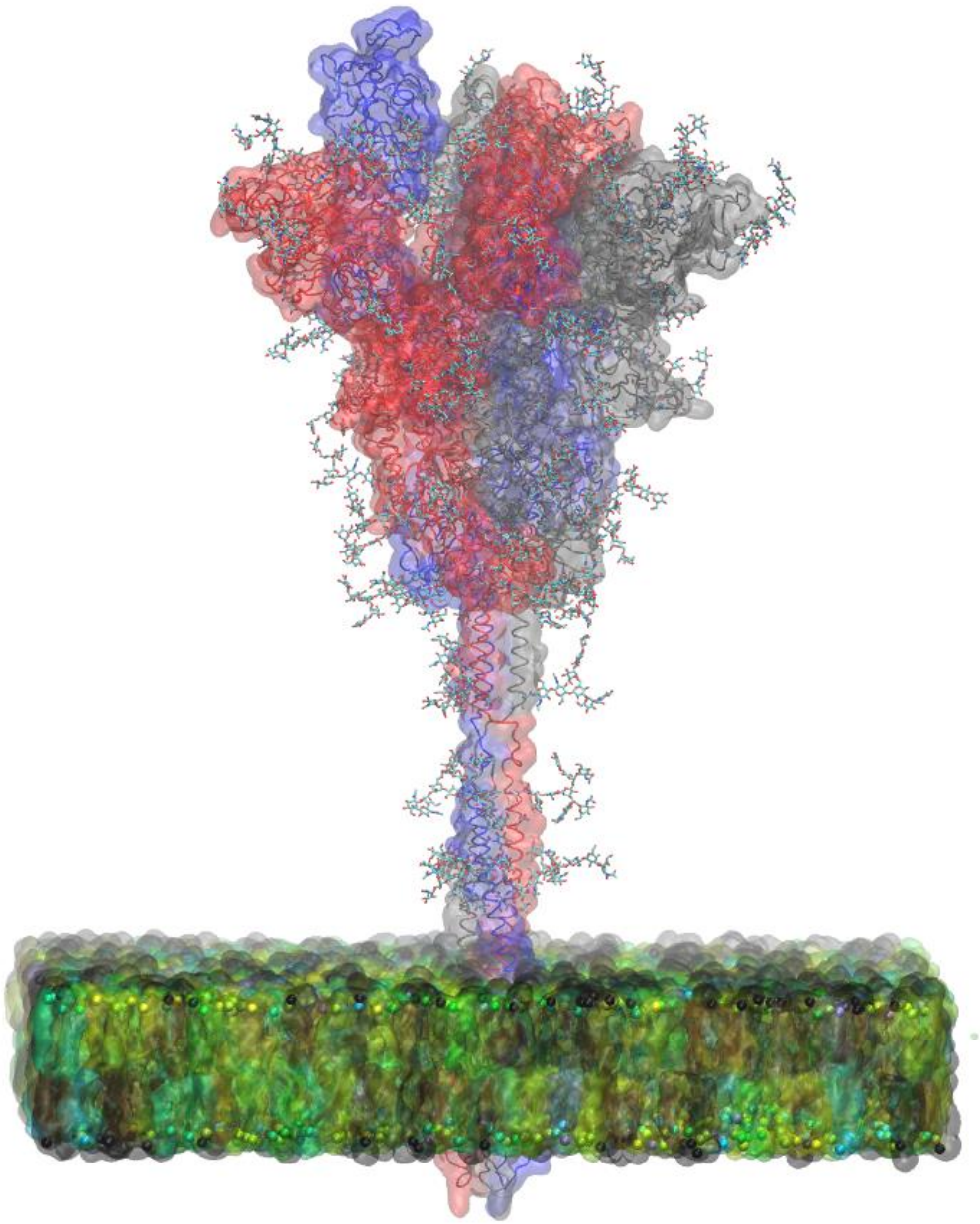
Modeling Spike-Antibody Complexes



Tutorial Contributors: Ashley L. Bennett | PhD
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[Tutorial Files are available on GitHub]

Table of Contents

Introduction

Proteins are essential biomolecules that perform a wide range of important functions in living organisms, such as catalyzing chemical reactions, transporting molecules, and providing structural support. The structure of a protein refers to sequence of amino acids that make up the protein and the three-dimensional arrangement of atoms in space.

The importance of protein structure lies in the fact that it directly influences protein function. The specific shape of a protein allows it to interact with other molecules, such as enzymes, receptors, or substrates, in a highly specific manner. A slight change in protein structure can have a significant impact on its function and may lead to a loss of activity or altered activity that can result in disease or other biological processes.

Protein structure is essential for protein function, and understanding protein structure is crucial for the development of new drugs, understanding genetic diseases, and advancing our understanding of biological processes at the molecular level. Molecular modeling is a biophysical method that uses computers to model protein structures and simulate their dynamic behavior. By modeling and simulating protein structure and dynamics, we can gain new insights in protein structure and function that is otherwise inaccessible through current experimental techniques.

The SARS-CoV-2 Spike protein is the primary target for SARS vaccine development. The Spike protein is composed of three identical subunits and is embedded in the membrane of the SARS-CoV-2 virus. The Spike protein binds to the ACE2 host cell receptor to initiate infection and is targeted by antibodies to neutralize the virus. Developing an understanding of the Spike protein structure is essential to developing effective therapeutics and vaccines. A common approach to vaccine development is to study and compare structures of the Spike protein, the Spike-ACE2 complex, and Spike-antibody complexes to determine the structural components necessary for viral infection effective neutralization by antibodies.

This video of the SARS-CoV-2 life cycle was made using computer models and simulations of the Spike protein and other SARS-CoV-2 viral components. This tutorial will provide a step-by-step guide to comparing Spike, Spike-ACE2, and Spike-antibody models using the VMD software. Not all topics can be covered in a short workshop so relevant links will be provided throughout the text for interested users to conduct further learning. The VMD website provides many tutorials that cover the many different functions of VMD and the associated NAMD simulation software.

Software

Installation

Installing VMD

1. Navigate to VMD software download page and select the most recent version that matches you system. Save the file in a directory of your choosing.
2. Double click on the file to launch the installer.
3. Follow the instructions to install VMD.

Other software

There is other visualization software freely available:

- ChimeraX
- PyMol

Simulation Software

The software used for simulations is separate from visualization software. Due to computational demand, these simulations typically run rather slowly on a laptop computer. It is better to use a GPU workstation or serve for running simulations. If you would like to check out some simulation software, NAMD is freely available, provides user documentation and tutorials, and integrates well with VMD. There are other simulation software suites available as well.

Tutorial Files

The files for this tutorial are available on the profile @ScientistAsh on GitHub.

These files are based on previously published models[1–3] that can be found on the CHARMM-GUI website.

References

(1) Woo, H.; Park, S.-J.; Choi, Y. K.; Park, T.; Tanveer, M.; Cao, Y.; Kern, N. R.; Lee, J.; Yeom, M. S.; Croll, T. I.; Seok, C.; Im, W. Developing a Fully Glycosylated Full-Length SARS-CoV-2 Spike Protein Model in a Viral Membrane. *J. Phys. Chem. B* **2020**, *124* (33), 7128–7137. https://doi.org/10.1021/acs.jpcb.0c04553.

(2) Choi, Y. K.; Cao, Y.; Frank, M.; Woo, H.; Park, S.-J.; Yeom, M. S.; Croll, T. I.; Seok, C.; Im, W. Structure, Dynamics, Receptor Binding, and Antibody Binding of the Fully Glycosylated Full-Length SARS-CoV-2 Spike Protein in a Viral Membrane. *J. Chem. Theory Comput.* **2021**, *17* (4), 2479–2487. https://doi.org/10.1021/acs.jctc.0c01144.

(3) Cao, Y.; Choi, Y. K.; Frank, M.; Woo, H.; Park, S.-J.; Yeom, M. S.; Seok, C.; Im, W. Dynamic Interactions of Fully Glycosylated SARS-CoV-2 Spike Protein with Various Antibodies. *J. Chem. Theory Comput.* **2021**, *17* (10), 6559–6569. https://doi.org/10.1021/acs.jctc.1c00552.

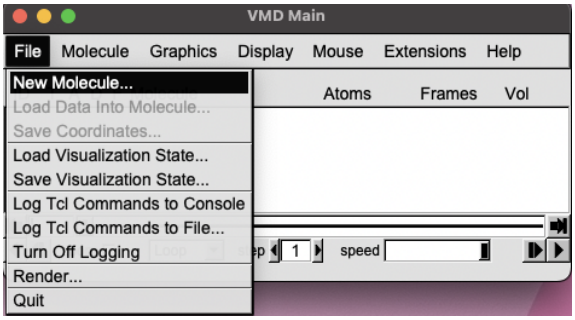
Working with a Spike-Membrane Model

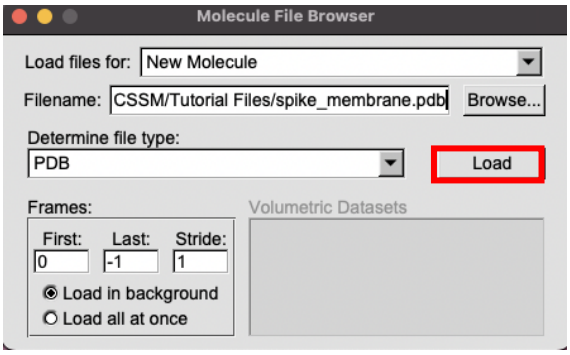
In this section you will learn the basic functions of VMD and the best visualization strategies for looking at the SARS-CoV-2 Spike-Membrane complex. We will start with loading the Spike-Membrane complex and displaying the Spike protein embedded in a membrane. The Spike protein is a homotrimer, composed of 3 identical subunits. The Spike protein also has glycans, or sugar molecules, that are added on to asparagine residues after the protein is translated.

Load the Spike-Membrane Complex

The first step is to load the Spike-Membrane complex. A pdb file, prot_glyc_memb.pdb[1], which contains the atomic coordinates for the SARS-CoV-2 Spike protein, glycans, and membrane.

1. Start a VMD session by double clicking on the VMD logo.
2. In the VMD Main window, choose `File` → `New Molecule…`
3. A Molecule File Browser window will appear. Use the `Browse…` button to find the file spike_membrane.pdb file. After selecting the file in the file browser window, you will be directed back to the Molecule File Browser window.
4. Press the `Load` button to load the molecule.



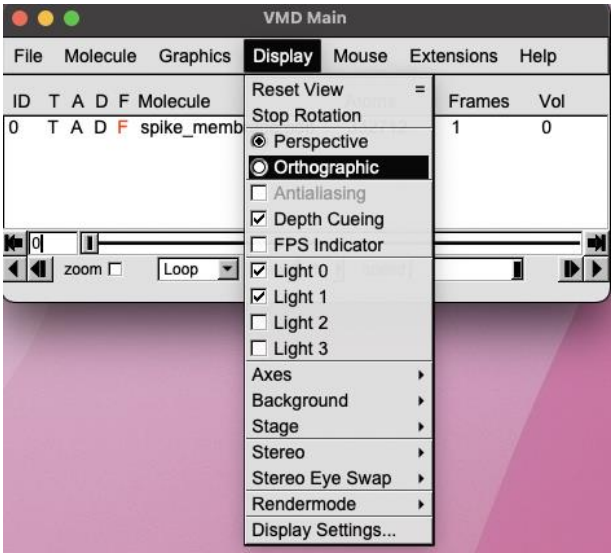


Now, the SARS-CoV-2 Spike protein, it's associated glycans, and a membrane will appear in the VMD OpenGL Display window. You may close the Molecular File Browser Window if you would life.

Protein Data Bank: The Protein Data Bank (PDB) is a database that contains the atomic coordinates for experimentally determined protein structures. Often the first step in modeling proteins is to find a structure of the protein, or a closely related protein, in the PDB. VMD can download a pdb file from the PDB website when the computer is connected to the internet. Just type the four letter PDB code of the protein in the Filename text entry op the Molecule File Browser Window and press the load button. VMD will download it automatically. You can browse the PDB here.

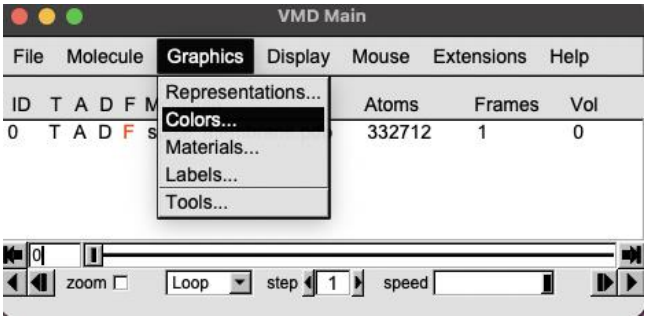
Changing the Spike-Membrane Complex Viewpoint

First, we will change the VMD display Settings. By default, VMD will display models in perspective mode. In perspective mode, things nearer the camera appear larger. Perspective projection provides strong size-based visual depth cues but, the displayed image will not preserve scale relationships and objects very close to the camera may appear distorted. Orthographic projection preserves scale and parallelism relationships between objects in the displayed image, but greatly reduces depth perception. We will first change to orthographic projection.



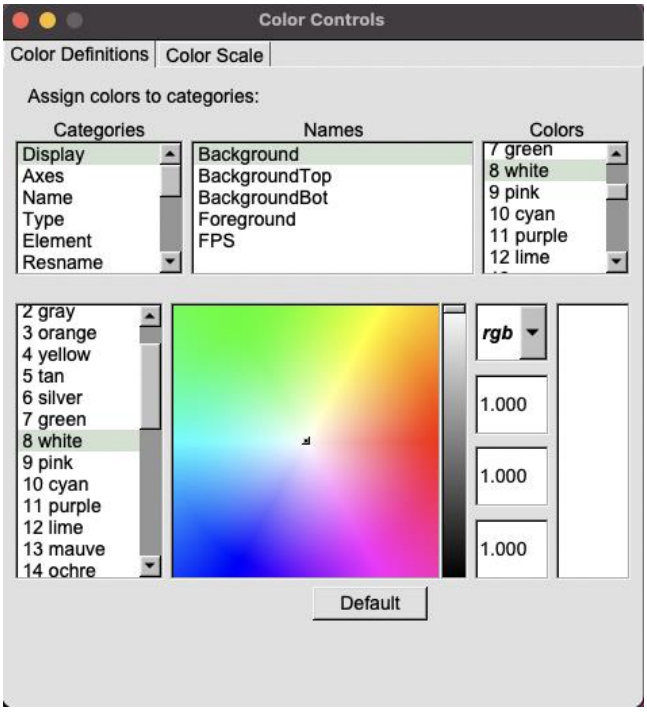
1. In the VMD Main window click `Display → Orthographic.` You should now see the molecule in orthographic projection.

Sometimes it is easier to see molecules when you change the background display to a different color. Now we will change the display background color to white.

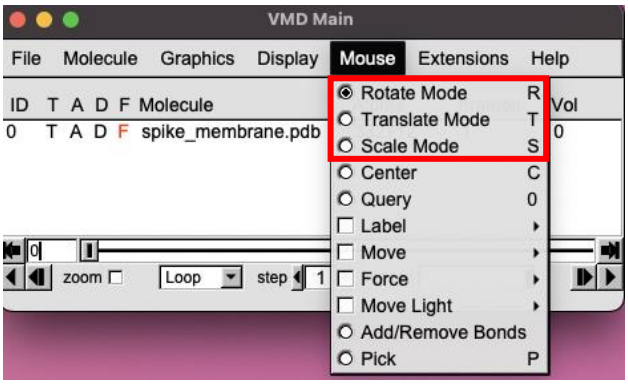


1. In the VMD Main window click `Graphics → Colors.` A new Color Controls window will pop up.

2. Select `Display` under the `Categories` column, `Background` under the `Names` column, and `8 white` under the Colors column. You should now see the background in the VMD OpenGL Display window turn white. You may select a different color if you wish.



To see the 3D structure of the Spike-Membrane complex, we will use the mouse in multiple modes to change the viewpoints. VMD allows users to rotate, scale, and translate the viewpoint of your model.



1. In the VMD OpenGL Display window, press the left mouse button down and move the mouse. Explore what happens. This is the rotation mode of the mouse and allows you to rotate the molecule around an axis parallel to the screen.

2. If you hold down the right mouse button and repeat the previous step, the rotation will be done around an axis perpendicular to the screen.

3. In the VMD Main window, look at the `Mouse` menu. Here you will be able to switch the mouse mode from `Rotation` to `Translation` or `Scale` modes.

4. Choose `Translation` mode and go back to the VMD OpenGL Display window. You can now model the molecule around when you hold the left mouse button down.

5. Go back to the `Mouse` menu and choose the `Scale` mode this time. This will allow you to zoom in or out by moving the mouse horizontally while holding the left mouse button down.

Note that these actions performed with the mouse only change the viewpoint and do not change any atomic coordinates associated with the loaded molecule.

Mouse Modes: Note that each mouse mode has its own characteristic cursor and its own shortcut key (r: `Rotate`, t: `Translate`, s: `Scale`). When you are in the OpenGL Display window, you can use these shortcut keys instead of `Mouse` menu to change the mouse mode.

Another useful option is the `Mouse` → `Center` menu item. It allows you to specify the point around which rotations are done.

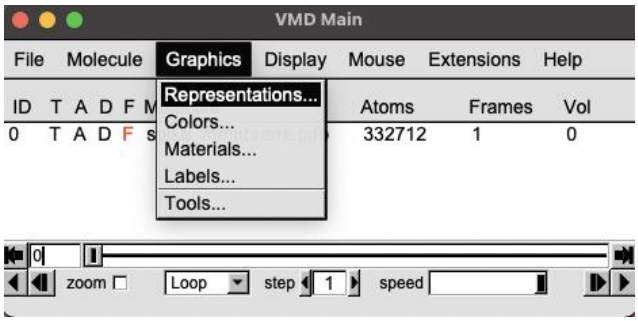
6. Select the `Center` menu item and pick one atom at one end of the Spike protein; The cursor should display a cross.

7. Press **r** to rotate the molecule with the mouse and see how your molecule moves around the point you have selected.

8. In the VMD Main window, select `Display` → `Reset View` menu items to return to the default view you can reset the view by pressing the "=" key when you are in the VMD OpenGL Display window.

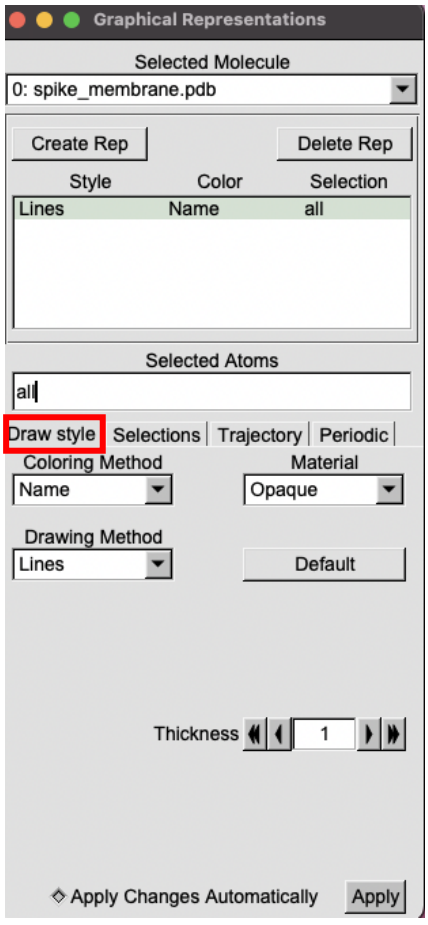
Building a Visualization of the Spike-Membrane Complex

VMD can display the Spike-Membrane complex in various ways using the `Graphical Representations` menu. Each representation is defined by four main parameters: the selection of atoms included in the representation, the drawing style, the coloring method, and the material. The selection determines which part of the Spike-Membrane complex is drawn, the drawing method defines the style the complex will be drawn in, the coloring method determines how the representation will be colored, and the material determines the effects of lighting, shading, and transparency on the representation. In this section, you will build a representation of the Spike-Membrane complex.



1. In the VMD Main window, choose the `Graphics → Representations…` menu item.

2. The Graphical Representations window will appear, and you will see highlighted in green the current default representation displaying the Spike-Membrane complex.



3. In the `Draw Style` tab, we can change the style and color of the representations.

4. Click on the `Drawing Method` and you will see a list of options. Choose `NewCartoon`, a popular method for representing the backbone atoms of the protein. You will now see the protein backbone only with helices are drawn as coiled ribbons, β-sheets are solid arrows, and all other structures are represented as tubes.

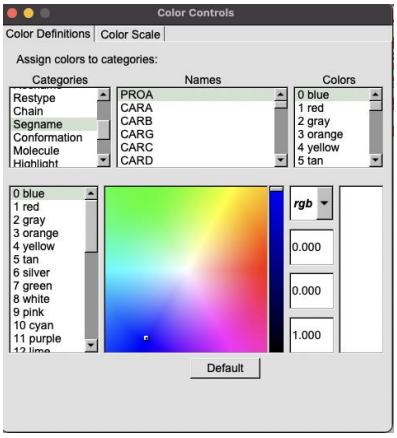
Drawing Methods: Each `Drawing Method` has its own parameters. For instance, change the `Thickness` of the lines, `Aspect Ratio`, or `Resolution` by using the controls on the lower right-hand-side corner of the Graphical Representations window. Click the `Apply` button to apply these changes. To reset the parameter values, click the `Default` button. Sometimes looking at several different methods is helpful for choosing the best drawing method for visualization.

Notice that the glycan and membrane atoms are no longer visible. Only the protein atoms can be represented with the NewCartoon drawing method.

5. In the Selected Atoms text box in the Graphical Representations window, type "protein" so that only the protein atoms are selected. Change the Coloring Method to SegName. This will render the Spike protein with each subunit colored differently.

You should now see the Spike protein with one subunit in blue, one subunit in red, and the third subunit in grey.

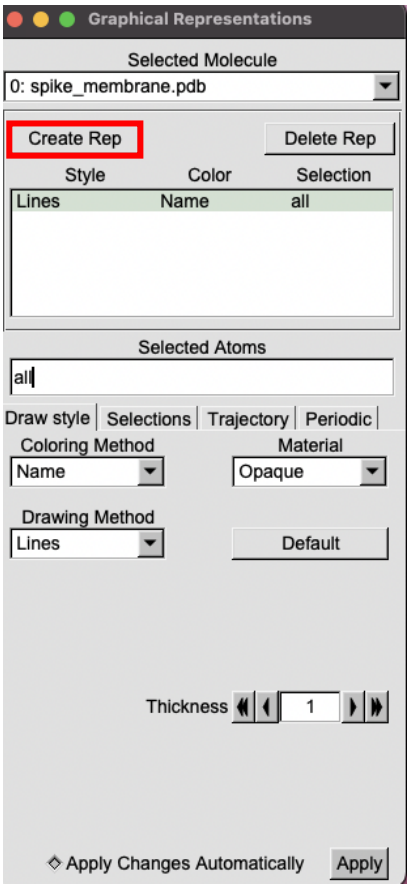
Color Mapping: Each Coloring Method can be adjusted from the default coloring parameters. If your subunit colors are different or you would like to use other colors, in the VMD Main window click on Graphics → Colors…. Under the Categories column select Segname and in the Names columns find PROA, PROB, and PROC and select the color you would like to use in the Colors column one at a time.



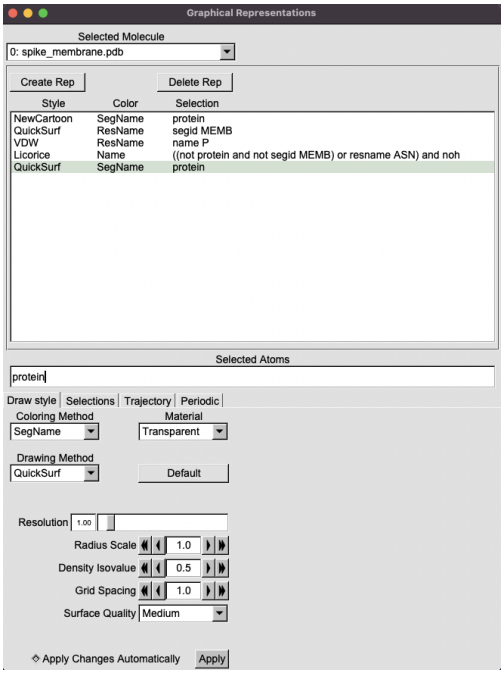
To visualize the glycans and membrane we need to create separate representations for them.

6. In the Graphical Representations window click the Create Rep button.

7. In the Selected Atoms text box type "segid MEMB" (you do not need to include the quotation marks) and hit the Enter key. Change the Drawing Method to QuickSurf, the Coloring Method to ResName, and the Material to Transparent. This will show the membrane in transparent space-filling spheres that allow you to visualize the volumetric distribution of the membrane while at the same time visualizing the insertion of the Spike protein into the membrane. The different colors demonstrate the different types of lipids that compose the membrane.



Drawing Methods: Note that while the NewCartoons drawing method only applies to protein molecules, most other drawing methods can be applied to any molecule. For example, to visualize the volumetric distribution of the protein you can render the protein selection in VDW.

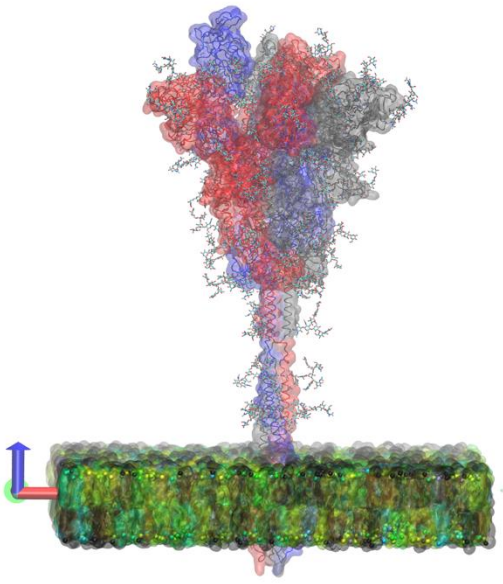


8. In the Graphical Representations window click the `Create Rep` button and in the `Selected Atoms` text box type "`name P`" and hit the `Enter` key. Change the `Drawing Method` to `QuickSurf` and the `Coloring Method` to `ResName`. Change the `Material` to `Opaque`. This will show phosphorous atoms of the lipid headgroups in the membrane in space-filling spheres.

9. In the Graphical Representations window click the `Create Rep` button and in the `Selected Atoms` text box type "`((not protein and not segid MEMB) or resname ASN) and noh`" and hit the `Enter` key. Change the `Drawing Method` to `Licorice` and the `Coloring Method` to `Name`. Change the `Material` to Opaque. This will show the glycan and asparagine residues as thick sticks colored according to atom name. The hydrogen atoms are omitted for simplification.

10. In the Graphical Representations window select the first representation for the protein and click the `Create Rep` button and in the `Selected Atoms` text box type "`not segid MEMB`" and hit the `Enter` key. Change the `Drawing Method` to `QuickSurf` and the `Coloring Method` to `SegName`. Change the `Material` to `Transparent`. This will show the volume of Spike protein within the Spike model fitting inside displayed volume.

You should now have a Spike-Membrane complex model that looks something like that shown below. It is okay if you used different coloring and drawing methods.

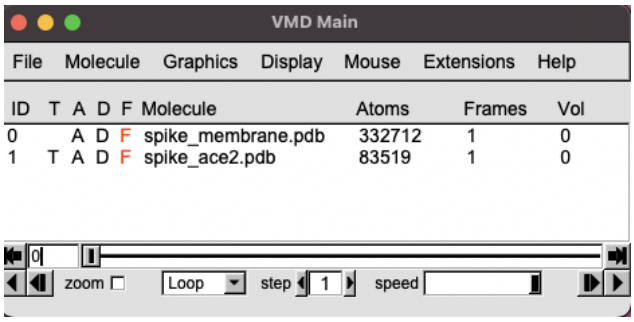


Examining the Spike-ACE2 Receptor Complex

SARS-CoV-2 viral infection is initiated when the Spike protein binds to the ACE2 receptor on the host cell. By examining the interface of the Spike-ACE2 complex, we can learn a tremendous amount of information about the structural mechanisms that allow this binding, and thus infection, to occur. In this section you will learn how to examine the Spike-ACE2 receptor complex.

Load the Spike-ACE2 Complex

The first step is to load the Spike-ACE2 complex. A pdb file, spike_ace2.pdb, which contains the atomic coordinates for the SARS-CoV-2 Spike protein, glycans, and ACE2 receptor.



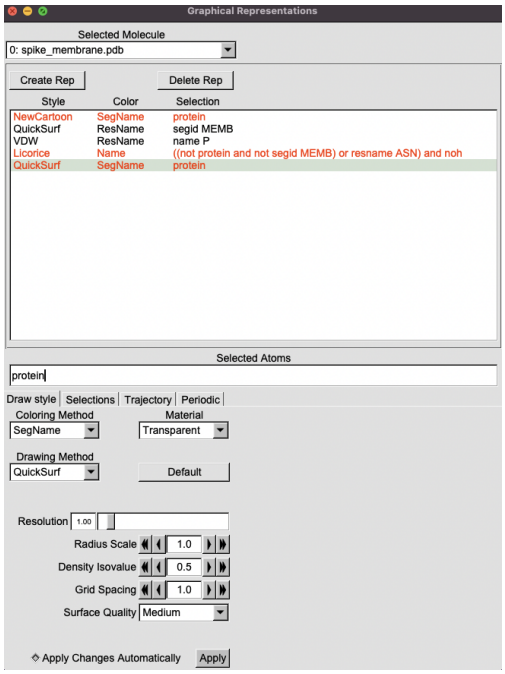
1. In the VMD Main window, choose `File` → `New Molecule`…. Select the spike_ace2.pdb file and click the `Load` button. There should now be two molecules loaded in the VMD Main window.

Top Molecule: Notice that there is the letter 'T' next to the spike_ace2.pdb molecule. This indicates that this molecule is the Top molecule, and the indicated actions will be performed on this molecule. To switch which molecule is the top molecule, double-click the blank space in the T column next to the molecule you wish to be Top molecule. The T should relocate to the new molecule. Note that your molecule ID numbers may be different than those observed in the above figure.

To visualize the Spike-ACE2 complex more easily, we need to hide the representation of the Spike we made in the previous section.

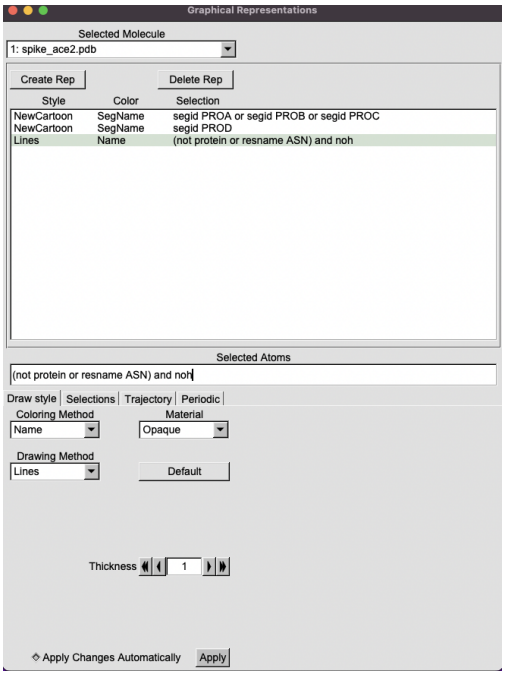
2. In the Graphical Representations window select the spike_membrane.pdb molecule in `Select Molecule` dropdown box.

3. Double-click on the protein and glycan representations to hide the representations.



Now we need to create a new visualization of the Spike-ACE2 complex.

4. In the Graphical Representations window switch to the spike_ace2.pdb molecule in the `Selected Molecule` dropdown box.

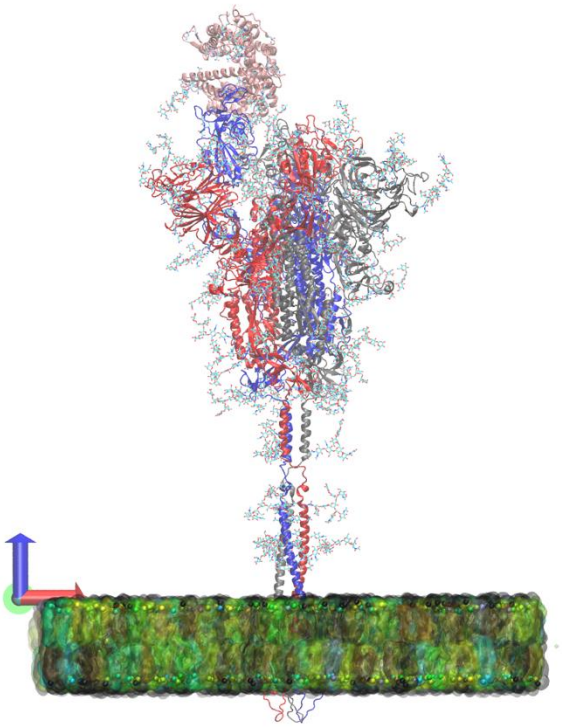


5. Change the `Selected Atoms` to "`segid PROA or segid PROB or segid PROC`", `Coloring Method` to SegName, `Drawing Style` to NewCartoon, and `Material` to Opaque. This will render the Spike protein backbone with all three subunits colored differently.

6. Click the `Create Rep` button to create a representation for the ACE2 receptor. Change the `Selected Atoms` to "`segid PROD`", `Coloring Method` to SegName, `Drawing Method` to `New Cartoon`, and `Material` to Opaque. This will render the ACE2 protein in pink.

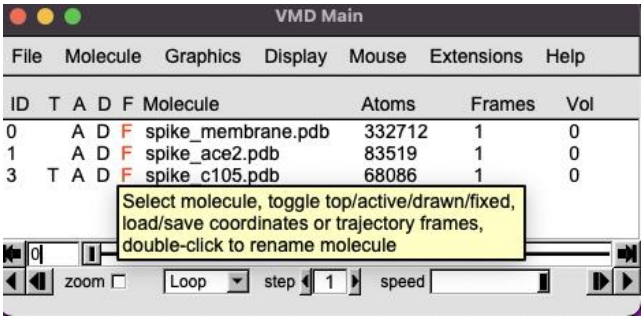
7. Click the `Create Rep` button to create a representation for the glycans. Change the `Selected Atoms` to "`(not protein or resname ASN) and noh`", the `Drawing Method` to `Lines`, `Coloring Method` to Name, and `Material` to Opaque. This will display the glycans and the asparagine residues as sticks colored by atom name without hydrogens.

You should now have a model of the Spike-Memrbane-ACE2 complex, like that shown in the image below.



Comparing the ACE2 and C105 Binding Sites

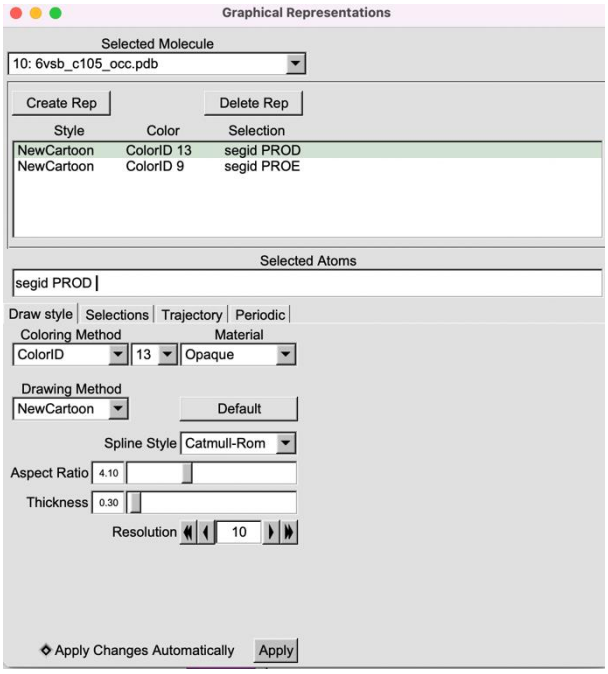
The ACE2 receptor binds to the receptor binding domain (RBD) of the Spike protein. C105 is a SARS-CoV-2 neutralizing antibody. In this section we will compare the Spike-ACE2 receptor complex with the Spike-C105 antibody complex to gain insight into how this antibody works to neutralize the SARS-CoV-2 virus. This structure of C105 antibody contains two chains, one heavy chain and one light chain, that both make important contacts with the Spike protein. We will look at how the two chains interact with the Spike protein



1. In the VMD Main window, choose `File` → `New Molecule`…. Select the spike_c105.pdb file and click the `Load` button. There should now be three molecules loaded in the VMD Main window. Make sure that this newly loaded molecule is indicate as the Top molecule.

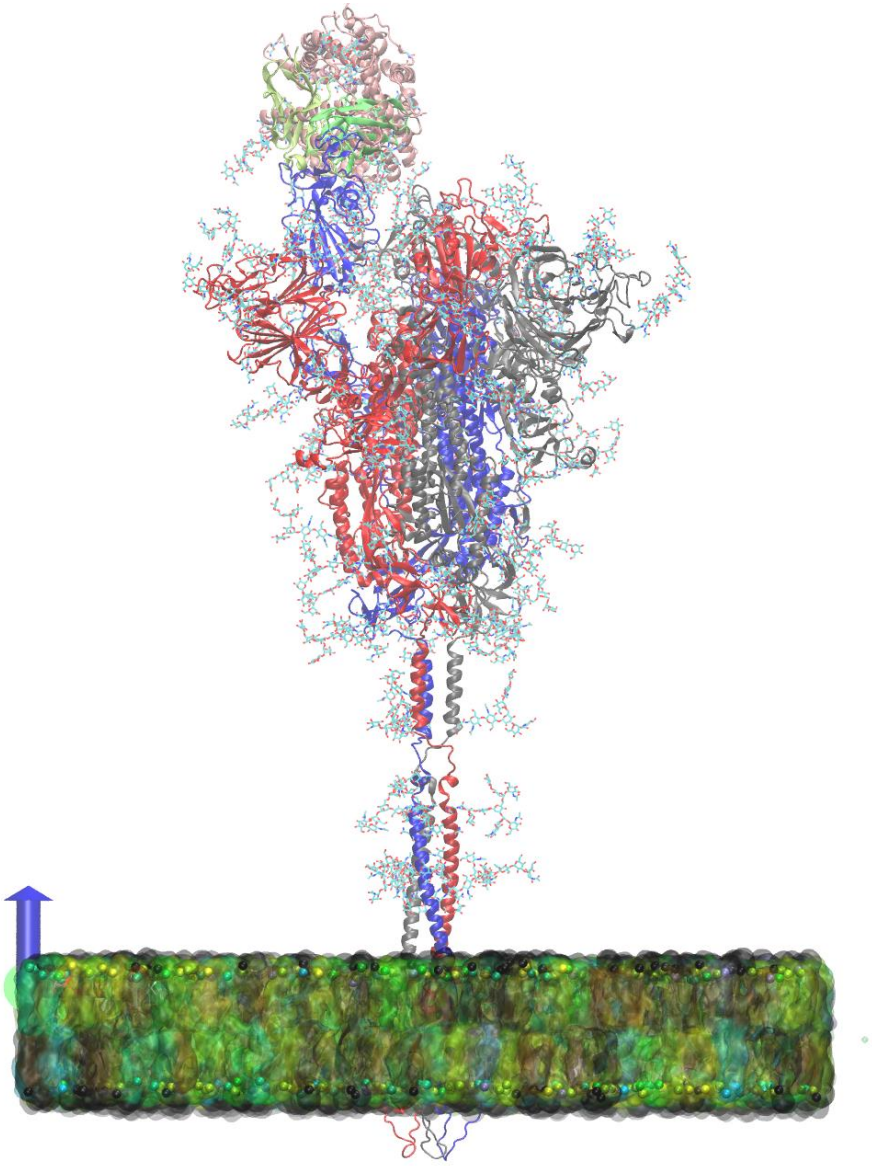
2. Switch to the spike_c105.pdb molecule in the `Selected Molecule` dropdown box.

3. Change the `Selected Atoms` to "`segid PROD`", `Coloring Method` to `ColorID 7`, `Drawing Style` to NewCartoon, and `Material` to `Brushed Metal`. This will render the protein backbone of the C105 antibody heavy chain in green.



4. Click the `Create Rep` button to create a representation for the C105 antibody light chain. Change the `Selected Atoms` to "`segid PROE`", `Coloring Method` to `ColorID 18`, `Drawing Method` to `New Cartoon`, and `Material` to `Brushed Metal`. This will render the C105 antibody light chain backbone in light chain in light green.

You should now have three molecules loaded and rendered such that you can visualize the Membrane-Spike-ACE2-C105 model as seen in the image below.



What should be readily apparent is that the C105 antibody is overlapping with the ACE2 receptor. The binding site for the C105 antibody overlaps with the binding site for the ACE2 receptor, thus acting as a blocking molecule. When the C105 antibody is bound to the Spike protein the ACE2 receptor cannot bind, preventing the SARS-CoV-2 from initiating infection. This is one structure-based mechanism by which the C105 antibody works to fight the SARS-CoV-2 virus.

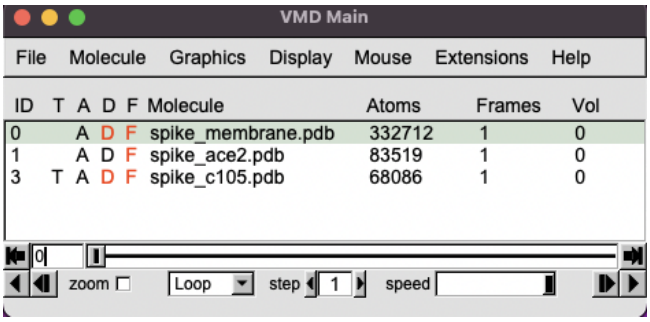
Examining Binding Sites

One extremely useful aspect of modeling is the ability to interrogate protein structures at atomic resolution. This way we can identify specific residues and interactions that are likely important for function. Residues that are identified as important for a specific function can then be mutated and tested for altered function in experiments. When looking at receptor and antibody binding, it is helpful to know which specific residues make up the binding sites. In this section we will examine the ACE2 receptor and C105 antibody binding sites on the Spike protein.

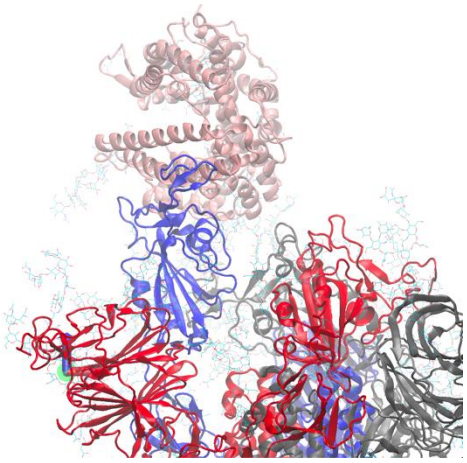
Compare Visualizations

When large complexes are loaded in VMD on a standard laptop, the `Rotation`, `Scale`, and `Translation` modes of the OpenGL Display window can be slow and cause much lag. To make examining the binding sites on Spike easier we will first deactivate the membrane representation and zoom in on the receptor binding domain (RBD) of the Spike protein.

1. In the VMD Main window, deactivate the Spike-Membrane and Spike-C105 antibody complexes by double clicking the D next to the molecule name. The D should turn red to indicate it is inactive. This will hide the membrane and antibody representations.



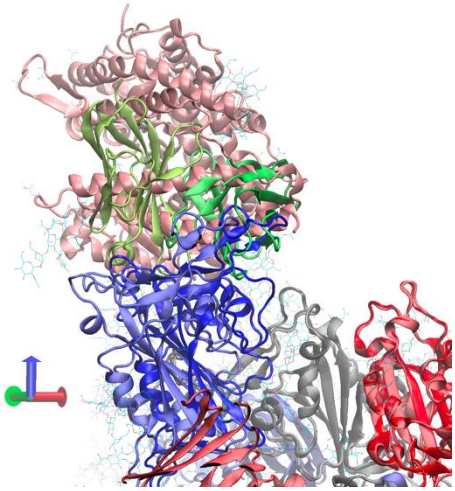
2. Use the `Scale`, `Translate`, and `Rotate` mouse modes to center the RBD-ACE2 interface. Your view may be from a different angle, and that is fine. Choose whatever zoom and angle is most comfortable for you.



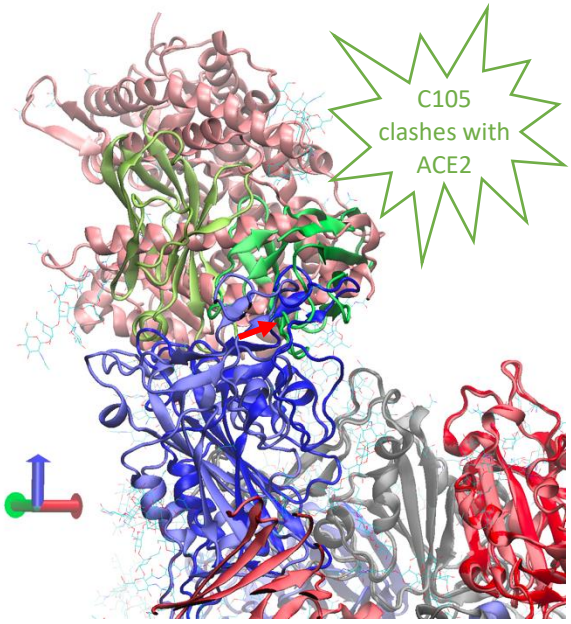
3. Double click the D button in the VMD main window to activate the Spike-C105 antibody complex.

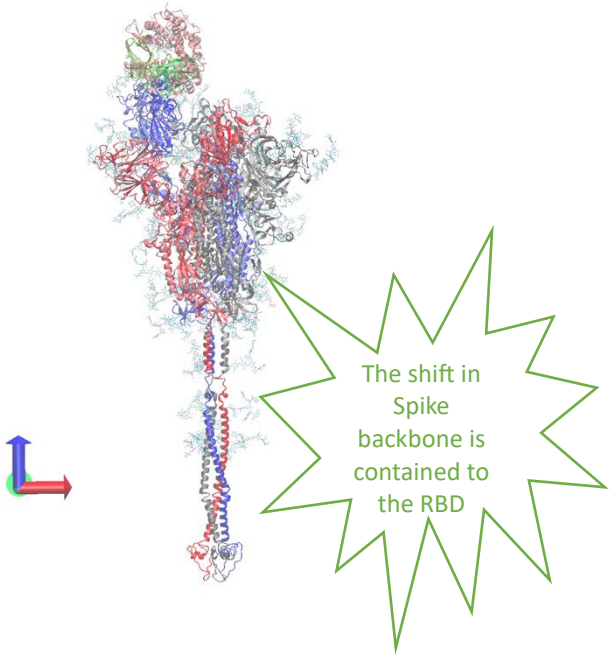
4. In the Graphical Representations window select the Spike-Ab complex and click the `Create Rep` button to create a representation for the antibody-bound Spike structure. Change the `Selected Atoms` to "`segid PROA or segid PROB or segid PROC`", `Coloring`

`Method` to `ColorID SegName`, `Drawing Method` to `New Cartoon`, and `Material` to `Brushed Metal`. This will render the antibody-bound Spike backbone in the same colors as in the already ACE2-bound Spike. You should see something like that shown at the right.



5. Rotate this zoomed in scene around to see how the RBDs align. Try turning the C105-bound Spike molecules on and off in the VMD Main window to observe how C105 antibody binding causes a slight shift in the position of the RBD. Zoom out on the larger complex to observe how the slight shift in RBD is not observed anywhere else in the Spike protein.





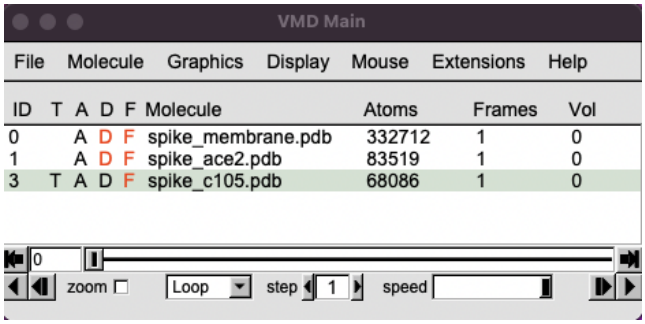
As you can see by working through the exercises above, the RBD is primary structural component of the Spike protein that moves upon C105 antibody binding. This antibody also partially overlaps with the ACE2 receptor when bound to the Spike protein, indicating that both the ACE2 and C105 antibody cannot be bound at the same time. By analyzing and comparing the structures of the ACE2-bound Spike with the C105 antibody-bound Spike we can determine that the C105 antibody works to neutralize the Spike protein in part by competing with ACE2 for binding to the Spike protein and by shifting the position of the RBD, thus altering the structure, and therefore function, of the Spike protein.

Finding Important Interactions

To leverage the structural analysis above to improve vaccine and drug design, we need to determine what residues are important in the Spike-C105 antibody complex. In this section, you will look at specific residues in the ACE2-Spike and C105 antibody-Spike binding sites to find interesting residues and interaction.

1. Double click the D button in the VMD main window to deactivate the Spike-ACE2 complex.

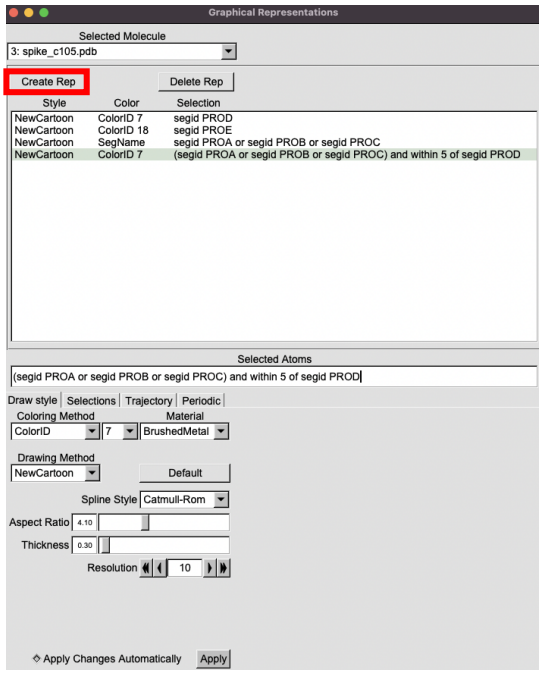
2. Use the `Scale`, `Translate`, and `Rotate` mouse modes to center the RBD-C105 antibody interface.



First, we need to create a representation for Spike residues that are within interaction distance of antibody residues.

3. In the Graphical Representations window select the Spike-C105 antibody complex, select first representation, and click the `Create Rep` button.

4. Change the `Selected Atoms` to "`segid PROA and within 5 of segid PROD`", `Coloring Method` to `ColorID 13`, `Drawing Method` to `NewCartoon`, and `Material` to `Brushed Metal`. This will color the Spike residues within 5Å of the C105 antibody heavy chain dark green.



Interaction distances: Different types of bonds have different interaction distances. While covalent and ionic bonds occur usually <3Å, electrostatic interactions can occur distances of >12Å. Selecting the proper distance cutoff for viewing an interacting partner is somewhat of an arbitrary choice but should be influenced by the type of interactions you would like to study, the research question you are considering, and feasibility of displaying/analyzing all residues. You can try to change you cutoff value to see the different residues you pick up.

5. In the Graphical Representations window select any of the representations and click the `Create Rep` button.

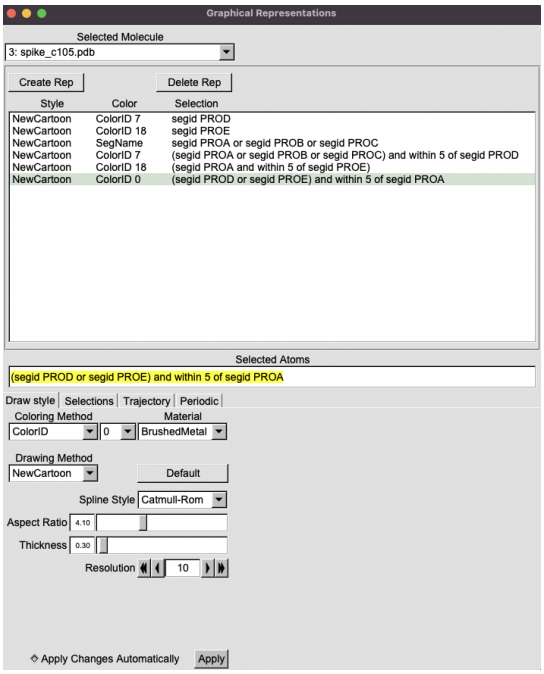
6. Change the `Selected Atoms` to "`segid PROA and within 5 of segid PROE`", `Coloring Method` to `ColorID 18`, `Drawing Method` to `NewCartoon`, and `Material` to `Brushed Metal`. This will color the C105 antibody light chain residues within 5Å of the Spike protein light green.

Antibody structures: Antibodies consist of four protein chains: two identical heavy chains and two identical light chain, held together by disulfide bonds. Light chains are smaller than heavy chains and contain one variable and one constant domain. The heavy chains contain one variable domain and three constant domains. The variable domains have high sequence variability and are responsible for the specificity of the antibody. The constant domains determine the antibody class and antibody belongs to. The structures shown throughout this tutorial show one heavy chain and one light chain for simplicity.

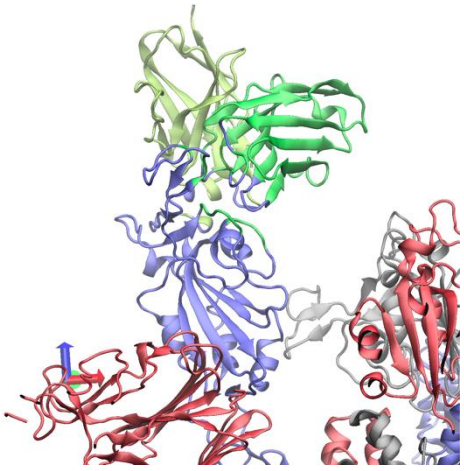
Now we need to create representations for the antibody residues that are within interaction distance of the Spike RBD.

7. In the Graphical Representations window select any of the representations and click the `Create Rep` button.

8. Change the `Selected Atoms` to "`(segid PROD or segid PROE) and within 5 of segid PROA`", `Coloring Method` to `ColorID 0`, `Drawing Method` to `NewCartoon`, and `Material` to `Brushed Metal`. This will color the C105 antibody residues within 5Å of the Spike protein blue. The Graphical Representations window should look like the figure shown to the right.

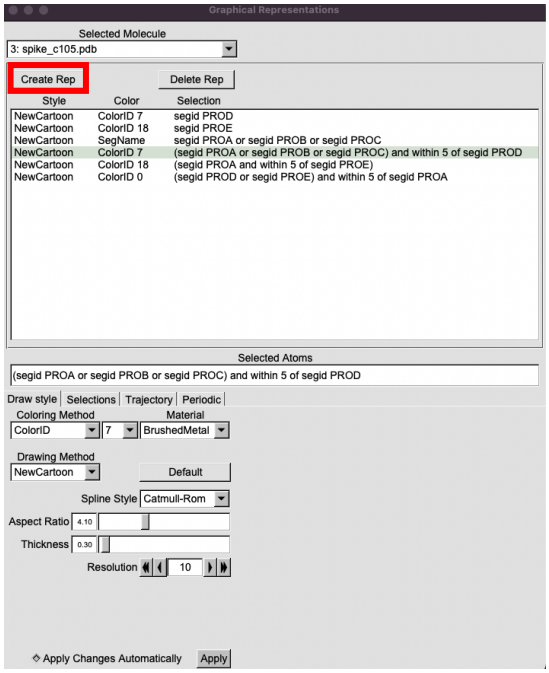


The OpenGL Display window should appear like the image to the right. Use the `Scale`, `Translate`, and `Rotate` mouse modes to explore the Spike RBD-C105 antibody interface.



This type of visualization can be helpful in identifying locations of contact between the Spike protein and antibodies. Identifying locations of contact in the Spike RBD-C105 antibody complex can help us to identify specific interactions that help to stabilize these complexes. We will now look at specific

interactions that help stabilize the Spike-C105 antibody complex. To do this, we need to look at the sidechains, which so far we have ignored.

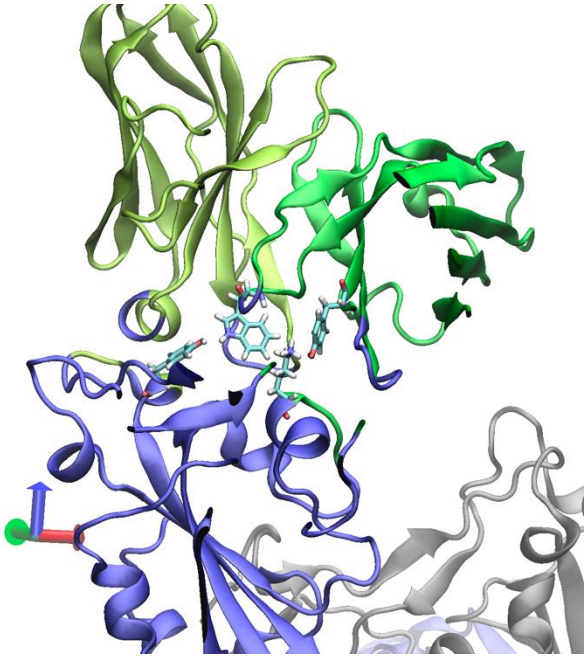


1. In the Graphical Representations window select the representation for the Spike residues that are within 5Å of the C105 antibody heavy chain. Click `Create Rep`.

2. Change the drawing paraemters to the following: `Selected Atoms` to "`segid PROA and (resid 505 or resid 417)`", `Coloring Method` to `Name`, `Drawing Method` to `Licorice`, and `Material` to `Brushed Metal`. This will display the Spike RBD residues tyrosine 505 and lysine 417 as sticks colored according to atom name.

3. Click `Create Rep` and change the drawing parameters to the following: `Selected Atoms` to "`segid PROD and (resid 101 or resid 33)`", `Coloring Method` to `Name`, `Drawing Method` to `Licorice`, and `Material` to `Brushed Metal`. This will display the C105 antibody heavy chain residues tryptophan 101 and tyrosine 33 as sticks colored according to atom name. The Graphical Representations window should look like the figure above.

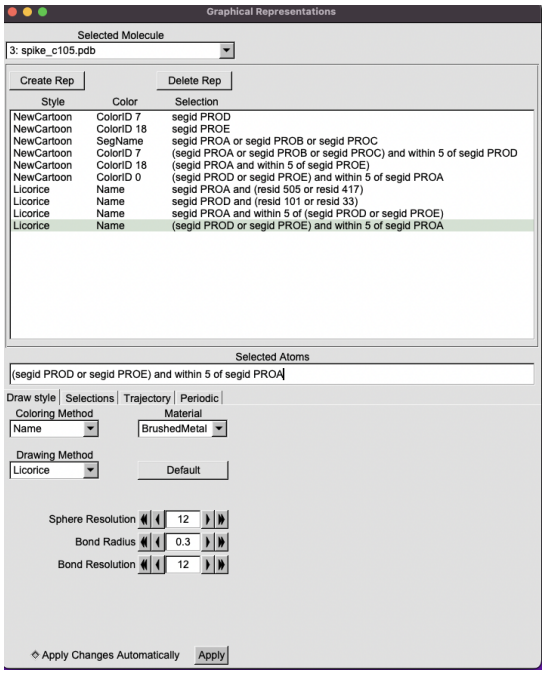
The OpenGL window should now display the Spike RBD-C105 antibody interface at the center with the two interactions we visualize shown below. Use the `Scale`, `Translate,` and `Rotate` mouse modes to further explore these interactions.

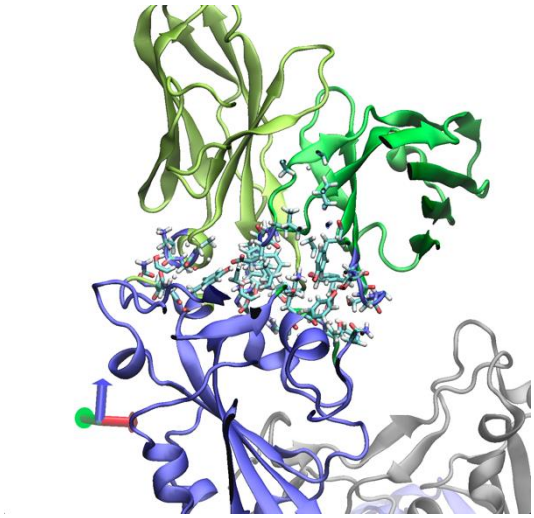


The blue coloring on the antibody structure and the green coloring on the Spike structure in the image above indicates that there are residues and interactions other than those shown involved in the binding interface. If you would like to further explore the binding interface, follow these instructions for building a representation for these other residues and use the `Scale`, `Translate,` and `Rotate` mouse modes to further explore these interactions.

1. Click `Create Rep` and change the drawing parameters to the following: `Selected Atoms` to "`segid PROA and within 5 of (segid PROD or segid PROE)`", `Coloring Method` to `Name`, `Drawing Method` to `Licorice`, and `Material` to `Brushed Metal`. This will display the Spike RBD residues within 5Å of the C105 antibody as sticks colored according to atom name.

2. Click `Create Rep` and change the drawing parameters to the following: `Selected Atoms` to "`(segid PROD or segid PROE) and within 5 of segid PROA`", `Coloring Method` to `Name`, `Drawing Method` to `Licorice`, and `Material` to `Brushed Metal`. This will display the C105 antibody residues within 5Å of the C105 antibody as sticks colored according to atom name. The Graphical Representations window should look like the figure above and the OpenGL Display window should look like the image to the right.





As you can see in the visualization, the complete set of interacting residues at the Spike RBD-C105 antibody binding site is complex. There are many residues interacting with each other. One of the difficulties in modeling is being able to sift through the overwhelming amount of data that can be extracted from these structures. By continuing to explore this interface you may be able to find interactions I have not already mentioned! Can you find any?

Unbonded atoms: When using the filter "within 5Å" you may notice in the model that some atoms appear to be disconnected from everything else in the structure and look like floating circles. This happens because not all atoms in the residue meet the filtering criteria "<5Å" so the other atoms are not shown.

Watching Trajectories

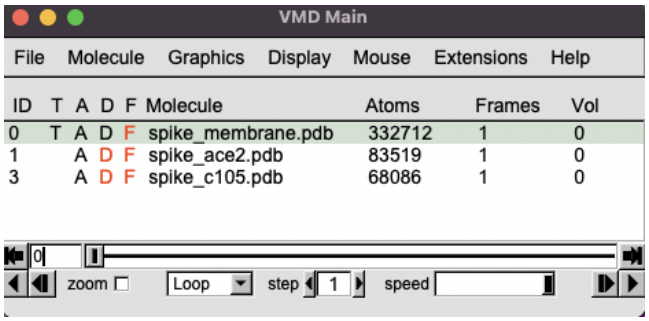
As you can see, examining single structures can be invaluable in providing insight into specific structural features in the Spike protein that are important for receptor and antibody binding. However, many proteins are also structurally dynamic and can exist in more than one structure. We call the set of structures a protein can exist in a conformational ensemble. Different structures in the conformational ensemble can have different functional states, including inactive, so understanding how proteins move between different structures is another important component of studying protein structures. We use computer simulation techniques to simulate our protein models and examine their movements. In this section we will load a molecular dynamics simulation of the Spike protein and examine the structural dynamics ins the Spike protein.

First we need to download the trajectory file.

1. Download the trajectory file from [CHARMM-GUI COVID-19 Archive] and save it to you desired location.

2. Decompress the download files by double-clicking.

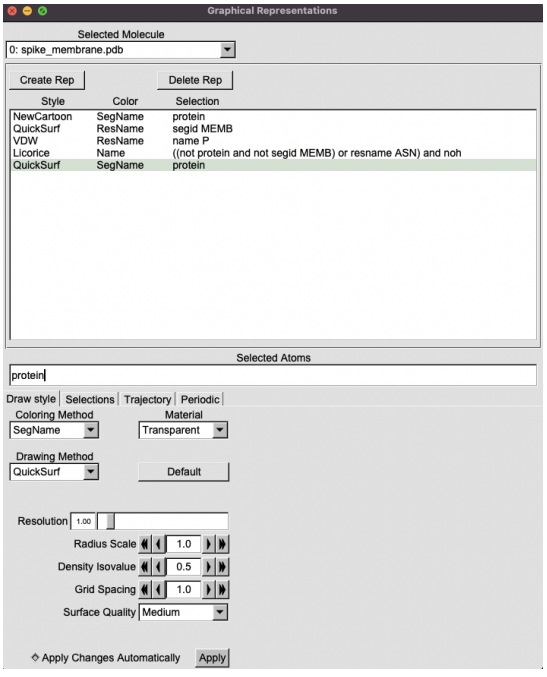
Now we need to load the trajectory. Trajectory files contain atomic velocities but no coordinates. Therefore, trajectory files must be loaded as data into a coordinate file.



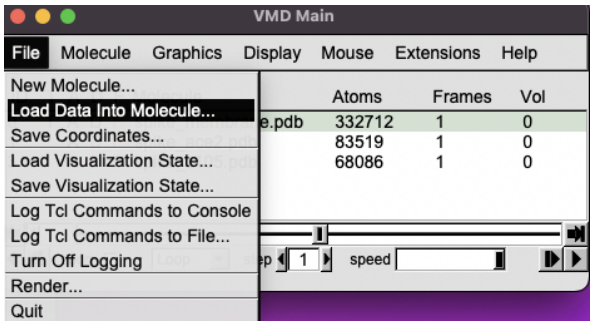
1. In the VMD main window deactivate the Spike-Ace2 receptor and Spike-C105 antibody complexes and select the Spike-Membrane complex.

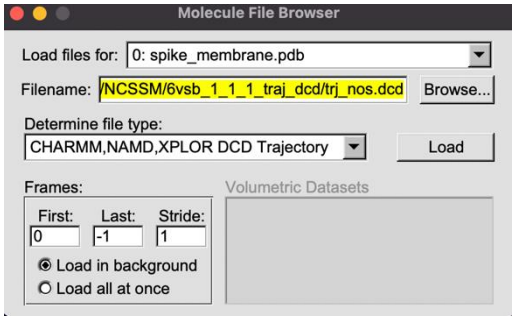
2. In the Graphical Representations window, reactivate all the representations you previously made for this model.

3. In the VMD Main window go to `File > Load Data Into Molecule…` (image on next page)

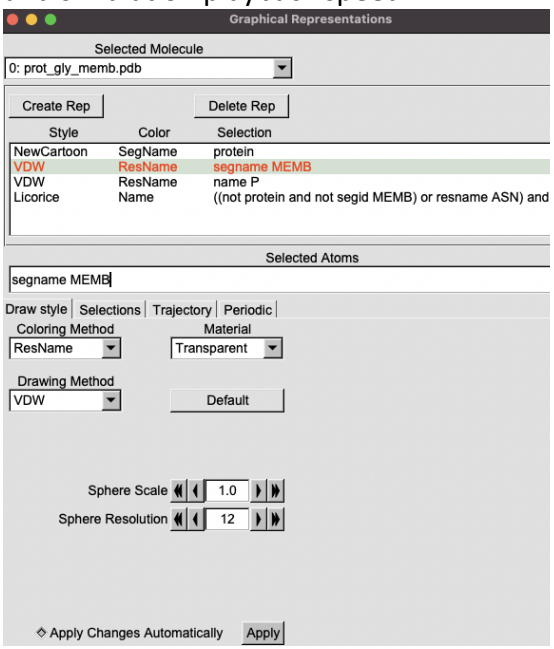


4. In the Molecule File Browser window select the trj_nos.dcd file, select DCD Trajectory as the file type, and click the Load button.



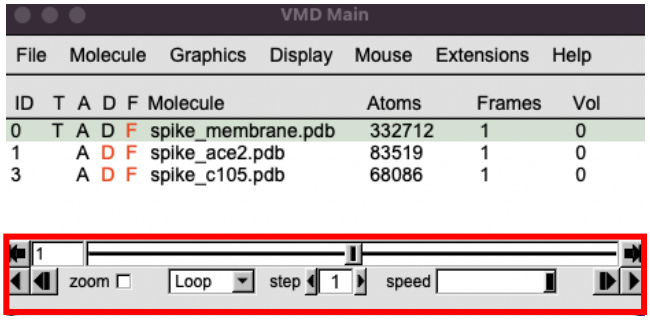


Loading speed: If you feel like the trajectory is loading too slowly, you can turn off the membrane representation (shown below) to increase the loading and simulation playback speed.



You should now be able to watch the simulation file load in. As you do so, you should see the Spike protein moving around relative to the membrane.

Use the trajectory player controls to play around with the trajectory and other loaded molecules as much as you like! What do you see? Do you notice anything interesting?



Conclusion

In this workshop we skimmed over how to use the VMD software to load and visualize Spike protein models and simulation files and how to examine these files to gain insight into the Spike function. The exercises covered in this workshop are only a small subset of the various computational tools and techniques that computational biophysicists use to explore and understand protein structures and function. Visit the VMD tutorial website to learn more about some of these other tools. There are also other software packages that are freely available (see section on Software) with many how-to articles online. You should try these out as well. You can learn more about the theory underlying these computational tools (as well as some experimental methods) at the Physical Lens on the Cell blog. And remember, you can always find interesting and important protein structure at the Protein Data Bank website.