Supporting Information

Fluorescent strip sensor for rapid determination of toxins

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Detailed information of the whole experimental procedure

Chemicals and Materials

OTA and other toxins were purchased from Sigma-Aldrich Company. N-hydroxy-succinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) were purchased from J&K Chemical Ltd. The nitrocellulose high-flow plus membrane and glass fiber membrane were from Whatman (Dassel, Germany). Semi-rigid polyethylene sheets and adhesive tape were from the local market of Wuxi, Jiangsu.

The aptamer and other two DNA probes used in this study were synthesized by Sangon Biotech (Shanghai) Co., Ltd. Aptamer and two DNA probes sequences were:

Aptamer: 5'-GAT CGG GTG TGG GTG GCG TAA AGG GAG CAT CGG ACA AAA AAA AAA AAA AAA AAA-NH_2-3'

Test line DNA probe 1: 5'-Biotin-CTA GCC CAC ACC CAC CGC ATT TCC CTC GTA GCC TGT-3'

Control line DNA probe 2: 5'-Biotin-TTT TTT TTT TTT TTT TTT-3'

Buffer 1 used in the experiments contained 200 mM Tris-HCl (pH 8.8), 100 mM KCl, 100 mM $(NH_4)_2SO_4$, 20mM and $MgSO_4$.

Preparation of the QD-aptamer conjugates

Into 69 μL QD solution, 16 μL 0.1mM EDC and 10μL 0.0125 mM NHS were added for reaction under stirring for 15 min in the dark. 5μL aptamer aqueous solution at 10μM was added and reacted at room temperature for 3 hours in the dark. The confirmation of the conjugation was carried out by the agarose gel electrophoresis. The concentration of the agarose gel was 2% and

the voltage was 10V/cm and the running time of the electrophoresis was 40 min.

Preparation of streptavidin-biotin DNA probe conjugates

Streptavidin was dissolved in the 0.01 mM phosphate-buffered saline (PBS) solution (pH 7.4) at 1 mg/mL. 5 μL 10 μg/mL streptavidin solution and 35μL DNA probe solution were mixed and reacted at 4 □ for 2 hours. The mixture was centrifugated at 6000 rpm with a centrifugal filter (cutoff 30 000, Millipore) for 30 min to remove excess DNA probes. After two washes with the PBS solution, the conjugates were dissolved in the same PBS solution for following using.

Based on the competitive recognition model we used, we determine the signal-to-noise (S/N) ratio for different QD-aptamer conjugates on the conjugate pad. For example, with a concentration of prepared QD-aptamer conjugates of 500 μmol/L as one time, we compared the S/N ratio results of a half, 1 time, 2 times and 3 times respectively. Results indicated that the S/N ratio came to the highest value when one time (500 μmol/L) conjugates was used, so 500 μmol/L was adopted as the best amount of the QD-aptamer conjugates for subsequent experiments.

Two frequently used running buffer were initially compared, 10% Triton X-100/ 6% sucrose/ 8% PEG/ in buffer ***1*** and 10% PVP/ 6% sucrose/ 8% PEG/ in buffer ***1***. We found that Triton X-100 gave an obvious tailing phenomenon of the QD-aptamer conjugates on the nitrocellulose membrane (See ESI). Comparatively, PVP gave relatively better results, and was used for further research. The PVP concentration was further used to control the running buffer viscosity and then to control the running buffer migration rate. To ensure a sufficient reaction time on the strip, 10% PVP in the running buffer 1 was selected for the experiments

Preparation of the fluorescent strip

A schematic diagram of the fluorescent strip is in Figure 1. The nitrocellulose membrane, the glass-fiber membrane (conjugated pad), sample pad and absorbent pad were laminated 2 mm with each other in sequence to ensure the solution migration and pasted onto the plastic backing plate as shown in Figure 1. The plate was cut into 4mm-wide strips with a strip cutter (model CM4000, Irvine, CA, USA). The streptavidin-DNA probe 1 conjugates (test) and streptavidin-DNA probe 2 conjugates (control) were dispersed on the nitrocellulose membrane

with a manual membrane-drawing tip. The strips with lines were dried at ambient temperature for 10 min. The QD-aptamer conjugate (5μL/strip) was added to the glass-fiber membrane to be used as a conjugated release pad and air-dried for 5 min. Integrated fluorescent strips were stored in a self-sealing plastic bag with desiccant at 4 ºC. For testing, the strips were inserted into the pretreated sample solution and incubated for 10 min to get the final results.

Assay procedure

Aliquots of 120μL sample solutions containing the designed concentration of OTA in running buffer were prepared. Strips were inserted into these standard OTA solutions for 10 min. Results were collected and analyzed by the special software (FR980 software, Shanghai FURI Science & Technology Co.,Ltd.).

Sample preparation

To test application of our developed strips in the real samples, strips were used to detect the OTA in the red wine samples. Red wines from China were all purchased from the supermarket. Samples made in China were used in this study. The red wine samples were treated with the solid phase extracted column as reported method. Spiked samples were prepared by adding different volumes of OTA standard solution to wine samples lacking OTA.

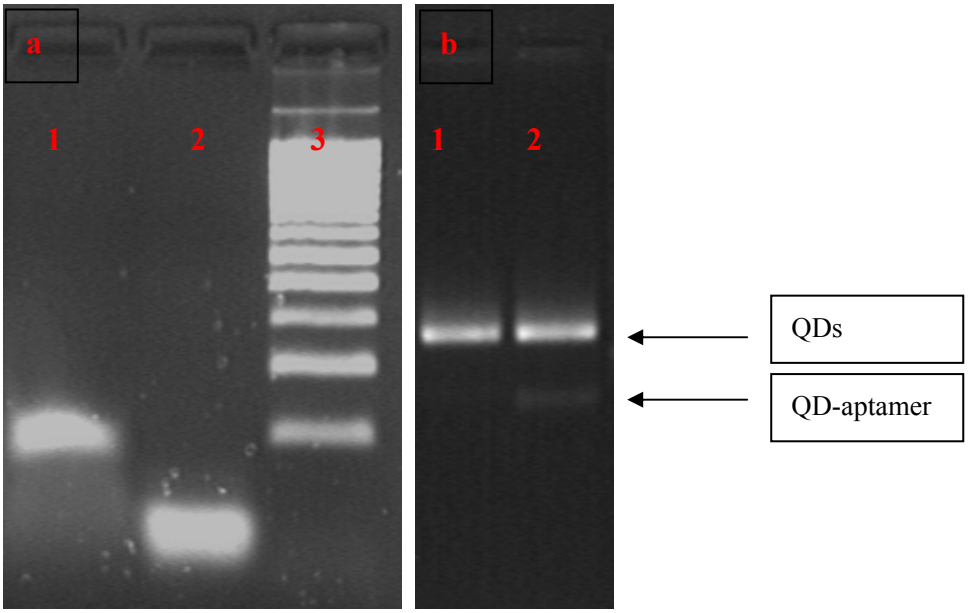

Figure S1. The agarose electrophoresis of the aptamer and the QD-aptamer conjugtes.

(Image a is the result with the EB stain. Lane 1: QDs-aptamer; lane 2: aptamer; lane 3: the marker; Image b is the result without EB stain and the fluorescence in the image is from QDs. Lane 1: QDs;

lane 2: QDs-aptamer conjugates. Concentration of the agarose gel: 2%)

From image a we can know that the mobility of the QD-aptamer is faster than that of the pure aptamer; And from image b we know that the mobility of the QD-aptamer is faster than that of the pure QDs. From all above results, both the EB stain and without EB stain results confirm the successful conjugation of the aptamer to the surface of the QDs.

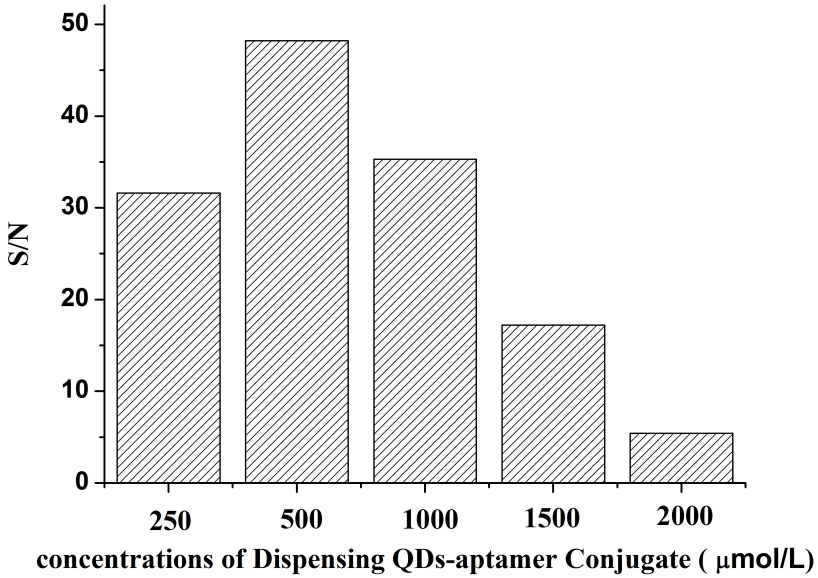

Figure S2. Effect of dispensing times of QD-aptamer conjugate on the signal-to-noise ratio for OTA detections.

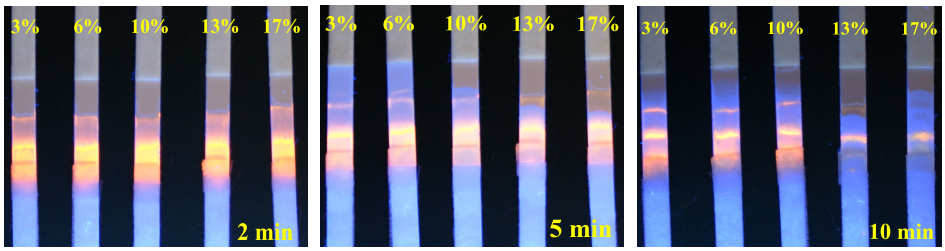

Figure S3. The concentration optimization of the mobility of the PVP buffer.

From the results shown in Figure 3S, we could see that there is almost no obvious difference among different conditions at the early 2 min, which may be due to the nonsufficient reactions. The test line was almost formed while the control line not. Till 5 min, the migration solution of 3%

and 6% PVP has already surpassed the membrane while the others still on the nitrocellulose membrane. Finally, at 10 min, we find that the both the control line and test line are formed from 3% to 13% PVP solutions while that of the 17% PVP solution is not. The un-formed control line is due to the high viscosity of the running buffer and the slow migration rate, which induce the short reaction time for the strip. From the above discussed results and ensuring the sufficient reaction time for the strip, 10% PVP in the running buffer 1 is selected for the experiments.

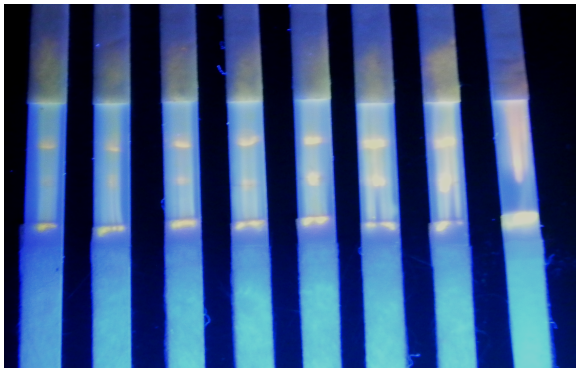

Figure S4. The strip detection results under the condition of 10% Triton X-100/6% sucrose/8% PEG/ in buffer *1* as the running buffer.

From results shown in Figure S4, we can find that the bands were not very clear under the condition of using Triton X-100 as the additive.

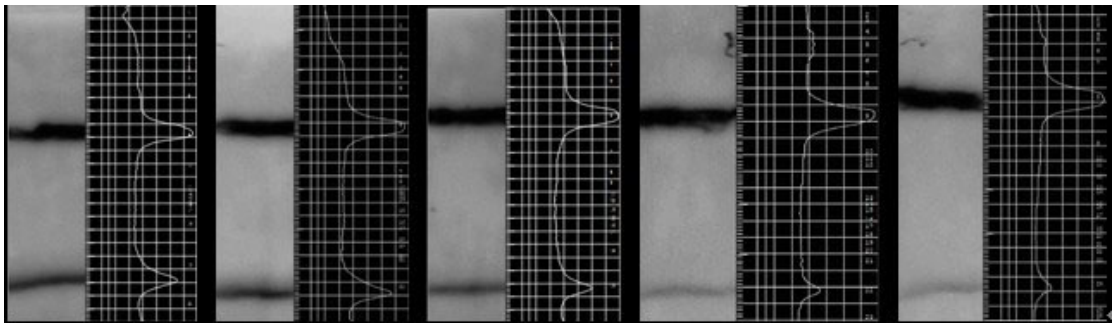

Figure S5. The scanned results of the strips at different concentrations by the software.

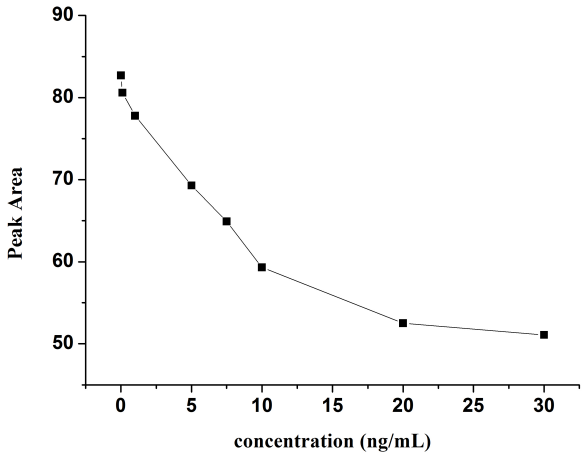

Figure S6. The detection results of strips in the full concentration range.

Table S1. The comparison results of the strip method and the HPLC method.

Sample Number	Strip results (n=4)	HPLC results (n=4)
Sample 1	4.7±0.2ng/mL	5. 3±0.4ng/mL
Sample 2	19.4±0.5ng/mL	18.9±0.3ng/mL
Sample 3	10.3±0.4ng/mL	10.7±0.6ng/mL
Sample 4	<1.9 ng/mL*	0.16±0.07ng/mL
Sample 5	<1.9 ng/mL*	0.94±0.1ng/mL

*The determined results were beyond the LOD of our method.

The HPLC determination of the OTA in the samples was carried out according to the previous method with little modifications. The prepared samples were dissolved in a 500 μL mobile phase consisting of acetonitrile/water/acetic acid (99: 99: 2) and filtered through 0.45 μm microfilter paper in to a 5mL screw-cap vial for subsequent HPLC analyses. The Separation was performed at ambient temperature at a flow rate of 1.0mL/min, with an injection volume of 20 μL for both standard solutions and coffee sample extracts. The fluorescence detector was operated at an excitation wavelength of 330nm and an emission wavelength of 460nm.

144