



# Engineering Covalently-Reactive Anti-SARS-CoV-2 Spike Nanobodies

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## Objective

We aimed to develop a covalently-reactive nanobody for the SARS-CoV-2 spike. These mutated, covalent nanobodies will act as long-term inhibitors for SARS-CoV-2 viruses by preventing its binding action.

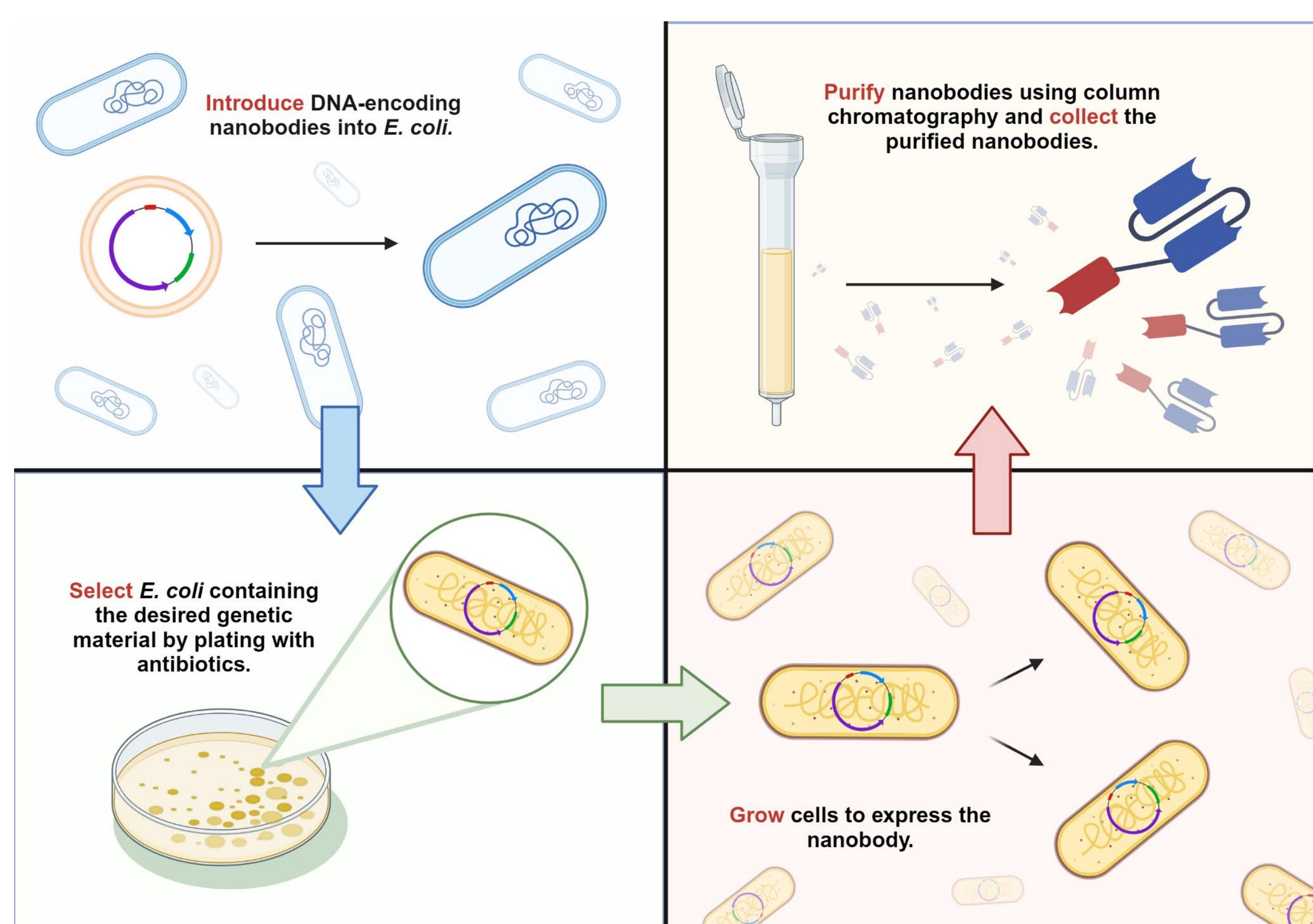
We aimed to express these nanobodies through recombinant protein expression in *E. coli* cell lines and purify them using His-tag column chromatography.

## Background

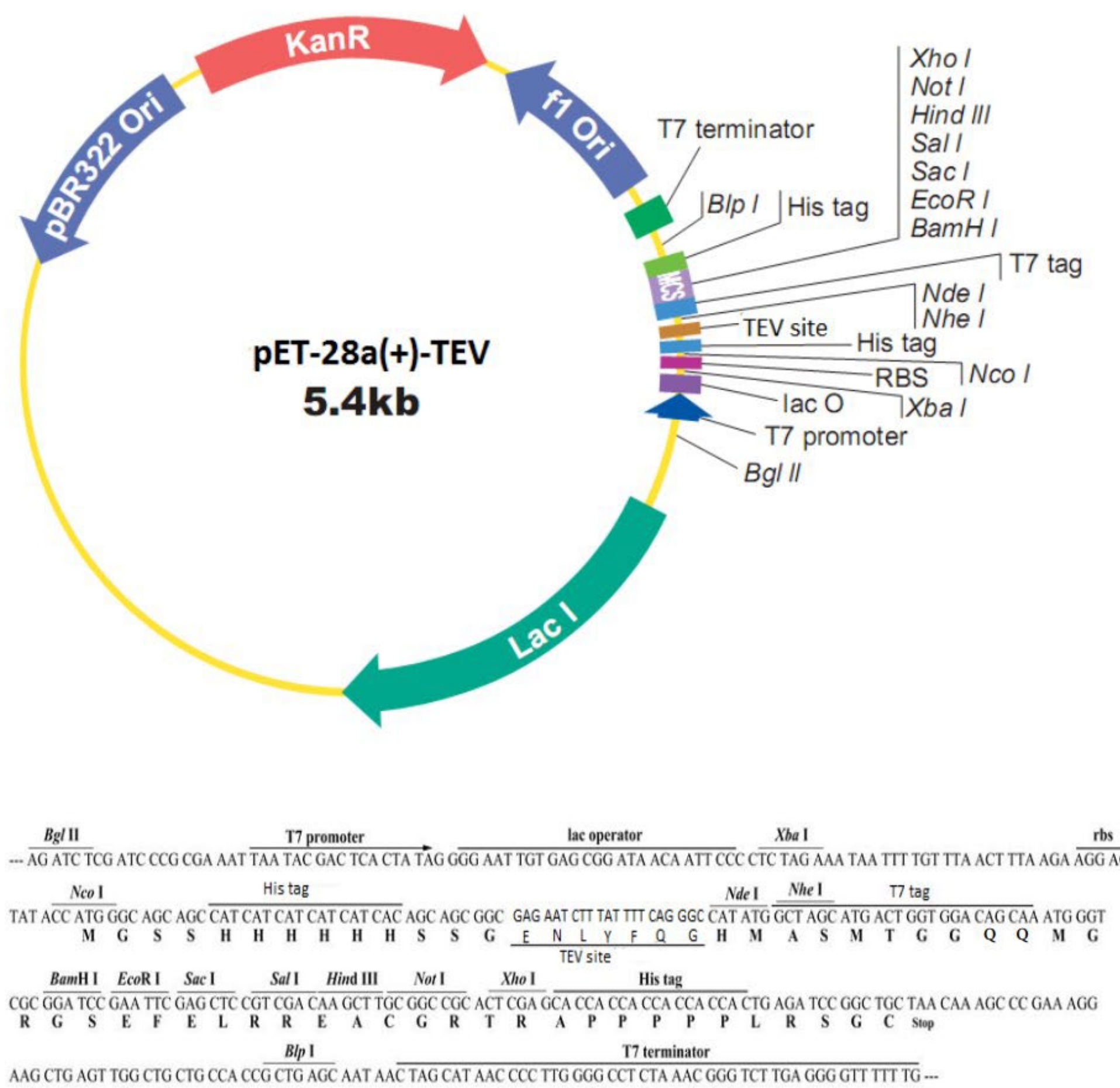
SARS-CoV-2 reproduces by exploiting host cell mechanisms, utilizing spike proteins that transition into an active conformation upon proximity to ACE-2 receptors on epithelial cells.

Our team explores engineered covalently-reactive nanobodies, modified with unnatural amino acids that bind irreversibly to the spike RBD of SARS-CoV-2. The covalent binding prevents the virus's interactions with the ACE-2 receptors on epithelial cells, which offers prolonged protection as opposed to small molecule drugs.

## Methods



**Figure 1.** Diagram depicting the main workflow of recombinant protein expression in *E. coli*.



### Wild-Type Sequence

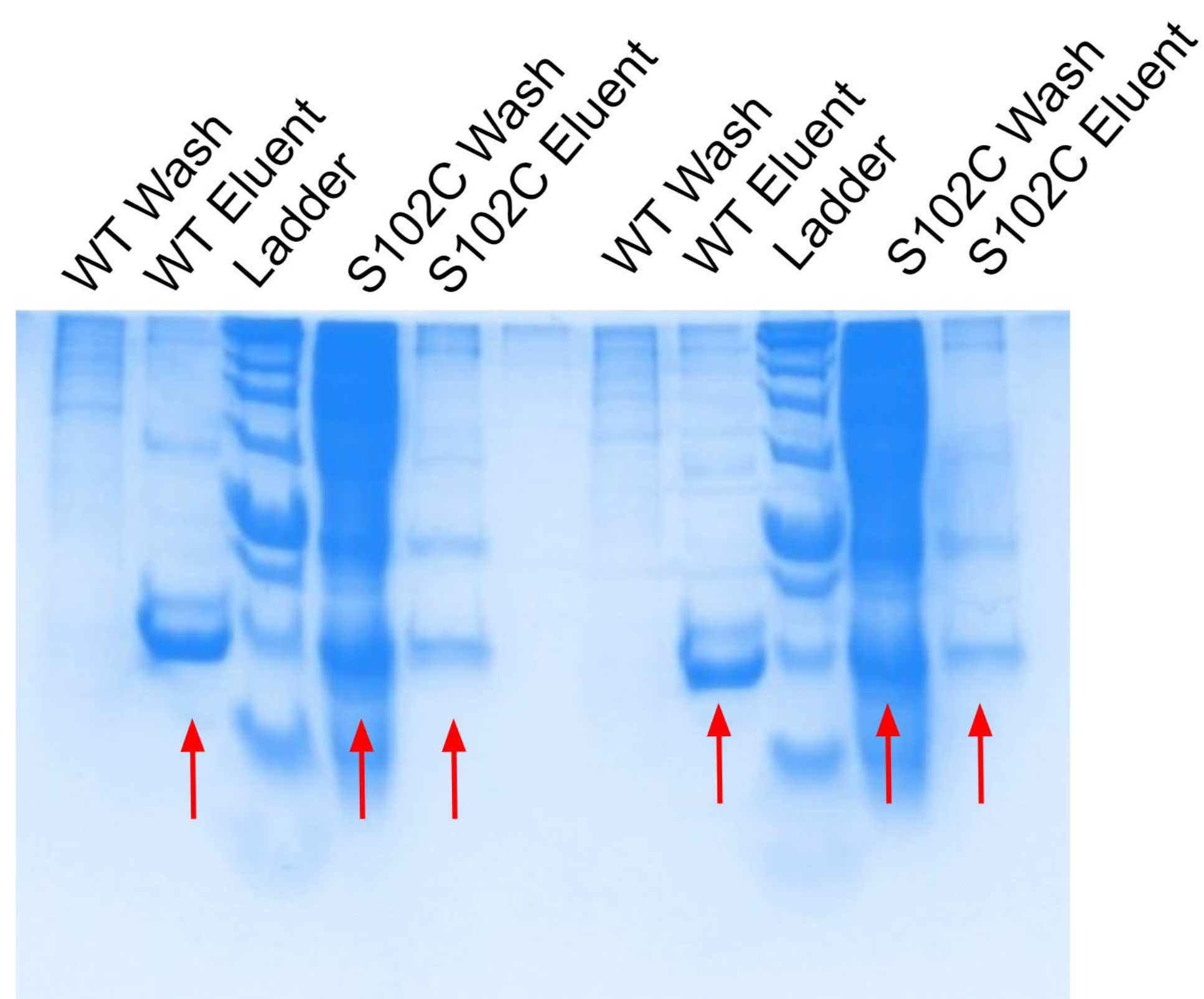
MQVQLVESGGGLVQAGGSLRLS**CAAS**GYIFGRNAMGWYRQAPGKERELVA  
GITRRG**S**ITYYAD**S**VKGRFTISRDNAKNTVYLQMNSLKPEDTAVYY**CAADPA**  
**S**PAYGDYWGQGTQVTV**SS**

### Mutated Sequence

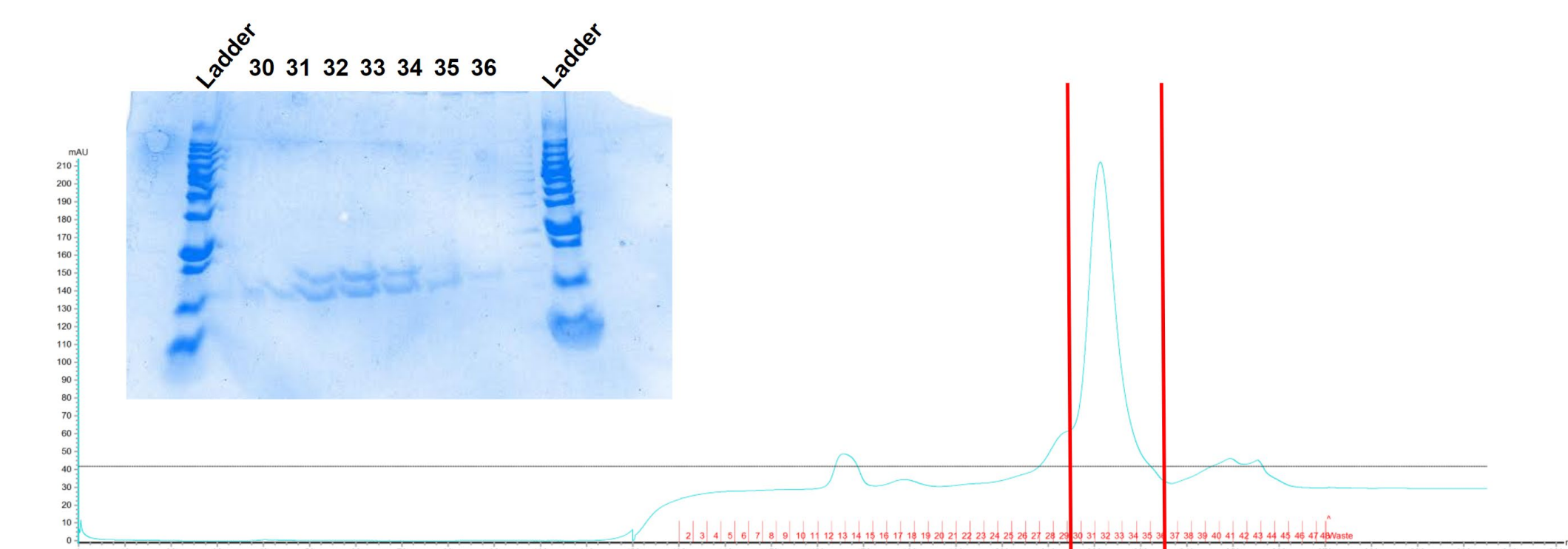
MQVQLVESGGGLVQAGGSLRLS**CAAS**GYIFGRNAMGWYRQAPGKERELVA  
GITRRG**S**ITYYAD**S**VKGRFTISRDNAKNTVYLQMNSLKPEDTAVYY**CAADPA**  
**C**PAYGDYWGQGTQVTV**SS**

**Figure 2.** Schematic diagram of plasmid used to express nanobodies in *E. coli* and wild-type and mutated nanobody sequences. S102C mutation is shown in red to green text.

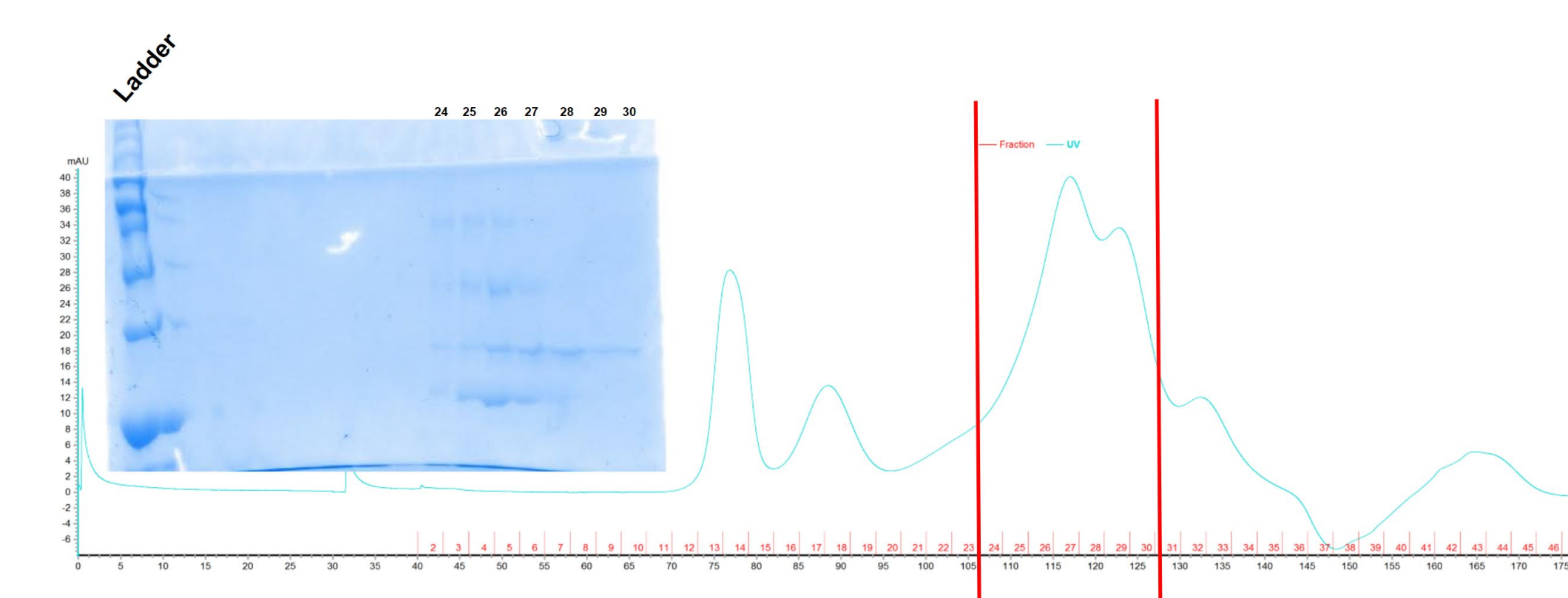
## Results



**Figure 3.** SDS-PAGE visualization of results from purification of nanobodies by His-tag interaction with Ni-NTA resin column. Arrow indicates the proteins of interest.



**SEC Purification Spectrum for WT Nanobody**



**SEC Purification Spectrum for Mutant Nanobody**

**Figure 4.** Results of the purification process using SEC chromatography. The red bars represent the fractions collected, which correspond to the lanes on the SDS-PAGE gel image.

## Conclusion and Future Direction

We successfully expressed and purified wild-type and mutated nanobodies with specificity to SARS-CoV-2 Spike. 12 mg of the wild-type nanobody and 4.5 mg of the mutated nanobody were recovered from 1 L culture.

We will assess the conjugation efficiency of the mutated nanobodies. We will perform a crosslinking assay to determine the level of association between the spike protein and nanobodies with covalent linkers attached. The proposed drug modality will greatly enhance the field of protein therapeutic design.

## Acknowledgements

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