



Rapid Detection of SARS-CoV-2 Antigens Using High-Purity Semiconducting Single-Walled Carbon Nanotube-Based Field-Effect Transistors

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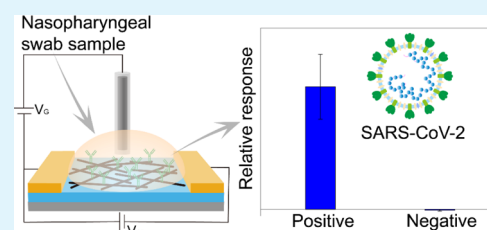
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Supporting Information

ABSTRACT: Early diagnosis of SARS-CoV-2 infection is critical for facilitating proper containment procedures, and a rapid, sensitive antigen assay is a critical step in curbing the pandemic. In this work, we report the use of a high-purity semiconducting (sc) single-walled carbon nanotube (SWCNT)-based field-effect transistor (FET) decorated with specific binding chemistry to assess the presence of SARS-CoV-2 antigens in clinical nasopharyngeal samples. Our SWCNT FET sensors, with functionalization of the anti-SARS-CoV-2 spike protein antibody (SAb) and anti-nucleocapsid protein antibody, detected the S antigen (SAg) and N antigen (NAg), reaching a limit of detection of 0.55 fg/mL for SAg and 0.016 fg/mL for NAg in calibration samples. SAb-functionalized FET sensors also exhibited good sensing performance in discriminating positive and negative clinical samples, indicating a proof of principle for use as a rapid COVID-19 antigen diagnostic tool with high analytical sensitivity and specificity at low cost.

KEYWORDS: COVID-19, SARS-CoV-2, rapid antigen testing, field-effect transistor, semiconducting carbon nanotube, biosensor



1. INTRODUCTION

Rapid detection of SARS-CoV-2 infection is critical for reducing morbidity and mortality of Coronavirus disease 2019 (COVID-19).¹ The current methodology in assessing the present infection of SARS-CoV-2 relies on nucleic acid amplification tests (NAATs) which detect the genetic material from SARS-CoV-2.² While NAAT-based tests demonstrate excellent sensitivity for detection of viral RNA, it is a high complexity test requiring specialized equipment and training, and current shortages mean results can take up to a week to be returned in some areas.

Testing for SARS-CoV-2 antigens is an appropriate addition to NAAT. Antigen detection, in general, is relatively inexpensive, can have a short turnaround time, and is amenable to point-of-care diagnostic methodologies. Currently existing rapid antigen testing tools in both laboratories and clinics are mostly based on lateral flow immunoassay (LFA) platforms.^{3–8} LFA is cheap and easily mass-produced, making it advantageous for rapid in-field detection of SARS-CoV-2 antigens.⁹ However, the sensitivity of LFA is generally not high enough to accurately screen COVID-19 patients.

Among several potentially useful detection methods for viral protein detection, field-effect transistor (FET)-based biosensing devices are advantageous as they offer high sensitivity, small size, and label-free and real-time detection.^{10–12} Recently, graphene-based FETs have been applied for COVID-19 detection. Zhang et al.¹³ have developed a label-free graphene-FET immunosensor that can identify and capture the SARS-CoV-2 spike protein S1 within 2 min with

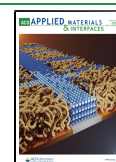
a limit of detection of 0.2 pM. The detection relies on the highly specific interaction between SARS-CoV-2 spike protein S1 and the SARS-CoV-2 spike S1 subunit protein antibody (CSAb) or human angiotensin-converting enzyme 2 (ACE2)-functionalized graphene surface. Seo et al.¹⁴ developed a SARS-CoV-2 viral detection platform with a graphene-based FET biosensing device functionalized with the anti-SARS-CoV-2 spike antibody. The reported COVID-19 FET sensor detects SARS-CoV-2 spike proteins in nasopharyngeal swabs without preprocessing of the samples and can detect SARS-CoV-2 when RNA is present at 2.4×10^2 copies/mL. Both graphene-based FET sensors showed promise for applications in COVID-19 diagnosis, albeit with lower sensitivity compared to NAAT but with a short detection time.

Herein, we developed a SARS-CoV-2 antigen (Ag) FET nanobiosensor by employing high-purity semiconducting (sc) single-walled carbon nanotube (SWCNT) functionalized with a specific antibody to access the presence of two SARS-CoV-2 structural proteins: spike protein (S antigen, SAg) and nucleocapsid protein (N antigen, NAg) (Figure 1). High-purity sc-SWCNTs offer high on-state conductance and high

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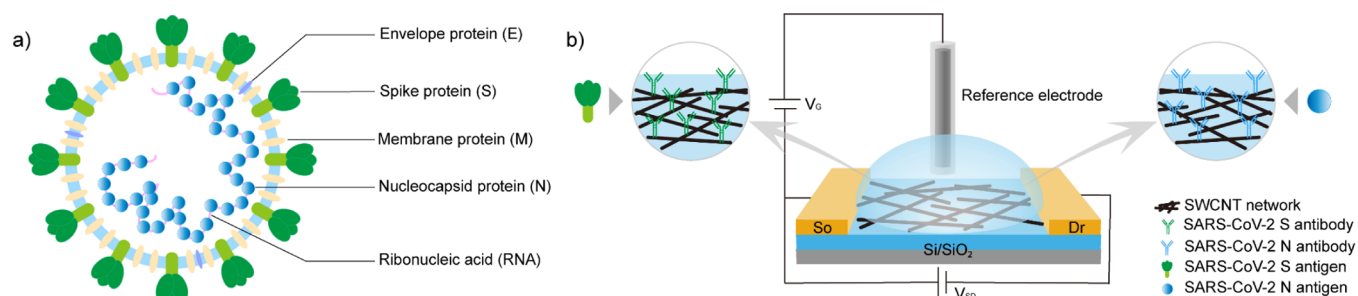


Figure 1. Detection of SARS-CoV-2 Ag using SWCNT-based FET biosensors. (a) Schematic structure of SARS-CoV-2 to demonstrate targeting proteins. (b) Schematic illustration of a liquid-gated SWCNT FET for detection of SARS-CoV-2 SAg and NAg. Interdigitated gold electrodes (yellow blocks) are configured as the source (So) and drain (Dr). Source-drain bias (V_{SD}) is 50 mV. Gate voltage (V_g) is applied through a silver/silver chloride reference electrode. Insets show SAB- and NAB-functionalized SWCNTs for specific detection of SAg and NAg, respectively.

on/off ratio for FETs, providing higher analytical sensitivity toward the target analyte compared to other carbon nanomaterials such as unsorted SWCNT and graphene. Sc-SWCNT FETs have demonstrated value as ultrasensitive biosensors including applications as aptasensors^{15,16} and label-free protein sensors.^{17–19} Moreover, the SWCNT is significantly cheaper and more widely available than CVD graphene films, thus lowering the cost of SWCNT FET sensors.

By integrating the anti-SARS-CoV-2 spike protein antibody (SAb) and anti-nucleocapsid protein antibody (NAb) with high-purity sc-SWCNTs, our SARS-CoV-2 Ag FET biosensor showed ultrasensitivity at an order of magnitude lower than other sensors to date and high analytical specificity toward SARS-CoV-2 SAg and NAg in calibration samples. Qualitative comparison of the NAAT and our SARS-CoV-2 Ag FET device indicated successful discrimination between positive samples and negative samples, suggesting their potential in COVID-19 diagnostics.

2. EXPERIMENTAL SECTION

2.1. Device Fabrication. Interdigitated gold electrodes were patterned on a Si/SiO₂ substrate using photolithography, forming 10 μ m channels. Semiconducting SWCNTs (IsoSol-S100, Raymor Industries Inc.) were prepared at 0.02 mg/mL in toluene and deposited between gold electrodes via dielectrophoresis (DEP) with an ac frequency of 100 kHz, applied bias voltage of 10 V, and bias duration of 120 s. The devices were annealed at 200 °C for 1 h before use.

The functionalization of the SARS-CoV-2 antibody on SWCNTs was achieved via 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)/N-hydroxysulfosuccinimide (sulfo-NHS) coupling. Specifically, 50 μ L of EDC/sulfo-NHS solution [50 mM/50 mM in 1 $\times$ phosphate-buffered saline (PBS), pH = 5.5] was first added to the devices to activate the carboxylic acid groups on SWCNTs. The devices were then rinsed with nanopure water and incubated with 2 μ L of 100 μ g/mL SARS/SARS-CoV-2 coronavirus spike protein (subunit 1) polyclonal antibody (Thermo Fisher Scientific, Waltham, MA, USA; Cat# PAS-81795) for 12 h at 4 °C for SAb-functionalized devices and 4 μ L of 50 μ g/mL anti-SARS-CoV-2 NP antibody (Clone# 6F10) (BioVision Inc., Milpitas, CA, USA; Cat# A2060) for NAB-functionalized devices. After a thorough rinse with nanopure water, the devices were soaked in a blocking buffer (0.1% Tween 20 and 4% polyethylene glycol in PBS) for 30 min to block unreacted surfaces. After blocking, the devices were rinsed again with nanopure water before any FET measurements.

2.2. Fluorescence Imaging. Fluorescence images were obtained using an Olympus 1X81/1X2-UCB microscope. The enhanced green fluorescent protein (EGFP) antibody (Antibodies-online Inc, Limerick, PA, USA) was immobilized on the SWCNT FET device using the same method as described in the “Device Fabrication”

section. EGFP protein solution (2 μ L, 10 μ g/mL) was then added to the device and incubated for 10 min at room temperature. Fluorescence images before and after the addition of EGFP were captured under an excitation of 489 nm. As a control, 2 μ L of 10 μ g/mL EGFP protein solution was also added to a bare SWCNT FET device with blocking. Fluorescence images of the bare SWCNT FET device before and after EGFP binding were also taken under an excitation of 489 nm.

2.3. UV–Vis–NIR Absorption Spectroscopy. sc-SWCNTs (100 μ L, 0.02 mg/mL) were drop-casted on a 1" $\times$ 1" quartz slide and heated at 200 °C to evaporate the solvent. UV–vis–NIR spectra of the sc-SWCNT were collected using a PerkinElmer LAMBDA 900 UV–vis–NIR spectrophotometer.

2.4. Atomic Force Microscopy. Atomic force microscopy (AFM) data were collected using a Bruker Multimode 8 AFM system with a Veeco Nanoscope IIIa controller in the tapping mode. The AFM image and height profiles were processed and obtained in Gwyddion.

2.5. X-ray Photoelectron Spectroscopy. X-ray photoelectron spectroscopy (XPS) data were generated on a Thermo ESCALAB 250 Xi XPS using monochromated Al K α X-rays as the source. A 650 μ m spot size was used, and the samples were charge-compensated using an electron flood gun.

2.6. Raman Spectroscopy. The XplorA Raman-AFM/TERS system was used to record all Raman spectra. The radial breathing mode (RBM) region was recorded using a 785 nm (100 mW) excitation laser operating at 1% power. D and G peak regions were recorded using a 638 nm (24 mW) excitation laser operating at 1% power.

2.7. FET Measurements. Liquid-gated FET device configuration was employed to study the FET transfer characteristics of SARS-CoV-2 antibody-functionalized FET devices for the detection of SARS-CoV-2 antigens. Nanopure water was used as the gating electrolyte. FET characteristic curves were recorded by collecting the source-drain current (I_d), while sweeping the gate voltage from +0.6 to −0.6 V versus a 1 M Ag/AgCl reference electrode with a fixed drain voltage of 50 mV.

A series of SARS-CoV-2 spike S1-His recombinant protein (Sino Biological, Beijing, China; Cat# 40591-V08H) solutions ranging from 0.55 μ g/mL to 55 μ g/mL and recombinant coronavirus nucleoprotein (BioVision Inc.; Cat# P1523) ranging from 0.016 μ g/mL to 16 μ g/mL were prepared in PBS. All protein solutions were tested from the lowest to the highest concentrations. For each measurement, 2 μ L of the protein solution was added to the antibody-functionalized devices and incubated for 2 min. The devices were then washed three times with nanopure water to remove any unbound protein and measured in nanopure water as the gating electrolyte.

The relative response (R) of each FET device was calculated using $R = \Delta I/I_0$ at $-0.5 V_g$, where $\Delta I = I_d - I_0$ and I_0 is the drain current in nanopure water before antigen exposure at $-0.5 V_g$. The final results reported were averaged relative responses of two to six devices with standard deviation (SD) as error bars. The number of devices (n) tested for each experiment was specified in the figure.

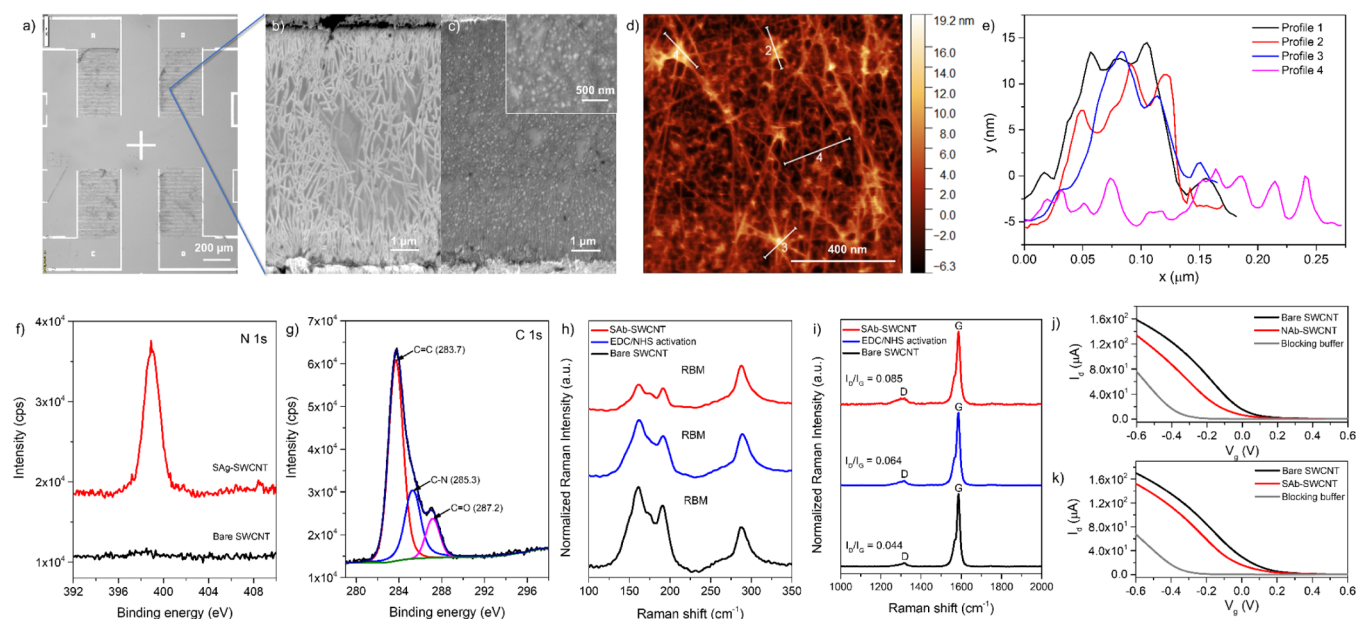


Figure 2. Characterizations of SARS-CoV-2 antibody-functionalized SWCNT FET devices. (a) Microscopic image of SWCNT FET devices. (b) SEM image of SWCNT networks deposited on a FET device and (c) after SAB functionalization. The inset shows a zoom-in view of SAB-functionalized SWCNTs. (d) AFM image of SAB-functionalized SWCNTs. (e) Height profiles of SAB immobilized on the SWCNT and bare SWCNTs. (f) High-resolution XPS spectra of N 1s of the bare SWCNT and antibody-functionalized SWCNT, and (g) C 1s of the antibody-functionalized SWCNT with deconvolutions of the overall signal. (h) RBM region and (i) D and G peak regions of Raman spectra of the SWCNT during antibody functionalization. The RBM region was recorded using a 785 nm excitation laser. All spectra were normalized to the Si peak at 507 cm^{-1} . D and G peak regions were recorded using a 638 nm excitation laser. All spectra were normalized to the G peak at 1587 cm^{-1} . (j) FET transfer characteristics of an SWCNT FET device during SAB functionalization and (k) NAb functionalization.

2.8. Clinical Sample Tests. Remnant nasopharyngeal swab samples tested by NAAT for SARS-CoV-2 RNA on emergency use authorized platforms for standard clinical care were used under the auspices of the University of Pittsburgh IRB study number 20040220. A total of 28 PCR-positive samples and 10 negative nasopharyngeal swab samples were tested. Each sample (10 μL) was added to the antibody-functionalized devices and incubated for 2 min. After 2 min, the sample was removed from the devices and the devices were washed three times with water. FET measurements were taken in water as the gating electrolyte.

The relative responses were calculated using the same method as previously described in “FET Measurements”. The final results reported were averaged relative responses of one to four devices with SD as error bars. The number of devices (n) tested for each sample is summarized in the Supporting Information.

3. RESULTS AND DISCUSSION

As shown in Figure 2a, the sensor chip contains four FET devices with interdigitated gold source and drain electrodes patterned on a Si/SiO₂ substrate. The channel length is 10 μm . To fabricate the SWCNT FET devices, sc-SWCNTs were deposited between interdigitated gold electrodes via DEP, forming dense and interconnected networks on the Si/SiO₂ surface (Figure 2b). UV–vis–NIR absorption spectroscopy was utilized to investigate the semiconducting content in the sc-SWCNTs (Figure S1), and the absence of the M₁₁ peak confirmed the high-purity semiconducting content.

SARS-CoV-2 SAB and NAb were conjugated onto sc-SWCNTs via EDC/sulfo-NHS coupling between the carboxylic acid groups on the SWCNT sidewalls and the amine groups on the antibody. The EDC/sulfo-NHS coupling of antibodies on sc-SWCNTs was visualized using the EGFP antibody and EGFP (Figure S2). The morphology of SAB on SWCNTs was characterized using scanning electron micro-

scopy (SEM) and AFM (Figure 2c,d). The height profiles indicated a 12–15 nm increase in height after SAB immobilization (Figure 2e). XPS provided complementary evidence for the integration of the antibody on SWCNTs. The high-resolution C 1s scan of the bare SWCNTs confirmed the presence of oxygenated defect sites on SWCNTs (Figure S3). The appearance of the N 1s peak and C–N (285.3 eV) peak after antibody coupling also suggested successful conjugation of the antibody on SWCNTs (Figures 2f,g and S3). Raman spectroscopy revealed the effect of antibody functionalization on SWCNTs. Two major peaks were observed in the RBM region. The peak that ranged from 125 to 225 cm^{-1} decreased in intensity during functionalization, suggesting a preference of antibody functionalization on SWCNTs with larger diameters (Figure 2h).²⁰ Meanwhile, the I_D/I_G ratio increased from 0.044 to 0.085, indicating an increase in the degree of functionalization on SWCNTs due to the covalent bonding of antibodies to the SWCNTs (Figure 2i). In FET transfer characteristics (Figure 2j,k), the shift of threshold voltage toward more negative gate voltages and the decrease in the device conductance in the p-type region also revealed the successful functionalization of SAB and NAB on sc-SWCNTs. Further shift of the threshold voltage and decrease in the conductance were detected after the addition of blocking buffer (0.1% Tween 20 and 4% polyethylene glycol) to prevent nonspecific binding. Meanwhile, the gate leakage current was negligible compared with the ON state source-drain current, suggesting good insulation between the gate and source-drain electrodes; therefore, no encapsulation was required for Au electrodes (Figure S4).

We first investigated the performance of our SARS-CoV-2 Ag FET biosensors using SARS-CoV-2 SAg and NAg in calibration samples. All FET transfer characteristics were

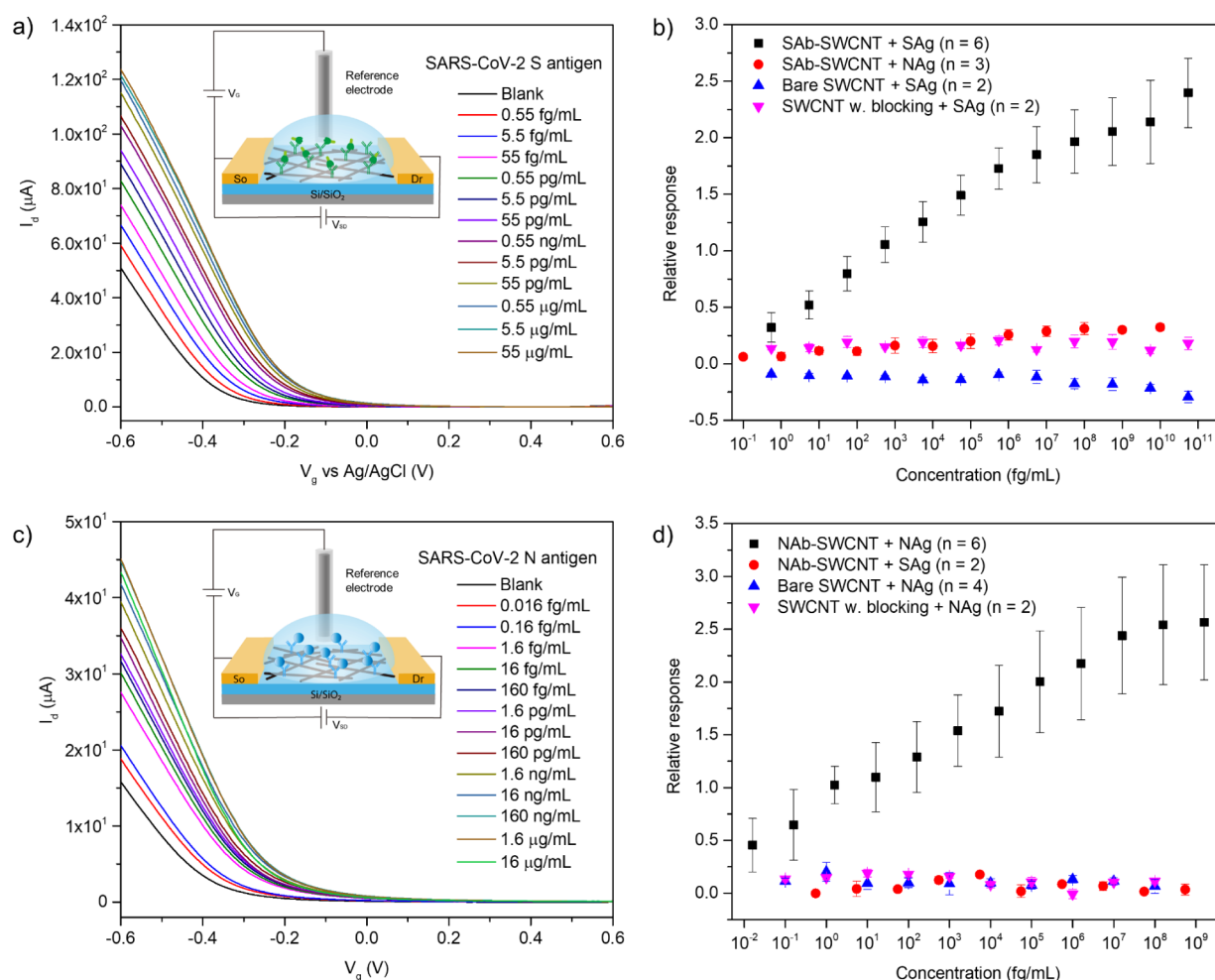


Figure 3. Detection of recombinant SARS-CoV-2 antigen proteins. (a) FET characteristic curves of SAb-functionalized SWCNT (SAb-SWCNT) FET devices upon exposure to increasing concentration of recombinant SARS-CoV-2 SAg. The inset shows the schematic illustration of FET configuration for SAg detection. (b) Calibration plot for SAg detection and nonspecific protein detection. (c) FET characteristic curves of NAb-functionalized SWCNT (NAb-SWCNT) FET devices upon exposure to increasing concentration of recombinant SARS-CoV-2 NAg. The inset shows the schematic illustration of FET configuration for NAg detection. (d) Calibration plot for NAg detection and nonspecific protein detection. All data points plotted in the calibration plots are mean $\pm$ SD. The number of devices (n) used for calculation is indicated in the parenthesis in the legend.

recorded by employing a liquid-gated FET configuration using nanopure water as the gating media to eliminate the impact of different ionic strengths on the sensing results. Figure 3a shows the I_d - V_g curve of SARS-CoV-2 SAb-functionalized FET devices for the detection of SAg. A shift toward more positive threshold voltages can be observed in the FET characteristics when only 0.55 fg/mL SAg was introduced to the device. The threshold voltage shifted further toward more positive values with increasing concentration of SAg (Figure S5a). This positive shift of the I_d - V_g curve can be attributed to the introduction of negatively charged SAg ($pI = 6.24$) near the SWCNT surface,²¹ inducing additional hole carriers, thus p-doping the SWCNTs.^{19,22} The calibration curve (Figure 3b) was constructed by plotting the relative response (R , $R = \Delta I/I_0$, where $\Delta I = I_d - I_0$ and I_0 is the drain current in nanopure water before antigen exposure at $-0.5 V_g$) against concentrations of SAg in a logarithmic scale, where the dynamic range of the SAg sensor can be determined to be 5.5 fg/mL to 5.5 pg/mL and the calibration sensitivity, defined as the slope of the linear region of the calibration curve, to be 0.25 by fitting the calibration curve (Figure S5b). Control experiments with

NAg demonstrated the high specificity of the SAb-functionalized devices. The low relative response of the bare SWCNT FET sensor with or without blocking toward SAg further indicated that the responses of the SAb-functionalized device toward SAg were indeed induced by the specific antigen-antibody interaction.

Similar to SAg detection, FET transfer characteristics of NAb-functionalized FET devices showed a consistent shift of the threshold voltage toward the more positive region with increasing NAg concentration (Figures 3c and S6a) due to the electrostatic gating effect. However, with the addition of positively charged NAg, the sensing mechanism is likely due to the neutralization of the positively charged antibody upon NAg binding.²³ By fitting the calibration curve, the dynamic range is found to be 16 fg/mL to 16 pg/mL and the calibration sensitivity is 0.22, displaying similar sensing performance to SAg detection (Figure S6b). Meanwhile, the NAb-functionalized FET devices showed minimal responses to nonspecific proteins, and SWCNT FET devices without NAb conjugation also did not respond to the addition of NAg, exhibiting the high specificity of the NAg sensor (Figure 3d).

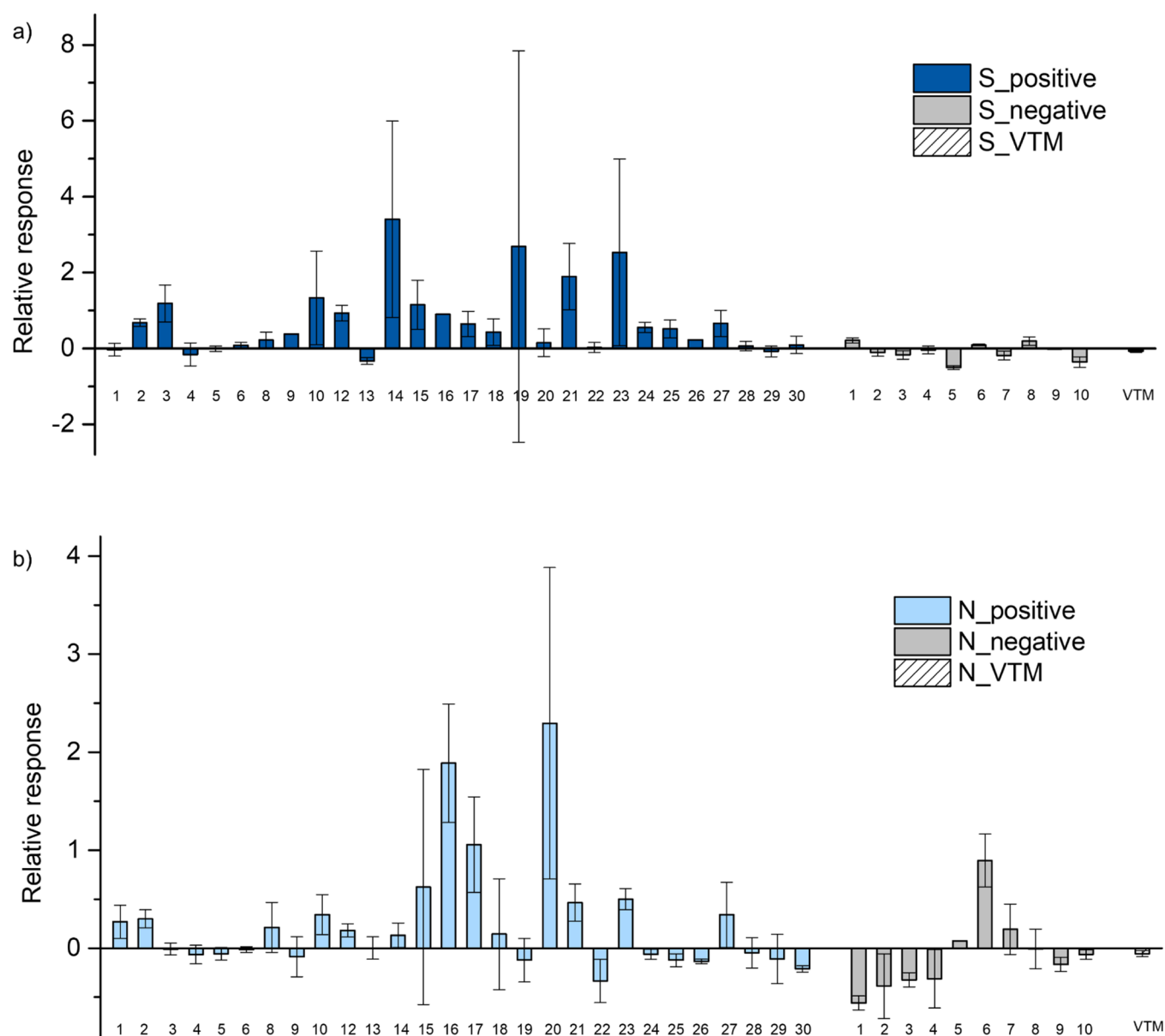


Figure 4. Clinical sample tests with (a) SAB- and (b) NAb-functionalized FET devices. All data plotted are mean $\pm$ SD. The number of devices (n) tested for calculation are summarized in Table S1.

Our SARS-CoV-2 Ag FET biosensors were then tested with clinical samples. A total of 28 NAAT positive samples and 10 NAAT negative samples were tested using both SAB- and NAb-functionalized FET biosensors. All clinical samples were nasopharyngeal swabs suspended in the viral transport medium (VTM), and the viral load of each sample was measured by approved FDA EUA NAAT assays. The existing literature indicates that the NAAT and antigen detection are biologically well correlated but not 100% concordant.^{24,25} The NAAT can detect RNA before a significant antigen is produced; additionally, the temporal course of the antigen versus RNA clearance after active infection is unclear. Figure 4 summarizes the relative responses of both SAB- and NAb-functionalized sensors for all samples and blank VTM. A total of 23 out of 28 SAB-functionalized FET devices responded positively to NAAT positive samples, consistent with what was observed previously, yielding a 17.8% false negative rate compared to the EUA NAAT. On the other hand, 7 out of 10 gave negligible or

negative responses toward NAAT-negative samples. The blank VTM, which contains Hank's balanced salt solutions, fetal bovine serum, gentamicin, and amphotericin B, only induced small negative relative response of the devices. Therefore, the negative responses and false positive responses from the NAAT negative samples may come from other biological species collected from the nasopharyngeal swab or represent the continued presence of the antigen after RNA clearance. The results suggested that our SAB-functionalized FET biosensor has the potential to work as a rapid clinical SARS-CoV-2 antigen detection diagnostic.

The NAb-functionalized FET devices demonstrated less effective discrimination between positive and negative samples as 15 out of 28 positive samples produced positive responses. Moreover, the induced responses are, in general, lower than those of SAB-functionalized devices. The less effective detection of NAg may be attributed to the lower concentration of available NAg present in the clinical samples. While SAg is

on the surface of SARS-CoV-2 virus, NAg, whose primary function is to form a capsid to protect the viral genome and enter the host cell, is released only when the virus enters the host cell.^{26–28} Without further sample processing, NAg might be limited within the virus or infected cell and therefore cannot be detected. For negative samples, although NAb-functionalized devices yielded the same false positive rate as SAB-functionalized devices, they had increased comparative responses to negative samples and blank VTM. This may indicate a higher susceptibility of NAb-functionalized devices to nonspecific binding of biomolecules or cross-reactivity of the antibody with other proteins. However, it is worth mentioning that the results for NAg detection are antibody-specific. Better sensing performance might be achieved using a different NAb.

4. CONCLUSIONS

In conclusion, we used SARS-CoV-2 antibody-functionalized SWCNT-based FET biosensors to assess the presence of the SARS-CoV-2 antigen in less than 5 min and at a few cents per test. Both SAB- and NAb-functionalized FET biosensors exhibited ultrasensitivity and high specificity toward their specific SARS-CoV-2 antigen in calibration samples. The limit of detection is determined to be 0.55 fg/mL for SAg and 0.016 fg/mL for NAg. Our SARS-CoV-2 Ag FET biosensor also achieved rapid detection of SARS-CoV-2 antigens in clinical samples without prior sample processing, and the results suggested that SAB-functionalized devices performed better than NAb-functionalized devices in discriminating COVID-19 positive and negative samples collected from nasopharyngeal swabs and are less susceptible to nonspecific species present in the clinical samples. Furthermore, our sc-SWCNT FET detection assay approach opens the opportunity for multiplex detection of not only viral antigens but also antibodies recognizing these antigens.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsami.0c22589>.

Experimental section, fluorescence images of the EGFP antibody-functionalized SWCNT FET device, UV–vis–NIR and XPS spectra of sc-SWCNT devices, fitting of calibration curves, and number of devices tested for clinical samples (PDF)

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Notes

The authors declare no competing financial interest.

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