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# Lipase-Supported Metal–Organic Framework Bioreactor Catalyzes Warfarin Synthesis

Wan-Ling Liu, Ni-Shin Yang, Ya-Ting Chen, Stephen Lirio, Cheng-You Wu, Chia-Her Lin,* and Hsi-Ya Huang*[a]

**Abstract:** A green and sustainable strategy synthesizes clinical medicine warfarin anticoagulant by using lipase-supported metal–organic framework (MOF) bioreactors (see scheme). These findings may be beneficial for future studies in the industrial production of chemical, pharmaceutical, and agrochemical precursors.

Biocatalysis uses natural catalysts such as enzymes to facilitate chemical transformations including organic synthesis.[1] The use of enzymes for biocatalytic reactions can provide numerous advantages including chemo-, regio-, and stereospecificity even under mild reaction conditions.[2,3] However, drawbacks, such as poor long-term stability under the conditioning process as well as the difficulties in recovering and recycling, often hinder its application.[4,5] To improve the mentioned shortcomings, enzyme immobilization on solid supports has been adapted as an effective alternative that enhances the enzyme functions and activities and results in an improvement in reusability, catalytic efficiency, and stability under drastic catalytic conditions.[6,7] For a decade, various solid supports, including nanoparticles,[8] polymers,[9,10] and mesoporous silica materials,[11,12] have been developed as enzyme immobilizing bioreactors. Among them, mesoporous silicate materials provided a high surface area with adequate pore size to retain and accommodate enzyme biomolecules as host materials.[13] Conversely, reviews on mesoporous silicate bioreactors have reported that they suffer from leaching during the reaction process due to the lack of specific interactions with enzyme molecules.[14] To achieve a strong interaction for enzyme immobilization, the support must be functionalized with a variety of functional groups; however, the outcome might result in decreasing the enzyme activity.[15] Thus, to maintain the enzyme activity, new immobilizers with improved effectiveness are needed upon functionalization.

Metal-organic frameworks (MOFs)—also known as porous coordination polymers—are a new class of crystalline porous materials that consist of metal or metal oxide corners connected by organic linkers.[16] These highly ordered crystalline materials show unique properties, such as high-surface area (up to thousands $m^2 g^{-1}$), porosity, and tunable pore sizes[17,18] and have attracted considerable attention in applications for gas storage,[19] heterogeneous catalysis,[20] sensors,[21] chromatographic separation,[22] drug delivery,[23] and so on.

In recent years, research has become active towards MOFs biocatalytic applications by using these materials as immobilized carriers.[24–30] In contrast to mesoporous silicate materials, owing to its high surface area, MOFs are capable of carrying sufficient organic functional moieties without post-synthetic modification; thus, these kind of porous materials can exhibit superior enzymatic catalysis with stable recyclability for immobilization supports. Several strategies including covalent bonding,[24,28] encapsulation,[25] and physical adsorption[26,27,29,30] were employed to immobilize biomolecules into the MOFs. Among them, physical adsorption, without any chemical modification, is the most convenient way; however, a mesoporous MOF or a chemical modification in the enzyme macromolecule is needed. To the best of our knowledge, no microporous MOFs combined with simple physical adsorption have been used in the immobilization of an enzyme.

Herein, we explore the porcine pancreatic lipase (PPL)—one of the most widely used enzymes in the biotransformation reaction for chemical and pharmaceutical industries—as a test enzyme[31,32] to evaluate the potential of microporous MOFs as solid supports. Several microporous MOFs (UiO-66(Zr), UiO-66-$NH_2$(Zr), and MIL-53(Al) and carbonized MIL-53(Al); Table 1 and Table S3 in the Suppoting Information) were employed to adsorb PPL with particle sizes ranging from 150–200 nm. The UiO-66(Zr) was synthesized using $ZrCl_4$ and 1,4-benzenedicarboxylic acid ($H_2BDC$), whereas UiO-66-$NH_2$ was constructed by using $ZrCl_4$ and 2-amino-1,4-benzenedicarboxylic acid ($H_2BDC$-$NH_2$). Meanwhile, the other types of MOF such as MIL-53(Al) were produced by using $Al(NO_3)_3 \cdot 9H_2O$ and $H_2BDC$, whereas the carbonized MIL-53(Al) was formed by MIL-53(Al) and was heated up to 800 °C (details of these MOFs are shown in the Supporting Information). The PPL has a dimension of about $4.6 \times 2.6 \times 1.1$ nm; thus, the diffusion and accessibility of this large molecule can be limited in the microporous MOFs materials. The adsorption of PPL was carried out by using a freshly synthesized MOFs solid and was immersed in a PPL solution of methanol and DMSO followed by mixing using a vortex for 1 h (Scheme 1, step 1), and subsequently centrifuged (6000 rpm, 5 mins) to give a PPL@MOF powder (Supporting Information).

[a] Dr. W.-L. Liu, N.-S. Yang, Y.-T. Chen, S. Lirio, C.-Y. Wu, Prof. C.-H. Lin, Prof. H.-Y. Huang
Chung Yuan Christian University
200, Chung-Pei Rd., Chung-Li, 320 (Taiwan)
E-mail: chiaher@cycu.edu.tw
hyhuang@cycu.edu.tw

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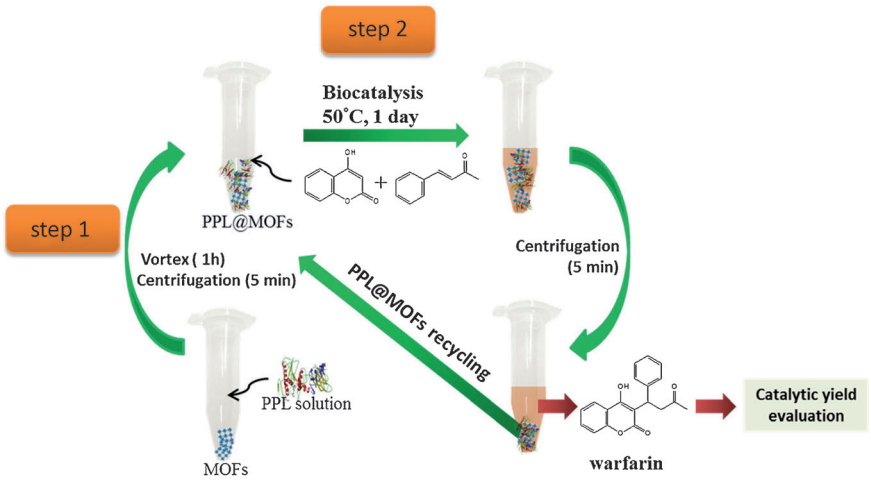
**Table 1.** Comparison of warfarin catalytic yields and loading capacities of PPL@MOFs or PPL@SBA-15.

| | Loading capacity[a] | Consecutive catalytic yield % (RSD %)[b] | | | | |
|---|---|---|---|---|---|---|
| | | 1st | 2nd | 3rd | 4th | 5th |
| in solution | – | 61.2 | – | – | – | – |
| UiO-66(Zr) | 202.4 | 86.9 (1.2) | 77.7 (1.2) | 73.0 (1.9) | 71.9 (1.9) | 70.6 (2.1) |
| UiO-66-$NH_2$(Zr) | 196.7 | 77.9 (1.0) | 74.7 (2.9) | 70.8 (2.4) | 67.2 (0.8) | 65.4 (0.4) |
| MIL-53(Al) | 196.1 | 79.0 (0.3) | 75.4 (1.8) | 73.4 (2.3) | 69.7 (0.6) | 67.4 (0.9) |
| carbonized MIL-53(Al) | 198.9 | 81.6 (1.6) | 79.4 (1.0) | 75.9 (1.2) | 72.4 (1.0) | 69.1 (1.1) |
| SBA-15 | 194.2 | 76.6 (0.1) | 74.6 (0.7) | 70.7 (0.8) | 66.5 (0.8) | 63.7 (0.8) |

[a] The unit is μmol PPL $g^{-1}$ support. [b] Values and relative standard deviation (RSD) were obtained from triplicate catalytic measurements.




**Scheme 1.** Warfarin synthesis catalyzed with PPL@MOFs.

As displayed in Scheme 1, step 1, a change in color for MOFs powder (for example, white UiO-66(Zr) solids turned into pale yellow; Figure S12, Supporting Information) was observed. The successful adsorption of PPL in different MOFs was confirmed by using spectroscopic techniques. In the FTIR sspectra, an amide bond (–CONH–) absorbance (1063, 1535, and 1676 $cm^{-1}$) was observed (Figures S5–S6, Supporting Information), which confirms the successful adsorption of PPL on the MOF's surface.

In addition, when using powder XRD spectra (Figure 1 and Figure S4 in the Supporting Information), an inherent characteristic peak still remains intact (the shift in the characteristic peaks in MIL-53(Al) was likely due to its strong breathing effect in different solvent environments (Figure S4, left images, Supporting Information). Moreover, when using SEM, the images (Figure S3, Supporting Information) showed no observable changes in the morphologies of MOFs after enzyme adsorption. With these results, successful adsorption of PPL on MOFs structure with good stability and crystallinity was developed.

The PPL@MOF bioreactor was then used to facilitate the Michael addition reaction of 4-hydroxycoumarin (A) and benzylideneacetone (B) (Scheme 1, step 2) to yield warfarin (C)—a common anticoagulant in the clinic[33]—which was determined by using capillary electrophoresis to assess the catalytic activity of the PPL@MOF bioreactor. The electropherograms have indicated that a very small amount of warfarin was produced either in the presence of UiO-66(Zr) MOFs or no PPL addition, whereas using a free PPL into the reactive medium (methanol; i.e., the usage of in-solution PPL) increases the amount of warfarin compound to 57.8% at room temperature (RT) for three days (Table S1, Supporting Information). Meanwhile, using PPL@UiO-66(Zr) as the catalyst yielded 76.3% of warfarin for the first-cycle and 58.3% for the fifth cycle (RT for 3 days; Table S1, Supporting Information), which was higher than the in-solution PPL. With these observations, the enhanced catalytic ability of the PPL enzyme after adsorption of PPL@UiO-66(Zr) as a biocatalyst with good reusability was confirmed.

Enzyme immobilization on solid supports is also regarded as a strategy in improving the enzyme activity even during drastic catalytic conditions. Thus, changing the reaction temperature was also evaluated in this study. When the reaction temperature was increased to 50 °C, no significant improvement was observed in PPL solution (~61.2% yield), whereas on the other hand, the PPL@UiO-66(Zr) produces 86.7% enhancement for the 1st cycle and 71.0% for the 5th cycle in a one-day reaction. (i.e., 1.41-fold enhancement when compared to in-solution PPL; Figure 2 and Table S1 in the Supporting Information).

When the reaction medium was changed to DMSO at 50 °C for a Michael addition reaction of 4-hydroxycoumarin and benzylideneacetone, a slight increase was observed in the production of warfarin for PPL@UiO-66(Zr) with 87.7% yield for the first cycle, which is relatively the same as for the free MOF with 87.1% yield (PPL in-solution). However, an abrupt change was observed in the fifth cycle with 13.2% yield (Table S1, Supporting Infortion). This is due to a decrease in the crystallinity of UiO-66(Zr), which was also confirmed in the PXRD spectra (Figure 1, bottom images). This spectra suggests that the catalytic ability of the enzyme is highly relative to the crystalline nature of the MOF material because it can lead to the high-order dispersion of enzyme in the MOFs support.

To explore the catalytic activity of MOFs, different kinds of microporous MOFs such as cage-type (UiO-66-$NH_2$(Zr)), tunnel-type (MIL-53(Al) and carbonized MIL-53(Al)) were used as solid supports for PPL adsorption (Scheme 1, step 1). In contrast to

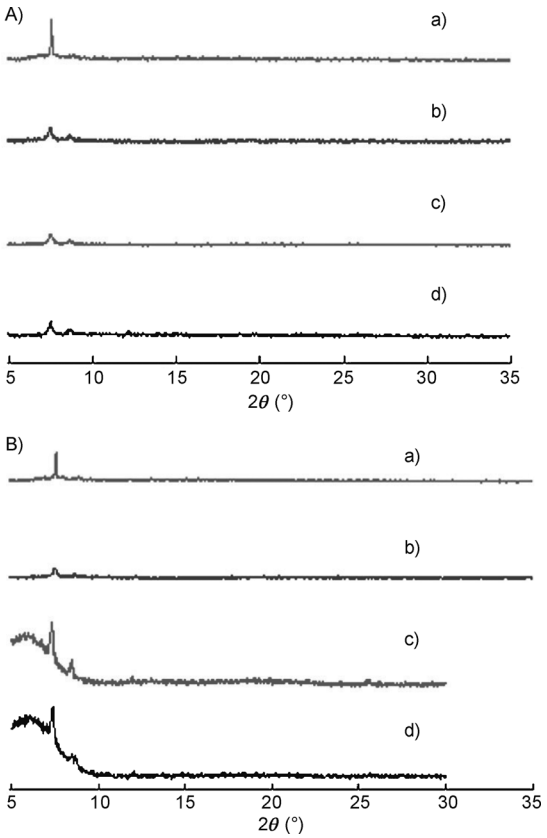



**Figure 1.** Powder XRD patterns of a) as-synthesized UiO-66(Zr), b) PPL@UiO-66(Zr), c) PPL@UiO-66(Zr) after first use, and d) PPL@UiO-66(Zr) after fifth use. Reaction solvent is methanol (A) and DMSO (B), respectively.

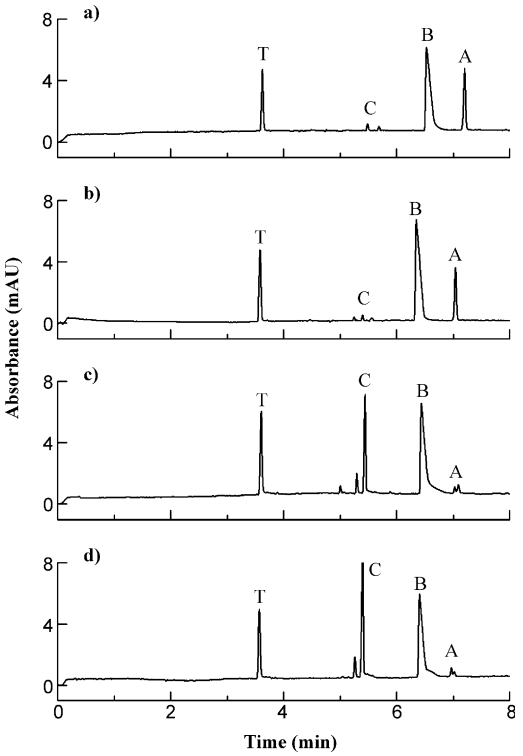



**Figure 2.** Electropherograms of the synthesized warfarin catalyzed by PPL@UiO-66(Zr): a) no PPL&UiO-66(Zr), b) UiO-66(Zr) only, c) in-solution PPL, and d) PPL@UiO-66(Zr) catalytic cycle 1. Capillary electrophoresis conditions: running buffer, 132.5 mM borax buffer and 15 mM SDS, pH 8.5; sample concentration: catalytic product diluted 100-fold with running buffer; capillary, 60 cm × 50 μm ID; separation voltage, 28 kV; normal injection, 0.5 psi for 3 s. T (thiourea), A (reactant, 4-hydroxycoumarin), B (reactant, benzylideneacetone), and C (product, warfarin).

free PPL, the adsorbed PPL enzyme on MOFs provided a superior catalytic activity in the synthesis of warfarin with almost the same activity when reused. As shown in Table 1, the catalytic activity of UiO-66-$NH_2$(Zr), MIL-53(Al), and carbonized MIL-53(Al) in producing warfarin for the first to fifth cycle ranges between 77.9 to 65.4%, 79.0 to 67.4%, and 81.6 to 69.1%, respectively. In comparison with UiO-66(Zr), the UiO-66-$NH_2$(Zr) carries hydrophilic amine moieties causing an 11% decrease in the production of warfarin, whereas 5% enhancement for carbonized MIL-53(Al) than the natural MIL-53(Al) [for first catalytic use]. In addition, it shows that the hydrophobicity of the natural UiO-66(Zr) and carbonized MIL-53(Al) were favourable on the adsorption of the hydrophobic lipase on the MOF's surface. Some reports have also indicated that the active sites could be exposed and the active stability is likely enhanced when lipase is attached on hydrophobic supports due to its structure transformation.[34] Thus, in achieving a good catalytic activity, functionalization is often needed in supporting the lipase, however, in this study it was eliminated when the MOFs were used as supports because of the wide range nature of MOFs.

SBA-15, a common mesoporous silica material was also used to compare the catalytic activity with the PPL@MOFs. The SBA-15 was also successful in PPL adsorption by mixing in a vortex for 1 h. The result showed a lower product formation of warfarin in the catalytic activity of PPL@SBA-15 ranging from 76.6 to 64.3% for the first to fifth cycle under the same reaction conditions (Table 1). The loading capacity of MOFs and SBA-15 was further investigated by the bicinchoninic acid protein assay (BCA) method, which shows 202.4 (UiO-66(Zr)), 196.7 (UiO-66-$NH_2$(Zr)), 196.1 (MIL-53(Al)), 198.9 (carbonized MIL-53(Al)), and 194.2 $\mu mol\,g^{-1}$(SBA-15), respectively (Table 1). The PPL loading capacities observed on these MOFs were consistent with their catalytic efficiency in the adduct formation of warfarin synthesis.

It is noteworthy to mention that the recovered MOFs after PPL adsorption for each cycle revealed in the SEM images have the same morphologies (Figure S3, Supporting Information). Table 2 summarizes the pore size and surface area of MOFs and PPL@MOFs after warfarin synthesis. When dried after the adsorption procedure, the enzymes form a conjugated impermeable layer thereby decreasing the surface area of the MOF. Compared to MOFs, an almost 99% decrease in the surface area was observed after PPL attachment, and remains the same even after the fifth cycle of PPL@MOFs, thereby indicating large enzyme coverage on the MOFs' surface with stable attachment after further catalytic cycles. Meanwhile, the surface area of PPL@SBA-15 contained a slight increase of about 5% after the fifth cycle. This is due to the pore size of SBA-15

**Table 2.** Pore size and surface area of MOFs.

| MOFs | As-synthesized MOF pore size [nm] | | PPL@MOF | PPL@MOF catalytic cycle 1 BET surface area [$m^2 g^{-1}$] | PPL@MOF catalytic cycle 5 |
|---|---|---|---|---|---|
| UiO-66(Zr) | 0.6, 1.0, 1.2 | 1052 (100%)[a] | 2.39 (0.23%) | 5.81 (0.52%) | 7.20 (0.68%) |
| UiO-66-$NH_2$(Zr) | 0.6, 1.2, 1.4 | 832 (100%) | 6.609 (0.79%) | 11.448 (1.37%) | 20.646 (2.48%) |
| MIL-53(Al) | 0.5 | 964 (100%) | 2.46 (0.26%) | 3.52 (0.37%) | 6.93 (0.72%) |
| carbonized MIL-53(Al) | 0.7 | 1089 (100%) | 10.24 (0.94%) | 10.94 (1.01%) | 20.24 (1.86%) |
| SBA-15 | 5.0 | 789 (100%) | 35.19 (4.46%) | 58.34 (7.39%) | 70.92 (8.99%) |

[a] Relative ratio = (surface area of PPL@supports/supports) × 100%.

(~5 nm) that causes PPL leakage during and after the catalytic cycle that disabled the entrapment of the PPL macromolecules within the mesopores. This assumption also agrees with the PPL loading capacity as well as the catalytic ability in warfarin synthesis using SBA-15 materials (Table 1). In contrast to SBA-15, the MOFs contained a larger surface area thereby increasing the amount of PPL molecules adsorbed in MOFs that leads to a higher catalytic activity and provides an excellent reusability in adduct formation of warfarin.

Enzyme storage and catalytic stability play an important role in a reaction. Thus, the storage time and catalytic stability between batch-to-batch produced PPL@MOFs were also studied. The PPL@MOFs showed no significant decay activity (over 65% warfarin yields obtained after 35 days stored at 4 °C; Figure S14, Supporting Information) as well as highly reproducible catalysis ability (lower than 3% relative standard deviation in product formation of warfarin obtained from PPL@MOFs prepared in three different batches; Table S3, Supporting Information). Lastly, the activity and stability of PPL@UiO-66(Zr)s used to catalyze the hydrolysis of *p*-nitrophenyl palmitate were also studied. The results revealed excellent hydrolysis efficiency and stability when reused (hydrolysis activity in the range of 100 to 67.5% within 5 reuses; Table S2, Supporting Information). With high enzyme activity as well as stable reusability, the usage of microporous MOFs to adsorb PPL macromolecules is highly competitive when compared to other common enzyme-immobilized supports reported so far.[35,36]

To summarize, we have demonstrated a potential crystalline microporous MOF material for enzyme adsorption bioreactors without any chemical modification on the MOF's surface or enzyme macromolecule. With the aid of simple vortex-assisted enzyme adsorption, the formation of PPL@MOFs particles provided an exceptional catalytic ability and reusability especially when using carbonized MOFs or hydrophobic MOFs as solid supports. This is also the first report on warfarin synthesis by enzyme adsorption as a biocatalyst (Table S4, Supporting Information) and herein successful clinical medicine synthesis by the novel MOF-biocatalyst was established. These findings would be beneficial for future studies in the industrial production of chemical, pharmaceutical, and agrochemical precursors. Simultaneously, this strategy to fabricate biocatalysts is green and sustainable.

## Experimental Section

### PPL adsorption onto MOFs

The solid support (2 mg, MOFs or SBA-15) was immersed into PPL solution (200 μL; 25 mg PPL, commercial source from Sigma (Lot #SLBC9250 V), dissolved in 100 μL MeOH and 100 μL DMSO), and the mixture was gently vortexed at RT for 1 h. The produced PPL adsorbed MOFs (PPL@MOFs or PPL@SBA-15) were washed two times with 200 μL MeOH prior to further catalysis and submission to other tests.

### Warfarin synthesis via in-solution PPL and PPL@MOFs

The traditional PPL in-solution catalysis procedure was performed by mixing PPL (5 mg), 4-hydroxycoumarin (reagent A, 1 mg), and benzylideneacetone (reagent B, 4.5 μL) in MeOH (100 μL) and then the mixture was stirred at 50 °C for 1 day. Similarly, the adsorbed PPL catalysis was performed by mixing PPL@MOFs (2 mg), reagent A (1 mg), and reagent B (4.5 μL) in MeOH (100 μL). The mixture was then stirred at 50 °C for 1 day. The warfarin product was separated from the solid MOF biocatalysts by 5 min centrifugation at 10000 rpm.

### Conditions for capillary electrophoresis

Separation of reagents and product was performed with a Beckman P/ACE™ MDQ CE system and the detector wavelength was set at 214 nm. Uncoated fused-silica capillaries (60 cm × 50 μm ID, with an effective length of 50 cm) were obtained from Polymicro Technologies (Phoenix, AZ, USA) and new capillary was flushed with 1 M sodium hydroxide and deionized water for 3 and 5 min, respectively.

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**Keywords:** green chemistry · lipase · metal–organic frameworks · physical adsorption · warfarin

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**Biochemistry**

*W.-L. Liu, N.-S. Yang, Y.-T. Chen, S. Lirio, C.-Y. Wu, C.-H. Lin,* H.-Y. Huang**

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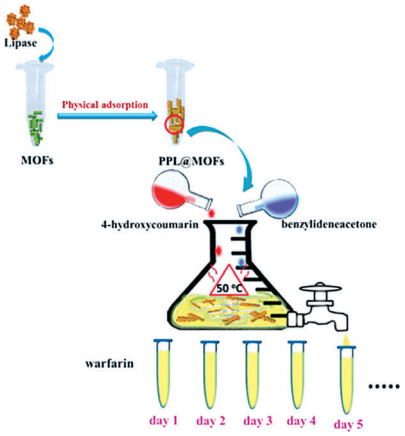
## Lipase-Supported Metal–Organic Framework Bioreactor Catalyzes Warfarin Synthesis




**Green chemistry**: A green and sustainable strategy synthesizes clinical medicine warfarin anticoagulant by using lipase-supported metal–organic framework (MOF) bioreactors (see scheme). These findings may be beneficial for future studies in the industrial production of chemical, pharmaceutical, and agrochemical precursors.