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CRISPR-Cas12a-Powered Dual-Mode Biosensor for Ultrasensitive and Cross-validating Detection of Pathogenic Bacteria

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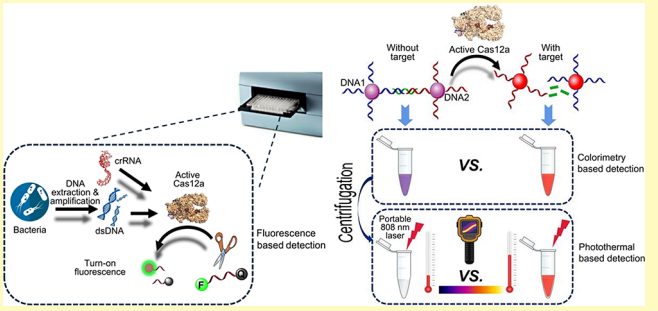
ABSTRACT: Pathogenic bacteria infection severely threatens human health and causes substantial medical and financial concern. Rapid, sensitive, specific, and reliable detection of pathogenic bacteria is crucial. In the current study, a CRISPR-Cas12a-powered dual-mode biosensor was developed for ultrasensitive and cross-validating detection of pathogenic bacteria. Simply, the amplicons of *Salmonella* (used as a model)-specific *invA* sequence triggered CRISPR-Cas12a-based indiscriminate degradation of single-stranded DNAs that were supposed to link two gold nanoparticle (AuNP) probe pairs, inducing an aggregation-to-dispersion change. This generated observable color changes that became even more apparent after centrifugation. The color changes can be discerned by naked eyes and recorded using a portable colorimeter. Meanwhile, the photothermal effect of CRISPR-Cas12-powered AuNPs was explored for the first time through 808 nm near-infrared irradiation such that quantitative measurement can be carried out by recording temperatures using a thermal camera. For both modes, a limit of detection of 1 CFU/mL and a dynamic range of detection from 10^0 to 10^8 CFU/mL were obtained, which were comparable with or better than previously reported methods. Our proposed biosensor demonstrated satisfactory selectivity for *Salmonella* against other interfering cells. Furthermore, this biosensor proved to be capable of *Salmonella* detection in food samples. Regarding the real applications, the result from each mode can be used for cross-validation. Only the case having doubly confirmed positive or negative results can be assured, which rendered a more dependable biosensing conclusion. This technology not only expands the reach of the CRISPR-Cas system in biosensing but also provides a general method for bacteria sensing with desirable sensitivity, specificity, and cross-validating capacity.



KEYWORDS: *CRISPR-Cas12a, trans-cleavage, colorimetric and photothermal detection, pathogenic bacteria detection, dual-mode detection, cross-validating detection*

Bacterial infectious diseases are growing public health concerns worldwide. Therefore, rapid, sensitive, specific, and cross-validating detection of pathogenic bacteria is crucial to human health in terms of disease prevention and control. Conventional methods for detecting bacteria largely count on culturing techniques and biochemical analysis, which are time-consuming and laborious.

CRISPR-Cas (clustered regularly interspaced short palindromic repeats, CRISPR; CRISPR-associated nuclease, Cas) is defined as an adaptive defense system in prokaryotes to fight against alien nucleic acids.[1] Apart from being used as useful tools in gene editing, CRISPR-Cas systems also show great potential in biosensing due to their outstanding sensitivity and specificity.[2−4] CRISPR-Cas12a represents nonspecific nucleic acid-cutting activities (also named *trans*-cleavage) upon the recognition of the target sequence by crRNA (CRISPR RNA). Using this feature, Doudna's team has developed a diagnostic system called "DETECTR" (DNA endonuclease-targeted CRISPR trans reporter), which enables rapid, specific, and sensitive human papillomavirus sensing.[5] Currently, CRISPR-Cas12a has been used to detect bacteria, viruses, regulatory RNA, and even small molecules.[6−9] However, all these CRISPR-Cas-based biosensors are based on fluorescent signal readouts or colorimetric signal readouts by using paper lateral flow assay, which is limited. So far, to our knowledge, in terms of CRISPR-Cas biosensors, other signal readout strategies have been barely reported.

Gold nanoparticles (AuNPs) have received increasing attention owing to their unique colorimetric and photothermal properties, which bear great potential in biosensor construction.[10−12] On one hand, AuNPs represent a color change along with aggregation and dispersion. This change could be distinguished by naked eyes.[11] On the other hand, AuNPs display a photothermal (PT) effect that absorbs and converts

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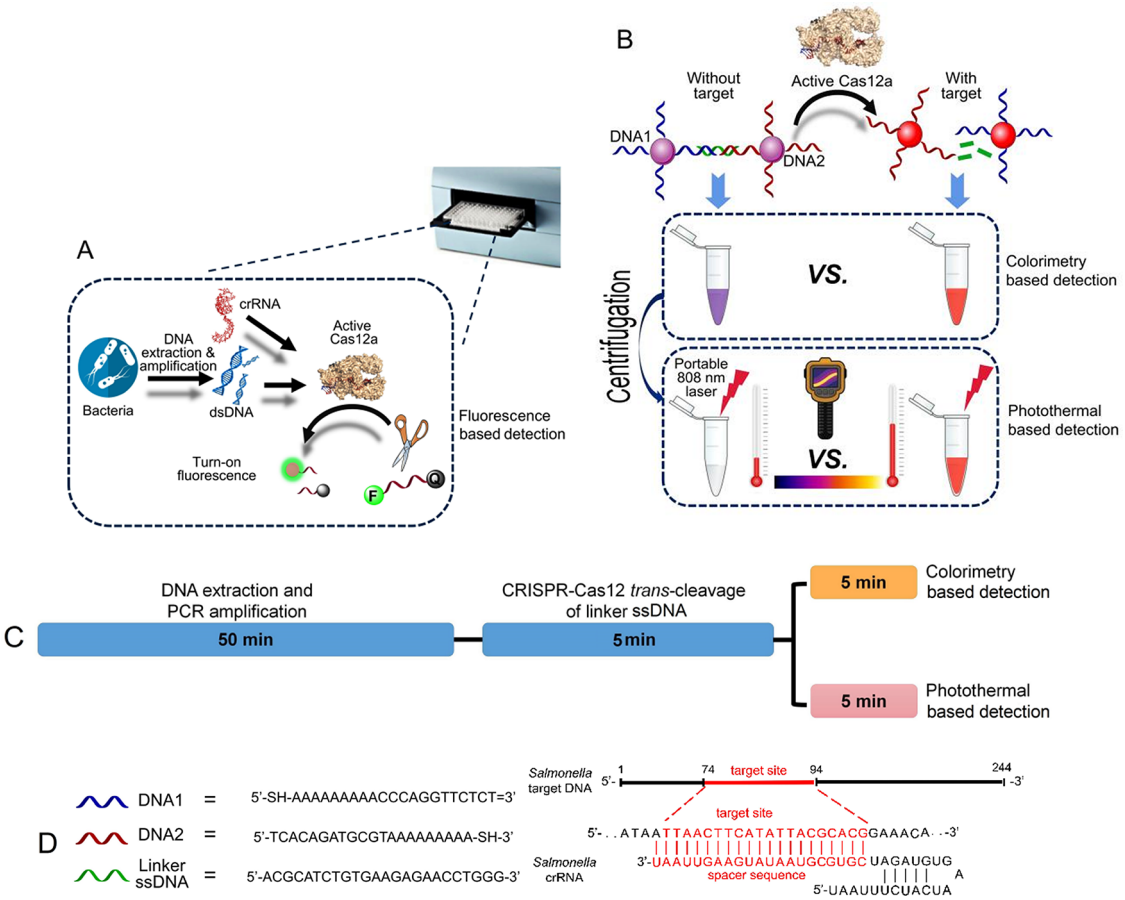
Scheme 1. Principle of the CRISPR-Cas12a-Powered Dual-Mode Biosensor for Ultrasensitive and Cross-validating Detection of Pathogenic Bacteria[a]



[a]*Salmonella* is used as a model for the bacterium. (A) Scheme of fluorescence-based bacterial detection by utilizing the *trans*-cleavage of CRISPR-Cas12a. F = FAM (fluorescein); Q = BHQ1 (black hole quencher 1). (B) Principle of the CRISPR-Cas12a-powered biosensor for colorimetry and photothermal-based detection. A portable colorimeter and a thermal camera can be used to record output signals. AuNPs-DNA1 and AuNPs-DNA2 pairs are cross-linked via base pairing with a linker ssDNA (green) with DNA1 (blue) and DNA2 (red). A portable NIR irradiator is used to generate an 808 nm laser. (C) Estimated time duration for each step of the proposed biosensor. (D) Sequences of linker ssDNA, DNA1 and DNA2 (left), and crRNA (right) used in this study.

the resonant light into thermal energy when certain materials are irradiated with a laser beam at a specific wavelength. When in the state of dispersion, AuNPs display a less intense PT effect with the illumination of a near-infrared (NIR) laser. However, under the aggregated state, AuNPs show a stronger PT effect.[13] Compared with the colorimetric methods, photothermal detection based on AuNPs is less interfered by color to perform quantitative analysis and is reported to have a lower limit of detection (LOD) value.[14] In addition, the plasmonic effect of AuNPs was leveraged by combining with Cas12a *trans*-cleavage activity for visual and colorimetric detection of virus.[11]

In this study, we intended to explore the colorimetric and photothermal features of AuNPs with CRISPR-Cas12a. For the first time, taking advantages of CRISPR-Cas biosensing and AuNP-based bioassays, we constructed a CRISPR-Cas12a-powered AuNP biosensor for dual-mode ultrasensitive and cross-validating detection of pathogenic bacteria. The platform not only harnessed the high sensitivity and specificity of Cas12a but also combined both colorimetric and photothermal readouts provided by AuNP-based bioassays. The proposed biosensor was successfully used for detecting the pathogenic bacteria *Salmonella* with the lowest LOD value (1 CFU/mL) and the range of detection (10^0–10^8 CFU/mL) for both signal readouts. Compared with other detection methods, this biosensor rendered higher detection sensitivity and a wider range of detection; meanwhile, the results can be obtained with a portable colorimeter and a thermal camera, which can be easily adapted to cross-validating detection.

EXPERIMENTAL SECTION

Materials and Reagents. Bacterial strains and human cells used in this study were possessed by the group. DNA oligonucleotides were chemically synthesized from Shanghai Sangon Biotech (China). The sequences and modifications of the DNAs are shown in Table S1. Also, 2× Taq PCR MasterMix II was purchased from Tiangen Biotech (China). LbCas12a (#M0653T) was purchased from New England Biolabs (USA).

Bacteria Culture and Counting. The Luria–Bertani (LB) broth medium was chosen to cultivate *Salmonella*. The liquid culture was maintained in a shaker at a speed of 160 rpm/min at 37 °C. After this, a few serial dilutions were prepared. Then, 100 μL of each sample was spread on Hektoen enteric (HE) agar plates at 37 °C for 24 h. Then, the numbers of colonies on three parallel Petri dishes were counted.

PCR Amplification of the *invA* Gene of *Salmonella*. The genomic DNA of each bacterial sample was extracted using a

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MiniBEST Bacteria Genomic DNA Extraction Kit (TaKaRa) according to the method of a previous report.[15] The PCR protocol for *invA* gene amplification was set as follows: 94 °C for 3 min, 28 cycles with 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s.

Synthesis of crRNA. The crRNA synthesis was strictly conducted as our previous work.[16]

Standard Curves, Sensitivity, and Selectivity of the CRISPR-Cas12a-Based Method for *Salmonella* Sensing. In a typical experiment, 200 nM LbCas12a, 300 nM crRNA, 50 nM fluorescent single-stranded DNA (ssDNA) reporter, and different concentrations of nucleic acids were incubated in a 100 μL reaction buffered with 50 mM Tris−HCl (pH 8.0), 100 mM NaCl, 10 mM $MgCl_2$, and 1 mM DTT. The assays were carried out with 484 nm excitation and 529 nm emission wavelengths at 37 °C. A Tecan plate reader was used to measure the fluorescent emission signals. For the specificity test, genomic DNA, extracted from seven different organisms, was amplified using *Salmonella* primers and then followed by CRISPR-Cas-based detection. Each data point was measured in triplicate.

Synthesis and Characterization of AuNPs. The synthesis of AuNPs was performed based on a previous publication.[12] The particle size was determined using a Hitachi HT7700 transmission electron microscope (TEM) and a Malvern ZS90 dynamic light scattering (DLS) instrument.

Preparation of DNA-Functionalized AuNPs. In a typical experiment, DNA1 (100 μM, 3 μL) was mixed with citrate-AuNPs (100 μL). The mixture was then placed at −20 °C for 2 h followed by thawing at ambient temperature. After thawing, the solution was washed three times with buffer A (5 mM HEPES buffer, pH 7.6) by centrifugation at 4 °C at 12,000 rpm/min for 30 min. Finally, the pellet was collected and resuspended in buffer B that was 10 mM HEPES (pH 7.6) supplemented with 300 mM NaCl.

Optimization of the Linker ssDNA Concentration. The linker ssDNA was optimized for the AuNP-based visual detection. A solution containing 25 μL of AuNP-DNA1, 25 μL of AuNPs-DNA2, and 50 μL of linker ssDNA with various concentrations was incubated at 37 °C for 10 min followed by a cooling process to ambient temperature. The sample easily precipitated after a mild centrifugation (5000 rpm for 3 min). The color of the solution was recorded, and the absorbance of each sample was scanned from 450 to 700 nm.

CRISPR-Cas12a-Powered Biosensor for Colorimetric Detection. A reaction mixture consisting of 200 nM Cas12a, 300 nM crRNA, 50 nM linker ssDNA, and target dsDNA (amplicons) with different concentrations was incubated in 5 mM HEPES buffer (pH 7.6) with 10 mM $MgCl_2$ and 150 mM NaCl for 25 min followed by 65 °C heating for 5 min for inactivation. This reaction mixture (50 μL) was then mixed with 25 μL of AuNPs-DNA1 and 25 μL of AuNPs-DNA2 solution at 37 °C for 10 min. The sample easily precipitated after a mild centrifugation (5000 rpm for 3 min). The color of the supernatant was recorded as an image, and the absorbance of each sample was scanned from 450 to 700 nm using an Infinite 200 plate reader (Tecan, Switzerland) or a Hach DR900 handheld colorimeter.

CRISPR-Cas12a-Powered Biosensor for Photothermal Detection. For a typical photothermal-based assay, a reaction mixture composed of 200 nM Cas12a, 300 nM crRNA, and 50 nM linker ssDNA together with varying concentrations of the target DNA was incubated in 5 mM HEPES buffer (pH 7.6) supplemented with 150 mM NaCl and 10 mM $MgCl_2$ at 37 °C for 25 min followed by a 65 °C heating for 5 min for inactivation. Then, 50 μL of reaction was then mixed with 25 μL of AuNPs-DNA1 and 25 μL of AuNPs-DNA2 solutions at 37 °C for 10 min. The sample easily precipitated after a mild centrifugation (5000 rpm for 3 min). The supernatant was irradiated with a portable 808 nm laser irradiator at 0.5 W/cm^2 for 5 min, and the temperature changes were monitored by a DT-980A thermal camera.

Detection of *Salmonella* in Food Samples Using the Proposed Biosensor. Each milk sample was used directly. The *Salmonella*-negative samples were treated by spiking with varying amounts of *Salmonella* cells, which were subjected to analysis using the proposed biosensor. The milk samples were placed with exposure to ambient environment. Then, 1 mL of aliquot of each sample was taken every 24 h within 4 days. Then, a fraction of each sample was used for plate counting, and part of it was subjected to analysis using the proposed biosensor.

RESULTS AND DISCUSSION

Design and Feasibility of the Proposed Biosensor. As illustrated in Scheme 1, the designed biosensor is built on the *trans*-cleavage of the CRISPR-Cas12a system together with the colorimetric and photothermal effects of AuNPs in different statuses. Specifically, the *Salmonella* genomic DNA is extracted and the invasion gene A (*invA*) gene is amplified followed by CRISPR-Cas12a processing. It is known that *invA* is a crucial virulence factor for cell invasion in intestinal epithelium.[17] It should be the most commonly used maker in *Salmonella* detection. Upon recognition of the target amplicons with designed crRNA, the *trans*-cleavage of CRISPR-Cas12a is activated and the fluorescent ssDNA reporter is indiscriminately degraded. When the ssDNA is doubly labeled with a fluorophore (F) at 5′ and a quencher (Q) at 3′ ends, the fluorescent signals would significantly enhance after Cas12a *trans*-cleavage. The enhanced fluorescent signals can be recorded using a microplate reader and used for quantitative detection of *Salmonella* (Scheme 1A). Additionally, a linker ssDNA is designed, which is able to hybridize with premade AuNPs-DNA probe pairs (AuNPs coated with DNA1 and DNA2, respectively) via annealing. In the absence of target amplicons, the linker ssDNA remains intact and AuNPs are maintained as the aggregation status with a purple color. While in the presence of the target amplicons, the linker ssDNA is cut off, and the AuNPs change from aggregation to dispersion. The dispersed AuNP solution exhibits a red color. This color change can be discerned by naked eyes. After a mild centrifugation, the aggregated AuNPs (without target amplicons) can be precipitated and the solution turns colorless, while the dispersed AuNPs (with target amplicons) remain in the solution as colloidal particles, displaying a red color (Scheme 1B). In addition, the supernatant of each sample is collected and irradiated by an NIR 808 laser. In the absence of target amplicons, the aggregated AuNPs are precipitated after centrifugation and the supernatant is devoid of AuNPs, where no obvious PT effect occurs. In contrast, when the target amplicons are present, the AuNPs are in the supernatant rather than in the precipitate after a mild centrifugation and therefore the supernatant fraction has a much stronger PT effect (Scheme 1B). These color-changing and photothermal effects can be monitored using a portable colorimeter and a thermal camera, respectively, for detection (Scheme 1B). The estimated time duration of each step of the proposed biosensor is shown in Scheme 1C, and around 90 min is needed to render a sample-to-result test. The sequences of DNA probes, linker ssDNA for AuNP conjugation, and crRNA are shown in Scheme 1D.

To begin with, AuNPs and thiolated DNA were conjugated to make AuNPs-DNA probes by a freezing method at −20 °C for 2 h. As shown in Figure S1, after thawing, the AuNP solution mixed with thiolated DNA exhibited excellent stability and thus represented a red color, which indicated that the surfaces of AuNPs were successfully coated with the DNA probes. In contrast, the naked AuNPs aggregated to precipitate thoroughly after freezing. Then, the concentrations of the linker ssDNA that hybridized with the AuNPs-DNA probes were optimized and the AuNPs were characterized by TEM

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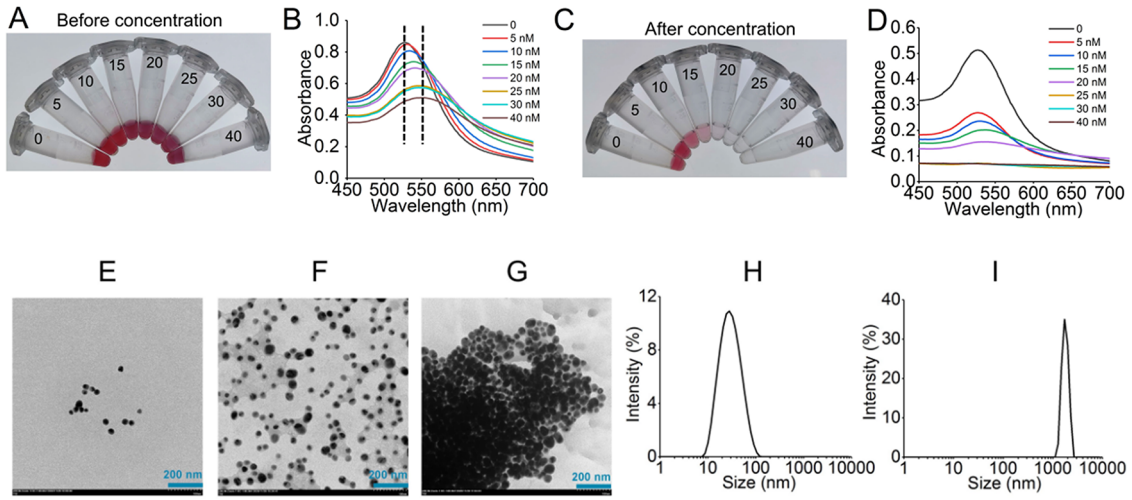

Figure 1. Optimization of the concentration of linker ssDNA and the characterization of AuNPs with different statues. (A) Red-to-purple color transitions and (B) UV−vis spectra of AuNPs with different concentrations of the linker ssDNA (nM) before concentration. (C) Color transitions and (D) UV−vis spectra of AuNPs with different concentrations of the linker ssDNA (nM) after concentration. TEM analysis of (E) naked AuNPs, (F) AuNPs-DNA probes, and (G) cross-linked AuNPs-DNA probes. DLS of naked (H) AuNPs and (I) cross-linked AuNPs-DNA probes.

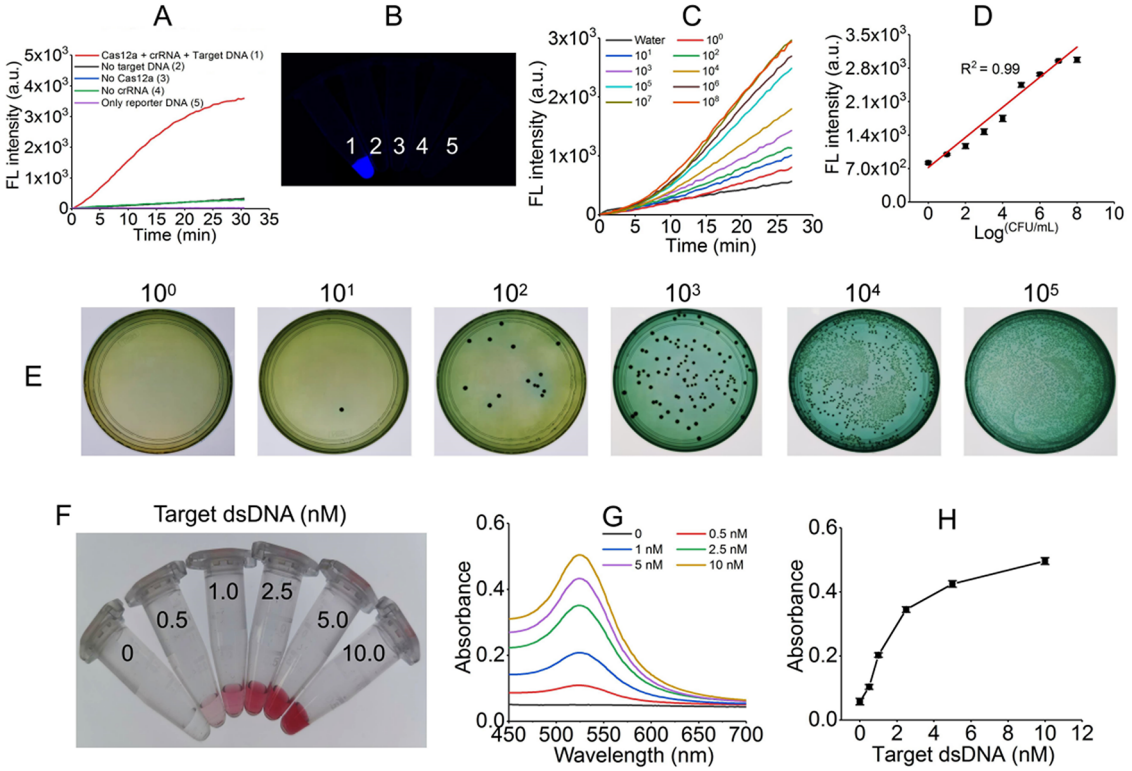

Figure 2. Designed CRISPR-Cas12a biosensor for quantitative detection of *Salmonella*. (A) Feasibility assessment by real-time fluorescent assay to confirm that the *trans*-cleavage of CRISPR-Cas12a can only be activated in the presence of the right target and crRNA. (B) Visual observation of samples under UV irradiation. Please note that the number of each sample corresponded with the samples in (A). (C) Dependence of fluorescence intensity on the *Salmonella* concentration. (D) Linear relationship between fluorescence intensity and the concentration of *Salmonella* over the range from 10^0 to 10^8 CFU/mL. (E) Plate counting method for *Salmonella* detection. (F) Color change and (G) UV−vis spectra of AuNPs corresponding to different concentrations of the target dsDNA. Each sample was centrifuged after reaction. (H) Linear relationship between UV−vis absorbance ($A_{520\ nm}$) and the concentration of dsDNA (data derived from (G)). The sequence of the target dsDNA is listed in the Supporting Information (DNA3).

and DLS. As shown in Figure 1A,B, the maximum absorbance (λ_{max}) shifted from 520 to 550 nm along with the linker concentration ranging from 0 to 40 nM in the presence of AuNPs-DNA probes. At a 25 nM linker ssDNA concentration, the redshift of the spectra of AuNPs-DNA probes halted, implying that the 25 nM linker induced a complete DNA hybridization (Figure S2). Moreover, when the linker concentration was 25 nM, the color of AuNPs completely changed to colorless, indicating a complete aggregation (Figure 1C,D). Therefore, this optimized concentration of linker

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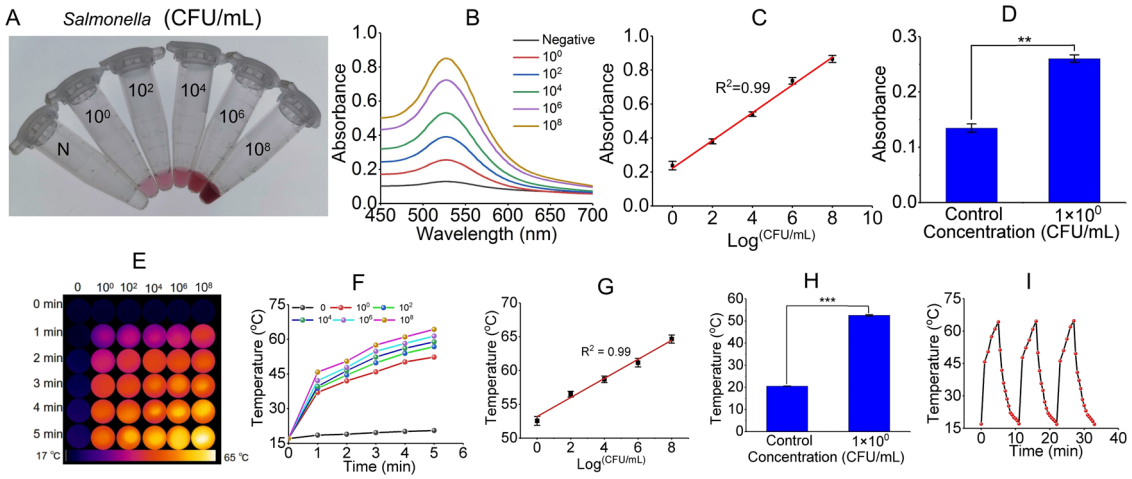

Figure 3. CRISPR-Cas12a-powered dual-mode biosensor for ultrasensitive detection of *Salmonella*. (A) Images for visual detection results of *Salmonella* using the CRISPR-Cas12a biosensor based on colorimetry. (B) UV−vis spectra of each sample in (A). (C) Linear relationship between the UV−vis absorbance ($A_{520\ nm}$) and the concentration of *Salmonella*. (D) LOD of the CRISPR-Cas12a-powered biosensor with colorimetric readouts. (E) Thermographs and (F) temperature profiles of the aqueous solution having different concentrations of *Salmonella* under NIR irradiation. (G) Linear relationship between the increased temperature and the concentrations of *Salmonella* (data derived from (G)). (H) LOD of the CRISPR-Cas12a-powered biosensor with photothermal readouts. (I) Temperature increasing and decreasing profiles of the AuNP solution with illumination on-and-off three times. N = negative control (without *Salmonella*).

ssDNA was used for the subsequent study. The TEM analysis and DLS were further conducted to observe the differences between dispersed and aggregated states of AuNPs (Figure 1E−I).

To test the feasibility of the CRISPR-Cas12a-based detection with regard to its *trans*-cleavage, some preliminary experiments were carried out. *Salmonella* genomic DNA was extracted, and the *invA* gene was amplified with PCR followed by CRISPR-Cas12a detection with different readouts. As shown in Figure 2A, the real-time fluorescent CRISPR-Cas12a assay was performed. Only when the target DNA and crRNA were both present that the *trans*-cleavage activity of Cas12a was triggered, inducing the degradation fluorescent ssDNA reporter and generating significantly enhanced fluorescent (FL) signals. The increased fluorescence could also be visualized under a portable UV irradiator (Figure 2B).

Before we tested the relationship between the fluorescence intensity and *Salmonella* concentration, the optimization of the LbCas12a/crRNA ratio related to the performance of the fluorescence detection was conducted. As shown in Figure S3, the result was denoted by the fluorescence (FL) intensity increase fold (I), which was equal to $\frac{\text{FL of group with target DNA}}{\text{FL of group without target DNA}}$. We therefore used 200 nM Cas12a and 300 nM crRNA for subsequent experiments since such concentrations gave rise to the greatest I value. We chose the *Salmonella*-specific *invA* gene as a target and applied conventional PCR for gene amplification (Figure S4). It was noted that the FL of CRISPR-Cas12 assay increased in a *Salmonella* concentration-dependent manner (Figure 2C). A linear relationship (R^2 = 0.99) was obtained between the FL and *Salmonella* concentration over the range from 10^0 to 10^8 CFU/mL (Figure 2D). For cross-validation, different concentrations of *Salmonella* were also detected with the "gold standard" conventional colony counting method (Figure 2E). Both fluorescent CRISPR-Cas12a assay and plate counting method showed the same trend and agreed well. In addition, we also tested our proposed CRISPR-Cas12a-powered biosensor with colorimetric readouts for nucleic acid detection. It was noticed that only when target dsDNA existed that the proposed biosensor was able to generate results of red color. Along with the increase in the concentration of dsDNA, the color becomes redder (Figure 2F). The corresponding UV−vis absorbance at 520 nm was recorded as shown in Figure 2G,H. These abovementioned facts implied a strong possibility of quantitative detection of *Salmonella* using the CRISPR-Cas12a system.

CRISPR-Cas12a-Powered Dual-Mode Biosensor for Ultrasensitive Detection of *Salmonella*. For *Salmonella* detection with the CRISPR-Cas12a-powered biosensor, different concentrations of *Salmonella* were extracted and amplified with specific *invA* gene primers followed by CRISPR-Cas12a-based detection. For the colorimetric analysis, there was an obvious color change between the negative and the positive samples (Figure 3A). The UV−vis absorbance was increased in a *Salmonella* concentration-dependent manner (Figure 3B). A linear relationship (R^2 = 0.99) was obtained over the range from 10^0 to 10^8 CFU/mL (Figure 3C). The LOD was 1 CFU/mL (Figure 3D). For NIR laser-driven photothermal detection, the supernatant of each sample after centrifugation was irradiated with an 808 nm laser generated by a portable irradiator for 5 min. As a result, a significant photothermal effect was observed (Figure 3E), resulting in an apparent temperature increase (Figure 3F). The increase in the temperature was dependent on the concentration of *Salmonella*, and a linear relationship (R^2 = 0.99) between the increased temperature and concentration of *Salmonella* over the range from 10^0 to 10^8 CFU/mL can be obtained (Figure 3G). The LOD was 1 CFU/mL (Figure 3H). The as-prepared AuNPs-DNA probes are able to resist long-term laser irradiation with no obvious change in the maximum temperature after repetitive heating and cooling processes three times (Figure 3I), indicative of robust photothermal stability. All the results demonstrated that our CRISPR-Cas12a-powered biosensor exhibited a great potential for dual-mode ultrasensitive detection of *Salmonella*. The sensitivity and range of detection of our proposed biosensor stood as the best compared with some recently reported methods for *Salmonella*

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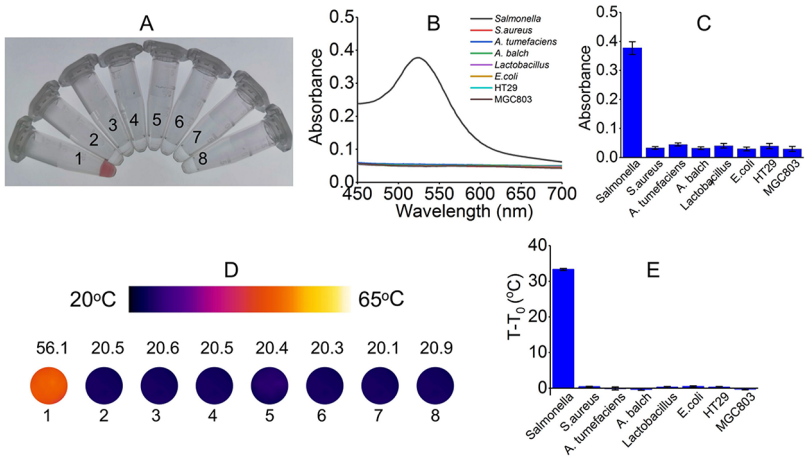

Figure 4. Selectivity test of the designed biosensor for *Salmonella* detection over other common cells. (A) Images for visual detection based on colorimetry. (B) UV−vis absorbance spectra of each sample in (A). (C) UV−vis absorbance values at 520 nm. (D) Thermographs and (E) temperature profiles of the aqueous solution in the presence of *Salmonella* or other cells. Please note that 1: *Salmonella*, 2: *Staphylococcus aureus*, 3: *Agrobacterium tumefaciens*, 4: *A. balch*, 5: *Lactobacillus*, 6: *Escherichia coli*, 7: HT29 cancer cells, 8: MGC803 cancer cells. T_0 represents the temperature of the solution without irradiation.

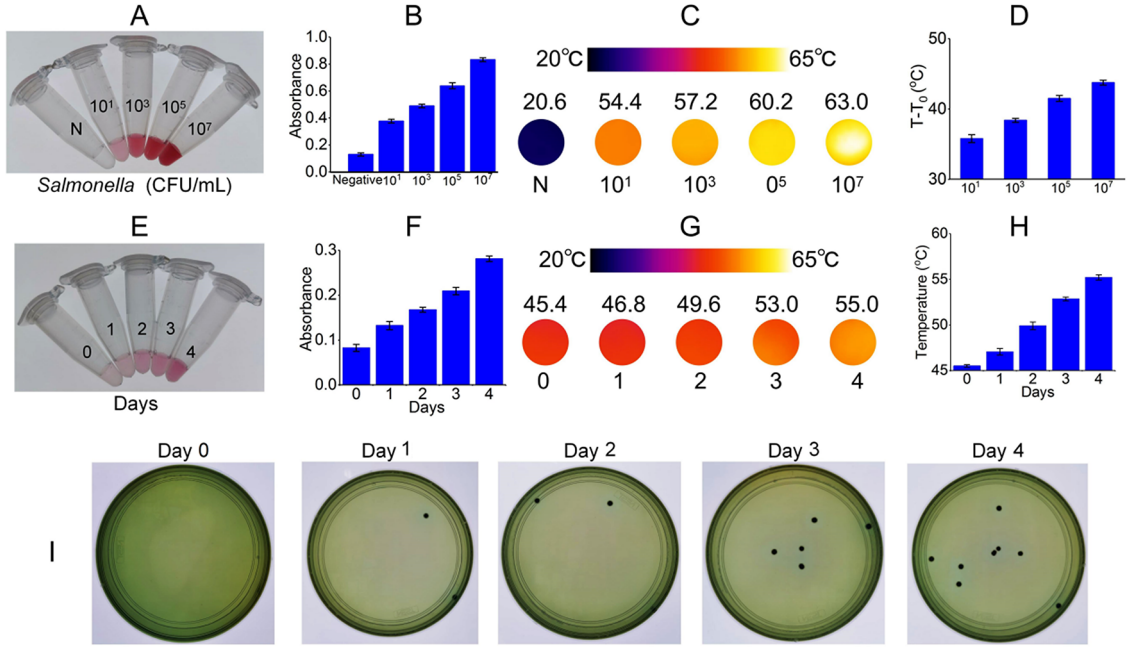

Figure 5. *Salmonella* detection in contaminated sour milk samples using the CRISPR-Cas12a-powered dual-mode biosensor. (A) Image and (B) UV−vis absorbance values at 520 nm for visual detection of different concentrations of *Salmonella* (CFU/mL) spiked in milk samples based on colorimetry. N = negative control (without *Salmonella*). (C) Thermographs and (D) temperature profiles of samples after 808 nm laser irradiation. (E) Image and (F) UV−vis absorbance values at 520 nm for visual detection of *Salmonella* in milk samples after exposure to ambient environment based on colorimetry. Here, 0−4 represents the days of exposure. (G) Thermographs and (H) temperature profiles of samples after 808 nm laser irradiation. (I) Results of samples (same as shown in (E)) subjected to analysis by the traditional plate counting method.

detection (Table S2). In terms of the sample-to-answer time, our biosensor required approximately 60 min, which was comparable with or better in comparison to most of the reported methods, as shown in Table S2.

Selectivity of the Designed CRISPR-Cas12a-Powered Dual-Mode Biosensor for *Salmonella* Detection. The selectivity of the proposed CRISPR-Cas12a-powered biosensor for *Salmonella* over some other interfering cells was also investigated. As shown in Figure 4A, only *Salmonella* triggered the *trans*-cleavage activity of Cas12a and subsequently the linker ssDNA was degraded, giving rise to a red color. The corresponding UV−vis absorbance spectra and the absorbance values at 520 nm are shown in Figure 4B,C, respectively. Similarly, photothermal-based detection was also carried out. As shown in Figure 4D, only the sample containing *Salmonella*, instead of other bacteria or human cells, showed a significantly increased temperature up to 56.1 °C. Figure 4E shows the histogram of changed temperatures. These results demonstrated the high specificity of the CRISPR-Cas12a-powered dual-mode biosensor for *Salmonella* detection.

Use of the Proposed CRISPR-Cas12a-Powered Dual-Mode Biosensor for Cross-validating Detection of *Salmonella* in Food Samples. Upon the successful detection of *Salmonella* in aqueous solution using our proposed CRISPR-

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Cas12a-powered biosensor, the performance of this biosensor was further investigated by testing daily food samples. The commercial milk samples were purchased from a local supermarket for testing. A series of known quantities of *Salmonella* samples (predetermined by a traditional plate counting method) were spiked into *Salmonella*-negative milk samples. As shown in Figure 5A−D, the colorimetric and photothermal readouts changed corresponding with the increase in terms of *Salmonella* concentration, indicating the feasibility of the CRISPR-Cas12a-powered biosensor for detecting *Salmonella* in food samples. A linear relationship can be obtained (Figure S5). We also placed fresh pure milk samples in a Petri dish and allowed them to be exposed to ambient environment so that the microorganisms in the air could be naturally inoculated in the milk samples. We collected part of each milk sample every 24 h for consecutive 4 days. The samples taken every day were tested using the traditional plate counting method and CRISPR-Cas12a-powered biosensor. As shown in Figure 5E−I, it can be seen that along with the increase in days, the colorimetric and photothermal signals changed correspondingly, which was in accordance with the results of the traditional plate counting method. It was also noted that the dual-mode results of this proposed biosensor agreed well with each other, which would be helpful in delivering a more reliable pathogenic bacterial detection outcome.[18]

CONCLUSIONS

In conclusion, we developed a novel CRISPR-Cas12a-powered biosensor, which was used for detection of pathogenic bacteria with high sensitivity and specificity by colorimetric and photothermal dual-signal modes. The performance of the proposed biosensor was outstanding as evidenced by its lowest LOD value (1 CFU/mL) and widest range of detection (10^0−10^8 CFU/mL) in buffered solutions for both colorimetric and photothermal signal modes. The developed biosensor was successfully applied for the detection of *Salmonella* in actual food samples. Our biosensor benefited from the sequence-targeting capability of the CRISPR-Cas12a system and the target-induced nonspecific DNase activity of Cas12a nuclease. The colorimetric and photothermal features of AuNPs were also leveraged in this designed biosensing system to generate visual readouts for quantitative detection. The colorimetric and photothermal signals can be simply recorded using portable devices. To our knowledge, for the first time, we realized a CRISPR-Cas-powered biosensor with both colorimetric and photothermal dual-signal modes for quantification. This is reminiscent of a number of recently developed CRISPR-Cas-based biosensors, most of which were based on fluorescent or colorimetric signals through a lateral flow immunochromatographic assay. By side-by-side comparison of dual-mode results of this proposed biosensor, a cross-validation detection can be achieved, leading to a more authentic decision. In summary, our proposed biosensor balances rapidity, sensitivity, specificity, and cross-validating capacity. This biosensor broadens the application of CRISPR-Cas12a in the field of molecular detection and is promising to be applied in a variety of occasions.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssensors.1c00686.

Experimental details of the freezing-based labeling method for AuNPs-DNA preparation (Figures S1 and S2), optimization of the LbCas12a reaction system (Figure S3), PCR amplification of the *invA* gene (Figure S4), linear relationship of the sensor in detecting milk (Figure S5), DNA oligonucleotides used in this study (Table S1), and comparison of different methods for *Salmonella* detection (Table S2) (PDF)

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Author Contributions

L.M. contributed in the conceptualization, investigation, project management, supervision, methodology, writing (re-

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view and editing), and funding acquisition. L.P. contributed in the experiment and data analysis. L.Y. contributed in writing (review and editing). G.L. contributed in the methodology and writing (review and editing). S.M. contributed in the project management, investigation, and writing (review and editing).

Notes

The authors declare no competing financial interest.

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