Discovery of new Covid infection mechanism offers clue to SARS-CoV-2 leap to humans

One of the best-known aspects of the Covid-19 pandemic is that the virus 'jumped' into people from animals – perhaps bats or pangolins – in a process known as zoonotic transfer.

What hasn't been clear to scientists is exactly how, from a mechanistic point of view, the virus moved between animal and human cells as part of that initial leap – and what tricks it might have used in its journey.

An international team led by scientists at the Rosalind Franklin Institute demonstrated that, despite prior uncertainty, SARS-CoV-2's spike protein can latch on to sugars known as sialic acids found on the surface of human host cells. That's in addition to the ACE2 protein that has long been known to attach to the receptor-binding domains (RBDs) that sit atop SARS-CoV-2's crown-like spikes.

Professor Ben Davis of the Rosalind Franklin Institute, one of the study's senior authors, says: 'Two of the ongoing mysteries of the coronavirus pandemic are the full mechanisms behind viral transmission and the origins of the zoonotic leap. While it's well known that the combination of RBD in the spike and ACE2 protein on the human host cell surface gives the virus one of its footholds, that always seemed unlikely to be enough to give it the flexibility it needs to control fully how it enters and then exits our cells to carry on infecting.

'In influenza, it's been known for a long time that the virus grabs hold of a sugar on the surface of human host cells called sialic acid, and then uses that as a way of getting into the cell. Flu treatments such as Relenza stop the virus from getting back out of the cell by inhibiting a particular enzyme and ensuring it gets stuck at the surface. Coronaviruses don't have an equivalent enzyme, but nevertheless it's been suggested that they might still use sugars as an early attachment point to grab hold of and get into human host cells. This therefore means that as viruses they may have to tread a much finer line when it comes to the balance of getting in and out of the cell.'

The team set out to investigate this by using nuclear magnetic resonance (NMR) spectroscopy and magnetization transfer techniques available at the Franklin. When it became clear that quantifying the complex sugar-pathogen interactions would not be possible with traditional versions of these techniques, the researchers developed a new, more sophisticated method, which they have called universal saturation transfer analysis (uSTA).

Study team member Ben Gaunt, a researcher in the Franklin's PhD programme, explains: 'Using traditional saturation transfer difference, which is an NMR spectroscopy technique, we were able to see that the sialic acid ligand was interacting with the SARS-CoV-2 spike protein, but not *how* it was interacting. We teamed up with our colleagues Professor Andrew Baldwin and Charles Buchanan in Oxford, who have been developing an algorithm for analysing the signal 'peaks' in mass spectrometry readouts of binding interactions, and combined this with complex mathematics – a modified version of what are called the Bloch-McConnell equations – to revamp the saturation transfer method.

'We've called the technique uSTA because it provides a potentially universal way of using saturation transfer analysis to quantify with much greater accuracy the binding interactions between protein and ligand, including structural and kinetic features. It's the first time this has been done in such complex systems, which is hugely exciting.'

Professor James Naismith, Director of the Rosalind Franklin Institute, adds: 'This new technique can now be used by scientists to shed light on other viral structures and answer extremely detailed questions. The work is an example of the unique technologies the Rosalind Franklin Institute was set up to develop.'

Using uSTA and extremely precise high-resolution imaging, the research team showed that the SARS-CoV-2 spike binds to sialic acid in key representative sugars and, unexpectedly, that the binding takes place in a part of the spike protein called the N-terminal domain. The results were confirmed using cryo-electron microscopy.

Professor Davis says: 'What's really interesting is that the N-terminal domain is the location for lots of the mutations that take place in the spike protein as the virus evolves rapidly. Sugar binding may help explain why this is happening.

'And, strangely, it's only the original strain of the virus that exhibits sialic acid binding. The subsequent variants of concern we've seen, such as alpha, beta, delta and omicron, get rid of this mechanism.'

Professor Davis suggests that sugar binding may have been necessary for the initial zoonotic leap into humans from animals, but could then be discarded – particularly if the feature is detrimental to the virus's mission of replication and infection within humans. This so-called 'crypticity' may also be of evolutionary benefit in other ways.

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