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MOLECULE OF THE MONTH: INFLUENZA NEURAMINIDASE

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Fighting the flu: atomic structures and the search for drugs and vaccines

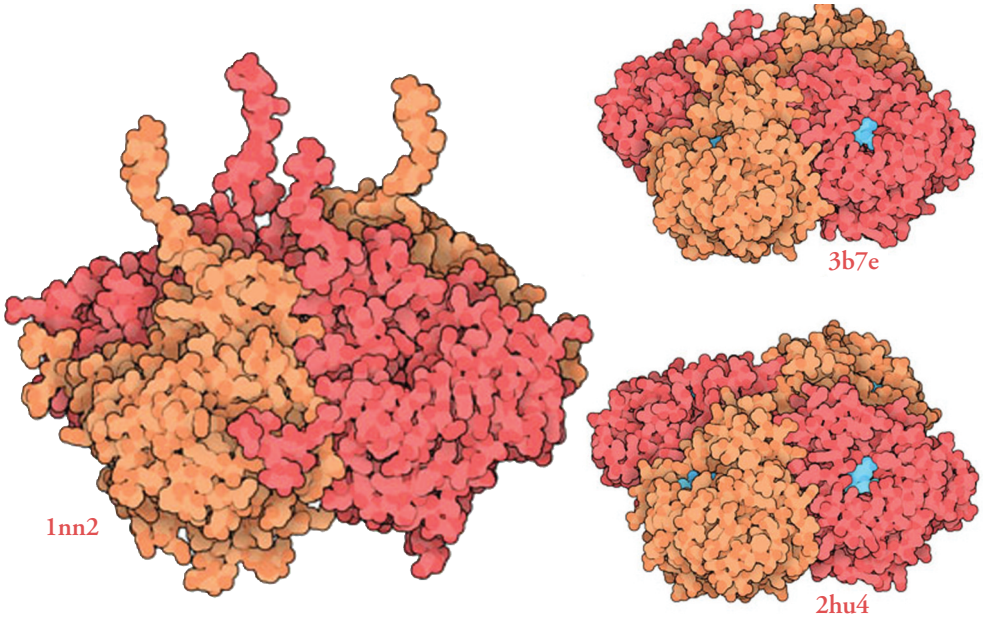
The influenza virus is continually changing. Every decade or so, a dangerous new strain appears and poses a threat to public health. This year, there has been an outbreak of a new strain of H1N1 flu, more commonly known as swine flu. The H1N1 designation refers to the two molecules that cover the surface of the virus: hemagglutinin and neuraminidase. Together, these two molecules control the infectivity of the virus. Hemagglutinin plays the starring role. As the virus approaches a cell, it binds to polysaccharide chains on the cell surface and then injects the viral genome into the cell. Neuraminidase, on the other hand, plays its major role after the virus leaves an infected cell. It ensures that the virus doesn't get stuck on the cell surface by clipping off the ends of these polysaccharide chains.

About the *RCSB PDB Molecule of the Month*

Using selected molecules from the PDB archive, each feature includes an introduction to the structure and function of the molecule, a discussion of its relevance to human health and welfare, and suggestions for viewing and accessing further details.

The *RCSB PDB Molecule of the Month* is read by students, teachers, and scientists worldwide at **www.pdb.org**.

This May 2009 edition was written and illustrated by David S. Goodsell (RCSB PDB and The Scripps Research Institute).



Clipping Sugars

Neuraminidase, shown on the left from PDB entry 1nn2, is composed of four identical subunits arranged in a square. It is normally attached to the virus surface through a long protein stalk (not shown). The active sites are in a deep depression on the upper surface. They bind to polysaccharide chains and clip off the sugars at the end. The surface of neuraminidase is decorated with several polysaccharide chains (seen extending upwards and downwards in this structure) that are similar to the polysaccharide chains that decorate our own cell surface proteins.

Pigs and People

As with hemagglutinin, neuraminidase comes in a variety of subtypes named N1-N9. These subtypes are defined by their interaction with antibodies: all of the variants within a given subtype will be neutralized by a similar set of antibodies. These subtypes are one of the causes of the continual effectiveness of influenza. Some of the subtypes promote infection in people, others promote infection in birds, and others target pigs and other mammals. As viruses spread and infect different organisms, they can mix and match different subtypes, randomly building new combinations and occasionally coming up with particularly lethal combinations.

Fighting Back

Two effective drugs are currently used to battle influenza infection: zanamivir (Relenza) and oseltamivir (Tamiflu). These drugs were discovered using the crystal structures found in the PDB. By studying the binding of molecules to the neuraminidase active site, researchers were able to design new drug molecules that mimic the natural substrates of the enzyme. These molecules bind tightly in the active site, and block its essential role in viral release. Two structures of these drugs are shown here. On the top right (PDB entry 3b7e) is zanamivir bound to neuraminidase from the "Spanish flu" virus that caused a pandemic in 1918. On the bottom right (PDB entry 2hu4) is oseltamivir bound to an avian flu virus.

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INFLUENZA NEURAMINIDASE

RCSB Protein Data Bank

The Protein Data Bank (PDB) is the single worldwide repository for the processing and distribution of 3D structure data of large molecules of proteins and nucleic acids. The RCSB PDB is operated by Rutgers, The State University of New Jersey and the San Diego Supercomputer Center and the Skaggs School of Pharmacy and Pharmaceutical Sciences at the University of California, San Diego–two members of the Research Collaboratory for Structural Bioinformatics (RCSB).

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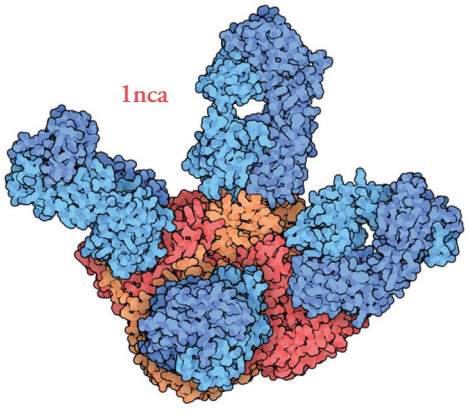
The RCSB PDB is a member of the worldwide PDB (**wwPDB; www.wwpdb.org**).

Antibodies and Vaccines

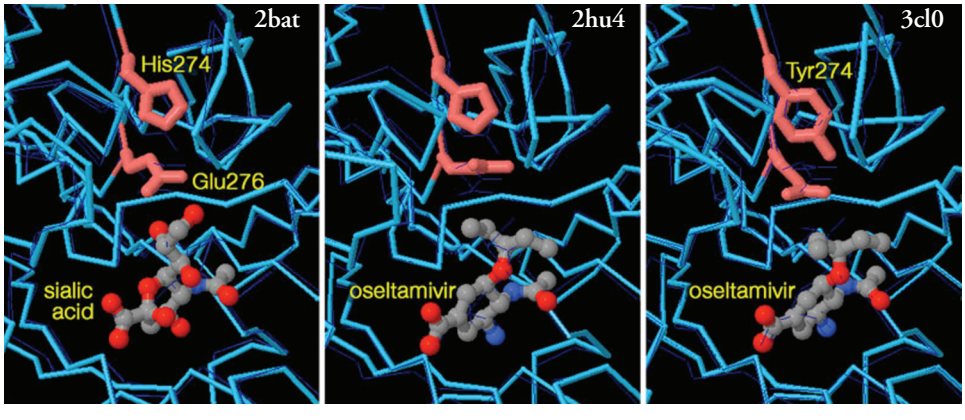
When we are infected with influenza, our immune system makes antibodies to fight the virus. The flu vaccine is a way to make the immune system ready for this protection, by challenging us with a weakened virus or harmless fragments of the virus, and spurring the immune system to make the proper antibodies before an infection. As you might expect, these antibodies recognize the proteins on the surface of the virus. The most effective ones are the ones against hemagglutinin, which block infection of new cells.



Antibodies against neuraminidase, like the ones shown here from PDB entry 1nca, can reduce the severity of the flu, playing a supporting role in the fight against the virus.

Exploring the Structure

When designing a drug, we walk a fine line. Drugs need to be different from the natural substrate of the enzyme so that the enzyme can't catalyze a reaction to destroy it. However, drugs must also be very similar to the natural substrate of the enzyme, so that they bind tightly and block the enzyme. It is also important to make drugs similar to the natural substrates in order to avoid drug resistance. An example of this problem is shown in the three structures below. The first structure shows neuraminidase with sialic acid in the active site (PDB entry 2bat). This structure shows us how the enzyme interacts with polysaccharides during its normal reaction. The second structure shows the binding of oseltamivir, one of the drugs used to fight influenza infection (PDB entry 2hu4). Notice that it is similar but not identical to sialic acid: it is slightly larger and it forces a glutamate (shown in pink) to swing upwards a bit towards a neighboring histidine (also shown in pink). The third structure is a drug resistant strain of the enzyme (PDB entry 3cl0). The histidine has mutated to a larger tyrosine, forcing the glutamate down against the drug. The drug still binds, but not nearly as tightly, so the polysaccharide substrates can easily displace it and the drug is no longer effective against the mutant virus. However, there is still plenty of room for the sialic acid to bind, so the enzyme still works for its normal function of viral release.



References:

1nn2: J.N. Varghese, P.M. Colman (1991) Three-dimensional structure of the neuraminidase of influenza virus A/Tokyo/3/67 at 2.2 Å resolution. *J.Mol.Biol.* **221**: 473-486.

3b7e: X. Xu, X. Zhu, R.A. Dwek, J. Stevens, I.A. Wilson (2008) Structural characterization of the 1918 influenza virus H1N1 neuraminidase *J.Virol.* **82**: 10493-10501

2hu4: R.J. Russell, L.F. Haire, D.J. Stevens, P.J. Collins, Y.P. Lin, G.M. Blackburn, A.J. Hay, S.J. Gamblin, J.J. Skehel (2006) The structure of H5N1 avian influenza neuraminidase suggests new opportunities for drug design. *Nature* **443**: 45-49

1nca: W.R. Tulip, J.N. Varghese, W.G. Laver, R.G. Webster, P.M. Colman (1992) Refined crystal structure of the influenza virus N9 neuraminidase-NC41 Fab complex. *J.Mol.Biol.* **227**: 122-148

2bat: J.N. Varghese, J.L. McKimm-Breschkin, J.B. Caldwell, A.A. Kortt, P.M. Colman (1992) The structure of the complex between influenza virus neuraminidase and sialic acid, the viral receptor. *Proteins* **14**: 327-332

3cl0: P.J. Collins, L.F. Haire, Y.P. Lin, J. Liu, R.J. Russell, P.A. Walker, J.J. Skehel, S.R. Martin, A.J. Hay, S.J. Gamblin (2008) Crystal structures of oseltamivir-resistant influenza virus neuraminidase mutants. *Nature* **453**: 1258-1261

Topics for Further Exploration

1. Structures are available for several of the 9 different subtypes of neuraminidase. Can you find examples of different ones?
2. Many structures of neuraminidase bound to inhibitors are available in the PDB. Can you find similarities and differences between them and sialic acid?

Additional Reading about Neuraminidase

1. P. M. Colman (1994) Influenza virus neuraminidase: structure, antibodies, and inhibitors. *Protein Science* **3**, 1687-1696.
2. M. von Itzstein (2007) The war against influenza: discovery and development of sialidase inhibitors. *Nature Reviews Drug Discovery* **6**, 967-974.