SUPPLEMENTARY INFORMATION

Molecular Dynamics Simulations Indicate the COVID-19 Mpro Is Not a Viable Target for Small-Molecule Inhibitors Design

Maria Bzówka[1#], Karolina Mitusińska[1#], Agata Raczyńska[1], Aleksandra Samol[1], Jack Tuszyński[2,3], Artur Góra[1*]

1) Tunneling Group, Biotechnology Centre, ul. Krzywoustego 8, Silesian University of Technology, Gliwice, 44-100, Poland
2) Department of Physics, University of Alberta, Edmonton, AB, T6G 2E1, Canada
3) DIMEAS, Politecnico di Torino, Corso Duca degli Abruzzi, 24, Turin, 10129, Italy

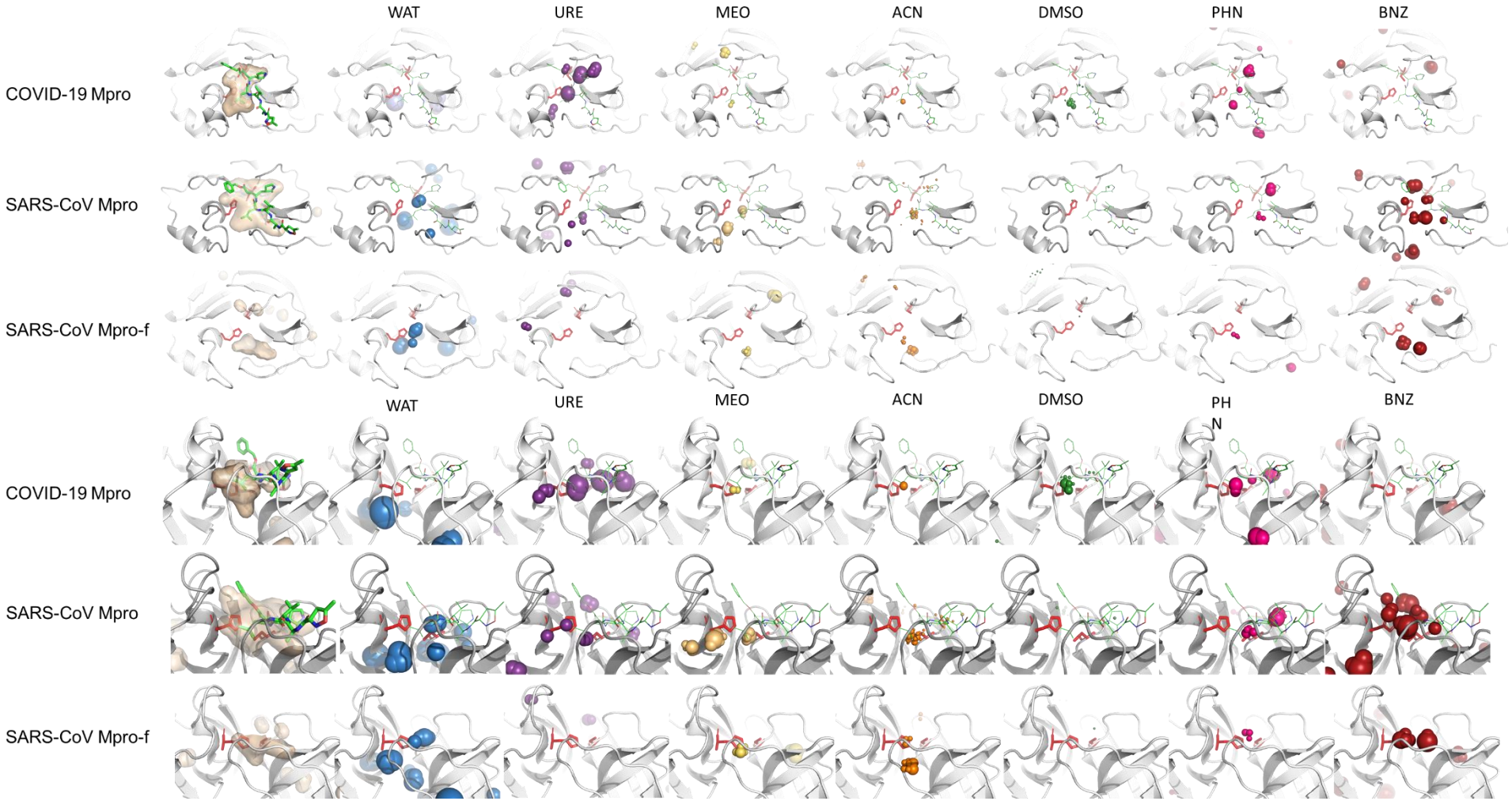
*Corresponding author
[#]These authors contributed equally to this work

Supplementary Table S1. Differences between COVID-2019 and SARS-CoV Mpros sequences. The last column shows differences in total energies (in kcal/mol) calculated as differences in Gibbs free energy folding between SARS-CoV Mpro and introduced single-point mutations as they are in COVID-19 Mpro structure.

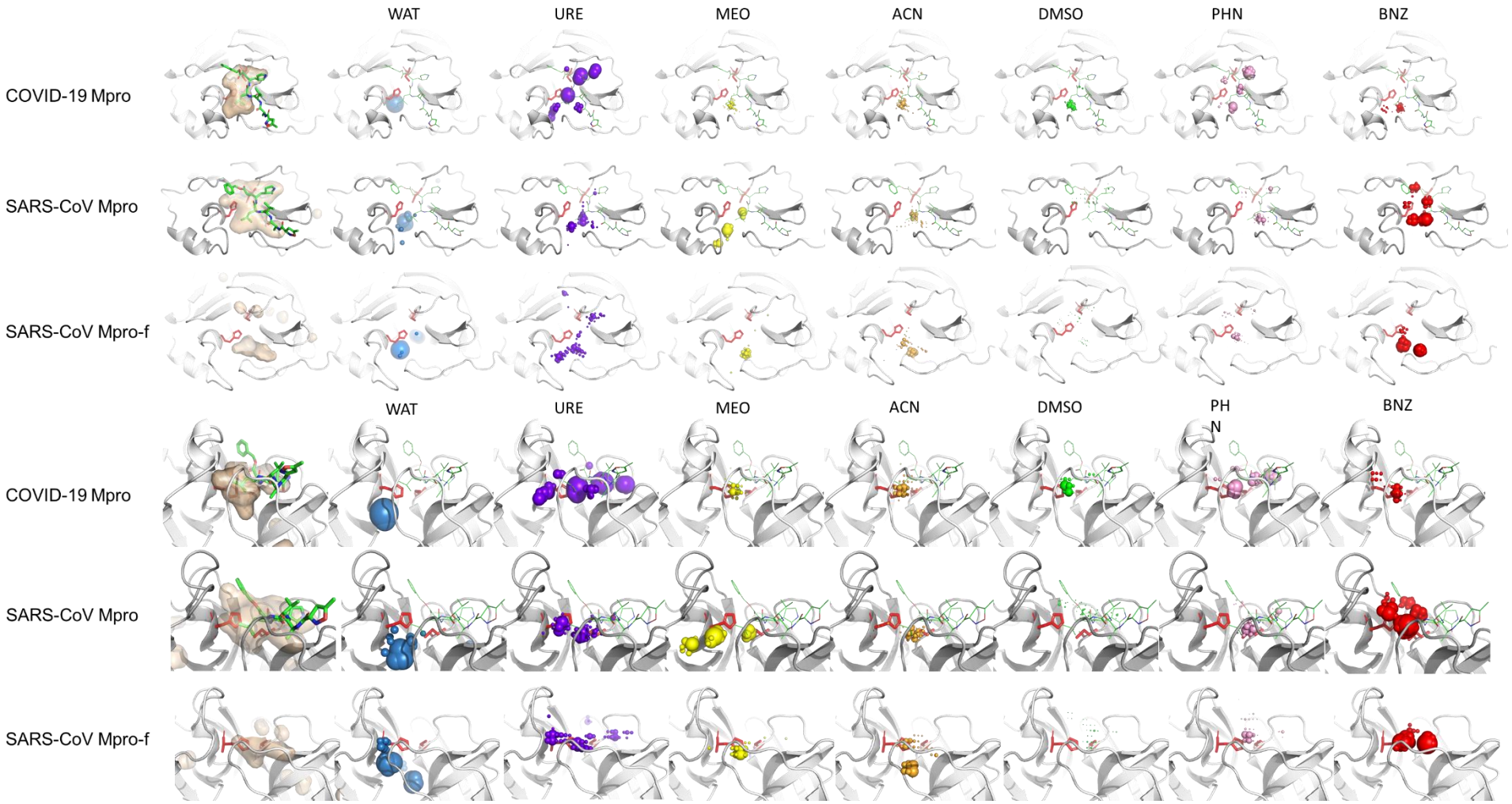
ID	COVID-19 Mpro	SARS-CoV Mpro	domain	buried/exposed (based on the NetSurfP calculations)	total energy differences [kcal/mol] (based on the FoldX calculations)
35	V	T	I	B	0.90
46	S	A	I	B	0.14
65	N	S	I	B(COVID)/ E(SARS)	0.32
86	V	L	I	B	2.76
88	K	R	I	E	-0.42
94	A	S	I	E	-0.41
134	F	H	II	E	-0.85
180	N	K	II	E	1.29
202	V	L	III	B	1.78
267	S	A	III	B	2.18
285	A	T	III	E	-0.37
286	L	I	III	E	-0.08
Changes in the protein’s sequence, not present in the crystallographic structure					
305	F	Q		E	
306	Q	G		E	

Supplementary Table S2. The volumes of the *Outer* pocket volumes [Å^3] in *time-window* mode.

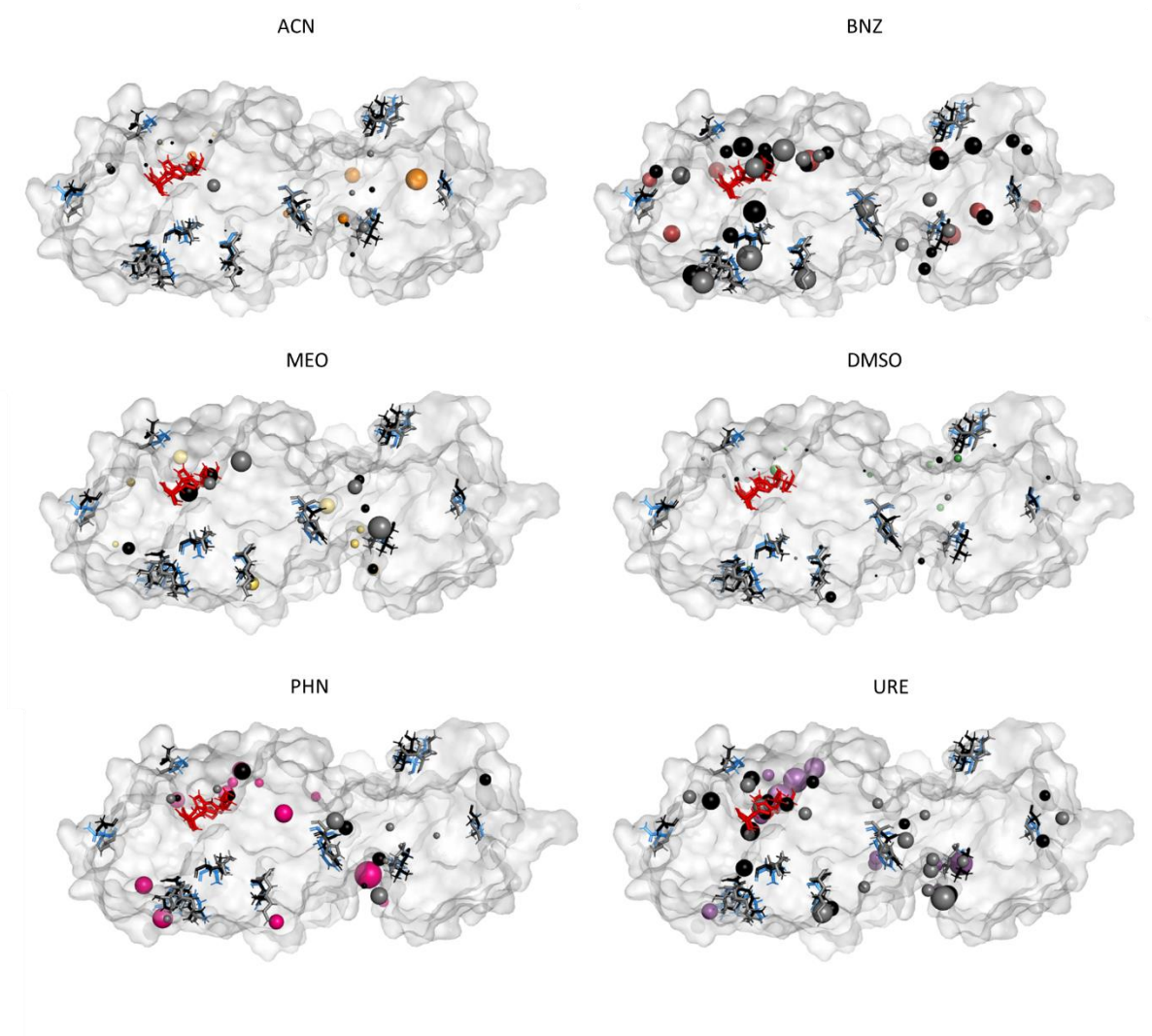
Time	COVID-19 Mpro	SARS-CoV Mpro	SARS-CoV Mpro-f
0-10ns	142	212	319
10-20ns	169	260	302
20-30ns	90	246	226
30-40ns	90	153	271
40-50ns	98	289	266
average	118	232	277



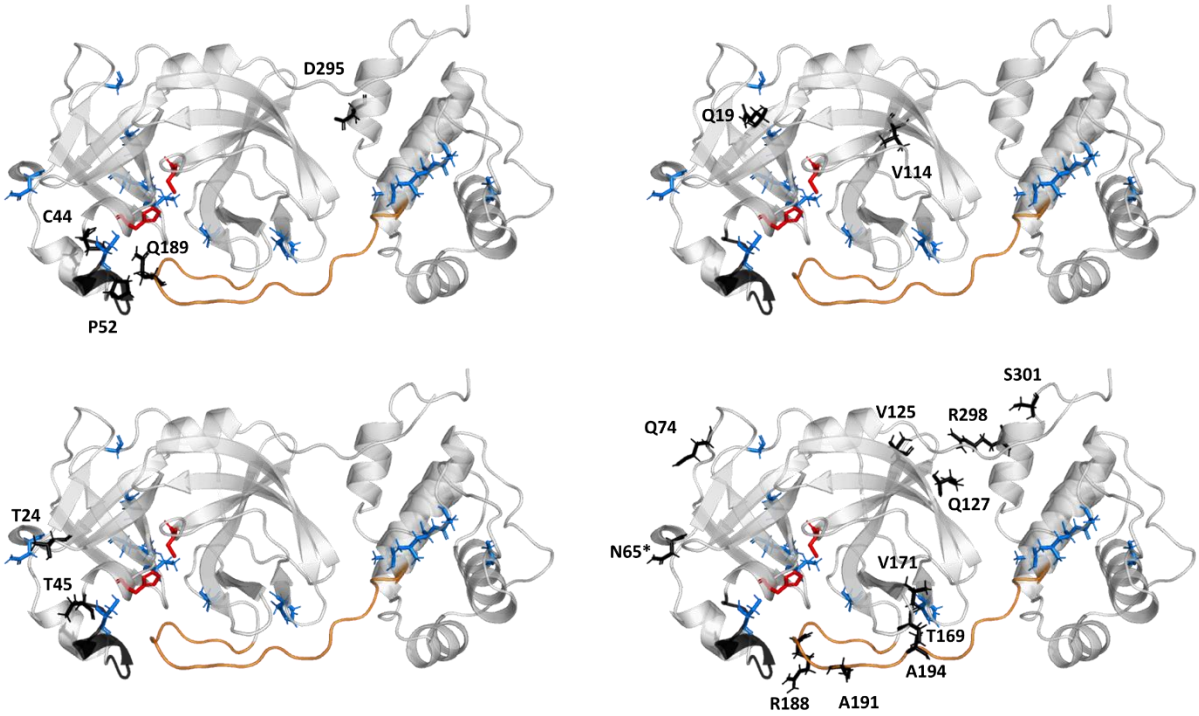
Supplementary Figure S1. Localisation of the global hot-spots of all analysed Mpros: COVID-19 Mpro, SARS-CoV Mpro and SARS-CoV Mpro-f. Hot-spots for individual cosolvents are represented by spheres, and their size reflects the hot-spots density. The colour-coding is as follows: purple - urea, green - DMSO, yellow - methanol, orange - acetonitrile, pink - phenol, red - benzene. The active site residues are shown as red sticks, the N3 inhibitor structure as green lines, and the proteins' structures are shown in cartoons representation.



Supplementary Figure S2. Localisation of the local hot-spots of all analysed Mpros: COVID-19 Mpro, SARS-CoV Mpro and SARS-CoV Mpro-f. Hot-spots for individual cosolvents are represented by spheres, and their size reflects the hot-spots density. The colour-coding is as follows: purple - urea, green - DMSO, yellow - methanol, orange - acetonitrile, pink - phenol, red - benzene. The active site residues are shown as red sticks, the N3 inhibitor structure as green lines, and the proteins' structures are shown in cartoons representation.



Supplementary Figure S3. Localisation of the global hot-spots of all analysed Mpros: COVID-19 Mpro, SARS-CoV Mpro and SARS-CoV Mpro-f. The structures of all three analysed Mpro structures are superposed and the colour-coding is as follows: orange, red, yellow, green, pink and purple hot-spots are from the COVID-19 Mpro, grey hot-spots from the SARS-CoV Mpro-f and black hot-spots from the SARS-CoV Mpro structure. The active site residues are shown as red sticks, the differing residues of the COVID-19 Mpro as blue sticks, and the proteins' structures are shown in surface representation.



Supplementary Figure 4. Localisation of the evolutionary correlated residues of Mpros (black sticks). The CMA analysis provided four groups of evolutionary correlated residues. The COVID-19 Mpro structure is presented as cartoon, the active site residues are shown as red sticks, the unique residues of the COVID-19 Mpro as blue sticks, the asterisks indicates unique residue that belong to evolutionary correlated residues. The loop C44-P52 is coloured black and F185-T201 loop is orange. Please note, that within one of the correlated groups (upper left) the residues from C44-P52 loop are correlated with Q189 from the linker loop and with residue from III domain.

Supplementary Table S3. FoldX results for the most energetically favourable potential mutations in the COVID-19 Mpro structure. Amino acids from the binding cavity are marked **bold**.

Mutation	Energy difference [kcal/mol]	buried/exposed (based on the NetSurfP calculations)
A260D	-3.67	E
Y154H	-2.04	E
T21I	-2.01	B
H41L	**-1.95**	**B**
Q127L	-1.89	E
A194P	-1.82	E
A129V	-1.75	B
Q306R	-1.73	E
H164L	**-1.71**	**B**
S301L	-1.68	E
V233L	-1.58	B
Q244P	-1.54	E
N53D	-1.51	E

Supplementary Table S4. FoldX results for binding cavity amino acids (7Å within the N3 inhibitor). Catalytic dyad is marked **bold**.

Mutation	Energy [kcal/mol]	buried/exposed (based on the NetSurfP calculations)
H41L	**-1.95**	**B**
H164L	-1.71	B
T169I	-1.49	E
T45S	-0.93	E
E47Q	-0.91	E
T24R	-0.84	E
S46P	-0.81	B
Q189L	-0.79	E
T26K	-0.78	E
C145F	**-0.77**	**B**
A191P	-0.58	E
H163Y	-0.55	B
E166Q	-0.43	E
L50R	-0.37	E
A193T	-0.36	E
H172L	-0.35	B
D187A	-0.35	E
V186F	-0.30	E
L141R	-0.28	B
N142Y	-0.22	B
N119Y	-0.08	E
S144A	-0.08	B
A173V	-0.02	B
T190I	0.05	E
Q192L	0.22	E
S147T	0.29	B
N28I	0.37	B
Y54F	0.40	B
C44S	0.41	B
M49L	0.42	B
V42L	0.74	B
Y118F	0.78	B
T25A	0.84	E
L27I	1.04	B
F140Y	1.09	B
F181Y	1.37	B
F185L	1.47	E
P52A	1.54	E
L167I	2.30	B
G143R	2.42	B
P39A	3.21	B
R40I	3.50	E
G146A	9.72	B

Supplementary Table S5. The final percentage concentration of particular cosolvents for both COVID-19 and SARS-CoV Mpros systems.

Cosolvent	Concentration [%]	Number of added molecules - COVID-19 Mpro	Number of added molecules – SARS-CoV Mpro	Number of added molecules – SARS-CoV Mpro-f
ACN	4.5	ACN: 450 WAT: 19712	ACN: 450 WAT: 19801	ACN: 450 WAT: 19858
BNZ	1.0	BNZ: 50 WAT: 19712	BNZ: 50 WAT: 19801	BNZ: 50 WAT :19858
DMSO	4.8	DMSO: 250 WAT: 19712	DMSO: 250 WAT: 19801	DMSO: 250 WAT: 19858
MEO	4.3	MEO: 550 WAT: 19712	MEO: 550 WAT: 19801	MEO: 550 WAT: 19858
PHN	1.2	PHN: 50 WAT: 19712	PHN: 50 WAT: 19801	PHN: 50 WAT: 19858
URE	4.4	URE: 300 WAT: 19712	URE: 300 WAT: 19801	URE: 300 WAT: 19858