Supporting Information

Target-Responsive DNA Hydrogel for Non-enzymatic and Visual Detection of Glucose

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S1 Acrydite phosphoramidite and Shinkai's receptor synthesis [1, 2]

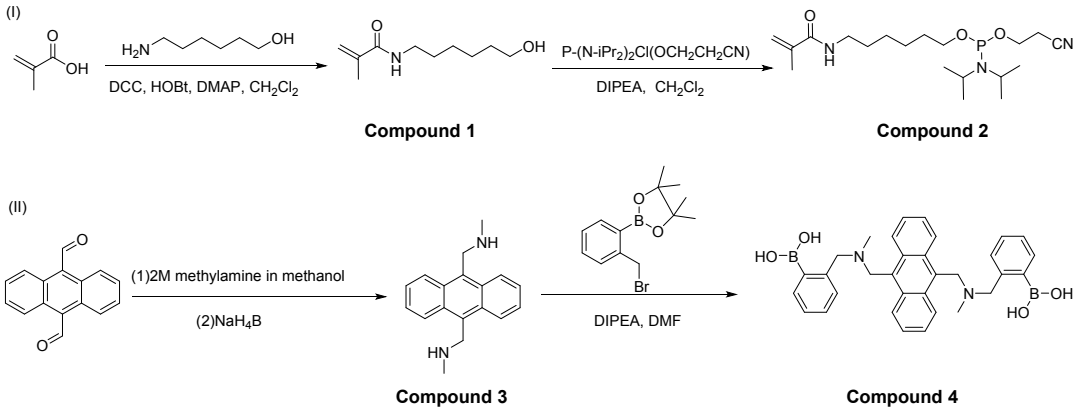

Figure S1 Synthetic route of acrylamine tethered phosphoramidite monomer and boronic acid derivative

Compound 1: In a 50 mL round-bottomed flask, a solution of 2-methylacrylic acid (440 mg, 5 mmol)， 6-amino-1-hexanol (585 mg, 5 mmol), DCC (1356 mg, 6 mmol) and HOBt (810 mg, 6 mmol) in 5 mL of DMF was mechanically stirred under a nitrogen atmosphere at room temperature overnight. The reaction mixture was filtered, and a 5-fold volume of water was added, followed by three 5-fold volume extractions using ethyl acetate. The organic phase was washed with saturated brine and dried over anhydrous Na_2SO_4. The solvent was removed with a rotary vacuum evaporator, and the oily crude product was purified by silica gel column chromatography (ethyl acetate:hexane=1:1, v/v) to obtain a colorless oil marked as Compound 1. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.92 (s, 1H), 5.66 (s, 1H), 5.29 (s, 1H), 3.63 (t, 2H), 3.29 (q, 2H), 1.94(s, 3H), 1.57-1.52 (m, 4H), 1.39-1.34 (m, 4H). ESI-MS (m/z)$[1+H]^+$ 186.2, Found: 186.6.

Compound 2: Compound 1 (213 mg, 1.15 mmol) was dissolved in 2 mL of anhydrous CH_2Cl_2 at 0 °C, and DIPEA (0.55 mL, 3.22 mmol) was slowly added under nitrogen. Then, 2-cyanoethyl-*N, N*-diisopropylchlorophosphoramidite (0.28 mL, 1.15 mmol) was added dropwise, and the reaction solution was mechanically stirred at 0 °C for 3 h under a nitrogen atmosphere. After removing the solvent under reduced pressure, the residual oily product was purified by silica gel column chromatography (ethyl acetate:hexane:trimethylamine=40:60:2, v/v/v) and dried to afford Compound 2. Compound 2 was modified onto normal DNA by a DNA synthesizer to obtain acrydite-DNA. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.83 (s, 1H), 5.65 (s, 1H), 5.29 (s, 1H), 3.85-3.76 (m, 2H), 3.65-3.55 (m, 4H), 3.32-3.27 (q, 2H), 2.65-2.62 (t, 2H), 1.954 (s, 1H), 1.61-1.53 (m, 4H),

1.41-1.34 (m, 4H), 1.18-1.16 (m, 12H). ^{31}P ($CDCl_3$): δ 147.26. ESI-MS (m/z)[1+H]$^+$ 386.2, Found: 386.4.

Compound 3: Anthracene-9,10-dicarboxaldehyde (234 mg, 1 mmol) was dissolved in 30 mL of methanol and cooled to 0 °C, and 2 M methylamine in methanol (1.5 mL, 3.0 mmol) was slowly added. Then, the reaction mixture was stirred at room temperature overnight under a nitrogen atmosphere. The reaction mixture was cooled to 0 °C, sodium borohydride (190 mg, 5 mmol) was added quickly, and then the reaction mixture was stirred for additional 6 h at room temperature. The resulting mixture was poured onto 10 mL of ice-water and the aqueous layer extracted with chloroform (3×30 mL). The organic phase was washed with saturated brine and dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (methanol : dichloromethane =1:10, v/v) to afford 1,1'-(anthracene-9,10-diyl)bis(N-methylmethanamine) marked as Compound 3. ^{1}H NMR (500 MHz, $CDCl_3$): δ8.40-8.38 (d, 4H), 7.55-7.53 (d, 4H), 4.69 (s, 4H), 2.68 (s, 6H), 1.51 (bs, 2H). ESI-MS (m/z)[1+H]$^+$ 265.2, Found: 265.1.

Compound 4: Compound 3 from the previous step (132 mg, 0.5 mmol) and 2-bromomethylphenylboronic acid pinacol ester (561 mg, 1.98 mmol) were dissolved in DMF (3.0 mL), followed by slow addition of DIPEA (0.264 mL, 1.49 mmol). Then, the reaction mixture was mechanically stirred at room temperature overnight, followed by heating at 40 °C for 2 h. The reaction mixture was diluted with 20 mL of water and then the aqueous layer extracted with chloroform (3×30 mL). The organic layers were collected together and dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (methanol : dichloromethane =1:20, v/v) to afford Compound 4 (Shinkai's receptor). ^{1}H NMR (500 MHz, $CDCl_3$): δ8.35-8.33 (m, 4H), 8.00-7.82 (m, 1H), 7.62-7.59 (m, 1H), 7.52-7.49 (m, 1H), 7.44-7.36 (m, 8H), 4.37 (s, 4H), 3.99 (s, 4H), 2.19 (s, 6H). ESI-MS ([Compound 4+glucose], m/z)[1+H]$^+$ 641.3, Found: 641.3.

S2 HPLC purification

After ethanol precipitation, the crude DNA products were purified by an LC 3000

semipreparative HPLC system with a reversed-phase C18 column. Acetonitrile and 0.01 M triethylamine acetate (TEAA) were chosen as mobile phase, and the gradient elution procedure is shown in Table S2.

Table S2 gradient elution procedure of HPLC purification

Component(%) / Time(min)	Acetonitrile	0.01 M TEAA
0	0	100
3	0	100
3.01	12	88
33	21	73

S3 Characterization of the result of the polymerization reaction

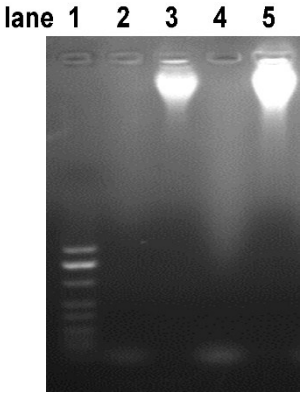

Figure S3 Characterization the result of the polymerization reaction by 2% agarose gel electrophoresis. Lane 1: low molecular DNA marker, lane 2: Strand A, lane 3: polymerization products of Strand A, lane 2: Strand B, lane 3: polymerization products of Strand B.

S4 Synthesis and modification of gold nanoparticles

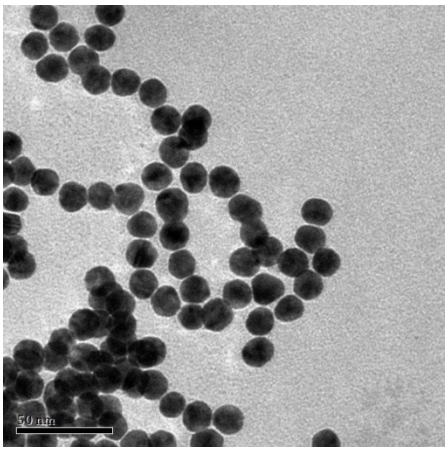

Figure S4 The characterization of 13 nm AuNPs by SEM, scale bar=50 nm.

S5 The feasibility investigated by fluorescence

First, FAM labelled Apt-linker at the 5' end and Dabcyl labelled Strand B at the 3' end were synthesized separately. Then, different DNA combination solution was denatured at 95 ^{o}C for 10 min, followed by hybridization at 25 ^{o}C for 1 h. At the same time, glucose and its ligand were incubated to form the glucose complex at 37 ^{o}C for 10 min. Then, three different solutions (glucose, Shinkai's receptor, and glucose complex) were each mixed with DNA solution, and incubated at 25 ^{o}C for 1 h. Then, fluorescence was detected for investigating the feasibility of competitive binding.

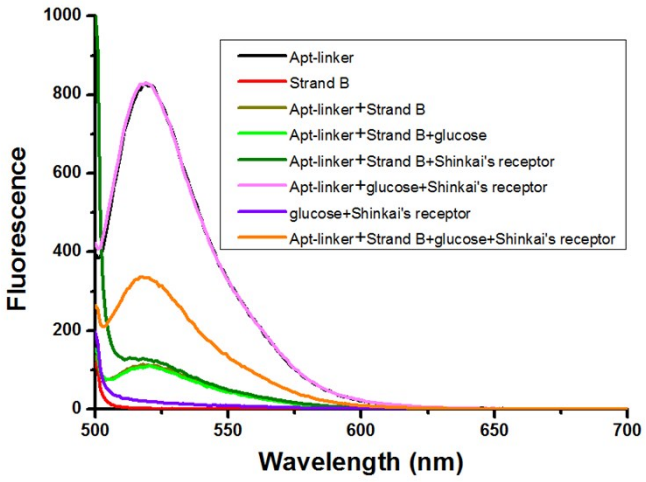

Figure S5 The results of the feasibility investigated by fluorescence. Apt-linker: 100 nM, Strand B: 500 nM; glucose: 10 mM, Shinkai's receptor: 200 μM.

S6 Optimization of reaction time

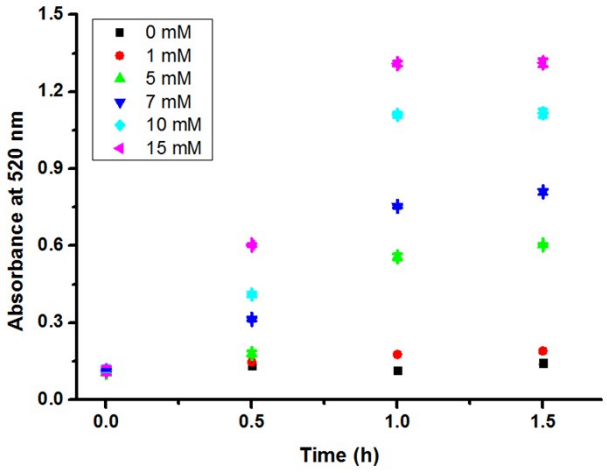

Figure S6 Optimization of the reaction time for detection. Six different concentrations of glucose (0 mM, 1 mM, 5 mM, 7 mM, 10 mM, 15 mM) were used, and the absorbance of AuNPs at 520 nm at four different incubation times (0 h, 0.5 h, 1 h, 1.5 h) was observed.

S7 Detection of glucose in serum

First, the glucose concentration in the fresh serum was detected by a glucose meter to have a concentration of 2.5 mM by the external standard method. Then serum samples spiked with different concentrations of glucose in Hepes buffer were incubated with Shinkai's receptor, followed by testing with the DNA hydrogel.

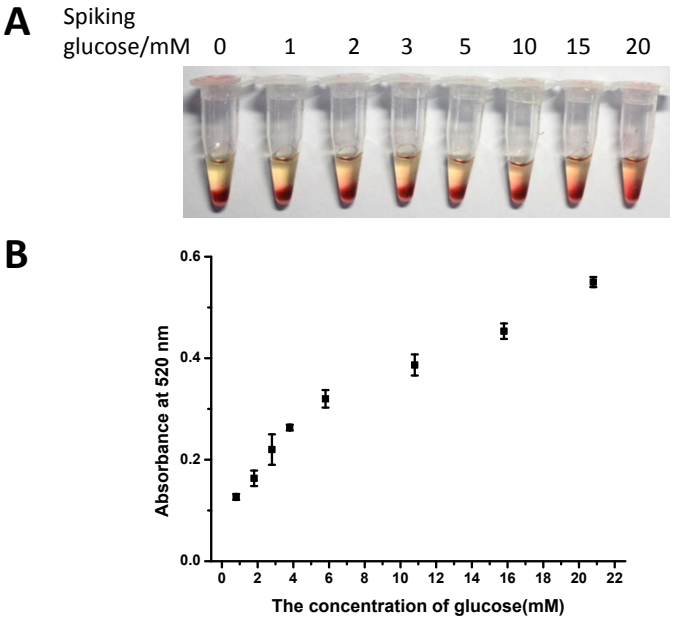

Fig. S7 Detection of glucose in 30% serum. (A) Image of DNA hydrogel response for different concentrations of glucose spiking in serum samples. (B) Absorbance of supernatant at 520 nm for each sample, excluding background.

References

1. Y. Ma, Y. Mao, D. Huang, Z. He, J. Yan, T. Tian, Y. Shi, Y. Song, X. Li and Z. Zhu, *Lab on A Chip*, 2016, **16**.
2. K.-A. Yang, M. Barbu, M. Halim, P. Pallavi, B. Kim, D. M. Kolpashchikov, S. Pecic, S. Taylor, T. S. Worgall and M. N. Stojanovic, *Nat. Chem.*, 2014, **6**, 1003-1008.