Catalytic Crosslinked Microparticles Self-Assembled from Enzyme-Nanoparticle Complexes

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Supporting Information

Materials and instruments

Lipase (*candida rugosa* lipase), dicyclopentadiene (DCPD), ruthenium-based 1st generation Grubbs' catalyst and Nile red were purchased from Sigma-Aldrich. Trichlorobenzene (TCB) was purchased from Fluka Chemicals. Compat-Able Protein Assay Kits and Coomassie blue (Bradford protein assay kit) were purchased from Thermo Fisher Scientific Inc. 8-Octanoyloxypyrene-1,3,6-trisulfonic acid trisodium salt (OPTS) was purchased from Markergene Technology Inc.

Zeta potential values were measured using a MALVERN Zetasizer Nano ZS instrument. Each sample was scanned three times and an average value was reported. Optical and fluorescence microscopy images were taken using an Olympus IX51 instrument and enzymatic activity assays were performed by plate fluorescence reader (SpectraMax M5). Confocal pictures were obtained on a Zeiss LSM 510 Meta microscope using 63X objective.

Nanoparticle preparation

Positively charged gold nanoparticles (AuNPs) were synthesized according to previous reports.[1] In brief, pentanethiol-coated AuNPs with a core diameter of 2 nm were synthesized using the Brust-Schiffrin two-phase method.[2] A Murray place-exchange reaction was used to obtain the quaternary ammonium functionalized AuNPs.[3] Oleic acid coated iron oxide nanoparticles with a core diameter of 10 nm were synthesized through the thermal

decomposition of $Fe(CO)_5$ in organic solvents and subsequent oxidation in air.[4]

Enzyme-immobilized microparticle (MP) preparation

0.72 μM of AuNPs functionalized with trimethylammonium tetraethylene glycol ligands were mixed with 0.36 μM of lipase in 200 μL of 5 mM phosphate buffer (pH 7.4). The oil phase was prepared through mixing 90 μL DCPD, 8 μL TCB and 2 μL of 5% Grubbs' catalyst in toluene. Immediately after preparing the oil phase, 5 μL of the oil phase was added to the mixture of lipase and AuNPs. After adding the oil phase, the solution was mechanically agitated via amalgamator. After 2 h, enzyme-immobilzed MPs were purified using a membrane bag.

Enzyme immobilization yield (enzyme quantification method):

The immobilization yield of the lipases was calculated by measuring the amount of residual enzymes in the solution of MPs using a Bradford protein assay. Free nanoparticles and any interfering substances were first removed using Compat-Able Protein Assay Kit and the calibration with a standard sample was carried out.

Enzyme activity assay

Enzyme activity assays in a 5 mM buffer solution were performed using OPTS as a substrate and fluorescence of products were analyzed over time using plate fluorescence reader ($\lambda_{ex}/\lambda_{em}$: 460/510 nm). The molarity of the OPTS was 0.2 M and calculated ionic strength was 1.2 M, 6-fold higher than that of 100 mM phosphate buffer. 4-nitrophenyl acetate was used as the substrate for enzyme activity assays in the mixture of acetone and water and the absorbance of products was observed at 450 nm. Washing processes were performed before the repeated reuse of biocatalysts as shown in **Figure S2**. The MPs and

enzyme-immobilized MCs in 100 μL water phase were washed by 4 L of water for 4 hour using 0.2 μm pore-sized membrane bag.

Figure S1. IR spectrum of enzyme-immobilized MPs, DCPD and lipase

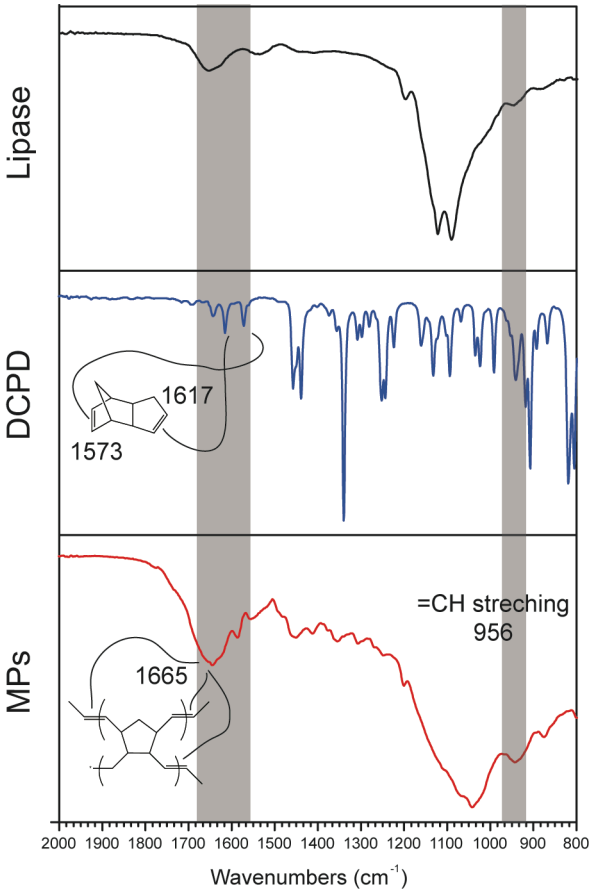
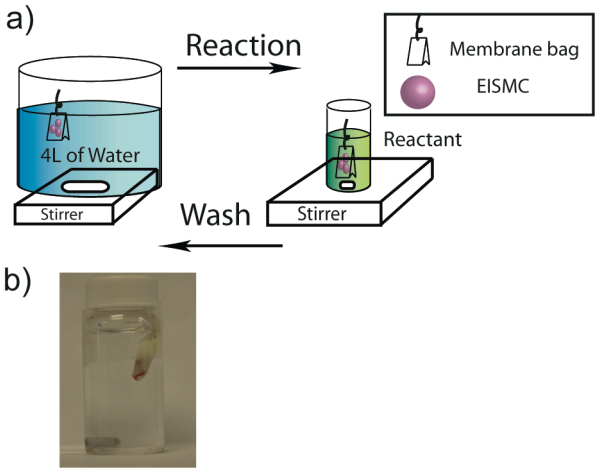


Figure S2. a) Schematic illustration of the repeated washing processes and enzyme activity assays of MPs for recycling test b) picture of 0.2 μm pore size membrane bag including the MPs



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