Supporting Information

Immunochromatographic Assay for Ultrasensitive Detection of Aflatoxin B_1 in Maize by Highly Luminescent Quantum Dot Beads

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1. Absorption and fluorescence spectra of CdSe/ZnS QDs.

The absorption and fluorescence spectra of core/shell nanocrystals (CdSe/ZnS QDs) are shown in Figure S1A and 1B. The CdSe/ZnS QDs shows a broad UV absorption spectrum while the emission spectrum of QDs presents a narrow fluorescence peak at 620 nm with full width at half maximum (FWHM) of 25 nm.

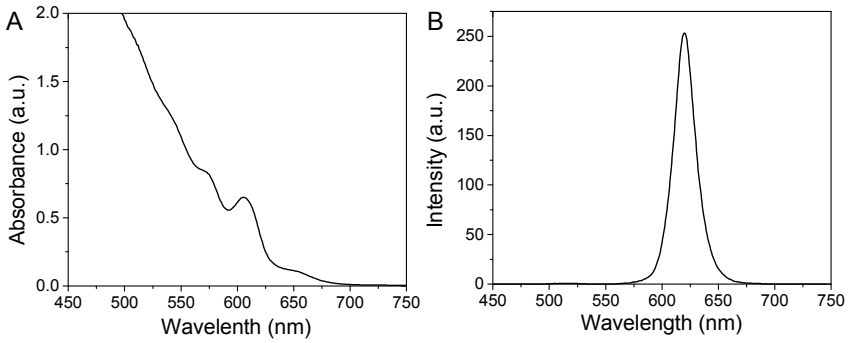

Figure S1. The absorption (A) and fluorescence (B) spectra of core/shell nanocrystals (CdSe/ZnS QDs) for QBs preparation.

2. The histogram of size distribution of QBs

The size distribution of the prepared QBs was displayed in Figure S2, the histogram of size distribution indicates the average diameter of these submicrobeads is 247 nm ±13 nm. Data were obtained by analyzing 60 prepared QBs visible in the TEM image.

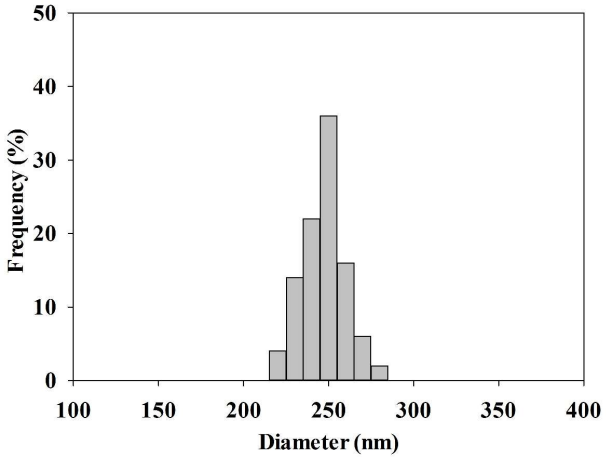

Figure S2: Size distribution of the prepared QBs.

3. The fluorescence spectra of CdSe/ZnS QDs and the prepared QBs.

To evaluate the fluorescence intensity enhancement of the QBs compared with the QDs, fluorescence intensity of both materials was monitored using a Hitachi F-4500 fluorescence spectrophotometer (Tokyo, Japan). As shown in Figure S3, the fluorescence intensity of QBs at concentration of 7.99 pmol L^{-1} (dissolved in ultrapure water) was basically the same as water soluble CdSe/ZnS QDs with carboxylic acid at 22.875 nmol L^{-1} (dissolved in ultrapure water). In the present study, Fluorescent enhanced factor (F_{FE}) is defined as the ratio of the concentration of QDs to that of QBs under their same fluorescence intensity (F_{FE}=$Con._{QD}$/ $Con._{QB}$=(22.875 nmol

L^{-1})/(7.99 pmol L^{-1})=2863). Hence, for same number of QB/QD particles, luminescence intensity of QBs was presumably 2863 times higher than QDs.

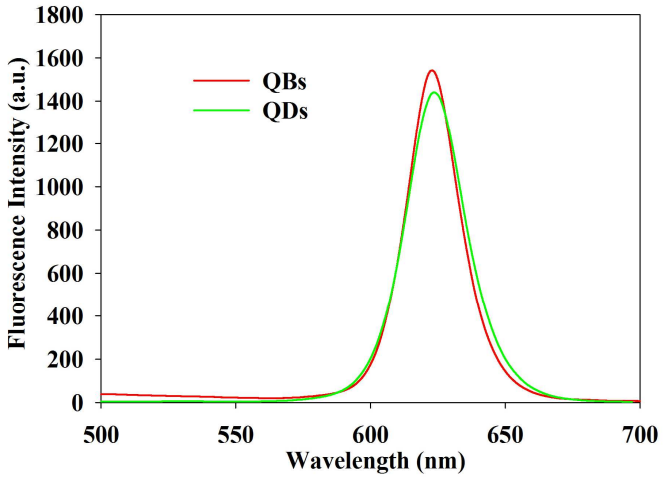

Figure S3. Photoluminescence spectra of QDs (22.875 nmol L^{-1}, dissolved in ultrapure water) and QBs (7.99 pmol L^{-1}, dissolved in ultrapure water). At the same molar concentration, luminescence intensity of QBs was presumably 2863 times higher than QDs.

4. Determination of the amounts of the target antibody in ascites

The amounts of the target antibody in ascites were quantified by a western blot combined with a gray-scale quantitative technology. Western blot was performed as previously described with slight modification.[1,2] Briefly, samples (10 μL) pre-mixed with 6×loading buffer (2 μL) were incubated at 95°C for 5 min. The ascites and purified antibody samples were separated by 12% of sodium dodecyl sulfate (SDS)-polyacrylamide gels using a Mini-Gel apparatus (Bio-Rad, Hercules, CA) and then transferred to reinforced polyvinylidene fluoride (PVDF) membrane. Blots were blocked with 5% (w/v) bovine serum albumin (BSA) in PBS (0.01 M, pH 7.4) for 1 h

and detected with horseradish peroxidase (HRP)-conjugated anti-mouse IgG (Jackson ImmunoResearch) and 3,3' diaminobenzidine substrate (Sigma, St. Louis, MO).

Protein concentrations of both ascites and purified antibody were estimated by using gray-scale quantitive technology. The ascites (6 mg mL^{-1}) and purified antibody (120 μg mL^{-1}, 60 μg mL^{-1}, 30 μg mL^{-1} and 15 μg mL^{-1}) were spotted onto PVDF membrane (Merck Millipore, Billerica, MA). As shown in Figure S4, the ascites (Lane 1) and purified antibodies of different concentrations (Lanes 2, 3, 4 and 5) demonstrated one band at 150 kDa. The amounts of the target antibody in ascites were quantified by analyzing the gray-scale of the bands. The standard curve was constructed by plotting the gray values of bands from the purified antibody against the corresponding concentrations of the purified antibody. The calculated equation was $y=11.311x-1420.3$ ($R^2=0.9513$). The amounts of the target antibody in ascites were calculated using the standard equation as 0.24 mg mL^{-1} (6 mg mL^{-1}×4%).

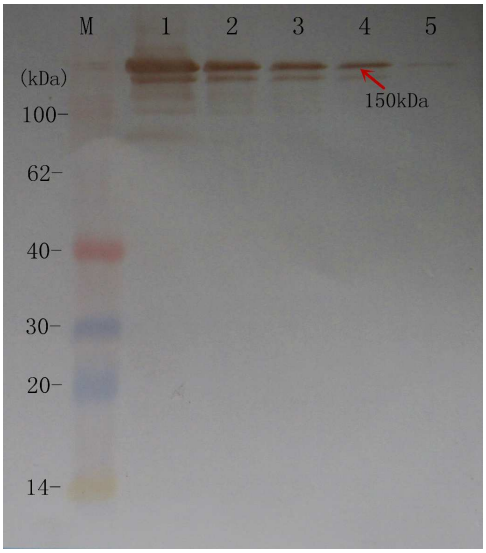

Figure S4. Western blot analysis of the ascites and the purified antibodies. Lane 1: the AFB_1 ascites (6 mg mL^{-1}). Lane 2: the purified antibody (120 μg mL^{-1}). Lane 3: the purified antibody (60 μg mL^{-1}). Lane 4: the purified antibody (30 μg mL^{-1}). Lane 5: the purified antibody (15 μg mL^{-1}).

5. Confirmation of the saturation concentration of the ascites conjugated with the QBs.

After estimating the amounts of the target antibody in ascites, the saturation concentration of the ascites labelled on the surface of QBs was investigated before conjugation. In this study, the amounts of the ascites of 10, 30, 60, 90, 120, 150, 200 or 300 μg were conjugated with 1 mg of QBs. As shown in Figure S5, the fluorescence intensity (FI) on the test line increased along with the concentration of the ascites ranged from 0 to 150 μg per mg QBs, and then it reached a plateau with further increase of ascites concentration. Therefore, 150 μg ascites per mg QBs was considered as the maximum amount of the ascites conjugated with the QBs.

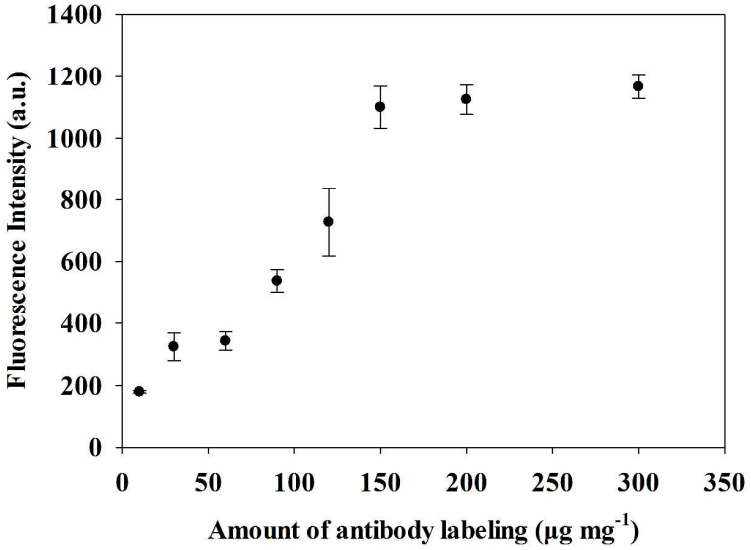

Figure S5. Optimization of the content of anti-AFB_1 ascites on the surface of QBs.

6. Optimization of the QBs strip parameters.

In this study, the concentration of AFB_1-BSA on the test line and the volume of QB-mAbs probe (36 μg mL^{-1}) which could directly affect the fluorescence signal and the analytical sensitivity of the strip, were optimized according to a similar "checkerboard titration" method. The inhibition ratios were used to further confirm the optimal combinations, where the inhibition ratios were obtained by $B/B_0 \times 100$, B_0 and B represent the FI_T/FI_C ratio of the negative sample and an AFB_1 spiked sample (10 pg mL^{-1}), respectively. As shown in Table S1, 0.4 mg mL^{-1} AFB_1-BSA on the test line and 5 μL of QB-mAbs probe (36 μg mL^{-1}) were the optimal combinations.

Table S1. Optimization of the concentration of AFB_1-BSA on the test line and the volume of QB-mAbs probe pre-mixed with the sample solution.

No.	Volume of QB-mAbs probe (μL)	Concentration of AFB_1-BSA (mg mL^{-1})	FI_T	FI_C	FI_T/FI_C	Inhibition Ratios (%)
1	3	0.4	880.49	527.87	1.67	37.91
2	4	0.4	1023.79	545.13	1.88	32.46
3*	5	0.4	1221.49	633.97	1.93	40.16
4	3	0.8	827.65	299.83	2.76	36.09
5	4	0.8	1171.05	365.67	3.2	38.38
6	5	0.8	1188.61	413.46	2.87	34.75
7	3	1.2	818.8	230.15	3.56	37.07
8	4	1.2	1024.67	315.78	3.24	23.64
9	5	1.2	1268.76	376.29	3.37	32.49

* The optimal combinations.

7. Stereogram of the strip

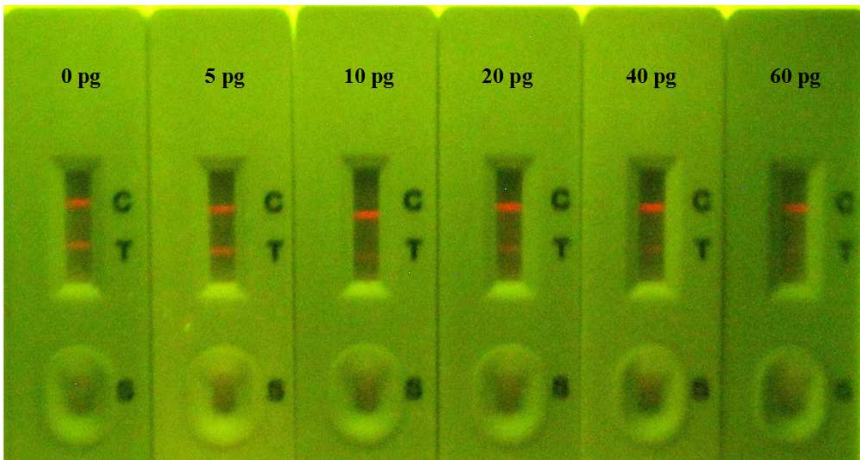

Figure S6. Stereogram of the immunoassay strips for detection of AFB_1. AFB_1 at the concentrations of 0, 5.0, 10, 20, 40 and 60 pg mL^{-1} were tested.

8. Standard curve of the QD-based ICA sensor

The anti-AFB_1 mAb labeled QD probe was prepared according to our previously reported method.[3] Briefly, 20 μL of water soluble CdSe/ZnS QDs (5.0 μmol L^{-1}) was

diluted with 1.0 mL 0.05 M borate buffer (pH 5.0), and then 9.6 μg EDC and 2.2 μg sulfo-NHS were added to activate the carboxyls. After reaction at room temperature for 30 min, the anti-AFB_1 mAbs (the reactant ratio of mAbs to QD was 10:1), which were purified from the ascetic fluid using ammonium sulfate precipitation, were added to the ester-activated QDs. The mixture pH was adjusted to 7.4, and reacted at room temperature for 40 min. Then, the 1000-fold molar of glucosamine hydrochloride was mixed with the solution to block excess carboxyl sites on the QD surface. After another 1 h, glucosamine hydrochloride was added to a final concentration of 2% (w/v), and the solution pH was adjusted to 4.5. The anti-AFB_1 mAb labeled QD conjugates were separated by centrifugation at 29,000g for 30 min at 4□. The pellet was re-dispersed with 5 mL 0.02 M phosphate buffer saline (PBS, pH 7.4) containing 0.1% (w/v) NaN3, was and was stored at 4°C for future use.

For QD-ICA sensor, the concentrations of AFB_1-BSA and donkey anti-mouse IgG antibodies on both lines were the same with those of QB-ICA method. To achieve the same fluorescence intensity on the test line with QB-ICA senor, the optimal volume of QD-mAbs probe (20 nmol L^{-1}) for QD-ICA was 4.0 μL. Moreover, a series of standard working solutions spiked to a final concentration of 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5 and 2.0 ng mL^{-1} with an AFB_1 stock solution (20 ng mL^{-1}) were prepared for the construction of the standard curve. Under the optimal conditions, as displayed in Figure S7, a regression equation y=-0.2230Ln(x)+0.3639 with a reliable correlation coefficient (R^2=0.9969) and a median inhibitory concentration (IC_{50}) of 0.54 ng mL^{-1} was obtained, where y is % B/B_0 and x is the AFB_1 concentration.

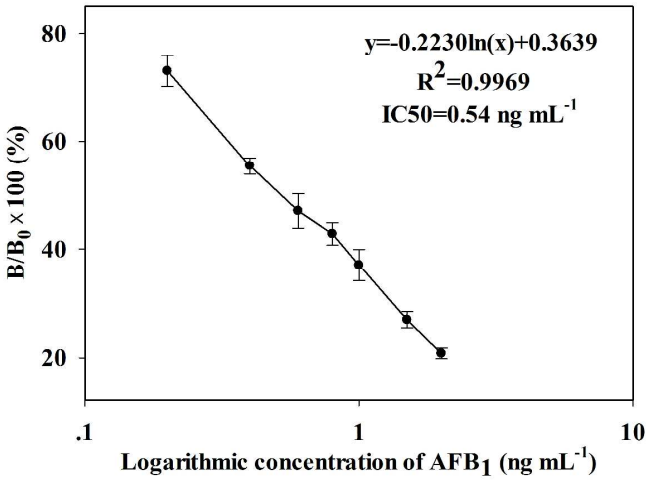

Figure S7. Optimized standard QD-based ICA sensor inhibition curve for AFB_1 was obtained by plotting $B/B_0 \times 100\%$ against the logarithm of AFB_1 concentration. B_0 and B represent the FI_T/FI_C ratio of the negative sample and a serial of AFB_1-spiked positive samples, respectively. Error bars were based on three duplicate measurements at different AFB_1 concentrations.

9. Consumption of antibodies between QB-ICA and QD-ICA sensor

The consumption of antibodies is an important factor that influences the sensitivity of competitive immunochromatographic assay. The fewer antibodies on each strip test mean the higher sensitivity of the strip test. Detailed calculation of the antibodies and probes for QB-ICA and QD-ICA sensor was described as following:

QB-ICA method

The density (ρ) of QBs is 1.8 g/cm^3, and the average diameter of QBs is 247 nm.

For every strip assay, the optimized volume of QB-mAbs probe (36 μg mL^{-1}) was 5 μL. The saturated labeled concentration of ascites per mg of QBs was 150 μg. The content of target antibodies in the ascites is 4%.

Mass (m, per QB) = ρ*V = (4/3) ρπR^^{3} =1.419 × 10^{-14} g.

Avogadro's constant (NA) = 6.02 × 10^^{23}/moL.

The amounts of 1 mg QBs=0.001 g/1.419 × 10^{-14} g = 7.05 × 10^^{10}.

Thus, mole number of 1 mg QBs = 7.05 × 10^^{10}/NA = 1.17 × 10^{-13} moL= 0.117 pmoL.

The mole number of QB-mAbs probe for each strip test = 5 × 10^{-3} × 0.036 × 0.117 = 0.021 fmoL.

The contents of target antibodies for each strip test = 5 × 10^{-3} × 150 × 0.036 × 0.04 = 1.08 ng

QD-ICA method

The optimized labeled mole ratio of the purified anti-AFB_1 mAbs and QDs was 10:1.

The molecular weight of mouse antibodies was 150000 Da.

For each strip assay, the optimized volume of QD-mAbs probe (20 nmol L^{-1}) was 4 μL.

Thus, mole number of QD-mAbs probe for each strip test = 4 × 10^{-6} × 20 = 80 × 10^{-6} nmoL = 80 fmoL.

The contents of anti-AFB_1 mAbs for each strip test = 10 × 80 × 150000 = 1.2×10^^{8} fg = 120 ng

Therefore, the amount of QB-mAbs probes for QB-ICA method is only 1/3810 of those using the QD as a signal probe (0.021fmoL /80 pmoL), while the content of target antibodies for QB-ICA is 111 folds lower than that of QD as a signal probe for the strip test (1.08 ng/120 ng).

Reference

(1) Lathrop, A.; Banada, P.; Bhunia, A. Differential Expression of InlB and ActA in *Listeria Monocytogenes* in Selective and Nonselective Enrichment Broths. *J. Appl. Microbiol.* **2008**, *104*, 627-639.

(2) Ding, Y. L.; Liu, M. Y.; Han, W.; Yang, S. L.; Liu, H.; Gong, Y. Application of Phage-Displayed Single Chain Antibodies in Western Blot. *Acta Biochim. Biophys. Sin.* **2005**, *37*, 205-209.

(3) Xu W., Xiong Y. H., Lai W. H., Xu Y., Li C. M., Xie M. Y. A Homogeneous Immunosensor for AFB_1 Detection Based on FRET Between Different-sized Quantum Dots. *Biosen. Bioelectron.* **2014**, *56,* 144–150.