"*Small Molecule Stabilization of non-native Protein-Protein Interactions of SARS-CoV-2 N Protein as a Mechanism of Action against COVID-19*" Fernández, J.F. and Lavecchia, M. J.

Supplementary Information

N Protein Sequence Alignments

SARS-CoV-2 and MERS-CoV

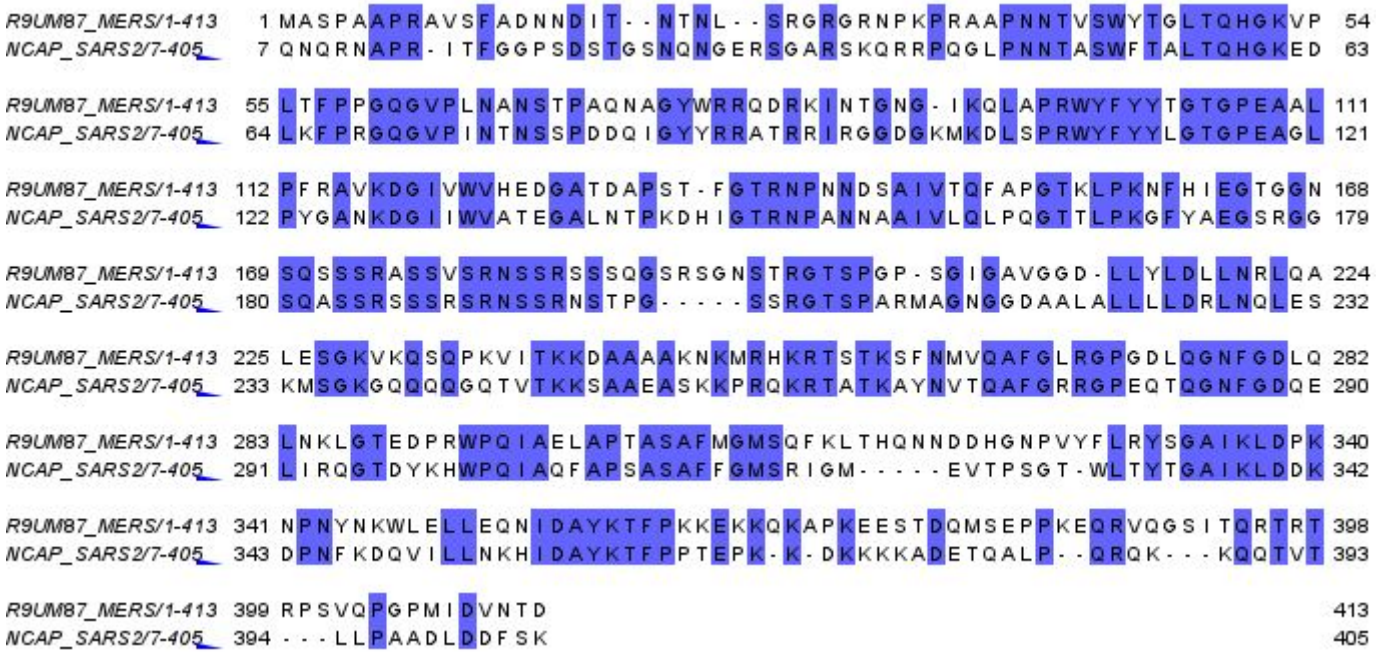


Figure S1. Sequence alignment between SARS-CoV-2 Nucleocapsid Protein (Uniprot: P0DTC9) and MERS-CoV analog (Uniprot: R9UM87) using Jalview. Pairwise alignment identity: 48,42%

SARS-CoV-2 and SARS-CoV

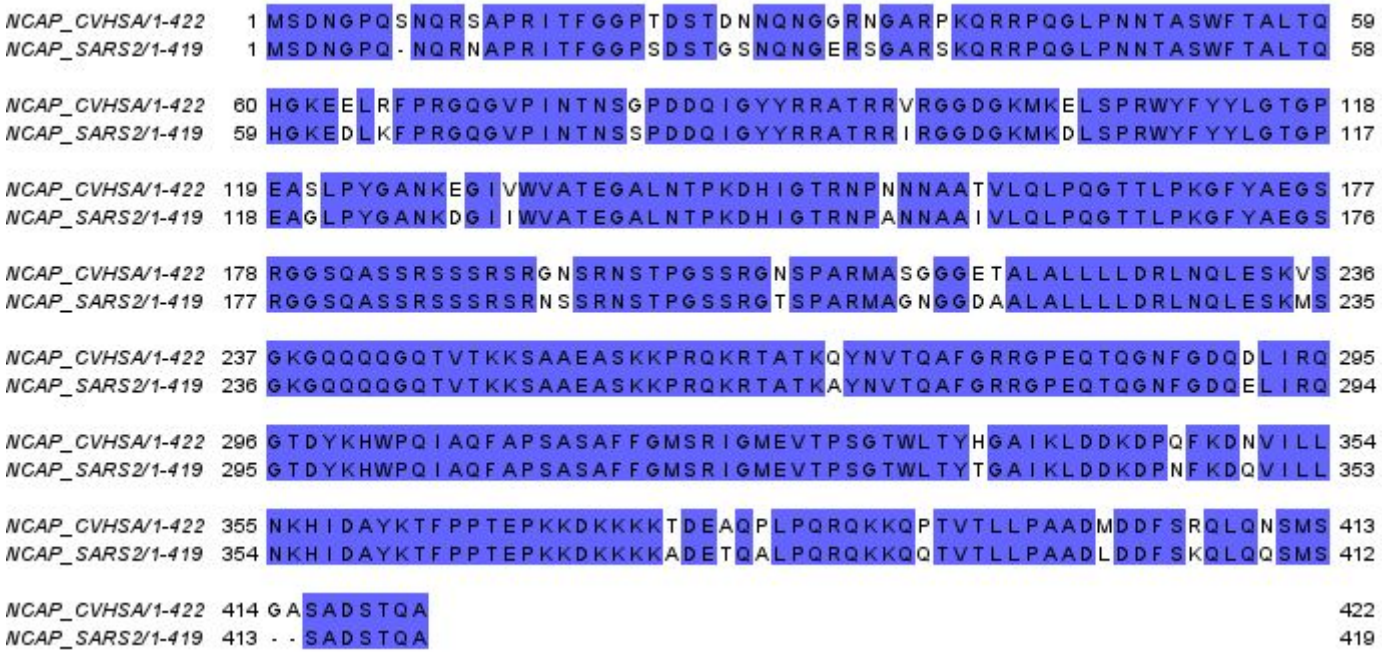


Figure S2. Sequence alignment between SARS-CoV-2 Nucleocapsid Protein (Uniprot: P0DTC9) and SARS-CoV analog (Uniprot: P59595) using Jalview. Pairwise alignment identity: 90,52%

Compounds Structures

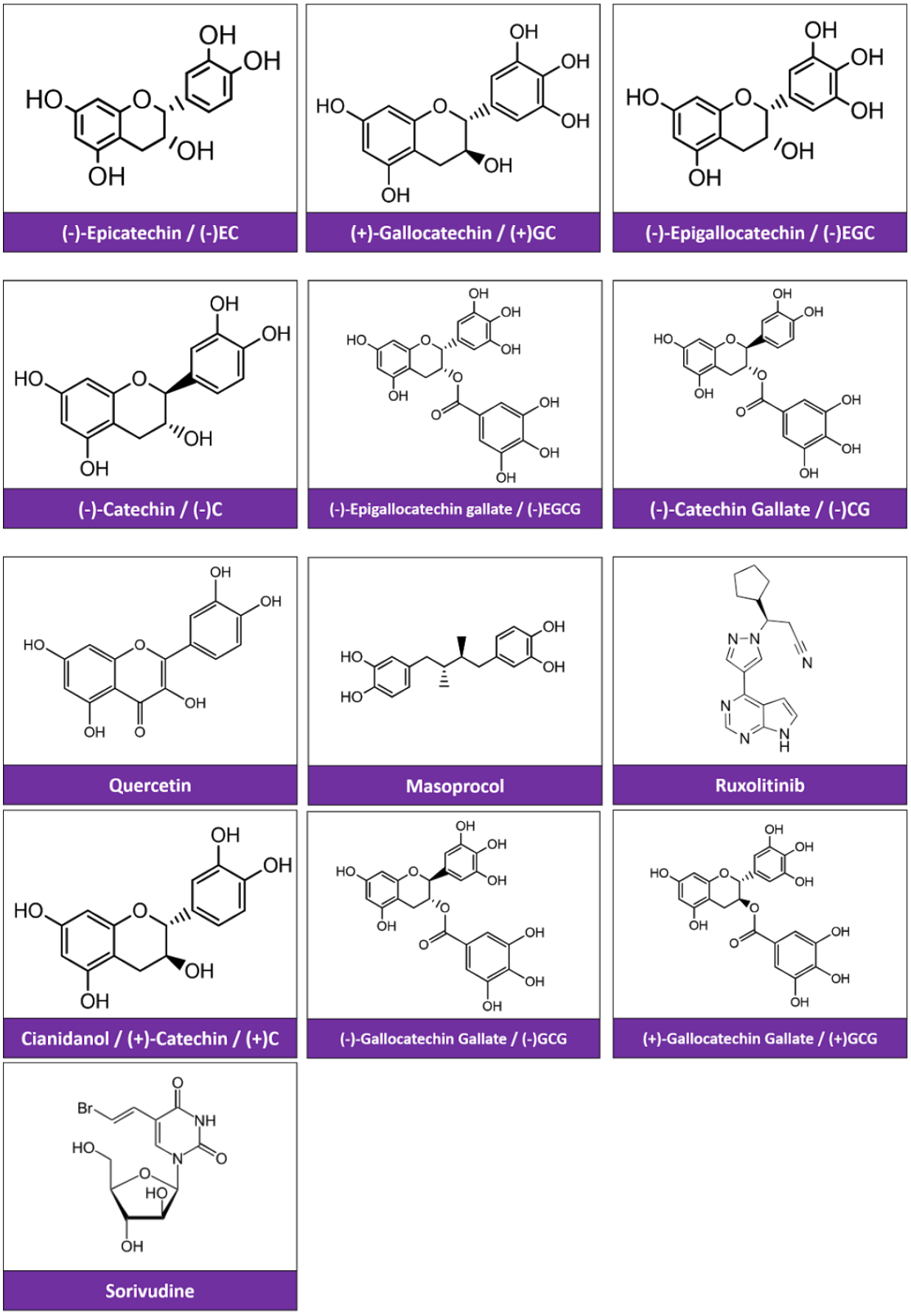


Figure S3. Structure of the compounds that stabilized at least two interfaces

Docking

Docking Results

Table S1. Summary of Docking Results. Compounds with the best consensus docking score of all three docked interfaces are displayed.

Molecule Name	Database	Score IF-1	Pos. IF-1	Score IF-2	Pos. IF-2	Score IF-3	Pos. IF-3	Score RNA	Pos. RNA	Position *Consensus* Docking
Rosmarinic acid	Polyphenol	-11,0	108	-11,6	312	-13,0	41	-8,5	1	1
Gallic acid 3-O-gallate	Polyphenol	-12,2	24	-12,4	75	-14,2	8	-6,4	151	2
Ignotine	Investigational	-12,3	21	-11,2	477	-10,9	437	-7,9	2	3
255734-04-4	Investigational	-11,0	116	-11,2	472	-13,3	23	-7,0	50	4
Butein	Polyphenol	-12,1	25	-11,8	195	-12,3	93	-6,4	164	5
4-Caffeoylquinic acid	Polyphenol	-11,4	59	-10,2	1334	-14,9	2	-6,2	253	6
Epigallocatechin	Investigational / Polyphenol	-11,2	83	-12,6	43	-12,4	82	-6,3	195	7
Tienamicina	Approved	-10,6	177	-11,9	182	-13,9	14	-6,2	245	8
1-Butylguanidinium	Polyphenol	-11,3	68	-9,3	2184	-14,0	13	-6,9	53	9
Cianidanol	Approved / Polyphenol	-12,2	23	-11,8	198	-12,8	52	-5,7	516	10
(+)-Gallocatechin 3-O-gallate	Polyphenol	-11,6	41	-12,9	21	-12,6	64	-5,5	650	11
(+)-Gallocatechin	Polyphenol	-11,7	37	-12,0	156	-12,9	46	-5,7	543	12
5-hidroxy-L-tryptophan	Approved	-11,8	35	-11,8	201	-10,5	596	-6,9	60	13
5-Br-2'-Deoxyuridine	Investigational	-12,0	30	-10,7	819	-10,7	503	-7,2	31	14
o6-bencylguaniane	Investigational	-12,5	18	-11,4	391	-10,8	462	-6,4	143	15
Diazepinomicin	Investigational	-9,0	760	-13,6	4	-10,7	525	-7,5	9	16
177975-08-5	Investigational	-9,7	414	-11,3	416	-13,1	33	-6,9	59	17
Ruxolitinib	Approved	-13,1	7	-9,9	1651	-11,1	346	-6,6	98	18
1-[Amino-(3,4-dichloroanilino)methylidene]-2-propan-2-ylguanidine	Investigational	-11,7	39	-11,1	550	-11,5	207	-6,6	97	19
Phenformin	Approved	-11,5	48	-10,9	673	-14,4	7	-5,4	830	20
Arbutamine	Approved	-9,6	482	-12,2	109	-12,9	47	-6,6	107	21
Arginin	Approved	-11,4	55	-11,4	380	-12,9	49	-5,9	386	22
Masoprocol	Approved	-12,1	26	-12,1	138	-13,2	28	-4,9	1359	23
Cyanidin	Polyphenol	-12,4	20	-11,1	541	-12,4	79	-5,6	607	24
3-Caffeoylquinic acid	Polyphenol	-10,5	198	-11,9	165	-13,4	21	-5,8	476	25
Feruloyl tartaric acid	Polyphenol	-10,5	199	-10,1	1403	-12,2	105	-7,2	21	26
(-)-Epicatechine	Investigational / Polyphenol	-11,6	46	-12,6	41	-12,2	112	-5,4	831	27
N-Butan-2-yl-1-(6-methyl-1H-benzimidazol-2-yl)piperidine-4-carboxamide	Investigational	-10,0	316	-11,7	266	-10,9	430	-7,4	15	28
Indalpine	Investigational	-10,2	272	-11,6	270	-11,9	138	-6,7	81	29
Etilevodapo	Investigational	-13,0	8	-11,4	396	-10,1	812	-6,0	315	30
ECGC	Investigational /	-8,6	1095	-11,6	320	-13,0	43	-7,1	32	31

	Polyphenol									
(-)-Catechin Gallate	Polyphenol	-8,9	798	-13,2	10	-12,4	83	-6,4	162	32
Caffeoyl tartaric acid	Investigational	-11,1	101	-11,0	619	-12,3	99	-6,1	259	33
Sedemetan	Investigational	-9,4	549	-11,3	454	-11,4	249	-7,4	13	34
Ceforanide	Approved	-10,3	245	-11,7	245	-10,3	731	-7,3	20	35
Vipadenant	Investigational	-10,0	339	-11,1	534	-11,6	195	-7,0	44	36
Valomaciclovir	Investigational	-11,3	70	-11,8	222	-13,1	29	-5,1	1090	37
Phloretin	Polyphenol	-10,2	269	-12,0	153	-10,8	460	-6,8	69	38
Solabegron	Investigational	-11,3	67	-11,3	421	-12,3	97	-5,7	509	39
Pexidartinib	Approved	-10,8	133	-10,5	984	-11,6	194	-6,7	82	40
Guanosina	Investigational	-11,5	47	-10,9	685	-10,2	795	-6,8	66	41
Vyvanse	Approved	-9,8	388	-12,2	107	-13,3	25	-5,6	569	42
Bendazac	Investigational	-11,2	89	-11,6	299	-9,7	1129	-6,9	54	43
5-Caffeoylquinic acid	Polyphenol	-10,0	323	-11,8	213	-11,5	223	-6,6	108	43
Debio-1347	Investigational	-9,7	445	-12,7	36	-11,2	296	-6,5	135	44
Procaterol	Investigational	-11,2	87	-11,8	214	-8,7	1957	-7,3	17	45
(-)-Catechin	Polyphenol	-11,2	75	-11,7	235	-12,2	108	-5,5	709	46
Triprolidine	Investigational	-10,6	179	-12,1	140	-10,5	592	-6,5	119	47
Polydatin	Approved	-8,7	1022	-12,2	113	-14,9	3	-5,4	787	48
Resveratrol 5-O-glucoside	Polyphenol	-8,7	1023	-12,2	114	-14,9	4	-5,4	788	49
Zolmitriptan	Approved	-10,8	139	-13,3	6	-11,7	185	-5,2	1000	51
(-)-Gallocatechin Gallate	Polyphenol	-7,4	2223	-13,2	11	-13,1	36	-6,3	131	55
Quercetina	Investigational	-10,9	124	-12,4	63	-11,2	310	-5,5	673	59
Brivudine	Approved	-13,8	1	-10,7	846	-9,9	1011	-5,1	1075	74
Trifluridine	Approved	-13,3	4	-10,1	1429	-11,4	241	-4,1	2262	165
Sorivudine	Approved	-13,1	7	-11,0	612	-8,7	1925	-4,6	1822	289
Compounds selected for MD prior to Polyphenol database addition										
Catechins selected for MD prior to Polyphenol database addition										
Catechins selected for MD after Polyphenol database addition										

Correlation with previously reported activities

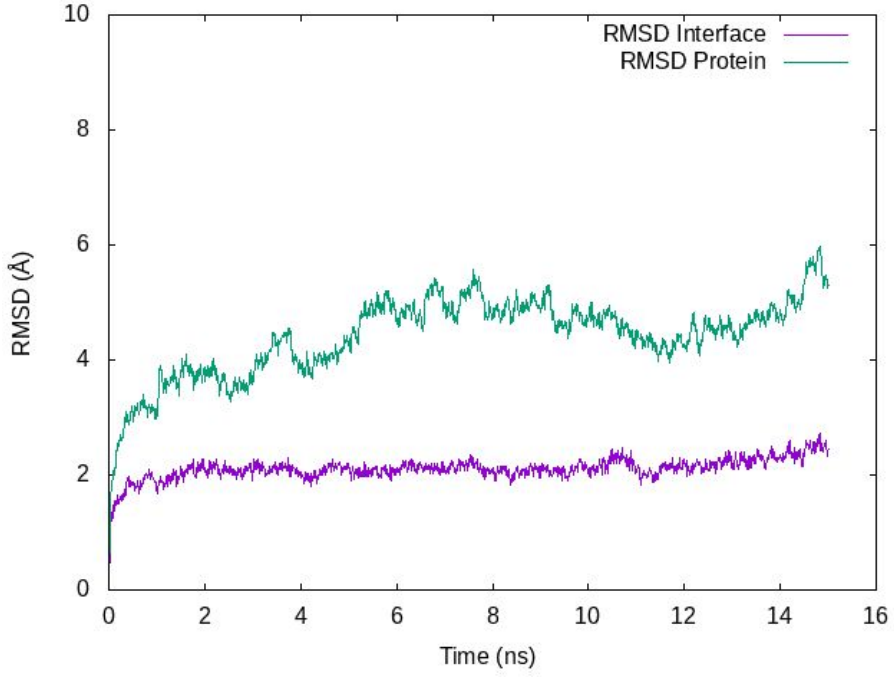
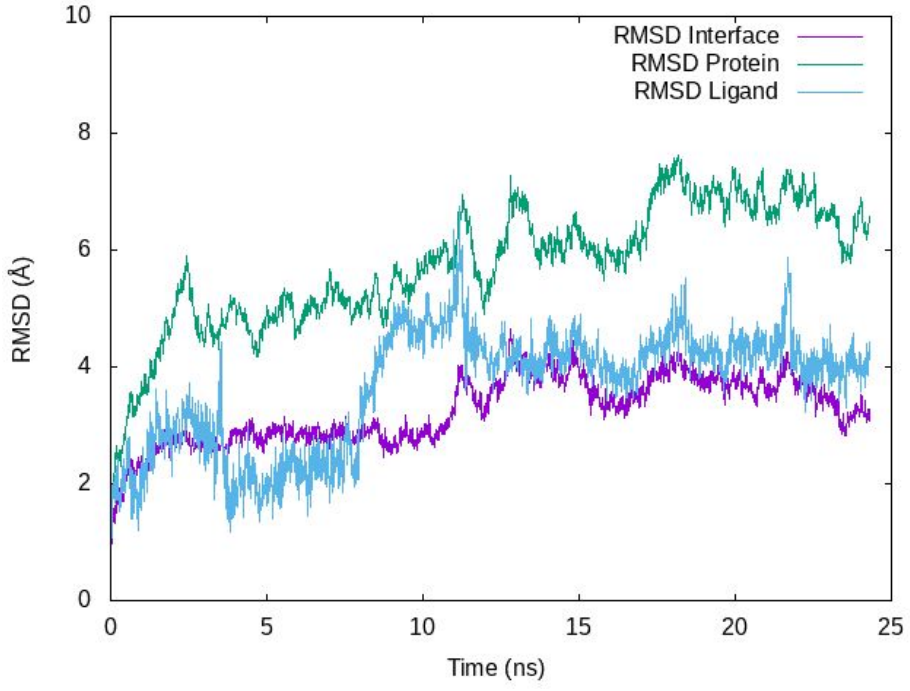
Table S2. Docking results of compounds included in Roh's work, where "Reported activity" classifies compounds according to their reported outcome.

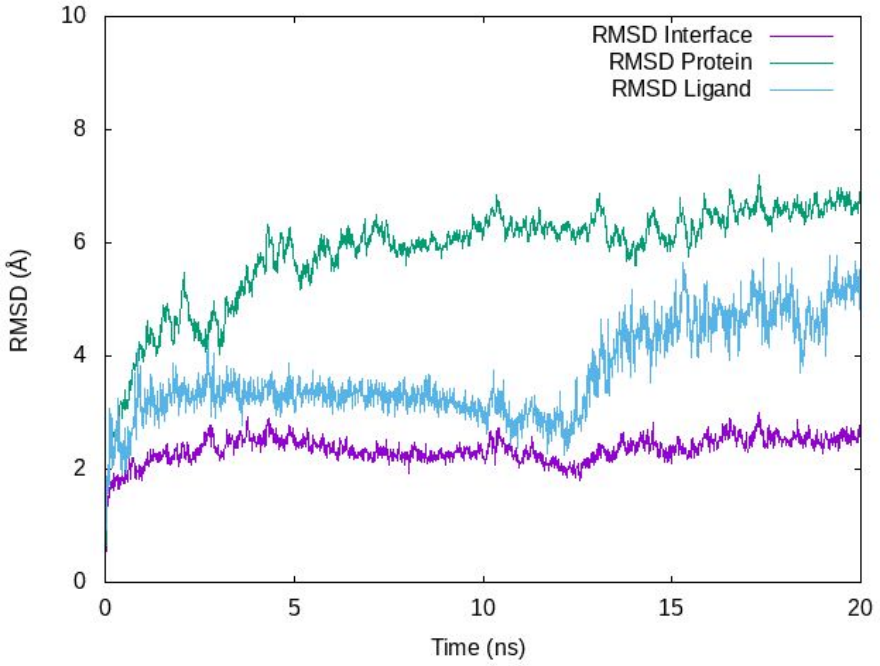
Molecule Name	Score IF-1	Position IF-1	Score IF-2	Position IF-2	Score IF-3	Position IF-3	Score RNA	Position RNA	Consensus Docking Position	Reported Activity
(-)-Catechin Gallate	-8,9	798	-13,2	10	-12,4	83	-6,4	162	32	Active
(-)-Galloatechin Gallate	-7,4	2223	-13,2	11	-13,1	36	-6,3	191	55	Active
Myricetin	-10,7	154	-12,5	55	-11,8	160	-5,2	976	63	Inactive
Quercetin	-10,9	125	-12,4	64	-11,2	311	-5,6	629	56	Inactive
(-)-Catechin	-11,2	75	-11,7	235	-12,2	108	-5,5	709	46	Inactive
Baicalein	-10,6	182	-11,5	355	-10,7	495	-5,8	455	112	Inactive
Glycitein	-7,4	2238	-11,1	523	-10,8	465	-4,3	2102	1275	Inactive
Kaempferol	-9,6	464	-10,8	706	-10,6	572	-5,2	1030	436	Inactive
Luteolin	-10,4	215	-10,8	707	-12,2	107	-5,5	683	104	Inactive
Morin	-10,3	251	-10,8	727	-10,7	508	-5,7	510	193	Inactive
Genistein	-10,8	145	-10,6	864	-11,0	376	-5,1	1155	248	Inactive
Rutin	-6,6	2973	-10,6	900	-7,0	3198	-4,1	2874	2763	Inactive
Naringenin	-9,2	657	-10,6	919	-11,1	358	-5,4	853	401	Inactive
Hesperidin	-5,9	3388	-10,0	1477	-7,6	2830	-3,5	2675	3085	Inactive
Daidzein	-9,5	515	-10,0	1558	-11,3	276	-4,5	1863	727	Inactive
Apigenin	-9,2	654	-9,5	2031	-10,8	479	-5,2	1058	719	Inactive
Naringin	-7,8	1921	-8,8	2645	-8,0	2577	-4,1	2301	2645	Inactive
Diosmin	-4,8	3717	-7,2	3474	-8,5	2099	-3,7	2570	3407	Inactive

Molecular Dynamics

Reported MERS-CoV Interfaces

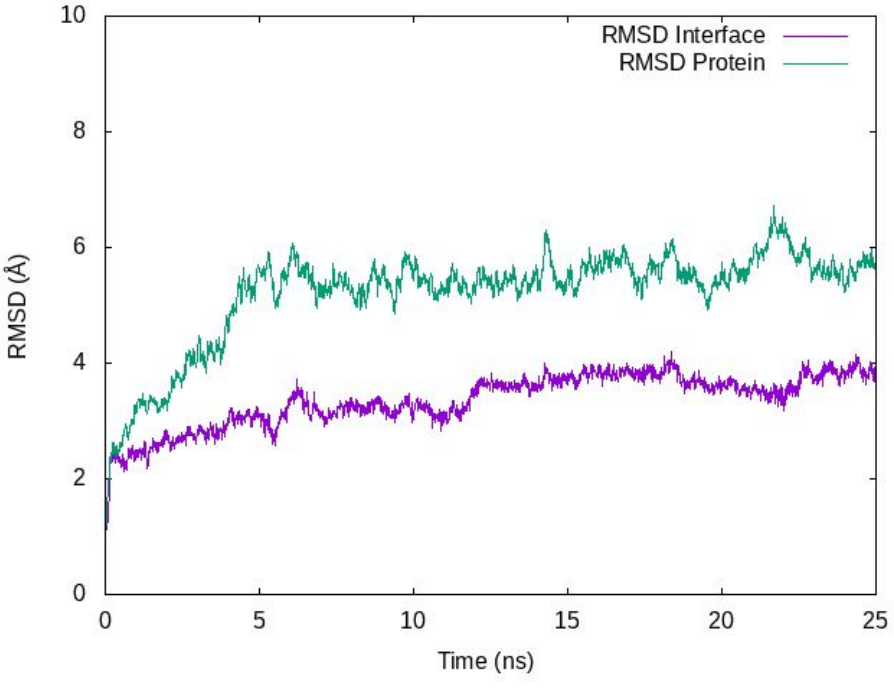
Table S3. RMSD profiles of the equilibration/production stage is shown for the reported MERS-CoV Interfaces. "RMSD Protein" was measured considering all residues in the complex aligned using the protein backbone. "RMSD Interface" were calculated aligning by the interface-forming residues and then measured using those same residues

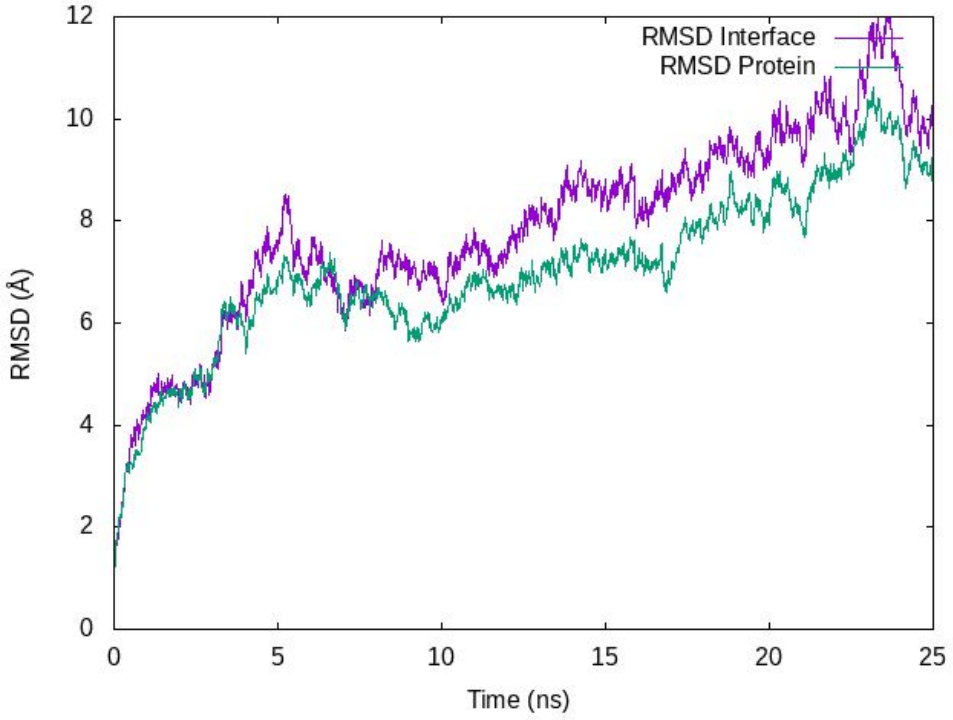
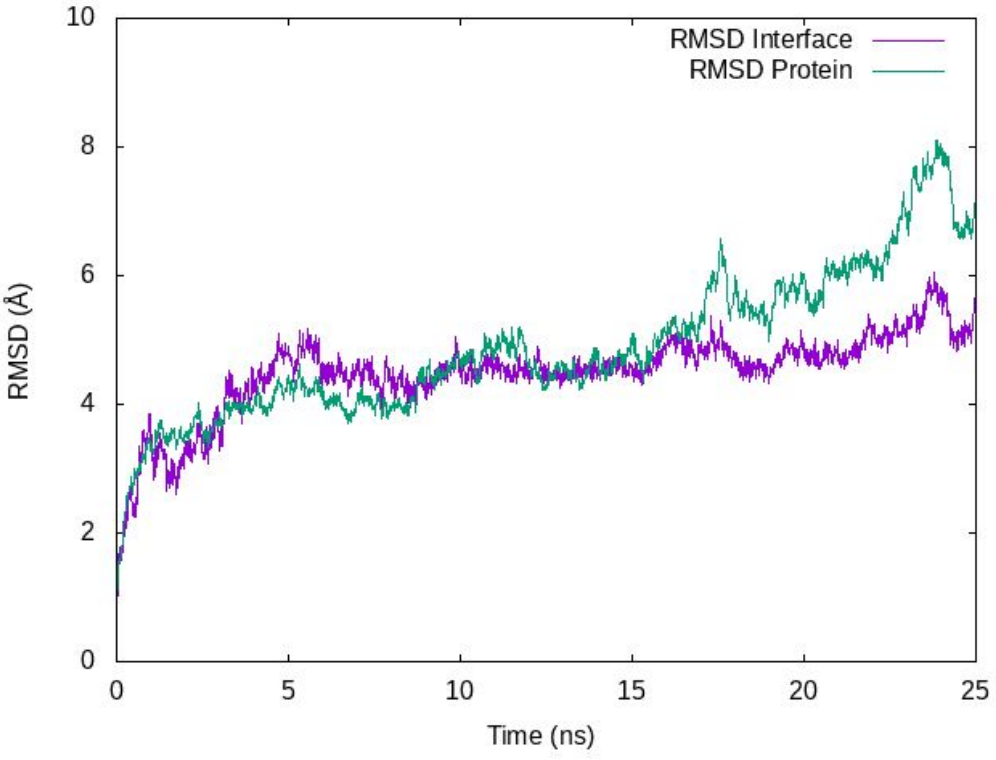
Ligand ID	RMSD
MERS-CoV Isolated	
P1/MERS-CoV	

P3/**MERS-CoV**	

SARS-CoV-2 Ligand-Free Interfaces

Table S4. RMSD profiles of the equilibration/production stage are shown for ligand-free interfaces. "RMSD Protein" was measured considering all residues in the complex aligned using the protein backbone. "RMSD Interface" were calculated aligning by the interface-forming residues and then measured using those same residues.

Ligand ID	**RMSD**
IF-1	

IF-2	 RMSD Interface RMSD Protein RMSD (Å) Time (ns) 0 2 4 6 8 10 12 0 5 10 15 20 25
IF-3	 RMSD Interface RMSD Protein RMSD (Å) Time (ns) 0 2 4 6 8 10 0 5 10 15 20 25

Interfaces and their Interaction with Zn^{2+}

*Table S5. RMSD profiles of the equilibration/production stage are shown for **IF-1** and **IF-2** interacting with Zn. "RMSD Protein" was measured considering all residues in the complex aligned using the protein backbone. "RMSD Interface" and "RMSD Ligand" were calculated aligning by the interface-forming residues and then measured using those same residues and Zn2+, respectively.*

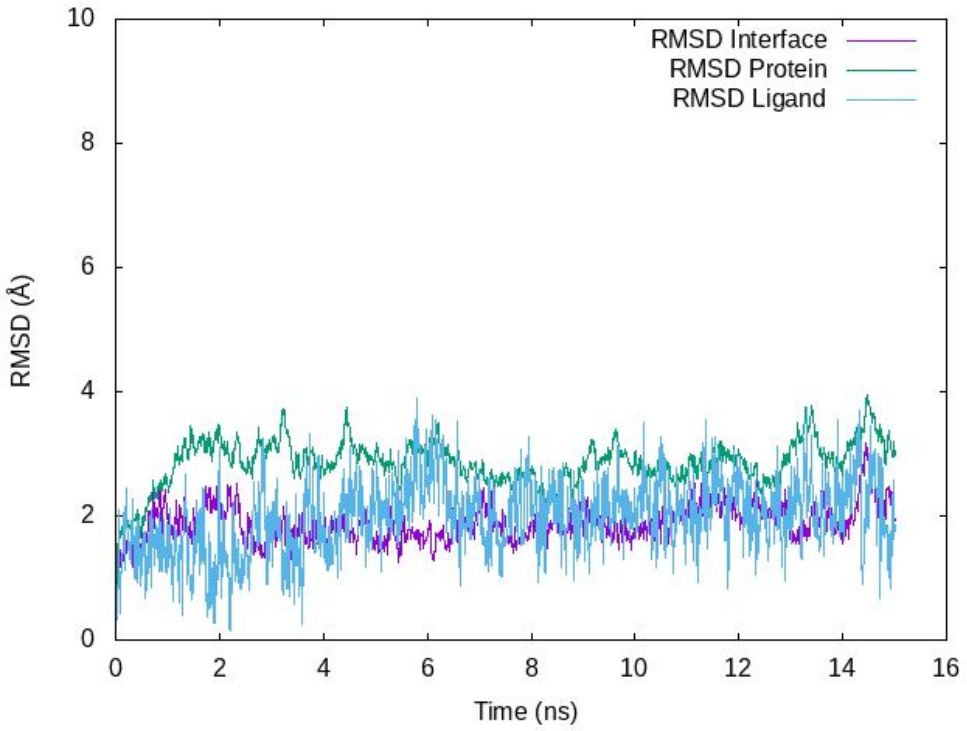
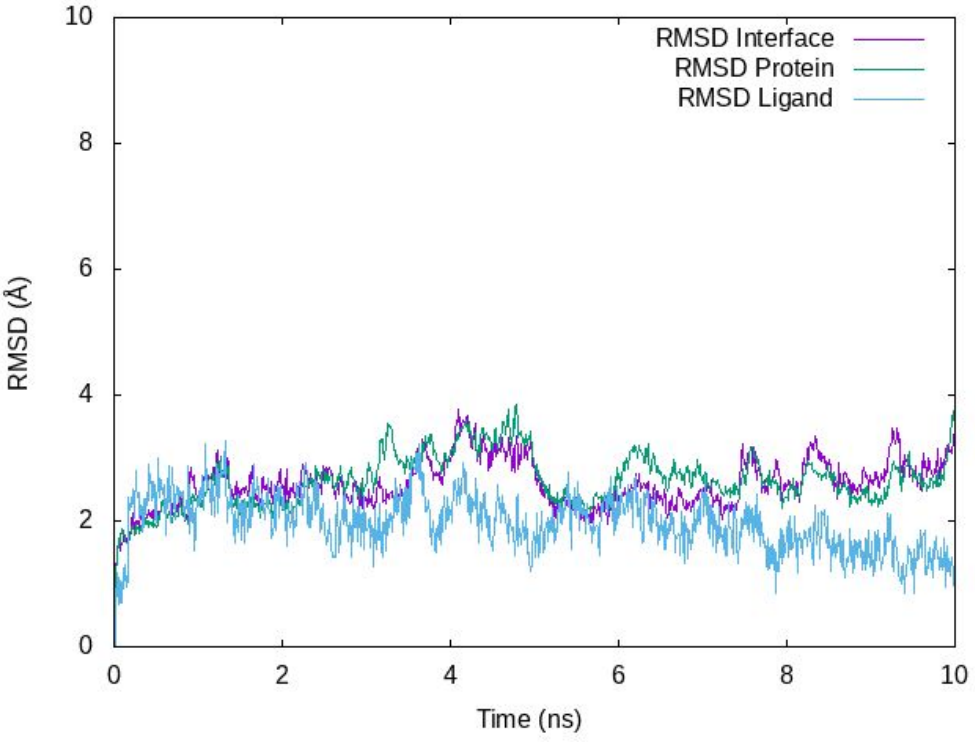
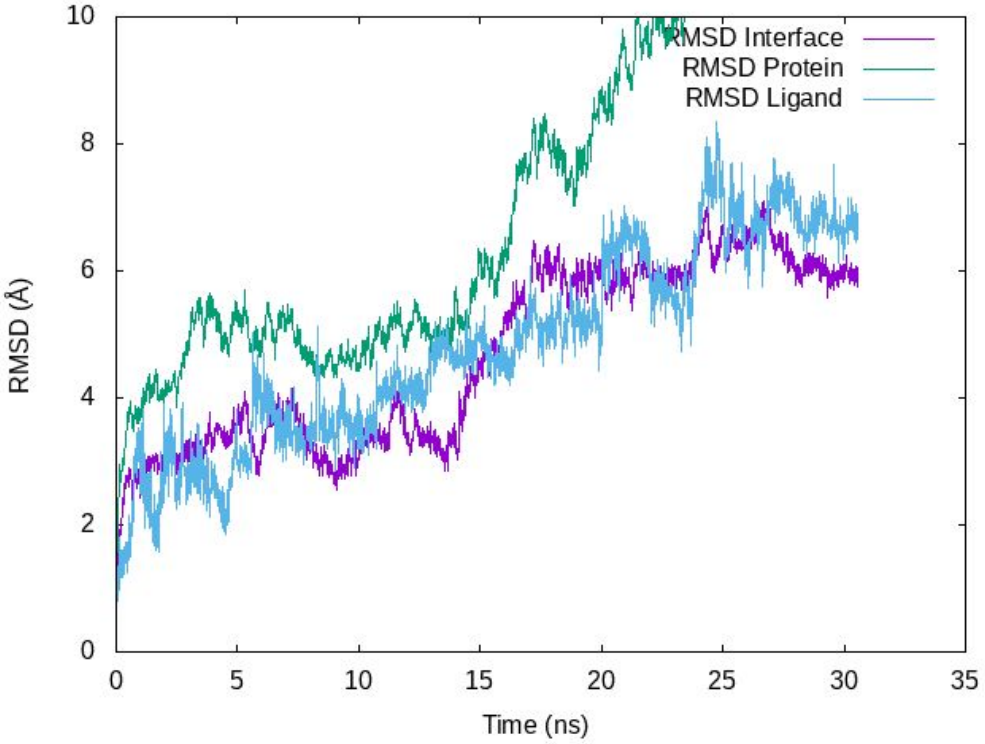
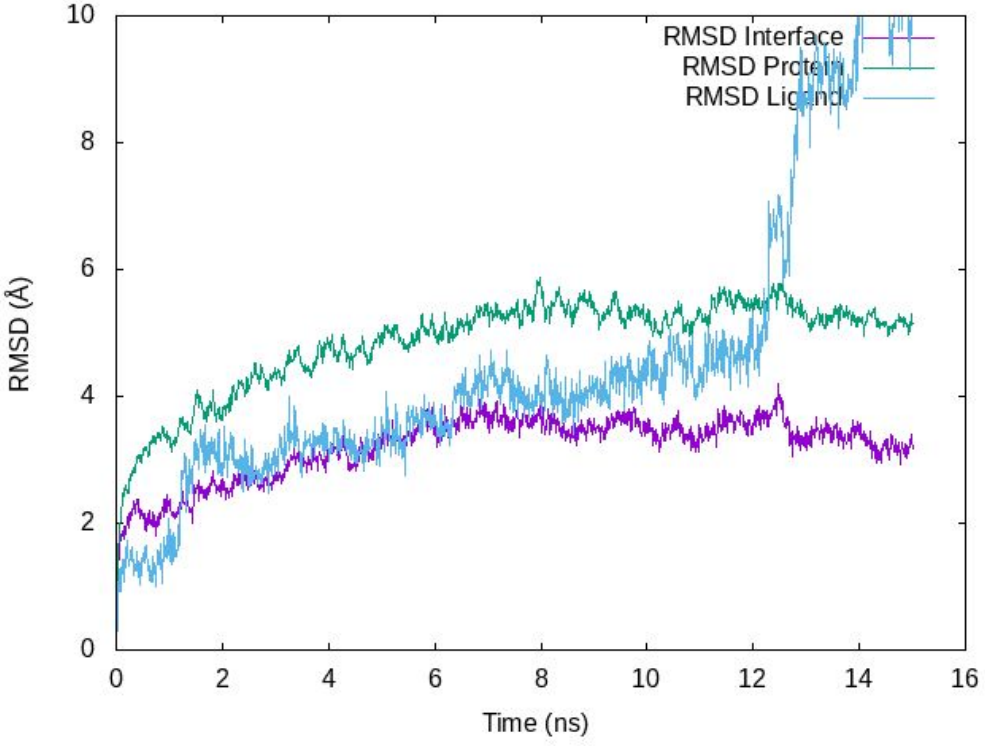
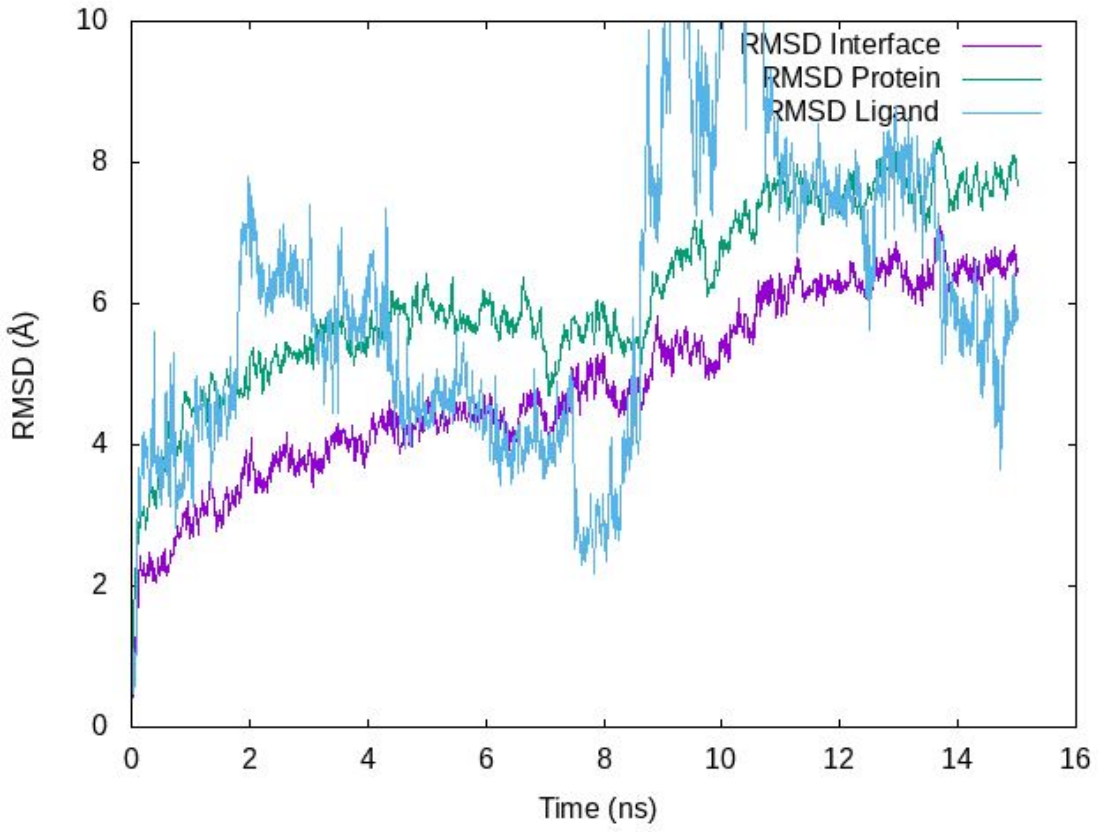
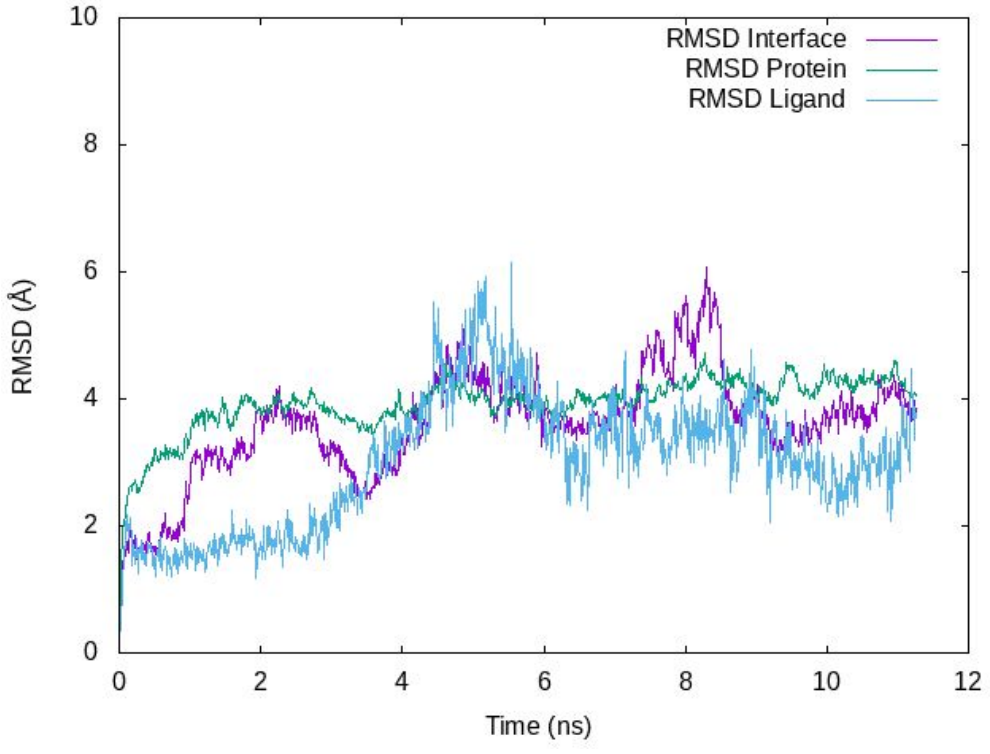
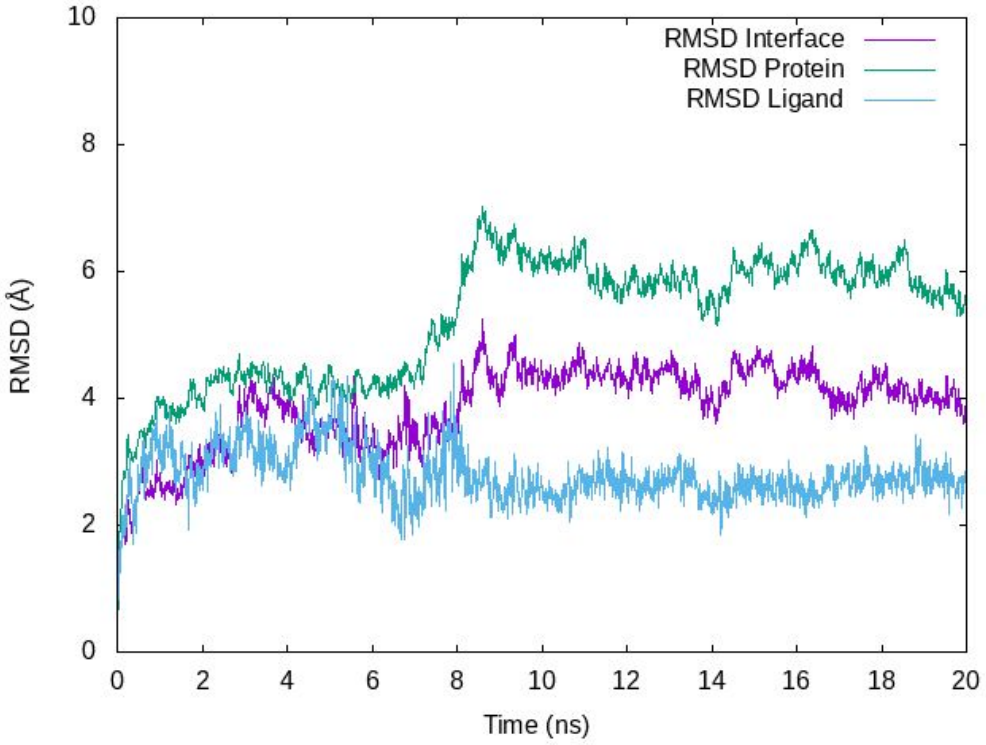
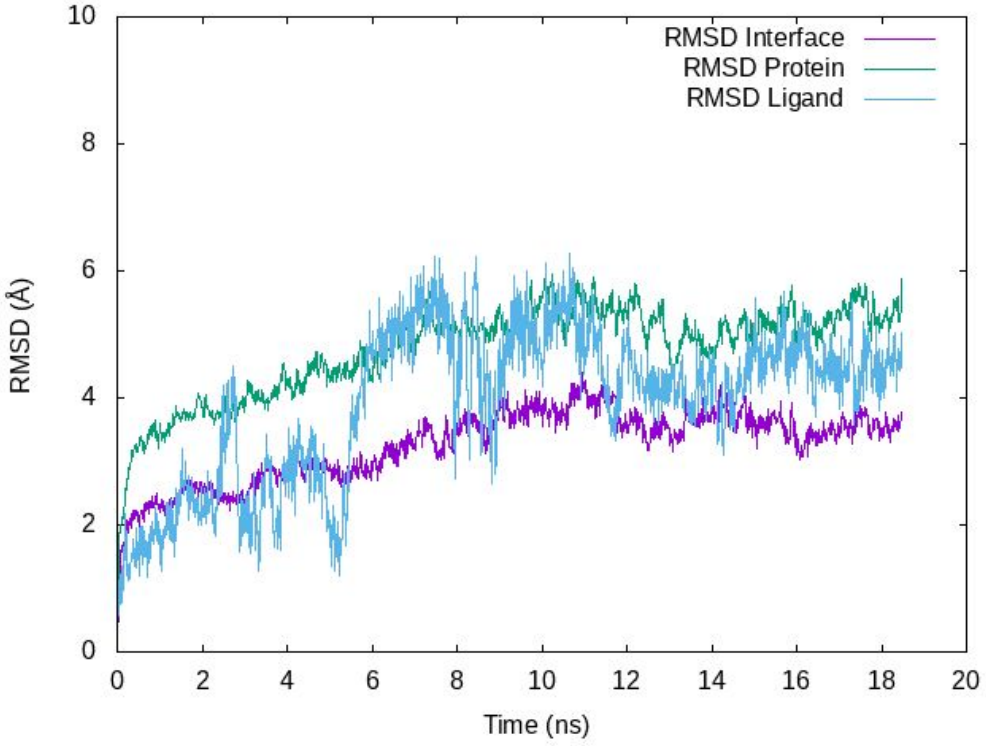
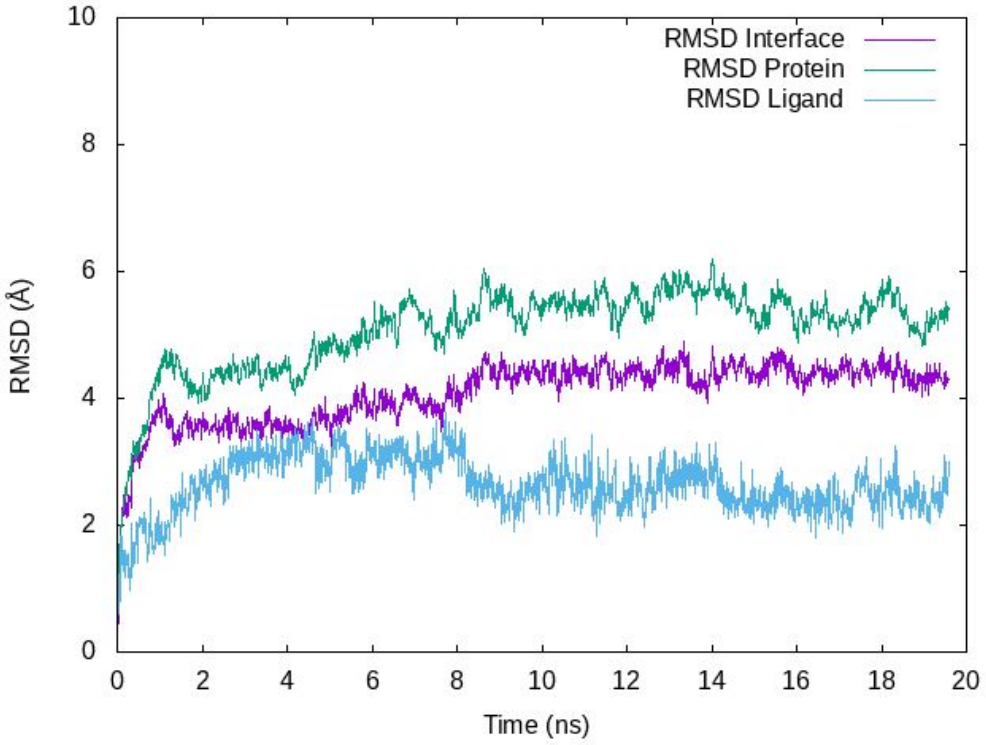
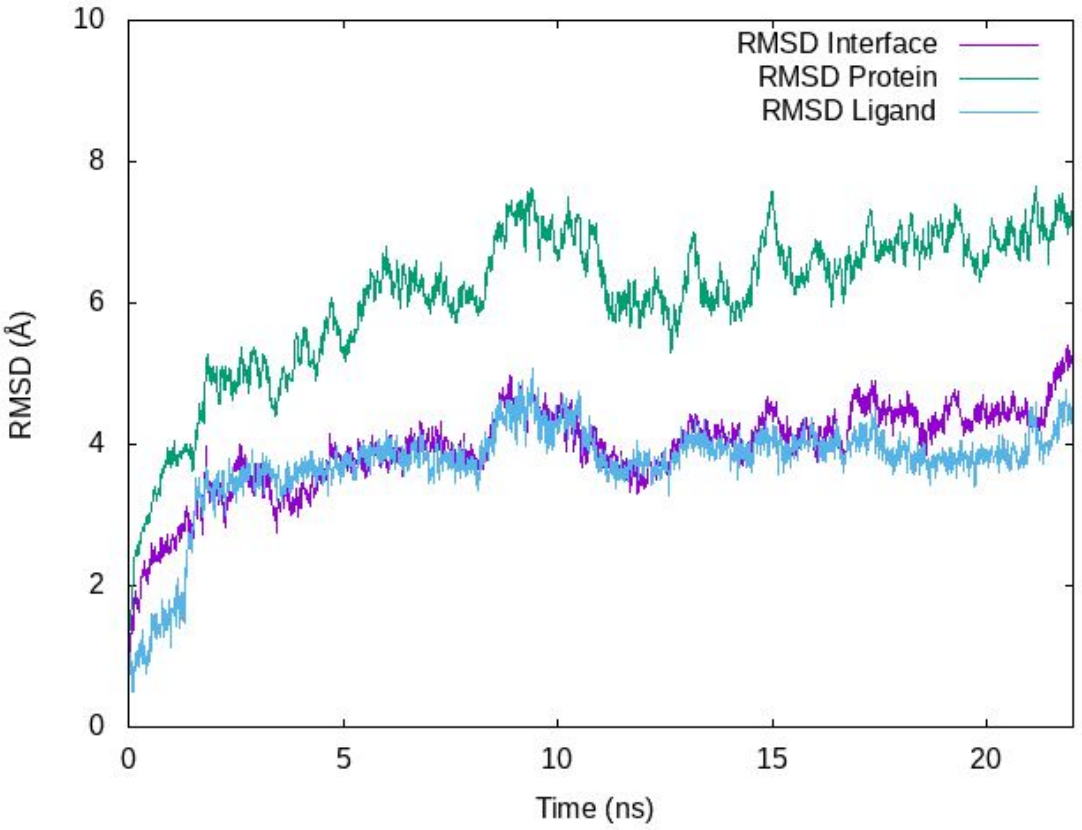
Ligand ID	RMSD
IF-1	
IF-2	

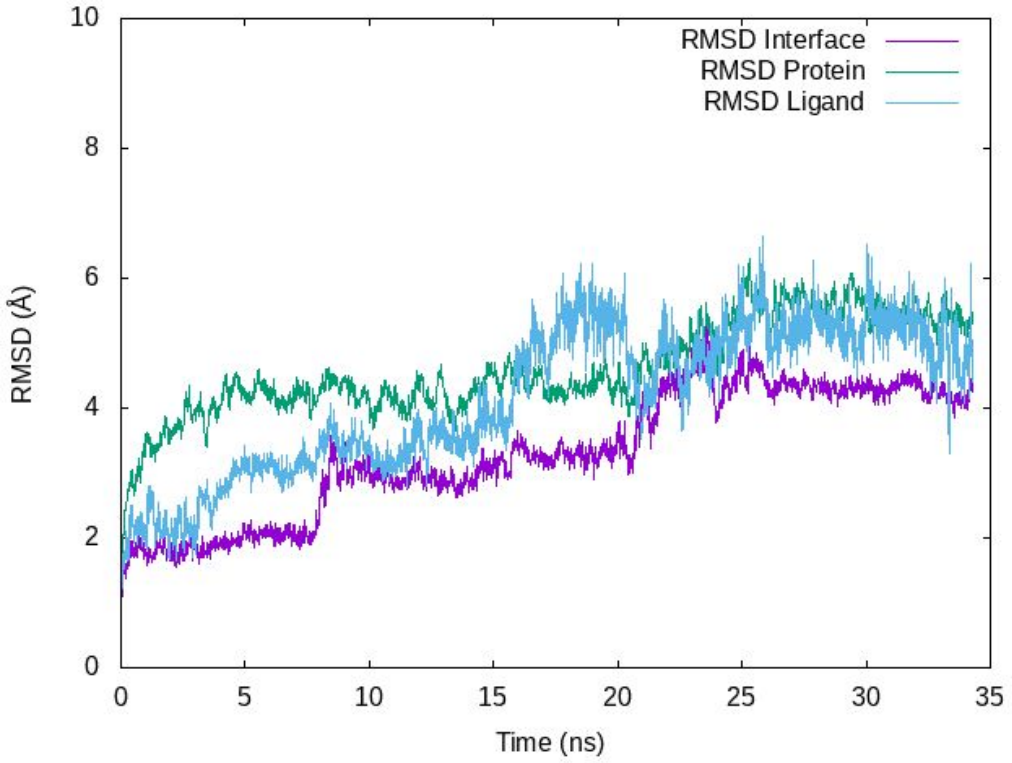
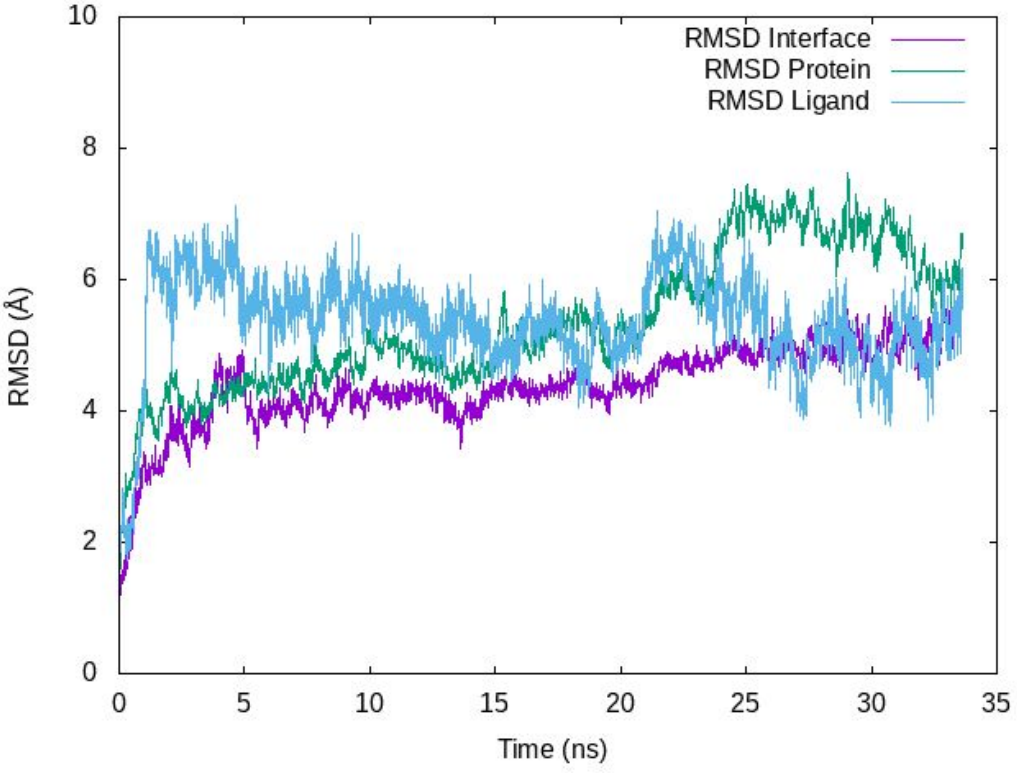
*Table S6. RMSD profiles of the equilibration/production stage are shown for each compound in **IF-1**. "RMSD Protein" was measured considering all residues in the complex aligned using the protein backbone. "RMSD Interface" and "RMSD Ligand" were calculated aligning by the interface-forming residues and then measured using those same residues and the ligand, respectively.*

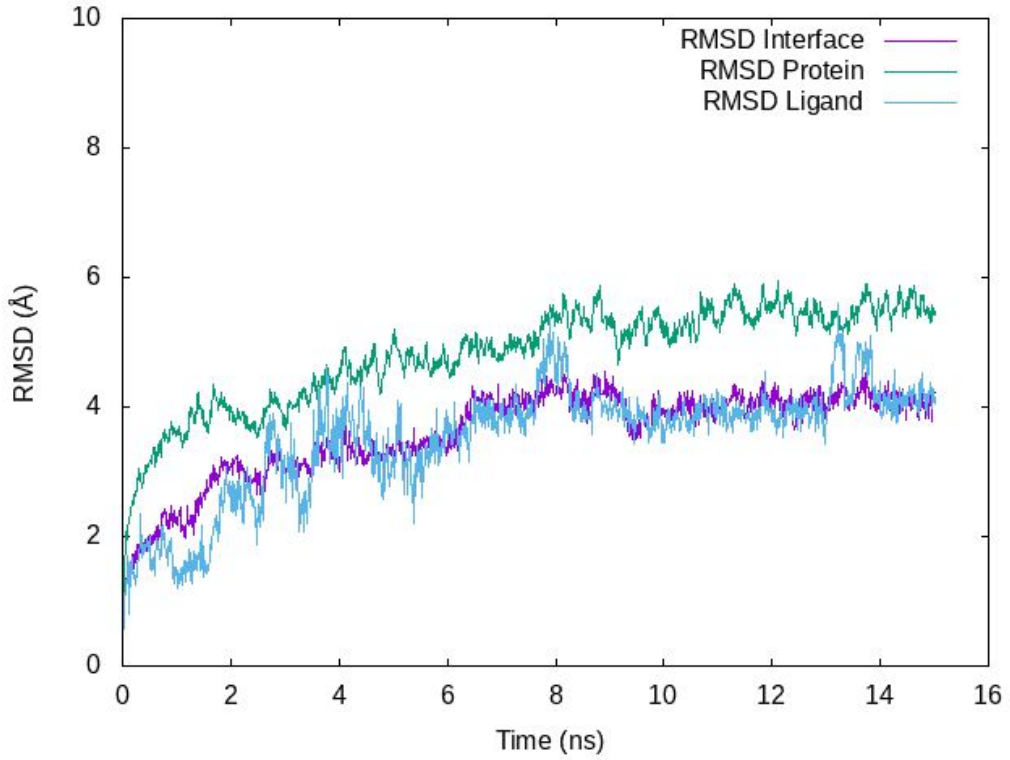
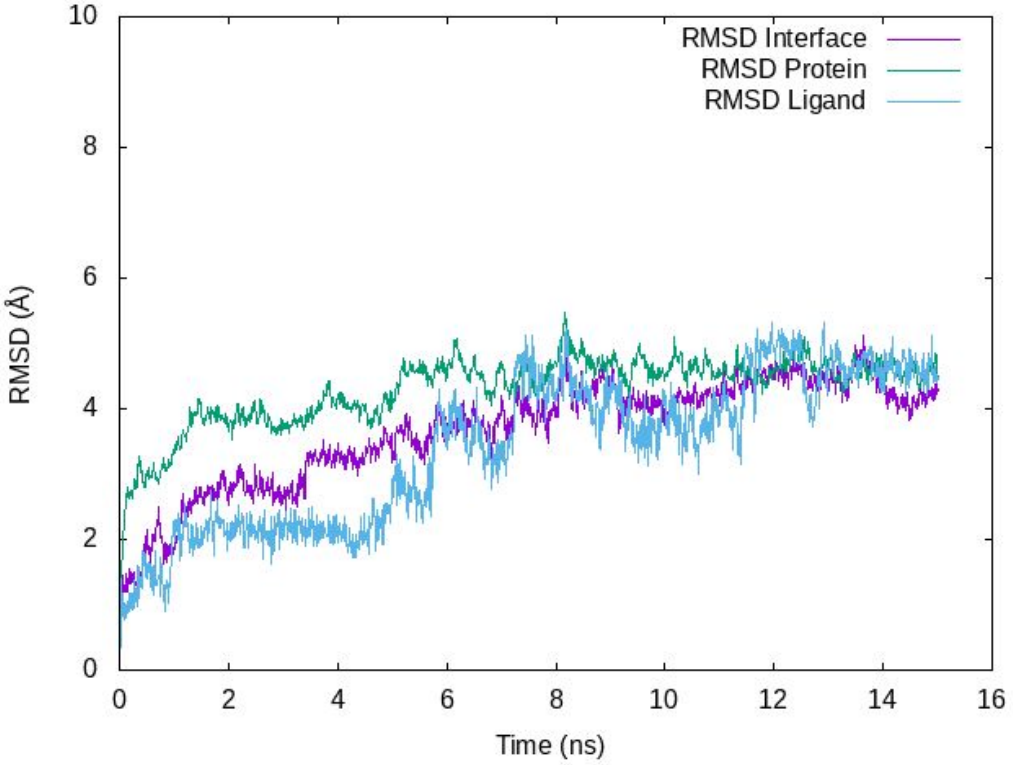
Ligand ID	RMSD
Masoprocol	
Trifluridine	

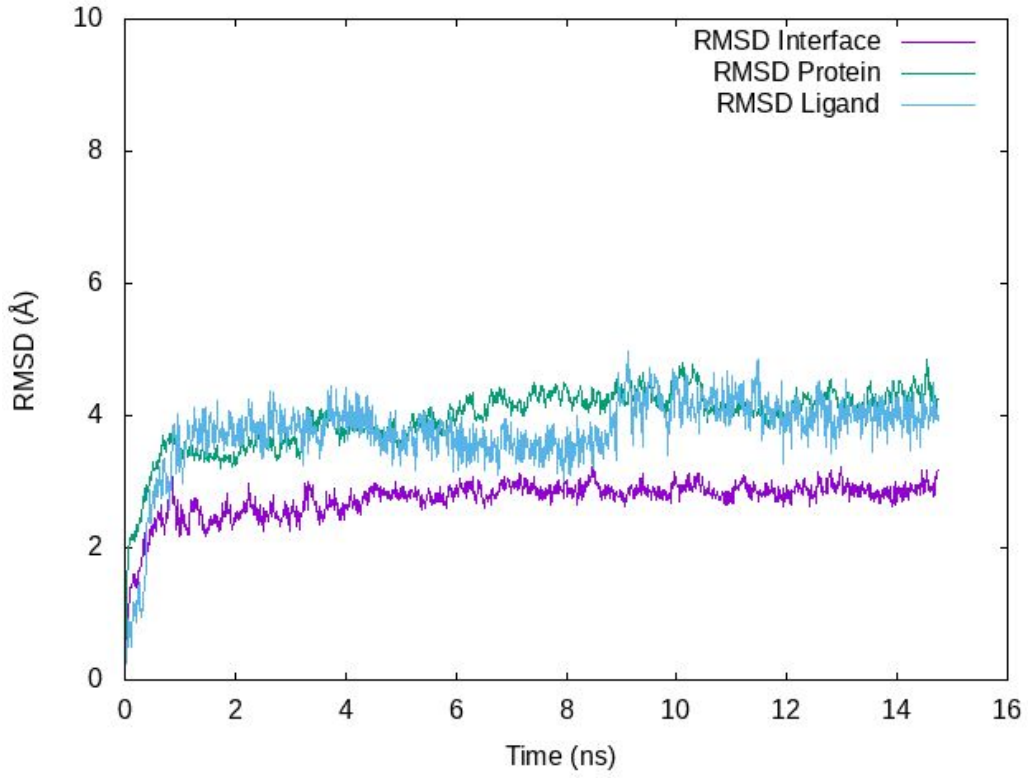
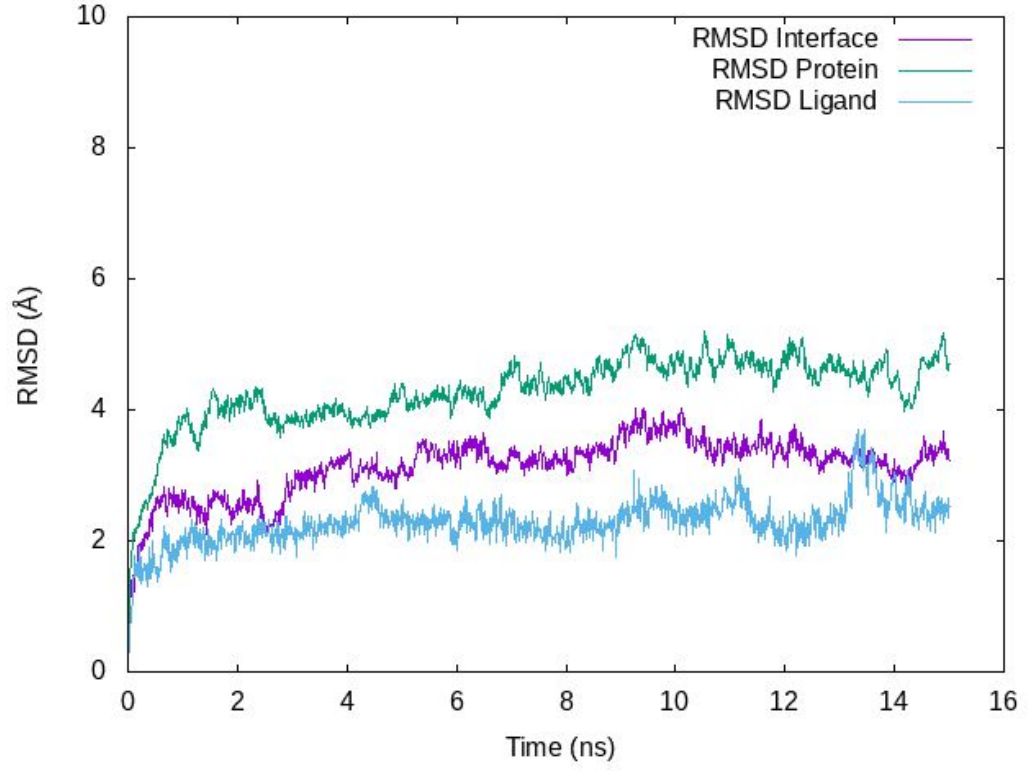
Brivudine	
(-)EGC	

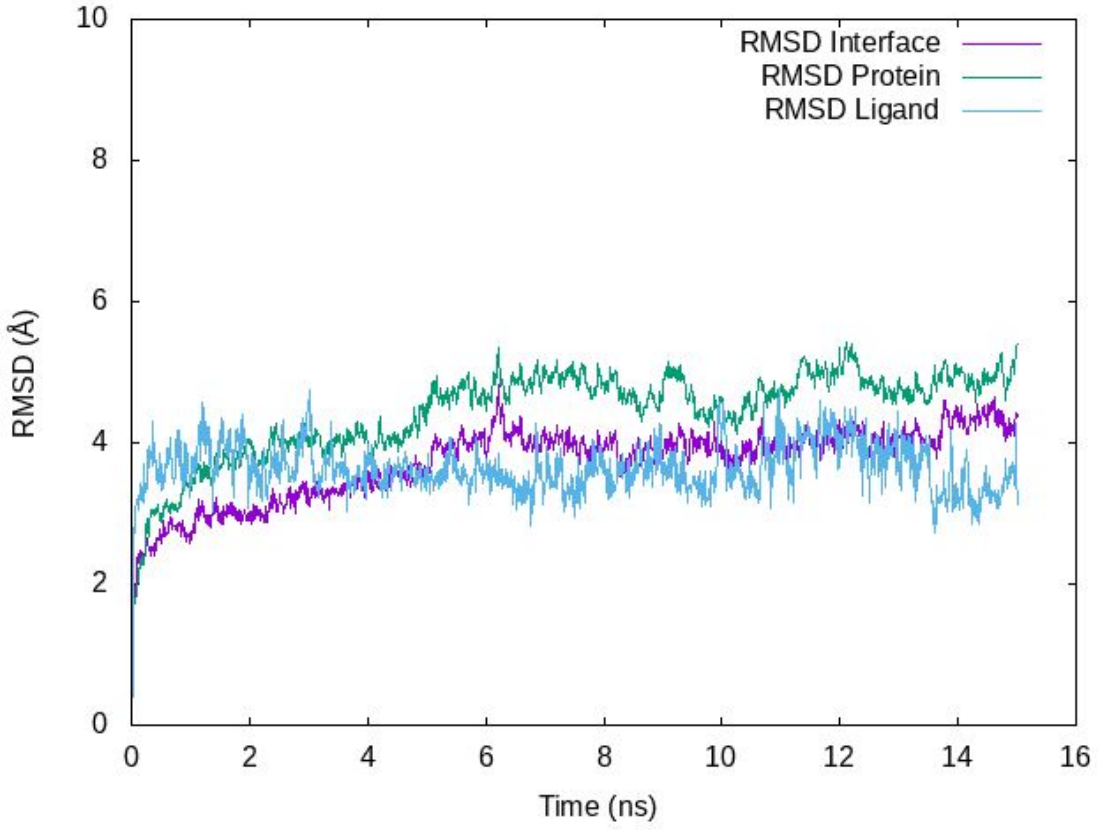
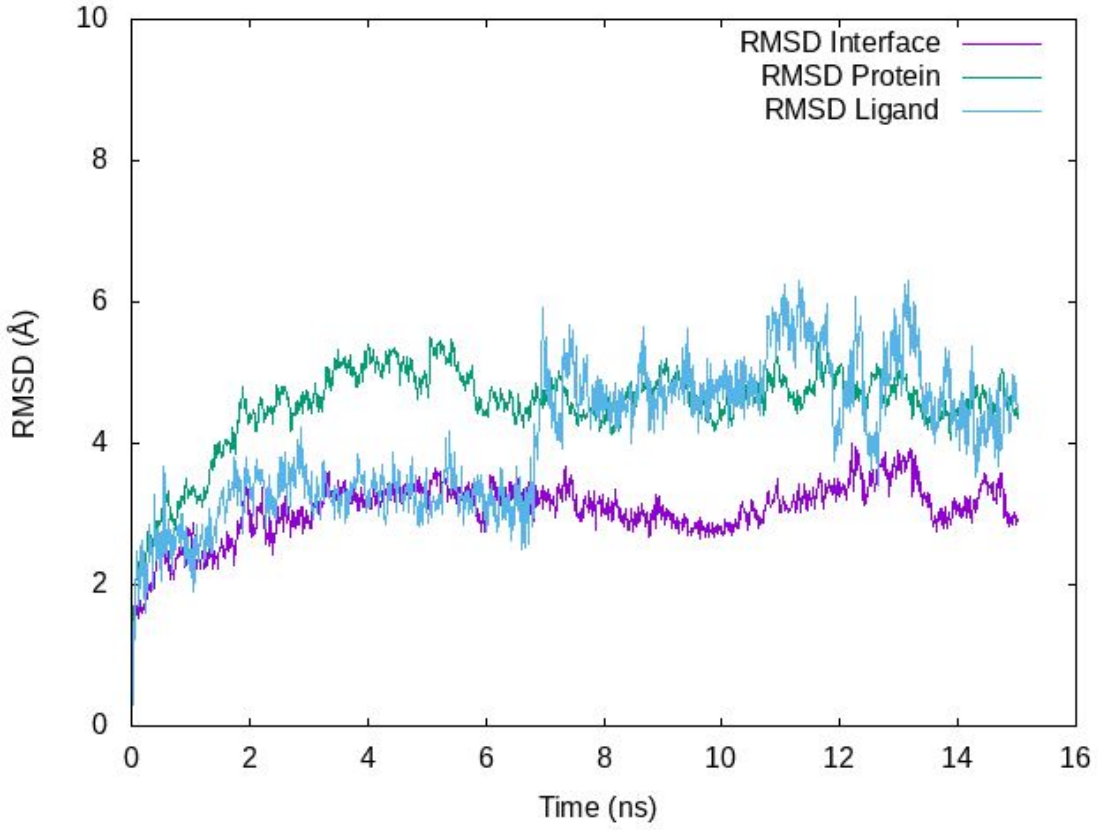
Quercetin	
Ruxolitinib	

(-)EC	 RMSD Interface RMSD Protein RMSD Ligand RMSD (Å) Time (ns) 0 2 4 6 8 10 12 14 16 18 20
(-)EGCG	 RMSD Interface RMSD Protein RMSD Ligand RMSD (Å) Time (ns) 0 5 10 15 20

Cianidanol	
Gallic acid 3-O-gallate	

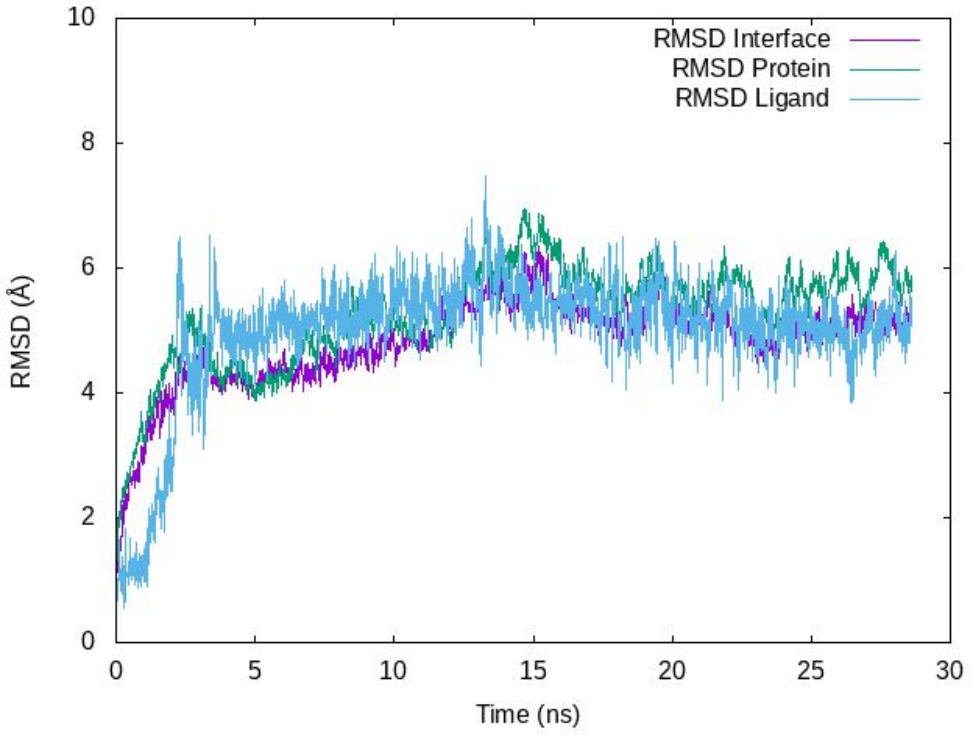
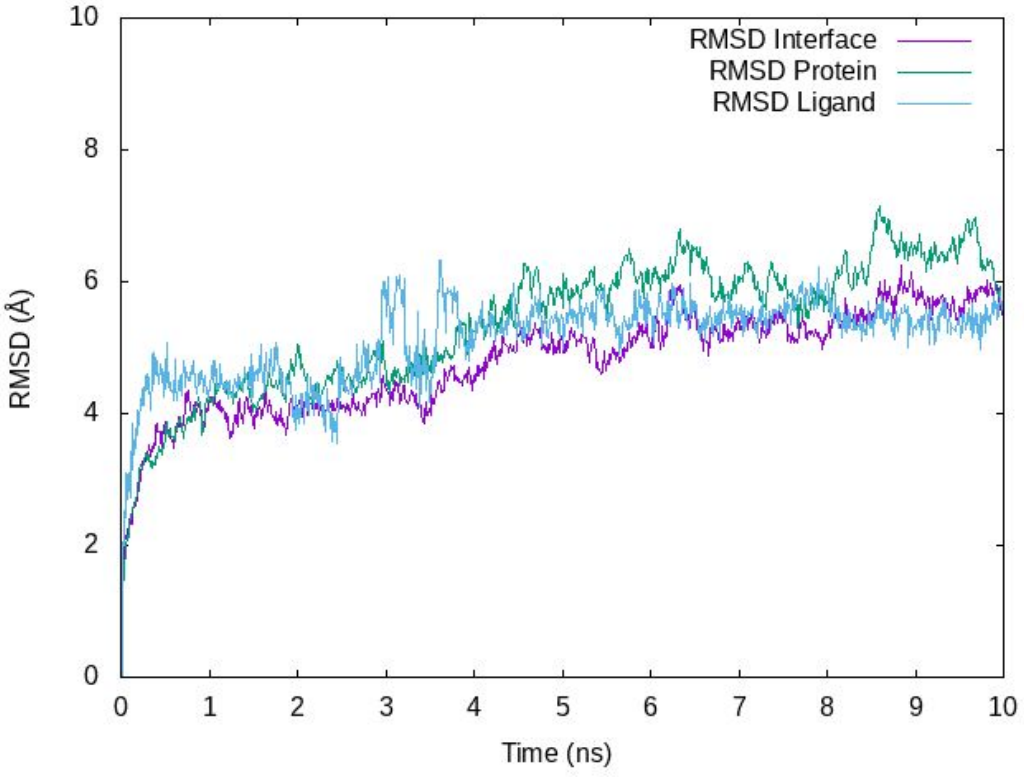
(+)GC	 RMSD Interface RMSD Protein RMSD Ligand RMSD (Å) Time (ns) 0 2 4 6 8 10 12 14 16
(+)GCG	 RMSD Interface RMSD Protein RMSD Ligand RMSD (Å) Time (ns) 0 2 4 6 8 10 12 14 16

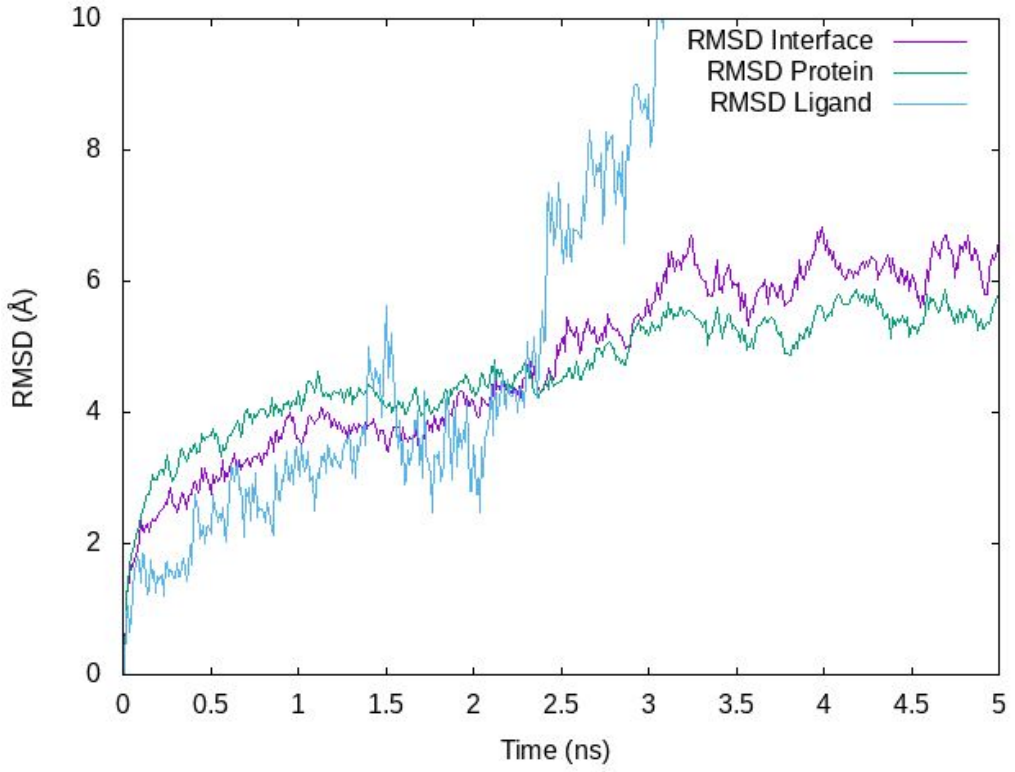
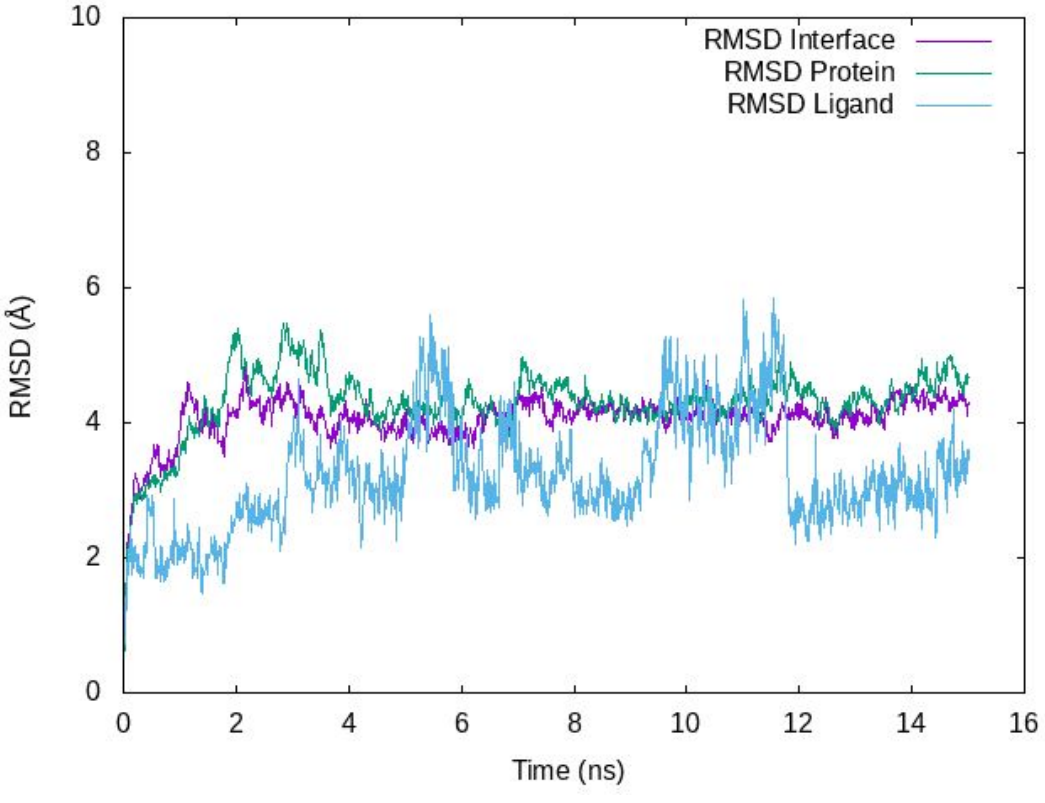
(-)C	 RMSD Interface RMSD Protein RMSD Ligand RMSD (Å) Time (ns) 0 2 4 6 8 10 12 14 16
(-)CG	 RMSD Interface RMSD Protein RMSD Ligand RMSD (Å) Time (ns) 0 2 4 6 8 10 12 14 16

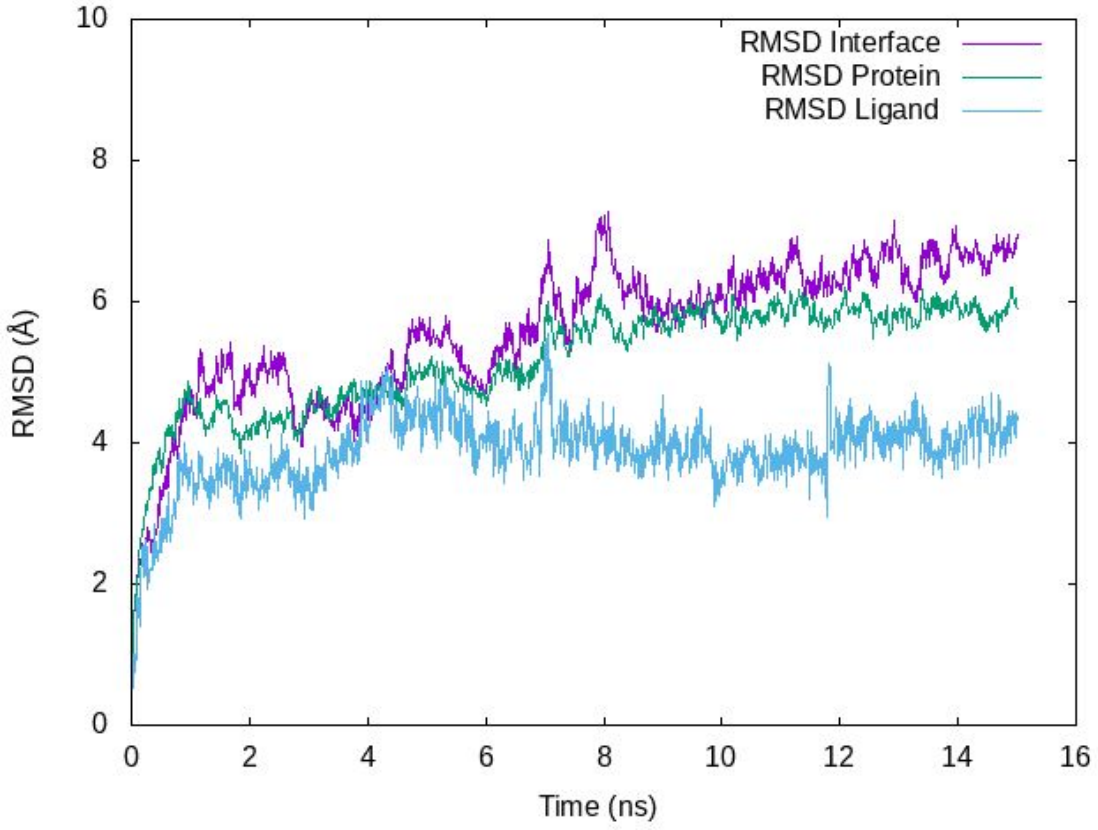
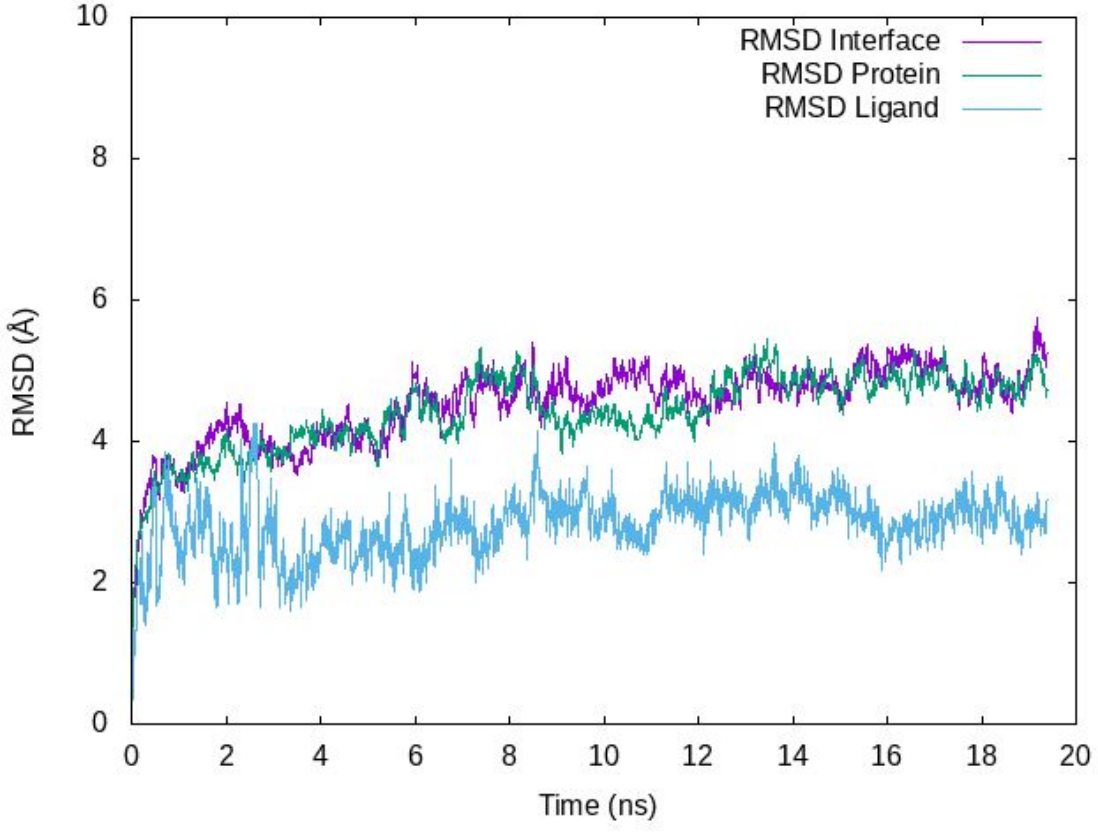
(-)GCG	
Sorivudine	

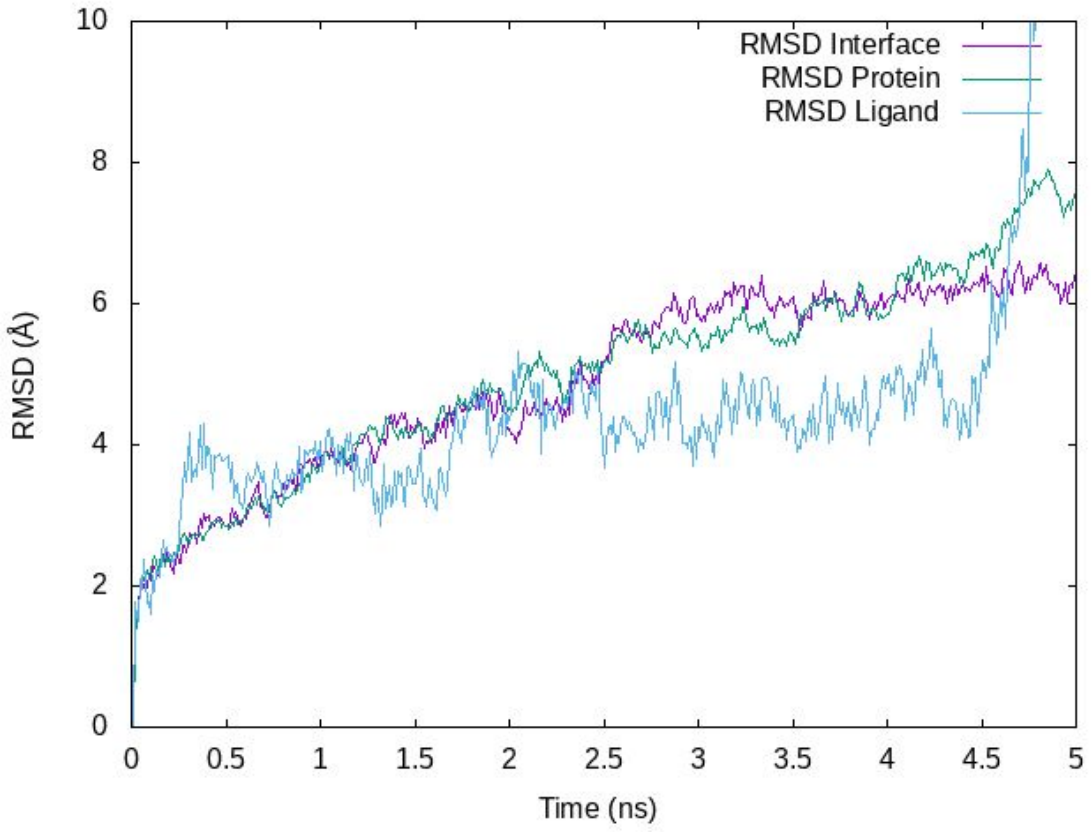
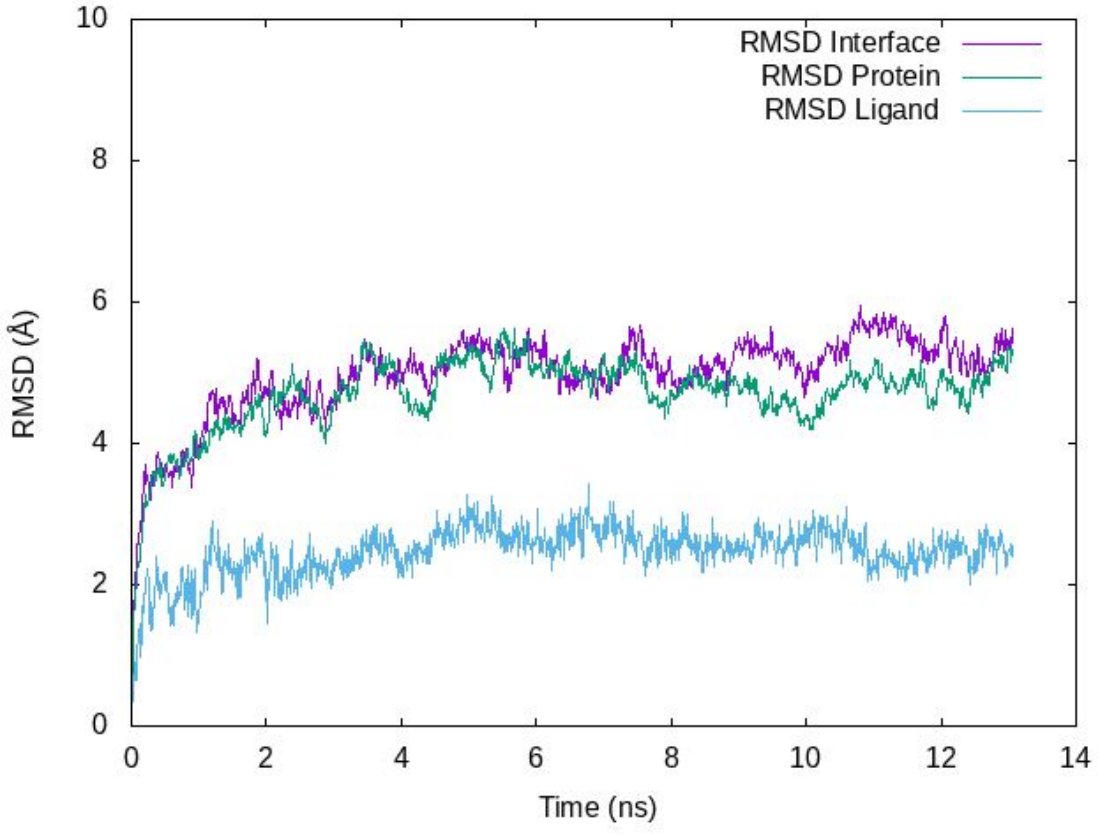
IF-2

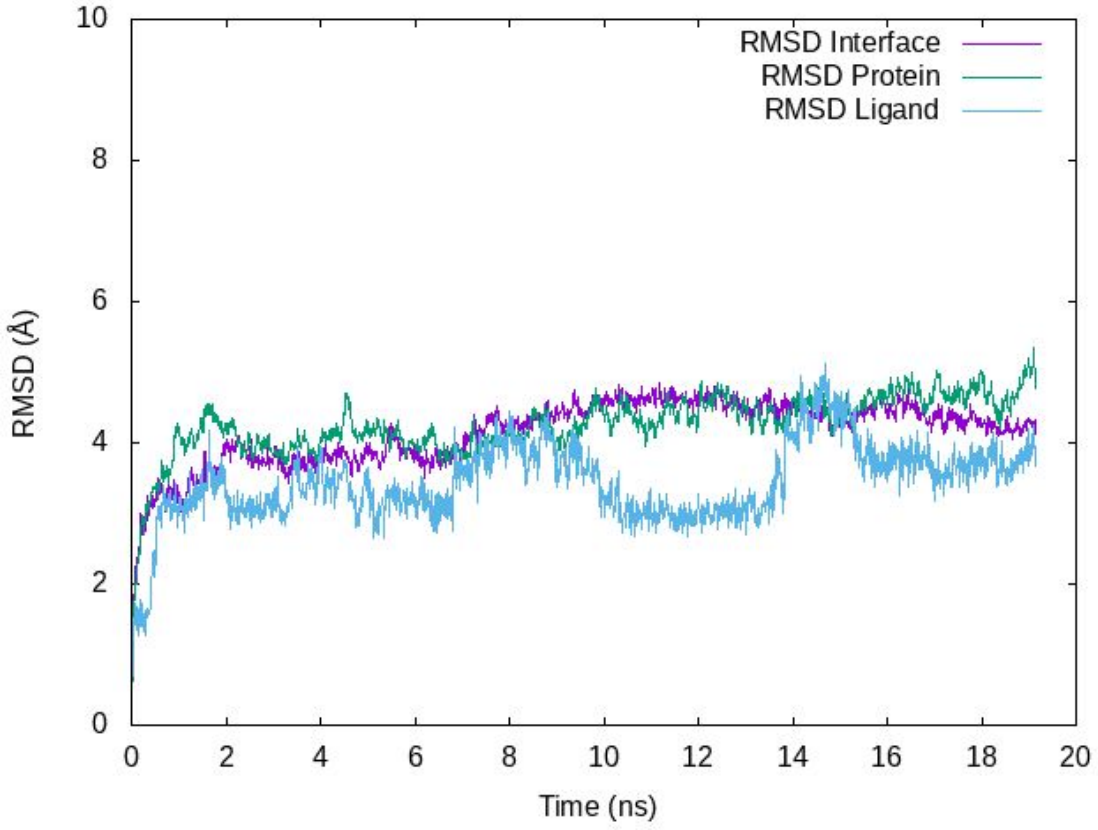
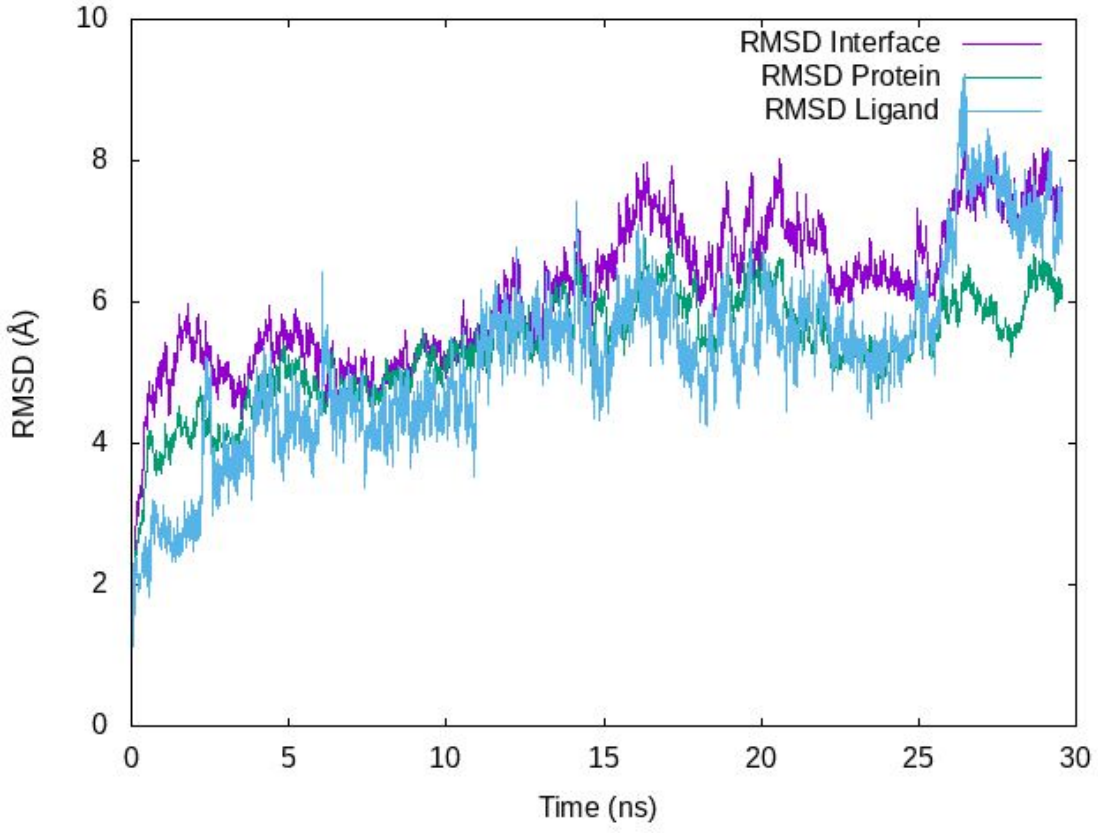
Table S7. RMSD profiles of the equilibration/production stage are shown for each compound in IF-2. "RMSD Protein" was measured considering all residues in the complex aligned using the protein backbone. "RMSD Interface" and "RMSD Ligand" were calculated aligning by the interface-forming residues and then measured using those same residues and the ligand, respectively.

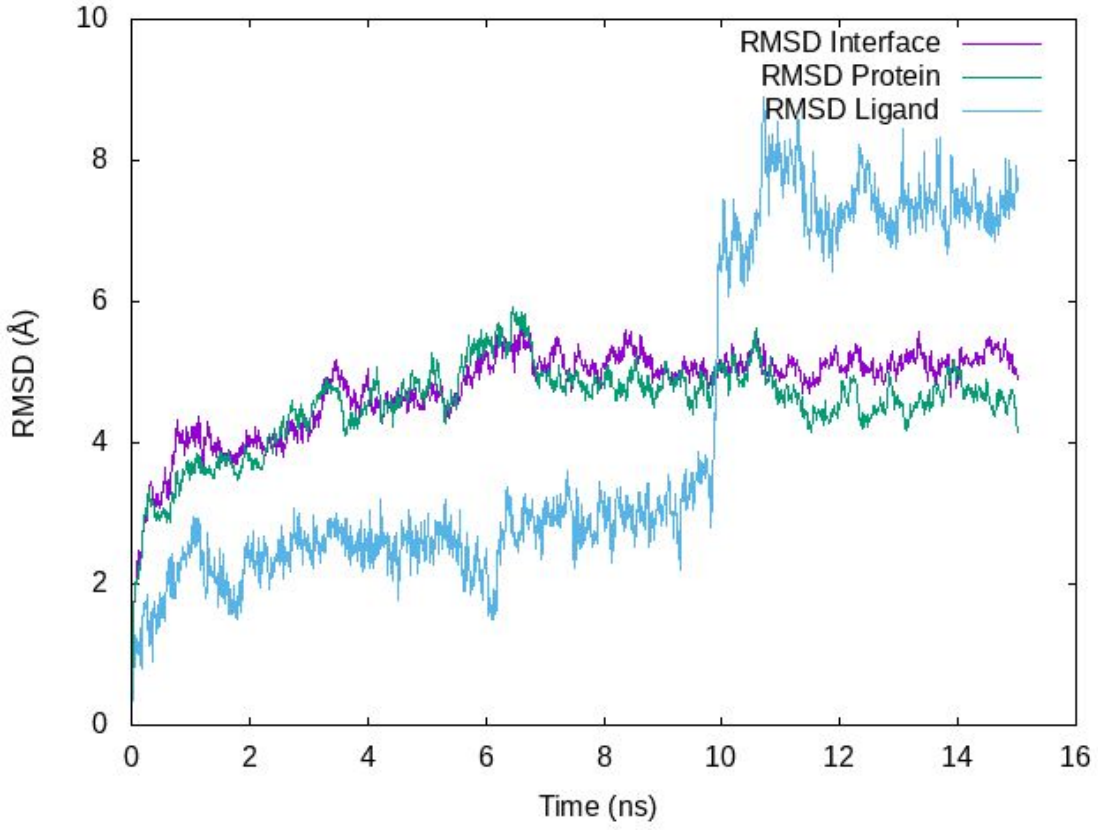
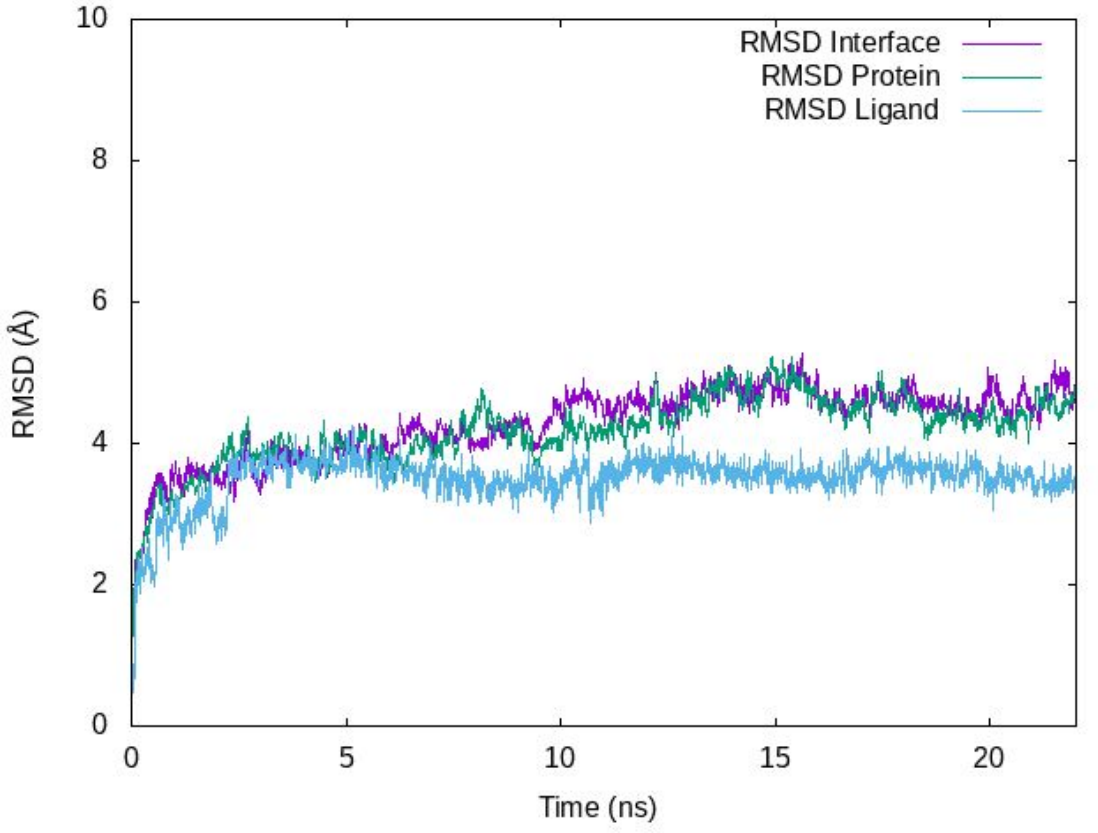
Ligand ID	RMSD
Masoprocol	
Phenformin	

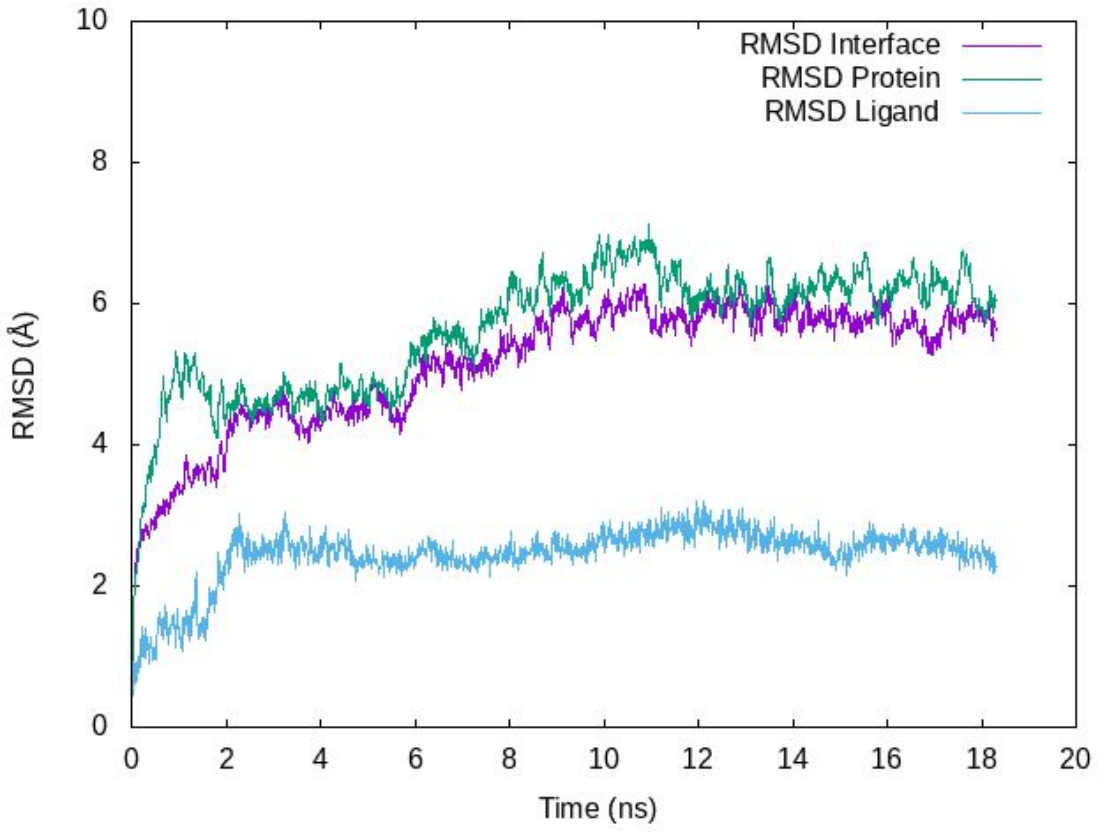
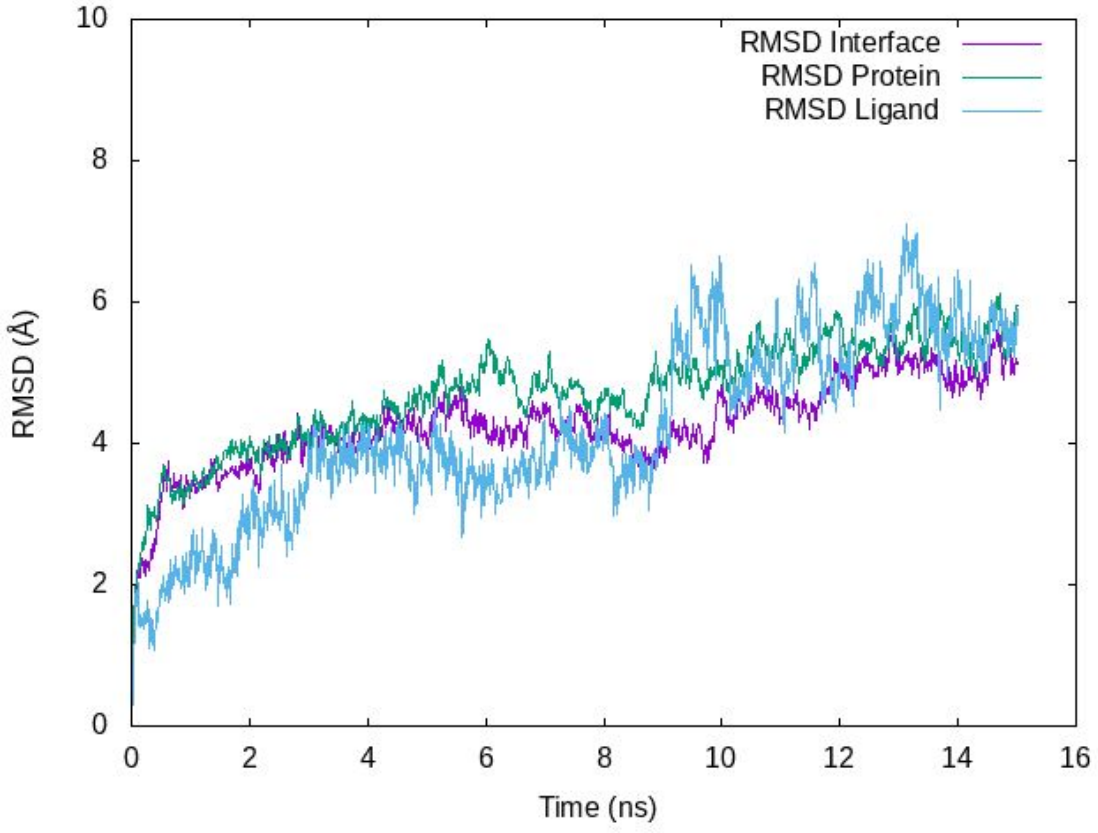
2-iminobiotin e	
(-)EGC	

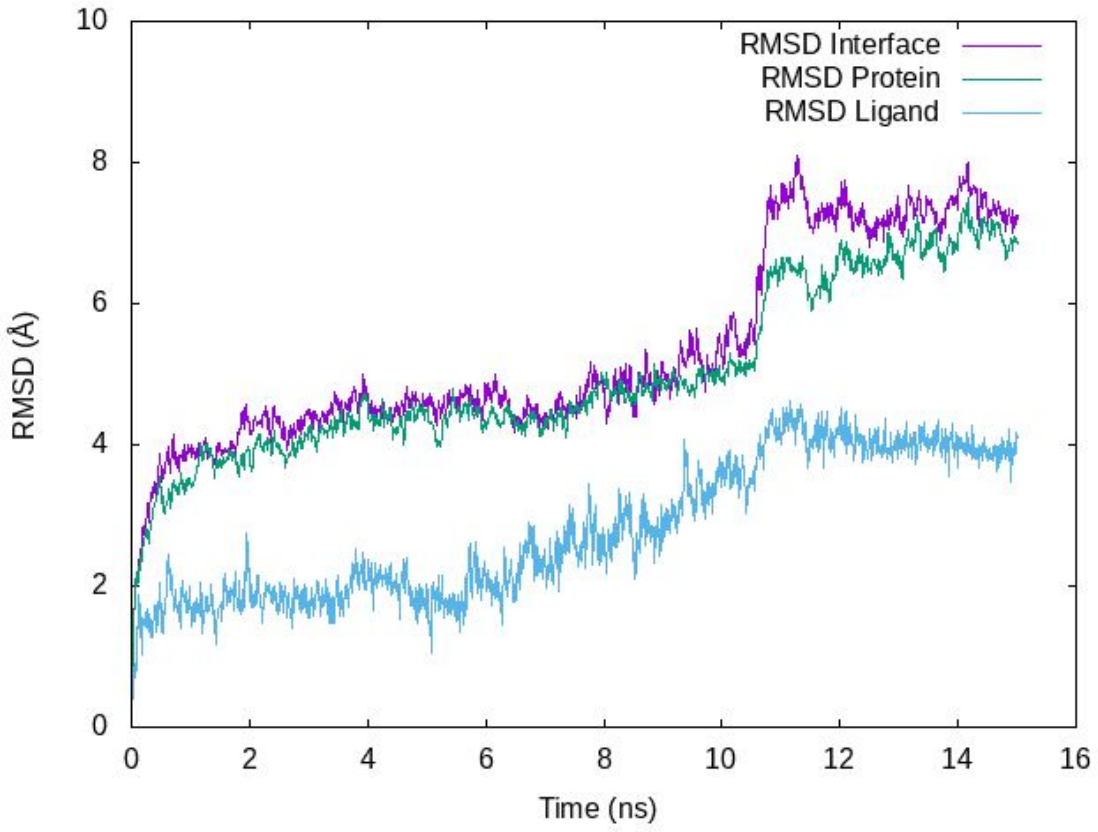
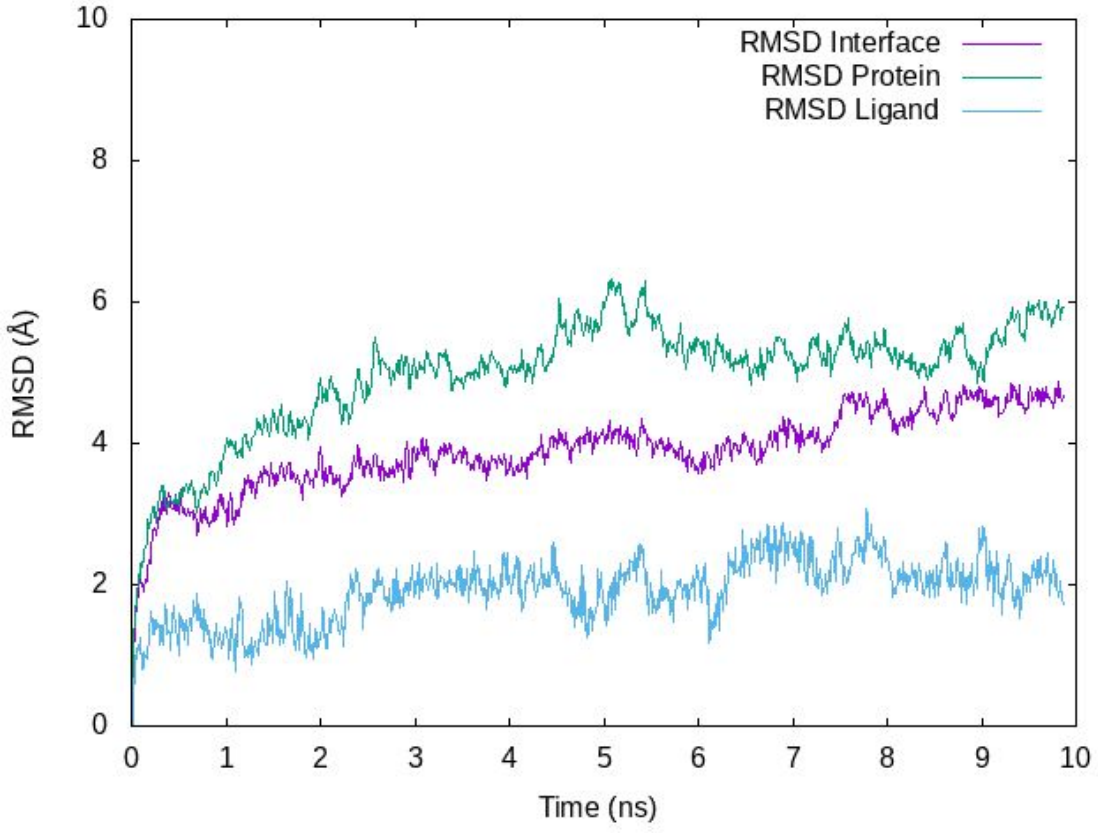
Quercetin	
Ruxolitinib	

o6-bencylguaniane	
(-)EC	

(-)EGCG	
Cianidanol	

Gallic acid 3-O-gallate	
(+)GC	

(+)GCG	 RMSD Interface RMSD Protein RMSD Ligand RMSD (Å) Time (ns) 0 2 4 6 8 10 12 14 16 18 20
(-)C	 RMSD Interface RMSD Protein RMSD Ligand RMSD (Å) Time (ns) 0 2 4 6 8 10 12 14 16

(-)CG	 RMSD Interface RMSD Protein RMSD Ligand RMSD (Å) Time (ns) 0 2 4 6 8 10 12 14 16
(-)GCG	 RMSD Interface RMSD Protein RMSD Ligand RMSD (Å) Time (ns) 0 1 2 3 4 5 6 7 8 9 10

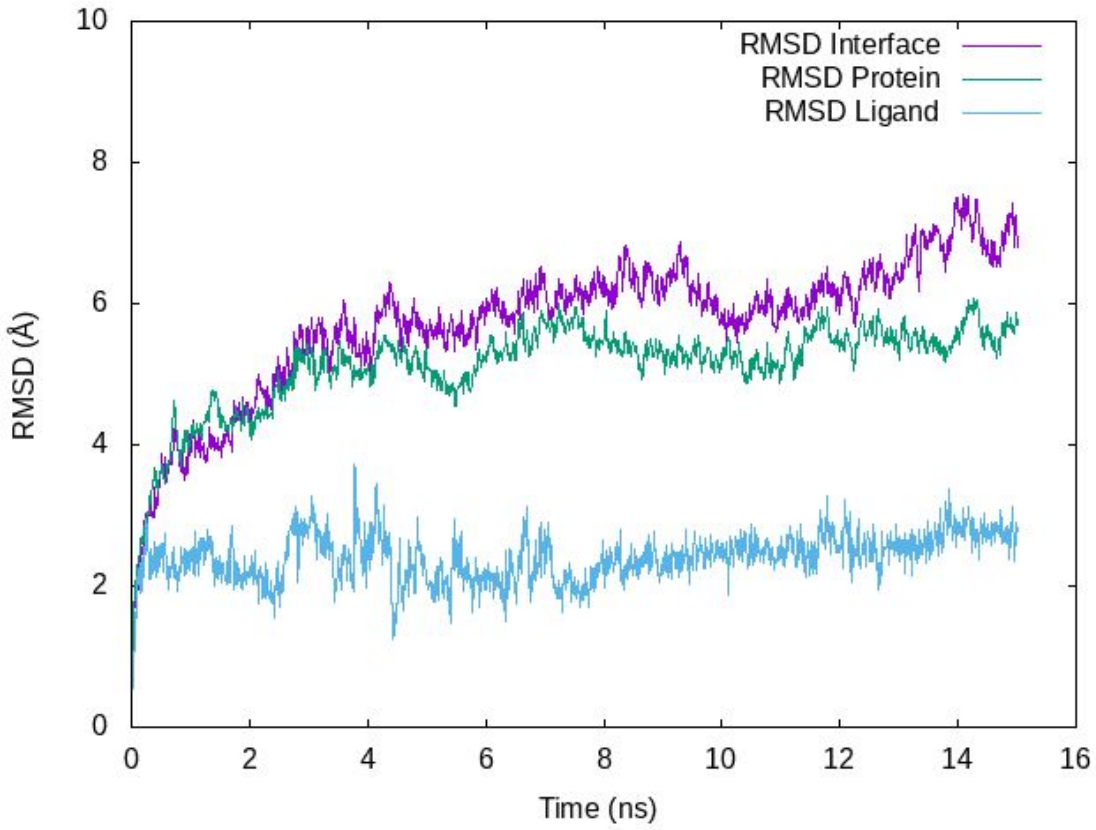
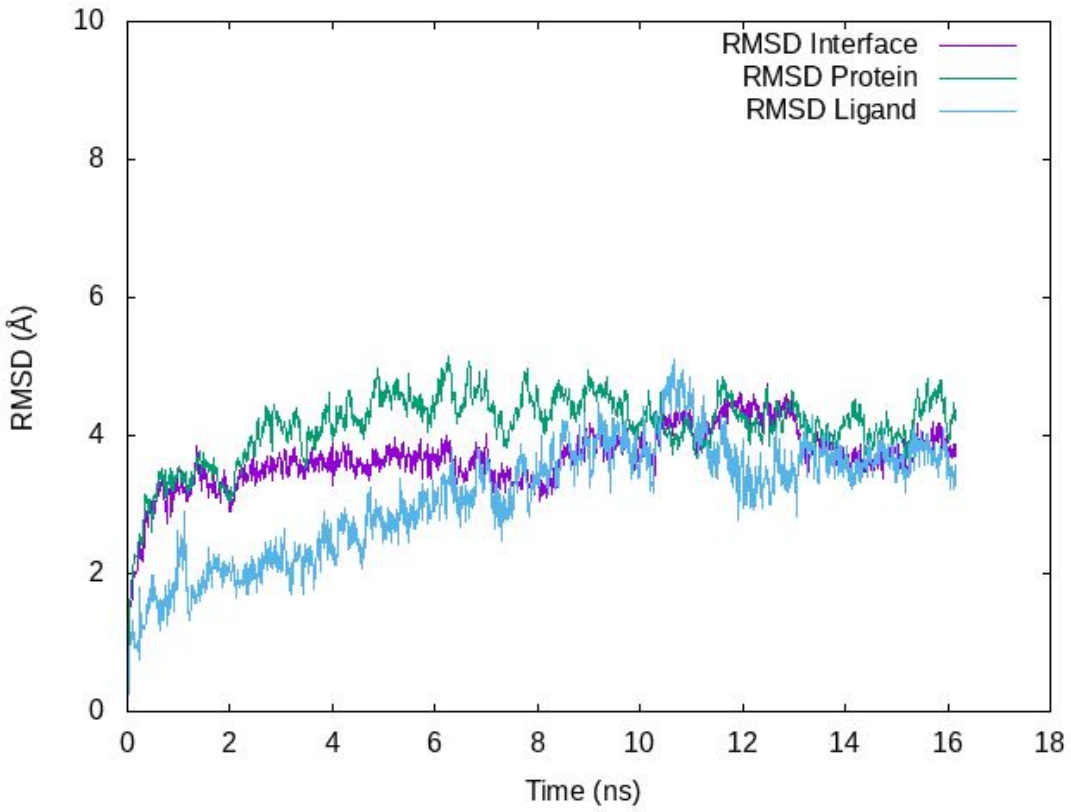
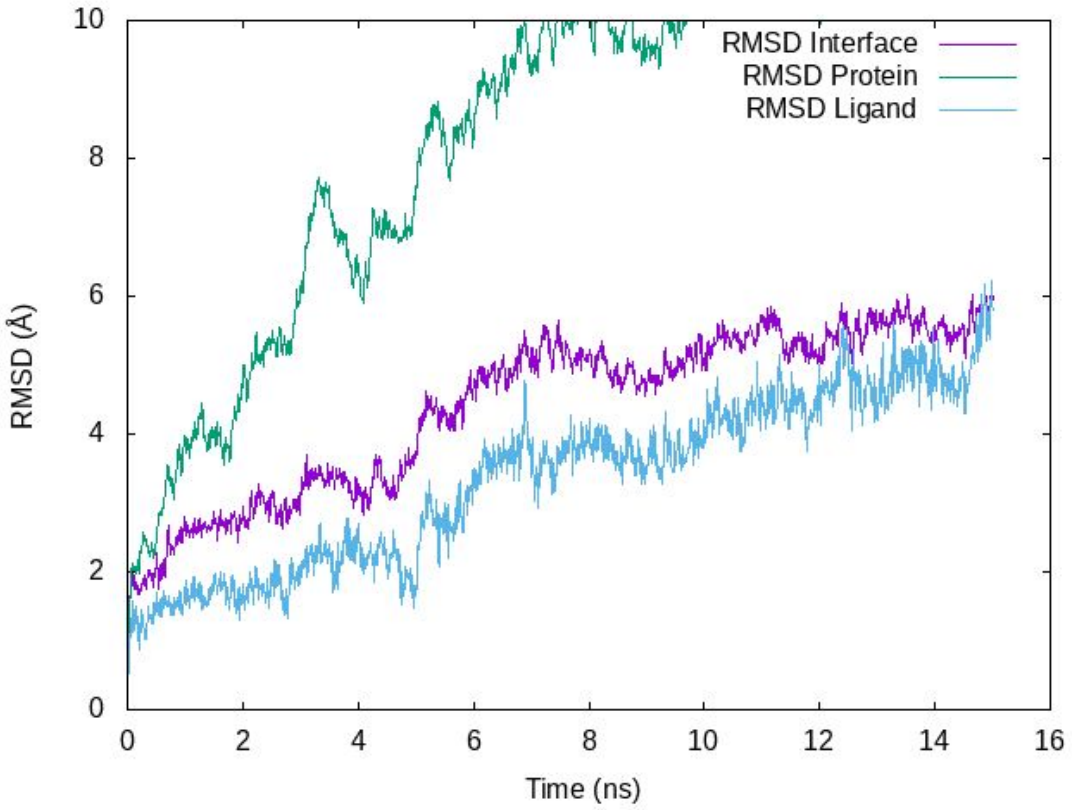
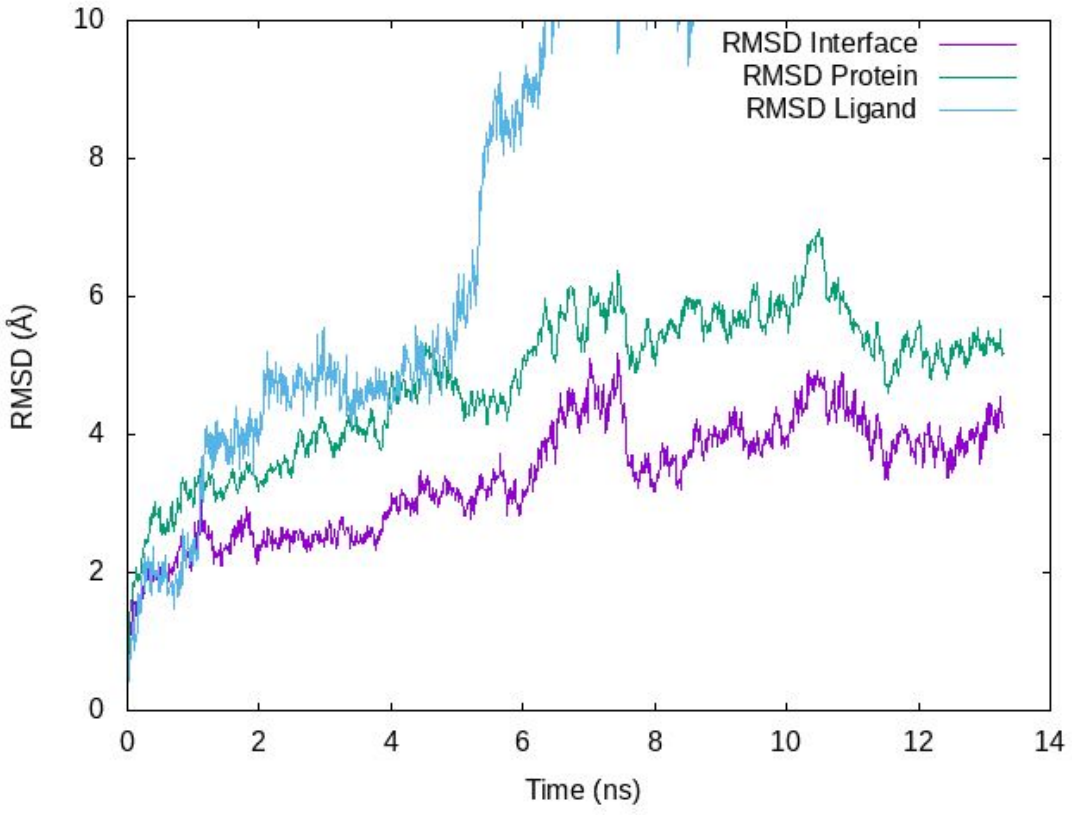
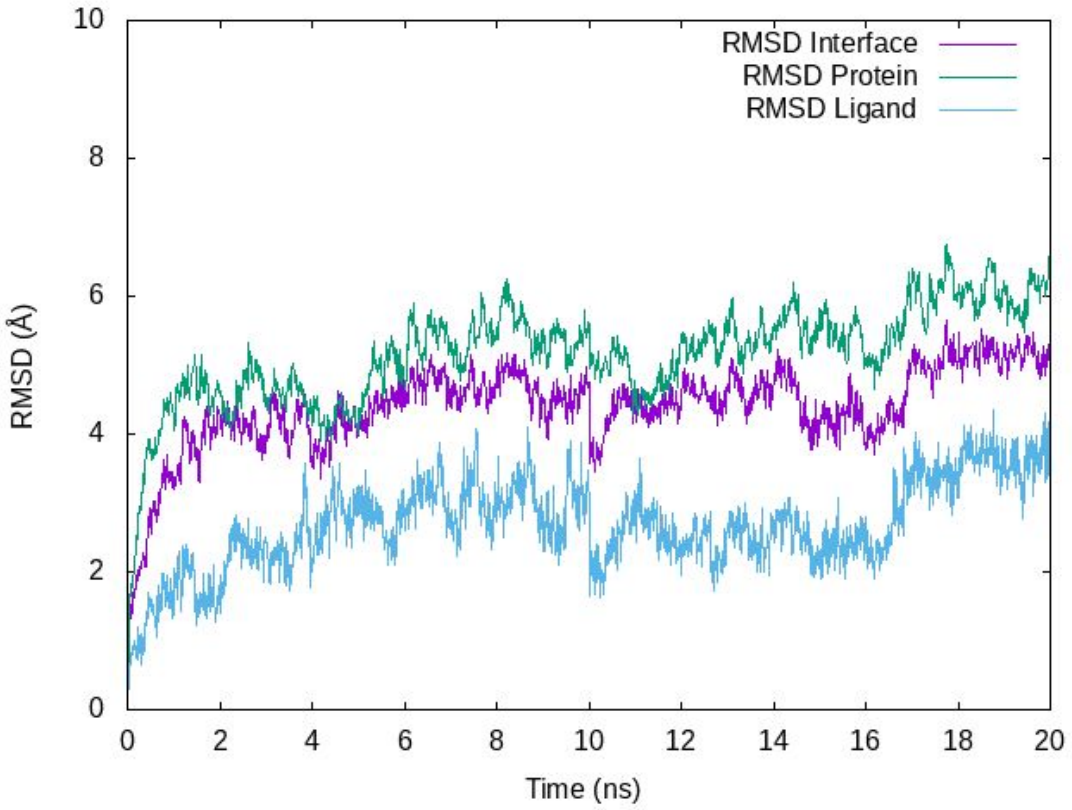
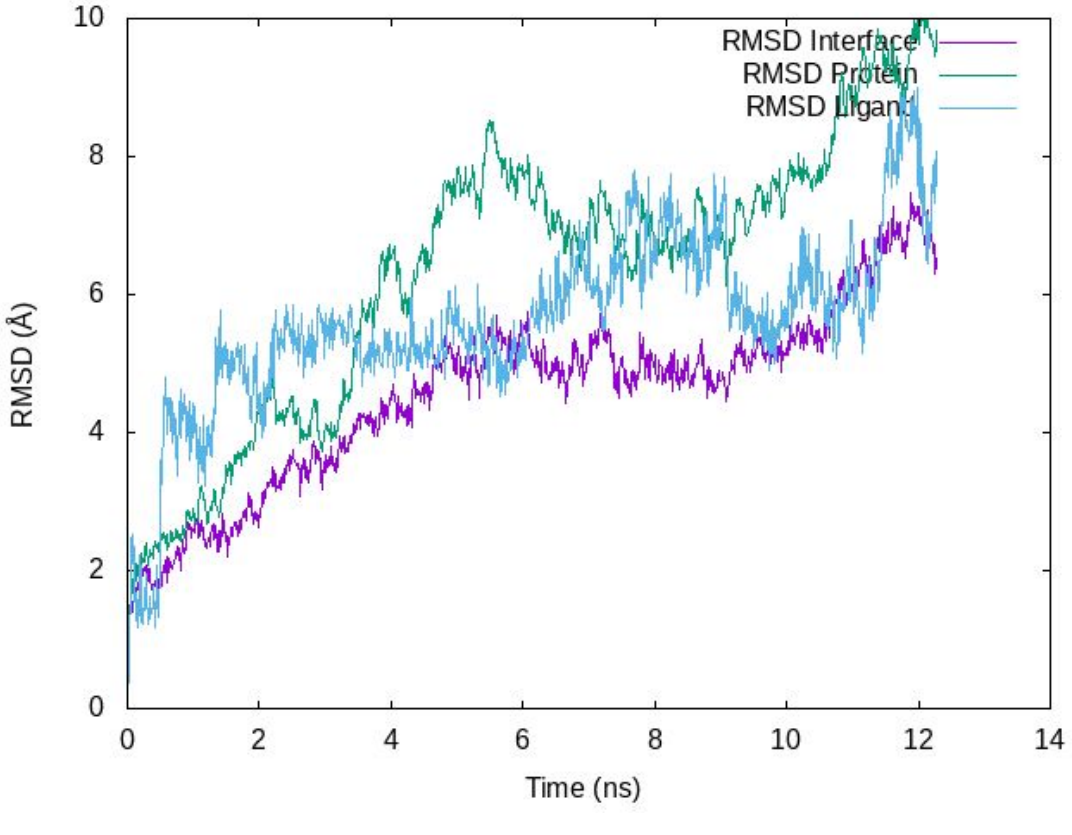
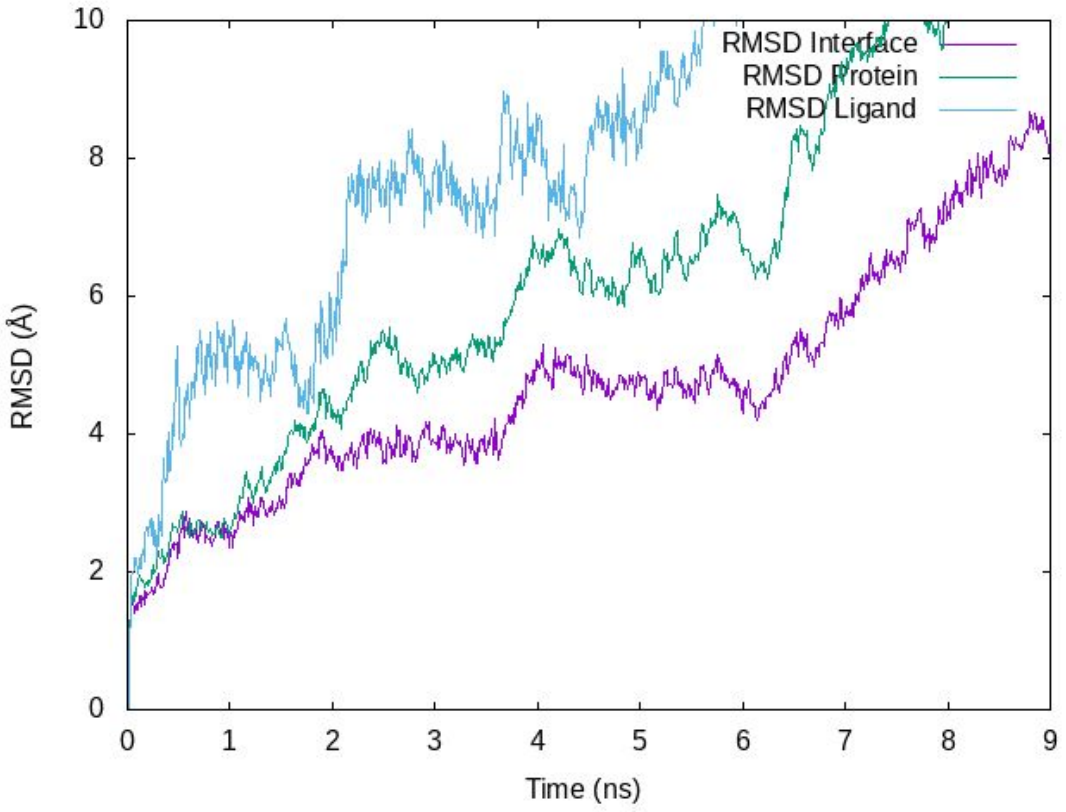
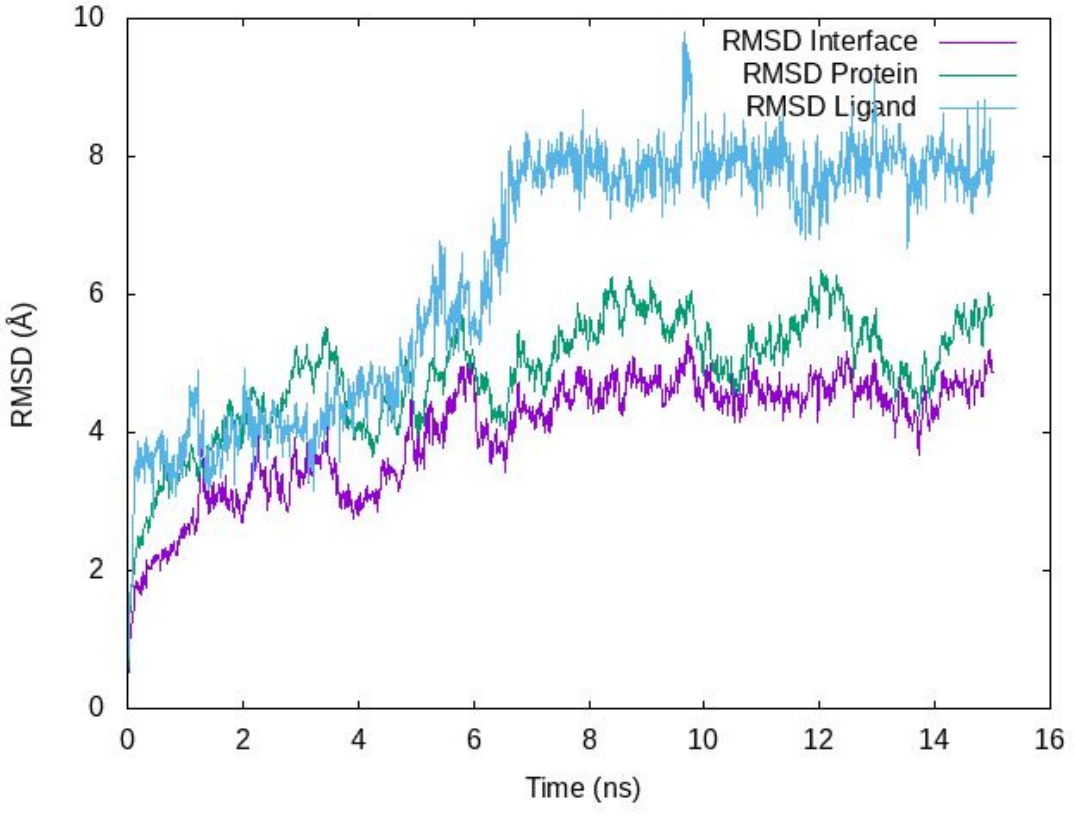
Sorivudine	 RMSD Interface RMSD Protein RMSD Ligand RMSD (Å) Time (ns) 0 2 4 6 8 10 12 14 16

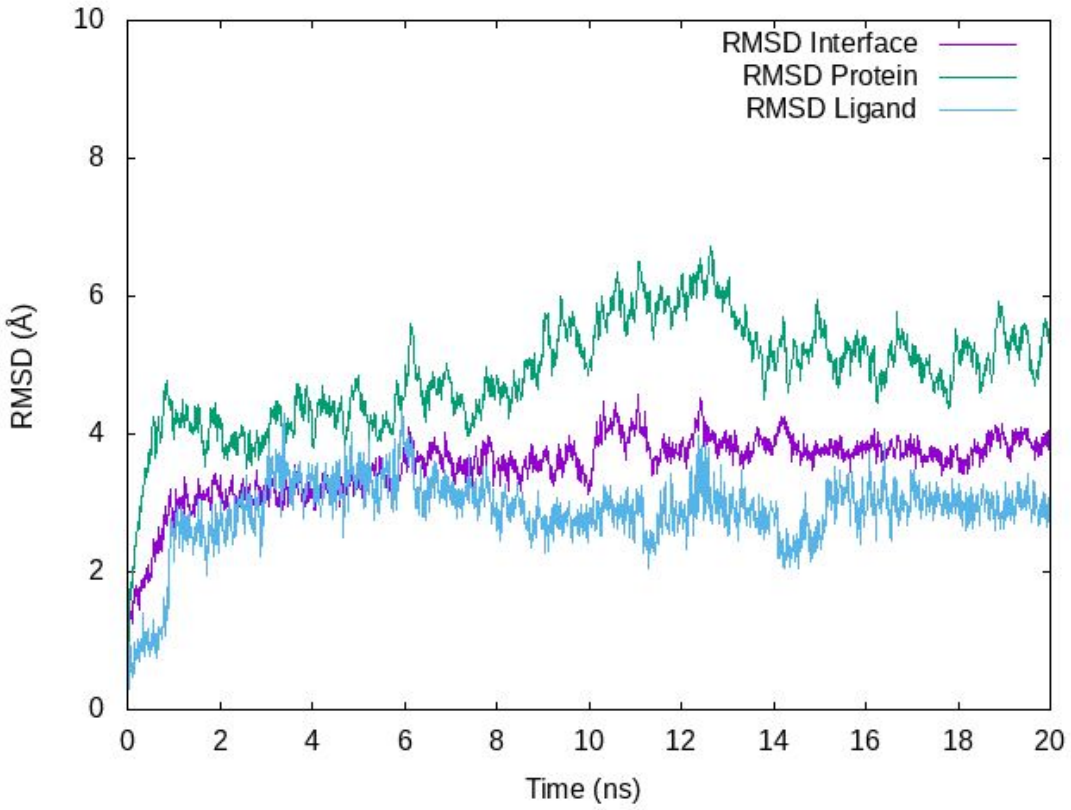
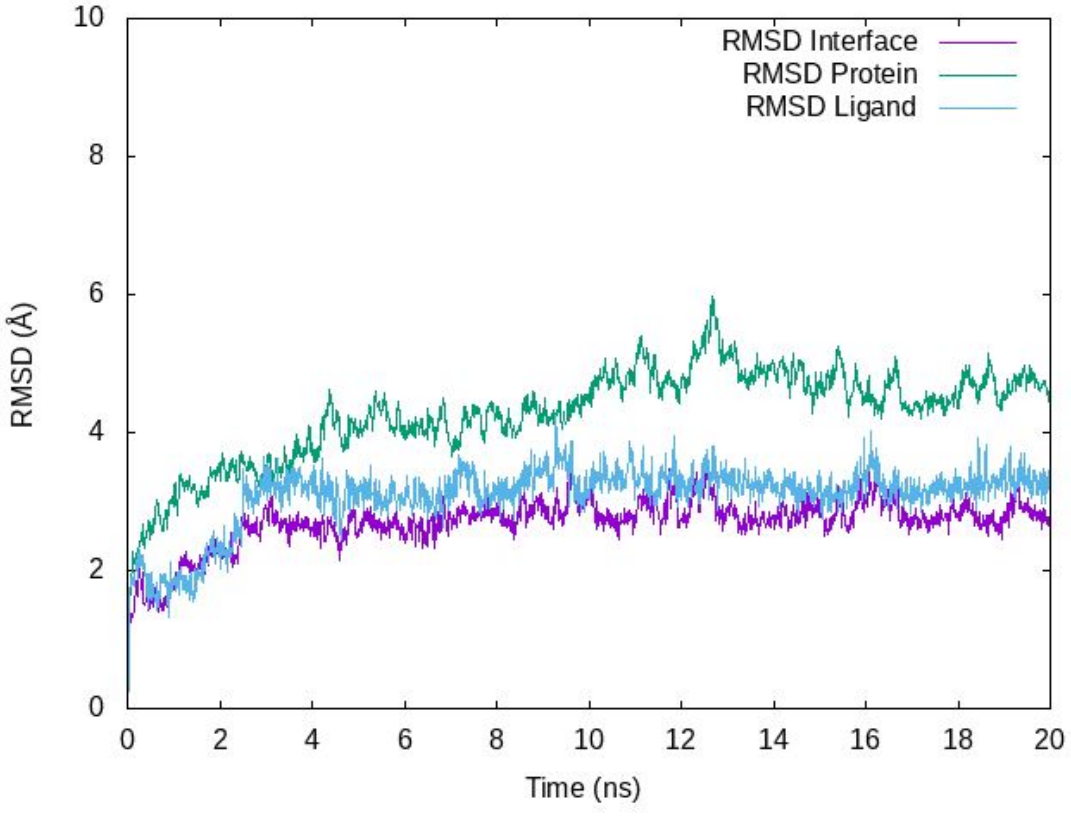
*Table S8. RMSD profiles of the equilibration/production stage are shown for each compound in **IF-3**. "RMSD Protein" was measured considering all residues in the complex aligned using the protein backbone. "RMSD Interface" and "RMSD Ligand" were calculated aligning by the interface-forming residues and then measured using those same residues and the ligand respectively.*

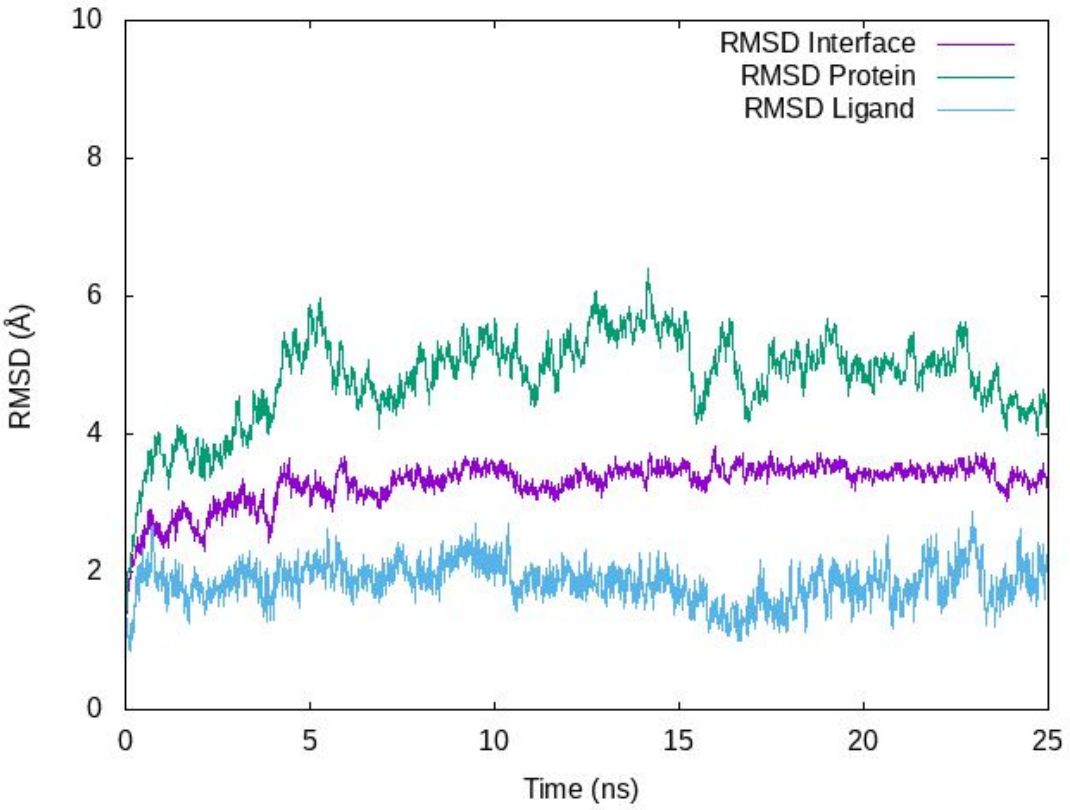
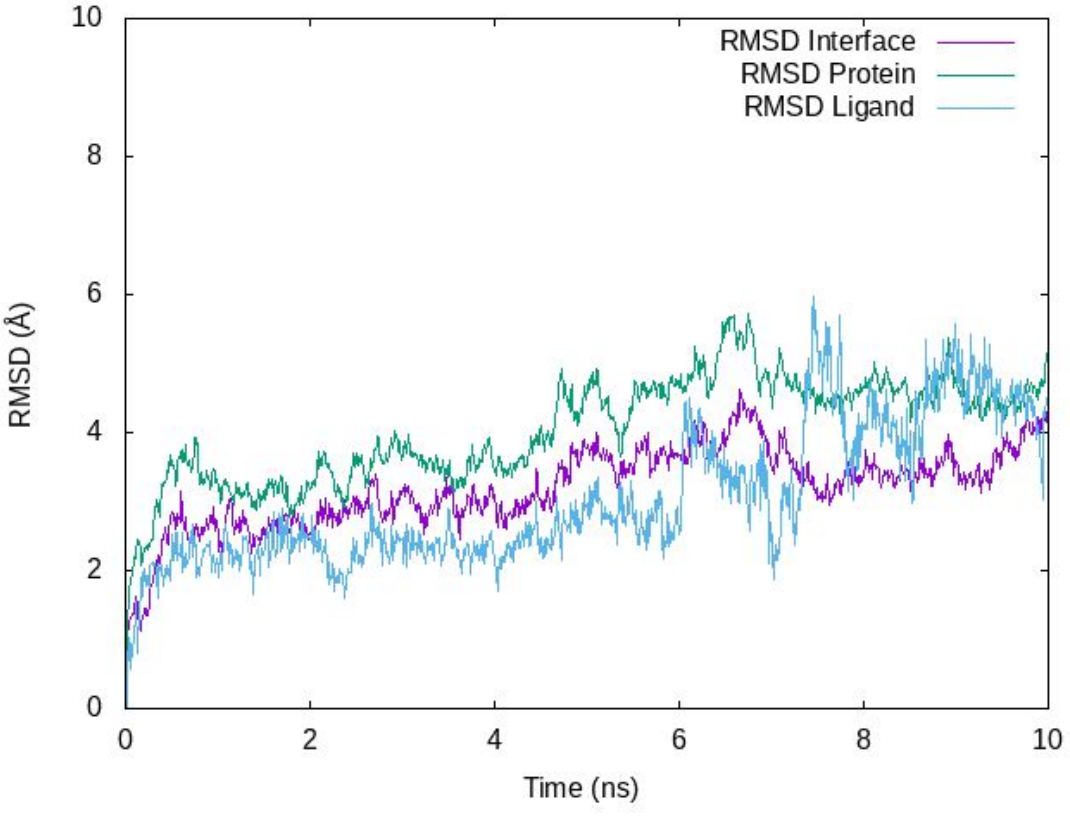
Ligand ID	RMSD
Masoprocol	

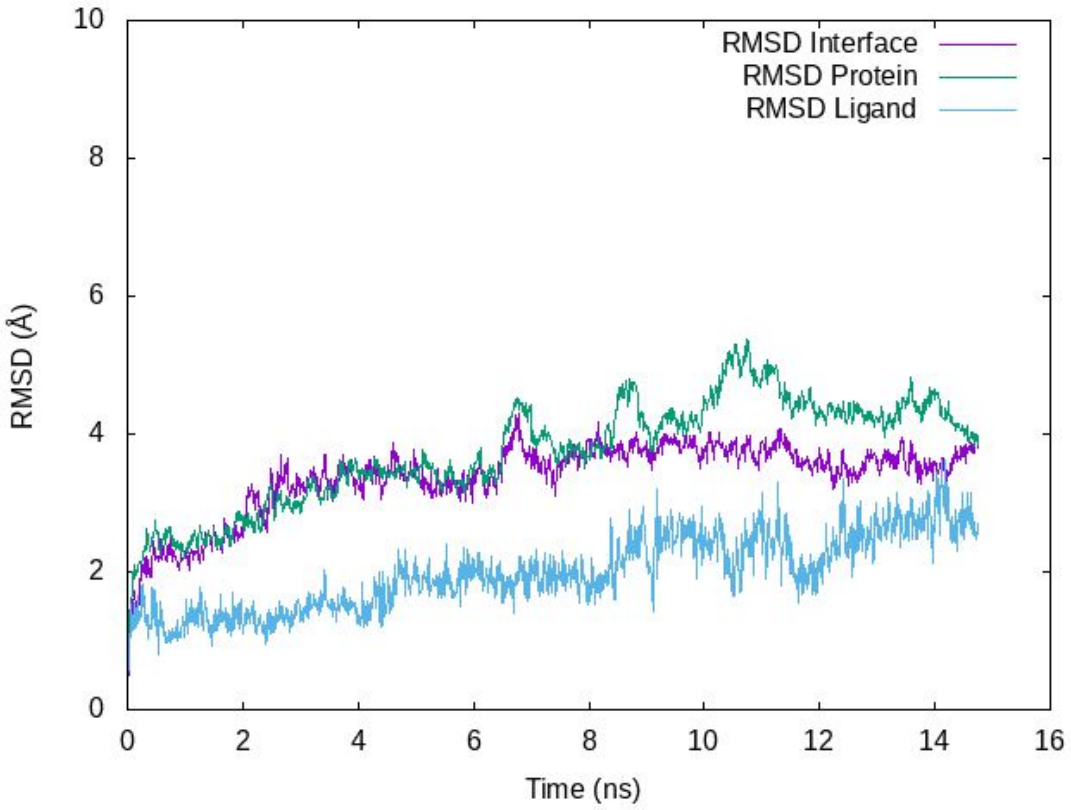
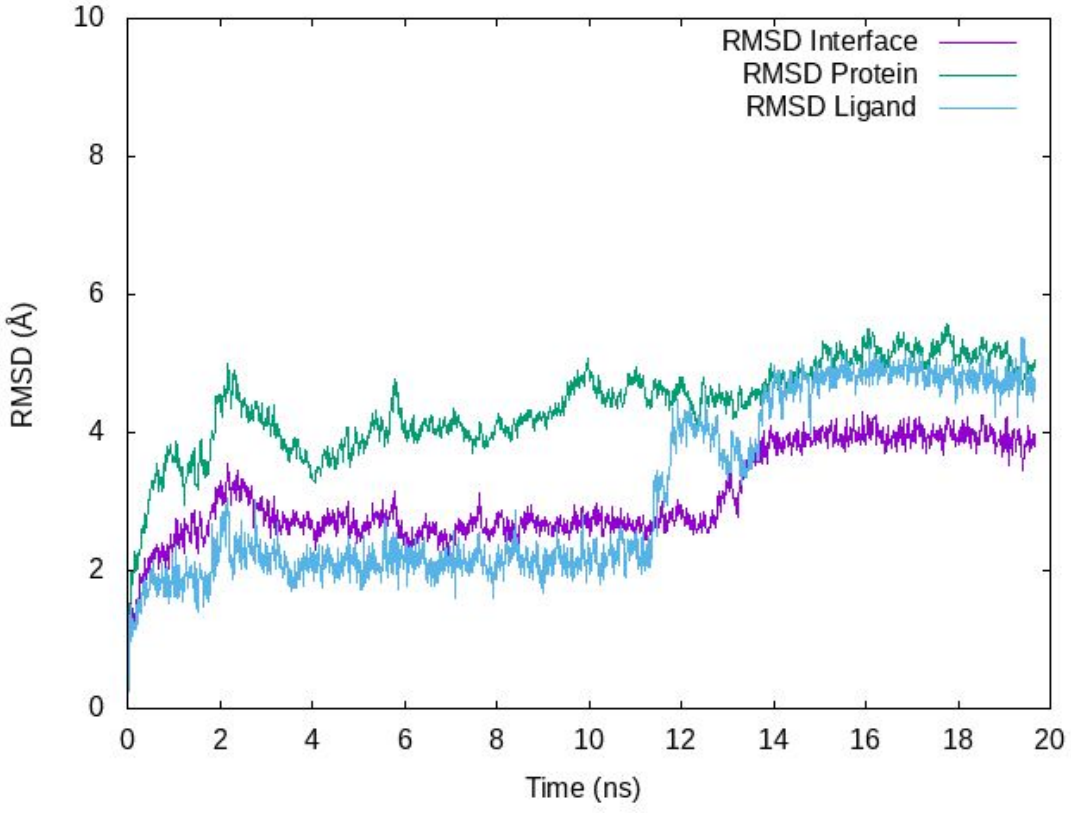
Phenformin	
Thienamycin	

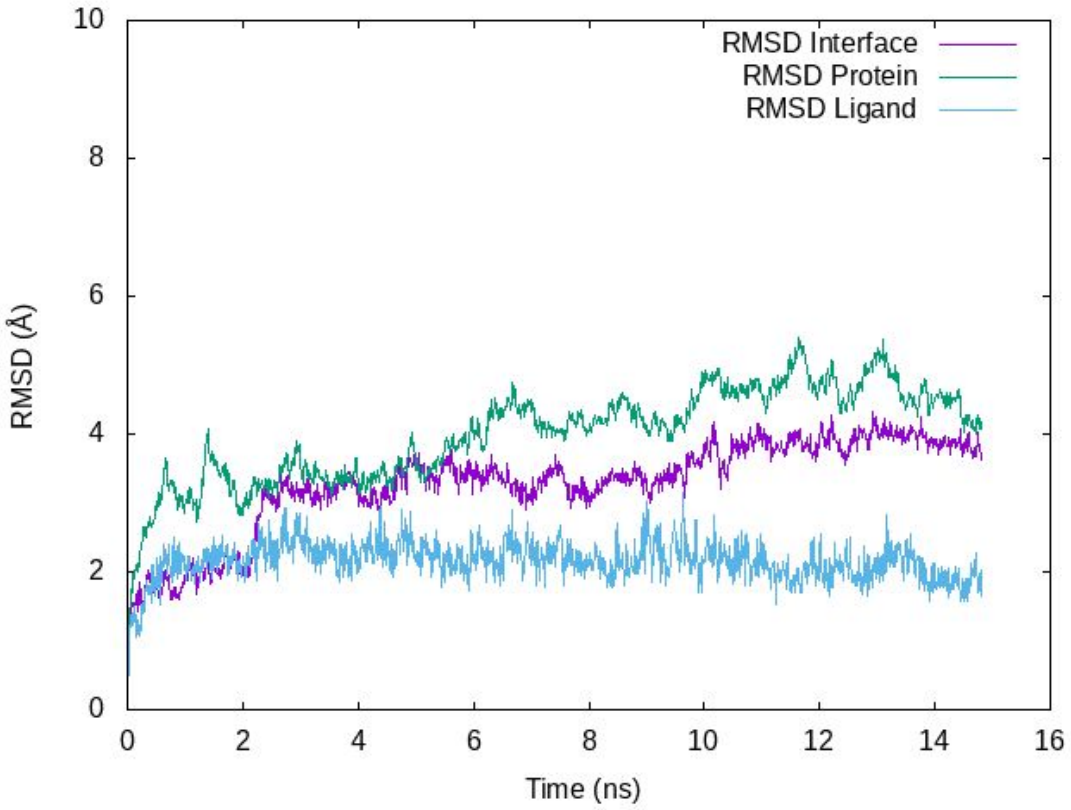
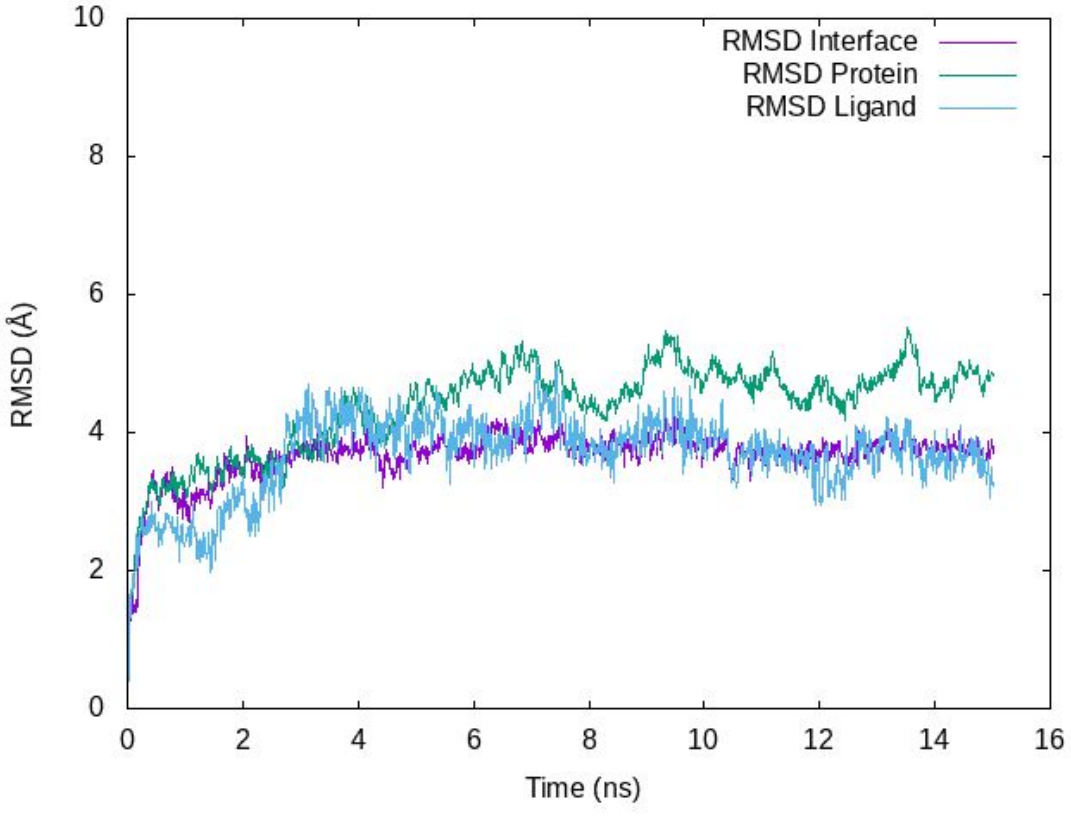
(-)EGC	
Quercetin	

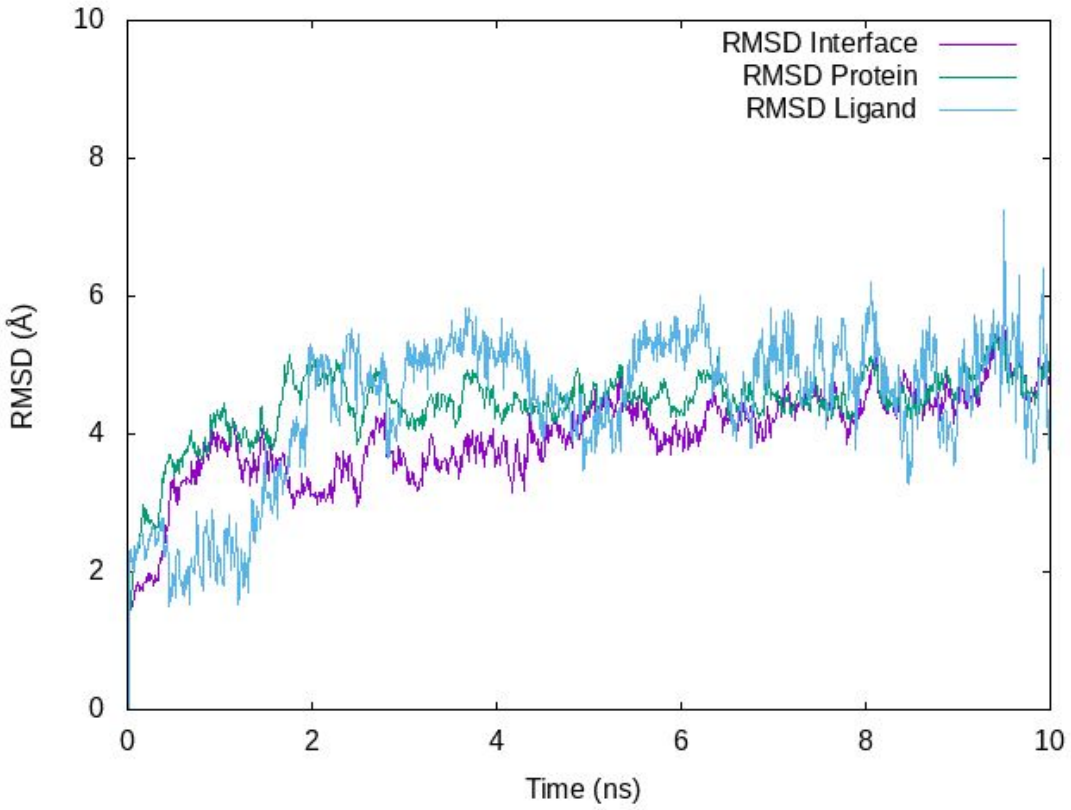
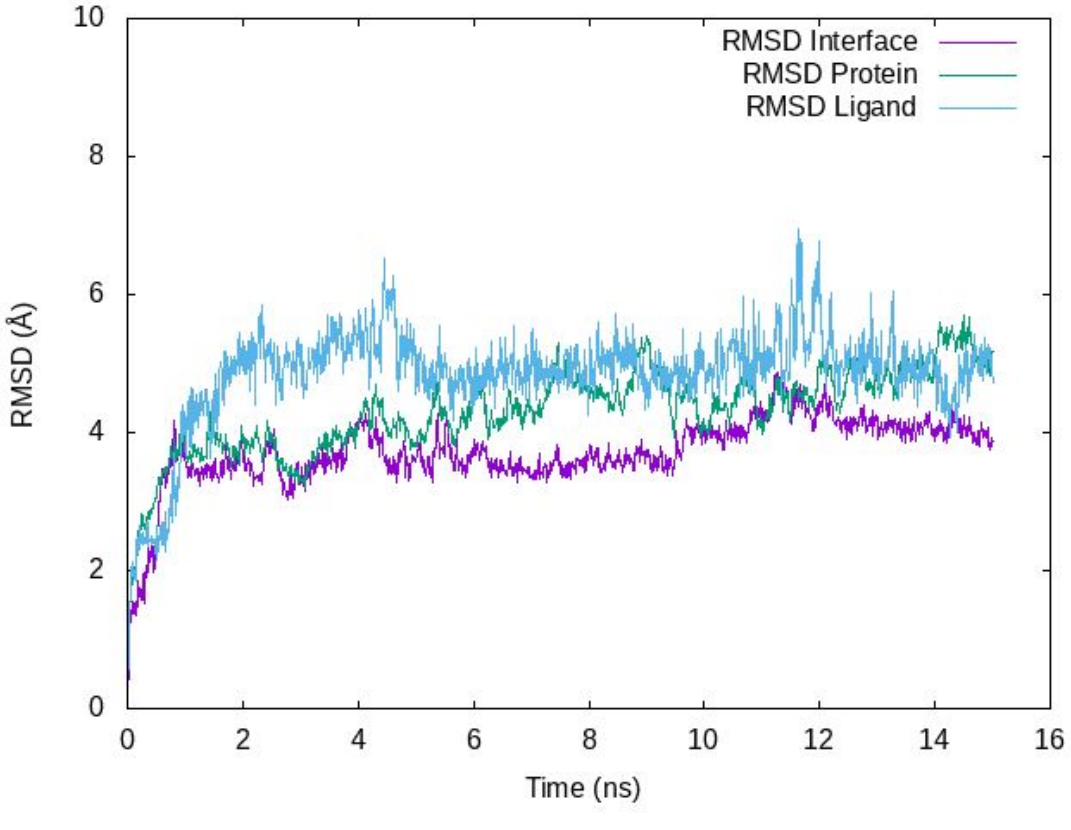
Ruxolitinib	
Polydatin	

(-)EC	 RMSD Interface RMSD Protein RMSD Ligand RMSD (Å) Time (ns) 0 2 4 6 8 10 0 2 4 6 8 10 12 14 16 18 20
(-)EGCG	 RMSD Interface RMSD Protein RMSD Ligand RMSD (Å) Time (ns) 0 2 4 6 8 10 0 2 4 6 8 10 12 14 16 18 20

Cianidanol	
Gallic acid 3-O-gallate	

(+)GC	 RMSD Interface RMSD Protein RMSD Ligand RMSD (Å) Time (ns) 0 2 4 6 8 10 12 14 16
(+)GCG	 RMSD Interface RMSD Protein RMSD Ligand RMSD (Å) Time (ns) 0 2 4 6 8 10 12 14 16 18 20

(-)C	 RMSD Interface RMSD Protein RMSD Ligand RMSD (Å) Time (ns) 0 2 4 6 8 10 12 14 16
(-)CG	 RMSD Interface RMSD Protein RMSD Ligand RMSD (Å) Time (ns) 0 2 4 6 8 10 12 14 16

(-)GCG	
Sorivudine	

MMPBSA/MMGBSA Energy Calculations

Per-residue energy decomposition for ligand/dimer energy in P1 and P3 **IF-MERS-CoV**

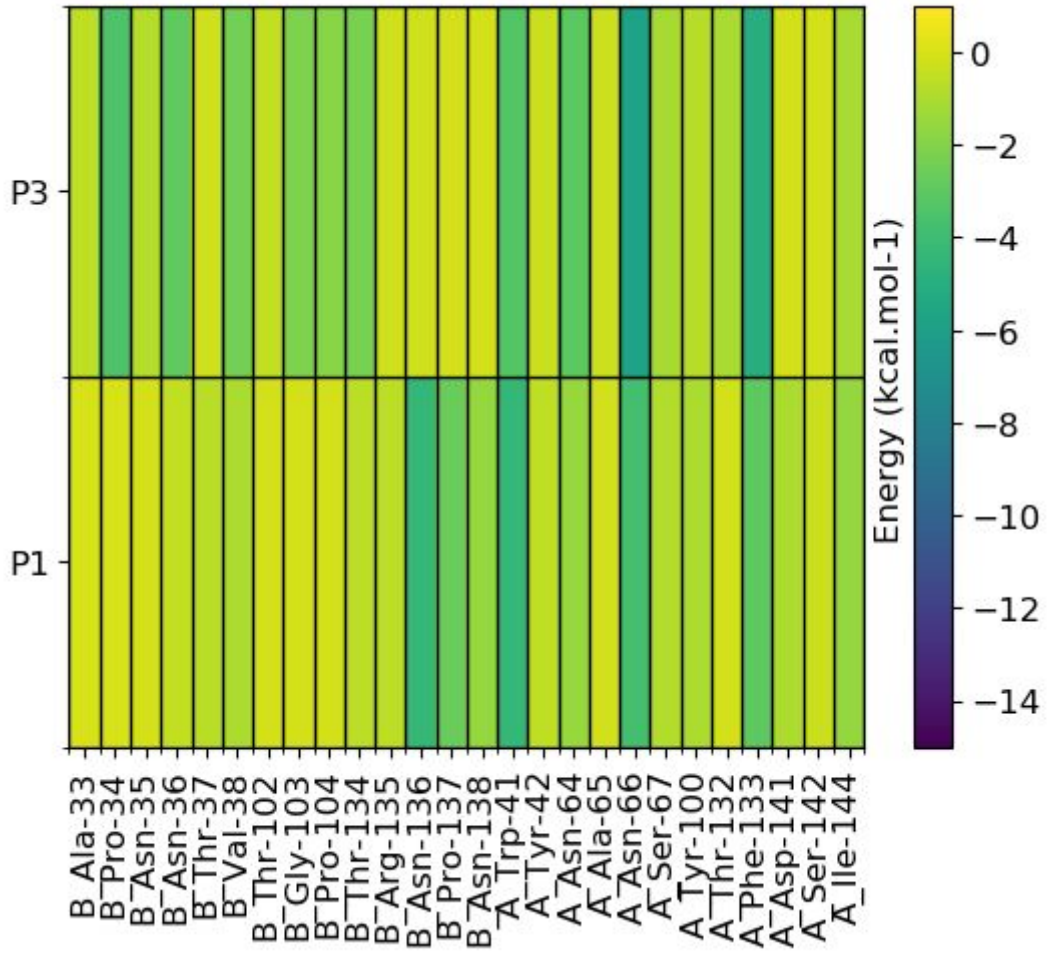


*Figure S4. Per-residue decomposition of ligand/dimer interaction energy in P1/**IF-MERS-CoV** and P3/**IF-MERS-CoV***

Ligand-Dimer Energy Calculations

Table S9. Interaction Energy calculations for each compound. Reported biological activity is mentioned, when available. Standard deviations are shown between parentheses.

Ligand	Interface	Lig-Monomer1 (kcal/mol)	Lig-Monomer2 (kcal/mol)	Lig-Dimer (kcal/mol)	Monomer-Monomer Interaction (kcal/mol)	TIE (kcal/mol)
Cianidanol	IF-3	-140 (7)	-51 (3)	-191 (7)	-50 (5)	-240 (8)
(-)EGC	IF-3	-61 (4)	-59 (3)	-120 (4)	-65 (4)	-185 (6)
Masoprocol	IF-3	-101 (5)	-28 (2)	-129 (5)	-56 (3)	-185 (6)
(+)GCG	IF-2	-56 (3)	-22 (2)	-77 (3)	-89 (8)	-166 (8)
(-)CG	IF-2	-45 (3)	-32 (2)	-78 (4)	-83 (7)	-162 (8)
(-)EC	IF-1	-46 (2)	-15 (5)	-60 (6)	-94 (6)	-154 (8)
(-)EC	IF-3	-78 (5)	-18 (2)	-95 (5)	-55 (4)	-150 (6)
(-)EGCG	IF-2	-30 (3)	-27 (3)	-57 (4)	-93 (4)	-150 (6)
(-)GCG	IF-1	-31 (2)	-29 (3)	-60 (4)	84 (6)	-144 (7)
Cianidanol	IF-1	-9 (2)	-23 (2)	-32 (3)	-110 (5)	-142 (6)
(+)GC	IF-1	-32 (5)	-11 (3)	-42 (3)	-96 (6)	-139 (7)
(+)GCG	IF-1	-39 (3)	-16 (6)	-55 (6)	-79 (9)	-134 (9)
(-)GCG	IF-2	-45 (2)	-20 (2)	-63 (3)	-69 (7)	-133 (8)
(-)CG	IF-1	-26 (2)	-35 (3)	-60 (4)	-69 (6)	-130 (6)
(+)GCG	IF-3	-36 (4)	-26 (2)	-61 (5)	-67 (4)	-129 (6)
(-)C	IF-1	-48 (2)	-13 (1)	-60 (2)	-67 (5)	-127 (5)
Quercetin	IF-1	-37 (4)	-12 (2)	-49 (4)	-77 (6)	-126 (6)
(-)C	IF-3	-31 (3)	-22 (2)	-53 (3)	-72 (4)	-125 (5)
Gallic acid 3-O-gallate	IF-1	-22 (5)	-31 (2)	-52 (5)	-72 (7)	-124 (7)
(+)GC	IF-2	-31 (3)	-19 (2)	-50 (3)	-70 (4)	-120 (5)
(-)EGCG	IF-1	-36 (4)	-37 (4)	-73 (6)	-45 (5)	-118 (7)
(-)EGCG	IF-3	-49 (2)	-20 (3)	-69 (3)	-47 (4)	-116 (5)
Ruxolitinib	IF-2	-15 (1)	-30 (2)	-44 (2)	-70 (5)	-114 (5)
(+)GC	IF-3	-42 (5)	-16 (4)	-58 (5)	-55 (4)	-113 (6)
(-)CG	IF-3	-35 (3)	-25 (2)	-60 (3)	-53 (4)	-113 (5)
(-)EC	IF-2	-30 (5)	-9 (2)	-37 (3)	-74 (5)	-110 (6)
(-)EGC	IF-2	-37 (4)	-11 (3)	-48 (4)	-55 (6)	-103 (7)
(-)C	IF-2	-22 (3)	-22 (2)	-44 (5)	-56 (6)	-100 (7)
(-)EGC	IF-1	-17 (2)	-28 (3)	-45 (4)	-52 (4)	-97 (6)
Sorivudine	IF-1	-7 (2)	-32 (4)	-39 (4)	-56 (6)	-95 (7)
Sorivudine	IF-3	-38 (4)	-3 (1)	-41 (4)	-49 (5)	-89 (6)

Quercetin	IF-2	-25 (2)	-16 (1)	-38 (2)	-50 (4)	-88 (5)
Cianidanol	IF-2	-18 (4)	-16 (2)	-34 (5)	-51 (4)	-84 (6)
Ruxolitinib	IF-1	-15 (1)	-15 (2)	-30 (2)	-51 (4)	-81 (5)
Sorivudine	IF-2	-16 (2)	-19 (4)	-36 (2)	-42 (4)	-78 (4)
Masoprocol	IF-2	-18 (2)	-15 (2)	-33 (2)	-43 (4)	-76 (5)
2-iminobiotin	IF-2	Complex Not Stabilized				
Brivudine	IF-1	Complex Not Stabilized				
Dexlansoprazole	IF-3	Complex Not Stabilized				
Gallic acid 3-O-gallate	IF-3	Complex Not Stabilized				
Masoprocol	IF-1	Complex Not Stabilized				
(-)GCG	IF-3	Complex Not Stabilized				
o6-bencylguaniane	IF-2	Complex Not Stabilized				
Phenformin	IF-2	Complex Not Stabilized				
Phenformin	IF-3	Complex Not Stabilized				
Polydatin	IF-3	Complex Not Stabilized				
Quercetin	IF-3	Complex Not Stabilized				
Ruxolitinib	IF-3	Complex Not Stabilized				
Thienamycin	IF-3	Complex Not Stabilized				

Per-residue Energy Decomposition for Ligands in **IF-2** with Lower Mon/Mon than the Isolated IF

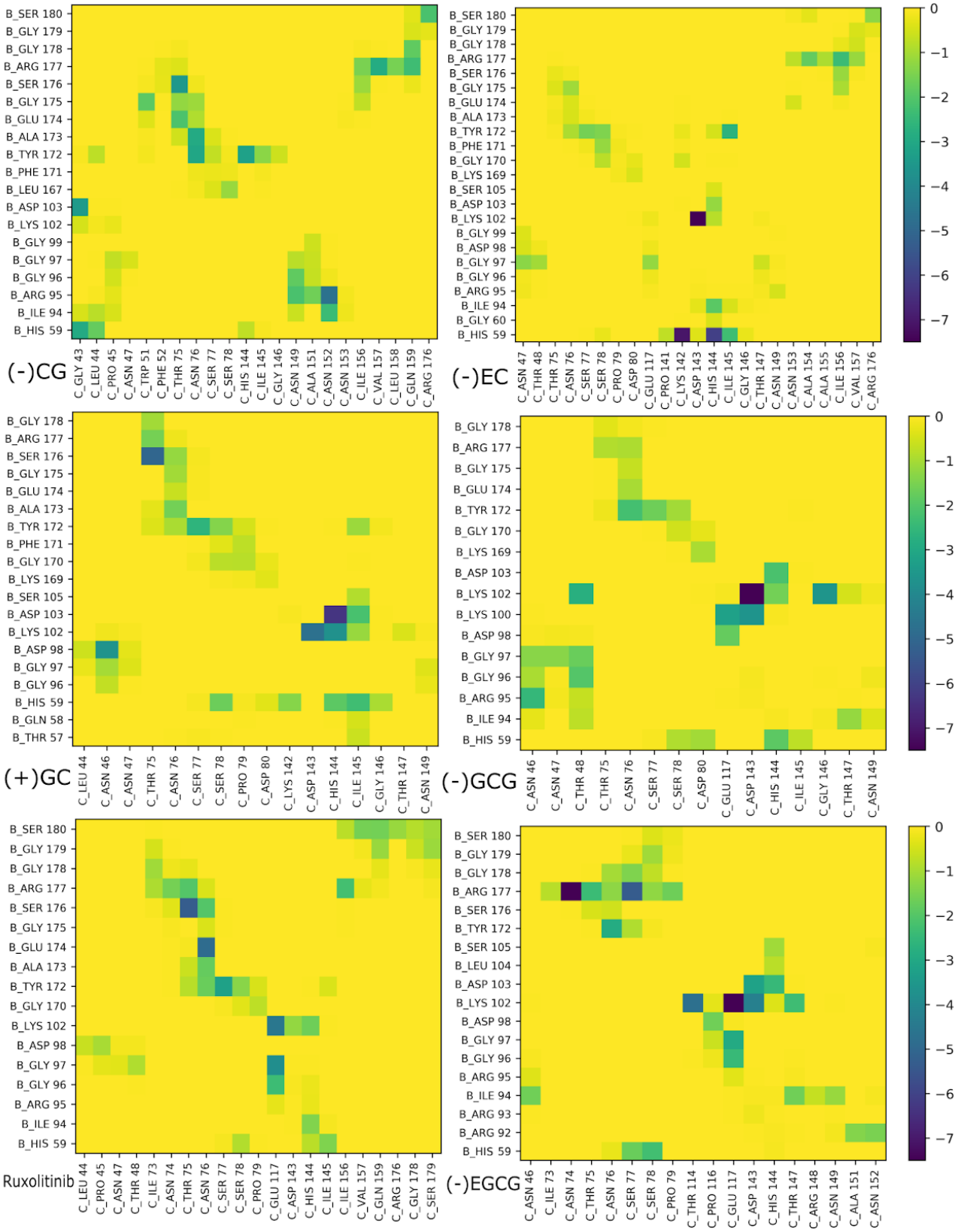


*Figure S5. Per-residue decomposition of Mon/Mon interaction energy in **IF-2** in selected ligands.*

Ligand-Dimer Interaction Visualization of the Most Stable Complexes

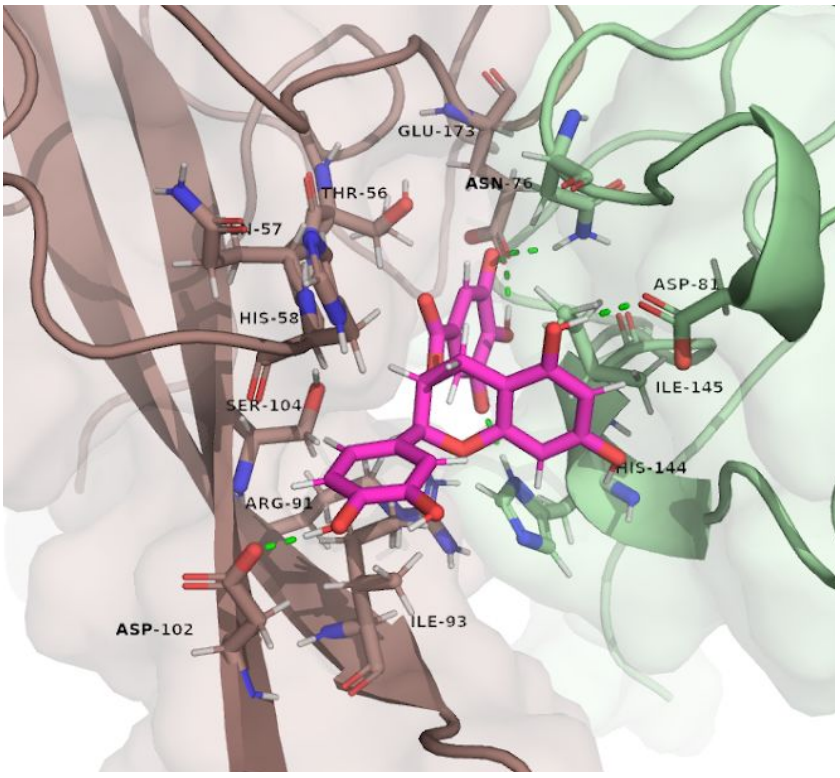


*Figure S6. (-)-Catechin Gallate in **IF-1***

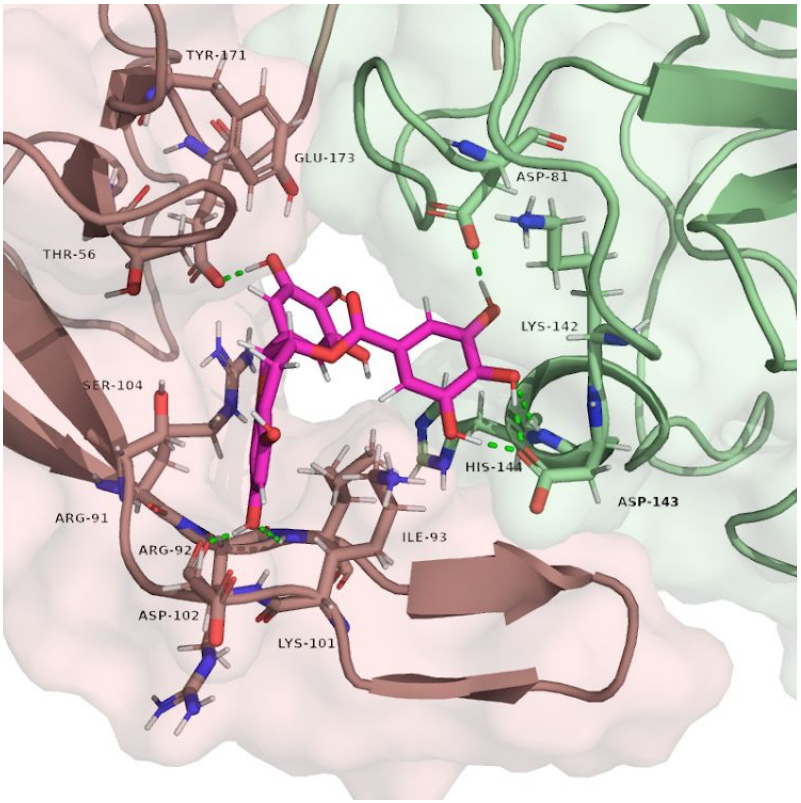


*Figure S7. (-)-Epigallocatechin Gallate in **IF-1***

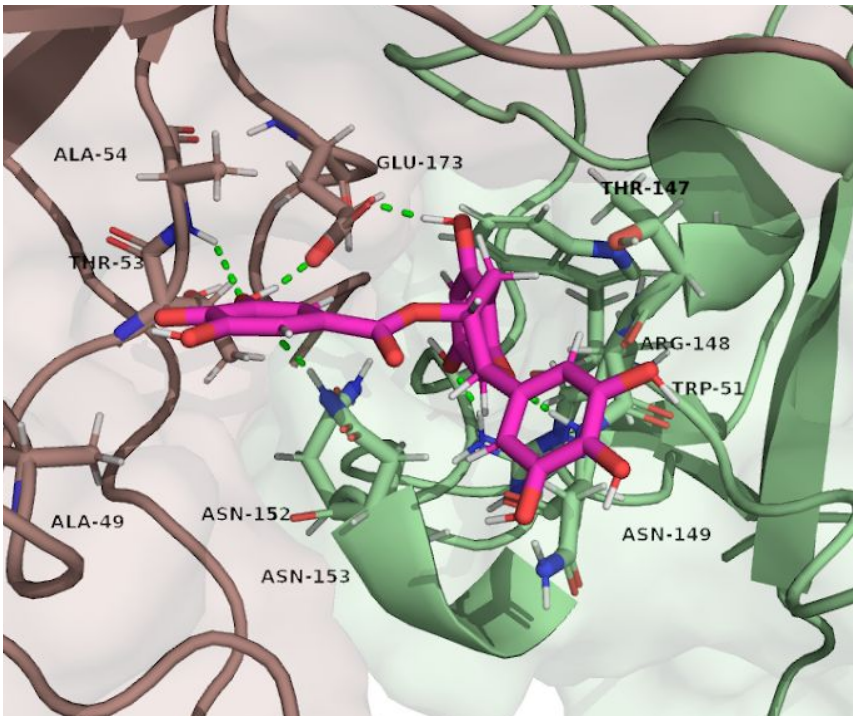


*Figure S8. (-)-Gallocatechin Gallate in **IF-1***

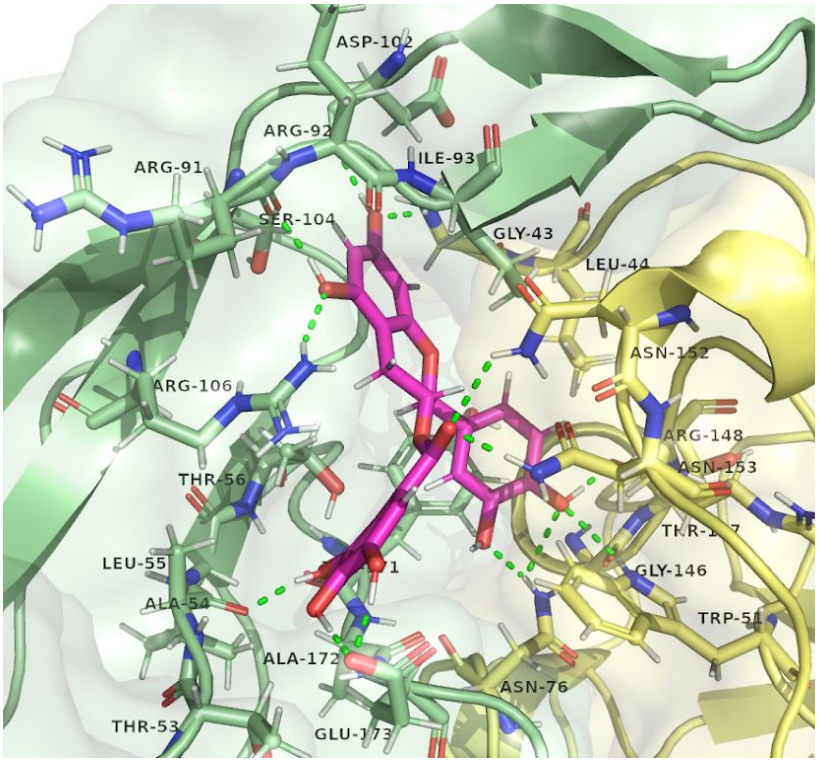


*Figure S9. (-)-Catechin Gallate in **IF-2***

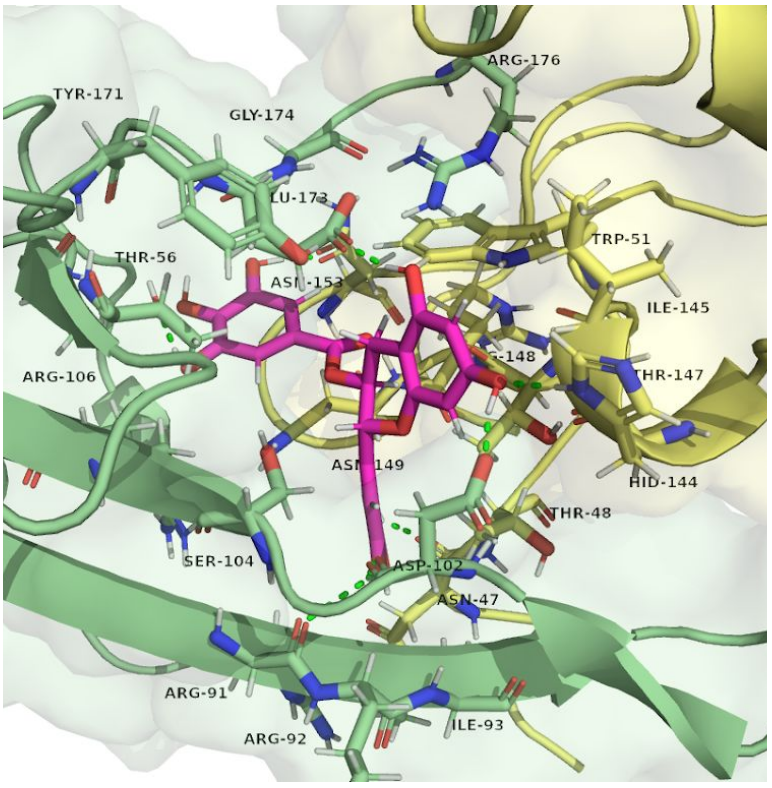


*Figure S10. (-)-Gallocatechin Gallate in **IF-2***

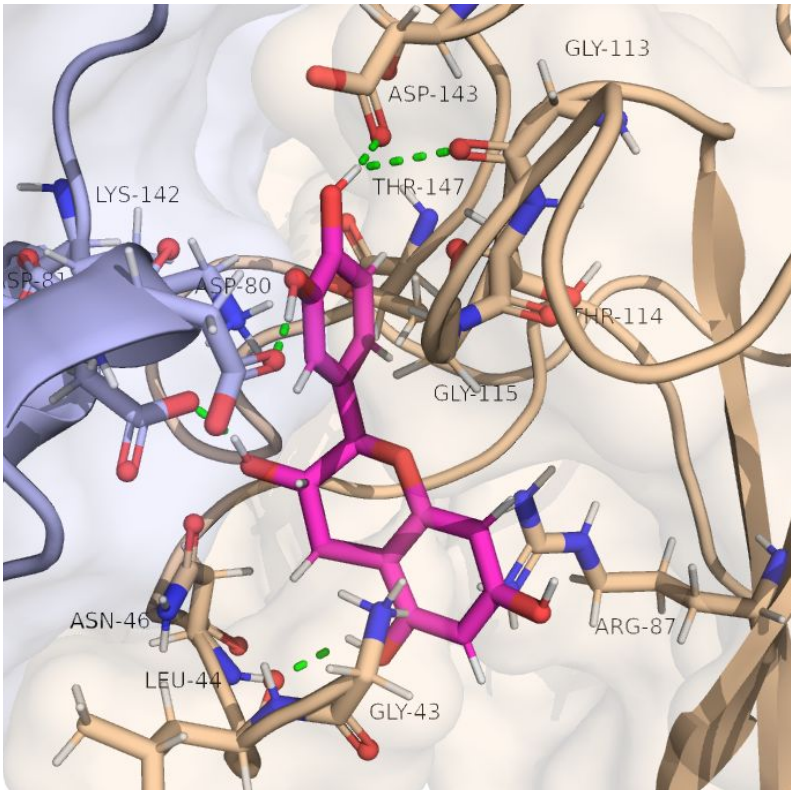


*Figure S11. Cianidanol in **IF-3***

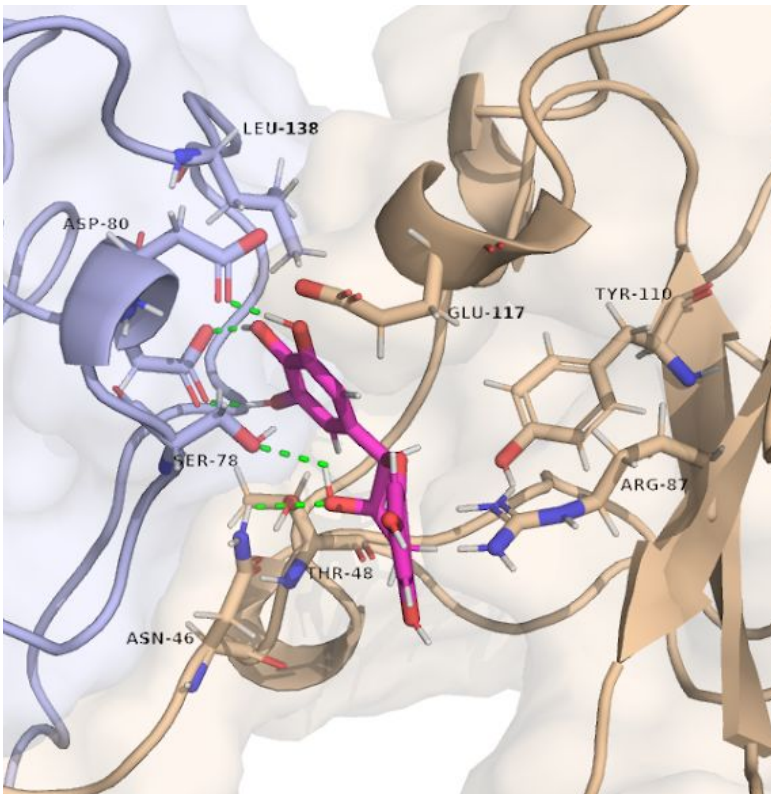


*Figure S12. (-)-Epigallocatechin in **IF-3***

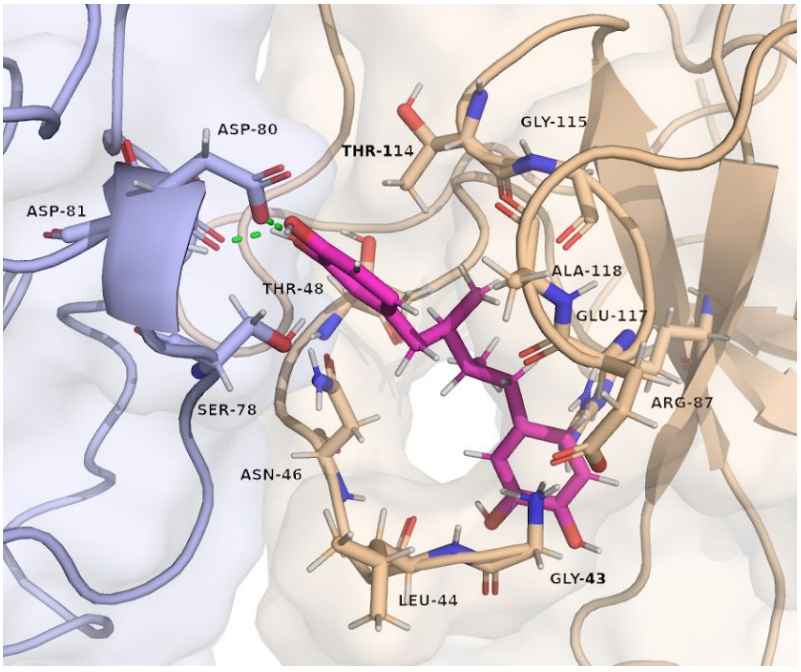


*Figure S13. Masoprocol in **IF-3***