Metal–Organic Frameworks

Protein-Structure-Directed Metal–Organic Zeolite-like Networks as Biomacromolecule Carriers

Huanrong Wang+, Lin Han+, Dong Zheng, Mingfang Yang, Yassin H. Andaloussi, Peng Cheng, Zhenjie Zhang, Shengqian Ma, Michael J. Zaworotko, Yifan Feng, and Yao Chen*

Abstract: Fabrication of zeolite-like metal–organic frameworks (ZMOFs) for advanced applications, such as enzyme immobilization, is of great interest but is a great synthetic challenge. Herein, we have developed a new strategy using proteins as structure-directed agents to direct the formation of new ZMOFs that can act as versatile platforms for the in situ encapsulation of proteins under ambient conditions. Notably, protein incorporation directs the formation of a ZMOF with a sodalite (sod) topology instead of a non-porous diamondoid (dia) topology under analogous synthetic conditions. Histidines in proteins play a crucial role in the observed templating effect. Modulating histidine content thereby influenced the resultant MOF product (from dia to dia + sod mixture and, ultimately, to sod MOF). Moreover, the resulting ZMOF-incorporated proteins preserved their activity even after exposure to high temperatures and organic solvents, demonstrating their potential for biocatalyst and biopharmaceutical applications.

In the past decades, zeolite-like metal–organic frameworks (ZMOFs, also referred to metal–organic zeolites, MOzs) have emerged as a metal–organic analogues of inorganic zeolites and are an important subclass of MOFs because they can integrate the merits of both MOFs and zeolites[1–3] such as, high porosity thanks to cage-like cavities,[2] designable pore window/shape,[4] customizable functionality,[5] and exceptional stability.[6] ZMOFs have become an active field of MOF chemistry as they offer potential utility for applications as diverse as gas storage/separation,[7] drug delivery,[8] sensing,[9] enzyme immobilization,[10–13] and so on.[14–16] Theoretically, the number of ZMOFs is vast, as reflected by the high number of hypothetical zeolite topologies and the modular nature of MOFs databases.[17,18] However, ZMOFs are still rare among the 75600 MOF entries in version 5.38 of the Cambridge Structural Database. This is largely because the synthesis of ZMOFs requires control of network topology and functionality. This is difficult because the assembly of tetrahedral building blocks (namely, the fundamental building blocks of zeolitic networks such as ZMOFs) tends to result in the default dia topology, which is not a zeolitic topology.

Template-directed synthesis has proved to be a powerful strategy to obtain new ZMOFs that are otherwise inaccessible.[19] For example, the presence of different templates, such as 1,2-diaminocyclohexane, imidazole, 1,3,4,6,7,8-hexahydro-2H-pyrimido[1,2-a]pyrimidine (HPP), afforded mer, sod, and rho Z-MOF topologies, respectively.[2–3] If the template(s) remains after MOF synthesis, additional functionalities, such as chirality,[20] catalysis,[21] and fluorescence,[22] can be transferred from templates to such “template@MOF” materials (@ = encapsulated by). Until now, template molecules have typically comprised small inorganic,[23] organic,[24] or organic–inorganic species[25] and the use of proteins as templating agents is yet to be reported. In polymer chemistry, proteins have been employed as removable templates to fabricate molecularly imprinted polymers (MIPs), which can be used for protein recognition.[26] These results inspired us to explore if proteins, especially bioactive enzymes, can indeed be used as templates to form new ZMOFs which can inherit the function of proteins (for example, catalysis, recognition, and sensing)[26]. In this study, we demonstrate the use of proteins as templates to direct the formation of a new ZMOF platform with zeolitic sodalite or sod topology (named hereafter ZPF-1, ZPF – zeolitic pyrimidine framework, Scheme 1). In contrast, the same experiment conducted in the absence of a protein template afforded the default dia network. ZPF-1 can thereby serve as a versatile platform for the in situ encapsulation of proteins with high loading capacity because the protein remains in the material after synthesis. We also investigated the mechanism of templating to study the role of histidines on proteins in directing the target ZMOF structure. The discovery of the templating effect of proteins and a more developed understanding of the mechanism should provide guidance for the design and synthesis of a broad range of functional porous materials that incorporate biomacromolecules.

Many MOFs are designed according to the “node and linker” approach and elaborated using “reticular chemistry”.[28] The key design concept in generating protein-encapsulating ZMOFs (for example, ZIF-8 and ZIF-90)[19–22] is the
tetrahedral geometry of the metal atoms (for example, Zn\(^{2+}\)) and a suitable subtended linker angle (for example, 142.4° for 2-R-imidazole, R = methyl or aldehyde). Pyrimidine derivatives are a basic building block of genetic material and possess structural similarity with the imidazole derivatives that generate ZIF-8 and ZIF-90. However, the use of pyrimidin-2-ol and zinc ions to generate ZMOFs has not been achieved by traditional synthesis methods, which afford the previously reported nonporous MOF (named herein DIA-1) with diamond morphology and dia topology (Supporting Information, Figure S1). Herein, inspired from the protein-template effect in the formation of MIPs, we show that use of proteins as templates to modulate the reactions of pyrimidin-2-ol and zinc cations is feasible, thereby preparing ZMOFs that encapsulate proteins.

Bovine serum albumin (BSA) was selected as a model protein in this study on the basis of its low cost and high solubility. Presence of BSA during synthesis resulted in a new crystalline phase (named herein ZPF-1) in aqueous solution at room temperature. Fourier transform infrared spectroscopy (FT-IR) (Supporting Information, Figure S2a) confirmed that successful encapsulation of BSA into ZPF-1 had occurred, indicated by the existence of characteristic peaks of BSA. Fluorescein isothiocyanate (FITC)-labeled BSA was also encapsulated by ZPF-1 and scanned by CLSM (confocal laser scanning microscopy, Supporting Information, Figure S2b) to further verify that successful encapsulation of BSA had indeed occurred. Calculations based on the Bradford method revealed that the loading of BSA is as high as 0.2365 gg\(^{-1}\). Powder X-ray diffraction (PXRD) and transmission electron microscopy (TEM) studies were conducted to further verify and investigate the template effect of BSA (Figure 1 and Supporting Information, Figure S3). Peak broadening observed in the PXRD pattern of BSA@ZPF-1 revealed the presence of nanometer-scale crystals, which was verified as such using TEM. As shown in Figure 1b, BSA@ZPF-1 formed particles approximately 80 nm in size and polyhedral morphology, whereas DIA-1 formed particles with dimensions on the order of 600 nm and prismatic morphology. Gas sorption data revealed the BSA@ZPF-1 exhibited a hierarchical structure with both ultra-micro pores originated from the structure nature of ZPF-1 and macro pores due to particle packing or defects (Supporting Information, Figure S4). Furthermore, the obtained MOF product was shown to be affected by the concentration of BSA. A mixture of BSA@ZPF-1 and DIA-1 was obtained when reducing the amount of BSA (less than 0.05 mgmL\(^{-1}\), Supporting Information, Figure S5). These results show that BSA indeed exhibits a template effect that enables the formation of ZPF-1.

On the basis of the templating effect of BSA, we studied if other proteins could also serve as templates. The template effect was indeed broadly observed for proteins, such as GOX (glucose oxidase), Mb (myoglobin), insulin, CAT (catalase), ACP (acid phosphatase), and G-IgG (goat anti-BSA IgG polyclonal antibody), all of which templated the formation of ZPF-1 (Supporting Information, Table S1), as verified by PXRD (Supporting Information, Figure S6) and TEM (Supporting Information, Figure S7). FT-IR spectroscopy results confirmed that proteins were successfully encapsulated into ZPF-1 (Supporting Information, Figure S8). This broad performance across a range of proteins prompted us to investigate the mechanism behind the templating effect. As the basic building blocks of proteins, amino acids were first tested. After screening all 20 natural amino acids, we found that only histidine templated the formation of ZPF-1 (Supporting Information, Figure S9) at room temperature from aqueous solution. Single crystal X-ray diffraction (SCXRD) data revealed that each zinc atom is four-coordinated in a tetrahedral geometry connected to bridging pyrimidin-2-ol ligands to generate a three-dimensional (3D) network with sod topology that resembles the structure of ZIF-8 and ZIF-90. Compared with the regular sod cages of ZIF-8 and ZIF-90, with inner cage diameters of approximately 7 Å, ZPF-1 possessed a smaller sod cage of approximately 6 Å diameter (Figure 2). PXRD analysis revealed that samples of protein@ZPF-1 matched the calculated pattern of ZPF-1 (Figure 1a and Supporting Information, Figure S6).

We subsequently discovered that the amount of added histidine can affect the outcome. PXRD (Supporting Information, Figure S10) tests revealed a mixture of DIA-1 and ZPF-1 when reducing the concentration of histidine. This effect is consistent with that observed for BSA. In order to exclude other possible factors that may affect the templating process (for example, anions and metal–ligand ratio), we varied the zinc salts (for example, ZnCl\(_2\), Zn(OAc)\(_2\), ZnSO\(_4\), and Zn(ClO\(_4\))\(_2\)) and the ratios of reactants with no influence on the topology of the final product (Supporting Information,
in histidine can form structural precursors of the sod network (Scheme 2). If the above assertion is correct, the amount of histidine in proteins (Supporting Information, Table S1) is likely to influence their templating effect. Thus, we treated BSA with diethyl pyrocarbonate (DEPC), a histidine-specific alkylating reagent (Figure 3a). As the alkylation reaction of DEPC progressed, the UV absorbance of BSA at 227 nm increased (Figure 3b). The absorbance at 227 nm had no obvious upward trend from 60 min to 120 min after DEPC treatment, indicating that the alkylation reaction had reached equilibrium at 60 min (Figure 3a,b). When DEPC-modified BSA was used as an additive in the reaction of pyrimidin-2-ol and zinc ions, DIA-1 was produced with the absence of ZPF-1 (Supporting Information, Figure S15), indicating the need for histidine during the formation of ZPF-1. Furthermore, we expressed glutathione S-transferase (GST), a protein with histidine, and recombinant GST (rGST), a histidine-free protein, in Escherichia coli to directly investigate the structure-directed role of histidine in proteins (Figure 3d). The purified GST and rGST were found to be approximately 99% pure as indicated by dodecyl sulfate, sodium salt (SDS)-Polyacrylamide gel electrophoresis (SDS-PAGE, Supporting Information, Figure S16). As predicted, GST produced the desired sod structure of ZPF-1, while rGST formed the structure of DIA-1 (Figure 3c and Supporting Information, Table S1). Besides, we found that the addition of histidine as a co-template in the preparation of protein@ZPF-1 can significantly increase the encapsulation efficiency of proteins in ZPF-1 (for example, BSA encapsulation increased up to 31% in BSA@ZPF-1). Supporting Information, Table S2). These results revealed that histidine indeed plays a crucial role in the templating process of ZPF-1.

The successful incorporation of proteins into ZPF-1 offers potential for applications, such as biocatalysis and biopharmaceutical formulation. CAT, a well-known biocatalyst, was selected as a representative enzyme to evaluate the performance of the protein@ZPF-1 platform. The catalytic activity of CAT@ZPF-1 was evaluated by tracking the change of H₂O₂ in solution over time (apparent rate constant, $K_{app}$, Supporting Information, Figure S17). The results revealed that CAT@ZPF-1 possesses a high activity ($K_{app} = 3.54 \times 10^{3} \text{ s}^{-1}$, Supporting Information, Figure S18). The confinement of enzymes in MOFs is expected to provide protection against stress. We exposed CAT@ZPF-1 and free CAT to heat (70°C), organic solvent (methanol and DMF), and trypsin (an endopeptidase that cuts off the carboxyl side of the lysine and arginine residues in the polypeptide chain). As shown in

![Figure 2](image_url) Structural diagram of ZPF-1 vs. ZIF-8. Color code: carbon (gray), hydrogen (white), nitrogen (blue), and oxygen (red).

![Figure 3](image_url) a) Reaction pathway of histidine imidazole groups with an excess of DEPC; b) UV/Vis spectra of BSA reacted with 10 mM DEPC recorded at 0, 10, 60, and 120 min; c) The PXRD patterns of products after adding CST or rGST; d) Modeled 3D structure of GST and rGST. His residues are shown in magenta. These His residues were mutated to Lys, shown in green.

![Scheme 2](image_url) A proposed process for the histidine-induced templating effect in the generation of ZMOFs for protein encapsulation.
highlight original activity after release from the MOF. These results contrast, G-IgG encapsulated by CAT@ZPF-1 exhibited 97% of its original activity after treatment (Figure 4d). In as structure-directed agents in order to tune the formation of biopharmaceutical formulation and storage.

Furthermore, as verified by a Bradford assay, the encapsulated G-IgG can be completely released from ZPF-1 after various treatments. We also directly characterized the enzymatic activity of CAT@ZPF-1 with high efficiency (0.32 gg/C0) after recycling catalytic experiments (Supporting Information, Figures S19 and S20).

As important biopharmaceuticals, antibodies are a fast-growing category of therapeutic proteins that often suffer from inferior biophysical stability. G-IgG antibody was selected to probe the potential of the ZPF-1 system to protect antibodies and serve as a new biopharmaceutical formulation platform. An enzyme-linked immunosorbent assay (ELISA) was used to determine antibody activity. Our results revealed that G-IgG can be successfully encapsulated by ZPF-1 with high efficiency (0.32 gg/C0), with the encapsulation process presenting no influence on antibody activity (Figure 4c). Furthermore, as verified by a Bradford assay, the encapsulated G-IgG can be completely released from ZPF-1 within 15 min.

Conflict of interest

The authors declare no conflict of interest.

Keywords: enzyme immobilization · metal–organic frameworks · protein structure · self-assembly · template synthesis · zeolite analogues

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