Supporting information

In situ synthesis of porous silica nanoparticles for covalent immobilization of enzymes

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1. Experimental

Materials: TEOS (GR), APTS (GR), Triton X-100 (GR), catalase from bovine liver, urea (GR) and proteinase K were purchased from Sigma-Aldrich, USA. Cyclohexane (AR), n-hexanol (AR), acetone (AR), ammonia solution (GR), hydrogen peroxide (30%), and hexaammonium heptamolybdate tetrahydrate (AR) were obtained from Sinopharm Chemical Reagent Co., Ltd. EDC•HCl and NHS were obtained from GL Biochem (Shanghai) Ltd.

Covalent Encapsulation of Catalase in Silica NPs: Catalase was firstly functionalized with APTS by the conventional EDC/NHS method. Briefly, 1 mL of 10 mg/mL catalase was mixed with 2 mL of 50 mM phosphate buffer (pH 7.5). Then 40 mg EDC•HCl and 50 mg NHS were added to the mixture and stirred for 30 min. Finally, 500 μL of APTS was added to the mixture and stirred vigorously under the room temperature for 24 hours to complete the reaction. The APTS-functionalized catalase (APTS-catalase) was purified on a G-25 column (GE, USA) and concentrated by ultra-filtration with Millipore Amicon

Ultra-50 filters. The APTS-functionalization was checked on an anion-exchange column(HiTrap Q HP-1ml).

APTS-catalases were encapsulated in silica NPs following the common reverse micro-emulsion method. Typically, the water-in-oil microemulsion was prepared by mixing cyclohexane (7.50 mL), Triton X-100 (1.77 mL), n-hexanol (1.80 mL), concentrated (~100 mg/ml) APTS-catalase solution (300 μL), and TEOS (100 mL).Then 25% ammonia (60 μL) was added to system to initiate the polymerization. After stirring the mixture for 24 hours for the complete polymerization, acetone (20 mL) was added to break up the microemulsion and precipitate the catalase@silica NPs. The NPs were obtained by centrifugation at 12000 rpm and washed several times with ethanol and deionized water.

Catalase Activity Assay: Catalase activity was determined by the Góth method. [21] Briefly, 1 mL of hydrogen peroxide was catalyzed by adding 0.2 mL of catalase (0.5 μM) for 1 min at 37°C, and then terminated by adding 1 mL of ammonium molybdate (32.4 mM), and cooled to 25°C for absorbance measurement. Since ammonium molybdate reacts with the residual H_2O_2 to form primrose stable complexes, the catalase activity can be determined by the absorbance of this complex at 405 nm (on a HITACHI U-3010 Spectrophotometer). One unit of enzymic activity is defined as the amount of enzyme that decomposes 1 mmol H_2O_2/s. Results were expressed as relative activity to free catalase. Protease K digestion for both free and encapsulated catalase were carried out in 50 mM Tris-HCl buffer (pH 7.5) at 37 °C with the final concentration of protease K of 0.1 mg/mL. At predetermined time points, aliquots of sample were removed for immediate catalase activity assay.

TEM images were obtained on a JEM 200CX microscope (JEOL, Japan). Samples were prepared by drop-coating onto a carbon-coated 300 mesh copper grid and dried under room temperature. The enzyme loading of catalase@silica was calculated from the nitrogen content of the NPs, which was determined by a Vario EL-III elemental analyzer (Elementar, German). BET experiments were carried out on a Automated Surface Area & Pore Size Analyzer (QUADRASORB SI), and samples were dried at 70°C before the measurements.

2. Protease K digestion of catalase grafted on the surface of silica NPs

To demonstrate that catalase is encapsulated inside silica NPs, we prepared silica NPs covalently coated with catalase (in the above procedure, APTS-catalase was added after the formation of silica NPs). The silica surface-grafted catalase can be digested by protease K in about one hour, as shown in Fig. S1. It demonstrates immobilizing on the surface of silica NPs could not provide protection from protease K digestion.

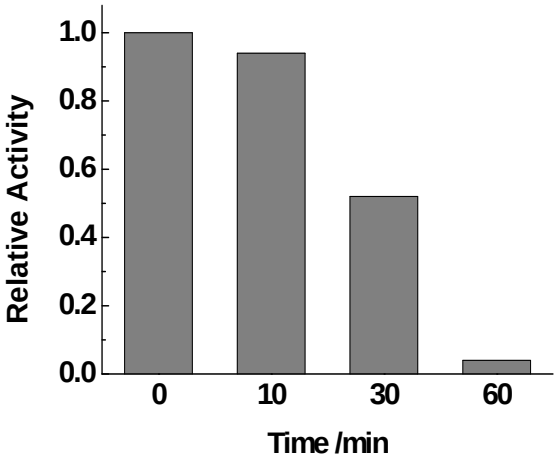

Fig. S1 The relative enzymatic activityof catalase grafted on the surface of silica NPs after protease K digestion for different times.