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论文题目： 基于CRISPR信号放大技术的可视化抗原检测方法研究

CRISPR-enabled signal amplification for visual antigen detection

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Abstract

Detection of pathogenic antigens for infectious diseases with high sensitivity is highly desirable. CRISPR systems, which are well known as a powerful toolset for genomic editing, have recently been utilized for empowering nucleic acid detection by exploiting their capability to selectively identify and cleave DNA/RNA under physiological conditions. However, whether CRISPR can be used to enhance immunological assays remains a key challenge, largely due to the difficulty of coupling immunological (antibody-antigen) reactions to CRISPR. In this study, we devise a gold nanoparticle probe as a connection hub to couple an immunological recognition process with the CRISPR-based signal amplification to enable high-sensitivity detection of a viral antigen. Upon the capturing of the viral antigen by the nanoparticle probe, the cleavage activity of the Cas12a protein of CRISPR is activated, which efficiently cleaves surrounding fluorescent reporter DNA molecules to generate fluorescent signals. This method can detect as low as 1 ng/ml of SARS-CoV-2 antigen protein in simulated samples, which is ~1000 times more sensitive than conventional lateral flow antigen testing and comparable to the sensitivity of PCR-based nucleic acids detection. The entire process can be completed within 40 minutes without the need for specialized equipment or operations. Thus, this CRISPR-enabled signal amplification method provides a low-cost and ready-to-use means for on-site rapid detection of viruses.

Keywords

CRISPR, novel coronavirus, immunogold, gold colloid, nanoprobe, isothermal amplification

基于 CRISPR 信号放大技术的可视化抗原检测方法研究

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论文摘要

近年来，发展针对传染病抗原的高灵敏度检测方法已经成为一项迫切的需求。CRISPR 系统作为一类强大的基因组编辑工具，可以在生理条件下选择性地识别和裂解 DNA/RNA，因此最近被研究用于等温核酸检测的信号放大。然而，CRISPR 能否用于抗原检测的信号放大仍然是一个关键挑战，这主要是由于将免疫学（抗体-抗原）反应与 CRISPR 系统结合起来存在困难。在本研究中，我们设计了一种金纳米粒子探针作为连接枢纽，将免疫学识别过程与基于 CRISPR 的信号放大结合，实现对病毒抗原的高灵敏度检测。纳米粒子探针捕获病毒抗原后，CRISPR 的 Cas12a 蛋白的裂解活性被激活，从而有效地裂解周围的荧光报告 DNA 分子，产生荧光信号。该方法可检测模拟样本中低至 1 ng/ml 的新冠病毒抗原蛋白，灵敏度是传统测流抗原检测试剂盒的约 1000 倍，与基于 PCR 的核酸检测灵敏度相当。整个过程可在 40 分钟内完成，无需专业设备或操作。因此，这种基于 CRISPR 信号放大的检测方法可以为现场快速检测病毒提供一种低成本、即用型手段。

关键词

CRISPR, 新冠病毒, 免疫金, 胶体金, 纳米探针, 等温扩增

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1. Introduction

Over the past decades, the emergence and global spread of airborne diseases, including SARS, influenza A, avian influenza, and the recent SARS-CoV-2-induced COVID-19, have inflicted substantial tolls on human health and the economy. The pathogens of these diseases are predominantly RNA viruses, capable of aerial transmission and primarily invading the human body via the respiratory system, thereby facilitating rapid and widespread contagion. Consequently, accurate and accessible detection of these pathogens is pivotal in curbing viral transmission, mitigating severe outcomes and mortality in susceptible populations, and maintaining societal and economic operations[1].

Viral detection methodologies are primarily bifurcated into two categories: nucleic acid detection, which pinpoints specific viral genomic sequences, and antigen detection, which typically employs immunological assays to identify viral proteins or entire viral entities. Each approach possesses its unique advantages and limitations, often necessitating a balance between sensitivity and practicality. For instance, polymerase chain reaction (PCR)-based nucleic acid detection is currently recognized as the gold standard for the identification of a wide range of pathogens, including SARS-CoV-2. PCR's ability to exponentially amplify a single target nucleic acid sequence results in unparalleled sensitivity, enabling the detection of infections in their early stages, including pre-symptomatic or asymptomatic cases[2]. This is crucial for effective disease control and prevention. However, PCR's primary drawback is the requirement for a laborious thermal cycling process (typically lasting 2-6 hours) under stringent temperature control. This confines its application to professional laboratories equipped with specialized instruments and staffed by trained personnel[3]. As a result, the surge in demand for large-scale nucleic acid testing during pandemics often surpasses the testing capacities of these institutions, leading to significant delays, often spanning several days, from sample collection to result reporting.

On the other hand, immunoassays of viral antigens present certain benefits. They utilize rapid, natural antigen-antibody immune recognition reactions that require no sample pretreatment, facilitating the development of point-of-care test (POCT) devices such as lateral

flow antigen test kits[4]. These kits enable facile home testing, delivering visually discernable results within 15 minutes. Their wide use during the COVID-19 pandemic has alleviated the burden on professional institutions and personnel[5]. Nonetheless, the sensitivity of these kits is constrained. A recent study evaluating the sensitivity of several commercially available SARS-CoV-2 antigen test kits[6] revealed that most kits fail to consistently yield positive results when the antigen concentration falls below 1 μg/ml. This limit of detection (LOD) approximates a Ct value (cycle threshold) of 25 in real-time quantitative PCR nucleic acid testing[7], which is ~1000 times away from the diagnostic threshold (Ct value of 35). This sensitivity limitation may result in false negatives in pre-symptomatic/asymptomatic cases, potentially impeding disease control and treatment. Overall, the pursuit of virus detection methods that balance high sensitivity with convenience continues to be desirable and challenging.

Recently, nucleic acid detection methods based on the Clustered Regularly Interspersed Short Palindromic Repeats (CRISPR)-Cas endonuclease systems have been developed, enabling isothermal signal amplification without the need for precise thermal cycling equipment[8]. Originating as an adaptive defense mechanism in natural prokaryotes, CRISPR-Cas systems can effectively identify and cleave foreign nucleic acids. This capability has led to their use as a versatile genome editing tool across various living organisms, earning two scientists the 2020 Nobel Prize in Chemistry[9]. Certain CRISPR-Cas systems, such as Cas12a and Cas13, are found capable of efficiently cleaving multiple nearby nucleic acid molecules upon binding to target sequences. This process has been utilized for isothermal nucleic acid detection with high sensitivity yet convenience[10-12]. However, their application in immunoassays is limited, primarily due to the difficulty in effectively translating immunological recognition signals to nucleic acids that can be processed by these systems.

Here, we report a viral antigen detection method that combines the immunological recognition ability of a gold nanoparticle (AuNP) probe with the signal amplification ability of a CRISPR system. The binding of the probe to the viral antigen leads to the activation of the Cas12a endonuclease, which efficiently cleaves surrounding fluorescent reporter DNA

molecules, producing visual fluorescent signals under UV light. This approach can identify as little as 1 ng/ml of SARS-CoV-2 antigen spiked in throat swab samples, demonstrating a sensitivity ~1000 times greater than conventional lateral flow antigen tests. The whole process can be completed within 40 minutes, requiring no specialized equipment or operations.

2. Materials and Methods

2.1. Materials and instruments

Gold nanoparticles (AuNPs) of 15 nm in diameter were purchased from BBI Solutions. DNA and RNA sequences, including initiator DNA (3' thiolated), guide RNA (gRNA), and reporter DNA (5'-FAM and 3'-BHQ1) were synthesized by Jie Li Biotechnology Co. (sequences listed in Table 1). Synthetic viral antigen standard (recombinant SARS-CoV-2 nucleocapsid protein, referred to as N protein), and monoclonal antibodies against N protein (capture and detection antibodies) were purchased from Sino Biological Company. Cas12a protein was purchased from NEB. Other materials include plastic centrifuge tubes and phosphate buffer solution (PBS, pH=7.2).

The experimental instruments include: ultraviolet (UV)-visible spectrometer (Cary100, Agilent), fluorescence spectrometer (FLS900, The Edinburgh Instruments), multimode microplate reader (Synergy H1, BioTek), benchtop pH meter (ORION 3-STAR, Thermo Scientfic), particle size analyzer (Zetasizer Nano ZS90, Malvern), thermal incubator, benchtop centrifuge, portable UV lamp, and a smartphone for photo recording.

Table 1. Nucleic acid sequences used in this study.

Name	Sequence (5'-3')
initiator DNA	TATCAGCTGTGGAACACCCAGGTAAACTTTTTTTTTTTTTTTTTTTTTTTT-SH
gRNA	UAAUUUCUACUAAGUGUAGAUCCUGGGUGUUCCACAGCUGA
reporter DNA	FAM-TTTTTTTTTT-BHQ1

SH, sulfhydryl group; FAM, a green fluorescence dye; BHQ1, Black Hole Quencher -1.

2.2. Preparation of nanoprobes

1 mL of AuNP solution (15 nm diameter of nanogold particles) was added to a 2 mL plastic centrifuge tube. The pH value of this solution was adjusted to ~9.6 by dropwise adding K_2CO_3 solution (0.1 M). 10 μL of 1 mg/ml detection antibody solution was added to the mixture, followed by a 30-minute incubation at room temperature with agitation. The solution was supplemented with thiolated initiator DNA to a final concentration of 500 nM and incubated at room temperature with shaking for 1 h. Then, over 4 hours, 1 M NaCl solution was incrementally added at minimum 20-minute intervals until achieving a final concentration of 0.1 M. The solution was then incubated with agitation at room temperature for another 4 hours. Bovine serum albumin (BSA) solution at a mass/volume concentration of 10% was added until the final concentration of BSA was 0.1%, and the incubation was carried out with shaking for 1 h at room temperature. To remove unbound antibodies and DNA strands, a centrifugal washing process was carried out: the solution was first centrifuged for 20 min with a centrifugal force of ~13,800 g; the supernatant was aspirated and removed by pipette; the precipitate was resuspended by adding 1 mL of PBS (pH=7.2) containing 0.1% BSA. This process was repeated three times. The final product was stored at 4°C for further use.

2.3. Preparation of antigen capture tube or microplate

The antibody coating buffer was prepared by adjusting the pH of the PBS buffer to 9.6 using a Na_2CO_3 solution. The capture antibody (initial concentration 1 mg/ml) was diluted using this buffer to 5 μg/ml. The diluted capture antibody solution was added to a 1.5 mL centrifuge tube (200 μl per tube) or a 96-well microplate (200 μl per well), sealed, and incubated overnight at 4°C. Following the incubation, the solution was discarded, and the tube or microplate was washed three times with PBS buffer (pH=7.2). 1.5 mL of PBS buffer containing 1% BSA (blocking buffer, pH=7.2) was added, and the tube or microplate was incubated at room temperature for 2 hours. After three additional washes with PBS buffer, the solution was discarded, and the tube or microplate was inverted on filter paper to absorb residual liquid, yielding the antigen capture tube or microplate.

2.4. Preparation of CRISPR-based signal reporting module

Cas12a was prepared at a final concentration of 50 nM and mixed with gRNA at a final concentration of 62.5 nM in a 50 μL solution of 1× NEB Buffer 2.1 (comprising 50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 100 μg/ml Recombinant Albumin, pH 7.9). This mixture was incubated at 37 °C for 10 minutes. Subsequently, the reporter DNA was introduced into the solution, achieving a final concentration of 200 nM. The solution was gently mixed at room temperature to yield the reporting reagent, which was subsequently stored at 4 °C.

2.5. Preparation of standard samples of SARS-CoV-2 N protein

2.5 ml PBS buffer was added to the tube containing 100 μg lyophilized SARS-CoV-2 N protein. The tube was sealed and left to stand for 10 minutes. It was then gently inverted several times until the protein was fully dissolved, forming a standard N protein sample solution of 40 μg/ml. The N protein solution was further diluted to various known concentrations using PBS buffer via serial dilution. For instance, adding 10 μl of the sample to 990 μl of PBS buffer results in a 1/100 dilution of the original concentration; mixing 200 μl of the sample with 200 μl of PBS results in a 1/2 dilution. Using this method, we prepared test samples with concentrations of 20, 16, 10, 8, 5, 4, 2.5, 2, 1.25, 1, and 0.5 ng/ml.

2.6. Detection of SARS-CoV-2 N protein

The materials and apparatus utilized for antigen detection are depicted in **Figure 1**. The nucleocapsid (N) protein sample of a predetermined concentration was harvested using a sampling swab and subsequently introduced into a 100 μL solution of the synthesized nanoprobe. The sample was thoroughly extracted from the swab into the solution. The resulting mixture was then fully transferred into the antigen capture tube. Following a 15-minute incubation period, the solution was decanted from the tube and subjected to three washes, each with 1.5 mL of phosphate-buffered saline (PBS). A 100 μL aliquot of the reporting reagent was added to the tube and incubated for an additional 20 minutes. The tube was then exposed to ultraviolet (UV) light in a dark environment, and the outcome was visually assessed. A positive result, indicative of the presence of a detectable viral antigen in the sample, was confirmed if the test sample solution exhibited a pronounced green

fluorescence relative to the blank control solution. Conversely, a negative result, suggestive of the absence of a viral antigen in the sample at detectable concentrations, was determined if no discernible fluorescence was observed. For quantitative analysis, the aforementioned procedure can be performed in an antigen capture microplate and the results can be read using a microplate reader.

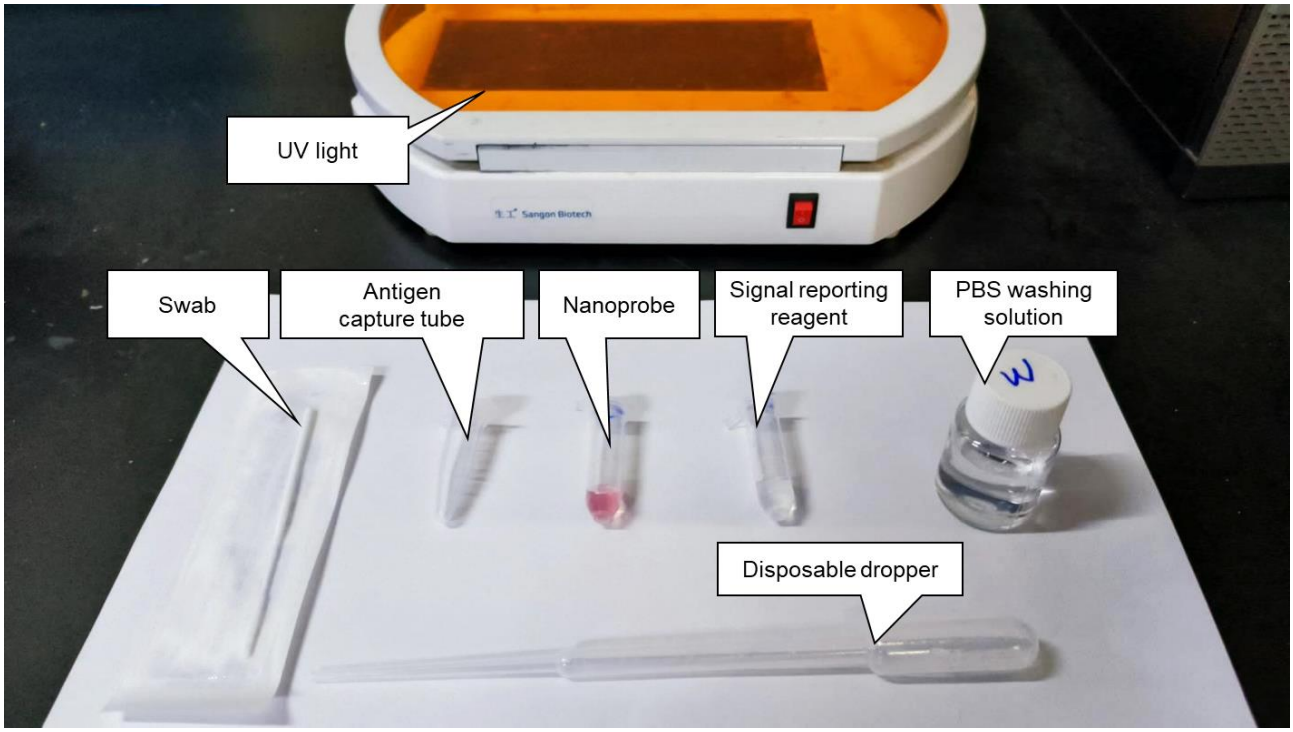

Figure 1. Materials and devices required for antigen detection using our method.

2.7. Data analysis

In this study, the mean and standard deviation (S.D.) were utilized to statistically assess the results. The standard deviation, calculated using the formula $S = \sqrt{\frac{\sum_{i=1}^{N}(X_i - \bar{X})^2}{N-1}}$, indicates the extent to which multiple test results deviate from the mean. Here, *N* represents the number of tests, and, $\bar{X}$ is the mean of *N* test results. Microsoft Excel was used to calculate the data and generate histograms. The AVERAGE function in Excel was used to automatically calculate the mean from multiple rows of data, while the STDEV function was used to calculate S.D. In the histogram created in Excel, the height of a column represents the mean, and the height of an error bar represents S.D. A short error bar indicates a small deviation from the mean in multiple test results, suggesting good reproducibility of the method.

3. Results

3.1. Design of the system

In our design, the antigen detection system, as depicted in **Figure 2**, is bifurcated into two modules: the first module is dedicated to immunological recognition, while the second module employs CRISPR-based technology for signal reporting.

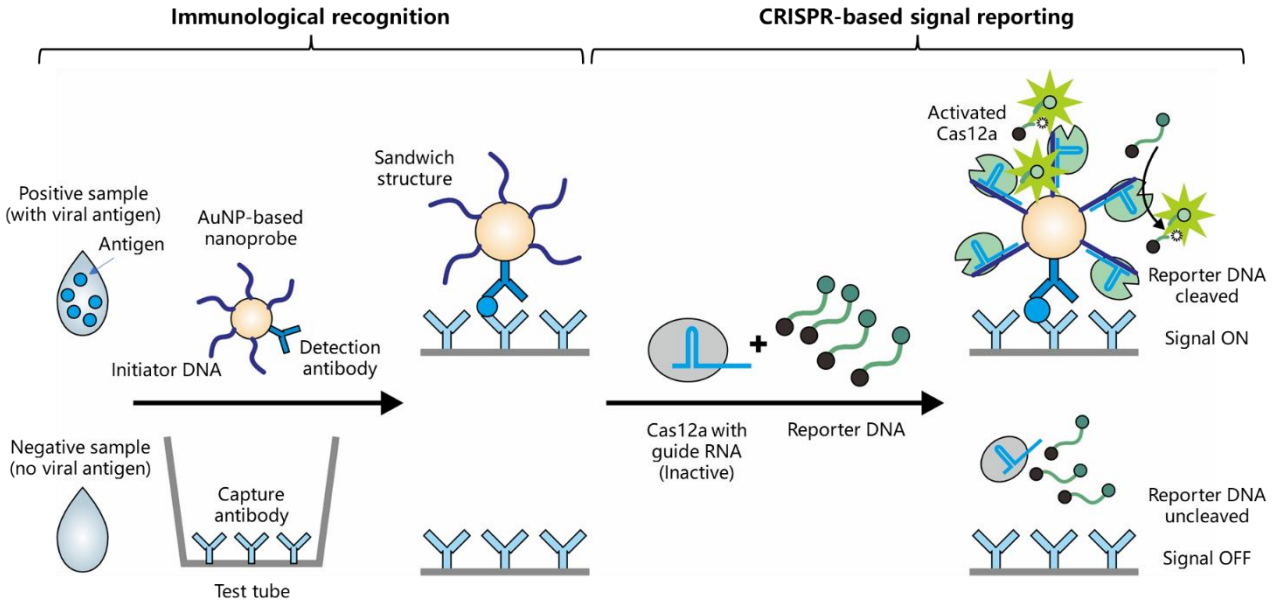

Figure 2. Schematic illustration of the antigen detection system.

The immunological recognition module, a key component of our system, comprises an antigen capture tube (or microplate) and a solution of nanoprobes. The antigen capture tube serves as a solid phase, coated with capture antibodies specifically targeting the viral antigen protein. The nanoprobes are gold nanoparticles (AuNPs) co-modified with detection antibodies and initiator DNA strands. In a standard detection procedure, the test sample is initially combined with the nanoprobes within the antigen capture tube. Should the target antigen be present in the sample, it binds to the detection antibody on the nanoprobe and is subsequently captured by the capture antibody through immunological interactions. This results in the formation of a sandwich structure immobilized on the inner surface of the tube (**Figure 2**). Conversely, in the absence of the antigen, this sandwich structure can not form, and any unbound nanoprobes are subsequently washed away. This process bears

resemblance to conventional immunoassays such as the enzyme-linked immunosorbent assay (ELISA). However, a distinguishing feature of our system is the use of initiator DNA strands, rather than enzymes, to trigger subsequent signal reporting.

The CRISPR-based signal reporting module is composed of the Cas12a protein, guide RNA (gRNA), and reporter DNA strands. Each Cas12a protein associates with a gRNA to form a ribonucleoprotein complex. This complex, guided by the gRNA, binds to the initiator DNA on the nanoprobes, which possesses a sequence partially complementary to the gRNA. This binding event triggers the cleavage activity of Cas12a, leading to the continuous cleavage of the reporter DNA strands. Each reporter DNA strand is modified with a fluorescent dye and a quencher at its respective ends. In the intact state of the reporter DNA, the proximity of the dye and quencher results in fluorescence quenching. However, upon cleavage by Cas12a, the dye and quencher become separated, restoring fluorescence. Therefore, the presence of nanoprobes carrying the initiator DNA, retained in the tube via immunological interactions, can be determined by measuring this fluorescence.

3.2. Construction and characterization of the nanoprobes

Upon the synthesis of the nanoprobes as delineated above, we embarked on their characterization employing transmission electron microscopy (TEM), UV-visible spectrophotometry, and particle size analysis. TEM imaging revealed the AuNPs as spherical black entities with an average diameter approximating ~15 nm. Post the conjugation of the detection antibodies and initiator DNA to the AuNPs, TEM imaging discerned low-contrast circular halos encircling the nanoparticles (**Figure 3a**), a phenomenon attributable to the adsorption of antibody proteins and DNA strands onto the nanoparticles. The optical absorption peak of the nanoparticles, post conjugation with antibodies and initiator DNA, manifested a redshift (shift towards longer wavelengths) in comparison to the naked AuNPs (**Figure 3b**). This shift is ascribed to the augmentation in particle size, a consequence of the surface plasmon resonance characteristics inherent to the gold nanoparticles. Particle size analysis (**Figure 3c-d**) indicated that the conjugation of detection antibodies escalated the hydrodynamic size of the nanoparticles from ~17 nm to ~30 nm, and the comprehensive

assembly of the nanoprobe with the initiator DNA culminated in a hydrodynamic size of ~47 nm. Collectively, these findings corroborate the successful adsorption of detection antibodies and initiating DNA onto the nanoprobe surface. In summary, we have successfully engineered a nanoprobe system encompassing an AuNP core, multiple copies of detection antibodies, and initiator DNA strands. We envisage that this system will facilitate the conversion of an immunological recognition signal into nucleic acids, thereby instigating the CRISPR-based signal amplification process.

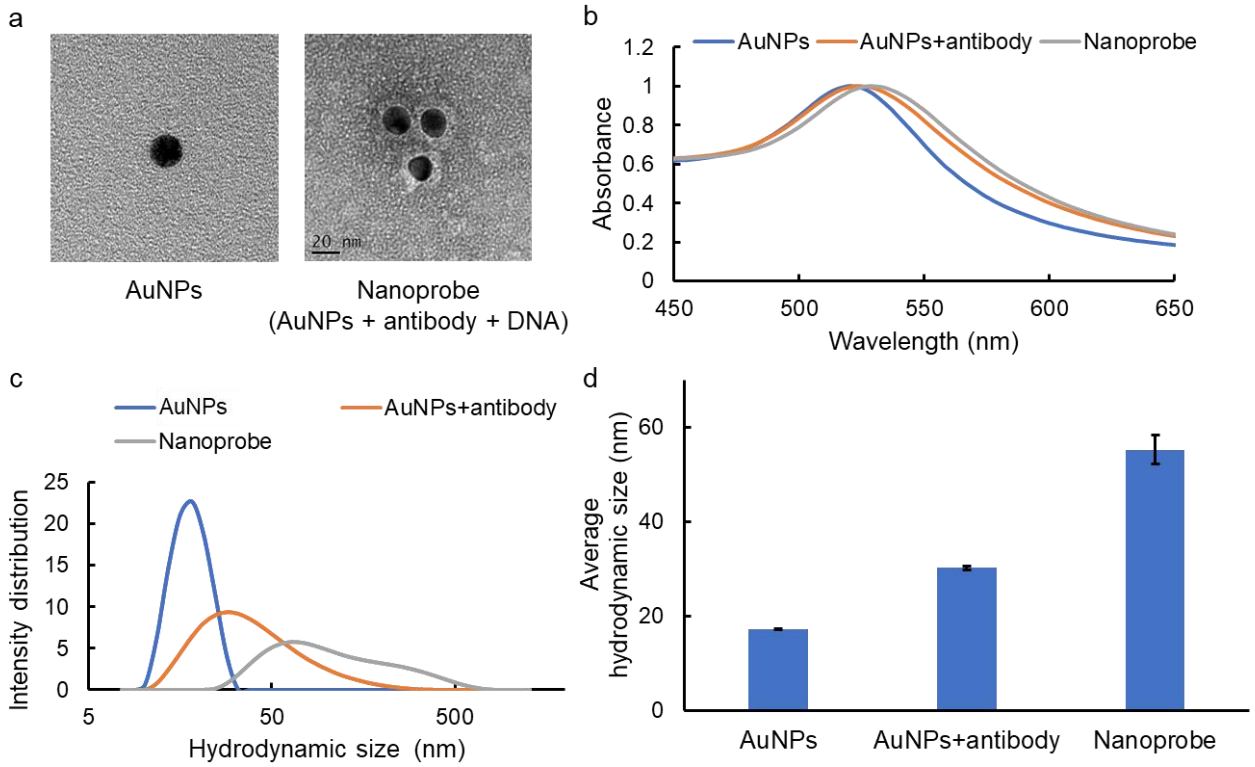

Figure 3. Characterization of the nanoprobes. (a) Transmission electron microscope (TEM) images of individual AuNPs (left) and the nanoprobes (right). (b) UV-visible absorption spectra of naked AuNPs (blue curve), AuNPs with capture antibodies (orange curve), and AuNPs with capture antibodies and initiator DNA (grey curve). (c) Hydrodynamic size distribution of AuNPs and subsequent addition of capture antibodies and initiating DNA, and (d) statistical analysis of average hydrodynamic size. Scale bar: 20 nm. Error bars, mean ± standard deviation (n=3 independent measurements).

3.3. Detection of standard samples

Upon the successful establishment of the nanoprobes, we embarked on assessing their potential for detecting the SARS-CoV-2 N protein. Standard testing samples were prepared by diluting the N protein in PBS solution to various concentrations (20, 16, 10, 8, 5, 4, 2.5, 2, 1.25, 1, and 0.5 ng/ml), with the PBS solution devoid of N protein serving as the blank control. To determine the optimal reaction time for the CRISPR-based signal reporting, we initially examined samples with low concentrations of N protein (1.25, 2, and 2.5 ng/ml). The test was conducted in a microplate coated with capture antibodies, and the fluorescence kinetics (fluorescence levels over time) were measured using a microplate reader. As depicted in **Figure 4a**, the samples with low concentrations exhibited a rapid increase in fluorescence levels within the initial 20 minutes of the reactions. Concurrently, the fluorescence of the blank control maintained a background level throughout the same duration. The contrast in fluorescence levels at the 20-minute mark was sufficient to distinguish between the blank control and the positive samples with low concentrations. Therefore, despite the continued increase in fluorescence levels beyond the 20-minute mark, we opted for a 20-minute reaction time for subsequent experiments, taking into account the requirement for swift detection.

Subsequently, we performed visual assessments on standard samples with varying N protein concentrations, utilizing a 20-minute CRISPR reporting reaction in test tubes. The test tubes containing N protein concentrations ≥1 ng/ml exhibited noticeable green fluorescence under UV light (**Figure 4b**), discernible to the naked eye. The fluorescence intensities of these solutions were quantitatively evaluated using a microplate reader, revealing a linear relationship between the fluorescence values and N protein concentrations within the range of 1-2000 ng/ml (**Figure 4c**). The linear regression correlation coefficient (R^2) was 0.996, approaching 1, indicative of an almost perfect linear relationship, suggesting our method's capability to quantitatively detect N protein within this concentration range. Moreover, the fluorescence level resulting from 1 ng/ml N protein surpassed that of the blank control plus three times the standard deviation. We deduced that the limit of detection (LOD) of our method was approximately 1 ng/ml (**Figure 4d**), significantly lower than the reported LOD

(~1 μg/ml) of the commercially available lateral flow antigen test kit. Consequently, our method demonstrated an enhancement in detection sensitivity by approximately 1000-fold.

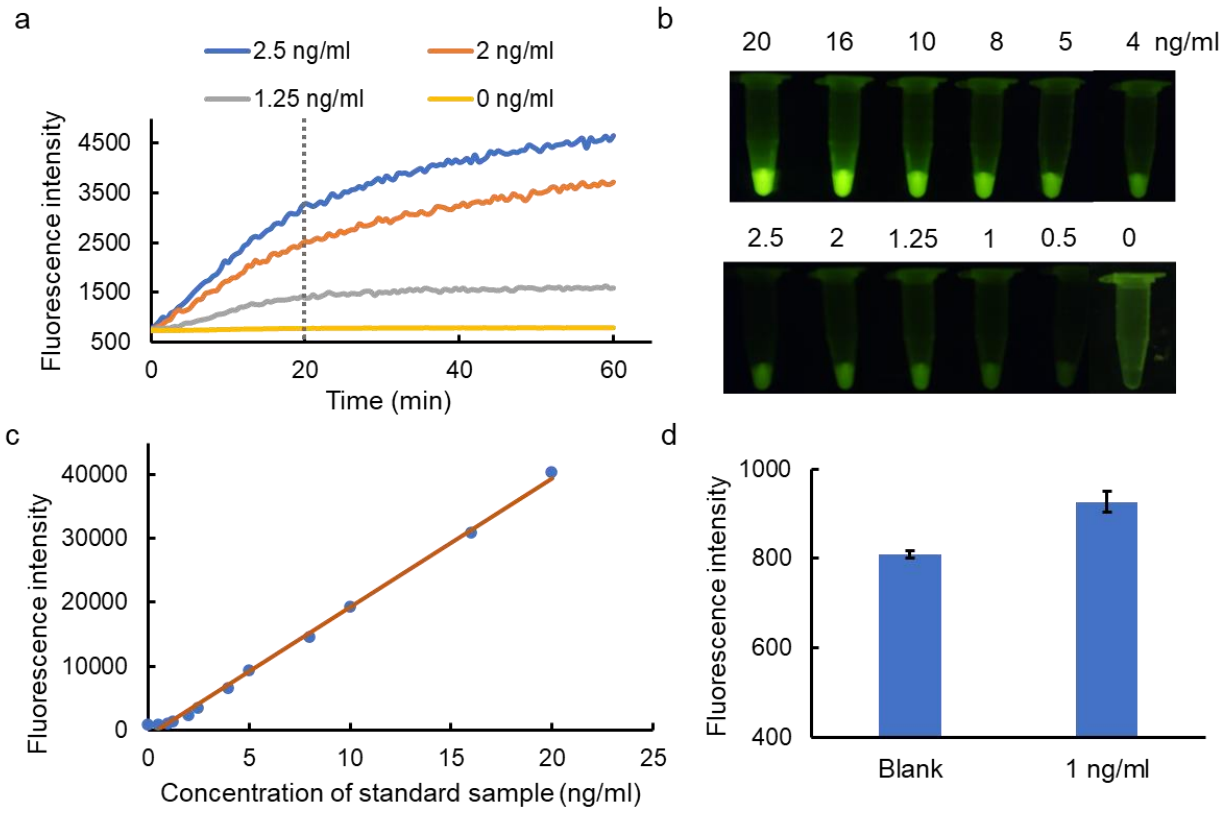

Figure 4. Validation of the system with standard samples. (a) Fluorescence kinetics resulting from samples with different N protein concentrations. (b) Photos of results in test tubes with different N protein concentrations under UV light. (c) Linear regression curve of resulting fluorescence intensities versus N protein concentrations. (d) Comparison of fluorescent levels between the 1 ng/ml N protein sample and the blank. Error bars, mean ± standard deviation (n=3 independent measurements).

We proceeded to assess the specificity of our detection approach by employing human serum albumin (HSA) as a negative control, in the absence of the N protein. The data revealed that the positive sample (N protein at 5 ng/ml) elicited conspicuous fluorescence under UV light. In contrast, the fluorescence emitted by HSA, even when present at a ten-fold higher concentration (50 ng/ml), was akin to that of the blank (**Figure 5**). This evidence substantiates the specificity of our method in detecting the SARS-CoV-2 N protein.

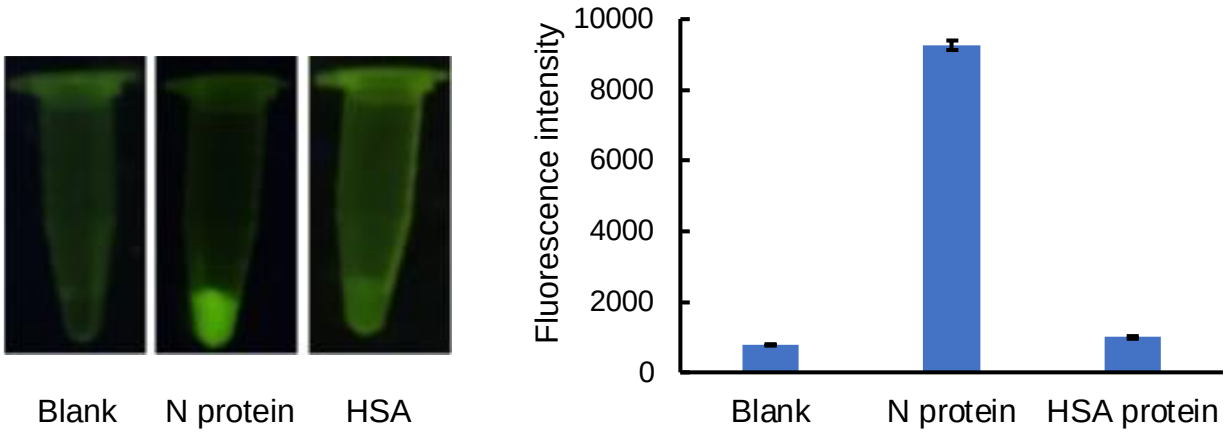

Figure 5. Validation of the specificity of this detection system. Photos of test tubes (left) and fluorescence intensity measurement (right) of blank, N protein sample (positive control), and HSA. Although the concentration of HSA (human serum albumin) is 10 times higher (50 ng/ml) than N protein, no significant fluorescence is observed. Error bars represent the standard deviation of three independent measurements.

3.4. Detection of spiked samples

To assess the robustness of our approach in real-world scenarios, we proceeded to examine saliva and nasal swab specimens spiked with the N protein. In the context of authentic biological samples, inherent proteases and nucleases may potentially degrade the biomolecular agents within the detection system. Additionally, the presence of extraneous proteins could lead to nonspecific adsorption on the nanoprobes, thereby compromising the precision of the detection. To address this, we introduced the N protein into the saliva or nasal swab collection fluids of healthy individuals to create the spiked samples (N protein final concentration 5 ng/ml). Our findings revealed that, utilizing our detection method (**Figure 6**), both spiked samples demonstrated vibrant fluorescence intensities on par with the standard sample. The fluorescence levels measured across the three groups did not exhibit significant disparity, underscoring the robustness of our approach in real-world sample detection and its potential applicability in the practical identification of the SARS-CoV-2 antigen.

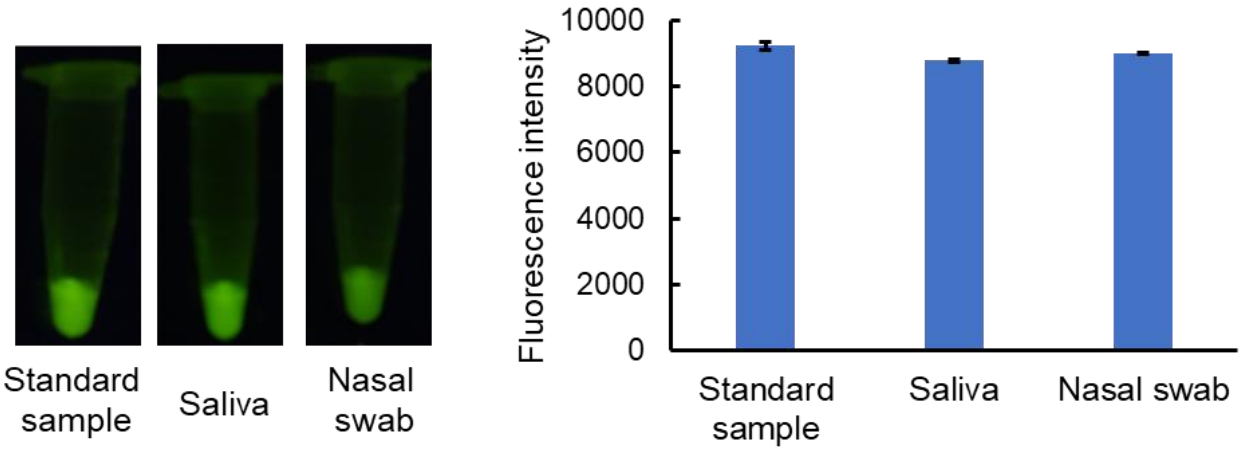

Figure 6. Detection of N protein in spiked samples. Photographs (left) and measured fluorescence values (right) of standard samples (N protein diluted with PBS), saliva, and nasal swab samples (spiked with 5 ng/ml N protein). Error bars, mean ± standard deviation (n=3 independent measurements).

3.5. Comparison with a commercial antigen test kit

Finally, we compared the sensitivity between our system and a commercially available SARS-CoV-2 antigen test kit. N protein samples were prepared at two distinct concentrations (1 μg/ml and 5 ng/ml) and subjected to both systems for testing. The detection results are presented in **Figure 7**. At a high N protein concentration of 1 μg/ml, the commercial kit exhibited a positive result, that is, both the control line (C line) and the test line (T line) on the strip turned red. Simultaneously, our method demonstrated bright fluorescence in the test tube, confirming the capability of both methods to detect N protein at this concentration. However, at a reduced N protein concentration of 5 ng/ml, the commercial kit displayed only the control line, with the test line remaining invisible. In contrast, our method continued to exhibit a discernible fluorescence signal in the test tube, thereby suggesting a superior sensitivity of our method over the commercially available SARS-CoV-2 antigen test kit.

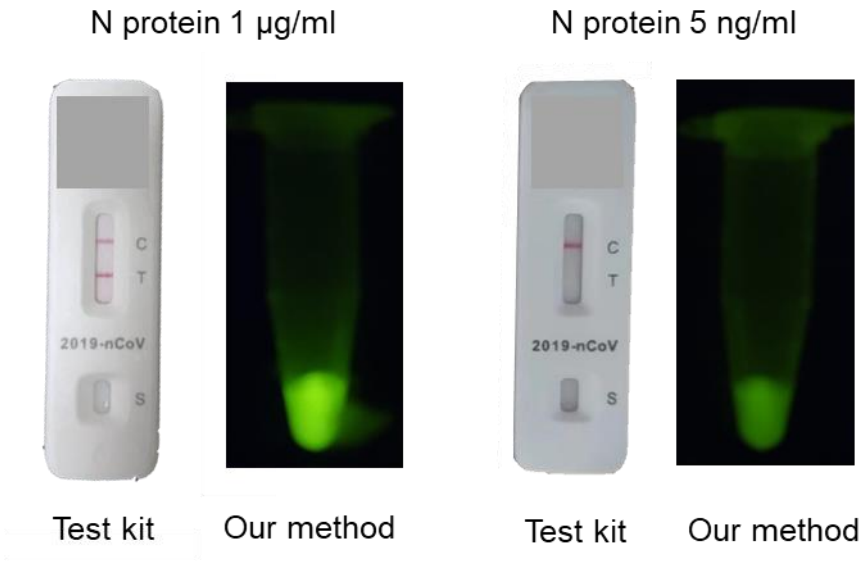

Figure 7. Comparison of our method with a commercial antigen test kit for SARS-CoV-2 N protein detection. At an antigen concentration of 1 μg/ml (left), both the commercial antigen test kit (red lines for T and C) and our method (visible green fluorescence) yielded positive results. At an antigen concentration of 5 ng/ml, the commercial antigen test kit showed a negative result (red line for C, no visible T line), while our method still yielded a positive result (visible green fluorescence).

4. Discussion and conclusions

In this study, we have engineered a visual viral antigen detection method by leveraging AuNP as a connection hub to couple immunological antigen recognition with CRISPR signal amplification. Each AuNP conjugates detection antibodies and DNA strands capable of activating Cas12a protein, forming a nanoprobe enabling the translation of immunological recognition signals for CRISPR system processing.

This detection approach offers several advantages. (1) It exhibits high sensitivity towards the viral antigen. By harnessing the signal amplification ability of the CRISPR system, this method achieved an LOD of ~1 ng/ml for the SARS-CoV-2 antigen protein, signifying an enhancement in sensitivity by ~1000 times compared to commercial antigen test kits. (2) It is both convenient and rapid. The entire testing procedure, which does not necessitate specialized equipment or personnel, can be completed within 40 minutes, thus outpacing

conventional real-time quantitative PCR testing. In future research, this method could be integrated with test strips and miniaturized fluorescence readers, thereby further augmenting testing convenience. In conclusion, this study provides a new approach to the development of immunological POCT methods that balance high sensitivity and convenience.

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6. Acknowledgments

6.1. Topic selection and background

In the past decades, the global spread of airborne diseases, including the most recent Covid-19 caused by the novel coronavirus, has brought enormous damage to both human health and economic growth. Hence, precise and convenient detection of pathogens is an urgent need for controlling the propagation and preventing severe cases and mortality. Through literature research, we learned that pathogen detection methods can be categorized into two types: nucleic acid detection, which identifies specific virus genome sequences, and antigen detection, typically employing immunological assays against viral proteins or whole viral particles. Each method has its pros and cons, often presenting a tradeoff between sensitivity and convenience. For example, PCR-based nucleic acid detection is highly sensitive but is limited by the dependence on expensive instruments and professionals. Antigen tests based on lateral flow immunoassays are fast, convenient, and inexpensive, but the sensitivity is inadequate. Thus, we asked the question of whether we can develop a new detection method that combines the convenience of lateral flow antigen tests and the sensitivity of PCR.

We also learned that CRISPR-Cas systems, known as powerful genome editing tools, have been recently harnessed for signal amplification, enabling non-PCR nucleic acid detection due to their ability to identify and cleave nucleic acids under ambient conditions. However, their application in the immunological detection of antigens is limited, primarily due to the difficulty in coupling immunological recognition with the CRISPR system.

According to the background, our team proposed the research topic, that is, to develop a visual antigen detection method based on CRISPR signal amplification technology, aiming to detect viral proteins with high sensitivity yet convenience.

6.2. Contributions of the team members

Hongxuan Fan contributed to the literature research, conceptual design of this study, performance of the antigen detection experiment, data analysis, and manuscript drafting. Hao Zheng contributed to the literature research, preparation of the materials, data analysis, and manuscript drafting.

6.3. Instructors

Yixin Chen is the science teacher of the team members at school. He directed the topic selection and literature research, coordinated the study, and revised the manuscript. Jiang Li is a professor at Shanghai Advanced Research Institute, Chinese Academy of Sciences. He is an instructor on the team. He provided theoretical and experimental guidance for the study, and revised the manuscript. All the tutorial works are unpaid.

6.4. Other supports

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论文选题来源与研究背景

在过去数十年中，我们经历了多轮空气传播疾病的全球蔓延，包括最近几年的新型冠状病毒大流行，给人类健康和经济增长带来了巨大损失。因此，精确、便捷地检测病原体是控制传播、预防重症和死亡的迫切需要。通过文献调研，我们了解到病原体检测方法可分为两类：一类是核酸检测，即识别特定的病毒基因组序列；另一类是抗原检测，通常采用针对病毒蛋白或整个病毒颗粒的免疫测定法。每种方法都各有利弊，通常需要在灵敏度和便利性之间做出妥协。例如，基于 PCR 的核酸检测灵敏度高，但受限于对昂贵仪器和专业人员的依赖。基于侧流免疫试剂盒的抗原检测快速、方便、便宜，但灵敏度不足。因此，我们提出：能否开发一种新的检测方法，将抗原检测试剂盒的便利性和 PCR 的灵敏度结合起来？

我们还了解到，CRISPR-Cas 系统作为一种强大的基因组编辑工具，在等温生理条件下可以有效识别和裂解核酸，因此最近已被研究用于核酸检测的信号放大，可实现不需要 PCR 的高灵敏度核酸检测。然而，它们在抗原免疫学检测方面的应用却很有限，主要原因是难以有效地将免疫学识别过程与 CRISPR 系统连接起来。

根据这一背景，团队提出了研究课题，即开发一种基于 CRISPR 信号放大技术的可视化抗原检测方法，旨在高灵敏且便捷地检测病毒蛋白。

队员的工作与贡献

樊泓萱参与了文献调研、概念设计、抗原检测实验、数据分析和论文初稿撰写等。

郑好参与了文献调研、材料准备、数据分析和论文初稿撰写等。

指导老师

陈易新，上海宋庆龄学校中学物理教师，是团队的科创活动指导老师。他指导了选题和文献研究，负责研究工作的统筹协调，并参与了论文修改。

李江，中国科学院上海高等研究院研究员，是团队的学术指导老师。他为研究提供了理论和实验指导，并参与了论文修改。

上述指导工作均无报酬。

其他支持

这项工作得到了中国科学院上海高等研究院实验室的支持。材料的 TEM 成像由在该实验室工作的焦凯博士完成。