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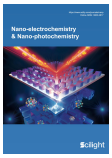

Review

CRISPR/Cas Powered Electrochemiluminescence Biosensing

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Abstract: In the face of fast-changing global public health threats, there is an urgent demand for novel detection platforms that combine high sensitivity with robust selectivity in real samples. Electrochemiluminescence (ECL), as a powerful analytical tool, offers excellent sensitivity and low background for biosensing. However, its reliance on traditional recognition elements limits the specificity and further applications in trace-level target detection. The emergence of CRISPR/Cas systems, with their programmable recognition and powerful signal amplification via nonspecific *trans*-cleavage activity, presents a transformative opportunity. In this review, we focus on the development of CRISPR/Cas-powered ECL biosensing platforms for overcoming these challenges. We first introduce the fundamental mechanisms and main integration strategies that couple the precise nucleic acid recognition and collateral cleavage of CRISPR/Cas with the sensitive light emission of ECL transduction. Subsequently, we systematically highlight the broad applicability of these hybrid platforms for detecting diverse targets, including pathogenic nucleic acids and small molecules, with direct relevance to disease diagnostics, environmental monitoring, and food safety. Finally, we discuss the current challenges and future prospects of these platforms, emphasizing their potential as next-generation tools for field-deployable, ultra-sensitive analysis in public health security.

Keywords: CRISPR/Cas; electrochemiluminescence; biosensing; signal amplification; public health

1. Introduction

Global public health security faces an increasingly complex landscape of threats, ranging from the rapid mutation of viral pathogens like SARS-CoV-2, the re-emergence of zoonotic diseases, to the insidious accumulation of environmental toxins and foodborne contaminants [1–4]. These biological and chemical hazards rarely exist in isolation; they often exist at ultralow concentrations within complex, heterogeneous matrices, including whole blood [5], soil [6], plant tissues [7], and wastewater, etc. [8]. While established laboratory techniques like polymerase chain reaction (PCR) and chromatography-mass spectrometry (GC-MS) provide definitive identification, the reliance on centralized infrastructure and labor-intensive sample pretreatment limits their utility for the rapid, on-site decision-making required in modern epidemiology and environmental monitoring [9,10]. Consequently, there is a pressing demand for detection platforms that combine laboratory-grade sensitivity with field-deployable potential [11].

Electrochemiluminescence (ECL) has emerged as a highly promising analytical technique capable of bridging this gap. As a light emission process triggered by electrochemical reactions at electrode surfaces, ECL uniquely integrates the advantages of electrochemistry and chemiluminescence, offering high sensitivity, a wide dynamic range, low background noise, and excellent spatiotemporal control [12,13]. Unlike conventional optical

methods such as fluorescence or colorimetry, ECL does not require an external light source for excitation, thereby effectively eliminating issues related to light scattering and autofluorescence, which are common challenges in analyzing complex biological and environmental samples [14]. These attributes have established ECL as a powerful platform for diverse applications, including life analysis, environmental monitoring, and food testing. Despite these inherent advantages, the overall performance of an ECL biosensor is fundamentally governed by the specificity and efficiency of its molecular recognition element. Conventional ECL systems often rely on antibody-antigen binding or nucleic acid hybridization, which may suffer from limited selectivity in distinguishing highly similar sequences (e.g., single-nucleotide polymorphisms) or insufficient sensitivity for trace-level targets [15,16]. To advance ECL toward next-generation, ultra-sensitive diagnostics, it is essential to integrate it with a programmable and amplifiable molecular recognition mechanism [17].

Originally discovered as an adaptive immune mechanism in prokaryotes, the clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) system has been repurposed beyond revolutionary gene-editing applications to serve as a versatile and programmable molecular detection platform [18]. Central to this functionality are Cas effector proteins, such as Cas12 and Cas13, which can be directed by a designed guide RNA (gRNA) to specifically recognize target nucleic acid sequences [19]. The system achieves exceptional specificity through the requirement for precise base-pairing between the gRNA and its target, enabling discrimination down to single-nucleotide mismatches. Upon target binding, certain Cas proteins exhibit nonspecific "*trans*-cleavage" (or collateral cleavage) activity, enabling them to indiscriminately degrade surrounding reporter nucleic acids. Such enzymatic turnover acts as a powerful biological amplifier; a single recognition event can catalyze the cleavage of thousands of reporter molecules, thereby converting a scarce molecular signal into a detectable chemical output [20,21]. The CRISPR/Cas system, with its high specificity, modular design, and inherent nucleic acid-triggered *trans*-cleavage activity, offers an ideal molecular engine to empower ECL sensing, enabling both precise target identification and substantial signal enhancement.

In this review, we present a comprehensive summarization of the development of CRISPR/Cas-powered ECL platforms. The main integration strategies and the mechanism of signal transduction are first introduced. Subsequently, the broad applicability of these systems in detecting varying targets is highlighted and systematically summarized. Finally, challenges and prospects are discussed for CRISPR/Cas-powered ECL platforms in public health monitoring.

2. Fundamentals and Signal Transduction Mechanisms of CRISPR/Cas Powered ECL Platforms

The CRISPR/Cas system acts as an adaptive immune mechanism in most archaea and bacteria, providing defense against invading foreign genetic elements such as phages and plasmids [22]. Based on the architecture of their effector complexes, CRISPR-Cas systems are broadly categorized into Class 1 (utilizing multi-subunit effector complexes) and Class 2 (relying on a single effector protein) [23]. The latter, due to its structural simplicity and programmability, has garnered significant attention in molecular diagnostics [24]. Key representatives of Class 2 include Type II (Cas9), Type V (Cas12), and Type VI (Cas13), each possessing distinct cleavage properties that define their application scope.

As shown in Figure 1, Cas9 is guided by a single-guide RNA (sgRNA) to recognize a protospacer sequence adjacent to an NGG protospacer adjacent motif (PAM) and introduces a site-specific double-strand break in dsDNA through coordinated cleavage by the HNH and RuvC domains, typically generating blunt ends [25]. This precise *cis*-cleavage behavior supports its widespread use in genome editing, whereas diagnostic formats that require built-in signal amplification more often favor effectors with target-activated collateral cleavage. Accordingly, Cas9-based sensing commonly relies on auxiliary amplification or enrichment modules rather than intrinsic *trans*-cleavage.

In contrast, Cas12 recognizes a TTTV PAM sequence and cleaves dsDNA using its RuvC domain to produce staggered ends. Moreover, upon target binding, Cas12 undergoes a conformational change that activates an indiscriminate *trans*-cleavage activity against surrounding single-stranded DNA (ssDNA) [26]. Similarly, Cas13 targets single-stranded RNA (ssRNA) guided by CRISPR RNA (crRNA), executing specific *cis*-cleavage of the target RNA, which subsequently triggers the *trans*-cleavage of collateral ssRNA molecules [27]. The programmable collateral activity of Cas12 and Cas13 serves as the fundamental mechanism for signal amplification in biosensing [28]. In addition, Cas12f (also known as Cas14), an ultra-compact Type V CRISPR effector, has recently emerged as a promising alternative for biosensing applications [29]. Owing to its remarkably small molecular size and relaxed PAM requirements, Cas12f enables flexible target selection and facilitates system miniaturization [30]. Similar to Cas12a, Cas12f exhibits collateral ssDNA cleavage activity upon target recognition, which can be exploited for signal amplification.

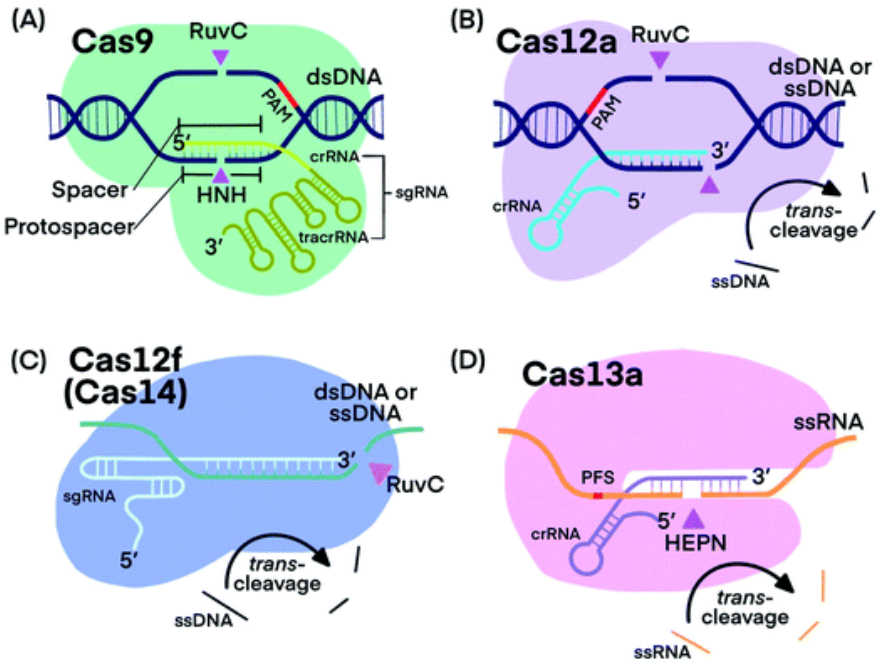

Figure 1. Fundamental components of CRISPR–Cas9 (**A**), -Cas12a (**B**), -Cas12f (**C**), and -Cas13a (**D**) systems. Reprinted with permission from ref. [31]. Copyright 2021 Royal Society of Chemistry.

Notably, these Cas effectors exhibit substantial differences in target preference, PAM/PFS requirements, collateral cleavage efficiency, and operational complexity, which directly affect their analytical performance and applicable scenarios in biosensor design. To facilitate the rational selection of appropriate Cas proteins for ECL biosensing, the key characteristics of representative Cas effectors are systematically summarized and compared in Table 1.

Table 1. Comparison of representative CRISPR/Cas effectors for ECL biosensing.

Cas Protein	Type	Target (*cis*)	Key Mechanistic Features	Typical Applications
Cas9	Type II	dsDNA	NGG PAM; HNH/RuvC *cis* dsDNA cleavage (blunt); *Trans*: None	Genome editing; Mutation calling; Target enrichment
Cas12a (Cpf1)	Type V	dsDNA	TTTV PAM; RuvC *cis* dsDNA cleavage (staggered); *Trans* (ssDNA): Strong	DNA pathogen detection; Biosensing;
Cas13a	Type VI	ssRNA	PFS (if applicable) HEPN *cis* ssRNA cleavage; *Trans* (ssRNA): Strong	RNA virus detection; Transcript profiling RNA biosensing
Cas12f (Cas14)	Type V	dsDNA	Relaxed PAM (ortholog-dependent); RuvC *cis* cleavage; *Trans* (ssDNA): Moderate	POCT devices; Portable Biosensors; Microfluidic systems

Following the fundamental principles of ECL and the unique molecular recognition and amplification capabilities of CRISPR/Cas systems, their integration creates a powerful synergy for biosensing. The analytical performance of these hybrid platforms critically depends on the design of the signal transduction, which dictates how the specific biochemical event (target-activated Cas *trans*-cleavage) is converted into a quantifiable ECL readout. Based on the function of the reporter nucleic acid and the resulting signal change, the dominant coupling strategies can be categorized into three primary mechanistic modes as illustrated in Figure 2.

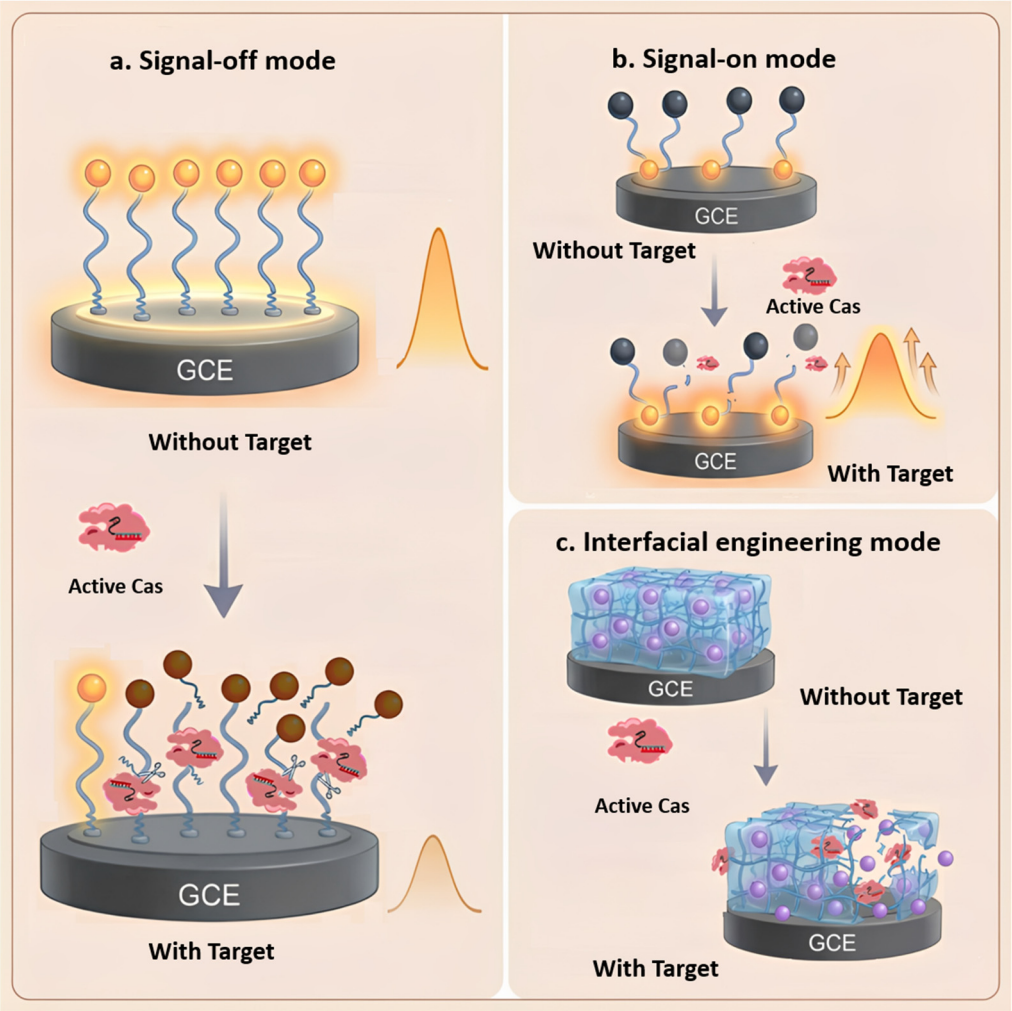

Figure 2. Three representative sensing modes of the CRISPR/Cas-powered ECL system.

2.1. Signal-Off Mode: Cleavage of Immobilized Reporters on the Electrode

In this direct and widely used configuration, single-stranded DNA (ssDNA) reporter molecules are covalently attached to the electrode surface. These reporters are often conjugated directly to the ECL luminophore (e.g., $Ru(bpy)_3^{2+}$) [32]. In the absence of the target, the intact reporter maintains a stable, high ECL signal. Upon target recognition, the activated Cas effector (typically Cas12a for DNA targets or Cas13a for RNA targets) initiates its indiscriminate *trans*-cleavage activity [33]. This enzymatic action cleaves and releases the ssDNA reporters along with their attached luminophores from the electrode interface into the bulk solution. The detachment of the emitters from the conductive surface dramatically diminishes the ECL intensity, yielding a "signal-off" response. This mode is appreciated for its operational simplicity and direct correlation between target concentration and signal decrease. However, it can be susceptible to false-positive decreases from non-specific surface degradation.

2.2. Signal-On Mode: Cleavage of a Quencher Immobilized on the Electrode Interface

In this strategy, the ECL luminophore is first drop-casted or covalently modified on the electrode. A ssDNA probe, serving as the substrate for Cas *trans*-cleavage, is then tethered to this luminophore. The distal end of this DNA probe is modified with an efficient ECL quencher molecule [34,35]. In the initial state, the proximity of the quencher to the surface-confined luminophore causes strong quenching, resulting in a suppressed or "off" ECL state. Target-triggered activation of the Cas protein (Cas12 or Cas13) initiates collateral cleavage activity, which specifically severs this ssDNA tether. The cleavage event releases the quencher from the electrode surface, thereby eliminating the quenching effect and restoring the ECL signal from the immobilized luminophores. This kind of design yields a "signal-on" response with a typically low background and a high signal-to-noise ratio, as the signal is generated directly at the electrode interface where the ECL reaction occurs [36–38].

2.3. Interfacial Engineering Mode: Cleavage-Triggered Nanostructural or Conformational Change

This approach focuses on engineering a responsive ECL-active interface where Cas-mediated cleavage triggers a macroscopic change in the electrode's properties. A typical design involves constructing or immobilizing a DNA hydrogel on the electrode surface, which incorporates the ECL emitters inside. This responsive interface is composed of cross-linked DNA strands that are susceptible to Cas *trans*-cleavage. It serves a dual purpose: firstly, it provides a high-capacity, three-dimensional matrix for the stable incorporation of ECL luminophores and the diffusion of necessary co-reactants, creating an optimal microenvironment for the ECL reaction. Secondly, the hydrophilic and densely charged nature of the DNA hydrogel can effectively form an anti-fouling interface, resisting the non-specific adsorption of proteins and other biomacromolecules from complex biological matrices, which is a significant advantage for real-sample sensing [39,40]. In the initial state, the intact interface may either facilitate or hinder electron transfer, generating a baseline ECL signal [41]. Target-induced activation of Cas leads to the cleavage of the component DNA strands, causing the dissolution of the entire interface, resulting in a pronounced change (either increase or decrease) in the ECL signal. This mode is particularly powerful as it translates a single biocatalytic cleavage event into the disruption of an entire nanostructure containing a multitude of signal-generating units, thereby achieving exceptionally high signal amplification and robust performance in challenging sample environments.

Despite the widespread application of these three signal transduction modes, their analytical performance and practical suitability differ substantially. The signal-off strategy is characterized by its straightforward architecture and facile fabrication process, making it attractive for rapid assay development and large-scale deployment. However, because signal attenuation can also arise from nonspecific surface degradation, probe desorption, or matrix interference, this mode is more prone to false-positive responses, particularly in complex biological samples. In contrast, signal-on systems convert target recognition into signal enhancement, generally providing superior signal-to-noise ratios and improved detection sensitivity, which is particularly critical for trace-level target analysis. However, the requirement for precise spatial arrangement among luminophores, quenchers, and DNA probes inevitably increases the complexity of probe design and surface modification, leading to higher optimization costs and reduced fabrication robustness. Interfacial engineering strategies emphasize the regulation of mass transfer and electron transport through responsive nanostructured interfaces. By integrating antifouling properties and three-dimensional signal amplification matrices, these platforms exhibit enhanced stability and reproducibility in real-sample analysis. Nevertheless, their multistep fabrication procedures and limited scalability remain major challenges for practical translation and large-scale manufacturing.

Therefore, the rational selection of signal transduction strategies should be guided by specific analytical requirements, including target concentration, sample complexity, operational environment, and device integration level. The key characteristics, advantages, and limitations of different strategies are systematically summarized in Table 2.

Table 2. Comparison of signal transduction modes in CRISPR/Cas-powered ECL biosensors.

Strategy	Signal Transduction Mechanism	Advantages	Limitations	Suitable Applications
Signal-off	Release/detachment of luminophore-labeled reporters	Simple design; Easy fabrication; Low cost	Prone to false positives; Limited robustness	Rapid screening; Quantitative assays
Signal-on	Dequenching of surface-confined luminophores	High S/N; Low background; High sensitivity	More complex probe design; Optimization-intensive	Trace-level quantitative detection
Interfacial engineering	Cleavage-triggered interface disassembly/assembly	High stability; Strong amplification; Antifouling	Multistep fabrication; Scalability limited	Complex matrices; Long-term monitoring

In summary, the integration of CRISPR/Cas biology with ECL analytical techniques provides multiple versatile pathways for signal transduction in biosensing. The selection among signal-off, signal-on, and interfacial engineering modes should be guided by the desired sensitivity, operational robustness, and specific application contexts. These transduction strategies constitute the cornerstone for the development of next-generation CRISPR–ECL biosensors and will be further elaborated in the following sections, focusing on practical applications in public health monitoring.

3. Biosensing Applications

3.1. Clinical Virological Diagnostics

Precise and early detection of viruses, particularly high-threat pathogens like HPV and SARS-CoV-2, is essential for clinical diagnostics and epidemiological surveillance [42–44]. While qPCR remains the gold standard, its reliance on specialized equipment and trained personnel limits its accessibility [45]. This section reviews recent applications of CRISPR/Cas-powered ECL platforms for the sensitive detection of various pathogens.

The dominant "signal-on" mode is effectively demonstrated in several HPV DNA detection methods. For example, charge-tunable Ru@SiO_2 nanoparticles were employed as ECL emitters. A DNA hairpin probe (HP) attached to the nanoparticles created strong electrostatic repulsion with the negatively charged electrode, suppressing the ECL signal. When target DNA activated Cas12a, it cleaved the HP, reducing the surface charge and allowing the nanoparticles to approach the electrode, thereby enhancing the ECL signal. The biosensor demonstrated high sensitivity with a detection limit of 3.4 fM for HPV-16 and successful application in real sample analysis [46]. By using the same ECL emitters, hybridization chain reaction (HCR) was coupled with CRISPR/Cas12a to realize the detection of hepatitis B virus (HBV) [47]. Similarly, Luo et al. constructed a CRISPR/Cas12a-based ECL imaging platform for the ultrasensitive detection of HPV-18 DNA [48]. It utilized polymer dots (Pdots) as ECL emitters and a target-activated Cas12a system to cleave a quencher-labeled hairpin DNA, resulting in an amplified "off-on" ECL imaging signal. The biosensor demonstrated high sensitivity with a detection limit of 5.3 fM and excellent specificity for HPV-18, showcasing its potential for clinical nucleic acid diagnostics. Another approach used L-methionine-stabilized gold nanoclusters (Met-AuNCs) as emitters, with the signal initially suppressed by a ferrocene-quencher labeled ssDNA [49]. Cas12a-mediated cleavage of this ssDNA released the quencher, leading to a "signal-on" response with a 0.48 pM detection limit for HPV-16 DNA.

A representative work exemplifying the "signal-off" mode demonstrated the synergy between CRISPR/Cas systems and high-efficiency polymer dot (Pdot) emitters for sensitive HPV-16 detection. As shown in Figure 3, PFBT Pdots with a large conjugated structure were prepared via nanoprecipitation as the ECL emitter. Benefiting from their high photostability and carboxyl-functionalized surface, this material achieved a relative ECL efficiency of 39.3% (relative to $[Ru(bpy)_3]^{2+}$), significantly outperforming many traditional inorganic and small-molecular luminophores. The authors further integrated these Pdots with the CRISPR/Cas12a system and the localized surface plasmon resonance (LSPR) effect of electrochemically deposited Au nanoparticles (depAuNPs) to construct a sensitive ECL biosensing platform. The biosensor was fabricated by assembling hairpin DNA on depAuNPs-modified electrodes. In the initial state, a single-stranded assistant DNA opens the hairpin DNA to bind Pdots-DNA on the electrode surface, resulting in intense ECL emission. Upon recognition of target HPV-16 DNA, the activated Cas12a digests the assistant DNA, which decreases the amount of Pdots-DNA bound to the biosensor, thereby reducing the ECL emission and achieving "signal-off" detection. This method achieved a detection limit as low as 3.2 fM for HPV-16 DNA. This work not only developed a strategy to regulate the surface binding of Pdots but also combined CRISPR-mediated signal amplification with the LSPR enhancement of noble metals, providing a powerful tool for sensitive clinical pathogen detection [50].

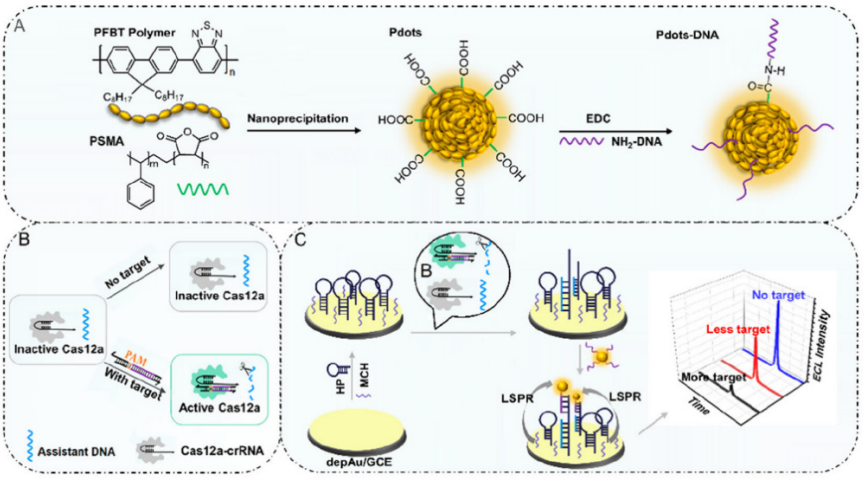

Figure 3. Schematic illustration for the construction and detection mechanism of a CRISPR/Cas12a-powered ECL biosensing platform. (**A**) The synthesis of polymer dots (Pdots) emitters. (**B**) Activation of CRISPR/Cas12a. (**C**) Au nanoparticle-mediated LSPR signal enhancement for sensitive DNA analysis. Reprinted with permission from ref. [50]. Copyright 2023 American Chemical Society.

In another work, a signal-on ECL imaging platform using hot exciton organic nanorods (BB NRs) was developed for HPV-16 detection. These BB NRs, featuring a hybridized local and charge-transfer (HLCT) state, achieved a high anodic ECL efficiency of 56.7%. The strategy utilized BHQ2-labeled ssDNA to initially quench the ECL signal via resonance energy transfer. Upon target recognition, activated Cas12a cleaved the DNA, releasing the quencher and restoring the intense emission. This method achieved a detection limit of 0.6 fM and combined CRISPR/Cas specificity with high-throughput imaging, providing a powerful tool for sensitive DNA analysis [51]. Beyond single-analyte detection, a unique "signal-switchable" strategy was developed for multiplexing. Using spherical nucleic acid (SNA) carriers loaded with carbon dots, the platform provided an "on" signal for HIV DNA via a sandwich hybridization and an "off" signal for HPV-16 DNA via Cas12a cleavage [52].

For RNA viruses, particularly Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the CRISPR-Cas13a system is uniquely advantageous due to its intrinsic RNA-targeting capability, either operating directly or in combination with transcriptional amplification steps. Wei et al. developed a highly sensitive ECL sensing platform termed "EDT-Cas" (entropy-driven triggered T7 amplification-CRISPR/Cas13a system) for the detection of the SARS-CoV-2 RdRp gene. In this strategy, the target sequence first initiated an entropy-driven cyclic amplification process, which exposed a concealed T7 promoter. T7 RNA polymerase subsequently recognized this promoter and generated large quantities of single-stranded RNA (RNA transcript), serving as the activator for Cas13a. Upon activation, Cas13a *trans*-cleaved the DNA reporter probes embedded with -U-U- RNA cleavage motifs immobilized on a AuNPs/$Ti_3C_2T_x$/Ru(II)-PEI-modified glassy carbon electrode (GCE) interface, resulting in the release of ferrocene labels and a corresponding signal-on ECL response. Owing to this multi-level cascade amplification, the platform achieved an ultralow limit of detection (LOD) of 7.39 aM and demonstrated reliable performance in pharyngeal swabs and environmental samples [53].

In parallel with Cas13a-based strategies, Cas12a has also been extensively integrated into SARS-CoV-2 ECL biosensing through diverse signal amplification architectures. For example, a self-enhanced ruthenium complex anchored on ZIF-8 was employed as an ECL emitter. By coupling double-stranded specific nuclease (DSN) and catalytic hairpin assembly (CHA) for signal amplification to activate Cas12a, a signal-on detection mode was demonstrated for the sensitive detection of SARS-CoV-2 RNA [54]. Similarly, Zhang et al. proposed a series of DNA nanostructure-mediated amplification strategies, including 3D DNA walkers on gold nanoparticles [55], pH-regulated regenerative DNA tetrahedrons [56], and reverse-regulation via DNA triple-helix structures [57] for the detection of the SARS-CoV-2 RdRp gene. Conclusively, these approaches demonstrated the flexibility of Cas12a-based ECL platforms in accommodating programmable DNA architectures for enhanced signal transduction.

Overall, these studies on viral nucleic acids detection underscore the core advantages of the CRISPR/Cas-ECL synergy: ultra-high sensitivity, excellent specificity enabled by CRISPR's programmable recognition, and operational simplicity. The choice of signal mode and the selection of high-performance ECL emitters provide a flexible toolkit to optimize for sensitivity, background noise, or multiplexing capability. Notably, successful detection in complex matrices like human serum and blood samples underscores the platform's robustness and clinical potential.

3.2. Food Safety and Environmental Monitoring

Foodborne pathogens and environmental pollutants pose significant threats to global public health and economic stability. However, their detection is often hindered by the complex compositions, low target concentrations, and interfering substances inherent in food and environmental matrices [58–60]. Here, we summarize recent CRISPR–ECL biosensors for foodborne pathogens and for chemical pollutants/toxins, with emphasis on interface design, real-sample validation, and analytical performance in food safety and environmental surveillance.

While food-borne diseases stem from various sources like viruses, toxins, and chemicals, pathogens remain a predominant factor [61]. Key pathogens, including *Salmonella typhimurium, Escherichia coli, Staphylococcus aureus,* and *Listeria monocytogenes*, are frequently linked to disease outbreaks. Thus, precise detection of these pathogens is vital to ensure food safety. For *Salmonella* detection, Zhang et al. reported a representative "signal-on" sensor in which Pt nanoclusters anchored on delaminated MXenes (Pt NCs/D-MXenes) served as co-reactant accelerators. Upon Cas12a activation, the enhanced generation of reactive oxygen species (ROS) significantly amplified the ECL response, enabling sensitive detection of *S. typhimurium* [62]. In contrast, our group developed a "signal-off" platform for *Salmonella* detection by coupling allosteric probe-triggered isothermal amplification with Cas12a-mediated cleavage, leading to the detachment of PCN-224 ECL emitters from the electrode surface and a corresponding signal decrease [63].

A similar signal-suppression paradigm has been applied to *Staphylococcus aureus* detection. Liu et al. introduced programmable T-junction DNA structures to initiate strand displacement amplification (SDA), thereby

increasing the number of Cas12a cleavage events and pushing the detection sensitivity to the femtomolar level [64]. Distinct from these cleavage-dependent strategies, Wu et al. proposed an alternative "signal-on" approach for *Listeria monocytogenes* by exploiting the high-affinity binding capability of catalytically inactive Cas9 (dCas9). Without relying on target-initiated cleavage, this binding-based recognition strategy achieved high-fidelity detection with single-base specificity, highlighting the versatility of CRISPR proteins in ECL signal regulation [65].

Beyond sensitivity improvement, bridging the gap between laboratory-based assays and on-site food safety testing remains a key challenge. To address this issue, microfluidic integration has emerged as a powerful means to automate and streamline CRISPR-ECL workflows. As shown in Figure 4, Mao et al. developed a modular microfluidic CRISPR/Cas13a–ECL sensor that integrated nucleic acid extraction, target recognition, and signal readout into a single platform for multichannel pathogen RNA analysis [66]. The device incorporated an immiscible filtration assisted by surface tension (IFAST) module, in which magnetic bead–captured RNA was transported across oil barriers under magnetic control, effectively replacing conventional centrifugation and pipetting. Following Cas13a-mediated *trans*-cleavage, signal readout was achieved using a multichannel closed bipolar electrode ECL (MCBPE-ECL) chip based on dry chemistry, with reagents pre-deposited on fiber substrates to simplify operation. This integrated system enabled the simultaneous detection of *Escherichia coli* and *Staphylococcus aureus* within 30 min, achieving an LOD of 0.372 fM for *E. coli* 16S rRNA and 63.8 cfu mL^{-1} in clinical blood samples.

A distinct advantage of CRISPR-ECL platforms is their adaptability to non-nucleic acid analytes—such as pesticides and biotoxins—via functional nucleic acids (FNAs). By employing aptamers to transduce ligand-binding events into specific DNA signals, these systems activate Cas effector *trans*-cleavage, thereby modulating ECL output for ultrasensitive environmental analysis.

Building on this transduction principle, a homogeneous CRISPR/Cas12a–ECL sensor was reported by Zheng et al. for N-nitrosodimethylamine (NDMA) detection [67]. As illustrated in Figure 5, by integrating a vertically ordered mesoporous silica film (VMSF)-modified ITO electrode with CRISPR-driven hyperbranched rolling circle amplification (HRCA), the system achieved triple signal amplification while effectively suppressing false positives arising from primer dimer formation. In the absence of NDMA, the formation of large dsDNA-$Ru(phen)_3^{2+}$ complexes was repelled by the VMSF, preventing signal generation. Conversely, the presence of NDMA inhibited the formation of long dsDNA, allowing free, positively charged $Ru(phen)_3^{2+}$ to diffuse through the nanopores and enrich at the electrode surface, generating a strong ECL signal. The selective gating effect of the VMSF enabled a "signal-on" response in the presence of NDMA, resulting in an impressive LOD of 5.33 pg mL^{-1}.

Despite such successes, broadly applying this platform to pesticide small molecules requires overcoming weak target binding and inefficient signal translation. Recent advances address these challenges through two main approaches: structure-based signal conversion and material-based signal enhancement.

Regarding signal conversion, researchers have engineered DNA nanostructures to bridge recognition and activation. For instance, Li et al. utilized a triple-helix DNA structure to translate acetamiprid binding into primer release, thereby initiating the subsequent CRISPR/Cas12a cascade reaction [68]. Similarly, the target acetamiprid triggered the release of an activator DNA from an aptamer complex, which then activated the *trans*-cleavage activity of Cas12a. This enzyme cleaved ferrocene-labeled DNA probes on the electrode surface, leading to a measurable recovery of the ECL signal generated from perylene-derived covalent organic framework (PTCA-COF) [69]. At the signal output interface, to overcome the low signal-to-noise ratio associated with pesticide small molecules, Peng et al. employed a signal-on strategy based on "in situ crystallization-induced (CIE) ECL" of Alq_3 microcrystals by coupling with Cas12a cleavage. This approach achieved highly sensitive signal activation [70]. In terms of material enhancement, recent studies leverage nanoconfinement to stabilize luminophores. For example, Cai et al. utilized HOF-14 to immobilize luminescent molecules [71], Shi et al. synthesized $Ir(ppy)_3$-functionalized zeolitic imidazolate framework-8 (Ir-ZIF-8) [72] as ECL emitters, and they successfully converted aggregation behavior—typically a cause of quenching—into an "aggregation-induced electrochemiluminescence (AIECL)" to improve the biosensing performance of the CRISPR/Cas-powered ECL platforms.

In conclusion, by integrating the programmable recognition and *trans*-cleavage activities of CRISPR effectors with diversified ECL signal regulation modes, highly sensitive and selective detection of foodborne pathogens, small-molecule contaminants, and environmental toxins was realized. Moreover, the incorporation of microfluidic automation, functional nucleic acid–based signal transduction, and advanced luminescent materials exploiting nanoconfinement or aggregation-induced effects has effectively improved the biosensing performance. Collectively, these advances underscore the methodological robustness and application potential of CRISPR–ECL platforms and position them as a promising foundation for next-generation, portable analytical technologies aimed at safeguarding food safety and public health.

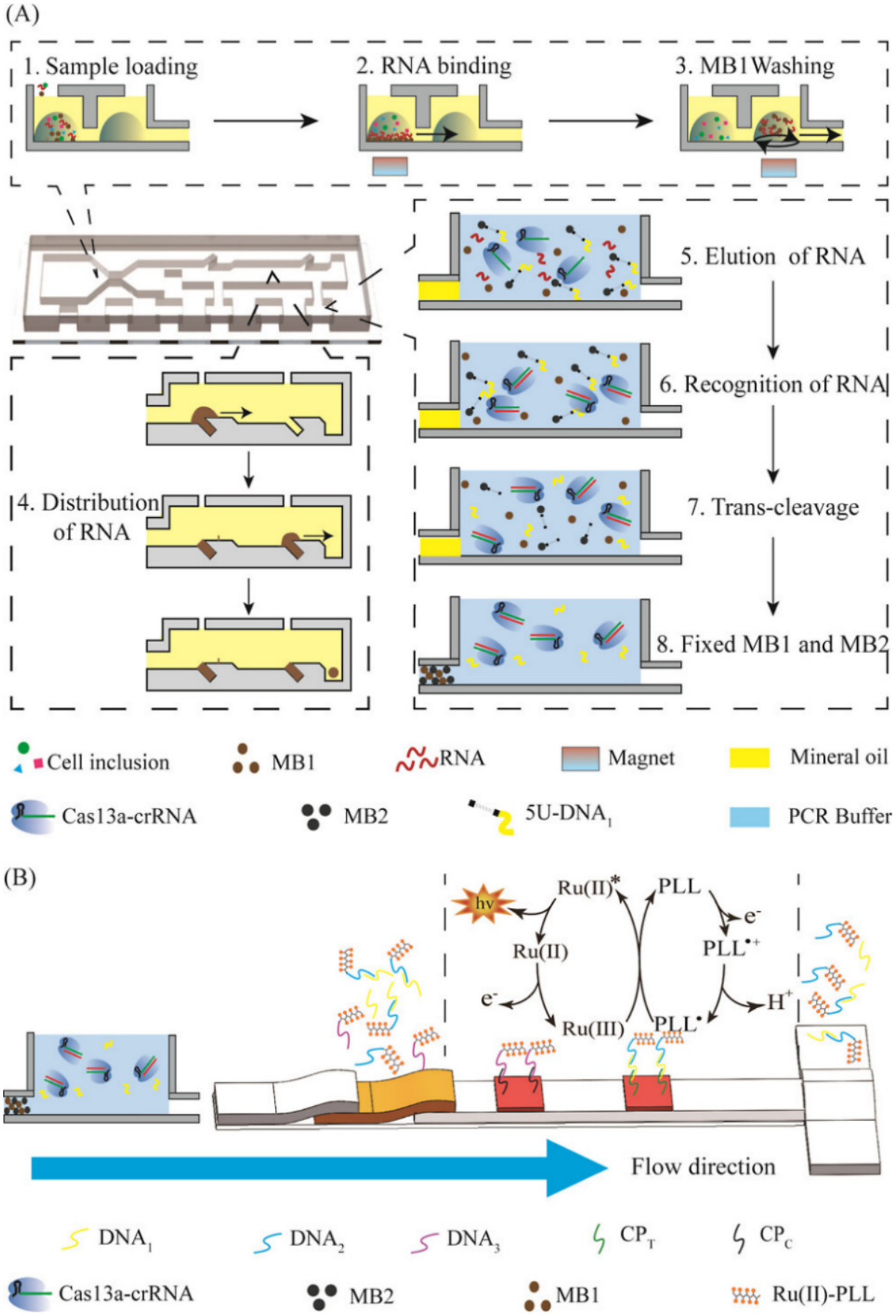

Figure 4. Modular microfluidic sensor integrating nucleic acid extraction, CRISPR/Cas13a reaction, and ECL detection. (**A**) Rapid RNA extraction and magnetic transport based on IFAST. (**B**) Fiber-assisted multichannel closed bipolar electrode (MCBPE) system for dry-chemistry ECL detection, enabling rapid and multiplexed pathogen screening. Reprinted with permission from ref. [66]. Copyright 2025 American Chemical Society.

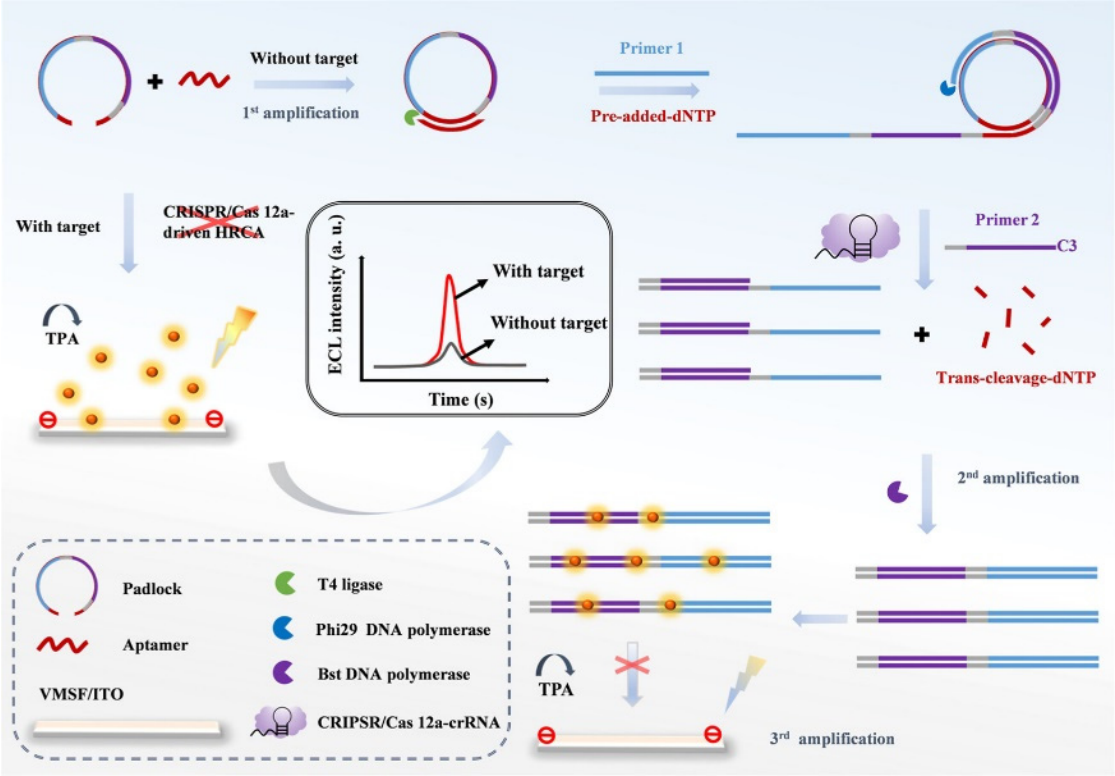

Figure 5. Principle of the ultrasensitive homogeneous ECL biosensor for N-nitrosodimethylamine (NDMA) detection utilizing a vertically ordered mesoporous silica film (VMSF)-modified electrode. Reprinted with permission from ref. [67]. Copyright 2025 American Chemical Society.

3.3. Zoonotic and Plant Disease Pathogens Detection

Extending beyond human clinical diagnostics, CRISPR–ECL platforms are increasingly applied to zoonotic and plant pathogens, supporting One Health surveillance and early agricultural warning. These targets are often present at ultralow levels in matrix-rich samples (animal tissues/fluids and crude plant extracts), where conventional workflows may be slow or operationally demanding [73–75]. Below, we highlight representative CRISPR–ECL designs for zoonotic bacteria and plant pathogens, emphasizing strategies that maintain selectivity and sensitivity in complex samples.

For the detection of the human-associated clade of *Streptococcus suis*, Hu et al. reported a signal-off mode CRISPR/Cas-ECL platform. This method utilized yttrium metal-organic frameworks (Y-BTC) as highly efficient ECL donors. Upon target-induced isothermal amplification, the activated Cas12a cleaved the DNA shell on gold nanoparticles (AuNPs), exposing bare AuNPs to be electrostatically adsorbed onto the Y-BTC surface. This proximity enabled electrochemiluminescence resonance energy transfer (ECL-RET), quenching the signal and achieving sensitive detection with a limit of 17 pM [76]. For the detection of rabies viral RNA, a strategy combining binding-induced isothermal amplification with DNA nanotweezers has been developed by Liu et al. [77]. By leveraging the *trans*-cleavage activity of Cas12a to cleave a trigger strand and induce the conformational opening of the nanotweezers, the platform successfully converted molecular recognition into a robust ECL signal response. Specifically, the detection was based on the Cas12a-dependent structural switching of the DNA tweezer: cleavage of the trigger strand opened the structure to generate a strong $Ru(bpy)_3^{2+}$ ECL signal, while an intact strand kept it closed for hemin binding and signal quenching. In another example, a signal on the CRISPR/Cas-ECL strategy was proposed for the detection of *Burkholderia pseudomallei.* It integrated a self-enhanced Au@PEI-ABEI@Pt porous hydrogel as a highly efficient emitter with target-triggered Cas12a activity. Initially, the ECL signal was quenched by dopamine-labeled DNA (DA-S1). Upon target recognition, activated Cas12a cleaved the DA-S1, restoring the ECL signal. This "signal-on" platform achieved a detection limit of 5 CFU mL^{-1} in serum, leveraging the hydrogel's catalytic properties and biocompatibility to maintain enzymatic activity on the electrode [78].

Huanglongbing (HLB), also known as citrus greening disease, represents one of the most devastating threats to global citrus production [79]. Its detection is of paramount importance due to the insidious nature, rapid spread, and catastrophic economic impact. The causal pathogen, *Candidatus Liberibacter asiaticus*, is phloem-limited and transmitted by the Asian citrus psyllid vector [80]. In the absence of a cure, robust detection technologies are not merely a diagnostic tool but an essential frontline defense for safeguarding orchards, protecting livelihoods, and

ensuring the sustainability of the citrus industry worldwide [81]. The primary challenge in early diagnosis lies in overcoming a low number of targets and severe matrix interference in crude plant extracts. To address these issues, our group has proposed two CRISPR/Cas-ECL approaches. We developed a one-pot platform by utilizing superparamagnetic beads coated with a mesoporous silica shell to encapsulate the ECL emitter $Ru(bpy)_3^{2+}$, which initially exhibited a low ECL signal due to the hindrance of electron transfer by the surface-conjugated single-stranded DNA. To improve the sensitivity, we coupled rolling circle amplification (RCA) with CRISPR. Upon recognition of the target, RCA generated abundant amplicons that activated the Cas12a to perform *trans*-cleavage of the surface DNA quenchers, restoring the electron transfer pathway and triggering a turn-on ECL signal [82]. On the other hand, to enhance the anti-interference capability of biosensors in complex matrices, we constructed a CRISPR-responsive smart DNA hydrogel triggered by rolling circle amplification (Figure 6). The DNA hydrogel, synthesized via cryo-polymerization, effectively encapsulates ECL emitters (AuAg nanoclusters) to build a robust anti-fouling barrier, physically blocking macromolecules and impurities in the plant matrix from reaching the electrode surface. Upon target recognition, the generated RCA amplicons efficiently activate the *trans*-cleavage activity of CRISPR/Cas12a. The activated Cas12a then acts as a specific de-crosslinker by digesting the DNA strands within the hydrogel network, triggering the degradation of the hydrogel. This structural collapse leads to the release of encapsulated AuAg nanoclusters and a subsequent decrease in ECL intensity, translating microscopic molecular recognition into macroscopic interfacial reconfiguration. This "signal-off" strategy offers a unique and highly sensitive solution for the analysis of the C*Las* Omp gene in complex plant samples [39].

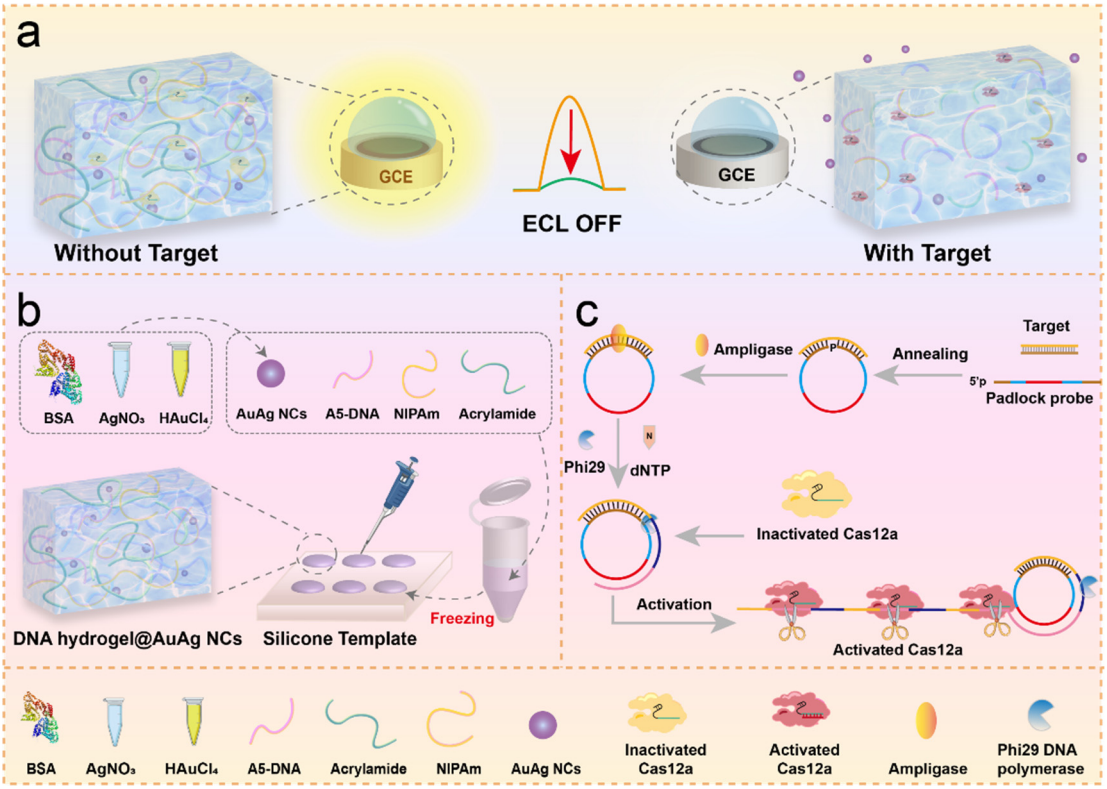

Figure 6. Schematic illustration of the ECL biosensor for C*Las* Omp gene detection using RCA and CRISPR/Cas12a-responsive smart DNA hydrogel: (**a**) Principle of the ECL biosensor; (**b**) Synthesis of DNA hydrogel@AuAg NCs; (**c**) Rolling circle amplification (RCA) of the target and subsequent activation of the CRISPR/Cas12a system. Reprinted with permission from ref. [39]. Copyright 2024 American Chemical Society.

Taken together, these advances highlight the strong potential of CRISPR–ECL platforms for the detection of zoonotic and plant disease pathogens. By integrating programmable CRISPR recognition with efficient ECL signal transduction, such systems enable sensitive, specific, and matrix-tolerant analysis. Looking forward, the continued development of robust interfaces, simplified workflows, and multiplexing capabilities is expected to further facilitate the translation of CRISPR–ECL sensors toward practical disease surveillance in agricultural and livestock settings. We summarized the analytical performance of representative CRISPR–ECL biosensors in this review in Table 3.

Table 3. Analytical performance of representative CRISPR–ECL biosensors.

Target	Signal Strategy	ECL emitter	LOD	Linear Range	Ref.
HPV-16 DNA	Signal-on	$Ru@SiO_2$	3.4 fM	10 fM–10 nM	[46]
HBV DNA	Signal-on	$Ru@SiO_2$	7.41 fM	10 fM–10 nM	[47]
HPV-18 DNA	Signal-on	PFBT Pdots	5.3 fM	10 fM–500 pM	[48]
HPV-16 DNA	Signal-on	Met-AuNCs	0.48 pM	1 pM–10 nM	[49]
HPV-16 DNA	Signal-off	PFBT Pdots	3.2 fM	5 fM–50 pM	[50]
HPV-16 DNA	Signal-on	BB NRs	0.6 fM	1 fM–10 nM	[51]
HIV DNA	Signal-on	Carbon Dots	30 fM	100 fM–1 μM	[52]
HPV-16 DNA	Signal-off	Carbon Dots	0.32 pM	1 pM–100 nM	[52]
SARS-CoV-2 RdRp gene	Signal-on	AuNPs/$Ti_3C_2T_X$/Ru(II)-PEI	7.39 aM	20 aM–1 pM	[53]
SARS-CoV-2 RNA	Signal-on	Ru-PEI/Au@ZIF-8	0.67 fM	1 fM–100 pM	[54]
SARS-CoV-2 RdRp gene	Signal-on	PEI-Ru@Ti_3C_2@AuNPs	12.8 aM	10–1000 aM	[55]
SARS-CoV-2 RdRp gene	Signal-on	Au-g-C_3N_4	43.7 aM	10 aM–10 pM	[56]
SARS-CoV-2 RdRp gene	Signal-off	GOAu-Ru	32.8 aM	0 aM–1 fM	[57]
Salmonella typhimurium	Signal-on	Pt NCs/D-MXenes (luminol/O_2)	6 CFU/mL	10–10^6 CFU/mL	[62]
Salmonella	Signal-off	PCN-224	37 CFU/mL	50–5 × 10^6 CFU/mL	[63]
Sa-16 SrDNA	Signal-off	PCN-224/CdS QDs	0.437 fM	1 fM–10 nM	[64]
Listeria monocytogenes hlyA gene	Signal-on	$Ru(bpy)_3^{2+}$-labeled probe	38 CFU/mL	10^{-5}–10^{-1} ng/μL	[65]
EGFR L858R mutant	Signal-on	$Ru(bpy)_3^{2+}$-labeled probe	0.1 pg/μL	10^{-5}–10^{-1} ng/μL	[65]
Escherichia coli 16S rRNA	Signal-off	$Ru(bpy)_3^{2+}$-based self-enhanced probe	0.372 fM	0.01–100 pM	[66]
Escherichia coli blood sample	Signal-off	$Ru(bpy)_3^{2+}$-based self-enhanced probe	63.8 CFU/mL	2.5 × 10^2–2.5 × 10^7 CFU/mL	[66]
N-nitrosodimethylamine	Signal-on	$Ru(phen)_3^{2+}$	5.33 pg/mL	10–10^7 pg/mL	[67]
Acetamiprid	Signal-on	ZIF-8@Pe	394 fM	1 pM–100 μM	[68]
Acetamiprid	Signal-on	PTCA-COF	2.7 pM	0.1 nM–100 μM	[69]
Acetamiprid	Signal-on	Alq_3MCs@PEI	0.79 fM	1.0 fM–1.0 nM	[70]
Microcystin-LR	Signal-off	ILu/HOF-14	0.275 pg/mL	0.5 pg/mL–0.5 μg/mL	[71]
Microcystin-LR	Signal-on	Ir-ZIF-8	1.2 pg/mL	0.01–50 ng/mL	[72]
Streptococcus suis	Signal-off	Y-BTC	17 pM	25 pM–50 nM	[76]
Rabies viral RNA	Signal-on	$Ru(bpy)_3^{2+}$-chitosan	2.8 pM	5 pM–5 nM	[77]
Burkholderia pseudomallei	Interfacial engineering	Au@PEI-ABEI@Pt	5 CFU/mL	0–1.5 × 10^8 CFU/mL	[78]
Candidatus Liberibacter asiaticus nrdB gene	Signal-on	MB/mSi/Ru/A	2 fM	10 fM–1 nM	[82]
Candidatus Liberibacter asiaticus Omp gene	Interfacial engineering	AuAgNCs	40 fM	50 fM–5 nM	[39]

4. Conclusions and Perspectives

The integration of CRISPR/Cas systems with ECL has established a versatile platform for ultrasensitive, highly specific, and programmable biosensing. By coupling CRISPR-mediated target recognition and *trans*-cleavage amplification with ECL's low background, broad dynamic range, and precise electrochemical control, CRISPR–ECL systems have demonstrated remarkable performance across clinical viral diagnostics, food safety, environmental monitoring, and plant and zoonotic disease pathogen detection. Flexible signal transduction strategies, including suppression, enhancement, and interface modulation, combined with advanced nanomaterials, further enhance the overall biosensing performance.

Despite these advances, broader translation faces critical challenges. Cas enzyme stability and batch-to-batch consistency remain limiting factors for large-scale deployment. Multistep nucleic acid amplification introduces operational complexity and contamination risks. Sensor performance in complex matrices is constrained by enzyme inhibitors, non-specific adsorption, and luminescence quenching. Moreover, integration levels vary

widely, and truly field-deployable point-of-care systems are hindered by issues in device standardization, reagent lyophilization, and cost.

Future development requires coordinated progress in molecular modules, sensing interfaces, and device engineering. Expanding the Cas toolbox with enhanced thermostability, shorter recognition motifs, and higher catalytic efficiency will enable single-nucleotide resolution, multiplexed detection, and direct RNA analysis. From the perspective of the ECL platform, developing a highly efficient ECL emitter with good aqueous stability alongside robust co-reaction accelerators will improve signal fidelity. Interface engineering strategies, such as MOF/COF confinement, single-atom catalysts, and DNA nanostructure-guided electrodes, can strengthen amplification while suppressing background noise [83].

Practical application will benefit from integrated microfluidic devices, reagent pre-packaging, and closed-tube workflows to achieve "sample-in, answer-out" operation. Coupling CRISPR–ECL with machine learning–based pattern recognition and data-driven calibration will facilitate multi-analyte profiling, automated decision-making, and intelligent analysis. With continued interdisciplinary innovation, CRISPR–ECL platforms are poised to evolve into next-generation biosensors capable of real-time, ultra-sensitive, on-site detection, providing powerful tools for public health, food safety, and agricultural biosecurity monitoring.

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Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

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