Supporting Information for

Design of Dendritic Large-Pore Mesoporous Silica Nanoparticles with Controlled Structure and Formation Mechanism in Dual-Templating Strategy

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1. Experiments

1.1 Materials

Cetyltrimethylammonium bromide (CTAB) and 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) were purchased from Sigma-Aldrich. Tetraethyl orthosilicate (TEOS), ferrocene carboxylic acid (FCA), hematoporphyrin dihydrochloride (HP), indomethacin (IDM), protoporphyrin disodium (NAPP) and sodium diethyldithiocarbamate (DDTC) were obtained from Tianjin Heowns Biochemical Technology Co., Ltd. 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) was received from Shanghai Bide Pharmatech Co., Ltd. Ibuprofen (IBU) and deferasirox (DFS) were purchased from Dalian Meilun Biotechnology Co. Ltd. Propantheline bromide (PB) was purchased from Shanghai Aladdin Bio-Chem Technology Co., Ltd. Neostigmine bromide (NB) and tetrasodium iminodisuccinate (IDS) were obtained from Shanghai Macklin Biochemical Co., Ltd. Tetrabutyl ammonium bromide (TAB) was purchased from Tianjin Guangfu Fine Chemical Research Institute. Doxorubicin hydrochloride (Dox) and photosensitizer IR780 were purchased from Beijing J&K Scientific Ltd. Hemoglobin (Hb, from bovine blood) and phosphotungstic acid were provided by Sigma Co. Ltd. Triethanolamine (TEA), methanol and ethanol were provided by Tianjin Kemiou Chemical Reagent. Ammonium nitrate (NH_4NO_3) and sodium chloride (NaCl) was received from Tianjin Fengchuan Chemical Reagent Co., Ltd. All the chemicals were used as received without further purification.

1.2. Synthesis of DLMSN

DLMSN was prepared via a one-step synthesis method by a dual-template method in aqueous solution using CTAB as the major template and an auxiliary template, TEOS as the silica source, and TEA as the catalyst and solubilizer for the auxiliary template. FCA, HP, IBU, IDM, DFS, DDTC, NAPP, IDS, DOTA, PB, NB and TAB were used as auxiliary templates to prepare DLMSN.

Briefly, the DLMSN was synthesized as follows. First, TEA (400.0 mg) were added to water (20 mL) under a magnetic stirring at 500 rpm at 80 ℃ in oil bath for 0.5 h. CTAB (304.0 mg) was added to the above TEA solution and stirred for another 1 h. Then, a certain amount of the auxiliary template was added to the above mixture and kept on stirring for 3 h. TEOS (3.2 mL) was then added and stirred for 20 min. The resulting solution was centrifuged at 15000 rpm for 20 min and the precipitate was washed several times with ethanol to remove the residual reactants to obtain DLMSN. The templates were removed

from the $DLMSN_{FCA}$ via extraction in 10 wt% ammonium nitrate ethanol solution at 80 ℃ for 6 h. This extraction process was repeated for three times. The templates in $DLMSN_{HP}$ were extracted with NaCl solution (1 wt%) in methanol at room temperature for 3 h. This extraction process was repeated for three times.

The MSN without using the auxiliary templates were also prepared and used as control. The preparation is as follows. CTAB (1.852 g) and TEA (0.24 g) were dissolved in water (80 mL) at 50 ℃ and intensively stirred (1000 rpm) for 1 h. TEOS/ethanol solution (40 wt%, 2.5 mL) was then added to the above solution by five injections (0.5 mL per injection) under vigorously stirring at 30 min intervals. The mixture was allowed to react for 24 h at 50 ℃ under intensively stirring (1000 rpm). The products were collected by centrifugation and washed for three times with ethanol to remove the residual reactants.

1.3. Characterizations

Transmission electron microscopy (TEM) images were obtained on a Hitachi HT7700 microscope, and scanning electron microscopy (SEM) images were obtained on a ZEISS GeminiSEM300 microscope. Nitrogen adsorption-desorption isotherms were performed at 77 K on a Micromeritics ASAP 2020 system. All the samples were activated at 423 K for 4 h in vacuum before measurements. The specific surface area was calculated by the Brunauer-Emmett-Teller (BET) method. The pore size was calculated by Density-Functional Theory (DFT) method. The particle size and size distribution were determined by dynamic light scattering method (DLS) on a Malvern Instrument (Malvern，Zetasizer Nano ZS90) in Milli-Q water at 25 ℃, and the sample concentration was ~1 mg/mL. The contents of FCA or HP in DLMSN were determined by inductively coupled plasma mass spectrometry (ICP-MS) (Thermo Electron Corporation, X7) or ultraviolet-visible-near infrared (UV-Vis-NIR) spectrophotometer (Hitachi, U-3900) after removal of CTAB.

In order to have an intuitive understanding of the micelle structure, the micelles prepared by coassembly of CTAB and different auxiliary templates were negatively stained by phosphotungstic acid and observed by TEM. These micelles were prepared by the same process as that for preparation of DLMSN nanoparticles except that TEOS was not added. A typical synthesis method of negatively stained specimens was performed as follows. The generated micelles were double diluted quickly, dropped on copper mesh for 1 min, and then a drop of 2% phosphotungstic acid was added on the carbon surface. After only 1 min the excess staining agent was removed by touching the grid with the edge of a piece of filter

paper. And then the solvent was evaporated under gentle heating by a lamp. Every sample processed was immediately examined under TEM which avoided the degradation of micelles. Here the CTAB concentration was 21 mM, which is above the surfactant critical micelle concentration. The CTAB micelles were also prepared used as control. Breifly, CTAB (304.0 mg) was added to water (20 mL) under a magnetic stirring at 500 rpm for 1 h at 80 ℃ in oil bath. The zeta potentials of these micelles were also measured by above Malvern Instrument.

1.4. Molecular dynamics (MD) simulations

The molecular dynamics (MD) simulations for modeling selfassembly process of CTAB and the coassembly process of IBU with CTAB micelles were conducted using GROMACS with OPLS-AA force field.[1] The topology of cetyltrimethylammonium ion was modified by applying the Wang and Larson charges to the polar group of the cetyltrimethylammonium unit.[2,3] First, we simulated the self-assemble process of the CTAB micelles. The CTAB unit was replicated 32 times to form the bilayer structure of CTAB. The bilayer model was then equilibrated and put in a $10\times10\times10$ nm^3 water box to reach the equilibrium state in a 5 ns MD process, where the PME electrostatics was utilized and the cut off distance for van der Waals interaction was set as 0.9 nm with period boundary condition.[1] Then after the self-assembly, the CTAB micelles was kept in the box by adding 12×27 IBU units randomly. Then the system was neutralized with Br^- atom and hydrated in the same box. Next, the system went through equilibrium and MD process for another 1 ns. Each bilayer unit contains 32 CTAB monomers and the whole system contains 27 bilayer unites by replicating the unit 3 times along x, y, z direction, respectively. The interaction energy variation is calculated based on the electrostatic energy and van der Waals interactions. The increasing of the interaction energy variation indicates the weaker interactions and vice versa.

1.5. Study of reaction conditions on the DLMSN formation

For study the dependence of pore structure of DLMSN on different feeding strategy, two different feeding strategies were used, where how to add CTAB and the auxiliary template was varied. The following processes for silica formation upon TEOS hydrolysis are the same as in Section 1.2. FCA was used as the auxiliary template and the mass ratio of FCA to CTAB is 0.3. In first strategy, CTAB powder (304.0 mg) was added to the TEA aqueous solution (136 mM, 20 mL), and the mixture was kept stirring for 1 h. FCA (91.2 mg) was then added to the mixture and stirred for another 3 h. In second strategy, CTAB (304.0 mg)

powder and FCA (91.2 mg) powder were simultaneously added into the TEA aqueous solution (136 mM, 20 mL), and the mixture were kept stirring for 3 h.

For study the dependence of pore structure of DLMSN on hydrolysis temperature of TEOS, FCA was used as the auxiliary template and the mass ratio of FCA to CTAB is 0.2. The hydrolysis temperature of TEOS was set at 70, 75, 80, 85, and 90 ℃.

For study the dependence of pore structure of DLMSN on hydrolysis time of TEOS, FCA was used as the auxiliary template and the mass ratio of FCA to CTAB is 0.2. The hydrolysis time of TEOS was set at 4, 7, 8, 10, 15, 20, 40, and 60 min. 0.5 mL of the samples were dispersed in ethanol at each time point. For TEM measurements, one drop of this solution was placed on a Cu grid covered with holey carbon when the reaction time was 4 min. The other samples were centrifuged at 15000 rpm for 20 min and the precipitate was washed one time with ethanol. The TEM samples were prepared as above. The average particle size, average diameter of pore mouth, and average length of branch for $DLMSN_{FCA}$ reflect the average of about 100 particles, pore mouth, and branch.

1.6. Measurements of remaining therapeutic agents in DLMSN and ROS measurement upon irradiation.

The content of FCA in $DLMSN_{FCA}$ was determined by ICP-MS after etching the silica in NaOH solution (1 M). The content of HP in $DLMSN_{HP}$ was determined by UV-visible spectrophotometer after etching the silica in HF solution (5 mM).

ROS level was detected by using an oxidant-sensitive dye DCFH-DA.[4,5] As the DCFH was an unstable agent, the DCFH solution used in the experiment was obtained by mixing DCFH-DA solution (20 mM, 15 μL) with NaOH solution (10 mM, 1200 μL) for 30 min. Then, phosphate buffer saline (PBS, pH 7.4, 10 mM, 4.8 mL) was added to form the stock solution for further use. To study the ROS level of $DLMSN_{FCA}$ under X-ray irradiation, the $DLMSN_{FCA}$ solution (100 μL) with different FCA concentrations (0.5, 1, 1.5 and 2 mM) were mixed with DCFH solution (100 μL) and irradiated with X-ray (RadSource RS-2000 PRO Biological System) at 0, 4, and 8 Gy. The fluorescence intensity of the DCFH was measured on a fluoro-spectrophotometer at an excitation wavelength of 488 nm. To study the ROS level of $DLMSN_{HP}$ under laser irradiation, $DLMSN_{HP}$ with different concentrations (24, 48, and 72 mg/mL, 100 μL) were used, which corresponds to the HP concentrations of 20, 40, and 60 μg/mL, respectively. They were mixed with DCFH solution (100 μL) and exposed under 633 nm laser irradiation at 2 W/cm^2 for 5 min. DLMSN nanoparticles without adding HP and HP solution were used as controls.

1.7. Selective drug loading for $DLMSN_{FCA}$ with different pore sizes.

Three theraputic drug molecules were used to investigate the selective drug loading for $DLMSN_{FCA}$, including Dox, photosensitizer IR780, and Hb. MSN and DLMSN were used in this section, which are summarized as silica nanoparticles. FCA was used as the auxiliary template and the mass ratio of FCA to CTAB is 0, 0.1, 0.2, and 0.3.

For loading of Dox (an anticancer drug), Dox (1 mg) was dissolved in Milli-Q water (2 mL). Then silica nanoparticles (1 mg) were dispersed in the Dox solution (2 mL) via sonication, and the mixture was stirred for 24 h at room temperature. The mixture was centrifuged at 15000 rpm for 20 min and washed with Milli-Q water to remove the free Dox. The amount of Dox loaded in the silica nanoparticles was obtained from the absorption of the supernatant solutions containing Dox molecules measured by UV-Vis spectroscopy at the wavelength of 480 nm. The Dox loading capacity of the DLMSN was calculated as the percentage of the mass of Dox related to the mass of silica nanoparticles.

For loading of IR780 (a photosensitizer), IR780 (1 mg) was dissolved in methanol (2 mL). Then silica nanoparticles (1 mg) were dispersed in the IR780 methanol solution (2 mL) via sonication, and the mixture was stirred for 24 h at room temperature. The mixture was centrifuged at 15000 rpm for 20 min to collect the sediment, which was washed with methanol to remove the residual free IR780. The amount of IR780 loaded in the silica nanoparticles was obtained from the absorption of the supernatant solutions containing IR780 molecules measured by UV-Vis spectroscopy at the wavelength of 780 nm. The IR780 loading capacity of the DLMSN was calculated as the percentage of the mass of IR780 related to the mass of silica nanoparticles.

For loading of Hb, Hb (1 mg) was dissolved in Milli-Q water (1 mL). Then silica nanoparticles (1 mg) were dispersed in the Hb solution via sonication. The resulting mixture was continuously shaken in a shaking bath at 160 shakes/min for 24 h at room temperature.

The mixture was centrifuged at 15000 rpm for 20 min to collect the sediment, which was washed three times with Milli-Q water to remove the residual free Hb. The amount of Hb loaded in the silica nanoparticles was obtained from the absorption of the supernatant solutions containing Hb molecules measured by UV-Vis spectroscopy at the wavelength of 412 nm. The Hb loading capacity of the DLMSN was calculated as the percentage of the mass of Hb related to the mass of silica nanoparticles.

2. Reference

[1] Farafonov, V. S.; Lebed, A.V. Molecular Dynamics Simulation Study of Cetylpyridinum Chloride and Ctyltrimethylammonium Bromide Micelles. *V. N. Karazin Kharkiv National University Bulletin. Chemical series*. **2016**, *27*, 25-30.

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3. Supplementary Figures

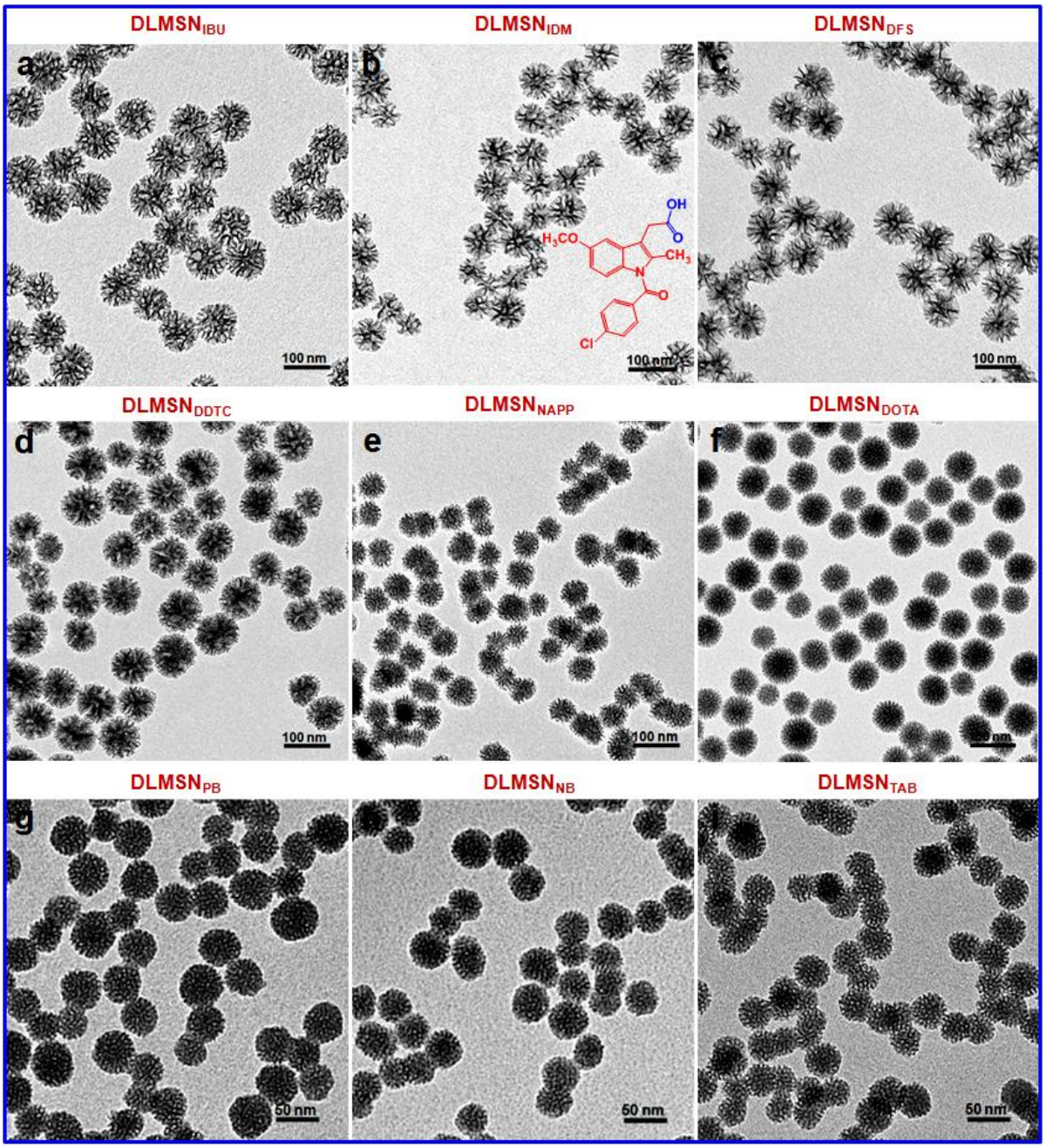

Figure S1. TEM images of (a) DLMSN$_{IBU}$, (b) DLMSN$_{IDM}$, (c) DLMSN$_{DFS}$, (d) DLMSN$_{DDTC}$, (e) DLMSN$_{NAPP}$, (f) DLMSN$_{DOTA}$, (g) DLMSN$_{PB}$, (h) DLMSN$_{NB}$, and (i) DLMSN$_{TAB}$. For (a), the mass ratio of IBU to CTAB is 0.23; for (b)−(i), the mass ratio of the auxiliary templates to CTAB is 0.3.

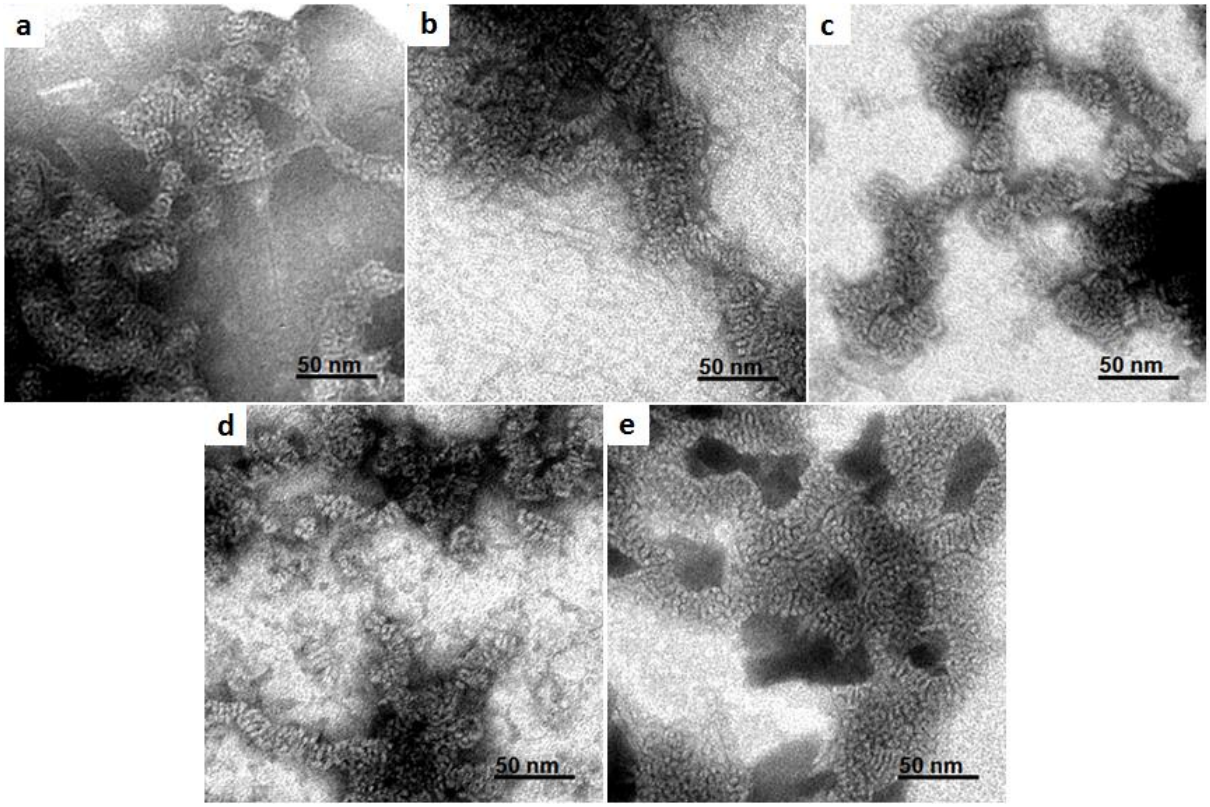

Figure S2. TEM images of CTAB micelles in presence of TEA (a) and the micelles coassembled by CTAB with the auxiliary templates (b-e). The auxiliary templates are (b) NB and (c-e) FCA. For (b), the mass ratio of NB to CTAB is 0.3; for (c)-(e), the mass ratio of the FCA to CTAB is 0.1, 0.2, and 0.3, respectively.

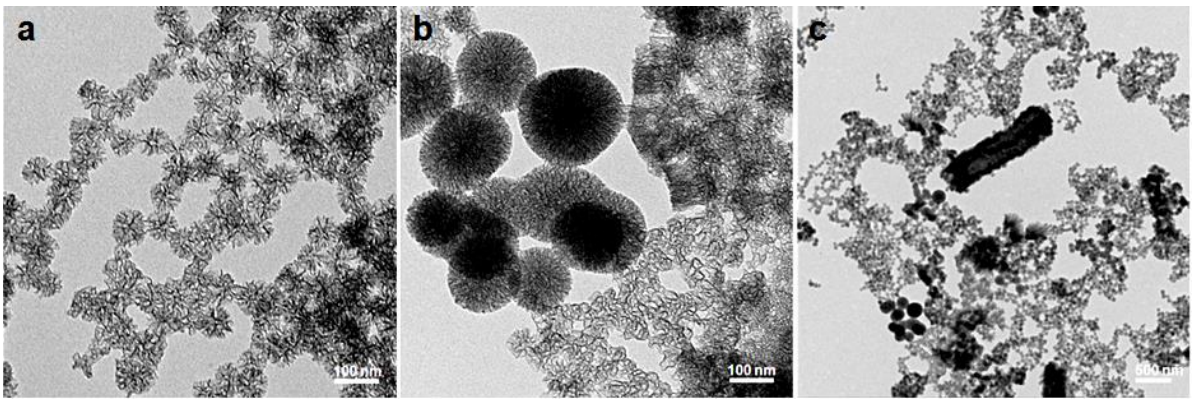

Figure S3. TEM images of $DLMSN_{FCA}$ prepared at the mass ratio of FCA to CTAB of 0.4. Particles with rather large size and irregular pores or porous sheets were obtained.

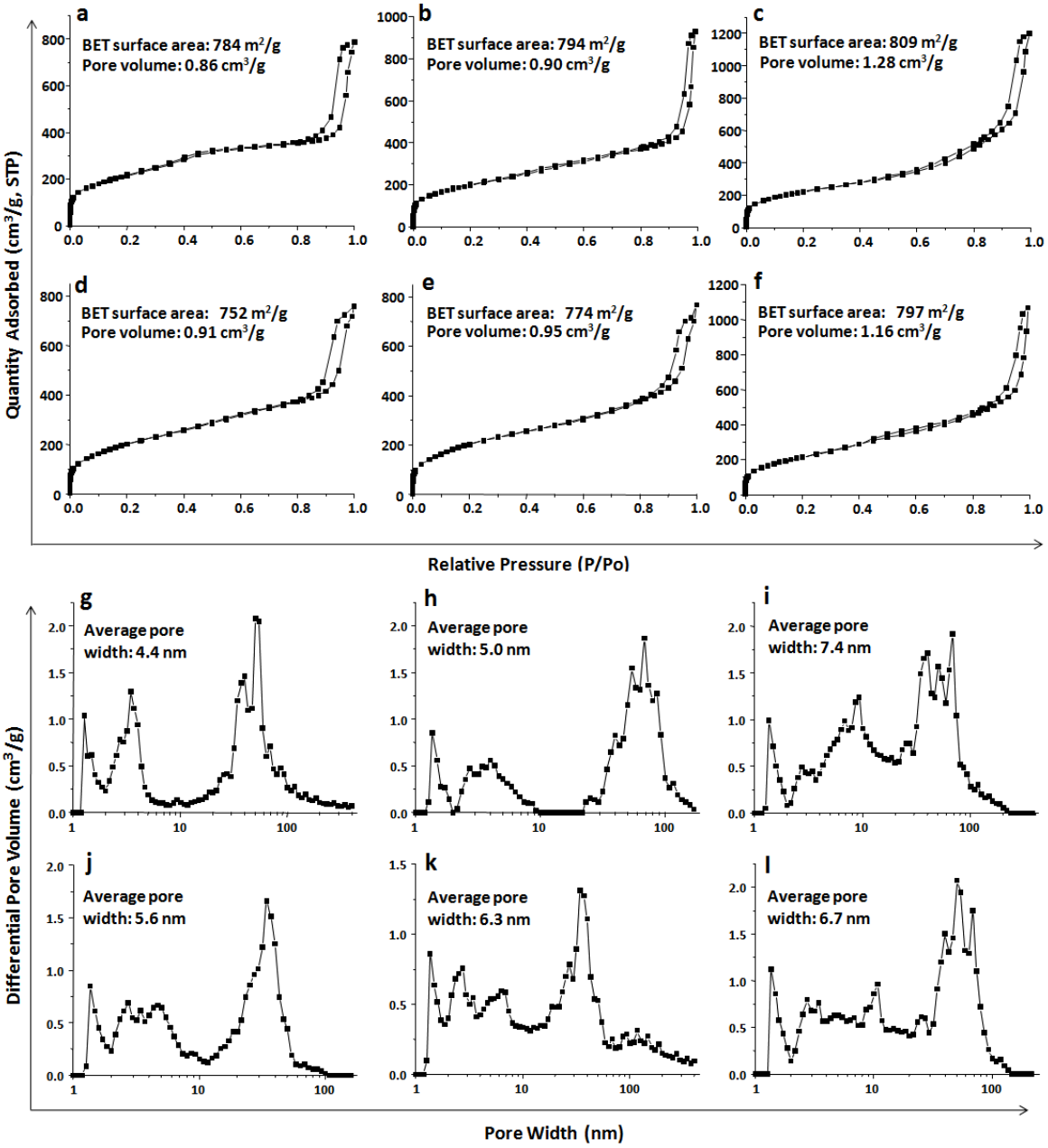

Figure S4. (a-c) N_2 adsorption-desorption isotherms and (g-i) pore size distributions for $DLMSN_{FCA}$. The mass ratio of FCA to CTAB is 0.1 for (a, g), 0.2 for (b, h), and 0.3 for (c, i), respectively. (d-f) N_2 adsorption-desorption isotherms and (j-l) pore size distributions for $DLMSN_{FCA}$. The mass ratio of HP to CTAB is 0.1 for (d, j), 0.2 for (e, k), and 0.3 for (f, l), respectively. All the DLMSN samples demonstrated a H3 type hysteresis loop of the type IV isotherm in the relative pressure range of 0.4–1.0, suggesting the presence of various-sized pores containing micro- and meso- pores.

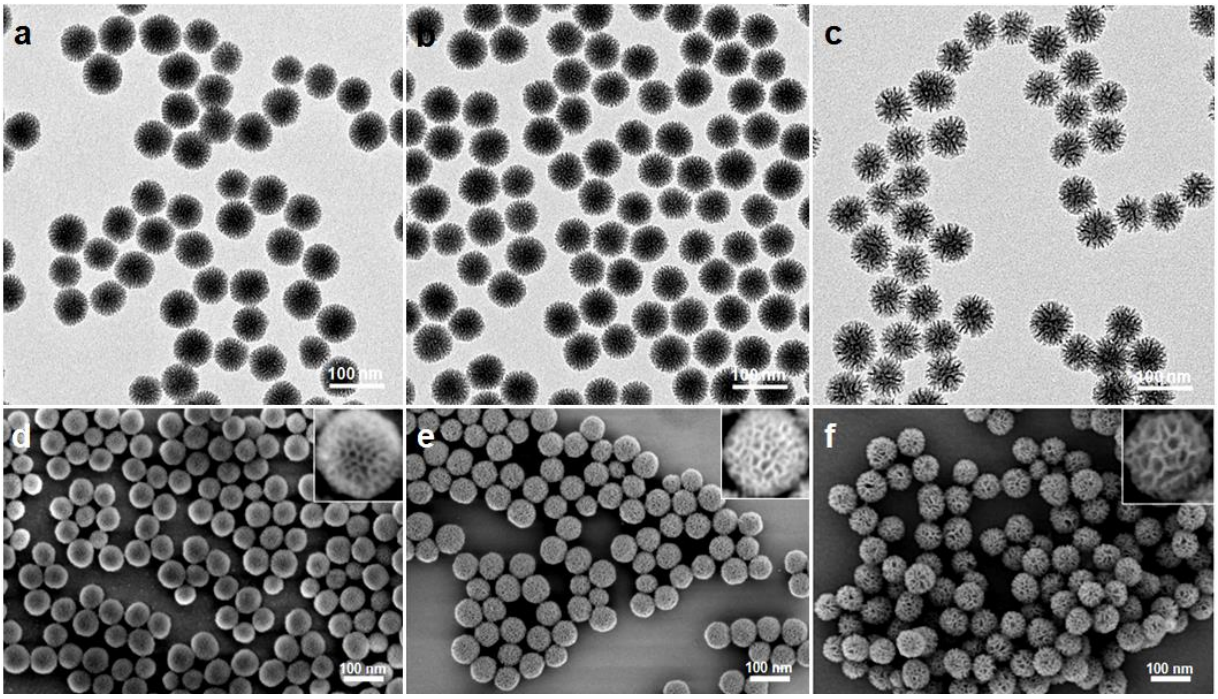

Figure S5. (a–c) TEM and (b–d) SEM images of $DLMSN_{HP}$. The mass ratio of HP to CTAB is 0.1 for (a, d), 0.2 for (b, e), and 0.3 for (c, f).

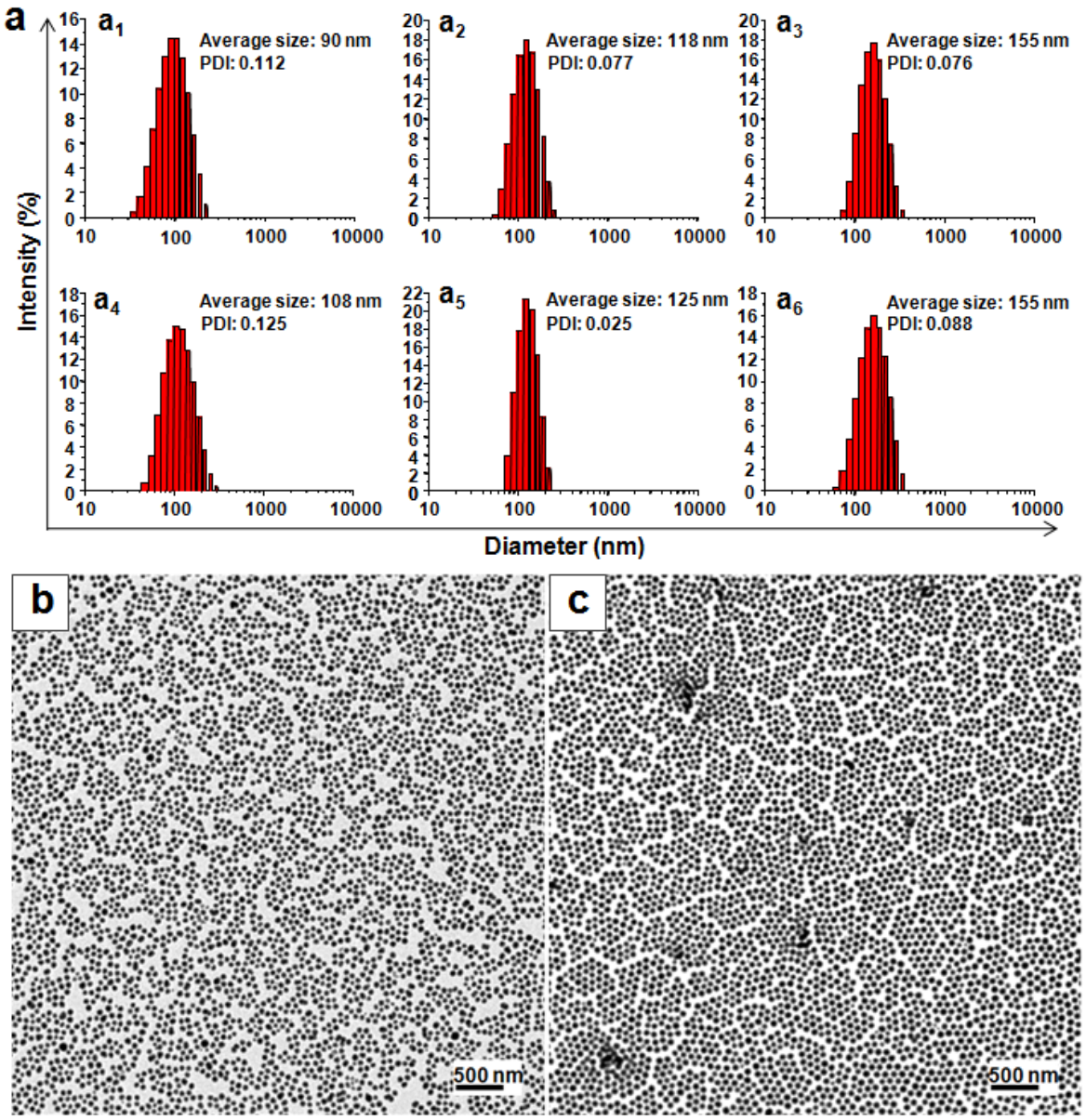

Figure S6. (a) Size distribution and (b, c) morphology of DLMSN for different mass ratio of the auxiliary template to CTAB. The auxiliary template is FCA for (a_1, a_2, a_3, and b) and is HP for a_4, a_5, a_6, and c). The mass ratios of FCA to CTAB for (a_1), (a_2), and (a_3) are 0.1, 0.2, and 0.3, respectively; the mass ratios of HP to CTAB for (a_4), (a_5), and (a_6) are 0.1, 0.2, and 0.3, respectively; the mass ratio of FCA to CTAB in (b) is 0.3; the mass ratio of HP to CTAB in (c) is 0.3.

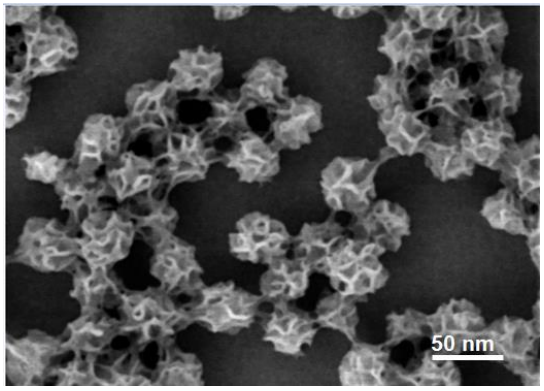

Figure S7. SEM image of $DLMSN_{FCA}$ with TEOS hydrolysis at 80 ℃ for 10 min. The mass ratio of FCA to CTAB is 0.2.

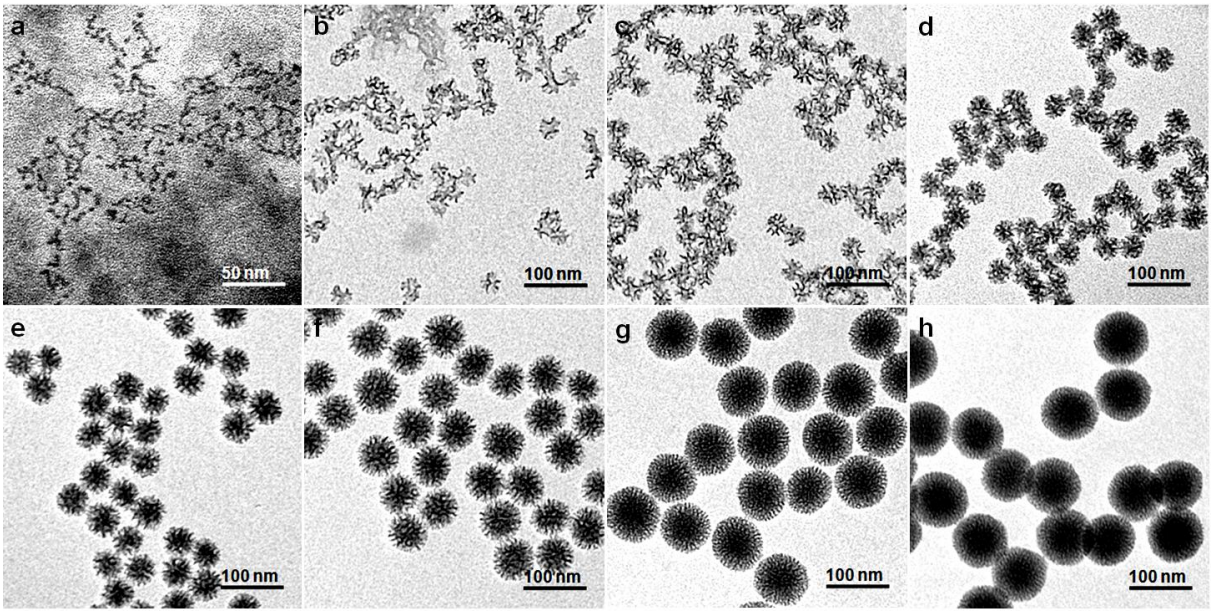

Figure S8. TEM images of $DLMSN_{FCA}$ for different hydrolysis time of TEOS at 80 ℃. From (a) to (h), the hydrolysis time is 4, 7, 8, 10, 15, 20, 40, and 60 min, respectively. The mass ratio of FCA to CTAB is 0.2.

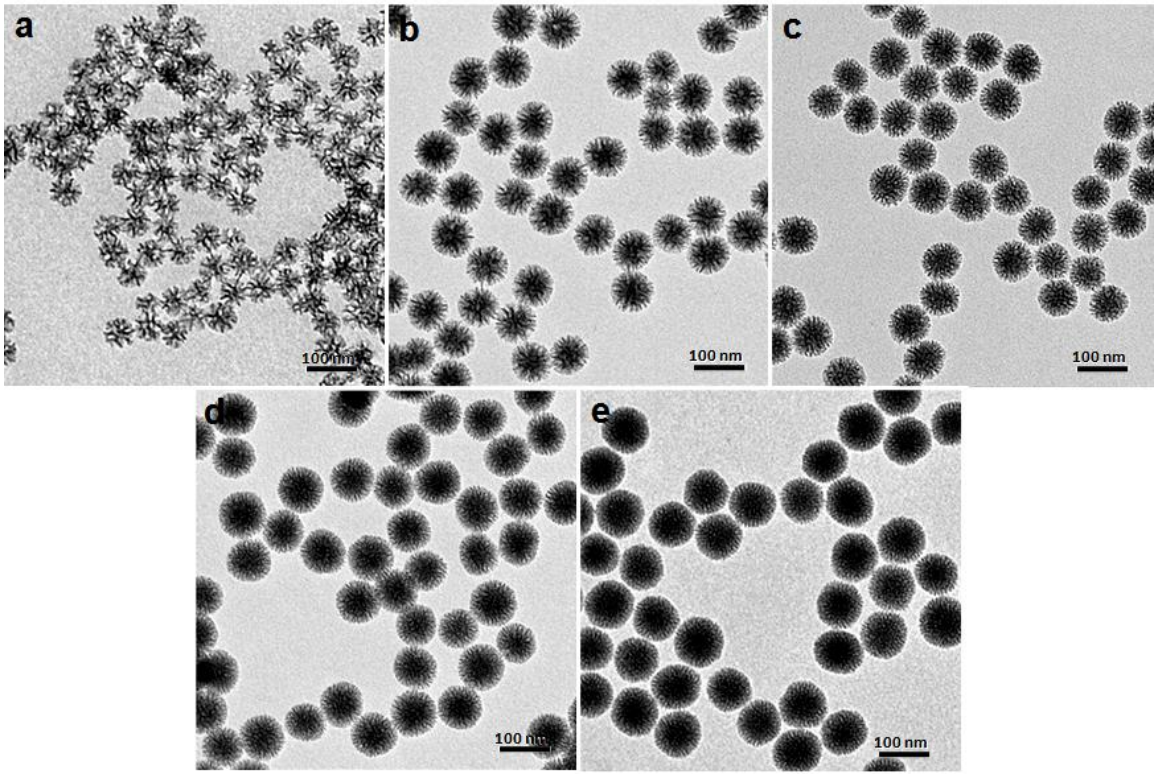

Figure S9. TEM images of $DLMSN_{FCA}$ for different hydrolysis temperature of TEOS at mass ratio of FCA to CTAB of 0.2 for hydrolysis time of 20 min. From (a)-(e), the hydrolysis temperature is 70, 75, 80, 85, and 90 ℃, respectively.

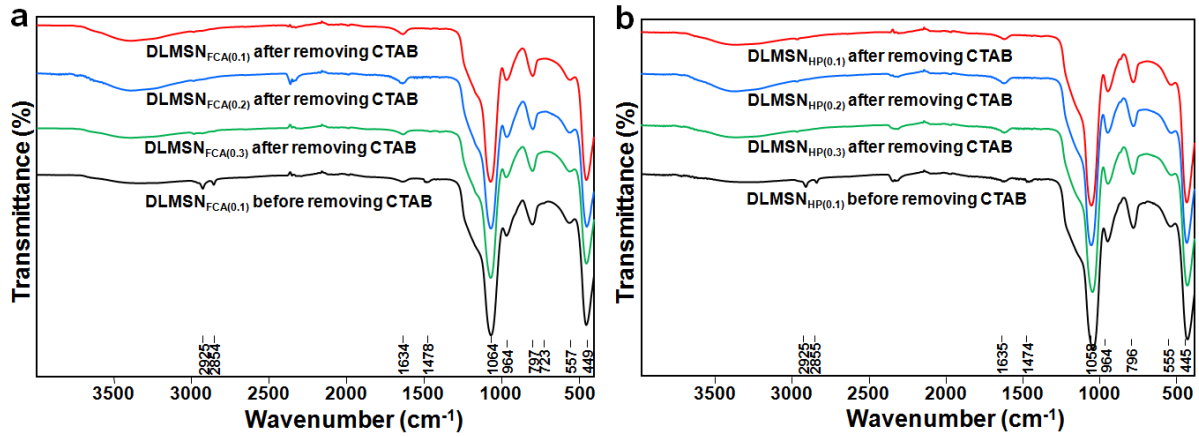

Figure S10. FT-IR spectra for (a) $DLMSN_{FCA}$ and (b) $DLMSN_{HP}$ for different mass ratio of the auxiliary template to CTAB. The number in the brackets indicates the mass ratio of the auxiliary template to CTAB.

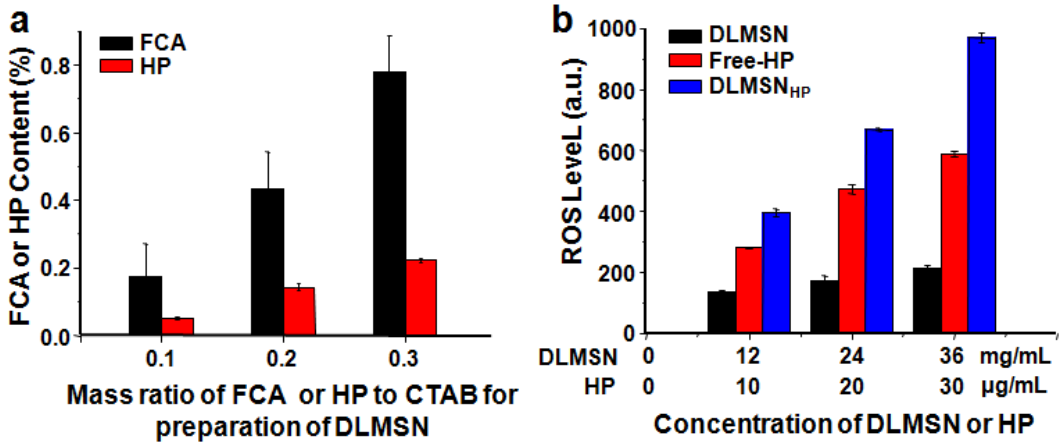

Figure S11. (a) The remaining content of the auxiliary templates (FCA and HP) in the $DLMSN_{FCA}$ and the $DLMSN_{HP}$ after removing CTAB. The mass ratio of the auxiliary template to CTAB is 0.1, 0.2, and 0.3. (b) The ROS level generated by the remaining HP in $DLMSN_{HP}$ upon exposure to 633 nm laser irradiation at 2 W/cm^2 for 5 min, using DLMSN without HP and free HP as controls.