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# Bio-functionalization of metal–organic frameworks by covalent protein conjugation†

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**Bioconjugation of functional proteins onto metal–organic frameworks (MOFs) has been achieved using activation of pendent linking groups of the organic linkers on the surface of MOFs. Fluorescent microscopy revealed successful conjugation of an enhanced fluorescent protein onto MOFs. In addition, *Candida-antarctica*-lipase-B-conjugated MOFs showed no loss of enantioselectivity and activity in transesterification of (±)-1-phenylethanol.**

Metal–organic frameworks (MOFs) are garnering more interest in material science. Thermally and mechanically stable MOFs with large void volumes have been useful in advanced applications such as selective catalysis, sensors, and high-efficiency gas storage.[1] Although diverse MOF materials have been prepared by solvothermal reactions between metal ions and organic linker compounds, organic linkers should possess adequate thermal stability because of their synthetic conditions.

To incorporate thermally unstable compounds into MOFs, the "postsynthetic covalent modification" was recently explored by several research groups.[2,3] For instance, Cohen and coworkers used IRMOF-3 (isoreticular metal–organic framework-3) as a starting MOF material for postsynthetic modification. IRMOF-3 is composed of Zn(II) ions and 2-amino-1,4-benzene dicarboxylic acid ($NH_2$-BDC). The two carboxylate groups of $NH_2$-BDC link $Zn_4O$ nodes but the amino group is intact during the IRMOF-3 formation. Thus, the amino groups are available for further modification of the framework. Cohen and Wang have shown that the amino groups can be modified by simple organic reactions and introduced several organic groups.[2] Since then many groups have reported modifications of MOFs using similar approaches, which require a special functional group, such as an amino group, for further modification.[3] Therefore, these approaches should be limited for the MOFs bearing such functional groups in the original linker molecules.

We have explored to overcome this limitation by using the linking groups of MOFs. In fact, typical organic linkers of the MOFs contain carboxylate groups. For example, a series of IRMOFs are composed of $Zn_4O$ nodes and dicarboxylate organic linkers.[1f] Either pendent $Zn_4O$ nodes or carboxylate groups should occupy the surface of the frameworks and thereby are exposed on the surface of IRMOFs. If pendent carboxylate groups are present on the surface of MOFs, then they could be activated and modified by simple organic reactions. We assumed that a certain amount of the carboxylate groups are exposed and can be activated by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) or dicyclohexyl carbodiimide (DCC). Then, the activated carboxylates can be conjugated with other compounds or even biomaterials, such as proteins. We exploited this idea of conjugating proteins to coordination polymers or MOFs with different structural architectures, such as one-, two- and three-dimensional (3D) structures. To our knowledge, the incorporation of functional proteins to MOFs has not yet been achieved. We first chose an enhanced green fluorescent protein (EGFP) as a model protein because its presence and the folding status of EGFP can be easily tracked by fluorescence microscopy.

A new indium(III)-based one-dimensional (1D) coordination polymer was prepared from the reaction between $In(NO_3)_3$ and 1,4-phenylenediacetic acid ($H_2$pda) in *N*,*N*-diethylformamide (DEF) (see ESI†). The crystal structure was shown in Fig. S1 and S2 (ESI†). The coordination mode of indium in the 1D-MOF is 8-coordinated by four carboxylates. The overall bonding scheme of the indium ion is very similar to those observed in several previous examples of 3D In-MOFs. The indium ion formed a pseudotetrahedral structure with four bridging pda ligands chelated in a bidentate manner. However, the bent geometry of methylene groups of the bridging pda ligands prevented the pseudotetrahedral motifs from expanding into a 3D network. Instead, it formed a 1D coordination polymer bearing a relatively large cavity between two negatively-charged indium ions and two parallel benzene rings

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† Electronic supplementary information (ESI) available: Experimental details, X-ray crystallographic information of 1D-MOF, PXRD patterns for activated and EGFP-conjugated MOFs, IR analysis of 1D-MOF, emission spectra and CLSM images of EGFP-MOFs, $N_2$ adsorption/desorption isotherms for the CAL-B-3D-MOF and measurements of the specific activities of CAL-B-MOFs. CCDC 789848. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c0cc03288c


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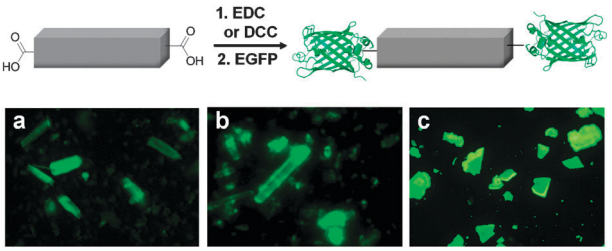


**Fig. 1** Schematic representation of the bioconjugation of the 1D-polymer, $[(Et_2NH_2)(In(pda)_2)]_n$, with EGFP. Fluorescence microscopic images of EGFP coated MOFs. (a) 1D + EGFP. (b) 2D + EGFP. (c) 3D + EGFP. An Olympus WIB filter set ($\lambda_{ex}$ = 460–490 nm; $\lambda_{em}$ > 515 nm) was used for recording the fluorescence.

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from the pda ligands. In fact, the counter cation, diethylammonium ion, is perfectly captured in this cavity. The diethylammonium ions were generated from the decomposition of solvent molecules, DEF, during the reaction. The 2D-MOF, $[Zn(bpydc)(H_2O)\cdot(H_2O)]_n$, is composed of zinc ions and 2,2′-bipyridine 5,5′-dicarboxylate (bpydc).[4] IRMOF-3 was used as the 3D-MOF.[5] All organic linkers of the MOFs contain carboxylate groups.

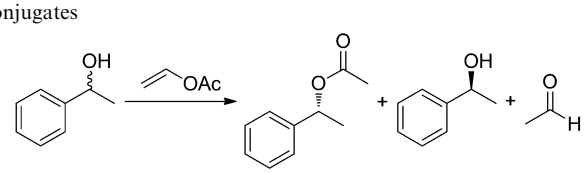
We attempted to conjugate MOFs with EGFP by treating EDC to activate the dangling carboxylate groups of MOFs (Fig. 1). We noticed, however, the 1D-polymer was only activated and conjugated with EGFP. Presumably, pda was rather easily activated in aqueous media because the aliphatic carboxylate is more reactive than the aromatic one.[6] In addition, this observation implies that no physical adsorption of EGFP to the MOFs occurs. For 2D- and 3D-MOFs, we activated the carboxylates by DCC in dichloromethane instead of EDC in an aqueous buffer. This activation step did not alter the crystallinity of the MOFs (Fig. S3 in ESI†).

For the following protein-conjugation step, we need to use a buffer (PBS, pH 7.3) as a reaction media in order to conjugate proteins because proteins are not soluble in most organic solvents. The use of a buffer could be problematic because MOFs, especially the 3D-MOF (IRMOF-3), may be not stable against water.[7] Therefore, we evaluated the stability of the 3D-MOF in water and PBS buffer by comparing PXRD patterns before and after soaking the 3D-MOF in water or PBS buffer. To our surprise, 1-h incubation of the 3D-MOF in a PBS buffer did not alter the PXRD pattern of the original phase while longer incubation (for 6 h) in PBS buffer or incubation in water changed the patterns (Fig. S4 in ESI†). We envision that this finding will broaden the scope of IRMOF-3 functionalization in an aqueous buffer solution. After activation of the MOFs, we successfully conjugated EGFP with the MOFs. However, the longer reaction period (> 1 h) for binding EGFP to 1D- and 3D-MOFs altered the structural integrity while the 2D-MOF remained unchanged (Fig. S3 in ESI†). The six-histidine tag attached to the N-terminal of EGFP may coordinate to the metal ions of the 1D- and 3D-MOFs and result in the structural alteration but not for the 2D-MOF. The different coordination environment ($N_2O_4$) of 2D-MOF is presumably more stable than those of the other MOFs. The presence of EGFP on the surface of the MOFs was confirmed by a fluorescence microscope. The EGFP-decorated crystals emitted a uniform green fluorescence (Fig. 1). In addition, the IR spectra of EDC- and EGFP-decorated 1D-MOF showed different patterns from that of 1D-MOF (Fig. S5 in ESI†). Thus, it can be concluded that EGFP was successfully introduced on the surface of the MOFs and the anchored EGFP is still functional.

Solid-state luminescence measurements also provided the characteristic spectrum of EGFP (Fig. S6 in ESI†). The surface modification of the MOFs was further confirmed by using a confocal laser scanning microscope (CLSM). Most green emissions from the EGFP-decorated MOFs were observed from the surface of crystals (Fig. S7 in ESI†). The amounts of EGFP coated on the MOFs were determined as 0.048, 0.052, 0.064 mg $g^{-1}$ of the 1D-, 2D-, and 3D-MOFs, respectively. To our knowledge, this is the first direct bioconjugation of coordination polymers or MOFs.

We also applied this strategy to conjugate MOFs with a functional protein, such as an enzyme. We chose CAL-B (*Candida antarctica* lipase B) that catalyzes hydrolysis or formation of an ester. CAL-B is one of the most widely used enzymes because of its high activity and selectivity.[8] CAL-B was conjugated to the three MOFs through the same coupling methods. The amounts of proteins decorated on MOFs were determined as 0.12, 0.17, and 0.18 mg $g^{-1}$ of the 1D-, 2D-, and 3D-MOFs, respectively (see Table 1). PXRD patterns showed that the structural integrity of frameworks was intact after CAL-B conjugation (Fig. 2). It is noteworthy to realize that even the hydrolytically unstable 3D IRMOF-3 maintained its framework structure during the conjugation. Presumably, hydrophobic CAL-B–protein conjugation enhances water-resistance of MOFs.[7] In addition, $N_2$ sorption measurement at 77 K for the CAL-B–3D-MOF exhibited very low Brunauer–Emmett–Teller (BET) surface area, 15.0 $m^2$ $g^{-1}$, compared with the activated native 3D-MOF which showed the BET surface area of 1605.1 $m^2$ $g^{-1}$ (Fig. S8 in ESI†). These data demonstrate the efficient outer surface functionalization of 3D-MOF by CAL-B instead of structural disintegration of 3D-MOF.

**Table 1** The catalytic activity and enantioselectivity of CAL-B–MOF conjugates




| Enzyme | The amount of CAL-B protein decorated/mg $g^{-1}$ | Specific activity/ μmol $min^{-1}$ $mg^{-1}$ | $E^a$ |
|---|---|---|---|
| Free CAL-B | n.a. | 0.037 ± 0.013[b] | >200 |
| 1D-MOF | n.a.[c] | n.d.[d] | n.a. |
| 1D + CAL-B | 0.12 | 15 ± 3.1 | >200 |
| 2D-MOF | n.a. | n.d. | n.a. |
| 2D + CAL-B | 0.17 | 22 ± 6.6 | >200 |
| 3D-MOF | n.a. | n.d. | n.a. |
| 3D + CAL-B | 0.18 | 39 ± 1.3 | >200 |

[a] $E$ = enantiomeric ratio as defined by C. S. Chen, Y. Fujimoto, G. Girdaukas and C. J. Sih, *J. Am. Chem. Soc.* 1982, **104**, 7294. [b] Errors are standard deviations for three measurements obtained from recycling experiments. [c] n.a. = not available. [d] n.d. = not detected.

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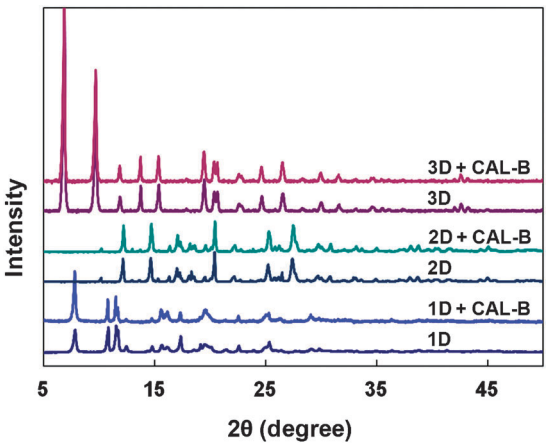


**Fig. 2** PXRD patterns of the 1D-, 2D- and 3D-MOFs, and CAL-B-conjugated 1D-, 2D- and 3D-MOFs.

To evaluate the activity of the immobilized CAL-B, we tested the catalytic activity in the transesterification of (±)-1-phenylethanol as a model reaction (Table 1). In addition, CAL-B has high enantioselectivity toward (*R*)-(+)-1-phenylethanol ($E = >200$).[9] We also measured the enantioselectivity of the immobilized CAL-B and the free CAL-B.

In addition, to examine the possibility of physical adsorption of CAL-B on the MOFs, we attempted to adsorb CAL-B on the MOFs without the activation step or after treatment of the activated MOFs by *n*-butylamine to block binding of CAL-B. The amount of physically adsorbed CAL-B was estimated by difference of the absorbances of the supernatant at 280 nm, before and after incubation. The amount of physically bound CAL-B was negligible because the difference was near the detection limit (~0.001) of a UV spectrophotometer. Besides, no transesterification activity of the MOFs was observed (Table S3 in ESI†). These results clearly indicate that no detectable amount of CAL-B was adsorbed on the MOFs.

We compared the specific activities of the CAL-B coated coordination polymers with a free CAL-B enzyme. In general, immobilized enzymes are more active in organic solvents than free enzymes and can be easily separated from the reaction mixture and re-used. We have measured and averaged the specific activities from three-times recycling experiments (Fig. S9, S10 in ESI† and Table 1). The recycling did not cause a significant decrease of the activity of CAL-B-coated coordination polymers indeed showed several hundred-fold higher activities and the same enantioselectivity of the product compared to the native CAL-B. Especially, the CAL-B on 3D-MOF showed about a $10^3$-fold higher activity than free CAL-B. In addition, the enantioselectivity of CAL-B coated on the three MOFs is as high as that of the free CAL-B. Despite the lack of a detailed rate enhancement mechanism at the moment, we speculate that the MOFs might provide confined spaces nearby the surface resided enzymes for substrates to contact enzymes more efficiently. The enhanced rate acceleration of 3D-MOF compared with 1D- and 2D-MOFs could be explained by the same speculation. In addition, the amino groups of IRMOF-3 presumably help to maintain the optimum pH for the enzymatic reaction because an addition of a weak base to an enzymatic reaction media can prevent a decrease of pH caused by formation of a by-product, such as acetic acid which can be generated by hydrolysis of vinyl acetate.[8a]

After CAL-B was incorporated onto 3D-MOF, the CAL-decorated 3D-MOF is still bearing unmodified amino groups. These amino groups may be served as further modification sites. Thus, the CAL-B-decorated 3D-MOF can be conjugated with another protein. We introduced EGFP (0.063 mg $g^{-1}$), which was activated by EDC, to the CAL-B-decorated 3D-MOF (Fig. S11 in ESI†) to form dual protein-conjugated MOF. The dual protein-conjugated 3D-MOF showed multi-functionality, *i.e.* fluorescence and transesterification activity (38 μmol $min^{-1}$ $mg^{-1}$).

These results clearly indicate that functional proteins can be decorated on MOFs without losing their functions. This approach is, therefore, an important step towards the functional modification of MOFs.

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