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Structural analysis of a peptide-based simplified model reproducing SARS-CoV-2 S RBD/ACE2 binding site

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Introduction

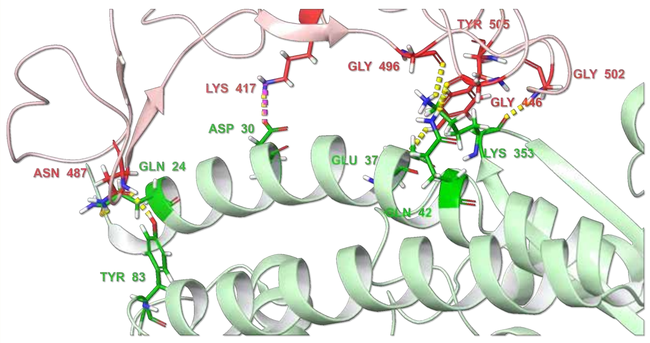
SARS-CoV-2, the pathogen causing the COVID-19 outbreak, is an RNA virus which infects lung cells through the binding of its surface glycoprotein spike (S) with the angiotensin-converting enzyme 2 (ACE2). The moiety of S involved in the interaction – namely receptor binding motif (RBM) – has been subjected to a remarkable number of point mutations throughout the rapid evolution of the virus. Therefore, the description of a model that individuates the interacting residues between S and ACE2 needs to be updated as soon as new variants of interest emerge. To this aim, the synthesis of peptide molecules reproducing short moieties involved in the interaction, rather than the production of the full protein construct, allowed the development of a simplified system that would afford to study the change in the SARS-CoV-2 S RBD/ACE2 binding related to the frequent mutations.[1-3]

In this work, we synthesized and studied the structure of short amino acid sequences, mimicking the two proteins' critical portions. Variations in the residues were easily managed through the one-point alteration of the sequences. Nuclear magnetic resonance (NMR) spectroscopy provides insights into ACE2 and SARS-CoV-2 S RBD deriving peptides' structures with their related variants (Alpha, Beta, and Gamma). Moreover, these sequences proved to be highly immunogenic, as many monoclonal antibodies target this moiety and give an individual susceptibility to the different variants. To study this effect, we used these peptides to functionalize the monomers of H-chain of human ferritin (hHFt), a protein able to assemble in an ordered 24-mer nanocage with high thermal and chemical stability in physiological conditions. The decoration of the outer surface of the nanocage with the S-derived epitopes can provide a tool able to elicit an immune answer in view of the development of novel vaccine strategies that can also be applied to other critical pathologies, for which there is no therapeutical strategy.

Results

Peptides

Analysis of the SARS-CoV-2 S/ACE2 complex



ACE2 $_{21}$I-Q$_{42}$ sequence

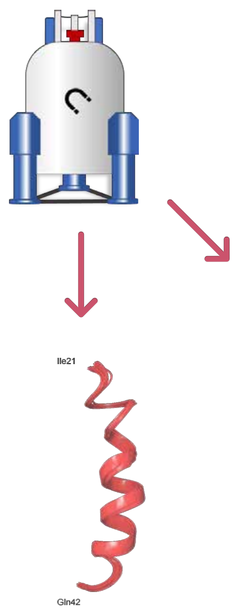
$_{21}$IEEQAKTFLDKFNHEAEDLFYQ$_{42}$

S RBD $_{482}$G-Q$_{506}$ sequences

Original (SMIMIC$_{OR}$)	$_{482}$GVEGFNCYFPLQSYGFQPTNGVGYQ$_{506}$
B.1.1.7 (SMIMIC$_{\alpha}$)	$_{482}$GVEGFNCYFPLQSYGFQPT**Y**GVGYQ$_{506}$
P.1/B.1.351 (SMIMIC$_{\beta\gamma}$)	$_{482}$GV**K**GFNCYFPLQSYGFQPT**Y**GVGYQ$_{506}$

Synthesis of the peptides and characterization by solution NMR

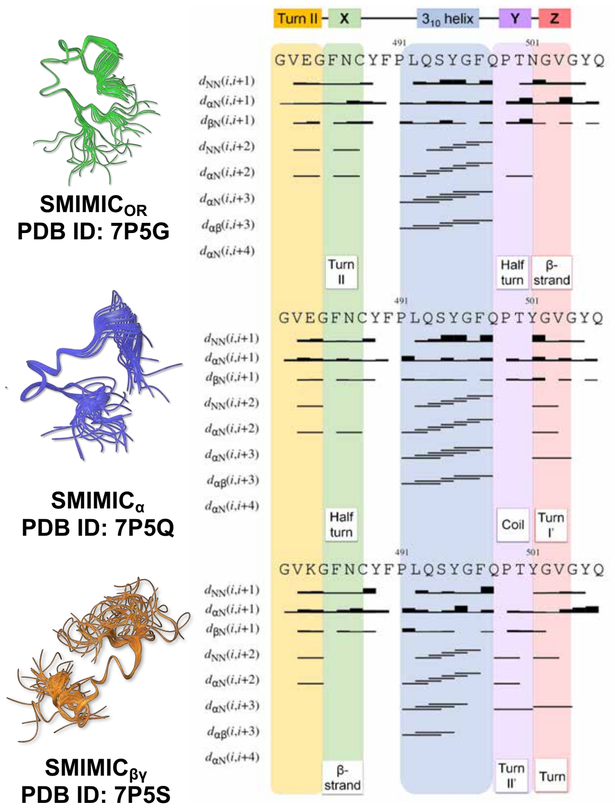
Solvent mixture: HFIP/water 50/50 v/v



ACE$_{21-42}$ PDB ID: 7P55

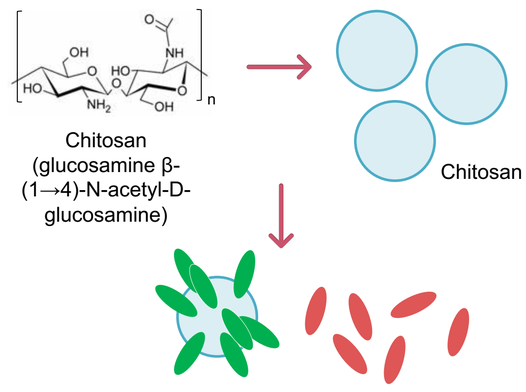
The peptide keeps the helix conformation, like in ACE2.

Effect of the mutations on the secondary structures



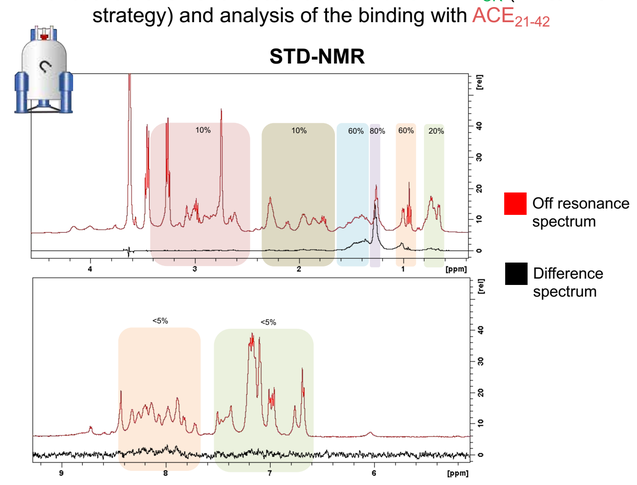
NOE connectivities bar plot shows a common pattern Turn II–X–3$_{10}$ helix–Y–Z for the three peptides, where the X, Y and Z conformations are affected by the mutated residues: in particular the mutation N501Y has a strong effect both on N and C terminus.

Analysis of the binding ACE$_{21-42}$/SMIMIC$_{OR}$



Functionalization of chitosan beads with SMIMIC$_{OR}$ (EDC/NHS strategy) and analysis of the binding with ACE$_{21-42}$

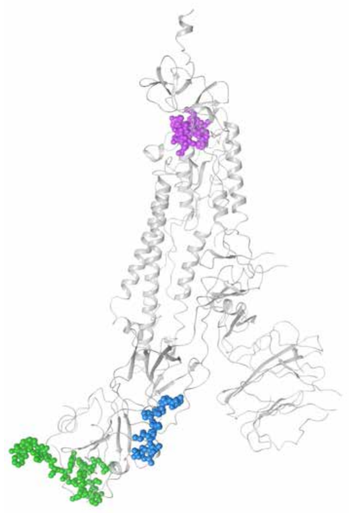
STD-NMR



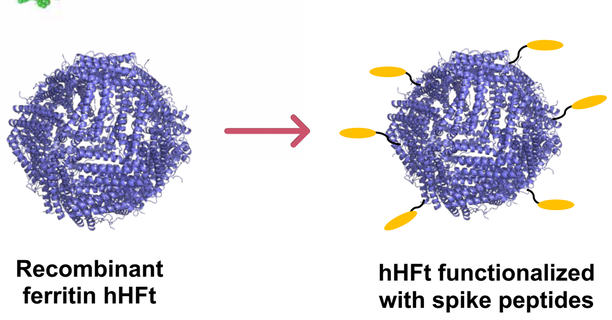
STD-NMR experiments on SMIMIC$_{OR}$ functionalized chitosan beads with ACE$_{21-42}$ (1:50 molar ratio) show a clear effect on the aliphatic protons of ACE$_{21-42}$, confirming the presence of binding driven by the peptide's side chains.

Fuctionalization of ferritin

Selection of the peptide sequences



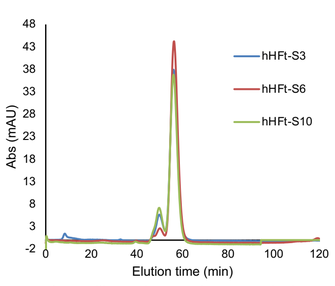
Name	Sequence	Note
S3	^{327}VRFPNITNL^{335}	Highly immunogenic and conserved epitope[4]
S6	$_{482}$GVEGFNSYFPLQ**S**YGFQPTNGVGYQ$_{506}$	SMIMIC$_{OR}$ with the mutation C494S to provide more stability
S7	$_{482}$GVEGFYSYFPLQ**S**YGFQPTYGVGYQ$_{506}$	SMIMIC$_{\alpha}$ with the mutation C494S to provide more stability
S8	$_{482}$GVKGFYSYFPLQ**S**YGFQPTYGVGYQ$_{506}$	SMIMIC$_{\beta\gamma}$ with the mutation C494S to provide more stability
S9	$_{482}$GVAGFNSYFPLR**S**YSFRPTYGVGHQ$_{506}$	482-506 residues from the Delta variant with the C494S mutation
S10	$_{896}$IPFAMQMAYRFNGIG$_{910}$	Highly immunogenic and conserved epitope[5]



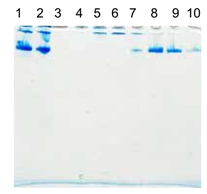
We engineered 10 plasmids of hHFt sequence including the S-deriving peptides (in the table above we reported only the most promising). The spike peptides were added at the **C-terminus** of hHFt and separated using a $(GGGGS)_3$ linker.[6-7]

Protein expression in E.coli and purification

The recombinant proteins were expressed following the previously described protocols[7], but the temperature of the thermal treatment was lowered from 75°C to 65°C due to their lower stability compared to the native hHFt.

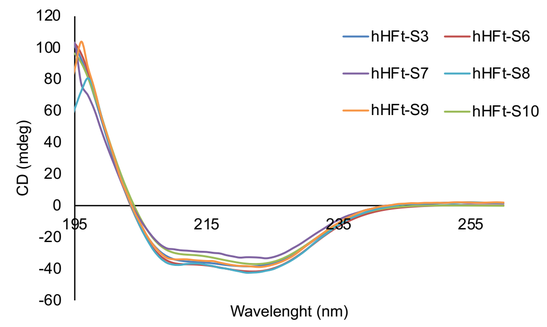


An illustrative size exclusion chromatography (SEC) run of hHFt-S3, S6 and S10 using a Superdex^{TM} S200 column, which manages to separate proteins from 10 to 600 kDa.



Native gel electrophoresis of hHFt-S10 after the SEC run: we loaded a native hHFt as standard (lane 1), the mixture loaded in the column (lane 2), and the product eluted from the column after 40 (lane 3), 48 (lane 4) 50 (5), 52 (6), 54 (7), 58 (8), 60 (9) and 62 (10) minutes.

CD

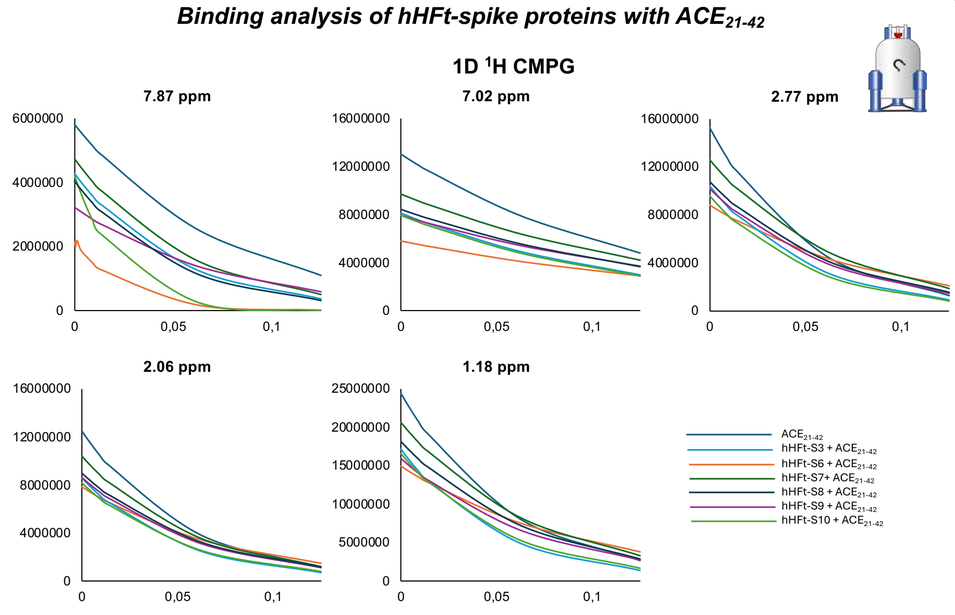


CD spectra of the modified ferritins in phosphate buffer at pH 7.4 suggest that the proteins tend to assume a helix structure, as also reported in a previous work of characterization of hHFt in native conditions[8].

Characterization of the products of gene expression by SEC, native PAGE, and CD spectroscopy reported that the hHFt proteins modified with the spike sequences behave similarly to the parent hHFt in native conditions, with the successful formation of the super-assembled nanocage.

Binding analysis of hHFt-spike proteins with ACE$_{21-42}$

1D ^{1}H CMPG



T2 (s)							
ppm	ACE$_{21-42}$	hHFt-S3 + ACE$_{21-42}$	hHFt-S6 + ACE$_{21-42}$	hHFt-S7 + ACE$_{21-42}$	hHFt-S8 + ACE$_{21-42}$	hHFt-S9 + ACE$_{21-42}$	hHFt-S10+ ACE$_{21-42}$
7.87	0.075±1.2e^{-3}	0.051±1.3e^{-3}	0.025±1.3e^{-3}	0.056±1.2e^{-3}	0.049±1.4e^{-3}	0.074±2.2e^{-3}	0.023±6.0e^{-4}
7.02	0.12±7.9e^{-4}	0.13±1.3e^{-4}	0.18±3.3e^{-4}	0.150±1.4e^{-3}	0.151±1.6e^{-3}	0.164±1.9e^{-3}	0.124±1.3e^{-3}
2.80	0.050±3.0e^{-4}	0.0517±4.8e^{-4}	0.088±8.5e^{-4}	0.066±4.6e^{-4}	0.065±5.4e^{-4}	0.065±5.5e^{-4}	0.051±4.8e^{-4}
2.06	0.053±3.4e^{-4}	0.0504±49e^{-4}	0.076±7.4e^{-4}	0.058±4.3e^{-4}	0.061±5.5e^{-4}	0.061±5.8e^{-4}	0.053±5.4e^{-4}
1.18	0.056±2.3e^{-4}	0.0502±3.2e^{-4}	0.61±6.1e^{-4}	0.068±3.5e^{-4}	0.068±4.3e^{-4}	0.071±4.6e^{-4}	0.055±3.5e^{-4}
Prot.	-	6.8e^{-3}±1.9e^{-4}	0.026±7.7e^{-4}	0.015±4.8e^{-4}	0.053±2.0e^{-3}	0.132±5.0e^{-3}	0.014±8.8e^{-4}

1D ^{1}H CPMG acquired with different delays from 0 to 0.125 ms of hHFt-spike proteins in the presence of ACE$_{21-42}$ peptide showed that respect to the reference value of T_2 measured on the free peptide (dark blue line in the plots, first column in the table), the T_2 of ACE$_{21-42}$ decreases in the presence of the proteins, resulting in a faster relaxation that indicates an interaction of the peptide with the protein.

Conclusions. In this poster, we report a methodology for studying large binding complexes using short peptide sequences. The starting model was the SARS-CoV-2 S RBD/ACE2 complex. We extracted two sets of peptide sequences mimicking the binding domains of the two proteins (including the mutations of the variants of concern in SARS-CoV-2 S) and studied the peptides' conformation in solutions. Moreover, we functionalized chitosan beads with SMIMICS$_{OR}$ and tested their binding potential with ACE$_{21-42}$ using STD-NMR, reporting a sizeable binding. With the aim of using these S-deriving sequences in a pharmacological active tool, we functionalized the human H chain of the ferritin (hHFt) with the spike peptides, which is a very interesting molecule for the drug delivery and for the development of immunoreactive devices. We successfully produced six modified hHFt sequences and tested their binding with ACE$_{21-42}$ using 1D CPMG. These preliminary results will be used as the basis for a thorough study of the potential of these macromolecules in novel vaccine development strategies.

References: [1]Grubaugh, et al. Cell 184 (2021) 1127–1132.; [2]Sanyaolu, et al. Therapeut. Adv. Infect. Dis. 8 (2021), 20499361211024372; [3]Buonocore, et al., Heliyon 8.11 (2022); [4]Bagherzadeh, et al. Scientific reports 12.1 (2022): 14017; [5]Tarke, et al. Cell 185.5 (2022): 847-859; [6]Ma, et al. " ACS Applied Bio Materials 4.3 (2021): 2654-2663; [7]Rodrigues, et al. " Pharmaceutics 13.10 (2021): 1621. [7]Lucignano, et al. International Journal of Biological Macromolecules (2024): 134373. [8]Lucignano, et al. Frontiers in Molecular Biosciences 10 (2023): 1008985.

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