Supporting Information

Endowing chloroplasts with artificial "cell walls" using Metal-Organic Frameworks

Lei Shi, Ailing Li, Weiwei Zhang, Haishan Wu, Yuwu Chi*

MOE Key Laboratory for Analytical Science of Food Safety and Biology, Fujian Provincial Key Laboratory of Analysis and Detection for Food Safety, and College of Chemistry, Fuzhou University, Fuzhou, Fujian, 350108, P. R China.

E-mail: y.w.chi@fzu.edu.cn

EXPERIMENTAL SECTION

Chemicals and Materials. 2-Methylimidazole (2-MeIM, 98%) was purchased from Macklin (Shanghai, China). Zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$, 99%) was obtained from Fuchen chemical reagent company (Tianjin, China). Percoll was received from Biosharp (Shanghai, China). Spinach was bought from a local market.

CH extraction from spinach leaves. The spinach leaves were placed in cool overnight to consume starch produced before. Then the clean and dry leaves without veins were homogenized in a cooled (4 °C) aqueous buffered solution (400 mM sucrose, 50 mM Tris-HCl and 10 mM NaCl) and grinded quickly for 2 min. The homogenate was filtered through 4-layer-gauze to remove large debris followed by centrifugation at ×300 g for 3 min to remove remaining cell debris and at ×1000 g for 5 min to acquire intact CH organelles. The intact CHs were dispersed in CEB for further use. Usually, the integrity of the obtained raw organelles are not satisfied (30%) but it can be significantly improved (95%) by centrifugating the raw organelles in Percoll. Firstly, 3 mL 80% Percoll solution was placed at the bottom of the centrifuge tube and then 3 mL 40% Percoll solution was laid on the 80% Percoll solution cautiously. At last, 1mL CH suspension was spread on the top. After the centrifugation at ×1500 g for 15 min, CHs with whole membranes laying between 40% and 80% Percoll were obtained and suspended in an aqueous buffered solution for further use.

Preparation of CH@ZIF-8. In a typical procedure, $Zn(NO_3)_2 \cdot 6H_2O$ (0.224g) and 2-methylimidazole (4.56g) were dissolved in 1.6 and 16 mL deionized water, respectively. Under gentle stirring, 2 mL of the purified CH suspension and the $Zn(NO_3)_2$ solution were added in drop into the 2-methylimidazole solution in turn. The mixed solution was further stirred gently for 20 min followed by incubation for 5

hours at room temperature. Then, the white deposit was separated via centrifugation at ×300 g for 3 min and washed by deionized water for 3 times. Finally, the product was dispersed in an aqueous buffered solution for further test.

Instruments. In order to verify the effectiveness of CH extraction and immobilization, the morphologies of CH, ZIF-8 and CH@ZIF-8 were investigated by the scanning electron microscopy (SEM) on a Nova Nano SEM 230 field-emission microscope. For obtaining ideal SEM images, the samples were prepared as follows: the organelles were fixed with 2.5% aqueous glutaraldehyde solution, then dropped and dried (at room temperature) on a silicon wafer. The CH@ZIF-8 and blank ZIF-8 powders obtained by vacuum-freeze drying their suspensions were stuck on conductive tapes and removing big particles with an argon stream. All SEM samples were coated with gold nanoparticles to improve their electronic conductivities.

In order to investigate the porous structures of the blank ZIF-8 and CH@ZIF-8 hybrid materials, nitrogen adsorption and desorption experiments for their dried samples were performed at 77 K (BET, Micromeritics ASAP 2020, USA). The pore size distribution was determined by the BJH (Barret, Joyner, Halenda) method and the specific surface area (SBET) was calculated by the Brunauer-Emmett-Teller (BET) method.

X-ray powder diffraction (XRD) patterns were measured by a X'Pert Pro MPD X. Thermogravimetry (TGA) measurement was carried out on a thermogravimetry analyzer (Netzsch TG209F3). Fluorescence microscopic images were obtained by an inverted Eclipse Ti−U microscope (Nikon, Japan) equipped with a color camera (DS-Ri1). Cross-sectional confocal microscopic images were recorded on a laser confocal fluorescence microscope (Nikon C2). Fluorescence signals for measuring oxygen were recorded by a USB2000 fluorescence spectrometer (Ocean Optic Inc., USA).

The simulated sunlight (AM 1.5G, 1000 W/m^2) was generated by a 500 W xenon lamp with a filter (Beijing Bo Fei Lai Technology Co., Ltd. China).

Measurement of O_2 produced in photosynthesis of CH@ZIF-8. Since oxygen is the well known by-product of chloroplasts' photosynthesis, the photosynthetic activity of chloroplasts can be estimated by the production of oxygen. Tris(2,2'-bipyridine) ruthenium (II) dichloride (abbreviated as "Ru(II)" hereafter) is a kind of fluorescence indicator, which is quite stable and sensitive to oxygen. The fluorescence of Ru(II) can be sensitively and selectively quenched by oxygen both in liquid and gas phases. Therefore, the production of oxygen produced by photosynthesis can be monitored by the quenching of the fluorescence of Ru(II)[1].

The photosynthetic activity studies of the CH@ZIF-8 in aqueous solution were undertaken in a home-made system consisting of a photosynthetic cell and a O_2 sensor by detecting the fluorescence quenching of ruthenium (Figure S1). Typically, the CH@ZIF-8 hybrids were dispersed in 10 mM $NaHCO_3$ aqueous solution to form photosynthetic reaction solution in the photosynthetic cell. Then the reaction solution was exposed to the simulated sunlight. The temperature of the photosynthetic reaction solution was maintained at 25 °C by a flow of cooling water during the light irradiation. After a period of photosynthetic reaction, the solution was pumped into a flow-through O_2 sensor based on quenching of Ru(II) fluorescence by O_2, where the variation of O_2 concentration was monitored. A 0.22-um filter was placed in front of the O_2 sensor, to prevent CH@ZIF-8 particles from entering the O_2 sensor. After 5 mL solution was pumped through the O_2 sensor, the pump was stopped and the fluorescence spectrum of Ru(II) in the sensing membrane was recorded for evaluating the concentration of O_2 in the solution. The portion of reaction solution flowing through the O_2 sensor (free of CH@ZIF-8 particles) was pumped back to the

photosynthetic cell to maintain the volume and components of the photosynthetic

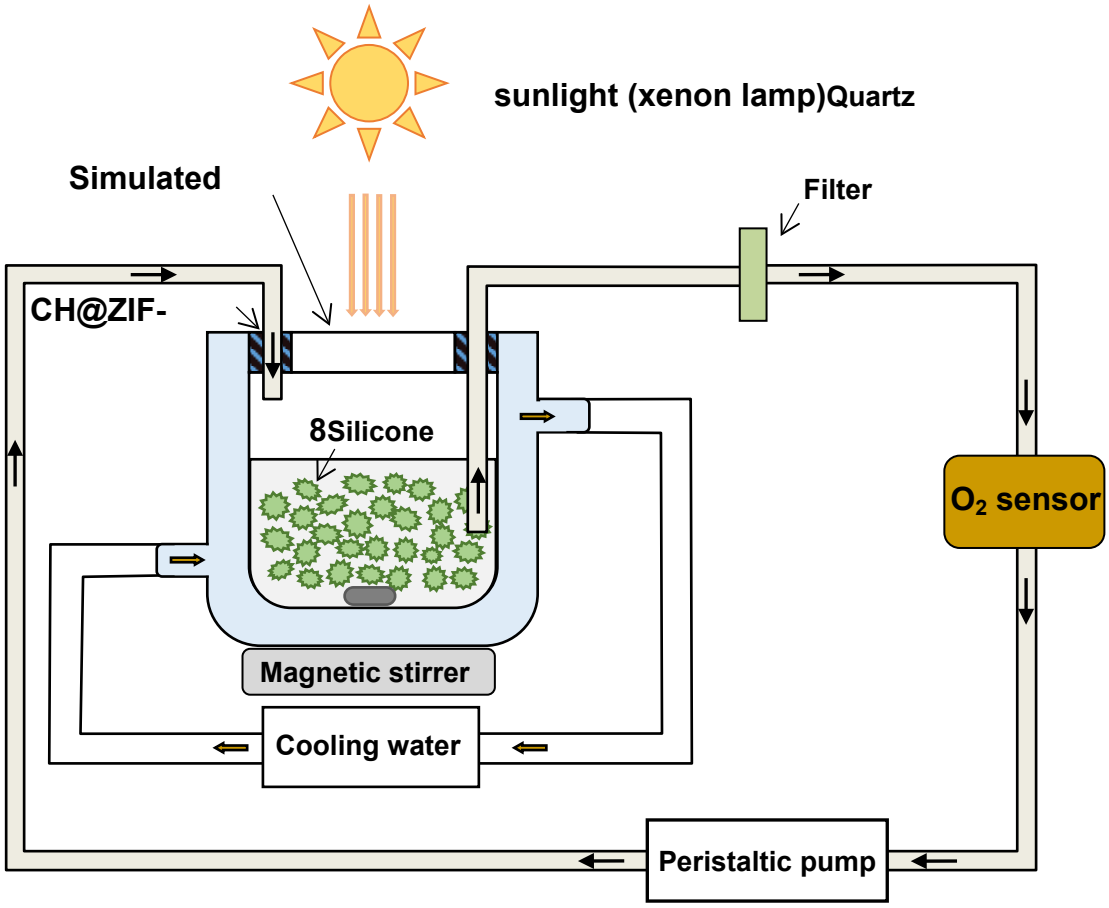

Figure S1. Schematic diagram of the system for evaluating the photosynthetic activity of CH@ZIF-8 hybrid materials.

reaction solution. The O_2 senor (as depicted in Figure S2) consisted of a sensing membrane, which was prepared by immersing a piece of Nafion membrane in 1 mM Ru(II) solution for 24 h; a piece of Teflon spacer, at the center of which a oval-shaped section was carved to accommodate a thin-layer of test solution (c.a 200 μm in thickness, and 10 μL in volume); a plexiglass cell body, on which a "L"-shaped inlet solution channel and a "L"-shaped outlet solution channel were respectively drilled, and a plexiglass optical window (Figure S2A). The sensing cell was assembled by screwing all parts together tightly via screw hole on them (left section of Figure S2B). In measurement of O_2 in solution, a Y-type of optical fiber was placed in front of the optical window, and Ru (Ⅱ) sensing membrane was excited by a LED light with a maximum wavelength at 450 nm, and the emission spectra of Ru(II) with maximum wavelength at 610 nm was collected by the USB 2000 modular spectrometer. In order to eliminate the interference from the excitation light, the signal light was passed through a LPF525 long-pass optical filter (Rayan Technology Co., Ltd.) with a cut-off wavelength rang of 200-505 nm before reaching the spectrometer (right section of Figure S2B). Finally, the device of the system for evaluating the photosynthetic activity of CH@ZIF-8 hybrid materials was shown in Figure S3.

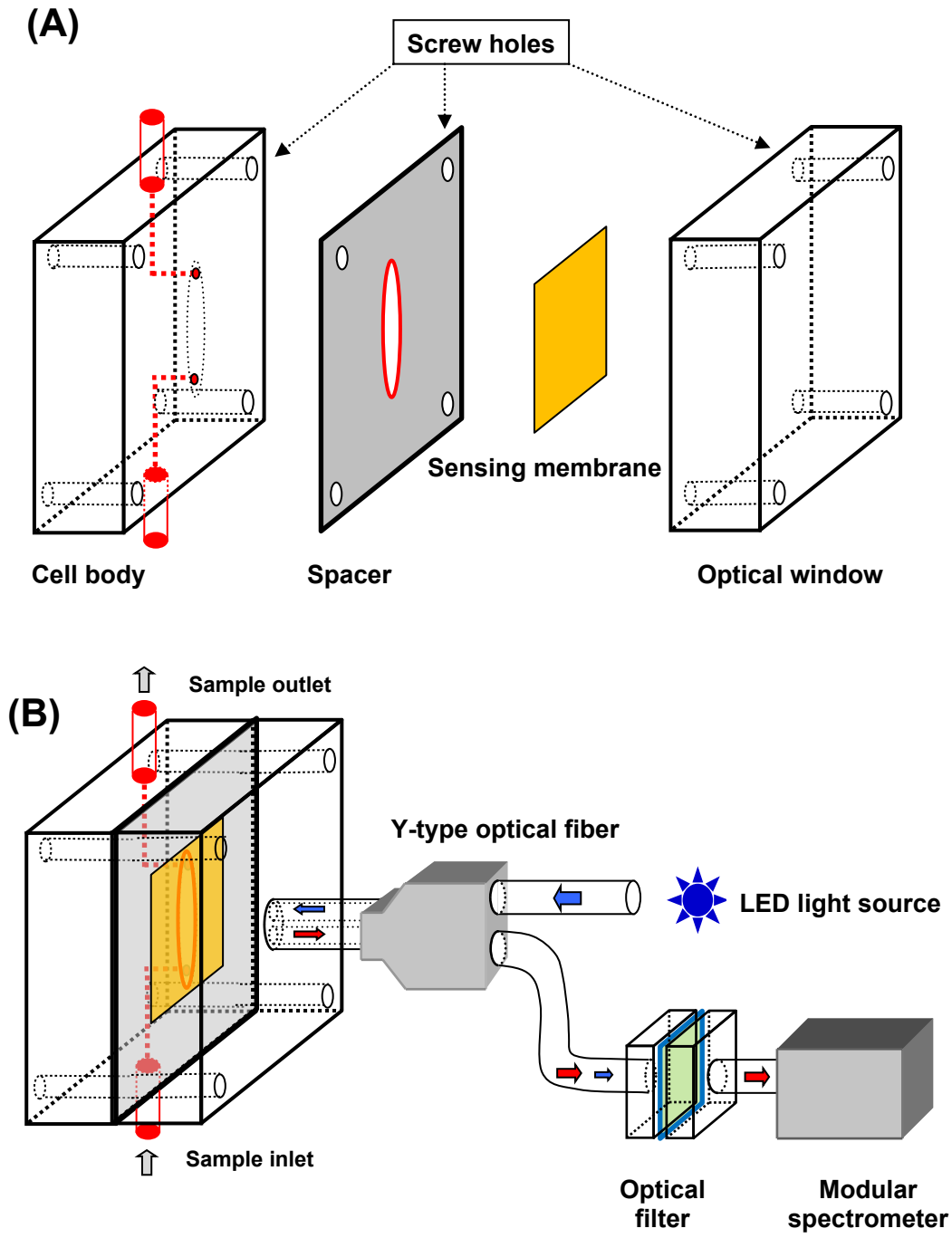

Figure S2. Illustration of the O_2 sensor. (A) The structure of O_2 sensing cell; (B) The setup of the optical fiber based O_2 sensor.

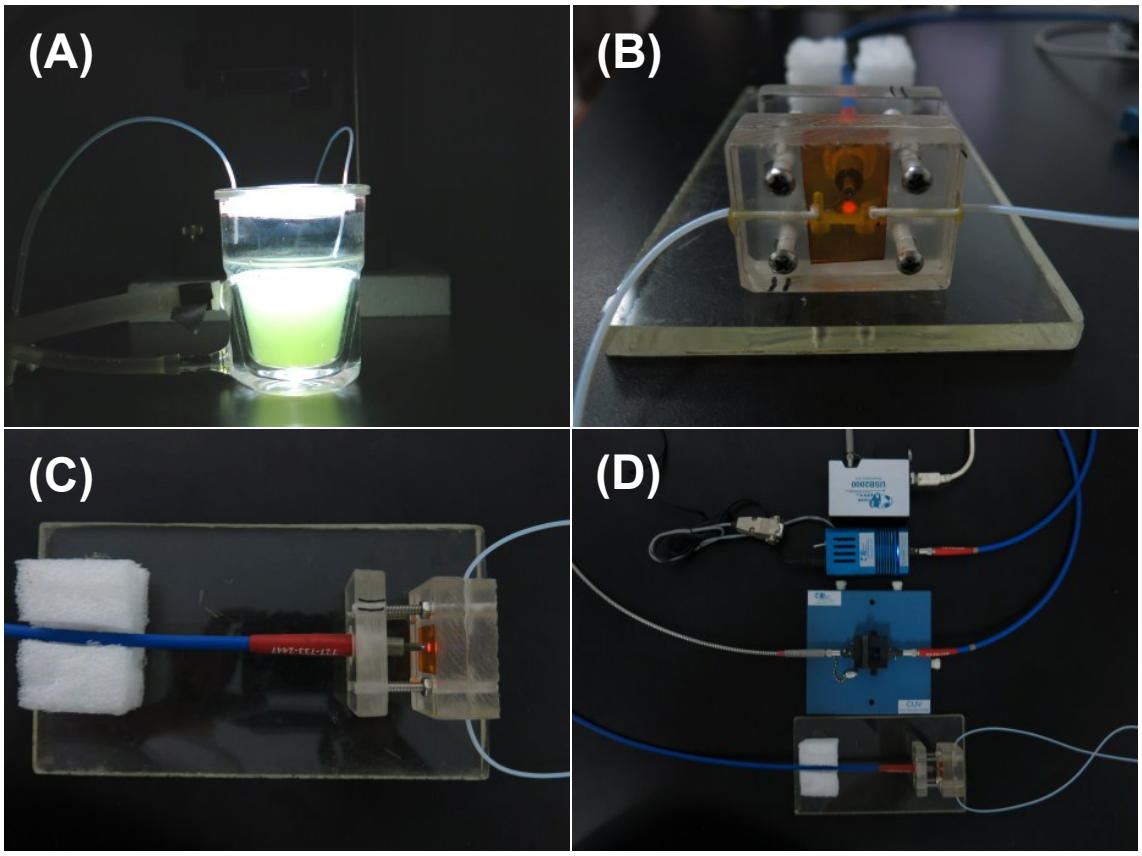

Figure S3. The main devices involved in the of system for evaluating the photosynthetic activity of CH@ZIF-8 hybrid materials. (A) photosynthetic cell containing the CH@ZIF-8 hybrid materials and $NaHCO_3$ solution; (B) and (C) The setup of flow-through O_2 sensor with a Ru(II)/Nafion sensing membrane; (D) The optical fiber sensing system for detection O_2 produced during the photosynthesis.

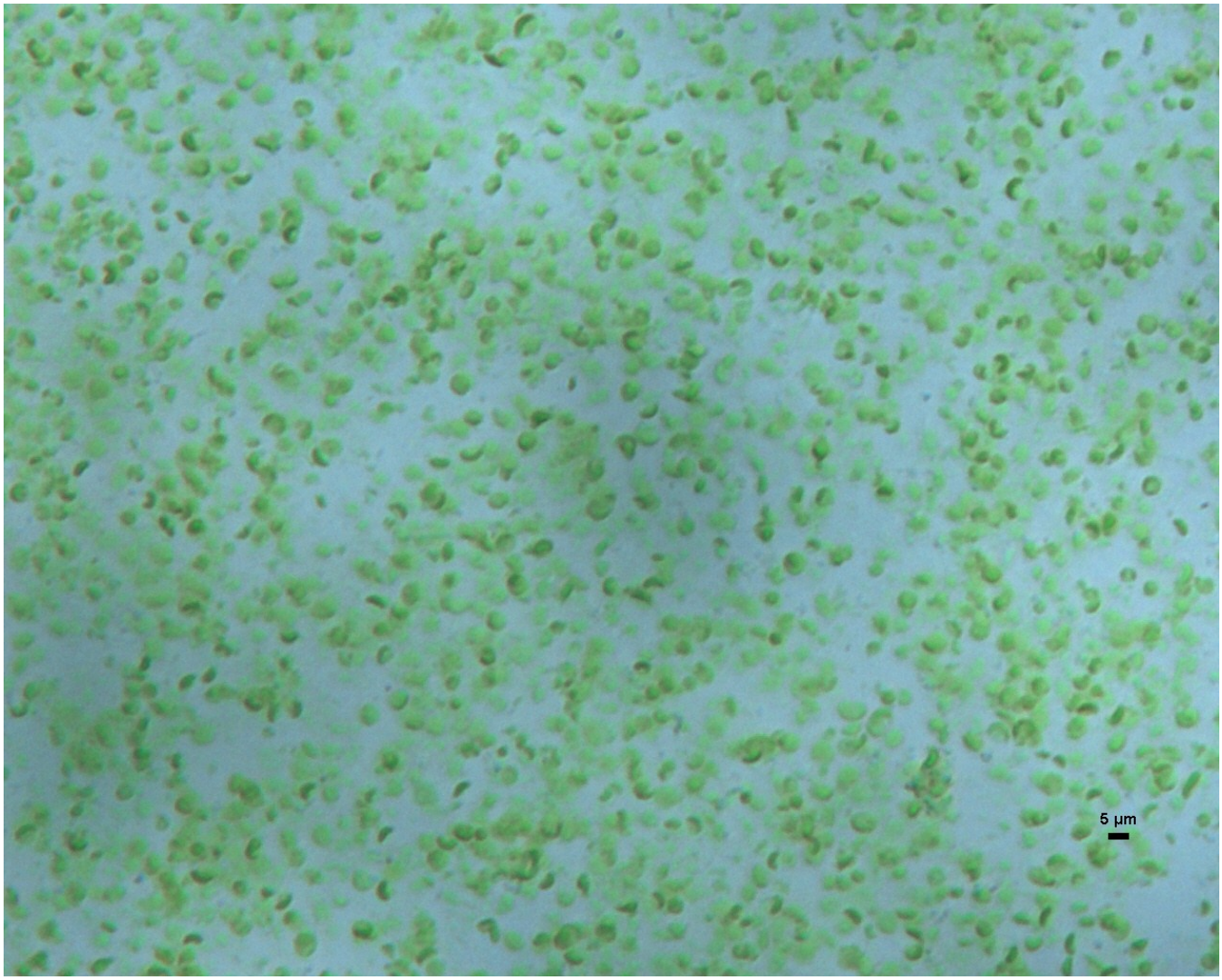

Figure S4. Microscopic image of the extracted CHs.

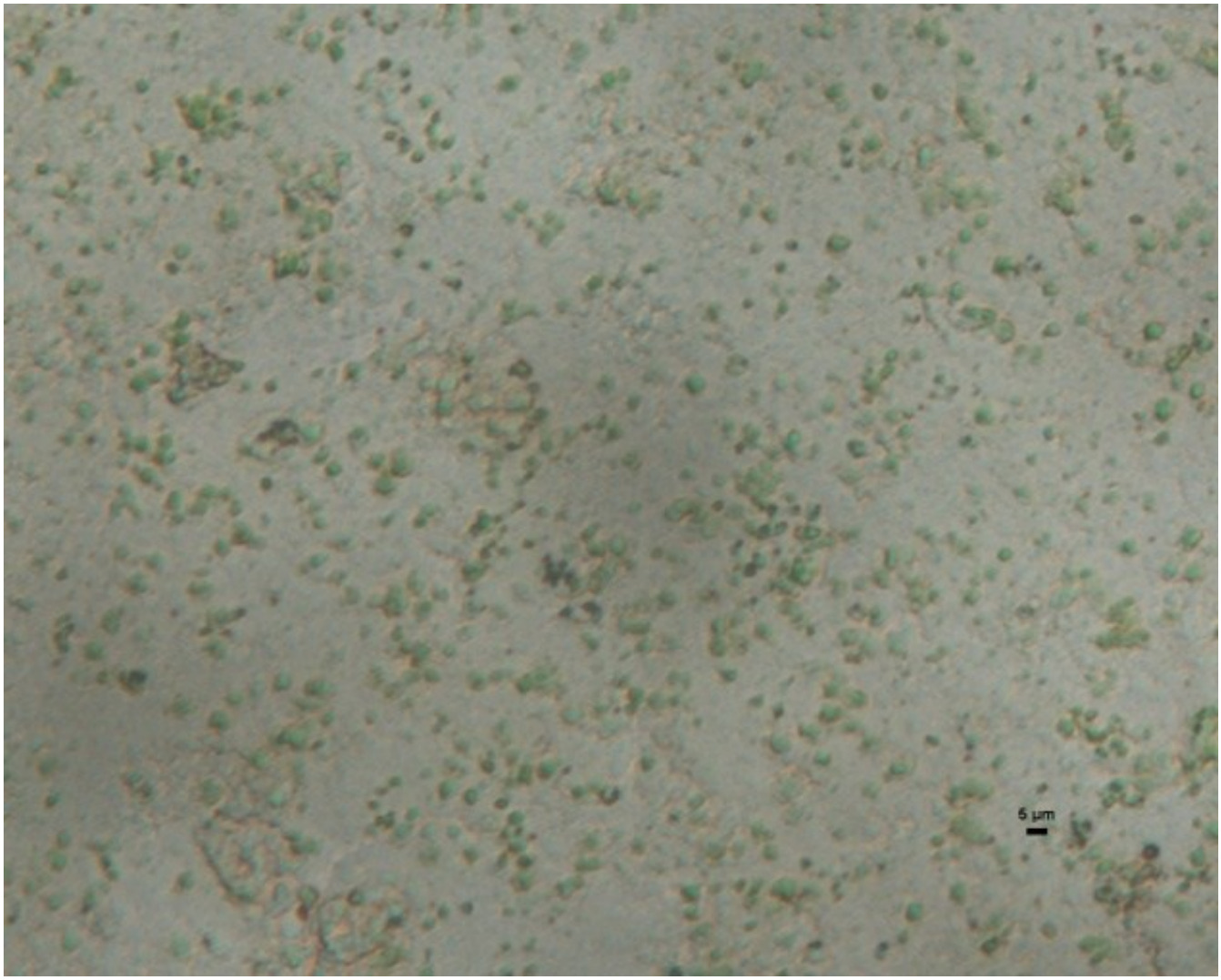

Figure S5. Microscopic image of the prepared CHs@ZIF-8.

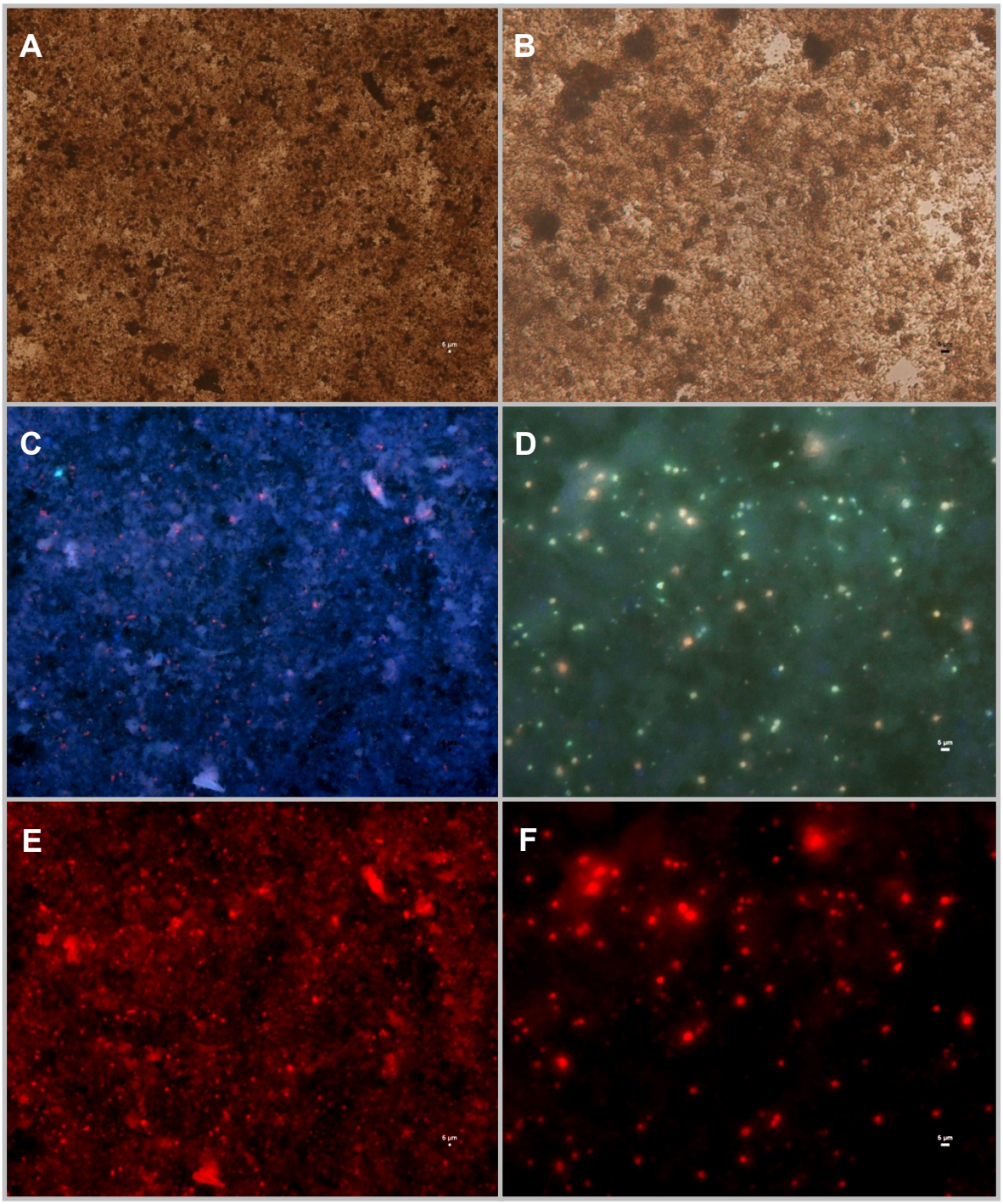

Figure S6. Fluorescence microscopic images of CH@ZIF-8 composites with a high particle density at 10- fold amplification (A, C and E) and 40-fold amplification (B, D and F) under white light (A and B), UV light (C and D) and green light (E and F).

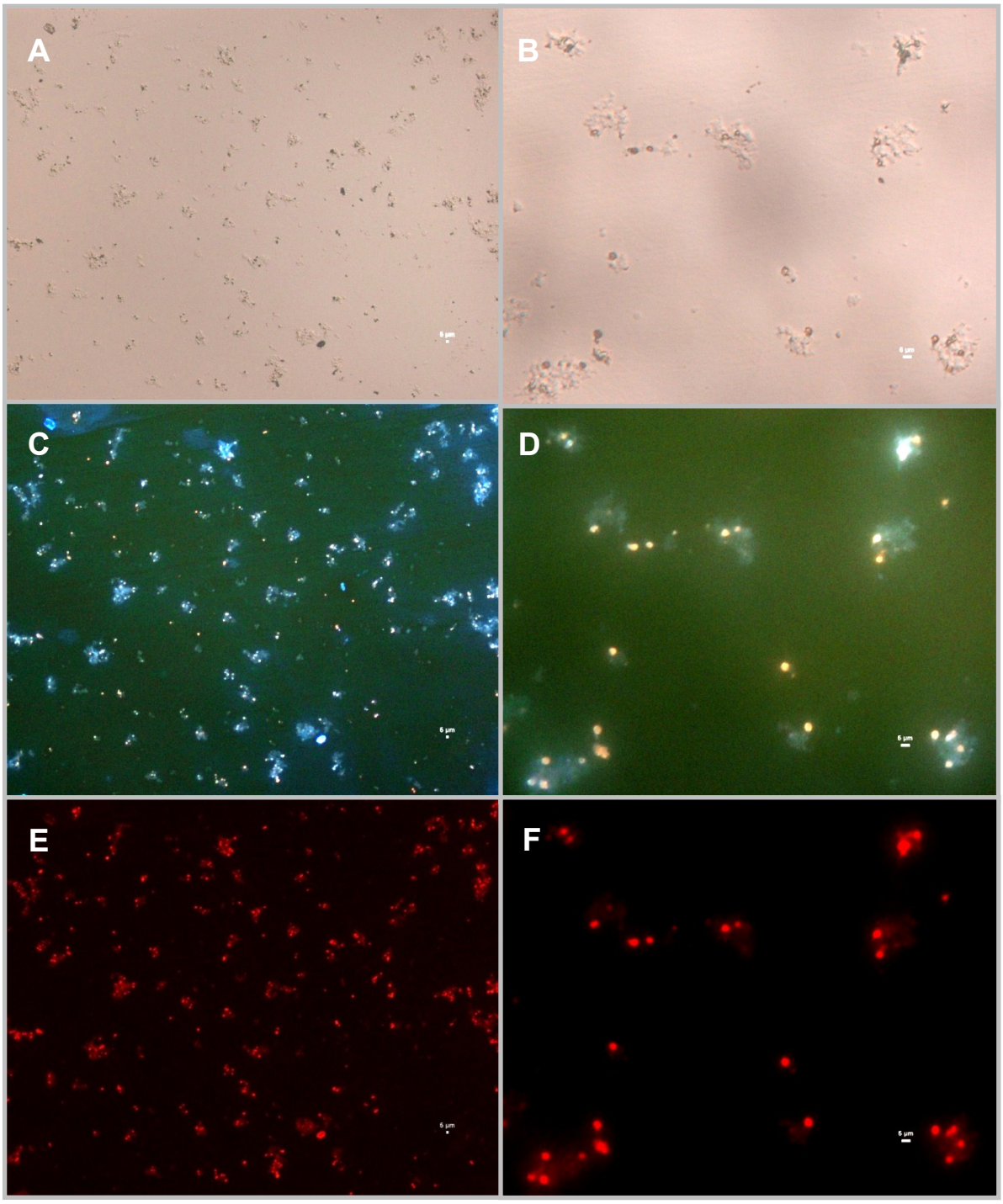

Figure S7. Fluorescence microscopic images of CH@ZIF-8 composites with a low particle density at 10- fold amplification (A, C and E) and 40-fold amplification (B, D and F) under white light (A and B), UV light (C and D) and green light (E and F).

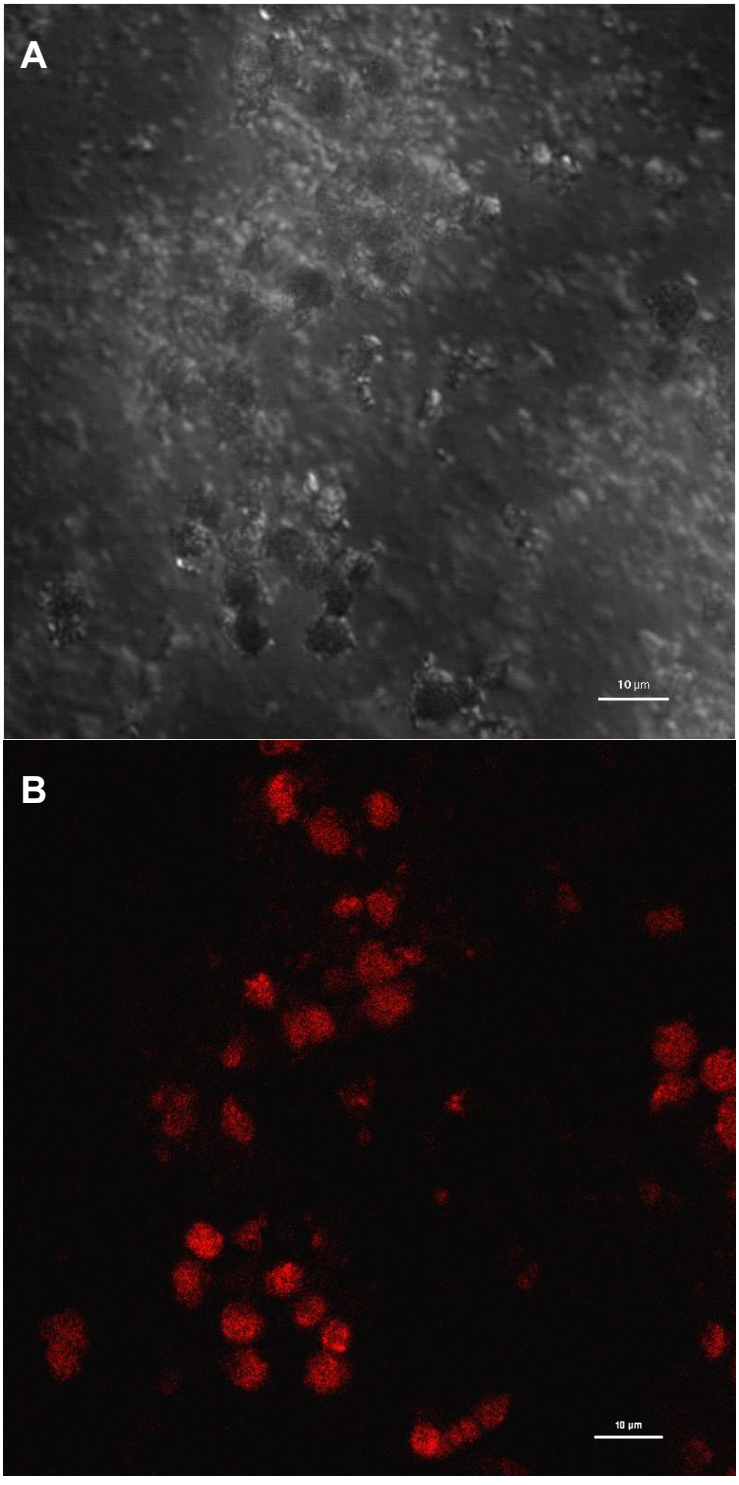

Figure S8. Confocal microscopic images of CH@ZIF-8: (A) Bright field microscopic image; (B) Fluorescence microscopic image excited by 405 nm laser.

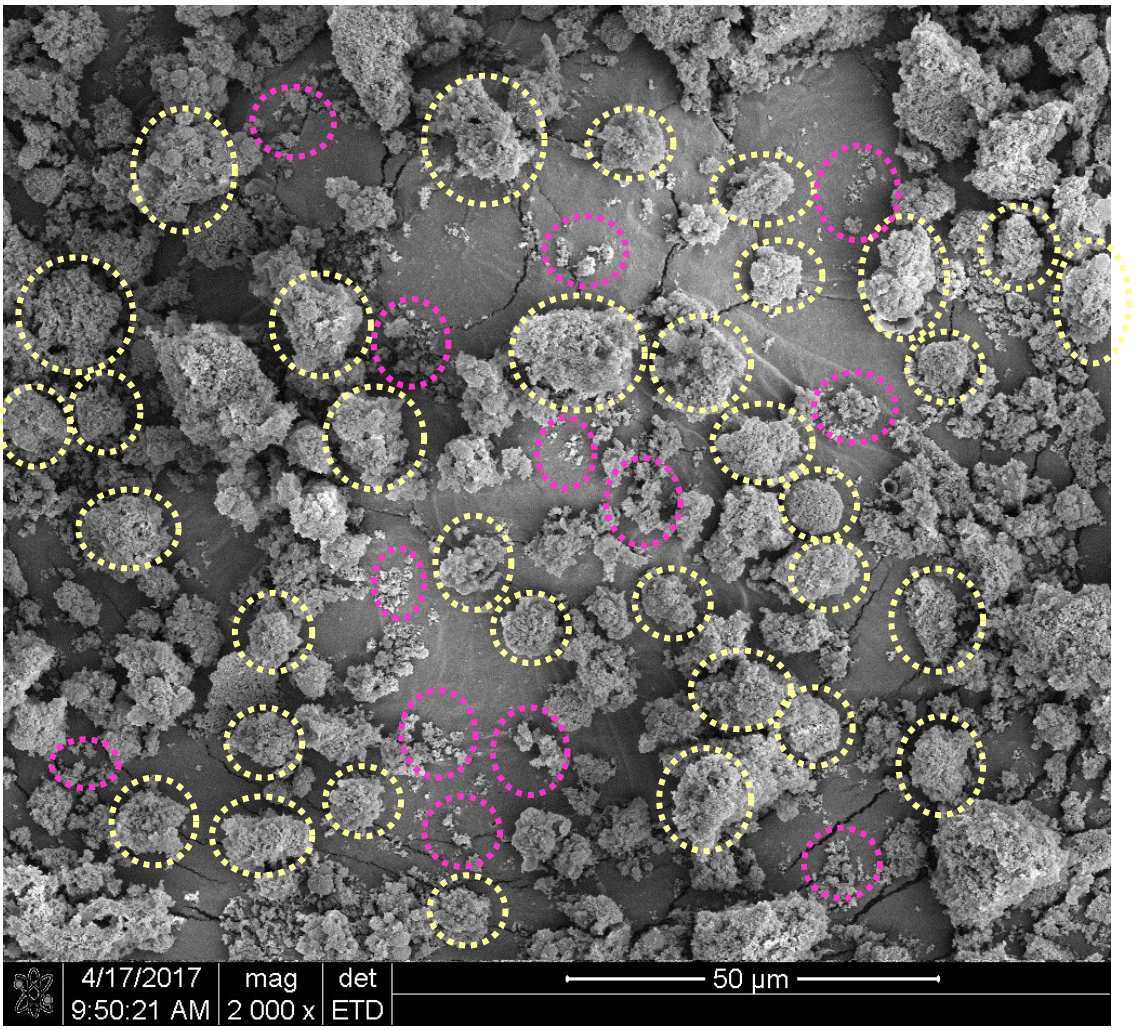

Figure S9. SEM images of CH@ZIF-8 particles over a wide scope. Various sizes of small aggregated ZIF-8 particles less than 1 μm (marked with pink circles) are randomly among CH@ZIF-8 particles (marked with yellow circles).

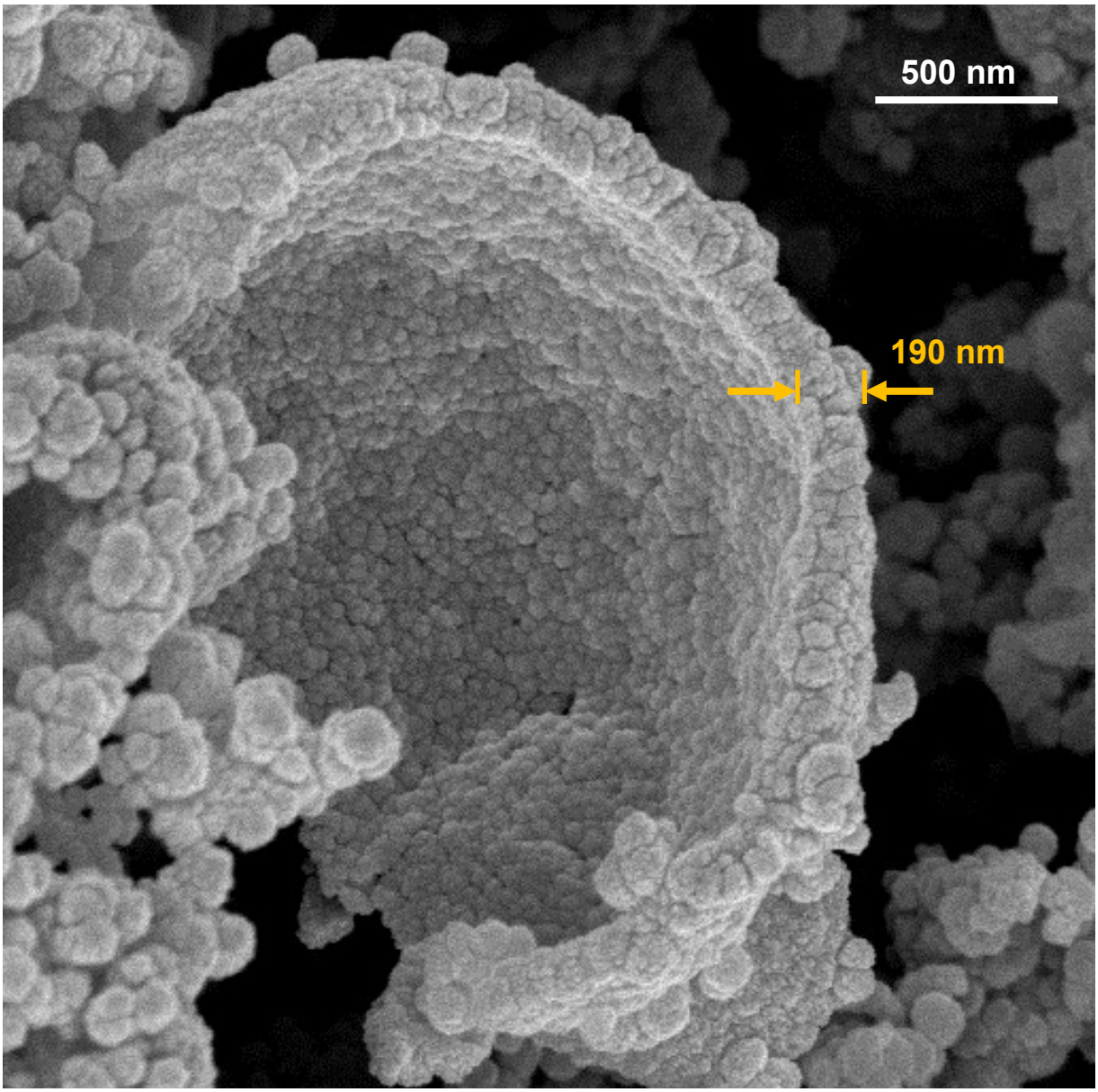

Figure S10. The thickness (< 200 nm) estimated for the "egg shell" of CH@ZIF-8.

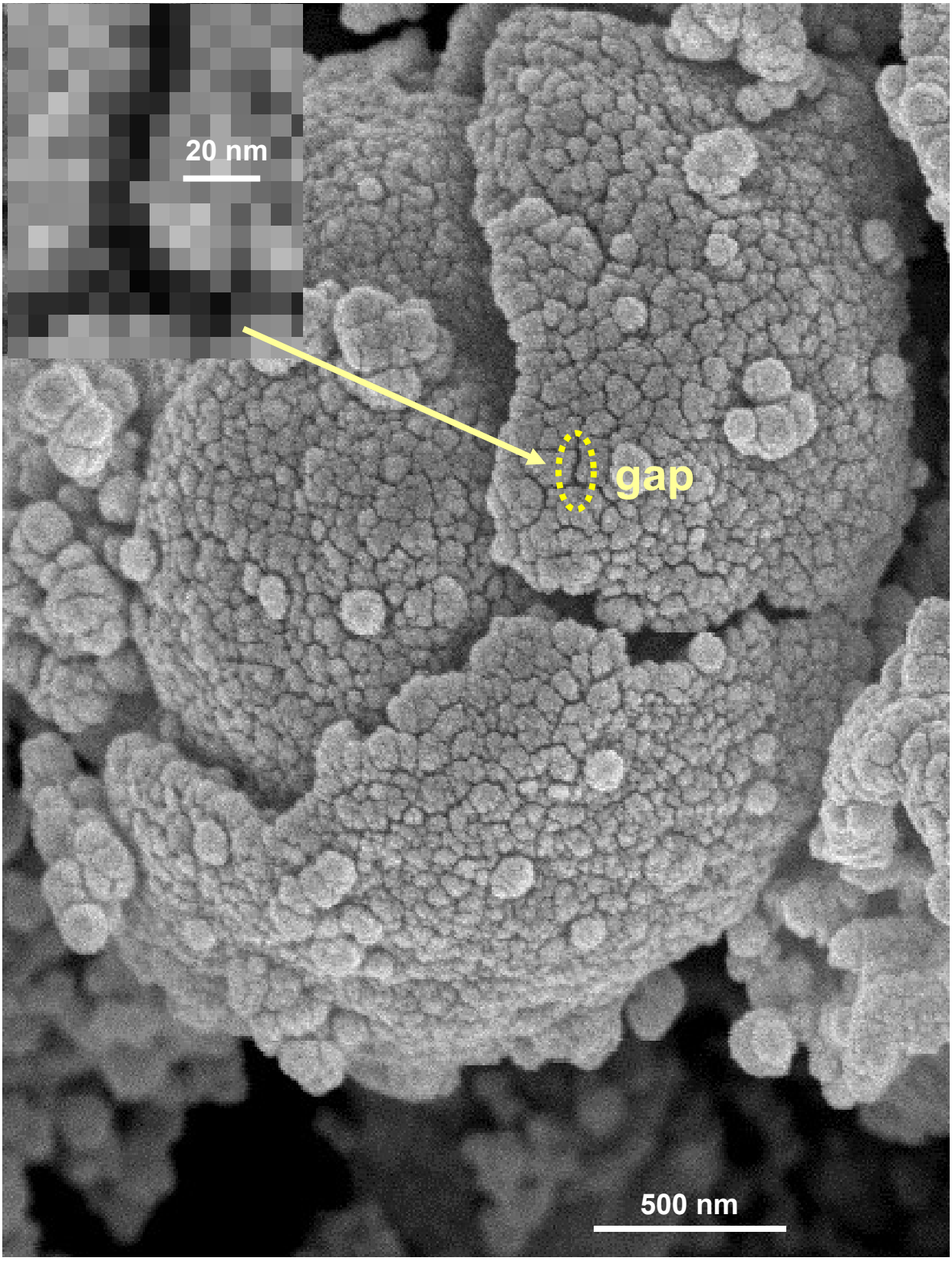

Figure S11. Gaps (<10 nm) in the "egg shell" of CH@ZIF-8.

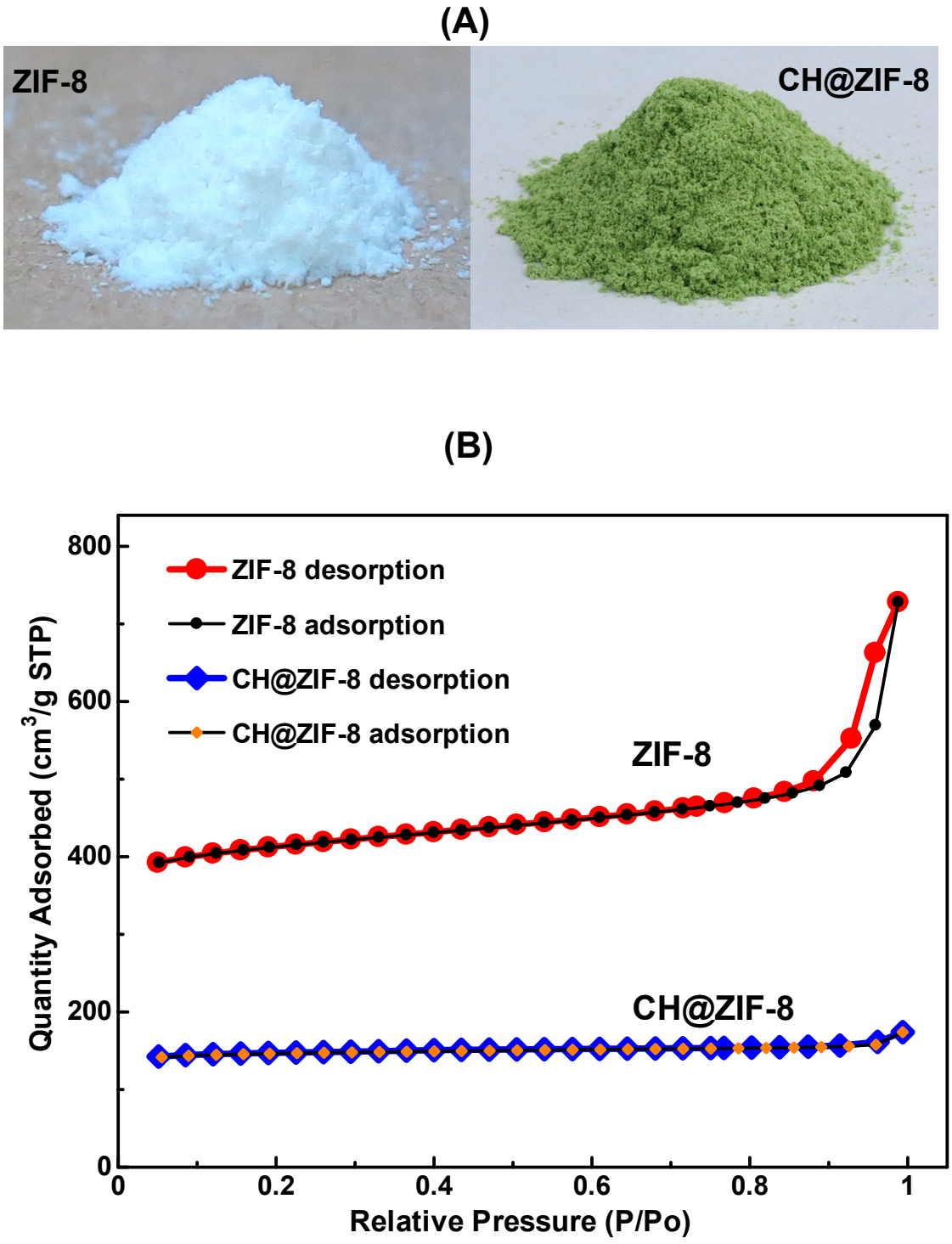

Figure S12. (A) Photos of ZIF-8 and CH@ZIF-8 powder; (B) N_2 adsorption-desorption isotherms of ZIF-8 and CH@ZIF-8.

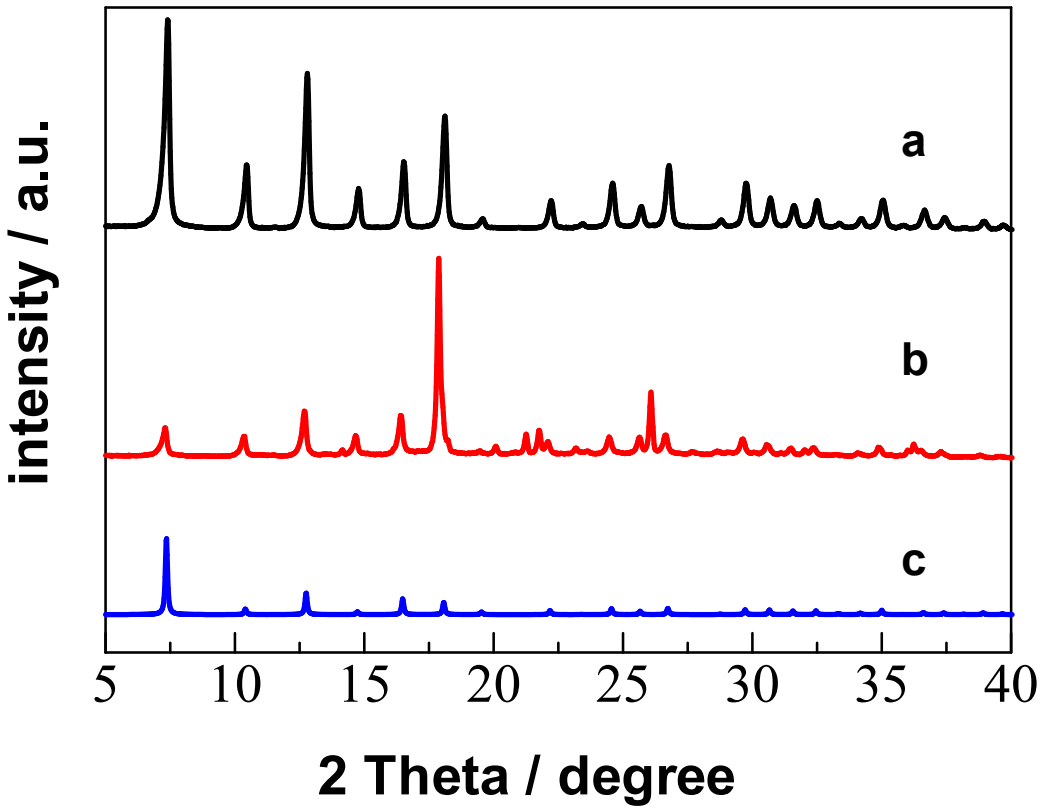

Figure S13. XRD data of ZIF-8 (a), CH@ZIF-8 (b), simulated ZIF-8 (c)

Estimating core-shell structure from BET

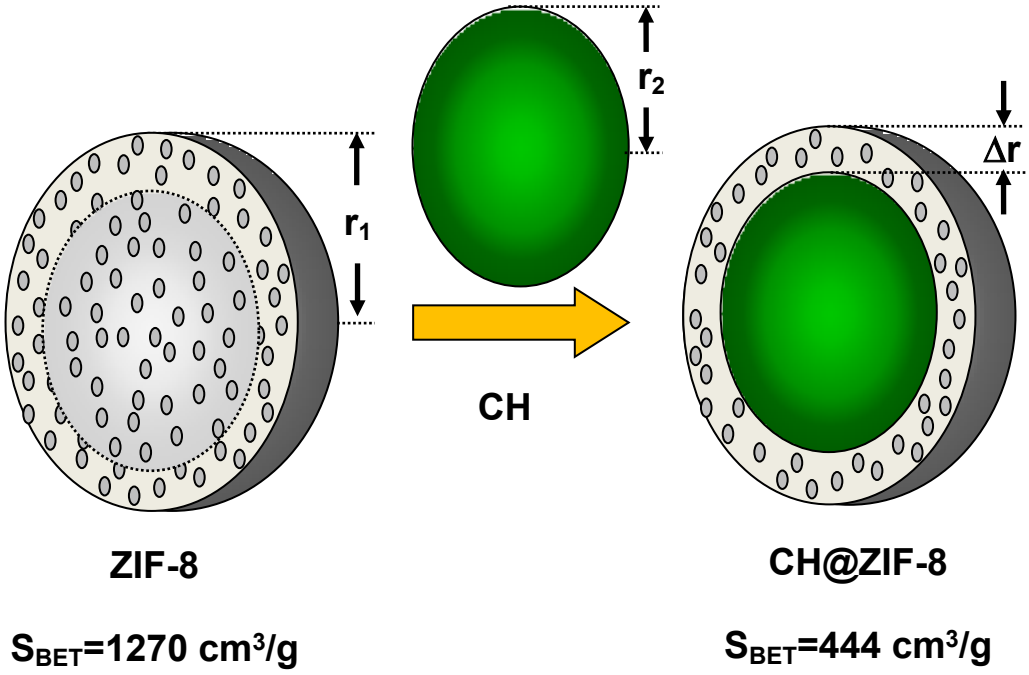

Figure S14. Model for estimating core and shell size of CH@ZIF-8 from BET.

The BET surface areas of dried ZIF-8 and CH@ZIF-8 are 1270 and 444 m^2/g, respectively. As shown in Figure S12, the synthesis of CH@ZIF-8 can be regarded equaling to following displace process: The core of a virtual ZIF-8 ball (with S_{SBE} of 1270 m^2/g) is displaced by CH (with very low S_{SBE}, ca. 0 cm^3/g), producing CH@ZIF-8 with apparent S_{SBE} of 444 m^2/g. If we assume that the dried CH and ZIF-8 have the similar density, then we can estimate the thickness of ZIF-8 coated at the surfaces of CH (i.e. the shell thickness of CH@ZIF-8). In this case, the decrease of BET surface area of ZIF-8 ball is linearly proportional to the decrease of ZIF-8 volume , then we have following equations:

$$\frac{V_{ZIF-8}-V_{CH}}{V_{ZIF-8}}=\frac{r_1^3-r_2^3}{r_1^3}=\frac{S_{BET}^{CH@ZIF-8}}{S_{BET}^{ZIF-8}}=\frac{444}{1270} \quad (1)$$

Then,

$$\Delta r = r_1 - r_2 = 0.1542 r_2 \quad (2)$$

Where V_{ZIF-8}, V_{CH}, r_1, r_2 are the volumes and semi-diameters of CH@ZIF-8 and CH, respectively. If CH has the diameter of 4.08 μm (detected by DLS), then the shell thickness of CH@ZIF-8 (Δr) is 314 nm, and the diameter of CH@ZIF-8 is 4.71 μm,

which is close to the value of 4.96 μm detected by DLS.

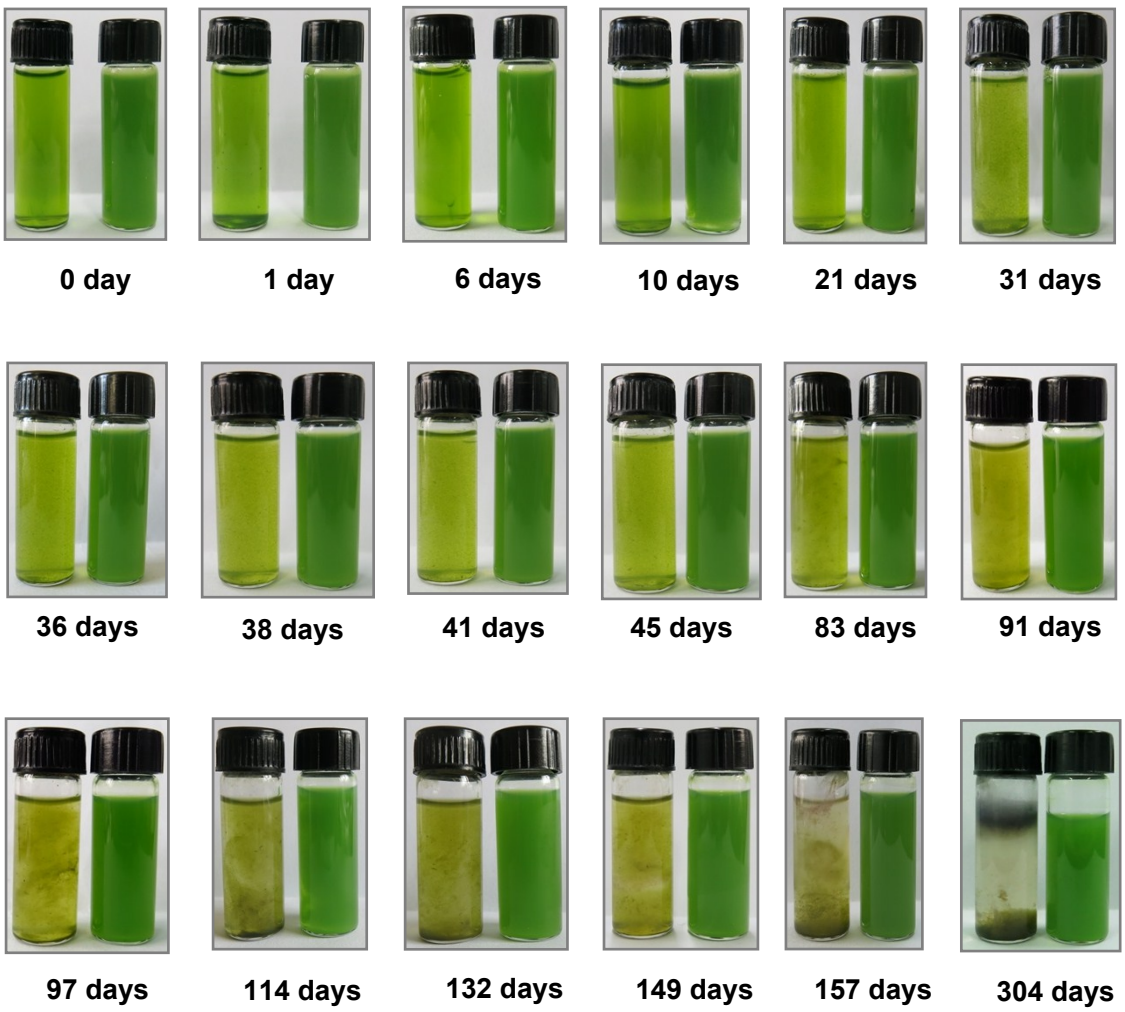

Figure S15. The stability of CH@ZIF-8 (right) and CH (left) solutions upon standing at 4 °C.

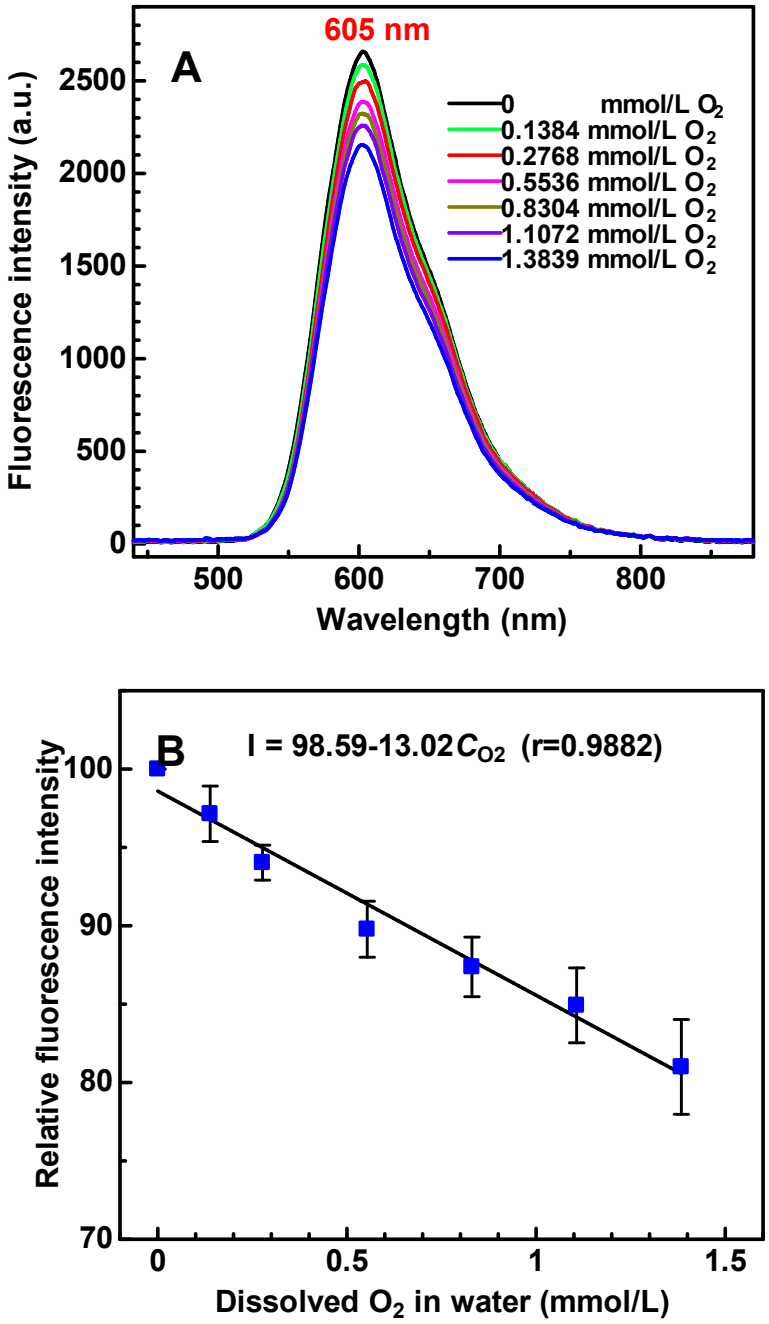

Figure S16. The fluorescent responses of the developed O_2 sensor to standard O_2 solutions with various concentrations (A) and the sensing calibration curve for the detection of O_2 in aqueous solution (B)

Detection of O_2 produced during photosynthesis of CH@ZIF-8.

From the calibration curve (Figure 9), a quantitative determination of O_2 concentration in water is obtained (Eq. S1):

$$I = 98.59 - 13.02C_{O_2} \quad \text{.....(S1)}$$

where I and C_{O2} are the fluorescence intensity recorded by sensor and the concentration of dissolved O_2 in water.

From Equation S1, the neat O_2 concentration produced in the solution can be obtained (Eq. S2):

$$\Delta C_{O_2} = \frac{I_{air}(1 - \frac{I}{I_{air}})}{13.02} = \frac{94.97 \times (1 - \frac{I}{I_{air}})}{13.02} = 7.294 \times (1 - \frac{I}{I_{air}}) \quad \text{.....(S2)}$$

Where ΔC_{O2}, I, I_{air} are respectively the neat O_2 concentration produced in the solution, fluorescent intensities obtained at "t" and 0 for the CH@ZIF solution initially saturate with air.

Table S1. Fluorescent detection of O_2 production from CH@ZIF-8 photosynthetic system during light illumination.

Reaction time (min)	I/I_{air}(%)[a]	O_2 production (mmol/L)
0	100	0
30	95.86	0.3018
60	92.09	0.5768
90	87.98	0.8767
120	87.16	0.9361
150	90.89	0.6646

[a] I, I_{air} are fluorescent intensities obtained at "t" and 0 for the CH@ZIF solution initially saturate with air.

References

[1] T. Ishiji, K. Kudo, M. Kaneko, Sensor Actuat. B-Chem. **1994**, 22, 205-210.