Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Alchemical Approach Performance in Calculating the Ligand-Binding Free Energy

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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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2ZNS  EANRTLIVTTILEEPYVMYRKSDKPLYGNDRFEGYCLDLLKELSNILGFIYDVKLVPDGKYGAQNDKGEWNGMVKELIDH
4DLD  --NRTLIVTTILEEPYVMYRKSDKPLYGNDRFEGYCLDLLKELSNILGFLYDVKLVPDGKYGAQNDKGEWNGMVKELIDH

2ZNS  RADLAVAPLTITYVREKVIDFSKPFMTLGISILYRKGTPIDSADDLAKQTKIEYGAVRDGSTMTFFKKSKISTYEKMWAF
4DLD  RADLAVAPLTITYVREKVIDFSKPFMTLGISILYRKGTPIDSADDLAKQTKIEYGAVRDGSTMTFFKKSKISTYEKMWAF

2ZNS  MSSRQQTALVRNSDEGIQRVLTTDYALLMESTSIEYVTQRNCNLTQIGGLIDSKGYGVGTPIGSPYRDKITIAILQLQEE
4DLD  MSSRQQSALVKNSDEGIQRVLTTDYALLMESTSIEYVTQRNCNLTQIGGLIDSKGYGVGTPIGSPYRDKITIAILQLQEE

2ZNS  GKLHMMKEKWWRGNGC-
4DLD  GKLHMMKEKWWRGNGCP
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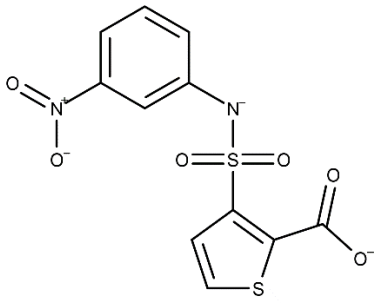
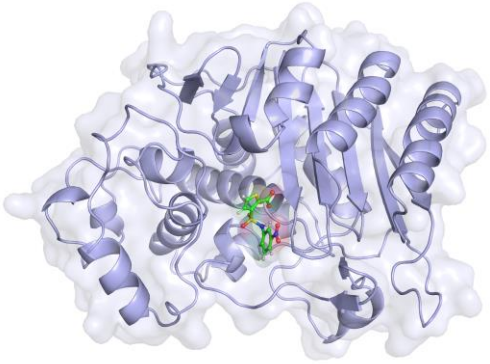
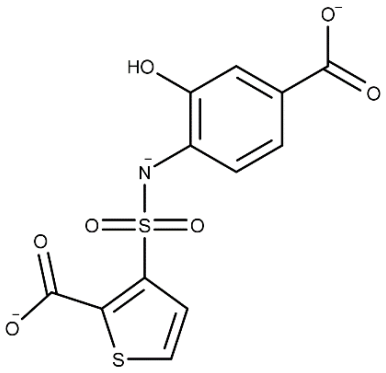
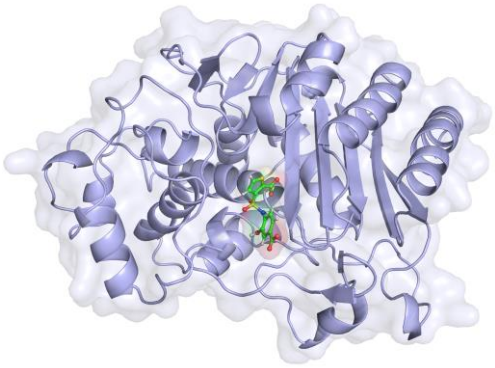
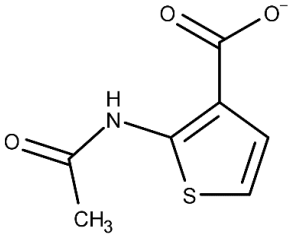
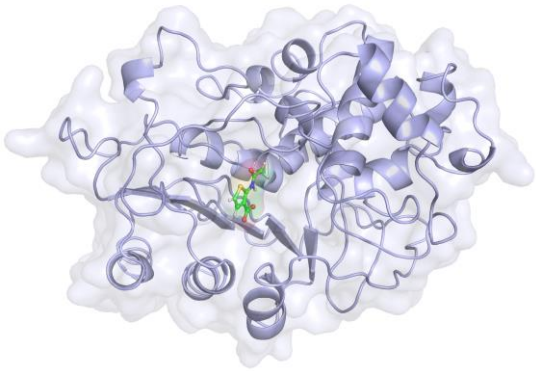
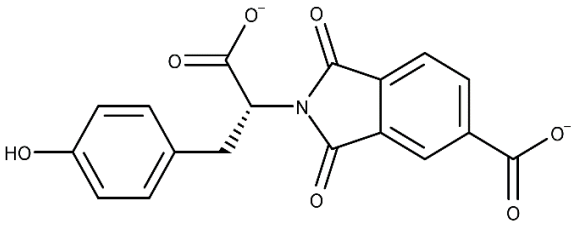
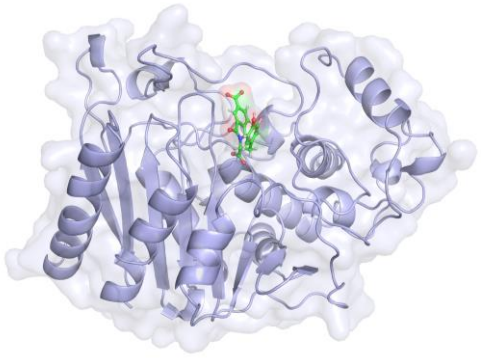
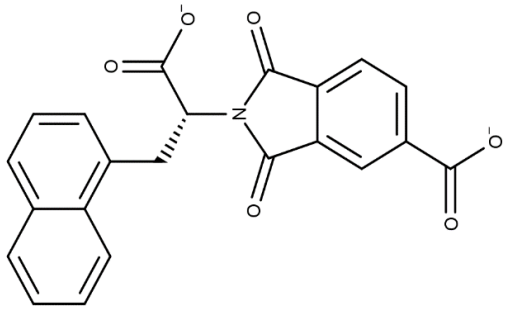
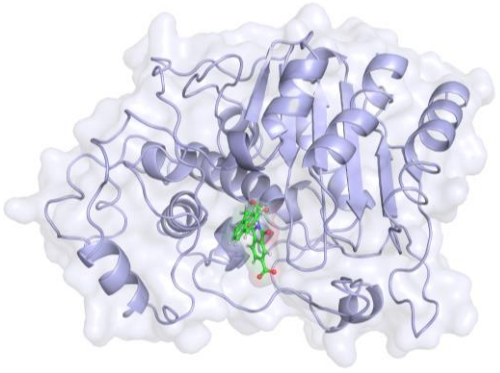
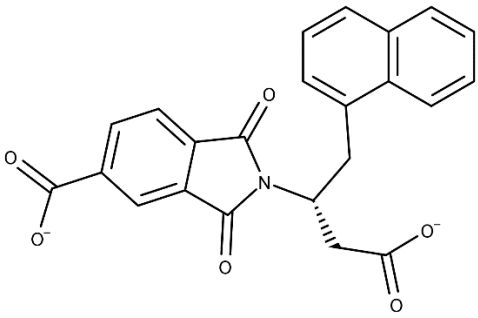
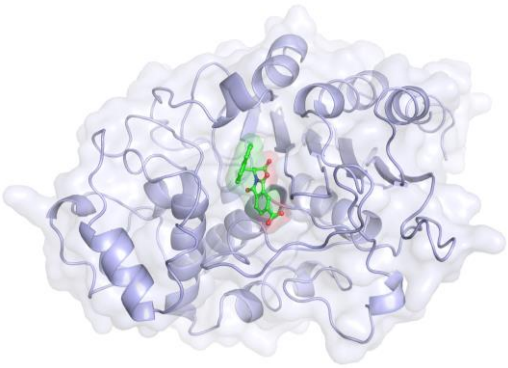
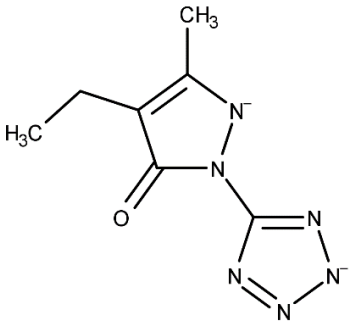
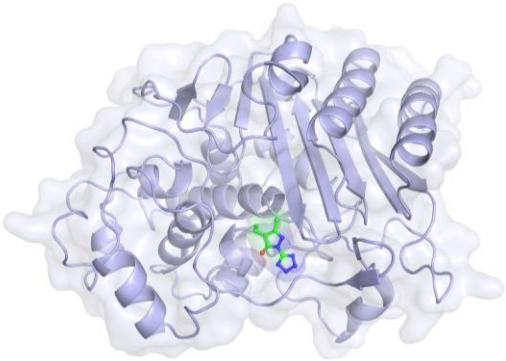
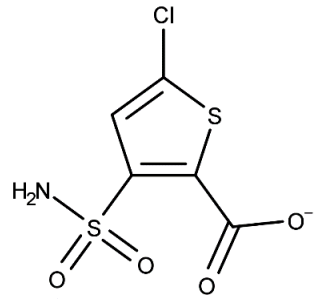
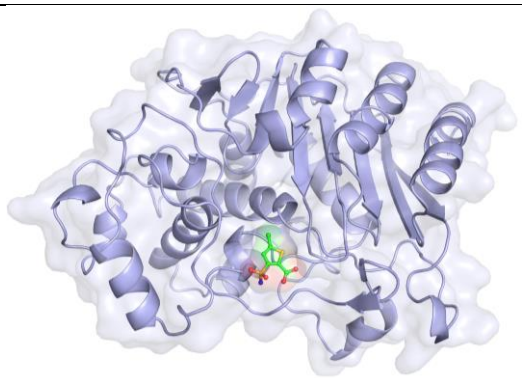
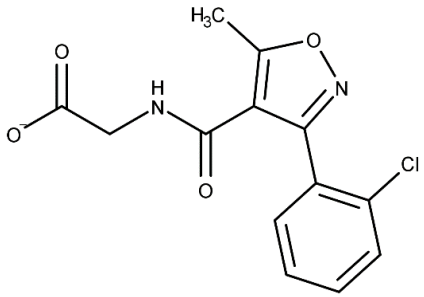
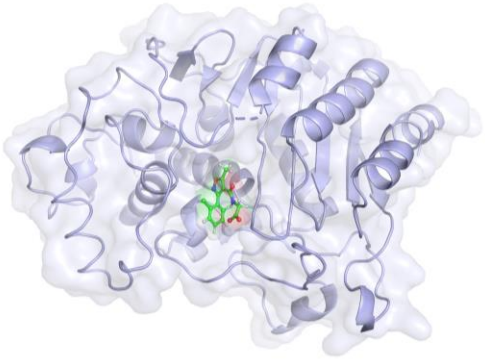
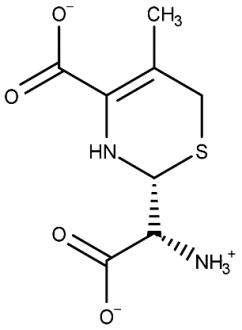
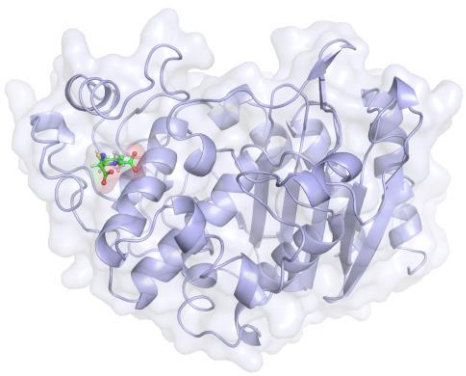
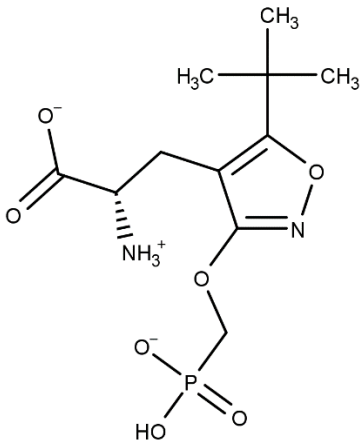
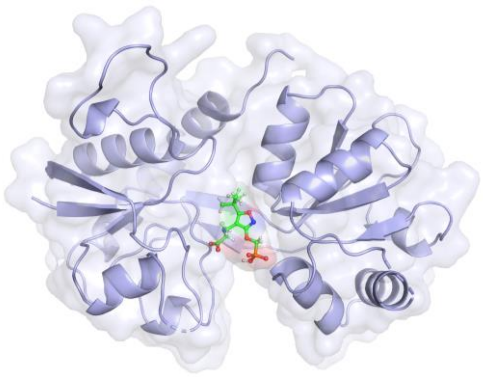
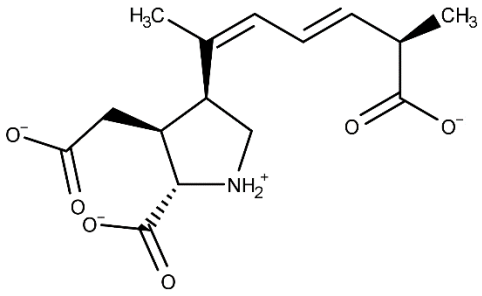
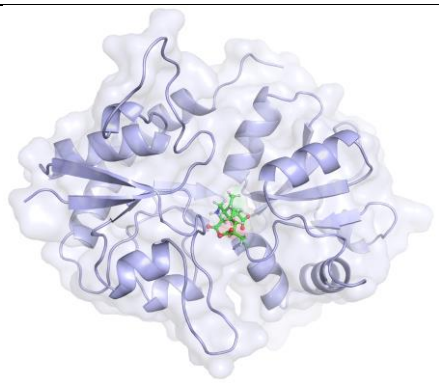
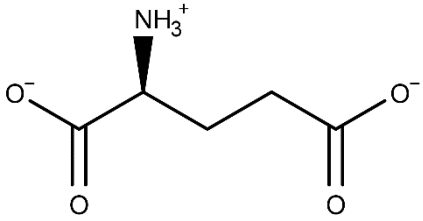
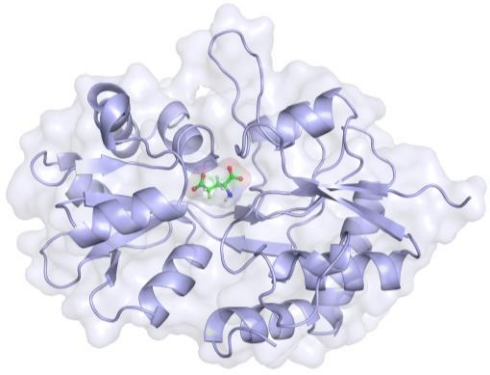
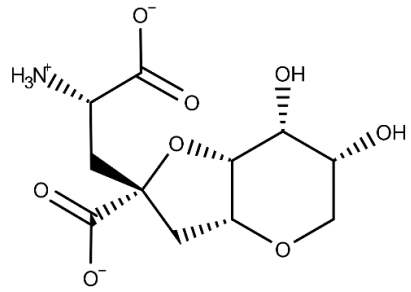
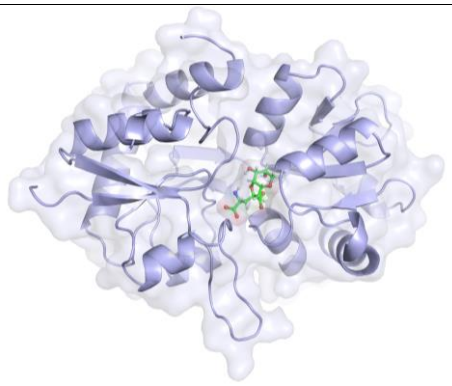
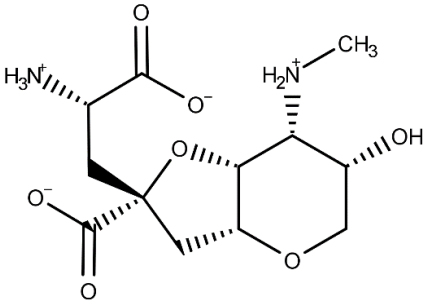
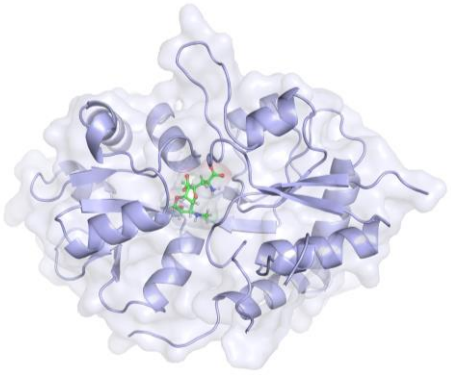
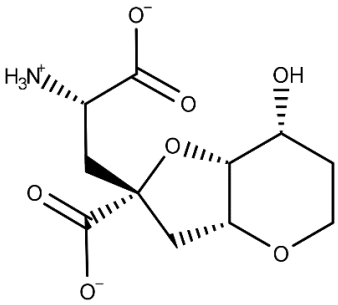
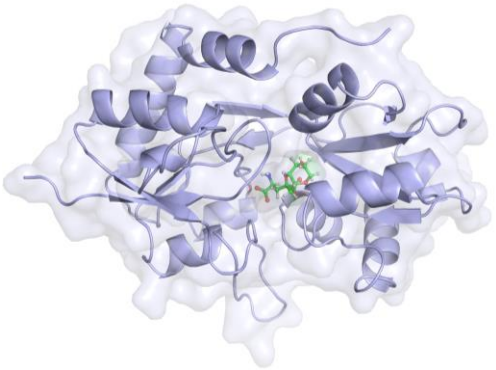
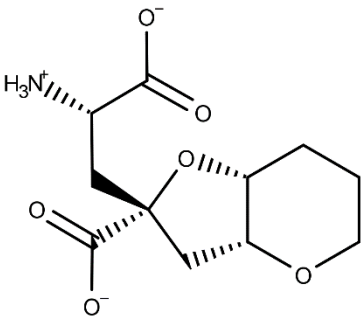
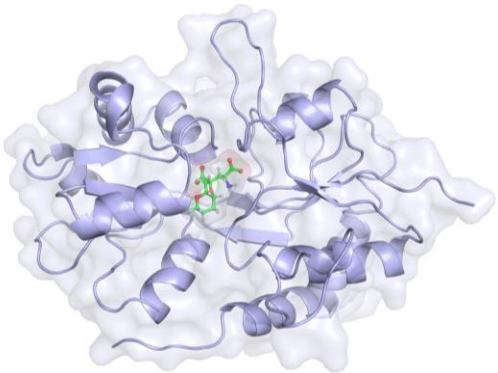
Table S1. 2D and 3D structure for all ligands of AmpC, GluK1, Hsp90 and SARS-CoV-2 Mpro systems

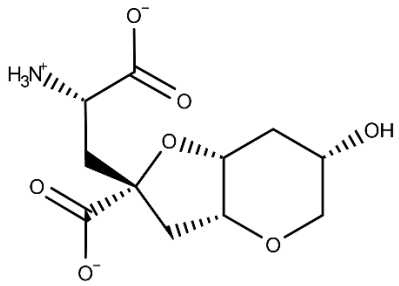
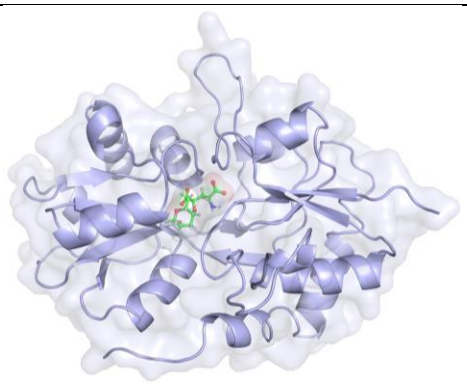
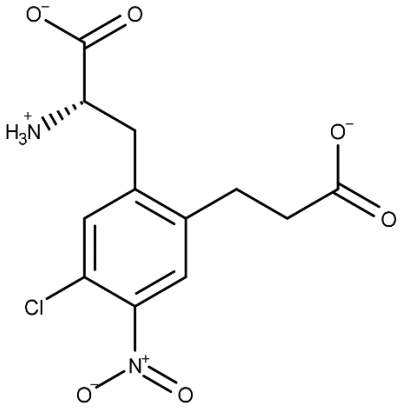
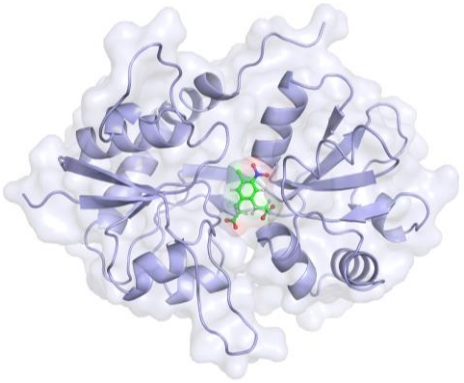
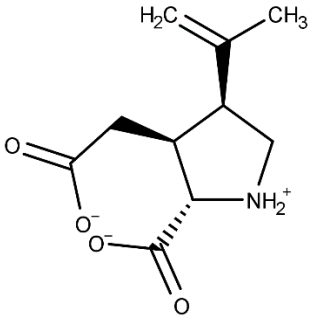
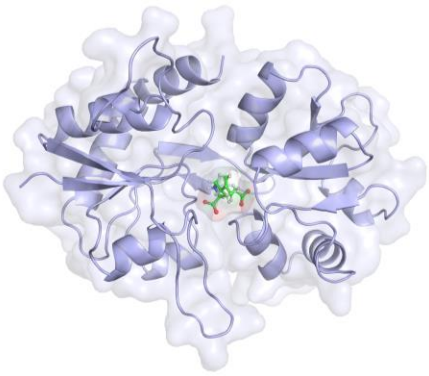
N⁰	PDB ID	2D Structure	3D Structure
1	1XGI		
2	1XGJ		
3	2HDU		

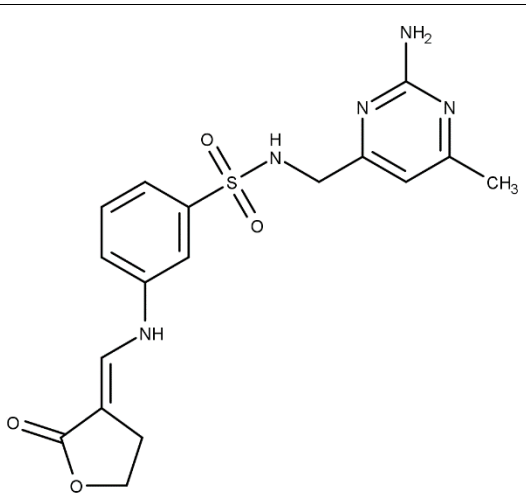
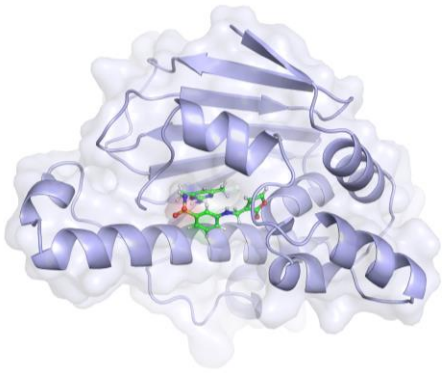
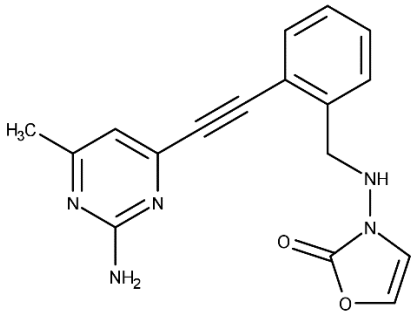
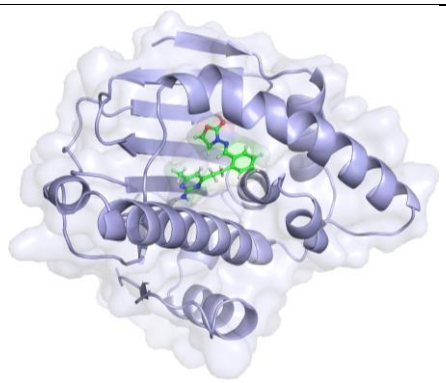
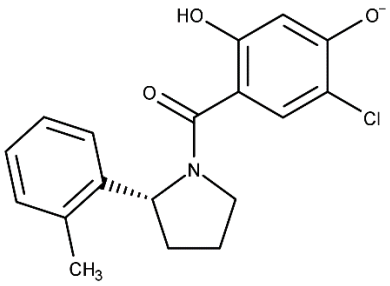
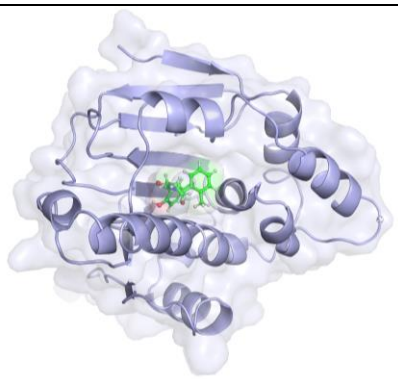
4	**2PU2**		
5	**2R9W**		
6	**2R9X**		
7	**3GR2**		

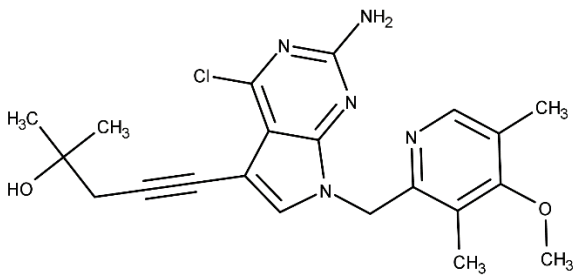
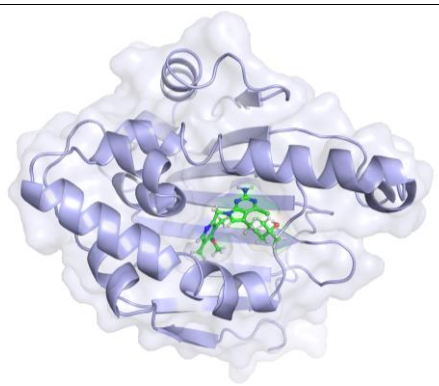
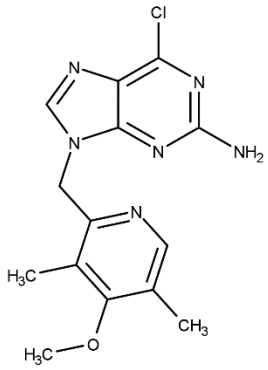
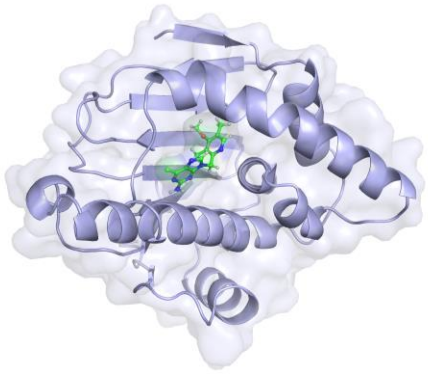
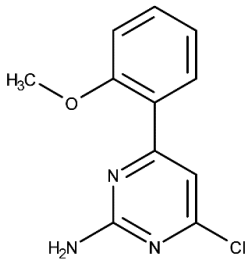
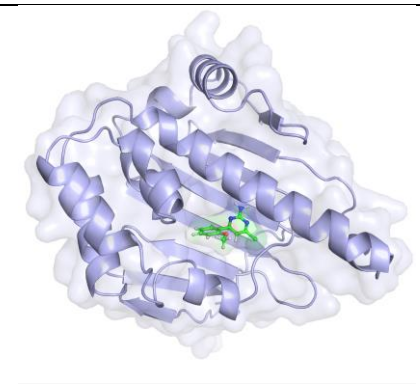
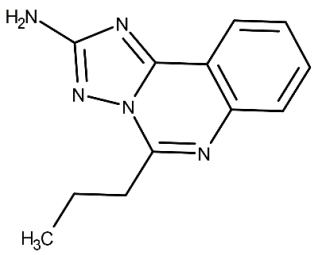
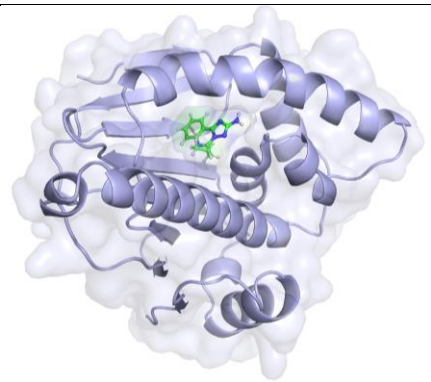
8	**4KZ3**		
9	**4KZ5**		
10	**4OKP**		

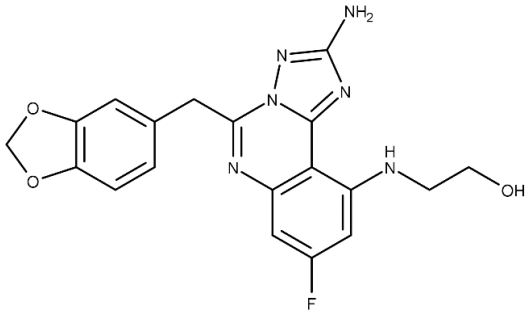
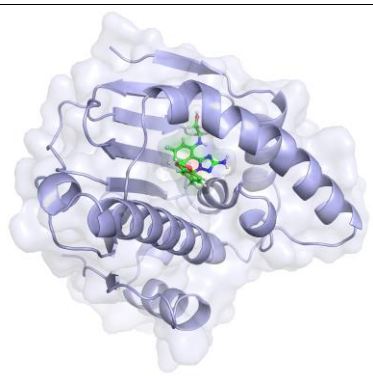
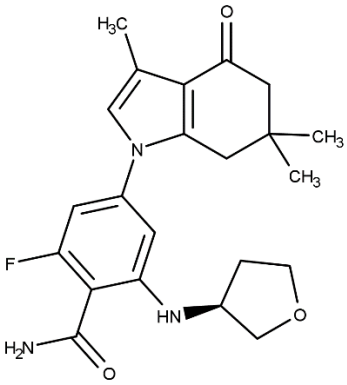
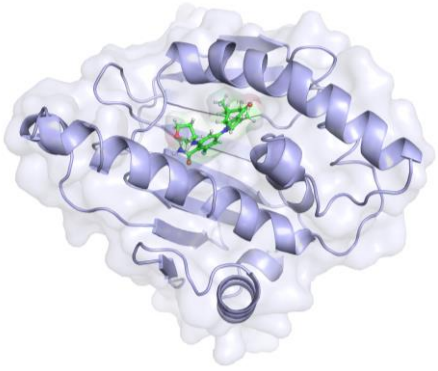
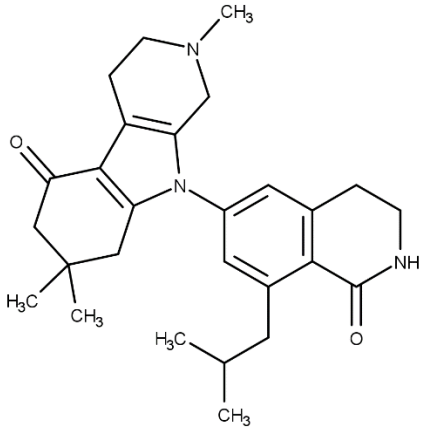
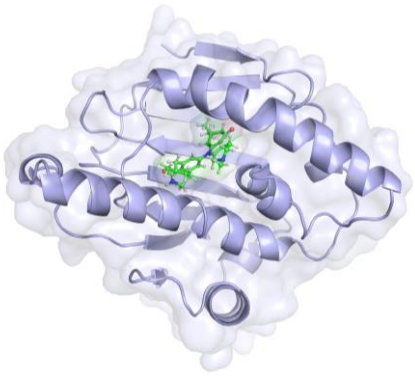
11	**1VSO**		
12	**2PBW**		
13	**2ZNS**		

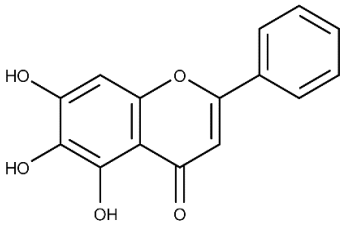
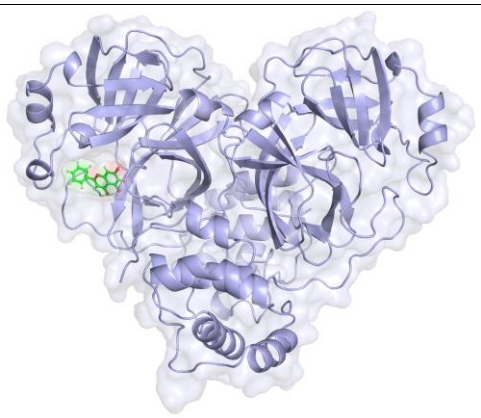
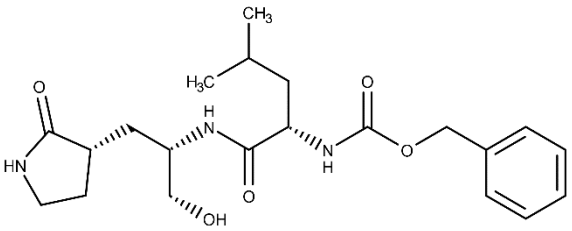
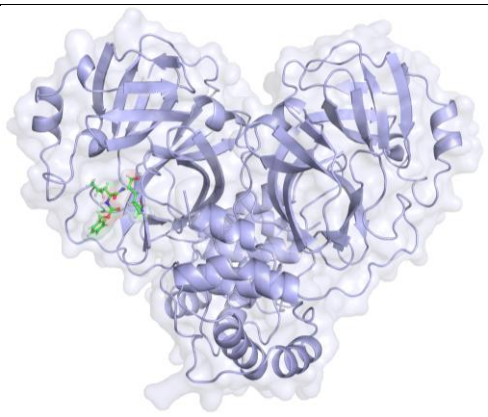
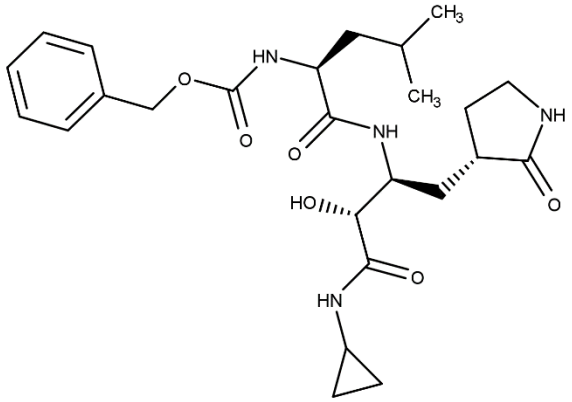
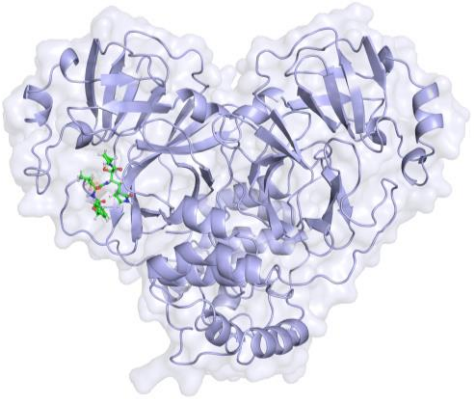
14	**2ZNU**		
15	**3FV1**		
16	**3FVN**		
17	**3FVG**		

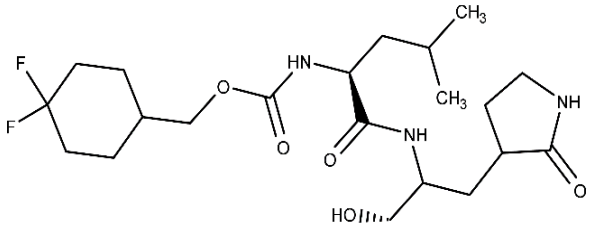
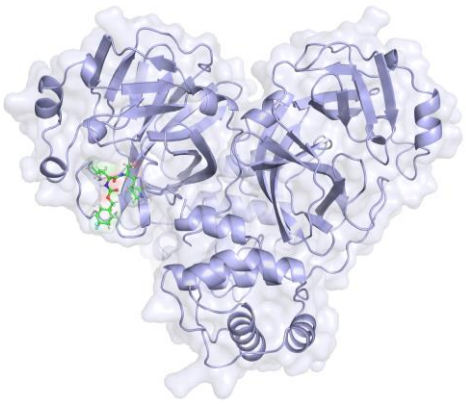
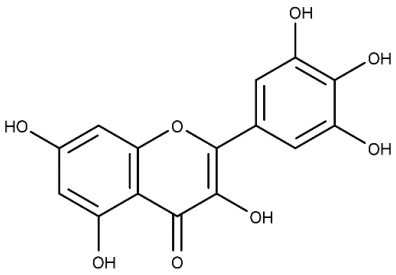
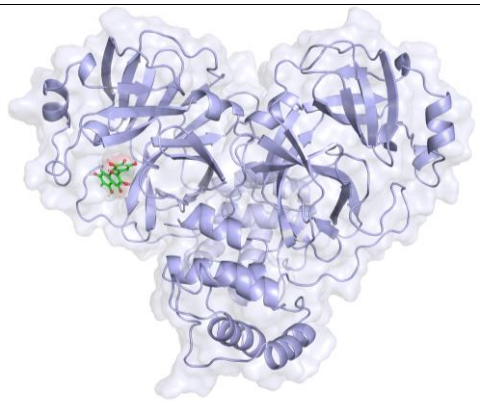
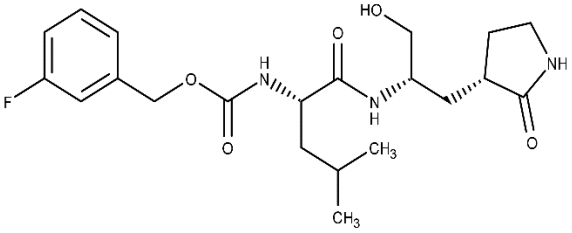
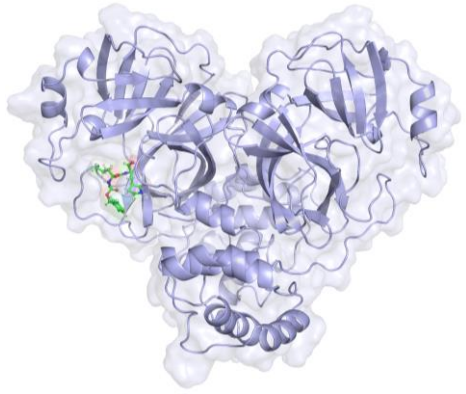
18	**3FVK**		
19	**4DLD**		
20	**4E0X**		

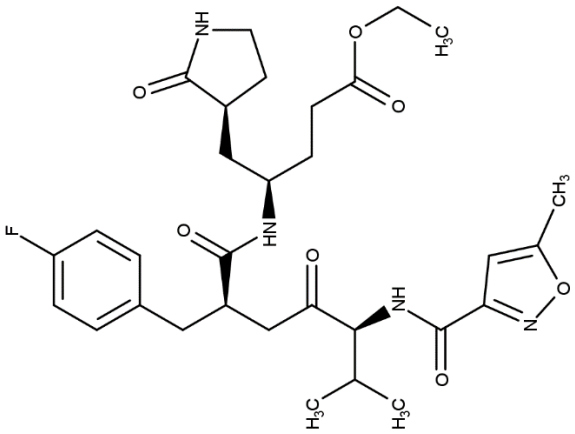
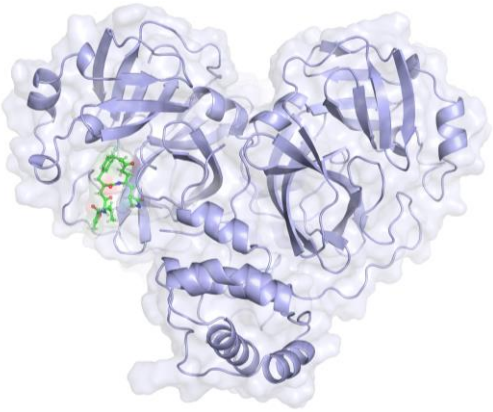
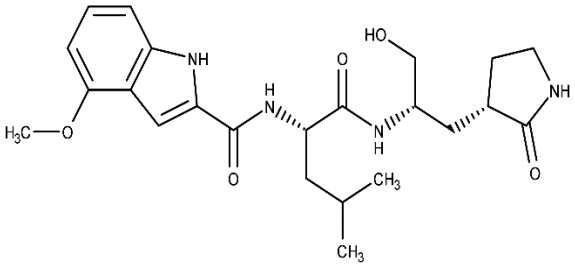
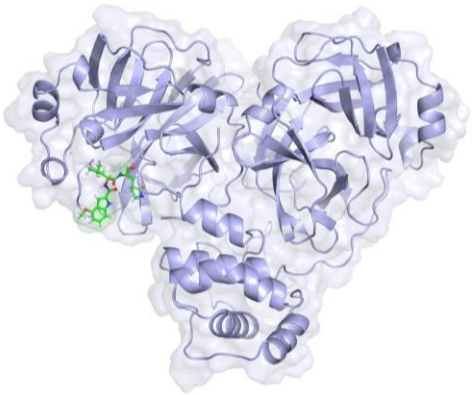
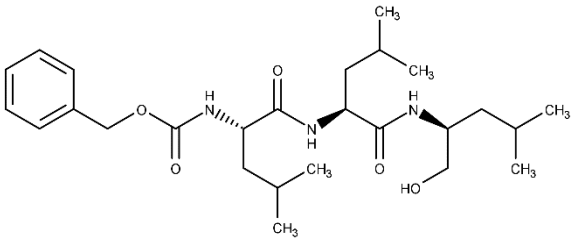
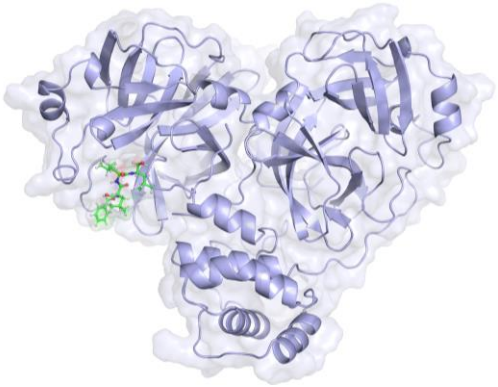
21	**2QG0**		
22	**2QG2**		
23	**3K97**		

24	**3NMQ**		
25	**3QDD**		
26	**3R4M**		
27	**4CWF**		

28	**4CWT**		
29	**4NH8**		
30	**4O07**		

31	**6M2N**		
32	**6WTT**		
33	**6XBG**		

34	**6XMK**		
35	**7B3E**		
36	**7LCR**		

37	**7L8I**		
38	**7LDL**		
39	**7NG3**		

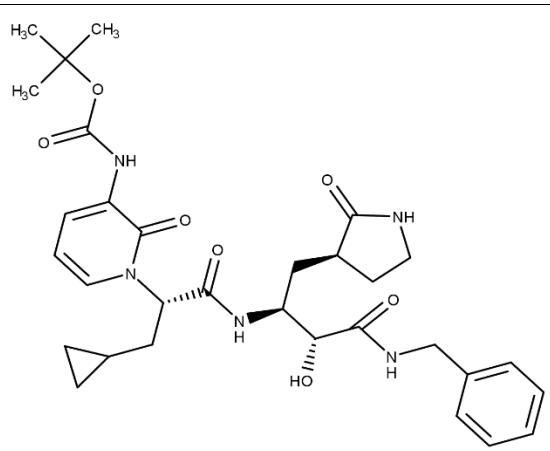
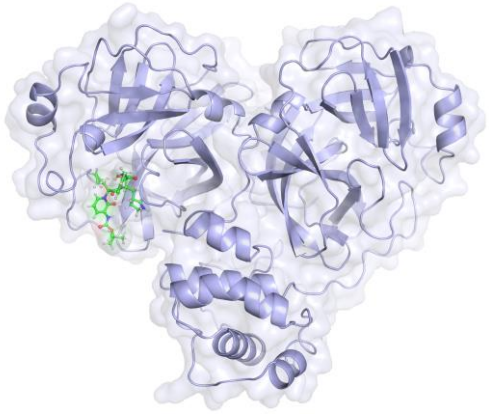
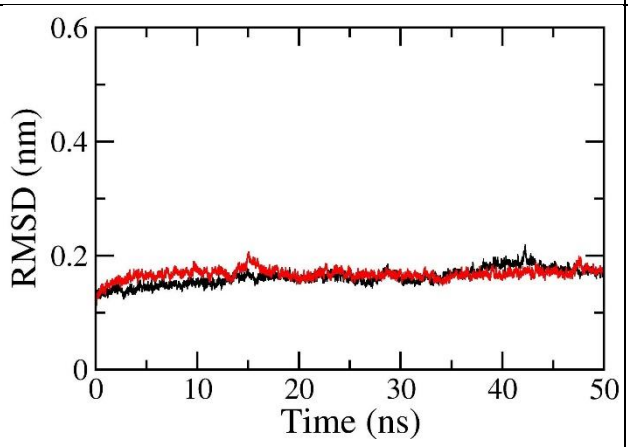
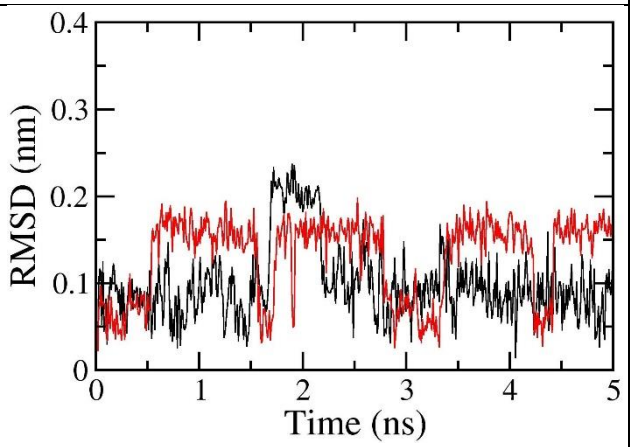
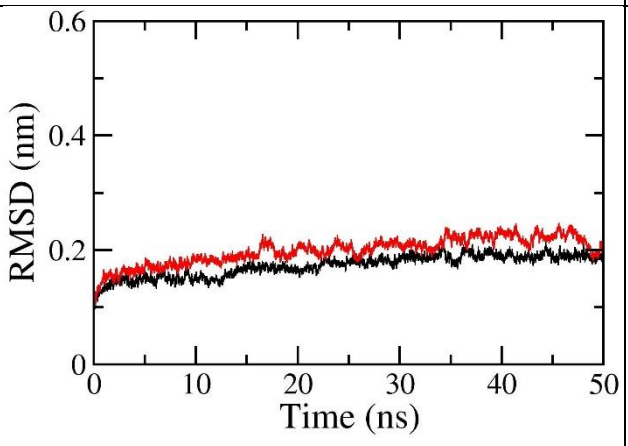
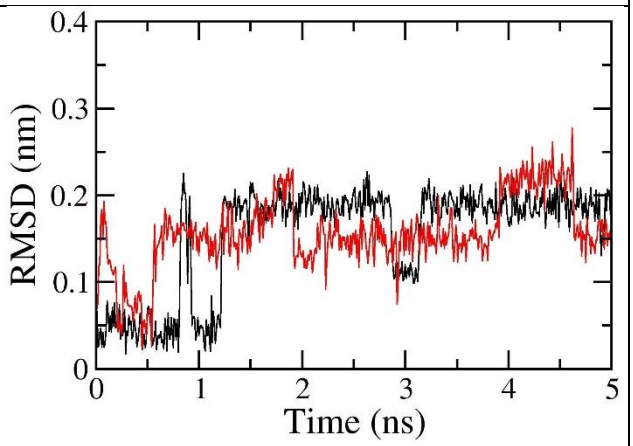
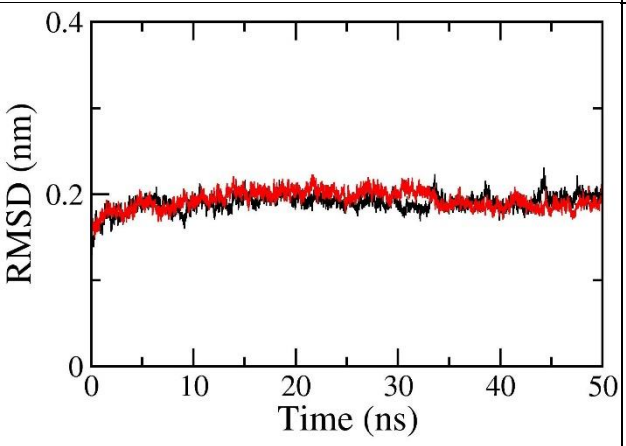
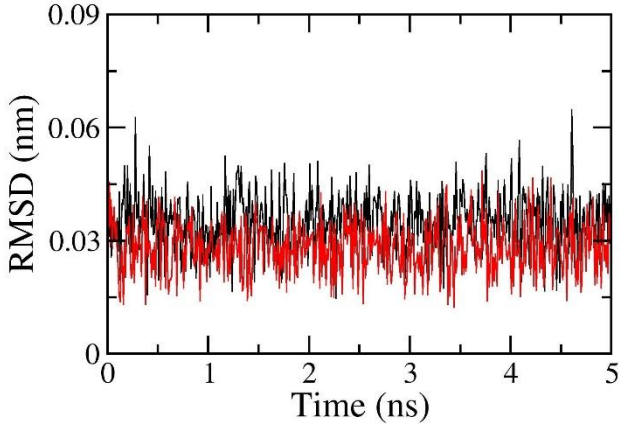
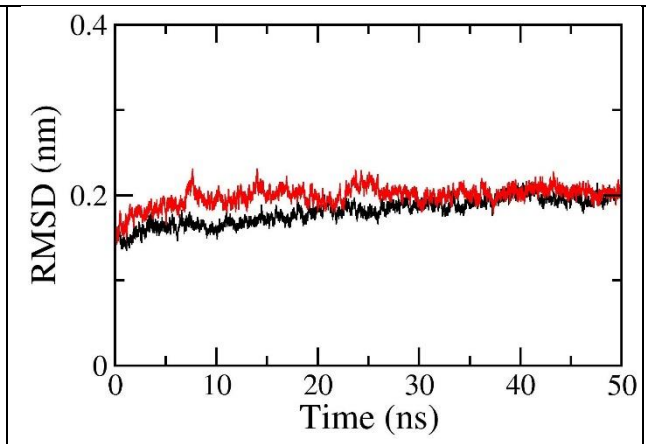
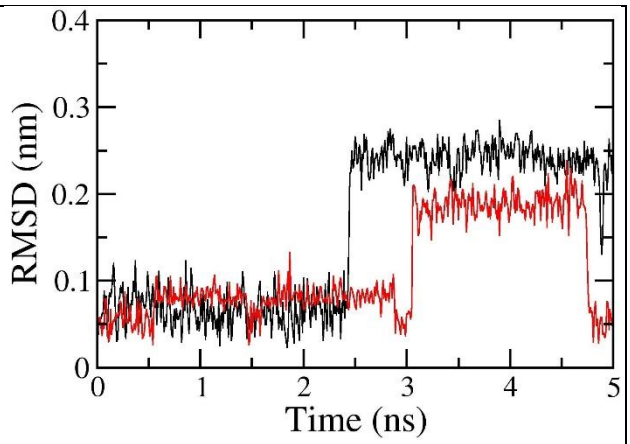
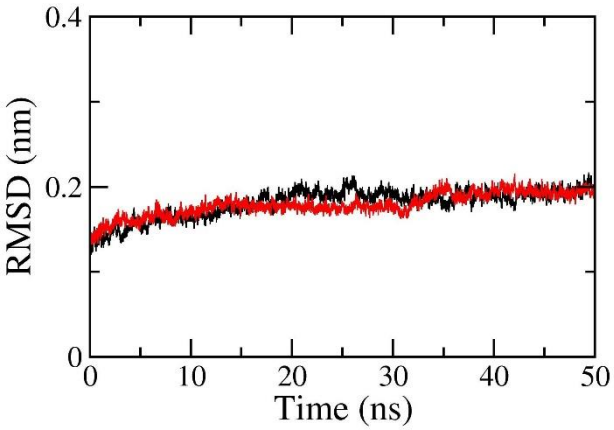
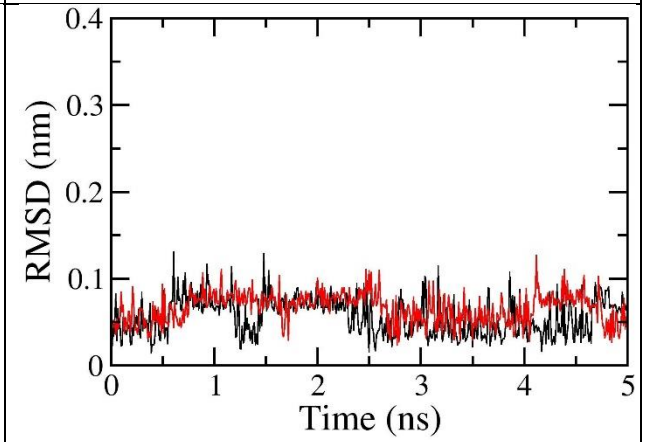
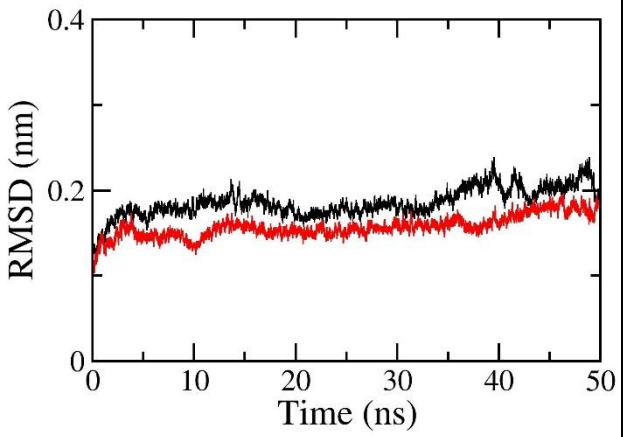
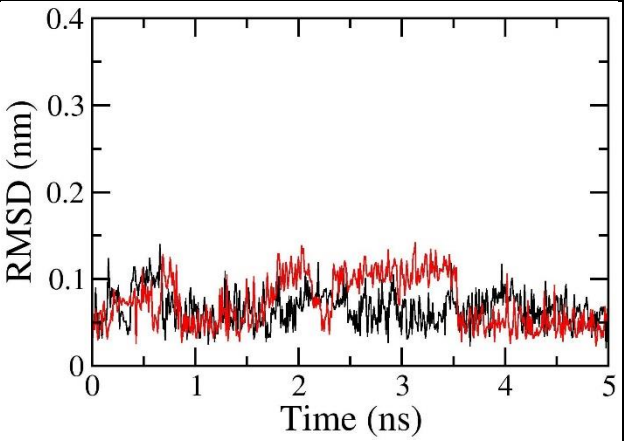
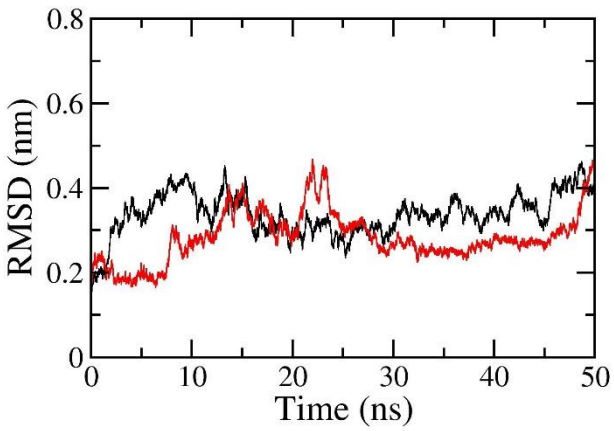
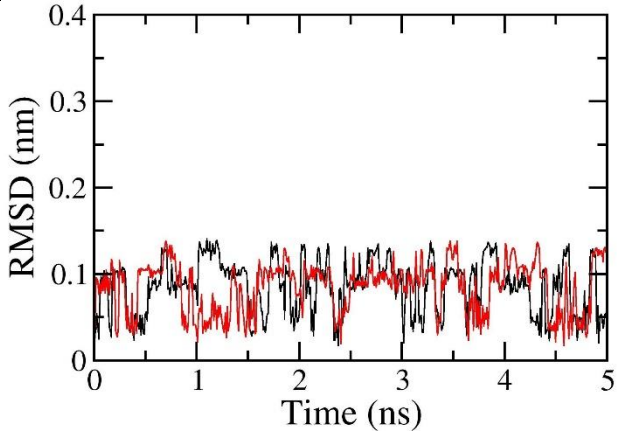
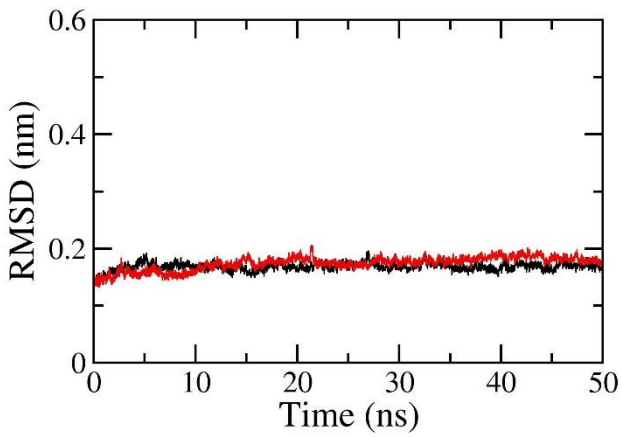
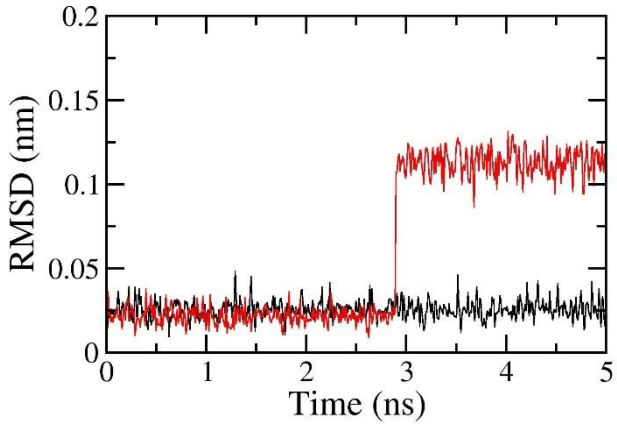
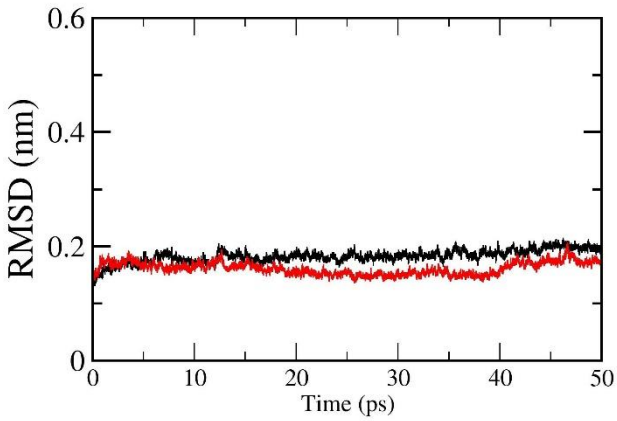
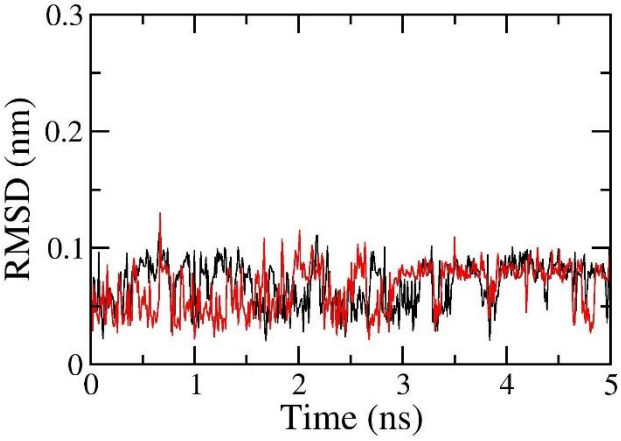
40	**8A4T**		

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

N⁰	PDB ID	Complex in Solution	Ligand in Solution
1	1XGI		
2	1XGJ		
3	2HDU		

4	2PU2		
5	2R9W		
6	2R9X		

7	3GR2		
8	4KZ3		
9	4KZ5		

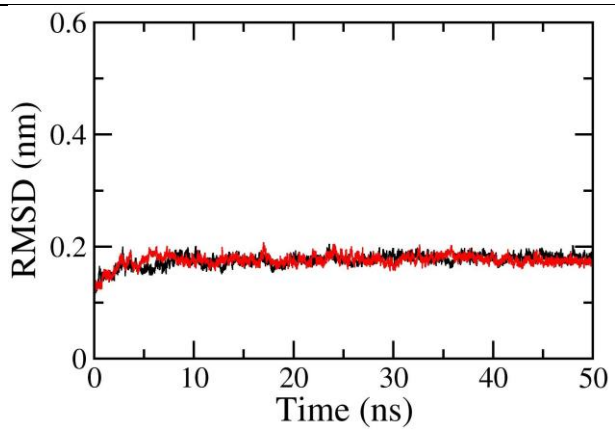
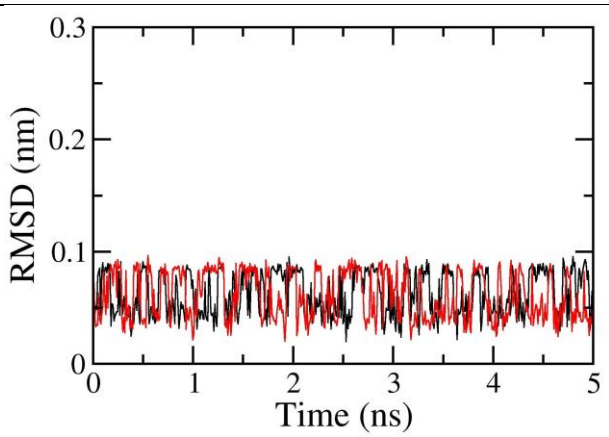
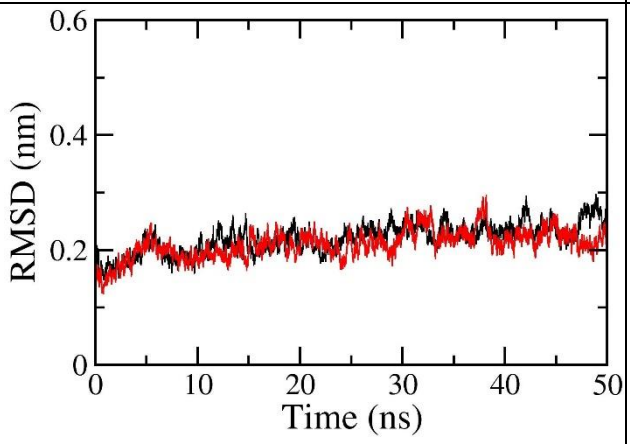
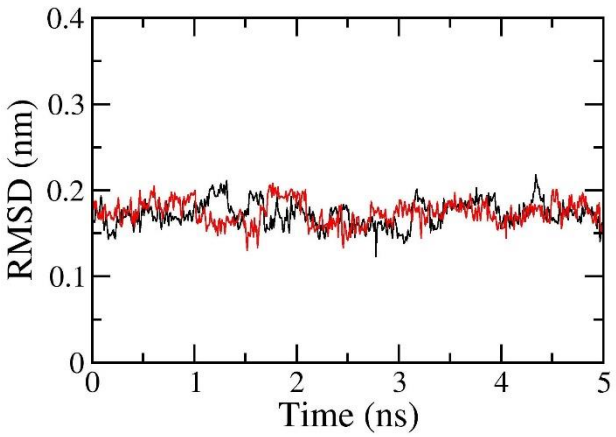
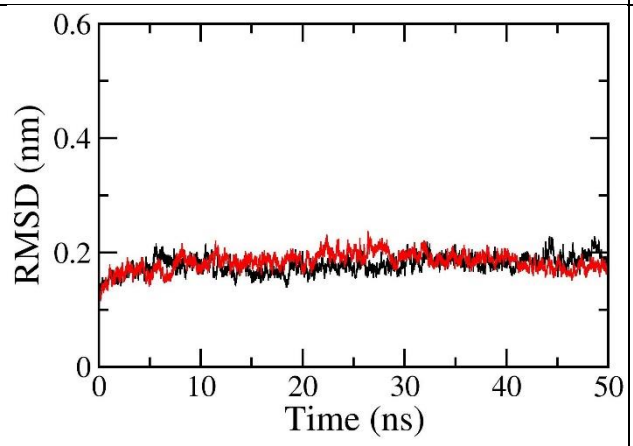
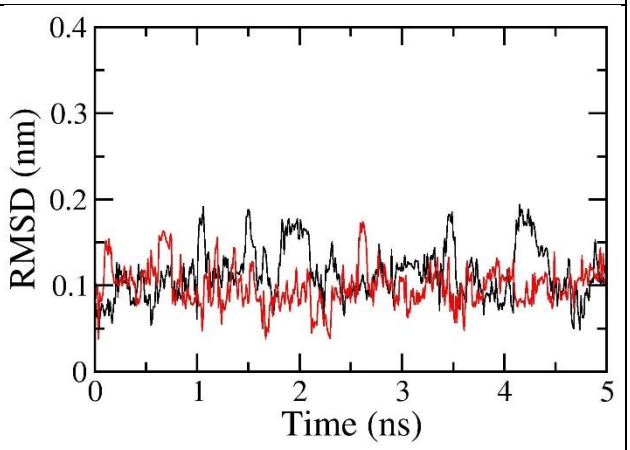
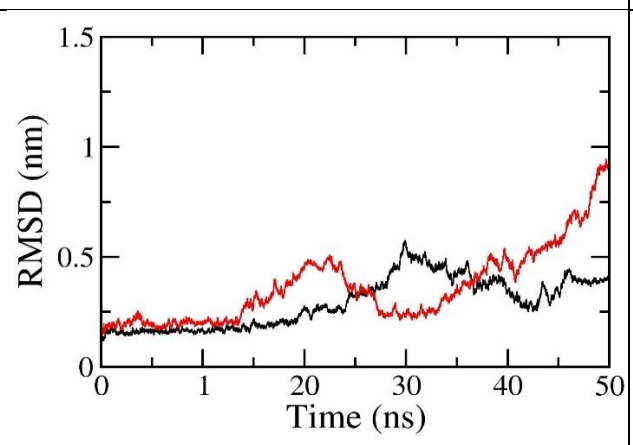
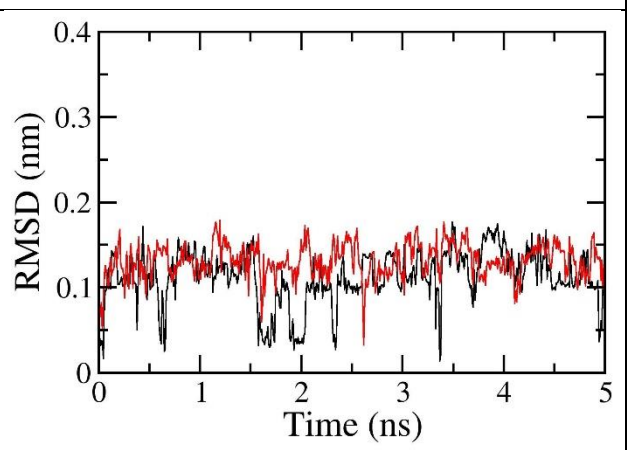
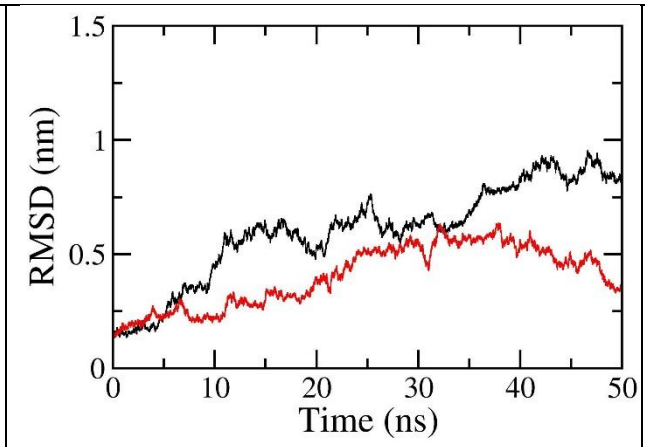
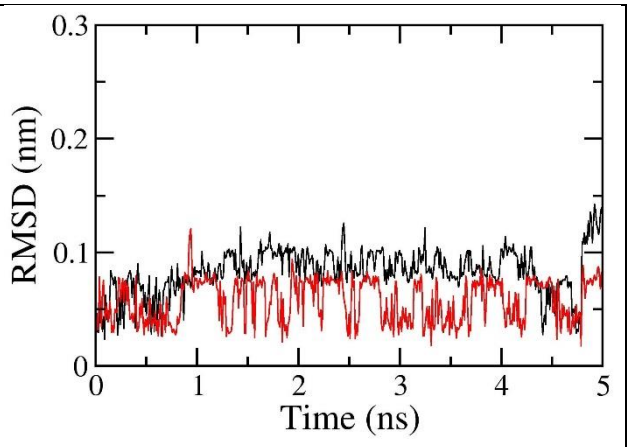
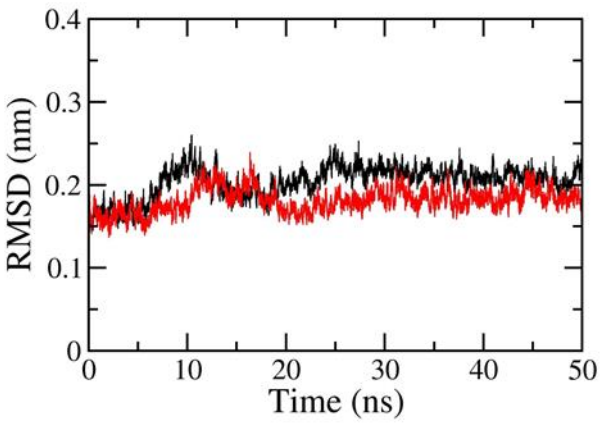
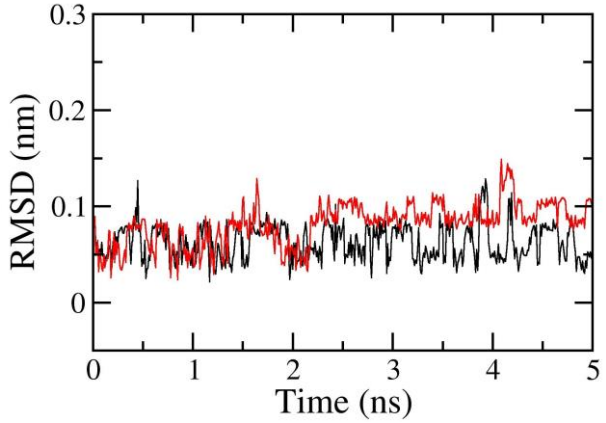
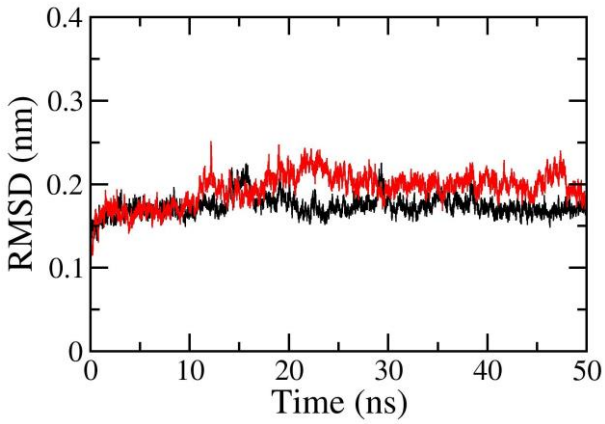
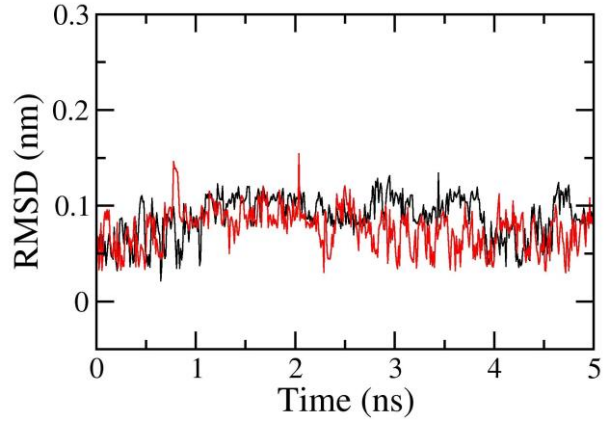
10	**4OKP**	 RMSD (nm) 0.6 0.4 0.2 0 0 10 20 30 40 50 Time (ns)	 RMSD (nm) 0.3 0.2 0.1 0 0 1 2 3 4 5 Time (ns)

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

N⁰	PDB ID	Complex in Solution	Ligand in Solution
1	1VSO		
2	2PBW		
3	2ZNS		

4	2ZNU		
5	3FKV		
6	3FVN		
7	3FVG		

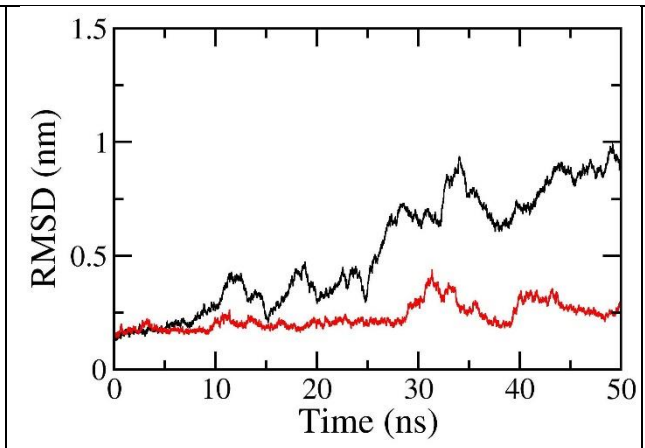
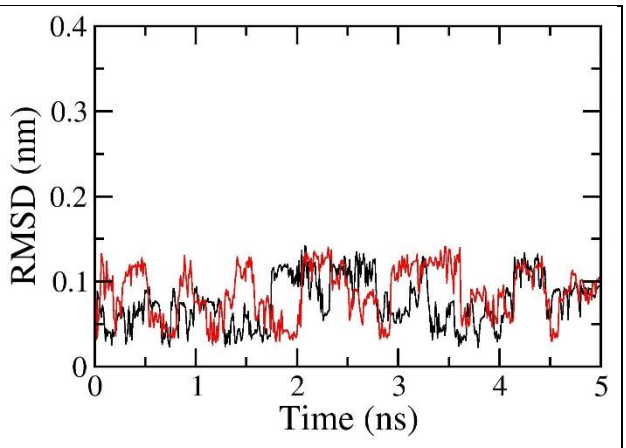
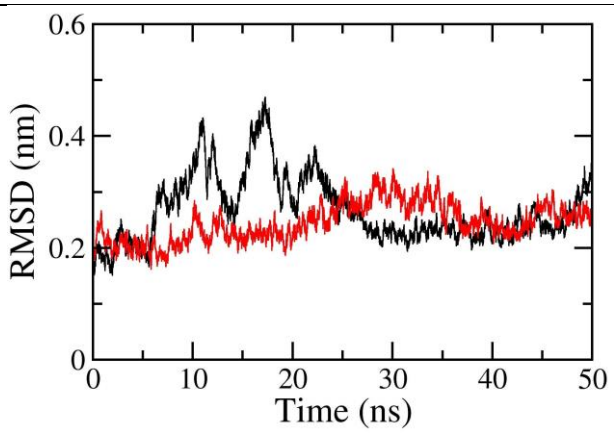
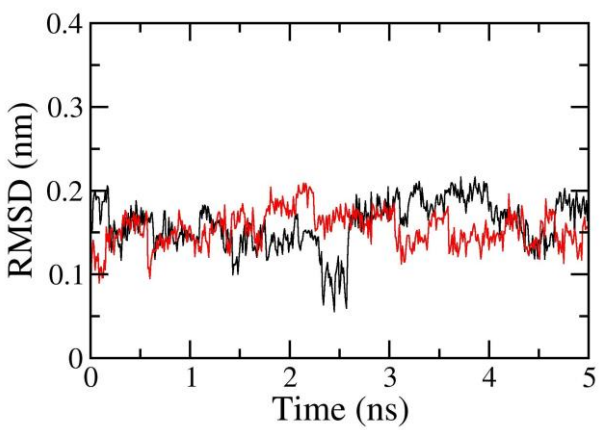
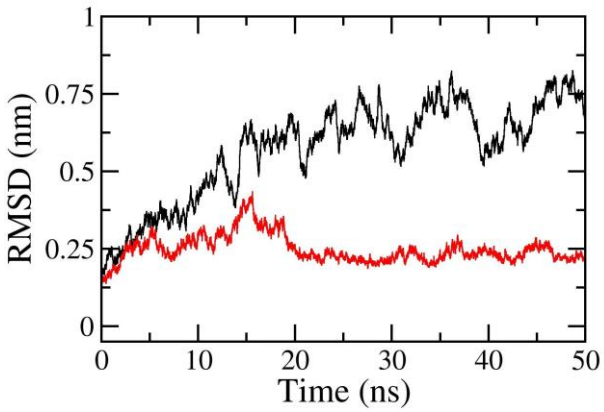
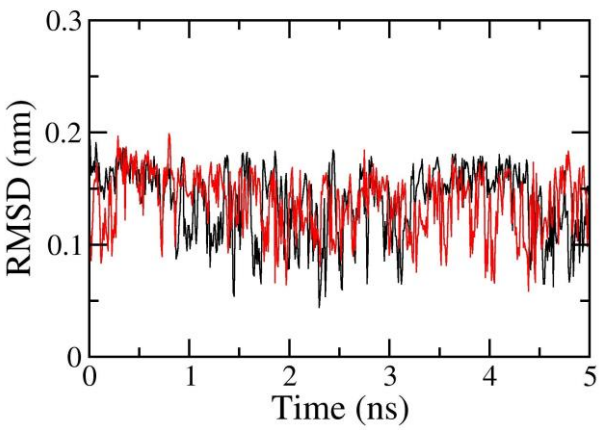
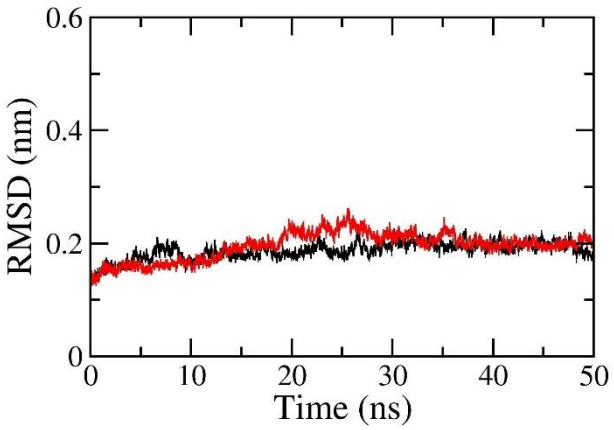
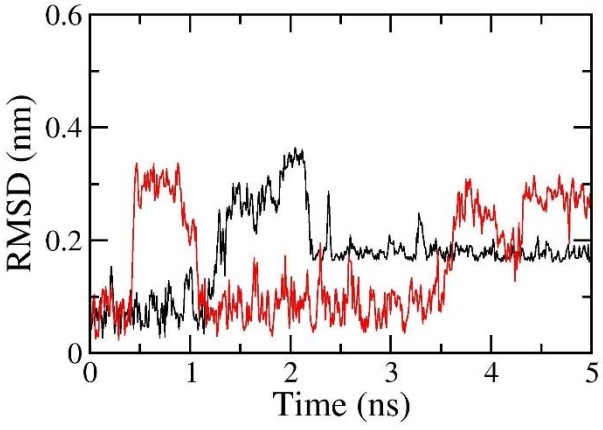
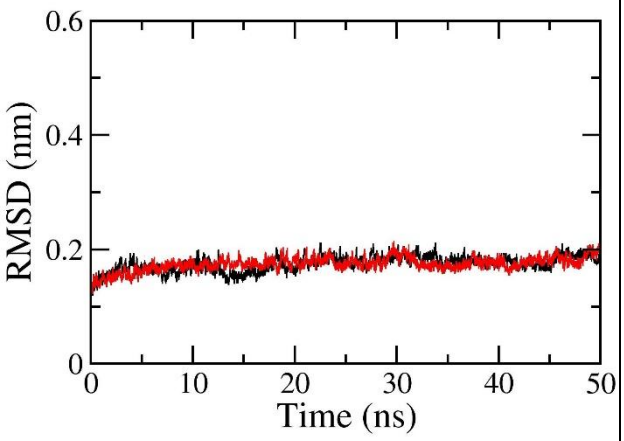
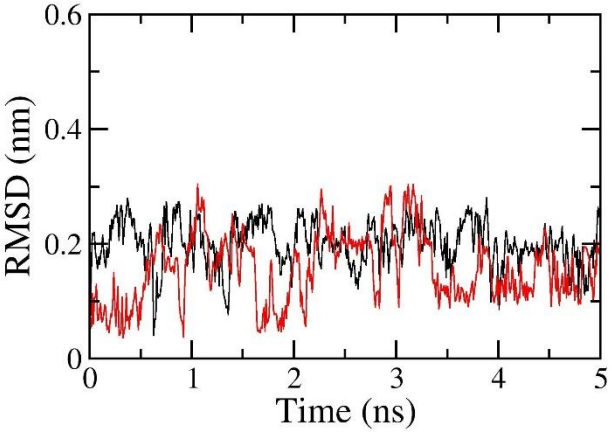
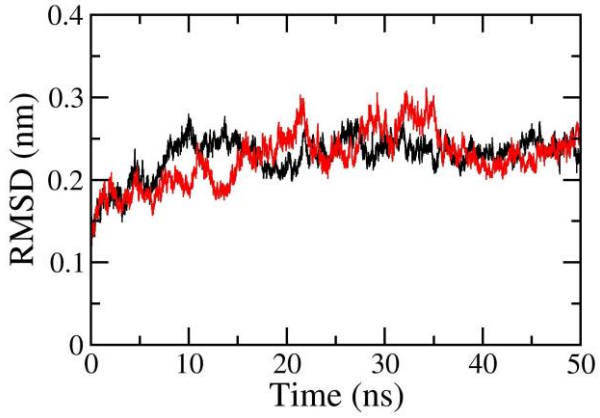
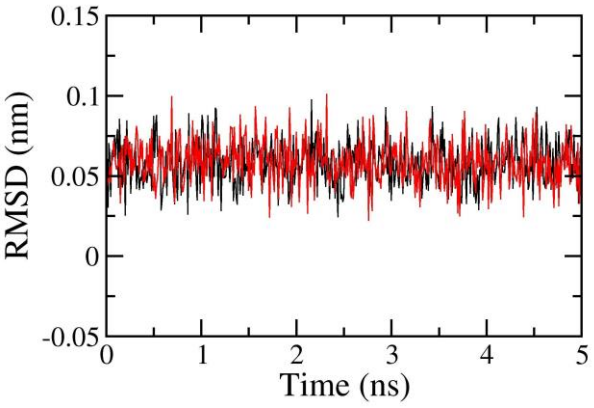
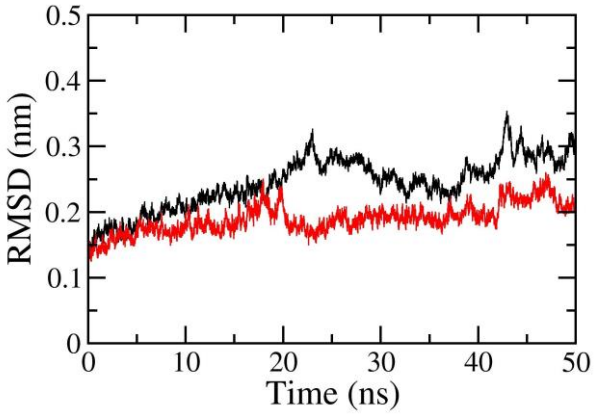
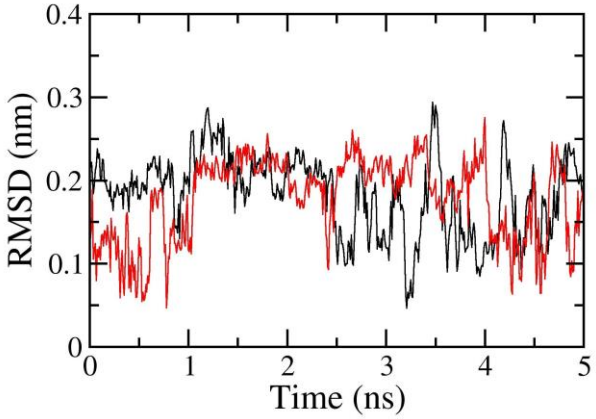
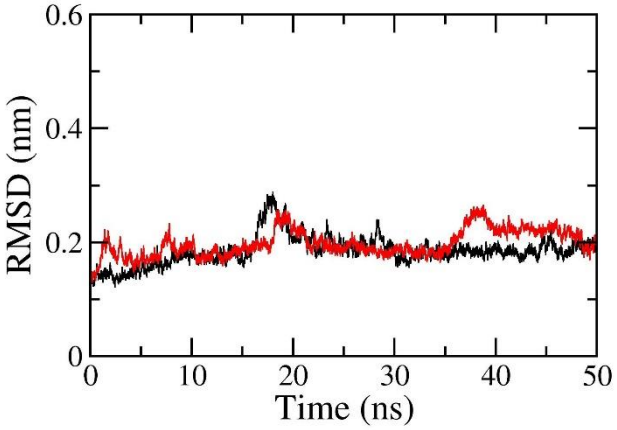
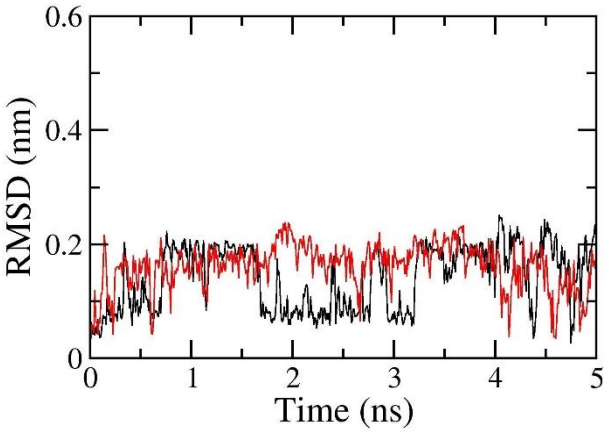
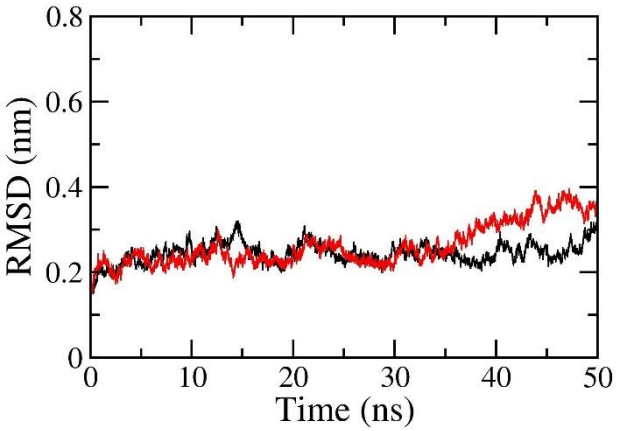
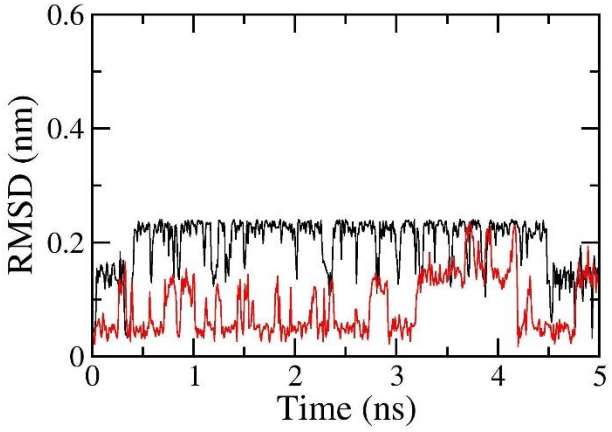
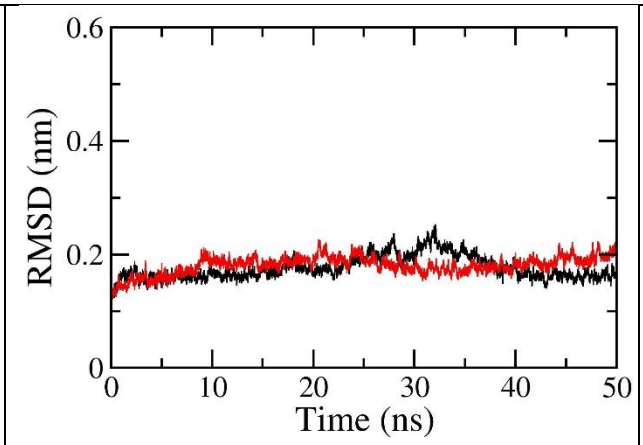
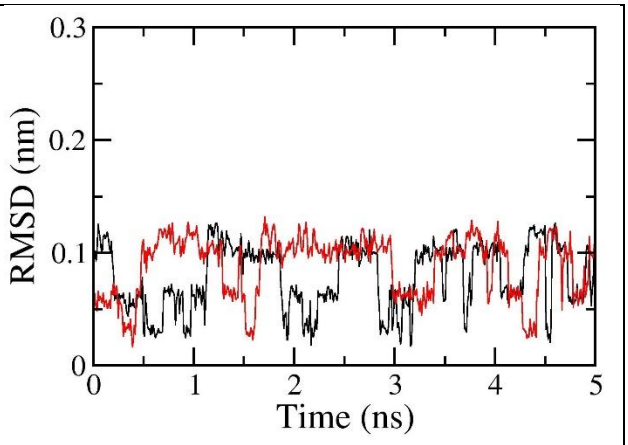
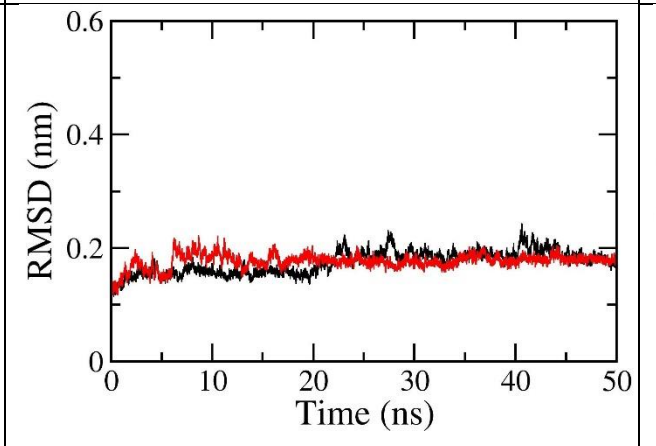
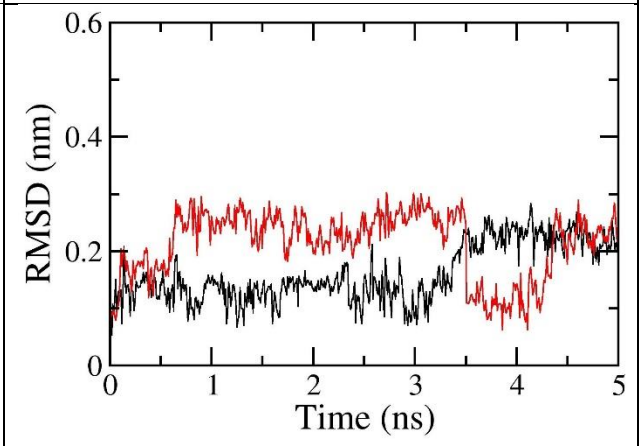
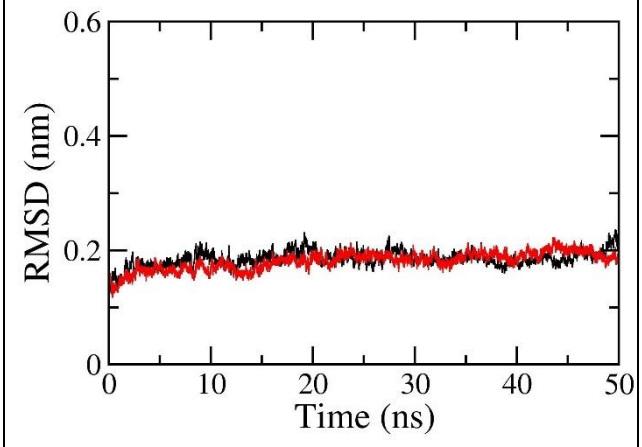
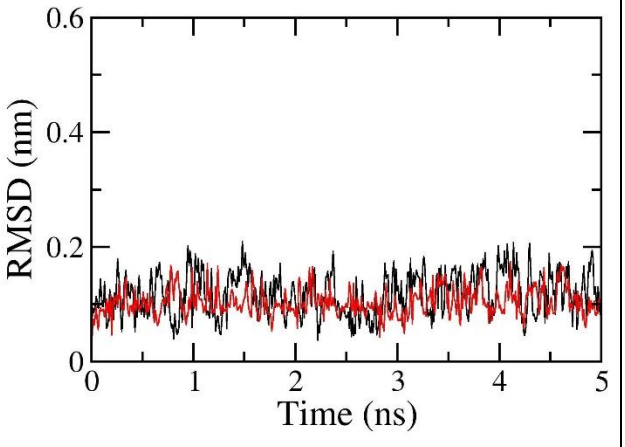
8	3VF1		
9	4DLD		
10	4E0X		

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

N⁰	PDB ID	Complex in Solution	Ligand in Solution
1	2QG0		
2	2QG2		
3	3K97		

4	3NMQ		S 
5	3QDD		
6	3R4M		

7	4CWF		
8	4CWT		
9	4NH8		

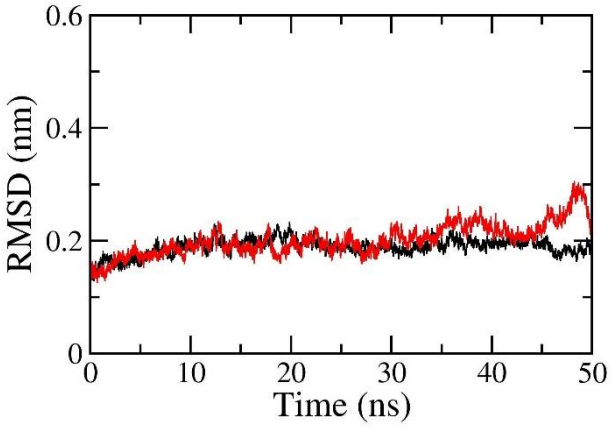
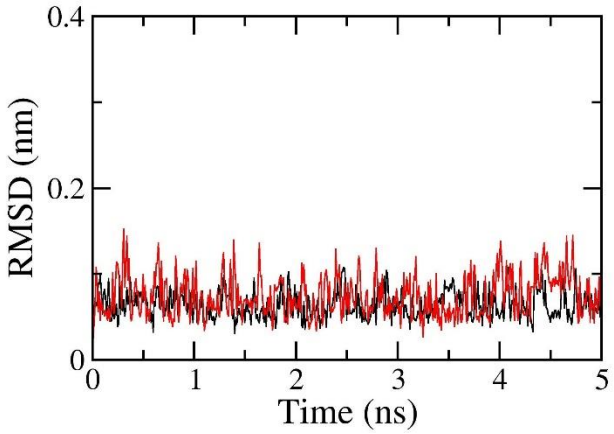
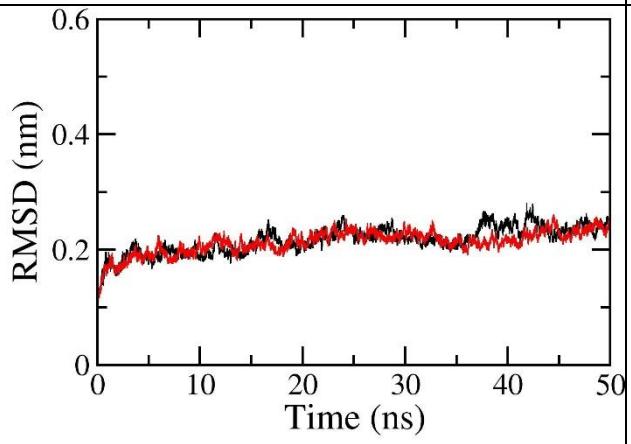
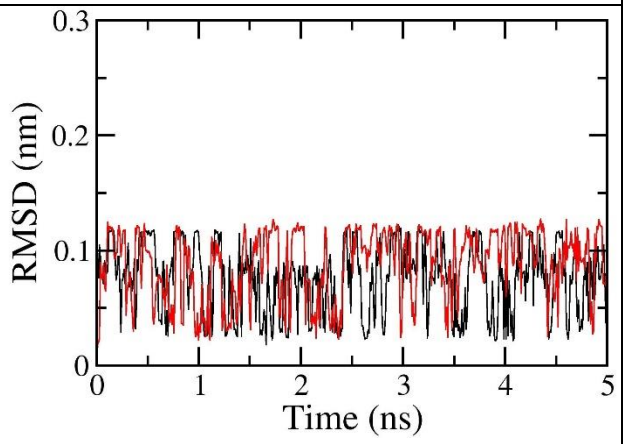
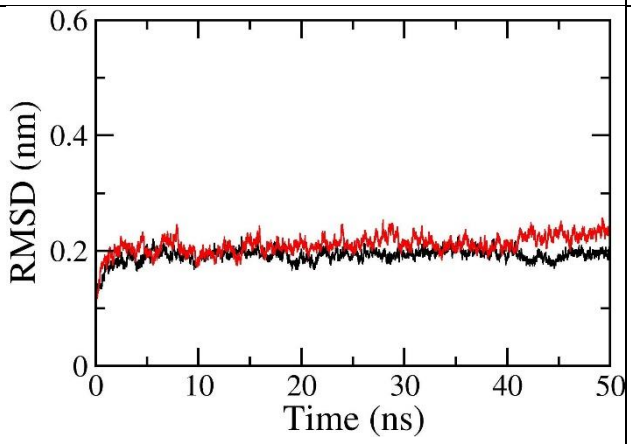
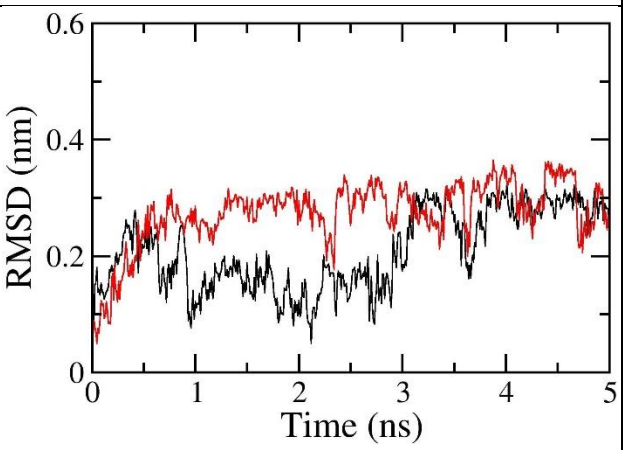
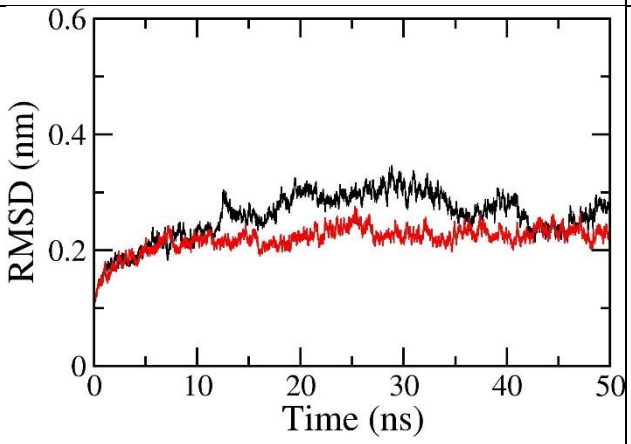
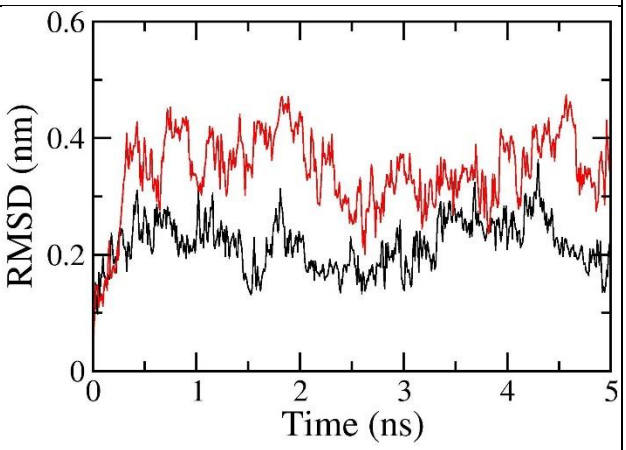
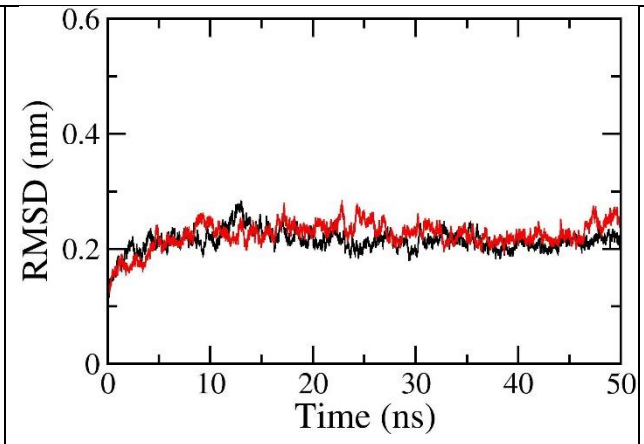
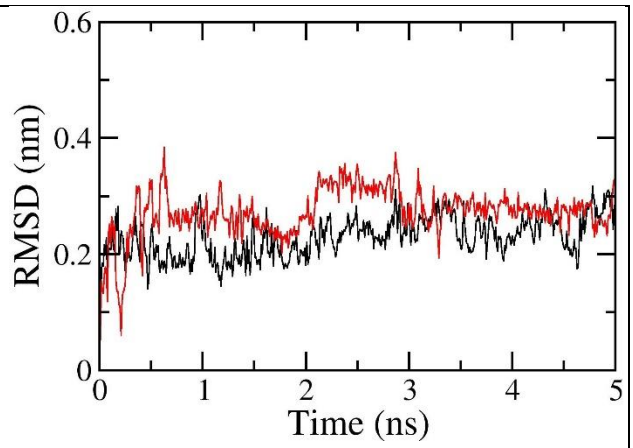
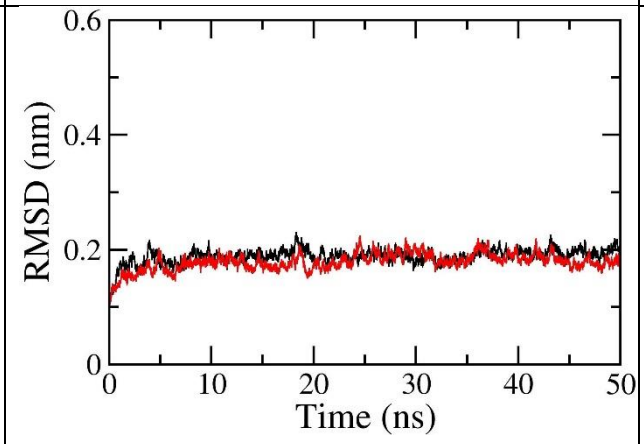
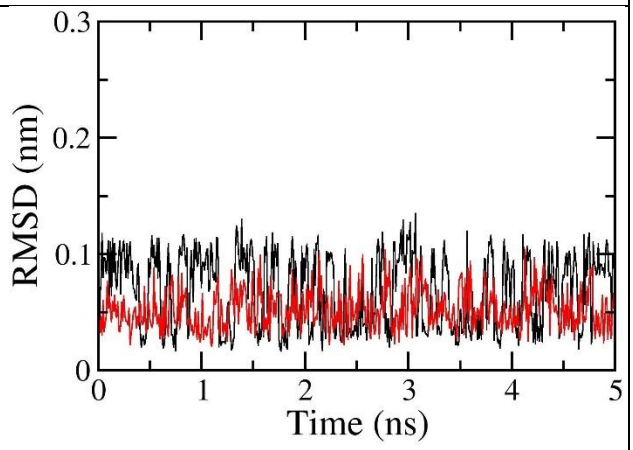
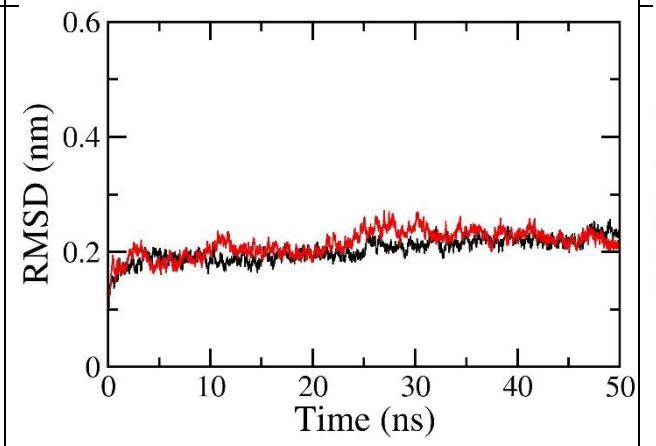
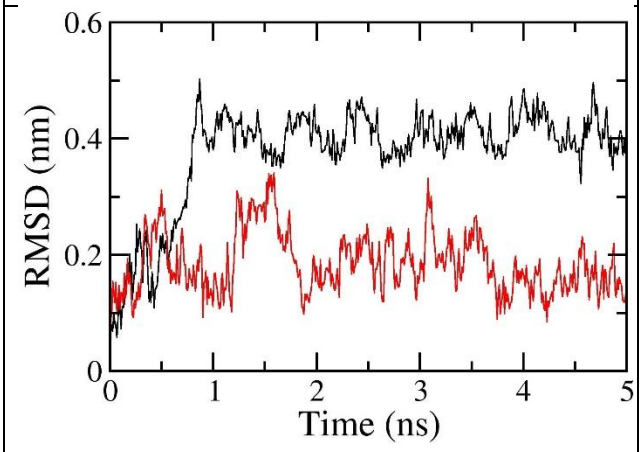
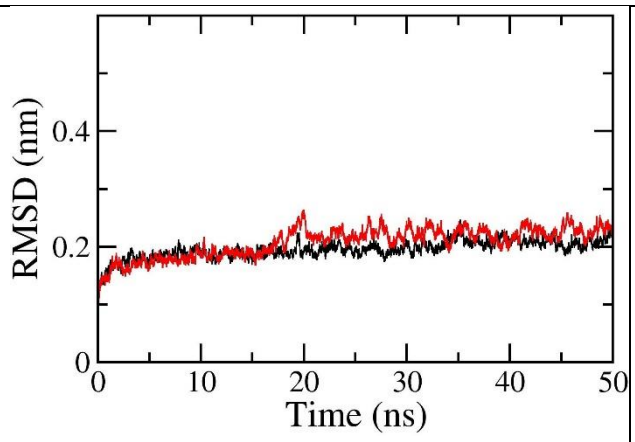
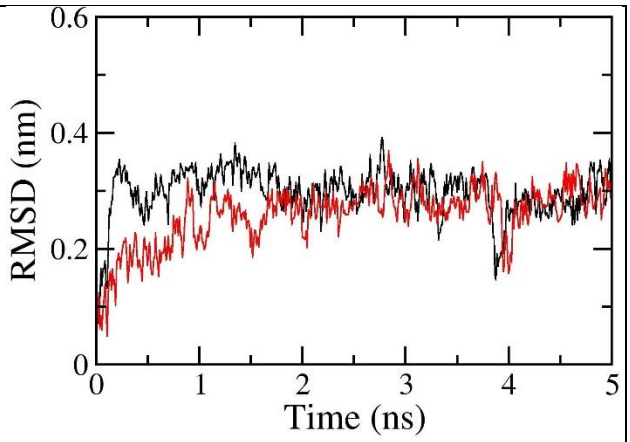
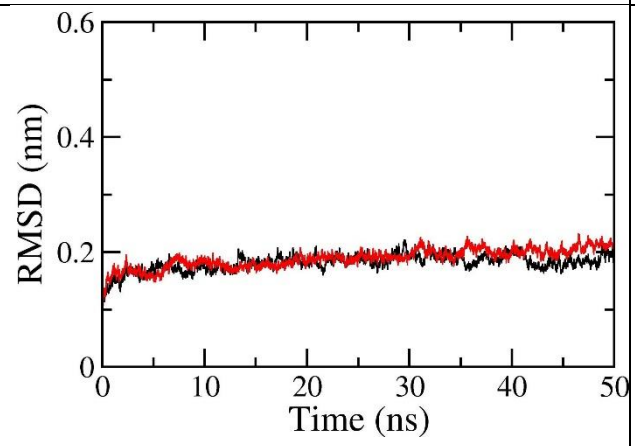
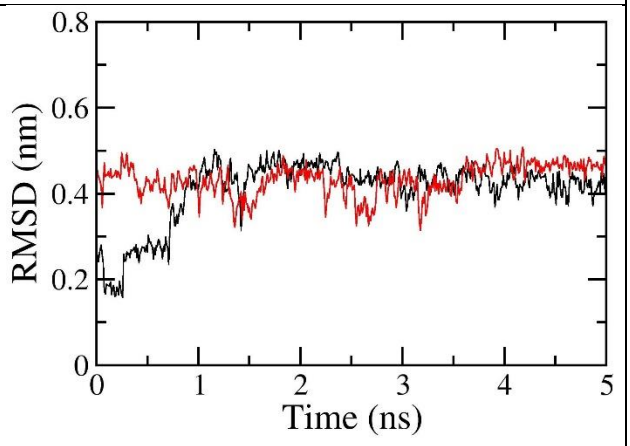
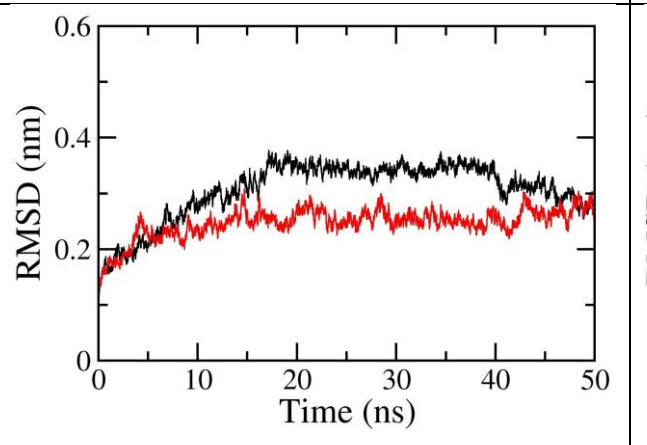
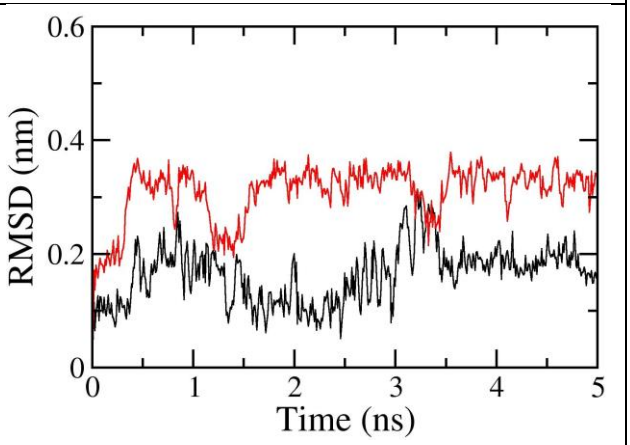
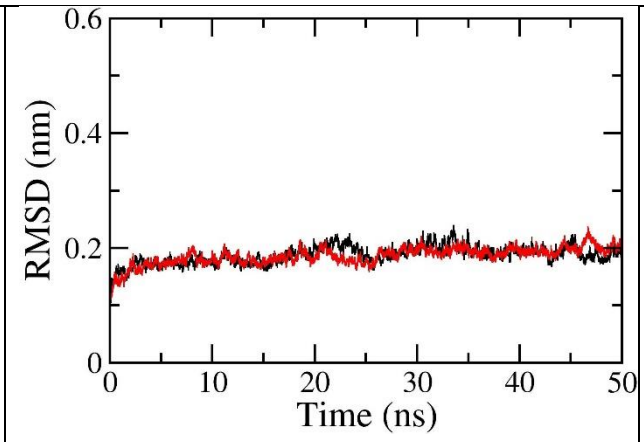
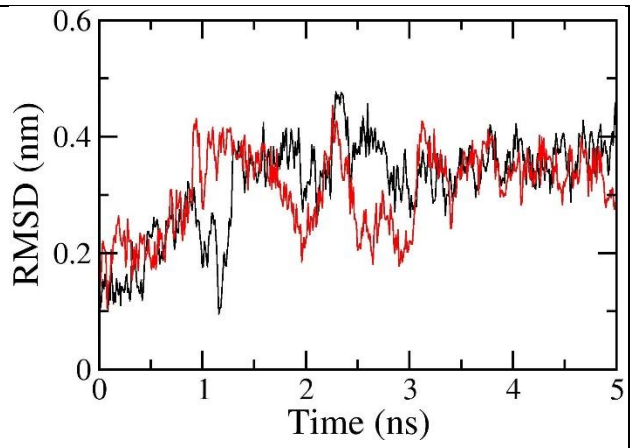
10	**4O07**	 RMSD (nm) 0.6 0.4 0.2 0 0 10 20 30 40 50 Time (ns)	 RMSD (nm) 0.4 0.2 0 0 1 2 3 4 5 Time (ns)

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

N⁰	PDB ID	Complex in Solution	Ligand in Solution
1	6M2N		
2	6WTT		
3	6XBG		

4	6XMK		
5	7B3E		
6	7L8I		

7	7LDL		
8	7NG3		
9	7CBT		

10	**8A4T**	 RMSD (nm) 0.6 0.4 0.2 0 0 10 20 30 40 50 Time (ns)	 RMSD (nm) 0.6 0.4 0.2 0 0 1 2 3 4 5 Time (ns)

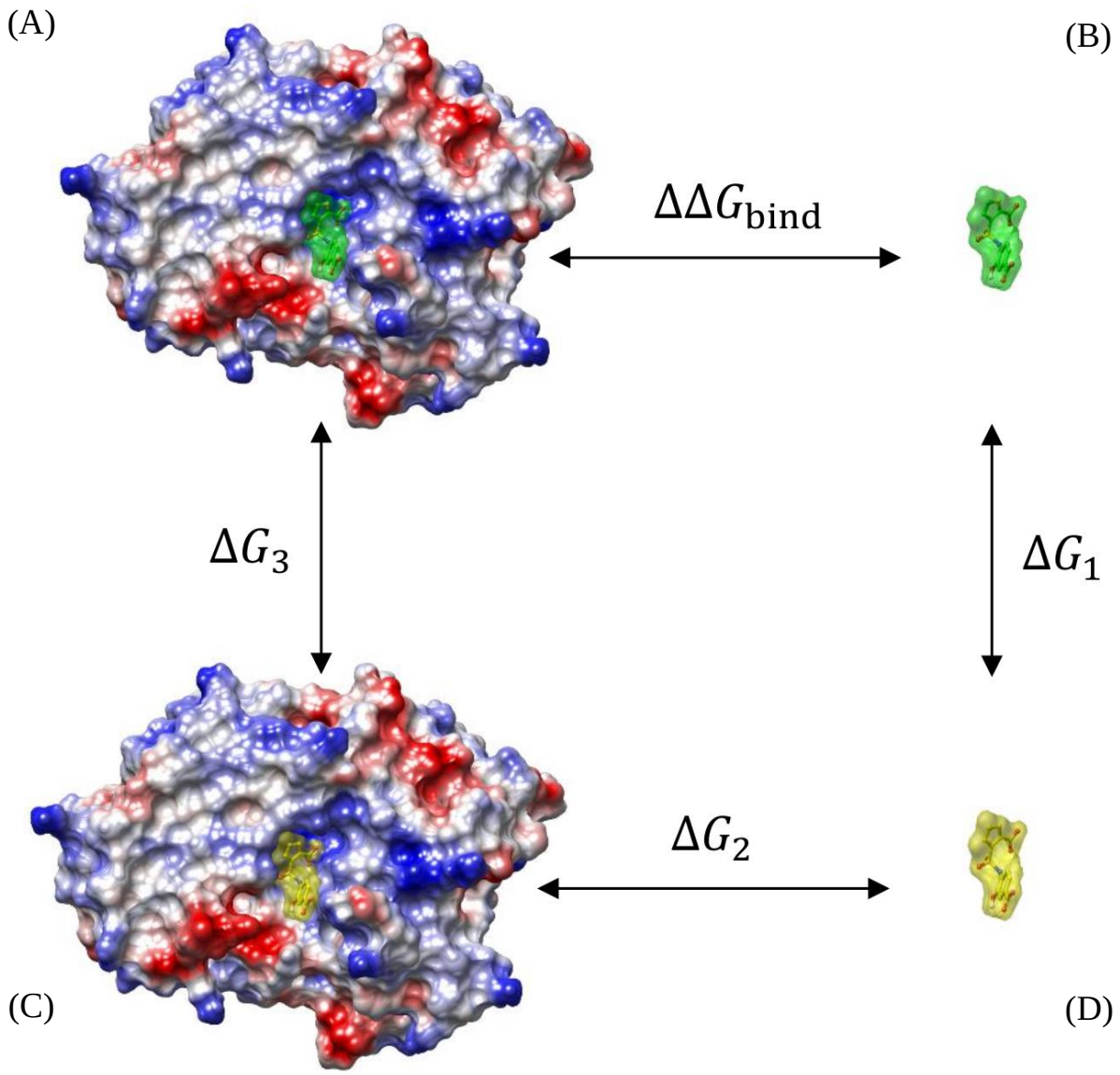


Figure S1. Thermodynamics diagram of FEP calculations. (A) full-interaction state of a ligand with surrounding protein and solvation; (B) full-interaction state of a ligand with surrounding solvation; (C) non-interaction state of a ligand with surrounding protein and solvation; (D) non-interaction state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

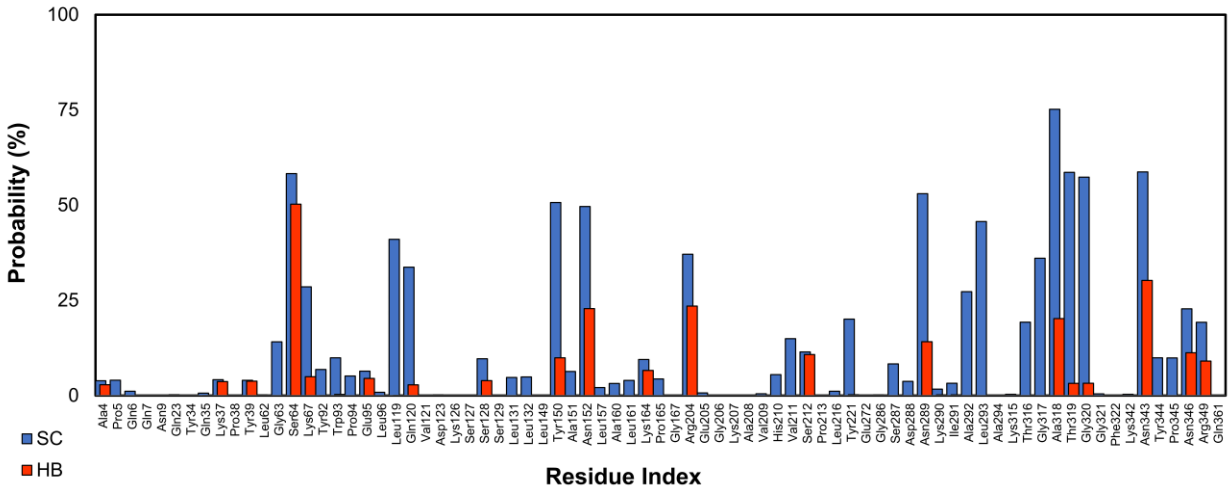


Figure S2. SC and HB Contacts between AmpC Residues and Inhibitors. The values were computed over the interval 25-50 ns of MD simulations.

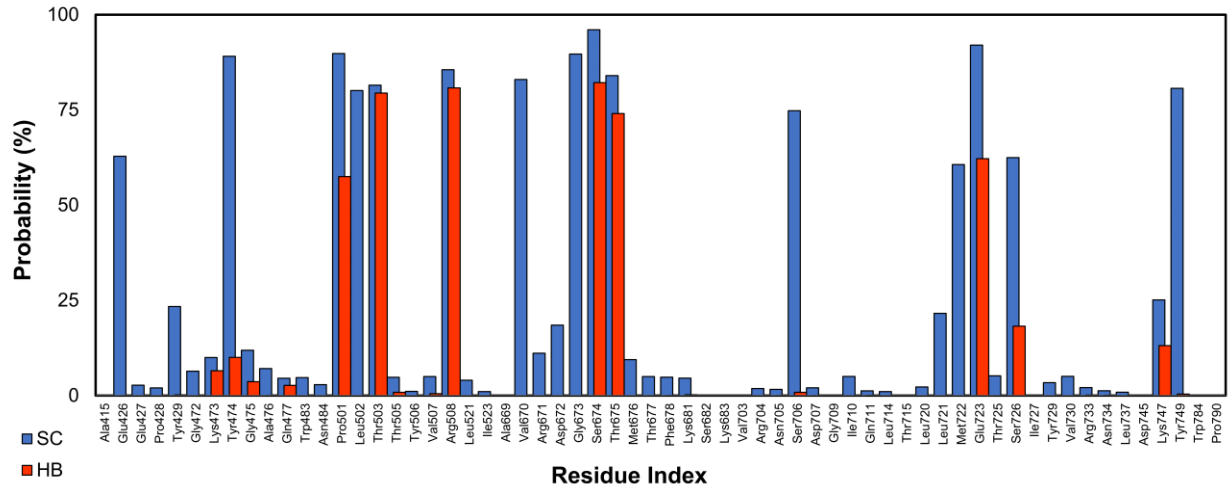


Figure S3. SC and HB Contacts between GluK1 Residues and Inhibitors. The values were computed over the interval 25-50 ns of MD simulations.

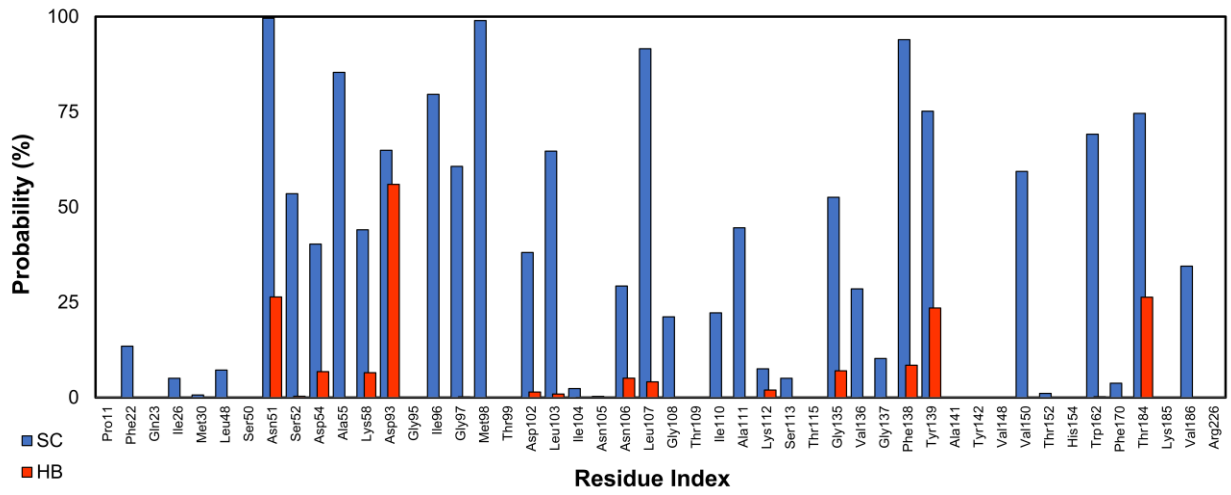


Figure S4. SC and HB Contacts between Hsp90 Residues and Inhibitors. The values were computed over the interval 25-50 ns of MD simulations.

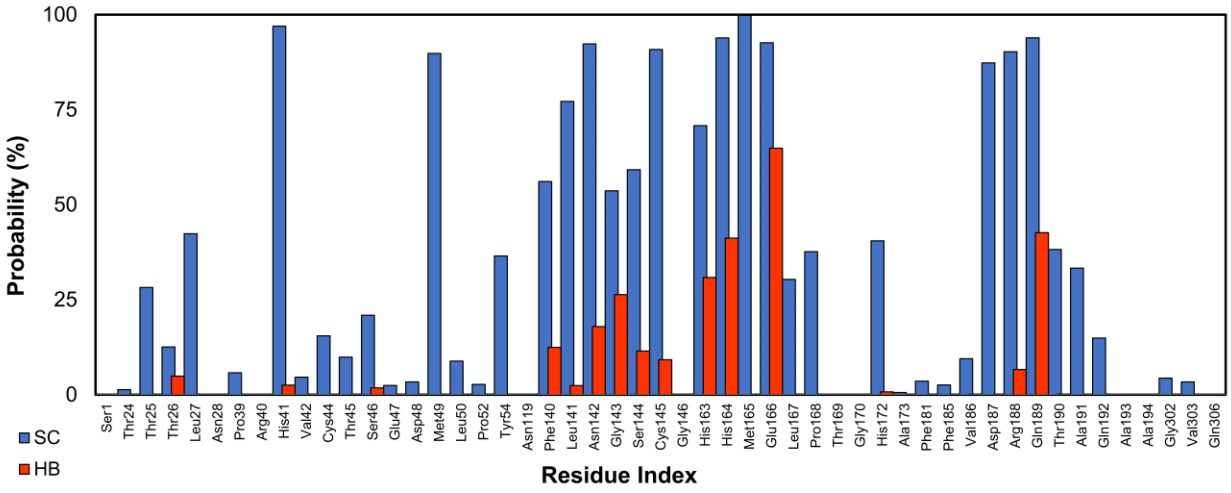


Figure S5. SC and HB Contacts between SARS-CoV-2 Mpro Residues and Inhibitors. The values were computed over the interval 25-50 ns of MD simulations.

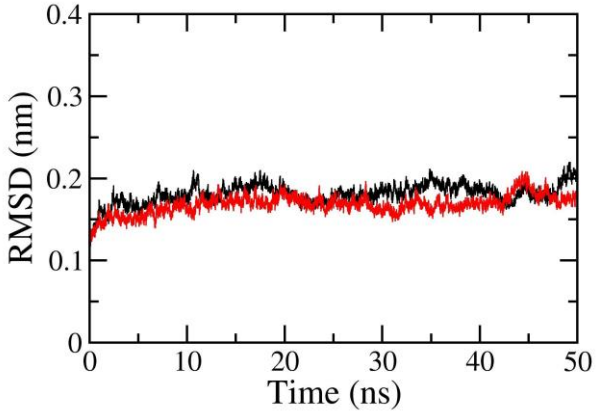
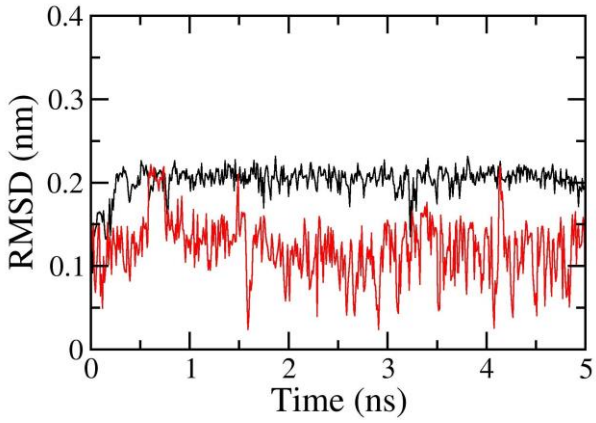
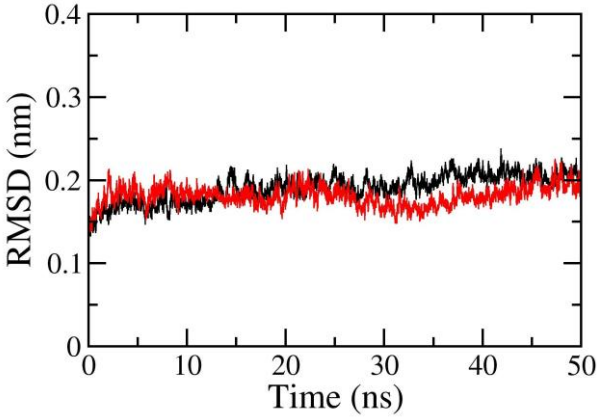
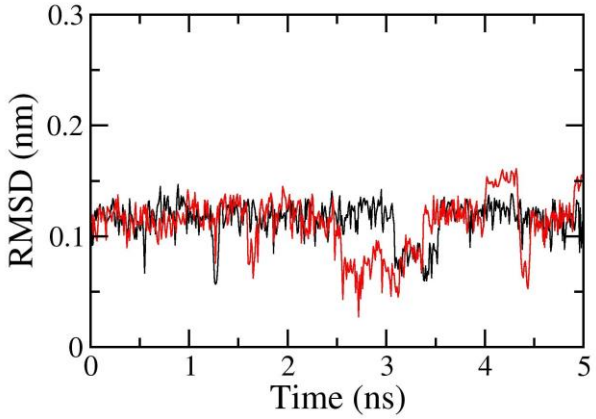
Table S6. Calculated versus Experimental Binding Affinities between AmpC, GluK1, Hsp90, SARS-CoV-2 Mpro and its inhibitors

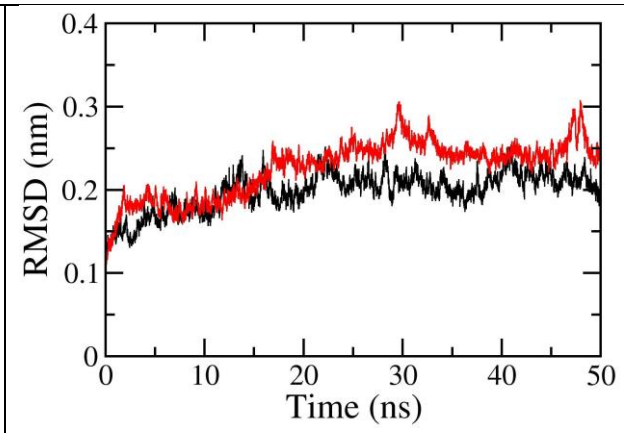
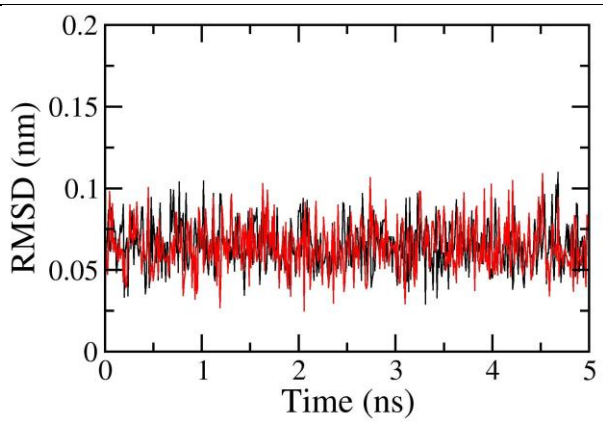
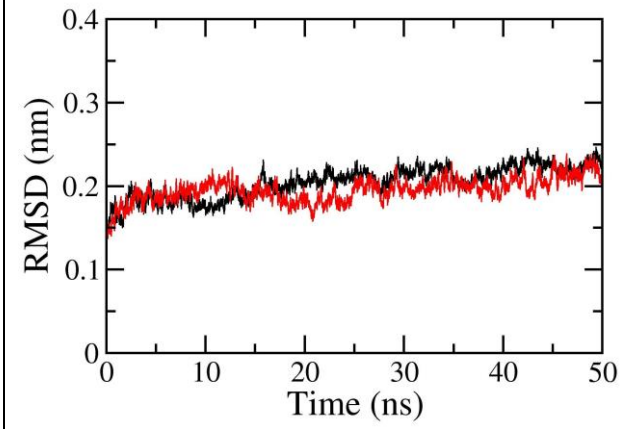
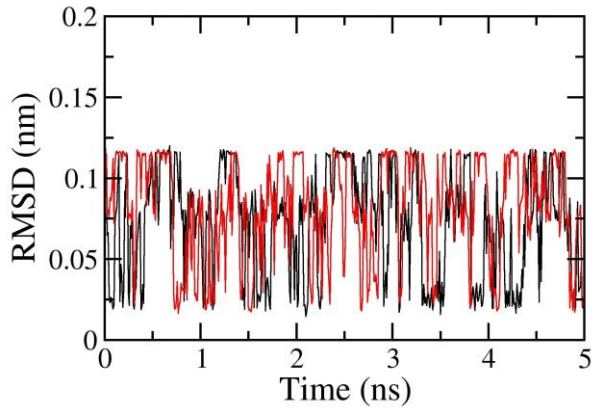
N⁰	PDB ID	PDB ID	ΔG_{cou}	ΔG_{vdW}	ΔG_{FEP}	ΔG_{EXP} [b]
1	AmpC	1XGJ	-2.41	-5.10	−7.50 ± 1.74	-8.24
2	GluK1	3FV1	-21.90	-0.78	−22.68 ± 0.24	-12.77
3	Hsp90	3K97	-2.74	-8.66	−11.40 ± 0.91	-10.98
4	SARS-CoV-2 Mpro	6M2N	-0.67	-5.83	−6.51 ± 0.64	-8.25

[b]The 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.

N⁰	PDB ID	Complex in Solution	Ligand in Solution
1	1XGJ		
2	3FV1		

3	3K97	 RMSD (nm) Time (ns)	 RMSD (nm) Time (ns)
4	6M2N	 RMSD (nm) Time (ns)	 RMSD (nm) Time (ns)

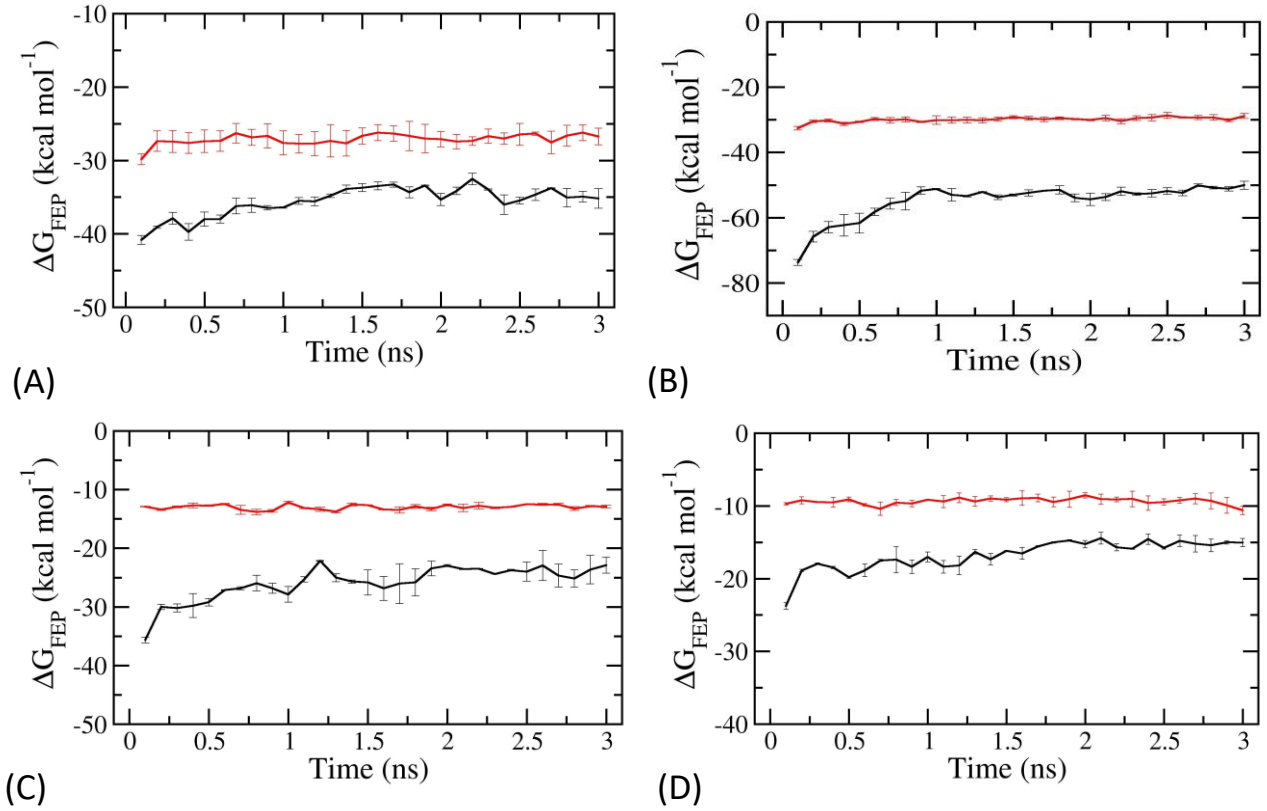


Figure S6. Time dependence of binding free energies for solvated complexes: (A) AmpC *(1XGJ)*, (B) GluK1 *(3FV1)*, Hsp90 *(3K97)*, SARS-CoV-2 Mpro *(6M2N*). The values collected in the equilibrium region were used to calculate ΔG_{FEP}. The error bars show the root mean square deviations.