Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
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Electronic Supplementary Information

Signal-accumulating DNAzyme-crosslinked hydrogel for colorimetric sensing of hydrogen peroxide

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1. Reagents

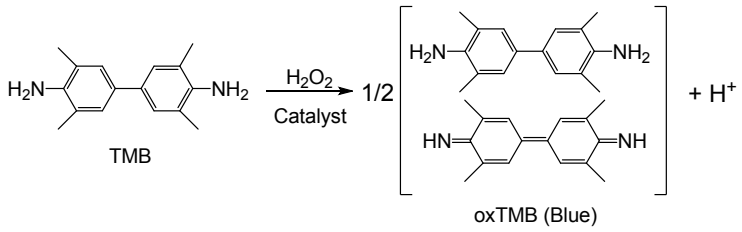
Hemin, Tris(Hydroxymethyl)aminomethane, TritonX-100, 2-[4-(2-Hydroxyethyl)-1-piperazinyl] ethanesulfonic acid, 2-Cyanoethyl diisopropyl chlorophosphoramidite, 3,3',5,5'-Tetramethylbenzi -dine were purchased from Sigma-Aldrich and stored in a refrigerator at 4℃. All other reagents were obtained from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). All solutions were prepared using ultrapure water. All DNA sequences were obtained from Sangon Biotech (Shanghai, China).

DNA sequences used in this work.

Name	DNA sequences
Acrydite-DNA	Acrydite-AAA AAA AAA AAA AAA A
4C15s	5'- AG GTG GGG AGG AGC GGG GTG CT -3'
T-4C15s-T	5'-TTT TTT TTT TTT TTT TAG GTG GGG AGG AGC GGG GTG CTT TTT TTT TTT TTT TT-3'

2. Reaction of H_2O_2-mediated TMB oxidation by catalyst (DNAzyme or Hemin alone).

H_2O_2-mediated TMB oxidation was carried out in working solution at room temperature. The working solution was prepared by mixing catalyst-solution and substrate-solution with 4:15 volume ratio. DNAzyme or Hemin-containing catalyst-solution was prepared as following (section 4). Substrate-solution was freshly prepared by adding appropriate H_2O_2 and TMB into C–P buffer (0.1M citrate–0.2M disodium hydrogen phosphate pH 4.4; 20mM KCl).

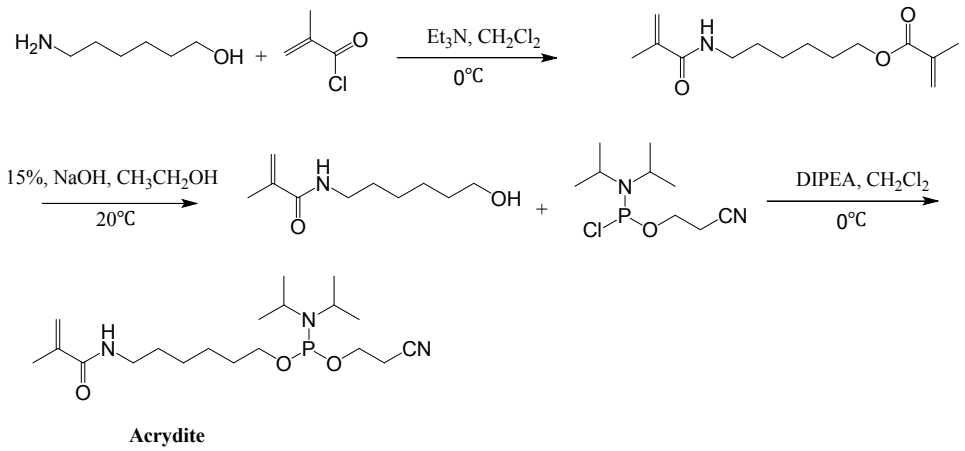


Scheme S1. Catalytic reaction of H_2O_2-mediated TMB oxidation.

3. Synthesis of acrydite

The synthesis of acrydite was similar to previous report (Scheme S2) [1]. 6-amino-1-hexanol (0.5g, 4.3mmol) was cooled at 0℃ in dichloromethane, Triehylamine (1.2ml, 8.6mmol) was added to the solution, and methacryloyl choride (1.35g, 12.7mmol) was added dropwise, the reaction solution was then stirred at 0℃ for 2 hours. After concentrated of the mixture, the mixture was poured into 5 mL ethanol and 15% sodium hydroxide (2 ml) for 1h. After evaporation of all solvents, the residue was added dichloromethane (10mL) and washed with sodium hydrogen carbonate solution (10mL, 5mL×2) and sodium chloride (10mL, 5mL×2). The resulting material was chromatographed on a silica gel using ethyl acetate to afford 6-hydroxyhexyl methacrylamide (0.64g, 80% yield). A solution 6-hydroxyhexyl methacrylamide (0.50 g, 2.70 mmol) in anhydrous CH_2Cl_2 (10 mL) was added slowly N,N'-Diisopropylethylamine (DIPEA) (0.98 g, 7.50 mmol). Then, 2-cyanoethyl diisopropyl chlorophosphoramidite (0.87 ml, 3.25 mmol) was added dropwise to the reaction solution and the mixture was stirred at 0 °C for 2

h. After removing the solvent, the residue was added dichloromethane (10 mL) and washed with sodium hydrogen carbonate solution (10 mL, 5 mL×2) and sodium chloride (10 mL, 5 mL×2) and dried with $MgSO_4$. The residue was chromatographed on a silica gel using a 40:60:3 ethyl acetate-hexane-triethylamine and dried to afford acrydite (0.62g, 60% yield) as colorless oil. The final product was coupled with DNA to obtain acrydite-DNA by DNA synthesizer. Acrydite: ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.85 (s, 1H), 5.67 (s, 1H), 5.30 (s. 1H), 3.80-3.75 (m, 2H), 3.70-3.50 (m, 4H), 3.35-3.25 (m, 2H), 2.65 (t, 2H), 1.95 (s, 3H), 1.68-1.52 (m, 4H) 1.47-1.30 (m, 4H) 1.22-1.15 (m, 12H)；ESI-MS, (m/z)：384.9 $[M-H]^-$, Molecular weight of Acrydite-DNA was determined by Sangon Biotech, Shanghai, China (Fig. S1).



Scheme S2. Synthetic route of acrydite.

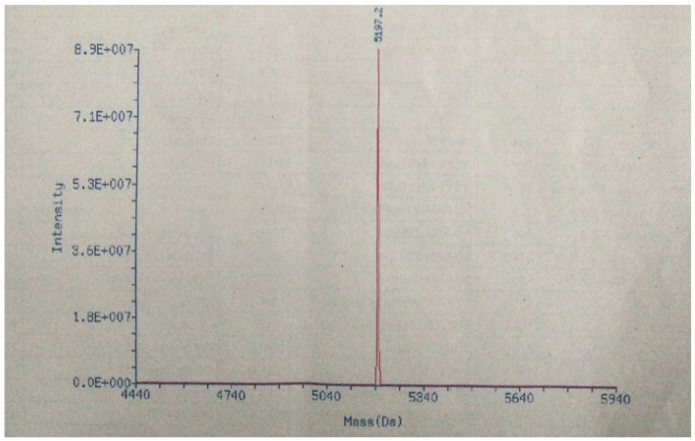


Fig. S1 Mass spectrum of acrydite-DNA.

3. Preparation of DNAzyme and hemin-solution

DNAzyme solution was prepared as described prevously.[2] 500μL of T-4C15s-T DNA (100 μM) in 40 K buffer (50 mM 2-morpholinoethanesulfonic acid hydrate pH 6.5; 40 mM potassium acetate; 100 mM Tris acetate;1% DMSO) was heated at 95℃ for 5 min, and then incubated at room temperature for 1h to form intermolecular G-quadruplex structure. Subsequently, the same volume of 40 KT buffer (50 mM 2-morpholinoethanesulfonic acid hydrate pH 6.5; 40 mM potassium acetate; 100 mM Tris acetate; 1% DMSO; 0.05% Triton X-100) was added into the solution and incubated for another 20 min at room temperature. Finally, 1.9 μL (0.64 mM) hemin

in DMSO was added into the G-quadruplex solution and incubated for 30 min at room temperature. The G-quadruplex DNA can complex with hemin to form DNAzyme. The final concentration of DNAzyme was 1.0 μM. The hemin solution was prepared by the addition of 1.9 μL(0.64 mM) hemin into 999 μL 40 KT buffer, and then incubated for 30 min at room temperature. The final concentration of hemin solution is 1.2 μM. The DNAzyme- and hemin-solution were used for the detection of H_2O_2 as shown in Fig. 1B.

4. Preparation of DNAzyme-crosslinked hydrogel

To prepare DNA-branched polyacrylamide, 4.7 μL of 4 mM acryldite-DNA solution was mixed with 3.2 μL 25% acrylamide in HEPES buffer (pH=7.0, 200 mM NaCl), and then made up to 8.6 μL solution. Copolymerization was initiated by adding 0.7 μL 2% APS and 0.7 μL 2% TEMED into the solution. Then, total 10.0 μL reaction mixture was immediately kept on vacuum for 15 min. The mole ratio of acryldite-DNA per acrylamide was about 1/600. On the other hand, 5.0 μL of 0.46 mM DNAzyme solution was prepared according to the protocol described above. The DNAzyme-crosslinked hydrogel was then prepared by mixing the 10 μL DNA-branched polyacrylamine solution and the 5 μL DNAzyme-solution. The hydrogel was then washed three times with HEPES buffer. The washing buffers were collected, and concentration of unbound DNAzyme in washing buffer was measured by using UV/Vis spectrophotometer (SHIMADZU, UV-2700). Thus, the amount of DNAzyme inside the gel was determined. While, the DNAzyme-crosslinked hydrogel can swell in the water and the volume of hydrogel will be larger after swelling equilibrium. We put 2 μL (2 mg) of DNAzyme-crosslinked hydrogel into 100 μL 40 KT buffer for 3 h, then picked the gel out from the 40 KT buffer. The weight of DNAzyme-crosslinked hydrogel was measured by one over ten-thousand analytical balance (GG, JJ124BC). Then, the volume of hydrogel grew from 2 μL (2 mg) to 3.0 μL (3.0 mg). Finally, the concentration of DNAzyme inside the gel was obtained (105.5 μM).

5. Visual detection limit of DNAzyme-crosslinked hydrogel

Visual detection limit of DNAzyme-crosslinked hydrogel was determined by the following experiment. DNAzyme-crosslinked hydrogel containing 105.5 μM cross-linked DNAzyme, was put into 100 μL reaction solution containing 0.2 mM TMB and different concentration of H_2O_2 (0, 0.3, 3, 9, 30, 60, 300μM) respectively. The reaction was then monitored with 10 min interval by digital camera. The result is shown in Fig. S2a. Similar parallel assay with free DNAzyme was also carried out, in which the same amount of DNAzyme was used (Fig. S2b).

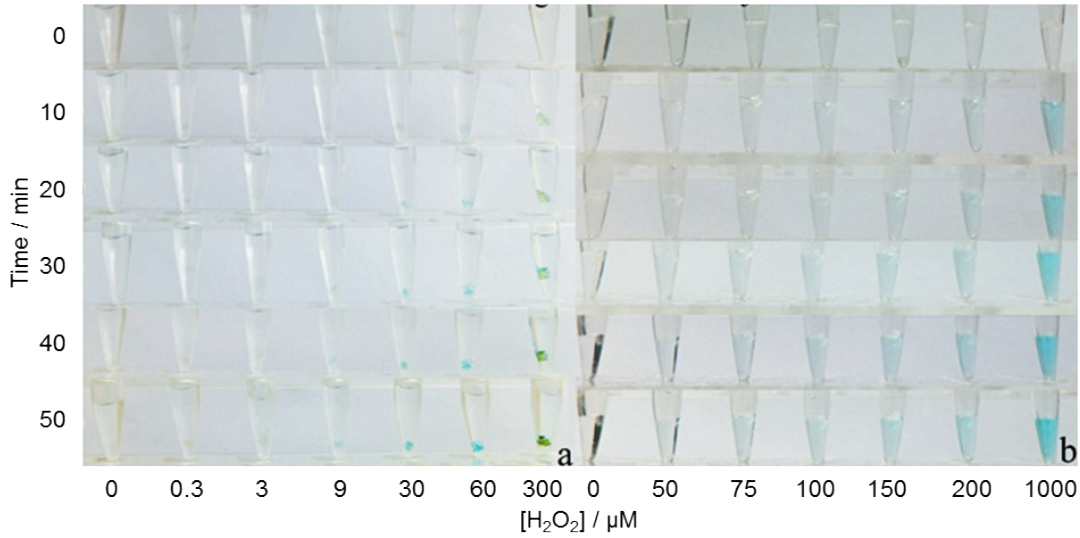


Fig. S2 Determination of visual detection limit using DNAzyme-crosslinked hydrogel (a) and free DNAzyme (b) for H_2O_2.

6. Reusability of DNAzyme-crosslinked hydrogel

We firstly put fresh DNAzyme-crosslinked hydrogel into reaction solutions (0.2 mM TMB and 0.2 mM H_2O_2) for 20 min. Subsequently, the hydrogel was taken out from reaction solution and soaked in 100 μL of 1% H_2SO_4 for 1 min. Then the buffer was removed and replaced with 100 μL C-P buffer, and incubated for 30min at room temperature. This process was repeated five times, and the results were recorded with digital camera (Fig. S3).

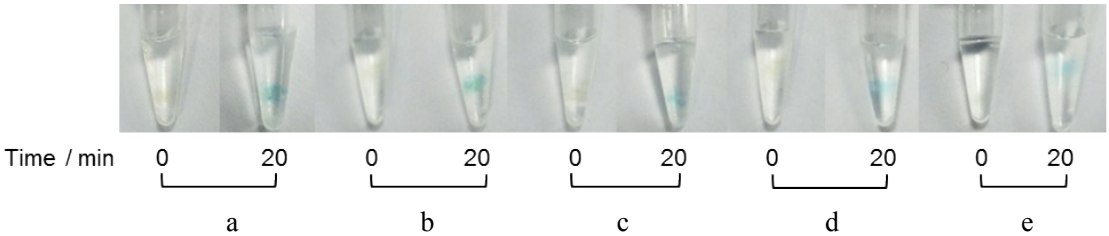


Fig. S3 Reusability of DNAzyme-crosslinked hydrogel. (a) Test with freshly prepared hydrogel. (b) Test with firstly regenerated hydrogel. (c) The second regeneration of DNA hydrogel with same concentration of TMB and H_2O_2. (d) The third regeneration of DNA hydrogel with same concentration of TMB and H_2O_2. (e) The fourth regeneration of DNA hydrogel with same concentration of TMB and H_2O_2. Reaction condition: [TMB] = 0.2 mM, [H_2O_2] = 0.2 mM.

7. Kinetic assay

Kinetic assay with DNAzyme-crosslinked hydrogel for H_2O_2 was carried out in reaction buffer (pH 4.4, 100 mM citrate; 200 mM disodium hydrogen phosphate; 0.2 mM TMB) at 20℃. The absorbance of products (oxTMB) was monitored by UV/Vis spectrophotometer at 652nm. Five small blocks of 105.5 μM DNAzyme-crosslinked hydrogels, which have almost the same volume (about 1.5 μL after swelling), were used. At each time point, reaction was stopped by taking the gel out from the reaction solution, and the gel was put into blank solution that without H_2O_2, followed by heating up to 70°C to convert the gel into a solution form, and the concentration of oxTMB inside the gel was then calculated. With the calculated concentration values at each time point (5 min, 10min, 15min, 20min, 30min), the time course of oxTMB-production inside the gel, was plotted as shown in Fig. 2B, and then initial velocity of oxTMB-production was calculated. This assay also repeated under 0.2 mM of TMB with various concentration of H_2O_2 (0.08 mM, 0.16 mM, 0.4 mM, 0.8 mM, 1.6 mM). Finally, Lineweaver-Burk plots were obtained according to the following Michaelis-Menten equation. Kinetic assay with free DNAzyme (T-4c15s-T) for H_2O_2 was also performed in parallel by changing the concentration of H_2O_2 (0.08 mM, 0.16 mM, 0.4 mM, 0.8 mM, 1.6 mM) under the same buffer condition (Fig. 3). In the reaction solution, the concentration of free DNAzyme was 0.83 μM，and the total reaction volume was 190 μL. In these experiments, the molar of DNAzyme crosslinked inside gel and that of free DNAzyme in solution was kept equal. Above experiments were repeated at least two times. Kinetic parameters were listed in Table S1.

$$\mathbf{V= V_{max}. [S] / ([S] + \boldsymbol{K}_m)}$$

V is the initial velocity, $\mathbf{V_{max}}$ is the maximum reaction velocity; $\boldsymbol{K}_\mathbf{m}$ is the Michaelis constant; **[S]** is the concentration of H_2O_2.

Table S1. Kinetic parameters obtained from Fig. 3. Free DNAzyme: reaction in a free DNAzyme (T-4c15s-T) solution; Inside-hydrogel: The oxTMB production inside the DNAzyme-crosslinked hydrogel.

Catalysis condition	$[E]_T$ / μmol L^{-1}	K_m / mmol L^{-1}	V_{max} / mol L^{-1} s^{-1}	k_{cat} / s^{-1}
Free DNAzyme	0.83	1.48±0.17	$(2.52±0.13)\times10^{-8}$	0.03±0.002
Inside-hydrogel	105.5	2.38±0.37	$(2.11±0.42)\times10^{-5}$	0.19±0.04

8. Effect of crosslinked DNAzyme-concentration inside hydrogel

In order to enhance the detection sensitivity of hydrogel, we increased the concentration of the cross-linked DNAzyme (T-4c15s-T) inside the gel by simultaneously increasing the mole ratio of acrydite-DNA/acrylamide (from 1/800, 1/600, 1/500, to 1/400) in DNA-branched polyacrylamide, and the effect of DNAzyme concentration inside gel was investigated (Fig. S4). The concentration of crosslinked DNAzyme inside the each of the four gels was determined as described above.

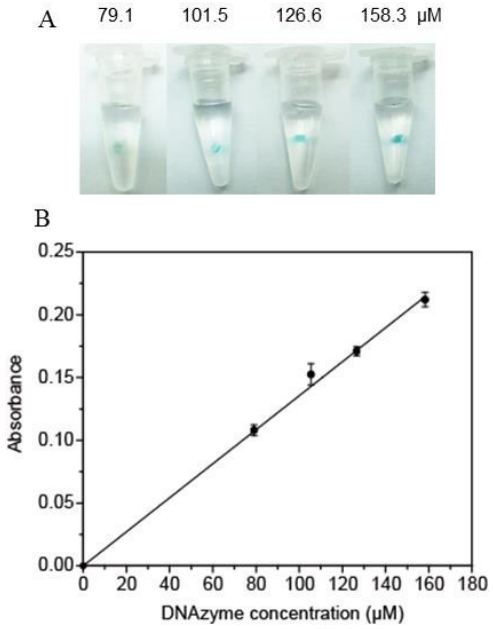


Fig. S4 Detection of H_2O_2 using DNAzyme-crosslinked hydrogel prepared with varying DNAzyme concentration inside hydrogel. (A) Color change of a hydrogel in the presence of TMB (0.2 mM) and H_2O_2 (3.0 μM) within 50 min. (B) Absorbance (652 nm) change of oxTMB with increasing the concentration of the DNAzyme inside the gel.

By using the DNAzyme-crosslinked hydrogel with the highest DNAzyme-concentration (158.3 μM), the visual detection limit was further determined. The result is shown in Fig. S5.

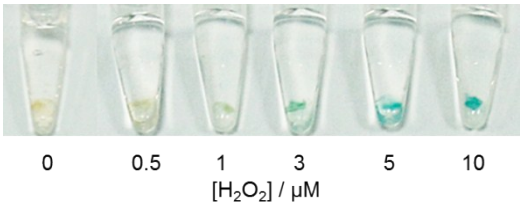


Fig. S5 Determination of visual detection limit by using the DNAzyme-crosslinked hydrogel with 158.3 μM of DNAzyme for H_2O_2. Reaction time: 50 min.

References

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2. M. Liu, T. Kagahara, H. Abe and Y. Ito, *Bull. Chem. Soc. Jpn.*, 2009, **82**, 99-104.