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CRISPR-Cas13a-powered electrochemical biosensors for RNA-based disease diagnostic and monitoring

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ABSTRACT

Nucleic acids serve as specific, selective, and sensitive components in molecular diagnostics, offering efficient and high-precision results. Unlike DNA, RNA expression reflects real-time cellular activity, allowing for the monitoring of disease progression, treatment response, or environmental influences. This makes RNA a superior biomarker due to its ability to enable early disease detection, provide higher specificity, allow non-invasive sampling, and offer high sensitivity for low-abundance targets. RNA-based biosensor innovations hold significant potential for detecting genetic diseases, such as cancer, and preventing viral infections. Electrochemical biosensors have become a fast and efficient alternative to gold-standard diagnostic methods, offering simplicity, rapid response, and suitability for clinical use, including point-of-care applications. Recent advancements have integrated the CRISPR-Cas13a system with electrochemical biosensors to enhance RNA detection sensitivity and specificity. The CRISPR-Cas system, an adaptive immune mechanism in bacteria, has been widely utilized for diagnostics. Cas13a is superior to other Cas proteins for RNA detection due to its high specificity, inherent signal amplification, and ability to detect low-abundance RNA without requiring reverse transcription or amplification steps. This review summarized recent progression of CRISPR/Cas 13a and its combination with electrochemical technique, including electrochemiluminescence (ECL) and photoelectrochemical (PEC) methods. The principles and advantages of CRISPR/Cas13a, electrochemical, ECL, and PEC technique for RNA detection are described. In electrochemical-based biosensors, Cas13a recognizes and binds to the target ssRNA, triggering its trans-cleavage activity, which indiscriminately cuts nearby RNA reporters. This process alters the electrochemical signal, enabling selective and sensitive RNA detection. Finally, several examples of CRISPR/Cas13a-based electrochemical biosensors are discussed, highlighting their potential as molecular diagnostic tools for RNA detection and emphasizing their advantages in sensitivity, specificity, and rapid detection capabilities.

1. Introduction

Nucleic acids are important biomolecules composed of long chains of nucleotide polymers, which consist of nitrogenous bases, sugars, and phosphate groups. These biomolecules serve to store, carry, and regulate the expression of genetic information within organisms. Based on the type of sugar they contain, nucleic acids are divided into deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) [1]. RNA plays a vital role as a carrier of genetic information encoded in DNA, as well as contributing to protein synthesis, in accordance with the central dogma of molecular biology. Various types of RNA, such as mRNA, circRNA, lncRNA, miRNA, and snRNA, play key roles in the physiological and pathophysiological processes of organisms [2–4]. RNA also acts as a mediator in genetic changes, transcription regulation, and

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post-transcriptional processes [5,6]. Moreover, RNA sequences contain specific genetic information that supports various cellular processes [7]. Dysregulation of RNA can lead to genetic disorders, including cancer [8–10]. In an analytical context, nucleic acids are often chosen for detection due to their better thermal stability compared to proteins or lipids, making them ideal for use in extreme environmental analyses. Unlike DNA, RNA expression reflects real-time cellular activity, allowing for monitoring of disease progression, treatment response, or environmental influences, making it a superior biomarker due to its ability to enable early disease detection, offer higher specificity, allow non-invasive sampling, and provide high sensitivity for low-abundance targets [11,12].

Innovations in RNA detection have great potential to support the early detection of genetic-related diseases and the prevention of pandemics caused by viruses [13,14]. This is further reinforced by the widespread spread of the SARS-CoV-2 virus, which caused the COVID-19 pandemic, causing million cases of infection and million deaths [15]. Additionally, RNA viruses, such as dengue, ebola, influenza, HIV, and zika, also pose significant public health threats [16–21]. The gold standard method for RNA detection in current clinical practice is quantitative reverse transcription polymerase chain reaction (RT-qPCR), which requires a reverse transcription process to convert RNA into DNA before amplification [22,23]. However, PCR-based methods involve lengthy thermal cycling, making them time-consuming, and rely on expensive instrumentation, limiting accessibility for resource-limited settings. Additionally, PCR requires multiple processing steps, increasing the risk of contamination and false positives, while its complex protocol makes multiplex detection challenging. The development of RNA detection methods is crucial to achieving sensitivity comparable to PCR and addressing issues related to the system [12]. As an alternative, electrochemical biosensors have shown great potential in molecular diagnostics [24,25]. These analytical devices integrate biological recognition elements into a transducer to detect interactions with analytes and convert them into electrical signals [26].

Electrochemical biosensors offer rapid RNA detection, often providing results within minutes to an hour, significantly reducing detection time compared to PCR. They operate with simpler, cost-effective instrumentation, making them more suitable for point-of-care testing. Additionally, the absence of complex sample preparation reduces operational costs while minimizing the risk of cross-contamination, as they eliminate the need for nucleic acid amplification. Furthermore, electrochemical biosensors can be engineered for multiplex detection by modifying electrode surfaces with specific probes, enabling simultaneous analysis of multiple RNA targets, which improves diagnostic efficiency compared to PCR-based methods that require extensive reagent optimization. The integration of electrochemical biosensors with clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas). CRISPR-Cas technology has advanced significantly, given CRISPR's ability to detect nucleic acids with high sensitivity and specificity [27]. RNA detection typically requires amplification and reverse transcription processes; however, one of the CRISPR variants, Cas13a, has a specific focus on detecting RNA sensitively, where even a small amount of RNA can activate Cas13a to perform trans-cleavage activity on a probe within the biosensor. This allows for more efficient and accurate RNA detection, aligning with its key advantage in facilitating early diagnosis. The use of this technology in electrochemical biosensors enhances the potential for rapid, accurate, and specific RNA detection in clinical diagnostic applications.

The CRISPR-Cas system is an adaptive immune mechanism found in bacteria and archaea to protect against viral or plasmid attacks [28]. During reinfection, Cas proteins guided by crRNA are able to recognize and cleave foreign DNA or RNA. In recent years, the CRISPR-Cas system has gained increasing popular in molecular diagnostics, especially with the discovery of trans-cleavage activity by the Cas13a protein, which is guided by gRNA and functions as nuclease enzyme capable of cleaving target RNA in a short time [29–32]. A key advantage of Cas13a is its trans-cleavage activity, which enables it to cleave both target RNA and nearby reporter or probe RNA, enhancing detection sensitivity without the need for nucleic acid amplification. Cas13a is highly specific to RNA, making it ideal for detecting RNA-based targets such as viruses, genetic biomarkers, and non-coding RNAs like miRNA [31]. Several Cas13a-based detection methods have been developed, including a Cas13a-responsive DNA hydrogel capillary sensor for RNA detection of SARS-CoV-2 [33], fluorescence quenching for detecting circROBO1 and BRCA as breast cancer biomarkers [34], fluorogenic RNA aptamers for monkeypox virus detection [35], lateral flow assays for visual SARS-CoV-2 detection [36], and polydisperse droplet digital CRISPR/Cas13a for detecting circular RNA and microRNA as cancer biomarkers [37]. In addition, the CRISPR-Cas13a system has been successfully integrated into electrochemical biosensors, improving specificity and sensitivity for RNA detection, including SARS-CoV-2 [38], SARS-CoV-2 L452R mutation [39], DENV-1 [40] and RNA [41,42].

Traditional electrochemical approaches often rely on redox indicators such as methylene blue (MB), ferrocene (Fc), or label-free detection [43] for biomolecule detection. However, these electrochemical reporters typically face challenges, such as relatively small signals compared to measurements using optical methods. To overcome this limitation, electrochemical sensors can be integrated with optical technique, such as Electrochemiluminescence (ECL) or Photoelectrochemical (PEC) method, to enhance measurement sensitivity [44]. ECL, in particular, has emerged as a highly sensitive detection method, offering signal amplification and rapid response times while maintaining the benefits of electrochemical techniques, including low noise levels and high specificity [45,46]. The integration of ECL with electrochemical techniques such as differential pulse voltammetry (DPV), cyclic voltammetry (CV), and electrochemical impedance spectroscopy (EIS) has proven effective for detecting various biomarkers. This includes p-Tau-181 protein as a biomarker for Alzheimer's [47] aptasensors for detecting acetamiprid [48], and CRISPR-Cas13a-based sensors for detecting brain natriuretic peptide (BNP) as a biomarker for heart failure [49] as well as MiR-17 as a biomarker for cancer and cardiovascular diseases [49] and *Leishmania infantum* [50]. The development of ECL-based electrochemical biosensors has also been applied in detecting detect Hepatitis A, B, and C virus DNA[51,52] and Cytokeratin 19 (CK19) as a biomarker for breast, lung, and liver cancer [53]. Additionally, CRISPR-Cas13a-based biosensors have been used for detecting *Escherichia coli* RNA [54] and matrix metalloproteinase-2 (MMP-2) as a biomarker for cancer [55].

On the other hand, the development of biosensors with the combination of PEC offers higher sensitivity and selectivity for nucleic acid detection due to the low background signal and photoexcited electron transfer, making them ideal for detecting trace amounts of RNA of more complex samples. Recently, PEC-based biosensor have been developed for detecting cancer-related RNAs, including miRNA-21 for various cancers [56,57], miRNA-155 for cancer progression [58], miRNA-122 for gastric cancer subtype identification [59], exosomal miRNA-92a-3p for colorectal cancer [60], long non-coding RNA HOTAIR for multiple cancers [61], an general circRNA detection has also been explored [62,63]. Furthermore, the integration with CRISPR/Cas13a has also successfully applied for pathogen detection [64] and miRNA detection [65,66]. In addition, the combination of ECL and PEC has been used to detect oxytetracycline [67].

Over the past few years, several review papers have explored the use of CRISPR/Cas13a for RNA detection. Granados-Riveron and Aquino-Jarquin (2021) provide a focused overview of CRISPR/Cas13-based strategies for ultrasensitive and specific detection of microRNAs, covering various biosensing platforms including fluorescence and electrochemical methods [68]. Zhao et al. (2022) provides a comprehensive overview of Cas13a's mechanisms and its applications in detecting diverse biological targets such as viruses, bacteria, and non-coding

RNAs, particularly through amplification-free platforms like SHERLOCK [31]. Additionally, a broader electrochemical perspective is offered by Clianta et al. (2024), covering both clinical diagnostics and food safety monitoring, and highlighting the modularity of CRISPR/Cas12a-based electrochemical biosensor designs [69]. Zhu et al. (2025) focuses on detection strategies for small non-coding RNAs such as miRNA, lncRNA, and circRNA using optical and electrochemical approaches [70]. Meanwhile, a separate 2025 publication by Karimi et al. (2025) highlights the utility of Cas13a for plant RNA virus detection, emphasizing attomolar sensitivity and isothermal amplification techniques, though it only briefly mentions the potential for adaptation to electrochemical platforms [71].

In contrast to these reviews, our work offers a broader application-driven and multimodal perspective by systematically comparing CRISPR/Cas13a-integrated electrochemical biosensors—including electrochemical, PEC, and ECL systems. Our focus lies not only in summarizing the mechanisms of signal transduction, but also in showcasing their implementation in viral and host RNA diagnostics. To our knowledge, this is the first review to comprehensively evaluate CRISPR/Cas13a-powered multi-mode electrochemical biosensors with a specific focus on diagnostic implementation and future directions. This review explores the development of CRISPR/Cas13a-based electrochemical biosensors for RNA detection, emphasizing their applications in clinical diagnostics and disease monitoring. It begins by discussing the importance of RNA in biological systems and the need for highly sensitive detection methods. The advantages of electrochemical biosensors, including their sensitivity, portability, and real-time detection capabilities are highlighted. Furthermore the review examine the CRISPR/Cas13a mechanism, particularly its trans-cleavage activity upon RNA recognition, and explore its integration with electrochemical techniques, such as square wave voltammetry (SWV), ECL, and PEC methods to enhance biosensor performance. Additionally, various signal amplification strategies, such as catalytic hairpin assembly (CHA) and tetrahedral DNA (Td) frameworksw are discussed for their role in improving detection performance. Finally, the review presents key case studies demonstrating the potential of these biosensors in detecting disease biomarkers, providing a comprehensive overview of their current applications and future prospects in RNA diagnostic.

2. CRISPR systems

CRISPR/Cas system was first reported as a series of direct repeat sequences separated by small spacers in the *E. coli* genome by a group of Japanese researchers in 1987 [72], and was later defined by Jansen et al. in 2002 [73,74]. CRISPR/Cas system is an adaptive immune system found in bacteria (40 %) and archaea (90 %) to defend against viral and plasmid attacks [75]. The CRISPR system operates through three distinct stages. First, during the adaptation stage, foreign nucleic acids are recognized and integrated into the spacer sequence of the CRISPR array, forming an immunological memory. Second, in the expression and maturation stage, CRISPR-RNA (crRNA) is transcribed from precursor crRNA (pre-crRNA) and processed into its mature form. Finally, in the interference stage, the Cas-CRISPR-crRNA complex identifies and cleaves the target foreign DNA or RNA, effectively neutralizing the threat [31,76]. The CRISPR/Cas system consists of one or more Cas effector proteins with nuclease activity, which require complementary base-pairing of crRNA or gRNA to the target DNA or RNA sequence. In most DNA-targeting CRISPR system, a protospacer adjacent motif (PAM), typically a short sequence of 2–4 nucleotides, is required at the 5′ or 3′ end of the target DNA to enable precise recognition. However, in RNA-targeting systems such as Type VI CRISPR/Cas13, a protospacer flanking sequence (PFS) may be required instead of a PAM. Additionally, some CRISPR/Cas systems do not require either PAM or PFS to recognize the target sequence. The accuracy of target recognition in the CRISPR/Cas system depends on the length of the gRNA (20–30 base pairs), while and the presence of similar or identical sequences within the genome influences specificity and the risk of off-target effects [77].

The CRISPR/Cas system is classified into two classes, each comprising multiple types and subtypes. Class 1 consists of complexes involving multiple effector proteins that work together to carry out target recognition and cleavage. On the other hand, class 2 is characterized by a single, multifunctional effector protein, making it more efficient and widely adopted in biotechnology and diagnostic due to its simplicity [78,79]. Class 2 is further categorized into three types, each with distinct Cas nucleases. Type II CRISPR/Cas9 is the most extensively used system in genome editing due to its precision and adaptability [80]. Additionally, Type V CRISPR/Cas12a, and type VI CRISPR/Cas13a, with Cas12a widely applied for DNA detection and Cas13a utilized for RNA-based diagnostic methods [81,82].

One of the key difference in the mechanisms of Cas9, Cas12a, and Cas13a in diagnostic utilization is lies in their nuclease activity (Fig. 1 and Table 1) Cas9 exhibits only cis-cleavage activity, which is carried out by the nuclease domain (NUC) after recognizing PAM on the target double-stranded DNA (dsDNA). The NUC of Cas9 consists of two catalytic sites: histidine-asparagine-histidine (HNH), which cleaves one strand of the target DNA that hybridizes with the gRNA, and resolvase C (RuvC), which cleaves the complimentary DNA strand, resulting in a double-strand break (DSB). In contrast, Cas12a and Cas13a exhibit both cis- and trans-cleavage activities. Cas12a primarly recognizes dsDNA, but its trans-cleavage occurs specific to single-stranded DNA (ssDNA), such as a reporter ssDNA [83,84]. The NUC activity of Cas12a is mediated by RuvC domain, which consists of three subdomains: RuvC I, which cleaves the target DNA strand hybridized with gRNA; RuvC II, which cleaves the complementary DNA strand, generating a DSB; and RuvC III, which facilitates trans-cleavage of non-specific ssDNA such as reporter molecule. On the other hand, Cas13a exclusively recognize single-stranded RNA (ssRNA), with its nuclease activity originating from the higher eukaryotes and prokaryotes nucleotide-binding (HEPN) domain. The HEPN domain is responsible for cis-cleavage, which specifically target the complementary ssRNA, and trans-cleavage, which non-specifically cleaves any non-target ssRNA, such as reporter RNA. This unique trans-cleavage activity is widely exploited in CRISPR-based detection platforms [85].

In addition to Cas9, which is widely used for DNA detection, Cas12a offers an additional advantage through its trans-cleavage activity, enabling the cleavage of labeled reporter DNA for signal amplification in biosensors [86]. Additionally, Cas14, a smaller Cas protein, facilitates integration into biosensor systems due to its compact size [87]. However, Cas14 is still under optimization, and its mechanism is not yet fully refined for practical biosensing applications. For RNA detection, Cas12 and Cas14 require reverse transcription to convert RNA into complementary DNA (cDNA) before recognition [88]. In contrast, type III-E CRISPR-Cas effectors (Cas7–11) can directly target and cleave RNA without the need for reverse transcription. However, Cas7–11 lacks trans-cleavage activity, limiting its use in biosensor signal amplification [89]. Another alternative is dead Cas9 (dCas9), a catalytically inactive variant modified into RCas9 for RNA targeting with the assistance of a PAMmer [90]. However, dCas9 has lower sensitivity and lacks cleavage activity, making it more suitable for RNA imaging or CRISPR interference (CRISPRi) rather than biosensing applications. Furthermore, Cas12a2 has been identified as an RNA-targeting protein, but its broad degradation activity across dsDNA, ssDNA, and RNA limits its application in biosensors [91]. Among RNA-targeting CRISPR systems, Cas13 (including Cas13a, Cas13b, Cas13d, Cas13x, and Cas13y) remains the most widely used due to its ability to recognize RNA and perform both target-specific cleavage and trans-cleavage for signal amplification [92,93]. As a result, ongoing advancements in RNA biosensors primarily focus on optimizing the CRISPR-Cas13 system to enhance sensitivity, specificity, and real-time detection capabilities.

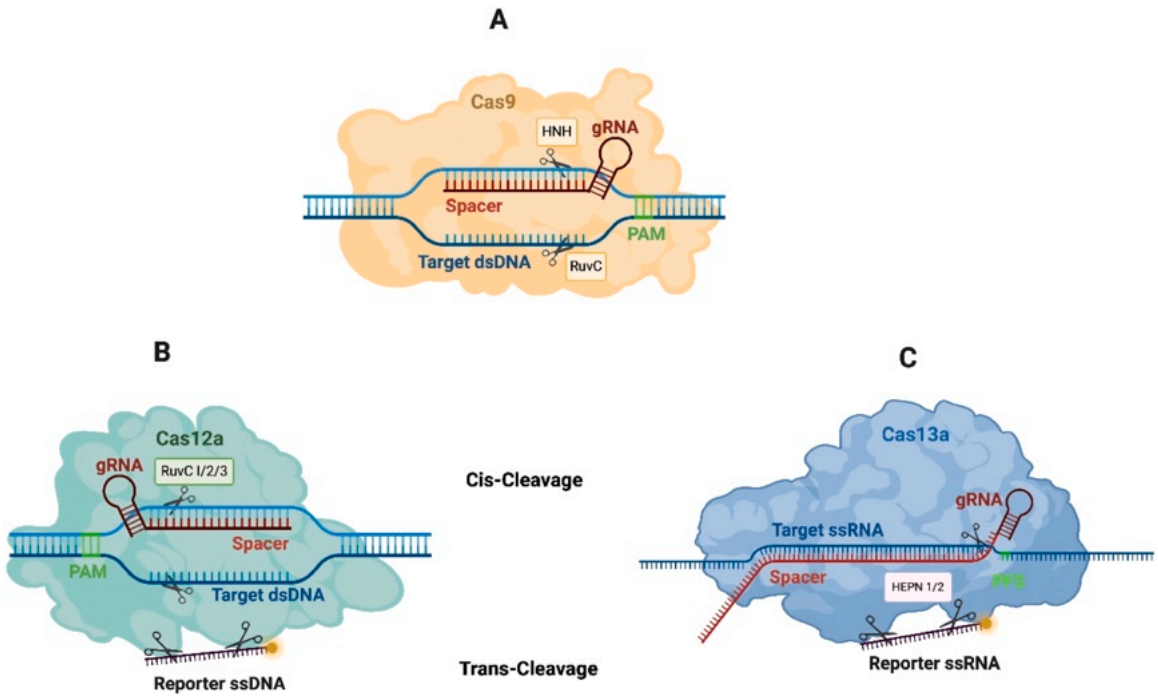

Fig. 1. Schematic of (A) Cas9, (B) Cas12a, and (C) Cas13a.

Table 1
Comparison Cas9, Cas12a, and Cas13a [20].

Type	II	V	VI
Protein effector	Cas9	Cas12a	Cas13a
Catalytic domain	HNH and RuvC	RuvC	HEPN
Pre-crRNA processing	No	Yes	No
TracrRNA	Yes	No	No
Spacer length	18 - 24 nt	18 - 24 nt	22 - 28 nt
PAM/PFS	3′, G-rich (NGG)	5′, T-rich, (TTTN)	3′, non G, PFS
Substrate	dsDNA	dsDNA, ssDNA	ssRNA
Cleavage pattern	Blunt	Staggered	Near U dan A
Collateral cleavage	No	ssDNA	ssRNA

2.1. CRISPR-Cas13

In the CRISPR system, Cas13a is an RNA-targeting enzyme with both cis- and trans-cleavage activity, similar to Cas12. It operates with high specificity by recognizing RNA targets through complementary base pairing between the guide RNA (gRNA) and the target RNA. Upon binding, Cas13 is activated and cleaves the target RNA at a specific site, triggering nonspecific cleavage of surrounding RNA molecules, a phenomenon known as trans-cleavage [94]. This property significantly enhances signal amplification, making Cas13 highly effective for RNA detection [95]. In electrochemical biosensors, Cas13 amplifies detection signals by cleaving reporter RNA, which can then be converted into electrochemical or optical responses, enabling rapid and highly sensitive RNA detection [41].

Cas13 consists of several subtypes, classified based on structural and functional differences (Table 2), with Cas13a (C2c2) being the most widely used in research and diagnostics. Cas13a was initially discovered in the Type VI CRISPR system and is known for its highly efficient trans-cleavage activity, making it well-suited for signal amplification in RNA biosensors. Cas13b, found in the Type V CRISPR system, exhibits similar activity but is more commonly applied in RNA expression modification rather than biosensing. Cas13c and Cas13d are newer variants within the Type VI system, but their trans-cleavage efficiency is lower, and their applications remain less explored. Due to its superior trans-cleavage activity, Cas13a is the preferred choice for RNA biosensor applications, whereas Cas13b and other variants are often used in RNA modification and editing. Additionally, protospacer flanking sequences (PFS) recognized by each Cas13 variant differ, influencing their target specificity. Cas13a typically recognizes 'AA' or 'AG' sequences, characterized by two highly conserved bases, while Cas13b and Cas13c prefer different sequence motifs, with Cas13c recognizing slightly longer sequences [31]. These distinctions in sequence recognition further determine their suitability for various diagnostic and gene regulation applications.

Cas13a has several variants, each with distinct characteristics based on its bacterial origin. LshCas13a, from *Leptotrichia shahii*, was one of the first identified variants and exhibits strong trans-cleavage activity, making it a model for early CRISPR-Cas13 studies [96]. LwCas13a, derived from *Leptotrichia wadei*, demonstrates high specificity for uracil (U)-containing RNA sequences, enabling precise cleavage while minimizing off-target effects, making it a preferred choice for CRISPR-based nucleic acid detection. LbCas13a, from *Lachnospiraceae* bacterium, is known for its superior stability and specificity, with a reduced risk of off-target activity, making it ideal for precision diagnostics. LbuCas13a,

Table 2
Differences in Cas13 types.

No	Component	Cas13a	Cas13b	Cas13c	Cas13d
1	Other names	C2c2	C2c2-like	C2c3-like	C2c5
2	Source of origin	*Lachnospiraceae bacterium*	*Leptotrichia shahii*	*Dysgonomonas mossii*	*Corynebacterium* spp.
3	PFS	1 base sequence (A, U, or C) or 2 base sequence (AA or AG)	PFS is more flexible with base variations	PFS preference may vary, longer	More variations on PFS
4	Trans-Cleavage activity	High; Target RNA and other RNA in system	High	Slightly lower than Cas13a and Cas13b	Low

also from *Lachnospiraceae* bacterium (*Leptotrichia buccalis*), exhibits high RNA cleavage efficiency, particularly in detection platforms [97]. Among these, LwCas13a and LbCas13a are the most widely used due to their advantages in precision, stability, and reliable detection performance.

Cas13 cleavage activity is initiated when the enzyme is guided by gRNA, which recognizes the target RNA through complementary base pairing with the protospacer flanking sequence (PFS), a specific motif located downstream of the target RNA. Once bound, Cas13 cleaves the phosphodiester bond in the RNA backbone through its ribonuclease (RNase) activity, which is mediated by the Higher Eukaryotes and Prokaryotes Nucleotide-binding (HEPN) domain. This domain contains catalytic residues that facilitate cleavage, aided by divalent metal ions such as Mg^{2+} or Mn^{2+}, which stabilize reaction intermediates and promote nucleophilic attack on the phosphodiester bond, resulting in RNA fragmentation. Once activated, Cas13 also randomly cleaves non-target RNA or reporter RNA through trans-cleavage activity, a key mechanism for signal amplification in RNA-based detection systems [98].

The CRISPR system has various applications in molecular diagnostics, as summarized in Table 3. One of the most widely used methods is Specific High Sensitivity Enzymatic Reporter Unlocking (SHERLOCK), which leverages Cas13's trans-cleavage activity. After recognizing and cleaving the target RNA, Cas13 indiscriminately cleaves surrounding RNA molecules, amplifying the detection signal. This system is widely applied in disease and biomarker detection, particularly using fluorescence and optical biosensors. Another approach, CRISPR-Cas13-Assisted resolution of microbial encounters and nucleic acids (CARMEN), integrates Cas13 with fluorescence detection techniques. In this system, RNA cleavage by Cas13 generates fluorescent signals, enabling the detection of RNA-based pathogens in environmental samples and microbial contamination. Additionally, the DNA endonuclease targeted CRISPR trans reporter (DETECTR), although primarily associated with Cas12, can also utilize Cas13 for RNA detection. DETECTR is often coupled with bioluminescence, electrochemical biosensors, or optical detection techniques, making it practical for point-of-care (POC) diagnostic applications [99].

The CRISPR/Cas13a (C2c2) system was discovered in 2015 [100] and contains a single ribonuclease effector that binds and processes crRNA to form an RNA-targeting complex guided by gRNA. Sturcturally, Cas13a is composed of two primary lobes called the crRNA recognition lobe (REC) and the nuclease lobe (NUC). The REC lobe consists of the helices-1 domain and the N-terminal domain (NTD), while the NUC lobe contains the HEPN1 domain, HEPN2 domain, helices-2 domain, and the linker connecting the two HEPN domains [101]. The mature crRNA contains a 3′ spacer and a 5′ handle, which is further divided into 5′ flank, 5′ stem, loop, 3′ stem, and 3′ flank, as illustrated in Fig. 2 [102]. Cas13a facilitates the maturation of pre-crRNA into crRNA, forming a functional complex that recognizes foreign RNA targets. Upon target recognition, Cas13a cleaves the target RNA through cis-cleavage and simultaneously degrades nearby single-stranded RNA (ssRNA) in a non-specific manner via trans-cleavage, a key feature of its RNA detection mechanism [103].

The trans-cleavage activity of Cas13a can be utilized in fluorescent and electrochemical biosensors (Fig. 3). Upon recognizing its RNA target, Cas13a undergoes activation, leading to non-specific cleavage of nearby single-stranded RNA (ssRNA), including reporter RNA (reRNA) (Heo et al., 2022). Notably, target RNA cleavage is not required for Cas13a activation, as binding alone can trigger trans-cleavage activity. The cleavage of reRNA is detected by a transducer, which converts the biochemical reaction into an electrical signal, a mechanism widely implemented in CRISPR-based biosensors such as SHERLOCK [85].

Table 3
Differences in CRISPR systems for molecular diagnostic applications.

No	System	SHERLOCK	CARMEN	DETECTR
1	Focus	Specific RNA detection with high sensitivity	Environmental detection (water, soil, etc.)	Rapid on-site detection
2	Detection technique	Fluorescence or optical	Fluorescence or optical	Electrochemical or optical
3	Application	Clinical diagnostics, pathogen or biomarker detection	Environmental monitoring, pathogen detection	POC, pathogen or biomarker detection

3. RNA as biomarker

RNA has long been at the centre of molecular biology, particularly in the central dogma that outlines the flow of genetic information, as introduced by Francis Crick in 1958. In general RNA encompasses two main functions which is information function and catalytic function [104]. It is a fundamental biomolecule composed of nucleotide chains linked by phosphodiester bonds [105]. Unlike DNA, which serves primarily as a genetic blueprint, RNA plays a dynamic role in gene expression and regulation. Structurally, RNA consists of ribose sugar, phosphate groups, and four nitrogenous bases: adenine (A), guanine (G), cytosine (C), and uracil (U), replacing thymine (T) in DNA. The presence of a hydroxyl (-OH) group at the 2′-carbon of ribose makes RNA more chemically reactive and less stable than DNA, contributing to its transient nature in cellular processes. Additionally, RNA can fold into complex secondary and tertiary structures, allowing it to function beyond genetic information storage, such as in enzymatic catalysis and molecular interactions [106,107]

RNA exists in various forms, each with distinct biological roles (Table 4). Messenger RNA (mRNA) serves as the intermediate between DNA and protein synthesis, carrying genetic instructions from the nucleus to the ribosome. Transfer RNA (tRNA) and ribosomal RNA (rRNA) are essential for translation, facilitating amino acid assembly into functional proteins. Beyond protein-coding RNA, non-coding RNAs (ncRNAs), including microRNA (miRNA), long non-coding RNA (lncRNA), and circular RNA (circRNA), regulate gene expression, epigenetics, and cellular signaling. These regulatory RNAs influence fundamental biological processes, including cell differentiation, immune responses, and disease progression, highlighting their importance as molecular biomarkers. In virology, RNA also serves as genetic material for various viruses, such as influenza, HIV, and SARS-CoV-2. RNA viruses mutate more rapidly than DNA viruses due to their replication depending on polymerases without proofreading abilities. In contrast, DNA viruses rely on DNA polymerase with proofreading capabilities. Therefore, RNA viruses evolve more quickly and are more pathogenic than DNA viruses [108]. Although RNA is unstable in its basic state, it can be easily detected and quantified even in very low abundance [109]. Compared to protein biomarkers, RNA biomarkers offer higher sensitivity and specificity, while also surpassing DNA biomarkers in providing dynamic information about cellular conditions and their regulatory processes [3].

As biomarker, RNA plays a crucial role in molecular diagnostics offering significant advantages such as the ability to detect pathogens at early stages of infection, providing specific information about viruses or bacteria, and monitoring gene expression in genetic diseases or cancer for more personalized therapy. Unlike DNA, which remains relatively stable, RNA expression dynamically fluctuates in response to cellular conditions, making it a more precise indicator of disease states, treatment responses, and environmental influences. RNA biomarkers are widely utilized in oncology, infectious disease monitoring, and neurological disorder detection; however, their clinical application is challenged by RNA's inherent instability, susceptibility to degradation, and the need for amplification in traditional detection methods. The degradation of RNA is primarily due to the ribose sugar structure, which makes it highly reactive and prone to hydrolysis, along with the presence of natural RNase enzymes that contribute to low thermal stability [119]. These factors necessitate careful sample handling and storage to

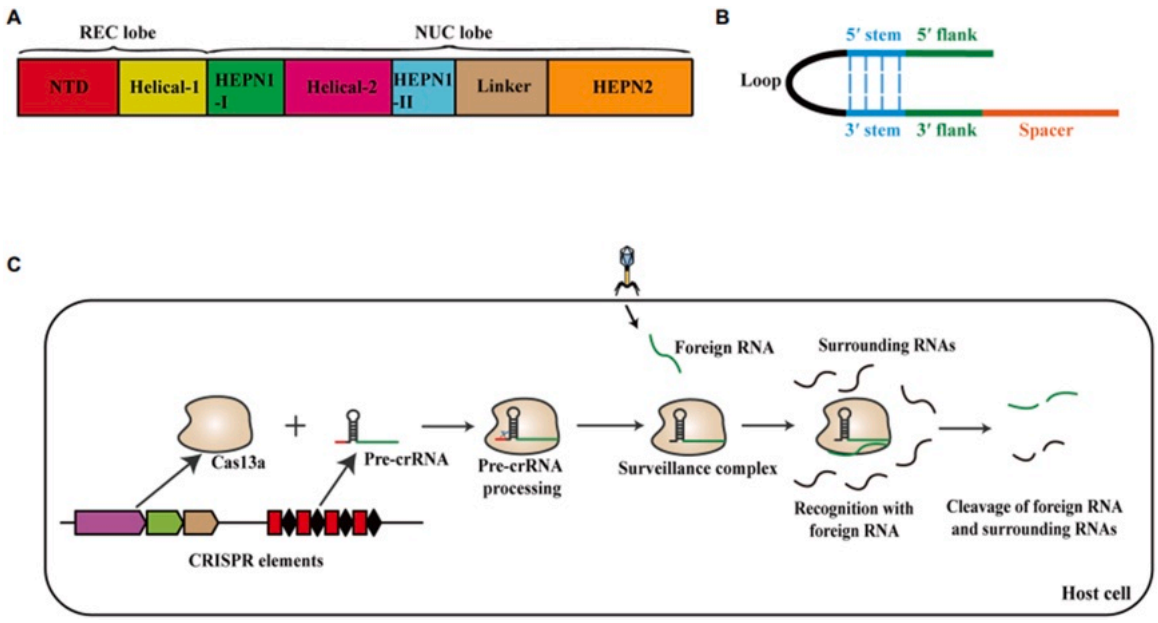

Fig. 2. (A) Schematic of Cas13a domains; (B) Schematic of crRNA structure maturation; (C) Mechanism of CRISPR/Cas13a system [31].

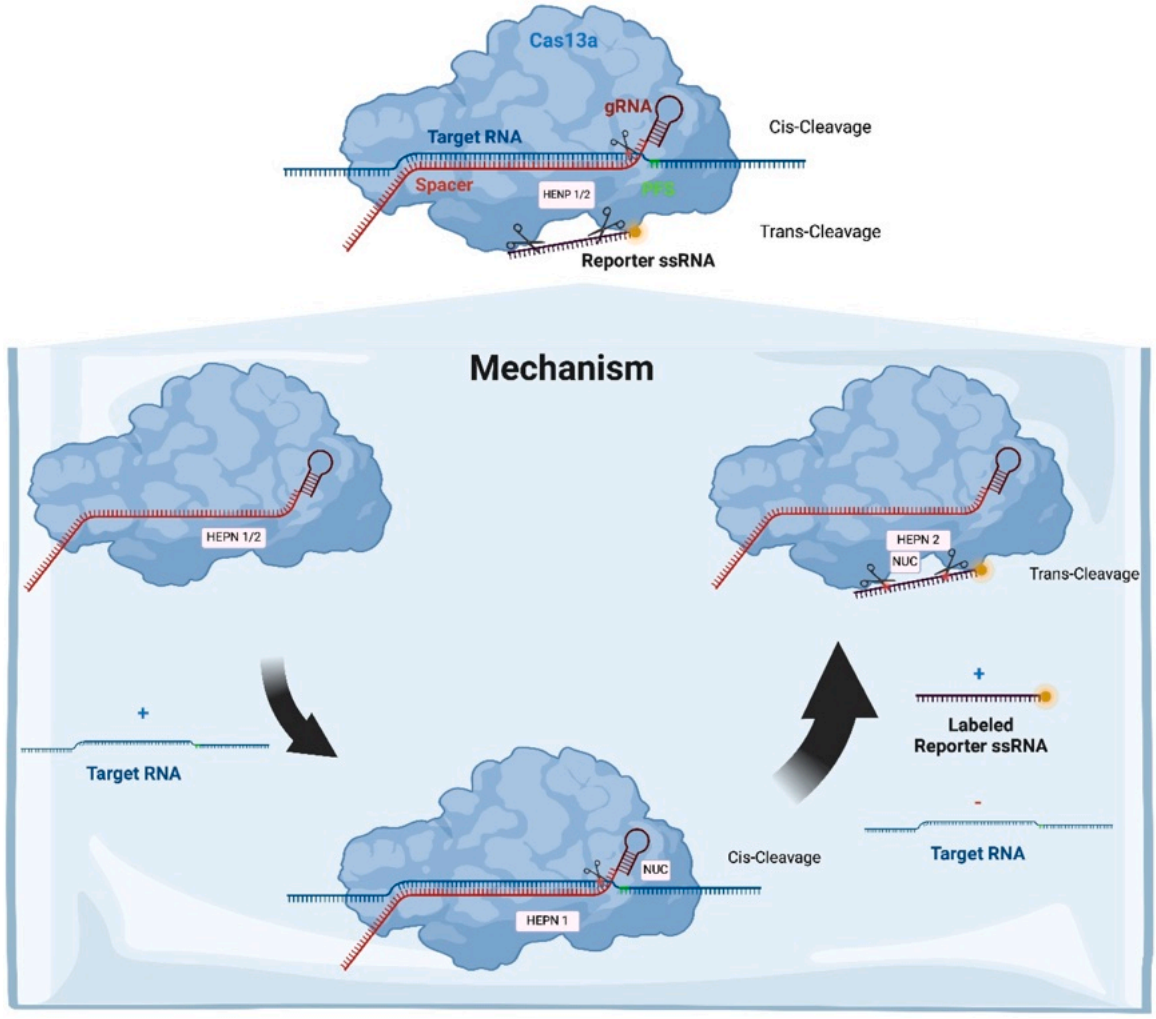

Fig. 3. Mechanism of CRISPR/Cas13a system activation for RNA detection.

Table 4
Variety of RNA types and their functions [110].

Name	Abbreviation	Distribution	Function	Diagnostic Relevance	Example of Target Detction
Messenger RNA	mRNA	All organisms	Protein coding	Biomarker for viral and bacterial gene expression, and cancer	two survivin (Sur) mRNA for human cancers [111]
Transfer RNA	tRNA	All organisms	Translation (pembawa asam amino)	Biomarker for cellular stress and metabolic disorders	tRNA fragments for epilepsy [112]
Ribosomal RNA	rRNA	All organisms	Translation (komponen ribosom)	Universal target for bacterial and fungal detection	16S rRNA for *E. coli* [113]
Small nuclear RNA	snRNA	Eukaryotes, and archaea	Pre-mRNA splicing	Genetic diseases and cellular abnormalities	U6 snRNA as a control for cellular RNA expression [114]
Small nucleolar RNA	snoRNA	Eukaryotes, and archaea	mRNA nucleotide modifications	Involved in cancer and leukemia	RNA SNORD1C for coloteral cancer [115]
Small interfering RNA	siRNA	Eukaryotes	Post-transcriptional regulation and transcriptional gene silencing	Specific gene expression detection and therapeutic applications	siRNA for therapeutic evaluation [116]
microRNA	miRNA	Eukaryotes	Regulation of mRNA stability and translation	Biomarker for various diseases (viral/cancer)	Cytomegalovirus (CMV) miRNAs (UL22A-5p and UL112-3p) for human herpesvirus [117]
Long Non-Coding RNA	lncRNA	Eukaryotes	Regulation of gene expression, epigenetics, and chromatin structure	Biomarker for cancer, neurodegenerative diseases	lncRNA HOTAIR for cancer progression and metastasi [61]
Circular	circRNA	Eukaryotes	Acts as a miRNA sponge, regulates transcription, and stabilizes RNA	Biomarker for infections and cancers	CircRNA SATB2 for early-stage lung cancer [118]

prevent contamination and degradation. To address these challenges, electrochemical biosensors provide a promising alternative, offering high sensitivity, rapid detection, and the ability to overcome RNA instability and sample contamination, making them an efficient and reliable tool for molecular diagnostics.

4. RNA based biosensor

A sensor consists of a chemical or biological receptor that specifically interacts with the target analyte and converts this recognition process into an analytically useful signal [120]. A sensor with a receptor specifically designed as a biological recognition element is called a biosensor [121]. Biosensors are reliable analytical devices for identifying and detecting target molecules. The application of biosensors for medical diagnostics has become widespread and more prominent, especially since the COVID-19 pandemic, due to the ability to do rapid measurement of viral infection [122].

This analytical device integrates biological recognition components with a physicochemical detector, consisting of a bioreceptor, transducer, and detector with a digital output [123]. Bioreceptors, which include antibodies, enzymes, aptamers, or nucleic acids (DNA or RNA), selectively bind to their complementary targets such as antigens, substrates, other DNA, or RNA [124]. Upon interaction with the target analyte, the bioreceptor generates a signal that is processed by transducer for a measurement. Transducers are classified into four types: electrochemical, optical, calorimetric, and mass [125,126]. Among these, electrochemical biosensors are widely recognized for their ability to efficiently integrate biological sensing with electrochemical analysis, making them a powerful tool for molecular diagnostics [81].

4.1. Electrochemical-based biosensors

Electrochemical biosensors offer high sensitivity and excellent selectivity in detecting targets with simple instruments and fast reaction times, in Fig. 4. The analytical method using electrochemical biosensors is generally based on electron transfer processes that occur on the electrode surface and electroactive materials within the electrolyte [127]. Electrode surfaces coated with bioreceptors are specifically capable of distinguishing between biomolecules and targets, and the interaction that occurs will be converted into distinct electrical signals, allowing both qualitative and quantitative detection of targets [128]. The electrical signal generated from electrochemical measurements will be proportional to the analyte concentration [25]. Signal conversion can take the form of current, potential, impedance, or ion charge. Popular detection methods include CV, SWV, DPV, and EIS [129,130].

There are three types of electrodes commonly used in electrochemical biosensors. The working electrode serves as the site where redox reactions occur and functions as a transducer element, with its reduction potential depending on the concentration of the analyte. The reference electrode acts as a comparator to measure the potential at the working electrode, with its reduction potential being independent of the analyte concentration. The counter electrode is responsible for conducting the entire current needed to balance the current at the working electrode [131,132].

Numerous studies have explored the detection of RNA using electrochemical-based biosensors, leading to significant advancements in their development. These innovations have enhanced the sensitivity, specificity, and practicality of RNA detection, making electrochemical biosensors a promising tool for molecular diagnostics. For example, Cheng et al. (2021) developed an electrochemical biosensor method to detect mRNA based on proximity-dependent surface hybridization chain reaction (HCR). All electrochemical measurements, including DPV, CV, and EIS were used to measure the response of the signals [133,134]. Thiolated oligonucleotides were immobilized on the surface of a gold electrode, then given a mixture containing the Fc-H1/H1 DNA probe (ferrocene-conjugated thiolated hairpin DNA), the H2 DNA probe (hairpin DNA without thiol), and the target mRNA. The target mRNA triggered the HCR to produce a long duplex DNA chain immobilized on the gold electrode, and produced a very stable ferrocene-based redox current. This label-free, isothermal, and non-enzymatic method successfully detected survivin mRNA with a detection limit of 3 fM [135].

In the same year, Peng et al. (2021) designed an electrochemical biosensor method to detect SARS-CoV-2 RNA by combining the signal amplification capabilities of catalytic hairpin assembly (CHA) and terminal deoxynucleotidyl transferase (TdT). The HP DNA-immobilized gold electrode was dripped with a mixture containing HP1, HP2, and target RNA, then given a mixture containing dNTP and TdT. The presence of target RNA can trigger CHA to produce a double-stranded product which then hybridizes with the HP DNA structure on the electrode surface to produce Y-shaped DNA with three protruding 3′ ends. These ends can be extended by the TdT enzyme to form a long single-stranded DNA product. The addition of $Ru(NH_3)_6^{3+}$ serves to increase the signal measured using DPV. This method was successfully detected SARS-CoV-2 RNA with a detection limit as low as 26 fM [136].

4.2. Electrochemiluminescence-based biosensors

ECL biosensors integrate the advantages of electrochemical and

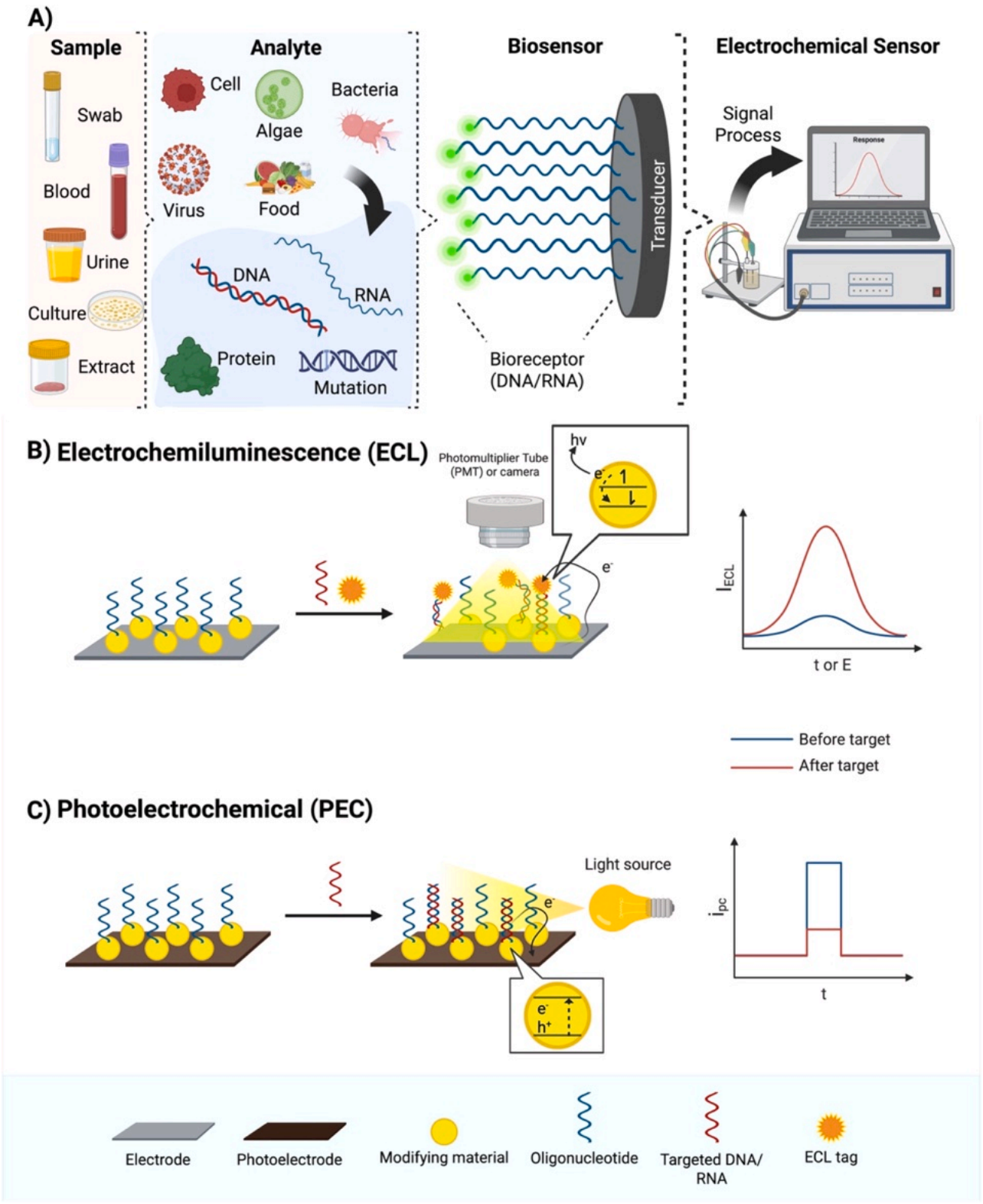

Fig. 4. Simple schematic of (A) a nucleic acid-based electrochemical biosensor in general, (B) ECL, and (C) PEC.

chemiluminescence technologies to quantify analytes [137–140]. The analytical technology using ECL biosensors not only inherits the high sensitivity and excellent selectivity of electrochemical biosensors, but also shows a wide dynamic range that originates from chemiluminescence biosensors [141,142]. In addition, ECL biosensors have no requirement for the external excitation light source resulting in near-zero background noises, which are superior to other spectroscopic technologies [143,144]. Therefore, ECL biosensors have been widely used in the field of DNA [51] and RNA detection [145].

Yang et al. (2024) developed a "signal-on-to-off" ECL biosensor for miRNA-21 detection by employing catalytic hairpin assembly (CHA) cycles. PdPt@SnS_2 nanosheets were prepared as both the luminophore

and co-reaction accelerator to effectively catalyze the reduction of $S_2O_8^{2-}$ to produce more $SO_4^{\bullet-}$ for boosting the ECL signal. In the absence of miRNA-21, the ECL behavior demonstrated a "signal-on" state. While miRNA-21 was present, the ECL strength exhibited a "signal-off" state affected by the quenching effect in CHA cycles, which was used to detect miRNA-21. As a result, the ECL biosensor displayed a wide detection range with miRNA in a range of 1 aM to 1 nM [146].

To further realize ultrasensitive RNA detection, various signal amplification strategies have been applied to enhance the sensitivity of ECL biosensors. For example, Xu et al. (2017) reported a novel ECL biosensor combining the 3D DNA walker-assisted signal amplification strategy and distance-controlled "on-off-super on" strategy for ultrasensitive detection of miRNA-141. First, the introduction of the 3D DNA walker in the ECL biosensor for target conversion and signal amplification was superior to traditional 1D and 2D walkers. Besides, the constructed "on-off-super on" strategy was based on the distance-induced surface plasma resonance between CdS QDs and Au NPs leading to the ECL quenching and enhancement, which exhibited remarkable capability in ultrasensitive miRNA-141 detection [147].

To improve the accuracy and reliability of RNA detection, ratiometric ECL biosensors have been developed to eliminate false positive and false negative signals caused by single-signal measurements. For instance, Huo et al. (2018) established a dual-wavelength ECL-RET ratiometric biosensor integrating with duplex-specific nuclease (DSN)-assisted signal amplification strategy for miRNA detection. Due to the excellent ECL-RET efficiency between Au NP-luminol-LDH donor and Au NCs acceptor, a dual-wavelength ECL emission was observed at 440 nm and 620 nm. When the target miRNA-107 was introduced, the ECL intensity increased at 440 nm and decreased at 620 nm. Taking advantage of the DSN-assisted target recycling amplification strategy, the proposed ECL ratiometric biosensor showed excellent performance for accurate and reliable miRNA detection [148].

4.3. Photoelectrochemical-based biosensors

PEC-based biosensors offer significant advantages over conventional electrochemical and ECL biosensors due to their unique mechanism of signal generation. Unlike electrochemical biosensors, which rely solely on redox reactions, and ECL biosensors, which require electrochemical excitation of luminophores, PEC biosensors use photoactive semiconductor materials (usually called photoelectrode) that generate photocurrents when exposed to light. The measurement of target analytes, whether through quantitative assessment or qualitative identification, is conducted by monitoring variations in the photoelectric signal. These fluctuations arise due to interactions between the analyte and the sensor interface, which influence charge transfer dynamics, light absorption efficiency, or electron-hole recombination processes [149]. This enables higher sensitivity and lower background noise, as the separation of excitation (light source) and detection (electrochemical signal) reduces interference from electrochemical side reactions [62,150,151]. Additionally, PEC biosensors can operate at lower applied potentials, minimizing damage to biological samples and increasing the stability of detection systems. To further enhance the performance of PEC biosensors, significant research efforts have been dedicated to the development of advanced photoelectrode materials. Researchers have explored nanostructured semiconductors [60], heterojunction-based composites [56], and plasmonic nanomaterials to improve light absorption, charge separation, and electron transfer efficiency.

Li et al. (2024) reports a signal-switchable PEC biosensor for the ultrasensitive detection of long non-coding RNA (lncRNA) in cancer cells, utilizing ZrO_2@CuO bimetallic oxides and T7 exonuclease (T7 Exo)-assisted signal amplification. The biosensor features TiO_2 nanodisks as the initial photoactive material, generating an anodic background signal, while ZrO_2@CuO introduces a cathodic photocurrent, enabling a polarity-switching mechanism to enhance sensitivity and eliminate false interference. The T7 Exo-assisted amplification facilitates target-induced DNA strand displacement, ensuring highly selective detection. This strategy achieved a detection limit of 0.12 fM and a linear detection range from 1 fM to 100 pM. The developed biosensor demonstrated high stability, excellent selectivity against mismatched sequences, and reproducibility over multiple assays, making it suitable for clinical applications [61].

To reduce the probability of false-negative diagnoses and ensure more accurate subtype identification, Zong et al. (2025) developed a dual-biomarker detection strategy for identifying AFP-producing gastric cancer (AFP-GC) using α-fetoprotein (AFP) and microRNA-122 (miRNA-122). The PEC-based biosensor operates on an AND logic gate strategy for the simultaneous detection of both biomarkers. It employs a CdTe quantum dots (CdTe QDs)/NiO/ITO photocathode, where exciton-plasmon coupling between CdTe QDs and Au nanoparticles enhances the photocurrent signal upon dual-target recognition. A catalytic hairpin assembly (CHA) signal amplification mechanism is used to improve sensitivity, ensuring a robust response only when both AFP and miRNA-122 are present. The biosensor demonstrates a detection limit of 6.22 pg/mL for AFP and 4 fM for miRNA-122, with a wide dynamic range. The system was successfully validated using serum samples from AFP-GC patients, effectively distinguishing them from common gastric cancer cases [59].

5. Application of CRISPR-Cas13a-based electrochemical biosensors in RNA detection

Following the discussion on the CRISPR-Cas13a system, RNA classification, and RNA-based biosensing strategies, this section focuses on the application of CRISPR-Cas13a-integrated electrochemical biosensors for RNA detection. These platforms leverage the unique trans-cleavage activity of Cas13a to produce detectable electrochemical signals upon target RNA recognition, enabling highly specific and sensitive detection without the need for nucleic acid amplification. Various electrochemical techniques, ECL, and PEC have been employed to construct these biosensors. Applications span from viral RNA detection and cancer biomarker monitoring to POC diagnostics, highlighting the translational potential of these systems in clinical and field settings. The following subsections provide representative case studies demonstrating the practical implementation and advantages of CRISPR-Cas13a-based electrochemical biosensing platforms.

5.1. Electrochemical biosensors

The superiority of electrochemical biosensors, coupled with Cas13a, in enhancing RNA detection sensitivity and specificity—particularly for detecting viruses and cancer biomarkers—has attracted significant attention from researchers worldwide, driving further advancements in biosensing technology. For example, Ma et al. (2024) developed a CRISPR-based electrochemical biosensor that combines CRISPR/Cas13a with a primer exchange reaction (PER), referred to as PER-E-CRISPR, for the sensitive detection of miR-21 without target amplification (Fig. 5a). The dual signal amplification process involves the binding of miR-21 by CRISPR/Cas13a and the hybridization of a short ssDNA with a concatenated PER sequence. When the miR-21 target is present, CRISPR/Cas13a cleaves the ribonucleotide site on hairpin 1 (HP1) and releasing a the trigger that open hairpin 2 (HP2) on the electrode surface, activating the concatenated PER that binds methylene blue-labeled single-stranded DNA (ssDNA-MB). The amplified signal is then detected using SWV. The assay showed a linear detection range for miR-21 from 10^{-13} to 10^{-7} M, with a detection limit of 30.2 fM [152].

Another advancement was made by Gong et al. (2024), who introduced an innovative approach by integrating the CRISPR/Cas13a system with a two-dimensional DNA nanoprobe (DNP)-based electrochemical biosensor for the sensitive and specific detection of B-type natriuretic peptide (BNP), a key biomarker for heart failure, a severe condition requiring rapid diagnosis. The processes begins with a BNP-specific

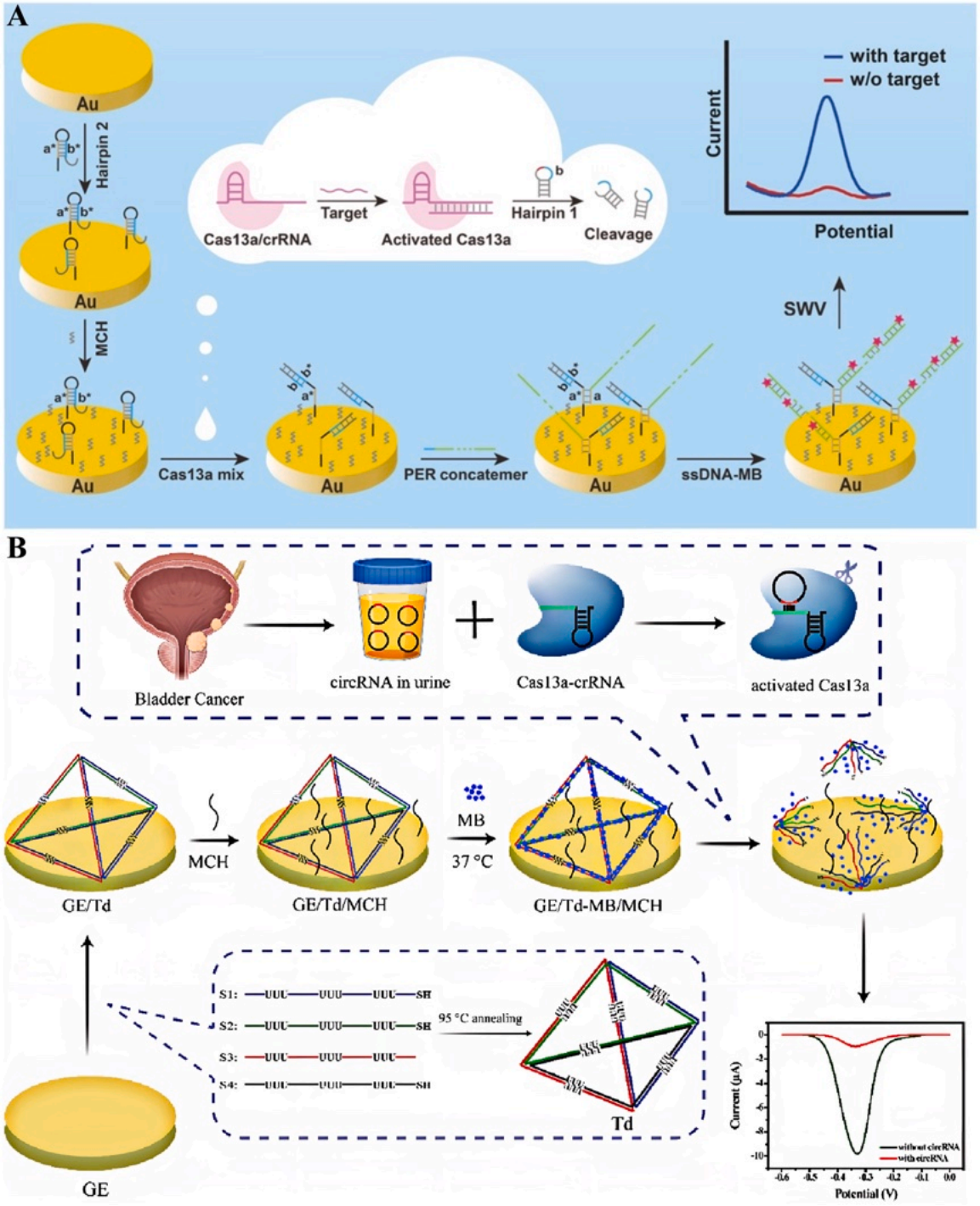

Fig. 5. (A) Principle of PER-E-CRISPR for MiR-21 detection (Reprinted by [152]) and (B) Schematic illustration of a CRISPR/Cas13a-based electrochemical biosensor combined with DNA Tetrahedron to detect circRNA (Reprinted by [42]).

aptamer binding to BNP, triggering the release of complementary DNA that acts as a template for RNA amplification by T7 RNA polymerase. The resulting RNA activates the CRISPR/Cas13a system, which cleaves target RNA at U–U base sites, initiating a strand displacement reaction on a gold electrode and causing the release of ferrocene-labeled DNA probes (D2 and D3), leading to a significant decrease in the electrochemical signal. In the absence of BNP, the aptamer remains inactive, preventing RNA amplification and signal alteration. This biosensor exhibits high sensitivity, with a detection limit of 0.74 aM, and can detect BNP in human serum samples with minimal interference, making it highly suitable for clinical applications and point-of-care monitoring. Furthermore the incorporation of an additional strand displacement reaction (TSDR)-based amplification enhances intermediate DNA release iteratively, further amplifying the detection signal. This strategy not only offers high speed and accuracy but also flexibility and effectiveness as a biomolecular detection platform, addressing the precision diagnostic needs for heart failure [153].

The development of a circRNA detection method in urine by Cheng et al. (2023) utilized an electrochemical biosensor based on CRISPR/Cas13a with a tetrahedral DNA (Td) framework. The Td structure enhances the analytical performance of the electrochemical biosensor by optimizing the nucleic acid chain density and minimizing steric

hindrance effects [154]. Uracil bases within the Td structure can be recognized by activated Cas13a, leading to the cleavage of phosphodiester bonds between uracil bases. The Td framework is immobilized on the gold electrode surface via Au-S bonds, while mercapto-1-hexanol (MCH) is used to block the active sites on the electrode surface. Subsequently, methylene blue (MB) is intercalated into the double-helix structure of the Td. The presence of circRNA activates Cas13a, which cleaves phosphodiester bonds between uracil bases within the Td, resulting in the destruction of the Td-MB complex and the release of intercalated MB molecules (Fig. 5b), which leads to a low electrochemical current peak. This strategy successfully detected bladder cancer-related circRNA in urine, achieving a detection limit as low as 0.089 fM [42].

On the other hand, the study conducted by Cui et al. (2021) developed a CRISPR/Cas13a electrochemical biosensor for miRNA-21 detection, incorporating the catalytic hairpin assembly (CHA) reaction to enhance signal amplification. In Fig. 6a, hairpin DNA 1 (H1) was immobilized on the surface of a gold electrode via Au-S bonding and coated with MCH to minimize nonspecific binding. When the target miRNA-21 is present, it hybridizes with the Cas13a-crRNA complex, activating Cas13a's trans-cleavage activity. This leads to the cleavage of Hairpin DNA 0 (H0), which contains an RNA sequence at the trans-cleavage site, releasing a secondary target (ST) DNA fragment. The ST fragment initiates the CHA reaction by hybridizing with H1, opening its structure and forming a complex with H2 labelled with MB. This cyclic process continuously release ST fragments, further amplifying the

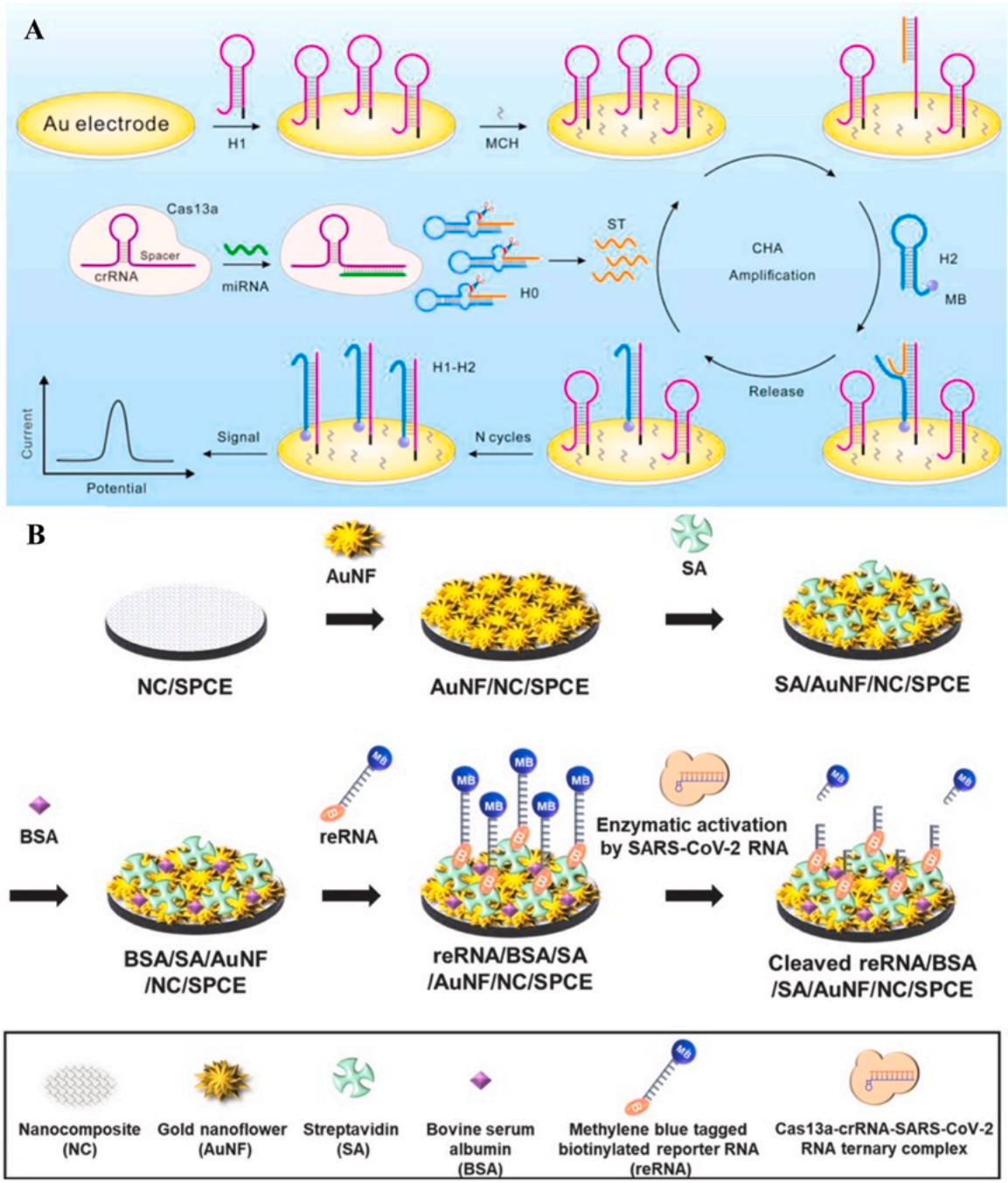

Fig. 6. (A) Illustration of the electrochemical biosensor scheme based on CRISPR/Cas13a with CHA reaction for detecting miRNA-21 (Reprinted by [155]) and (B) Illustration of the electrochemical biosensor scheme based on CRISPR/Cas13a for detecting SARS-CoV-2 (Reprinted by [38]).

signal. The accumulation of MB on the electrode surface generates an electrochemical signal proportional to the miRNA-21 concentration. The developed electrochemical biosensor demonstrated a broad linear detection range from 10 fM to 1 nM, with a detection limit of 2.6 fM, highlighting its high sensitivity and potential for molecular diagnostics and clinical applications [155].

Among the most researched electrochemical biosensor approaches for viral RNA detection is the CRISPR/Cas13a-based strategy for SARS-CoV-2 detection. Heo et al. (2022) developed a rapid, sensitive, and amplification-free nucleic acid detection method utilizing the trans-cleavage activity of CRISPR/Cas13a, coupled with a nanoparticle-structured electrode (Fig. 6b) methylene blue (MB)-labeled reporter RNA (reRNA) was immobilized on the electrode surface using a streptavidin-biotin system, while bovine serum albumin (BSA) was applied to block uncoated active sites. The viral RNA was extracted from saliva swab samples using a lysis buffer. The RNA was dissolved in a Cas13a-crRNA complex specifically designed to recognize the SARS-CoV-2 RNA sequence based on the crRNA's complementary binding to the target region through the PFS. Upon activation, Cas13a triggered non specific clevage of single-stranded RNA (ssRNA) leading to

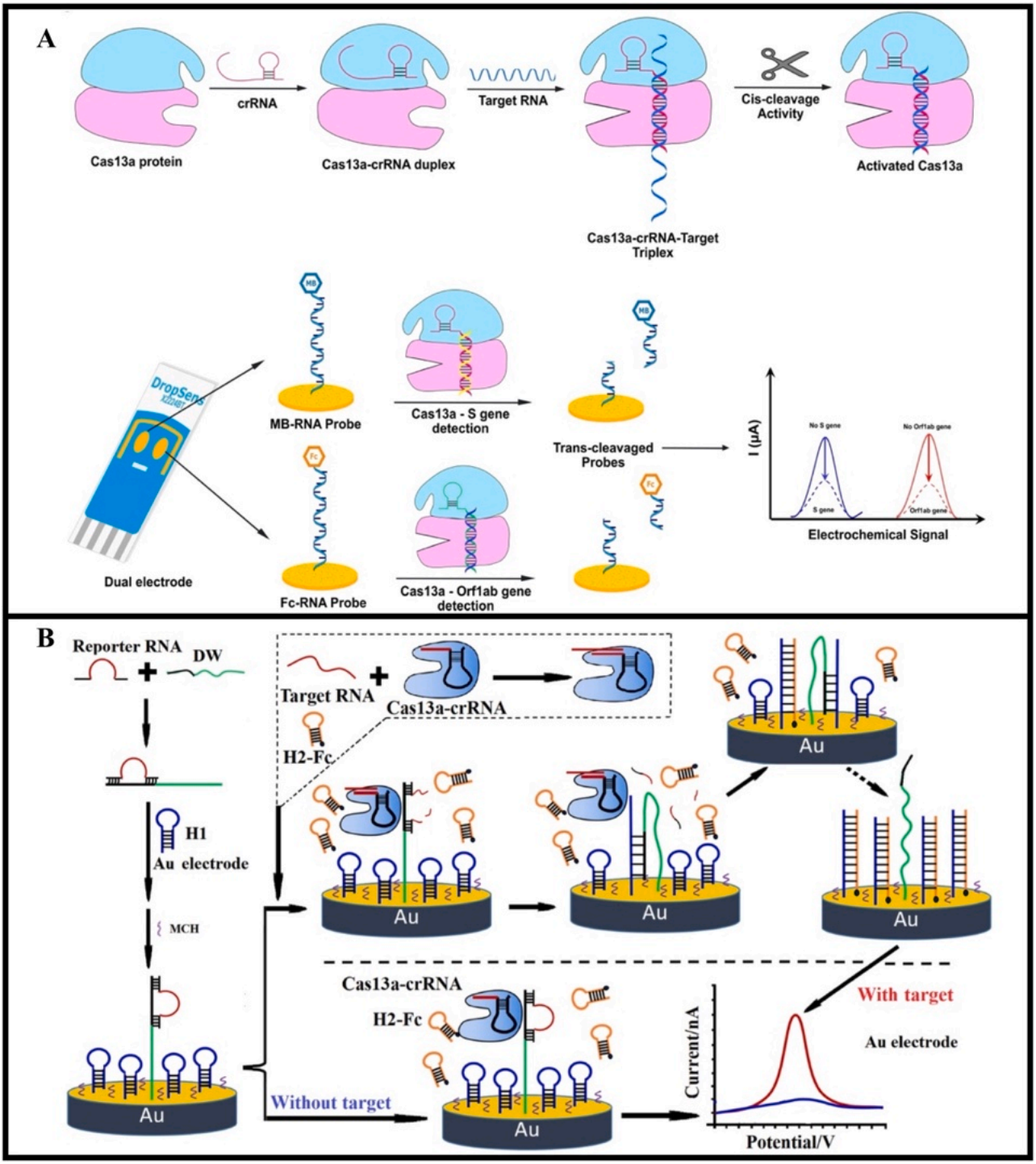

Fig. 7. Illustration of (A) the CRISPR/Cas13a-based electrochemical biosensor scheme for detecting SARS-CoV-2 (Reprinted by [156]) and (B) the CRISPR/Cas13a-based electrochemical biosensor scheme with CHA reaction for detecting DENV-1 (Reprinted by [40]).

collateral cleavage of reRNA into short RNA fragments and the release of MB labels from the electrode surface. This caused a reduction in electron transfer from the redox probe on the electrode surface, resulting in a decreased peak current measured by DPV. The biosensor exhibited exceptional sensitivity, capable of detecting the ORF and S genes of SARS-CoV-2 at remarkably low levels of 4.4×10^{-2} fg/mL and 8.1×10^{-2} fg/mL, respectively [38].

Expanding on the advancements in SARS-CoV-2 detection, Kashefi-Kheyrabadi et al. (2023) developed an electrochemical biosensor utilizing CRISPR/Cas13a and a screen-printed gold electrode (SPGE) with dual working electrodes. Unlike the study by Heo et al. (2022), this research employed thiolated reRNA immobilized via Au–S bonds to enhance detection efficiency. As shown in Fig. 7a, the reRNA was labeled with methylene blue (MB) and ferrocene (Fc), while nonspecific adsorption was prevented using mercaptohexanol (MCH). This system effectively detected the ORF1ab and S genes in both synthetic and clinical samples with remarkable sensitivity. The LoD reached 4.5 ag/μL for the ORF1ab gene and 2.5 ag/μL for the S gene. This enhanced performance underscores the potential of this biosensor platform for accurate and sensitive molecular diagnostics of SARS-CoV-2, offering a promising tool for both clinical and point-of-care testing applications [156].

Beyond SARS-CoV-2 detection, electrochemical biosensors integrated with CRISPR/Cas13a have also been applied to detect other RNA viruses, such as dengue virus (DENV-1). Wang et al. (2021) developed an electrochemical biosensor method combining CRISPR/Cas13a with DNA amplification via CHA. In this study, RNA samples of DENV-1 were used. RNA samples from DENV-1 were analyzed using a biosensor prepared by assembling reRNA to hybridize with the swing arm of a DNA walker (DW) and H1 on the surface of a gold electrode (Fig. 7b). The presence of DENV-1 RNA triggers trans-cleavage activity of CRISPR/Cas13a system, which leading to the cleavage of reRNA and the subsequent release of the swing arm of the DW. This released swing arm acts as an initiator for CHA, which occurs on the electrode surface through hybridization. The Opened H1 then hybridizes with ferrocene-labeled hairpin 2 (H2-Fc), allowing multiple H2-Fc molecules to be captured

Table 5
Electrochemical biosensors based on CRISPR/Cas13a.

Target	Electrode	Electrode modification	Nucleic acid amplification method	Measurement technique	LoD	Sample	Refs.
DENV-1	Gold	H1 & silenced DW	CHA	ACV	0.78 fM	Human serum RNA extraction	[40]
SARS-CoV-2	SPCE	-	Isothermal	SWV	~100 copies/ μL	Novel coronavirus nucleic acid standar	[157]
SARS-CoV-2	SPCE	AuNF/NC & biotin-ssRNA-MB	-	DPV	4.4×10^{-2} fg/mL (ORF gene) and 8.1×10^{-2} fg/mL (gen S)	Spiked RNA from artificial saliva	[38]
SARS-CoV-2 RNA	SPCE	-	RT-RAA	SWV	low as 1.66 aM	Novel Coronavirus Nucleic Acid Standard	[158]
SARS-CoV-2 RNA	SPGE	Microfluidic device	-	SWV	10 aM	nasopharyngeal swab clinical samples	[159]
SARS-CoV-2 L452R mutation	Gold	Composite Mxene-AuNP & MB-ssRNA-tertiolasi	-	SWV	1 fM	Nasopharyngeal swab	[39]
SARS-CoV-2	SPGE	Thiolated reRNA	-	SWV	2.5 ag/μL (gen S) & 4.5 ag/μL (gen Orf1ab)	RNA isolation and extraction	[156]
SARS-CoV-2	SPCE	DNA tetrahedron	-	SWV	89.86 aM (gen RdRp)	Swab faring	[160]
miRNA-19b & miRNA-201 as tumor biomarkers	Electrochemical cell	-	-	DPV	2 pM	Human serum	[161]
miRNA-19b & miRNA-201 as tumor biomarkers	Electrochemical cell	-	-	Ampero-metry	10 pM	Human serum	[162]
miRNA-21 as lung, breast, and stomach cancer biomarkers	Gold	H1 & MCH	CHA	DPV	2.6 fM	Human serum	[155]
miRNA-17, miRNA-155, miRNA-19b, miRNA-210, TTF-1 mRNA, and EGFR mRNA as a non-small cell lung cancer (NSCLC) biomarkers	SPGE	Thio-DNA	CHDC	SWV	50 aM	Human blood	[163]
miRNA-19b as a tumor biomarkers	mSPCE	Electrodeposition of AuNP & tetrahedral DNA framework	-	Amperometry	10 pM	Artificial serum sample	[41]
circRNA as a bladder cancer biomarkers	Gold	DNA tetrahedron	-	SWV	0.089 fM	Urine	[42]
miR-21	Gold electrode	-	-	EIS, CV, SWV	30.2 fM	Plasma samples	[152]
Yersinia pestis, F. tularensis, Chlamydia psittaci, B. mallei, B. pseudomallei, and Brucella melitensis.	Screen-printed carbon electrode (SPCE) on a CHI 760E	Multi-chamber electrochemical microfluidic chip (MEM Chip)	RPA	SWV and Optical	1 pM (25 min) or 30 zM (45 min with assistance of RPA)	Mouse organs (heart, liver, spleen, lungs, kidneys) and powders (flour, sugar, milk)	[64]
BNP as a heart failure biomarker	Gold	Fc-labeled DNA	-	DPV	0.74 fM	Human serum	[153]
miRNA-21 as colorectal cancer biomarker	Gold	MB-labelled HP probe	Nicking-mediated DNA cascade reaction (NDCR)	DPV, EIS	8.26 fM	Blood plasma	[164]

by the electrode, generating an electrochemical signal measured using alternating current voltammetry (ACV). This strategy successfully detected DENV-1 RNA extracted from samples, demonstrating a broad linear detection range from 5 fM to 50 nM, with a detection limit as low as 0.78 fM. This approach showcases the potential for highly sensitive and specific RNA virus detection through innovative biosensor technologies [40]. Key developments of electrochemical biosensors using CRISPR-Cas13a are shown in Table 5.

5.2. ECL biosensors

Researchers have further explored the integration of ECL technology to enhance detection sensitivity and signal stability. By coupling CRISPR/Cas13a with ECL, biosensors can achieve highly specific and amplified signal responses, making them particularly useful for clinical diagnostics. Wei et al. (2023) developed an innovative method for SARS-CoV-2 detection using an ultrasensitive isothermal amplification system called the 'Entropy-driven triggered T7 amplification-CRISPR/Cas13a system' (EDT-Cas). As shown in Fig. 8, this system leverages RNA amplification by T7 RNA polymerase and Cas13a cleavage activity to detect the RdRp gene of SARS-CoV-2. The Ti_3C_2Tx-based ECL signal molecules provide unique advantages in improving detection sensitivity and electrode surface modification. The process begins with entropy-driven amplification to form a T7 promoter, which subsequently triggers large-scale RNA transcription by T7 RNA polymerase. The transcribed RNA is then recognized by the Cas13a/crRNA complex, which cleaves the target RNA and initiates collateral cleavage of a DNA reporter probe containing -U-U- sequences immobilized on the electrode surface. This cleavage releases ferrocene (Fc) molecules, leading to significant changes in the ECL signal. The EDT-Cas system integrates three key stages: isothermal amplification, RNA transcription, and CRISPR/Cas13a cleavage, enabling rapid, sensitive, and efficient detection of SARS-CoV-2. To validate the biosensor, two sample types were tested: (1) SARS-CoV-2 RdRp gene spiked into healthy human throat swabs and environmental samples at various concentrations and (2) SARS-CoV-2 sprayed onto pork and food packaging, stored for 10 days, and subsequently tested. The results demonstrated a recovery rate of 96.70 %–104.56 % for the RdRp gene, confirming the biosensor's capability to detect the target gene in both human and environmental samples. The method achieved a detection limit as low as 7.39 aM and reliably identified the RdRp gene in clinical samples. Additionally, the system exhibited excellent reliability and stability, effectively detecting SARS-CoV-2 in throat swabs and environmental samples using the EDT-Cas system [165].

Expanding the application of CRISPR/Cas13a-based ECL biosensors beyond viral RNA detection, Liu et al. (2024) developed a dry chemistry-based ECL gene sensor for RNA detection without the need for amplification. The sensor employs an ECL probe based on poly-(L-lysine) (PLL) covalently bound to the luminescent agent Ru(II), amplifying signals without additional reagents on a cloth-based closed bipolar electrode-ECL (C-BPE-ECL) chip. Cas13a's trans-cleavage enhances signal detection, while the dual-recognition mechanism of CRISPR/Cas13a ensures high specificity. This sensor demonstrated high sensitivity (range: $10–10^5$ fM, detection limit: 0.65 fM) and successfully detected RNA from *E. coli* O157:H7 within 20 min in urine and blood samples. Its efficient design reduces material consumption, supports multi-channel detection, and includes a portable ECL analyzer for automated data collection and analysis. Cas13a cleavage is activated in the presence of the target, cutting molecular beacon (MB) probes via Cas13a trans-cleavage (Fig. 9a). The cleaved products are magnetically separated and applied to a hybridization reaction-based DNA sensor system and a self-enhanced ECL system (Fig. 9b and c). In the hybridization reaction, cleaved products form S2-P complexes and are directed to the detection pad zone, where they bind to CP2 to form S2-P-CP2

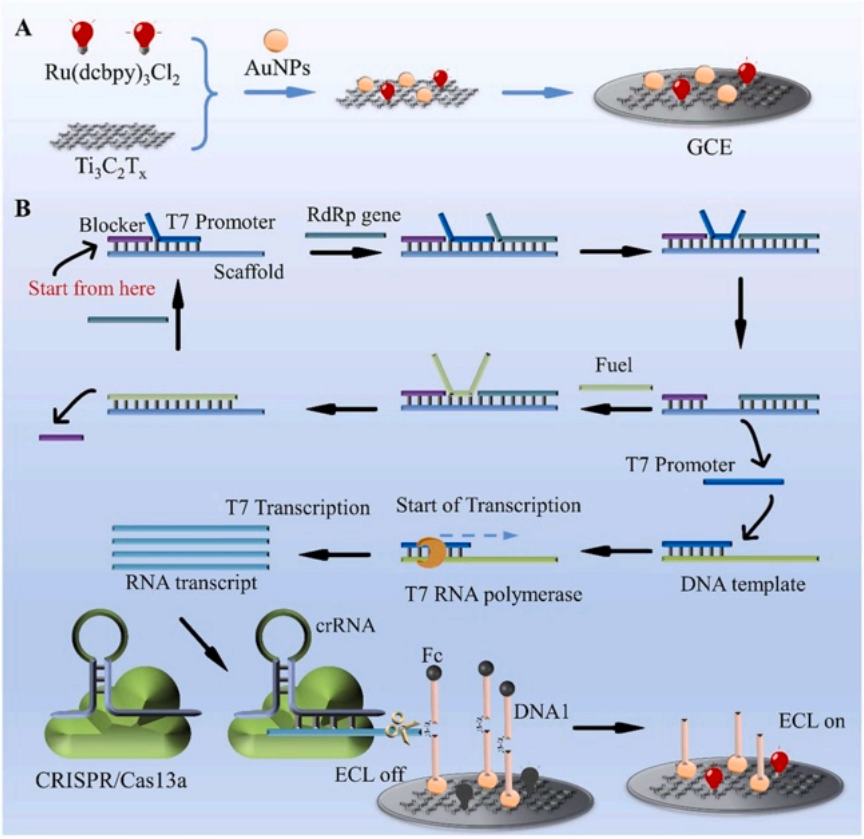

Fig. 8. EDT-Cas system based on ECL for RdRp gene detection of SARS-CoV-2 (Reprinted by [165]).

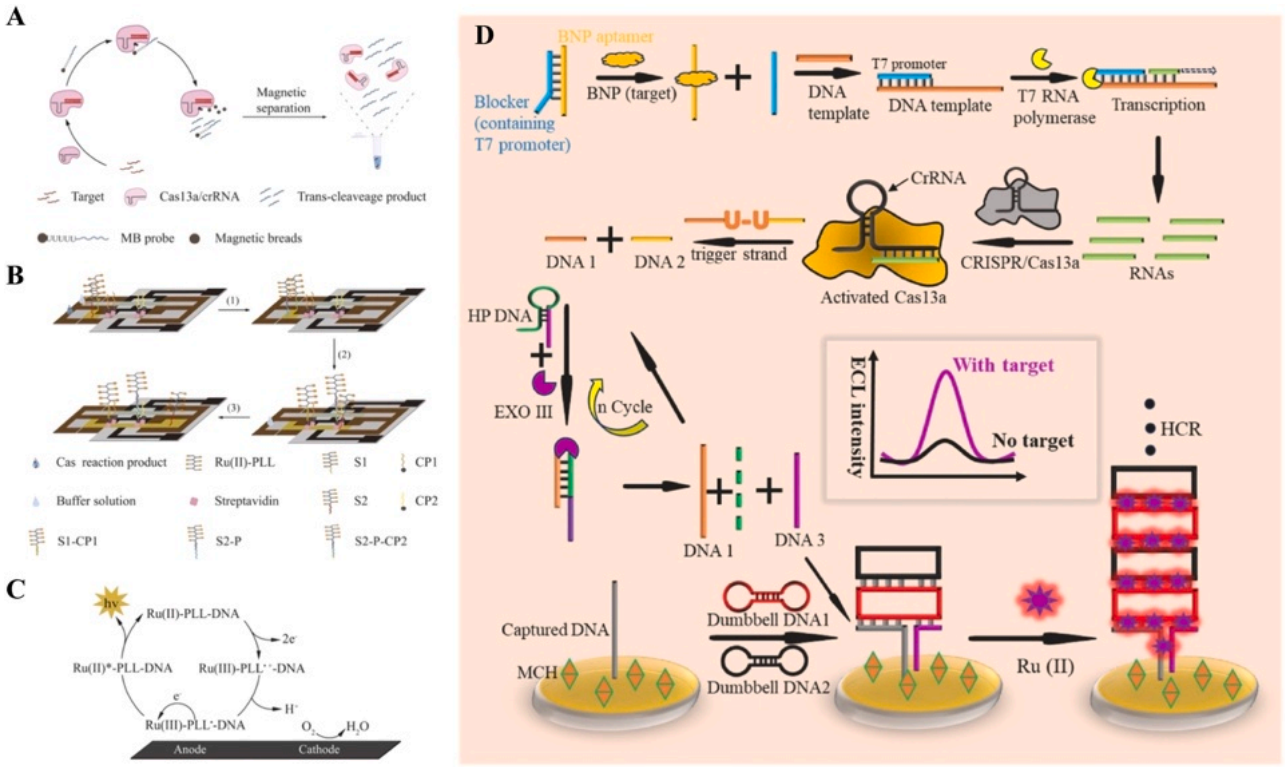

Fig. 9. Demonstrating the working principle of the DC-ECL-CRISPR gene sensor to detect RNA. A) Mechanism of the CRISPR/Cas13a reaction, B) Mechanism of the hybridization reaction, C) Principle of the ECL reaction based on the Ru(II)-PLL-DNA system (Reprinted by [54]). D) Schematic illustration of T7 RNA polymerase-assisted amplification in the CRISPR/Cas13a system to detect BNP using the ECL sensor (Reprinted by [49]).

complexes. If the target is absent, no hybrid complexes form. Simultaneously, S1 probes (self-enhanced ECL probes) flow to the control zone and form S1-CP1 complexes with CP1. After the hybridization reaction, the ECL reaction is triggered by applying a specific voltage to the driving electrode, generating an ECL signal in the detection zone. The T/C signal intensity positively correlates with the target concentration. In this system, poly-(L-lysine) (PLL) acts as an intra-molecular co-reactant for Ru(II), a mechanism confirmed through cyclic voltammetry and ECL experiments. This innovative design facilitates highly sensitive and specific RNA detection in a portable and efficient format [54].

Beyond bacterial RNA detection, ECL biosensors coupled with CRISPR/Cas13a have also been explored for detecting biomarkers associated with cardiovascular diseases. Zhang et al. (2024) focused on the detection of B-type natriuretic peptide (BNP), an important biomarker for heart failure (HF), which is challenging to detect due to its low concentration and short half-life in blood (Fig. 9d). This study utilized a CRISPR/Cas13a-based ECL sensor platform assisted by T7 RNA polymerase for amplification. The system integrates hairpin-exonuclease III (Exo III) amplification and a dumbbell-shaped hybridization chain reaction (HCR). The BNP aptamer strand in the dual Blocker/aptamer BNP structure specifically binds to BNP, releasing a single-stranded Blocker containing a T7 promoter sequence. This Blocker strand binds to the DNA template, generating large amounts of RNA through T7 RNA polymerase amplification. The transcribed RNA activates CRISPR/Cas13a, which cleaves the trigger strand to produce DNA1 and DNA2. The co-amplification reaction between DNA1 and DNA hairpin through the Exo III mechanism generates a short DNA3 strand, which plays a key role in exponentially opening the dumbbell structure of DNA1/DNA2 via the dumbbell-shaped HCR technique. The HCR product enables the integration of Ru(II) luminescent molecules, producing a strong ECL signal on the sensor platform. This system achieved a detection limit as low as 3.2 fg/mL and demonstrated recovery rates ranging from 98.4 % to 103 % in human serum samples, making it a promising tool for early heart failure detection through the synergy of multiple amplification strategies and CRISPR/Cas13a specificity [49]. Table 6 presents reported developments of CRISPR-Cas13a-integrated ECL biosensors.

5.3. PEC biosensors

PEC biosensors have emerged as a powerful tool for RNA detection, offering highly sensitive photocurrent responses and minimal background noise. By coupling CRISPR/Cas13a with PEC technology, researchers have developed innovative biosensors capable of achieving high specificity and efficiency for nucleic acid detection in clinical and environmental applications. For instance, Zhang et al. (2024) developed a PEC-based biosensor for detecting miRNA-21 by integrating CRISPR/Cas13a, nanozyme catalysis, and an organic photoelectrochemical transistor (OPECT) system (Fig. 10). An indium tin oxide (ITO) electrode was modified with a $CdIn_2S_4/SiW_{12}$ hybrid semiconductor material to enhance photoelectrochemical performance. The presence of the miRNA-21 target activated the CRISPR/Cas13a system, leading to the cleavage of DNArU (a single-stranded DNA containing multiple uracil ribonucleotides). This cleavage event triggered the release of glucose oxidase (GOx), which produced hydrogen peroxide (H_2O_2). In the presence of SiW_{12}, H_2O_2 reacted with 4-chloro-1-naphthol (4-CN) to form benzo-4-chloro-hexadienone (4-CD), resulting in signal suppression. This strategy achieved an ultra-low detection limit of 0.53 fM, demonstrating exceptional sensitivity for miRNA-21 detection [170].

Similarly, Jiang et al. (2023) developed a PEC biosensor for miRNA-21 detection, utilizing MoS_2@AuNPs-modified ITO electrode. The electrode surface was functionalized with capture DNA (SH-DNA) via Au-S bonds, followed by passivation with MCH to minimize nonspecific interactions (Fig. 11). A probe containing uracil ribonucleotide sequences (biotin–rU–DNA) was designed as a CRISPR/Cas13a target. In the absence of miRNA-21, the probe remained bound to SH-DNA,

Table 6
ECL biosensors based on CRISPR/Cas13a.

Targetz	Electrode	Electrode modification	Amplification method	Measurement technique	LoD	Sample	Refs.
MiRNA-17	Screen-printed bipolar electrode (BPE)	-	Isothermal exponential amplification reaction (EXPAR)	ECL	1×10^{-15} M	RNA extracted from human cancer cell lines (MDA-MB-231, MCF-7, HepG2) and a normal liver cell line (LO2)	[166]
SARS-CoV-2 RdRp gene	glassy carbon electrode (GCE)	AuNPs Ti_3C_2Tx/Ru (II)-PEI	entropy-driven cyclic amplification	CV, EIS, and ECL	7.39 aM	human pharyngeal swabs and environmental samples	[165]
miRNA-21	GCE	-	-	CV and ECL	0.53 fM		[167]
E. coli O157:H7 RNA	closed bipolar electrode-ECL (C-BPE-ECL) lateral flow chip	-	-	ECL	0.65 fM	clinical human urine and blood samples	[54]
Matrix metalloproteinase-2 (MMP-2)	GCE	-	EXPAR	ECL	12.8 aM	MMP-2 in complex biological samples	[55]
Brain natriuretic peptide (BNP)	Gold Electrode	-	HCR	ECL, EIS, CV	3.2 fg/mL	human serum samples	[49]
MiR-17	Gold Electrode	-	HCR	ECL, EIS, CV	14.38 aM	complex biological samples (cell lysates)	[168]
E. coli 16S rRNA	multichannel closed bipolar electrode	CPT & CPC	-	ECL	63.8 cfu/mL	human blood samples	[169]

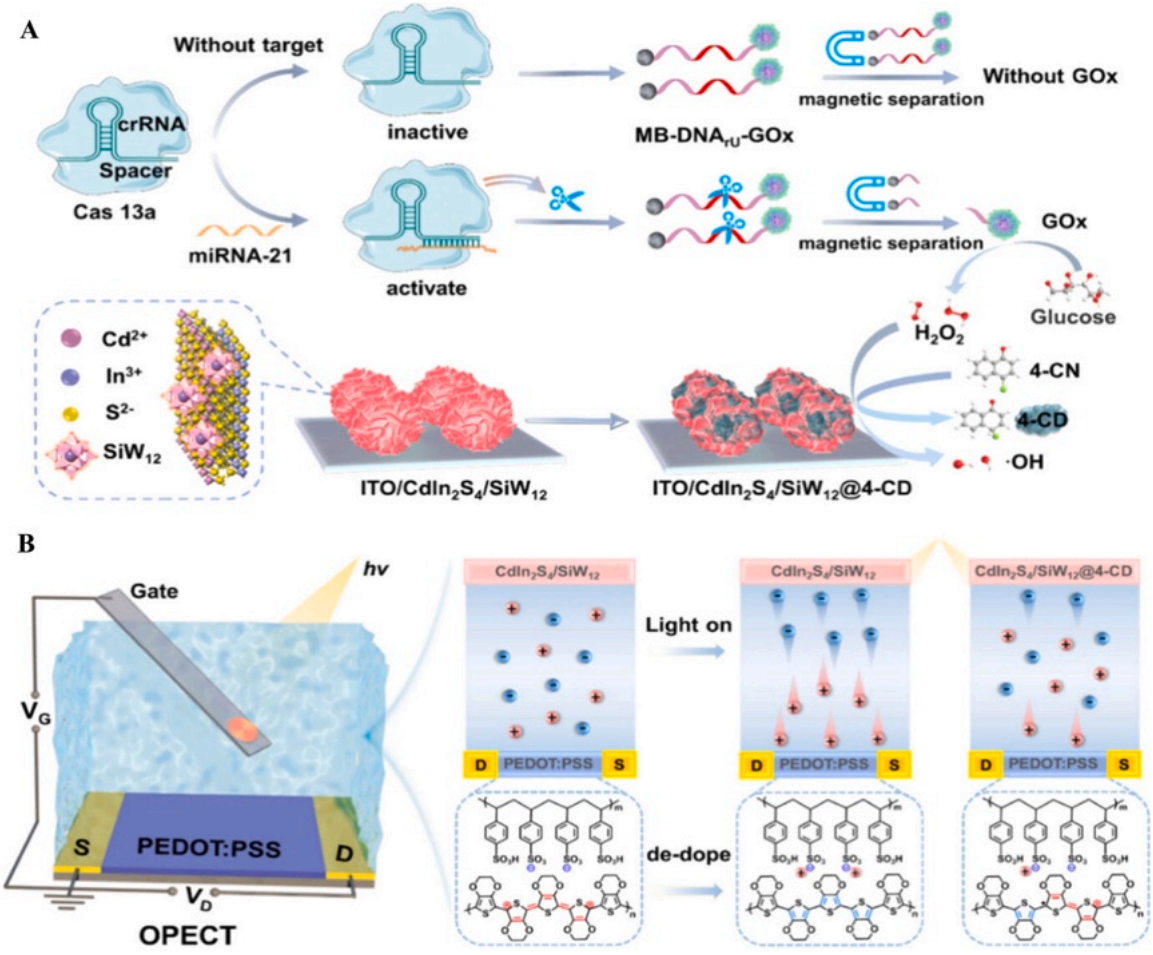

Fig. 10. (A) CRISPR/Cas13a-assisted nanozyme cascade reaction at the ITO/CdIn$_2$S$_4$/SiW$_{12}$ gate, and (B) OPECT configuration its associated regulation mechanism [170].

allowing streptavidin to attach to the electrode surface, which inhibited electron transfer and generated a low photocurrent signal. However, when miRNA-21 was present, Cas13a was activated, leading to cleavage of the probe and reducing its hybridization with SH-DNA, thereby increasing electron transfer and significantly enhancing the PEC signal. This biosensor exhibited a linear detection range of 1 fM to 5 nM, with a detection limit of 1 fM, making it a highly effective platform for direct RNA detection without requiring DNA conversion [65]. Table 7 highlights reported developments of CRISPR-Cas13a-assisted PEC biosensors.

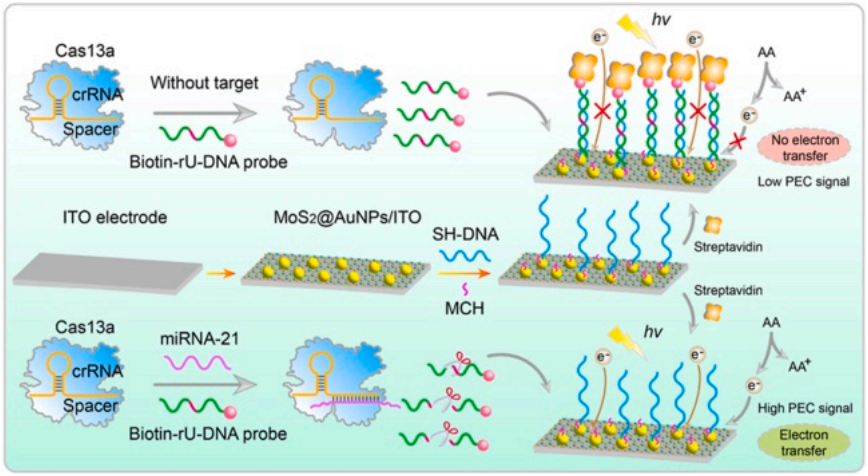

Fig. 11. Schematic of the Cas-PEC biosensor for the specific and direct assay of miRNA-21 (Reprinted by [65]).

Table 7
CRISPR/Cas13a-based PEC biosensors.

Target	Electrode	Electrode modification	Amplification method	Measurement technique	LoD	Sample	Refs.
miRNA	ITO electrode	MoS_2@AuNPs	-	PEC	1 fM	Human serum samples	[65]
MiRNA-21	FTO glass (Fluorine-doped Tin Oxide glass)	Branched-TiO_2 Nanorods (B-TiO_2 NRs)	-	PEC	9 fM	Human serum samples	[66]
miRNA-21	ITO electrode	$CdIn_2S_4/SiW_{12}$@4-CD	-	OPECT (organic photoelectrochemical transistor)	0.53 fM	Human serum samples	[170]
circRNA	ITO electrode	T-COF/Ag_2S QDs	-	PEC	0.5 fM	Whole blood of lung cancer patients	[63]

6. Challenges and future prospect

Despite significant progress in the development of CRISPR-Cas13a-based electrochemical biosensors, several fundamental challenges remain before their widespread clinical application can be realized. Cas13a offers a distinct advantage over DNA-targeting CRISPR systems by directly recognizing and cleaving RNA targets, thus eliminating the need for reverse transcription into cDNA. This reduces reagent complexity, shortens assay times, and lowers the overall cost—key benefits when developing rapid and accessible diagnostic platforms. Electrochemical detection further complements this simplicity by enabling miniaturized, low-power, and cost-effective readouts that are compatible with portable and even disposable formats. However, the translation of these molecular systems into robust diagnostic tools still faces critical hurdles. Achieving high analytical specificity—particularly in discriminating highly homologous RNA sequences—remains a persistent challenge, often requiring intensive optimization of guide RNA sequences and reaction conditions. Off-target trans-cleavage activity can also compromise assay selectivity and increase false-positive rates, particularly in complex biological matrices. Additionally, maintaining sensor stability and reproducibility over time and across production batches continues to be a bottleneck, particularly when immobilizing biomolecules onto electrode surfaces. Integration into portable formats also demands expertise in microfluidic engineering, as sample handling, reagent delivery, and signal detection must be seamlessly automated in a miniaturized environment. To address these limitations, lab-on-chip technologies are increasingly being explored. A notable example is the CRISPR-Cas13a-powered ECL microfluidic chip developed by Zhou et al., which achieved a limit of detection as low as 1 fM while maintaining a compact, user-friendly format [166]. Such systems exemplify the potential of integrated, amplification-free CRISPR biosensors to meet the performance, portability, and usability standards required for point-of-care diagnostics.

The integration of artificial intelligence (AI) and the Internet of Things (IoT) into CRISPR-Cas13a-based biosensor platforms is poised to revolutionize molecular diagnostics by enhancing both analytical performance and operational accessibility. AI technologies can be strategically employed across various stages of biosensor development, from the in silico optimization of guide RNA sequences to real-time data interpretation and predictive modeling. Deep learning and machine learning algorithms are increasingly utilized to predict RNA secondary structures, minimize off-target effects, and select Cas13a target regions with maximal cleavage efficiency. For instance, Zhang et al. (2023) demonstrated a data-driven framework that significantly improved the predictive power of CRISPR-Cas13a activity by training neural networks on large-scale kinetic datasets, allowing for accurate forecasting of detection performance and reducing empirical trial-and-error processes [171]. Such tools are invaluable for accelerating assay development while maintaining high sensitivity and specificity, particularly when targeting diverse RNA viruses or low-abundance biomarkers.

On the other hand, IoT-enabled biosensing systems are gaining traction due to their ability to bridge laboratory-grade performance with real-world usability. These platforms can integrate wireless connectivity, mobile-device interfaces, and cloud-based analytics to enable remote access, centralized data management, and instant diagnostic feedback. A prominent example is the work of Weidmann et al. (2025), who developed a compact, smartphone-interfaced fluorescence detection system for isothermal molecular assays [172]. This system utilizes a low-power Bluetooth module to transmit raw fluorescence data from a miniaturized sensor (based on the AS7341 multispectral chip) to a smartphone application, which in turn uploads the information to a secure cloud server for automated result processing. The diagnostic outcome is then displayed on the user's device within minutes, enabling sample-to-result molecular detection outside of traditional laboratory

settings. The device demonstrated high accuracy for SARS-CoV-2 detection using RT-RPA, with lyophilized reagents and CE-IVDD certified components, making it suitable for field use and scalable manufacturing.

These emerging digital biosensing frameworks align well with the evolving concept of "smart diagnostics"—integrated systems that combine biochemical specificity with algorithmic intelligence and digital connectivity. By merging AI-guided CRISPR-Cas13a biosensor design with IoT-enabled electrochemical or optical signal processing, future diagnostic tools could achieve rapid, automated decision-making, enabling not only individual diagnostics but also population-level disease surveillance. Moreover, smartphone integration supports user-friendly interfaces, geo-tagged epidemiological data logging, and even interoperability with electronic health records, thereby promoting precision public health.For example, Hu et al. (2025) developed a portable, smartphone-integrated RT-RPA-assisted CRISPR-Cas12a/Cas13a device that still achieves ultra-sensitive RNA detection (0.5 copies/μL) within 15 min [173]. This system uses lyophilized reagents, sample-in-result-out automation, and mobile control via a dedicated app, making it particularly suitable for field use or resource-limited settings. Such platforms combine with CRISPR/Cas13a system significantly reduce reagent consumption and instrumentation needs compared to, i.e., RT-qPCR, thereby facilitating rapid diagnostics without sacrificing analytical performance. In this context, integrating CRISPR-Cas13a with lab-on-chip and smartphone-based electrochemical readouts could enable next-generation, amplification-free biosensors with enhanced portability, automation, and connectivity. This opens up future prospects for decentralized molecular diagnostics, particularly for infectious disease detection, surveillance, and management in remote or underserved regions.

Another important prospect of CRISPR-Cas13a-based electrochemical biosensors is the advancement of multiplexed detection systems capable of identifying multiple RNA targets in a single assay [174]. This functionality is particularly advantageous for diagnosing complex infectious diseases or cancer, where co-detection of distinct biomarkers enhances diagnostic accuracy and clinical relevance. Kashefi-Kheyrabadi et al. (2023) developed an amplification-free multiplexed CRISPR-Cas13a electrochemical biosensor to detect two SARS-CoV-2 genes—S and Orf1ab—using redox-tagged probes (methylene blue and ferrocene) on a dual-electrode platform [156]. The biosensor demonstrated ultra-sensitive detection limits of 2.5 ag/μL (26.2 copies/μL) for S and 4.5 ag/μL (53.5 copies/μL) for Orf1ab, with high specificity, even distinguishing single-nucleotide mismatches and unrelated viral RNAs. Complementing this, Mao et al. (2025) introduced a fully integrated modular microfluidic system that combines nucleic acid extraction, CRISPR-Cas13a detection, and digital signal output to simultaneously detect Influenza A virus (IAV) and Respiratory Syncytial Virus (RSV) RNA in clinical throat swab samples [169]. Their platform achieved limits of detection of 6.6 copies/μL for IAV and 8.4 copies/μL for RSV, with robust signal separation across dual microchambers and orthogonal fluorescence channels. These studies highlight how CRISPR-Cas13a multiplexed biosensing platforms—whether electrochemical or microfluidic—can be effectively designed to distinguish between closely related RNA targets in a single run, thereby enabling more efficient, accurate, and comprehensive molecular diagnostics.

As CRISPR-based biosensing platforms continue to advance, the integration of multiple signal transduction strategies—particularly electrochemical, ECL, and PEC methods—has emerged as a powerful approach to enhance analytical performance. These hybrid systems leverage the distinct strengths of each technique: the high sensitivity and simplicity of electrochemical detection, the low background and signal amplification of ECL, and the light-driven signal enhancement and polarity-switching capabilities of PEC. Such combinations enable improved signal-to-noise ratios, broader dynamic ranges, and built-in dual-mode confirmation that increases analytical reliability. A notable example is the CRISPR-Cas12a-based biosensor developed by Zheng et al. (2024), which employs catalytic hairpin assembly (CHA) and Cas12a-mediated cleavage to release methylene blue-loaded liposomes for dual EC and PEC detection [171]. This system achieved an exceptionally low limit of detection of 0.11 aM and demonstrated high selectivity against single-base mismatches, enabling accurate RNA target quantification over a wide concentration range. The dual-mode output not only enhances detection confidence but also mitigates false-positive results, which is crucial for clinical diagnostics. With continued refinement, such multi-modal CRISPR biosensing platforms offer a compelling route toward highly sensitive, selective, and portable lab-on-chip systems suitable for real-world point-of-care applications.

7. Consclusion

CRISPR/Cas13a-based electrochemical biosensors have emerged as powerful tools for the sensitive and specific detection of RNA, offering unprecedented potential in various diagnostic fields. This technology has demonstrated remarkable sensitivity, with detection limits often reaching the femtomolar range or lower, making it ideal for applications in clinical diagnostics, infectious disease detection, and cancer biomarker identification. The integration of CRISPR/Cas13a with electrochemical methods has greatly enhanced the performance of biosensors. For instance, SWV has been effectively used to detect RNA targets by measuring the current changes when CRISPR/Cas13a cleaves the RNA, providing highly sensitive and rapid analysis. In this context, SWV detects the activation of Cas13a's trans-cleavage activity, where the target RNA triggers collateral cleavage of nearby RNA sequences, leading to a measurable electrochemical response. In integration with ECL, the CRISPR/Cas13a-induced cleavage activates luminophores, such as ruthenium or luminol, which emit light when excited, allowing for highly sensitive detection with minimal background noise. This method benefits from the high specificity of CRISPR/Cas13a, as it cleaves target RNA precisely, triggering strong, reliable light emission. In PEC based biosensors, CRISPR/Cas13a cleaves RNA, influencing the flow of photoinduced electrons, which amplifies the detection signal. This system not only enhances sensitivity but also provides additional versatility by coupling CRISPR/Cas13a's RNA-cleaving activity with light-driven electrochemical processes, further increasing the potential for rapid, sensitive, and selective RNA detection.

Key studies have highlighted the versatility and promise of this technology. Gong et al. (2024) developed a CRISPR/Cas13a-based electrochemical biosensor for BNP detection, achieving exceptional sensitivity in human serum, while Cheng et al. (2023) demonstrated the potential for circRNA detection in urine, offering a non-invasive approach for cancer diagnostics. Wei et al. (2023) combined CRISPR/Cas13a with entropy-driven amplification and ECL to detect SARS-CoV-2, achieving ultra-sensitive results. Dong et al. (2023) further advanced the field with a portable CRISPR/Cas13a-based biosensor for pathogen detection, highlighting the applicability of this technology for point-of-care testing (POCT) and rapid diagnostics.

Despite these advancements, several challenges remain for the widespread adoption of CRISPR/Cas13a-based electrochemical biosensors. Issues such as biosensor stability, scalability, and non-specific binding require further optimization. Nevertheless, the progress made so far underscores the immense potential of this technology to revolutionize molecular diagnostics and precision medicine. As research continues, these biosensors could become more accessible, cost-effective, and reliable, providing invaluable tools for early disease detection, personalized medicine, and global health monitoring.

CRediT authorship contribution statement

Salma Nur Zakiyyah: Writing – original draft, Visualization, Investigation, Formal analysis. **Irkham:** Writing – review & editing, Validation, Supervision, Funding acquisition, Conceptualization. **Karina Salsabiila Putri Sima:** Resources, Investigation, Data curation.

Clianta Yudin Kharismasari: Writing – review & editing, Visualization, Data curation. **Mengzhen Xi:** Writing – review & editing, Data curation. **Shabarni Gaffar:** Validation, Supervision. **Mehmet Ozsoz:** Writing – review & editing, Validation, Supervision, Conceptualization. **Francesco Paolucci:** Validation, Supervision, Methodology. **Giovanni Valenti:** Writing – review & editing, Validation, Supervision. **Yeni Wahyuni Hartati:** Writing – review & editing, Validation, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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