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Aptamer-based biosensing platforms for saxitoxin and tetrodotoxin: Advances, challenges, and future perspectives in food safety and environmental monitoring

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ABSTRACT

Saxitoxin (STX) and tetrodotoxin (TTX) are among the most potent marine neurotoxins, posing severe risks to public health and the seafood industry. Their high toxicity, structural diversity, and occurrence in complex aquatic and food matrices pose significant challenges for reliable detection and quantification. Conventional instrumental analysis methods offer high sensitivity and specificity but require costly instrumentation, skilled personnel, and time-consuming sample preparation. Immunoassays, while faster, may suffer from limited recognition of toxin congeners. In recent years, aptamers, synthetic single-stranded DNA or RNA sequences have emerged as promising alternatives to antibodies for toxin recognition. Aptamer-based biosensing platforms offer advantages in terms of stability, reproducibility, ease of modification, and scalability. A broad range of detection techniques has been developed, including optical, electrochemical and hybrid systems, often incorporating nanomaterials and signal amplification strategies to achieve ultralow detection limits in food and environmental samples. Yet, the path from promising laboratory prototypes to reliable field tools remains challenging, particularly when matrix effects compromise sensor robustness. This review provides a comprehensive overview of aptamer selection strategies for STX and TTX, recent advances in biosensing technologies, and the performance of various platforms in different matrices. Key challenges, including matrix effects, technological feasibility, and the need for compliance with official regulations, are discussed. Finally, perspectives for developing robust, field-deployable aptasensors are outlined, emphasizing their potential to enable rapid, sensitive, and cost-effective toxin monitoring for food safety and environmental protection.

1. Introduction

Saxitoxin (STX) and tetrodotoxin (TTX) are among the most potent neurotoxins known, both capable of blocking voltage-gated sodium channels (VGSCs). This blockade leads to severe neurotoxic effects, including paralysis and, in extreme cases, death [1]. Despite their structural differences, these toxins share a similar mechanism of action but with varying affinities for different VGSC isoforms [2]. Furthermore, both STX and TTX belong to broad families of structurally related congeners that can also differ significantly in toxicity. Due to their toxicity, these two families are key targets for detection in food safety and environmental monitoring applications. However, the structural variability of the congeners complicates their detection and toxicological assessment [3].

Given the high toxicity and propensity of STX and TTX to accumulate and contaminate aquatic ecosystems, the development of rapid and sensitive detection methods is crucial. The mouse bioassay (MBA), although still an official method in some countries, is one of the most traditional techniques to detect STX and TTX. However, it raises ethical concerns and lacks specificity [4]. Conventional instrumental techniques, such as high-performance liquid chromatography (HPLC) and liquid chromatography–mass spectrometry (LC–MS), offer excellent sensitivity but require complex instrumentation and specialized personnel [5,6]. Immunoassays, based on antibodies, offer rapid and specific detection but may fail to detect certain toxic congeners of the STX and TTX families due to structural differences that limit antibody recognition [7,8].

Against these backdrops, aptamers have emerged as promising

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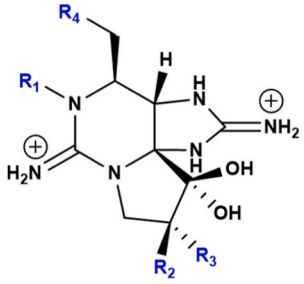
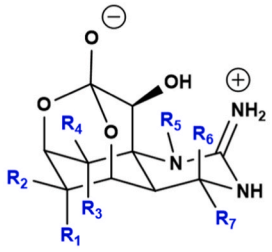
alternative biorecognition molecules for the development of biochemical assays. These single-stranded oligonucleotides, selected via Systematic Evolution of Ligands by Exponential Enrichment (SELEX), can rival antibodies in affinity while providing advantages in terms of production cost, reproducibility, stability, modification flexibility, and scalability [9]. Their versatility has enabled the development of a wide range of aptamer-based biosensing platforms for the detection of STX and TTX in seafood as well as other marine and freshwater samples. A wide variety of format and signal transduction strategies have been explored. These include optical, electrochemical, and hybrid platforms, which show great promise in terms of sensitivity, selectivity, and suitability for complex matrices such as seafood and environmental samples [10–12].

Several challenges must still be addressed to ensure consistent and reliable performance of aptamer-based biosensing platforms. These include the impact of physicochemical factors (e.g., temperature, pH), matrix interferences, the structural diversity of toxin congeners, and the intrinsic conformational flexibility of aptamers, which while beneficial for target recognition, may lead to variability in signal output.

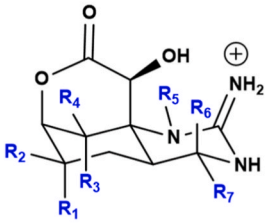
This review provides a comprehensive overview of the current state of aptamer-based biosensing platforms for the detection of STX and TTX. It describes several biosensing strategies, highlights their advantages over other analysis methods, and examines their applications in food safety and environmental monitoring. This review also discusses the limitations and challenges that must be addressed to enhance their practical applicability and commercial viability. By providing a comprehensive analysis of these systems, this review aims to support future research and drive the development of more efficient, sensitive, and field-deployable detection systems.



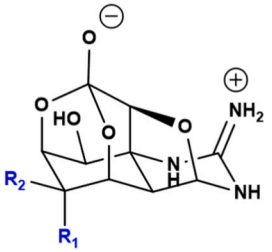
Compound	R_1	R_2	R_3	R_4
Saxitoxin	H	H	H	$CONH_2$
Neosaxitoxin	OH	H	H	$CONH_2$
Decarbamoyl saxitoxin	H	H	H	H
Decarbamoyl neosaxitoxin	OH	H	H	H
Gonyautoxin 1	OH	OSO_3^-	H	$CONH_2$
Gonyautoxin 2	H	OSO_3^-	H	$CONH_2$
Gonyautoxin 3	H	H	OSO_3^-	$CONH_2$
Gonyautoxin 4	OH	H	OSO_3^-	$CONH_2$
Gonyautoxin 5 B1	H	H	H	$CONHSO_3^-$
Gonyautoxin 6 B2	OH	H	H	$CONHSO_3^-$
Decarbamoyl gonyautoxin 1	OH	OSO_3^-	H	H
Decarbamoyl gonyautoxin 2	H	OSO_3^-	H	H
Decarbamoyl gonyautoxin 3	H	H	OSO_3^-	H
Decarbamoyl gonyautoxin 4	OH	H	OSO_3^-	H
C1	H	OSO_3^-	H	$CONHSO_3^-$
C2	H	H	OSO_3^-	$CONHSO_3^-$
C3	OH	OSO_3^-	H	$CONHSO_3^-$
C4	OH	H	OSO_3^-	$CONHSO_3^-$



Compound	R_1	R_2	R_3	R_4	R_5	R_6	R_7
TTX	OH	CH_2OH	H	OH	H	H	OH
4-*epi*TTX	OH	CH_2OH	H	OH	H	OH	H
6-*epi*TTX	CH_2OH	OH	H	OH	H	H	OH
11-deoxy TTX	OH	CH_3	H	OH	H	H	OH
6,11-deoxyTTX	H	CH_3	H	OH	H	H	OH
4-*epi*-11-deoxy TTX	OH	CH_3	H	OH	H	OH	H
11-norTTX-6(*R*)-ol	H	OH	H	OH	H	H	OH
11-norTTX-6(*S*)-ol	OH	H	H	OH	H	H	OH



Compound	R_1	R_2	R_3	R_4	R_5	R_6	R_7
8-*epi*-5,6,11-trideoxyTTX	H	CH_3	OH	H	H	H	OH
1-hydroxy-8-*epi*-5,6,11-trideoxyTTX	H	CH_3	OH	H	OH	H	OH



Compound	R_1	R_2
4,9-anhydroTTX	OH	CH_2OH
4,9-anhydro-11-deoxyTTX	OH	CH_3
4,9-anhydro-6-*epi*-TTX	CH_2OH	OH

Fig. 1. Schematic representation of the PSP toxins and TTXs chemical structures.

2. Chemical and toxicological similarities and differences between STX and TTX

STX is a small, non-protein neurotoxin with a tricyclic guanidinium structure, produced primarily by marine dinoflagellates of the genera *Alexandrium*, *Gymnodinium*, and *Pyrodinium* [13]. Due to its polar functional groups, it is hydrophilic and highly water-soluble, which facilitates its dispersion in aquatic environments. STX accumulates in bivalve mollusks, which act as vectors of contamination along the food webs. More than 40 natural congeners of STX have been identified (Fig. 1), including gonyautoxins (GTXs), decarbamoyl, and N-sulfocarbamoyl analogues, which vary in both polarity and toxicity [14]. TTX, similarly, is a small, non-protein neurotoxin with a tricyclic structure and high polarity, owing to the presence of hydroxyl and guanidinium groups. It is biosynthesized by symbiotic bacteria, mainly of the genera *Pseudomonas* and *Vibrio*, which inhabit marine and amphibious organisms such as pufferfish, newts, and certain crustaceans. TTX accumulates selectively in tissues such as liver, ovaries, and skin, where concentrations are typically the highest [15,16]. Over 30 analogues of TTX have been reported (Fig. 1), including 4-*epi*TTX, 11-deoxyTTX, and 5,6,11-trideoxyTTX among others, which exhibit varying degrees of toxicity and recognition in bioanalytical assays [17].

From a pharmacological perspective, both STX and TTX exert their neurotoxic effects by binding to VGSCs, thereby blocking sodium ion influx and disrupting the initiation and propagation of action potentials (Fig. 2). This mechanism underlies a wide range of symptoms, including tingling, numbness, muscle weakness, and, in severe cases, respiratory paralysis and death. The clinical manifestations depend on the dose and the route of exposure, which is typically oral in cases of foodborne intoxication [18]. Despite both toxins inhibit VGSCs, they display different affinities for particular channel isoforms. Molecular docking and computational pharmacology studies have shown that STX forms stable interactions with NaV1.4, confirming it as a primary target in muscle tissue [1]. TTX exhibits greater selectivity for NaV1.1, NaV1.3, and NaV1.7, which are involved in neuronal excitability and pain perception [19]. These biochemical and pharmacological nuances are essential for understanding toxin-specific symptoms.

3. Conventional analysis methods for STX and TTX

Bioassays were the first methods developed for the detection of several families of marine toxins, including STX and TTX, by assessing their toxic effects in biological systems. The MBA involves intraperitoneal injection followed by mortality recording. The assay functions on the principle that STX and TTX block VGSCs, leading to neuromuscular paralysis and eventually death at sufficiently high doses. By recording the time to onset of symptoms and the survival of the animals, the assay provides a measure of the toxins' presence and toxic potency. The MBA has long been an official method in many countries, and still is in some of them. However, concerns about ethical implications, low specificity, and variable reproducibility have driven the search for alternative methods [20].

Among cell-based approaches, the Neuro-2a cell-based assay (CBA) utilizes murine neuroblastoma cells to detect STX and TTX by measuring the inhibition of VGSCs [21–24]. In this assay, Neuro-2a cells are co-exposed to ouabain and veratridine, which synergistically induce cytotoxicity by disrupting sodium homeostasis. The presence of VGSC-blocking toxins such as STX or TTX inhibits this cytotoxic effect, resulting in a dose-dependent increase in cell viability. Thus, the assay indirectly quantifies toxin activity by measuring the protective effect against OV-induced toxicity [22]. While this assay has shown high sensitivity to STX and TTX, it is not without limitations. For example, Nicolas et al. [25] demonstrated that co-occurring marine toxins like palytoxin can alter membrane potential in ways that confound interpretation, reducing assay specificity. Interferences from the matrix is also a challenging issue. Also exploiting Neuro-2a cells, Automated Patch Clamp (APC) systems have been set up for TTX [23,26] and STX [24]. This automated device, in which a single cell is immobilized on a chip, its membrane potential is controlled, and ionic currents are recorded, has been widely used by the pharmaceutical industry and is now being exploited as a bioanalytical tool for sample screening and toxin quantification purposes.

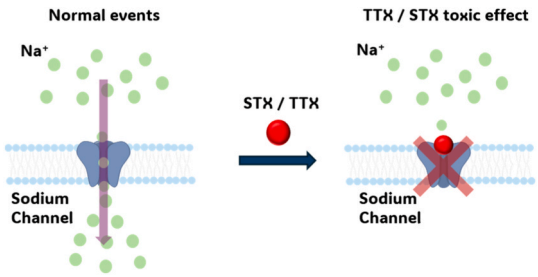


Fig. 2. Schematic representation of the STX and TTX toxicity mechanism.

In Europe, the official method for the control of Paralytic Shellfish Poisoning (PSP) toxins, the STX group, in bivalve mollusks is based on high-performance liquid chromatography with fluorescence detection (HPLC–FLD) following pre-column oxidation [27], as described by the Association of Official Analytical Chemists (AOAC) [28]. This method offers excellent specificity and sensitivity, and it is able to identify not only STX but also STX congeners. For TTX, there is not an official method, but liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) has become the most widely used and reliable instrumental approach. LC–MS/MS, and also high-resolution mass spectrometry (HRMS), provide accurate and sensitive qualitative and quantitative results, allowing high-throughput and trace-level detection of TTX analogues. Compared to FLD detection, mass spectrometry does not require derivatization, simplifying the analytical workflow. The structural similarity among TTX analogues ensures consistent ionization responses, and TTX itself is often used to quantify them [29]. Despite their high analytical performance, instrumental analysis methods are not without drawbacks. They require costly equipment, skilled operators, and complex sample preparation procedures.

Immunoassays and immunosensors have been extensively explored for the detection of STX and TTX, offering high specificity and sensitivirties. These platforms exploit antibodies that may cross-react with multiple congeners within the toxin family, depending on the structural similarity of epitopes. This cross-reactivity can be advantageous when broad-spectrum detection is needed but poses challenges when detecting analogues of differing toxicological relevance [30–39]. Ideally, cross-reactivity should correlate with the toxicological potency of each congener, allowing the immunosensing approach to reflect the true hazard posed by the sample. This approach aligns with the concept of Toxicity Equivalency Factors (TEFs), which are used to express the relative toxicity of congeners with respect to a reference compound. When antibodies do not reflect these toxic equivalencies either by over or under reacting with certain analogues, quantification becomes less reliable [32]. An important asset in the field of immunosensing approaches for the detection of toxins has been the integration of magnetic beads, which have been successfully employed in both colorimetric immunoassays [34] and electrochemical immunosensors [35]. Their high surface to volume ratio enhances the immobilization efficiency of antigens or antibodies, leading to improved binding kinetics and assay sensitivity [40]. Magnetic beads also allow for rapid and efficient separation using magnetic fields, which simplifies washing steps and minimizes matrix interferences, an essential feature when analyzing complex seafood samples.

4. Aptamers for STX and TTX

Aptamers are single-stranded oligonucleotides (DNA or RNA) that

fold into unique three-dimensional structures, allowing them to bind target molecules with high specificity and affinity. Thanks to their synthetic accessibility, chemical stability and ease of modification, aptamers have become valuable tools in diagnostics and biosensing, offering several advantages over antibodies [41]. Their selection is carried out through a process known as SELEX, which involves iterative rounds of binding, partitioning, and amplification to enrich high-affinity sequences [42]. This process defines the binding properties and functional performance of the resulting aptamers.

Classical SELEX procedures can be suboptimal when selecting aptamers for small, chemically simple molecules like STX and TTX, due to limited binding epitopes. To overcome this, several modified SELEX strategies have been developed. Immobilized Metal-ion affinity Chromatography SELEX (IMC–SELEX) immobilizes the aptamer library on metal-affinity supports, such as Ni-NTA resin, rather than the target itself. This approach was successfully used by Zhou et al. [9] to isolate a high-affinity STX aptamer (K_d = 19 nM) from a G-quadruplex library, with molecular simulations revealing key hydrogen bonding and van der Waals interactions. Another strategy is Graphene Oxide SELEX (GO–SELEX), which takes advantage of the strong affinity between single-stranded DNA and GO to separate unbound from bound sequences, eliminating the need for target immobilization (Fig. 3). The random sequence library is incubated with GO, resulting in the adsorption of the ssDNA on its surface. Then, the toxin is incubated, and the sequences able bind to it are released, recovered, and amplified. The amplified ssDNA is then used for the next round. A counter selection step can be added using a structurally related molecule, e.g. GTX, to avoid potential subsequent cross-reactions. This method was successfully employed to select STX-binding aptamers, which were later tested with mussels using a localized surface plasmon resonance (LSPR) sensor [43]. Also of interest is the strategy called Multiple–SELEX, which enables the simultaneous selection of aptamers against multiple targets. Gu et al. [44] used this technique to generate aptamers for STX, TTX, and domoic acid, achieving K_d values in the 44–62 nM range.

In an innovative deviation from traditional SELEX-based protocols, Li et al. [45] employed a computational repurposing strategy, selecting DNA aptamers for TTX with high thermal stability (T_m > 50 °C) as templates. Candidate aptamers were screened via molecular docking, refined through molecular dynamics, and experimentally validated using microscale thermophoresis (MST). Two initial aptamers, Tv-51 and AI-57, exhibited nanomolar affinity for TTX, and their binding sites were further characterized through spontaneous binding simulations. Guided by these structural insights, two optimized variants Tv-46 and AI-52 were engineered to enhance binding characteristics. While Tv-46 maintained comparable affinity with improved specificity, AI-52 demonstrated both enhanced affinity and selectivity toward TTX. Although promising, these aptamers have yet to be evaluated in complex biological matrices.

A major limitation shared by most SELEX approaches is the use of buffer systems during selection, which do not accurately reflect the complexity of real-world matrices such as food extracts or biological fluids. This discrepancy can compromise the performance of aptamers in practical applications. To overcome this, a strategy known as Real Sample-Assisted SELEX has been proposed. Ding et al. [46] demonstrated that conducting SELEX in milk allowed the selection of aptamers with significantly improved binding affinity and detection performance for the antibiotic sarafloxacin. Specifically, they used 20-fold diluted milk from the seventh screening round onward, along with magnetic beads as supports and appropriate washing buffers to remove interferences. Therefore, milk components never entered the PCR amplification step. The aptamer selected in real milk outperformed its buffer-selected counterpart in terms of binding strength, conformational responsiveness, and detection sensitivity. Despite the promise of this approach, no studies to date have reported the use of real sample matrices such as seawater or seafood extracts during the SELEX process for STX or TTX. This gap highlights a clear opportunity for future research: integrating real matrices during SELEX could significantly improve aptamer robustness and reliability under field conditions, enhancing their practical application.

5. Aptasensing systems for the detection of STX and TTX

Aptamer-based biosensing systems have recently emerged as highly promising tools for the detection of STX and TTX, offering significant advantages in terms of sensitivity, selectivity, and potential for on-site deployment. As previously mentioned, despite their structural differences, both toxins pose comparable analytical challenges, primarily due to their small molecular size and potent toxicity at trace levels. Recent studies have highlighted a wide array of aptasensing systems ranging from optical and electrochemical approaches to hybrid platforms frequently incorporating advanced nanomaterials, signal amplification techniques, and engineered aptamers. These systems are described below and summarized in Table 1.

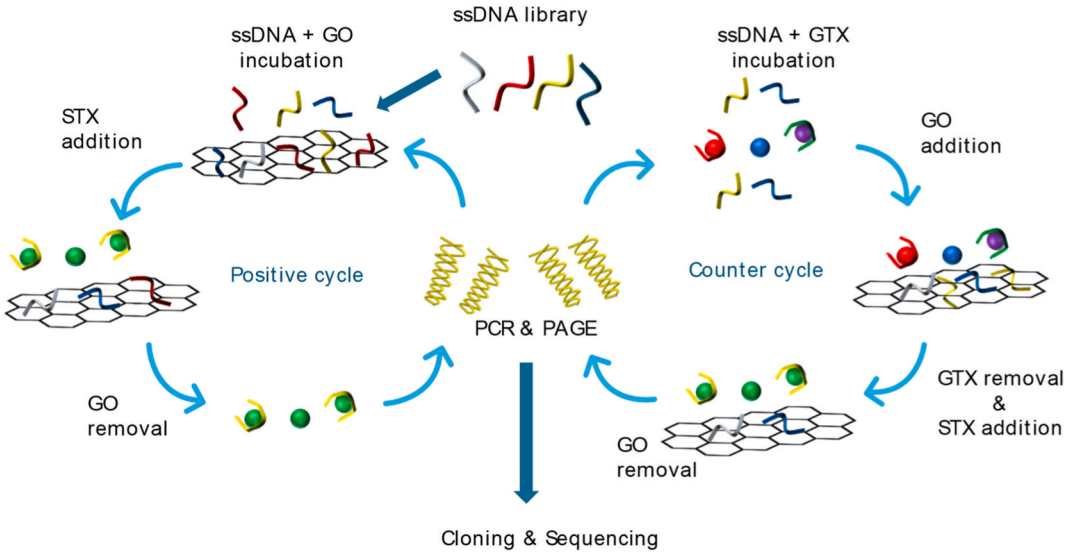


Fig. 3. Schematic representation of the GO-SELEX for STX aptamer inspired by Ref. [43].

Table 1
Main characteristics and performance of the aptasensing systems for STX and TTX.

Target	Apt. name	Sequence (5′-3′)	Detection method	LOD (nM)	Matrix	Recovery (%)	Ref.
STX	M-30f	TTG AGG GTC GCA TCC CGT GGA AAC AGG TTC ATT G	Colorimetry	4.25×10^{-2}	Scallop	106–114	47
			Colorimetry	1.42×10^{-1}	Seawater Scallop	98–114	48
			Fluorescence	6.00	Shellfish	106–111	50
			Spectrometry	11.70	Shellfish	109–104	56
			Differential Pulse Voltammetry	1.00	Shellfish	84–112	65
			Differential Pulse Voltammetry	1.00×10^{-3}	Shellfish	85–112	66
			Impedance	1.00	–	–	72
	APT^{STX1}	CCG TGG AAA CAT GTT CAT TGG GCG CAC TCC GCT TTC TGT AGA TGG CTC TAA CTC TCC TCT	Spectrometry	3.30×10^{-2}	Fish Crustacean	96–105	61
			Differential Pulse Voltammetry	0.38	Mussel	73–121	64
			Square Wave Voltammetry	1.56×10^{-2}	Freshwater	–	67
			Potentiometry	0.05	Mussel	111	74
	STX apt	GGT ATT GAG GGT CGC ATC CCG TGG AAA CAT GTT CAT TGG GCG CAC TCC GCT TTC TGT AGA TGG CTC TAA CTC TCC TCT	Colorimetry	1.00×10^{-5}	Seawater	–	12
			Fluorescence	1.17	Shellfish	94–103	10
	APT^{STX}	AAA AAA AAA A TGG GGA GTA GGG ACA GGA GGT GG	Fluorescence	6.00×10^{-1}	Shellfish	82–103	51
			Spectrometry	13.00	Shellfish	78–150	57
	Hp DNA	TTG AGG GTC GCATCC CGT GGA AACAGG TTC ATT GTT TCC ACG GGA TGC AGT ATG	Spectrometry	1.20×10^{-2}	Lake water	95–102	11
	45e-1	CTC GGG GGC GCG GTT GAT CGG AGA GGG	Spectrometry	1.67	Seawater	97–106	9
	STX aptamer	TAG GGA AGA GAA GGA CAT ATG ATG GCA CAA GGC CTC ATC AAT CGG TAT ACG GGT TGA CTA GTA CAT GAC CAC TTG A	Surface Plasmon Resonance	8.22	Mussel	96–116	43
	APT-9	CTT CTT CTT TTG AGG GTC GCA TCC CGT GGA AAC AGG TTC ATT GTT CTT CTT C	Square Wave Voltammetry	0.92	Seawater	94–111	68
	STX aptamer	TTT TTT TGG GGA GTA GGG ACA GGA GGT GG	Differential Pulse Voltammetry	1.00	Clam Shrimp	71–94	69
TTX	TTX apt	TCA AAT TTT CGT CTA CTC AAT CTT TCT GTC TTA TC	Colorimetry	2.19×10^{-1}	Pufferfish Clam	–	49
			Fluorescence	8.46×10^{-2}	Pufferfish Clam	91–116	54
			Fluorescence	8.31×10^{-4}	Clam Shellfish	100–117	55
			Fluorescence	1.90×10^{-2} (FL)	Pufferfish	98–106 (FL)	59
			Spectrometry	2.50×10^{-2} (SERS)	Clam	100 -106 (SERS)	
			Differential Pulse Voltammetry	9.90×10^{-2} (DPV)	Fish	98–99 (DPV)	60
			Spectrometry	0.12 (SERS)		98–99 (SERS)	
	TTX apt	AAA AAT TTC ACA CGG GTG CCT CGG CTG TCC	Fluorescence	7.4×10^{-2}	Human serum	97–106	52
			Fluorescence	1.1×10^{-2}	Pufferfish	100–106	53
			Fluorescence	3.07	Shellfish	85–101	10
			Spectrometry	7.50×10^{-2}	Pufferfish	96–99	58
			Impedance	0.62	–	–	71
	TTX apt D3	ATA CCA GCT TAT TCA ATT TAA TGC GGG GTG AGG CTC AAT CAA GGA AAG ATA TAA GTA AGC AAA AAG GTC AAA CAA GGG CGA GAT AGT AAG TGC AAT CT	Colorimetry	0.97	Pufferfish	94–109	33
			Lateral Flow Assay	0.94	Pufferfish	–	31

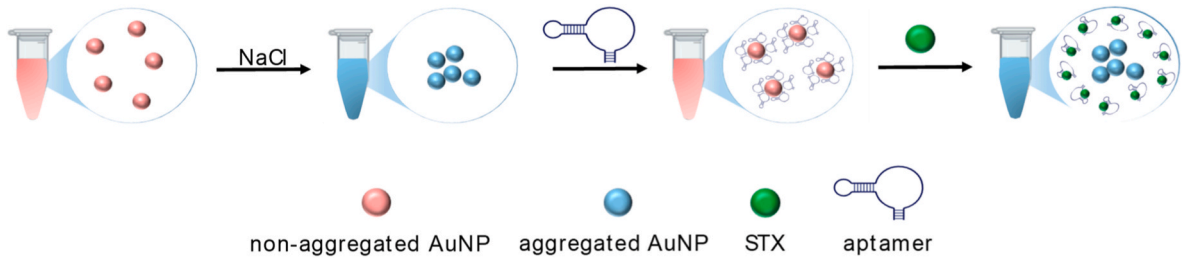


Fig. 4. Schematic representation of the colorimetric aptamer-based assay for STX inspired by Ref. [12].

5.1. Optical systems

Optical aptasensors exploit the interaction between light and matter to transduce aptamer–toxin binding events into measurable optical signals. These methods encompass a broad range of strategies, each offering unique advantages in terms of sensitivity, signal diversity, and potential for multiplexing. Their versatility enables both simple screening formats and highly sensitive platforms capable of detecting trace toxin levels in complex samples.

5.1.1. Colorimetric assays

Among optical methods, colorimetric strategies stand out for their simplicity and visual interpretability, making them suitable for rapid, low-cost screening. Gold nanoparticle (AuNP)-based systems have been widely employed, leveraging the optical properties of AuNPs and their tendency to aggregate under certain ionic conditions. In the absence of stabilizing agents, the addition of NaCl induces aggregation of AuNPs, resulting in a visible color change from red to blue (Fig. 4). However, when AuNPs are functionalized with aptamers, these molecules adsorb onto the nanoparticle surface and stabilize the colloid, preventing aggregation even in the presence of salt. Upon the addition of the target toxin, the aptamers preferentially bind to it, detaching from the AuNP surface. This displacement exposes the nanoparticles to the destabilizing effect of NaCl, triggering aggregation and a measurable color shift. This mechanism allows for a highly sensitive and specific detection system. Notably, Qiang et al. [12] reported a limit of detection (LOD) of 1×10^{-5} nM for STX, validated in seawater, using this strategy. While very simple and highly effective, this method requires careful optimization of ionic conditions, as the interaction between AuNPs and NaCl may be affected by proteins or other ions present in complex sample matrices.

Alternative colorimetric strategies for toxin detection have recently exploited the peroxidase-mimicking properties of nanomaterials to enable label-free and highly sensitive assays. In this context, Zhao's group [47] developed an aptamer-based assay that integrates a hybridization chain reaction (HCR) with AuNP nanozyme catalysis. In this system, STX competes with a biotinylated complementary strand to bind the aptamer immobilized on magnetic beads. When STX is present, it displaces the aptamer, triggering HCR and generating long double-stranded DNA polymers. These DNA structures enhance the peroxidase-mimicking activity of AuNPs, which are dispersed in solution and interact with the negatively charged DNA polymers via surface adsorption. This interaction promotes the formation of locally dense nanozyme clusters that catalyze the colorimetric oxidation of 3,3,5,5-tetramethylbenzidine (TMB) in the presence of H_2O_2, producing a strong blue color measurable at 652 nm. Moreover, magnetic separation steps were included to isolate the aptamer-target complexes before signal development, thereby removing matrix interferences. This system provided an LOD of 42.46 pM in scallop samples. Building on a similar strategy, Li et al. [48] developed another system for STX detection. In this system, the aptamer is initially hybridized with a complementary DNA (cDNA) strand, forming a duplex that remains in solution and does not interact with the AuNP surface. In the presence of STX, the toxin binds to the aptamer and induces a conformational change that displaces the cDNA. The released cDNA, now single-stranded, adsorbs onto the surface of AuNPs, enhancing their peroxidase-like catalytic activity. This activation enables efficient oxidation of TMB in the presence of hydrogen peroxide, generating a quantifiable colorimetric signal. This mechanism yielded a LOD of 0.1423 nM and was validated in both seawater and scallop samples, demonstrating high reproducibility and simplicity.

A similar principle but different nanomaterial was employed by Liu et al. [49] for TTX detection using a ZrFe metal-organic framework (MOF) with intrinsic peroxidase-like properties. In this system, the aptamer suppresses MOF catalytic activity through surface adsorption. In the presence of TTX, the aptamer preferentially binds the toxin, detaching from the MOF and thereby restoring its catalytic function. As with the AuNP system, the reactivated MOF catalyzes the oxidation of TMB in the presence of H_2O_2, generating a measurable blue signal. This method achieved an LOD of 0.07 ng/mL (0.219 nM) and demonstrated strong performance in pufferfish muscle and clam matrices.

5.1.2. Fluorescence assays

Fluorescence-based detection strategies have been widely employed for STX and TTX analysis with aptamers. In many of these aptasensing systems, the binding of the target molecule induces a structural change in the aptamer (e.g., hairpin to linear), altering the spatial proximity between a fluorophore and a quencher or affecting the hybridization of the aptamer with a complementary strand, thereby modulating fluorescence emission. Nevertheless, Cheng et al. [50] reported an exception to this general mechanism by designing a temperature-assisted "turn-on" fluorescent aptasensor for STX detection that does not rely on substantial aptamer conformational change. In their system, fluorescence is quenched at low temperatures due the close proximity between hexachlorofluorescein (HEX) and a quencher BHQ1 (black-hole quencher 1) at the ends of a structured aptamer. Upon heating to 61 °C, the aptamer unfolds, separating the fluorophore from the quencher and leading to fluorescence emission. In the presence of STX, the aptamer binds the toxin in its unfolded state and fails to refold upon cooling, resulting in a sustained fluorescence signal ("turn-on"). This approach enables effective detection (LOD = 6.0 nM) despite minimal structural rearrangement upon target recognition and demonstrates that aptasensing can still be achieved via thermal control of folding equilibria. The assay was successfully validated in spiked shellfish samples, showing recoveries of 106–111 %. Recently, Zhu et al. [51] proposed a sophisticated strategy for STX detection, integrating quantum dots (QDs) with Fe_3O_4@Au–Pt nanozymes within an inner filter effect (IFE) framework. In this system, the nanozyme catalyzes the oxidation of TMB, generating a colored product that quenches QD fluorescence through the IFE mechanism. This configuration achieved an LOD of 0.6 nM, delivering results in less than 30 min and demonstrating high recovery rates (82–102 %) in shellfish samples.

Parallel innovations have emerged for TTX sensing. In an earlier study, Lan et al. [52] developed a simple, cost-effective, label-free fluorescent sensor using berberine as a reporter molecule. Upon TTX binding, the aptamer undergoes a conformational switch from a random coil to a compact neck ring structure, altering the local

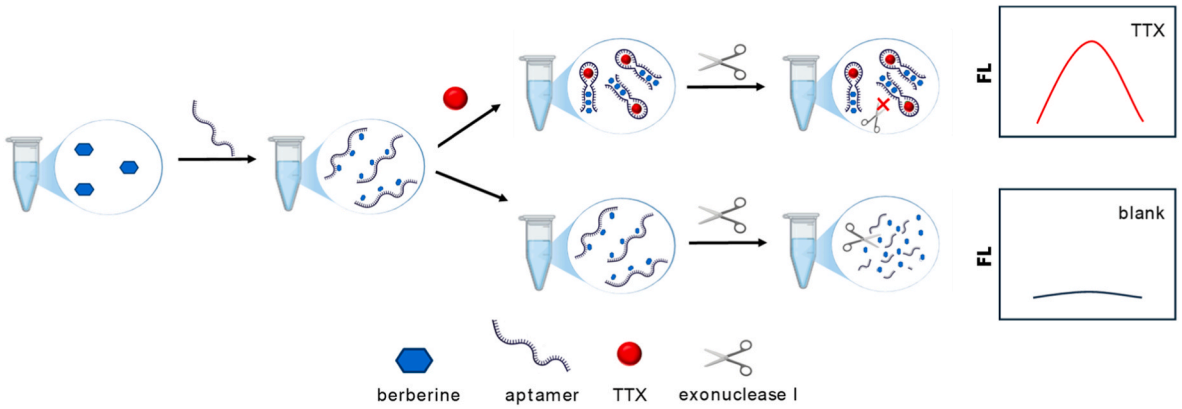


Fig. 5. Schematic representation of the exonuclease I-assisted fluorescence aptamer-based assay for TTX inspired by Ref. [52].

microenvironment and enhancing the fluorescence of berberine (Fig. 5). This system achieved an LOD of 0.074 nM and demonstrated high recovery rates (97–106 %) in spiked human serum, with minimal matrix interference. Building upon this concept, the same group introduced a more sensitive approach by incorporating an enzyme-assisted signal amplification mechanism [53]. Specifically, Exonuclease I (Exo I) was used to selectively degrade unbound aptamers, while aptamer–TTX complexes resisted enzymatic digestion due to their looped conformation, thereby preserving the berberine fluorescence. This enhancement enabled a markedly lower LOD of 0.011 nM, with successful validation in spiked pufferfish tissue samples.

Further expanding the capabilities of MOF-based fluorescent sensing, Liu et al. [54] developed a ratiometric aptasensing system using a multifunctional ZrFe-based MOF. This label-free system leverages the intrinsic fluorescence (445 nm) of this nanomaterial and its peroxidase-mimetic catalytic conversion of *o*-phenylenediamine (OPD) to its oxidized fluorescent product OPDox (555 nm), producing a dual-emission response. Upon TTX binding, the aptamer dissociates from the MOF surface, restoring enzymatic activity and reactivating fluorescence at 555 nm, while quenching the 445 nm signal, yielding a built-in calibration mechanism. The sensor achieved an LOD of 0.027 ng/mL (0.085 nM) and was validated in pufferfish and clam matrices with excellent recoveries. While this system offers high analytical performance, it requires fluorometric instrumentation and precise control of pH and ionic strength, which may limit its application in field conditions.

Triple-cycle oligonucleotide amplification platforms have demonstrated remarkable sensitivity improvements in aptamer-based detection systems. Zhang et al. [55] introduced a fluorescence-based system for TTX detection that integrates strand displacement amplification (SDA), catalytic hairpin assembly (CHA), and a magnetic bead-based aptamer–cDNA competition assay. In this system, the presence of TTX prevents hybridization between the aptamer and a cDNA strand immobilized on magnetic beads, allowing the free cDNA to initiate downstream amplification. Through sequential SDA and CHA reactions, a DNA sequence complementary to a fluorogenic hairpin probe is generated. This probe is labeled with a fluorophore (FAM) at the 5′ end and a quencher (BHQ1) at the 3′ end. Upon hybridization with the amplified target, the hairpin structure unfolds, separating fluorophore and quencher and thereby restoring fluorescence. The resulting signal increases proportionally with TTX concentration. The assay achieved an LOD of 8.31×10^{-4} nM and showed excellent recovery (100–117 %) in spiked clam and shellfish samples, with strong agreement to commercial ELISA kits.

Within the domain of multiplex detection, Dou et al. [10] developed a fluorescent FRET-based biosensor utilizing zirconium-based nanoscale MOFs (NMOFs) and tetramethylrhodamine (TAMRA)-labeled aptamers for the simultaneous detection of STX and TTX in shellfish samples. The design leveraged the overlap between the MOF emission and TAMRA absorption to create a robust FRET response upon target binding. This ratiometric platform achieved LODs of 1.17 nM for STX and 3.07 nM for TTX, and exhibited strong pH independence, excellent specificity against other marine toxins, and stable performance over 21 days.

5.1.3. *Spectroscopic methods*

Raman spectroscopy and Surface-Enhanced Raman Spectroscopy (SERS) exploit the plasmonic enhancement of Raman scattering signals due to plasmonic resonance induced by metallic nanostructures, enabling ultrasensitive molecular detection. Raman spectroscopy itself measures inelastic scattering of light caused by molecular vibrations, providing a molecular fingerprint. Cheng et al. [56] were the first to implement this technique for STX aptamer-based analysis, developing

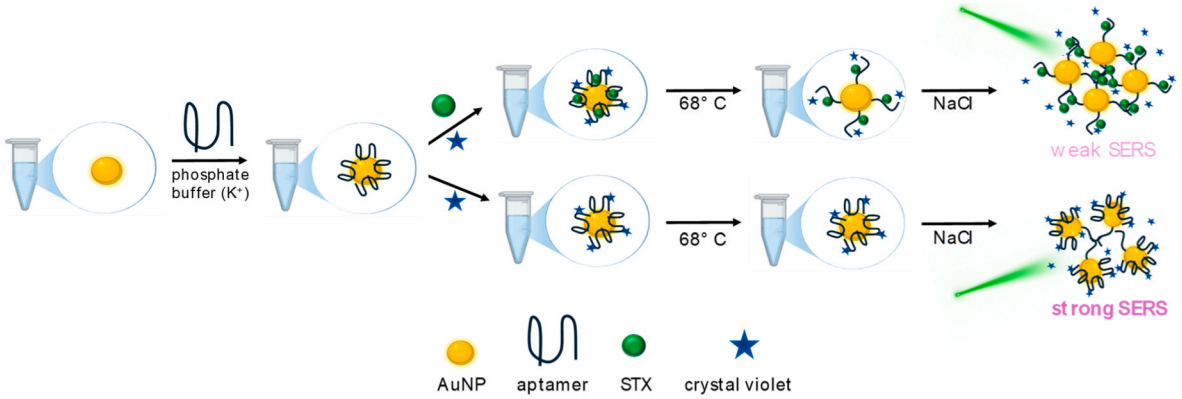


Fig. 6. Schematic representation of the SERS aptamer-based method for STX inspired by Ref. [56].

an aptamer-functionalized AuNP system incorporating crystal violet as a Raman reporter (Fig. 6). In the absence of STX, the aptamer formed a G-quadruplex structure that strongly bound the dye, yielding an intense SERS signal. STX did not trigger a conformational switch under ambient conditions but reduced the thermal stability of the aptamer. Upon heating, this destabilization promoted unfolding of the G-quadruplex, releasing the reporter and thereby decreasing the Raman signal. This system achieved an LOD of 11.7 nM and demonstrated effective performance in shellfish matrices. Building upon this, Bai et al. [11] introduced an innovative signal amplification strategy by integrating rolling circle amplification (RCA) into the sensing platform. In their system, the presence of STX triggered RCA, generating long single-stranded DNA strands capable of hybridizing with specially designed core–shell SERS probes (Au@4-NTP@SiO_2). These probes featured strong Raman reporter molecules and efficient plasmonic enhancement, significantly amplifying the SERS signal. This approach enabled highly sensitive detection of STX, achieving an LOD of 0.012 nM. The platform was validated with spiked lake water samples, showing recoveries ranging from 95 to 102 %, with good reproducibility. Li et al. [57] developed an alternative SERS-based system that integrates Pt/Au nanozymes, magnetic separation, and a catalytic reaction involving $HAuCl_4$ and H_2O_2. Validated in seafood matrices, the sensor achieved an LOD of 13 nM. However, the observed variability in recovery rates (78–150 %) indicates a need for further optimization to enhance reliability. These advancements in STX detection highlight the versatility of aptamer-based SERS systems, particularly when combined with nanomaterial-driven amplification strategies.

Regarding SERS-based aptasensors for TTX, Yin et al. [58] reported a self-assembled AgNP monolayer array with a ratiometric calibration scheme. The platform employs 4-mercaptobenzonitrile (MBN) as a Raman reporter in the biologically silent region (2228 cm^{-1}), while the silicon peak at ~520 cm^{-1} serves as an internal reference to correct signal fluctuations. Upon binding with TTX, the aptamer–nanoparticle complex dissociates, releasing the reporter and reducing the Raman signal in a concentration-dependent manner. The system achieved an LOD of 0.024 ng/mL (0.075 nM), with an RSD of 8.8 %, and was successfully validated in spiked pufferfish muscle tissue.

Integrated platforms combining multiple signal outputs such as fluorescence and SERS are increasingly employed to improve analytical performance in complex food matrices. These "dual-mode" strategies provide two independent yet complementary signal outputs, thereby reducing the probability of measurement inaccuracies. Liu et al. [59] developed a facile dual-mode aptasensing system based on AuNPs embedded in a MOF and Cy3-labeled aptamers, enabling simultaneous fluorescence and SERS readouts. Upon binding to TTX, the Cy3-aptamer–TTX complex dissociates from the MOF surface, leading to fluorescence restoration and concurrent SERS signal attenuation. This bidirectional response yielded LODs as low as 6 pg/mL (0.019 nM) (fluorescence) and 8 pg/mL (0.025 nM) (SERS). The method was validated in spiked and naturally contaminated pufferfish and clam samples, demonstrating excellent recovery (96–106 %), reproducibility (CV < 5.5 %), and specificity. While dual-mode strategies demand more instrumentation, they improve reliability and reduce the risk of false-positive or false-negative outcomes. A comparable strategy was proposed by Yao et al. [60] who engineered an aptasensing system based on Ag@Cu_2O nanoparticles functionalized with TTX-specific aptamers and assembled onto MXene nanosheets, a new class of 2D material that possesses large surface area, excellent electrical conductivity, and chemical stability. The platform could be interrogated through two complementary readout mechanisms, either electrochemically, via the distinct redox peaks at −0.13 V (Cu^+) and +0.17 V (Ag^0), or spectroscopically, exploiting the SERS response of 4-aminothiophenol (4-ATP) enhanced by the plasmonic properties of the Ag@Cu_2O core–shell structure. This doble readout versatility yielded LODs of 31.6 pg/mL (0.099 nM) (electrochemical) and 38.3 pg/mL (0.120 nM) (ratiometric SERS), and was successfully validated in spiked fish tissue extracts, showing high recovery and specificity. The integration with MXene nanosheets further contributed to signal stability and ratiometric normalization.

Biolayer Interferometry (BLI) and spectroscopic ellipsometry have also emerged as promising label-free approaches, particularly for STX sensing. BLI measures changes in optical thickness of the sensor surface caused by binding, which produces a shift in the interference pattern of reflected light, while spectroscopy ellipsometry measures changes in the polarization state of light from a surface as a result of binding. Zhou et al. [9] developed a BLI-based aptasensing system employing IMC–SELEX for aptamer selection, specifically designed to preserve the native conformation of STX during the selection process. The resulting device demonstrated excellent stability and reproducibility under controlled conditions; however, its performance in complex sample matrices has yet to be evaluated (Fig. 7). Similarly, Caglayan et al. [61] implemented an Attenuated Internal Reflection Spectroscopic Ellipsometry (AIR–SE) platform, achieving high sensitivity (LOD = 0.033 nM) and demonstrating effective performance in fish and crustacean samples (Fig. 8). Although the method entails considerable instrumental requirements, it presents a compelling label-free alternative with strong potential for

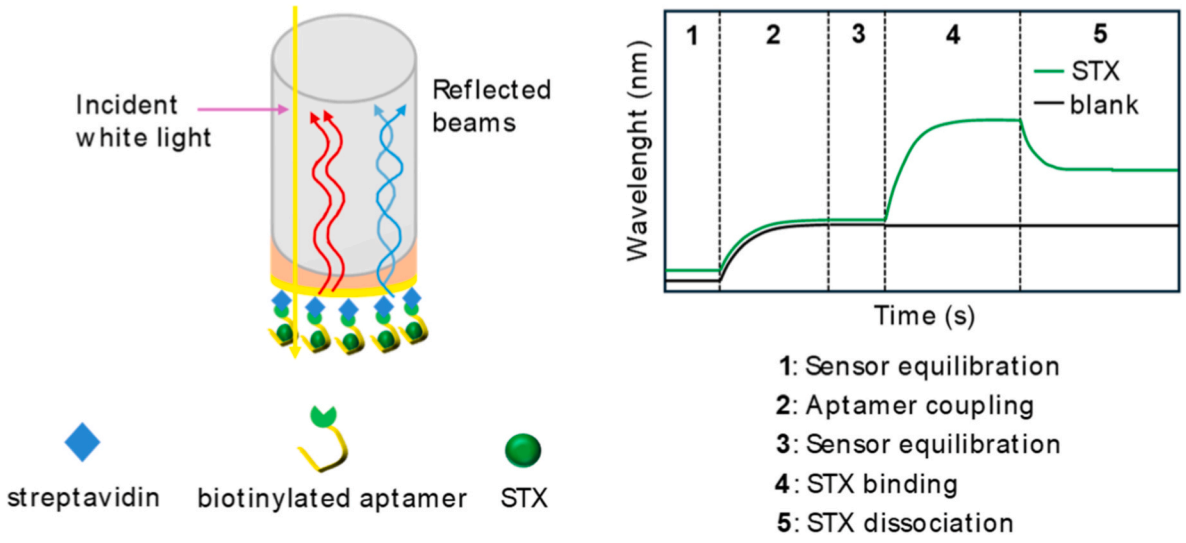


Fig. 7. Schematic representation of the BLI aptamer-based method for STX inspired by Ref. [9].

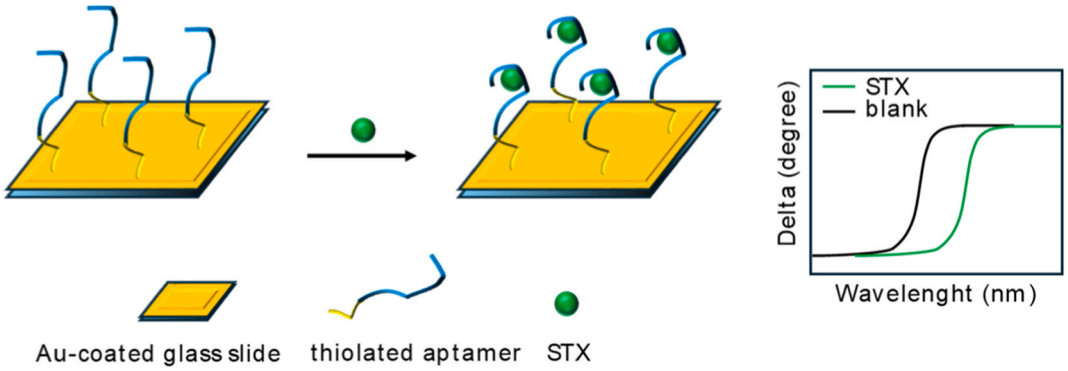


Fig. 8. Schematic representation of the AIR-SE aptamer-based method for STX inspired by Ref. [61].

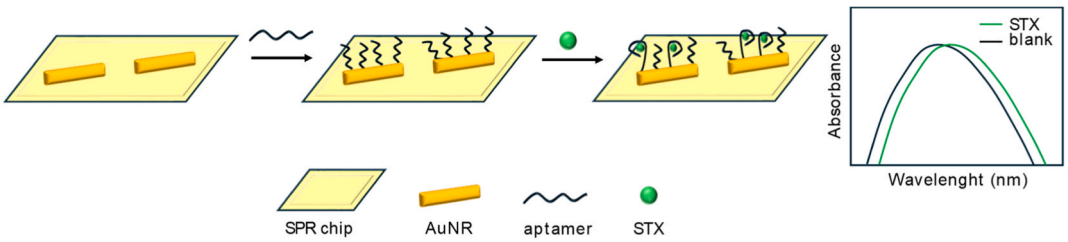


Fig. 9. Schematic representation of the aptamer–based SPR biosensor for STX inspired by Ref. [43].

high-precision applications.

5.1.4. Surface plasmon resonance (SPR) biosensors

Within the plasmonic domain, Ha et al. [43] developed a localized surface plasmon resonance (LSPR) biosensor based on gold nanorods (ANRs) functionalized with STX-specific aptamers selected via GO-SELEX. Upon toxin binding, the aptamer–STX interaction induces measurable shifts in the LSPR peak, enabling quantitative detection (Fig. 9). Although its LOD of 8.22 nM was relatively modest, the sensor was successfully validated in contaminated mussel samples.

5.1.5. Lateral-flow assays (LFAs)

Field-deployable strategies have been explored for the detection of TTX and STX. Among them, LFAs stand out for their portability, low cost, and visual readout capabilities. Bruno [62] developed a competitive aptamer–QD LFA for the detection of these toxins. Due to biosafety restrictions, the assay was validated using BSA-toxin conjugates instead of free toxins, which limits the direct applicability of the results to real-world matrices. Fluorescent signal intensity at the test line was analyzed using NIH ImageJ software, and although empirical LODs were constrained by the molecular weight of the BSA conjugates (typically 1–10 μg), theoretical calculations suggested that LODs as low as 4.5 ng could be achieved with pure toxins. Despite this limitation, the platform shows strong promise for on-site diagnostics with minimal instrumentation.

Aptamer–antibody hybrid strategies have recently gained attention for their ability to combine the high affinity of aptamers with the well-established specificity of monoclonal antibodies. Shkembi et al. [33] developed a sandwich assay for TTX detection in which a monoclonal anti-TTX antibody served as the capture element, while a biotinylated aptamer selected through capture-SELEX assisted by Next-Generation Sequencing (NGS), a high-throughput technique for identifying aptamer candidates with optimal binding profiles, functioned as the detection probe. This configuration exploited the cage-like molecular structure of TTX, which enables simultaneous recognition by both antibody and aptamer. The assay demonstrated high sensitivity, with an LOD of 310 pg/mL (0.97 nM), and excellent reproducibility (CV < 5 %). It was successfully validated in both spiked and naturally contaminated pufferfish extracts, showing high recovery and strong agreement with a magnetic bead-based immunoassay. The same group recently translated the assay into a LFA format for rapid on-site analysis [31]. The test employs an AuNP-conjugated monoclonal antibody as the reporter, and

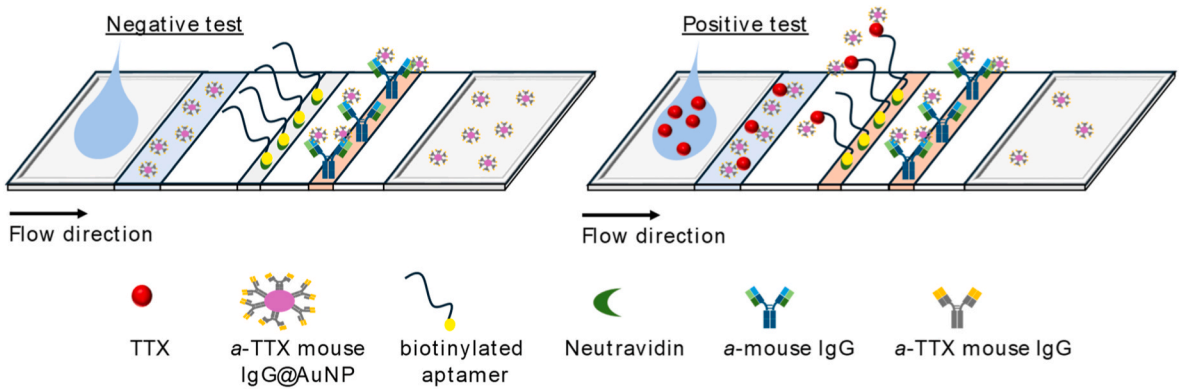


Fig. 10. Schematic representation of the aptamer–antibody hybrid LFA for TTX inspired by Ref. [31].

an immobilized aptamer on the membrane as the capture element (Fig. 10). In the presence of TTX, a sandwich complex forms at the test line between the antibody, the toxin, and the aptamer, generating a visible signal. The system was optimized for analytical parameters including sensitivity, specificity, stability, and reproducibility, and was applied to contaminated pufferfish extracts. Comparative analysis with established methods such as CBA and LC–MS/MS confirmed the accuracy and reliability of the approach.

5.2. Electrochemical systems

Electrochemical aptasensors have emerged as a powerful strategy for the detection of analytes, offering a combination of high sensitivity, operational simplicity, and strong potential for miniaturization. By transducing aptamer–target interactions into measurable electrical signals, these platforms enable quantitative, and often portable solutions. A diverse range of sensor architectures has been proposed, varying in electrode design, signal amplification strategies, and surface functionalization methods.

5.2.1. Amperometric/voltammetric biosensors

Amperometric/voltammetric biosensors detect changes in current arising from redox processes at the electrode surface, often mediated by electroactive probes. These systems are widely applied due to their sensitivity and quantitative readout [63]. A widely adopted approach involves the functionalization of aptamers with methylene blue (MB) as an electrochemical indicator. Hou et al. [64] developed an aptasensor for STX detection by modifying a gold electrode with a self-assembled monolayer of mercaptoacetic acid-functionalized multi-walled carbon nanotubes (MWCNTs). These nanotubes were used to immobilize the STX-specific aptamer and electrostatically anchor MB, which served as a redox probe. Upon STX binding, the aptamer undergoes a conformational change that hinders electron transfer between MB and the electrode surface, resulting in a concentration-dependent decrease in electrochemical signal obtained by differential pulse voltammetry (DPV). The system achieved an LOD of 0.38 nM and was validated in spiked mussel samples, demonstrating high recovery and analytical precision. Zheng et al. [65] implemented a similar strategy by immobilizing a MB-labeled STX-specific aptamer onto a bare gold electrode. However, in this case, they co-immobilized a short DNA strand to force MB molecules away from electrode surface, which restricted the electron transfer. In the presence of STX, the MB-aptamer-STX complex adopts a folded conformation, bringing MB closer to the electrode surface, thereby promoting electron transfer. This conformational switch results in an increased electrochemical peak by DPV, achieving an LOD of 1 nM in buffer. Although the sensor showed promising recovery rates (84–112 %) in spiked shellfish samples, further validation with broader real-world matrices would be necessary. In a related work, Li et al. [66] reported a highly sensitive electrochemical aptasensor for STX based on a gold nanopillar array electrode functionalized with MB-labeled aptamers. The sensing strategy was the same, but the ordered nanopillar structure significantly enhanced the electroactive surface area and facilitated even more the electron transfer, resulting in a remarkably low LOD of 0.001 nM. The sensor demonstrated excellent selectivity and was successfully validated in spiked shellfish samples, yielding recovery rates between 85 % and 112 %. Park et al. [67] developed a highly sensitive aptasensor by combining a round-type micro-gap electrode (RMGE) with porous platinum nanoparticles (pPtNPs), which enhanced both surface area and signal transduction. The detection relied on square wave voltammetry (SWV), and the biosensor achieved a very low LOD of 0.0156 nM in freshwater samples, demonstrating excellent sensitivity and selectivity. While the design offers remarkable analytical performance, the complexity of electrode fabrication and the requirement for precise calibration may limit its widespread adoption. Alternative approaches exploited DNA nanostructures to optimize aptamer orientation and target recognition. Qi et al. [68] combined DNA nanotetrahedra with aptamer–triplex motifs immobilized on screen-printed electrodes (Fig. 11). This spatially organized configuration promoted efficient aptamer orientation and reduced steric hindrance, facilitating high-affinity recognition of STX. The binding event was transduced into measurable current variations by SWV, generating clear response curves with good linearity. The aptasensor achieved an LOD of 0.92 nM in seawater, with excellent selectivity against other marine toxins and satisfactory recovery in spiked samples. However, its applicability to food matrices remains to be explored. Zeng et al. [69] developed a ratiometric aptasensor incorporating silver nanoparticles and potassium ferricyanide. The platform produces two differential pulse voltammetric signals, one from AgCl (≈0.13 V) and one from Prussian Blue (≈0.19 V) and uses their intensity ratio as the analytical metric. This dual-signal output improved stability and quantification accuracy, minimizing background interference. Validated in clams and shrimp, the sensor demonstrated robust reproducibility (RSD <10 %) and achieved an LOD of 1 nM.

5.2.2. Impedimetric biosensors

Impedimetric biosensors monitor changes in interfacial properties at the electrode surface, particularly charge transfer resistance (Rct), which is altered upon target binding. This label-free technique allows sensitive detection without the need for redox labels [70]. One of the first applications to TTX detection was described by Fomo et al. [71]. A glassy carbon electrode was coated with a conductive film of polyaniline doped with poly (4-styrenesulfonic acid) (PANI/PSSA) (Fig. 12). An

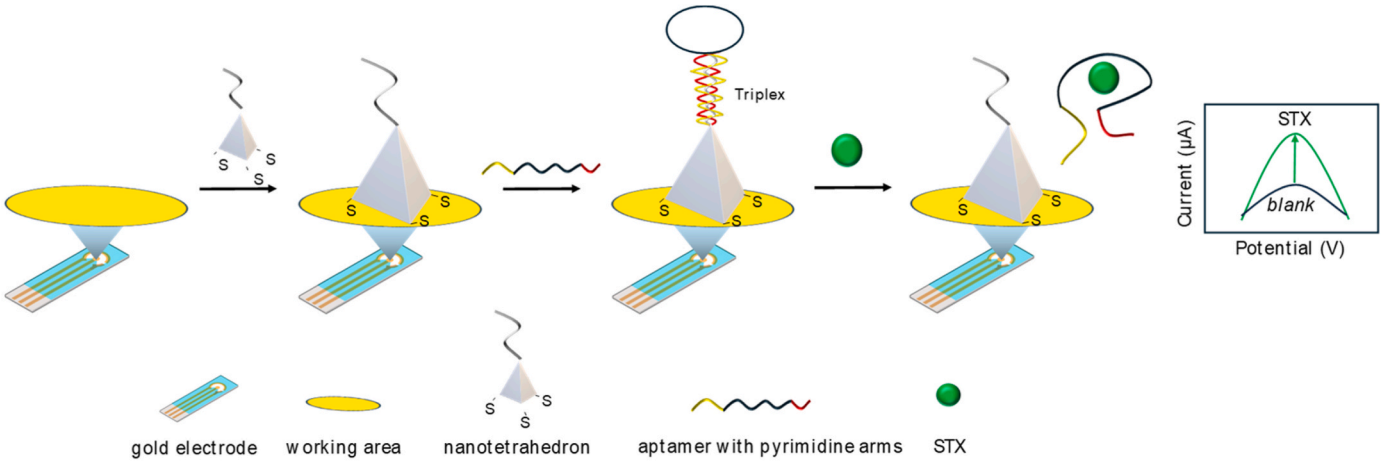


Fig. 11. Schematic representation of the aptamer–based voltammetric biosensor for STX inspired by Ref. [68].

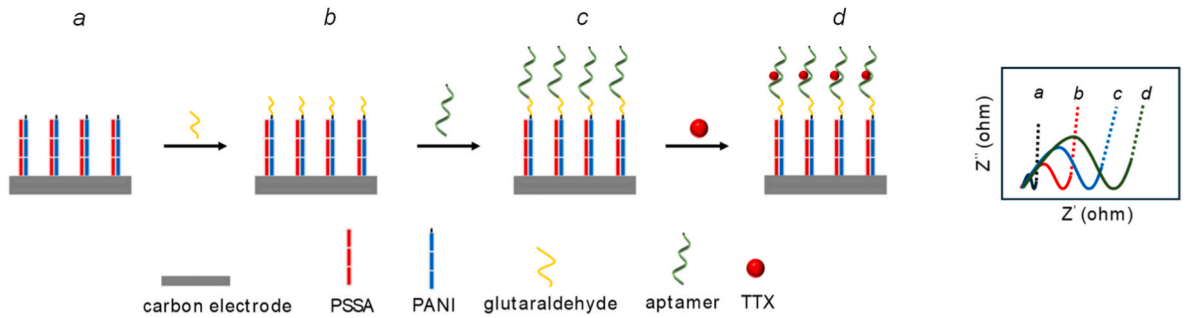


Fig. 12. Schematic representation of the aptamer–based impedimetric biosensor for TTX inspired by Ref. [71].

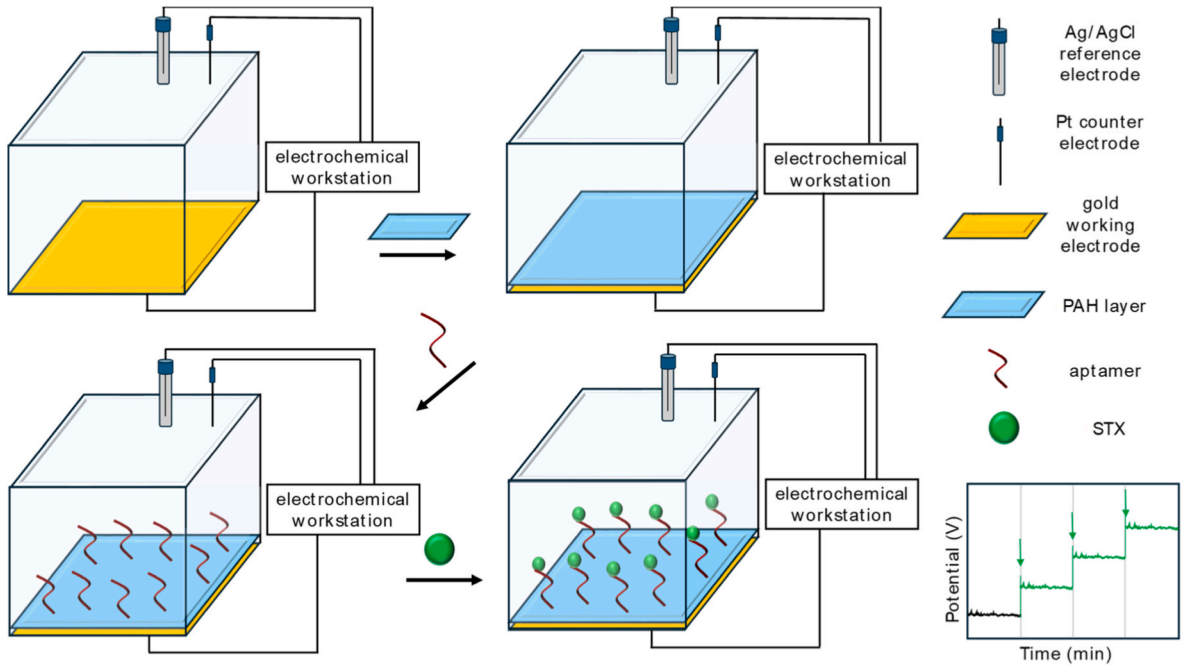


Fig. 13. Schematic representation of the aptamer–based potentiometric biosensor for STX inspired by Ref. [74].

amine terminated anti-TTX aptamer was then covalently attached to this layer via glutaraldehyde cross-linking. When TTX binds to the surface-immobilized aptamer, the local environment of the conductive polymer is altered, which hinders electron transfer between the electrode and the redox probe in solution. In the EIS spectrum, this appears as an increase in the charge-transfer resistance in the Nyquist plot. By monitoring how Rct changes with TTX concentration, the researchers built a calibration curve and achieved quantitative detection without the need for fluorescent or enzymatic labels. The sensor reached an LOD of 0.623 nM and had a dynamic linear range from 0.720 to 3.35 nM. This approach was later extended to STX detection. Serrano et al. [72] developed an aptasensor utilizing thiol-modified M-30f aptamers immobilized on a gold electrode. The sensor detected changes in Rct upon STX binding, achieving an LOD of 0.3 μg/L (1 nM). While the system demonstrated high selectivity and reproducibility in aqueous samples, its applicability to more complex matrices has yet to be investigated.

5.2.3. *Potentiometric biosensors*

Potentiometric biosensors measure variations in potential at the electrode–electrolyte interface generated by the aptamer–target interaction. This simple and cost-effective transduction mode provides a valuable alternative to amperometric/voltammetric and impedimetric platforms, although it may suffer from lower selectivity, sensitivity and reproducibility [73]. A representative example was reported by Noureen et al. [74], who introduced an electrolyte–insulator–semiconductor aptasensor for STX detection (Fig. 13). The device was modified with a positively charged poly(allylamine hydrochloride) (PAH) layer, which facilitated aptamer immobilization in a flat orientation within the Debye length, thereby reducing charge screening effects. Upon STX binding, aptamer conformational changes induced local charge redistribution, producing measurable shifts in capacitance–voltage curves and constant-capacitance responses. The sensor exhibited a linear response in the range of 0.5–100 nM with an LOD as low as 0.05 nM. Beyond sensitivity, the system showed strong selectivity against structurally related toxins, including dinophysistoxin, pectenotoxins, and yessotoxin. The platform also demonstrated good operational stability, maintaining consistent responses for up to nine days of storage. Importantly, its applicability to real matrices was validated in mussel extracts, achieving recovery rates between 91 % and 116 %.

5.3. *Comparative analytical performance of aptasensing systems*

It is challenging to make a well-founded comparison of the analytical performance of aptasensing systems for STX and TTX due to the wide diversity of designs reported, the fact that many of them remain at the proof-of-concept stage, and the frequent lack of comprehensive characterization and/or validation. Moreover, studies do not consistently

report the same analytical parameters, which further complicates direct comparison. Nevertheless, some general assumptions and overarching observations can still be drawn.

When comparing sensitivities, no clear performance advantage can be attributed to either optical or electrochemical platforms. The lowest LOD (10^{-5} nM) has been reported for the strategy exploiting AuNP aggregation [12], which is, in fact, a very simple colorimetric format. Other highly sensitive systems, which have attained LODs in the 10^{-3}–10^{-4} nM range, include signal enhancement strategies, such as DNA amplification [55] or nanostructured electrodes [66]. Regarding nanomaterials, also interesting are MOFs, which have been integrated into both colorimetry [49] and fluorescence [10,54,59] systems to take advantage of their intrinsic catalytic activity or fluorescence properties. Enzyme-assisted signal amplification has also been proved to decrease the LODs [53]. In contrast, certain spectrometric methods have yielded the highest LODs (>10 nM) [56,57], followed by LSPR-based systems (8 nM) [43]. Indeed, label-free formats may not provide sufficient sensitivity, but their main advantage lies in their simplicity.

In relation to selectivity, approaches that exploit structural switching of the aptamer, such as MB-labeled electrochemical probes [64,65] or fluorescence-based hairpin systems [52,53], generally yield strong discrimination due to binding-induced conformational changes. Ratiometric [10,54,58,69] or dual-signal [59,60] readouts, which provide two independent signal outputs, can further enhance selectivity. However, ratiometric approaches demand a stable internal reference and dual-signal formats require additional instrumentation.

Regarding robustness in front of matrix compounds, many systems perform well in buffer but show signal suppression or drift when analyzing complex matrices such as shellfish or pufferfish extracts. Systems relying on nanomaterial aggregation [12] or catalytic activity [54] tend to be more susceptible to non-specific signals due to changes in ionic strength. Nevertheless, the use of magnetic beads as supports have been demonstrated to remove, or at least mitigate, matrix effects [46,47,55,57].

Due to their similar mechanisms of action and potential co-occurrence, multiplex detection of STX and TTX may be desirable. However, only one example has been reported for the simultaneous detection of these two toxin families [10].

Finally, in terms of portability, despite the strong potential for miniaturization of electrochemical biosensors, it is clear that LFAs take the lead. These devices, particularly those validated with natural samples [31], have proven to be excellent diagnostic tools for on-site analysis, being extremely simple to use.

6. Challenges and future insights

6.1. Matrix-dependent performance variability

A critical factor influencing the practical deployment of aptamer-based biosensors for STX and TTX detection is their analytical performance across diverse sample matrices. Many scientific works developing aptasensing systems do not carry out full validations; they often perform only limited testing with a small number of real samples. However, comprehensive validation is essential, since while buffer-based assays often demonstrate exceptional sensitivity, their reliability frequently diminishes in complex biological or environmental samples due to matrix effects. In marine water, for instance, high ionic strength and the presence of divalent cations may interfere with aptamer folding or disrupt nanomaterial stability, particularly in AuNP-based colorimetric systems. Conversely, freshwater matrices may introduce organic contaminants or humic substances that compromise signal fidelity. Biological tissues such as pufferfish muscle, shellfish, or scallop tissue introduce further challenges, including protein fouling, enzymatic degradation of aptamers, and non-specific adsorption phenomena. There is still very limited truly mechanistic analysis of matrix effects, and their impact is usually inferred only from whether recovery rates and LODs remain within acceptable ranges. A systematic, side-by-side benchmarking of the same aptasensing system across different matrices is still lacking yet would be essential to identify which specific matrix components drive deviations in sensitivity, accuracy, precision, and selectivity.

Moreover, current mitigation strategies remain largely empirical and are mostly restricted to sample dilution, magnetic separation during washing steps, and “dual-signal” approaches. However, such enhancements sometimes increase assay complexity, reducing their suitability for rapid or decentralized deployment. Therefore, it is crucial to develop strategies that effectively minimize matrix effects while preserving the ease of use and operational simplicity of the developed biosensing systems and analytical procedures. Such approaches may focus on improving sample pretreatment through clean-up of extracts and/or preconcentration of target toxins (e.g. through centrifugation, filtration, and/or solid-phase extraction), or on enhancing the intrinsic sensitivity and robustness of the sensing platforms themselves. In this regard, not only the detection technique, but also the key features of the aptamer can be improved. The use of real-sample SELEX, including complex biological or environmental matrices in some selection rounds, could yield aptamers with higher resilience to matrix-induced interferences and able to operate under realistic conditions. Moreover, the intrinsic affinity and stability of an aptamer can be enhanced through strategies such as truncation, site-directed mutagenesis, point mutation, splitting, extension, structure-switching design, and exonuclease-assisted engineering. For instance, Zheng et al. [75] used rational site-directed mutagenesis and truncation to optimize a STX aptamer, obtaining a shorter, quadruplex-forming variant with enhanced structural stability and higher binding affinity. Finally, the use of standard addition or matrix-matched calibration could also provide more robust compensation for matrix-induced effects.

6.2. Technological accessibility and practical applicability

While many of the platforms reviewed exhibit ultralow LODs and high selectivity, their technological demands vary considerably, impacting real-world feasibility, particularly in resource-constrained settings. Colorimetric assays and LFAs offer excellent accessibility due to their visual readout, low cost, and minimal equipment requirements, making them suitable for on-site screening in low- and middle-income regions. In the case of LFAs, portable readers may also help in provided semi-quantifications besides the qualitative results after visual inspection. Fluorescence-based methods and spectroscopic techniques necessitate advanced optical instrumentation and skilled personnel, which may limit scalability in routine food safety and environmental applications. Electrochemical aptasensors, especially those employing nanostructured electrodes, provide a balance between sensitivity and portability but may still require specialized techniques for the fabrication of miniaturized devices and controlled operating conditions. In general, only a small number of studies report validation using natural samples and comparison with reference methods. Besides, these studies are scattered, highly heterogeneous, and often constrained to proof-of-concept evaluations. This scarcity underscores the gap between technological potential and practical readiness.

To accelerate the transition from laboratory prototypes to field-ready aptasensing systems, several complementary strategies can be pursued. The development of multiplexed detection platforms would allow monitoring of multiple toxins simultaneously, increasing analytical throughput. Microfluidics systems integrating sample clean-up could help manage complex matrices. Portable and low-cost instrumentation can facilitate decentralized analyses, expanding accessibility in resource-limited settings. Collectively, these innovations suggest a strong trajectory toward deployable aptasensing systems in the near future.

6.3. Compliance with regulatory thresholds

The analytical sensitivity of aptasensors must also be contextualized within the framework of established regulatory thresholds. For STX, both the European Commission and the United States Food and Drug Administration (USA FDA) prescribe a maximum tolerable level of approximately 800 μg/kg in shellfish [76]. For TTX, the European Food Safety Authority (EFSA) suggests safety thresholds of 44 μg/kg [77]. The majority of aptasensors discussed in this review demonstrate LODs ranging from as low as 10 fM to approximately 13 nM. This highlights their potential not only for compliance verification but also for early warning and preventative screening. Nonetheless, it should be noted that many of these LODs are reported under controlled conditions or spiked matrices. As mentioned above, exhaustive comprehensive validation in naturally contaminated real-world samples remains limited, raising concerns about inter-batch variability, matrix interference, and robustness under field conditions.

Broader validation campaigns, as well as harmonization and standardization protocols, are necessary before these technologies can be adopted for regulatory compliance or commercial use. For an aptasensing system to be used as a screening method, it has to be capable of rapidly classifying samples as suspect or compliant, with a very low false-negative rate at the decision limit. Single-laboratory validation is required, benchmarked against HPLC-FLD or LC-MS/MS. This validation should establish the relevant matrices and toxin levels corresponding to regulatory decision limits, and evaluate key performance parameters, including selectivity/specificity, sensitivity, LOD, cut-off, detection capability, trueness, recovery, precision, ruggedness/robustness, and stability. Following single-laboratory validation, a multi-laboratory validation should be conducted once a Standard Operating Procedure (SOP), including Quality Assurance/Quality Control (QA/QC) criteria, has been written and agreed upon. The method must comply with relevant regulatory and standard-setting frameworks, such as the European Commission, Codex Alimentarius, AOAC INTERNATIONAL, and the US FDA. Early engagement with regulatory authorities is essential, as their feedback will help ensure that the method meets expectations and improve the likelihood of eventual acceptance.

7. Conclusions

Aptamer-based biosensors offer a promising route for the rapid and sensitive detection of STX and TTX in food and environmental samples. Their adaptability to different transduction strategies and compatibility with nanomaterial-based enhancements have enabled analytical performance that can meet or surpass regulatory limits. However, challenges remain in translating these advances into robust, field-ready tools. Performance variability in complex matrices, limited large-scale validation, and the absence of standardized protocols continue to restrict widespread adoption. Future work should focus on integrating real-sample conditions into aptamer selection, improving sensor stability in variable environments, and streamlining fabrication processes. Addressing these aspects will be key to moving from proof-of-concept devices to reliable systems capable of supporting routine monitoring and safeguarding public health.

CRediT authorship contribution statement

Avazbek Abduvakhidov: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Mònica Campàs:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Abduvakhidov reports travel was provided by European Commission. Campas reports travel was provided by European Commission. If there are other authors, they 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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Data availability

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

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