Triple-Probe DNA Framework-Based Transistor for SARS-CoV-2 10-in-1 Pooled Testing

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ABSTRACT: Accurate and population-scale screening technology is crucial in the control and prevention of COVID-19, such as pooled testing with high overall testing efficiency. Nevertheless, pooled testing faces challenges in sensitivity and specificity due to diluted targets and increased contaminations. Here, we develop a graphene field-effect transistor sensor modified with triple-probe tetrahedral DNA framework (TDF) dimers for 10-in-1 pooled testing of SARS-CoV-2 RNA. The synergy effect of triple probes as well as the special nanostructure achieve a higher binding affinity, faster response, and better specificity. The detectable concentration reaches 0.025−0.05 copy μL−1 in unamplified samples, lower than that of the reverse transcript-polymerase chain reaction. Without a requirement of nucleic-acid amplification, the sensors identify all of the 14 positive cases in 30 nasopharyngeal swabs within an average diagnosis time of 74 s. Unamplified 10-in-1 pooled testing enabled by the triple-probe TDF dimer sensor has great potential in the screening of COVID-19 and other epidemic diseases.

KEYWORDS: pooled testing, field-effect transistor, COVID-19, DNA nanostructure

INTRODUCTION

As of January 2022, the global coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has induced 330 million infectious cases and 550 thousand deaths. The day-by-day increasing number of infected patients puts a momentous burden on public healthcare systems, resulting in a lack of medical resources and a crash of numerous economies.1−3 The interpersonal transmission and community spread model demands the development of easily operated diagnosis technology which can quickly identify infected individuals from a wide population for preventing the outbreak. Pooled testing with high detection throughput is the most frequently used method for population-scale screening, especially in regions with a population prevalence below 10%.4−6 However, during the sample pooling process, the viral load of the positive sample decreases relative to the increasing sample volume when mixed with negative samples, frequently causing false-negative results.6 The additional introduction of biomolecules and contaminations also raise the difficulty to accurately capture the targets. Thus, sensitivity and accuracy are the critical requirements in pooled testing. The quantitative reverse transcript polymerase chain reaction (qRT-PCR), as the gold-standard nucleic-acid detection technology, is the most convincing and widely employed method for COVID-19 testing. Nevertheless, it requires extraction and amplification procedures (>2 h) as well as skilled technicians and specific laboratories and equipment.7−9 Meanwhile, other established nucleic-acid testing methodologies face challenges in sensitivity and accuracy, which increase the difficulties when testing pooled samples.10−22 Therefore, it is urgently required to develop a rapid, sensitive, and easily operated method for SARS-CoV-2 nucleic-acid pooled testing.

The aptamer field-effect transistor (apt-FET) sensor is a pervasive method for tracing biological analytes owing to its relatively high sensitivity, specificity, and easily fabricated features.23−26 Considerable efforts have been made to improve the sensitivity, which mainly focus on the sensing materials, the design of the DNA probes, and the exploitation of new sensing mechanisms. Hwang et al. put forward a “deformed graphene channel” strategy and significantly improved the sensitivity, which led to a limit of detection (LoD) of single-stranded DNA (ssDNA) down to 10−19 mol L−1.27 A graphene field effect transistor (g-FET) sensor modified with multiprobe DNA nanomaterials may achieve the sensitivity requirement in...
pooled testing. Graphene is a single atomic-layer sensing material with high carrier mobility. The charge carriers (holes or electrons) in graphene are confined and exposed in a narrow range that is close to the surface which makes graphene ultrasusceptive to surrounding electrostatic variation and generates low intrinsic electrical noise. These combined

Figure 1. Triple-probe TDF dimer g-FET sensor for SARS-CoV-2 RNA testing. (a) Workflow and schematic diagram of the triple-probe TDF dimer g-FET sensor for SARS-CoV-2 RNA testing. The enlarged diagram is the g-FET sensing surface modified with a triple-probe TDF dimer. (b) Digital photograph of a working device. (c) Optical microscope image of the g-FET channel. (d) AFM image of the sensing surface after triple-probe TDF dimer immobilization (measured in fluid).

Figure 2. SARS-CoV-2 RNA testing. (a) Triple-probe TDF dimer structure, viral Genome map, and targeted regions of three probes. (b) Transfer curve measurement of adding different concentrations of target RNA ($I_d$–$V_g$ response curve). (c) Real-time $|\Delta I_d/I_d|$ response upon different concentrations of target RNA (red line, modified with triple-probe TDF dimer; gray line, without immobilized probes). (d) $|\Delta I_d/I_d|$ responses of single- and triple-probe TDF dimer g-FET sensors to different concentrations of target RNA. (e) $|\Delta I_d/I_d|$ responses of the triple-probe TDF dimer g-FET sensor to SARS-CoV-2 target RNA (0.05 copy $\mu$L$^{-1}$) and nontarget RNA (samples II, III, and IV: 0.5 copy $\mu$L$^{-1}$). All RNA samples were dissolved in full saliva with the addition of 12.5 mM Mg$^{2+}$, and the pH is 7.0–7.4. The error bars of parts d and e are defined by the standard deviation of the results from at least 3 parallel experiments.
features make graphene a sensitive material for biosensing applications. However, until now, the limit of detection (LoD) has ranged from $10^{-15}$ to $10^{-9}$ mol L$^{-1}$, which does not meet the sensitivity demand of pooled testing.

Creatures with multitentacle anatomies formed through thousands of years of evolution because this structure remarkably improves their sensitivity to smell and ability to catch and hunt. Inspired by these creatures, researchers have developed many multiprobe sensors that can notably increase the capture efficiency, resulting in higher detection sensitivity and selectivity. Nevertheless, these multiprobe sensors still cannot realize single- or few-molecule detections. Recent advances in structural DNA nanotechnology offer an accurate and controllable method to synthesize various DNA nanostructures with specific functions, which can improve the binding affinity to targets by designing different configurations in biodetection.

Here, we develop a g-FET sensor modified with a triple-probe tetrahedral DNA framework (TDF) dimer for SARS-CoV-2 RNA testing and modulate the sensing process at the molecular scale. The synergy of the triple probe improves the binding affinity and enables g-FET sensors to directly detect 0.025–0.05 copy µL$^{-1}$ (0.4–0.8 $×$ $10^{-9}$ mol L$^{-1}$) SARS-CoV-2 RNA in artificial saliva without amplification. The sensor exhibits 100% overall percent agreement (OPA) with RT-PCR results within an average diagnosis time of 74 s when testing nasopharyngeal swab samples. Because of its high sensitivity and accuracy, the sensor enables 10-in-1 nucleic-acid pooled testing, showing great values in population-scale screening of COVID-19 and other infectious diseases.

**RESULTS AND DISCUSSION**

The workflow of the triple-probe TDF dimer g-FET sensor for SARS-CoV-2 RNA testing is illustrated in Figure 1a. The sensor is configured from a liquid-gated g-FET with a polydimethylsiloxane (PDMS) chamber on the graphene surface (enlarged diagram). Each working sensing area (Figure 1c) is 30 × 150 μm$^2$ (W × L). The designed DNA nanostructure is a probe-tunable TDF dimer with an edge length of 7 bp (∼2.6 nm of theoretical length), and then, one, two, or three probes are anchored (Figure S2a) on the top tetrahedron. All of the DNA sequences (5′⋯3′) are listed in Table S1 (Supporting Information). The synthesized TDF monomer and dimer nanostructures are analyzed by 10% polyacrylamide gel electrophoresis; most of the lanes only have a major band, and the yield is calculated as ∼60% (Figure S2b), which can confirm the successful synthesis of the TDF dimer nanostructure.

The TDF dimers are immobilized onto the g-FET device (Figure S3) through molecule linkers: 1-pyrenebutanoic acid succinimidyl ester (PASE). The Raman spectrum (Figure S1a–c) demonstrates that PASE is modified on the graphene surface through π⋯π interaction and remains homogeneous after PASE modification. An atomic force microscopy (AFM) image measured in fluid reflects that TDF dimers are anchored on the sensing surface in an orderly manner (Figure 1d). AFM images (Figure S4) measured in air show the morphology of the sensing surface, and the roughness of the surface observably increases when graphene is modified with PASE (2.0–2.5 nm) and is immobilized with triple-probe TDF dimer (5.8–7.0 nm) compared to that (0.8–1.2 nm) of the intrinsic graphene surface. Moreover, the appearances of the N 1s peak and P 2p peak (Figure S5a,b) in X-ray photoelectron
spectroscopy (XPS) measurements after modification also verify that the PASE and TDF dimer are anchored on the graphene sensing surface. In the transfer curve measurement of the device, the interaction between PASE and graphene induces an n-doping effect on graphene, giving rise to a negative offset (8–20 mV) of $V_{\text{Dirac}}$ (as the liquid-gate voltage $V_{\text{lg}}$ when the drain-source current $I_{\text{ds}}$ reaches its minimum). After TDF dimer immobilization, $V_{\text{Dirac}}$ further shifts negatively by 22–32 mV. All of these results demonstrate the successful modification of PASE and TDF dimer on the g-FET sensing surface.

The three probes designed in this work target SARS-CoV-2 RNA at the ORF1ab gene (nt 13377–13404), RdRp gene (nt 15469–15494), and E gene (nt 26332–26358) regions, respectively (Figure 2a). The transfer curve measurement ($I_{\text{ds}}$ vs $V_{\text{lg}}$) of the triple-probe TDF dimer g-FET device shows the response when adding the IVT RNA solutions (Figure 2b); the $V_{\text{Dirac}}$ exhibits a continues negative shift signal to $\sim$60 mV when the RNA solution concentration changes from 0.025 to 1000 copy $\mu$L$^{-1}$. Compared to the $I_{\text{ds}}$ vs $V_{\text{lg}}$ responses of single- and dual-probe TDF dimer devices to IVT RNA (Figure S7a,b), the $V_{\text{Dirac}}$ of the triple-probe TDF dimer presents an at least 2-fold signal enhancement. In addition, the $V_{\text{Dirac}}$ of the triple-probe TDF dimer device exhibits a $\sim$10 mV negative offset when 0.025 copy $\mu$L$^{-1}$ target RNA solution is added (Figure 2b), which demonstrates that the g-FET sensor modified with the triple-probe TDF dimer is highly sensitive.

The real-time responses ($I_{\text{ds}}$ vs $t$) of the triple-probe TDF dimer g-FET device to target RNA solution are recorded (Figure 2c) with different concentrations (from 0.025 to 1000 copy $\mu$L$^{-1}$). Remarkable electrical response signals ($|\Delta I_{\text{ds}}|/I_{\text{ds0}}$ decrease) are detected within 5 min even when the target RNA solution concentration is 0.025 copy $\mu$L$^{-1}$, and the response time is shorter than that (10–15 min) of dual- and single-probe TDF dimer devices (Figure S7c,d). For the g-FET device without immobilized probes, no obvious response signals (Figure 2c) are observed even when 1000 copy $\mu$L$^{-1}$ target RNA solution is added (gray line). In

Figure 4. Clinical validation of the triple-probe TDF dimer g-FET sensor. (a) Real-time $|\Delta I_{\text{ds}}|/I_{\text{ds0}}$ response of P1 and P1. The inset is the diagnosis time for P1–P14. (b) $|\Delta I_{\text{ds}}|/I_{\text{ds0}}$ response upon an addition of clinical samples (P1–P14, F1–F6, and H1–H10). (c) Confusion matrix summarizing the assay discrimination performance between positive and negative swab samples. (d) Real-time $|\Delta I_{\text{ds}}|/I_{\text{ds0}}$ response upon various diluted concentrations (1%, 10%, and 100%) of P8. (e) Comparison of this assay with other reported methods for SARS-CoV-2 RNA detection. (f) Work流程 illustration for the 10-in-1 pooled sample testing strategy. (g) Real-time $|\Delta I_{\text{ds}}|/I_{\text{ds0}}$ response of the 10-in-1 pooled negative sample (M1) and positive sample (M9). (h) $|\Delta I_{\text{ds}}|/I_{\text{ds0}}$ response upon the addition of clinical 10-in-1 pooled samples (negative, M1–M7; positive, M8–M14).
| detection method          | analyte type                      | target                          | sample or medium | amplification | LoD                  | response time | clinical validation | pooled testing | ref |
|---------------------------|-----------------------------------|---------------------------------|------------------|---------------|----------------------|---------------|--------------------|----------------|-----|
| qRT-PCR                   | viral RNA                         | ORF1ab and N gene               | sputum samples   | yes           | 10 copy/reaction     | 75 min        | yes                | no             | 49  |
| qRT-PCR                   | viral RNA                         | RdRp1, RdRp2, E gene, N gene, N1, N2, and N3 | nasopharyngeal swabs | yes           | 5 copy/μL N3; 10 for N1, N2, E; 50 for RdRp1, RdRp2 | 60–90 min | yes                | no             | 50  |
| qRT-PCR (China NMPA)      | viral RNA                         | ORF1ab and N gene               | nasopharyngeal swabs | yes           | 0.6–3.2 copy/μL     | >120 min       | yes                | no             | 3   |
| qRT-PCR (US CDC)          | viral RNA                         | N1, N2, and N3                  | nasopharyngeal swabs | yes           | 1–3.2 copy/μL       | >120 min       | yes                | no             | 51  |
| 384 RT-PCR method         | viral RNA                         | N1, N2 gene                     | nasopharyngeal swabs | yes           | 5 copy/μL           | 73.2 min       | yes                | no             | 52  |
| qRT-PCR                   | viral RNA                         | RdRp/helicase (Hel), S, and N gene | nasopharyngeal swabs | yes           | 11.2–21.3 copy/reaction | ~60 min | yes                | no             | 9   |
| iLACO (RT-LAMP)           | synthetic RNA                     | ORF1ab gene                     | nasopharyngeal swabs | yes           | 10 copy/reaction     | 15–40 min      | yes                | no             | 53  |
| early detection RT-LAMP   | viral RNA                         | N gene                          | nasopharyngeal swabs | yes           | 118.6 copy/reaction  | 30–40 min      | yes                | no             | 54  |
| RT-LAMP/Cas12 DETECTR assay | synthetic RNA                     | E and N gene                    | nasopharyngeal swabs | yes           | 10 copy/μL          | 45 min         | yes                | no             | 12  |
| AIOD-CRISPR-Cas12a assay  | viral RNA                         | N gene                          | nasopharyngeal swabs | yes           | 5 copy/μL           | 20–40 min for extraction; 20 min for reaction | yes                | no             | 14  |
| RT-LAMP                   | viral RNA                         | ORF1ab, E and N gene            | throat swab specimens | yes           | 1 copy/μL           | 30 min         | yes                | no             | 55  |
| RPA/SHERLOCK assay        | synthetic RNA                     | S and ORF1ab gene               | Hybrid detect assay buffer | yes           | 10 copy/μL          | 60 min         | no                 | no             | 56  |
| CRISPR/SHERLOCK assay     | viral RNA                         | S, N and ORF1ab gene            | nasopharyngeal and throat swabs | yes           | 42 copy/μL          | >60 min        | yes                | no             | 13  |
| RT-RAA assay              | viral RNA                         | S, ORF1ab gene                  | nasopharyngeal swabs | yes           | 10 copy/reaction for S gene | 20–25 min | yes                | no             | 10  |
| Exo-1QRT-RPA assay        | viral RNA                         | N gene                          | nasopharyngeal swabs | yes           | 7.74 copy/μL        | 20–25 min      | yes                | no             | 11  |
| electrochemical DPV       | viral RNA                         | ORF1ab, N gene                  | saliva or nasopharyngeal swabs | no           | 0.2 copy/μL         | 190 min        | no                 | no             | 17  |
| electrochemical DPV       | viral RNA                         | S, N gene                       | nasopharyngeal swabs | yes           | 1 copy/μL           | <120 min       | yes                | no             | 15  |
| current−voltage electrochemical assay | viral RNA | N gene | nasopharyngeal swabs | no | 6.9 copy/μL | 30 min for extraction, 5 min for detection | yes                | no             | 16  |
| ELISA                     | antibody                          | human IgM and IgG serum         | human IgM and IgG reaction mixture | no | N.A. | 60–180 min | yes                | no             | 57  |
| chemiluminescent immunoassay | antibody                          | human IgM and IgG serum         | reaction mixture | no | 4.6 μM | 48 min | no                 | no             | 58  |
| MALDI-MS                  | virus antigen                     | SARS-CoV-2 S-protein            | nasopharyngeal swabs | no | N.A. | >20 min | yes                | no             | 18  |
| FET-based biosensor       | antigen                            | SARS-CoV-2 S-protein            | nasopharyngeal swabs | no | 0.242 copy/μL | >1 min | yes                | no             | 46  |
| electrochemical DPV       | antigen | SARS-CoV-2 S-protein | saliva | no | 16.66666 copy/μL | 10–30 s | no | no | 59 |
| TDF dimer g-FET assay     | viral RNA                         | ORF1ab, RdRp and E gene         | nasopharyngeal swabs | no | 0.025–0.05 copy/μL | 1–4 min (74 s in average) | yes | yes | this work |
addition, from the $|\Delta I_d/I_{ds0}|$ response values (Figure 2d) of the triple-probe TDF dimer g-FET sensor, the calculated LoD reaches 0.01 copy $\mu$L$^{-1}$ (Figure S9). Then, the $|\Delta I_d/I_{ds0}|$ responses of the single- and dual-probe TDF dimer device to target RNA solution are also measured under the same conditions; the response values of the triple-probe TDF dimer device are at least twice that of the single-probe device (Figure 2d, Figure S7e). This is mainly attributed to the high probability and matching rate of the triple probe, which enables more efficient binding to target RNA and then showcases a larger $|\Delta I_d/I_{ds0}|$ response value and shorter response time in $I_{ds0}−t$ measurements as well as a larger $V_{\text{Dirac}}$ offset in $I_{ds0}−V_{lg}$ measurements. Moreover, we measured the $I_{ds0}−V_{lg}$ and $I_{ds0}−t$ response of the device modified with ssDNA probes (Figure S8). The ssDNA probes contain three corresponding types of probes mixed at a 1:1:1 ratio. It is found that $V_{\text{Dirac}}$ negative offset is negligible, and the $|\Delta I_d/I_{ds0}|$ response values are smaller than those of triple-, dual-, and single-probe TDF dimer g-FET devices.

Then, we measured the $I_{ds0}−t$ response values by adding three nontargeted RNA samples with different concentrations (0.5, 5, 50, and 100 copy $\mu$L$^{-1}$) in artificial saliva, including MERS-CoV RNA (sample II), SARS-CoV RNA (sample III), and human RNA (sample IV). The $|\Delta I_d/I_{ds0}|$ response values of nontargeted samples (Figure 2e, Figures S10 and S11) are negligible compared to that of the target sample (sample I: SARS-CoV-2 RNA solution). Therefore, we conclude that the triple-probe TDF dimer modified g-FET sensor exhibits an excellent detection performance with ultrasensitivity, short response time, and high specificity. By replacing the probes on the top tetrahedra of the triple-probe TDF dimers, we also measured the transfer curve and real-time response of the sensor upon different concentrations of cDNA (extracted from a confirmed COVID-19 case reverse transcribed cDNA). From the $|\Delta V_{\text{Dirac}}|/|\Delta V_{\text{Dirac,max}}|$ and $|\Delta I_d/I_{ds0}|$ response values (Figure S12), we find that the sensors also have excellent detection performances when testing the cDNA samples, which shows a much broader applicability of the sensor.

Biodetection includes biorecognition and signal transduction processes. The efficient recognition rate to target RNA and the signal transduction process is the fundamental reason that the triple-probe TDF dimer g-FET sensor achieves such an excellent detection performance. The recognition upon target RNA is essentially a DNA hybridization reaction, and the binding affinity between the DNA probe and target RNA is the crucial factor in this process. The signal transduction process can be amplified via the FET device and reflected in the electrical measurement, which is mainly revealed in the $V_{\text{Dirac}}$ offset of transfer curve test and the current change of real-time measurement. The DNA hybridization reaction can induce charge accumulation on the graphene surface and then cause a doping effect on graphene, and the monolayer graphene with high mobility enables an efficient and sensitive signal transduction process.

We chose another two types of DNA probes (Figure 3a,b) as control experiments and tested the $I_{ds0}−V_{lg}$ responses to target IVT RNA. One probe type is the single-probe TDF dimer, and the other one is three types of ssDNA probes mixed with a 1:1:1 ratio. Meanwhile, we calculate the binding affinity by using the normalized response of $\Delta V_{\text{Dirac}}/\Delta V_{\text{Dirac,max}}$ (the correlation between $\Delta V_{\text{Dirac}}/\Delta V_{\text{Dirac,max}}$ and target RNA concentration is described by the Hill–Langmuir model).
where \( \Delta V_{\text{Dirac,max}} \) is the maximum \( \Delta V_{\text{Dirac}} \) denoted \( \Delta V_{\text{Dirac,max}} = V_{\text{Dirac,max}} - V_{\text{Dirac,0}} \) (\( V_{\text{Dirac,0}} \) and \( V_{\text{Dirac,max}} \) refer to the offset of adding the zero and maximum concentration of target RNA solution, respectively); \( A \) is the saturation response coefficient of the sensing system; and \( n \) is the Hill coefficient corresponding to the binding cooperativity. The pseudo-\( K_D \) of the triple-probe TDF dimer sensor is 0.1–0.3 \( \times 10^{-15} \) mol \( \mu L^{-1} \) (\( \sim 0.01 \) copy \( \mu L^{-1} \)) and is calculated from the fitted curve (Figure 3d), which is 3 and 7 orders of magnitude lower than those of the single-probe TDF dimer sensor and ssDNA sensor, respectively. The fitted \( I_{ds} - V_g \) responses (Figure 3e) of triple- and single-probe TDF dimer and ssDNA probe modified sensors are obtained by using the standard deviation of 8 parallel measurements, and the results reveal that the triple-probe TDF dimer sensor has an offset of \( \Delta V_{\text{Dirac}} \) larger than those of the other two types of sensors. The testing repeatability of the three different types of DNA probe sensors (Figure 3f) also indicates that the triple-probe TDF dimer sensor has a remarkable signal compared to the other two types of sensors. Furthermore, we also measured the transfer curve response and real-time test (Figures S6 and S7) of the other three control types of DNA probe sensors (dual- and single-probe TDF dimer and ssDNA probes). These results reveal that the triple-probe TDF dimer g-FET sensor exhibits a better sensing ability than the others. The synergy effect of the triple probe enables a higher binding affinity and shorter biorecognition time compared to the dual and single probe. Besides, unlike ssDNA probes, the existence of the TDF dimer structure can effectively prevent the probe DNA intertwining with each other and avoid nonspecific biomolecule adsorption on the graphene surface. Thus, the special structure of the TDF dimer as well as the triple probes, combined with efficient signal transduction of g-FET, gives rise to ultrasensitive detection. The confocal fluorescence microscopy measurement indicates that the device modified with the triple-probe Cy3-conjugation (Cy3: a fluorescent dye Cyanine3, rather than a probe) TDF dimer has a stronger fluorescence intensity than the device modified with ssDNA Cy3-conjugation (Figure 3c). The main reason is that ssDNAs on the sensing surface are easily entangled with each other and tend to adsorb laterally on the graphene surface. The confocal microscopy measurement also demonstrates that the modification manner of the DNA probes on the graphene surface was consistent with the schematic diagram depicted in Figure 3a,b. Furthermore, we tested 14 nasopharyngeal swabs (P1–P14) collected from SARS-CoV-2 positive patients with the cycle-threshold (Ct) of RT-PCR ranging from 24.9 to 40.5, 6 samples (P1–F6) from fever clinic patients, and 10 samples (H1–H10) from healthy volunteers. The real-time \( |\Delta I_{ds}/I_{ds0}| \) response upon P1 and F1 (Figure 4a) and a histogram of statistical responses (Figure 4b) from all clinical samples are also recorded. Although the nasopharyngeal swabs include large amounts of nonspecific biomolecules and contaminations, SARS-CoV-2 RNA from positive samples (P1–P14) can be detected by this sensor and showed considerable signals \( (|\Delta I_{ds}/I_{ds0}| > 0.8\%) \), whereas weak signals \( (|\Delta I_{ds}/I_{ds0}| < 0.1\%) \) are monitored (Table S2) when testing the negative samples (F1–F6) and healthy samples (H1–H10). The testing results of 30 nasopharyngeal swabs exhibit 100% OPA with RT-PCR results, as well as 0.93 sensitivity and 1.00 specificity (Figure 4c), indicating that this triple-probe TDF dimer g-FET sensor can clearly identify the positive and negative samples. Moreover, the response time for diagnosing COVID-19 positive samples (P1–P14) is shortened to 1–4 min with an average of 4 s (inset of Figure 4a) with the \( |\Delta I_{ds}/I_{ds0}| \) response value reaching 3 times that of negative patients (Figure S13a). The \( |\Delta I_{ds}/I_{ds0}| \) responses (Figures S13b,c and S14) upon other clinical samples (P3–P4, F3–F6, and H1–H10) are also measured and recorded in the Supporting Information. In addition, to verify that this sensor is more sensitive than the qRT-PCR assay, we tested the \( I_{ds}-t \) response of the sensor to diluted clinical samples (P8: Ct = 30, diluted from 1/10 to 1/100 times) sequentially (Figure 4d, Figure S15). The result reveals that this sensor still achieves an observable \( |\Delta I_{ds}/I_{ds0}| \) response (0.54%) even when the sample is diluted by 1/10 times, which indicates that the triple-probe TDF dimer g-FET sensor has higher sensitivity than the qRT-PCR assay. Compared with the commercial COVID-19 detection kits\(^8\) (Table S4) and other established methodologies, such as qRT-PCR,\(^9,49,50,12\) the US CDC and China National Medical Products Administration (NMPA)-approved qRT-PCR,\(^5,31\) assay, the reverse transcription loop-mediated isothermal amplification (RT-LAMP),\(^12,53–55\) assay, the clustered regularly interspaced short palindromic repeats (CRISPR),\(^13,14,56\) assembly, the electrochemical (EC),\(^15–17\) assay, and other methods,\(^12,16,46,57–59\) this triple-probe TDF dimer g-FET sensor assay exhibits a shorter response time and higher LoD in COVID-19 viral RNA detection (Figure 4e, Table 1). The above testing results demonstrate that the sensor exhibits a rapid and sensitive detection capability, which can solve the extrinsic problems of having insufficient sensitivity and being a time-consuming process, thus enabling 10-in-1 pooled testing. The workflow of pooled testing is illustrated in Figure 4f. First, 200 \( \mu L \) portions of each nasopharyngeal swab sample are collected from 10 healthy individuals and then mixed in one pool as the negative samples (M1–M7). For the 10-in-1 positive pooled samples (M8–M14), 200 \( \mu L \) portions of each nasopharyngeal swab sample are collected from 9 healthy individuals and mixed with 200 \( \mu L \) portions of each nasopharyngeal swab sample from positive samples (P8–P14) in one pool, respectively. The real-time \( |\Delta I_{ds}/I_{ds0}| \) responses of M1 and M9 (Figure 4g) and M2–M4, M8, and M10 (Figure S16) are also recorded by this sensor. All of the samples (M8–M14) mixed with one positive sample exhibit larger \( |\Delta I_{ds}/I_{ds0}| \) responses from 2.69% to 10.58%, while the negative mixture samples (M1–M7) only generate weak responses from 0.056% to 0.228% (Figure 4h, Table S3). These 10-in-1 pooled testing results reveal that the sensor can rapidly and remarkably distinguish the pooled samples mixed with 1 positive nasopharyngeal swab. This sensor provides an accurate and easily operated detection method that exhibits great practical value in rapid identification of COVID-19 infected individuals from a wide population and in realizing large-scale screening of infectious epidemic diseases.

**CONCLUSIONS**

In this work, we develop a g-FET sensor modified with a triple-probe TDF dimer that realizes unamplified testing of samples from COVID-19 infected individuals as well as 10-in-1 pooled testing of different types of clinical samples.
samples. Compared with existing technologies, this sensor achieves shorter diagnosis time and higher sensitivity via improving the binding efficiency by the synergy effect; it overcomes extrinsic defects when employed in 10-in-1 pooled testing. The sensor can identify the positive pooled samples despite its low viral load, which reveals great value in solving the problem of point-of-care detection and population-scale screening for COVID-19, as well as the mutated strains of SARS-CoV-2 virus and other infectious diseases via replacing the probes. Moreover, the sensor can be developed into a comprehensive platform when integrated with a portable microelectronic system, which can rapidly and accurately monitor the COVID-19 patients in airports, rail stations, and other public places with huge visitor flow rates. Rapid, portable, easily operated 10-in-1 nucleic-acid pooled testing, which increases the testing throughput and reduces the consumption of medical resources, can alleviate the burden on public healthcare services.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.nanolett.2c00415.

Detailed information on the experimental description, LoD and RSD calculations, supplementary tables, Raman spectrum, synthesis and characterization of the TDF dimer, AFM image of surface morphology, XPS data, control experimental measurements, and clinical testing data (PDF).

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Author Contributions
D.W. conceived of the ideas, supervised the project, and provided the foundational support. Y.W. performed the research, did the experiments, analyzed the data, drew the figures, and prepared the manuscript. L.W. designed the TDF nanostructure, supplied the SARS-CoV-2 IVT RNA samples, and provided guidance for the ideas. C.D. and D.K. assisted in data analysis and provided help for the figures. Y.C. assisted in fluorescence measurements. M.G. prepared the clinical swap samples. D.J. and D.W. revised the manuscript. Y.L. supported the research and commented on the manuscript. All authors have given approval to the final version of the manuscript.

Notes
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