Supplementary Material (ESI) for Chemical Communications

Supporting Information

Enhanced catalytic DNAzyme for label-free colorimetric detection of DNA

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Experimental Details

Materials: Hemin and twelve single-strand DNAs (S1, 5' CGA TTC GGT ACT GGC TCA AAA TGT GGA GGG T 3'; S2, 5' AGG GAC GGG AAG AAA GAT AAT GCG CAT GCT CAA 3'; S3, 5' TTG AGC ATG CGC ATT ATC TGA GCC AGT ACC GAA TCG 3'; S1', 5' CTG GCT CAA AAT GTG GAG GGT 3'; S2', 5' AGG GAC GGG AAG AAA GAT AAT 3'; S3', 5' ATT ATC TGA GCC AG 3'; S1'', 5' GGT ACT GGC TCA AAA TGT GGA GGG T 3'; S2'', 5' AGG GAC GGG AAG AAA GAT AAT GCG CAT 3'; S3'', 5' ATG CGC ATT ATC TGA GCC AGT ACC 3'; S1''', 5' CGA TTC CGA TTC GGT ACT GGC TCA AAA TGT GGA GGG T 3'; S2''', 5' AGG GAC GGG AAG AAA GAT AAT GCG CAT GCT CAA GCT CAA 3'; S3''', 5' TTG AGC TTG AGC ATG CGC ATT ATC TGA GCC AGT ACC GAA TCG GAA TCG 3') were purchased from Sangon Biotechnology Co., Ltd (Shanghai, China). Triton X-100 and 2,2-azinobis(3-ethylbenzothiozoline)-6-sulfonic acid (ABTS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 30% H_2O_2 was purchased from Sinopharm Group Chemical Reagent Co., Ltd (Shanghai, China). The stock solution of hemin (5 mM) was prepared in DMSO, stored in the dark at –20 □, and diluted to the required concentration with the binding buffer (25 mM HEPES, pH 7.4, 20 mM KCl, 200 mM NaCl, 0.05% (w/v) Triton X-100, 1% (v/v) DMSO). The stock solutions (60 mM) of ABTS and H_2O_2 were prepared in water. 18 MΩ water

purified by a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout.

Instrumentation: A Cary 500 Scan UV-Vis-NIR Spectrophotometer (Varian, Harbor City, CA, USA) was used to record the absorption spectra of $ABTS^{\cdot+}$ at room temperature in the wavelength range from 390 to 500 nm.

Preparation and treatment of hemin/G-quartet DNAzyme: The DNAzyme was prepared as reported previously.[1] Briefly, 5 OD of S1 (or S1', S1'', S1''') and S2 (or S2', S2'', S2''') were prepared in the TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.4). The two DNA solutions were mixed at 1:1 volume ratio, and heated at 88 □ for 10 min to dissociate any intermolecular interaction, then gradually cooled to room temperature. An equal volume of the hybridization 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 mixture. At room temperature, this mixture was allowed to fold overnight, and then incubated with hemin of same molar concentration for over 12 h to form the hemin/G-quartet complex. Finally, the DNAzyme was treated with S3 (or S3', S3'', S3''') overnight at room temperature.

Colorimetric measurement: Just before use, the stock solution of ABTS was diluted to 6 mM with the detection buffer (25 mM HEPES, pH 8.0, 20 mM KCl, 200 mM NaCl, 0.05% Triton X-100, 1% DMSO). To 980 μL of 6 mM ABTS solution was added 10 μL of 60 mM H_2O_2. To this mixture was quickly added 0.5 μM DNAzyme (final concentration). The absorption spectra of the reaction mixture within 4 min were recorded by the UV-Vis spectrophotometer.

References:

1. Y. Xiao, V. Pavlov, R. Gill, T. Bourenko and I. Willner, *ChemBioChem*, 2004, **5**, 374.