Repurposing of FDA-approved Drugs against Active Site and Potential Allosteric Drug Binding Sites of COVID-19 Main Protease-Supporting Material

Merve Yuce[1], Erdem Cicek[2], Tugce Inan[1], Aslihan Basak Dag[3], Ozge Kurkcuoglu[1] and Fethiye Aylin Sungur[2]

[1]Istanbul Technical University, Department of Chemical Engineering, Istanbul, Turkey

[2]Istanbul Technical University, Computational Science and Engineering Division, Informatics Institute, Istanbul, Turkey

[3]Istanbul Technical University, Department of Molecular Biology and Genetics, Istanbul, Turkey

Supporting Information

Table S1: List of residues predicted by GNM and Residue Network Model.

Hinge residues located between the dynamic domains	Residues with high-frequency fluctuations in fast modes of GNM	Hub residues predicted by residue network model
R4, A7, P9, S10, V20, L27, C38, P39, C44, E55, D56, V68, R76, Q83, N84, V86, K90, Y101, Q107, F112, A116, P122, G124, Q127, F134, G138, G143, G146, S147, N151, D155, H163, E166, G170, G183, A194, T199, V202, V204, W207, Y209, V212, R217, L227, Y237, T243, D248,S254, L282, E288, T292, R298, G302	M6, A7, F8, P9, V13, V18, N28, G29, C38, P39, V114, L115, A116, C117, G120, G146, S147, V148, Y161, M162, H164, G174, T175	G2, F3, A7, F8, E14,G15, S113, S139, F140, H163, D197, T199, N203, Y209, E290, F291

Table S2: MM/GBSA energy components and active site residues forming H bond interaction with hit compounds.

Compound		MM/GBSA binding free energy (kcal/mol)					H-Bond Residues
		E_{vsw}	E_{ele}	E_{GB}	E_{surf}	ΔG_{bind}	
Antrafenine	subunit A	-40.99 ±4.62	-9.45 ±4.00	25.87 ±3.98	-5.35 ±0.60	**-29.92** ±4.54	T26, N142, H164, Q189, T190
	subunit B	-40.64 ±4.42	-5.23 ±3.58	23.52 ±3.58	-5.30 ±0.50	**-27.66** ±3.74	S46, N142, C145, E166, R188, T190, Q192
Nilotinib	subunit A	-57.30 ±5.45	-12.6 ±5.26	33.40 ±4.60	-6.77 ±0.65	**-43.35** ±4.61	H41, S46, N142, E166, Q189, T190, Q192
	subunit B	-42.74 ±7.70	-11.39 ±4.91	44315 ±5.75	-5.25 ±0.90	**-30.31** ±6.73	N142, E166, Q189, Q192, S1*
Quinupristin	subunit A	-53.23 ±6.41	-10.13 ±4.94	33.45 ±5.10	-5.94 ±0.75	**-35.85** ±5.20	H164, E166, Q189, T190, Q192
	subunit B	-48.25 ±9.35	-13.81 ±5.81	36.89 ±7.06	-5.78 ±1.06	**-30.95** ±7.58	S46, N142, G143, Q189
Paritapirevir	subunit A	-60.54 ±5.08	-12.17 ±5.89	43.26 ±5.05	-6.36 ±0.53	**35.82** ±4.71	N142, G143, S144, C145, H164, E166, Q189
	subunit B	-56.78 ±5.54	-20.48 ±5.55	51.81 ±4.95	-6.75 ±0.55	**-32.2** ±5.35	H41, N142, G143, E166, Q189, S1*
Entrectinib	subunit A	-56.12 ±4.36	-10.44 ±3.64	32.32 ±3.43	-6.15 ±0.46	**-40.37** ±4.51	T25, H41, N142, S144, H164, E166, Q189, T190, Q192, S1*
	subunit B	-46.08 ±5.10	-8.42 ±3.53	27.25 ±3.43	-5.57 ±0.63	**-32.81** ±4.91	S46, N142, H164, E166, Q189, T190
Rutin	subunit A	-55.67 ±4.20	-14.61 ±4.64	35.05 ±4.13	-6.47 ±0.5	**-41.70** ± 4.2	T25, T26, H41, N142, G143, S144, C145, H163, H164, E166, Q189, S1*
	subunit B	-46.54 ±4.97	-9.62 ±5.60	27.72 ±4.72	-5.62 ±0.54	**-34.05** ±5.38	T25, T26, H41, N142, S144, H163, H164, E166, R188, Q189
Ivermectine	subunit A	-39.90 ±4.72	-6.12 ±3.60	25.44 ±4.11	-4.95 ±0.61	**-25.52** ±3.92	H41, S46, H164, E166, R188, Q189, T190, Q192
	subunit B	-62.50 ±7.11	-12.25 ±4.80	39.01 ±5.19	-7.58 ±0.85	**-43.32** ±6.98	T25, T26, S46, N142, E166, R188, Q189, T190, Q192

Table S2: continued from previous page

Compound		MM/GBSA binding free energy (kcal/mol)					H-Bond Residues
		E_{vdw}	E_{ele}	E_{GB}	E_{surf}	ΔG_{bind}	
Dihydroergotamine	subunit A	-43.98 ±6.02	-10.17 ±5.01	27.80 ±3.87	-4.73 ±0.57	**-31.07** ±7.18	S46, H164, E166, Q189, T190, Q192
	subunit B	-60.48 ±3.28	-18.64 ±3.69	38.35 ±2.76	-6.36 ±0.26	**-47.13** ±3.41	H41, N142, G143, E166, Q189, T190
Digitoxin	subunit A	-66.09 ±6.86	-12.00 ±5.04	41.17 ±5.80	-7.87 ±0.81	**-44.79** ±6.18	T25, T26, H41, S46, N142, G143, C145, E166, Q189, T190
	subunit B	-43.43 ±6.87	-15.28 ±6.38	34.38 ±5.99	-5.23 ±0.79	**-29.55** ±7.33	T25, T26, H41, S46, N142, Q189
Rifapentine	subunit A	-37.13 ±7.01	-2.41 ±2.97	19.97 ±4.72	-4.07 ±0.84	**-23.73** ±5.31	H41, S46, N142, E166, Q189, T190
	subunit B	-56.65 ±5.81	-9.15 ±4.17	31.72 ±4.04	-5.79 ±0.48	**-39.96** ±5.53	H41, S46, N142, E166, R188, Q189
Selinexor	subunit A	-50.76 ±3.48	-20.04 ±2.97	33.90 ±2.63	-6.29 ±0.36	**-43.19** ±3.34	H41, N142, G143, S144, C145, H163, H164, E166, R188, Q189, T190, Q192
	subunit B	-48.43 ±3.08	-18.82 ±3.72	33.10 ±3.41	-6.13 ±0.31	**-40.29** ±3.03	H41, N142, G143, S144, C145, H163, H164, E166, Q189, T190, Q192
Bromocriptine	subunit A	-57.50 ±5.23	-11.69 ±3.29	30.15 ±3.01	-6.11 ±0.47	**-45.15** ±5.03	H41, E166, Q189, Q192
	subunit B	-55.74 ±5.14	-10.96 ±3.65	44346 ±3.78	-6.04 ±0.43	**-42.68** ±5.00	H41, S46, N142, E166, Q189, Q192
Diosmin	subunit A	-54.07 ±5.16	-11.74 ±4.57	29.46 ±4.54	-6.04 ±0.59	**-42.39** ±4.56	H41, S46, N142, H164, E166, R188, Q189, T190, Q192
	subunit B	-63.52 ±4.21	-20.04 ±4.24	38.71 ±3.50	-7.2 ±0.33	**-52.06** ±4.95	T25, T26, H41, N142, G143, S144, C145, H163, H164, E166, Q189, T190, Q192
Elbasvir	subunit A	-50.86 ±5.20	-11.00 ±6.32	37.29 ±5.77	-5.62 ±0.65	**-20.29** ±4.69	H41, E166, Q189, Q192
	subunit B	-51.15 ±9.35	-12.89 ±8.76	35.56 ±8.08	-6.39 ±1.28	**-33.76** ±8.63	H41, S46, N142, E166, Q189, Q192

Table S3: MM/GBSA energy components and potential allosteric site 1 residues forming H bond interaction with hit compounds.

Compound	MM/GBSA binding free energy (kcal/mol)					H-bonded residues
	E^{vdw}	E_{ele}	E_{GB}	E_{surf}	ΔG_{bind}	
Bromocriptine	-43.43 ±6.17	-20.38 ±7.34	37.62 ±8.06	-4.80 ±0.80	**-30.99** ±5.57	N72, K97, S121, K12*, K97*, K100*, D153*, Y154*
Nilotinib	-36.36 ±2.87	-17.74 ±3.62	29.88 ±3.41	-4.20 ±0.23	**-28.42** ±2.75	A70, N72
Elbasvir	-69.32 ±5.00	-43.14 ±11.18	69.54 ±9.48	-8.17 ±0.58	**-51.08** ±5.89	K12, N72, K97, Y154*, E14*, N72*, K97*, S121*
Dihydroergotamine	-42.73 ±10.22	-26.66 ±10.83	43.68 ±12.81	-4.78 ±1.20	**30.49** ±8.45	K97, K97*
Digitoxin	-59.38 ±5.64	-25.15 ±14.70	49.79 ±12.54	-7.00 ±0.75	**-41.74** ±6.40	K12, E14, N72, K97, S121, P122, K12*, E14*, N72*, K97*, S121*, Y154*
Diosmin	-52.62 ±4.97	-19.76 ±7.95	42.29 ±6.98	-6.45 ±0.53	**-36.56** ±5.17	E14, K97, S121, P122, S123, K12*, E14*, D153*, Y154*
Selinexor	-31.97 ±2.84	-17.34 ±6.39	28.43 ±5.86	-4.02 ±0.29	**-24.90** ±3.03	K97, K97*
Entrectinib	-44.39 ±4.52	-15.31 ±6.62	32.87 ±6.35	-5.69 ±0.62	**-32.48** ±4.41	E14, A70, N72, K97, S121

Table S4: MM/GBSA energy components and potential allosteric site-2 residues forming H bond interaction with hit compounds.

Compound	**MM/GBSA binding free energy (kcal/mol)**					**H-bonded residues**
	E^{vdw}	E_{ele}	E_{GB}	E_{surf}	ΔG_{bind}	
Bromocriptine	-62.02 ±3.98	-12.88 ±7.73	42.02 ±7.38	-6.74 ±0.40	**-39.62** ±4.39	K5, K137, L287, E288, K137*, G283*, S284*
Nilotinib	-55.68 ±4.12	-28.49 ±4.31	53.93 ±4.15	-6.90 ±0.44	**-37.13** ±3.79	K5, W207, G283, F3, K5*, K137*
Elbasvir	-71.86 ±4.72	-23.43 ±10.19	61.12 ±9.83	-8.20 ±0.52	**-42.37** ±4.80	K137, W207, G283, S284, A285, K137, L282*, G283*
Dihydroergotamine	-59.44 ±3.64	-11.90 ±4.44	37.82 ±4.33	-6.93 ±0.30	**-40.44** ±4.23	K137, S284, E288, F3*, K5*, W207*, S284*
Digitoxin	-57.25 ±5.09	-17.43 ±6.47	53.79 ±6.10	-7.12 ±0.63	**-28.01** ±4.38	K5, K137, G283, S284, A285, E288, E290, F3*, R4*, L282*, G283*, S284*
Diosmin	-54.30 ±5.16	-28.16 ±6.21	52.66 ±5.60	-7.13 ±0.48	**-36.94** ±4.75	K137, S284, L287, E288, F3*, R4*, K5*, W207*, L282, S284
Selinexor	-51.24 ±2.24	-2.95 ±2.94	21.50 ±2.33	-6.17 ±0.18	**-38.86** ±2.50	W207, R4*, K5*, K137*
Entrectinib	-59.00 ±4.14	-20.42 ±5.78	43.49 ±3.81	-7.03 ±0.46	**-42.93** ±6.40	K5, K137, E288, E290, F3*, R4*, K5*, S284*

Table S5: H-bond fraction of active site residues of subunit A with hit compounds. The given value corresponds to sum of the all H bond interactions between residue atoms and compound atoms.

ligand	**T24**	**T25**	**T26**	**H41**	**S46**	**N142**	**G143**	**S144**	**C145**	**H163**	**H164**	**E166**	**R188**	**Q189**	**T190**	**Q192**	**S1***
bromocriptine	0	0	0	0.48	0	0	0	0	0	0	0	0.28	0	13.72	0	0.04	0
rifapentine	0	0	0	0.04	0.08	2.04	0	0	0	0	0	1.92	0	1.44	0.28	0	0
nilotinib	0	0	0	6.36	0.04	3.44	0	0	0	0	0	3.4	0	6	3.36	14.56	0
paritapirevir	0	0	0	0	0	12.88	29.04	0.04	0.28	0	0.04	61.28	0	2	0	0	0
dihydroergotamine	0	0	0	0	0.36	0	0	0	0	0	7.88	9.16	0	5.48	4.2	6.64	0
quinupristin	0	0	0	0	0	0	0	0	0	0	0.04	1	0	29.36	2.08	3.76	0
digitoxin	0.4	0.2	24.92	10.4	0.48	2.72	0.88	0	0.04	0	0	0.84	0	0.04	0.32	0	0
antrafenine	0	0	61.76	0	0	0.36	0	0	0	0	0.84	0	0	0.4	0.04	0	0
rutin	0	1.32	0.2	0.96	0	5.52	8.6	9.96	2.88	2.12	14.16	1.64	0	0.92	0	0	0.12
diosmin	0	0	0	0.92	0.32	0.04	0	0	0	0	1.24	6.64	0.08	4.72	10.88	17.72	0
selinexor	0	0	0	2.88	0	0.2	6.72	2.28	16.16	0.04	0.88	0.2	0.08	0.04	2.12	7.8	0
entrectinib	0	0.56	0	0.64	0	2.88	0	0.08	0	0	43.04	9.32	0	0.48	0.04	0.04	0.08
ivermectin	0	0	0	0.24	0.28	0	0	0	0	0	0.52	0.04	5.96	7.04	3.16	3.8	0
elbasvir	0.84	0.04	2.4	0	0.44	15.76	0.08	0	0	0	0	0	0	4.24	0.24	0	0

Table S6: H-bond fraction of active site residues of subunit B with hit compounds. The given value corresponds to sum of the all H bond interactions between residue atoms and compound atoms.

ligand	**T24**	**T25**	**T26**	**H41**	**S46**	**N142**	**G143**	**S144**	**C145**	**H163**	**H164**	**E166**	**R188**	**Q189**	**T190**	**Q192**	**S1***
bromocriptine	0	0	0	0.6	0.12	0.04	0	0	0	0	0	5.28	0	8.48	0	0.04	0
rifapentine	0	0	0	11.28	0.12	0.08	0	0	0	0	0	30.68	0.2	0.96	0	0	0
nilotinib	0	0	0	0	0	0.4	0	0	0	0	0	1.2	0	3.96	0	0.04	0.04
paritapirevir	0	0	0	0.12	0	9.28	86.12	0	0	0	0	15.2	0	2.56	0	0	13.96
dihydroergotamine	0	0	0	3.84	0	36.4	23.24	0	0	0	0	28.2	0	10.32	5.84	0	0
quinupristin	0	0	0	0	2	9.28	1.12	0	0	0	0	0	0	6.68	0	0	0
digitoxin	0	1.16	0.44	0.96	1.28	11.76	0	0	0	0	0	0	0	0.24	0	0	0
antrafenine	0	0	0	0	1.48	0.08	0	0	0.12	0	0	0.48	0.44	0.04	1.24	7	0
rutin	0	0.2	25.56	10.2	0	0.32	0	0.04	0	0.6	6.56	0.2	2.56	6.76	0	0	0
diosmin	0	0.08	0.24	3	0	0.04	9.04	7.56	22.16	1.44	30.32	21.8	0	5.32	2.24	0.08	0

selinexor	0	0	0	0.04	0	0.16	3.44	1.4	16.32	0.12	15.8	0.72	0	0.08	3.6	16	0
entrectinib	0	0	0	0	11.16	0.36	0	0	0	0	0.12	10.68	0	6.6	0.04	0	0
ivermectin	0	0.12	7.64	0	0.96	2.04	0	0	0	0	0	55.92	0.96	2.92	25.96	11.04	0
elbasvir	0	0.04	0	0	2.04	18	0	0	0	0	0	0	0	24.52	0.28	0	0

Table S7: H-bond fraction of potential allosteric site 1 residues with hit compounds. The given value corresponds to sum of the all H bond interactions between residue atoms and compound atoms.

ligand	**K12**	**E14**	**A70**	**N72**	**K97**	**S121**	**P122**	**S123**	**Y154**	**K12***	**E14***	**N72***	**K97***	**K100***	**S121***	**D153***	**Y154***
bromocriptine	0	0	0	7.52	0.6	0.04	0	0	0	0.08	0	0	1.84	3.6	0	42.04	0.52
nilotinib	0	0	48.08	2.6	0	0	0	0	0	0	0	0	0	0	0	0	0
elbasvir	14.84	0	0	9.76	5.6	0	0	0	7.84	0	69.16	0.08	0.36	0	2.24	0	0
dihydroergotamine	0	0	0	0	0.48	0	0	0	0	0	0	0	33.2	0	0	0	0
digitoxin	0.04	1.96	0	0.8	14.96	0.24	0.2	0	0	1.88	0.04	10.84	18.56	0	3.56	0	2.8
diosmin	0	13.64	0	0	0.48	1.32	2.08	3.68	0	0.08	0.08	0	0	0	0	0.6	3.44
selinexor	0	0	0	0	48.28	0	0	0	0	0	0	0	0.92	0	0	0	0
entrectinib	0	0.04	6.04	3.72	0.2	4.64	0	0	0	0	0	0	0	0	0	0	0

Table S8: H-bond fraction of potential allosteric site 2 residues with hit compounds. The given value corresponds to sum of the all H bond interactions between residue atoms and compound atoms.

ligand	**K5**	**K137**	**W207**	**G283**	**S284**	**A285**	**L287**	**Q288**	**Q290**	**F3***	**R4***	**K5***	**K137***	**W207***	**L282***	**G283***	**S284***
bromocriptine	3.44	0.16	0	0	0	0	0.24	39.52	0	0	0	0	0.12	0	0	0.12	0.28
nilotinib	0.28	0	8.52	0.12	0	0	0	0	0	3.44	0	19.52	0.2	0	0	0	0
elbasvir	0	14.68	0.16	3.04	12.52	27	0	0	0	0	0	0	2.2	0	2.36	0.04	0
dihydroergotamine	0	0.04	0	0	0.2	0	0	0.12	0	0.52	0	0.08	0	10.48	0	0	0.04
digitoxin	1.2	0.16	0	1.84	5.72	0.04	0	32.72	4.04	0.84	0.56	0	0	0	6.16	0.16	0.96
diosmin	0	3.84	0	0	2.6	0	7.88	17.56	0	47.2	0.16	0.04	0	11.84	65.56	0	1.08
selinexor	0	0	5.64	0	0	0	0	0	0	0	2.08	67.64	0.0.4	0	0	0	0
entrectinib	1.48	1	0	0	0	0	0	16.76	12.12	1.2	3.76	17.4	0	0	0	0	0.08

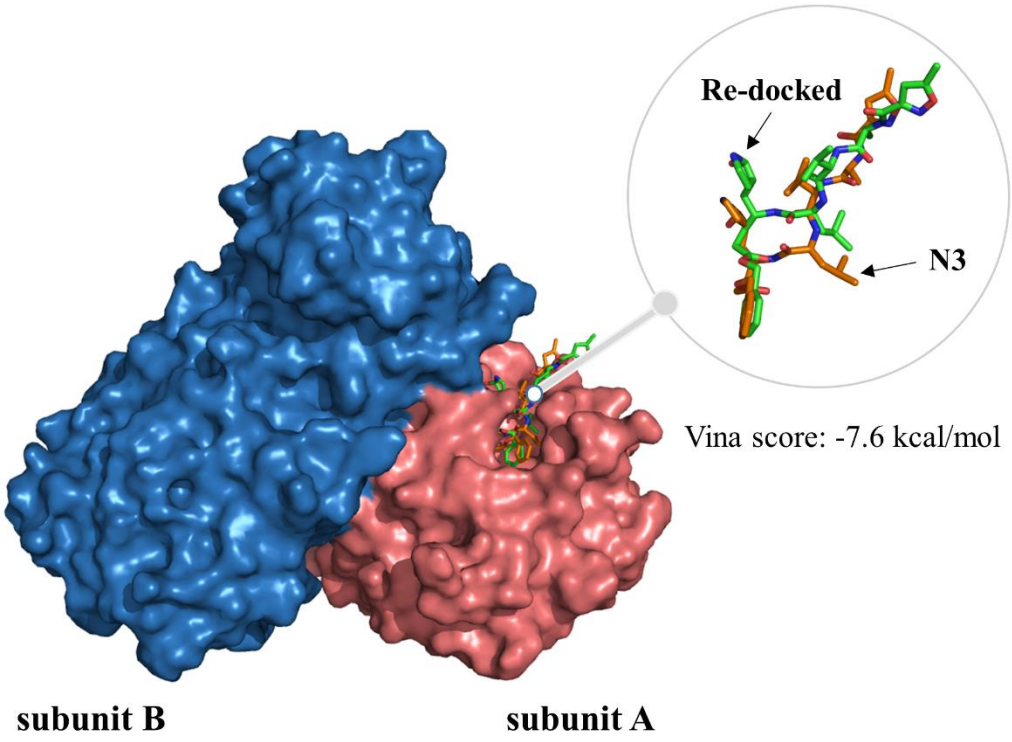

Figure S1: Re-docking validations for N3. Crystallographic (orange) and re-docked (green) N3 inhibitor of the SARS-CoV-2 M^{pro} inside its binding pocket (PDB ID:6lu7). Here, re-docked N3 within the active site cavity of M^{pro} is in a similar position, and interacts with similar residues, as in the native structure.

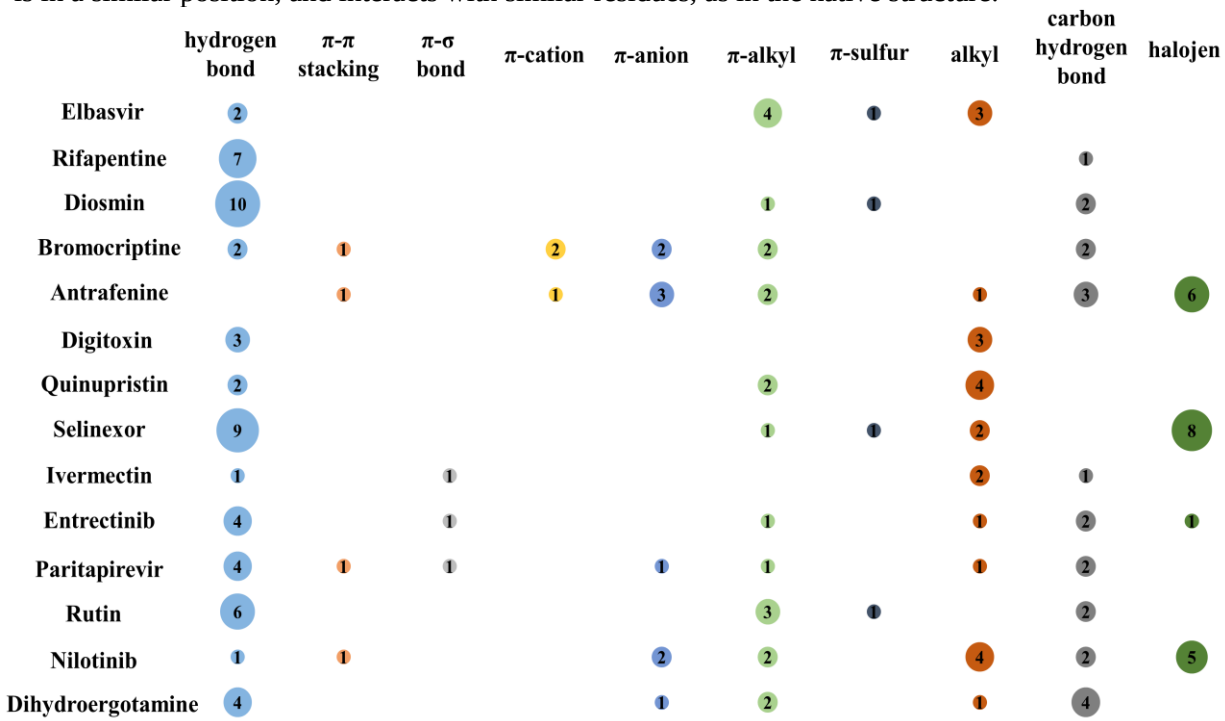

Figure S2: Number of interactions for 14 most promising hit drugs with the active site residues of SARS-CoV-2 M^{pro}. Bubble scaling is from 1 to 10 interactions.

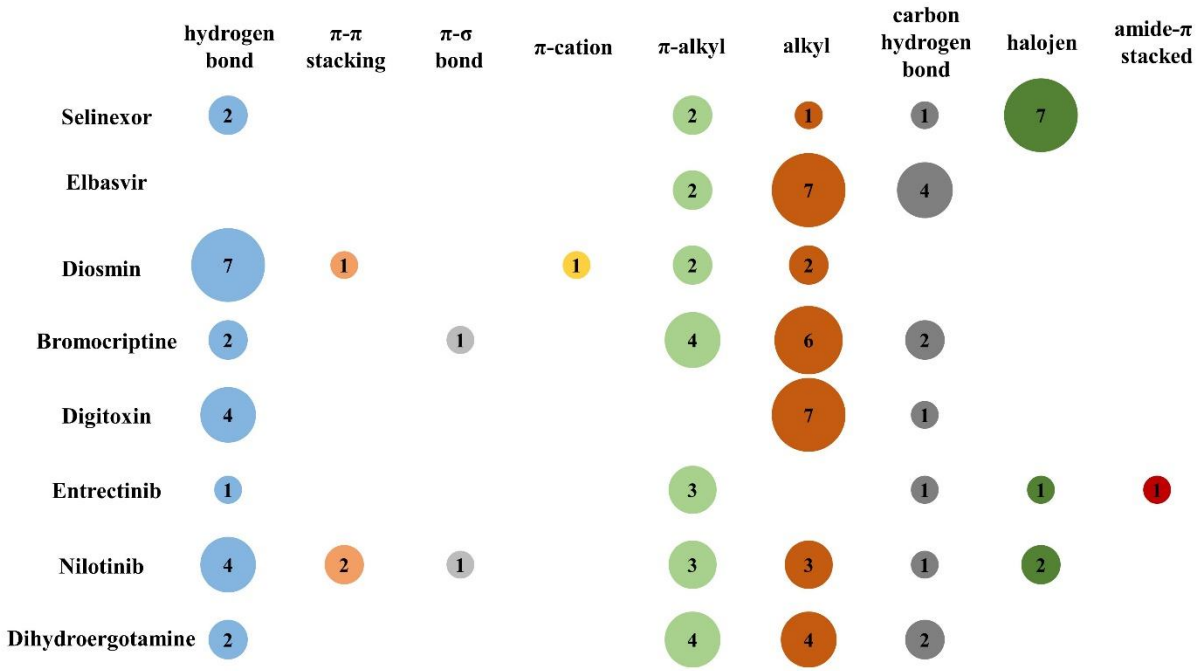

Figure S3: Number of interactions for 8 most promising hit drugs with potential allosteric site 1 residues of SARS-CoV-2 M^{pro}. Bubble scaling is from 1 to 10 interactions.

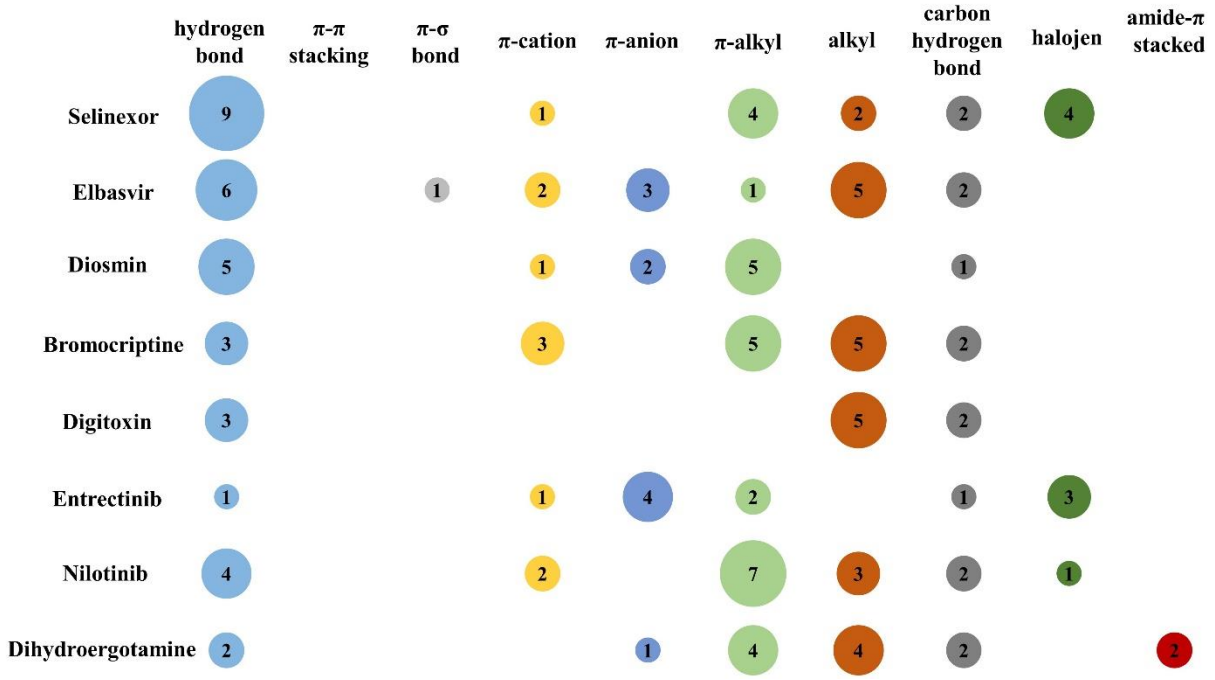

Figure S4: Number of interactions for 8 most promising hit drugs with potential allosteric site 2 residues of SARS-CoV-2 M^{pro}. Bubble scaling is from 1 to 10 interactions.

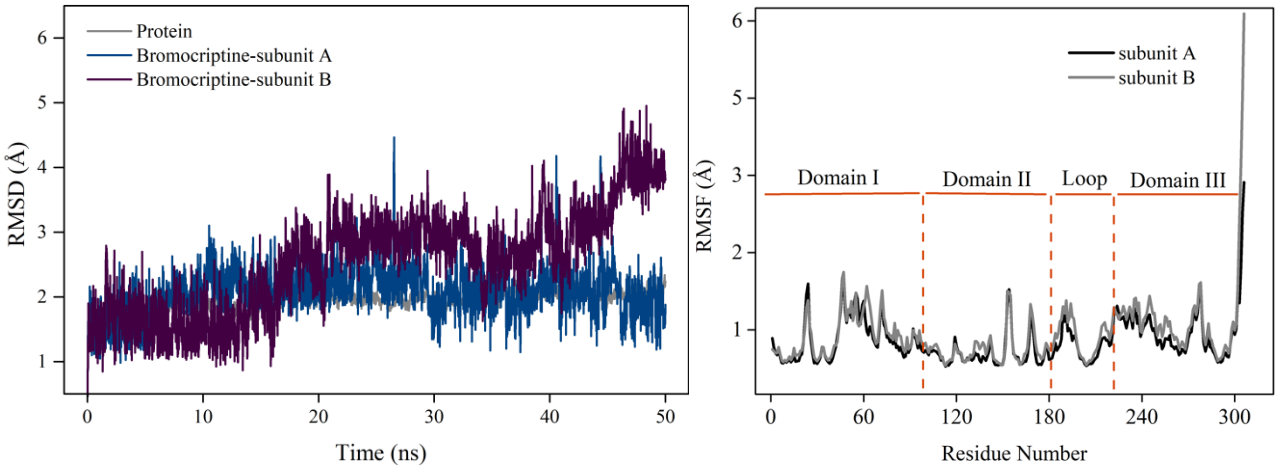

Figure S5: Protein and ligand RMSD (left) and RMSF (right) of SARS-CoV-2 M^{pro} in complex with bromocriptine.

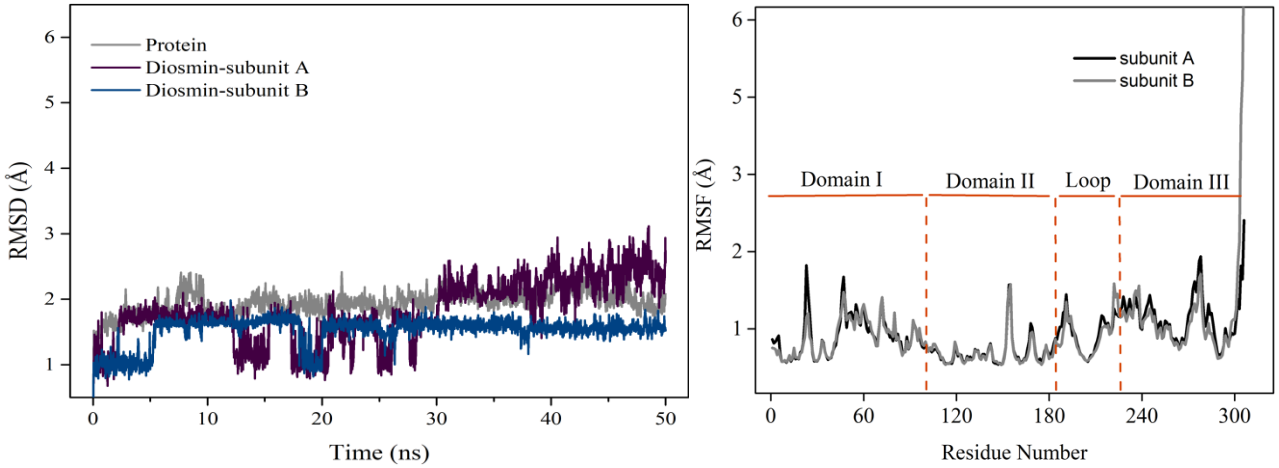

Figure S6: Protein and ligand RMSD (left) and RMSF (right) of SARS-CoV-2 M^{pro} in complex with diosmin.

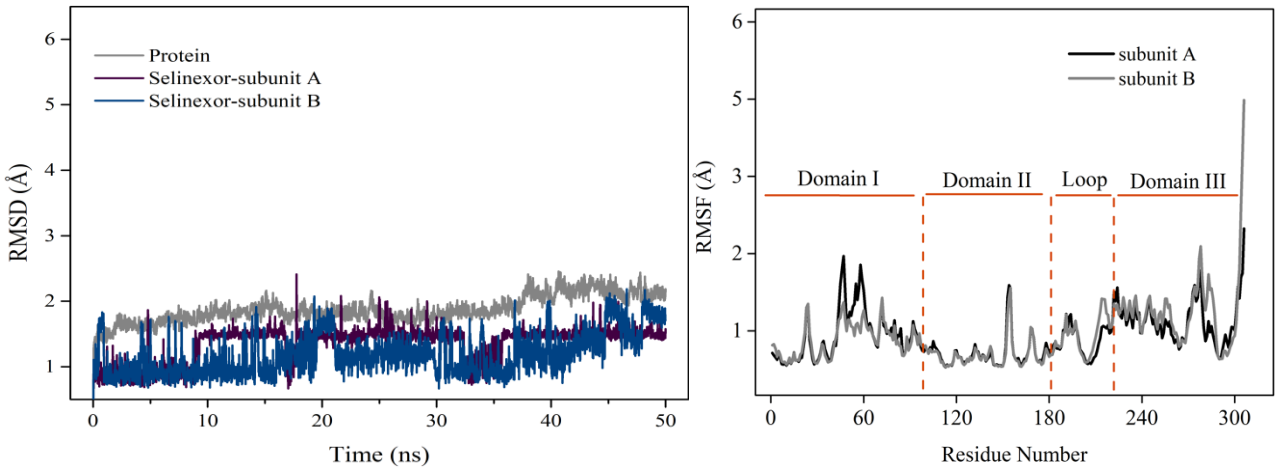

Figure S7: Protein and ligand RMSD (left) and RMSF (right) of SARS-CoV-2 Mpro in complex with selinexor.

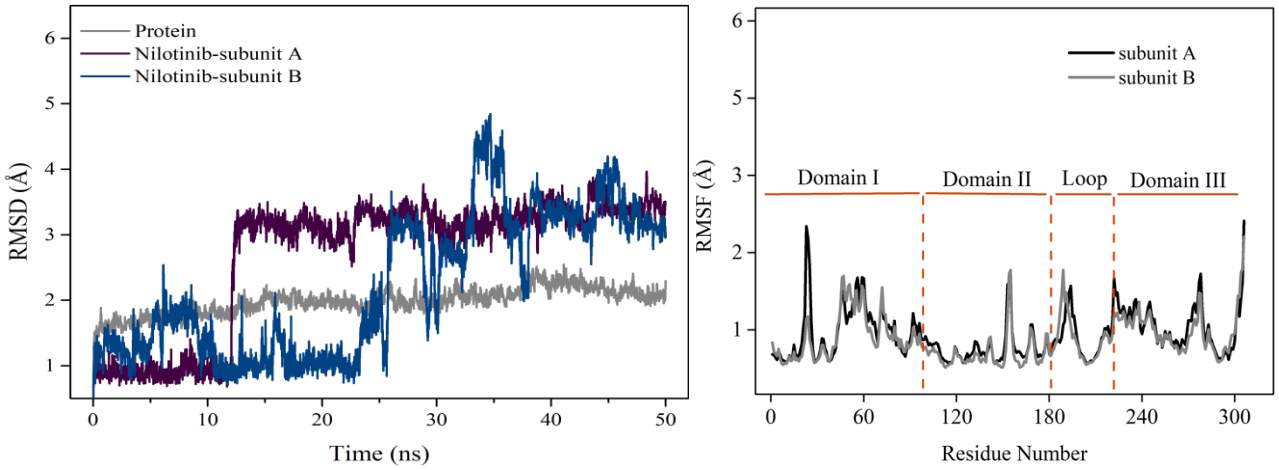

Figure S8: Protein and ligand RMSD (left) and RMSF (right) of SARS-CoV-2 Mpro in complex with nilotinib.

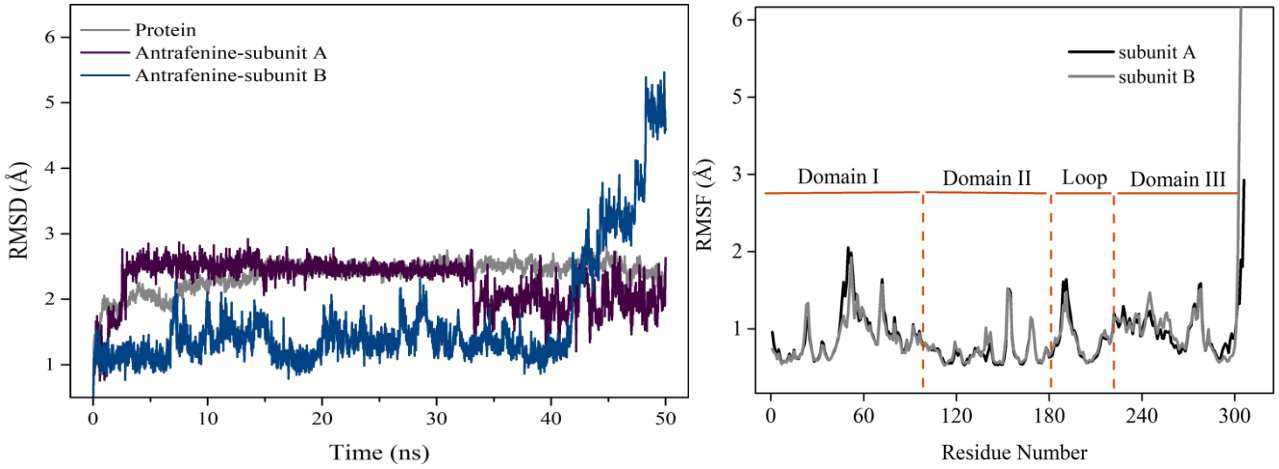

Figure S9: Protein and ligand RMSD (left) and RMSF (right) of SARS-CoV-2 Mpro in complex with antrafenine.

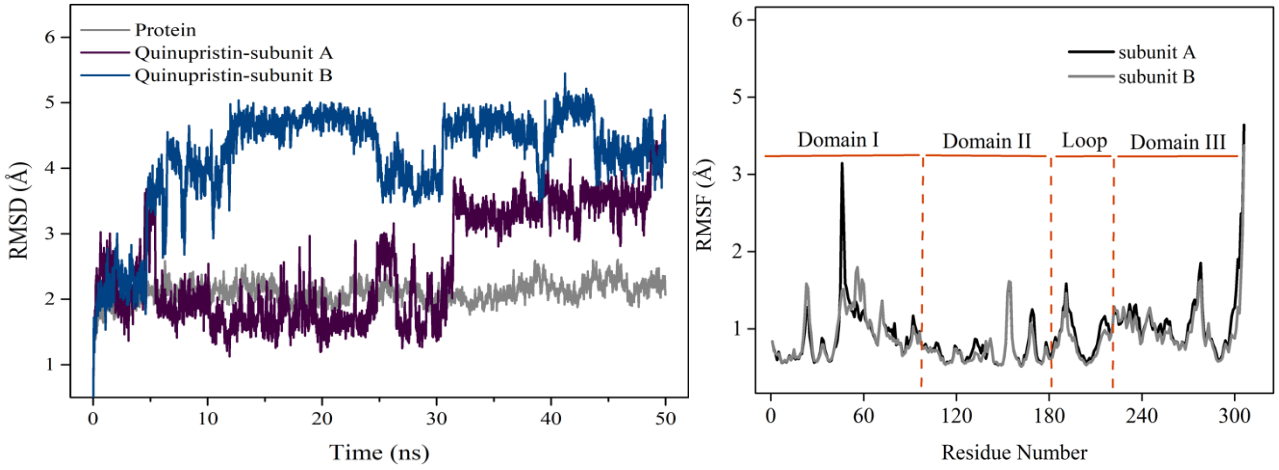

Figure S10: Protein and ligand RMSD (left) and RMSF (right) of SARS-CoV-2 Mpro in complex with quinupristin.

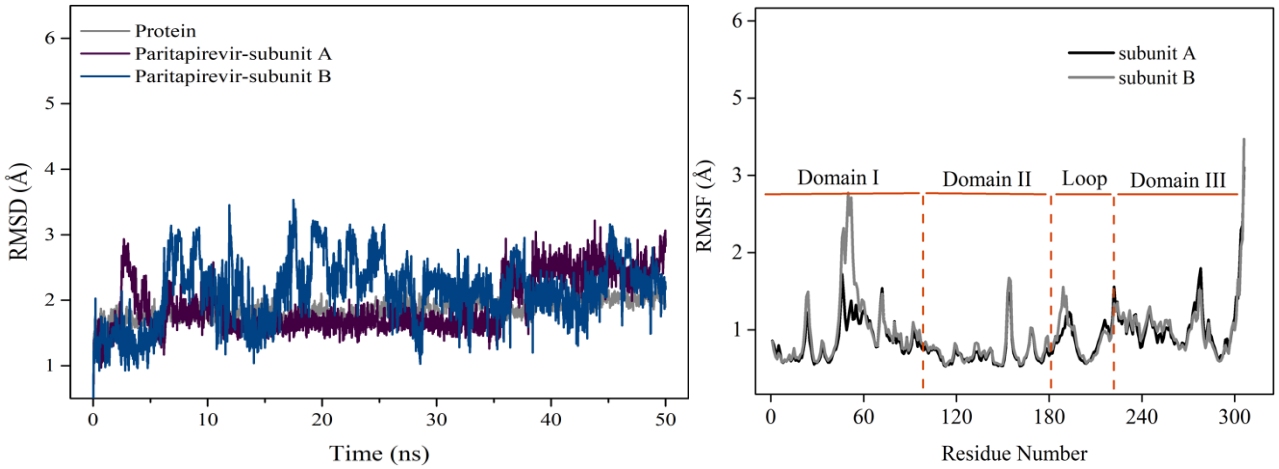

Figure S11: Protein and ligand RMSD (left) and RMSF (right) of SARS-CoV-2 Mpro in complex with paritapirevir.

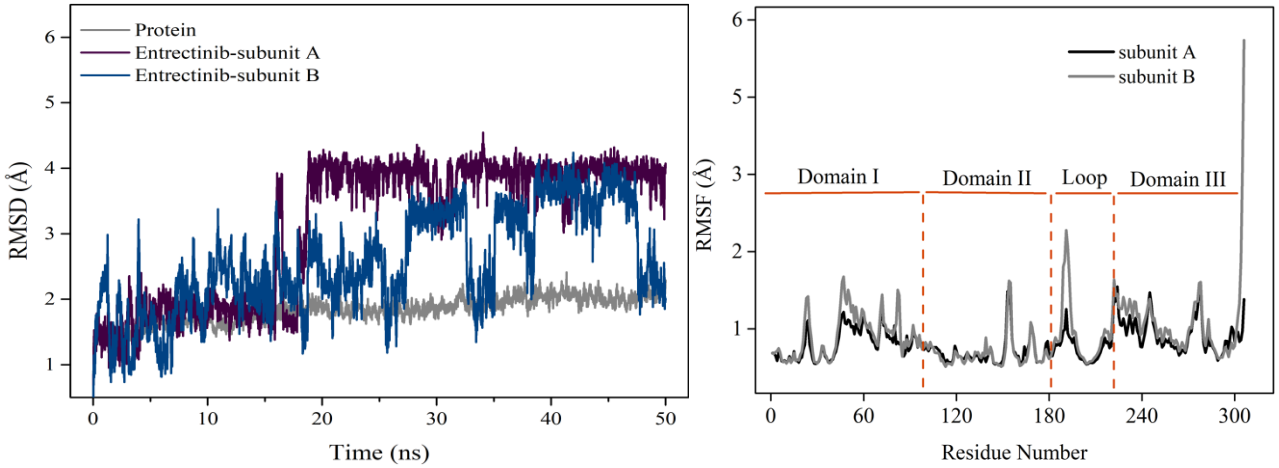

Figure S12: Protein and ligand RMSD (left) and RMSF (right) of SARS-CoV-2 Mpro in complex with entrectinib.

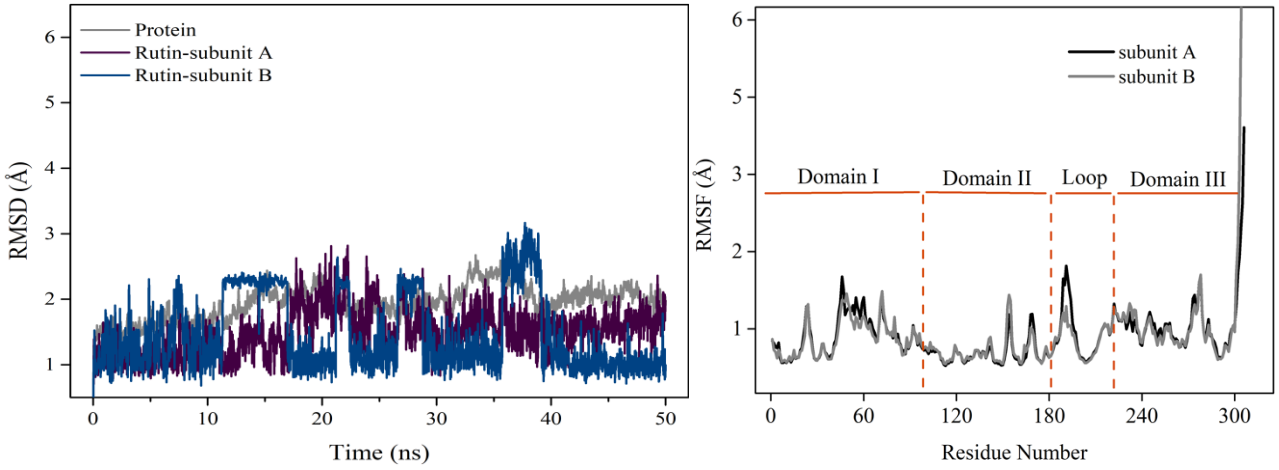

Figure S13: Protein and ligand RMSD (left) and RMSF (right) of SARS-CoV-2 Mpro in complex with rutin.

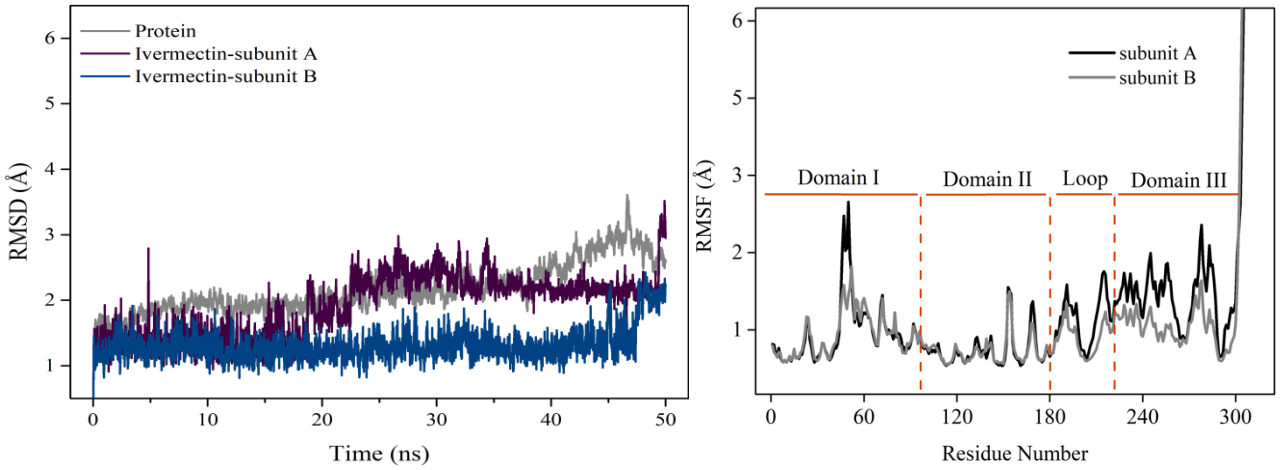

Figure S14: Protein and ligand RMSD (left) and RMSF (right) of SARS-CoV-2 Mpro in complex with ivermectine.

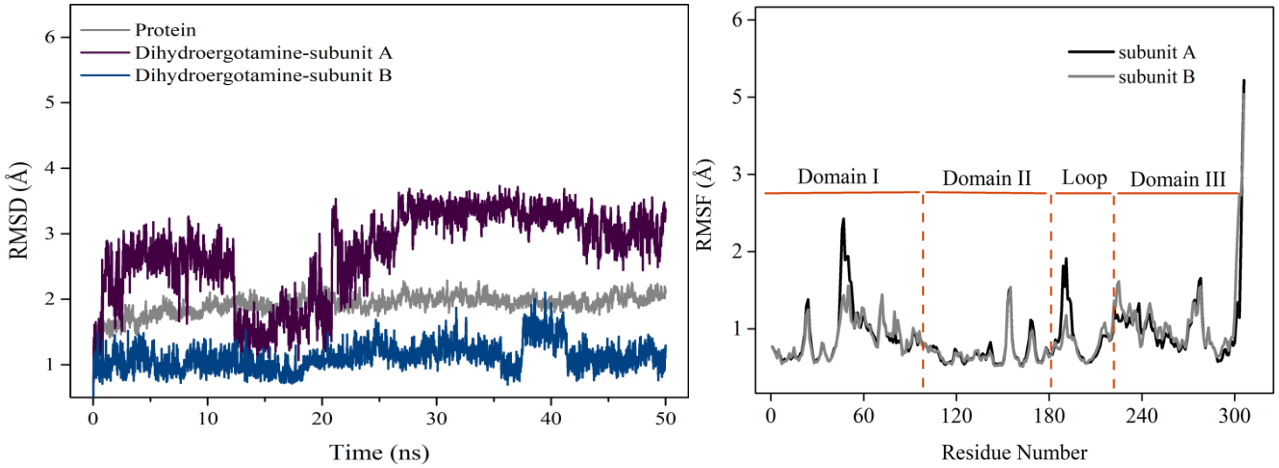

Figure S15: Protein and ligand RMSD (left) and RMSF (right) of SARS-CoV-2 Mpro in complex with dihydroergotamine.

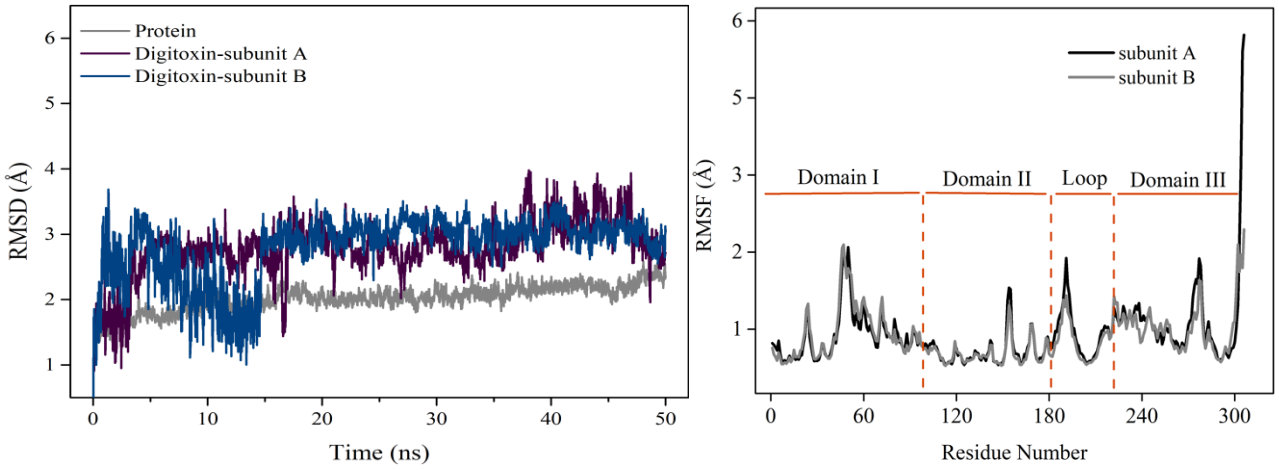

Figure S16: Protein and ligand RMSD (left) and RMSF (right) of SARS-CoV-2 Mpro in complex with digitoxin.

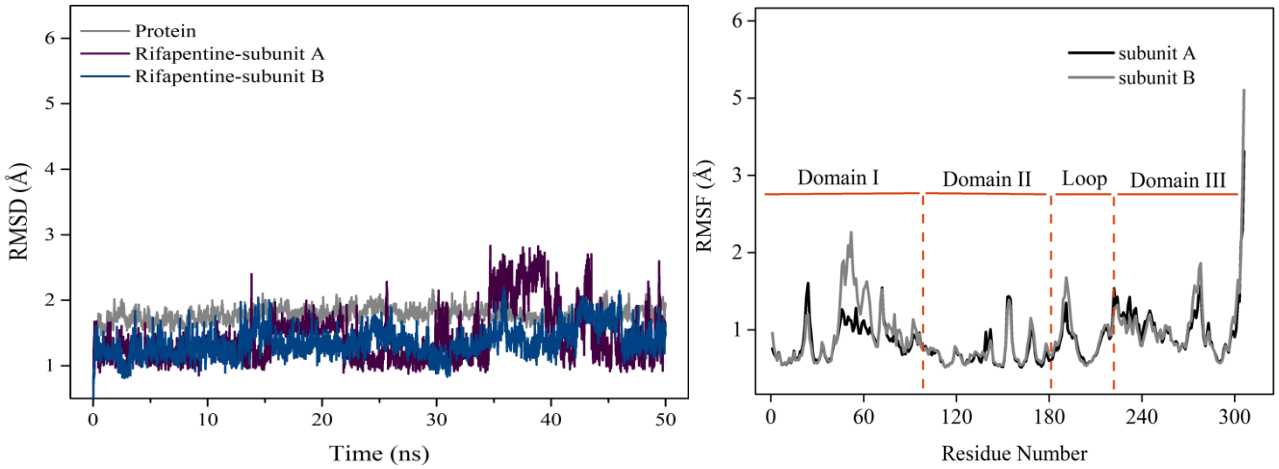

Figure S17: Protein and ligand RMSD (left) and RMSF (right) of SARS-CoV-2 Mpro in complex with rifapentine.

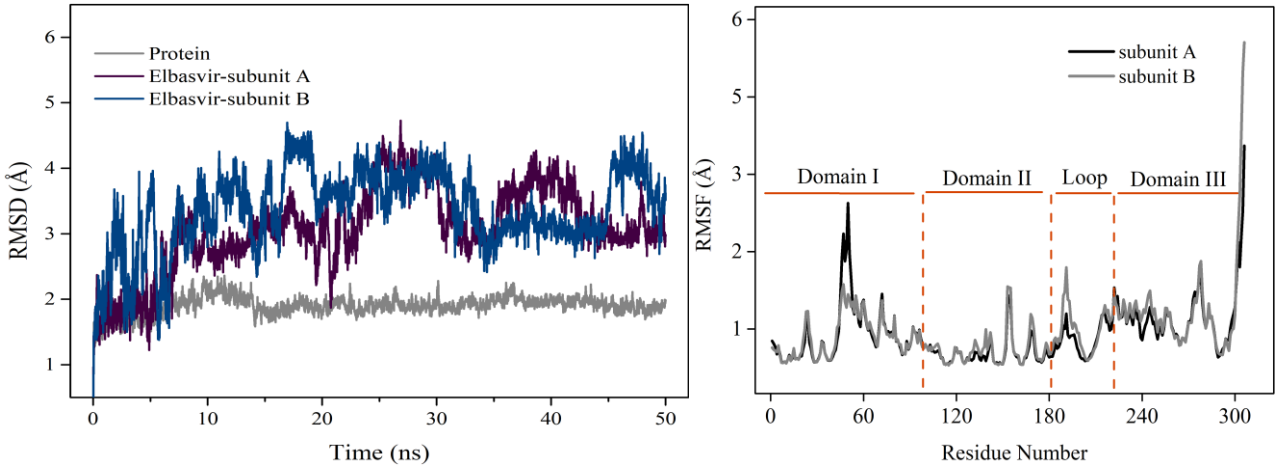

Figure S18: Protein and ligand RMSD (left) and RMSF (right) of SARS-CoV-2 Mpro in complex with elbasvir.

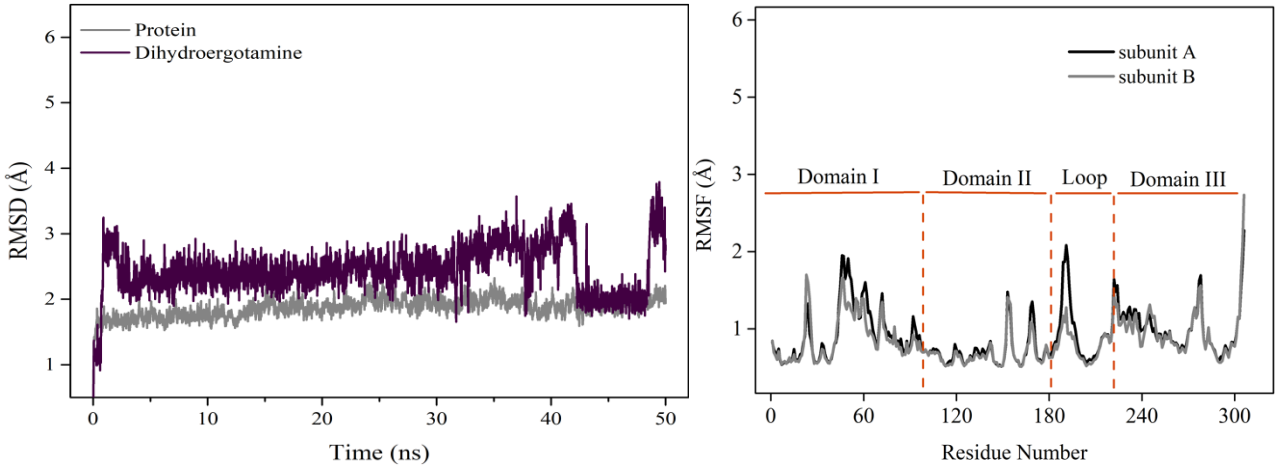

Figure S19: Protein and ligand RMSD (left) and RMSF (right) of the predicted allosteric site-1 of SARS-CoV-2 Mpro in complex with dihydroergotamine.

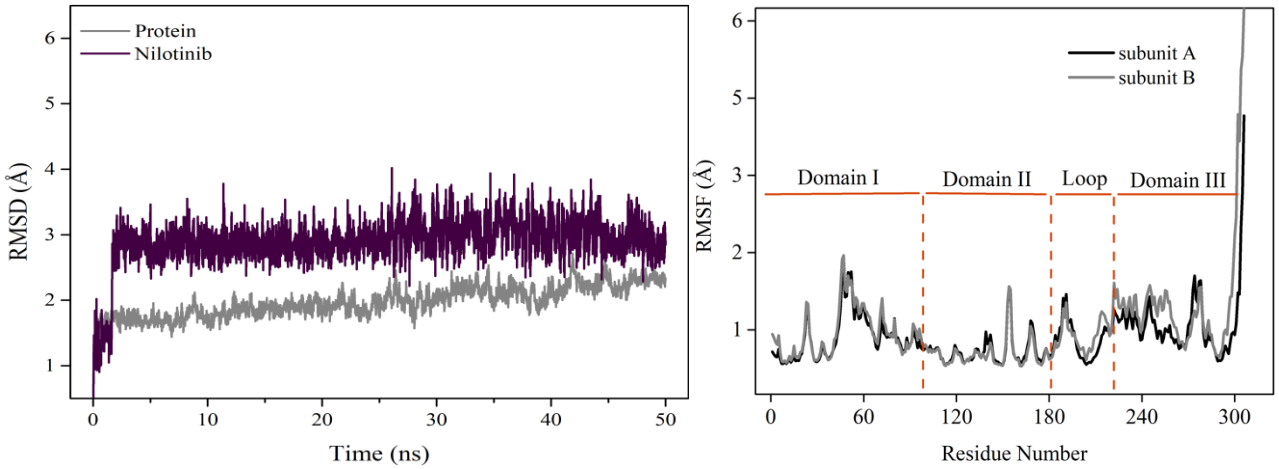

Figure S20: Protein and ligand RMSD (left) and RMSF (right) of the predicted allosteric site-1 of SARS-CoV-2 Mpro in complex with nilotinib.

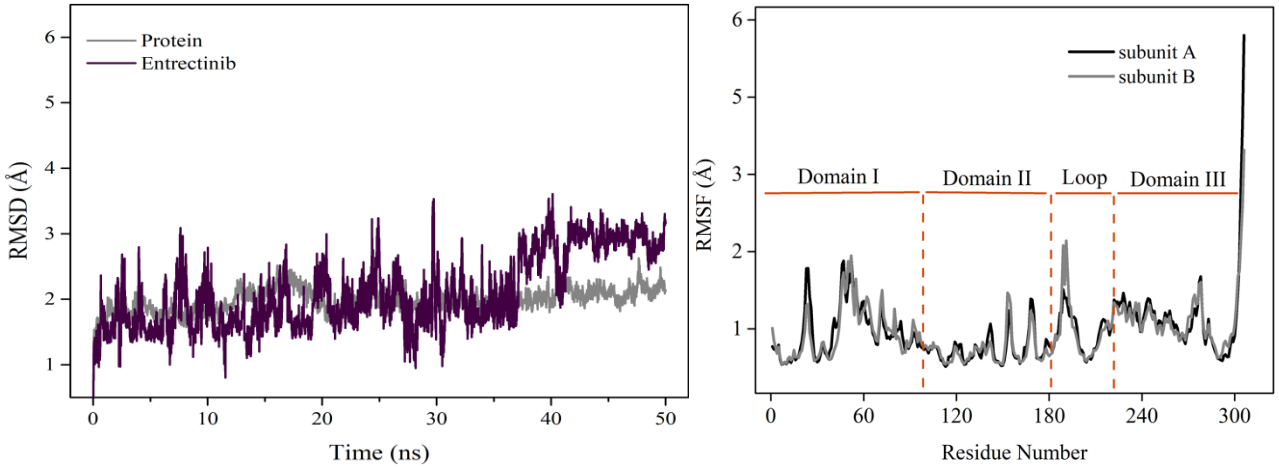

Figure S21: Protein and ligand RMSD (left) and RMSF (right) of the predicted allosteric site-1 of SARS-CoV-2 Mpro in complex with entrectinib.

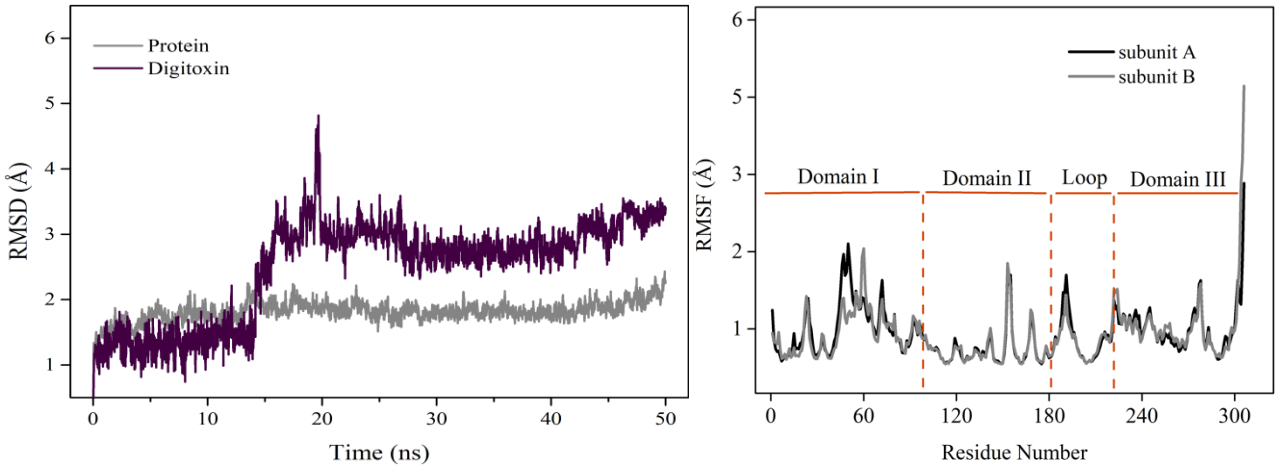

Figure S22: Protein and ligand RMSD (left) and RMSF (right) of the predicted allosteric site-1 of SARS-CoV-2 Mpro in complex with digitoxin.

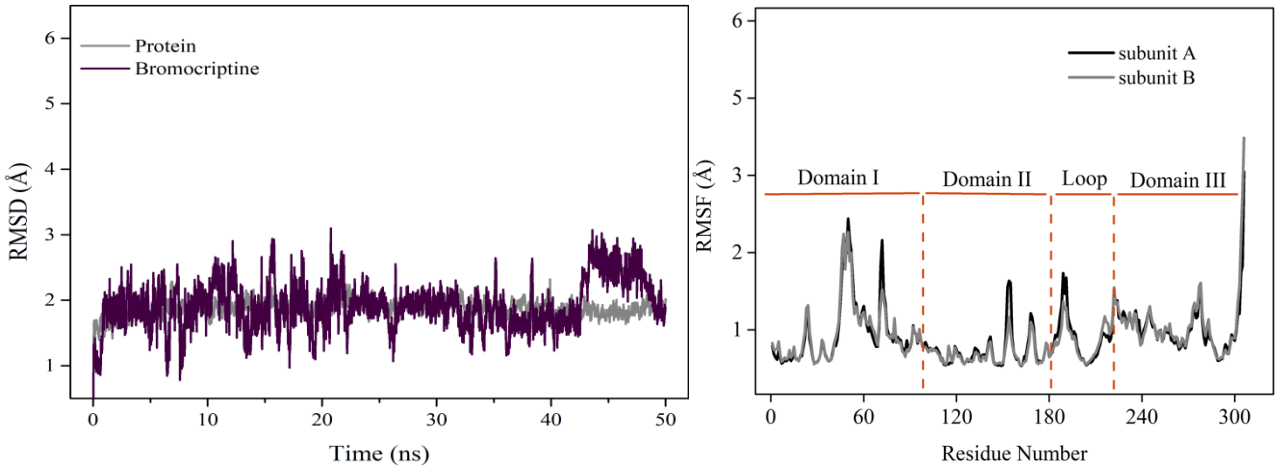

Figure S23: Protein and ligand RMSD (left) and RMSF (right) of the predicted allosteric site-1 of SARS-CoV-2 Mpro in complex with bromocriptine.

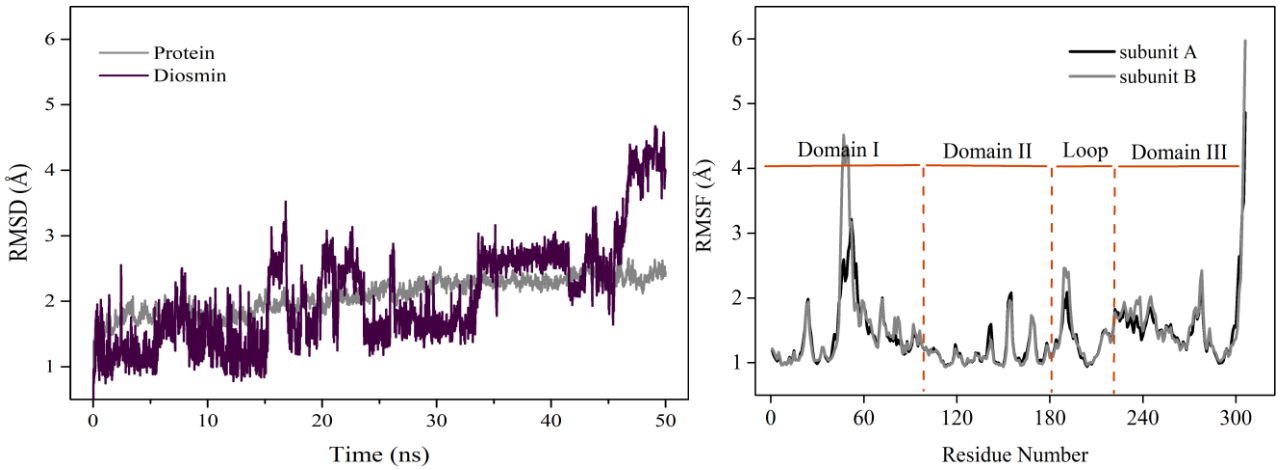

Figure S24: Protein and ligand RMSD (left) and RMSF (right) of the predicted allosteric site-1 of SARS-CoV-2 Mpro in complex with diosmin.

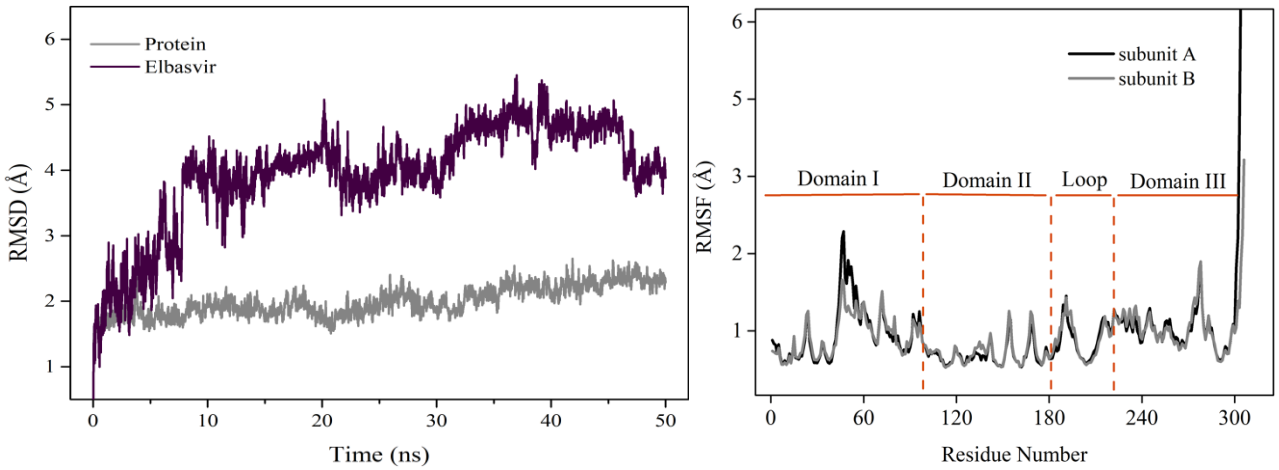

Figure S25: Protein and ligand RMSD (left) and RMSF (right) of the predicted allosteric site-1 of SARS-CoV-2 Mpro in complex with elbasvir.

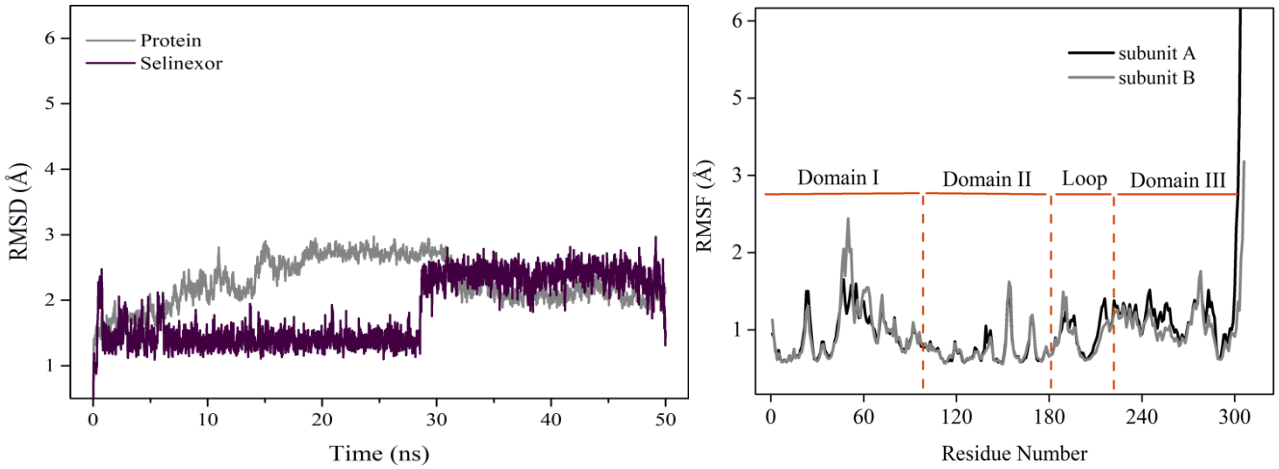

Figure S26: Protein and ligand RMSD (left) and RMSF (right) of the predicted allosteric site-1 of SARS-CoV-2 Mpro in complex with selinexor.

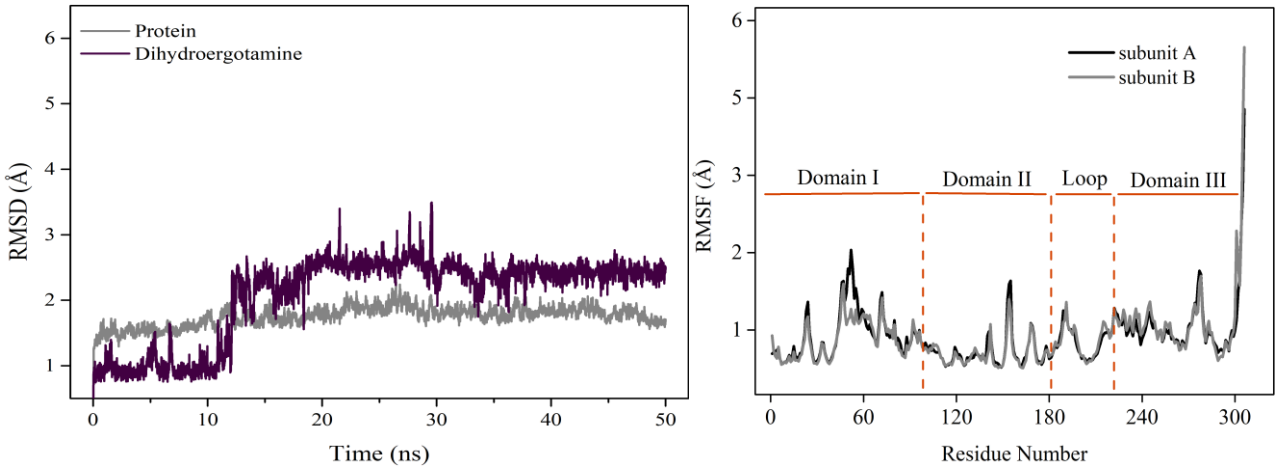

Figure S27: Protein and ligand RMSD (left) and RMSF (right) of the predicted allosteric site-2 of SARS-CoV-2 Mpro in complex with dihydroergotamine.

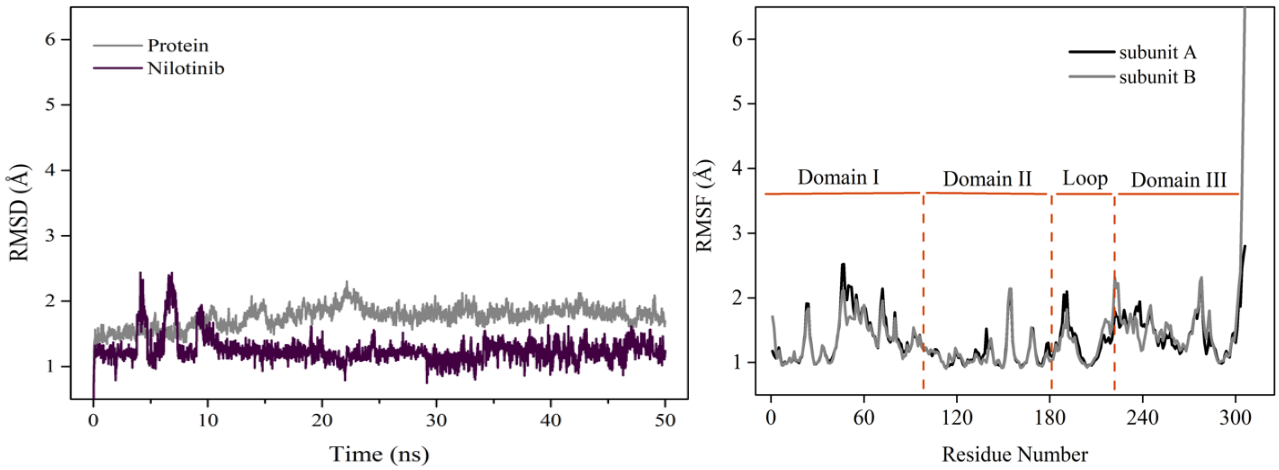

Figure S28: Protein and ligand RMSD (left) and RMSF (right) of the predicted allosteric site-2 of SARS-CoV-2 Mpro in complex with nilotinib.

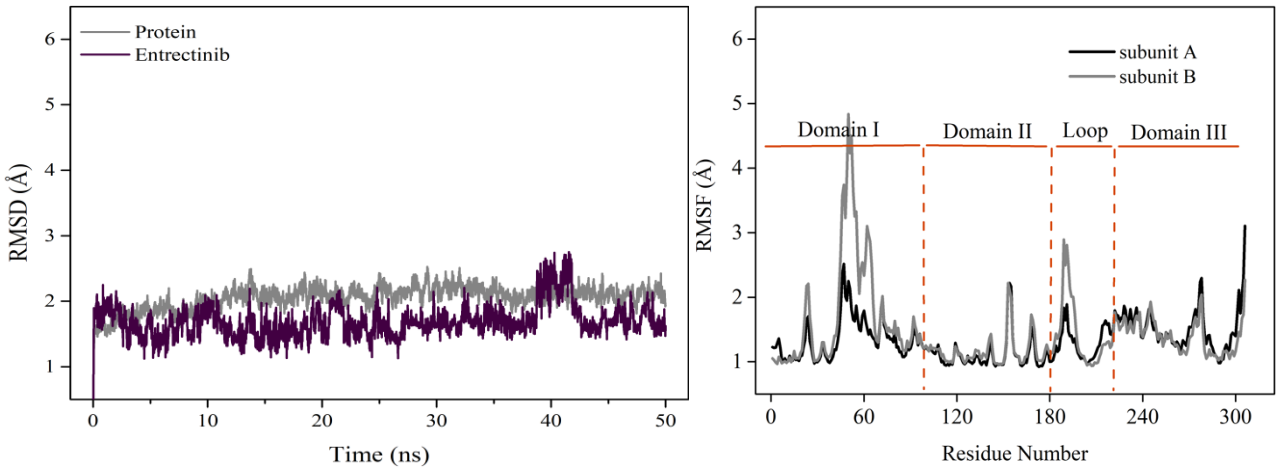

Figure S29: Protein and ligand RMSD (left) and RMSF (right) of the predicted allosteric site-2 of SARS-CoV-2 Mpro in complex with entrectinib.

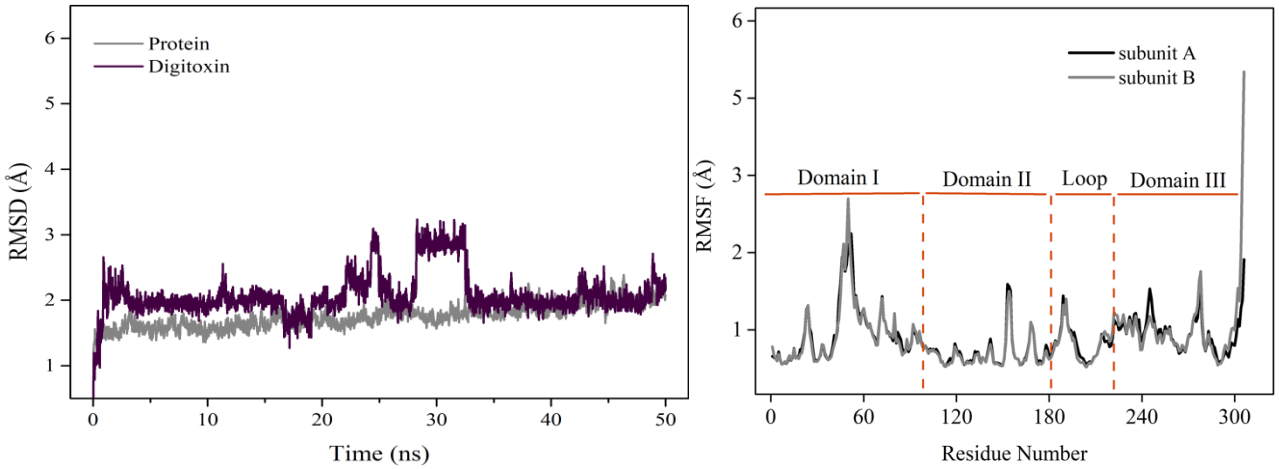

Figure S30: Protein and ligand RMSD (left) and RMSF (right) of the predicted allosteric site-2 of SARS-CoV-2 Mpro in complex with digitoxin.

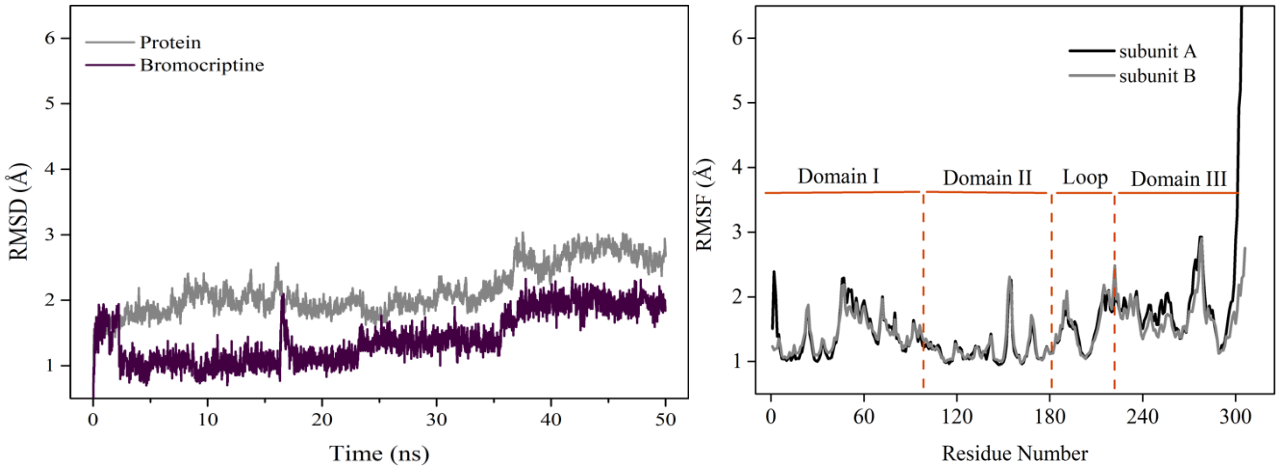

Figure S31: Protein and ligand RMSD (left) and RMSF (right) of the predicted allosteric site-2 of SARS-CoV-2 M^{pro} in complex with bromocriptine.

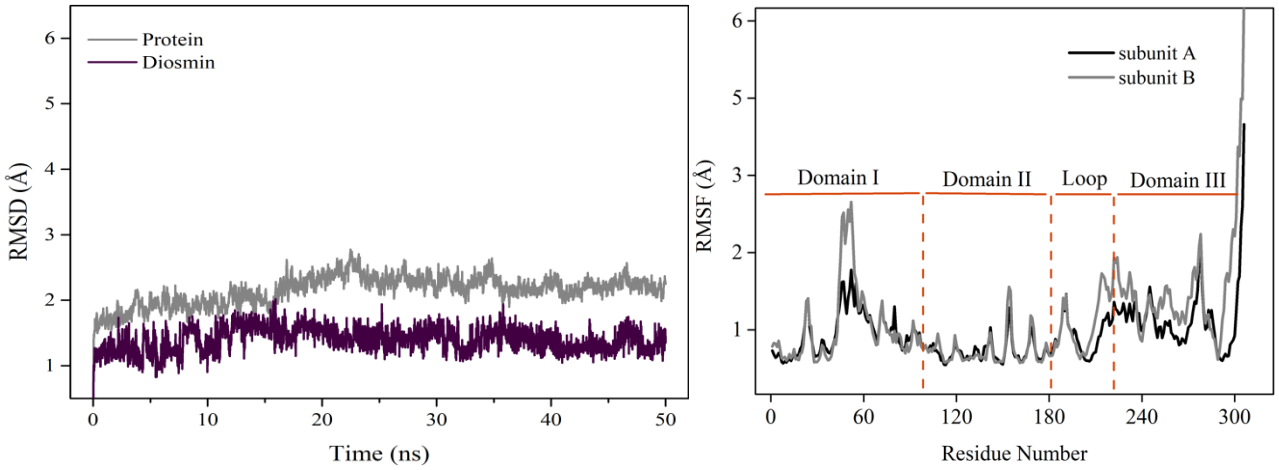

Figure S32: Protein and ligand RMSD (left) and RMSF (right) of the predicted allosteric site-2 of SARS-CoV-2 M^{pro} in complex with diosmin.

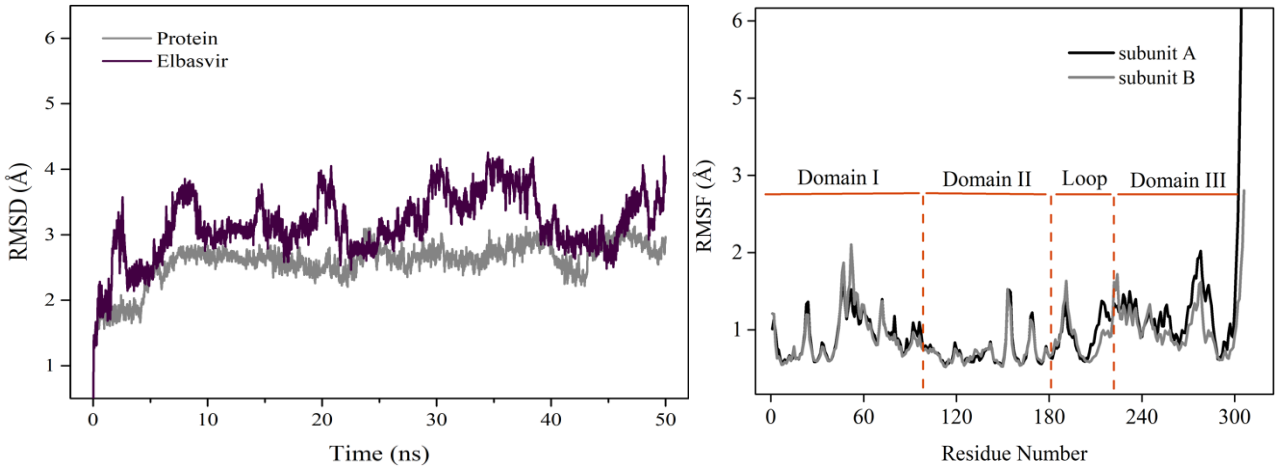

Figure S33: Protein and ligand RMSD (left) and RMSF (right) of the predicted allosteric site-2 of SARS-CoV-2 Mpro in complex with elbasvir.

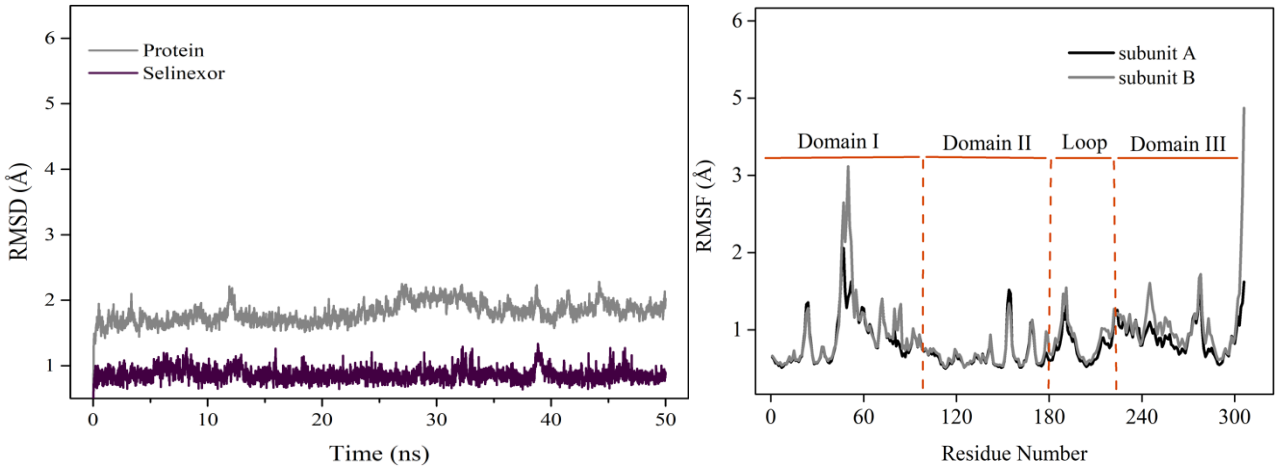

Figure S34: Protein and ligand RMSD (left) and RMSF (right) of the predicted allosteric site-2 of SARS-CoV-2 Mpro in complex with selinexor.