# First comprehensive structural data on a key flu protein—it's central to infection

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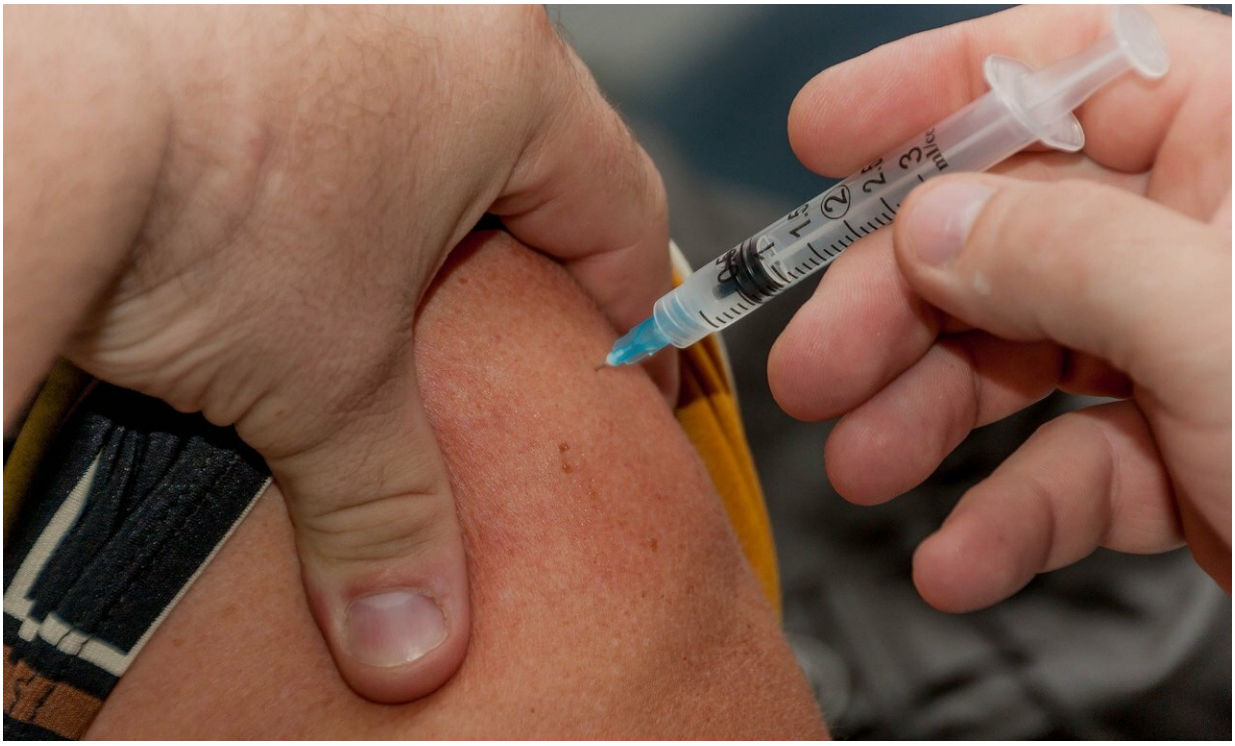

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Influenza A viruses spawn epidemics, global pandemics and sweeping outbreaks that kill millions of birds, yet only now have flu scientists obtained comprehensive, high-resolution structural data on a protein that is key to the very survival of an influenza virus.

The highlighted protein is RNA polymerase, which transcribes and replicates the viral genome in the nucleus of host cells.

Dr. Ervin Fodor and colleagues at Oxford University have shed new light on the protein in groundbreaking research that also has uncovered new sites for antiviral drugs that can take aim at vulnerable targets in RNA polymerase, a protein Fodor calls FluPolA.

"All these sites are well-conserved regions," Fodor said in an email, referring to possible new flu-drug targets that could function across a range of flu viruses. "FluPolA is one of the most conserved proteins in flu in general. Hence, it is a good target for antiviral drug development."

FluPolA is a major driver in the flu infection process. Without it, a flu virus can't copy its genetic information and commandeer that of the host cells. But more data had been collected over the years on the structure of FluPolA derived from an influenza A virus circulating among bats, Fodor had found, than FluPolA in H3N2, which frequently affects vast swaths of the human population. There also wasn't much FluPolA structural information for H5N1, a noteworthy flu menace in birds.

Fodor and his Oxford colleagues have now cast a bright spotlight on FluPolA and its multiple functions by revealing the arrangement of atoms within the critical protein for both H3N2 and H5N1. The team achieved new insights into the structure of FluPolA for both viruses by using X-ray crystallography and cryo-electron microscopy.

Writing in a recent issue of the journal *Nature*, Fodor defines the viruses involved in the FluPolA research as the human flu virus, A/1968/H3N2, the strain that circled the globe in the Hong Kong flu pandemic 51 years ago. The other FluPolA for which the team obtained high-resolution structural data is A/duck/Fujian/H5N1, a 2002 strain—a particularly nasty killer of domesticated birds.

New hurdles had to be surmounted to provide what is now an extensive amount of data on FluPolA for both H3N2 and H5N1, the *Nature* paper revealed.

"There were many technical difficulties to overcome," Fodor said. "The influenza A virus polymerase—FluPolA—is difficult to express and purify in large amounts. One issue is that three subunits need to be co-expressed and purified. A second issue is that FluPolA is particularly unstable, it has highly flexible domains which make crystallization-based approaches very difficult.

"We opted for an H3N2 polymerase as a representative of a human influenza virus; H3N2 strains currently circulate in the human population, although we went for an old strain from 1968, which our groups studied previously," he said.

"We picked an H5N1 avian FluPolA because we wanted to compare avian and human influenza A virus polymerases—and we also studied this particular avian strain before. Human and avian FluPolA differ in sequence and we wanted to know exactly how these differences affect the structure. Avian FluPolA works poorly in human cells and the avian virus needs to adapt to the mammalian cell environment which involves adaptive mutations in FluPolA," Fodor said.

Influenza A viruses—as well as their B counterparts—have a genome made up of eight RNA segments. These strands carry all the information a flu virus needs to replicate in a host. The RNA segments are copied by RNA polymerase.

The flu is a respiratory illness in humans that can be caused by A or B viruses. B influenza causes infection only in humans. Both types can cause serious respiratory complications. Influenza A viruses have driven major epidemics as well as global pandemics. The deadly 1918 flu, the

worst in recorded history, was caused by an A influenza virus.

Statisticians at the U.S. Centers for Disease Control and Prevention estimate that anywhere from 291,000 to 646,000 people die annually around the world because of seasonal flu-related complications.

H5N1, often referred to as an HPAI, or highly pathogenic avian influenza, primarily affects birds. Other HPAIs affect birds as well. The spread of H5N1, which is lethal in domestic birds, can have tremendous economic consequences. Knowing more about how structure influences function can lead to new ways of disrupting the infection process in humans and birds, global health experts say.

Among the vulnerable sites that are potential antiviral targets in influenza A viruses, are those that hinge on a key discovery in the Oxford research: FluPolA exists as a monomer or dimer, a small molecule or molecular complex.

"We identified several sites, including the FluPolA dimerization interface and a binding site for viral RNA," Fodor said of possible antiviral sites. "These sites could be targeted by small compounds. The idea is that if such compounds prevented FluPolA dimerization or RNA binding, these would act as antivirals," he said.

"We also identified a nanobody, a small antibody, that binds at a particular site on FluPolA, preventing FluPolA dimerization. When we express this nanobody in cells, the cells produce less virus, indicating it is inhibitory."

**More information:** Haitian Fan et al. Structures of influenza A virus RNA polymerase offer insight into viral genome replication, *Nature* (2019). DOI: 10.1038/s41586-019-1530-7