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Review

Boosting Enzyme Activity in Enzyme Metal−Organic Framework Composites

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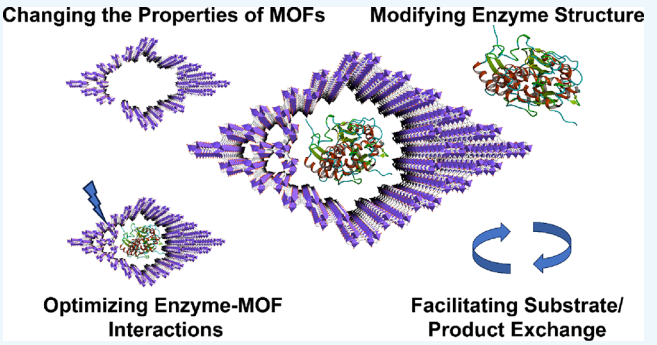
ABSTRACT: Enzymes, as highly efficient biocatalysts, excel in catalyzing diverse reactions with exceptional activity and selective properties under mild conditions. Nonetheless, their broad applications are hindered by their inherent fragility, including low thermal stability, limited pH tolerance, and sensitivity to organic solvents and denaturants. Encapsulating enzymes within metal−organic frameworks (MOFs) can protect them from denaturation in these harsh environments. However, this often leads to a compromised enzyme activity. In recent years, extensive research efforts have been dedicated to enhancing enzymatic activity within MOFs, leading to the development of new enzyme−MOF composites that not only preserve their catalytic potential but also outperform their free counterparts. This Review provides a comprehensive review on recent developments in enzyme−MOF composites with a specific emphasis on their enhanced enzymatic activity compared to free enzymes.



KEYWORDS: *metal−organic framework, enzyme, enzyme−MOF composites, encapsulation, activity enhancement*

1. INTRODUCTION

Enzymes, proteins that function as biocatalysts, have evolved distinctive 3D structures over millions of years. They possess the ability to catalyze a wide range of chemical reactions with high efficiency and selectivity, making them widely used in various industries.[1] However, enzymes are extremely delicate, susceptible to structural disruption due to variations in temperature and chemical environment, resulting in reduced enzymatic activity and selectivity.[2] This limitation hinders their extensive industrial applications.

The global demand for stable and sustainable enzymes continues to grow, leading to a discernible shift toward designing and producing materials that can effectively encapsulate enzymes for commercial applications.[3] Immobilizing enzymes onto solid supports emerges as a solution, not only enhancing their stability but also enabling reusability for enzyme catalyst development.[4,5] However, the efficacy of enzymes is profoundly influenced by these supports, given the enzymes' sensitivity to their microenvironment and interactions with the support materials.[6]

A variety of materials such as silica, graphene oxide, and polymers have been explored as potential supports for enzyme immobilization, targeting large-scale industrial applications.[7] Recently, metal−organic framework (MOF)-based materials have gained significant attention due to their versatile combination of metal nodes and organic ligands, making them ideal for various applications.[8] One kind of MOF is zeolitic imidazolate frameworks (ZIFs), which exhibit structural similarities to traditional aluminosilicate zeolites. Notably, zeolitic imidazolate framework 8 (ZIF-8), comprising zinc ions and imidazolate ligands, has found various applications, owing to its excellent structural and textural properties.[9]

MOFs can serve as effective encapsulation materials, shielding enzymes from adverse conditions including high temperatures, extreme pHs, and organic solvents. Enzyme−MOF composites can be prepared via two closely related synthetic approaches: 1) the co-precipitation method, which normally employs additional capping agents such as biocompatible polymers to promote composite formation, and 2) the biomimetic mineralization method, which does not require any additives, and the MOF can grow on the surface of enzymes in aqueous solutions.[2,10] Recently, the Doonan group and the Liang group showcased representative research works of encapsulating enzymes in MOFs through the biomimetic mineralization method, which showed much higher enzyme

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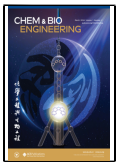

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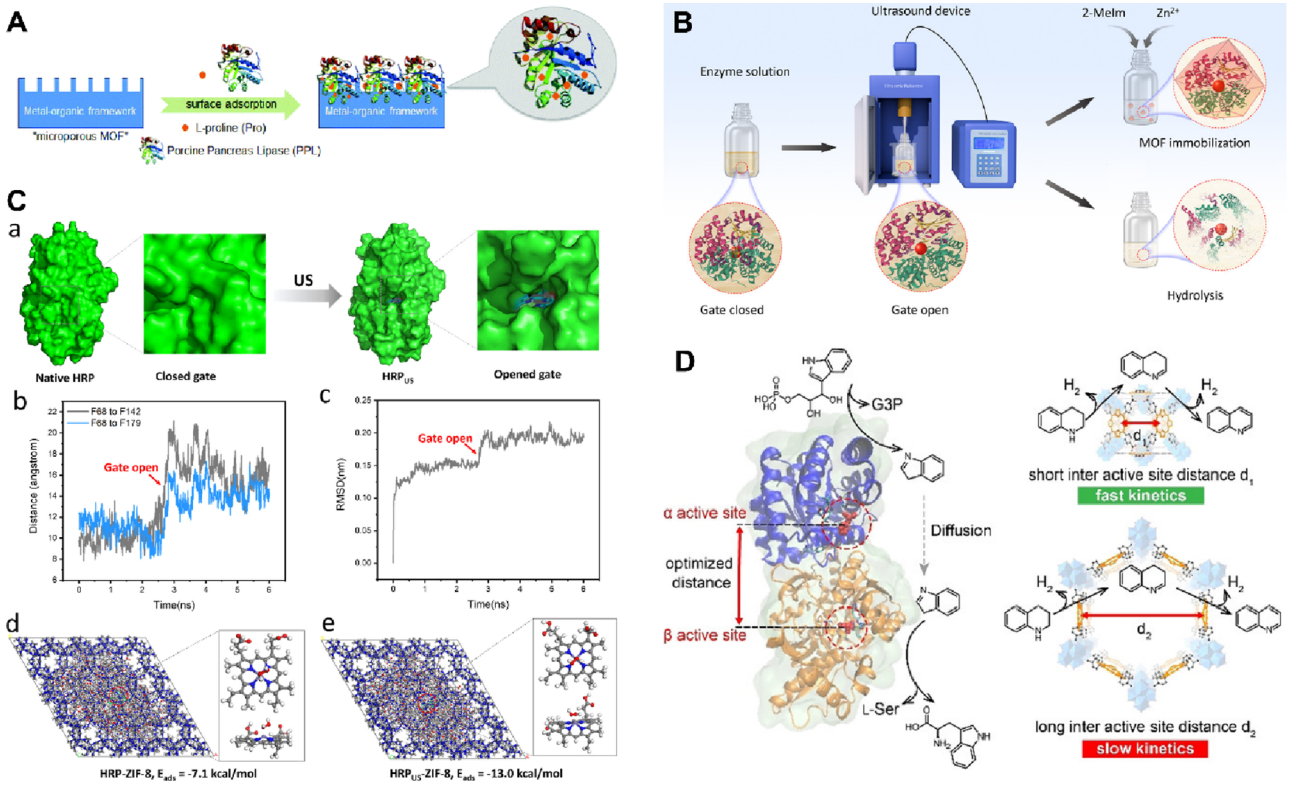

Figure 1. (A) Schematic representation of the immobilization of PPL and Pro on a microporous MOF. Adapted from ref 23 with permission. Copyright 2021 Royal Society of Chemistry. (B) Schematic depicting the "locking" of the ultrasound-induced active conformation of metalloenzymes in MOFs. (C) MD simulations of the conformational change of HRP after ultrasound treatment: (a) surface representations of HRP before and after ultrasound treatment (dark-blue, N; light-blue, C; red, Fe); (b) distance of F68 to F142 and to F179 in the HRP structure during ultrasound pretreatment; (c) RMSD of HRP structure during ultrasound pretreatment; (d) HRP-ZIF-8 and (e) HRPUS-ZIF-8 after adsorption of H_2O_2, and their top- and side-view structures of the heme after adsorption of H_2O_2. (B) and (C) reprinted from ref 24 with permission. Copyright 2022 American Chemical Society. (D) Illustration of enzyme-inspired discussion between interactive site distance in porphyrin MOFs and their catalytic efficiency of THQ dehydrogenation driven by visible light. Left: enzyme distance oriented to two subsequent steps in tryptophan synthesis. The drawing is based on the crystal structure of tryptophan synthase (Protein Data Bank: 1A5S). Right: THQ dehydrogenation is catalyzed by porphyrin MOF. The distances between porphyrin linkers are determined by various MOFs structures. Adapted from ref 25 with permission. Copyright 2020 Wiley-VCH Verlag GmbH & Co..

stability after encapsulation, compared to the co-precipitation method.[10−13] Moreover, MOFs offer readily tunable chemical and structural features such as size, shape, pore size, surface area, and structural flexibility.[14] For example, some pioneer works reported by the Yang group generalized the synthesis of size-tunable enzyme−MOF composites[15,16] and offered insights into the orientation of enzymes encapsulated in MOFs,[17,18] which are important for the design of MOF-based enzyme immobilization systems. Nevertheless, when enzymes are encapsulated within MOFs, a common challenge arises: reduced enzymatic activity, mainly due to the constrained enzyme conformation and limited substrate accessibility. Over the past few years, several strategies have emerged to enhance the activity of enzymes encapsulated in MOFs, which can be categorized into four main approaches: 1) modifying enzyme structure, 2) tuning MOFs structure, 3) optimizing enzyme−MOF interactions, and 4) facilitating substrate and product exchange.

This Review offers an overview of recent advancements in enzyme−MOF composites, providing a thorough summary and critical analysis of recent strategies employed to enhance the enzymatic activity of these composites. It also introduces pertinent industrial applications of enzyme−MOFs composites and highlights the emerging trends of MOF-based biocatalysts.

2. RECENT ADVANCES IN ENHANCING ENZYMATIC ACTIVITY IN ENZYME−MOF COMPOSITES

MOFs with encapsulated enzymes, known as enzyme−MOF composites, present new opportunities to improve the inherent fragility of enzymes. However, MOF encapsulation often leads to compromised enzyme activity due to the decreased flexibility of enzyme structures.[14,19,20] Consequently, enhancing enzyme catalytic performance in enzyme−MOF composites remains a critical challenge. This work provides a comprehensive summary of recent research endeavors dedicated to improving the performance of enzyme−MOF composites. It explores four major strategies, including the control and modification of enzyme molecular structures, MOF structures, enzyme−MOF interactions, and substrate and product exchange.

2.1. Modifying Enzyme Structure. Recently, controlling and modifying enzyme structures have emerged as a popular approach for enhancing enzyme activity, owing to the abundance of functional groups and intramolecular hydrogen bonds within enzyme molecules.

2.1.1. Engineering Enzyme Active Sites. The active site of an enzyme is the region that binds to substrate molecules, playing a crucial role in enzyme catalytic activity.[21] In recent years, engineering enzyme active sites has surfaced as a

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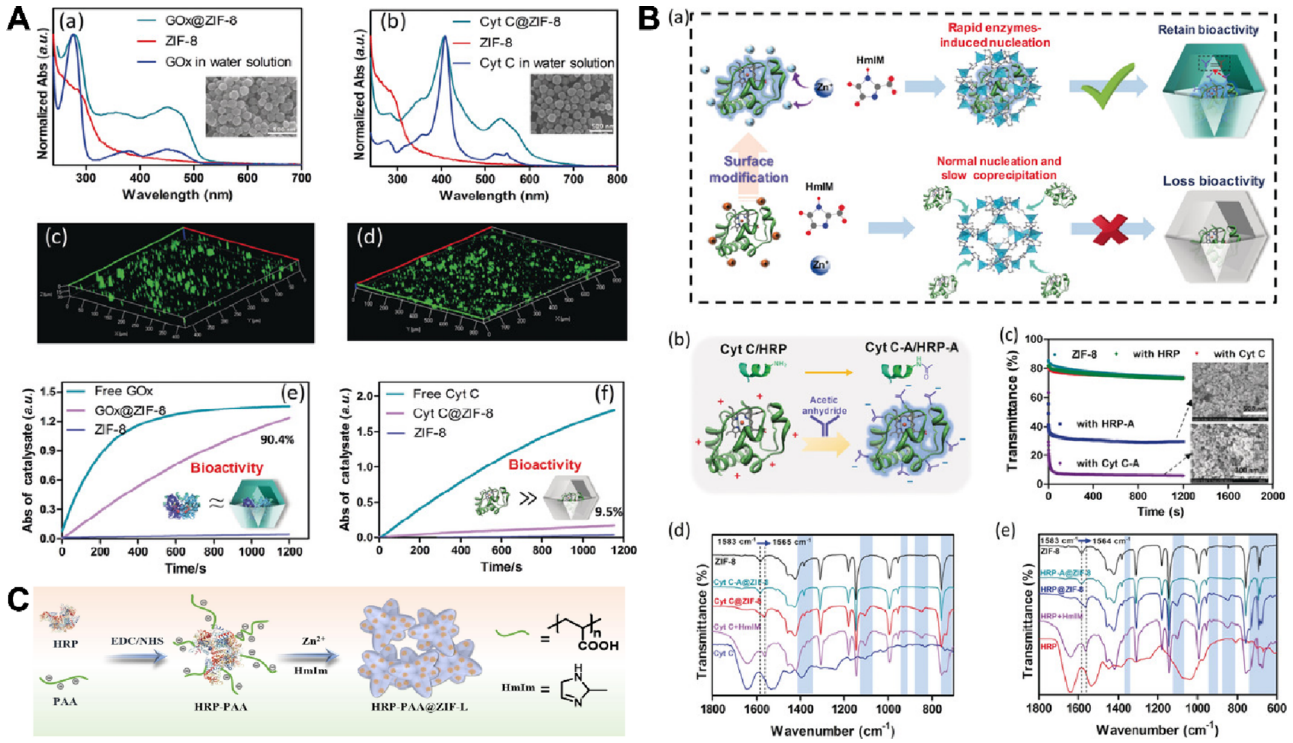

Figure 2. (A) Characterization of enzymes@ZIF-8 and its bioactivity conversion. Solid UV−vis absorption spectra of GOx@ZIF-8 (a) and CytC@ZIF-8 (b). 3D-CLSM profile of the distribution of GOx (c) and CytC (d) in ZIF-8. The conversion of the enzymatic activity of GOx@ZIF-8 (e) and CytC@ZIF-8 (f). Conditions: 0.2 mM glucose for GOx@ZIF-8; 0.2 mm H_2O_2 for CytC@ZIF-8. (B) (a) Embedding patterns controllable by enzyme-surface modifications enabling modulation of the bioactivity of the embedded enzymes; (b) chemical modification of CytC and HRP altering the surface charge from positive to negative; (c) time-dependent transmittance of the synthetic process with different enzymes. SEM images of the CytC-A- and HRP-A-triggered ZIF-8 nucleation (the inset). ATR-FTIR analysis of CytC/Cyt C-A@ZIF-8 (d) and HRP/HRP-A@ZIF-8 (e). Adapted from ref 28 with permission. Copyright 2019 Wiley-VCH. (C) Schematic diagram of the synthesis of HRP-PAA@ZIF-L. Adapted from ref 29 with permission. Copyright 2022 Elsevier.

prominent approach for enhancing enzyme catalytic performances.

Opening enzyme active sites is an effective approach. Vaidya et al. managed to open the active sites of lipase in lipase−ZIF-8 composites using a surfactant (sodium dodecyl sulfate, SDS) modulation method, which enhanced lipase activity by 3-fold compared to its free counterparts.[22] The changed enzyme structural conformation after modulation was confirmed by using Fourier transform infrared spectroscopy (FTIR). In another study reported by Lirio et al., the active site of lipase was opened by the co-immobilization of L-proline in MOF−1,4-NDC (Al) (Figure 1A), leading to an improved enzymatic activity.[23] This can be attributed to the enhanced enantioselectivity provided by the chiral environment of the amino acid.

Ultrasound treatment can also open enzyme active sites and improve enzyme activity; however, enzymes upon ultrasound treatment often become unstable and more susceptible to losing activity due to their high-energy conformation.[24] Nevertheless, the combination of an ultrasound treatment and a MOF could address the issue of enzyme instability. Using ultrasound treatment, Liang et al.[24] successfully "locked" enzymes in their activated forms using a one-pot immobilization on ZIF-8 and improved the activity of various enzymes by up to 5.3-fold compared to their native counterparts (Figure 1B). The change in enzyme active site and structural conformation after ultrasound treatment was confirmed by solid-state UV−vis, electron paramagnetic resonance (EPR), circular dichroism (CD) spectroscopy, Fourier-transform infrared (FTIR) spectroscopy, and molecular dynamics (MD) simulations (Figure 1C). The distance between F68 to F142 and between F179 and F68 in the horseradish peroxidase (HRP) structure as well as the sharply elevated root-mean-square deviation (RMSD) at around 3 ns after simulated ultrasound treatment suggested the opening of the HRP active site (Figure 1C).

Apart from opening active sites, the distance between active sites has also proven to be critical for achieving a high catalytic efficiency. Gong et al. studied the correlation between the distances of inter active sites and the photocatalytic performance using a number of porphyrin MOFs, revealing that a smaller distance could lead to a higher catalytic activity (Figure 1D).[25] This study provides insights for the design of highly active muti-enzyme−MOF complexes.

2.1.2. Enzyme Biochemical Modification. Enzymes can be activated through various biochemical modifications using polymers and amino acids.[26] Nadar et al. enhanced lipase activity by its conjugation with amino acid (proline), followed by a co-immobilization in ZIF-8.[27] The lipase−proline-ZIF-8 composite exhibited 135% enhanced enzymatic activity compared to that of native lipase. This enhancement can be attributed to the ability of the amino acid to preserve the active conformation of the enzyme, as evidenced by the similarity in the secondary structure of lipase in both its free and immobilized forms when proline was present. Zou et al. used 2-methyl-1*H*-imidazole-4-carboxylic acid to chemically modify lipase, and the enzyme catalytic activity was 1.3-fold higher than that of free lipase.[19] The modified enzyme was further immobilized in ZIF-8, and the α-helix content was increased

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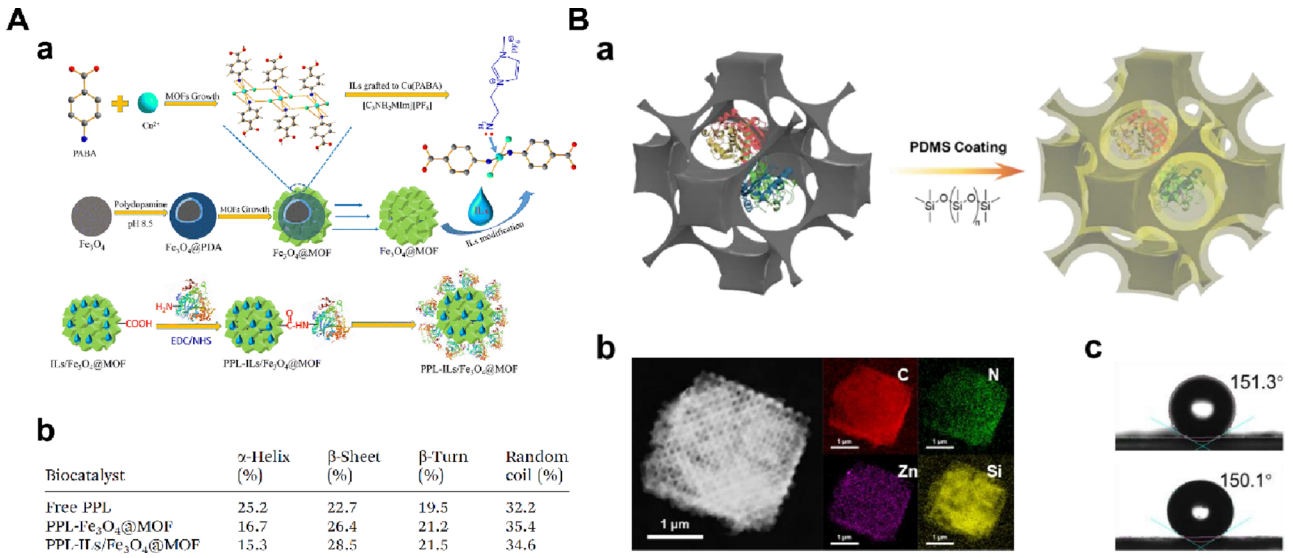

Figure 3. (A) (a) Synthesis route of ionic liquid modified Fe_3O_4@MOF and the covalent immobilization of lipase on ionic liquid modified Fe_3O_4@MOF; (b) percentage of the secondary structure of enzymes. Adapted from ref 30 with permission. Copyright 2023 Royal Society of Chemistry. (B) Preparation of 3DOM-C@TLL@PDMS and the catalytic performance of different biocatalysts in biodiesel production: (a) schematic illustration of preparation of 3DOM-C@TLL@PDMS; (b) HAADF-STEM image and corresponding EDS mapping of 3DOM-C@TLL@PDMS; (c) static water contact angle (upper, ~151.3°) and static glycerin contact angle (lower, ~150.1°) of 3DOM-C@TLL@PDMS. Adapted from ref 33 with permission. Copyright 2023 Elsevier.

from 14.3% to 18.9% according to circular dichroism (CD) spectroscopy results.

In another study, Chen et al. reported that enzymes tended to lose activity when encapsulated in ZIF-8 through coprecipitation, due to the unfolding effects and competing coordination caused by the 2-methylimidazole ligand (Figure 2A).[28] Typically, enzymes with an isoelectric point (pI) higher than the pH of the reaction solution, and thus a positive charge, exhibit a more pronounced loss of activity. This reduction in activity is often due to denaturation caused by 2-methylimidazole, which tends to displace Zn^{2+}, leading to slower encapsulation within the framework. However, Chen et al. successfully enhanced the biofunctionality of enzyme−MOF composites by chemically modifying the amino acids on the surfaces of enzymes using acetic anhydride (Figure 2B). This chemical modification increased the negative surface charge of enzymes, significantly accelerating the encapsulation process. These findings offer valuable insights for the development of robust enzyme−MOF composites with excellent activity, applicable to a wide range of industries, including food, pharmaceutical, biosensing, and biofuel sector.

Other than amino acids, short-chain polymers can also be used for enzyme modifications to improve enzymatic activity in enzyme−MOF composites. For example, Zhou et al. chemically grafted short-chain polyacrylic acid (PAA) onto HRP via EDC/NHS reaction to improve enzyme activity and encapsulated the modified enzyme using ZIF-L (Figure 2C).[29] The modified enzyme−MOF composite exhibited a higher activity than that of its unmodified form, possibly due to the enhanced electrostatic interactions between the enzyme and substrate and the reduced molecular diffusion resistance.

2.2. Changing the Properties of MOFs. Modulating the hydrophobicity of MOFs is a powerful technique for enhancing the activity of lipase−MOF composites, as the lipase active center becomes accessible when the hydrophobic lid opens.[30−32] Suo et al. encapsulated lipase in ionic-liquid [1-aminopropylimidazolium and bis(trifluoromethylsulfonyl)-imine]-modified Cu-MOF, and the immobilized enzyme exhibited 2.1-fold higher activity than free enzyme (Figure 3Aa).[30] The presence of ionic liquid grafted material led to multiple interactions with the immobilized enzyme, and the enzyme conformation changes were observed by using circular dichroism (CD) spectroscopy. However, enzyme encapsulated in both modified and unmodified MOFs showed a 9−10% reduced α-helix content compared to the free enzyme (Figure 3Ab). Despite the drop of α-helix content, lipase encapsulated in ionic-liquid-modified-MOF showed an 0.8% decrease in random coil content compared to that encapsulated in unmodified MOF (Figure 3Ab). In another study, Hu et al. studied the impact of MOF hydrophobicity on the activity of encapsulated lipase by modifying UiO-66 using polydimethylsiloxane (PDMS) coating.[31] The immobilized lipase showed remarkably enhanced activity after the hydrophobic modification of UiO-66. This improvement might be explained by the enhanced hydrophobic interactions between the MOF and enzyme, facilitating the opening of the lipase active center. However, the conformational change of enzyme active centers was not characterized, and further studies are required to understand the phenomenon. Similarly, Zhou et al. also employed a PDMS coating approach to increase the hydrophobicity of ZIF-8 to enhance the catalytic activity of the immobilized lipase (Figure 3B).[33] The reported postimmobilization hydrophobic modification method was effective, and a 1.8-fold higher initial reaction rate was achieved after applying a PDMS coating on enzyme−MOF composites. Nevertheless, the mechanism underpinning the elevated enzymatic activity should be further explored by using both experimental and computational methods.

Recent research has shown that zirconium-based MOFs with improved electroconductivity exhibited enhanced catalytic activity,[34] due to the redox hopping between periodically arranged chemically equivalent sites.[35] The ability to modulate the conductivity of MOFs opens avenues for creating highly efficient enzyme−MOF composites, making them ideal for

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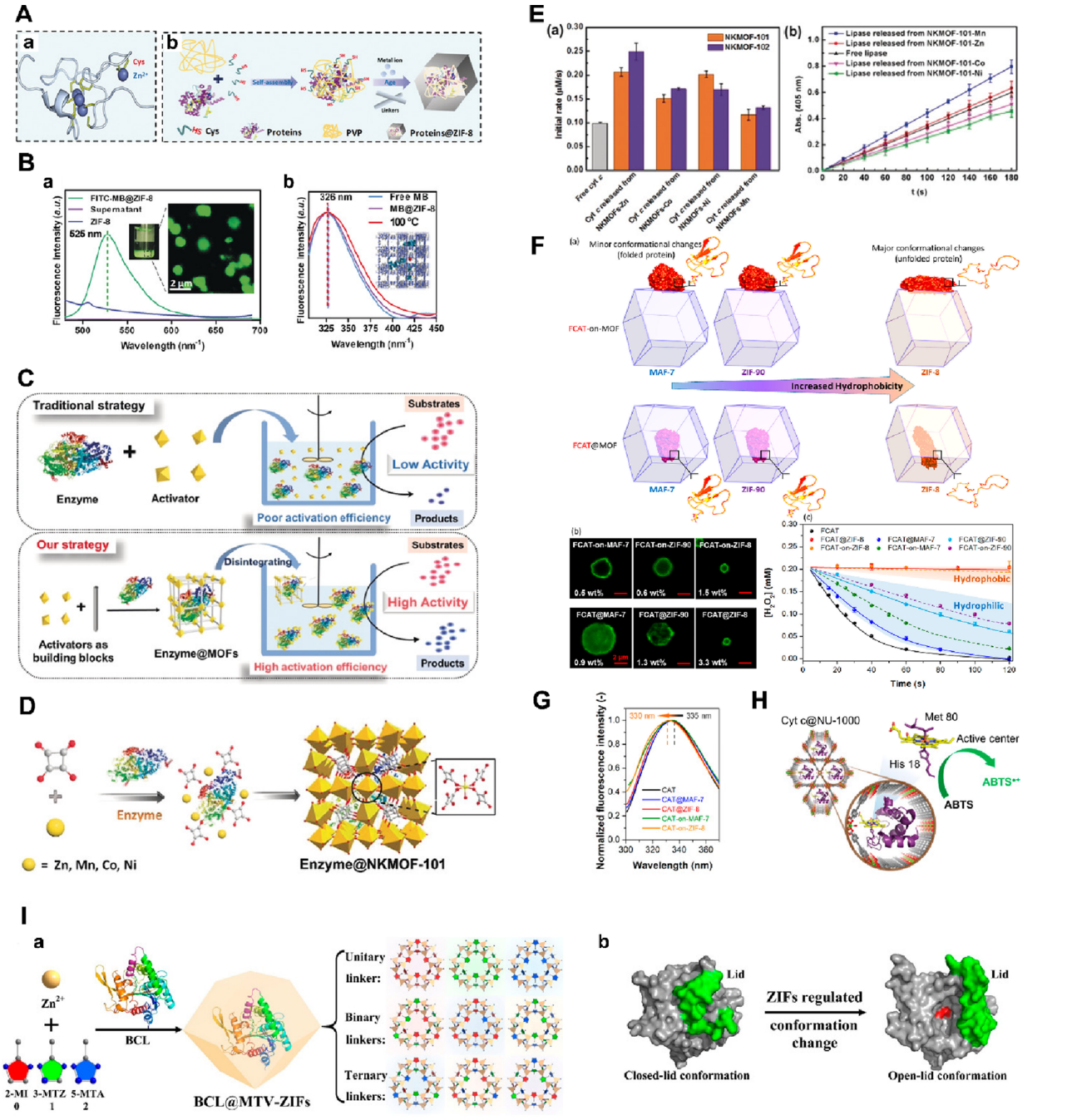

Figure 4. (A) (a) Illustration of the Zn^{2+} binding by metallothionein. (b) Schematic representation of the amino-acid-boosted one-pot embedding strategy. (B) (a) Fluorescence emission profile of ZIF-8 and FITC-MB@ZIF-8 (inset: confocal laser scanning microscopy image of FITC-MB@ZIF-8 (excitation wavelength: 488 nm)). (b) Fluorescence spectra of free MB and MB@ZIF-8 and that obtained after heating the MB@ZIF-8 to 100 °C (1 h). Adapted from ref 47 with permission. Copyright 2019 Wiley-VCH. (C) Illumination of MOFs as platforms to construct customized enzyme formulations with boosted activity. (D) Illustration of the enzyme encapsulation process by NKMOF-101. (E) (a) The initial rate of free Cyt c and Cyt c released from NKMOF-101-M and NKMOF-102-M; (b) catalytic curves of free lipase and lipase released from NKMOF-101-M. Test conditions: 1 mM p-NPA. Adapted from ref 41 with permission. Copyright 2020 Wiley-VCH. (F) (a) Effects of MOFs with varying degrees of hydrophobicity on CAT -on- or @MAF-7, ZIF-90, and ZIF-8; (b) confocal laser scanning microscopy (CLSM) of CAT -on-/@MAF-7, ZIF-90, and ZIF-8 showing differences in localization, particularly among the encapsulated samples; (c) activity data for CAT -on-/@MAF-7, ZIF-90, and ZIF-8 compared with the free enzyme. Reprinted from ref 2 with permission. Copyright 2021 American Chemical Society. (G) Fluorescence spectra of CAT and different FCAT/MOF composites. Measurements were carried out with a CAT concentration of 1 μM in 0.1 M Tris-HCl buffer (pH 8). Reprinted from ref 42 with permission. Copyright 2019 American Chemical Society. (H) Schematic illustration of Cyt c encapsulated in the mesopores of MOF NU-1000 and its oxidation of ABTS. The atom color code is as follows: yellow, C; red, S; orange, Fe; blue, N of the heme protein active site; purple, amino acid chain connected to the active center. Reprinted from ref 43 with permission. Copyright 2020 American Chemical Society. (I) (a) Synthesis of BCL@MTV-ZIF-8; (b) closed-lid conformation and open-lid conformation of BCL regulated by MTV-ZIFs. The α-helix lid and catalytic site of BCL are colored green and red, respectively. Reprinted from ref 44 with permission. Copyright 2021 American Chemical Society.

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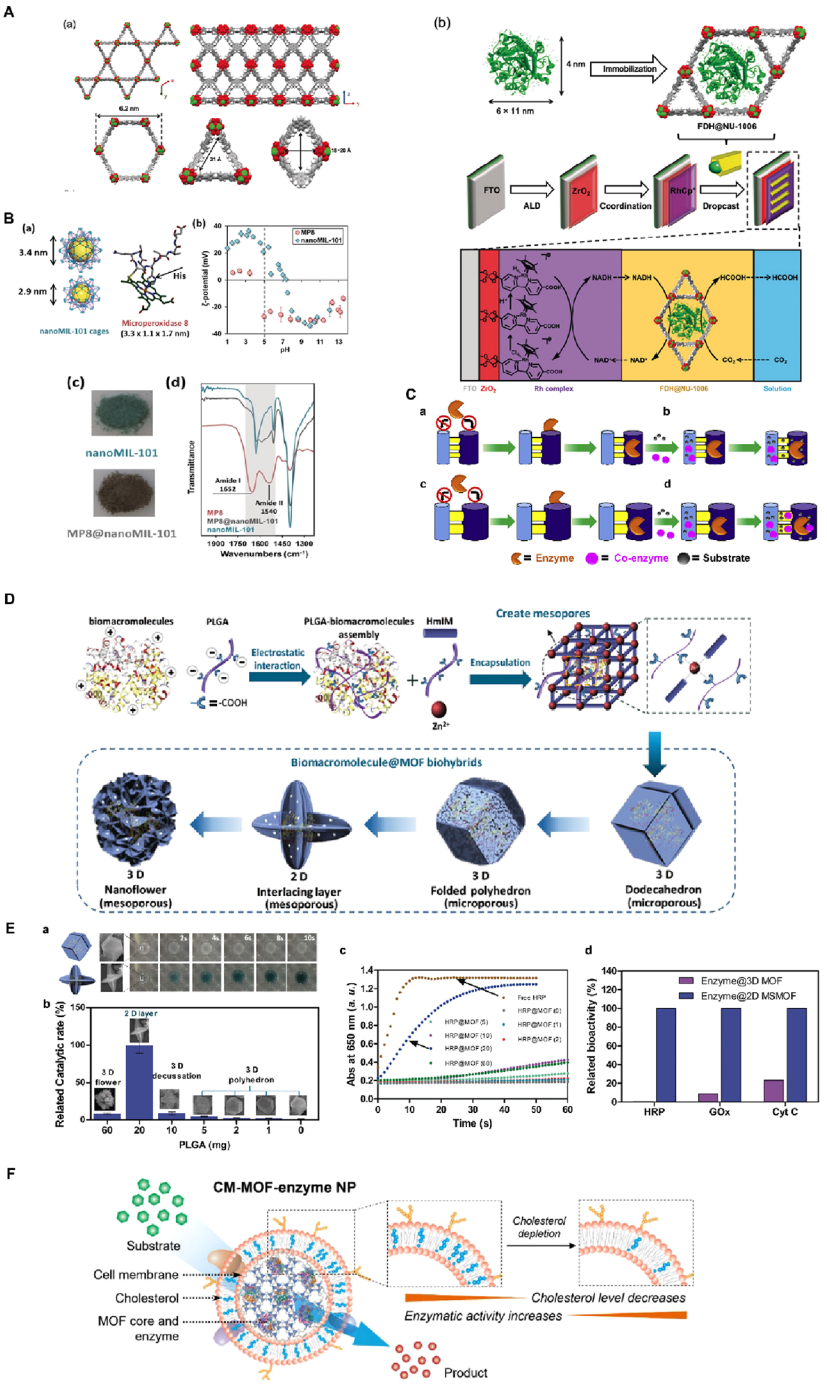

Figure 5. (A) (a) Crystal structure of MOF NU-1006; (b) schematic illustration of FTO electrode modification method and the bioelectrocatalytic reaction mechanism for CO_2 reduction. (B) (a) Cages of nanoMIL-101 (yellow sphere indicates cage volume) and molecular structure of microperoxidase-8; (b) z-potential measurements of nanoMIL-101 (blue diamonds) and MP8 (red circles) as a function of pH; (c) images of the powder of nanoMIL-101 (top) and MP8@nanoMIL101 (bottom); (d) FT-IR spectra of MP8 (red), MP8@nanoMIL-101 (gray), and nanoMIL-101 (blue). Adapted from ref 48 with permission. Copyright 2019 Wiley-VCH. (C) Schematic representation of immobilization of enzymes and coenzyme-mediated reactions in interconnected hierarchical channel systems. Reprinted from ref 52 with permission. Copyright 2018 Elsevier. (D) Schematic representation of the *in situ* encapsulation of biomacromolecules within a deformable MOF by using a PLGA modulator. (E) (a) The visual catalytic process of enzyme@3D microporous MOF (top) and enzyme@2D MSMOF (bottom) (condition: 5 mM H_2O_2); (b) effect of the architecture of the MOF coating on bioactivities (condition: 0.2 mM H_2O_2); (c) catalytic kinetic curve of different HRP@MOF biohybrids (condition: 0.2 mM H_2O_2); (d) bioactivity of enzyme@3D MOFs and enzyme@2D MSMOFs. Adapted from ref 53 with permission. Copyright 2020 Wiley-VCH. (F) Schematic diagram of enhancing the enzymatic activity of cell-membrane-coated enzyme-loaded MOF NPs (denoted as "CM-MOF-enzyme NPs") by reducing the membrane cholesterol (Chol) content. Adapted from ref 56 with permission. Copyright 2022 Wiley-VCH.

various applications in the electrochemical, photoelectrochemical, and electronic fields.

2.3. Optimizing Enzyme−MOFs Interactions. *2.3.1. Pre-nucleation of Metal Ions.* Inspired by the metal cation

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accumulating pattern of metallothioneins, metal ions serve as enzyme activators for the construction of enzyme−MOF composites with boosted enzymatic activities.[36−38] Motivated by this concept, Lyu et al. reported an *in situ* synthesis of immobilized Cyt c in ZIF-8, with an improved enzymatic activity.[39] The possible interaction between the zinc ion and the enzyme offered Cyt c a higher substrate affinity toward H_2O_2 molecules, which resulted in a 10-fold increase in its apparent activity. In another work, Chen et al. developed an amino acid (cysteine)-boosted MOF-based enzyme encapsulation strategy for enhancing enzymatic activity (Figure 4A). Given cysteine's inherent ability to gather metal ions through coordination interactions, it was hypothesized that cysteine could promote the accumulation of prenucleation clusters of MOF organic ligands and metal ions around the enzyme molecules. This would in turn expedite the encapsulation of enzymes within MOFs. However, a challenge arises in enriching enzyme surfaces with cysteine, due to the relatively weak interactions between enzyme and cysteine. To address this, polyvinylpyrrolidone (PVP), a biocompatible macromolecule, was chosen as a capping agent because of its proven capability in forming a complex with both the enzyme and the amino acid.[39,40] The successful encapsulation of the enzyme in ZIF-8 was confirmed by fluorescence at a wavelength of 525 nm, which is attributed to the fluorescein-isothiocyanate (FITC)-labeled enzyme present in the enzyme−MOF composites (Figure 4B). Based on their findings, the cysteine/PVP-induced prenucleation of metal ions around enzymes is a viable method for encapsulating various enzymes, resulting in high activity and enzyme loading capacity.

In another study, An et al. reported a platform technology for the *in situ* assembling of enzymes and metal ion activators into enzyme−MOF composites (Figure 4C,D).[41] The inclusion of metal activators not only preserves the enzyme's native conformation but also enhances its activity by 251%. The significant boost in activity was ascribed to the improved affinity between the enzymes and their substrates (Figure 4E). The binding of the metal activator with the enzyme was verified by dispersive X-ray spectroscopy (EDX), and this weak metal−enzyme interaction was further confirmed by the gradual removal of metal ions from the enzyme through multiple washing for a long time.

2.3.2. Hydrophilic/Hydrophobic Interactions. The hydrophobicity of the MOFs affects the enzymatic activity of enzymes encapsulated inside. Liang et al. reported that enzymes encapsulated in hydrophobic ZIF-8 displayed a reduced catalytic activity and low stability (against high temperatures, proteases, and organic solvents), while those encapsulated in hydrophilic MAF-7 or ZIF-90 maintained high activity and stability (Figure 4Fa).[2,42] It is noteworthy whether the catalase encapsulated inside or adsorbed onto the surface of ZIF-8 remained inactive, as evidenced by the consistent H_2O_2 level (Figure 4Fbc). The solid-state UV−visible spectra revealed that the heme-binding point was not modified after encapsulation in MOFs, indicating that (HmIM) did not inhibit the active site of the enzyme. However, the blue-shifted λ_{max} for catalase on ZIF-8 from 335 to 330 nm (compared to the free enzyme) from fluorescence spectra suggested that the tertiary structure of catalase was perturbed after absorption on the ZIF-8 surface, which explained why the adsorbed catalase lost its activity (Figure 4G). ATR-FTIR spectra along with the corresponding second derivative spectra suggested that both the encapsulated and absorbed catalase on ZIF-8 aggregated due to the hydrophobicity of the MOF. In another study, Chen et al. reported that the hydrophilic/hydrophobic interactions between the organic linker of MOFs (NU-1000) and the enzyme cytochrome c (Cyt c) can potentially alter enzyme structure, resulting in an enhanced enzyme catalytic performance (Figure 4H).[43] This is likely due to the enhanced accessibility of the enzyme active center to the substrates, as the enzyme structure change around the active site was observed using both experimental techniques (electron paramagnetic resonance (EPR) spectroscopy and solid-state UV−vis), and MD simulations. This study provides new insights into improving enzymatic activity by manipulating the hydrophilicity and hydrophobicity of MOFs.

Li et al. doubled the catalytic activity of encapsulated lipase from Burkholderia cepacia (BCL) in ZIFs by continuously tuning the enzyme−MOF interaction through modulating the hydrophilicity within the pore microenvironment.[44] By variation of the ratio of the ZIF linkers, the enzyme−MOF interaction was continuously tuned along with the hydrophilicity change of the MOF microenvironment (Figure 4Ia). Changes in enzyme conformation post-encapsulation were characterized using FTIR spectra and fluorescence studies, revealing a transition in the enzyme structure from a closed-lid conformation to an open-lid conformation as the MOF hydrophilicity increased (Figure 4Ib). Furthermore, their study identified an optimal point for enzyme activity when the hydrophobicity of the ZIF linkers.

Hydrophobicity also has a significant impact on enzyme activity in covalent organic frameworks (COFs). To optimize enzyme activity, Ma's group incorporated diverse hydrophilic organic functional groups, such as −OMe, −OH and −ONa into the crystalline porous COFs one-dimensional channels.[45,46] This modification resulted in an enhancement of enzyme performance, which was correlated with an augmentation in hydrophobicity. These studies provide insights into the complicated relationship between enzymes and their crystalline porous supports.

2.4. Facilitating Substrate and Product Exchange.

2.4.1. Preconcentration of Substrates. Increasing the substrate concentration results in a higher rate of enzyme-catalyzed reactions. Based on this concept, Chen et al. improved the activity of formate dehydrogenase encapsulated in mesoporous NU-1006 by the pre-concentration of reaction substrate,[48] as evidenced by the selective adsorption of nicotinamide adenine dinucleotide (NADH) rather than NAD^+ (Figure 5A). This finding paved the way for crafting highly efficient and environmentally sustainable electroenzymatic reaction systems for carbon dioxide reduction. In another study, Gkaniatsou et al. reported a significant catalytic activity improvement of microperoxidase-8 (peroxidase-type enzyme) when encapsulated in MIL-101(Cr), which was attributed to the pre-concentration of reactants through charge matching between the dye and the MOF (Figure 5B).[49] Working in synergy with the enzyme, the mesoporous MOF matrix selectively boosted the oxidation reaction of dyes through charge-based selective adsorption of reactants.

2.4.2. Enhanced Substrate/Product Diffusion. Increasing the pore size of materials is one of the common methods for enhancing substrate/product diffusion. One possible approach to enlarging the pore sizes of MOFs is through the induction of defects. Hu et al. reported a microfluidic-assisted synthesis of enzyme-loaded defective ZIF-8.[50] By varying the concentrations of the MOF precursor, structural defects were

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Table 1. Summary of Recent Applications of Enzyme−MOF Composites

Applications	Enzyme	MOF	Enzyme functions/reactions	Advantages	Ref
Catalysis	Glucose oxidase	ZIF-8, PCN-224(Fe)	Glucose oxidation	high stability, construction of multienzyme system for cascade reaction	57−61
	Catalase	UiO-66, ZIF-8, ZIF-90, MIL-101	Reduction of hydrogen peroxide	Retained enzyme activity at harsh conditions; preserved capability for adsorbed molecules	53,62−66
	Lipase	UiO-66, ZIF-67, MOF-74, ZIF-8	Hydrolysis reactions	Protective activity against harsh condition; integration for cascade reaction	67−71
	Cytochrome *c*	NKMOF-101/102, ZIF-8, PCN-222, UiO-66	Removal of hydrogen peroxide; oxidation of ABTS	Boosted enzymatic activity compared with free enzyme	41,72,73
	Cellulase	UiO-66-NH_2, ZIF-8	Cellulose hydrolysis	Improved enzyme stability and shelf life; tolerance to ionic liquid	74,75
	β-Galactosidase	UiO-66-NH_2, ZIF-8	Hydrolysis of cellobiose	Increased resistance to protease and acidic conditions	76
	Formate dehydrogenase	NU-1006, MIL-125-NH_2	CO_2 fixation, formic acid production	Enhanced activity for efficient CO_2 fixation	77,78
	Horseradish peroxidase	ZIF-8	Evaluations of enzyme activities	Enhanced activity, thermostability, and recyclability	50,79
	Catechol oxidase	MOF-818	Oxidation of catechol	Similar activity to the oxidase with specificity	80
Electrochemical catalysis	Pepsin	ZIF-8 modified with Ni^{2+}	Oxygen evolution reaction	Stabilization and reduced overpotential for reaction	81
	Formate dehydrogenase	In(III)-Ni MOF, NU-1006	CO_2 fixation, formic acid production	Enhanced CO_2 absorption and stability; higher yield compared with free enzymes	82,83
Biosensing	Glucose oxidase	MAF-2, Fe-MIL-88B-NH_2, Cu-BTC, ZIF-8	Glucose detection	Enhanced sensing activity, improved thermostability, and low detection limit	84−87
	Horseradish peroxidase	ZIF-8, ZIF-90, amorphous ZIFs	Signal generation for quantification	Protective effect on enzyme; combination or cascade with other enzymes	88−91
	Cholesterol oxidase	PCN-333	Cholesterol detection	Improved stability and sensitivity	92
Biomedicine	Glucose oxidase	ZIF-8, PCN-224, Cu-TCPP(Fe)	Consumption of endogenous glucose and oxygen	High catalytic activity to induce starvation and synergize with prodrug; Low biotoxicity	93−96
	Catalase	ZIF-8, MOF-808, MIL-101-NH_2	Removal of toxic molecule; generation of oxygen in hypoxia	Preserved enzymatic activity in physiological conditions	97−99
	Tyrosinase	PCN-333(Al)	Activate prodrug for cancer therapy	High activity and stability in acid condition	100
	Superoxide dismutase	ZIF-zni	Relieve oxidative stress	Protection against acidic conditions; high therapeutic efficacy	101
	L-Methioninase	UiO-66	Consumption of L-methionine in cells	Improved enzyme stability	102

successfully generated with tunable pore size from approximately 1 to 6 nm through defect engineering. The glucose oxidase encapsulated in defect-induced ZIF-8 showed an enzyme loading of 8.45% w/w and retained more than 98% of its activity, while those encapsulated in ZIF-8 only retained less than 10% of the enzymatic activity. Although the MOF prepared via microfluidic methods exhibits diminished crystallinity, the XRD peaks remained discernible. Upon inducing additional coordination defects, enzyme-amorphous MOF composites were successfully synthesized, as evidenced by a complete absence of distinct XRD peaks.[51] The mesopores (1−10 nm) generated in this disordered and fuzzy structure of MOFs greatly improved the activity of encapsulated enzymes due to enhanced substrate/product diffusion. In another study, Li et al. expanded the pore apertures of a set of Zr-based MOFs,[52] by maintaining precise control over the torsional angles associated with the linkers. The activity of the immobilized enzyme was observed to be dependent on the size of the open channels. This effect was attributed to the varying diffusion rates of substrates and coenzymes as well as the accessibility of the immobilized enzymes (Figure 5C). This approach allows for the accommodation of desired enzymes in larger channels, while still providing sufficient space for substrates and coenzymes to diffuse through smaller channels and bridging windows.

The morphology of MOFs can also be tuned to enhance the substrate/product diffusion. Chen et al. significantly increased the enzyme activity in 2D MOFs by shortening the diffusion path of substrates and enlarging the pore channel of MOFs.[53] Using g-poly-L-glutamic acid (PLGA), a peptide modulator, the ZIF-8 material can be shaped into a 2D mesoporous layer from various 3D microporous structures (Figure 5D). The catalytic efficiency and catalytic power of enzyme@2D MOFs were significantly higher than those of enzyme@3D MOFs (Figure 5E), indicating that the accessibility of the encapsulated enzyme was enhanced, thus boosting the activity of enzyme−MOF composites.

The substrate/product exchange in enzyme−MOF composites can be enhanced by combining them with carbon-MOF composite materials. For example, Liu et al. encapsulated HRP in hydrophilic carbon-ZIF-67(Co) composite materials, and the apparent substrate affinity has been increased by 75%.[54] The carbon-ZIF-67 composite exhibited an equilibrium of hydrophilic and hydrophobic properties, establishing a localized microenvironment conducive to interactions between the active sites of the immobilized enzyme molecules and substrates. Consequently, this enhanced the substrate affinity of the immobilized enzyme. This study is beneficial for the development of biocatalysis and biosensing.

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Additionally, MOFs have attracted increasing interest for the encapsulation of therapeutic enzymes in the pharmaceutical industries. To interface these enzyme−MOFs composites with biological systems better, coating enzyme−MOFs composites with cell membranes has emerged, enabling the biomimicking properties of MOFs.[55] Wang et al. reported that the enzymatic activity of enzyme encapsulated in cell-membrane-coated MOFs can be significantly improved by reducing the membrane cholesterol content (Figure 5F).[56] They revealed that a deficiency in cholesterol decreased the arrangement of lipid molecules, leading to the generation of vacant spaces within the membranes of living cells and an increase in membrane permeability. Consequently, this makes the substrates more readily available to the enclosed enzymes, enabling the products to diffuse out of the membrane more easily. In another study, by using a single-step interfacial biomineralization approach, Bell et al. presented the coating of a poly(ether sulfone) (PES) hollow fiber membrane with an enzyme-embedded ZIF-8 layer to facilitate the transport of educts and products during the reaction, which improved the enzymatic activity by 50%.[57]

3. APPLICATIONS OF ENZYME−MOF COMPOSITES

Over the past few decades, there has been a surge in research on enzyme immobilization using MOFs, due to their facile synthesis and porous structures that promote substrate diffusion. This encapsulation not only ensures robust protection but also enhances stability. The protective efficacy of MOFs largely depends on the integrated structure of the MOF and the enzyme. Therefore, to maximize the encapsulation efficiency, the porosity of MOFs should be compatible with the size of the enzymes. Further exploration of host−guest interactions between MOFs and enzymes is essential for designing composites suitable for practical applications. Moreover, it is imperative to address the cost challenge associated with the large-scale production of enzyme−MOF composites for industrial applications. Recent advancements have focused on developing new enzyme−MOF composites and their manufacture methods for various applications such as catalysis, sensing, and biomedicine.[2] Drawing recent research conducted from 2017 to the present work, we summarize illustrative examples of enzyme−MOF composites and their applications in Table 1.

For catalytic enzymes, a wide range of materials has been explored for enzyme immobilization to improve stability and reusability, thus making it possible to develop cost-effective catalysts.[103,104] With the advances of reticular chemistry and materials engineering of MOFs, enzyme−MOF composites have attracted tremendous research interest in the field of catalysis and biocatalysis, including cellulose hydrolysis,[74] carbon dioxide fixation,[77] and hydrogen peroxide removal.[41] Such studies represent the potential of enzyme−MOF composites in the food industry and pollution treatment.

Moreover, new strategies to control enzyme distribution within MOFs have facilitated the mass production of enzyme−MOF composites, striking a balance between the cost and commercial viability. Feng et al. utilized dissociation equilibrium of MOFs to generate defects for enzyme immobilization. Several enzymes, including lipase, catalase, and glucose oxidase, have been successfully immobilized in UiO-66, showing good enzymatic activities and stability. Furthermore, this approach offers broad applicability, presenting a cost-effective alternative to the ball milling method for industrial MOF-based composite production.[67]

Recently, a particular focus on the fabrication of MOF−enzyme-composite-based devices has emerged.[105] Bell et al. reported a facile method of interfacial crystallization to integrate enzyme-encapsulated MOF crystals onto the reactive shell-side of hollow fiber membranes.[57] Although the catalytic activity of hollow fibers is inferior to that of free enzymes, they represent high stability and sustainability. Notably, the enzymatic activity was enhanced when subjected to convective flow transport. Additionally, Hu et al. employed microfluidic gradient mixing to synthesize a variety of enzyme−MOF composites, comparing their performance to those synthesized using conventional bulk solution methods.[50] The on-chip laminar flow facilitated the formation of detects and multimodal pores in the composites, thus improving substrate permeation and enzyme stability, especially under thermal stress.

Enzyme−MOF composites have been widely explored as electrochemical catalysts for green energy production via oxygen/hydrogen evolution reactions.[106] Yang et al. immobilized pepsin on the ZIF-8 surface using metal ions as enzyme anchors.[107] Among the four types of metal ions tested, Ni^{2+} demonstrated optimal binding capacity and stability for pepsin. The pepsin-immobilized ZIF-8 composites attained a high oxygen evolution efficiency with remarkable conductivity and activity in neutral solutions.

Enzyme−MOF composites have also attracted wide interests in biosensing owing to the enzyme's high sensitivity and selectivity toward analytes.[108] Over the past decades, many glucose-sensing devices have been developed by coupling enzyme−MOF composites with colorimetric or fluorescent indicators.[109] Recently, Xu et al. encapsulated glucose oxidase *in situ* in an oxygen-sensitive and luminescent MOFs for ratiometric sensing of glucose.[84] The composites, free of additional indicator, possess a detection limit of 1.4 μM glucose as well as improved shelf life and thermostability.

In addition, incorporating enzyme−MOF composites in conventional biological detection methods has demonstrated promising avenues in the fields of biosensors and analytical assays. For instance, Zhang et al. reported a hybrid platform of enzyme ZIF-8 complexes for regenerative and catalytic digital detection of RNA (EZ-READ).[88] The system was composed of magnetic beads and HRP@ZIF-8 composites with a DNA probe as a linker. Upon hybridization of RNA targets, the DNA linker simultaneously regenerated RNA and liberated the composites for the signal enhancement of RNA detection. Such applications of enzyme−MOF composites in RNA detection assays offer more robust and reliable measurements compared to traditional RNA detection assays.

Enzyme−MOF composites have also demonstrated potential as biomimetic reactors to treat inflammatory disease and activate prodrugs for cancer treatment.[28,93] Lian et al. synthesized a tyrosinase−MOF nanoreactor capable of converting the prodrug paracetamol to a cytotoxic compound. This process promoted oxidative stress within tumor cells, leading the inhibition of tumor growth *in vivo*.[100] The mesoporous structure of the MOF with a diameter of 5.5 nm pores can accommodate tyrosinases, protecting them from the acidic environment in tumors, while maintaining their efficacy in catalyzing prodrug conversion. Such prodrug-encapsulated enzyme−MOF nanoreactors are envisioned as versatile platforms for cancer treatment and other therapeutic

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interventions. In addition, superoxide dismutase, an antioxidant enzyme, was entrapped into ZIF-zni to mitigate reactive oxygen species for the treatment of inflammatory disease.[101] Encapsulation in MOFs slows degradation of enzymes in various pH conditions that mimic the environment of the gastrointestinal tract, providing a new oral therapeutic for treating inflammatory colon disease.

Recent advancements in enzyme−MOFs composites highlight their significant potential for practical applications. However, for translation from the laboratory to market, several critical factors need to be addressed, including cost-effectiveness, scalability in production, stringent quality control, and a comparative evaluation against existing products in the market. By conducting further fundamental studies on both enzymes and MOFs, along with the development of novel fabrication technologies and exploration of new applications, we can foresee the future commercialization of enzyme−MOF composites.

4. CONCLUSIONS AND FUTURE PERSPECTIVES

Extensive research has been dedicated to enhancing the enzymatic activity of enzyme−MOF composites, especially to counteract the potential reduction in enzymatic activity post-encapsulation within MOFs. Common strategies include modifying the structure of both the enzyme and MOF, fine-tuning the interactions between the enzyme and MOF, and facilitating substrate and product exchange. Adjusting the hydrophobic interaction between enzymes and MOFs through enzyme/MOF functionalization and engineering enzyme active sites stand out as the most popular techniques for maximizing enzymatic activity. Moreover, enzyme−MOF composites find diverse potential applications, ranging from biocatalysts and biosensors to applications in biomedicine.

Looking ahead, enzyme−MOF beads represent a burgeoning category of promising materials, showcasing favorable properties and high scalability and opening opportunities to a wide array of large-scale industrial applications. MOFs can be structured into beads or granules for many industrial applications, as their great performance observed at the laboratory scale often fails to extend to pilot or industrial-scale applications due to their microcrystalline structure.[110] For example, granulation can be applied to shape MOF powders into beads with the presence of binders such as sucrose and dimethylformamide (DMF).[111] Moreover, MOF−polymer composite beads can be prepared using a polymerization method, which adopts the cross-linking of biocompatible, biodegradable poly(acrylic acid) (PAA) and sodium alginate monomers and calcium ions.[112] This simple, scalable, and nontoxic method can be used to synthesize MOF beads with properties such as surface areas that are similar to those of their parent MOF materials, offering potential for a wide range of large-scale industrial applications or pilot-scale demonstrations.

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Notes

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