**Supporting information**

# Rational Synthesis of Hierarchical Magnetic Mesoporous Silica Microspheres with Tunable Mesochannels for Enhanced Enzymes Immobilization

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## Experimental Section

*Materials and reagents*

$FeCl_3$, trisodium citrate, sodium acetate, ethanol, ethylene glycol, cyclohexane, isopropanol, concentrated aqueous ammonia solution (28%), and triethanolamine (TEA) were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Cetyltrimethylammonium chloride (CTAC), tetraethyl orthosilicate (TEOS), and dopamine were purchased from Alfa Aesar (Heysham, Lancs, UK). Xanthine oxidase (XOD) and catalase (CAT) were supplied by Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water (18.2 MΩ) was prepared using a Milli-Q water purification system (Millipore, Bedford, MA, USA). All chemicals were of analytical grade and were used without further purification.

*Synthesis of $Fe_3O_4$@n$SiO_2$ microspheres*

Superparamagnetic $Fe_3O_4$ particles were synthesized by a modified solvothermal reaction. Typically, $FeCl_3$ (0.65 g) and trisodium citrate (0.2 g) were dissolved in ethylene glycol (20 mL) under magnetic stirring, and sodium acetate (1.8 g) was gradually added. The mixture was stirred for 10 h, transferred into a 50-mL Teflon-lined stainless-steel autoclave, heated at 200°C for 10 h then cooled to room temperature. The obtained black product was washed with ethanol and deionized water three times and dried at 45°C under vacuum for 12 h prior to subsequent use.

Silica-coated $Fe_3O_4$ microspheres were prepared according to the Stöber method as follows: as-synthesized $Fe_3O_4$ particles (0.1 g) were homogeneously dispersed in a mixture of ethanol (40 mL) and deionized water (10 mL), and 1.2 mL of concentrated aqueous ammonia solution (28%) was added and the mixture sonicated for 1 h. After pre-stirring for 15 min at 30°C, TEOS (0.4 mL) was added dropwise and stirring continued for 4 h.

The obtained microspheres were collected, washed with ethanol and deionized water, and dried at 45°C under vacuum for 12 h.

*Synthesis of MMSM-5IPA*

Core-shell structure microspheres with large dendritic mesopores were prepared using an improved approach of water/oil biphase system. Briefly, $Fe_3O_4$@n$SiO_2$ microspheres (0.1 g) prepared as described above were dispersed in a mixture of deionized water (60 mL), CTAC (3.0 g) and TEA (0.15 mL) and sonicated for 1 h, then stirred at 65 °C for 1 h. Cyclohexane (20 mL) containing 5% TEOS and 2.5% isopropanol was then added carefully to form a biliquid phase system. Self-assembly was performed at 65 °C for 12 h with gentle mechanical stirring, and products were collected by magnetic separation and washed with ethanol and water three times. To remove the template and organic phase, the obtained particles were extracted and refluxed with hydrochloric acid/ethanol solution at 75°C for 12 h three times. Finally, the obtained sample was dried under vacuum at 45 °C overnight prior to further use.

*Synthesis of MMSM-5IPA@PDA*

PDA modification of the interior of the pore walls was carried out using the procedure in which MMSM-5IPA (0.04 g) were redispersed in ethanol (10 mL) and 10 mM TRIS-HCl buffer (10 mL) and sonicated for 30 min. Deionized water (30 mL) containing 40 mg dopamine hydrochloride was then added while gently stirring and the mixture reacted for 3 h. The obtained products (MMSM-5IPA@PDA) were washed with deionized water thoroughly.

*Immobilization of XOD, CAT, and enzymes activity assays*

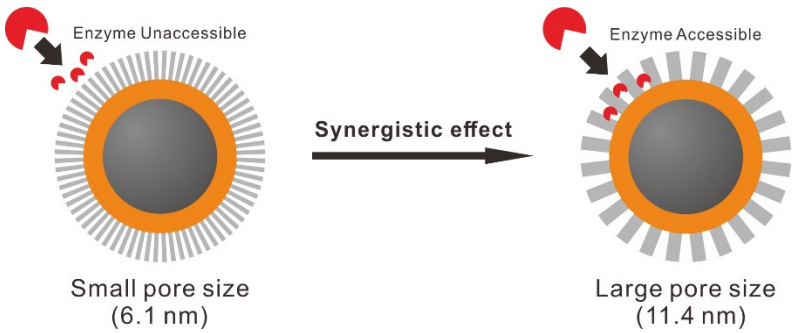
MMSM-5IPA@PDA (1.0 mg) were first washed and equilibrated in 50 mM PBS buffer (pH 7.4) for 1 h, then 1000 μL of PBS buffer containing XOD ( 15 μg) or CAT (10 μg) were added and incubated at 4 °C for 12 h with shaking. MMSM-5IPA@PDA immobilized with XOD or CAT were collected by magnetic separation, washed with deionized water to eliminate non-specific adsorption, and stored in the buffer at 4 °C until needed.

To determine the activity of XOD, the reaction mixtures containing 50 μL XOD solution (15 μg /mL) and 750 μL PBS was initiated by adding 200 μL xanthine (0.5 mM). The absorbance was measured at 290 nm each second at room temperature. Within 90 s (the initial rate), the curve of the slope of the absorbance reflected the enzyme activity. Similarly, the activity of CAT was determined by using the hydrogen peroxide ($H_2O_2$) as substrates and record at 240 nm. Briefly, 50 μL CAT (10 μg/mL) mixed with 1000 μL $H_2O_2$ (20 mM) rapidly and then record the enzymatic kinetics at UV 240 nm for the 60s.

*Measurements and characterization*

The morphology of samples was investigated using field-emission scanning electron microscopy (FE-SEM) on a Hitachi S-4800 microscope (Tokyo, Japan). Transmission electron microscopy (TEM) images were collected with a Tecnai 12 microscope (Eindhoven, The Netherlands) operating at 120 kV. For TEM measurements, the sample was dispersed in ethanol and dropped onto a carbon film Cu grid and dried thoroughly before measurement. X-ray diffraction (XRD) patterns were recorded on a Bruker D8 Advance X-ray diffractometer (Karlsruhe, Germany) equipped with Cu Kα radiation and operated at 40 kV and 40 mA with a step of 0.02°. Nitrogen adsorption-desorption isotherms were measured at 77 K using a Micromeritics ASAP 2020 M+C system (Norcross, GA, USA). Samples were degassed at 423 K for 10 h under vacuum before

testing. The total pore volume was calculated from the amount adsorbed at a maximum relative pressure ($P/P_0$) of 0.99. The Barrett-Joyner-Halenda (BJH) method was conducted to calculate the sample pore size from the desorption branches of the isotherms. The Brunauer-Emmett-Teller (BET) method was used to calculate the specific surface area. Fourier transform infrared (FTIR) spectra were recorded on a TENSOR 27 spectrophotometer (Bruker, Ettlingen, Germany) with a spectral width of 4000–400 $cm^{-1}$. X-ray photoelectron spectroscopy (XPS) was performed on an Escalab 250Xi X-ray photoelectron spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Magnetic properties of samples were investigated using superconducting quantum interference device (SQUID) magnetometry.




**Scheme S1**. The mesopores size can be enlarged by the synergetic effect of surfactant concentrate and amphiphilic agent IPA for enhancing the accessibility of biomacromolecules

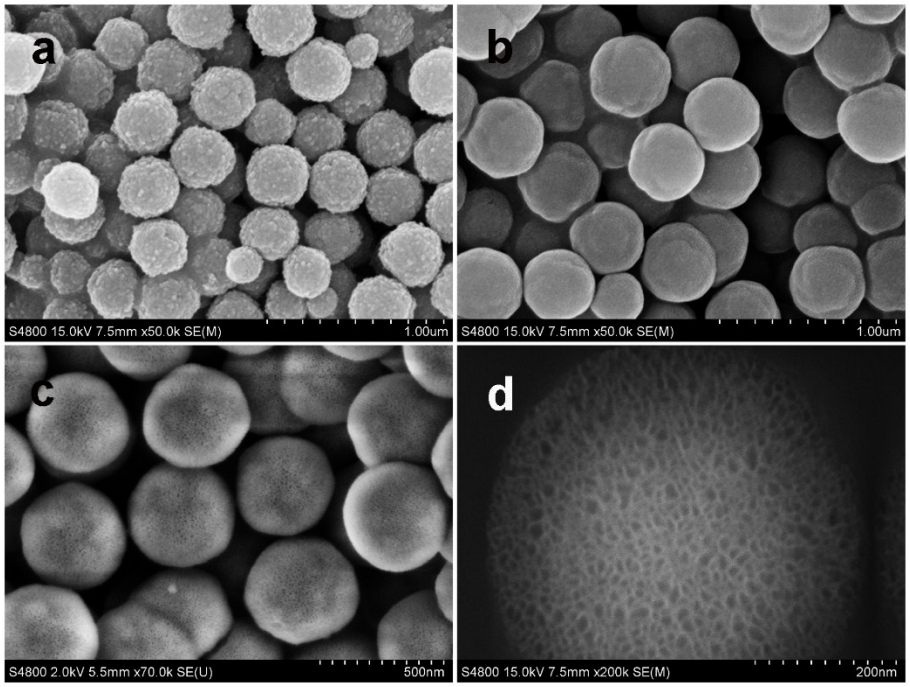



**Figure S1** FE-SEM images of $Fe_3O_4$ nanoparticles (a), $Fe_3O_4$@n$SiO_2$ microspheres (b), and MMSM-5IPA (c, d).

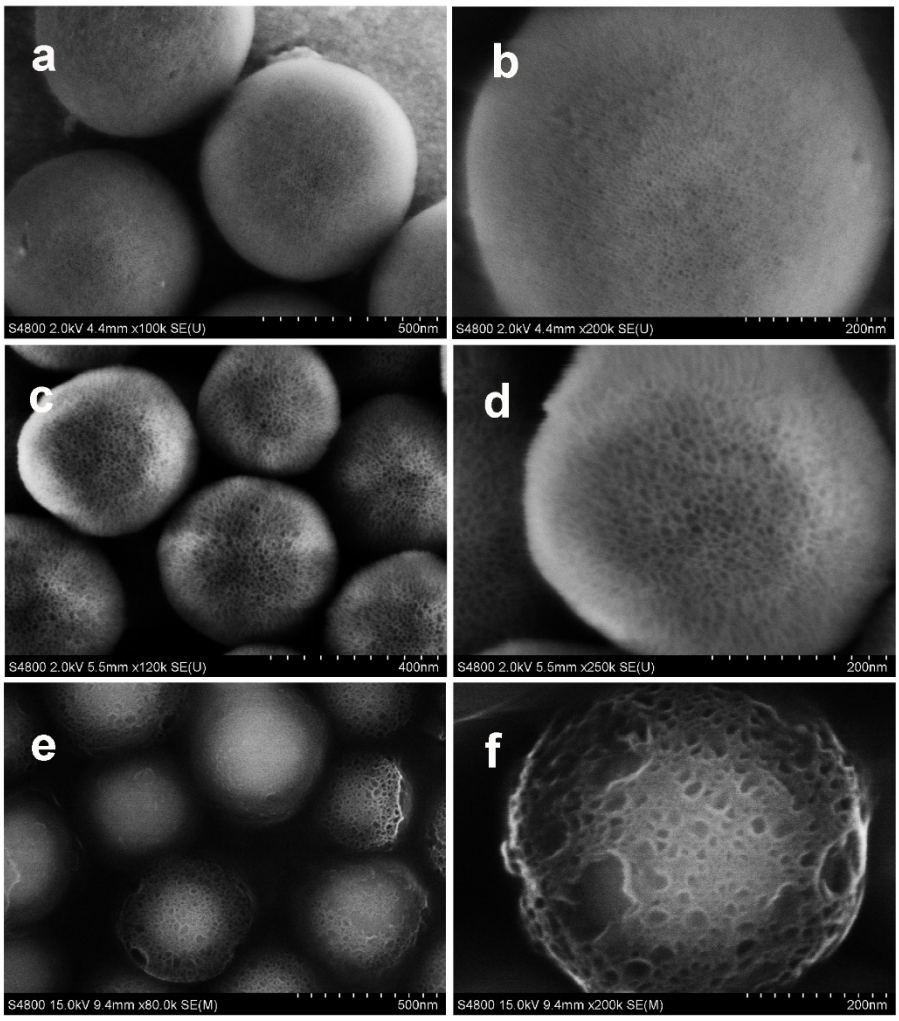



**Figure S2** FE-SEM images of MMSM-1 (a, b), MMSM-5 (c, d), and MMSM-10 (e, f) synthesized with different CATC concentration of 1, 5, and 10 wt%, respectively.

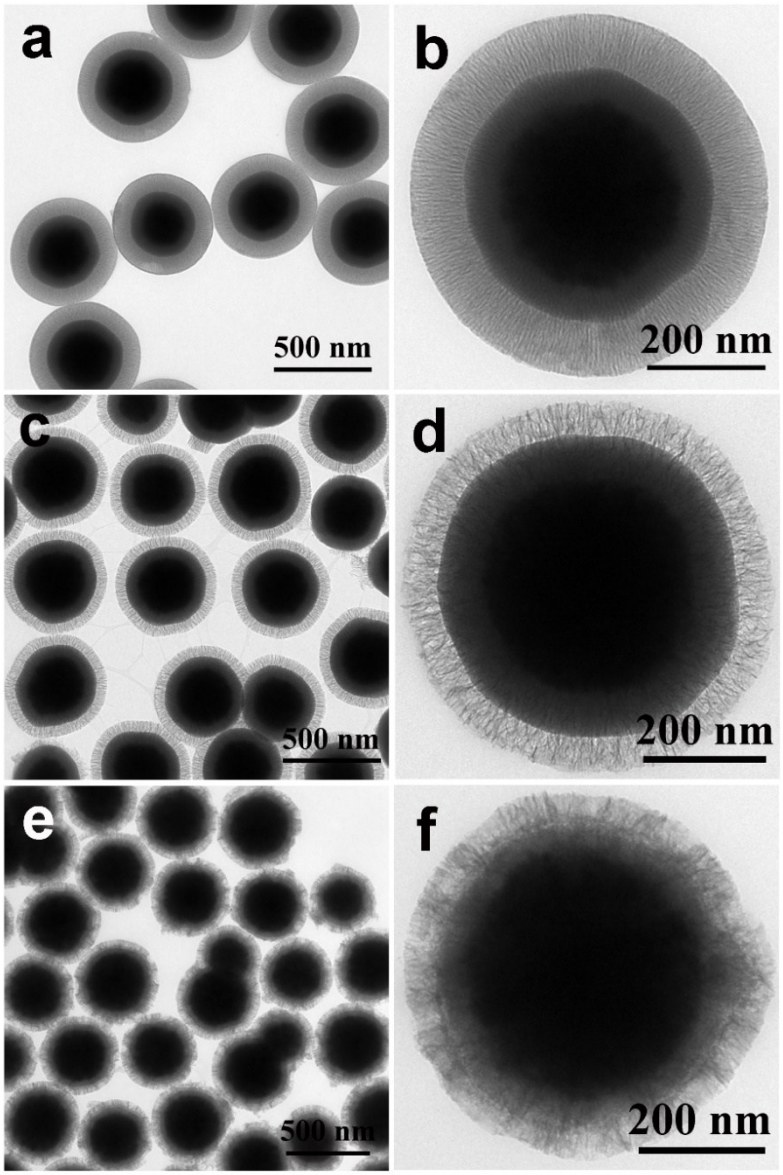
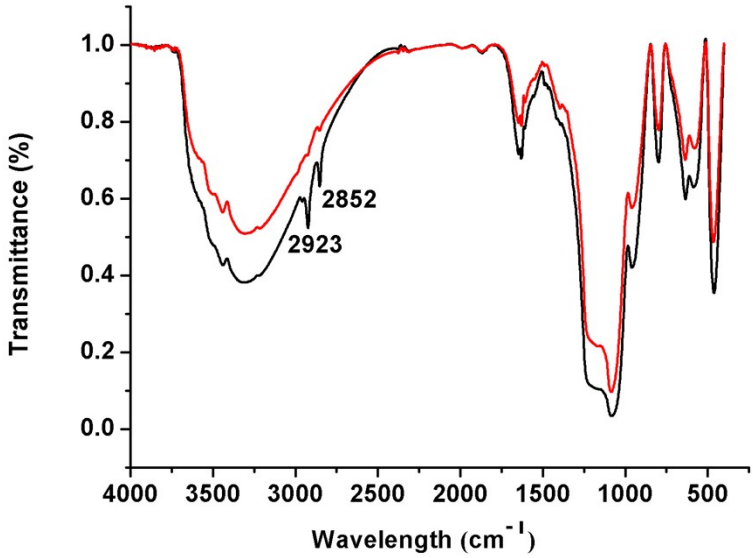



**Figure S3** TEM images of MMSM-1 (a, b), MMSM-5 (c, d), and MMSM-10 (e, f) synthesized with different CATC concentration of 1, 5, and 10 wt%, respectively.

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130 **Figure S4** FT-IR spectra of $Fe_3O_4$@n$SiO_2$@CTAC/$SiO_2$ microspheres without extraction
131 of HCl/ethanol (black) and MMSM-5IPA particles after extraction with HCl/ethanol for
132 twice (red).

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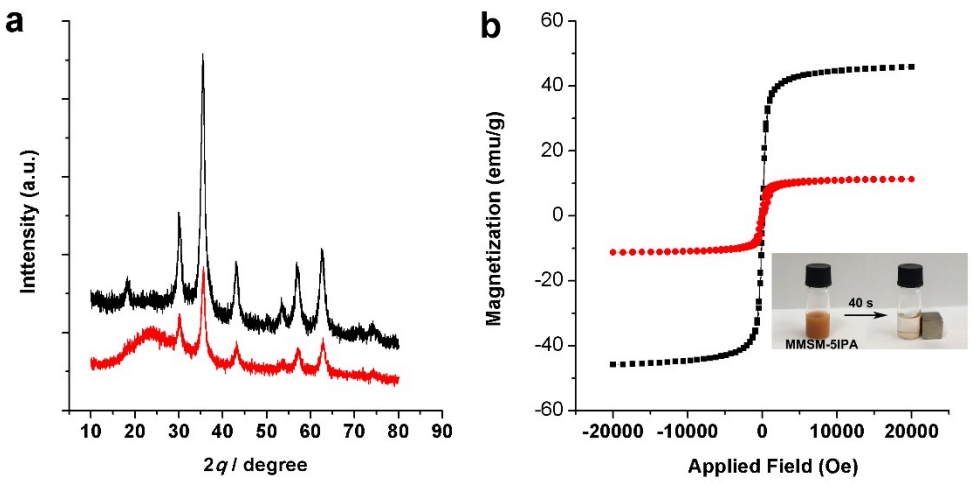


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136 **Figure S5** Wide-angle X-ray diffraction spectra (a) and magnetic hysteresis loops (b) of

137 $Fe_3O_4$ (black) and MMSM-5IPA (red). Inset is the separation process of the MMSM-5IPA

138 by a magnet.

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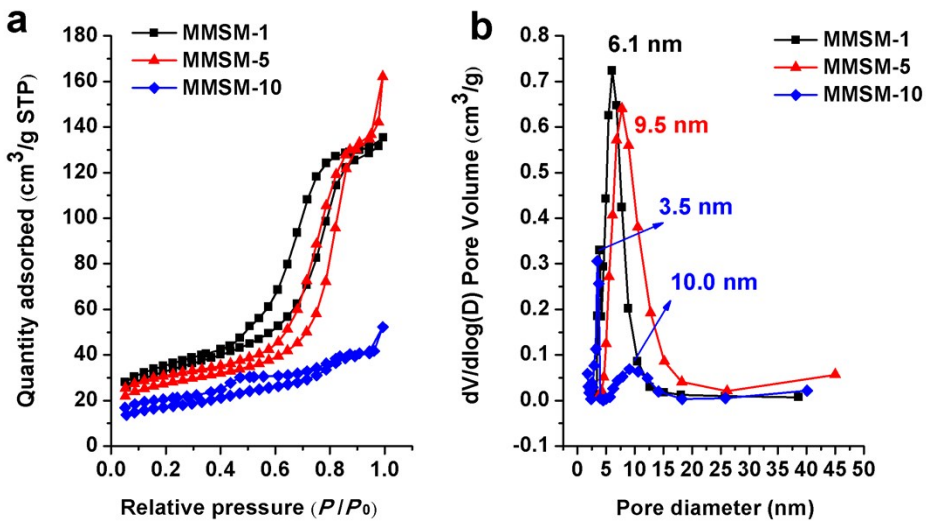


**Figure S6** Nitrogen adsorption-desorption isotherms (a) and pore size distribution curves (b) of MMSM-1, MMSM-5, MMSM-10 microspheres with different with different CATC concentration of 1, 5, and 10 wt%, respectively.

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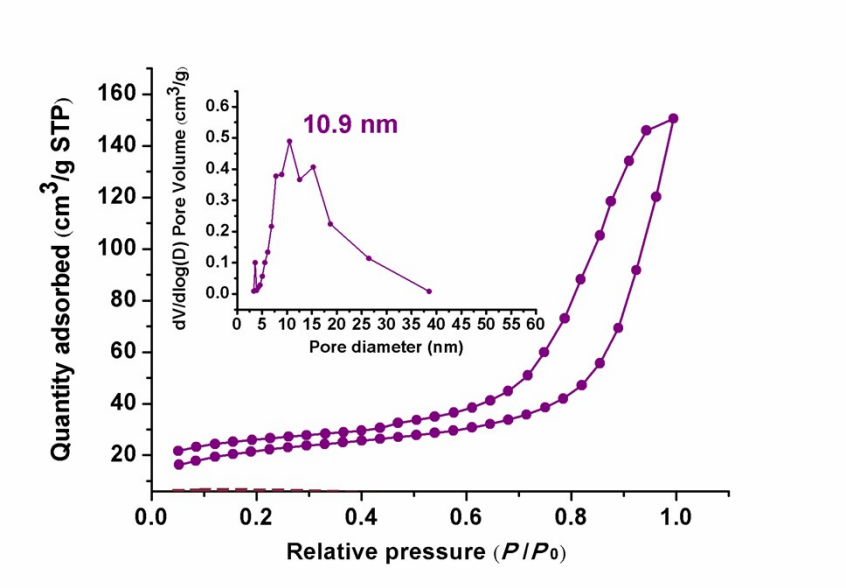


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149 **Figure S7** Nitrogen adsorption-desorption isotherms of MMSM-5IPA@PDA

150 microspheres synthesized in ethanol aqueous solution. Inset is pore size distribution curve.

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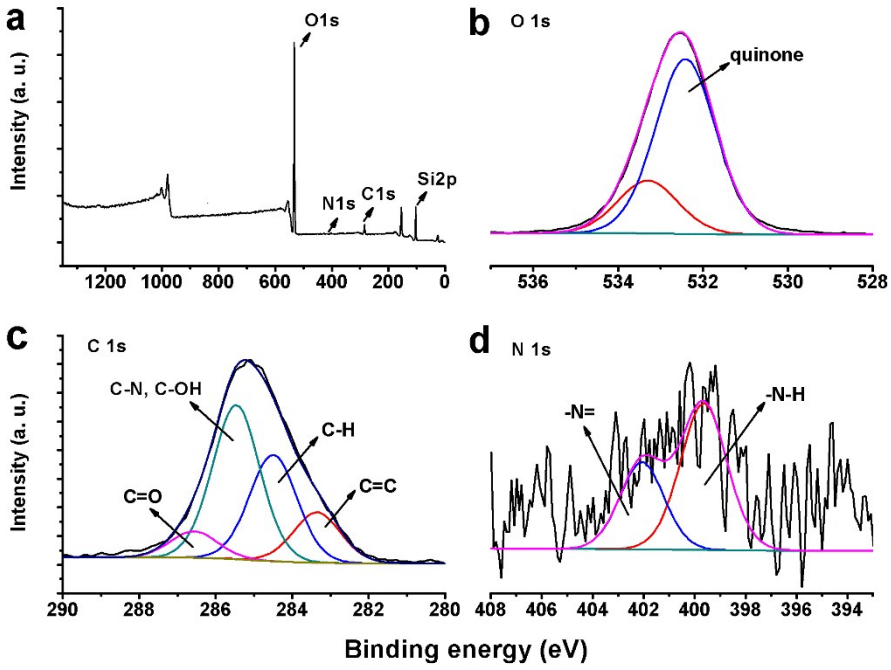


**Figure S8** XPS analysis of MMSM-5IPA@PDA survey scan (a) with high-resolution spectra of C 1s, O 1s, and N 1s. In O 1s spectra, peaks at 533.2 and 532.4 eV belong to quinone and catechol groups of PDA (b). The peaks in the C 1s spectrum at 286.4, 285.8, 284.5, and 283.4 eV correspond to C=O bonds (C-N and C-OH), C-H, and aromatic C, respectively (c). In the N 1s spectrum, the peak at 399.8 eV has a small shoulder at 402.2 eV, and was attributed to -NH- and -N= (d).

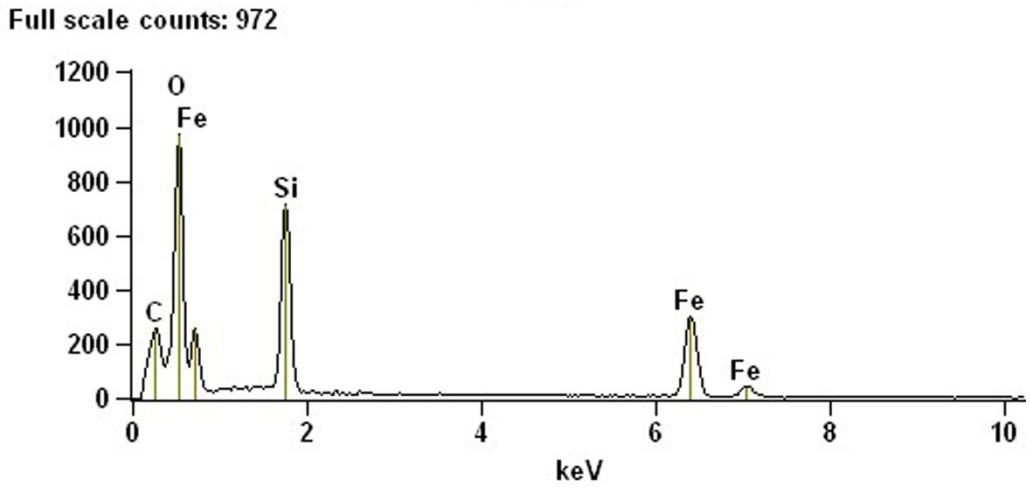


**Figure S9** EDX spectra of MMSM-5IPA@PDA.