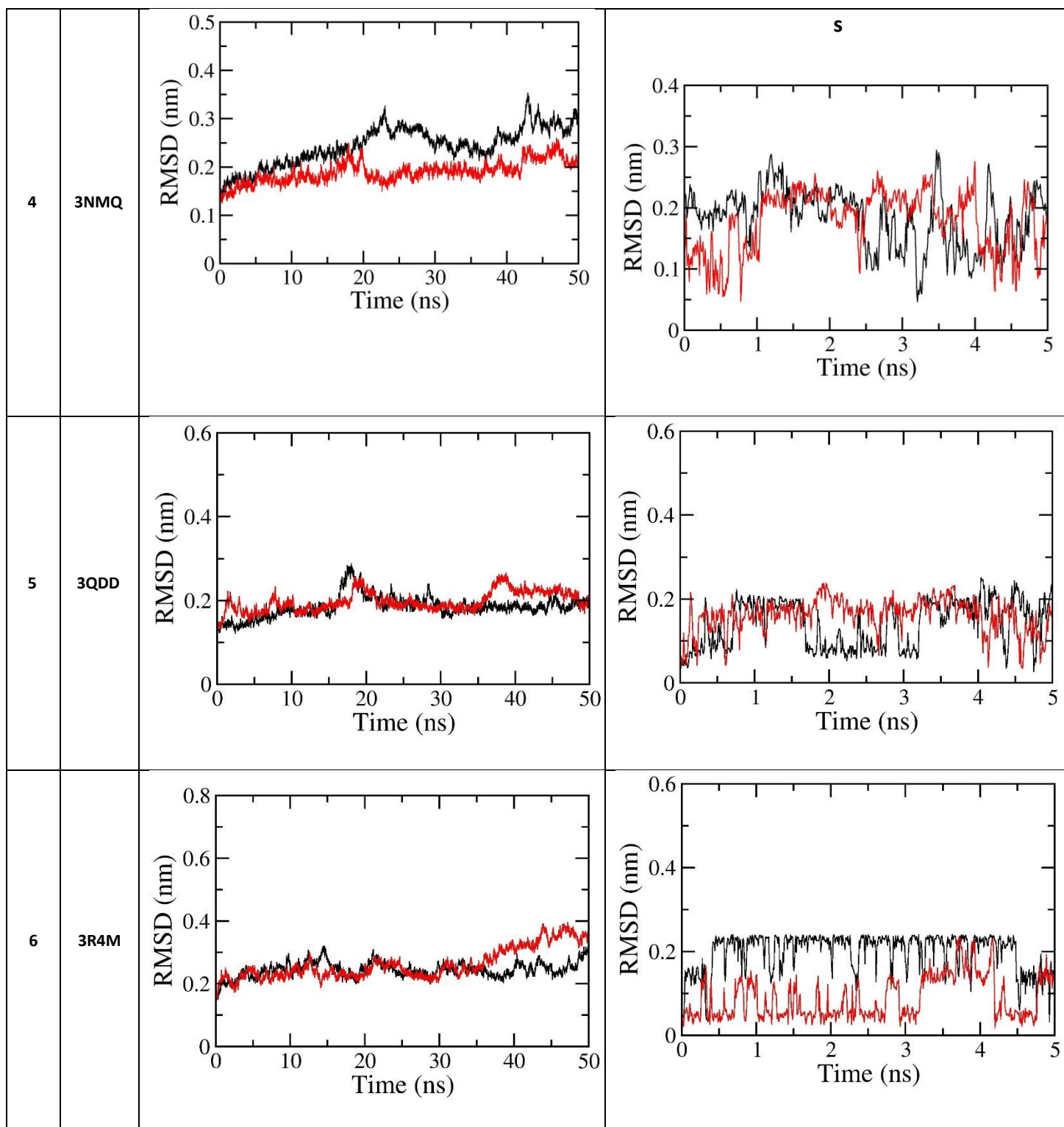
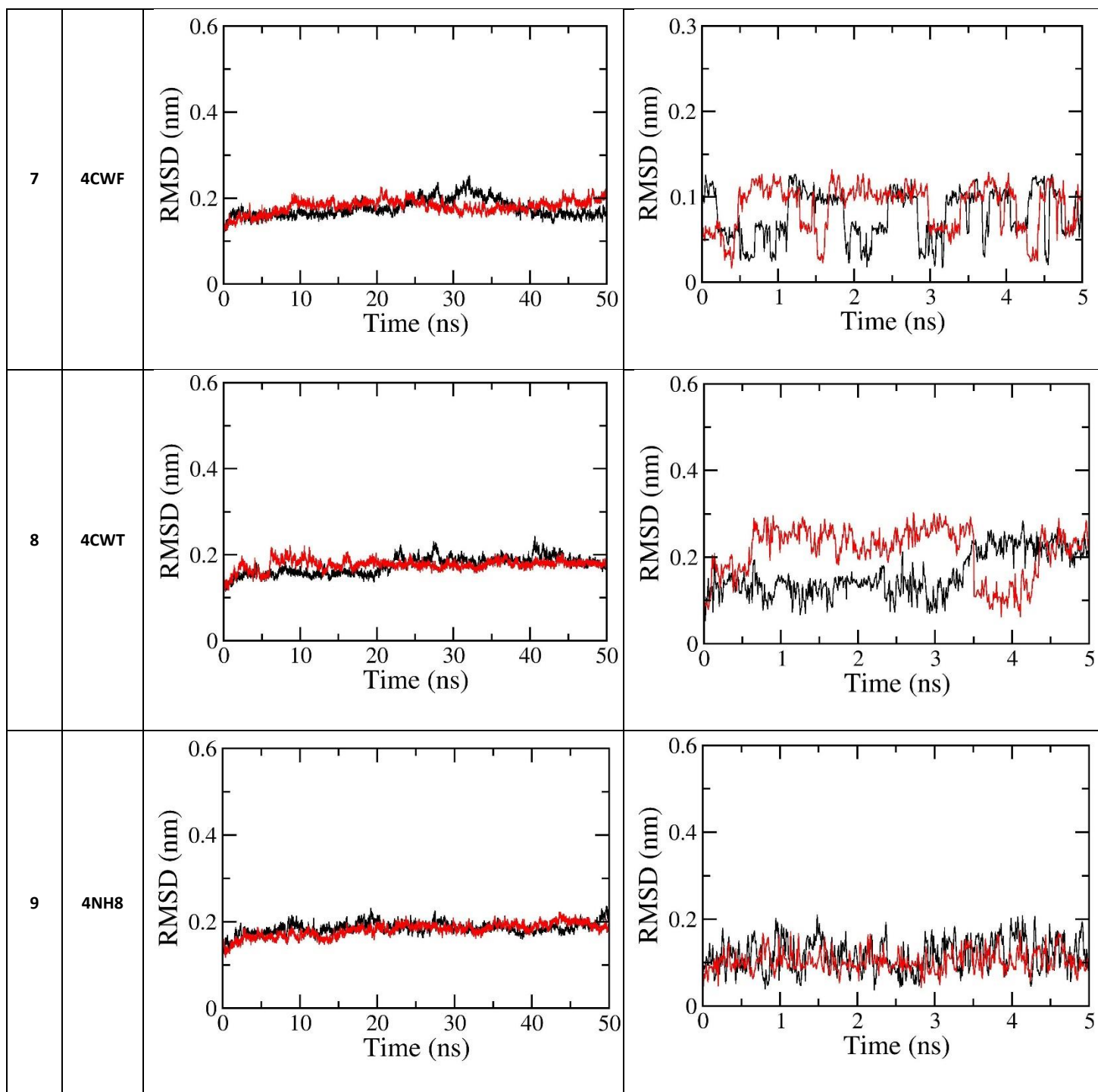


Table S4. All-atom RMSD of Hsp90 + ligand and ligand in solution systems.

N ^o	PDB ID	Complex in Solution	Ligand in Solution
1	2QG0		
2	2QG2		
3	3K97		





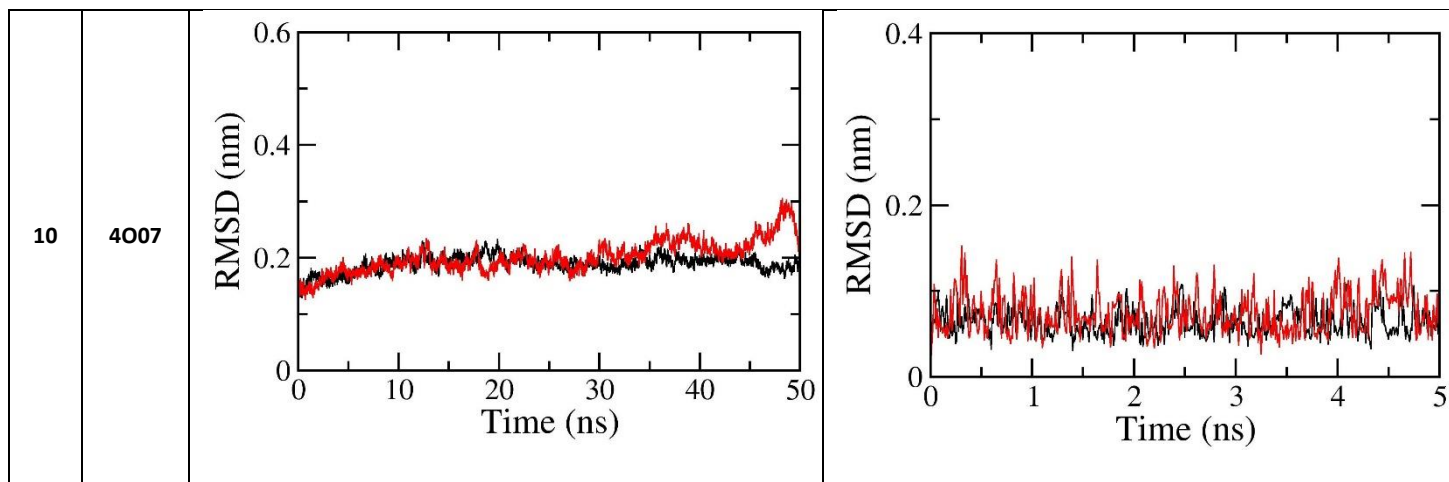
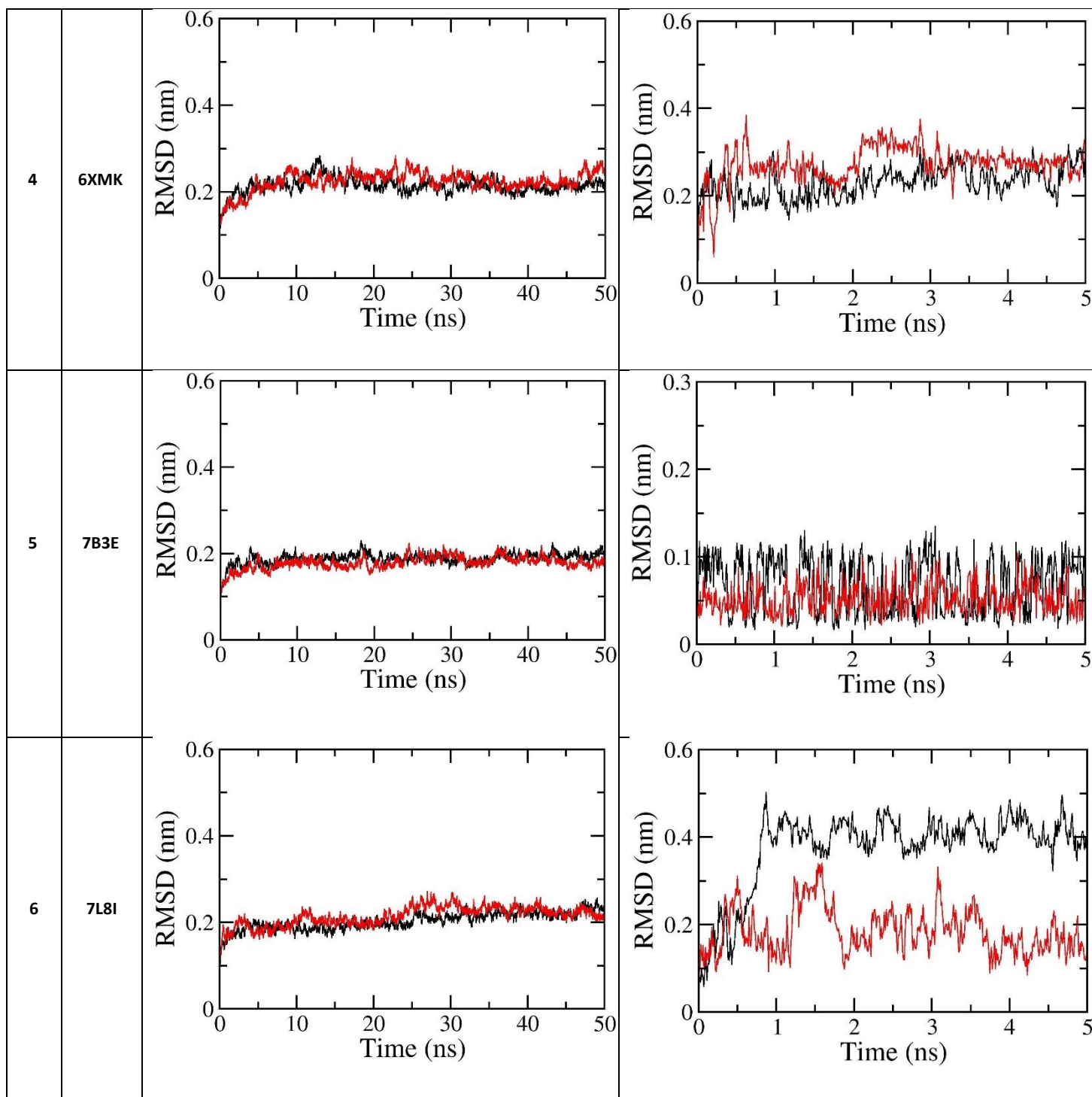
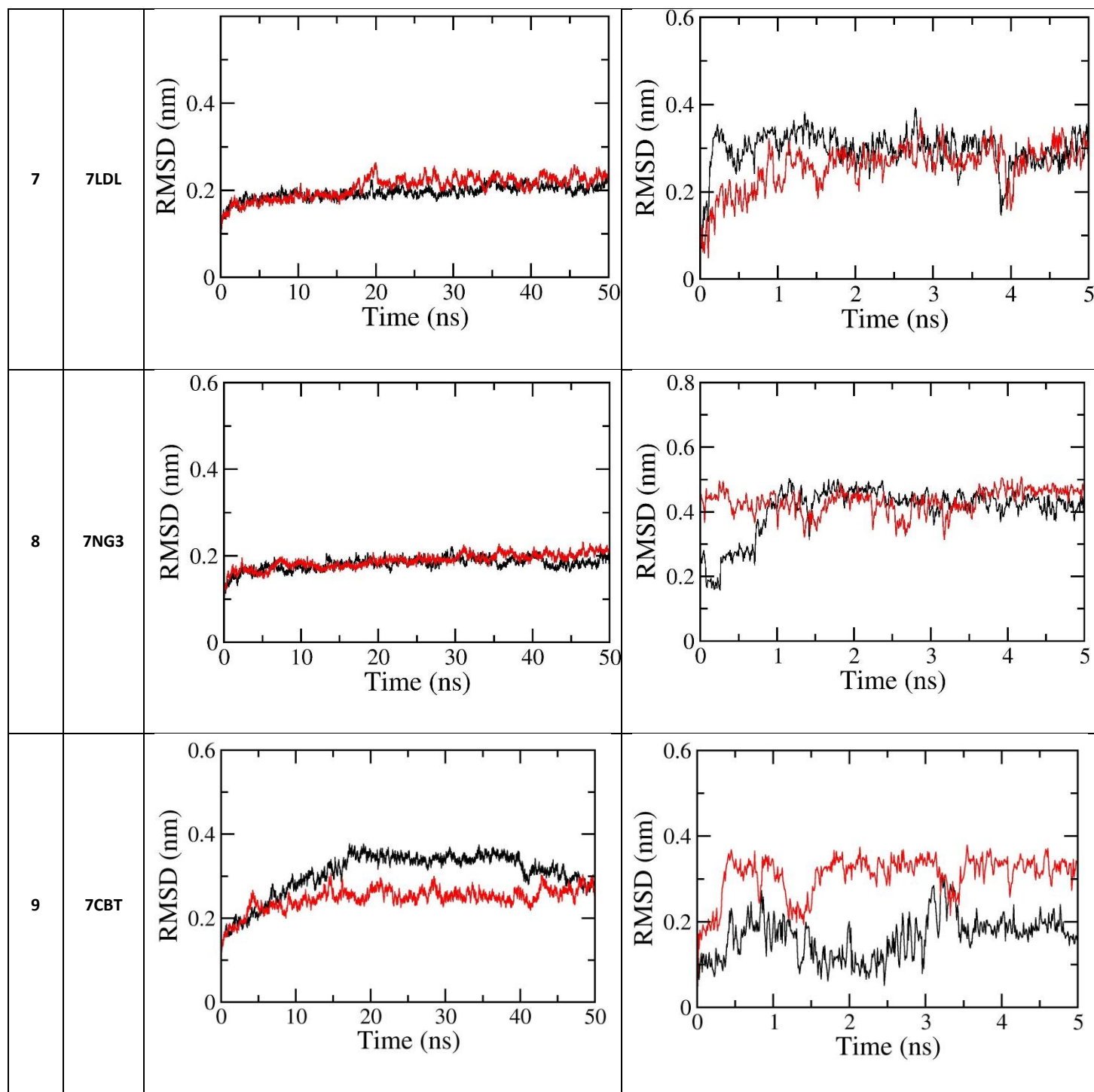
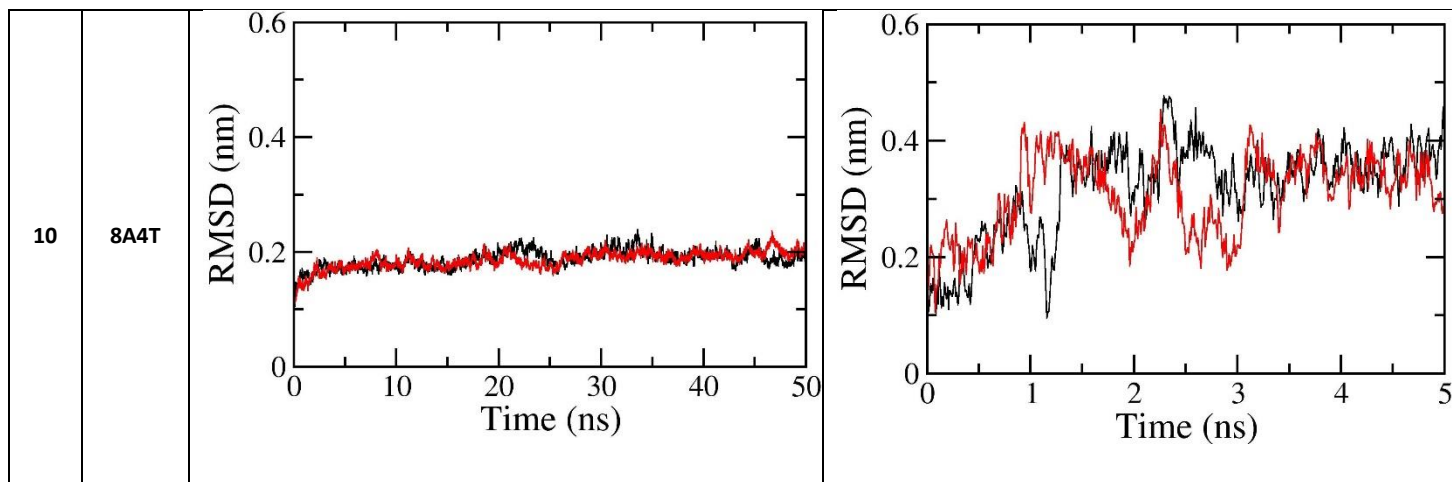


Table S5. All-atom RMSD of SARS-CoV-2 Mpro + ligand and ligand in solution systems.

N°	PDB ID	Complex in Solution	Ligand in Solution
1	6M2N		
2	6WTT		
3	6XBG		







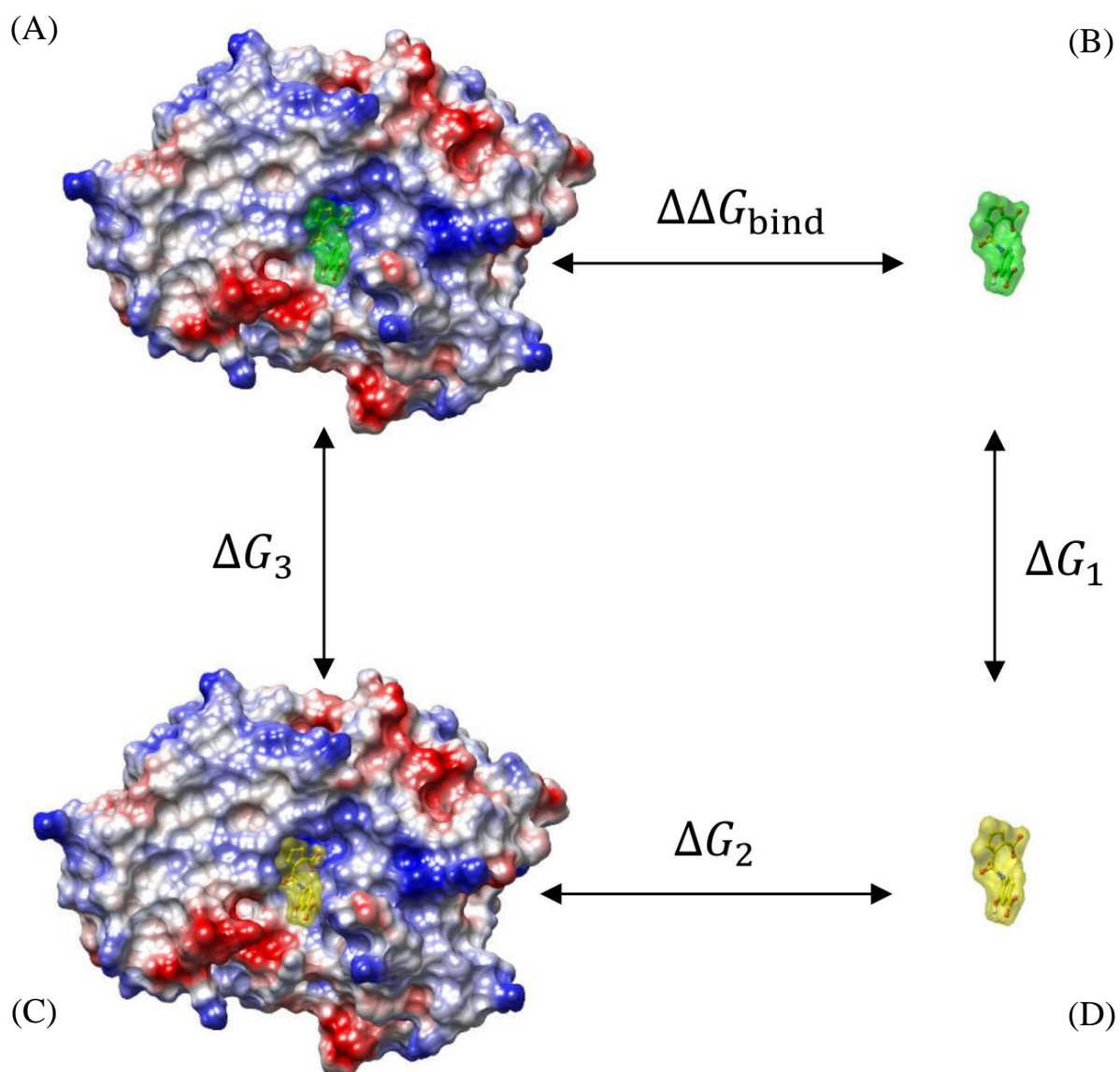


Figure S1. Thermodynamics diagram of FEP calculations. (A) full-interaction state of a ligand with surrounding protein and solvation; (B) full-interaction state of a ligand with surrounding solvation; (C) non-interaction state of a ligand with surrounding protein and solvation; (D) non-interaction state of a ligand with surrounding solvation.

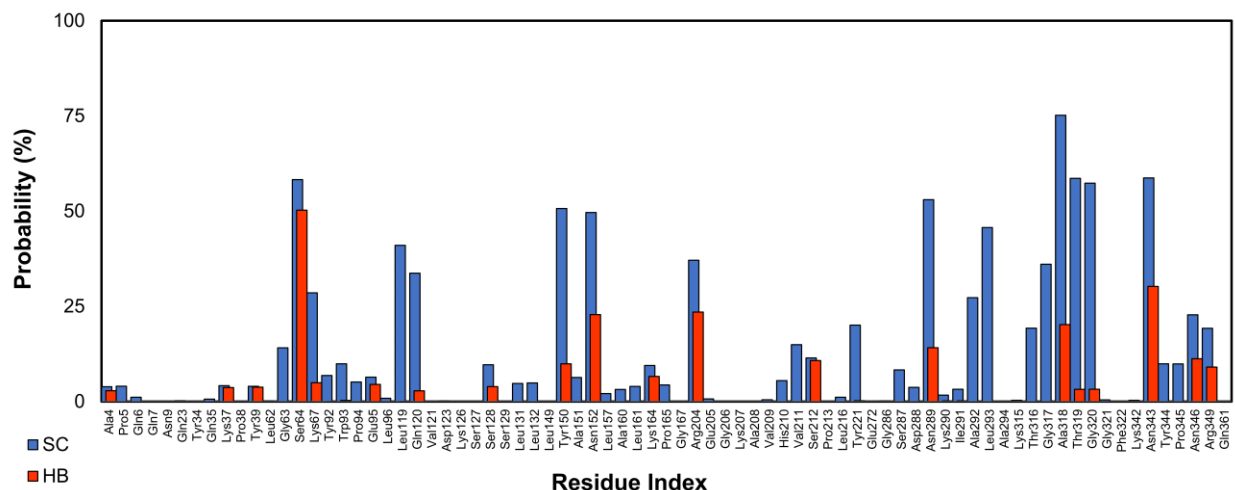


Figure S2. SC and HB Contacts between AmpC Residues and Inhibitors. The values were computed over the interval 25-50 ns of MD simulations.

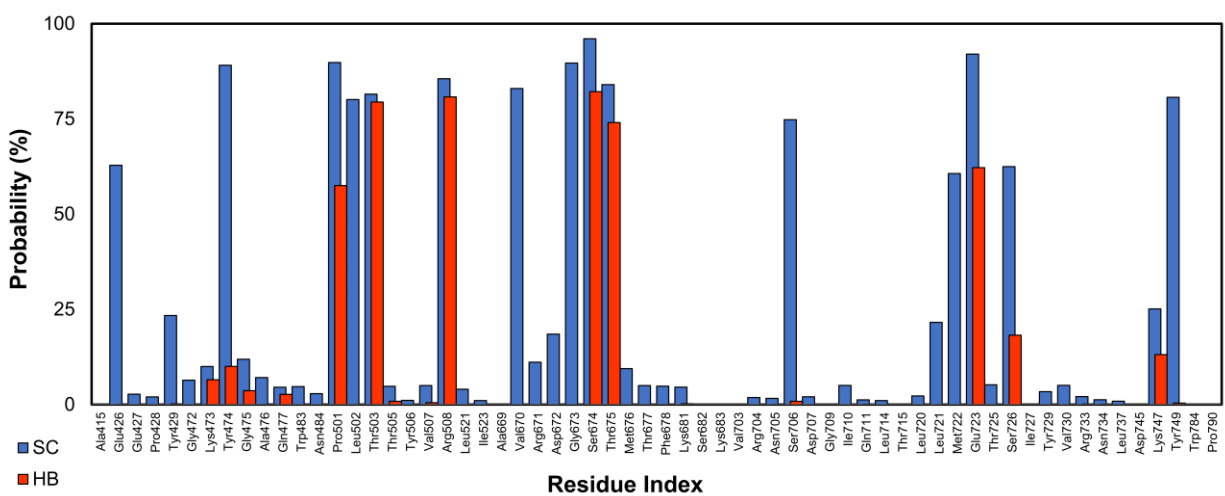


Figure S3. SC and HB Contacts between GluK1 Residues and Inhibitors. The values were computed over the interval 25-50 ns of MD simulations.

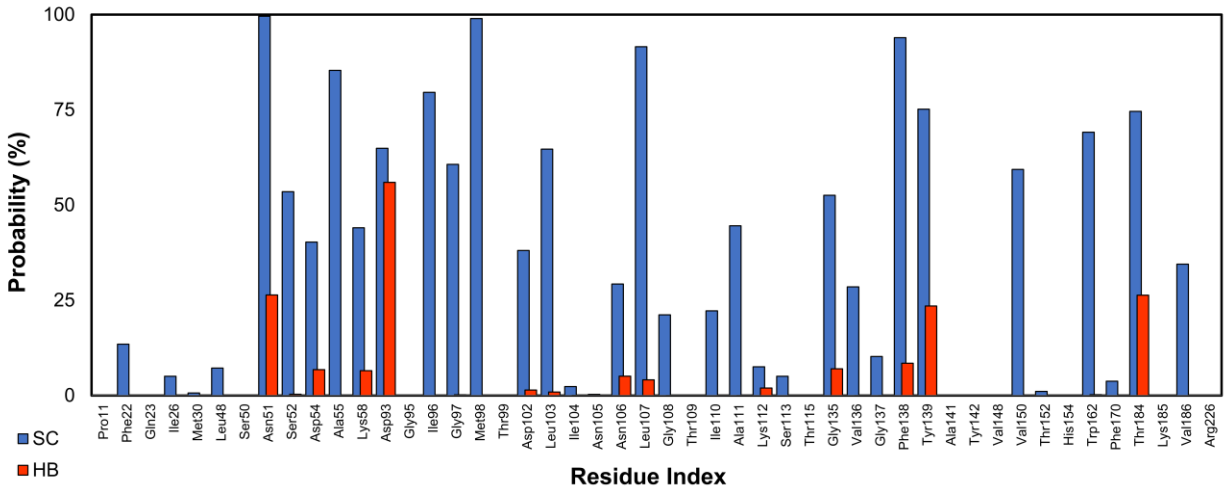


Figure S4. SC and HB Contacts between Hsp90 Residues and Inhibitors. The values were computed over the interval 25-50 ns of MD simulations.

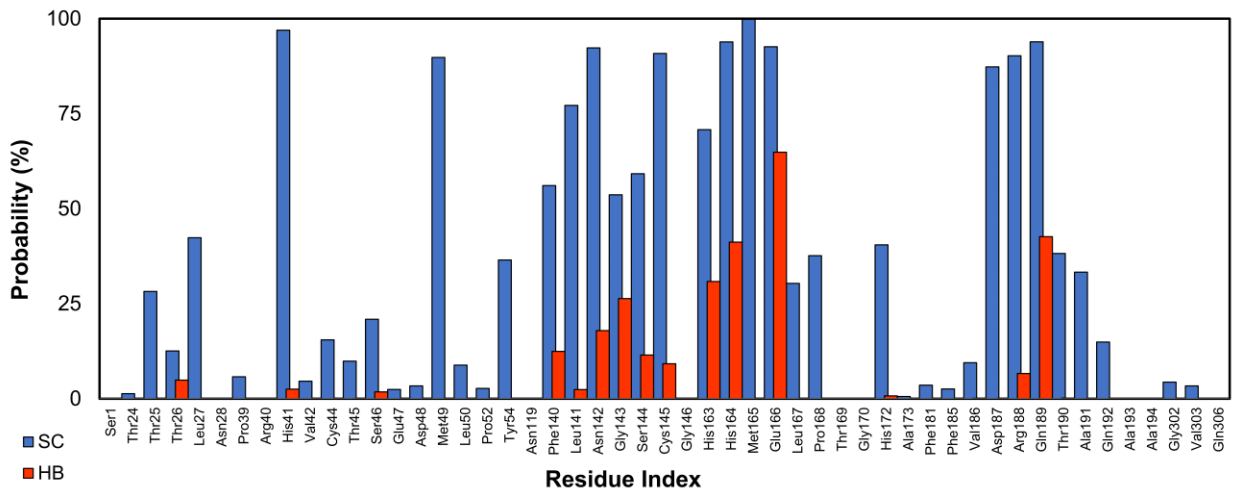


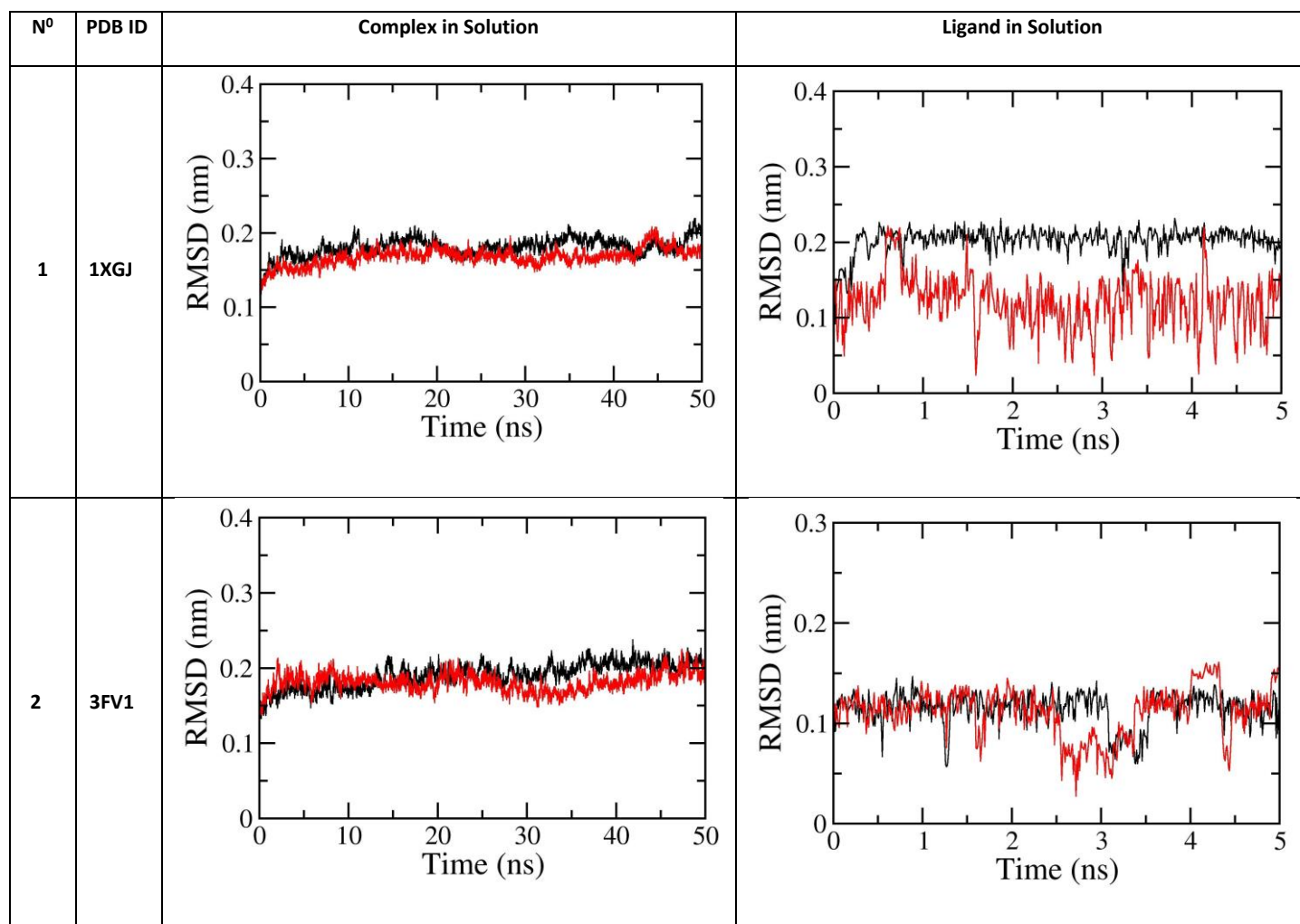
Figure S5. SC and HB Contacts between SARS-CoV-2 Mpro Residues and Inhibitors. The values were computed over the interval 25-50 ns of MD simulations.

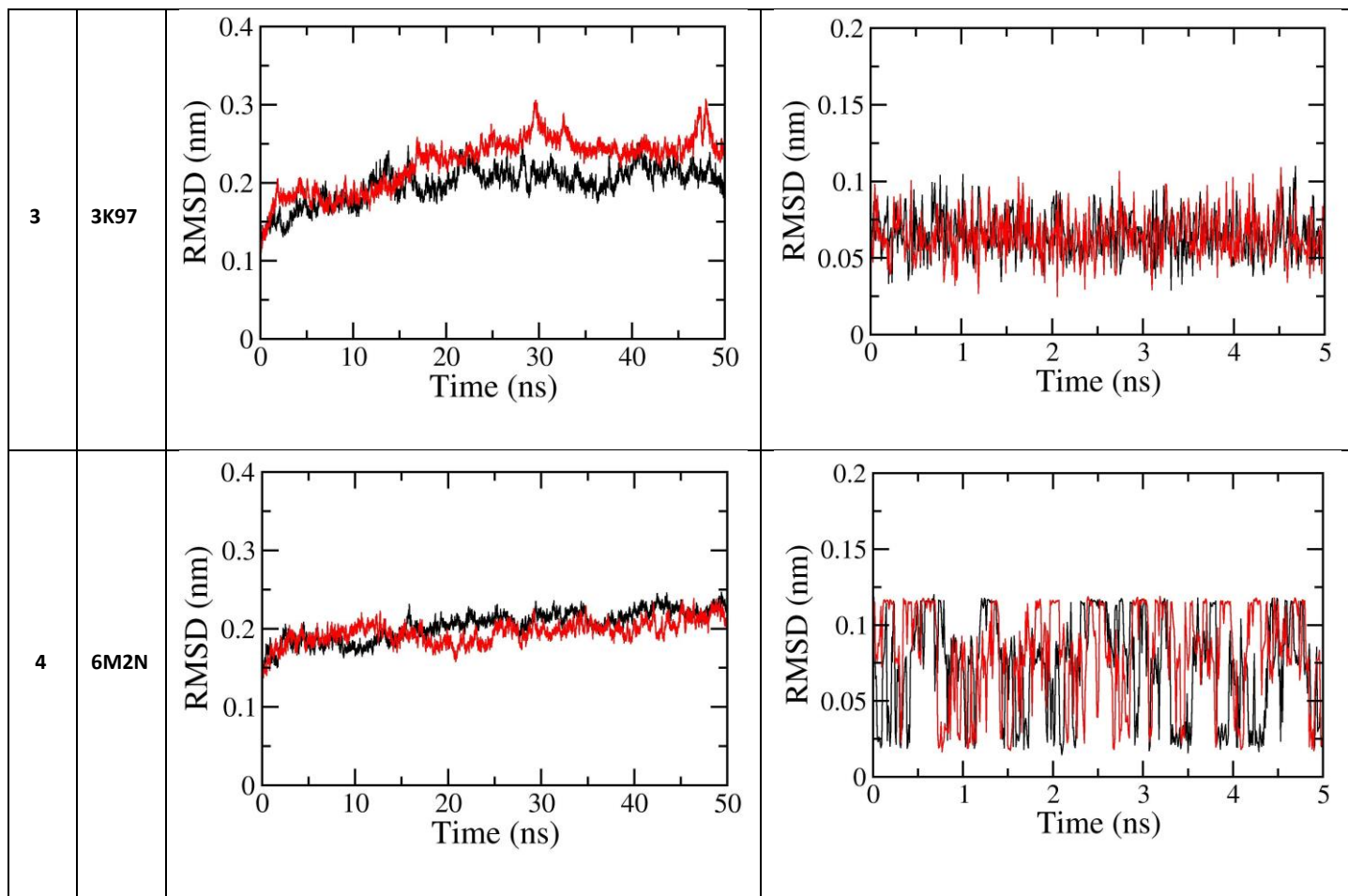
Table S6. Calculated versus Experimental Binding Affinities between AmpC, GluK1, Hsp90, SARS-CoV-2 Mpro and its inhibitors

N ^o	PDB ID	PDB ID	ΔG_{cou}	ΔG_{vdW}	ΔG_{FEP}	ΔG_{EXP}^b
1	AmpC	1XGJ	-2.41	-5.10	-7.50 ± 1.74	-8.24
2	GluK1	3FV1	-21.90	-0.78	-22.68 ± 0.24	-12.77
3	Hsp90	3K97	-2.74	-8.66	-11.40 ± 0.91	-10.98
4	SARS-CoV-2 Mpro	6M2N	-0.67	-5.83	-6.51 ± 0.64	-8.25

^bThe experimental binding free energies were computed based on the inhibition constant k_i . The computed error was obtained using the bootstrapping method.

The unit is kcal mol⁻¹.

Table S7. All-atom RMSD of AmpC, GluK1, Hsp90, SARS-CoV-2 Mpro + ligand and ligand in solution systems.



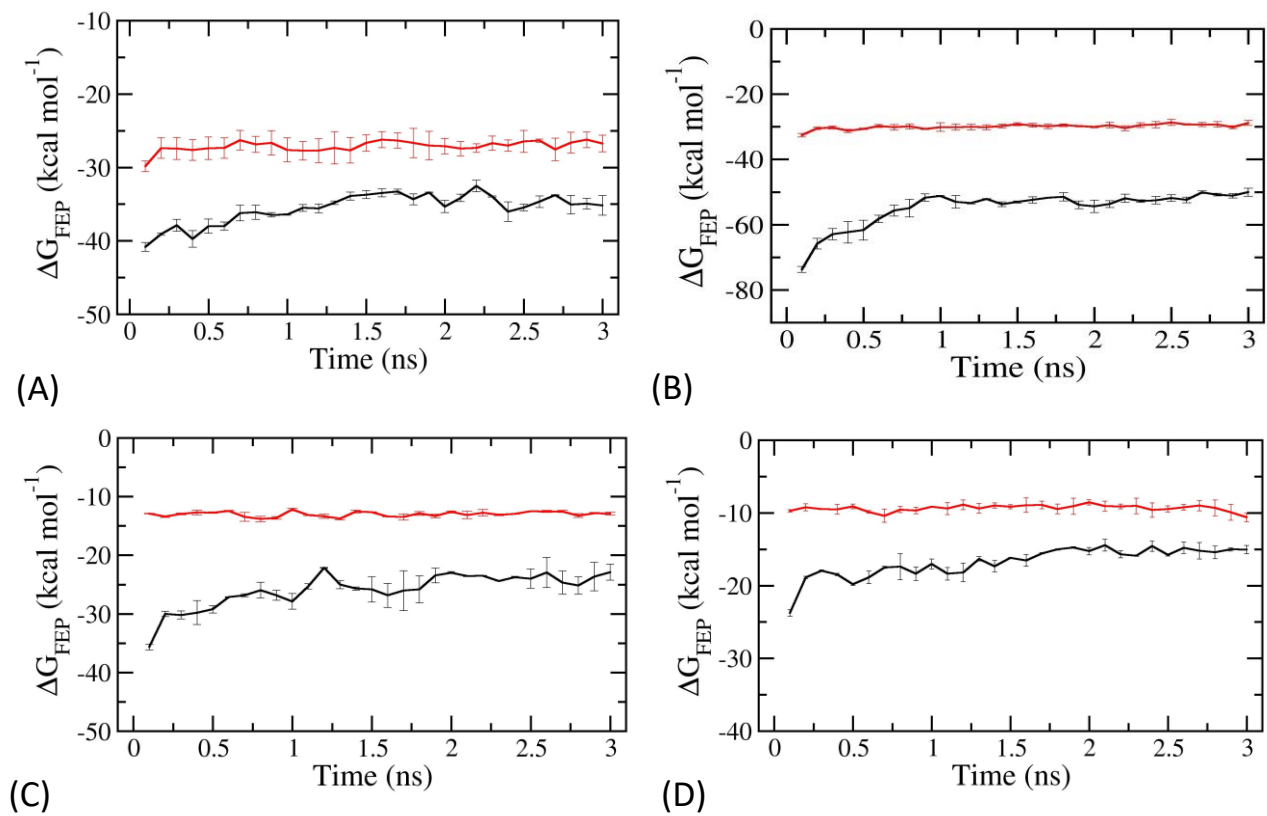


Figure S6. Time dependence of binding free energies for solvated complexes: (A) AmpC (*1XGJ*), (B) GluK1 (*3FV1*), Hsp90 (*3K97*), SARS-CoV-2 Mpro (*6M2N*). The values collected in the equilibrium region were used to calculate ΔG_{FEP} . The error bars show the root mean square deviations.