Flu Fighters: The Ever-Evolving Need for New Flu Vaccines

Instructor Guide

Target Audience
This modular activity can be used for students at varying levels, ranging from high school to upper level college biology. It is designed for biology students who are somewhat familiar with the differences between cells and viruses, and have had an introduction to DNA and protein structure. It can be used as a practical exercise for introducing a variety of biological concepts, including: genetic drift, vaccine design, immunology, and epidemiology.

Activity Summary
Main Activity: Students will utilize NCBI tools to explore antigenic drift by comparing amino acid sequences of the hemagglutinin (HA) protein from several different strains of human (H1N1) influenza, and then map the location of key mutations on the 3D structure of the HA protein. They will then compare the amino acid sequences of the HA protein from human influenza (H1N1) to that of avian influenza (H5N1) to discover the degree of similarity between viruses that have undergone antigenic shift. Finally, students will find and align amino acid sequences of HA protein from measles virus isolates to compare the degree of similarity observed for HA proteins from a virus that is known to mutate less frequently.

Throughout this activity, students will explore the biological basis for the need for more frequent vaccination against influenza vs measles.

Extension activities: For more advanced students, the following activities can also be done:

1. Design a strategy to develop a universal vaccine for influenza.
2. Calculate R0 for H5N1, and use this value to predict the likelihood of a pandemic and how that would change with vaccination status.

Learning objectives:
By the end of this lesson, students will be able to:

- Utilize the NCBI resources that are relevant for identifying and aligning multiple amino acid sequences, and for visualizing key amino acids on the 3D structure of a protein.
- Generate a hypothesis about the genetic basis for a seasonal vaccine against human influenza (H1N1).
- Identify important factors that must be considered when designing a vaccine against a protein antigen.
- Evaluate the potential of current flu vaccines to protect against avian Influenza (H5N1).
- Explain the reasons for requiring only a single measles vaccine in contrast to requiring yearly influenza vaccines to maintain immunity.

Materials Needed
Computers for each student/group of students, and access to the internet to utilize NCBI tools.

Background Information

Before leading the activity, instructors can use the below resources to prepare for class and/or assign the resources to students.

- *Influenza virion and HA protein structures* (Figure 1):
  Influenza is a (-)ssRNA enveloped virus with two major proteins extending from the envelope, hemagglutinin (HA) and neuraminidase (NA), that function in critical steps of the viral life cycle. HA mediates virus binding to sialic acid on host cells whereas NA promotes cleavage of sialic acid to promote viral exit. The structure of HA is complex, where a single HA molecule is a trimer composed of three identical monomers, where each monomer contains two polypeptides, HA1 and HA2, covalently attached by a disulfide linkage. HA1 is located at the N-terminus, and forms the head, whereas HA2 is located at the C-terminus, and forms the stem of each HA monomer.

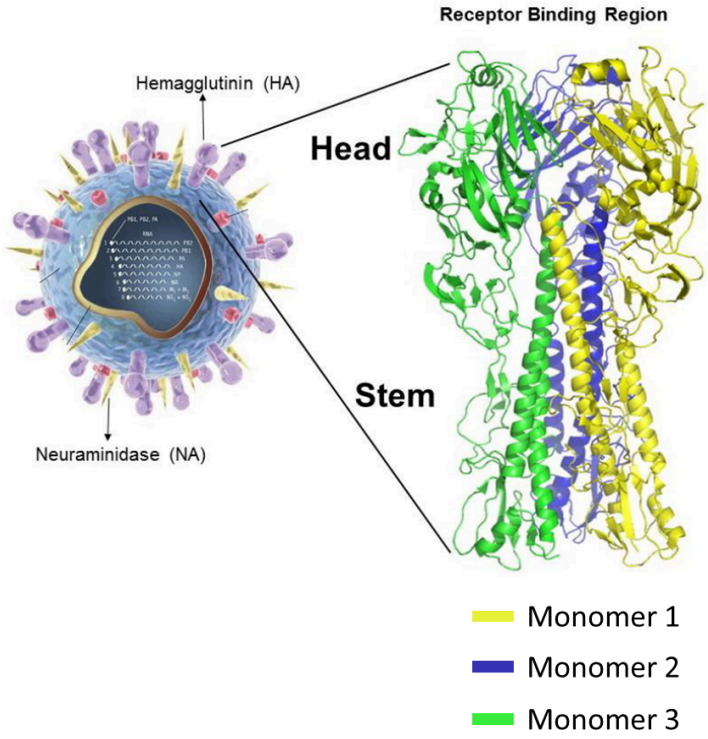


Figure 1. Influenza Virion and Hemagglutinin (HA) Structures. (A) An influenza virion, illustrating the segmented (-)ssRNA genome and envelope with protruding hemagglutinin (HA) and neuraminidase (NA) proteins. (B) Trimeric HA protein consisting on three identical monomers, each containing a head and stem region.

Modified from: Jiang, Z. et al. (2015). Antibody Cross-Reactivity to Hemagglutinin Protein Antigens Demonstrates Feasibility for Development of a "Universal" Influenza A Synthetic Peptide Vaccine. The 24th American Peptide Symposium. DOI:10.17952/24APS.2015.036

*Refer to the Protein Data Bank for additional structural details about HA protein.

- *Flu vaccines*:
  This TedEd video explains why you have to get a flu shot every year. Refer to the review article containing information about the evolution of influenza viruses as they relate to the need for frequent vaccination.
- *Antigenic drift vs antigenic shift*:
  This video about antigenic drift and antigenic shift from the CDC defines and compares both concepts.
- *Vaccines and vaccine design*:
  Use this comprehensive review about vaccines, vaccine design, the immune response following vaccines, and herd immunity to learn more.
- *Herd immunity* (Figure 2):

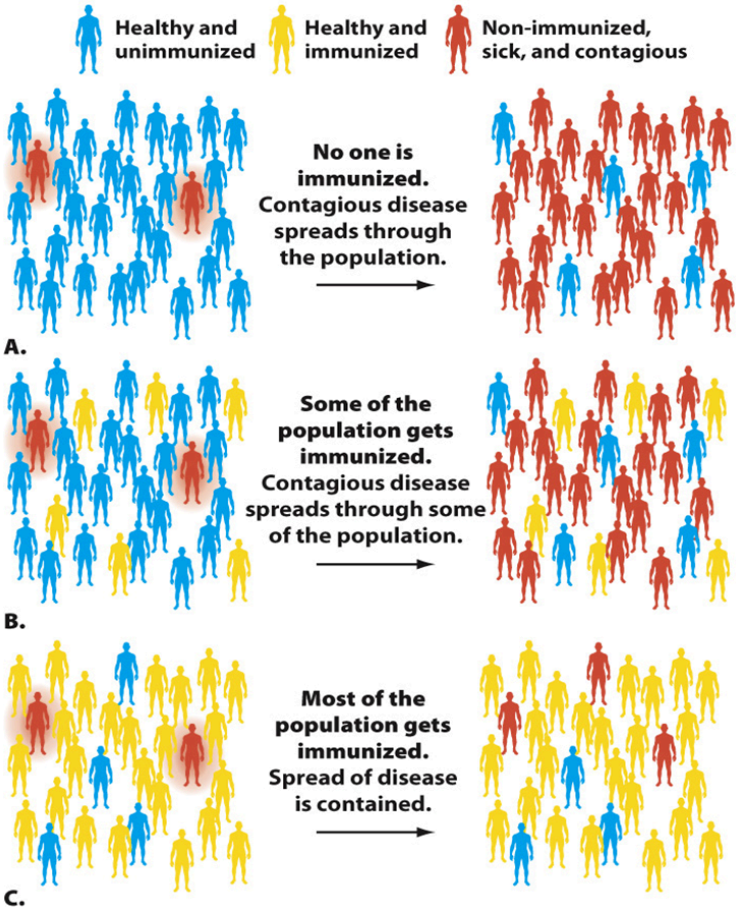


Figure 2. Schematic Diagram Depicting Herd Immunity. When an increasing number of individuals within a given population of healthy individuals (blue) are immunized (yellow), less disease transmission (red) is observed. Taken from Microbiology: The Human Experience, 2nd edition, Figure 17.2.

- *Generating protective immunity:*
  Innate and adaptive immune responses are both important for generating protective immunity against viruses. One critical component of the adaptive immune response that contributes to anti-viral immunity is the production of antibodies, or proteins secreted by B cells that bind directly to viral surface proteins and block the viral replication pathway at several places (Figure 3).

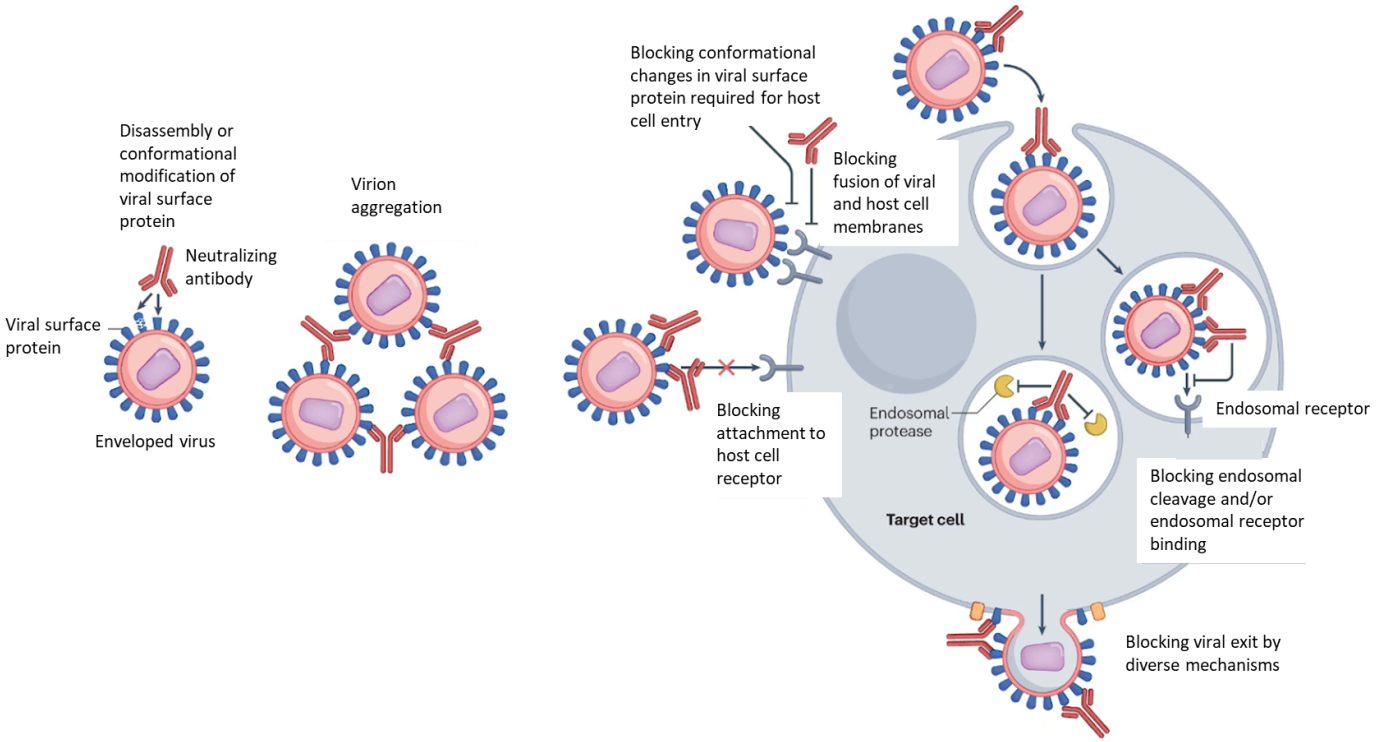


Figure 3. Effects of Neutralizing Antibodies on Viral Replication. Antibodies generated by B cells of the adaptive immune system bind to viral surface proteins, which can promote aggregation of virions, blocking viral entry by preventing binding to host cell surface receptors, by blocking viral uncoating in the endosome by inhibiting endosomal cleavage and/or endosomal receptor binding, or by blocking viral exit.

Modified from: Burton, D.R. Antiviral neutralizing antibodies: from in vitro to in vivo activity. Nat Rev Immunol 23, 720–734 (2023). https://doi.org/10.1038/s41577-023-00858-w.

- *Pandemics*:
  When we think of influenza pandemics, we tend to think of the great pandemic in 1918 (also called Spanish flu) because of the number of people that died at the time, which is estimated to be between 40-50 million people. However, there have been other influenza pandemics throughout history, each one with a devastating death toll. Although we refer

to those events simply as flu pandemics, it is important to recognize that it may not have been the same virus responsible for every pandemic (Figure 3).

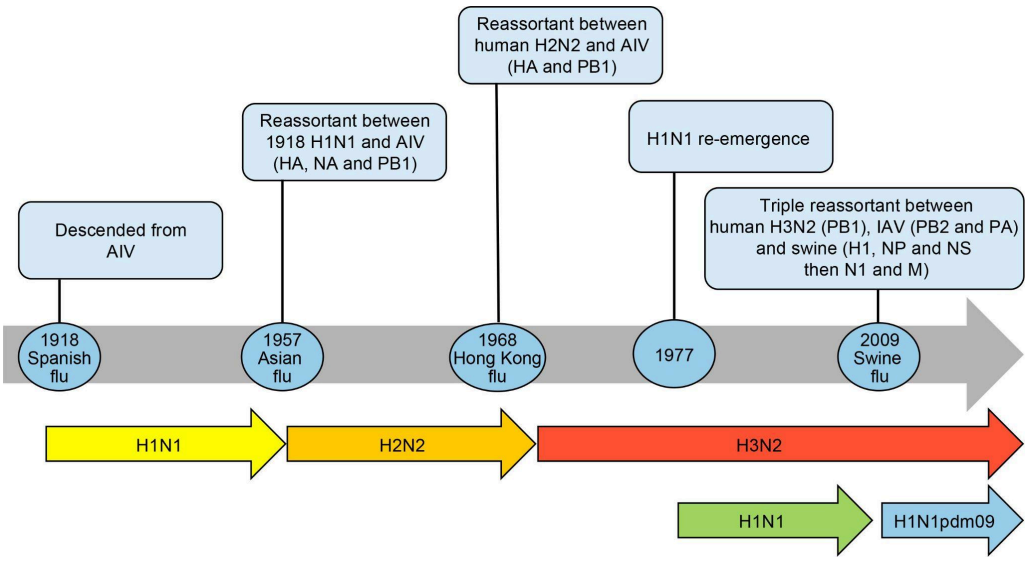


Figure 4. Influenza Pandemics Throughout History. The influenza pandemics throughout history and the viruses that caused each.

For more information, refer to this video containing a more in-depth explanation about pandemics and the next generation of influenza vaccines.

- *Avian influenza (H5N1):*
  H5N1 has the potential to become pandemic in humans if the virus successfully finds a way to transmit from human to human. The Centers for Disease Control (CDC) offers up-to-date information on the current status of H5N1 in the United States. The potential for avian influenza to become the next pandemic is also discussed there. While transmission of this virus from an infected bird to a person is not unheard of, the spread of H5N1 to cows and the subsequent human case represents one of several concerning developments in recent years.

- *Measles virus*:
  In contrast to the influenza vaccine which is needed yearly, a single measles vaccine (consisting of a primary immunization and single booster) is needed to achieve long-term immunity. As part of this activity, students will be asked to formulate a hypothesis to identify possible factors that contribute to this difference. There is not necessarily one correct hypothesis, as both antigenic variation and the immune response contribute. For primary literature articles on both of these topics, refer to the links above.

- *Additional concepts that may need to be reviewed with students:*
  - Central dogma (DNA →RNA→Protein)
  - Codons

- Single letter abbreviations for amino acids

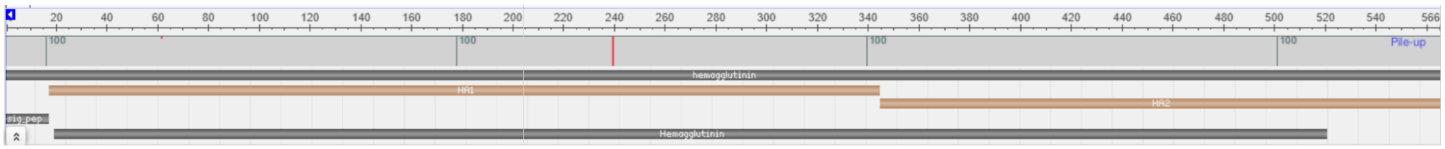
Activity Details

- *How the flu virus protein sequences were selected:*
  In order to make the flu sequences relevant, they were pulled from the last 10 years of ones used to make the flu vaccine. These are sequences against the protein hemagglutinin. We have included the sources below, the CDC has links to multiple years, we have just included this year for brevity, as well as 2 other sources.

  Accession numbers for vaccine from CDC that were also used in a primary literature article regarding influenza vaccines from 2010-2020 and listed below. You can access the sequences by typing the accession numbers in the NCBI protein database or by assessing the entire collection here.

  - 2025, 2024 - A/Wisconsin/67/2022 (H1N1)pdm09-like virus - WBO08838.1
  - 2024. 2025 - A/Victoria/4897/2022 (H1N1)pdm09-like virus - WEY08903.1
  - 2022 - A/Hawaii/70/2019 (H1N1)pdm09-like virus - QLF80309.1
  - 2019 - A/Michigan/45/2015 (H1N1)pdm09-like - QBL89789.1
  - 2011 - A/California/7/2009 - QFR38303.1
  - 2009 - A/Brisbane/59/2007 (H1N1)-like - AHG96683.1
  - 2007 - A/New Caledonia/20/1999 (H1N1) - AGQ47728.1

  To confirm the amino acid residues that constitute the head domain and the stem domain, H1 sequences were aligned in Protein BLAST, and the MSA Viewer was used to visualize the amino acid regions for HA1 (“head”) and HA2 (“stem”) as seen below in brown.



- *H1N1 vs H5N1 sequence comparison:*
  Advanced students can pull multiple H5N1 sequences for a search from the NCBI protein database so they can see the variability of HA between H1N1 and H5N1, and assess the variability in H5N1 as well. For lower-level students, we have provided instructions of how to add the H5N1 sequence to their H1N1 alignment to compare. The H5N1 sequence is available at accession number: ABW90135.1.

- *Measles sequences:*
  Advanced students should search the NCBI protein database for sequences from isolates across the globe and compare it to the original sequence (AAC60733). However,

this may be too advanced for some levels of teaching so we have included a measles sequence collection with sequences that can be aligned using NCBI COBALT.

- *How the iCn3D structure was generated*:
  A 3D H1N1 hemagglutinin structure from the Protein Data Bank (4LXV) was generated using NCBI iCn3D and the HA H1N1 sequence (WEY08903) was compared to the 3D structure mentioned above using the sequence to structure function in iCn3D by copying the sequence in for the comparison. The differences between the H1N1 sequences were highlighted in pink on the alignment and were grouped together in the diff_test tab (in case they disappear). The students should see that they are on looking at the head region, which is where the vaccine antigen is currently located. If you want to learn more about iCn3D the NCBI outreach group has a learning activity about how to use this program.

Possible discussion questions

1. How might differences in the viruses contribute to the types and/or frequencies of mutations observed for influenza vs measles?
2. Generate a hypothesis about the genetic basis of the pandemic potential of avian Influenza (H5N1).

Extension Activities

1. Explore R_0 - public health officials are concerned about the introduction of new diseases for which there are no vaccines or treatments. Thinking about H5N1, how many people would need to be vaccinated to protect the general public against this disease? Discuss what could happen if there isn't a vaccine.

   Additional resources and ideas:
   a. Overview of R_0 and cautions about its use from the CDC, and more general overview with R_0 values for several common diseases.
   b. Primary literature article where an R_0 for H5N1 was calculated.
   c. You can also have students look for other sources, and potentially use different R_0.

2. Universal Vaccine - currently the flu vaccine is a trivalent or quadrivalent vaccine consisting of HA from a H1N1 influenza A strain, H3N2 influenza A strain and an influenza B strain. So we don't have to make a new vaccine every year, scientists are trying to create a Universal vaccine.

   Additional resources and ideas:
   a. NIH news article about universal vaccine trials
   b. Review article about a universal influenza vaccine

c. TedEd video about a universal vaccine for influenza
d. You can also have students search for additional sources.

Possible assignment for students

Now that you understand how a universal vaccine would work, and based on what you learned about the H1N1 vaccine, pull several different flu virus sequences from NCBI, align them, and decide on what area of the protein you would target for a universal vaccine and why. Write this up in a brief summary (~1 page) including an image of the amino acid sequence area. More advanced students could actually go into vaccine design.