**Electronic Supplementary Information**

# A Facile High-speed Vibration Milling Method to Mass Production of Water-dispersible Silicon Quantum Dots for Long-term Cell Imaging

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## 1. Experimental section

## Materials and apparatus

p-type (phosphorus doped) Si chips was purchased from Sigma. Ultrapure water (over 18 MU cm) from a Milli-Q reference system (Millipore) was used throughout. UV-visible and fluorescence spectra were obtained using Hitachi U-3010 and F-4500 spectrophotometers, respectively. FT-IR spectra (4000–400 cm-1) in KBr were collected on a Varian Excalibur 3100 FTIR spectrometer. TEM and HRTEM images were taken on a JEOL JEM-2100F transmission electron microscope at an acceleration voltage of 200 kV. XPS was performed using ESCALAB 250 spectrometer with a mono X-Ray source Al Kα excitation (1486.6 eV). Binding energy calibration was based on C1s at 284.6 eV. Zeta potentials were recorded on Zetasize 3000 HS (Malvern, UK) Dynamic light scattering (DLS) measurements were made on a Zetasizer nano ZS (ZEN3600) instrument (Malvern, England). A model

HI-98128 pH-meter (Hanna Instruments Inc., USA) was employed for pH measurements. The fluorescence images of the HeLa cells were acquired with a Nikon A1R confocal and multiphoton system using 488 nm laser and an oil immersion 60× objective lens. The absorbance for MTT analysis was recorded on a microplate reader (BIO-TEK Synergy HT, USA) at 490 nm.

**Preparation of succinic acid acyl peroxide:** A one-step procedure was used to synthesis of succinic acid acyl peroxide . Briefly, a fine powder of succinic anhydride (40 g) was added to ice cold hydrogen peroxide (8%, 100 mL) and stirred for 30 min until all the powder dissolved and a white gel-like solution formed. The solution was filtered with a 0.45 mm PTFE membrane filter (PALL) and the solid was washed with a small amount of cold water and air-dried for 10 min. The white peroxide product, m.p. 106 ℃ (literature m.p. 105–107 ℃) was transferred to a glass vial and vacuum-dried at room temperature for 24 h. Approximately 24 g of succinic acid acyl peroxide was obtained.

**Preparation of water dispersible Si QDs**

Water dispersible SiQDs were synthesized by the HSVM method. In summary, 500 mg of p-type (phosphorus-doped) Si chips were initially cleaned in hydrofluoric acid (HF, 20%) for 5 min to remove surface oxides. Then, HF treated Si chips were placed in a stainless steel milling container along with stainless steel milling balls. In a glove box under nitrogen atmosphere, the container was filled with approximately 3 g of succinic acid acyl peroxide, which was capsuled and shaken vigorously for 3 h (SPEX 8000 Mixer/Mill, USA). Thus, an ultrafine powder was produced. This ultrafine powder was well dispersed in ultrapure water by ultrasonic treatment. The large particles were removed through filtering using 0.22 mm membranes. The water-dispersible SiQDs were then obtained (Scheme 1a). The ultrasonic and filtering processes were repeated four times to collect SiQD solutions (Scheme 1b). Next, the obtained SiQD solution was dialyzed against 10:1 (v/v) distilled water–acetone solution several times to remove any organic residues not associated with the SiQDs. Afterward, the obtained SiQDs solution was frozen and subjected to high vacuum to

remove the ice. The modified SiQD powder was then obtained.

**Quantum Yield Estimates.** photoluminescence quantum yields of the SiQDs solution were determined by comparing the integrated emission from the nanocrystals to quinine sulfate in 0.1 M $H_2SO_4$ solutions of matched absorbance. Samples were diluted so that they were optically thin. The emission spectra for quantum yield measurements were collected using a F-4500 spectrophotometers.

**pH Stability.** The pH of the micelle-encapsulated Si dispersions was varied by the dropwise addition of NaOH or HCl. The pH was monitored with a HI-98128 pH-meter.

**Temperature Study.** Micelle-encapsulated Si QDs were dispersed in water and heated. Samples of the QD solution were extracted at temperatures ranging from ambient to 100 °C, and the fluorescence was measured using a spectrofluorometer.

**Photostability Comparison of SiQDs and FITC in water.** The photoluminescence intensity of FITC and SiQDs was adjusted to the same value to compare the photostability of SiQDs and FITC in water. The two samples were then continuously irradiated for different time intervals using a 450 W xenon lamp at 365 nm.

**Cytotoxicity assay**

MTT Assay of Cell Viability. Human epithelial cervical cancer cells (Hela) cells (in Dulbecco′ s modified eagle medium (DMEM) medium) were dispersed in 96-well plates (90 μL in each well containing $1 \times 10^4$ cells per well). 20 μL of SiQDs (200 μg/mL) solution as that used in the following cellular imaging was added to each well. Incubation was carried out for 1, 6, 12, , 24 and 48 h in a humidified atmosphere at 37 °C with 5% $CO_2$. The cell medium solutions were exchanged by 100 μL of fresh medium, followed by the addition of 20 μL MTT (5 mg/mL) solution to each well. The cell plates were then incubated at 37 ºC in 5% $CO_2$ for 4 hours. The culture medium was discarded and 100 μL dimethylsulfoxide was added. Absorbance was measured at 570 nm. The absorbance measured for an untreated cell population under the same experimental conditions was used as the reference point to establish 100% cell viability.

**Long-term Cell imaging.** HeLa cell was cultured in culture media (DMEM/F12 supplemented with 10% FBS, 50 unit/mL of penicillin, and 50 μg/mL of streptomycin) at 37 °C in a humidified incubator containing 5% $CO_2$. HeLa cells were seeded in a 35 mm culture plate at a density of $10^4$ cells/well in culture media. After 24 h, the cells were incubated with 20 μL of SiQDs (200 μg/mL) or FITC (200 μg/mL) in culture media for 60 min at 37 °C. The cells were washed using PBS and then fixed with 4% paraformaldehyde in PBS for 20 min. The fixed HeLa cells were carefully washed thrice with PBS. The cells were irradiated and imaged using NIS-element analysis AR 4.13.00 with a cooled CCD camera at 100 ms intervals for each color automatically. Windows for SiQDs and FITC were collected from 500 nm to 530 nm, respectively.

**2. Table S1, the yield of SiQDs obtained from different reaction times.**

| Reaction time (h) | 1 h | 2 h | 3 h | 4 h | 5 h |
|---|---|---|---|---|---|
| The yield of obtained SiQDs ( mg) | 31 mg | 56 mg | 80 mg | 79 mg | 82 mg |

**3. Figure S1, the temperature-dependent FL emission of the as-prepared SiQDs.**