Supporting information for

Encapsulation of enzyme in metal ion-surfactant nanocomposites for catalysis in highly polar solvents

Materials

Lipase from *Candida rugose* (CRL) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Novozyme 435 (lipase B from *Candida Antarctica* and immobilized on a macroporous acrylic resin) and Lipozyme TLIM (lipase from *Thermomyces lanuginosus* and immobilized on silica granulation) were purchased from Novozymes (Durham, NC, USA). NaDC was obtained from Sinopharm Chemical Reagent Company (Shanghai, China). Cobalt chloride was purchased from Guangdong Chemical Reagent Engineering-technological Research and Development Center (Guangzhou, China). Vitamin E was obtained from Tokyo Chemical Industry (Tokyo, Japan). Succinic anhydride was purchased from Linfeng Chemical Reagent Company (Shanghai, China). Dopamine and vitamin E succinate were obtained from Aladdin Reagent Company (Shanghai, China). Other chemicals were of analytical grade.

Methods

CRL-MSNC synthesis

The preparation of CRL-MSNC is shown schematically in Fig. 1. In a typical experiment, a solution (10 mL) containing both NaDC (10 mmol/L) and CRL (0.18 mg/mL) was added to a water solution consisting of cobalt chloride (10 mL; 20 mmol/L). The mixture was stirred for 0.5 h at room temperature, followed by two cycles of centrifugation at 8000 rpm for 15 min and washing to obtain CRL-MSNC.

PDA@CRL-MSNC synthesis

CRL-MSNC was first synthesized by mixing the enzyme, NaDC, and cobalt ion

solutions together as described, followed by incubation in freshly prepared dopamine solution (2 mg/mL) in Tris buffer (10 mM; pH 8.0) for 24 h at room temperature to allow the self-polymerization of PDA in aqueous solution.

MSNC synthesis

The synthesis of MSNC without enzyme followed the same procedure as that used for the preparation of CRL-MSNC, but in the absence of enzyme solution. A solution (10 mL) containing NaDC (10 mmol/L) was added to a water solution consisting of cobalt chloride (10 mL 20 mmol/L). The mixture was stirred for 0.5 h at room temperature, followed by two cycles of centrifugation at 8000 rpm for 15 min and washing to obtain pure MSNC.

ICP-MS analysis

Inductively coupled plasma mass spectrometry (ICP-MS) was performed on a X Series ICP-MS, Thermo Fisher Scientific Inc.

Protein encapsulation ratio

The protein contents in the crude enzyme solutions or encapsulated enzyme preparations were determined according to the Bradford method, which used bovine serum albumin as the standard. The assay mixture consisted of 4 mL of Bradford reagent and 1 mL of test solution. The absorbance was read after 3 min standing at 595 nm. The protein encapsulation ratio of the CRL-MSNC or PDA@CRL-MSNC preparations was calculated indirectly from the difference between the amount of enzyme introduced into the reaction mixture and the amount of enzyme in the filtrate after encapsulation.

SEM and TEM

The morphologies of the samples were characterized by SEM (S-3400 II; Hitachi, Tokyo, Japan) using an accelerating voltage of 20 kV with fit magnification. For TEM, sample preparation was accomplished by dispersing the composite in ethanol with

ultrasound and then placing a well-dispersed droplet on a copper grid. Samples were then dried and analyzed on a JEM-200CX transmission electron microscope (JEOL, Tokyo, Japan). The energy dispersive X-ray spectroscopy mapping of PDA@CRL-MSNC was conducted on a JEOL JEM-2010 high-resolution TEM.

FTIR spectra

Analysis of the chemical functional groups of CRL-MSNC and PDA@CRL-MSNC were conducted by means of FTIR spectroscopy (Nicolet Is5; Thermo Fisher Scientific). Each sample was ground thoroughly with potassium bromide, and the resulting powder was pressed to form a transparent pellet using a hydraulic press. FTIR spectra were collected in transmission mode between 800 cm^{-1} and 3400 cm^{-1} at a resolution of 2 cm^{-1}.

TGA

TGA in air was performed on a TGA Q500 thermogravimetric analyzer (TA Instruments, New Castle, DE, USA). The sample was heated from room temperature to 400°C at a rate of 10°C/min under air atmosphere.

Enzymatic activity assay in water solution

The activity of CRL embedded in the MSNC and PDA@MSNC in water solution was determined by a standard method using pNPB as the substrate. Lipase (100 μL) or immobilized lipase (with 100 μL water) was added to 2.87 mL of 50 mM phosphate buffered saline (PBS; pH 7.0) solution. After pre-incubation at 30°C for 3 min in a water bath, 30 μL of 100 mM pNPB solution in DMSO was added. After stirring for 5 min, the mixture was centrifuged at 4000 rpm for 3 min to separate the supernatant and determine the absorbance at 405 nm on a UV/Vis spectrophotometer. One unit of lipase activity was defined as the amount of enzyme releasing 1 μmol of *p*-nitrophenol per minute.

Enzyme stability in polar organic solvents

CRL-MSNC and PDA@CRL-MSNC were incubated in polar organic solvents containing 100% ethanol, 100% methanol, or 100% DMSO for 5 min. After centrifugation at 4000 rpm for 3 min and washing with deionized water, CRL-MSNC and PDA@CRL-MSNC were subjected to the previously described enzymatic assays.

Enzyme reuse in water solution

The recycling use of enzyme catalysts was assessed by adding CRL-MSNC and PDA@CRL-MSNC with equivalent amounts of protein in 2.87 mL of 50 mM PBS (pH 7.0). After pre-incubation at 30°C for 3 min, 30 μL of 100 mM pNPB solution in DMSO was added to the reaction. After stirring for 5 min, the mixture was centrifuged at 4000 rpm for 3 min to separate the supernatant and determine the absorbance at 405 nm on a UV/Vis spectrophotometer. The precipitate was washed twice with PBS and centrifuged at 4000 rpm for 2 min. The recovered enzyme composite was used for the next batch of enzymatic reactions. This procedure was repeated 10 times to determine the reusability of the enzyme catalysts.

Lipase-catalyzed synthesis of vitamin E succinate

Enzymatic esterification was performed using 0.2 mmol of vitamin E, 1 mmol of succinic anhydride, and 400 μL free CRL or an equivalent quantity (in terms of protein) of CRL-MSNC (20 mg) or PDA@CRL-MSNC (40 mg) in 5 mL organic solvent in a tightly closed 10 mL plastic tube. The tube was incubated in a water bath at 55°C and agitated with mechanical stirring at 800 rpm. Novozyme 435 and Lipozyme TLIM (40 mg) at the same protein concentrations were also used for the synthesis of vitamin E succinate.

High-performance liquid chromatography (HPLC) analysis

Quantitative analysis of the reactants and products were conducted using a HPLC system from Agilent Technologies (Santa Clara, CA, USA). A reversed-phase column (Amethyst C18-P; 250 mm × 4.6 mm, 5-μm diameter) was used, and the reactants and products were detected by UV/Vis detector (G1314F1260VWD) at 285 nm. A mixture

of methanol/acetic acid [50/0.3 (v/v)] was used as an eluent at 36°C, with a flow rate of 1 mL/min. Esterification activity was determined by calculating the transformation of the substrate (vitamin E) to ester.

Enzyme reuse in DMSO

The recycling use of the enzyme catalysts was assessed by adding CRL-MSNC or PDA@CRL-MSNC with equivalent concentrations of protein in 5 mL organic solvent containing 0.2 mmol vitamin E and 1 mmol succinic anhydride. After stirring for 4 h, the mixture was centrifuged at 10,000 rpm for 10 min. Quantitative analysis of the supernatant was conducted by HPLC, and the precipitate was used for the next batch of enzymatic reactions. This procedure was repeated 10 times to determine the reusability of the enzyme catalysts.

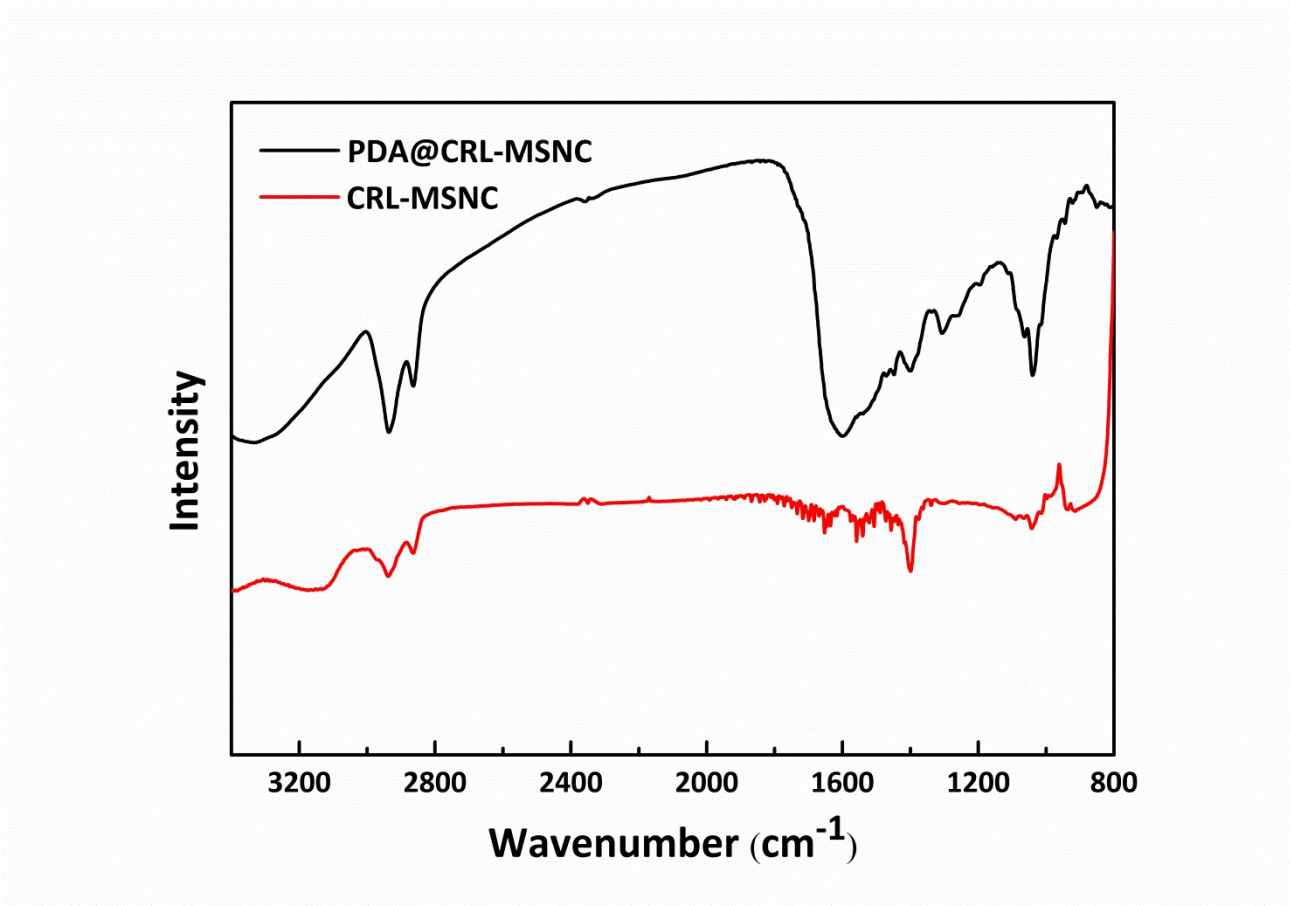

Figure S1. FTIR spectra of CRL-MSNC and PDA@CRL-MSNC.

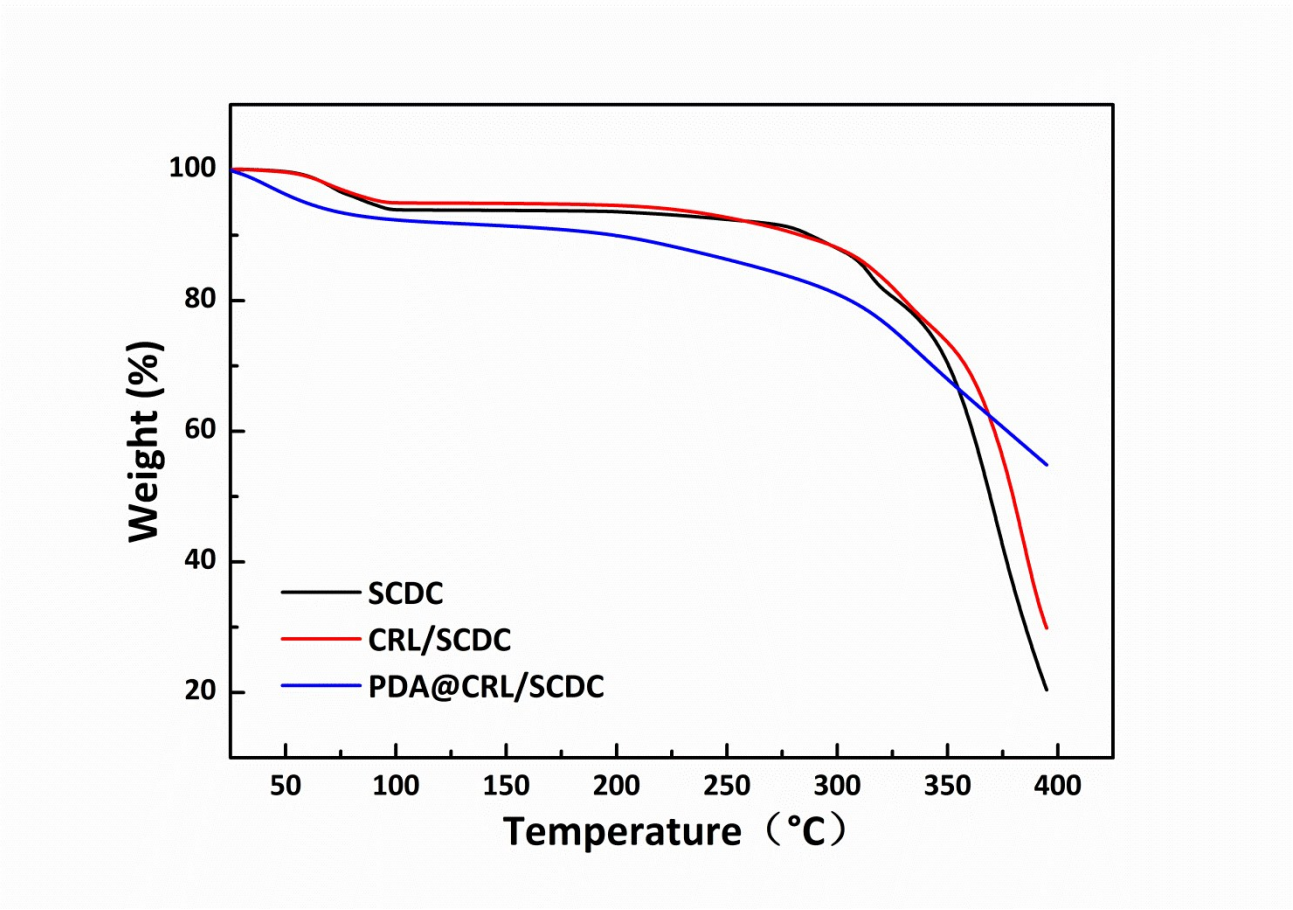

Figure S2. TGA analysis of MSNC, CRL-MSNC, and PDA@CRL-MSNC.

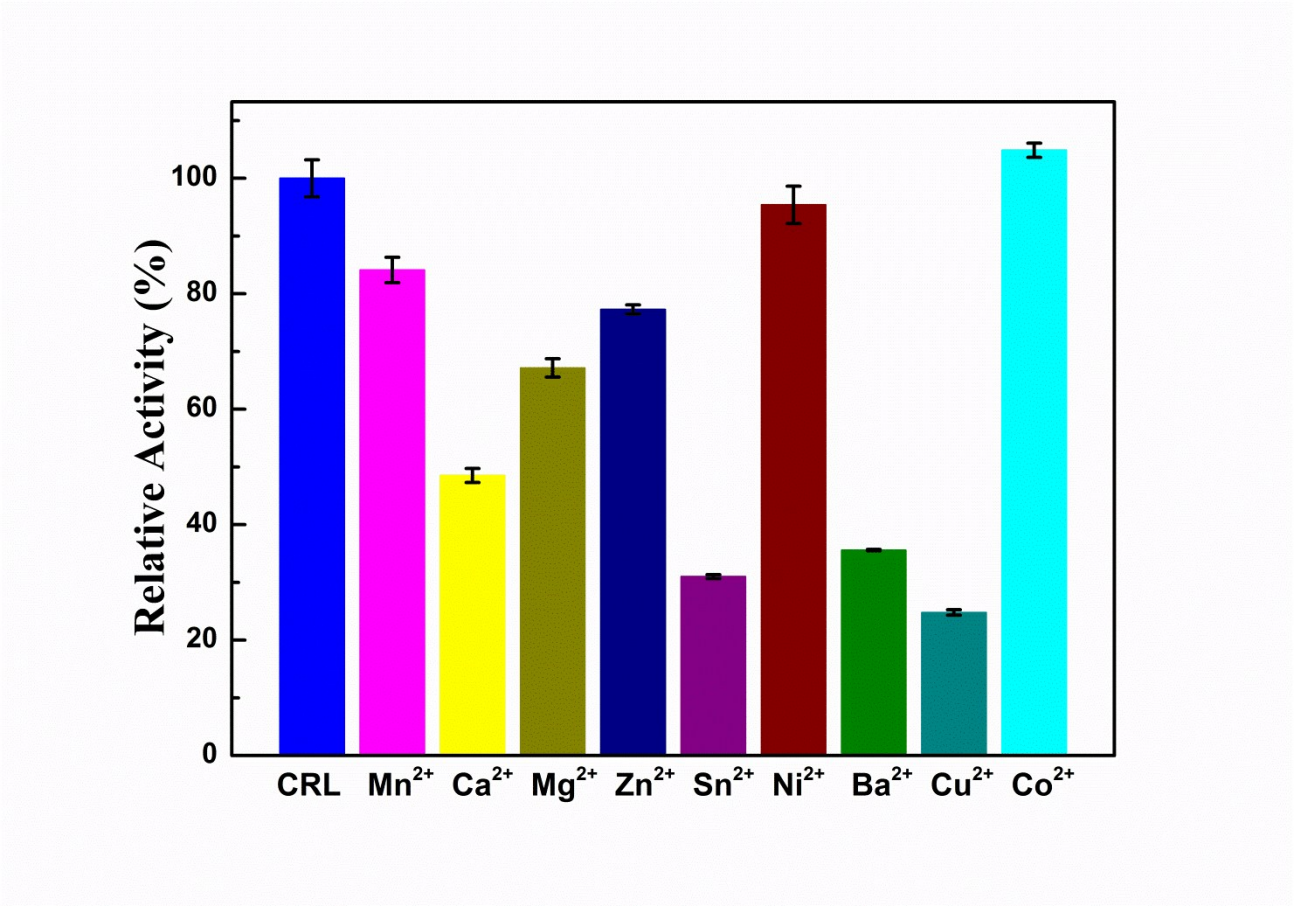

Figure S3. Relative activity of CRL-MSNC prepared by different metal ions.

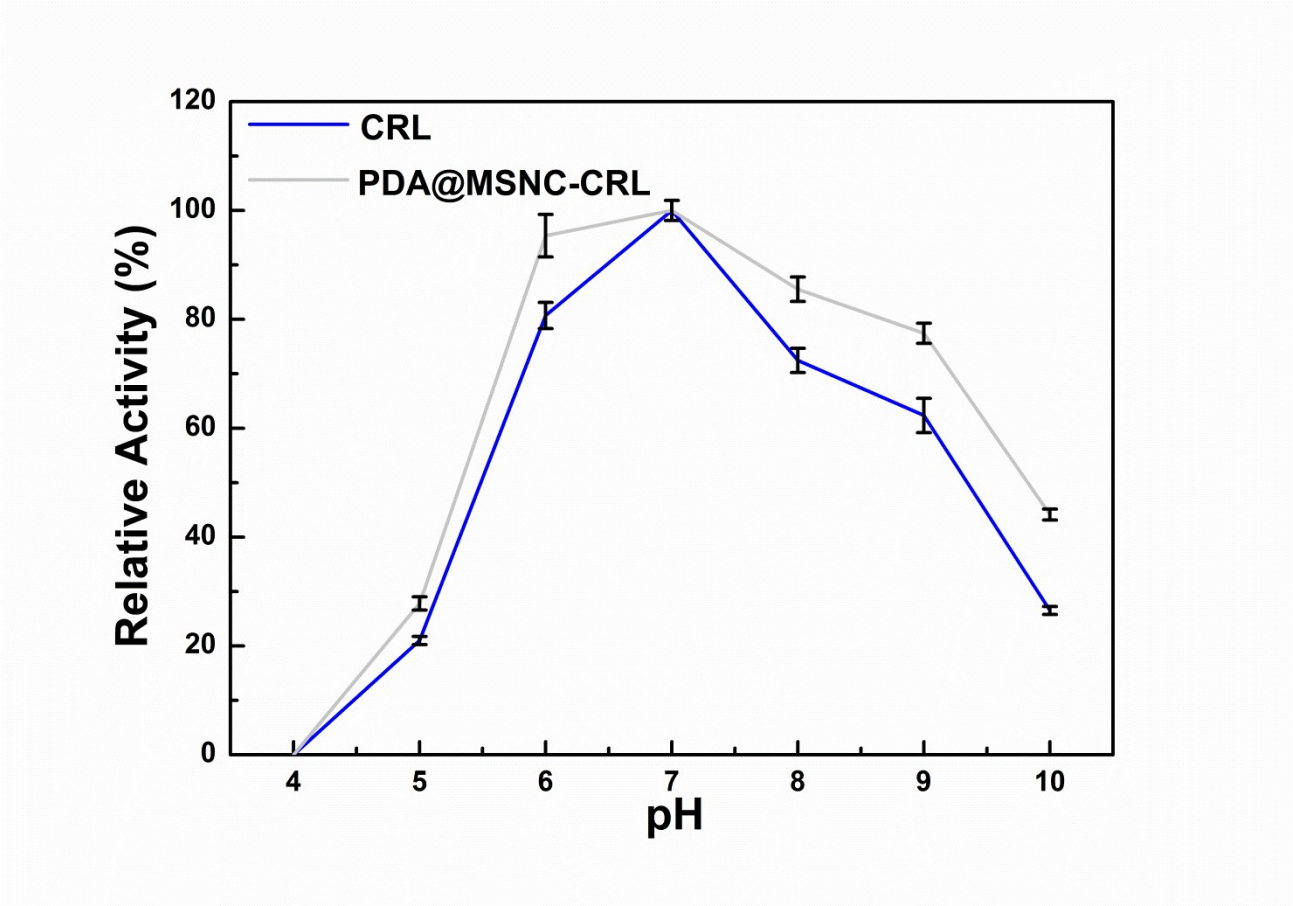

Figure S4. Effect of pH on the relative activity of free CRL and PDA@CRL-MSNC. (100 mM citrate buffer solution for pH 4.0–5.0, 100 mM phosphate buffer for pH 6.0–8.0, and 100 mM Na_2CO_3-$NaHCO_3$ buffer solution for pH 9.0–10.0)

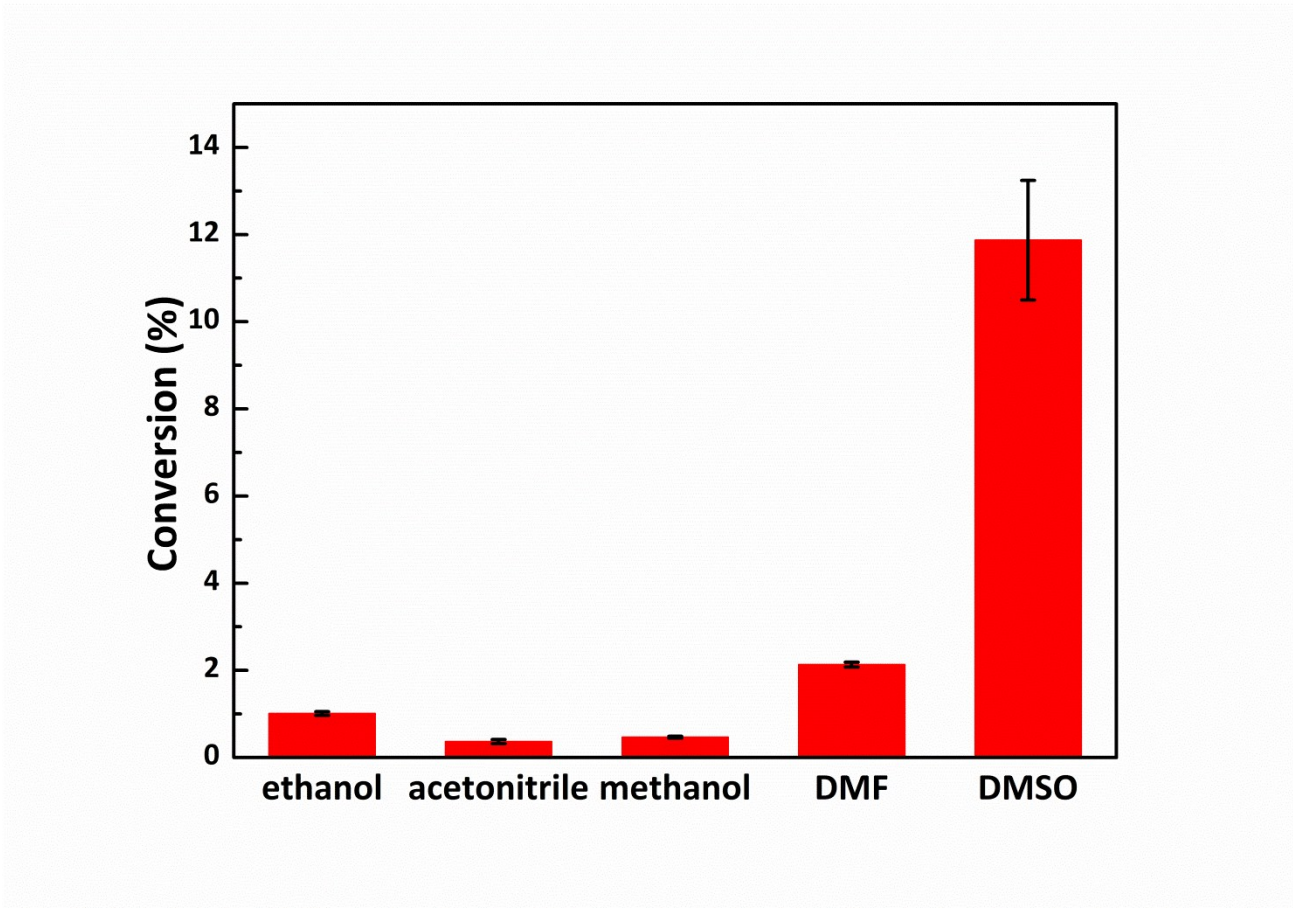

Figure S5. Effect of different organic solvents on the yield of vitamin E succinate catalyzed by free CRL.

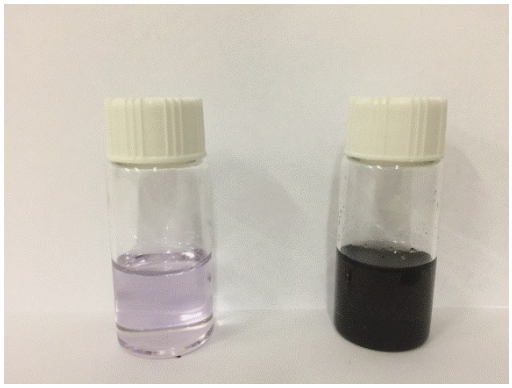

Figure S6. Appearance of CRL-MSNC (left) and PDA@CRL-MSNC (right) in DMSO following enzymatic reactions.