Supporting Information

Binding characteristic of various antibody formats against Aflatoxins

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Supplementary Data

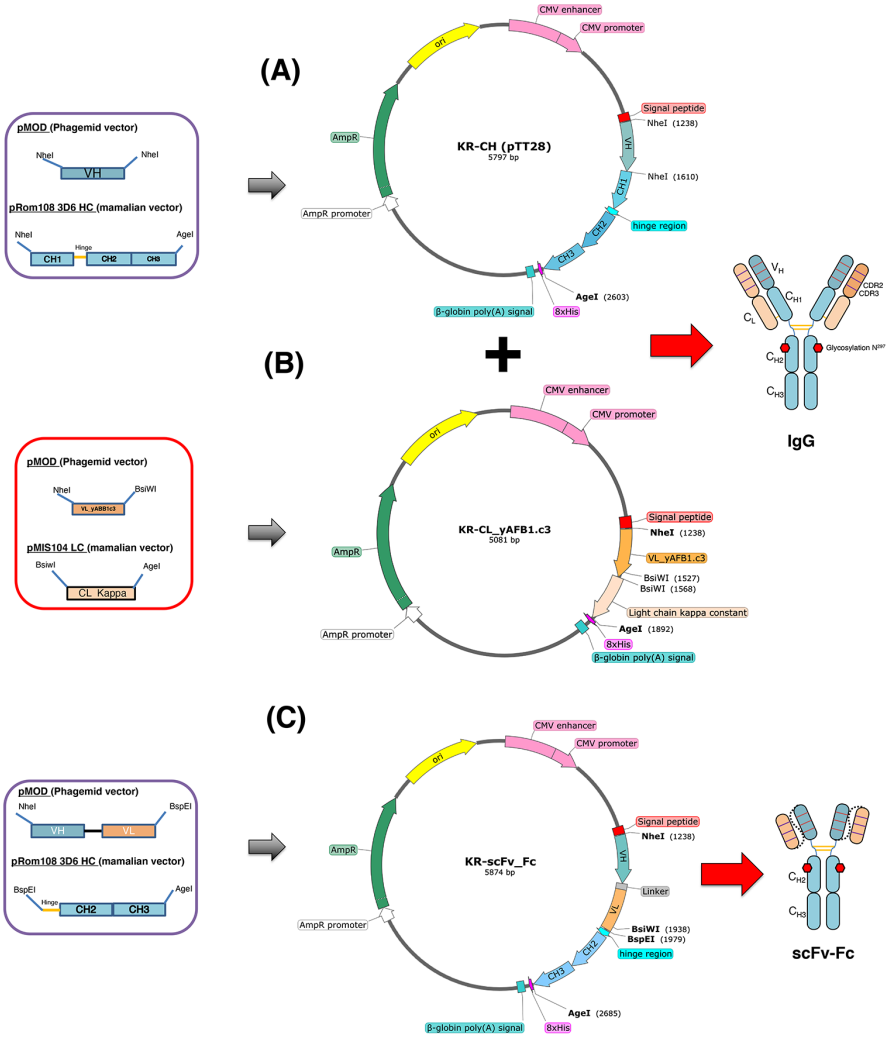

Figure S1. Diagram of IgG and scFv-Fc expression vectors in this study. The heavy chain vector; pKR-CH (A) was constructed by cloning VH genes and constant heavy chain (CH) between restriction sites *Nhe*I/*Nhe*I and *Nhe*I/*Age*I of pTT28 vector, respectively. The VL yAFB1.c3 genes and constant kappa light chain was cloned via *Nhe*I/*BsiW*I and *BsiW*I/*Age*I, respectively to construct a kappa light chain vector; pKR-CL_yAFB1.c3 (B). The mammalian expression vector pKR-scFv-Fc (C) was constructed by cloning scFv genes and Fc region into pTT28 vector, via *Nhe*I/*BspE*I and *BspE*I /*Age*I, respectively.

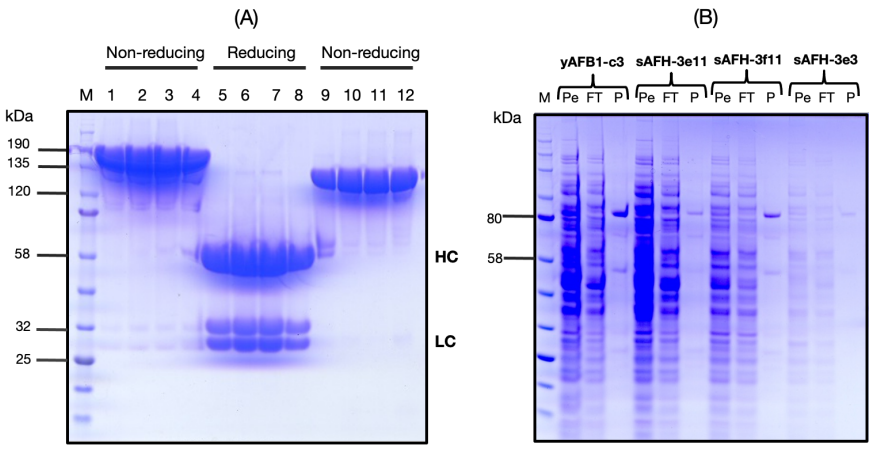

Figure S2. SDS-PAGE analysis of purified scFv-Fc, IgG, and scFv-AP. Fifteen microliters per lane of proteins were analyzed on 12% polyacrylamide gels and stained with coomassie blue. (A) A non-reducing purified IgG (lane 1-4) and scFv-Fc (lane 9-12) appeared at the size of approximately 150 kDa and 120 kDa, respectively. Purified IgG in reducing condition (lane 5-8) shows heavy (HC) and light chains (LC) at approximately 60 kDa and 30 kDa, respectively. M, Precision Plus Protein Dual Color Standards; Lane 1, 5, 9 yAFB1-c3; Lane 2, 6, 10 sAFH-3e11; Lane 3, 7, 11 sAFH-3f11; Lane 4, 8, 12 sAFH-3e3. (B) Hexa-histidine affinity chromatography- purified fractions from periplasmic extracts of scFv-AP clones yAFB1-c3, sAFH-3e11, sAFH-3f11 and sAFH-3e3 expressed from *E.coli* TG1. The purified scFv-AP antibodies are shown at the size of approximately 80 kDa. M, Precision Plus Protein Dual Color Standards; lane Pe, periplasmic fraction; lane FT, flow-through fraction; lane P, purified scFv antibody.

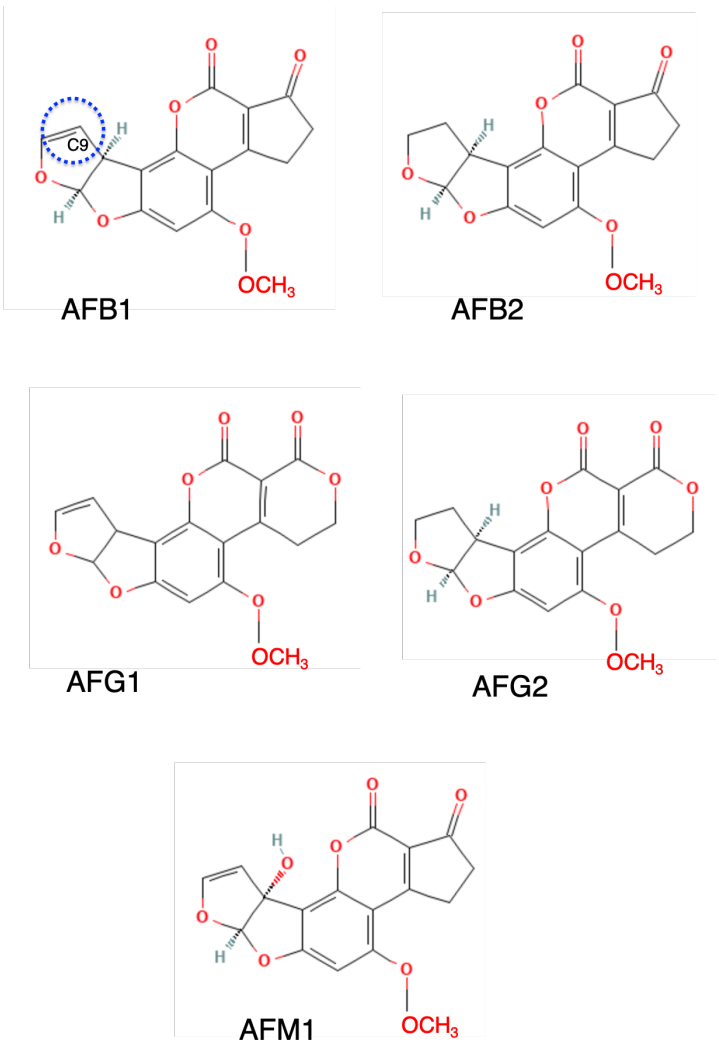

Figure S3. Chemical structures of different types of aflatoxins. The four major types of aflatoxins are B1, G1, B2 and G2. Aflatoxin M1 is the hydroxylated metabolite of aflatoxin B_1. The blue circle indicated the area around C9 where the antibodies likely bind.

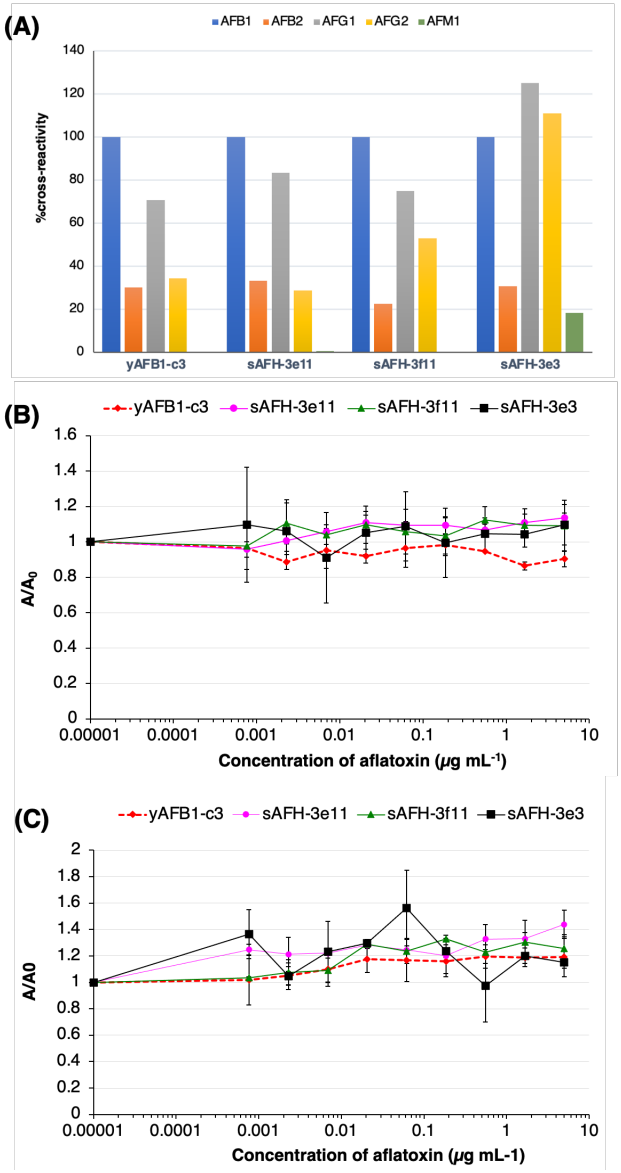

Figure S4. Cross-reactivity of scFv-AP against other aflatoxins and non-related mycotoxins. The competitive ELISA was performed using four different clones of scFv-AP in the presences of various concentrations of mycotoxins. (A) The histogram of data from Table 2, illustrating % cross-reactivity of scFv-AP against AFB1(blue), AFB2 (orange), AFG1 (grey), AFG2 (yellow) and AFM1(green). Competitive ELISA against Zearalenone (B) and Ochratoxin (C) are shown. The data is expressed as an average of triplicate measurement of absorbance at 405 nm with standard deviations. No-cross reactivity was detected.

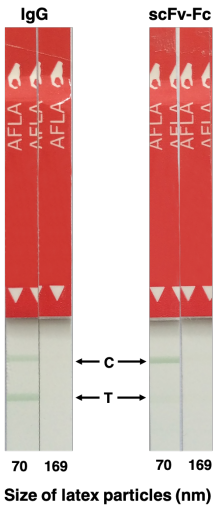

Figure S5. LFIA analysis illustrating the optimal condition of the antibody-latex conjugation. Different size of latex microspheres (70 and 169 nm) conjugated with 0.05 mg mL^{-1} of antibodies (IgG and scFv-Fc) were tested for LFIA. The strip was coated with anti-mouse IgG as control line; C and AFB1-BSA as Test line; T. The green color indicated proper binding conditions.

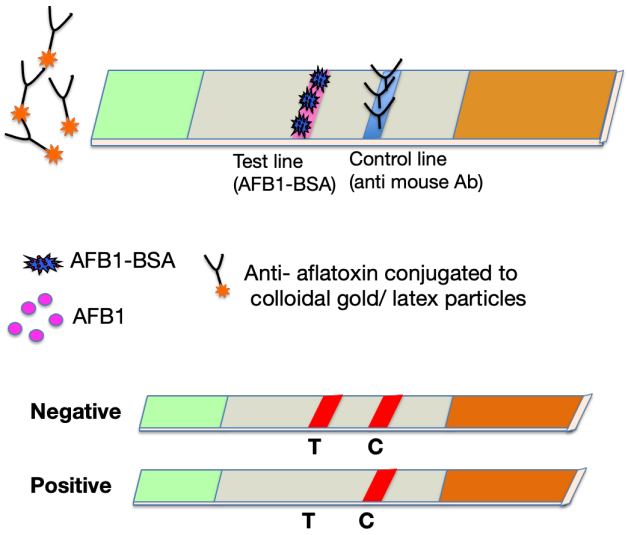

Figure S6. Schematic illustration of a lateral flow assay for the detection of aflatoxin. The visualization of bound antibody at the test line (AFB1-BSA) and control line (anti mouse IgG) was obtained using colloidal gold- (red line) or latex particles- (green line) conjugated anti-aflatoxin IgG. Fading or absence of test line indicated positive result due to the competition of contaminated toxins.