Supporting Information

A Gold Nanoparticle-Based Lateral Flow Immunosensor for Ultrasensitive Detection of Tetrodotoxin

Yue Li[a,b], Xinxin Xu[a,b], Liqiang Liu[a,b*], Hua Kuang[a,b], Liguang Xu[a,b], Chuanlai Xu[a,b*]

[a]*State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, People's Republic of China;* [b]*International Joint Research Laboratory for Biointerface and Biodetection, and School of Food Science and Technology, Jiangnan University, Wuxi, People's Republic of China;*

[*]Corresponding author. Email: murel@163.com; xcl@jiangnan.edu.cn;

Methods

Preparation of antigens

The immunogen (TTX-KLH) was prepared using a modified formaldehyde method[1]. Briefly, 1 mg TTX in 200 μL of acid methanol was coupled to 4 mg KLH in 1 mL of 0.05 M carbonate buffer (CB, pH 9.6) in the presence of 72 μL 37% formaldehyde. After stirring at 37℃ for 4 d, the reaction mixture was dialyzed against 0.01 M phosphate buffer solution (PBS, pH 7.4). The coating antigen (TTX-BSA) was synthesized in the same way.

Production of antibodies against TTX

Animal studies in this work were performed according to institutional ethical guidelines and approved by the Committee on Animal Welfare of Jiangnan University. Female BALB/c mice (6–8 weeks old, purchased from Qinglongshan Laboratory Animal Co., Ltd. Nanjing, China) were immunized subcutaneously with 40 μg/mouse of TTX-KLH emulsified with an equal volume of complete Freund's adjuvant. One month later, booster immunizations were administered with 20 μg/mouse of TTX-KLH emulsified

with an equal volume of incomplete Freund's adjuvant at three weeks intervals. The titer and sensitivity of the serum were measured by ic-ELISA. The most responsive mouse was given an intraperitoneal injection of 10 μg TTX-KLH in 100 μL normal saline and sacrificed for cell fusion. The spleen cells were fused with SP2/0 in the presence of 1 mL PEG 1500. After three rounds of subcloning using the limiting dilution method, the obtained hybridoma was expanded in BALB/c mice[2]. Finally, the mAbs were purified using Protein-G affinity chromatography according to the manufacturer's instructions. Animal studies were performed in accordance with institutional ethical guidelines and approved by the Committee on Animal Welfare of Jiangnan University.

Ic-ELISA procedure

The ic-ELISA was performed by following a previously-reported protocol[3]. Typically, 100 μL of 0.1 μg mL^{-1} TTX-BSA in 0.05 M CB (pH 9.6) was added to 96-well microplates and incubated for 2 h at 37°C to coat the wells. The coated plates were washed three times with 0.01 M PBS containing 0.05% (v/v) Tween-20 (PBST, pH 7.4) to remove excess unbound TTX-BSA. The washed plates were then blocked with 200 μL/well of gelatin blocking buffer including 0.2% (m/v) gelatin in CB (pH 9.6) for 2 h at 37°C. After washing three times, the TTX-BSA-coated plates were incubated with 50 μL/well of various concentrations of TTX standard solution in PBS and 50 μL/well of appropriate diluted antibody in PBS containing 0.1% (m/v) gelatin (PBSG) for 30 min at 37°C. After washing again, 100 μL/well of HRP-labeled goat anti-mouse IgG diluted 1:5,000 in PBSG was added and the plate was then incubated for a further 30

min at 37°C. After four washes with PBST, an enzyme-catalyzed reaction was carried out by adding 100 μL/well of fresh substrate solution (citrate buffer (100 mL of 0.1 M citrate phosphate buffer containing 18 μL of 30% H_2O_2, pH 5.0) and TMB solution (glycol containing 0.06 % (m/v) TMB) with a ratio of 5/1) and stopped after a 15 min incubation at 37°C by addition of 50 μL/well of 2 M sulfuric acid. The absorbance values at 450 nm were measured using a microplate reader. All experiments were performed in triplicate.

Synthesis of GNPs

GNPs were synthesized using trisodium citrate reduction of $HAuCl_4$ in water[4, 5]. Briefly, 2 mL of freshly-prepared trisodium citrate solution (10 g L^{-1}) was rapidly added to a boiling $HAuCl_4$ solution (100 mL, 0.25 mM) under vigorous stirring. In a few minutes, the color of the reaction solution changed from blue to wine-red. After that, the solution was kept boiling for a further 5 min and then allowed to cool to room temperature and stored at 4°C before use. The morphology and size of GNPs was determined by TEM.

LC-MS/MS analysis

Five grams of sample homogenate were mixed with 11 mL of 1% acetic acid-methanol solution (v/v) and stirred for 2 min. Following ultrasonic extraction in a 50°C water bath for 15 min, the sample was centrifuged at 8,000 × *g* for 5 min and the supernatant was collected. The residue was reextracted with a further 11 mL of 1% acetic acid-methanol. The supernatants were combined and made up to 25 mL with 1% acetic acid–methanol. After freezing at -20°C for 30 min, the extract was then centrifuged at 8,000

×*g* for 5 min, then 5 mL of supernatant was diluted into 20 mL PBS, followed by adjusting the pH to 7–8 using sodium hydroxide solution. Subsequently, the extract was purified using an immunoaffinity column. TTX was eluted from the cartridge with 5 mL of 2% acetic acid-methanol and blown dry. The residue was dissolved in 1 mL of formic acid solution (0.1%)–acetonitrile solution (1+1) and filtered through a 0.22 μm nylon filter prior to injection into LC-MS/MS. Separation was performed based on gradient elution using 5 mmol/L ammonium acetate containing 0.01% acetic acid (v/v) (mobile phase A) and acetonitrile (mobile phase B). Multiple reaction monitoring was carried out with positive ion mode at m/z 320 > 162 and m/z 320 > 302 (precursor ion > production).

Caption:

Fig. S1 Chemical structure of TTX.

Fig. S2 Crucian and clam samples were verified as TTX-negative by LC-MS/MS.

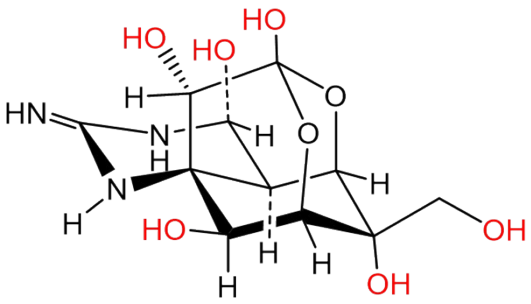
Fig. S3 Optimization of pH (8.4 and 5.3) for GNP-antibody probe preparation. Evaluation of GNP-mAb probes were performed by testing TTX in PBS using immunosensor strip (T1 and T2 represent the T line antigen concentrations at 0.2 and 0.5mg mL^{-1}, respectively. 1 and 2 represent the TTX standard concentrations at 0 and 5 ng mL^{-1}, respectively). Each test was repeated thrice.

Fig. S4 Optimization of surfactants in suspension buffer. S1, S2, S4, S11, S12, S13, S14, and S15 represent suspension buffer respectively containing PVP, PEG, BSA, Tween-20, Brij 35, Triton X-100, Rhodasurf®, and CAP. **(A)** Determination of TTX in PBS (1 and 2 represent the TTX standard concentrations at 0 and 5 ng mL^{-1}, respectively). **(B)** Determination of TTX in matrix (1 and 2 represent the TTX standard concentrations at 0 and 10 ng mL^{-1}, respectively). Each test was repeated thrice.

Fig. S5 Evaluation of antigen concentration of T line by testing TTX standard in blank matrix (T1 and T2 represent the T line antigen concentrations at 0.5 and 1 mg mL^{-1}, respectively. 1 and 2 represent the TTX standard concentrations at 0 and 10 ng mL^{-1}, respectively). Each test was repeated thrice.

Fig. S6 The cross-reactivity of the immunosensor assay (1, 2, 3, and 4 represent the blank, STX, PTX, and NEO, respectively). Each test was repeated thrice.

Fig. S7 TTX analysis by LC-MS/MS.



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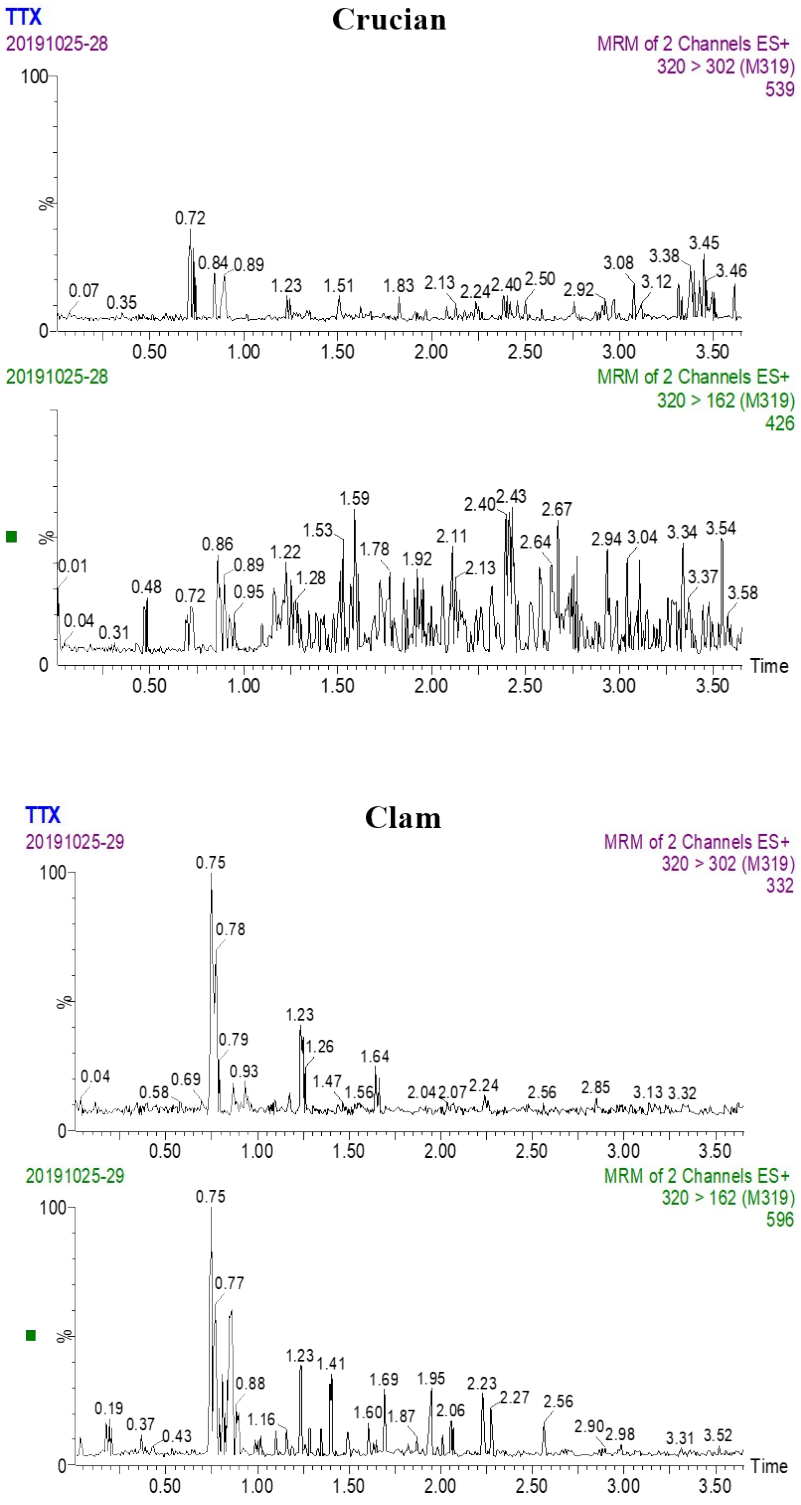

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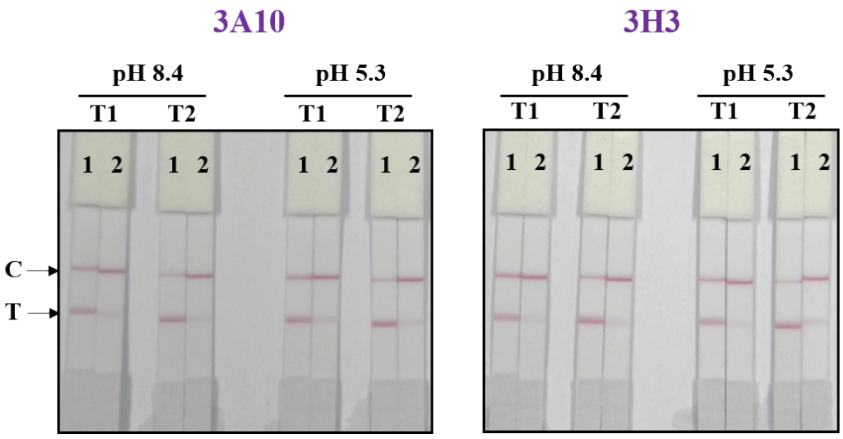

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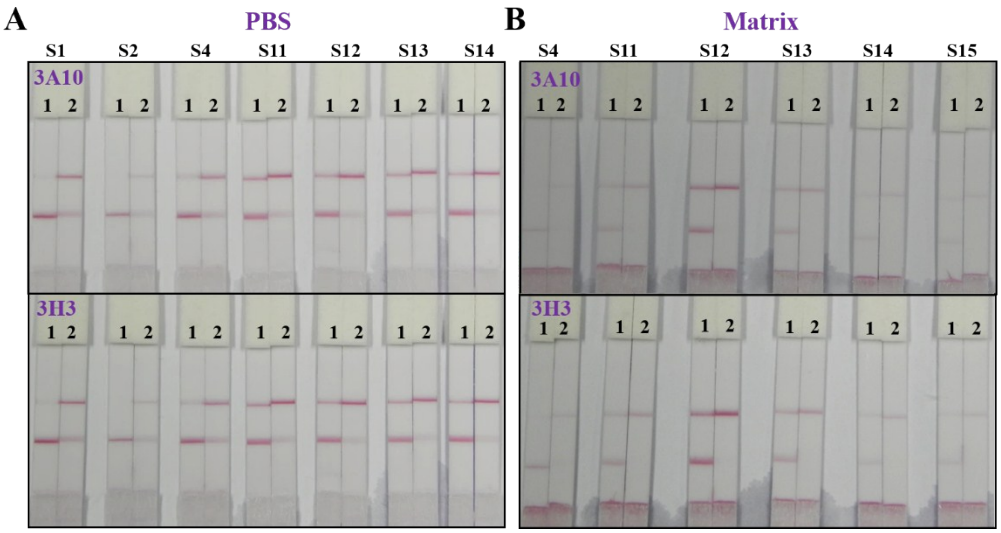

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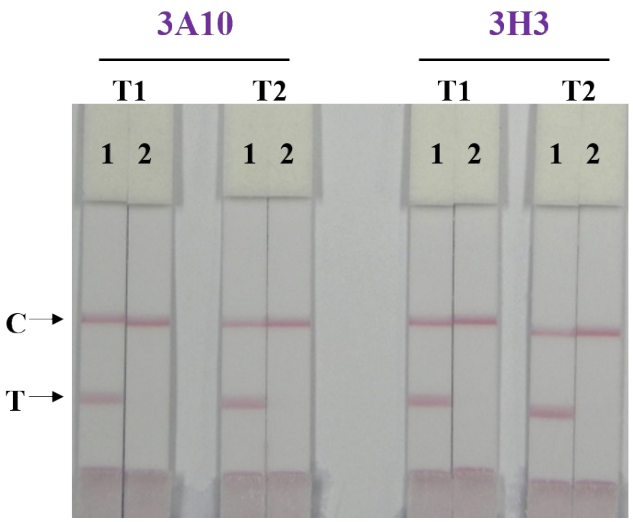

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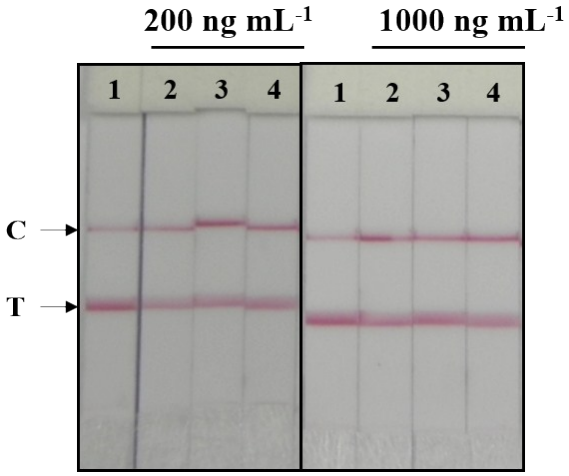

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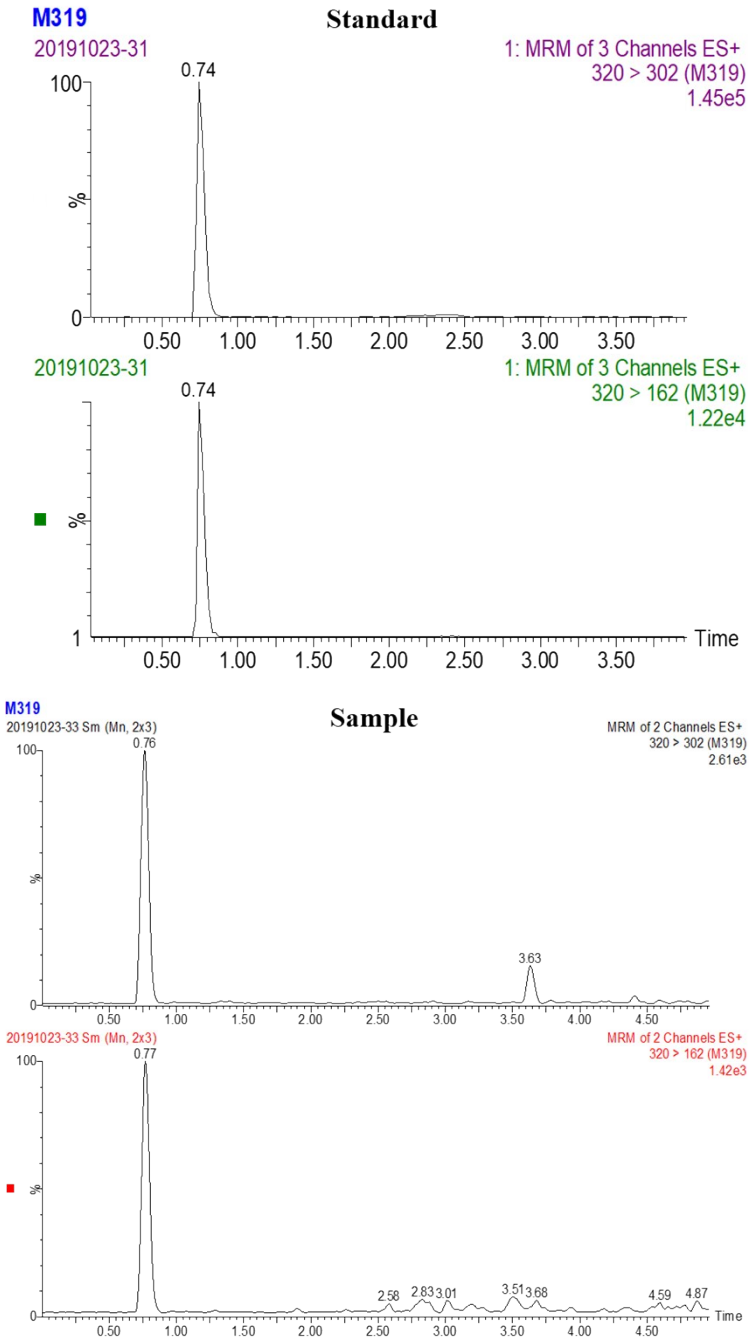

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Reference:

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