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CRISPR-Cas12a coupled with universal gold nanoparticle strand-displacement probe for rapid and sensitive visual SARS-CoV-2 detection

Sitong Liu a, Tie Xie b, Xiaojing Pei a,*, Shujing Li a, Yifan He a, Yigang Tong b, Guoqi Liu c

a College of Chemistry and Materials Engineering and Institute of Cosmetic Regulatory Science, Beijing Technology and Business University, Beijing 100048, PR China
b College of Chemistry and Materials Engineering, Beijing Technology and Business University, Beijing 100048, PR China
c Biotecnovo (Beijing) Co., LTD, Beijing Economic and Technological Development Zone, Beijing, PR China

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ABSTRACT

Point of care (POC) diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are particularly significant for preventing transmission of coronavirus disease 2019 (COVID-19) by any user at any given time and place. CRISPR/Cas-assisted SARS-CoV-2 assays are viewed as supplemental to RT-PCR due to simple operation, convenient use and low cost. However, most current CRISPR molecular diagnostics based on fluorescence measurement increased the difficulty of POC test with need of the additional light sources. Some instrument-free visual detection with the naked eye has limitations in probe universality. Herein, we developed a universal, rapid, sensitive and specific SARS-CoV-2 POC test that combines the outstanding DNase activity of Cas12a with universal AuNPs strand-displacement probe. The oligo trigger, which is the switch the AuNPs of the strand-displacement probe, is declined as a result of Cas12a recognition and digestion. The amount of released AuNPs produced color change which can be visual with the naked eye and assessed by UV-Vis spectrometer for quantitative detection. Furthermore, a low-cost hand warmer is used as an incubator for the visual assay, enabling an instrument-free, visual SARS-CoV-2 detection within 20 min. A real coronavirus GX/P2V instead of SARS-CoV-2 were chosen for practical application validation. After rapid virus RNA extraction and RT-PCR amplification, a minimum of $2.7 \times 10^2$ copies/mL was obtained successfully. The modular design can be applied to many nucleic acid detection applications, such as viruses, bacteria, species, etc., by simply modifying the crRNA, showing great potential in POC diagnosis.

1. Introduction

Rapid and accurate viral detection is critical for infectious status assessment, decision making, and subsequent control of viral dissemination. COVID-19 pandemic caused by the severe acute respiratory disease coronavirus 2 (SARS-CoV-2), posed an unprecedented threat to worldwide public health and economies. Diagnostic tools that are rapid, accurate, specific, sensitive are urgently necessary to control the spread of SARS-CoV-2 [1,2]. While nucleic acid-based tests are considered to be more sensitive regarding serological tests but present gold standard qRT-PCR-based assays with limitations such as requirement for professional operation, sophisticated reagents and instrumentation [3]. Point of care diagnosis are particularly significant for the diagnosis of COVID-19, and can be applied in quantitative testing of SARS-CoV-2 loads in samples by any user at any given time and place [4]. The CRISPR/Cas nucleases are programmed with CRISPR RNAs (crRNAs) and exhibit target-dependent indiscriminate endonuclease activity, leading to trans-cleavage nearby single-strand nucleic acids [5,6]. CRISPR assays showed outstanding competence and have been viewed as augmenting RT-PCR [7,8]. For instance, J. Collins et al. reported a face mask with a lyophilized CRISPR sensor for wearable, noninvasive detection of SARS-CoV-2 at room temperature within 90 min [9]. Ge et al. constructed a serious of CRISPR/Cas12a photo-electrochemical biosensors based on nanomaterials [10–12]. Yet, most current CRISPR molecular diagnostics based on trans-cleavage activity engineered the fluorescent ssDNA reporters [5,13–15]. Additional light sources of fluorescence increase the difficulty of POC applications. Instrument-free, visual detection with the naked eye is one important

* Corresponding author.
E-mail address: pxj@btbu.edu.cn (X. Pei).

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key for nucleic acids detection at the point of care [16–23]. Lateral flow strips based on CRISPR-Cas12a and-Cas13a are commonly used for visual readout for simple and sensitive detection of SARS-CoV-2 with the advantages of simple operation and low cost [24–27]. However, it’s more appropriate for antigen and antibody tests, and nucleic acid test is difficult to achieve signal changes by base complementary pairing hybridization [28,29]. In addition, they cannot be used for quantitative detection, which is crucial for monitoring disease progression in patients.

Gold nanoparticles (AuNPs) are highly efficient visual readout that has been widely used for sensitive nucleic acids detection due to their outstanding optical characteristics benefiting from the localized surface plasmon resonance [28,30]. Moreover, excellent properties of low cost, good biocompatibility and easy surface chemistry has attracted considerable interest in both academia and industry [31,32]. Mirkin and coworkers are the pioneers for developing the DNA-functionalized gold nanomaterials for sequence-specific gene detection [33]. Following numerous significant efforts has been made based on outstanding optical characteristics of AuNPs [28,34–40]. However, most of AuNPs probes with limitations in universality, which need to be redesigned when detecting different targets, thus increasing the cost and time spent on optimizing of the experimental conditions [41,42].

We herein developed a universal, rapid, specific and sensitive SARS-CoV-2 detection that combines the specific recognition and trans-cleavage ability of Cas12a with AuNPs strand-displacement probe. The AuNPs strand-displacement probe was ingeniously designed as sandwich-structured nanocomplex composed of DNA-functionalized AuNP, linker DNA, and DNA-functionalized magnetic bead. The sandwich structure’s overhang region acts as a toehold hybridized with an oligo Trigger to initiate the strand displacement for releasing AuNPs. The modular design can be applied to a number of nucleic acid detection applications, including the detection of viruses, bacteria, species, etc., by simply modifying the crRNA. We validated the feasibility by taking SARS-CoV-2 ORF1a as the model target and investigated the amount of products of SARS-CoV-2 ribonucleic acid template.

2. Material and methods

2.1. Materials and reagents

Chloroauric acid (HAuCl₄), trisodium citrate, Na₂HPO₄, NaCl and NaH₂PO₄ are obtained from Sinopharm Chemical Reagent Co., Ltd. Streptavidin-modified magnetic bead (Dynabeads™ MyOne™ Streptavidin T1, 10 mg/mL) is purchased from Thermo Fisher Scientific. All HPLC-purified DNA oligonucleotides and crRNA are purchased from Sangon Biotech Co., Ltd. (Shanghai, China). The sequences of the oligonucleotides are listed in Tables S2–S4. The diethylpyrocarbonate (DEPC)-treated water was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). 1 M Cas12a and Buffer 3.1 (pH 7.9) containing NaCl (100 mM), Tris-HCl (50 mM), MgCl₂ (10 mM), DTT (1 mM), were purchased from New England Biolabs (Beijing, China). RNase inhibitors were purchased from Takara Biotechnology Co., Ltd. (Dalian, China). The RNase-free environment throughout the experiments using DEPC-treated water and RNase-free tips and tubes. GX/P2V beta coronavirus isolated by using vero E6 cells were provided from Yiqiang Tong’s laboratory.

2.2. Instrumentation

Ultraviolet-visible (UV–vis) absorption spectrum were measured with Nanodrop one Thermo Fisher Scientific. Fluorescence measurements were carried out at 25 °C using BIO-GENER Q3200 real-time fluorescence PCR instrument.

2.3. Preparation of AuNPs

AuNPs were synthesized by reducing HAuCl₄ with sodium citrate, as described in our previous work [28]. All glassware was cleaned with freshly prepared aqua regia and rinsed thoroughly with water. Specifically, 4 mg HAuCl₄ powder with 40 mL water were mixed to make a 0.1 mg/mL HAuCl₄ solution. The water bath was used for incubation heating with parameters set as 100 °C, 250 rpm. After the temperature rises to 100 °C, 200 mL of HAuCl₄ solution were added in a 250 mL three-necked flask and placed in water bath. When the temperature of the liquid rises to 100 °C, 3 mL of 38.8 mM sodium citrate solution were added. When the solution changed from purple to wine red, continue to heat and reflux for 40 min. Then, the three-necked bottle were taken out and dropped to room temperature, the resulting solution were transfer to the 500 mL reagent bottle and stored at a 4 °C for use. The particle size, as determined by transmission electron microscopy (TEM), was 22.9 ± 2.4 nm (Fig. S1).

2.4. Functionalisation of AuNPs

The aging salt approach was used to functionalize AuNPs at a temperature of 25 °C [43]. Specifically, 100 μL of 10 μM L-Au was transferred into a microcentrifuge tube, and 10 μL of 10 mM TCEP was added to activate the thiol-modified DNA. After incubation at 25 °C for 1 h, 1 mL of the above prepared gold nanoparticles was added to the TCEP-treated thiol DNA, and the resulted mixture was stored at 4 °C for 16 h. Subsequently, 100 μL of 50 mM PBS (pH 7.6) was added with gentle shaking. The resulted phosphate concentration was 5 mM. Then, with gentle vibration, 100 μL of 1 M NaCl was added. The reaction solution was placed in the dark for 24 h. Excess DNA was then got rid of by centrifugation at 4500 rpm for 7 min. After the supernatant has been removed, the red precipitate was washed three times with deionized water and then finally redispersed in fresh PBS.

2.5. Preparation of AuNPs strand-displacement probe

The method of modifying the oligo Trigger on the surface of AuNPs is as follows: after diluting the AuNPs to be functionalized by a certain number, add the diluted Linker, 75 °C, 1500 rpm, 5 min, anneal to 37 °C, add L-MB DNA, continue annealing to 25 °C. The nucleotide sequences not involved in hybridization were removed by centrifugation, dispersed with PBS buffer, and then 10 mg/mL streptavidin-modified magnetic beads were added, and the reaction was shaken at 25 °C and 1500 rpm. Magnetic separation was performed after 30 min, the AuNPs that were not successfully connected in the supernatant were then washed with PBS buffer and removed, and placed at 4 °C for later use.

2.6. Visual SARS-CoV-2 detection

Specifically, the detection system includes 0.5 μL 20 μM crRNA (500 nM), 0.5 μL 10 μM Cas12a (250 nM), 2 μL 5 μM oligo Trigger (500 nM), 14 μL Buffer 3.1, 1 μL RNase inhibitor reagent, and 50 μL AuNPs displacement probe, and the brackets are the concentrations in the detection system. Specifically, crRNA, Cas12a, Buffer 3.1, RNase inhibitor and 2 μL of 100 nM target were added into the centrifuge tube, then add 2 μL of 5 μM oligo Trigger (500 nM); the resulting mixture was reacted at 37 °C for 1.5 h. Then, 50 μL of AuNPs strand displacement probe was added, and reaction for 30 min at 25 °C. The naked eye and the nanodrop was used for ultraviolet absorption detection.
2.7. AuNPs strand-displacement probe with different concentrations of oligo Trigger

Oligo Trigger were replaced with different concentrations (4 μM, 1 μM, 500 nM, 100 nM, 50 nM, 10 nM, 5 nM, 1 nM, 0), and the detection was performed according to the method in Visual SARS-CoV-2 detection section. The naked eye comparison was performed first, and then the UV absorption detection was performed using nanodrop.

2.8. Oligo Trigger concentration at 1 μM, 500 nM, 100 nM for SARS-CoV-2 detection

Different concentrations (1 μM, 500 nM, 100 nM, respectively) of oligo Trigger, Buffer 3.1 (12 μL, 14 μL, 12 μL), 0.5 μL of 10 μM Cas12a (250 nM), 0.5 μL of 20 μM crRNA (500 nM), 2 μL of different concentration gradient targets and 1 μL RNase inhibitor were added in a series of Eppendorf tubes. The resulting mixture was put into reaction at 37 °C for 1.5 h. Then, 50 μL of AuNPs strand-displacement probe was added, and reaction for 30 min at 25 °C. The naked eye comparison was performed first, and then the UV absorption detection was performed using nanodrop.

2.9. One-tube assay

Mix 0.5 μL of 10 μM Cas12a protein (250 nM), 0.5 μL of 20 μM crRNA (500 nM), 2 μL of different concentrations of target (100 nM, 10 nM, 1 nM, 100 pM, 0), 2 μL of 5 μM oligo Trigger (500 nM), 14 μL of Buffer 3.1 and 1 μL of RNase inhibitor were added to the lids of a series of Eppendorf centrifuge tubes. Add 50 μL of AuNPs strand-displacement probe to the bottom of the centrifuge tube. The CRISPR reaction occurs by incubating the centrifuge tube’s cap with a hand warmer for 1.5 h. The solution in the centrifuge tube was then centrifuged to the bottom of the centrifuge tube and reacted at room temperature for 30 min. The naked eye comparison was performed first, and then the UV absorption detection was performed using nanodrop. For reaction time investigation, combine two groups of 0.5 μL of 10 μM Cas12a protein (250 nM), 0.5 μL of 20 μM crRNA (500 nM), 2 μL of different concentrations of targets (100 nM, 0), 2 μL of 5 μM oligo Trigger (500 nM), 14 μL of Buffer 3.1 and 1 μL of RNase inhibitor were added to the lids of a series of Eppendorf tubes. Add 50 μL of AuNPs strand-displacement probe to the bottom of the centrifuge tube, incubate the top of the centrifuge tube with a hand warmer, and the CRISPR reaction occurs. The top of one group of centrifuge tubes was reacted for 15 min, after that the solution in the centrifuge tube was centrifuged to the bottom, reacted at room temperature for 30 min, and photographs were taken every 5 min to observe the color difference between the samples and the control group. The top of another set of centrifuge tubes was reacted for 10 min, after that the solution in the centrifuge tube was centrifuged to the bottom, and reacted for 10 min at room temperature. The naked eye comparison was performed first, and then the UV absorption detection was performed using nanodrop.

2.10. Specificity experiment

Combine 2 μL of different coronavirus targets (100 nM), 0.5 μL of 10 μM Cas12a protein (250 nM), 0.5 μL of 20 μM crRNA (500 nM), 2 μL of 5 μM oligo Trigger (500 nM), 14 μL of Buffer 3.1 and 1 μL of RNase inhibitor was added to the lids of a series of Eppendorf tubes. Add 50 μL of AuNPs strand-displacement probe to the bottom of the centrifuge tube. Add 50 μL of AuNPs strand-displacement probe to the bottom of the centrifuge tube. The CRISPR reaction occurs by incubating the centrifuge tube’s cap with a hand warmer for 1.5 h. The solution in the centrifuge tube was then centrifuged to the bottom of the centrifuge tube and reacted at room temperature for 30 min. The naked eye comparison was performed first, and then the UV absorption detection was performed using nanodrop.

2.11. GX/P2V Viral RNA extracting and analysis of clinical specimens

20 μL Si-OH MBs, 200 μL lysis buffer, and 200 μL virus fluid were added into 1.5-ML Eppendorf tubes. After a brief vortex to mix, the mixture was incubated for 5 min at 25 °C. Following magnetic separation, remove the supernatant and wash it 2 times with freshly-prepared 80% ethanol solution to remove the supernatant. After the ethanol has completely evaporated, add the amplification solution to disperse the magnetic beads for the amplification reaction. The extracted GX/P2V viral RNA was recognized by Cas12a as targets after RT-PCR. Specifically, 27 μL of 2 × PCR Master Mix, 1 μL of 20 μM forward primers, 1 μL of 20 μM reverse primers, and extracted viral RNA were added to a 200 μL PCR tube and brought to 50 μL with water. The RT-PCR procedure (2 min at 94 °C, 2 min 30 s at 94 °C, 30 s at 55 °C, 20 s at 72 °C, 32cycles) was performed.

3. Results and discussion

Scheme 1 illustrates the typical assay workflow of Cas12a target recognition, ssDNA collateral DNase activities on oligo Trigger, and oligo Trigger on AuNPs strand-displacement probes. The AuNPs strand-displacement probes is based on an ingeniously designed sandwich-structured nanocomplex composed of DNA-functionalized AuNP, linker DNA, and DNA-functionalized magnetic bead. The sandwich structure’s overhang region acts as a toehold hybridized with an oligo Trigger to initiate the strand displacement for releasing AuNPs. The lengths of the double helix regions and the toehold were designed to ensure that the oligo Trigger-induced strand-displacement reaction occurs, releasing AuNPs from the AuNPs strand-displacement probe. After magnetic separation, the released AuNPs in the tube can be visual with the naked eye. SARS-CoV-2 ORF1a gene encodes replicase polyprotein 1a, which play an important role in virus RNA transcription and replication. ORF1a gene sequence of 110 base pairs at the location from 13328 to 13437 in the genome map was selected as the model target. Specifically, the addition of targets unleashes the indiscriminative ssDNAse properties of the Cas12a/crRNA complex. As a result, the oligo Trigger would be entirely degraded as a single-stranded substrate of Cas12a. In the presence of targets, Cas12a degrades oligo Trigger, lightening the color of the AuNPs solution. Therefore, target concentration is inversely proportional to the amount of released AuNPs. The amount of target can be visible with the naked eye and assessed by UV-visible spectroscopy for quantitative detection simultaneously. The operation was considerably simplified by combining quick AuNPs strand displacement and Cas12a cleavage as a one-pot reaction in a single tube. A low-cost hand warmer has been directly used as its incubator for instrument-free assay of SARS-CoV-2 detection in routine applications. The AuNPs strand-displacement probes is based on an ingeniously designed sandwich-structured nanocomplex composed of DNA-functionalized AuNP (9 bp L-Au), 21 bp linker DNA, and DNA-functionalized magnetic bead (11 bp L-MB), and toehold (21 nt), respectively [44]. We investigated the preparation efficiency using the ratio of the absorbance value of AuNPs of sandwich-structured nanocomplex and L-Au modified after subtracting blanks. Results suggested that the preparation efficiency is 94.6 % (Table S1). To evaluate the feasibility of the proposed strategy for SARS-CoV-2 detection, we investigated the amount of oligo Trigger on AuNPs strand-displacement probes. The oligo Trigger hybridized with toehold strand of AuNPs strand-displacement probe resulting in the release of AuNPs, producing a pink color of the supernatant in the tube. The effect of oligo Trigger concentration on AuNPs strand-displacement probe was explored when the oligo Trigger concentration increased, resulting in color and absorption change (Fig. 1). Results showed that the oligo Trigger concentration has a good linear correlation between 10 nM and 500 nM. The released AuNPs efficiency reaches 97% when oligo Trigger at 1 μM using the
ratio of the absorbance value of released AuNPs and sandwich-structured nanocomplex after subtracting blanks (Table S1).

Taking the ORF1a fragment as an example, the analytical performance of different concentrations of oligo Trigger was explored. Fix the concentration of oligo Trigger was 1 \( \mu \text{M} \), the target concentration gradient was 100 nM, 50 nM, 20 nM, 10 nM, 1 nM and the blank. With
that the oligo Trigger as the substrate of Cas12a DNase activity, 1 µM is excessive to produce obvious difference due to small changes of targets. To verify our hypothesis and improve the sensitivity, the oligo Trigger was reduced to 500 nM, and the concentration gradients were carried out. There was a significant color and the absorbance gradient difference with concentration of 100 nM, 10 nM, 1 nM, 100 pM and the blank, and the sensitivity as low as 1 nM was successfully achieved without amplification strategy (Fig. 2AB and S2AB). It is speculated that the low concentration of oligo Trigger as the substrate of Cas12a, remaining less oligo Trigger producing signal difference. All results suggested that when the concentration of oligo Trigger is 500 nM, the naked-eye and absorbance detection are obviously distinguished.

The operation procedure was further simplified by combining the AuNPs strand displacement and Cas12a cleavage as a one-pot reaction in a single tube. Rapid AuNPs strand-displacement was performed at room temperature, and Cas12a cleavage reaction was triggered with hand warmer incubation. Specifically, the strand-displacement reagent mixture of AuNPs was added to the bottom of a 0.2 mL PCR tube, and Cas12a reaction solution was added within the tube lid (Fig. 3A). After that, the tube was gently capped to prevent the Cas12a reagent from falling into it. The tube was placed in a foam pipe rack with a household handwarmer on top for heating. After 30 min of trans-cleavage reaction of Cas12a, the reaction solution is manually thrown to the bottom of the tube, and the AuNPs strand-displacement reaction occurs at room temperature. Taking the novel coronavirus ORF1a fragment as an example, there was a significant difference between the samples of 100 nM, 10 nM, 1 nM, 100 pM and the blank, and the sensitivity as low as 1 nM was successfully achieved without amplification strategy (Fig. 3B and S3). Compared with the step-by-step reaction of the instrument with strict temperature control, the sensitivity of the integrated hand warmer reaction is reduced, the approximate 1 nM sensitivity achieved with the proposed method is among the high level without involving the amplification strategy or signal enhancement for CRISPR visible assay (Table 1).

To verify our hypothesis and improve the sensitivity, the oligo Trigger concentrations are 1000 nM, the effect of different target molecule concentrations 100 nM, 50 nM, 20 nM, 10 nM, 1 nM, 0 on AuNPs strand displacement probe; (A) the histogram of the absorbance peak value of 521 nm of the concentration gradient change; (B) the image of the strand displacement reaction; (C) the histogram of the peak of 521 nm of the concentration gradient, and (D) the strand displacement reaction image. When the oligo Trigger concentrations are 100 nM, 100 pM, 50 pM, 10 pM, 0 on AuNPs strand displacement probe; (E) the histogram of the peak of 521 nm absorbance changes of the concentration gradient, and (F) the strand displacement reaction image. n = 3 replicates, bars represent mean ± S.D. * P < 0.05, ** P < 0.01, *** P < 0.0001, n.s. not significant.
further applied to detect SARS-CoV-2 from practical samples. Because GX/P2V is more than 85% genetically similar to SARS-COV-2 and is not pathogenic to humans, GX/P2V is used to clinically validate the safety of experimental operators [18,45]. 110 bp gene sequence at the location from 26719 to 26828 in the GX/P2V genome map were selected as a model target. Using an equivalent volume of lysis buffer and 15 μL of 25 mg/mL magnetic beads, GX/P2V viral RNA was isolated from 200 μL of cell supernatant. After that, the composite we obtained was used directly as a template to amplify the target DNA. The positive samples’ fluorescence signal becomes stronger as the incubation duration in -creases (Fig. 5 and S6). The sensitivity of $2.7 \times 10^2$ copies/mL was successfully obtained. To the best of our knowledge, the approximate 2.7 × 10^2 copies/mL sensitivity achieved with the proposed method is at high level among the different CRISPR visible detection (Table 1). These results strongly suggested the proposed method has potential in practical sample applications.

4. Conclusions

In summary, we developed a universal, rapid, specific, sensitive and user-friendly SARS-CoV-2 detection module that combines the specific target recognition and trans-cleavage ssDNA ability of Cas12a with AuNPs strand-displacement probe. For one-tube assay, the Cas12a was placed on the top of the centrifuge tube, and the AuNPs strand-displacement reaction every 5 min; (D) the color image of Cas12a reaction for 15 min, strand displacement for 30 min and Cas 12a reaction for 10 min, strand displacement for 10 min n = 3 replicates, bars represent mean ± S.D. *P < 0.05, * *P < 0.01, * ** *P < 0.0001, n.s. not significant.

Table 1
Comparison the proposed method with different CRISPR visible detection.

| Targets                        | Signal read-out                        | Limit of detection | Linear range              | Time   | Reference |
|--------------------------------|----------------------------------------|--------------------|---------------------------|--------|-----------|
| Nucleic Acid                   | naked eye under blue light             | 10 aM with RPA     | 10-10^7 aM                | 30 min | [20]      |
| genetically modified organisms | color change of gold nanorods          | 1 nM with RAA      | 0.1 – 40 wt%              | NA     | [46]      |
| SARS-CoV-2                     | SYBR Green I and lateral flow strip    | 7.659 copies/μL with RT-RPA | 10-0.1 copies/μL | 15-20 min | [47] |
| genetic markers                | color readout                          | 10 aM with LAMP    | 10 aM-10 M                | NA     | [31]      |
| miRNAs et al                   | colorimetric gene-sensing              | 10 copies/ul with PCR or RPA | 10-10^7 copies/ul. | 1 h    | [30]      |
| SARS-CoV-2                     | color change and naked eye             | 100 copies/μL with LAMP | 10^2-10^5 copies/μL | 35 min | [48]      |
| SARS-CoV-2                     | naked-eye colorimetry                  | 59 copies/ reaction with LAMP | 10^2-10^4 copies/ reaction | NA     | [49]      |
| SARS-CoV-2                     | color change                          | 3.2 nM             | 2.5-100 nM                | 30 min | [50]      |
| SARS-CoV-2                     | visual detection                       | 3 copies with RPA  | 3-3 × 10^5 cps            | 20 min | [16]      |
| SARS-CoV-2                     | Fluorescent signal read-out            | 100 fM             | 100 fM-100 nM             | 4000 s | [51]      |
| SARS-CoV-2                     | naked eye                              | 100 pM             | 100 pM-100 nM             | 20 min | This work |
A low-cost hand warmer is used as an incubator of visual detection to detect clinical samples within 20 min, enabling an instrument-free, visual SARS-CoV-2 detection at the point of care. A sensitivity as low as 1 nM was successfully achieved without an amplification strategy. A real coronavirus GX/P2V instead of SARS-CoV-2 were chosen for practical applications.

**Fig. 4.** Specificity analysis for SARS-CoV-2. (A) Sequence alignment of the SARS-CoV-2 target region (ORF1a gene) and the corresponding regions on other human coronaviruses. (B) the image of the strand displacement reaction; (C) the histogram of the absorbance peak value of 521 nm of the concentration gradient change. n = 3 replicates, bars represent mean ± S.D. *P < 0.05, * *P < 0.01, * ** *P < 0.0001, n.s. not significant.

**Fig. 5.** Real-samples applications. (A) Extracted GX/P2V viral RNA. (B) the absorbance curve at different concentrations; (C) the histogram of absorbance peaks of 521 nm at different concentrations, and (D) the images of tube. n = 3 replicates, bars represent mean ± S.D. *P < 0.05, * *P < 0.01, * ** *P < 0.0001, n.s. not significant.
application validation. After rapid virus RNA extraction and RT-PCR amplification, a minimum of 2.7 × 10^5 copies/mL can still be obtained, showing great potential in clinical diagnoses. By simply changing the crRNA, the modular architecture can be used for a variety of nucleic acid detection applications, such as the detection of viruses, bacteria, species, etc. This method broadens the signal readout library of Cas12a probes and provides a rapid, sensitive, point-of-care assay for SARS-CoV-2 in the post PCR applications.

CRediT authorship contribution statement

Sitong Liu: Methodology, Investigation, Data curation. Tie Xie: Methodology, Investigation, Data curation. Xiaojiao Pei: Writing – original draft, Writing – review & editing, Supervision. Shuqing Li: Supervision, Writing – review & editing. Yifan He: Supervision, Writing – review & editing. Yigang Tong: Conceptualization, Resources, Supervision. Guoqiu Liu: Conceptualization, Resources, Supervision.

Declaration of Competing Interest

Sitong Liu and Xiaojiao Pei declare that a patent relative the study has been filed. The remaining authors declare no competing interests.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.snb.2022.133009.

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Sitong Liu is a Master’s degree candidate majoring in chemistry at Beijing Technology and Business University. He graduated with a bachelor’s degree in 2021 from Beijing Technology and Business University. His current research interest is CRISPR probes on nucleic acid detection.

Tie Xie is a Master’s degree candidate majoring in chemistry at Beijing University of Chemical Technology. He graduated with a bachelor’s degree in 2016 from Beijing University of Chemical Technology. His current research interest is CRISPR probes on nucleic acid detection.