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Fe_3O_4–Au@mesoporous SiO_2 microspheres: an ideal artificial enzymatic cascade system†

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Cite this: *Chem. Commun.*, 2013, **49**, 4643

Received 24th January 2013,
Accepted 29th March 2013

DOI: 10.1039/c3cc40622a

www.rsc.org/chemcomm

An artificial enzymatic cascade system with high activity and stability is engineered based on Fe_3O_4–Au@mesoporous SiO_2 (MS) microspheres, which possess both intrinsic glucose oxidase (GOx)- and peroxidase-mimic activities.

Enzyme mimics have attracted sustained attention because they possess many advantages compared with natural enzymes, such as low cost of preparation and purification, high stability to environmental conditions, and difficulty in degeneration and inactivation.[1] Recently, Fe_3O_4 magnetic nanoparticles (NPs), which were generally considered to be biologically inert, have been discovered to possess intrinsic peroxidase-mimic activity.[2] Afterwards, peroxidase-mimic activities were reported for CeO_2 NPs,[3] FeS nano-sheets,[4] V_2O_5 nanowires[5] and single walled carbon nanotubes.[6] Very recently, small Au NPs were found to exhibit glucose oxidase (GOx)-mimic activity. In the presence of oxygen, they can catalytically oxidize glucose and produce H_2O_2.[7] Enzyme mimics possess some properties similar to natural enzymes such as selectivity, specificity and high activity, and have been used to replace enzymes in biomedical applications, such as immuno-assays,[3] detection of DNA,[8] and other biological applications as well.[9]

Exploring the ''static'' enzyme mimics is not enough to simulate intelligent architectures, just like combining protein, nucleic acid and lipid alone is not sufficient to form a functional cell.[10] One desirable but challenging topic is the assembly of various nanomaterials together into ordered functional systems, a common phenomenon in nature, but much more difficult to achieve with artificial components. To mimic complex enzyme systems in nature, Willner *et al.* fabricated enzyme cascades by linking GOx and horseradish peroxidase (HRP) to the DNA scaffolds for biocatalysis.[11] The organized enzyme cascades were found to proceed more effectively than homogeneous mixtures of GOx and HRP, due to a high local concentration of the reactive components confined in the vicinity of biocatalysts.[12] This ordered enzyme system also inspired the synthesis of other organized biosystems for bio-applications.[13] Natural enzymatic cascade systems also have the intrinsic drawbacks similar to enzymes. Therefore, engineering of enzyme-mimic NPs for artificial enzymatic cascade systems is a logical implementation to simulate the biocatalysis system, which will be significant in bionics, biosensing and biomedicine applications. However, enzyme mimics have not been used to simulate complex enzyme systems, especially cascade enzyme-catalytic reactions. In addition, the enzyme-mimic NPs reported are limited by low activity and stability due to their heterogeneous distribution and easy aggregation during catalytic reactions.[14]

Herein, we report novel, discrete and monodisperse Fe_3O_4–Au@mesoporous SiO_2 (MS) microspheres with both high GOx- and peroxidase-mimic activities. The superparamagnetic Fe_3O_4 cores provide high peroxidase-mimic activity and make the artificial enzymatic system easily recyclable. On the surface of Fe_3O_4 cores, ultrafine Au NPs with high GOx-mimic catalytic activity are dispersed. Furthermore, Fe_3O_4–Au particles are encapsulated in MS shells to hinder the aggregation and keep them stable even under harsh conditions. Meanwhile, small active molecules are allowed to diffuse in and out of the MS shells.[15] Based on these functional units, the Fe_3O_4–Au@MS microspheres as robust nanoreactors can catalyze a self-organized cascade reaction, which includes oxidation of glucose by oxygen to yield gluconic acid and H_2O_2, and then oxidation of 3,3,5,5-tetramethylbenzidine (TMB) by H_2O_2 to produce a colour change. To the best of our knowledge, it is reported for the first time that both glucose oxidase- and peroxidase-mimic activities are combined in one nanoparticle to simulate the enzymatic cascade system without the help of natural enzymes.

The preparation of Fe_3O_4–Au@MS microspheres is described in Scheme 1. Firstly, the water dispersible superparamagnetic Fe_3O_4 particles were synthesized *via* a hydrothermal method

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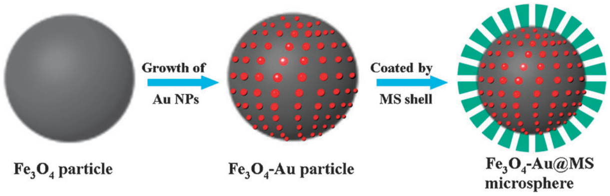
† Electronic supplementary information (ESI) available: Experimental details and relevant figures. See DOI: 10.1039/c3cc40622a



Scheme 1 The synthesis route to Fe_3O_4–Au@MS microspheres.

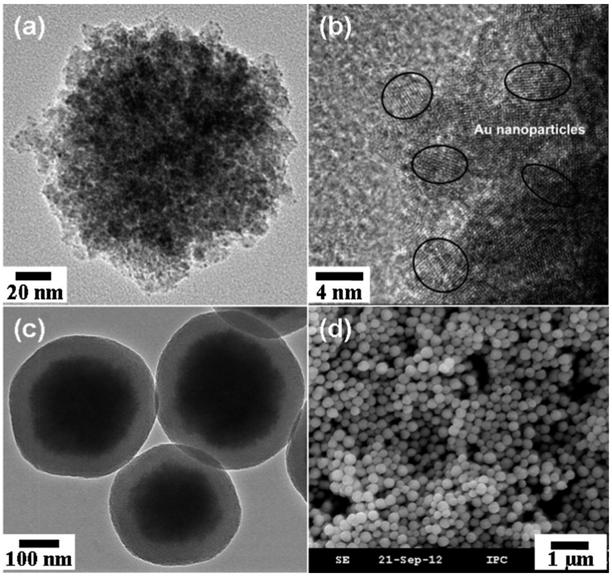


Fig. 1 (a) TEM image of Fe_3O_4–Au particles. (b) High-resolution TEM image of Fe_3O_4–Au particles. (c) TEM image of Fe_3O_4–Au@MS microspheres. (d) SEM image of Fe_3O_4–Au@MS microspheres.

described in the literature with minor modifications (see Experimental section in the ESI†).[16] Secondly, Fe_3O_4–Au particles with highly dispersed Au NPs on the surfaces were prepared by reducing auric chloride ions with $NaBH_4$ in the presence of Fe_3O_4 particles. Finally, through a surfactant-assembled sol–gel process with TEOS as the SiO_2 source and cetyltrimethylammonium bromide (CTAB) as the structure-directing agent, a mesostructured CTAB–SiO_2 composite layer was formed on the Fe_3O_4–Au particles. After the removal of the CTAB template by acetone extraction, Fe_3O_4–Au@MS microspheres were obtained.

Scanning electron microscopy (SEM) images show that the Fe_3O_4 particles have a nearly spherical shape and uniform size with a rough surface (Fig. S1a, ESI†). Transmission electron microscopy (TEM) images indicate that the obtained magnetite particles are loose clusters composed of nanocrystals with a size of about 7–10 nm (Fig. S1d, ESI†). Fourier transform infrared (FT-IR) spectra show that Fe_3O_4 particles are capped by citrate groups exhibiting excellent dispersibility in polar solvents such as water and ethanol, which favour the subsequent coating or modification with other NPs or polymers (Fig. S2, ESI†). For the preparation of Fe_3O_4–Au particles, the citrate groups on the surface of the magnetite particles act as stabilizing agents by providing anchoring sites to allow the formation of highly dispersed AuNPs. The TEM images of Fe_3O_4–Au particles obtained clearly show that their surfaces are evenly covered by ultrafine Au NPs (Fig. 1a and b). A wide-angle XRD pattern of the Fe_3O_4–Au microspheres shows the characteristic broad diffraction peaks corresponding to the cubic-phase Au and spinel Fe_3O_4 NPs, which further prove the attachment of Au NPs and the well-retained magnetite phase (Fig. S3, ESI†). According to the Debye–Scherrer formula, the sizes are calculated to be 4.2 and 7.2 nm for Au and magnetite nanocrystals respectively, in good agreement with the TEM observations. We have analyzed the content of Au in Fe_3O_4–Au particles using an energy dispersive spectrometer (Fig. S4, ESI†). The Au content is 6.54% (wt%).

To stabilize these Fe_3O_4–Au particles and keep their high enzyme-mimic activity during enzyme-catalyzed reactions, we intended to induce a MS layer on the surface of Fe_3O_4–Au particles in our study. The obtained Fe_3O_4–Au@MS microspheres (Fig. 1d) exhibit a more regular spherical shape with smooth surfaces due to the MS shells of the Fe_3O_4–Au@MS microspheres, compared with Fe_3O_4 particles (Fig. S1b, ESI†). N_2 sorption–desorption isotherms of the Fe_3O_4–Au@MS microspheres show representative type-IV curves, suggesting cylindrical pores with a narrow pore size distribution at 3.8 nm (Fig. S5, ESI†). The BET surface area is calculated to be as high as 251.8 $m^2\ g^{-1}$ (Fig. S5, ESI†). In addition, Fe_3O_4–Au@MS microspheres show a superparamagnetic behaviour according to the hysteresis loops (Fig. S6, ESI†). No remanence or coercivity was detected at room temperature as the Fe_3O_4 particles are composed of ultrafine magnetite nanocrystals, which have been proved by TEM and XRD. Fe_3O_4–Au@MS microspheres with superparamagnetic property can remain homodispersed in solution instead of aggregating, which is beneficial for diffusing substrates to interact with catalysts. By simply using a magnet, the Fe_3O_4–Au@MS microspheres can be completely separated from mixture within 1 min because of the high magnetite content (Fig. S6, ESI†). The excellent dispersibility, high stability and superparamagnetic properties of the Fe_3O_4–Au@MS microspheres make them an ideal material for liquid-phase catalysis.

In our study, we proved that Fe_3O_4–Au@MS microspheres possessed both intrinsic GOx- and peroxidase-mimic activities, and engineered an artificial enzymatic cascade system with high activity and stability based on this nanostructure. The peroxidase-mimic activity of Fe_3O_4–Au@MS microspheres in solution was evaluated first. It was found that the Fe_3O_4–Au@MS microspheres could catalytically oxidize TMB in the presence of H_2O_2, producing H_2O and the oxidation of TMB (oxTMB) with blue color [eqn (1)]:

$$H_2O_2 + \mathrm{TMB} \xrightarrow{Fe_3O_4-Au@MS} H_2O + \mathrm{oxTMB} \tag{1}$$

As shown in Fig. S7 (ESI†), a typical blue colour reaction was observed after the Fe_3O_4–Au@MS microspheres were added into the TMB–H_2O_2 solution (pH = 4.0) for 10 min. Removing the microspheres using an external magnetic field, we found that the major absorbance peaks of the reaction mixture appeared at 370 and 652 nm, which originated from oxTMB. Additional control experiments showed that neither H_2O_2–TMB nor Fe_3O_4–Au@MS microspheres/TMB could introduce any colour change (Fig. S7, ESI†). It proved that Fe_3O_4–Au@MS

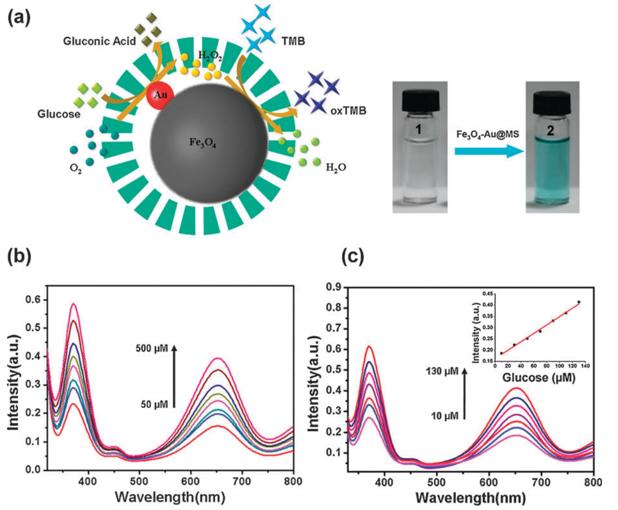


Fig. 2 (a) The principle of the artificial enzymatic cascade system based on Fe_3O_4–Au@MS microspheres, (b) typical H_2O_2 concentration response curves (pH = 4.0; reaction time was 10 min), (c) typical glucose concentration response curves (pH = 4.0; reaction time was 30 min). The linear range for glucose was from 1×10^{-5} to 1.3×10^{-4} mol L^{-1}.

microspheres exhibited intrinsic peroxidase-mimic activity. In addition, the relationship between the absorbance of oxTMB and H_2O_2 concentration was further studied. From Fig. 2b, we can see that the absorbance of the reaction mixture intensified with increasing amounts of H_2O_2.

It was reported that small Au NPs possessed intrinsic GOx-mimic activity and could catalyze the oxidation of glucose with the cosubstrate oxygen (O_2), producing gluconate and hydrogen peroxide (H_2O_2).[7] In our study, we suggested that Au NPs of Fe_3O_4–Au@MS microspheres would act as GOx [eqn (2)]:

$$\text{Glucose} + O_2 \xrightarrow{Fe_3O_4-Au@MS} \text{Gluconic acid} + H_2O_2 \quad (2)$$

We have already proved that the Fe_3O_4–Au@MS microspheres could act as effective peroxidase mimics. And H_2O_2 is the main product of the glucose oxidase (GOx)-catalyzed reaction. Therefore, a self-organized cascade reaction could be realized using Fe_3O_4–Au@MS microspheres only. The proposed principle for the artificial enzymatic cascade system is described as follows (Fig. 2a). In Fe_3O_4–Au@MS microspheres, glucose was oxidized by the cosubstrate oxygen (O_2) in solution catalyzed by Au NPs, yielding gluconic acid and H_2O_2. H_2O_2 was confined in the microsphere, and formed high local concentration. It was directly catalyzed by Fe_3O_4 to oxidize TMB, and had no time to diffuse. Owing to the oxidation of TMB, the blue color reaction occurred, demonstrating that an effective artificial enzymatic cascade system had been formed in solution. Control experiments indicated that neither glucose/TMB nor Fe_3O_4–Au@MS microspheres/TMB can lead to any color change (Fig. S7, ESI†). The effect of pH on the catalytic activity of this artificial enzymatic cascade system was investigated. The velocity of the cascade reaction was the highest at pH 4.0 (Fig. S8, ESI†). At this value of pH, it can be seen that the absorbance of the reaction mixture became stronger with the increasing concentration of glucose in a linear relationship from 10 to 130 μM (Fig. 2c) with a detection limit of 0.5 μM (Fig. S9, ESI†), after 30 min of reaction. It is proved that the activity and stability of Fe_3O_4–Au@MS are much higher than the mixture of Fe_3O_4 and Au particles (Fig. S10, ESI†). The activity and structure of Fe_3O_4–Au@MS microspheres can be maintained after 6 cycles of reaction. As control, serious aggregation of Au NPs on the surface of Fe_3O_4–Au particles occurred after only one cycle of reaction (Fig. S11, ESI†).

In summary, we have prepared Fe_3O_4–Au@MS microspheres, which possess both intrinsic glucose oxidase (GOx)- and peroxidase-mimic activities and can mimic the complexity and function of the enzymatic cascade system. Based on the color reaction caused by the artificial enzymatic cascade system, Fe_3O_4–Au@MS microspheres can be used to target and visualize tumor tissues as cell probes, detect biomolecules and catalyze biochemical reactions. This work can also inspire the studies for constructing various nanocomponents together into organized functional systems.

This work was supported by the National Natural Science Foundation of China (no. 51202260, 81171454, 61171049, 61178035, 81000667).

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