

Exact tailoring of an ATP controlled streptavidin-binding aptamer

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Experimental section

1. Materials

DNA sequences were synthesized and purified by Sunbiotech Co. Ltd. (Beijing, China). Streptavidin coated sepharose beads (Streptavidin Sepharose High Performance) were obtained from GE Healthcare Bio-Sciences AB (GE Healthcare, Sweden). Adenosine triphosphate (ATP) was obtained from Beijing Xinjingke Biotechnology (Beijing, China). Guanosine triphosphate (GTP), uridine triphosphate (UTP) and Cytidine triphosphate (CTP) were purchased from Sigma-Adirich (St. Louis, MO). 20×SYBR-green I (20 mg/mL) in dimethylsulfoxide (DMSO) was obtained from Fanbo Biochemicals (Beijing, China), 1×SYBR-green I (20 mg/mL) was prepared by diluting 20×SYBR-green I with DMSO. RPMI 1640 medium (Gibico) and fetal calf serum were purchased from Life Technologies Corporation (USA). Plasma was obtained from Beijing Red Cross Blood Center. Prior to use, non-soluble matter was removed by centrifugation at 10,000 rpm for 15 min. The buffer used for all experiments, unless otherwise indicated, was Tris-HCl buffer with 25 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 3 mM MgCl₂ and 0.02% Tween20, pH 7.4. The deionized water was prepared on a UPHW-III-90T UP water purification system (Chengdu, China). All the fluorescence measurements were recorded by SpectraMax M5 (Molecular. Devices Corporation, USA).

2. The binding ability of Fused Allosteric Aptamer

100 μL of 0.5 μM FAA (FAA-1 or FAA-2) was incubated with 1mM ATP and 10 μL of streptavidin coated beads at 25°C for 20 min with slight shaking. After incubation, the sample was transferred to an empty column and washed with 50 μL of Tris-HCl buffer. Then the streptavidin coated beads was transferred to a 96-well plate (Costar), and added 10 μL 1 \times SYBR green I and 90 μL Tris-HCl buffer. After mixing, the fluorescence intensity was measured by SpectraMax M5 with excitation at 485 nm and emission at 525 nm.

3. CD spectra measurement

CD spectra (220-300 nm) were collected on a Jasco J-815 circular dichroism spectropolarimeter (JASCO, Japan) at a rate of 500 nm/min in a fused quartz cell (400 μL 1 cm). Measurements were carried out in Tris-HCl buffer at room temperature. The CD spectra were applied background subtraction, and smoothed.

4. Fluorescence response of FAA-2 to different concentrations of ATP

100 μL of 0.5 μM FAA-2 was incubated with different concentrations of ATP and 10 μL of streptavidin coated beads at 25 °C for 20 min with slight shaking. After incubation, the sample was transferred to an empty column and washed with 50 μL of Tris-HCl buffer. Then the streptavidin coated beads was transferred to a 50 μL centrifuge tube and added 10 μL 1 \times SYBR green I. After shaking, the fluorescence was observed under UV-light and photographed. Then the sample was transferred to a 96-well plate (Costar) and added 90 μL of Tris-HCl buffer. After mixing, the fluorescence intensity was measured by SpectraMax M5 with excitation at 485 nm and emission at 525 nm

5. Selectivity of FAA-2

100 μL of 0.5 μM FAA-2 contained 10 μL of streptavidin coated beads was incubated with 1 mM ATP, GTP, UTP or CTP at 25 °C for 20 min with slight shaking. After incubation, the sample was transferred to an empty column and washed with 50 μL of Tris-HCl buffer. The streptavidin coated beads were transferred to a 96-well plate and then added 10 μL of 1 \times SYBR green I and 90 μL of Tris-HCl buffer. After mixing, the fluorescence intensity was measured by SpectraMax M5 with excitation at 485 nm and emission at 525 nm.

6. Measurement of apparent equilibrium dissociation constant (Kd)

The affinity of FAA-2 to streptavidin was measured by incubating different of FAM labeled FAA-2 with 2 μ L streptavidin-coated beads in the present of 2mM ATP at 25°C for 20 min with slight shaking. After incubation, the concentrations of free FAA-2 were determined by measuring the fluorescence intensity of supernatant. The streptavidin coated beads were washed with 100 μ L binding buffer in the present of 2mM ATP, then the bound FAA-2 were eluted with 150 μ L of 5mM biotin. The amounts of bound aptamers were calculated from the fluorescence intensities of the eluates. The apparent K_d of FAA-2 to streptavidin was obtained by fitting the amount of bound aptamers against the concentration of free aptamers to the equation $Y = B_{max} X / (K_d + X) + Ns * x$, using SigmaPlot (Jandel, San Rafael, CA).

7. Response of FAA-2 to ATP in biological sample

100 μ L of 0.5 μ M FAA-2 was incubated with 0 or 1 mM ATP and 10 μ L of streptavidin coated beads in 30% human plasma, 30% RPMI 1640 medium (with/without 10% fetal calf serum) at 25°C for 20 min with slight shaking. After incubation, the sample was transferred to an empty column and washed with 50 μ L of Tris-HCl buffer. The streptavidin coated beads were transferred to a 96-well plate and then added 10 μ L of 1 \times SYBR green I and 90 μ L Tris-HCl buffer. After mixing, they were measured on a 96-well plate (Costar) by SpectraMax M5 with excitation at 485 nm and emission at 525 nm.

8. Regeneration of streptavidin coated beads

100 μ L of 0.5 μ M FAA-2 was incubated with 1 mM ATP and 10 μ L of streptavidin coated beads at 25°C for 20 min with slight shaking. After incubation, the sample was transferred to an empty column and washed with 50 μ L of Tris-HCl buffer. The streptavidin coated beads was transferred to a 96-well plate, and then added 10 μ L of 1 \times SYBR green I and 90 μ L Tris-HCl buffer. After mixing, they were measured on a 96-well plate (Costar) by SpectraMax M5 with excitation at 485 nm and emission at 525 nm. The beads were transferred to an empty column and washed with 50 μ L of 10% DMSO. Regeneration of the streptavidin coated beads was performed by washing with 2 \times 50 μ L of Tris-HCl buffer. Then the regenerated streptavidin coated beads were incubated with FAA-2 and ATP mixture and treated as described above for additional 5 times.

Table S1. Sequences of the DNA strands used.

| Name | Sequences(From 5' to 3') |
|-------|--|
| FAA-1 | ACCTGGGGGAGTATACCGCTGTGTGACGCAACACTTGCGGAGGAAGGT |
| FAA-2 | ATACCTGGGGGAGTATACCGCTGTGTGACGCAACACTAGCGGAGGAAGGTAT |

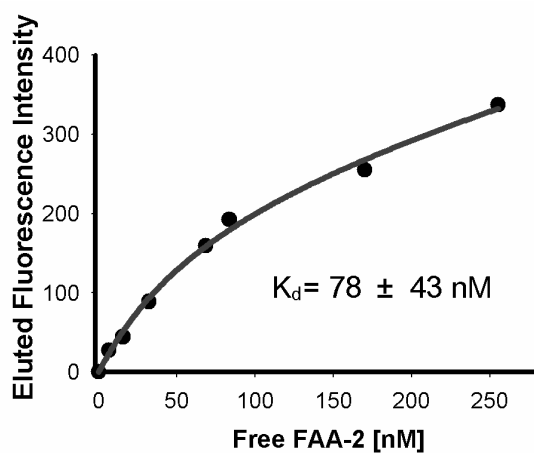


Fig S1 Binding curves of FAA-2 on streptavidin-coated beads in the presence of 2mM ATP.

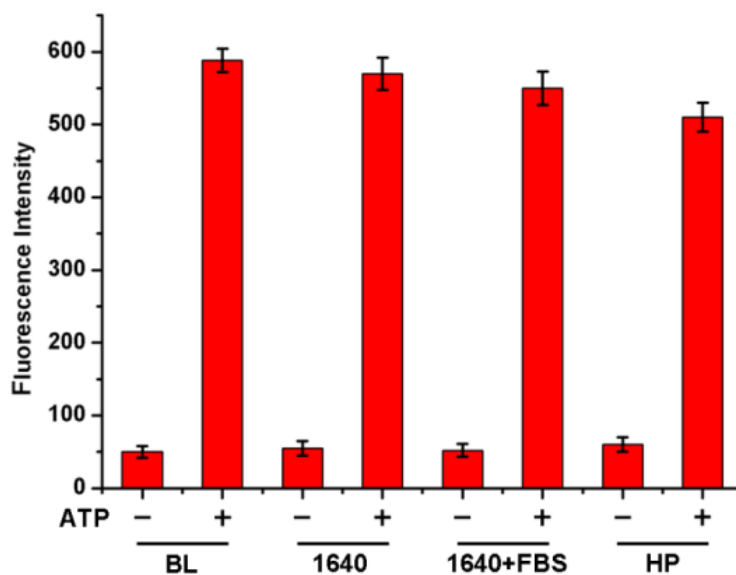


Fig. S2 Response of FAA-2 (0.5 μ M) to 1 mM ATP in biological samples (BL: Tris-HCl buffer; 1640: 30% RPMI 1640 cell culture medium; 1640+FBS: 30% RPMI 1640 medium with 10% fetal calf serum; HP: 30% human plasma).

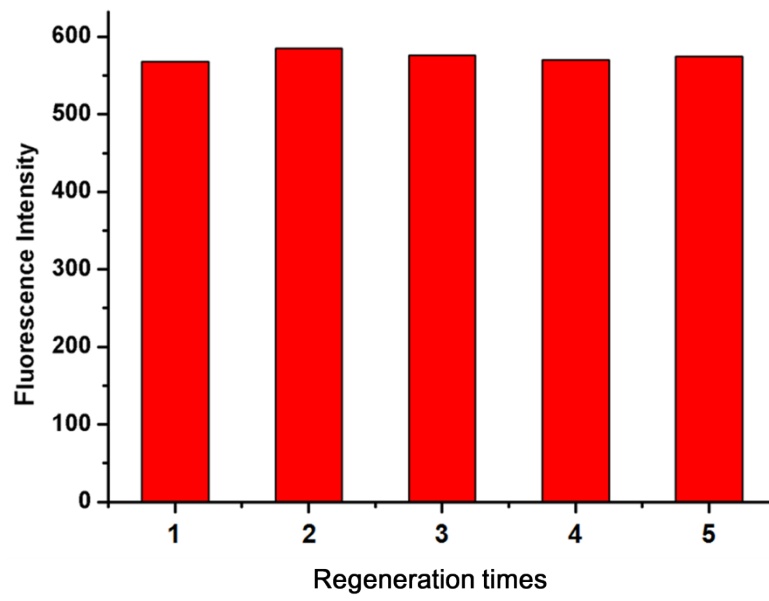


Fig. S3 The binding of FAA-2 on the reused streptavidin coated beads in the present of 1 mM ATP.