Ultrasensitive ELISA+ enhanced by dendritic mesoporous silica nanoparticles

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Supporting information

1. Experimental section

1.1 Chemicals

Cetyltrimethylammonium chloride (CTAC) solution (25 wt% in H_2O), tetraethylorthosilicate (TEOS), triethanolamine (TEA), 3-aminopropyltriethoxysilane (APTES), phosphate buffered saline (PBS), glutaraldehyde (50 wt% in H_2O), cyclohexane, chlorobenzene, horseradish peroxidase (HRP) and toluene were purchased from Sigma-Aldrich. Guinea pig polyclonal to insulin (2nd antibody) was obtained from Abcam Australia Pty Ltd. Human insulin ELISA kit was purchased from Life Technologies (Australia Pty Ltd). All chemicals were used as received without purification. Deionized water (DI water) used for all experiments was obtained from a Milli-Q system (Millipore, Bedford, MA).

1.2 Materials Synthesis

Synthesis of DMSN-1:[1] In a 100 mL round bottom flask, 24 mL of CTAC solution (25 wt%), 0.18 g of TEA and 36 mL of water were mixed and stirred at 60 °C for 1 h. Then, 4 mL of TEOS and 16 mL of cyclohexane (20 v/v %) were mixed and added to the solution. The solution was kept at 60 °C under a stirring speed of 150 rpm (12 h). The products were obtained after calcination at 550 °C for 5 h.

Synthesis of DMSN-2&3:[2] In a typical synthesis, 4.8 mL CTAC (25% water solution), 0.04 g TEA and 7.2 mL Milli-Q water were mixed to form the water phase and stirred at 60 °C for 1 h. Then 4 mL of premixed oil phase (containing 3.5 mL chlorobenzene and 0.5 mL TEOS) was added to the bottom of water phase. The mixture was stirred at 60 °C under a stirring speed of 150 RPM (12 h) and 500 RPM (12 h) for the synthesis of MSN-2 and MSN-3, respectively. The solid samples were centrifuged at 15 000 RPM for 15 min and washed with ethanol for three times. In the final step, the products were obtained after calcination at 550 °C for 5 h.

1.3 Materials characterization

Nitrogen-sorption isotherms of the samples were obtained by a Micromeritics Tristar II 3020 system at 77 K. Before the measurements, the bare/amino modified samples were degassed at 100/80 °C for at least 8 h in vacuum. The pore size distributions were calculated by the density functional theory (DFT) method.[1] The total pore volume and surface area were calculated by using typical Brunauer–Emmett–Teller(BET) method. TEM images were directly taken with a JEOL 1010 microscope operated at 100 kV by dispersing the samples on a Cu grid covered with carbon films. The SEM images were obtained using JEOL JSM 7800 field-emission scanning electron microscope (FE-SEM) operated at 1 kV using gentle bean mode. The sample was dispersed in ethanol and then dropped to an aluminum foil piece and attached to the conductive carbon tape. Next, the sample was dried in vaccum over at 60 °C for 12 hours. Fourier transform infrared (FTIR) spectra were collected with ThermoNicolet Nexus 6700 FTIR spectrometer equipped with Diamond ATR (attenuated total reflection) Crystal. For each spectrum, 320 scans were collected at a resolution of 4 cm^{-1} over the range of 400–4000cm^{-1}. The ζ potential and dynamic light scattering (DLS) measurements were carried out in PBS at 25 °C using a Zetasizer Nano-ZS from Malvern Instruments. UV-vis transmittance spectra were measured with a Shimadzu UV-2450 double beam spectrophotometer. The absorbance of HRP and anti-insulin are at 401 and 278 nm, respectively.

1.4 Amino modification of DMSN

1 g of DMSNs were dispersed in 30 mL of APTES (4 mmol) toluene solution, and refluxed at 110 °C for 18 h. The products were collected by centrifugation and washed with ethanol for 3 times. The powder was dried at room temperature overnight.

1.5 Immobilization of HRP and 2nd antibody

The HRP immobilization method was modified from literatures:[3, 4] 2 mg of DMSNs was suspended in 1 mL of PBS buffer (pH 7.4), then 1 mL of 2.5% glutaraldehyde was added and the mixture was stirred for 1 h in room temperature. The mixture was centrifuged and washed 3 times with PBS to remove the excess reagent. In the next step, the precipitate was resuspended with PBS and 2 mg of HRP was added. After stirring for 23 h, the solution was centrifuged and the precipitate was washed to remove unbound HRP. All the supernatants were collected for UV test to measure the HRP concentration. The resulting samples were named as DMSN-1-H, DMSN-2-H and DMSN-3-H.

The 2nd antibody immobilization method was modified from literatures:[3, 4] After immobilized with HRP for 6 h, 1 mL of 0.2 mg/mL anti-insulin was added into the solution and further stirring for 17 h. The products were centrifuged and washed to remove unbound HRP and anti-insulin. All the supernatants were collected for UV test to measure the levels of HRP and anti-insulin. The resulting samples were named as DMSN-1-H-A, DMSN-2-H-A and DMSN-3-H-A.

1.6 The activity measurement of immobilized HRP

The activity measurement of immobilized HRP was conducted by using TMB/H_2O_2 as substrates, and the results were compared with free HRP. DMSNs-H solutions were prepared in PBS with different HRP concentration (0-0.25 μg mL^{-1}). Then, mixed with TMB/H_2O_2 (give a total volume of 50 μL) in a 96-well microplate and incubated at 25 °C for 10 min followed by 50 μL of 1 M HCl to stop the reaction. The optical density (O.D.) was read at 450 nm by a microplate reader.

1.7 Ultrasensitive ELISA

The standard insulin solutions with ultra-low concentrations (1 ~ 1/2000 times of LOD of commercial ELISA kit =6.86 ~ 3.43x10^{-3} pg mL^{-1}) were prepared freshly. They were stepwise diluted from the standard solution in the ELISA kit (containing 168 pg mL^{-1} of insulin in human serum).

ELISA$^+$ was conducted following the instruction of traditional ELISA kit by simply replacing the anti-insulin HRP with functionalized DMSNs-H-A. Typically, 50 μL of each standards/control/samples was added into the wells together with 50 μL of anti-insulin HRP, incubating for 30 min at room temperature. For the ELISA$^+$, 50 μL (4 μg mL^{-1}) of functionalized DMSNs-H-A were employed to replace the anti-insulin HRP. The liquid was decanted thoroughly and the wells were washed 4 times with the diluted wash solution before adding 100 μL stabilized chromogen. After 15 min, 100 μL of stop solution (1 M HCl) was added and the plate was measured by reading the absorbance at 450 nm using a Synergy HT microplate reader within 1 h. For the control experiments, PBS was used to replace insulin contained serum as a negative control. All the standards/control/samples were run in duplicate.

The standard curve of ELISA$^+$ was built by using the above procedure to test a series of insulin samples in low concentrations (20-100 fg ml^{-1}). To test the recovery rate, 1 μl of insulin (1 fg) was spiked to 50 μl (3 fg) of insulin standard solution, and it became sample *x* with 4 fg of insulin theoratically. Then, sample *x* was tested by ELISA$^+$ and calculated according to the standard curve. The recovery rate is the difference between calculated value and theoratical value (4 fg). Samples were run in duplicate.

2. Table and Figures

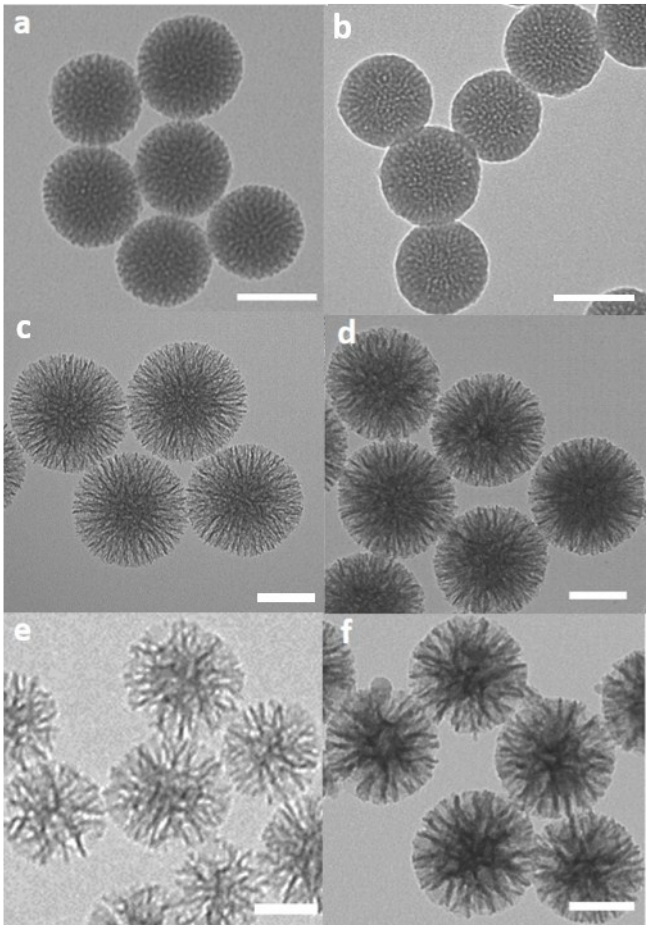

Figure S1 TEM images of (a) DMSN-1, (b) DMSN-1-NH_2, (c) DMSN-2, (d) DMSN-2-NH_2, (e) DMSN-3; and (f) DMSN-3-NH_2. Scale bar: 100 nm.

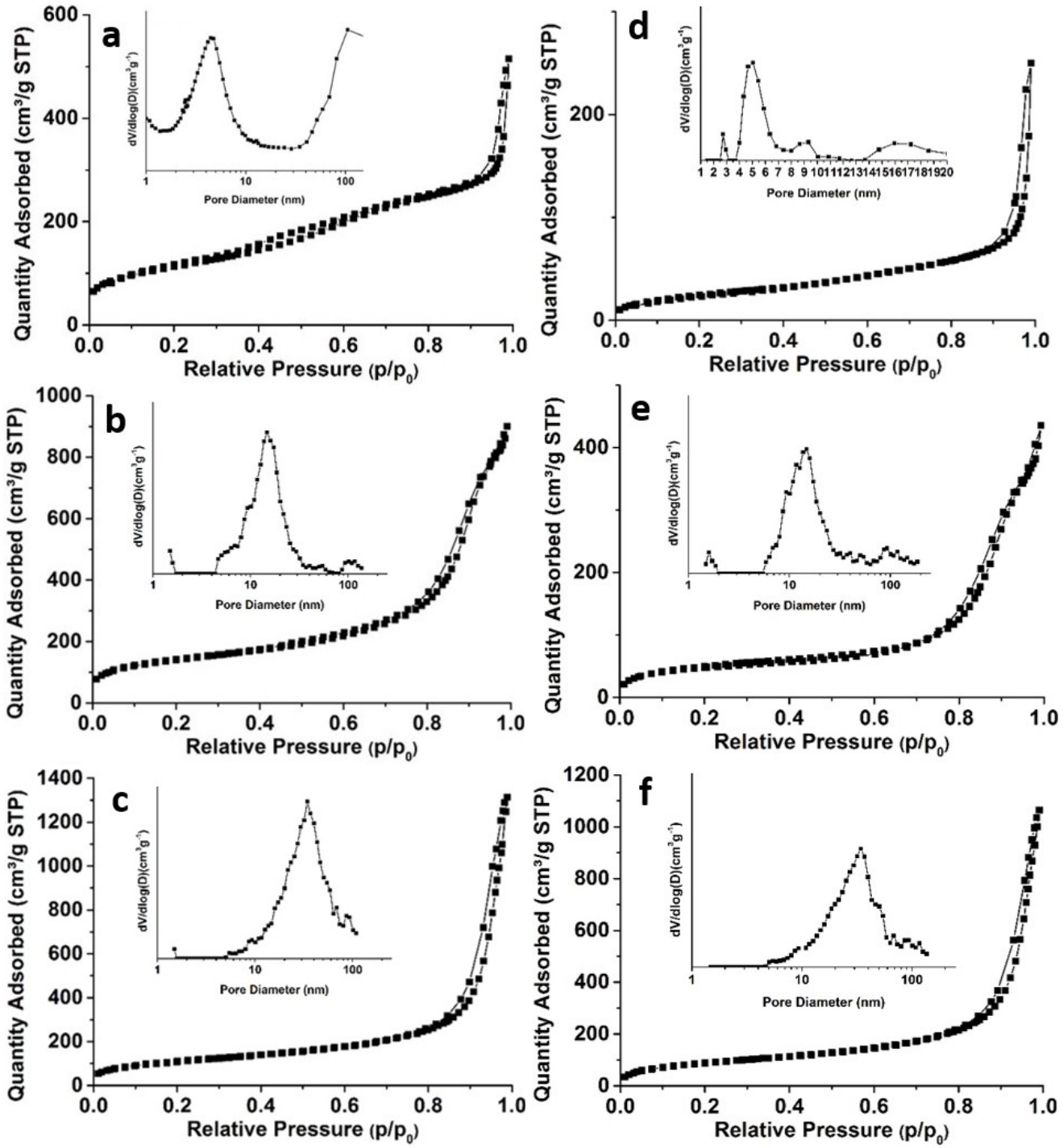

Figure S2 Nitrogen adsorption-desorption isotherms and pore size distributions of (a) DMSN-1, (b) DMSN-2, (c) DMSN-3 and amino modified (d) DMSN-1-NH_2, (e) DMSN-2-NH_2, (f) DMSN-3-NH_2.

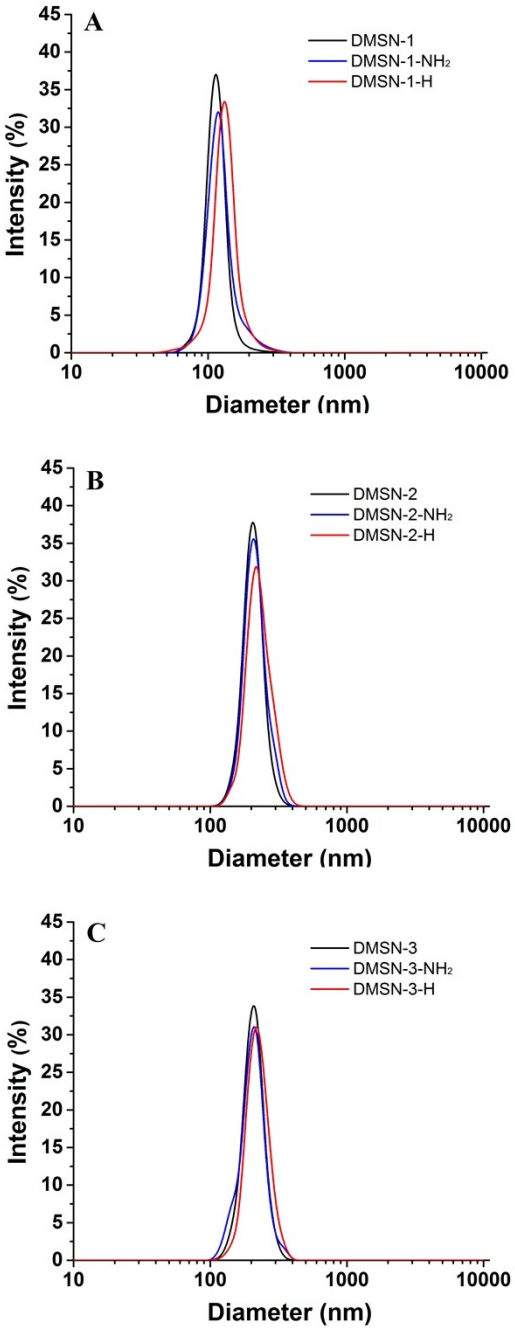

Figure S3 Particle size distribution curves measured by DLS for (A) DMSN-1, DMSN-1-NH_2, DMSN-1-H; (B) DMSN-2, DMSN-2-NH_2, DMSN-2-H; and (C) DMSN-3, DMSN-3-NH_2, DMSN-3-H.

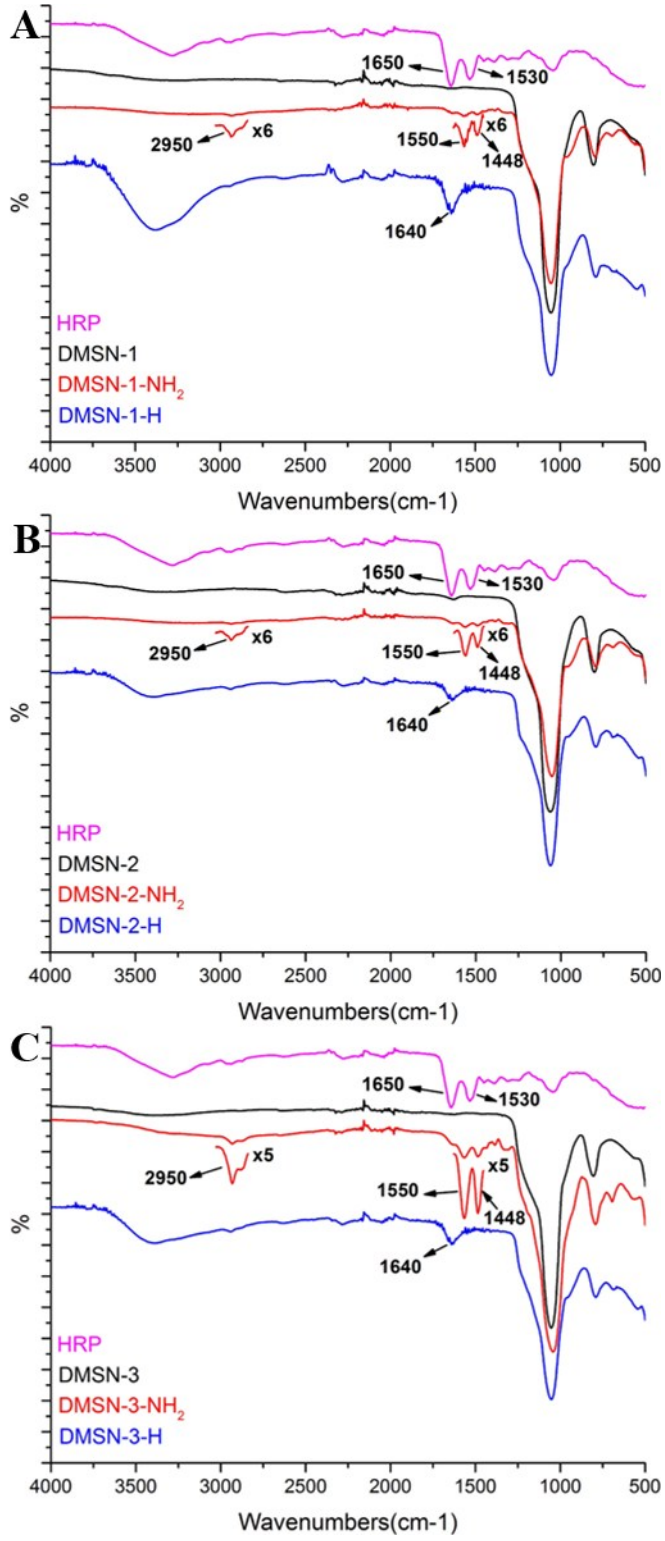

Figure S4 FTIR spectra of (A) DMSN-1, (B) DMSN-2 and (C) DMSN-3 at different steps: bare materials, with amino modification, with HRP loading. The FTIR spectrum of free HRP is also included. The peaks at 2950, 1550 and 1448 cm^{-1} in DMSN-NH_2 are enlarged and shown as insert below.

Table S1. Surface area, pore volume, pore size and ζ potential of materials

Sample	BET surface area (m^2/g)	Pore volume (cm^3/g)	DFT pore size (nm)	ζ potential
DMSN-1	396	0.80	6.9	-25
DMSN-1-NH_2	91	0.39	4.9	36
DMSN-2	484	1.39	14.9	-18.4
DMSN-2-NH_2	176	0.67	14.5	14
DMSN-3	389	2.03	34.2	-27.3
DMSN-3-NH_2	323	1.65	34.2	26

Table S2. HRP loading of materials

	DMSN-1	DMSN-2	DMSN-3
Loading Amount ($mg\ g^{-1}$)	283±10	390±9	382±15

Table S3. HRP and 2nd antibody loading of materials

Loading Amount ($mg\ g^{-1}$)	DMSN-1	DMSN-2	DMSN-3
HRP	288±9	392±12	377±8
Antibody	57±5	60±4	55±4

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4. L. L. Chen, Z. J. Zhang, P. Zhang, X. M. Zhang and A. H. Fu, *Sensor Actuat B-Chem*, 2011, **155**, 557-561.