Supporting Information

Light Controlled Reversible Inversion of Nanophosphor-stabilized Pickering Emulsions for Biphasic Enantioselective Biocatalysis

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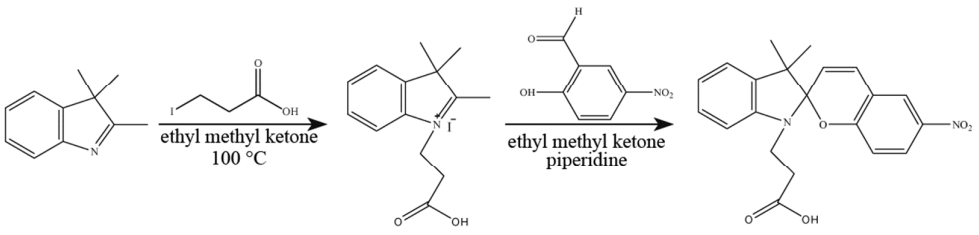
Experimental section:

Reagents and materials: Nanopure water (18.2 MΩ; Millpore Co., USA) was used in all experiments and to prepare all buffers. Tetraethylorthosilicate (TEOS), (3-aminopropyl)triethoxysilane, NaOH, NH_4F, oleic acid, 1-Octadecene, mandelic acid, mandelonitrile, 2, 3, 3-trimethylindolenine, 3-iodopropanoic acid, and 5-nitrosalicylaldehyde were purchased from Sigma-Aldrich. $YCl_3 \cdot 6H_2O$, $YbCl_3 \cdot 6H_2O$, $TmCl_3 \cdot 6H_2O$ were provided by Aladdin Reagent (Shanghai, China). All chemical agents were of analytical grade and used directly without further purification.

Syntheis of carboxy containing spiropyrans (Sp-COOH): During the synthesis, all the reaction vessels were wrapped in aluminum foil to ensure the reaction was performed in the dark. The Sp-COOH [1'-(β-carboxyethyl)-3', 3'-dimethyl-6–nitrospiro[indoline-2', 2-chromane] (denoted as Sp-COOH) was synthesized as shown in Scheme S1. 2,3,3-Trimethylindole-nine (0.06 mol), 3-iodopropanoic acid (0.06 mol), and ethyl methyl ketone (5 mL) were heated under nitrogen at 100 °C for 3 h. The resulting solid material was dissolved in water and the solution was washed with chloroform. Evaporation of water gave l-(β-carboxyethyl)-2, 3, 3-tri-methylindolenine iodide. This iodide (0.04 mol), 5-nitrosali-cylaldehyde (0.04 mol), and piperidine (3.8 mL, 0.04 mol) were dissolved in ethyl methyl ketone, and the red solution was refluxed for 3 h. On standing overnight, the product precipitated as a yellow crystalline powder. This was filtered and washed with methanol to give the product SP-COOH. The ^{1}H NMR spectrum of SP-COOH is shown in Figure S1 in the Supporting Information. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ = 1.0–1.3 (6H; 2C***H***$_3$), 2.6 (2H; C***H***$_2$COO), 3.4–3.5 (2H; C***H***$_2$N), 5.9–6.0 (2H; olefinic protons), 6.6–8.2 (aromatic protons).



Scheme S1. Synthesis of the carboxy containing spiropyran compound.

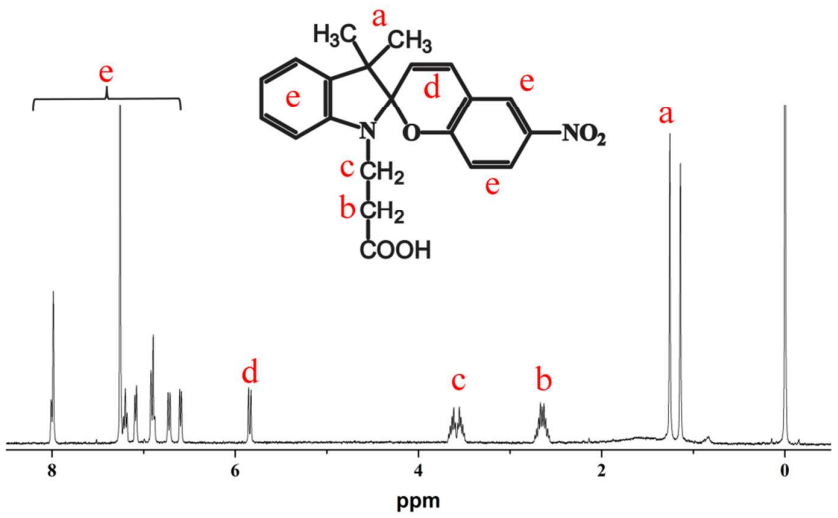
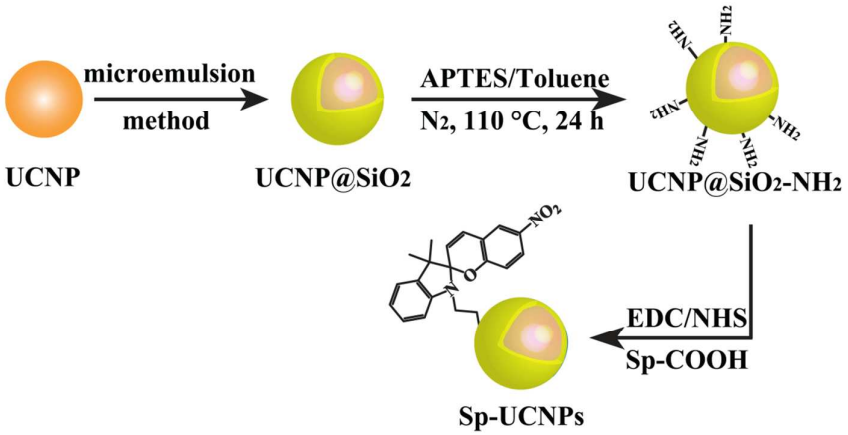


Figure S1. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS) spectra of the synthesized Sp-COOH.

Synthesis of upconversion nanoparticles (UCNPs): β–$NaYF_4$: Yb/Tm (25/0.3 mol %) nanocrystals were synthesized according a modified method as previously reported. In a typical experiment, YCl_3, $YbCl_3$, and $TmCl_3$ were mixed with 12 mL oleic acid and 15 mL octadecene in a 100 mL flask. The solution was heated to 140 °C to form a homogeneous solution and remove residual water and oxygen. The temperature was then cooled to room temperature with a gentle flow of high-pure nitrogen gas through the reaction flask. A 10 mL methanol solution containing NaOH (2.5 mmol) and NH_4F (4 mmol) was slowly added into the flask and stirred for 30 min. The solution was slowly heated to remove methanol, degassed at 100 °C for 30 min, and then heated to 300 °C and maintained for 1.5 h under high-pure nitrogen protection. After the solution was cooled naturally, resulting nanoparticles were precipitated out by the addition of ethanol, collected by centrifugation, washed with ethanol for several times, and finally re-dispersed in 4 mL cyclohexane.



Scheme S2. Schematic illustration of the synthesis of spiropyran conjuagated upconversion nanolamps.

Synthesis of silica coated upconversion nanoparticles (UCNPs@SiO_2): CO-520 (0.1 mL), cyclohexane (6 mL) and UCNPs solution in cyclohexane (4 ml, 0.01M) were mixed and stirred for 10 min. Then CO-520 (0.4 mL) and ammonia (0.08 mL, 28 wt %) were added and the container was sealed and sonicated for 20 min until a transparent emulsion was formed. TEOS (0.04 mL) was then added into the solution, and the solution was rotated for two days at a speed of 600 rpm. Silica/UCNPs were precipitated by adding acetone, and washed with ethanol/water (1:1 v/v) twice and then dried at 70°C in vacuum.

Synthesis of amino functionalized UCNPs@SiO_2 (UCNPs@SiO_2-NH_2): UCNPs@SiO_2 (100 mg) were added to dry toluene (60 mL) and suspended as a result of stirring and sonication. (3-aminopropyl)triethoxysilane (APTES) (100 µL) was added to the mixture using a micropipette. The solution was placed under N_2 and heated under reflux for 24 hours. The nanoparticles were removed from solution by centrifugation and washed with toluene, THF, and EtOH, sequentially. The material was dried for 24 hours using vacuum. The presence of amino groups was confirmed by FTIR spectroscopy using the KBr pellet method (Figure S2).

Synthesis of spiropyran conjugated UCNPs@SiO_2 (Sp-UCNPs): During the synthesis, all the reaction vessels were wrapped in aluminum foil to ensure the reaction was performed in the dark. SP-COOH (200 mg, 0.526 mmol) was firstly dissolved in 30 mL dimethylformamide (DMF). Then EDC (500 mg) and NHS (400 mg) in 4 mL MES buffer (pH 6.0, 20 mM) were added to the solution. After agitating overnight at temperature in the dark, the solution was mixed 100 mg

UCNPs@SiO_2-NH_2 in 4 mL MES buffer. The resulting solution was stirred at room temperature in dark for 48 h and then centrifuged at 12000 rpm to separate the precipitate. The resulting black was sonicated and extensively washed three times with DMF and ethanol to remove the physisorbed SP-COOH.

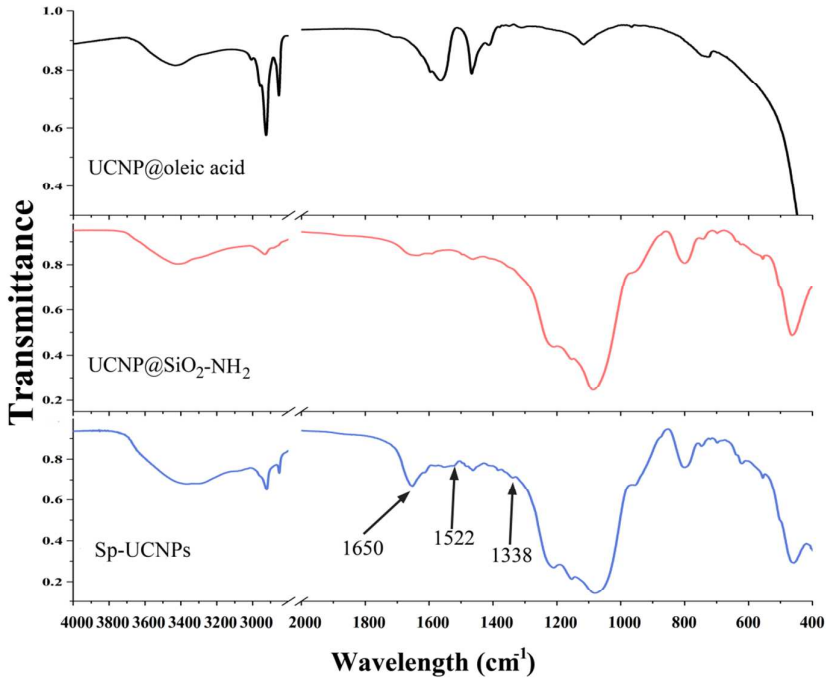


Figure S2. FTIR spectra of oleic coated UCNPs, UCNPs@SiO_2-NH_2, and Sp-UCNPs respectively.

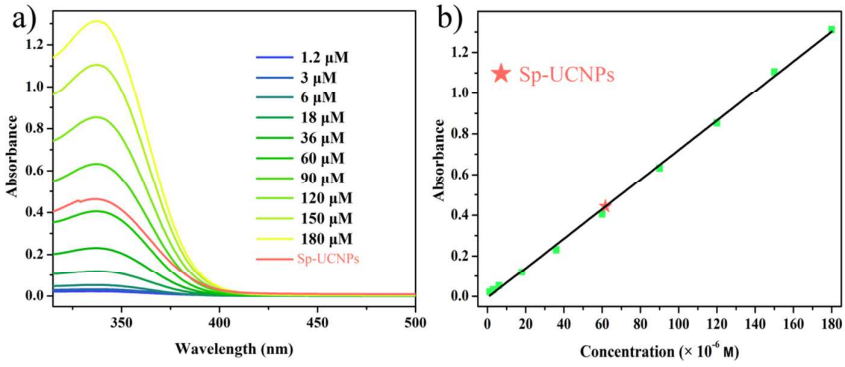


Figure S3. The grafting amount of spiropyrans on UCNP@SiO_2-NH_2 was calculated using the UV-vis absorption spectroscopy. a) Absorption spectra of the carboxyl-containing spiropyrans with different concentrations of 1.2 - 180 μM in THF. b) The absorbance at 337 nm as a function of spiropyran concentration. The UV-Vis absorption of spiropyran in the Sp-UCNPs was obtained by subtraction of the absorbance contributed by UCNPs. The spiropyran content (red star point) of Sp-UCNPs (0.3 mg/mL in THF) was determined to be 7.6 wt%.

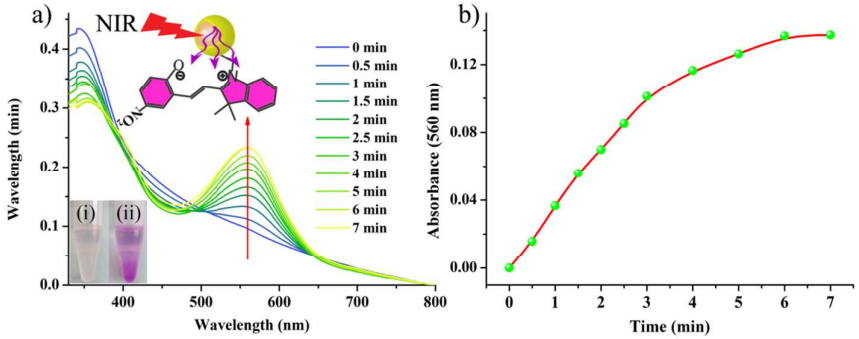


Figure S4. a) UV-vis spectra of Sp-UCNPs (0.1 mg/mL in toluene) irradiated by NIR laser for different time. Inset (i) and (ii) photographs of colloid suspensions of Sp-UCNPs in toluene before and after NIR irradiation, respectively. b) The absorption at 560 nm subtraction of the absorbance contributed by UCNPs is depended on the time of irradiation.

Preparation of Sp-UCNPs stabilized Pickering Emulsion: Typically, 1.5 mL of deionized water was added into a 4 mL EP tube. Then, 1.5 mL of oil (toluene or ethyl acetate) containing 0.015 g Sp-UCNP was then added into the tube. After vigorously shaking with Vortex Genie 2 (Scientific Industries) at 80%×50 Hz for about 4 min, Sp-UCNPs stabilized w/o emulsions could be obtained. For NIR induced emulsion inversion, after illuminating with 980 nm NIR laser in dark at 2 W for 7 min until the colloids became violet color, the solution was then homogenized by Vortex Genie 2 for about 4 min to achieve o/w emulsions. For visible light induced emulsion inversion, after exposing to ambient visible light for about 5 min until the colloids became colorless, the solution was then homogenized by Vortex Genie 2 for about 4 min to achieve w/o emulsions.

Characterization of Emulsions: The emulsion type was determined with an optical microscope after dilution with water or oil and, where necessary, by addition of the water-soluble fluorescein isothiocyanate labeled dextran (Dex-FITC). Furthermore, the emulsion type was determined by measuring the conductivity of the continuous phase using a conductivity meter (DDS-11A, Rex, Shanghai). Typically, the Pickering emulsion containing toluene and 0.01 M NaCl aqueous solution (1:1, v/v) was prepared as above. The conductivity of the continuous phase was measured after the formation of different type of Pickering emulsion. If the conductivity is above 1000 μs/cm, the emulsion type is O/W, and if the conductivity is below 1 μs/cm, the emulsion type is W/O (as shown Figure S7).

Characterization of Water Contact Angles (WCA): For the contact angle measurement, the particles were deposited on a filter paper by filtration and dried. Enough particles were used to

form a dense pack to eliminate the influence of the substrate. Water contact angles were measured a contact angle goniometer (DSA, KRUSS GMBH, Germany) by the sessile water drop method with 2 μL water drops. The WCA values were recorded after 3 s from droplet deposition. The surface was then dried and illuminated with the appropriate wavelength of light, and the next set of contact angle measurement was taken. Reported data are averages of at least 10 measurements at different places on the each sample.

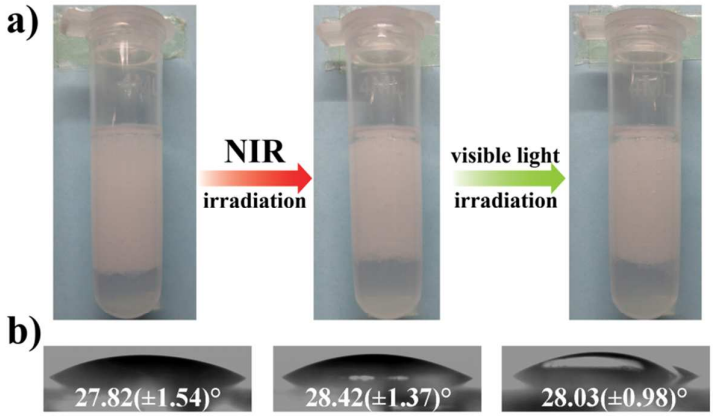


Figure S5. a) Photographs of Pickering emulsions stabilized by UCNPs@SiO_2 treated with different wavelength of light. b) Water contact angles of UCNPs@SiO_2 treated with the corresponding wavelength of light used in a). No light-induced changes were observed during the whole process.

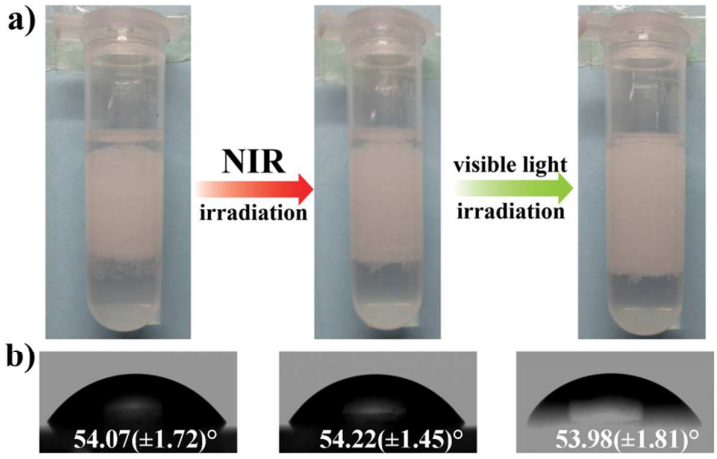


Figure S6. a) Photographs of Pickering emulsions stabilized by UCNPs@SiO_2-NH_2 treated with different wavelength of light. b) Water contact angles of UCNPs@SiO_2-NH_2 treated with the corresponding wavelength of light used in a). No light-induced changes were observed during the whole process.

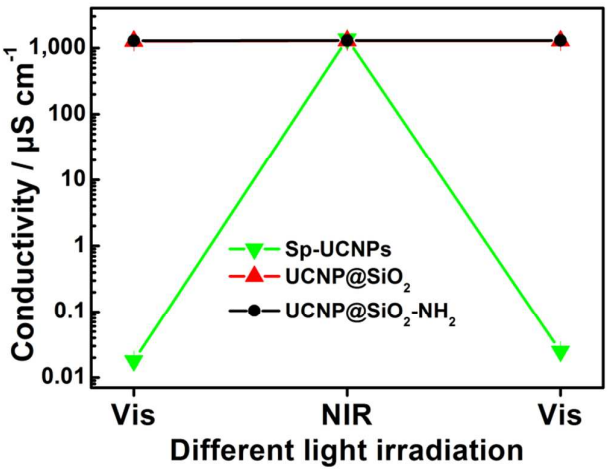


Figure S7. The plot shows the conductivity variations of Pickering emulsions of toluene and 0.01 M aqueous NaCl (1:1) stabilized by Sp-UCNPs, UCNP@SiO_2 and UCNP@SiO_2-NH_2 particles upon different light irradiation. Error bars were estimated from three independent measurements.

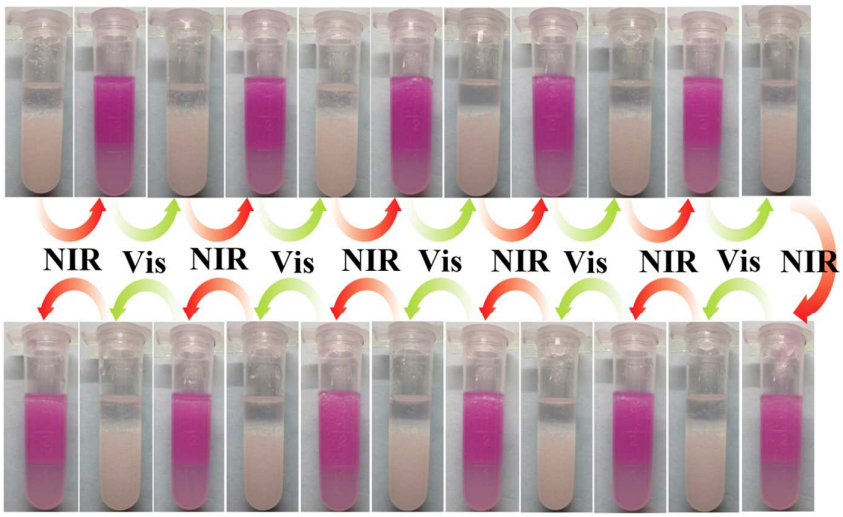


Figure S8. Photographs of successive NIR/visible (Vis) light controlled Sp-UCNPs stabilized Pickering emulsion inversion using **toluene** as the oil phase (ten cycles). The recycle process needed neither change in temperature nor addition of any auxiliary chemicals.

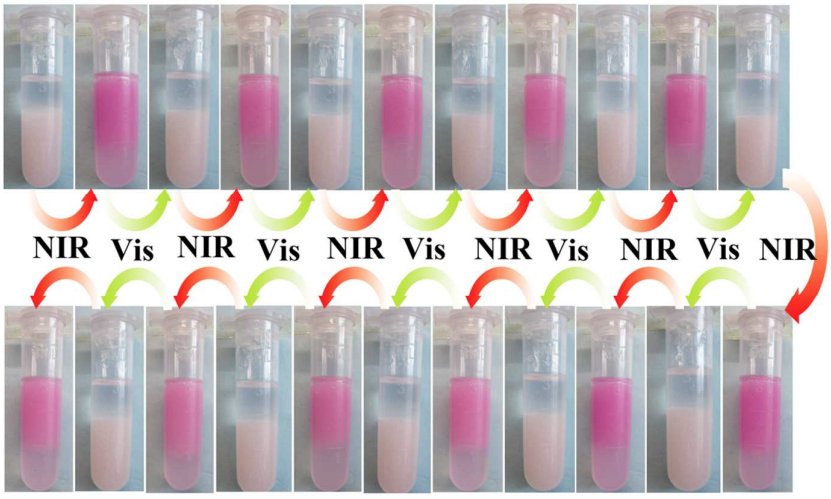
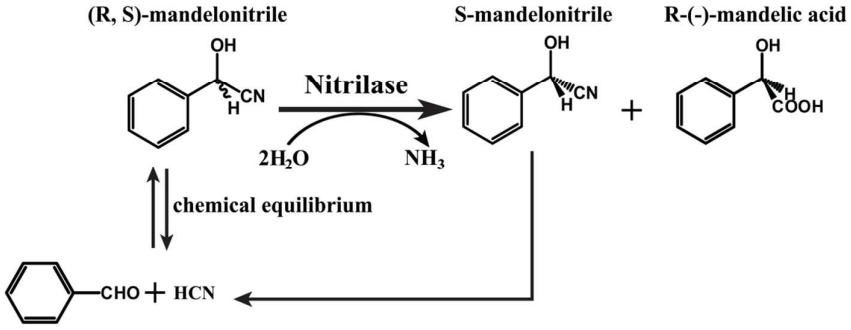


Figure S9. Photographs of successive NIR/visible (Vis) light controlled Sp-UCNPs stabilized Pickering emulsion inversion using **ethyl acetate** as the oil phase (ten cycles). The recycle process needed neither change in temperature nor addition of any auxiliary chemicals.

Microorganism and Culture Conditions: Alcaligenes faecalis ATCC 8750 (ATCC 8750) was used as the biocatalyst. The culture medium contained (per liter) 10 g of L-Glutamic acid, 5 g of yeast extract D-3, 5 g of peptone, 1.2g of KH_2PO_4, 0.8 g of K_2HPO_4, 0.2 g of $MgSO_4 \cdot 7H_20$, 30 mg of $FeSO_4 \cdot 7H_2O$, 1 g of NaCl and 3 g of n-butyronitrile (pH 7.2). A culture that had first been grown in 3 ml of culture medium was incubated to 100 ml of the same medium in a 500 ml flask and cultured at 32°C with reciprocal shaking (250 rpm). After 18 h of culture, cells were harvested by centrifugation at 9,000 rpm at 5°C, and then washed with 0.05 M potassium phosphate buffer, pH 7.0, containing 1 mM dithiothreitol (DTT) and 0.1 mM EDTA. The scanning electron microscopy (SEM) of ATCC 8750 is shown in Figure S9.



Scheme S3. Reaction mechanism for R-(-)-mandelic acid production from mandelonitrile by alcaligenes faecalis ATCC 8750.[44]

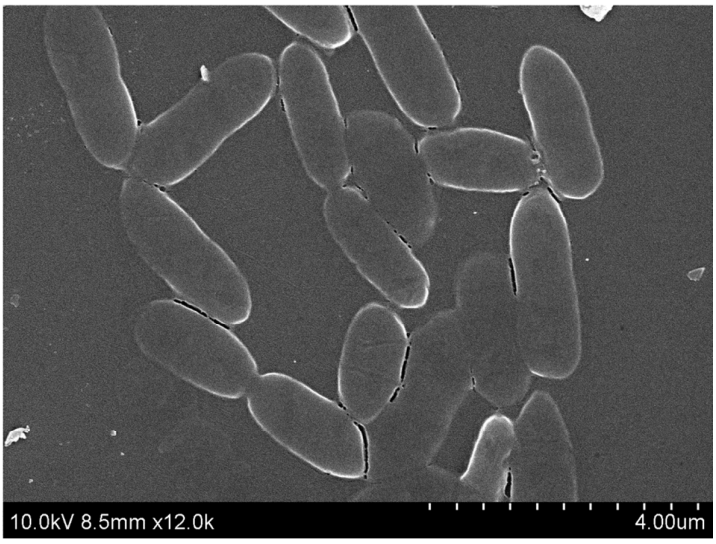


Figure S10. SEM image of Alcaligenes faecalis ATCC 8750.

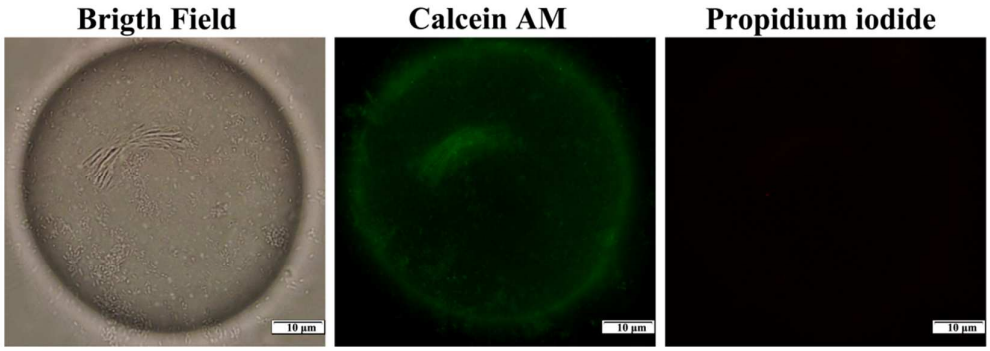


Figure S11. Fluorescence microscopy images of Alcaligenes faecalis ATCC 8750 immobilized in Sp-UCNPs stabilized Pickering emulsion. The microbes were co-stained with calcein AM and propidium iodide after immobilization. The appearance of significant green fluorescence and negligible red fluorescence indicated that most of the encapsulated cells were still viable.

Assessment of the Catalytic Performance of Alcaligenes faecalis ATCC 8750: Typically, for Pickering emulsion based catalysis, certain amount (shown in Figure 4) of mandelonitrile was added to the above Pickering emulsion containing 2 mg/ml microbe in the aqueous phase. The reactions were carried out on the Vortex Genie 2 with 50%×50 Hz at 32 °C. To determine the substrate conversion efficiency, a 5 µL aliquot of the toluene solution was removed at different reaction times and analyzed by UV-vis techniques utilizing the calibration curve of mandelonitrile in toluene (absorbance at λ=289 nm, shown in Figure S11). For reactions in biphasic reactions without Pickering emulsions, a solution of mandelonitrile in 1.5 mL toluene was simply added to 1.5 mL aqueous solution containing 2 mg/ml microbe. Then the mixture was shaken on the Vortex Genie 2 at 32 °C. The substrate conversion efficiency was determined similarly to that of

Pickering emulsion based systems. The hydrolysis of mandelonitrile in monophasic system was performed in 1.5 mL aqueous solution containing 2 mg/ml microbe and 5% (v/v) of methanol as cosolvent. Then the mixture was shaken on the Vortex Genie 2 at 32 °C. Samples were then taken and extracted with toluene for determining the substrate conversion efficiency. In the above systems, 0.1 M potassium phosphate buffer (pH 8.0) was used as the aqueous phase. All reactions were repeated at least three times. Similar conditions were maintained for determining the specific activity of formation of mandelic acid. The reaction mixtures were shaken for 30 min and then stopped by centrifugation (9000 rpm, 4 °C for 10 min). Then the product mandelic acid was determined by the UV-vis techniques utilizing the calibration curve of mandelic acid in potassium phosphate buffer (absorbance at λ=254 nm, shown in Figure S14). The specific activity was defined as the amount of dry cell weight (DCW) that produced 1μmol of mandelic acid per minute under above assay conditions (shown in Table S1).

Table S1. The specific activity (μmol·min^{-1}·mg^{-1} DCW) of Alcaligenes faecalis ATCC 8750 in different reaction systems with different initial concentration of mandelonitrile.*

	50 mM	100 mM	300 mM
Pickering emulsion	0.145	0.256	0.547
Biphasic	0.027	0.045	0.092
Monophasic	0.186	0.108	0.021

*The specific activity was defined as the amount of dry cell weight (DCW) that produced 1μmol of mandelic acid per minute under above assay conditions.

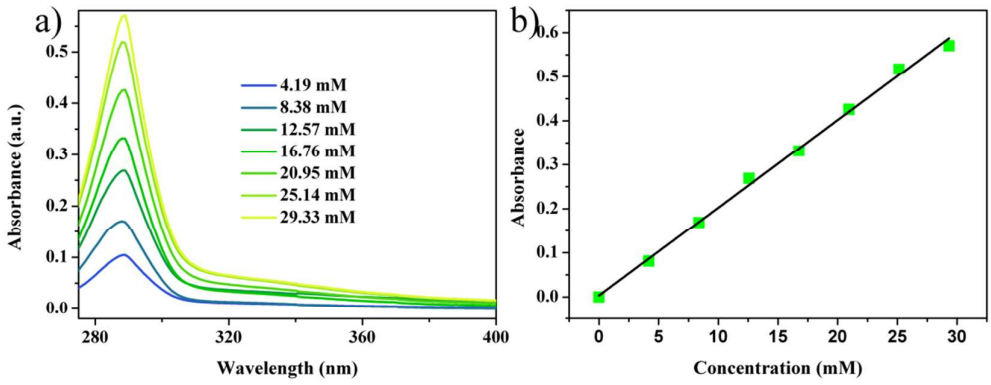


Figure S12. a) Absorption spectra of the mandelonitrile with different concentrations of 0 - 30 μM in toluene. b) The absorbance at 289 nm as a function of mandelonitrile concentration.

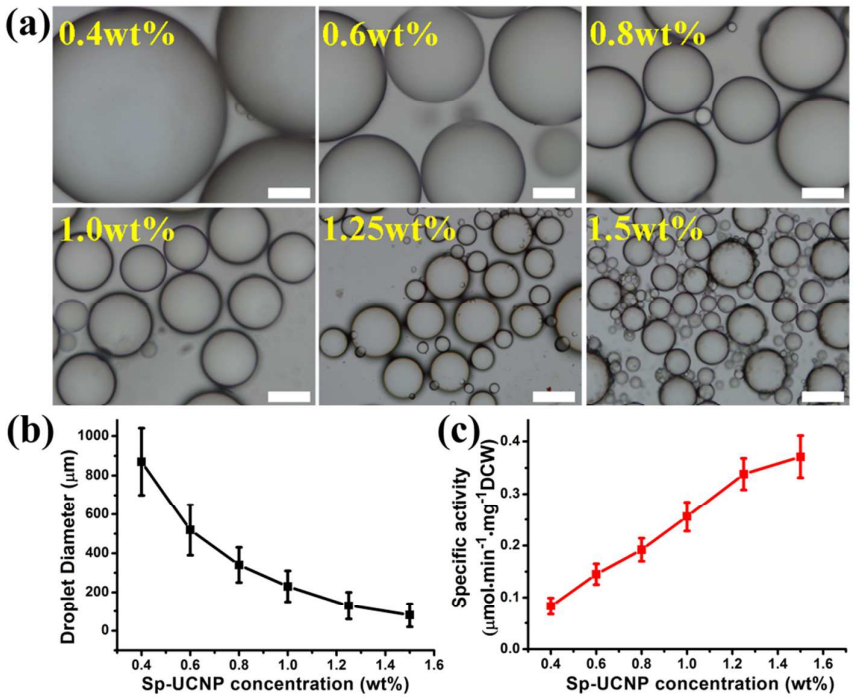


Figure S13. (a) Microscopy images of the formulated w/o Pickering emulsion using different amounts of Sp-UCNPs (scale bar: 200 μm). (b) Plots of mean droplet diameter corresponding to (a). The diameter values were statistically measured by microscopy and calculated based on at least 100 droplets. (c) Plots of the specific activity of Alcaligenes faecalis ATCC 8750 immobilized in the w/o Pickering emulsion corresponding to (a); the initial substrate concentration was 100 mM; ATCC 8750 concentration was kept the same; the organic solvent in all cases was toluene.

Kinetic study of Alcaligenes faecalis ATCC 8750 biocatalysis in Pickering emulsion: Typically, mandelonitrile at different concentrations (50 mM, 75mM, 100mM, 150mM, 200mM, 250mM, 300mM, 350mM and 400mM) were added into the ATCC 8750 loaded Pickering emulsions to start the reaction. The initial reaction rates ($mM \cdot min^{-1}$) were evaluated by linear regression of the experimental data from a plot of the product mandelic acid concentration versus time. The slope of the curve during the first 10 min was defined as the initial reaction rate. The apparent kinetic parameters Michaelis constant-K_m and the maximum reaction rate-V_{max} were calculated according to the Lineweaver-Burk equation (Equation 1):

$$\frac{1}{\nu} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}} \quad (1)$$

Where ν is the initial velocity, V_{max} is the maximal reaction velocity and S is the concentration of substrate. Figure S13 showed the plot of the reciprocal of the reaction rate, ν ($mM \cdot min^{-1}$), versus the reciprocal of the substrate concentration, [S] (mM). Accordingly, the 1/ν and 1/[S] were related

to the Equation (2):

$$\frac{1}{v} = 150.063\frac{1}{[S]} + 0.45746 \quad (2)$$

Thus, K_m was calculated as 328.035 mM and V_{max} as 2.186 mM·min^{-1}. The diffusive limitations and substrate partition between the dispersed phase and continuous phase may contribute to the high apparent K_m value. [1]

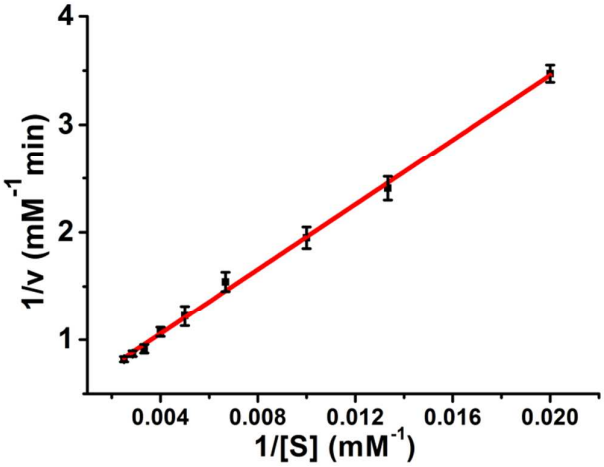
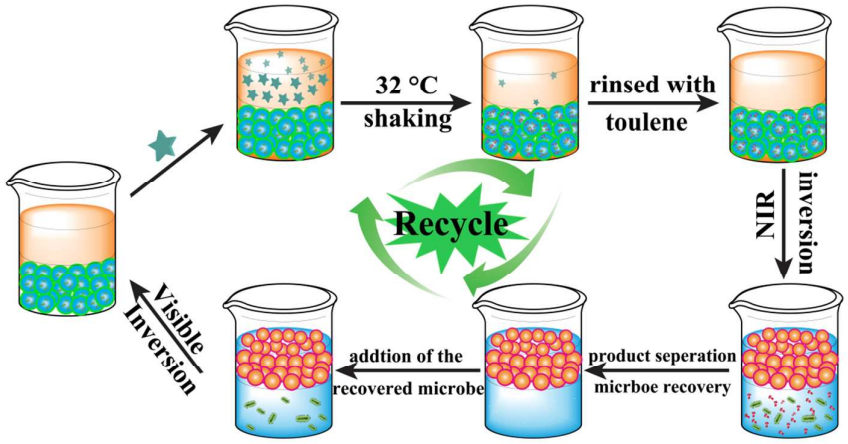


Figure S14. Lineweaver-Burk plot of the reciprocal of initial rate (1/v) versus the reciprocal of the substrate concentration (1/[S]) for the determination of kinetic parameters K_m and V_{max} of Alcaligenes faecalis ATCC 8750 loaded in the Pickering emulsion. Error bars were estimated from three independent measurements.



Scheme S4. Schematic illustration of the recycle catalytic reaction process in nanolamp-stabilized Pickering emulsions.

Recycle catalytic reaction in nanolamp-stabilized Pickering emulsions: The catalytic reaction was followed with the same procedure as the assessment of hydrolysis of mandelonitrile described as above. 100 mM mandelonitrile was used as the initial substrate concentration in this procedure.

As shown in the Scheme S4, at the end of each catalytic cycle (4 hours), the toluene phase of the Pickering emulsion was carefully without broking the Pickering emulsion removed by pipette, followed by addition of fresh toluene for 3 times until no yellow color of mandelonitrile was observed. Then NIR or UV (control experiments) irradiation was used to transform the w/o emulsion to o/w emulsion and the extra aqueous was quickly resolved in the bottom layer. Next, the aqueous solvents were removed and the biphasic system was rinsed several times with fresh solvents. The product and biocatalyst were separated through centrifugation. Then, the recovered microbes were dispersed in fresh solvents and introduced again to the system. Finally, the o/w emulsion was transformed to w/o emulsion by visible light and new substrate was added to restart a new cycle. The product concentration was determined by the UV-vis techniques utilizing the calibration curve of mandelic acid in potassium phosphate buffer (absorbance at λ=254 nm, shown in Figure S14). In the above systems, 0.1 M potassium phosphate buffer (pH 8.0) was used as the aqueous phase. All reactions were repeated at least three times.

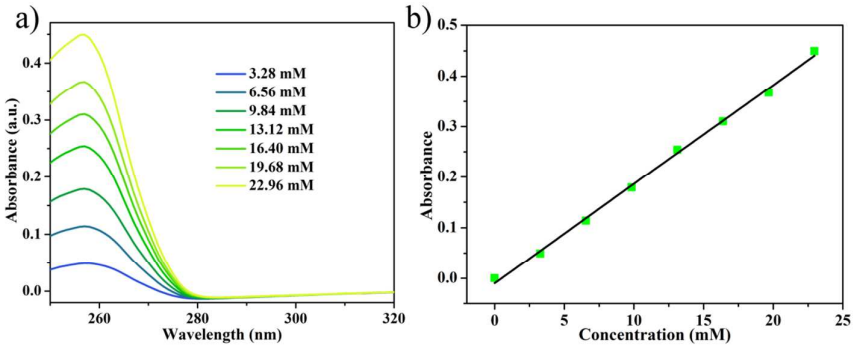


Figure S15. a) Absorption spectra of the mandelic acid with different concentrations of 0 - 25 μM in pH 8.0, 0.1 M potassium phosphate buffer. b) The absorbance at 254 nm as a function of mandelic acid concentration.

Enantioselectivity: High performance liquid chromatography (HPLC) was carried out on a Shimadzu CLASS-VP V6.14SP1 instrument to analyze the enantioselectivity, and the liquid chromatography was equipped with a Daicel Chiralcel OD-Hcolumn. The enantioselectivity was expressed as the enantiomeric excess (e.e.). The enantioselectivity of the reaction was calculated by use of the following equation (MA represents mandelic acid):

$$\text{e.e.}(\%) = \frac{[(R)MA] - [(S)MA]}{[(R)MA] - [(S)MA]} \times 100$$

Table S2. The recycling biocatalytic performance of Alcaligenes faecalis ATCC 8750 in Pickering emulsion for the asymmetric hydrolysis of racemic mandelonitrile.

Cycle number	Enantioselectivity (e.e., %)
1	98.3±0.4
2	99.1±0.2
3	97.8±0.3
4	98.5±0.2
5	98.8±0.5
6	98.1±0.2
7	97.5±0.4
8	97.8±0.3
9	98.2±0.2
10	98.5±0.4

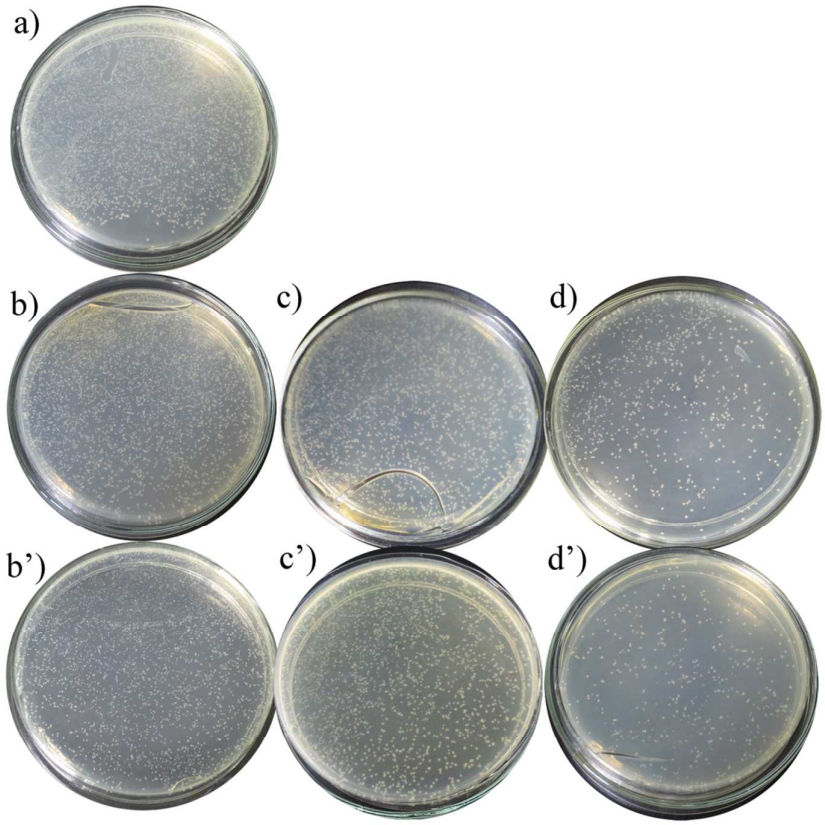


Figure S16. (a) Showed the colonies of ATCC 8750 used before catalysis as positive control. (b) and (b') showed the colonies of ATCC 8750 recovered from the NIR/visible light controlled Pickering emulsions at the fifth and tenth cycle, respectively. (c) and (c') showed the colonies of ATCC 8750 recovered from the UV/visible light controlled Pickering emulsions at the fifth and tenth cycle, respectively. (d) and (d') showed the colonies of ATCC 8750 recovered from the biphasic systems at the fifth and tenth cycle, respectively. About 94.3%-(b), 87.1%-(b'), 82.6%-(c), 65.2%-(c'), 4.9%-(e) and 1.3%-(e') of the bacteria remained after corresponding reaction cycles.

References for Supporting Information:

(1) (a) Banerjee, A.; Kaul, P.; Banerjee, U. C. *Appl. Microbiol. Biotechnol.* **2006**, *72*, 77; (b) Xue, Y. P.; Xu, M.; Chen, H. S.; Liu, Z. Q.; Wang, Y. J.; Zheng, Y. G. *Org. Process. Res. Dev.* **2013**, *17*, 213.