CORONAVIRUS

# A new tool to probe SARS-CoV-2 variants

## Virus-like particles offer a new way to investigate genetic variation in SARS-CoV-2

By **Bryan A. Johnson**[1] *and* **Vineet D. Menachery**[1,2,3,4]

Although efforts have been made to understand the biology of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a major focus has been on investigating genetic variation in the virus. However, progress is hampered by the need to perform experiments involving SARS-CoV-2 in biosafety level 3 (BSL3) laboratories, which require substantial training for safe operation. On page 1626 of this issue, Syed *et al.* (*1*) offer an alternative to using live virus, introducing a new SARS-CoV-2 virus-like particle (VLP) system. The authors innovate on previous VLP systems by incorporating a reporter construct to study infection (*1, 2*). Illustrating the system's utility, they use VLPs to characterize mutations in SARS-CoV-2 variants of concern.

SARS-CoV-2 VLPs are created by expressing the four structural proteins—spike, membrane, envelope, and nucleocapsid—in a packaging cell line (*2*). Upon expression, VLPs consisting of these four proteins and a lipid membrane self-assemble and are released from the cell (*3*). Despite resembling SARS-CoV-2 morphologically, traditional VLPs cannot be used to study the effect of a mutation on fitness because they lack genetic material to deliver to target cells (*2*). Syed *et al.* introduced a key innovation. They first identified the SARS-CoV-2 packaging signal, a genetic marker used to identify full-length genomes for packaging into the virion (*4*). This packaging signal was incorporated into the 3' untranslated region of a luciferase reporter plasmid, causing the resulting transcripts to be packaged within VLPs. Syed *et al.* show that VLPs deliver these luciferase reporters to target cells, allowing the resulting signal to be used as a proxy for SARS-CoV-2 infection. Thus, the effects of particular mutations on the strength of the luciferase signal can be used to determine modulation of SARS-CoV-2 infection (see the figure).

In the broader context of studying SARS-CoV-2 genetic variation, VLPs represent a middle ground between two commonly used methodologies: infectious clones and pseudovirus vectors. SARS-CoV-2 infectious clones are the gold standard because they create recombinant virus, incorporating mutations anywhere in the genome (*5*). However, using SARS-CoV-2 infectious clones is technically challenging and creates live SARS-CoV-2 that requires BSL3 laboratories for study. This limits the use of SARS-CoV-2 infectious clones to laboratories with access to such facilities and willingness to invest in developing a specialized skill set.

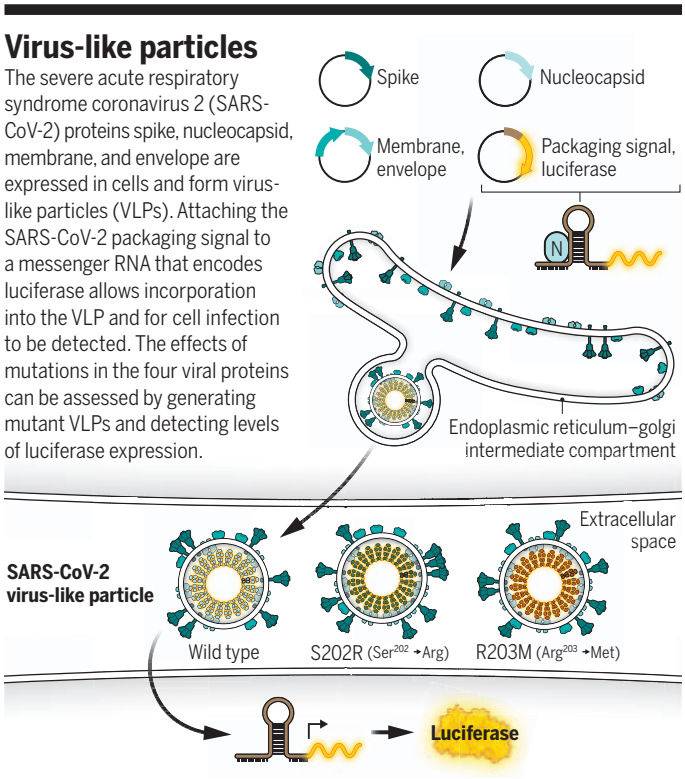
Pseudovirus systems are the leading alternative to using SARS-CoV-2 infectious clones. In these systems, SARS-CoV-2 spike protein is expressed in cells along with a noncoronavirus packaging system and a reporter gene, with the most common being lentivirus-based (*6*). Like the VLPs developed by Syed *et al.*, pseudoviruses self-assemble, incorporating spike proteins on their surface and packaging reporter messenger RNA (mRNA) (*6*). The primary advantage of pseudovirus systems is their ease of use, allowing rapid analysis of spike mutations. Pseudoviruses can be generated in the widely available 293T cell line by simply expressing a small number of proteins (*6*). Additionally, because pseudoviruses replace replication genes, they do not undergo continued amplification in target cell lines (*6*). This makes them safe to use in BSL2 laboratories, which are available to most researchers. However, the only SARS-CoV-2 protein incorporated into pseudoviruses is spike. Because substantial genetic variation occurs outside of spike, the pseudovirus systems have limited applicability to study SARS-CoV-2 variants.

The SARS-CoV-2 VLPs used by Syed *et al.* offer researchers several advantages over pseudoviruses. Rather than relying on the packaging machinery of another virus, VLPs use SARS-CoV-2 proteins and recapitulate packaging, assembly, and release, as occurs in genuine virus infection (*3*). In principle, this allows the effects of variant mutations on these processes to be studied. Similarly, because all four structural proteins are incorporated into SARS-CoV-2 VLPs, additional genetic variation can be captured. Like pseudoviruses, VLPs do not undergo subsequent rounds of replication, allowing them to be used safely in BSL2 laboratories.

Illustrating the utility of SARS-CoV-2 VLPs, Syed *et al.* characterized several nucleocapsid mutations. SARS-CoV-2 nucleocapsid is a hotspot for coding mutations, particularly within its serine-rich (SR) motif (*7, 8*). Although its exact function is unclear, the SR motif has many phosphorylated amino acids and is located within a region of intrinsic structural disorder (*7, 9, 10*). Using their SARS-CoV-2 VLP system, Syed *et al.* analyzed the effects of several common nucleocapsid

### Virus-like particles

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) proteins spike, nucleocapsid, membrane, and envelope are expressed in cells and form virus-like particles (VLPs). Attaching the SARS-CoV-2 packaging signal to a messenger RNA that encodes luciferase allows incorporation into the VLP and for cell infection to be detected. The effects of mutations in the four viral proteins can be assessed by generating mutant VLPs and detecting levels of luciferase expression.




GRAPHIC: V. ALTOUNIAN/*SCIENCE*

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mutations and found that several enhanced infection, including those present in the Alpha, Gamma, and Delta variants (*7*). These data are consistent with findings using SARS-CoV-2 infectious clones (*1, 11*).

The finding that nucleocapsid mutations enhance SARS-CoV-2 infection has important implications. To date, most studies of SARS-CoV-2 genetic variation have focused on spike (*12*). This is understandable, because spike binds to the host cell receptor angiotensin-converting enzyme 2 (ACE2), and is thus the primary determinant of infection (*13*). Additionally, because spike is the target of available vaccines, determining if mutations affect protection is a pressing question (*12*). However, recent studies suggest that nucleocapsid mutations lead to enhanced virulence and fitness, highlighting the need to characterize genetic variation elsewhere in the viral genome (*11*). Because SARS-CoV-2 VLPs recapitulate enhancement of infection by these nucleocapsid mutations, they can be used to characterize mutations in emerging variants, such as deletion of amino acids 31 to 33 in the nucleocapsid protein of the Omicron variant.

Although a promising platform, there are limitations of this SARS-CoV-2 VLP system. Only the four structural proteins are present. Thus, like pseudoviruses, the scope of variation that can be captured is limited. For example, variant mutations in the viral replication machinery cannot be examined with VLPs (*14*). Additionally, while allowing for safe use in BSL2 laboratories, the inability of VLPs to undergo continued replication makes them unsuitable to study virulence or transmission. Furthermore, although data presented by Syed *et al.* suggest that enhancement of infection by VLPs and live SARS-CoV-2 are correlated, additional work is needed to determine how closely VLPs model infection. As SARS-CoV-2 evolves, it is critical that the effects of new mutations are characterized. ■


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BIOCATALYSIS

# Teaching natural enzymes new radical tricks

## Cytochromes P450 were engineered to conduct abiotic, stereoselective radical reactions

*By* **Yang Zhang** *and* **Paweł Dydio**

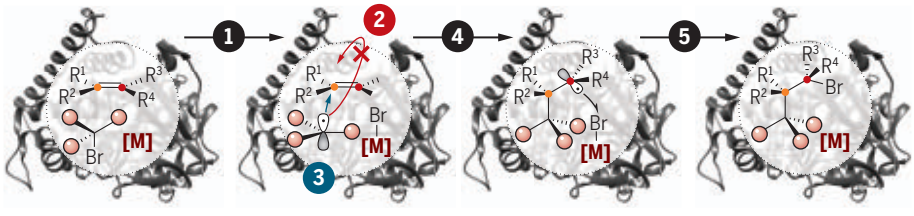
Chemical reactions that are difficult to execute with small-molecule catalysts can at times be executed selectively and efficiently with natural enzymes (*1*), reengineered enzymes (*2*), or even "artificial" enzymes (*3*). Organic reactions that occur through free-radical intermediates are particularly difficult to control with small-molecule catalysts, but their enzymatic counterparts are rare as well (*4*). On page 1612 of this issue, Zhou *et al.* (*5*) reported an elegant and powerful strategy to repurpose cytochrome P450, heme-based enzymes that natively conduct selective oxidation reactions, to catalyze an atom-transfer radical cyclization (ATRC), a free-radical reaction that thus far was only known to proceed unselectively in the presence of small-molecule catalysts. Directed evolution that iteratively accumulates constructive mutations to the protein scaffold (*2*, *3*) created variants of P450 enzymes that execute highly stereocontrolled ATRC. These results open a new forefront in unnatural radical biocatalysis (*4*).

Free-radical reactions that quickly build up molecular complexity, such as atom-transfer radical additions, are of fundamental importance in organic synthesis. However, the control of enantio- and diastereoselectivity of the formed products represents a long-standing challenge (*6*) and limits their utility in the synthesis of fine chemicals such as pharmaceuticals and agrochemicals. The intrinsic difficulty in controlling stereoselec-

### From oxidation to radical reactions

Zhou *et al.* used directed evolution to repurpose P450 enzymes, which natively perform selective oxidations, so that they perform stereoselective atom-transfer radical cyclization reactions instead. Different evolved enzymes were created so that different stereoisomers could be produced.

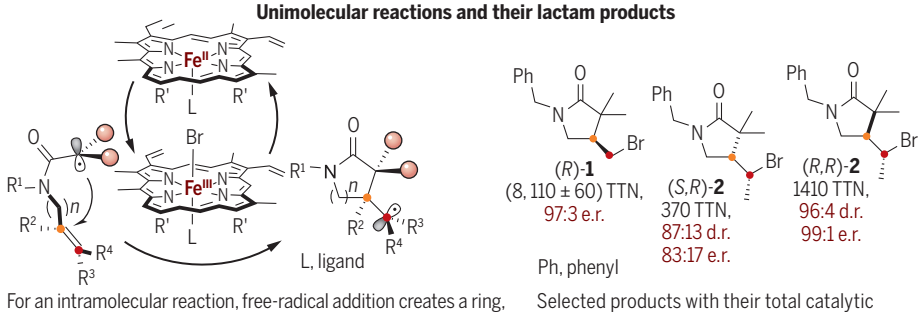
**Enzymatic free-radical addition**




1 Free-radical formation
2 Unfavored attack (→)
3 Favored attack (→)
4 Carbon-carbon bond formation
5 Carbon-bromine bond formation

A free radical forms when the heme metal center [M] abstracts Br from one substrate. The enzyme positions the double bond of the other substrate so that free-radical addition occurs from one side. Attack from the other side (shown as X) is prevented so that the process is stereoselective; R's are various substituents.

**Unimolecular reactions and their lactam products**




For an intramolecular reaction, free-radical addition creates a ring, and this intermediate cyclization step is enabled by a bromine shuttling mediated by an Fe redox center of the heme cofactor.

Selected products with their total catalytic turnover numbers (TTNs) and enantiomeric (e.r.) and diastereomeric (d.r.) ratios are shown.

GRAPHIC: KELLIE HOLOSKI/*SCIENCE* BASED ON Y. ZHANG AND P. DYDIO