

## Design and Study of Novel Peptide Inhibitors against the SARS-Coronavirus Spike Protein

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### Introduction

Severe Acute Respiratory Syndrome (SARS) is an acute respiratory illness caused by infection with a novel coronavirus (SARS-CoV). Infection by SARS coronaviruses requires fusion of the viral and cellular membranes, which is mediated by the viral envelope Spike (S) glycoprotein and receptors on the target cell. The S protein contains two hydrophobic repeat regions, denoted HRN and HRC, which oligomerize the S glycoprotein into a trimer in the native state, and when activated collapse into a six-helix bundle structure driving fusion of the host and viral membranes. We and others have previously reported that the HR regions of SARS-CoV S protein can associate to form a very stable helical six-stranded structure and residues 902–950 in HRN and 1151–1185 in HRC were identified to be crucial for their interaction [1-4].

Due to the severity and mortality (10%) witnessed in the fall of 2003 during the spread of the SARS-CoV pandemic, and the current lack of effective agents for the antiviral therapy of SARS-CoV infection, it has become imperative to learn as much as possible about this virus and the ability to prevent future infection. As successfully used in fusion inhibitor design for HIV [5], peptides derived from HRC can bind to the transiently exposed HRN coiled-coil trimer and block the formation of the six-helical bundle (Fig. 1), which ultimately leads to a loss of membrane-fusion activity. In this study, HRN (902-950) peptide of SARS-CoV S protein was chosen as the target for testing the interaction of HRC analogs.

The 36-residue HRC peptide (1150-1185) was chosen as the region to design a series of HRC analogs, in order to increase their stability and binding affinity with HRN. These substitutions /modifications involved: (1) increasing helical propensity (HRC2 and HRC4); (2) increasing hydrophobicity in the hydrophobic core (HRC1 and HRC3); and (3) introducing a lactam bridge (HRC5). The sequences of these peptides are shown in Figure 2.

### Results and Discussion

To examine the helical structure changes of the HRC analogs, we analyzed each peptide by circular dichroism spectroscopy (CD). Under physiological conditions, these analogs are well folded, soluble, have higher  $\alpha$ -helical content and are more stable than native HRC as expected (in Fig. 3A and Table 1).

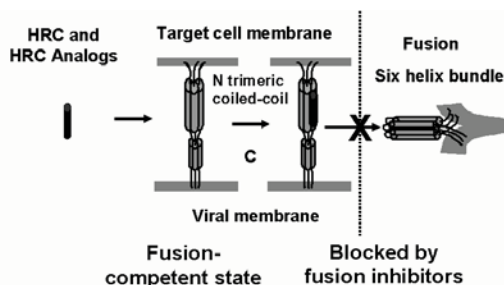


Fig. 1. Schematic model illustrating the action of SARS-CoV fusion inhibitors that target HRN.



native PAGE. The results are shown in Figure 4 and Table 1. HRC or HRC analogs alone showed a band in the lower part of the gel. With the exception of HRC4, the mixture of HRC analogs and HRN showed two bands: the lower one had the same position as isolated HRC analog, and the upper band is higher order oligomeric complex formed by HRN and HRC analog. HRN alone showed no band because it carries a net positive charge under the native electrophoresis conditions, and consequently does not enter the gel.

Table 1. Summary of HRC analogs and interaction with HRN

Peptide Name	[ $\theta$ ] <sub>222</sub> <sup>a</sup>		% $\alpha$ helix <sup>b</sup>		T <sub>m</sub> <sup>c</sup> (°C)	Complex with HRN		Complex formation tested by Native PAGE
	Benign	50%TFE	Benign	50%TFE		T <sub>m</sub> (°C)	$\Delta$ T <sub>m</sub> (°C)	
HRC	-21370	-29720	61	85	37	85	0	+
HRC 1	-32060	-31510	92	90	57	74	-11	+
HRC 2	-27060	-29740	77	85	48	86	+1	+
HRC 3	-30900	-31450	88	90	66	75	-10	+
HRC 4	-28560	-30990	82	89	74	59	-26	-
HRC 5	-27770	-32320	80	92	41	88	+3	+

a. [ $\theta$ ]<sub>222</sub> is the mean residue molar ellipticity (deg cm<sup>2</sup> dmol<sup>-1</sup>) measured at 222 nm in a 100 mM PBS, pH 7.0, in the absence (benign) or presence of 50% TFE (v/v). Concentration of peptides was 70  $\mu$ M. b. %  $\alpha$  helix was calculated from [ $\theta$ ]<sub>222</sub> based on an ellipticity value for 100%  $\alpha$ -helical content derived from the equation,  $XH_n = XH_\infty (1 - k / n)$ , where  $XH_\infty$  is -37,400, the wavelength dependent constant,  $k$ , is 2.5, and  $n$  is the number of residues in the helix. c. T<sub>m</sub> is the temperature at which there is a 50% decrease in fraction folded compared to the fully folded coiled-coil as determined by CD at 5 °C.

## Conclusions

1. The substitutions/modifications have increased the HRC  $\alpha$ -helical structure and stability. As shown in Table 1, the results from CD and native PAGE suggested HRC2 and HRC5 have increased binding affinity with HRN.
2. The two analogs, HRC2 and HRC5, will now be tested as peptide fusion inhibitors in an antiviral activity assay.

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## References

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