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Supplementary Information

G-quadruplex-based DNAzyme for sensitive mercury detection with the naked eye

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Experimental Details

Materials. Two G-quadruplex DNAs (HT-DNA: 5'-TTA GGG TTA GGG TTA GGG TTA GGG TTA-3', AGRO100: 5'-GGT GGT GGT GGT TGT GGT GGT GGT GG-3') and hemin were purchased from Sangon Biotechnology Co., Ltd (Shanghai, China). Before use, these oligonucleotides were dissolved in 25 mM Tris-Ac buffer (pH 8.0), and quantified by using UV−Vis absorption spectroscopy with the following extinction coefficients (ε_{260nm}, M^{-1} cm^{-1}): A = 15400, G = 11500, C = 7400, T = 8700. The stock solution of hemin (5 mM) was prepared in DMSO, stored in the dark at –20 ℃, and diluted to the required concentration with aqueous buffer.

Preparation of hemin−G-quadruplex complexes modulated by Hg^{2+}. A solution of 80 nM HT-DNA (in 25 mM Tris−Ac, pH 8.0) were heated at 88 ℃ for 10 min, and gradually cooled to room temperature. Then, to this solution was added an equal volume of the Tris-Ac buffer (25 mM Tris-Ac, pH 8.0, 20 mM KAc, 100 mM NaAc, 0.05% Triton X-100), allowing the folding of HT-DNA for 40 min into the G-quadruplex structure. Then, an equal volume of 8 nM hemin was added and incubated for 1 h to form the hemin−HT-DNA complex. Finally, an equal volume of different concentrations of Hg^{2+} (in Tris−Ac buffer) was added, and this mixture was allowed to incubate at room temperature for further 20 min.

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Visual detection. In a typical experiment, 980 μL of 400 μM TMB solution (25 mM MES−Ac, pH 4.5, 20 mM KAc) was added into a clear glass vial, followed by 10 μL of the hemin-G-quadruplex complexes prepared above. Then, 10 μL of 200 mM H_2O_2 was quickly added to initiate the TMB−H_2O_2 reaction, allowing this reaction for 10 min at 37 ℃. Finally, the reaction was terminated by addition of 200 μL HCl (1 M). When the solution color ultimately turned to yellow, the photograph of the reaction mixture was taken using a digital camera. All photographs were used without further processing.

Supplementary Figures

It is found pH of detection solution has different influence on DNAzyme activity and hemin catalysis (Fig. S1). At pH 4.5, the hemin−HT-DNA DNAzyme functions well,

Fig. S1 Effect of pH of the reaction solution on the DNAzyme activity and the background imparted by hemin catalysis.

whereas the background is the lowest. Acidic conditions have proven to favor the TMB oxidation. This explains why the DNAzyme-catalyzed reaction is promoted when pH decreases. However, for hemin catalysis, another factor need to consider.

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The uncomplexed hemin is directly exposed to the solvent. In particular, it is subject to dimerization in aqueous solution. This significantly influences the intrinsic activity of hemin. That is, the change of ambient environment has a bigger influence on the intrinsic activity of hemin than that of DNAzyme. Presumably, a solvent environment of pH 4.5 furthest inhibits the hemin activity, albeit favorable for TMB oxidation. As a result, this condition confers the lowest background imparted by hemin catalysis. This pH is thus chosen in the following experiments.

Within 10 min reaction, the DNAzyme activity increases with the concentration of H_2O_2 (Fig. S2A). However, a little precipitate is observed when the concentration of H_2O_2 is too high. This phenomenon is more obvious if the reaction time increases to 30 min. In view of these factors, 2 mM H_2O_2 is chosen. Similarly, the precipitate is also observed when the concentration of TMB is too high (Fig. S2B). Therefore, 0.4 mM TMB is optimal.

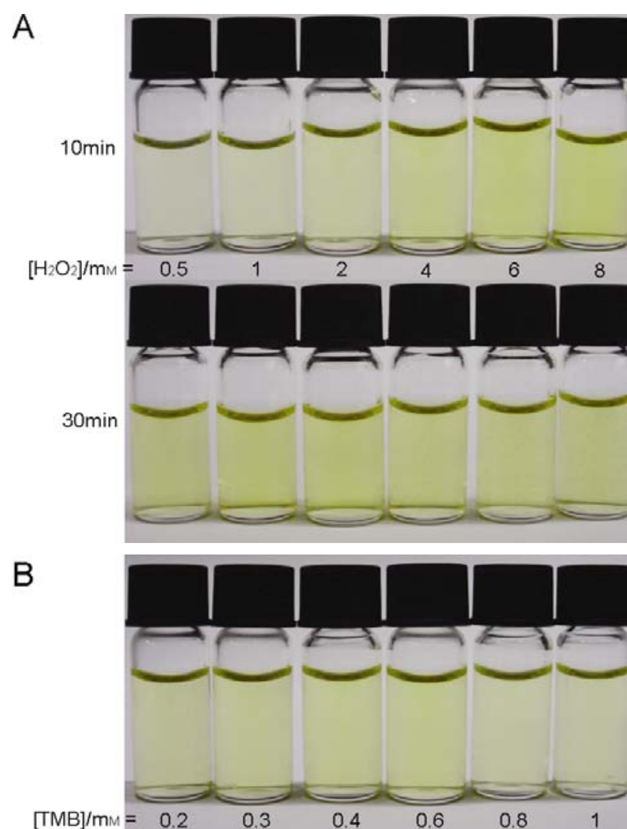

Fig. S2 Effect of the concentrations of H_2O_2 (A) and TMB (B) on the DNAzyme activity.