Protein Antivirals by Rapid Redesign of Tertiary Structures (PARROTS)

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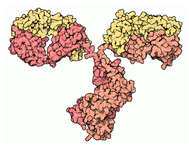
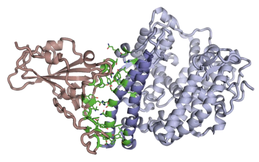
Background

One million deaths to date from COVID-19 highlight the urgent need for a method to rapidly develop mutation-resistant antiviral drugs.





Antiviral therapeutics

Monoclonal antibodies bind to the spike protein and prevent virus from entering cells
- Established manufacturing pipelines
- Not resistant to viral mutation

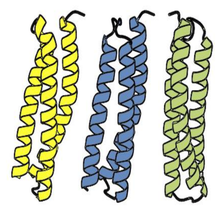
Receptor traps outcompete the receptor-antigen binding interaction to neutralize the infection
- Resistant to viral mutation
- Too large (>200 KDa)
- Development requires structural information

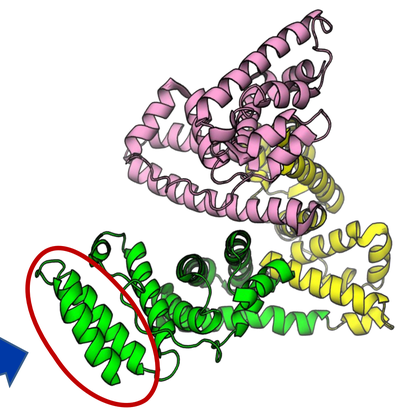
There is a need for a method to rapidly build antiviral therapeutics that overcomes the limitations of monoclonal antibodies and receptor traps.

Objective

We propose a new, rapid, and generalizable pipeline for engineering small (<66 KDa) and easy-to-produce alternatives to established antiviral protein therapeutics.

PARROTS (Protein Antivirals by Rapid Redesign of Tertiary Structures) replaces native human serum albumin (HSA) helical bundles with computationally-designed helical bundles that are designed to tightly bind to viral antigens: **HSA-traps**.

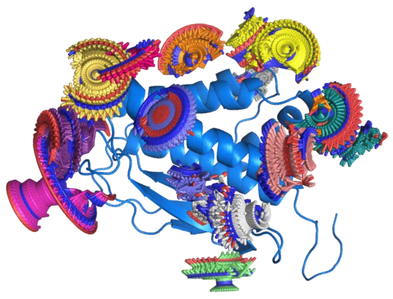




The alpha helices in HSA that are replaced with the designed helical bundle are circled in red.

Potential HSA-trap benefits
- High solubility (50 mg/mL, blood)
- 3-week serum half-life
- Modular helical architecture
- Prior safe use in drug delivery

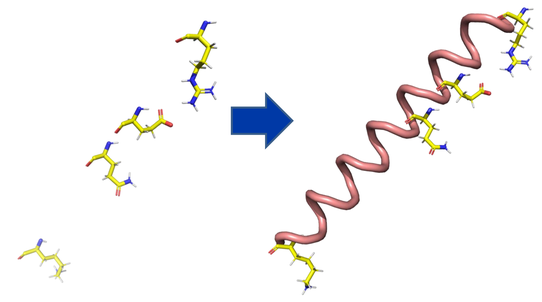
1



Potential idealized hydrogen bonds that can be formed with a viral antigen. The monkeypox antigen is shown here.

We generate antigen-binding helices that include as many **ideal hydrogen bonds** as possible with the antigen.

Sequence logo compiling designed binding helices for the monkeypox viral antigen.



Example binding helix generated by inpainting.

We apply **inpainting**, a recent method built on generative adversial neural networks [1], to identify the designed binding helices with best closure geometry and antigen interactions.

We then redesign and optimize binding helix sidechains using **Protein MPNN**, a protein design method using machine learning [2].

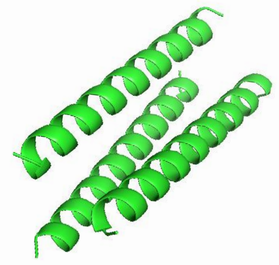
Binding Helix → Helical Bundle → Loops → Optimize Interface

2

The **MakeBundle Rosetta mover**, based around Crick helix parameters which define helical backbone geometries [3], was used to generate variant polyalanine helical bundles into which the binding helices were inserted.

Altered parameters

Omega0	Major helix twist per residue	0, 0.5, 1, 1.5 radians
Radius	Major helix residue	5.5, 6, 6.5, 7, 7.5, 8, 8.5 angstroms
Length	Number of residues	31, 32, 33, 34 residues



Helical bundle generated by the MakeBundle mover

The binding helices were inserted into the new helical bundles using a custom alignment algorithm that we developed.

Move binding helix to one of the bundle helices → Rotate binding helix so the binding site faces outward → Insert binding helix into the helical bundle

The best helical bundles were selected by docking the viral antigen and scoring the complex before loop design.

3

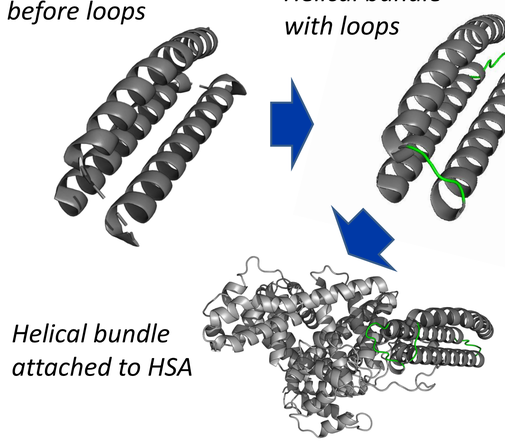
We used the **Rosetta Remodel mover** [4] to apply cyclic coordinate descent and kinematic closure to design loops within the helical bundles and connect the HSA scaffold to the helical bundles, for three total new loops.

The number of residues in the loops was determined by the distance between helices (one residue per 3.25 Å). For each helical bundle, after generating the first loop, the best structures were selected by total score, and the next loop was designed. The final systems made were scored to find the best ones for the viral antigen.

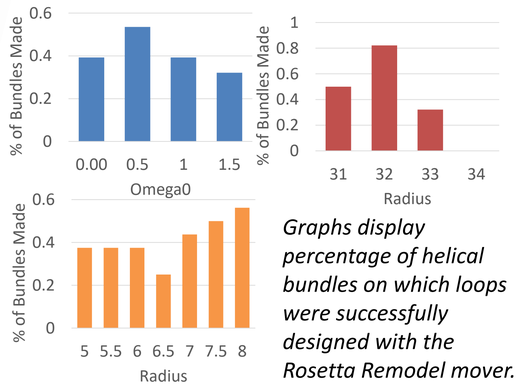
Helical bundle before loops

Helical bundle with loops

Helical bundle attached to HSA



Percentage of Bundles with Successful Loops



Graphs display percentage of helical bundles on which loops were successfully designed with the Rosetta Remodel mover.

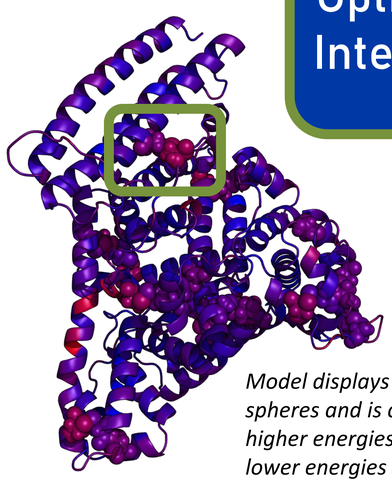
4

The interface formed by HSA and the bundle was **designed** with Protein MPNN [2], **minimized**, and **repacked** to improve interactions.

To increase the stability of the HSA-trap, a we introduced a disulfide bond between HSA and the helical bundle with Rosetta's **DisulfideMover [4]**.

The new disulfide bond created with the mover, boxed in green, had similar energies as existing disulfide bonds in the system *(right)*.

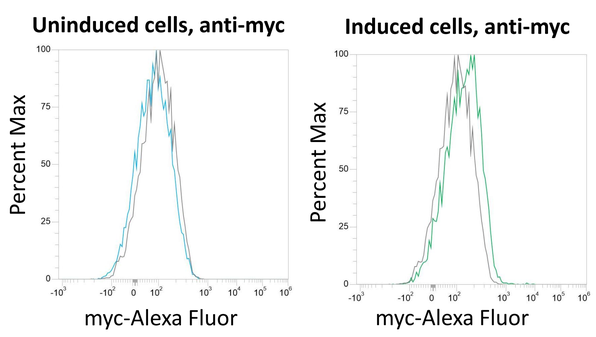
After these computational steps, 100-1000 HSA-traps will be chosen for testing by binding experiments on the surface of yeast and *in vitro*. We will affinity mature the top binders to further improve the binding affinity with the viral antigen.



Model displays disulfides as spheres and is colored with higher energies in red and lower energies in blue

Experimental Analysis

Flow cytometry analysis of yeast-displayed HSA

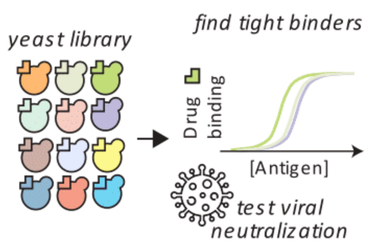


Wild-type HSA yeast surface display. Galactose is required to express and surface display the HSA, and the HSA is disulfide bonded to the yeast surface via the native yeast protein Aga2.

Flow cytometry analysis was performed on wild-type HSA on the **surface of yeast** to establish a positive control for screening future designed HSA-trap libraries. The colored lines show samples that were stained by fluorescently labeled antibodies (**green** for anti-myc-AlexaFluor 488, **yellow** for anti-HA-R-PE). As negative controls, the **blue** lines show uninduced samples and the **gray** lines show unstained samples. Experiments using the HA tag best highlight the expression of full-length HSA. Also, the negative controls highlight there is minimum background noise.

Future Applications

The PARROTS pipeline will output hundreds of HSA-trap candidates. We will perform:
- Pooled yeast surface display to study HSA trap-antigen binding
- Further computational refinement of the HSA-trap interface with viral antigen
- Binding affinity measurements of HSA-traps *in vitro* with surface plasmon resonance
- Pseudovirus neutralization assays to determine HSA-trap efficacy



The antivirals market is projected to increase to $60 billion by 2028. HSA-traps developed using PARROTS stand to become long-lasting, easy-to-develop, and scalable antiviral therapeutics. Beyond viral antigens, **HSA can be applied to several challenging targets including multi-pass membrane proteins and inaccessible solid tumors.**

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2. Dauparas, J., Anishchenko, I., Bennett, N., Bai, H., Ragotte, R. J., Milles, L. F., ... Baker, D. (2022). Robust deep learning based protein sequence design using ProteinMPNN. *bioRxiv*. doi:10.1101/2022.06.03.494563
3. Leman, J. K., Weitzner, B. D., Lewis, S. M., Adolf-Bryfogle, J., Alam, N., Alford, R. F., ... Bender, B. J., Blacklock, K., Bonet, J., Boyken, S. E., Bradley, P., Bystroff, C., Conway, P., Cooper, S., Correia, B. E., ... Bonneau, R. (2020). Macromolecular modeling and design in Rosetta: recent methods and frameworks. *Nature methods*, *17*(7), 665–680. https://doi.org/10.1038/s41592-020-0848-2
4. Huang, P. S., Ban, Y. E., Richter, F., Andre, I., Vernon, R., Schief, W. R., & Baker, D. (2011). RosettaRemodel: a generalized framework for flexible backbone protein design. *PloS one*, *6*(8), e24109. https://doi.org/10.1371/journal.pone.0024109