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Current biosensing strategies based on in vitro T7 RNA polymerase reaction

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ABSTRACT

Recently, a unique behavior of T7 RNA polymerase has expanded its functionality as a biosensing platform. Various biosensors utilizing T7 RNA polymerase, combined with fluorescent aptamers, electrochemical probes, or CRISPR/Cas systems, have been developed to detect analytes, including nucleic acids and non-nucleic acid target, with high specificity and low detection limits. Each approach demonstrates unique strengths, such as real-time monitoring and minimal interference, but also presents challenges in stability, cost, and reaction optimization. This review provides an overview of T7 RNA polymerase's role in biosensing technology, highlighting its potential to advance diagnostics and molecular detection in diverse fields.

1. Introduction

RNA molecules play vital and diverse regulatory roles within cells by interacting with various nucleic acids, proteins, and small molecules. Inspired by this natural versatility, researchers have begun engineering RNA molecules with new biological functions.[1–3] In recent years, synthetic biology has made considerable progress, developing innovative synthetic RNA elements capable of regulating gene expression in vivo, primarily in bacterial and yeast systems, setting the stage for scalable and programmable cellular behaviors.[4–7] Current challenges in this emerging field include utilizing computational and directed evolution approaches to improve the complexity of engineered RNA systems and expanding their application to mammalian systems.[8,9] Additionally, there are ongoing efforts to design RNA molecules that can act as sensors for both intracellular and environmental cues, as well as probes for studying biological networks and key components of engineered cellular regulatory systems.[10,11] Therefore, understanding RNA synthesis is important in synthetic biology research.

Since the introduction of in vitro enzymatic processes for RNA synthesis, T7 RNA polymerase has become widely used in both research and commercial settings. Derived from the T7 bacteriophage, this enzyme catalyzes RNA synthesis from DNA in the 5' → 3' direction.[12] The process involves several stages, including promoter recognition, promoter elongation, transcription, and termination. T7 RNA polymerase is highly specific to its promoter, transcribing only DNA located downstream of a T7 promoter sequence. Its unique characteristics have made it an invaluable tool across a variety of fields, from uncovering basic biological mechanisms to producing recombinant proteins, developing RNA-based therapeutics, and advancing biosensing technologies.[13–15]

T7 RNA polymerase is widely utilized in nucleic acid research, especially in biosensing applications, due to its strong and specific activity. A key advantage of T7 RNA polymerase over DNA polymerase is its ability to continuously and isothermally produce multiple RNA chains from a single DNA template.[16,17] This makes it valuable in both direct and indirect biosensing. In indirect biosensing, T7 RNA polymerase generates RNA for subsequent use where it can produce crRNA for biosensing purposes. In direct biosensing, which either capture biomolecules or result from the interaction between a DNA template and an analyte. These capabilities make T7 RNA polymerase a crucial tool in RNA-based biosensing technologies.

The aim of this study is to review the available biosensors based on T7 RNA polymerase for robust and efficient detection of various analytes. Several biosensors utilizing T7 RNA polymerase, combined with fluorescent aptamers, electrochemical probes, or CRISPR/Cas systems, have been developed for detecting various analytes. Additionally, the advantages and challenges associated with these methods will be

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discussed. This review provides a comprehensive overview of the current state of T7 RNA polymerase-based biosensor technology, offering guidance for future research and development in the field.

2. In vitro T7 RNA Polymerase as Biosensor

T7 RNA polymerase reactions are central to RNA synthesis, using T7 RNA polymerase derived from the T7 bacteriophage to transcribe RNA from a DNA template. The process of transcription by T7 RNA polymerase involves three key stages[18–20]: promoter recognition, transcription, and termination (Fig. 1). First, T7 RNA polymerase specifically recognizes and binds to a short, well-defined promoter sequence, typically located upstream of the gene to be transcribed. This promoter sequence is crucial for initiating transcription and ensures that the enzyme only transcribes DNA located downstream of the T7 promoter. Once bound, the polymerase begins RNA synthesis by incorporating nucleotides complementary to the DNA template in the 5′ to 3′ direction. The enzyme transitions into the elongation phase after clearing the promoter, during which it synthesizes a continuous RNA strand while maintaining a stable elongation complex. T7 RNA polymerase is highly processive during elongation, capable of producing long RNA molecules without dissociating from the template. Termination occurs when the polymerase reaches a specific termination signal or the end of the template, leading to the release of the newly synthesized RNA transcript. This streamlined and efficient process, devoid of additional transcription factors, makes T7 RNA polymerase an ideal enzyme for controlled in vitro transcription. Its processivity and precision make it an essential tool in various applications.[21,22]

T7 RNA polymerase exhibits a unique ability to transcribe RNA from single-stranded DNA (ssDNA) templates, though it requires a double-stranded DNA (dsDNA) promoter for transcription initiation.[23–25] This specificity arises because the enzyme must first bind to a fully duplexed promoter region, typically extending from about −17 to +1 relative to the transcription start site. Once bound and transcription is initiated, T7 RNA polymerase can continue elongation using ssDNA as the template, making it highly versatile. This allows for efficient RNA synthesis in situations where only a minimal portion of the template needs to be double-stranded, simplifying experimental designs and reducing template preparation requirements. The polymerase's capability to function on ssDNA templates after promoter binding also enhances its utility in synthetic biology and biosensing applications, where custom RNA sequences can be produced efficiently.

DNA-based biosensing plays a critical role in preventive measures against dangerous biomolecules and pathogen infections. However, conventional DNA-based biosensors often suffer from limitations in sensitivity and specificity.[26,27] The use of in vitro T7 RNA polymerase reactions offers signal amplification through transcription, significantly enhancing both sensitivity and specificity. One of the key benefits is the ability to control and fine-tune reaction conditions, which allows for precise and isothermal RNA synthesis without the need for complex cellular machinery.[28,29] Detection systems can be developed at the pre-, during, and post-transcription stages of T7 RNA polymerase activity. In the pre-transcription stage, the analyte interacts with the DNA template, influencing the transcription reaction. During transcription, the competition between T7 RNA polymerase and the analyte (e.g., proteins or biomolecules) can also be utilized for biosensing applications. The significant differences in transcription products between the pre- and during-transcription stages result in a highly sensitive detection platform. In post-transcription-based T7 RNA polymerase biosensing, the system relies on RNA transcription products acting as triggers that interact with the analyte. Various interactions, such as RNA aptamer-biomolecule, RNA-DNA, and RNA-RNA interactions, can occur during this stage, enabling the development of biosensing applications with high sensitivity and specificity. In addition, compared to in vivo T7 RNA polymerase reactions, in vitro T7 RNA polymerase offers several advantages, particularly in biosensing applications.[20] In vitro reactions eliminate cellular interference, thereby reducing noise and variability in biosensing outputs. Moreover, in vitro T7 RNA polymerase can be easily integrated with other biosensing platforms to detect a wide range of analytes with high specificity and sensitivity. This flexibility and scalability make in vitro T7 RNA polymerase particularly well-suited for the development of efficient, responsive, and programmable biosensors.

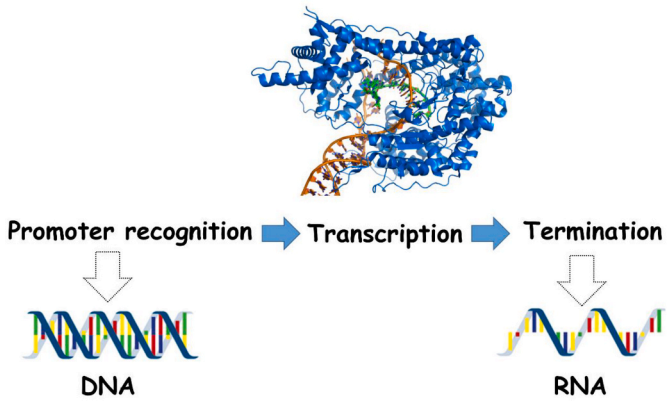

Fig. 1. T7 RNA polymerase transcription process.

3. Biosensor of T7 RNA Polymerase

3.1. T7 RNA polymerase based on fluoroscence

In vitro T7 RNA polymerase biosensing based on fluorescence operates by leveraging the enzyme's ability to transcribe RNA from a DNA template, producing RNA molecules that interact with fluorescent aptamers or dyes to generate a measurable signal.[30] The principle involves a DNA template containing a T7 promoter sequence that T7 RNA polymerase specifically recognizes and binds to, initiating transcription. Besides being used downstream as a biosensing platform, the T7 promoter has also been utilized in detection circuits for analyte sensing. As RNA is synthesized, it can be designed to include sequences that bind to fluorescent molecules, such as light-up RNA aptamers, which emit fluorescence upon binding. This fluorescence-based approach allows for real-time monitoring of transcription, as the fluorescence intensity increases proportionally with the amount of RNA produced. This method provides a powerful tool for tracking RNA synthesis and quantifying T7 RNA polymerase activity without complex equipment. Several T7 RNA polymerase-based fluorescence biosensors are shown in Table 1.

The in vitro T7 RNA polymerase-based biosensing strategy with fluorescence output employs a split T7 promoter-based isothermal transcription amplification system for rapid and highly specific detection of SARS-CoV-2.[31] T7 RNA polymerase is central to this approach, as it initiates transcription only when the target RNA forms a complete double-stranded T7 promoter within a three-way junction structure. This design enables T7 RNA polymerase to transcribe a light-up RNA aptamer, which, when combined with a fluorogenic dye, produces a strong fluorescence signal. The system detects SARS-CoV-2 RNA in less than 30 min at 37 °C, achieving high sensitivity (96.7 %) and specificity (100 %) in clinical tests with 60 samples. By targeting two loci on the SARS-CoV-2 genome, this strategy enhances detection sensitivity down to 102 copies/μL (167 aM), providing a rapid, single-enzyme method suitable for point-of-care diagnostics.

Another T7 RNA polymerase-based biosensing strategy with fluorescence output uses a high-throughput iSpinach fluorescent aptamer-based system to monitor in vitro transcription by T7 RNA polymerase in real-time.[32] The key role of T7 RNA polymerase in this setup is to initiate transcription from a DNA template, producing RNA transcripts that subsequently bind to the iSpinach aptamer, leading to a fluorescence signal when combined with the DFHBI dye (Fig. 2). This

Table 1
T7 RNA polymerase-based fluoroscence biosensors.

Biosensing Strategy	Analyte Type	Limit of Detection	References
Split T7 promoter-based isothermal transcription amplification	SARS-CoV-2 RNA (variants) and bacterial 16S rRNA	10^2 copies/μL	31
Molecular beacon-based isothermal detection via T7 RNA polymerase-aided target regeneration	Sequence-specific DNA	10 pM	17
iSpinach fluorescent aptamer-based real-time monitoring of in vitro transcription	RNA synthesis/ transcription reactions	0.75–25 μg/ mL	32
Aptamer-based switching system for communication of non-interacting proteins	Non-interacting proteins	10 nM	33
Label-free detection of T4 Polynucleotide Kinase activity via malachite green aptamer	T4 Polynucleotide Kinase (PNK) activity	Sub-femtomolar range	34
Aptamer-regulated in vitro transcription for protein biosensing	Proteins (e.g., cytokines IFN-γ, IL-6, TNF-α)	2–10 nM	35

fluorescence-based system enables continuous monitoring of RNA production, allowing rapid optimization of transcription conditions. The method achieves high sensitivity, with fluorescence intensities that correlate closely with RNA yields, offering a direct readout of T7 RNA polymerase activity.

Several in vitro T7 RNA polymerase-based strategies with fluorescence output have also been demonstrated previously.[17,33–35] Challenges in this method include fluorescent aptamers or dyes, which often require precise folding to emit signals, potentially failing to interact consistently with RNA due to secondary structures or environmental factors, thereby affecting signal quality.

3.2. T7 RNA polymerase based on electrochemical biosensor

In vitro T7 RNA polymerase-based electrochemical biosensing operates by leveraging the enzyme's ability to transcribe RNA from a DNA template upon the presence of a target molecule, such as DNA or RNA.[36] When the target binds to a specific probe on the sensor, it triggers the formation of a double-stranded region containing a T7 promoter. T7 RNA polymerase recognizes this promoter and transcribes RNA, which is then used as an amplified signal. The RNA products interact with detection elements leading to an electrochemical response. This response, typically measured as changes in current or potential, correlates with the concentration of the target, enabling highly sensitive detection. The specificity of T7 RNA polymerase ensures a low signal-to-noise ratio, making this method ideal for detecting nucleic acids and other biomolecules in applications like diagnostics. Several T7 RNA polymerase-based electrochemical biosensors are shown in Table 2.

The electrochemical output-based in vitro T7 RNA polymerase biosensing has been employed with a homogeneous target-initiated transcription amplification system for ultrasensitive electrochemical detection of pathogenic DNA.[38] The role of T7 RNA polymerase is

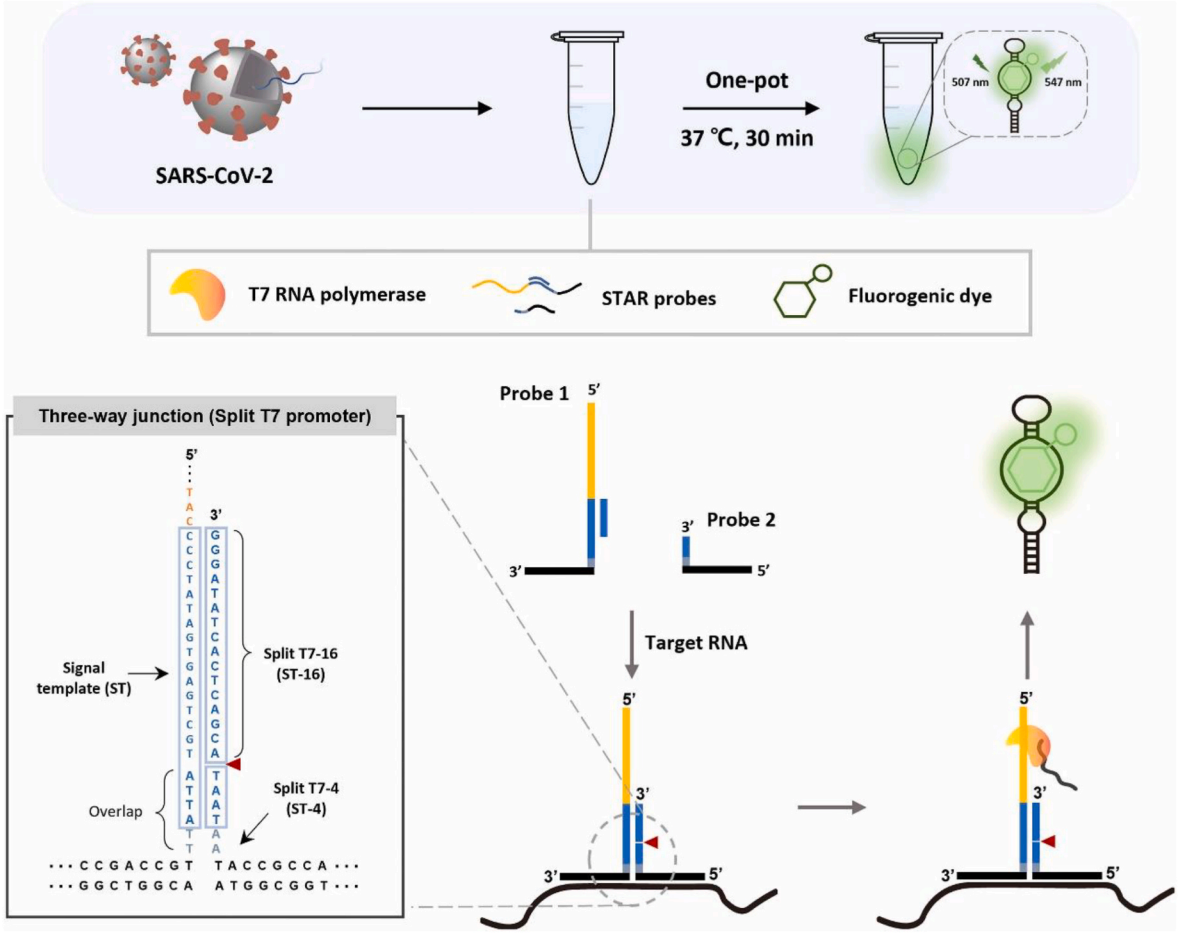

Fig. 2. Schematic images representation of T7 RNA polymerase-based fluoroscence biosensors. Reproduced with permission from Ref. 31.

Table 2
T7 RNA polymerase-based electrochemical biosensors.

Biosensing Strategy	Analyte Type	Limit of Detection	References
Defective T junction-induced transcription amplification with T7 RNA polymerase	DNA from Group B Streptococci (GBS)	0.4 fM (Synthetic DNA); 400 copies of GBS DNA	37
Homogeneous target-initiated transcription amplification (HTITA) strategy	Pathogenic DNA (Salmonella *invA* gene)	0.97 fM; 1000 copies of Salmonella DNA	38
Proteolysis-triggered transcription amplification for electrochemical detection	Protease biomarker (MMP-2)	7.1 fM for MMP-2	39
Electrochemical biosensor integrating NASBA for viral RNA detection	Viral RNA (HIV, Zika, Influenza)	~200 copies/μL for viral RNA	40

critical in amplifying the detection signal. After the target DNA binds to a specially designed hairpin probe, the conformational change allows for a primer extension reaction, which produces double-stranded DNA containing a T7 promoter. T7 RNA polymerase recognizes this promoter and transcribes numerous RNA molecules from the downstream DNA template. These RNA products then hybridize with probes on the biosensor's surface, facilitating enzyme-amplified electrochemical readout. This cascade of amplification significantly boosts the detection sensitivity, enabling the system to detect DNA at extremely low concentrations, down to 0.97 fM.

Another electrochemical output-based in vitro T7 RNA polymerase biosensing method employs a defective T-junction-induced transcription amplification system, harnessing T7 RNA polymerase for ultrasensitive electrochemical detection of DNA (Fig. 3).[37] T7 RNA polymerase plays a crucial role by driving transcription amplification once the defective T-junction, formed upon target DNA binding, creates a double-stranded promoter region. This promoter region allows T7 RNA polymerase to generate abundant single-stranded RNA products, which subsequently hybridize with biotinylated probes on the biosensor surface, leading to a measurable electrochemical signal. The DTITA approach enables high signal amplification while maintaining low background noise, resulting in a detection limit as low as 0.4 fM for synthetic DNA. The method is also robust, showing consistent performance across assays and successfully detecting Group B Streptococcus DNA in clinical samples without PCR.

Various electrochemical methods based on in vitro T7 RNA polymerase have been demonstrated previously.[39,40] The use of in vitro T7 RNA polymerase-based electrochemical biosensing presents several challenges despite its advantages in sensitivity and specificity. One major issue is maintaining the stability and activity of T7 RNA polymerase during the reaction, as enzyme degradation or inhibition can affect the reliability of the biosensor. Another challenge lies in minimizing nonspecific binding, which can lead to background noise and reduce the accuracy of the detection.

3.3. T7 RNA polymerase based on colorimetric

In vitro T7 RNA polymerase biosensing based on colorimetric detection is a powerful and sensitive technique used to detect specific target molecules by exploiting the enzyme's transcriptional activity. In this method, T7 RNA polymerase transcribes RNA from a DNA template in a controlled, cell-free environment when a target molecule, such as a DNA sequence, RNA, or a protein like a protease, is present. The transcribed RNA then triggers a colorimetric reaction, often involving materials or enzymatic systems that produce a visible color change. The color change occurs due to interactions between the RNA and colorimetric reagents, such as the aggregation of nanoparticles or the activation of chromogenic substrates, which shifts the color of the solution from one hue to another, commonly from red to blue. This process allows for easy, rapid, and highly sensitive detection of target molecules with minimal equipment, as the color change can be observed by the naked eye or measured using simple devices. The in vitro nature of this system ensures precise control over the reaction conditions, improving specificity and minimizing interference from other biological components. Overall, T7 RNA polymerase-based colorimetric biosensing is widely used in diagnostics and research due to its simplicity, scalability, and versatility. Several T7 RNA polymerase-based colorimetric biosensors are shown in Table 3.

The utilization of T7 RNA polymerase in an in vitro transcription/

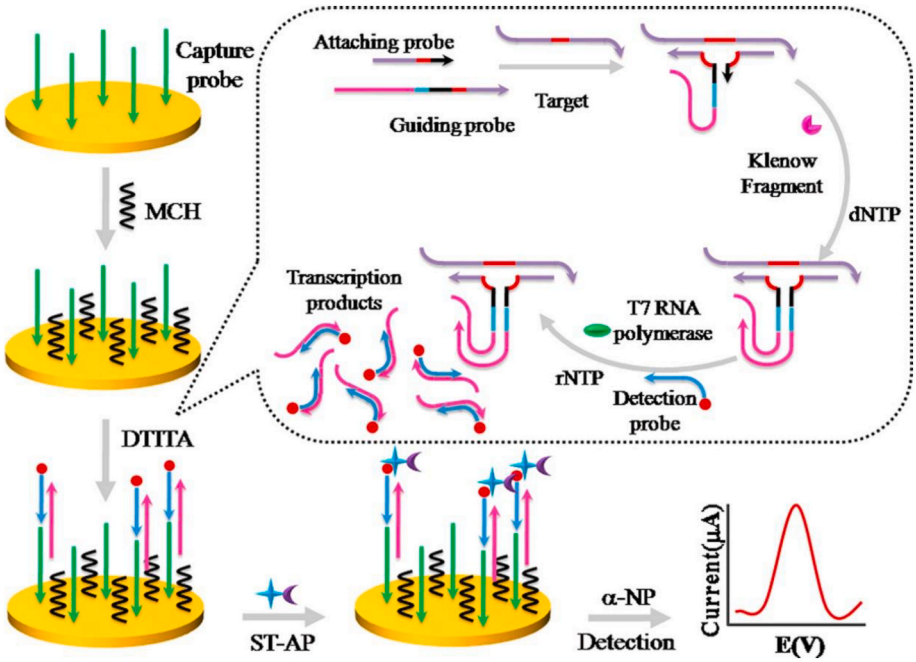

Fig. 3. Schematic images representation of T7 RNA polymerase-based electrochemical biosensors. Reproduced with permission from Ref. 37.

Table 3
T7 RNA polymerase-based colorimetric biosensors.

Biosensing Strategy	Analyte Type	Limit of Detection	References
Split T7 switch-mediated cell-free protein synthesis system	Target nucleic acids (HPV DNA, SARS-CoV-2)	10 pM	41
Toehold switch-mediated riboregulatory assay with NASBA	SARS-CoV-2 viral RNA	Around 100 copies of synthetic COV-2 RNA	42
Proteolysis-responsive transcription coupled with spherical nucleic acids	Protease biomarkers (MMP-2, thrombin, HCV)	3.3 pM for MMP-2	43
Toehold RNA biosensor with NASBA and IVTT	SARS-CoV-2 RNA (including variants)	667 RNA copies; 110 aM	2

translation assay, coupled with a cell-free protein synthesis platform, enables efficient RNA detection with a colorimetric output.[42] By leveraging the specificity of T7 RNA polymerase for its promoter, the assay allows precise transcription of viral RNA in synthetic samples, ultimately amplifying the signal for detection. One key advantage of this approach is the ability to operate at room temperature, making it cost-effective and accessible for use in resource-limited settings, particularly in developing countries. The system has shown high sensitivity, detecting RNA concentrations as low as 110 aM, corresponding to approximately 667 RNA copies per reaction. This platform offers a low-cost (~0.26 euro per test) solution for nucleic acid diagnostics, with potential for further optimization to reduce costs and increase scalability.

Another colorimetric biosensing strategy based on T7 RNA polymerase uses a split T7 switch-mediated cell-free protein synthesis (CFPS) system to detect target nucleic acids with enhanced sensitivity and specificity.[41] The key role of T7 RNA polymerase is to initiate transcription only when the split T7 promoter forms a complete double-stranded structure in the presence of target nucleic acids, enabling precise control and reducing background noise, which was a limitation in previous toehold switch systems (Fig. 4). Operating under isothermal conditions, the system achieves a low detection limit of 10 pM, significantly improving detection efficiency. T7 RNA polymerase's selective transcription initiation enhances the platform's versatility, allowing for both fluorescent and colorimetric output, making it adaptable for various diagnostic applications. The one-pot setup, enabled by T7 RNA polymerase, simplifies the process and makes it suitable for on-site diagnostics.Fig. 5

Several in vitro T7 RNA polymerase-based colorimetric methods have also been demonstrated before.[43,44] One of the key challenges in in vitro T7 RNA polymerase biosensing based on colorimetric detection lies in maintaining the system's sensitivity and specificity while minimizing background noise. Achieving highly selective transcription that only occurs in the presence of the target molecule is critical, as unintended or "leaky" transcription can lead to false positives and reduce the accuracy of the assay. Additionally, ensuring that the colorimetric response is robust and easily distinguishable can be difficult, particularly in complex samples where other substances might interfere with the reaction or obscure the color change. Another challenge is optimizing the stability of the reagents, such as the RNA transcribed by T7 RNA polymerase, which may degrade over time, affecting the reliability of the detection.

3.4. T7 RNA polymerase integrated with CRISPR/Cas system

In vitro T7 RNA polymerase biosensing integrated with CRISPR/Cas systems represents a promising new frontier in biosensing, leveraging the unique strengths of both T7 RNA polymerase for transcriptional amplification and CRISPR/Cas systems for specific target recognition and signal amplification. The CRISPR/Cas system, known for its precise targeting capabilities, has recently emerged as a robust biosensing platform capable of detecting nucleic acids with high specificity and sensitivity.[45–48] In these systems, T7 RNA polymerase transcribes RNA from a DNA template when a specific target sequence or analyte triggers activation, producing RNA that is subsequently detected or cleaved by CRISPR-associated proteins like Cas12a or Cas13a. This dual-enzyme approach enables significant signal amplification, often generating a detectable readout—such as fluorescence or electrochemical changes—that correlates directly with the target concentration. The CRISPR/Cas system's adaptability to various signal outputs, such as fluorescent, electrochemical, and colorimetric, makes it versatile for diverse applications, including pathogen detection, environmental monitoring, and diagnostics.[49–54] Despite its newness as a biosensing platform, the integration with T7 RNA polymerase in a cell-free, in vitro setup demonstrates a powerful, modular approach to sensitive, real-time detection, paving the way for innovative diagnostic tools. Several T7 RNA polymerase-based colorimetric biosensors are shown in Table 4.

The CRISPR/Cas system integrated with T7 RNA polymerase has been applied for SARS-CoV-2 detection through an "entropy-driven triggered T7 amplification-CRISPR/Cas13a system" (EDT-Cas), combining T7 RNA polymerase and CRISPR/Cas13a to enhance detection sensitivity.[56] T7 RNA polymerase plays a crucial role by initiating transcription of target SARS-CoV-2 RNA after the entropy-driven cyclic amplification creates a T7 promoter. This process generates abundant single-stranded RNAs (ssRNAs), which further activate CRISPR/Cas13a,

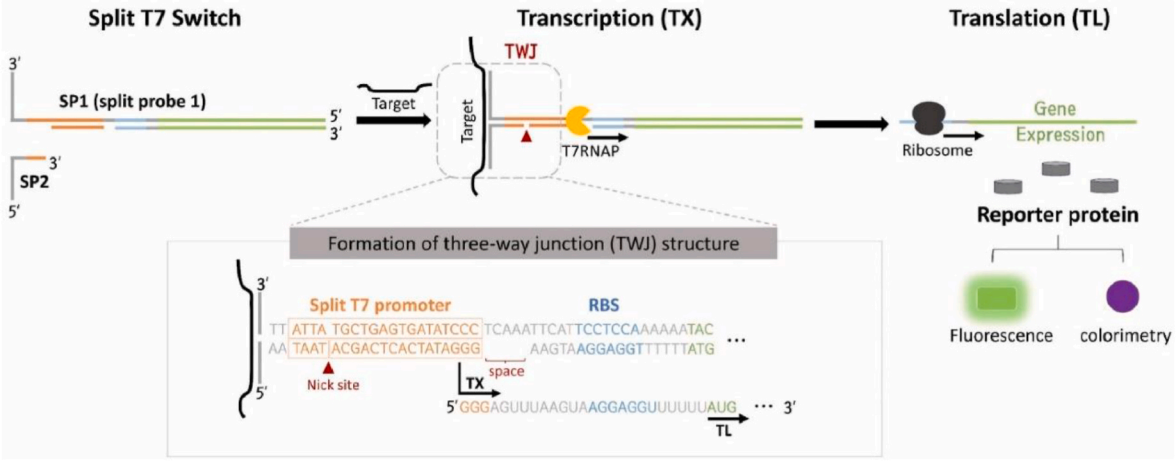

Fig. 4. Schematic images representation of T7 RNA polymerase-based colorimetric biosensors. Reproduced with permission from Ref. 41.

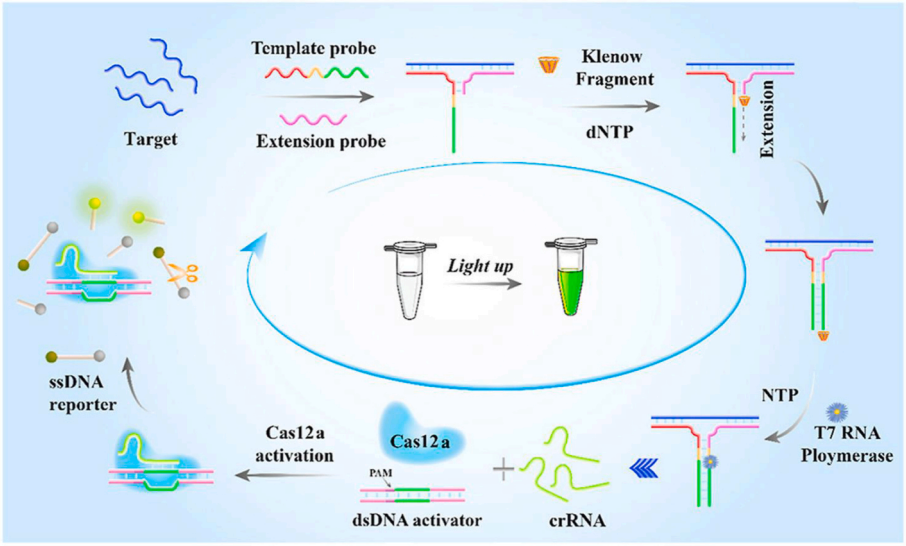

Fig. 5. Schematic images representation of T7 RNA polymerase-based CRISPR/Cas biosensors. Reproduced with permission from Ref. 61.

Table 4
T7 RNA polymerase-based CRISPR/Cas biosensors.

Biosensing Strategy	Analyte Type	Limit of Detection	References
CRISPR/Cas12a with Target-Protected Transcription Amplification	miRNA, proteins, exosomes	0.75 fM (miRNA), around 5 pg/mL (protein), around 2.5×10^7 particles/μL (exosome)	55
Entropy-Driven T7 RNA Polymerase + CRISPR/Cas13a for SARS-CoV-2	SARS-CoV-2 viral RNA	7.39 aM	56
Hyper-Branching Rolling Circle Amplified CRISPR/Cas13a	miRNA	200 aM	57
Bis-Enzyme Cascade CRISPR-Cas12a Platform for miRNA	miRNA	1 pM	58
Electrochemical Detection of BNP via CRISPR/Cas13a + Chain Substitution	B-Type Natriuretic Peptide (BNP)	0.74 aM	59
T7 RNA Polymerase and CRISPR-Cas12a for MMP-2 Detection	Matrix Metalloproteinase-2 (MMP-2)	0.189 pg/mL	60
CRISPR/Cas12a with Self-Supply crRNA and Transcription Amplification	Nucleic acids (DNA and RNA)	417 fM	61
T7 RNA Polymerase-Assisted CRISPR/Cas13a for BNP Detection	B-Type Natriuretic Peptide (BNP)	3.2 fg/mL	62

enabling specific cleavage of reporter probes on the electrode surface. The resulting ECL signal amplification allows for the detection of SARS-CoV-2 RNA at extremely low concentrations, with a detection limit of 7.39 aM, demonstrating high sensitivity and repeatability.

Another biosensing strategy, using a CRISPR/Cas12a nucleic acid sensing platform enhanced by T7 RNA polymerase-driven transcription amplification, has been applied for highly sensitive nucleic acid detection.[61] T7 RNA polymerase plays a critical role in this system by initiating transcription once the target nucleic acid forms a three-way junction structure with proximity probes. This structure enables T7 RNA polymerase to produce numerous crRNA molecules, which then assemble with Cas12a and dsDNA activators, forming a complex that triggers Cas12a's *trans*-cleavage activity. This cascade effect amplifies the fluorescence signal significantly, allowing for detection limits as low as 41.7 amol. This dual-step amplification approach not only enhances the platform's sensitivity but also reduces nonspecific background signals, resulting in highly precise nucleic acid detection applicable to both DNA and RNA targets.

Several paper[55,57–60,62,63] In vitro T7 RNA polymerase biosensing based on CRISPR/Cas systems presents unique challenges, particularly due to the dual-enzyme mechanism, which involves both T7 RNA polymerase and CRISPR-associated proteins like Cas12a or Cas13a. This dual-enzyme setup contributes to higher operational costs, as both enzymes are expensive to produce and purify, and it also requires precise reaction conditions to maintain optimal activity, adding complexity to the workflow. Furthermore, the use of two enzymes can be time-consuming, as it requires sequential steps where T7 RNA polymerase transcribes the target RNA, which then activates the CRISPR/Cas reaction for signal detection. Ensuring compatibility between these enzymatic activities—especially in a controlled, cell-free environment—demands meticulous optimization of conditions like temperature, buffer composition, and ion concentrations to avoid loss of sensitivity or false-positive signals.

4. Conclusion

In vitro T7 RNA polymerase-based biosensing strategies, focusing on fluorescence, electrochemical, colorimetric, and CRISPR/Cas system integrations, have been demonstrated. Each of these platforms showcases the unique ability of T7 RNA polymerase to produce targeted RNA sequences in controlled reactions, achieving high sensitivity and specificity in biosensing applications. The versatility of T7 RNA polymerase, whether coupled with fluorescent aptamers, electrochemical sensors, colorimetric reagents, or CRISPR-based systems, highlights its critical role in advancing diagnostic technologies for nucleic acid detection. While these methods offer promising detection capabilities, challenges such as reaction optimization, cost, and stability must be addressed to facilitate their broad adoption in practical and point-of-care settings. This study reviews the potential of T7 RNA polymerase biosensors as robust tools for future applications in diagnostics and environmental monitoring, guiding further research and innovation in the field.

CRediT authorship contribution statement

David Septian Sumanto Marpaung: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ayu Oshin Yap Sinaga:** Writing – review & editing, Writing – original draft, Resources, Methodology, Conceptualization. **Damayanti Damayanti:** Writing – review & editing, Writing – original draft. **Taharuddin Taharuddin:** Writing – review & editing, Writing – original draft. **Setyadi Gumaran:** Writing – review & editing, Writing – original draft.

Availability of data and material

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Declaration of competing interest

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

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