Supporting Information

De Novo Design of Potential Peptide Analogs against Main Protease of Omicron Variant using *in silico* Studies

Stanly Paul M.L.[c], Sonia Kumari[d], Tamás A. Martinek*[ab] and Elizabeth Sobhia M *[d]

[a.]Department of Medical Chemistry, University of Szeged, Dóm tér 8, H-6720 Szeged, Hungary

[b.]ELKH-SZTE Biomimetic Systems Research Group, University of Szeged, Dóm tér 8, H-6720 Szeged, Hungary. Email: martinek.tamas@med.u-szeged.hu

[c.]Institute of Pharmaceutical Analysis, University of Szeged, Eotvos u. 6, G-6720 Szeged, Hungary. Email: stanley.szte@gmail.com

[d.]Department of Pharmacoinformatics, National Institute of Pharmaceutical Education and Research (NIPER), Sector-67, S.A.S. Nagar, Mohali 160062, India. Email: mesophia@niper.ac.in

Table of Contents   **Page number**
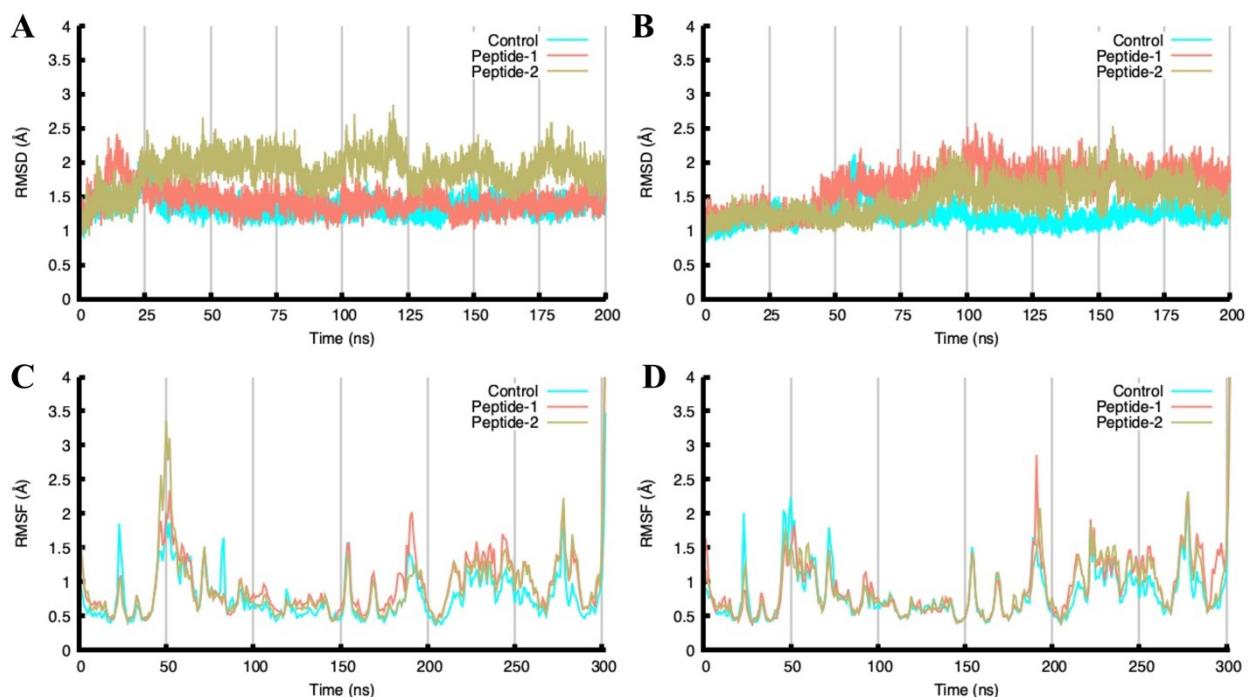
Figure S1. (A) RMSD of Mpro$^{(SARS-CoV2)}$ while binding with control peptide (cyan), peptide-(I)(salmon) and peptide-(II) (olive) in MD simulations (B) RMSD of Mpro$^{(Omicron)}$ while binding with control peptide (cyan), peptide-(I) (salmon) and peptide-(II) (olive) in MD simulations (C) RMSF of Mpro $^{(SARS-CoV2)}$ while binding with control peptide (cyan), peptide-(I) (salmon) and peptide-(II) (olive) in MD simulations (D) RMSF of Mpro$^{(Omicron)}$ while binding with control peptide (cyan), peptide-(I) (salmon) and peptide-(II) (olive) in MD simulations.
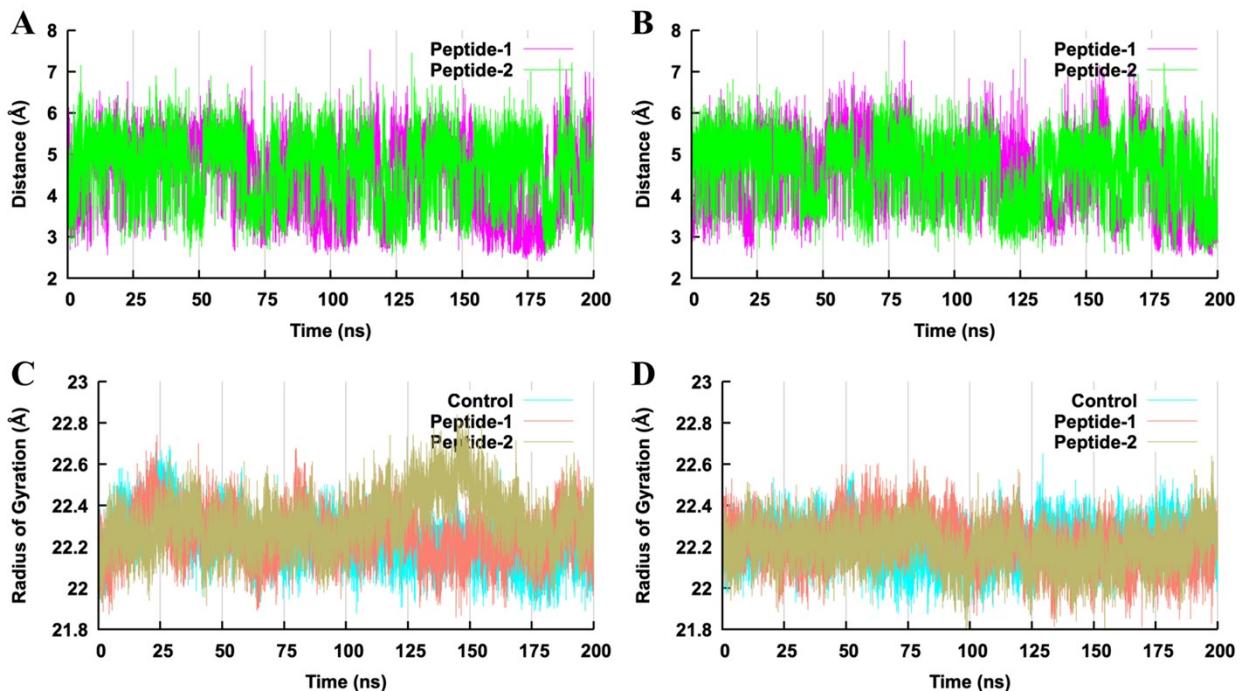
Figure S2. (A) Salt bridge distance between Mpro $^{(SARS-CoV-2)}$ Glu166 and Lys528 Peptide(I) (magenta), Peptide(II) (green) during 200ns MD simulations. (B) Salt bridge distance between Mpro $^{(Omicron)}$ Glu166 and Lys528 Peptide(I) (magenta), Peptide(II) (green) during 200ns MD simulations. (C) The radius of gyration of Mpro $^{(SARS-CoV2)}$ during the 200ns MD simulations while interacting with control peptide (cyan), Peptide(I) (salmon) and Peptide(II) (olive) (D) Radius of gyration of Mpro $^{(Omicron)}$ during the 200ns MD simulations while interacting with control peptide (cyan), Peptide(I) (salmon) and Peptide(II) (olive).
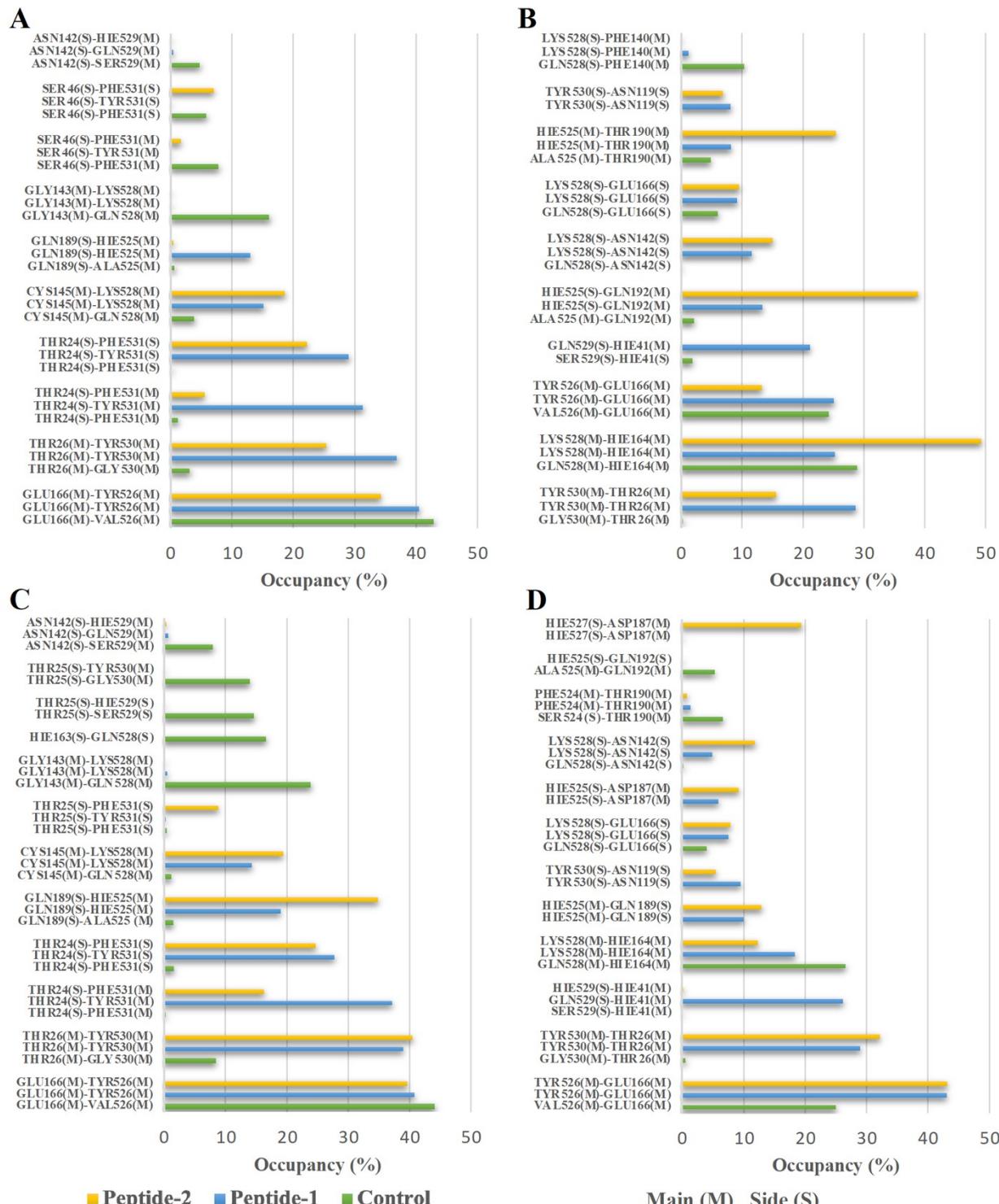
Figure S3. Histogram for hydrogen bond occupancy between pairs (peptide-protein)in SARS-CoV2(A,B) and Omicron (C, D); (A) Peptide/peptide mimics acting as hydrogen bond "Acceptor" and Mpro[(SARS-CoV2)] as hydrogen bond "Donor"; (B) Peptide/peptide mimics acting as hydrogen bond "Donor" and Mpro[(SARS-CoV2)] as hydrogen bond "Acceptor"; (C) Peptide/peptide mimics acting as hydrogen bond "Acceptor" and Mpro[(Omicron)] as hydrogen bond "Donor"; (D) Peptide/peptide mimics acting as hydrogen bond "Donor" and Mpro(Omicron) as hydrogen bond "Acceptor".
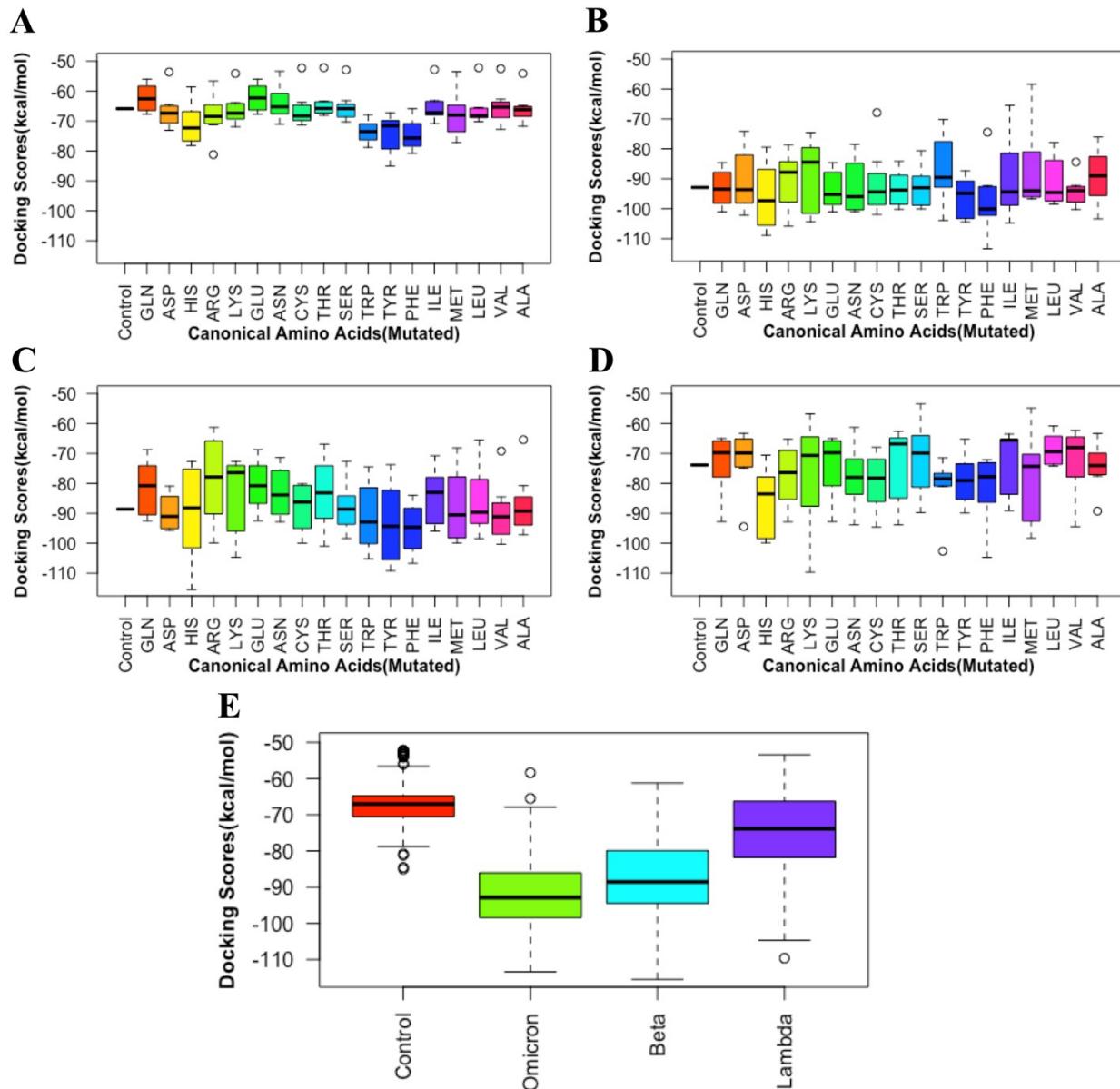
Figure S4. X-axis denotes the canonical amino acids (except proline and glycine) and Y-axis denotes docking scores. The standard deviation of the (A)Mpro [(SARS-CoV-2)]-peptide/peptide-analogs, (B) Mpro [(Omicron)]-peptide/peptide-analogs, (C) Mpro [(Beta)]-peptide/peptide-analogs, (D) Mpro [(Lambda)]-peptide/peptide-analogs docking scores were obtained by using 8 replicates (20 canonical amino acids (except Proline and glycine) were mutated on 8 amino acid peptides. (E) Comparison of docking scores across variants (significant difference observed (P-value < 0.005).
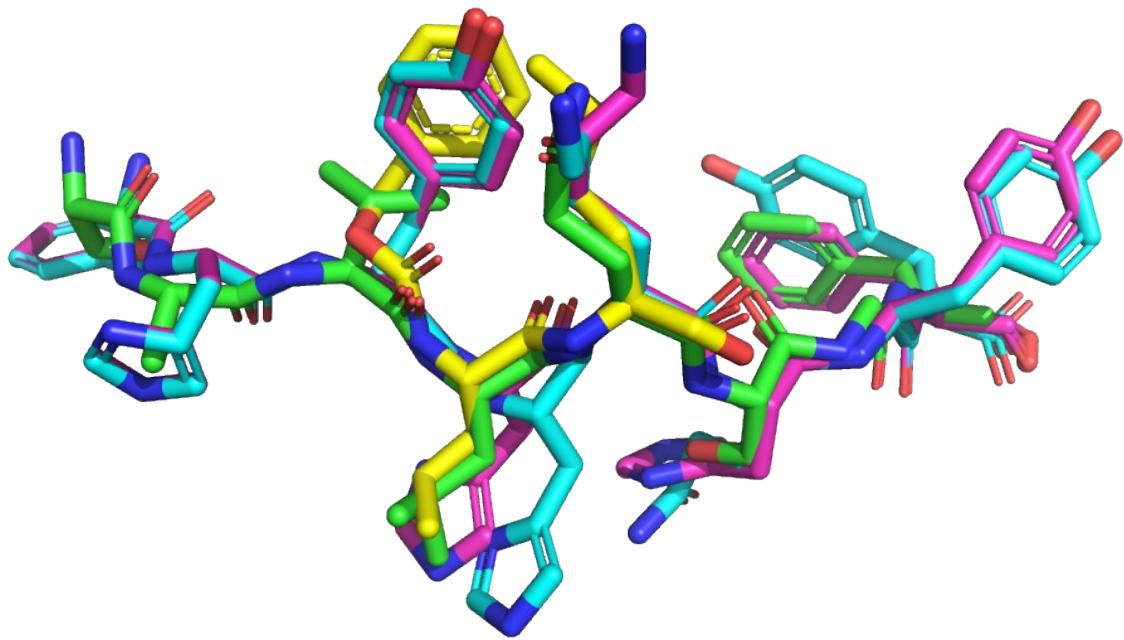
Figure S5. Superimposition of reference peptide(green), peptide-1(cyan) and peptide-2(pink with calpeptin(yellow)
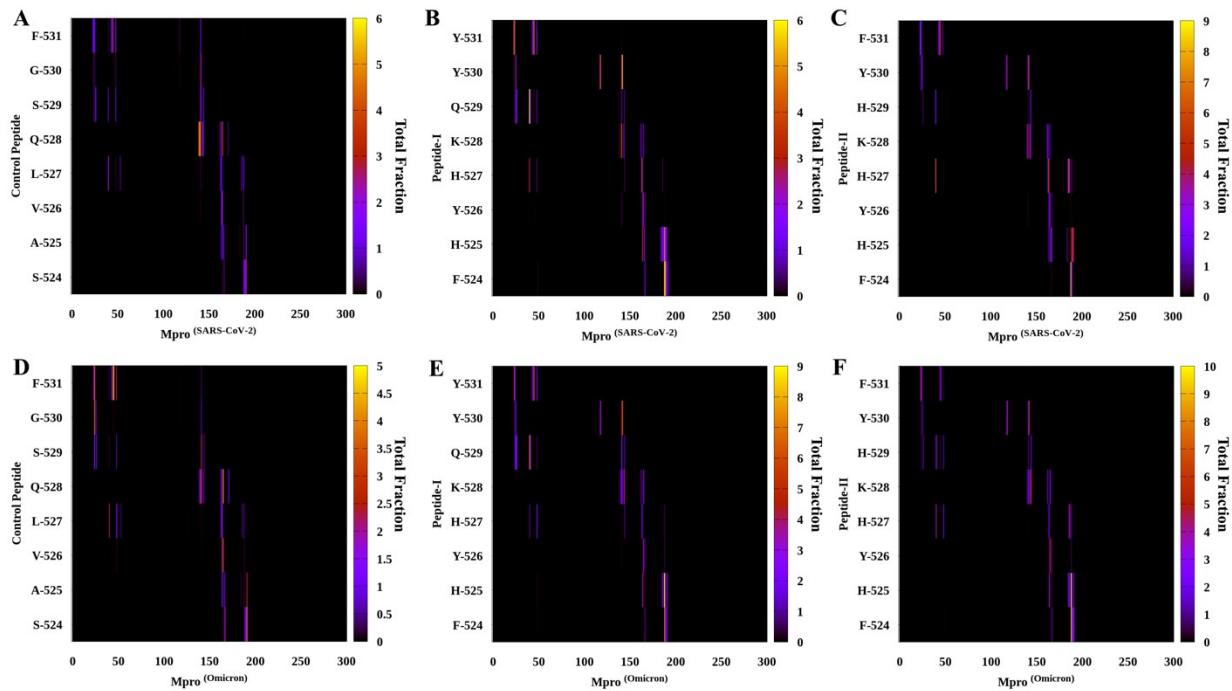
Figure S6. Contact information within 4Å in between (Mpro^(SARS-CoV-2)) with peptide substrate(A), Peptide-1(B) and Peptide-2(C). Native contacts between Mpro^(Omicron) with peptide substrate(D), Peptide-1(E) and Peptide-2(F).

Table S1. Initial docked pose interactions of substrate peptide(control) with Mpro[(SARS-CoV2, Omicron, Beta, Lambda)] variants. "*" denotes hydrogen bonds.

Peptides	Interactions	Peptide residues	Control	Distance (Å)	Omicron	Distance (Å)	Beta	Distance (Å)	Lambda	Distance (Å)
		Peptide	**Mpro (Protein)**							
Reference Peptide	Hydrogen Bond	524:Ser	189:Gln 168:Pro 190:Thr	3.2 3.3 3.4	190:Thr 168:Pro 191:Ala	3.7 3.9 3.9	190:Thr 168:Pro 191:Ala	3.4 3.5 3.7	168:Pro 190:Thr	3.5 3.6
		525:Ala	190:Thr* 189:Gln 188:Arg	2.9 3.1 3.0	190:Thr* 166:Glu 188:Arg 189:Gln 167:Leu 192:Gln 168:Pro	2.9 3.3 3.3 3.4 3.8 3.9 4.0	190:Thr* 189:Gln 188:Arg 167:Leu 166:Glu 165:Met	3.0 3.5 3.6 3.6 3.7 4.0	190:Thr* 166:Glu 189:Gln 188:Arg 165:Met 167:Leu	2.9 3.3 3.4 3.5 3.9 3.9
		526:Val	166:Glu*	2.8	166:Glu* 165:Met	3.3 3.4	166:Glu* 165:Met 189:Gln	3.4 3.5 3.6	166:Glu* 165:Met	3.1 3.5
		527:Leu	41:Hie 49:Met 165:Met	3.1 3.3 3.3	49:Met 164:His 41:Hie 165:Met	3.5 3.6 3.7 4.0	189:Gln* 49:Met 41:Hie 164:His	3.2 3.8 3.8 3.9	164:His 49:Met 41:Hie 165:Met	3.6 3.7 3.8 4.0
		528:Gln	63:Hie* 143:Gly* 140:Phe* 145:Cys*	2.8 3.0 3.1 3.2	143:Gly* 163:Hie* 140:Phe* 166:Glu* 145:Cys* 144:Ser 141:Leu 142:Asn 164:His	2.9 3.0 3.0 3.1 3.2 3.3 3.6 3.6 3.7	143:Gly* 140:Phe* 163:Hie* 144:Ser 145:Cys* 166:Glu 141:Leu 142:Asn	3.0 3.0 3.0 3.2 3.3 3.4 3.6 3.7	166:Glu* 143:Gly* 140:Phe 142:Asn 144:Ser* 145:Cys 41:Leu	3.0 3.0 3.1 3.1 3.1 3.3 3.5
		529:Ser	142:Asn 25:Thr 143:Gly 145:Cys 41:Hie 26:Thr 27:Leu	3.0 3.5 3.7 3.8 3.8 3.9 4.0	41:Hie 143:Gly 142:Asn 27:Leu 145:Cys 25:Thr 26:Thr	3.4 3.5 3.6 3.9 3.9 4.0 4.0	41:Hie 143:Gly 49:Met 42:Asn	3.2 3.8 3.9 4.0	41:Hie 143:Gly 145:Cys 142:Asn 26:Thr	3.2 3.3 3.7 3.8 3.9
		530:Gly	26:Thr*	2.8	26:Thr* 143:Gly 25:Thr	2.9 3.3 3.6	26:Thr 143:Gly 25:Thr	2.9 3.3 3.6	26:Thr* 143:Gly 25:Thr	2.9 3.3 3.7
		531:Phe	24:Thr 26:Thr 142:Asn	3.0 3.3 3.8	24:Thr 26:Thr	3.3 4.0	46:Ser 24:Thr 25:Thr	3.3 3.5 3.6	24:Thr 46:Ser 49:Met	3.3 3.3 4.0
Peptide-(I)	Hydrogen Bond	525:His	-	-	190:Thr	2.9	188:Arg	3.0	190:Thr	2.8
		526:Tyr	166:Glu	3.3	-	-	166:Glu	3.4		

		527:His					164:His	3.0		
		528:Lys	143:Gly 145:Cys	2.9 3.4	143:Gly 145:Cys	2.9 3.3	140:Phe 143:Gly 145:Cys 166:Glu	3.0 3.0 3.1 2.9	140:Phe 166:Glu	3.0 2.9
		529:His	-	-	-	-	-	-	143:Gly	3.1
		530:Tyr	26:Thr 25:Thr	2.8 3.6	26:Thr 25:Thr		26:Thr	2.9	-	-
	Pi-Pi	527:His	41:His	3.0	41:His	3.1	-	-	-	-
	Salt bridge	528:Lys	166:Glu	3.1	-	-	166:Glu	2.9	166:Glu	2.9
Peptide-(II)	Hydrogen Bonds	525:Hie	190:Thr	2.9	190:Thr	2.9	190:Thr	2.8	188:Arg	2.9
		526:Tyr	166:Glu	3.0	166:Glu	3.4			166:Glu	3.4
		528:Lys	143:Gly 145:Cys	2.8 3.3	143:Gly 145:Cys	2.9 3.2	140:Phe 166:Glu	3.0 2.9	140:Phe 143:Gly 145:Cys	3.0 2.9 3.3
		529:His					143:Gly	3.1	46:Ser	3.1
		530:Tyr	26:Thr 25:Thr	2.7 3.5	26:Thr 25:Thr	2.7 3.5			26:Thr	3.0

Table S2. Tukey or Dunnett multiple comparison testing made in between the average docking scores of substrate peptide(control) and canonical amino acid mutant peptides.

Type	P adjacent			
	Wild type	**Omicron**	**Beta**	**Lambda**
GLN-Control	0.9989987	1	0.9976458	1
ASP-Control	1	1	1	1
HIS-Control	0.9415269	0.9999999	1	0.7247772
ARG-Control	0.9999976	1	0.9210504	0.9999999
LYS-Control	1	0.9999927	0.9999881	1
GLU-Control	0.9985704	1	0.9900602	1
ASN-Control	0.9999999	1	0.9998998	0.9999998
CYS-Control	1	1	1	0.9999525
THR-Control	1	1	0.9999434	1
SER-Control	1	1	1	1
TRP-Control	0.4072177	0.9980674	1	0.9989491
TYR-Control	0.2558095	0.9999998	0.9999884	0.9999882
PHE-Control	0.1944254	0.9999844	0.9992989	0.9968224
ILE-Control	1	1	0.999999	1
MET-Control	0.9999997	0.9992334	1	0.999997
LEU-Control	1	1	1	0.9999806
VAL-Control	1	1	1	1
ALA-Control	1	0.9999989	1	1

Table S3. Tukey or Dunnett multiple comparison testing made in between the average docking scores of (SARS-CoV2/Wild Type (WT)) and SARS CoV2 variants [Omicron, Beta and Lambda].

	difference	lower	upper	p adjacent
Omicron-WT	-16.592434	-20.145196	-13.039672	0.0000000
Beta-WT	-8.390461	-11.943222	-4.837699	0.0000000
Lambda-WT	1.164474	-2.388288	4.717236	0.8331979

Table S4. Mpro sequence data collection for SARS-CoV2 variants

Variant	Virus name	Accession ID	Collection date	Submission Date	Length	Location	Originating lab
Alpha (B.1.1.7)	hCoV-19/Japan/HiroYH02/2021	EPI ISL_6756515	8/2/21	11/26/21	29763	Asia/Japan/Tokyo	The Virology lab, Hiroshima University
Beta (B.1.351)	hCoV-19/Japan/TY27-328-P0/2021	EPI ISL_5416540	--/07/21	10/21/21	29764	Asia/Japan/Tokyo	Department of Virology I, National Institute of Infectious Diseases
Gamma (P.1)	hCoV-19/Japan/TY30-974-P0/2021	EPI ISL_6228367	--/08/21	11/12/21	29768	Asia/Japan/Tokyo	Department of Virology I, National Institute of Infectious Diseases
Delta (B.1.617.2)	hCoV-19/Japan/TKYS01334/2021	EPI ISL_6832166	10/25/21	11/29/21	29769	Asia/Japan/Tokyo	Tokyo Metropolitan Institute of Public Health
Omicron (BA.1)	hCoV-19/Sweden/OmicronCoV_Isolate_1/2021	EPI ISL 10866182	--/--/21	3/9/22	29,822	Europe/ Sweden	Swedish national genomic surveillance program of SARS-CoV-2
Omicron (BA.2)	hCoV-19/ Cabo Verde/FG-AG0104/2022	EPI ISL 14721358	7/22/22	8/29/22	29,660	Africa/ Cabo Verde/ Fogo	Virology Laboratory of Fogo
Omicron (BA.2.12.1)	hCoV-19/ India/DL-GSL_MAMC_LNH/2022	EPI ISL 12643348	4/30/22	5/11/22	29,862	Asia/India/Delhi	Department of Microbiology, MAMC & Genome Sequencing Lab, Lok Nayak Hospital
Omicron (BA.4)	hCoV-19/Andorra/AND-255 221941418801-GC/2022	EPI ISL 14551163	6/29/22	8/19/22	29,636	Europe / Andorra / Andorra	Laboratori d'anàlisis clíniques, Hospital Nostra Senyora de Meritxell
Omicron (BA.5)	hCoV-19/Andorra/AND-259 221941419101-GC/2022	EPI ISL 14551152	7/3/22	8/19/22	29,622	Europe / Andorra / Andorra	Laboratori d'anàlisis clíniques, Hospital Nostra Senyora de Meritxell
Theta (P.3)	hCoV-19/Angola/CERI-KRISP-K012704/2021	EPI ISL_2492682	3/18/21	6/11/21	29,811	Africa / Angola / Luanda	Instituto Nacional de Investigação em Saúde
Zeta (P.2)	hCoV-19/Argentina/C97133/2020	EPI ISL_778843	11/20/20	1/7/21	29,896	South America / Argentina	Servicio Virosis Respiratorias-Departamento Virología-INEI
Epsilon (B.1.427)	hCoV-19/Anguilla/603	EPI ISL_2478962	3/19/21	6/10/21	29,782	North America /	The Caribbean Public Health Agency

Variant	Virus name	Accession ID	Collection date	Submission Date	Length	Location	Originating lab
	00/2021					Anguilla	
Eta (B.1.525)	hCoV-19/Algeria/17885/2021	EPI ISL 12156746	3/1/21	4/20/22	29,311	Africa / Algeria / Algiers	NIC, Viral Respiratory Unit
Kappa (B.1.617.1)	hCoV-19/Angola/CERI-KRISP-K012705/2021	EPI ISL_2493071	3/21/21	6/11/21	29,842	Africa / Angola / Luanda	Instituto Nacional de Investigação em Saúde
Iota (B.1.526)	hCoV-19/Afghanistan/2870x0198 23487_23597/2021	EPI ISL_4572806	4/7/21	9/29/21	29,721	Asia / Afghanistan	Afghanistan
Mu (B.1.621)	hCoV-19/Argentina/INEI109824/2021	EPI ISL_7751221	7/9/21	12/17/21	29,902	South America / Argentina / CABA	Servicio Virosis Respiratorias-Departamento Virologia-INEI
Lambda (C.37)	hCoV-19/Argentina/INEI096534/2020	EPI ISL_2158693	11/8/20	5/19/21	29,792	South America / Argentina / Ciudad Autonoma de Buenos Aires	Servicio Virosis Respiratorias-Departamento Virología-INEI

Table S5. Binding free energy of peptide and peptide-analogs against Mpro [SARS-CoV-2 and Omicron]

Energy	Mpro[SARS-CoV2]_ Control	Mpro[Omicron]_ Control	Mpro[SARS-CoV2]_ Peptide(I)	Mpro[Omicron]_ Peptide(I)	Mpro[SARS-CoV2]_ Peptide(II)	Mpro[Omicron]_ Peptide(II)
ΔE_{VdW}	-72.41	-76.00	-97.51	-100.40	-93.98	-92.06
ΔE_{Eel}	-59.77	-64.96	-224.66	-230.73	-197.40	-251.61
ΔE_{Egb}	94.43	99.14	262.25	268.68	233.13	284.88
ΔE_{Esurf}	-9.29	-9.92	-12.15	-12.33	-11.62	-11.76
ΔG_{Gas}	-132.19	-140.97	-322.17	-331.14	-291.39	-343.68
ΔG_{Solv}	85.13	89.21	250.09	256.34	221.50	273.12
ΔG_{Total}	**-47.05**	**-51.75**	**-72.0**	**-74.79**	**-69.88**	**-70.56**