Peptide Scaffold Antidotes and Vaccines for SARS-CoV-2 and Biodefense

ANDRE WATSON
CEO, LIGANDAL INC.
APRIL 16, 2020

Ligandal has designed a prospective antidote and vaccine for SARS-CoV-2 (the novel coronavirus) to be formulated and developed in response to the worldwide pandemic. This approach utilizes peptide scaffolds (think of them as nanorobots) that are designed with supercomputing and AI to mimic the critical immune binding and responsive elements of the virus, in addition to being designed to serve as rapid-response antidotes to an ongoing infection. In essence, this approach is expected to aid in eliminating the virus from an already-infected host, while bolstering the immune response.

In other words, the approach creates vaccines and antidotes against SARS-CoV-2, whereby the damage of SARS-CoV-2 / COVID-19 is offset, and whereby the viral immune cloaking mechanisms of the virus are eliminated, while the peptides simultaneously display immuno-epitopes in order to instill an ultra-specific immune response, in comparison to existing vaccine and therapeutic options which may not adequately form an immune response, and frequently treat symptomatic conditions without addressing the root cause of viral propagation and infection.

In the present instance, we mimic the viral binding elements of SARS-CoV-2 (“COVID”) to competitively displace ACE2 from the spike protein receptor binding domain (RBD), in order for the virus to no longer display what we hypothesize to be an ACE2 “cloak” from the immune system, while also offsetting the need for alternative therapeutic solutions, because the peptide scaffolds will expose the virus for the host’s own immune system to learn and deal with while also presenting key B cell and T cell epitopes for bolstering immune response.

This approach is fully synthetic, which lends itself to rapid prototyping, personalized approaches for various viruses, and scale-up on the order of days or weeks instead of years. We have successfully completed peptide synthesis and are now engaging in a series of studies to determine binding affinity for ACE2 and CD147, in addition to immune recognition of the “peptide scaffolds” by antibodies from recovered COVID-19 patient blood.

This is a potential breakthrough medical moment. A new approach to curing the cause, versus treating the symptoms, has been made possible by the tragic coronavirus pandemic. COVID has made possible for a new way of thinking about medicine and healthcare, through creating atomically-precise, genetically tailored, peptide and drug/gene delivery nanosystems for broad therapeutic, vaccine, and preventative medical purposes.

This, coupled with the virus’ other mechanisms for burying antibody immuno-epitope sites, poses a significant problem in eliminating the virus from the body. These peptide scaffolds are designed to be ultra-high-affinity, fully synthetic constructs that can serve as competitive displacers to the viral entry mechanisms, as well as preventing ACE2 from forming its cloak.
around the virus. Furthermore, the scaffolds are designed to act as immuno-boosters, presenting precise antibody immuno-epitopes as well as T cell receptor (TCR) immuno-epitopes against the key viral immunogenic sites.

PLEASE SEE THE FOLLOWING VIDEO OF THE CONTENTS OF THIS PAPER:
https://drive.google.com/file/d/1x1tEi-TGphy5XH1rb3toAF6PnOpSkHIS/
To begin, please see Ligandal’s prior work on **Rapid Simulation and Design of Synthetic Peptide Mini-Scaffold Vaccines for Novel Coronavirus (SARS-CoV-2) and Pandemic Preparedness** for rationale, and a [video](#) explaining our strategy for aligning a SWISS-MODEL structure of SARS-CoV-2 (the novel coronavirus)\(^{\text{i}}\) to its binding site with Gibbs free energy plots of key interacting residues,\(^{\text{ii}}\) as well as comparing to the Veesler Lab cryo-EM structures\(^{\text{iii}}\) of the viral spike protein in open and closed conformations. We worked with Dr. Jinbo Xu to simulate our proprietary sequences in their final, folded conformations.\(^{\text{iv}}\)
On February 11, we correctly predicted the binding domain of SARS-CoV-2 to the ACE2 receptor. Shown is an overlap of our structural modeling of ACE2 bind to a SWISS-MODEL simulated spike protein (red and blue/multicolored, respectively), in comparison to PDBID 6M0J showing a crystal structure via X-ray crystallography of the SARS-CoV-2 receptor binding domain (RBD) bound to ACE2 (both yellow).

Predicted alignment of SWISS-MODEL SARS-CoV-2 Spike RBD to ACE2.
Actual alignment of SARS-CoV-2 RBD bound to ACE2 (PDB ID 6M0J).
Overlay of alignments of the simulated vs. X-ray crystallographic structures.

Whole spike (SWISS-MODEL) overlaid with the simulated and X-ray crystallographic binding clefts.
Shown are SEQ12 peptide scaffolds built upon a C60 buckminsterfullerene. This particular sequence is designed to mimic the binding cleft of the virus to ACE2, whereby it is anticipated to competitively displace the virus from ACE2 receptors and soluble ACE2. The fullerene substrate acts to multivalently display a tetrad or greater amount of each peptide.
The vaccine/antidote “scaffolds” are designed to prevent the viral spike protein (green) from binding to ACE2 (red).
Shown are folded states 0, 4 and 9 of SEQ10, SEQ11 and SEQ12, whereby the scaffolds is inhibiting the binding cleft of ACE2 (red).
PEPTIDE SCAFFOLDS ARE DESIGNED TO INHIBIT THE VIRUS FROM BINDING TO ACE2, WHILE EXPOSING ITS IMMUNE RESPONSIVE SITES FOR ANTIBODY AND TCR GENERATION.

Top: Tetravalent SEQ11 bound to a buckminsterfullerene substrate, in the bound conformation to ACE2.
Bottom: Tetravalent SEQ12 bound to buckminsterfullerene substrate, in the bound conformation to ACE2.
Buckminsterfullerene (C60) with peptide scaffolds bound to soluble ACE2 receptor.
Peptide scaffold SEQ13 (purple) bound to viral spike protein at its ACE2-binding site, preventing binding to ACE2, while allowing for presentation of antibody immuno-epitopes on the viral protein surface. Shown also is the SARS-CoV-2 spike protein (green) with T cell immuno-epitopes (pink).
Ligand peptide scaffolds assembled upon buckminsterfullerene, with ACE2 shown in red. The peptide scaffolds can act as rapid antidotes to the SARS-CoV-2 coronavirus, preventing the virus from binding to and entering cells, as well as exposing the sophisticated immune cloaking mechanisms, whereby the virus gets coated in soluble ACE2, in order to displace soluble ACE2 from the virus and allow for natural immune recognition of the viral surface.
An individual peptide scaffold bound to ACE2 can serve as a competitive inhibitor to viral entry.

Predicted thermodynamics of binding and dissociation constant of SEQ12_center0 with ACE2:

<table>
<thead>
<tr>
<th>Protein-protein complex</th>
<th>ΔG (kcal mol⁻¹)</th>
<th>K_d (M) at 37.0 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE2 with SEQ12_center0</td>
<td>-16.1</td>
<td>4.7E-12</td>
</tr>
</tbody>
</table>
Wildtype SARS-CoV-2 (coronavirus) SWISS-MODEL truncated structure, bound to ACE2 receptor based on alignment to SARS-CoV-1; binding residues are colored green, repulsive residues are colored orange, neutral residues are colored yellow.\textsuperscript{vi,vii}
Additionally, we report a finding that antibodies found against fragments of the virus, such as those against the WHOLE-SPIKE or spike fragment (reference PDB ID 6W41 for a human antibody against a spike RBD https://www.rcsb.org/structure/6W41) are actually not neutralizing antibodies against the fully-formed virus… See the whole spike overlapping with the antibody; therefore, the wrong immune responses may actually be detrimental to vaccine development.
Chaotic binding simulations of SEQ10 bound to ACE2 in 9 possible states via an electron cloud Heisenberg Uncertainty approach.
Chaotic binding simulations of SEQ11 bound to ACE2 in 9 possible states via an electron cloud Heisenberg Uncertainty approach.
Chaotic binding simulations of SEQ12 bound to ACE2 in 9 possible states via an electron cloud Heisenberg Uncertainty approach.
Veesler Lab Cryo-EM atomic resolution structure of SARS-CoV-2 in open conformation with simulated binding cleft based on prior alignment of SWISS-MODEL structure.
One folded state of SEQ12 with ACE2.
Two folded states (center0 and center9) of SEQ12 with ACE2.

Chaotic assortment of 9 folded states (center0 – center9) with ACE2, probably showing reasonable average folding and locations of all possible folded states given Heisenberg Uncertainty Principle.
SWISS-MODEL spike protein aligned to SARS-CoV-1 binding site, with binding residues colored green.
ACE2 bound to synthetic peptide antidote.
ACKNOWLEDGEMENTS:

We thank Dr. Jinbo Xu for granting access to RaptorX,4 and simulation of scaffolds based on Ligandal’s sequences.

CONFLICTS OF INTEREST:
Andre Watson is a board member and shareholder in Ligandal, Inc.

i SWISS-MODEL:

ii PDBePISA:


iv RAPTORX:

v PRODIGY: