Supporting information

Fluorescence immunoassay based on the enzyme cleaving ss-DNA to regulate the synthesis of histone-ds-poly(AT) templated copper nanoparticles

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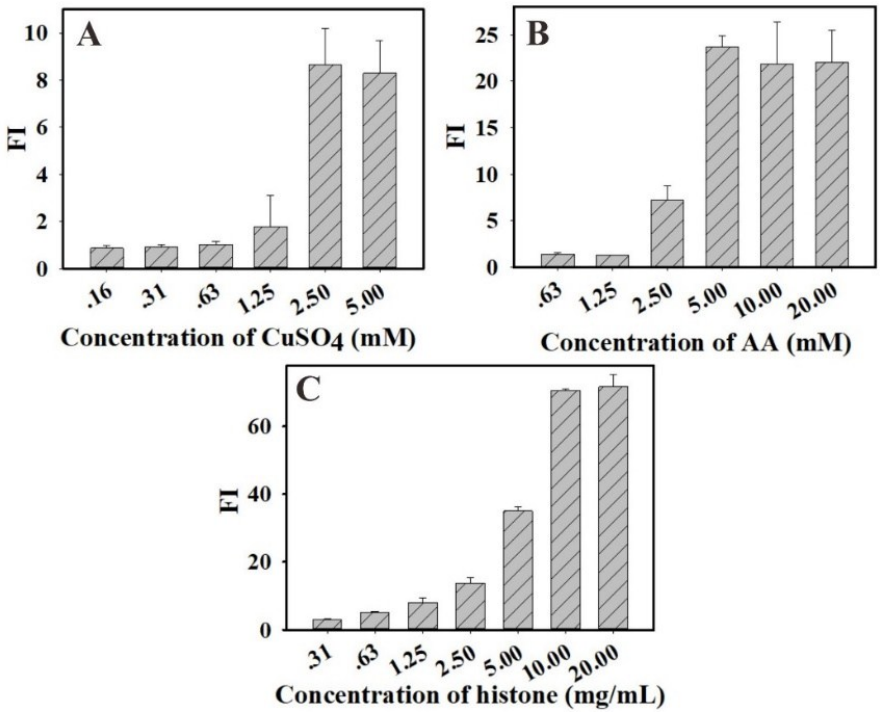
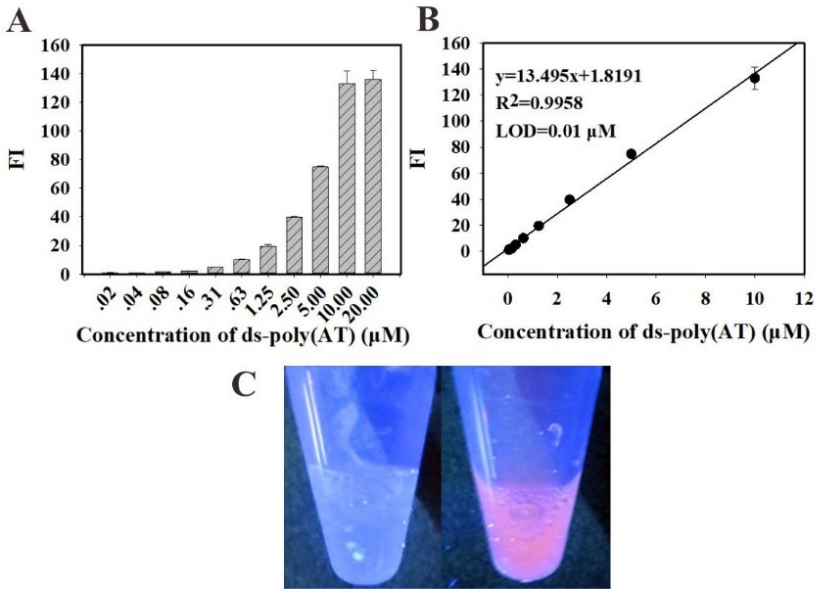


Figure S1. Optimization of synthesis CuNPs. **A.** Optimization of $CuSO_4$ in the presence of 2.50 mM of AA, 5 mg/mL of histone with 5 µM of ds-poly(AT). **B.** Optimization of AA in the presence of 2.50 mM of $CuSO_4$, 5 mg/mL of histone with 5 µM of ds-poly(AT). **C.** Optimization of histone in the presence of 2.50 mM of $CuSO_4$, 5 mM of AA with 5 µM of ds-poly(AT).



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43 **Figure S2. A.** Optimization of ds-poly(AT) in the presence of 2.50 mM of $CuSO_4$, 5

44 mM of AA, 10 mg/mL of histone. **B.** Calibration curve of ds-poly(AT) for synthesizing

45 of CuNPs. **C.** Photo show of CuNPs in the absence (left) and presence (right) of $CuSO_4$.

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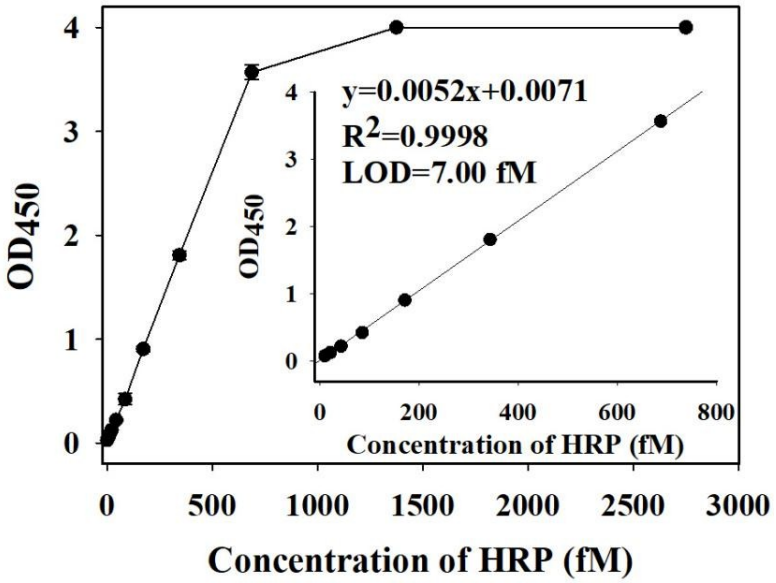

Figure S3. Absorbance changes upon the interaction of TMB with different concentration of HRP ranging from 0.67 fM to 2750 fM. The LOD value of the HRP to TMB was calculated as 7.00 fM, which was defined as the lowest concentration of HRP that generated a higher absorbance than the blank absorbance plus 3 standard deviations.

HRP-based conventional immunoassay for AFB_1 detection

The 96-well microplates were first coated with 100 μL of protein G (25 μg/mL) in bicarbonate buffer (0.10 M, pH 8.60) at 4 °C overnight and then blocked with 1 mg/mLof BSA solution at 37 °C for 1 h. After washing three times with PBS buffer containing 0.05% Tween-20 (PBST, pH 7.40), 100 μL of anti-AFB_1 mAbs (75 ng/mL) was added and incubated at 37 °C for 1 h. After washing with PBST three times, 50 μL of HRP@AFB_1 solution (0.50 μg/mL) and 50 μL of sample solution were added into each plate well. After incubation at 37 °C for 60 min, the microplates were washed with PBST five times, and then 100 μL of TMB solution was added. After incubation for 15 min at room temperature, the reaction was terminated with 50 μL of 2 M H_2SO_4, and the absorbance was measured at 450 nm using a microplate reader.

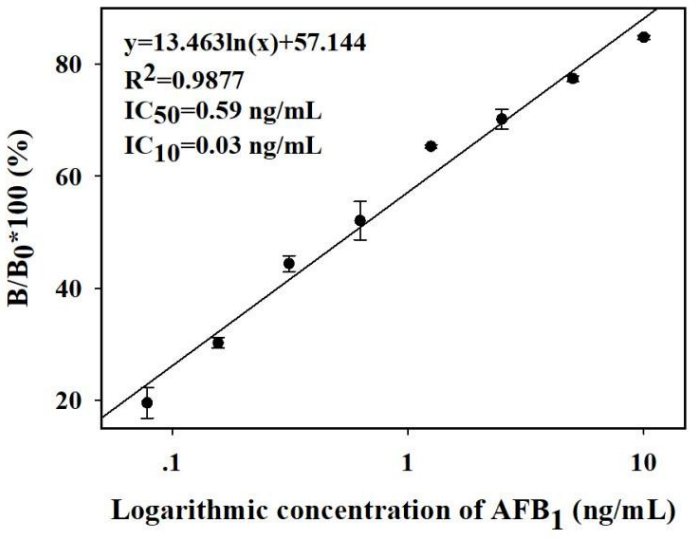

Figure S4. Calibration curve of the conventional immunoassay. The dynamic linear range of AFB_1 concentration is 0.04 - 10 ng/mL. Vertical bars indicate the standard deviation (n = 3).

Table S1. The values of FI in the checkerboard titration.

Dilution ratio of GOx-AFB_1 (588 μg/mL)	Dilution ratio of anti-AFB_1 mAbs (500 μg/mL)				
	2000	4000	8000	16000	32000
200	1.03	1.43	1.16	1.54	6.27
400	1.04	1.00	2.47	3.73	6.36
800	1.36	1.37	2.51	3.86	6.14
1600	3.77	3.98	4.19	4.20	6.25
3200	4.45	6.39	6.40	6.42	6.57

UPLC-FLD Analysis Method

The reliability and practicability of the developed fluorescence immunoassay was further confirmed by UPLC-FLD. Samples pretreatment and UPLC-FLD operation were conducted according to the national standard GB 5009.96-2016 (China) with some modification. Briefly, corn samples (1g) was extracted by ultrasound with 5 mL of 60% (v/v) methanol/water for 20 min. After centrifugation at 6000 rpm for 15 min, 3 mL of supernatant solution was further diluted three folds with 6 mL PBS. Extraction of samples were filtered with 0.22 μM cellulose membrane and spiked with different concentrations of AFB_1. Then, samples were cleaned by immune affinity column and further employed for UPLC-FLD analysis. Chromatographic separation was achieved with an ACQUITY UPLC BEH C18 (1.70 μm 2.10 × 100 mm) column using mobile phase methanol-water (30-70), Ex365 nm and Em436 nm. The flow rate was 0.20 mL/min, column temperature was 30℃ and the injection volume was 1 μL in the full injection mode. The monitoring appearance time was chosen as 2.731 min (quantitation time, standards) and 2.745 (qualitative time, samples).