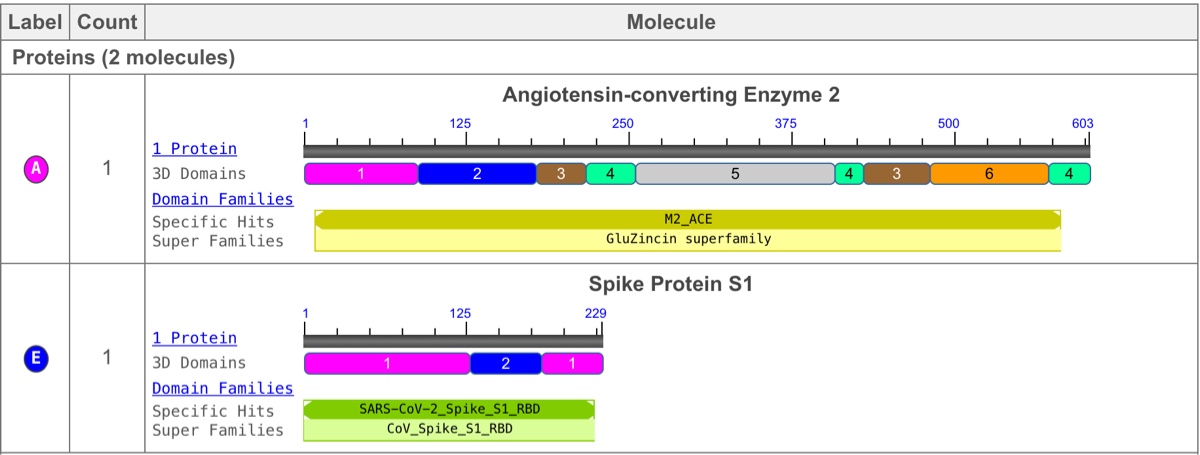
Finding and annotating aligned structures

This procedure shows a method for aligning the spike proteins from two structures and using the alignment to show where an antibody binds to the spike protein relative to the site where ACE2 binds to the spike protein. Aligning structures is helpful because it puts the structures in the same position making them easier to compare. You will also learn how to add annotations and how to save a link to your annotated structures so you can use them again.

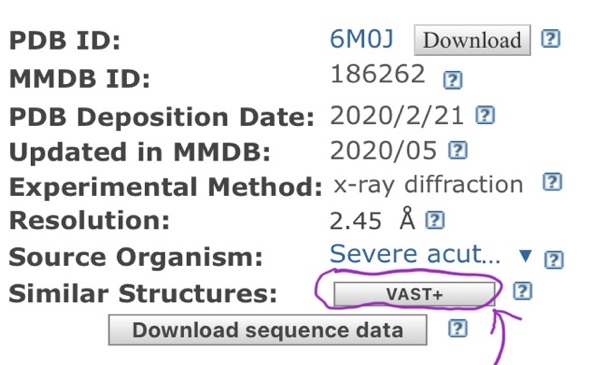
1. Go to the NCBI and search for 6M0J. (Note: this PDB ID contains a zero, not the letter O).
2. Click the title of the structure. In this case, the title is “*Crystal Structure of SARS-CoV-2 spike receptor-binding domain bound with ACE2.*”

This structure contains a small portion of the SARS-CoV-2 spike protein and the ACE2 protein. Different letters are used to identify the protein chains in this structure.

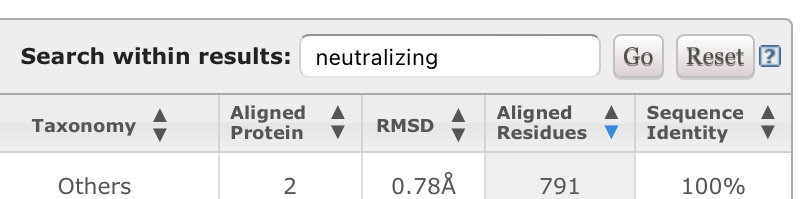
1. Use the information in the table to determine which protein chain (A or E) corresponds to the Receptor Binding Domain (RBD) from the spike protein and which protein chain corresponds to the human ACE2 protein. Record your answers in the data sheet.



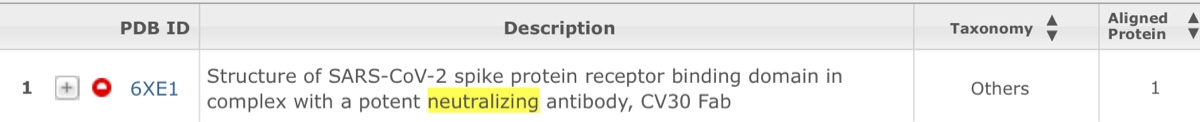
1. Scroll up to see the top of the page. Click the Vast+ button.



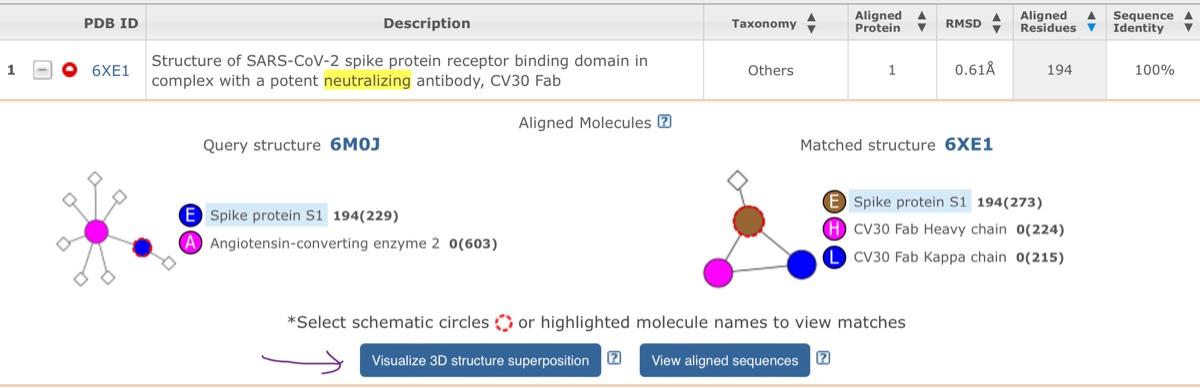
1. Type “neutralizing” in the “Search within results” area and click Go. This will allow you to search through the VAST+ results for aligned structures that contain neutralizing antibodies.



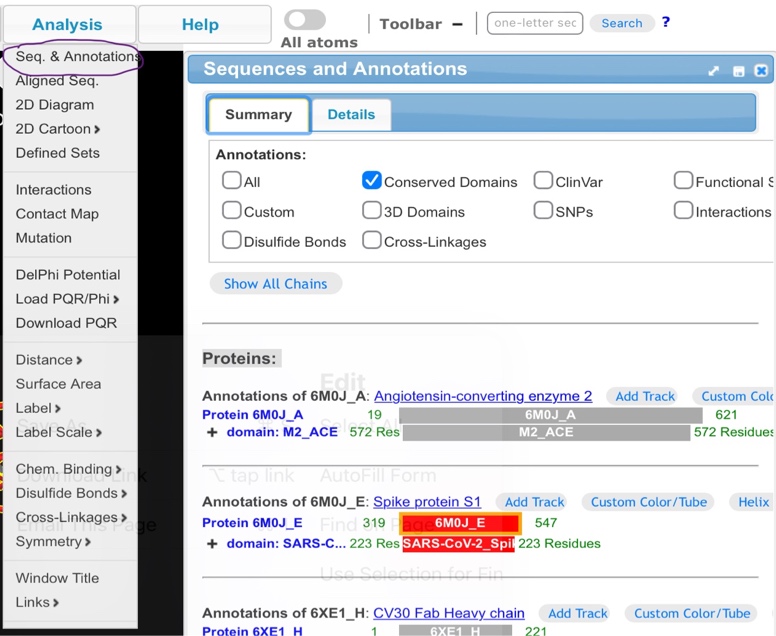
We are searching for neutralizing antibodies because those are the antibodies that are most likely to be protective, at least against some variants of SARS-CoV-2.

1. Look through the table and find the row with 6XE1 (below). We will use 6XE1 for this example. Towards the end of this part, you will look at an alignment to a structure that contains a different neutralizing antibody. 
2. Click the + button on the left side of the PDB ID to view a diagram showing the protein chain from 6M0J aligned to a protein chain from the structure in the table (in this case the protein chain is the spike protein from 6XE1).
3. A diagram of the protein chains in the structure will open below the row.

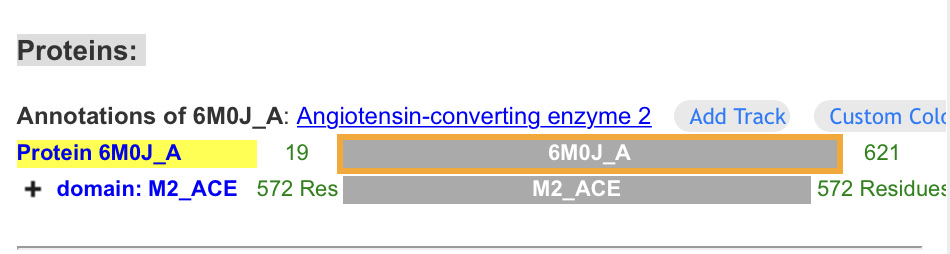
This diagram shows that 6M0J contains the spike protein and ACE2. It also shows that 6XE1 contains the spike protein and the Fab portion of the CV30 antibody. The blue highlighting shows the aligned protein chains. In this case, the aligned chains are from the spike protein.



1. Click Visualize 3D structure superposition to view the aligned structures in iCn3D.
2. Annotate the structure to make it easier to identify and see where the ACE2 protein and antibody proteins are binding to the spike protein.
   1. Open the Analysis menu and choose Seq. & Annotations.

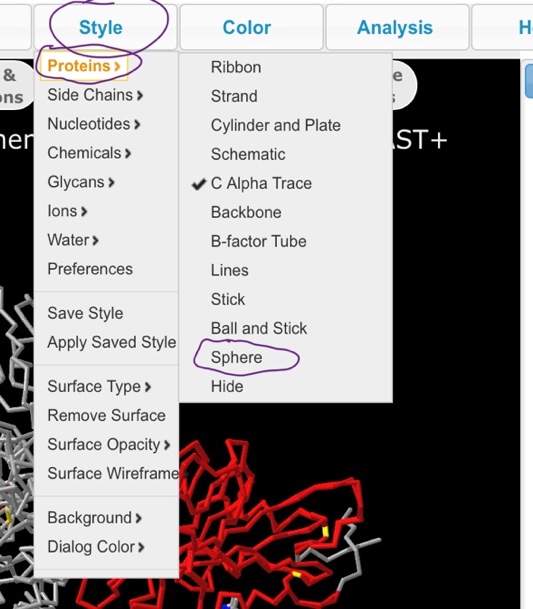


* 1. Click the name of the ACE2 protein chain (6M0J\_A). This action selects the chain and highlights it in yellow.



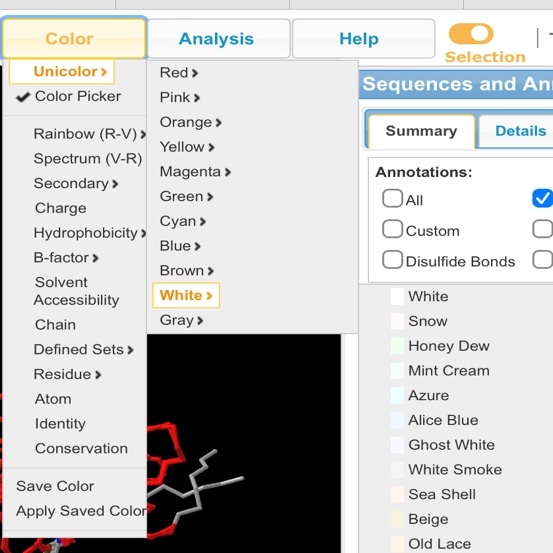
* 1. Open the Style menu. Choose Proteins, and then choose Sphere.

Selecting a protein chain and choosing the Sphere drawing style will cause atoms in the selected protein (ACE2) to be drawn as spheres.



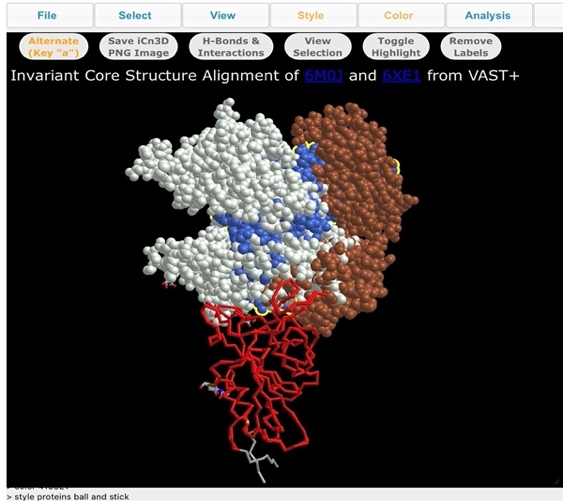
* 1. Open the Color menu. Choose a light color.

This will change the color of any selected protein chain (ACE2) to a light color.



Annotating is the process of adding extra information to a document or image. Sometimes images are annotated with labels. In the case of molecular structure models, annotations can include colors and drawing styles.

* 1. Annotate the heavy and light chains of the antibody.
  2. Select the heavy chain of the antibody and use the same process as above to change the drawing style to spheres and the color to brown.
  3. Select the light chain. Change the drawing style to spheres and the color to blue.



1. Compare the structure of the spike protein bound to ACE2 with the structure of the spike protein bound to an antibody by doing the following:
   1. Press the “A” key on your keyboard or click the Alternate button to alternate between the structure containing ACE2 and the structure that contains an antibody.

If ACE2 and the antibody bind to the same part of the spike protein, then the antibody is likely to block binding to ACE2 and prevent infection.

* 1. Does this antibody look like it would interfere with the ability of the spike protein to bind to ACE2?

Graphical user interface, application

Description automatically generated

1. Return to the VAST+ table at the NCBI (step 6).
2. Choose a different structure from the VAST+ table. This will contain a different neutralizing antibody.
3. Record the PDB ID for your new structure alignment.
4. Annotate ACE2, and the antibody in your new alignment, as you did in steps 10c – 11 to determine if the antibody in your new structure might neutralize SARS-CoV-2 by blocking binding to ACE2.
5. Open the Style menu and choose white.
6. Alternate between the structures and save one image showing the spike protein bound to ACE2 and one image showing the spike protein bound to your new antibody.
7. Paste the images of your spike protein and ACE2 and your new antibody and the spike protein in your data sheet.
8. Open the File menu and choose Share link.
9. Copy the Lifelong Short URL and save it in your notes.

Graphical user interface, text, application

Description automatically generated