Supporting Information

Enzyme-responsive Nanocontainer As an Intelligent Signal-Amplification Platform for Multiple Proteases Assay

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Experimental Section

Materials: Tetraethylorthosilicate (TEOS), (3-aminopropyl)-trimethoxysilane (APTES), Rodamine B were purchased from Sigma-Aldrich. Glutaraldehyde and N-cetyltrimethylammonium bromide (CTAB) were obtained from Alfa Aesar. Glucose oxidase was purchased from Sangon Biotech Co. (shanghai, China). FAD was got from USB CO. (USA). Trypsin, protease K, chymotrypsin and human serum albumin (HSA) were purchased from Sigma-Aldrich (USA). All the chemicals were used as received without further purification. Nanopure water (18.2MΩ; Millpore Co., USA) was used in all experiments and to prepare all buffers.

The preparation of apo-GOX: The basic procedure for apo-GOX preparation was followed as previously described[1]. GOX (20 mg) was dissolved in 1 mL of 0.1 M PBS buffer (pH 6.4), and added into 20 mL of the as-prepared ammonium sulfate solution (Satd. $(NH_4)_2SO_4$ solution at 25°C was acidified to pH 1.4 with 7% H_2SO_4). The solution was mixed through stirring for 1h at 4°C and moved to centrifuge tube. The yellow supernatant was removed after centrifugation at 13000 rpm for 30 min at 4°C. The precipitate was then redissolved and neutralized by adding 2 mL of 2.5 M sodium acetate solution at 4 °C. The neutralized solution was subjected to one more cycle of acidified salt treatment, centrifugation and neutralization. The final product was dissolved in 0.1 M PBS buffer (pH 6.4). The concentration of apo-GOX was estimated by comparing the absorbance at 278 nm with the absorption of standard solution of GOX solution.

Synthesis of amino-modified MSNs: N-Cetyltrimethylammonium bromide (CTAB, 1.00 g, 2.74 mmol) was dissolved in 480 mL of deionized water. Sodium hydroxide aqueous solution (2.00 M, 3.50 mL) was introduced to the CTAB solution and the temperature of the mixture was adjusted to 353 K. Tetraethoxysilane (TEOS, 5.00 mL, 22.4 mmol) was added dropwise to the surfactant solution under vigorous stirring. The mixture was allowed to react for 2 h to give rise to a white

precipitate. This solid crude product was filtered, washed with deionized water and ethanol, and dried under high vacuum to yield the as-synthesized MCM-41. To remove the surfactant template (CTAB), 1.50 g of the as-synthesized MCM-41 was refluxed for 6 h in a methanolic solution of 1.50 mL HCl (37.2%) in 150 mL methanol. The resulting material was filtered and extensively washed with deionized water and ethanol. The surfactant-free MCM-41 material was placed under high vacuum with heating at 333 K to remove the remaining solvent from the mesopores.

MCM-41 (1.00 g) was refluxed for 20 h in 80.0 mL of anhydrous toluene with 1.00 mL (5.67 mmol) of 3-aminopropyltrimethoxysilane to yield the 3-aminopropyl-functionalized MSNs (NH_2-MSNs) material. The solid product was filtered, washed with anhydrous toluene, and ethanol, and dried in air.

Synthesis of CHO-MSNs and FAD-MSNs: CHO-MSN was synthesized using reported method with slight modification[2]. 100mg of NH_2-MSNs was dispersed in 10mL SSC buffer (25 mM sodium citrate, 150 mM NaCl, pH 7.4), 50 μL of glutaraldehyde (50%) was added and stirring at room temperature for 10h. The solid product was filtered, washed with water three times. Then it was redispersed in 10mL SSC buffer, followed by adding 25mg NH_2-FAD and stirring at room temperature for 24h. The final production was treated with $NaBH_3CN$ for 1h followed by washing with water.

Rhodamine B loading and dye release experiments: The loading of rhodamine B (RhB) inside the mesopores of FAD-MSNs was achieved as follows. The purified FAD-MSN was incubated in the phosphate-buffered saline (25 mM, 1 mM $CaCl_2$, 25 mL, pH=7.4) of RhB (5 mg) for 24 h at room temperature to reach loading saturation. Then the pore entrances of the MSNs loaded with RhB were capped upon adding apo-GOX with a final concentration of 1 mg/mL, allowing the interaction of construction GOX on the surface of MSNs. The protein-capped RhB-loaded MSNs were centrifuged and repeated washing with phosphate-buffered saline to remove RhB molecules and the uncapped apo-GOX from the exterior surface of the material. All the washing solutions were collected, and the loading of RhB was calculated from the difference in the concentration of the initial and left dyes in the Supernatant. The loading percentage was approximately 3.2% of mesoporous silica in weight. RhB-loaded GOX-MSNs (10 mg) material was dispersed in 25 mL of phosphate-buffered saline and the mixture was stirred slowly at 37 °C. Actually, most of the nanoparticles were located at the corner of container. Upon addition of protease, a sample (200μl)

was taken from the suspension at indicated time. The nanoparticles were separated from the sample by centrifugation at 3000 rpm for 0.5 min. The released dye in the supernatant was monitored via the absorbance band of the dye centered at 554 nm. Then the separated nanoparticles and dye solution were added back to original suspension for the next test. For protease detection, the measurement was performed at 90 min.

Measurements and Characterizations: FT-IR analyses were carried on a Bruker Vertex 70 FT-IR Spectrometer. X-ray measurements were performed on a Bruker D8 FOCUS Powder X-ray Diffractometer using Cu Ka radiation. TEM images were obtained with JEOL JEM-2010EX transmission electron microscope with a tungsten filament at an accelerating voltage of 200kV. N_2 adsorption–desorption isotherms were recorded on a Micromeritics ASAP 2020M automated sorption analyzer. The specific surface areas were calculated from the adsorption data in the low pressure range using the BET model and pore size was determined following the BJH method. UV-vis spectroscopy was carried out with a JASCO V-550 UV/vis spectrometer.

In the experiments, the MCM-41 particles were synthesized using a base-catalyzed sol-gel procedure. Low-angle XRD patterns of the as-prepared silica nanoparticles shows three low-angle reflections typical of a hexagonal array as presented in Fig. S1. The morphologies and microstructures of the MSN and protein-capped-MSNs are clearly revealed by SEM and TEM images. The synthesized MSNs showed the spherical nanoparticles with an average size of 100 nm in diameter (Fig. S2, S3). Highly ordered hexagonal array and streak structural features could be easily observed from the corresponding inserted HRTEM image. Compared with the uncapped MSN nanoparticles (FAD-MSNs), no clear difference in shape and average diameter was observed. However, a border appeared after anchoring the protein on the surface of the nanoparticles indicating the efficient grafting of apo-GOX to the nanoparticles as well as the blocking of the pores of the nanoparticles.

FTIR spectroscopy was used to monitor the surface functionalization of MSNs (Fig. S4). The emerging absorption band at around 1530 cm^{-1} in the sample NH_2-MSNs demonstrates the efficient functionalization of MSNs with amino group. Compared with NH_2-MSNs, there is a new absorption band at around 1700 cm^{-1} in the sample CHO-MSNs, which can be assigned to vibrations of the carboxyl groups contained within the attached glutaraldehyde molecules. After grafting FAD cofactor to MSNs, the band at 1700 cm^{-1} disappeared due to the reaction between

FAD-NH_2 and the aldehyde group on the CHO-MSNs. The efficient grafting of GOX onto mesoporous silica was validated by the appearance of an enhanced band at 1562 cm^{-1}, which is characteristic of amide vibration of proteins. Signals below 1500 cm^{-1} in all of the spectra can be mainly attributed to the silica framework. Changes in pore volume and diameter were investigated by nitrogen sorption experiments (Fig. S5). BET specific surface values, pore volumes, and pore size calculated from the N_2 adsorption-desorption isotherms for NH_2-MSNs, FAD-MSNs and GOX-MSNs are listed in Table S1. The adsorption-desorption isotherms of MSN-NH_2 showed a typical Type IV curve with a specific surface area of 1059 m^2g^{-1}, an pore diameter of 2.69 nm and a narrow pore distribution (Fig. S6). The silica particles functionalized with the FAD group (FAD-MSNs) showed the same type curve as NH_2-MSNs but with a little decline at the pore diameter and a obviously decline at pore volume, which indicated the formation of gatehandles. However, compared with FAD-MSNs, the pore size and the pore volume of GOX-MSNs-dye decreased dramatically. These results demonstrated that apo-GOX could be tightly attached on the nanoparticles and block the pores efficiently.

Reference

1. K Sugawara, N Kamiy, G Hirabayashi, H Kuramitz, *Electroanalysis*, 2006, **18**, 1001 - 1006
2. T Georgelin, S Bombard, J M Siaugue, V Cabuil, *Angew. Chem. Int. Ed.* 2010, **49**, 8897 –8901

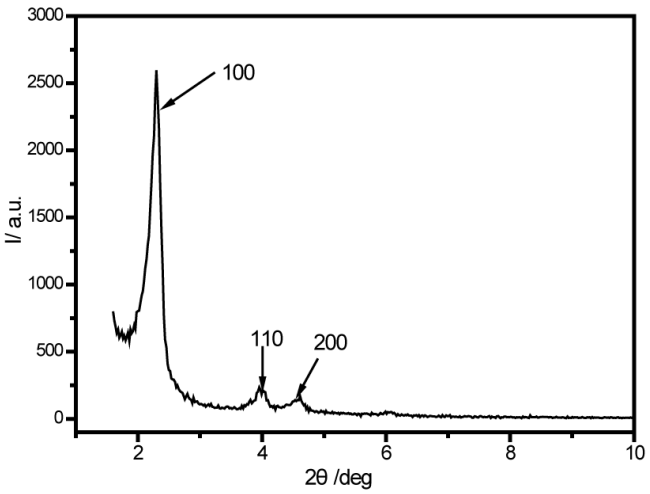

Fig. S1 Low angle X-ray patterns of the as-prepared MCM-41.

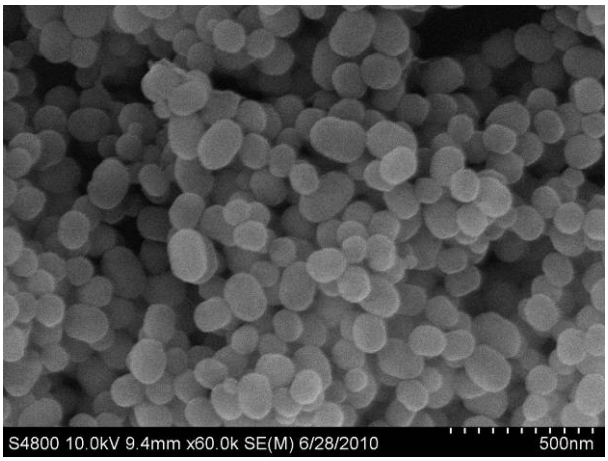

Fig. S2 SEM image of the as-synthesized MCM-41.

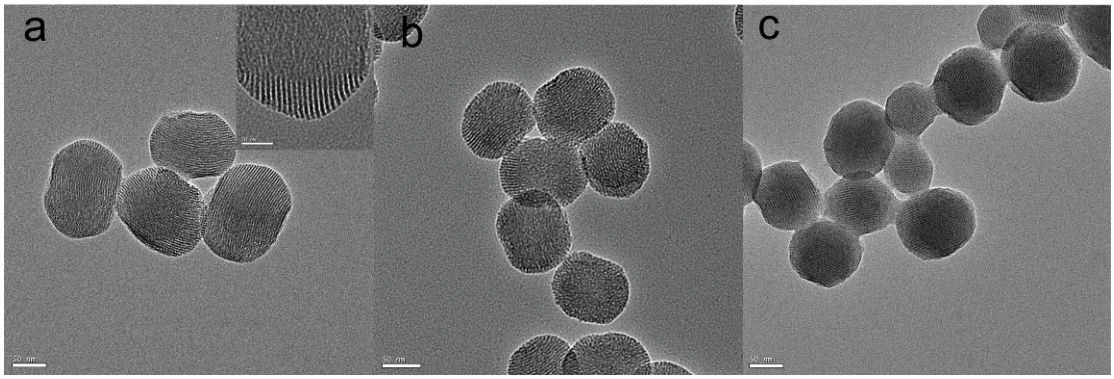

Fig. S3 TEM image of MSNs (a), FAD-MSNs (b) and protein capped MSNs (c).

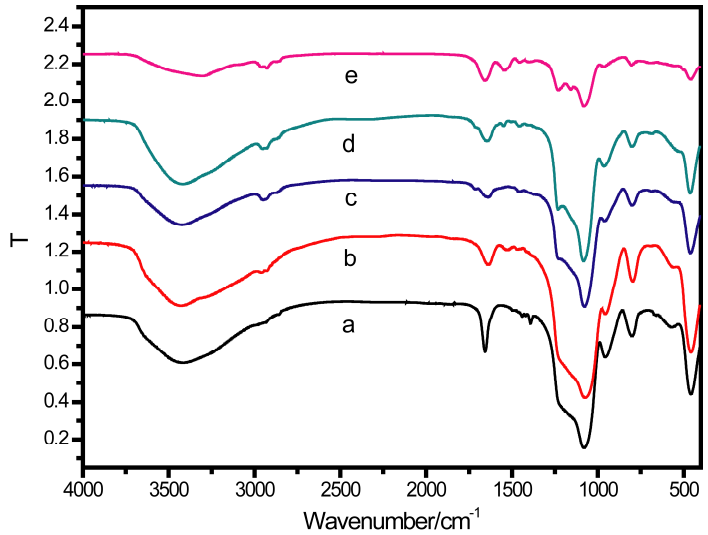

Fig. S4 FTIR spectra of the samples: (a) MCM-41,(b) NH_2-MSNs, (c) CHO-MSNs, (d) FAD-MSNs and (e) GOX-MSNs.(the spectra is shifted 0.4(n-1).)

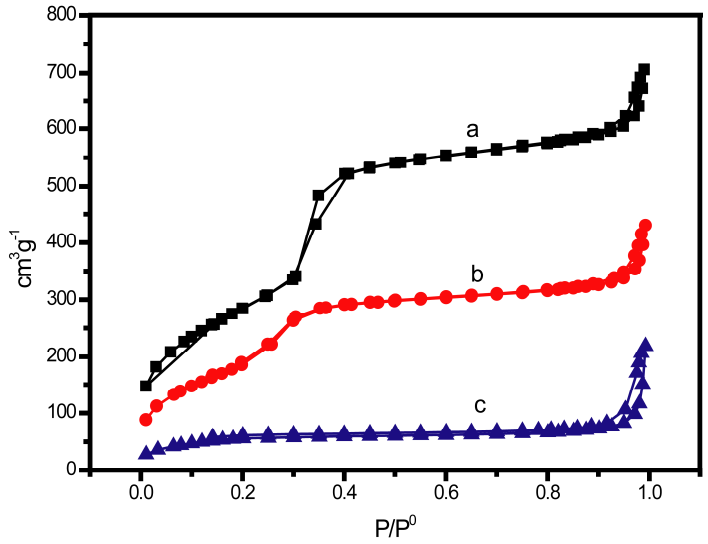

Fig. S5 N_2 adsorption and desorption isotherms of the samples (a) NH_2-MSNs, (b) FAD-MSNs,

and (c) RhB loaded GOX-MSNs.

Table S1 The pore diameter and pore volume of NH_2-MSNs, FAD-MSNs and RhB loaded GOX-MSNs.

	NH_2-MSNs	FAD-MSNs	GOX-MSNs
S_{BET} [m^2g^{-1}]	1059.20	839.06	181.75
Pore volume [cm^3g^{-1}]	0.96	0.55	0.15
Pore diameter [nm]	2.69	2.61	—

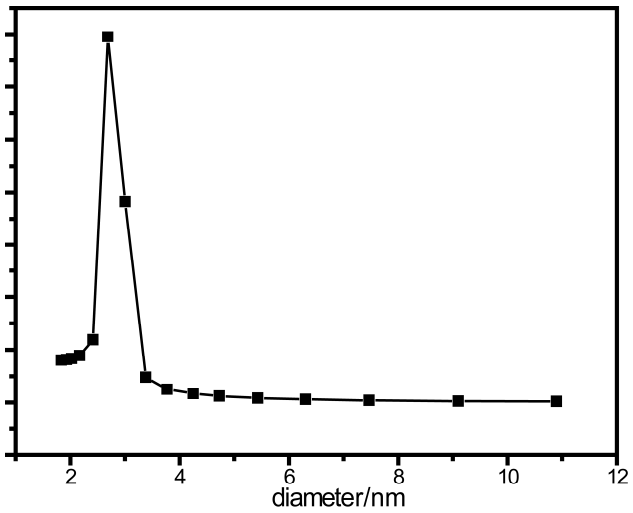

Fig. S6 Pore diameter distribution of NH_2-MSNs

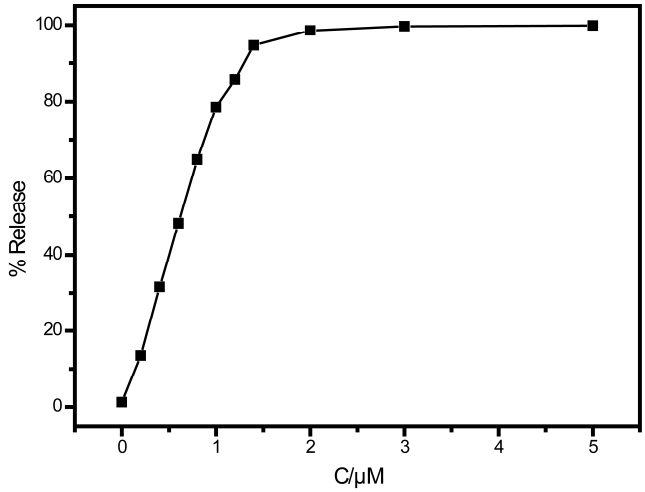

Fig. S7 The accumulated release amounts upon different concentrations of protease K.(the measurement is performed at 90min.)

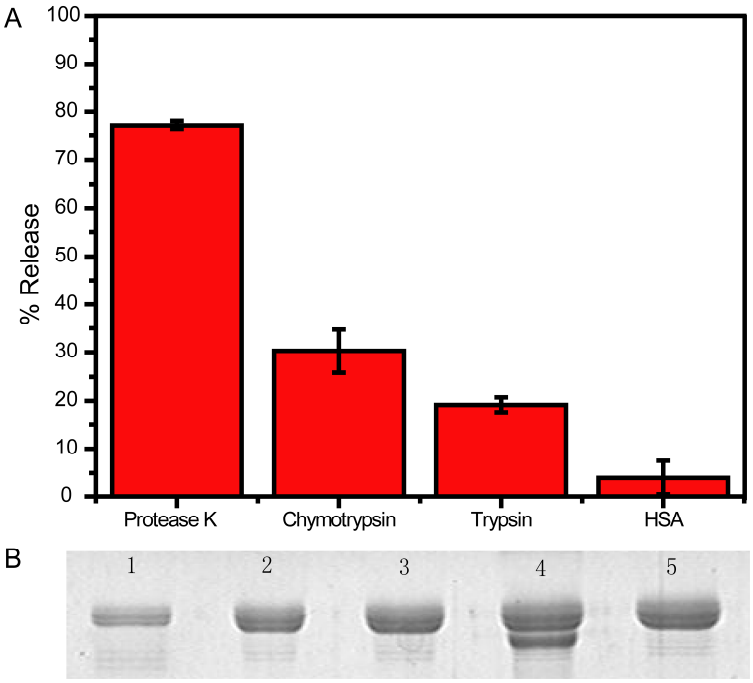

Fig. S8 A) The accumulated release amounts of RhB by different proteases at the concentration of 1 μM. B) gel electrophoresis of GOX upon different proteases at the same condition.(1) protease K, (2) chymotrypsin, (3) trypsin, (4) HSA, (5) Blank.

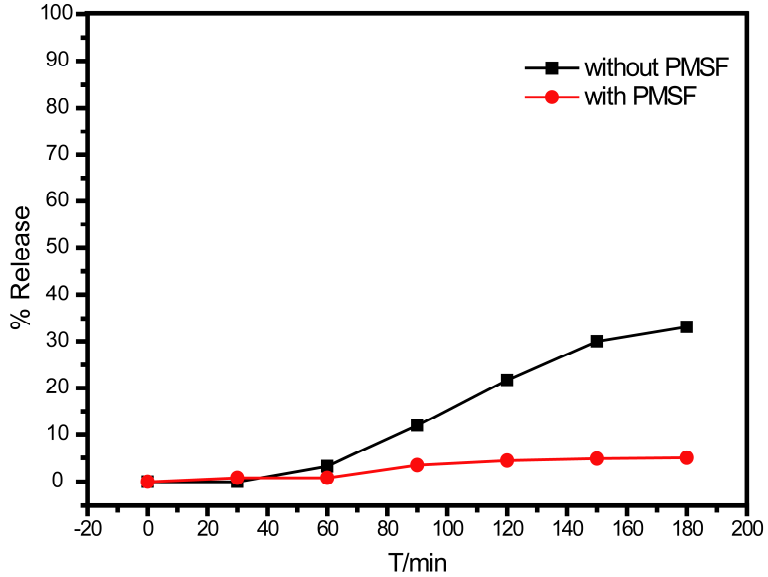

Fig. S9 Chymotrypsin induced release profile of rhodamine B from GOX-MSNs with and without inhibitor (PMSF).