Applying Nextstrain and iCn3D to Modify and Expand an Existing Activity for Undergraduate Students Characterizing Potential Binding of Antibodies to Mutations in the Pathogens Influenza, Respiratory Syncytial Virus (RSV), or Enterovirus D68

ERICA LANNAN[1]*, ALLYSON STERLING[2], YAN HU[3], SANDRA PORTER[4]

[1]*Department of Natural Sciences, Prairie State College, Chicago Heights, IL 60411*

[2]*Department of Biology, Regis University, Denver, CO 80221*

[3]*Department of Biology, Worcester State University, Worcester, MA 01602*

[4]*Digital World Biology LLC, Seattle, WA 98107*

**elannan@prairiestate.edu*

Abstract: Antibodies are proteins that can protect against disease using a variety of mechanisms, including binding to pathogens and targeting them for destruction. Structural modeling of antibody binding to the SARS-Cov-2 spike protein and how mutations might allow viruses to escape antibody neutralization has been previously investigated in Antibody Engineering Hackathons. The procedure for investigating immune escape can be used for students in affordable and accessible Course-Based Undergraduate Research Experiences (CUREs). In this work, we adapted and expanded the SARS-Cov-2 protocol to address new pathogens, including hookworms, Respiratory Syncytial Virus (RSV), Influenza, and Enterovirus D68. We found each presented unique challenges; however, these challenges present opportunities for student research. We describe how modifications to the SARS-Cov-2 protocol designed for SARS-CoV-2 could allow students to investigate the impact of mutations in each of these pathogens when binding to antibodies.

Keywords: iCn3D, Nextstrain, SAbDab, hookworm, enterovirus D68, RSV, Influenza, SARS-CoV-2

Introduction

Antibodies are important components of the immune system that help protect against disease. One of the functions of the antibody is neutralization, where the antibody blocks the pathogen's ability to target cellular structures. The strength of antibody binding to a particular pathogen is determined through chemical bonds and interactions that together influence the specificity of the interaction. Monoclonal antibodies are a pure collection of antibodies with identical amino acid sequences [1]. Previous hackathon teams worked to create bioinformatics activities for students involving SARS-CoV-2 and monoclonal antibodies [2-4]. In these activities, students can identify locations where antibodies bind and neutralize the SARS-CoV-2 Spike protein using 3D modeling tools (iCn3D) [2, 3, 5]. Furthermore, students can find and input new variant sequence information [6], align these variant sequences to sequences from known 3D structures, and identify mutations within the antibody binding site of the Spike protein [2, 5]. Finally, using the mutations and interactions tools within iCn3D, students can make predictions regarding the strength of antibody binding to new variants through the gain or loss of various bonds [2, 5].

The instructions and instructor guides developed through earlier hackathons [2-4] for SARS-CoV-2 were used in Bioinformatics courses at Shoreline Community College (Fall 2022), a Cellular and Molecular Biology course at Prairie State College (Spring 2023 and Fall 2023), and a Microbiology and Immunology course at Regis University (Spring 2024). The freely available databases and software employed in this project make modern research techniques available to a greater number of students and allow students to perform state-of-the-art analyses from their home or classroom computers. iCn3D [5] is used in this project because it is a free,

open-source, web-based application that does not require students to download any files. It is also the only application allowing users to share links to the annotated structure models, an important feature for evaluating student work.

Feedback from students and faculty indicated that they wanted to explore different antibody-pathogen interactions rather than continue indefinitely with SARS-Cov-2, allowing students to take ownership of the project and follow their specific interests. In this study, we broadened the approach and expanded this procedure to other pathogens and their neutralizing antibodies. To achieve this goal, we needed two important pieces of information: 1) the presence of a particular pathogen and mutation data in the Nextstrain database [6] and 2) 3D structural information for an antigenic protein in a complex with an antibody. Structures of antibody-protein complexes are deposited by researchers in the Protein Data Bank (PDB) (rcsb.org) [7] and compiled in the Structural Antibody Database (SAbDab) [8]. We describe the resources we found for four new pathogens (hookworms, Respiratory Syncytial Virus (RSV), Influenza, and Enterovirus D68), our initial results, and the challenges posed by each as they can be applied for use in student research projects.

Methods

Antibody Engineers Hackathon

The 3rd Annual Antibody Engineering Hackathon took place August 7-10th, 2023. During this event, small groups of individuals worked collaboratively and virtually to solve problems related to antibodies that could then be utilized in undergraduate classroom settings and share their results with the larger group [4]. Our group focused on exploring how we could adapt the bioinformatics approach, described previously for SARS-CoV-2 [2-4], to determine how the SARS-CoV-2 protocol may need to be adjusted to examine the impact of mutations on neutralizing antibodies, potentially resulting in escape from neutralization by antibodies. Our goal was to assist faculty who might be developing student projects or CUREs based on this procedure and their own interests.

Using SAbDab to Find PDB Antibody-Pathogen Structures

The first step of the SARS-CoV-2 protocol [2] involves locating structure files that contain antibodies that are bound to proteins from specific pathogens. SAbDab contains antibody structural data available as PDB files and is a useful source for antibody structures because the structures are standardized and annotated [8]. To adapt the SARS-CoV-2 protocol to different pathogens, we searched SAbDab for the following pathogens: influenza, hookworms (*Necator americanus, Ancylostoma ceylanicum, and Ancylostoma duodenale*), RSV, and Enterovirus D68. Then, we downloaded all available SAbDab data to a Microsoft Excel file. Available data includes the PDB file name, antibody heavy and light chain names associated with the NCBI Molecular Modeling Database (MMDB) [9], antigen names associated with the MMDB [9], organism, species where the antibody heavy and light chain was made, antigen species, references to publication(s), notes about the structure, and more [8].

Alignment of Sequence Data to Structure Data

The next step from the SARS-CoV-2 protocol [2] was to align protein sequence data from antigenic variants to the pathogen protein sequence in the structure. Using sequence data available in the Nextstrain database [6] for each of the new pathogens we analyzed, we aligned the mutation data to the structure using tools in iCn3D [5]. For influenza, sequence data was also obtained from the Influenza Virus Database. The Influenza Virus Database will be redirected to the NCBI Virus site in Fall 2024 [10]. Viral sequences can also be found through the Influenza Virus Database with a variety of filters such as nucleotide or amino acid sequence, filter for a keyword or strain name, the influenza subtype (A, B, or C), the host, the country, protein, H and N subtypes, sequence length, collection date, and release date [10].

Results and Discussion

We narrowed down a list of potential new organisms based on the availability of data and the interests of the group members. SAbDab was used in conjunction with information found in Pubmed to identify known structures for the three pathogens (RSV, Enterovirus D68, and Influenza virus) [8].

Hookworm

Parasitic hookworms may be found in contaminated soil and infect the intestines of over 400 million people globally [11]. These parasites are responsible for the loss of over 4 million disability-adjusted life years (DALY) and cause economic losses exceeding $100 billion annually [12, 13]. None of the hookworms of interest (*Necator americanus, Ancylostoma ceylanicum, and Ancylostoma duodenale*) were found in the Nextstrain database [6]. Furthermore, no structures containing hookworm proteins associated with any monoclonal antibody were detected in the SAbDab database [8]. Using the PDB database (rscb.org) [7], we identified two structures related to immune responses, *Stichodactyla helianthus* toxin (ShK)-like immunomodulatory peptide from hookworm *Ancylostoma caninum* (main host is Canids) (PDB: 2MD0) and the filarial worm *Brugia malayi* (PDB: 2MCR) [14]. Structure alignments were completed with those two peptides in iCn3D using VAST+ based on TM-align (Figure 1) [5]. Areas where the aligned sequences are highly similar (red) (Figure 1) may be good targets for antibodies in the future, as they are highly conserved regions among different hookworm species.

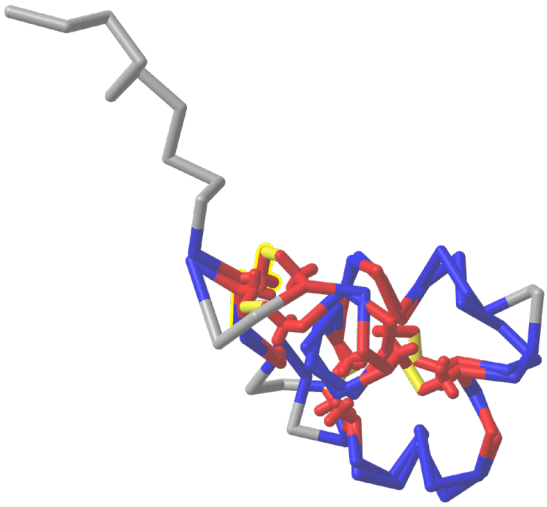

Fig. 1. ShK-like immunomodulatory peptide alignment of PDB files 2MD0 (Ancylostoma caninum) and 2MCR (Brugia malayi (filarial worm)). Red = identical amino acids, blue = different amino acids, gray = amino acids that are only found in one of the structures. Yellow = disulfide bonds https://structure.ncbi.nlm.nih.gov/icn3d/share.html?M4tnYB3gCYjoTsJa7

RSV

RSV is a non-segmented, enveloped, negative-sense RNA virus that causes cold-like symptoms that are typically mild but can be severe, particularly in the very young or immunocompromised [15]. Healthy infants under eight months of age can be given monoclonal antibodies (mAb) to prevent severe RSV symptoms [16]. Three monoclonal antibodies appear in the literature for this purpose: palivizumab, the original mAb studied for use in infants [17-19]; motavizumab, a 2nd generation derivative of palivizumab [20, 21]; and nirsevimab, a recently approved mAb, commercially known as Beyfortus [20, 22-25]. Motavizumab is not currently licensed for use due to adverse skin reactions [26, 27]. All of these antibodies bind to the Fusion Glycoprotein (F) of RSV [28, 29], which is highly conserved in RSV [30].

Using the SAbDab database [8], nine PDB structures were identified in August 2023. As of May 2024, forty-eight structures were available (Table 1). Of these structures, 64.5% contained an antibody bound to the RSV F protein F, and 14.5% contained an antibody bound to the RSV Glycoprotein (G). The remaining PDB structures included either only antibody structures or only F protein structures (Table 1). Using the Nextstrain database [6], we saw little variability in the F protein [31], so we predict that the binding region(s) will not change greatly. The G protein of RSV does mutate frequently [31], but structures of G protein-antibody complexes were not available in the SAbDab database at the time of this study (August 2023) [8].

Table 1. RSV Structures Available in SAbDab May 2024

# PDB IDs	Antigen protein	Antibody species
23	F	Human
5	F	Mouse
3	F	Llama
7	G	Human
2	F only, no antibody	Mouse
2	N/A Antibody only	Llama
2	N/A Antibody only	Cow
1	N/A Antibody only	Human
1	preF	Human
2	Other	

Structures of the RSV G protein complexed with antibodies are now available in the SAbDab database [8] and could be used for future student projects. The seven unique PDB structures in the SAbDab database [8] contain five unique neutralizing antibodies [32-34] (Table 2). Two of the structures (PDB: 5WNA (Fig. 2a) and 6BLI) include multiple copies of antibodies (3D3 and CB002.5, respectively) binding to multiple G proteins [32, 34]. One structure (PDB: 5WN9) includes the heavy chain only (antibody 2D10) bound to the G protein [32]. The other structures (PDB: 5WNB, 6UVO, 7T8W, and 6BLH (Fig. 2b)) include three different antibodies (3D3, 3G12, 3G12, and CB017.5, respectively), with each heavy and light chain in combination with a single, unique conformational state of the G protein [32-34]. The 7T8W PDB structure shows a mutated G protein bound to the 3G12 antibody [33]. To simplify the evaluation of real-time mutations for student projects, the single antibody-antigen complexes will allow students to most easily assess whether a mutation found in the Nextstrain database [6] is likely to result in loss of neutralizing activity for any of the known structures following previous protocols [35].

Table 2. RSV G Protein Containing Structures Available in SAbDab May 2024

PDB ID	Antibody name	# Antibodies	# G proteins	Reference
5WN9	2D10	H chain only	1	[32]
5WNA	3D3	2	2	[32]
5WNB	3D3	1	1	[32]
6BLH	CB017.5	1	1	[34]
6BLI	CB002.5	4	4	[34]
6UVO	3G12	1	1	[32]
7T8W	3G12	1	1, mutated	[32]

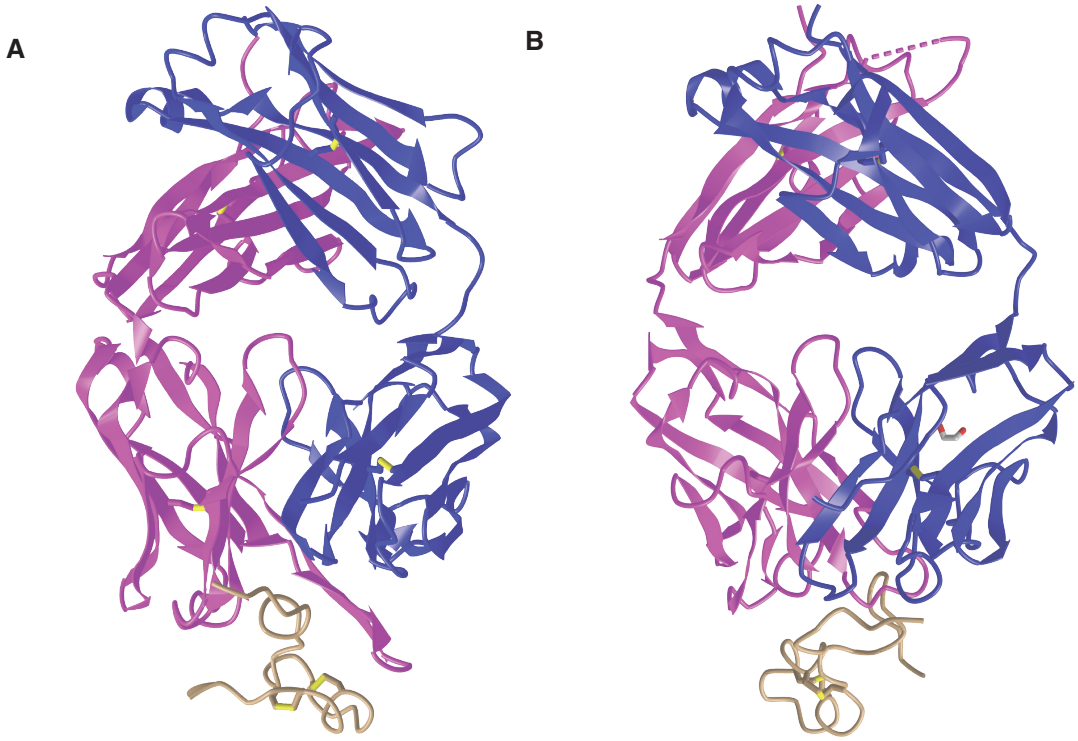

Fig. 2: Representative PDB structures showing RSV G protein and antibody complexes binding to specific portions of the G protein. (A) 5WNA: Structure of antibody 3D3 bound to the central conserved region of RSV G protein. https://structure.ncbi.nlm.nih.gov/icn3d/share.html?NDTWQ34p1pbgHPCh7 Pink = heavy chain of the antibody, Blue = light chain of the antibody, Brown = RSV G protein. (B) 6BLH: Structure of RSV G central conserved region bound to antibody Fab CB017.5 https://structure.ncbi.nlm.nih.gov/icn3d/share.html?xsQadbUuKn6CJRuz9 Pink = heavy chain of the antibody, Blue = light chain of the antibody, Brown = RSV G protein.

Influenza

Influenza A is a segmented, enveloped, negative-sense RNA virus that causes influenza. This highly contagious virus is categorized into subtypes by the Hemagglutinin (HA) and Neuraminidase (NA) glycoproteins present on the surface of the virus [36]. The mutations associated with this virus lead to frequent changes in HA/NA sequences [37] and loss of protection from previous antibodies. Thus, finding vaccines effective against the differing strains of the virus is critical due to the economic and health impacts of influenza infections [38-41].

Using the SAbDab database [8], we found many structures of antibodies associated with influenza (268 unique identifiers). We selected one PDB structure for further study, a "Broadly reactive antibody" that binds with the hemagglutinin protein (HA1) from the 2009 Pandemic strain (PDB: 4M5Z) (Fig. 3a) [42].

To see if the term “Broadly reactive antibody” was still accurate for current strains of influenza, we first looked for sequence information in the Nextstrain database [6]. Unfortunately, direct links to Genbank accession numbers were unavailable at the time of this study. To avoid manually entering all of the mutations, we searched elsewhere for sequence information on isolates. The NCBI's Influenza Virus Database provides sequence information on influenza that is linked directly to accession numbers for the gene(s) of interest [10]. As a proof of concept, we searched for sequences isolated from 2023 using the parameters of influenza type A, human host, any country, H1, N1, full length only, and collapsed identical sequences. The first sample that contained a full genome sequence and accession number in the list was from an H1N1 influenza strain (OQ615380) [43]. We aligned the HA protein sequence (WEI46805.1) from this strain to the hemagglutinin protein from the 2009 Pandemic strain (PDB: 4M5Z) in iCn3D using BLAST (Fig. 3b/c) [5].

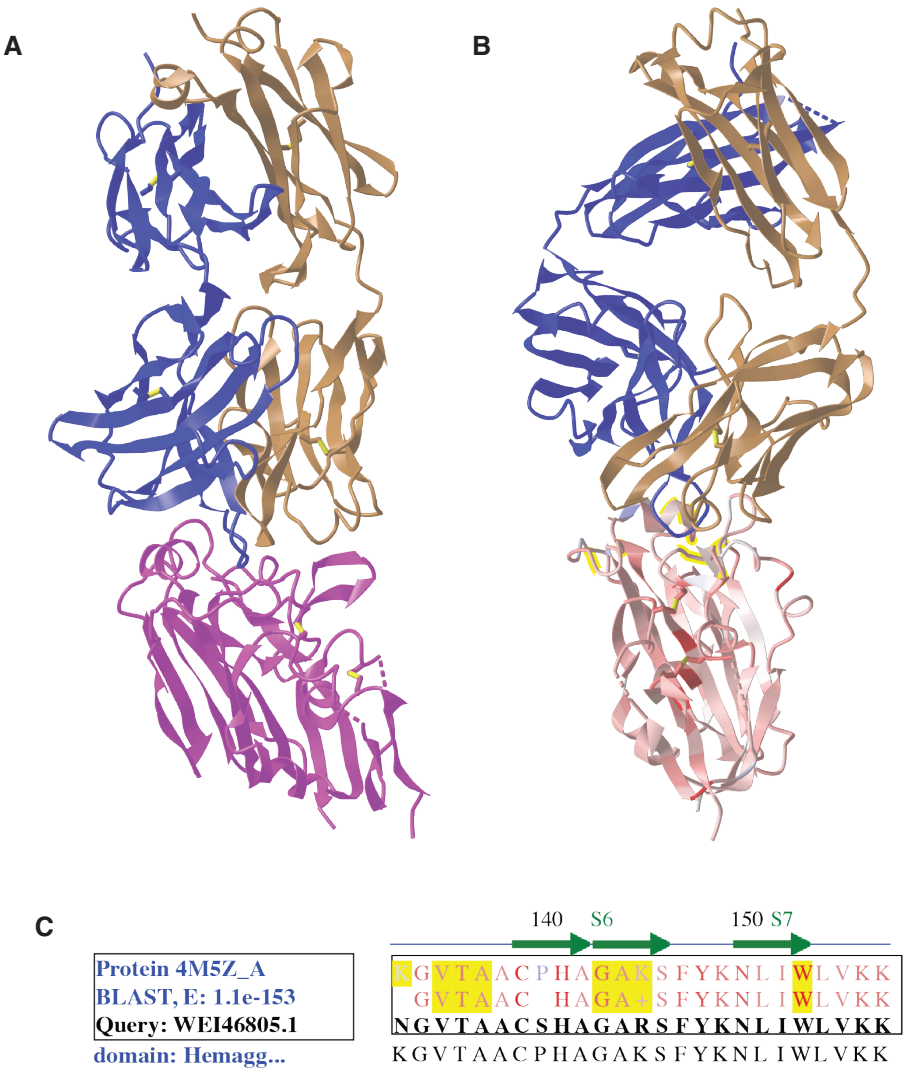

Fig. 3: Representative PDB structures showing Influenza HA1 protein and antibody complexes. (A) 4M5Z: Crystal structure of the broadly neutralizing antibody 5J8 bound to 2009 pandemic influenza hemagglutinin, HA1 subunit [5]. Pink = HA1 subunit, Blue = heavy chain of the antibody, Brown = light chain of the antibody. (B) HA protein sequence from variant (WEI46805.1) aligned to the HA structure in 4M5Z, with antibody binding domain amino acids highlighted in yellow [5]. On HA (bottom): the intensity of red represents the similarity and importance of the aligned amino acids, and Blue = different amino acids. https://structure.ncbi.nlm.nih.gov/icn3d/share.html?Ej7qWpxs48aQPGpd6 (C) Amino acid sequence alignment with amino acids in the antibody-binding domain highlighted yellow. The amino acid numbers are indicated along the top, and the green arrows indicate elements of secondary structure (S6 = sheet 6 and S7 = sheet 7). The top pink sequence is from the HA protein of 4M5Z. A blue color is used to indicate amino acids that differ (mutations) between the HA protein in 4M5Z and the amino acids in the third sequence, from the variant (WEI46805.1) (shown in black). The sequence between the 4M5Z hemagglutinin and the variant shows amino acids that are found in the same position in both HA proteins. An empty space is used when the amino acids differ and are not chemically similar. A "+" indicates that the two amino acids are different but chemically similar. The bottom sequence (black) is the consensus sequence of the hemagglutinin domain.

The HA1 sequences were very similar, as indicated by the red/pink coloration of the aligned amino acids (Fig. 3b). After verifying that both sequences came from the H1 version of hemagglutinin, we used the Select by Distance function in iCn3D [5] to identify the antibody binding region on the HA protein [2,35]. Within this binding region, most of the amino acids remained the same (Fig. 3c). We chose instances where a lysine (K) was replaced by an arginine (R) and a glutamine (Q) by a glutamic acid (E) for further investigation. The lysine (K) to arginine (R) mutation at position 145 appears to change interactions significantly (Fig. 4a). A pi-cation interaction between HA1 K145 and Y100 on the antibody heavy chain is lost, along with the following bonds from K145 to the light chain: hydrogen bonds to G29 and K31; hydrophobic interactions with T30, K31, V32, and N66; and a salt bridge ionic interaction with K51 (Fig. 4a). The glutamine (Q) to glutamic acid (E) mutation at 192 does not change interactions with the amino acids in the antibody, as indicated by the lack of different interaction lines (Fig. 4b) and only one hydrophobic interaction occurs at N32 of the heavy chain. As a consequence, this individual change is unlikely to have an effect on HA-antibody interactions. Although many amino acid interactions appear to be maintained within the predicted binding domain, and the change at 192 did not have any effect, significant changes did occur when amino acid 145 was mutated. Thus, we predict that the antibody would not be able to neutralize this variant of the virus. However, the loss of this interaction would need to be experimentally confirmed in a wet lab setting.

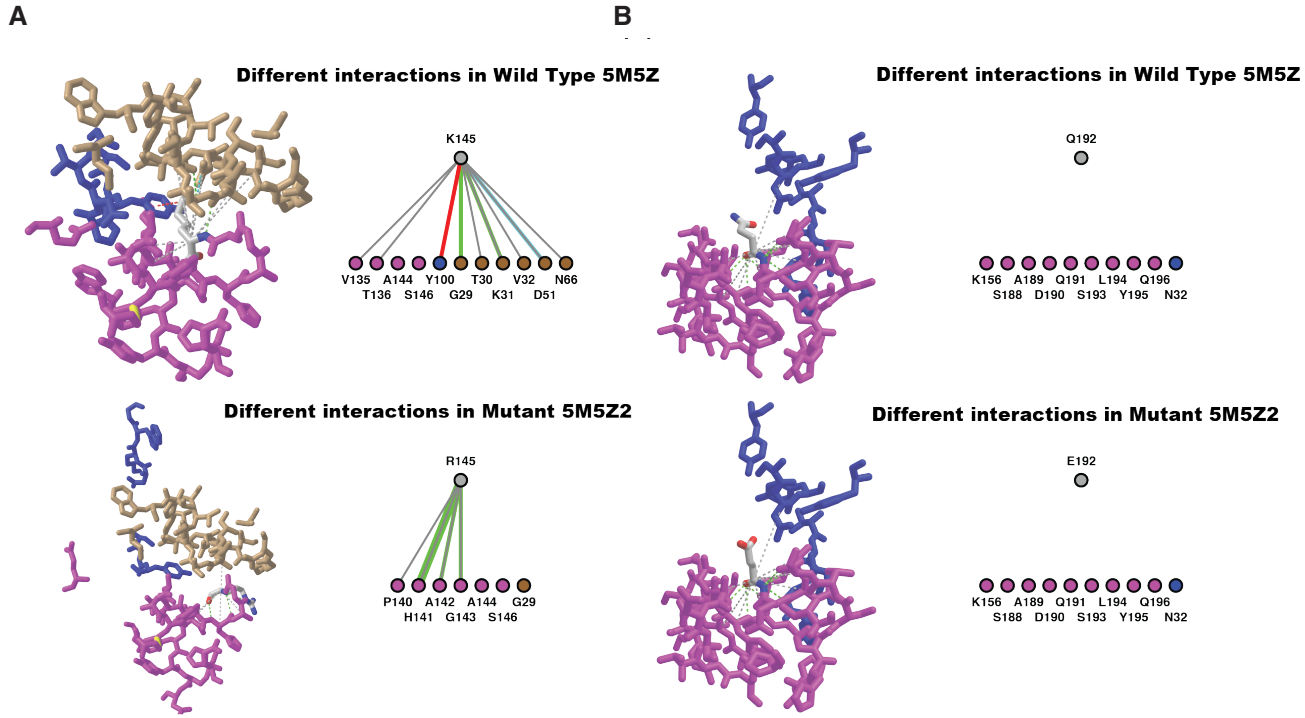

Fig. 4: Impact of mutations on predicted binding between antibody 5J8 and Influenza HA1 PDB structure 4M5Z. (A) HA1 amino acid K145 interactions with antibody 5J8. Top original K145. Bottom variant R145. Right: The color of the lines indicates the type of bond or chemical interaction. Green: H-bonds, Cyan: Salt Bridge/Ionic, Grey: Contacts, Red: π-Cation. Pink amino acids: HA1 Blue: heavy chain of the antibody. Brown: light chain of the antibody. https://structure.ncbi.nlm.nih.gov/icn3d/share.html?4RN9GsSVFZjdFZP96 (B) Neither Q192 (original) nor E192 (the variant) show different interactions with amino acids in the antibody, indicating that the binding is the same in both structures. Top original Q192. Bottom variant E192. https://structure.ncbi.nlm.nih.gov/icn3d/share.html?qFKdLueWN8ukBfD57

Enterovirus D68

Enterovirus D68 is a single, positive-stranded RNA picornavirus that typically causes cold-like symptoms, including runny nose, sneezing, coughs, and body aches [44]. Severe symptoms such as difficulty breathing or even a polio-like illness with extremity weakness, difficulty swallowing, or facial weakness can occur [44]. Children and infants are particularly at risk for disease, which can be spread by coughs, sneezes, or touching a contaminated surface [44]. Currently, no specialized treatments for this infection exist [44], so understanding the binding between potential monoclonal antibody therapeutics and Enterovirus proteins would benefit both basic research endeavors and have potential clinical implications.

The capsid of Enterovirus D68 contains four proteins: Viral Protein (VP) 1, VP2, VP3, and VP4. VP1 is the most variable protein, followed by VP2, VP3, and then VP4 [6,45]. Eight PDB structures were identified through the SAbDab database [8] in August 2023. The same eight structures remained the only available structures in May 2024 in the SAbDab database [8]. Three of the eight structures included different conformational states of the complex when the pathogen was bound with the 2H12 antibody. These three structures included all four of the viral capsid proteins (PDB: 7EBZ (Fig. 5a), 7ECY, and 7EBR) [46]. Three of the eight structures contained both antibodies and multiple capsid proteins (PDB: 6WDS, 6WDT [47], and 7EC5 [46]). The two structures where the antibody contacts only one of the capsid proteins (PDB: 6AJ9 and 6AJ7 (Fig. 5b) [48]) were the easiest to assess whether a mutation found in the Nextstrain data would be likely to result in loss of binding [35].

Table 3. Enterovirus D68 Capsid Protein Containing Structures Available in SAbDab May 2024

PDB ID	Antibody name	Capsid proteins	Reference
6WDT	EV68-228	VP1, 3	[47]
6WDS	EV68-159	VP1-3	[47]
7EBZ	2H12	VP1-4	[46]
7ECY	2H12	VP1-4	[46]
7EBR	2H12	VP1-4	[46]
7EC5	8F12	VP1, 3 genome polyprotein	[46]
6AJ7	15C5	VP3	[48]
6AJ9	11G1	VP1	[48]

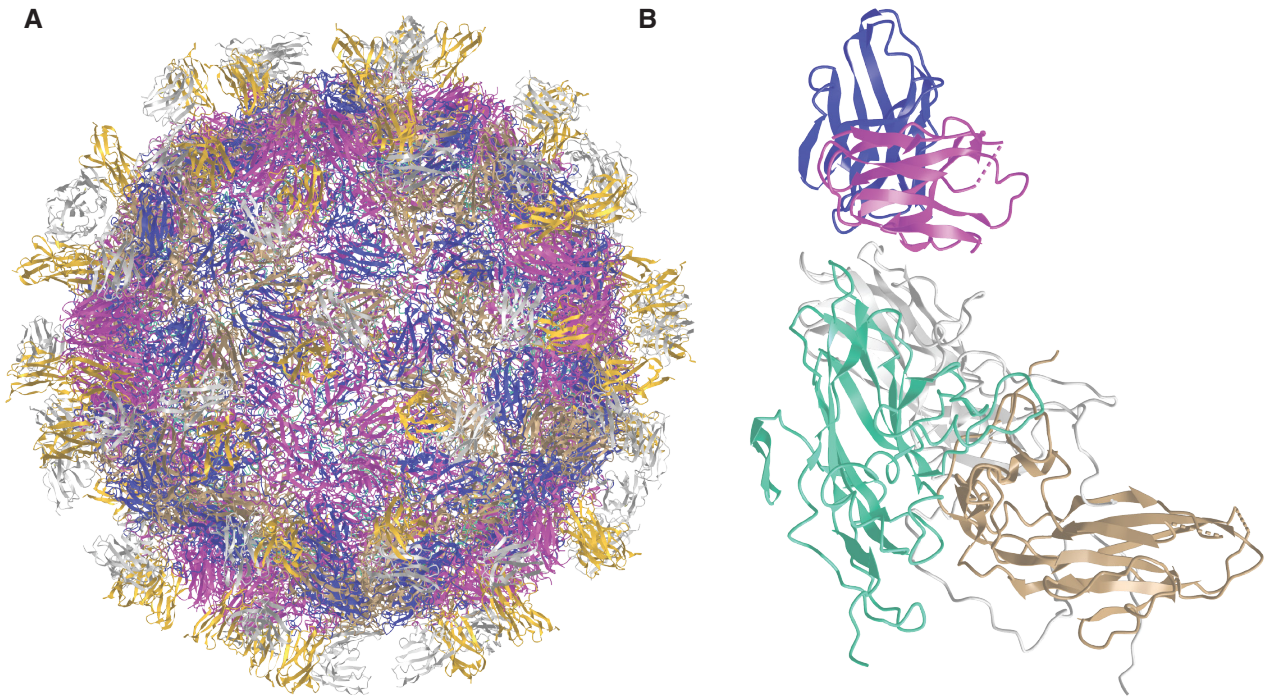

Fig. 5: Representative PDB structures showing Enterovirus D68 capsid proteins and antibody complexes. (A) 7EBZ: EV-D68 complete capsid structure in complex with 2H12 Fab. Pink: Capsid protein VP1, Blue: VP3, Brown: VP2, Green: VP4, Grey: Antibody heavy chain, Gold: Antibody light chain. https://structure.ncbi.nlm.nih.gov/icn3d/share.html?EJ3g1tq5QNXEvTKN6 (B) 6AJ7: Three EV-D68 capsid proteins in complex with antibody (Fab 15C5). Pink: Light chain of the antibody, Blue: Heavy chain of the antibody, Grey: VP3, Green: VP2, Brown: VP1. https://structure.ncbi.nlm.nih.gov/icn3d/share.html?1JMtcnqnRkzw62NQ8

Conclusion

Detailed instructions and an instructor guide are available for the original and updated protocols for analyzing the effect of mutations on antibody binding with SARS-Cov-2 [2,35]. In this work, we identified a number of specific modifications to that protocol that were needed in order to predict whether mutations in different pathogens beyond SARS-Cov-2 will be neutralized by an antibody. First, the sequence of the reference strain of the pathogen is needed. Furthermore, that pathogen should mutate rapidly and have a large number of variants within the proteins being studied, which are most likely to be antigens found on the surface of the pathogen that can interact with neutralizing antibodies. The variant protein sequence information is needed in NCBI accession number format, or a FASTA sequence, for alignment purposes. Some pathogen variant sequence information can be found on the website Nextstrain.org, but other pathogens may need to be found at the NCBI or other databases.

Additionally, PDB files containing pathogen protein/antibody complexes are needed in order to analyze the structures using iCn3D [5]. PDB files for many pathogen proteins can be found using SAbDab [8] or other databases. These structures are needed in order to align variant sequences, identify if mutations are happening within the antibody binding region on the pathogen protein, and identify any disruptions to the antibody-antigen bonds and interactions. Furthermore, we found that it can be very difficult to analyze the antibody-antigen interactions if the structure is composed of a complex of proteins. Thus, single pathogen proteins/antibody structures are preferred whenever possible. Finally, we also saw that the way the structures may be annotated with colors and chain lettering in the NCBI Molecular Modeling Database (MMDB) [9] might result in confusion, as in Figure 5a versus 5b, that can be addressed by changing color annotations in iCn3D [5].

To use this project with students, we recommend that instructors advise students to focus on structures that contain a single antibody-antigen interaction. Additionally, students need to be able to correctly identify and distinguish between the heavy and light chains of the antibody and the pathogen target. Finally, we highly recommend that instructors require students to submit the url of the structure alignments. The ability to look at the same structure as the student greatly helps with troubleshooting and identifying the source of errors. Our results demonstrate that the SARS-CoV-2 protocols can be applied to other pathogens, provided that the following conditions are met: the sequence information for the pathogen is available, the pathogen proteins of interest have a high level of mutability, and neutralizing antibody structures (PDB files) are available. A "quick start" guide is located under supplemental materials to give instructors additional information on the websites and protocols used.

Acknowledgments. This work was supported by the National Science Foundation (NSF) under the award DUE 2055036.

Disclosures. The authors declare no conflicts of interest.

Supplemental Materials. Please see https://micronanoeducation.org/wp-content/uploads/2024/09/2340461-supplemental-information-quickstart-guide.pdf

References

[1] J. P. Manis, "Overview of therapeutic monoclonal antibodies," UpToDate. Accessed: Aug. 25, 2024. [Online]. Available: https://medilib.ir/uptodate/show/3970

[2] S. Porter, U. Hilgert, E. Lannan, and M. Stieber, "Evaluating the potential for immune escape: how likely is an antibody to protect against a specific SARS-CoV-2 variant?," *Antibody Engineering*, 2022, doi: 10.25334/82D0-AT65.

[3] S. Porter and T. Smith, "Combining iCn3D and NextStrain to create a novel undergraduate research experience around SARS-CoV-2 variants and commercial antibodies," *Front. Genet*., vol. 14, 2023, doi: 10.3389/fgene.2023.1024063.

[4] S. Porter et al., "Igniting Creativity: Hackathons for Developing Undergraduate Research Projects in Antibody Engineering," *J. Adv. Technol. Educ. J ATE*, vol. 2, no. 2, Jul. 2023, doi: 10.5281/zenodo.8124853.

[5] J. Wang et al., "iCn3D: From Web-Based 3D Viewer to Structural Analysis Tool in Batch Mode," *Front. Mol. Biosci.*, vol. 9, Feb. 2022, doi: 10.3389/fmolb.2022.831740.

[6] J. Hadfield et al., "Nextstrain: real-time tracking of pathogen evolution," *Bioinformatics*, vol. 34, no. 23, pp. 4121–4123, Dec. 2018, doi: 10.1093/bioinformatics/bty407.

[7] H. M. Berman et al., "The Protein Data Bank," *Nucleic Acids Res*., vol. 28, no. 1, pp. 235–242, Jan. 2000, doi: 10.1093/nar/28.1.235.

[8] J. Dunbar et al., "SAbDab: the structural antibody database," *Nucleic Acids Res*., vol. 42, no. (Database Issue), pp. D1140-6, Jan. 2014, doi: 10.1093/nar/gkt1043.

[9] T. Madej et al., "MMDB and VAST+: tracking structural similarities between macromolecular complexes," *Nucleic Acids Res*., vol. 42, no. Database issue, pp. D297–D303, Jan. 2014, doi: 10.1093/nar/gkt1208.

[10] Y. Bao et al., "The Influenza Virus Resource at the National Center for Biotechnology Information | Journal of Virology," *J. Virol*., vol. 82, no. 2, Jan. 2008, doi: 10.1128/jvi.02005-07.

[11] CDC, "About Hookworm," Soil-Transmitted Helminths. Accessed: Aug. 25, 2024. [Online]. Available: https://www.cdc.gov/sth/about/hookworm.html

[12] J. Hamory, E. Miguel, M. Walker, M. Kremer, and S. Baird, "Twenty-year economic impacts of deworming," *Proc. Natl. Acad. Sci*., vol. 118, no. 14, p. e2023185118, Apr. 2021, doi: 10.1073/pnas.2023185118.

[13] A. Montresor, P. Mwinzi, D. Mupfasoni, and A. Garba, "Reduction in DALYs lost due to soil-transmitted helminthiases and schistosomiasis from 2000 to 2019 is parallel to the increase in coverage of the global control programmes," *PLoS Negl. Trop. Dis.*, vol. 16, no. 7, Jul. 2022, doi: 10.1371/journal.pntd.0010575.

[14] S. Chhabra et al., "Kv1.3 channel-blocking immunomodulatory peptides from parasitic worms: implications for autoimmune diseases," *FASEB J.*, vol. 28, no. 9, pp. 3952–3964, Sep. 2014, doi: 10.1096/fj.14-251967.

[15] CDC, "Respiratory Syncytial Virus (RSV)," Respiratory Syncytial Virus Infection (RSV). Accessed: Aug. 25, 2024. [Online]. Available: https://www.cdc.gov/rsv/index.html

[16] CDC, "Immunizations to Protect Infants," Respiratory Syncytial Virus Infection (RSV). Accessed: Aug. 25, 2024. [Online]. Available: https://www.cdc.gov/rsv/immunizations-protect-infants/index.html

[17] A. L. Rogovik, B. Carleton, A. Solimano, and R. Goldman, "Palivizumab for the prevention of respiratory syncytial virus infection," *Can. Fam. Physician*, vol. 56, no. 8, pp. 769–772, Aug. 2010.

[18] T. F. Feltes et al., "Palivizumab prophylaxis reduces hospitalization due to respiratory syncytial virus in young children with hemodynamically significant congenital heart disease," *J. Pediatr.*, vol. 143, no. 4, pp. 532–540, Oct. 2003, doi: 10.1067/s0022-3476(03)00454-2.

[19] "Palivizumab, a humanized respiratory syncytial virus monoclonal antibody, reduces hospitalization from respiratory syncytial virus infection in high-risk infants. The IMpact-RSV Study Group," *Pediatrics*, vol. 102, no. 3 Pt 1, pp. 531–537, Sep. 1998.

[20] M. Sun et al., "Monoclonal Antibody for the Prevention of Respiratory Syncytial Virus in Infants and Children: A Systematic Review and Network Meta-analysis," *JAMA Netw. Open*, vol. 6, no. 2, Feb. 2023, doi: 10.1001/jamanetworkopen.2023.0023.

[21] K. L. O'Brien et al., "Efficacy of motavizumab for the prevention of respiratory syncytial virus disease in healthy Native American infants: a phase 3 randomised double-blind placebo-controlled trial," *Lancet Infect. Dis.*, vol. 15, no. 12, pp. 1398–1408, Dec. 2015, doi: 10.1016/S1473-3099(15)00247-9.

[22] J. M. Jones et al., "Use of Nirsevimab for the Prevention of Respiratory Syncytial Virus Disease Among Infants and Young Children: Recommendations of the Advisory Committee on Immunization Practices - United States, 2023," *MMWR Morb. Mortal. Wkly. Rep.*, vol. 72, no. 34, pp. 920–925, Aug. 2023, doi: 10.15585/mmwr.mm7234a4.

[23] S. B. Drysdale et al., "Nirsevimab for Prevention of Hospitalizations Due to RSV in Infants," *N. Engl. J. Med.*, vol. 389, no. 26, pp. 2425–2435, Dec. 2023, doi: 10.1056/NEJMoa2309189.

[24] L. L. Hammitt et al., "Nirsevimab for Prevention of RSV in Healthy Late-Preterm and Term Infants," *N. Engl. J. Med.*, vol. 386, no. 9, pp. 837–846, Mar. 2022, doi: 10.1056/NEJMoa2110275.

[25] M. P. Griffin et al., "Single-Dose Nirsevimab for Prevention of RSV in Preterm Infants," *N. Engl. J. Med.*, vol. 383, no. 5, pp. 415–425, Jul. 2020, doi: 10.1056/NEJMoa1913556.

[26] T. F. Feltes, H. M. Sondheimer, R. M. R. Tulloh, K. M. Jensen, G. A. Losonsky, and M. P. Griffin, "A Randomized Controlled Trial of Motavizumab Versus Palivizumab for the Prophylaxis of Serious Respiratory Syncytial Virus Disease in Children With Hemodynamically Significant Congenital Heart Disease" *Pediatr. Res.*, vol. 70, pp. 186–191, Aug. 2011, doi: https://doi.org/10.1203/PDR.0b013e318220a553.

[27] American Academy of Pediatrics Committee on Infectious Diseases and American Academy of Pediatrics Bronchiolitis Guidelines Committee, "Updated guidance for palivizumab prophylaxis among infants and young children at increased risk of hospitalization for respiratory syncytial virus infection," *Pediatrics*, vol. 134, no. 2, pp. e620-638, Aug. 2014, doi: 10.1542/peds.2014-1666.

[28] S. Johnson et al., "Development of a humanized monoclonal antibody (MEDI-493) with potent *in vitro* and *in vivo* activity against respiratory syncytial virus," *J. Infect. Dis*., vol. 176, no. 5, pp. 1215–1224, Nov. 1997, doi: 10.1086/514115.

[29] Q. Zhu et al., "A highly potent extended half-life antibody as a potential RSV vaccine surrogate for all infants," *Sci. Transl. Med*., vol. 9, no. 388, p. eaaj1928, May 2017, doi: 10.1126/scitranslmed.aaj1928.

[30] J. S. McLellan, W. C. Ray, and M. E. Peeples, "Structure and function of respiratory syncytial virus surface glycoproteins," *Curr. Top. Microbiol. Immunol*., vol. 372, pp. 83–104, 2013, doi: 10.1007/978-3-642-38919-1_4.

[31] K. Kistler, "RSV-A Adaptation." Accessed: Aug. 25, 2024. [Online]. Available: https://blab.github.io/atlas-of-viral-adaptation/rsv_a/

[32] S. O. Fedechkin, N. L. George, J. T. Wolff, L. M. Kauvar, and R. M. DuBois, "Structures of respiratory syncytial virus G antigen bound to broadly neutralizing antibodies," *Sci. Immunol*., vol. 3, no. 21, Mar. 2018, doi: 10.1126/sciimmunol.aar3534.

[33] S. O. Fedechkin, N. L. George, A. M. Nuñez Castrejon, J. R. Dillen, L. M. Kauvar, and R. M. DuBois, "Conformational Flexibility in Respiratory Syncytial Virus G Neutralizing Epitopes," *J. Virol*., vol. 94, no. 6, Feb. 2020, doi: 10.1128/JVI.01879-19.

[34] H. G. Jones et al., "Structural basis for recognition of the central conserved region of RSV G by neutralizing human antibodies," *PLoS Pathog*., vol. 14, no. 3, Mar. 2018, doi: 10.1371/journal.ppat.1006935.

[35] A. Sterling, E. Lannan, and S. Porter, "Evaluating the potential for immune escape: how likely is an antibody to protect against a specific SARS-CoV-2 variant? Version 2.0," *Antibody Engineering*, doi: 10.25334/Q7KG-5V20.

[36] A. Hashem, "Prospects of HA-Based Universal Influenza Vaccine," *BioMed Res. Int*., vol. 414637, 2015, doi: 10.1155/2015/414637.

[37] K. Kistler, "H3N2 Adaptation." Accessed: Aug. 25, 2024. [Online]. Available: https://blab.github.io/atlas-of-viral-adaptation/h3n2/

[38] C. de Courville, S. M. Cadarette, E. Wissinger, and F. P. Alvarez, "The economic burden of influenza among adults aged 18 to 64: A systematic literature review," *Influenza Other Respir. Viruses*, vol. 16, no. 3, pp. 376–385, May 2022, doi: 10.1111/irv.12963.

[39] A. E. Macias et al., "The disease burden of influenza beyond respiratory illness," *Vaccine*, vol. 39, pp. A6–A14, Mar. 2021, doi: 10.1016/j.vaccine.2020.09.048.

[40] A. Gordon and A. Reingold, "The Burden of Influenza: a Complex Problem," *Curr. Epidemiol. Rep*., vol. 5, no. 1, pp. 1–9, 2018, doi: 10.1007/s40471-018-0136-1.

[41] G. A. Willis et al., "The impact of influenza infection on young children, their family and the health care system," *Influenza Other Respir. Viruses,* vol. 13, no. 1, pp. 18–27, Jan. 2019, doi: 10.1111/irv.12604.

[42] M. Hong et al., "Antibody recognition of the pandemic H1N1 Influenza virus hemagglutinin receptor binding site," *J. Virol*., vol. 87, no. 22, pp. 12471–12480, Nov. 2013, doi: 10.1128/jvi.01388-13.

[43] E. W. Sayers, M. Cavanaugh, K. Clark, J. Ostell, K. D. Pruitt, and I. Karsch-Mizrachi, "GenBank," *Nucleic Acids Res*., vol. 48, pp. D84–D86, 2020, doi: 10.1093/nar/gkz956.

[44] CDC, "About Enterovirus D68," Non-Polio Enterovirus. Accessed: Aug. 25, 2024. [Online]. Available: https://www.cdc.gov/non-polio-enterovirus/about/about-enterovirus-d68.html

[45] K. Kistler, "Enterovirus D68 Adaptation." Accessed: Aug. 25, 2024. [Online]. Available: https://blab.github.io/atlas-of-viral-adaptation/enterovirusd68/

[46] C. Zhang et al., "Functional and structural characterization of a two-MAb cocktail for delayed treatment of enterovirus D68 infections," *Nat. Commun*., vol. 12, no. 2904, Aug. 2021, doi: 10.1038/s41467-021-23199-5.

[47] M. R. Vogt et al., "Human antibodies neutralize enterovirus D68 and protect against infection and paralytic disease," *Sci. Immunol*., vol. 5, no. 49, Jul. 2020, doi: 10.1126/sciimmunol.aba4902.

[48] Q. Zheng et al., "Atomic structures of enterovirus D68 in complex with two monoclonal antibodies define distinct mechanisms of viral neutralization," *Nat. Microbiol*., vol. 4, no. 1, pp. 124–133, Jan. 2019, doi: 10.1038/s41564-018-0275-7.