

How Remdesivir and Molnupiravir Interact with MERS-CoV

Lauren Hall, New Hampshire Academy of Science, 2021

Abstract

With the worldwide attention that coronavirus disease 2019 (COVID-19) has received over the past year, renewed attention has been focused on the related illness, Middle East Respiratory Syndrome (MERS). The causal infectious agent for MERS is also a coronavirus (MERS-CoV). In previous studies, the antiviral medications remdesivir and molnupiravir have been shown to have therapeutic benefits for patients infected with MERS-CoV. This study sought to better understand how remdesivir and molnupiravir interact with the MER-CoV RNA-dependent RNA polymerase, and where the antiviral medications most likely bind within the nsp7, nsp8, and nsp12 protein complex that composes the RNA-dependent RNA polymerase. The interactions between molnupiravir and remdesivir and another coronavirus, severe acute respiratory syndrome coronavirus (SARS-CoV), were modeled to give more information about the behavior of the antiviral medications across the complete Coronaviridae family. AutoDock Vina was used to model the interaction between the protein complex and each of the antiviral drugs. Both molnupiravir and remdesivir docked in nearly the same location for both MERS-CoV and SARS-CoV, suggesting that molnupiravir and remdesivir could have a similar effect on the two viruses. Both of the antiviral medications docked to the nsp12 protein in MERS-CoV and SARS-CoV, suggesting that the nsp12 protein is particularly susceptible to binding by antiviral agents, and that when the drugs are bound to the protein, the effectiveness of the polymerase is inhibited. The results from this study could lead to the development of new antiviral medications that can better target the identified site of binding within the RNA-dependent RNA polymerase of both MERS-CoV and similarly structured coronaviruses.

Introduction

Over the past two years, coronavirus disease 2019 (COVID-19) has blown through the population and caused the death of more than 600,000 people in the United States alone by the end of August 2021, according to the Centers for Disease Control and Prevention. Despite the increasing availability of vaccines, the need for improved medical care for patients with the disease remains.

The enormous attention that COVID-19 has received within the past year has helped to shed light on a related illness: Middle East Respiratory Syndrome (MERS). Similar to COVID-19, MERS is caused by a coronavirus called the Middle East Respiratory Syndrome Coronavirus (MERS-CoV). As of the end of 2021, no vaccines have been fully developed to combat the spread of MERS. Instead, antiviral drugs have risen to the forefront in the fight against MERS. These antiviral drugs include remdesivir and molnupiravir.

Gilead, a biotechnology company, first developed remdesivir (GS-5734) in 2009 to treat the Ebola virus. Remdesivir soon was viewed by many to have the potential to combat a multitude of viruses including MERS-CoV. According to a study conducted by the National Institutes of Health's Rocky Mountain Laboratories, macaques, a monkey that inhabits parts of North Africa and Asia, were treated with remdesivir (E de Wit, et al). The study contained three groups: monkeys that got treated before being infected with MERS-CoV, monkeys that got treated after being infected with MERS-CoV, and untreated monkeys (E de Wit, et al). The monkeys that got treated before being infected showed barely any symptoms compared to the untreated monkeys (E de Wit, et al). This study demonstrates that remdesivir has the potential to be hugely beneficial to humans in the fight against MERS-CoV (E de Wit, et al).

Molnupiravir (MK-4482) is a drug that was developed in 2003 by Emory University and is seen as a potential treatment for MERS and SARS. Some studies have been conducted on the effect of molnupiravir on SARS-CoV-2, and molnupiravir was shown to have reduced the symptoms in a number of the patients infected with SARS-CoV-2 (Florian Kabinger, et al).

This study seeks to understand where remdesivir and molnupiravir bind to MERS-CoV. The MERS-CoV proteins nsp7, nsp8, and nsp12 were selected to dock with molnupiravir and remdesivir, as these essential proteins carry out much of the replication and transcription duties through extensive interaction (Robert N. Kirchdoerfer, et al). The nsp12 protein is an RNA-dependent RNA polymerase, and nsp7 and nsp8 are cofactors. The structure of nsp12 has been compared to that of a cupped hand with fingers, a palm, and a thumb. Furthermore, the comparison between how the two antiviral drugs interact with MERS-CoV and SARS-CoV helped to better understand the potential use cases of these drugs.

Methods

Obtaining Protein + Antiviral Drug Structures

The primary structures of the nsp7, nsp8, and nsp12 proteins were obtained using a combination of models from the National Center for Biotechnology Information (NCBI). Using a protein structure prediction platform, Robetta, the primary structures were subsequently folded and able to be used to perform further investigations (Minkyung Baek, et al). A structure of the nsp12, nsp8, and nsp7 proteins of SARS-CoV (PDB ID: 6NUR) was retrieved from the RCSB PDB Data Bank. To get the correct protein structures, Chimera, a protein visualization platform, and Sublime Text, a code editor, were used (Eric Petterson, et al; Jon Skinner). The Protein Data Bank in Europe provided information and models of the drugs remdesivir and molnupiravir, which were each electronically assembled using Avogadro, a molecular editor and visualizer.

Binding the Proteins

Once having the folded protein and assembled drug structures, the various protein structures (nsp7, nsp8, and nsp12) were bound together in all possible varieties to calculate the

most stable binding position for all three proteins. Haddock, an application that docks proteins together and calculates the binding affinity, was the primary source through which the protein structures were bound together (G.C.P van Zundert, et al). All combinations of two out of the three protein structures were docked. Once the two proteins were docked, the proteins were renumbered using Rosie, a Rosetta application that renumbers protein structures, so that they could be clearly differentiated later on when viewing them on molecular visualization systems (S. Lyskov, et al). Subsequently, all of the bound two-protein structures were bound to the not-yet-bound protein using Haddock (G.C.P van Zundert, et al). Along with the docked protein structures, Haddock provided the energy statistics and RMSD value for each structure.

Binding the Protein Structures to the Drugs

The molecular docking software, AutoDock Vina, was used to analyze how molnupiravir and remdesivir docked with the various combinations of proteins. When preparing the proteins for docking, the water molecules were removed and the polar hydrogens and Kollman charge were added. AutoDock Vina helped to transfer the pdb file format of the protein structure into a pdbqt file (O. Trott, et al). The ligands were also prepared using AutoDock Vina. The various protein structures and the antiviral drugs were then docked using AutoDock Vina's command-line application. After running the appropriate commands for docking, "log" files containing the information about the docking and a pdbqt file of the antiviral drug in the calculated position were output.

Viewing the Docked Proteins and Drugs

After using Autodock Vina to dock the proteins and drugs, pyMOL, an application used to view molecules, was utilized to view how the drugs interacted with the protein structures (O. Trott, et al; "The PyMOL Molecular Graphics System," 2021). AutoDock Vina produced 10 positions of the drug as well as a binding affinity for each of them. PyMOL allowed for easy toggling across all of these positions. Furthermore, by viewing the proteins and the drugs docked together, a comparison between how the two drugs interacted with the same protein structure could be made. PyMOL also made comparing how the drugs interacted with the same proteins in SARS-CoV and MERS-CoV easier.

Results

nsp8 docked with the nsp7 and nsp12 structure	nsp12 docked with the nsp7 and nsp8 structure	nsp7 docked with the nsp12 and nsp8 structure
---	---	---

Cluster 9		Cluster 3		Cluster 6	
HADDOCK score	268.8 +/- 25.7	HADDOCK score	280.4 +/- 33.1	HADDOCK score	77.5 +/- 9.7
Cluster size	4	Cluster size	4	Cluster size	8
RMSD from the overall lowest-energy structure	26.1 +/- 0.1	RMSD from the overall lowest-energy structure	11.3 +/- 0.1	RMSD from the overall lowest-energy structure	0.6 +/- 0.4

Fig. 1: Energy values of nsp7, nsp8, and nsp12 when bound in three different combinations.

The nsp7 protein docked with the nsp12 and nsp8 structure had the lowest RMSD value at 0.6 +/- 0.4. The least stable protein structure was the nsp8 protein docked with the nsp7 and nsp12 structure, with an RMSD value of 26.1 +/- 0.1. Thus, the nsp7 docked with the nsp12 and nsp8 structure was the primary structure with which to bind to remdesivir and molnupiravir.

Protein Structure	MERS-CoV nsp12		MERS-CoV nsp7nsp8		MERS-CoV nsp8nsp12		MERS-CoV nsp7nsp12	
Antiviral Drug	Remdesivir	Molnupiravir	Remdesivir	Molnupiravir	Remdesivir	Molnupiravir	Remdesivir	Molnupiravir
Binding Affinity (kcal/mol)	-6.8	-4.8	-5.2	-5.5	-6.2	-5.5	-6.8	-5.5
(ranked from lowest	-6.4	-4.7	-5.1	-4.8	-6.2	-4.8	-6.4	-5.4
to highest)	-6.3	-4.7	-5.1	-4.5	-6.1	-4.5	-6.4	-5.3
	-6.3	-4.6	-4.8	-4.4	-5.9	-4.4	-6	-5.1
	-6.3	-4.6	-4.7	-4.2	-5.9	-4.2	-5.9	-5.1
	-6.2	-4.5	-4.6	-4.1	-5.8	-4.1	-5.9	-5
	-6	-4.5	-4.5	-4.1	-5.8	-4.1	-5.8	-4.8
	-5.8	-4.5	-4.5	-4.1	-5.8	-4.1	-5.8	-4.8
	-5.8	-4.4	-4.3	-4	-5.8	-4	-5.6	-4.6
Protein Structure	MERS-CoV nsp8-nsp7nsp12		MERS-CoV nsp7-nsp8nsp12		MERS-CoV nsp12-nsp8nsp7		SARS-CoV nsp12nsp8nsp7	
Antiviral Drug	Remdesivir	Molnupiravir	Remdesivir	Molnupiravir	Remdesivir	Molnupiravir	Remdesivir	Molnupiravir
Binding Affinity (kcal/mol)	-6.4	-5.8	-7.5	-5.3	-6.2	-5.2	-6.5	-5.7
(ranked from lowest	-6.1	-5.5	-6.3	-5.2	-6.1	-5.1	-6.2	-5.5
to highest)	-5.9	-5.4	-6.1	-5.2	-6.1	-5.1	-6.2	-5.5
	-5.8	-5.3	-6.1	-5	-5.8	-5.1	-6	-5.4
	-5.8	-5.2	-6	-5	-5.8	-5	-5.7	-5.2
	-5.8	-5.2	-5.9	-4.9	-5.7	-5	-5.7	-5.1
	-5.7	-5.2	-5.8	-4.8	-5.6	-4.7	-5.7	-5.1
	-5.7	-5.1	-5.7	-4.7	-5.5	-4.6	-5.6	-5
	-5.7	-5.1	-5.5	-4.6	-5.5	-4.6	-5.5	-5

Fig. 2: The binding affinities (kcal/mol) of each of the different protein structures bound with each of the drugs (remdesivir and molnupiravir). These binding affinities were obtained from the docking with AutoDock Vina. “nsp7-nsp8nsp12” denotes that the structure was created by docking the nsp7 protein with the nsp8 and nsp12 protein structure.

Overall, remdesivir had a lower binding affinity with the protein structures and an average binding affinity of -6.45 kcal/mol. Molnupiravir had an average binding affinity of -5.41 kcal/mol. When docked with the most stable three-protein structure, the nsp7 protein bound to the nsp8 and nsp12 structure, remdesivir had a binding affinity of -7.5 kcal/mol and molnupiravir had a binding affinity of -5.3 kcal/mol. When docked with the SARS protein complex, remdesivir had a binding affinity of -6.5 kcal/mol and molnupiravir had a binding affinity of -5.7 kcal/mol.

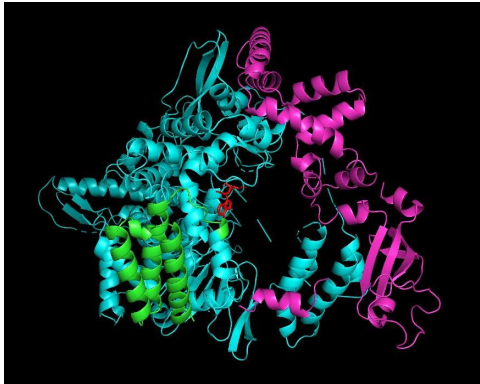
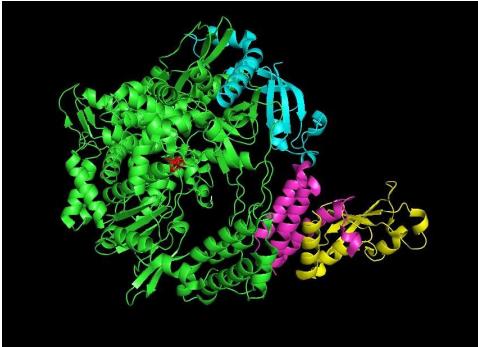
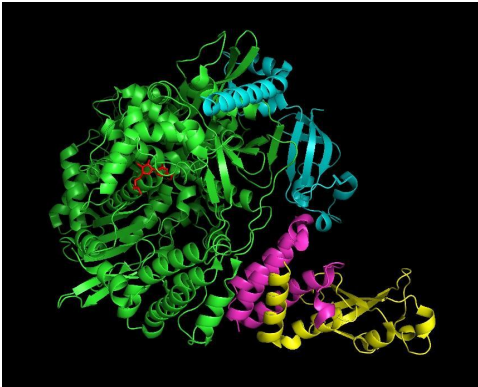
	MERS-CoV	SARS-CoV
Remdesivir		
Molnupiravir		

Fig. 3: The most stable docking position of remdesivir and mulupiravir in the nsp7, nsp8, and nsp12 protein structure in MERS-CoV and SARS-CoV. This graphical representation of the docking was created on pyMOL. The red is the ligand and the multiple colors of the MERS-CoV and SARS-CoV protein structures represent the different protein chains. In the first column for MERS-CoV, nsp12 is light blue, nsp7 is green, and nsp8 is purple. In the second column for SARS-CoV, nsp12 is green, nsp8 is light blue and yellow, and nsp7 is purple.

Remdesivir and molnupiravir bound to the MERS-CoV nsp7, nsp8, and nsp12 protein complex in nearly the exact same location: the inner pocket of the nsp12 protein. Furthermore, these two antiviral drugs docked to SARS-CoV in nearly the exact same location.

Discussion

The predicted docking locations of both remdesivir and molnupiravir when bound with MERS-CoV suggest that the two drugs have a similar effect on MERS-CoV. Furthermore, both remdesivir and molnupiravir dock with MERS similarly to SARS. As seen in Figure 3,

remdesivir and molnupiravir fit into the “palm” part of the nsp12 protein of both MERS-CoV and SARS-CoV.

The large structure of nsp12 compared to nsp7 and nsp8 suggests that nsp12 has a larger role in viral genome replication and transcription (Kyung H. Choi, et al). Remdesivir and molnupiravir would likely inhibit nsp12 from carrying out its functions properly because both remdesivir and molnupiravir find the most stable position tucked right into the “palm” of nsp12. Furthermore, when nsp12 adjusts to fit either remdesivir and molnupiravir, nsp12’s interaction with nsp7 and nsp8 is also likely to adjust slightly as well. All three of the proteins are essential for MERS-CoV to carry out key functions: transcription and replication. Therefore, molnupiravir and remdesivir are likely to affect those functions of MERS-CoV because of their docking site to the nsp12 protein. Because of how similar MERS-CoV’s structure is to that of SARS-CoV, molnupiravir and remdesivir are likely to affect SARS-CoV’s nsp7, nsp8, and nsp12 protein structure and function in similar ways.

In the future, more research should be done to study how other upcoming therapeutics, such as ribavirin, interact with MERS-CoV (David R. Strayer, et al). Remdesivir and molnupiravir are just two of the many drugs that could help to alleviate the symptoms of MERS and perhaps even prevent deaths. Further research is necessary to build a more precise understanding of how antiviral medications in use today interact with MERS-CoV. This information would be highly beneficial in the development of more powerful antivirals effective against MERS-CoV and other types of coronaviruses

Sources

- "About COVID-19." *Centers of Disease Control and Prevention*, U.S. Department of Health & Human Services, 24 May 2021, www.cdc.gov/coronavirus/2019-ncov/your-health/about-covid-19/basics-covid-19.html. Accessed 29 Aug. 2021.
- Al-Osail, Aisha M, and Marwan J Al-Wazzah. "The history and epidemiology of Middle East respiratory syndrome corona virus." *Multidisciplinary respiratory medicine* vol. 12 20. 7 Aug. 2017, doi:10.1186/s40248-017-0101-8
- Bailey-Elkin, Ben A. "Crystal Structure of the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Papain-like Protease Bound to Ubiquitin Facilitates Targeted Disruption of Deubiquitinating Activity to Demonstrate Its Role in Innate Immune Suppression." *Journal of Biological Chemistry*, vol. 289, no. 50, Dec. 2014, <https://doi.org/10.1074/jbc.M114.609644>. Accessed 29 Aug. 2021.
- Carlson, Robert, editor. "Middle East Respiratory Syndrome (MERS) Vaccines." *Coronavirus Today*, 25 Aug. 2021, www.coronavirustoday.com/mers-vaccines. Accessed 29 Aug. 2021.
- Choi, Kyung H. "Viral polymerases." *Advances in experimental medicine and biology* vol. 726 (2012): 267-304. doi:10.1007/978-1-4614-0980-9_12

- David R. Strayer, Robert Dickey and William A. Carter, "Sensitivity of SARS/MERS CoV to Interferons and Other Drugs Based on Achievable Serum Concentrations in Humans", *Infectious Disorders - Drug Targets* 2014; 14(1) .
<https://doi.org/10.2174/1871526514666140713152858>
- Eastman, Richard T., et al. "Remdesivir: A Review of Its Discovery and Development Leading to Emergency Use Authorization for Treatment of COVID-19." *ACS Central Science*, vol. 6, no. 5, 4 May 2020, pp. 672-83, <https://doi.org/10.1021/acscentsci.0c00489>. Accessed 29 Aug. 2021.
- Gordon, Calvin J., and Egor P. Tchesnokov. "The antiviral compound remdesivir potently inhibits RNA-dependent RNA polymerase from Middle East respiratory syndrome coronavirus." *Journal of Biological Chemistry*, vol. 295, no. 15, Apr. 2020, <https://doi.org/10.1074/jbc.AC120.013056>. Accessed 29 Aug. 2021.
- Hewens-Martin, Yella. "How do SARS and MERS compare with COVID-19?" *Medical News Today*, 10 Apr. 2020, www.medicalnewstoday.com/articles/how-do-sars-and-mers-compare-with-covid-19. Accessed 29 Aug. 2021.
- Kabinger, F., Stiller, C., Schmitzová, J. *et al.* Mechanism of Molnupiravir-induced SARS-CoV-2 mutagenesis. *Nat Struct Mol Biol* (2021). <https://doi.org/10.1038/s41594-021-00651-0>
- Katella, Kathy. "5 Things To Know About the Delta Variant." *Yale Medicine*, 26 Aug. 2021, www.yalemedicine.org/news/5-things-to-know-delta-variant-covid. Accessed 29 Aug. 2021.
- Kirchdoerfer, R.N., Ward, A.B. Structure of the SARS-CoV nsp12 polymerase bound to nsp7 and nsp8 co-factors. *Nat Commun* **10**, 2342 (2019).
<https://doi.org/10.1038/s41467-019-10280-3>
- "Merck Announces Supply Agreement with U.S. Government for Molnupiravir, an Investigational Oral Antiviral Candidate for Treatment of Mild to Moderate COVID-19." *Merck*, Merck Sharp & Dohme, 2021, www.merck.com/news/merck-announces-supply-agreement-with-u-s-government-for-molnupiravir-an-investigational-oral-antiviral-candidate-for-treatment-of-mild-to-moderate-covid-19/. Accessed 29 Aug. 2021.
- "Middle East Respiratory Syndrome (MERS)." *Centers for Disease Control and Protection*, U.S. Department of Health & Human Services, 2021, www.cdc.gov/coronavirus/mers/about/index.html. Accessed 29 Aug. 2021.
- Min, Jung Sun et al. "A Cell-Based Reporter Assay for Screening Inhibitors of MERS Coronavirus RNA-Dependent RNA Polymerase Activity." *Journal of clinical medicine* vol. 9, 8 2399. 27 Jul. 2020, doi:10.3390/jcm9082399
- "Molecular Docking Tutorial: AUTODOCK VINA - PART 1." *Youtube*, uploaded by Sanket Bapat, 20 Mar. 2020, youtu.be/Sux91FJ3Xe8. Accessed 29 Aug. 2021.

- "Reduction in Infectious SARS-CoV-2 in Treatment Study of COVID-19 with Molnupiravir." *Conference on Retroviruses and Opportunistic Infections*, CROI Foundation/IAS-USA, 2021,
www.croiconference.org/abstract/reduction-in-infectious-sars-cov-2-in-treatment-study-of-covid-19-with-molnupiravir/. Accessed 29 Aug. 2021.
- "Remdesivir prevents MERS coronavirus disease in monkeys." *National Institutes of Health*, U.S. Department of Health and Human Services, 13 Jan. 2020,
www.nih.gov/news-events/news-releases/remdesivir-prevents-mers-coronavirus-disease-monkeys. Accessed 29 Aug. 2021.
- Strayer, David R et al. "Sensitivity of SARS/MERS CoV to interferons and other drugs based on achievable serum concentrations in humans." *Infectious disorders drug targets* vol. 14,1 (2014): 37-43. doi:10.2174/1871526514666140713152858
- "UniProtKB - A0A0G2RC73 (A0A0G2RC73_CVHOC)." *UniProt*,
www.uniprot.org/uniprot/A0A0G2RC73. Accessed 29 Aug. 2021.
- Yazdanpanah, Yazdan, and Benoît Guéry. "Les antirétroviraux ont-ils une place dans le traitement du syndrome respiratoire aigu sévère?" [Antiretroviral drugs in severe acute respiratory syndrome]. *Presse médicale (Paris, France : 1983)* vol. 35,1 Pt 2 (2006): 105-7.
doi:10.1016/s0755-4982(06)74531-6
- G.C.P van Zundert, J.P.G.L.M. Rodrigues, M. Trellet, C. Schmitz, P.L. Kastiris, E. Karaca, A.S.J. Melquiond, M. van Dijk, S.J. de Vries and A.M.J.J. Bonvin. "The HADDOCK2.2 webserver: User-friendly integrative modeling of biomolecular complexes." *J. Mol. Biol.*, 428, 720-725 (2016).
- Minkyung Baek, Frank DiMaio, Ivan Anishchenko, Justas Dauparas, Sergey Ovchinnikov, Gyu Rie Lee, Jue Wang, Qian Cong, Lisa N. Kinch, R. Dustin Schaeffer, Claudia Millán, Hahnbeom Park, Carson Adams, Caleb R. Glassman, Andy DeGiovanni, Jose H. Pereira, Andria V. Rodrigues, Alberdina A. van Dijk, Ana C. Ebrecht, Diederik J. Opperman, Theo Sagmeister, Christoph Buhlheller, Tea PavkovKeller, Manoj K Rathinaswamy, Udit Dalwadi, Calvin K Yip, John E Burke, K. Christopher Garcia, Nick V. Grishin, Paul D. Adams, Randy J. Read, David Baker. Accurate prediction of protein structures and interactions using a 3-track network. *Science* 10.1126/science.abj8754 (2021); doi: <https://doi.org/10.1126/science.abj8754>
- Pettersen, Eric F et al. "UCSF Chimera--a visualization system for exploratory research and analysis." *Journal of computational chemistry* vol. 25,13 (2004): 1605-12.
doi:10.1002/jcc.20084
- Lyskov S, Chou FC, Conchúir SÓ, Der BS, Drew K, Kuroda D, Xu J, Weitzner BD, Renfrew PD, Sripakdeevong P, Borgo B, Havranek JJ, Kuhlman B, Kortemme T, Bonneau R, Gray JJ, Das R., "Serverification of Molecular Modeling Applications: The Rosetta Online Server That Includes Everyone (ROSIE)". *PLoS One*. 2013 May 22;8(5):e63906. doi: 10.1371/journal.pone.0063906. Print 2013. Link

O. Trott, A. J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, *Journal of Computational Chemistry* 31 (2010) 455-461

The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC.

"COVID Data Tracker Weekly Review." Centers for Disease Control and Prevention, U.S.

Department of Health & Human Services, 27 Aug.

2021, www.cdc.gov/coronavirus/2019-ncov/covid-data/covidview/index.html. Accessed 31 Aug 2021.

E de Wit et al. Prophylactic and therapeutic remdesivir (GS-5734) treatment in the rhesus macaque model of MERS-CoV infection. *PNAS* DOI: 10.1073/pnas.1922083117.

Skinner, Jon. Sublime Text. Sublime Text, Sublime HQ Pty Ltd, www.sublimetext.com/. Accessed 31 Aug. 2021.