

Controllable immobilization of enzymes in metal-organic frameworks for biocatalysis

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In this issue of *Chem Catalysis*, Yang and co-workers describe the preparation of enzyme@metal-organic framework composites for efficient biocatalysis under both weakly acidic and basic conditions. This work unambiguously provides a general and green co-precipitation method of orderly immobilizing enzymes in metal-organic frameworks, which will be instructive for research in both academia and industry.

As a result of the pursuit of green and sustainable synthesis of chemicals by both academia and industry, biocatalysts based on enzymes have been considered an ideal platform; however, cell-free enzyme catalysis has not yet been widely adopted in industrial applications, even after years of research, mainly because of the unsatisfactory efficiency, long-term stability, and recyclability.¹ The introduction of solid support for enzyme immobilization could help to protect enzymes from deactivation and increase their stability and recyclability to provide a solution for the issues encountered in practical enzyme catalysis, and it has therefore attracted great attention.² Although different kinds of solid supports have been investigated for enzyme immobilization, the design of new host matrices for enzymes remains highly needed. For example, inorganic porous materials are easily available but lack structural flexibility, and organic porous materials can be readily designed with a variety of documented synthesis options, but most of them tend to be structurally amorphous. It has been suggested that the crystalline nature and uniform pore of the host can benefit the oriented loading of enzyme molecules and facilitate the structure-property study in biocatalysis.³

Metal-organic frameworks (MOFs) are an emerging class of crystalline porous materials from the coordination of metal nodes or clusters and organic ligands. Different from other traditional host materials, MOFs have intrinsic advantages in terms of their high surface areas, tunable pore sizes and structures, functionalizable pore walls, and diverse architectures, making them promising candidates for enzyme immobilization, which has been rapidly developed in the last decade.^{4,5} Currently, enzyme immobilization in MOFs is still limited by two aspects: (1) the necessary match between enzyme molecular dimension and the MOF pore size and (2) the narrow pH range for the enzyme@MOF composite to survive. Luckily, increasing efforts have been made to overcome these limitations; for example, Zn²⁺, imidazolate, and enzyme co-precipitation composites get over the size limitation of enzyme molecules and are stable under basic conditions.⁶ Enzyme@UIO-MOFs were found to be stable under acid conditions but still have a restriction on enzyme size.⁷ Recent work reported a polymer-ZIF hybrid that is stable under both acidic and basic conditions and excels in terms of size limitation because enzymes are present on the surface of ZIF,⁸ however, the procedure for preparing the resultant polypepti-

de@ZIF-8/ZIF-L hybrid does require special expertise, thus impeding its broad applicability. Considering the different kinds of enzymes and large number of MOFs, as well as the wide pH range for optimal biocatalysis performance, it is critical to develop a composite system that is stable under both acidic and basic conditions yet generally adaptable for various enzymes with different surface charges, molecular weights, and/or molecular sizes.

In this issue of *Chem Catalysis*, Yang and co-workers⁹ co-precipitated Ca²⁺, organic carboxylate ligand (1,4-benzenedicarboxylic acid [BDC], biphenyl-4,4'-dicarboxylic acid [BPDC], or 2,2'-diaminobiphenyl-4,4'-dicarboxylic acid [NH₂-BPDC]), and enzyme (lysozyme [lys], lipase, glucose oxidase [GOx], or horseradish peroxidase [HRP]) into crystalline MOFs under ambient conditions (Figure 1A). Interestingly, both powder X-ray diffraction and scanning electronic microscopy analyses confirmed that varying the starting amounts of three reagents could tune the morphology while keeping the crystal structure unchanged. Four differently sized enzymes were readily incorporated into MOFs to afford a series of enzyme@MOF composites that overcame the obstacle of size limitation in immobilizing enzymes in solid supports. Meanwhile, the loading amounts of enzymes, determined by the bicinchoninic acid method, were easily altered. Accordingly, confocal fluorescence spectroscopy was employed to map the presence of enzymes within the composites. Significantly, all enzyme@MOFs demonstrated high efficiencies in catalyzing the respective model reactions. More importantly, the catalytic performance of the enzymes was well

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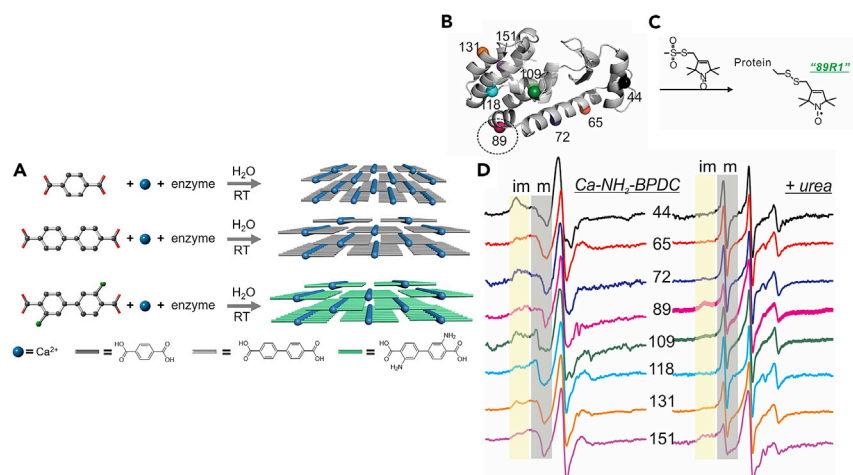


Figure 1. Preparation of enzyme@Ca-MOF composites and analysis of enzyme backbone dynamics

(A) Schemes for the preparation of three enzyme@MOF composites.

(B) Eight surface sites of T4L.

(C) SDSL of a representative site (89C) of T4L with R1.

(D) EPR spectra of all labeled sites in the absence (left) and presence (right) of urea for lys@Ca-NH₂-BPDC.

Adapted from Yang and co-workers.⁹

maintained in enzyme@MOF under both weakly acidic and basic conditions, and the efficiency was higher in enzyme@Ca-NH₂-BPDC composites than in others under the same conditions. In order to discern the intrinsic connection between structure and function, the authors utilized site-directed spin labeling (SDSL)-electron paramagnetic resonance (EPR) spectroscopy to probe the enzyme backbone dynamics at the residual level regardless of the background matrices. For example, lys@Ca-NH₂-BPDC exhibited higher dynamics than Ca-BPDC and Ca-BDC on multiple protein sites (Figures 1B–1D). Therefore, the authors speculated that the reason for the different catalytic performances was the difference in the enzyme backbone flexibility, which is presumably affected by the spatial environment,¹⁰ for the three kinds of MOFs. Meanwhile, six catalytic cycles for each lipase@Ca-MOF were conducted, and more than 90% relative activity was retained, indicating the superior stability and reusability of enzyme@MOF catalysts.

As a noteworthy progression in the field of enzyme@porous materials, the work by Yang and co-workers also inspires a number of new opportunities. From the perspective of material synthesis, the extension from Ca²⁺ and terephthalic acid MOFs to other MOF systems could be predictable given the large number of MOFs and their flexible characters. In addition, among four different sizes of enzymes immobilized, only GOx represents a large enzyme, and it is foreseeable that more large enzymes and even customer-designed enzymes could be applied in the co-precipitation strategy. Those will make the co-precipitation approach as general as the authors stated in their article, thus moving enzyme immobilization a step forward. From the perspective of catalysis, extension to other efficient, specific, and significant reactions can be anticipated on the basis of the character of enzyme catalysis and its broad scope. Rarely exploited cascade reactions taking advantage of both enzymes and MOFs will be highly desired. Moreover, techniques or combined measurements could be developed for a

deeper understanding of the interactions between enzymes and porous materials, namely establishing a clear relationship between structure and property and explaining insightful mechanisms for catalysis, especially as they relate to the synergistic effect of the composite. Furthermore, although a pH range of 3.7–9.5 has been investigated, how to broaden the pH of reaction conditions for enzyme@porous material composites to a more compatible range, and thus boost their resistance to both acidic and basic conditions, still needs improvement in materials design through the balancing of MOFs and enzymes. After all, challenges and opportunities coexist in both the design of enzyme@porous materials and the subsequent catalysis applications, which will bring a new era to the industrialization of enzyme catalysis.

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An enzyme-mimicking inorganic catalyst for effective nitrogen photofixation

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Drawing inspiration from natural enzymes is a meaningful strategy to develop high-performance artificial catalysts. In this issue of Chem Catalysis, the Tsang group mimic FeMoco in natural nitrogenase to design [Fe-S₂-Mo] motifs on MoS₂ for N₂ photofixation, which rank the highest energy efficiency among all photocatalytic systems reported to date.

Natural nitrogenase is capable of catalyzing N₂ reduction with superior activity and selectivity in global nitrogen cycle. Researchers over the past decades have endeavored to elucidate the mechanism of nitrogenase as a means of designing catalysts for efficient N₂ fixation under benign conditions to replace the energy-intensive Haber-Bosch process.¹ Nevertheless, the synthesis of artificial catalysts with enzyme-like performance failed to make a satisfying breakthrough in the 20th century, which is possibly attributed to the following two aspects.² The first aspect is intrinsic complexity of nitrogenase catalysis. Generally, N₂ is reduced in catalytic MoFe protein of nitrogenase, which needs the energy input from Fe protein via complex processes of adenosine 5'-triphosphate (ATP) hydrolysis and electron delivery (Figure 1A).^{3,4} The energy transduction and electron transfer during N₂ reduction remain obscure because of the structural and chemical diversity of nitrogenase, which hinders the design

of functional catalysts. The second aspect is technical restriction for atomic-level fabrication and characterization of catalysts. Limited by the immature technologies, the precise regulation and fine characterization of catalysts at atomic scale was hard to implement at that time, further constraining the exploration of highly effective artificial catalysts. Encouragingly, in last decade, the flourish of nanotechnology successfully realizes the atomic-level engineering of artificial catalysts. Specifically, burgeoning single-atom catalysts with well-defined atomic and electronic structures are promising candidates to mimic natural enzymes.⁵ Besides, advanced characterization tools, such as spherical aberration-corrected electron microscopy and synchrotron radiation-based techniques, play key roles in identifying the structural and catalytic properties of catalysts.⁶ More importantly, emerging photocatalysis and electrocatalysis drive reactions by optical and electric energy, respectively, which

offer innovative approaches to accomplish the energy input during reactions.

In this issue of *Chem Catalysis*, Tsang et al. are inspired by MoFeco in natural nitrogenase and successfully fabricate Fe-modified two-dimensional single-layered MoS₂ (Fe-sMoS₂) for the conversion of N₂ to NH₃ (Figure 1B).⁷ Three percent of Fe-sMoS₂ (3 wt % of Fe loading) catalyst exhibits superior activity and selectivity for NH₃ at 270°C under visible-light illumination. Impressively, the solar-to-NH₃ energy-conversion efficiency reaches 0.24%, which is the highest efficiency among all the reported photocatalytic systems so far. What's more, a considerable NH₃ yield is also sustained when applying solar furnace without extra thermal energy input during N₂ reduction, indicative of the great potential of Fe-sMoS₂ catalysts for the application in practical agricultural production. In this work, the existence state of Fe atoms on Fe-sMoS₂ catalysts is systematically investigated from experimental and theoretical perspectives. High-angle annular dark-field scanning transmission electron microscopy and atomic-resolved electron energy-loss spectroscopy are performed for 3% Fe-sMoS₂, illustrating that Fe atoms are uniformly dispersed and deposited at the position of Mo or S sites in MoS₂. Combining with the

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