Electronic Supplementary Information

Photocatalytic Oxidation of TMB with the Double Strand DNA-SYBR Green I Complex for Label-Free and Universal Colorimetric Bioassay

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Materials.

3,3',5,5'-Tetramethylbenzidine (TMB), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 2-(N-morpholine) ethanesulfonate (MES) were from Aladdin (Shanghai, China). Sodium hydroxide, hydrochloric acid, hydrogen peroxide (H_2O_2), dimethyl sulfoxide (DMSO) and citrate were purchased from Kelong Reagent Co. (Chengdu, China). *O*-phenylenediamine (OPD), 3,3'-diaminobenzidine (DAB) and peroxidase from horseradish were obtained from J&K Scientific (Beijing, China). Ethidium bromide (EB) was purchased from Alfa Aesar (USA). Thrombin from bovine plasma was obtained from Sigma-Aldrich (St. Louis, MO, USA). SYBR Grenn I (SG, 10000×) and Amplex Red (AR) was from Life Technology (American). The concentration of 10000 × SG solution is calculated to be 19.6 mM according to the research from Vitzthum, et al in 2004.[S1] Gel Red (GR, 10000 ×) were purechased from Biotium (American). Oligonucleotides were provided by Shanghai Sangon Biotech Co., Ltd. (Shanghai, China).

Methods.

The sequences of DNA used in this work were given in Table S1. To obtain a typical photocatalytic system, two complementary ssDNAs and SG were mixed and diluted to 2 mL with Citrate or MES buffer (pH 4.5). The final concentrations of dsDNA and SG are 100 nM and 3.92 μM (2×), respectively. The mixture was incubated for 15 min and irradiated with blue LED for another 15 min.

Table S1 The sequences of used DNA in this work.

No.	Type	Sequence
1	10 base pair	5'-ACA CAC ACA C-3'
	Complementary	5'-GTG TGT GTG T-3'
2	20 base pair	5'-ACA CAC ACA CAC ACA CAC AC-3'
	Complementary	5'-GTG TGT GTG TGT GTG TGT GT-3'
3	30 base pair	5'-ACA CAC ACA CAC ACA CAC ACA CAC ACA CAC-3'
	Complementary	5'-GTG TGT GTG TGT GTG TGT GTG TGT GTG TGT-3'
4	40 base pair	5'-ACA CAC ACA CAC ACA CAC ACA CAC ACA CAC ACA CAC ACAC-3'
	Complementary	5'-GTG TGT GTGT GTG TGT GTG TGT GTG TGT GTG TGT GTG TGT-3'
5	50 base pair	5'-ACA CAC ACA CAC ACA CAC ACA CAC ACA CAC ACA CAC ACA CAC ACA CAC AC-3'
	Complementary	5'-GTG TGT GTG TGT GTG TGT GTG TGT GTG TGT GTG TGT GTG TGT GTG TGT GT-3'
6	Mercury aptamer I	5'-CGC ATT CAG GAT TCT CTA CTC GTA-3'
	Mercury aptamer II	5'-TTC GTG TTG TGT TTC CTG TTT GCG-3'
7	Target DNA	5'-GAG CAT ACA TAG GGT TTC TCT TGG TTT-3'
	Target DNA probe	5'-AAA CCA AGA GAA ACC CTA TGT ATG CTC-3'
	Single base mismatch	5'-GAG CAT ACA TAG GCT TTC TCT TGG TTT-3'
	Three base mismatch	5'-GAG CAT ACA TAG CCA TTC TCT TGG TTT-3'
8	Hairpin structure	5'-CGT CCC GTC CCC TGC CAC GGT CTG AGA GGA CGG GAC G-3'
9	Cocaine aptamer I	5'-AGA CAA GGA AAA-3'
	Cocaine aptamer II	5'-TCC TTC AAT GAA GTG GGT CG-3'
10	Thrombin aptamer	5'-GGT TGG TGT GGT TGG-3'
	Complementary	5'-CCA ACC ACA CCA ACC-3'

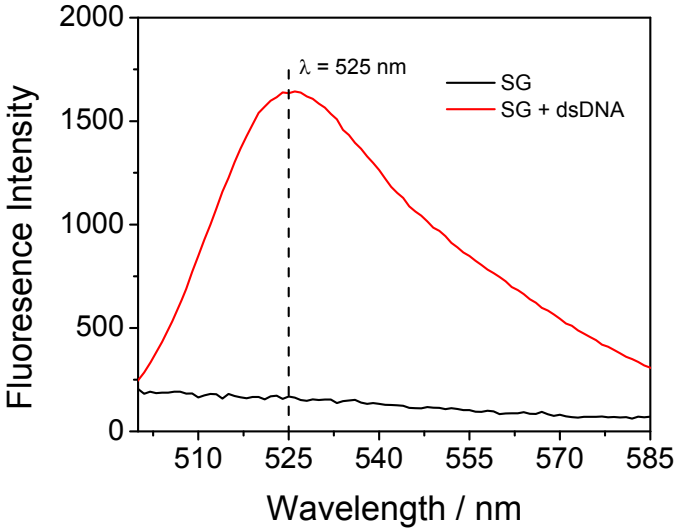

Fig. S1 Fluorescence spectra of SG and DNA-SG complex confirming the intercalation of SG into the grooves of dsDNA. Experimental conditions: dsDNA, 100 nM; SG concentration, 3.92 μM; and excitation wavelength, 485 nm.

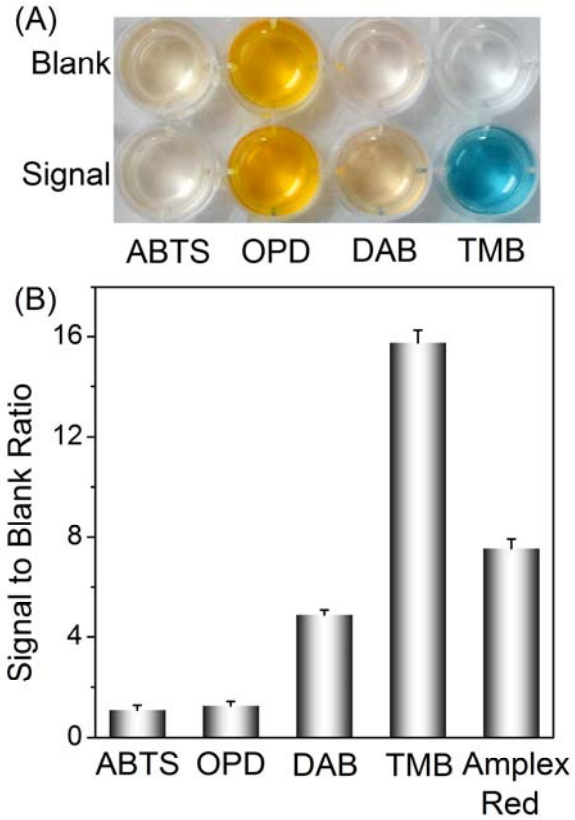

Fig. S2 The photooxidase could catalyze the oxidation of various oxidase substrates. Experimental conditions: dsDNA, 100 nM; SG concentration, 3.92 μM;; Incubation time, 15 min; and irradiation time, 10 min.

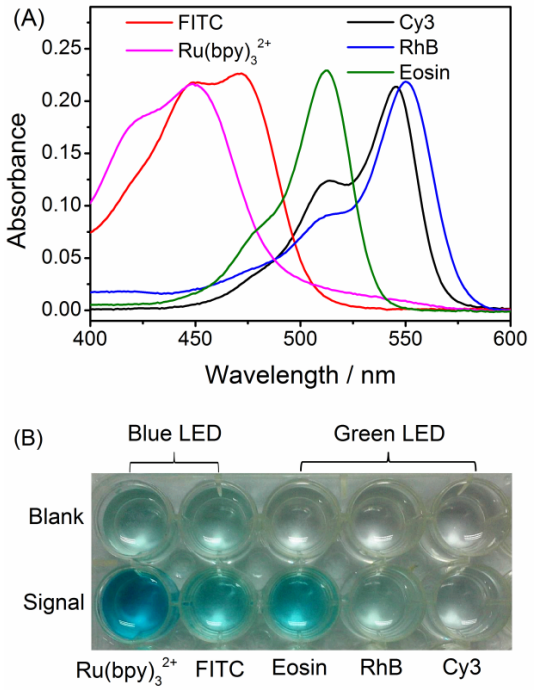

Fig. S3 (A) Oxidation of TMB catalyzed by light-irradiated dyes; and (B) the corresponding absorption spectra of the dyes. The concentrations of dyes were all 100 nM and the concentration of TMB was 200 mg/L.

For detection of DNA, Hg^{2+} and cocaine, the DNA/aptamers were incubated with the target analyte and 3.92 μM SG for 15 min in 0.1 M citrate buffer solution (pH 4.5) with the final solution volumes of 2 mL. The detailed sensing conditions were list in Table S2. After formation of DNA-EB complex, TMB was added (200 mg/L), and the resultant solution was irradiated with blue LED (460-470 nm, 3 W, Yichuang Technology Co., Zhuhai, China) for 15 min. The blue LED array was placed in the above of the reaction solutions (Fig. S4). The sensing media were also 0.1 M citrate buffer (pH 4.5) and the mixture was irradiated for 15 min. For detection of thrombin, the thrombin aptamer was incubated with the complementary sequence and 3.92 μM SG firstly for 15 min. Then, thrombin was added to the solutions and incubated for 15 min. TMB was added to the resultant solutions, and followed by the

LED irradiation. After photoreaction for 15 min, the colors were observed by naked-eye and also recorded by a camera equipped in an OPPO R817 cellular phone. The absorbance values were determined with a UV-5200PC spectrophotometer (Shanghai Yuanxi Instrument Co., Ltd., Shanghai, China).

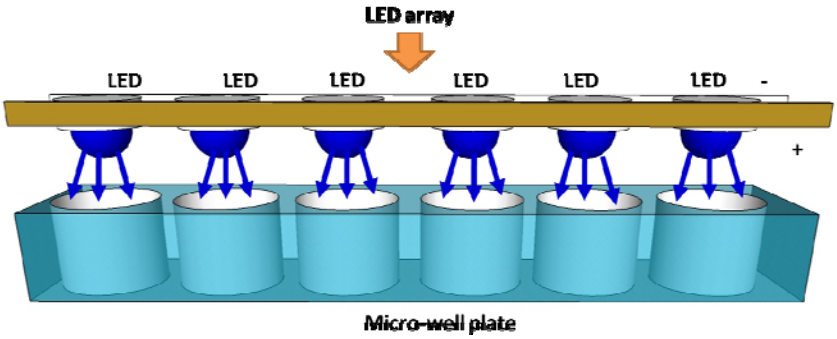

Fig. S4 Schematic illustration of the set-up of LED-irradiated photoreaction.

Table S2 Sensing conditions for detection of Hg^{2+}, DNA, cocaine and thrombin.

Analyte	Reaction solution
BRCA1 gene	100 nM DNA probe + DNA target+ 3.92 μM SG in pH 4.5 citrate buffer
Thrombin	100 nM thrombin aptamer + 100 nM complementary sequence + 3.92 μM SG + thrombin in pH 4.5 citrate buffer
Cocaine	100 nM Cocaine aptamer I, II + 3.92 μM SG in pH 4.5 citrate buffer
Hg^{2+}	100 nM Hg^{2+} aptamer I, II + 3.92 μM SG in 2-(N-morpholine) ethanesulfonate buffer (pH4.5)

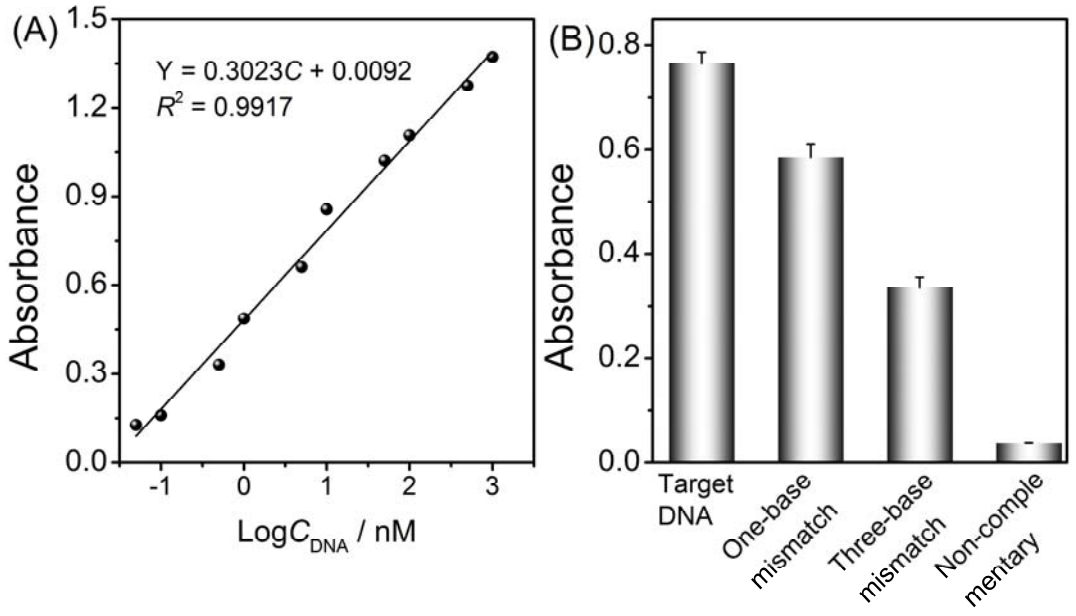

Fig. S5 Analytical performance for DNA detection: (A) calibration graph of detection with absorbance measurements and (B) Selectivity evaluation of the DNA sensor

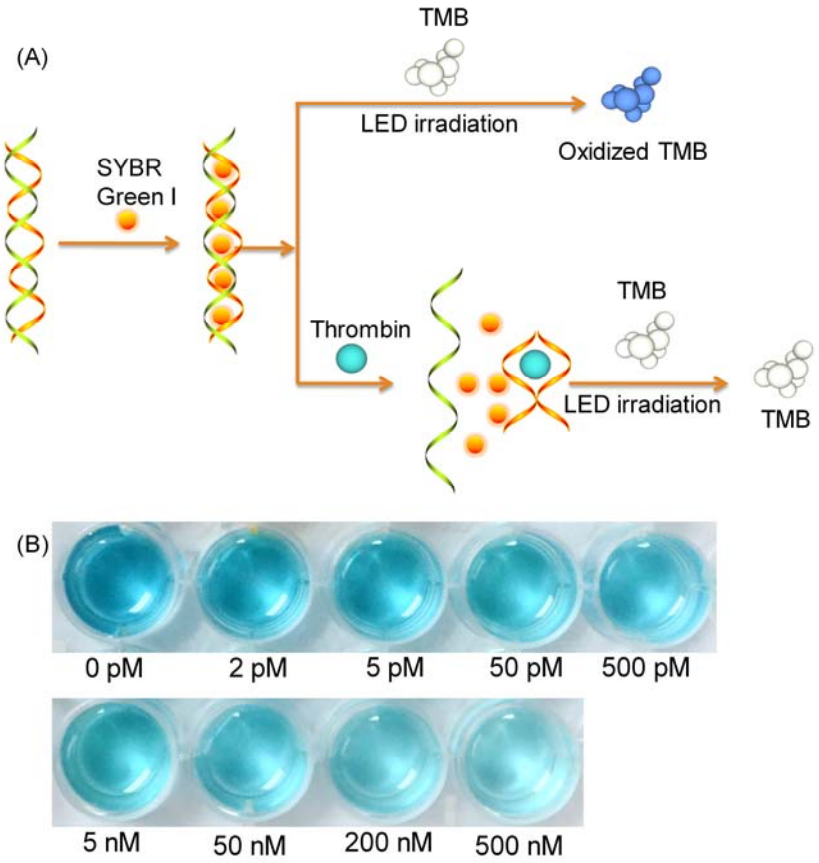

Fig. S6 Design and performance of the visual sensor based on photocatalytic activity of dsDNA-SYBR green complex for thrombin detection: (A) the scheme of the thrombin biosensor; and (B) photographs of the visual detection of 2 pM-500 nM thrombin.

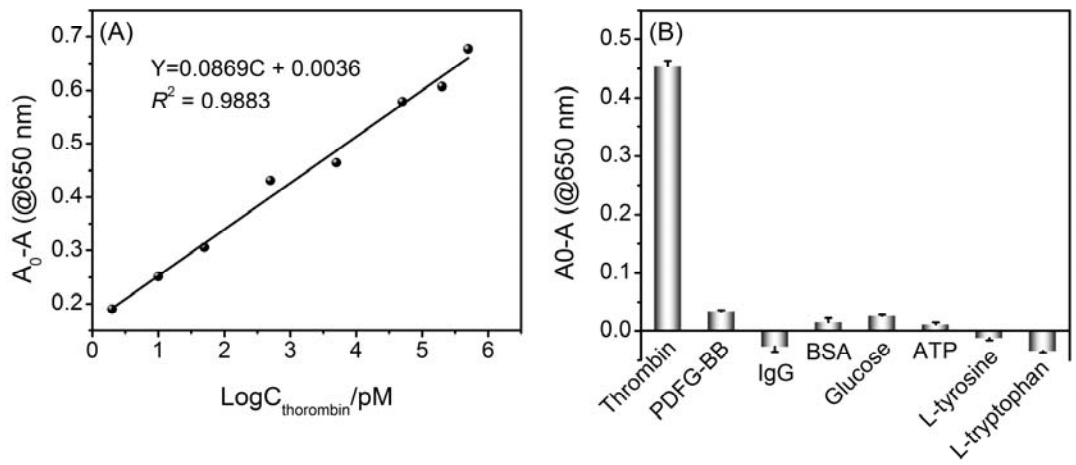

Fig. S7 Analytical performance for thrombin sensing: (A) calibration graph of thrombin detection with absorbance measurements and (B) selectivity evaluation of the thrombin sensor (10 nM, other proteins: 1 μM).

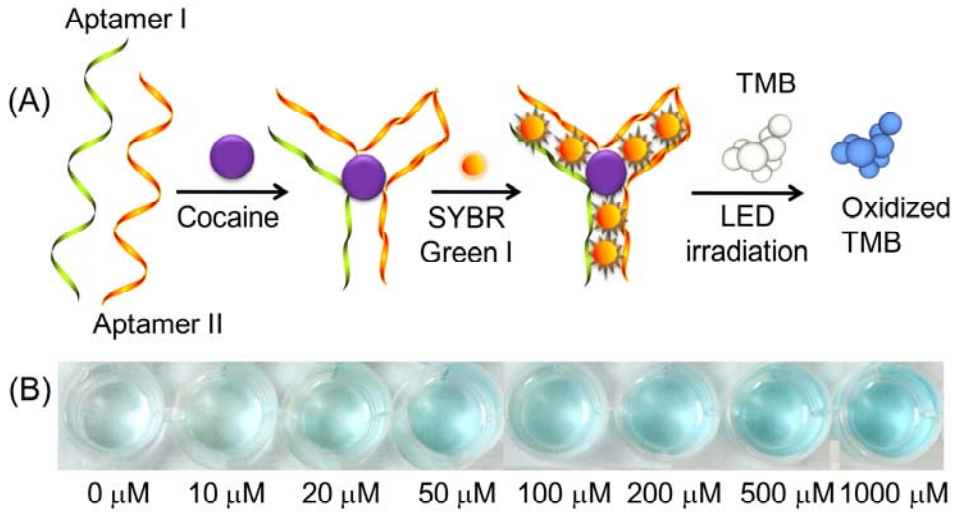

Fig. S8 Design and performance of the dsDNA-SYBR green-based visual sensor for small molecule (cocaine): the cocaine aptamer was splited into two DNA strands, which were separated in the absence of cocaine.[S2,S3] The presence of cocaine glued the two strands, resulting in the formation of dsDNA structure. Addition of SYBR green activated the photocatalytic activity of dsDNA-SYBR green complex, which could catalyze the oxidation of TMB to give color readout (Fig. S8A). The resultant color change allows visual detection of cocaine down low to 20 μM (Fig. S8B).

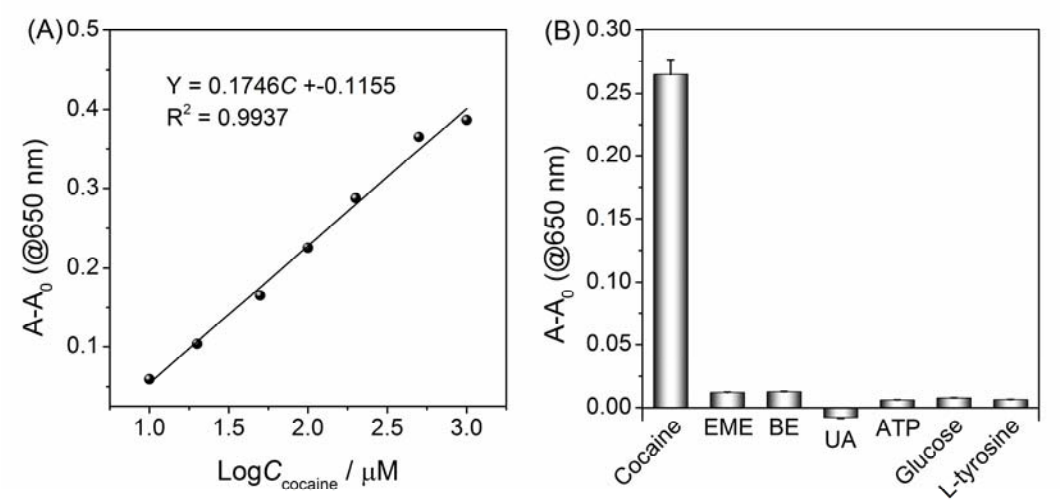

Fig. S9 Analytical performance for cocaine sensing: (A) calibration graph of cocaine detection with absorbance measurements and (B) selectivity evaluation of the cocaine sensor (100 μM each). EME: ecgonine methyl ester; BE: benzoyl ecgonine; UA: uric acid.

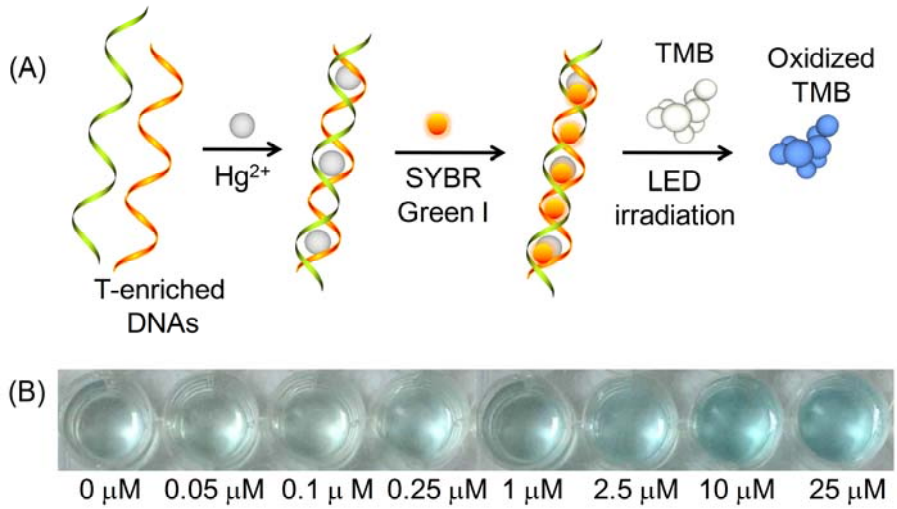

Fig. S10 Design and performance of the dsDNA-SYBR green-based visual sensor for metal ions (Hg^{2+}): (A) the scheme of the Hg^{2+} biosensor; and (B) photographs of the visual detection of 0.05-25 μM of Hg^{2+}. Two T-enriched ssDNAs (Hg^{2+}-specific aptamer) were employed as the probe. In the presence of Hg^{2+}, DNA duplex was generated due to the formation of T-Hg-T mismatch. Then the introduction of SYBR green would result in the intercalation of SYBR green into the grooves of duplex, i.e., formation of dsDNA-SYBR green complex that can photo-catalyze the oxidation of TMB to produce color change.

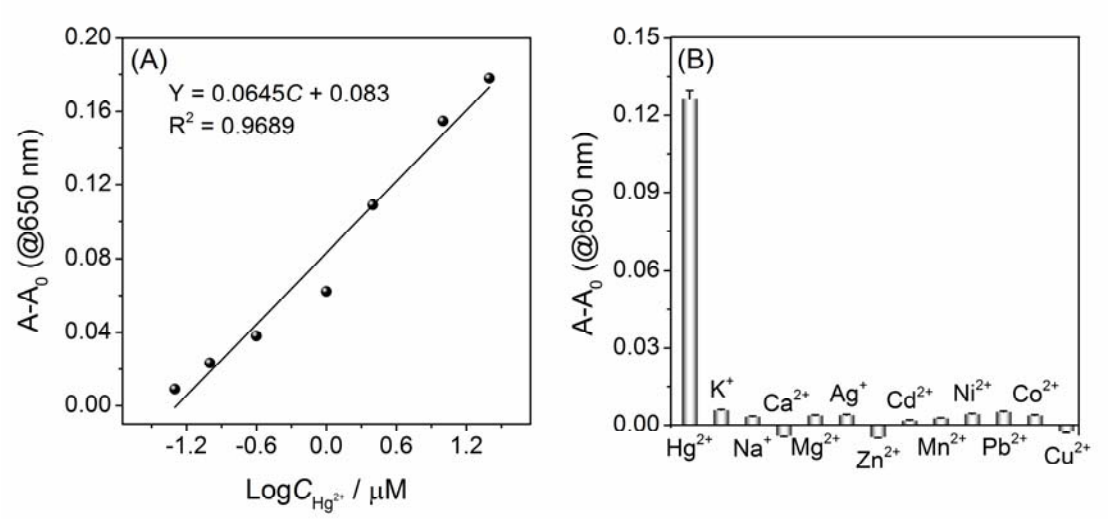

Fig. S11 Analytical performance for Hg^{2+} sensing: (A) calibration graph of Hg^{2+} detection with absorbance measurements and (B) selectivity evaluation of the Hg^{2+} sensor (2.5 μM each).

Table S3. Oberable detection limits and measurable detection limits for DNA, thrombin, cocaine, and Hg^{2+}.

	observable detection limit	measurable detection limit
DNA	0.1 nM	0.02 nM
Thrombin	0.5 nM	0.84 pM
Cocaine	20 μM	2.3 μM
Hg^{2+}	0.25 μM	25 nM

Table S4. Comparison of the general characteristics and performances of dsDNA-SYBR green, DNAzyme, nanozyme, and HRP in bioassays.

Catalyst	Description	Oxidant	Bioassay
dsDNA-SG	Random dsDNA sequences with SG intercalated in, photo-irradiation	Dissolved oxygen	Label-free, universal
DNAzyme	Specific G-quadruplex-based DNA, metal ion and hemin intercalated in	H_2O_2	Label-free, universal[a]
Nanozyme	Limited to a series of nanomaterials such as Fe_3O_4 and graphene oxide	Mostly H_2O_2	Labeled, not universal
HRP	The prototype of various DNAzymes, nanozymes, and the photooxidase in nature	H_2O_2	Labeled, not universal

a: it can be made label-free and universal, but the G-quadruplex structure must be included.

References

[S1] H. Zipper, H. Brunner, J. Bernhagen, F. Vitzthum, Nucleic Acids Res., 2004, 32, 22e136.

[S2] J. Zhang, L. H. Wang, D. Pan, S. P. Song, F. Y. C. Boey, H. Zhang and C. H. Fan, *Small*, 2008, **4**, 1196-1200.

[S3] X. L. Zuo, Y. Xiao and K. W. Plaxco, *J. Am. Chem. Soc.*, 2009, **131**, 6944-6945.