Optical Tracking of the Interfacial Dynamics of Single SARS-CoV-2 Pseudoviruses

Yi-Nan Liu, Zhen-Ting Lv, Si-Yu Yang, and Xian-Wei Liu*

ABSTRACT: The frequent detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in healthcare environments, accommodations, and wastewater has attracted great attention to the risk of viral transmission by environmental fomites. However, the process of SARS-CoV-2 adsorption to exposed surfaces in high-risk environments remains unclear. In this study, we investigated the interfacial dynamics of single SARS-CoV-2 pseudoviruses with plasmonic imaging technology. Through the use of this technique, which has high spatial and temporal resolution, we tracked the collision of viruses at a surface and differentiated their stable adsorption and transient adsorption. We determined the effect of the electrostatic force on virus adhesion by correlating the solution and surface chemistry with the interfacial diffusion velocity and equilibrium position. Viral adsorption was found to be enhanced in real scenarios, such as in simulated saliva. This work not only describes a plasmonic imaging method to examine the interfacial dynamics of a single virus but also provides direct measurements of the factors that regulate the interfacial adsorption of SARS-CoV-2 pseudovirus. Such information is valuable for understanding virus transport and environmental transmission and even for designing anticontamination surfaces.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the ongoing coronavirus disease 2019 (COVID-19) pandemic. In addition to direct transmission through respiratory droplets from infected persons, SARS-CoV-2 can also be transmitted indirectly through fomites. Environmental surfaces can be contaminated by SARS-CoV-2 adsorption from virus-containing droplets, aerosols, sputum, and feces. Recently, SARS-CoV-2 RNA was frequently detected on surfaces in healthcare environments, in accommodations, and even in untreated wastewater. A recent meta-analysis summarized the published results of SARS-CoV-2 RNA contamination on surfaces in different environments, revealing that 21% of the testing surfaces in testing laboratories and 17% of the testing surfaces in COVID-19 patient rooms were positive in PCR tests. The viable virus can be isolated for up to 28 days on common surfaces (glass, stainless steel, etc.) by postinoculation and was also found on the surface of the cold-chain food package. All of these reports have raised concerns about the potential for SARS-CoV-2 to be transmitted through environmental fomites, although the transmission modes of SARS-CoV-2 are not fully understood. Therefore, studying the adsorption of SARS-CoV-2 to environmental surfaces is crucial to develop methods and design materials for minimizing the potential risk of fomite transmission.

Many efforts have been made to study the interfacial process of viruses. Techniques such as atomic force microscopy (AFM), and quartz crystal microbalance (QCM) measurements have been applied to study virus adsorption, which has improved our understanding of the interfacial processes of viruses. QCM technology provides information on viruses deposited onto a surface or removed from a surface. This ensemble-based measurement suffers from several drawbacks. It is challenging to resolve individual adsorption events. In addition, the soft nature of viruses deviates from the rigid-particle premise of Sauerbrey's equation. The obtained frequency shifts cannot be directly applied to the adsorption strength of viruses. More importantly, a bias in the attachment efficiency can occur at a high ionic strength due to aggregate formation. AFM has a limited throughput and is challenging for real-time observation. Furthermore, most previous studies have used bacteriophages as model viruses, while research on the surface binding of enveloped viruses remains limited. SARS-CoV-2 is structured with a lipid envelope and protruding spike proteins on its surface. Recent studies have indicated that the protruding spike proteins of SARS-CoV-2 govern interactions with surfaces and hosts. These features are quite different from those of bacteriophages. Therefore, the available literature on the
interfacial adsorption of other viruses cannot provide sufficient information for us to understand the interfacial behavior of SARS-CoV-2, but this understanding is urgently needed to effectively control its environmental transmission.

In this study, we tracked the interfacial dynamics of single SARS-CoV-2 pseudoviruses using plasmonic imaging technology. The SARS-CoV-2 pseudoviruses express the spike protein of SARS-CoV-2 with external features (spike proteins and lipid bilayer envelopes) similar to those of SARS-CoV-2. Thus, such pseudoviruses are the ideal surrogate viruses to investigate the interfacial behavior of SARS-CoV-2. Recently, we developed novel protocols based on plasmonic imaging technology for studying bacterial activity and quantifying bacterial adhesion strength.26−28 Unlike traditional methods, plasmonic imaging is extremely sensitive to the vertical position of individual viruses and capable of high-throughput imaging of single viruses and bacteria.29,30 Using this capability, we probed the interfacial behavior of pseudoviruses under varied conditions and studied the adsorption dynamics at a microscopic scale. We also observed the adsorption process in different simulated scenarios, such as saliva, lung fluid, and surface water. Therefore, the information obtained can improve our understanding of surface contamination by SARS-CoV-2 and is valuable for designing new anticontamination materials.

# MATERIALS AND METHODS

**Pseudovirus and Adsorption Conditions.** The SARS-CoV-2 pseudovirus was purchased from Genewiz (Suzhou, China). The pseudovirus is a surrogate of SARS-CoV-2 that was prepared using the lentiviral vector to express the spike protein of SARS-CoV-2. Because of the similar size and viral envelope of lentiviruses, such pseudoviruses have an outer structure that is similar to that of SARS-CoV-2 but do not contain the sequence of SARS-CoV-2. Because of the similar size and viral envelope of SARS-CoV-2, the envelope of the pseudovirus lacks the E and M structural di

of SARS-CoV-2 and are replication-de cient. The main difference between the pseudovirus and SARS-CoV-2 is that the envelope of the pseudovirus lacks the E and M proteins of SARS-CoV-2. For the virus adsorption experiments under different ionic strengths, phosphate-buffered saline (PBS) solutions of different concentrations (ionic strengths: 23, 77, and 160 mM) were used. For the virus adsorption experiments in the simulated scenarios, saliva, lung fluid, and surface waters were used as the adsorption media. The simulated saliva contained 0.82 mM MgCl₂, 1 mM CaCl₂, 3.3 mM KH₂PO₄, 3.8 mM K₂CO₃, 5.6 mM NaCl, 10 mM KCl, and 1.6 g/L mucin, with the pH adjusted to 7.4.31 The simulated lung fluid contained 2.2 mM NH₄Cl, 10.95 mM NaCl, 1.5 mM CaCl₂, 2H₂O, 1.1 mM Na₂HPO₄, 32.1 mM NaHCO₃, 0.6 mM sodium citrate, 2.5 mM glycerol, and 0.2 g/L dipalmitoylphosphatidylcholine.32 For the simulated surface water, 10 mg/L humic acid was dissolved in 0.16X PBS solution. Humic acid was purified before use.33 Among the chemicals used in the media, humic acid, mucin, and albumin were purchased from Sigma-Aldrich (St. Louis, MO, USA), Sangon Biotech (Shanghai, China), and Absin Bioscience Inc. (Shanghai, China), respectively. The other chemicals were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

**Plasmonic Imaging Setup.** The plasmonic imaging system was built on an inverted optical microscope, as described previously (Ti-E, Nikon, Japan).30 A 660 nm superluminescent light-emitting diode (SLD-26-HP, Superlum, Ireland) was applied as the light source. A CCD camera was utilized to record plasmonic images. BK-7 glass coverslips were coated with 2 nm Cr and 48 nm Au and used as the sensing chips. Before use, the chips were coated with a self-assembled monolayer (SAM). Briefly, the chips were immersed in 1 mM 11-aminooctadecanethiol hydrochloride aqueous solution or 11-mercapto-1-undecanethiol hydrochloride aqueous solution overnight to be functionalized with amino or carboxylic acid groups.

**Plasmonic Imaging of Virus Interfacial Adsorption.** The sensing chip was placed onto the object stage of the inverted microscope. The concentrated pseudoviruses were resuspended in specific adsorption media (final concentration: 2.5 × 10⁹ virions/mL) and placed onto a sensing chip (40 μL). Then, the plasmonic images were recorded immediately at a frame rate of 106.7 frames per second for 100 s. The detection limit of plasmonic imaging technology has been determined to be 30 nm for polystyrene particles.34

**Interface Energy Calculations.** The interface energy between the adsorption surface and virus (ΦDLVO+steric) was calculated from the sum of the van der Waals potential energy (Φvlv), double-layer potential energy (Φdl), and steric energy (Φsteric):21

\[
Φ_{DLVO+steric}(h) = Φ_{vlv}(h) + Φ_{dl}(h) + Φ_{steric}(h)
\]

The van der Waals potential between the virus and sensing chip can be calculated using

\[
Φ_{vlv}(h) = -\frac{A}{6h}\left[1 + \left(\frac{14h}{\lambda}\right)^{-1}\right]
\]

where h is the distance between the sensing chip and virus, A is the Hamaker constant, r represents the radius of the virus, and λ represents the characteristic wavelength of the van der Waals interaction.

Similarly, the double-layer potential energy can also be obtained from the interfacial physicochemical properties of the virus and surface, which is given by

\[
Φ_{dl}(h) = 6πA\left(\frac{kT}{\epsilon}\right)\epsilon\tanh\left(\frac{\epsilon\eta}{4kT}\right)\tanh\left(\frac{\epsilon\eta'}{4kT}\right)e^{-\frac{h}{ds}}
\]

in which ε is the dielectric constant of the adsorption medium, κ is the Debye−Hückel parameter, and η, and η', represent the zeta potentials of the virus and sensing chip, respectively.

Given the nanoscale roughness on the surface of the SARS-CoV-2 pseudovirus, the steric interaction was also taken into consideration. The steric interaction comprises the osmotic (Φoms) and elastic interaction energy (Φel), as given by

\[
Φ_{oms}(h) = \frac{2πρ_{s}N_{A}}{V}\left[\frac{1}{2} - x(h)\delta(d - h)^{2}\right]
\]

where

\[
x(h) = \frac{2}{3}\left[1 - \frac{1}{6}\left(\frac{h}{d}\right)^{3} - \left(\frac{h}{2d}\right)^{3}\right] + \frac{h}{d}\ln\left(\frac{h}{d}\right)
\]

\[
Φ_{el}(h) = \frac{3G_{max}r^{2}}{ρ_{p}\delta(d + r)^{2} - r^{3}}
\]

in which Φ, δ, dp, Mω, and Gmax are the volume fraction, length, density, molecular weight, and maximum surface concentration of the spike protein, respectively. Nω is Avogadro’s constant, V is...
the volume of the water molecule, and $\chi$ is the Flory–Huggins solvency parameter.

The parameters used in the interface energy calculation are given in Table S1. Among these parameters, the zeta potentials of the viruses and sensing chips were measured using a Zetasizer (ZEN3600, Malvern Instruments Co, UK) and a streaming potential analyzer (SurPASS3 Anton Paar, Austria), respectively. The other parameters were obtained from the literature. The roughness of the sensing chips (0.3 nm; measured by AFM) was neglected in the above calculation.

**Data Processing.** The plasmonic image processing and interface energy calculation were performed using custom-written MATLAB (The MathWorks, USA) code. The plasmonic intensity profiles of single viruses were extracted from plasmonic images and smoothed by a moving average filter to reduce the shot noise. The vertical positions of the viruses were obtained using the equation

$$I_z = I_0 \exp\left(-\frac{z}{L}\right)$$

where $I_z$ is the plasmonic intensity of the virus at the vertical position $z$, $I_0$ is the plasmonic intensity of the virus that firmly attaches to the surface, and $L$ is the decay length constant of the evanescent field (100 nm).

The equilibrium desorption constant ($K$) of the virus was defined as the ratio between the number of single-virus desorption events ($N_d$) and the number of adsorption events under certain conditions to assess the affinity of the virus to the surface:

$$K = \frac{N_d}{N_a}$$

Molecular docking between the spike protein and mucin was performed using the ClusPro web server based on the PIPER program. The structures of the spike protein and mucin were obtained from Protein Databank entries 6VXX and 6RBF, respectively. The lowest free energy change, $\Delta G_{\text{Lowest}}$, was calculated from the most populated docked structure. The simulated docked structure was generated using the PyMOL Molecular Graphics System (Version 1.3, Schrödinger, LLC).

**Analysis.** The association and dissociation processes of mucin to the $-COOH$ surface were measured by using a surface plasmon resonance (SPR) instrument (4500, Biosensing Instrument Inc., USA) at a flow rate of 100 $\mu$L/min. The equilibrium dissociation constant ($K_D$) was obtained from fitting of the SPR response to the Langmuir model. The roughness of the Au-coated sensing chip was determined using an atomic force microscope (Demension Icon AFM, Bruker, USA). The contact angles of the sensing chips were measured by using a JC2000G contact-angle system (Zhongchen Co., China).

## RESULTS AND DISCUSSION

**Tracking the Interfacial Dynamics of Single SARS-CoV-2 Pseudoviruses.** To investigate the interfacial behavior of SARS-CoV-2, we tracked the adsorption of single SARS-CoV-2 pseudoviruses using a surface plasmon resonance imaging technique. Surface plasmon resonance (SPR) originates from the oscillation of free electrons at a metal–dielectric interface. Electron oscillations form surface plasmons that propagate along the interface and produce an evanescent wave that decays exponentially near the interface, which makes the metal–dielectric interface highly sensitive to subtle differences in refractive index. When viruses attach to the surface, they scatter the surface plasmon wave and produce a plasmonic scattering image with high contrast. Since the surface plasmons decay exponentially from the surface into the solution, one can image single viruses with high throughput and track the vertical position with a resolution of 0.1 nm (Figure 1a). To simulate the external features of SARS-CoV-2, we used the SARS-CoV-2 pseudovirus to study the interfacial dynamics of viruses. We placed the solution of SARS-CoV-2 pseudovirus onto sensing chips coated with a $-COOH$ ending SAM and...
recorded the plasmonic images simultaneously (ionic strength 160 mM). The pseudoviruses collided onto the sensing chip, which could be captured by a CCD camera with a parabolic pattern in the image due to the scattering of surface plasmons by the virus. Since the size of the virus is approximately 70 nm, which presents a challenge for other label-free optical imaging technologies, we used the difference images by subtracting the first frame from the subsequent frames. To avoid the interference of virus aggregates, we determined the plasmonic intensity distribution of adsorbed viruses at an ionic strength of 160 mM (the highest ionic strength used in this study) and excluded particles with outlier intensities beyond the 3-fold standard deviation interval (Figure S1). By extracting the plasmonic intensity of individual viruses from the image sequences, we can obtain the corresponding vertical position−time relationship during the collision and differentiate the transient and stable adsorptions of viruses onto the surface (Figure 1b−d and Videos S1 and S2), indicating that the plasmonic imaging technique is capable of tracking virus interfacial processes.

**Electrostatic-Force-Dependent Adsorption Process of SARS-CoV-2 Pseudoviruses.** Since the interfacial adsorption of viruses usually occurs in solution, we therefore explored the effect of solution and surface chemistry on the interfacial behavior of SARS-CoV-2 pseudoviruses (Figure 2a). We imaged and counted virus adsorption in PBS solution with ionic strengths ranging from 23 to 160 mM onto −NH2- or −COOH-coated surfaces (Figure 2b,c). The results suggest that a high ionic strength favors virus adsorption. For the −NH2 surface, the adsorption rate (8.8 × 10⁵ μm² s⁻¹) at an ionic strength of 160 mM was 6.5 times higher than that at an ionic strength of 23 mM (1.3 × 10⁵ μm² s⁻¹). Meanwhile, the adsorption rates on the −NH2 surface were also superior to those on the −COOH surface at the same ionic strengths (Figure 2d,e). Although many forces, such as van der Waals and hydrophobic forces, are involved, electrostatic forces are still the dominant factor in virus−surface interactions.

To further study the interfacial process of the virus, we determined the diffusion velocity by analyzing the vertical position at different times (Figure 3a−c). With increasing ionic strength, the near-surface diffusion velocity of the pseudoviruses increased correspondingly from 790.7 ± 96.3 to 1633.1 ± 108.0 nm/s on the −NH2 surface and from 520.0 ± 121.1 to 1450.5 ± 100.2 nm/s on the −COOH surface; the results from statistical tests are given in Tables S2 and S3. The symbols in (d) and (e) indicate the significance levels: *, P < 0.05; **, P < 0.01; ***, P < 0.001.

**Figure 2.** Electrostatic-force-dependent deposition of SARS-CoV-2 pseudoviruses. (a) Schematic diagrams of the electrostatic force-dependent deposition rate. (b, c) Plasmonic images of the surfaces taken after a 4 s adsorption of the pseudoviruses (scale bar: 5 μm). Kinetics of SARS-CoV-2 pseudovirus adsorption on (d) −NH2 and (e) −COOH surfaces.

**Figure 3.** Electrostatic-force-dependent near-surface diffusion of SARS-CoV-2 pseudoviruses. (a) Schematic diagrams of electrostatic force-dependent near-surface diffusion velocity. Near-surface vertical position variations of SARS-CoV-2 pseudoviruses (b, at an ionic strength of 160 mM on a −NH2 surface; c, at an ionic strength of 23 mM on the −COOH surface). Near-surface diffusion velocity of SARS-CoV-2 pseudoviruses on (d) −NH2 and (e) −COOH surfaces at various ionic strengths. The symbols in (d) and (e) indicate the significance levels: *, P < 0.05; **, P < 0.01; ***, P < 0.001.
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Figure 4a. The viruses on the (b) −NH2 and (c) −COOH surfaces at various ionic strengths. Vertical equilibrium positions of adsorbed pseudoviruses on (e) −NH2 and (f) −COOH surfaces at various ionic strengths.

way ANOVA, no statistical significance), which can be attributed to the weakened electrostatic force. These results indicate that the diffusion velocity is dependent on the electrostatic force. Although the rough outer surface of the virus also affects the diffusion process, given the stable viral structure under the adsorption conditions, the contribution of roughness to the enhanced near-surface diffusion velocities can be excluded.

We also analyzed the interfacial potential energy from a thermodynamic perspective. Since SARS-CoV-2 possesses spikes with a length of ca. 20 nm, the effect of the nanoscale roughness of SARS-CoV-2 was included in the calculation of the interface energy by introducing steric interactions (Figure S4). The calculation results provide an explanation of why the electrostatic interaction dominates the profiles of the interfacial potential energy. Under the adsorption conditions, the virus and surface are both negatively charged (Table S1). As the ionic strength is increased, the electrostatic repulsive forces from the electrical double layer are substantially inhibited, resulting in a gradual decrease in the energy barrier in the interface energy curves. The trends in the adsorption rates and diffusion velocities under different conditions were found to be consistent with the results of the thermodynamic calculations. The increase in ionic strength compresses the electric double layer. The −NH2 surface is less negatively charged than the −COOH surface. Both of the above factors facilitate the adsorption of SARS-CoV-2.

Adsorption Equilibrium Position of SARS-CoV-2 Pseudoviruses. To reveal the microscopic interaction process between the SARS-CoV-2 pseudovirus and the surface, we quantified the equilibrium positions of individual viruses. As mentioned above, virus collisions have two distinct features: stable adsorption and transient adsorption (Figure 1c,d). We randomly selected and analyzed more than 100 collision events for each adsorption condition, which exhibited different features of interfacial behavior (Figure 4a). The viruses on the −NH2 surface at higher ionic strength were more likely to be stably absorbed and had a smaller probability of detaching (Figure 4b).

For example, the probability of repulsion events on the −NH2 surface at an ionic strength of 160 mM (15.3 ± 3.3%) was less than half of that on the −COOH surface at the same ionic strength (35.0 ± 5.0%). This was also half of the probability of repulsion events occurring on the same surface at an ionic strength of 23 mM (33.5 ± 4.9%; Figure 4c). We also calculated the equilibrium desorption constant to quantify the difference in the adsorption behavior under different adsorption conditions (Table S4). The equilibrium desorption constant is the ratio between the desorption event number and the adsorption event number, which reflects the affinity of viruses to a surface under specific conditions. The equilibrium desorption constants were also found to be substantially different under different adsorption conditions, indicating the contribution of the electrostatic force to the interfacial behavior of adsorbed viruses.

The stably adhered viruses exhibited a balance in the attractive and repulsive forces. We then determined the vertical equilibrium position under different conditions by extracting the plasmonic intensity of the stably attached virus and found that the equilibrium positions were dependent on the ionic strength (Figure 4d). The attached viruses on the −NH2 surface at an ionic strength of 160 mM had a considerably lower equilibrium position (30.4 ± 3.5 nm) in comparison to those on the −COOH surface at the same ionic strength (40.5 ± 3.8 nm), indicating that the impaired electrostatic repulsive force resulted in strong binding between the virus and the surface (Figure 4e,f). Taken together, the above results validated the role of the electrostatic force in the interface interaction between SARS-CoV-2 pseudoviruses and surfaces. Our study provides solid evidence at the single-particle level that negatively charged surfaces can inhibit the adsorption of SARS-CoV-2 pseudoviruses. This information will be beneficial for designing anticontaminating personal protection equipment (PPE) with surfaces coated by negatively charged functional groups.

Virus–Surface Interactions in Different Scenarios. Both the case study and animal experiments have revealed the potential risk of SARS-CoV-2 transmission through contaminated surfaces. Virus-containing droplets and lung
liquid are important sources of surface contamination.\textsuperscript{43} Furthermore, because of the frequent detection of SARS-CoV-2 RNA in wastewater, the potential risk of transmission via wastewater discharge into natural water exists. We therefore examined the adsorption of SARS-CoV-2 pseudoviruses in practical scenarios by using simulated saliva, lung fluid, and surface water. Unlike the mineral media used above, such simulated media contain a large quantity of mucin, biosurfactant, and humic acid. These organic substances enhance bacterial adhesion by forming conditioning films on surfaces and reducing the electrostatic repulsive force;\textsuperscript{44,45} however, little is known about the influence of these components on SARS-CoV-2 adsorption.

We probed both the virus adsorption kinetics and equilibrium positions in the above scenarios. In comparison with the control group (in PBS solution with an ionic strength of 23 mM), the pseudoviruses in simulated saliva exhibited a higher adsorption rate (18.14 × 10\textsuperscript{4} μm\textsuperscript{-2} s\textsuperscript{-1}) and near-surface diffusion velocity (1976.1 ± 134.4 nm/s). They bound more tightly to the surface (vertical equilibrium position: 20.6 ± 2.9 nm). The near-surface diffusion velocities of pseudoviruses in simulated lung fluid and surface water were determined to be 1394.1 ± 203.2 and 978.2 ± 135.1 nm/s, respectively. The vertical equilibrium positions in lung fluid and surface water were determined to be 36.9 ± 3.3 and 79.5 ± 4.4 nm, respectively (Figure 5). These results indicate that the adsorption of SARS-CoV-2 pseudoviruses to a −COOH surface can be enhanced in simulated scenarios such as saliva, lung fluid, and surface water, which helped us understand the high risk of SARS-CoV-2 potential fomite transmission in practical environments.

We next determined the role of the organic components in the virus–surface interaction. The main difference between the formula of simulated saliva and those for other media lies in the addition of mucin. Hence, we further studied the interfacial behavior of SARS-CoV-2 pseudovirus in simulated saliva without mucin. The adsorption of SARS-CoV-2 pseudovirus in this medium was impaired (Figure S5). Without mucin in simulated saliva, the deposition rate of the SARS-CoV-2 pseudovirus decreased to 49.7%, the ratio of stable adsorbed viruses decreased to 53.2%, and the corresponding vertical equilibrium position of the viruses was elevated 2.53 times (from 20.6 ± 3.0 to 51.9 ± 4.6 nm). All of the results above validated the important role of mucin in the adsorption enhancement of SARS-CoV-2 pseudovirus.

To determine the reason for the adsorption enhancement by mucin, we conducted interfacial adsorption experiments using SPR. The SPR sensorgram indicates that mucin can steadily bind to the −COOH sensing chip (K\textsubscript{D} = 8.55 g/L; Figure S6), implying that mucin can form a conditioning film over the sensing surface. After the surface was coated with mucin, the hydrophilicity of chips remained stable (water contact angle: 39.0 ± 0.7° for the −COOH surface and 37.4 ± 3.0° for the mucin binding surface), while the zeta potential of the −COOH surface increased from −30.7 to −22.7 mV. The SARS-CoV-2 pseudovirus is negatively charged. Therefore, the mucin coating weakened electrostatic repulsion and promoted virus adsorption. We also simulated the interactions between the pseudovirus and mucin. As the spike protein is the protruding structure of SARS-CoV-2, we predicted the interactions between the spike protein and mucin by using a diagram of molecular docking. The docking results indicate that mucin has the potential to bind the S\textsuperscript{1} domain of the S1 subunit of the spike protein (Figure S7; ΔG\textsubscript{docking} = −768.9 kcal/mol). Altogether, mucin can improve the adsorption of SARS-CoV-2 pseudovirus by both screening the negative charge and molecular bridging.

**Significance of This Work.** The detection of SARS-CoV-2-contaminated surfaces has raised wide concerns on the potential risk of fomite transmission of SARS-CoV-2 in indoor environments.\textsuperscript{3,4,11,42,46} In addition, the finding of SARS-CoV-2 RNA in wastewater samples also reveals the possibility that the viruses can spread to natural aquatic environments from the discharge and leakage of wastewater, especially in low-sanitation countries.\textsuperscript{6,8,47,48} Therefore, the investigation into SARS-CoV-2 interfacial interactions is of great significance for both transmission prevention and risk assessment of the environmental transmission of SARS-CoV-2. In this study, we probed the interfacial dynamics of the SARS-CoV-2 pseudovirus at the single-virus level. We found the dominant role of electrostatic forces in the adsorption of SARS-CoV-2 pseudovirus, which improved our understanding of virus–surface interactions. Although artificial surfaces were used as sensing chips, which are not real environmental interfaces for pseudovirus adsorption, we demonstrated here that this technique could be used to answer some potential fundamental questions for SARS-CoV-2 transmission at the single-virus level by artificial surface coating. The substantial enhancement in interfacial interactions in different simulated scenarios, especially in saliva, also underlines the risk of SARS-CoV-2 surface contamination and the importance of sanitization. Furthermore, given the prominent effect of the electrostatic force, the superficial modification of negatively charged functional groups could be a potential strategy for designing PPE to minimize virus contamination. Considering its severe infectivity and long-term survival on a
surface, the transmission risk of SARS-CoV-2 through fomites needs to be prudently managed, and more studies are warranted to shed light on viral contamination via environmental fomites at the molecular level.

**ASSOCIATED CONTENT**

* Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.0c06962.

Additional figures for the plasmonic intensity distribution and plasmonic imaging of the pseudoviruses under different adsorption conditions, the interface energy and electrostatic potential of different adsorption conditions, the adsorption process of different adsorption conditions, the role of mucin in the adsorption enhancement of SARS-CoV-2 pseudovirus, SPR sensing for the binding of mucin to a −COOH surface, and a simulated complex of the spike protein and mucin and additional tables about the parameters used for the interface energy calculation, the results from statistical tests, and the equilibrium desorption constants under different conditions (PDF)

Plasmonic imaging of the stable adsorption of single SARS-CoV-2 pseudoviruses (AVI)

Plasmonic imaging of the transient adsorption of single SARS-CoV-2 pseudoviruses (AVI)

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**Author Contributions**

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**Notes**

The authors declare no competing financial interest.

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