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CRISPR-Based Biosensors: Mechanisms of Biomarker Recognition and Detection of Non-Nucleic Acid Targets

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Abstract. In fields such as the early diagnosis of diseases, food safety monitoring, and environmental pollutant screening, the requirements for the sensitivity, specificity, and efficiency of detection technologies are extremely stringent. Traditional detection techniques such as HPLC and ELISA are cumbersome, time-consuming, and costly, and cannot meet the needs of on-site rapid testing and trace target screening. Under this backdrop, the CRISPR-Cas system, with its high targeting specificity and programmability, has brought new directions to the development of biosensors, particularly promoting technological innovations in recognition mechanisms and signal amplification for the detection of non-nucleic acid targets. This study focuses on CRISPR-Cas system-based biosensors, analyzing their structural composition, comparing the detection approaches for nucleic acid and non-nucleic acid targets, and conducting a performance comparison with traditional technologies. Studies have shown that this sensor combines high sensitivity and specificity with low cost and rapid detection, is capable of detecting both nucleic acid and non-nucleic acid targets, and thus has broad application prospects. However, it still has some limitations, such as the complexity of the indirect recognition process and the constraints on signal amplification. The integration of signal amplification, AI, nanotechnology, and microfluidics in the future is expected to break through bottlenecks and achieve industrial applications.

Keywords: CRISPR/Cas system, biosensor, non-nucleic acid targets, substance detection

1. Introduction

Situated within the domain of molecular diagnostics, an isothermal amplification approach—premised on Recombinase Polymerase Amplification coupled with Clusteblue Regularly Interspaced Short Palindromic Repeats (RPA-CRISPR)—has emerged as an alternative for field-deployable analyses whose reliance upon sophisticated apparatuses remains minimal. From instances observed in developing methodological constructs, researchers have delineated a quantitative detection paradigm wherein real-time calibration and fluctuation regulation are facilitated during operational runs. Reliance upon signal origination in such protocols can be attributed to integrated RPA-CRISPR/Cas12a systems configured for single-tube reaction environments; not merely convenience but also systematized coherence between different catalytic phases has been prioritized by these design choices. Screenings conducted of crRNA sequences corresponding to suboptimally positioned protospacer adjacent motif (PAM) sites constitute another structural layer within this

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detection platform. Further, optimization efforts directed toward constituent reaction parameters afford kinetic equilibrium—amplificatory processes juxtaposed against nuclease-mediated cleavage reactions manifesting mutual moderation under specified conditions. Visible within empirical results,an adaptable analytical architecture anchored in RPA-CRISPR praxis thus materializes. To domains contiguous with aquacultural surveillance or alimentary safety evaluations may such technology demonstrably extend, offering multiplex utility for pathogen identification where immediacy and logistical austerity pblueominate [1,2].

Fungal toxin contamination poses a continuous threat to global food safety, thereby generating an urgent need for rapid, sensitive, and on-site applicable detection technologies. Biosensors based on the clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 12a (CRISPR/Cas12a) system have emerged as a highly promising technical solution to meet this demand [3].

Subject to investigation in the present inquiry are the scopes by which biosensory constructs grounded in CRISPR modalities for substance detection may be developed and operationalized, as well as modes through which measurement systems derived from CRISPR schematics could undergo deployment. Centeblue upon those bio-detection assemblies wherein CRISPR-Cas architectures serve as the fundamental apparatus, mechanisms of analyte identification, along with latent capacities for extended application, come under scrutiny. Comprising primary concerns are analyses undertaken with respect to structural constituents configuring the sensor body itself; emphasis is placed upon elucidation of exactitude within target localization processes mediated centrally via CRISPR-Cas machinery, whose functional integration with molecular recognition domains—aptameric or immunoglobular entities being representative examples—together with modules responsible for signal mediation (fluorescence-based conduits, electrochemical arrangements among them),receives extensive examination [4]. Comparison drawn between molecular signaling routes used in sensing nucleic acid and non-nucleic acid analytes demonstrates: requisite becomes apparent for introduction of an intermediary recognition subunit—the role assumed here by aptamer-typified ligands or antibody fragments poised to transduce native targets into surrogate nucleic sequences, to induce subsequent activation of Cas effectors. Quantifiable readouts then emerge through enzymatic cleavage directed by these proteins, it can be seen from this system design. Parallel assessment executed vis-à-vis legacy technological counterparties yields insights regarding respective methodological advantages and constraints. Through juxtaposition of approaches differing both in technical base and implementation sequence, premier constructional principles dictating the ultimate sensitivity, selectivity, and robustness characterizing such sensors become discernible. Outlined thereby, within closing considerations, are critical parameters indispensable for advancement toward point-of-care diagnostics marked by stability as well as reliability against environmental fluctuations—elements needing precedence throughout development frameworks destined for medical field adaptation.

2. Overview of CRISPR-based biodetection technology

2.1. Biosensors based on CRISPR technology (CRISPR bio-detection technology)

The CRISPR-Cas system is essentially a powerful molecular system guided by RNA molecules that can precisely target and act on specific DNA or RNA sequences. The functionality relies on the synergistic interaction between two core components: Cas proteins (CRISPR-associated proteins) and guide RNA (gRNA). Here, Cas proteins act as "molecular scissors" capable of recognizing and cleaving nucleic acids, responsible for performing specific nucleic acid processing functions, while

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the guide RNA (gRNA) performs the "navigation" function by carrying a complementary base sequence that targets the nucleic acid of interest. Utilizing the principle of strict complementary base pairing to precisely identify and bind to specific sites on target DNA or RNA molecules, thereby recruiting Cas proteins to the specific site and enabling them to function in a targeted manner. For example, common Cas proteins such as Cas9 and Cas12 can specifically cleave target nucleic acids, This also constitutes its core mechanism of action in the field of gene editing. In interdisciplinary fields like biosensing, however, the cleavage activity of Cas proteins is ingeniously harnessed to amplify detection signals [1]. The reason this system is described as "powerful" lies in its exceptional programmability and high specificity. Programmability is reflected in the ability to flexibly adjust the target by simply altering the base sequence of the guide RNA, enabling the targeting of nearly any nucleic acid sequence and adaptation to diverse application scenarios [5,7]. The high specificity is attributed to the strict base complementary pairing between the guide RNA and the target nucleic acid. Additionally, some Cas proteins recognize the PAM site (protospacer adjacent motif) near the target sequence, creating a dual-layered safeguard that effectively prevents the misidentification and binding of non-target sequences. Its targeting precision far exceeds that of traditional nucleic acid manipulation tools, which also lays a core foundation for its successful expansion from the field of gene editing to multiple fields such as biological detection [1,7].

2.2. Advantages of CRISPR-based biosensors

Traditional methods for detecting non-nucleic acid targets (such as proteins and small molecules) primarily rely on techniques like HPLC and ELISA. Problems such as long detection cycles, complicated operation procedures, and high detection costs are prevalent. It is difficult to meet the practical needs of rapid screening and on-site detection [8]. The CRISPR-Cas system (such as Cas9, Cas12, etc.) was initially developed as a gene-editing tool capable of precisely identifying nucleic acid sequences. When integrated with biosensors, they can convert target recognition signals into directly detectable signals, such as fluorescence or electrochemical signals. Meanwhile, it retains its inherent excellent precise targeting ability, which helps the sensor achieve signal visualization and quantification, and further realizes a detection effect with high sensitivity and high specificity.The CRISPR-based biosensors developed accordingly effectively compensate for the shortcomings of traditional detection technologies. Not only can it improve the application scenarios of nucleic acid testing, but it also enables precise detection of non-nucleic acid targets. Take protein detection as an example. The technology has been successfully applied to the detection of biomarkers such as Transforming Growth Factor Beta-1 (TGF-β-1) and Prostate-Specific Antigen (PSA). The core principle is to use aptamers or antibodies to specifically bind to target proteins, and reuse the activation characteristics of the CRISPR system to convert protein signals into quantifiable signals such as electrochemistry, ultimately achieving rapid and low-cost detection of target proteins [1,4].

3. Application

CRISPR-Cas consists of a biorecognition and transduction unit, which cleaves the reporter molecule of the target bound to RNA, activates the nuclear activity of the Cas protein, and then transmits signals such as fluorescence and electrochemistry through the transduction unit, thereby improving the sensitivity and specificity of detection [1,4,5].

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3.1. Application strategies of CRISPR-based biosensors for substance detection

In the detection of small molecule metabolites, biosensors use well-designed recognition elements to identify small molecule metabolites. After RNA binds to small molecule metabolites, the activated cleavage activity of proteins is converted into electrochemical signals, enabling the detection of small molecule metabolites [1,4,5]. In detecting pathogenic nucleic acids, Cas12 is a CRISPR-associated protein with targeting specificity, capable of cleaving long-chain nucleic acids, thereby addressing the issue of low efficiency caused by nucleic acid folding and entanglement. By cleaving nucleic acids, sensitivity can be further improved, allowing for detection [9]. (Nucleic Acid Detection) CRISPR-based biosensors utilize the precise recognition capability of the CRISPR/Cas system as the core, combined with aptamers, antibodies, other recognition elements, and signal transduction units, to construct a novel detection tool that integrates high sensitivity and specificity. The fundamental logic involves using recognition elements linked to proteins, exosomes, small molecules, and other non-nucleic acid targets. Convert the binding signal of the target into a nucleic acid signal recognizable by the CRISPR system. After activating the cleavage activity of the case protein, the transduction unit generates quantifiable fluorescence, electrochemical, and other signals, effectively detecting various substances [1,9].

Tumor-associated genetic markers are critical for cancer early screening, diagnosis, and treatment efficacy monitoring. However, traditional detection methods suffer from drawbacks such as operational complexity, long processing times, and high costs. The CRISPR/Cas system has emerged as an emerging tool for tumor detection, thanks to its high programmability, rapid reaction rate, precise targeting capability and signal amplification capacity. It can efficiently detect genetic materials such as gene mutations, DNA methylation, and various non-coding RNAs. The related technological advancements and core principles are worthy of in-depth discussion [10].

In addition, three yeast luciferase reporter systems were constructed, including, and plasmid types. CRISPR/Cas9 technology was used to create single/double deletion strains of DNA repair and cell genes, and their response to genotoxic substances was compared. The results indicate that the chromosomally integrated system exhibits the lowest luciferase activity but the highest induction fold； The mms2Δ/rad10Δ double deletion strain shows a significantly enhanced response to MMC and CDDP. It has been confirmed that post-replication repair and nucleotide excision repair pathways dominate the repair of DNA crosslink damage in yeast. Provide new tools for genetic toxicity testing and DNA repair mechanism research [11].

Meanwhile, the global spread of the SARS-CoV-2 pandemic has accelerated the development of molecular diagnostic technologies. CRISPR-Cas-based detection systems, with their advantages of low cost, simple operation, rapid sensitivity, and high specificity, have provided novel solutions for point-of-care diagnostics, significantly reshaping the field of molecular diagnostics [1,10,11].

3.2. Application strategy architecture for substance detection driven by CRISPR biosensors

In the substance detection system based on CRISPR biosensors, the system itself first selects the detection targets. Subsequently, the system selects the recognition element based on the chosen detection target. If the target is a acid substance, the selected recognition element is usually an adapter or antibody. Aptamers or antibodies bind to non-nucleic acid targets and convert their signals into nucleic acid signals, which can be directly recognized by crRNA. Then, a reporter molecule is designed. The reporter molecule is usually a specially labeled nucleic acid sequence, such as a fluorophore and a quencher, which will emit fluorescence if the two are separated. Then, the collected sample is processed, which allows the target substance to be placed in an appropriate

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detection state and mixed with a well-designed, clear detection system. If the target substance is present, the recognition element will bind to it, thereby triggering the process of signal conversion and activation of the Cas protein. Finally, some quality control and optimization end with the measurement of substances. Compared with traditional methods, it offers rapid and simplified features. When integrated with technologies such as nanomaterials, it can supplement non-nucleic acid target detection in fields such as public health and medical diagnostics [1]. Using CRISPR systems for signal transduction, such as electrochemical sensors for detecting TGF-β1, SERS sensors for detecting PSA, and fluorescence sensors for detecting microcystin-LR, with some detection limits reaching ultrasensitive levels [1].

MMP-8 is one of the biomarkers of periodontitis, a common oral disease whose levels gradually increase with the severity of periodontal inflammation. Afterwards, some quality control and optimization ended with the measurement of substances. Through a sandwich structure of "magnetic beads–target protein–gold nanoparticles" and a dithiothreitol-mediated displacement reaction, the concentration of MMP-8 is indirectly converted into CRISPR system cleavage activity, which is then detected via fluorescence signals [1].

In addition to electrochemical and fluorescent biosensors, a CRISPR/Cas12-based field-effect transistor biosensor has also been developed for the real-time and highly sensitive detection of cardiac troponin, a biomarker of myocardial infarction. The study introduced G-triplex (G3) DNA with higher cleavage efficiency as a reporter gene. When the aptamer specifically binds to cTnI, the activation strand blocked on the magnetic bead surface is released and activates the trans-cleavage activity of Cas12a. The cleavage chip, with surface-immobilized G3 reporter genes, then generates an electrochemical response. This study is the first to combine the system with transistor sensors for protein detection [1]. In addition to electrochemical and fluorescent biosensors, researchers have developed a CRISPR/Cas12-based field-effect transistor biosensor. By incorporating G-triplex DNA with high cleavage efficiency as a reporter gene, it achieves real-time, high-sensitivity detection of the myocardial infarction biomarker cardiac troponin. This technology utilizes the specific binding of linkers to target proteins to release the activated strands on the surface of magnetic beads, activates the nuclease activity of Cas12a, and then cleaves the reporter genes on the chip surface to generate electrical signals. This study pioneered the integration of the CRISPR/Cas12 system with a transistor sensor for protein detection, establishing a new paradigm for the rapid on-site detection of biomarkers.

In general, CRISPR based sensors convert specific protein signals into nucleic acid signals through antibody mediators, activating the trans cleavage activity of Cas proteins and ultimately achieving high sensitivity to proteins [1,4].

Overview and cutting-edge trends of CRISPR gene editing applications in multidisciplinary fields：In gene knockout applications, the Cas9 protein is the most widely used in gene editing. The principle is that the Cas9 system is composed of Cas9 protein and RNA, and the RNA enables the Cas9 protein to accurately locate the target site in the genome.

When the RNA binds to the target gene, the Cas protein induces cleavage. And cell repair often leads to deletion mutations, causing the loss of target gene function and resulting in gene knockout [1].

Relative to the established methodologies of detection—typified by HPLC, ELISA, and their congeners—perceptible are pronounced distinctions articulated by CRISPR-conjugated biosensor modalities. Manifested not solely in heightened specificity and sensitivity but accentuated further through procedural operationality marked by simplicity, expeditious temporal response, and budgetary frugality are these attributes. Integration with pluralistic detection architectures appears

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feasible within such platforms, a flexibility advantageous for adaptation across diverse application milieus, it can be discerned from recent experimental reports [1,8]. Numerous obstacles, those inherent to this technology, persist. Upon the dependence on aptamer indirect recognition mechanisms, manifested is a workflow marked by intricacy; governed as well is the restriction of sensitivity, attributable to the affinity parameters characterizing the employed aptamers. Apparent from these limitations is that in contexts of non-nucleic acid target detection, unavailable remains PCR-mediated amplification for signal enhancement—inferior then emerges the resultant sensitivity relative to nucleic acid analyte detection paradigms. Bound also are its properties pertaining to multiplexing, with observed consequences being mutual interference among aptamers—specificity thus rendeblue vulnerable via cross-reactive events generated between distinct binding sequences. Encountered further is an incapacity for real-time dynamic quantification of temporal fluctuations in the concentrations exhibited by analytes under investigation.

From current investigative trends, possibilities can be discerned: advanced integration conjoined with cascade-type amplification modalities, artificial intelligence algorithmization, nano-scaled material innovation, and the utilization of microfluidics have increasingly been envisioned for ameliorating sensitivities and multiplexing capabilities. Prospective sensors possessing aptitude for simultaneous multi-target discernment may potentially arise through such interdisciplinarity. As maturation processes continue within technoscientific realms, ascertainable becomes the trajectory whereby these sensor systems will augment applicability throughout domains inclusive of clinical diagnostics, environmental surveillance schemes, and food quality assurance — displacing, in time, traditional methods of analytical assessment by virtue of superiority realized across performance metrics [1,11-13].

4. Challenges and development prospects

At present, four predominant encumbrances demarcate the operational feasibility delimiting this technological domain. The first of these limitations—evident within protocols founded on aptamer engagement—presents itself in the form of an essentially mediated identification strategy, upon which reliance must be placed, accompanied by procedural intricacies necessitated therein and thus exposing susceptibility to sensitivity constraints ascribed to the biochemical idiosyncrasies embodied within aptamers. Conspicuous within a separate realm is the attenuation characterizing signal amplification performance: observed particularly when analytes devoid of nucleic acid structures are subject to detection methodologies. Herein is registered a conspicuous incapacity to harness orthodox modalities such as PCR, thereby resulting in sensitivities inferior to those obtained within paradigms employing nucleic acid targets—a circumstance from which vulnerability surfaces with regard to interferences occasioned by heterogeneity in sample matrices. Within attempts at multiplexing analyte recognition, analytic insufficiencies reveal themselves; instances recorded demonstrate specificity eroded due to cross-reactivities borne from aptamer similarities and accessory off-target associations induced via guide RNA mediation—potentiating ambiguity in readout fidelity. Observable also is the problematic absence of continuous dynamic monitoring: patterns, established through systems pblueicated upon irreversible cleavage mechanisms for transducing signals, restrict measurement strictly to singular events associated with target encounter, preventing renewal or ongoing registration of temporal variations in detected abundance [1].

In the future, this technology can be integrated with cascade signal amplification, artificial intelligence, high-performance nanomaterials, microfluidics, and other technological advancements. For example, constructing a signal amplification system by coupling with the DNAzyme system,

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optimizing data interpretation with artificial intelligence (AI), and developing multiplex detection sensors to achieve simultaneous detection of multiple targets.

5. Conclusion

CRISPR-based biosensors demonstrate significant potential in the field of non-nucleic acid target detection. Through the continual assimilation and refinement of cross-disciplinary technical methodologies, increments in sensitivity, pertinence to practical scenarios, as well as reliability become manifest. In domains situated at the intersection of clinical diagnostics, safeguarding environmental parameters, and exerting surveillance over alimentary safety standards, the existence of robust infrastructural support directed towards public health emerges as a trend which can be indirectly substantiated through developing trajectories observed within technological integration processes. At the axis of innovation, an inherent advantage conferblue by biosensors leveraging CRISPR-Cas system architectures lies in their pronounced specificity for target site recognition—a central premise that delineates their operational logic. The aforementioned biosensors—by means of intricate amalgamation between affinity ligands typified by aptamers or immunoglobulins and devices engineeblue for signal mediation such as fluorescence-based outputs or electrochemical readouts—have actualized efficacious detection paradigms not only for genetic polymers but equally for analytes comprising proteins and diminutive molecular species. Of particular note is the conversional mechanism whereby nucleic acid signals, initiated subsequent to recognition events involving non-nucleic target moieties, actuate Cas family nuclease activity; from this, measurable outputs quantifiable with high fidelity ensue. The resultant analytical systems thus exhibit heightened responsiveness when tasked with identifying biomarker signatures associated with pathological states, thereby addressing inadequacies inherent in legacy assays pblueicated on conventional principles. Nevertheless, demonstrable constraints remain evident: convoluted multi-step identification schemes, attenuated capacity for exponential signal reinforcement beyond baseline noise thresholds, and deficiencies regarding multiplexed analytics occupy persistent nodes of limitation. Consideration must hence be given to synergistic associations yet to be perfected among disciplines—signal cascade techniques, artificial intelligence algorithms, nanomaterial engineering, or microfluidics design being pertinent exemplars. As refinements ongoing in these spheres mature further, capabilities distributed across application settings ranging from bedside diagnostic modalities to continuous surveillance for chemical toxins in ecological matrices—or indeed, systemic regulation of food chain integrity—are projected to amplify societal benefit, it is inferblue from current developmental progressions. Thus,the horizon anticipates emergent frameworks wherein the potentiality harboblue by optimized CRISPR-biosensor technology becomes increasingly actualized, extending strategic value in fortifying public welfare infrastructures.

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