Supporting Information

Lipase-entrapped colloidosomes with light-responsive wettability for efficient and recyclable Pickering interfacial biocatalysis

Dingyi Yang, Qi Zeng, Kaiwen Tan, Haoyue Hou, Xingyuan Fang, Chenlong Guo, Hao Yuan and Tao Meng*

[a.] School of Life Sciences and Engineering, Southwest Jiaotong University, Chengdu, Sichuan, 610031, PR China

[b.] E-mail: taomeng@swjtu.edu.cn (T. Meng)

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Experimental Procedures

1. Materials. Lipase (CALB) from Candida sp. expressed in Aspergillus niger and Fluorescein isothiocyanate (FITC) are purchased from Sigma-Aldrich. Anatase TiO$_2$ nanoparticles (5 nm) are purchased from Ningbo Jiweina New Material Technology Co., Ltd. (Ningbo, China). Butanol, Coomassie brilliant blue G 250, phosphoric acid and octane are obtained from Kelong Chemical Reagents Co. Ltd. (Chengdu, China). Nile red is purchased from Aladdin Industrial Corporation (Shanghai, China). Medium chain triglycerides with a density of 0.93 g/ml (MCT, C8:C10=50:50) are purchased from a local supermarket (Chengdu, China). Sodium dihydrogen phosphate (NaH$_2$PO$_4$) is purchased from Guangdong Guanghua Technology Co., Ltd. Disodium hydrogen phosphate (Na$_2$HPO$_4$) is gotten from Tianjin Zhiyuan Chemical Reagents Co., Ltd. Anhydrous ethanol, sodium hydroxide, phenolphthalein and acetone are obtained from Chengdu Shiyang Chemical Reagents Co., Ltd. Deionized water is utilized throughout the study. All the chemicals are of analytical grade.

2. Synthesis of lipase-entrapped TiO$_2$ colloidosomes (LTCs). LTCs are synthesized according to the method of constructing lipase-entrapped hollow colloidosomes reported previously.[1] For immobilized CALB in colloidosomes, 1 mL of lipase solution (commercially available CALB solution diluted 4 times with water), mix with 2 mL of TiO$_2$ dispersion with nanoparticles diameter of 5 nm (10 wt.%) in a 50 mL polyethylene (PE) tube thoroughly, and then adding 30 mL of butanol, are emulsified by centrifugal disperser at 8000 rpm for 1 min (S-10, Ningbo Xinzhi Biotechnology Co., Ltd.). Stand for 24 h. After that, LTCs can be obtained and freeze-drying (SCIENTZ-12N, Ningbo Xinzhi Biotechnology Co., Ltd.).

3. The enzyme loading amount and encapsulation rate of lipases in colloidosomes. The encapsulation rate of lipase in colloidosomes is measured through the Bradford method.[2] More specifically, the system for preparing LTCs use the above step, centrifuge (6000 rpm, 5 min) and remove the butanol phase (Sorvall ST 16R), and then 10 mL up water added to is LTCs, centrifuge, and keep the supernatant. 1 mL of supernatant is added into 5 mL of Coomassie Blue solution (0.2% w/v) and mix homogenously. Allowing the mixture to react for 3 min, the absorbance values are measured at 595 nm and compared to a CALB standard curve to determine the protein concentration. The encapsulation rate of lipases in colloidosomes is calculated

by the equation as shown below, where m_i (mg) is the initial amount of enzyme used for immobilization, m_s (mg) is the amount of enzyme in supernatant.

Encapsulation rate = $(m_i - m_s)/m_i \times 100\%$

The enzyme loading amount (mg/g) of the colloidosomes is calculated using the following formula, where m (g) is the weight of the colloidosomes.

Enzyme loading amount = $(m_i - m_s)/m$

4. Evaluation of the surface wettability of TiO$_2$ nanoparticles and LTCs. The three-phase contact angle of TiO$_2$ nanoparticles and LTCs at the oil-water interface are measured by using the dip-coating method. To obtain compact TiO$_2$ nanoparticles films on a glass slide (5 × 5 mm), the glass slide is dip-coated into a 1% (w/v) TiO$_2$ nanoparticles dispersion in water using a withdrawal speed of 14 cm min^{-1}. The treated glass slide is dried at 25 °C for 30 minutes. To ensure the glass slide is fully covered by nanoparticles, the above deposition process is repeated. Subsequently the glass slide is placed at the bottom of a transparent quartz vessel. Octane is then poured into the vessel. A 3 µL water droplet is carefully placed on the disk surface. The appearance of the water droplet on the substrate is photographed when the droplet is stationary. All of the contact angles that are measured through water are the arithmetic average of at least five measurements on the same sample. The evaluation of the surface wettability of LTCs is the same as that described above.

5. Assessment of light-responsive performance of Pickering emulsions based on LTCs. The prepared slide with LTCs film is placed at the bottom of a transparent quartz container, octane is poured into the container, and a 3 µL water droplet is carefully placed on the surface of the slide. Then, the transparent quartz container is illuminated with an ultraviolet light lamp (ZF-1, Hangzhou Qiwei Instrument Co., Ltd.), and the appearance of the droplets is photographed when TCA did not continue to change. Removes the slide and place it at 25 °C to dry. Then the TCA is re-measured. All of the contact angles measured through octane are the arithmetic average of at least five measurements on the same sample. Typically, the light-responsive PIB system consists of octane and PBS (pH = 7.0), the concentration of LTCs and oil-water ratio are set to 0.75 wt.% and 5:5, and the emulsion is prepared according to the procedure described above and left for 30 minutes at room temperature. Then, the emulsion is irradiated with ultraviolet light (254 nm, 6 w) until it is completely demulsified (Stirring to disperse the emulsions). Then leaving in the dark for 10 minutes and homogenized again for 2 minutes to re-form the emulsion.

6. Assessment of catalytic performance of light-responsive PIB system based on LTCs. Typically, LTCs are used to construct PIB system, which uses octane as the oil phase and PBS (pH = 7.0) as the aqueous phase. Octane (1.0 mL) containing MCT (5 vol%) is added to the aqueous phase (1.0 mL) containing LTCs (0.75 wt.%). Following homogenization, the reaction is carried out in Electric heating constant temperature air drying oven (DHG-9070A, Shanghai One Instrument Science Instrument Co., Ltd.) without stirring at 37 °C. After the required reaction time, 1.0 mL of inactivator (water : ethanol : acetone = 1:1:1) adds to terminate the hydrolysis reaction, and the octanoic acid and decanoic acid generated during the reaction titrate with 0.05 mol·L^{-1} NaOH solution. The titration is vortexed and shaken several times to ensure complete reaction. The calculation of conversion rate is as follows, where C is the conversion rate, V_{NaOH} (mL) is the net consumption of the titrated NaOH solution, c_{NaOH} (mol·L^{-1}) is the concentration of NaOH solution, c_S (mol·L^{-1}) is MCT concentration, V_S (mL) is the amount of MCT:

$C = (V_{NaOH} \times c_{NaOH})/(c_S \times V_S) \times 100\%$

The enzyme activity is defined as: the amount of enzyme that is required to catalyze the hydrolysis of MCT to produce 1 µmol of fatty acid per minute at 37 °C at pH 7.0, which is an enzyme activity unit, symbol by U (U·mg^{-1}). The enzyme activity formula is as follows, c (mol·L^{-1}) is the concentration of NaOH solution, ΔV (mL) is the net consumption of NaOH solution generated

during the titration, m (mg) is the amount of enzyme added, t (min) is the reaction time:

$U = (c \times \Delta V)/(m \times t)$

7. Assessment of the recyclability of light-responsive PIB system based on LTCs. To investigate the hydrolysis reaction of LTCs in oil-in-water Pickering emulsion, the recyclability is studied. Hydrolysis of MCT is performed as described above. After each cycle reaction is completed, the Pickering emulsion is demulsified with ultraviolet light (Stirring to disperse the emulsions), and the oil phase products in the upper layer are directly separated, and the LTCs are used for the next cycle. Then, after adding the new substrate, homogenization is carried out for 2 min for a new cyclic reaction. The new reaction batch are carried out under same conditions as the previous (40 min per cycle). After each cycle, collect the supernatant and use titration to determine relative enzyme activity. The relative enzyme activity (%) we use to investigate the recyclability of the Pickering emulsion stabilized by LTCs, which is defined as the percentage of residual enzyme activity in the Nth reaction cycle and the enzyme activity in the first reaction cycle. The formula is as follows:

Relative enzyme activity (%) = $U_{Nth} / U_{first} \times 100\%$

8. Assessment of optical characteristics of LTCs. Light is an electromagnetic wave, so the optical transport process inside LTCs can be studied using electromagnetic theory, and the optical properties of LTCs can be determined by solving Maxwell's equations.[3] In this study, the Maxwell equations are solved using the Finite-Difference Time-Domain (FDTD) method, and all FDTD cases are implemented using FDTD solutions. By calculating the results, the electric field intensity distribution is obtained, thus reflecting the intensity of light absorption. The simulation model is shown in figure 3f, where the diameter of TiO_2 nanoparticles is set to 5 nm. In fact, the LTCs contain a large number of TiO_2 nanoparticles, calculations would be too complicated, the computer conditions are not capable of achieving realistic calculations. Therefore, we calculate the electric field distribution of only 3-layer TiO_2 nanoparticles. Light is emitted along the axis, the wavelength of light we chose to be 100~400 nm, and the material of all particles we define as anatase titanium dioxide.

9. Characterization techniques. Scanning electron microscope (SEM, ZEISS sigma500, Germany) is used to characterize the morphology of LTCs. Samples is prepared that is 1% (w/v) colloidosome dispersion, dropped on a piece of silicon oxide slides, and dried at 25 °C for 30 min, sprayed with a gold coating to remove supernumerary electrons. Using BET analyze the LTCs (ASAP2460, America). Transmission electron microscope is used to characterize the morphology of TiO_2 nanoparticles (TEM, JEOL-JEM2100F, Japan). Confocal laser scanning microscope (CLSM, Nikon-A1R, Japan) is used to record microscopic images with the excitation wavelength of 488 nm. Before tested, lipases are labeled with fluorescein isothiocyanate (FITC) and Oil phase by Nile red tag. Fluorescence inverted microscope (IX71-A12FL/PH, Olympus, Japan) is used to excite the green fluorescence of FITC-labelled CALB at 488 nm to obtain a fluorescence image. Optical microscope (Phoenix Co. Ltd., XSP-24, China) is used to observe the surface morphological characteristics of the emulsion droplets.

Results and Discussion

10. Formation mechanism diagram of LTCs.

Figure S1 shows the self-assembling mechanism for the fabrication of LTCs. The lipases and TiO_2 nanoparticles are evenly dispersed into the water phase, and the outer phase is butanol. After emulsification, the water emulsion droplets containing the TiO_2 nanoparticles and lipases are suspended in butanol. These TiO_2 nanoparticles spontaneously are absorbed at the emulsion interface, driven by the minimization of the total interfacial free energy.[4] Because water has the appropriate dissolubility in butanol, after a while, a small amount of water diffuses into surrounding butanol, making the emulsion dispersed phase volume smaller, the surface area reduced, and the original nanoparticle layer is compressed, which led to the nanoparticles are closely packed and wrapped on the surface of droplets. As water continues to diffuse into butanol, TiO_2

nanoparticles and lipases in water accumulate into shell, forming hollow and multilayer colloidosomes where the enzyme is confined in porous shell. And the lipase in the butanol phase can be negligible, because the lipase cannot cross the water-butanol interface.[1] The powder form of LTCs obtained after lyophilization.
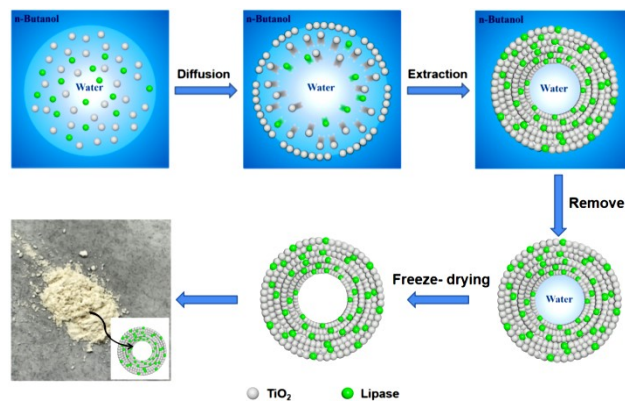

Figure S1. Formation mechanism diagram of LTCs.

11. Effect of TiO$_2$ nanoparticle concentration (C_{TiO2}) on the morphology of colloidosomes.

In the process of preparing colloidosomes, the moderate concentration of nanoparticles is essential for the formation of regular colloidosomes.[1] As shown in Figure S2a-d, when C_{TiO2} is low, colloidosomes with sphericity can be formed. As the concentration increases, the colloidosomes exhibit better spherical structure and dispersion. Further increasing the C_{TiO2} leads to larger size of colloidosomes, which is not conducive to stabilize Pickering emulsion. As shown in Figure S2f and S2g, the average size of LTCs (C_{TiO2} = 15 wt.% and 20 wt.%) are 2.99 µm and 6.59 µm respectively. Moreover, when the concentration is very high, a large amount of TiO$_2$ nanoparticles dispersed in the water phase aggregate together, causing disordered precipitation of TiO$_2$ nanoparticles. And the effect of C_{TiO2} on the mean diameters of the colloidosomes is shown in Figure S2e. Therefore, the C_{TiO2} is 10 wt.% that formed a regular shape of colloidosomes, which is more conducive to subsequent experiments. That is, the system of preparing colloidosomes is: 2 mL of TiO$_2$ dispersion (10 wt.%) and 1 mL of PBS solution fully mixed, then add 30 mL of butanol.

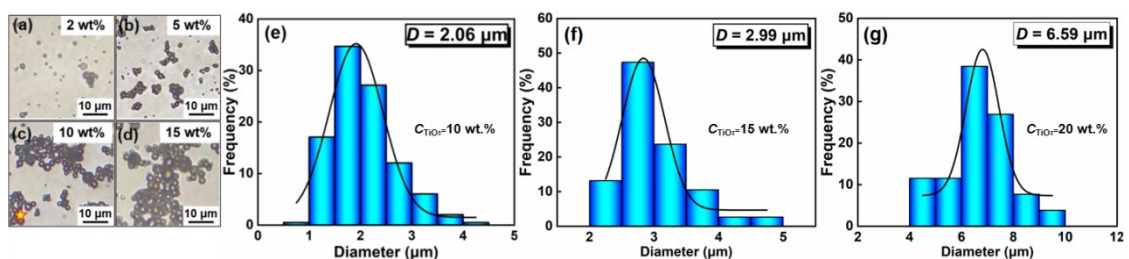**Figure S2.** (a-d) Optical microscopy images of LTCs with varied TiO$_2$ nanoparticles concentrations. C_{TiO2} (wt.%): (a) = 2 wt.%, (b) = 5 wt.%, (c) = 10 wt.%, (d) = 15 wt.%. (e-g) the size distributions of LTCs (C_{TiO2} = 10 wt.%, 15 wt.% 20 wt.%), the D is average size, the scale bar means 10 µm.

12. FT-IR spectra of LTCs.

To further confirm the successful assembly of lipase in LTC, as shown in Figure S3, the amide III band at 1220 cm^{-1} ~ 1330 cm^{-1} is the characteristic absorption peak of the protein, which provides direct evidence for successful loading of the lipase.
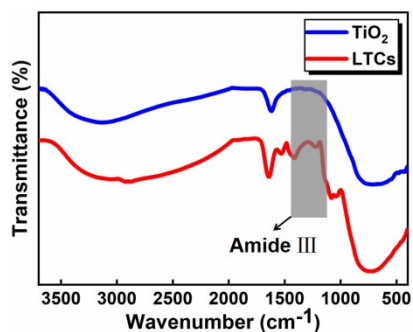
Figure S3. FT-IR spectra of LTCs and TiO$_2$ nanoparticles.

13. The BET analysis of LTCs.

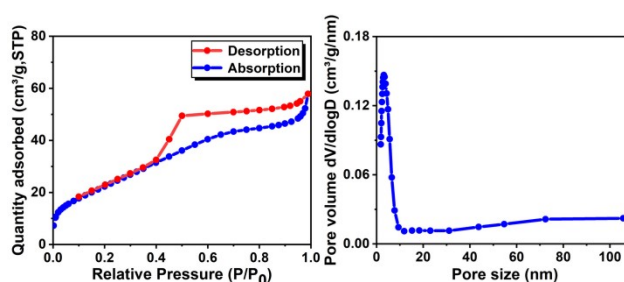The pore structure of LTCs is analyzed through nitrogen adsorption-desorption isotherms, as depicted in Figure S3.

Figure S4. The nitrogen adsorption-desorption isotherms and pore size distribution of LTCs.

14. Transmission electron microscope (TEM) image of TiO$_2$ nanoparticles.

To observe the aggregation state of TiO$_2$ nanoparticles, we employ transmission electron microscopy to examine a dispersion of TiO$_2$ nanoparticles at certain concentration, as shown in Figure S3.
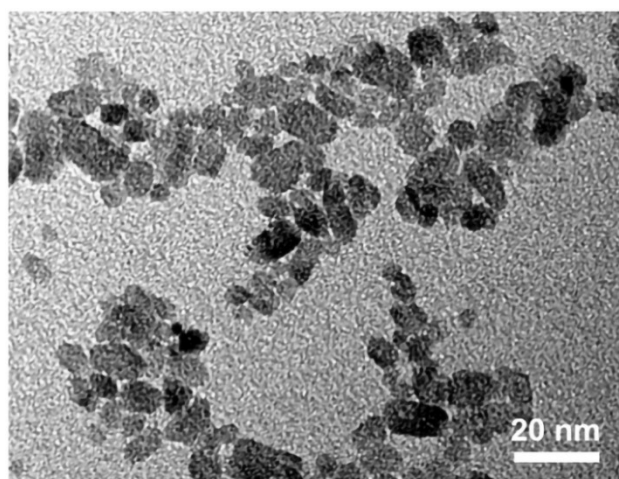

Figure S5. TEM image of TiO$_2$ nanoparticles, the scale bar means 20 nm.

15. Evaluation of the leakage of lipase from LTCs in water.

In order to investigate the leakage of lipase during use of LTCs, the LTCs are subjected to a 24-hour immersion experiment in water (Figure S5). The computational results indicate that the loading amount (13.59 mg/g) of lipase within LTCs remains virtually unchanged over a 24-hour period. And the fluorescent images show that the FITC-labelled CALB

encapsulated in colloidosomes does not diffuse into water over time, and the spherical shape of LTCs remains intact in the experiment.
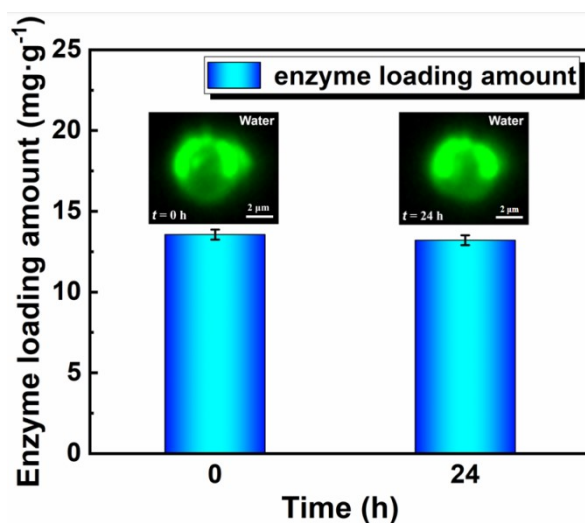

Figure S6. Immersion experiment of FITC-labelled CALB from LTCs in water, fluorescent images and enzyme loading amount taken at 0h and 24h, the scale bar means 2 μm.

16. Effect of different TiO$_2$ concentrations on the encapsulation efficiency of LTCs.

To evaluate the effect of different TiO$_2$ concentrations on the encapsulation efficiency of LTCs, we test the encapsulation efficiency of LTCs prepared with different TiO$_2$ concentrations (2wt.%, 5wt.%, 10wt.% and 15wt.%). As shown in Figure S7, the encapsulation efficiency of enzyme for the colloidosomes increases from 75% to 92% with the concentration of TiO$_2$ nanoparticles from 2 wt.% to 10 wt.%. This is attributed to the higher concentration of TiO$_2$ nanoparticles leading to the more stable encapsulation of enzymes.
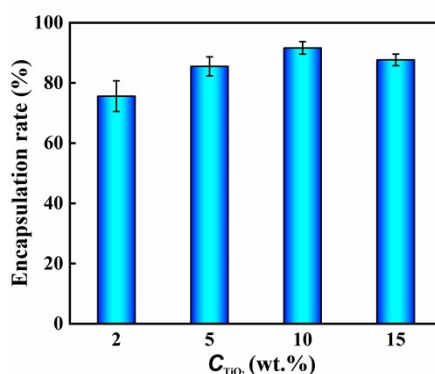

Figure S7. Encapsulation efficiency of LTCs at different TiO$_2$ concentrations (C_{TiO_2}=2 wt.%, 5 wt.%, 10 wt.%, 15 wt.%).

17. Effect of different TiO$_2$ concentrations on the enzyme loading of LTCs.

As shown in Fligure S8, the enzyme loading amount increases from 8.69 mg/g to 53.66 mg/g with the C_{TiO_2} from 15 wt.% to 2 wt.%. This is attributed to an increase in the concentration of TiO$_2$ nanoparticles leading to an increase in the weight of LTCs. This proves that the immobilization enzyme loading amount (mg/g) in this study can be optimized through the concentration of TiO$_2$ nanoparticles to support the construction of a more efficient biocatalytic system.
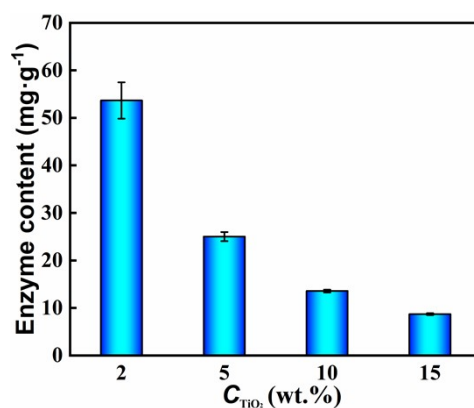
Figure S8. Enzyme loading amount of LTCs at different TiO_2 concentrations (C_{TiO_2}=2wt.%, 5wt.%, 10wt.%, 15wt.%).

18. Evaluation of the surface wettability of TiO_2 nanoparticles and LTCs.

In order to prove that the amphiphilic lipase changes the wettability of colloidosomes, we measure the three-phase contact angle of TiO_2 nanoparticles before and after loading lipase. As is seen in Figure S6, the contact angle of the LTCs is 73°, which is bigger than that of the TiO_2 nanoparticles is 8°, making LTCs have a suitable wettability to stabilize oil-in-water Pickering emulsion.
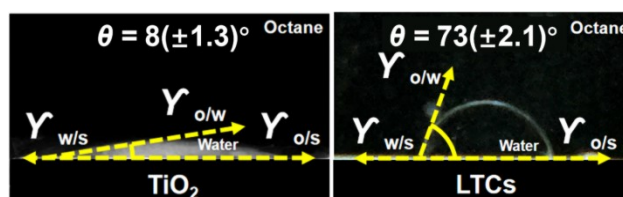

Figure S9. Three-phase contact angle of TiO_2 nanoparticles and LTCs.

19. Effect of different enzyme concentrations on the tri-phase contact antennae (TCA) of LTCs.

As shown in Figure S10, the three-phase contact angle (TCA) of LTCs increases from 47° to 67° with the concentration of enzyme from 1.2 mg/mL to 2.4 mg/mL. This is attributed to an increase in amphiphilic lipase concentration that brought the wettability of LTCs closer to 90°.
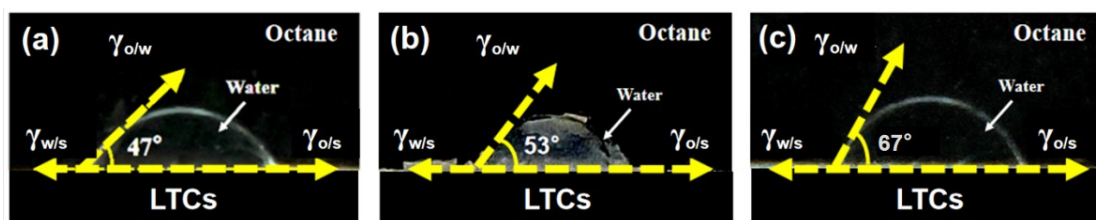

Figure S10. TCA of LTCs at the different enzyme concentrations (C_{enzyme}=1.2 mg/mL, 1.6 mg/mL, 2.4 mg/mL).

20. Evaluation of Pickering emulsion types based on LTCs.

To ascertain the type of stable Pickering emulsion formed by LTCs, we characterize the emulsion using laser confocal scanning electron microscopy, with the oil phase labeled by Nile Red.
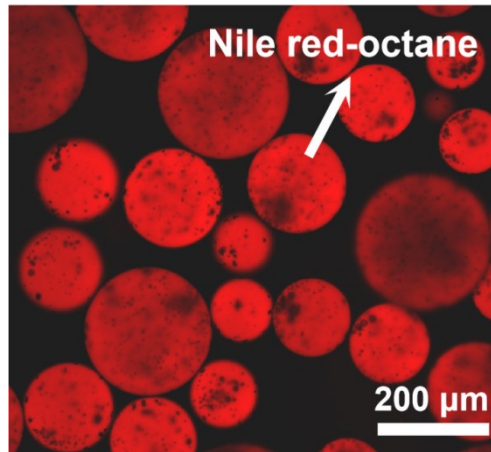
Figure S11. CLSM images of Pickering emulsions stabilized by LTCs, red represents the Nile red-stained oil phase, the scale bar means 200 μm.

21. Effect of LTCs concentration (C_{LTCs}) and oil-water ratio on the diameters of Pickering emulsion droplets.

The concentration of the emulsifier and oil-water ratio is crucial factors that affect the interface area of Pickering emulsions, as they is adjusted to increase the reaction area and improve catalytic efficiency[4]. As shown in Figure S12a1-a4 and S12c, the size of the Pickering emulsions progressively decreases (309 μm to 143 μm) with an increase in concentration of LTCs (0.25 wt.% to 0.75 wt.%). However, when the concentration of LTCs is further increased to 1.00 wt.%, the size of the Pickering emulsions remains almost unchanged (145 μm). According to the theory of restricted aggregation,[4] the size of emulsions decreases as the concentration of the emulsifier increases, more particles need to be removed in the coalescence of droplets is due to adsorption of more emulsifier at the interface, resulting in the formation of a more stable Pickering emulsion. Once the emulsifier reaches saturation, the size of the emulsions no longer decreases. Similarly, as shown in Figure S12b1-b4 and S12d, the size of the Pickering emulsions progressively decreases (450 μm to 139 μm) with a decrease in the oil-water ratio (7:3 to 5:5). However, when the oil-water ratio further decreases to 4:6, the size of the Pickering emulsions increases to 175 μm. Furthermore, according to the mechanism of the three-dimensional viscoelastic particle network,[6] soild particles in the continuous phase can slow down or inhibit the aggregation of the emulsions. However, a lower concentration of LTCs due to an increase in water results in an unstable three-dimensional network, which also explains why the size of the emulsions at a 1.00 wt.% concentration of LTCs remains unchanged.
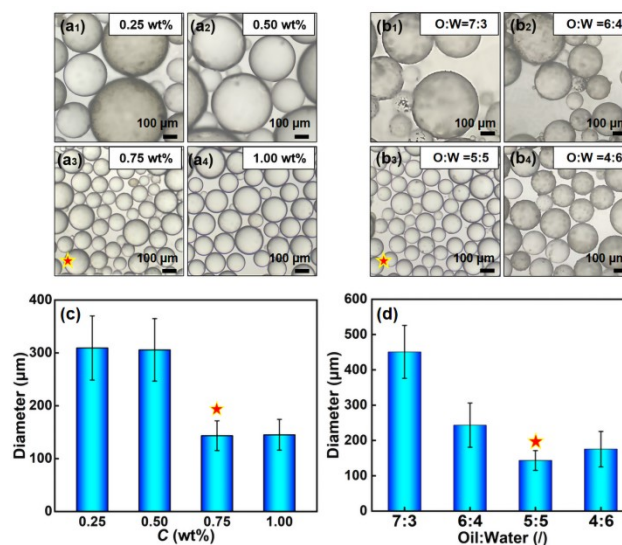
Figure S12. (a_1-a_4) Micrographs of Pickering emulsions formed under different concentrations of LTCs and (c) corresponding droplet diameter distributions (oil-to-water ratio of 5:5). (b_1-b_4) Micrographs of Pickering emulsions formed under different water-oil ratios and (d) corresponding droplet diameter distributions (LTCs concentration of 0.75 wt.%), the scale bar means 100 μm.

22. Evaluation of Stability of Pickering emulsion.

To investigate the stability of the Pickering emulsion stabilized by LTCs (C_{LTCs} = 0.75 wt.%, octane : water=5:5), we allow the emulsion to stand at room temperature (25 ℃) for 60 days, monitoring changes in the morphology and size of the emulsion droplets, as depicted in Figure S9.
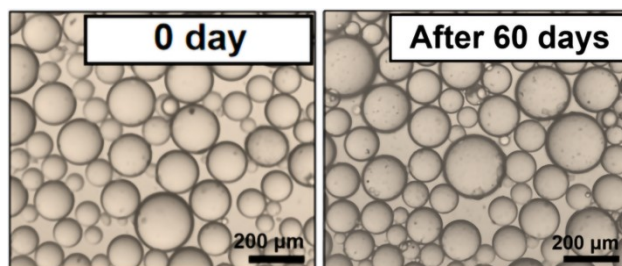

Figure S13. Optical microscopy images of Pickering emulsions at the 0 day and 60 days, the scale bar means 200 μm.

23. Assessment of demulsification process of light-responsive Pickering emulsions.

As shown in Figure S14, the Pickering emulsions stabilized by LTCs are gradually demulsified during UV irradiation within 30 minutes.
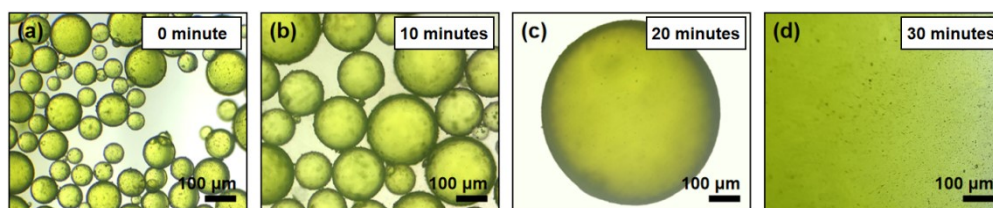

Figure S14. Micrograph of the light-responsive demulsification process of a Pickering emulsion, with the oil phase (MCT) stained with curcumin, the scale bar means 100 μm.

24. Evaluation of the effect of agitation on demulsification of light-responsive Pickering emulsions.

To determine the light-responsive behavior of the emulsion, we observe the changes in the morphology and size of the emulsion under the condition of only stirring, as shown in Figure S10. The results indicate that there are no significant changes in the morphology and size of emulsions before and after 30 minutes of stirring.
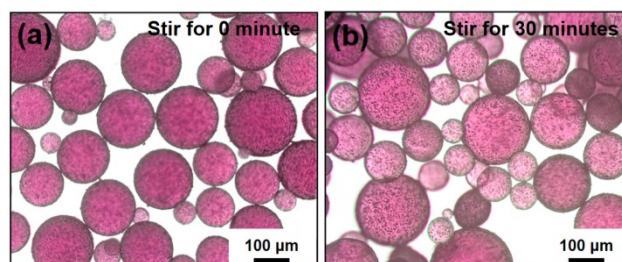
Figure S15. Optical microscopy images of Pickering emulsions stabilized by LTCs before and after 30 min of stirring, Nile Red-labeled oil phase, the scale bar means 100 μm.

25. Enzymatic kinetics studies of PIB system.

We further analyzed the enzymatic reaction kinetic parameters of the PIB system by adjusting the substrate concentration (Figure S16). Michaelis-Mentenin constants, including k_m (affinity of enzyme to substrate), V_{max} (maximum reaction rate of the reaction when the active site of the enzyme is saturated with substrate), and turnover number k_{cat}, are given in Table S1.
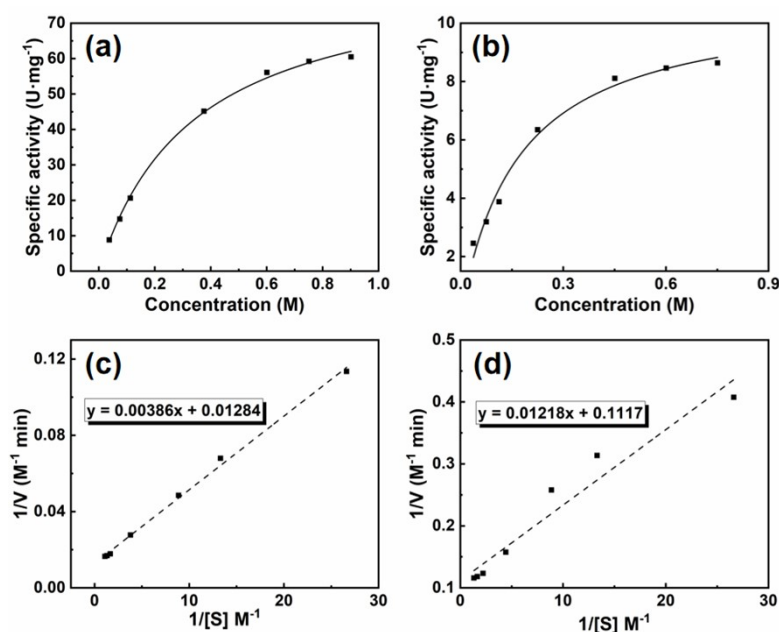

Figure S16. Enzymatic kinetics studies of PIB (a, c) and two-phase systems (b, d).

Note: The kinetic parameters Michaelis constant k_m and the maximum reaction rate V_{max} were calculated using the Lineweaver-Burk equation:

$$\frac{1}{V} = \frac{k_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

where V is the initial reaction rate, and $[S]$ is the concentration of the substrate.

Table S1. Comparison of enzymatic kinetics parameters of PIB and two-phase systems.

	k_m (M)	V_{max} (M·min^{-1})	k_{cat} (min^{-1})
PIB (LTCs)	0.3006	7.7882×10^{-5}	12.6121
Biphasic (Free lipase)	0.1090	8.9526×10^{-6}	1.4498

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