Enhancing Carbon Capture Efficiency with a Large-Sized Bionic Jellyfish-Carbonic Anhydrase Complex

Supporting information

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Chemicals.

Carbonic anhydrase (bCA, from bovine erythrocytes) was purchased from Innochem (China). Beijing Plastic Factory No. 7 (China) provided the low-density polyethylene (LDPE) film. *ε*-Polylysine (*ε*-PLL) was purchased from Babel Tide Technology Co. (China). Sigma-Aldrich Chemical Co. (USA) supplied isopropyl thioxanthone (ITX), glycidyl methacrylate (GMA), glutaraldehyde (GA), *p*-nitrophenyl acetate (*p*-NPA), *p*-nitrophenol (*p*-NP), and fluorescein isothiocyanate (FITC). Shaanxi Fast Gas Co. (China) provided standard gases of CO_2/N_2 (0.15/0.85 vol). All of the other reagents were of analytical grade.

Experiment

The preparation process of Bj-CA.

Preparation of "tentacles" GMA/*ε*-PLL with varying lengths. Poly(lysine) hydrochloride with molecular weights of 0.7–1.5, 1–3, 2–4, 3–7, and 7–15 kDa was dissolved in 4 mL of deionized water, and equivalent gradient concentrations of GMA/deionized water (1.58–2.37×10^{-6} M) were added. The pH was adjusted to 4.5 using a 1 M hydrochloric acid solution and agitated for 12 hours at 60 °C and 250 rpm. Lastly, the GMA that had not reacted was taken out by spinning it at 8000 rpm three times in a 10 kDa ultrafiltration tube to obtain the GMA/*ε*-PLL "tentacles".

GMA/*ε*-PLL "tentacle" grafting. Using a "photo induced surface control/active grafting" method, the "tentacles" were grafted on inert "umbrella" films. This method is divided into two steps: 1) inoculating the photoinitiator isopropylthioanthrone dormant species (ITXSP) onto the surface of the "umbrella" through a UV-induced extraction-hydrogen coupling reaction;

2)grafting the "tentacle" layer onto the "umbrella" by generating surface radicals by ITXSP, which initiates graft polymerization under visible light irradiation. The specific operation is as follows: ITX/acetone saturation solution was applied evenly on both sides of the LDPE diaphragm, and ITXSP resting seeds were sown on the LDPE diaphragm surface (L-ITXSP) utilizing the "sandwich" structure illustrated in Figure 1(a) under 6 minutes of exposure to ultraviolet mercury light to establish sites for "tentacular" development. After evenly coating both sides of L-ITXSP with the L-PGMA/ε-PLL polymer solution, the "sandwich" structure was employed to activate the resting species of ITXSP under visible light irradiation (1 h) to graft the "tentacles" of GMA/ε-PLL onto the L-ITXSP surface to generate L-PGMA/ε-PLL (Figure 1(a)). After grafting, the membrane surface was thoroughly cleansed with deionized water.

Fixation of "stinging cell" CA. Immerse L-PGMA/ε-PLL in a 5% (v/v) aqueous glutaraldehyde (GA) solution. L-GA membranes were obtained after a 12-hour incubation at 35 °C at 200 rpm. Finally, the membranes were incubated for 12 hours in a 0.1 mg/mL CA/PBS buffer solution (0.05 M, pH=8). The "stinging cell" CA was successfully covalently immobilized in the "tentacle" PGMA/ε-PLL, referred to here as Bj-CA.

Structural characterization of Bj-CA.

A custom-designed (100 μm strip and SUST) photo mask was created to characterize the successful grafting of the "tentacle" L-PGMA/ε-PLL. The patterned L-PGMA/ε-PLL brush layer was created using the "sandwich structure" in Figure 1(a). The changes in the apparent morphology of the LDPE films before and after grafting were examined using an in situ microscope (VHX-7000, Keenes, Japan). X-ray photoelectron spectroscopy (XPS, Thermo Scientific K-Alpha+, America) was used to qualitatively analyze the elemental species and the different chemical valence states of the atoms of the substances. At each reaction step, the water contact angle of the membrane surface was measured using a video-optical contact angle meter (CD, DSA100B, KRUSS, and Germany).

Enzyme activity determination.

The Bradford technique is used to calculate enzyme load. 100 μL CA/PBS solution (0.05 M, pH = 8) with a concentration gradient of 0.05–0.12 mg/mL was placed in a 2 mL centrifuge tube, and 990 μL of Komas Brilliant Blue staining solution was added to stain the free CA. The standard curve of concentration versus absorbance was plotted. Take 100 μL of the remaining enzyme solution of the immobilized enzyme system in a 2 mL centrifuge tube and repeat the

above operation. Standard curves were used to quantify enzyme concentration and the quantity of leftover enzymatic solution, and enzymatic loading was obtained by difference.

The esterase technique was used to assess enzyme activity. Plot the concentration/absorbance standard curve at 400 nm of gradient concentration *p*-NP/PBS(0.01−0.045 mM, pH=8) using a UV-Vis spectrophotometer (UV-2600, Shimadzu, China). Bj-CA membrane was put in 3 mL PBS buffer, followed by 100 μL *p*-NPA/acetonitrile (0.01 M). After shaking the reaction system at 35 ℃, 300 rpm for 3 minutes, the absorbance of the solution system at 400 nm was measured, and the concentration of catalytic hydrolysis product *p*-NP was calculated by the standard curve. To exclude the influence of *p*-NPA autolysis, a blank control experiment was conducted in this work, in which the concentration of the hydrolysis product *p*-NP was evaluated in a buffer without adding an enzyme. The difference was utilized to calculate the actual quantity of substrate decomposition catalyzed by the enzyme. As the unit of enzyme activity, the quantity of enzyme necessary to release 1 μmol of *p*-NP per minute was chosen. The relative catalytic performance of immobilized CA under different conditions was characterized by relative activity (Eq. S-1).

$$Relative\ activity(\%) = \frac{Enzyme\ activity\ of\ each\ condition}{Enzyme\ activity\ of\ optimum\ condition} \times 100\% \quad \text{(Equation S-1)}$$

Effect of structure on immobilized CA activity.

To investigate the effect of structure on the activity of immobilized CA. In this study, the immobilization of CA was achieved by adsorption (hydrogen bonding between ε-NH_2 of ε-PLL and -COOH and -OH of CA) and covalent binding (-CHO of GA and -NH_2 of CA) interactions, respectively. Three sets of L-PGMA/ε-PLL and L-GA membranes were prepared separately and then immersed in CA/PBS buffer (0.05 M, pH = 8). By incubation for 12 h at 35 °C, the adsorption-immobilized CA (A-CA) and covalently immobilized CA (Bj-CA) membranes were obtained. Then, the enzymatic activities of A-CA and Bj-CA were measured by enzyme activity assay. Furthermore, this study incubated free CA into PBS buffer (pH = 8, 0.05 M) for the same time as immobilized CA at 35 °C to exclude the effect of the enzyme immobilization process on the enzyme activity of CA.

pH dependence and stability testing of immobilized CA.

Optimal catalytic pH. The catalytic activity of Bj-CA under different conditions was measured by the enzyme activity assay method described above and characterized by Eq. S-1,

respectively when the pH of the PBS buffer in the immobilized CA catalytic system was set to 6~10 at 35 °C.

pH-resistance characteristics. To investigate whether the pH resistance of immobilized CA was enhanced, free and immobilized CA were incubated in PBS buffer (0.05 M) at pH=6 and pH=10 for 10, 20, 30, 40, 50, and 60 min, respectively, and then measured according to the enzyme activity assay described above. The enzyme activity was characterized by relative activity (Eq. S-1).

Storage stability. Free CA and immobilized CA were each stored at 25 °C、40 °C and 0 °C. Residual activitiy were determined according to the enzyme activity assay described above. Enzyme activity was measured by relative activity (Eq. S-1).

Effect of mass transfer on the activity of immobilized CA and the flexible mass transfer characteristics of the "tentacles".

A single-factor experiment of grafting brush length and grafting density was developed in this work to thoroughly explore the effect of mass transfer on Bj-CA enzymatic activity. When the GMA concentration was 2.37×10^{-6} M, the *ε*-PLL molecular weights were adjusted to 0.7-1.5, 1-3, 2-4, 3-7, and 7-15 kDa. The relative enzyme activities of Bj-CA were calculated using Eq. S-1 to explore the influence of grafting brush length on mass transfer efficiency. An equivalent gradient concentration of GMA/deionized water ($1.58\text{-}2.37\times10^{-6}$ M) was produced to produce Bj-CA. The relative enzyme activities of immobilized CA were evaluated using eq. S-1 to explore the influence of grafting brush density on the enzyme activities of Bj-CA.

Additionally, particle exclusion tests were used to describe the flexible mass transfer capabilities of the "tentacle" PGMA/*ε*-PLL. Carboxylated Fe_3O_4 magnetic microspheres with a size of 200 nm were uniformly coated on a slide, and then FITC-stained L-PGMA/*ε*-PLL was placed on the slide with a coverslip to create a spatial barrier to the dense tentacle layer. The L-PGMA/*ε*-PLL surface was examined using laser confocal fluorescence microscopy (LSCM) using a 480 nm laser. A permanent magnet was put in the center of the stage, and the aggregation state and location of carboxylated Fe_3O_4 magnetic microspheres were controlled by unidirectionally altering the relative positions of the permanent magnet and stage. Finally, LSCM conducted a raster scan of the specified region to capture changes in the fluorescence layer of the "tentacles" over time.

CO_2 capture performance of free CA and Bj-CA and their operational stability.

In this study, the actual CO_2 capture effect of free CA and Bj-CA in aqueous solution was investigated to evaluate the application value of Bj-CA in CO_2 capture. Additionally, the reliability of operation of Bj-CA was examined through straightforward recovery and reuse. The gas used in the experiments was CO_2/N_2 0.15/0.85 (v/v) standard gas. Bj-CA/free CA was first placed in a 50 mL centrifuge tube with 40 mL PBS buffer (0.05 M, pH=9) and preheated in a water bath (35 ℃) for 5 min. The gas flow rate at the inlet was controlled by a gas flow meter at 15 mL/min, and the volume concentration of CO_2 in the outlet gas was measured by a CO_2 infrared analyzer (AW-T6, Zhaowei, China). The CO_2 capture efficiency of the reaction system was calculated using Eq. S-2.

$$CO_2\ absorption\ rate = Q_{inlet}\, y_{CO_2,\ inlet} - Q_{outlet}\, y_{CO_2,\ outlet} \quad \text{(Equation S-2)}$$

Q_{inlet} and Q_{outlet} are the inlet and outlet gas molar flow rates (mol/min), respectively, calculated from the gas volume flow rate (mL/min) and the gas concentration (mol/mL). $y_{CO_2,\ inlet}$ and $y_{CO_2,\ outlet}$ are the molar fractions of carbon dioxide at the gas inlet and outlet, respectively.

Operational Stability. The experiment was carried out as previously reported and was characterized by relative enzyme activity (Eq. S-1). CA was recovered after each batch procedure using a simple enzyme membrane extraction. After rinsing the enzyme membrane with PBS buffer (0.05M, pH=9), it was introduced to a fresh test mixture and consecutively cycled until enzyme activity dropped below 60%.

Results and discussion

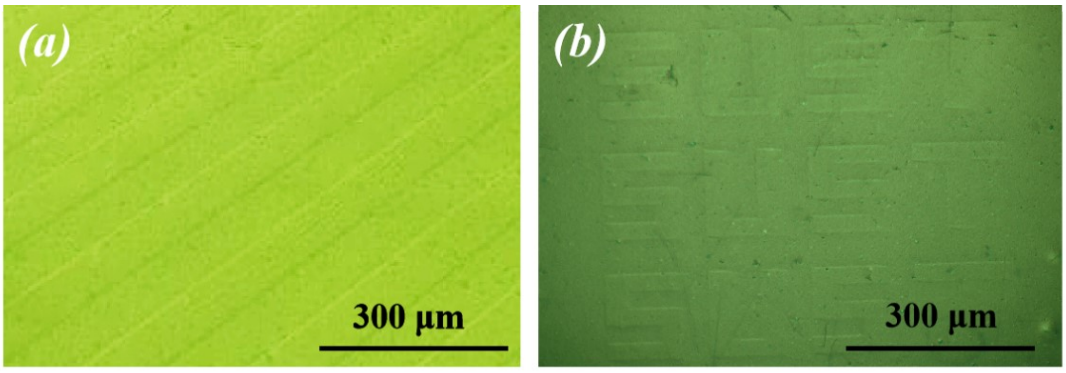


Figure S1 Patterned L-PGMA/ε-PLL observed under 3D microscope (a) stripe-like; (b) SUST letters

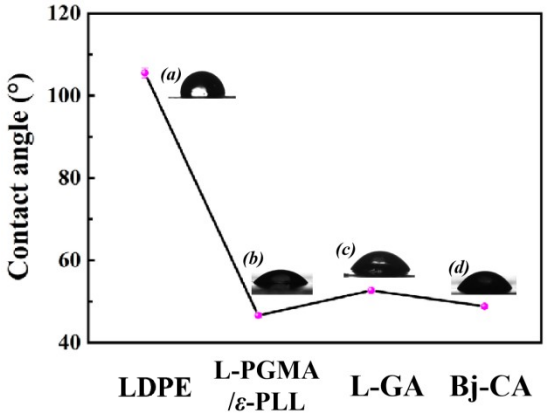


Figure S2 Static water contact angle of (a) LDPE, (b) L-PGMA/ε-PLL, (c) L-GA, and (d) Bj-CA

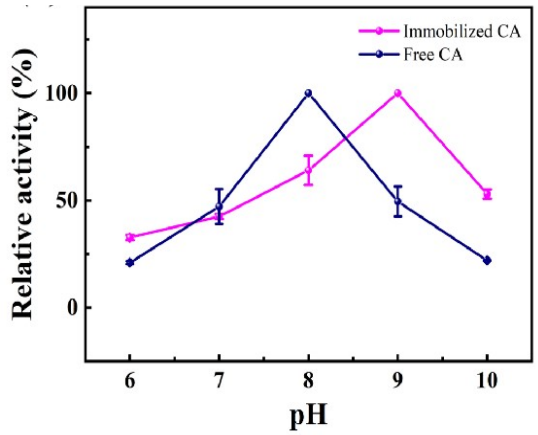


Figure S3 The optimal pH of Bj-CA and free CA (T=35 ℃)

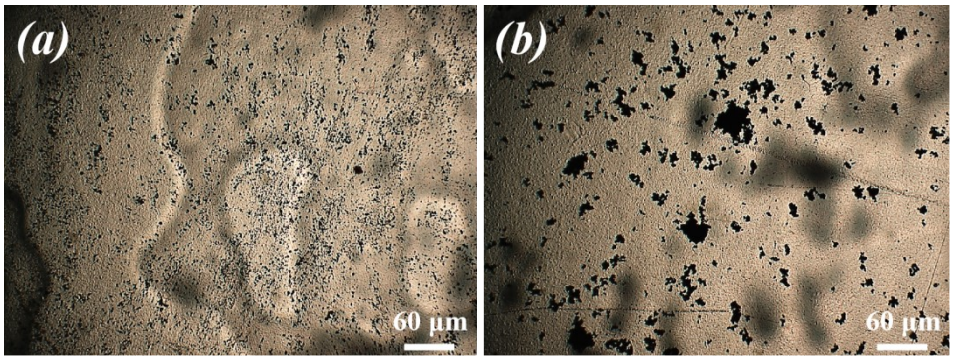


Figure S4 The structure of Fe_3O_4 magnetic microspheres under observed under 3D microscope：(a)Discrete and (b)aggregated states

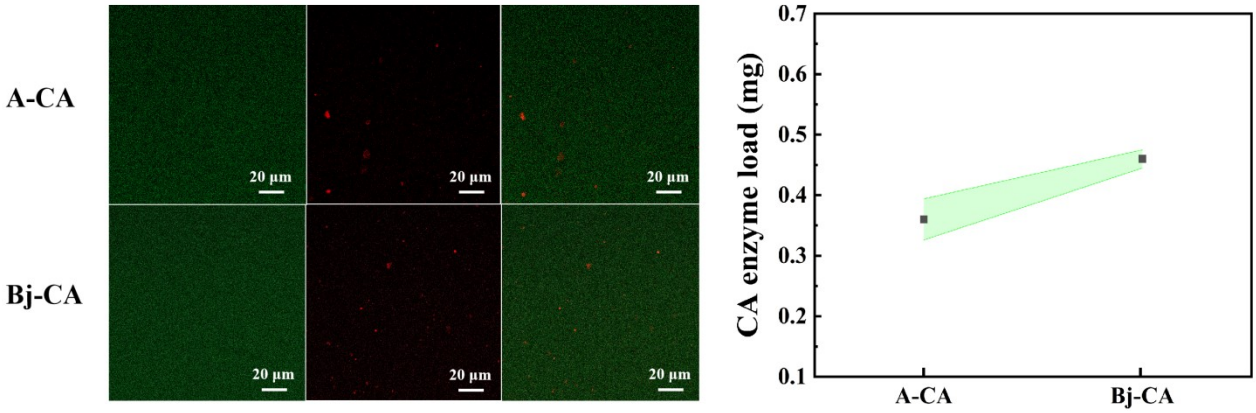
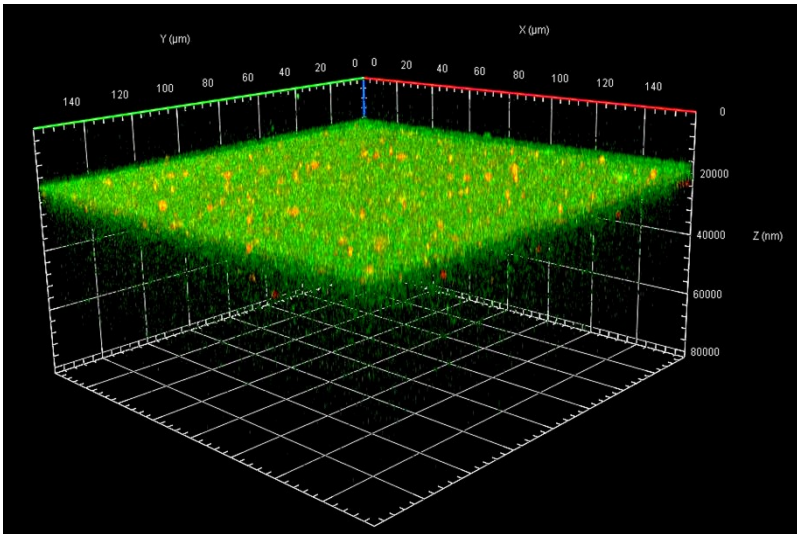


Figure S5(a) Distribution of immobilized A-CA versus Bj-CA after Rhodamine B staining; (b) CA loading of A-CA versus Bj-CA when the concentration of GMA is 2.37×10^{-6} mol/L and the molecular weight of GMA/ε-PLL is 30-70 kDa

As can be seen from Figure S4(a), both adsorption-immobilized CA (A-CA) and covalently-immobilized CA (Bj-CA) after rhodamine staining successfully appeared in the green brush layer structure, which can be a preliminary proof of the successful immobilization of CA. In addition, as shown in Figure S4(b), when the concentration of GMA was 2.37×10^{-6} mol/L and the molecular weight of GMA/ε-PLL was 30-70 kDa, both A-CA and Bj-CA possessed the corresponding enzyme loading, which further proved the successful immobilization of CA. Thank you for bringing this to our attention.



Figrue S6 Orientation of tentacles in Bj-CA under LSCM after staining L-GMA/ε-PLL and CA by FITC and Rhodamine B, respectively

Table S1 Operational stability of conventional CA mass transfer enhancement strategies

Substrate	Immobilization method	Recycling method	Number of cycles	Relative enzyme activity	Ref.
Biosilica matrix	Encapsulation	Centrifugation	4	87%	[1]
Hydrophilic textile support materials	Encapsulation	Filter	10	59%	[2]
Mesoporous silica anoparticles	Covalent connection	Centrifugation	8	41%	[3]
Fe_3O_4 magnetic microspheres	Covalent coupling	Magnetism	8	40%	[4]
ZIF-8	Adsorption	Centrifugation	9	85%	[5]
ZIF-8	Encapsulation	Centrifugation	6	64%	[6]

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