Electronic Supplementary Information

Crackled Nanocapsules: "Imperfect" Structure for Enzyme Immobilization

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Experimental section

Materials

Trishydroxymethyl aminomethane (Tris) was purchased from Sigma-Aldrich Co., Ltd. Glycerol carbonate, dimethyl carbonate, acetone, tert-butyl alcohol and cyclohexanol were all obtained from Tianjin Damao Chemical Reagent Factory. Anhydrous ethanol was purchased from Tianjin Guangfu Technology Development Co., Ltd. Coomassie Blue (G-250) was purchased from Beijing Dingguo Biological Technology Co., Ltd. All other reagents were used without further purification.

Preparation of ZIF-8 Nanocrystals

All the samples of ZIF-8 nanocrystals were prepared in aqueous solution. An aqueous solution of 4.8 mL, Zn $(NO_3)_2 \cdot 6H_2O$ (200 mg, 0.14 mM) was quickly added to an aqueous solution of 8 mL Hmim (2 g, 3 mM). Immediately, the resulting solution was stirred at 600 rpm for 15 min. Then, the solution was centrifuged at 8000 rpm for 3 min and washed three times with water. Finally, the ZIF-8 nanocrystals were acquired.

Preparation of Crackled Organosilica Nanocapsules (CONs)

Four types of CONs were synthesized. Briefly, the ZIF-8 nanocrystals (20 mg) as synthesized above were dispersed in 3 mL of deionized water. After dispersion, the above solution was sonicated for 5 min. Then, different amounts of TEOS were added with the final concentrations of 65, 80, 96, and 128 mM, respectively. The mixture was shaken for 20 min. Then APTES was added to fix the final concentrations of 9.97, 12.39, 14.96 and 19.94 mM, respectively. The mixtures were shaken on the shaker for nearly 4 h followed by centrifugation at 3000 rpm for 1 min and washed twice with water. The CONs were obtained after removing the templates through EDTA

treatment (5 mL, 50 mM, pH 7.0). The four types of CONs were denoted as CONs-1, CONs-2, CONs-3, CONs-4, respectively.

Characterizations

TEM and SEM were conducted to examine the morphology and wall thickness of the CONs. X-ray diffraction (XRD) analysis of powdered samples was performed using a RINT 2500 V X-ray powder diffractometer. The diffraction conditions were: Cu Kα (λ=1.5406 Å) as a ray source, operating voltage 40 kV, operating current 300 mA, scanning range 5-80°, scanning speed 5° min^{-1}. Fourier transform infrared spectrometer (FTIR) was used to determine the chemical composition of the CONs. The scanning range was 450-4000 cm^{-1} with a resolution of 1.93 cm^{-1}. The CRL concentration was measured by U3010 UV-Vis Spectrophotometer (Hitachi, Japan). Nitrogen sorption isotherms of the ZIF-8/CONs and CONs at 77.3 K were determined by Micromeritics ASAP 2020. The sample was degassed at 90 °C for 8 hours before measurement. The pore size was calculated using the Barrett-Joyner-Helenda (BJH) method and the specific surface area was calculated using the Brunauer-Emmett-Teller (BET) method.

Loading Capacity and Immobilization Efficiency

The as-synthesized CONs (CONs-1 to CONs-4, CONs/ZIF-8) were dispersed in 3 mL CRL solution (50 mM, pH 7.0, Tris-HCl buffer). The concentration of CRL in solution was 1 mg mL^{-1}. The mixture was shaken for 20 min and centrifuged at 3000 rpm for 1 min. After that, the samples were washed twice with deionized water, and a series of immobilized CRL were acquired.

The amount of immobilized CRL was calculated by the ratio of the mass of immobilized CRL to the mass of CONs. The immobilization efficiency was the percentage of CRL immobilized on the CONs to the amount of CRL initially added.

The concentration of CRL solution was determined by Coomassie brilliant blue method. The amount of CRL loading was calculated according to Equation (S1), and the immobilization efficiency was calculated according to Equation (S2):

$$\text{Loading capacity (mg g}^{-1}\text{supports)}=\frac{(m-C_1V_1)}{W\times 1000} \quad \text{(S1)}$$

$$\text{Immobilization efficiency (\%)}=\frac{(m-C_1V_1)}{m}\times 100\% \quad \text{(S2)}$$

where m (mg) was the mass of CRL initially added, V_1 (mL) and C_1 (mg mL^{-1}) were the volume of the supernatant and the concentration of CRL in the supernatant after enzyme immobilization, W (mg) was the mass of different CONs or CONs/ZIF-8.

Enzyme Adsorption Kinetics

The adsorption kinetics of CRL on CONs and ZIF-8/CONs were demonstrated according to Langmuir and Freundlich models based on the following equations.

Langmuir model:

$$\frac{C}{Q}=\frac{1}{bQ_m}+\frac{1}{Q_m}C \quad \text{(S3)}$$

Freundlich model:

$$\frac{Q}{Q_m}=kC^{1/n} \quad \text{(S4)}$$

or

$$\ln Q=\frac{1}{n}\ln C+\ln kQ_m \quad \text{(S5)}$$

where C, Q, and Q_m were the equilibrium concentration of CRL, the adsorption amount of CRL at a certain time, and the maximum adsorption amount of CRL, respectively. b was the adsorption equilibrium constant, n and k were the experimental parameters.[S1]

Enzyme Activity

2 mg mL^{-1} *p*-nitrophenyl palmitate (*p*-NPP) (1 mL) in n-hexane was added to 2 mL of the CONs dispersion. The mixed solution was shaken on the shaker for 10 min, then 1 mL of 0.5 M Na_2CO_3 was added to the mixture to stop the reaction. After the supernatant was diluted 10 times, the absorbance at 410 nm was measured with a UV spectrophotometer (Hitachi U-3010). The product *p*-nitrophenol (*p*-NP) concentration was then calculated from the measured absorbance values.

Reaction Kinetic Constants

To determine kinetic parameters, free CRL and immobilized CRL were incubated in a series of reaction solutions with various concentrations of *p*-NPP (0.5 mg mL^{-1} to 5 mg mL^{-1}). Then, the reaction rates were measured according to the section of "Assay of Enzyme Activity". The maximum reaction rate (V_{max} (mmol (L min)$^{-1}$)) of the free CRL and immobilized CRL, as well as the Michaelis constant (K_m (mmol L^{-1})) were calculated by the Michaelis-Menten equation (S6):

$$\frac{1}{V} = \frac{K_m}{V_{\max}} \times \frac{1}{[\mathrm{S}]} + \frac{1}{V_{\max}} \tag{S6}$$

where V (mmol (L min)$^{-1}$) was the initial reaction rate for different concentrations of substrate; [S] (mmol L^{-1}) was the initial concentration of the reactants; V_{max} was the maximum reaction rate; K_m was the Michaelis constant. The k_{cat} were calculated by the equation (7)：

$$k_{cat} = \frac{M_{p-NPP}}{e \times 60} \tag{S7}$$

where k_{cat} was turnover number (s^{-1}); $M_{p\text{-}NPP}$ was the amount of *p*-NPP degraded by CRL (mmol (L min)$^{-1}$) and e was the amount of CRL molecules (mmol L^{-1}).

Leakage Ratio and Stabilities

60 mg CRL@CONs-2 and CRL@ZIF-8/CONs-2 were added to 20 mL Tris-HCl buffer (50 mM, pH 7.0) with mixing for 40 h. Then, the concentration of CRL in

supernatant was checked by the Bradford's method. The leakage ratio of CRL could be obtained by the following formula (S8):

$$\text{Leakage rate} = \frac{C_i V_i}{M_T} \times 100\% \quad \text{(S8)}$$

Where, C_i, V_i and M_T represent the concentration of CRL in buffer solution, the buffer solution volume, and CRL initially immobilized on the adsorbents, respectively.

The influences of temperature, pH and recycling stability on the activity of immobilized CRL were investigated. The effects of temperature, pH and recycling stability on the activity of immobilized CRL were investigated. The temperature stability of free and immobilized CRL in Tris-HCl buffer solution (50 mM, pH 7.0) was evaluated by measuring the activities of residual enzyme, after being incubated in water bath at different temperatures (20, 30, 40, 50, 60, 70 ℃) for 3 hours. The pH stability of free and immobilized CRL was measured by the residual activity of CRL, after incubation for 3 hours in different pH buffers (4.0-10.0). The buffers used were NaAc-HAc buffer solution (pH 4.0-5.0), PBS (pH 6.0-7.0) and Tris-HCl buffer solution pH (8.0-10.0), respectively. In addition, the recycling stability of CRL@CONs was checked. After each reaction was completed, the immobilized lipase was recovered by centrifugation, washed repeatedly with Tris-HCl buffer (50 mM, pH 7.0), and used for the next catalytic reaction. The ratio of the activity of the immobilized CRL after recycling to its initial activity indicates the recycling stability. In all stability assessments, the initial activities of free CRL and the immobilized CRL were assumed to be 100%, and the relative CRL activity was the ratio of remaining

CRL activity to the initial CRL activity.

Reusability of CRL@CONs-2

The reusability of CRL@CONs-2 was investigated in the transesterification reaction. Typically, glycerol and DMC in a molar ratio of 1:10 were mixed with CRL@CONs. The above mixture was incubated at a temperature of 60 ℃ and a shaking speed of 170 rpm for 24 hours. When the reaction was completed, the immobilized enzyme was collected by centrifugation after each reaction, repeatedly washed with Tris-HCl buffer (50 mM, pH 7.0) and used for the next catalytic reaction cycle. The new round of reactions was the same as the previous round of reactions. Quantitative analysis of the substrate was conducted by gas chromatography.

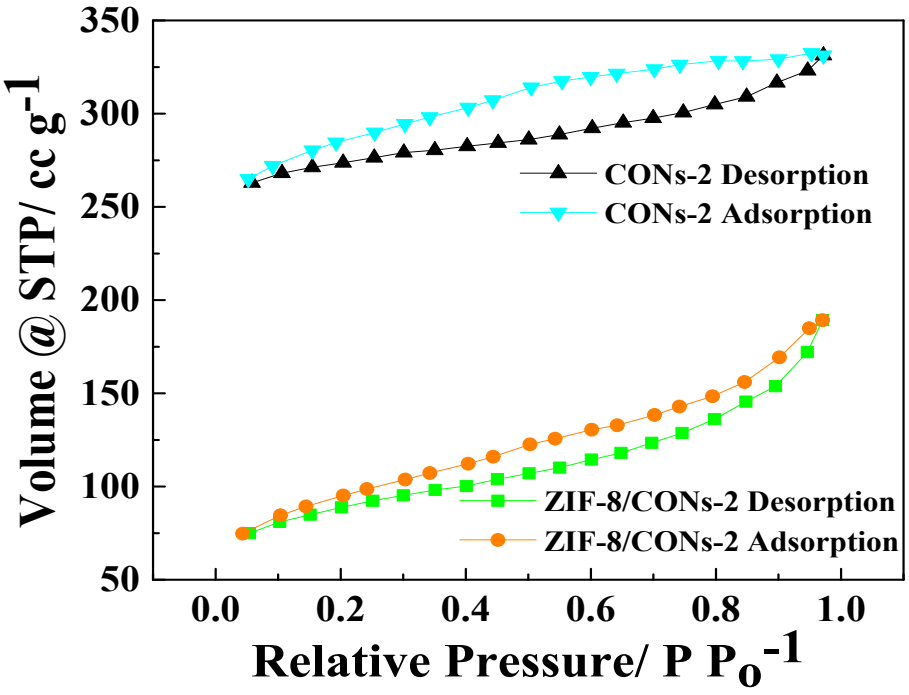

Fig. S1 The N_2 adsorption–desorption isotherm of ZIF-8/CONs-2 and CONs-2.

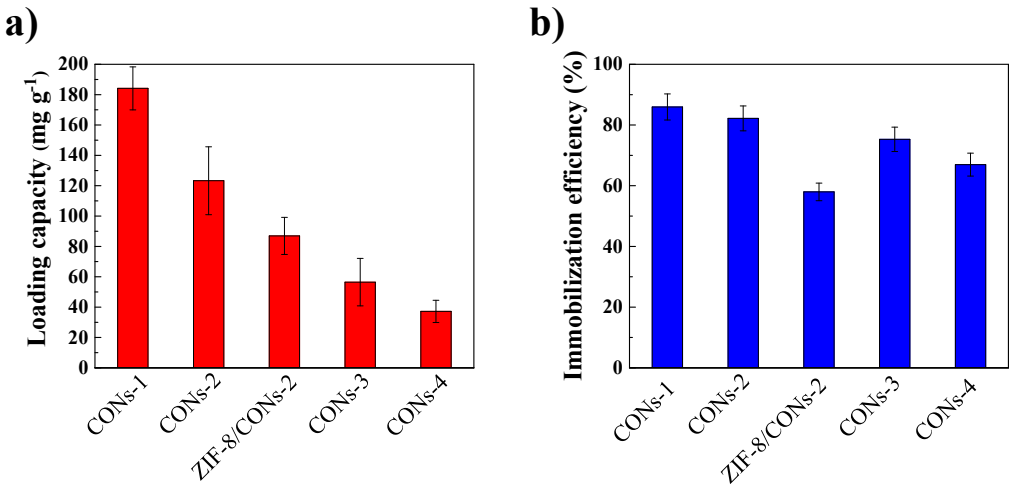

Fig. S2 (a) Immobilization efficiency and (b) loading capacity of CRL on CONs-1, CONs-2, ZIF-8/CONs-2, CONs-3 and CONs-4.

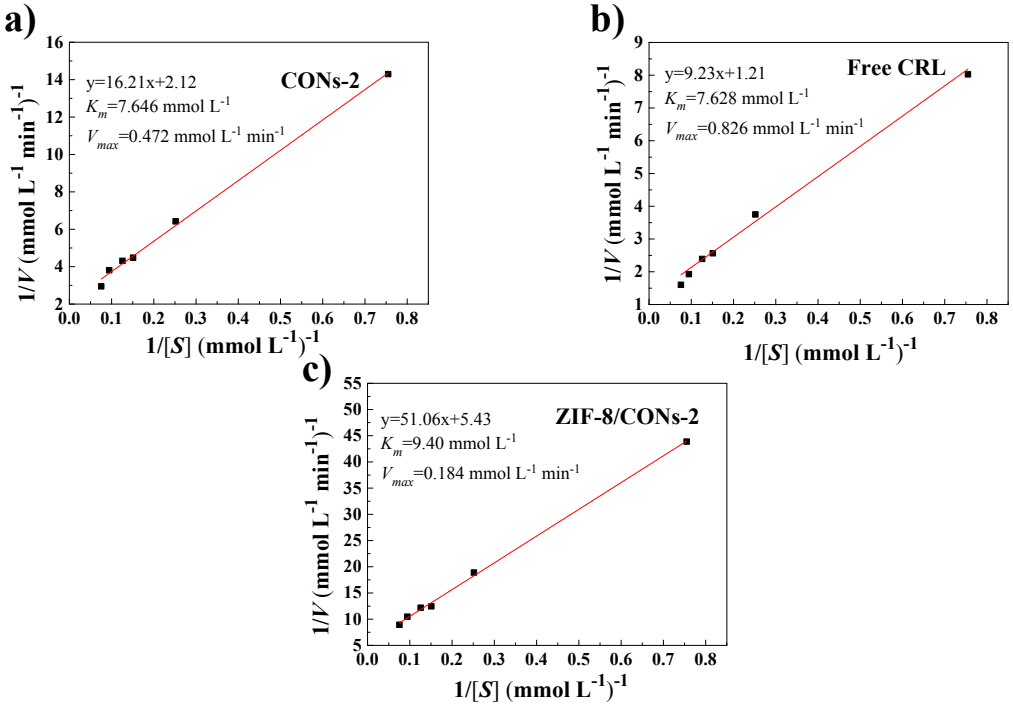

Fig. S3 Kinetic constants of free CRL and CRL@CONs-2, CRL@ZIF-8/CONs-2

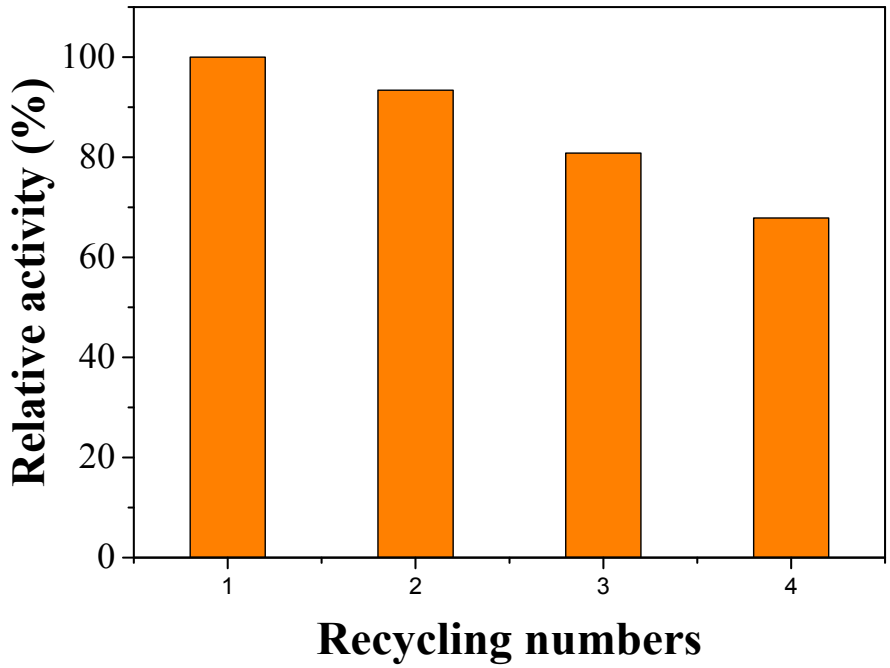

Fig. S4 Recycling stability of CRL@CONs-2 in the hydrolysis of *p*-NPP.

Table S1 List of opening ratio and wall thickness of CONs prepared under different TEOS and APTES concentrations.

	TEOS concentration (mM)	APTES concentration (mM)	Opening ratio (%)	Thickness (nm)
CONs-1	65	15	100	8
CONs-2	80	19	95	11
CONs-3	96	23	47	22.5
CONs-4	128	30	0	43

Table S2 Kinetic constants of free CRL, CRL@CONs-2 and CRL@ZIF-8/CONs-2.

	Free CRL	CONs	ZIF-8/CONs
K_m (mM)	7.628	7.464	9.401
V_{max} (mM min^{-1})	0.826	0.472	0.184
k_{cat} (s^{-1})	0.551	0.383	0.212
k_{cat}/K_m (mM^{-1} s^{-1})	13.844	19.963	44.344

References:

S1. W. Zhang, Q. Li, J. Cong, B. Wei and S. Wang, *Polymers*, 2018, **10**, 216.