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Supplementary Information

Encapsulation of collagenase within biomimetically mineralized metal–organic frameworks: designing biocomposites to prevent collagen degradation

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The supplementary experiments and/or results are described below.

1. Fluorescent labeling of enzyme and CLSM analysis

Approximately 10 mg of bacterial collagenase were dispersed in 1 mL of a carbonate-bicarbonate buffer (0.1 M, pH 9.2). A solution of fluorescein isothiocyanate (FITC) was prepared by dissolving 2 mg of fluorescent dye in 1 mL of anhydrous dimethyl sulfoxide. The fluorescent labeling was conduct as follows: 50 μL of the FITC solution was gradually added to the enzyme solution under gentle agitation, and then the reaction mixture was incubated in the dark for 8 h at about 4 °C. After incubation, the FITC-labeled enzyme was recovered by passing the mixture through ultrafiltration membranes with nominal molecular weight limit of 30,000 (Amicon Ultra-0.5 centrifugal filter devices). Solvent exchange was performed three times by reconstituting the concentrate to the original sample volume with ultrapure water and then repeating the filtration process. The concentration/solvent-exchange cycles aimed at removing buffer salts and unreacted FITC molecules from the enzyme mixture. Next, the FITC-labeled enzyme concentrate was used in the preparation of a fresh batch of collagenase@ZIF-8 composite (adopting the same synthetic conditions and purification steps described in the article).

The presence of FITC-labeled enzymes in the MOF composite was examined via confocal laser scanning microscopy (CLSM). Powder samples were analyzed on a Leica TCS SPE microscope using the following settings: laser excitation wavelength, 488 nm; laser intensity (AOTF control), 10%; emission bandwidth, 500–600 nm; ×100/1.4 NA in oil.

2. UV-vis spectroscopy – Calibration curve model

A stock solution composed of bacterial collagenase (0.5 $mg \cdot mL^{-1}$) and excess of 2-methylimidazole (70 $mg \cdot mL^{-1}$) in ultrapure water was prepared in a 25-mL volumetric flask.

Aliquots were extracted from the stock to obtain diluted solutions containing collagenase in the following concentrations ($\mu g \cdot mL^{-1}$): 210; 180; 150; 120; 90; 60; 30. The concentration of 2-methylimidazole in the diluted solutions was kept at 70 $mg \cdot mL^{-1}$ by using aqueous solution of 2-methylimidazole (70 $mg \cdot mL^{-1}$) as diluent to achieve the total diluted volumes. Fundamentally, the organic ligand was employed in large excess to produce standard solutions that mimicked the composition of the original supernatant (from the biomimetic synthesis). The standard solutions were analyzed using a Perkin Elmer Lambda 50 spectrophotometer. Absorbance spectra were collected in the range of 200–800 nm with a resolution of 1 nm and scan speed of 141.2 $nm \cdot min^{-1}$. The results are shown in Fig. S1.

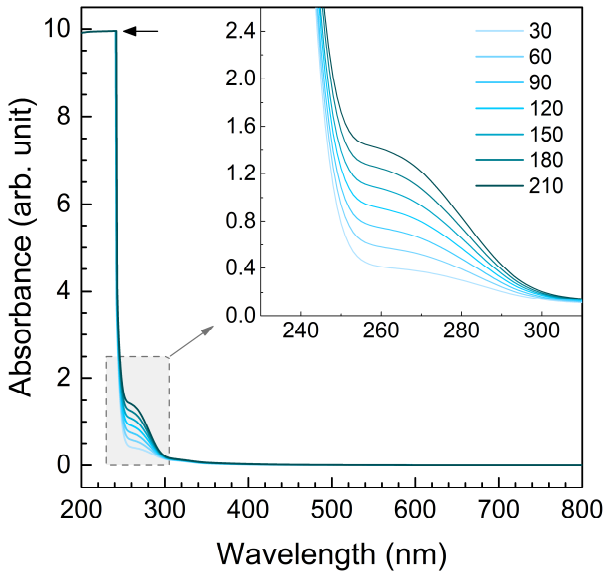

Fig. S1. (a) Concentration-dependent absorption spectra of collagenase in aqueous solution of 2-methylimidazole. Collagenase concentration range, 30–210 $\mu g \cdot mL^{-1}$. The black arrow indicates a saturated band corresponding to the excess of 2-methylimidazole in the solutions.

To construct the calibration curve, each standard solution had the absorbance monitored at 258 nm.

Additionally, aqueous solutions of individual components used in the biomimetic synthesis of ZIF-8 (or related procedures) were prepared for recording the absorption spectrum of each analyte to support the spectroscopic investigation (Fig. S2). Collagenase showed a distinct absorption band peak at 258 nm, which did not overlap with peaks of other components.

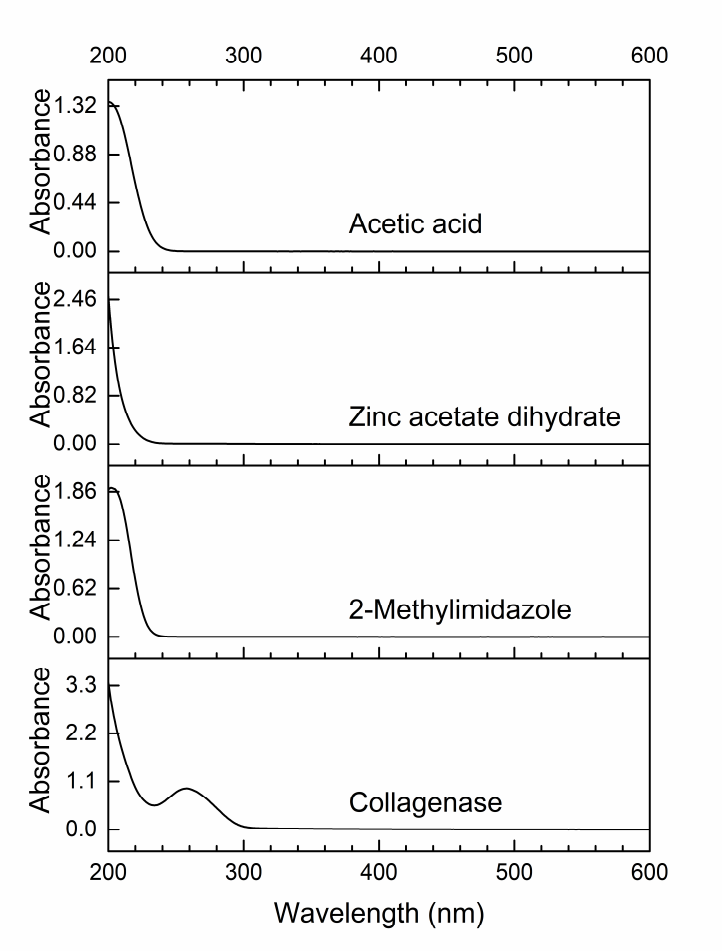

Fig. S2. Absorption spectrum of individual components used in the biomimetic synthesis of ZIF-8 (or related procedures). Each analyte was dissolved in ultrapure water.

3. UV-vis spectroscopy – Stability of bacterial collagenase in water over time

An investigation was conducted to verify if the absorbance by bacterial collagenase in water remained constant over time. This was a requisite for estimating the amount of residual

enzyme in the supernatant based on its typical absorption peak at ≈ 258 nm. First, an aqueous solution of bacterial collagenase (0.5 mg·mL^{-1}, 25 mL) was prepared in a volumetric flask. A dilute solution (180 µg·mL^{-1}, 25 mL) was then obtained and transferred to a beaker, where its pH was measured immediately with a digital pH-meter. A 1-mL aliquot was extracted from the diluted solution and transferred to a quartz cuvette to monitor the absorbance of collagenase over time. A spectrum was recorded every 30 min for 6 h, using the same scanning parameters mentioned above. In parallel, the remainder of the diluted collagenase solution (180 µg·mL^{-1}, 24 ml) had its pH adjusted to 5 by adding acetic acid (0.1 M) gradually. After adjusting the pH, a 1-mL aliquot of the solution was transferred to a quartz cuvette and analyzed over a 6-h period. The result of this investigation is shown in Fig. S3.

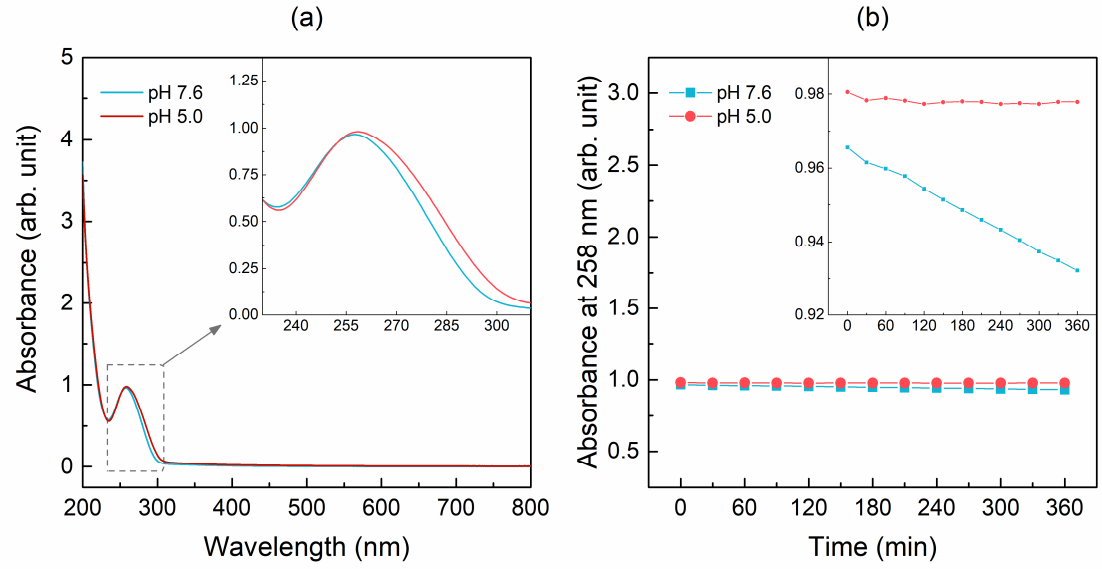

Fig. S3. (a) Absorption spectra of bacterial collagenase in water (180 µg·mL^{-1}) at different pH. (b) Absorbance at 258 nm by bacterial collagenase in water over a 6-h period.

The absorption spectra of collagenase in water with pH 7.6 and 5.0 were comparable (Fig. S3(a)). In aqueous solution with initial pH of 7.6, the absorbance at 258 nm (by collagenase) decreased linearly over a 6-h period (Fig. S3(b)). However, the final measured absorbance (A =

0.9322) was only 3.5% lower than the initial one (A = 0.9656), which suggested that the absorbance at 258 nm was still reliable for assessing the amount of residual collagenase in the supernatant by means of UV-vis spectroscopic data. The absorbance at 258 nm remained practically constant for the collagenase solution with pH adjusted at 5 (Fig. S3(b)). Monitoring the absorbance by the enzyme at pH 5.0 was relevant for estimating the amount of enzyme released via pH-triggered release procedure, which generated an aqueous phase with similar pH value.

4. Calculation of lattice parameter

The lattice parameter (a) for the biomimetically mineralized ZIF-8 crystal was determined through equation 1, which suits the special case of cubic crystal structures, where all of the constants are equal and we only refer to a.[1] In equation 1, λ is the wavelength of the incident X-ray beam (1.5406 Å in this case), θ is the Bragg angle and h, k and ℓ represent the Miller indices.

$$a = \frac{\lambda}{2 \cdot sin\,\theta} \cdot \sqrt[2]{h^2 + k^2 + \ell^2} \quad (1)$$

The parameters substituted in the equation 1 are informed in Table S1.

Table S1. Parameters used in the calculation of the lattice parameter of the biomimetically mineralized ZIF-8

Peak positon (2θ)*	Attributed plane (hkℓ) †	Obtained lattice parameter (Å)
7.32	(011)	17.07
10.40	(002)	17.00
12.72	(112)	17.03
14.72	(022)	17.01
16.44	(013)	17.04
18.04	(222)	17.02

* Obtained from the XRD pattern of the collagenase@ZIF-8 composite

† The planes were attributed to the peaks based on information reported in the literature previously [2,3]

The averaged value indicates a lattice parameter of 17.03 Å ± 0.02.

5. Pore structure parameters

The Brunauer–Emmett–Teller model surface area (S_{BET}) and total pore volume (V_{total}) calculated from the nitrogen isotherms are shown in Table S2.

Table S2. Pore structure characteristics of the obtained products

Material	Single point surface area ($m^2 \cdot g^{-1}$)	S_{BET} ($m^2 \cdot g^{-1}$)	V_{total} ($cm^3 \cdot g^{-1}$)
As-synthesized ZIF-8	1267	1222	0.57
Biomimetically mineralized ZIF-8	1280	1237	0.70

6. Supplementary illustrations

To illustrate the size of *Clostridium* collagenases, ribbon representations of two known crystal structures were created using a molecular graphics software (RasMol[4] version 2.7.5). The used three-dimensional structural data were freely available for download on the Protein Data Bank (PDB) website (deposition codes 2Y3U[5] and 4AR1[6]).

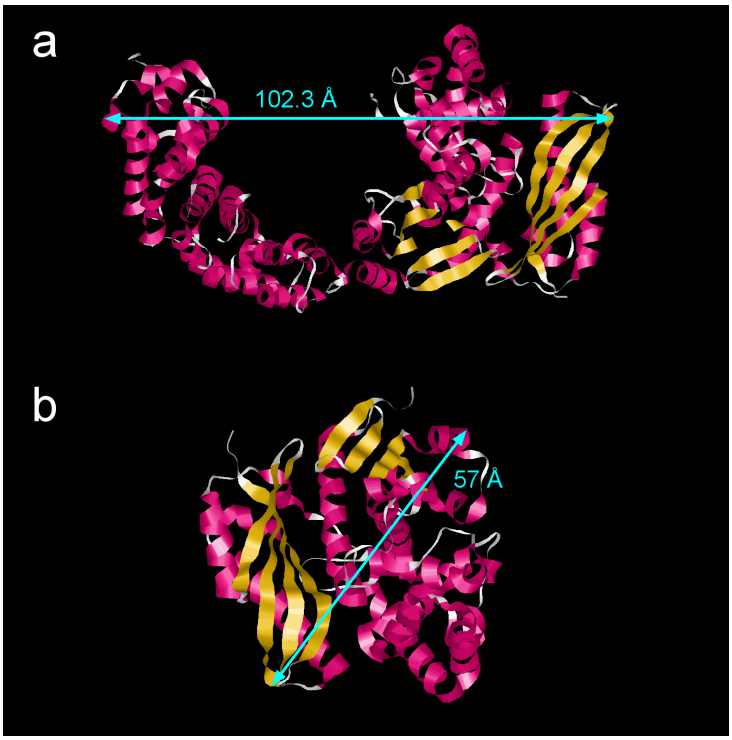

Fig. S4. Ribbon representation of (a) collagenase G from *Clostridium histolyticum* and (b) peptidase domain of collagenase H from *Clostridium histolyticum*. The calculated distances are presented in angstroms (Å). Crystal data were obtained from the PDB website with the codes 2Y3U and 4AR1.

Supplementary references

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