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Electronic Supporting Information

Split Aptazyme-Based Catalytic Molecular Beacon for Amplified Detection of Adenosine

Jin Huang, Yong He, Xiaohai Yang, Kemin Wang*, Ke Quan, Xiaoping Lin.

State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Key Laboratory for Bio-Nanotechnology and Molecular Engineering of Hunan Province, Hunan University, Changsha 410082, P. R. China

Chemicals and Materials

Oligonucleotides used in this work were synthesized and purified by Takara Biotechnology Co. Ltd. (Dalian, China), and their sequences are shown in Table 1 in the main text. Adenosine and the analogures were purchased from Sigma-Aldrich Chemical Co. Ltd. All work solutions were 50 mM HEPES (200 mM NaCl, pH 7.0). The pH measurements were carried out using an Orion 3 Star pH meter (Thermo Fisher, USA). All solutions were prepared with Milli-Q water (18.2 MΩ.cm) from a Millipore system.

Fluorescence measurement

Fluorescence measurements were carried out on a Hitachi F-7000 fluorescence spectrometer (Hitachi. Ltd., Japan). The excitation wavelength was set at 494 nm (slit 5 nm), and the emission spectra were collected from 510 nm to 650 nm (slit 5 nm). The fluorescence intensity at 521 nm was used to evaluate the performances of the proposed assay strategy.

Procedure for conditions optimizing

1. **Zn^{2+} concentration:** 0 or 1 mM adenosine were incubated with 100 nM SA1 and SA4 in the buffer with different Zn^{2+} concentrations (50 mM HEPES , 200 mM NaCl, 10 mM $MgCl_2$, pH 7.0) for 30 min at 25 ^{o}C. Then, 300 nM MB were added into the above solution and incubated for another 2 h. Finally, the fluorescence emission intensity at 521 nm was measured.
2. **Mg^{2+} concentration:** 0 or 1 mM adenosine were incubated with 100 nM SA1 and SA4 in the buffer with different Mg^{2+} concentrations (50 mM HEPES , 200 mM NaCl, 0.5 mM $ZnCl_2$, pH 7.0) for 30 min at 25 ^{o}C. Then, 300 nM MB were added into the above solution and incubated for another 2 h. Finally, the fluorescence emission intensity at 521 nm was measured.
3. **SA sequences:** 0 or 1 mM adenosine were incubated with 100 nM different split-aptazyme pairs in the buffer (50 mM HEPES , 200 mM NaCl, 10mM $MgCl_2$, 0.5 mM $ZnCl_2$, pH 7.0)

for 30 min at 25 ^{o}C. Then, 300 nM MB were added into the above solution and incubated for another 2 h. Finally, the fluorescence emission intensity at 521 nm was measured.

4. **Concentration ratio of SA to MB:** 0 or 1 mM adenosine were incubated with 100 nM SA2 and SA6 in the buffer (50 mM HEPES , 200 mM NaCl, 10mM $MgCl_2$, 0.5 mM $ZnCl_2$, pH 7.0) for 30 min at 25 ^{o}C. Then, different concentrations of MB were added into the above solution and incubated for another 2 h. Finally, the fluorescence emission intensity at 521 nm was measured.

Procedure for adenosine detection

Different concentrations of adenosine were incubated with 100 nM SA2 and SA6 in the buffer (50 mM HEPES , 200 mM NaCl, 10 mM $MgCl_2$, 0.5 mM $ZnCl_2$, pH 7.0) for 30 min at 25 ^{o}C. Then, 400 nM MB were added into the above solution and incubated for another 2 h. Finally, the fluorescence emission intensity at 521 nm was measured.

Procedure for selectivity

Adenosine and its analogues were incubated with 100 nM SA2 and SA6 in the buffer (50 mM HEPES , 200 mM NaCl, 10 mM $MgCl_2$, 0.5 mM $ZnCl_2$, pH 7.0) for 30 min at 25 ^{o}C separately. Then, 400 nM MB were added into the above solution and incubated for another 2 h. Finally, the fluorescence emission intensity at 521 nm was measured.

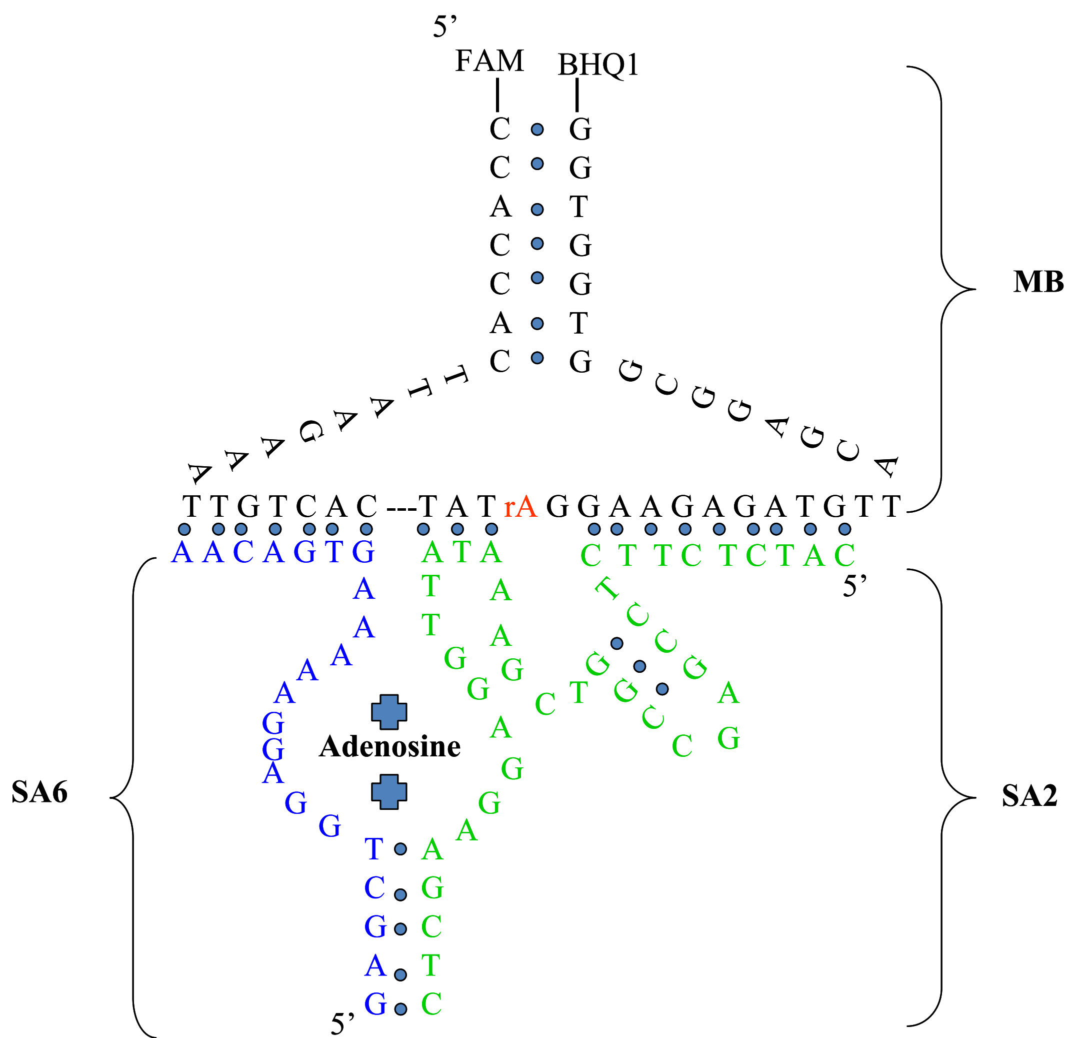

Figure S1. The primary and the proposed secondary structure of the adenosine detection system built on MB, SA2 and SA6.

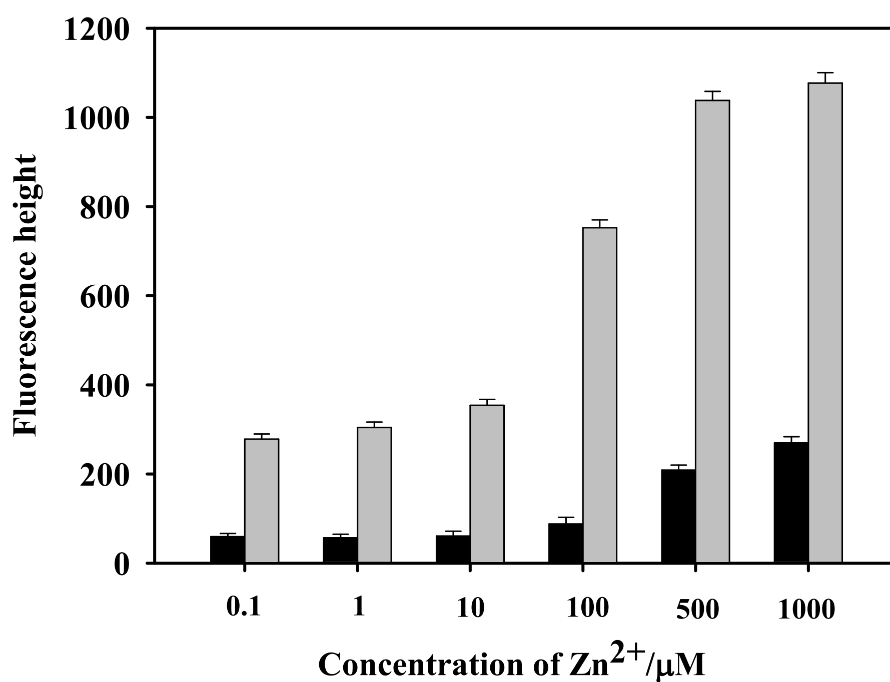

Figure S2. Optimization of concentration of Zn^{2+}. (Black and gray denote fluorescence background and signal, respectively).

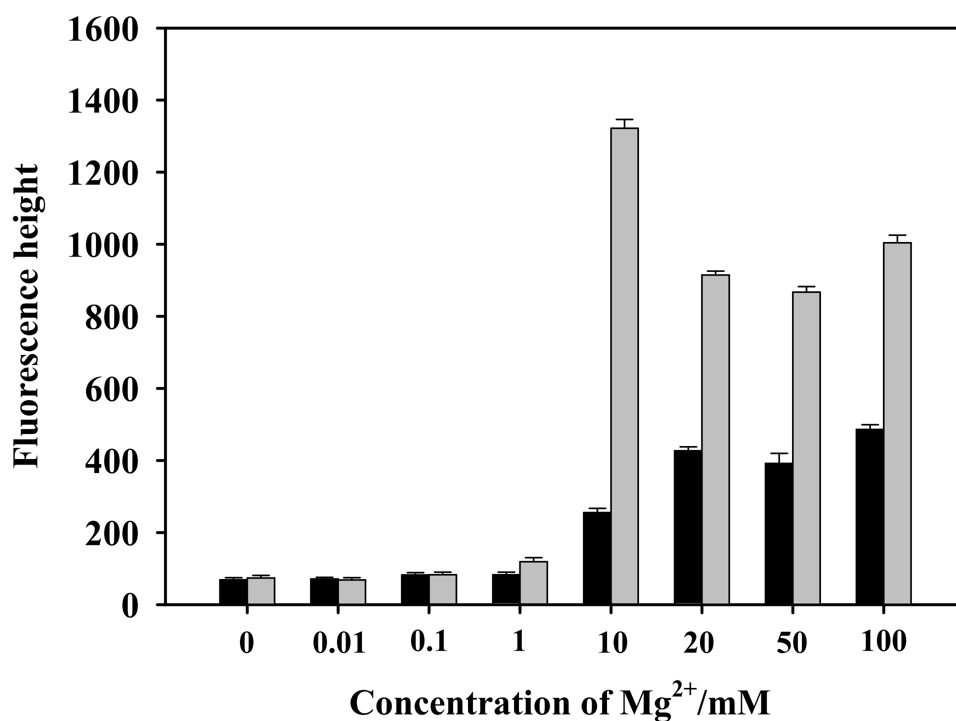

Figure S3. Optimization of concentration of Mg^{2+}. (Black and gray denote fluorescence background and signal, respectively).