Electronic Supporting Information

An Immunoassay for Ochratoxin A Using Tetramethylrhodamine Labeled Ochratoxin A as a Probe Based on Binding Induced Fluorescence Change

Yapiao Li,[1,2] Ning Zhang,[1] Hailin Wang,[1,2] Qiang Zhao[1,2*]

1. State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

2. University of Chinese Academy of Sciences, Beijing 100049, China

* Corresponding author

Tel: +86-10-62849892. Fax: +86-10-62849892.

E-mail: qiangzhao@rcees.ac.cn

Table S1 Comparison of a few immunoassays for OTA.

Method	Detection limit (nM)	Linear range (nM)	Ref.
Competitive fluorescence assay based on FRET between QDs and rhodamine	0.002	0.02-0.12	[17]
Enzyme-linked immunosorbent assay using a nanobody-AviTag fusion protein	0.07	0.13-1.73	[18]
Surface plasmon resonance with amplification using colloidal gold immunoconjugates	0.15	NA[a]	[19]
Enzyme-linked immunosorbent assay	0.3	0.08-1.14	[22]
Competitive fluorescence assay using TMR-conjugated OTA probe	1	2-31.2	this work
Label-free impedimetric immunosensor	1.2	2.5-50	[20]
Direct fluorescence assay relying on fluorescence from the dianionic form of OTA bound with antibody	1.2	NA	[15]
Fluorescence polarization immunoassay	2	0.6-3.7	[23]
Direct FRET fluorescence assay using intrinsic fluorescence of OTA and antibody	2.5	NA	[14]
Lateral flow immunoassay	2.5	NA	[21]

a NA means not available.

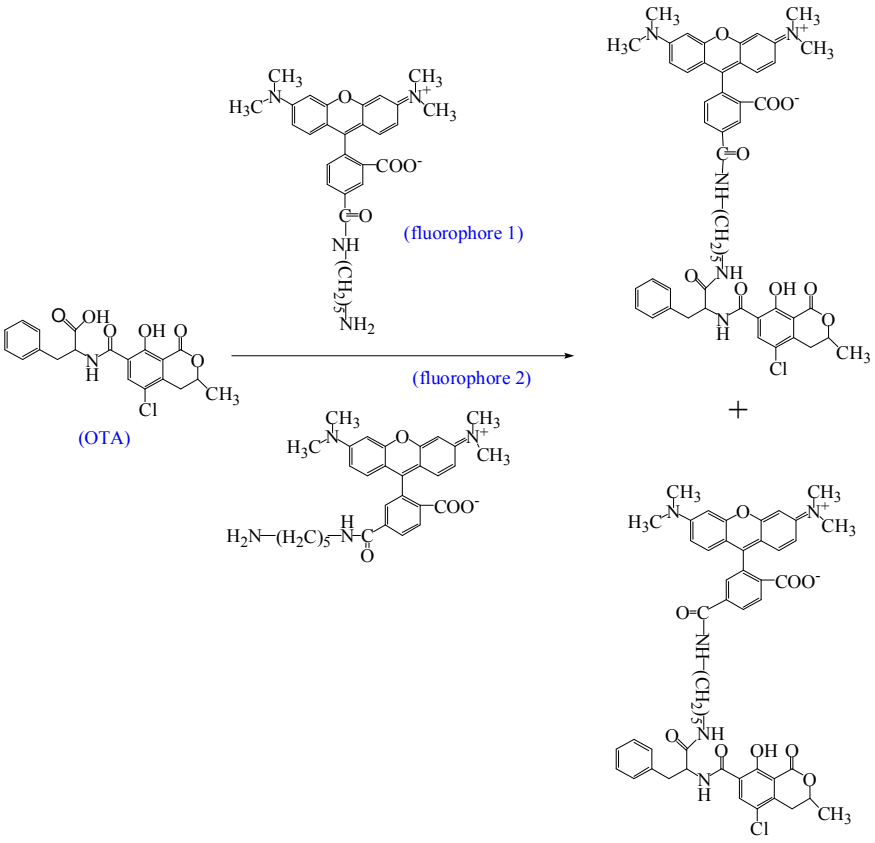


Fig. S1. Schematic for preparation of TMR-OTA probes. The TMR fluorophores contained two isomers (fluorophore 1 and fluorophore 2).

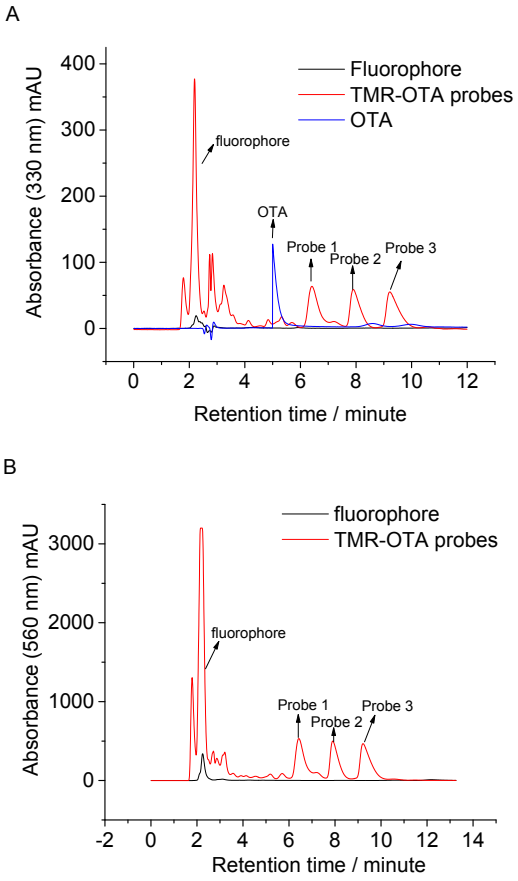
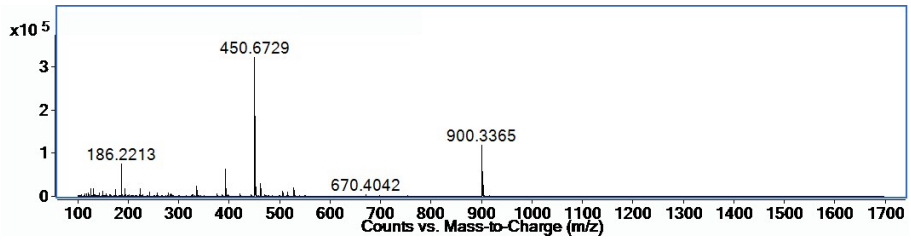
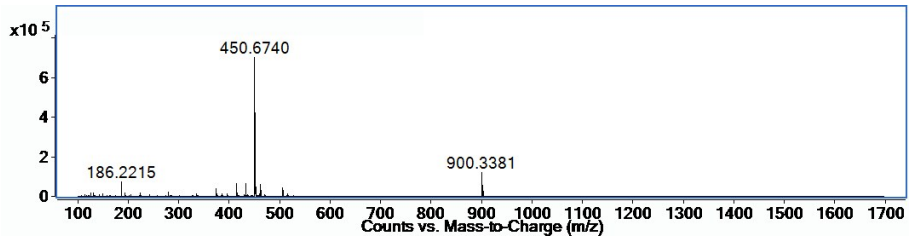


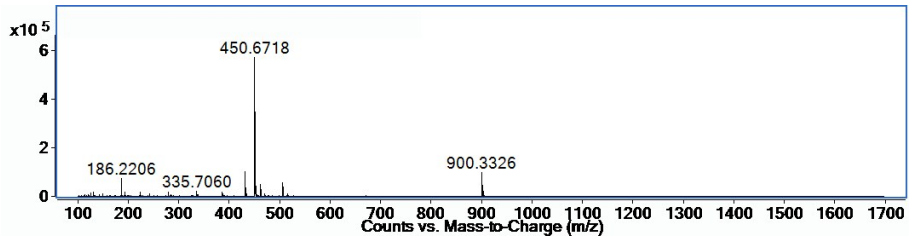
Fig. S2. Chromatograms of fluorophore TMR (black), OTA (blue) and TMR-OTA probes (red) obtained from fluorophore TMR and OTA (absorbance at 330 nm (A) and 560 nm (B) using a UV detector). The fluorescent probes of OTA with retention time of 6.3 minute, 8 minute and 9.3 minute, were denoted as TMR-OTA Probe 1, TMR-OTA Probe 2, and TMR-OTA Probe 3, respectively.



(a)



(b)



(c)

Fig. S3. Mass spectra of TMR-OTA Probe 1 (a), TMR-OTA Probe 2 (b), and TMR-OTA Probe 3 (c). The total ion chromatograms of the obtained three TMR-OTA probes of OTA all showed the presence of a molecular ion of m/z 900.3365, 900.3326 and 900.3381 ($C_{50}H_{50}N_5O_9Cl$, calculated m/z, 900.337), corresponding to the $[M+H]^+$ adduct.

The qualitative analysis was carried out by an Agilent 1290 UHPLC system coupled with a 6540 quadrupole time-of flight (Q-TOF) mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Zorbax Eclipse Plus C18 column (2.0 mm × 50 mm i.d., 1.8 μm particle size, Agilent Technologies, Palo Alto, CA) was used for separation. Mobile phase consisted of two solvents: 1‰ formic acid aqueous solution (solvent A) and methanol (solvent B). Isocratic elution of 60% B was used for

UHPLC separation. The column temperature was set at 30 °C, and the flow rate was 0.25 mL/min.

The Q-TOF mass spectrometer was operated in positive chemical ionization mode. Nitrogen was used for both nebulization and desolvation. The nebulization gas was set at 35 psi. The flow rate of desolvation gas was 10 L/min. Source temperature was set at 350 °C and the flow rate was 12 L/min. Capillary voltage was set at 3500 V. Nozzle Volt-age was set at 0V. High purity nitrogen (99.999%) was used as the collision gas. 25 eV collision energy was applied in targeted MS/MS mode.

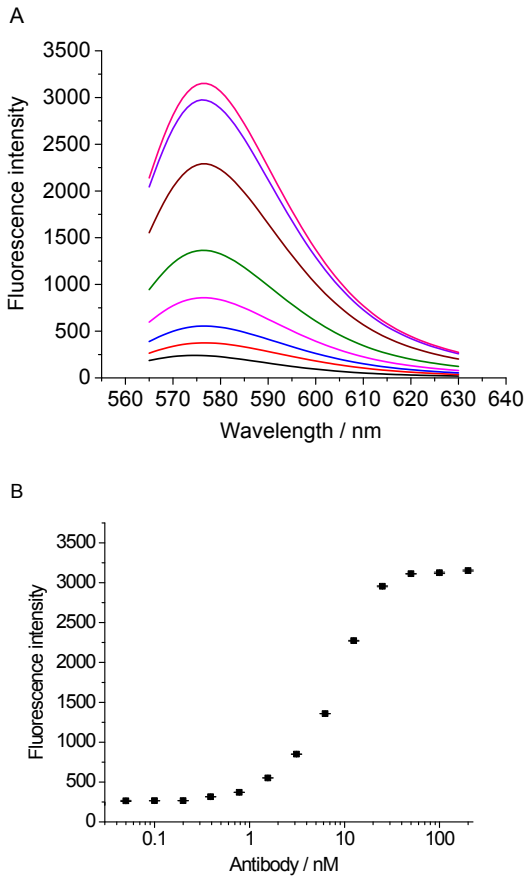


Fig. S4. (A) Typical fluorescence emission spectra of the TMR-OTA Probe 2 (10 nM) in the presence of various concentrations of antibody specific to OTA. From the bottom curve to the top curve, the corresponding concentrations of antibody were 0, 0.78, 1.56, 3.12, 6.25, 12.5, 25, and 100 nM. (B) The fluorescence intensity of TMR-OTA Probe 2 (10 nM) in the presence of various concentrations of antibody specific to OTA.

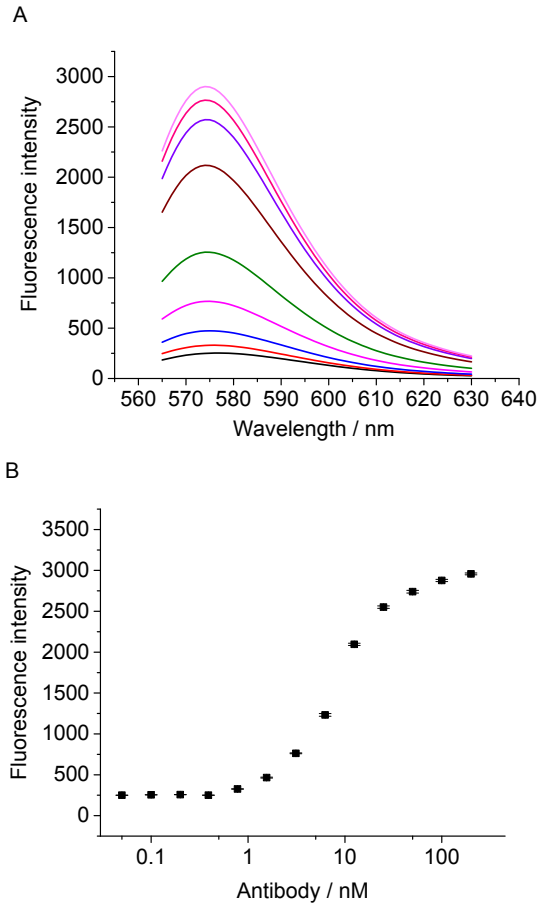


Fig. S5. (A) Typical fluorescence emission spectra of the TMR-OTA Probe 3 (10 nM) in the presence of various concentrations of antibody specific to OTA. From the bottom curve to the top curve, the corresponding concentrations of antibody were 0, 0.78, 1.56, 3.12, 6.25, 12.5, 25, 50, 100 nM. (B) The fluorescence intensity of TMR-OTA Probe 3 (10 nM) in the presence of various concentrations of antibody specific to OTA.

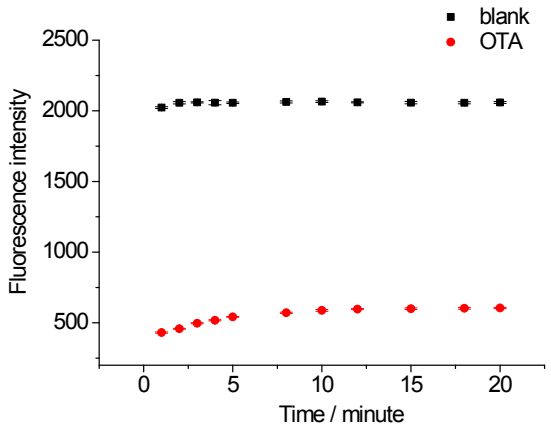


Fig. S6 Effect of incubation time on the fluorescence signal of blank sample and OTA sample (10 nM) in the assay for OTA using TMR-OTA Probe 1.

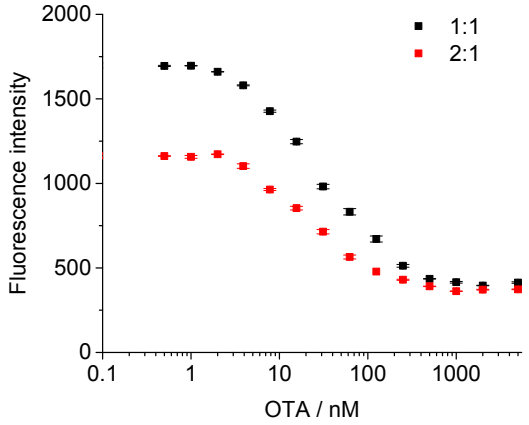


Fig. S7. Effect of the ratios of the TMR-OTA Probe 1 and antibody on the detection for OTA. Black: 10 nM probe 1 and 10 nM antibody. Red: 10 nM Probe 1 and 5 nM antibody.

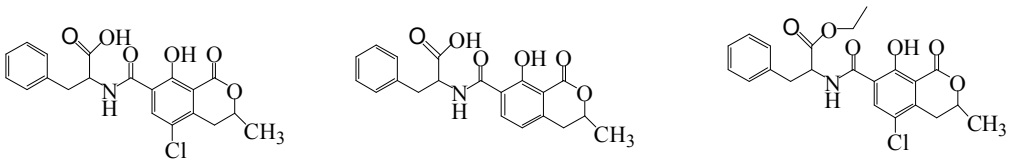


Fig. S8. Differences of OTA, OTB and OTC in structures.

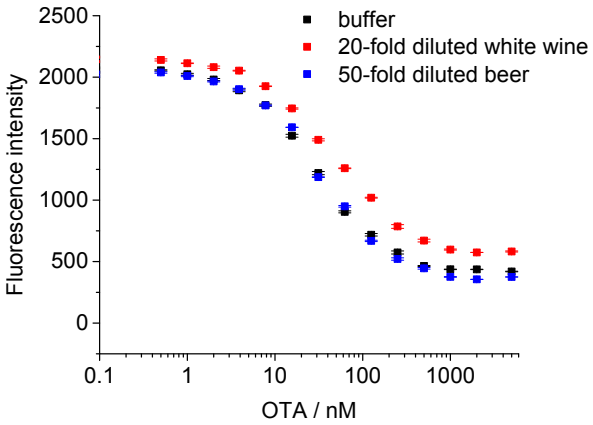


Fig. S9. Fluorescence responses corresponding to various concentrations of OTA in binding buffer, 20-fold diluted white wine and 50-fold diluted beer. In the assay, the solution contained 10 nM Probe 1 and 10 nM antibody.