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# Fabricate Covalent Organic Framework Capsules with Commodious Microenvironment for Enzymes

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**KEYWORDS:** covalent organic frameworks, capsule, immobilization, biomacromolecules, biocatalysis

**ABSTRACT:** Enzyme immobilization has demonstrated itself to be a favorable protocol to promote industrialization of biomacromolecules. Despite tremendous efforts to develop new strategies and materials to realize this process, maintaining enzyme activity is still of formidable challenge. Herein, we created a sacrificial templating method, using metal-organic frameworks (MOFs) as sacrificial templates to construct hollow covalent organic framework (COF) capsules for enzyme encapsulation. This strategy can provide capacious microenvironment to unleash enzyme molecules. The improved conformational freedom of enzymes, enhanced mass transfer and protective effect against external environment ultimately boosted the enzymatic activities. We also found that this strategy possesses high versatility that is suitable for diverse biomacromolecules, MOF templates and COF capsules. Moreover, the dimensions, pore sizes and shell thickness of COF capsules can be conveniently tuned, allowing for customizing bioreactors for specific functions. For example, co-encapsulation of different enzymes with synergistic functions were successfully demonstrated using this bioreactor platform. This study not only opens up a new avenue to overcome the present limitations of enzymatic immobilization in porous matrixes, but also provides new opportunities for construction of bio-microdevices or artificial organelles based on crystalline porous materials.

## INTRODUCTION

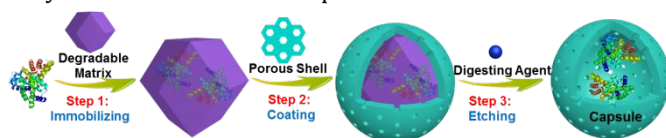
As natural catalysts, enzymes possess fascinating characteristics, such as high efficiency, regio- and stereoselectivity, allowing for continual advances in biotechnological applications.<sup>1,2</sup> However, the intrinsically fragile nature of enzymes greatly impedes their industrialization. Enzyme immobilization has become a favorable strategy with increased stability and reusability, easy operation, energy conservation and continuous-flow production, prompting the application of enzymes.<sup>3-11</sup> Despite these merits, most confined enzymatic systems still confront great challenges. For instance, tightly packed enzymes within host materials typically lack conformational freedom that may greatly affects molecular recognition.<sup>12,13</sup> Mass transfer of substrates or products can also be a limiting factor, and thus internally-adsorbed enzymes may display substantially reduced activity.<sup>14,15</sup> Moreover, traditional incorporation methods such as adsorption often suffer from low loading or high leaching due to weak interaction or pore size mismatching.<sup>16</sup>

Exploration of new platforms to overcome these challenges for optimal enzyme performance in confined space is, therefore, in urgent demand.

Compartmentalization of biomacromolecules is a common phenomenon in living systems.<sup>17,18</sup> The eukaryotic cell is a prototypical example whereby the unique biochemical microenvironment allows the spatial control of various functions. Such enclosed units confer physical protection against external disturbance, while maintaining a certain degree of freedom for the internal contents.<sup>19,20</sup> Inspired by nature, the design and construction of biomimetic 'smart capsules' can be a promising approach to overcome the challenges of confined enzymatic systems.<sup>21-23</sup> Organic polymer and inorganic nanoparticles are materials commonly used to prepare capsules through various approaches.<sup>18,24,25</sup> However, they may suffer from irregular structure or lack of chemical functionality.<sup>26,27</sup> Covalent organic frameworks (COFs) are an emerging class of crystalline porous materials with high and well-defined porosity, high stability, and readily engineered

functionality, allowing them as excellent hosts for enzyme encapsulation.<sup>28-31</sup> However, the harsh synthetic conditions required in the fabrication of COFs make it impractical to directly make COF capsules encapsulating biomacromolecules through a “one-pot” strategy.<sup>32-34</sup> The sister material of COFs, metal-organic frameworks (MOFs) can in-situ encapsulate biomacromolecules to form biomacromolecule@MOF (@ = encapsulating) with high loading and good protection.<sup>35-38</sup> However, this often results in a penalty to lose the conformational freedom of enzymes, leading to a detrimental effect on enzyme activity. Many reports including our recently work have proved that biomacromolecule@MOF can be easily digested to release biomacromolecules without losing their bioactivities which render MOFs as perfect sacrificial templates to make COF capsules.<sup>37</sup> Combining the benefits of both COFs and MOFs to form ‘smart capsules’ may open up new opportunities to greatly enhance the performance of confined enzyme: i) the MOF encapsulation can protect enzymes from harsh synthetic conditions, such as those amenable to COF formation, allowing for COFs to directly coat on enzyme@MOF particles; ii) the high porosity of COF shells then allows for subsequent digestion of MOF cores while facilitating mass transfer of substrates resulting in active, conformationally unhindered enzymes within a protective COF capsule.

Herein, we created a versatile approach to fabricate biomimetic ‘smart COF capsules’ for the encapsulation of active biomacromolecules. Biomacromolecules are first encapsulated within digestible MOFs via in-situ encapsulation to form biomacromolecules@MOF which can protect biomacromolecules and act as templates for the growth of biomacromolecule@MOF@COF core-shell structures. Finally, the MOF core was etched away to liberate the biomacromolecules within the hollow COF capsule (Scheme 1). Such a commodious microenvironment in the formed biomacromolecule@COF offers protective effects while maintaining the conformational freedom of biomacromolecules and facilitating the mass transfer of substrates/products, which is essential for high activity or synergistic effect of different enzymes. This study opens up a new avenue to overcome the present limitations of enzymatic immobilization in porous matrixes.

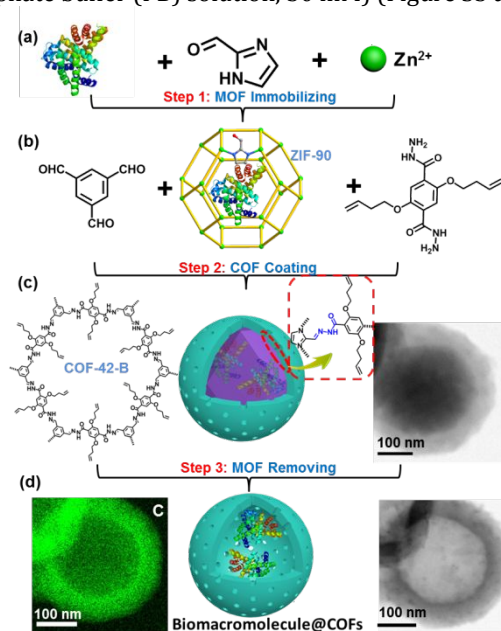


**Scheme 1.** Schematic illustration of the stepwise approach to construct biomacromolecule@capsule.

## RESULTS AND DISCUSSION

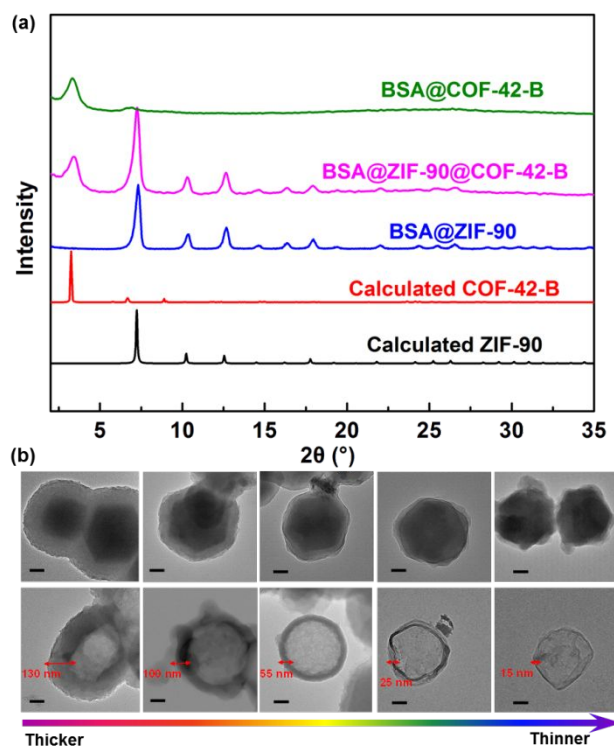
**Materials Preparation and Characterizations.** A supporting matrix is required for COF capsule formation in order to protect enzymes against harsh synthetic conditions and to act as a template. ZIF-90 has been previously shown to efficiently load various biological active macromolecules

(e.g. enzymes) and protect them against harsh conditions while also being feasibly digested under mildly acidic conditions.<sup>35-37</sup> Thus, ZIF-90 is a perfect candidate to demonstrate the concept due to the presence of unreacted aldehyde groups in the ligand 2-imidazolecarbaldehyde (ICA), which could be anticipated in the amine-aldehyde condensation reaction involved in a subsequent COF formation, and therefore allows the MOF particles as templates in the formation of COF shells (Figure 1). In order to maintain the activity of biomacromolecules during the synthesis process, an acid-stable COF that can be synthesized under mild conditions is then required for the construction of capsules, for which COF-42 was chosen.<sup>39,40</sup> COF-42 was synthesized via the room temperature condensation of 1,3,5-triformylbenzene (TB) and 2,5-Bis(ethoxy)terephthalohydrazide (BETH) under acetic acid catalysis. However, because protic acids could decompose the enzyme@MOF complexes, a MOF-compatible catalyst, Sc(OTf)<sub>3</sub>,<sup>41</sup> was used instead of acetic acid. Various structural analogues of the BETH monomer were screened to find one that produced a crystalline COF-42 product (Table S1, Figure S1 and S2). It was found that only 2, 5-Bis(butenyloxy)terephthalohydrazide (BBTH) succeeded to afford a crystalline COF-42 analogue (named as COF-42-B) which was stable under acid condition (e.g. pH = 5, phosphate buffer (PB) solution, 50 mM) (Figure S3 and S4).



**Figure 1.** Illustration of the synthetic route of biomacromolecule@COF capsules. (a) Immobilizing biomacromolecules in ZIF-90 via an in-situ encapsulation method; (b) A one-pot reaction of ZIF-90 with COF monomers; (c) Illustration of the core-shell structure of biomacromolecule@ZIF-90@COF-42-B (middle), structure of COF-42-B (left), a TEM image of the core-shell structure (right); (d) Illustration of the capsule structure of biomacromolecule@COF-42-B (middle), EDS carbon

distribution (left) and a TEM image (right) of biomacromolecule@COF-42-B capsule.



**Figure 2.** Characterizations of BSA@COF-42-B capsule. (a) PXRD patterns of products in different synthesis stage. BSA@ZIF-90 (blue), BSA@ZIF-90@COF-42-B (purple), BSA@COF-42-B (green), calculated pattern of ZIF-90 (black) and COF-42-B (red); (b) TEM images showing BSA@ZIF-90@COF-42-B precursors (upper) and BSA@COF-42-B capsules (below) with different shell thickness tuned by adding different amounts of BSA@ZIF-90 (from left to right, 6.0 mg, 9.0 mg, 12.0 mg, 15.0 mg and 18.0 mg). Scale bar: 100 nm.

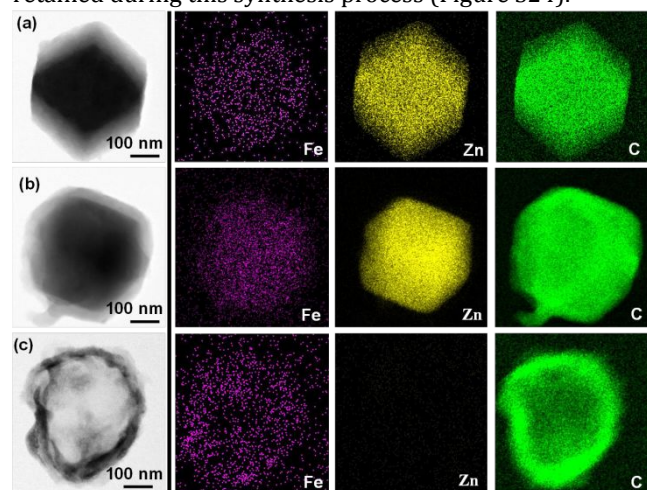
In order to verify the construction strategy of COF capsules, BSA (Bovine Serum Albumin) was chosen as a model biomacromolecule on the basis of its low cost and high solubility. BSA@ZIF-90 was first prepared via the in-situ encapsulation method according to literature.<sup>36</sup> The encapsulation efficiency of BSA was measured to be as high as 99%, using a standard Bradford Assay, and the loading percentage was calculated to be 0.22 g BSA per g ZIF-90 (Figure S5). Subsequently, the as-prepared BSA@ZIF-90 was mixed with TB, BBTH and Sc(OTf)<sub>3</sub> in 1, 4-dioxane/mesitylene (3:1 v/v) for 30 min. PXRD patterns, TEM images, showed a combination of two crystalline phases (i.e. ZIF-90 and COF-42-B) (Figure 2 and S6), indicating the existence of BSA@ZIF-90@COF-42-B. FT-IR results further verified the successful encapsulation of BSA (Figure S7). Our previous result has demonstrated that ZIF-90 can be digested in aqueous solution with pH < 7.<sup>37</sup> We then exposed the as-prepared BSA@ZIF-90@COF-42-B to a PB solution (pH = 5, 50 mM). The progress of digesting

reaction was monitored by TEM, which showed a clear transformation from the core-shell structure to a hollow capsule within 1 h. Tracking characteristic PXRD peaks of ZIF-90 afforded a result consistent with TEM data (Figure S8 and S9). Energy dispersive spectrometer (EDS) element mapping further demonstrated the successful construction of COF-42-B capsules (Figure 1d). Porosity of these materials was found to be retained during the synthetic procedure (Figure S10). N<sub>2</sub> sorption data collected at 77 K revealed the Langmuir surface areas of 1390 m<sup>2</sup>/g, 610 m<sup>2</sup>/g, 436 m<sup>2</sup>/g and 835 m<sup>2</sup>/g, BET surface areas of 723 m<sup>2</sup>/g, 367 m<sup>2</sup>/g, 235 m<sup>2</sup>/g and 530 m<sup>2</sup>/g, pore size distribution centered around 0.9 nm, 1.8 nm, 1.2 nm, and 1.8 nm for BSA@ZIF-90, COF-42-B, BSA@ZIF-90@COF-42-B and BSA@COF-42-B, respectively. Moreover, it was found that the thickness of the COF-42-B shell could be readily adjusted by altering the amount of added BSA@ZIF-90. Upon increasing the amount of BSA@ZIF-90, the PXRD reflection peaks assigned to ZIF-90 exhibited increased intensity relative to the intensity of the COF-42-B peaks (Figure S11), and TEM images showed the COF shell varying from 130 nm to 15 nm (Figure 2b). A control experiment was further performed to test if BSA can be directly incorporated into COF-42-B via the traditional pore adsorption method. The attempt failed because the channel size of COF-42-B was much smaller than the molecule size of BSA (Figure S12).

**Exploration of the generality of capsule fabrication strategy.** In order to explore the generality of this synthetic strategy, we examined the use of ZPF-2 (ZPF = Zeolitic Pyrimidine Framework) as the sacrificial template, instead of ZIF-90. ZPF-2 with a *sod* topology was synthesized from Zn(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O and 2-Hydroxy-5-fluoropyrimidine (Figure S13) in aqueous solution at room temperature.<sup>42</sup> Herein, for the first time, we demonstrated that ZPF-2 can efficiently incorporate biomacromolecules via in-situ encapsulation method. As revealed by TEM, PXRD, and FT-IR (Figure S14-S16), ZPF-2 can efficiently encapsulate BSA and serve as a sacrificial template to yield hollow BSA@COF-42-B capsules. Additionally, we found that digesting rate of ZPF-2 was faster than ZIF-90 and the MOF cores was completely removed in 10 min (Figure S17). Moreover, we found ZIF-8 can also be applied to fabricate COF capsules via a similar approach as ZIF-90 and ZPF-2 (Figure S18-S20). However, only a relatively small amount of ZIF-8 could be incorporated within COF-42-B because increased amounts of ZIF-8 inhibited the formation of COF-42-B. We also explore whether other COF materials can be used to make COF capsules. A hollow COF capsule containing BSA was successfully fabricated by COF-43-B (Figure S21 and S22).<sup>39</sup> This stepwise strategy has, therefore, proven to be an adaptable method to fabricate biomacromolecules-containing hollow COF capsules that could foreseeably be modified for use in highly specific applications.

**Biocatalysis application of COF capsules.** On the basis of the successful encapsulation of BSA in COF capsules, we further explored whether enzymes can be trapped within

COF capsules. CAT is a well-known multimeric protein with four subunits, which can catalyze the breakdown of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) to generate  $\text{O}_2$  and  $\text{H}_2\text{O}$ , protecting cells from the oxidative damage caused by hydrogen peroxide.<sup>43</sup> In order to immobilize CAT within COF capsules, ZPF-2 was selected as the model sacrificial MOF. EDS mapping was performed to confirm the successful encapsulation of CAT within the capsules. As shown in Figure 3, Fe, the characteristic element present in CAT, was uniformly dispersed in all samples; indicative of the successful encapsulation of CAT. Additionally, confocal images further verified the uniform dispersion of CAT in all samples (Figure S23), and circular dichroism (CD) and UV-Vis spectra confirmed that the structure of CAT was retained during this synthesis process (Figure S24).



**Figure 3.** Bright field images (left) and EDS elemental mapping (right) of Fe, Zn and C for (a) CAT@ZPF-2, (b) CAT@ZPF-2@COF-42-B and (c) CAT@COF-42-B.

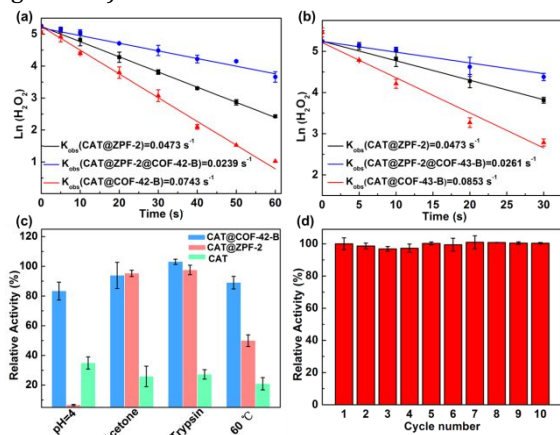
Factors such as particle size and capsule shell thickness were also investigated to optimize the catalytic activity of this novel enzyme-incorporated system. A series of CAT-loaded ZPF-2 crystals with particle sizes ranging from 100 nm to 8000 nm were synthesized (Figure S25). TEM images were used to confirm particle size uniformity for each sample (Figure S26a). Catalytic activity towards  $\text{H}_2\text{O}_2$  was evaluated via the FOX method (Figure S27 and S28). With the same amount of enzymes, CAT@ZPF-2 achieved the highest  $K_{\text{obs}}$  of  $0.0473 \text{ s}^{-1}$  with a particle size of  $\sim 1500 \text{ nm}$ . COF capsule shell thickness was also tuned by varying the amount of added CAT@ZPF-2 core (with particle size of  $\sim 1500 \text{ nm}$ ). The results reveal that the activity of enzyme@COF-42-B capsules obeyed a Gaussian distribution when shell thickness was modified (Figure S29), and the best performance was achieved with a shell thickness of  $\sim 60 \text{ nm}$  ( $K_{\text{obs}} = 0.0743 \text{ s}^{-1}$ ) (the loading capabilities was  $1.66 \text{ g/g}$ ). Generally, the thicker capsule shell is, the higher mass transfer resistance exists. Besides thickness, capsule size of COF-42-B also plays a crucial role in the activity of encapsulated enzymes. Thereafter, we

prepared a series of COF capsules in the range of 350-1500 nm, with a capsule thickness of  $\sim 60 \text{ nm}$  (Figure S26b-g), and found that capsules of  $\sim 1500 \text{ nm}$  exhibited the best performance (Figure S30). Thus, the optimized COF capsules of  $\sim 1500 \text{ nm}$  size and  $\sim 60 \text{ nm}$  thickness were used for further study.

The biocatalytic assay revealed that CAT@ZPF-2@COF-42-B before MOF etching presented 49% lower enzyme activity than CAT@ZPF-2, which should be ascribed to the high mass transfer resistance in CAT@ZPF-2@COF-42-B. After etching treatment, the generated CAT@COF-42-B capsule demonstrated a dramatic 58% improvement of activity compared to CAT@ZPF-2, which could be 210% when compared to CAT@ZPF-2@COF-42-B (Figure 4a). Negligible leaching of CAT was detected during the etching process (Figure S31). A further comparison of the kinetics of CAT@ZPF-2 and CAT@COF-42-B revealed that their  $V_{\text{max}}$  and  $K_{\text{M}}$  were  $2.73 \mu\text{M/s}$  and  $0.0625 \text{ mM}$ , and  $3.98 \mu\text{M/s}$  and  $0.0531 \text{ mM}$ , respectively (Figure S32). Although their activities are lower than free CAT (Figure S33), the results show significantly boosted biocatalytic activity for CAT@COF-42-B capsule structure after the removal of MOFs, indicating the unleashing CAT molecules from the tightly packed MOF composite to the commodious microenvironment of COF capsules is beneficial for enzymatic performance. Additionally, we also investigate the CAT-loaded COF-43-B capsules which showed a similar trend as COF-42-B (Figure S34). After optimization, the activity of CAT@COF-43-B exceeded CAT@COF-42-B and showed an increase up to 81% compared with that of CAT@ZPF-2 (Figure 4b) (loading capabilities was  $1.49 \text{ g/g}$ ). This result is probably due to the larger pore size of COF-43-B ( $3.2 \text{ nm}$ ) compared with COF-42-B ( $1.9 \text{ nm}$ ) (Figure S35) that facilitate the mass transport of substrates and products. These results indicate that the synthetic strategy can be applied as a versatile and tunable approach for on-demand preparation of high efficient bioreactor based on COF capsules.

**Exploration of protection effect and reusability of COF capsules.** The protection effect of the formed COF capsules was examined under various perturbation conditions, such as acid, proteases, acetone and high temperature (Figure 4c). CAT@COF-42-B prepared via CAT@ZPF-2 was selected as a representative. First, we assessed its acid tolerance in disodium hydrogen phosphate-citric acid buffer ( $100 \text{ mM}$ ,  $\text{pH} = 4$ ) for 30 min. CAT@COF-42-B was seen to retain 85% of its original activity while free CAT retained only 35%, and CAT@ZPF-2 lost almost all activity ( $>99\%$ ) due to the leaching of CAT resulted from dissolution of ZPF-2 under the acidic condition (Figure S36a). COF-42-B was shown to protect CAT with 95% of original activity retained after exposure to acetone for 60 min. By contrast, free CAT retained only 25% of activity. A similar result was observed after exposure to protease trypsin ( $6 \text{ mg/mL}$ ) for 60 min: CAT@COF-42-B retained  $\sim 100\%$  of its activity, while free CAT retained only 25% of activity. Next, the influence of heating was evaluated.

After treatment under 60 °C for 10 min, free CAT and CAT@ZPF-2 retained only 20% and 50% of original activity, respectively, while CAT@COF-42-B retained 88% of original enzymatic activity. These results revealed that CAT@COF-42-B capsules demonstrated superior protective effect that surpassed its counterparts such as free CAT and CAT@ZPF-2. Furthermore, we investigated the catalytic durability and stability of CAT@COF-42-B, which is an important criterion for heterogeneous biocatalysts. CAT@COF-42-B can be readily recovered without significant loss of activity for at least 10 cycles (Figure 4d), and negligible leakage was detected during recycling experiments (Figure S36b). In comparison, traditional porous materials such as SBA-15 and active carbon were used to immobilize CAT via the conventional adsorption method. Their loading capabilities were demonstrated to be 0.21 g/g, and 0.09 g/g, respectively (Figure S37 and S38). The formed CAT composites exhibited much lower enzyme loading or activity compared with the CAT@COF capsule (~1.6 g/g, Figure S39), and exhibited no recyclability (Figure S40).

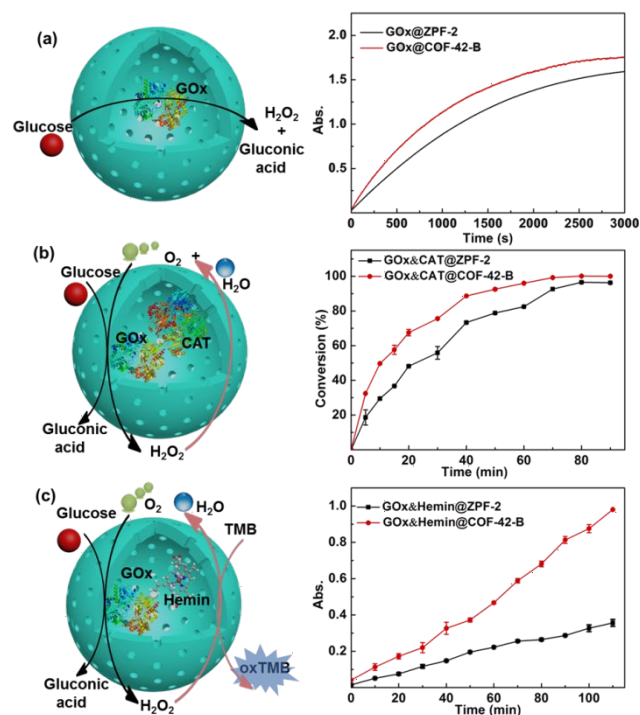


**Figure 4.** Biological activity of CAT@COF capsules. (a) Kinetics of degradation of H<sub>2</sub>O<sub>2</sub> for CAT@ZPF-2 (black), CAT@ZPF-2@COF-42-B (blue) and CAT@COF-42-B (red). (b) Kinetics of degradation of H<sub>2</sub>O<sub>2</sub> for CAT@ZPF-2 (black), CAT@ZPF-2@COF-43-B (blue) and CAT@COF-43-B (red). (c) Activity percentage of CAT, CAT@ZPF-2 and CAT@COF-42-B after treatment under various harsh conditions. (d) Recycling experiments of CAT@COF-42-B.

#### Cascade reactions conducted in COF capsule systems.

In practical applications, two or more enzymes are often required to cooperate with each other for advanced functions.<sup>44</sup> Fabricating multi-enzyme architectures has garnered increasing attention with great potential in the field of biocatalysis and advanced microdevice, such as 'artificial organelles'.<sup>27,45,46</sup> For example, GOx can spontaneously oxidize glucose into gluconic acid, meanwhile producing H<sub>2</sub>O<sub>2</sub> which can serve as the substrate of CAT. Combining these two enzymes represents a model cascade reaction of a biologically relevant system.<sup>47</sup> To prove the concept of cascade reactions in COF capsule, GOx

was firstly encapsulated in COF-42-B capsules like CAT. We found that GOx@COF-42-B using ZPF-2 as a sacrificial template exhibited an improved activity (~170%) compared to GOx@ZPF-2 (Figure 5a). We co-encapsulated GOx and CAT into COF-42-B and examined their cascade reaction effect. The cascade catalytic efficiency was evaluated by the detection of glucose consumption using the 3,5-dinitrosalicylic acid (DNS) method (Figure S41).<sup>48</sup> Compared to GOx@ZPF-2, GOx&CAT@ZPF-2 exhibited a much higher glucose consumption rate attributed to the participation of CAT. As shown in Figure 5b, GOx&CAT@COF-42-B composite produced a 130% consumption rate of glucose compared to its counterpart (GOx&CAT@ZPF-2). These results demonstrated the synergistic effect between the two enzymes was significantly enhanced after unleashed from ZPF-2 to the commodious COF capsule. To further prove the concept of cascade reactions in COF capsule, another multi-enzyme bioreactor based on GOx&Hemin@COF-42-B was evaluated spectroscopically using 3,3',5,5'-Tetramethylbenzidine (TMB) and glucose as substrates, accompanying by a colorimetric change detectable at 650 nm (Figure 5c). The results revealed that GOx&Hemin@COF-42-B showed an enhanced activity of 180% compared to its counterpart (GOx&Hemin@ZPF-2), and further validated COF capsule can provide the freedom to multiple enzymes for better cascade performance, which implying potential applications in the fabrication of bioreactors or advance microdevices.



**Figure 5.** Catalytic performance of various systems. (a) GOx@ZPF-2 and GOx@COF-42-B activity using a colorimetric

assay based on the oxidation of ABTS by a Cyt *c* coupled system ( $\lambda = 660$  nm). (b) GOx&CAT@ZPF-2 and GOx&CAT@COF-42-B activity based on the kinetic consumption of glucose. (c) GOx&Hemin@ZPF-2 and GOx&Hemin@COF-42-B activity using a colorimetric assay.

## CONCLUSION

In conclusion, we created a facile three-step approach to fabricate novel COF capsules to encapsulate various biomacromolecules which cannot be easily incorporated by COFs via traditional approaches (i.e. adsorption method). Firstly, biomacromolecules are first encapsulated within digestible MOFs via in-situ encapsulation to form biomacromolecule@MOF systems. This composite can further act as templates for the growth of biomacromolecule@MOF@COF core-shell structures. Finally, the MOF cores were etched away to unleash the tied biomacromolecules within the hollow COF capsule. This facile, scalable and effective strategy afforded high-performance COF bioreactors, which can maintain enzyme conformational freedom, stabilize the enzyme and meanwhile allow effective diffusion of the substrates and products. The COF capsules can provide capacious internal environments for the optimal performance of the encapsulated biomacromolecules with good protection effect against external perturbations. The encapsulated enzymes in COF capsules exhibited excellent reusability which surpassed the traditional porous materials such as SBA-15. In addition, this synthetic platform exhibits high versatility and tunability: different sacrificial MOF templates (e.g. ZIF-90, ZIF-8 and ZPF-2) and COF shells (COF-42-B and COF-43-B), as well as various enzymes (e.g. CAT, GOx) were used in this study; various factors such as the COF shell thickness and pore size could be fine-tuned to optimize the performance the bioreactors. We also demonstrated that multiple enzymes could be easily co-encapsulated within one COF capsule using this synthetic strategy and act as advanced bioreactors capable of efficient cascade reactions. Such diverse advances may inform the further understanding of enzyme behavior in both *in vivo* and *in vitro* systems, and expand the possibilities for the construction of microdevices, artificial organelles, and even artificial cells.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website. Detailed experimental procedures, material, FT-IR, <sup>1</sup>H NMR, PXRD, TEM, and so on.

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### Notes

The authors declare no competing financial interest.

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## REFERENCES

- (1) Schmid, A.; Dordick, J. S.; Hauer, B.; Kiener, A.; Wubbolts, M.; Witholt, B. Industrial Biocatalysis Today and Tomorrow. *Nature* **2001**, *409* (6817), 258-268.
- (2) Prasad, S.; Roy, I. Converting Enzymes into Tools of Industrial Importance. *Recent Pat. Biotechnol.* **2018**, *12* (1), 33-56.
- (3) Kuchler, A.; Yoshimoto, M.; Luginbuhl, S.; Mavelli, F.; Walde, P., Enzymatic Reactions in Confined Environments. *Nat. Nanotechnol.* **2016**, *11* (5), 409-420.
- (4) Sheldon, R. A.; van Pelt, S. Enzyme Immobilisation in Biocatalysis: Why, What and How. *Chem. Soc. Rev.* **2013**, *42* (15), 6223-6235.
- (5) Sirisha, V. L.; Jain, A.; Jain, A. Enzyme Immobilization: An Overview on Methods, Support Material, and Applications of Immobilized Enzymes. *Adv. Food Nutr. Res.* **2016**, *79*, 179-211.
- (6) Lian, X.; Fang, Y.; Joseph, E.; Wang, Q.; Li, J.; Banerjee, S.; Lollar, C.; Wang, X.; Zhou, H.-C. Enzyme-MOF (metal-organic framework) Composites. *Chem. Soc. Rev.* **2017**, *46* (11), 3386-3401.
- (7) Li, P.; Modica, J. A.; Howarth, A. J.; Vargas, L. E.; Moghadam, P. Z.; Snurr, R. Q.; Mrksich, M.; Hupp, J. T.; Farha, O. K. Toward Design Rules for Enzyme Immobilization in Hierarchical Mesoporous Metal-Organic Frameworks. *Chem* **2016**, *1* (1), 154-169.
- (8) Huang, S.; Kou, X.; Shen, J.; Chen, G.; Ouyang, G. Armoring the Enzymes with Metal-Organic Frameworks. *Angew. Chem. Int. Ed.* **2020**. <http://dx.doi.org/10.1002/anie.201916474>.
- (9) Yang, X. Y.; Li, Z. Q.; Liu, B.; Klein-Hofmann, A.; Tian, G.; Feng, Y. F.; Ding, Y.; Su, D. S.; Xiao, F. S. "Fish-in-Net" Encapsulation of Enzymes in Macroporous Cages as Stable, Reusable, and Active Heterogeneous Biocatalysts. *Adv. Mater.* **2006**, *18*, 410-414.
- (10) Fernandez-Lafuente, R. Stabilization of Multimeric Enzymes: Strategies to Prevent Subunit Dissociation. *Enzyme Microb. Technol.* **2009**, *45* (6-7), 405-418.
- (11) Pang, Y.; Li, H.; Farmakes, J.; Xiao, F.; Chen, B.; Ma, S.; Yang, Z. How Do Enzymes Orient When Trapped on Metal-Organic Framework (MOF) Surfaces? *J. Am. Chem. Soc.* **2018**, *140* (47), 16032-16036.
- (12) Johnson, B. J.; Algar, W. R.; Malanoski, A. P.; Ancona, M. G.; Medintz, I. L. Understanding Enzymatic Acceleration at Nanoparticle Interfaces: Approaches and Challenges. *Nano Today* **2014**, *9* (1), 102-131.
- (13) Sun, Q.; Pan, Y.; Wang, X.; Li, H.; Farmakes, J.; Aguila, B.; Yang, Z.; Ma, S. Mapping Out The Degree of Freedom of Hosted Enzymes in Confined Spatial Environments. *Chem* **2019**, *5*, 1-12.
- (14) Klermund, L.; Castiglione, K. Polymersomes As Nanoreactors for Preparative Biocatalytic Applications: Current Challenges and Future Perspectives. *Bioproc. Biosyst. Eng.* **2018**, *41* (9), 1233-1246.
- (15) Hanefeld, U.; Gardossi, L.; Magner, E. Understanding Enzyme Immobilisation. *Chem. Soc. Rev.* **2009**, *38* (2), 453-468;
- (16) An, H. D.; Li, M. M.; Ga, J.; Zhang, Z. J.; Ma, S. Q.; Chen, Y. Incorporation of Biomolecules in Metal-Organic Frameworks for Advanced Applications. *Coordin. Chem. Rev.* **2019**, *384*, 90-106.
- (17) Rodriguez-Arco, L.; Kumar, B. V. V. S. P.; Li, M.; Patil, A. J.; Mann, S. Modulation of Higher-Order Behaviour in Model Protocell Communities by Artificial Phagocytosis. *Angew. Chem. Int. Ed.*



2019, 58 (19), 6333-6337.

(18) Godoy-Gallardo, M.; York-Duran, M. J.; Hosta-Rigau, L. Recent Progress in Micro/Nanoreactors toward the Creation of Artificial Organelles. *Adv. Healthcare Mater.* **2018**, 7 (5), 1700917-1700951.

(19) Lu, A. X.; Oh, H.; Terrell, J. L.; Bentley, W. E.; Raghavan, S. R. A New Design for An Artificial Cell: Polymer Microcapsules with Addressable Inner Compartments That Can Harbor Biomolecules, Colloids or Microbial Species. *Chem. Sci.* **2017**, 8 (10), 6893-6903.

(20) Einfalt, T.; Witzigmann, D.; Edlinger, C.; Sieber, S.; Goers, R.; Najer, A.; Spulber, M.; Onaca-Fischer, O.; Huwyler, J.; Palivan, C. G. Biomimetic Artificial Organelles with In Vitro and In Vivo Activity Triggered by Reduction in Microenvironment. *Nat. Commun.* **2018**, 9, 1127-1138.

(21) Borodina, T.; Markvicheva, E.; Kunizhev, S.; Moehwald, H.; Sukhorukov, G. B.; Kreft, O. Controlled Release of DNA from Self-Degrading Microcapsules. *Macromol. Rapid Comm.* **2007**, 28, 1894-1899.

(22) Huo, J.; Aguilera-Sigalat, J.; El-Hankari, S.; Bradshaw, D. Magnetic MOF Microreactors for Recyclable Size Selective Biocatalysis. *Chem. Sci.* **2015**, 6, 1938-1943.

(23) Zyzin, M. V.; Ramos-Cabrer, P.; Carregal-Romero, S. Encapsulation of Enzymes in Porous Capsules via Particle Templating. *Methods Mol. Biol.* **2020**, 2100, 227-241.

(24) Renggli, K.; Baumann, P.; Langowska, K.; Onaca, O.; Bruns, N.; Meier, W. Selective and Responsive Nanoreactors. *Adv. Funct. Mater.* **2011**, 21 (7), 1241-1259.

(25) Xu, Z.; Xiao, G.; Li, H.; Shen, Y.; Zhang, J.; Pan, T.; Chen, X.; Zheng, B.; Wu, J.; Li, S.; Zhang, W.; Huang, W.; Huo, F. Compartmentalization Within Self-Assembled Metal-Organic Framework Nanoparticles for Tandem Reactions. *Adv. Funct. Mater.* **2018**, 28 (34), 1802479 -1802488.

(26) Shi, J.; Jiang, Y.; Wang, X.; Wu, H.; Yang, D.; Pan, F.; Su, Y.; Jiang, Z. Design and Synthesis of Organic-Inorganic Hybrid Capsules for Biotechnological Applications. *Chem. Soc. Rev.* **2014**, 43 (15), 5192-5210.

(27) Schoonen, L.; van Hest, J. C. M. Compartmentalization Approaches in Soft Matter Science: From Nanoreactor Development to Organelle Mimics. *Adv. Mater.* **2016**, 28 (6), 1109-1128.

(28) Sun, Q.; Tang, Y.; Aguila, B.; Wang, S.; Xiao, F.-S.; Thallapally, P. K.; Al-Enizi, A. M.; Nafady, A.; Ma, S. Enhancement Reaction Environment Modification in Covalent Organic Frameworks for Catalytic Performance. *Angew. Chem. Int. Ed.* **2019**, 58 (26), 8670-8675.

(29) Sun, Q.; Fu, C.-W.; Aguila, B.; Perman, J.; Wang, S.; Huang, H.-Y.; Xiao, F.-S.; Ma, S. Pore Environment Control and Enhanced Performance of Enzymes Infiltrated in Covalent Organic Frameworks. *J. Am. Chem. Soc.* **2018**, 140 (3), 984-992.

(30) Sun, Q.; Aguila, B.; Lan, P. C.; Ma, S. Tuning Pore Heterogeneity in Covalent Organic Frameworks for Enhanced Enzyme Accessibility and Resistance against Denaturants. *Adv. Mater.* **2019**, 31 (19), 1900008-1900014.

(31) Zhang, S.; Zheng, Y.; An, H.; Aguila, B.; Yang, C.-X.; Dong, Y.; Xie, W.; Cheng, P.; Zhang, Z.; Chen, Y.; Ma, S. Covalent Organic Frameworks with Chirality Enriched by Biomolecules for Efficient Chiral Separation. *Angew. Chem. Int. Ed.* **2018**, 57 (51), 16754-16759.

(32) Ma, T.; Kapustin, E. A.; Yin, S. X.; Liang, L.; Zhou, Z.; Niu, J.; Li, L.-H.; Wang, Y.; Su, J.; Li, J.; Wang, X.; Wang, W. D.; Wang, W.; Sun, J.; Yaghi, O. M. Single-Crystal X-Ray Diffraction Structures of Covalent Organic Frameworks. *Science* **2018**, 361 (6397), 48-52

(33) Kandambeth, S.; Venkatesh, V.; Shinde, D. B.; Kumari, S.;

Halder, A.; Verma, S.; Banerjee, R. Self-Templated Chemically Stable Hollow Spherical Covalent Organic Framework. *Nat. Commun.* **2015**, 6, 6786-6795.

(34) Feng, X.; Ding, X.; Jiang, D. Covalent Organic Frameworks. *Chem. Soc. Rev.* **2012**, 41 (18), 6010-6022.

(35) Liang, K.; Ricco, R.; Doherty, C. M.; Styles, M. J.; Bell, S.; Kirby, N.; Mudie, S.; Haylock, D.; Hill, A. J.; Doonan, C. J.; Falcaro, P. Biomimetic Mineralization of Metal-Organic Frameworks as Protective Coatings for Biomacromolecules. *Nat. Commun.* **2015**, 6, 7240-7247.

(36) Shieh, F.-K.; Wang, S.-C.; Yen, C.-I.; Wu, C.-C.; Dutta, S.; Chou, L.-Y.; Morabito, J. V.; Hu, P.; Hsu, M.-H.; Wu, K. C. W.; Tsung, C.-K. Imparting Functionality to Biocatalysts via Embedding Enzymes into Nanoporous Materials by A De Novo Approach: Size-Selective Sheltering of Catalase in Metal-Organic Framework Microcrystals. *J. Am. Chem. Soc.* **2015**, 137 (13), 4276-4279.

(37) Feng, Y.; Wang, H.; Zhang, S.; Zhao, Y.; Gao, J.; Zheng, Y.; Zhao, P.; Zhang, Z.; Zaworotko, M. J.; Cheng, P.; Ma, S.; Chen, Y. Antibodies@MOFs: An In Vitro Protective Coating for Preparation and Storage of Biopharmaceuticals. *Adv. Mater.* **2019**, 31 (2), 1805148-1805154.

(38) Wu, X.; Ge, J.; Yang, C.; Hou, M.; Liu, Z. Facile Synthesis of Multiple Enzyme-Containing Metal-Organic Frameworks in A Biomolecule-Friendly Environment. *Chem. Commun.* **2015**, 51 (69), 13408-13411.

(39) Uribe-Romo, F. J.; Doonan, C. J.; Furukawa, H.; Oisaki, K.; Yaghi, O. M. Crystalline Covalent Organic Frameworks with Hydrazone Linkages. *J. Am. Chem. Soc.* **2011**, 133 (30), 11478-11481.

(40) Ding, S.-Y.; Cui, X.-H.; Feng, J.; Lu, G.; Wang, W. Facile Synthesis of -C=N-Linked Covalent Organic Frameworks under Ambient Conditions. *Chem. Commun.* **2017**, 53 (87), 11956-11959.

(41) Matsumoto, M.; Valentino, L.; Stiehl, G. M.; Balch, H. B.; Corcos, A. R.; Wang, F.; Ralph, D. C.; Marinas, B. J.; Dichtel, W. R. Lewis-Acid-Catalyzed Interfacial Polymerization of Covalent Organic Framework Films. *Chem* **2018**, 4 (2), 308-317.

(42) Galli, S.; Masciocchi, N.; Tagliabue, G.; Sironi, A.; Navarro, J. A. R.; Salas, J. M.; Mendez-Linan, L.; Domingo, M.; Perez-Mendoza, M.; Barea, E. Polymorphic Coordination Networks Responsive to CO<sub>2</sub>, Moisture, and Thermal Stimuli: Porous Cobalt(II) and Zinc(II) Fluoropyrimidinolates. *Chem-Eur. J.* **2008**, 14 (32), 9890-9901.

(43) Tehrani, H. S.; Moosavi-Movahedi, A. A. Catalase and Its Mysteries. *Prog. Biophys. Mol. Bio.* **2018**, 140, 5-12.

(44) Shi, J.; Wu, Y.; Zhang, S.; Tian, Y.; Yang, D.; Jiang, Z. Bioinspired Construction of Multi-Enzyme Catalytic Systems. *Chem. Soc. Rev.* **2018**, 47 (12), 4295-4313.

(45) Graefe, D.; Gaitzsch, J.; Appelhans, D.; Voit, B. Cross-Linked Polymersomes As Nanoreactors for Controlled and Stabilized Single and Cascade Enzymatic Reactions. *Nanoscale* **2014**, 6 (18), 10752-10761.

(46) Chang, F. P.; Chen, Y. P.; Mou, C. Y. Intracellular Implantation of Enzymes in Hollow Silica Nanospheres for Protein Therapy: Cascade System of Superoxide Dismutase and Catalase. *Small* **2014**, 10 (22), 4785-4795.

(47) Ma, Y.; Zhao, Y.; Bejjanki, N. K.; Tang, X.; Jiang, W.; Dou, J.; Khan, M. I.; Wang, Q.; Xia, J.; Liu, H.; You, Y.-Z.; Zhang, G.; Wang, Y.; Wang, J. Nanoclustered Cascaded Enzymes for Targeted Tumor Starvation and Deoxygenation-Activated Chemotherapy without Systemic Toxicity. *ACS Nano* **2019**, 13 (8), 8890-8902.

(48) Yong, Y.; Su, R.; Liu, X.; Xu, W.; Zhang, Y.; Wang, R.; Ouyang, P.; Wu, J.; Ge, J.; Liu, Z. Lectin Corona Enhances Enzymatic Catalysis on The Surface of Magnetic Nanoparticles. *Biochem. Eng. J.* **2018**, 129, 26-32.

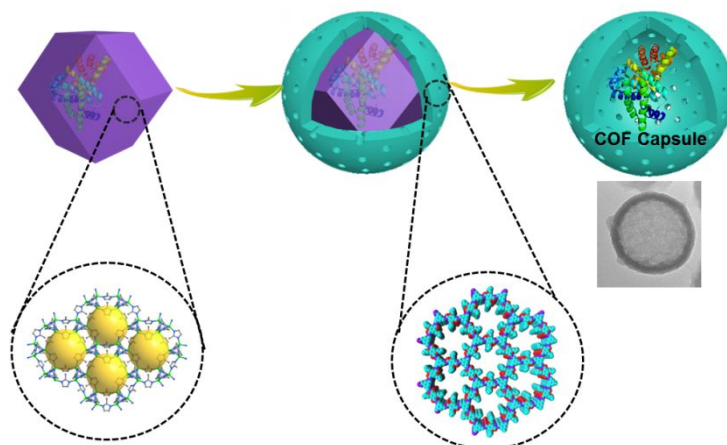


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