Supporting Information

A Label-Free and Colorimetric Turn-on Assay for Coralyne based on Coralyne-induced Formation of Peroxidase-mimicking Split DNAzyme

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Reagents and Instruments

Oligonucleotides were synthesized by SBS Genetech. Co., Ltd. (Shanghai, China). Hydrogen peroxide, ABTS, coralyne, hemin, TritonX-100, palmatine and berberine were purchased from Sigma–Aldrich. HEPES, NaOH, $MgCl_2$, NaCl, KCl were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All other reagents were of analytical grade and were used without further purification or treatment. Double distilled water (DDW) was used throughout the measurements. UV–vis absorbance measurements were performed on a Shimadzu UV-2600 Spectrophotometer (Japan).

Procedure of Assay

In the coralyne assay, strand A and strand B (15 μL, 5 μM) were hybridized with each other in 50 mM HEPES buffer, and the solution was heated at 90 °C for 10 min and gradually cooled to room temperature. Then, an appropriate concentration of coralyne was added into the DNA solution, and the

mixture was incubated at 37 °C for 40 min, allowing coralyne induced poly(A) to form the homo-adenine DNA duplex. An equal volume of the 2 × HEPES buffer (50 mM HEPES, pH 7.4, 40 mM KCl, 400 mM NaCl, 0.1% Triton X-100, 2% DMSO) was added to the DNA solutions, and the DNA sequences were allowed to fold for 40 min at room temperature. Then, 1.0 μM of hemin solutions was added, and the mixture was incubated at room temperature for 1 h to form the hemin–G-quadruplex DNAzymes. Colorimetric analysis utilizing G-quadruplex DNAzyme was performed in the ABTS-H_2O_2 reaction system at room temperature. 3.0 mM of ABTS and 2.0 mM of H_2O_2 were added. Then, the absorption spectrum of the reaction product $ABTS^{\bullet+}$ was recorded by a Shimadzu UV-2600 Spectrophotometer after the reaction had run for 5 min. The absorbance at 420 nm was used for quantitative analysis.

Table S1 The oligonucleotide sequence

Oligonucleotide	Sequence
Strand A	5'-GGGGGGC$(A)_{20}$TTT$(A)_{20}$CGGGGGG-3'
Strand B 1	5'-TTTTTTTTAAATTTTTTTT-3'
Strand B 2	5'-TTTTTTTTTTAAATTTTTTTTTT-3'
Strand B 3	5'-TTTTTTTTTTTTAAATTTTTTTTTTTT-3'
Strand B 4	5'-TTTTTTTTTTTTTTTTAAATTTTTTTTTTTTTTT-3'

The red region of strand A is the coralyne recognition sequence. The blue region of strand A is the peroxidase-like split G-quadruplex-hemin DNAzym sequence.

Table S2 Results for the detection of coralyne in real samples

$C_{Original}$ (μM)	C_{Added} (μM)	C_{Found} (μM)	Recovery (%)
0.50	1.00	1.52	102.0
1.50	3.00	4.40	96.67

Concentrations: ABTS, 3 mM; H_2O_2, 2 mM; Hemin, 1 μM.

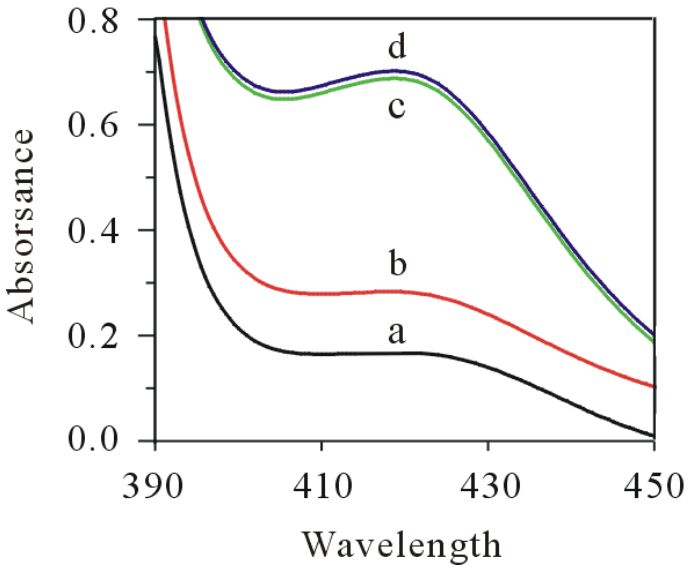

Fig. S1 UV-vis absorption spectra of the ABTS-H_2O_2 reaction system containing 1 μM hemin in the presence of (a) strand A/strand B, (b) strand A, (c) strand A/strand B with 2 μM coralyne and (d) strand A with 2 μM coralyne.

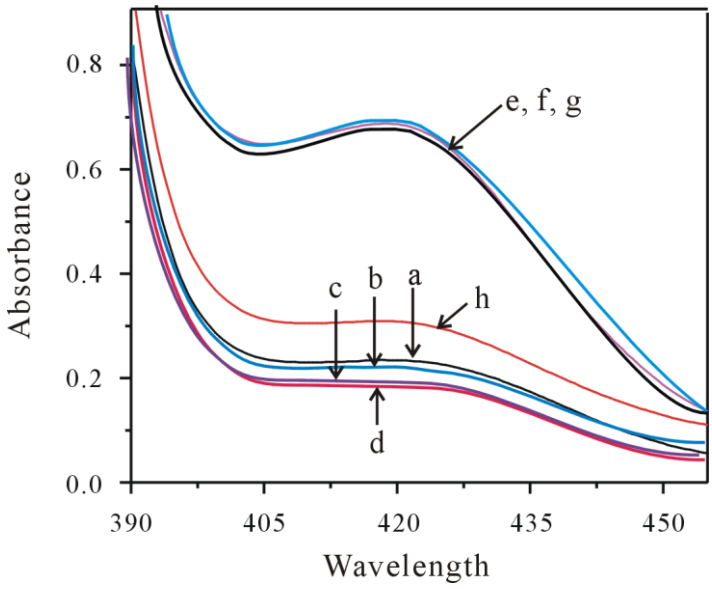

Fig. S2 UV-vis absorption spectra of the ABTS-H_2O_2 reaction system containing 1 μM hemin in the presence of (a) strand A/strand B 1, (b) strand A/strand B 2, (c) strand A/strand B 3, (d) strand A/strand B 4, (e) strand A/strand B 1 with 2 μM coralyne, (f) strand A/strand B 2 with 2 μM coralyne, (g) strand A/strand B 3 with 2 μM coralyne, (h) strand A/strand B 4 with 2 μM coralyne.

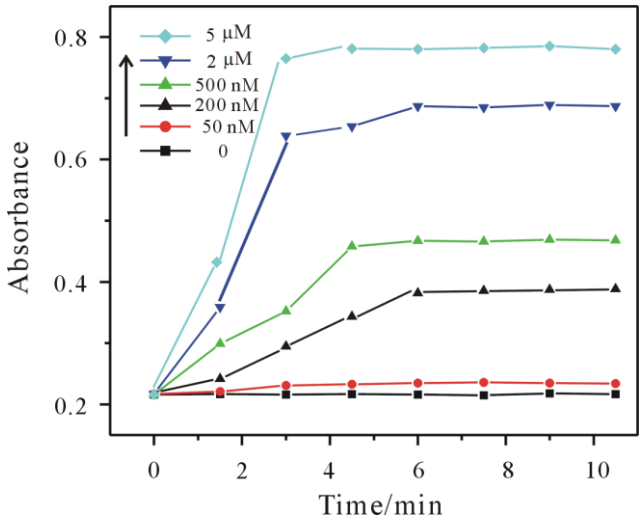

Fig. S3 The plot of the absorbance at 420 nm of the ABTS-H_2O_2 reaction system containing 1 μM hemin versus time, with various concentrations of coralyne. (The data were recorded at the interval of 1.5 min.)