Electronic Supplementary Material (ESI) for Chemical Communications

Electronic Supporting Information for

A Label-Free, Direct and Noncompetitive FRET Immunoassay for Ochratoxin A Based on Intrinsic Fluorescence of an Antigen and Antibody Complex

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Materials and Methods

Reagents. Ochratoxin A (OTA) and ochratoxin B (OTB) were purchased from Sigma-Aldrich Chemical Co. (Yongin, Korea) and Santa Cruz Biotechnology Inc. (CA, USA), respectively. Bovine serum albumin (BSA), mouse monoclonal antibody (Mouse-MAb), Tween 20, and MES salt were obtained from Sigma-Aldrich Chemical Co. Anti-OTA antibody was purchased from Abcam Inc. (Cambridge, England). HPLC-grade methanol was obtained from Merck Chemical Corp. (Darmstadt, Germany). Veratox for ochratoxin was obtained from Neogen Co. (MI, USA). The 0.45 μm syringe filter was obtained from Gelman Science (MI, USA). All other chemicals and organic solvents used were of reagent grade or better. Standard solutions containing various concentrations of OTA in methanol were prepared for further assay.

Preparation of FRET immunoassay for OTA detection. For the OTA FRET immunoassay, 0.5 μL anti-OTA stock solution was added to the fluorescence measurement cell along with 180 μL 25 mM MES buffer (pH 6.0) containing 0.3% Tween 20. After the addition of 20 μL of various concentrations OTA in methanol, the solution was incubated at room temperature for 20 min followed by the fluorescent measurement.

Determination of specificity in the FRET immunoassay. In order to determine the specificity of the FRET immunoassay system, independent solutions were prepared containing OTA (20 and 100 ng/ml), and either 50 μg/mL BSA or 100 μg/mL of Mouse-MAb, and 10 μg/mL anti-OTA. In addition, solutions containing 10 μg/mL anti-OTA and 20 or 100 ng/mL OTB were subjected to the FRET immunoassay.

Sample preparation. To determine OTA concentrations in wheat grain samples that were spiked with 20 and 100 ng/mL OTA, the samples were extracted using 60% methanol in water (v/v) and a 0.45 μm syringe filter. The extracts were then diluted ten times with MES buffer to yield an OTA solution with a final methanol concentration of 10%. The solution obtained by mixing the OTA and anti-OTA solutions was incubated at room temperature for 20 min and subjected to a fluorescent measurement.

Instrumentation. UV–vis absorption spectra were recorded by using a DU 800 UV–visible spectrophotometer (Beckman Coulter, Brea, CA). The fluorescence intensities of all samples were recorded by using a LS 55 Fluorescence Spectrometer (PerkinElmer, Waltham, MA). The emission spectra were recorded in the wavelength range of 300-600 nm with excitation at 280 nm and slit widths for excitation and emission of 10 nm.

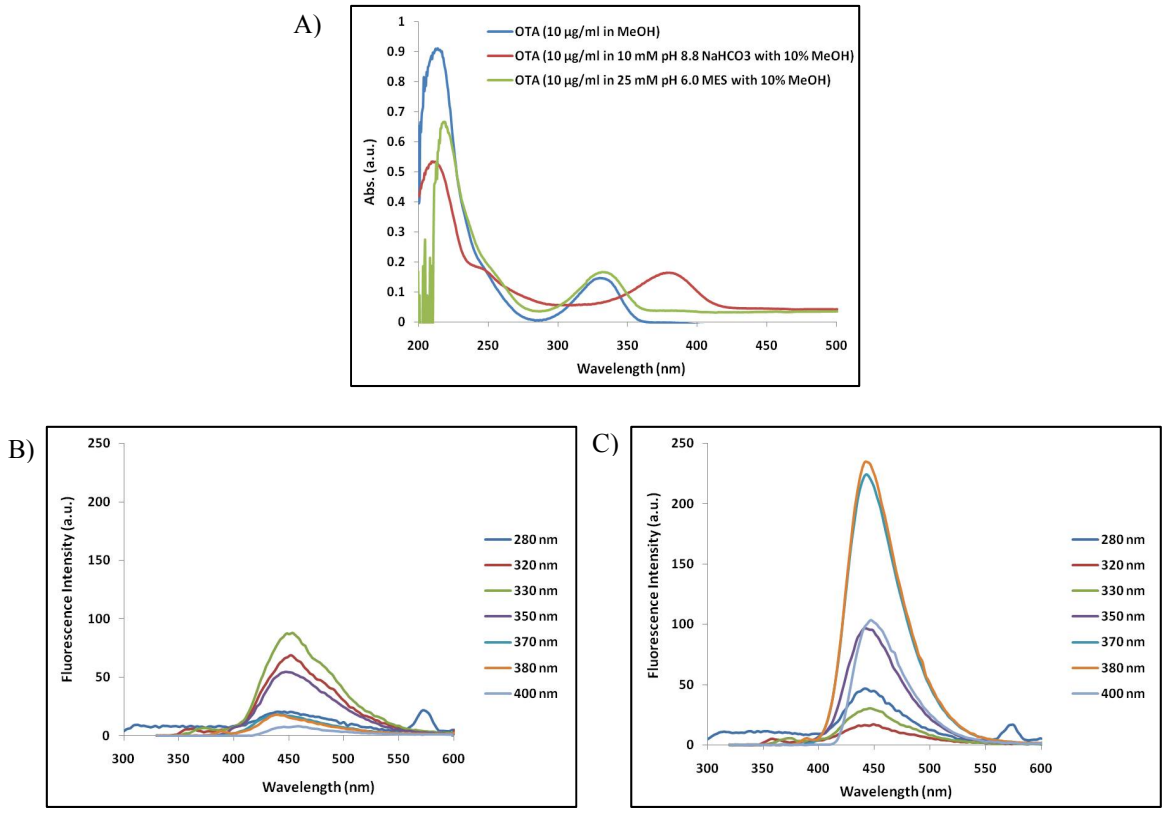


Figure S1. Absorption spectra (A) of ochratoxin A (OTA, 10 µg/ml) in methanol (MeOH), 10 mM $NaHCO_3$ buffer (pH 8.8) with 10% MeOH, and 25 mM MES buffer (pH 6.0) with 10% MeOH; Fluorescence spectra of OTA (0.1 µg/ml) in 25 mM MES buffer (pH 6.0) with 10% MeOH (B) and 10 mM $NaHCO_3$ buffer (pH 8.8) with 10% MeOH (C) upon excitation at 280, 320, 330, 350, 370, 380, and 400 nm.

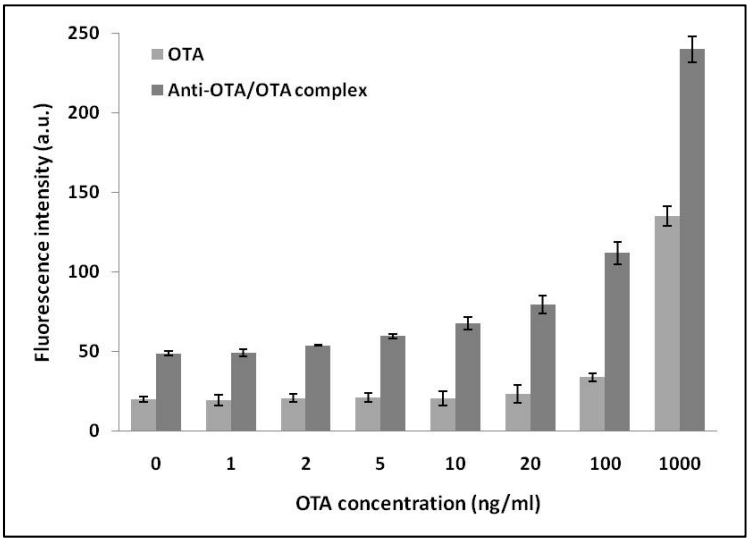
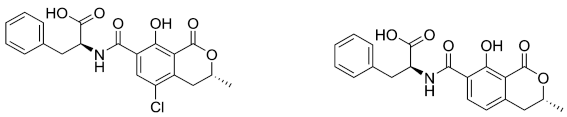


Figure S2. Fluorescence intensities of the anti-OTA/OTA complex (dark gray) and free OTA (light gray) at 450 nm as a function of OTA concentrations (0, 1, 2, 5, 10, 20, 100, and 1000 ng/mL). The fluorescence spectra were produced with excitation at 280 nm. The Error bars indicate standard deviations of data from experiments performed in triplicate.



OTA **OTB**

Figure S3. Chemical structure of OTA and OTB

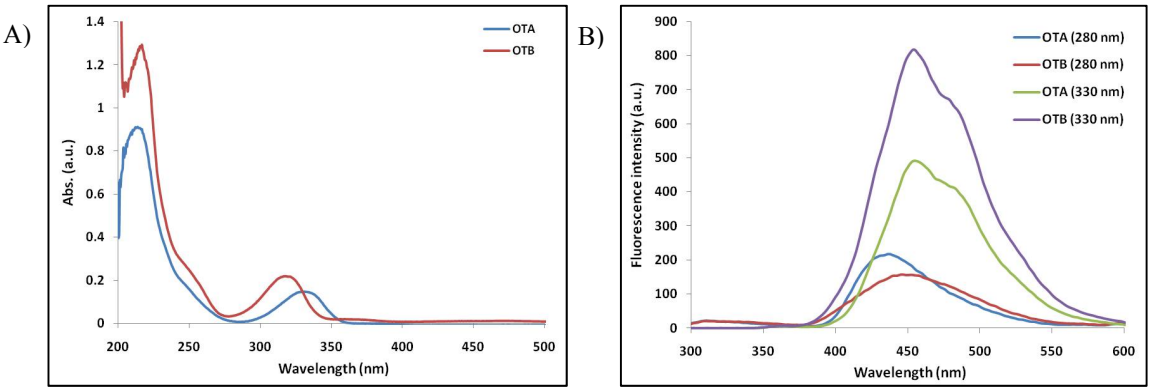


Figure S4. (A) Absorption spectra of 10 μg/mL OTA and OTB in methanol. (B) Fluorescence spectra of 1 μg/mL OTA and OTB in methanol with excitation at 280 nm and 330 nm.

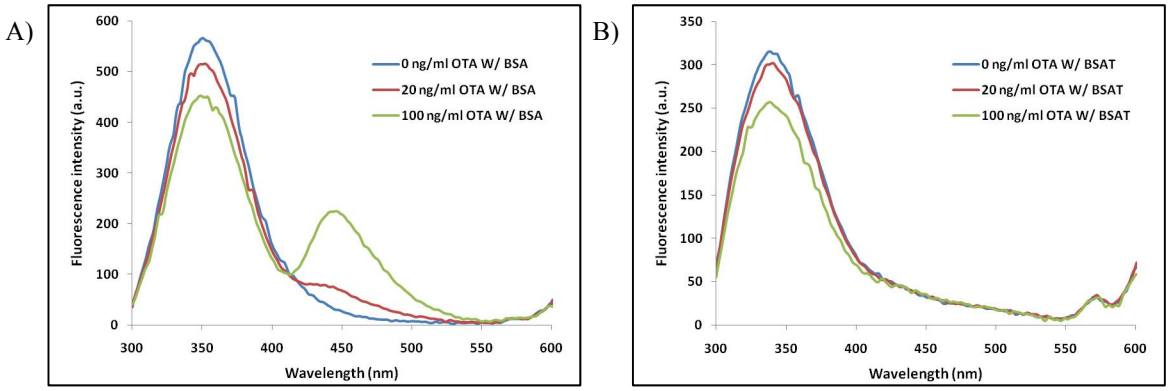
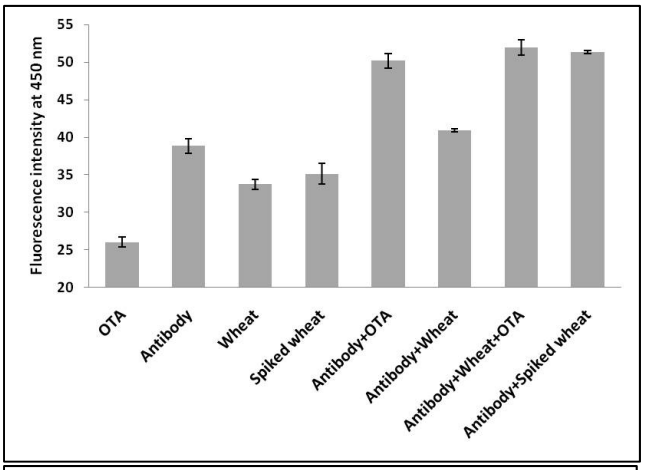


Figure S5. Fluorescence spectra of 0, 20, and 100 ng/mL OTA coupled with (A) 50 μg/mL BSA and (B) 50 μg/mL BSA with 0.3% Tween 20 (BSAT) in the 25 mM MES buffer (pH 6.0) (λ_{ex} = 280 nm).

A)



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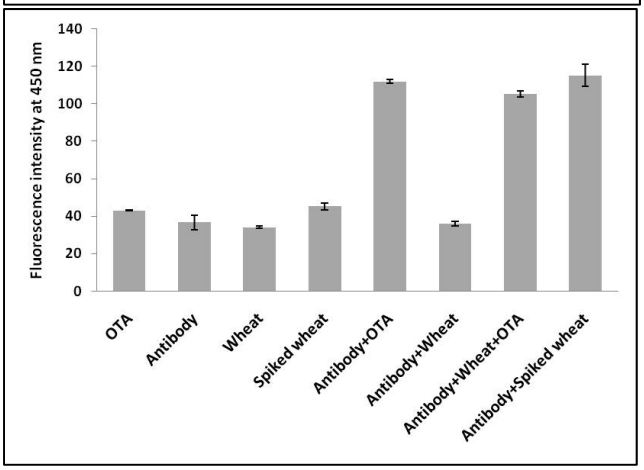

Figure S6. Detection of 20 ng/mL (A) and 100 ng/mL (B) OTA in the spiked wheat grain samples in reference to the control OTA by using the FRET immunoassay (λ_{ex} = 280 nm, λ_{em} = 450 nm). The Error bars indicate standard deviations of data from experiments performed in triplicate.