Supporting Information

A new continuous fluorometric assay for acetylcholinesterase activity and inhibitor screening with emissive core-shell silica particles containing tetraphenylethylene fluorophore

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1. Experimental Section

General. ^{1}H-NMR and ^{13}C-NMR were collected on Bruker Avance 400-MHz spectrometer. Mass spectra were determined with Bruker Apex IV FTMS. The X-ray photoelectron spectroscopy data were obtained with an ESCALab220i-XL electron spectrometer from VG Scientific using 300W AlKα radiation; the base pressure was about 3×10^{-9} mbar; the binding energies were referenced to the C1s line at 284.8 eV from adventitious carbon. Scanning electron micrographs were taken with Hitachi S-4800 microscope equipped with a digital camera. Samples for the SEM were prepared by dropping diluted silica particles solution on silicon, and the solvent was evaporated under ambient conditions and then sputtered with Pt. TEM images were recorded on a JEOL JEM-1011 electron microscope at an accelerating voltage of 100 kV. Samples for the TEM were prepared by dropping diluted silica particles solution on carbon-coated copper grids and the solvent was evaporated under ambient conditions. ξ-potential measurements were performed at 25 ^{0}C with a Nano ZS (Malvern) equipped with a solid-state He–Ne laser (λ = 633 nm). Fluorescence spectra were measured on a Hitachi F-4500 spectrophotometer at 37 °C, and absorption spectra were recorded on a Hitachi U-3010 spectrophotometer at room temperature.

AChE (from *electrophorus electricus*) and tacrine were purchased from Sigma-Aldrich, and neostigmine bromide was purchased from TCI. Alkaline phosphatase (AP) was purchased from Alfa Aesar. Glucose oxidase (GOx) was purchased from Amersco and trypsine was purchased from BioDee Company, China. Stock solutions of emissive silica particles, tacrine, and neostigmine bromide were prepared with pure water. The commercially available AChE (2.0 U/mL) containing Tris buffer salt was dissolved with pure water and used directly. Pure water was obtained with a Millipore filtration system. The sample solutions containing phosphate-buffered saline (PBS) (50 mM, pH 8.5) buffer for spectral studies were further prepared.

"Inhibition efficiency" was calculated with the equation: $(F_{(\text{inhibitor})} - F_0) / (F_{(\text{no inhibitor})} - F_0)$, in which the F(inhibitor) and F(no inhibitor) refer to the fluorescence intensity at 470 nm of solution containing silica partciles [27.6 μg/mL in 50 mM PBS buffer

solution, pH=8.5], [AChE]=1.0 U/mL, [compound **2**]= 4.0 μM in the absence and presence of different concentrations of neostigmine bromide (0.5, 1.0, 5.0, 10, 50 nM) or in the absence and presence of different concentrations of tacrine (5.0, 10, 20, 50, 250 nM), respectively. F_0 refers to the fluorescence intensity of the ensemble at 470 nm in the absence of AChE and the corresponding inhibitors.

Preparation of Compound 1. Compound **3** (0.59 g, 1.57 mmol) and Cs_2CO_3 (1.53 g, 4.7 mmol) were stirred in CH_3CN (20 mL) under nitrogen atmosphere. After refluxing for 1.0 hrs, 3-chloropropyltriethoxysilane (3.02 mL, 12.56 mmol) was added and the reaction was refluxing for 48 hrs. After the reaction, the solvent was removed under reduced pressure and the residue purified by the silica gel column chromatography with petroleum ether (60 – 90 °C) and CH_2Cl_2 (2:1, v/v) as eluent to afford compound **1** (0.87 g) as a yellow oil in 95 % yield. ^{1}H-NMR ($CDCl_3$, 400 MHz, ppm) δ= 7.11-7.02 (10H, m), 6.96-6.88 (4H, m), 6.86-6.63 (4H, m), 3.89-3.82 (6H, m), 3.75-3.73 (3H, m), 3.54-3.51 (2H, m), 1.87 (2H, m), 1.24-1.21 (9H, m), 0.77-0.73 (2H, m). ^{13}C-NMR ($CDCl_3$, 100 MHz, ppm) δ= 158.00, 157,56, 144.408, 144.34, 139.82, 139.62, 136.53, 136.28, 132.58, 131.50, 128.51, 128.40, 128.36, 127.73, 127.62, 126.23, 113.73, 113.61, 113.16, 113.06, 69.73, 58.48, 58.39, 55.11, 47.54, 26.60, 22.89, 18.40, 18.36, 9.25, 6.60. HR-MS (EI): calcd. for $C_{36}H_{42}O_5Si$: m/z 582.2802; found: 582.2809.

Preparation of silica particles derived from compound 1.[3] Compound **1** (23.3 mg, 0.04 mmol) was added to a mixture of ethanol (28.9 mL), ammonium hydroxide (0.3 g, 0.35 mL) and distilled water (1.4 mL), and the reaction mixture was stirred at room temperature for 3.0 hrs. Then, TEOS (1.01 g, 4.85 mmol) was added to the above reaction mixture, followed by stirring at room temperature in dark for additional 24 hrs. The obtained nanoparticles were separated by centrifugation and washed three times with ethanol and five times with pure water. Finally, the dispersion of silica nanoparticles in pure water (5 mL) was prepared and stored in a refrigerator at 4 ^{o}C before being used. A portion of stock solution (0.5 mL) was centrifuged and the residue was dried under vacuum to obtain white powdered material (2.7 mg). Thus, the concentration of the stock solution of silica nanoparticles was calculated to be 5.4 mg/mL.

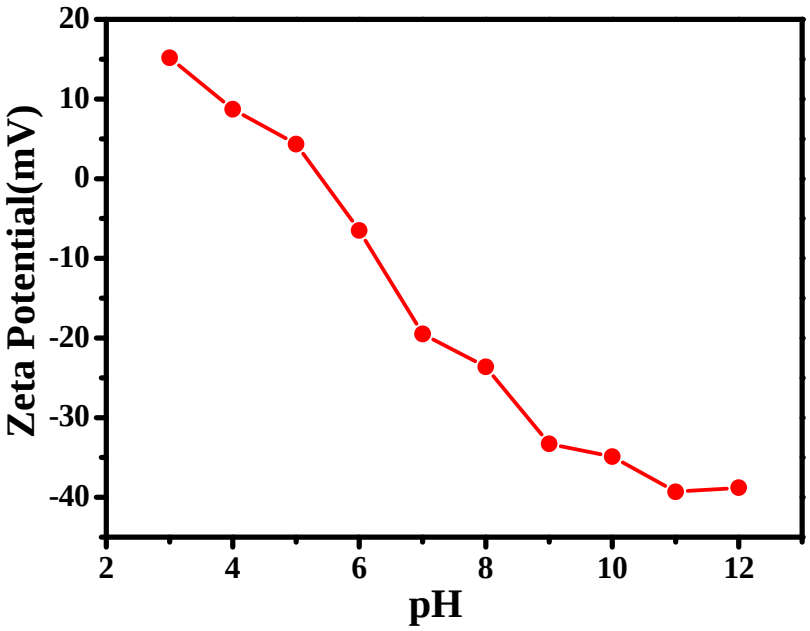

Figure S1. Variation of ξ potentials of silica particles vs pH values of solutions.

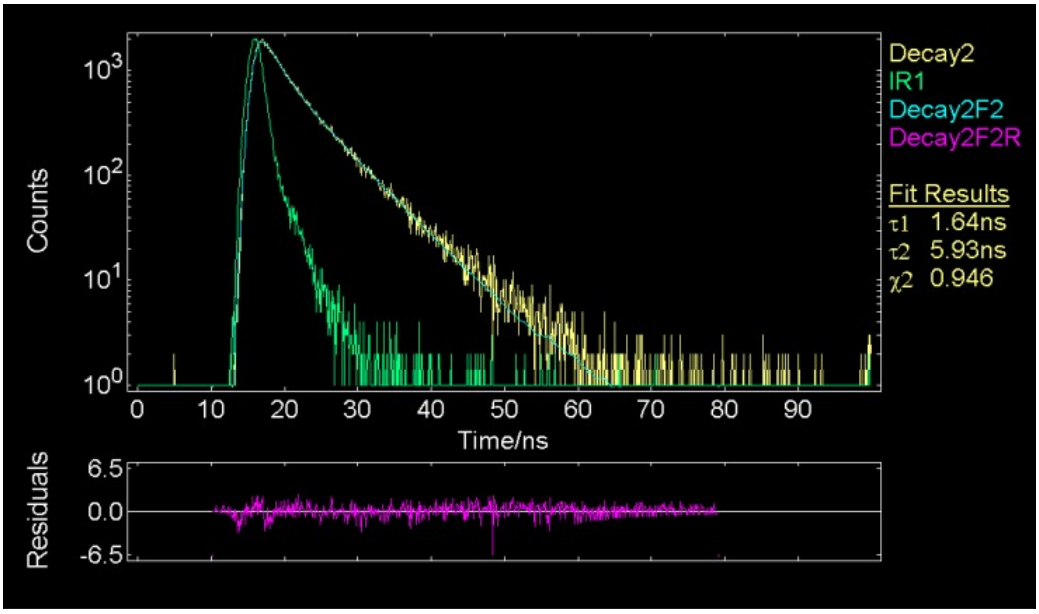

Figure S2. The fluorescence decay profile of the emissive silica particles.

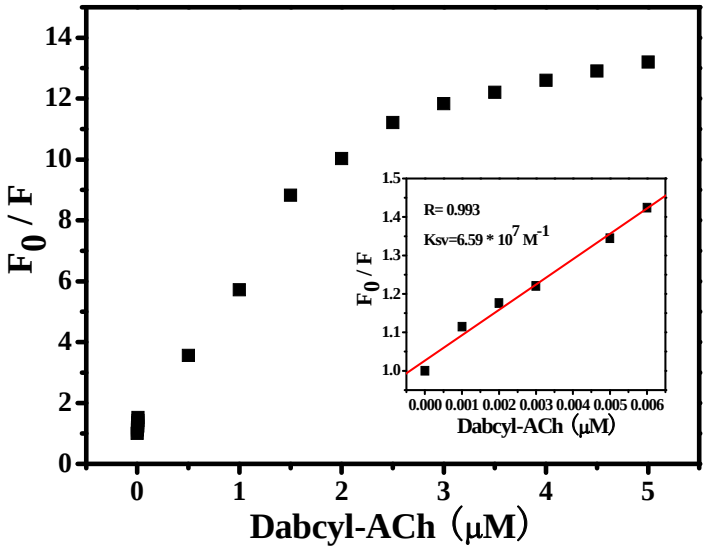

Figure S3. The Stern-Volmer plot of silica particles quenched by compound **2**; the inset shows the fit linear of the fluorescence ratios (F_0/F) at 470 nm against the concentrations of **2** (0, 1, 2, 3, 5, 6 nM); the excitation wavelength was 334 nm.

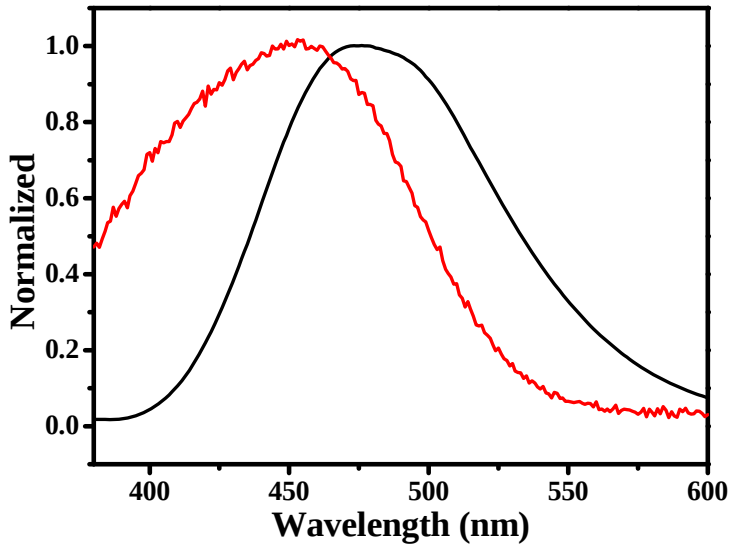

Figure S4. The normalized fluorescence spectrum of the silica particles (black line) and UV-Vis absorption (red line) spectrum of compound **2**.

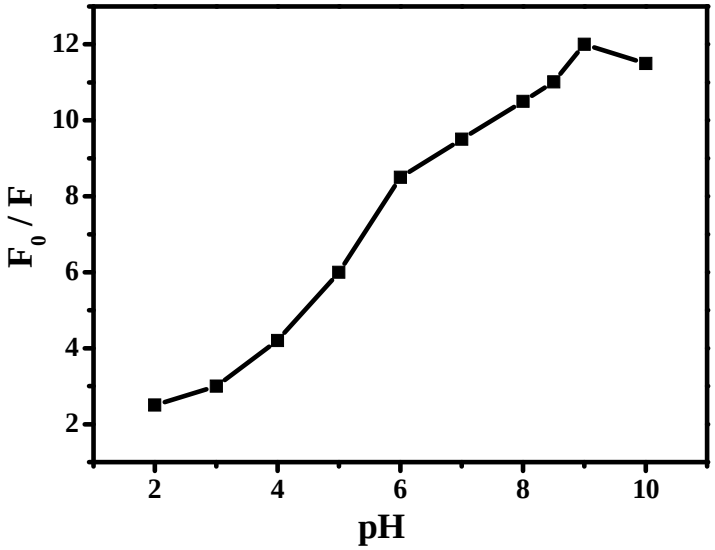

Figure S5. Variation of the fluorescence intensity ratio at 470 nm *vs.* the pH of the solutions for the ensemble of silica particles (27.6 μg/mL) and compound **2** (4.0 μM); the F_0 and F are the fluorescence intensity at 470 nm in the absence and presence of compound **2**; the excitation wavelength was 334 nm.

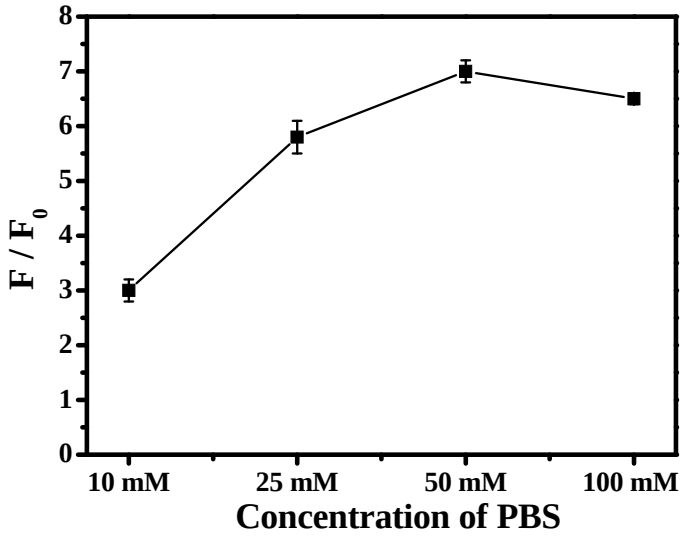

Figure S6. The variation of fluorescence intensity ratio at 470 nm for the ensemble solutions of silica particles and **2** before and after addition of AChE; the F_0 and F refer to the fluorescence intensity at 470 nm ($\lambda_{exc.}$ = 334 nm) of the silica particles (27.6 μg/mL) and compound **2** (4.0 μM) in phosphate buffer solutions (pH = 8.5) containing AChE (2.0 U/mL) before and after incubation at 37 ^{o}C for 30 min..

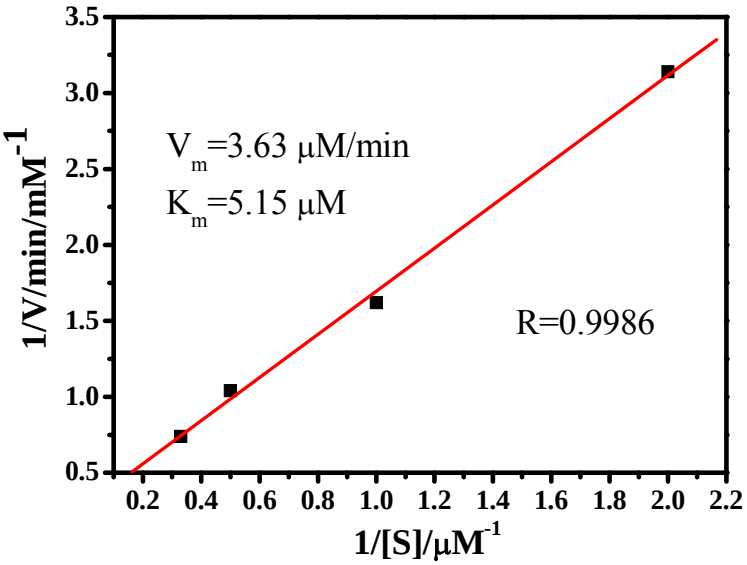

Figure S7. Lineweaver-Burk plot for the hydrolysis of compound **2** catalyzed by AChE (1.0 U/mL) in the presence of silica particles (27.6 μg/mL in 50 mM PBS, pH = 8.5); the concentration of compound **2** varied from 0.5 to 3 μM; the fluorescence intensity was monitored at 470 nm, and the excitation wavelength was 334 nm.

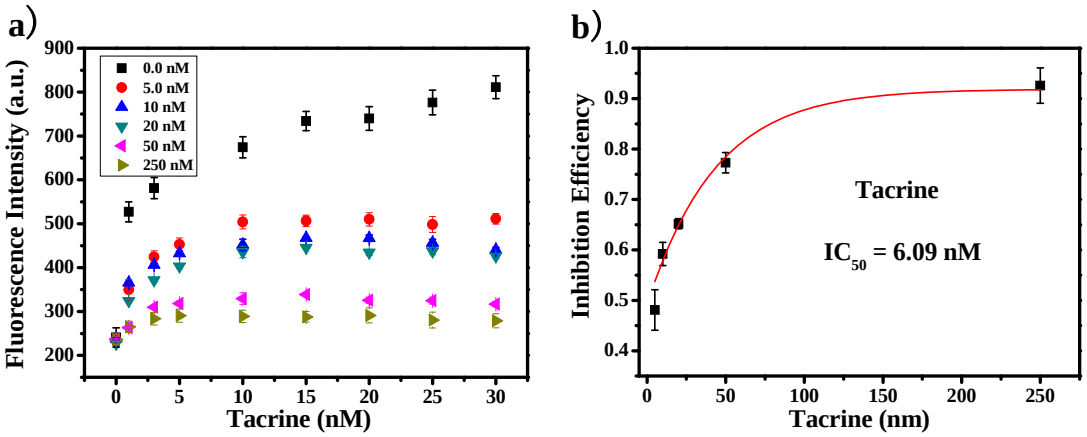

Figure S8. a) Plot of the fluorescence intensity at 470 nm *vs.* the hydrolysis time for the ensemble of silica particles (27.6 μg/mL) and compound **2** (4.0 μM) containing AChE (1.0 U/mL) in the presence of different concentrations of tacrine (5.0, 10, 20, 50, 250 nM) in 50 mM PBS buffer solution (pH = 8.5) at 37 ^{o}C; b) plot of the inhibition efficiency for AChE *vs.* the concentration of the inhibitor.

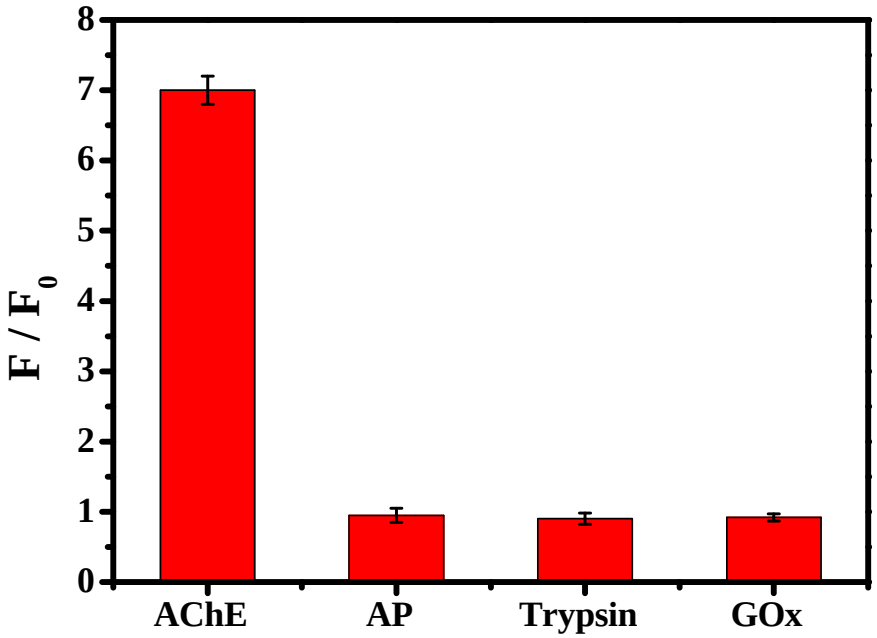

Figure S9. The variation of the fluorescence intensity ratio at 470 nm for the ensemble of silica particles and **2** before and after the addition of different enzymes; the F_0 and F refer to the fluorescence intensity at 470 nm ($\lambda_{exc.}$ = 334 nm) of the silica particles (27.6 μg/mL) and compound **2** (4.0 μM) in 50 mM phosphate buffer solutions (pH = 8.5) containing either AChE (2.0 U/mL) or AP (2.0 U/mL) or trypsin (8 μg/mL) or GOx (2.0 U/mL) before and after incubation at 37 ^{o}C for 30 min.

Reference:

1. F. D. Feng, Y. L. Tang, S. Wang, Y. L. Li and D. B. Zhu, *Angew. Chem. Int. Ed.*, 2007, **46**, 7882–7886.

2. W. X. Xue, G. Z. Zhang, D. Q. Zhang and D. B. Zhu, *Org. Lett.*, 2010, **12**, 2274-2277.

3. M. Faisal, Y. N. Hong, J. Z. Liu, Y. Yu, J. W. Y. Lam, A. J. Qin, P. Lu and B. Z. Tang, *Chem. Eur. J.*, 2010, **16**, 4266-4272.