Supporting Information

Pickering Emulsion as an Efficient Platform for Enzymatic Reactions without Stirring

Lijuan Wei,[†] Ming Zhang,[†] Xiaoming Zhang,[†] Hongchuan Xin,[‡] and Hengquan Yang*[,†]

[†]School of Chemistry and Chemical Engineering, Shanxi University, Taiyuan 030006, China
[‡]Key Laboratory of Biofuels, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao 266101, China

Experimental section

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Chemicals. Native Lipase B from *Candida antarctica* (CALB) was purchased from Novozymes. (R, S)-1-phenylethyl acetate (98%), (*R, S*)-α-methylbenzyl butyrate (98%) and (*R, S*)-1-octen-3-yl acetate (97%) were purchased from Adamas Reagent Co., Ltd, (China). (*R, S*)-2-octanol acetate (98%) and (*R, S*)-4-methyl-2-pentanol acetal from Alfa Aesar. $(MeO)_3SiCH_3$ (98%) and acetyl chloride (98%） were purchased from Aladdin (China). Cyclohexane (AR), n-hexanol (AR), tetraethyl orthosilicate (TEOS, AR) phosphoric acid (85%), Coomassie Brilliant Blue G-250 (CBB), bovine serum albumin (BSA) and ammonia ($NH_3 \cdot H_2O$, 25 wt %) were purchased from Sinopharm Chemical Reagent Co., Ltd, (China). Triton X-10 (TX-10, 98%) was obtained from Guangzhou Reagent Company (China). Deionized water was used in this study.

SiO_2 emulsifier preparation. SiO_2 nanospheres were synthesized according to our previous procedure.[1] 1.5 g of as-synthesized SiO_2 nanospheres (dried at 120 °C for 4 h) was dispersed into toluene (8 mL). Into this suspension, a mixture of 4.5 mmol $(MeO)_3SiCH_3$ and 4.5 mmol $N(C_2H_5)_3$ were added. After heating under reflux for 4 h under a N_2 atmosphere, the solid material was isolated by centrifugation, washed four times with toluene and dried, yielding methyl-modified silica particles as emulsifier.

Labelling enzyme with Rhodamine B. 2 mL native CALB was dispersed into 2 mL PBS (phosphate buffer: 0.1 M Na_2HPO_4-0.1 M NaH_2PO_4, pH 7.0), leading to a suspension. 100 μL (0.3 mg ml^{-1}) Rhodamine B was added to the above suspension. After stirring 12 h at room temperature in the dark, the solid were isolated by centrifugation. After being washed four times with PBS, Rhodamine B-labelling CALB was obtained.

Characterization. Samples for transmission electron microscope (TEM) observations were prepared by dispersing the sample powder in ethanol using ultrasound and then allowing a drop of the suspension to evaporate on a copper gird covered with a holey carbon film. TEM images of methyl-modified SiO_2 nanospheres were obtained on a JEOL-JEM-2000EX instrument (operated at 120 kV). Energy-dispersive X-ray spectrum (EDX) was achieved on a field emission transmission electron microscope equipped with an energy dispersive spectrometer (FEI Tecnai G2 F20, 200 kV). Nitrogen-sorption experiments were performed at -196 °C on a Micromeritics ASAP2020 system. Before measuring, the sample was out gassed at 120 °C under vacuum for 6 h. The surface area was calculated from the adsorption branch in the relative pressure range of 0.05–0.15 using the Brunauer–Emmett–Teller (BET) method. X-ray photoelectron spectrum (XPS) were recorded on a Kratos Axis Ultra DLD, and the C 1s line at 284.9 eV was used as a reference. The contact angles of water in air on silica particle disks were measured using a Krüss DSA100 instrument. Before measurement, the powder sample was compressed into a disk with a thickness of approximately 1 mm (ca. 2 MPa). A drop of water (1 μL) was injected on the sample disk. The appearance of the water drop was recorded at ca. 0.1 s with a digital camera. Contact angle was determined by a photo goniometric method. The size of emulsion droplets was examined using an optical microscopy (XSP-8CA, Shanghai, China) equipped with 10 × magnification lens. Gas chromatography (GC) analysis was carried out on an Agilent 7890A instrument (Agilent-113-2532, Chiral Cyclodex-B, 30 m × 320 μm × 0.25 μm), with a flame ionization detector.

Determination of protein content (Bradford method). Coomassie Brilliant Blue G-250 (100 mg, CBB) and 100 mL of phosphoric acid (85% w/v) were dispersed into 50 mL of ethanol (95%). The resulting solution was then diluted to 1 L with deionized water. The final composition of this solution is 0.01% (w/v) Coomassie Brilliant Blue G-250, 4.7% (w/v) ethanol, and 8.5% (w/v) phosphoric acid.

Protein assay. A UV-vis standard curve for Bovine Serum Albumin (BSA) assay was obtained using different protein concentrations with 0.02, 0.04, 0.06, 0.08 and 0.1 mg mL^{-1}. 1 mL of a sample solution was added into 4 mL of CBB solution. The resultant mixture was incubated for 10 min at room temperature for UV-vis determination (595 nm). The blank experiment was also conducted in the absence of protein.

Determination of the amount of enzyme adsorbed onto the emulsifier. The experimental procedure of the Pickering emulsion preparation is the same with the reaction systems except the absence of reactant. After standing for 6 h, the Pickering emulsion was subjected to demulsification through centrifugation, to separate the aqueous solution. The concentration of CALB in this aqueous solution was determined using UV-vis spectroscopy, as the aforementioned procedure. The amount of enzyme adsorbed onto the emulsifier was calculated through comparing the enzyme concentrations before and after adsorption.

Calculations of the surface coverage C

$$C = m_p D/4\rho_p V_d d_p$$

where m_p is the mass of solid particle emulsifier, D is the average diameter of an emulsion droplet, ρ_p is the density of the solid particle emulsifier (2.65 g cm^{-3}), V_d is the volume of water and d_p is the solid particle diameter.[2]

Calculations of the conversion and enantiomeric excess of ester (ee_s) and alcohol (ee_p).

$$ee_s = \frac{[\text{ester}]S - [\text{ester}]R}{[\text{ester}]S + [\text{ester}]R} \times 100\%$$

$$ee_p = \frac{[\text{alcohol}]R - [\text{alcohol}]S}{[\text{alcohol}]R + [\text{alcohol}]S} \times 100\%$$

$$\text{Conv. } \% = ee_s/(ee_p + ee_s) \times 100\%$$

Reference

Reference

(1) Kong, D.; Zhang, C.; Xu, Z.; Li, G.; Hou Z.; Lin, J. Tunable photoluminescence in monodisperse silica spheres. *J. Colloid Interface Sci.*, **2010**, *352*, 278-284.

(2) Gautier, F.; Destribats, M.; Perrier-Cornet, R.; Dechézelles, J. F.; Giermanska, J.; Héroguez, V.; Ravaine, S.; Leal-Calderon, F.; Schmitt, V. Phys. Chem. Chem. Phys. 2007, 9, 6455.

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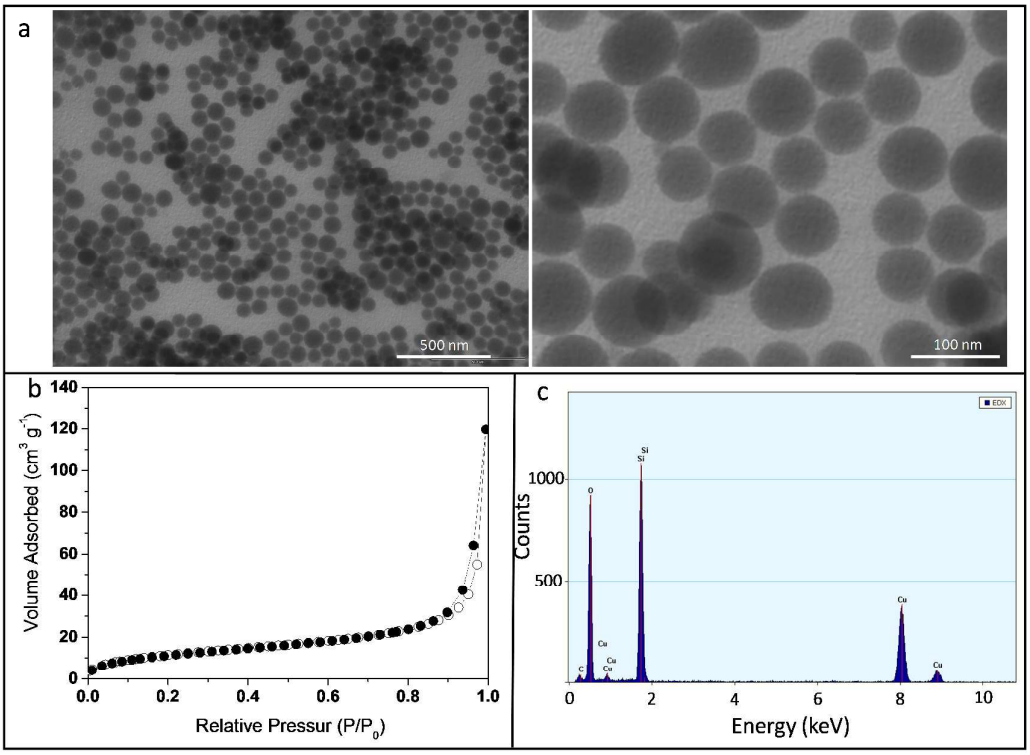

Figure S1. TEM images (a), N_2 adsorption-desorption isotherms (b) and energy-dispersive X-ray spectrum (EDX) (c) of the emulsifier.

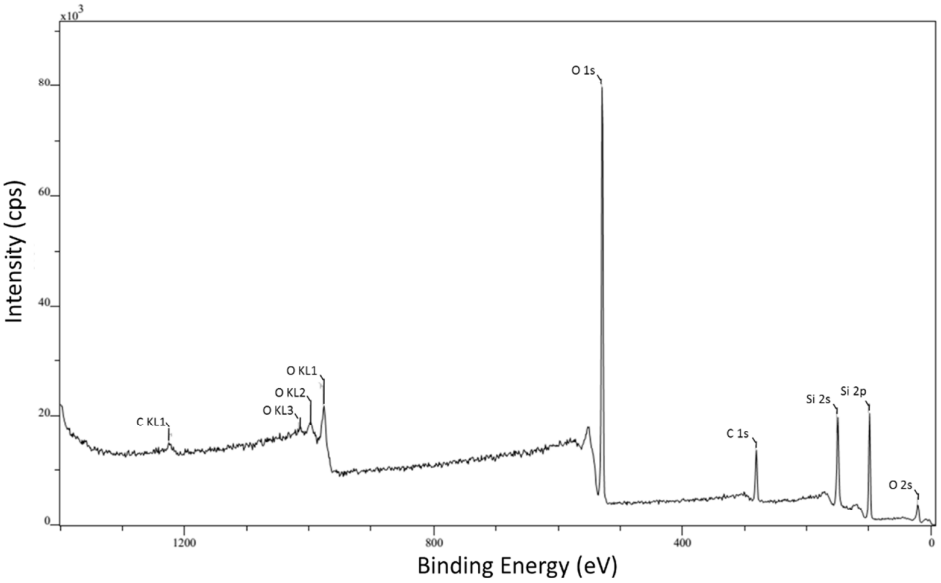

Figure S2. X-Ray photoelectron spectrum (XPS) of the emulsifier.

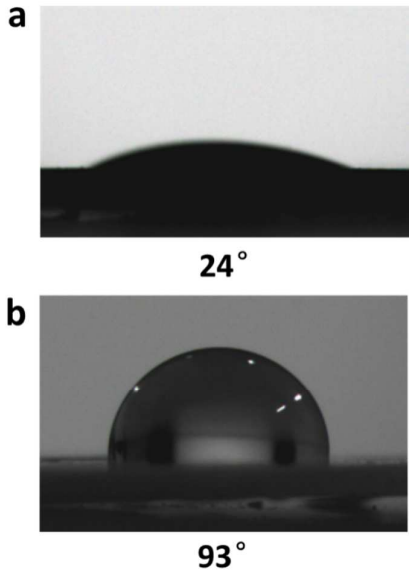

Figure S3. Water contact angle of the unmodified silica pellet (a) and the emulsifier pellet (b).

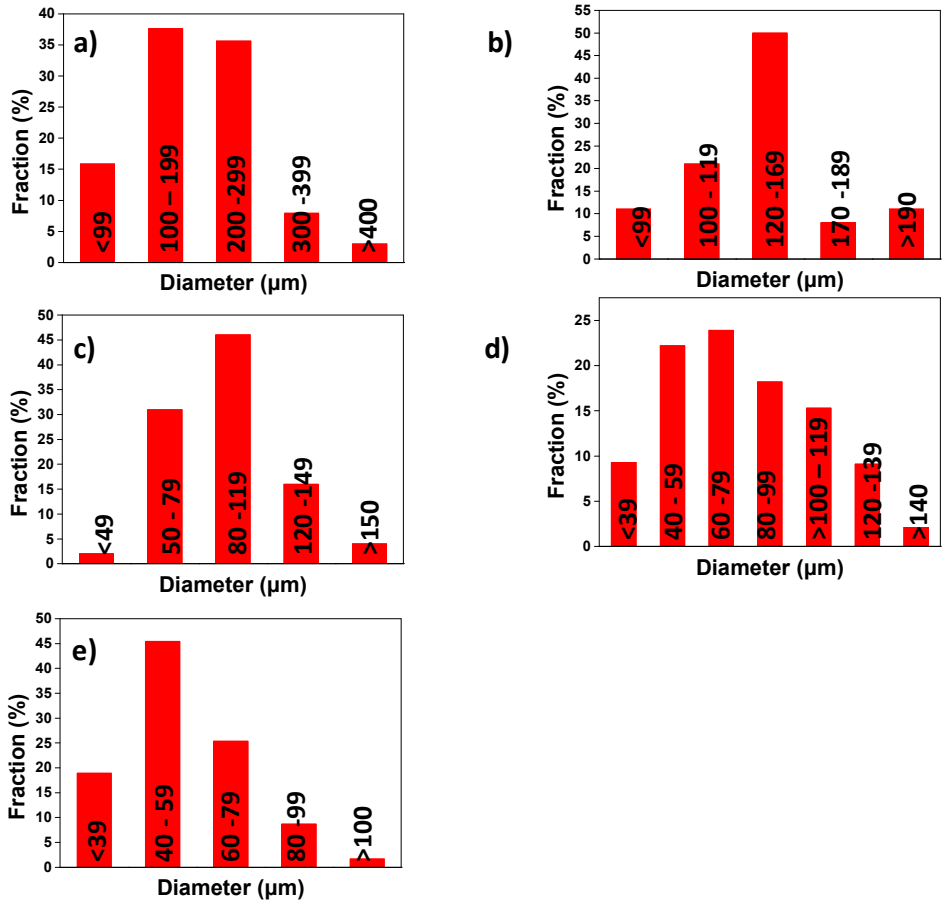

Figure S4. Amount of emulsifier (wt.%) distributions of the Pickering emulsion systems with different amounts of emulsifier: (a) 0.25 wt %, (b) 0.5 wt %, (c) 1 wt %, (d) 2 wt %, (e) 3 wt %.

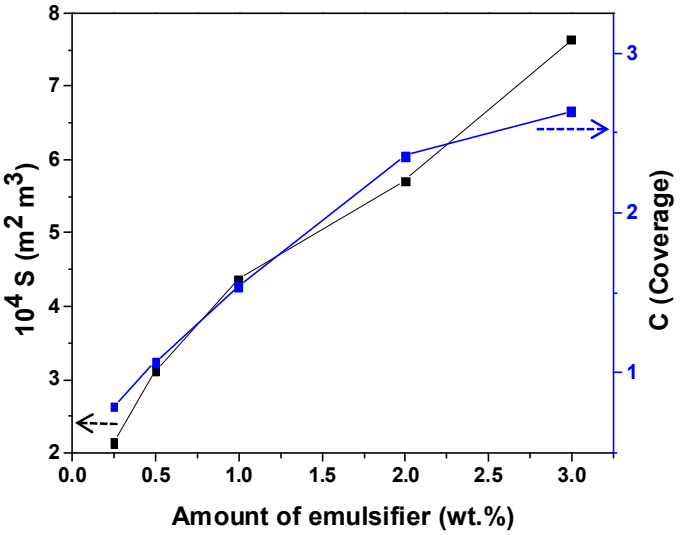

Figure S5. Oil−water interfacial area (S) and surface coverage (C) of emulsifier versus the emulsifier amount.