Supplementary Materials

Article

Polycations as aptamer-binding modulators for sensitive fluorescence anisotropy assay of aflatoxin B1

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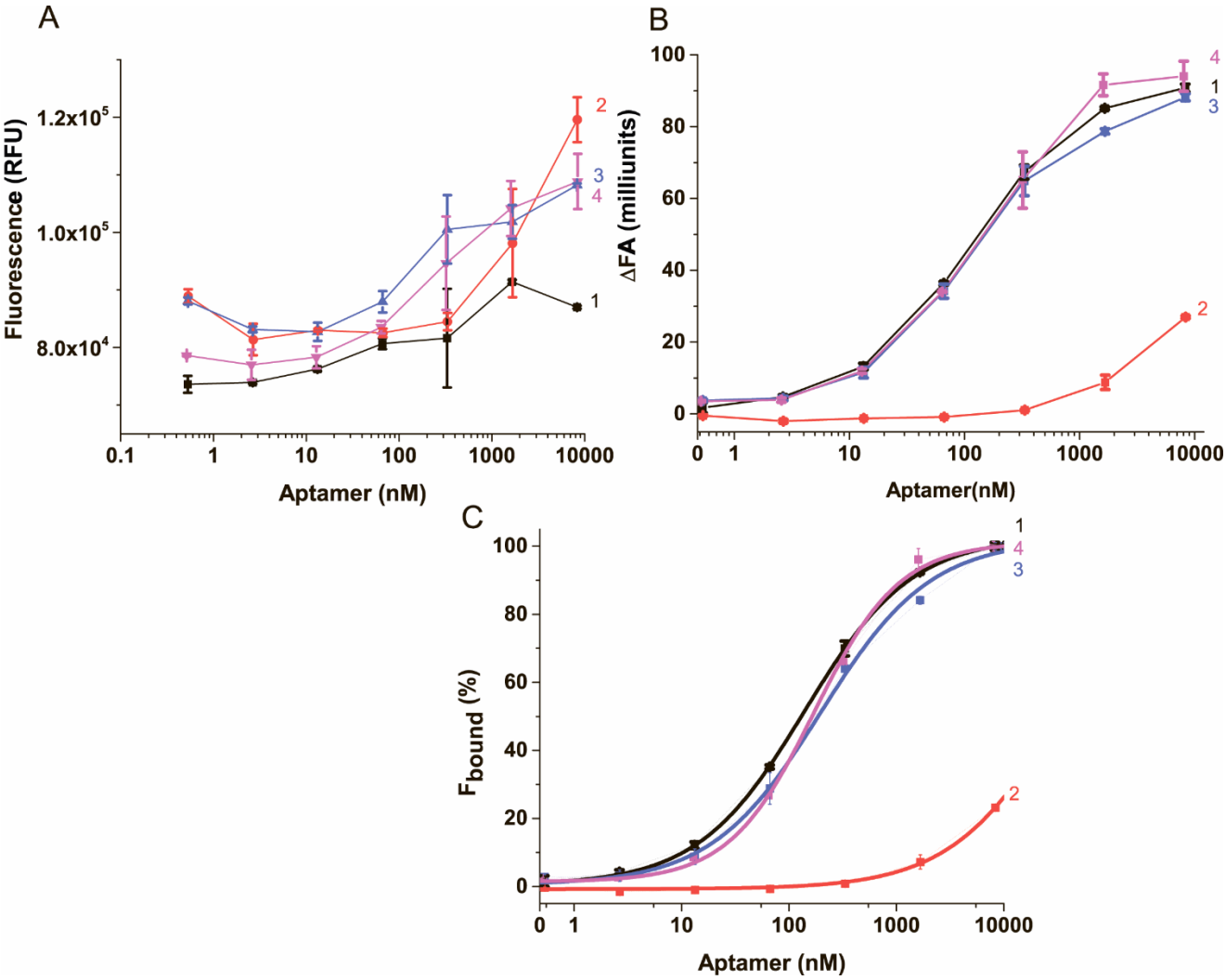

Figure S1. The dependencies of fluorescence (A), the change of fluorescence anisotropy (B), and the percentage of the bound fraction(C) of AFB_1-EDF on the concentration of the untruncated stem-loop aptamer in different buffers: 1 – 20 mM Tris-Acetate, 100 mM Na Acetate, 10 mM $MgAcetate_2$, 10 mM $CaCl_2$, pH 8.4; 2 – 20 mM Tris- Acetate, 100 mM NaAcetate, pH 8.4; 3 – 20 mM Tris- Acetate, 20 mM $MgAcetate_2$, pH 8.4; 4 – 20 mM Tris-Acetate, 100 mM NaAcetate, 20 mM $MgAcetate_2$, pH 8.4 (n=3)

Table S1. The comparison of dissociation constants of the anti-AFB1 loop-stem aptamer in different buffer compositions

#	Buffer composition	K_D (nM)
1	20 mM Tris-Acetate, 100 mM NaAcetate, 10 mM $MgAcetate_2$, 10 mM $CaCl_2$, pH 8.4	145.0±5.3
2	20 mM Tris-Acetate, 100 mM NaAcetate, pH 8.4	30500±4200*
3	20 mM Tris-Acetate, 20 mM $MgAcetate_2$, pH 8.4	190±28.1
4	20 mM Tris-Acetate, 100 mM NaAcetate, 20 mM $MgAcetate_2$, pH 8.4	173.1±9.5

*the K_D value was estimated with an assumption that maxFA is the same as for the buffer#1 composition

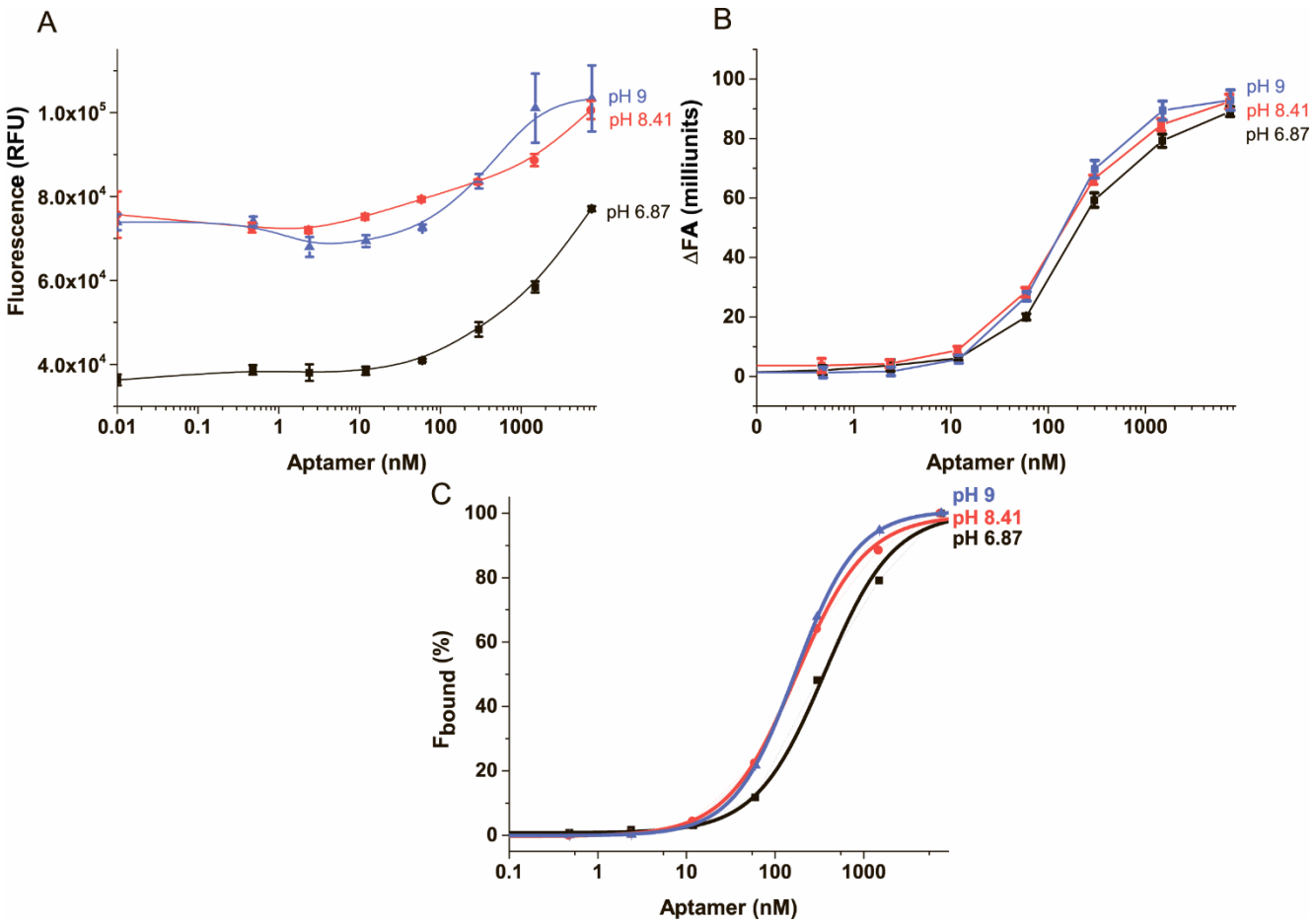

Figure S2. The dependencies of fluorescence (A), fluorescence anisotropy (B), and the percentage of bound fraction (C) of AFB1-EDF on the concentration of initial aptamer at different pH (n=3)

Table S2. The comparison of dissociation constants of the initial anti-AFB1 loop-stem aptamer at different pH

pH	Buffer composition	K_D (nM)
pH 6.8	20 mM Tris-Acetate, 100 mM NaAcetate, 20 mM $MgAcetate_2$	362.5±33.5
pH 8.4		173.1±9.5
pH 9		168.8±1.1

Synthesis of the aptamer-protein complexes

500 µL of 4 µM prepared 5'-biotinylated aptamer 38nt in TB was mixed with 500 µL of 4 or 8 µM streptavidin in TB. Then the obtained stock solutions were incubated for 1 h at room temperature with gentle stirring. After the series of consecutive 5-fold dilutions were prepared and mixed with an equal volume of AFB1-EDF (a final concentration of 5 nM). Each well contains 100µL of solution.

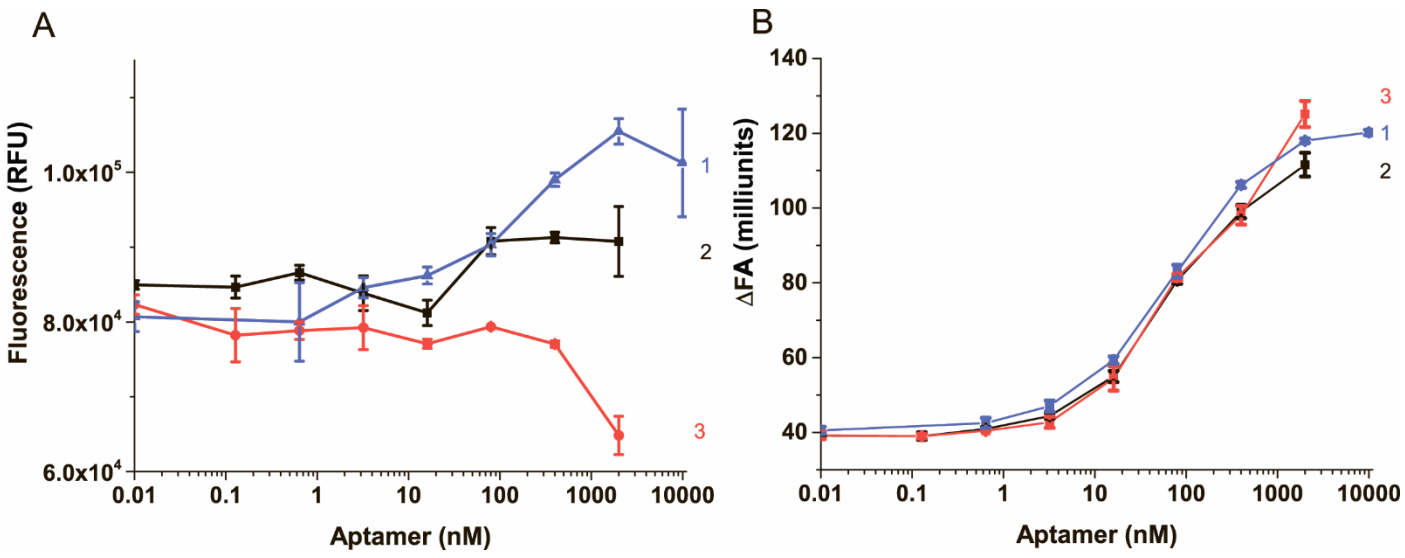

Figure S3. The dependencies of fluorescence (A) and change of fluorescence anisotropy (B) of AFB1-EDF in the presence of aptamer 38nt (1) and its conjugates with streptavidin at ratio 2:1 (2) and 1:2 (3). (n=3)