Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

Rational Design of an Optical Adenosine Sensor by Conjugating a DNA Aptamer with Split DNAzyme Halves

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Experimental Section

Chemicals and Materials

Unless otherwise mentioned, all chemicals were purchased from Bio Basic Inc. (BBI) through Shanghai Sangon Bioengineering Technology and Services Co., Ltd. (Shanghai, China). Hemin stocking solution (5 mM) was prepared in DMSO, which was then stored in a freezer and shielded from light. 30% hydrogen peroxide (H_2O_2) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

DNA oligonucleotides with the following sequences were custom-synthesized and PAGE (polyacrylamide gel electrophoresis) purified by Shanghai Sangon Bioengineering Technology and Services Co., Ltd. (Shanghai, China).
L-strand (5'-3'): **GGGTTGGG***TCTTTCAGTCCGTCA*ACCTGGGGGAGTATTGCGGAGGAAGGT**GGGTAGGG**;
S-strand (5'-3'): *TGACGGACTGAAAGA*

Bold bases correspond to the G-quadruplex halves, italic bases represent two complementary sequences responsible for the formation of a rigid duplex spacer in the assembled sensor, and underlined is the sequence of an anti-adenosine aptamer.

Assay method

A solution containing pre-annealed (95°C for 2 min, 65°C for 5 min, 50°C for 10 min, 37°C for 10 min, and room temperature for at least 30 min) L and S-strands (final concentrations are 0.1 μM) and adenosine in a 50 mM Tris-HCl buffer (pH 7.4) supplemented with 10 mM KCl, 0.1 M NaCl and 5 mM $MgCl_2$ was allowed to stay at room temperature for 30 minutes after the addition of adenosine. 1.1 μL of hemin solution (10 μM in DMSO, diluted from a 5 mM stocking solution) was then introduced to the above solution mixture. The resulting solution was then vortex-stirred several times to obtain a hemin-intercalated G-quadruplex within the sensor structure. ABTS (final concentration is 0.5 mM) and H_2O_2 (final concentration: 1.2 mM) were finally added to the system one hour after the addition of hemin. Final volume of a sample was ensured to be 110 μL by supplementing with buffer solution when necessary for absorbance measurements in a microcuvette.

Oxidation reactions of ABTS were carried out in the aforementioned buffer containing 50 mM Tris-HCl, 10 mM KCl, 0.1 M NaCl and 5 mM $MgCl_2$, and the final concentrations for ABTS and H_2O_2 were 0.5 mM and 1.2 mM, respectively. Kinetical absorbance-time curves were recorded on a U-2910 UV-Vis spectrophotometer (Hitachi, Japan) by continuously monitoring the characteristic absorbance of the oxidation product (a cationic radical: $ABTS^{\cdot+}$) at 418nm for 8 min with the starting absorbance adjusted to zero by the control software. Inaccessible time gaps between sample mixings and absorbance measurements were controlled to be constant and as short as possible to guarantee inter-data consistency (15 seconds in all cases).

Fig. S1 Kinetical absorbance-time curves of the adenosine assay in response to the other three nucleoside analogues including (a) cytidine, (b) uridine and (c) guanosine. Note that the absorbance-time curve for guanosine with a concentration larger than 500 μM shows a slower increase of absorbance at the beginning. The reason for this phenomenon is still unknown, but it seemed to have a relationship with the relatively low solubility of guanosine as observed in our experiments. White precipitates of undissolved guanosine at intended concentrations larger than 500 μM were visually distinguishable in the buffers. We thus excluded data points for larger guanosine concentrations in Fig. 2.