Uncovering the role of membrane sugars in flu infection

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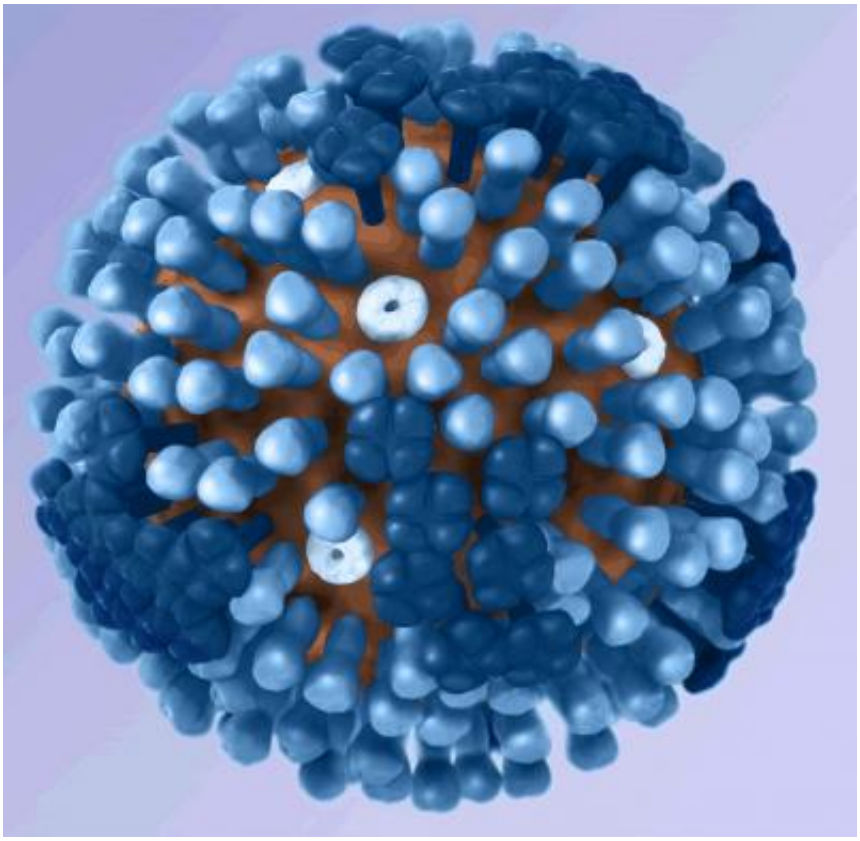

A 3-D image of a flu virus. Credit: Center for Disease Control

The flu virus relies on using human cells to reproduce and spread. But before it even gets to the cell surface, the virus must navigate the tall, dense forest of sugar-coated proteins on the cell surface known as the glycocalyx. New research from Stanford reveals how a class of

particularly bushy proteins in this forest, called mucins, could hinder the flu's progression.

In the study, published in the *Proceedings of the National Academy of Sciences* on May 26, chemistry graduate students and co-lead authors Bette Webster and Corleone Delaveris made synthetic versions of wild mucins and anchored them to lab-made membranes. They found that increasing the density of mucins on the surface inhibited two major steps of influenza A infection. These findings help explain the cell's natural response to infection and provide a foundation to better understand and treat the flu and other respiratory viruses.

"This fundamental research gives us and other scientists a leg-up in being able to develop treatments for the flu and other viruses," said Webster. "How do you treat a virus if you don't know how it's infecting a cell? And how do you improve an existing therapy if you don't know why it's working?"

Webster, a student in the lab of chemistry professor Steven Boxer, and Delaveris, a student in the lab of Baker Family Co-Director of Stanford ChEM-H Carolyn Bertozzi, began collaborating after a conversation over breakfast at the 2017 Chemistry-Biology Interface Retreat. The pair recognized their shared interest in how mucins on cell membranes affect viral infection.

Mucins drew their interest for two big reasons. First, when cells are infected with viruses, they often begin making a lot more mucins. Though the genes involved in the process have mostly been identified, why cells do this is unknown. Second, mucins often contain a specific sugar, called sialic acid, that could tether the virus to a cell and help position it to bind to receptors on the cell surface.

Once a virus is successfully bound, the cell membrane folds back on

itself to swallow the virus, along with the mucins and other cell surface sugars in the vicinity, to make a small bubble inside the cell, called an endosome. In the next vital step for viral infection, the virus fuses with the shell of this endosome to release its genetic material into the cell.

The researchers wanted to deconvolute the ways that the length of mucins, their density on the surface, and the amount of sialic acid could each help or inhibit membrane binding and fusion. Wild mucins are structurally varied in how long they are and what sugars decorate their branches, so the team made a series of short and long mucins that either contained or lacked sialic acid. They then planted them sparsely or densely on their membrane mimics and introduced the flu virus to watch binding and fusion separately.

They found that a dense coat of mucins hindered binding and slowed down fusion. While the exact density needed to cause these changes varied based on the length of individual mucins, they observed these virus-impeding shifts in both short and long mucins.

Further experiments showed that when there is ample space between them, mucins of various lengths can relax on themselves and settle atop the cell surface resulting in a thin mucin coat. When the mucins are planted close together, however, they extend straighter from the surface and create a thicker barrier that the incoming virus must navigate. Combined with previous research, this finding supports a theory that cells make more mucins in response to infection to make a thicker barrier that impedes the flu virus during binding and fusion.

"What's amazing is that we've found evidence for how a cell protects itself from infection," said Delaveris. "Protection mechanisms, like bulking up the glycocalyx in this case, can instruct therapeutic design in the future."

The researchers hope that their method and findings will help in tackling other viruses that infect individuals through places like the lungs and respiratory tracts, which have mucin-rich mucous coatings. "There is increasing interest in the mechanisms by which respiratory viruses make their first point of contact with their hosts," said Bertozzi. "What we learned from these studies of flu virus may have relevance to other viruses that must navigate a mucus layer while engaging cell surface ligands, including SARS-CoV-2"

More information: Corleone S. Delaveris et al. Membrane-tethered mucin-like polypeptides sterically inhibit binding and slow fusion kinetics of influenza A virus, *Proceedings of the National Academy of Sciences* (2020). DOI: 10.1073/pnas.1921962117

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