Fluorescence quenching in luminescent porous silicon nanoparticles for the detection of intracellular Cu^{2+}

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Experimental

Preparation of LPSiNPs: The single side polished, (100) oriented, and p-type Si wafers (boron doped, 8~10 Ω cm resistivity, purchased from Hefei Kejing Materials Technology Co. Ltd., China) were boiled in 3:1 (v/v) concentrated H_2SO_4/30% H_2O_2 for 30 min and then rinsed copiously with Milli-Q water (≥18 MΩ cm resistivity). The PSi samples (1.54 cm^2) with about 30 μm thick porous layer were prepared by electrochemically etching in an ethanolic HF solution (HF (40%)/ethanol (1:1 v/v)) at 20 mA/cm^2 for 45 min. After the sonication in water to detach the porous layer and filtration with 0.45 μm filtration membrane, LPSiNPs samples were prepared for next experiments.

Microwave-assisted synthesis of UA-LPSiNPs: The single-mode heating microwave system NOVA made by Preekem of Shanghai in China was used for the microwave-assisted synthesis of UA-LPSiNPs. The fresh prepared PSi samples was immersed in pure UA, followed by sonication and microwave irradiation. After 40-min heating at 120 °C, 24-h dialysis in water (60,000 Da molecular weight cut-off)

and filtration with 0.45 μm filtration membrane, UA-LPSiNPs were prepared for next experiments.

Fluorescent sensing of Cu^{2+}: $CuCl_2$ was used for Cu^{2+} sensitivity studies. A stock solution of $CuCl_2$ (1 mmol/L) was prepared, and then various concentrations were obtained by serial dilution of the stock solution. For Cu^{2+} detection, solutions with different concentrations of Cu^{2+} ions were added into 2 mL of UA-LPSiNPs (20 μg/mL) solution. Meanwhile a reagent blank experiment was carried out. The PL spectra of the test solution (F) and the blank solution (F_0) were collected using a fluorescence spectrometer with the excitation at 365 nm, and then $\Delta F = F_0 - F$ was calculated. Under the same conditions, the following chlorinates were used to evaluate the specificity of UA-LPSiNPs nanoprobe: K^+, Na^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Fe^{2+}, and Mn^{2+}.

Cell viability assays: Hela cells (~3×10^5 cell/mL) were dispersed within 96-well plates to a total volume of 100 μL/well and maintained at 37 °C in a 5% CO_2/95% air incubator for 24 h. Then the culture media was removed and the cells were incubated in culture medium containing the as-prepared UA-LPSiNPs with different concentrations for 24 h and washed with the culture medium. An amount of 100 μL of the new culture medium containing MTT (10 μL, 5 mg/mL) was then added, followed by incubating for 4 h to allow the formation of formazan dye. After removing the medium, 150 μL DMSO was added to each well to dissolve the formazan crystals. Absorbance was measured at 570 nm in a microplate photometer. Cell viability values were determined (at least three times) according to the following formulae: cell viability (%) = the absorbance of experimental group/the absorbance of blank control group × 100%.

Cellular imaging: Hela cells were plated onto 30-mm cell culture coverslips and incubated with UA-LPSiNPs (20 μg/mL) for 24 h. The attached nanoparticles were washed three times with PBS solution (pH 7.4), and cells samples were monitored using LSCM (Leica TCS SP5, Germany) with the excitation at 405 nm. After the uptkae of UA-LPSiNPs, Helas cells were incubated in PBS solution (pH 7.4) containing 20 μmol/L and 100 μmol/L Cu^{2+} for 15 min, respectively. Finally cell

samples were washed by PBS solution, and monitored using LSCM under the same working conditions.

Instruments and methods: UV-Vis adsorption spectra were recorded by a Shimadzu UV-2450 spectrophotometer. PL measurements were performed using a Perkin-Elmer LS55 fluorescence spectrometer. FTIR spectra were recorded using Bruker Vertex 70 spectrometer at 0.25 cm^{-1} resolution. Analysis of nanoparticles size was performed using Malvern Zetasizer Nano ZS DLS measurements. SEM images were taken by JEOL JSM-7600F scanning electron microscope with the accelerating voltage of 15 kV. TEM images were taken by JEOL JEM-2100 UHR transmission electron microscope with the accelerating voltage of 200 kV. Multiscan MK3 microplate photometer (Thermo Scientific) was used to monitor the absorbance of Hela cells during MTT assays.

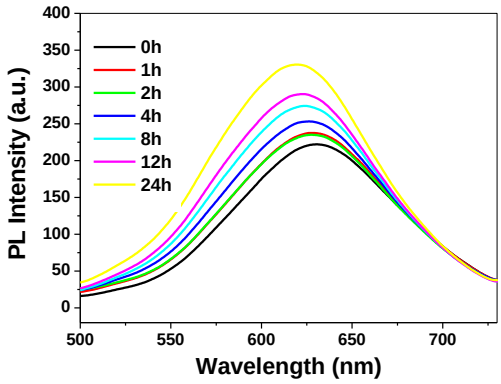

Figure S1. PL spectra of UA-LPSiNPs incubated in PBS solution (pH 7.4) at 37 °C for different hours, with an excitation of 380 nm.

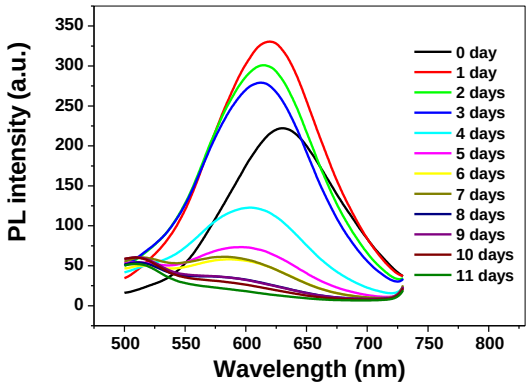

Figure S2. PL spectra of UA-LPSiNPs incubated in PBS solution (pH 7.4) at 37 °C for different days, with an excitation of 380 nm.

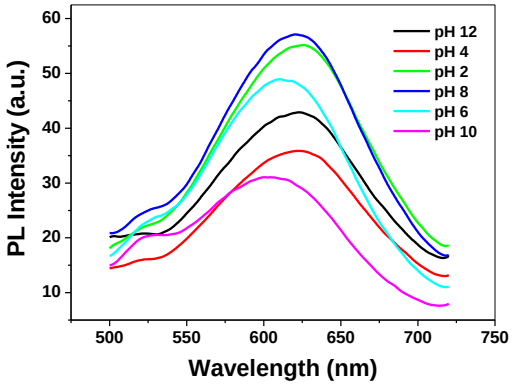

Figure S3. PL spectra of UA-LPSiNPs incubated in aqueous solution with different pH values, with an excitation of 380 nm.

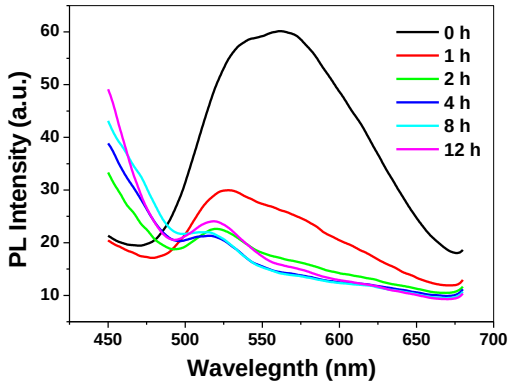

Figure S4. PL spectra of bare LPSiNPs incubated in PBS solution (pH 7.4) at 37 °C for different hours, with an excitation of 365 nm.

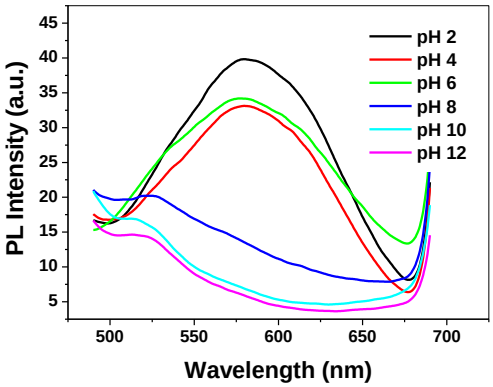

Figure S5. PL spectra of bare LPSiNPs incubated in aqueous solution with different pH values, with an excitation of 365 nm.

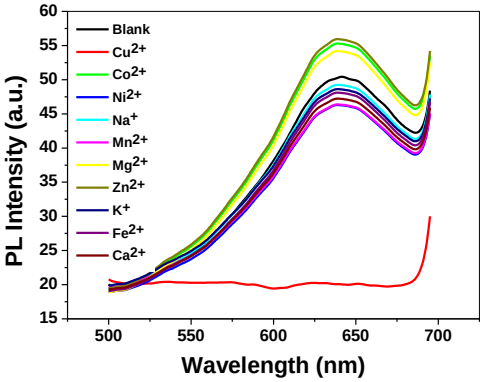

Figure S6. PL spectra of UA-LPSiNPs incubated in aqueous solution (pH 7.4) containing different metal ions (20 μmol/L), with an excitation of 365 nm.

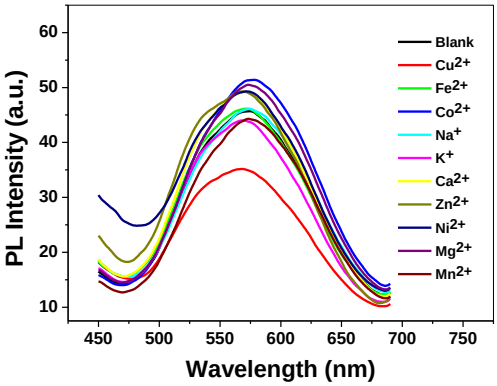

Figure S7. PL spectra of bare LPSiNPs incubated in aqueous solution (pH 7.4) containing different metal ions, with an excitation of 365 nm.

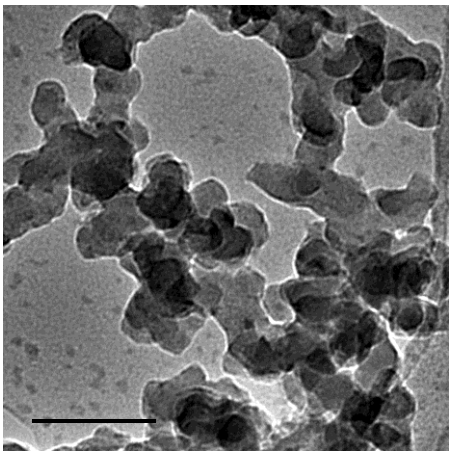

Figure S8. TEM image of UA-LPSiNPs.

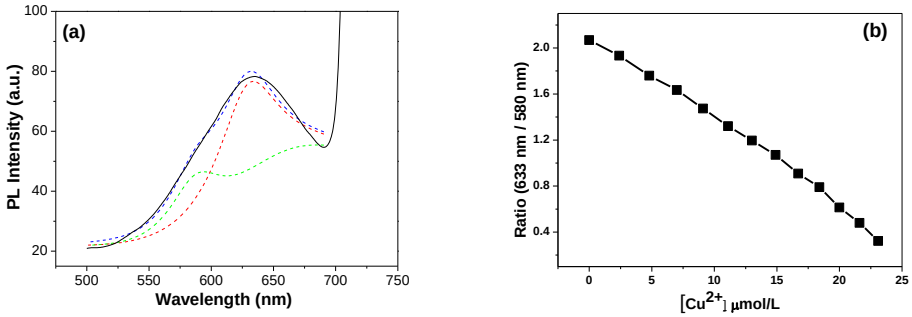

Figure S9. (a) multi-gaussian fitting of PL spectra of UA-LPSiNPs, (b) the relationship between the ratio of 633 nm/580 nm and the concentration of adding Cu^{2+}.

According to FTIR spectra of UA-LPSiNPs in Fig.1a, we found that ν_{Si-OH} stretching mode at around 3434 cm^{-1}, and δ_{Si-OH} bending mode at 1638 cm^{-1} didn't change, however $\nu_{Si-O-Si}$ stretching mode at 1085 cm^{-1} became stronger, which demonstrated that slight oxidation of hydrogen-terminated silicon surfaces happened during microwave-assisted heating [1]. Therefore, PL peak of LPSiNPs at 633 nm can be attributed to hydrogen-terminated silicon surfaces, and the component at approximately 580 nm can be attributed to the slightly oxidation of porous silicon surfaces [2]. Multi-gaussian fitting of PL spectra using the peak at 633 nm and the component at 580 nm was calculated in Fig. S9. The relationship between the ratio of PL intensity (633 nm/580 nm) and the concentration of copper ions indicated that PL

quenching at 633 nm was more sensitive for copper ions than that at 580 nm. That is, oxidation of LPSiNPs would decrease the sensitivity of PL quenching for copper ions.

Reference:

[1] B. Xia, W. Y. Zhang, W. Y. Bao, C. Dong, J. F. Zhang, and J. S. Shi, *Phys. Status Solidi A*, 2012, **209**, 2247-2250.

[2] Z. H. Kang, Y. Liu, C. H. A. Tsang, D. D. D. Ma, X. Fan, N.-B. Wong, and S.-T. Lee, *Adv. Mater.*, 2009, **21**, 661-664.