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Aptamers for aflatoxin M1: from aptasensing technology to commercialization

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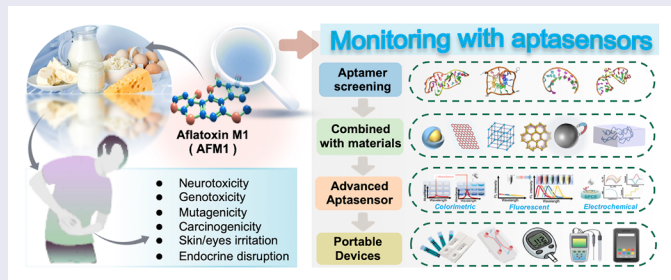
ABSTRACT

Aflatoxin M1 (AFM1) contamination in milk and dairy products poses a significant global concern due to its severe health risks to humans. Sensitive and specific detection of AFM1 has emerged as the most effective strategy for mitigating these health risks. Aptamers are regarded as ideal biorecognition elements for AFM1 monitoring owing to their small size, ease of production, excellent affinity and specificity, high stability, and low immunogenicity. Herein, we review the advancements in SELEX-based aptamer selection and the recent development of aptasensors for AFM1 from 2020 to 2025 for the first time. Additionally, the progress in signal transduction strategies, underlying mechanisms, and the design of portable devices are highlighted. Furthermore, we critically examine and summarize the prospects, challenges, and future trends in aptasensor technology, with a particular emphasis on its potential for real-world application and commercialization.

KEYWORDS

Aptasensor; aflatoxin M1; food safety; signal transduction; commercialization

GRAPHICAL ABSTRACT



Introduction

Antibodies, usually recognized as classic receptors, are responsible for target recognition via specific antibody-antigen interactions (Zhang and Guo 2022a; Jones et al. 2020). Over the past few decades, antibody-based immunoassays have been established as the gold standard in biomedical and biotechnology fields, including biosensing, therapy, and diagnosis (Li et al. 2020; Xu et al. 2021). Notably, specific antibodies have been developed and approved as affinity reagents for targeting Alzheimer (Van Dyck 2018), cancer (Kumar et al. 2024), and SARS-CoV-2 (Ju et al. 2023). In recent years, there has been a growing interest in antibody-based immunoassays from biomedical research to food safety control (Jia et al. 2021; Tang et al. 2020). Classic immunoassay techniques, such as enzyme-linked immunosorbent assays (ELISA) and lateral flow immunoassays (LFIA), are widely employed for screening purposes and are commercially

available (Zhao et al. 2021; Xing et al. 2020). However, the high production costs of antibodies and their limited stability in complex environments restrict their broader applications (Hou et al. 2022; Zhao et al. 2022). More importantly, the preparation of antibodies against small molecules remains a rigorous challenge due to their low immunogenicity and the weak immune response elicited in animals (Talan et al. 2018). Therefore, the exploration of promising alternatives is of great significance.

Aptamers, often referred to as chemical antibodies, exhibit excellent affinity and specificity for target biomolecules, even superior to antibody (Dixon, Williams, and Pretorius 2021; Mao et al. 2022; Li et al. 2022). Unlike antibodies, aptamers are nucleic acid molecules selected *in vitro* by systematic evolution of ligands by exponential enrichment (SELEX) technique, rather than undergoing the complicated animal experiments *in vivo*. This makes aptamers more convenient to produce than antibodies (Krissanaprasit et al. 2021; Yang et al.

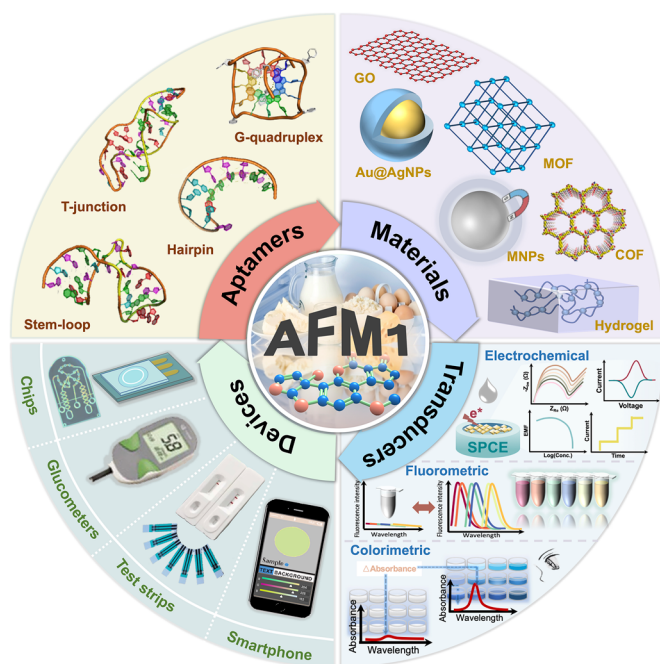


Figure 1. Comprehensive overview of aptamer sensing technologies toward AFM1. Firstly, as specific recognition element, aptamers can form various secondary structures like hairpin, stem-loop, G-quadruplex, and T-junction, etc. Furthermore, the structural basis and binding events between the aptamer and its target can be characterized by calculation programs. Interestingly, the embedding of functionalized nanomaterials like graphene oxide (GO), Au@AgNPs, metal-organic frameworks (MOFs) and covalent organic frameworks (COFs) into aptasensors can significantly improve the signal transduction and signal amplification. The commonly used signal transduction strategies include fluorescent, colorimetric, and electrochemical signal generation. Notably, the integrating of portable devices with these aptasensors can achieve rapid, portable, and high-throughput analysis, which will accelerate their commercialization.

2023; Guo and Wang 2024). As illustrated in Figure 1, aptamers can form various secondary structures, such as hairpin, stem-loop, G-quadruplex, and T-junction. Computational tools are often employed to elucidate the structural basis and binding mechanisms between aptamers and their targets. These interactions can induce conformational changes in aptamers, leading to the generation of detectable optical and electrochemical signals. Notably, advanced nanomaterials, such as metal-organic frameworks (MOFs), covalent organic frameworks (COFs) and MXenes, have been integrated into aptasensors to enhance signal transduction and amplification. Furthermore, the combination of aptasensors with portable devices has been developed for high-throughput analysis, which will be discussed in detail in the following section. Besides, aptamers also exhibit additional advantages, including low production cost, high stability, minimal batch-to-batch variation, non-immunogenicity, easy modification, and the ability to target a wide range of molecules (proteins, cell, tissue, small molecules like mycotoxins) (Ni et al. 2021; Zon 2022; Pundir et al. 2022). Accordingly, aptamers are increasingly regarded as ideal alternatives to antibodies, and aptasensors have gained unprecedented attention across various fields (Dunn, Jimenez, and Chaput 2017; Zhang and Guo 2022b). Aflatoxin M1 (AFM1), a hydroxyl metabolite of aflatoxin B1 (AFB1), contaminates milk and dairy products when lactating animals consume AFB1-contaminated feeds (Yu et al. 2023;

Tang et al. 2022). Despite strict regulations by many countries and organizations, with maximum residue levels (MRLs) for AFM1 in milk and dairy products ranging from 0.025 $\mu\text{g}/\text{kg}$ to 0.5 $\mu\text{g}/\text{kg}$ (Zhang et al. 2021a; Kaur et al. 2022), AFM1 contamination remains a persistent global food safety issue. Due to its severe health risk, the International Agency for Research on Cancer (IARC) has classified AFM1 as a group 1 carcinogen (Chen et al. 2023a; Oduola et al. 2022; Qiao et al. 2021). Over the past decade, aptasensor technologies have achieved remarkable progress in detecting AFM1 for food safety applications (Ahmadi et al. 2022; Guo et al. 2020; Zhang, Guo, and Wang 2023).

Several reviews have addressed various aspects of aptasensing for aflatoxins. For instance, Liu et al. (2020) provided an overview of electrochemical biosensing for aflatoxins, including a brief discussion on aptasensors. Zahra et al. (2022) and Zavvar et al. (2022) reported on the development of the smartphone and CRISPR based aptasensor, respectively, with limited examples of their application to AFM1. Another comprehensive review focused on visual methods for aflatoxin detection, emphasizing immunological approaches over aptasensors (Wang et al. 2022). Additionally, our group has extensively investigated aptasensor technologies for mycotoxins, with a previous review highlighting advancements in fluorescent aptasensing for aflatoxins (Zhang and Guo 2022b). However, a detailed and systematic discussion on aptamer selection and aptasensor technologies for AFM1 is still lacking. In this review article, we first discuss recent advancements in aptamer selection. Afterward, we provide an in-depth analysis of aptasensor technologies for the analysis of AFM1, categorized by signal transduction mechanisms. Besides, the unique advantages, challenges and limitations of aptasensors are also discussed. By offering a comprehensive overview of aptasensor development for AFM1 detection, this review aims to provide valuable insights into current trends and future direction in the field.

Aptamers as novel recognition elements for AFM1

Over the past three decades, antibody-based immunoassays have dominated biomedical detection and food safety monitoring through established platforms such as ELISA and LFIA (Pan et al. 2021; Fang et al. 2020; Bao et al. 2021; Su, Zhao, and Dou 2020; Xiong et al. 2020). These methods demonstrate adequate sensitivity for AFM1 screening in dairy products, with the initial development of competitive ELISA for milk AFM1 detection dating back to 1990 (Nieuwenhof, Hoolwerf, and Van Den Bedem 1990). However, inherent limitations associated with antibodies, including high production costs, reduced stability in complex matrices, and technical challenges in generating immune responses against low molecular weight toxins like AFM1, have motivated the exploration of alternative recognition molecules (Ma, Ó'Fágáin, and O'Kennedy 2020; Peltomaa et al. 2022; Chen et al. 2023b; Gandhi et al. 2018). This scientific landscape has driven the emergence of nucleic acid aptamers as promising alternatives, establishing them as next-generation biorecognition elements in food safety monitoring.

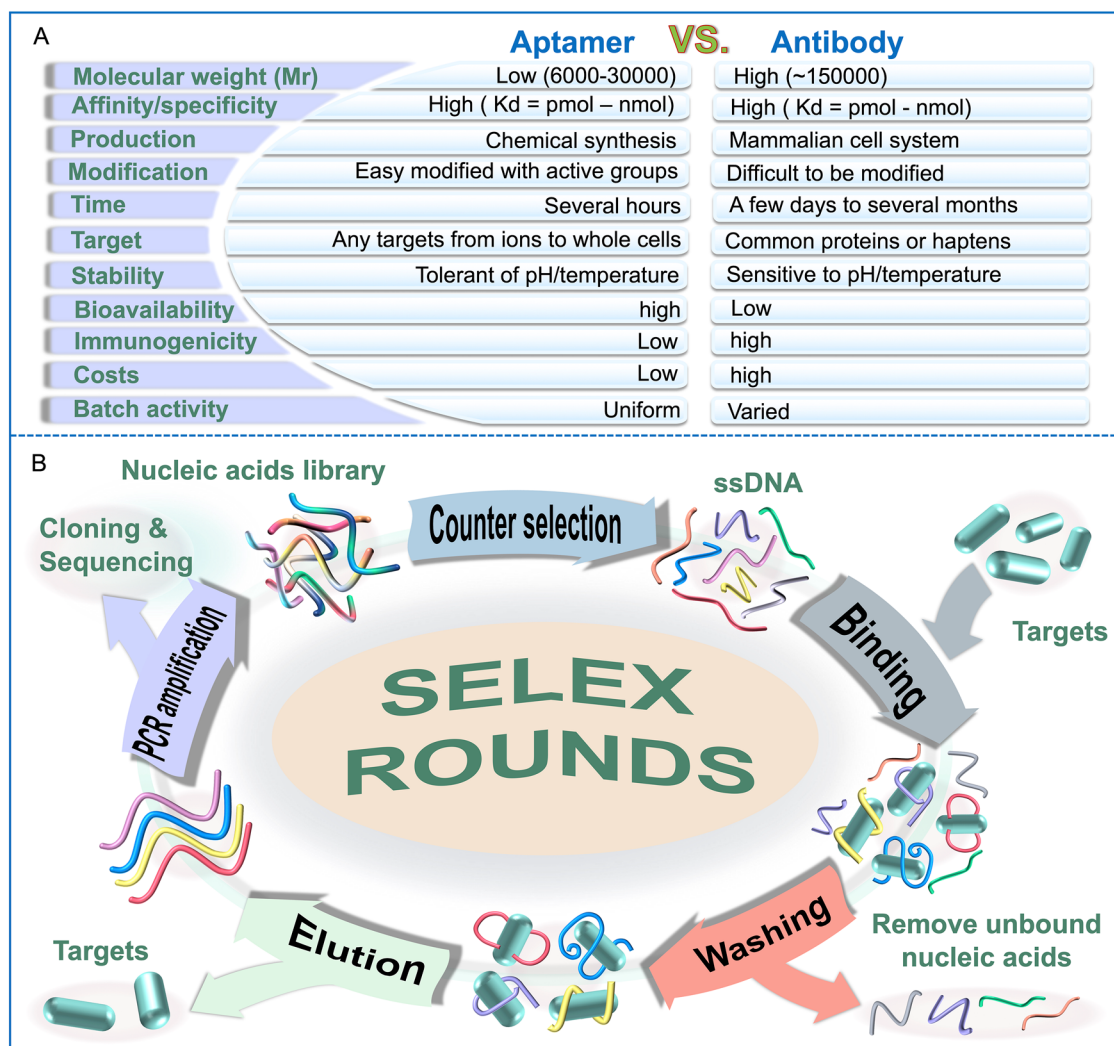


Figure 2. (A) Summarization of the major characteristics on aptamers compared with antibodies. (B) Schematic illustration of the SELEX-based aptamer screening technologies for small molecules.

Aptamers, often termed “chemical antibody,” are short single-stranded DNA/RNA oligonucleotides that exhibit excellent affinity and specificity for their target biomolecules (Eivazzadeh-Keihan et al. 2022). As summarized in Figure 2A, aptamers offer distinct advantages over conventional antibodies: (i) cost-effective chemical synthesis with minimal batch variation, (ii) superior thermal and chemical stability under harsh processing conditions, (iii) reversible denaturation properties, and (iv) enhanced structural flexibility for precise recognition of small-molecule targets (Zon 2022; Li et al. 2021). The *in vitro* selection process through SELEX further eliminates biological variability inherent in animal-derived antibodies (Hou et al. 2022).

The SELEX methodology, as depicted in Figure 2B, begins with a diverse DNA library containing 10^{14} – 10^{18} unique sequences, each comprising a central random sequence flanked by fixed primer regions at both ends. These libraries are incubated with the target molecule, allowing specific sequences to form single-stranded DNA (ssDNA)/target complexes. The bound ssDNAs are then separated from the unbound population. Subsequently, the selected ssDNAs are amplified via polymerase chain reaction (PCR) to generate

an enriched pool through multiple cycles. Finally, the enriched ssDNAs are characterized for affinity, specificity, and structural properties, with the most promising sequence identified as the aptamer for the target (Li et al. 2023a). The resultant aptamers usually folded into unique three-dimensional structures stabilized by base pairing and base-stacking interactions. These structures, which include stems, hairpin, loop, multiloop, G-quadruplexes, bulge, and pseudoknot, enable aptamers to specifically recognize their targets through hydrogen bonding, hydrophobic interactions, van der Waals forces, aromatic stacking, and electrostatic complementarity (Liu et al. 2022a).

The first electrochemical aptasensor for AFM1 detection, developed by Nguyen et al. (2013), utilized an aptamer (Apt-1) with the sequence “ACT GCT AGA GAT TTT CCA CAT.” However, details regarding its affinity, specificity, and selection protocol were not provided in their work. Subsequently, Malhotra et al. (2014) successfully isolated a specific AFM1-binding aptamer (Apt-2) through conventional SELEX methodology. The identified sequence, “ATC CGT CAC ACC TGC TCT GAC GCT GGG GTC GAC CCG GAG AAA TGC ATT CCC CTG TGG TGT TGG

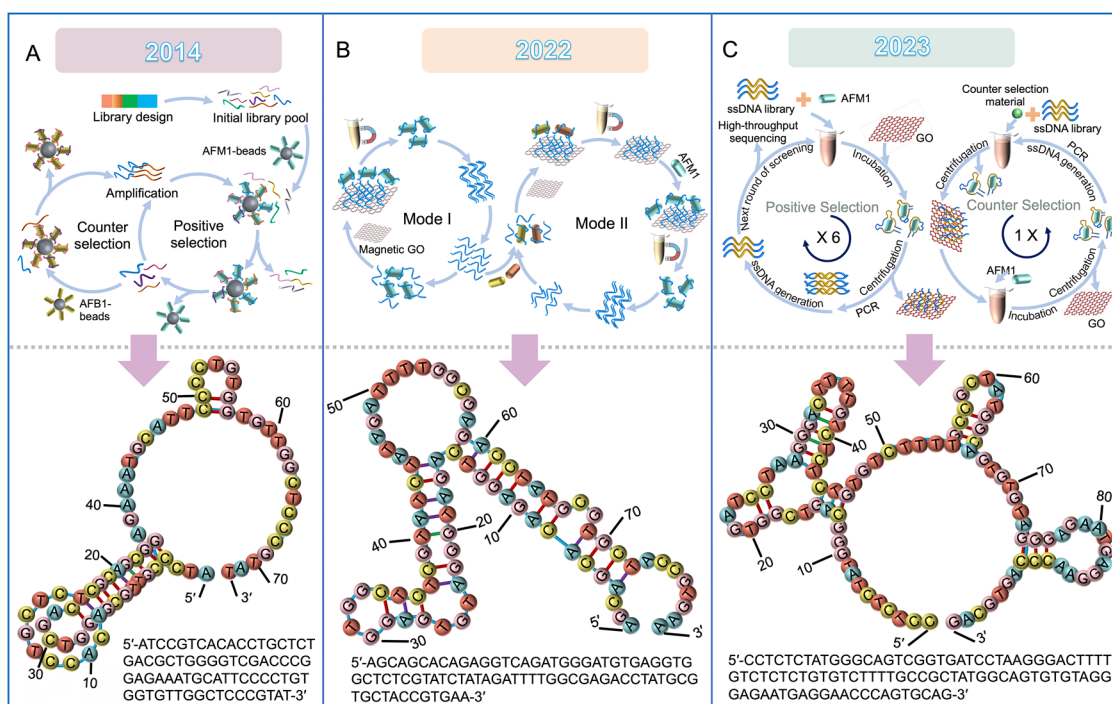


Figure 3. Advancements in aptamer selection by SELEX toward AFM1. (A) Schematic diagram of the conventional SELEX to select specific aptamer for AFM1 together with the sequences and secondary structure (Malhotra et al. 2014). (B) Mechanism representation of the magnetic GO-SELEX technique for screening aptamer together with the sequences and secondary structure (Liu et al. 2022b). (C) Schematic illustration of the GO-SELEX strategy for aptamer selection *in vitro* together with the sequences and secondary structure (Wei et al. 2023). All the illustrations were recreated according to the cited references.

CTC CCG TAT,” demonstrated high affinity for AFM1 with a dissociation constant (K_d) of 35 nM. As illustrated in Figure 3A, this sequence exhibited remarkable specificity when implemented in a colorimetric aptasensor platform. Over the past decade, Apt-1 and Apt-2 have been the primary aptamers used in AFM1 biosensor development. However, these pioneering aptamers lack the conserved structural motifs (e.g., G-quadruplexes) associated with enhanced target binding. This limitation, coupled with insufficient characterization data in initial reports, has constrained their widespread adoption compared to AFB1 detection systems.

The conventional SELEX generally suffer from the barriers like time-consuming procedures, low efficiency, and weak binding affinity. To address this challenge, Liu et al. (2022b) innovatively incorporated functionalized magnetic graphene oxide (GO) into the SELEX process (Figure 3B). The magnetic GO, characterized by its large surface area, thermal stability, and photoelectric properties, facilitated the screening of high enrichment aptamers. The resulting aptamer (Apt-3) possessed a significantly higher affinity for AFM1, with a K_d of 8.12 nM. Molecular docking studies revealed that the binding mechanism involved hydrogen bonding and hydrophobic interactions. A colorimetric aptasensor was subsequently developed to validate the practical application of Apt-3. In a similar protocol, Wei et al. (2023) optimized the SELEX process by employing a DNA library with a five-segment distribution, which reduced the prevalence of simple structures and shortened screening cycles (Figure 3C). The selected aptamer (Apt-4), featuring hairpin and stem-loop structures, demonstrated strong binding affinity for AFM1, with a K_d of 109 nM. A colorimetric

aptasensor based on Apt-4 also showed high performance in AFM1 detection. While current applications remain limited, the development of Apt-3 and Apt-4 demonstrates substantial progress in AFM1 recognition capabilities, providing critical insights for future biosensor development.

Nanomaterials as active elements of advanced aptasensors

Nanomaterials (1–100 nm in at least one dimension) act as active elements in aptasensors by dynamically driving sensing processes through their intrinsic physicochemical properties. The term “active” reflects their direct roles in target recognition, signal generation, and amplification, enabled by nanoscale attributes such as high surface-to-volume ratios, quantum confinement effects, and tunable surface chemistry (Hejji et al. 2023; Xie et al. 2020; Wu et al. 2020; Khoshbin et al. 2022; Wang et al. 2019). As illustrated in Figure 4, various nanomaterials, such as nonmetallic, metallic, magnetic, and organic frameworks, have been extensively studied for their unique architectures and properties (Wei et al. 2023; Cao et al. 2024; Guo et al. 2021; Rahmani et al. 2021). These materials actively contribute through three mechanisms: (i) catalytic or plasmonic effects that convert molecular interactions into detectable signals, (ii) enhancement of electron transfer kinetics at sensing interfaces, and (iii) provision of high-density functional sites for aptamer immobilization and target recognition (Li et al. 2023b; Sharma et al. 2021; Su et al. 2021; Zhang et al. 2021b; Che et al. 2021; Zahra et al. 2023; Báez et al. 2021; Amiri, Nekouieian, and Saberi 2021).

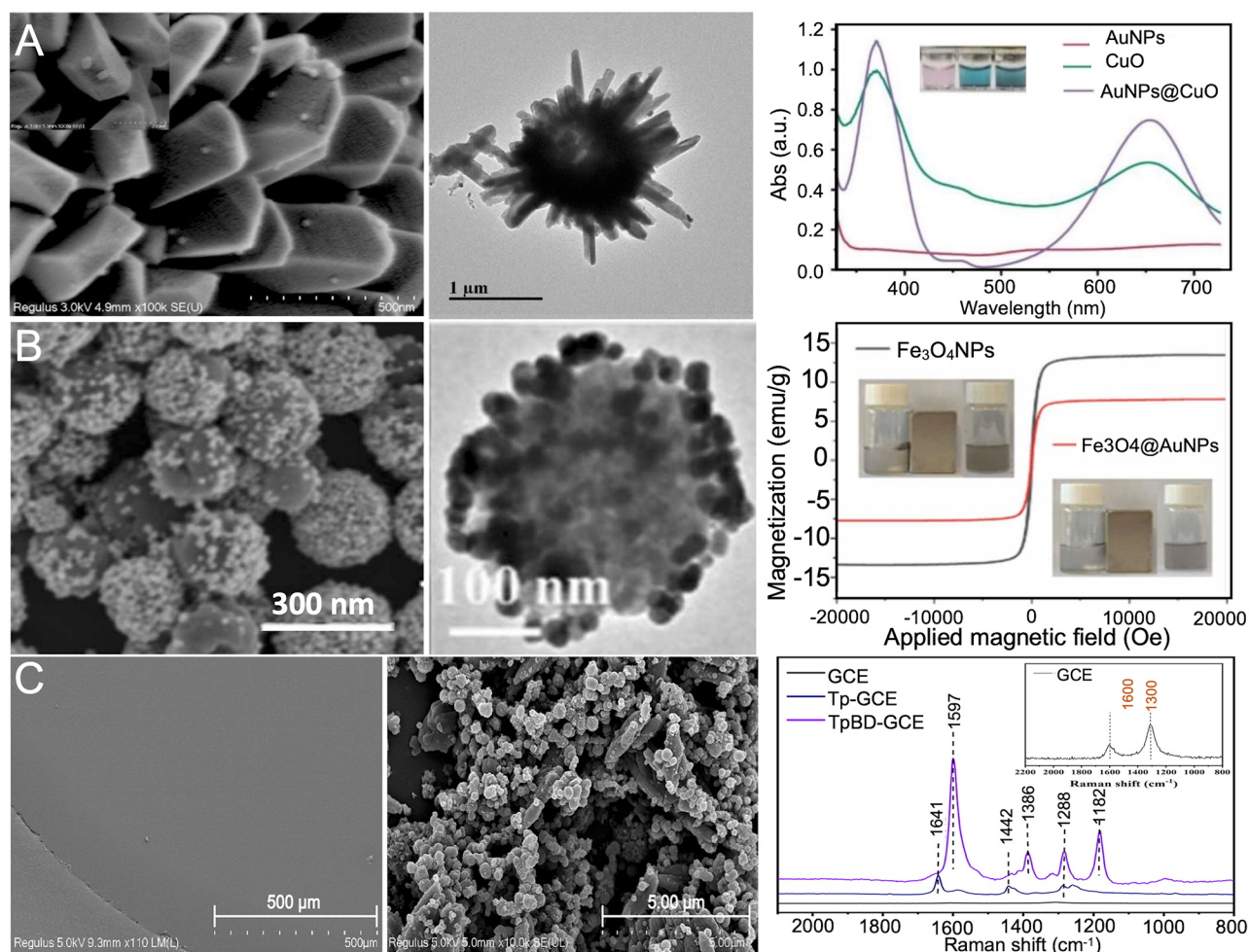


Figure 4. Various unique architectures and properties of nanomaterials. (A) SEM and TEM images of AuNPs@CuO, UV-Vis diagrams to verify the catalytic ability of the composite materials (Wei et al. 2023). Copyright 2023, American Chemical Society. (B) SEM and TEM image of Fe₃O₄@AuNPs, magnetic hysteresis curves of Fe₃O₄NPs and Fe₃O₄@AuNPs (Cao et al. 2024). Copyright 2023, Elsevier. (C) SEM images and Raman spectroscopy of GCE and TpBD-GCE (Guo et al. 2021). Copyright 2020, Elsevier.

Noble metal nanoparticles, particularly gold nanoparticles (AuNPs), actively enhance optical signals through their excitation of localized surface plasmon resonance (LSPR). In the work of Jalalian et al. (2021), aptamer-functionalized AuNPs actively regulated nanoparticle dispersion states in response to AFM1 binding, inducing visible color shifts proportional to target concentration. This dynamic signal modulation achieved a 15-fold sensitivity improvement compared to conventional methods (Lerdsri et al. 2021). Magnetic nanoparticles similarly exhibited active roles through their dual functionality: their superparamagnetism enabled rapid target isolation from complex matrices, while their high surface-to-volume ratio facilitated dense aptamer loading for enhanced binding efficiency (Guo et al. 2021).

With the development of two-dimensional (2D) materials like GO, the field of optical biosensing substrates has experienced significant progress. GO and reduced graphene oxide (rGO) exhibit atomic-level coordination and oxygen-rich surface groups that actively mediate electron transfer, enabling ultrasensitive electrochemical detection of AFM1 at sub-ng/L levels (Chrouda et al. 2022; Ahmadi et al. 2022). Upon the success of graphene-based materials, emerging 2D nanomaterials such as MXenes have recently captured the attention of

researchers. MXenes combine the structural advantages of 2D materials with unique properties including metallic conductivity, exceptional hydrophilicity, inherent biocompatibility, and highly modifiable surfaces. These characteristics make MXenes as promising candidates for next-generation sensing platforms. Although MXene-based aptasensors for AFM1 specifically remain unexplored, their success in detecting structurally analogous mycotoxins demonstrates substantial potential (Shoaib et al. 2024; Shelash Al-Hawary et al. 2024). For instance, MXenes (Ti₃C₂T_x)/aptamer hybrids achieved an ultralow limit of detection (LOD) of 0.8 pg/mL for AFB1 detection through synergistic field-effect transistor signal enhancement (Zhu et al. 2025). The combination of MXenes' large surface area (facilitating high-density aptamer loading) and tunable surface chemistry (enabling precise biorecognition interfaces) creates opportunities for developing novel AFM1 detection strategies that could surpass current graphene-based approaches.

Notably, advanced porous materials like COFs and MOFs extend beyond mere structural scaffolding by integrating catalytically active functionalities into their architectures (Jahangiri-Dehaghani, Zare, and Shekari 2020). Yang et al. (2024) demonstrated that polyethyleneimine-functionalized

MOFs simultaneously immobilized aptamers and amplified electrochemical signals via their inherent redox activity. Similarly, TpBD COFs enhanced sensor stability and electron conversion efficiency through covalent interactions with cDNA probes, confirming their active participation in signal generation (Song et al. 2021; Zhang et al. 2022). These advancements illustrate the evolution of nanomaterials from passive substrates to multifunctional components that directly dictate sensor performance through optical, catalytic, and electronic properties. Recent innovations further highlight synergistic combinations of nanomaterials with molecular recognition elements. For instance, Hybrid systems integrating molecularly imprinted polymers (MIPs) with aptamer-nanomaterial complexes exploit complementary recognition mechanisms, where nanomaterials actively enhance both signal amplification and specificity (Yang et al. 2024). This paradigm shift has driven remarkable improvements in AFM1 detection, achieving sensitivity enhancements from ng/mL to pg/mL ranges while addressing selectivity and throughput limitations.

The integration of engineered nanomaterials into aptasensing platforms has driven breakthrough advancements in analytical performance for AFM1 detection, fundamentally transforming their role from passive substrates to active, multifunctional elements within sensing architectures. As material science progresses, the hotspot is increasingly shifting toward the rational design of nanomaterials with tailored active properties, such as programmable catalytic activity, tunable plasmonic responses, and stimuli-responsive behaviors. This evolution highlights nanomaterials as critical components in next-generation food safety systems, addressing complex matrix challenges and redefining AFM1 detection standards.

Advanced aptasensors for AFM1 detection

Optical aptasensing of AFM1

Optical aptasensors have emerged as promising tools for AFM1 detection due to their simplicity, rapid response, and potential for point-of-care (POC) testing. Among these, colorimetric and fluorescent-based systems dominate the field due to their operational simplicity and adaptability. However, the practical implementation of these methods faces challenges, including limited sensitivity and procedural complexity. These limitations highlight the need for innovative engineering approaches to meet the increasingly stringent regulatory requirements for mycotoxin monitoring. This section provides a critical analysis of the evolving optical aptamer sensing paradigm, with particular emphasis on breakthroughs in signal amplification strategies, nanomaterial integration, and structural optimization.

Colorimetric aptasensors are the simplest optical sensing strategies, as they rely on visible color changes detectable by the naked eye or a microplate reader. Early colorimetric systems exploited the plasmonic properties of AuNPs, where AFM1 binding induced aptamer conformational changes, triggering nanoparticle aggregation accompanied by characteristic red-to-blue color shifts. Jalalian et al. (2021) demonstrated this principle through salt-induced AuNP aggregation with an LOD of 0.1 ng/mL (Figure 5A), while Lerd Sri et al. (2021)

achieved enhanced sensitivity (0.05 ng/mL) through optimized aptamer orientation on AuNP surfaces (Figure 5B). Nevertheless, these systems faced limitations in complex matrices due to inconsistent aggregation kinetics and susceptibility to ionic interference. Subsequent innovations integrated enzymatic amplification to address sensitivity constraints. As shown in Figure 5C, Abnous et al. (2021) pioneered a CRISPR-Cas12a-coupled rolling circle amplification (RCA) system, where AFM1 inactivated Cas12a to protect RCA-generated primers, inducing sequence-specific AuNP aggregation. By synergizing enzymatic cascades with peroxidase-mimetic AuNPs, they achieved dual signal amplification and an unprecedented LOD of 50 fg/mL, a three-order-of-magnitude improvement over conventional methods. Such advancements demonstrate that enzymatic amplification mechanisms can effectively address sensitivity constraints in colorimetric platforms.

In contrast to colorimetric approaches, fluorescent aptasensors, particularly those based on Förster resonance energy transfer (FRET), offer enhanced sensitivity and multiplexing capabilities (Zhang and Guo 2022b). In early FRET-based systems, aptamers were typically labeled with fluorophores at their termini, while quenchers were strategically positioned on complementary strands to suppress fluorescence through proximity-induced FRET mechanisms. Qiao et al. (2021) previously reported a conformation-switching FRET aptasensor for quantitative monitoring of AFM1 contamination in raw milk. As depicted in Figure 6A, this dual-labeled architecture exploited a competitive hybridization strategy between a carboxyfluorescein (FAM)-labeled AFM1-specific aptamer and a carboxy-*tert*-methyl rhodamine (TAMRA)-conjugated cDNA. The initial formation of the aptamer-cDNA duplex established a FRET-quenched state (donor-acceptor distance < 8 nm), effectively suppressing FAM fluorescence through static quenching. Upon AFM1 recognition, the aptamer underwent structural rearrangement to form a stable AFM1-aptamer complex, which triggered the displacement of cDNA. This conformational change resulted in dose-dependent fluorescence signal recovery through separation of the FRET pair. The optimized system demonstrated a detection limit of 0.5 ng/mL for AFM1.

The detection of AFM1 in dairy products presents significant analytical challenges due to matrix-induced interference, particularly from milk components that cause signal suppression through nonspecific cDNA displacement and fluorophore photobleaching. To overcome these limitations in conventional fluorescence-based detection systems, Sameiyan et al. (2021) engineered a breakthrough fluorescent aptasensor for AFM1 detection in milk using molybdenum disulfide nanosheets (MoS₂ NSs). As shown in Figure 6B, MoS₂ nanosheets served as a fluorescence quencher for FAM-labeled cDNA instead of organic quenchers (e.g., TAMRA). Based on the high surface-to-volume ratio of 2D MoS₂ nanosheets, the platform achieved label-free aptamer immobilization while preventing nonspecific cDNA separation through strong π - π stacking interactions. Moreover, different from previous designs, the aptasensor employed a dual aptamer-cDNA assembly configured in a bivalent binding mode, a structural innovation that enhanced stability by

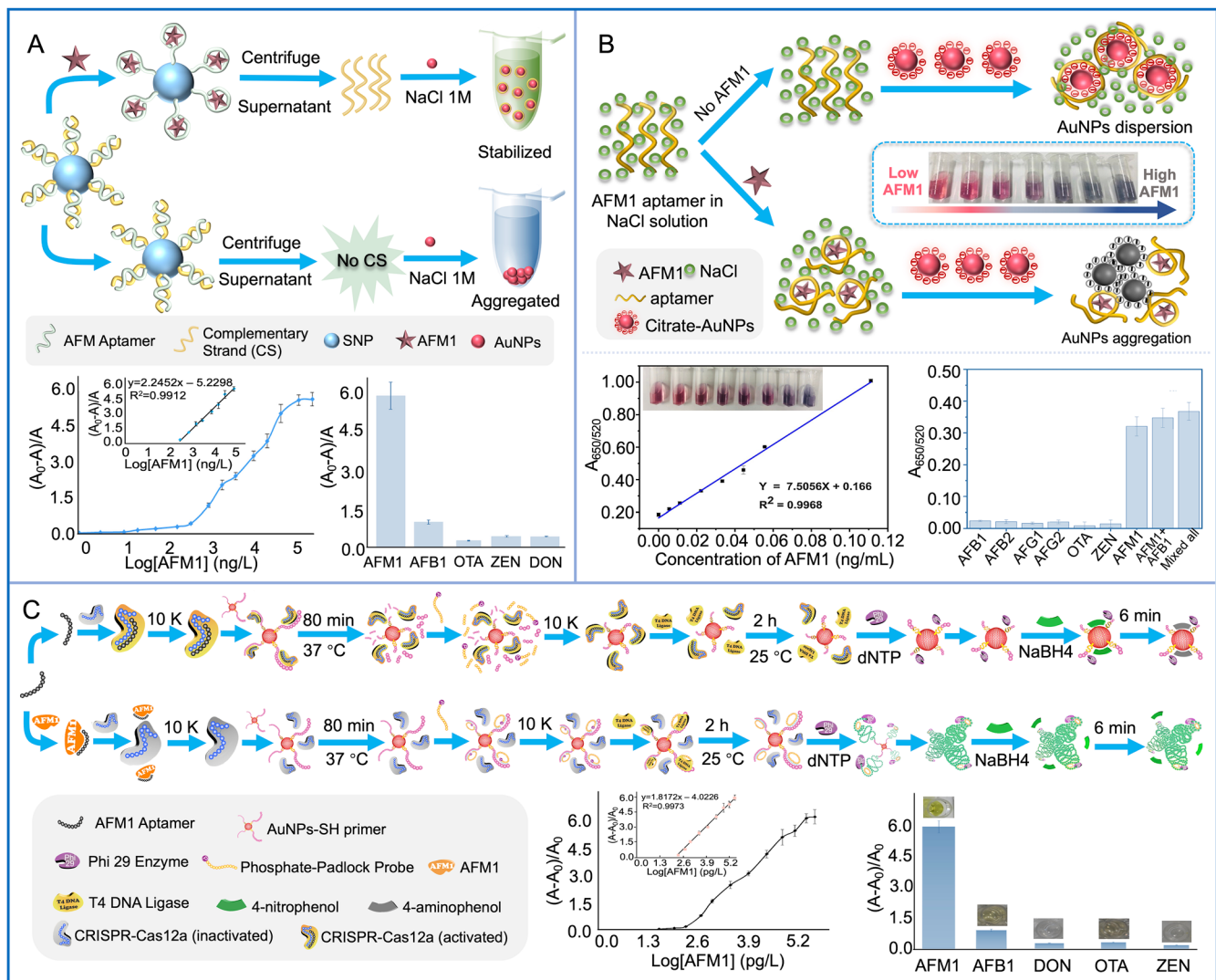


Figure 5. (A) Schematic diagram of unique design of the colorimetric aptasensor for detection of AFM1 in milk based on salt-induced AuNPs aggregation and aptamer/SNPs (Lerdsri et al. 2021). Copyright 2021, Elsevier. (B) Schematic illustration of the colorimetric aptasensor for visualized analysis of AFM1 in milk based on salt-induced AuNPs aggregation and LSPR change of AuNPs (Jalalian et al. 2021). Copyright 2020, Elsevier. (C) Working principle of the colorimetric aptasensing of AFM1 in milk based on aptamer/AuNPs/CRISPR-Cas12a/RCA platform (Abnous et al. 2021). Copyright 2021, Elsevier.

preventing cDNA dissociation and structural degradation during target recognition. The synergistic integration of nanomaterial-enhanced quenching and optimized probe architecture enabled sensitive AFM1 quantification with an LOD of 0.164 ng/mL. The platform exhibited exceptional reliability in complex milk systems, with robust recovery rates of 94.7–108.9%, effectively addressing matrix interference challenges. This dual-optimization strategy demonstrated substantial improvements in both sensitivity and applicability in mycotoxin monitoring for food safety. Parallel work by Ge et al. (2023) introduced DNA double-cross nanostructures with spatially separated Cy3/Cy5-BHQ2 pairs, enabling simultaneous detection of AFM1 and AFB1 through target-specific aptamer release. As shown in Figure 6C, two specific aptamers, labeled with Cy3 and Cy5, were self-assembled with BHQ2-labeled cDNAs to form a DNA double-cross nanostructure. Such configuration can cause the fluorescent signal quenching. Upon binding to AFM1 and AFB1, the aptamers released from the cDNA, resulting in the recovery of fluorescent signals. Accordingly, highly

sensitive detection was achieved with LODs of 6.24 pg/mL for AFM1 and 9.0 pg/mL for AFB1.

The pursuit of ultra-sensitive detection has driven the integration of fluorescent aptasensors with advanced amplification strategies. For example, Niazi et al. (2020) reported a novel fluorescent aptasensor for amplified detection of AFM1 via RCA technique. Notably, graphitic carbon nitride ($g-C_3N_4$) nanosheets were employed as fluorescence quenchers due to their GO-like structure and superior size variability compared to GO (Figure 6D). On the other hand, Ln^{3+} -doped nanoparticles were used as fluorescent probes instead of traditional fluorophores, reducing background signals and improving sensitivity. This approach achieved a detection sensitivity enhanced by four orders of magnitude, with an LOD down to femtogram level (19.4 fg/mL). However, enzymatic amplification processes, such as those involving DNA ligase and polymerase, often result in complex procedures and susceptibility to degradation in complicated matrices. To overcome these limitations, a non-enzymatic fluorescent aptasensor was developed for AFM1 detection based on

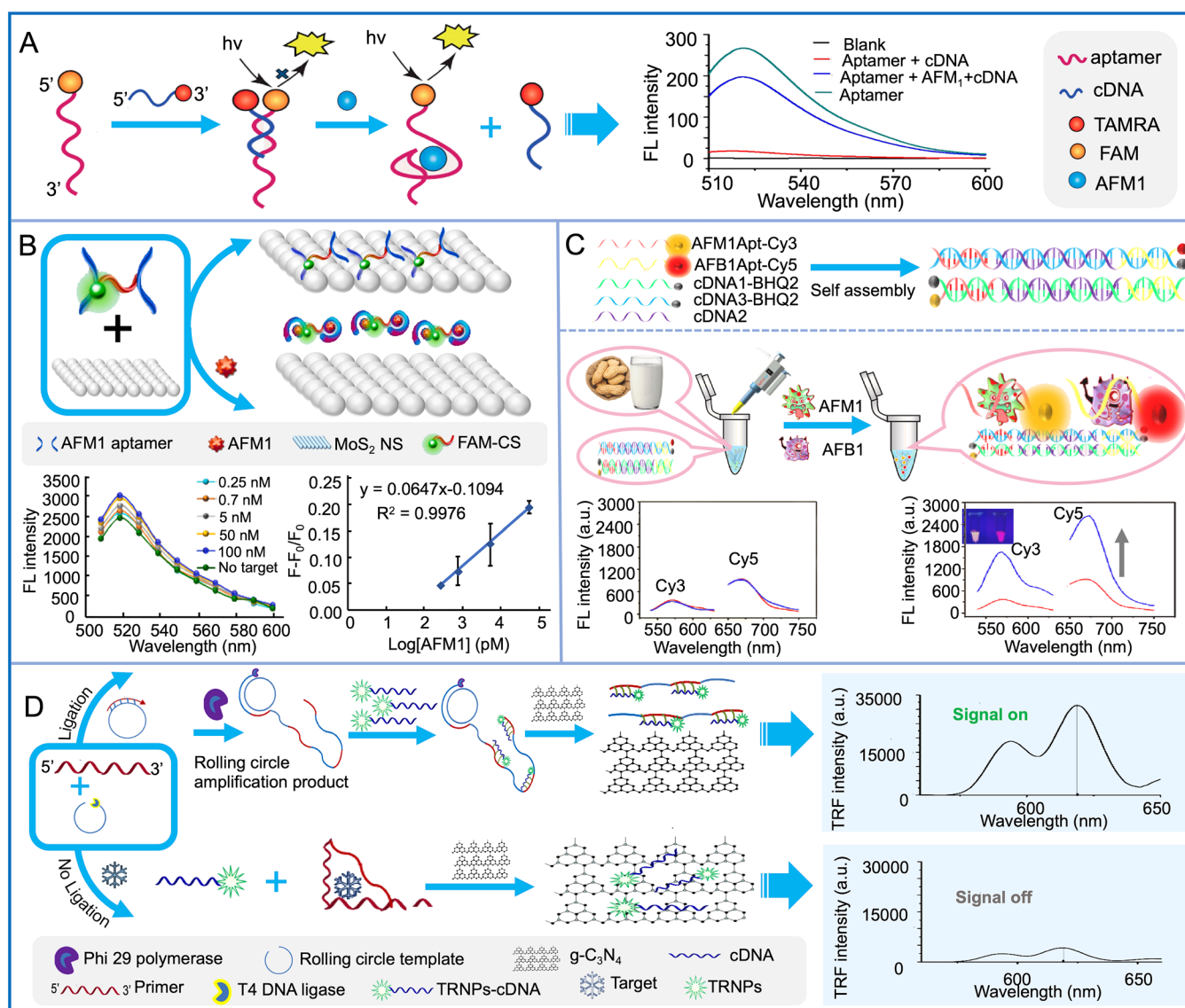


Figure 6. (A) Mechanism representation of the fluorescent aptasensing of AFM₁ in milk based on target-induced fluorescence quenching/recovery (Qiao et al. 2021). Copyright 2021, Creative Commons Attribution License (CC BY). (B) Schematic illustration of the fluorescent aptasensor for AFM₁ determination in milk through bivalent binding aptamer-cDNA and MoS₂ nanosheets (Sameiyan et al. 2021). Copyright 2021, Elsevier. (C) Schematic illustration of the fluorescent aptasensor for simultaneous recognition of AFM₁ and AFB₁ via a dual cross DNA nanostructure (Ge et al. 2023). Copyright 2023, Elsevier. (D) Schematic diagram of the fluorescent aptasensor platform to monitor AFM₁ in milk based on g-C₃N₄ as fluorescence quencher and RCA technique (Niazi et al. 2020). Copyright 2020, Elsevier.

DNAzyme and DNA walker amplification (Wang et al. 2023). In this design, the aptamer was inserted into the DNAzyme to prevent nuclease degradation. The binding of AFM₁ was found to enhance the structural stability of the DNAzyme, thereby activating its catalytic activity. This activation facilitated the release of a G-quadruplex probe, which subsequently interacted with berberine, leading to a significant enhancement in the fluorescent signal (Figure 7A). Notably, Aran and Bayraç (2023) introduced functionalized DNA hydrogels as three-dimensional (3D) sensing platforms, where aptamer-cDNA crosslinking provided spatial confinement to reduce background noise. The hydrogel's interconnected porous architecture facilitated enhanced mass transport kinetics, enabling both rapid analyte diffusion and equipment-free visual detection with an LOD of 0.56 ng/mL. This enzyme-free design achieved picogram-level detection limits without

requiring ligases or polymerases, emphasizing its practicality for POC applications. Furthermore, the system indicated notable advantages in cost efficiency, structural stability, and mechanical flexibility, highlighting its potential for scalable deployment in resource-limited settings. By integrating signal amplification through spatial organization with user-friendly readout capabilities, this innovation represents a significant advancement in bridging the gap between sensitivity and real applications.

Beyond conventional optical modes, emerging hybrid systems combine magnetic responsiveness with optical readouts for enhanced practicality. Cao et al. (2024) utilized functionalized AuNPs and AgNPs to produce intense and non-interfering surface enhancement of Raman scattering (SERS) signal for detection of AFB₁ and AFB₂. Additionally, aptamer-modified Fe₃O₄@AuNPs were used to induce color

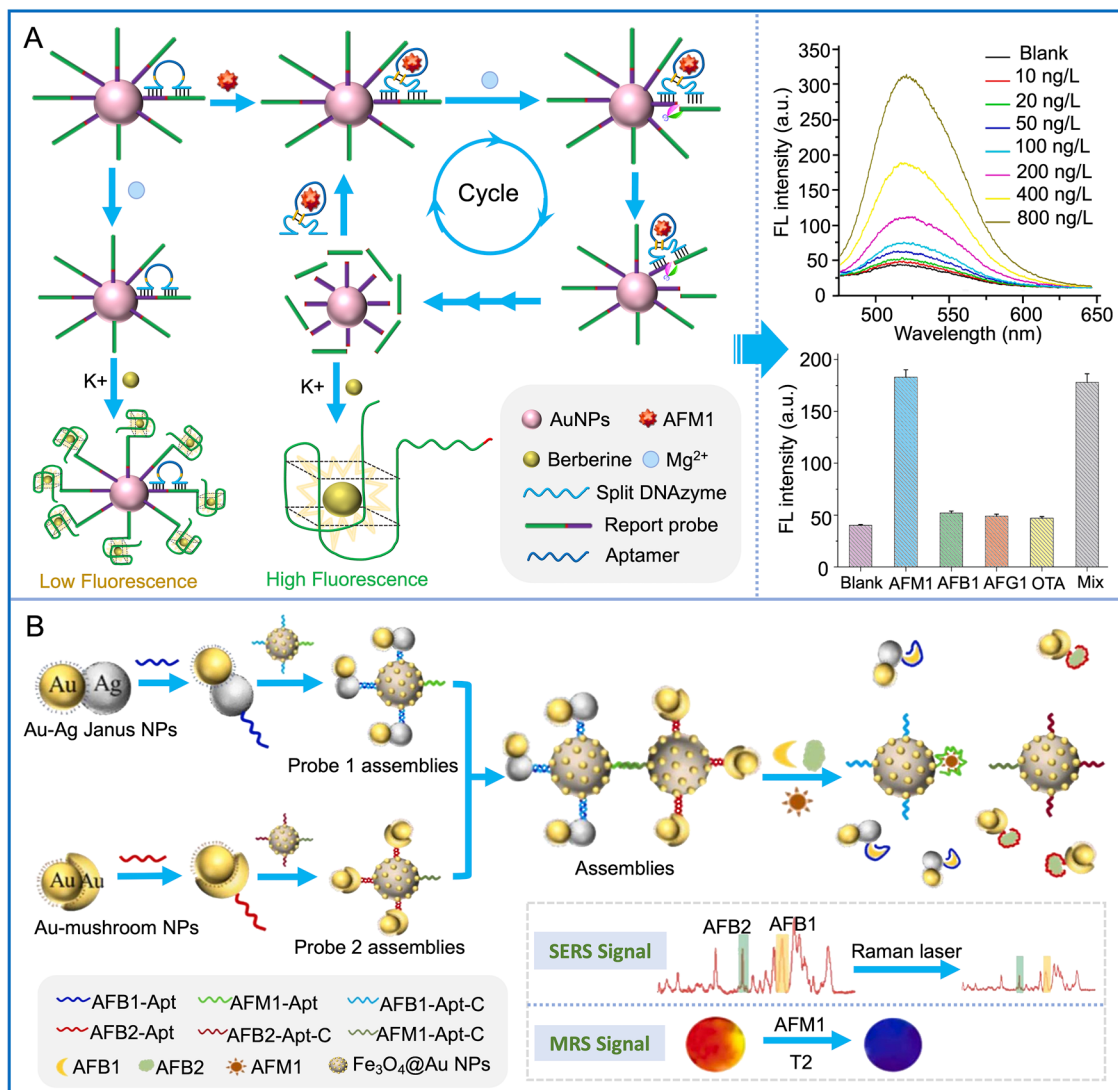


Figure 7. (A) Working principle of the special design for fluorescent aptasensor for AFM1 detection in milk by aptamer/AuNPs/DNAzyme/DNA walker signal amplification strategy (Wang et al. 2023). Copyright 2022, Elsevier. (B) Schematic illustration of dual-mode aptasensor for simultaneous detection of AFB1, AFB2, and AFM1 based on surface enhanced Raman scattering (SERS) and magnetic nanoprobe (Cao et al. 2024). Copyright 2023, Elsevier.

changes in MRS signals for AFM1 detection. The existence of AFM1 destroyed DNA-NPs assemblies, causing the $\text{Fe}_3\text{O}_4\text{@AuNPs}$ from aggregation to dispersion status, reducing transverse relaxation time (T2) values and altering the color change of MRS signal. This colorimetric aptasensor achieved highly sensitive detection of AFM1 with an LOD 420 fg/mL (Figure 7B). These systems reveal the synergistic potential of magnetic and optical modalities. However, challenges in signal standardization and inter-platform reproducibility need to be further addressed for scalable applications.

Despite significant advancements, critical limitations hinder the translation of optical aptasensors to real-world settings. Fluorescent systems often suffer from fluorophore photobleaching and aptamer-labeling-induced affinity loss, while colorimetric methods remain constrained by inherent sensitivity ceilings. The integration of hybrid materials (e.g., noble metal nanostructures, 2D materials) with multi-modal signal amplification strategies (e.g., enzymatic/non-enzymatic cascades, SERS/MRS) presents a promising frontier. However, the lack of universal design principles for nanomaterial-aptamer

interfaces and standardized synthesis protocols poses significant translational barriers. Future efforts should focus on mechanistic studies of signal transduction, scalable nanomaterial functionalization, and rigorous validation in real-world matrices to realize the full potential of optical aptasensors for AFM1 monitoring.

Electrochemical aptasensing of AFM1

Electrochemical biosensors have gained prominence in food safety monitoring, particularly for POC testing, owing to their rapid response, operational simplicity, cost-effectiveness, and high sensitivity (Bernalte et al. 2020; Bi et al. 2021; Kurup, Mohd-Naim, and Ahmed 2022; Umaphathi et al. 2022; Negahdary 2020). Driven by the integration of biomolecular recognition elements and engineered nanomaterials, the field of electrochemical aptasensing has witnessed transformative advancements in AFM1 detection. Early electrochemical platforms relied on the inherent specificity of aptamers immobilized on conventional electrodes, yet faced limitations in sensitivity and signal stability (Hou et al. 2019;

Kékedy–Nagy and Ferapontova 2019). A pivotal breakthrough emerged with the incorporation of conductive nanomaterials such as rGO and AuNPs, which enhanced electron transfer kinetics and provided high surface areas for aptamer immobilization. For instance, Ahmadi et al. (2022) developed a label-free electrochemical aptasensor for the detection of AFM1 in milk. This aptasensor utilized a pencil graphite electrode (PGE) modified with rGO and AuNPs. The rGO/AuNPs nanocomposite facilitated the self-assembly of aptamers on the sensing surface, resulting in a detection range of 0.5–800 ng/L with an LOD of 0.3 ng/L. Similarly, Hui et al. (2022) designed an innovative electrochemical aptasensor using DNA-controlled gold-silver core-shell nanoparticles (Au@Ag conjugates) on a gold electrode. The sensing mechanism relied on the conformational change of the aptamer upon binding to AFM1, which disrupted the Au@Ag conjugates and reduced the adsorption of methylene blue (MB) on the electrode surface. This led to a measurable decrease in electrochemical signals, enabling the detection of AFM1 with a wide linear range (0.05–200 ng/mL) and an LOD of 0.02 ng/mL. The aptasensor was successfully applied to detect AFM1 in various milk samples, including cow, goat, and sheep milk, highlighting its practical applicability in food safety monitoring.

Despite the successful application of AuNPs, AgNPs, graphene etc. in highly sensitive detection of AFM1 (Jalalian et al. 2018; Hamami, Mars, and Raouafi 2021), their aggregation tendency remains a persistent challenge requiring further optimization. To solve the problem, polymers such as pectin and polyaniline have been used as stabilizers to optimize electrode interfaces. For example, a novel electrochemical aptasensor was exploited for the ultrasensitive detection of trace levels of AFM1 based on aptamer/pectin-AuNPs/GO composites film (Chrouda et al. 2022). In this design, pectin served dual roles as a stabilizer for nanoparticles and signals enhancer. This sensing platform achieved a remarkable LOD down to 0.2 ng/L for AFM1, with a rapid detection of only 20 min, making it highly suitable for POC testing of food mycotoxins.

The quest for ultra-sensitive detection has further driven the adoption of porous crystalline materials, such as COFs and MOFs, which offer tunable porosity and high-density binding sites (Xue et al. 2023; Huang et al. 2024; Lv et al. 2021). For example, Pang et al. (2020) introduced a pioneering approach using TpBD, a representative COF, to modify a glassy carbon electrode (GCE). Their design incorporated a primer-AuNPs-aptamer complex as the molecular recognition element, wherein the primer triggered RCA process to generate DNAzymes for catalytic signal amplification. This strategy, when integrated with an ELISA-inspired protocol, achieved sensitive detection of AFM1 in milk with an LOD of 0.15 ng/mL (Figure 8A). However, conventional physical deposition methods for COF modification often suffer from nanomaterial desorption during electrochemical operations. To overcome this limitation, their group further proposed an *in-situ* growth protocol to covalently anchor TpBD COFs onto GCE surfaces (Guo et al. 2021). In this optimized system, aptamers were immobilized on magnetic nanoparticles for target recognition, while retaining the same LOD of 0.15 ng/mL for

AFM1 in milk. Parallel advancements in MOF-based aptasensors have demonstrated comparable advancements. MIL-101(Fe), an iron-containing MOF, has shown exceptional catalytic performance in biosensing applications (Zorainy et al. 2021). Jahangiri-Dehaghani, Zare, and Shekari (2020) engineered a composite material by incorporating platinum nanoparticles (PtNPs) into MIL-101(Fe) (MIL-101(Fe)/PtNP) for GCE modification. Subsequent functionalization with amino-terminated aptamers yielded an electrochemical aptasensor with ultrahigh sensitivity, achieving an LOD of 2 pg/mL for AFM1 detection in milk. These hierarchical frameworks address historical limitations of conventional carbon and gold electrodes by simultaneously enhancing aptamer loading capacity and charge transfer efficiency.

Recent advances in electrochemical sensing have focused on dual-recognition systems to overcome limitations in specificity and sensitivity for detecting AFM1 (Zhou et al. 2021; Elfadil et al. 2021). A notable innovation by Yang et al. (2024) integrated aptamers with molecularly imprinted polymers (MIPs) on a hierarchical nanomaterial interface. As illustrated in Figure 8B, their platform employed polyethyleneimine-functionalized metal-organic frameworks (PEI/MIL-100(Cr)) immobilized on gold nanoparticle-modified glassy carbon electrodes (AuNPs/GCE). The dual recognition mechanism combined MIP cavities for structural complementarity to AFM1 with aptamers for selective binding, while a signal amplification probe (cApt-Au@PEIM) enhanced electron transfer efficiency. This synergistic design achieved a broad linear detection range (0.01–200 nM) and a remarkably low LOD of 0.07 nM, demonstrating the efficacy of MOF-MIP hybrids in amplifying sensor response. Such innovations highlight the transition from single-element sensing to multifunctional interfaces, where hierarchical material design and multi-recognition mechanisms collectively enhance performance.

Upon advances in aptamer engineering, split aptamers, artificially cleaved probes engineered by dividing parent aptamers into functional fragments, have emerged as a transformative strategy to overcome the susceptibility of single-signal electrochemical biosensors to environmental interference in complex matrices (Shoab et al. 2025). In a pioneering study by Li et al. (2024), a dual-signal ratiometric aptasensor was developed for ultrasensitive detection of AFM1 by using split aptamer fragments (S1 and S2) functionalized with ferrocene (Fc) and MB redox tags, integrated onto a rGO/AuNP-modified electrode interface. The sensing mechanism exploited target-induced structural reorganization: AFM1 binding triggered displacement of the Fc-labeled complementary strand (CS1), resulting in a dual-response signal, a suppressed Fc current and a synergistically amplified MB signal. This self-referencing mechanism effectively corrects for matrix effects and nonspecific interactions, addressing a critical limitation of single-signal platforms. The aptasensor achieved a detection limit of 0.015 µg/L in buffer and 0.05 µg/L in milk, outperforming many conventional single-output systems. The split aptamer design enhanced binding specificity by reducing structural flexibility compared to full-length aptamers, while the optimized splitting strategy preserved high affinity for AFM1. Coupled with the nanomaterial-enhanced electrode interface, this approach

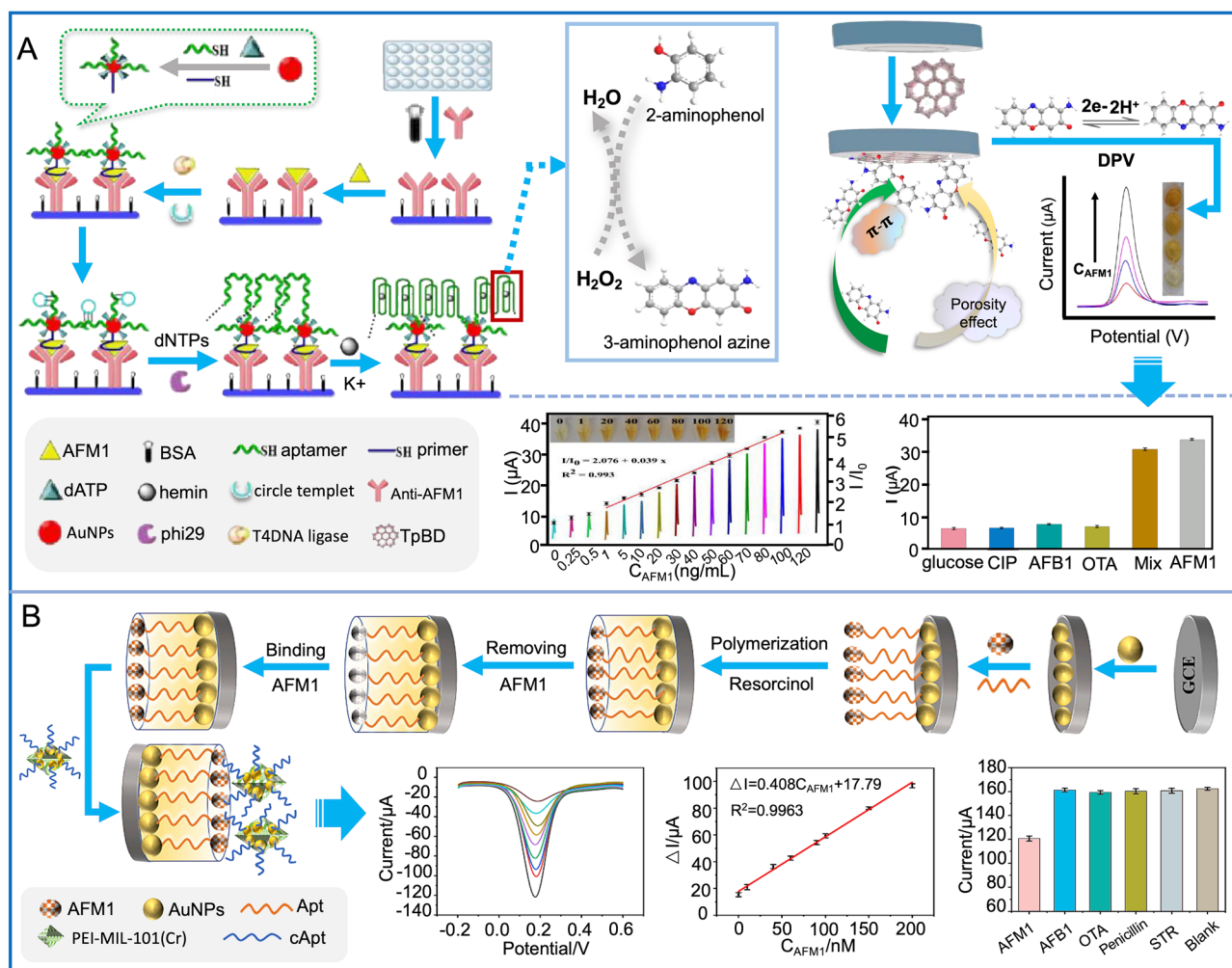


Figure 8. Representative electrochemical aptasensors toward AFM1: (A) Schematic illustration of the electrochemical sensing for detection of AFM1 in milk by using aptamer/antibody/COFs/RCA platform (Pang et al. 2020). Copyright 2020, Elsevier. (B) Schematic illustration of the electrochemical approach for detection of AFM1 in milk by using MIP and aptamer as dual-recognition elements (Yang et al. 2024). Copyright 2023, Elsevier.

established reliable quantification of AFM1 in real milk samples without extensive pretreatment. These results demonstrate the transformative potential of split aptamer-based ratiometric systems in food safety monitoring, offering a pioneering framework toward robust, interference-resistant detection platforms for low-abundance contaminants in complex biological matrices.

In general, electrochemical sensing has been widely acknowledged as a powerful technique for AFM1 monitoring. However, several limitations and shortcomings remain to be addressed: (i) High-performance substrate electrodes are critical for providing sufficient binding sites for DNA modification and ensuring fast electron transfer. The commonly used Au and carbon electrodes are lack of stability and repeatability. The screen-printed carbon electrodes (SPCEs), which offer outstanding stability and parallelism, hold promise for improving analytical performance and will be discussed in the following section. (ii) While significant progress has been made, some electrochemical aptasensor still show limited sensitivity, the intelligent design of sensing interfaces, integration of functionalized nanomaterials, and implementation of appropriate signal amplification strategies are essential for further improving sensitivity and signal transduction. (iii) Despite significant

advancements in developing high-performance electrochemical aptasensors for AFM1 detection, their transition to practical commercialization requires integration with miniaturized portable platforms, including personal glucose meter (PGM)-based systems, paper-based analytical devices, and smartphone-coupled detection interfaces.

Application of aptasensors with portable devices

The evolution of aptasensor integration with portable platforms marks a paradigm shift in analytical methodologies, particularly for on-site detection of contaminants like AFM1 in dairy products. Early advancements in this field focused on addressing the limitations of conventional ELISA kits, which suffer from antibody dependency, prolonged workflows, and restricted multiplexing capabilities. A key breakthrough came from He et al. (2020), who developed a microfluidic chip-based aptasensor by integrating specific aptamers into a tripartite DNA-functionalized Fe₃O₄@Au magnetic nanoparticle system. This design effectively addressed interference from complex matrices, enabling the simultaneous detection of multiple targets. The aptamers recognized their respective targets, leading to the release of the

aptamer-target complex, and triggering RCA in the presence of phi29 polymerase. By utilizing three cDNA sequences with different lengths of RCA products, the system distinguished and quantified signals for kanamycin (KANA), 17 β -estradiol (E2), and AFM1 with low LODs of 0.32 pg/mL, 6.8 pg/mL, and 0.95 pg/mL, respectively. Notably, the device achieved target response within just 3 min, demonstrating its potential for rapid on-site detection. The innovation lies in its ability to minimize matrix interference through magnetic separation and signal amplification, establishing a foundation for high-throughput, multi-analyte detection in complex samples.

Following this, researchers explored electrochemical aptasensors to improve sensitivity and simplicity. Hamami, Mars, and Raouafi (2021) developed an antifouling electrochemical aptasensor using SPCEs modified with polyethylene glycol (PEG) and AuNPs (PEG/AuNPs/SPCEs) for AFM1 detection in milk. The aptamer was covalently immobilized on the surface of PEG/AuNPs/SPCEs, and capacitive signals were measured to quantify the binding event of AFM1, and further contributing to quantitative monitoring of AFM1 (Figure 9A). This approach achieved a detection sensitivity of 7.14 pg/mL, comparable to fluorescence-based systems. The design effectively minimized nonspecific adsorption while simplifying the detection process, highlighting the potential of electrochemical systems for field applications. Subsequent advancements by Ramalingam, Elsayed, and

Singh (2023) integrated GQDs and AuNPs on SPCEs, broadening the linear detection range to 100 pM–2 nM and reducing the testing time to 20 min (Figure 9B). Although this modified system exhibited slightly higher LOD of 3.97 nM, it demonstrated an effective compromise between analytical performance and operational practicality for portable device engineering.

The integration of aptasensors with portable devices, such as PGMs, test strips, and smartphones, represents a transformative advancement in detection technology. These hybrid systems utilize established hardware infrastructure to reduce costs while enabling real-time data analysis and wireless connectivity. However, current implementations still face challenges in signal consistency, reproducibility across different sample types, and the absence of standardized calibration protocols. Future development should focus on optimizing interfacial compatibility between biological sensors and portable devices, achieving robust, user-friendly systems that meet regulatory requirements for food safety monitoring.

Commercialization of aptasensors

The commercialization of aptasensors for AFM1 detection faces substantial technical and operational barriers, with no market-ready devices currently reported in scientific literature or available on commercial platforms. This gap

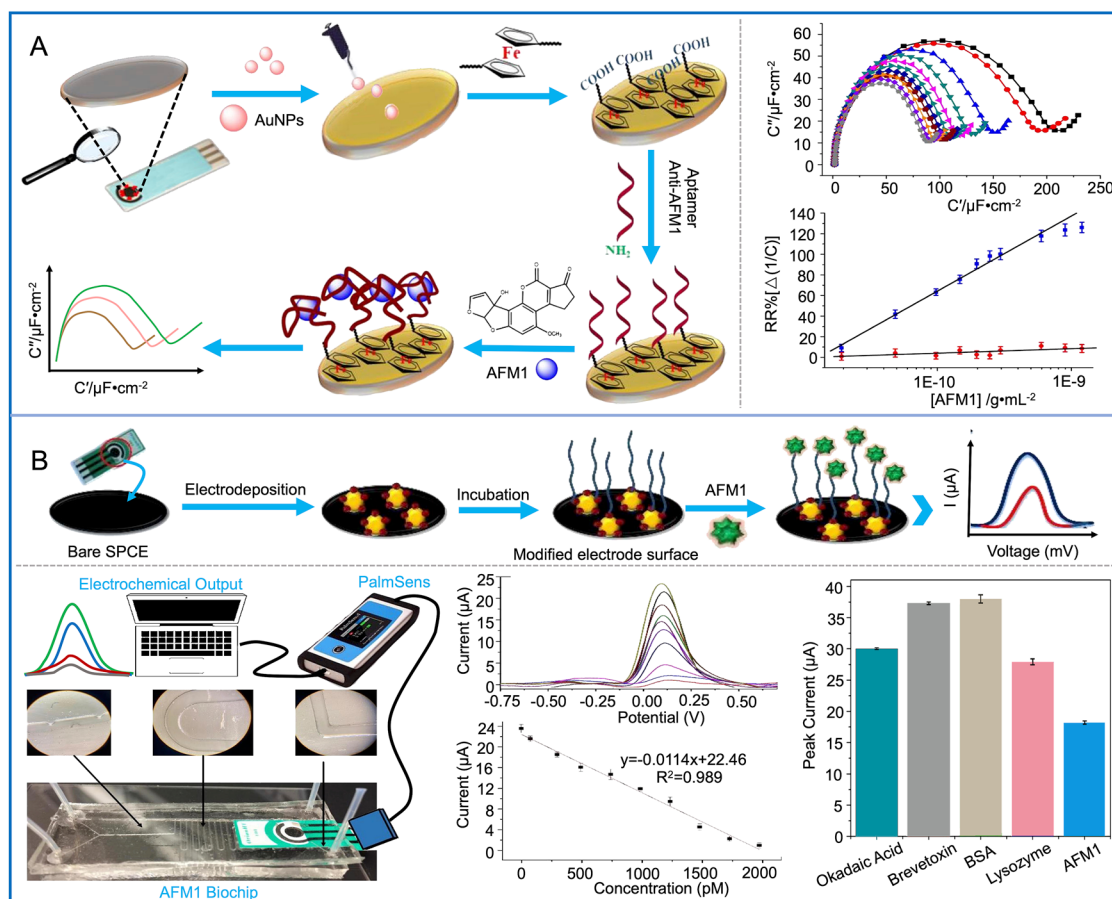


Figure 9. Advanced aptasensors integrated portable devices for AFM1 detection: (A) Working principle of the electrochemical nanoaptasensor for detection of AFM1 in milk via aptamer/PEG/AuNPs/SPCEs (Hamami, Mars, and Raouafi 2021). Copyright 2021, Elsevier. (B) Schematic illustration of the electrochemical aptasensor platform to monitor AFM1 in milk by using aptamer/GQDs/AuNPs/SPCEs (Ramalingam, Elsayed, and Singh 2023). Copyright 2022, Elsevier.

highlights the challenges in translating laboratory-scale aptamer-based assays into standardized, robust, and economically viable technologies. Primary limitations include maintaining long-term stability, ensuring reproducibility across diverse environmental and matrix conditions (e.g., dairy products and processed foods), and scaling up manufacturing processes without compromising performance (Ardalan and Ignaszak 2024; Al Mamun et al. 2021). Although aptasensors exhibit theoretical advantages in rapidity, portability, and cost-efficiency, their transition to commercial platforms remains hindered by technical immaturity, particularly in aptamer development and aptasensor engineering.

Current methods for AFM1 detection, such as ELISA and HPLC, remain industry standards due to validated reproducibility across laboratories. However, these techniques exhibit limitations in portability, operational complexity, and equipment costs, restricting their utility for on-site monitoring. In contrast, aptasensors have the potential to overcome these limitations through their adaptability to miniaturized formats and the possibility of automation. Despite these advantages, aptasensors for AFM1 have not yet achieved the level of validation and reproducibility necessary for widespread industrial adoption. This discrepancy underscores the need for comprehensive studies to benchmark aptasensor performance against established methods while addressing matrix interference and environmental variability.

A critical bottleneck in the development of AFM1 aptasensors is the relative immaturity of AFM1-specific aptamers compared to those targeting other mycotoxins, such as AFB1 and ochratoxin A (OTA). Current aptamer screening protocols often produce sequences with suboptimal binding affinity or cross-reactivity, partly due to inadequate validation of selection methodologies. Advances in computational tools, including *in silico* aptamer design and machine learning-driven optimization, could accelerate the discovery of high-affinity aptamers with enhanced stability in complex matrices. Simultaneously, aptasensor engineering must address the tradeoffs between performance and cost. While the integration of nanomaterials, such as GO and AuNPs, improves sensitivity, enabling detection limits as low as 0.01 ng/mL in prototypes, it also increases production costs. Achieving commercial viability will require innovative fabrication techniques, such as roll-to-roll printing or modular microfluidic designs, to reduce material costs and facilitate scalable manufacturing.

Further challenges arise from the stringent regulatory requirements for food safety testing. For example, the European Union's maximum residue limit for AFM1 in milk is set at 0.05 µg/kg, requiring aptasensors that exhibit exceptional precision and reliability under real-world conditions. Although current prototypes show promise, they lack sufficient long-term stability data and robustness against variables such as temperature fluctuations and pH changes. Systematic validation across diverse food matrices, including milk, cheese, and infant formula, is essential to demonstrate consistency and accuracy. Collaborative efforts among academic researchers, industry stakeholders, and regulatory bodies will be crucial in establishing standardized testing protocols and certification pathways.

In conclusion, the successful commercialization of AFM1 aptasensors depends on addressing interconnected technical and economic challenges. It is essential to prioritize the development of high-affinity aptamers through advanced screening strategies, optimize sensor architectures for cost-effective scalability, and conduct rigorous multi-laboratory validation studies. By addressing these issues, aptasensors have the potential to become transformative tools for on-site mycotoxin detection, providing rapid and reliable solutions to protect global food supply chains and public health (Wang et al. 2024).

Conclusions and outlook

As a typical mycotoxin contaminant in milk and dairy products, AFM1 poses great risks to global food safety and public health. Therefore, the development of rapid, sensitive, and cost-effective detection platforms for AFM1 is both crucial and urgent. Aptamers, often termed “chemical antibodies,” offer obvious advantages over traditional antibodies, including smaller size, higher affinity, ease of modification, and lower cost. Recent advancements in aptasensors using optical (e.g., fluorescence, colorimetry) and electrochemical signal transduction mechanisms have achieved remarkable analytical performance, with LODs spanning from fg/mL to ng/mL (Table 1). These platforms have been validated against gold-standard methods such as HPLC and ELISA, demonstrating comparable accuracy in real-sample analyses (e.g., raw milk, cheese). Notably, innovations in nanomaterials (e.g., quantum dots, noble metals) and nucleic acid amplification techniques (e.g., HCR, RCA) have significantly enhanced sensitivity and selectivity, positioning aptasensors as promising alternatives for on-site monitoring.

Despite these achievements, there are still some vital scientific limitations and challenges to be overcome: (i) As mentioned above, while advanced materials (e.g., fluorescent nanomaterials, Au/Ag nanoparticles) and DNA-based amplification strategies (e.g., hybridization chain reaction, HCR; RCA) have improved detection limits, the exploration of synergistic material combinations (e.g., MXene-graphene hybrids) and emerging technologies (e.g., CRISPR-Cas12a-assisted signal amplification) could further enhance sensitivity and robustness. (ii) It can be realized that the publications of AFB1 aptasensors far exceed that of AFM1 research. Other commonly used optical and electrochemical technologies also exhibit promising spaces for AFM1 detection, including SERS, photoelectrochemical (PEC), ratiometric optical and electrochemiluminescent signal response. Additionally, dual-mode aptasensors have gradually gained increasing attention since they can eliminate background signals, provide more accurate results and achieve high-throughput data. (iii) In recent decade, few studies pay attention to the aptamer screening of AFM1. Numerous reports are rarely focused on the aptasensor technologies development rather than the mechanism exploration. Nevertheless, the conformational change, binding kinetics and environmental conditions are of great significance in the interactions between the aptamer and target. Fascinatingly, the recent discovery of two novel aptamers against AFM1

Table 1. Summarization of optical and electrochemical aptasensors for detection of AFM1 in milk and dairy products.

Aptasensor type	Transduction principle	Sensing interface	Target	Dynamic range	LOD	Samples	Reference
Fluorescent Aptasensor	Fluorescent quenching/recovery	Aptamer-FAM/cDNA-TAMRA	AFM1	1–100 ng/mL	0.5 ng/mL	Milk	(Qiao et al. 2021)
Fluorescent Aptasensor	Fluorescent quenching/recovery	Bivalent binding aptamer-cDNA	AFM1	0.7–10 nM	0.5 nM	Milk	(Sameiyan et al. 2021)
Fluorescent Aptasensor	FRET	Apt-DNA probes/Cy3-BHQ2/Cy5-BHQ2	AFM1/AFB1	0.01–200/0.01–150 ng/mL	6.24/9.0 pg/mL	Peanut/pure milk	(Ge et al. 2023)
Fluorescent Aptasensor	Fluorescent quenching/recovery	Aptamer/g-C ₃ N ₄ nanosheet/RCA product/TRFNPs-cDNA	AFM1	0.0001–0.5 ng/mL	19.4 fg/mL	Milk	(Niazi et al. 2020)
Fluorescent Aptasensor	G4 generated from DNA walker amplification to bind with berberine	Aptamer/DNAzyme/report probe/AuNPs/berberine	AFM1	0.01–0.8 ng/mL	6.40 pg/mL	Milk	(Wang et al. 2023)
Fluorescent Aptasensor	Laser-induced Fluorescence/Microfluidic chip	Au-Fe ₃ O ₄ @PP@CDT@AP	KANA/E2/AFM1	1–10 ⁴ /20–2000/3–50 pg/mL	0.32/6.8/0.95 pg/mL	Milk	(He et al. 2020)
Fluorescent Aptasensor	Fluorescent quenching/recovery	PEG@Mn _{0.002} Ta ₃ S ₆ NSs/TAMRA-Apt-B1/FAM-Apt-M1	AFB1/AFM1	0.0025–500/0.001–500 nM	2.47/1 pM	Milk/human serum	(Yadav et al. 2023)
Fluorescent Aptasensor	FRET	Graphene oxide/Cy3-Apt-B1/Cy5-Apt-M1	AFB1/AFM1	0.01–300/0.05–500 ng/mL	8.7/20.1 pg/mL	Peanut/milk	(Cai et al. 2024)
Visual/Fluorescent aptasensor	Visualizing gel-to-sol transition/Fluorescent quenching/recovery	FAM-aptamer/BHQ1-cDNA/DNA hydrogel	CHL/AFM1	0.1–1000/1–200 nM	1.0/1.7 nM	Milk	(Aran and Bayraç 2023)
Colorimetric Aptasensor	Salt-induced AuNPs aggregation	Aptamer/AuNPs/NaCl	AFM1	0.078–10 mg/mL	0.078 ng/mL	Milk	(Liu et al. 2022b)
Colorimetric Aptasensor	AuNPs@CuO catalyze TMB to generate ox TMB	AuNPs@CuO-apt/cDNA@Fe ₃ O ₄ /TMB	AFM1	0.5–500.0 ng/mL	0.50 ng/mL	Milk powder	(Wei et al. 2023)
Colorimetric aptasensor	Salt-induced AuNPs aggregation	AuNPs/aptamer/CS (dsDNA)-modified SNPs/NaCl	AFM1	0.3–75 ng/mL	0.03 ng/mL	Milk	(Jalalian et al. 2021)
Colorimetric aptasensor	Salt-induced AuNPs aggregation	AuNPs/72-Mers ssDNA/NaCl	AFM1	0.005–0.100 ng/mL	0.002 ng/mL	Milk	(Lerdri et al. 2021)
Colorimetric aptasensor	CRISPR-Cas12a and peroxidase-mimic activity of AuNPs	Primer-modified AuNPs/CRISPR-Cas12a/RCA/4-nitrophenol	AFM1	0.0002–0.3 ng/mL	0.05 pg/mL	Milk	(Abnous et al. 2021)
Colorimetric Aptasensor	Salt-induced AuNPs aggregation/paper-based microfluidic devices	AuNPs/aptamer/NaCl	AFM1	1 pM–1 μM	3 pM	Buffalo milk	(Kasoju et al. 2020)
SERS/MRS dual-mode aptasensor	Fe ₃ O ₄ @AuNPs aggregation	Au-Ag Janus NPs/Au-mushroomNPs/Fe ₃ O ₄ @AuNPs-aptamer	AFB1/AFB2/AFM1	0.01–10/0.001–5/0.05–0.10 ng/mL	3.45/0.31/0.42 pg/mL	Maize meal (AFB1/AFB2)/Milk (AFM1)	(Cao et al. 2024)
Electrochemical aptasensor	Resistance charge transfer change induced by aptamer/AFM1 recognition	AuNPs/rGO-PGE	AFM1	0.5–800 pg/mL	0.3 pg/mL	Milk	(Ahmadi et al. 2022)
Electrochemical aptasensor	Current change induced by aptamer/AFM1 recognition	ferrocene-labeled signal probe/aptamer/magnetic nanoparticles/TpBD-GCE	AFM1	0.5–80 ng/mL	0.15 ng/mL	Skimmed milk/milk powder/milk beverage	(Guo et al. 2021)
Electrochemical aptasensor	Current change induced by aptamer/AFM1 recognition	aptamer/pectin-AuNPs/GO/GCE	AFM1	0.01–1 ng/mL	0.2 pg/mL	Full fat pasteurized milk/raw cow milk	(Chrouda et al. 2022)
Electrochemical aptasensor	Electrochemical impedance spectroscopy	BSA/Apt/PtNP/MIL-101(Fe)/GCE	AFM1	0.01–80 ng/mL	2 pg/mL	Milk powder/pasteurized milk	(Jahangiri-Dehaghani, Zare, and Shekari 2020)

(Continued)

Table 1. Continued.

Aptasensor type	Transduction principle	Sensing interface	Target	Dynamic range	LOD	Samples	Reference
Electrochemical aptasensor	Current signal response induced by MIP and aptamer dual-recognition	cApt-Au@PEIM/AFM1/MIP-Apt/AuNPs/GCE	AFM1	0.01–200 nM	0.07 nM	Cow/sheep/goat milk	(Yang et al. 2024)
Electrochemical aptasensor	Current change induced by aptamer/AFM1 recognition	cDNA2-Au@Ag/Apt-Au@Ag/cDNA1/GE	AFM1	0.05–200 ng/mL	0.02 ng/mL	Cow/goat/sheep milk	(Hui et al. 2022)
Electrochemical aptasensor	Capacitive signal response induced by aptamer/AFM1 recognition	aptamer/FcTGL/AuNPs/SPCE	AFM1	20–300 pg/mL	7.14 pg/mL	Pasteurized cow milk	(Hamami, Mars, and Raouafi 2021)
Electrochemical aptasensing	Current change induced by RCA protocol/ aptamer recognition/ELISA	primer-AuNPs-aptamer/antibody/COF-TpBD-GCE	AFM1	0.5–80 ng/mL	0.15 ng/mL	Skim milk/milk powder/milk beverage	(Pang et al. 2020)
Electrochemical aptasensor	Current change induced by aptamer/AFM1 recognition	aptamer/GQDA-SPEC	AFM1	0.1–2 nM	3.97 nM	Partially skimmed bovine milk	(Ramalingam, Elsayed, and Singh 2023)
Electrochemical aptasensor	Current change induced by aptamer/AFM1 recognition	KCC-1-nPr-NH ₂ -DPA/Cys A/AuNPs/Aptamer/GCE	AFM1	10 fM–0.1 μM	10 fM	Pasteurized milk	(Kordasht and Hasanzadeh 2020)

opens up a new horizon for rapid development of aptasensors. (iv) The selectivity investigation plays an important role in the validation of developed aptasensors, especially for the aflatoxin subtypes with structural analogues like AFB1, AFG1, AFM2, etc. Therefore, evaluating of the influence of interferences on the aptamer affinity and specificity is essential. (v) Milk samples are well-known characterized by complicated matrix systems, which contain varieties of nutrient components, such as proteins, fats, sugars, vitamins, etc. The feasibility and practicality of developed aptasensors in milk and dairy products remains a big challenge. Therefore, more efforts should be made to investigate the variations of different kinds of milk on the developed aptasensors. (vi) The stability and folding characteristics may be influenced by the special conditions like temperature, pH, ionic strength, etc., thereby affecting the detection performance. Hence, the optimization of the SELEX protocols for specific aptamers that can undergo various matrices is crucial. Therefore, greater efforts should be directed toward addressing the technical barriers and developing products that make these methods and devices more suitable and convenient for POC testing of AFM1, thereby enhancing the safety of milk and dairy products.

Author contribution statement

Xiaodong Guo: Investigation, Conceptualization, Methodology, Writing – original draft. Mengke Zhang: Writing – original draft, Methodology, Formal analysis, Conceptualization. Xin Wen: Methodology, Formal analysis, Data curation. Genxi Zhang: Investigation, Methodology, Formal analysis. Tao Zhang: Methodology, Formal analysis, Data curation. Xinhui Lou: Investigation, Formal analysis, Data curation. Mengzhi Wang: Funding acquisition, Methodology, Writing – review & editing. Marie-Laure Fauconnier: Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. Kaizhou Xie: Supervision, Writing – review & editing, Formal analysis, Data curation, Conceptualization.

Declaration of interest statement

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.

Disclosure statement

No potential competing interest was reported by the authors.

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Data availability

No data was used for the research described in the article.

Abbreviations and nomenclatures

ELISA	Enzyme-linked immunosorbent assays
LFIA	Lateral flow immunoassay
SELEX	Systematic Evolution of Ligands by Exponential Enrichment
AFM1	Aflatoxin M1
AFB1	Aflatoxin B1
MRL	Maximum Residue Limit

IARC	International Agency for Research on Cancer
ssDNA	Single-stranded DNA
PCR	Polymerase chain reaction
GO	Graphene oxide
FRET	Förster resonance energy transfer
FAM	carboxyfluorescein
TAMRA	carboxy- <i>tert</i> -methyl rhodamine
cDNA	Complementary DNA
dsDNA	Double-stranded DNA
POC	Point-of-care
MoS ₂	Molybdenum disulfide
RCA	Rolling circle amplification
MRS	Magnetic relaxation switch
2D	two-dimensional
rGO	Reduced graphene oxide
LOD	Limit of detection
OTA	ochratoxin A
LSPR	Localized surface plasmon resonance
SERS	surface enhancement of Raman scattering
3D	three-dimensional
PGE	Pencil graphite electrode
MB	Methylene blue
COFs	Covalent organic frameworks
GCE	Glass carbon electrode
MOFs	Metal-organic frameworks
PtNPs	Platinum nanoparticles
Fc	ferrocene
MIPs	Molecularly imprinted polymers
SPCEs	Screen printed carbon electrodes
PGMs	personal glucose meters.

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