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# Rift widens over structure of HIV's molecular anchor

**Studies of a potential vaccine target bolster claims that an earlier paper was flawed.**

**Brendan Borrell**

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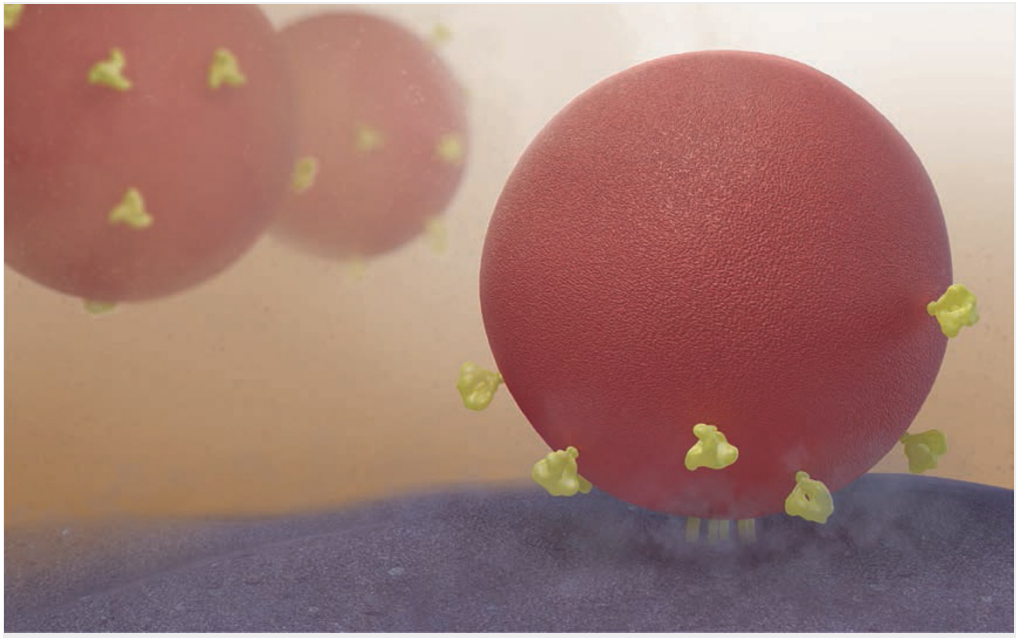

REF. 7

The structure of a protein known informally as the HIV trimer is among the most highly prized goals of structural biology.

To infect a human cell, HIV sports protrusions that hook on to the cell's surface. If researchers could find a way to block this process, they might have a way to develop a long-awaited vaccine. But the architecture of this molecular harpoon, which is called envelope glycoprotein and known informally as the HIV trimer, has been the centre of controversy since the summer, when scientists questioned the most precise description of it ever published. Now three studies have been published, two of them today, all of which agree with one another and differ from that earlier analysis, leading to calls for the paper to be retracted.

"I give no weight to the previous paper," says structural biologist Marin van Heel of Leiden University in the Netherlands. "They are throwing up a lot of smoke screens, not releasing the data, and not retracting the paper. The next step is due with these new structures."

The lead author of the earlier study, structural biologist Youdong Mao of the Dana-Farber Cancer Institute in Boston, Massachusetts welcomes the new data but says it has no bearing on the validity of his study. "Our team is firmly standing behind our earlier work," he says.

**Small wonder**

Refining the three-dimensional architecture of HIV's surface proteins with increasing resolution has been a goal for the past decade, as such knowledge can help to guide vaccine design. Because these proteins are inherently unstable, structural biologists seeking to visualize them have had to introduce stabilizing mutations and to freeze the structures in place using liquid nitrogen. This imaging technique is called cryo-electron microscopy (cryo-EM).

The dispute over the proteins' architecture began in early June, when Mao and his colleagues published cryo-EM reconstructions at a resolution of 6-ångströms in *Proceedings of the National Academy of Sciences*[1]. According to their study, the tip of the spike seemed to have a cavity at its centre.

However, several scientists, including Sriram Subramaniam at the National Cancer Institute in Bethesda, Maryland, disagreed with the

results, claiming that no virus particles were even present in the ten raw images that the team later shared. “There was nothing to see,” says Subramaniam, “It was like ‘Where’s Waldo?’ Where’s the particle?”

According to structural biologist Richard Henderson at the MRC Laboratory of Molecular Biology in Cambridge, Mao’s group seem to have obtained their results by averaging more than 5,000 grainy images and aligning them to a lower-resolution reference model. Although the reference method is widely used in cryo-EM, it has the potential to create the illusion of a particle where none exists.

In a Perspective subsequently published in *PNAS*, Henderson pointed out that one can reconstruct Einstein’s face by aligning 1,000 images of white noise[2]. He suspects that such an unintentional error crept in here, although Mao, in his team’s published response, wrote that they “took specific measures to avoid reference bias”[3].

However, because Mao and colleagues have not shared their entire dataset and methods with their critics, it is impossible to know exactly what was going on. “We cannot yet prove categorically that their work is nonsense,” says Henderson,” but I personally have no doubt that it is.”

**New data**

On 23 October, Subramaniam and his colleagues published their own cryo-EM-based reconstruction of the surface proteins in *Nature Structural and Molecular Biology*[4], revealing a very different architecture.

Rather than finding a cavity at the centre of the spike, they identified three helices (corkscrew-shaped structures) concealed within the spike in its inactivated state. When the surface proteins make contact with the surface of a cell from the human immune system, the outer components of the spike swivel around these helices and prepare for invasion in a manner strikingly similar to that seen in influenza viruses.

Now two further studies, published online today in *Science*[5, 6], have used cryo-EM and X-ray crystallography to elucidate the surface proteins in even greater detail. Their results bolster the findings by Subramaniam's team.

“This is a frame shift in how we think about vaccine design,” says structural biologist Andrew Ward at the Scripps Research Institute in La Jolla, California, an author on both of the *Science* studies. “Now that we have a better view of what the immune system sees, we can make better hypotheses and designs.”

In an email to *Nature*, Mao wrote that he is glad to see the new data coming out, but argues that the differences in the structures are simply due to different ways the molecules were engineered for imaging, not a flaw in their technique. “A full understanding of the [glycoprotein] structure likely will require scientist to capture all different ‘snapshots’ under different contexts and ‘connect’ them together.”

As a consequence of the controversy, structural biologists hope that, as with genetic sequence data, the publication of raw micrographs will become standard. Subramaniam, for instance, has released all 4,713 of his images, an unprecedented move. “We need to learn from this,” says van Heel, ”and see how we can improve deposition in databases for better quality control.”

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## References

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