Supporting Information

Specific Detection of Cancer Cells through Aggregation-Induced Emission of a Light-Up Bioprobe

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EXPERIMENTAL SECTION

Instrumentation

Probe purification was performed with a Hitachi L-2000 reverse-phase high performance liquid chromatography (HPLC, Hitachi Corp., Japan) equipped with a C18 analytical column. UV-Vis absorption spectra were obtained using a Cary 50 Bio Spectrophotometer (Varian Inc., CA, USA) equipped with a xenon flash lamp. Emission spectra were recorded with a Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon Inc., USA). Excitation wavelength was 352 nm and the emission spectra were corrected against PMT response. Excitation and emission slit widths were both of 8 nm. Quartz cuvettes with 3 mm path length and 3 mm window width were used for the emission measurements. Unless otherwise specified, all spectra were taken at an ambient temperature of 20 ºC in 20 mM phosphate buffer at pH 7.4. Cell imaging was performed using a Nikon Eclipse Ti fluorescence microscope (Nikon Corp., Japan).

Materials

The amino functional group modified Ramos cell specific aptamer (5'-H_2N-AAC ACC GGG AGG ATA GTT CGG TGG CTG TTC AGG GTC TCC TCC CGG TG-NH_2-3') was synthesized and Ultra-PAGE purified by Sangon Biotechnology Co. Ltd. (Shanghai, China). Nuclease S1 was purchased from Sangon Biotechnology Co., Ltd. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was purchased from J&K Scientific Co., Ltd. (Beijing, China). Other chemicals were purchased from Sigma-Aldrich (USA) or Alfa Aesar (USA) and used as received. Organic solvents

(analytical grade) were freshly dried and distilled before use. All stock and buffer solutions were prepared with water purified with Milli-Q A10 (Millipore, Billerica, MA, USA).

Cell culture

All the cells used in this work were cultured in cell flasks separately according to the instructions from the American Type Culture Collection. The cell line was grown to confluence in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 100 IU/mL of penicillin-streptomycin. The cells were maintained at 37 ºC in a humidified atmosphere (95% air and 5% CO_2). The cell densities were determined using a hemocytometer, and this was performed prior to each experiment. A 1.0 mL suspension of 2×10^5 cells dispersed in RPMI 1640 cell medium was centrifuged at 1500 rpm for 5 min, washed with phosphate buffer 5 times, and resuspended in 1.0 mL of the cell medium.

Probe synthesis

Synthesis of compound 4: Diphenylmethane (**2**) (2.02 g, 12.02 mmol) was dissolved in anhydrous THF (30 mL). A solution of n-butyllithium (10.00 mmol) in hexane (4.0 mL, 2.5 M in hexane) was added at 0 ºC under a nitrogen atmosphere. The resulting orange-red solution was stirred for 1 hour at 0 ºC. Then, 4-benzoylbenzoic acid (**3**) (2.71 g, 12.00 mmol) was added and the mixture was stirred for 6 hours while allowing the temperature to rise gradually to room temperature. The solution turned

from orange-red to green gradually. The reaction was quenched by the addition of an aqueous solution of ammonium chloride, and the organic layer was extracted with EtOAc (3 × 100 mL). The organic layers were combined, washed with saturated NaCl solution and dried with anhydrous $MgSO_4$. After solvent evaporation and drying under vacuum, the crude product (compound **4**) was obtained without further purification.

Synthesis of compound 5: The resulting compound **4** crude product was dissolved in anhydrous toluene (80 mL) in a Schlenk flask fitted with a Dean-Stark trap. A catalytic amount of p-toluenesulfonic acid (PTSA) was added and the mixture was refluxed for 6 hours. The resulting brown residue was cooled to room temperature and washed with $NaHCO_3$ (10%, 50 mL). The organic layer was dried with anhydrous $MgSO_4$ and evaporated under vacuum. The crude product was purified by a silica gel column using a mixture of CH_2Cl_2 / methanol (10 : 1) as eluent. After drying under vacuum, compound **5** (1.27 g, 38%) was obtained as a white powder. ^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) = 12.83 (s, 1H), 7.74 (d, 2H), 7.13 (m, 9H), 7.09 (d, 2H), 6.99 (d, 6H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) = 171.98, 149.80, 143.16, 143.04, 143.01, 142.73, 139.86, 131.42, 131.27, 129.64, 127.89, 127.72, 126.98, 126.79. MALDI-TOF-MS: m/z calculated for $C_{27}H_{20}O_2$: 376.2; found: 376.2.

Synthesis of compound 1: Compound **5** (100 mg, 0.27 mmol), pyridine (0.1 mL), and *N*,*N'*-disuccinimidyl carbonate (77 mg, 0.30 mmol) were dissolved in CH_3CN (30

mL). The mixture was refluxed for 3 hours. After solvent evaporation, the residue was poured into a saturated NaCl solution and extracted with EtOAc (3 × 50 mL). The organic layers were combined and evaporated under vacuum. The crude product was purified by a silica gel column using a mixture of EtOAc / petroleum ether (1 : 1) as eluent. After drying under vacuum, compound **1** (0.10 g, 79%) was obtained as a white powder. ^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) = 7.69–7.71 (d, 4H), 7.39–7.42 (t, 5H), 6.96–6.99 (t, 10H), 3.34 (s, 4H). ^{13}C-NMR (100 MHz, DMSO-d_6): δ (ppm) = 175.54, 166.60, 155.97, 147.93, 147.72, 147.59, 147.44, 144.37, 136.95, 135.91, 135.77, 134.84, 133.31, 133.29, 133.11, 132.40, 132.18, 127.31, 30.71. MALDI-TOF- MS: m/z calculated for $C_{31}H_{23}NO_4$: 473.2; found: 496.3 (M + Na).

Synthesis of the TPE-aptamer: The amino functional group modified Ramos cell specific aptamer (100 μM) was dissolved in $NaHCO_3$ (0.1 M, 250 μL) aqueous solution. Compound **1** (1.00 mM) was dissolved in DMSO (250 μL). The two solutions were mixed thoroughly, and the mixture was incubated at 37 ºC for 24 hours. The mixture was centrifuged, and the supernatant was purified by reverse phase HPLC. The collected product was vacuum-dried to yield colorless solid. Deionized water was added to yield a stock solution of desired concentration. The **TPE-aptamer** sample solution was stored at 4 ºC before use.

Detection of cancer cells

Different amounts of the Ramos cells (0, 100, 200, 500, 1000, 2000, 3000, 4000, 5000, 10000, 15000 and 20000, respectively) were added to the solution of **TPE-aptamer** (final concentration: 2.5 μM) in 20 mM phosphate buffer at pH 7.4 (total sample volume: 100 μL). The samples were mixed briefly, and the emission spectra were recorded.

Cancer cell recognition in biological fluid: **TPE-aptamer** (final concentration: 2.5 μM) and different amounts of Ramos cells (0, 1000, 2000, 5000 and 10000, respectively) were added to 80 μL human serum (total sample volume: 100 μL). The samples were mixed briefly, and the emission spectra were recorded.

Cell imaging

Ramos, CCRF-CEM and HeLa cells (10,000 cells each) were added to the solution of **TPE-aptamer** (final concentration: 2.5 μM) in 20 mM phosphate buffer at pH 7.4 (total sample volume: 50 μL). The samples were incubated at 4 ºC for 30 min in the dark, and then placed in a glass dish for the cell imaging study using a fluorescence microscope**.**

Cell cytotoxicity assay

The cell cytotoxicity of the **TPE-aptamer** was investigated via the MTT assay. The cells were seeded at a density of 5~10 × 10^4 per well in a 96-well microplate, stabilized for 24 h, and then exposed to the concentration gradient of the **TPE-**

aptamer (0, 0.75, 1.5, 2.5, 3.5 and 5 μM, respectively). After 24 h incubation, 3-(4, 5-dimethylthiazol-2- yl)-2, 5-diphenyltetrazolium bromide (MTT) was added (10 □L/well at 5 mg/mL concentration), and the cells were incubated for 4 h. The supernatant was removed, 150 μL DMSO was added and the UV-vis absorption at 570 nm was determined.

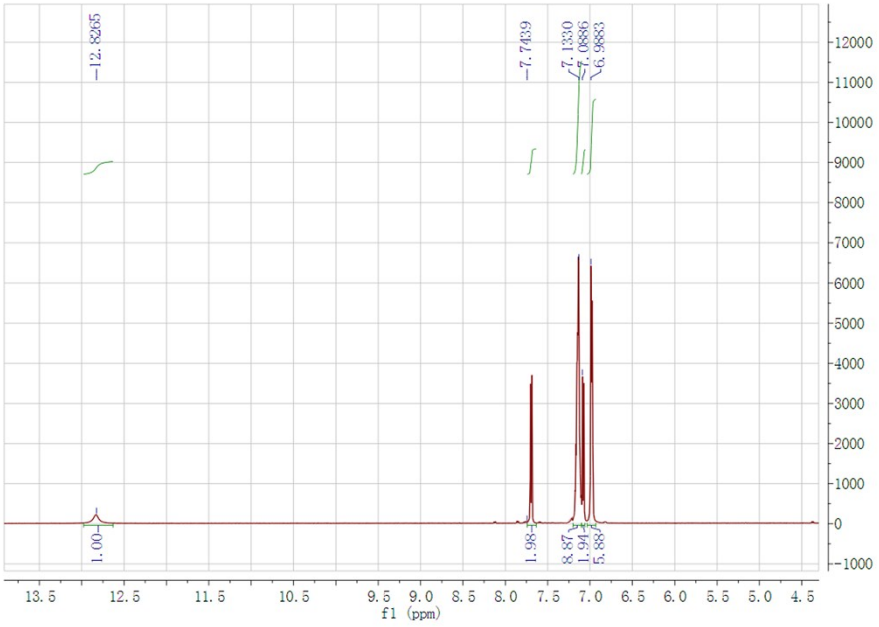

Figure S1. [1]H-NMR spectrum of compound **5**.

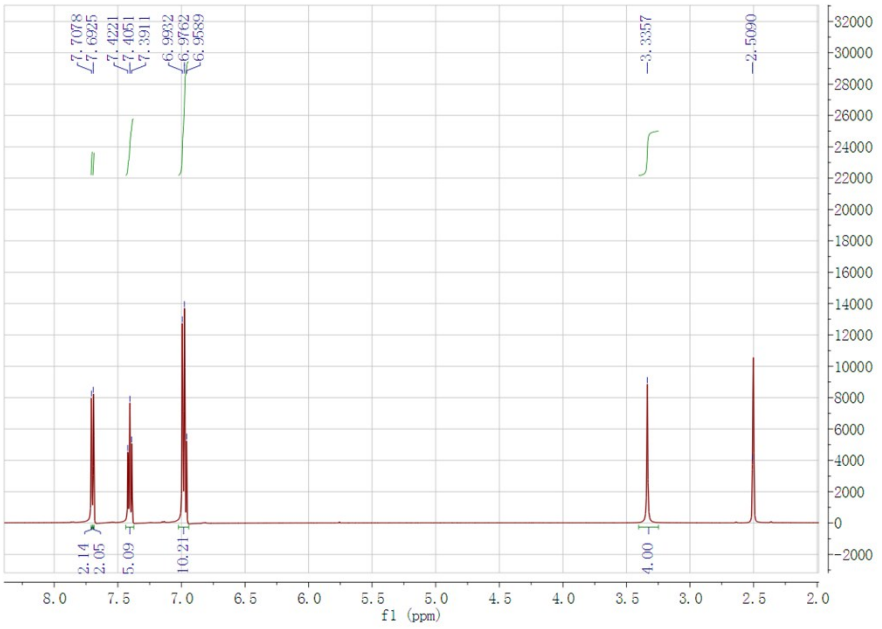

Figure S2. [1]H-NMR spectrum of compound **1**.

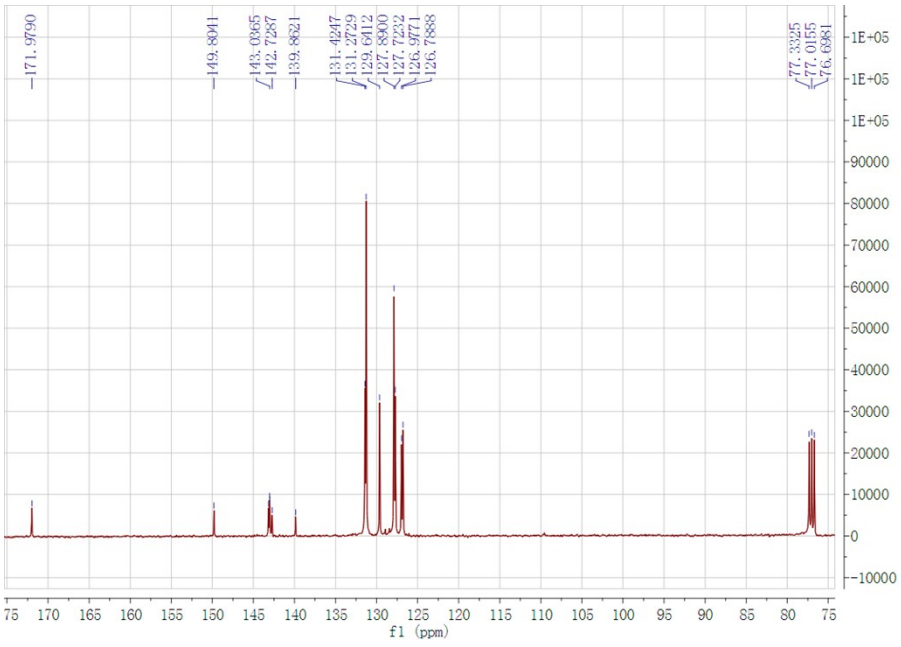

Figure S3. [13]C-NMR spectrum of compound **5**.

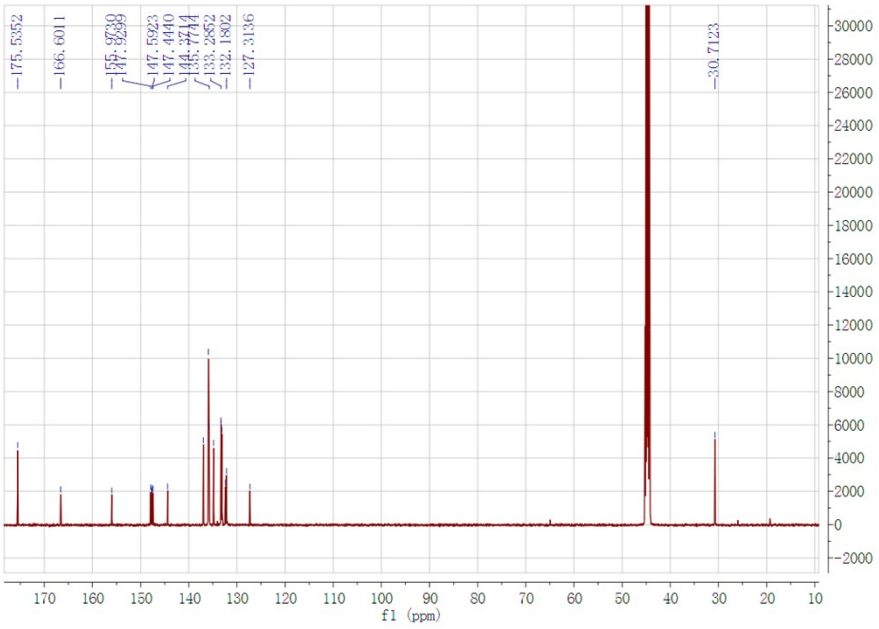

Figure S4. ^{13}C-NMR spectrum of compound **1**.

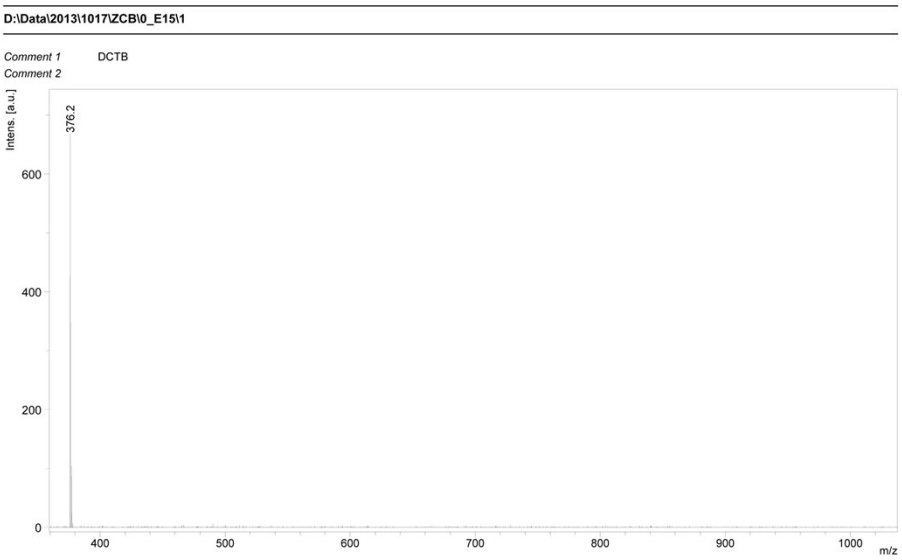

Figure S5. MALDI-TOF-MS of compound **5**.

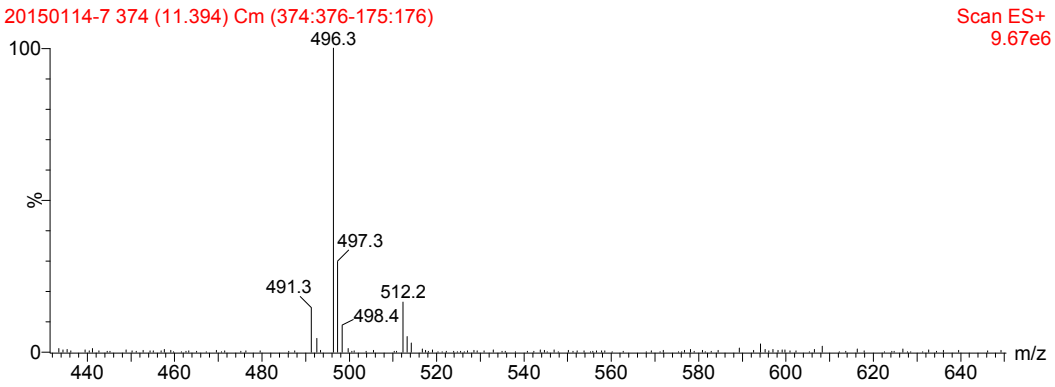

Figure S6. MALDI-TOF-MS of compound **1**.

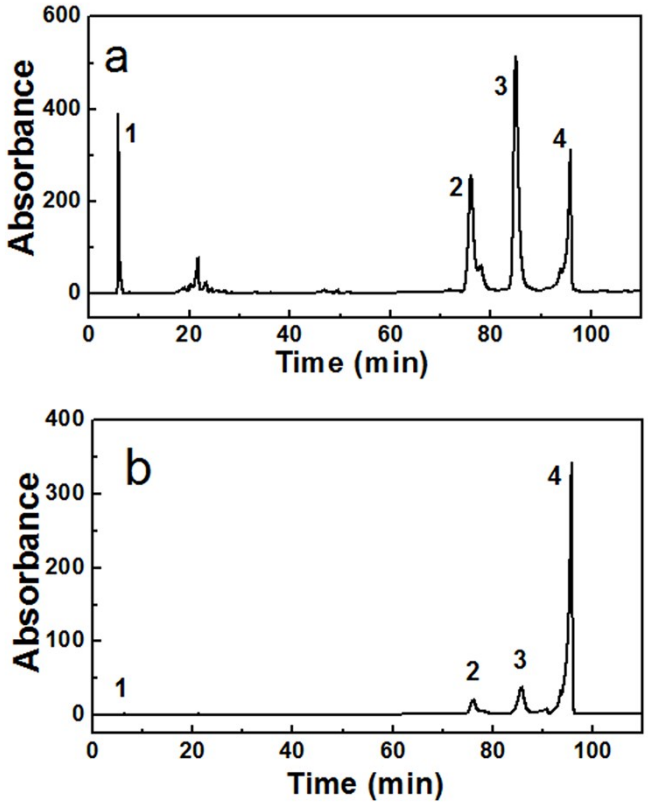

Figure S7. Reverse phase HPLC chromatograms of the crude product: (**a**) absorption at 260 nm; (**b**) absorption at 350 nm.

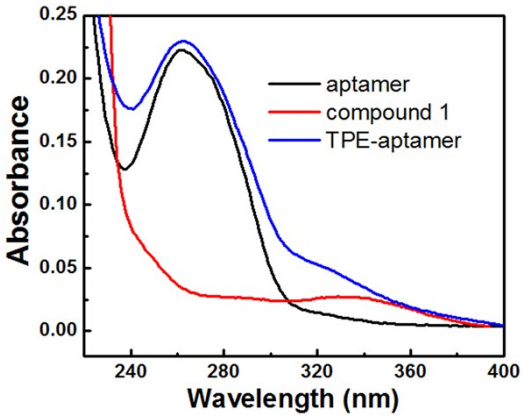

Figure S8. UV-vis absorption spectra of the aptamer (2.5 μM in H_2O), compound **1** (5 μM in DMSO) and the **TPE-aptamer** (2.5 μM in H_2O).

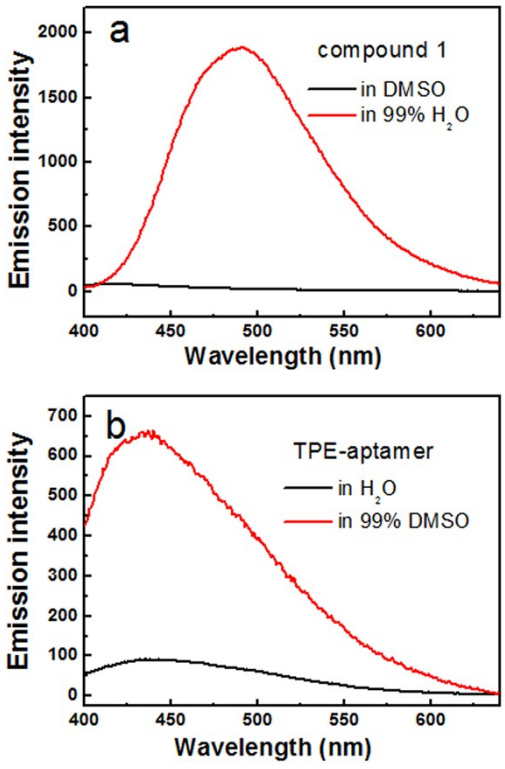

Figure S9. Emission spectra of (**a**) 5 μM compund **1** and (**b**) 2.5 μM **TPE-aptamer** in different solvents.

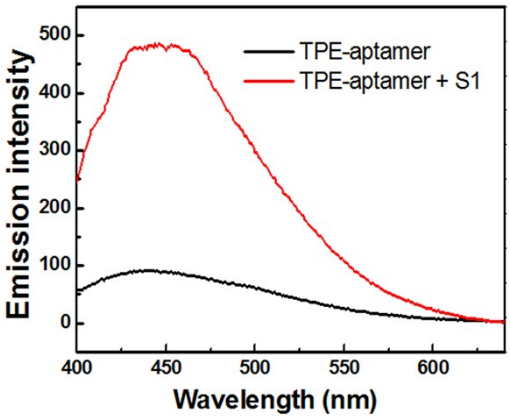

Figure S10. Emission spectra of 2.5 μM TPE-aptamer (black) and the TPE-aptamer digested by nuclease S1 (red) in 20 mM phosphate buffer at pH 7.4.

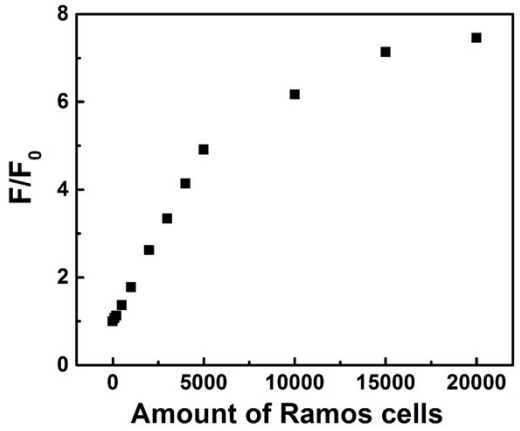

Figure S11. Plot of the emission intensity changes of the **TPE-aptamer** at 470 nm after the addition of the Ramos cells. F and F_0 represent the fluorescence emission intensity after and before the addition of the Ramos cells.

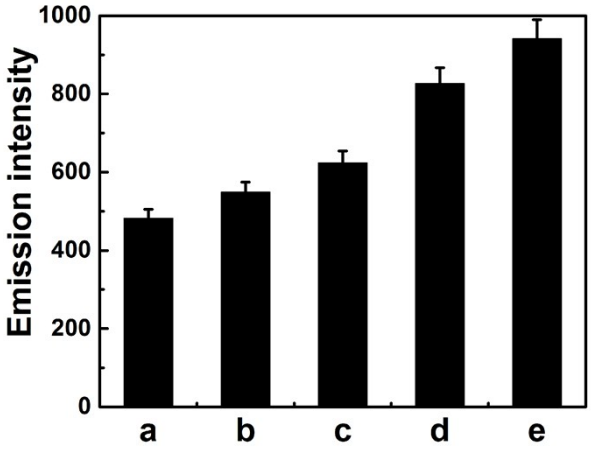

Figure S12. Changes in emission intensity of the **TPE-aptamer** at 470 nm against the amount of Ramos cells added to the human serum sample mixture. Amount of the cell added, columns a-e: 0, 1000, 2000, 5000 and 10000, respectively.

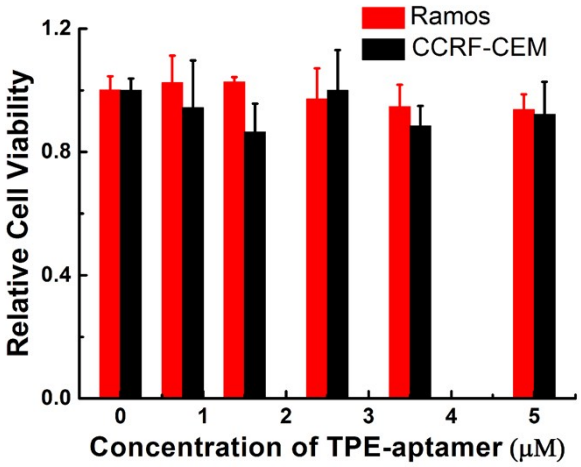

Figure S13. MTT assay, cytotoxicity of the **TPE-aptamer** at different concentrations (probe concentration: 0, 0.75, 1.5, 2.5, 3.5 and 5 μM).