

SARS-CoV-2 Variant Screening Using a Virus-Receptor-Based Electrical Biosensor

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Cite This: *Nano Lett.* 2022, 22, 50–57



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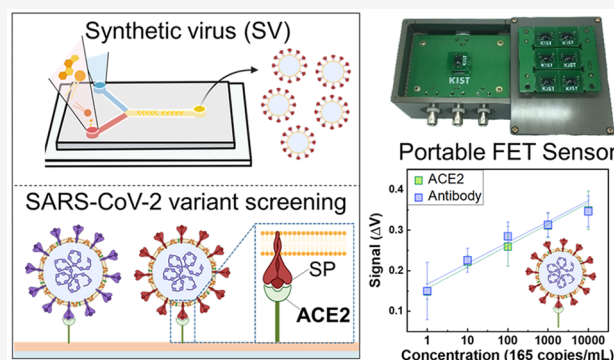
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ABSTRACT: SARS-CoV-2 variants are of particular interest because they can potentially increase the transmissibility and virulence of COVID-19 or reduce the effectiveness of available vaccines. However, screening SARS-CoV-2 variants is a challenge because biosensors target viral components that can mutate. One promising strategy is to screen variants via angiotensin-converting enzyme 2 (ACE2), a virus receptor shared by all known SARS-CoV-2 variants. Here we designed a highly sensitive and portable COVID-19 screening biosensor based on the virus receptor. We chose a dual-gate field-effect transistor to overcome the low sensitivity of virus-receptor-based biosensors. To optimize the biosensor, we introduced a synthetic virus that mimics the important features of SARS-CoV-2 (size, bilayer structure, and composition). The developed biosensor successfully detected SARS-CoV-2 in 20 min and showed sensitivity comparable to that of molecular diagnostic tests (~165 copies/mL). Our results indicate that a virus-receptor-based biosensor can be an effective strategy for screening infectious diseases to prevent pandemics.

KEYWORDS: SARS-CoV-2, variant screening, ACE2, virus receptor, biosensor



INTRODUCTION

Since the onset of the coronavirus disease 2019 (COVID-19) pandemic, many biosensors have been developed to contain the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that is responsible.^{1–4} The polymerase chain reaction (PCR) is a molecular diagnostic test and has been the leading diagnostic tool for confirming COVID-19 patients due to its high diagnostic accuracy.^{5–7} There are biosensors available for screening individuals who are negative for COVID-19.^{8–10} For example, the lateral flow assay is a widely used screening tool that can detect viral proteins onsite.^{11–13} However, even though biosensors have been successfully deployed to containing COVID-19, their performance at screening variants is questionable.

SARS-CoV-2 has mutated over time, and numerous variants have been identified.^{14,15} In particular, multiple researchers have recognized the biological significance of the spike protein mutation because it is responsible for the initial infection process.^{16,17} Li et al.¹⁶ analyzed over 100 variants to investigate the correlation between infectivity and spike protein mutation; they found that mutations of the spike protein in the reaction-binding domain and glycosylation region can diminish the binding affinity with antibodies. Accordingly, antibody-based biosensors have clear limitations in screening variants. On consideration that a fraction of false negatives can lead to a

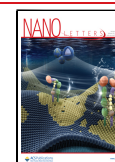
catastrophic containment failure of COVID-19, devising a screening strategy for SARS-CoV-2 variants is necessary.¹⁸

Angiotensin-converting enzyme 2 (ACE2) is the main receptor used by SARS-CoV-2 for cellular entry.¹⁹ Infection of SARS-CoV-2 begins when the spike protein binds to ACE2. Therefore, multiple researchers have explored how mutations in the spike protein affect the binding event with ACE2. Ozono et al.¹⁷ studied the binding affinity of ACE2 with five variants having global spread and mutations in the spike protein. Four of the five variants showed increased binding affinity with ACE2, and the D614G mutation in particular showed a 34% increase. Considering that the D614G mutation has rapidly replaced the wild type, we can infer that variants with a higher binding affinity with ACE2 are more contagious.²⁰ Accordingly, implementing ACE2 in the biosensor can be an effective strategy for screening variants with high transmissibility. However, ACE2 has a relatively low binding affinity with the spike protein in comparison to antibodies.^{11,21}

Received: August 12, 2021

Revised: December 18, 2021

Published: December 28, 2021



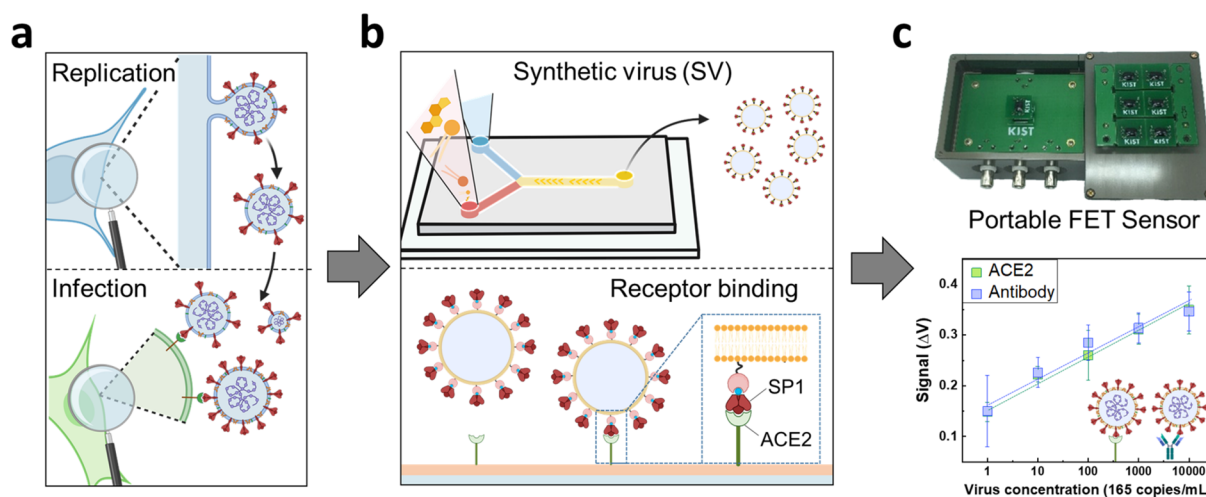


Figure 1. Overview of the development of the virus-receptor-based portable electrical biosensor: (a) replication and infection process of SARS-CoV-2; (b) microfluidic synthesis of the synthetic virus and receptor-based sensing strategy; (c) portable electrical biosensor and its sensing performance.

Recently, there has been increasing efforts in the development of ACE2-based biosensors. Yang et al.²² reported that an ACE2-based biosensor can provide sensitivity comparable with that of PCR. Even though their biosensor itself provided a high limit of detection (17.7 pM), the combination with a machine-learning technique improved the sensitivity down to 80 copies/mL in 5 min. The study clearly implies that ACE2 can be an effective biorecognition element to provide PCR-comparable sensitivity. There is another interesting study regarding an on-site-friendly ACE2 based biosensor. The research team led by Landry reported a carbon-nanotube-based colloidal biosensor.²³ Previous SARS-CoV-2 biosensors require bulky equipment such as lasers and probe stations. A colloidal sensing system, on the other hand, can be very small as long as a portable readout strategy is developed.

Developing a screening platform for the highly pathogenic SARS-CoV-2 is a challenge. Such a highly contagious virus can be only handled at biosafety level 3 and 4 facilities.²⁴ In addition, optimizing the recognition reaction with SARS-CoV-2 is difficult due to its intrinsic heterogeneity,^{25–28} which can affect the sensing signal. For this aspect, a synthetic virus is an excellent tool for providing a controlled binding environment because its physical properties, such as the size, structure, and composition, can be tuned. With the proper molecular design, safe and quality-controlled synthetic viruses can be a fruitful approach for biosensor development.

Here, we developed a virus-receptor-based COVID-19 biosensor for screening SARS-CoV-2 variants. Specifically, we employed a dual-gate field-effect transistor (FET) biosensor to provide high sensitivity. We also introduced a synthetic virus engineered at the molecular level to construct a portable electrical biosensor for detecting SARS-CoV-2 via ACE2. We found that our platform provides a sensitivity comparable with that of molecular diagnostic tests.

RESULTS AND DISCUSSION

Our study was focused on developing a biosensor for screening SARS-CoV-2 variants. The construction of such a biosensor depends on (i) employing a recognition element that can capture mutated virus components, (ii) obtaining quality-controlled virus samples, and (iii) gaining access to biosafety level 3 facilities. We used a virus receptor as a recognition

element. We also introduced a synthetic virus strategy that allows a supply of quality-controlled samples and the development of a biosensor outside of biosafety level 3 facilities. The combination of ACE2 and an electrical biosensor allows the rapid and sensitive detection of the synthetic viruses. The sensing results for SARS-CoV-2 screening indicated that our approach provides sensitivity comparable to that of molecular diagnostic tests even in portable form.

Figure 1 presents the research outline. In the human body, SARS-CoV-2 is replicated from host cells with their plasma membrane as an envelope. The heterogeneous virus then infects target cells through ACE2. Since ACE2 is the main doorway for SARS-CoV-2 variants,^{19,29–31} employing this receptor for biosensor development can be an effective screening strategy for variants.^{11,32–34} We introduced a synthetic virus, which we used to successfully develop and validate a virus-receptor-based biosensor. Our results showed that ACE2 can be a useful recognition element for developing highly sensitive biosensors to SARS-CoV-2 variants.

Molecular Design of Synthetic SARS-CoV-2. Synthetic SARS-CoV-2 was fabricated by using a microfluidic device and a subsequent bioconjugation strategy (Figure 2a). Note that our synthetic virus strategy was not focused on fabricating an identical and biologically functional SARS-CoV-2. Instead, we focused on designing lipid-based particles with spike proteins that provide structurally the same initial binding events without infectivity. A lipid membrane can provide fluidity and a dynamic undulation of spike proteins, which is an important factor for virus infection processes.^{35–38} We designed our synthetic viruses by using microfluidics, which allowed us to modulate the molecular details, such as the size, lipid membrane composition, and protein density (Figure S1).

Maleimide-functionalized liposomes were prepared by using a microfluidic device.³⁹ The prepared liposomes were then conjugated with thiolated avidin and biotinylated spike proteins sequentially. Figure 2b presents the increase in size of the liposomes upon bioconjugation. As expected, the conjugations with thiolated avidin and biotinylated spike protein increased the average hydrodynamic radii by 12.5 and 11 nm, respectively. The increased size indicates successful conjugation of the target biomolecules, considering the sizes of thiolated avidin and biotinylated spike proteins (69 and 78.6 kDa, respectively). The measured ζ potential shown in Figure 2c also supports the

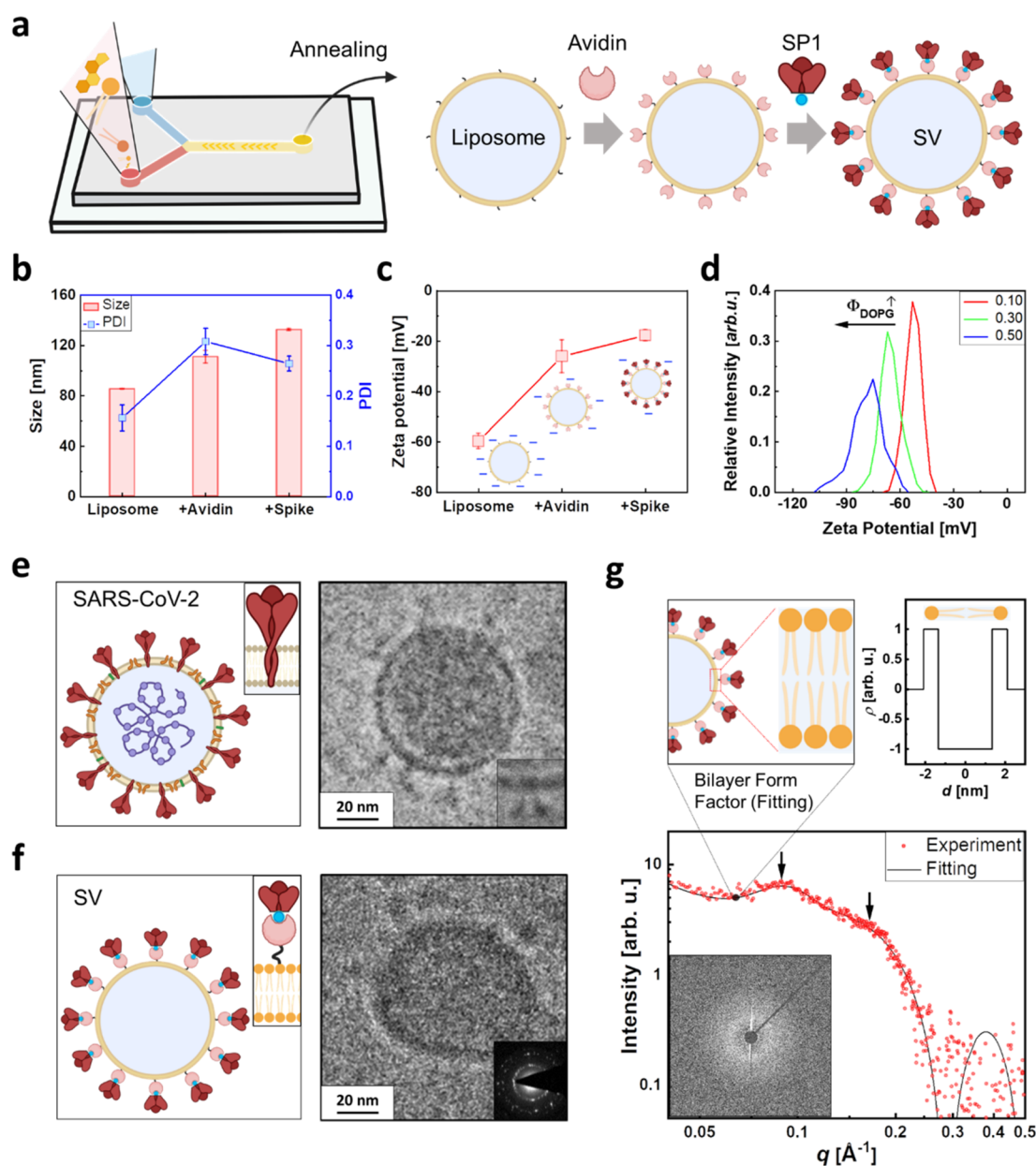


Figure 2. Preparation and structural characterization of the synthetic virus. (a) Microfluidic fabrication of the synthetic virus. (b) Changes in the size of the synthetic virus during its fabrication. The increased size indicates a successful conjugation. (c) Changes in the ζ potential upon bioconjugation of the avidin and spike proteins during fabrication of the synthetic virus. The negative charge of the lipid membrane was screened as avidin and spike proteins were conjugated. (d) Changes in the ζ potential with increasing composition of the negatively charged lipid (DOPG). Cryo-EM study of (e) SARS-CoV-2 and (f) the synthetic virus. In both Cryo-EM images, spike proteins (SARS-CoV-2) and sp1-avidin complexes (synthetic virus) are visible at the periphery. It is noteworthy that the Cryo-EM image of SARS-CoV-2 has a darker contrast inside, presumably due to the highly charged mRNA at its core. (g) SAXS scan of the synthetic virus and form factor fitting results indicating the presence of a bilayer structure.

successful conjugation. The initial liposome showed a ζ potential of -60 mV due to the incorporation of 20 mol % of negatively charged phospholipids (1,2-dioleoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (DOPG)). Upon addition of the thiolated avidin and biotinylated spike proteins, the ζ potential gradually shifted toward a positive value (-26 and -18 mV, respectively), which indicated that the surface potential of the vesicle was changed by having target biomolecules at the membrane. To underpin the capacity of the molecular control for the synthetic virus, we also prepared a set of liposomes with different DOPG contents ($\Phi_{DOPG} = 0.1$ – 0.5). The ζ potential shown in Figure 2d clearly indicates a gradual shift toward a negative surface potential as the Φ_{DOPG} increased.

To explore whether the synthetic virus resembled important structural features of SARS-CoV-2, structural characterizations were performed. Figure 2e,f shows representative cryogenic electron microscopy (cryo-EM) images of SARS-CoV-2 and the synthetic virus, respectively. Figure 2e shows SARS-CoV-2 with clearly visible ellipsoidal spike proteins at its periphery and a bilayer structure (dark rim). Importantly, the core of the virus has a dark contrast, presumably due to highly charged mRNA inside (29,903 base pair). Figure 2f shows the synthetic virus with the globular-shaped sp1-avidin complex. The synthetic virus also has a clear bilayer structure at its rim. We also imaged the selected area electron diffraction pattern of the synthetic virus; the inset image shows the tail packing structures

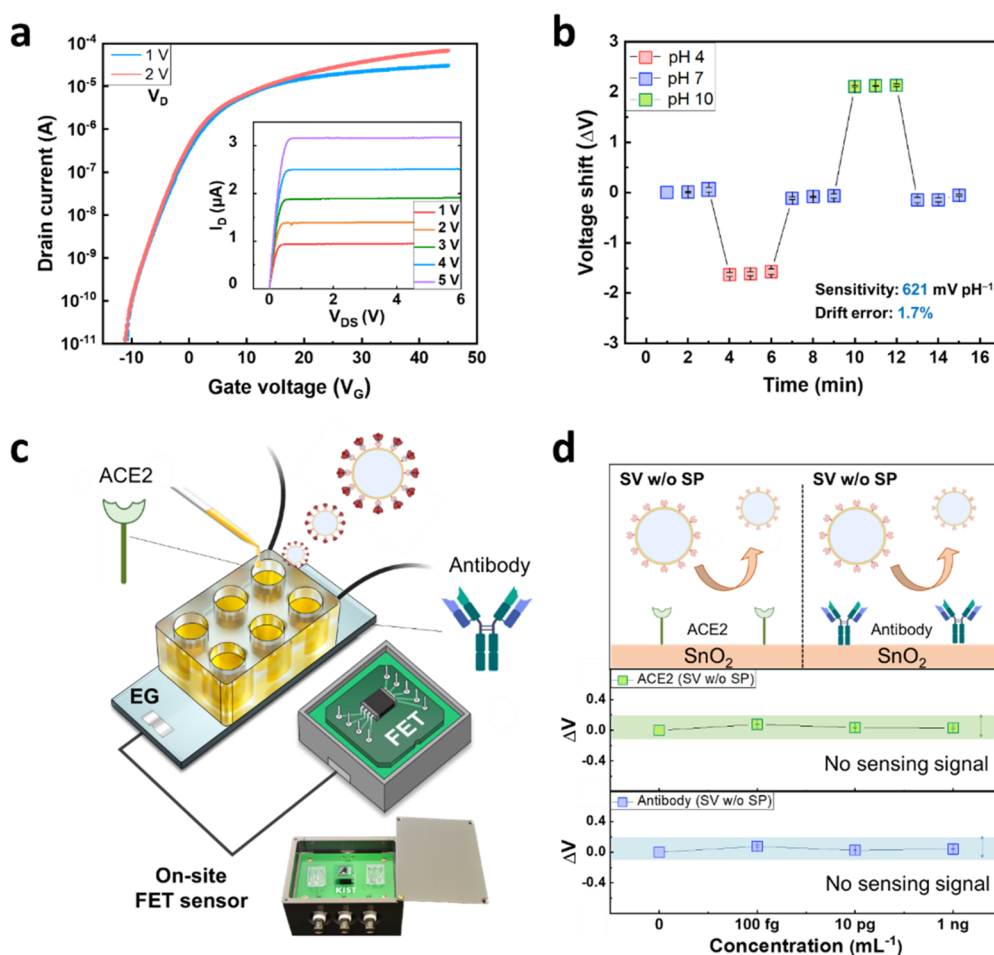


Figure 3. Electrical performance of the biosensor. (a) Transfer and output curves in bottom-gate sweep mode showing high on/off current ratios and a good subthreshold swing. (b) Real-time hysteresis and voltage drift in three different pH buffer solutions. The pH sensitivity and drift error were determined by calculating the voltage shifts at the reference current (1 nA). (c) Schematic of the experimental setup. Each well in the extended gate (EG) contains 100 μ L of the samples. (d) Nonspecific binding test of the synthetic virus without target spike proteins showing no detectable signal increase even at 1 ng/mL. Each sample was measured three independent times, and the data were expressed as the mean value and standard deviation.

characteristic of lipids, which prove that the virus is composed of lipids. Interestingly, the number density of sp1 domains in the synthetic virus was similar to that of SARS-CoV-2.²⁸

One of the most important structural components of an enveloped virus is the lipid bilayer. Synchrotron small-angle X-ray scattering (SAXS) was used to characterize the presence of a lipid bilayer in the synthetic virus. Figure 2g shows SAXS scans of the synthetic virus. The inset shows a 2D scattering image with a smeared ring near the beam stop, which represents the bilayer form factor of the sample. We fitted scattering data (red dots) with the calculated bilayer form factor (black line) at a thickness of 4.3 nm. The integrated 1D scattering curve in Figure 2g shows a nice fitting up to $q < 0.3$, where the large q region showed the typical low signal to noise data. The fitting results unambiguously show the presence of a bilayer in the samples. Note that a weak and broad bump was observed around $q = 0.09$ and $0.18 \text{ \AA}^{-1}$ (black arrows), indicating that a few vesicles adhered to each other when the concentrated synthetic virus was being prepared.⁴⁰

Electrical Performance of Portable Biosensor. We fabricated and optimized a portable FET biosensor for SARS-CoV-2 detection. Figure 3a shows the transfer and output curves of the electrical biosensor. The electrical performance of the portable biosensor showed a good subthreshold swing and high

on/off current ratio. The real-time hysteresis and drift from three different pH buffer solutions were investigated to explore the device sensitivity and stability. Figure 3b shows that our portable device had a sensitivity of 621 mV/pH, which is 10 times higher than the Nernst limit.^{41–46} In addition, repetitive buffer exchange did not alter the voltage shift significantly, which emphasizes the reliable performance of the device and minimal drift even in portable form.

Figure 3c shows the schematic of the virus measurement setup. We conjugated ACE2 and the antibody in different wells of the disposable extended gate (EG), which was connected to the packaged FET biosensor.⁴¹ It was important to test whether our sensing surface induced a rupture of lipid vesicles (synthetic virus and SARS-CoV-2), which would lead to nonspecific binding.^{47–49} Therefore, liposomes without the spike protein were prepared and measured at different concentrations. As shown in Figure 3d, there were no significant voltage shifts even at 1 ng/mL, which indicated that our surface chemistry effectively prevented the rupture of liposomes.

Sensing Performance. The sensing performance of the fabricated portable biosensor was evaluated (Figure 4a). We analyzed the voltage shift (ΔV_{ref}) at the reference current (1 nA) after the sample incubation for 20 min. A spike protein is a glycosylated type I membrane protein in SARS-CoV-2, which

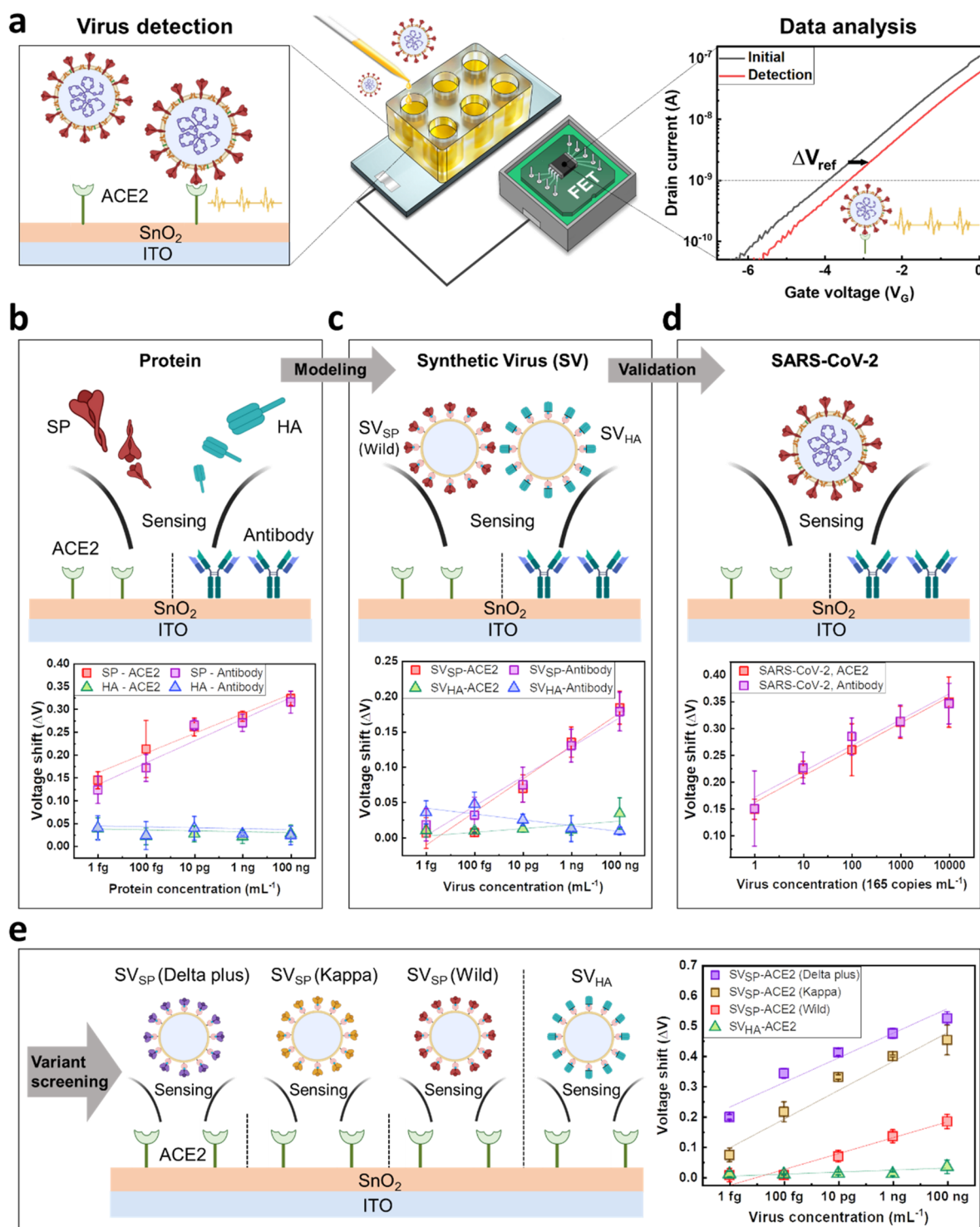


Figure 4. Sensing performance of the biosensor. (a) Schematic of the experimental setup. Voltage shifts at the reference current (10^{-9} A) after 20 min sample incubation were monitored. Sensing performance of the receptor-based portable electrical biosensor: (b) protein (SP, spike protein; HA, hemagglutinin); (c) wild-type synthetic virus (SV_{SP} , synthetic virus with spike protein; SV_{HA} , synthetic virus with hemagglutinin); (d) wild-type SARS-CoV-2. (e) Sensing performance of the receptor-based portable electrical biosensor for variants. Synthetic viruses with spike proteins from delta plus (purple) and kappa (light brown) variants and wild-type (red) were spiked into $1\times$ PBS and measured. Each data point was acquired after three independent measurements and was expressed as the mean value and standard deviation.

plays a crucial role in the initial infection of target cells.⁵⁰ Therefore, it is important to validate whether our biosensor can selectively respond to spike proteins. We chose hemagglutinin of H1N1 influenza as a control due to symptoms similar to those of COVID-19. Distinguishing such viruses is an important

criterion for COVID-19 screening.⁵¹ The sensing results of spike and hemagglutinin are shown in Figure 4b. As shown in the schematics, two different soluble proteins were measured using ACE2 and antibody as recognition elements. In our biosensor, proteins were detected down to the 1 fg/mL level. It was found

that ACE2 and antibody provide similar sensitivities (21.93 and 21.99 mV/dec, respectively) for spike protein in our device. Also, both recognition elements show no sensing response at all hemagglutinin concentrations, revealing an excellent specificity.

Next, synthetic viruses with different surface viral proteins were used to evaluate the device's performance at the supramolecular level (Figure 4c). Overall, the sensing results for the synthetic virus and viral proteins were similar. Both ACE2 and antibody showed comparable sensitivities with a negligible sensing response to synthetic H1N1 up to 100 ng/mL. Our biosensor distinguished the synthetic virus with the spike protein (wild type) from synthetic H1N1 when the concentration was above 10 pg/mL. Higher voltage shifts (30.5 and 24.78 mV/dec for ACE2 and antibody, respectively) were observed with the synthetic viruses than with the viral proteins. We attributed the greater sensing response to the large size of the synthetic viruses, which allowed more matter to interact with the electron channel of the device.⁵² To verify if our biosensor can be compatible with biological fluid, we measured different concentrations of wild-type SARS-CoV-2 in 1% nasal fluid. We confirmed that our biosensor provides a similar sensing performance at 1% nasal fluid (Figure S6).

Next, SARS-CoV-2 was measured to validate the performance of our portable electrical biosensor. Figure 4d shows the sensing results for SARS-CoV-2. As was observed with the synthetic virus, both ACE2 and antibody provided the lowest detectible concentrations, similar to those of molecular diagnostic tests (the lowest detection was 0.1 PFU or 165 copies/mL).⁵³ The results clearly showed that ACE2 can be used with the FET-based biosensor to provide a sensing performance comparable with that of antibodies.

Finally, to validate variant screening performance, synthetic viruses with delta plus and kappa variants were prepared and measured with the biosensor (Figure 4e). As a result, not only the sensing response but also higher voltage shifts in comparison with wild-type SARS-CoV-2 were observed. The higher binding affinity of variants with ACE2 may contribute to the increase in sensing response from the biosensor.¹⁷

CONCLUSION

In this study, we introduced a highly sensitive and portable COVID-19 screening biosensor based on a virus receptor. The FET biosensor was developed and optimized by using a synthetic virus. We engineered synthetic viruses to resemble the important structural features of SARS-CoV-2, such as a bilayer structure and the size. The performance of the developed biosensor was validated by using proteins and synthetic viruses. The corresponding limits of detection (LoDs) were 1 fg/mL and 10 pg/mL, respectively. To evaluate the specificity, we chose hemagglutinin of H1N1 because influenza and COVID-19 have similar symptoms. The signals from hemagglutinin-based samples were clearly distinguished from those of spike-protein-based samples for both antibody and ACE2 up to 100 ng/mL. The optimized biosensor was finally tested against SARS-CoV-2 and showed a LoD of ~165 copies/mL. Notably, ACE2 and antibody showed no discernible signal difference in our study.

Our results demonstrate the potential of virus receptors as a recognition element in biosensors for screening variants. Our virus-receptor-based FET screening system that is leveraged by synthetic viruses can be a useful tool for screening upcoming SARS-CoV-2 variants.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.nanolett.1c03108>.

Detailed materials and methods (fabrication of the synthetic virus, structural characterization of the virus, fabrication of the portable FET biosensor, surface treatment of the extended gate, SARS-CoV-2 specimen collection and inactivation, measurement setup, and statistical analysis), size and lipid composition control of liposomes, SAXS scan of SARS-CoV-2 and fitting results, Cryo-EM image of the synthetic virus, optimization of ACE2 immobilization for SARS-CoV-2 screening, ELISA result of spike proteins with ACE2, and sensing performance of the biosensor at 1% nasal fluid with wild-type synthetic virus (PDF)

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Author Contributions

S.P., H.K., and K.H.L. conceived the idea. H.K. designed and fabricated the synthetic virus. H.K. performed structure analyses of synthetic and real SARS-CoV-2. S.P. and K.H.L. developed a portable electrical biosensor and K.W. performed sensing of synthetic and real SARS-CoV-2. J.-M.K. and H.-J.J. prepared inactivated SARS-CoV-2 specimen. S.P., H.K., Y.J., and K.H.L. wrote the manuscript. All authors read and endorsed the manuscript.

Author Contributions

[†]S.P. and H.K. contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by Basic Science Research Program through the National Research Foundation (NRF) funded by the Ministry of Science and ICT (MSIT) (2020R1A2C2009291) and Ministry of Education (2020R1A6A3A01100429) of the Republic of Korea. This work was also supported by a Korea Medical Device Development Fund grant funded by the Korean government (the Ministry of Science and ICT, the Ministry of Trade, Industry and Energy, the Ministry of Health & Welfare, the Ministry of Food and Drug Safety) (Project Number: 1711138210, KMDF_PR_20200901_0115-01). This research was partially supported by the Korea Institute of Science and Technology (KIST) (Institutional 2E31130, 2E3115C) and KU-KIST Graduate School of Converging Science and Technology, Korea University.

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