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Computationally-designed miniproteins showing neutralization activity against SARS-CoV-2

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As the COVID-19 pandemic demonstrated, the need for robust antiviral therapies against SARS-CoV-2 remains substantial. A promising strategy to meet this demand is through the development of proteins tailored for enhanced binding affinity to crucial viral targets, such as epitopes located on their fusion proteins. Among these targets is the Spike protein's receptor binding domain (RBD), which interacts with the human receptor ACE2 protein, initiating the viral entry process. Targeting the RBD epitope that binds to ACE2 is a promising strategy to fight COVID-19 and is used here as a model target to validate our approach.

In this work, we implemented a computational pipeline leveraging artificial intelligence-based computational design methodologies, RFDiffusion and ProteinMPNN, to de novo design miniproteins targeting the RBD epitope that interacts with ACE2. The most promising designs were selected based on a combination of relevant criteria, including metrics derived from the protein structure prediction tool AlphaFold2 and properties like surface hydrophobicity and shape complementarity to the target.

We expressed in vitro selected designs and evaluated their binding affinity to the RBD using Bio-layer Interferometry and Yeast Display assays. Proteins demonstrating favourable binding affinity to the RBD were subsequently subjected to neutralization assays, evaluating the proteins' ability to inhibit SARS-CoV-2 infection.

We observed that two of the selected designs can bind to the RBD and neutralize viral infection, thus successfully demonstrating that this computational framework is able to design proteins that are tailor-made to interact with specific epitopes. This paves the way for the next round of design, where the most promising candidates are being optimized using strategies that consider the oligomerization tendency observed in some of the designs tested.