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DNAzyme Crosslinked Hydrogel: A New Platform for Visual Detection of Metal Ions

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Reagents:

All the oligonucleotides used in the work are listed in Table S1 and were synthesized on a 12-Column DNA Synthesizer (PolyGen GmbH), and purified by HPLC (Agilent 1100). HEPES, TEMED, APS and Acrylamide were purchased from Sigma (Shanghai, China). 2-Cyanoethyl diisopropyl chlorophosphoramidite was purchased from Chem Genes (Wilmington, US). Other reagents were purchased from Sinopharm chemical Reagent (Shanghai, China).

Table S1. DNA sequences of DNAzyme and Substrate used in this work

Enzyme39	5'-CCG AGG TAA GCC TGG GCC TCT TTC TTT TTA AGA AAG AAC-3'
Substrate28	5'-AGC TTC TTT CTA ATA CGG CTT ACC TAG G-3'
Mutated Substrate	5'-CCT AGG TAA GCC GTA TTA GAA AGA AGC T-3'
Enzyme35	5'-GGT AAG CCT GGG CCT CTT TCT TTT TAA GAA AGA AC-3'
Substrate24	5'-AGC TTC TTT CTA ATA CGG CTT ACC-3'
Enzyme37	5'-G AGG TAA GCC TGG GCC TCT TTC TTT TTA AGA AAG AAC-3'
Substrate26	5'-AGC TTC TTT CTA ATA CGG CTT ACC TA -3'

Preparation of AuNPs:

The AuNPs (13 nm diameter) were prepared by following a previously reported procedure.[1] About 0.5% BSA were added into AuNPs solution and stirred overnight. Then, AuNPs were centrifuged down at 14000 rpm. After removing supernatant, AuNPs were resuspended in water.

Melting Temperature of DNAzyme/Substrate

Metlting Temperatures of Enyzme35/Substrate24, Enyzme37/Substrate26, Enyzme39/Substrate28 were measured in PBS with 500 mM NaCl. Melting profiles were monitored at 260 nm on a UV spectrophotometer (Agilent 8453) equipped with a Peltier temperature controller. The melting profile was monitored starting from 85 °C at -2 °C/min interval. The DNAzyme forms an intermolecular duplex with a Tm at around 64 °C (FigS1a). The Triplex domain of the Enzyme/substrate by itself was unstable and no obvious melting point was observed under our experimental condition. This result strongly suggested that after cleavage, the hydrogel is only crosslinked by the triplex domain and would not be stable at room temperature. The melting temperatures of Enyzme35/Substrate24, Enyzme37/Substrate26, Enyzme39/Substrate28 were found to be 30, 35 and 43 °C respectively.

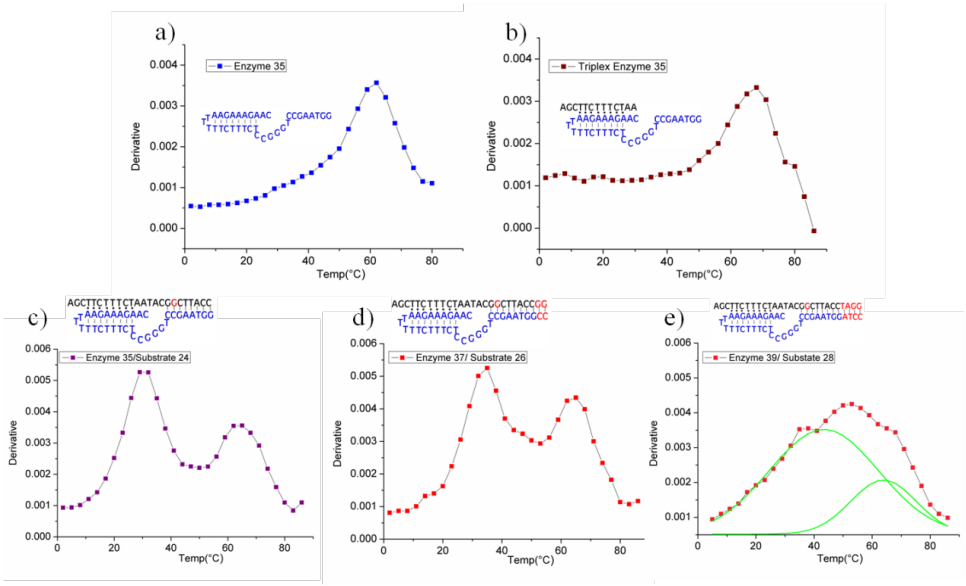
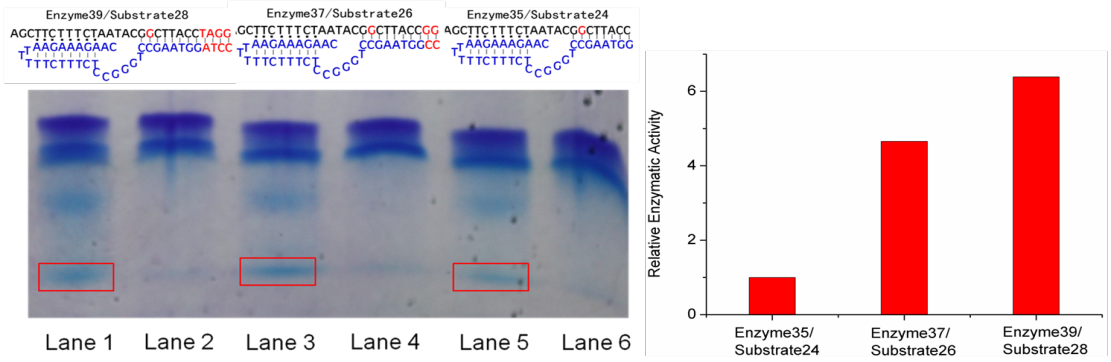


Fig. S1 Melting Temperature measurement (Tm) of different DNAzyme/Substrate pairs with different duplex domain lengths. a) DNAzyme35; b) Triplex 35; c) DNAzyme35/Substrate24; d) DNAzyme37/Substrate26; e) DNAzyme39/ Substrate28.

DNAzyme Cleavage Efficiency.

The cleavage efficiency of the all enzymes were investigated. Different DNAzyme/Substrate pairs were incubated at the concentration ratio of 20 μM:10 μM at room termperature for 1 hour before PAGE analysis. As shown in Fig.S2(a), the cleavage efficiency of the DNAzyme increased with the extention of the duplex domain. The DNA bands related to the cleaved product were analyzed by ImageJ to evalute the relative enzymatic activity of each DNAzyme/Substrate pair. As indicated by Fig.S2(b), the relative enzymatic activity of Enzyme37/ Substrate26 was as high as 4.5 times activity of Enzyme35/Substrate24 while Enzyme39/Substrate28 was as high as 6 times that of the Enzyme35/Substrate24. Such a significant improvement in enzyme activity was likely due to the efficient binding of the enzyme and substrate as a result of increase in binding affinity.

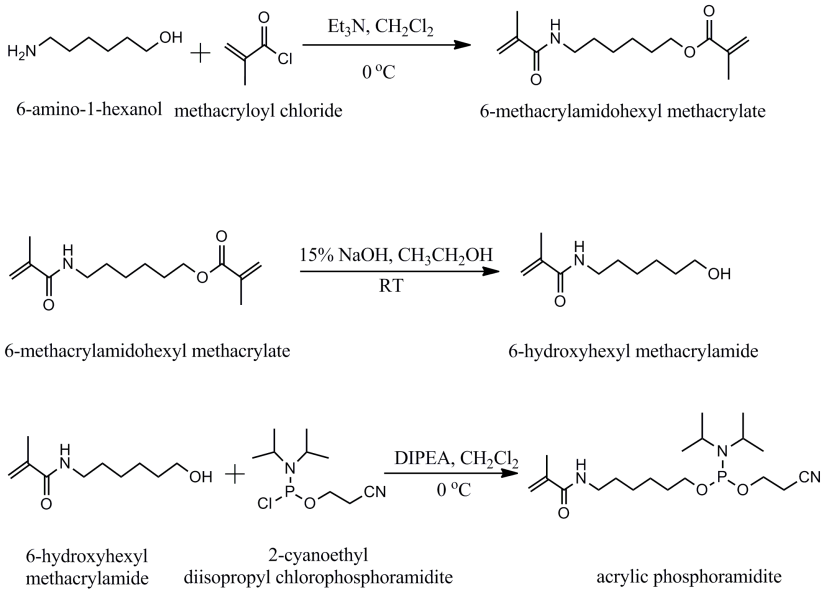


a) b)

Fig. S2 a) Cleavage efficiency assay of DNAzyme/Substrate pairs with different duplex domain length. Lane 1: DNAzyme39/Substrate28 with 20 μM Cu^{2+}; Lane 2: DNAzyme39/Substrate28; Lane 3: DNAzyme37/Substrate26 with 20 μM Cu^{2+}; Lane 4: DNAzyme 37/Substrate26; Lane 5: DNAzyme 35/Substrate 24 with 20 μM Cu^{2+}; Lane 6: DNAzyme35/Substrate24. b) Relative enzymatic activity of band enclosed by red pane calculated by ImageJ. The assay buffer contained 10 μM Substrate and 20 μM DNAzyme and 50 mM HEPES, 1.5 M NaCl, and 50 μM ascorbate.

Synthesis of acrylic phosphoramidite

To graft DNAzyme and Substrate onto the branch linear polyacrylamide, acrylic phosphoramidite was first synthesized. The synthesis of acrylic phosphoramidite is shown in **Scheme S1**. Briefly, 6-amino-1-hexanol (1 g, 8.53 mmol) and triethylamine (2.36 mL, 17 mmol) was cooled to 0 °C. Methacryloyl chloride (2.67 g, 2.55 mmol) was added dropwise, and the mixture was stirred at 0 °C for 2 hours. After evaporation of all solvents, the residue was dissolved in 10 mL ethanol and 15% sodium hydroxide (4 ml) was added into the solution. The solvent was evaporated, and 6-hydroxyhexyl methacrylamide was chromatographed on a column of silica gel G using ethyl acetate. N,N' Diisopropylethylamine (DIPEA) (0.98 g, 7.50 mmol) was added slowly to a solution containing 6-hydroxyhexyl methacrylamide (0.50 g, 2.70 mmol) in anhydrous CH_2Cl_2 (10 mL) at 0 °C. Then, 2-cyanoethyl diisopropyl chlorophosphoramidite (0.87 ml, 3.25 mmol) was added dropwise, and the reaction mixture was stirred at 0 °C for 2 h. After removing the solvent, the residue was purified by column chromatography (ethyl acetate/hexane/triethylamine 40:60:3) and dried to afford the title compound as a colorless oil. The final product was coupled with normal DNA to obtain acrydite-DNA by DNA synthesizer. ^{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 (m, 3H), 1.68-1.52 (m, 4H) 1.47-1.30 (m, 4H) 1.22-1.15 (m, 12H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 168.4, 140.2, 119.1, 117.7, 63.5, 58.3, 42.9, 39.5, 31.0, 29.5, 26.6, 25.6, 24.6, 20.3, 18.7.



Scheme S1. Synthesis of acrylic phosphoramidite.

Synthesis of acrydite-DNA

Acrylic phosphoramidite obtained from the last step was used to couple to DNAzyme and Substrate, respectively, on a DNA synthesizer to obtain acrydite-DNAzyme and acrydite-Substrate. As Fig. S3 shows, MALDI-TOF mass analysis indicated successful synthesis of the acrydited-DNA.

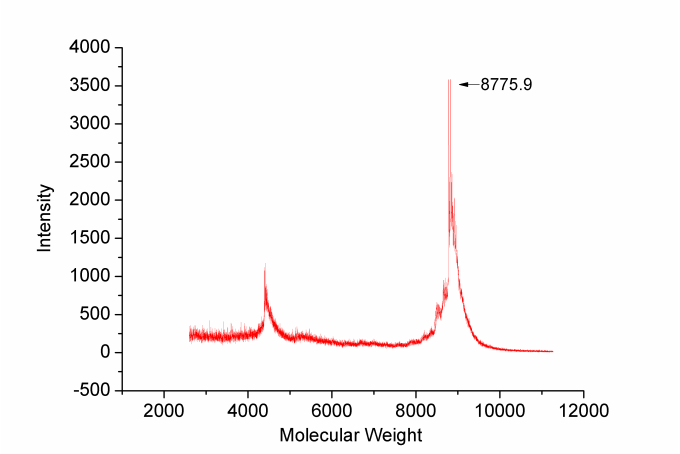


Fig. S3. MALDI-TOF characterization of acrydite- Substrate DNA. The calculated molecular weight is 8776.

Preparation of DNA side-chain polymer: 16 μL 3 mM Acrydite-DNA were separately mixed with 8 μL 25% acrylamide in 24 μL 100 mM HEPES buffer (pH=7.0, 3 M NaCl). Then, 0.7 μL 10% APS and 0.7 μL 10% TEMED were added in the solution. The solution was immediately kept in a vacuum to remove air and the polymerization reaction took about 10 minutes to complete. Under this condition, it is estimated that there is a DNA chain in every 600 repeat units.

Preparation of DNAzyme-Hydrogel: 48 μL of DNA side-chain polymer solution was mixed with 4 μL BSA coated AuNPs to obain a pink colored homogeneous DNA side chain polymer solution. AuNPs doped DNAzyme crosslinked Hydrogel was then formed by mixing the two types of DNA side-chain polymer solution in equal amounts (5 μL + 5 μL). Gel dissolution experiments were done at at 37 °C in HEPES Buffer (50 mM, pH=7.0 1.5 M NaCl, 3% H_2O_2).

1. Z. Zhu, C. C. Wu, H. P. Liu, Y. Zou, X. L. Zhang, H. Z. Kang, C. J. Yang and W. H. Tan, *Angew. Chem. Int. Ed. Engl.*, 2010, **49**, 1052-1056.