Electronic Supplementary Information

Dye-Concentrated Organically Modified Silica Nanoparticles as a Ratiometric Fluorescent pH Probe by One- and Two-Photon Excitation

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Experimental

General Procedure: All reagents were purchased from Aldrich and used as received. Chemical structures were identified by ¹H NMR (Varian Gemini-300, 300 MHz) and electrospray ionization (ESI) mass spectroscopic analysis (Themo Finnigan LCQ Advantage mass spectrometer). Transmission electron microscopy was performed using a JEOL JEM-100cx electron microscope, operating at an accelerating voltage of 80 kV. UV-vis absorption and one-photon excited fluorescence were measured using a Shimadzu UV-3101 PC spectrophotometer and a Jobin-Yvon Fluorog FL-311 spectrofluorometer, respectively. Relative fluorescence quantum yields (Φ_r) were estimated using coumarin 540A in methanol as a reference. The spectra of two-photon excitation cross-section (σ_2) were recorded by using a mode-locked Ti:sapphire laser (Mira from Coherent, 130 fs pulses at a repetition rate of 76 MHz), along with a standard solution of Rhodamine 6G (100 μ M in methanol) as a calibration standard.¹⁰ The pH-dependence measurements were performed in citric acid-phosphate buffer solution, prepared by mixing aqueous solutions of citric acid (0.1 mol L⁻¹) and Na₂HPO₄ (0.2 mol L⁻¹). Ratios of fluorescence intensities (*R*) at 580 and 460 nm as a function of pH were theoretically fitted by using the following equation to extract the pK_a value:

$$R = \frac{I_{580}}{I_{460}} = \frac{F_P(580)10^{-\text{pH}} + F_N(580)10^{-\text{pK}_a}}{F_P(460)10^{-\text{pH}} + F_N(460)10^{-\text{pK}_a}}$$
(1)

, where $F_P(\lambda)$ and $F_N(\lambda)$ designate fluorescence intensities of the endpoint species (protonated, *P*, and neutral, *N*, respectively) at the considered wavelength (λ).

2-Bromo-6-(6-hydroxyhexyloxy)naphthalene (1): To a mixture of 6-bromo-2-naphthol (4 g, 17.9 mmol), K_2CO_3 (5 g, 36.2 mmol), and a catalytic amount of 18-crown-6 in 10 mL of DMF, 6-chloro-1-hexanol (4 g, 29.3 mmol) was added at room temperature. The reaction mixture was stirred at 30 °C for 1 day and poured into excess water and dichloromethane. The organic phase was washed several times with the KOH solution (1 N) and purified by column chromatography on a silica gel. The eluting impurities were

removed using dichloromethane and then the product was collected by eluting with ethyl acetate/*n*-hexane (1/1 by volume). Yield 5.1 g (88%). ¹H NMR (300 MHz, CDCl₃): δ 7.90 (1H, d, *J*=1.8 Hz), 7.63 (1H, d, *J*=9.0 Hz), 7.58 (1H, d, *J*=8.7 Hz), 7.48 (1H, dd, *J*=1.8, 8.7 Hz), 7.15 (1H, dd, *J*=2.4, 9.0 Hz), 7.07 (1H, d, *J*=2.4 Hz), 4.05 (2H, t, *J*=6.5 Hz), 3.67 (2H, t, *J*=6.5 Hz), 1.90-1.35 (8H, m). MS (ESI): calcd for C₁₆H₁₉BrO₂, *m*/*z*=323.06 (M+H⁺); found, *m*/*z*=323.3 (M+H⁺).

4-[2-{6-(6-hydroxyhexyloxy)naphthalen-2-yl}vinyl]pyridine (NVP): A mixture of 1 (2.5 g, 7.7 mmol), 4vinylpyridine (1.22 g, 11.6 mmol), Pd(OAc)₂ (0.14 g, 0.62 mmol), tri-*o*-tolylphosphine (0.48 g, 1.6 mmol), triethylamine (Et₃N, 2 mL), and tetrahydrofuran (THF, 8 mL) was introduced into a pressure tube, under argon atmosphere. The reaction mixture was refluxed for 3 days. After evaporation of Et₃N and THF, the crude product was purified by column chromatography on a silica gel. The impurities were removed by successive elution using dichloromethane and ethyl acetate. Then, the product was collected by eluting with THF. Yield 1.1 g (41%). ¹H NMR (300 MHz, CDCl₃): δ 8.58 (2H, d, *J*=5.1 Hz), 7.82 (1H, s), 7.76-7.67 (3H, m), 7.46-7.37 (3H, m), 7.16 (1H, dd, *J*=1.8, 9.3 Hz), 7.12 (1H, d, *J*=1.8 Hz), 7.07 (1H, d, *J*=16.5 Hz), 4.09 (2H, t, *J*=6.5 Hz), 3.68 (2H, t, *J*=6.5 Hz), 1.90-1.47 (8H, m). MS (ESI): calcd for C₁₆H₁₉BrO₂, *m/z*=348.19 (M+H⁺); found, *m/z*=348.3 (M+H⁺). UV absorption: λ_{max}(DMSO/water=1/1 by volume)/nm 337 (ε/M⁻¹cm⁻¹ 13 520) and 387 (11 150).

Organically Modified Silica Nanoparticle (OSNP) Preparation: A mixture of NVP (52 mg, 0.15 mmol), (3-isocyanatopropyl)triethoxysilane (74 mg, 0.30 mol), and a catalytic amount of dibutyltin diacetate in 3 mL THF was refluxed in a pressure tube for 1 day. After cooling, 30 μ L NH₄OH (28.0~30.0%, J. T. Baker) was added and the mixture was stirred for 10 h at room temperature. After syringe filtering by a membrane filter (0.2 μ m pore size) and five-times dilution in THF, 0.2 mL solution was added into AOT (0.22 g)/1-butanol (0.4 mL)/water (20 mL) micelle under vigorous stirring. The sol-gel reaction was carried out at room temperature for 2 days, and AOT and 1-butanol were completely removed by dialyzing the dispersion against water in a 12~14 kDa cutoff cellulose membrane for 48 h.

Supporting data



Fig. S1 Normalized one-photon excitation spectra for the 600-nm emission of the NVP solution in DMSO/water (=1/1 by volume) in neutral (solid line), basic (dashed line), and acidic (circle) conditions.



Fig. S2 Absorption and PL spectra of partially protonated solution, by adding a small amount of citric acid; excitation at 370 nm (solid line) and 430 nm (dashed line).



Fig. S3 Normalized absorption and PL (excited at 370 nm) spectra of the OSNP dispersion in neutral water (solid line) and in neutral water/DMSO mixture (1/1 by volume, dashed line).



Fig. S4 Normalized excitation spectra of the OSNP dispersion at pH 7 for emissions at 450 (circle) and 600 nm (solid line).