Synthesis, conformational analysis and biological activity evaluation of novel antiviral peptides blocking the SARS-CoV-2 cell-entry

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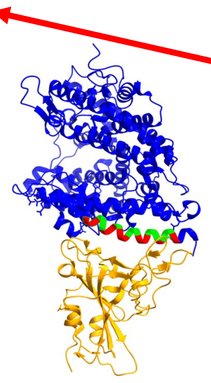
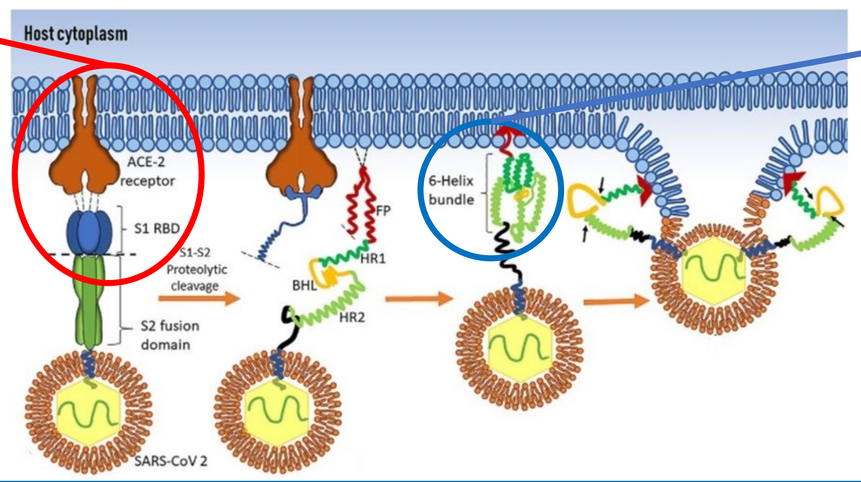
Introduction

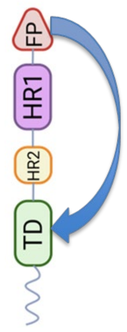
ACE2-RBD interaction

The infection pathway starts when the RBD of the viral spike protein interacts with the ACE2, which acts as a host receptor for the RBD expressed on the host cell surface.

Most of ACE2 residues involved in the interaction with RBD are located in the α-helix α1, namely the portion ACE2(24-42).
In particular, the key residues are: Q24, T27, D30, K31, H34, E35, E37, D38, Y41 e Q42







IFP-MPER interaction

Once the subunit S1 is primed by the proteases, the Fusion Peptide (FP) is free to insert into the host membrane, then the HR1 and HR2 start to interact each other forming the so-called 6-Helix Bundle (6-HB), pulling the viral and the host membrane closer. [1]

The Internal Fusion Peptide (IFP) plays a key role in the bundle formation because it interacts with the Membrane Proximity External Region (MPER) contained in the Transmembrane Domain (TD). [2]

Design and antiviral activity of the novel peptides

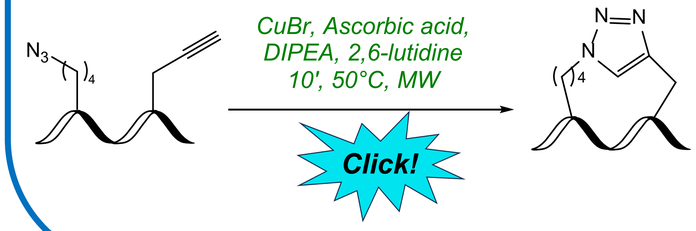
Possible antiviral peptide ?

ACE2(24-42): capable of interacting with RBD…

…but

Computational studies have shown that α1 unfolds rapidly and therefore the binding with RBD is weak.[3]

Cu(I) catalyzed Azide-Alkyne Cycloaddition (CuAAC) was exploited to obtain conformational stable analogs [4,5]



Peptide	Sequence	IC_{50} (mean ± SD) μM[a]
	Ac-QAKTFLDKFNHEAEDLFYQ-NH_2	2.80±2.68
	Ac-QAKTFLDKFNHEAEDLFYQ-NH_2	0.71±2.11
ACE2(24-42)	Ac-QAKTFLDKFNHEAEDLFYQ-NH_2	>100
	Ac-QAKTFLDKFNHEAEDLFYQ-NH_2	0.20±1.11
	Ac-QAKTFLDKFNHEAEDLFYQ-NH_2	2.79±1.96
P1	Ac-QAKT-NHCHCO-LDK-NHCHCO-NHEAEDLFYQ-NH_2	>100
	Ac-QAKTFLDKFNHEAEDLFYQ-NH_2	8.33±3.60
	Ac-QAKTFLDKFNHEAEDLFYQ-NH_2	9.43±2.44
P2	Ac-QAKTFLDK-NHCHCO-NHE-NHCHCO-EDLFYQ-NH_2	>100
	Ac-QAKTFLDKFNHEAEDLFYQ-NH_2	97.94±21.84
	Ac-QAKTFLDKFNHEAEDLFYQ-NH_2	46.83±9.97
P3	Ac-QAKTFLDKFNHE-NHCHCO-EDL-NHCHCO-YQ-NH_2	8.89±6.03
	Ac-QAKTFLDKFNHEAEDLFYQ-NH_2	>100
	Ac-QAKTFLDKFNHEAEDLFYQ-NH_2	1.70±0.52
P4	Ac-QAKT-NHCHCO-LDK-NHCHCO-NHE-NHCHCO-EDL-NHCHCO-YQ-NH_2	>100
	Ac-QAKTFLDKFNHEAEDLFYQ-NH_2	5.93±9.74

[a]IC_{50} values are measured by inhibitory viral plaque reduction assay (PRA) on Vero E6 cells.

Peptide	Sequence	IC_{50} (mean ± SD) μM[a]
	Ac-SGWTFGAGAALQIPFAMQMAYRF-NH_2	0.20±1.11
	Ac-WTFGAGAALQIPFAMQMAYRF-NH_2	0.71±2.11
	Ac-FGAGAALQIPFAMQMAYRF-NH_2	0.20±1.11
	Ac-AGAALQIPFAMQMAYRF-NH_2	2.79±1.96
	Ac-SGWTFGAGAALQIPFAMQMAY-NH_2	8.33±3.60
	Ac-SGWTFGAGAALQIPFAMQM-NH_2	9.43±2.44
	Ac-SGWTFGAGAALQIPFAM-NH_2	97.94±21.84
	Ac-WTFGAGAALQIPFAMQMAY-NH_2	46.83±9.97
	Ac-FGAGAALQIPFAMQM-NH_2	>100
	Ac-LQIPFAMQMAYRF-NH_2	1.70±0.52
	Ac-FAMQMAYRF-NH_2	5.93±9.74

[a]IC_{50} values are measured by inhibitory viral plaque reduction assay (PRA) on Vero E6 cells.

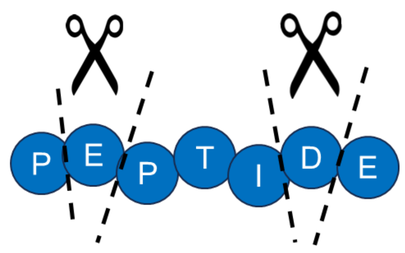
Possible antiviral peptide ?

IFP peptide: capable of interacting with MPER…

…but

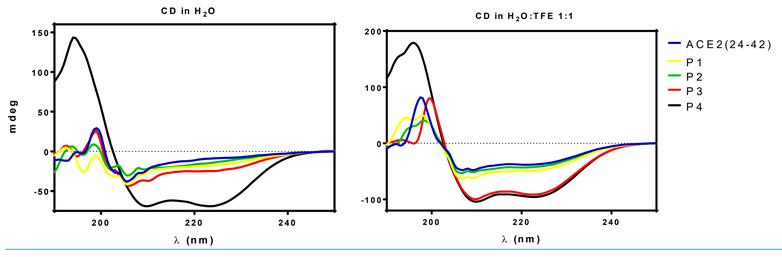
IFP has a promising antiviral activity against SARS-CoV-2 (IC_{50} = 2,80 ± 2,68 μM) but it is a 23mer peptide.

Shortening the IFP to find the minimal active sequence



Conformational analysis

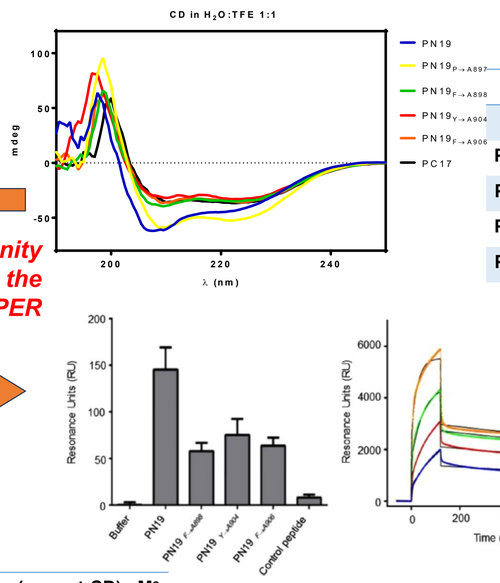
CD spectra of the peptides to evaluate their secondary structure. The experiment were performed both in neat H_2O and in H_2O:TFE 1:1 mixture. Simulation of percentage of secondary structure in H_2O:TFE (1:1) and in H_2O (in bracket) evaluated using online tool BestSel.



Peptide	α-helix	β-strand	β-turn	Random coil
ACE2(24-42)	63.3 (8.8)	23.6 (15.1)	13.1 (14.7)	0.0 (61.3)
P1	40.1 (5.4)	13.5 (22.9)	8.5 (16.9)	37.9 (54.7)
P2	37.3 (7.7)	15.7 (25.8)	10.2 (14.6)	36.8 (51.9)
P3	57.0 (34.6)	32.8 (31.7)	5.2 (25.1)	5.0 (8.6)
P4	96.2 (49.5)	0.0 (1.5)	3.8 (7.0)	0.0 (42.0)

Results

Peptides affinity constant with the sequence MPER by SPR



Ala-scan and conformational analysis

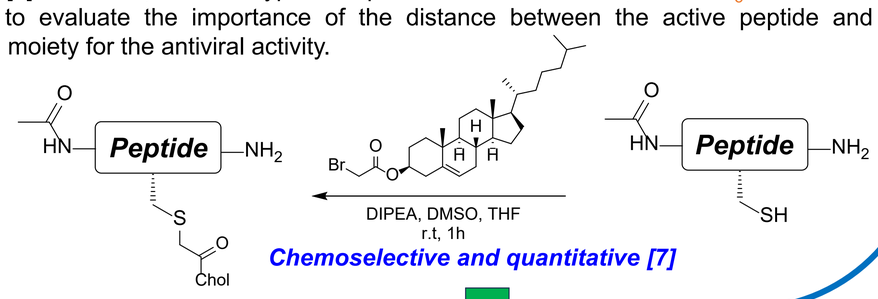
Ala-scan of the peptide PN19 to evaluate the importance of each residue for the antiviral activity and CD spectra to find a correlation between secondary structure and biological activity.

Peptide	Sequence	IC_{50} (mean ± SD) μM	α-helix	β-strand	β-turn	random
PN19	Ac-FGAGAALQIPFAMQMAYRF-NH_2	0.20±1.11	56,3	19,2	13,1	11,5
$PN19_{P→A897}$	Ac-FGAGAALQI*A*FAMQMAYRF-NH_2	0.24 ± 0.10	61,3	28,5	10,2	0
$PN19_{F→A898}$	Ac-FGAGAALQIP*A*AMQMAYRF-NH_2	57.64 ± 66.15	26,2	22,1	13,8	37,9
$PN19_{Y→A904}$	Ac-FGAGAALQIPFAMQMA*A*RF-NH_2	28.32 ± 47.96	50,7	5,4	12,3	31,6
$PN19_{F→A906}$	Ac-FGAGAALQIPFAMQMAYR*A*-NH_2	2,65 ± 4.45	47,4	26,0	26,6	0
PC17	Ac-SGWTFGAGAALQIPFAM-NH_2	97.94 ± 21.84	23,5	21,7	38,9	15,9

Surface Plasmon Resonance (SPR) experiments to measure the affinity constant of the peptides with the sequence MPER25c (CLIDLQELGKYEQYIKWPWYIWLGF). Only the peptide PN19 has a well establish interaction with MPER peptide, with a ***K_D= 9,44 nM***

Derivatization with Cholesterol

Synthesis of cholesteryl analogs of peptide PN19, to exploit the lipidic rafts present in the viral membrane.[6] Moreover, different types of spacers were introduced, i.e. PEG_5 and/or GSGSG pentamer, to evaluate the importance of the distance between the active peptide and the cholesterol moiety for the antiviral activity.



Chemoselective and quantitative [7]

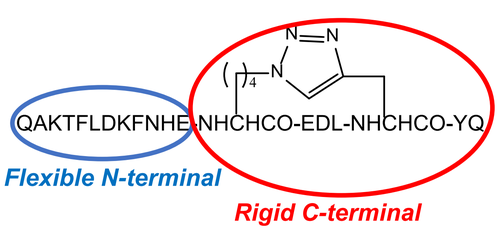
Antiviral activity evaluation

Peptide	Sequence	IC_{50} (mean ± SD) μM[a]
[C^1(chol)]PN19	Ac-C(chol)FGAGAALQIPFAMQMAYRF-NH_2	0.38 ± 0.11
[C^{20}(chol)]PN19	Ac-FGAGAALQIPFAMQMAYRFC(chol)-NH_2	0.08 ± 0.04
[C^1(chol)]PN19-spacer	Ac-C(chol)-GSGSG-FGAGAALQIPFAMQMAYRF-NH_2	0.98 ± 0.12
[C^{25}(chol)]PN19-spacer	Ac-FGAGAALQIPFAMQMAYRF-GSGSG-C(chol)-NH_2	0.74 ± 0.34
[C^1(chol)]PN19-PEG_5	Ac-C(chol)-*PEG_5*-FGAGAALQIPFAMQMAYRF-NH_2	3.70 ± 1.01
[C^{20}(chol)]PN19-PEG_5	Ac-FGAGAALQIPFAMQMAYRF-*PEG_5*-C(chol)-NH_2	0.14 ± 0.12
[C^1(chol)]PN19-spacer-PEG_5	Ac-C(chol)-*PEG_5*-GSGSG-FGAGAALQIPFAMQMAYRF-NH_2	1.75 ± 0.71
[C^{25}(chol)]PN19-spacer-PEG_5	Ac-FGAGAALQIPFAMQMAYRF-GSGSG-*PEG_5*-C(chol)-NH_2	0.04 ± 0.02

[a]IC_{50} values are measured by inhibitory viral plaque reduction assay (PRA) on Vero E6 cells.

Conclusions

The stabilizing effect increased by shifting the bridge position towards the C-terminal part or by increasing the number of stapled, but the too high rigid structure appears to hamper the antiviral activity.
In this scenario, the peptide **P3** represents a model peptide, with a flexible N-terminal and rigid C-terminal part. [8]



The studies on shortened sequences of the IFP peptide allows to find a shorter and more active peptide termed **PN19** with a nanomolar IC_{50}. Moreover, this screening point out the key role played by the aromatic residues in the sequence, as confirmed also by the Ala-scan.[9]

The addition of a cholesterol moiety in the N-terminal part of the peptide PN19 increases dramatically the antiviral activity, reaching nanomolar range of IC_{50}. On the other hand, the cholesterol moiety in the C-terminal is detrimental. Regarding the distance between cholesterol and the active peptide sequence, only the addition of the pentamer GSGSG alone seems to decrease the activity.

References

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