Electronic Supporting Information

Bovine serum albumin-coated quantum dots as a cytoplasmic viscosity probe in a single living cell

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Experimental details

1. Materials

Cadmium 2,4-pentanedionate (98%) was purchased from Alfa Aesar. Tri-*n*-octylphosphine (TOP), tributylphosphine (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. Octadecylphosphoric acid (ODPA) was purchased from Strem Chemicals. Glutathione (GSH, reduced form), potassium *t*-butoxide, $ZnEt_2$ (1M hexane solution), and tetrahydrofuran were purchased from Wako Pure Chemical Industries. Polyethylenimine (branched, average molecular weight, 25,000) and BSA (bovine serum albumin) were purchased from Sigma Aldrich. EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride), sulfo-NHS (N-hydroxysulfosuccinimide), and PEG-8000 (polyethylene glycol, average molecular weight, 8000) were purchased from Dojindo, Molecular BioSciences, and MP Biomedicals, respectively. Other reagents and solvents were analytical grade.

EGFP (6xHis) was overproduced in *Escherichia coli* BL21 (DE3) Star transformed pRSET-EGFP, and purified by HisTrap HP and HiPrep 16/60 Sephacryl S-200 HR from GE Healthcare Life Sciences.

2. Measurement instruments

Fluorescence spectra of QDs were measured with a spectrofluorometer FP-8000 (JASCO Corporation) at the excitation wavelength of 488 nm. Absolute viscosities of BSA and PEG aqueous solutions were measured by using m-VROC (Nihon Rufuto Co., Ltd.) at 37 ^{o}C. Zeta potentials for QDs were taken with Nano-ZS (Malvern) at pH = 7.4 (10 mM PBS). FCS autocorrelation curves of QDs in bulk aqueous solution were measured by using a compact FCS

(C9413-01MOD, Hamamatsu Photonics) with a 473 nm semiconductor laser as an excitation light source. Confocal fluorescence images and FCS autocorrelation curves for HeLa cells were taken with a LSM510-ConfoCor3 system (Carl Zeiss) with C-Apochromat 40×/1.20W Korr as objective. EGFP and QDs were excited with a 488 nm Ar-ion laser. Emission signals were collected through a band-pass filter (505-550 nm) for EGFP or a long-pass filter (580 nm) for QDs.

3. Synthesis of CdSe/ZnS QDs

QDs were synthesized by a modified method 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 300 mg of octadecyphosphoric acid were loaded into a 25 mL of three-necked flask and heated at 300 ^{o}C under an argon atmosphere. At this temperature 0.5 mL of TOP was added to the flask and stirred for 30 min. Then the temperature was increased to 350 ^{o}C and 1 mL of the stock solution of Se (100 mg/ mL TBP) was swiftly added, and the growth of QD particles was monitored by their fluorescence spectra. When the emission maximum of the QDs reached to 610 nm, the flask was removed from the heater and the temperature of the solution was lowered to 60 ^{o}C. Then ca. 10 mL of ethanol was added to precipitate CdSe QDs. QD precipitates were separated by centrifuge and resolved to 10 mL of chloroform. A chloroform solution of the QDs was loaded to a 25 mL of three-necked flask, and then 3 g of HDA were added to the flask. After evaporation of chloroform under reduced pressure, the mixture was heated to 180 ^{o}C, and 0.25 mL of a Zn-S stock solution (2.5 mL of $ZnEt_2$ solution + 0.5 mL of hexamethyldisilathiane + 7 mL of TOP) was dropwisely added under vigorously stirring. Then the temperature of the solution was lowered to 100 ^{o}C and the solution was stirred for 1 hour. After cooling to room temperature, ca. 10 mL of ethanol was added to the solution to precipitate CdSe/ZnS QDs. The CdSe/ZnS QDs were separated by centrifuge and resolved to 20 mL of tetrahydrofuran.

4. Preparation of GSH-coated QDs

One mL of a tetrahydrofuran solution (1 μM) of CdSe/ZnS QDs was mixed with 1 mL of 100 mg/mL GSH aqueous solution. The resulting precipitates of GSH-coated QDs were separated by centrifugation and washed with 2 mL of water twice to remove the organic solvent. To make the QDs soluble to water, 10 mg of potassium *t*-butoxide and 2 mL of water were added. The aqueous solution of GSH-QDs was sonicated for 5 min using a bath-type sonicator. To remove excess GSH and potassium *t*-butoxide, the GSH-coated QD solution was passed through a size-exclusion column (Sephadex G-25, GE Healthcare) using a 10 mM PBS (pH= 7.4).

5. Preparation of PEI-coated QDs

One mL of the tetrahydrofuran (ca. 1 μM) solution of CdSe/ZnS QDs was mixed with 100 mg of PEI under sonication for 5 min using a bath-type sonicator. The organic solvent of the QD solution was removed by using a rotary evaporator. Then 2 mL of water was added to the QD solution under sonication. The resuspended solution of PEI-coated QDs was successively passed through a 0.45 μm and 0.2 μm membrane filter. Free PEI was removed by passing the PEI-coated QD solution through a 50 kDa centrifugal membrane-filter with PBS buffer.

6. Preparation of BSA-coated QDs

BSA was conjugated by using an EDC/sulfo-NHS coupling as follows. Ten μL of 10 mM EDC solution was added to 1 mL of an aqueous solution of GSH-coated QDs (1 μM, pH =7.4). Then, 20 μL of 10 mM sulfo-NHS solution was added to the solution. After 1 hour, 100 μL of BSA solution (100 mg/mL) was added and incubated for 2 hours at room temperature. BSA-coated QD solution was passed through a 100 kDa centrifugal membrane-filter to remove free BSA and free coupling agents.

7. Incorporation QDs to living cells

A beads loading method was used for incorporation of QDs to living cells according to the reported procedure.[2] In briefly, HeLa cells were cultured on φ35 mm glass bottom dishes at 40-50 % confluent. The medium was removed just before beads loading of QDs into cells. Twenty μL of the concentrated BSA-QD solution (ca. 10 μM) was dropped on the glass bottom dishes. Immediately, siliconized beads were sprayed from top of the dish and the dish was rocked 5 times. The beads were removed by rinse with a PBS solution twice. Then medium was added to the dish for incubation at 37 ^{o}C.

8. FCS measurements

FCS uses the fluctuations of the fluorescence intensity in a tiny confocal volume to determine the diffusion times of fluorescent particles. Fluctuations in the fluorescence intensities *I(t)* can be analyzed by using the autocorrelation function *G(τ)*:

$$G(\tau) = \frac{< I(t)I(t+\tau) >}{< I(t) >^2} \qquad (1)$$

where the symbol <> stands for the ensemble average and $I(t+\tau)$ is the fluorescence intensity in single photon counting method obtained from the detection volume at delay time τ. The curve fitting for the multicomponent model is given by:

$$G(\tau) = 1 + \frac{1 - f + f\exp(-\tau/\tau_t)}{N(1-f)} \sum_i y_i \left(1 + \frac{\tau}{\tau_i}\right)^{-1} \left(1 + \frac{\tau}{s^2\tau_i}\right)^{-1/2} \quad (2)$$

where y_i and τ_i are the fraction and diffusion time of component I, respectively. N is the number of fluorescent molecules in the detection volume defined by the beam waist ω_0 and the axial radium z_0, s is the structure parameter representing the ratio of ω_0 and z_0. The detection volume made by ω_0 and z_0 was approximated as a cylinder. f is the average fraction of triplet state molecules and τ_t is triplet relaxation time.

Fluorescence autocorrelation curves for QDs in aqueous solutions and cells were accumulated ten times for each measurement. The diffusion constants of BSA-QD (D_{QD}) for the aqueous solutions and cells were calculated from the diffusion constant of Rh6G, D_{Rh6G} (280 $\mu m^2/s$)[3] as follows:

$$\frac{D_{QD}}{D_{Rh6G}} = \frac{\tau_{Rh6G}}{\tau_{QD}} \quad (3)$$

The hydrodynamic diameter d_{QD} of QDs can be calculated by the following equation based on the Stokes-Einstein relationship ($D=k_BT/6\pi\eta r$) :

$$d_{ST} = d_{ST} \times \frac{\tau_{QD}}{\tau_{ST}} \quad (4)$$

where d_{ST} is the diameter of a standard particle, and τ_{QD} and τ_{ST} are the diffusion times of QDs and the standard particles, respectively. As a standard particle, we used fluorescent latex beads (20 nm in diameter, Molecular Probe, Inc.).

References

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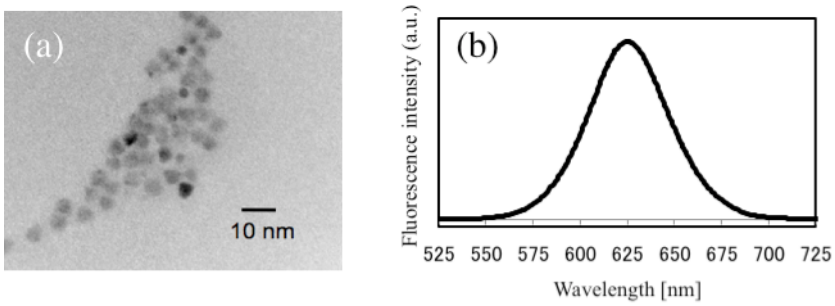

Figure S1. (a) Transmission electron microscope image of TOPO-capped CdSe/ZnS QDs. (b) Fluorescence spectrum of the QDs in chloroform at the excitation of 488 nm.

Table S1. Characterization data for QDs used in this study

	PEI-QD	GSH-QD	BSA-QD
Hydrodynamic diameter (nm)	12.9	6.6	9.1
Zeta potential (mV)	39.6	-22.8	-11.3
Emission peak (nm)	625	629	627

Table S2. Viscosity and diffusion constants of BSA-QDs in aqueous solution at 37 ^{o}C

Aqueous solutions	Viscosity (cP)	Diffusion constants ($\mu m^2/s$)
Pure water	0.73	10.0
BSA (200 mg/ mL)	1.93	4.98
BSA (300 mg/ mL)	2.82	3.00
PEG-8000 (50 mg/ mL)	1.42	7.06
PEG-8000 (100 mg/ mL)	2.85	3.43
PEG-8000 (200 mg/ mL)	7.14	1.55

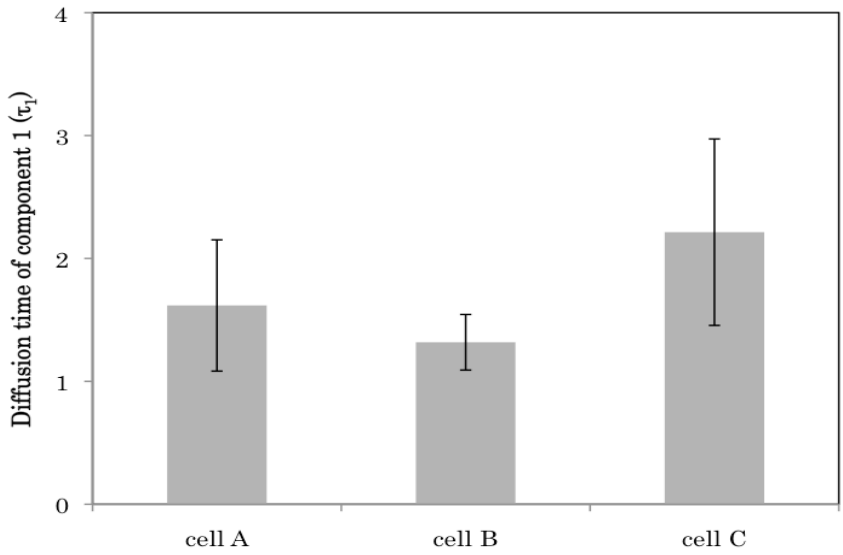

Figure S2. Diffusion times of the fast component (τ_1) of BSA-QDs in the cytoplasm of HeLa cells.