Supplementary Materials (ESI) for Chemical Communications

# A quantum dot-based ratiometric pH sensor

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## *Experimental details*

### *1. Materials*

Cadmium 2,4-pentanedionate (98 %) was purchased from Alfa Aesar. Stearic acid, potassium *t*-butoxide, glutathione (GSH, reduced form), and $ZnEt_2$ (1M hexane solution) were purchased from Wako Chemicals (Japan). Tri-n-octylphosphine (TOP), tri-butylphosphine (TBP), tri-n-octylphosphine oxide (TOPO), hexamethyldisilathiane, and hexadecylamine (HDA) were purchased from Tokyo Kasei (Japan). Selenium (powder, 99.999%) was purchased from Sigma-Aldrich. Dimethylcadmium ($CdMe_2$, 10 wt% in hexane) was purchased fro Strem Chemicals. Fluorescein-5-isothicyanate was purchased from Invitrogen.

*Se (TBP) and Cd-Zn-S stock solution:* All procedures were performed under argon atmosphere. 100 mg of selenium (powder) was added to 1mL of TBP at room temperature. Selenium was easily dissolved to TBP by sonication using a bath-type sonicator (D150H, Delta). 2 mL (2 mmol) of $ZnEt_2$ (1M hexane solution) and 0.57 mL (0.5 mmol) of $CdMe_2$ were added to 7.0 mL of TOP. Then, 0.52 mL (2.5 mmol) of hexamethyldisilathiane was added to the $CdMe_2$-$ZnEt_2$/TOP solution. All stock solutions were stored in argon atmosphere.

### *2. Synthesis of CdSe/CdZnS QD (600 nm)*

CdSe QDs were first synthesized by using cadmium 2,4-pentanedionate as a Cd precursor.[1] The mixture of 1 g of TOPO, 3 g of HDA, 66 mg (0.2 mmol) of cadmium 2,4-pentanedionate and 250 mg of stearic acid was loaded into a 25 mL of three-necked flask and heated at 250 $^oC$ under argon atmosphere. After stirring for 30 min at 250 $^oC$, 1 mL (1 mmol) of the stock solution of Se (80 mg/ 1mL TOP) was swiftly added, and the temperature of the solution was lowered to 200 $^oC$. At this temperature, the growth of the CdSe QDs was monitored by measurements of their fluorescence spectra. When the emission maximum reached to 585 nm, the three-necked flask was removed from the heater and the temperature of the solution was lowered to 60 $^oC$. Then ca. 10 ml of ethanol was added for the precipitation of CdSe QDs. The QD precipitation was separated by centrifuge and was resolved to 10 mL of chloroform. The CdSe solution was loaded to a 25 mL of three-necked flask, and then 1 g TOPO and 3 g HDA were added

to the flask and chloroform was evaporated under reduced pressure. After evaporation of chloroform, the mixture was heated to 200 $^{o}$C, and 0.25 ml of a Cd-Zn-S stock solution was slowly added under stirring. Then temperature of the solution was lowered to 100 $^{o}$C and the solution was stirred for 5 hrs at this temperature. After the temperature was lowered to RT, QDs were precipitated by addition of excess methanol and separated by centrifuge. CdSe/CdZnS QDs were resolved to 20 mL of tetrahydrofuran.

### *3. Preparation of GSH coated QD (GSH-QD)*

To 1 mL of the CdSe/CdZnS QDs solution prepared by the above method, 1 mL of GSH (200 mg/ mL) was added, and the mixture was heated to 60 $^{o}$C. The resulting precipitates of GSH-coated QDs (GSH-QDs) were separated by centrifugation and washed with 2 mL of water two times to remove tetrahydrofuran. To the QD precipitates, 10 mg of potassium *t*-butoxide and 2 mL of water were added. The aqueous solution of QDs was sonicated for 5 min using a bath-type sonicator. The QD aqueous solution was passed through a 0. 2 μm membrane filter. Excess GSH and potassium *t*-butoxide were removed by the dialysis using PBS buffer. The concentration of GSH-QDs was estimated by using fluorescence correlation spectroscopy. The particle number of the QDs in confocal volume was determined, and the concentration of QDs was determined using an aqueous solution (20 nM) of rhodamine 6G as a reference. For the conjugation of FITC, the PBS buffer of the GSH-QDs solution was exchanged with water by using a size-exclusion column (Nap$^{TM}$-5 column, Sephadex G-25, GE Helthcare). The pH of the GSH-QD solution (0.1 μM) was adjusted by using 100 mM phosphate buffers (pH = 6, 6.5, 7, 7. 5 and 8).

### *4. Preparation of FITC conjugated QD (FITC-QD)*

FITC (4 mg) was dissolved to 1 mL of dimethyl sulfoxide. To 1 mL of GSH-QDs (1 μM in water), 2.5 ~ 10 μL of the FITC solution (10 mM) was added under stirring. After 30 min stirring, the solution was passed through a size-exclusion column (Nap$^{TM}$-5 column, Sephadex G-25, GE Helthcare) using water as an eluent. The concentration of FITC conjugated QDs (FITC-QD) was estimated by using the fluorescence correlation spectroscopy. The pH of the FITC-QD solution (0.1 μM) was adjusted by using 100 mM phosphate buffers (pH = 6, 6.5, 7, 7. 5 and 8).

### *5. Preparation of FITC conjugated GSH (FITC-GSH)*

GSH (10 mg) was dissolved to 1 mL of water. To this solution, 5 μL of the FITC solution (10 mM) was added under stirring. After 30 min stirring, the solution was passed through a size-exclusion column (Nap$^{TM}$-5 column, Sephadex G-25, GE Helthcare) using water as an eluent. This operation was repeated

two times to remove free GSH and unconjugated FITC products. The pH of the GSH-QD solution (0.1 μM) was adjusted by using 100 mM phosphate buffers (pH = 6, 6.5, 7, 7. 5 and 8).

### *6. Vesicle preparation*

To a 10 mL test tube, 10 mg of soybean phospholipids (Nacalai Tesque, Japan) was added and dissolved by 1 mL of chloroform. Then chloroform was evaporated under reduced pressure. To the test tube containing the soybean phospholipids, 10 mL of water was added and the tube was sonicated for 10 min using a tip-type sonicator. To make large sizes of vesicle suspensions, the freezing-thawing-sonication method[2] was used. The sonicated lipid suspension (10 mL) was frozen at 196 $^{o}$C by gently shaking in liquid nitrogen and left to thaw at RT for 15 min. The thawed milky solution was sonicated in a bath-type sonicator doe 30 s at RT. This freezing-thawing-sonication was repeated three times. The hydrodynamic size of the lipid vesicle was determined to be ca. 100 nm in diameter by using a dynamic light scattering apparatus (Malvern, Nano-ZS). 1 mL of the vesicle suspension was diluted by 3 mL of phosphate buffers (10 0 mM, pH = 6, 6.5, 7, 7.5, and 8).

### *7. Fluorescence measurements*

Fluorescence spectra were measured with a FP-6200 spectrofluorometer (JASCO) using 1cm × 1cm quartz cells with an excitation wavelength at 460 nm. Fluorescence autocorrelation curves were measured by using a compact FCS system (C9413-01MOD, Hamamatsu Photonics) with a 473 nm semiconductor laser as an excitation light source.

Fluorescence lifetime measurements: a femtosecond pulse from a regenerative amplifier (Libra HE, Coherent) was lead to an Optical parametric amplifier (OPerA Solo, Coherent), which generates 60 fs pulse at 1 kHz with wavelength tuneable in the infrared region. The beam was directed to two BBO crystals to generate third harmonics pulse with the wavelength of 390nm and FWHM of 5 nm. Samples contained in 1cm cells were excited with a ~5mm spot size. Power density of the excitation beam was set to be sufficiently 1cm × 1cm (5 μJ/cm$^2$) to avoid any saturation effect. Emission was collected though an analyzer whose polarization direction is perpendicular to that of the excitation beam. Time–resolved spectral was obtained using a photon-counting streak camera (Hamamatsu C4780) through a 250 mm monochromator (250is, Chromex). The instrumental response function is about 0.7 ns.

**References**

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2) U. Pick, *Arch. Biochem. Biophys*. 1981, **212**, 186.