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REVIEW

Application of pre-amplification-based CRISPR-Cas nanostructured biosensors for bacterial detection

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ABSTRACT

Bacterial infections are one of the primary triggers of global disease outbreaks. Traditional detection methods, such as bacterial culture and PCR, while reliable, are limited by their time-consuming procedures and operational complexity. In recent years, the CRISPR-Cas system has demonstrated significant potential in gene editing and diagnostics due to its high specificity and precision, offering innovative solutions for bacterial detection. By integrating pre-amplification techniques, the CRISPR-Cas system has substantially enhanced detection sensitivity, particularly excelling in detecting low-concentration target bacteria. This review summarizes the principles and application examples of CRISPR-Cas-based fluorescence, electrochemical, lateral flow, and colorimetric nanostructured biosensors developed over the past three years, categorizing them according to their recognition methods (e.g. bacterial genomes, aptamers, antibodies). It systematically explores the broad application prospects of these sensors in medical diagnostics, environmental monitoring, and food safety assessment. Additionally, this review discusses future research directions and potential development prospects, providing new insights and technical support for the rapid diagnosis and treatment of bacterial infections.

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Article highlights

- For the first time, the article systematically integrates pre-amplification techniques with CRISPR/Cas systems, significantly enhancing the sensitivity and specificity of bacterial detection. This integration is a distinctive feature of the study.
- In reviewing fluorescence signals, the article provides a more systematic and in-depth analytical framework based on different mechanisms such as bacterial genomic DNA, aptamers, and immune recognition.
- The article particularly emphasizes the role of nanomaterials in signal amplification and detection performance, while also exploring their synergistic effects with CRISPR/Cas systems.
- The article not only discusses the practical applications of biosensors in bacterial detection but also thoroughly analyzes their limitations and challenges, offering a more comprehensive and practical perspective.
- The article incorporates the latest research advancements in the field, ensuring the timeliness and cutting-edge nature of the content, while also proposing future research directions to guide subsequent studies.
- The article reviews research that combines interdisciplinary fields such as CRISPR/Cas systems, nanomaterials, and signal amplification technologies, demonstrating the potential of multidisciplinary research.
- Through detailed classification and systematic analysis, the article provides readers with a clear research framework, making it easier to understand and apply.
- The article offers important references for the development and optimization of bacterial detection technologies.

1. Introduction

Bacterial infections annually trigger millions of disease outbreaks globally [1], often involving multiple bacteria in a single sample, some of which can cause substantial illness even at low concentrations [2]. Traditional detection methods, including bacterial culture and enrichment [3,4], typically necessitate 3 to 5 days for conclusive results [5]. While enzyme-linked immunosorbent assay (ELISA) [6] and polymerase chain reaction (PCR) [7] have expedited detection to 1–2 days, they require complex procedures, sophisticated nucleic acid extraction, and expensive equipment. Especially in diagnosing clinical infectious diseases, accurately determining whether the infecting bacteria are viable or dead is crucial for formulating treatment plans. Therefore, there is an urgent need for an accurate, rapid, and sensitive detection method to distinguish between viable and dead bacteria to assist in early clinical diagnosis.

The diversity of bacterial detection technologies stems from the unique biological characteristics of bacteria and the specific detection requirements. Currently, the main technical approaches include genome extraction [8], immune recognition [9], and aptamer recognition [10], each with its distinct advantages and applicable scenarios. Genome extraction technology achieves detection by amplifying specific sequences within the bacterial genome, offering extremely high sensitivity and specificity, and is capable of detecting bacteria at very low concentrations. However, its complex operational procedures and the need for sophisticated equipment limit its application in rapid on-site detection. Based on the classic antigen-antibody interaction, immune recognition enables highly sensitive detection of target bacteria through techniques such as ELISA or immunofluorescence. Its mature technological framework and the extensive availability of commercial antibodies make it an essential tool for rapid screening [11]. Compared to traditional antibodies, aptamers offer broader applicability, enabling precise detection without immunogenic structures on bacteria. Aptamer recognition is a particular detection method based on nucleic acid molecules, where aptamers, selected through the Systematic Evolution of Ligands by Exponential Enrichment (SELEX) technology, can bind with high affinity and specificity to surface antigens or proteins of target bacteria [12].

The CRISPR-Cas system, recognized for its high specificity and precision in gene editing, was originally an integral part of the bacterial immune system [13]. It comprises multiple arrays of regularly spaced short palindromic repeats (CRISPR) and associated proteins (Cas). As a multifaceted and utility-driven suite of enzymatic editing tools within biological sciences [14–16], it has garnered extensive interest in applications ranging from genome editing and bioengineering [17] to diagnostic technologies [18]. However, in practical applications, the concentration of target bacteria is often low, and direct detection may face challenges of insufficient sensitivity. To address this issue, pre-amplification technologies (such as PCR and isothermal amplification) have been introduced, significantly enhancing the sensitivity and reliability of detection by amplifying target nucleic acids [19]. Pre-amplification technology enriches low-concentration targets to detectable levels and reduces interference from nonspecific signals by incorporating specific primers or probes, thereby improving detection specificity [20]. Furthermore, combining amplification products with the unique optical, electrical, and magnetic properties of signal molecules further enhances the performance of biological recognition elements. Among these, nanomaterials, as signal molecules, offer high surface area, tunable optical properties, and excellent electrical performance, providing robust technical support for signal amplification and multifunctional integration [21]. In 2017, Feng Zhang's research group developed the SHERLOCK platform, which harnesses the power of recombinase polymerase amplification (RPA) amplicons to engage the nuclease activity of CRISPR-Cas13a. This activation process facilitates the high-sensitivity detection of pathogens like Zika and dengue viruses, utilizing a paper-based diagnostic system. Concurrently, the DETECTR system, developed by Doudna and colleagues, has employed loop-mediated isothermal amplification (LAMP) amplicons to activate CRISPR-Cas12a, enabling precise fluorescence-based detection of specific DNA sequences, such as those from HPV16 or HPV18 [22,23]. CRISPR-Cas14a also can bind to nucleic acid targets and cleave single-stranded DNA (ssDNA) [24]. In comparison, CRISPR-Cas9, directed by a small-guide RNA (sgRNA), specifically targets and cleaves double-stranded DNA (dsDNA), while the nuclease-inactive CRISPR-dCas9 variant can bind to DNA under sgRNA guidance without leading to DNA strand cleavage [25].

CRISPR-Cas technology has emerged as a highly promising tool for nucleic acid detection, leveraging its proteins' unique tunable nuclease activity [26]. This versatile system has been employed in nanostructured biosensors to detect various targets, including bacteria, viruses, RNA, and small molecules [27–33]. Cas9 [34], dCas9 [35], Cas12a [36], Cas13a [37], and

Cas14 [38] proteins have been utilized as recognition elements for target, which are then converted into various detection signals such as fluorescent, electrochemical, colorimetric, and electrochemiluminescent (ECL). These versatile analytical tools have penetrated numerous fields, including but not limited to medical diagnostics, environmental monitoring, food safety assessment, and bioprocess optimization [39].

2. CRISPR-Cas based fluorescent nanostructured biosensors

The genomic DNA of target bacteria can be amplified through PCR or isothermal amplification techniques. Meanwhile, the target bacteria can also be specifically recognized by antibodies and aptamers, which are then converted into nucleic acid signals for further amplification. In the CRISPR-Cas system, the CRISPR-Cas12a protein, guided by CRISPR RNA (CrRNA), binds to the amplified products, activating its trans-cleavage activity. As illustrated in Figure 1, when the ends of single-stranded DNA/RNA (ssDNA/ssRNA) are modified with a fluorescent group (FAM) and a quencher group (BHQ), FAM is initially quenched by BHQ through fluorescence resonance energy transfer (FRET). Upon cleavage of the ssDNA, the fluorescent group regains its fluorescence, potentially indicating the presence of the target nucleic acid molecule [40–43]. This system enables accurate target recognition and amplified signal transduction [44]. Researchers have also proposed various enhancements to improve the performance of fluorescent nanostructured biosensors within the CRISPR-Cas system. These improvements include the integration of PCR amplification, isothermal amplification techniques, enzymes, visualization methods, and nanomaterials. The synergistic combination of these technologies has significantly enhanced the speed, sensitivity, and simplicity of detecting bacteria.

Nanostructured biosensor detection of bacteria generally involves recognizing genomic DNA, Genomic DNA amplification of target signals can be achieved through various techniques. Zhou et al. extracted genomic DNA from *Salmonella*, amplified it via PCR, and combined it with plintR ligase to form DNA products that activate both the cis and trans cleavage functions of CRISPR-Cas12a, thereby generating a fluorescent signal. This method has successfully attained a detection threshold of 10 colony-forming units (CFU) for *Salmonella*, simultaneously allowing for the discrimination between live and dead bacteria and the identification of multiple serotypes [45].

PCR requires nucleic acid amplification across three distinct temperatures, escalating the assay's cost and complexity. Conversely, emerging isothermal nucleic acid amplification methods, including LAMP, RPA, recombinase-assisted amplification (RAA), nuclease-assisted amplification (NEAA), heterogeneous chain reaction (HCR), primer exchange reaction (PER), and salting-out rolled-circle amplification (SRCA), dispense with the need for costly temperature-control equipment and are comparatively more uncomplicated to execute. Combining amplified products formed through isothermal amplification technology with the CRISPR-Cas system, highly specific, sensitive, and user-friendly nanostructured biosensors have been developed, making them ideal for rapid on-site detection. In 2020, Wang *et al*. utilized RPA technology to swiftly DNA structure, effectively triggering the cleavage function of CRISPR-Cas12a, allowing for the detection of 1 CFU/mL of *Escherichia coli* (*E. coli*) O157:H7 and *Staphylococcus aureus* (*S. aureus*) within 50 minutes [36]. More recently, in 2022, Bia et al. employed NEAA to amplify the genome, detecting 80 CFU/mL of Salmonella in eggs within a mere 20 minutes [46].

Nanomaterials offer a rapid method for bacterial detection and may enhance the early diagnosis of bacterial infections, especially in resource-limited environments [47]. In 2022, Zhou *et al*. integrated functionalized quantum dots with RAA-CRISPR -Cas12a to reduce the signal-to-noise ratio, allowing the detection of *S. aureus* at concentrations as low as 5.4×10^2 CFU/mL [48]. Liu *et al*. integrated RAA-CRISPR-Cas12a with ZIF-8@FLS fluorescent nanocomposites for the sensitive detection of *Salmonella typhimurium* (*S. typhimurium*), achieving a remarkably low detection limit of 1.3×10^2 CFU/mL and enabling sample detection within 2 hours [49]. Li *et al*. integrated the target recognition capabilities of LAMP and dCas9

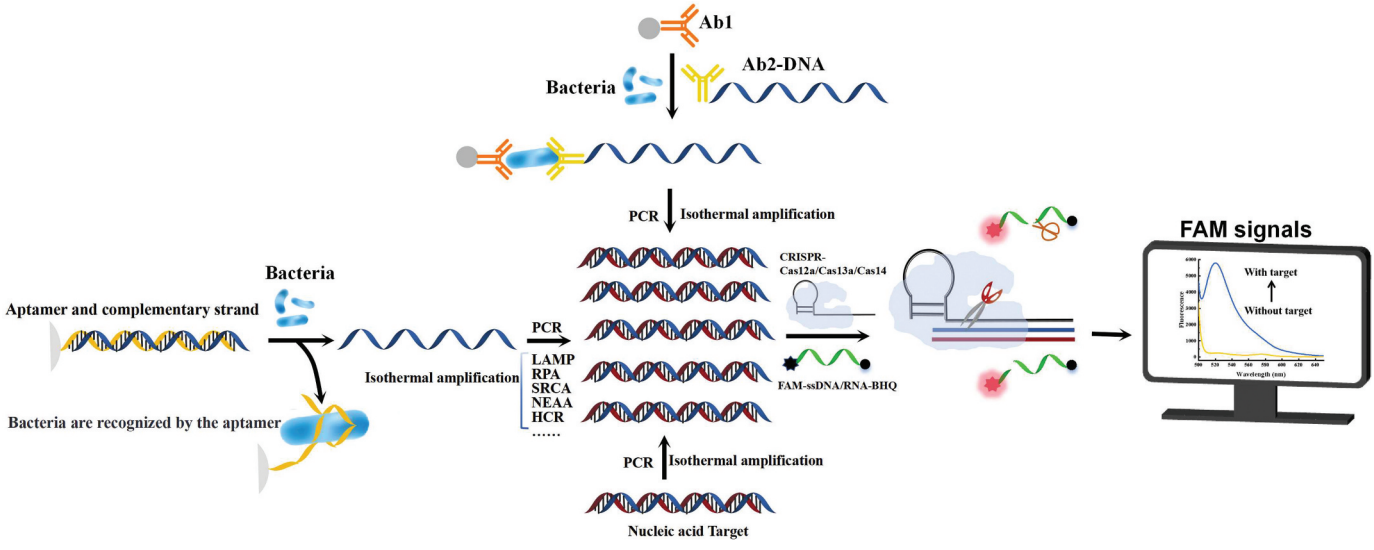

Figure 1. Principle of CRISPR-Cas-based fluorescent nanostructured biosensor.

to fabricate a portable, visual, bimodal nanostructured biosensor, which employs a combination of fluorescence and colorimetric signals for the visual detection of foodborne pathogens and is accompanied by a custom 3D-printed fluorescence reader and a handheld colorimeter for on-site analysis [50]. Other pre-amplification CRISPR-Cas nanostructured biosensors using the genome as a template are shown in Table 1 [4,51–58].

Due to antibodies' specific recognition of antigens, the fluorescent probes prepared by immobilizing antibodies on the surface of carbon dots exhibit excellent specificity and sensitivity in bacterial detection. By using magnetic nanoparticles modified with capture antibodies (MNPs@Ab1) and RCA primers linked to specific antibodies (primer@Ab2), target bacteria are isolated through immunomagnetic separation, and detection is achieved by combining rolling circle amplification (RCA) with CRISPR-Cas12a signal amplification. This method demonstrates a wide linear range for detecting *S. typhimurium*, with a detection limit as low as 1.93×10^2 CFU/mL [59].

Aptamers are short ssDNA or ssRNA molecules capable of forming specific three-dimensional structures for targeted binding to proteins or cells. Leveraging their unique biological and chemical properties, aptamers have demonstrated significant value in developing diagnostic reagents and therapeutic agents. Compared to antibodies, aptamers offer minimal batch-to-batch variation, lower production costs, and ease of modification. By combining aptamers with carbon dots that feature easily modifiable surfaces and excellent fluorescence properties, highly specific fluorescent probes with superior detection limits can be constructed, providing an efficient tool for the specific identification of pathogenic bacteria [60]; Shen *et al*. utilized a variant probe that incorporated the structural domain of an aptamer and the structural domain of the T7 promoter for RNA transcription and amplification. They combined this with the side-branching cleavage activity of the CRISPR-Cas13a system to detect *Salmonella* in milk samples and serum across a range of concentrations from 1 to 10^5 CFU [61]. Qiao *et al*. utilized the interaction between *Pseudomonas. aeruginosa* (*P. aeruginosa*) and its aptamer on a DNA three-way junction (TWJ) structured probe to trigger the migration of double-stranded DNA branches, thereby activating CRISPR-Cas14a1. The method detected target bacteria at concentrations ranging from 10 to 10^5 CFU/mL within 30 minutes, with a detection limit as low as 3.4 CFU/mL [62]. By integrating aptamer-based magnetic separation with bifunctional HCR-scaffolded multivalent aptamers and the activity of CRISPR-Cas12a, ultrasensitive detection of *Salmonella* was achieved, with a linear range of 0–10^7 CFU/mL and a detection limit of 2 CFU/mL [63].

Fluorescent nanostructured biosensors facilitate the introduction of reporter nucleic acid molecules bearing fluorescent groups into the reaction system, enabling highly sensitive detection of target nucleic acid molecules with broad applicability. Nonetheless, current research indicates that most nucleic acid signal amplification strategies employing the trans-cleaving activity of CRISPR-Cas systems typically utilize 5–15 base pair ssDNA fused with fluorescent reporters. The susceptibility of these short-sequence ssDNA to degradation can lead to the premature release of the fluorescent signal, potentially resulting in false-positive outcomes. Future research should focus on the strategic design of reporter molecule DNA length and conformation to enhance stability and reduce the incidence of false-positive results. When faced with complex samples that contain interfering fluorescent signals, such as biological fluids and soil, which can disrupt the performance of fluorescent nanostructured biosensors, it is crucial to optimize sensitivity and accuracy within these complex matrices. This can be achieved through sample pretreatment or the incorporation of highly sensitive fluorescent nanomaterials, enabling more reliable detection in challenging environments.

3. CRISPR-Cas-based electrochemical nanostructured biosensors

Electrical signal fluctuations, including voltage and current, are typically correlated with the concentrations of target analytes. The CRISPR-Cas system has been successfully integrated into electrochemical biosensing, primarily by establishing a direct

Table 1. CRISPR-Cas12a-based fluorescent bacterial nanostructured biosensors.

Sample	Detection time	Bacteria Name	Amplification method	Bio-Recognition	Signal Source	Detection Range	Detection Limit	References
Milk, Pure drinking water	> 1 h	*B. cereus*	RAA	Genomic DNA	FAM	101–10^8 CFU/mL	500 CFU/mL	[4]
Natural food sample	> 1 h	*L. monocytogenes*	RAA	Genomic DNA	FAM	0.68×10^{-13} M – 0.68×10^{-19} M	0.68 aM	[51]
Enoki mushroom	> 1 h	*L. monocytogenes*	LAMP	Genomic DNA	FAM	100–10^6 CFU/mL	1 CFU/mL	[52]
Milk	> 1 h	*S. typhimurium*	RAA	Genomic DNA	FAM	$8.7–8.7 \times 10^4$	2 CFU/mL	[53]
Aaliva	> 1 h	*M. tuberculosis*	LAMP	Genomic DNA	FAM	50 fg – 5 ng	10 copies (50 fg)	[54]
Milk	> 1 h	*Salmonella*	RPA	Genomic DNA	FAM	$2.58 \times 10^1 – 2.58 \times 10^4$ CFU/mL	0.2 CFU/mL	[55]
Serums	> 1 h	*S. typhimurium*	RPA	Genomic DNA	FAM	$3 \times 10^3 – 3 \times 10^5$ CFU/mL	3×10^3 CFU/mL	[56]
Beef	45 min	*V. parahemolyticus*, *E. coli*, *S. aureus*	RPA	Genomic DNA	FAM	10–10^6 copies	10 copies	[57]
Rice flour, fresh white flour, glutinous rice flour	> 1 h	*B. gladioli*	RPA	Genomic DNA	FAM	100–10^6 CFU/mL	10^1 CFU/mL	[58]

correlation between electrical signals and target concentrations, thereby facilitating the quantitative detection of these targets [64,65]. Upon recognition of the genomic sequence or aptamer/antibody, it is converted into amplified products, thereby catalyzing the trans-cleavage activity of the CRISPR-Cas protein. This process involves cleaving the methylene blue (MB)-labeled single-stranded DNA/RNA immobilized on the electrode surface, releasing MB molecules and reducing the electrochemical signal, enabling precise quantification of the target in the test sample (Figure 2).

He et al. extracted DNA from *S. typhimurium* and initially amplified it using PCR, followed by a secondary amplification through the activation of CRISPR-Cas12a, which targets the side-branch cleavage of a signal hairpin DNA probe. This process triggers the release of an electrochemical marker, resulting in a marked reduction in current. The method achieves a detection limit of 55 CFU/mL for *S. typhimurium* [66]. Li *et al*. employed RAA technology to amplify genomic DNA. They combined it with CRISPR-Cas12a for the rapid and ultra-sensitive detection of *Listeria monocytogenes* (*L. monocytogenes*), achieving a detection limit as low as 0.68 aM [51]. Huang *et al*. immobilized a sulfhydryl-modified reporter probe (SH-ssDNA-MB) on the surface of a gold nanoparticle-modified electrode via an Au-S bond. In the presence of *S. aureus*, the amplification of genomic DNA by the SRCA method activated the trans-cleavage activity of CRISPR-Cas12a, inducing the cleavage of SH-ssDNA-MB from the Gold nanoparticle (AuNPs)-modified electrode, leading to a decrease in the current signal [67].

Electrochemical aptamer-based nanostructured biosensors achieve sensitive detection of analytes by transducing the changes in charge transfer, which occur at the interface of a conductive matrix due to the specific binding of an aptamer to its target, into discernible electrical signals. Such nanostructured biosensors offer the advantages of simplicity, quick response, and high sensitivity. The interaction with the *S. aureus* aptamer initiates the trans-cleavage activity of CRISPR-Cas12a. This enzyme nonspecifically cleaves bystander single-stranded DNA sequences attached as electrochemical labels on the electrode surface, altering the electron transfer from the labels and changing the electrochemical readout [68].

DNA metallization is the process where metal nanowires are formed by exploiting the electrostatic attraction between negatively charged DNA and positively charged metal ions, following the application of a reducing agent or an electric potential. This method facilitates the development of highly sensitive nanostructured biosensors. The nanostructured biosensor's integration of dual electrochemical signals facilitates self-calibration, as the target is determined by calculating the ratio, thereby diminishing interferences. This approach leads to precise analytical results and enhances reliability. The ratiometric nanostructured biosensor developed by Zheng *et al*., utilizing the IFc/IMB format, achieves 100% sensitivity in detecting *Salmonella* and shows consistency with the results of real-time fluorescence quantitative PCR (RT-qPCR) [69].

Electrochemical nanostructured biosensors can directly analyze clinical samples, such as urine, serum, and whole blood. Ideally, these sensors facilitate rapid, precise, and highly stable continuous bacteria monitoring within complex matrices. However, The detection duration for electrochemical nanostructured biosensors employing the CRISPR-Cas system exceeds 1 hour. Priority should be given to enhancing their response time and sensitivity to advanced CRISPR-Cas-based electrochemical nanostructured biosensors. Achievements in this area may stem from employing novel and efficacious electrode materials, refining sensor architecture, and incorporating sophisticated signal augmentation methods. Nanomaterials can significantly bolster sensor responsiveness and expedite detection due to their distinctive electrochemical attributes. Beyond material innovation, the sensor's structural configuration and overall design are paramount. Integration with microfluidics allows for rapid mixing and processing of samples in confined spaces, significantly accelerating the detection process. This is particularly critical for biological samples that require immediate processing.

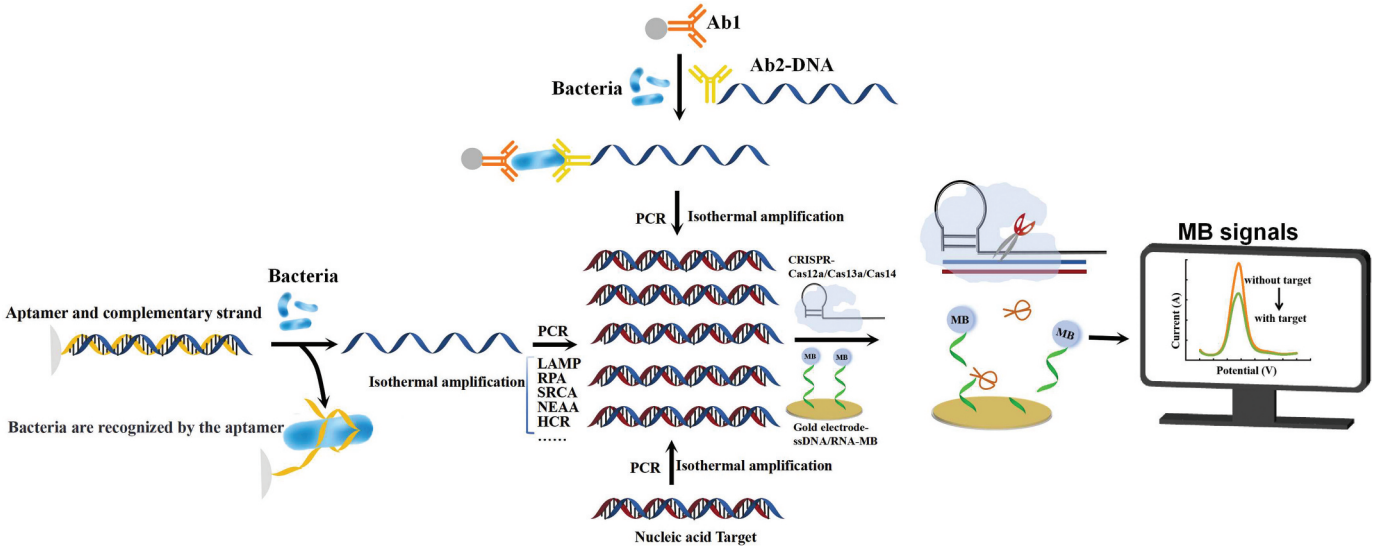

Figure 2. Principle of CRISPR-Cas-based electrochemical nanostructured biosensor.

Simultaneously, applying intelligent algorithms and data processing technologies facilitates efficient data analysis and result interpretation, enabling operators to obtain reliable test results without sophisticated instrumentation.

4. CRISPR-Cas-based lateral flow assay (LFA) nanostructured biosensor

LFA (Lateral Flow Assay) is a rapid and portable detection platform that has recently gained widespread popularity. The team led by Zhang Feng developed SHERLOCKv2, integrating nucleic acid test strips as a signal reporting system, offering exceptional convenience for Point-of-Care Testing (POCT) applications.They engineered the probe by attaching two small molecule labels, FAM and biotin, at each end. The commercial nucleic acid test strips they employed utilize anti-FAM antibodies conjugated with colloidal gold, while the test line on the NC membrane is coated with streptavidin to capture biotin. Originally, these test strips were designed to detect nucleic acid amplification products containing both FAM and biotin via a sandwich assay.In a groundbreaking innovation, Zhang Feng's team reimagined the interpretation approach for these test strips. They repurposed the quality control line of the sandwich assay test strip as the detection line, enabling the determination of whether the probe has been cleaved [70]. The integration of isothermal amplification, CRISPR-Cas12a technology, and lateral flow chromatography through the use of test strips makes the assay cost-effective. It enables visual detection of bacteria in clinical or food samples (Figure 3).

Lee *et al*. utilized a ssDNA probe labeled with biotin and FAM. On the test (T) line, streptavidin binds the tagged ssDNA probes, resulting in a red appearance. Conversely, ssDNA cleaved by CRISPR-Cas12a fails to immobilize on the T line. The cleavage activity of the ssDNA probe is contingent upon LAMP amplicon production, which allows for the visual distinction of test results as either positive or negative [71].

Wang *et al*. amplified the *Mycobacterium tuberculosis* (*M. tuberculosis*) complex (MTC) using LAMP, followed by CRISPR-Cas12a activation, successfully detecting 10 copies (50 fg of the MTC genomic template) via a lateral flow sensor [54]. Similarly, Zhu *et al*. employed lateral flow immunochromatographic strips to read the results after 15 minutes of RPA amplification and 10 minutes of CRISPR-Cas12a cleavage, achieving rapid detection and typing of *Helicobacter pylori* (*H. pylori*). The process takes only 25 minutes, with

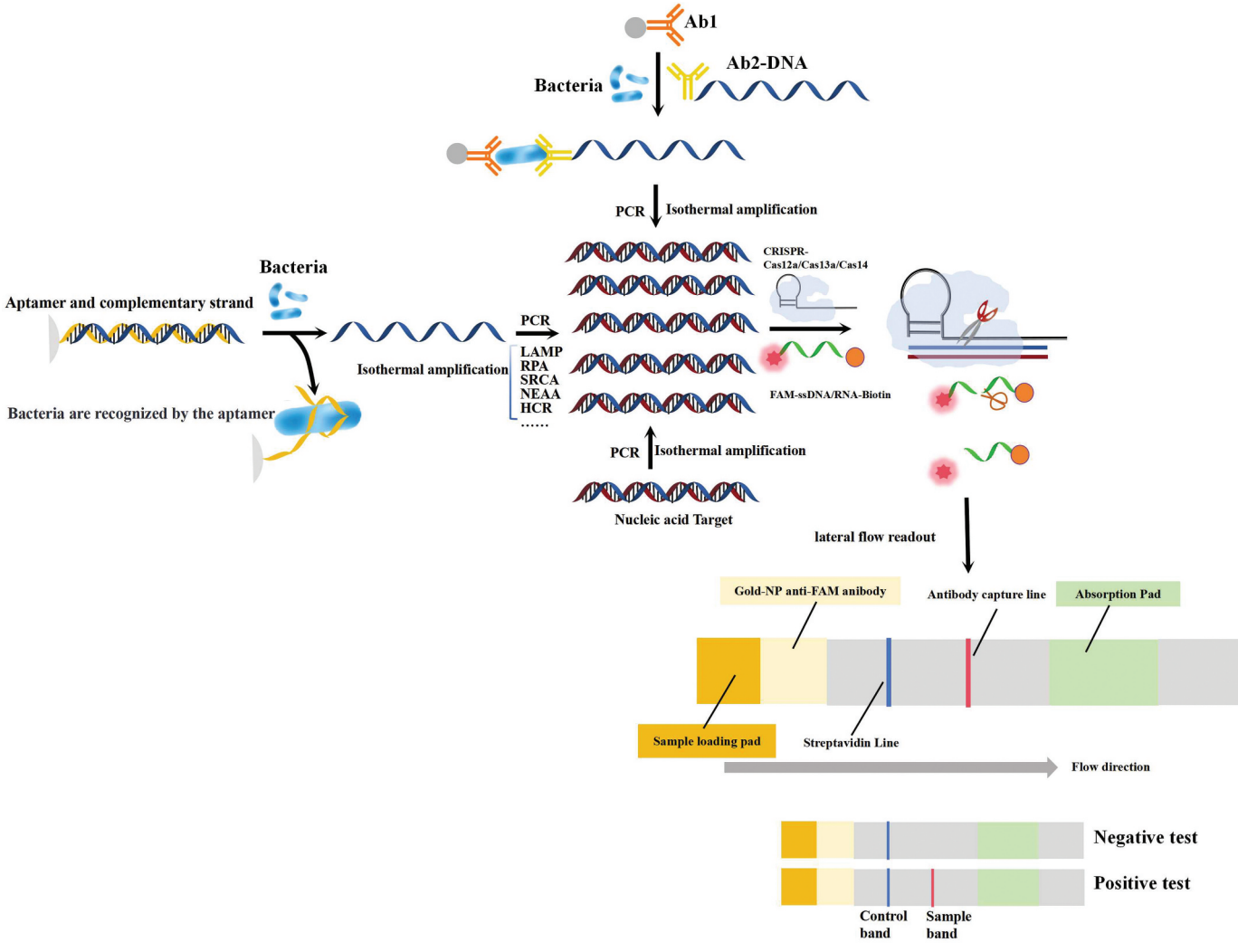

Figure 3. Principle of CRISPR-Cas-based LFA nanostructured biosensor.

a sensitivity as low as 2 ng/μL [72]. Qiu *et al*. developed a CRISPR-Cas12a-mediated nucleic acid detection platform named CRISPR-CLA, which integrates CRISPR-Cas12a-based nucleic acid detection with LAMP technology. By applying CRISPR-CLA, they successfully achieved accurate detection of *Neisseria faecalis* (*N. faecalis*) using a novel species-specific gene called pbr1, detecting as low as 100 fg of double-stranded DNA in sputum samples [73]. Employing functionalized quantum dots facilitates the integration of lateral flow assay with fluorescence detection. Zhou *et al*. devised a cost-effective, straightforward, and sensitive method for detecting *S. aureus*. This method combines RAA, CRISPR-Cas12a technology, and lateral flow fluorescence with functionalized quantum dots. In their approach, CRISPR-Cas12a-mediated activation initiates trans-cleavage activity, digesting the non-complementary biotinylated DNA probe to the capture probe anchored on the test line (T line). This action causes the T line to emit no fluorescence signal on the LFA nanostructured biosensor. Visual inspection or a fluorescent strip reader can measure the fluorescence intensity of both the T and control lines [48].

The high specificity of the CRISPR-Cas system, combined with the convenience of traditional lateral flow assays, provides a rapid, accurate, and cost-effective detection method. This technology harnesses the CRISPR-Cas system's ability to identify specific nucleic acid sequences and generate a detectable signal in as little as several minutes from sample processing to result in readout. Such rapidity is crucial for clinical and field settings where immediate decision-making is paramount. Moreover, its simplicity and portability render it ideal for low-resource areas and urgent field-based testing, significantly broadening its potential applications. Nevertheless, a significant limitation of conventional lateral flow assays is their design, which typically supports the detection of only one target substance, thus restricting their utility when concurrent identification of multiple pathogens or analytes is necessary. To address this challenge, researchers should aim to advance the development of multiple lateral flow test paper sensors and expand the application range of LFA through various innovative means. Specific innovations include configuring multiple test lines on the same test paper, each dedicated to a specific target, enabling simultaneous multi-target detection.

Additionally, diverse detection markers were adopted to enhance the sensitivity and applicability of the reagents. Furthermore, integrating these multi-detection solutions into intelligent, automated platforms enables advanced quantitative analysis and data processing, significantly enhancing detection accuracy and ease of operation.

5. CRISPR-Cas-based colorimetric nanostructured biosensor

Colorimetric nanostructured biosensors, widely utilized across fields like biomedicine, food industry, and environmental sciences, are preferred for their high sensitivity and selectivity, ease of handling, and visible detection with the naked eye. In recent years, significant advancements have been achieved in developing CRISPR-Cas-based colorimetric nanostructured biosensors. For instance, in 2021, Chen *et al*. demonstrated the translation of sequence information into peroxidase-mimicking activity by a G-quadruplex DNAzyme nanostructure, enabling CRISPR-Cas-based colorimetric detection of *Vibrio parahaemolyticus* (*V. parahaemolyticus*) at a concentration as low as 9.8 CFU/mL [74]. Wei *et al*. utilized a silver ion (Ag^+) aptamer, serving both as a trans-cleavage substrate in the CRISPR-Cas12a system and as a modifier for the Ag/TMB chromogenic reaction. By integrating RPA technology, they achieved visual detection of *Methicillin-Resistant Staphylococcus aureus* (MRSA) with a detection concentration as low as 8 CFU/mL [75]. In 2024, Shang *et al*. introduced a novel droplet microfluidics-based assay coupled with a colorimetric strategy integrating RAA and CRISPR-Cas13a. This approach enables the precise and sensitive detection of a broad spectrum of bacteria by altering the color of the droplets, boasting a sensitivity threshold of as low as 101 copies/μL [76].

CRISPR-Cas-based colorimetric nanostructured biosensors offer a rapid, precise, and user-friendly detection method, ideally suited for resource-limited settings or where immediate on-site analysis is imperative. Despite the widespread use of gold nanoparticles in colorimetric nanostructured sensors, their high cost has constrained broader application. Although CRISPR-based colorimetric techniques boast substantial benefits in bacterial detection, they may encounter signal instability and diminished accuracy in discerning low-concentration samples. To surmount these hurdles, researchers must delve into creating innovative nanomaterials and refine detection systems and signal processing algorithms to enhance sensitivity and stability.

Moreover, the majority of colorimetric nanostructured biosensors are single-use. Consequently, the advancement of reusable reagents and the creation of continuous monitoring sensor systems are pivotal for cost reduction, curbing chemical waste production, and fulfilling the demand for real-time tracking of sample dynamics. To encapsulate, such enhancements are set to propel significant advancements in colorimetric nanostructured biosensors, broadening their applicability across diverse fields.

6. CRISPR-Cas-based magnetic relaxation switching (MRS) nanostructured biosensor

The MRS method, based on magnetic nanoparticles (MNPs), represents an emerging rapid detection technique. It combines nuclear magnetic resonance (NMR), immunoassay, and nanotechnology to achieve a high signal-to-noise ratio and immunity to signal interference from sample matrices. This method applies to the trace detection of hazardous substances in food and environmental samples [77,78]. In 2022, Shen *et al*. obtained the genomic DNA of Salmonella typhimurium through PCR and controlled the binding of MNPs of two different sizes using the CRISPR-Cas12a system. They employed smaller MNPs as magnetic probes to achieve MRS output. The detection limits for Salmonella in spiked samples of pure bacterial solution and chicken extract were 1.3×10^2 CFU/mL and 1.8×10^3 CFU/mL, respectively [79]; Wen *et al*. accomplished the detection of *Salmonella* at concentrations as

low as 53 CFU/mL using a cascade-conjugated technique that employs terminal deoxynucleotidyl transferase (Tdt)-mediated signal amplification. This method leverages UDG's resistance to contamination, the specificity of PCR primers, the CRISPR-Cas12a system's accurate recognition abilities, and magnetic probe signals that remain unaffected by the sample matrices [80].

MRS nanostructured biosensors that leverage the CRISPR-Cas system exemplify state-of-the-art biotechnological detection. By harnessing the CRISPR-Cas enzyme's unparalleled targeting accuracy, these sensors can trigger a reaction among magnetic particles when detecting specific nucleic acid sequence variations, thereby enabling the sensitive and specific identification of target molecules. Nevertheless, MNPs frequently require enhanced cross-linking or exhibit nonspecific binding to the target, which can impede effective interaction with the target molecule, leading to diminished detection signals. Additionally, nonspecific binding can introduce background noise, compromising the accuracy of detection outcomes. Consequently, refining the synthesis of MNPs or enhancing their surface functionalization is imperative to achieve greater surface activity and biocompatibility. Developing innovative chemical cross-linking approaches is crucial for ensuring a robust and specific connection between MNPs and targets. Furthermore, computational simulations and high-throughput screening methods can optimize the binding dynamics between MNPs and targets, boosting the sensors' performance.

Microarray-based high-throughput MRS detectors are now available. They feature compact, portable, and efficient designs ideal for field applications and resource-constrained settings. As a result, developing novel CRISPR-Cas high-throughput MRS sensors has emerged as a pivotal avenue for future research.

7. CRISPR-Cas-based surface-enhanced Raman scattering (SERS) nanostructured biosensor

SERS is a spectroscopic technique that leverages the surface effects of metallic nanostructures to amplify the Raman scattering signals of molecules adsorbed onto their surfaces. This technique is highly regarded for its optical sensing capabilities, offering single-molecule level sensitivity and molecular fingerprinting spectroscopy, all while exhibiting a remarkable insensitivity to quenching [81]. For example, by leveraging SERS, Ma *et al*. developed an integrated microfluidic paper-based analytical device (μPAD) based on recombinase RPA and CRISPR-Cas, creating an RPA-Cas12a-μPAD sensor for ultrasensitive and instantaneous detection of pathogenic bacteria [82]. Wang *et al*. utilized the CRISPR-Cas9 system to identify and unwind target gene amplicons induced by RPA accurately. They then bound many AuNS@Ag to the intermediate bridge structure on the test strip's test line (T line). They obtained recognizable SERS signals using a smartphone-integrated portable Raman spectrometer. This method can detect Staphylococcus aureus at concentrations as low as 1 CFU/mL, with the entire analysis process completed in just 45 minutes [83].

SERS technology, when integrated with the gene-editing prowess of the CRISPR-Cas system, provides a spectroscopic tool for bacterial detection. The strength of the SERS signal hinges on the substrate preparation's quality, with factors like metal nanoparticle size, shape, distribution, and interactions with the substrate material significantly affecting signal reproducibility and stability. To bolster the reliability of CRISPR-Cas-based SERS sensors, researchers must employ a range of optimization tactics, including meticulous control over nanoparticle characteristics and enhancing substrate uniformity via surface modifications and self-assembly. By implementing these thorough strategies, SERS sensor performance is expected to improve substantially, broadening their applicability in environmental monitoring, food safety, biomedical diagnostics, and beyond.

8. CRISPR-Cas-based nanostructured biosensor for other signals

Electrochemiluminescence (ECL), which integrates electrochemistry with chemiluminescence, has garnered attention in bioanalysis and clinical diagnostics because of its exceptional features, including simple device requirements, low background noise, high sensitivity, and a broad dynamic range [84]. Wang *et al*. developed a method wherein *Salmonella* triggers an isothermal amplification reaction, activating CRISPR-Cas12a and releasing the ECL emitter from the porphyrin metal-organic framework (MOF, PCN-224), resulting in a decrease in the ECL signal. This method achieves a detection limit for *Salmonella* as low as 37 CFU/mL [85]. Although CRISPR-Cas-based ECL sensors show great potential in biodetection, they still face challenges and problems in their practical application and further development. The stability and reproducibility of the sensor are critical technical challenges because any small environmental changes may affect the cleavage efficiency of the CRISPR-Cas system and the intensity of the ECL signal, thereby affecting the accuracy of the detection results. To optimize the conditions of the electrochemiluminescence reaction, one must consider the selection of electrode materials, electrolyte composition, excitation voltage, and current to ensure stable luminescence signals.

Due to its high resolution and spatial controllability, photothermal sensing has garnered significant attention in optical imaging applications, including disease treatment and diagnosis, environmental monitoring, and more. It effectively addresses the limitations of traditional sensors, such as susceptibility to interference, high cost, inconvenient portability, and reliance on specific operating procedures. The silver nanoparticle-mediated DNA metallization strategy can employed in the CRISPR-Cas12a system to generate a photothermal signal for detection, which can be easily captured using a portable infrared camera, thereby reducing dependence on sizable analytical equipment. Gu *et al*. integrated CRISPR-Cas12a with silver particles deposited in situ on DNA structures as a signaling mechanism for photothermal detection. This approach enabled them to quantify, visualize, and detect *S. aureus* and *L. monocytogenes* with high sensitivity, reaching as low as 1 CFU/mL [86]. Photo-responsive temperature change sensors, which rely on photo-thermal conversion,

overcome the limitations of conventional sensors and offer an alternative approach with greater potential for various applications. Consequently, there is a need to develop high-performance photo-thermal conversion materials that exhibit exceptional photo-thermal properties and stability.

Biolayer Interferometry (BLI) utilizes a probe-based nanostructured biosensor to enable direct detection of samples, eliminating the requirement for fluorescent or isotopic labeling. The instrument directs white light onto the nanostructured biosensor's surface, subsequently capturing the reflected light. The reflected spectrum, which encompasses various frequencies, is modulated by the thickness of the sensor's optical film layer, forming an interference pattern [87]. Qian *et al*. developed a real-time, sensitive, and rapid digital system employing CRISPR-Cas-BLI to detect the ALP gene in *E. coli* DH5α. The CRISPR nanostructured biosensor, immobilized on the biolayer, could recruit the target DNA to the sensor surface, thereby altering its optical thickness. This change in thickness resulted in modifications to the interference pattern and the response signal of BLI, achieving a detection limit of 1.34 pg for *E. coli* DH5α [88]. The CRISPR-Cas-based BLI system is a promising approach for the rapid and sensitive detection of target DNA analytes. With the advancement of miniaturization and integration technologies, future BLI devices are anticipated to become more portable and cost-effective, thereby expanding their applicability in clinical diagnostics and field testing scenarios.

Personal glucose meters (PGM) are among the most widely used devices. Using the ability of convertase enzymes to catalyze the hydrolysis of sucrose to glucose, researchers use PGMs to detect proteins, small organic molecules, metal ions, and nucleic acid molecules [89–91]. Zhou *et al*. demonstrated that a combination of glucose signaling, RAA, CRISPR-Cas12a reaction, enzyme reaction, and PGM reading can detect *Salmonella* at a 5 CFU/mL concentration in complex food samples [92]. Portable nanostructured biosensors are easy to operate and can be used in various working environments, facilitating scientific research, public health monitoring, and food safety management while offering the capability to respond to emergencies. Consequently, there is a growing need to develop more portable, noninvasive, low-cost, and multifunctional monitoring technologies. CRISPR-Cas nanostructured biosensors, which can be based on personal glucose meters, have the potential to support personalized medicine by monitoring an individual's genetic variants or disease markers, thereby enabling the provision of a customized treatment plan for the patient.

9. Multimodal nanostructured biosensors based on the CRISPR-Cas system

Multimodal nanostructured biosensors typically integrate multiple sensing signals, either simultaneously or sequentially, with the aim of cross-validating assay results to achieve optimal sensitivity and accuracy. Xu *et al*. employed the RPA technique to amplify the target signal, activating the nonspecific degradation of ssDNA by CRISPR-Cas12a. The simultaneous emission of fluorescence and electrochemical signals enabled the detection of as few as two copies per reaction of the positive reference plasmid. This level of sensitivity is on par with that of the traditional PCR method [93]. Wang *et al*. developed a CRISPR-Cas9-based dual-mode platform that combined SERS and colorimetry. This platform could detect *S. aureus* with detection limits as low as 1 CFU/mL within 45 minutes [34].

Multimodal sensors integrate multiple sensing techniques to enable comprehensive and accurate capture of environmental information, demonstrating significant potential for efficient bacterial detection. However, one of the challenges current technologies face is signal interference, which directly affects the accuracy of the signal output. To address this, researchers must develop innovative sensing mechanisms and sensitive materials to eliminate such interference and enhance sensor performance.

Regarding data processing, the signals collected by multimodal sensors require meticulous analysis and processing. The study and application of machine learning algorithms can effectively extract valuable information from complex signals. Additionally, introducing feedback control algorithms can help optimize human-robot interaction and refine intelligent robot control. Integrating these technologies enhances the sensors' intelligence level and lays a solid foundation for achieving more accurate biological detection and intelligent control.

10. Conclusion

This review systematically explores the application of CRISPR-Cas system-based nanostructured biosensors in bacterial detection and highlights their significant potential in medical diagnostics, food safety, and environmental monitoring. By integrating pre-amplification techniques, providing a detailed classification of signal mechanisms, conducting an in-depth analysis of the role of nanomaterials, and discussing practical applications and challenges, we offer a comprehensive and innovative perspective on research in this field. (1) Unlike previous reviews, our study emphasizes combining pre-amplification techniques with the CRISPR-Cas system and nanomaterials for bacterial detection. This approach significantly enhances the sensitivity and specificity of detection, a distinctive feature of our research. (2) Our review provides a detailed classification of various signal mechanisms, focusing on fluorescence signals. We innovatively categorize fluorescence signals based on bacterial genomic DNA, aptamers, and immune recognition, offering a more structured and in-depth analysis. (3) While previous reviews have extensively discussed CRISPR-Cas-driven nano biosensors, our study specifically highlights the role of nanomaterials in signal amplification and detection performance, as well as their synergistic effects with the CRISPR-Cas system; (4) Our review dedicates a section to discussing the applications of these biosensors in real-world bacterial detection scenarios, along with their limitations and challenges. This provides a more balanced and application-oriented perspective; (5) We incorporate the latest research progress in this field to ensure the timeliness and relevance of the review. We hope these innovative insights will inspire further research, promoting the widespread application of this technology in bacterial detection and related

fields, thereby making greater contributions to human health, food safety, and environmental protection.

11. Future perspective

Bacterial nanostructure biosensors based on the CRISPR-Cas system exhibit immense potential for application in medical diagnostics, food safety, and environmental monitoring. However, with the continuous advancement of technology and the expansion of application scenarios, numerous challenges remain to be overcome while presenting new opportunities. Below are the key directions for future research and development:

(1) Distinguishing between live and dead bacteria is crucial for diagnosing and treating infectious diseases. Future research should focus on developing biomarkers to identify cell membrane integrity (such as compounds or proteins specific to membrane permeability) and designing improved CRISPR-Cas nanostructure biosensors that produce distinct signal outputs for live and dead bacteria. Additionally, integrating nanoparticles as carriers and utilizing encapsulation techniques to penetrate bacterial walls to identify and differentiate live and dead bacteria, generating different signal responses, will further enhance detection accuracy and practicality.
(2) Current bacterial detection methods often require the extraction of genomic DNA, which is time-consuming and complex, limiting their application in rapid diagnostics and POCT. Aptamers, such as ssDNA or ssRNA sequences with high affinity, specificity, and ease of modification, show great promise. Future research should further optimize the screening and refinement of aptamers, leveraging SELEX technology and computational simulation methods (such as molecular dynamics simulations, docking algorithms, and machine learning models) to accelerate aptamer development. A deeper understanding of the interactions between aptamers and bacteria, improving their stability and affinity, will significantly enhance detection efficiency and convenience.
(3) With the continuous optimization of CRISPR-Cas technology, developing nanostructure biosensors capable of simultaneously detecting multiple bacteria becomes feasible. This multi-target detection capability will aid in improving early disease diagnosis rates and advancing precision medicine. By generating distinct signals for each bacterium, doctors can provide personalized treatment recommendations based on tailored detection protocols, improving treatment outcomes. Future research should further explore technical pathways for multi-target detection, optimizing signal output and data analysis methods to meet the demands of complex sample detection.
(4) Creating more efficient CRISPR variants is key to enhancing the performance of bacterial biosensors. By optimizing these variants' targeting efficiency and specificity, detection sensitivity and accuracy can be significantly improved. Additionally, engineering PAM sequences to expand their recognition range will enable the detection of a broader spectrum of bacteria. Introducing multiplex CRISPR systems or dual-verification mechanisms can enhance detection reliability, reducing false positives or negatives. These improvements advance the application of bacterial biosensors in medical diagnostics, food safety, and environmental monitoring and provide stronger technical support for rapid and precise bacterial detection.
(5) Miniaturization and portability are core trends in developing bacterial nanostructure biosensors. Micro-nano manufacturing technologies to develop compact, rapid, on-site detection systems can meet the need for immediate testing in resource-limited or emergency situations. Future research should focus on enhancing sensor integration and portability, reducing operational complexity. Specific technical pathways include combining the CRISPR-Cas system with microfluidic or paper-based microfluidic technologies to develop low-cost, portable devices; utilizing smartphone cameras or fluorescence detection modules for real-time visual readouts; and improving stability and storage performance through lyophilized CRISPR-Cas components for field use. Additionally, integrating real-time monitoring with wireless technology will enable remote operation and continuous biological process monitoring, while establishing wireless sensor networks will facilitate real-time big data analysis, supporting research and decision-making, thereby promoting widespread application in medical, environmental, and food safety fields.
(6) Incorporating artificial intelligence and machine learning technologies will significantly enhance the intelligence and automation of bacterial nanostructure biosensors. By optimizing detection processes and data analysis through intelligent algorithms, the reliability and accuracy of sensors will be further improved. With wireless communication technology, remote operation and real-time data transmission of sensors can be achieved, making them suitable for long-term monitoring scenarios.
(7) Alongside technological development, environmental sustainability is a crucial direction for future research. Developing eco-friendly materials and manufacturing processes to reduce environmental impact is key to the long-term application of bacterial nanostructure biosensors. Future research should focus on the life-cycle management of sensors, promoting their application in protecting human health, maintaining the environment, and advancing sustainable development. Through green manufacturing and sustainable design, sensors will better serve the long-term development goals of society and the environment.

Author contribution statement

Hehua Zhang: Writing – original draft, Data curation, Writing – review & editing. Hongmin Guo: Writing – review & editing, Formal analysis. Hongzhi Pan: Conceptualization, Funding acquisition, Supervision, Visualization, Writing – original draft, Writing – review & editing.

Disclosure statement

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Christou L. The global burden of bacterial and viral zoonotic infections. Clin Microbiol And Infect. 2011;17(3):326–330. doi: 10.1111/j.1469-0691.2010.03441.x
2. Xing G, Zhang W, Li N, et al. Recent progress on microfluidic biosensors for rapid detection of pathogenic bacteria. Chin Chem Lett. 2022;33(4):1743–1751. doi: 10.1016/j.cclet.2021.08.073
3. Shang Y, Xiang X, Ye Q, et al. Advances in nanomaterial-based microfluidic platforms for on-site detection of foodborne bacteria. TrAC Trends Analytical Chem. 2022;147:116509. doi: 10.1016/j.trac.2021.116509
4. Xing G, Shang Y, Wang X, et al. Multiplexed detection of foodborne pathogens using one-pot CRISPR/Cas12a combined with recombinase aided amplification on a finger-actuated microfluidic biosensor. Biosens Bioelectron. 2023;220:114885. doi: 10.1016/j.bios.2022.114885
5. Mooijman KA, Pielaat A, Kuijpers AFA. Validation of EN ISO 6579–1 - microbiology of the food chain - horizontal method for the detection, enumeration and serotyping of salmonella - part 1 detection of salmonella spp. Int J Food Microbiol. 2019;288:3–12. doi: 10.1016/j.ijfoodmicro.2018.03.022
6. Jain S, Chattopadhyay S, Jackeray R, et al. Highly sensitive detection of salmonella typhi using surface aminated polycarbonate membrane enhanced-elisa. Biosens Bioelectron. 2012;31(1):37–43. doi: 10.1016/j.bios.2011.09.031
7. Maurischat S, Baumann B, Martin A, et al. Rapid detection and specific differentiation of Salmonella enterica subsp. enterica enteritidis, typhimurium and its monophasic variant 4,[5],12: i: – by real-time multiplex PCR. Int J Food Microbiol. 2015;193:8–14. doi: 10.1016/j.ijfoodmicro.2014.10.004
8. Wang Q, Wen Y, Li Y, et al. Ultrasensitive electrochemical biosensor of bacterial 16S rRNA gene based on polyA DNA probes. Anal Chem. 2019;91(14):9277–9283. doi: 10.1021/acs.analchem.9b02175
9. Li Y, Xiong Y, Fang L, et al. An electrochemical strategy using multifunctional nanoconjugates for efficient simultaneous detection of Escherichia coli O157: H7 and Vibrio cholerae O1. Theranostics. 2017;7(4):935–944. doi: 10.7150/thno.17544
10. Léguillier V, Heddi B-O, Vidic J-O. Recent advances in aptamer-based biosensors for bacterial detection. Biosensors (Basel). 2024;14(5):210. doi: 10.3390/bios14050210
11. Güven E, Azizoglu R-O. The recent original perspectives on nonculture-based bacteria detection methods: a comprehensive review. Foodborne Pathog Dis. 2022;19(7):425–440. doi: 10.1089/fpd.2021.0078
12. Chen S, Zhang L, Yuan Q, et al. Current advances in aptamer-based biomolecular recognition and biological process regulation. Chem Res Chin Univ. 2022;38(4):847–855. doi: 10.1007/s40242-022-2087-9
13. Horvath P, Barrangou R. Crispr/cas, the immune system of bacteria and Archaea. Science. 2010;327(5962):167–170. doi: 10.1126/science.1179555
14. Cong L, Ran FA, Cox D, et al. Multiplex genome engineering using CRISPR/Cas systems. Science. 2013;339(6121):819–823. doi: 10.1126/science.1231143
•• **This article has provided me with a lot of inspiration regarding the future applications of the CRISPR/Cas system.**
15. Li Y, Li S, Wang J, et al. Crispr/cas systems towards next-generation biosensing. Trends Biotechnol. 2019;37(7):730–743. doi: 10.1016/j.tibtech.2018.12.005
16. Amitai G, Sorek R. CRISPR-Cas adaptation: insights into the mechanism of action. Nat Rev Microbiol. 2016;14(2):67–76. doi: 10.1038/nrmicro.2015.14
17. English MA, Soenksen LR, Gayet RV, et al. Programmable crispr-responsive smart materials. Science. 2019;365(6455):780–785. doi: 10.1126/science.aaw5122
18. Gootenberg JS, Abudayyeh OO, Lee JW, et al. Nucleic acid detection with CRISPR-Cas13a/C2c2. Science. 2017;356(6336):438–442. doi: 10.1126/science.aam9321
19. Zhang L, Jiang H, Zhu Z, et al. Integrating CRISPR/Cas within isothermal amplification for point-of-care assay of nucleic acid. Talanta. 2022;243:123388. doi: 10.1016/j.talanta.2022.123388
20. Stougaard M, Ho YP. DNA-based nanosensors for next-generation clinical diagnostics via detection of enzyme activity. Expert Rev Mol Diagn. 2014;14(1):1–3. doi: 10.1586/14737159.2014.863151
21. Chang Y, Xia N-O, Huang Y, et al. In situ assembly of nanomaterials and molecules for the signal enhancement of electrochemical biosensors. Nanomaterials (Basel). 2021;11(12):3307. doi: 10.3390/nano11123307
22. Kellner MJ, Koob JG, Gootenberg JS, et al. SHERLOCK: nucleic acid detection with CRISPR nucleases. Nat Protoc. 2019 Oct;14(10):2986–3012. doi: 10.1038/s41596-019-0210-2
•• **This literature pioneered the detection method using SHERLOCK technology, setting a precedent for applying isothermal amplification-CRISPR/Cas technology in detection.**
23. Engreitz J, Abudayyeh O, Gootenberg J, et al. CRISPR tools for systematic studies of RNA regulation. Cold Spring Harb Perspect Biol. 2019;11(8). doi: 10.1101/cshperspect.a035386
24. Aquino-Jarquin G. CRISPR-Cas14 is now part of the artillery for gene editing and molecular diagnostic. Nanomed: Nanotechnol, Biol Med. 2019;18:428–431. doi: 10.1016/j.nano.2019.03.006
25. Gootenberg JS, Abudayyeh OO, Kellner MJ, et al. Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. Science. 2018;360(6387):439–444. doi: 10.1126/science.aaq0179
26. Barrangou R, Marraffini Luciano A. CRISPR-Cas systems: prokaryotes upgrade to adaptive immunity. Mol Cell. 2014;54(2):234–244. doi: 10.1016/j.molcel.2014.03.011
27. Chen M, Luo R, Li S, et al. Paper-based strip for ultrasensitive detection of OSCC-Associated salivary MicroRNA via CRISPR/Cas12a coupling with IS-Primer amplification reaction. Anal Chem. 2020;92(19):13336–13342. doi: 10.1021/acs.analchem.0c02642
28. Lee RA, Puig HD, Nguyen PQ, et al. Ultrasensitive crispr-based diagnostic for field-applicable detection of plasmodium species in symptomatic and asymptomatic malaria. Proc Natl Acad Sci. 2020;117(41):25722–25731. doi: 10.1073/pnas.2010196117
29. Peng L, Zhou J, Liu G, et al. CRISPR-Cas12a based aptasensor for sensitive and selective ATP detection. Sensors Actuators B: Chem. 2020;320:128164. doi: 10.1016/j.snb.2020.128164
30. Xiong Y, Zhang J, Yang Z, et al. Functional DNA regulated CRISPR-Cas12a sensors for point-of-care diagnostics of non-nucleic-acid targets. J Am Chem Soc. 2020;142(1):207–213. doi: 10.1021/jacs.9b09211
31. Chen JS, Ma E, Harrington LB, et al. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. Science. 2018;360(6387):436–439. doi: 10.1126/science.aar6245

32. Knott GJ, Doudna JA. CRISPR-Cas guides the future of genetic engineering. Science. 2018;361(6405):866–869. doi: 10.1126/science.aat5011
33. Huang CH, Lee KC, Doudna JA. Applications of CRISPR-Cas enzymes in cancer therapeutics and detection. Trends Cancer. 2018;4(7):499–512. doi: 10.1016/j.trecan.2018.05.006
34. Wang J, Jiang H, Chen Y, et al. CRISPR/Cas9-mediated SERS/colorimetric dual-mode lateral flow platform combined with smartphone for rapid and sensitive detection of staphylococcus aureus. Biosens Bioelectron. 2024;249:116046. doi: 10.1016/j.bios.2024.116046
35. Guk K, Keem JO, Hwang SG, et al. A facile, rapid and sensitive detection of MRSA using a crispr-mediated DNA FISH method, antibody-like dCas9/sgRNA complex. Biosens Bioelectron. 2017;95:67–71. doi: 10.1016/j.bios.2017.04.016
36. Wang YQ, Ke YQ, Liu WJ, et al. A one-pot toolbox based on Cas12a/crRNA enables rapid foodborne pathogen detection at Attomolar Level. ACS Sens. 2020;5(5):1427–1435. doi: 10.1021/acssensors.0c00320
37. Zhang T, Zhou W, Lin X, et al. Light-up RNA aptamer signaling-CRISPR-Cas13a-based mix-and-read assays for profiling viable pathogenic bacteria. Biosens Bioelectron. 2021;176:112906. doi: 10.1016/j.bios.2020.112906
38. Wei Y, Tao Z, Wan L, et al. Aptamer-based Cas14a1 biosensor for amplification-free live pathogenic detection. Biosens Bioelectron. 2022;211:114282. doi: 10.1016/j.bios.2022.114282
39. Jayanthi V, Das AB, Saxena U. Recent advances in biosensor development for the detection of cancer biomarkers. Biosens Bioelectron. 2017;91:15–23. doi: 10.1016/j.bios.2016.12.014
40. Tian T, Shu B, Jiang Y, et al. An ultralocalized Cas13a assay enables universal and nucleic acid amplification-free single-molecule RNA diagnostics. ACS Nano. 2021;15(1):1167–1178. doi: 10.1021/acsnano.0c08165
41. Wang Y, Peng Y, Li S, et al. The development of a fluorescence/colorimetric biosensor based on the cleavage activity of CRISPR-Cas12a for the detection of non-nucleic acid targets. J Hazard Mater. 2023;449:131044. doi: 10.1016/j.jhazmat.2023.131044
42. Fonfara I, Richter H, Bratovič M, et al. The crispr-associated DNA-cleaving enzyme Cpf1 also processes precursor CRISPR RNA. Nature. 2016;532(7600):517–521. doi: 10.1038/nature17945
43. Swarts DC, van der Oost J, Jinek M. Structural basis for Guide RNA processing and seed-dependent DNA targeting by CRISPR-Cas12a. Mol Cell. 2017;66(2):221–233.e4. doi: 10.1016/j.molcel.2017.03.016
44. Dai Y, Wu Y, Liu G, et al. CRISPR mediated biosensing toward understanding cellular biology and point-of-care diagnosis. Angew Chem Int Ed. 2020;59(47):20754–20766. doi: 10.1002/anie.202005398
45. Zhou C, Li W, Zhao Y, et al. Sensitive detection of viable salmonella bacteria based on tertiary cascade signal amplification via splintR ligase ligation-pcr amplification-CRISPR/Cas12a cleavage. Anal Chim Acta. 2023;1248:340885. doi: 10.1016/j.aca.2023.340885 Epub 2023 Jan 23.
• **This reference, by incorporating splintR ligase ligation and the CRISPR/Cas12a system, has achieved significant breakthroughs in detection sensitivity and specificity, providing a technical reference for detecting similar pathogens.**
46. Bai L, Wang L, Huang S, et al. Rapid, visual, and sequence-specific detection of salmonella in egg liquid with vis-neaa, a CRISPR/Cas12 empowered new strategy. J Agr Food Chem. 2022;70(7):2401–2409. doi: 10.1021/acs.jafc.1c06715
47. Lou D, Fan L, Cui Y, et al. Fluorescent nanoprobes with oriented modified antibodies to improve lateral flow immunoassay of cardiac troponin I. Anal Chem. 2018;90(11):6502–6508. doi: 10.1021/acs.analchem.7b05410
48. Zhou BQ, Ye QH, Li F, et al. CRISPR/Cas12a based fluorescence-enhanced lateral flow biosensor for detection of staphylococcus aureus. Sens Actuat B-Chem. 2022;351. doi: 10.1016/J.Snb.2021.130906
49. Zhao Y, Yu Q, Duan M, et al. Locking-fluorescence signals regulated CRISPR/Cas12a biosensor based on metal–organic framework for sensitive detection of salmonella typhimurium. J Agric Food Chem. 2024 Nov 20;72(46):25987–25996. doi: 10.1021/acs.jafc.4c07681
50. Li Y, Qiao J, Zhao Z, et al. A CRISPR/dCas9-enabled, on-site, visual, and bimodal biosensing strategy for ultrasensitive and self-validating detection of foodborne pathogenic bacteria. Food Front. 2023;4(4):2070–2080. doi: 10.1002/fft2.286
51. Li F, Ye Q, Chen M, et al. An ultrasensitive CRISPR/Cas12a based electrochemical biosensor for listeria monocytogenes detection. Biosens Bioelectron. 2021;179:113073. doi: 10.1016/j.bios.2021.113073
52. Lee S-Y, Kim U, Kim Y, et al. Enhanced detection of listeria monocytogenes using tetraethylenepentamine-functionalized magnetic nanoparticles and LAMP-CRISPR/Cas12a-based biosensor. Anal Chim Acta. 2023;1281:341905. doi: 10.1016/j.aca.2023.341905
53. Wu SY, Yuan J, Xu A, et al. A lab-on-a-tube biosensor combining recombinase-aided amplification and CRISPR-Cas12a with rotated magnetic extraction for detection. Micromachines-Basel. 2023;14(4). doi: 10.3390/mi14040830
54. Wang Y, Li J, Li S, et al. LAMP-CRISPR-Cas12-based diagnostic platform for detection of mycobacterium tuberculosis complex using real-time fluorescence or lateral flow test. Microchim Acta. 2021;188(10):347. doi: 10.1007/s00604-021-04985-w
55. Xia L, Yin J, Zhuang J, et al. Adsorption-free self-priming direct digital dual-crRNA CRISPR/Cas12a-assisted chip for ultrasensitive detection of pathogens. Anal Chem. 2023;95(10):4744–4752. doi: 10.1021/acs.analchem.2c05560
56. Kasputis T, He Y, Ci Q, et al. On-site fluorescent detection of sepsis-inducing bacteria using a graphene-oxide CRISPR-Cas12a (GO-CRISPR) system. Anal Chem. 2024;96(6):2676–2683. doi: 10.1021/acs.analchem.3c05459
57. Liu H, Wang JB, Zeng HJ, et al. RPA-Cas12a-fs: a frontline nucleic acid rapid detection system for food safety based on CRISPR-Cas12a combined with recombinase polymerase amplification. Food Chem. 2021;334. doi: 10.1016/j.foodchem.2020.127608
58. Zheng JL, Liu L, Li XM, et al. Rapid and simple detection of Burkholderia gladioli in food matrices using RPA-CRISPR/Cas12a method. Foods. 2023;12(9). doi: 10.3390/Foods12091760.
59. Xiang X, Xing G, Liu Y, et al. Immunomagnetic separation combined with RCA-CRISPR/Cas12a for the detection of salmonella typhimurium on a figure-actuated Microfluidic biosensor. J Agric Food Chem. 2023;71(36):13518–13526. doi: 10.1021/acs.jafc.3c03799
•• **Article ingeniously converts immune recognition signals into nucleic acid signals and triggers the combined use of isothermal amplification reactions with the CRISPR/Cas system, demonstrating significant novelty.**
60. Zhou J, Fau - Rossi J, Rossi J. Aptamers as targeted therapeutics: current potential and challenges. Nat Rev Drug Discov. 2017;16(3):181–202. doi: 10.1038/nrd.2016.199
61. Shen J, Zhou X, Shan Y, et al. Sensitive detection of a bacterial pathogen using allosteric probe-initiated catalysis and CRISPR-Cas13a amplification reaction. Nat Commun. 2020;11(1):267. doi: 10.1038/s41467-019-14135-9
62. Yu C, Liu Y, Zhang W, et al. A three-way junction probe triggered CRISPR/Cas14a1 enhanced EXPonential amplification reaction for sensitive Pseudomonas aeruginosa detection. Anal Methods. 2025;17(2):284–290. doi: 10.1039/d4ay01728e
63. Qiao Z, Xue L, Sun M, et al. Highly sensitive detection of Salmonella based on dual-functional hcr-mediated multivalent aptamer and amplification-free CRISPR/Cas12a system. Anal Chim Acta. 2023;1284:341998. doi: 10.1016/j.aca.2023.341998
• **This article presents a biosensor that utilizes aptamer recognition followed by isothermal amplification to activate CRISPR/Cas12a, serving as an exemplary model for such applications.**
64. Dai Y, Somoza RA, Wang L, et al. Exploring the trans-cleavage activity of CRISPR-Cas12a (cpf1) for the development of a universal electrochemical biosensor. Angew Chem Int Ed Engl. 2019 Nov 25;58(48):17399–17405. doi: 10.1002/anie.201910772
65. Bruch R, Baaske J, Chatelle C, et al. CRISPR/Cas13a-powered electrochemical microfluidic biosensor for nucleic acid amplification-free

miRNA diagnostics. Adv Mater. 2019;31(51):e1905311. doi: 10.1002/adma.201905311 PubMed PMID: 31663165; eng.
66. He Y, Jia F, Sun Y, et al. An electrochemical sensing method based on CRISPR/Cas12a system and hairpin DNA probe for rapid and sensitive detection of salmonella typhimurium. Sensors And Actuators B: Chem. 2022;369:132301. doi: 10.1016/j.snb.2022.132301
67. Huang LQ, Yuan N, Guo W, et al. An electrochemical biosensor for the highly sensitive detection of Staphylococcus aureus based on SRCA-CRISPR/Cas12a. Talanta. 2023;252. doi: 10.1016/j.talanta.2022.123821
68. Liu X, Bu S, Feng J, et al. Electrochemical biosensor for detecting pathogenic bacteria based on a hybridization chain reaction and CRISPR-Cas12a. Anal Bioanal Chem. 2022;414(2):1073–1080. doi: 10.1007/s00216-021-03733-6
69. Zheng S, Yang Q, Yang H, et al. An ultrasensitive and specific ratiometric electrochemical biosensor based on SRCA-CRISPR/Cas12a system for detection of salmonella in food. Food Control. 2023;146:109528. doi: 10.1016/j.foodcont.2022.109528
70. Gootenberg JS, Abudayyeh OO, Kellner MJ, et al. Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. Science. 2018 Feb 15;360(6386):439–444. doi: 10.1126/science.aaq0179
71. Lee S-Y, Oh S-W. Lateral flow biosensor based on LAMP-CRISPR/Cas12a for sensitive and visualized detection of salmonella spp. Food Control. 2023;145:109494. doi: 10.1016/j.foodcont.2022.109494
72. Zhu Y, Lin C, Xu H, et al. Establishment and methodological evaluation of a method for rapid detection of Helicobacter pylori and virulence genes based on CRISPR-Cas12a. Infect Drug Resist. 2023;16:435–443. doi: 10.2147/IDR.S398098
73. Qiu XT, Xu S, Liu XP, et al. CRISPR/Cas12a-based diagnostic platform accurately detects Nocardia farcinica targeting a novel species-specific gene. Front Cell Infect Mi. 2022:12. doi: 10.3389/Fcimb.2022.884411
74. Chen X, Wang L, He F, et al. Label-free colorimetric method for detection of Vibrio parahaemolyticus by trimming the G-Quadruplex DNAzyme with CRISPR/Cas12a. Anal Chem. 2021;93(42):14300–14306. doi: 10.1021/acs.analchem.1c03468
75. Wei L, Wang Z, Wang J, et al. Aptamer-based colorimetric detection of methicillin-resistant staphylococcus aureus by using a CRISPR/Cas12a system and recombinase polymerase amplification. Anal Chim Acta. 2022;1230:340357. doi: 10.1016/j.aca.2022.340357
76. Shang Y, Xing G, Lin J, et al. Multiplex bacteria detection using one-pot CRISPR/Cas13a-based droplet microfluidics. Biosens Bioelectron. 2024;243:115771. doi: 10.1016/j.bios.2023.115771
77. Hou S, Huang C, Zhang D, et al. Broad-spectrum portable magnetic relaxation switching immunosensor with gold-functionalized magnetic nanoprobes for the sensitive detection of multiple pyrethroids. J Hazard Mater. 2023;451:131141. doi: 10.1016/j.jhazmat.2023.131141
78. Wang X, Pei K, Sun H, et al. A magnetic relaxation switch sensor for determination of 17β-estradiol in milk and eggs based on aptamer-functionalized Fe3O4@Au nanoparticles. J Sci Food Agric. 2021;101(13):5697–5706. doi: 10.1002/jsfa.11224
79. Shen YF, Jia F, He YW, et al. A CRISPR-Cas12a-powered magnetic relaxation switching biosensor for the sensitive detection of Salmonella. Biosens Bioelectron. 2022;213. doi: 10.1016/J.Bios.2022.114437
80. Wen J, Ren L, He Q, et al. Contamination-free V-shaped ultrafast reaction cascade transferase signal amplification driven CRISPR/Cas12a magnetic relaxation switching biosensor for bacteria detection. Biosens Bioelectron. 2023;219:114790. doi: 10.1016/j.bios.2022.114790
81. Langer J, Jimenez de Aberasturi D, Aizpurua J, et al. Present and future of surface-enhanced raman scattering. ACS Nano. 2020;14(1):28–117. doi: 10.1021/acsnano.9b04224
82. Zhuang J, Zhao Z, Lian K, et al. Sers-based CRISPR/Cas assay on microfluidic paper analytical devices for supersensitive detection of pathogenic bacteria in foods. Biosens Bioelectron. 2022;207:114167. doi: 10.1016/j.bios.2022.114167
83. Wang J, Jiang H, Chen Y, et al. CRISPR/Cas9-mediated SERS/colorimetric dual-mode lateral flow platform combined with smartphone for rapid and sensitive detection of staphylococcus aureus. Biosens Bioelectron. 2024;249:116046. doi: 10.1016/j.bios.2024.116046
84. Maar RR, Zhang R, Stephens DG, et al. Near-infrared photoluminescence and Electrochemiluminescence from a remarkably simple boron difluoride Formazanate dye. Angew Chem Int Ed. 2019;58(4):1052–1056. doi: 10.1002/anie.201811144
85. Wang C, Zhang Y, Liu S, et al. Allosteric probe-triggered isothermal amplification to activate CRISPR/Cas12a for sensitive electrochemiluminescence detection of Salmonella. Food Chem. 2023;425:136382. doi: 10.1016/j.foodchem.2023.136382
86. Gu X, Tang Q, Kang X, et al. A portable CRISPR-Cas12a triggered photothermal biosensor for sensitive and visual detection of Staphylococcus aureus and listeria monocytogenes. Talanta. 2024;271:125678. doi: 10.1016/j.talanta.2024.125678
87. Li C, Zhan W, Yang Z, et al. Broad neutralization of SARS-CoV-2 variants by an inhalable bispecific single-domain antibody. Cell. 2022;185(8):1389–1401.e18. doi: 10.1016/j.cell.2022.03.009
88. Qiao S-P, Liu Z-N, Li H-C, et al. Construction of a CRISPR-Biolayer interferometry platform for real-time, sensitive, and specific DNA detection. Chembiochem. 2021;22(11):1974–1984. doi: 10.1002/cbic.202100054
89. Zhang J, Shen Z, Xiang Y, et al. Integration of solution-based assays onto lateral flow device for one-step quantitative point-of-care diagnostics using personal glucose meter. ACS Sens. 2016;1(9):1091–1096. doi: 10.1021/acssensors.6b00270
90. Xiang Y, Lu Y. Using commercially available personal glucose meters for portable quantification of DNA. Anal Chem. 2012;84(4):1975–1980. doi: 10.1021/ac203014s
91. Wu T, Yang Y, Cao Y, et al. Bioinspired DNA–inorganic hybrid nanoflowers combined with a personal glucose meter for onsite detection of miRNA. ACS Appl Mater & Interface. 2018 Dec 12;10(49):42050–42057. doi: 10.1021/acsami.8b15917
92. Zhou C, Huang D, Wang Z, et al. CRISPR Cas12a-based "sweet" biosensor coupled with personal glucose meter readout for the point-of-care testing of Salmonella. J Food Sci. 2022 Sep;87(9):4137–4147. doi: 10.1111/1750-3841.16287 PubMed PMID: 35986652.
93. Xu J, Ma J, Li Y, et al. A general RPA-CRISPR/Cas12a sensing platform for Brucella spp. detection in blood and milk samples. Sensors And Actuators B: Chem. 2022 Aug 1;364:131864. doi: 10.1016/j.snb.2022.131864