Supporting Information

Multimodal Detection of a Small Molecule Target Using Stimuli-Responsive Liposome Triggered by Aptamer-Enzyme Conjugate

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EXPERIMENTAL DETAILS

Chemicals and Materials

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. Phosphatidylcholine 2-acetylhydrolase (PLA_2, MW = 18 kDa) from honey bee venom, 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), gadopentetic acid (Gd-DTPA) 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.

All oligonucleotides used in this study were purchased from Integrated DNA Technologies Inc. (Coralville, IA) with the following sequences (from left to right: 5′ to 3′).

Biotin-modified DNA (Biotin-DNA) for cocaine, and uranium psensors:

TCACAGATGAGTAAAAAAAAAAAA-biotin

Thiol-modified DNA (Thiol-DNA) for cocaine and UO_2^{2+} sensors:

HS-AAAAAAAAAAAAGTCTCCCGAGAT

Cocaine aptamer (Coc-Apt):

TTTTTTACTCATCTGTGAATCTCGGGAGACAAGGATAAATCCTTCAATGAAGT GGGTCTCCC

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

Liposome buffer: pH 7.6, 25 mM HEPES, 150 mM NaCl, 5 mM KCl, 1mM $MgCl_2$, 1mM $CaCl_2$

Formulation of Smart Liposome

Smart liposomes were prepared by the film hydration/extrusion method. The liposome is consist of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy-(polyethylene glycol)-1000] (DPPE-PEG, ammonium salt) in a molar ratio of 9.5 : 0.5. DPPC and DPPE-PEG (95:5 mol %, 3.6 μmol total lipid) were co-dissolved 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 different signaling agents (0.8 mg/mL). The lipids mixture 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 molecules were removed by size exclusion chromatography on a G-25 column (GE Healthcare) using liposome preparation buffer as eluent. The concentration of the lipids in each sample was measured at the fluorescence emission of 627 nm using Nile Red stained liposome samples. The

release profile of stimuli-responsive liposome was tested to optimize the experimental conditions in 10 mM pH 7.4 HEPES buffer solution containing 150 mM NaCl and 150 μM $CaCl_2$. The liposome samples were centrifuged at 16,110 *g* for 5 min and the supernatant was collected for future experiments. The standard lipids concentration for the prepared liposome solution was tuned to be ~5 mg/mL for future experiments.

Preparation of DNA-PLA_2 Conjugate

The DNA−PLA_2 conjugate was synthesized by the maleimide-thiol reaction using hetero-bifunctional linker sulfo-SMCC. 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 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 unreacted thiol-DNA, the solution was purified by Amicon-30K by 8 times using PBS buffer. Since the surface amine groups in PLA_2 are not associated with the active sites, the conjugation will still preserve the catalytic activity of the enzyme.

Activity Assay of DNA-PLA_2 Conjugate

In order to quantitatively estimate the enzymatic activity of DNA-PLA_2 and free PLA_2, the Invitrogen EnzChek® Phospholipase A_2 Assay Kit as a simple fluorescent method was applied to monitor the activity. The EnzChek® PLA_2 Assay used Red/Green

BODIPY PC-A2 (1-O-(6-BODIPY 558/568-Aminohexyl)-2-BODIPY FL C5-Sn-Glycero-3-Phosphocholine) as the PLA_2 substrate. After incubation with the substrate, the PLA_2 activity is monitored by the intensity increase of a single wavelength at ~510 nm. Four PLA_2 samples were tested: 1. free PLA_2 of 1 U/mL concentration (~5 nM); 2. free PLA_2 of 1 U/mL mixed with 25 μM thiolated DNA; 3. DNA-PLA_2 conjugates prepared by ~5 nM PLA_2 and excess thiolated DNA; 4. Negative control without PLA_2. Kinetics curves were monitored for 15 min with excitation at 470 nm and emission at 510 nm. Fluorescence spectra were measured after mixing the substrate with PLA_2 samples for 15 min.

Characterizations

Dynamic light scattering (DLS) was performed using a Malvern Zetasizer Nano ZS instrument (Malvern Instruments, UK).

Cryo-TEM samples of liposomes were prepared using a Vitrobot according to a literature protocol. Briefly, a drop of liposome solution was applied onto a TEM grid, the grid was then blotted and immediately plunge-frozen into ethane slush cooled by liquid nitrogen. The as-prepared sample onto TEM grid was then transferred onto a cryo holder via Gatan CT3500 Cryotransfer system, which was pre-cooled with liquid nitrogen. The images were obtained on a JEOL 2100 transmission electron microscope at an acceleration voltage of 80 kV.

Supernatants of Gd-DTPA-loaded smart liposomes were placed in a 96-well plate and imaged by using Allegra 3T MRI scanner at a repetition time (TR) of 2000 ms and inversion time from 22 ms to 1600 ms. T1-weighted images obtained at different

inversion times were used to fit T1 relaxation time corresponding to different cocaine concentrations by using following equation:

$$S(t) = S_0 (1 - 2\exp(-TI/T1) + \exp(-TR/T1)),$$

where S_0: magnetization, TI: inversion time, TR: repetition interval, S(t): relaxivity (signal from amplitude of free induction decay).

ADDITIONAL FIGURES

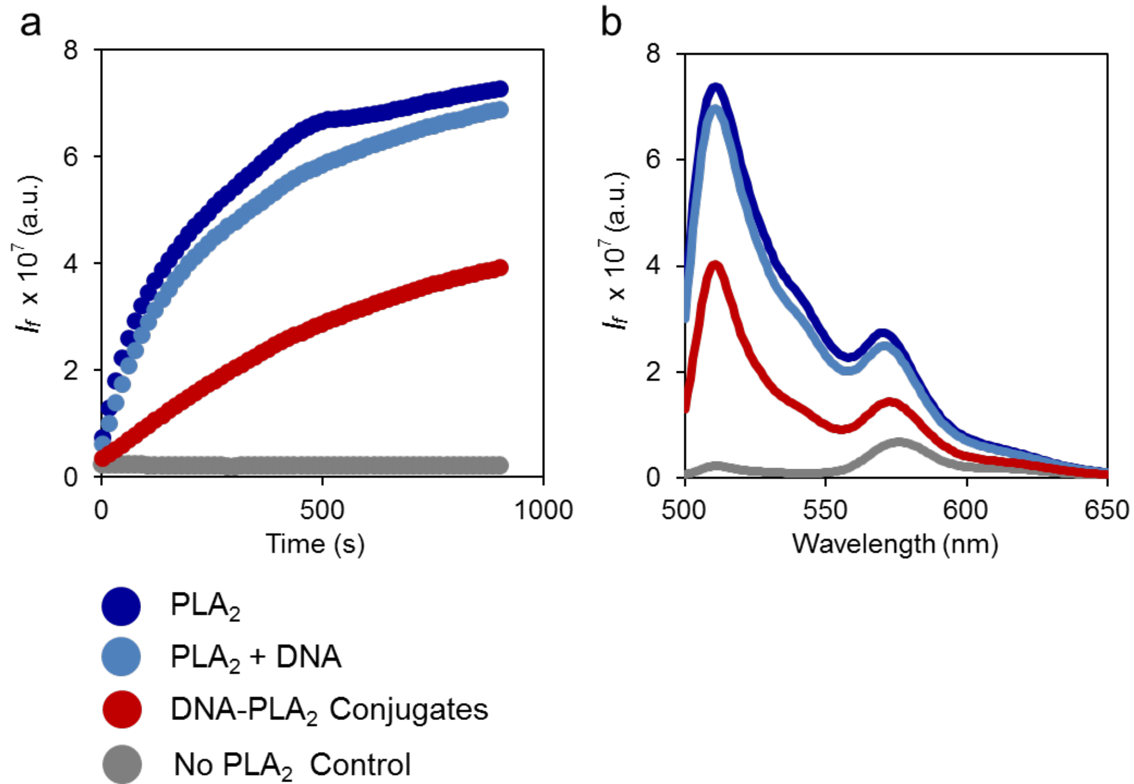

Figure S1. Kinetic study (a) and fluorescence spectra (b) showing the enzymatic activity of four PLA_2 samples. Dark blue: substrate with 1 U/mL PLA_2 (~5 nM); Light blue: substrate with 1 U/mL PLA_2 and 25 μM thiolated DNA; Red: substrate with DNA-PLA_2 conjugates (~5 nM PLA_2 reacted with excess amount of thiolated DNA); Grey: substrate without PLA_2 as negative control. Kinetic curves were monitored for 15 min with excitation at 470 nm and emission at 510 nm. Fluorescence spectra were measured after mixing substrate with PLA_2 samples for 15 min.

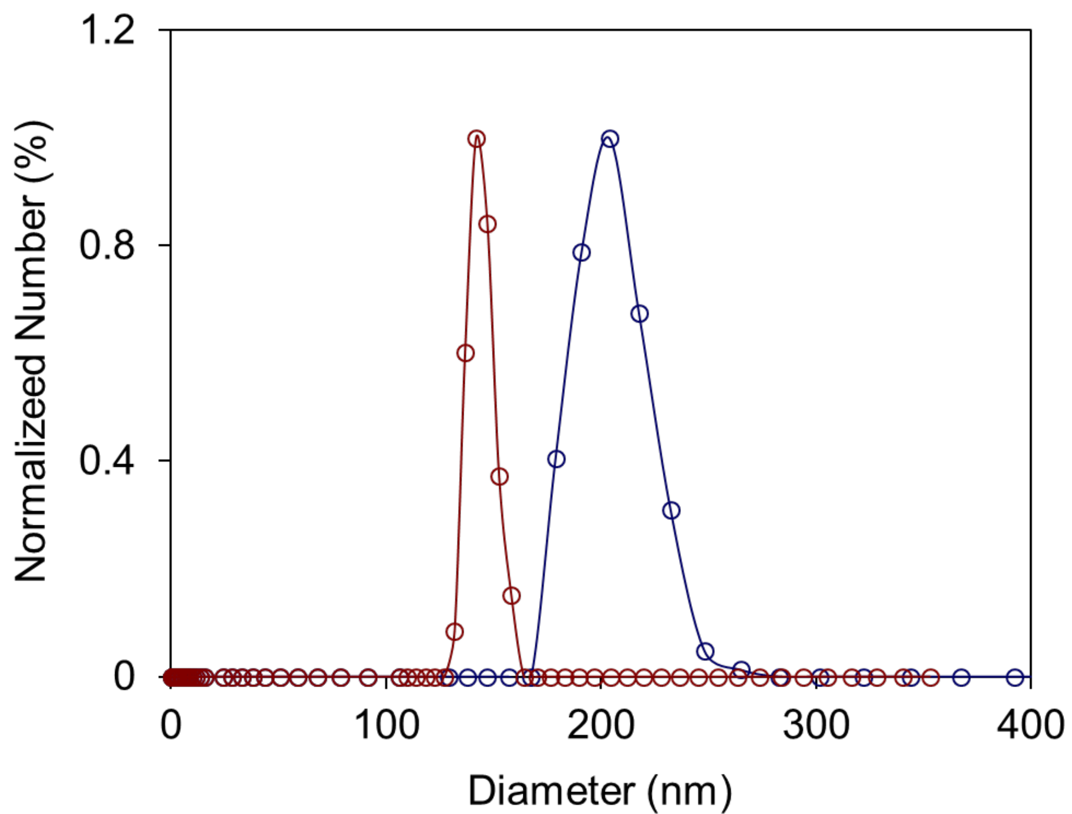

Figure S2. DLS results of smart liposome samples diluted in buffer (black) and PLA_2-containing buffer (red). The hydrodynamic radius of smart liposome increased in the presence of PLA_2.

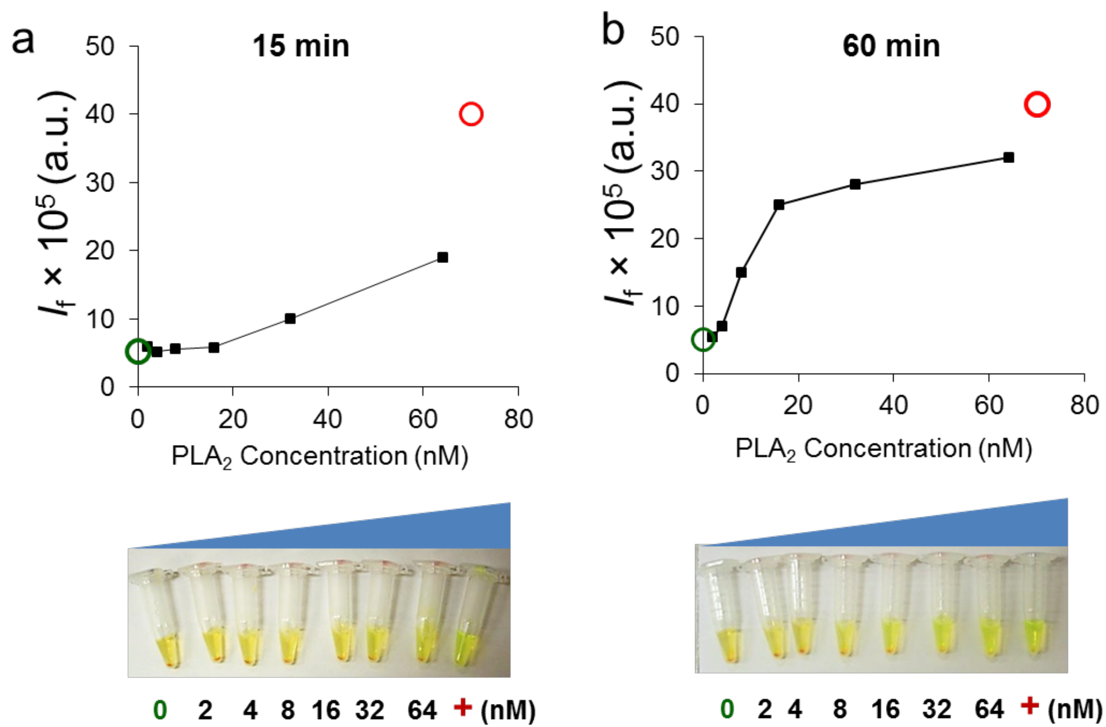

Figure S3. PLA_2 enzymatic activity tests of PLA_2 with the reaction time of 15 min (a) and 1 hour (b). Top: calibration curve of the fluorescence intensity of hydrolyzed liposomal uranin treated with PLA_2 of different concentration concentrations. Bottom: corresponding photograph images. Sample with green label is no PLA_2 negative control and sample with red label is positive control with uranin fully released under surfactant and heat treatment.

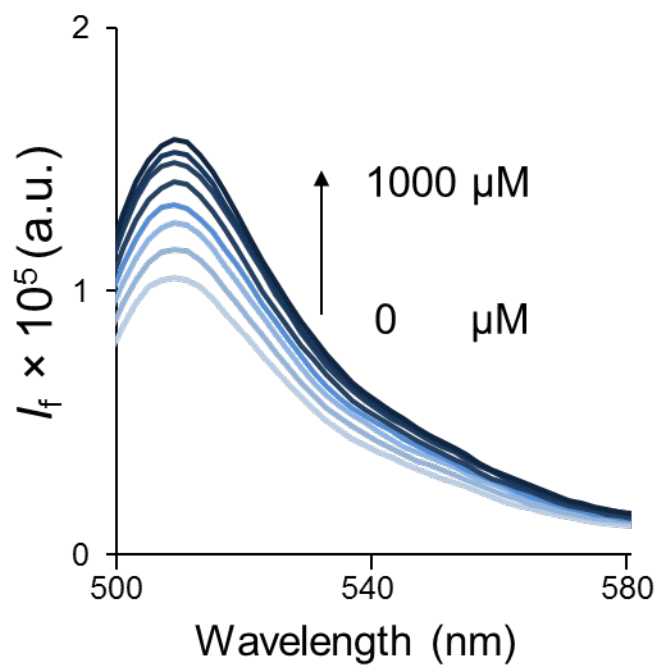

Figure S4. Design and performance of fluorescence detection of cocaine. Fluorescence spectra of the supernatants of smart liposome samples treated with released DNA–PLA_2 conjugates upon the addition of cocaine at different concentrations.

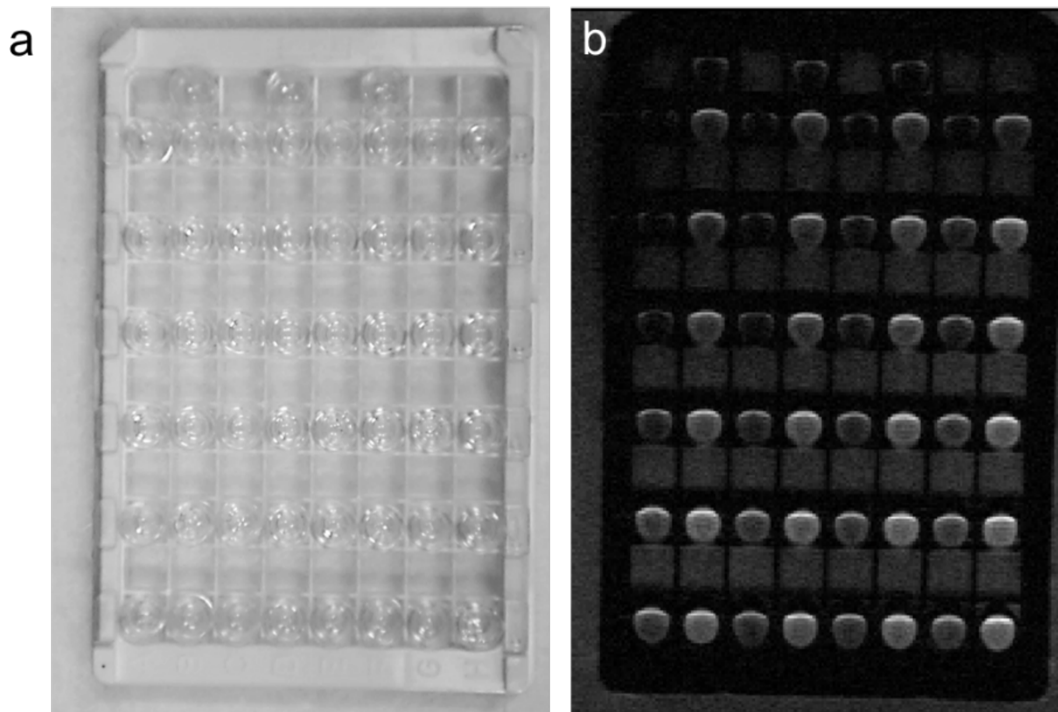

Figure S5. Representative photograph (a) and MRI T1-weighted image (b) of a 96-well plate with a series of samples corresponding to different cocaine concentrations.

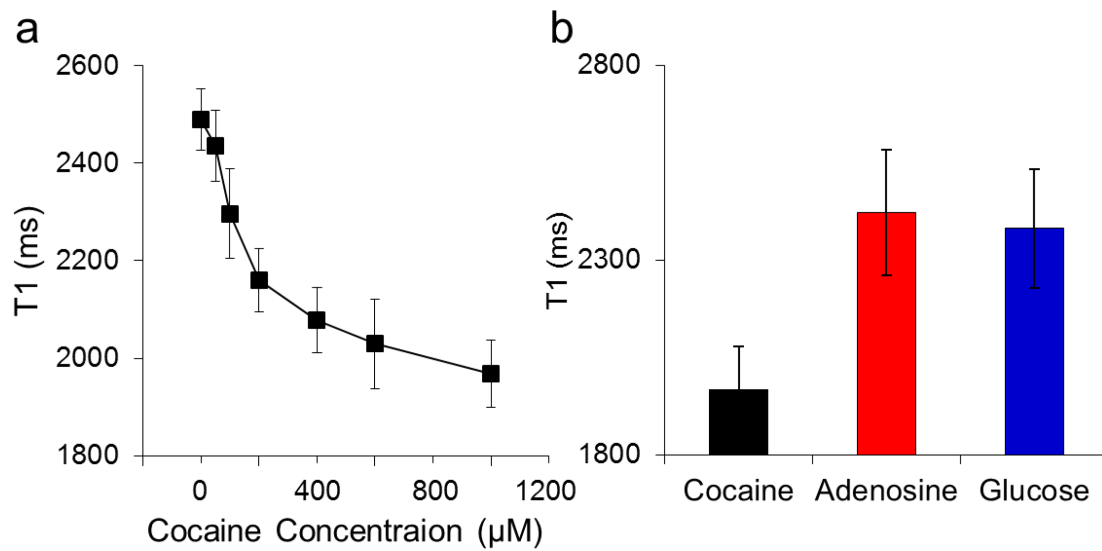

Figure S6. (a) Calibration curve of the T1 relaxation time of different smart liposome samples in the presence of different cocaine concentrations. (b) Selectivity of the MRI detection of cocaine over adenosine and glucose plotting with T1 relaxation time.