Supporting Information

Robust Enzyme-Silica Composites Made from Enzyme Nanocapsules

Materials

N-acryloxysuccinimide, acrylamide (AAM), N,N'-methylenebisacrylamide (BIS), ammonium persulfate (APS), tetramethylethylenediamine (TEMED), HEPES, sodium acetate, acetic acid, sodium phosphate monobasic monohydrate, sodium phosphate dibasic, Pluronic®P123, tetramethyl orthosilicate (TMOS), 1,4-bis(triethoxysilyl)benzene (BES), (3-aminopropyl) trimethoxysilane (AMS), paraoxon, 4-nitrophenyl laurate, glucose, 3,3',5,5'-tetramethylbenzidine (TMB), bicinchoninic acid (BCA), tartaric acid, $CuSO_4$, dimethyl sulfoxide (DMSO), sodium dodecyl sulfate (SDS), lipase from thermomyces lanuginosus, horseradish peroxidase (HRP) and glucose oxidase (GOX) from Aspergillus niger were purchased from Sigma-Aldrich and were used as received. N-(3-aminopropyl) methacrylamide was purchased from Polymer Science, Inc.

Instruments

Fourier Transformed Infrared Spectroscopy (FT-IR) was acquired with JASCO FT/IR-420 spectrometer. TEM images were obtained on a Philips EM-120 TEM instrument. Particle size and zeta potential were measured with Zetasizer Nano-ZS (Malvern Instruments Ltd., UK). UV-Visible adsorption was acquired with a Bechman Coulter DU®730 UV/Vis Spectrophotometer. Fluorescence intensities were measured with a Tecan GENios Multifunction microplate reader. N_2 adsorption–desorption isotherms were obtained on an ASAP 2020 pore analyzer at 77 K under continuous adsorption condition. Brunauer-Emmett-Teller (BET) and Barrett-Joyner-Halenda (BJH) methods were used to determine the surface area, pore size distribution.

Characterizations

DLS measurement: DLS experiments were performed with a Zetasizer Nano-ZS (Malvern Instruments Ltd., UK) equipped with a 10-mW helium-neon laser (λ = 632.8 nm) and thermoelectric temperature controller. Measurements were taken at 173° scattering angle. The samples are in a pH 8.5 50 mM HEPES buffer with a protein concentration of 0.5 mg/mL at 25 °C.

TEM measurement: TEM images were obtained on a Philips EM-120 transmission electro microscopy. For nanocapsules imaging, 10 µL 0.5 mg/mL nOPH is dropped on a copper grid. After 2 min, the solution is drawn off from the edge of the grid with filter paper. 5 µL of 1% pH=7.0 phosphotungstic acid (PTA) solution was immediately added on top of the grid. After another 5 min, the grid is washed 3 times with DI-water and allowed to dry in air. The grid is then stored for TEM observation. For enzyme-silica composite imaging, nOPH-silica suspension was dried on a copper grid and directly used for TEM observation without further staining. TEM images are acquired with an acceleration voltage of 120 kV.

Preparation

OPH expression and purification: OPH from *Pseudomonas diminuta* was expressed by E. coli strain BL21 carrying pET28-derived expression vector. Bacterial cells were grown in a LB and $CoCl_2$ were added at the induction step to a final concentration of 1 mM. Subsequent enzyme extraction were purified by gel filtration with Sepharose® 6B (Sigma Aldrich) and ion exchange with DEAE–Sephadex® (Sigma Aldrich) at 4 °C in 50 mM HEPES buffer (pH 8.5) according to the method described by Omburo[1].

EGFP expression and purification: EGFP was expressed by E. coli strain BL21 carrying pET28-derived expression vector with His-tag on C-terminal. Bacterial cells were grown in a LB and EGFP extraction were purified by Hispur™ Ni-NTA column (Thermo Scientific) and further desalted on a G25 desalting column (GE Healthcare).

Preparation of nanocapsules: OPH was modified by N-acryloxysuccinimide at a molar

ratio of 1 : 5 in 50 mM HEPES buffer at pH 8.5. The acryloylation reaction took place at 4 °C for 2 hours. Acryloylated OPH was further reacted for 4 hours at 4 °C with monomers acrylamide (AAM) and N-(3-aminopropyl) methacrylamide hydrochloride (APM), crosslinker N,N'-methylenebisacrylamide (BIS), initiated by ammonium persulfate (APS) and tetramethylethylenediamine (TEMED). The molar ratio of acryloylated OPH : AAM : APM : BIS was 1 : 2500 : 500 : 200. The resulted nanocapsules were dialyzed against 50 mM HEPES buffer at pH 8.5 and further purified with Sephadex G-75 to remove the unreacted monomers, initiators and enzymes. Lipase, EGFP and GOX nanocapsules were synthesized in similar method. Briefly, lipase, EGFP and GOX were dialyzed against 10mM phosphate buffer at pH 7.0 and acryloylated by N-acryloxysuccinimide at molar ratio of 1 : 5. Subsequently, AAM, APM and BIS were introduced and initiated by APS and TEMED at the same molar ratio as preparation of nOPH. The resulted nLipase, nEGFP and nGOX were purified by dialysis and Sephadex G-75.

Synthesis of enzyme-silica composites: 0.2 g of P123 was dissolved in 10 ml of phosphate buffer (pH = 4.7) at room temperature until the solution became transparent, and then nanocapsules were added. After stirring for 0.5 h, 0.278 mL tetramethyl orthosilicate (TMOS) was added and stirred for 24 h. The resulted precipitates were centrifuged and washed with 50mM pH 8.5 HEPES buffer. The products were then extracted in ethanol/buffer solution for 24 h to remove the P123. The synthesis of the nanocomposites from other silica precursors, 1,4-bis(triethoxysilyl)benzene (BES) and (3-aminopropyl) trimethoxysilane (AMS), was conducted in the same approach by replacing 20% molar ratio of TMOS to BES or AMS. nLipase-silica, nEGFP-silica and nGOX-silica nanocomposites were prepared by same method with the same amount of P123 and precursors. The resulted precipitates were centrifuged, washed with 10mM pH 7.0 phosphate buffer. The products were then extracted in ethanol/buffer solution for 24 h to remove the P123.

Protein concentration, activity and stability

BCA protein content quantification: All the protein content in solution was determined by bicinchoninic acid (BCA) colorimetric protein assay. Briefly, a tartrate buffer (pH 11.25) containing 25 mM BCA, 3.2 nM $CuSO_4$, and appropriately diluted protein/nanocapsules was incubated at 60 °C for 30min. After the solution was cooled to room temperature, absorbance reading at 562 nm was determined with a UV-Vis spectrometer. OPH, lipase, EGFP and GOX solutions with known concentration were used as standards. For the protein content in the enzyme-silica nanocomposites, the difference between the total amount of protein/nanocapsules added and the amount of protein/nanocapsules remained in the supernatant after precipitation was used as the protein content in the composites.

OPH activity assay: 10 μl of paraoxon (75 mM) solution in DMSO was added to 1.0 ml of HEPES buffer solutions (50 mM, pH 8.5) containing the native enzyme, nanocapsule and enzyme-silica composites with same amount of OPH respectively. The mixture was placed in a quartz cuvette and the absorbance change was monitored at 405 nm under room temperature. One unit is defined as hydrolysis reaction to release 1.0 μmol 4-nitrophenol per minute.

Lipase activity assay: 100 μl of 4-nitrophenyl laurate (10 mM) solution in DMSO was added to 1.0 ml of phosphate buffer solutions containing the native lipase, the nanocapsule and enzyme-silica composites with the same amount of lipase respectively. The mixture was placed in a quartz cuvette and the absorbance change was monitored at 405 nm under room temperature. One unit is defined as hydrolysis reaction to release 1.0 μmol 4-nitrophenol per minute.

EGFP fluorescence test: All fluorescence spectra were recorded on Tecan GENios Multifunction microplate reader at room temperature. 488 nm and 535nm were chosen as the excitation and emission wavelength respectively.

GOX activity assay: Glucose, TMB and HRP were dissolved in sodium acetate buffer (50mM, pH 5.1) at a final concentration of 1.72%, 0.5 mM and 0.01mg/ml respectively. The native enzyme, the nanocapsules and enzyme-silica composites with the

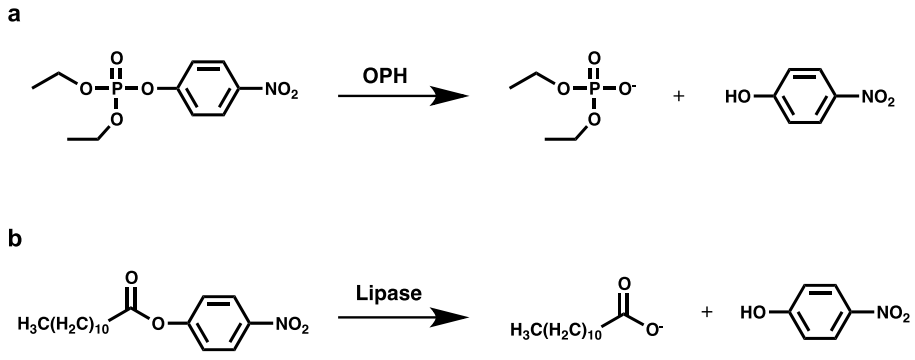
same amount of GOX were added to the assay solution respectively. The mixture was placed in a quartz cuvette and the absorbance change was monitored at 655 nm under room temperature. One unit is defined as oxidation of 1.0 μmole of β-D-glucose to D-gluconolactone and H_2O_2 per minute.

Thermal and organic solvent stability assay for OPH: For thermal stability, activities of native OPH, OPH nanocapsules and nOPH-silica were measured after incubation at 55 °C for 1-4 h. For organic solvent stability, activities of native OPH, nOPH and nOPH-silica incubated in the solutions with different volume fractions of DMSO were measured. The activity was expressed in the percentage relative to the initial activities.

Thermal stability activity assay for lipase: Activities of native lipase, lipase nanocapsules and nLipase-silica at different temperatures were measured in buffer solutions at pH 7.0 from 30 to 90 °C. The activity was expressed in the percentage relative to the maximal activity value at 40 °C.

Thermal and SDS stability assay for EGFP: For thermal stability, fluorescence of native EGFP, EGFP nanocapsules and nEGFP-silica were measured after incubation at 75 °C for 1-4 h. For surfactant stability, activities of native EGFP, nEGFP and nEGFP-silica incubated in the solutions with 0.5% SDS at 55 °C were measured. The fluorescence intensity was expressed in the percentage relative to the initial fluorescence intensity.

Supplemental figures



Scheme S1. Schematic illustration of a) OPH catalyze the hydrolysis of paraoxon; b) lipase catalyze

the hydrolysis of 4-nitrophenyl laurate

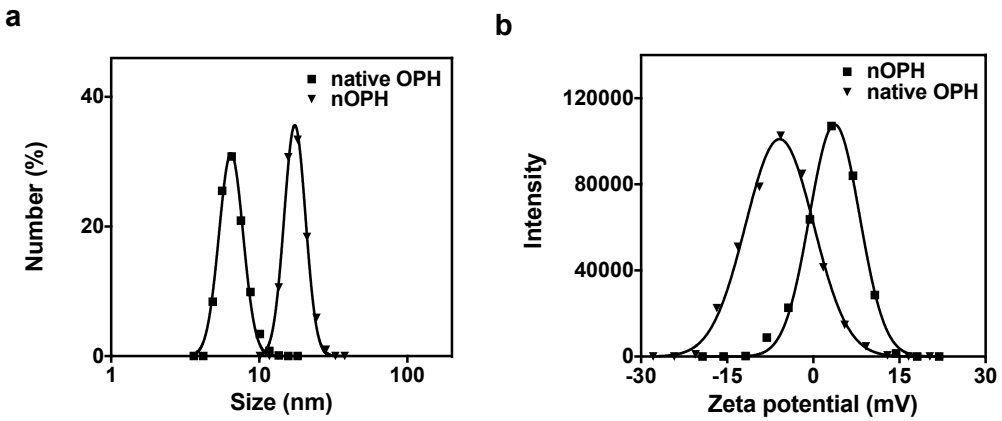


Fig. S1 a) DLS for native OPH and OPH nanocapsule; b) Zeta potential for native OPH and OPH nanocapsule.

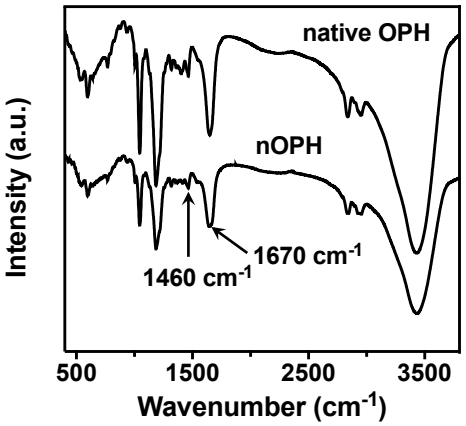


Fig. S2 FTIR spectra of native OPH and OPH nanocapsule.

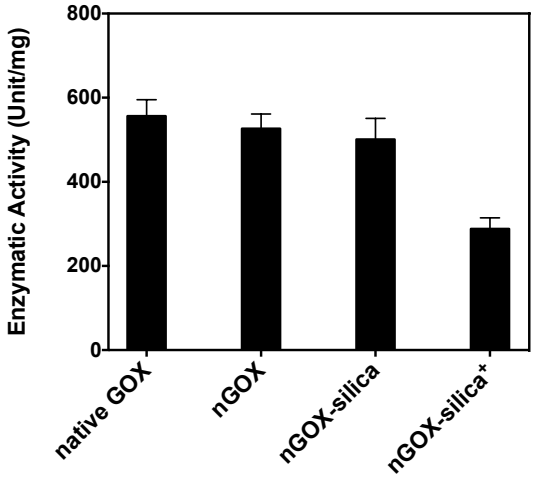


Fig. S3 Enzymatic acitivity of GOX, GOX nanocapusle and their enzyme-silica composite.

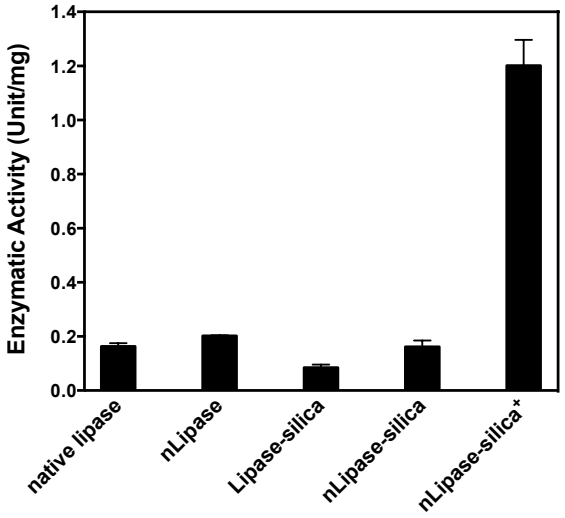


Fig. S4 Enzymatic acitivity of lipase, lipase nanocapusle and their enzyme-silica composite.

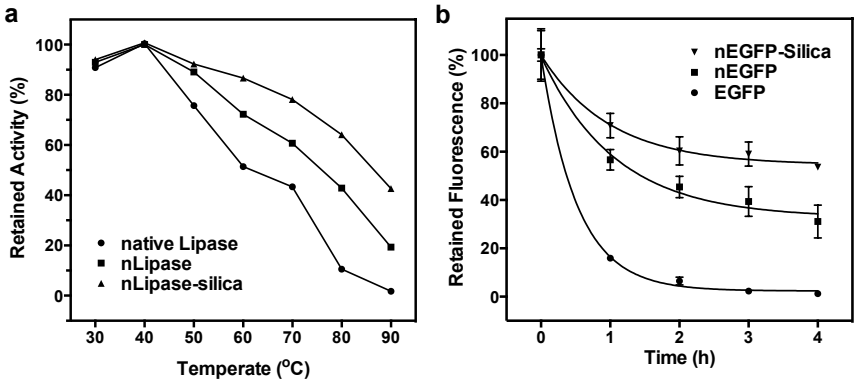


Fig. S5 (a) Relative activity of native lipase, nLipase and nLipase-silica at different temperature; b) relative activity of native EGFP, nEGFP and nEGFP-silica at 75 °C.

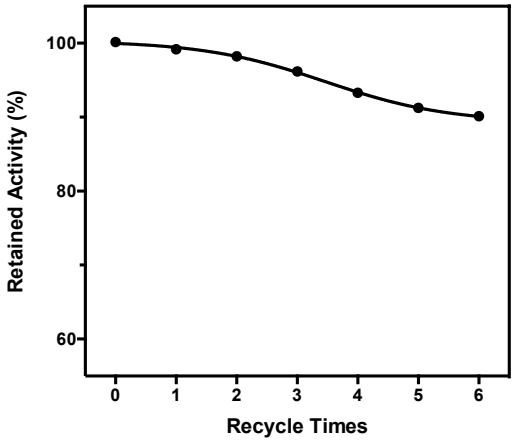


Fig. S6 The recycling durability of nLipase-silica composite.

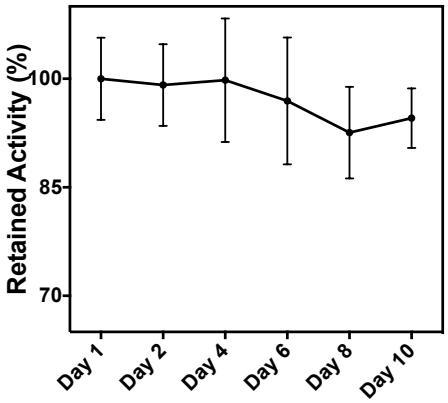


Fig. S7 The long-term storage stability of nLipase-silica composite.

[1] G. A. Omburo, J. M. Kuo, L. S. Mullins, F. M. Raushel, *J. Biol. Chem.* **1992**, *267*, 13278.