

Supplementary Information

Stöber Strategy to Multifluorescent Organosilica Nanocrystal

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Supporting Information

Materials

All chemicals, including Vinyltriethoxysilane (VTES) (CP), ammonium hydroxide (AR), sodium dihydrogen phosphate (AR), dibasic sodium phosphate (AR), hydrochloric acid (AR), toluene (AR), tetrahydrofuran (THF) (CP) and EtOH (AR) were used as received without any further purification. Fluorescein isothiocyanate (FITC) (HPLC), Rhodamine B (RhB) (HPLC), vinyltriethoxysilane (VTES) (97%), γ -mercaptopropyltriethoxysilane (MPTES) and aminopropyltriethoxysilane (APTS) were purchased from Sigma-Aldrich, and ultrapure water was used for all experiments.

Preparation of organosilica nanocrystals (OSNCs)

In a typical process, a certain amount of VTES and $\text{NH}_3\cdot\text{H}_2\text{O}$ were dispersed into solution containing 30 mL EtOH and 45 mL H_2O under magnetic stirring. A white solid product composed of OSNCs and amorphous SiO_2 were obtained by evaporating the liquid under negative pressure after keeping stirred at R. T. for 24 h. The purification of OSNCs was realized through an extraction process from the white solid product using THF as solvent. In experiment, the different $\text{H}_2\text{O}/\text{EtOH}$ volume ratio and the volume concentration of ammonia (80 mM, 120 mM, 160 mM, 220 mM) and VTES (6 mM, 9 mM, 15 mM) were adjusted to investigate the influence of experimental condition to the formation of OSNCs.

Preparation of FRET system

100 μL OSNC-1 and 10 μL APTS were dissolved in 20 mL toluene and stirred for 24 h under N_2 atmosphere at 95 $^\circ\text{C}$ to get the amino modified OSNC-1. The toluene solution was distilled in vacuum and re-dispersed in 20 mL ethanol, named solution A. 1.8 mg FITC was dissolved with 20 mL ethanol and named solution B. Different ratio between solution A and solution B was adopted to synthesis the FRET system with different detection range. Typically, set OSNC-1:FITC=1:1 as an example, 1 mL of solution A and 1 mL of solution B were measured accurately and dispersed in 3 mL of ethanol. Then, the FRET-based pH probe was prepared after stirring for 24 h in dark. For pH detection, 200 μL of pH probe solution was added into PBS solution (0.1 M) with different pH value which was prepared beforehand, and fluorescence of the mixture was recorded. Different ratio between solution A and solution B was adopted to accommodate the detection range.

Characterization:

X-ray diffraction (XRD) patterns of all samples were collected in the range 10-80° (2θ) using a Rigaku D/max 2550 diffractometer (Cu Kα radiation, λ =1.5406 Å), operated at 40 kV and 100 mA. The transmission electron microscopy (TEM) was conducted on a JEOL JEM-2100EX electron microscope, operated at an accelerating voltage of 200 KV. The instrument employed for XPS studies was a Perkin-Elmer PHI 5000C ESCA system with AlK_α radiation operated at 250 W. The shift of the binding energy due to relative surface charging was corrected using the C1s level at 284.6 eV as an internal standard. The one-photon fluorescence emission spectra were measured on luminescence spectrometry (RF-5301PC) at room temperature. The CIE (1931) coordinates were calculated from the fluorescence data using the CIE1931xy.V.1.6.0.2a software package. The colors of the fluorescence emissions were identified by the CIE colorimetry system. Any color could be described by the chromaticity (x, y) coordinates on the CIE diagram. Images of live cells were acquired under an OLYMPUS Fluo View FV1000 confocal fluorescence microscope laser with a 60× oil-immersion objective lens.

Quantum yield:

Quantum yield of OSNCs was calculated by reference method with the equation as follows:

$$\phi_u = \phi_s \times \frac{F_u}{F_s} \times \frac{A_s}{A_u} \times \frac{n_u^2}{n_s^2}$$

Here, ϕ means quantum yield, F means the integration of fluorescent intensity, A means the maximum absorbance, n means the refractive index, u means the sample to be tested, s means the standard reference.

We choose RhB as the fluorescence quantum yield standard ($\phi_s=69\%$ in dilute ethanol solution). By using the equation above, the quantum yield of OSNC-3 is calculated with 25.12%. And similarly, the quantum yields of OSNC-1 (460 nm) and OSNC-2 (530 nm) are further calculated as 22.38% and 24.74%, using Quinine sulfate ($\phi_s=54\%$ in 0.1 M H₂SO₄) and Fluorescein ($\phi_s=79\%$ in 0.1 M NaOH) as the QY standards, respectively.

Immunofluorescent labeling:

Hela cells were cultured (37 °C, 5% CO₂) on a cover glass with 10% heat-inactivated FBS overnight. 2 mL Hela, add 5 uL OSNC-3 solution, cultured at R.T. for 2 h, washed with PBS solution. Samples were examined by argon laser ($\lambda_{\text{excitation}} = 488$ nm) with 100 mW power under a confocal laser microscope (Leica, TCS-SP5). Detection window is 515–550 nm. The excitation wavelength and detection window are 404 nm and 440–475 nm, respectively, when OSNC-2 is used for cell labeling. Images were captured and processed with image analysis software.

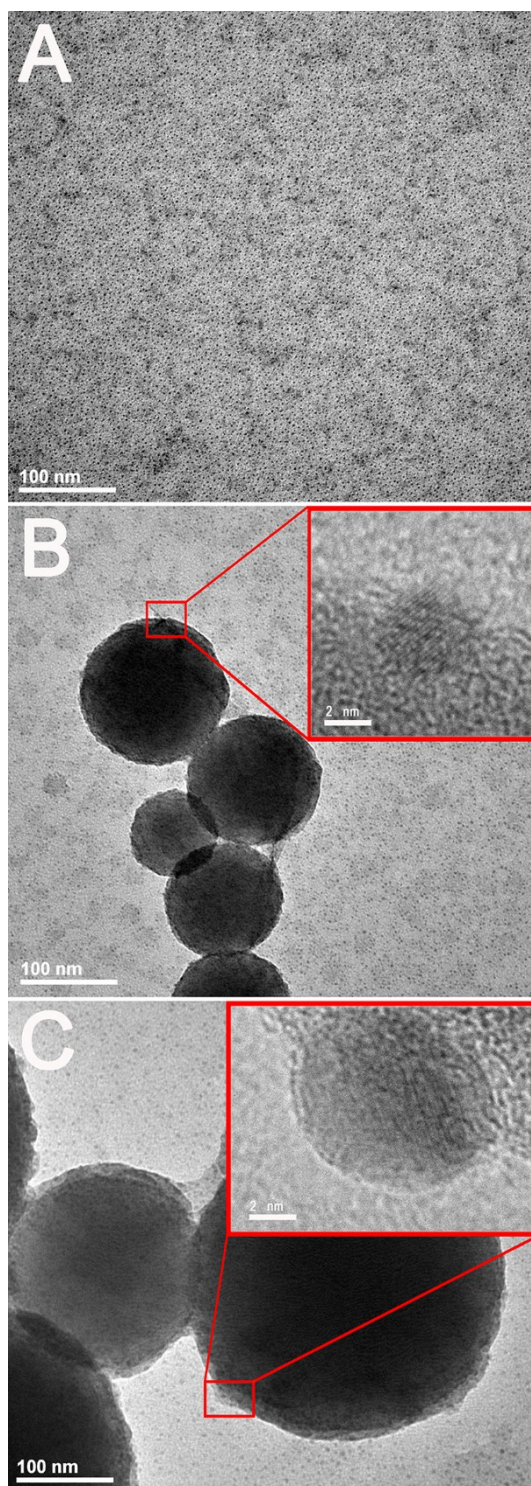


Figure S1. TEM images of crude products synthesized with specific concentrations of VTES of (A) 6 mM, (B) 9 mM and (C) 15 mM, which are named as CP1, CP2 and CP3, respectively. (Synthesis condition: $V_{\text{H}_2\text{O}/\text{EtOH}} = 1.5$, $C_{\text{Ammonia}} = 60$ mM)

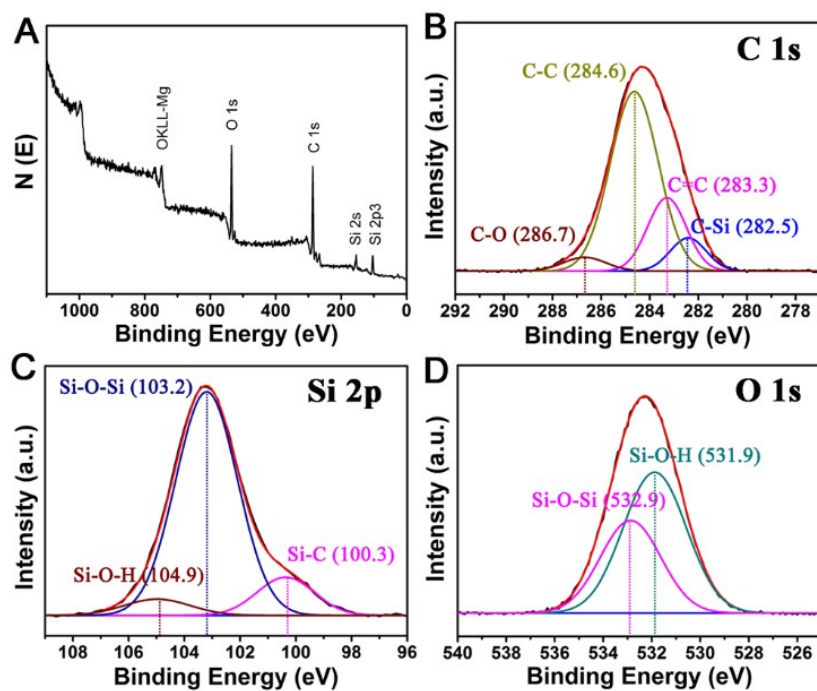


Figure S2. XPS spectra of OSNCs: (A) survey spectrum; (B) C 1s, (C) Si 2p and (D) O 1s regions.

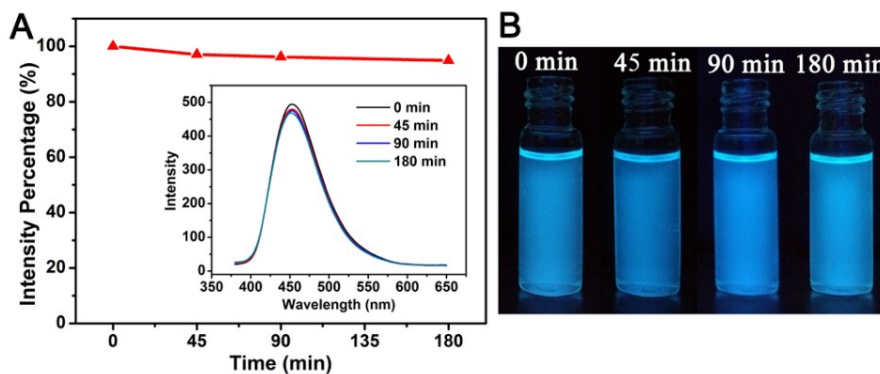


Figure S3. (A) Photo-stability of OSNC-1 under the irradiation of 300 W UV light for 0 to 3 h; (B) Optical images of OSNC-1 water solution under the UV-light irradiation. Inset in (A) is the emission spectra of OSNC-1.

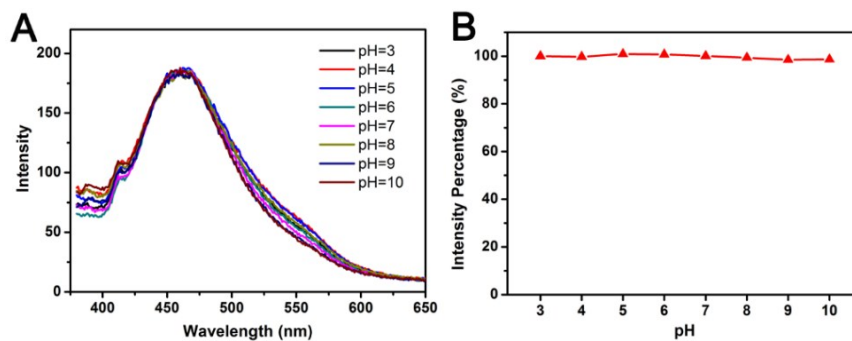


Figure S4. Fluorescence spectra of OSNC-1 in PBS (0.1 M) with different pH value.

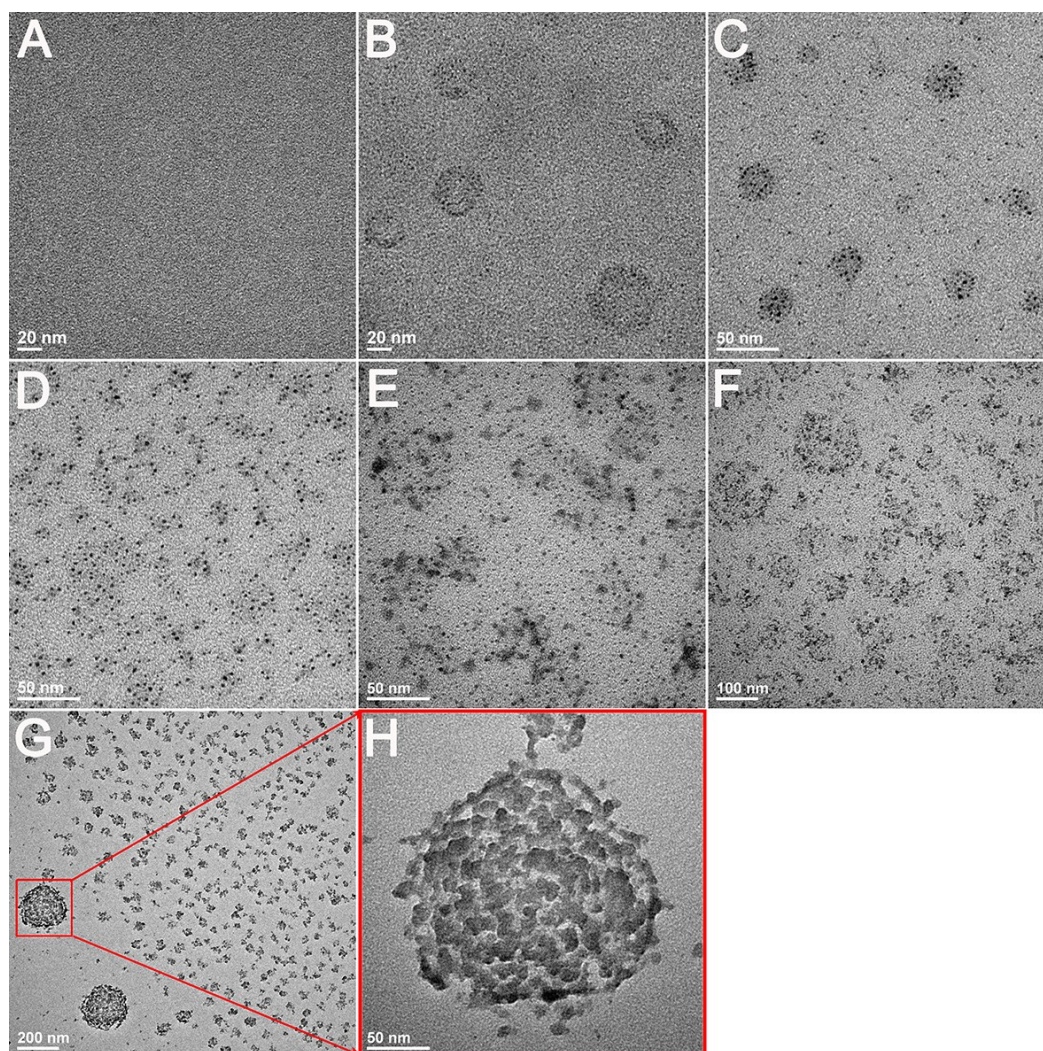


Figure S5. TEM images of crude products synthesized with different H₂O/EtOH volume ratios: (A) pure EtOH, (B) 0.25, (C) 0.67, (D) 1.5, (E) 4, (F) 8 and (G) 14. Image (H) is the magnification of image (G) within red square. (Synthesis condition: the concentration of VTES and ammonia are 6 mM and 160 mM, respectively.)

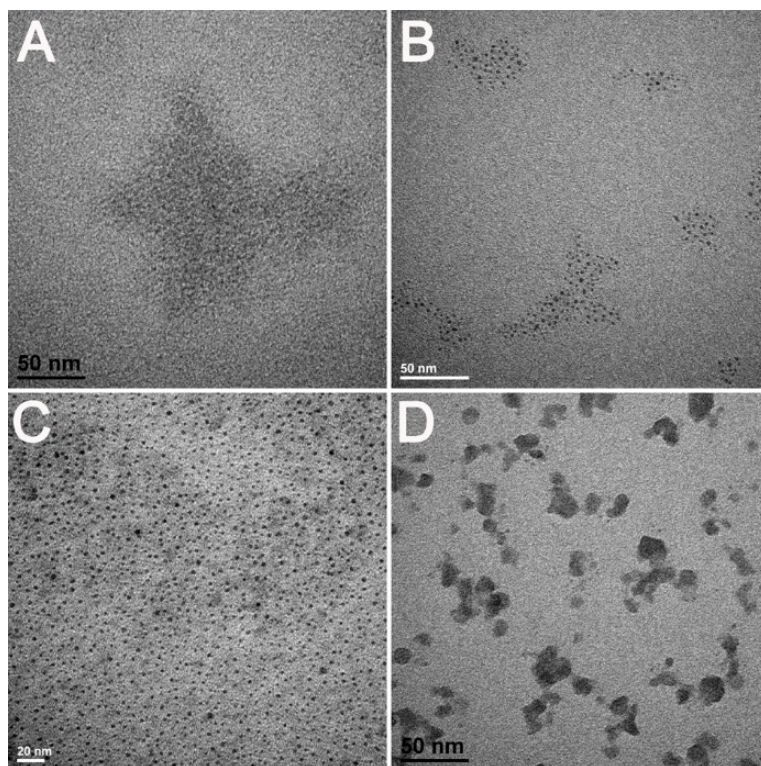


Figure S6. TEM images of crude products synthesized with different concentrations of ammonia: (A) 80 mM, (B) 120 mM, (C) 160 mM, (D) 220 mM. (Synthesis condition: the concentration of VTES is 6 mM and the H₂O/EtOH volume ratio is 1.5.)

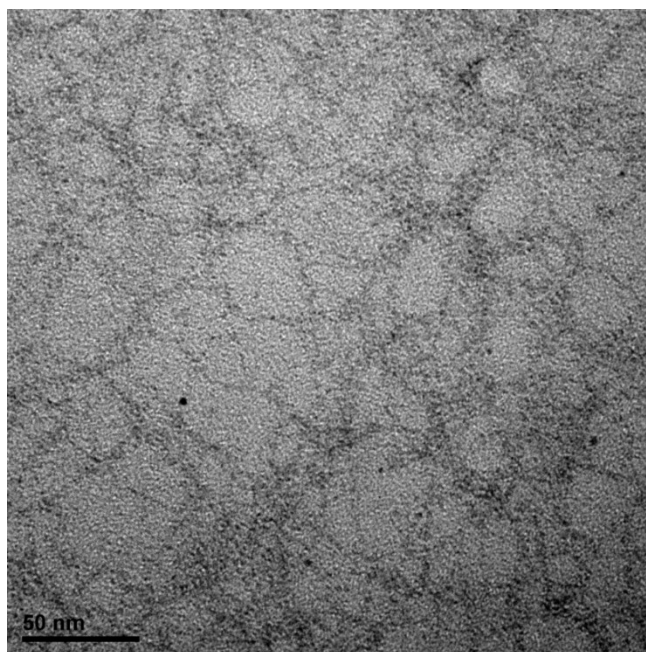


Figure S7. TEM images of the crude products synthesized with MPTES. (Synthesis condition: C_{silica precursor} = 9 mM, V_{H₂O/EtOH} = 1.5, C_{Ammonia} = 60 mM)

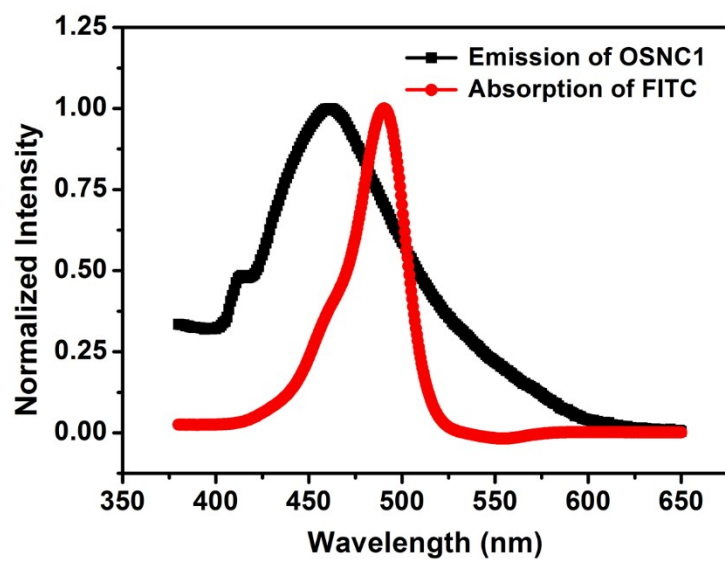


Figure S8. The emission spectrum (black) of OSNC1 and absorption spectrum (red) of FITC.