A robust colorimetric aptasensor for label-free detection of marine toxins based on tyrosine-capped gold nanoparticles

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1. Reagent and setup

Sodium chloride (NaCl), $HAuCl_4 \cdot 4H_2O$ (>99%), KOH, sodium citrate, L-tyrosine, absolute ethanol were procured from Aladdin (Shanghai, China). The sequence for PbTx-2 aptamer BT10 (5'-GGC CAC CAA ACC ACA CCG TCG CAA CCG CGA GAA CCG AAG TAG TGA TCA TGT CCC TGC GTG-3') and the sequence for OA aptamer OA34 (5'-GGT CAC CAA CAA CAG GGA GCG CTA CGC GAA GGG TCA ATG TGA CGT CAT GCG GAT GTG TGG-3') were obtained from previous studies [1, 2] and then synthesized by Sangon Biotech (Shanghai, China). The PbTx-2 solid was dissolved in ethanol to obtain a stock solution with a concentration of 100 μM for further use. All aqueous solutions used in this work were prepared with ultrapure water (MilliQ, Millipore).

Transmission Electron Microscope (TEM, HT-7700, Japan) was employed to observe the shape and the disperse status of AuNPs. A multi-mode microplate reader (SpectraMax Paradigm, Molecular Devices, USA) was applied to record the ultraviolet-visible (UV-vis) absorption spectra of AuNPs.

2. Synthesis of Cit-AuNPs and Tyr-AuNPs

Tyr-AuNPs were synthesized according to Pabudi's work that tyrosine was used as a reducing and capping agent [3]. In detail, 6 mg L-tyrosine and 17 mg KOH were weighed and then added

into a 300 mL aqueous solution followed by heated to boiling. Under vigorous boiling conditions, 2 mL $HAuCl_4$ solution with a mass fraction of 1% was added into the above solution with continuous heating for a further 20 min. The obtained wine red solution was cooled to room temperature and further kept at 4℃ for future use. As for Cit-AuNPs, the detailed synthesis method can be seen in our previous work [4]. Briefly, 100 mL of 0.01% $HAuCl_4$ solution was added to a round-bottom flask and then heated to boiling condition. Then, 4 mL of 1% sodium citrate solution was added rapidly to the above solution and further heated for 15 minutes. The obtained solution was also cooled to room temperature and further kept at 4℃ for future use.

3. Zeta potential and ITC measurement

3.1 Zeta potential measurement

Table S1. Zeta potential of Cit-AuNPs and Tyr-AuNPs

Type	Zeta Potential
Cit-AuNPs	-33.9 mV
Tyr-AuNPs	-39.0 mV

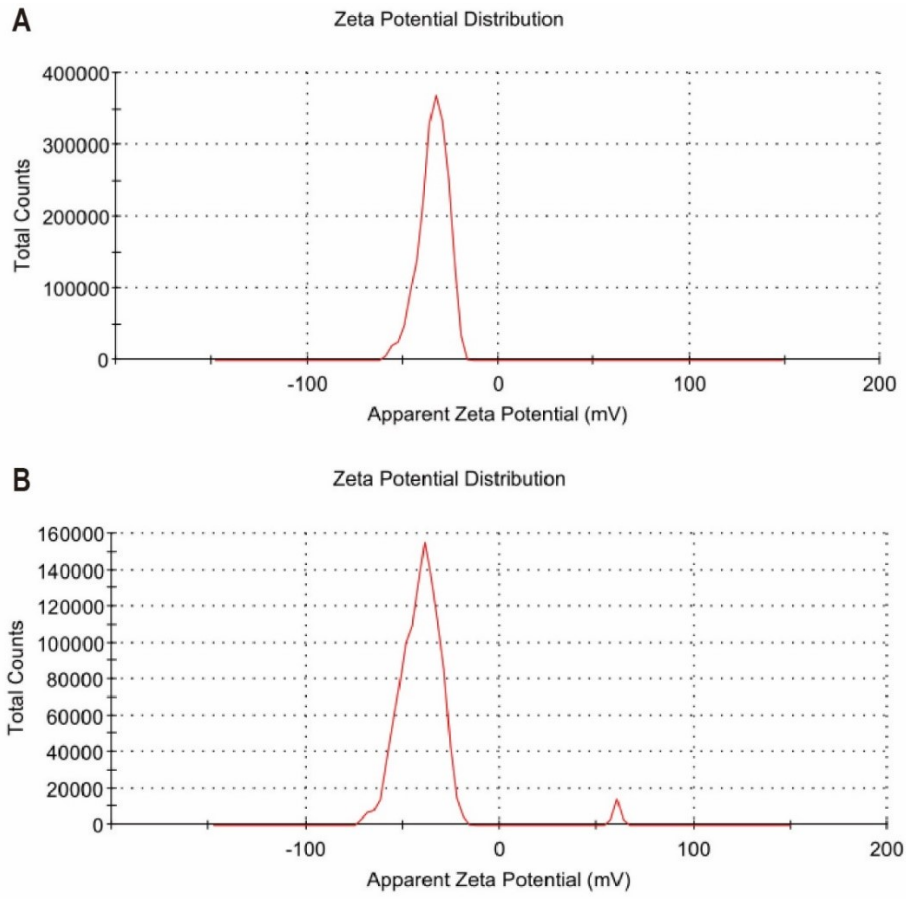

Fig. S1 The zeta potential distribution of Cit-AuNPs (A) and Tyr-AuNPs (B).

3.2 ITC measurement

The PbTx-2 samples were diluted to a concentration of 10 μM using pure water and loaded into the ITC syringe. Cit-AuNPs and Tyr-AuNPs solutions were centrifuged (14000 rpm, 20 min) to remove excess citrate acid or tyrosine acid. The obtained precipitates were dissolved in the buffer (pure water containing 10% v/v ethanol, matching the buffer of PbTx-2) to a concentration of around 10 nM and loaded into the sample cell. The volume of each injection was 4 μL except for the first injection which was 2 μL and the spacing between each titration was 180 s.

4. Optimization of the sensing conditions of OA detection

As for OA detection, several parameters including the concentration of the aptamer (OA34), the concentration of NaCl, and the time of aggregation were all optimized to obtain the best performance of the detection system. As shown in Fig. S2-A, the ability to protect AuNPs from aggregation is stronger as the concentration of OA34 increases. To ensure the highest sensitivity for PbTx-2 detection, $A_{650\,nm}$ / $A_{520\,nm}$ was used to evaluate the degree of aggregation. As shown in Fig. S2-B, the ratio changes slowly when the concentration of OA34 is over 2 μM indicating the decreasing sensitivity when the concentration is over 2 μM. Therefore, 2 μM was chosen as the optimal aptamer concentration. Concentrations of NaCl were optimized to evaluate its effect on the aggregation of AuNPs with 0 μM and 0.3 μM BT10. As shown in Fig. S2-C, with the NaCl concentrations increasing from 2% (w/w) to 6% (w/w), the DOR increases gradually and then decreases when the concentration reaches 3.6% (w/w). Therefore, 3.6% (w/w) NaCl was chosen as the best concentration to realize the largest difference. The effect of time after adding NaCl was also investigated and the spectra was recorded every 1 minute and the DOF was also calculated. As shown in Fig. S2-D, the DOR increases in the range of 0-10 min and then decreases. Considering rapid detection, 10 min was chosen as the best time.

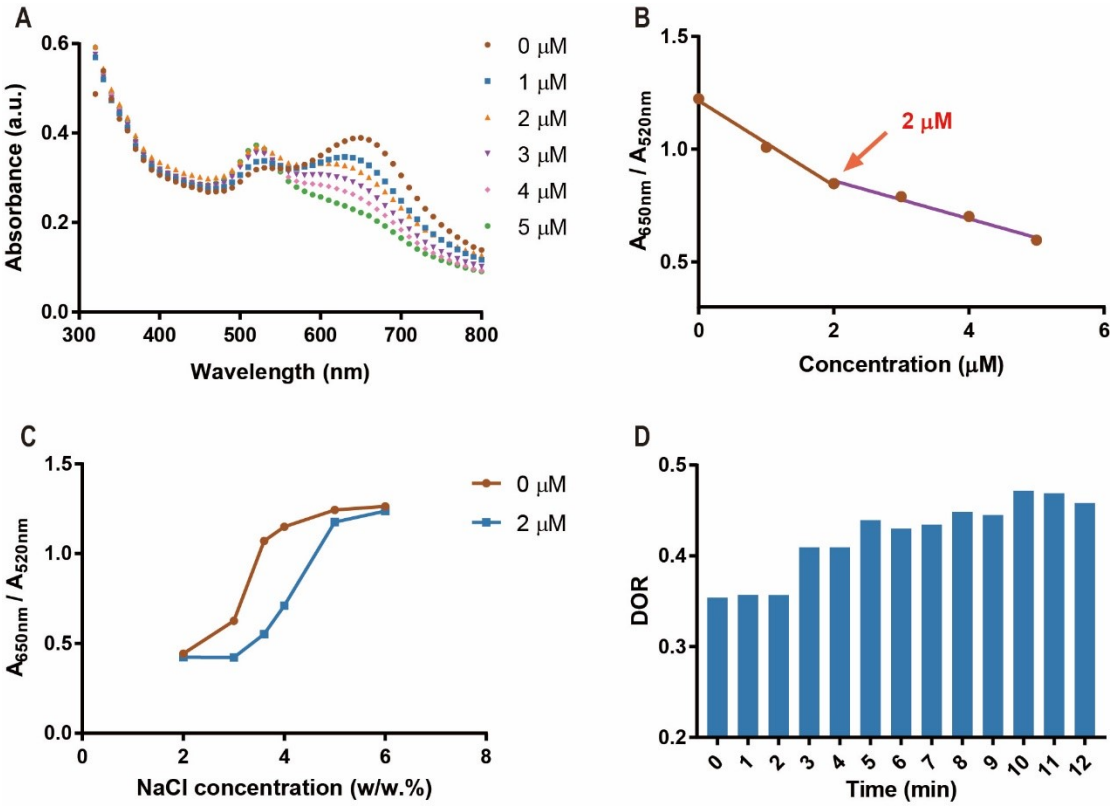

Fig. S2 (A) The spectra of AuNPs under different concentrations of OA34 after adding 10 μL 10% NaCl. (B) The $A_{650\ nm}$ / $A_{520\ nm}$ of AuNPs under different concentrations of OA34. (C) The $A_{650\ nm}$ / $A_{520\ nm}$ of AuNPs with 0 μM and 2 μM OA34 under different concentrations of NaCl. (D) Effect of time after adding NaCl on the values of DOR.

5. Comparison with other work

Table S2. The comparison of other methods for marine toxins detection

Analyte	Detection method	Label type	LOD (ppb)	Linear range (ppb)	Simplicity	Ref.
PbTx-2	Cardiomyocyte-based potential sensor	-	1.55	1.40-358.04	No	[5]
PbTx-2	Fluorescent aptasensor	Quantum dots	0.56	0-2	No	[6]
PbTx-2	Colorimetric aptasensor	Label-free	2.25	50-4000	Yes	This work
OA	HepG2-based biosensors	-	10.20	0-100	No	[7]
OA	Electrochemical aptasensor	Phosphorene-gold nanocomposite	0.006	8.05-201.25	No	[8]

OA	Colorimetric aptasensor	Label-free	5.19	50-4000	Yes	This work

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