Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Getting in on the action: New tools to see SARS-CoV-2 infect a cell

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In this issue of *Cell Chemical Biology*, Miao et al. develop probes for live cell tracking of SARS-CoV-2. The probes reveal the endocytic pathway for viral entry. Unexpectedly, the antiviral compound BafA1 traps the virus on the cell surface, highlighting the power of super-resolution imaging in live cells.

To hijack a cell, a virus must bind and enter. Observing viral entry is important both for understanding the virulence of a virus and for understanding how therapeutics block that entrance. But observing the viral entry is a challenge owing to the small size of viruses and their movement through the cell over time. This is true for SARS coronavirus 2 (SARS-CoV-2), the infamous virus responsible for COVID-19.

The imaging probes reported by Miao et al.[1] offer up a front-row seat to the infection process of SARS-CoV-2. Viral entry takes time, and the entry proteins must be imaged over time along the infection pathway. Robust organic dyes are ideal for live imaging, but their attachment to proteins in live cells requires additional techniques. Miao et al.[1] used stable organic dyes to label the host receptor protein, angiotensin converting enzyme 2 (ACE2), and the viral receptor binding domain (RBD) (see Figure 1A). The probes show the viral pathway beginning at the membrane surface, passing through the endocytic pathway, and entering the cell at the late endosome.

Like many viruses, SARS-CoV-2 must be proteolyzed prior to viral insertion into the membrane. Early studies by Hoffmann et al.[2] speculated the virus could insert into the plasma membrane independent of endocytosis. But more recent studies by Kreutzberger et al.[3] suggested that the endocytic pathway is necessary. Equally importantly, Meng et al.[4] showed that the new omicron variants have evolved to rely on the enzymes in the endocytic pathway for viral priming. The more recent variants are more infectious in healthy individuals than the earlier variants, suggesting that the endocytic pathway is evolving to be more important. Early variants, however, were more infectious in aged individuals and those with underlying conditions, presumably from an increased exposure to the endocytic pathway.[5,6]

In a second set of experiments, Miao et al.[1] applied their probes to test the effects of antiviral compounds on viral

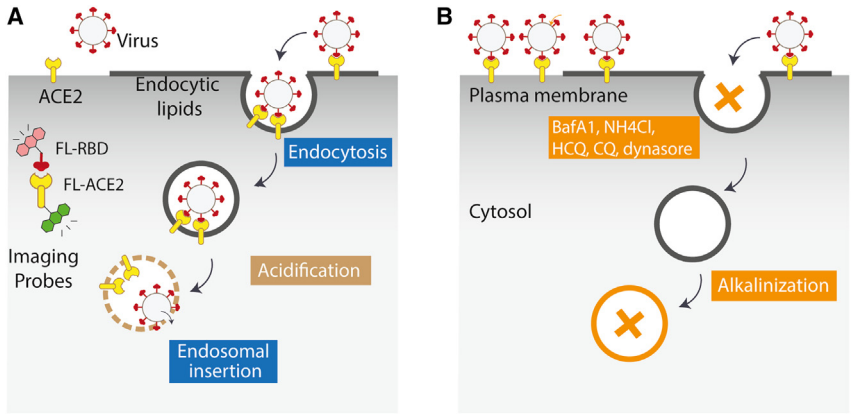

Figure 1. SARS-CoV-2 trapping model on the plasma membrane

(A) The spike protein of SARS coronavirus 2 (SARS-CoV-2) is shown as a red cartoon coating the surface of the virus. The host receptor for the virus, angiotensin converting enzyme 2 (ACE2), is shown in yellow. During infection, ACE2 moves into endocytic lipids where the virus binds and the complex is endocytosed. During endocytosis, the vesicle becomes acidic, triggering the virus to insert into the endosomal membrane and release its content into the cells (small black arrow). Organic dyes attached to ACE2 (FL-ACE2) and the spike receptor binding domain (FL-RBD) serve as probes for establishing viral entry in live cells.[1] (B) Antiviral surface trapping model of SARS-CoV-2. The drugs bafilomycin A1 (BafA1), ammonium chloride (NH_4Cl), hydroxychloroquine (HCQ), chloroquine (CQ), and dynasore are shown blocking ACE2's entrance into the endocytic pathway (orange X). For BaFA1 in particular, the bound virus accumulates on the surface and does not enter the endosome. The alkaline endosome (orange circle) is shown empty because the virus fails to reach the endosome. Other drugs reduce but do not eliminate endocytosis.

progression through the cell. The results are interesting and unexpected as they show an added mechanism previously unrecognized at the membrane surface. Bafilomycin a1 (BafA1), ammonium chloride (NH_4Cl), chloroquine (CQ), and hydroxychloroquine (HCQ) are antiviral compounds that are thought to trap the virus in the late endosome through alkalinization mechanisms. BafA1 specifically is thought to inhibit a proton pump responsible endosomal acidification.[7] The alkalinity blocks a critical proteolytic step, presumably in the late endosome, which leaves the virus unable to enter the cell.[3] Naturally, the live cell tracking system developed by Maio et al.[1] is ideal for observing the entrapment of SARS-CoV-2 in the endosome. Unexpectedly, when Miao et al.[1] treated the cells with 100 nM BafA1, the late endosomes were almost completely devoid of viral probe. Rather, the treatment trapped the virus on the cell surface (Figure 1B). How BafA1 traps the virus on the surface is unclear, but a lack of cutting in the endosome is unlikely, since the virus fails to reach the late endosome.

BafA1 is not unique; the ratio of endosomal to surface fluorescence was significantly decreased for HCQ, CQ, NH4Cl, and dynasore.[1] HCQ was used extensively during COVID-19. Recently, HCQ was shown to decrease ACE2's entrance into the endocytic pathway, and this reduced infection of a SARS-CoV-2-pseudotyped virus in mammalian cells.[5] Likewise, dynasore, a blocker of clathrin-mediated endocytosis, blocked pseudotyped infection into mammalian cells.[8] Combined, these studies demonstrate the importance of imaging along the endocytic pathway to understand the mechanism of antiviral compounds.

In addition to the structured illumination microscopy (SIM) super-resolution imaging used by Miao et al.,[1] the two-color labeling with organic dyes will be useful for peeling back a second layer of nanoscopic regulation on the plasma membrane using direct stochastic reconstruction microscopy (dSTORM). dSTORM has ~5 times the resolution of SIM, and it can be used to track proteins moving nanoscopic distances (<250 nm) between endocytic and non-endocytic lipids on the plasma membrane.[9]

The tools Miao et al.[1] report are timely and will facilitate basic research and drug discovery needed to solve the challenges in the fight against COVID-19. Understanding the effect of drugs on the membrane is important for understanding their mechanism of action and developing potent therapeutics. Many drugs exert an effect through the plasma membrane.[10] Having the proper tools to understand this potential mechanism is critical both at the cellular and nanoscopic levels.

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