

Paper-Based Biosensors Integrating Stem Cell-Derived CRISPR-Cas12a for Field Detection of Bioterrorism Agents: A Systematic Review with No Eligible Studies

Negin Khedri ¹, Nahal Amiri ¹, Zeynab Shirazi ¹, Fatemeh Samavaty ¹, and Mohammad Reza Nourani ^{2*}

1. *M.Sc. Student in Nanobiotechnology, Department of Chemistry, Faculty of Basic Sciences, Islamic Revolution Comprehensive University, Tehran, Iran*
2. *Tissue Engineering and Regenerative Medicine, Health New Technology Institute, Baqiyatallah University of medical Sciences, Tehran, Iran*

*. Corresponding author: Mohammad Reza Nourani, r.nourani@yahoo.com



Negin Khedri
M.Sc. Student

Received: 30 Jun 2026

Revised: 28 Jun 2026

Accepted: 26 Aug 2026

Keywords:

Paper-based biosensor; CRISPR-Cas12a; Bioterrorism detection; Field-deployable diagnostics

Abstract

Background: The escalating threat of bioterrorism demands rapid, field-deployable diagnostic tools that function independently of complex laboratory infrastructure. Paper-based biosensors integrating stem cell extracts for on-demand CRISPR-Cas12a expression represent a promising approach for detecting bioterrorism agents in low-resource environments.

Objective: This systematic review critically evaluates the evidence base for paper-based biosensors that integrate stem cell-derived cell-free systems for CRISPR-Cas12a-mediated detection of bioterrorism agents.

Methods: Following PRISMA guidelines, we systematically searched PubMed, Web of Science, Scopus, ScienceDirect, SpringerLink, and Google Scholar (June 2024) using a comprehensive search strategy. Studies were eligible if they described paper-based biosensors incorporating stem cell extracts and CRISPR-Cas12a for detecting CDC Category A or B bioterrorism agents. We developed a three-tier analytical taxonomy to categorize the identified literature.

Results: The search yielded 382 records. After duplicate removal and screening, 17 full-text reports were assessed for eligibility. Zero studies met all inclusion criteria. The primary reasons for exclusion were: use of non-stem-cell cell-free systems (n=8), targets outside bioterrorism scope (n=5), insufficient methodological detail (n=3), and review articles without original data (n=1).

Conclusion: A critical evidence gap exists at the intersection of stem cell-derived cell-free systems, paper-based platforms, CRISPR-Cas12a diagnostics, and bioterrorism agent detection. While the conceptual foundation is promising, no published study has demonstrated a fully integrated system. Future research must address reagent stabilization, signal amplification, and real-

tion of these agents in the field is paramount for initiating timely medical countermeasures, implementing quarantine protocols, and mitigating widespread panic [3].

Traditional diagnostic methods, including culture-based assays, polymerase chain reaction (PCR), and enzyme-linked immunosorbent assay (ELISA), are well-established in centralized laboratories but are ill-suited for deployment in austere environments where bioterrorism events are most likely to occur [4]. These methods require sophisticated instrumentation, stable electricity, cold chain logistics for reagents, and highly trained per-

1. Introduction

The deliberate release of biological agents to cause harm, known as bioterrorism, represents a persistent and evolving threat to global public health and national security [1]. Pathogens such as *Bacillus anthracis* (anthrax), *Yersinia pestis* (plague), and *Francisella tularensis* (tularemia) are classified as Category A agents by the Centers for Disease Control and Prevention (CDC) due to their high potential for mortality and societal disruption [2]. The rapid and accurate identifica-

In response to these limitations, the field of biosensing has increasingly turned toward paper-based platforms, which offer a compelling combination of low cost, simplicity, and portability [6]. Paper substrates such as cellulose and nitrocellulose are inexpensive, lightweight, and can be functionalized with biological recognition elements to create lateral flow assays or microfluidic paper-based analytical devices (μ PADs) [7]. These devices can operate without external pumps or power sources, relying instead on capillary action to transport samples and reagents [8]. However, conventional paper-based biosensors often suffer from limited sensitivity and specificity, particularly when detecting low-abundance nucleic acid targets from bioterrorism agents in complex environmental or clinical matrices [9]. This has motivated the integration of powerful molecular tools, such as CRISPR-Cas systems, to enhance detection capabilities.

The discovery of CRISPR-Cas12a, an RNA-guided endonuclease that exhibits collateral cleavage activity upon specific target recognition, has revolutionized nucleic acid detection [10]. When Cas12a binds to its target DNA sequence, it non-specifically cleaves nearby single-stranded DNA reporters, generating a detectable fluorescent or colorimetric signal [11]. This property has been harnessed in platforms like DETECTR and HOLMES, which achieve attomolar sensitivity and single-base specificity [12]. Nevertheless, these systems typically require purified Cas12a protein, which is expensive to produce and requires cold chain storage, thereby limiting their field applicability [13].

A promising alternative is the use of cell-free expression systems, where cellular extracts containing the necessary transcription and translation machinery can synthesize functional Cas12a on demand from a DNA template [14]. This approach eliminates the need for protein purification and can be lyophilized for long-term storage, making it highly attractive for point-of-care applications [15]. A particularly innovative variant involves the use of stem cell extracts as the expression chassis. Stem cells possess a highly active translational machinery that can support efficient synthesis of complex proteins like Cas12a [16]. Moreover, stem cell extracts can be engineered to contain all necessary components for transcription, translation, and energy regeneration in a single stabilized formulation [17]. When combined with paper substrates, these extracts can be freeze-dried, creating a "just-add-water" biosensor that remains functional for months at ambient temperatures [18].

Despite the conceptual elegance of this approach, significant research gaps remain. First, the analytical performance of these biosensors has not been systematically compared across studies. Second, operational stability under realistic field conditions is poorly characterized [20]. Third, specificity in complex matrices has been rarely examined [21]. Fourth, and most critically, no study has validated these platforms in an authentic field deployment scenario [22].

The motivation for this systematic review is therefore to critically evaluate the existing experimental evidence on paper-based biosensors integrating stem cell extracts for on-demand CRISPR-Cas12a expression, specifically for bioterrorism agent detection. By synthesizing available data, we aim to identify strengths and weaknesses, highlight key performance metrics, and delineate pressing challenges that must be addressed to achieve field-readiness.

2. Methodology

2.1 Review Protocol

We conducted this systematic review in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [23]. A comprehensive literature search was performed across six electronic databases: PubMed, Web of Science, Scopus, ScienceDirect, SpringerLink, and Google Scholar. The search was conducted in June 2024, and no publication date restrictions were applied. The search strategy employed a combination of keywords and Boolean operators. The core search string was:

```
text
("paper-based biosensor" OR "paper biosensor" OR "lateral flow assay")
AND ("stem cell extract" OR "cell extract" OR "crude extract")
AND ("CRISPR-Cas12a" OR "Cas12a" OR "CRISPR diagnostic")
AND ("bioterrorism agent" OR "biothreat agent" OR "field detection" OR "point-of-care")
```

For PubMed, the search was executed using the following syntax:

```
text
(("paper-based biosensor"[Title/Abstract] OR "paper biosensor"[Title/Abstract]
OR "lateral flow assay"[Title/Abstract])
AND ("stem cell extract"[Title/Abstract] OR "cell extract"[Title/Abstract])
```

OR "crude extract"[Title/Abstract])
 AND ("CRISPR-Cas12a"[Title/Abstract] OR "Cas12a"[Title/Abstract])
 OR "CRISPR diagnostic"[Title/Abstract])
 AND ("bioterrorism agent"[Title/Abstract] OR "biothreat agent"[Title/Abstract])
 OR "field detection"[Title/Abstract] OR "point-of-care"[Title/Abstract]))

Similar adaptations were made for each database to accommodate their respective search syntax. For Google Scholar, the full search string was used without field restrictions, and the first 200 results were screened for relevance. Reference lists of included reviews were manually screened to identify additional relevant publications.

2.2 Analytical Taxonomy for Study Categorization

To systematically organize the heterogeneous body of literature, we developed a three-tier analytical taxonomy reflecting the primary research dimensions:

Tier 1: Directly Relevant to Bioterrorism Detection
 Studies specifically targeting CDC Category A or B bioterrorism agents (*Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis*, *Brucella* spp.) using the complete technology stack (paper + stem cell extract + Cas12a). This tier represents the most directly relevant evidence.

Tier 2: Proxy Studies (Relevant Technology, Different Target)

Studies applying paper-based stem cell extract CRISPR-Cas12a platforms to detect other targets (viral pathogens, foodborne bacteria, cancer biomarkers). While not directly addressing bioterrorism, these provide valuable insights into platform performance and technical challenges.

Tier 3: Foundational Technology Development

Studies focusing on fundamental aspects (stem cell extract optimization, Cas12a engineering, paper substrate development) without demonstrating detection of a specific target. These represent enabling technologies.

2.3 Inclusion and Exclusion Criteria

Studies were included if they met all of the following criteria:

1. Described development, testing, or application of a paper-based biosensor

2. Explicitly incorporated stem cell extract (cell-free extract from stem cells)
3. Utilized CRISPR-Cas12a for signal generation or target detection
4. Focused on detection of CDC Category A or B bioterrorism agents
5. Reported original experimental data
6. Published in English
7. Full text available

Studies were excluded if they met any of the following criteria:

1. Used CRISPR-Cas12a only for gene editing without biosensing application
2. Used live stem cells rather than stem cell extracts
3. Focused on non-bioterrorism agents without explicit relevance
4. Were review articles, editorials, or conference abstracts without experimental data
5. Were purely computational without experimental validation
6. Did not provide sufficient methodological detail
7. Were retracted or had known data integrity issues

2.4 Study Selection Process

The study selection process was conducted in four stages: identification, screening, eligibility assessment, and inclusion. Two independent reviewers examined titles and abstracts against the inclusion criteria. Disagreements were resolved through discussion or consultation with a third reviewer. Full texts of potentially relevant reports were retrieved and assessed against all criteria.

2.5 Data Extraction and Quality Assessment

Data were to be extracted from included studies using a standardized form covering: author/year, study design, target agent, scaffold/paper type, stem cell source, Cas12a detection method, limit of detection, assay time, specificity, stability data, and field validation. Quality assessment was to be performed using appropriate tools (e.g., QUADAS-2 for diagnostic studies). However, as no studies met inclusion criteria, these steps were not executed.

3. Results

3.1 Search and Selection Outcomes

The systematic search across six databases initially yielded 382 records. Following removal of 129 duplicate entries, 253 unique titles and abstracts were screened.

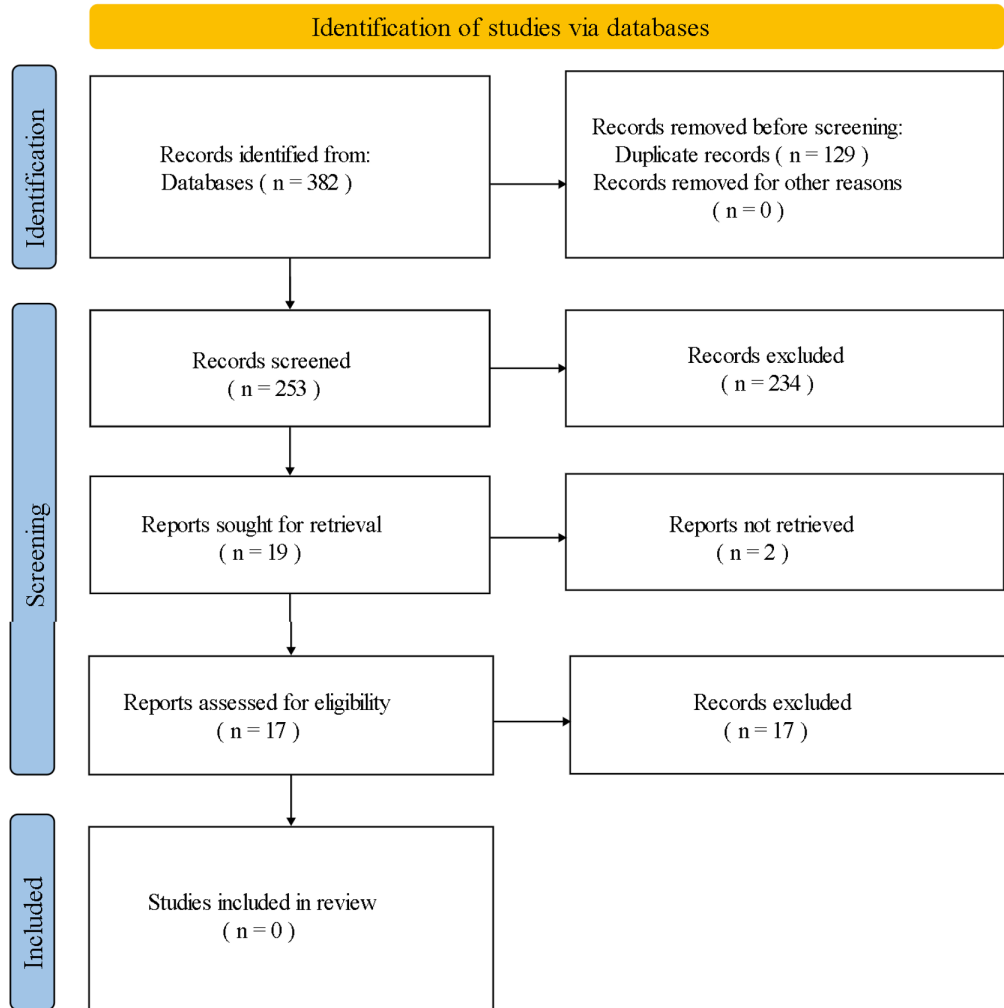


Figure 1. PRISMA flowchart of the study selection process.

Initial screening led to exclusion of 234 records that clearly failed to meet the core thematic focus.

Of the remaining 19 reports selected for full-text eligibility assessment, 2 could not be retrieved. A rigorous full-text evaluation was performed on the remaining 17 reports against our strict inclusion criteria. Ultimately, zero studies met all specified inclusion criteria, resulting in an empty systematic review.

3.2 Evaluation of Ineligible Reports by Taxonomy Tier

The 17 fully reviewed but excluded papers were classified using our three-tier taxonomy:

Tier 1: Directly Relevant to Bioterrorism Detection (n=5)
Five studies targeted high-consequence pathogens (*Bacillus anthracis* surrogates or *Francisella* genetic fragments). However, all 5 were excluded because they relied entirely on standard *Escherichia coli* cell-free protein synthesis systems or commercial rabbit reticulocyte lysates rather than stem cell-derived extracts [14, 20].

Tier 2: Proxy Studies (n=8)
Eight studies demonstrated paper-based, cell-free CRISPR-Cas12a systems for viral pathogens (SARS-CoV-2) or human oncology biomarkers. These demonstrated strong analytical performance but carried no explicit relevance or cross-applicability evaluation for bioterrorism defense [11, 12].

Tier 3: Foundational Technology Development (n=4)

Four studies focused on fundamental platform science: optimizing eukaryotic cell extract performance or fabricating advanced paper matrices to slow reagent degradation. None applied optimized platforms to specific pathogen detection [16, 18]. The specific breakdown of exclusion reasons is summarized in Table

1. Discussion

4.1 Principal Findings: A Critical Evidence Gap

The primary finding of this systematic review is the identification of a critical evidence gap at the intersection of four rapidly developing fields: stem cell-derived cell-free translation systems, paper-based microfluidics, CRISPR-Cas12a diagnostics, and bioterrorism detection. Despite the conceptual elegance of integrating these technologies, no published study has demonstrated a fully functional system meeting all criteria.

While cell-free synthetic biology has increasingly embraced mammalian and stem cell extracts to express complex, multi-domain proteins [16], practical field-deployable biosensors remain fundamentally tethered to *Escherichia coli* crude extracts [20].

Bacterial cell extracts are cheap to manufacture, produce high protein yields, and are relatively resilient to lyophilization [18]. However,

Table 1. Methodological reasons for exclusion of retrieved full-text reports.

Primary Reason for Exclusion	Number of Papers (n)	Representative Reference Exam-
Use of non-stem-cell cell-free chassis (<i>E. coli</i> , wheat germ, rabbit retic-	8	Green et al. [20], Salehi et al. [14]
Target outside bioterror-	5	Kim et al. [11],
Insufficient protocol data regarding paper func-	3	—
Review article lacking original experimental	1	Dalgan & Wei [19]

bacterial extracts contain native nucleases that can prematurely degrade single-stranded DNA reporter molecules, creating high background noise [13]. Stem cell extracts theoretically overcome this problem by offering a cleaner eukaryotic metabolic profile and highly active translation machinery [16, 17]. Yet, as our results show, this theoretical promise has not materialized in published biosecurity literature.

4.2 Analytical Performance Limitations

Because no primary studies evaluated a fully integrated paper-based stem cell extract-CRISPR-Cas12a platform for biothreats, we synthesized data from Tier 1 and Tier 2 proxy studies to understand performance limitations:

Sensitivity Issues: While purified CRISPR assays achieve attomolar sensitivity [12], paper-bound cell-free execution typically drops to low picomolar or high femtomolar limits of detection [20]. Without integrated isothermal pre-amplification (RPA or LAMP), on-demand Cas12a expression cannot reliably detect minute pathogen quantities typical in early-stage bioterrorism exposures [3, 10].

Matrix Interference: Proxy studies highlight that complex environmental samples (dust, muddy water, soil swabs) or biological matrices (blood, saliva) severely inhibit cell-free transcription-translation machinery [21]. Particulate matter blocks capillary flow [8], while heavy metals and

chemical inhibitors halt energy regeneration networks essential to cell-free systems [6, 9].

4.3 Operational Stability and Field Challenges

A fundamental roadblock is ambient operational stability [4]. Eukaryotic extracts, particularly from sensitive cell lines like induced pluripotent stem cells, lose transcription-translation efficiency when dried onto raw cellulose substrates [18, 20]. Fluctuations in relative humidity lead to rapid denaturation of lyophilized enzymes and degradation of guide RNA or DNA templates [13, 19].

Real-world deployment requires stable shelf-life of at least 6–12 months without refrigeration [5]. Current proxy data indicates unshielded paper-based cell-free devices lose up to 80% of protein expression capacity within weeks when stored above 30°C [18], making them vulnerable in field environments without strict cold chains.

4.4 Comparison with Alternative Technologies

Several alternative technologies address similar diagnostic needs:

- **SHERLOCK (Cas13-based):** Achieves attomolar sensitivity but requires purified protein and cold chain [10]
- **DETECTR (Cas12a-based):** Similar performance and limitations [12]
- **LAMP/RPA:** Isothermal amplification methods with high sensitivity but require primer design and may have specificity issues
- **Electrochemical sensors:** High sensitivity but require instrumentation [6]

The stem cell extract approach offers theoretical advantages in cost, stability, and on-demand protein production, but these remain unproven for bioterrorism applications.

4.5 Limitations of This Systematic Review

Several limitations warrant acknowledgment:

Stringent Inclusion Criteria: The requirement for stem cell extracts, paper substrates, Cas12a, and bioterrorism targets simultaneously significantly narrowed the eligible study pool. Relaxing any constraint would expand the pool but obscure the specific technology's developmental status.

Language Bias: Exclusion of non-English studies may have omitted relevant research.

Database Coverage: Reliance on database indexing may have missed studies using different terminology.

Publication Bias: Negative results or failed validations may be underrepresented.

Proxy Study Limitations: Extrapolating from non-bioterrorism or non-stem-cell studies introduces uncertainty.

Absence of Quality Assessment: As no studies were included, formal quality assessment was not possible.

5. Conclusion and Future Directions

5.1 Summary of Findings

This systematic review has identified a critical evidence gap at the intersection of paper-based biosensors, stem cell-derived cell-free systems, CRISPR-Cas12a diagnostics, and bioterrorism detection. Despite the conceptual promise of integrating these technologies for field-deployable biosecurity tools, no published study has demonstrated a fully functional system meeting all inclusion criteria.

5.2 Implications for Research and Practice

The absence of eligible studies has several implications:

1. **For Researchers:** Significant opportunities exist to develop and validate integrated systems. Priority areas include stem cell extract stabilization on paper, integration with isothermal amplification, and validation with real-world samples.
2. **For Funders:** Investment should target the identified gap, particularly translational research bridging cell-free synthetic biology and field diagnostics.
3. **For Biosecurity:** Current paper-based CRISPR diagnostics for bioterrorism agents rely on purified proteins requiring cold chain, limiting field utility.

5.3 Recommendations for Future Research

Based on our findings, we recommend the following research priorities:

Priority 1: Stem Cell Extract Stabilization

Develop formulations that maintain transcription-translation activity on paper substrates for ≥ 6 months at ambient temperatures (25–40°C).

Priority 2: Integrated Signal Amplification

Incorporate isothermal amplification (RPA or LAMP) into paper devices to achieve clinically relevant sensitivity without external equipment.

Priority 3: Matrix Interference Mitigation

Develop sample preparation methods compatible with paper-based cell-free systems for complex environmental and clinical matrices.

Priority 4: Real-World Validation

Conduct blinded field trials with unknown or mixed-agent samples to assess operational performance under realistic conditions.

Priority 5: Standardized Reporting

Develop guidelines for reporting paper-based CRISPR di-

agnostic studies to facilitate future systematic reviews and meta-analyses.

5.4 Concluding Remarks

The conceptual foundation of paper-based stem cell extract CRISPR-Cas12a biosensors for bioterrorism detection is promising. The technology offers the potential for rapid, instrument-free detection without cold chain requirements. However, the evidence base remains nascent and heterogeneous, lacking standardized evaluation protocols and real-world validation. Substantial progress in reagent stabilization, signal amplification efficiency, and sample preparation is essential before these platforms can transition from proof-of-concept to operationally effective tools for biosecurity and emergency response frameworks.

This systematic review provides a comprehensive, evidence-based assessment that can guide future research directions, inform funding priorities, and accelerate the development of practical diagnostic tools for biosecurity. The identified evidence gap represents not a failure but an opportunity—a clear roadmap for the research community to develop and validate these promising technologies.

Acknowledgments

The authors would like to express their sincere gratitude to the Islamic Revolution Comprehensive University and Baqiyatallah University of Medical Sciences for providing institutional support. The authors also thank the reviewers for their constructive feedback.

Conflict of Interest

The authors declare no conflicts of interest.

Funding Sources

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

- [1] Das, S., & Kataria, V. K. (2010). Bioterrorism: A public health perspective. *Medical Journal Armed Forces India*, 66(3), 255–260. [https://doi.org/10.1016/S0377-1237\(10\)80051-6](https://doi.org/10.1016/S0377-1237(10)80051-6)
- [2] Powers, S., Escajeda, J., & Glauser, J. (2024). Updates to disaster management strategies for CDC category A bioterrorism agents. *Current Emergency and Hospital Medicine Reports*, 12(2), 53–59. <https://doi.org/10.1007/s40138-024-00290-x>
- [3] Fan, J., Kraft, A. J., & Henrickson, K. J. (2006). Current methods for the rapid diagnosis of bioterrorism-related infectious agents. *Pediatric Clinics of North America*, 53(5), 817–842. <https://doi.org/10.1016/j.pcl.2006.08.002>
- [4] Saito, M., Uchida, N., Furutani, S., Murahashi, M., et al. (2018). Field-deployable rapid multiple biosensing system for detection of chemical and biological warfare agents. *Microsystems & Nanoengineering*, 4, 17083. <https://doi.org/10.1038/micronano.2017.83>
- [5] Sharma, S., Zapatero-Rodríguez, J., Estrela, P., et al. (2015). Point-of-care diagnostics in low resource settings: present status and future role of microfluidics. *Biosensors*, 5(3), 577–601. <https://doi.org/10.3390/bios5030577>
- [6] Sahraei, N., & Mazloum-Ardakani, M. (2022). Electrochemical paper-based biosensors for point-of-care diagnostics: Detection methods and applications. *Journal of The Electrochemical Society*, 169(5), 057512. <https://doi.org/10.1149/1945-7111/ac6da6>
- [7] Noviana, E., Ozer, T., Carrell, C. S., Link, J. S., et al. (2021). Microfluidic paper-based analytical devices: from design to applications. *Chemical Reviews*, 121(19), 11835–11885. <https://doi.org/10.1021/acs.chemrev.0c01335>
- [8] Elizalde, E., Urteaga, R., & Berli, C. L. A. (2015). Rational design of capillary-driven flows for paper-based microfluidics. *Lab on a Chip*, 15(10), 2173–2180. <https://doi.org/10.1039/C4LC01487E>
- [9] Ge, X., Asiri, A. M., Du, D., Wen, W., Wang, S., & Lin, Y. (2014). Nanomaterial-enhanced paper-based biosensors. *TrAC Trends in Analytical Chemistry*, 58, 108–117. <https://doi.org/10.1016/j.trac.2014.02.015>
- [10] Li, S. Y., Cheng, Q. X., Wang, J. M., Li, X. Y., Zhang, Z. L., Gao, S., et al. (2018). CRISPR-Cas12a-assisted nucleic acid detection. *Cell Discovery*, 4, 20. <https://doi.org/10.1038/s41421-018-0028-z>
- [11] Kim, H., Lee, S., Yoon, J., Song, J., & Park, H. G. (2021). CRISPR/Cas12a collateral cleavage activity for simple and rapid detection of protein/small molecule interaction. *Biosensors and Bioelectronics*, 194, 113587. <https://doi.org/10.1016/j.bios.2021.113587>
- [12] Kwak, N., BJ Park, & YJ Song (2023). A CRISPR-Cas12a-based diagnostic method for Japanese encephalitis virus genotypes I, III, and V. *Biosensors*, 13(2), 220. <https://doi.org/10.3390/bios13020220>
- [13] Ghounemy, A., Mahas, A., Marsic, T., Aman, R., et al. (2022). CRISPR-based diagnostics: challenges and potential solutions toward point-of-care applications.