Supporting information for

Temperature-responsive enzyme-polymer nanoconjugates with enhanced catalytic activities in organic media

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Materials

The following reagents were purchased from Sigma-Aldrich: Bovine serum albumin (BSA), *Candida rugosa* lipase (CRL), *Candida sp.* lipase (CALB), cytochrome c (Cyt C), Pluronic® F-127, Dess-Martin periodinane (1,1,1-tris(acetyloxy)-1,1-dihydro-1,2-benziodoxol-3-(1H)-one), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 4-nitrophenyl butyrate. Purpald (4-amino-3-hydrazino-5-mercapto-1,2,4-triazole, 99+%), formaldehyde (37% w/w in aqueous solution), sodium metaperiodate (98%), sodium cyanoborohydride (95%), hexanoic acid, palmitic acid, n-butyl alcohol and n-octanol were purchased from Alfa Aesar.

Aldehyde-end Functionalization of Pluronic F-127

Five times molar excess of Dess-Martin periodinane was added to a room-temperature solution of Pluronic F-127 in dichloromethane (10 mg/mL) and stirred overnight. The solvent was then evaporated by a rotary evaporator to obtain a viscous solution. The product was precipitated in cold diethyl ether and dried under vacuum at room temperature. The product was characterized by FT-IR spectroscopy and the Purpald colorimetric assay to confirm the conversion of the hydroxyl groups into aldehyde functionalities (determined as 30% conversion for hydroxyl groups).

Protein Conjugation

A solution of protein at the concentration of 5-10 mg/mL in aqueous solution (phosphate buffer, 10 mM, pH 7.0) was slowly added to a stirred solution of aldehyde-functionalized Pluronic in phosphate buffer (10 mM, pH 7.0), both at room temperature. The molar ratio of the aldehyde groups in the polymer to the amine groups in lysines on the protein in the reaction mixture was 1.1:1. After 2 hours of reaction, sodium cyanoborohydride ($NaCNBH_3$, 10% w/w of aldehyde-functionalized pluronic) was added to the mixture followed by stirring for 15 hours at room temperature. The mixture then was dialyzed against phosphate buffer (10 mM, pH 7.0) to remove unreacted reagents.

Transmission Electron Microscopy (TEM)

The dry powder of the protein-Pluronic conjugate obtained by lyophilization was dissolved in toluene (10 μg/mL of protein concentration). 10 μL of this solution was placed on the carbon-coated grid. After the toluene evaporated, 10 μL of sodium phosphotungstate aqueous solution (1%, pH 7.0) was applied to stain the sample. The excess of the liquid was removed after 2 minutes and the sample was dried for 24 hours at room temperature before making TEM measurements.

Enzymatic Activity Assay

The hydrolytic activities of CRL, CALB, and their conjugates in aqueous solution were determined by a standard method using 4-nitrophenyl butyrate (*p*-NPB) as the substrate at room temperature. Briefly, *p*-NPB was first dissolved in acetone and then diluted with phosphate buffer (10 mM, pH 7.0) containing 1.25% (w/v) Triton X-100, giving a final concentration of 0.5 mM. The reaction was started by adding 50 μL of the enzyme solution (50 μg/mL in 10 mM phosphate buffer, pH 7.0) to 950 μL of the substrate solution; the increase in absorbance was detected at 348 nm by using a UV/Vis spectrophotometer (Shimadzu UV2550). The peroxidase activity of cytochrome c in aqueous solution was measured by using ABTS and H_2O_2 as the substrate at room temperature. In a typical measurement, the assay mixture consisted of 960 μL of ABTS aqueous solution (2.8 mg/mL) and 20 μL of Cyt C or Cyt C-conjugate in aqueous solution (protein concentration: 0.1 mg/mL). The enzymatic reaction was initiated by adding 20 μL of H_2O_2 aqueous solution (0.3% v/v of H_2O_2 in water), and the increase of absorbance at 415 nm was measured by using a UV/Vis spectrophotometer (Shimadzu UV2550). The esterification activity of lipase in toluene was measured at 40 $^{\circ}$C using standard methods. For CRL and its conjugate, palmitic acid and n-octanol were used as substrates; for CALB and its conjugate, hexanoic acid and n-butyl alcohol were used. During a reaction run, a dry power of native enzyme or enzyme conjugate with the same protein content of 0.5 mg was added into 5 mL of toluene solution containing 0.1 mol/L of fatty acid and 0.1 mol/L of alcohol. Then the reaction mixture was shaken at 40 $^{\circ}$C in a horizontal shaker set to 200 rpm. The reaction was terminated by diluting with 10 mL of ethanol/acetone (1:1, v/v) after 20 min to determine the initial activity under saturated substrate conditions. The remaining free fatty acid in the reaction mixture was determined by titration with 0.05 mol/L of NaOH using phenolphthalein as the

indicator. Conversion of 1 μmol of fatty acid per minute in the assay conditions was defined as one enzyme unit activity (U ·mg^{-1}).

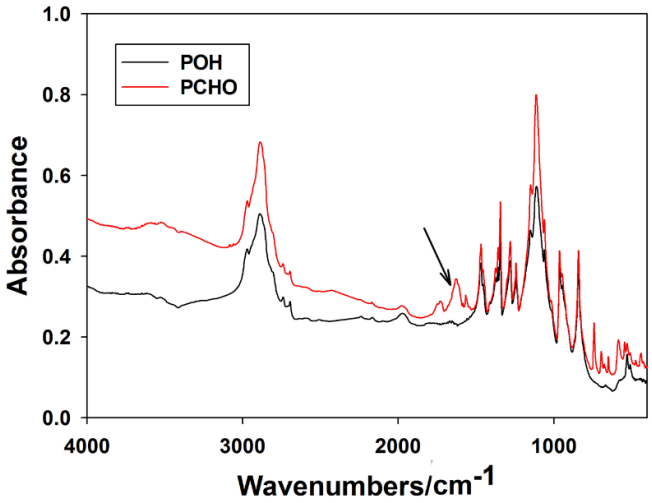

Fig. S1 FT-IR spectra of Pluronic F-127 (POH) and aldehyde-functionalized Pluronic (PCHO). (Arrow indicates the signal corresponding to C=O vibration)

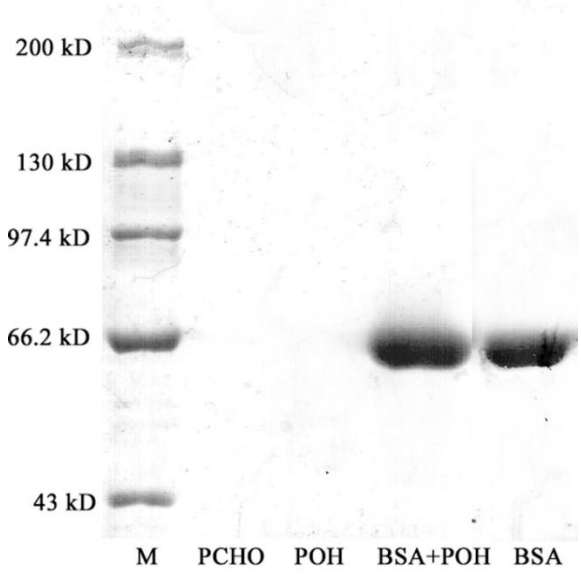

Fig. S2 Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis of modified Pluronic (PCHO), Pluronic F-127 (POH), mixture of BSA and pluronic F-127 (BSA+POH) and BSA. (M: protein molecular weight marker).

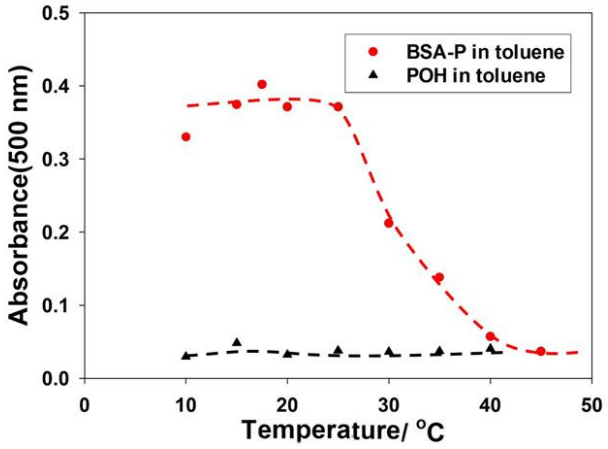

Fig. S3 Absorbance at 500 nm of toluene solution containing the BSA-Pluronic conjugate or Pluronic (1% w/v) at different temperatures.

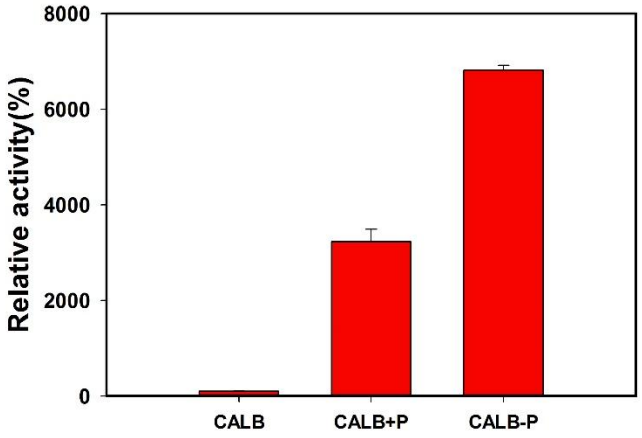

Fig. S4 The activities of CALB+P (CALB mixed with Pluronic F127) and CALB-P (CALB-Pluronic conjugate) compared to CALB (native enzyme) at the same protein content in toluene at 40 °C.

Table S1. Size of the dissolved conjugates measured by dynamic light scattering (DLS) (Malvern Nano ZS90) in toluene at the concentration of 0.1 mg/mL at room temperature.

Sample	BSA conjugate	CRL conjugate	CALB conjugate	Cyt C conjugate
Size (nm)	42±23	31±13	32±10	27±15