

Supporting Information

Crystal surface reactivity of Esterase@Zeolitic Imidazolate Framework Biocomposites

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S1. General Methods and Materials

Materials and reagents. All chemical reagents and solvents were commercially obtained from Sigma-Aldrich and were used without further purification.

Powder X-Ray Diffraction (PXRD) analysis. PXRD data were collected on a Bruker D2 PHASER diffractometer equipped with a Cu anode ($K\alpha$ radiation, $\lambda = 1.5418 \text{ \AA}$), collecting with a 1 mm slit in the $5\text{--}35^\circ$ 2θ range with steps of 0.02° and a time between each step of 1 s.

Fourier Transformed Infrared (FTIR) analysis. FTIR spectra were recorded on a THERMO NICOLET IR200 equipped with an ATR module.

Nitrogen adsorption isotherms. Nitrogen adsorption isotherms were measured at 77 K on a Micromeritics 3Flex volumetric instrument. Prior to measurement, samples were heated at 393 K for 12 h and outgassed to 10^{-1} Pa in a Micromeritics SmartVacPrep Module.

Thermogravimetric Analysis (TGA). TGA experiments were performed in a METTLER TOLEDO equipment from the Scientific Instrumentation Centre (CIC) of the University of Granada. In a typical analysis, TGA measurements were conducted in an oxidative atmosphere (air). The weight change of the samples was recorded from 30 to 950 $^\circ\text{C}$ with a ramp rate of 5°C min^{-1} .

Scanning Electron Microscopy (SEM) analysis. SEM images were obtained on a JEOL JSM-6490LV Microscope system from the Central Research Support Services of the University of Malaga.

Transmission Electron Microscopy- Energy Dispersive X-Ray Spectrometry (TEM-EDX). TEM-EDX images were obtained in a HAADF Thermo Fisher Scientific TALOS F200X in the Scientific Instrumentation Centre (CIC) of the University of Granada.

Z potential analysis. Z potential data was obtained on a Litesizer DLS 500 Anton-Paar employing Omega Cuvettes made from polycarbonate and filled with 600 μL of sample.

X-ray Photoelectron Spectroscopy (XPS). XPS analyses were obtained using a Kratos Axis Ultra-DLD instrument, located at the X-ray Photoelectron Spectroscopy Unit of the Scientific Instrumentation Center (CIC) at the University of Granada.

Diisopropylfluorophosphate (DIFP) degradation experiments. Simulant nerve agent degradation experiments were followed in an Agilent 8860 Gas Chromatograph. This chromatograph has a 16 port autosampler, a HP-5 column (50 m length, 0.320 mm diameter and 1.05 μm thickness) and a FID detector.

Esterase activity determination. Esterase activity was evaluated by using a colorimetric method which employs indoxyl acetate as a substrate that is converted into indigo blue. The concentration of the enzymatic product was calculated by UV-vis spectroscopy at $\lambda = 620 \text{ nm}$ using a Nanoquant model Infinite M200 Pro with 24-well plate.

^1H and ^{31}P Nuclear Magnetic Resonance Spectroscopy (NMR). NMR data was recorded on a 400 MHz BRUKER Nanobay Avance III HD High-Definition spectrometer.

S2. Protocols.

S2.1. Synthetic procedures

S2.1.1. Synthesis of Esterase@ZIF biocomposites.

Esterase@ZIF biocomposites were synthesized by varying the composition of zinc salt, 2-methylimidazole (mImH) and esterase in a fixed volume of water. In this way, we have carried out the biomineralization of three different Zeolitic Imidazolate Framework (ZIF) crystalline phases around a pig liver esterase (EC 3.1.1.1), which are sod ZIF-8, ZIF-CO₃-1 (abbreviated as ZIF-C) and ZIF-L. For this purpose, we prepared the following stock solutions:

- Zn(OAc)₂·2H₂O solution (160 mM). 0.3512 g of Zn(OAc)₂·2H₂O is dissolved in 10 mL of H₂O milli-Q.
- Zn(NO₃)₂·6H₂O solution (100 mM). 0.297 g of Zn(NO₃)₂·6H₂O is dissolved in 10 mL of H₂O milli-Q.
- mImH solution-1 (2560 mM). 2.1018 g of 2-methylimidazole is dissolved in 10 mL of H₂O milli-Q.
- mImH solution-2 (1000 mM). 0.821 g of 2-methylimidazole is dissolved in 10 mL of H₂O milli-Q.
- Esterase solution (26.3 mg/ml). 52.6 mg of esterase is dissolved in 2 mL of H₂O milli-Q.

In a typical experiment, the synthesis is carried out in 2 mL Eppendorf tubes (final reaction volume 2 mL), and 6 replicates for each Esterase@ZIF material are made. For each replicate, we prepared a “metal” and a “Ligand+esterase” solution in H₂O milli-Q according to tables S1-2.

Table S1. Volumes (μL) of stock solutions to synthesize Esterase@ZIF-8_120nm and Esterase@ZIF-C biocomposites

Sample	Metal Solution		Ligand+esterase solution		
	Zn(OAc) ₂ ·2H ₂ O	H ₂ O	mImH solution-1	H ₂ O	Esterase
Esterase@ZIF-8_120nm	500	500	500	176	324
Esterase@ZIF-C	910	90	234	442	324

Table S2. Volumes (μL) of stock solutions to synthesize Esterase@ZIF-L biocomposite.

Sample	Metal Solution		Ligand+esterase solution		
	Zn(NO ₃) ₂ ·6H ₂ O	H ₂ O	mImH solution-2	H ₂ O	Esterase
Esterase@ZIF-L	500	500	400	276	324

“Metal” solution was added to “Ligand+esterase” solution slowly but steadily and mixed 3 times by inversion. The resulting solutions (Table S3) were then incubated at room temperature for 24 h in static conditions. Then, the samples were centrifuged at 15,000 rpm for 5 min and the first supernatant was collected for protein quantification using Bradford assay (to calculate encapsulation efficiency, see S2.2.1). The samples were washed with 1 mL of Milli-Q water and resuspended by mixing with a spatula and 10 s of vortexing afterwards. The washing was repeated 3 times in total. Finally, the samples were air dried at room temperature for 24 h.

Table S3. Final concentrations of Zinc salt, mImH and esterase in each Esterase@ZIF synthesis.

Sample	Zn (mM)	mImH (mM)	Esterase (mg/mL)
Esterase@ZIF-8_120nm	40	640	4.26
Esterase@ZIF-C	72.8	299	4.26
Esterase@ZIF-L	25	200	4.26

S2.1.2. Synthesis of ZIF-8_150nm

0.47 g of zinc nitrate hexahydrate, $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (1.8 mmol), were dissolved in 10 mL of methanol and 10 mL of milli-Q water. Besides, 1.0 g of 2-methylimidazole, mImH (60 mmol), was dissolved in 10 mL of methanol. The two solutions were mixed and stirred for 2 h at room temperature. The resulting solid was recovered by centrifugation (10,000 rpm x 10 min) and washed once with methanol. The final product was dried at 110 °C overnight.¹

S2.1.3. Synthesis of ZIF-L.

0.540 g of zinc nitrate hexahydrate, $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (1.8 mmol), were dissolved in 40 mL of milli-Q water and 1.3 g of 2-methylimidazole (15.8 mmol) were dissolved in a separate solution of 40 mL of milli-Q water. Both solutions were mixed and stirred for 24 hours at room temperature. The resulting white solid was washed with water (3 x 20 mL) and recovered by centrifugation (4,000 rpm, 10 min). Finally, the sample was air dried at room temperature for 24 h.

S2.1.4. Synthesis of ZIF- CO_3 -1.

0.264 g of zinc nitrate hexahydrate, $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (0.87 mmol), and 0.074 g of 2-methylimidazole (0.87 mmol) were dissolved in 17.6 mL of a mixture of DMF/ H_2O (10:1, v/v). The solution was placed in a 23 mL Teflon-lined autoclave and the reaction was heated at 140 °C for 24h. The resulting white solid was washed with DMF (3 x 20 mL) and MeOH (3 x 20 mL) and recovered by centrifugation (4,000 rpm, 10 min). Finally, the sample was air dried at room temperature for 24 h.

S2.1.5. Synthesis of BSA@ZIF biocomposites.

Similar to Esterase@ZIF biocomposites (see S2.1.1.), BSA@ZIF biocomposites were synthesized by varying the composition of zinc salt, 2-methylimidazole (mImH) and Bovine Serum Albumin (BSA) in a fixed volume of water. We prepare the following stock solutions:

- $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ solution (160 mM). 0.3512 g of $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ is dissolved in 10 mL of H_2O milli-Q.
- mImH solution-1 (2560 mM). 2.1018 g of 2-methylimidazole is dissolved in 10 mL of H_2O milli-Q.
- BSA solution (20 mg/mL). 100 mg of BSA is dissolved in 5 mL of H_2O milli-Q.

In a typical experiment, the synthesis is carried out in 2 mL Eppendorf tubes (final reaction volume 2 mL), and 6 replicates for each BSA@ZIF material are made. For each replicate, we prepared a “metal” and a “Ligand+BSA” solution in H_2O milli-Q according to table S4.

Table S4. Volumes (μL) of stock solutions to synthesize BSA@ZIF-8_1.2 μm and BSA@ZIF-C biocomposites

Sample	Metal Solution		Ligand+BSA solution		
	$\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$	H_2O	mImH solution-1	H_2O	BSA
BSA@ZIF-8_1.2 μm	200	800	500	467	33
BSA@ZIF-C	350	650	145	531	324

“Metal” solution was added to “Ligand+BSA” solution slowly but steadily and mixed 3 times by inversion. The resulting solutions (Table S5) were then incubated at room temperature for 24 h. Then, the samples were centrifuged at 15,000 rpm for 5 min and the first supernatant was collected for protein quantification using Bradford assay (to calculate encapsulation efficiency). The sample was washed with 1 mL of Milli-Q water and resuspended by mixing with a spatula and 10 s of vortexing afterwards. The washing was repeated 3 times in total. Finally, the samples were air dried at room temperature for 24 h.

Table S5. Final concentrations of Zinc salt, mImH and BSA in each BSA@ZIF synthesis.

Sample	Zn (mM)	mImH (mM)	BSA (mg/mL)
BSA@ZIF-8_1.2 μm	40	640	0.33
BSA@ZIF-C	28	185	3.24

Additionally, we synthesized BSA@ZIF-8 biocomposite with smaller particle size.² In this case, we added 200 mg of BSA to 10 mL of a mImH aqueous solution 1400 mM. The mImH/BSA solution was allowed to age for 1 h. Then, we added 10 mL of zinc acetate dihydrate, $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$, aqueous solution 20 mM and this mixture was kept at room temperature for 16 h. When the reaction finished, we centrifuged at 5,000 rpm (30 min) and the supernatant was collected. This supernatant was further centrifuged at 14,000 rpm for 30 min and the resulting white precipitated was collected. This white solid was washed with water and allowed to air dry in order to obtain BSA@ZIF-8_120nm nanoparticles.

S2.1.6. Synthesis of Esterase@ZIF-8_60nm

In order to biomineralize the crystalline phase of sod ZIF-8 with a smaller particle size, we increased the ligand-to-metal ratio. For this purpose, we prepared the following stock solutions:

- $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ solution (160 mM). 0.3512 g of $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ is dissolved in 10 mL of H_2O milli-Q.
- mImH solution-1 (2560 mM). 2.1018 g of 2-methylimidazole is dissolved in 10 mL of H_2O milli-Q.
- Esterase solution (26.3 mg/ml). 52.6 mg of Esterase is dissolved in 2 mL of H_2O milli-Q.

In a typical experiment, the synthesis is carried out in 2 mL Eppendorf tubes (final reaction volume 2 mL), and 6 replicates are made. For each replicate, we prepared a “metal” and a “Ligand+esterase” solution in H_2O milli-Q according to table S6.

Table S6. Volumes (μL) of stock solutions to synthesize Esterase@ZIF-8_60nm biocomposite.

Sample	Metal Solution		Ligand+esterase solution		
	Zn(OAc) ₂ ·2H ₂ O	H ₂ O	mlmH Solution-1	H ₂ O	Esterase
Esterase@ZIF-8_60nm	200	800	676	0	324

“Metal” solution was added to “Ligand+esterase” solution slowly but steadily and mixed 3 times by inversion. The resulting solutions (Table S7) were then incubated at room temperature for 24 h in static conditions. Then, the samples were centrifuged at 15,000 rpm for 5 min and the first supernatant was collected for protein quantification using Bradford assay (to calculate encapsulation efficiency, see S2.2.). The samples were washed with 1 mL of Milli-Q water and resuspended by mixing with a spatula and 10 s of vortexing afterwards. The washing was repeated 3 times in total. Finally, the samples were air dried at room temperature for 24 h.

Table S7. Final concentrations of Zinc salt, mlmH and Esterase in Esterase@ZIF-8_60nm synthesis.

Sample	Zn (mM)	mlmH (mM)	Esterase (mg/mL)
Esterase@ZIF-8_60nm	16	865	4.26

The encapsulation efficiency of this new material is 84 % and possesses a loading capacity of 0.20 mg esterase/mg biocomposite.

S2.2. Evaluation of protein encapsulation efficiency.

S2.2.1. Encapsulation efficiency from the supernatant.

We evaluated protein (BSA or esterase) encapsulation of each Protein@ZIF biocomposite material using the Bradford assay. In this case, we have quantified the encapsulated protein by difference with the protein remaining in the supernatants after each synthesis. After 24 h of synthesis, the solid product is separated via centrifugation (15,000 rpm for 5 min) and the supernatant (~ 2 mL) is recovered by micropipette. 33.3 μL of this supernatant is mixed with 1 mL of Bradford reagent. This mixture is shaken gently and incubated for 30 min at room temperature. Finally, we measured the absorbance at 590 nm in a UV-Vis spectrophotometer. All the experiments were performed in sixfold to calculate the encapsulation efficiency (EE) and protein loading capacity (LC) (see Table S8).

$$EE (\%) = \frac{[Total\ Protein\ added] - [Protein\ in\ supernatant]}{[Total\ Protein\ added]} \times 100$$

$$LC = \frac{mg\ protein\ encapsulated}{mg\ biocomposite}$$

S2.2.2. Encapsulation efficiency from digested ZIFs.

We also evaluated the encapsulation efficiency for Protein@ZIF materials by digesting the ZIF component of the synthesized Protein@ZIF biocomposite materials. For this purpose, 5 mg of each Protein@ZIF material was incubated in 1 mL of citrate buffer solution (0.1 M, pH = 5.5) for 24 h. Then, 33.3 μL of this solution is mixed with 1 mL of Bradford reagent. This mixture is shaken gently and incubated for 30 min at room temperature. Finally, we measure the absorbance at 590 nm in a UV-Vis

spectrophotometer. All the experiments were performed in sixfold to calculate the encapsulation efficiency (EE) (see Table S8).

$$EE (\%) = \frac{[Protein\ in\ supernatant\ after\ digestion]}{[Total\ Protein\ added]} \times 100$$

Table S8. Comparison of encapsulation efficiency and loading capacity for Protein@ZIF biocomposites between the supernatant and digested experiments

Sample	Supernatant		Digested	
	EE (%)	LC	EE (%)	LC
Esterase@ZIF-8_120nm	76 ± 2	0.37 ± 0.01	11*	0.06*
Esterase@ZIF-C	96 ± 1	0.47 ± 0.03	87	0.43
Esterase@ZIF-L	74 ± 2	0.37 ± 0.01	76	0.38
BSA@ZIF-8_1.2µm	99 ± 1	0.05 ± 0.01	-	-
BSA@ZIF-8_120nm	70 ± 3	0.44 ± 0.20	-	-
BSA@ZIF-C	35.5 ± 4.7	0.11 ± 0.04	-	-

* Digested values calculated for Esterase@ZIF-8_120nm biocomposite does not match with the supernatant because of greater stability of sod ZIF-8 phase in citrate buffer (see results from TGA).

S2.3. Nerve agent simulant degradation.

S2.3.1. DIFP degradation by ZIFs and Protein@ZIF biocomposites

The hydrolytic degradation of nerve agent simulant diisopropylfluorophosphate (DIFP) was evaluated using gas chromatography-flame ionization detector (GC-FID) analysis. In a typical experiment, we incubated 2.5 µL of DIFP (0.015 mmol) with Esterase@ZIFs, BSA@ZIFs and ZIFs materials using a DIFP:ZIF ratio of 1:6 (0.084 mmol of ZIF), together with 1.08 µL dimethylformamide (DMF, 0.015 mmol, used as an internal reference) in 0.5 mL of Tris-HCl buffer solution (0.1 M, pH = 7.4). The reaction was stirred at room temperature, and DIFP degradation was monitored via GC-FID analysis at different times (Table S9).

Therefore, for the Esterase@ZIFs and BSA@ZIFs hybrids, we considered the proportion of ZIF present in the hybrids previously calculated (see Table S8).

Table S9. Half-life times and kinetic constants values for diisopropylfluorophosphate degradation reaction.

Sample	t _{1/2} (min)	Kinetic constant
Esterase@ZIF-8_120nm	8.0	0.054 min ⁻¹
Esterase@ZIF-8_60nm	2.1	0.154 min ⁻¹
ZIF-8_150nm	40	0.0224 min ⁻¹
Esterase@ZIF-C	7.2	0.044 min ⁻¹
ZIF-C	1382	0.0005 min ⁻¹
Esterase@ZIF-L	30	0.017 min ⁻¹
ZIF-L	75	0.0065 min ⁻¹
BSA@ZIF-8_1.2µm	174	0.0041 min ⁻¹
BSA@ZIF-8_120nm	22	0.0283 min ⁻¹
BSA@ZIF-C	78	0.0101 min ⁻¹
Esterase	2490	0.014 M ⁻¹ ·min ⁻¹

S2.3.2. Esterase+ZIFs physical mixture.

In order to determine the synergistic effect on Esterase@ZIF biocomposites, we carried out DIFP degradation experiments of the physical mixture between ZIFs and esterase.

In a typical experiment, we incubated 2.5 μL of DIFP (0.015 mmol) with a mixture of esterase + ZIF using the same proportions of each component as in Esterase@ZIF experiments, together with 1.08 μL dimethylformamide (DMF, 0.015 mmol) in 0.5 mL of Tris-HCl buffer solution (0.1 M, pH = 7.4). The reaction was stirred at room temperature, and DIFP degradation was monitored via GC-FID analysis at different times.

S2.3.3. Hot filtration test.

To demonstrate the heterogeneity of the process, we performed hot filtration tests. For this purpose, in the middle of the reaction course between Esterase@ZIF biocomposites and DIFP, the reaction solid was filtered.

In a typical experiment, we incubated 2.5 μL of DIFP (0.015 mmol) with Esterase@ZIF biocomposites (0.084 mmol of ZIF), together with 1.08 μL dimethylformamide (DMF, 0.015 mmol, used as an internal reference) in 0.5 mL of Tris-HCl buffer solution (0.1 M, pH = 7.4). When DIFP degradation is around 50 %, this solution is filtered. After this, the presence of DIFP in the buffer solution was monitored via GC-FID analysis at different times.

S2.3.4. Control experiments

We also carried out some control experiments to demonstrate that DIFP degradation is carried out by the studied materials.

On the one hand, we checked DIFP concentration in Tris-HCl buffer solution throughout the whole experiment. In a typical experiment, we incubated 2.5 μL of DIFP (0.015 mmol) with 1.08 μL dimethylformamide (DMF, 0.015 mmol, used as an internal reference) in 0.5 mL of Tris-HCl buffer solution (0.1 M, pH = 7.4). The presence of DIFP in the buffer solution was monitored via GC-FID analysis at different times.

On the other hand, we monitored the possible degradation of DIFP by ZIFs precursors: 2-methylimidazole and $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$. In a typical experiment, we incubated 2.5 μL of DIFP (0.015 mmol) with 0.084 mmol of mImH/ $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$, together with 1.08 μL dimethylformamide (DMF, 0.015 mmol, used as an internal reference) in 0.5 mL of Tris-HCl buffer solution (0.1 M, pH = 7.4). The presence of DIFP in the buffer solution was monitored via GC-FID analysis at different times.

S2.4. Enzymatic Activity.

S2.4.1. Determination of Michaelis-Menten constant for AChE and Esterase enzymes.

The enzymatic activity was determined by the indoxyl acetate colorimetric method.³ In this method, the concentration of the enzymatic product, indigo blue, was determined via UV-vis spectroscopy at a wavelength of 620 nm, with the molar extinction coefficient (ϵ) set at 22,140 $\text{M}^{-1}\text{cm}^{-1}$. This approach was chosen over the traditional Ellman method due to the susceptibility of acetylthiocholine to nucleophiles, such as oximes or imidazolate ligands, which can result in false-positive readings.

In a typical experiment, 24-well culture plates were filled with 925 μL of Tris-HCl (0.1 M, pH = 7.4) buffer solution, 25 μL of an Esterase/AChE aqueous solution (75 U/mL); and they were incubated for 30 min at 37 °C. Afterwards, we added 50 μL of indoxyl acetate

solution in isopropanol with different concentrations (10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M, $2.5 \cdot 10^{-4}$ M, $5 \cdot 10^{-4}$ M, $7.5 \cdot 10^{-4}$ M, 10^{-3} M, $2.5 \cdot 10^{-3}$ M, $5 \cdot 10^{-3}$ M, $7.5 \cdot 10^{-3}$ M and 10^{-2} M) and the mixtures were incubated for an additional 30 min. Finally, 3.33 mL of dimethyl sulfoxide (DMSO) was added to solubilize the enzymatic product (indigo blue) and stop the reaction. The absorbance of the solutions was measured at $\lambda = 620$ nm.

S2.4.2. Inhibition of enzymatic activity by diisopropylfluorophosphate.

We evaluated Esterase and AChE inhibition by nerve agent simulant diisopropylfluorophosphate (DIFP) under simulated biological conditions. For this purpose, we incubated free Esterase/AChE solutions with different concentrations of DIFP. In a typical experiment, 24-well culture plates were filled with 825 μ L of Tris-HCl (0.1 M, pH = 7.4) buffer solution, 25 μ L of an Esterase/AChE aqueous solution (75 U/mL); and they were incubated for 30 min at 37 °C. Then, we added 100 μ L of aqueous DIFP solution with different concentrations ($5 \cdot 10^{-7}$ M, 10^{-6} M, $5 \cdot 10^{-6}$ M, 10^{-5} M, 10^{-4} M, 10^{-3} M, 10^{-2} M and 10^{-1} M) and this mixture was incubated for 1 h. Afterwards, 50 μ L of indoxyl acetate solution in isopropanol (3.0 mM) was added and the mixtures were incubated for an additional 30 min. Finally, 3.33 mL of dimethyl sulfoxide (DMSO) was added to solubilize the enzymatic product (indigo blue) and stop the reaction. The absorbance of the solutions was measured at $\lambda = 620$ nm. The corresponding inhibition percentage was calculated according to the following equation:

$$\text{Inhibition (\%)} = 100 - \left(\frac{\text{Inh. Enzymatic Activity}}{\text{Free Enzymatic Activity}} \right) \times 100$$

Inh. Enzymatic Activity = Enzymatic activity for Esterase/AChE incubated with DIFP at different concentrations

Free Enzymatic Activity = Enzymatic activity for free Esterase/AChE at 37 °C.

S2.4.3. Retained enzymatic activity after Esterase biomineralization.

We determined the retained enzymatic activity after Esterase@ZIF biocomposites synthesis. To do this, we first determined the enzymatic activity of free Esterase at 37 °C. In a typical experiment, 24-well culture plates were filled with 925 μ L of Tris-HCl (0.1 M, pH = 7.4) buffer solution, 25 μ L of an Esterase aqueous solution (75 U/mL) and they were incubated for 1 h at 37 °C. Then, 50 μ L of indoxyl acetate solution in isopropanol (3.0 mM) was added and the mixtures were incubated for an additional 30 min. Finally, 3.33 mL of dimethyl sulfoxide (DMSO) was added to solubilize the enzymatic product (indigo blue) and stop the reaction. The absorbance of the solutions was measured at $\lambda = 620$ nm.

Secondly, we determined the enzymatic activity of Esterase@ZIF biocomposites. We incubated Esterase@ZIFs materials in 1 mL of Tris-HCl (0.1 M, pH = 7.4) buffer solution (1 h at 37 °C) so that the final concentration of Esterase in the suspension was 1.87 U/mL (we took into account the Esterase loading capacity in each Esterase@ZIF calculated by the Bradford assay; see Table S8). Afterwards, 50 μ L of indoxyl acetate solution in isopropanol (3.0 mM) was added and the mixtures were incubated for an additional 30 min. Then, we centrifuged this suspension, and the supernatants were added to 24-well culture plates. Finally, 3.33 mL of dimethyl sulfoxide (DMSO) was added to solubilize the enzymatic product (indigo blue) and stop the reaction. The absorbance of the solutions was measured at $\lambda = 620$ nm.

Additionally, we have also performed enzymatic activity assays for Zeolitic Imidazolate Frameworks (ZIF-8_150nm, ZIF-L and ZIF-C) as control experiments. In a typical

experiment, we incubated ZIFs materials in 1 mL of Tris-HCl (0.1 M, pH = 7.4) buffer solution (1 h at 37 °C) so that the final concentration of each ZIF in the suspension was the same as ZIF concentration in Esterase@ZIF experiments (see Table S8). Afterwards, 50 µL of indoxyl acetate solution in isopropanol (3.0 mM) was added and the mixtures were incubated for an additional 30 min. Then, we centrifuged this suspension and added the supernatants to 24-well culture plates. Finally, 3.33 mL of dimethyl sulfoxide (DMSO) was added to solubilize the enzymatic product (indigo blue) and stop the reaction. The absorbance of the solutions was measured at $\lambda = 620$ nm.

$$\text{Retained Enzymatic Activity after Synthesis (\%)} = \frac{\text{Enzymatic Activity Bio/ZIFs}}{\text{Free Enzymatic Activity}} \times 100$$

Enzymatic Activity Bio/ZIFs = Enzymatic activity for Esterase@ZIF biocomposites or ZIFs materials at 37 °C

Free Enzymatic Activity = Enzymatic activity for free Esterase at 37 °C

S2.4.4. Reactivation of the enzymatic activity by 2-methylimidazole.

We evaluated the reactivation of the enzymatic activity of AChE by 2-methylimidazole linker (mlmH) under simulated biological conditions. For this purpose, we incubated 50 % DIFP inhibited AChE solutions with mlmH at different concentrations. In a typical experiment, 24-well culture plates were filled with 725 µL of Tris-HCl (0.1 M, pH = 7.4) buffer solution, 25 µL of AChE aqueous solution (75 U/mL) and 100 µL of DIFP aqueous solution corresponding to 50% inhibited enzyme (see S2.4.2); and they were incubated for 1 h at 37 °C. Then, we added 100 µL of mlmH solution with different concentrations (10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M, 10^{-3} M, 10^{-2} M and 10^{-1} M) and this mixture was incubated for 1 h. Afterwards, 50 µL of indoxyl acetate solution in isopropanol (3.0 mM) was added and the mixtures were incubated for an additional 30 min. Finally, 3.33 mL of dimethyl sulfoxide (DMSO) was added to solubilize the enzymatic product (indigo blue) and stop the reaction. The absorbance of the solutions was measured at $\lambda = 620$ nm. The corresponding reactivation percentage was calculated according to the following equation:

$$\text{Reactivation (\%)} = \left(\frac{\text{React. Enzymatic Activity} - \text{Enzymatic Activity 50\%}}{\text{Free Enzymatic Activity} - \text{Enzymatic Activity 50\%}} \right) \times 100$$

React. Enzymatic Activity = Enzymatic activity for AChE incubated with mlmH at different concentrations

Enzymatic Activity 50 % = Enzymatic activity for 50% inhibited AChE at 37 °C.

Free Enzymatic Activity = Enzymatic activity for free AChE at 37 °C.

S2.4.5. Retained enzymatic activity with temperature.

Here we studied how temperature affects the enzymatic activity of free Esterase and Esterase@ZIF biocomposites. Regarding free esterase, 24-well culture plates were filled with 925 µL of Tris-HCl (0.1 M, pH = 7.4) buffer solution, 25 µL of an Esterase aqueous solution (75 U/mL) and they were incubated at two different times (30 min and 1 h) and different temperatures (60 °C, 70 °C, 80 °C and 90 °C). Then, 50 µL of indoxyl acetate solution in isopropanol (3.0 mM) was added and the mixtures were incubated for an additional 30 min. Finally, 3.33 mL of dimethyl sulfoxide (DMSO) was added to solubilize the enzymatic product (indigo blue) and stop the reaction. The absorbance of the solutions was measured at $\lambda = 620$ nm.

Regarding the Esterase@ZIF biocomposites, we incubated them (1.87 U/mL Esterase final concentration) in 1 mL of Tris-HCl (0.1 M, pH = 7.4) buffer solution at two different times (30 min and 1 h) and different temperatures (60 °C, 70 °C, 80 °C and 90 °C). Then, 50 µL of indoxyl acetate solution in isopropanol (3.0 mM) was added and the mixtures were incubated for an additional 30 min at room temperature. Finally, 3.33 mL of dimethyl sulfoxide (DMSO) was added to solubilize the enzymatic product (indigo blue) and stop the reaction. The absorbance of the solutions was measured at $\lambda = 620$ nm. The corresponding retained enzymatic activity with temperature was calculated according to the following equation:

$$\text{Retained Enzymatic Activity with Temperature (\%)} = \frac{\text{Enzymatic Activity Temp.}}{\text{Free Enzymatic Activity}} \times 100$$

Enzymatic Activity Temp. = Enzymatic activity for free Esterase or Esterase@ZIF biocomposites at a specific temperature (60, 70, 80 and 90 °C).

Free Enzymatic Activity = Enzymatic activity for free Esterase or Esterase@ZIF biocomposites at 37 °C.

S2.4.6. Retained enzymatic activity at Inhibitory Concentrations.

We then evaluated Esterase@ZIF biocomposites retained enzymatic activity towards DIFP inhibitory concentrations IC50 ($5 \cdot 10^{-7}$ M) and IC90 (10^{-4} M). In a typical experiment, we incubated 850 µL of Tris-HCl (0.1 M, pH = 7.4) buffer solution, 1.87 U/mL of each Esterase@ZIF material and 100 µL of DIFP aqueous solution ($5 \cdot 10^{-6}$ M for IC50 and 10^{-3} M for IC90) for 1 h at 37 °C. Afterwards, 50 µL of indoxyl acetate solution in isopropanol (3.0 mM) was added and the mixtures were incubated for an additional 30 min. Then, we centrifuged this suspension and added the supernatants to 24-well culture plates. Finally, 3.33 mL of dimethyl sulfoxide (DMSO) was added to solubilize the enzymatic product (indigo blue) and stop the reaction. The absorbance of the solutions was measured at $\lambda = 620$ nm. The corresponding retained enzymatic activity percentage was calculated according to the following equation:

$$\text{Retained Enzymatic Activity at IC (\%)} = \frac{\text{Enzymatic Activity IC}}{\text{Enzymatic Activity Bio}} \times 100$$

Enzymatic Activity IC = Enzymatic activity for Esterase@ZIF biocomposites at different DIFP Inhibitory Concentrations.

Enzymatic Activity Bio = Enzymatic activity for Esterase@ZIF biocomposites at 37 °C.

We also performed control experiments studying the retained Esterase enzyme activity against DIFP inhibitory concentrations IC50 ($5 \cdot 10^{-7}$ M) and IC90 (10^{-4} M) using a physical mixture of ZIFs and Esterase. In a typical experiment, we incubated 25 µL of an Esterase aqueous solution (75 U/mL) and the different ZIFs in 825 µL of Tris-HCl (0.1 M, pH = 7.4) buffer solution (1 h at 37 °C) so that the final concentration of each ZIF (ZIF-8_150nm, ZIF-L or ZIF-C) in the suspension was the same as ZIF concentration in Esterase@ZIF experiments. Then, we added 100 µL of DIFP solution ($5 \cdot 10^{-6}$ M for IC50 and 10^{-3} M for IC90) and this mixture was incubated for 1 h. Then, we centrifuged this suspension and added the supernatants to 24-well culture plates. Afterwards, 50 µL of indoxyl acetate solution in isopropanol (3.0 mM) was added and the mixtures were incubated for an additional 30 min. Finally, 3.33 mL of dimethyl sulfoxide (DMSO) was added to solubilize the enzymatic product (indigo blue) and stop the reaction. The

absorbance of the solutions was measured at $\lambda = 620$ nm. The corresponding retained enzymatic activity percentage was calculated according to the following equation:

$$\text{Retained Enzymatic Activity at IC (\%)} = \frac{\text{Enzymatic Activity IC}}{\text{Free Enzymatic Activity}} \times 100$$

Enzymatic Activity IC = Enzymatic activity for Esterase+ZIF physical mixture at different DIFP Inhibitory Concentrations.

Free Enzymatic Activity = Enzymatic activity for free Esterase at 37 °C.

S2.4.7. Detoxification experiments

In this case, additional enzymatic assays were carried out to compare Esterase@ZIFs and ZIFs ability to reduce the inhibitory effect of DIFP on AChE activity. In a typical experiment, 0.084 mmol of Esterase@ZIFs (Esterase@ZIF-8_120nm, Esterase@ZIF-L and Esterase@ZIF-C) were mixed with DIFP (0.029 M) in 0.5 mL of Tris-HCl (0.1 M, pH = 7.4) and stirred at room temperature. After different time frames (10 min, 30 min, 60 min, 4 h, 12 h and 24 h) the supernatant was separated from the solid by centrifugation (15,000 rpm $\times$ 5 min) and diluted 580 times. Then, a 24-well culture plate was filled with 825 μ L of Tris-HCl (0.1 M, pH = 7.4), 25 μ L of an aqueous solution of AChE (75 U/mL), and 100 μ L of the diluted supernatants. The mixtures were then incubated for 1 h at 37 °C. Afterward, 50 μ L of indoxyl acetate solution in isopropanol (3.0 mM) was added and the mixtures were incubated for an additional 30 min. Finally, 3.33 mL of DMSO was added to solubilize the enzymatic product (indigo blue) and the absorbance of the solutions at $\lambda = 620$ nm was collected. Additional experiments were carried out to estimate the enzymatic activity of uninhibited AChE and 50 % inhibited AChE with DIFP, following the same protocol as in the inhibition assays (see S2.4.2.). The corresponding detoxification percentage was calculated according to the following equation:

$$\text{Detoxification (\%)} = \left(\frac{\text{Enzymatic Activity Detox.} - \text{Enzymatic Activity 50\%}}{\text{Free Enzymatic Activity} - \text{Enzymatic Activity 50\%}} \right) \times 100$$

Enzymatic Activity Detox. = Enzymatic activity for AChE after incubation with Esterase@ZIFs/ZIFs

Enzymatic Activity 50 % = Enzymatic activity for 50% inhibited AChE at 37 °C.

Free Enzymatic Activity = Enzymatic activity for uninhibited AChE at 37 °C.

The same experiment was carried out with ZIF-8_150nm. In this case, 0.084 mmol of ZIF-8_150nm were mixed with DIFP (0.029 M) in 0.5 mL of Tris-HCl (0.1 M, pH = 7.4) and stirred at room temperature.

S3. Experimental results

S3.1. Characterization of Esterase@ZIF biocomposites

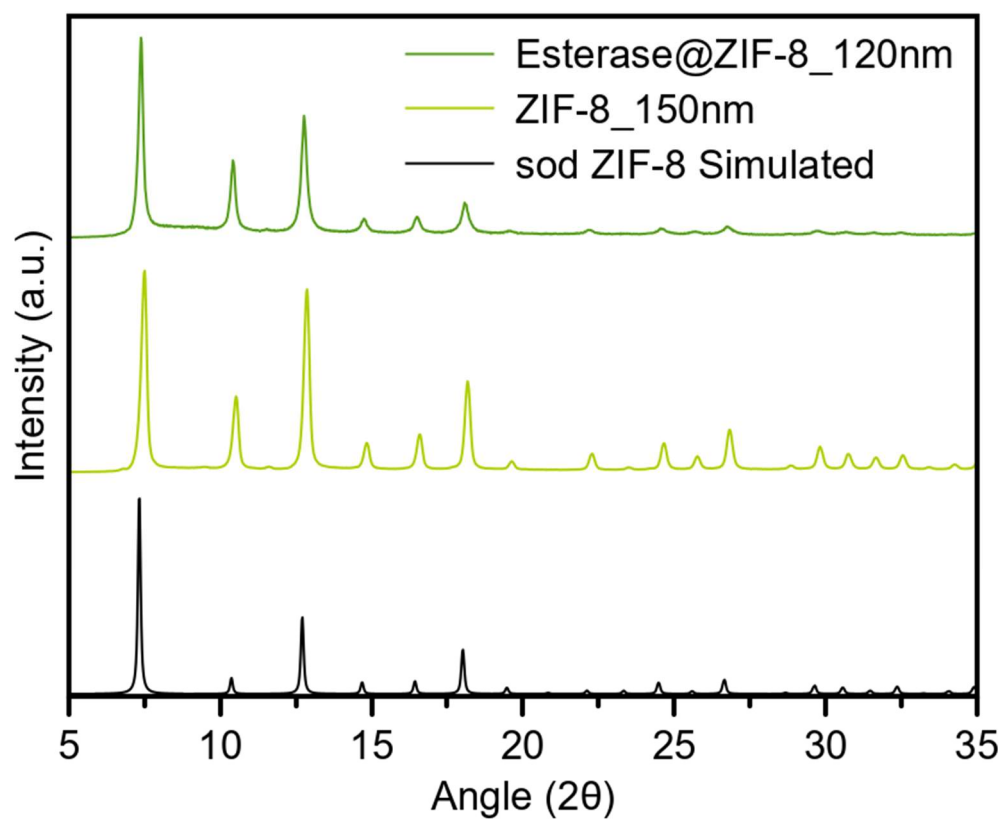


Figure S1. Powder X-Ray Diffraction patterns of Esterase@ZIF-8_120nm biocomposite (experimental, green), ZIF-8_150nm (experimental, light green) and simulated sod ZIF-8 (calculated, black).

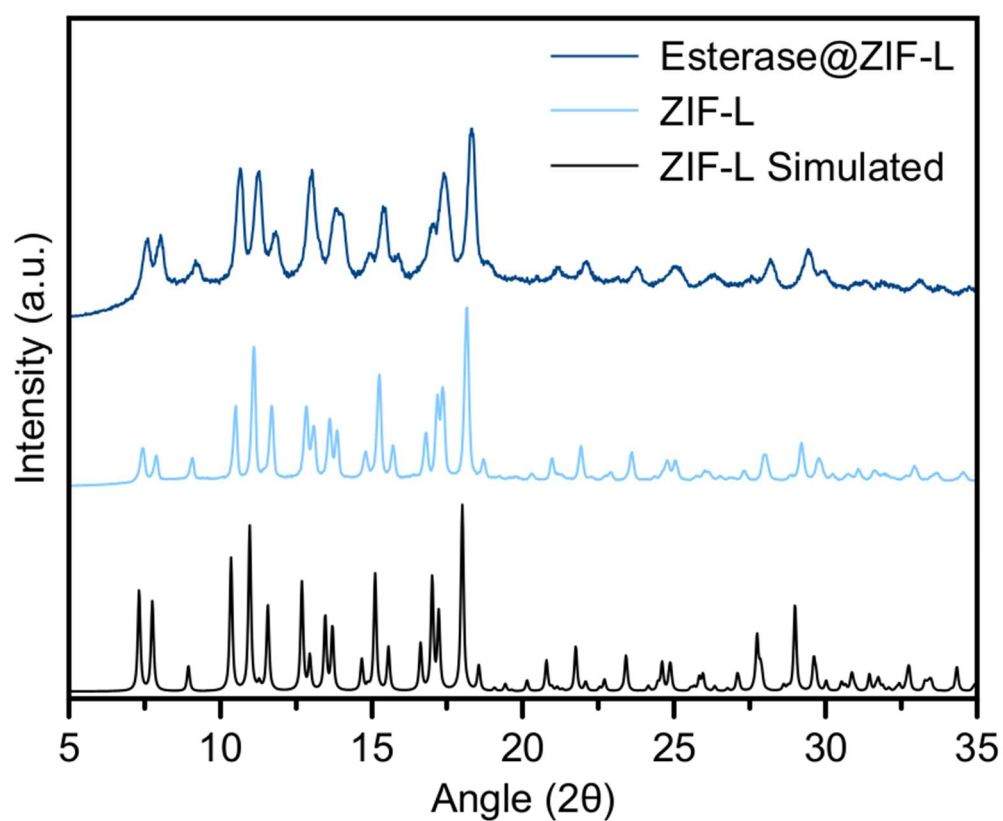


Figure S2. Powder X-Ray Diffraction patterns of Esterase@ZIF-L biocomposite (experimental, dark blue), ZIF-L (experimental, light blue) and simulated ZIF-L (calculated, black).

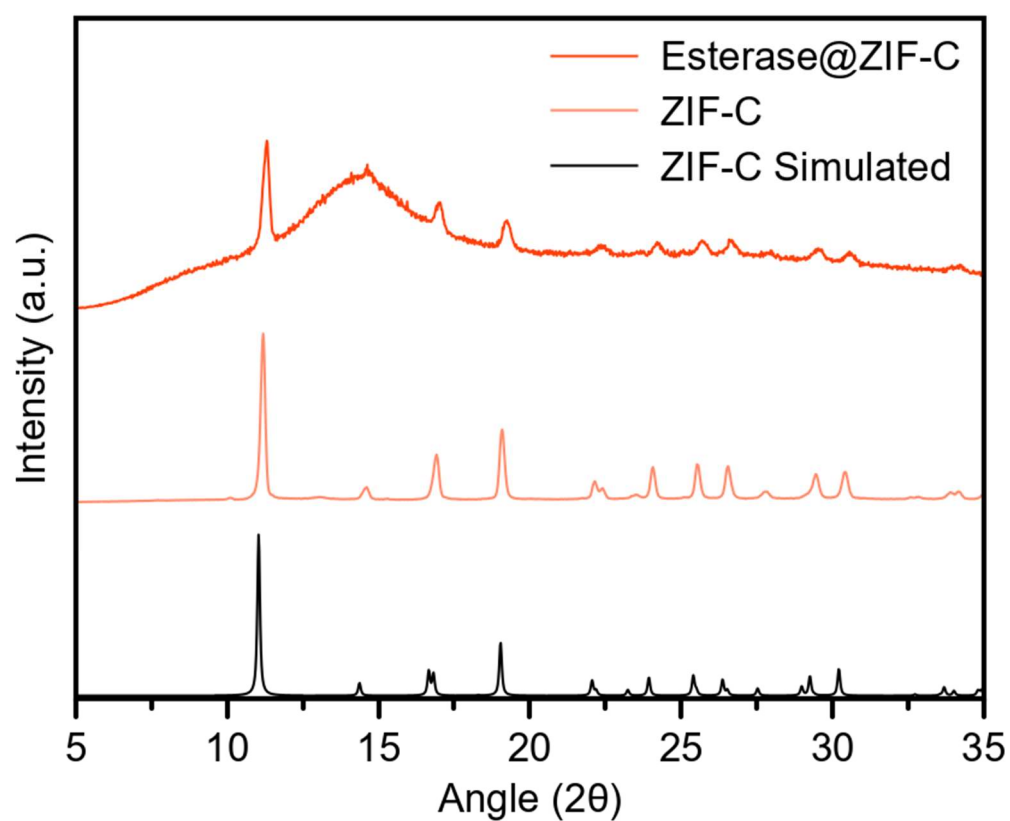


Figure S3. Powder X-Ray Diffraction patterns of Esterase@ZIF-C biocomposite (experimental, red), ZIF-C (experimental, light red) and simulated ZIF-C (calculated, black).

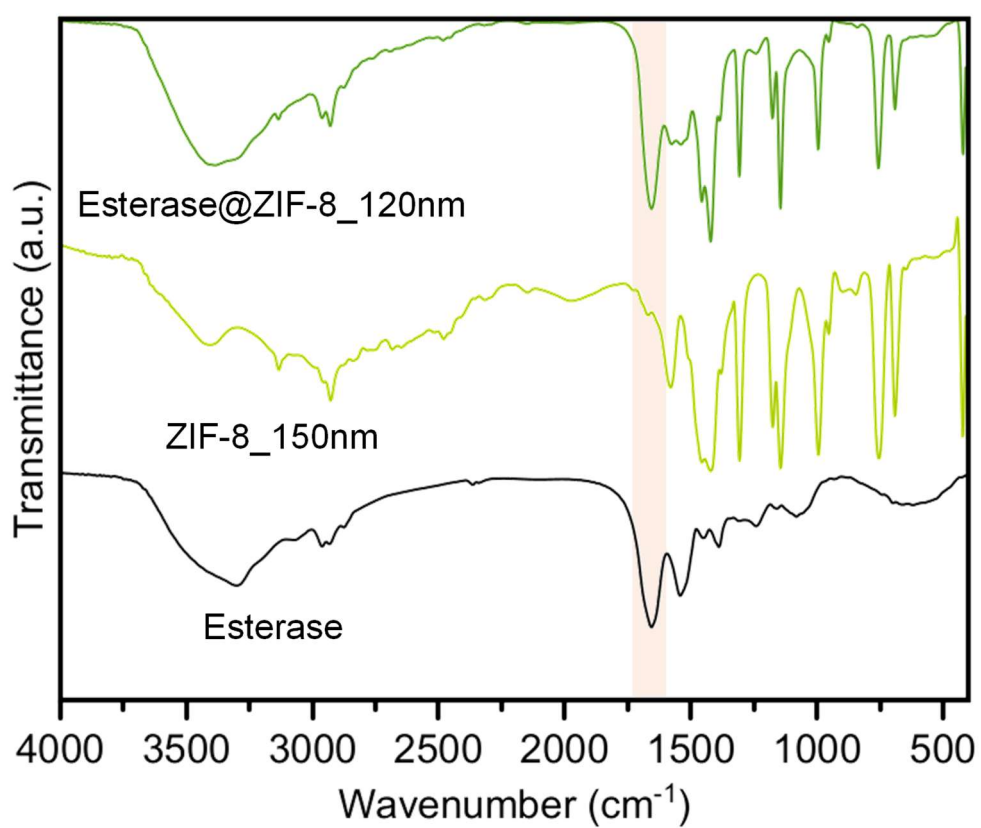


Figure S4. Fourier Transformed Infrared spectra (FTIR) of Esterase (black), ZIF-8_150nm (light green) and Esterase@ZIF-8_120nm (dark green) materials. The analysis of the FTIR data confirms the presence of the characteristic Amide I band (1700-1610 cm⁻¹) of the peptide backbone of Esterase highlighted in light orange.

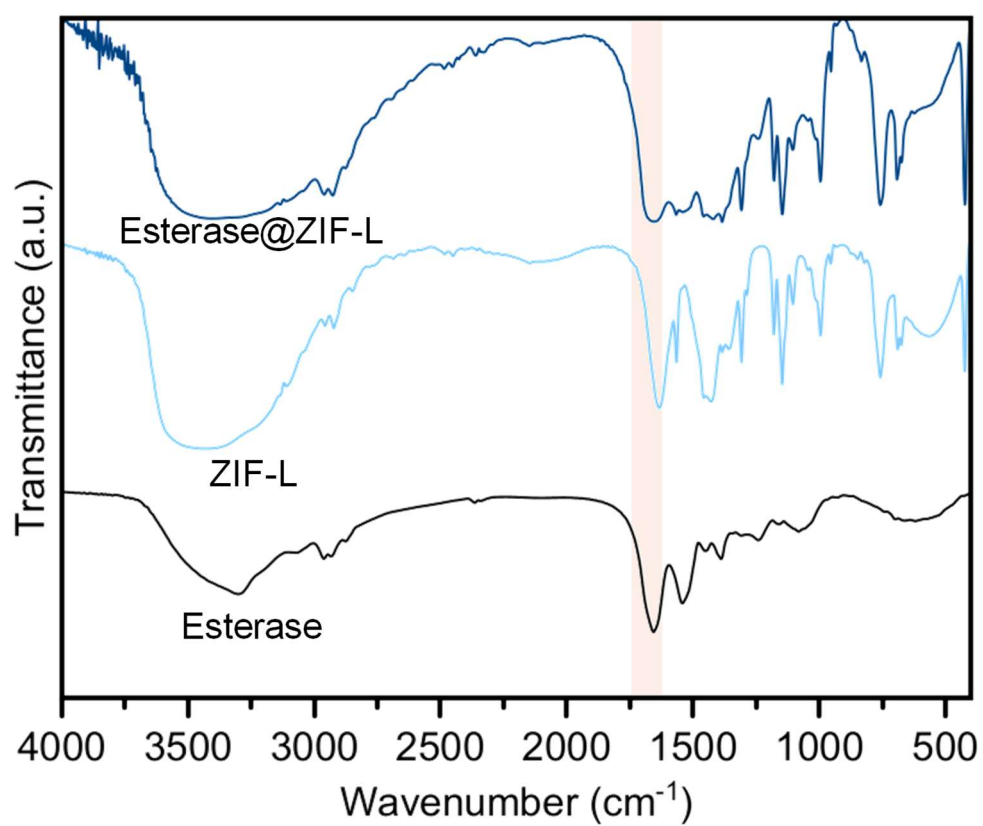


Figure S5. Fourier Transformed Infrared spectra (FTIR) of Esterase (black), ZIF-L (light blue) and Esterase@ZIF-L (dark blue) materials. The analysis of the FTIR data confirms the presence of the characteristic Amide I band (1700-1610 cm⁻¹) of the peptide backbone of Esterase highlighted in light orange.

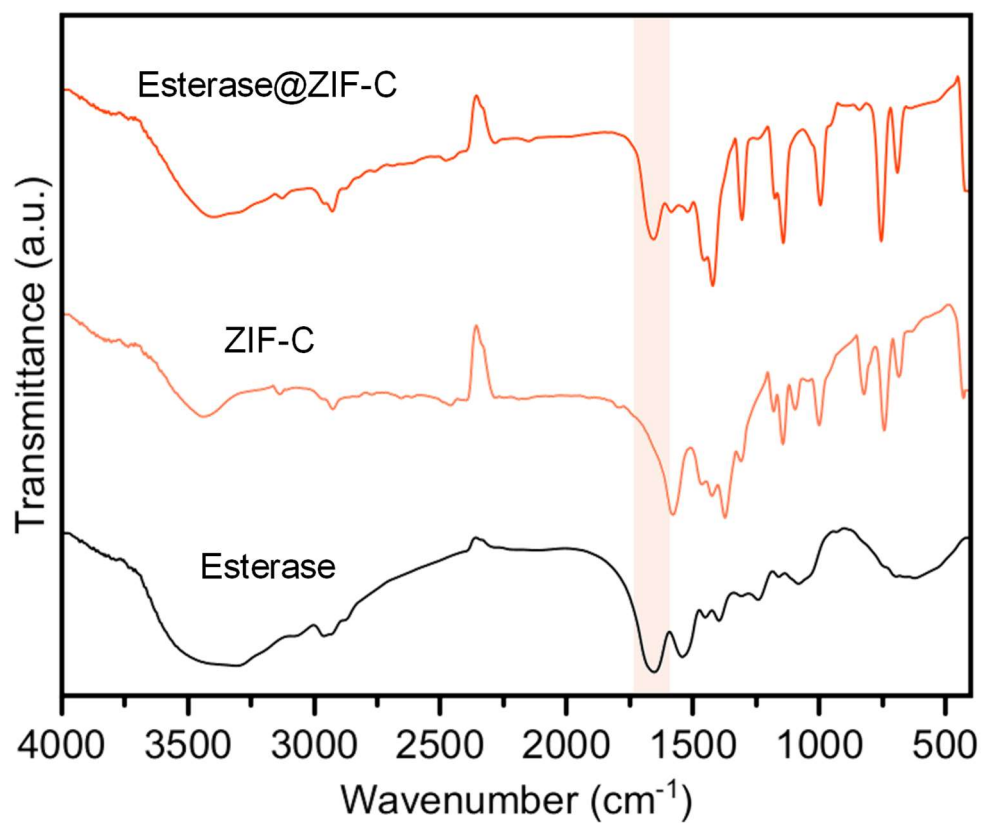


Figure S6. Fourier Transformed Infrared spectra (FTIR) of Esterase (black), ZIF-C (light red) and Esterase@ZIF-C (red) materials. The analysis of the FTIR data confirms the presence of the characteristic Amide I band ($1700\text{-}1610\text{ cm}^{-1}$) of the peptide backbone of Esterase highlighted in light orange.

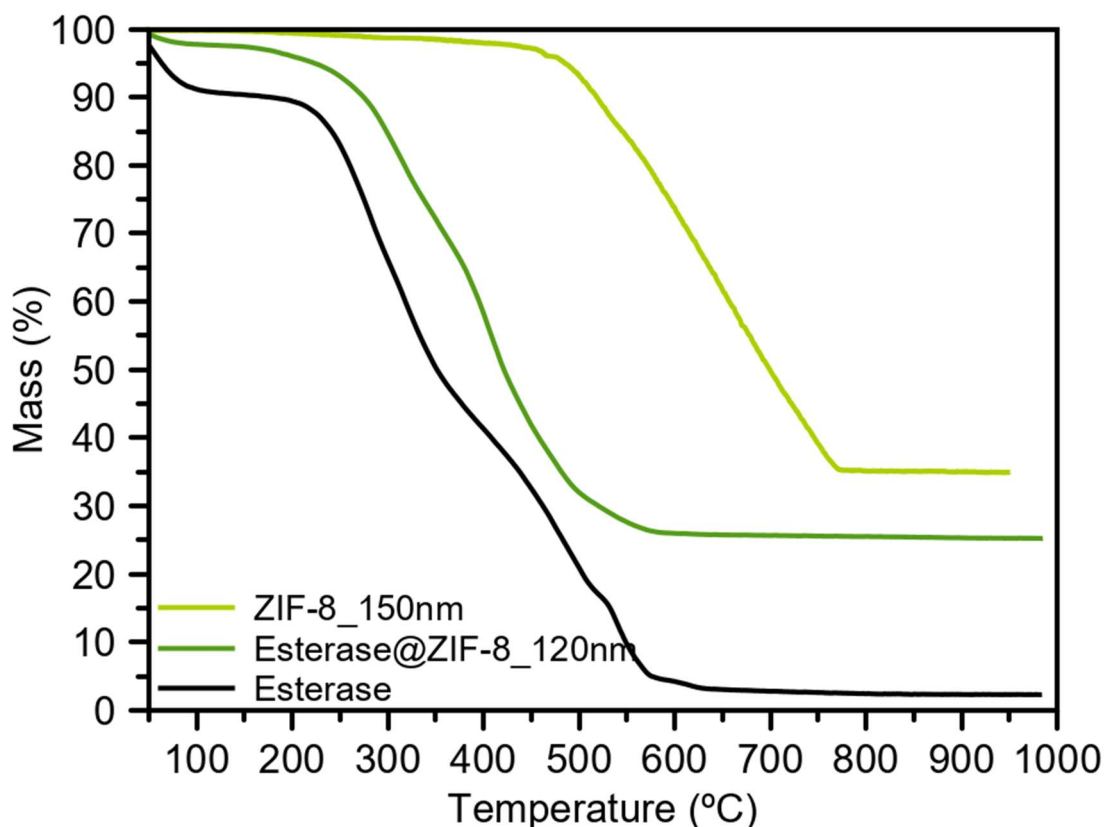


Figure S7. TGA profiles of ZIF-8_150nm (light green), Esterase@ZIF-8_120nm (green) and Esterase (black) in a range from 30 to 950 °C under an oxidative atmosphere (air) with a ramp rate of 5 °C min⁻¹.

From thermogravimetric analyses of Esterase, ZIF-8_150nm and Esterase@ZIF-8_120nm biocomposite we calculated the percentage of Esterase loading in the biomineralized materials. The mass loss associated with Esterase and ZIF-8_150nm was taken into account as a function of their decomposition temperatures. Finally, we also compared Esterase loading in TGA with the Bradford assay obtaining very similar results (Table S10).

Table S10. Decomposition temperature and mass loss for Esterase, ZIF-8_150nm and Esterase@ZIF-8_120nm.

	T _{Decomposition} (°C)	ΔMass _{150-950°C} (mg)	Mass loss (%)	% Loading (TG)	% Loading (Brad.)
ZIF-8_150nm	625	8.0	65	-	-
Esterase@ ZIF-8_120nm	411	8.3	70	35	37
Esterase	280	8.6	88	-	-

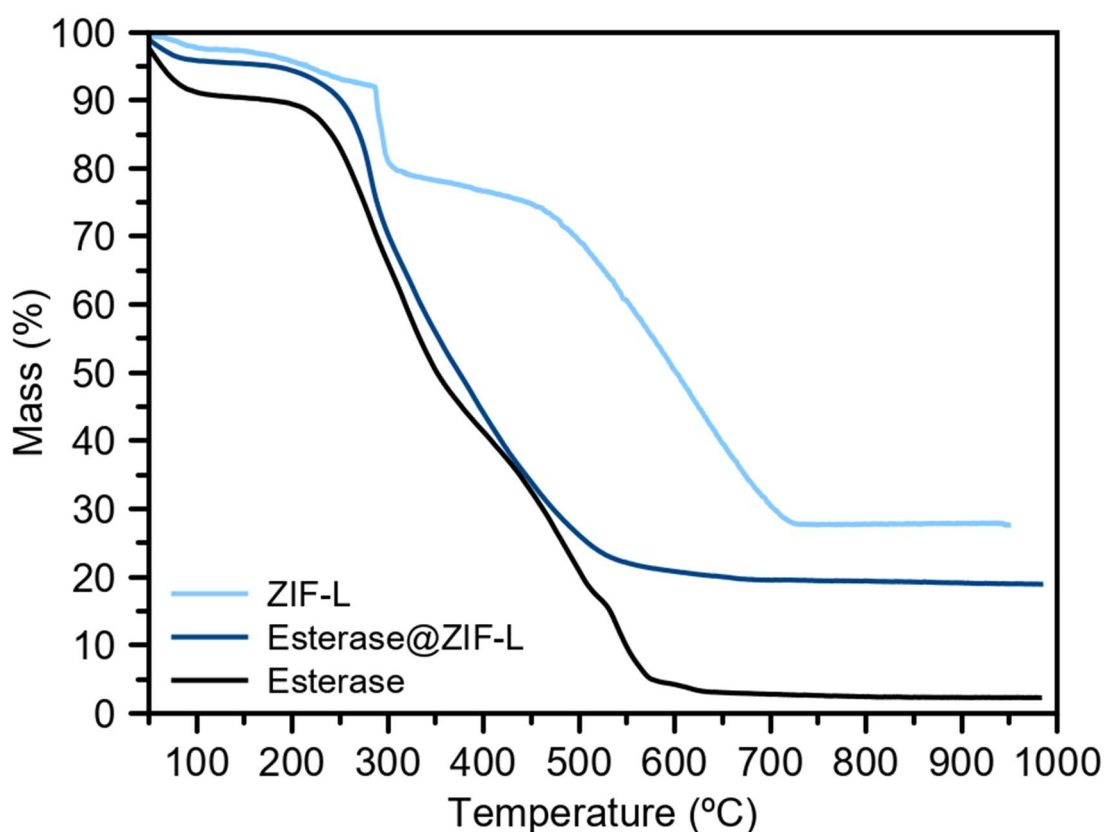


Figure S8. TGA profiles of ZIF-L (light blue), Esterase@ZIF-L (dark blue) and Esterase (black) in a range from 30 to 950 °C under an oxidative atmosphere (air) with a ramp rate of 5 °C min⁻¹.

From thermogravimetric analyses of Esterase, ZIF-L and Esterase@ZIF-L biocomposite we calculated the percentage of Esterase loading in the biomineralized materials. The mass loss associated with Esterase and ZIF-L was taken into account as a function of their decomposition temperatures. Finally, we also compared Esterase loading in TGA with the Bradford assay obtaining very similar results (Table S11).

Table S11. Decomposition temperature and mass loss for Esterase, ZIF-L and Esterase@ZIF-L.

	T _{Decomposition} (°C)	ΔMass _{150-950°C} (mg)	Mass loss (%)	% Loading (TG)	% Loading (Brad.)
ZIF-L	550	6.3	70	-	-
Esterase@ZIF-L	330	7.0	76	36	37
Esterase	280	8.6	88	-	-

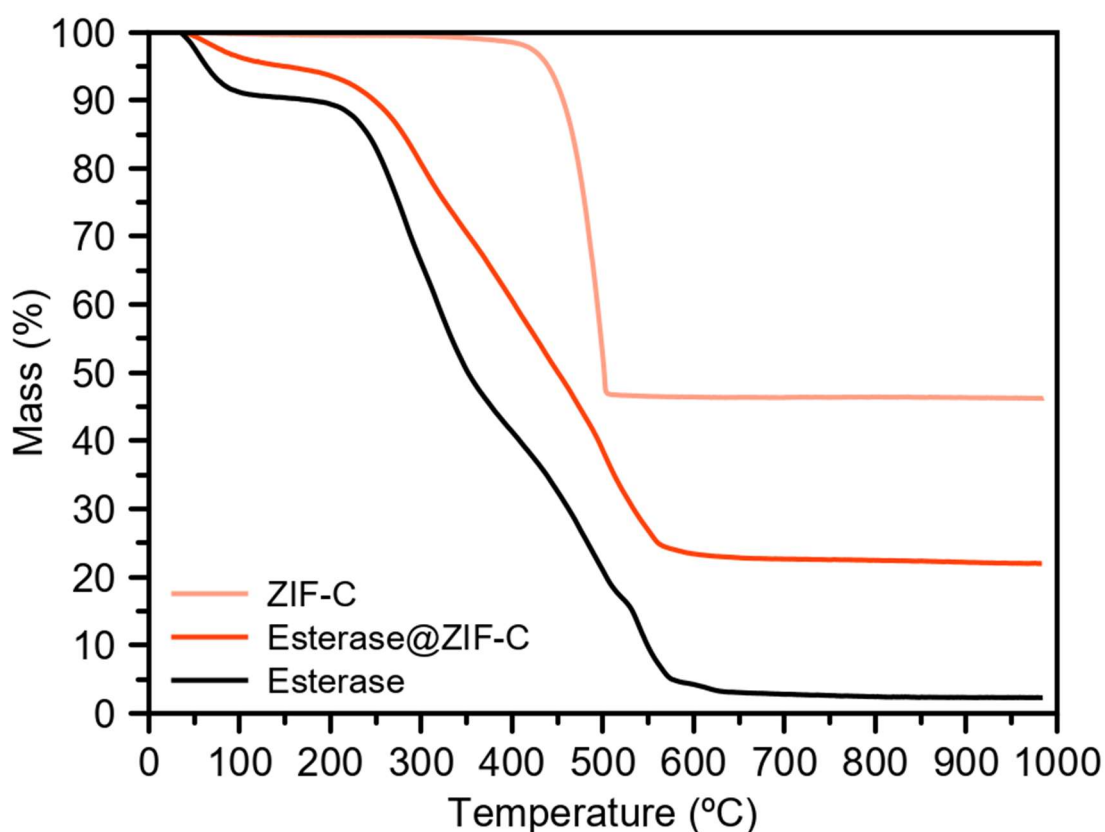


Figure S9. TGA profiles of ZIF-C (light red), Esterase@ZIF-C (red) and Esterase (black) in a range from 30 to 950 °C under an oxidative atmosphere (air) with a ramp rate of 5 °C min⁻¹.

From thermogravimetric analyses of Esterase, ZIF-C and Esterase@ZIF-C biocomposite we calculated the percentage of Esterase loading in the biomineralized materials. The mass loss associated with Esterase and ZIF-C was taken into account as a function of their decomposition temperatures. Finally, we also compared Esterase loading in TGA with the Bradford assay obtaining very similar results (Table S12).

Table S12. Decomposition temperature and mass loss for Esterase, ZIF-C and Esterase@ZIF-C.

	T _{Decomposition} (°C)	ΔMass _{150-950°C} (mg)	Mass loss (%)	% Loading (TG)	% Loading (Brad.)
ZIF-C	490	5.2	54	-	-
Esterase@ZIF-C	370	7.5	73	41	47
Esterase	280	8.6	88	-	-

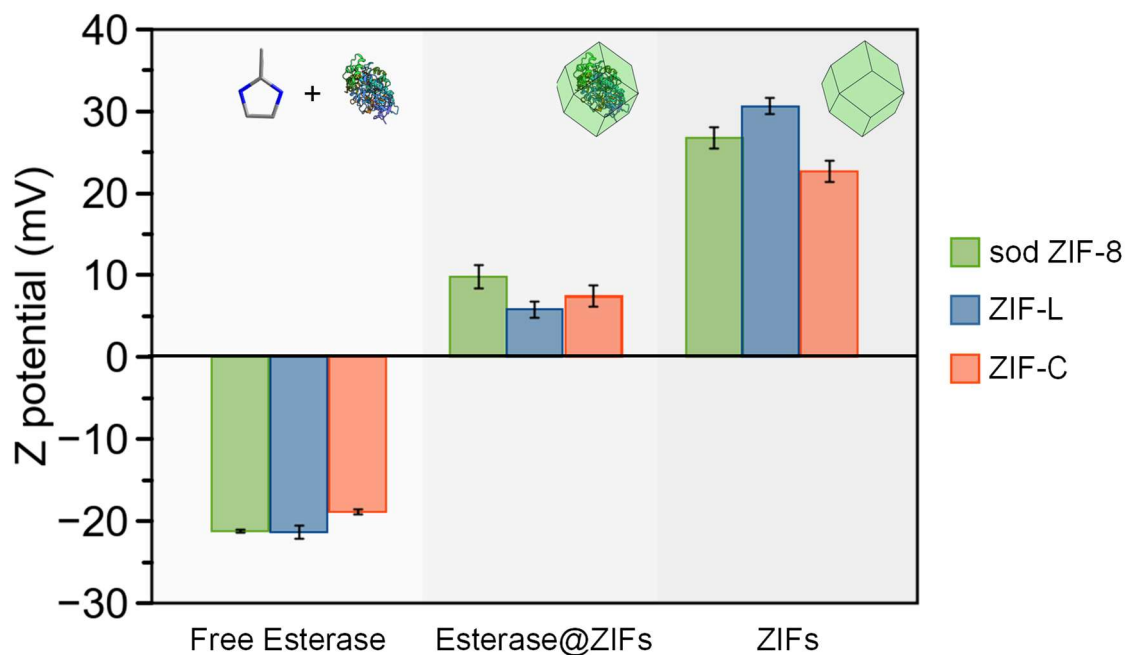


Figure S10. Z potential values of free Esterase in precursor ligand solutions (left), Esterase@ZIFs biomineralized (middle) and Zeolitic Imidazolate Frameworks alone (right).

Since Esterase has an isoelectric point of 5.0⁴, in an aqueous solution (pH $\approx$ 7) it possesses a net negative charge. When Esterase is added to a basic 2-methylimidazole ligand solution (see synthetic section S2.1.1.), the surface charge of this protein becomes even more negative (green, blue and red bars for the protein + ZIF-8_150nm, ZIF-L and ZIF-C ligand solutions, respectively). After the addition of the metal solution and biomineralization of the ZIFs, the net charge of the particles in all cases is positive due to the Zn ions in their crystalline structural surface. We can appreciate that ZIFs mineralized without Esterase have a more positive Z potential value because of the absence of this enzyme.

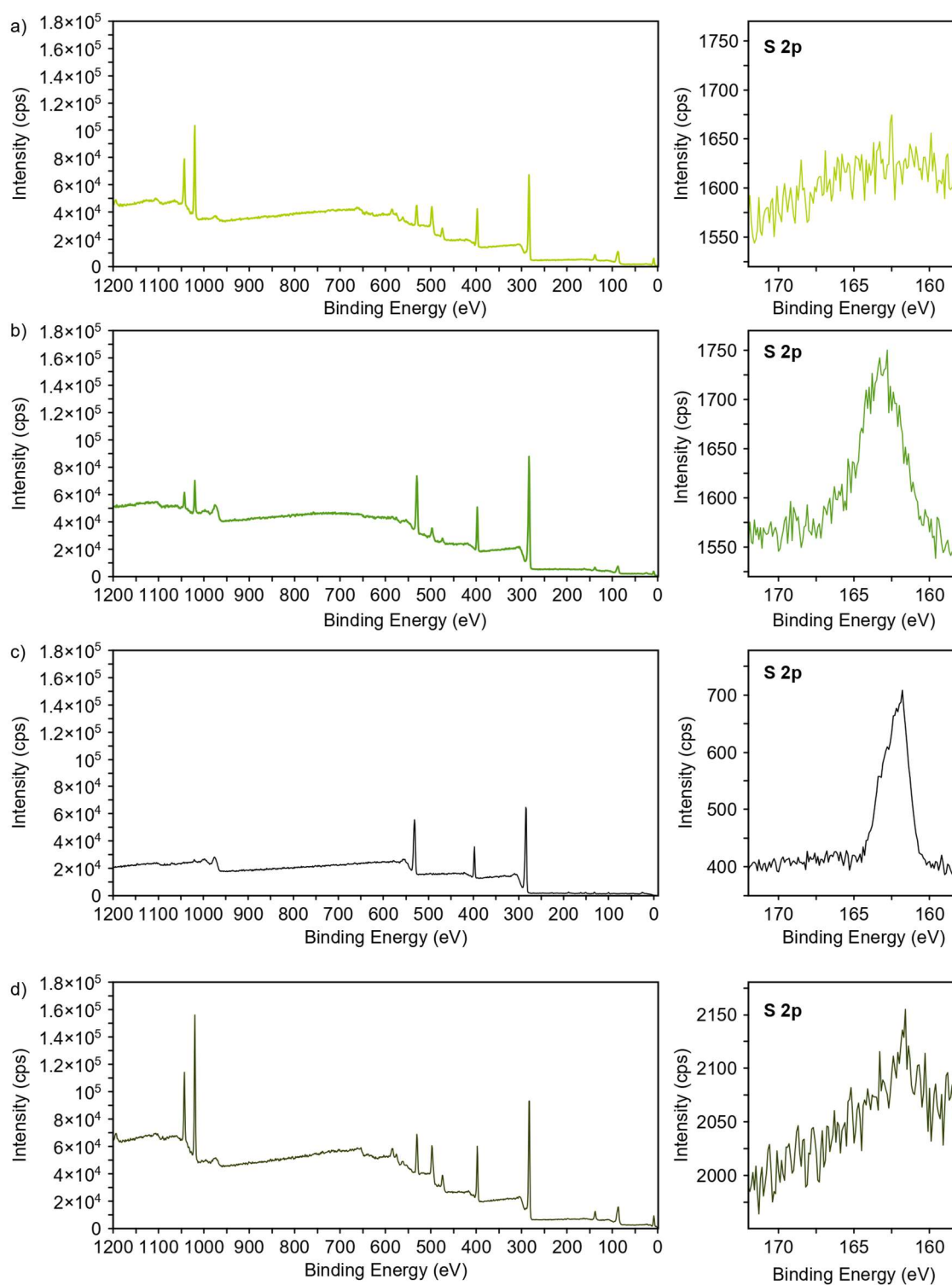


Figure S11. Experimental X-ray Photoelectron Spectroscopy (XPS) analysis together with the peak associated with the broadened S 2p signal of (a) ZIF-8_150nm, (b) Esterase@ZIF-8_120nm, (c) esterase and (d) ZIF-8_150nm+esterase physical mixture.

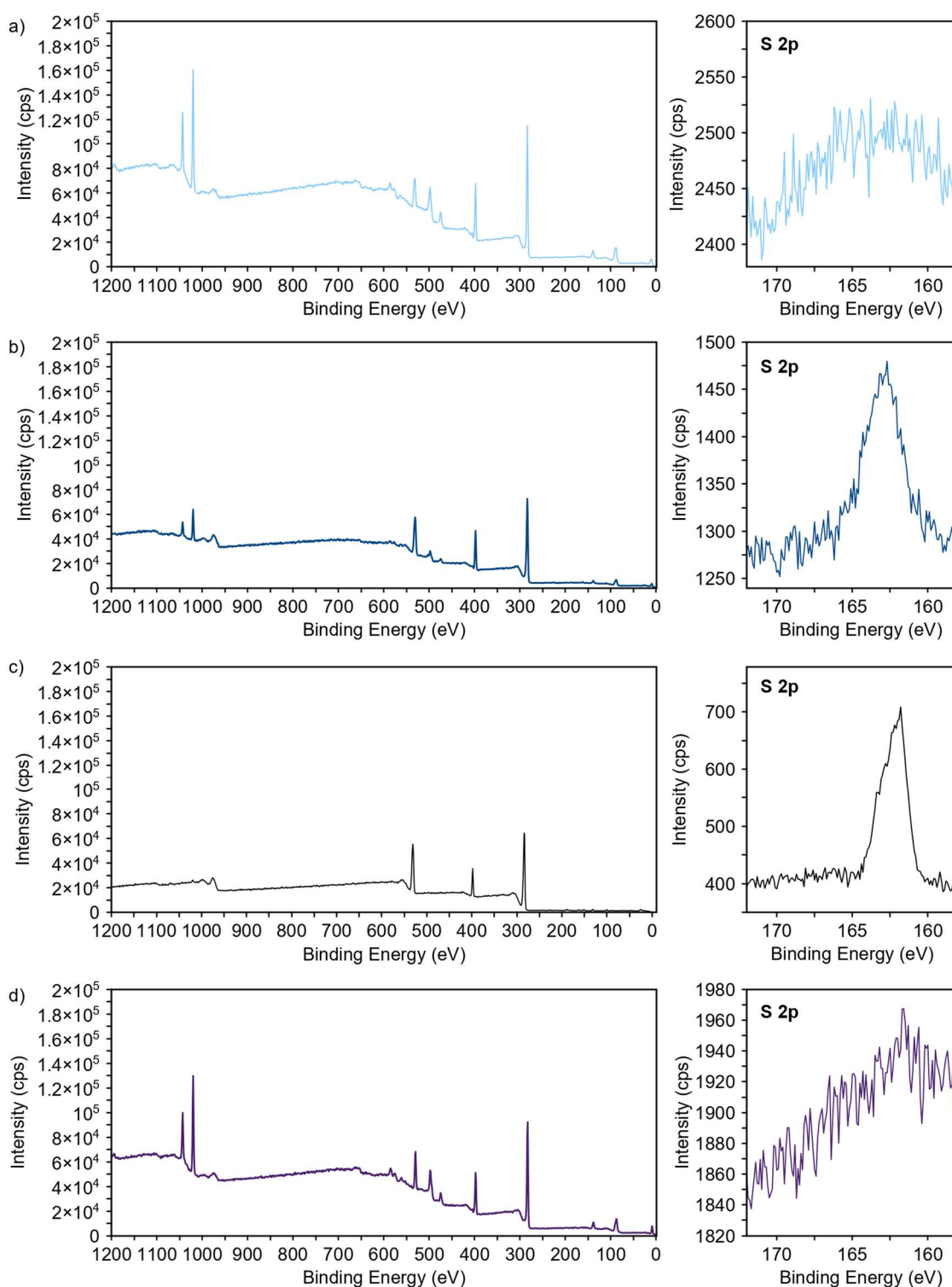


Figure S12. Experimental X-ray Photoelectron Spectroscopy (XPS) analysis together with the peak associated with the broadened S 2p signal of (a) ZIF-L, (b) Esterase@ZIF-L, (c) esterase and (d) ZIF-L+esterase physical mixture.

Table S13. Quantification in wt% of the different elements from XPS analysis (10 nm depth).

Sample	C	O	N	Zn	S
ZIF-8_150nm	50.2	6.72	16.06	27.02	0
Esterase@ZIF-8_120nm	60.3	17.11	13.79	8.3	0.48
Esterase+ZIF-8_150nm Physical Mixture	49.56	8.99	14.75	26.33	0.37
ZIF-L	50.76	8.97	14.76	25.51	0
Esterase@ZIF-L	57.41	17.34	15.83	8.84	0.58
Esterase+ZIF-L Physical Mixture	52.05	10.01	15.15	22.57	0.22
ZIF-C	42.3	9.93	10.74	37.03	0
Esterase@ZIF-C	50.74	10.64	17.15	20.76	0.71
Esterase+ZIF-C Physical Mixture	50.66	15.92	10.98	21.58	0.52
Esterase	62.92	21.8	11.27	0.92	0.45

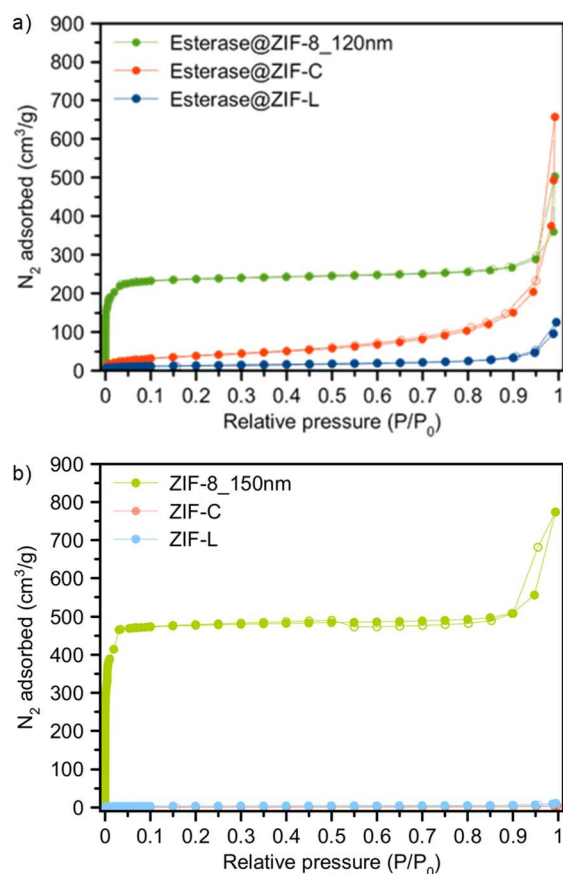


Figure S14. Nitrogen adsorption isotherms at 77 K of (a) Esterase@ZIF biocomposites and (b) ZIFs alone. Each sample was activated at 120 °C for 12 h under dynamic vacuum. The adsorption and desorption process of each N₂ isotherm are represented by filled and empty dots, respectively.

Table S14. BET surface area of Esterase@ZIF biocomposites in m² per gram of total material and per gram of MOF and their comparison to the ZIF original phases.

	S_{BET} (m ² /g)	S_{BET} (m ² /g) normalized to MOF mass	S_{BET} (m ² /g _{MOF})
Esterase@ZIF-8_120nm	950	1460	-
Esterase@ZIF-C	120	240	-
Esterase@ZIF-L	45	120	-
ZIF-8_150nm	-	-	1890
ZIF-C	-	-	2
ZIF-L	-	-	11

BET values obtained are referred to the Biocomposites taking into account both the ZIF and Esterase mass (column 1). We have carried out the calculation of the BET surface area related to the amount of ZIF present in the total material (column 2) and comparison to ZIF materials alone (column 3). To do this, we have considered the percentage of ZIF of each biocomposite (see Table S8).

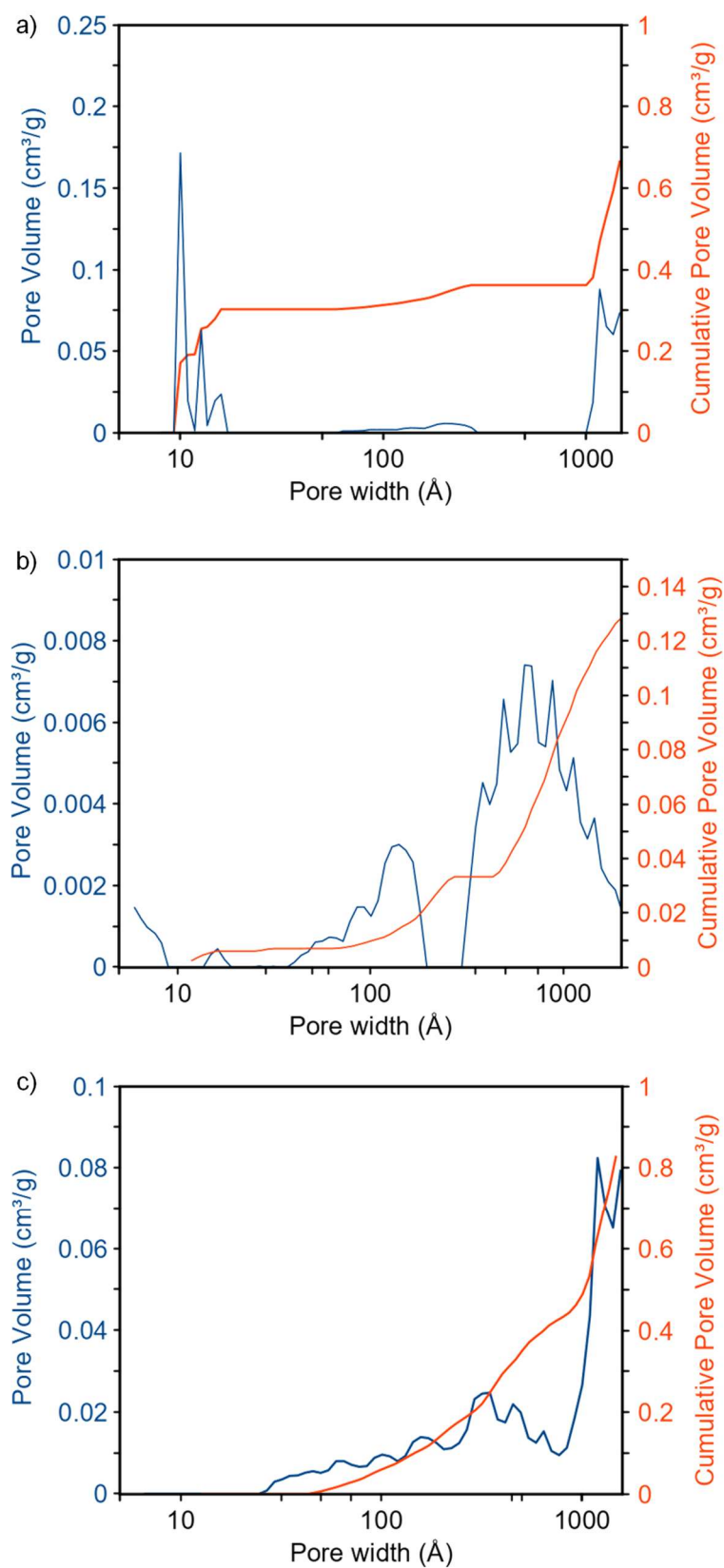


Figure S15. Pore volume distribution and cumulative pore volume calculated by Density Functional Theory from the experimental nitrogen adsorption isotherm curves for (a) Esterase@ZIF-8_120nm, (b) Esterase@ZIF-L and (c) Esterase@ZIF-C biocomposites.

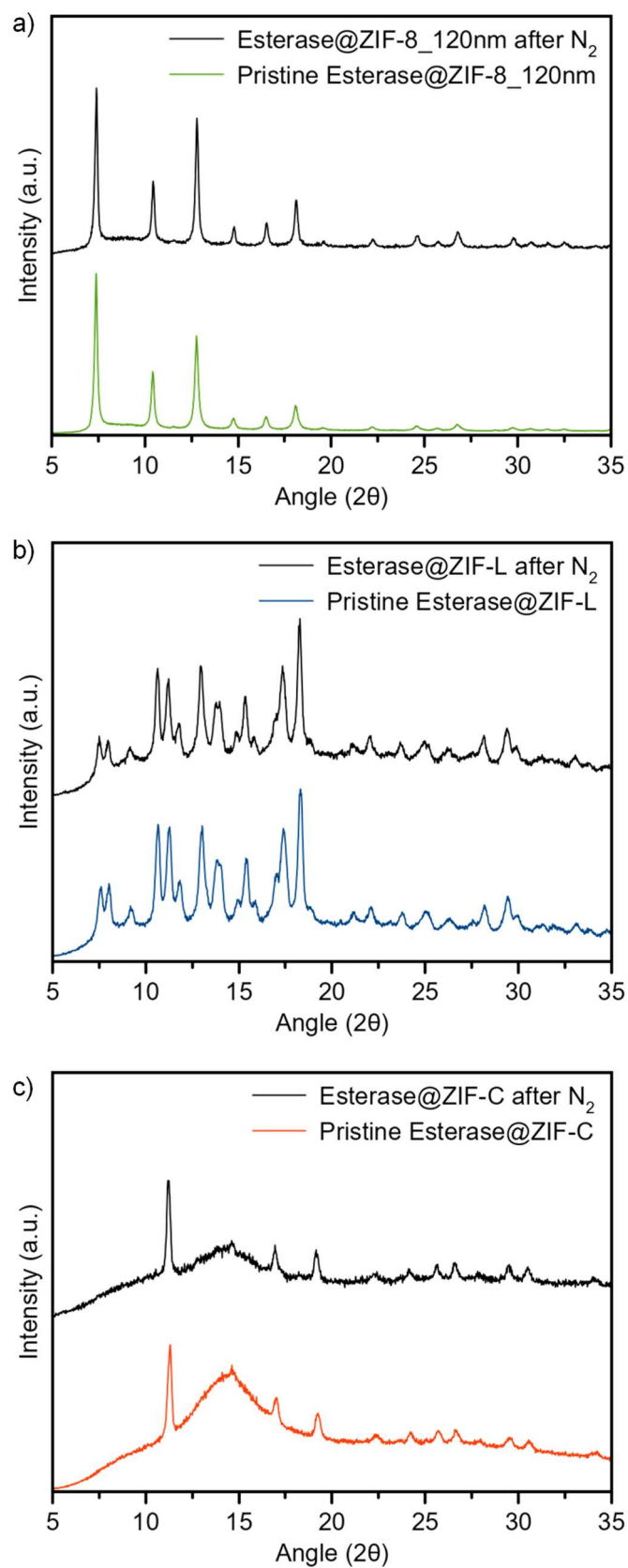


Figure S16. Powder X-Ray Diffraction patterns of (a) Esterase@ZIF-8_120nm, (b) Esterase@ZIF-L and (c) Esterase@ZIF-C biocomposites after nitrogen adsorption isotherm protocol. The comparison between each material before and after the activation protocol indicates that crystal phase is well maintained for each Esterase@ZIF.

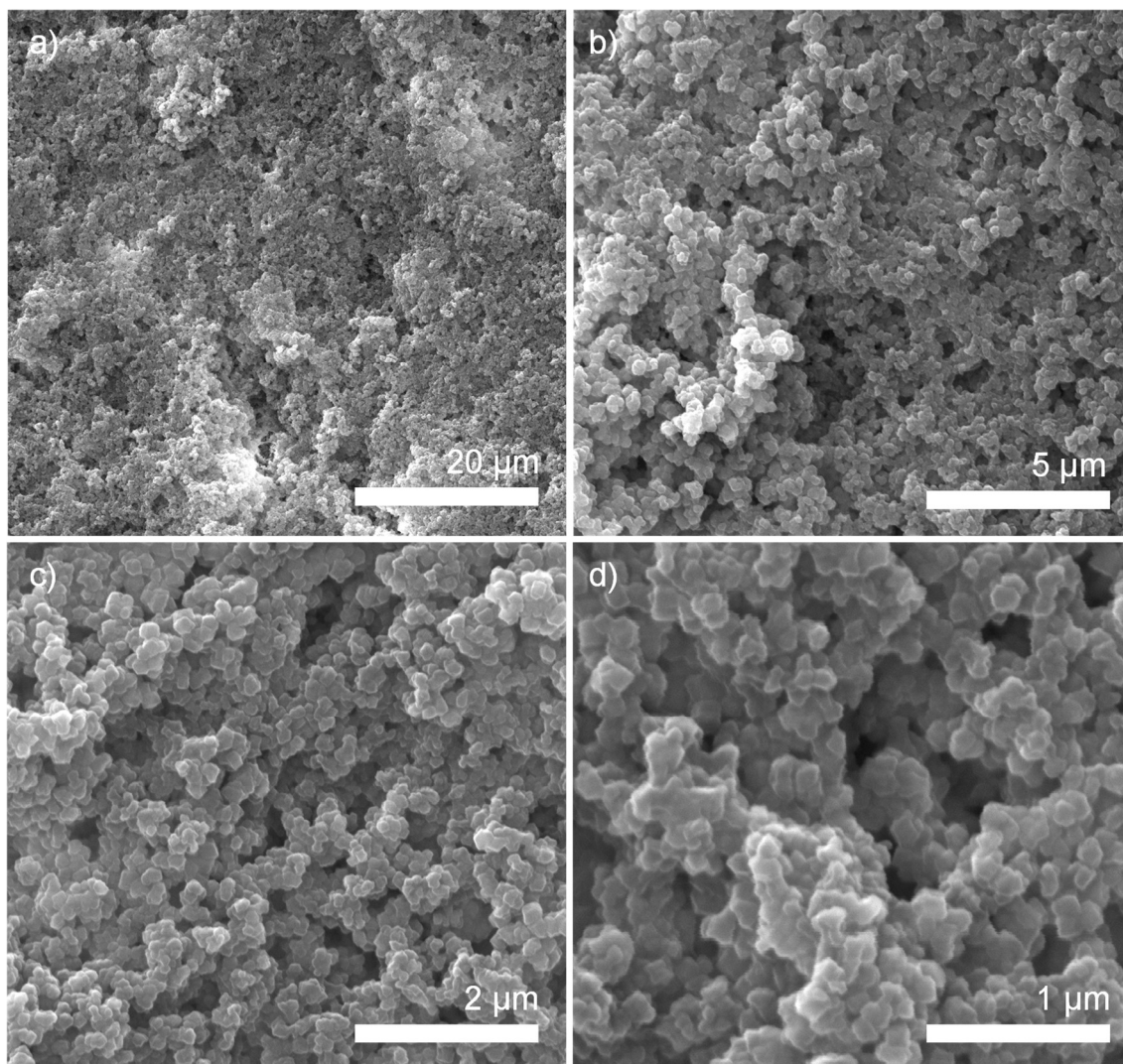


Figure S17. Scanning Electron Microscopy (SEM) images of Esterase@ZIF-8_120nm at different magnifications: a) 20 μm , b) 5 μm , c) 2 μm and d) 1 μm .

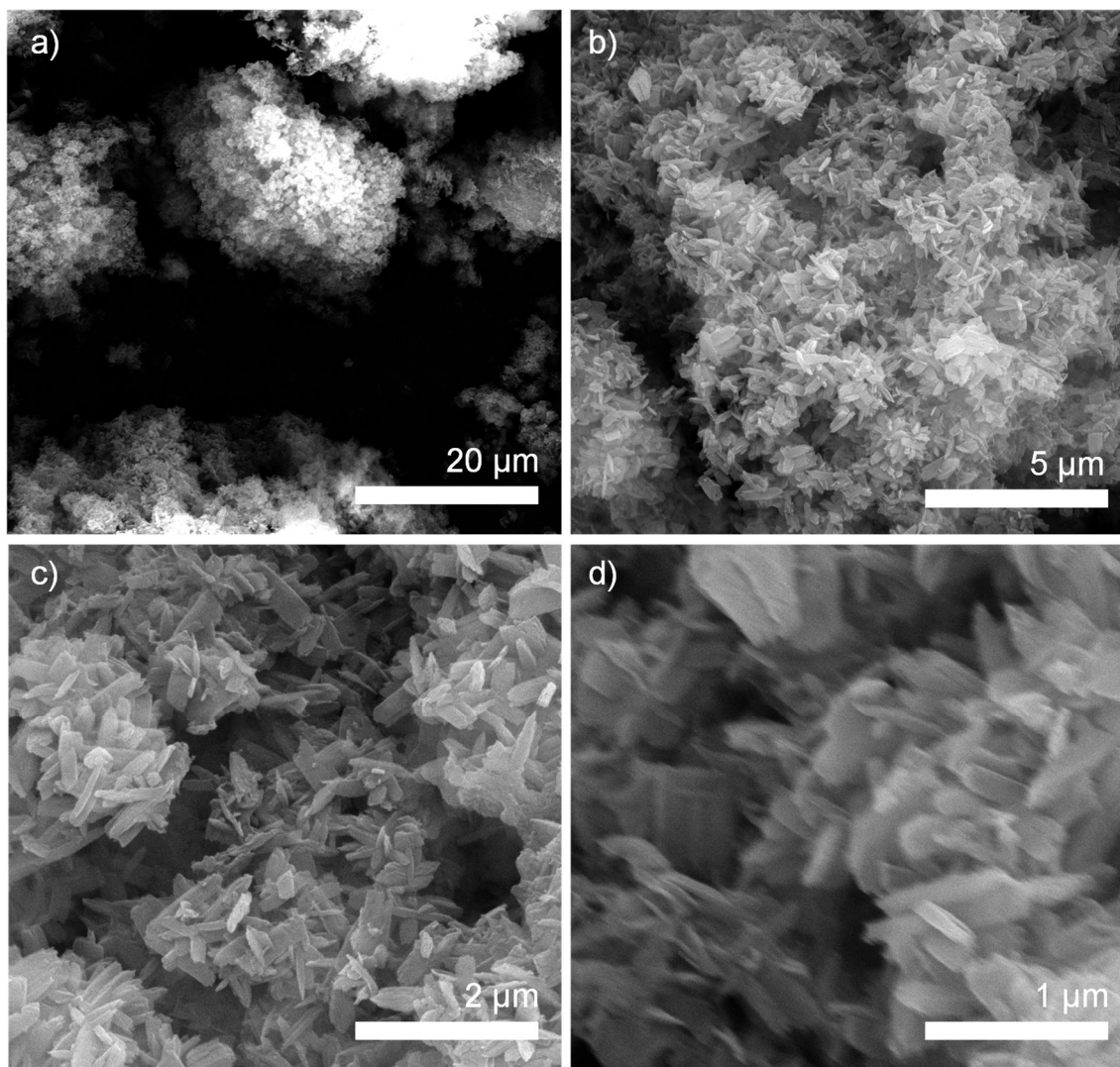


Figure S18. Scanning Electron Microscopy (SEM) images of Esterase@ZIF-L at different magnifications: a) 20 μm, b) 5 μm, c) 2 μm and d) 1 μm.

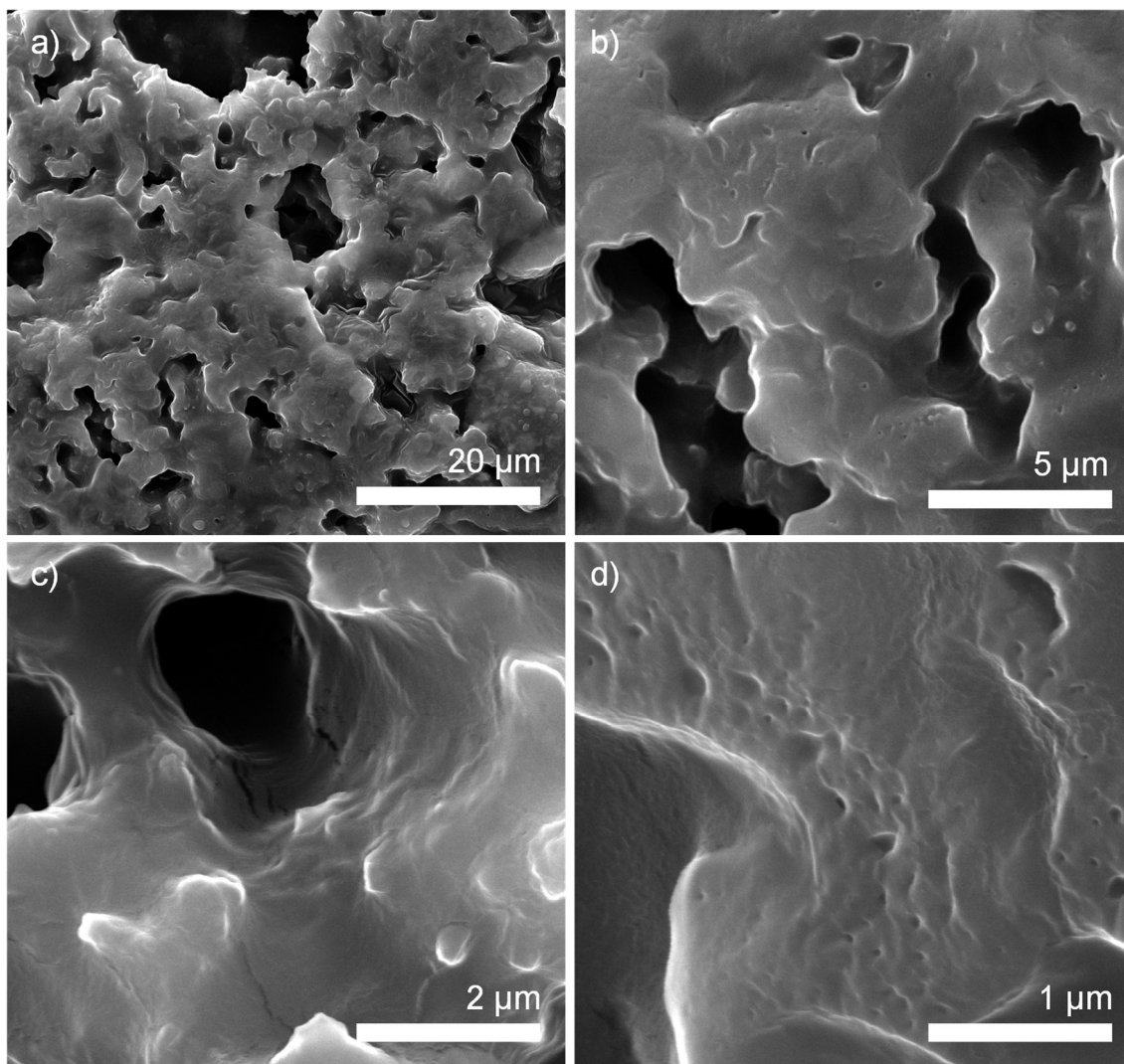


Figure S19. Scanning Electron Microscopy (SEM) images of Esterase@ZIF-C at different magnifications: a) 20 μm, b) 5 μm, c) 2 μm and d) 1 μm.

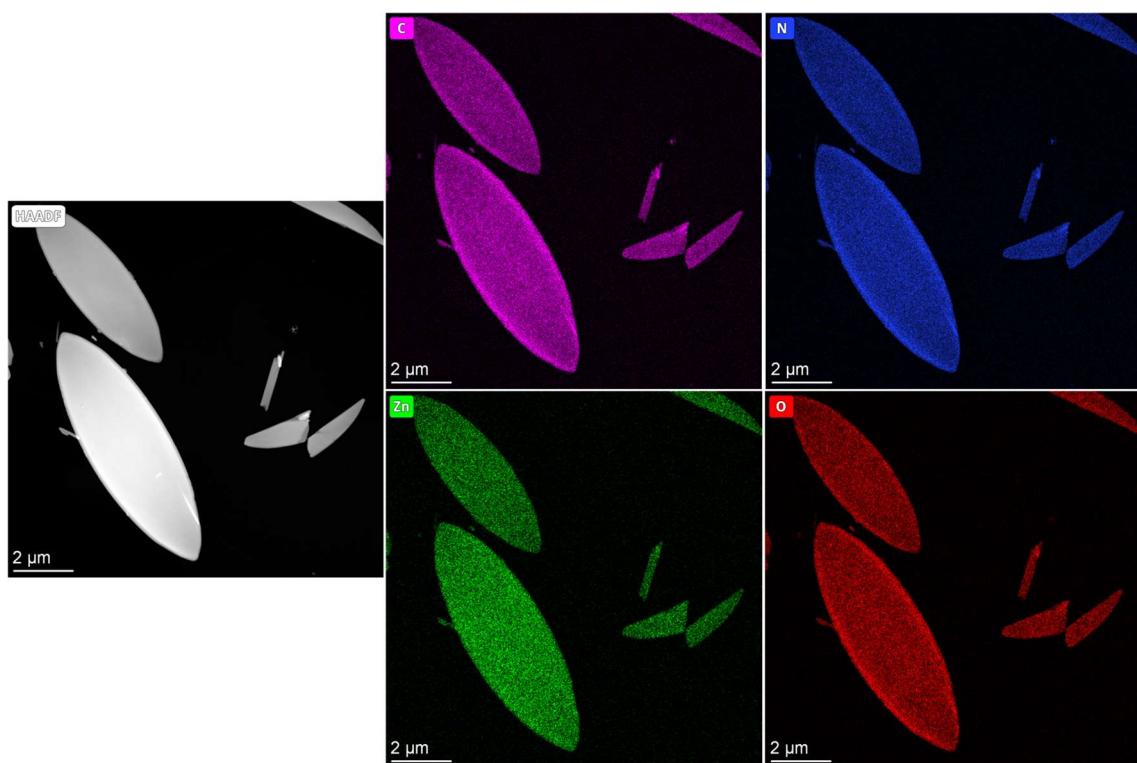


Figure S20. Transmission Electron Microscopy-Energy Dispersive X-ray spectroscopy (TEM-EDX) elemental maps of ZIF-L microparticles.

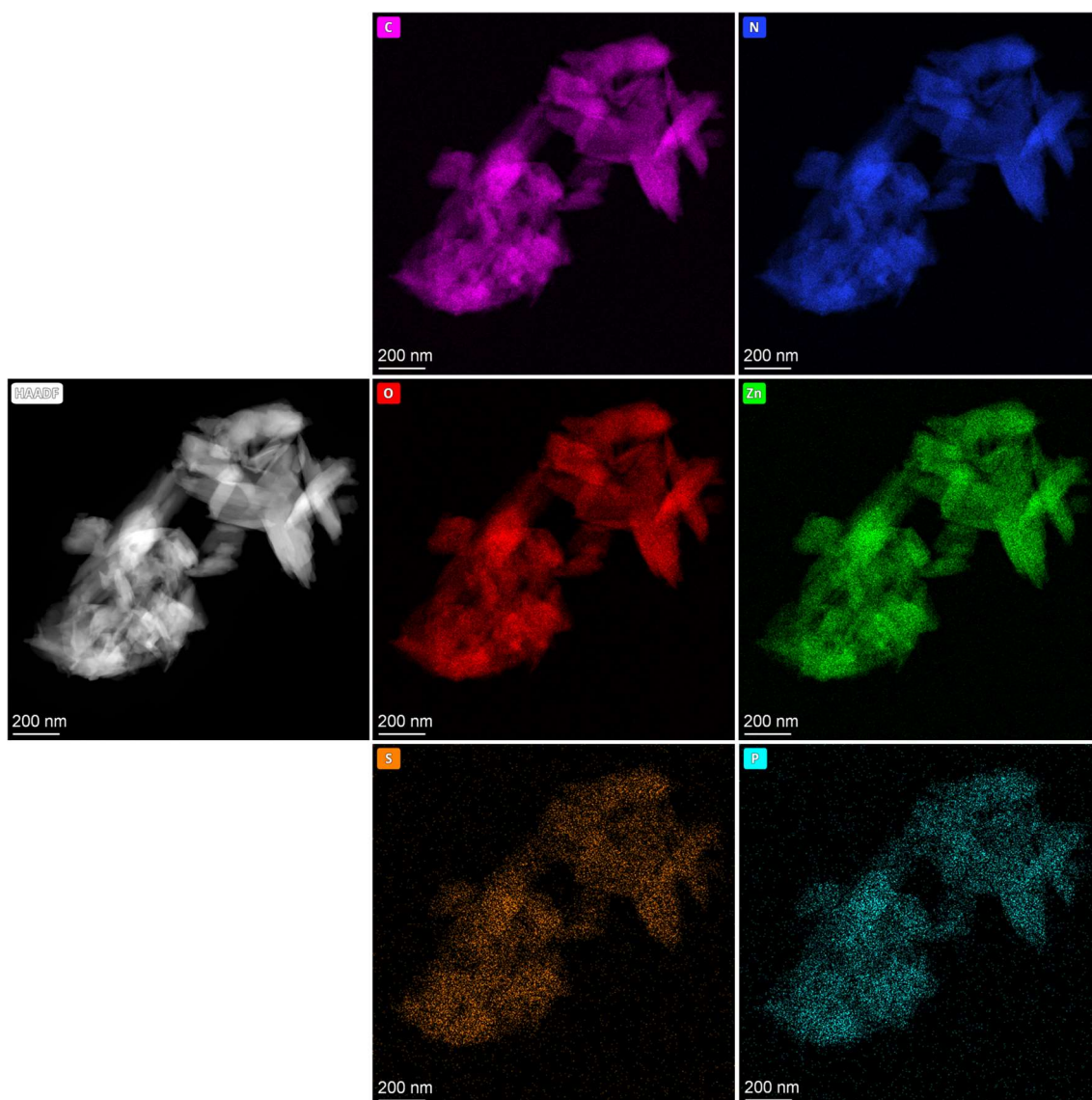


Figure S21. Transmission Electron Microscopy-Energy Dispersive X-ray spectroscopy (TEM-EDX) elemental maps of Esterase@ZIF-L nanoparticles.

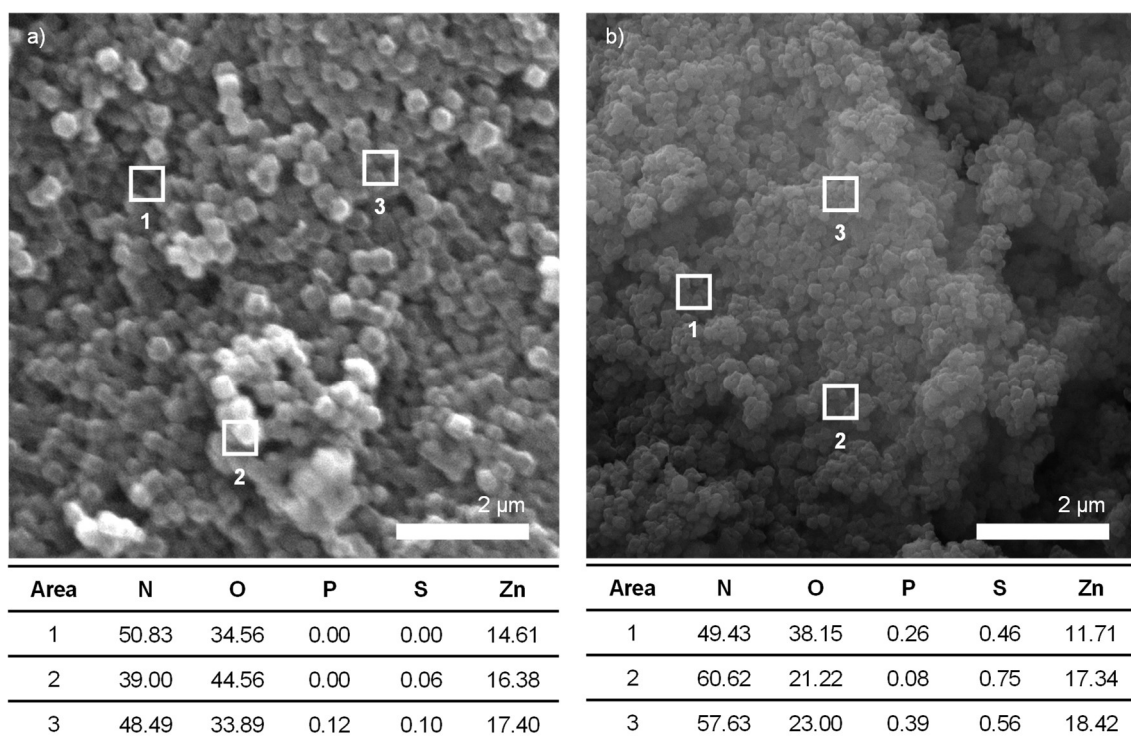


Figure S22. Scanning Electron Microscopy-Energy Dispersive X-ray spectroscopy (SEM-EDX) elemental analysis of (a) ZIF-8_150nm and (b) Esterase@ZIF-8_120nm.

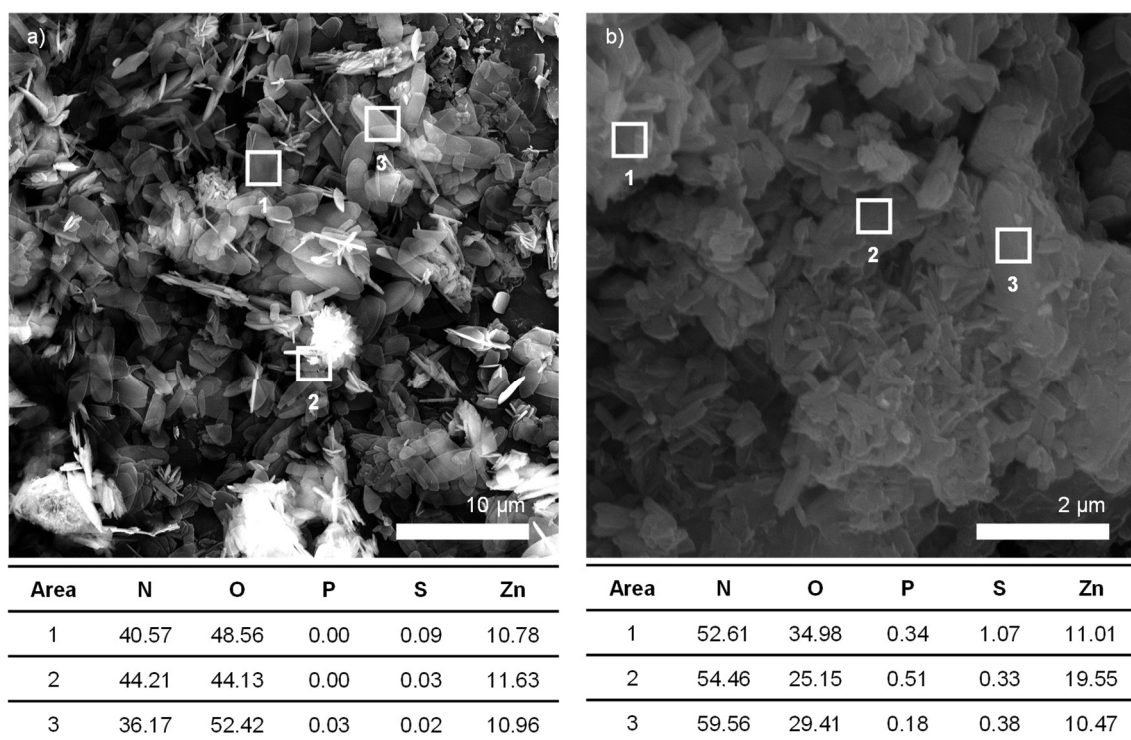


Figure S23. Scanning Electron Microscopy-Energy Dispersive X-ray spectroscopy (SEM-EDX) elemental analysis of (a) ZIF-L and (b) Esterase@ZIF-L.

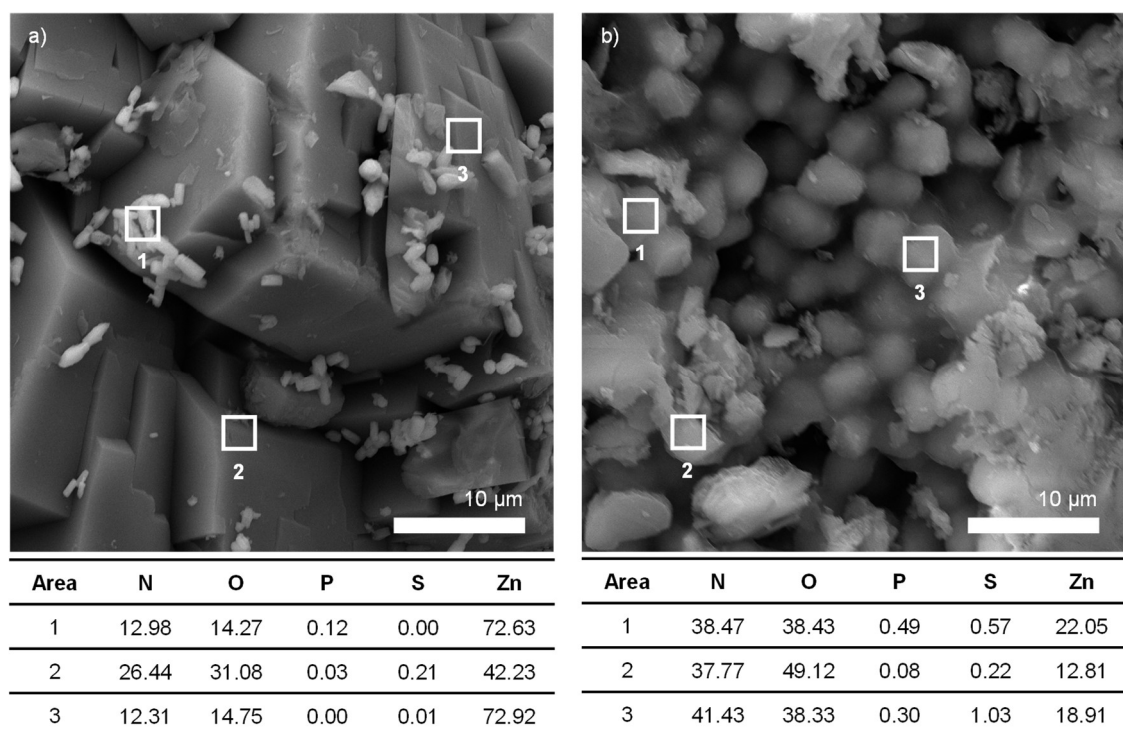


Figure S24. Scanning Electron Microscopy-Energy Dispersive X-ray spectroscopy (SEM-EDX) elemental analysis of (a) ZIF-C and (b) Esterase@ZIF-C.

S3.2. Characterization of BSA@ZIF biocomposites

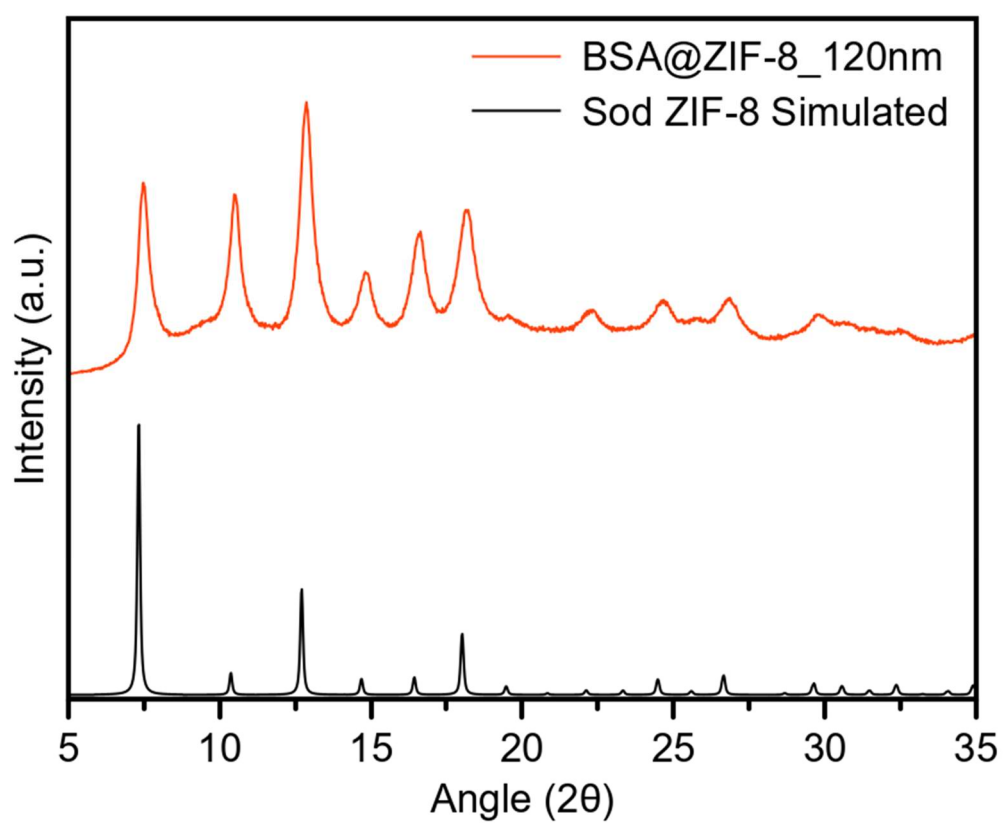


Figure S25. Powder X-Ray Diffraction pattern of BSA@ZIF-8_120nm biocomposite (experimental, red; sod ZIF-8 calculated, black).

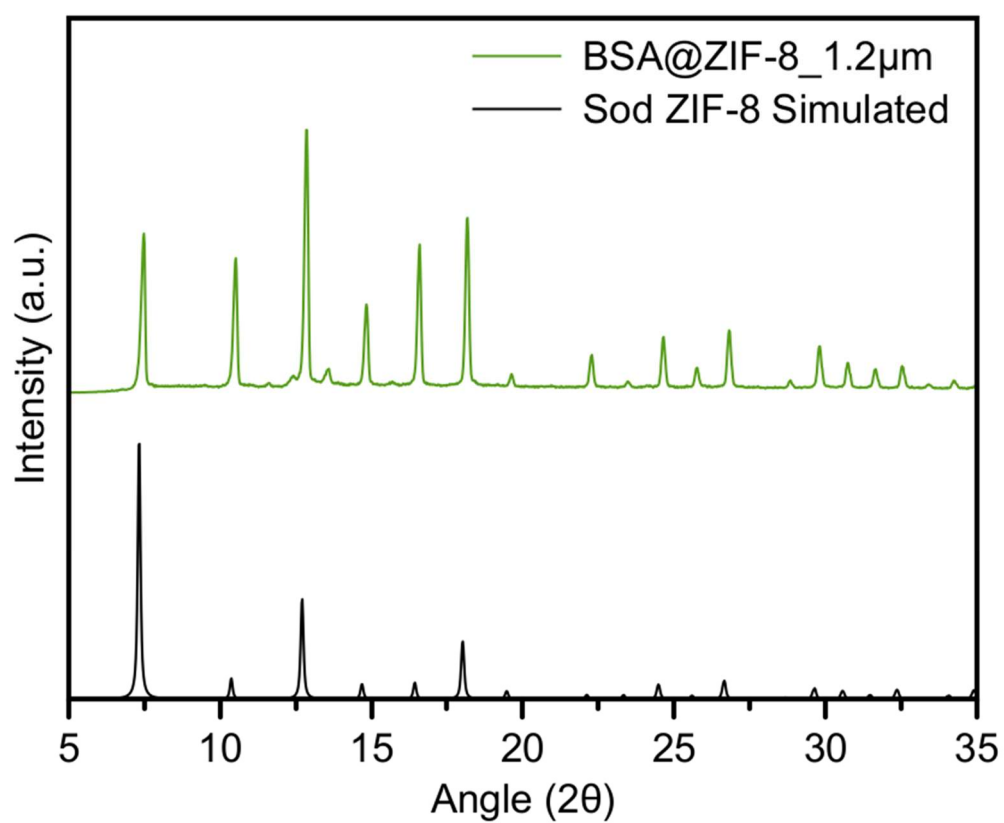


Figure S26. Powder X-Ray Diffraction pattern of BSA@ZIF-8_1.2 μ m biocomposite (experimental, green; sod ZIF-8 calculated, black).

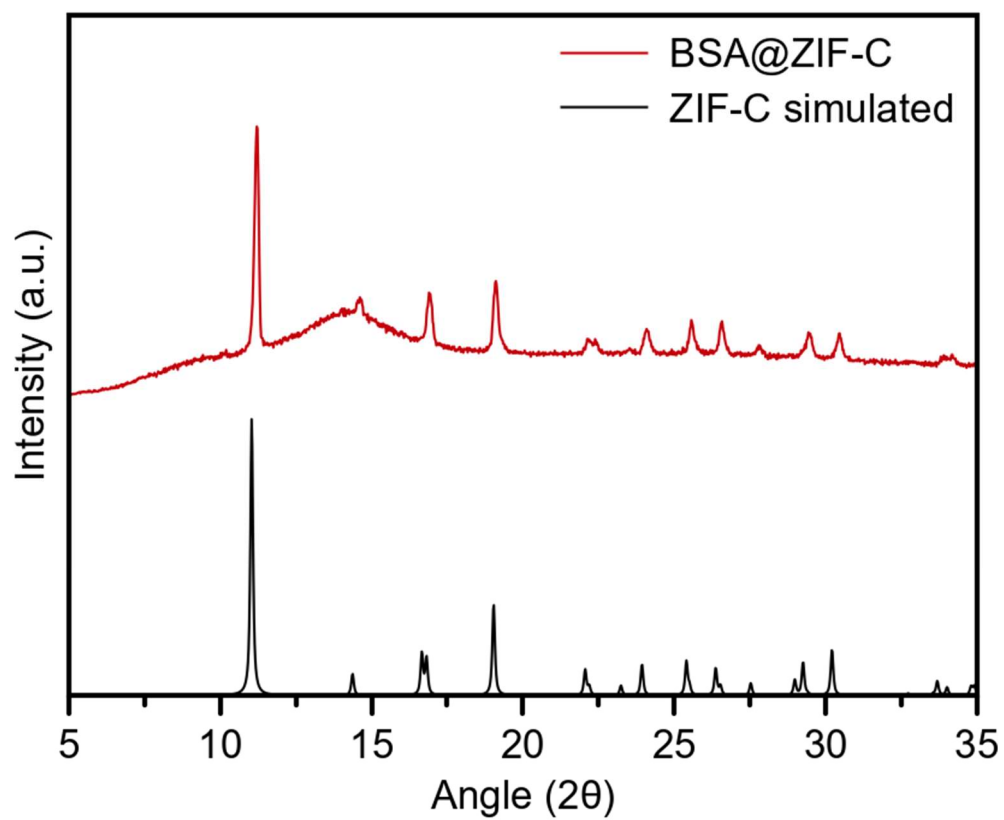


Figure S27. Powder X-Ray Diffraction pattern of BSA@ZIF-C biocomposite (experimental, dark red; ZIF-C calculated, black).

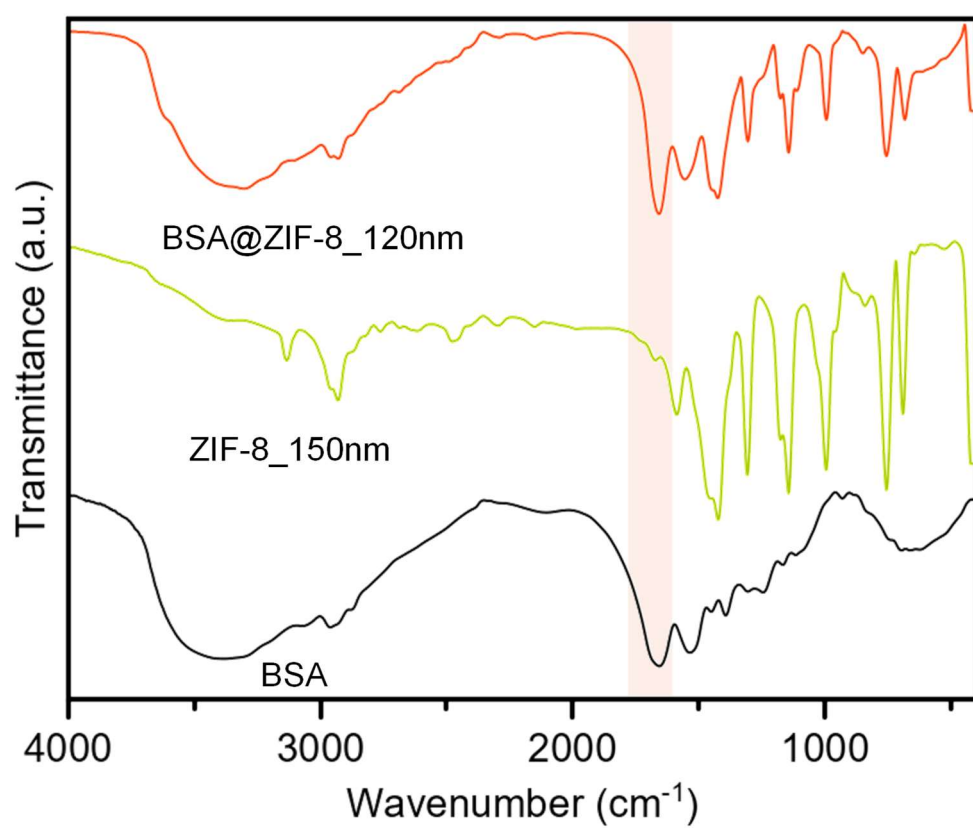


Figure S28. FTIR of BSA (black), ZIF-8_150nm (light green) and BSA@ZIF-8_120nm (red) materials. The analysis of the FTIR data confirms the presence of the characteristic Amide I band (1700-1610 cm⁻¹) of the peptide backbone of BSA highlighted in light orange.

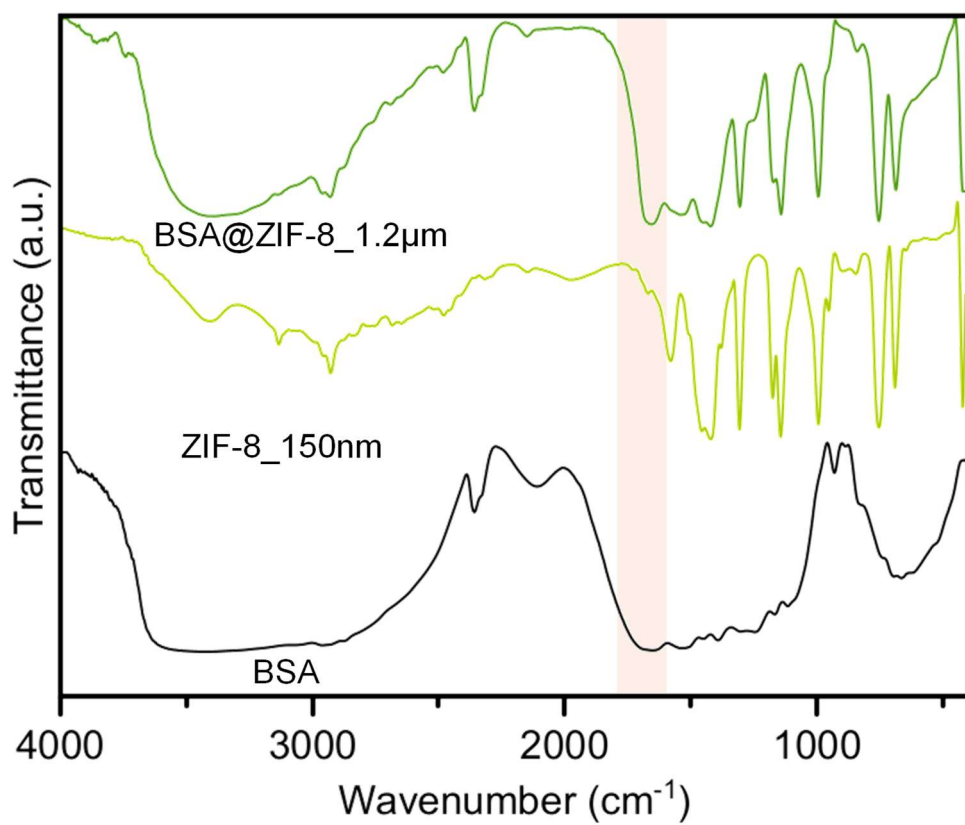


Figure S29. FTIR of BSA (black), ZIF-8_150nm (light green) and BSA@ZIF-8_1.2 μm (dark green) materials. The analysis of the FTIR data confirms the presence of the characteristic Amide I band (1700-1610 cm^{-1}) of the peptide backbone of BSA highlighted in light orange.

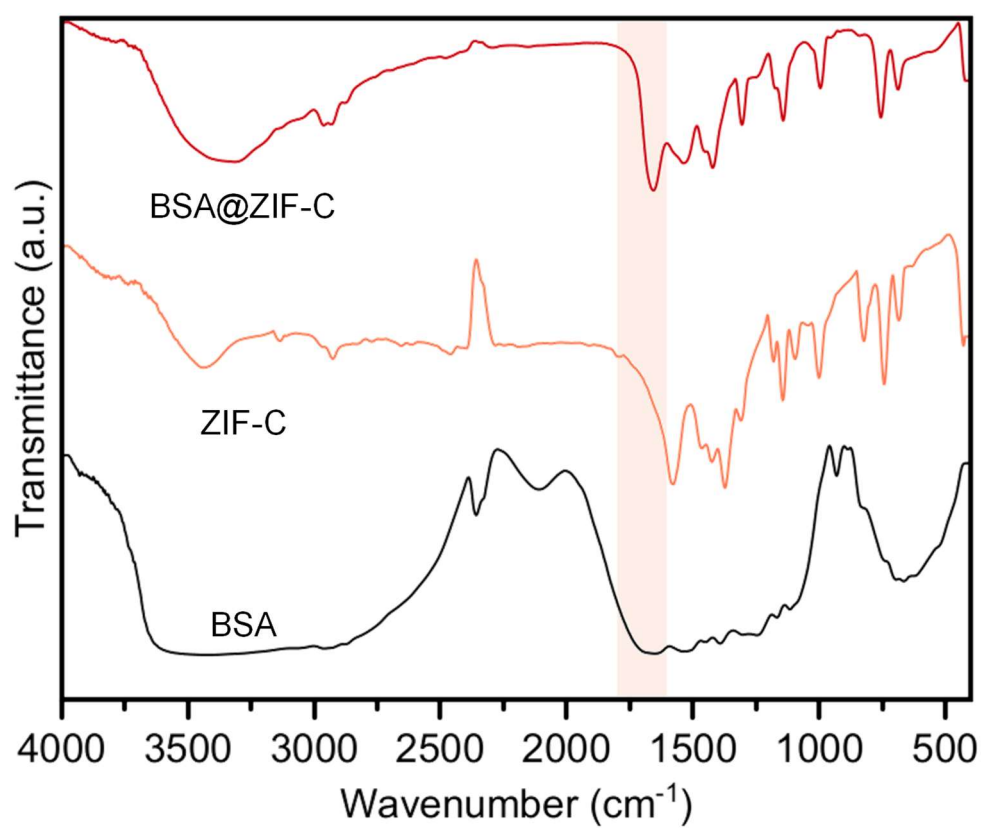


Figure S30. FTIR of BSA (black), ZIF-C (light red) and BSA@ZIF-C (dark red) materials. The analysis of the FTIR data confirms the presence of the characteristic Amide I band ($1700\text{--}1610\text{ cm}^{-1}$) of the peptide backbone of BSA highlighted in light orange.

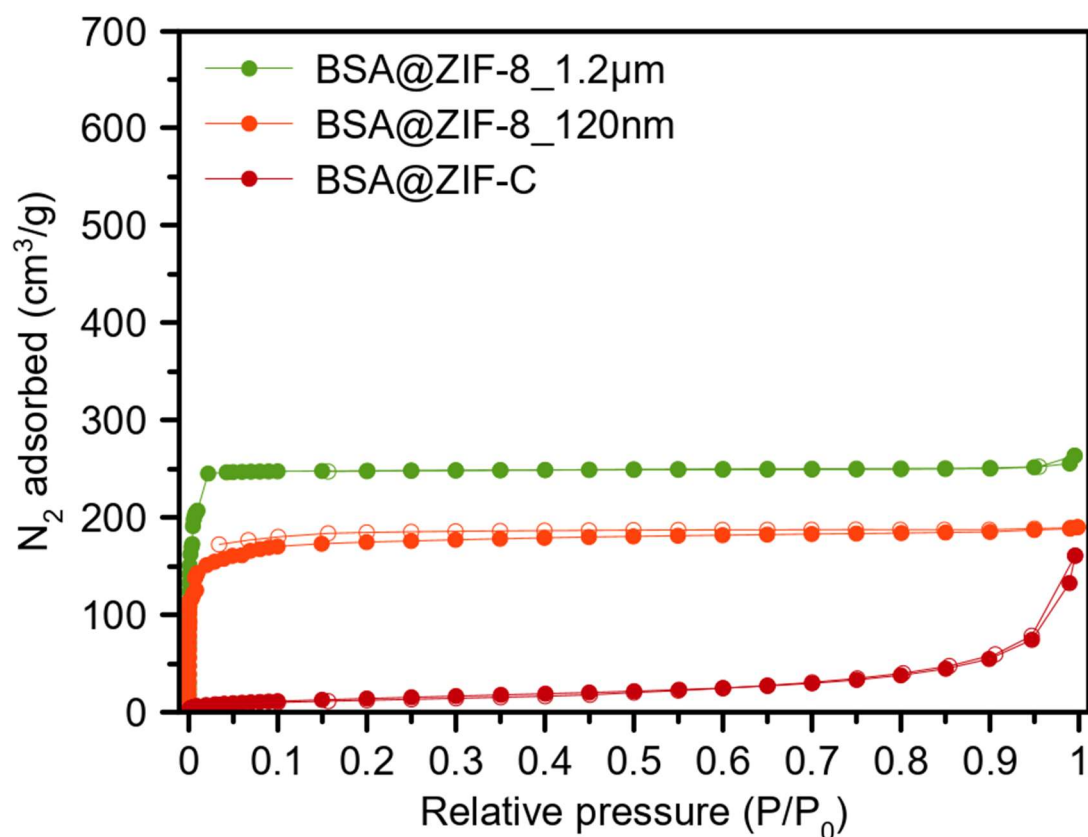


Figure S31. Nitrogen adsorption isotherm at 77 K of BSA@ZIF-8_1.2µm (green), BSA@ZIF-8_120nm (light red) and Esterase@ZIF-C (dark red) biocomposites. Each sample was activated at 120 °C for 12 h under dynamic vacuum. The adsorption and desorption process of each N₂ isotherm are represented by filled and empty dots, respectively.

Table S15. BET surface area of BSA@ZIF biocomposites in m² per gram of total material and per gram of MOF and their comparison to the ZIF original phases.

	$S_{BET} (m^2/g)$	$S_{BET} (m^2/g)$ normalized to MOF mass	$S_{BET} (m^2/g_{MOF})$
BSA@ZIF-8_1.2µm	1031	1610	-
BSA@ZIF-C	42	63	-
BSA@ZIF-8_120nm	680	1214	-
ZIF-8_150nm	-	-	1890
ZIF-C	-	-	2

BET values obtained are referred to the Biocomposites taking into account both the ZIF and BSA mass (column 1). We have carried out the calculation of the BET surface area related to the amount of ZIF present in the total material (column 2) and comparison to ZIF materials alone (column 3). To do this, we have considered the percentage of ZIF of each biocomposite (see Table S8).

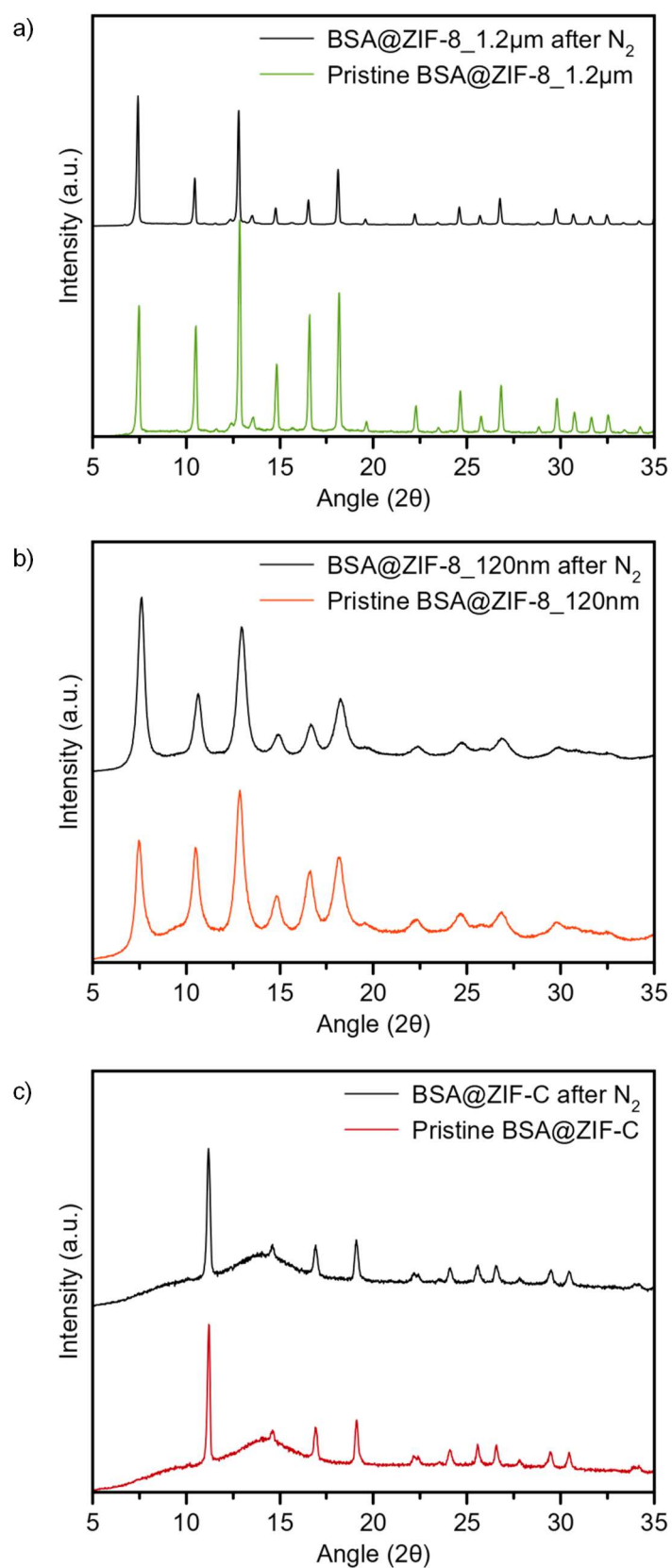


Figure S32. Powder X-Ray Diffraction patterns of (a) BSA@ZIF-8_1.2 μm and (b) BSA@ZIF-C biocomposites after nitrogen adsorption isotherm protocol. The comparison between each material before and after the activation protocol indicates that crystal phase is well maintained.

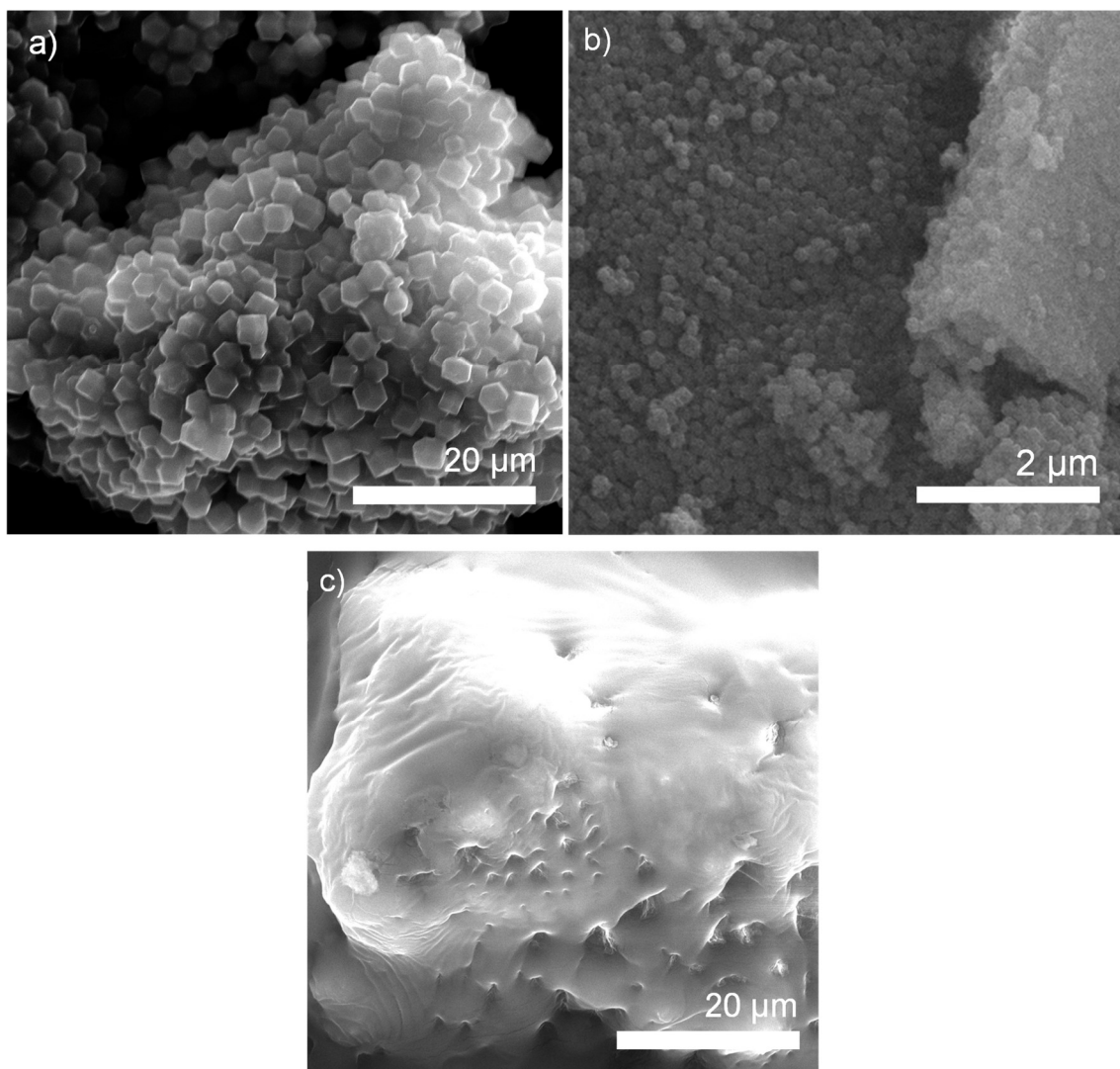


Figure S33. Scanning Electron Microscopy (SEM) images of (a) BSA@ZIF-8_1.2 μm , (b) BSA@ZIF-8_120nm and (c) BSA@ZIF-C.

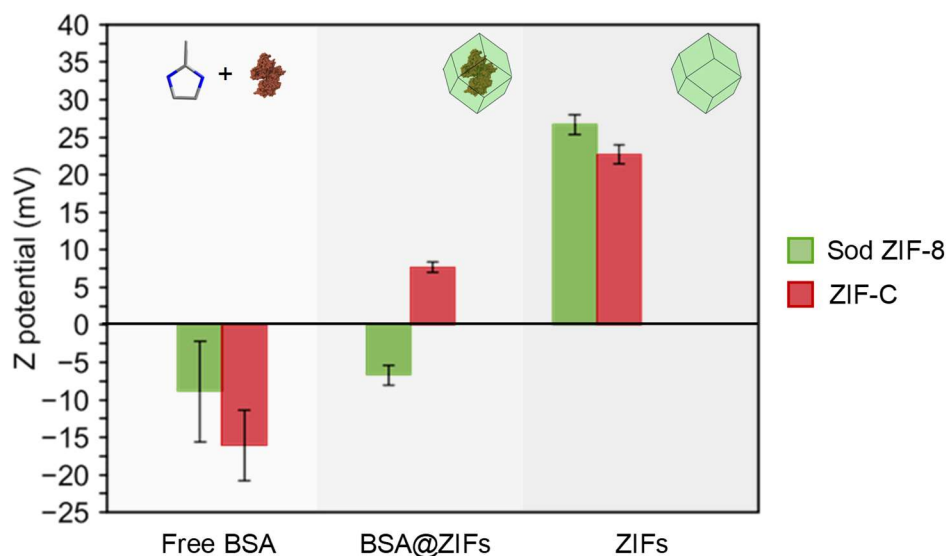


Figure S34. Z potential values of free BSA in precursor ligand solution (left), BSA@ZIF biomineralized (middle) and Zeolitic Imidazolate Frameworks alone (right).

Since BSA has an isoelectric point of 4.7⁵, in an aqueous solution (pH $\approx$ 7) it possesses a net negative charge. When BSA is added to a basic 2-methylimidazole ligand solution (see synthetic section S2.1.5.), the surface charge of this protein becomes even more negative (green and red bars for the BSA + ZIF-8_150nm and BSA+ZIF-C ligand solutions, respectively). After the addition of the metal solution and biomineralization of the ZIFs, the net charge of the particles changes due to the Zn ions in their crystalline structural surface (BSA@ZIF-8_120nm, green; and BSA@ZIF-C, dark red).

S3.2. Synergistic interplay of esterase and ZIF on biocomposite function

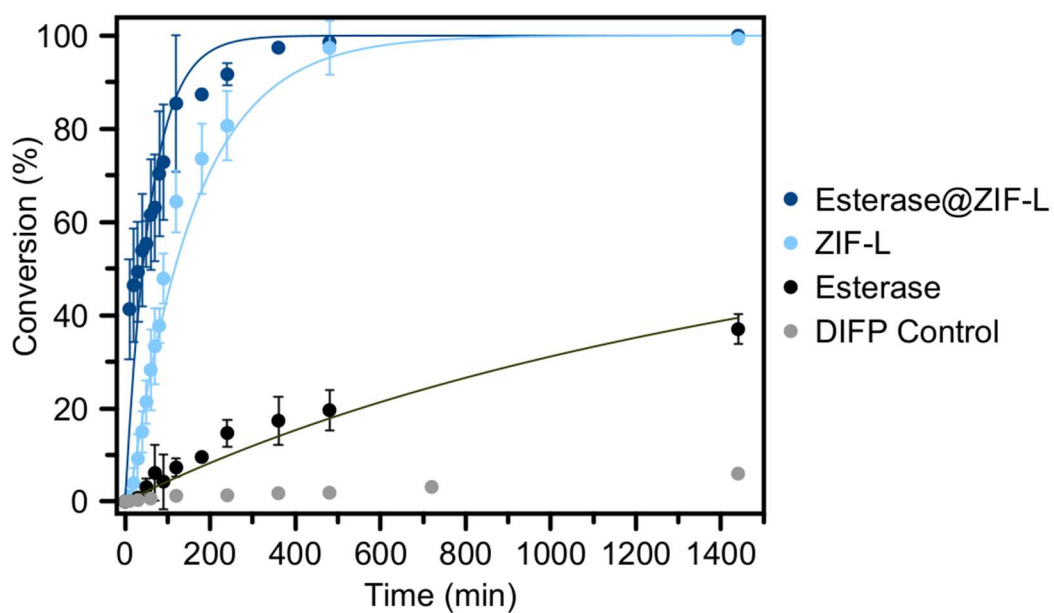


Figure S35. Profiles of diisopropylfluorophosphate hydrolytic degradation by Esterase, ZIF-L and Esterase@ZIF-L materials in simulated biological conditions. Experimental conditions: DIFP (0.029 M), 0.084 mmol of ZIF (in the case of Esterase, we considered the same amount of Esterase employed in the Esterase@ZIF material), DMF (0.029 M, internal reference) and Tris-HCl (0.1 M, pH = 7.4, 0.5 mL) at room temperature. Profile of DIFP degradation in the absence of these materials is represented in grey dots.

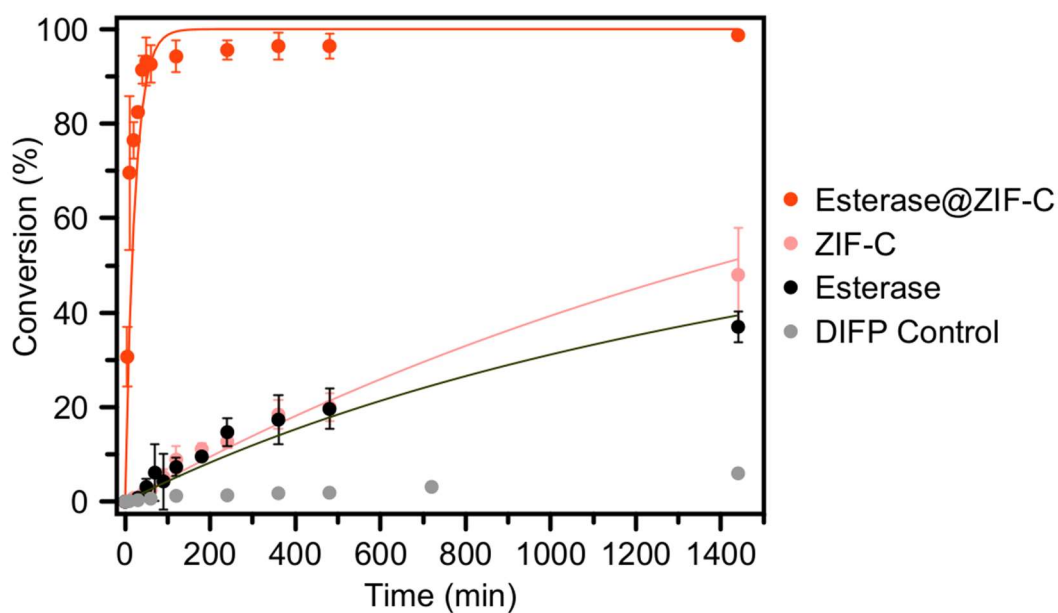


Figure S36. Profiles of diisopropylfluorophosphate hydrolytic degradation by Esterase, ZIF-C and Esterase@ZIF-C materials in simulated biological conditions. Experimental conditions: DIFP (0.029 M), 0.084 mmol of ZIF (in the case of Esterase, we considered the same amount of Esterase employed in the Esterase@ZIF material), DMF (0.029 M, internal reference) and Tris-HCl (0.1 M, pH = 7.4, 0.5 mL) at room temperature. Profile of DIFP degradation in the absence of these materials is represented in grey dots.

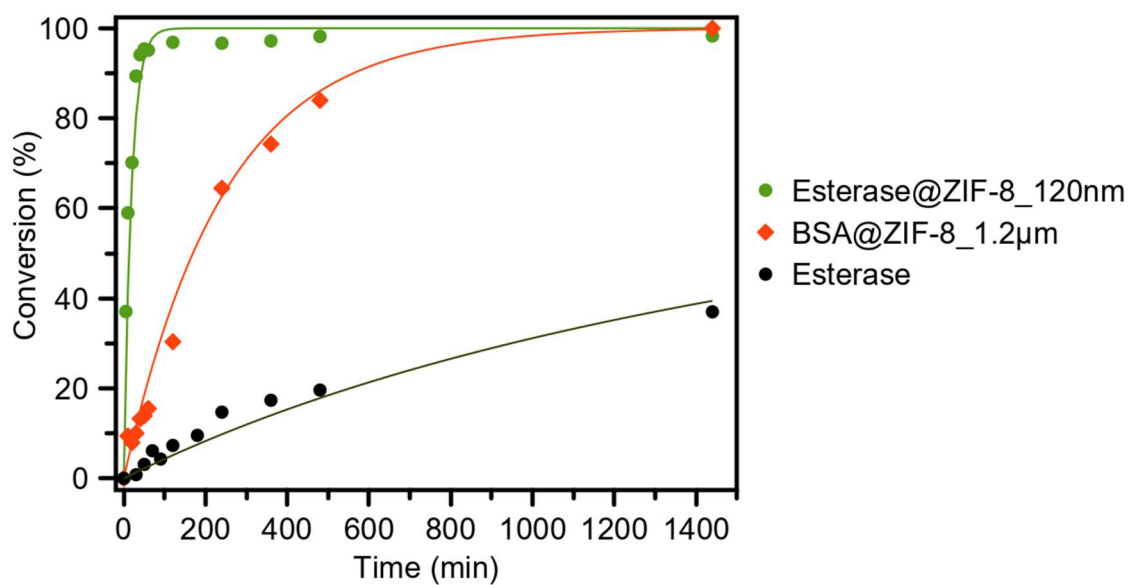


Figure S37. Profiles of diisopropylfluorophosphate hydrolytic degradation by Esterase@ZIF-8_120nm, BSA@ZIF-8_1.2μm and free Esterase in simulated biological conditions as control experiments. Experimental conditions: DIFP (0.029 M), 0.084 mmol of ZIF, DMF (0.029 M, internal reference) and Tris-HCl (0.1 M, pH = 7.4, 0.5 mL) at room temperature.

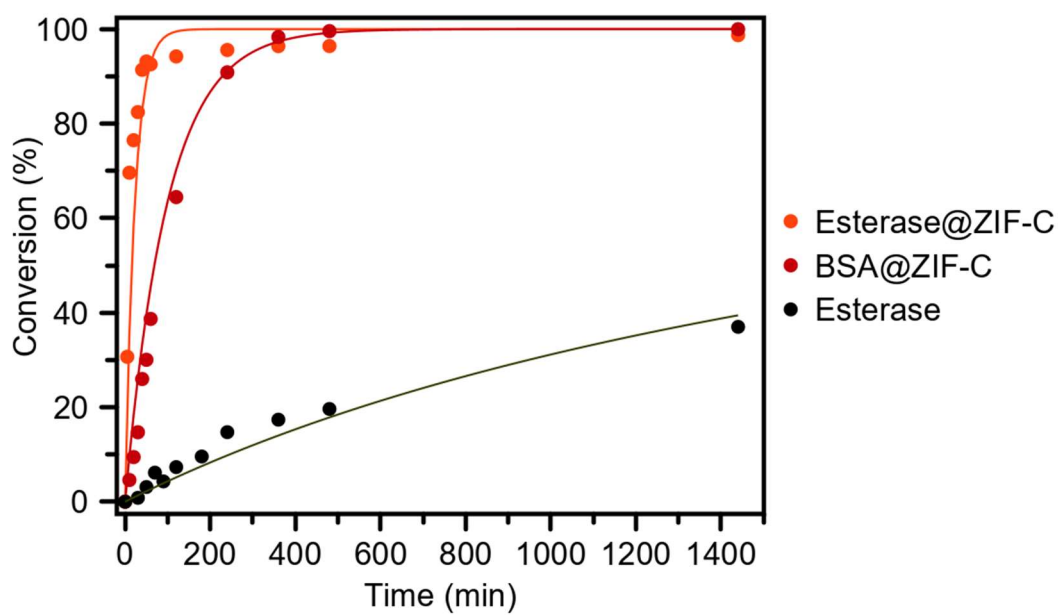


Figure S38. Profiles of diisopropylfluorophosphate hydrolytic degradation by Esterase@ZIF-C, BSA@ZIF-C and free Esterase in simulated biological conditions as control experiments. Experimental conditions: DIFP (0.029 M), 0.084 mmol of ZIF, DMF (0.029 M, internal reference) and Tris-HCl (0.1 M, pH = 7.4, 0.5 mL) at room temperature.

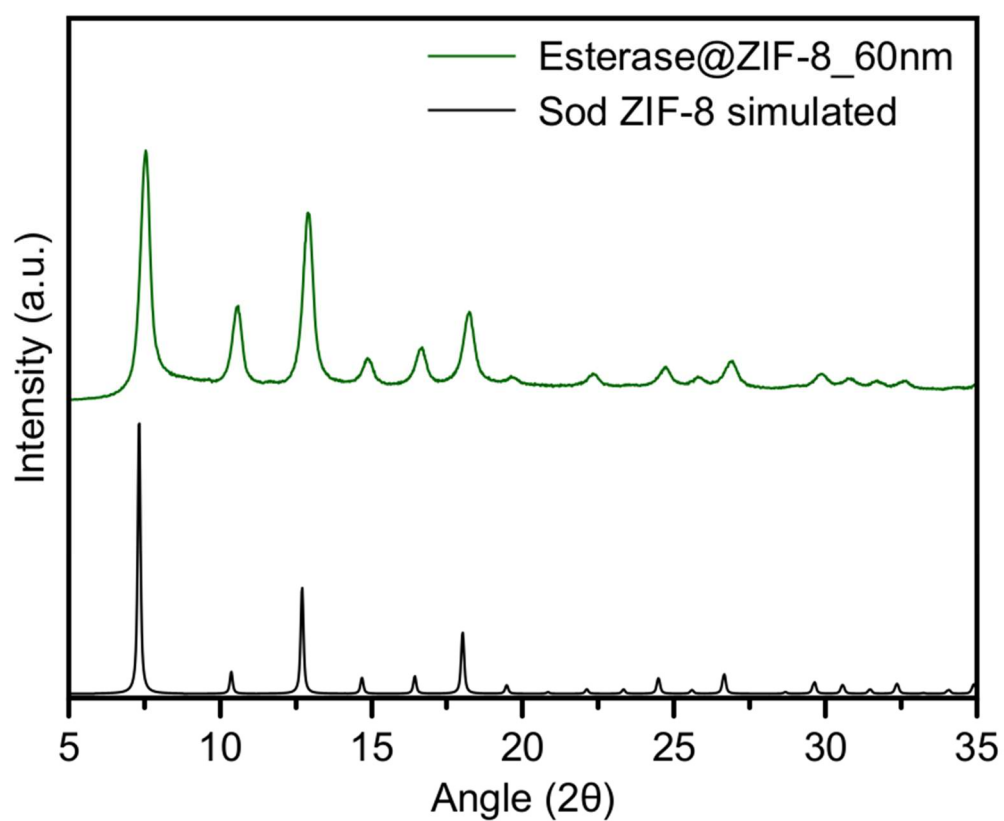


Figure S39. Powder X-Ray Diffraction pattern of Esterase@ZIF-8_60nm biocomposite (experimental, dark green; sod ZIF-8 calculated, black).

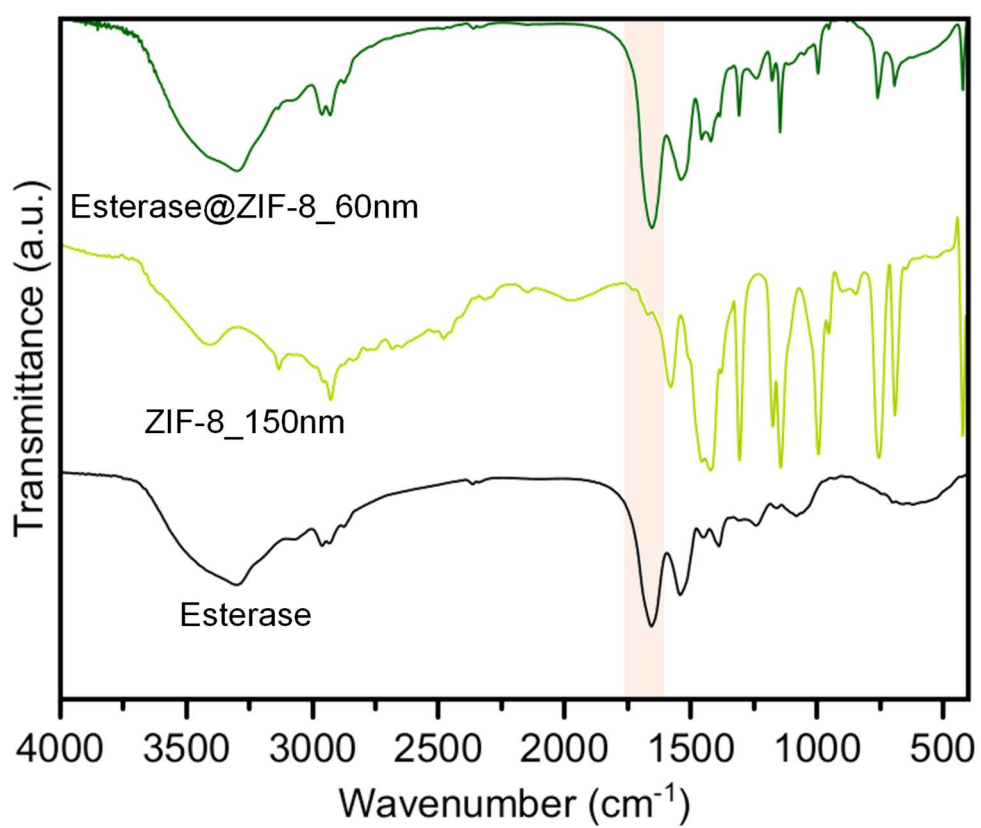


Figure S40. Fourier Transformed Infrared spectra (FTIR) of Esterase (black), ZIF-8_150nm (light green) and Esterase@ZIF-8_60nm (dark green) materials. The analysis of the FTIR data confirms the presence of the characteristic Amide I band (1700-1610 cm⁻¹) of the peptide backbone of Esterase highlighted in light orange.

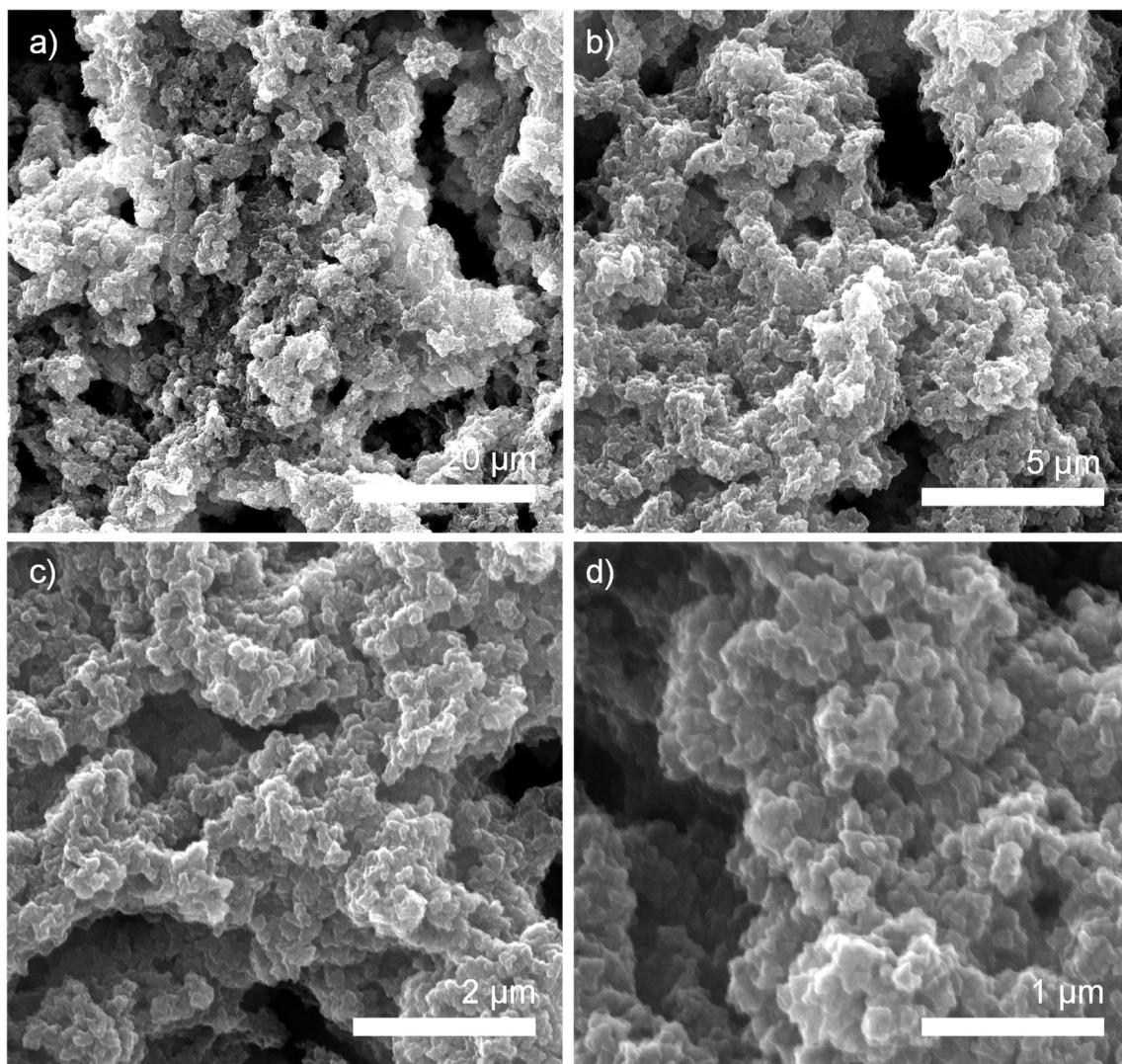


Figure S41. Scanning Electron Microscopy (SEM) images of Esterase@ZIF-8_60nm at different magnifications: a) 20 μm , b) 5 μm , c) 2 μm and d) 1 μm .

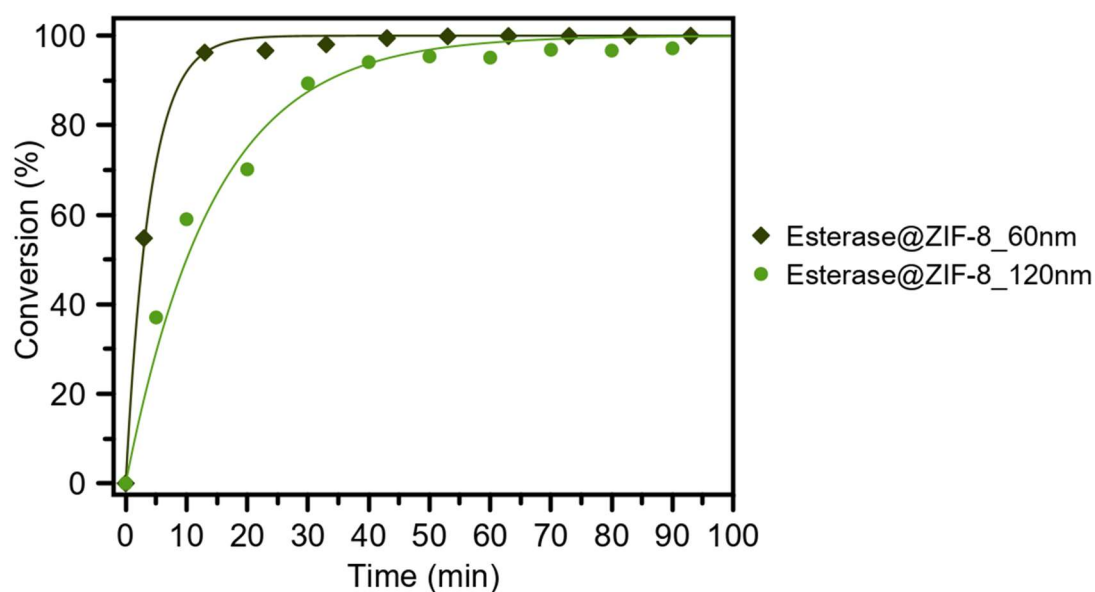


Figure S42. Profiles of diisopropylfluorophosphate hydrolytic degradation by Esterase@ZIF-8 biocomposites of two different particle size: Esterase@ZIF-8_60nm and Esterase@ZIF-8_120nm in simulated biological conditions. Experimental conditions: DIFP (0.029 M), 0.084 mmol of ZIF (we considered the amount of ZIF employed in each Esterase@ZIF material), DMF (0.029 M, internal reference) and Tris-HCl (0.1 M, pH = 7.4, 0.5 mL) at room temperature.

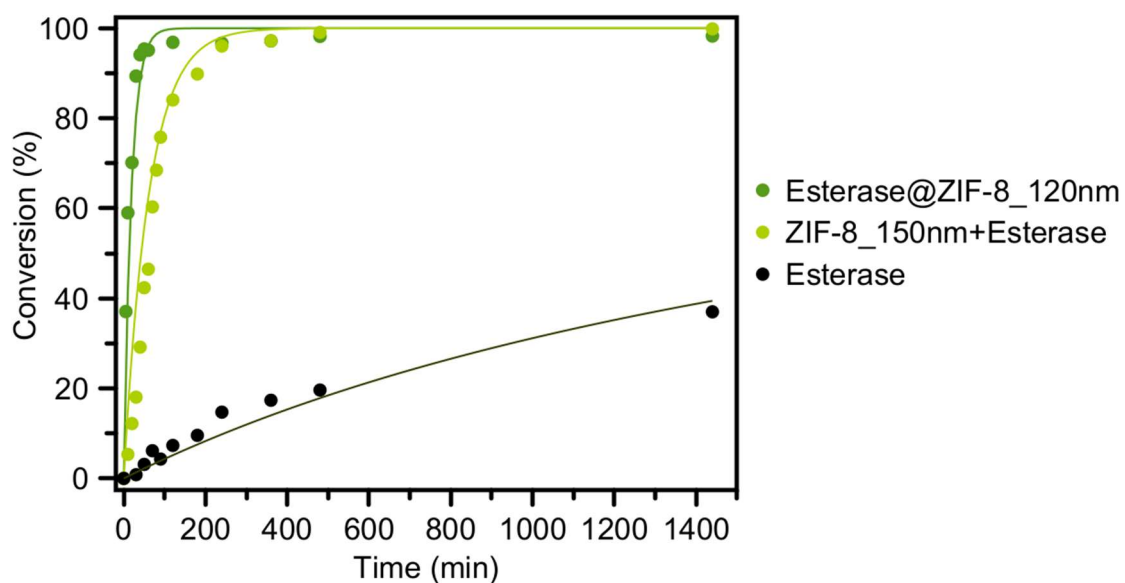


Figure S43. Profiles of diisopropylfluorophosphate hydrolytic degradation by Esterase@ZIF-8_120nm, ZIF-8_150nm + Esterase physical mixture and free Esterase in simulated biological conditions. Experimental conditions: DIFP (0.029 M), 0.084 mmol of ZIF (in the case of ZIF-8_150nm+Esterase physical mixture, we considered the amount of sod ZIF-8 and Esterase employed in the Esterase@ZIF-8_120nm material), DMF (0.029 M, internal reference) and Tris-HCl (0.1 M, pH = 7.4, 0.5 mL) at room temperature.

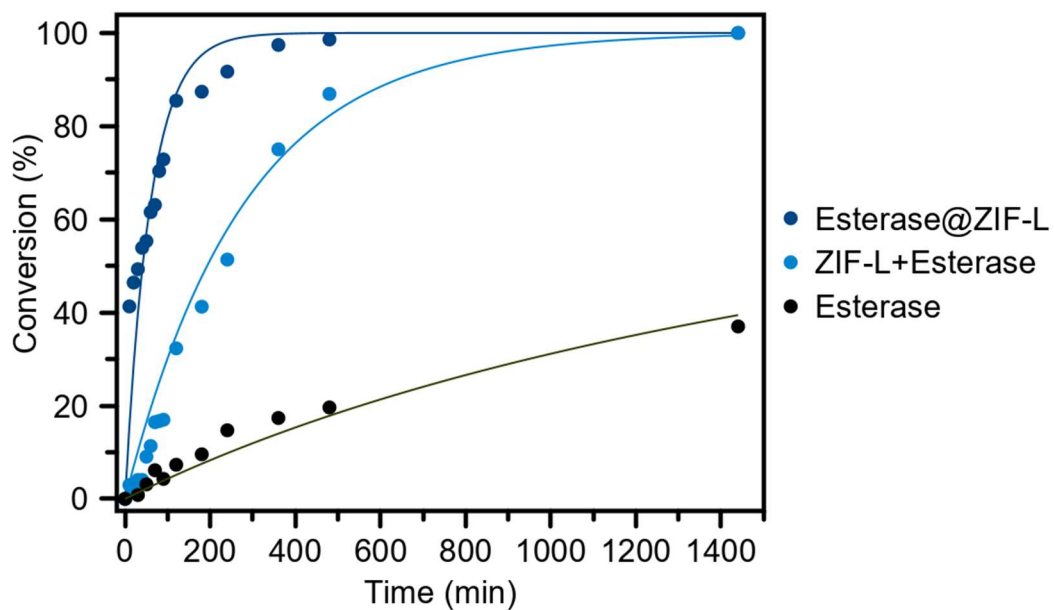


Figure S44. Profiles of diisopropylfluorophosphate hydrolytic degradation by Esterase@ZIF-L, ZIF-L + Esterase physical mixture and free Esterase in simulated biological conditions. Experimental conditions: DIFP (0.029 M), 0.084 mmol of ZIF (in the case of ZIF-L+Esterase physical mixture, we considered the amount of ZIF-L and Esterase employed in the Esterase@ZIF-L material), DMF (0.029 M, internal reference) and Tris-HCl (0.1 M, pH = 7.4, 0.5 mL) at room temperature.

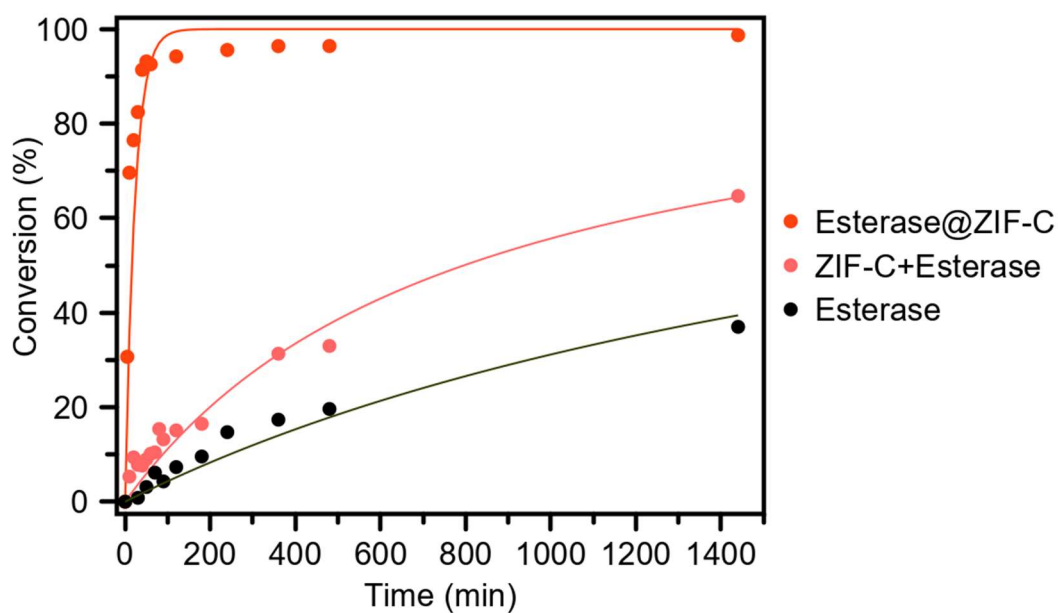


Figure S45. Profiles of diisopropylfluorophosphate hydrolytic degradation by Esterase@ZIF-C, ZIF-C + Esterase physical mixture and free Esterase in simulated biological conditions. Experimental conditions: DIFP (0.029 M), 0.084 mmol of ZIF (in the case of ZIF-C+Esterase physical mixture, we considered the amount of ZIF-C and Esterase employed in the Esterase@ZIF-C material), DMF (0.029 M, internal reference) and Tris-HCl (0.1 M, pH = 7.4, 0.5 mL) at room temperature.

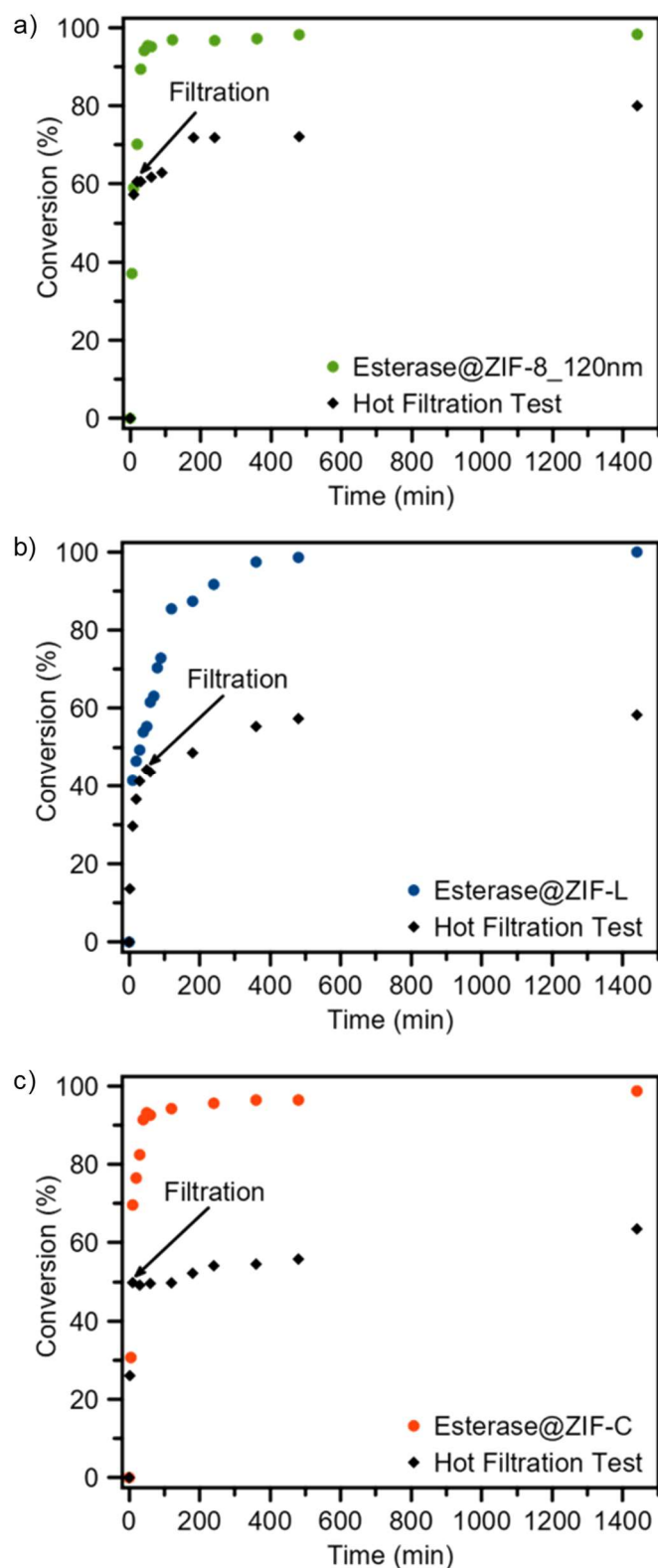


Figure S46. Hot filtration tests profiles of diisopropylfluorophosphate (DIFP) hydrolytic degradation by (a) Esterase@ZIF-8_120nm, (b) Esterase@ZIF-L and (c) Esterase@ZIF-C materials in simulated biological conditions. Experimental conditions: DIFP (0.029 M), 0.084 mmol of ZIF, DMF (0.029 M, internal reference) and Tris-HCl (0.1 M, pH = 7.4, 0.5 mL) at room temperature.

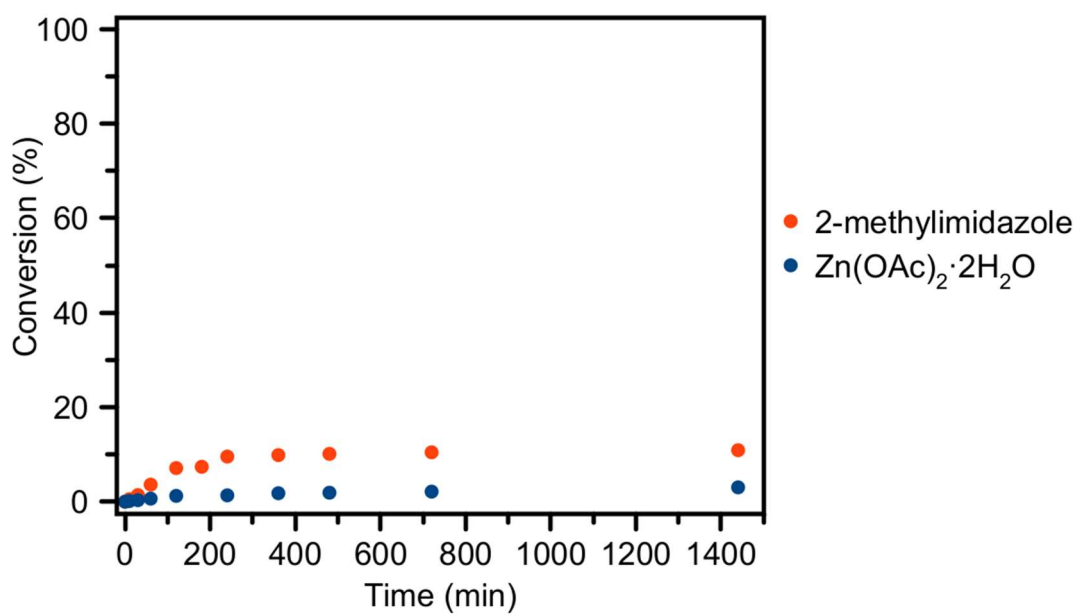


Figure S47. Profiles of diisopropylfluorophosphate hydrolytic degradation by 2-methylimidazole and Zn(OAc)₂·2H₂O control experiments in simulated biological conditions. Experimental conditions: DIFP (0.029 M), 0.029 M of 2-methylimidazole/Zn(OAc)₂·2H₂O, DMF (0.029 M, internal reference) and Tris-HCl (0.1 M, pH = 7.4, 0.5 mL) at room temperature.

S3.3. Esterase enzymatic activity of biocomposites

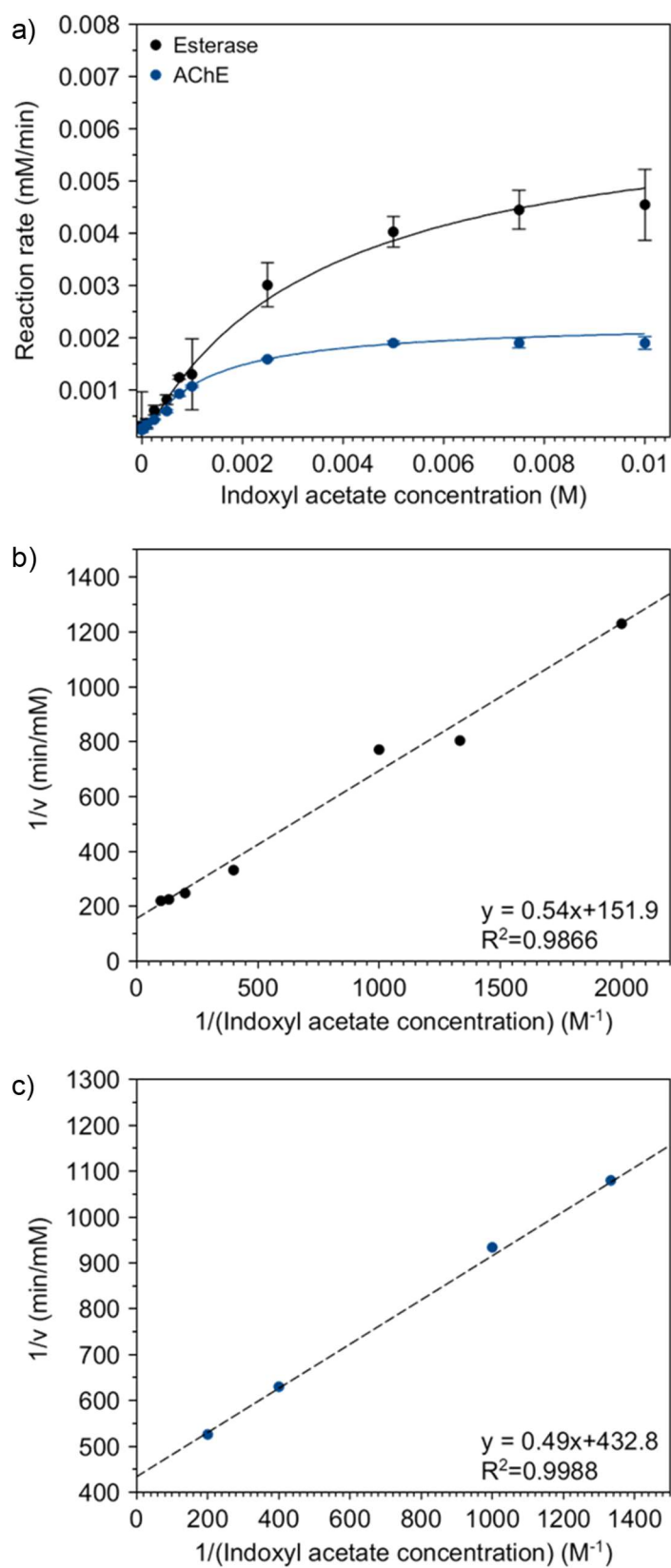


Figure S48. (a) Michaelis-Menten kinetic for Esterase (black) and AChE (blue) for indoxyl acetate test. Lineweaver-Burk double reciprocal plot adjustment for (b) Esterase and (c) AChE enzymes. See S2.4.1. for further experimental details.

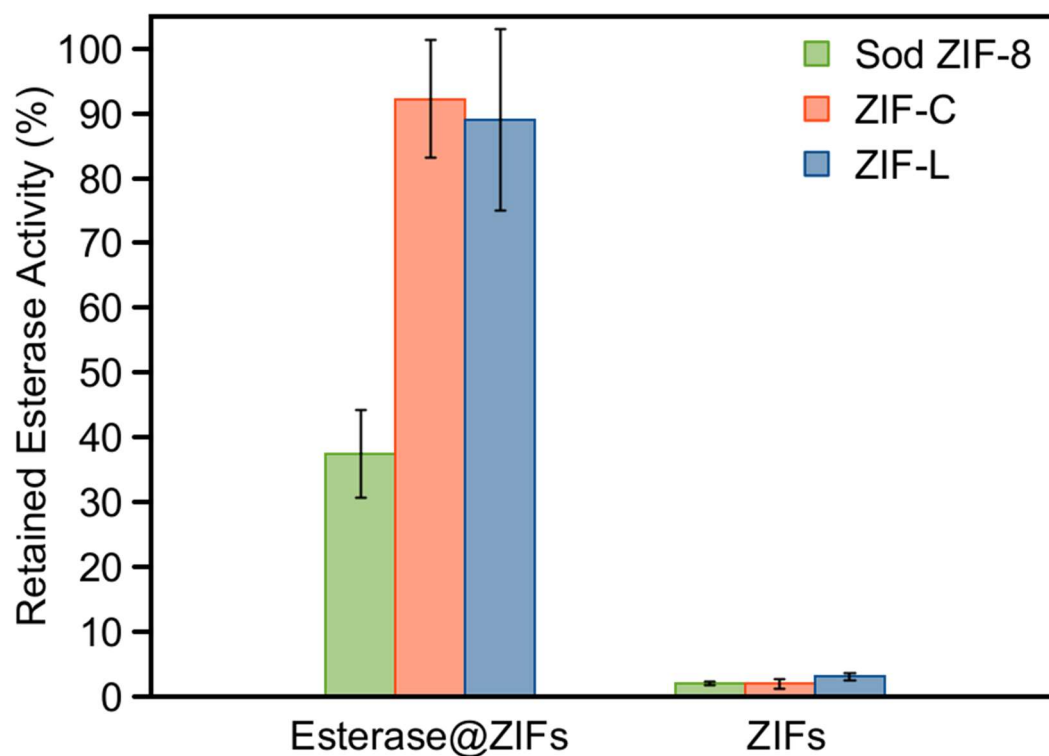


Figure S49. Retained enzymatic Esterase activity of Esterase@ZIFs after the biomineralization process. The enzymatic activity of the three new biocomposites (Esterase@ZIF-8_120nm, Esterase@ZIF-C and Esterase@ZIF-L) was compared to the enzymatic activity of free Esterase at 37 °C. In addition, we also compared the enzymatic activity of ZIFs (without Esterase) as control. See S.2.4.3. for further experimental details.

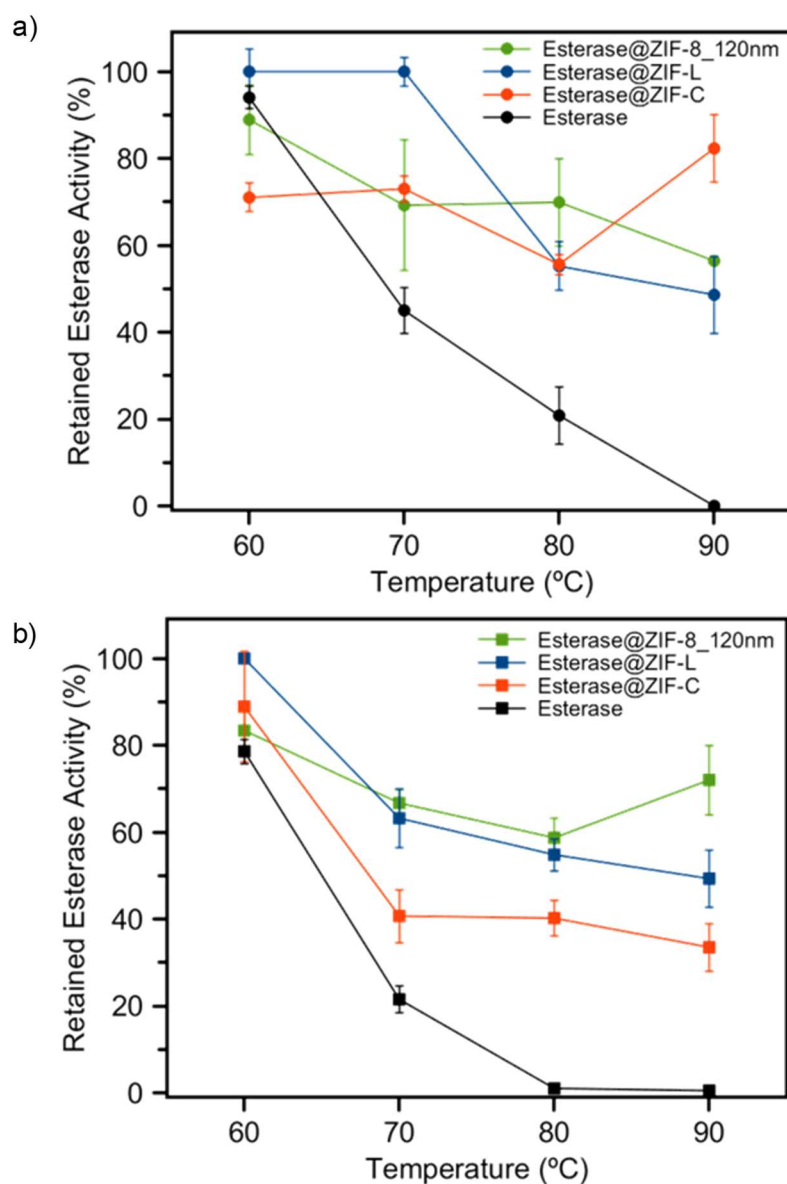
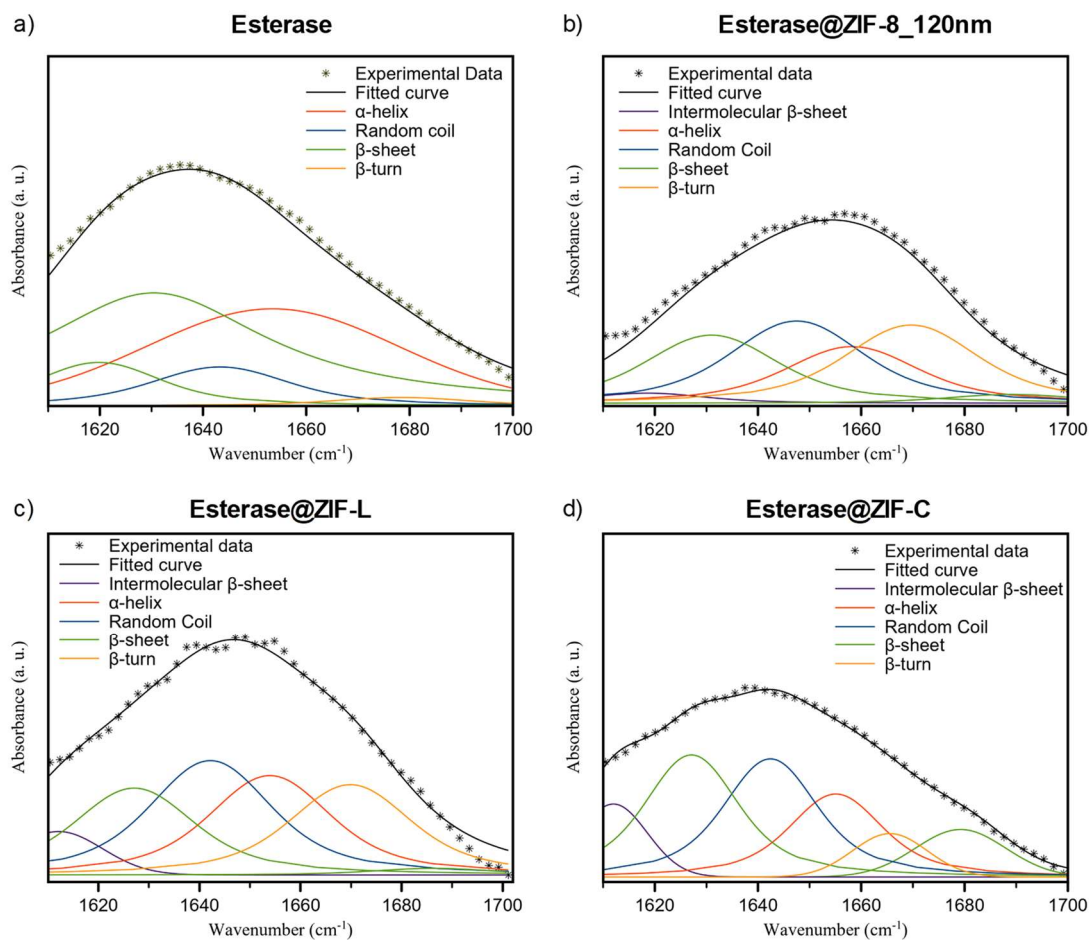


Figure S50. Retained enzymatic activity of free Esterase, Esterase@ZIF-8_120nm, Esterase@ZIF-L and Esterase@ZIF-C materials after incubation at different temperatures for (a) 30 min and (b) 1 h. See S.2.4.5. for further experimental details.

Table S16. Protein secondary structure assignment according to deconvoluted peaks

Average Position (cm ⁻¹)	Assignment	Reference
1600-1620	Intermolecular β -sheet	5 ⁶
1620-1640, 1680-1700	β -sheet	5-10 ⁶⁻¹¹
1640-1650	Random coil	6,8 ^{7,9}
1648-1660	α -helix	6,8 ^{7,9}
1660-1680	β -turn	5-8 ⁶⁻⁹

**Figure S51.** ATR-FTIR spectra and the corresponding second derivative ATR-FTIR spectra of (a) Esterase (b) Esterase@ZIF-8_120nm, (c) Esterase@ZIF-L and (d) Esterase@ZIF-C showing the structural content of intermolecular β -sheet, α -helix, random coil, β -sheet and β -turn. The structural contents were determined by the Voigt function fitted in Origin software.**Table S17.** Protein secondary structure assignment according to deconvoluted peaks

	β -sheet (%)	β -turn (%)	α -helix (%)	Random coil (%)	Intramolecular β -sheet (%)
Free Esterase	56.9	2.0	32.1	9.0	0
Esterase@ZIF-8_120nm	25.2	25.9	18.7	27.2	3.0
Esterase@ZIF-C	36.9	6.3	19.4	27.8	9.6
Esterase@ZIF-L	22.1	21.4	23.6	27.1	5.8

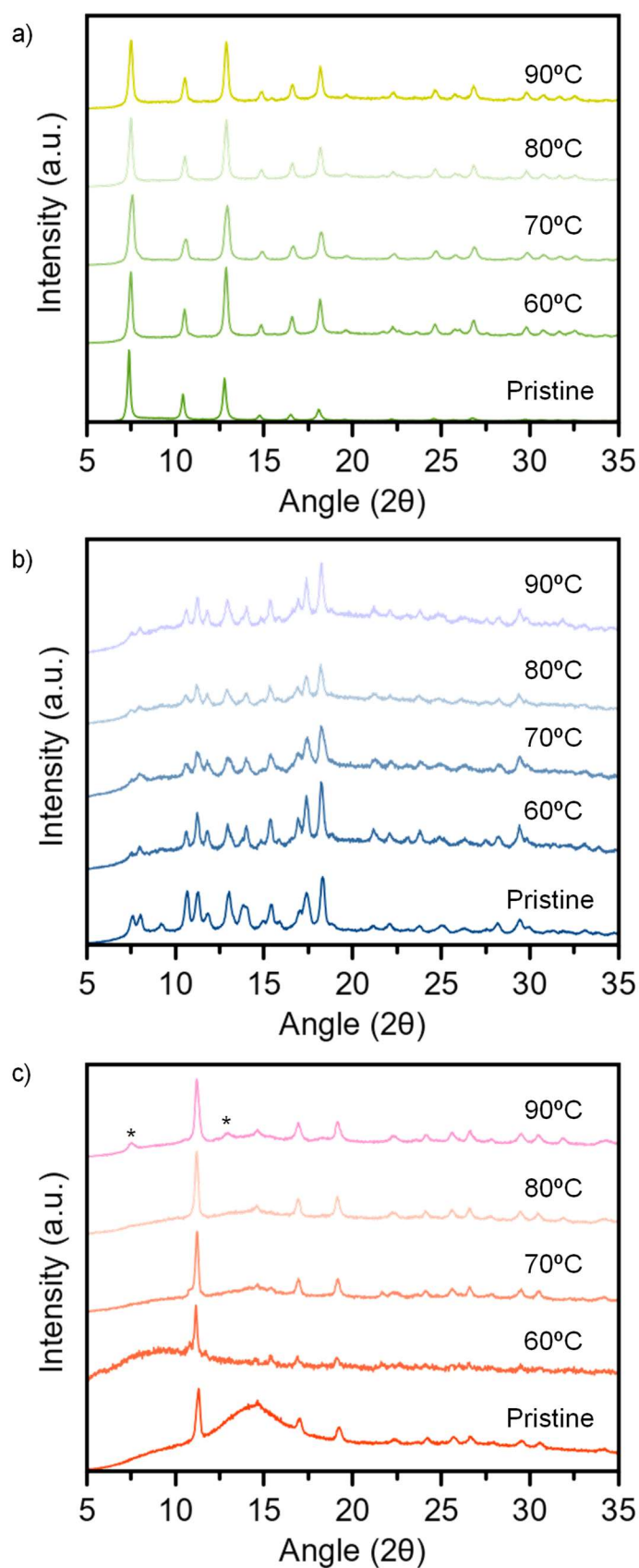


Figure S52. Powder X-Ray Diffraction patterns of (a) Esterase@ZIF-8_120nm, (b) Esterase@ZIF-L and (c) Esterase@ZIF-C biocomposites after different incubation temperatures. Experimental conditions: Esterase@ZIF (1.87 U/mL of Esterase) and Tris-HCl (0.1 M, pH = 7.4, 1 mL) incubated at different temperatures for 1 h. Asterisk: appearance of sodalite phase.

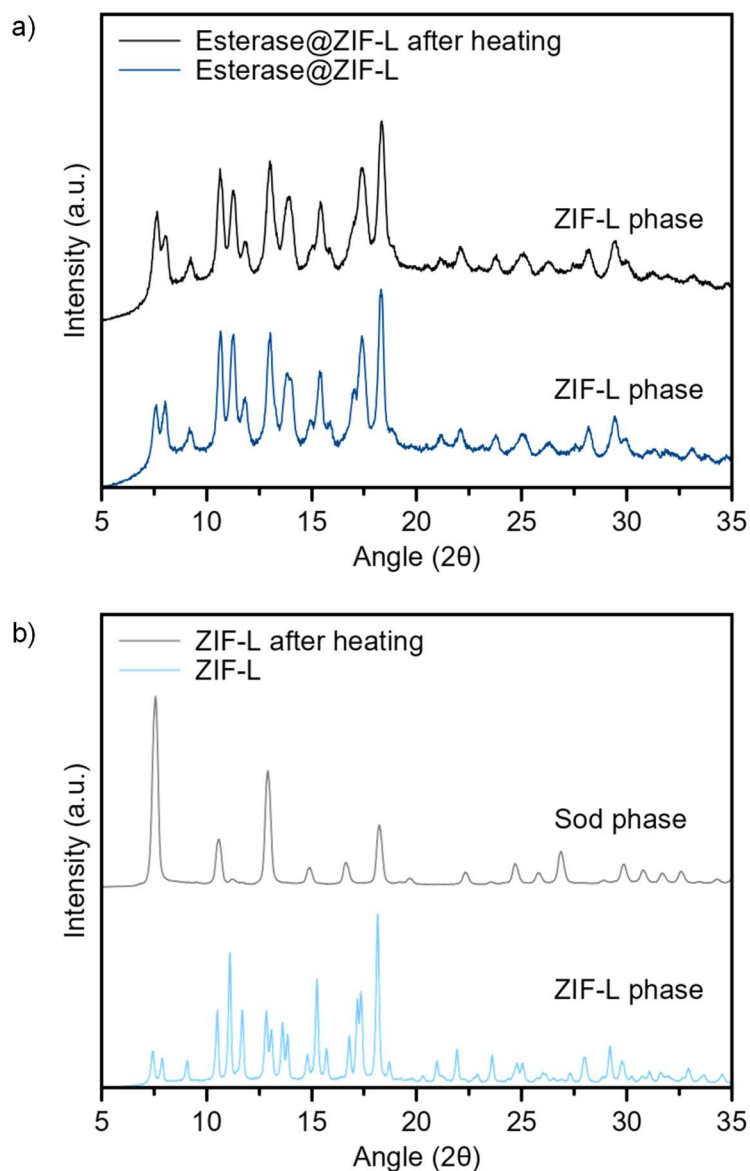


Figure S53. Powder X-Ray Diffraction patterns of (a) Esterase@ZIF-L and (b) ZIF-L materials after incubation in EtOH at 60 °C. Experimental conditions: ZIF-L or Esterase@ZIF-L (50 mg) were dispersed in ethanol (5 mL) and heated to 60 °C for 72 h. This was left to cool before separation by centrifuge at 6000 rpm for 10 min. The samples were dried at 60 °C overnight. The results indicate that ZIF-L crystalline phase is well maintained in the Esterase@ZIF biocomposite, probably due to a stabilization with the esterase encapsulation. In the ZIF-L material alone, there is a crystal phase change to the more stable sodalite phase as reported in literature^[12].

S3.4. DIFP induced ZIF crystal surface degradation.

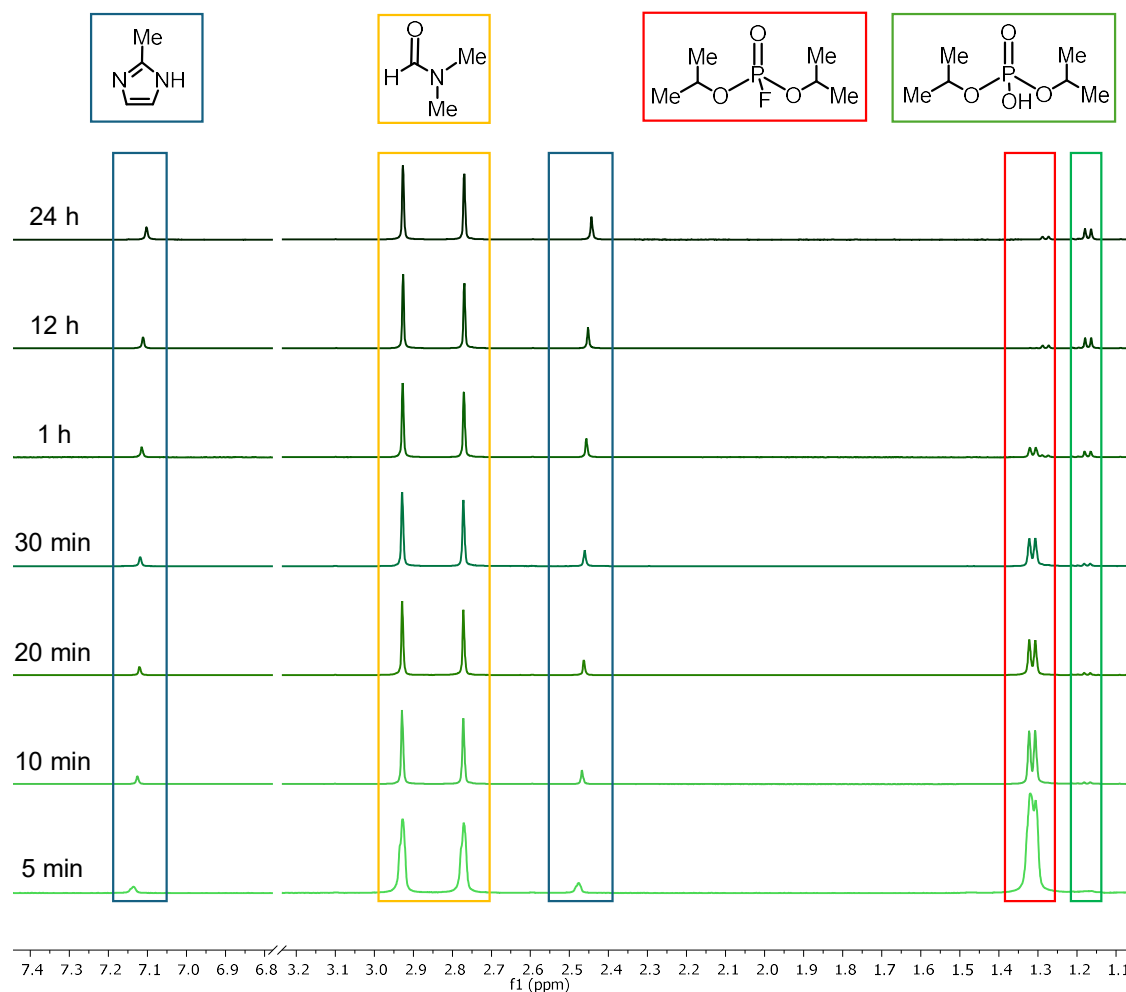


Figure S54. ^1H -NMR follow-up of diisopropylfluorophosphate (DIFP) hydrolysis and ZIF structural degradation with concomitant mlmH release by Esterase@ZIF-8_120nm biocomposite. Experimental conditions: DIFP (0.029 M), Esterase@ZIF-8_120nm (0.084 mmol of sod ZIF-8), dimethylacetamide (0.029 M, internal reference) and Tris-DCI (0.1 M, pD = 7.8, 0.5 mL) at room temperature.

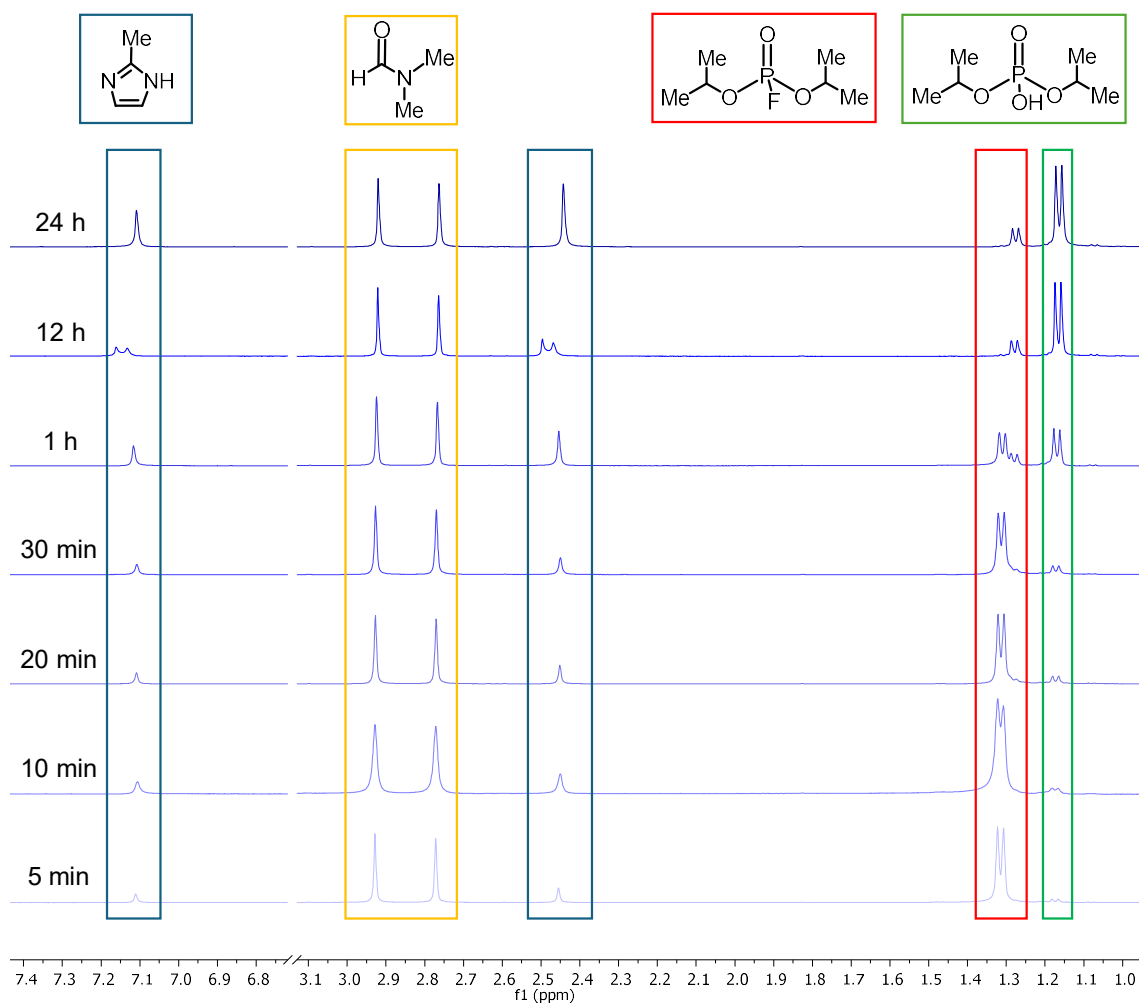


Figure S55. ^1H -NMR follow-up of diisopropylfluorophosphate (DIFP) hydrolysis and ZIF structural degradation with concomitant mlmH release by Esterase@ZIF-L biocomposite. Experimental conditions: DIFP (0.029 M), Esterase@ZIF-L (0.084 mmol of ZIF-L), dimethylacetamide (0.029 M, internal reference) and Tris-DCI (0.1 M, pD = 7.8, 0.5 mL) at room temperature.

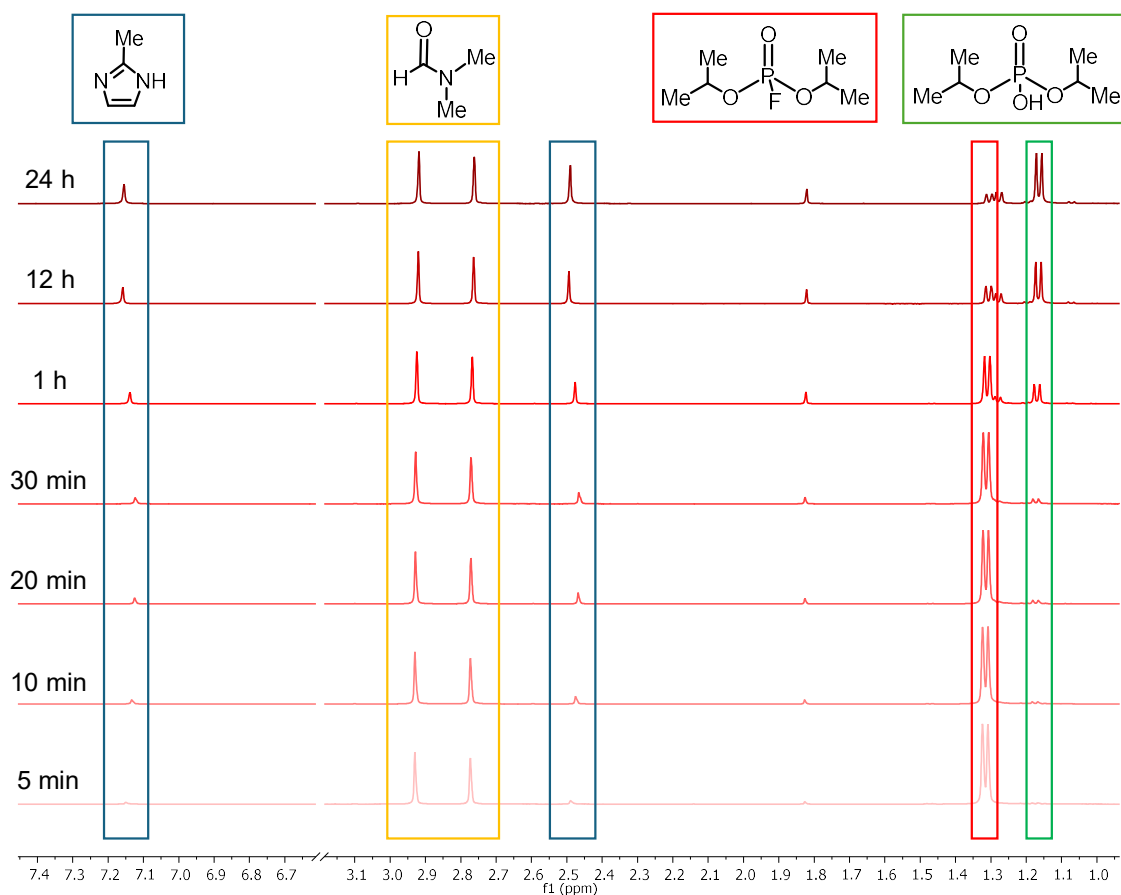


Figure S56. ^1H -NMR follow-up of diisopropylfluorophosphate (DIFP) hydrolysis and ZIF structural degradation with concomitant mlmH release by Esterase@ZIF-C biocomposite. Experimental conditions: DIFP (0.029 M), Esterase@ZIF-C (0.084 mmol of ZIF-C), dimethylacetamide (0.029 M, internal reference) and Tris-DCI (0.1 M, pD = 7.8, 0.5 mL) at room temperature.

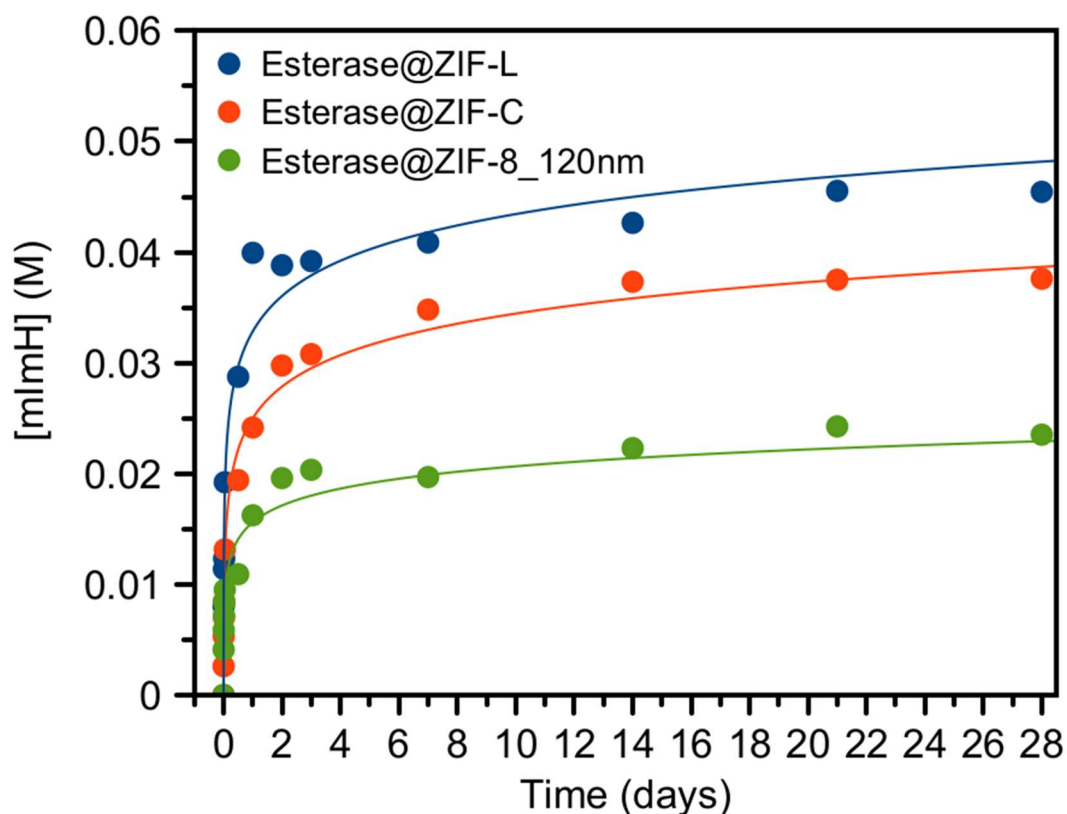


Figure S57. 2-methylimidazole release profile for Esterase@ZIF-8_120nm (green), Esterase@ZIF-L (blue) and Esterase@ZIF-C (red) along the reaction of biocomposites with diisopropylfluorophosphate (DIFP) over several days. Experimental points were collected by ^1H -NMR follow-up. Experimental conditions: DIFP (0.029 M), Esterase@ZIF (0.084 mmol of ZIF), dimethylacetamide (0.029 M, internal reference) and Tris-DCI (0.1 M, pH = 7.8, 0.5 mL) at room temperature.

Table S18. Concentration of 2-methylimidazole (mlmH) release by Esterase@ZIF biocomposites along different times.

Time (days)	Concentration of mlmH release (mM)		
	Esterase@ZIF-8_120nm	Esterase@ZIF-L	Esterase@ZIF-C
0.0035	4.1	8.0	2.6
0.0070	5.9	8.3	5.3
0.014	7.2	11.4	7.0
0.021	8.4	12.3	8.5
0.042	9.5	19.2	13.2
0.5	10.9	28.7	19.4
1	16.2	39.9	24.2
2	19.6	38.8	29.8
3	20.3	39.2	30.8
7	19.7	40.9	34.8
14	22.3	42.7	37.3
21	24.3	45.5	37.5
28	23.5	45.5	37.6

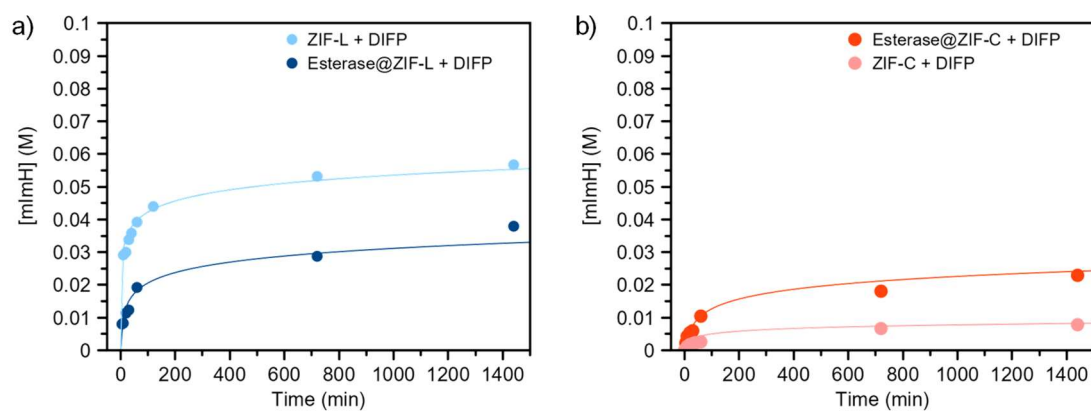


Figure S58. Comparison of 2-methylimidazole release profile for (a) Esterase@ZIF-L/ZIF-L and (b) Esterase@ZIF-C/ZIF-C materials during DIFP hydrolysis. Experimental points were collected by ^1H -NMR follow-up at different times. Experimental conditions: DIFP (0.029 M), 0.084 mmol of ZIF, dimethylacetamide (0.029 M, internal reference) and Tris-DCI (0.1 M, pD = 7.8, 0.5 mL) at room temperature.

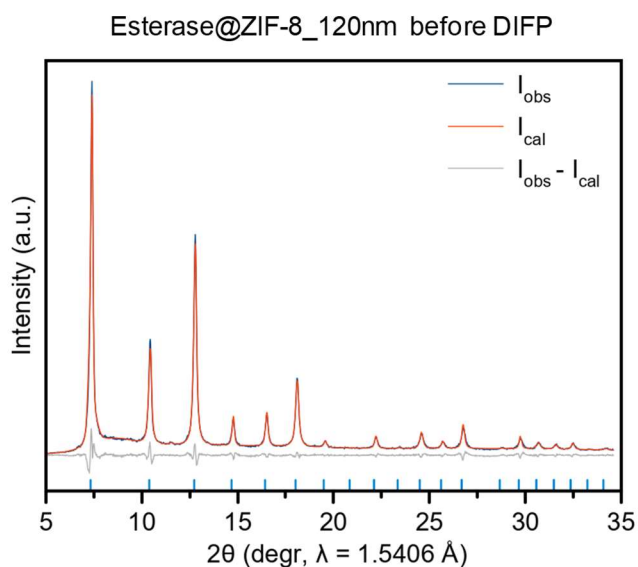


Figure S59. Graphical result of the whole powder pattern refinement carried out with the Le Bail method ($R_p = 0.05738$; $R_{wp} = 0.07395$) on the PXRD pattern of Esterase@ZIF-8_120nm before DIFP treatment in terms of observed, calculated and difference traces (blue, red and grey, respectively). The positions of the Bragg reflection are indicated by blue ticks. Cell parameters are summarized in Table S19.

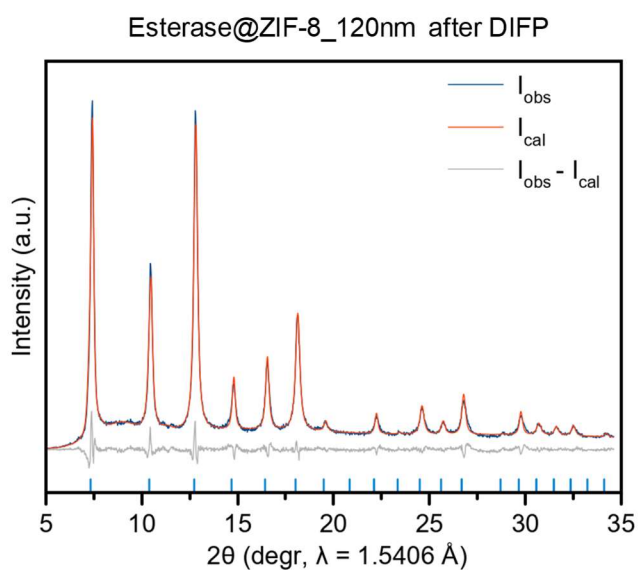


Figure S60. Graphical result of the whole powder pattern refinement carried out with the Le Bail method ($R_p = 0.05633$; $R_{wp} = 0.07152$) on the PXRD pattern of Esterase@ZIF-8_120nm after DIFP treatment in terms of observed, calculated and difference traces (blue, red and grey, respectively). The positions of the Bragg reflection are indicated by blue ticks. Cell parameters are summarized in Table S19.

Table S19. Cell parameters for Esterase@ZIF-8_120nm before and after DIFP treatment determined by PXRD Le Bail analysis. Crystalline domain size was estimated using a Lorentzian-convolution of Bragg peaks performed with the software Topas v3.

	Cell Parameter (Å)	Error (Å)	CS_L (nm)	Error_L (nm)
Esterase@ZIF-8_120nm Before DIFP	17.034	0.001	82	1
Esterase@ZIF-8_120nm After DIFP	17.031	0.002	54	1

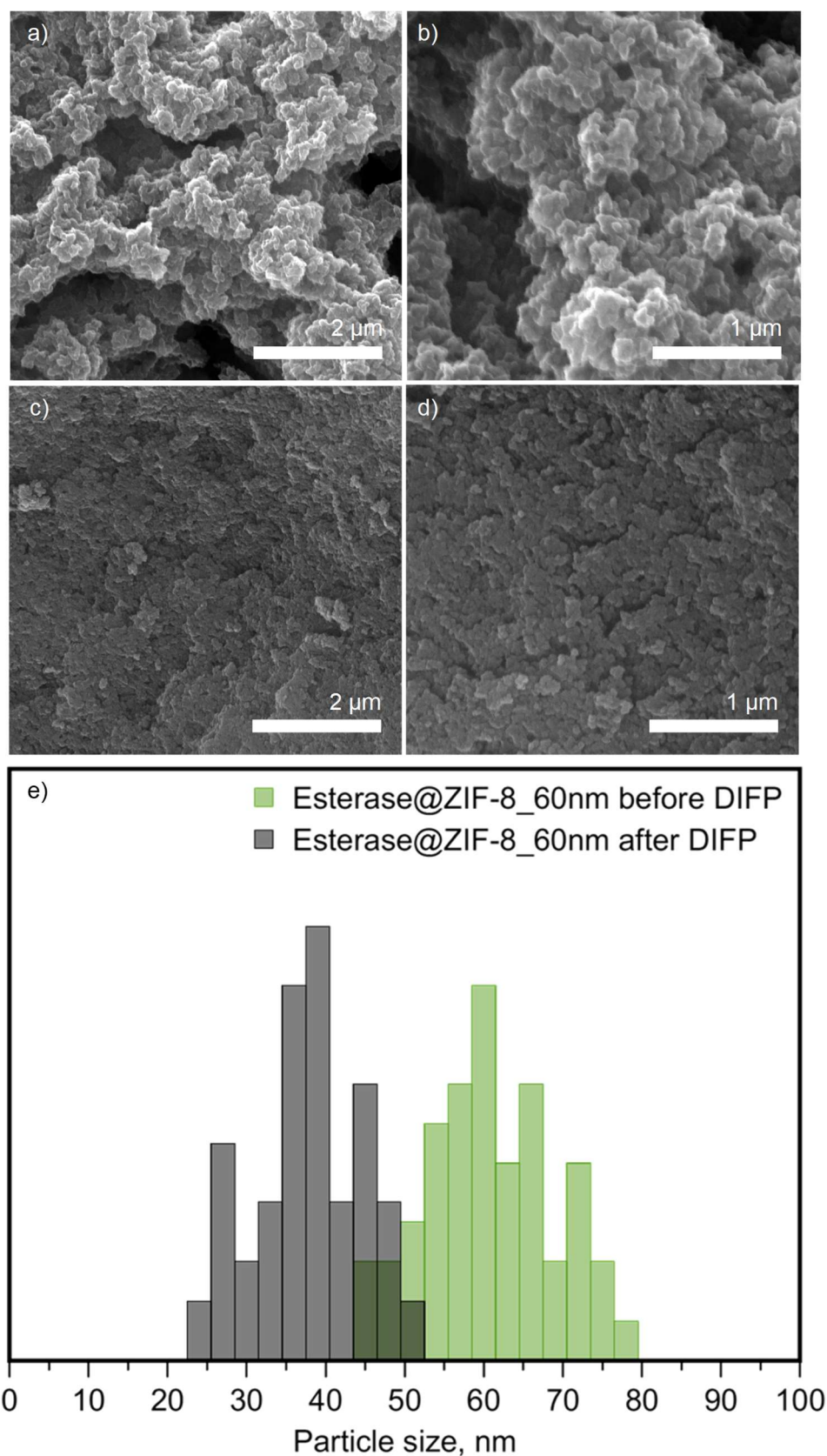


Figure S61. Scanning Electron Microscopy (SEM) images of Esterase@ZIF-8_60nm (a, b) before and (c, d) after DIFP reaction at different magnifications. (e) Particle size distribution of Esterase@ZIF-8_60nm before (green bars) and after (black bars) DIFP incubation. Experimental conditions: DIFP (0.029 M), Esterase@ZIF-8_60nm (0.084 mmol of sod ZIF-8) and Tris-HCl (0.1 M, pH = 7.4, 0.5 mL) at room temperature.

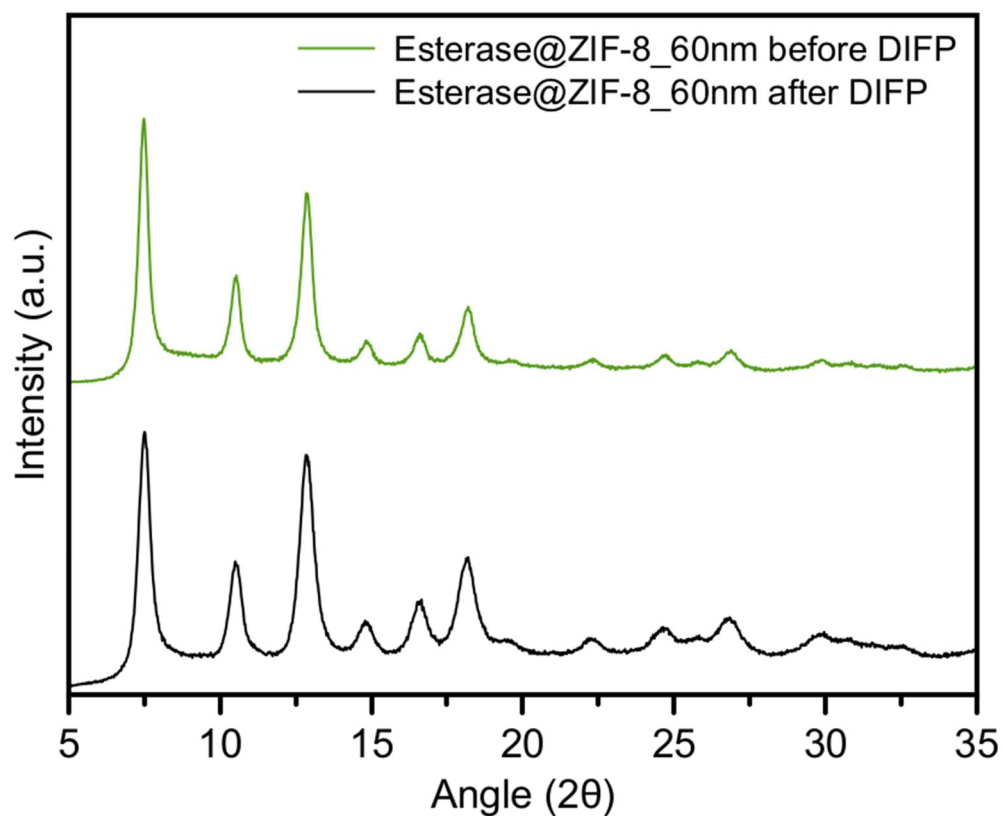


Figure S62. Powder X-Ray Diffraction patterns of Esterase@ZIF-8_60nm biocomposite before and after DIFP reaction. The comparison between each material before and after the activation protocol indicates that there is a slight widening of peaks due to particle size diminution. Experimental conditions: DIFP (0.029 M), Esterase@ZIF-8_60nm (0.084 mmol of ZIF) and Tris-HCl (0.1 M, pH = 7.4, 0.5 mL) at room temperature.

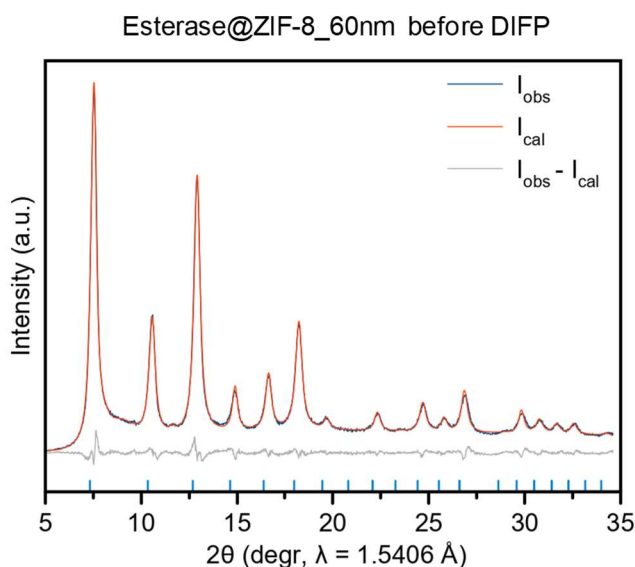


Figure S63. Graphical result of the whole powder pattern refinement carried out with the Le Bail method ($R_p=0.03647$; $R_{wp}=0.04754$) on the PXRD pattern of Esterase@ZIF-8_60nm before DIFP treatment in terms of observed, calculated and difference traces (blue, red and grey, respectively). The positions of the Bragg reflection are indicated by blue ticks. Cell parameters are summarized in Table S20.

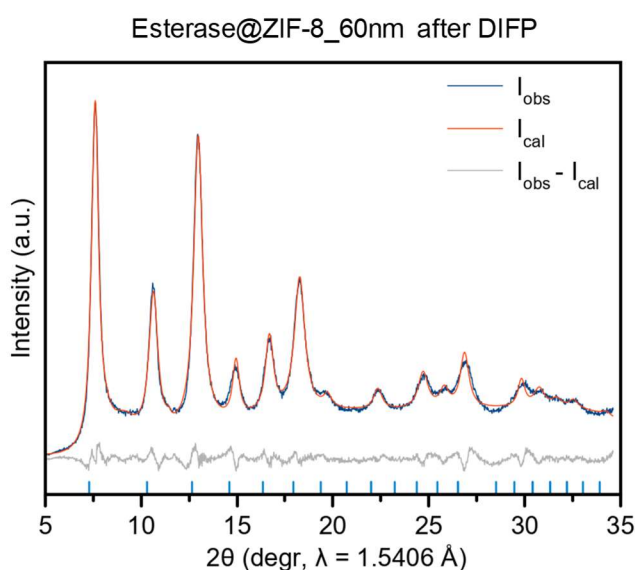


Figure S64. Graphical result of the whole powder pattern refinement carried out with the Le Bail method ($R_p=0.05633$; $R_{wp}=0.07152$) on the PXRD pattern of Esterase@ZIF-8_60nm after DIFP treatment in terms of observed, calculated and difference traces (blue, red and grey, respectively). The positions of the Bragg reflection are indicated by blue ticks. Cell parameters are summarized in Table S20.

Table S20. Cell parameters for Esterase@ZIF-8_60nm before and after DIFP treatment determined by PXRD Le Bail analysis. Crystalline domain size was estimated using a Lorentzian-convolution of Bragg peaks performed with the software Topas v3.

	Cell Parameter (Å)	Error (Å)	CS_L (nm)	Error_L (nm)
Esterase@ZIF-8_60nm Before DIFP	17.077	0.002	27.4	0.3
Esterase@ZIF-8_60nm After DIFP	17.129	0.004	18.2	0.3

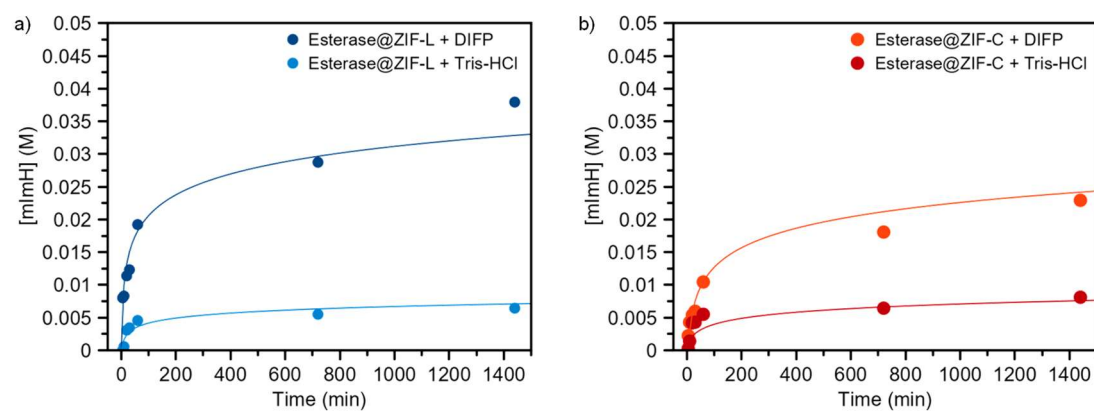


Figure S65. Comparison of 2-methylimidazole release profile for (a) Esterase@ZIF-L and (b) Esterase@ZIF-C biocomposites in the presence and absence of DIFP. Experimental points were collected by ^1H -NMR follow-up at different times. Experimental conditions: DIFP (0.029 M), 0.084 mmol of ZIF, dimethylacetamide (0.029 M, internal reference) and Tris-DCI (0.1 M, pD = 7.8, 0.5 mL) at room temperature.

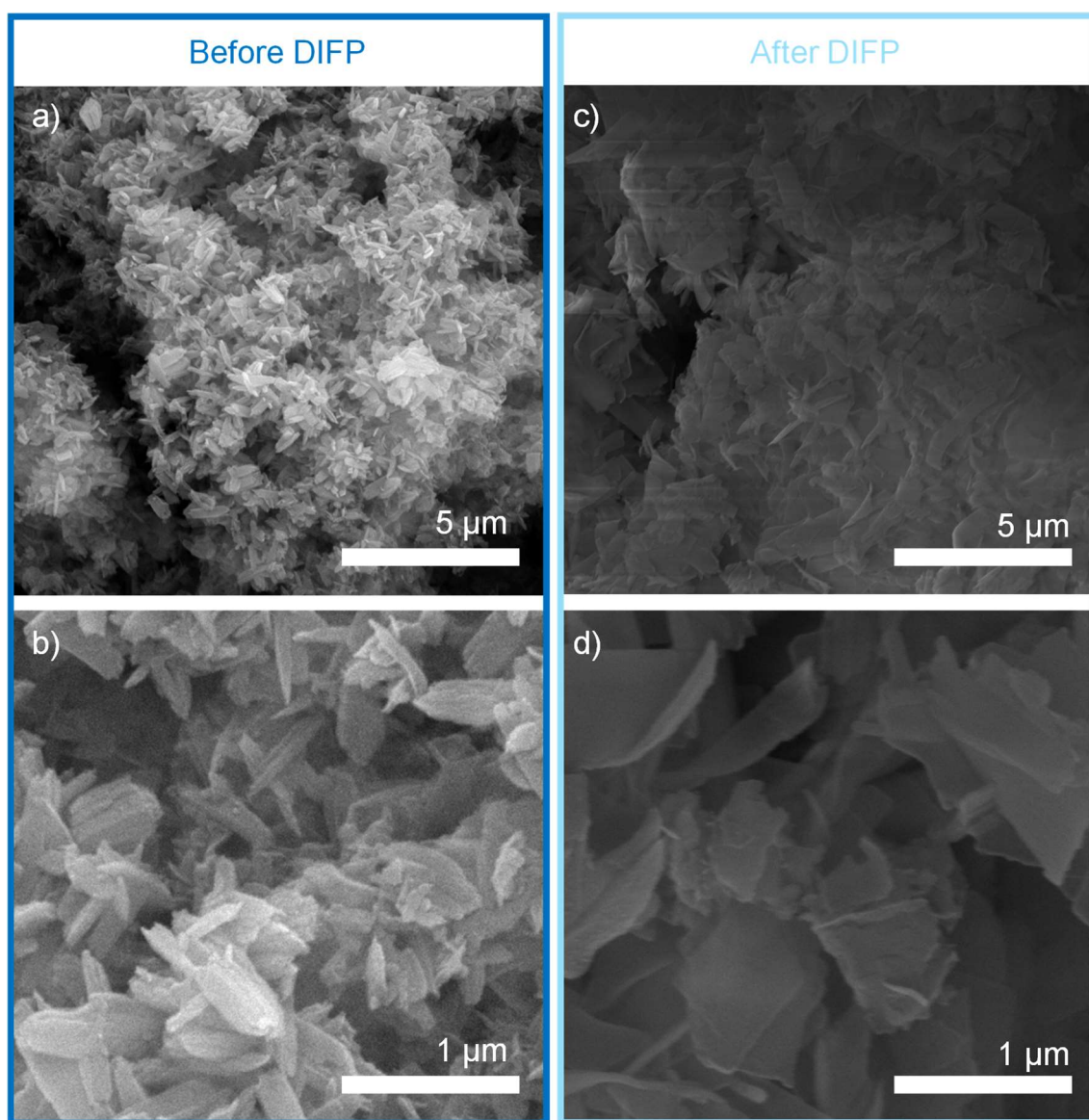


Figure S66. Scanning Electron Microscopy (SEM) images of Esterase@ZIF-L (a, b) before and (c, d) after DIFP reaction at different magnifications. Experimental conditions: DIFP (0.029 M), Esterase@ZIF-L (0.084 mmol of ZIF-L) and Tris-HCl (0.1 M, pH = 7.4, 0.5 mL) at room temperature.

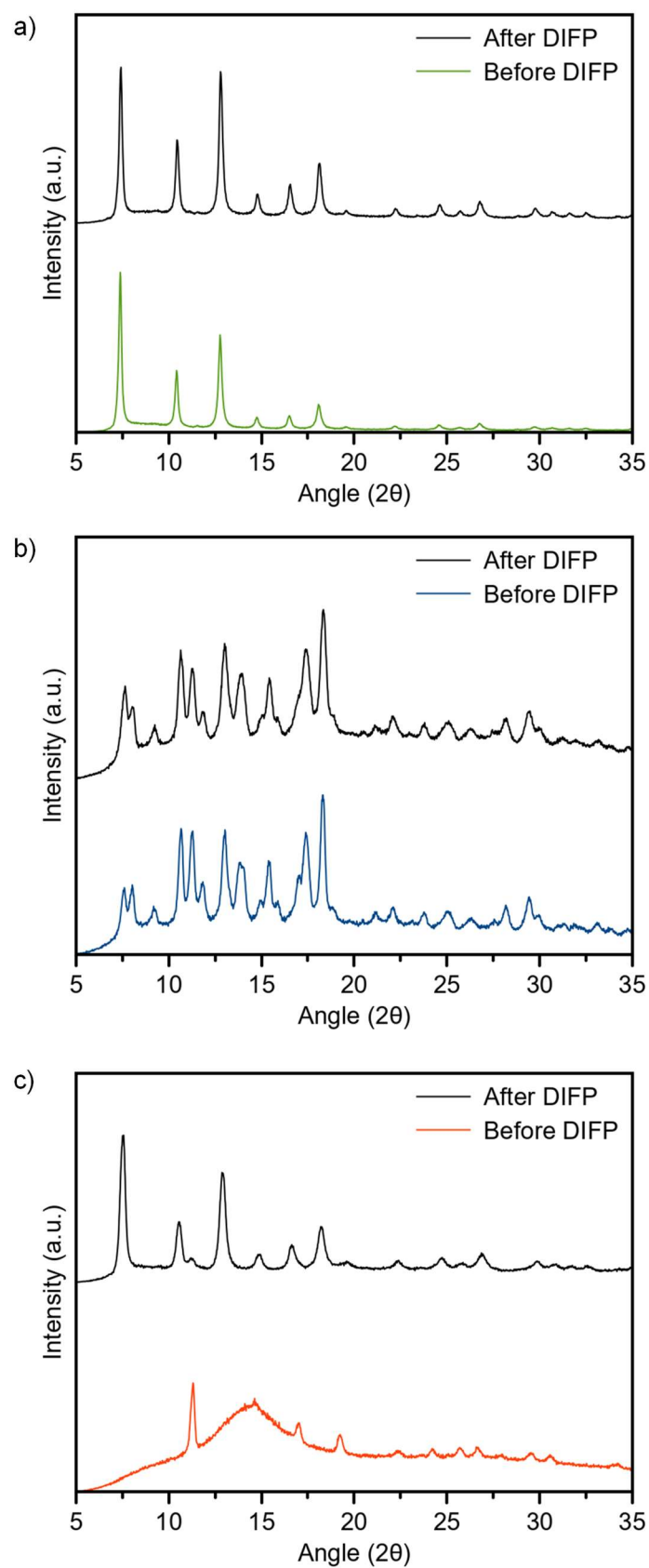


Figure S67. Powder X-Ray Diffraction patterns of (a) Esterase@ZIF-8_120nm, (b) Esterase@ZIF-L and (c) Esterase@ZIF-C biocomposites before and after DIFP reaction. Experimental conditions: DIFP (0.029 M), Esterase@ZIF (0.084 mmol of ZIF) and Tris-HCl (0.1 M, pH = 7.4, 0.5 mL) at room temperature.

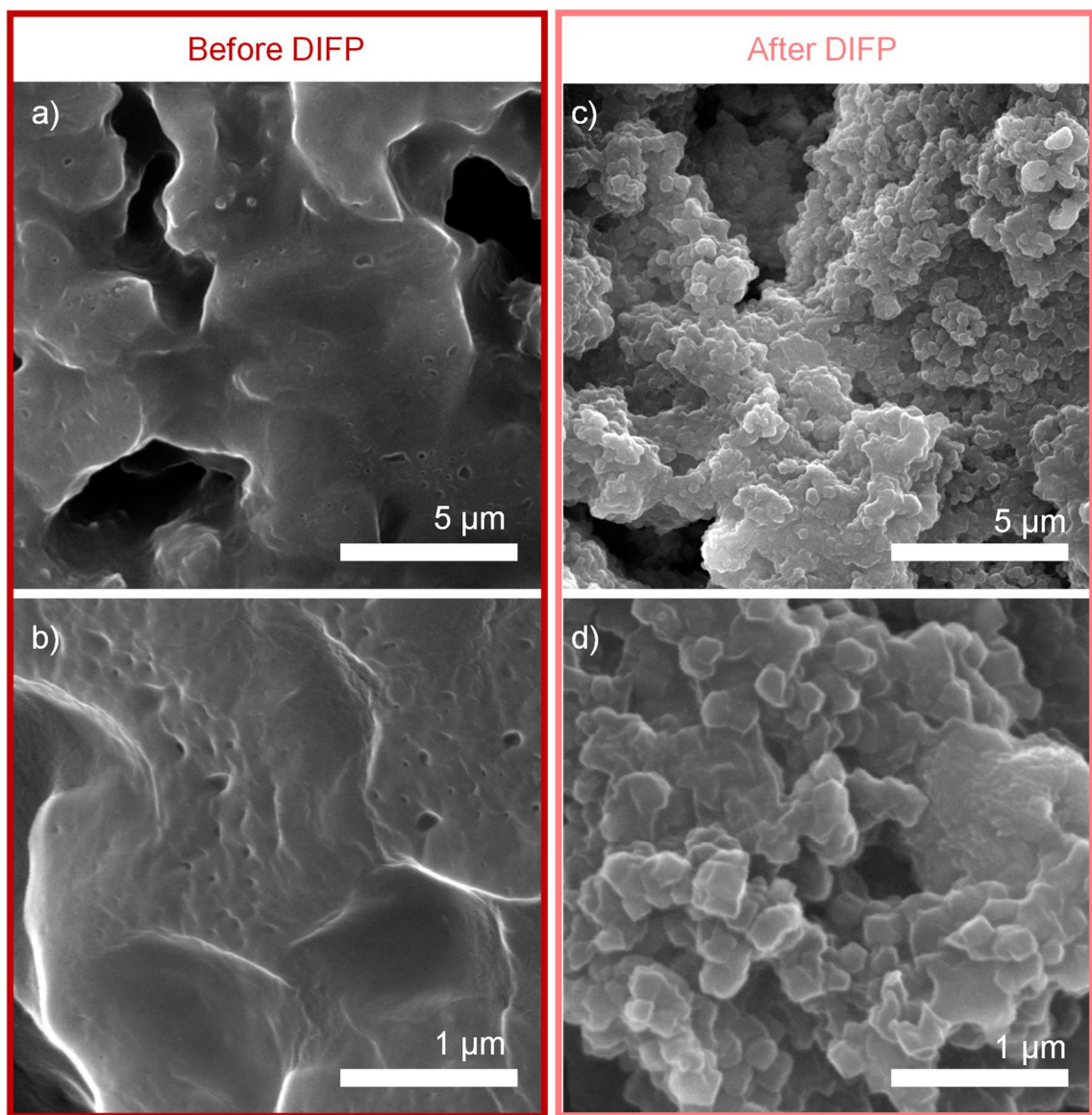


Figure S68. Scanning Electron Microscopy (SEM) images of Esterase@ZIF-C (a, b) before and (c, d) after DIFP reaction at different magnifications. Experimental conditions: DIFP (0.029 M), Esterase@ZIF-C (0.084 mmol of ZIF-C) and Tris-HCl (0.1 M, pH = 7.4, 0.5 mL) at room temperature.

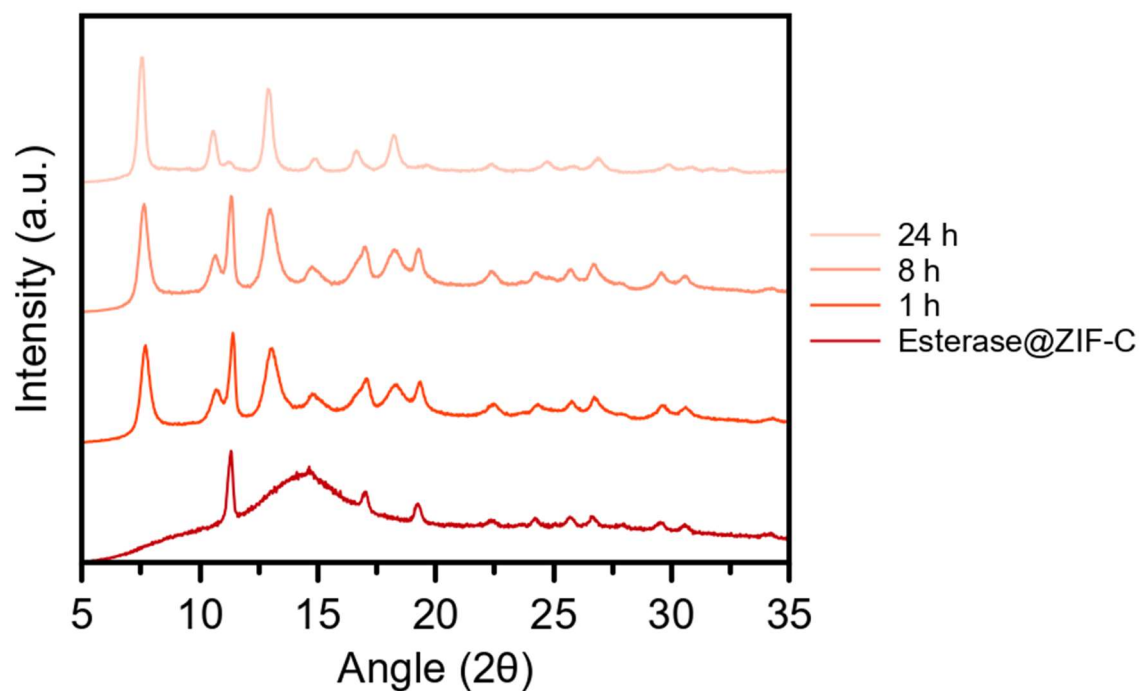


Figure S69. Powder X-Ray Diffraction patterns of Esterase@ZIF-C biocomposite along DIFP reaction at different times. We can observe a crystal phase transition from ZIF- CO_3 -1 to sodalite. Experimental conditions: DIFP (0.029 M), Esterase@ZIF-C (0.084 mmol of ZIF-C) and Tris-HCl (0.1 M, pH = 7.4, 0.5 mL) at room temperature.

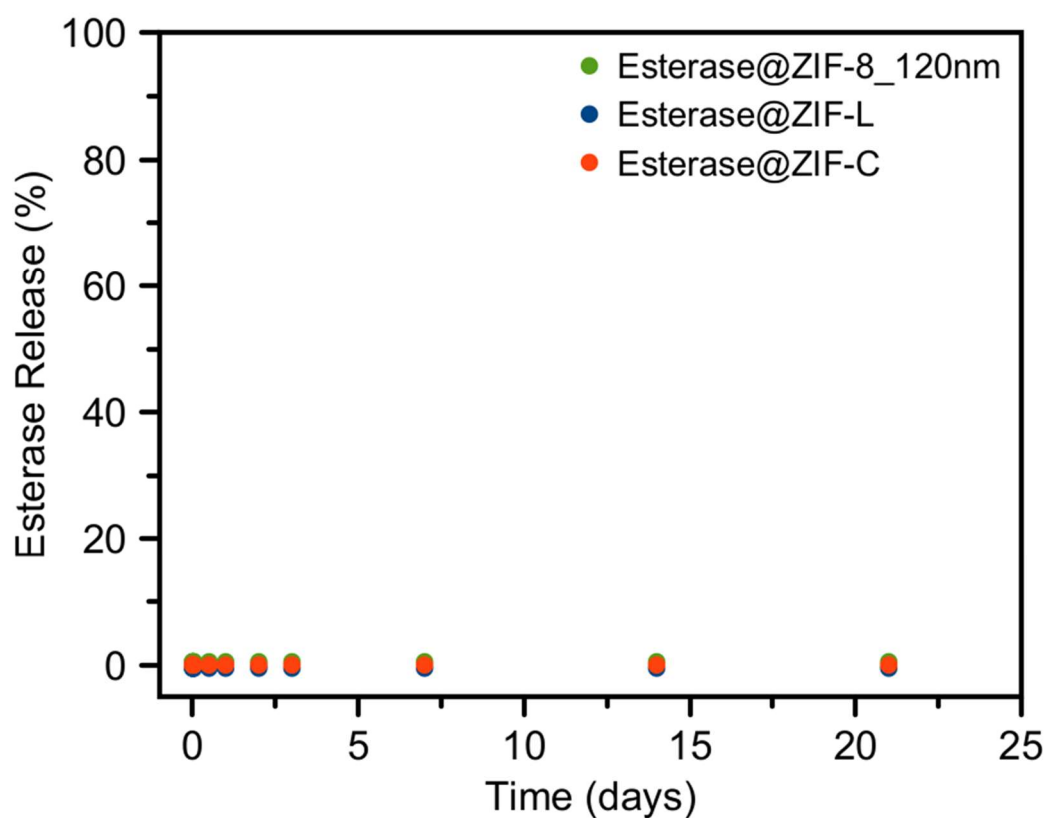


Figure S70. Esterase release profile for Esterase@ZIF-8_120nm (green), Esterase@ZIF-L (blue) and Esterase@ZIF-C (red) along the reaction of biocomposites with diisopropylfluorophosphate (DIFP) over several days. Experimental points were collected by UV-vis follow-up using the Bradford assay (see experimental protocol in S2.2.). Experimental conditions: DIFP (0.029 M), Esterase@ZIF (0.084 mmol of ZIF) and Tris-HCl (0.1 M, pH = 7.4, 0.5 mL) at room temperature.

S3.4. Detoxification ability of Esterase@ZIF biocomposites

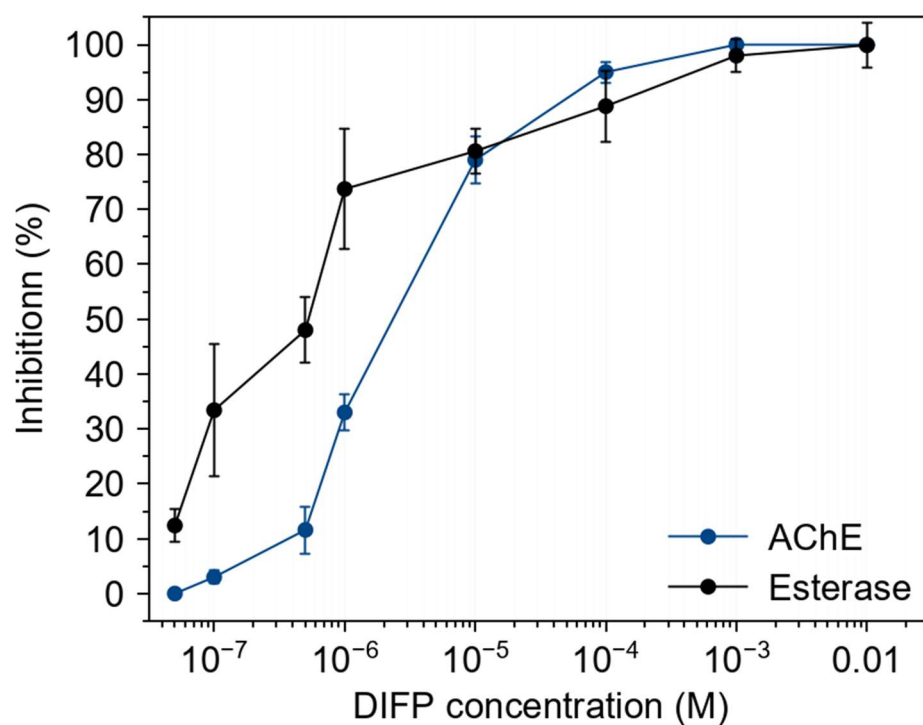


Figure S71. Esterase (black) and AChE (blue) inhibition percentage of their enzymatic activity by diisopropylfluorophosphate (DIFP) at different concentrations under simulated biological conditions (Tris-HCl buffer solution, 0.1 M, pH = 7.4). See S2.4.2. for further experimental details.

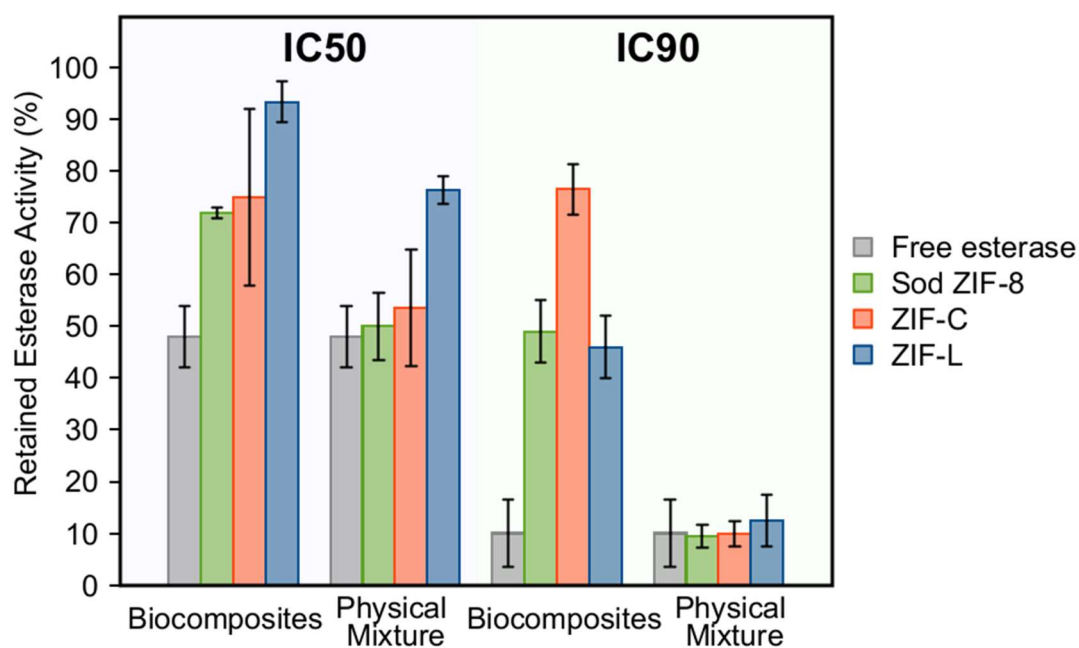


Figure S72. Retained enzymatic Esterase activity of free Esterase, Esterase@ZIFs and ZIFs+Esterase physical mixture after incubation with diisopropylfluorophosphate at Inhibitory Concentrations (IC) IC50 and IC90. The enzymatic activity of the three biocomposites was compared to each biocomposite enzymatic activity at 37 °C. See S2.4.6. for further experimental details.

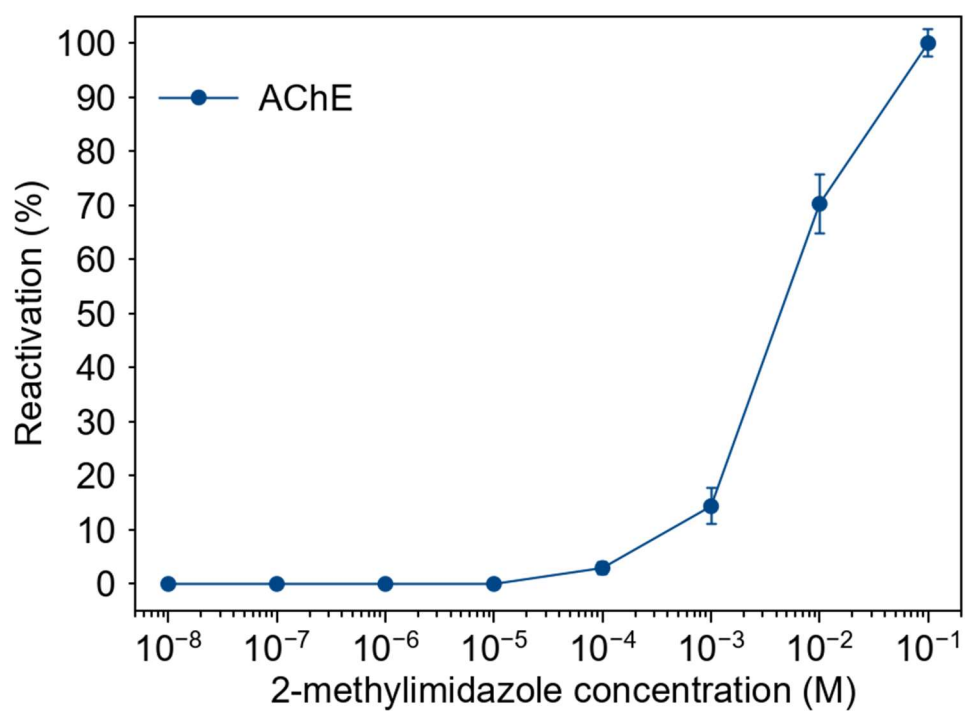


Figure S73. AChE (blue) reactivation percentage of its enzymatic activity by mlmH linker at different concentrations under simulated biological conditions (Tris-HCl buffer solution, 0.1 M, pH = 7.4). See S2.4.4. for further experimental details.

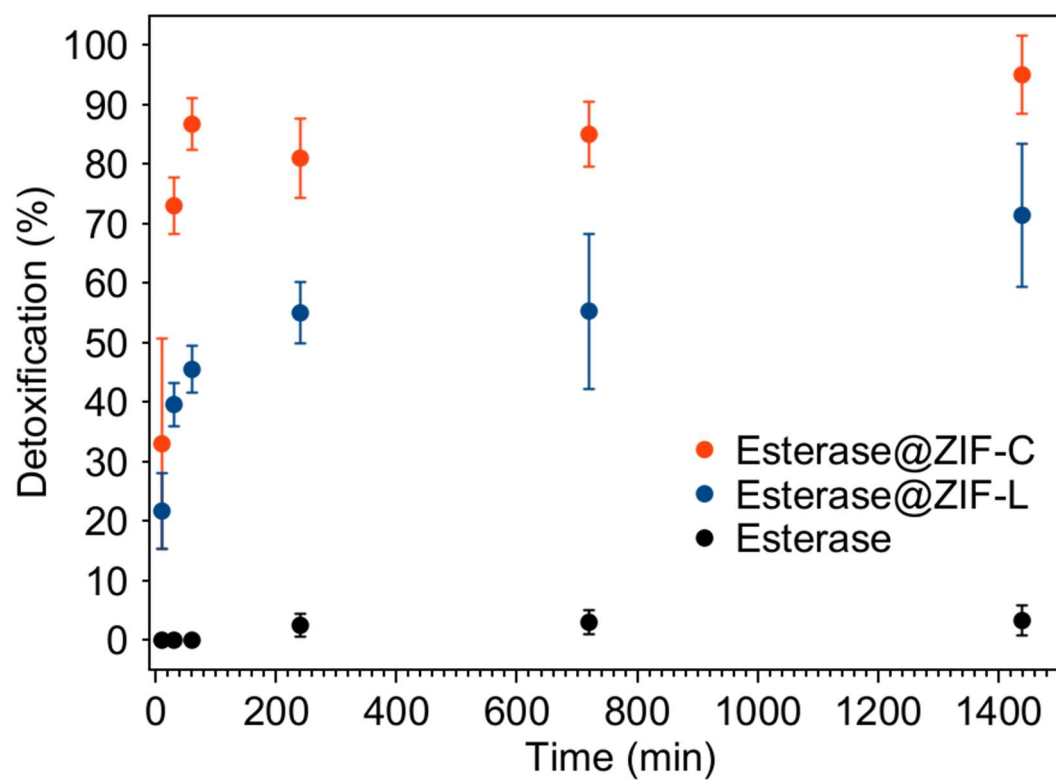


Figure S74. Detoxification profiles for Esterase@ZIF-C (red), Esterase@ZIF-L (blue) and Esterase (black) under simulated biological conditions (Tris-HCl, 0.1 M, pH = 7.4). See S2.4.7. for further experimental details.

S4. References

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