Electronic Supplementary Material (ESI) for Analytical Methods.
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Electronic Supporting Information for

Specific antibody-induced fluorescence quenching for direct and label-free immunosaaay

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Experimental Details & Methodology

Material and Instrumentation. All reagents were of analytical grade. Anti-Aflatoxin B_1 (AFB_1) antibody 1C11, 3G1 and antibody 1H2 were produced in our laboratory. AFB_1, bovine serum albumin (BSA), ovalbumin (OVA), rabbit anti-mouse polyclone antibody (product #: M7023) and alumina were purchased from Sigma (St. Louis, MO, USA). 2, 6-Di-O-methyl-β-cyclodextrin was obtained from Yuanye Biotech Co. (Shanghai, China). The fluorescence spectrophotometer (F-4500) was purchased from Hitachi. The peanut samples were grinded with food processer, which was obtained from JOYOUNG Co. (JYL-C090, Shandong, China).

Fluorescence scanning of AFB_1 standard. The AFB_1 standard dissolved in methanol was stored in the concentration of 10 μg/mL. Then, it was diluted with methanol-water to different concentrations, which the experiment needed. During the scanning of the fluorescence, the parameters of the fluorescence spectrophotometer were as follows: excitation light, 365nm, optical grating, 2.5 nm; transmitting light optical grating, 10 nm. During the following fluorescence measuring in the following experiment, the parameters were the same as these.

Fluorescence Enhance. The fluorescence enhancer 2, 6-Di-O-methyl-β-cyclodextrin was employed to enhance the intrinsic fluorescence signal of AFB_1. For each milliliter of AFB_1, 500μL 0.01 M 2, 6-Di-O-methyl-β-cyclodextrin was utilized. After adding this enhancer, the fluorescence signal of AFB_1 was scanned immediately.

Immunoreaction between AFB_1 and antibodies and proteins. After measured the fluorescence of the mixture of AFB_1 and fluorescence enhancer, the specific antibodies, non-specific antibodies or non-specific proteins dissolved in water were added into the mixture and shake up to enable them react with AFB_1. Certain times later, the fluorescence of the product was measured and evaluated.

Production of the alumina column. This column was produced in our lab. Shell of the syringe (1 mL) was soaked in methanol overnight to eliminate the fluorescence effect of the shell. Then the solid alumina (1 mL) was added to fulfill the dried shell. This column was used to clean the peanut sample extraction.

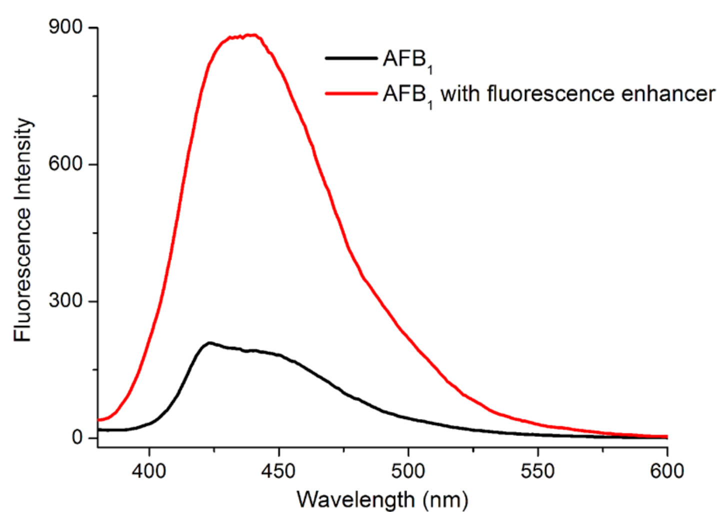

Figure S1. The fluorescence spectra comparison of AFB_1 and AFB_1 with the fluorescence enhancer. AFB_1 was of the concentration of 20 ng/mL dissolved in 10% methanol-water.

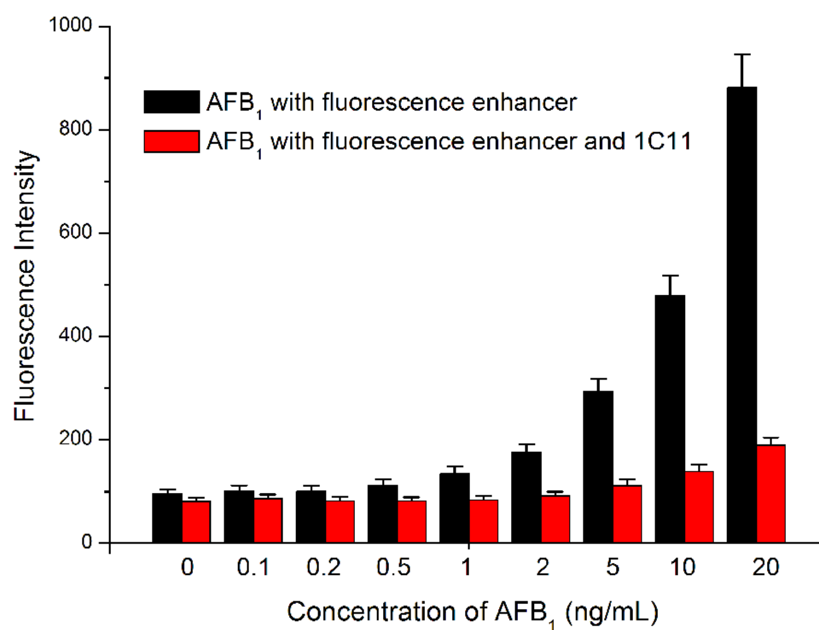

Figure S2. The fluorescence intensity value of serial concentrations of standard AFB_1 at 440 nm before and after the immureation with 1C11.

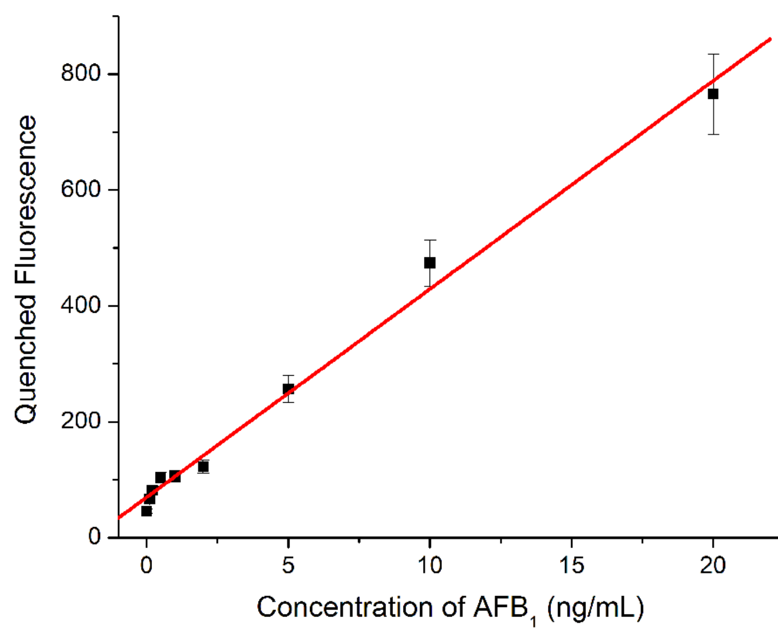

Figure S3. The standard curve of the quenched fluorescence intensity to different concentrations of AFB_1 in peanut sample matrix, y=35.95x+69.63, r^2=0.9959.