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Construction of Electrochemical Biosensors Based on the CRISPR/Cas12 System for Applications

Hongqu Liang[1], Liu Huang[1], Peng Yan[1], Fa Huang[2], Hongchao Zhang[2], Qisheng Luo[1*]

[1]Department of Neurosureery, Affiliated Hospital of Youjiang Medical University for Nationalities, Baise, China
[2]Graduate School, Youjiang Medical University for Nationalities, Baise, China
Email: *Luoqish@126.com

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Abstract

The current major issue in improving detection sensitivity and selectivity is to design an electrochemical sensor that does not require PCR amplification for nucleic acid identification and measurement. Because of their great sensitivity, precision, and simplicity of downsizing, electrochemical biosensors have emerged as a research hotspot in the field of nucleic acid detection. The CRISPR/Cas12 system has emerged as a potent tool for nucleic acid detection due to its powerful cleavage activity and selectivity. Specific electrode changes combined with the CRISPR/Cas12 system can greatly improve the performance of electrochemical biosensors. In this study, the design concepts of electrochemical biosensors based on the CRISPR/Cas12 system and their application advancements in nucleic acid detection are discussed.

Keywords

CRISPR, Cas12, Electrochemical Biosensor

1. Introduction

Nucleotide-polymers, which are composed of bases, sugars, and phosphate residues, constitute what are known as nucleic acids. Their primary job is to convey, preserve, and express an organism's genetic information. Nucleic acids are classified into two different categories: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) based on the types of bases and different kinds of sugars that they contain [1]. In addition to proteins and other tiny molecules, targets in organisms are also capable of being detected with nucleic acids as analytes [2] [3]. With the possible exception of RNA, nucleic acids are more thermally stable than other

protein targets. As a result, the use of nucleic acid-based assays is favorable since the analytes in the sample can survive even after heat exposure or various other procedures [4]. Furthermore, there is little chance of contamination with nucleic acid-based detection methods due to their great sensitivity and specificity [5] [6]. Scientists have created and employed a variety of techniques to detect nucleic acids, some of which include biosensors, lateral flow analysis (LFA), polymerase chain reaction (PCR), and clusters of regularly interspaced short palindromic repeats (CRISPR)-related systems (CRISPR/Cas) [7] [8]. These methods have been widely used in various fields.

In-depth discussions of the CRISPR method's benefits over PCR, LFA, and other tests have been written about in the literature [9] [10]. First off, the CRISPR technique has excellent specificity and sensitivity. Even at low concentrations, it has a high degree of precision for detecting target sequences. For example, Lee *et al*. [9] demonstrated that the CRISPR method combined with PCR could enable the detection of a wide range of oncogenes with high sensitivity (<0.01%) and accuracy, surpassing conventional targeted deep sequencing methods. Similarly, Liu *et al*. [10] developed a highly sensitive and rapid SARS-CoV-2 detection method using a portable CRISPR/Cas13a-based lateral flow assay, achieving a lower limit of detection (LOD) than q-PCR. These studies highlight the higher sensitivity and specificity of the CRISPR method compared to conventional detection methods.

The CRISPR method also provides a rapid detection method. It allows the detection of target sequences in a much shorter time compared to other methods. For example, Qiu *et al*. [11] developed a CRISPR-based nucleic acid detection platform that combined PCR amplification with CRISPR/Cas12a-based detection to achieve rapid detection of Nocardia nosocomial. Similarly, Zhang *et al*. [12] developed a CRISPR/Cas system-based assay for detecting Mycobacterium tuberculosis, which required an average of 90 minutes to report the results compared to the gold-standard culture method that required one month. These studies demonstrated the time-saving advantages of the CRISPR method for nucleic acid detection. Another advantage of the CRISPR method is its versatility. It can be applied to detect a wide range of targets, including viruses, bacteria, and genetic mutations. For example, Wang *et al*. [13] developed an ultrasensitive PCR-based CRISPR/Cas13a method for the detection of Helicobacter pylori, demonstrating the promising wide range of applications for the detection of slow-growing pathogens and highlighting the versatility of the CRISPR method. This versatility allows the CRISPR method to be adapted to various diagnostic needs.

As nucleic acid detection technology advanced, biosensing technology also advanced quickly. Biosensors are widely employed in many different industries and have the ability to detect substrates with great sensitivity and selectivity [14]. Furthermore, biosensors can be used in conjunction with other detecting techniques to see what kind of response they elicit. Recognizing detection substrates and translating the data from these substrates into electrical signals for readout are capabilities of electrochemical biosensors [15]. Electrochemical biosensors are

widely used in different fields due to their high sensitivity and easy miniaturization.

Electrochemical biosensors have undergone development and enhancement in recent times to enhance their performance, including limit of detection (LOD). One way to achieve selectivity and specificity is to integrate the technique with the CRISPR/Cas12 system. CRISPR/Cas12-based electrochemical biosensors for nucleic acid detection were first developed by Dai *et al*. [16] using the cis-action of Cas12 for the detection of human papillomavirus-16 (HPV-16) and fine virus B19 (PB-19), as well as the use of square wave voltammetry (SWV) measurements using methylene blue (MB) as an indicator of signaling. The findings demonstrated that both viruses may be detected with good sensitivity and selectivity using the approach. Consequently, the electrochemical biosensor approach based on CRISPR/Cas12 has the potential to offer an accurate nucleic acid detection method for identifying target nucleic acids.

This review discusses the application of CRISPR/Cas12-based electrochemical biosensors and electrode modifications that can improve the working mechanism of biosensors for the detection of target nucleic acids with LOD and selectivity. The discussion includes an examination of the various strategies employed to improve the selectivity and sensitivity of these biosensors, such as the use of nanomaterials, signal amplification strategies, and the optimization of the CRISPR/Cas12 system itself. We also consider the challenges that remain, such as the need for further miniaturization, integration into portable devices, and the development of user-friendly interfaces for non-expert users. Ultimately, the paper aims to provide a comprehensive overview of the current state of electrochemical biosensors based on the CRISPR/Cas12 system and to chart a course for future research and development in this exciting and rapidly evolving field. By doing so, it is hoped that these advancements will contribute to the broader goal of improving healthcare diagnostics and making molecular testing more accessible and affordable worldwide.

2. Biological Sensors

A biosensor is a device that converts biological information from analytes into analytical signals by using biochemical interactions to identify the analytes. The core components of the apparatus consist of a transducer, which acts as a carrier of signals and translates the signals the bioreceptor generates into signals readable by a detector, and a bioreceptor, also referred to as a biorecognition element, which performs the role of an analyte recognizer [17]. Biosensors employ a variety of signal transduction technologies, including thermal, optical, piezoelectric, magnetic, electrochemical, and microgravity techniques [18] [19].

2.1. Electrochemical Biosensors

Of the signal transduction techniques discussed, electrochemical-based biosensors have been extensively developed for nucleic acid detection because of their

high sensitivity, straightforward analytical process, quick response times, affordability, ease of downsizing, and mobility [20] [21]. The mechanism of these biosensors involves electrical signals in which biological information is converted into electrical signals such as current, voltage, conductance, and impedance (e.g., **Figure 1**) [22]. The electrochemical examination of biosensors involves the use of measurement techniques such as aerometric, voltammetric, conductometric, and impedance approaches [23].

In terms of nucleic acid detection, electrochemical biosensors have been used to detect viruses such as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), Zika virus (ZIKV), and human papillomavirus (HPV) [24]-[26]. Other studies of electrochemical biosensors have been reported, including the detection of genetic mutations that lead to breast cancer [27].

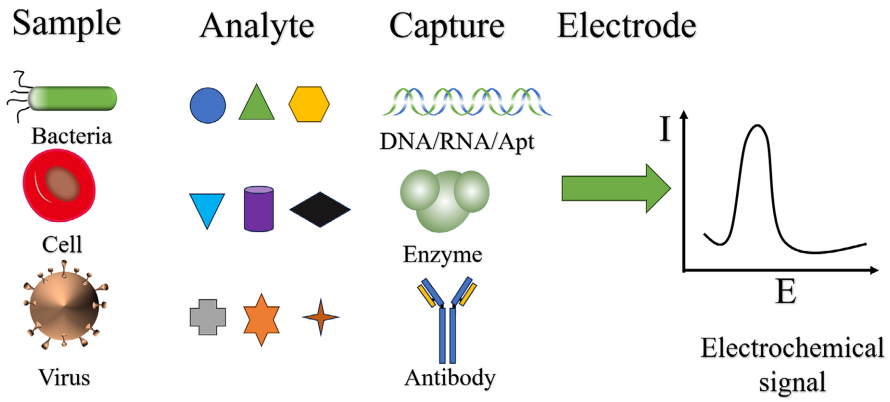


Figure 1. The principle of electrochemical biosensor.

2.2. Electrode Modification

Electrochemical analysis hinges on the interplay of three fundamental components: the working electrode (WE), the reference electrode (RE), and the auxiliary or counter electrode (CE). The WE is the epicenter of the analytical process, where the analyte undergoes a reduction-oxidation (redox) reaction. This electrode is crucial for the detection and quantification of target molecules, as it facilitates the electron transfer that is the hallmark of electrochemical sensing.

The RE, characterized by its stable and known half-cell potential, serves as a benchmark for measuring the potential of the WE. It provides a reliable reference point, ensuring that the measurements taken are accurate and reproducible. The stability of the RE is paramount, as any fluctuations in its potential could lead to erroneous readings. The CE, also known as the auxiliary electrode, plays a supportive role in the electrochemical cell. It helps to maintain the balance of the diffusion current at the WE by providing a pathway for the flow of electrons. This ensures that the WE remains in a steady state, which is essential for consistent and reliable measurements.

In the context of electrochemical biosensors, these electrodes are not just important; they are the linchpin of the system. They control the flow of electrons and

the interaction with biological agents, such as enzymes, antibodies, or nucleic acids. The design and optimization of these electrodes directly impact the sensitivity, selectivity, and response time of the biosensor. For instance, the modification of the WE with specific biorecognition elements, such as immobilized enzymes or DNA probes, can greatly enhance the selectivity of the sensor toward the target analyte. Similarly, the choice of materials and surface treatments for the RE and CE can influence the overall performance of the electrochemical cell [15].

One of the factors affecting the rate of electron flow at the WE is the component of the material. Each material produces a different sensitivity to the electrode and duration of analytical detection. One of the most commonly used materials as WE is carbon [28]. Carbon is used as a WE because of its good electronic conductivity, high chemical and thermal stability, and ease of modification [29]. Gold is also widely used as a WE, especially for the detection of nucleic acids, because of its high electrical conductivity, stability, and ease of immobilization with bio-organic materials [28] [30]. For the detection of nucleic acids, WE are usually modified to improve the performance of biosensors, for example, the electrodes are modified using gold nanoparticles (AuNPs). AuNPs have a large specific surface area, which increases the electron flow and can be used as a mediator to immobilize mercapturic sequences onto the electrodes through gold-sulfur bonding (Au-S), which leads to a better conductivity of the electrodes and biosensors. AuNPs can also be used to enhance the conductivity of the electrodes and biosensors, sensitivity, and selectivity [31]. Various types of molecular organic skeletons (MOFs) are also used as enhancers of signal response in sensors. MOFs are porous structures consisting of metal ions linked by organic ligands. This structure provides them with a well-defined framework and a sizable surface area, making them suitable for a wide range of applications. MOFs are highly tunable, allowing pore size and chemical functionality to be adjusted to meet specific biosensor requirements. In addition, their noteworthy attribute is their specific selectivity, which is an important aspect of biosensor development, including the use of CRISPR/Cas systems [32] [33].

2.3. Biomolecular Immobilization Technology

In biosensors, especially in electrochemical applications, immobilization techniques are key to binding biomolecules to the electrode surface. Immobilization ensures that the biomolecule interacts with the target analyte and facilitates the detection and quantification of specific molecules. Immobilization methods involve physical and chemical adsorption on the surface of the material, especially for applications that require its biological activity. The surface may contain reactive groups such as -OH, -COOH, or NH_2, as well as other reactive groups such as azides, alkynes, and -SH, all of which can be used to covalently bind biomolecules. Several immobilization techniques are used for biosensors, including physical adsorption (non-covalent), van der Waals force interactions, electrostatic interactions, hydrogen bonding, and hydrophobic bonding interactions. Upon adsorption,

the electrochemical biosensor-specific target (the object to be detected) is recognized by a biological component (bioreceptor) and detected by an electrochemical transducer that generates a measurable signal. The molecules can be cross-linked with each other. Covalent bonds include amine, sulfhydryl, and carboxyl bonds. Covalent bonds provide greater stability than physical adsorption. However, some physical adsorption processes may involve pairs of molecules with very strong affinity interactions approaching the strength of covalent bonding forces, such as biotin binding to streptavidin or antigen-antibody binding [34].

Several biomolecular immobilization techniques have also been applied to CRISPR biosensors [35]-[37], and in Cas9, biotinylating of polyethylene glycol (PEG) and streptavidin as well as the formation of amine bonds with the reactive ester, N-hydroxy succinimide (NHS), have been successfully developed on quartz slides [38]. The amine-based formation method of the CRISPR-Cas system has been used in several studies due to its significant advantage in selectively directing the formation of specific complexes with guide RNAs [38]. As an example of the inactivated Cas9 (dCas9) system, screen-printed electrode biosensors are based on covalently immobilized unlabeled graphene oxide. The carboxyl groups on graphene oxide were activated using a mixture of N-(3-dimethylaminopropyl) ethylenediamine carbodiimide hydrochloride (EDC) and N-hydroxy succinimide (NHS). The electrochemical response was successfully determined with high selectivity and accuracy after the addition of target DNA to real blood samples [39].

The type of biomolecule, the sensor's intended use, and the required stability and sensitivity all influence the immobilization approach selection. Every strategy has benefits and drawbacks, so researchers select the one that best suits their requirements. To obtain the optimal biosensor performance, it is frequently necessary to fully optimize the immobilization procedure, taking into account the impact of the DNA reporter probe's density on the electrode surface. In order to influence the incorporation of immobilization techniques and promote cleavage activity, ideal circumstances for the hydroxyl groups and phosphate backbone are essential. Significantly less signal change can be observed at high densities, and the density of ss-DNA implies that Cas is less accessible to ss-DNA as a result of spatial site blockage, which restricts trans-cleavage activity. Because of Cas's significant hindrance to its diffusion in the test solution, the concentration of Cas also influences nucleic acid endonuclease activity, which falls with increasing Cas concentration. Another important component influencing ss-DNA activity is its density. Longer ss-DNA results in variable cleavage efficiency because the carbon chain length affects the electrostatic interaction between the phosphate backbones of the ss-DNA probes immobilization processes. The length of ss-DNA also has an influence [16].

3. CRISPR/Cas System-Based Biosensors

CRISPR is homologous repetitive DNA sequences, also known as clustered spaced short palindromic repeats (exogenous DNA sequences: e.g. viruses, plasmids,

etc.). The palindromic sequences were first discovered in E. coli by Japanese scholars in 1987 [40], and together with Cas proteins, bacteria, and archaea, they form the CRISPR/Cas system, which performs natural immune functions. Researchers have suggested modifying the CRISPR/Cas system outside of bacterial cells or archaea for use in particular gene editing sectors because of its capacity to identify and cut exogenous DNA strands. This is done by carefully choosing and chopping the DNA strands that the system targets.

3.1. CRISPR/Cas System

According to its structural composition, CRISPR system can be According to its structural composition, CRISPR system can be categorized into six types. According to their structural composition, they can be categorized into six types: Class I (types I, III, IV) consists of multiple Cas proteins and crRNAs forming a complex that performs cleavage synergistically. A complex consisting of multiple Cas proteins and crRNA performs cleavage synergistically; Class II (types II, V, VI) consists of a single Cas protein Class II (types II, V, VI) consists of a single Cas protein that can perform cleavage alone [41]. In contrast to type I, type II Cas proteins require only one protein for cleavage and ancillary cleavage activities and play important roles in DNA or RNA editing, tracking, knockdown, and nucleic acid detection in the following areas [42].

The main ones widely used mainly in full gene editing systems are CRISPR/Cas9, CRISPR/Cas12, and CRISPR/Cas13. This paper focuses on an overview of electrochemical biosensors for CRISPR/Cas12.

3.2. CRISPR/Cas12

The CRISPR/Cas12 system is known to have a mechanism for cleaving non-target single-stranded DNA (ssDNA), which is known as trans cleavage. Trans-cleavage occurs after cis-cleavage has taken place (e.g., **Figure 2**) [43]. The ability of the CRISPR/Cas12 complex to perform cis-cleavage has been used by researchers to detect organismal nucleic acids as an indication of the presence of the target DNA, such as the study conducted by He *et al*. in 2020. In this study, a biosensor based on the CRISPR/Cas system was developed that can be used to detect African swine fever virus (ASFV) by attaching fluorescent and bursting groups to ssDNA as an indicator of the presence of the target DNA. ssDNA cleavage results in the generation of a fluorescent signal that can be read to determine the presence of the target DNA [44].

Compared to CRISPR/Cas9 and CRISPR/Cas13, the CRISPR/Cas12 system is more advantageous in detecting the presence of target nucleic acids because it possesses two cleavage activities (*i.e.*, trans and cis activities), either of which can be used as an indicator. At the same time, since the CRISPR/Cas9 system does not have a trans-cutting activity, it is not easy to combine the CRISPR/Cas9 system with a variety of other assays compared to CRISPR/Cas12, and therefore, there is less advantage in using the CRISPR/Cas9 for the assay [45] [46]. On the other

hand, CRISPR/Cas13 targets cutting RNA, which is more unstable than DNA. CRISPR/Cas12 overcomes this problem by utilizing reverse transcriptase to convert RNA into complementary DNA [47].

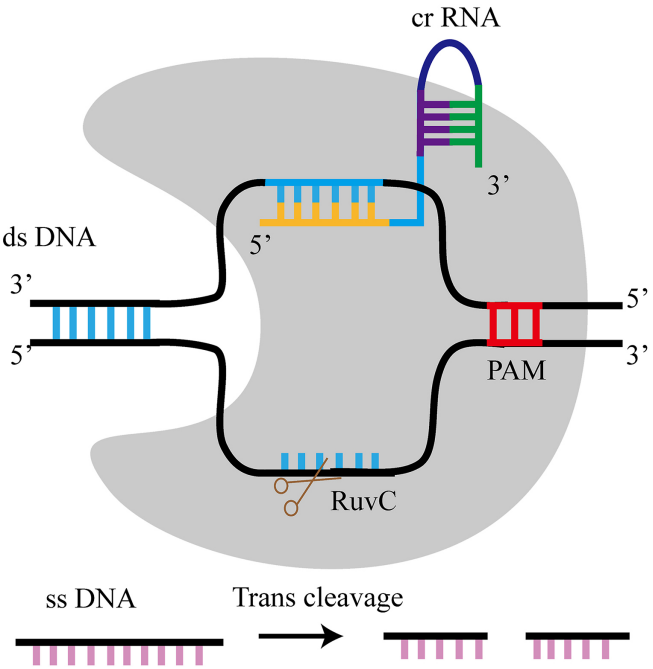


Figure 2. CRISPR/Cas12 recognizes the PAM of targeted dsDNA and specifically cuts the dsDNA, then cuts the non-targeted ssDNA (trans-cleavage).

3.3. CRISPR/Cas12-Based Electrochemical Biosensors

Research has indicated that the integration of the CRISPR/Cas12 system with electrochemical biosensors significantly enhances the selectivity and specificity in detecting target analytes. The selectivity and specificity in detecting the analytes of interest are achieved through the design of specific or requisite crRNAs, which enable the targeted and distinct identification of the analytes in question. One of the potential issues in applying the CRISPR/Cas12 system to electrochemical biosensors is the instability of the crRNA within the system. The fusion of the CRISPR/Cas12 system with electrochemical biosensing technology represents a significant advancement in the field of analytical chemistry. This innovative approach leverages the highly specific nuclease activity of the Cas12 protein, guided by the complementary crRNA, to cleave the target ssDNA sequences (e.g., **Figure 3**). The high-fidelity recognition conferred by the crRNA-Cas12 complex is a critical factor that underpins the superior selectivity and specificity of this biosensing platform.

Furthermore, the integration of CRISPR/Cas12 with electrochemical biosensors also opens up new avenues for multiplexed detection, where multiple target analytes can be identified simultaneously. This capability is particularly valuable in complex biological samples, such as clinical specimens, where the simultaneous

detection of various biomarkers can provide a more comprehensive assessment of disease states or infection profiles.

In conclusion, while the CRISPR/Cas12 system offers unparalleled opportunities for the development of highly specific and sensitive electrochemical biosensors, it is essential to continue refining the system to ensure the stability and robustness of the crRNA component. Advances in this area will not only bolster the analytical performance of these biosensors but also pave the way for their practical application in diverse fields, including clinical diagnostics, environmental monitoring, and food safety assessment [45].

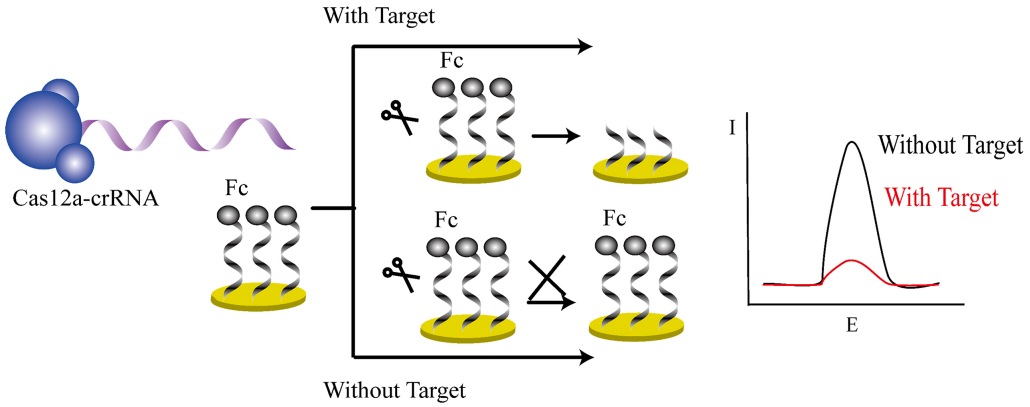


Figure 3. Schematic diagram of Fc-ssDNA cleavage by CRISPR/Cas12a.

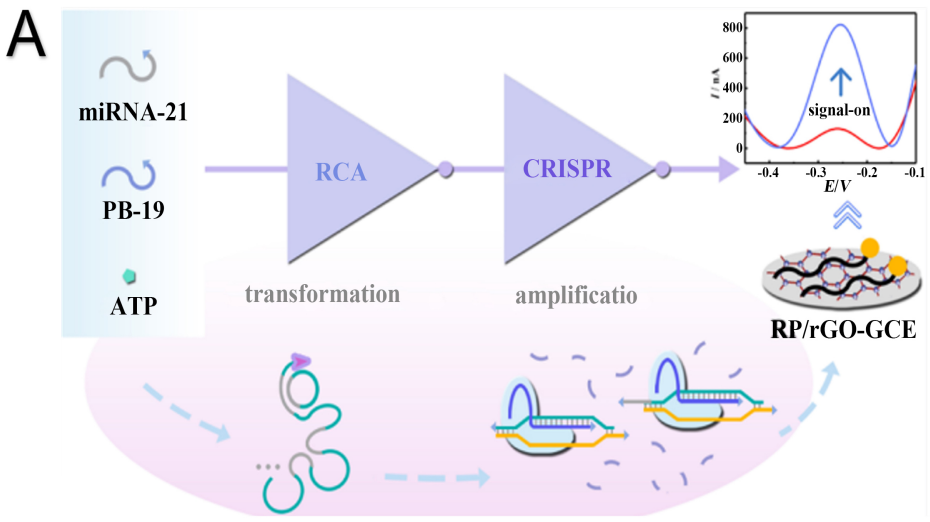
To overcome the instability of RNA in CRISPR/Cas biosensors, several strategies have been proposed in the literature. These strategies aim to enhance the stability and efficiency of RNA molecules in the design and function of CRISPR-based biosensors. One approach is to optimize the design of crRNA used in CRISPR/Cas systems. Patsali *et al.* [48] discussed the need to improve the efficiency and safety of the CRISPR/Cas system, including addressing concerns related to gRNA design, such as off-target activity and recombination. These studies suggest that optimizing the design and maturation of gRNAs can enhance the stability and functionality of the CRISPR/Cas system. In addition, the stability and signal amplification of RNA molecules in CRISPR-based biosensors can be enhanced by utilizing amplification techniques such as strand displacement amplification (SDA). Zhang *et al.* [49] described the combination of secondary strand displacement amplification with CRISPR/Cas12a effectors that were used for rapid and sensitive measurement of 8-oxo guanine DNA glycosylase. This method combines the advantages of the SDA and CRISPR/Cas systems to achieve a rapid, isothermal assay.

3.4. Application of CRISPR/Cas12-Based Electrochemical Biosensors

The advantages of CRISPR/Cas12-based electrochemical biosensors in improving selectivity and specificity have attracted the attention of many researchers from

all over the world, thus driving scientists to further investigate biosensing technologies. Qin *et al*. used reduced graphene oxide (rGO)-modified glassy carbon electrodes to detect miRNA-21 and PB -19. Using a combination of blocker probes and reporter probes (labeled with MB), this biosensor achieved a detection limit of 0.83 aM for miRNA-21 (e.g., **Figure 4(A)**) [37]. To detect the E gene of SARS-CoV-2, another study utilizing carbon electrodes was conducted by Wu *et al*. This study used a screen-printed carbon electrode (SPCE) modified with cerium oxide (CeO_2) nanorods, polyallylamine hydrochloride (PAH), and ferrocene (Fc)-labeled ssDNA to improve the sensitivity of the biosensor. The detection limit of this biosensor was 5.0×10^{-11} ng/μL (e.g., **Figure 4(B)**) [50].

In the research of CRISPR/Cas12-based electrochemical biosensors, the research based on gold electrodes is also one of the important directions. An example of a study using gold electrodes is the study presented by Liu *et al*. for the detection of SARS-CoV-2 antigen. This study used a gold electrode modified with DNA probes. The biosensor was evaluated based on the EIS technique and its detection limit was 0.077 ng/mL (e.g., **Figure 4(C)**) [51]. Another example is the detection of Cardiac Troponin I (cTnI) associated with immediate diagnosis of myocardial injury and cardiovascular disease proposed by Chen *et al*. The aptamer hybridizes to its partially complementary DNA (probe 2, P2) and is then modified on magnetic nanoparticles. When cTnI is present, cTnI binds to the aptamer and releases P2. The released P2 hybridizes with crRNA and triggers the trans-cutting activity of CRISPR/Cas12a, as shown. The detection limit was as low as 10 pg/mL with a linear range of 100 pg/mL to 50,000 pg/mL and was successfully applied to human serum samples [52]. Dai *et al*. also presented a party for the detection of human papillomavirus 16 (HPV-16) and fine virus B19 (PB-19) (e.g., **Figure 4(D)**) [16]. Assessment of biosensors utilizing SWV technology and having a 50 pM detection limit. Furthermore, an E-CRISPR array mediated aptamer was created having a detection limit of 0.2 nM for the protein known as transforming growth factor β1 (TGF-β1).



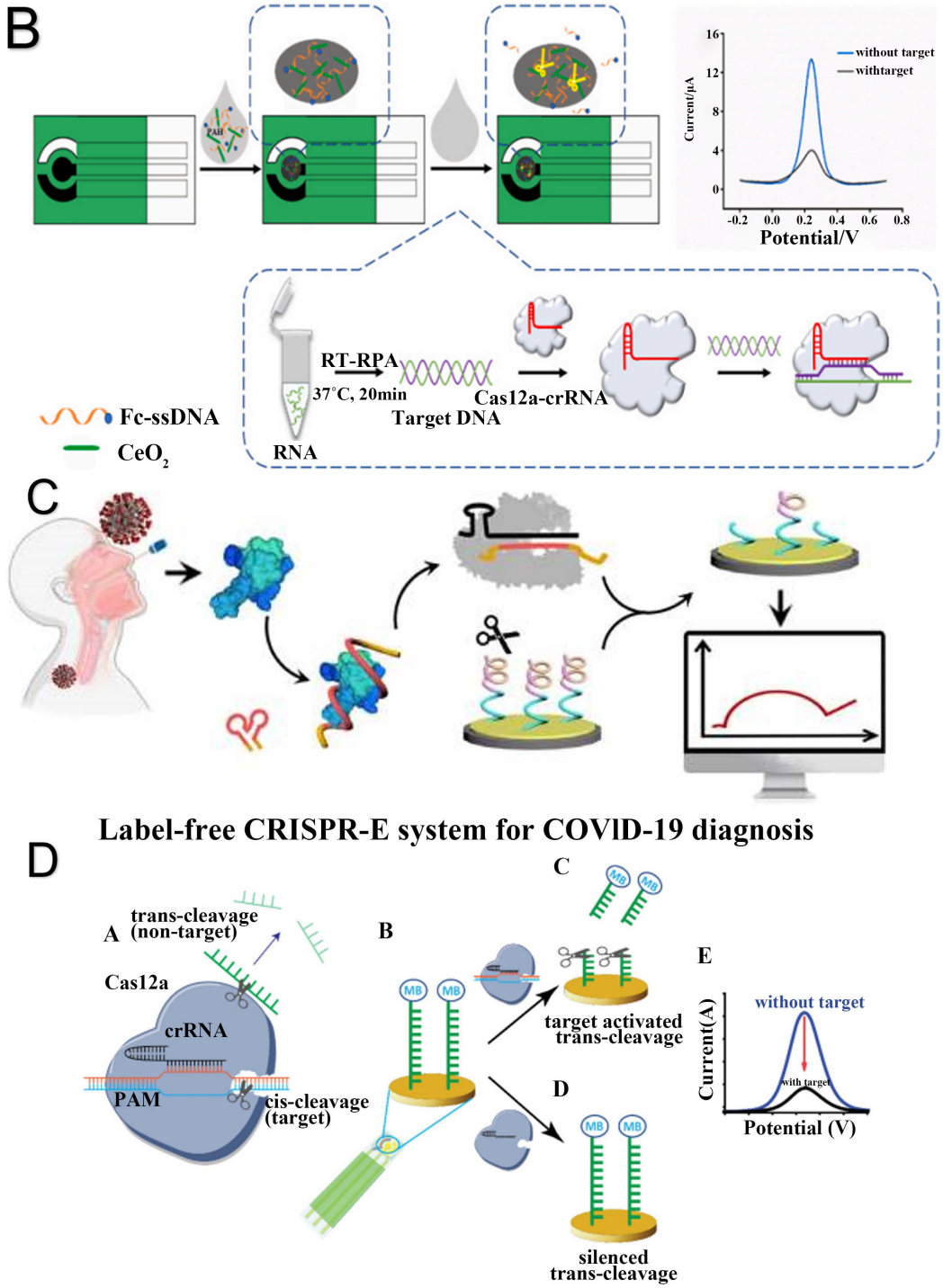
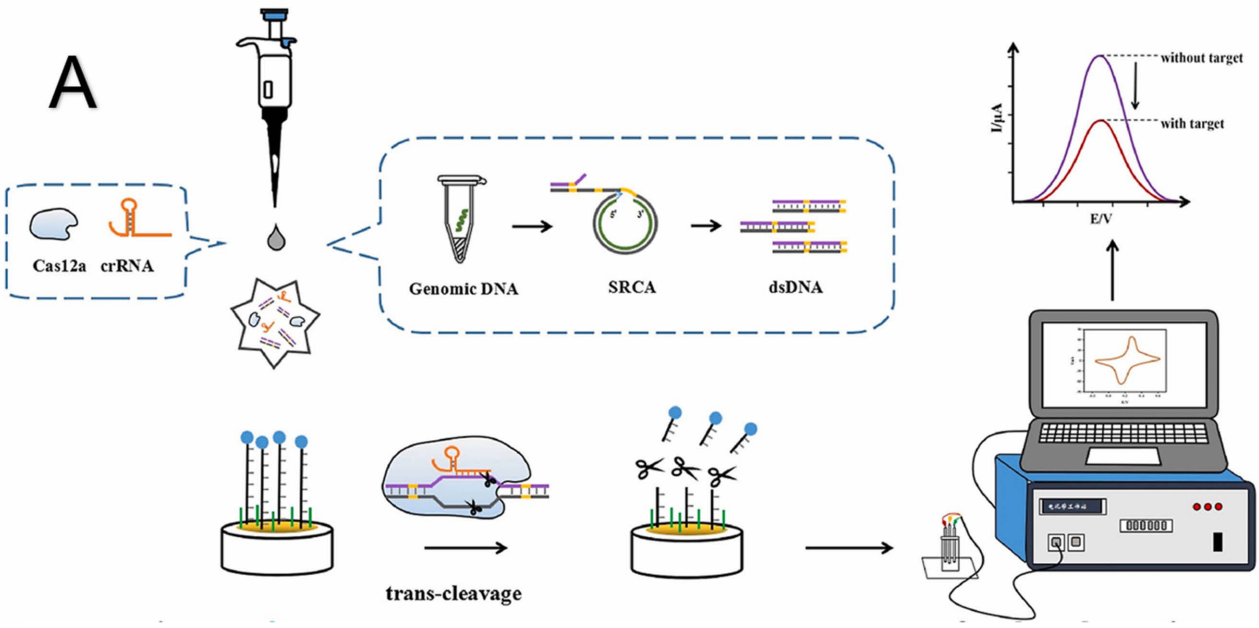


Figure 4. Detection principle of each electrochemical sensor (A) Universal and Programmable Rolling Circle Amplification-CRISPR/Cas12a-Mediated Immobilization-Free Electrochemical Biosensor [37]; (B) Ultrasensitive SARS-CoV-2 diagnosis by CRISPR-based screen-printed carbon electrode [50]; (C) CRISPR-Cas12a-mediated label-free electrochemical aptamer-based sensor for SARS-CoV-2 antigen detection [51]; (D) Exploring the Trans-Cleavage Activity of CRISPR-Cas12a (cpf1) for the Development of a Universal Electrochemical Biosensor [16].

The advantages of CRISPR/Cas12a-based electrochemical biosensors in improving

selectivity and specificity and their applications in disease markers, environmental health, and food safety have attracted the attention of many researchers around the world, especially for the detection of foodborne pathogens. For example, Huang *et al*. developed a novel electrochemical biosensor based on jumping rolled-ring amplification (SRCA) coupled with a CRISPR/Cas12a system for accurate detection of *S. aureus* (e.g., **Figure 5(A)**) [53]. The strategy uses methylene blue as the electrochemical signaling molecule and -SH modified signaling reporter probe (SH-ssDNA-MB) immobilized on the surface of a glassy carbon electrode modified with gold nanoparticles via Au-S bond. When the solution to be tested contains Staphylococcus aureus, the double-stranded DNA obtained by SRCA can be specifically recognized by the Cas12a/crRNA complex and in this way activates the Cas12a trans-cutting activity, which specifically recognizes and cleaves SH-ssDNA-MB, resulting in the MB moving away from the electrode surface and the decrease of the electrochemical signals. Under optimal conditions, the detection limit for S. aureus was 3 CFU/mL, respectively. Li *et al*. introduced the trans-cutting activity of CRISPR/Cas12a into an electrochemical biosensor (E-CRISPR) and combined it with recombinant enzyme-assisted amplification (RAA) to establish a cost-effective, specific, and ultrasensitive method (e.g., **Figure 5(B)**) [54]. Extracted *Listeria monocytogenes* DNA was subjected to RAA amplification to generate a large amount of ds DNA, which was subsequently bound to CRISPR/Cas12a-crRNA to activate the trans-cutting activity of CRISPR/Cas12a. When *Listeria monocytogenes* was present in the solution to be tested, the trans-cutting activity of CRISPR/Cas12a was activated to cut ss DNA on the electrode surface, and the MB modified on the tip was far away from the electrode surface, and the electrochemical signals were significantly altered, thus realizing the detection of *Listeria monocytogenes*, and the detection limit of this biosensor was 26 CFU/mL.



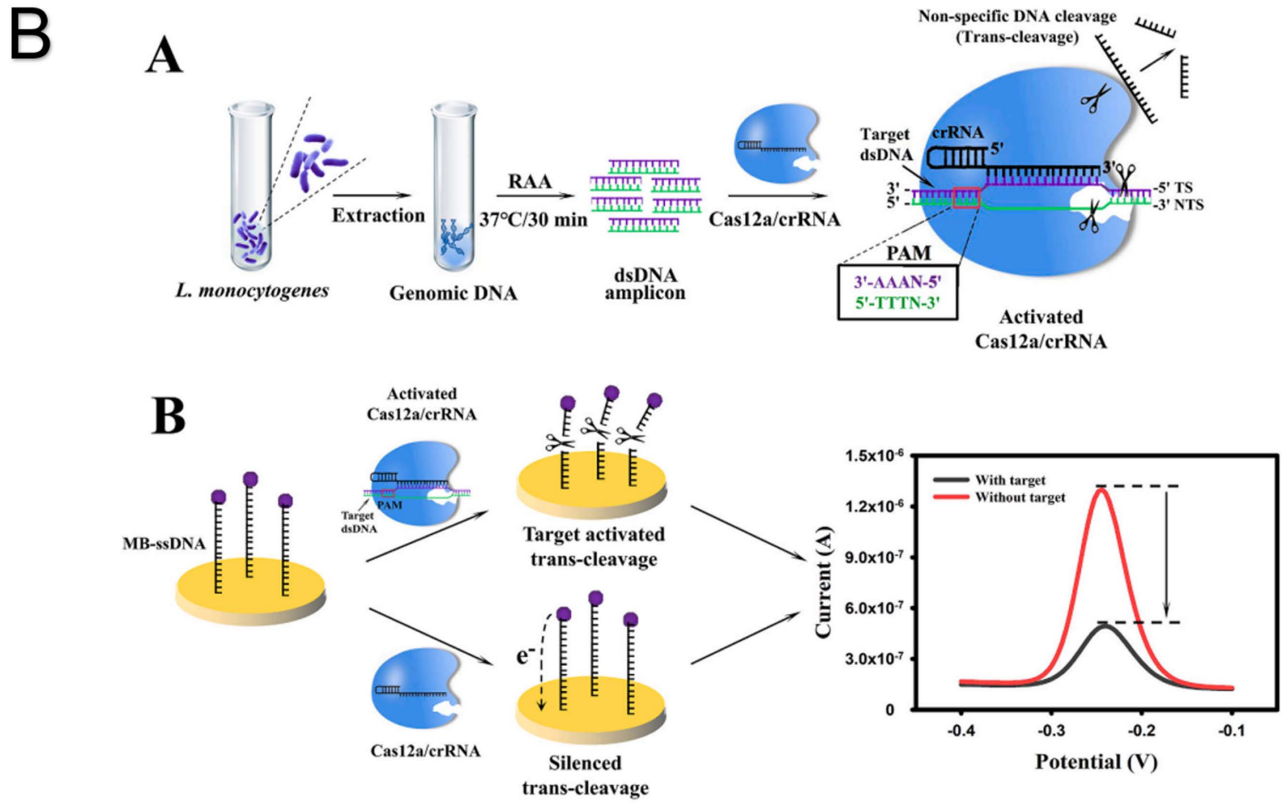


Figure 5. Principles of foodborne pathogen detection by electrochemical sensors (A) An electrochemical biosensor for the highly sensitive detection of Staphylococcus aureus based on SRCA-CRISPR/Cas12a [53]; (B) An ultrasensitive CRISPR/Cas12a based electrochemical biosensor for Listeria monocytogenes detection [54].

In addition to this, there are other CRISPR/Cas12-based sensing strategies for the detection of foodborne pathogens (e.g. **Table 1**).

Table 1. CRISPR/Cas12-based sensing strategies for the detection of foodborne pathogens.

Detection Method	Target	CRISPR/Cas	LOD	Reference
Electrochemical biosensor	*E. coli*	Cas12a	5.02 CFU/mL	[55]
Electrochemical biosensor	*S. typhimurium*	Cas12a	55 CFU/mL	[56]
Electrochemiluminescence	*Salmonella*	Cas12a	37 CFU/mL	[57]
Fluorescence	*S. aureus*	Cas12a	1.50 CFU/mL	[58]
Photothermal	*S. aureus*	Cas12a	1 CFU/mL	[59]
Colorimetric	*S. aureus*	Cas12a	5 CFU/mL	[60]
Fluorescence	*S. aureus*	Cas12a	4×10^3 fg/μL	[61]
Flow test strips	*S. aureus*	Cas12a	10 - 100 copies	[62]
Single-tube detection	*S. aureus*	Cas12a	5 copies	[63]
Fluorescence	*E. coli*	Cas9	40 CFU/mL	[64]
Fluorescence	*E. coli*	Cas12a	1 CFU/mL	[65]
Lateral flow	*E. coli*	Cas12a	1 CFU/mL	[66]

Continued

One-pot	*E. coli*	Cas12a	1 CFU/mL	[67]
Detection Method	Target	CRISPR/Cas	LOD	Reference
G-Quadruplex	*S. enterica*	Cas12a	20 CFU/mL	[68]
Fluorescence	*S. enterica*	Cas12a	5 CFU/mL	[69]

4. Conclusions and Outlook

The integration of CRISPR/Cas12 with electrochemical biosensors has been a groundbreaking development in the molecular diagnostics landscape. The synergy between the precision of the CRISPR/Cas12 system and the sensitivity of electrochemical detection platforms has led to the creation of a new generation of biosensors that are pushing the boundaries of what is possible in nucleic acid detection.

The use of modified carbon-based electrodes in these biosensors has been particularly noteworthy. Carbon, with its high surface area, chemical stability, and electrical conductivity, serves as an ideal substrate for electrode modification. By employing techniques such as chemical vapor deposition, electrochemical deposition, or surface functionalization, researchers have been able to enhance the performance of these electrodes, achieving attomolar (aM) level detection sensitivity—a level of sensitivity that was previously unattainable with traditional methods.

This level of sensitivity is not just a scientific milestone; it represents a significant leap forward in practical applications. In the medical field, for instance, such sensitive detection capabilities can lead to the early identification of diseases, including those at the genetic level. Early detection is crucial for the initiation of timely treatment, potentially improving patient outcomes and reducing healthcare costs. Moreover, the environmental monitoring sector stands to benefit significantly from these advances. The ability to detect minute traces of environmental pollutants or pathogens can help in the rapid assessment of contamination and the implementation of remediation strategies.

As we look to the future, the potential for innovation in the field of CRISPR/Cas12-driven electrochemical biosensors is immense. The ongoing research into novel electrode materials and modification techniques is expected to further refine the sensitivity, selectivity, and response time of these sensors. This could lead to the development of portable, user-friendly devices that can be deployed in a wide range of settings, from clinical laboratories to remote field locations. The transformative impact of these biosensors on disease diagnosis and monitoring cannot be overstated. By providing fast and accurate test results, they have the potential to revolutionize personalized medicine. The ability to tailor treatments to an individual's unique genetic makeup is a cornerstone of modern healthcare, and the development of sensitive and specific biosensors is a key enabler of this approach. Furthermore, the role of these sensors in early disease detection and continuous

monitoring is set to greatly enhance the efficiency of disease management. This is particularly important for chronic conditions, where ongoing monitoring is necessary to adjust treatments and manage the disease effectively. The molecular diagnostics market is on the cusp of a major transformation, driven by the highly targeted nature of Cas nucleases and cutting-edge sensor technology. As our understanding of the CRISPR/Cas system deepens, and with the potential discovery of novel Cas effectors, the diversity and functionality of biorecognition components will expand. This expansion will open up new possibilities for unraveling the complexity of biorecognition and developing even more sophisticated biosensors.

In conclusion, the fusion of CRISPR/Cas12 technology with electrochemical biosensors represents a paradigm shift in nucleic acid detection. It is a field that is rich with potential, promising to deliver significant advancements in healthcare, environmental science, and beyond. As research continues to push the boundaries of what is possible, we can expect to see these transformative biosensors playing a central role in a wide array of applications, enhancing our ability to detect, understand, and respond to the molecular world around us.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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