Supplementary Information

Nanoreactor：A Novel Platform for Protein Determination

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Experiments details

1. Reagents:

Thrombin aptamer probe (TBA: 5'-NH_2-$(CH2)_6$-TTTTTTTTTT***GGTTGGTGTGGTTGG***-3') used in the experiment was obtained from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). Thrombin from bovine plasma and 3,3',5,5'-Tetramethylbenzidine (TMB) were purchased from Sigma-Aldrich (St. Louis, MO). 3-glycidoxypropyltrimethoxysilane (GOPTS) was bought from Shanghai Aladdin Chemistry Co. Ltd.. all the other chemicals were of analytical reagent grade and were used as received without further purification. Solutions were prepared with deionized water processed with a Milli-Q ultra-high purity water system (Millipore, Bedford, MA, USA). Unless otherwise stated, all the reagents were prepared with deionized water.

2. Fe_3O_4 nanoparticle synthesis and function

2.1 Fe_3O_4 nanoparticle synthesis

Fe_3O_4 nanoparticles were prepared by a hydrothermal method.[1] $FeCl_3 \cdot 6H_2O$ (1.35 g, 5 mmol) was dissolved in ethylene glycol (40 ml) to form a clear solution, followed by the addition of NaAc (3.6 g) and poly(vinylpyrrolidone) (PVP-K30, molecular weight: 40 000) (1.0 g). The mixture was stirred vigorously for 30 min and then sealed in a teflon-lined stainless-steel autoclave (50 ml capacity). The autoclave was heated to and maintained at 200 °C for 8 h, and allowed to cool to room temperature. The black products were washed several times with ethanol and dried at 60 °C for 6 h.

2.2 Fe_3O_4 @SiO_2 nanoreactor synthesis

The as-prepared Fe_3O_4 nanoparticles (20 mg) were dispersed in a mixture containing 100 ml ethanol, 86.4 ml deionized water, 9.0 ml concentrated ammonia and 4.5 ml tetraethyl orthosilicate (TEOS) and stirred at room temperature for 6 h.[2] Subsequently, the resulted microspheres were washed several times with ethanol and deionized water and dispersed in ethanol (1.0 $mg.ml^{-1}$). The preparation of nanochannel-filled Fe_3O_4@SiO_2 core–shell nanoreactors were simply through etching treatment. Briefly, Fe_3O_4@SiO_2 core–shell nanoparticles were dispersed in 4 M hydrochloric acid (HCl), and allowed to stir for different times at room temperature. Then they were separated with external magnet and washed with water.

2.3 Fe_3O_4 @SiO_2 nanoreactor functionalization

According to the literature,[3] 5 mg as-synthesized nanoreactor dispersed in 50 ml ethanol solution (96%, V/V) and then 1 ml GOPTS was added. The mixture was oscillated and incubated at 37 °C for 6 h. After three-step separation by using an external magnet, the resulting product was dissolved in coupling buffer (0.1 M sodium carbonate, pH=10, 50 ml) and then reacted for another 24 h with TBA (10 μM, 450 μl) at 50 °C. Followed by the separation to remove the free TBA, the complexes were redispersed in coupling buffer and continued the reaction with cysteine (1 ml, 1 M) for at least 6 h to block excess epoxy groups. Wash the particles thoroughly with coupling buffer and dispersed in PBS buffer (10 mM 4 ml). The functionalized nanoreactor was kept under 4 °C for later use.

3. Nanoreactor's catalytic activity investegation

25 μl (0.5$mg.ml^{-1}$) nanoreactors with different etching times were added to microtubes separately. Each microtube was added with 380 μl NaAc-HAc buffer solution (0.2 M pH=4.0), 10 μl TMB solution (16 mM in ethanol) and 10 μl H_2O_2 (0.88 M in water) to form a homogeneous mixture. The mixtures were incubated immediately at 40 °C for 20 min. The UV–vis spectra were measured by a Cary 500 scan UV vis-NIR spectrophotometer (Varian) by monitoring the absorbance change at 656 nm.

4. Colorimetric detection of thrombin

The standard solution of thrombin was prepared in water. Different concentrations of thrombin were obtained by diluting standard solutions. The following procedure was used to study the concentration-dependent experiments: To detect thrombin, 20 μl functionalized nanoreactors, 10 μl KCl (2 M), 20 μl PBS (10 mM), and 50 μl different concentrations of thrombin were mixed

together and placed for 40 min at room temperature. After that, 380 µl NaAc-HAc buffer solution (0.2 M pH=4.0), 10 µl TMB solution (10 mM in ethanol) and 10 µl H_2O_2 (0.88 M in water) were sequentially added to the above mixture and placed at 40 °C for another 1 h. The absorbance were measured by UV vis-NIR spectrophotometer.

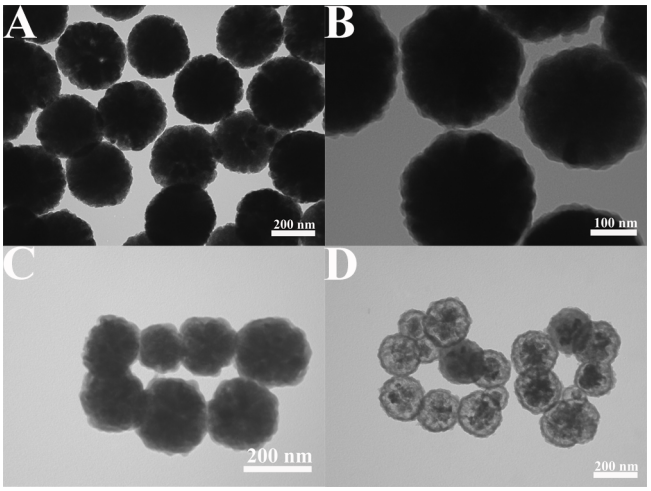

Fig. S1. TEM of as-prepared Fe_3O_4 nanospheres (A) and Fe_3O_4@SiO_2 core–shell nanoparticles (B). Fe_3O_4@SiO_2 core–shell nanoparticles after HCl etching for (C) 45 min and (D) 75 min.

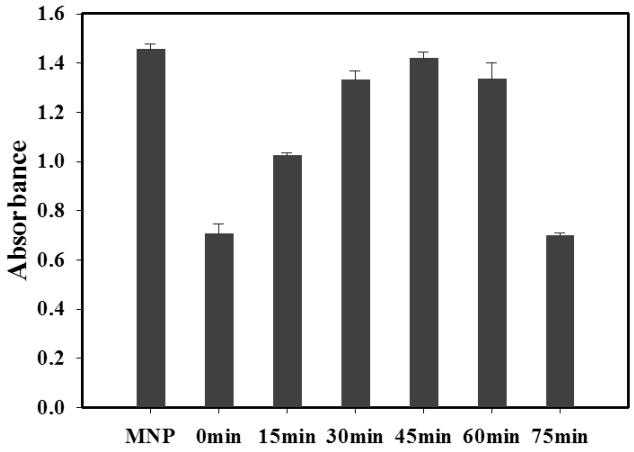

Fig. S2. The catalytic activity investigation of bare Fe_3O_4 nanospheres and Fe_3O_4@SiO_2 core–shell nanoparticles before and after HCl etching for 15 min, 30 min, 45 min, 60 min and 75 min.

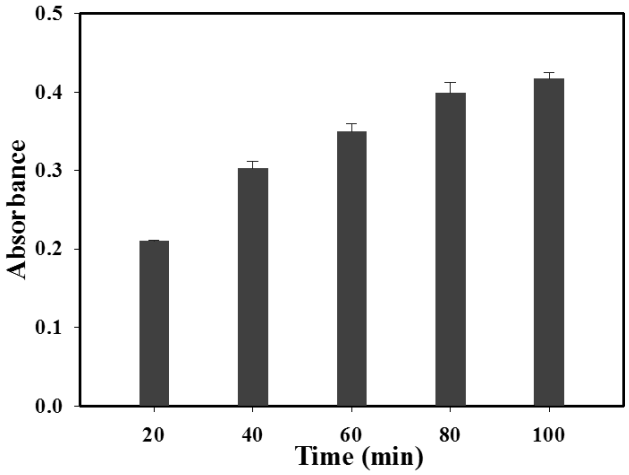

Fig. S3. The reaction time of catalysis in the presence of target. The catalytic reaction was allowed to maintain at 40 °C.

1. S. Guo, S. Dong E. Wang, *Chem. Eur. J.*, 2009, **15**, 2416.
2. L. Zhang, S. Guo S. Dong, *J. Biomed. Nanotechnol.*, 2009, **5**, 586.
3. G. T. Hermanson, *Bioconjugate Techniques*, 2008, 616.