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Aptamer-based Biosensors: Emerging Tools for Next-generation Diagnosis of Strategic Parasitic Diseases

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ABSTRACT

Parasitic diseases impose a profound global health and economic burden, particularly in resource-limited settings where rapid and accurate diagnostic infrastructure is lacking. Traditional diagnostic methods often fall short in sensitivity, specificity, and point-of-care adaptability. Aptamers—single-stranded DNA or RNA oligonucleotides selected in vitro via systematic evolution of ligands by exponential enrichment (SELEX)—have emerged as highly stable, cost-effective, and versatile biorecognition elements capable of binding diverse targets with high affinity and specificity. This review explored the mechanisms of aptamer-based biosensors (aptasensors) and their emerging applications in diagnosing strategic parasitic infections, including malaria, leishmaniasis, Chagas disease, human African trypanosomiasis, and giardiasis. Despite challenges, such as nuclease degradation and SELEX-related biases, aptamer-based biosensors demonstrate significant potential as reliable, sensitive, and specific tools for next-generation diagnosis and monitoring of parasitic diseases.

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Introduction

Parasites, a diverse group of organisms ranging from single-celled protozoa to complex multicellular invertebrates, inhabit a vast array of ecological niches and engage in intricate, often detrimental, relationships with their hosts. These organisms can reside externally (ectoparasites) or internally (endoparasites), establishing associations characterized by varying durations and host specificity. Their complex life cycles frequently involve multiple hosts and distinct developmental stages, underscoring the biological sophistication that enables their survival and propagation [1].

The global impact of parasitic infections is profound and far-reaching. In 2010, the World Health Organization (WHO) estimated that foodborne parasitic diseases alone were responsible for approximately 137,000 deaths and contributed to 15 million disability-adjusted life years (DALYs), highlighting the significant burden on global health [2]. The consequences of parasitic diseases extend beyond their direct pathology in human and animal hosts, imposing substantial economic burdens. These losses are largely attributable to diminished productivity within both human workforces and livestock populations. This reduction stems from widespread morbidity and mortality associated with parasitic infections, which not only compromise individual health but also escalate healthcare expenditures and diminish agricultural yields. The economic ramifications are particularly acute in resource-limited settings and vulnerable communities, where the delicate balance between public health and economic stability is easily disrupted. Consequently, effective control and prevention strategies are paramount to mitigate the extensive socio-economic impact of these neglected tropical diseases [3].

Despite considerable scientific advancements in the prevention, diagnosis, treatment, and control of infectious diseases, the eradication of many parasitic pathogens remains a formidable challenge. This persistence is largely due to the remarkable adaptability, evolutionary capacity, and complex biological mechanisms employed by these organisms.

The continuous emergence of novel diagnostic and therapeutic strategies is therefore essential to combat these persistent threats effectively. Within this context, the development of innovative diagnostic tools is crucial for timely and accurate disease detection, enabling prompt intervention and improved patient outcomes. Emerging technologies, such as aptamer-based biosensors, are poised to revolutionize the next generation of diagnostics for these strategically important parasitic diseases [4]. These biosensors can provide rapid, sensitive, and specific detection of parasites, facilitating timely treatment and improving patient outcomes. The integration of molecular diagnostics and point-of-care testing is expected to revolutionize the approach to diagnosing parasitic infections, particularly in endemic regions [5, 6].

Aptamers: A Novel Class of Biorecognition Elements

Aptamers, a groundbreaking class of nucleic acid-based molecules, were first conceptualized and developed in 1990 by two independent research groups. These are typically single-stranded deoxyribonucleic acid (ssDNA) or ribonucleic acid (ssRNA) sequences, ranging from 20 to 80 nucleotides in length. Their unique three-dimensional structures enable them to selectively bind to a diverse array of targets with high affinity and specificity. This remarkable versatility allows aptamers to recognize a broad spectrum of molecules, including but not limited to proteins, metal ions, viruses, and even small molecules, like toxins [7, 8]. The systematic generation of aptamers is primarily achieved through an iterative in vitro selection process known as systematic evolution of ligands by exponential enrichment (SELEX). This sophisticated methodology involves multiple rounds of selection, typically seven to ten, where nucleic acid libraries are incubated with the target molecule. Sequences that exhibit high binding affinity and specificity are amplified, and this process is repeated until aptamers with desired binding characteristics are isolated. Consequently, these aptamers often demonstrate dissociation constants in the nano- to picomolar range, a performance metric highly comparable to that of high-affinity antibodies. Aptamers are increasingly recognized as compelling alternatives to traditional antibody-based biorecognition elements, offering several distinct advantages that position them favorably for next-generation diagnostic and therapeutic applications. A principal advantage lies in their production method: Aptamers are generated via chemical synthesis. This synthetic route bypasses the biological variability inherent in antibody production, ensuring consistent batch-to-batch functionality and reliability [9]. Furthermore, the synthesis timeline for aptamers is considerably shorter, typically under three months, in contrast to the approximately six months required for the generation of monoclonal antibodies. This accelerated production also translates to greater cost-effectiveness, as aptamer synthesis does not necessitate expensive cell

culture or animal models that significantly inflate the cost of antibody production.

Beyond production advantages, aptamers possess inherent stability properties. They exhibit the remarkable ability to renature and regain their native structure and function even after exposure to denaturing conditions. This resilience facilitates extended storage and transport at ambient temperatures, a capability generally unattainable with antibodies, which are often sensitive to environmental fluctuations. Their significantly smaller molecular size, approximately 10 to 100 times smaller than antibodies, also confers an advantage in biological applications by enhancing tissue penetration [10]. Moreover, aptamers can be chemically modified at their 5' or 3' termini, or along their sugar-phosphate backbone, to significantly improve their stability [11]. These modifications can reduce susceptibility to degradation by nucleases and minimize renal clearance, thereby extending their half-life and making them more amenable to in vivo applications and therapeutic use.

Finally, aptamers can be readily conjugated with a variety of labels, such as biotin, digoxigenin, or fluorophores, without compromising their target affinity or specificity [12]. Leveraging these properties, aptamers demonstrate broad applicability, facilitating their incorporation into numerous diagnostic platforms, including flow cytometry, a variety of biosensing technologies, and enzyme-linked aptamer-sorbent assays (ELASA) [13].

Aptamer-based Biosensors: Mechanisms and Signal Transduction

Aptamer-based biosensors, often termed aptasensors, represent a sophisticated class of analytical devices that harness the exquisite molecular recognition capabilities of aptamers for the detection of specific target molecules. These sensors are rapidly emerging as powerful tools across diverse fields, including clinical diagnostics, environmental monitoring, and food safety, owing to their inherent advantages of high specificity, exceptional sensitivity, and remarkable adaptability. The fundamental architecture of an aptamer-based biosensor typically comprises three essential components:

The aptamer: This serves as the primary biorecognition element. Immobilized on a surface or in solution, the aptamer selectively binds to its designated target analyte with high affinity. The transducer: This component is responsible for converting the molecular recognition event–the binding of the aptamer to its target–into a measurable physical or chemical signal.

The signal output: The signal generated by the transducer is then processed and presented as an output, which can be optical, electrochemical, mass-based, or other measurable phenomena, depending on the chosen transduction strategy.

The core principle underlying aptasensor operation is the conformational change exhibited by the aptamer upon target binding. This change in structure can significantly alter the aptamer's physical or electrochemical properties. These alterations are then detected and quantified by the transducer, thereby providing a sensitive and specific means to measure the concentration of the target analyte within a given sample [14].

Diagnosis Application of Aptamer in Parasitic Disease

Aptamers hold substantial promise as innovative molecular tools for improving the management of parasitic diseases, which remain a major source of morbidity and mortality worldwide. Despite notable progress in prevention and treatment, several protozoan parasites continue to threaten global health—particularly in low- and middle-income regions where diagnostic infrastructure and access to therapeutics are often limited. At the beginning of the twenty-first century, hundreds of millions of individuals in developing regions were affected by parasites. These include *Trypanosoma brucei* (African trypanosomiasis), *Trypanosoma cruzi* (Chagas disease). In parallel, multiple *Leishmania* species are responsible for a spectrum of clinical manifestations, including cutaneous, mucocutaneous, and visceral leishmaniasis [15]. These distinct disease forms vary in tissue tropism and disease severity, which complicates diagnosis and necessitates stratified therapeutic and monitoring approaches.

In addition to trypanosomatids and leishmaniasis, malaria remains one of the most consequential parasitic infections. Caused primarily by *Plasmodium falciparum* and *Plasmodium vivax*, malaria continues to impose a severe burden on health systems globally, being associated with approximately 600,000 deaths and 200 million cases annually [16]. Collectively, these infections illustrate both the biological diversity of parasitic pathogens and the persistent challenges they create for surveillance, clinical management, and public health planning.

Over the past decade, rapid advances in parasite biology and a deeper understanding of host–parasite interactions—accelerated by modern "omics" approaches (e.g. genomics, transcriptomics, proteomics, and metabolomics)—have enabled the identification of novel

molecular targets and the rational design of improved diagnostics and therapeutics. These developments have expanded opportunities for precision medicine strategies, including the detection of disease-specific biomarkers and the emergence of more targeted drug discovery pipelines.

Nonetheless, parasitic infections remain life-threatening in many endemic settings, largely due to ongoing transmission, incomplete treatment efficacy, drug resistance in some contexts, and the continued limitations of current diagnostic workflows. Therefore, there is an ongoing and urgent need for new strategies that (i) enable accurate and timely diagnosis, (ii) improve monitoring of treatment response, and (iii) support the discovery of different targets and novel therapeutic modalities. In this regard, aptamer-based technologies—including aptamer-driven biosensors—are emerging as promising platforms that can address these requirements by providing sensitive, selective, and potentially point-of-care-compatible detection of parasite-derived biomarkers.

Common Parasitic Diseases Targeted by Aptamer-based Biosensors

Malaria

The limitations of existing diagnostic methods underscore the urgent need for innovative technologies that can provide rapid and quantitative results. Electrochemical aptamer-based sensors present a promising solution by employing aptamers as recognition elements that undergo conformational changes upon binding to their target, resulting in measurable electrochemical signals [17]. In a groundbreaking study, Yang et al. developed an electrochemical sensor specifically designed for quantifying the malaria biomarker *P. falciparum* lactate dehydrogenase (PfLDH). Initially, the authors selected three aptamer variants and fabricated a fluorescence aptasensor to evaluate their performance. Through optical techniques, they assessed the responsiveness of these aptamer variants, ultimately leading to the selection of the most effective candidates for electrochemical testing. Among the variants tested, the P11-30 aptamer exhibited the highest signal change, demonstrating its superior binding characteristics. This variant was chosen for further development due to its rapid response and stability in phosphate-buffered saline (PBS). The resulting electrochemical sensors showed significant sensitivity to PfLDH, with a detection range suitable for clinical applications. Specifically, the P11-30 (CTACTGTTGATATGAGTGATAGGGCGGCGC) variant demonstrated a dissociation constant (Kd) of 136±17 nM and a dynamic range of 18.7–1200 nM. These findings indicate that the sensor can effectively detect PfLDH concentrations relevant to clinical scenarios, making it a viable option for point-of-care applications in malaria diagnostics. Furthermore, the sensor proved effective in measuring PfLDH concentrations in diluted human blood, highlighting its potential for real-world diagnostic applications [18].

Leishmaniasis

In the study by Martín et al. the authors investigated the development of DNA aptamers that specifically recognize the H2A histone protein of *Leishmania infantum*. In this work, the aptamers AptLiH2A#1 and AptLiH2A#2 could detect the LiH2A protein from *L. infantum* promastigotes at a limit of detection (LOD) corresponding to approximately 7500 parasites. This was established using enzyme-linked oligonucleotide assay (ELONA) assays, indicating the sensitivity of the aptamers in recognizing the target protein in complex biological samples. The aptasensor demonstrated a linear detection range for the recombinant *L. infantum* H2A protein (rLiH2A) from 25 ng to 100 ng per well, with a LOD of 10 ng of rLiH2A per well [19].

The *L. infantum* GP63 protein (LiGP63) is another major surface antigen and virulence factor involved in promastigote adhesion to macrophages and the intracellular survival of amastigotes. Using SELEX, LiGP63-specific DNA aptamers were generated and characterized. From an initial set of 20 candidate sequences, 14 aptamers were found by confocal fluorescence microscopy and flow cytometry to bind more than 70% of *L. infantum* promastigotes, with distinct subcellular localization patterns. Subsequent dot-blot screening narrowed the selection to five aptamers, which were further validated using an aptamer-linked immobilized sorbent assay for the detection of endogenous LiGP63 in promastigote lysates. The selected aptamers recognized recombinant LiGP63 with binding affinities spanning 0.3–2.1 µM. Functionally, pretreatment of promastigotes with LiGP63Apt-4 (TTACCAGCTTATTCAATTCGTCGGTGGGTGGGTCGGGTGGGGACGAAGTGACGTTTCAAGATAGTAAGTGCAATAT), LiGP63Apt-27 (TTACCAGCTTATTCAATTGCGGGGGGGGGGGGGGGGTGGAGGGGGTTGTAACTGTGAGAGATAGTAAGTGCAATCT), and LiGP63Apt-28 (ATACCAGCTTATTCAATTGTACGCGTGTGGGGGTGGGGGGGCGGGTTCATACGGTGAAGATAGTAAGTGCAATCT) significantly reduced adhesion to and infection of RAW 264.7 macrophages.

Moreover, conjugation of LiGP63Apt-4 and LiGP63Apt-28 to liposomes significantly improved targeting to *L. infantum* promastigotes relative to plain liposomes, supporting their potential for future nanoparticle-based drug delivery strategies. Collectively, these findings highlight LiGP63-binding aptamers as promising candidates for diagnostic and therapeutic development in leishmaniasis [20].

In another study, an ultrasensitive turn-on fluorescent probe was developed by integrating an aptamer with a chitosan–carbon dot (chitosan-CD) nanocomposite. The carbon dots used in this system were synthesized from Quercus extract using a microwave-assisted method, and the resulting nanocomposite was characterized by multiple microscopic and spectroscopic techniques. Before aptamer incorporation, the chitosan-CD platform exhibited strong fluorescence emission, whereas the addition of the aptamer resulted in complete fluorescence quenching. This probe enabled selective detection and quantification of *L. infantum* through recognition of poly (A)-binding protein (PABP) on the parasite surface. The biosensor showed high sensitivity and specificity, with a LOD of 94 cells/mL and linear response ranges of 188–750 cells/mL and 3000–6000 cells/mL. Its selectivity was further confirmed against interfering pathogens, including *T. brucei* and *Staphylococcus aureus*, as well as against proteins, such as LiIF2α, LiP2a, and BSA. Overall, the findings suggest that chitosan-based carbon dot nanocomposites provide a promising platform for the development of highly selective and sensitive turn-on fluorescent probes for the early detection of *L. infantum*, with potential value for future clinical applications [21].

Human African trypanosomiasis and Chagas disease

Human African trypanosomiasis (HAT) lacks fast, robust, and affordable diagnostics suitable for large-scale screening. Aptamer-based sensors (aptasensors) are being explored as an alternative because aptamers can bind *Trypanosoma* parasites or their surface proteins with high specificity and can be coupled to diverse signalreadout platforms. Several RNA aptamers have been selected against *T. brucei* VSGs and live bloodstream-stage parasites, including RNA 216, which binds infective parasites and localizes to the flagellar pocket [22].

In a key study targeting Chagas disease, Nagarkatti et al. developed a non-polymerase chain reaction (PCR), non-serological enzyme-linked aptamer (ELA) assay to detect *T. cruzi* excreted secreted antigens (TESA) in host plasma, offering a novel diagnostic tool for this strategic parasitic disease. Using a SELEx-based approach with negative selection against host proteins and the related parasite *Leishmania donovani*, they identified a highly specific, serum-stable RNA aptamer, Apt-L44, which binds to a conserved target across multiple *T. cruzi* strains. The resulting ELA assay successfully detected circulating parasite biomarkers in infected mice during both the acute phase (7 to 28 days post-infection) and the chronic phase (55 to 230 days post-infection). By targeting active parasite-secreted antigens rather than host antibodies, this aptamer-based biosensing platform overcomes the limitations of conventional serology (such as cross-reactivity and the inability to assess cure) and PCR (which suffers from fluctuating parasitemia), demonstrating great potential for next-generation diagnostics and treatment monitoring [23].

Zelada-Guillén et al. developed an ultrasensitive, real-time potentiometric aptasensor designed to detect the variable surface glycoprotein (VSG) of African trypanosomes (*T. brucei*), and the causative agent of African trypanosomiasis (sleeping sickness). By pairing the exceptional potentiometric transduction capabilities of single-walled carbon nanotubes (SWCNTs) with a highly nuclease-resistant, 2'-F-modified RNA aptamer, the researchers successfully addressed the common limitation of aptamer degradation in biological fluids. To bypass tedious sample preparation, they utilized a tailored isotonic buffer containing a redox-buffering agent, ferricyanide/ferrocyanide ($[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$), which minimized blood matrix interference and signal drift. This label-free platform achieved an extraordinary detection limit of 300 aM in buffer and successfully identified VSG directly in diluted clinical blood samples down to 10 pM (4 fM in the electrochemical cell) within 2 to 10 minutes. Exhibiting high selectivity against abundant serum proteins and the capacity to recognize multiple conserved, isotypic VSG variants, this robust and cost-effective biosensor represents a major advancement toward point-of-care, real-time diagnostics for strategic parasitic infections [24].

Giardiasis

A representative example of aptamer-based biosensor development for parasitic disease diagnosis is provided by the electrochemical aptasensor designed for the detection of Giardia intestinalis cyst recombinant protein. In this study, a high-affinity DNA aptamer, referred to as "cyst 1" (5′-TCA GTC ATT ATG TAT GCT TGA TCA CTT ATC CGT CTG CCG T-3′) with kd=7.98 nM, was selected using the SELEX process, ensuring strong specificity toward cyst-stage antigens. The selected aptamer was subsequently integrated into an electrochemical

sensing platform, where it functioned as the biorecognition element immobilized on an electrode surface. Upon binding of the target protein, aptamer–analyte interactions induced measurable changes in the electrochemical signal, enabling sensitive and selective detection. The resulting aptasensor demonstrated robust analytical performance, with a wide linear range of 0.1 pg mL^{-1} to 1000 ng mL^{-1} and a low detection limit of 0.41 pg mL^{-1}, including high sensitivity and specificity, and was successfully validated in spiked tap water samples, underscoring its applicability in real-world matrices. Notably, this cyst-specific aptamer complements a previously reported trophozoite-targeting aptamer, allowing discrimination between distinct developmental stages of *G. intestinalis*. This work exemplifies how rational aptamer selection combined with electrochemical transduction can yield versatile, stage-specific diagnostic tools, highlighting the broader potential of aptamer-based biosensors for next-generation detection and monitoring of parasitic infections [25].

Conclusion

Despite their significant potential, aptamer technology currently faces notable challenges, including the susceptibility of RNA aptamers to nuclease-mediated degradation, complexities and biases inherent in the SELEX process, and ongoing difficulties with target accessibility, non-specific binding, and in vivo delivery. To overcome these barriers and successfully transition from bench to bedside, future research is increasingly focusing on the integration of artificial intelligence and machine learning to optimize aptamer design, which promises to enhance specificity, stability, and affinity while reducing development time and costs. Furthermore, advancements in nanomaterials, microfluidics, and wearable biosensor platforms, combined with efforts to refine SELEX methodologies, improve structural understanding of aptamer-target interactions, and develop biocompatible delivery systems, are expected to establish aptamers as reliable, cost-effective, and versatile alternatives to conventional affinity reagents for next-generation diagnostics and precision medicine. Continued interdisciplinary research and technological innovation are essential for refining aptamer-based platforms, facilitating their emergence as dependable, cost-effective alternatives to traditional affinity reagents. With ongoing progress, aptamers are poised to significantly impact clinical diagnostics, targeted therapeutics, and precision medicine in the near future.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors contribution's

Investigation and data curation: Hajar Ziaei Hezarjaribi; Writing the original draft: Rabeeh Tabaripour; Review and editing: Rezvan Yazdian Robati and Mahdi Fakhar; final approval: All authors.

Conflict of interest

The authors declared no conflicts of interest.

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