Part 1: The changing shapes of the SARS-CoV-2 spike protein

1. What was the PDB ID for the structure of the SARS-CoV-2 spike protein in the closed conformation? 6VXX
2. What was the PDB ID for the structure of the SARS-CoV-2 spike protein in the open conformation? 6VSB
3. Capture and paste images of the SARS-CoV-2 spike protein in open and closed conformations in the table below. The table size can be adjusted to accommodate the images but keep the image height below 3 inches.

| Open conformation | Closed conformation |
| --- | --- |
|  |  |

1. Paste an image of the SARS-CoV-2 spike protein, in an open conformation, below and circle the receptor binding domain.



Part 2: Finding and annotating aligned structures

1. For PDB ID 6M0J, enter the requested information in the table below.

| Protein chain | Name of protein |
| --- | --- |
| A | Angiotensin-convertin Ezyme 2 (ACE2) |
| E | Spike protein S1 RBD |

1. In the alignment between structures 6M0J and 6XE1, what protein is found in both structures and aligned? Covid Spike RBD
2. What is the name of the antibody in 6XE1? CV30
3. After viewing the two annotated structures, does it look like the antibody in 6XE1 would interfere with the ability of the spike protein to bind to ACE2? Yes
4. What is the PDB for other structure you investigated? (Part D) 7MF1
5. Paste the image of your new structure alignment with the spike protein bound to ACE2 and the image of your new antibody bound to the spike protein in the table below. Resize the images to be less than 3 inches tall.

| Spike protein bound to ACE2 | Spike protein bound to new antibody |
| --- | --- |
|  |  |

1. Did the new antibody look like it might block binding to ACE2?

Maybe, doesn’t seem to bind the exact same place as Spike

1. Paste the Lifelong Short URL for your annotated structure below.

https://structure.ncbi.nlm.nih.gov/icn3d/share.html?JwMn1tV6AzifJee39

Part 3: Identifying amino acid contacts

1. What aligned structures did you work with? 6M0J and 6XE1
2. Paste an image below that shows the names of the aligned protein chains.

6MOJ\_E & 6XE1\_E

1. Paste images below that show all the amino acids in the spike protein that your antibody contacts. These amino acids should appear with yellow highlights.

Paste image as in the instructions

1. Which amino acids (letter and #) from both ACE2 and the antibody contact the spike protein at the same location? Which amino acids (letter and #) from only 1 of the 2 sequences (ACE2 or antibody) contact the spike protein?

6M0J only - G114, Y117, G164, Q166, T168, N169

6XE1 only - R71, T83, G84, D88, Y89, R125, K126, N128, Y141, G144, S145

Both - K85, Y121, L123, F124, A143, F154, N155, Y157, Q161, G170, Y173

Part 4: Find emerging Sars-CoV-2 variants using NextStrain.org & identify mutations

NOTE- answers will vary if students are all selecting different variants from Nextstrain. However, students should have something similar to below.

1. Record information from the sample you chose to investigate, in part B, in the table below.

| Sample ID\* | USA/DE-CDC-LC1098616/2024 |
| --- | --- |
| Collection date | 2024\_02\_14 |
| S1 mutations | 52 |
| Country | USA |
| Host | Homo sapiens |
| Clade | 23l (BA.2.86) |
| Emerging Lineage | Yes |
| GenBank accession | PP414559 |

\*This is the number at the top of the sample information. It is found above the collection date.

1. Enter the Genbank accession number for the spike protein sequence from your sample.

WWQ16339.1

Part 5: Predict the effect of spike mutations on antibody binding

NOTE- answers will vary if students are all selecting different antibodies and different variants from Nextstrain (part 4). However, students should have overall results similar to the format below.

1. What is the PDB ID for your assigned antibody? 7KMG
2. What is the name of your assigned antibody? Ly-cov555
3. What is the name of the drug that contains your antibody? Bamlanivimab
4. What is the sequence ID for your variant protein sequence? WWQ16339.1
5. Paste images below showing the highlighted amino acids in the antibody binding site in the aligned structures.
6. For each mutation in the antibody binding site, what is the original amino acid and position and the amino acid at that position. Example: E484 is replaced by an A

G339, K356, S371, S373, S375, T376….

Binding site differences: L455S, N481K, V483-, E484K, F486P

1. Investigate the potential impact of each mutation listed above on antibody binding.
2. For each pair of original and mutant amino acids, paste images below that show the interactions that would be found between the original amino acid and the antibodies, spike protein, and oxygens and the interactions that would be found when the original is replaced by the mutant amino acid.

Example:

| Original amino acid | Mutant amino acid |
| --- | --- |
| Picture |  |

1. Prepare a table as shown below and complete the information for each mutated amino acid in the antibody binding site.

Example table:

|  | E484 | Kind of bond | A484 | Kind of bond |
| --- | --- | --- | --- | --- |
| Amino acid name | Glutamic acid |  | Alanine |  |
| Heavy chain interaction(s) | R50 | Salt bridge and contacts | None |  |
|  | Y101 | Contacts | None |  |
| Light chain interaction(s) | R96 | Salt bridge | None |  |
| Oxygen interactions | O97 | Hydrogen bond, contacts | None |  |

Blank amino acid table (copy/paste this table to use for any other amino acids investigated)

|  |  | Kind of bond |  | Kind of bond |
| --- | --- | --- | --- | --- |
| Amino acid name |  |  |  |  |
| Heavy chain interaction(s) |  |  |  |  |
|  |  |  |  |  |
| Light chain interaction(s) |  |  |  |  |
| Oxygen interactions |  |  |  |  |

1. Discuss how each antibody-binding site mutation is likely to impact the ability of the antibody to bind to the SARS-CoV-2 spike protein. (ie, does it lose interactions? Or gain interactions?)