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Supporting Information

"Synthesis of Hybrid Fe_3O_4/silica/NiO Superstructures and their Application as Magnetically Separable High-Performance Biocatalysts"

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Synthesis and modification of the hybrid nanoparticles of Fe_3O_4/silica/NiO supersptructure

General consideration. Any reagent including $FeCl_3$, sodium oleate, oleic acid, Igepal® CO-520, cyclohexane, NH_4OH, tetraethylorthosilicate, oleylamine, $Ni(acac)_2$, trioctylphosphine oxide (TOPO), 3-aminopropyltriethoxy-silane (APTMS) and 2-[methoxy(polyethylenoxy)propyl]trimethoxysilane (MPEOPS) was used as purchased without any purification. Analyses of transmission electron microscopy (TEM) were conducted with JEOL JEM-2010. Scanning tunneling microscopy (SEM) was carried out with LEO SUPRA 55 (Carl Zeiss, Germany). Magnetic properties of nanoparticles were measured using superconducting quantum interference device (SQUID) magnetometer (Quantum Design, MPMS5XL), which is equipped with a 5 T superconducting magnet. X-ray diffraction patterns were obtained by using X-Ray Diffractometer (18kW) (Mac Science, Japan). UV absorption and fluorescence were observed by using V670 UV-Visible-NIR spectrophotometer (JASCO).

Silica shell deposition on Fe_3O_4 nanoparticle. Fe_3O_4 nanoparticles having 12nm of core size were synthesized through the previously reported procedure. Polyoxyethylene(5)nonylphenyl ether (8 ml, 17.4 mmol, Igepal CO-520, containing 50 mol% hydrophilic group, Aldrich) was dispersed in a round bottom flask containing cyclohexane (170 ml) by sonication. Next, 35 mg of Fe_3O_4 nanoparticles dispersed in cyclohexane were added to the reaction solution. The resulting mixture was vortexed until the mixture became transparent. An ammonium hydroxide solution (30 %, 1.3 ml) were added to the reaction mixture to form a transparent suspension. Lastly, tetraethylorthosilicate (3 ml,, TEOS) was added, and stirred for 12 hr. The resulting silica nanospheres containing Fe_3O_4 nanocores were collected by magnetic decantation. The collected nanospheres were redispersed in EtOH and recovered by using a magnet.

The dispersion of the nanosphere into EtOH suspension and magnetic separation was repeated three times for the purification.

Preparation of the hybrid nanoparticles of Fe_3O_4/silica/NiO supersptructure (HNP). 3-Aminopropyltriethoxy-silane (5 ml, APTMS) was added to the 85 ml of toluene suspension containing silica nanospheres containing Fe_3O_4 nanocores (336 mg) and stirred for 12 hr at room temperature. The resulting silica nanospheres functionalized with amine groups were collected by the centrifugation. The collected nanospheres were purified by repeating the re-dispersion into EtOH and centrifugation. For the assembly of NiO, 30 mg of the silica nanospheres containing Fe_3O_4 nanocores, which have amine groups at the surface, were mixed with NiO nanoparticles (10 mg) in a THF/$CHCl_3$ mixture suspension. The resulting particles were collected by the centrifugation at 3000 rpm and purified by repeating the re-dispersion in hexane and centrifugation twice. The isolated solids were heated up with 5 ºC/min heating rate in a furnace and annealed in air condition for 2 hrs at 400 ºC, resulting in the hybrid nanoparticles of Fe_3O_4/silica/NiO supersptructure (**HNP**).

Preparation of the PEG-modified hybrid Fe_3O_4/silica/NiO nanoparticle (PEG-HNP). HNPs and 2-[methoxy(polyethylenoxy)propyl]trimethoxysilane (MPEOPS) were mixed in an ethanol ssuspension. Then small amount of NH_4OH solution was added to the suspension to initiate the reaction and stirred for 12 hr. The resulting PEG-modified hybrid Fe_3O_4/silica/NiO nanoparticle (**PEG-HNP**) were recovered by using the magnet and washed twice with EtOH.

Enzyme immobilization and evaluation of their catalytic activities

Preparation of His-tagged epoxide hydrolase. The recombinant *Escherichia coli*

containing the epoxide hydrolase gene of *Rhodotorula glutinis* and plasmid pGro7 was cultured in LB medium with 50 μg/ml ampicilne and 20 μg/ml chloramphenicol. When the OD600 of the culture reaches 0.5 at 37^{o}C, the epoxide hydrolase gene was expressed by induction with 1 mM IPTG and 4 mg/ml L-arabinose. After 24 h incubation at 15^{o}C, the cells were harvested and then disrupted by sonication. The epoxide hydrolase enzyme was separated by using Ni-sepharose column.

Enzyme immobilization. For the immobilization of *R. glutinis* epoxide hydrolase, 100 μg of the epoxide hydrolase were added to 1 ml of 100 mM phosphate buffer containing 4.8 mg **PEG-HNP**s and then incubated for 30 min with mild shaking at 25 ^{o}C. After washing twice with buffer to remove unbound proteins, the immobilized epoxide hydrolase was used for the enantioselective hydrolysis reactions. The enzyme loading ranges were 10.9-13.0 μg/mg **PEG-HNP**s. The retention of the enzymatic activity after immobilization was determined by comparing the relative initial rates of the same amounts of free and immobilized epoxide hydrolases.

Repeated-batch reuse of the immobilized enzyme. Repeated reuse of the immobilized epoxide hydrolase was carried out in successive batch mode for the enantioselective hydrolysis of racemic styrene oxide for preparing enantiopure (*S*)-styrene oxide. After the completion of enantioselective resolution reaction, the immobilized epoxide hydrolase was recovered from the reaction medium by magnetic bar. For the next reuse, new reaction buffer with 20 mM racemic styrene oxide was added to the immobilized enzymes. After each reaction, the aqueous sample was extracted with the same volume of cyclohexane, and then the styrene oxide in the cyclohexane phase was analyzed by chiral GC.

Analyses. The enantiomeric excess (*ee*) and yield for enantiopure styrene oxide were determined by chiral GC with a fused silica capillary β-DEX-250 column (60 m length,

0.25 mm ID, and 0.25 μm film thickness, Supelco Inc.) and a FID detector. The column, injector, and detector temperatures were 100, 220, and 220 °C, respectively. The enantiomeric excess was determined by the following equation, *ee*(%) = [((*S*)-epoxide – (*R*)-epoxide)/((*S*)-epoxide + (*R*)-epoxide)] x 100. The amount of the released protein from the **PEG-HNP**s after the each reaction was determined by Bradford and micro bicinchoninic acid (BCA) protein assay method.