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Magnetic-metal organic framework (magnetic-MOF): A novel platform for enzyme immobilization and nanozyme applications

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Abstract

Enzymes are nature's catalysts which are highly selective and efficient. Recently, metal organic frameworks (MOFs) have captivated great attention as an attractive porous support matrix for immobilization of enzymes. An outstanding improvement in catalytic efficiency, enhanced chemical/thermal stability, easy accessibility to active sites, promising recyclability and high enzyme loading are some of the fascinating features of enzyme-MOF composites. Due to low density and high dispersion, the handling and separation of enzyme-MOF composites is challenging. Hence, metal organic framework with magnetic properties (magnetic−MOF) can be a potential candidate as it possesses sophisticated structural design integrated with magnetic properties. Specifically designed magnetic−MOF offers novel properties such as devisable composition, large surface area, easy loading and rapid collection which make it potential amenable for enzyme immobilisation (magnetic-enzyme−MOF). This article highlights the different strategies for enzyme immobilization such as physical adsorption, covalent binding, co-ordination bonding and *de novo* encapsulation method. It further discusses about the artificial enzyme properties of magnetic-MOF coupled with enzyme to extend its application in biosensor.

Keywords: Magnetic, metal organic frameworks, enzyme, immobilization strategies, nanozyme.

Abbreviations

APTES- (3-Aminopropyl)triethoxysilane; BDC- Benzenedicarboxylic Acid; BET- Brunauer, Emmett and Teller; BJH- Barrett–Joyner–Halenda; BSA- Bovine serum albumin; BTC- Benzene-1,3,5-tricarboxylic acid; CalB- Candida Antarctica lipase B; CP- Chlorophenols; DFT- Density Functional Theory; DMF- Dimethylformamide; DMSO- Dimethylsulfoxide; DOTA- 1,4,7,10-tetraazacyclododecane N,N',N'',N'''-tetraacetic acid; DOX- Doxorubicin; DUT- Durban University of Technology; EDC- 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide; EDTA- ethylene diamine tetra acetic acid; GOx- Glucose oxidase; HAADF-STEM- High angle annular dark field-scanning transmission electron microscopy; HKUST- Hong Kong University of Science and Technology HRP- Horseradish peroxidase; K_m- Michaelis–Menten kinetic constant; MALDI-matrix-assisted laser desorption/ionization; MNP- Magnetic Nano Particles; MOF- Metal Organic Frameworks; NHS- N-hydroxysuccinimide; OPD- *o*-phenylenediamine; PDA- Polydopamine; PVP- Polyvinylpyrrolidone; SEM- Scanning electron microscope; TMB- 3,3',5,5'-tetramethylbenzidine; XRD- X-ray Diffraction; ZIF- Zeolitic Imidazolate Framework.

Introduction

In the drive towards sustainable manufacturing of chemicals, enzymes are one of the favourable options which are naturally evolved, highly efficient, biocompatible, biodegradable biocatalysts. They exhibit some interesting properties such as high activity, high selectivity and operation under mild physiological pH and temperature in greener solvent (usually water) for synthesis [1–4]. Despite all these benefits, industrial application of enzymes is often challenged by lack of operational/chemical stability, storage stability, difficult recovery and re-usability. Immobilization of the enzyme has been recognized as an important tool to overcome these drawbacks. With immobilization, the solid form of enzyme can be retained, that ameliorates the handling which is otherwise difficult in liquid formulation of enzyme [5–7]. Also, the enzyme stability enhances in the immobilized form, which sustains under both storage and operational conditions like denaturation by heat or organic solvents or by autolysis. Immobilization facilitates enzyme separation and re-use, thereby minimising or eliminating protein contamination in reaction medium which significantly reduces the cost, thus, enabling its industrial applicability in continuous, fixed-bed operation [8,9]. Additionally, reusability of immobilized enzyme results in higher catalyst productivities [10].

Enzyme immobilization on solid support has been already reviewed by Zdarta et al., Brady et al. and Chao et al. [11–13]. In recent years, porous structured nanomaterials have been adapted as the promising alternative carrier for enzyme immobilization. These porous materials offer various advantages such as high surface area, tailored pore-size distribution and controllable pore geometry. Amongst various available porous nanomaterials, mesoporous silica is the most extensively explored porous carrier for enzyme immobilization [14,15]. However, the lack of specific interaction between enzyme molecules and mesoporous silica material results into leaching during reaction process which significantly

affects the recovery and reusability. Also, the synthesis of hierarchical porous mesoporous silica materials is complicated in many aspects [16–18]. The catalytic performance of enzyme is affected probably due to the deactivation of enzymes and diffusion restrictions during immobilization procedure [19,20].

Apart from mesoporous silica based enzyme immobilization supports, there is an organic-inorganic hybrid platform which has been used for enzyme immobilization. This platform incorporates MOF and organic-inorganic crystals nanoflowers for immobilization of enzymes. For immobilization enzyme in nanoflowers, the synthesis of carriers and immobilization of enzymes are integrated in one step [21–24]. Using different types of inorganic (metal ions) crystals, various enzymes like alpha amylase [25], horseradish peroxidase (HRP) [26–28], α-chymotrypsin [29], catalase [30], glucose oxidase [31], glucoamylase [32], trypsin [33], urease [34], lipase [35] and many others have been prepared in the form of enzyme-inorganic crystal hybrid nanoflowers. They exhibited better catalytic properties, storage stability and excellent recyclability [36]. Although the enzyme-inorganic hybrid nanoflowers resulted in greatly enhanced activity and stability, the formation process of hybrid nanoflowers is quite slow, and incubation period in the reaction mixture being nearly 3 days at room temperature. This time-consuming process has critically limited their widespread use in real applications.

In the consideration of foregoing, MOF has emanated as the potential hybrid organic-inorganic crystalline material which has showed promising applications in the vast field of catalysis [37,38], separation [39,40], drug/enzyme carriers [41–43], energy conversion/storage [44,45]. It possesses some unique characteristics such as large pore volumes, tunable pore sizes with well-defined cavities, high surface areas, crystallographic structure and designable organic ligands [46,47]. These remarkable features of MOF make it an attractive support platform for the immobilization of enzymes. Also, ultra-high porosity

and large hierarchical surface area of MOF provides high loading capacity and strong affinity towards enzyme molecules to avert leaching [48]. However, the recovery of such an enzyme-MOF composite is very difficult due to its nanometer size, high dispersity and low density which restrict the recovery and reusability of biocatalyst [49,50]. In order to address the separation issue and facile handling of MOF, the researchers have employed new strategies incorporating magnetic nanoparticles with enzyme and MOF composite (magnetic-enzyme-MOF) [51–54]. These multifunctional magnetic hybrid MOFs have been a topic of growing interest because they possess both the magnetic properties of metal or metal oxides (mostly Fe_3O_4) and the diverse properties of MOFs in one material. The presence of magnetic nanoparticles has dual advantages. The most obvious potential benefit of the magnetic supported MOF is in the separation and handling of MOFs embedded with enzymes. Also, they have some unique features such as high surface area (as compared to bare magnetic nanoparticles), easy enzyme loading, rapid collection from reaction mixture and devisable composition [55–57]. In addition to this, magnetic-MOF exhibit enzyme-like characteristics to mimic the structures and functions of natural enzymes. These structures are commonly referred as nanozymes.

This review emphasises on the different strategies such as physical adsorption, chemical binding, co-ordination bonding and *de novo* method for enzyme immobilization using magnetic−MOF as a platform. Under each category, the preparation and special properties of magnetic-enzyme-MOF have been discussed in the form of examples. Further, this article also includes overview of magnetic−MOF as a nanozyme coupled with natural enzyme for application in the sector of biosensors. Finally, this review addresses the current challenges of magnetic-MOFs synthesis as well as possible applications in different field.

Magnetic−MOF for enzyme immobilization

The enzyme immobilization can be achieved either by simultaneous formation and immobilization in magnetic−MOF assembly or by immobilization of enzyme onto pre-synthesised magnetic−MOF by physical interactions or chemical bonding [58]. We have divided the immobilization strategies into three parts: (i) physical adsorption onto magnetic−MOF, (ii) covalent/co-ordination bonding with magnetic−MOF, (iii) co-ordination bonding and (iv) *de novo* encapsulation method. The list of different enzyme immobilized onto magnetic−MOF is summarized in Table 1. In all the examples of enzyme immobilization, different metal ions were used as inorganic component; 2-methylimidazole, H_2BDC and H_3BTC were commonly employed organic linkers during the formation of MOF around the magnetic nanoparticles (generally Fe_3O_4). The selection of methodology must be based on preserving the enzyme activity, efficiency and stability. In case of encapsulation method for catalytic applications, it is important to consider the effect of selection of counterpart of framework which may affect the substrate diffusion and/or selectivity. Even MOF crystalline size holds importance, as diffusion time increases with the square of the distance travelled. In addition to that, a proper balance of counter parts of MOF systems must be considered to maximize catalyst performance of immobilized enzyme as the enzymes are highly sensitive to external microenvironment.

Physical adsorption

This is the simple, cost-effective, faster route and most widely used method for protein immobilization. In the past decade, tremendous efforts have been focused on the preparation of porous nanomaterials as a support for enzymes [10,59–62]. The large surface area, pore diameters and uniform pore size of MOFs have been investigated for MOF to be a potential support for enzyme immobilization. Further, MOFs can exhibit strong interactions (hydrophilic/hydrophobic interaction and electrostatic interactions) with enzymes that prevent

their leakage during operation [63]. Trypsin (a protein hydrolysing enzyme) is the most frequently used enzyme for protein digestion. In this process, the highly efficient and complete digestion of all proteins is undoubtedly significant for accurate peptide identification and quantification. However, the procedure of conventional protein digestion in solution is time-consuming, and protease autolysis might produce non-targeted peptides. Considering these factors, Huo and team developed a novel immobilized enzyme magnetic−MOFs made up of Fe_3O_4@PDA-ZIF-90 for immobilization of trypsin. In most of the cases, hydrophilic materials are favourable to preserve the enzyme activity. Considering this fact, authors have introduced polydopamine (PDA) as a hydrophilic medium prior to enzyme immobilization. Magnetic ZIF-8 core-shell formations was done by a two-step synthesis protocol wherein the outer shell of 2-methylimidazole was deposited around pickering-stabilized hydrogel core UiO-66/Fe_3O_4. The performance of the immobilized trypsin was evaluated by using BSA protein. It was observed that the immobilized trypsin exhibits adequate digestion efficiency with the sequence coverage of 80% within a minute. On the other hand, free trypsin showed lesser digestion (70%) even after 12 h incubation. Further, the same group demonstrated the possibility of use of similar strategy for microcapsulation of *Candida antarctica* lipase B (CalB). The ease of magnetic recovery of CalB@MOF microcapsule was tested for catalytic recyclability during the transesterification which showed with 80% activity recovery even after six catalytic cycles without enzyme leaching [64].

These results highlights easy separation of magnetic−enzyme−MOF composite, convenient operation and excellent structural and thermal stability. However, the efficient binding of of protein molecules into small aperture of MOF requires size compatibility. Size variation may lead to low enzyme loading and thereby affecting enzyme recyclability, limiting the implementation of this method.

Covalent bonding

Mostly, in adsorption, there is high probability of enzyme leaching out from the support material which causes loss as well as low operational stability of enzyme. On the other hand, covalent binding can enhance the catalytic performance by multi-point stabilization of the active conformation [65,66]. Also, it shows excellent reusability performance due to chemical bonding with support [67–69]. In typical post synthetic modification procedure, MOF surfaces present a wide array of free and reactive functional groups (for example carboxyl, amino, hydroxyl groups etc.) that can be activated for coupling with reactive groups on the surface of the enzyme [70]. Recently, Samui and co-workers synthesized magnetic nanoparticle embedded amine-functionalized magnetic-MIL-88B and further activated by N-(3- dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) to immobilize lipase. The activation allows magnetic−MOF to react with the amino groups of enzyme to form covalent bond. The loading amount of lipase (*i.e.* immobilization yield) on magnetic−MOFs was 280 mg/g with the activity recovery of 86%. In FE-SEM analysis, the immobilized material exhibited bipyramidal hexagonal prism like structure with the size of 80 nm. Further, the lipase immobilized magnetic-MIL-88B presented wider pH (6-10) tolerance and enhanced thermal stability (30-60°C) than free form. The Michaelis–Menten studies showed that kinetic constant (K_m) was greatly reduced as compared to free enzyme, indicating greater affinity towards substrate. At the end, they observed suitable stability of immobilized lipase after multiple cycles of use and retains almost 84% activity after fifth cycle [71]. Various researchers immobilized lipase on different supports such as magnetic nanoparticle [72], chitosan magnetic nanoparticles [73], silica coated magnetic nanoparticle functionalized by glutaraldehyde [74] and EDC [75]. It was found that lipase immobilized on magnetic-MIL-88B showed superior catalytic properties as compared to other supports.

Following this work, Zhai and group came up with similar strategy but different approach which is illustrated in Figure 1. Authors have prepared Fe_3O_4@DOTA-ZIF-90 support for covalent enzyme immobilization *via* Schiff base reaction between the free aldehyde groups present on the surface of MOFs and free amino groups of the enzyme molecules. In multistep preparation procedure, pre-functionalized magnetic nanoparticles (NH_2-Fe_3O_4) were prepared followed by 1,4,7,10-tetraazacyclododecane N,N',N'',N'''-tetraacetic acid (DOTA, as a bifunctional reagent) functionalization which chelates Zn^{2+} metal ion. This magnetic material with free reactive aldehyde groups was incorporated by imidazolate-2-carboxyaldehyde. The prepared magnetic MOF material has novel characteristics such as i) high magnetic response (1.01 emu g^{-1}) for easy separation, ii) ultrahigh surface area (565.21 m^2g^{-1}) for high enzyme loading amounts and iii) excellent structural and thermal stability for prolonged lifespan. The trypsin was covalently grafted onto the surface of the magnetic−MOF that exhibits satisfactory digestion efficiency within a minute with 80% sequence coverage which is higher than that of the free trypsin (70%) which takes 12 h for digestion. The prepared biocatalyst was easily separated with external magnet and reused for multiple cycles [76].

This method showed an excellent potential for enzyme immobilization. However, it involves functionalization of pre-synthesized MOF which makes this method more complicated. Additionally, post-synthetic functionalization methods significantly reduce the porosity that ultimately affects the microenvironment around the enzyme and enzyme loading onto support.

Metal–ion affinity co-ordination bonding

Metal-ion affinity based enzyme immobilization represents a relatively new technique that is primarily appropriate for selective adsorption of enzyme on to the surface-exposed metal ion clusters of magnetic MOFs [77]. Binding of protein (enzyme) is based on interaction between electron donating groups present on protein surface and metal ion present on *via* one or more

co-ordination bonding [78,79]. In one of the studies, Wang and colleagues synthesized core-shell microsphere of Fe_3O_4@MOF by growing MIL-100 (Fe) (ferrous based MOF involves Fe ion and H_3BTC linker) onto mesoporous carboxyl-functionalized Fe_3O_4 nanoparticles. The core-shell microspheres possess high specific surface area 137.27 m^2 g^{-1} with pore size 7.07 nm. These values reveal that the surface area of prepared material is much higher than magnetic nanoparticles and many other magnetic porous materials as well. This highly porous support presents the large density of active sites in which the constitutional metal nodes have free or exchangeable co-ordination positions. *Candida rugosa* lipase was immobilized on the Fe_3O_4@MOF microspheres by two different routes (Figure 2): (a) covalent bonding through EDC/NHS and (b) metal–ion affinity interactions (with Zn^{2+}). It is noteworthy that the *Candida rugosa* lipase immobilized *via* metal–ion affinity exhibited higher activity recovery than covalent strategy. However, the covalently bonded lipase was found to be more thermally stable (higher residual activity) as compared to free and metal–ion affinity bounded enzyme. The authors attributed to this thermo-stability the multipoint bond formation between lipase and support, which led to enhancement in the enzyme rigidity. In a nut shell, the immobilized lipase remains active at a wide pH and temperature range as compared to free enzyme. Also, they exhibited significantly improved reusability (tested upto 10 cycles) and storage stability [80].

In another approach, Zhao and co-authors prepared a hierarchically structured MOF shell to form robust Fe_3O_4@polydopamine coated copper based MOF *via* multistep synthetic procedure which is highlighted in Figure 3. The presence of polydopamine can help to induce growth of MOFs and to maintain ultrahigh specific area of MOFs. Although the diameter of trypsin (3.8 nm) is larger than the pore size of copper-based MOF, the strong electrostatic interactions (co-ordination bonds) between MOFs (copper ions) and enzyme molecules can be attributed for high loading of enzyme on the surface of composite. Further, it was

employed for the digestion of protein (Cytochrome *c* as a model protein) and analysed by matrix-assisted laser desorption/ionization time-off light mass spectrometry. The efficiency of immobilized trypsin was found to be improved and showed 77% sequence coverage corresponding to 13 matched peptides. Further, similar magnetic support was used for immobilization of α-chymotrypsin and applied for protein digestion. The outcome suggested that the Fe_3O_4@polydopamine copper based MOF material can be implemented as a universal support to immobilize various enzymes (particularly for protein digesting enzymes) for better efficiency and stability as compared to the free form of enzyme [81].

Such metal ion affinity based enzyme immobilization onto core-shell magnetic composite showed potential applications in various sectors. The immobilized enzyme exhibited significantly improved reusability, storage stability and remained active at a wide range of pH and temperature as compared to free counterpart.

***de novo* encapsulation method**

The aforementioned methods involved enzyme immobilization after synthesis of magnetic-MOFs. These post synthetic immobilization approach involves physical adsorption/co-ordination binding enzymes directly into MOF pores, certainly restrict the immobilization due to large size of enzyme compared to the much smaller aperture and pores size of MOFs. Sometimes enzyme may undergo structural and conformational changes that could compromise biological activity [48,82]. An alternative strategy was developed by Hou and co-workers based on the biomineralization. Authors have encapsulated biomolecules within the MOF by a simple *de novo* approach without significant impact upon the inherent porosity of the MOF. In the experimental procedure, enzyme and MOF precursors (ligands and metal ions, and eventually additives) along with citrate coated magnetic nanoparticles were mixed together in solution, resulting in the crystallization of magnetically active MOF particles with embedded enzymes, either at the particle surface or as a core-shell material in a single step.

The major limitation of the other methodologies can be overcome by using *de novo* method as these magnetic−MOFs are synthesised under mild conditions (preferably aqueous solutions and room temperature) to preserve enzyme structure and catalytic activity [83,84]. This is the major reason for zeolitic imidazolate frameworks (ZIFs) to have gained great attention, which can be easily formed under mild biocompatible conditions [85,86].

The *de novo* encapsulation of enzyme along with magnetic nanoparticles within MOF was studied by Hou and co-workers for simultaneous immobilization of glucose oxidase (GOx) and magnetic nanoparticles (as an artificial peroxidase enzyme) within ZIF-8. The GOx embedded mZIF-8 was synthesized by simply mixing citrate coated magnetic nanoparticles with zinc, 2-methylimidazole and polyvinylpyrrolidone (PVP) solution containing GOx in one pot which is illustrated in Figure 4. They used PVP to assist the dispersion and stabilization of the protein in alcoholic solution. The prepared magnetic GOx-mZIF-8 was used as a rapid and facile colorimetric sensor for glucose detection that showed linear relationship in the range of 5.0 to 150.0 μM. It is important to note that the encapsulated GOx showed limit of detection lower than the one in reports. Also, the mZIF-8 embedded GOx showed excellent stability in a wide pH and temperature range. At the end, the residual activity even after 12 successive cycles of reuse was highly significant. The results suggested that the immobilized GOx within magnetic ZIF-8 exhibited better stability under unfavourable environment. This phenomenon might be due to the co-ordination between the enzyme and Zn^{2+}, which could protect the enzyme within the MOF. The facile one pot synthesis approach allows to encapsulate enzyme in MOFs which not only prevents leaching but also extends the thermal stability [87].

In another approach of preparation of magnetic multi-enzyme nanosystems, pre-synthesised HKUST-1@Fe_3O_4 nanoparticles were mixed with enzyme mixture of GOx and HRP *via* a layer-by-layer self-assembly method. There are three different synthetic routes for spatially

co-localization of HRP and GOx at different sites of HKUST-1 (*i.e.* inner layer, outer shell, or randomly distributed GOx) which are illustrated in Figure 5. It was observed that when GOx was immobilized in the outer shell and HRP on the inner layer (*i.e.* bienzyme nanosystems in the sequence of GOx-HRP-HKUST-1-Fe_3O_4), the specific activity was quite high. After immobilization of two enzymes, the resultant biocatalyst exhibited improved kinetic parameters such as K_m and V_{max} as compared to free enzyme mixture. Further, they showed better thermal stability (at a high temperature of 95°C, free enzymes lost their activity completely while the immobilized enzyme retained 21% residual activity), chemical tolerance as well as storage stability as compared to the free form of enzyme [88].

Hierarchical channel-type MOFs with pores can protect the enzyme even under harsh conditions or unfriendly environments. Moreover, the confinement of enzymes in 3D microenvironments is known to increase stability against toxic solvents, strongly acidic or basic media and temperature. Also, the channels/windows are available for diffusive transport of reactants and products to and from the encapsulated enzyme molecules. In addition to that, it prevents enzyme from leaching to obtain higher degree of recyclability. However, it provides relative hindrance for diffusive transport of substrates, when the enzyme is bound deep within the MOF. In this regard, synthetic conditions should be controlled in such a way that the desired enzyme molecules should fit into the outer layer making the enzymes are easily accessible for interaction with reactants.

Magnetic−MOF based nanozyme

Nanomaterial-based artificial enzymes, defined as ‘‘nanozymes’’ have attracted enormous interest as they possess some unique properties such as excellent robustness, stability, and low-cost production with easy scale-up, which are critically for an alternative to natural enzymes [89,90]. A variety of materials including cyclodextrins, porphyrins, metal complexes polymers, dendrimers and other biomolecules have been extensively explored for

their enzyme mimicking properties [91]. Certain nanomaterials, such as cerium, silver, gold, platinum nanoparticles etc. have been found to exhibit enzyme-like activities. The great advances have been made in this area due to the tremendous progress in nano-research and the unique characteristics of nanomaterials [92]. These nanozymes have got wide applications in an array of fields, including biosensing, immunoassays, detection of biomolecules such as DNA, proteins, cells, and small molecules including glucose [93].

Recently, a number of organic and inorganic materials have been recognized as enzyme mimics. The potential of creating highly selective catalytic pockets and diffusion-favoured hierarchical structures in MOFs has also been explored in the review given by Zhang and co-workers. MOF-based nanozymes were considered to be a great potential in achieving high sensitivity for the detection of a target analytes (for example, most common target H_2O_2) because of their high catalytic activity and presence of most abundant redox sites [94]. However, these nanomaterials exhibit low affinity towards H_2O_2 while giving relatively high Michaelis–Menten constant (K_m) values. Also, the separation of these materials is very complicated which mostly involves centrifugation and filtration procedures. To overcome these problems, Hou and co-authors introduced robust magnetic ZIF-8 (mZIF-8) which exhibited peroxidase-like properties. To prepare core−shell mZIF-8, citrate coated Fe_3O_4 was mixed with alcoholic solution of zinc and 2-methylimidazole. The peroxidase-like activity of mZIF-8 was verified by oxidation of *o*-phenylenediamine (OPD) in the presence of H_2O_2 to produce the orange colour products. Under optimal conditions, the oxidation of OPD was enhanced with linearly increasing concentration in the range of 1.2 μM to 0.1 mM of H_2O_2. This indicated that there is no influence on the peroxidase-like activity of mZIF-8 for wide range of H_2O_2. In kinetic studies, the K_m value for mZIF-8 with H_2O_2 as substrate was found to be lower than that of HRP, indicating stronger affinity of mZIF-8 towards H_2O_2 [87].

Recently, encapsulation of transition-metal nanoparticles into carbon or non-metallic heteroatom-doped carbon materials has received increasing attention because the carbon layers can help to protect metal nanoparticles and prevent them from agglomeration with adjacent nanoparticles. Dong and group realised the potential of encapsulating cobalt nanoparticles (CoNPs) into NH_2-MIL-88(Fe) MOFs-derived magnetic carbon (MC) as an enzyme mimicking peroxidase activity. The CoNPs/MC composites were prepared by carbonization of NH_2-MIL-88, followed by in situ reduction of Co^{2+} by sodium borohydrate. The peroxidase like activity of this magnetic−MOF was attributed to the efficient decomposition of H_2O_2 to form reactive oxygen which further reacted with chromogenic agent such as 3,3',5,5'-tetramethylbenzidine (TMB). There was no influence of pH and temperature on the peroxidase like activity of nanomaterial, indicating a promising nanozyme candidate. The high peroxidase-like activity of CoNPs/MC could be successfully applied in biosensing for the detection of glucose in serums. This magnetic-MOF showed linear relationship in the glucose range of 0.25 to 30 μM. Also, it showed high selectivity for glucose detection in biosensing system [95].

Another enzyme mimicking concept been reported by Wu and co-workers. The authors synthesized Fe based Fe_3O_4@MIL-100 which showed remarkable peroxidase-like activity, that was used in colorimetric detection of cholesterol. In the detection procedure, the cholesterol was oxidized by cholesterol oxidase to produce H_2O_2 in the presence of oxygen. The concentration of H_2O_2 was measured by colour change from the converted TMB (Figure 6). It showed linear correlation to cholesterol concentration ranging from 2 to 50 μM with a LOD of 0.8 μM. Also, catalytic system showed high selectivity and reusability for cholesterol and could be applied in the determination of cholesterol in serum [96].

As the nanozyme-based magnetic−MOF material is heterogeneous in nature, combining it with homogeneous catalyst (soluble enzymes) will provide complementary means to address

some inherent drawbacks of enzymes such as low stability, difficult in recovery and reusability. These intrinsic properties of magnetic−MOF will lead to a new wave of novel biocatalysts for extensive applications in various sectors such as biocatalysis, biosensors and biomedical.

Summary and future prospects

As a support for stabilizing enzymes against unfavourable and harsh environmental conditions, MOFs are an attractive matrix support on account of their well-documented chemical and thermal stability. In addition, these is a huge library of known frameworks and the characterization of their respective properties and inherent stabilities paired with high porosity. Despite numerous advantages of magnetic−MOFs, there is still a scope for the real-world application of magnetic−MOFs-enzyme composites in biocatalysis. Several critical issues, such as incorporation of polysaccharides as a component of magnetic−MOFs, mild reaction conditions and use of biocompatible solvents (aqueous environments) during the immobilization of enzyme. It will keep the enzymes in active form during the immobilization procedure, need to be addressed. Also, the selection of synthetic methods of MOF is determined by the organic and inorganic counterparts that depend on the biocatalytic applications. Further development is necessary which might open up new avenues in the rational design of multifunctional magnetic hybrid materials as a platform for enzyme immobilization. This can be utilized in significant advancement for potential applications in the field of biotechnology, and bio-banking.

The vast majority of these mesoporous MOFs remain relatively unstable under various conditions (e.g. solvents, pH, water etc.) for a prolonged period of time. This drastically hampers their practical use as enzyme immobilization matrices under real conditions. Therefore, improvement in the structural stability of MOF is needed to fully exploit the potential of this immobilization strategy and produce industrially viable bio-composite

materials. Post synthetic modification of magnetic MOFs can be another practical approach for MOF modification to extend applications.

Still, there is limited literature available on the synthesis of magnetic–MOFs for enzyme immobilization, especially; controlling the growth of MOF crystals on magnetic nanoparticles remains a challenge. It is necessary to get the fundamental knowledge and deep understanding of the MOF interaction with enzyme while considering the functional and magnetic properties of magnetic nanoparticles.

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List of Figures

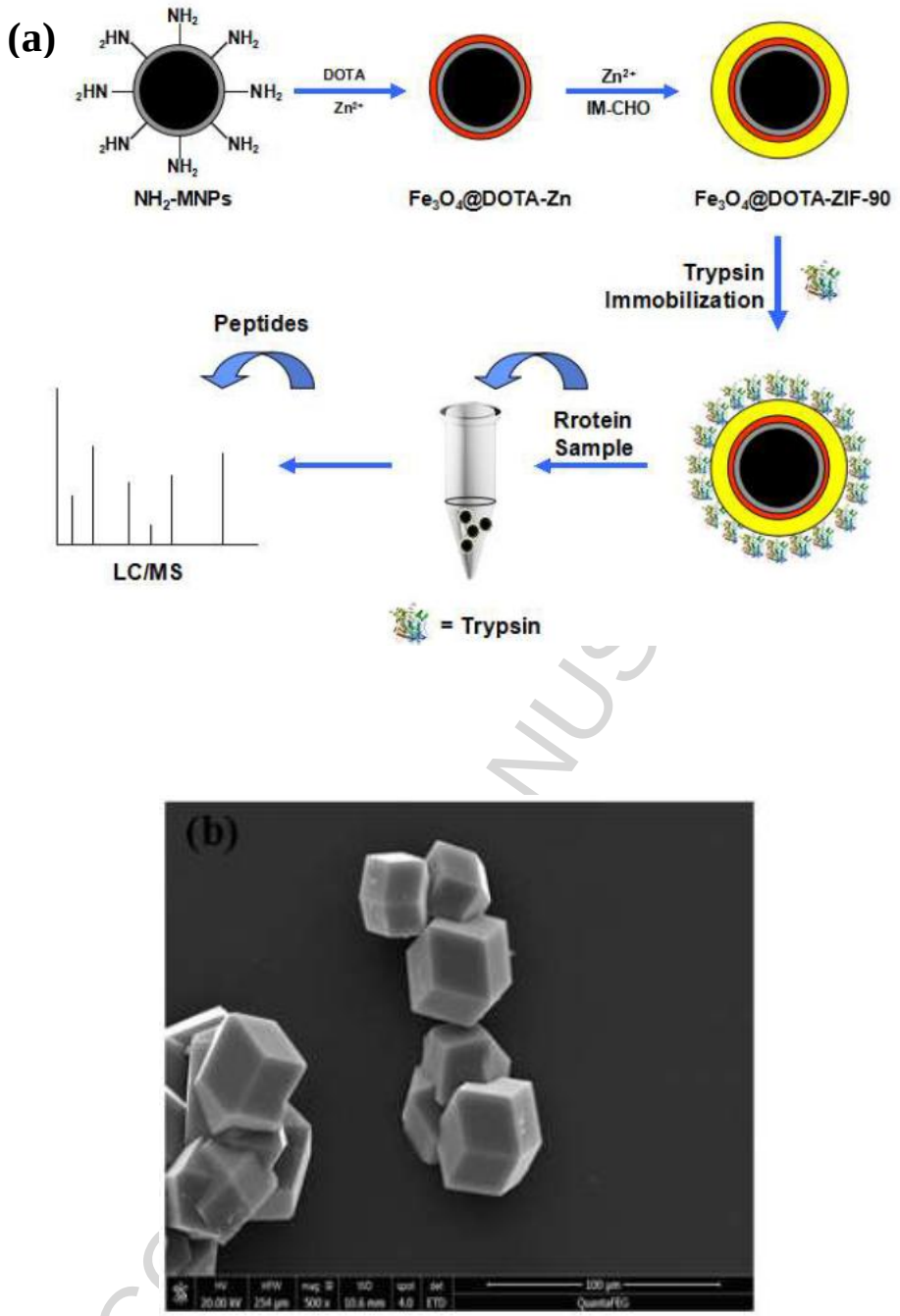


Figure 1 (a) Schematic of the preparation procedures and (b) SEM images of Fe_3O_4@DOTA-ZIF-90-trypsin (Copyright © 2015, Elsevier, reprinted with permission) [76].

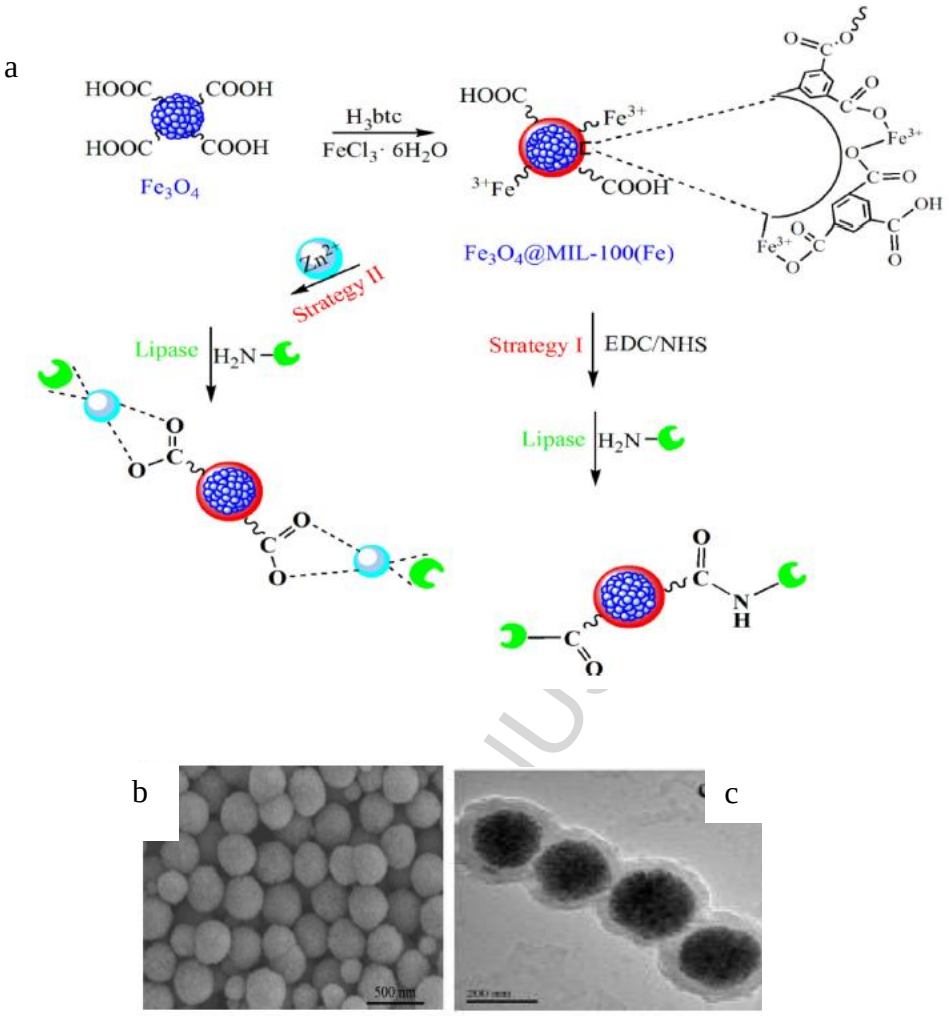


Figure 2 (a) The different strategies of immobilization of lipase onto Fe_3O_4@MIL-100 (Fe) microspheres. (b) SEM image (c) TEM image of immobilized lipase onto microspheres. (Reproduced from [80])

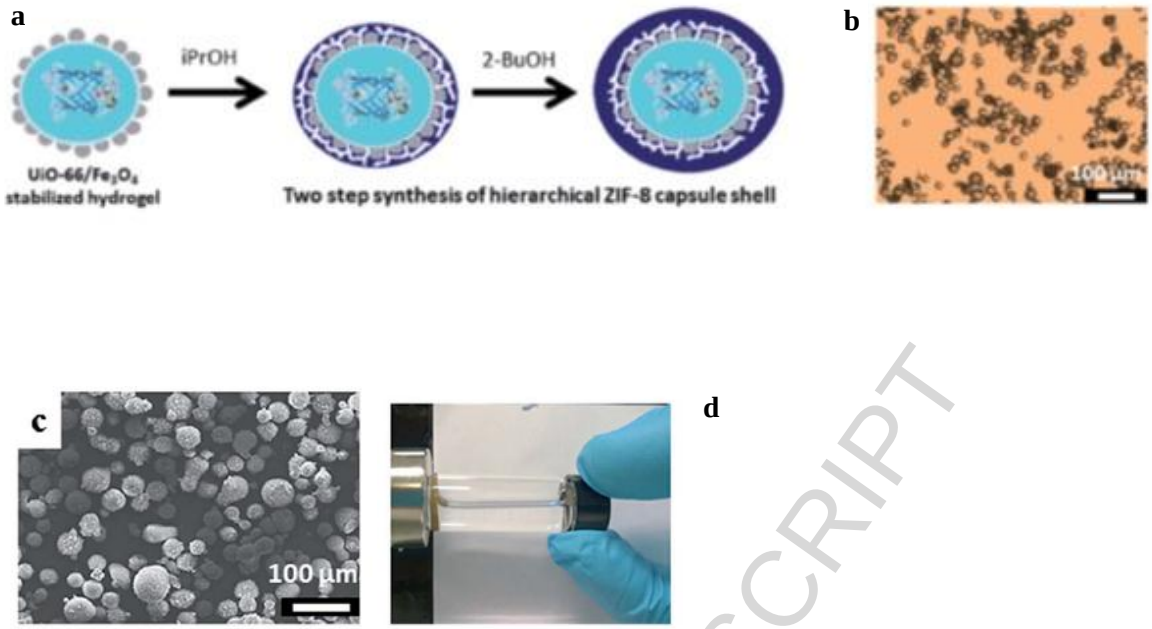


Figure 3 (a) Schematic illustration of preparation, (b) optical microscopy image agarose hydrogel droplets, (c) SEM image of the magnetic−MOF microcapsules.(d) the magnetic recovery of the capsules (Reproduced from [64]).

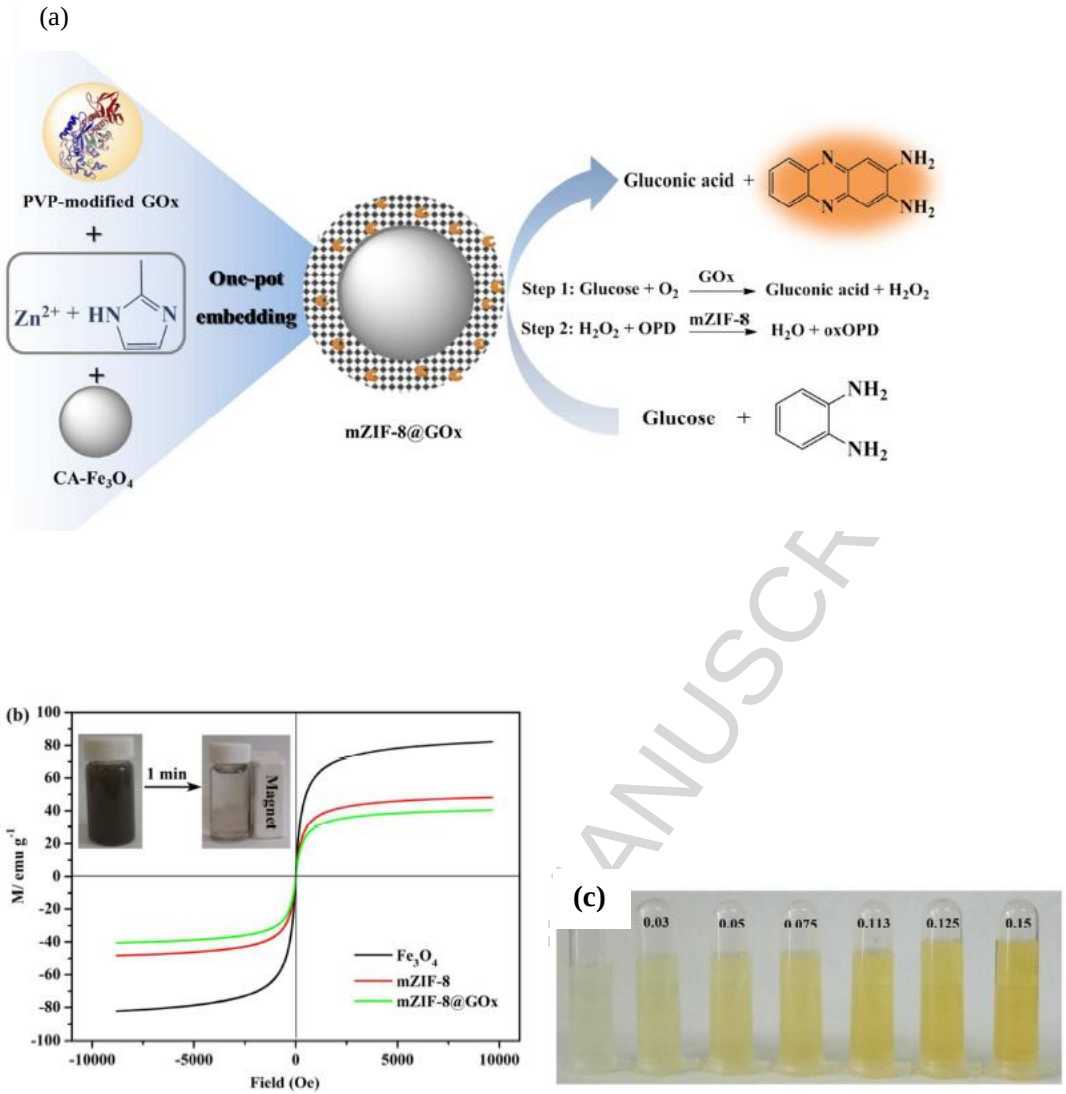


Figure 4 Schematic illustration of mechanism of glucose detection by GOx embedded mZIF-8, (b) hysteresis loops of Fe_3O_4, mZIF-8, and mZIF-8@GOx and magnetic separation of magnetic−MOF biocatalyst (inset). (c)Colorimetric change of OPD solutions containing mZIF-8@GOx with respect to glucose concentration (Copyright © 2015, Royal Society of Chemistry, reprinted with permission) [87]

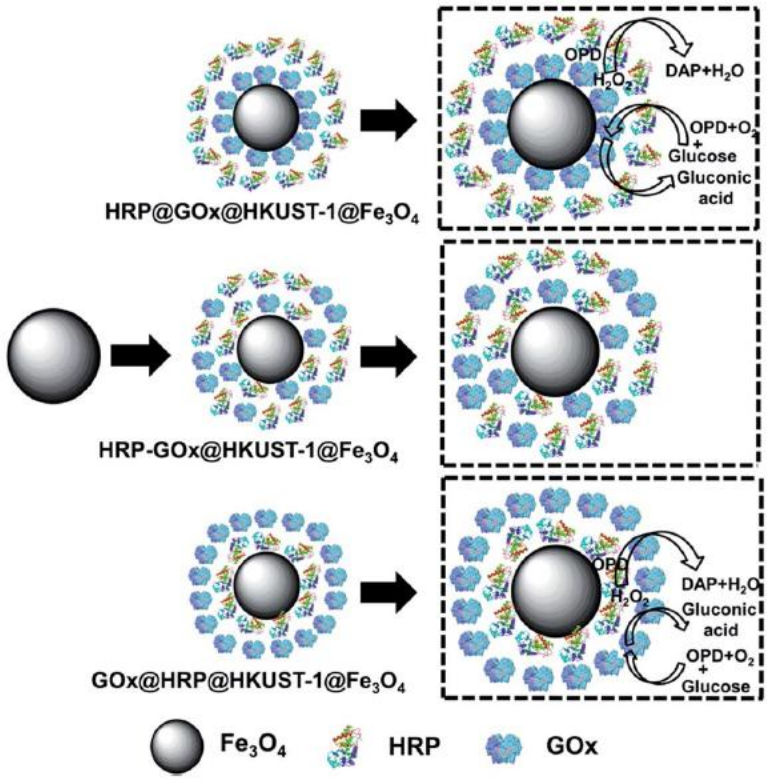
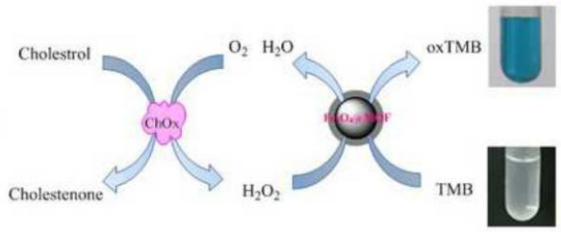


Figure 5 Schematic illustrations of the three different strategies for the positional assembly and spatial co-localization of GOx and HRP and their cascade biocatalysis reaction (Reproduced from [88])

a



b

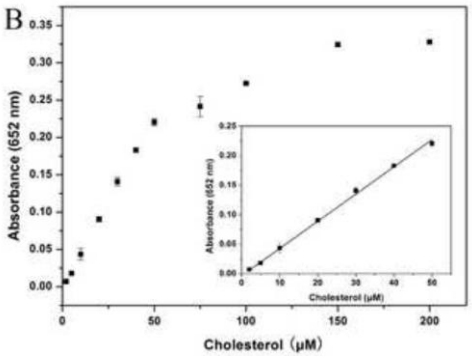


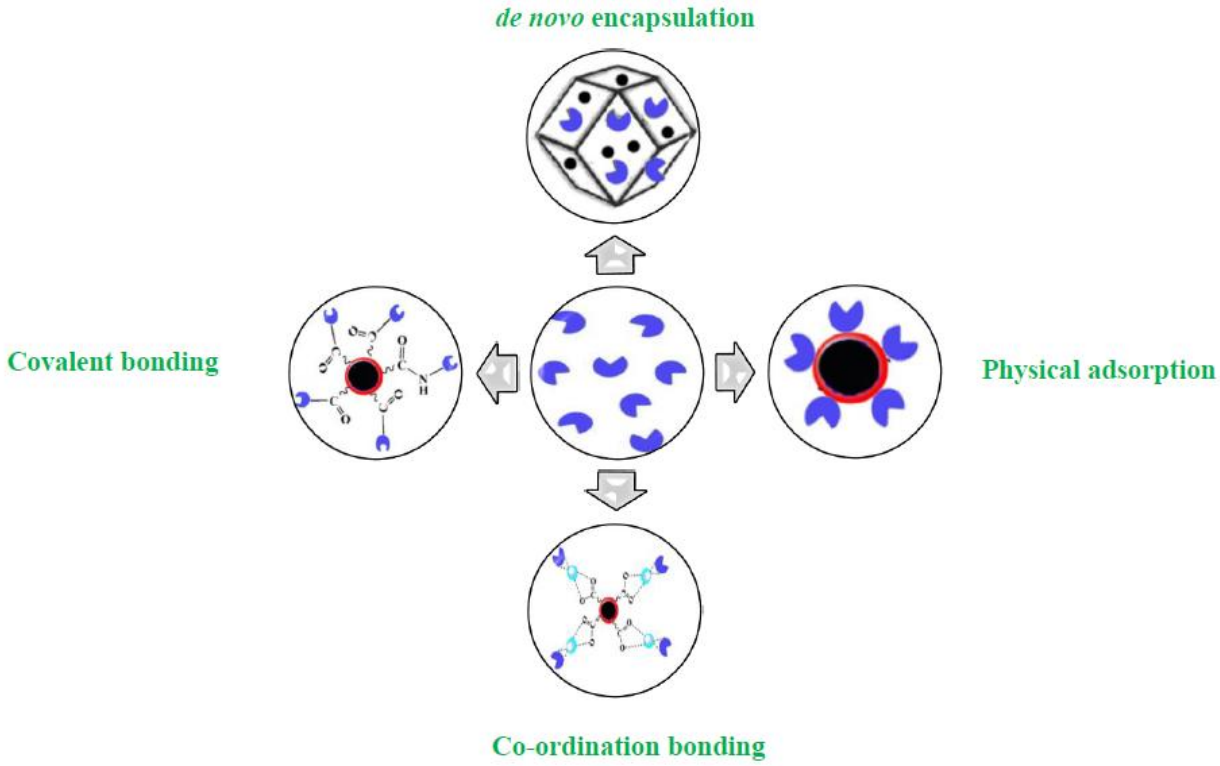
Figure 6 (a) Schematic illustration of colorimetric detection of cholesterol using cholesterol oxidase and magnetic MIL-100(Fe), (b) calibration plot corresponding to cholesterol concentration (Copyright © 2017 Elsevier B.V. All rights reserved, reprinted with permission) [96].

Table 1 Enzyme immobilization by using various magnetic (Fe_3O_4)–MOFs.

Metal	Ligand	Coating agent	Enzyme	Mode of immobilization	Ref.
Zn^{2+}	2-methylimidazole	Citric acid	GOx	Biomineralization	[97]
Fe^{3+}	H_3BTC	EDTA-2Na	Lipase	Metal–ion affinity	[80]
Fe^{3+}	H_3BTC	EDTA-2Na	Lipase	Covalent binding	[80]
Fe^{3+}	NH_2-BDC	-	*Candida Rugosa* Lipase	Physical adsorption	[71]
Zr^{4+}	H_2BDC	Agarose	CalB Lipase and Trypsin	Physical adsorption	[64]
Cu^{2+}	H_3BTC	Dopamine	Trypsin	Physical adsorption	[81]
Cu^{2+}	H_3BTC	Citric acid	GOx and HRP	Layer by layer physical adsorption	[88]
Fe^{3+}	H_3BTC	Citric acid	Cholesterol oxidase	Physical adsorption	[96]
Zn^{2+}	2-imidazolecarboxaldehyde	Amino group	Trypsin	Covalent binding	[98]

Graphical Abstract

The enzyme immobilization of onto magnetic-MOF can be strategies into three part: (i) physical adsorption onto magnetic MOF, (ii) covalent/co-ordination bonding with magnetic MOF, (iii) metal–ion affinity co-ordination bonding and (iv) *de novo* encapsulation method.



Highlights

► The robust magnetic-MOFs can be used as enzyme immobilization platform.

► The strategies for fabricating enzyme within magnetic-MOF have been explored.

► New emerging trends in magnetic-MOF as a nanozyme are disclosed.