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Supplementary Information

Aggregates of Silicon Quantum Dots as a Drug Carrier: Selective Intracellular Drug Release Based on pH-responsive Aggregation /Dispersion

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Experimental methods

Materials

Toluene, allylamine, sodium hydroxide (NaOH), hydrochloric acid (HCl), hydrofluoric (HF) acid and Rhodamine 6G were purchased from Wako Pure Chemicals Co. (Osaka, Japan). Silicon tetrabromide ($SiBr_4$), hexachloroplatinic acid (H_2PtCl_6 ($6H_2O$)), and doxorubicin (DOX) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Argon (Ar) and hydrogen (H_2) gas were purchased from Takachiho Chemical Industrial Co., Japan. Dulbecco's modified eagle medium (DMEM) and fetal bovine serum (FBS) were purchased from Life Technologies (Carlsbad, CA, USA). Phosphate-buffered saline (PBS) was purchased from Lonza (Basel, Switzerland). The Cell Counting Kit-8 was purchased from Dojindo (Kumamoto, Japan).

Synthesis and surface modification of Si-QDs

Si-QDs were synthesized via plasma-assisted decomposition of $SiBr_4$, as described in our previous report.[1] Briefly, we used Ar as a carrier gas to introduce precursor $SiBr_4$ and H_2 into a plasma chamber. Radio frequency (13.56 MHz) plasma was used to decompose $SiBr_4$ and induce a nucleation of Si. The synthesized Si-QDs were collected by a glass flask cooled with liquid nitrogen (N_2) and stored under N_2 atmosphere. The surface of the synthesized Si-QDs

were modified with allylamine via a Pt-catalyzed hydrosilylation reaction, as previously described.[2,3] In brief, surface oxide layer of Si-QDs, which was inevitably formed during the synthetic procedure and/or following storage period, was removed by HF acid (5 wt%). Following this, 10 mg of Si-QDs were dispersed in 10 ml of toluene, mixed with 1.0 ml of allylamine and 40 μl of 0.05 M $H_2PtCl_6(6H_2O)$ in isopropyl alcohol, and then stirred at 70 ˚C for 15 h in a nitrogen atmosphere with reflux. After removing solvents by evaporation, obtained amine-modified Si-QDs were dispersed in PBS at a concentration of 1.0 mg/mL. UV-visible absorption and photoluminescence (PL) spectra of Si-QD dispersion were measured by a UV-vis-NIR spectrometer (U-4100; Hitachi, Tokyo, Japan) and a spectrofluorometer (FP-6500; Jasco, Tokyo, Japan). The quantum yield of Si-QDs was measured by using Rhodamine 6G as a reference. Infrared (IR) spectra of as-synthesized and amine-modified Si-QDs were measured by an ATR-IR Spectrometer (FT/IR6100; Jasco, Tokyo, Japan).

Fabrication of DOX-loaded aggregates of Si-QDs

Prior to the fabrication of DOX-loaded Si-QD aggregates, the pH of the amine-modified Si-QD dispersion (1.0 mg/mL in PBS) was adjusted to 3.0 by HCl to stabilize Si-QDs via

electrostatic repulsion. It should be noted that even at pH 3.0, Si-QDs did not necessarily disperse as single Si-QD, possibly because of irreversible aggregation during the synthetic procedures and/or electrostatic interaction between surface amines and inevitably formed surface oxide layer. 0.7 mg of DOX was added to 3 mL of the dispersion and stirred for at least 30 min. Then, pH of the dispersion was gradually increased to 7.5 by the addition of NaOH to induce the aggregation of Si-QDs. Subsequently, the dispersion was dialyzed against PBS using a dialysis membrane (MWCO = 3.5 kDa; Thermo Fisher Scientific Inc., Waltham, MA, USA) for 1 day to remove free DOX. The size of the formed aggregates was measured by dynamic light scattering (DLS) (Zetasizer Nano-ZS; Malvern Instruments Ltd, Worcestershire, UK). DLS allows us to evaluate size of the aggregates in solution "as is" without drying the solvent, which sometimes induces further aggregation of particles. The amount of loaded DOX was determined using optical absorbance at 485 nm from DOX measured by the UV-vis-NIR spectrometer. We measured the absorption spectrum of DOX solutions with several known concentrations prior to the experiment and used these data as a reference.

Measurement of DOX release profile from Si-QD aggregates

We transferred 1.0 mL of the dispersion of DOX-loaded Si-QD aggregates into a dialysis

membrane tube. Then, the tube was immersed in 40 mL of PBS, in which the solution pH was adjusted to various values by HCl, and then stirred at 37°C. Because only released DOX passed through the membrane, we were able to evaluate the amount of released DOX by measuring the concentration of DOX in PBS outside the dialysis membrane. We determined the DOX concentration by the fluorescent intensity at 580 nm from DOX measured by the spectrofluorometer (excitation wavelength was 485 nm). We collected 100 μL of the sample at each time point during the release experiments and measured the fluorescent intensity at 580 nm to monitor the release profile of DOX. The fluorescent spectrum of DOX solutions with several known concentrations was also measured and used as a reference. We conducted three independent experiments for each condition. The averaged value was used as data and the standard deviation was used as an error bar.

Observation of the intracellular fate of DOX-loaded Si-QD aggregates

HepG2 human liver carcinoma cells were grown and maintained in DMEM containing 10% FBS at 37 °C in 5% CO_2. For confocal microscopic observation, HepG2 cells were seeded on 35-mm glass bottom dishes (Iwaki, Funabashi, Japan) at a density of 5.0×10^3 cells/cm^2 and grown in DMEM containing 10% FBS. Cells were maintained in a humidified incubator at 37 °C in 5% CO_2 overnight. HepG2 cells were then exposed to 2.0 mL of culture

medium containing DOX-loaded Si-QD aggregates (100 μg/mL). Since the aggregates contained 8.3 w/w% DOX, the drug concentration in the media was 8.3 μg/ml. This concentration was low enough to avoid severe cytotoxicity, as shown in the following cell viability assay. After 1.5 and 48 h exposure, cells were washed twice with PBS and observed via confocal laser scanning microscopy (CLSM) (TCS-SP2; Leica, Wetzlar, Germany) with a 63× oil lens. For a co-localization study, HepG2 cells were first incubated with 100 μg/mL of Si-QDs for 2 h, washed twice with PBS, and then further incubated with Lyso-Tracker Red (1.0 mM in PBS) for 20 min. After washed twice with PBS, the cells were observed via CLSM. A diode laser (405 nm) with a detection range of 430-530 nm was used for the Si-QD and an Ar laser (488 nm) with a detection range of 500-700 nm was used for DOX. For Lyso-Tracker red, a Kr laser (568 nm) with a detection range of 600–800 nm was used.

Cell viability assay

Anticancer activity of DOX-loaded Si-QD aggregates was evaluated by a cell viability assay. HepG2 cells were seeded on a 96-well microplate (BD Biosciences, Franklin Lakes, NJ, USA) at a density of 1.0×10^4 cells per well and kept overnight at 37 °C in 5% CO_2. Culture medium containing DOX-loaded Si-QD aggregates, Si-QD aggregates without DOX loading, or free DOX at various concentrations was loaded into each well and incubated for 48 h. We

used the Cell Counting Kit-8 to measure the cell viability in terms of mitochondrial function. The absorbance at 450 nm by formazans, produced by active mitochondria, was measured with a microplate reader (MPR A4i; Tosoh, Tokyo, Japan). The cell viability was calculated as the ratio of the absorbance value to that of the control. We conducted six independent experiments per particle/DOX concentration. The average value of six data points was plotted as a cell viability value and the standard deviation was used as an error bar.

References

1. P. Shen, N. Uesawa, S. Inasawa, Y. Yamaguchi, *J. Mater. Chem*. 2010, **20**, 1669-1675.
2. J. H. Warner, A. Hoshino, K. Yamamoto, R. D. Tilley, *Angew. Chem. Int. Ed.* 2005, **44**, 4550-4554; *Angew. Chem.* **2005**, *117*, 4626-4630.
3. S. Ohta, P. Shen, S. Inasawa, Y. Yamaguchi, *J. Mater. Chem.* 2012, **22**, 10631-10638.

Supplementary Figures

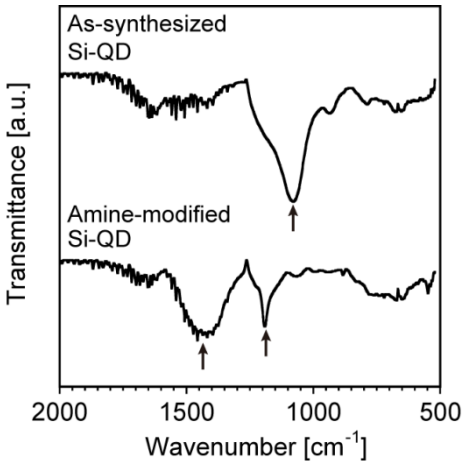

Fig. S1 ATR-IR spectra of as-synthesized and amine-modified Si-QDs. Arrows indicate peaks from stretching vibration of Si-O-Si (1100 cm^{-1}), stretching vibration of CH_2-NH_2 (1200 cm^{-1}), and vibrational scissoring of Si-C (1460 cm^{-1}). As-synthesized Si-QDs showed the peak at 1100 cm^{-1}, suggesting that the surface is covered by surface oxide layer. On the other hand, after the amine modification, new peaks at 1200 cm^{-1} and 1460 cm^{-1} emerged in the spectrum, confirming the formation of covalent bonds between allylamine molecules and Si-QD surface.

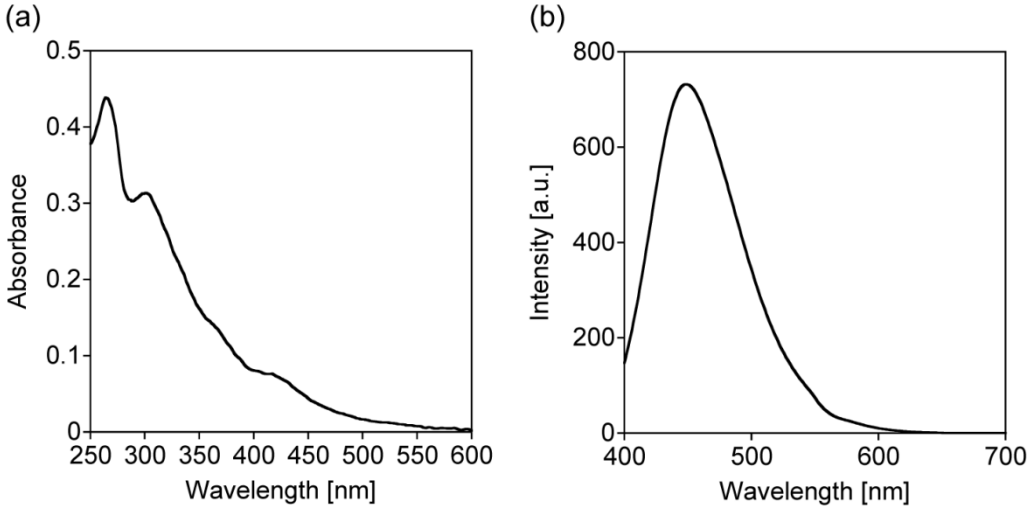

Fig. S2 UV-visible absorption (a) and PL (b) spectra of amine-modified Si-QDs dispersed in water. The excitation wavelength for PL measurement was 360 nm.

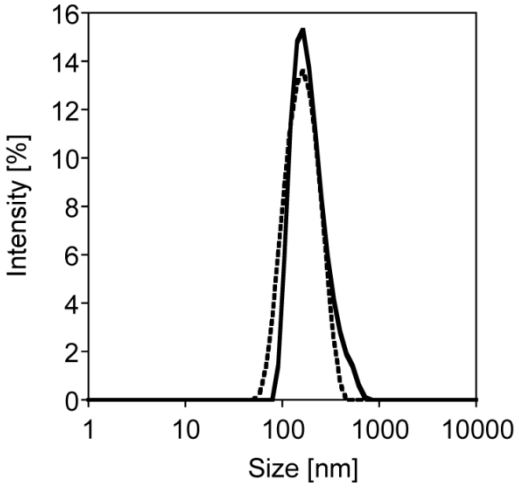

Fig. S3 Size distribution of the Si-QD aggregates right after the synthesis (dashed line) and after 1 month storage (solid line) measured by DLS.

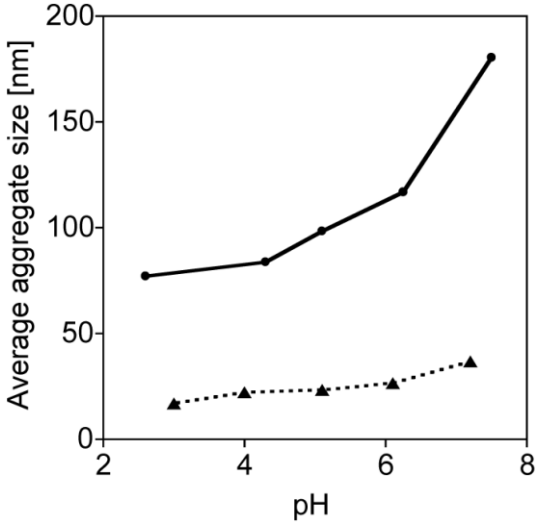

Fig. S4 pH dependence of the aggregate size of Si-QDs at 25 °C (circles) and 60 °C (triangles).

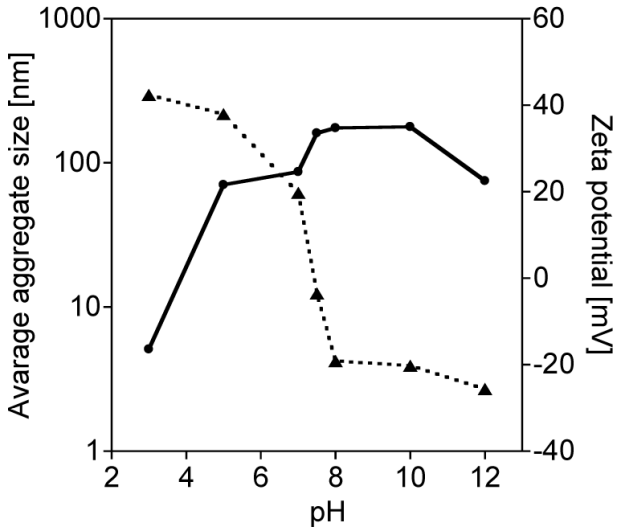

Fig. S5 Effect of pH on size (circles) and zeta potential (triangles) of the Si-QD aggregates. pH range examined here is wider than that in Fig. 2a. Batch of Si-QD was also different.

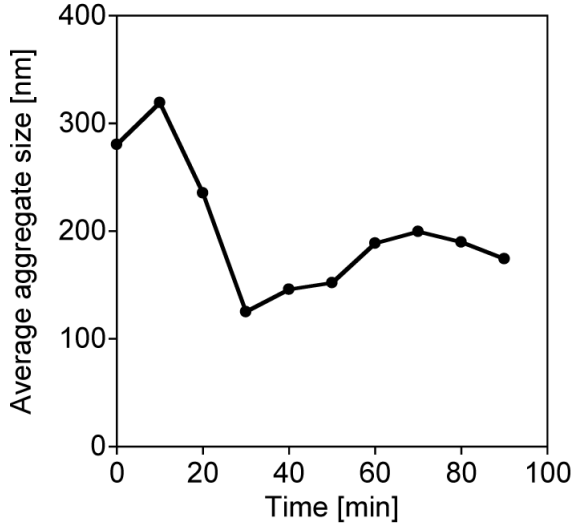

Fig. S6 Time change of the size of Si-QD aggregates at pH 5.0. pH of the Si-QD dispersion was changed from 7.5 to 5.0 by the addition of hydroclolic acid (HCl). The aggregate size was monitored by DLS right after the HCl addition.

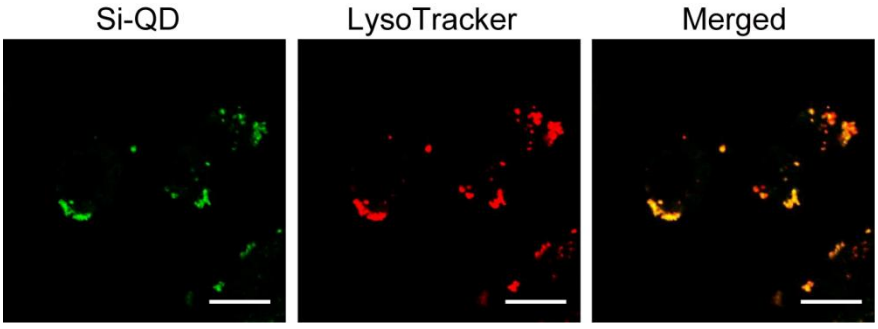

Fig. S7 Confocal microscopic images of HepG2 cells exposed to Si-QD aggregates for 4hours. HepG2 cells were also labeled with LysoTracker red, which selectively labels late endosomes/lysosomes. The signal from Si-QDs was overlapped with that from LysoTracker-red, indicating that Si-QDs were localized in late endosomes/lysosomes. It should be noted that DOX is not loaded into Si-QD aggregates in this experiment in order to avoid the fluorescence overlap with LysoTracker-red.

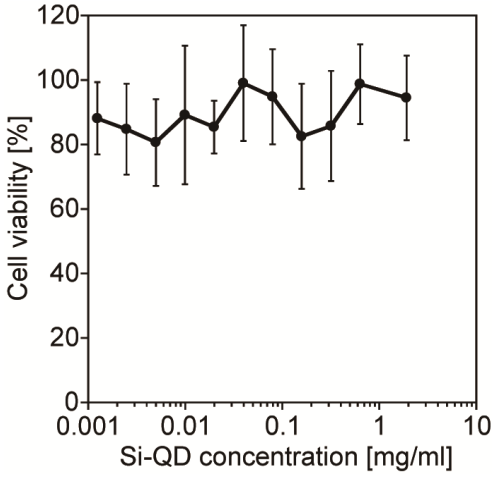

Fig. S8 Cell viability assay for HepG2 cells exposed to Si-QD aggregates without DOX loading. The concentration range examined here (1.2×10^{-3} to 2.0 mg/mL of Si-QD) corresponded to the concentration of Si-QDs used in the cell viability assay for DOX-loaded Si-QD aggregates (Fig. 3 in the main text).