Electronic Supplementary Material (ESI) for Analyst
This journal is © The Royal Society of Chemistry 2013

Supporting Information

A new fluorometric turn-on assay for alkaline phosphatase and inhibitor screening based on aggregation and deaggregation of tetraphenylethylene molecules

Xinggui Gu, Guanxin Zhang,* Zhuo Wang, Wenwen Liu, Le Xiao, Deqing Zhang *

Contents

This journal is © The Royal Society of Chemistry 2013

Synthesis and characterization:

Synthesis of compound 3. $TiCl_4$ (6.2 mL, 56 mmol) was added dropwise under N_2 atmosphere to a stirred suspension of Zn powder (7.3 g, 112 mmol) in dry THF (100 mL) below -10 ^oC, and the reaction was allowed to proceed under reflux for 2 h. A solution of 4,4'-bis(methoxy)benzophenone (3.40 g, 14.0 mmol) and 4-hydroxybenzophenone (2.8 g, 14.0 mmol) in THF (50 mL) was added to the suspension of the titanium reagent, and the reaction was allowed to proceed under reflux for 5 h. After reaction, the solvent was evaporated under reduced pressure and the residue was extracted with CH_2Cl_2 (3×50 mL). The combined organic layer was dried over Na_2SO_4, filtrated and the solvent was removed under reduced pressure. The crude product was separated with column chromatography on silica gel with petroleum ether (60−90 ^oC) / CH_2Cl_2 (4:1, v/v) as eluent to afford **2** as a white solid (1.77 g, 30%). M.p. 73.4-75.3 ^oC. ^{1}H-NMR (400 MHz, acetonitrile-d_3): δ 7.14-7.08 (m, 3H), 7.04-7.01 (m, 2H), 6.96-6.90 (m, 4H), 6.86-6.83 (m, 3H), 6.70-6.63 (m, 4H), 6.58-6.56 (m, 2H), 3.71 (s, 3H), 3.69 (s, 3H). ^{13}C-NMR (100 MHz, acetonitrile-d_3): δ 158.13, 158.09, 155.30, 139.32, 136.65, 136.57, 132.29, 132.23, 131.06, 127.66, 126.10, 114.47, 113.01, 112.97, 54.81, 54.79. HR-MS: calcd. for $C_{28}H_{24}O_3$ (M^+): 408.1725; Found: 408.1730.

Synthesis of compound 2. Compound **3** (1.02 g, 2.5 mmol) and NaH (0.12 g, 5.0 mmol) were dissolved in 100 mL of dry THF under an argon atmosphere, and diethyl chlorophosphate (0.73 mL, 5.0 mmol) was added, and the solution was stirred at room temperature for 24 hours. The reaction was quenched with H_2O (10 mL) and the solvent was evaporated under reduced pressure, and the crude mixture was extracted with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was chromatographed on silica gel with petroleum ether (60−90 ^oC)/ethyl acetate (2:1, v/v) as eluent to afford **3** as a white solid (1.18 g, 87%). M.p. 95.4-97.2 ^oC. ^{1}H-NMR (400 MHz, dichloromethane-d_2): δ 7.16-7.12 (m, 3H), 7.08-7.02 (m, 4H), 7.00-6.95 (m, 6H), 6.68 (t, 4H), 4.22-4.14 (m, 4H), 3.76 (s, 3H), 3.74 (s, 3H), 1.36-1.32 (m, 6H). ^{13}C-NMR (100 MHz, dichloromethane-d_2): δ 158.33, 158.28, 149.04, 148.97, 144.12, 141.17, 140.57, 138.26, 136.16, 132.52, 132.43, 131.25, 127.73, 126.20, 119.22, 119.17, 113.05, 112.95, 64.58, 64.52, 55.04, 15.94, 15.88. HR-MS: calcd. for $C_{32}H_{33}O_6P$ $(M)^+$: 544.2015; Found: 544.2023.

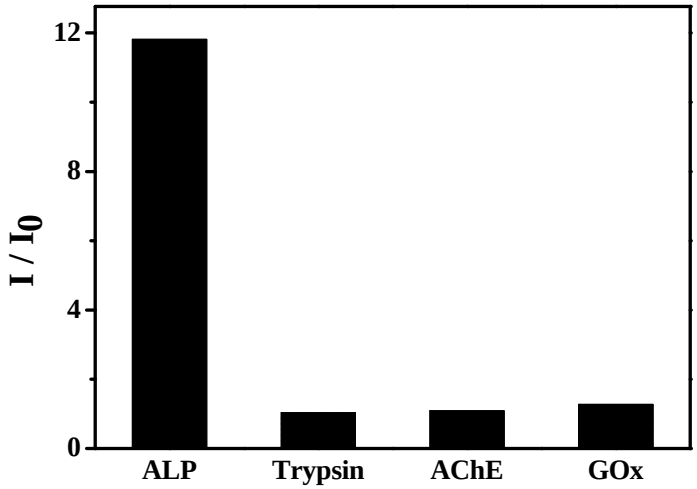

Figure S1. Variation of the fluorescence intensity at 480 nm for the solution of **1** [10 μM in Tris-HCl (10 mM) buffer solution, pH = 7.4] after separate incubation with ALP [2 U], Trypsin [10 mg/mL], AChE [1 U] and GOx (glucose oxidase, 10 mg/mL) for 15 min.; the excitation wavelength was 350 nm.

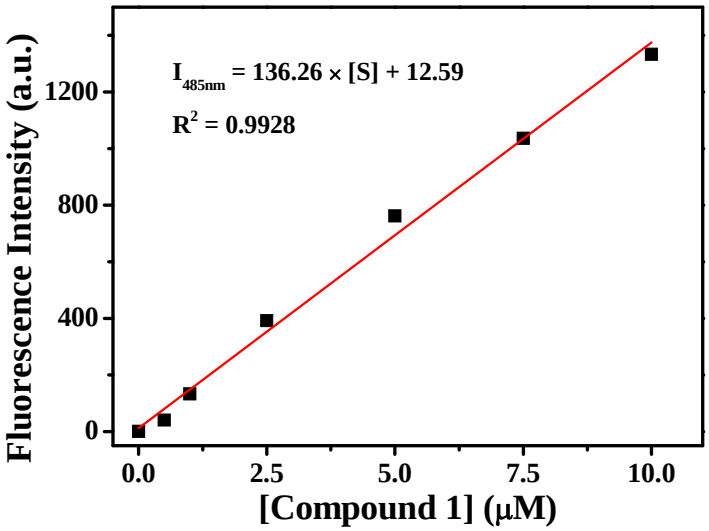

Figure S2. Variation of the fluorescence intensity of the solution of **1** (at 480 nm) after incubation with ALP (500 mU/mL) for 5.0 min. at 25 ^{o}C vs. the initial concentration of **1**; Tris-HCl buffer solution (10 mM, pH = 7.4) was employed; the fluorescence intensity at 480 nm was monitored and the excitation wavelength was 350 nm.

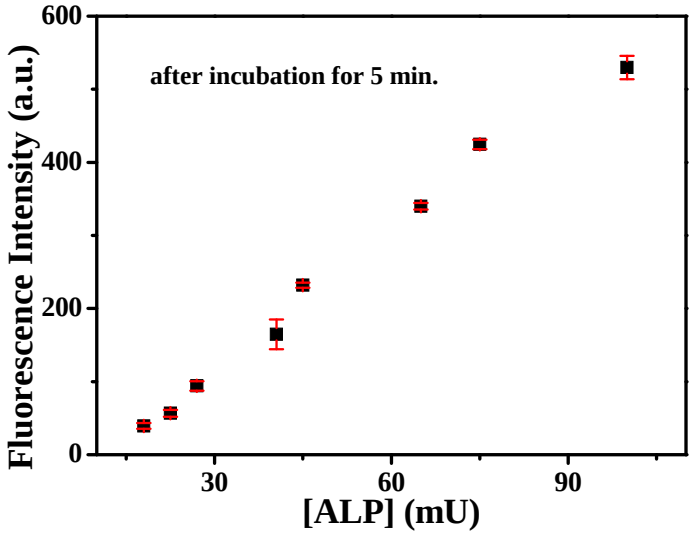

Figure S3. The plot of the fluorescence intensity for **1** [10 μM in Tris-HCl (10mM) buffer solution, pH = 7.4] at 480 nm vs. the amounts of ALP (from 0 to 100 mU/mL) upon incubation with ALP at 25 ^{o}C for 5.0 min.; the excitation wavelength was 350 nm.

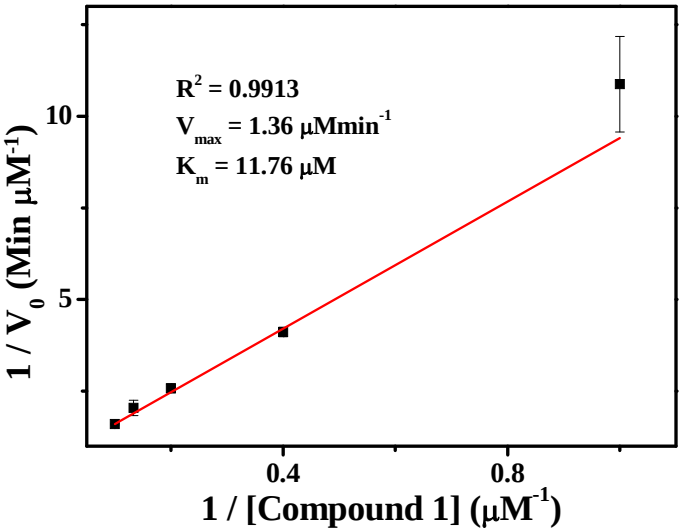

Figure S4. Lineweaver-Burk plot for the hydrolysis of **1** by ALP (75 mU/mL) in Tris-HCl (10 mM) buffer solution, pH = 7.4, 25 ^{o}C]; the concentration of **1** was 1.0, 2.5, 5, 7.5 and 10.0 μM; the fluorescence intensity was monitored at 480 nm and the excitation wavelength was 350 nm.

This journal is © The Royal Society of Chemistry 2013

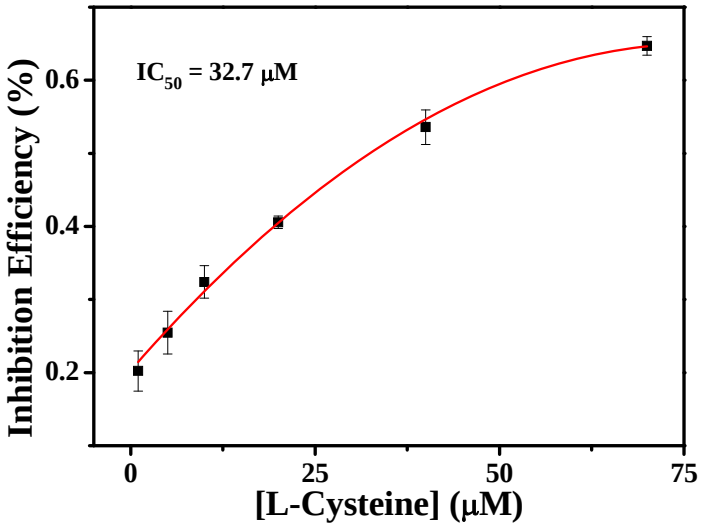

Figure S5. The plot of the inhibition efficiency of *L*-cysteine toward ALP vs. the concentration of *L*-cysteine; the measurements were performed with **1** [10 μM in Tris-HCl (10 mM) buffer solution, pH = 7.4, 25 ^{o}C], ALP (100 mU/mL), and different concentrations of *L*-cysteine (0, 1, 5, 10, 20, 40 and 70μM).

Cell Culture. Hela cells were maintained in DMEM (Gibco) medium containing 10% fetal bovine serum (Gibco) at 37 ^{o}C under a 5% humidified CO_2 atmosphere. Cells were pre-plated on substrates for 20 hours before each experiment.

Cytotoxicity assay. Using the Cell Counting Kit-8 (Dojindo), we measured the reduction of dehydrogenases in viable cells to show the cytotoxicity of probes. Hela cells in a 96-well plate (20000cells/well) were incubated with **1** (50 μM) for 30 minutes, followed by washing the cells with PBS twice. We add 100 μL of medium, containing 10 μL CCK-8 solutions, to each well of the plate and incubated the plate for 2.5 hours in the incubator. We measured the absorbance at 450 nm using a microplate reader (Ecan Infinite M200).

Cytotoxicity Assay. We found that Hela cells treatment with probes maintained almost 100% viability. From these data, it can be concluded that the probes is not toxic to cells and can be safety used for further studies.

Microscopy and imaging. We acquired images on a Leica inverted microscope (DMI 6000) equipped with a digital monochrome camera (Leica DFC 350 FX) and the software provided by Leica. Phase contrast and fluorescence micrographs were obtained with a 20× objective.

This journal is © The Royal Society of Chemistry 2013

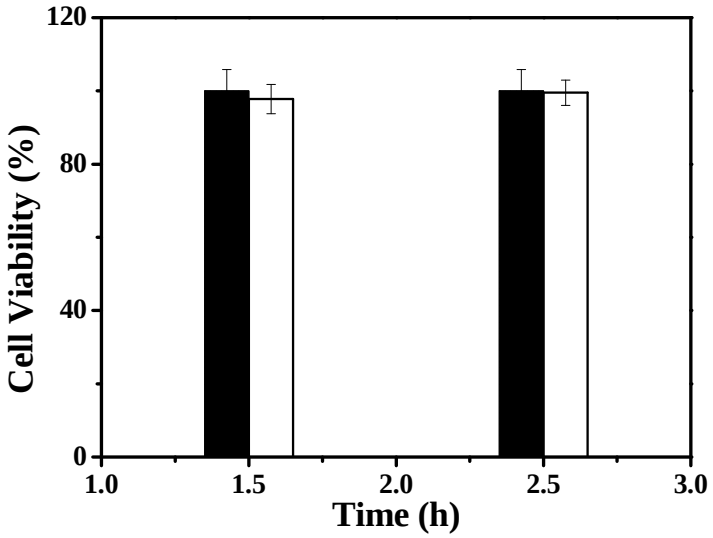

Figure S6. Cell viability of HeLa cells treated without (■) and with (□) **1** (50 μM) for different time periods. No cytotoxic effect was observed for the cells incubated with **1** for 1.5 h even after the treatment for 2.5 hrs.