Targeting the SARS-CoV-2 fusion mechanism: potent peptide inhibitors of the IFP-MPER interaction

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MPER peptides exhibit strong antiviral activity with nanomolar IC_{50}

The MPER-derived peptide Spike(1196-1220), rich in aromatic residues (Phe, Tyr, Trp), was synthesized via Induction-Assisted SPPS (IA-SPPS). To identify the minimal active sequence, we progressively shortened it from the N-terminus.

Peptide	Sequence	IC_{50} μM	CC_{50} μM	R (%)
Spike(1196-1220)	Ac-SLIDLQELGKYEQYIKWPWYIWLGF-NH_2	1.92 ± 0.67	>100	67
Spike(1198-1220)	Ac-IDLQELGKYEQYIKWPWYIWLGF-NH_2	1.10 ± 0.21	>100	68
Spike(1200-1220)	Ac-LQELGKYEQYIKWPWYIWLGF-NH_2	0.99 ± 0.71	>100	59
Spike(1202-1220)	Ac-ELGKYEQYIKWPWYIWLGF-NH_2	0.40 ± 0.31	52 ± 8	58
Spike(1204-1220)	Ac-GKYEQYIKWPWYIWLGF-NH_2	0.08 ± 0.07	>100	53
Spike(1206-1220)	Ac-YEQYIKWPWYIWLGF-NH_2	0.11 ± 0.05	84 ± 11	51
Spike(1208-1220)	Ac-QYIKWPWYIWLGF-NH_2	0.94 ± 0.48	>100	48
Spike(1210-1220)	Ac-IKWPWYIWLGF-NH_2	0.40 ± 0.13	>100	48
Spike(1212-1220)	Ac-WPWYIWLGF-NH_2	0.03 ± 0.02	>100	>100
Spike(1214-1220)	Ac-WYIWLGF-NH_2	5.47 ± 1.02	>100	38

Shortening the C-terminus boosts antiviral activity, with Spike(1212-1220) being most potent, while removing Trp1212 drastically reduces antiviral activity, underscoring the importance of aromatic residues.

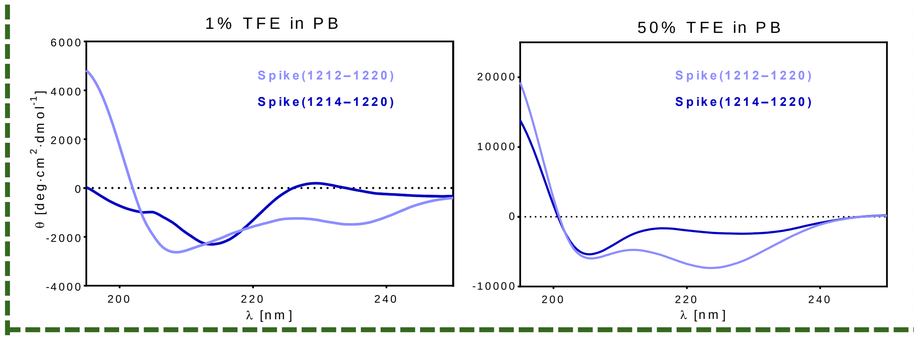
Aromatic residues and sequence specificity are crucial for biological activity

Peptide	Sequence	IC_{50} μM	CC_{50} μM	R (%)
Spike(1212-1218)	Ac-WPWYIWL-NH_2	4.68 ± 1.34	82 ± 10	-
Spike(1196-1211)	Ac-SLIDLQELGKYEQYIK-NH_2	20.08 ± 9.01	>100	76
Spike(1204-1220) scramble	Ac-WKFQYGPWGIIWLYKEY-NH_2	45.92 ± 7.56	>100	-
[Ala[1212,1214,1217]]Spike(1212-1220)	Ac-APAYIALGF-NH_2	40.90 ± 7.40	>100	75

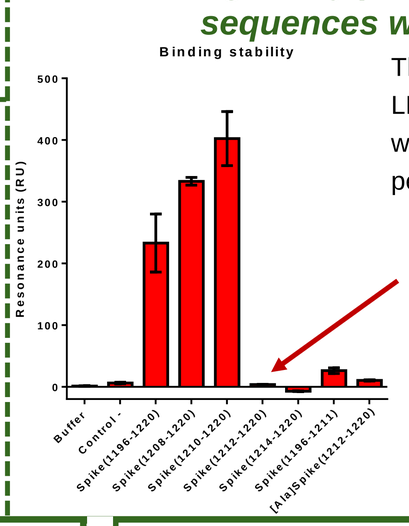
CD analysis revealed α-helical conformation propensity with aromatic side-chains interactions

1% TFE in PB: All peptides showed spectra of random-coil conformation.
50% TFE in PB: All peptides adopted an α-helical secondary structure, except for Spike(1212-1218) and Spike(1204-1220) scramble.

N-terminal truncation lowered the R-value, confirming the α-helix is mainly in the N-terminus. Indeed, Spike(1196–1211) had strong helicity (R = 75%); alanine substitution gave R-value = 65% (Ala penalty = 0 kcal/mol) [2].



SPR binding assay proves the interaction of MPER sequences with Fusion peptide



The fusion peptide [Cys[891]]Spike(861-891), LPPLLTDEMIAQYTSALLAGTITSGWTFGAC was immobilized onto the chip, while MPER peptides were used as analytes.

Spike(1212-1220) did not yield any signal, probably due to peptide aggregation

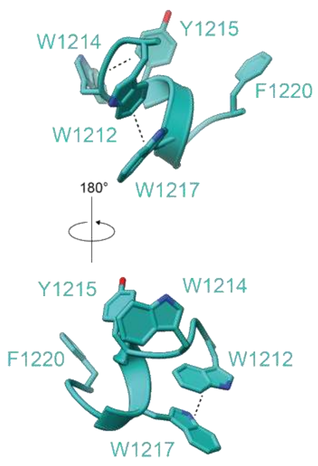
PB: 10 mM phosphate buffer, pH 7.4

π - π Stacking interactions were confirmed by high-resolution structure NMR

The high resolution structures of Spike(1212-1220) and Spike(1214-1220) were determined by NMR in both DMSO/H_2O and *n*-dodecylphosphocholine (DPC) [3,4].

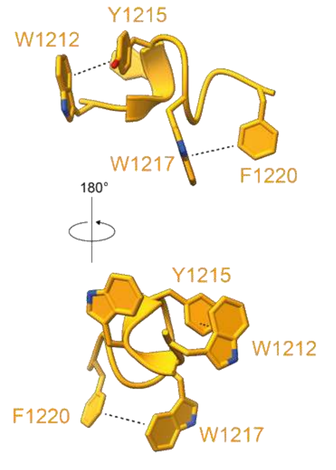
Spike(1212-1220)

π-π: W1212-W1217 parallel, W1214-Y1215 perpendicular

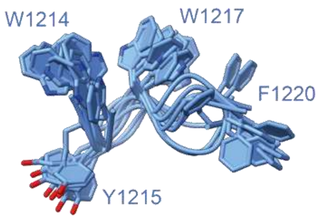


Spike(1212-1220) with DPC

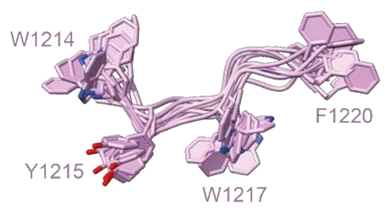
π-π: W1212-Y1215 parallel, W1217-F1220 perpendicular

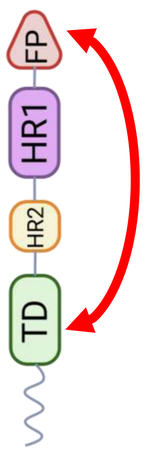


Spike(1214-1220)

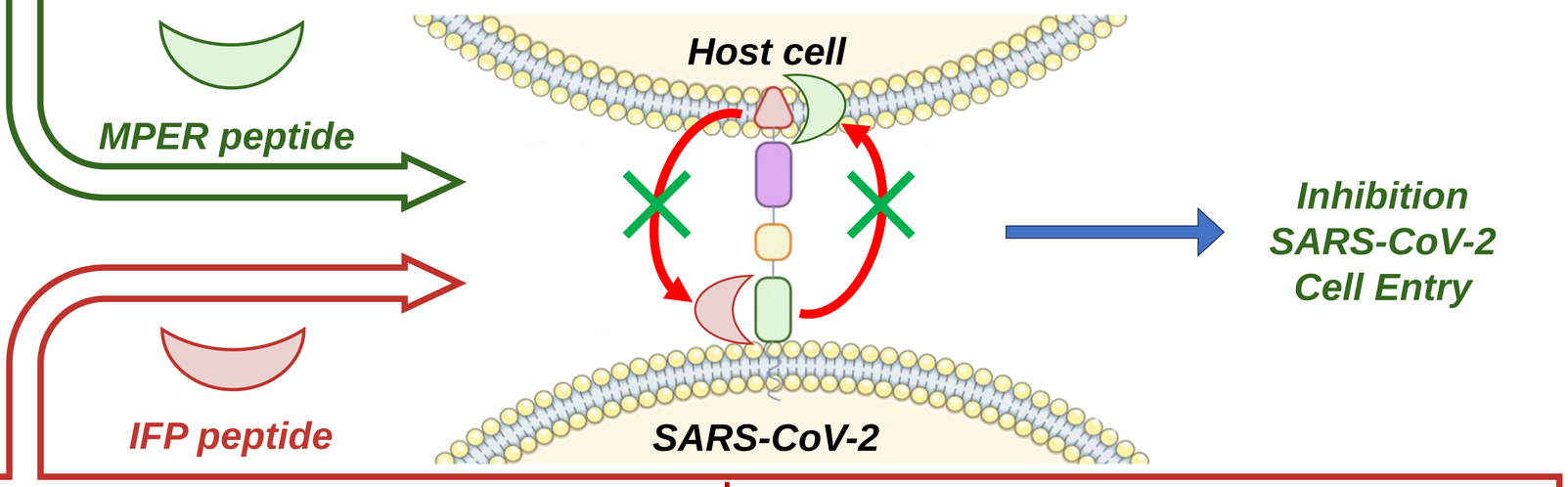


Spike(1214-1220) with DPC



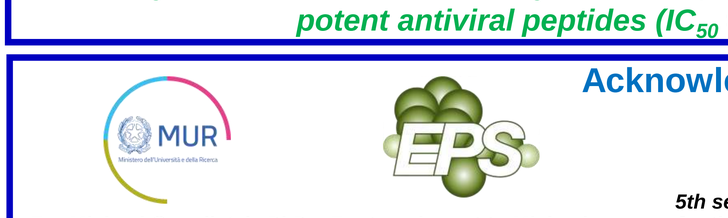


The spike protein is a trimeric surface protein anchored by its Transmembrane Domain (TD) and split into S1 and S2 subunits. S1 carries the Signal Peptide and Receptor Binding Domain for host cell recognition. After protease priming of S1, the Fusion Peptide (FP) inserts into the host membrane. Then, Heptad Repeats 1 and 2 (HR1, HR2) form a 6-Helix Bundle (6-HB), drawing the membranes together to create a pore for viral RNA entry. The formation of this bundle is mediated by the interaction between the Membrane Proximity External Region (contained in TD) and Internal Fusion Peptide (contained in FP) [1].



Enhancing antiviral activity by targeting lipid rafts via cholesterol functionalization

The IFP peptide Spike(888–906), designated PN19, exhibits potent antiviral activity against SARS-CoV-2 (IC_{50} = 0.49 μM) [5]. To target the lipid rafts present on the viral membrane, cholesteryl analogs of PN19 were synthesized [6].



Chemoselective and quantitative [7]

C-terminal cholesterol modification resulted in superior antiviral activity compared to N-terminal modification.

Peptide	Sequence	IC_{50} μM	CC_{50} μM
PN19	Ac-FGAGAALQIPFAMQMAYRF-NH_2	0.49 ± 0.15	>100
[C[1](chol)]PN19	Ac-C(chol)FGAGAALQIPFAMQMAYRF-NH_2	0.38 ± 0.11	>100
[C[20](chol)]PN19	Ac-FGAGAALQIPFAMQMAYRFC(chol)-NH_2	0.08 ± 0.04	>100

The linker length between PN19 and the cholesterol moiety plays a secondary role in antiviral activity

Several PN19 analogs bearing different linkers were synthesized. In particular, the pentapeptide spacer GSGSG and the PEG_6 linker were employed, either singularly or in combination. Both N- and C-terminal modifications were explored.

Peptide	Sequence	IC_{50} μM	CC_{50} μM
[C[1](chol)]PN19-spacer	Ac-C(chol)-GSGSG-FGAGAALQIPFAMQMAYRF-NH_2	0.98 ± 0.12	>100
[C[25](chol)]PN19-spacer	Ac-FGAGAALQIPFAMQMAYRF-GSGSG-C(chol)-NH_2	0.74 ± 0.34	>100
[C[1](chol)]PN19-PEG_6	Ac-C(chol)-PEG_6-FGAGAALQIPFAMQMAYRF-NH_2	3.70 ± 1.01	>100
[C[20](chol)]PN19-PEG_6	Ac-FGAGAALQIPFAMQMAYRF-PEG_6-C(chol)-NH_2	0.14 ± 0.12	>100
[C[1](chol)]PN19-spacer-PEG_6	Ac-C(chol)-PEG_6-GSGSG-FGAGAALQIPFAMQMAYRF-NH_2	1.75 ± 0.71	>100
[C[25](chol)]PN19-spacer-PEG_6	Ac-FGAGAALQIPFAMQMAYRF-GSGSG-PEG_6-C(chol)-NH_2	0.04 ± 0.02	>100

Amino acid sequence is crucial for biological activity

Peptide	Sequence	IC_{50} μM	CC_{50} μM
[C[10](chol)]PN9 scramble	Ac-RFQAYMFMAC(chol)-NH_2	>100	>100
[C[15](chol)]PN9-spacer-PEG_6 scramble	Ac-RFQAYMFMA-GSGSG-PEG_6-C(chol)-NH_2	>100	>100

N-terminal truncation confirms that the active region of PN19 is the aromatic-rich C-terminal

The shorter sequences were selected from a previous study based on their length and retention of biological activity [5]. In particular, PN13 and PN9 were selected, along with PN8, which served as the first inactive peptide.

Peptide	Sequence	IC_{50} μM	CC_{50} μM
PN13	Ac-LQIPFAMQMAYRF-NH_2	1.70 ± 0.52	>100
PN9	Ac-FAMQMAYRF-NH_2	5.93 ± 9.74	>100
[C[1](chol)]PN13	Ac-C(chol)-LQIPFAMQMAYRF-NH_2	1.20 ± 1.04	>100
[C[14](chol)]PN13	Ac-LQIPFAMQMAYRF-C(chol)-NH_2	0.28 ± 0.31	>100
[C[1](chol)]PN9	Ac-C(chol)-FAMQMAYRF-NH_2	3.44 ± 0.90	>100
[C[10](chol)]PN9	Ac-FAMQMAYRF-C(chol)-NH_2	0.27 ± 0.31	>100
[C[9](chol)]PN8	Ac-AMQMAYRFC(chol)-NH_2	15.02 ± 3.04	>100
[C[1](chol)]PN13-spacer-PEG_6	Ac-C(chol)-PEG_6-GSGSG-LQIPFAMQMAYRF-NH_2	21.14 ± 10.12	>100
[C[19](chol)]PN13-spacer-PEG_6	Ac-LQIPFAMQMAYRF-GSGSG-PEG_6-C(chol)-NH_2	0.37 ± 0.31	>100
[C[1](chol)]PN9-spacer-PEG_6	Ac-C(chol)-PEG_6-GSGSG-FAMQMAYRF-NH_2	16.08 ± 8.04	>100
[C[15](chol)]PN9-spacer-PEG_6	Ac-FAMQMAYRF-GSGSG-PEG_6-C(chol)-NH_2	0.14 ± 0.11	>100
[C[14](chol)]PN8-spacer-PEG_6	Ac-AMQMAYRF-GSGSG-PEG_6-C(chol)-NH_2	6.03 ± 3.05	>100

Conclusions

- ***IFP-MPER interaction is key for membrane fusion and a therapeutic target.***
- ***MPER truncation identified Spike(1212-1220) as potent inhibitor, stabilized by π - π stacking.***
- ***Cholesterol conjugation to IFP peptide (PN19) boosts antiviral activity, with C-terminal lipidation superior to N-terminal.***
- ***Linker length has minimal effect, but both amino acid sequence and aromatic C-terminus are crucial for antiviral activity.***

Our study led to two novel cell-entry inhibitors, Spike(1212-1220) and [C[25](chol)]PN19-spacer-PEG_6 as potent antiviral peptides (IC_{50} = 0.03, 0.04 μM, respectively) [8,9].

Perspectives

WORK IN PROGRESS

Studies are ongoing in order to increase the antiviral activity further into the nanomolar range

STAY TUNED

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