Supporting Information

Translating Molecular Detection into a Temperature Test Using Target-Responsive Smart Thermometer

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1. Materials and methods

The brand name of the portable thermometer used for the tests in this work was Super-Fast Thermapen (ThermoWorks, UT). The NIR laser pointer (808 nm, 1 W) was purchased from BOB LASER Co., LTD (China).

Streptavidin-coated magnetic beads (1 μm in average diameter) and Amicon-10K/30K centrifugal filters were purchased from Bangs Laboratories Inc. (Fishers, IN) and Millipore Inc. (Billerica, MA), respectively. Phospholipase A_2 (PLA_2, MW = 14.5 kDa) from honey bee venom, Indocyanine green (ICG), sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC), Tris(2-carboxyethyl)phosphine hydrochloride (TCEP), 2-(N-morpholino)ethanesulfonic acid (MES), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and other chemicals were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). Phospholipids including 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-1000] (ammonium salt, DPPE-PEG) were purchased from Avanti Polar Lipids. The following oligonucleotides were purchased from Integrated DNA Technologies, Inc. (Coralville, IA): from left to right: 5′ to 3′

Biotin-modified DNA (Biotin-DNA) for cocaine, and uranium sensors:
TCACAGATGAGTAAAAAAAAAAA-biotin
Thiol-modified DNA (Thiol-DNA) for cocaine and uranium sensors:
HS-AAAAAAAAAAAGTCTCCCGAGAT
Cocaine aptamer (Coc-Apt):
TTTTTTACTCATCTGTGAATCTCGGGAGACAAGGATAAATCCTTCAATGAAGTGGGTCTCC
C
UO_2^{2+}-dependent DNAzyme (39E):
CACGTCCATCTCTGCAGTCGGGTAGTTAAACCGACCTTCAGACATAGTGAGT
Substrate of the UO_2^{2+}-dependent DNAzyme (39S):
ACTCATCTGTGAACTCACTATrAGGAAGAGATGGACGTGATCTCGGGAGAC
Buffers used in this work:
PBS Buffer: pH 7.3, 0.1 M sodium phosphate, 0.1 M NaCl, 0.05% Tween-20
HEPES buffer: pH 7.4, 0.01 M HEPES, 0.1 M KCl, 0.001 M MgCl, 0.05% Tween-20
MES Buffer: pH 5.5, 0.05 M MES, 0.2 M NaCl, 0.05% Tween-20
Liposome preparation buffer: pH 7.6, 25 mM HEPES, 150 mM NaCl, 5 mM KCl, 1mM $MgCl_2$, 1mM $CaCl_2$

The detection device for the target-responsive liposome integrated thermometer system (TRESTM) was fabricated using a self-made photothermal chamber (Scheme 1). The NIR laser pointer and 96-well colorless plate were firmly fixed at the top and the bottom of sample holders in the chamber, respectively. The output power of NIR laser pointer was 1 W, and the height of the pointer to the bottom was controlled to have an illumination area of ~1.5 mm in diameter. All NIR-laser irradiations were carried out at room temperature (25 ^{o}C) under atmospheric conditions. After laser irradiation, a super-fast thermapen with 3-second readings was used to detect the temperature change. *Caution! NIR laser light is extremely dangerous to eyes. Always use NIR laser blocking protection glasses when measuring the temperature!*

2. Synthesis and characterization of DNA-PLA_2 conjugate

The DNA−PLA_2 conjugate was synthesized by the maleimide-thiol reaction using heterobifunctional linker sulfo-SMCC (Figure S1a). Briefly, 30 μL of 1 mM thiol-DNA, 2 μL of 1 M PBS buffer (pH 5.5), and 2 μL of 30 mM TCEP were mixed and incubated at room temperature for 1 hour. Then, the thiol-DNA was purified by Amicon-10K using PBS buffer by 8 times. For PLA_2 conjugation, 200 μL of 5 mg mL^{-1} PLA_2 in PBS buffer was mixed with 1 mg of sulfo-SMCC. After vortexing for 5 minutes, the solution was placed on a shaker for 1 hour at room temperature. The mixture was then purified by Amicon-10K using PBS buffer by 8 times. The purified solution of sulfo-SMCC-activated PLA_2 was mixed with the above solution of thiol-DNA. The resulting solution was kept at room temperature for 48 hours. To remove un-reacted thiol-DNA, the solution was purified by Amicon-30K 8 times using PBS buffer.

The purified DNA−PLA_2 conjugate was characterized by UV-Vis absorption spectra and PAGE images. As shown in Figure S1b, the UV absorption spectra of the purified DNA-PLA_2 conjugate overlaped well with the sum of its two components, DNA and PLA_2. Figure S1c and S1d show the PAGE images of the above conjugation products. The DNA was modified with FAM (fluorescein) so that the DNA and DNA−PLA_2 conjugate could be fluorescently imaged. In another gel, PLA_2 and DNA−PLA_2 conjugate were stained by Coomassie brilliant blue. The DNA−PLA_2 conjugate exhibited a broad fluorescent band that migrated very slowly, while free PLA_2 was invisible in this fluorescent image. Correspondingly, in the protein-stained image, the band for PLA_2 was located between 10K and 20K, which is in agreement with the molecular weight of PLA_2 (14.5 kDa). Upon conjugation with DNA, the migration of the DNA−PLA_2 conjugate band was less than that of PLA_2 because the conjugation increased the molecular weight of the enzyme.

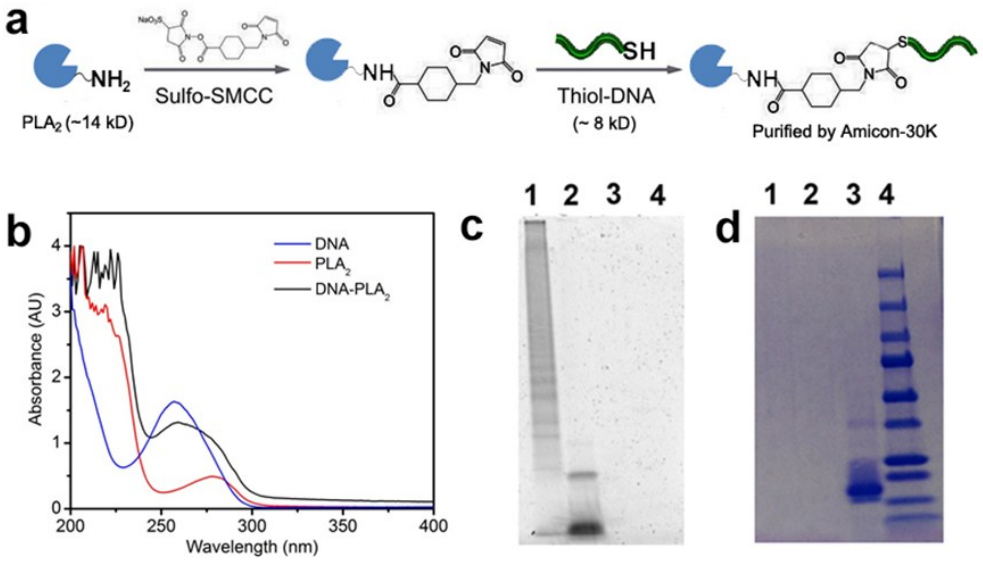

Figure S1. (a) The conjugation of DNA and PLA_2 by the heterobifuntional linker (sulfo-SMCC). (b) UV-Vis absorption spectra of the thiol-DNA, PLA_2, and DNA−PLA_2 conjugate. Native PAGE (4-20% gradient gel) fluorescence (c) and protein-staining (d) images for the conjugation products. lane 1: DNA−PLA_2 conjugate; lane 2: thiol-DNA; lane 3: PLA_2; lane 4: molecular weight markers.

3. Synthesis and characterization of liposome-encapsulated ICG

Liposome-encapsulated ICG were prepared by the film hydration/extrusion method. DPPC and DPPE-PEG (95:5 mol %, 3.6 μmol total lipid) were codissolved in chloroform. The organic solvent was eliminated under nitrogen, and the lipid film was placed under vacuum overnight. The dried film was then hydrated with liposome preparation buffer containing ICG (0.8 mg/mL, lipid/dye molar ratio of 260). The ICG-containing dispersion was freeze thawed 6 times, and incubated at 37 °C overnight. After that, the solution was extruded 10 times through double-stacked 200-nm pore size polycarbonate membranes using an extruder (Avanti Polar Lipids, Inc.) to yield small unilamellar vesicles. Free dye was removed by size exclusion chromatography on a G-25 column (GE Healthcare) using liposome preparation buffer as eluent. The purified liposome-ICG had a mean diameter of 155 nm as measured by dynamic light scattering (Malvern Zetasizer Nano ZS, UK), as shown in Figure S2. The PLA_2 catalyzed the hydrolysis of the ester bond at the *sn*-2 position of phospholipids, which led to the increase of the average hydration diameter.

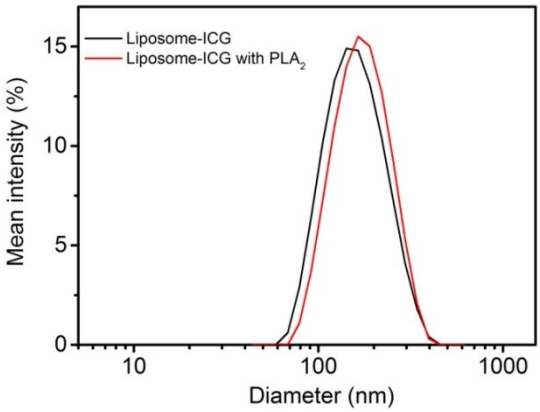

Figure S2. Size distribution of liposome-ICG before (black line) and after (red line) incubated with PLA_2 for 1h.

4. Standardize the concentration of liposome-ICG for thermo-sensor

In our TRESTM system, the liposome-ICG was used as an "enzyme substrate" for signal amplification. The concentration of liposome-ICG for each assay was standardized by UV-Vis absorption spectra. According to a standard curve of free ICG (Figure S3), the equivalent ICG concentration in liposome-ICG sample was determined to be about 0.25 mg mL^{-1} based on the UV absorption intensity (780 nm) of fully decomposed liposomes by heat treatment. The ICG encapsulation efficiency (EE) was calculated to be about 31%, which was expressed according to the following formula: EE (%) = (concentration of encapsulated ICG/concentration of initially added ICG) × 100 (%).

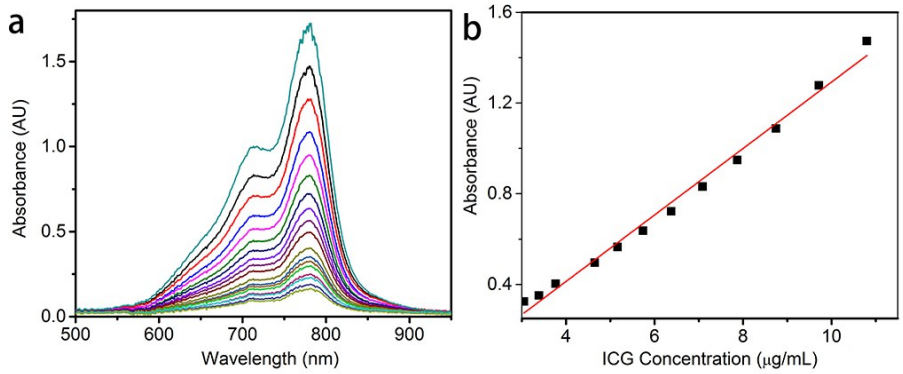

Figure S3. (a) Standard curve for quantification of ICG concentration in liposome-ICG used for thermo-sensor. (b) The UV absorption intensity at 780 nm was plotted as a linear function of ICG concentration.

5. Procedures for cocaine and UO_2^{2+} detection using the TRESTM system

For the cocaine sensor, a portion of 1 mL 1 mg/mL solution of MBs was placed close to a magnetic rack for 1 minute. The clear solution was discarded and replaced by 1 mL of PBS buffer. This buffer exchange procedure was repeated twice. Then, 12 μL 0.5 mM Biotin-DNA was added to the MBs solution and well mixed for 30 minutes at room temperature. After that, the MBs were washed twice using PBS buffer to remove excess biotin-DNA. Later, 12 μL of 0.5 mM cocaine aptamer were added to the MB solution and well mixed for 30 minutes at room temperature. After three times washing using PBS buffer to remove excess DNA, 200 μL of 5 mg/mL DNA−PLA_2 conjugate was added to the solution and well mixed at room temperature for 30 minutes. Excess DNA−PLA_2 conjugate was washed off by PBS buffer three times and was recycled for further use by condensing the washing solutions using an Amicon-30K. The DNA−PLA_2 immobilized MBs were then dispersed in 1 mL of PBS Buffer. After the removal of PBS buffer, the MBs contained in each 100 μL aliquot of this solution were well mixed with 100 μL of cocaine samples in PBS buffer for 30 minutes. After that, the solution was separated and the supernatant was incubated with 100 μL of liposome-ICG solution prepared as above. After 2 hours incubation, 180 μL of the supernatant was transferred into a 96-well colorless plate. Immediately after NIR laser irradiation for 1 min (1 W, ~1.5 mm in diameter for illumination area), the solution temperature was measured by thermometer.

For UO_2^{2+} DNAzyme sensor, the MBs were soaked in HEPES Buffer with additional 100 mM NaCl overnight before use to ensure the removal of the phosphate anions that adsorbed on the surface of MBs during storage, because MBs were purchased as solutions in PBS buffer and phosphate anions can

compete with the binding between UO_2^{2+} and the DNAzyme. The detection method was the same as cocaine detection except that all the PBS buffers were substituted by the pH 5.5 MES buffer.

6. Optimized procedure for cocaine detection

Many factors can affect the performance and results of the cocaine sensor system. The effects of incubation time of liposome-ICG with released PLA_2 (Figure S4a), and NIR laser irradiation time (Figure S4b) were investigated and optimized.

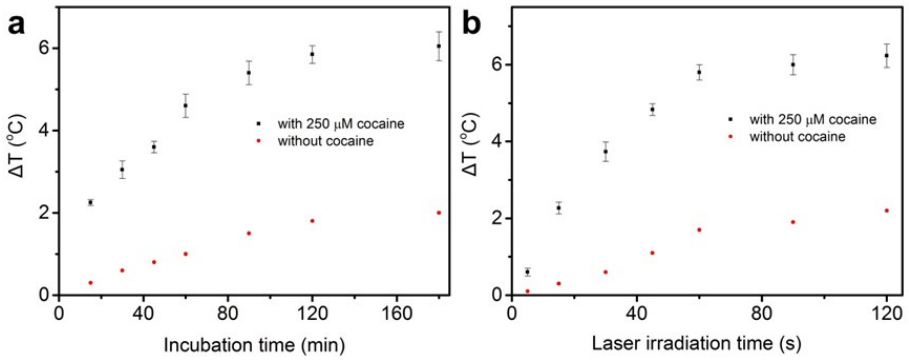

Figure S4. Optimize the procedure for cocaine detection. a, Effect of incubation time of liposome-ICG with released DNA−PLA_2 conjugate. b, Effect of NIR Laser irradiation time.

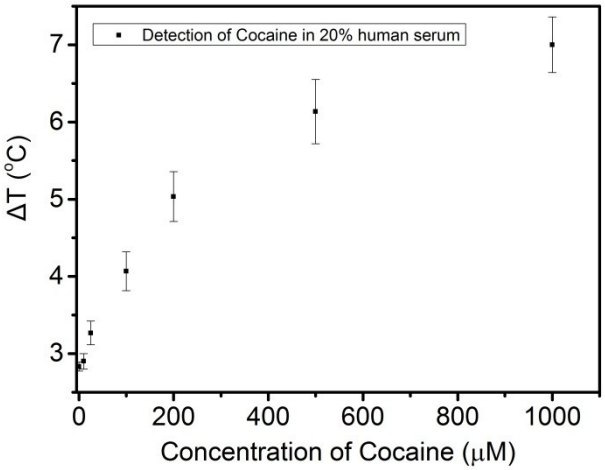

Figure S5. Detection of different concentrations of cocaine in 20% human serum using TRESTM system.

7. Storage Stability Evaluation of liposome-encapsulated ICG

For the end user's freedom in choosing the time of using the sensor system, the shelf-life of liposome-encapsulated ICG was investigated. Typically, a series of liposome-ICG was prepared, stored at 4 °C, and subjected to cocaine detection. The temperature response for 250 μM cocaine was recorded up to 4 weeks. As shown in Figure S6, after refrigerated storage for one month, our TRESTM system performed more than 80% of the initial sensitivity. The improved stability of lipsome-ICG was in agreement with previous reports tested by fluorescence spectra, because the encapsulated ICG molecules were protected from environmental aqueous solution in the liposome, which permitted

prolonged periods of storage without significant leakage or degradation. These results indicated the potential of liposome-encapsulated ICG for practical biosensing.

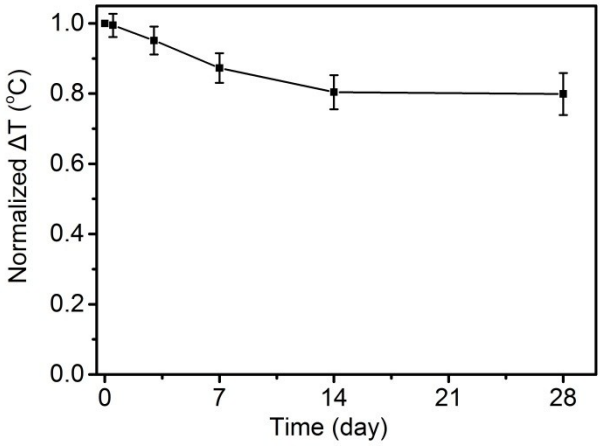

Figure S6. Detection of 250 μM cocaine using TRESTM system by the liposome-ICG after different periods of storage at 4 °C.