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A Label-Free Colorimetric Aptasensor Containing DNA Triplex Molecular Switch and AuNP Nanozyme for Highly Sensitive Detection of Saxitoxin

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Abstract Saxitoxin (STX), one of the most toxic paralytic shellfish poisons discovered to date, is listed as a required item of aquatic product safety inspection worldwide. However, conventional detection methods for STX are limited by various issues, such as low sensitivity, complicated operations, and ethical considerations. In this study, an aptamer-triplex molecular switch (APT-TMS) and gold nanoparticle (AuNP) nanozyme were combined to develop a label-free colorimetric aptasensor for the rapid and highly sensitive detection of STX. An anti-STX aptamer designed with pyrimidine arms and a purine chain was fabricated to form an APT-TMS. Specific binding between the aptamer and STX triggered the opening of the switch, which causes the purine chains to adsorb onto the surface of the AuNPs and enhances the peroxidase-like activity of the AuNP nanozyme toward 3,3',5,5'-tetramethylbenzidine. Under optimized conditions, the proposed aptasensor showed high sensitivity and selectivity for STX, with a limit of detection of 335.6 pmol L^{-1} and a linear range of 0.59–150 nmol L^{-1}. Moreover, good recoveries of 82.70%–92.66% for shellfish and 88.97%–106.5% for seawater were obtained. The analysis could be completed within 1 h. The proposed design also offers a robust strategy to achieve detection of other marine toxin targets by altering the corresponding aptamers.

Key words saxitoxin; colorimetric aptasensor; aptamer; triplex molecular switch; AuNP nanozyme

1 Introduction

Shellfish toxins are toxic metabolites produced by harmful algae. These toxins can be easily accumulated in the aquatic food chain, including seawater, shellfish, and fish, and can cause serious pollution and health risks. According to their different symptoms and mechanisms of action, shellfish toxins can be classified as paralytic shellfish poisons (PSP), diarrheic shellfish poisons, amnesic shellfish poisons, neurotoxic shellfish poisons, and azaspiracid shellfish poisons (Oshima *et al*., 1987; Steidinger, 1993). Saxitoxin (STX) is characterized by extreme toxicity, and is one of the most toxic PSP biotoxins discovered to date. Its toxicity is 1000 times that of sodium cyanide and 80 times that of cobra venom. STX can cause paralytic shellfish poisoning and even death in severe cases (Oshima *et al*., 1987; Steidinger, 1993; Wiese *et al*., 2010). The median lethal dose (LD50) of STX to mouses is 10 μg kg^{-1}. Given its toxic properties, STX has been listed as a required item of aquatic product safety inspection worldwide (Chang *et al.*, 1997). The World Health Organization stipulates that the concentration of STX in edible parts of shellfish should not exceed 80 μg per 100 g (Fraga *et al.*, 2012). Several methods, such as mouse bioassay (Park *et al.*, 1986), high-performance liquid chromatography (Bates *et al.*, 1975; Turner *et al.*, 2009), and enzyme-linked immunoassay (Li *et al.*, 2020), are employed to detect STX. However, these methods are limited by a number of issues, such as time-consuming procedures, high costs, complicated operations, and ethical considerations (Bates *et al.*, 1975; Park *et al.*, 1986; Turner *et al.*, 2009; Li *et al.*, 2020). Therefore, the development of simple and rapid methods for the highly sensitive detection of STX is very important.

Aptamers are oligonucleotide molecules and are generated by *in vitro* screening through Systematic Evolution of Ligands by EXponential enrichment (SELEX). These molecules show high affinity and specificity toward their targets (Nutiu *et al.*, 2005), which include toxins (Moez *et al.*, 2019), pathogenic bacteria (Abdelrasoul *et al.*, 2020), antibiotics (Zhang *et al.*, 2020), and heavy metals (Tian *et al.*, 2017). The anti-STX aptamer was selected by Handy *et al.* (2013) and optimized to achieve higher affinity by Zheng *et al.* (2015); these studies lay an excellent foundation for the construction of novel aptasensors for STX detection

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(Wang *et al.*, 2020).

Colorimetric aptasensors represent a new research hotspot and show great potential in visual and high-throughput detection. AuNPs are promising nanomaterials widely used as convenient transducers to construct various colorimetric aptasensors on account of their size-dependent surface plasmon resonance properties and specific surface area (Storhoff *et al.*, 1998; Lee *et al*., 2008). The peroxidase-like catalytic ability of these materials enables their application as super nanozymes in biosensing assays (Gao *et al.*, 2007; Yang *et al.*, 2019). For instance, Yang *et al.* (2020) developed a colorimetric aptasensor based on the enhanced peroxidase-like activity of ssDNA-assisted AuNP nanozyme to catalyze 3,3',5,5'-tetramethylbenzidine (TMB) and achieved the highly sensitive and selective detection of aflatoxin B1.

The DNA triplex is specifically formed by a third homopurine or homopyrimidine DNA strand bonded to a DNA duplex (Wang *et al.*, 2015; Ni *et al.*, 2018; Qi *et al.*, 2020). This molecule can be combined with aptamers to fabricate an aptamer-triplex molecular switch (APT-TMS) (Gao *et al*., 2017; Wang *et al.*, 2019; Zhao *et al.*, 2019). In our previous study, an electrochemical aptasensor based on a DNA nanotetrahedron and APT-TMS was developed to realize the sensitive and rapid detection of STX (Qi *et al.*, 2020). In this work, APT-TMS showed excellent ability to improve the sensitivity of the aptasensor.

In this study, an aptamer, TMS, and AuNP nanozyme were combined to develop a label-free colorimetric aptasensor for the rapid and highly sensitive detection of STX. An anti-STX aptamer designed with two pyrimidine arms and a purine chain were fabricated to form APT-TMS. STX triggered the opening of the switch, and enhanced the catalytic properties of the peroxidase-like AuNP nanozyme corresponded to the release of the ssDNA purine chains.

2 Materials and Methods

2.1 Materials

Glacial acetic acid and chloroauric acid ($HAuCl_4$) were purchased from Sinopharm Chemical Reagent Co., Ltd. Hydrogen peroxide (H_2O_2, 30%) was purchased from Tianjin Dingshengxin Chemical Industry Co., Ltd. Sodium citrate tribasic dihydrate was purchased from Beijing Jinming Biotechnology Co., Ltd. TMB was purchased from Solarbio Science & Technology Co., Ltd. STX, neoSTX, gonyautoxin 1/4 (GTX 1/4), and okadaic acid (OA) were purchased from the National Research Council of Canada.

All oligonucleotide sequences were synthesized by Sangon Biotech Co., Ltd. The anti-STX aptamer was first selected by Handy *et al.* (2013) and then optimized to achieve higher affinity by Zheng *et al.* (2015). The oligonucleotides are as follows:

Purine chain: 5'-GAAGAAGAAGAA-3';

Aptamer with two pyrimidine arms: 5'-CTTCTTCTTCTT*TTGAGGGTCGCATCCCGTGGAAACAGGTTCATTG*TTCTTCTTCTTC-3';

Aptamer with one arm: 5'-CTTCTTCTTCTTT*TGAGGGTCGCATCCCGTGGAAACAGGTTCATTG*NNNNNNNNNNNNN-3'.

2.2 Instruments

UV-Vis absorption spectra were recorded with a UV-2550 spectrophotometer (Shimadzu, Japan) and Multiskan Sky microplate reader (Thermo Scientific, USA). Transmission electron microscopy (TEM) images were acquired by a JEM-2100 system (JEOL, Japan). AuNPs were concentrated using a 5425 centrifuge (Eppendorf, Germany).

2.3 Synthesis of AuNPs

Synthesis of the citrate-capped AuNPs was performed *via* the trisodium citrate reduction method. Briefly, 1 mL of 1% $HAuCl_4$ solution and 95 mL of ultrapure water were heated to boiling under vigorous magnetic stirring in a round-bottomed flask. Then, 4 mL of 1% sodium citrate solution was added to the flask under constant heating and stirring. The color of the solution gradually changed from colorless to wine red. The AuNP solution was heated for another 10 min, slowly cooled, and kept at 4℃ away from light. The resultant AuNPs were characterized by UV-Vis spectroscopy and TEM and then concentrated by centrifugation (12000 r min^{-1}, 10 min).

2.4 Formation of the Aptamer-Triplex Molecular Switch

APT-TMS was constructed according to our previously reported method (Qi *et al.*, 2020). First, the anti-STX aptamer was attached with two pyrimidine arms (hereafter denoted aptamer-pyrimidine arms). Next, 6 μL of 100 μmol L^{-1} aptamer-pyrimidine arms, 6 μL of 100 μmol L^{-1} purine chain, and 10.5 μL of ultrapure water were mixed in the buffer of 20 mmol L^{-1} NaCl+2.5 mmol L^{-1} $MgCl_2$ (pH 6.5). Finally, the mixture was heated at 95℃ for 5 min and then slowly cooled to 25℃ to allow the purine chains and aptamer-pyrimidine arms to form APT-TMS in the solution.

2.5 Feasibility Verification of the Colorimetric Aptasensor

The formation and opening of APT-TMS were verified. First, 100 μmol L^{-1} aptamer with two pyrimidine arms or aptamer with one arm, 100 μmol L^{-1} purine chain, and ultrapure water were mixed in the buffer. The mixture was heated at 95℃ for 5 min and then slowly cooled to 25℃ to form triple-strand (APT-TMS) and double-strand hybrid systems. Finally, 30 μL of the triple-strand and double-strand hybrid system mixtures were incubated with 70 μL of a TMB/H_2O_2 mixture. Pure AuNPs were used as a control. The absorption spectra of the mixtures at 500−750 nm were recorded.

The developed colorimetric aptasensor was characterized to confirm whether it was well developed and applicable to STX detection. Briefly, 1.5 μL of samples containing different concentrations of STX (*i.e*., 0, 37.5, 70, and 150 nmol L^{-1}) were mixed with 8.5 μL of APT-TMS. After 60 min of incubation, 50 μL of 10 nmol L^{-1} AuNPs and 45 μL

of ultrapure water were added to the assay solution. A solution without APT-TMS was mixed with the AuNPs as a control. After 15 min of incubation, 30 μL of the reaction solution was added to 70 μL of the TMB/H_2O_2 mixture. The absorption spectra of the mixtures at 500–750 nm were then recorded.

2.6 Optimization of the Key Parameters of the Developed Aptasensor

The concentrations of TMB and H_2O_2 and the pH of acetic acid were optimized. First, 20 nmol L^{-1} STX was incubated with the prepared APT-TMS for 60 min to allow STX to bind with the anti-STX aptamer on APT-TMS. Then, 5 μL of the STX and APT-TMS mixture were mixed with 50 μL of 10 nmol L^{-1} AuNPs and 45 μL of ultrapure water. After incubation for 15 min, 70 μL of the mixture was added to 30 μL of the above reaction solution containing a certain gradient concentration of TMB (*i.e.*, 0, 0.125, 0.25, 0.5, 1, and 2 mmol L^{-1}), a certain gradient concentration of H_2O_2 (*i.e.*, 0, 5%, 10%, 15%, and 20%), and acetic acid of a certain pH (*i.e.*, 2.0, 3.0, 4.0, 5.0, 6.0, and 7.0). The absorption spectra of the mixtures at 500–750 nm and their corresponding absorption values at 650 nm were recorded.

2.7 Detection of Saxitoxin Using the Constructed Aptasensor

APT-TMS was incubated with the buffer/sample containing a certain concentration of STX and then added with AuNPs to allow the released ssDNAs to adsorb onto the surface of the particles. The addition of TMB/H_2O_2 to the mixture generated different colorimetric signals under the catalysis of AuNP nanozyme. The absorption spectra of the mixtures at 500–750 nm were recorded. The corresponding absorbance at 650 nm was used to calculate the signal change rate with the formula $(A-A_0)/A_0 \times 100\%$. A means the absorbance with various concentration of STX; A_0 means the absorbance without STX.

2.8 Pretreatment of Shellfish and Seawater Samples

Pretreatment of scallop samples was conducted according to the method reported by Ramalingam *et al.* (2019). Scallop samples were purchased from a local supermarket. The meat was removed from the shells, and all edible parts were homogenized. Then, 250 mg of the homogenate was added to 1 mL of 50% methyl alcohol and vortexed for 5 min. The vortexed sample was centrifuged for 5 min at 4000 r min^{-1}, and the supernatant was collected and heated at 75℃ for 5 min. The vortexed sample was centrifuged once more for 5 min at 4000 r min^{-1}, and the supernatant was obtained and stored at −20℃ for further experiments. Seawater samples were centrifuged for 20 min at 5000 r min^{-1} and then passed through a 0.22 μm filter. The filtrates were collected and stored at 4℃ in the dark before further analysis.

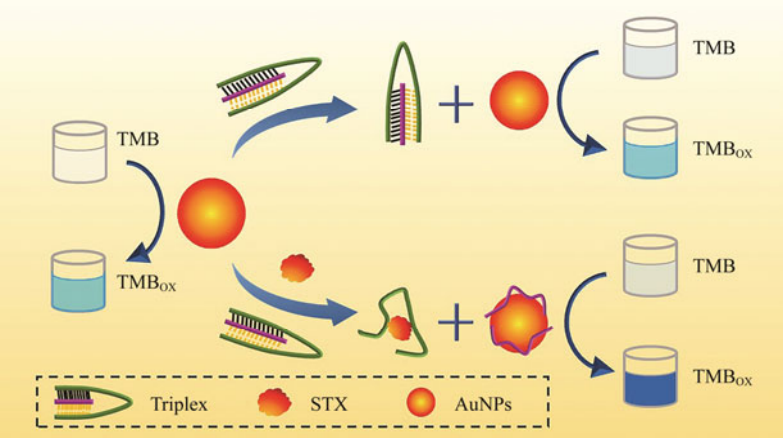
2.9 Statistical Analysis

The signal change rate (%) was calculated by the formula $(A-A_0)/A_0 \times 100\%$. Significant analysis was performed by the *t*-tests and analyzed by the *P* value.

3 Results and Discussion

3.1 Principle of the Colorimetric Aptasensor for Saxitoxin Detection

Scheme 1 presents the principle of the colorimetric aptasensor. Incubation of the aptamer containing pyrimidine arms at its two terminals with purine chains forms APT-TMS *via* Watson-Crick and Hoogsteen hydrogen bonds. In the presence of STX, the aptamer at the top of APT-TMS is specifically recognized by STX, which induces a conformational change and triggers the opening of the switch. The purine chains are then released and adsorbed onto the surface of the AuNPs. Adsorption of ssDNAs confers negative charges to the surface of the AuNPs and stabilizes the ·OH radicals of H_2O_2, thereby enhancing the electrostatic interaction between AuNPs and TMB. Thus, the peroxidase-like activity of the AuNP nanozyme is enhanced (Wang *et al.*, 2014; Hizir *et al.*, 2016; Yang *et al.*, 2020). This approach allows the quantitation of STX. The concentration of STX is positively correlated with the enhancement of the peroxidase-like activity of the nanozyme and increased absorbance of the solution. The analysis process could be completed within 1 h.



Scheme 1 Schematic illustration of the label-free colorimetric aptasensor containing a DNA triplex molecular switch and gold nanoparticle (AuNP) nanozyme for the highly sensitive detection of saxitoxin (STX).

3.2 Characterization of the AuNPs

The prepared citrate-capped AuNPs were characterized by UV-Vis spectroscopy and TEM. As shown in Fig.1(a), the synthesized AuNPs exhibited strong absorbance at 520 nm, as evidenced by the sharp absorption peak obtained at this wavelength. TEM revealed that the AuNPs are spherical in shape and dispersed evenly with an average size of approximately 13 nm. These results indicate the successful synthesis of the AuNPs. The concentration of the prepared AuNPs was measured using the UV-Vis spectroscopy at room temperature and calculated to be 3.28 nmol L^{-1} *via* the Lambert-Beer law ($\varepsilon = 2.4 \times 10^8$ L mol^{-1} cm^{-1}). The AuNPs were concentrated to a final concentration of 10 nmol L^{-1} for further experiments.

3.3 Verification of the Feasibility of the Colorimetric Aptasensor

The formation of the APT-TMS structure was verified. As shown in Fig.1(b), when the double-strand hybrid system (duplex) coexists with the AuNPs, free DNA terminals could be adsorbed onto the surface of the AuNPs to form nanozyme with enhanced catalytic performance. Obvious differences in the catalytic performance of the pure AuNPs and duplex-AuNPs may be observed. Our design aimed to form a triple-strand hybrid system (APT-TMS). In theory, APT-TMS cannot be adsorbed onto the surface of AuNPs, so no significant difference in the catalytic performance of the pure AuNPs and APT-TMS-AuNPs is expected. As shown in Fig.1(c), the catalytic performance of duplex-AuNPs was much higher than that of APT-TMS-AuNPs and AuNPs, thereby indicating that APT-TMS, rather than the duplex system, had been successfully formed.

The feasibility of the colorimetric aptasensor for STX detection was determined. As shown in Fig.1(d), the peroxidase-like activity of the AuNP nanozyme obviously increased, and gradient signal increments were observed as the concentration of STX increased. These findings demonstrate that the binding of STX to the aptamer disassembles APT-TMS and dramatically enhances the catalytic performance of AuNP nanozyme. The results confirm that the proposed aptasensor may be used for STX detection.

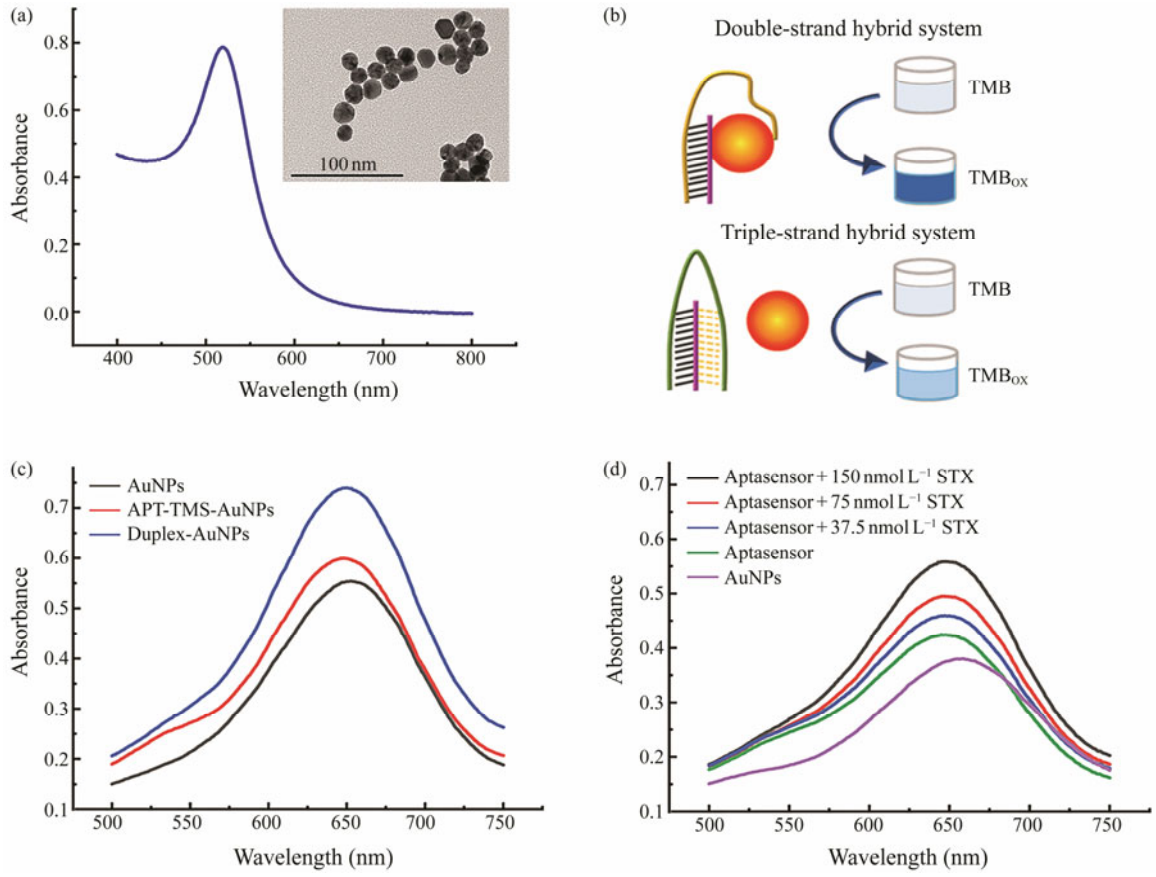


Fig.1 Characterization of AuNPs and feasibility verification of the proposed aptasensor. (a) UV-Vis spectrum and TEM imaging of the AuNPs; (b, c) Schematic illustration and characterization of the APT-TMS; (d) Feasibility verification of the colorimetric aptasensor towards STX.

3.4 Optimization of the Key Parameters of the Developed Aptasensor

Previous reports revealed that ssDNA/AuNP nanozyme show higher peroxidase-like activity than pure AuNPs and that the catalytic process between the AuNPs and TMB/H_2O_2 follows the Michaelis-Menten model (Liu *et al.*, 2014; Hizir *et al.*, 2016; Yang *et al.*, 2020). Thus, the optimal concentrations of TMB and H_2O_2 and pH of acetic acid were determined to obtain the ideal experimental conditions for

STX detection *via* the proposed aptasensor.

Figs.2(a, b) shows that the color intensity and absorbance of the test solution at 650 nm increased and then reached a plateau as the concentration of TMB increased to 1.0 mmol L^{-1}. Thus, 1.0 mmol L^{-1} TMB was selected for subsequent experiments. Figs.2(c, d) reveals that the color intensity and absorbance of the test solution at 650 nm increased and then reached a plateau as the concentration of H_2O_2 increased to 15%. Hence, 15% H_2O_2 was selected for further experiments. Finally, Figs.2(e, f) shows that the color intensity and absorbance of the test solution at 650 nm peaked when the pH of acetic acid was 4.0. This finding may be attributed to the inhibition of the transduction of H_2O_2 into H_2O and O_2 at lower or higher pH (Wang *et al.*, 2010; Wang *et al.*, 2017). Therefore, acetic acid with pH 4.0 was selected for the next experiments.

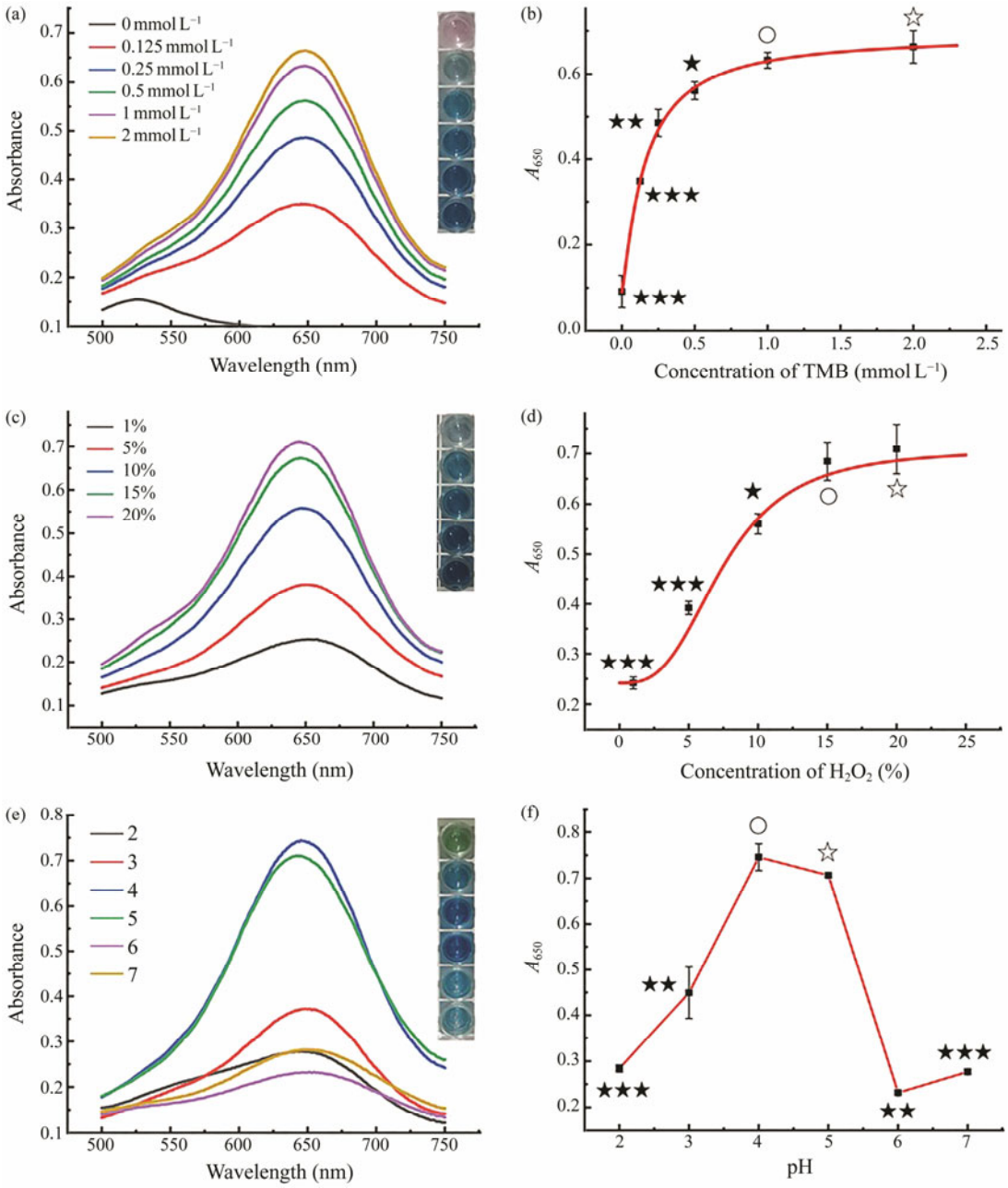


Fig.2 Optimization of key parameters of the developed aptasensor. (a, b) Optimization of the concentration of TMB; (c, d) Optimization of the concentration of H_2O_2; (e, f) Optimization of the pH values of acetic acid. Significance analysis was characterized by *P* value using two-tailed *t*-tests. The symbol '○' denotes control group, '☆' denotes $P>0.05$, '★' denotes $P<0.05$, '★★' denotes $P<0.01$, '★★★' denotes $P<0.001$.

3.5 Detection of Saxitoxin

Under the optimal conditions determined above, the colorimetric aptasensor based on APT-TMS and AuNP nanozyme was used for STX detection. As shown in Fig.3(a), increases in STX concentration caused the absorption peak at 650 nm to increase gradually. The signal change rates were calculated for quantification, and a good linear relationship with the logarithm of the STX concentration in the range of 0.59−150 nmol L^{-1} was obtained (Fig.3(b)). The li-

mit of detection (LOD) was calculated to be 335.6 pmol L^{-1}. As shown in Table 1, the LOD of the sensor proposed in this study is comparable with those of previously reported aptamer-based methods (Hou *et al.*, 2016; Gao *et al.*, 2017; Caglayan *et al.*, 2020; Qi *et al.* 2020; Qiang *et al.*, 2020) and much lower than those of other aptasensors, such as a temperature-assisted fluorescent aptasensor (Cheng *et al.*, 2018), an LSPR aptasensor (Ha *et al.*, 2019), and a SERS aptasensor (Cheng *et al.*, 2019). Indeed, the LOD achieved by the proposed sensor is much lower than the current standard for STX in shellfish (80 μg (100 g)$^{-1}$) (Fraga *et al.*, 2012).

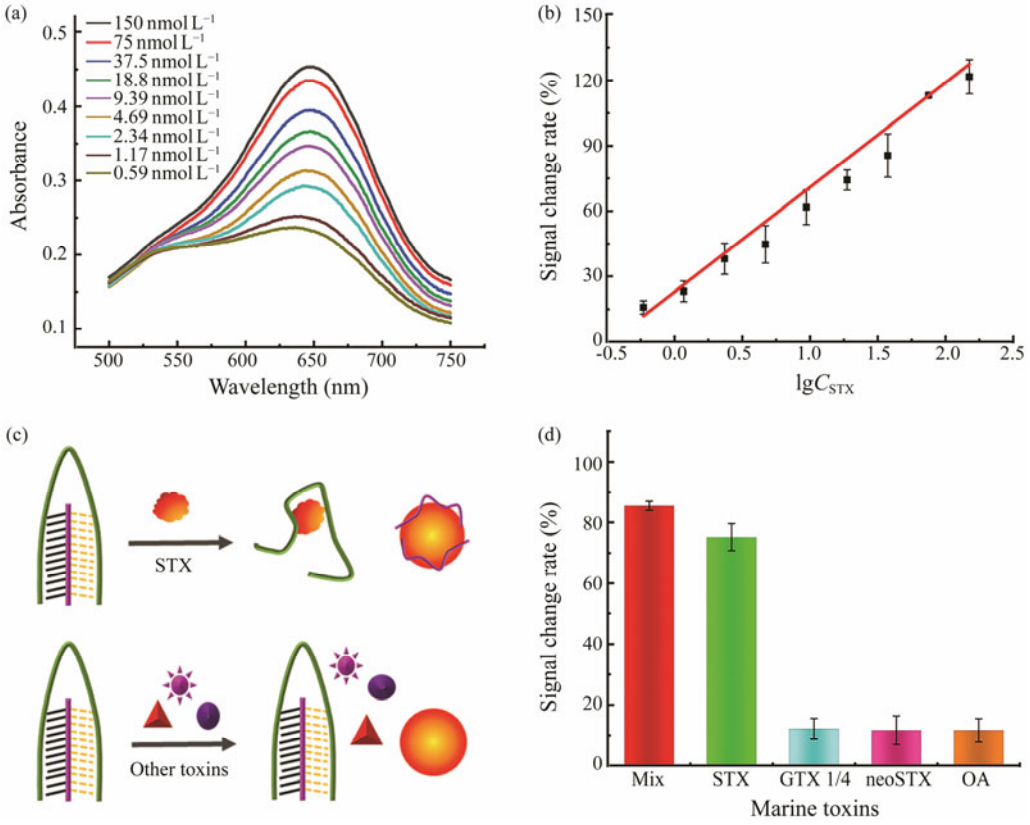


Fig.3 Detection of STX using the colorimetric aptasensor. (a) Absorption spectra with gradient concentrations of STX; (b) Analytical curves of the colorimetric aptasensor. C_{STX}, concentration of STX, unit: nmol L^{-1}. The linear equation is $y = 48.00x + 22.98$, $R^2 = 0.9919$; (c) Schematic illustration of the high selectivity of the colorimetric aptasensor; (d) Selective verification of the colorimetric aptasensor. GTX 1/4, gonyautoxin 1/4; neoSTX, neosaxitoxin; OA, okadaic acid.

Table 1 Comparison of aptasensors for STX detection reported in the literature

Methods	Linear range	Linear range (nmol L^{-1})†	LOD	LOD (nmol L^{-1})†	Reference
AuNPs-based colorimetric aptasensor	10 fmol L^{-1}–0.1 μmol L^{-1}	0.01–100	3 fg mL^{-1}	0.01	Qiang *et al.*, 2020
Temperature-assisted fluorescent aptasensor	0–24 ng mL^{-1}	0–80	1.8 ng mL^{-1}	6.0	Cheng *et al.*, 2018
Biolayer interferometry-based aptasensor	100–800 ng mL^{-1}	334–2676	0.5 ng mL^{-1}	1.67	Gao *et al.*, 2017
LSPR aptasensor	5–10000 μg L^{-1}	16.7–33445	2.46 μg L^{-1}	8.23	Ha *et al.*, 2019
SERS aptasensor	10–200 nmol L^{-1}	10–200	3.51 ng mL^{-1}	11.7	Cheng *et al.*, 2019
AIR-SE-based aptasensor	0.01–1000 nmol L^{-1}	0.01–1000	0.01–0.11 ng mL^{-1}	0.03, 0.37	Caglayan *et al.*, 2020
Amperometric aptasensor	0.9–30 nmol L^{-1}	0.9–30	0.38 nmol L^{-1}	0.38	Hou *et al.*, 2016
APT-TMS-based electrochemical aptasensor	2–400 nmol L^{-1}	2–400	0.92 nmol L^{-1}	0.92	Qi *et al.*, 2020
APT-TMS-based colorimetric aptasensor	0.59–150 nmol L^{-1}	0.59–150	335.6 pmol L^{-1}	0.3356	This work

Note: † Converted to nmol L^{-1} for easy comparison.

3.6 Analysis of Sensing Performance

STX and other shellfish toxins (*i.e.*, GTX 1/4, neoSTX, and OA) were analyzed using the constructed colorimetric aptasensor to determine the selectivity of the latter to STX. The selectivity of the aptasensor is mainly dependent on the selectivity of the anti-STX aptamer. In theory, the anti-STX aptamer can only bind with STX, which means other shellfish toxins should not cause the disassembly of TMS (Fig.3(c)). As shown in Fig.3(d), samples containing 20 nmol L^{-1} STX alone and a mixture of 20 nmol L^{-1} STX + 20 nmol L^{-1} GTX 1/4 + 20 nmol L^{-1} neoSTX + 20 nmol L^{-1} OA led to significant signal change rates; none of the other shellfish toxins resulted in obvious signal change rates, which demonstrates that the proposed colorimetric aptasensor possesses excellent selectivity for the discrimination of STX from other shellfish toxins.

The proposed aptasensor was applied to analyze STX in

shellfish and seawater samples to verify its accuracy toward STX in real samples. Pretreated STX-spiked shellfish and seawater samples with STX concentrations of 5, 20, and 60 nmol L^{-1} were analyzed using the developed colorimetric aptasensor. As shown in Table 2, the recoveries of STX in spiked shellfish and seawater samples were in the range of 82.70%–92.66% and 88.97%–106.5%, respectively, and with low RSDs. These values indicate that the aptasensor has good accuracy and repeatability and may also be used to detect STX in actual samples.

Table 2 Recovery studies of the colorimetric aptasensor in shellfish and seawater samples

Samples	Spiked (nmol L^{-1})	Analyzed (nmol L^{-1})	Recoveries (%)	RSD (%)
Shellfish	5	4.135 ± 0.07916	82.70	1.914
	20	17.92 ± 1.742	89.61	9.721
	60	55.60 ± 4.173	92.66	7.505
Seawater	5	4.449 ± 0.3404	88.97	7.651
	20	18.32 ± 1.489	91.61	8.128
	60	63.90 ± 3.064	106.5	4.795

4 Conclusions

In summary, the APT-TMS was successfully produced, and a label-free colorimetric aptasensor based on this switch and AuNP nanozyme was developed for the highly sensitive and selective detection of STX. APT-TMS demonstrated sensitive responses to STX. The specific binding between STX and the aptamer led to the opening of APT-TMS, and the released purine chains were adsorbed onto the surface of AuNPs to form the AuNP nanozyme. STX was quantified by measuring the colorimetric signals generated by the AuNP nanozyme. The developed colorimetric aptasensor demonstrated a good linear relationship between the signal responses and the logarithm of the STX concentration in the linear range of 0.59–150 nmol L^{-1}, a LOD of 335.6 pmol L^{-1}, good selectivity toward STX, and good practicability for analyzing STX in shellfish and seawater samples. The sensor showed good recoveries of 82.70%–92.66% for shellfish and 88.97%–106.5% for seawater. The simple analytical protocol offered by the developed aptasensor reflects its applicability to the high-throughput screening of multiple samples. The results of this study provide a new method for the development of aptasensors to monitor other targets *via* the alteration of aptamer sequences.

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References

Abdelrasoul, G. N., Anwar, A., Mackay, S., Tamura, M., Shah, M. A., Khasa, D. P., *et al*., 2020. DNA aptamer-based non-faradaic impedance biosensor for detecting *E. coli*. *Analytica Chimica Acta*, **1107**: 135-144, DOI: 10.1016/j.aca.2020.02.004.

Bates, H. A., and Rapoport, H., 1975. A chemical assay for saxitoxin, the paralytic shellfish poison. *Journal of Agricultural and Food Chemistry*, **23** (2): 237-239, DOI: 10.1021/jf60198a016.

Caglayan, M. O., and Stünda, Z., 2020. Saxitoxin aptasensor based on attenuated internal reflection ellipsometry for seafood. *Toxicon*, **187**: 255-261, DOI: 10.1016/j.toxicon.2020.09.005.

Chang, F. H., Anderson, D. M., Kulis, D. M., and Till, D. G., 1997. Toxin production of alexandrium minutum (Dinophyceae) from the bay of plenty, New Zealand. *Toxincon*, **35** (3): 393-409, DOI: 10.1016/S0041-0101(96)00168-7.

Cheng, S., Zheng, B., Yao, D., Kuai, S., Tian, J., Liang, H., *et al*., 2018. Study of the binding way between saxitoxin and its aptamer and a fluorescent aptasensor for detection of saxitoxin. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **204**: 180-187, DOI: 10.1016/j.saa.2018.06.036.

Cheng, S., Zheng, B., Yao, D., Wang, Y., Tian, J., Liu, L., *et al*., 2019. Determination of saxitoxin by aptamer-based surface-enhanced raman scattering. *Analytical Letters*, **52** (6): 902-918, DOI: 10.1080/00032719.2018.1505900.

Fraga, M., Vilariño, N., Louzao, M. C., Campbell, K., Elliott, C. T., Kawatsu, K., *et al*., 2012. Detection of paralytic shellfish toxins by a solid-phase inhibition immunoassay using a microsphere-flow cytometry system. *Analytical Chemistry*, **84** (10): 4350-4356, DOI: 10.1021/ac203449f.

Gao, L., Zhuang, J., Nie, L., Zhang, J., Zhang, Y., Gu, N., *et al*., 2007. Intrinsic peroxidase-like activity of ferromagnetic nanoparticles. *Nature Nanotechnology*, **2** (9): 577-583, DOI: 10.1038/nnano.2007.260.

Gao, S., Zheng, X., and Wu, J., 2017. A biolayer interferometry-based competitive biosensor for rapid and sensitive detection of saxitoxin. *Sensors and Actuators B: Chemical*, **246**: 169-174, DOI: 10.1016/j.snb.2017.02.078.

Gao, X., Li, H., Zhao, Y., and Jie, G., 2019. Triple-helix molecular switch-based versatile "off-on" electrochemiluminescence and fluorescence biosensing platform for ultrasensitive detection of lipopolysaccharide by multiple-amplification strategy. *Biosensors and Bioelectronics*, **143**: 111602, DOI: 10.1016/j.bios.2019.111602.

Ha, S. J., Park, J. H., Lee, B., and Kim, M. G., 2019. Label-free direct detection of saxitoxin based on a localized surface plasmon resonance aptasensor. *Toxins*, **11** (5): 274, DOI: 10.3390/toxins11050274.

Handy, S. M., Yakes, B. J., Dgrasse, J. A., Campbell, K., Elliott, C. T., Kanyuck, K. M., *et al*., 2013. First report of the use of a saxitoxin-protein conjugate to develop a DNA aptamer to a small molecule toxin. *Toxicon*, **61**: 30-37, DOI: 10.1016/j.toxicon.2012.10.015.

Hizir, M. S., Top, M., Balcioglu, M., Rana, M., Robertson, N. M., Shen, F., *et al*., 2016. Multiplexed activity of perAuxidase: DNA-capped AuNPs act as adjustable peroxidase. *Analytical Chemistry*, **88** (1): 600-605, DOI: 10.1021/acs.analchem.5b03926.

Hou, L., Jiang, L., Song, Y., Ding, Y., Zhang, J., Wu, X., *et al*., 2016. Amperometric aptasensor for saxitoxin using a gold electrode modified with carbon nanotubes on a self-assembled monolayer, and methylene blue as an electrochemical indicator probe. *Microchimica Acta*, **183**: 1971-1980, DOI: 10.1007/s00604-016-1836-1.

Lee, J. H., Wang, Z., Liu, J., and Lu, Y., 2008. Highly sensitive and selective colorimetric sensors for uranyl (UO_2^{2+}): Development and comparison of labeled and label-free DNAzyme-gold nanoparticle systems. *Journal of the American Chemical Society*, **130**: 14217-14226, DOI: 10.1021/ja803607z.

Li, J., and Persson, K. M., 2020. Quick detection method for paralytic shellfish toxins (PSTs) monitoring in freshwater. *Chemosphere*, **265**: 128591, DOI: 10.1016/j.chemosphere.2020.128591.

Liu, Y., Wang, C., Cai, N., Long, S., and Yu, F., 2014. Negatively charged gold nanoparticles as an intrinsic peroxidase mimic and their applications in the oxidation of dopamine. *Scientia Sinica*, **49**: 7143-7150, DOI: 10.1007/s10853-014-8422-x.

Moez, E., Noel, D., Brice, S., Benjamin, G., Pascaline, A., and Didier, M., 2019. Aptamer assisted ultrafiltration cleanup with high performance liquid chromatography-fluorescence detector for the determination of OTA in green coffee. *Food Chemistry*, **310**: 125851, DOI: 10.1016/j.foodchem.2019.125851.

Ni, Z., Ye, T., Yu, Y., Gao, L., Fei, Y., Li, Q., *et al*., 2018. Triplex-forming oligonucleotide as a lighting-up switch for a DNA abasic site-binding fluorescent ligand. *Journal of Luminescence*, **198**: 193-197, DOI: 10.1016/j.jlumin.2018.02.011.

Nutiu, R., and Li, Y., 2005. *In vitro* selection of structure-switching signaling aptamers. *Angewandte Chemie International Edition*, **117**: 1085-1089, DOI: 10.1002/anie.200461848.

Oshima, Y., Hasegawa, M., Yasumoto, T., Hallegraeff, G., and Blackburn, S., 1987. Dinoflagellate *Gymnodinium catenatum* as the source of paralytic shellfish toxins in tasmanian shellfish. *Toxicon*, **25** (10): 1105-1111, DOI: 10.1016/0041-0101(87)90267-4.

Park, D. L., Adams, W. N., Graham, S. L., and Jackson, R. C., 1986. Variability of mouse bioassay for determination of paralytic shellfish poisoning toxins. *Journal of Association of Official Analytical Chemists*, **69** (3): 547-550, DOI: 10.1007/BF02060843.

Qi, X., Yan, X., Zhao, L., Huang, Y., Wang, S., and Liang, X., 2020. A facile label-free electrochemical aptasensor constructed with nanotetrahedron and aptamer-triplex for sensitive detection of small molecule: Saxitoxin. *Journal of Electroanalytical Chemistry*, **858**: 113805, DOI: 10.1016/j.jelechem.2019.113805.

Qiang, L., Zhang, Y., Guo, X., and Gao, Y., 2020. A rapid and ultrasensitive colorimetric biosensor based on aptamer functionalized Au nanoparticles for detection of saxitoxin. *RSC Advances*, **10** (26): 15293-15298, DOI: 10.1039/D0RA01231A.

Ramalingam, S., Chand, R., Singh, C. B., and Singh, A., 2019. Phosphorene-gold nanocomposite based microfluidic aptasensor for the detection of okadaic acid. *Biosensors and Bioelectronics*, **135**: 14-21, DOI: 10.1016/j.bios.2019.03.056.

Steidinger, K. A., 1993. Some taxonomic and biologic aspects of toxic dinoflagellates. In: *Algal Toxins in Seafood and Drinking Water*. Falconer, I. R., ed., Academic Press, 1-28, DOI: 10.1016/B978-0-08-091811-2.50006-X.

Storhoff, J., Elghanian, R., Mucic, R. C., Mirkin, M. C. A., and Letsinger, R. L., 1998. One-pot colorimetric differentiation of polynucleotides with single base imperfections using gold nanoparticle probes. *Journal of the American Chemical Society*, **120**: 1959-1964, DOI: 10.1021/ja972332i.

Tian, J., Song, Y., Zhang, S., Liu, C., Jia, C., and Wang, M., 2017. Fe (III)-based metal-organic framework-derived core-shell nanostructure: Sensitive electrochemical platform for high trace determination of heavy metal ions. *Biosensors and Bioelectronics*, **94**: 358-364, DOI: 10.1016/j.bios.2017.03.014.

Tolle, F. T., Brandle, G. M., Matzner, D., and Mayer, G., 2015. A versatile approach towards nucleobase-modified aptamers. *Angewandte Chemie International Edition*, **54**: 10971-10974, DOI: 10.1002/anie.201503652.

Turner, A. D., Norton, D. M., Hatfield, R. G., Morris, S., Algoet, M., and Lees, D. N., 2009. Refinement and extension of AOAC Method 2005.06 to include additional toxins in mussels: Single-laboratory validation. *Journal of AOAC International*, **92** (1): 190-207, DOI: 10.1093/jaoac/92.1.190.

Wang, C., Chen, D., Wang, Q., and Tan, R., 2017. Kanamycin detection based on the catalytic ability enhancement of gold nanoparticles. *Biosensors and Bioelectronics*, **91**: 262-267, DOI: 10.1016/j.bios.2016.12.042.

Wang, J., Wang, Y., Rang, W., Xue, J., Zhou, B., Liu, L., *et al*., 2014. Colorimetric determination of 8-hydroxy-2'-deoxyguanosine using label-free aptamer and unmodified gold nanoparticles. *Microchimica Acta*, **181**: 903-910, DOI: 10.1007/s00604-014-1173-1.

Wang, N., Zhu, L., Wang, D., Wang, M., Lin, Z., and Tang, H., 2010. Sono-assisted preparation of highly-efficient peroxidase-like Fe_3O_4 magnetic nanoparticles for catalytic removal of organic pollutants with H_2O_2. *Ultrasonics Sonochemistry*, **17**: 526-533, DOI: 10.1016/j.ultsonch.2009.11.001.

Wang, Q., Yang, Q., and Wu, W., 2020. Ensuring seafood safe to spoon: A brief review of biosensors for marine biotoxin monitoring. *Critical Reviews in Food Science and Nutrition*, **3**: 1-13, DOI: 10.1080/10408398.2020.1854170.

Wang, X., Jiang, A., Hou, T., and Li, F., 2015. A versatile label-free and signal-on electrochemical biosensing platform based on triplex-forming oligonucleotide probe. *Analytica Chimica Acta*, **890**: 91-97, DOI: 10.1016/j.aca.2015.06.059.

Wang, Y., Yao, L., Ning, G., Wu, Y., Wu, S., Mao, S., *et al*., 2019. An electrochemical strategy for tetracycline detection coupled triple helix aptamer probe with catalyzed hairpin assembly signal amplification. *Biosensors and Bioelectronics*, **143**: 111613, DOI: 10.1016/j.bios.2019.111613.

Wiese, M., D'Agostino, P. M., Mihali, T. K., Moffitt, M. C., and Neilan, B. A., 2010. Neurotoxic alkaloids: Saxitoxin and its analogs. *Marine Drugs*, **8** (7): 2185-2211, DOI: 10.3390/md8072185.

Yang, T., Luo, Z., Tian, Y., Qian, C., and Duan, Y., 2019. Design strategies of AuNPs-based nucleic acid colorimetric biosensors. *Trends in Analytical Chemistry*, **124**: 115795, DOI: 10.1016/j.trac.2019.115795.

Yang, Y., Yin, Y., Li, X., Wang, S., and Dong, Y., 2020. Development of a chimeric aptamer and an AuNPs aptasensor for highly sensitive and specific identification of Aflatoxin B1. *Sensors and Actuators: B. Chemical*, **319**: 128250, DOI: 10.1016/j.snb.2020.128250.

Zhang, Y., Du, X., Deng, S., Li, C., and Deng, R., 2020. Dual triple helix-aptamer probes for mix-and-read detecting antibiotics in fish and milk. *Journal of Agricultural and Food Chemistry*, **68** (35): 9524-9529, DOI: 10.1021/acs.jafc.0c03801.

Zhao, L., Qi, X., Yan, X., Huang, Y., Liang, X., Zhang, L., *et al*., 2019. Engineering aptamer with enhanced affinity by triple helix-based terminal fixation. *Journal of the American Chemical Society*, **141** (44): 17493-17497, DOI: 10.1021/jacs.9b09292.

Zheng, X., Hu, B., Gao, S. X., Liu, D. J., Sun, M. J., Jiao, B. H., *et al*., 2015. A saxitoxin-binding aptamer with higher affinity and inhibitory activity optimized by rational site-directed mutagenesis and truncation. *Toxicon*, **101**: 41-47, DOI: 10.1016/j.toxicon.2015.04.017.

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