Electronic Supplementary Information

Experimental section

Synthesis of MSPs

The MSPs are prepared according to the reported procedure [1]. The typical synthetic procedure is as following. 16 ml of *n*-hexane was mixed with 80 ml of H_2O under vigorous stirring for 30 min at 38 ^oC. A solution containing 4 g of triblock copolymer poly(ethylene glycol)$_{20}$-poly(propylene glycol)$_{70}$-poly(ethylene glycol)$_{20}$ (Pluronic P-123), 50 ml of H_2O and 21 ml of HCl (37%) was added stirring for 15 min. 10.5 ml of tetraethoxysilane (TEOS) was dropped into the suspension and stirred for 20 h. Then the mixture was aged at 100 ^oC for 24 h. Finally, the solid product was recovered, washed, dried, and calcined at 550 ^oC for 6 h.

Synthesis of fluorescein isothiocynante (FITC)-labeled insulin

The FITC labeled insulin was prepared according to the reported procedure [2]. 200 mg of insulin was dissolved in 50 ml of sodium carbonate buffer (0.1 M, pH 9), and 2.5 ml of FITC in DMSO (1 mg/ml) was added in 5 μl aliquots while gently stirring in dark for 2 h at 10 ^oC. Then NH_4Cl (2.5 ml, 1M) was added into the solution to quench the excess FITC. After stirring for another 1 h, the solution was dialyzed in phosphate buffered saline (PBS, pH 7.2) for 2 days and freeze dried to yield FITC labeled insulin (FITC-Ins).

Insulin storage

FITC-Ins was loaded to the MSPs by the immersion method. 80 mg of silica and 40 mg of FITC-Ins were dispersed into 8 ml of 0.01M HCl solution and gently stirred for 24 h at 10 ^oC in dark. After centrifugation, the precipitation was washed twice by PBS (pH 5.7) and frozen dried, named as Ins-MSPs. Here, the pH value of PBS is 5.7 because insulin is nearly insoluble in water in the pH value range from 4.5 to 6.5. All the upper clear supernatants were collected and determined of the insulin concentrations by measuring insulin absorbance at 276 nm using an UV spectrophotometer. The amount of loaded FITC-Ins in MSPs was calculated from the decrease in insulin concentration.

Fabrication of enzyme multilayers coated MPSs (EMC-MSPs)

The Ins-MPSs were firstly dispersed into 10 mg/ml poly(ethylenimine) (PEI, Mw 50-100 kDa) in PBS (pH 5.7) and gently stirred for 20 min. After being centrifuged and washed with PBS (pH 5.7), the particles were mixed with GA (0.025% in PBS at pH 5.7) overnight. After washing with PBS (pH 5.7), the particles were re-dispersed into 4 mg/ml CAT or GOD solutions in PBS (pH 7.2) for 10 h, followed by three washings with PBS (pH 7.2). The GA and enzyme were alternatively adsorbed to a desired number (*n*) of enzyme layers. The samples were stored in PBS (pH 7.2) at 4 ^{o}C and named as Ins-EMC$_n$-MSPs.

Activity assay of the system

For activity assay, GOD coated MSPs (GOD-MSPs) via the same method without insulin was constructed individually. To measure the activity, 2.4 ml of *o*-dianizidine dihydrochloride solution (1.7 mg in 25 ml of PBS at pH 7.0), 0.5 ml of β-D-glucose solution (0.55 M) and 0.1 ml of horse radish peroxidase (1.7 mg in 5 ml of H_2O with ice water cooling) were mixed at 25 ^{o}C. The reaction begins immediately after 0.1 ml of the sample solution was added, and the light absorption was measured at 436 nm with UV spectrophotometer. The control experiment on free GOD solution was also performed with the same GOD amount as that cross-linked in GOD-MSPs. The amount of GOD cross-linked in each layer was determined by comparing the absorbance of the solutions before and after cross-linking on UV spectrophotometer at 280 nm.

Insulin release

A certain amount of Ins-EMC$_n$-MSPs was dispersed in 10 ml of PBS (pH 7.2) at various glucose concentrations stirring gently at 37 ^{o}C. The released insulin amount was determined via centrifuging the suspension at defined time intervals and measuring the absorbance of the upper clear solution at 276 nm on UV spectrophotometer.

The insulin release study by stepwise glucose treatment was performed following the above method except that the glucose buffer solution (200 mg/mL) and

non-glucose buffer solution were introduced every 1 h alternatively.

Characterization

TEM (Field Emission Transmission Electron Microscopy) analysis was conducted on JEM 2100F electron microscope operated at 200 KV. SEM (Scanning Electron Microscopy) analysis was conducted on JEOL JSM6700F electron microscope. The confocal images were obtained on Olympus FV1000-IX81 confocal microscopy. The UV-vis absorbance spectra were obtained on Shimadzu UV-2550. Nitrogen adsorption-desorption and pore size distributions were obtained at 77 K on Micromeritics Tristar 3000 analyzer after the samples being pre-treated at 120 ^{o}C for dehydration. The surface areas and pore volumes were calculated by the Brunauer-Emmett-Teller (BET). The δ-potentials were measured on a Zetasizer, NICOMP^{TM} 380 ZLS (USA).

References

1. Y. Zhu, W. Shen, X. Dong and J. Shi, *J. Mater. Res.* 2005, **20**, 2682-2690.
2. Y. Zhao, B. G. Trewyn, I. I. Slowing and V. S. Y. Lin, *J. Am. Chem. Soc.* 2009, **131**, 8398-8400.

Table S1.Pore structure parameters of MSPs before and after the insulin storage.

	BET surface area (m^2/g)	BET Pore volume (cm^3/g)	BJH Pore size (nm)	Particle size range [a] (μm)
MSPs	482.5	1.1	12.1	1.0~2.0
Ins-MSPs	38.0	0.1	12.0	1.0~2.0

[a] The particle sizes were obtained from TEM.

Table S2. δ-potentials of MSPs, Ins-MSPs, and the PEI-modified Ins-MSPs (PEI-Ins-MSPs).

Materials	δ-potential (mV)
MSPs	-34.37
Ins-MSPs	-19.79
PEI-Ins-MSPs	+24.76

It can be concluded from the δ-potential results that the positively charged PEI was absorbed onto the negatively charged Ins-MSPs via electrostatic interaction.

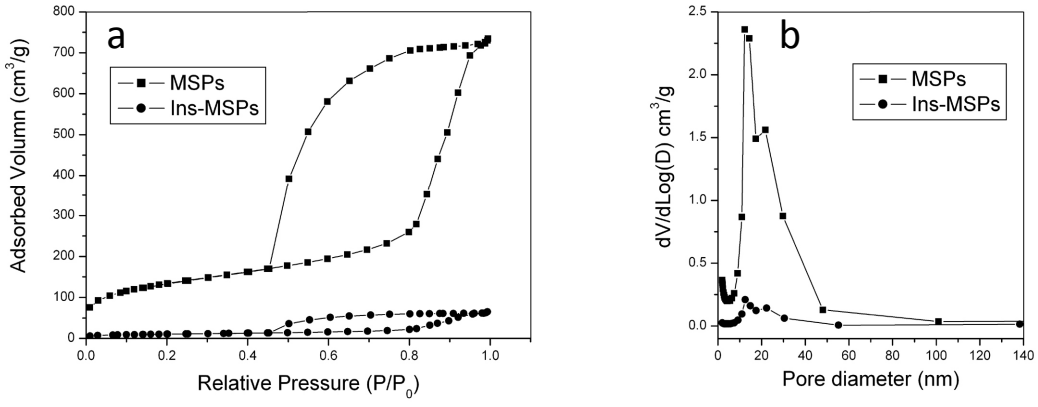

Figure S1. N_2 sorption isotherms (a) and the corresponding pore size distributions (b) of the MSPs before and after the insulin storage.

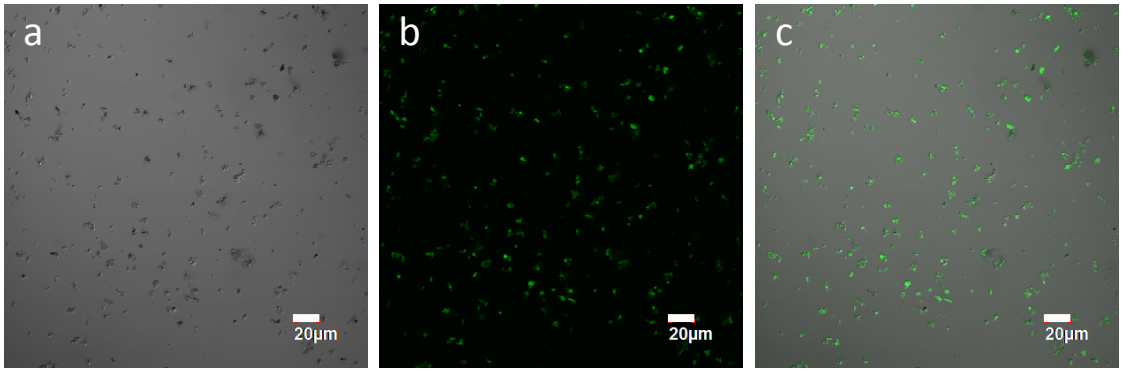

Figure S2. Confocal images of FITC-Ins-MSPs: (a) bright field image, (b) fluroscent confocal image, (c) merged image.

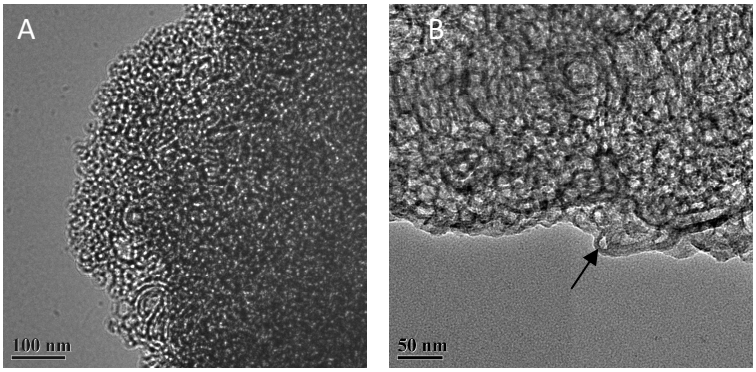

Fig. S3 TEM images of the Ins-MSPs before (A) and after (B) the coating of enzyme multilayers.

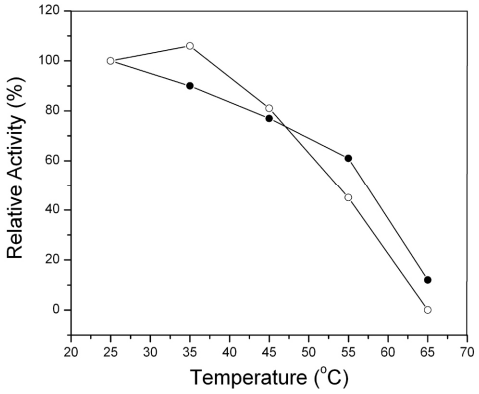

Fig. S4. Enzyme thermostability of EMC-MSPs (●) and free GOD (○).

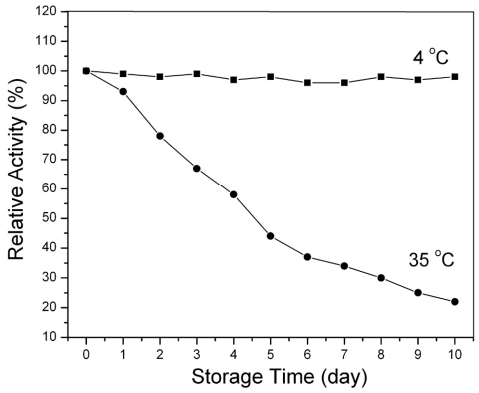

Fig. S5 Enzyme storage stability of EMC-MSPs at 4 °C (■) and 35 °C (●).

The samples were exposed to a certain temperature for 10 min, and immediately followed by an activity assay. Free GOD in solution (Fig. S4) shows a maximum activity at 35 °C and then a quick decrease at elevated temperatures, whereas the activity of GOD immobilized on EMC-MSPs shows a continuous decrease from its maximum at 25 °C at a significantly lower rate than free GOD. After incubated at

65 °C for 10 min, GOD immobilized on the particles still retains about 12% of its activity, while free GOD loses all its activity after the same treatment. These results are similar to those found for GOD alternatively deposited with polyelectrolyte on polystyrene particles via a layer-by-layer technique.[15] To evaluate the effect of storage time on the enzyme stability of EMC-MSPs, EMC-MSPs were incubated in PBS (pH 7.2) at 4 °C and 35 °C for designated time periods up to 10 days. Fig. S5 shows that the activity of EMC-MSPs keeps constant for 10 days at 4 °C. In contrast, the activity of that stored at 35 °C dropped continuously during the incubation and only 22% was left on the 10th day. The results demonstrate that the EMC-MSPs fabricated by the present approach should better be stored in relatively low temperature.