**Answer key**

Worksheet 1

**[Answer Keys](https://sciencecases.lib.buffalo.edu/collection/submit.html)**

**The answer key should repeat any questions in the case and for each provide a reasonable answer. In cases that do not include standard questions but instead require students to perform a task (draw a diagram, graph some data, write a brief essay), the key should make explicit what the task is trying to accomplish and provide guidelines for what constitutes an acceptable response and/or a sample.**

**Questions**

1. Viruses are considered to be non-living, yet they replicate. Do they follow the central dogma? Explain your answer with reference to the viral life cycle.

In general viruses do follow the central dogma, however they are using the machinery of the host cell. Virus infection introduces the nucleic acid into the host cell. For DNA viruses, the viral DNA molecule enters the nucleus to be replicated and transcribed. For RNA viruses, the RNA genome is copied by a viral-encoded reverse transcriptase to create a DNA version, which then enters the nucleus to be replicated and transcribed. The RNA molecules are transcribed in the nucleus and then translated in the cytoplasm on ribosomes. Proteins, such as the spike (S) protein pass into the rough endoplasmic reticulum and through the Golgi apparatus where glycosylation of the protein occurs.

1. The SARS CoV-2 virus infects human cells, but appears to have originally come from bats and probably an intermediate species? What might lead to a virus changing its host species or ability to infect other species?

Mutations in the viral genome, specifically the spike protein that attaches to cells, may provide an avenue for viral infection into other species. Different host species may also have different attachment protein sequences (ACE-2 for the spike protein).

1. How do you think that mRNA introduced into volunteers in the vaccination trial will produce an immune response?

The idea behind Moderna’s (and other companies) approaches is that the mRNA encoding the viral spike (S) protein is injected (using vesicles that fuse with the cells) into the host. Cells that take up the mRNA then use this to translate the protein in the cytoplasm. The protein matures as it moves through the endomembrane system becoming glycosylated by the Golgi apparatus and ultimately being expressed on the outside of the cells. This presentation of the host produced spike protein, without the other viral proteins, will hopefully produce an immune response against the virus.

Worksheet 2

**Questions:**

1. What are some advantages and disadvantages of using repurposed drugs, antibodies and vaccines in an outbreak?

Repurposed drugs have been previously approved for use by the FDA (or other governing body) and should be considered relatively safe, if used properly. This is termed off-label use. If effective (and they would need to be evaluated), they may speed up the short-term treatment of an outbreak.

Antibodies from recovered patients may also provide a short-term treatment. This is a form of passive immunity that has been used previously, including during the 1918 influenza epidemic.

Vaccines are a long-term solution that will require testing for safety and efficacy.

1. What are some of the cellular or viral targets for the repurposed drugs (see your completed Table 1)?

Some of the drugs that may be useful include nucleotide analogs (e.g., remdisiver), protease inhibitors (lopinavir), inhibition of Golgi apparatus function (hydroxychloroquine) and reverse transcriptase inhibitors. Baloxavir marboxil is a viral endonuclease inhibitor. All aspects of the viral life cycle may be targeted. In general, fewer side effects would be observed if the target is viral specific and not important for the host cell function.

1. Why is it recommended that you receive a seasonal influenza vaccination each year?

The seasonal flu is caused by a number of different viruses and each one mutates. This means that the vaccine that you receive this year is against the most recent outbreak (previous year) and may not be totally effective against the current strains.

1. How might the approach by Moderna (injecting mRNA) speed up the process of vaccination? How will the B-cell use the mRNA to produce and present the Spike protein?

Moderna’s approach (and other companies) of injecting mRNA allows the host to produce the spike protein and build an immune response without having been infected with attenuated or dead virus or requiring the long-term development of a protein based injection. The B-cell will use the mRNA, like a normal cellular mRNA, to produce the spike protein, which will undergo maturation (glycosylation) as it moves to the B-cell plasma membrane.

Worksheet 3

**Questions**

1. Why did the authors need to use B0AT1 protein to determine their protein structure even though the B0AT1 protein is not involved in the interaction between SARS-CoV-2 RBD and ACE-2 that they were trying to learn about?

The single transmembrane structure of the ACE-2 protein cannot be viewed in previous structures. The B0AT1 protein does interact with the ACE-2 protein in some cells, and is a transmembrane protein. The interaction between these two proteins allowed for visualization of the transmembrane domain of ACE-2.

1. Describe the non-covalent interactions between the S protein and the ACE-2 protein (Figure 5 of Yan *et al*., 2020) that ensure the attachment of the virus to the cell.

The S protein - ACE-2 interactions, as visualized in Yan *et al.* (2020) involve hydrogen and ionic bonds and van der Waals interactions.

1. Based on worksheet Figure 4, what are the main differences in the observed interactions between the ACE-2 PD and the SARS S glycoprotein RBD versus the SARS-CoV-2 S glycoprotein RBD. Discuss the possible functional implications of the structural differences you listed.

|  |  |  |
| --- | --- | --- |
| SARS-CoV-2 S protein RBD | SARS S protein | implications |
| K417 | D404 | Disrupts ionic bonds by altered charge |
| Q493 | N479 |  |
| L455 | Y442 | Size; hydrophobicity |
| F456 | L443 | Size; hydrophobicity |
| Y473 | F460 | Potential amino acid modification |

In text

Fill out Table 1 with the identities of the proteins corresponding to each label.

|  |  |
| --- | --- |
| 6M17A & C | Sodium-dependent neutral amino acid transporter |
| 6M17B & D | Angiotensin-converting enzyme 2 (ACE-2) |
| 6M17E & F | SARS-CoV-2 receptor binding domain |

What chemicals are included in the structure you are viewing? *By clicking on the highlighted chemical, a new window will open with the identity of that chemical. You can close the window after you get the information.*

Answer: water, N-acetyl-D-glucosamine, leucine and zinc (other structures have shown Cl in the ACE-2 protein as well, but not this structure.

Worksheet 4

**Questions**

Based on the information in Box, use the symbols under the aligned sequences to answer the following two questions.

 Example alignment

pdb|6M17|E RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNS-ASFSTF 59

AAS75868.1:306-527 RVVPSGDVVRFPNITNLCPFGEVFNATKFPSVYAWERKKISNCVADYSVLYNS-TFFSTF 59

YP\_003858584.1:310-528 RVTPTTEVVRFPNITQLCPFNEVFNITSFPSVYAWERMRITNCVADYSVLYNSSASFSTF 60

BBJ35999.1:1-186 RVQPQDTVVRFPNITNLCPFSEVFNATTFASVYAWNRKRISNCVADYSVLYNS-TSFSTF 59

AGZ48795.1:1-179 -------------------------------------------VADYSVLYNS-TSFSTF 16

AGZ48785.1:1-179 -------------------------------------------VADYSVLYNS-TSFSTF 16

ANA96090.1:310-513 RVSPSTEVVRFPNITNRCPFDRVFNASRFPSVYAWERTKISDCVADYTVLYNS-TSFSTF 59

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 K99

pdb|6M17|E KCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWN 119

AAS75868.1:306-527 KCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPGQTGVIADYNYKLPDDFMGCVLAWN 119

YP\_003858584.1:310-528 QCYGVSPTKLNDLCFSSVYADYFVVKGDDVRQIAPAQTGVIADYNYKLPDDFTGCVIAWN 120

BBJ35999.1:1-186 QCYGVSSTKLNDLCFTNVYADSFVVRGDEVRQIAPGQTGVIADYNYKLPDDFTGCVLAWN 119

AGZ48795.1:1-179 KCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPGQTGVIADYNYKLPDDFLGCVLAWN 76

AGZ48785.1:1-179 KCYGVSVTKLNDLCFSNVYADSFVVKGDDVRQIAPGQTGVIADYNYKLPDDFLGCVLAWN 76

ANA96090.1:310-513 KCYGVSPSKLIDLCFTSVYADTFLIRSSEVRQVAPGETGVIADYNYKLPDEFTGCVIAWN 119

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 Y135 Q156 F168

pdb|6M17|E SNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGF 179

AAS75868.1:306-527 TRNIDATSTGNYNYKYRYLRHGKLRPFERDISNVPFSPDGKPCTP-PALNCYWPLNDYGF 178

YP\_003858584.1:310-528 TNSLDSSN----EFFYRRFRHGKIKPYGRDLSNVLFNPSGGTCSA-EGLNCYKPLASYGF 175

BBJ35999.1:1-186 SRNQDASTSGNFNYYYRIWRSEKLRPFERDIAHYDYQVGTQF---------KSSLKNYGF 170

AGZ48795.1:1-179 TNSKDSSTSGNYNYLYRWVRRSKLNPYERDLSNDIYSPGGQSCSA-VGPNCYNPLRPYGF 135

AGZ48785.1:1-179 TNSKDSSTSGNYNYLYRWVRRSKLNPYERDLSNDIYSPGGQSCSA-VGPNCYNPLRPYGF 135

ANA96090.1:310-513 TAKQDQG-----QYYYRSSRKTKLKPFERDLSSDEN--------------GVRTLSTYDF 160

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 TN182/3

pdb|6M17|E QPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNF 223

AAS75868.1:306-527 YTTTGIGYQPYRVVVLSFELLNAPATVCGPKLSTDLIKNQCVNF 222

YP\_003858584.1:310-528 TQSSGIGFQPYRVVVLSFELLNAPATVCGPKQSTELVKNKCVNF 219

BBJ35999.1:1-186 YSSAGDSHQPYRVVVL---------------------------- 186

AGZ48795.1:1-179 FTTAGVGHQPYRVVVLSFELLNAPATVCGPKLSTDLIKNQCVNF 179

AGZ48785.1:1-179 FTTAGVGHQPYRVVVLSFELLNAPATVCGPKLSTDLIKNQCVNF 179

ANA96090.1:310-513 YPTVPIEYQATRVVVLSFELLNAPATVCGPKLSTQLIKNQCVNF 204

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1. Are the seven sequences identical, similar, or unrelated over the partial RBD? Briefly justify your answer based on your analysis of the primary structure of these domains.

The sequences are similar, not identical. Unrelated sequences should not have been part of the FASTA file. The BLASTp search will identify sequences that are identical or similar over the length of the sequence searched.

1. How conserved are the amino acids implicated in the interaction between SARS-CoV-2 (the partial RBD sequence here) and ACE-2 proteins? Include in your answer a short discussion on the similarities and differences in the physicochemical properties of the amino acids at these six positions.

Most of the amino acids identified by Yan *et al*. (2020) are not well conserved in these sequences. The exception is the Y135 position which is conserved in most (but not all) of the examples provided above.

1. How might the differences in the SARS-CoV-2 S protein sequences identified in this exercise indicate a difference in the attachment of the virus to the host cell?

The amino acids indicated as being important from the Yan *et al.* (2020) paper are not well conserved in the SARS S protein RBD or the bat RBD sequences. This could indicate that the change in amino acid sequences allowed the virus to attach more effectively to the human ACE-2 protein and allow for infection.

**Supplemental assessment**

**Sample Alignment**

 **Q24 D30 H34 Y41**

**NP\_001358344.1 MSSSSWLLLS--LVAVTAAQSTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEEN 58**

**XP\_018874749.1:1-805 MSGSSWLLLS--LVAVTAAQSTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEEN 58**

**XP\_016798468.1:1-805 MSGSSWLLLS--LVAVTAAQSTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEEN 58**

**ABU54053.1:1-805 MSGSFWFLLS--LVAVTAAQSTTEDRAKTFLDKFNHEAEDLSHESSLASWEYNTNISDEN 58**

**ADN93471.1:1-805 MSGSFWLLLS--LVAVTAAQSTTEDEAKKFLDKFNSKAEDLSYESSLASWDYNTNISDEN 58**

**XP\_017505746.1:12-805 -------------VAVTAAQSTSDEEAKTFLEKFNSEAEELSYQSSLASWNYNTNITDEN 47**

**XP\_007431942.2:29-827 -----WLCLIWSLVVLAVAQ-DVTQEAAEFLMQFDVRADDLYYDASIASWNYNTNITEEN 54**

**Q56NL1.1:1-805 MSGSFWLLLS--FAALTAAQSTTEELAKTFLETFNYEAQELSYQSSVASWNYNTNITDEN 58**

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 **M83**

**NP\_001358344.1 VQNMNNAGDKWSAFLKEQSTLAQMYPLQEIQNLTVKLQLQALQQNGSSVLSEDKSKRLNT 118**

**XP\_018874749.1:1-805 VQNMNNAGDKWSAFLKEQSTLAQMYPLQEIQNLTIKLQLQALQQNGSSVLSEDKSKRLNT 118**

**XP\_016798468.1:1-805 VQNMNNAGDKWSAFLKEQSTLAQMYPLQEIQNLTVKLQLQALQQNGSSVLSEDKSKRLNT 118**

**ABU54053.1:1-805 VQKMDEAGARWSAFYEEQSKLAKDYPLEEIQNSTVKLQLQILQQSGSPVLSEDKSKRLNS 118**

**ADN93471.1:1-805 VQKMDEAGAKWSAFYEEQSKLAKNYPLEEIQNDTVKRQLQILQQSGSPVLSEDKSKRLNS 118**

**XP\_017505746.1:12-805 VQKMNVAGAKWSTFYEEQSKIAKNYQLQNIQNDTIKRQLQALQLSGSSALSADKNQRLNT 107**

**XP\_007431942.2:29-827 AIKMHEADAVFSDFYEEASRNASKFNVNHITDETIRLQINLLQNGPTE---SSTKDQLNT 111**

**Q56NL1.1:1-805 AKNMNEAGAKWSAYYEEQSKLAQTYPLAEIQDAKIKRQLQALQQSGSSVLSADKSQRLNT 118**

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**NP\_001358344.1 ILNTMSTIYSTGKVCNPDNPQECLLLEPGLNEIMANSLDYNERLWAWESWRSEVGKQLRP 178**

**XP\_018874749.1:1-805 ILNTMSTIYSTGKVCNPNNPQECLLLEPGLNEIMANSLDYSERLWAWESWRSEVGKQLRP 178**

**XP\_016798468.1:1-805 ILNTMSAIYSTGKVCNPNNPQECLLLEPGLNEIMANSLDYNERLWAWESWRSEVGKQLRP 178**

**ABU54053.1:1-805 VLNAMSTIYSTGKVCRPNNPQECLLLEPGLDNIMGTSKDYSERLWAWEGWRAEVGKQLRP 178**

**ADN93471.1:1-805 ILNAMSTIYSTGKVCKPNNPQECLLLEPGLDNIMGTSKDYNERLWAWEGWRAEVGKQLRP 178**

**XP\_017505746.1:12-805 ILNTMSTIYSTGKVCNPGNPQECSLLEPGLDNIMESSKDYNERLWAWEGWRSEVGKQLRP 167**

**XP\_007431942.2:29-827 VLRKMSTIYSTGTVCKQDYPFDCLPLEPGLDDIMANNWDYSERLWAWEGWRADVGKKMRP 171**

**Q56NL1.1:1-805 ILNAMSTIYSTGKACNPNNPQECLLLEPGLDNIMENSKDYNERLWAWEGWRAEVGKQLRP 178**

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**NP\_001358344.1 LYEEYVVLKNEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVEHTFEEIKPLYE 238**

**XP\_018874749.1:1-805 LYEEYVVLKNEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVEHTFEEIKPLYE 238**

**XP\_016798468.1:1-805 LYEEYVVLKNEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVEHTFEEIKPLYE 238**

**ABU54053.1:1-805 LYEEYVVLKNEMARGYHYEDYGDYWRRDYETEESPGPGYSRDQLMNDVERILTEIKPLYE 238**

**ADN93471.1:1-805 LYEEYVVLKNEMARGYHYEDYGDYWRRDYETEESSGPGYSRDQLMKDVDRIFTEIKPLYE 238**

**XP\_017505746.1:12-805 LYEEYVVLKNEMARANHYEDYGDYWRGDYEAEGANGYNYSRDHLIEDVEHIFTQIKPLYE 227**

**XP\_007431942.2:29-827 LYESYVELKNKYARLRGYADYGDYWRANYEVDSPPEYQYKRAELMTDVENTFQEIKPLYE 231**

**Q56NL1.1:1-805 LYEEYVALKNEMARANNYEDYGDYWRGDYEEEWTGGYNYSRNQLIQDVEDTFEQIKPLYQ 238**

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**NP\_001358344.1 HLHAYVRAKLMNAY-PSYISPIGCLPAHLLGDMWGRFWTNLYSLTVPFGQKPNIDVTDAM 297**

**XP\_018874749.1:1-805 HLHAYVRAKLMNAY-PSYISPIGCLPAHLLGDMWGRFWTNLYSLTVPFGQKPNIDVTDAM 297**

**XP\_016798468.1:1-805 HLHAYVRAKLMNAY-PSYISPIGCLPAHLLGDMWGRFWTNLYSLTVPFGQKPNIDVTDAM 297**

**ABU54053.1:1-805 HLHAYVRTKLMDTY-PFHISPTGCLPAHLLGDMWGRFWTNLYPLTVPFGQKPNIDVTDAM 297**

**ADN93471.1:1-805 HLHAYVRAKLMDTY-PLHISPTGCLPAHLLGDMWGRFWTNLYPLTVPFGQKPNIDVTDAM 297**

**XP\_017505746.1:12-805 HLHAYVRAKLMDNY-PSHISPTGCLPAHLLGDMWGRFWTNLYPLTVPFRQKPNIDVTDAM 286**

**XP\_007431942.2:29-827 HLHAYVRRHLYKRYGPGLINPKGSLPAHLLGDMWGRFWTNLYPLMVPYPNKPNIDVTSAM 291**

**Q56NL1.1:1-805 HLHAYVRAKLMDTY-PSRISRTGCLPAHLLGDMWGRFWTNLYPLTVPFGQKPNIDVTDAM 297**

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 **K353R357**

**NP\_001358344.1 VDQAWDAQRIFKEAEKFFVSVGLPNMTQGFWENSMLTDPGNVQKAVCHPTAWDLGKGDFR 357**

**XP\_018874749.1:1-805 VDQAWDAQRIFKEAEKFFVSVGLPNMTQGFWENSMLTDPGNVQKAVCHPTAWDLGKGDFR 357**

**XP\_016798468.1:1-805 VDQAWDAQRIFKEAEKFFVSVGLPNMTQGFWENSMLTDPGNVQKAVCHPTAWDLGKGDFR 357**

**ABU54053.1:1-805 VNQGWDANRIFKEAEKFFVSVGLPNMTEGFWNNSMLTEPGDGRKVVCHPTAWDLGKDDFR 357**

**ADN93471.1:1-805 LNQGWDANRIFKEAEKFFVSVSLPKMTEGFWNKSMLTEPGDGRKVVCHPTAWDLGKGDFR 357**

**XP\_017505746.1:12-805 VNQTWDANRIFKEAEKFFVSVGLPKMTQTFWENSMLTEPGDGRKVVCHPTAWDLGKHDFR 346**

**XP\_007431942.2:29-827 VEKKWTVDSIFKAAEHFFVSIDLFNMTEGFWKNSMLEEPKDGRKVVCHPTAWDMGKKDYR 351**

**Q56NL1.1:1-805 VNQNWDARRIFKEAEKFFVSVGLPNMTQGFWENSMLTEPGDGRKVVCHPTAWDLGKGDFR 357**

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**Questions**

1. Based on the multiple sequence alignment, which amino acids from Worksheet 4 Table 2 might be:
	1. common in all of the species ACE-2 sequences

 K353 & R357 are common in the sequences shown

Y41 is common in all but one sequence

* 1. might be more unique to species closely related to humans?

The gorilla and chimpanzee sequences shown in this alignment are conserved at these particular amino acids

1. A species of *Rhinolophus* is believed to have been the host species for the SARS-CoV-2 virus. At these particular amino acids, how similar are the human and *Rhinolophus* species?

In the sample alignment above, there are two *Rhinolophus* sequences (ABU54053 and ADN93471). They only conserve the K353 and R357 amino acids of the amino acids identified in the structure. The ADN93471 sequence also has the Y41 sequence.

Supplemental ethics question

**Assignment:**

**Compare the personal choice to not vaccinate an individual with the failure to participate in social distancing during a pandemic. What are the personal and social ramifications of these choices for the person making the choice as well as others in that community? Do you consider these two choices to be equivalent? Can you think of any circumstance that would make you consider a different choice? Explain your response in a brief (500 -1000 word) essay.**

**Answers will vary based on the students. The goal of this essay is to provide the students with an opportunity to read (supplemental reading can be provided, see Teaching Notes) about social distancing and vaccinations. Students can find their own papers on these topics as well. The length can be adjusted based on the students involved.**

**One suggestion for the writing guide is to have three parts to the writing - a brief, general background on the topics being covered (social distancing vs. vaccination), a pros section for both and a cons section for both. Optionally, students can provide a personal statement. When one of the authors of this case assigns papers of this nature, the accuracy of the background and pros and cons is graded along with all of the writing format. The personal statement is graded for the writing quality, but not for the opinion.**