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Merging MOF Chemistry & Biocatalysis: A Perspective for Achieving Efficient Organic Synthetic Processes and Applications in the Chemical Industry?

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Biocatalysis has emerged in recent decades toward a widely applied catalysis technology in the chemical industry. In particular the fine chemicals and pharmaceuticals industries benefit from the advantages of biocatalysis, which made its way to a dominating industrial core technology for manufacturing chiral molecules. However, often biocatalysis is still to a certain extent away from having solved all challenges being needed for a "perfect industrial process technology". Among existing challenges are, e.g., stability under process conditions and easy separation of the catalyst from the reaction mixture with the additional option of recyclability. Here metal-organic framework (MOF) structures offer unique advantages, which can be beneficial for biocatalysis. A particular valuable option is integration of enzymes into MOF-subunits, thus having a potential positive impact on stability (by reducing the tendency of unfolding) and enabling compartmentalization as a beneficial strategy in, e.g., chemoenzymatic synthesis. In this "*Perspective*"-article, first the state of the art in biocatalysis is briefly summarized together with current challenges. Then, a short overview about current research achievements in "merging" MOF chemistry and biocatalysis is given as well as an outlook written from a biocatalysis practitioner's view, how MOF-enzyme hybrid systems can play a major role in future process development to overcome existing hurdles of enzymatic catalysis.

1. Introduction

Biocatalysis has emerged in recent decades toward a widely applied catalysis technology in the chemical industry.[1] In particular the fine chemicals and pharmaceuticals industry today benefits from the advantages of biocatalysis, including, among others, the outstanding stereoselectivity, which is of utmost importance in these industries.[2] Thus, biocatalysis made its way to a dominating industrial core technology type for manufacturing chiral molecules such as drugs and their intermediates. It is noteworthy that while still not being a "standard tool" in many organic chemistry labs, in the chemical industry biocatalysis is used in these fields (fine chemicals and pharmaceuticals) as a matured and routine manufacturing technology.[3] Impressive productivities can be obtained and many processes run at substrate loading enabling a highly competitive production of the desired target molecules. Thus, biocatalysis often outperforms "classic" chemical routes for chiral molecules such as kinetic resolution based on diastereomeric salt pair formation and even asymmetric chemocatalysis.

However, in spite of many success stories biocatalysis is still far away from solving all challenges being needed for a "perfect industrial process technology". As a selected example, the limitations of biocatalysis become evident when moving from the high value-low volume field of pharmaceuticals and fine chemicals to the bulk fields of commodity and specialty chemicals. Here, industrialization of biocatalysis still is in its infancy.

What are the reasons for this contradictory impact of biocatalysis in these fields? When it comes to the production of commodity chemicals, certainly the key advantage of using enzymes in pharma, which is stereoselectivity, is not relevant anymore. However, stability and recyclability of a catalyst in general are very important criteria, as the prices for catalysts (based on the same mass) are usually much higher than those for substrates. Thus, finding ways to significantly increase the stability of an enzyme catalyst is of utmost importance to give biocatalytic processes a perspective for commercialization in this field of bulk chemicals. There are some success stories for efficient immobilization, however, mostly related to pharmaceuticals as a product class belonging to the higher price segment. For example, the β-lactam antibiotic intermediate 6-APA is produced on >10.000 tons scale (see also chapter 2.1. below) and immobilization turned out as a key issue for achieving economical favorable data. It should be added that besides achieving a high stability, immobilization

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is connected with another key advantage being very relevant in the field of commodity chemicals, which is product and catalyst separation.

Obviously, the challenges lying ahead to move biocatalysis to an efficient technology for commodity chemicals production are also related to tools for immobilization and tailor-made materials. Metal-organic frameworks (MOFs) emerged as exciting tool in this field of enzyme immobilization,[4] which could contribute to overcome existing limitations of enzyme catalysts.

This "*Perspective*"-article on the potential of metal-organic framework-enzyme composites ("*Enzyme@MOF*" composites) as a new generation of biocatalysts centers on the following issues:

1. A brief review on the importance of enzymes and their drawbacks for industrial purposes.
2. An overview of reasons why "*Enzyme@MOF*" could serve as a new promising generation of biocatalysts and opportunities for the chemical industry.
3. A brief review session what has been done so far in the field of MOF-immobilized enzymes.
4. A summary and future outlook.

It should be added that this perspective is written by authors from a research group with a background in organic synthesis with enzymes and resulting biocatalytic process development, whose view on combining MOFs with enzyme catalysts is, thus, more focused on the synthetic application side with the goal to gain answers for questions such as:

- Where are opportunities for MOFs in biocatalysis?
- Why is it beneficial to use MOFs rather than other immobilisates and immobilization technologies, or in other words, how can enzyme catalysis particularly benefit from MOFs?
- Can MOFs contribute to solve the open challenges in biocatalysis that remained, at least in part, over decades and could not have been solved by other methods?
- What areas (products, reaction types) might be of particular value and potential in "merging" enzyme catalysis and MOF chemistry?

The following will attempt to provide answers to these questions. Since these answers are given from a very personal perspective, being aware that this might not reflect a general opinion, this "*Perspective*"-article is intended to serve more as a basis for further discussion of this topic area rather than a definitive "prediction". What can be said for sure, however, is that it is worth also from the view of a process chemist being active in biocatalysis to combine these two fascinating fields of biocatalysis and MOFs.

2. Importance of Enzymes and Their Drawbacks for Industrial Purposes

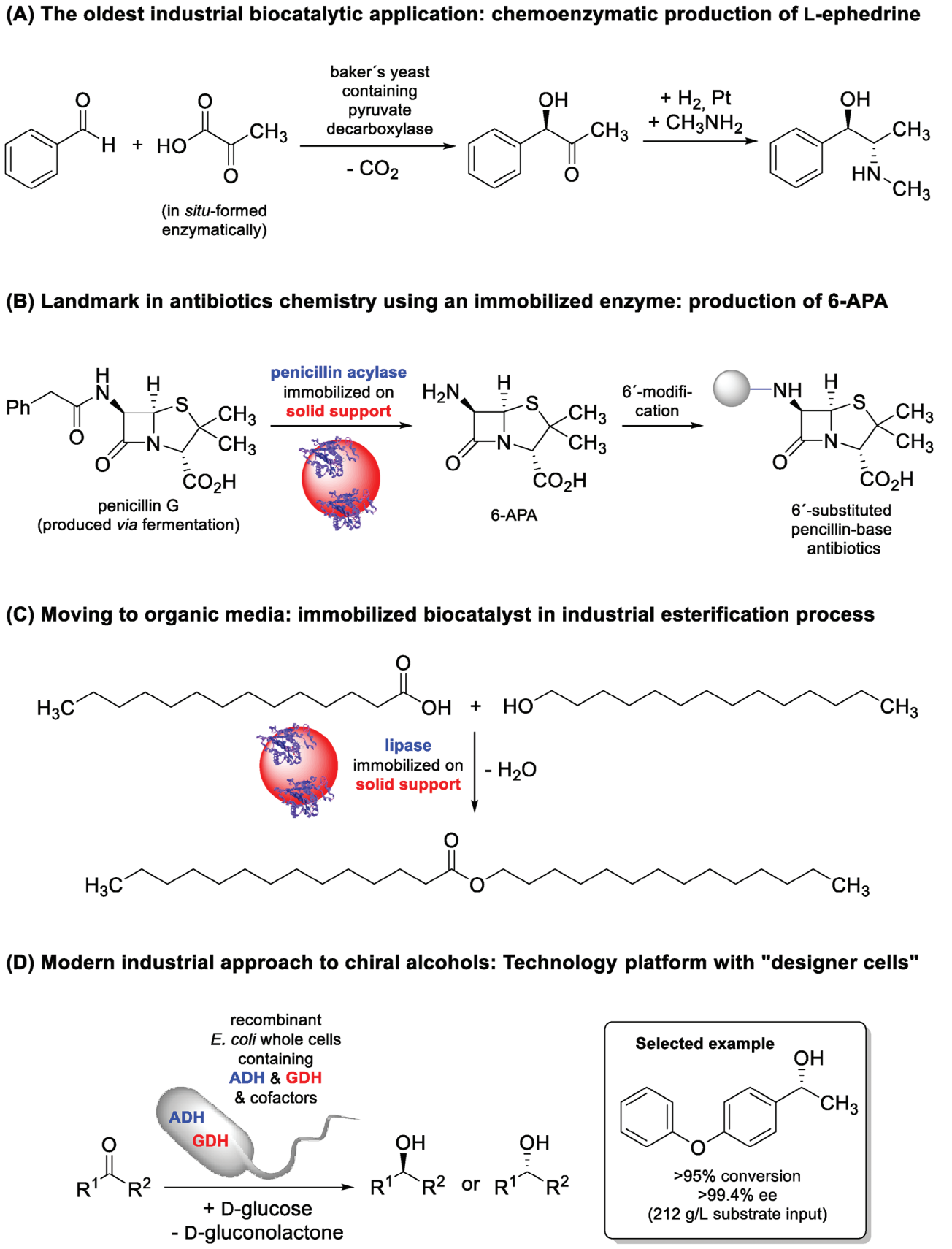
2.1. Summary of Importance of Enzymes in Industrial Processes

A major reason why enzyme catalysis made it to a dominating manufacturing technology in the fine chemicals and pharmaceuticals industry is the excellent stereoselectivity that enzymes show in such applications.[1] However, it is not stereoselectivity alone but also that this goes hand in hand with high process efficiency, which enables the production of such compounds with both, excellent enantiomeric access of >99% *ee* (being a typical regulatory criterion demanded for approval of drugs) and high substrate loading, being above 100 g L^{-1} for many biocatalytic processes, thus fulfilling industrial demands in terms of economy. Furthermore, another "selling point" for using enzymes is the suitability to tolerate many functional groups, thus avoiding the need of protecting group chemistry. As a result, the waste generated by such processes can be much lower, which contributes – besides sustainability – also to a more favorable economy of the overall process.

A few selected examples are given below to illustrate this enormous potential of enzyme catalysis for industrial applications from different perspectives, and representative solutions for achieving technical feasibility are shown (**Scheme 1**). When selecting these representative processes, the focus was on those process types that also provide interesting opportunities for the use of MOFs in combination with enzymes as a beneficial process solution (and this theme is discussed more in detail in Chapter 3 on perspectives and challenges of MOF-enzyme hybrid systems for industrial use as a new generation of biocatalysts).

Representing to the best of our knowledge the first industrial process of biocatalysis, baker´s yeast has been directly used as a biocatalyst for converting pyruvate (being available in molasses as a waste stream) with benzaldehyde (Scheme 1, part (A)).[5,2] This "*Umpolung*" reaction catalyzed by a pyruvate decarboxylase as biomass forms (*R*)-phenylacetcarbinol, which is then chemically converted with methylamine to the corresponding imine, followed by subsequent hydrogenation to L-ephedrine. This early industrial example, dating back to the 1930s and established by Knoll AG, already underlined the high efficiency of enzyme catalysis and its suitability for industrial production of chiral chemical products required in particular from the pharmaceutical industry. It is noteworthy that although many decades have passed, this process is still being applied in industry, thus also reflecting its high competitiveness.

In the following decades, industry became more and more interested in the application of enzyme technology to solve production challenges of chemicals on technical scale. A particular focus was on the use of isolated and immobilized enzymes rather than native whole cells (as in case of baker´s yeast in the L-ephedrine process outlined above). The emphasis upon the utilization of isolated enzymes as defined "molecular catalyst entity" for bioprocesses showed a high similarity to typical organic-synthetic process development with chemocatalysts. Accordingly, also the solutions to overcome hurdles when working with chemocatalysts have then been applied in an analogous way to enzymes, which is, among others, immobilization. Advantages of immobilizing enzymes (as well as chemocatalysts) in technical processes are not only reduction of catalyst costs by means of re-using the catalyst, but also simplification of the overall process design as a major benefit: Since immobilization of the catalyst leads to heterogenization, separation of product from catalyst becomes much easier, leading to the reduction of unit operation steps. A further advantage of immobilization, in particular in the field of biocatalysis, besides practical separation and re-use of enzymes and process simplification, is the higher stability of the resulting



Scheme 1. Representative examples of technical process technologies using enzyme catalysts.

immobilized enzyme compared to "free" enzyme in many cases. Clearly this advantage is also of interest when considering MOFs as an option to immobilize enzymes.

An industrial biocatalytic milestone highlighting the power of enzyme immobilization is the production of (+)-6-aminopenicillanic acid (6-APA) being a key intermediate for production of semisynthetic penicillin-derived β-lactam antibiotics (Scheme 1, part (B)).[6,7] The immobilized penicillin acylase enables a highly efficient hydrolysis of penicillin G (obtained readily via fermentation). This process is applied on a >10.000 tons scale to provide 6-APA for further derivatization and formation of semisynthetic penicillin-based antibiotics via chemical 6´-modification.

While this process is conducted in water, immobilized enzymes have also turned out to be suitable as catalysts in pure organic reaction media. Whether aqueous or organic solvents represent the reaction medium of choice depends on the type of substrate, the nature of the reaction and, last but not least, also on the type of enzyme. An example showing the advantages of biotransformations when being conducted in pure organic reaction medium is the direct esterification catalyzed by a lipase. Lipases are enzymes being capable to convert acids and alcohols into esters (in organic medium under removal of water) or, vice versa, hydrolyze esters to acids (which favorably proceeds in water as reaction medium). Thus, by designing the right reaction medium, the equilibrium of reaction can be shifted towards the desired direction. The direct esterification of an acid with an alcohol is of high interest for industry as a sustainable way to produce esters from non-activated acids, and has been developed to a technical process under solvent-free neat conditions for fatty acid ester production from a fatty acid and fatty alcohol (Scheme 1, part (C)).[8,9] Once again immobilization is a key criterion for an economic process as the catalyst can then be re-used and in addition easily separated from the reaction medium. Accordingly, the design of tailor-made immobilized enzymes is a prerequisite for such a successful catalyst. This also makes MOFs a promising heterogeneous system for such a purpose due to, e.g., the opportunity to embed enzymes into the 3D-network of MOFs (see also chapter 3 below).

Besides hydrolases, many other enzyme classes have been successfully used in the chemical industry, in particular for the synthesis of chiral products for applications in the pharmaceutical industry. Among them, enzymes catalyzing redox reactions play a major role. Representative examples of enantioselective biotransformations are the reductive amination of α-keto acids to α-amino acids,[10,11] the transamination of ketones to chiral amines,[12,13] and the reduction of ketones to chiral alcohols.[14,15] It is noteworthy that while the first two reaction types are still challenging reactions in the field of chemocatalysis, enantioselective metal-catalyzed hydrogenation of ketones has early emerged as a highly efficient and technically feasible process technology for production of chiral alcohols. Its importance is underlined by successful industrial transformations as well as having been awarded as a Nobel Prize technology.[16] However, even this excellent benchmark of asymmetric Noyori-type hydrogenation technology can be met using enzyme catalysis as demonstrated by numerous biotransformations for ketone reduction, which have been developed in particular in the last two decades.[17–19]

Among various reasons, why biocatalytic ketone reduction has emerged as an alternative to the well-established asymmetric metal-catalyzed hydrogenation, the design of tailor-made cells ("*designer cells*") is one of them, which also underlines how synthetic chemistry with enzymes benefits from the impressive progress in molecular biology. By means of this technology, enzymes can be produced in recombinant form with high overexpression, enabling the design of tailor-made *E. coli* cells. These cells are capable to be produced in highly economic form by high-cell density fermentation, containing the desired enzymes, here an alcohol dehydrogenase (ADH) and for in situ-cofactor recycling in high amount a glucose dehydrogenase (GDH). Based on such cells, the resulting highly efficient reduction processes run at high substrate loading of >100 g L^{-1} and lead to excellent conversion and enantioselectivity (thus, making such processes also of interest for industrial purpose). Such syntheses were developed using readily available, non-toxic and cheap D-glucose as a reducing agent in a pure aqueous system. A selective example is given in Scheme 1, part (D).[18]

These very few selected examples (among many successful examples of biocatalysis in the last decades with many of them demonstrated on technical scale) also show that complementary solutions have been found, which turned out to be "the key" to overcome each of the case-by-case limitations in "tailor-made form", thus enabling the realization of industrially feasible process technologies. The type of solution depends, as outlined above, on the type of challenge and limitation to be overcome, and therefore has to be developed on a case-dependent base.

It should be added that in recent years, significant attention has been given to the improvement of enzyme properties through the technique of enzyme immobilization.[20] This approach has been extensively reviewed and widely acknowledged as a powerful technology for the development of various industrial processes.[21] Hence, the selection of an appropriate support material plays a crucial role in enhancing the efficiency during the biocatalytic process. The support material provides a stable and favorable environment for the immobilized enzyme, influencing its activity, stability, and overall performance. Factors such as surface chemistry, porosity, mechanical strength, and compatibility with the enzyme should be carefully considered when selecting a support material.[20,22]

2.2. Summary of Drawbacks of Enzymes, Resulting Challenges To Be Solved & Opportunities For Solutions by MOFs

In spite of all the impressive features and advantages of enzyme catalysis, also drawbacks exist. In order to overcome and avoid these drawbacks, heterogenization of enzymes by their integration into MOF structures (thus, harnessing the advantages MOFs can offer) and the use of such hybrid systems would represent a promising concept. In the following, some typical (selected) drawbacks of enzymes are (very) briefly summarized, which should serve as a basis for a discussion, how embedding enzymes into MOF structures can contribute to overcome these existing hurdles.

A typical limitation in biocatalytic organic synthesis, is insufficient process stability of enzymes. This drawback can be rationalized by the very different reaction conditions of enzymes under native conditions (in a living cell) and under organic-synthetic process conditions. While typical substrate concentrations under native cellular biosynthetic conditions are low (often being below 10 mM or even 1 mM), from an organic process chemist´s perspective an ideal process should be able to be operated at high substrate loading of typically 1,000 mM or even higher. For the economy of a technical process such a high substrate loading plays an essential role. However, such high substrate loadings, as well as typical reaction conditions associated with organic synthesis such as the presence of organic solvents, elevated reaction temperature or unfavorable pH can be harmful for enzymes. Thus, identifying solutions for improvement of an enzyme's stability under organic-synthetic process conditions is still a challenge, and immobilization in general is a valuable option for achieving this goal. Therefore, attaching enzymes to MOF surfaces or embedding them into MOF pores could serve as such a valuable option.

Although the above described limitations are related to the reaction conditions of the biotransformation itself, also the reaction conditions of the synthesis of the substrate for the biotransformation or the further derivatization of its product can have an impact on the enzyme's performance. This is the case in cascade processes when multiple reactions are combined in a one-pot fashion. While one can expect similar reaction conditions when combining enzymatic reaction steps (as in case of fermentation processes), the combination of chemocatalytic and biocatalytic reactions steps often differ in required reaction conditions strongly from each other. Such combinations of reactions with "catalysts from different worlds" are challenging in many cases.[20] If such reaction steps for their desired combination turned out to be not compatible with each other, compartmentalization represents a potential solution. By spatial separation of, e.g., the catalyst entities in different compartments, combinations of such reactions being incompatible with each other in original form can be achieved. As for such a needed compartmentalization, MOF structures represent highly promising structures due to their fine-tuning with respect to size (thus, excluding certain components with a larger size to reach then the enzyme in the MOF) as well as hydrophobic or hydrophilic properties, which avoids, e.g., the contact with certain undesired components (substrates, intermediates, products, solvents). As this field of chemoenzymatic one-pot synthesis[20] is an emerging research area (due to the enormous potential to realize unique multi-step processes in a highly economic and sustainable mode, reducing the number of product isolations, work-up steps, solvent consumption and waste production), utilizing MOFs as a "problem solver" in this field would be of high interest.

In conclusion, the above-described drawbacks of enzymes in organic synthesis, at least in part, could in principle be overcome by MOF-embedded enzymes and some of these opportunities and promising perspectives are further described in the subsequent chapter 3.

3. "*Enzyme@MOF*" Composites: Perspectives & Challenges for Industrial Use as a New Generation of Biocatalysts

3.1. "*Enzyme@MOF*" Composites: Perspectives

A major breakthrough would be to embed enzymes in material structures that prevent enzyme destabilization while maintaining its high activity. Although at first glance it seems to be contradictory to keep enzymes flexible (which is needed for efficient catalysis) on the one hand and to make them rigid on the other hand (thus, preventing embedded enzymes from unfolding, to make them stable), MOF materials have one property which could be the "key" to exactly match these two criteria. MOF structures enable a fine tuning of the pore-sizes for entrapping enzymes as well as channels for substrate and product diffusion (by making them, on demand, more or less hydrophobic in a tailor-made fashion). Therefore, one could imagine a MOF design being tailor-made for enzymes in a way, that the following criteria are met: (i) perfect catalysis; (ii) no or negligible mass transfer limitation; (iii) suppression of defolding, thus maintaining enzyme stability.

Among further advantages of MOF is the suitability to design them for proper use in different solvents, thus making applications in water as well as organic media possible. A fulfilled criterion of stability by MOF-entrapment might also allow to reach novel process conditions being not reachable with enzymes in their native environment and even not with "classic" immobilized enzymes.

A further major application field can be seen in the area of chemoenzymatic one-pot synthesis.[23] Being a young field of process research, in recent years this concept gained increasing interest. In particular conducting such processes in water as non-toxic and environmentally friendly solvent, which in addition is cheap, is also attractive for economic purpose. In such processes, enzymes and man-made chemocatalysts are combined, thus enabling to run multiple reactions in one-pot. Consequently, work-up of the intermediates is not needed, leading to reduced amounts of needed solvents and a substantial reduction of waste. A prerequisite for efficiently combining multiple catalytic reactions is compatibility, and achieving it is in particular a challenge when catalysts from different "worlds of catalysis" shall be combined, such as enzymes and chemocatalysts. Here "*Enzyme@MOF*" composites as novel catalysts have the potential to make a substantial contribution and to overcome existing long-standing hurdles. For example, by embedding enzymes within MOFs in combination with a tailor-made pore size allowing diffusion of substrate but not of critical components (e.g., the chemocatalyst of the other reaction step), a direct contact of enzymes with critical components and, thus, deactivation can be avoided. These unique properties of MOF also differs from standard immobilization tools for enzymes, which "only" attach enzymes at the surface, thus not avoiding a direct contact with critical, homogeneously dissolved reaction components.

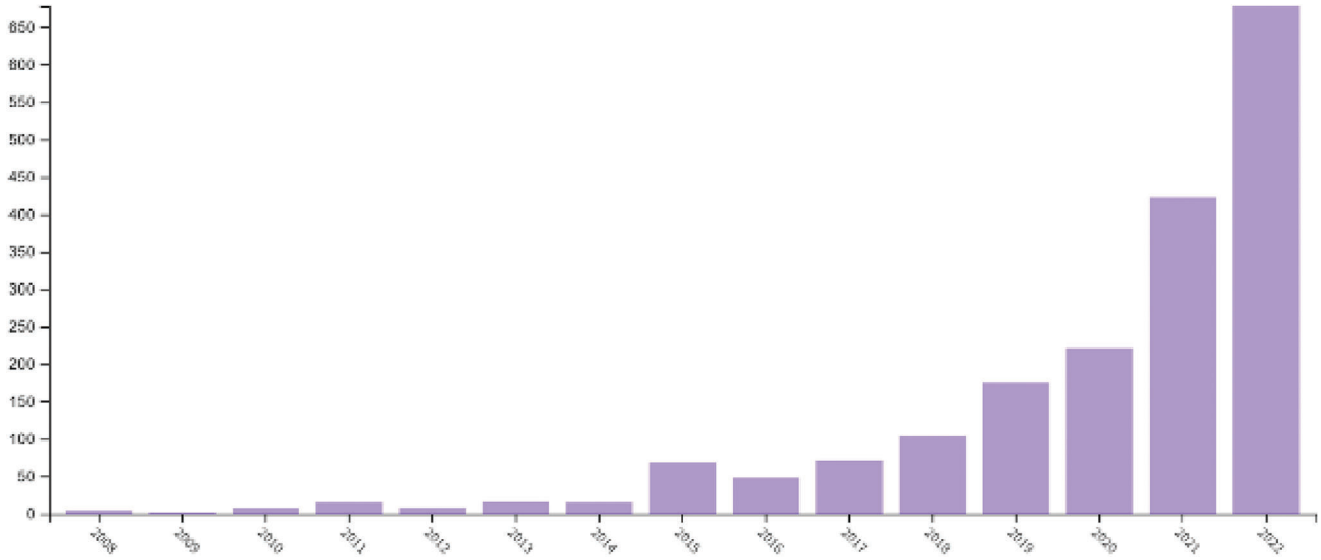


Figure 1. Annual publications in MOF research related to enzymes. Bibliometric analysis of Web of Science (WoS) database (https://www.webofscience.com).

3.2. "*Enzyme@MOF*" Composites: Challenges

In order to realize the potential of MOF-containing enzymes as new generation of biocatalysts for applications in organic synthesis, various prerequisites have to be fulfilled. One future challenge in this research area is related to analytics. Analytical methodologies being readily accessible in "standard organic labs" to provide comprehensive and fast characterization of such new generation of biocatalysts are needed. Such analytical tools should lead to a rapid determination if enzymes are immobilized "in" versus "on" MOFs, since such a different type of immobilization could lead to different results in organic synthetic applications. Besides availability of such analytical tools in organic (biocatalysis) synthetic labs, reproducibility, and robustness of preparation of "*Enzyme@MOF*" composites are a further challenge. Integration of enzymes, typically obtained in recombinant form, into MOF structures require robust and at the same time practical protocols in order to find a broad application among organic chemists. And as catalysis with "*Enzyme@MOF*" composites is a young and emerging research field, more synthetic data are needed in order to be able to evaluate the full potential of this type of catalysts in organic synthesis. In addition, studies on leaching and deactivation kinetics of enzymes being immobilized with MOFs would be valuable for further process development as well as information about abrasion and catalyst leaching under operational process conditions.

When having a look on all these criteria requiring competencies from many different fields (e.g., molecular biology and microbiology for enzyme design and preparation, material science and physics for MOF preparation and characterization, organic chemistry for synthetic applications) it becomes evident that interdisciplinary research is needed. Thus, a further need is bundling such competencies in interdisciplinary consortia in order to fully exploit the high potential of this "*Enzyme@MOF*" technology.

4. State of the Art of "*Enzyme@MOF*" Composites: Where Are We Now? What has been Solved? What Not?

4.1. General Development of the Research Area of "*Enzyme@MOF*" Composites

The concept of enzyme immobilization for industrial purpose has been intensively studied since the 1950ies. It involved also the concept of embedding enzymes in polymer matrices. Since then, a broad range of versatile immobilization methods, which, in part, have also been applied on technical scale, have been established.

A more recently developed, albeit very promising immobilization concept for biocatalysts is the heterogenization of enzymes by means of metal organic frameworks (MOFs) as component for heterogenization, thus forming "*Enzyme@MOFs*" immobilizes. The increasing scientific impact of "*Enzyme@MOFs*" as a research area is illustrated by a bibliometric analysis utilizing the Web of Science (WoS) database, which shows an exponentially growing number of scientific papers containing the keywords "MOF" and "Enzyme" (**Figure 1**).

As outlined above in chapter 3.1, among immobilization techniques MOFs have great advantages. Due to their modular structure, which consists of metal centers and organic linkers, MOFs can be varied to a broad extent. This allows researchers to adopt MOF to the specific demand of the applied enzyme and also enables the immobilization of enzymes that would face deactivation by conventional immobilization methods.[24]

4.2. Overview about Enzymes which had already been Immobilized Using MOFs

In recent years, numerous "*Enzyme@MOF*" composites have demonstrated promising results, with improvements in catalytic

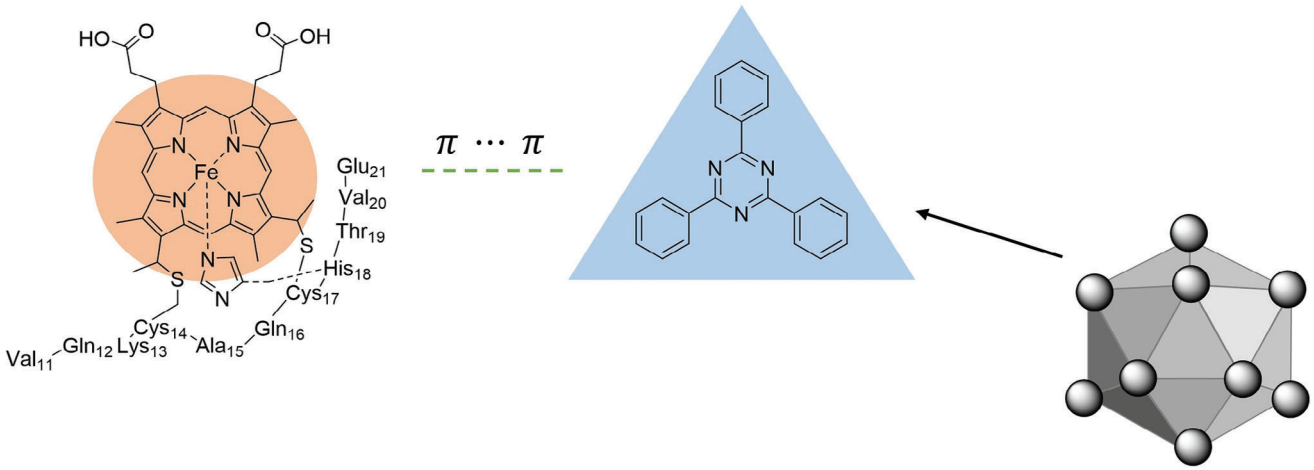


Figure 2. Interaction between heme group of MP-11 with triazene and benzene groups of Tb-mesoMOF.[45]

efficiency highlighted in various examples. A general overview of "*Enzyme@MOF*" composites, which have been prepared and utilized for various types of biotechnology applications up to now, is provided in **Table 1**.

However, with respect to applications in biocatalysis it has to be stated that most examples were performed on an analytical scale (and not preparative scale with product isolation). Therefore, the activity of the enzyme was investigated but applications in organic synthesis by means of such "*Enzyme@MOF*" as catalysts are still rare. Only in a few cases biocatalytic reactions have been conducted with characterization of the reaction progress by determining, e.g., the conversion of such transformations, e.g., by analysis via GC.

It is noteworthy that with respect to the type of "*Enzyme@MOF*" composites, various options have been considered in previous work for the heterogenization of the enzyme by means of a MOF structure (Table 1). While some of the enzyme have been encapsulated within the MOF structure (and the related entries are marked with green colored background in Table 1), others are heterogenized through a non-covalent attachment at the surface of the MOFs (red colored background). Further options of heterogenization consists of a covalent linkage or cross-linking strategy (orange colored background) or an in situ-formation of the "*Enzyme@MOF*" composite (blue colored background).

4.3. Enzymes "on MOF" Versus "in MOF": Overview about Different Types of MOF-Immobilized Enzymes

In recent years, a range of studies for "*Enzyme@MOF*" immobilization strategies have been performed and in the following some general advantages of this concept as well as different utilization of the "*Enzyme@MOF*" concept in terms of immobilizing enzymes "on MOF" versus "in MOF" are discussed.

Compared to other immobilization strategies, the "*Enzyme@MOF*" concept offers several advantages, such as higher surface areas and tunable pore size of the MOFs, which can contribute to improved thermal and chemical stability of the enzymes and less leaching of enzymes.[44] The reduced leaching of enzymes in case of "*Enzyme@MOF*" was explained by Ma *et al.*, who also investigated other porous materials, by strong interactions between the organic compounds of the MOF and the enzyme molecule.[45] In case of MP-11@Tb-mesoMOF, the heme group of MP-11 interacts by $\pi\cdots\pi$ interaction with the triazene and benzene group of the ligand (TATB), thus preventing leaching (**Figure 2**).

However, in spite of successful examples of this "*Enzyme@MOF*" concept, so far there is a lack of examples for its utilization in preparative processes, as most reactions are performed on an analytical scale. Either activation assays are used, as for ADHs,[35] ALDHs,[36] laccase,[42] catalase[37] or GOx/CPO[34] or only low substrate loadings are investigated. There is a limited number of examples of synthetic applications, in which a conversion or yield is given. In addition, typically reported processes so far have been done at low substrate loadings, which is unfavorable for utilization at enlarged, in particular industrial scale. Typical substrate concentrations are between the μM[29] and few mM[26] range. One of the few examples with industrially relevant substrate concentration is the synthesis of isoamyl acetate running at 2 M or 4 M, respectively, in combination with a very low amount of immobilisate (5 mg, 17 w/w% enzyme loading). Although a loss of activity was observed after immobilization with only 30% of relative activity, this may be, however, outperformed by the enhanced stability of the enzyme as "*Enzyme@MOF*" composite compared to the one of the free enzyme.

Since immobilized enzymes show improved solvent, temperature, as well as pH stability and can be easily recovered, they have attracted considerable interest in recent years.[46–49] As for the "*Enzyme@MOF*" concept, in principle there are two main strategies for immobilizing enzymes using MOFs, "*on MOFs*" or "*in MOFs*". These strategies can be further divided into surface attachment or covalent bonding for immobilization "*on MOFs*" or encapsulation and *in-situ* synthesis for immobilization "*in MOFs*". (**Figure 3**).[46,50,51]

A widely used immobilization strategy is the surface attachment due to its low costs, easy handling, and compatibility with a broad range of enzymes. Enzymes are immobilized by weak interactions such as Van-der-Waals forces, hydrogen bonds, hydrophobic interactions and

Table 1. Enzymes immobilized on MOFs using different immobilization strategies.

entry	enzymes	MOFs	pore size [Å]	Enzyme loading[a] [%]	reaction	Method	comparison free enzyme	Reference
1	GDH[c]	ZIF-7, -8, -67, -68, 70	2.9 – 13.1	0-31	NADH production	abs. 340 nm	n. a.	[25]
2	Lipase	HKUST-1	5.0, 10.6, 12.4	n.a.	ester formation	GC	17-fold increase reaction rate	[26]
3	lipase	UiO-66	8.5	>100.	Warfarin synthesis	electropherogram	1.2 increase, stable for 35 days in 4°C	[27]
4	lysozyme	meso-ZIF-8	72 – 450	27	Antimicrobial activity	abs. 450 nm	16-fold reduced activity, higher temperature stability	[28]
5	MP-11[e]	[Cu(BPDC)(DABCO)	27, 30.5, 55	5	ox. methylene blue	abs. 664 nm	10-fold increase	[29]
6	Lipase	IRMOF-3	11.2, 14.5	0.02	transesterification	GC	>1000-fold increase	[30]
7	CAL-B	UiO-66-NH_2	8	1	transesterification	GC	13-fold increase	[31]
8	trypsin	MIL-88B-NH_2(Cr)	n.a.	n.a.	Cleavage BSA	LC-MS	2-fold increase	[32]
9	SEH[d]	UiO-66-NH_2		1	enantiomeric hydration	GC	Activity remained in organic solvent and 45°C	[33]
10	CPO/GOx[f,g]	UiO-66-NH2	8, 11	0.02-0.04	chlorination	abs. 285 nm	54% increase	[34]
11	ADH[b]	PCN-333(Fe)	55	36	NADH production	abs. 340 nm	20% increase	[35]
12	ADH	Fe-BTC	23	5-20	NADH production	abs.340 nm	60% increase	[36]
13	catalase	ZIF-90	10	5	H_2O_2 consumption by xylenol method	abs. 560 nm	Sheltering from proteinases	[37]
14	tyrosinase	HKUST-1	5, 11, 13.5	>100	oxidation L-DOPA	abs. 475 nm	10-fold higher half life	[38]
15	MP-11	Tb-TATB	9	3	ox. 3,5-DTBC[e]	abs.420 nm	4-fold increase	
16	Esterase	NU-1000	31	18	ester formation	HPLC	30-fold increase	[21]
17	CAL-B	MOF-74 (Ni)	11	0.8	transesterification	GC	>2.24 fold increase	[40]
18	CAL-B	ZIF-8	41.7, 122.4	n.a	transesterification	GC	Improved properties	[41]
19	laccase	Fe-BTC	23	2	oxidation ABTS	abs. 405 nm	n.a..	[42]
20	catalase	ZIF-8	41.7, 122.4	12, 20	H_2O_2 concentration	abs. 240 nm	10 cycle recycling with small decrease	[43]

[a] amount of enzyme bound to MOF in w%; [b] alcohol dehydrogenase; [c] glucose dehydrogenase; [d] soybean epoxide hydrolase, [e] Microperoxidase; [f] Glucoseoxidase; [g] Chloroperoxidase, [e] 3,5-Di-tert-butylcatechol. green:encapsulation; red: surface attachment; orange: covalent linkage/crosslinking; blue: in situ formation.

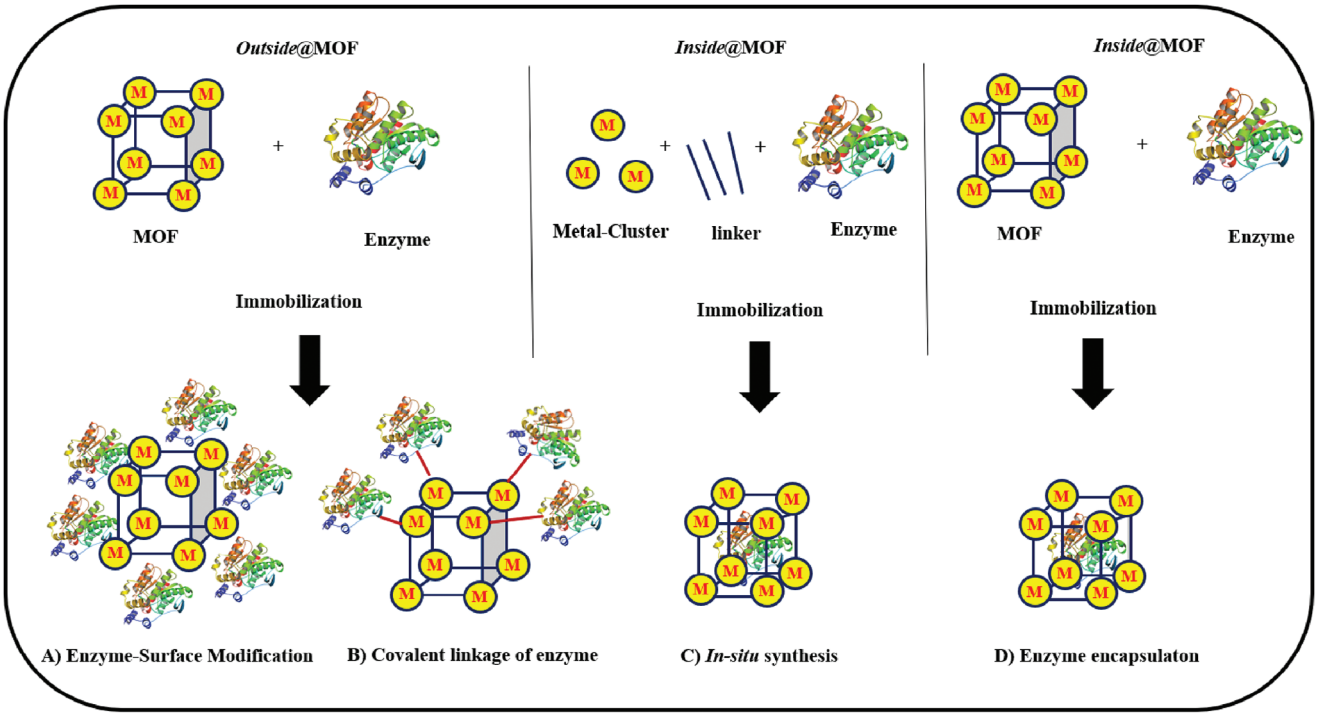
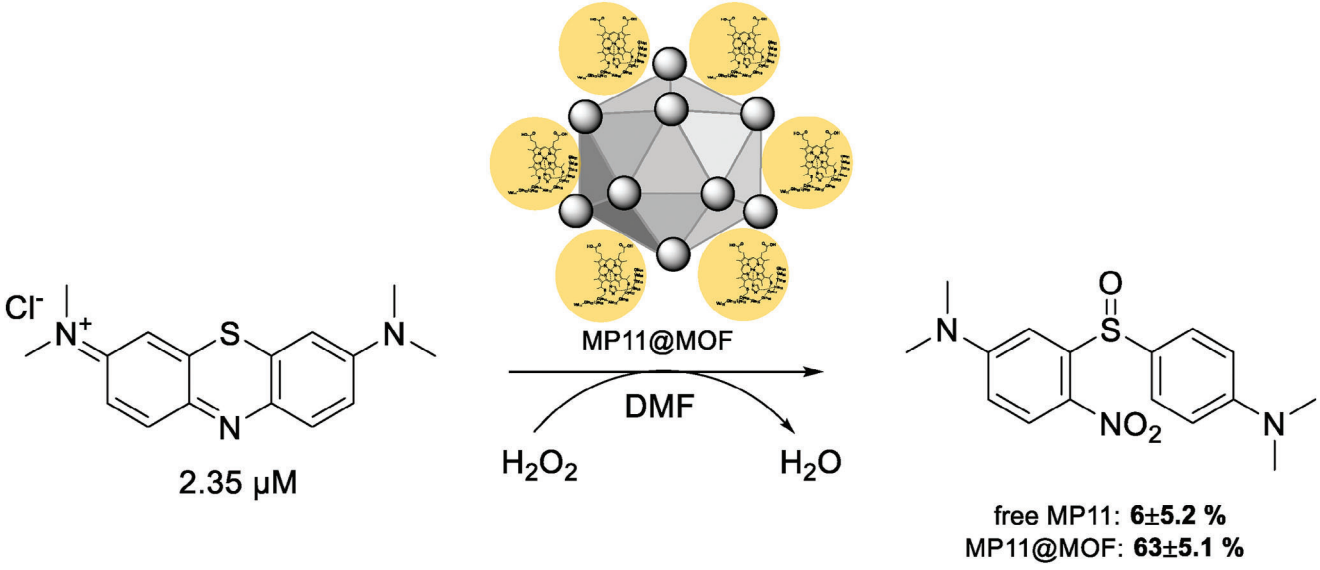


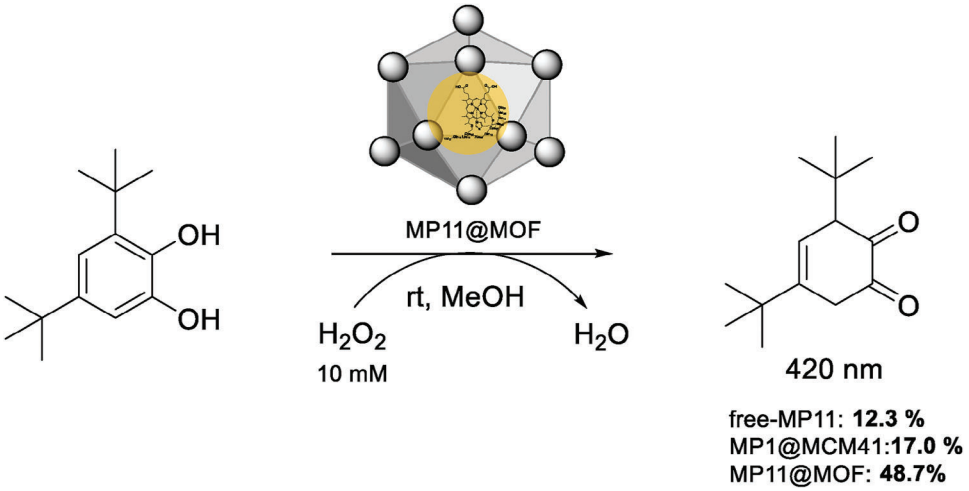
Figure 3. Different immobilization strategies for the formation of "*Enzyme@MOF*" composites: A) surface attachment, B): covalent linkage, C) in situ synthesis, D) encapsulation.

charge-charge interaction.[52–55] Therefore, it is not only prominent in MOF immobilization but also for e.g. polyacrylates[56–58] or styrene-divinylbenzene co-polymers.[59–61] The removal of the enzymes can be easily achieved by adjusting the pH or temperature.[46] However, enzyme leaching may pose a major drawback.[62]

A proof-of-concept for surface attached "*Enzyme@MOF*" was reported by Balkus et al. with the immobilization of a peroxidase (MP-11) on $[Cu(BPDC)(DABCO)]_n$ MOF, which resulted in increased activity of the enzyme (**Scheme 2**). Stability in organic solvent was investigated whereby the conversion was 10-fold increased with MOF-associated enzyme (Scheme 2).[29] Since free MP-11 tends to aggregate in solution, the accessibility of the heme in the active site is limited, which affects its activity.[63] Therefore the enzyme immobilization enables higher stability and better conversions. Microperoxidases are prepared by the



Scheme 2. Oxidation of methylene blue by MP11@MOF by Balkus et al.[29]



Scheme 3. Oxidation of 3,5-di-*tert*-butyl-catechol by MP11@MOF from Ma et al.[39]

proteolytic digestion of cytochrome c by, e.g., pepsin for the formation of MP-11.[64,65]

In another example, Ma et al. immobilized MP-11 inside of Tb-TATB through a post-synthetic encapsulation. By encapsulation of MP-11 its aggregation was overcome leading to a 4-fold increase of conversion (**Scheme 3**).[39] In addition, mesoporous silica (MCM-41) showed a significant decrease in catalytic activity in this example, resulting in no significant increase in conversion. Thus, in this case a significantly higher conversion was obtained by means of a MOF, while conventional strategies such as porous silica did not increase the conversion drastically.

In both examples, *Outside@MOF* (Balkus et al.) and *Inside@MOF* (Ma et al.) lead to an increase of conversion in comparison to the experiment with free enzyme and conventional immobilization strategies, although it has to be stated that yet only low substrate loadings were investigated. For industrial application, an increase of substrate loading would be needed. Thus, also a comparison of "*Enzyme@MOF*" with "free" enzyme and conventional immobilization strategies under these modified conditions (e.g., at high substrate loading) would further contribute to determine the impact of "*Enzyme@MOF*" concept for large scale applications.

Encapsulating enzymes in MOFs can lead to higher solvent or temperature stability as undesired unfolding of enzymes is suppressed.[35] In addition, Tsung et al. showed higher protease stability for in situ-synthesized "*Enzyme@MOFs*". In situ-formation can overcome the limitation of pore size for encapsulation of enzymes[66] and the smaller pockets can be used to inhibit contact with proteinases.[37] While larger molecules such as proteinases are not able to touch and therefore decompose the enzyme, small substrates such as H_2O_2 are still able to diffuse through the framework, thus conducting the desired biotransformation (**Scheme 4**).

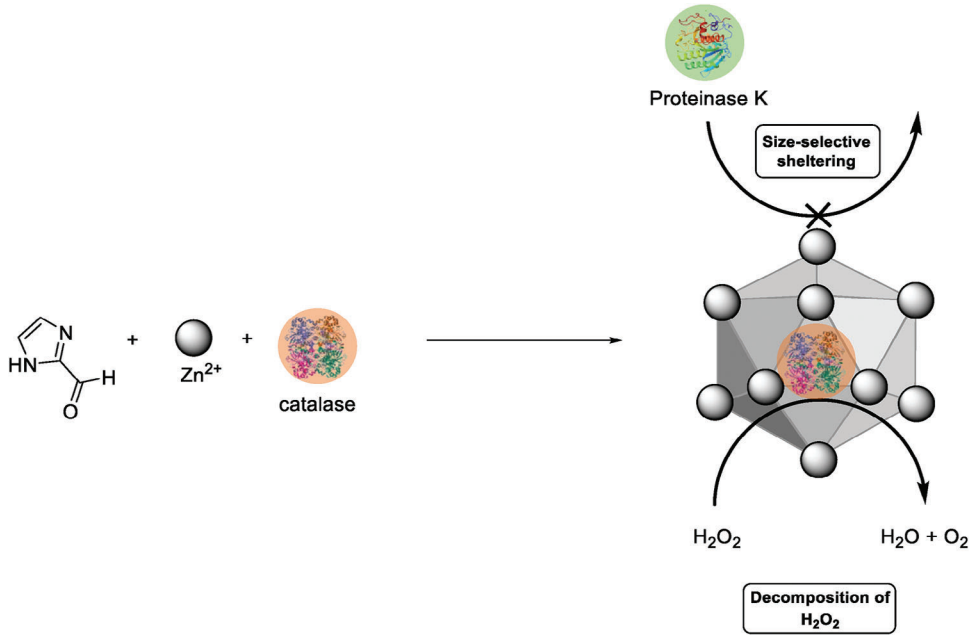
Furthermore, enzymes can be used to induce nucleation of MOFs.[67] In the case of ZIF-8, proteins with a low isoelectric point (pI) (< 7) initiated the nucleation when precursors were below supersaturation while when using enzymes with high pI (> 7), an initiation of nucleation was not observed.[68]

The in situ-formation of "*Enzyme@MOFs*" has also an impact in the crystal and pore size. Patterson et al. showed in their study that increasing protein concentration leads to a reduced crystal size when investigating different precursor loadings and ratios.[69]

Using this strategy a high diffusion barrier and mass transfer limitation remains as a significant drawback compared to free enzymes or other immobilization strategies, respectively.[70,71] In situ-strategies for enzyme immobilization have been implemented utilizing liposomes,[70] agarose[71] and encapsulated strategies by sol-gels.[72–75] Another immobilization method is the covalent linkage of enzymes on MOF-materials (*Outside@MOF*). The covalent binding of enzymes often requires activation of the amino or carboxy functions by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), *N,N'*-dicyclohexylcarbodiimide (DCC) (**Scheme 5**) or glutaraldehyde (**Scheme 6**). This ensures a stronger binding and consequently leaching of enzymes to less extent.[30,50]

However, denaturation is more pronounced compared to other immobilization strategies.[70] Other examples for immobilization are based on the use of activated resins such as sepabeds,[76–78] cellulose[79–81] or mesoporous material.[82] Lou et al. showed the enantioselective hydrolysis of 1,2-epoxyoctane by the use of covalent bound SEH@UiO-66-NH_2, whereby the stability in organic solvent, pH-stability, thermostability and storage stability was increased.[33]

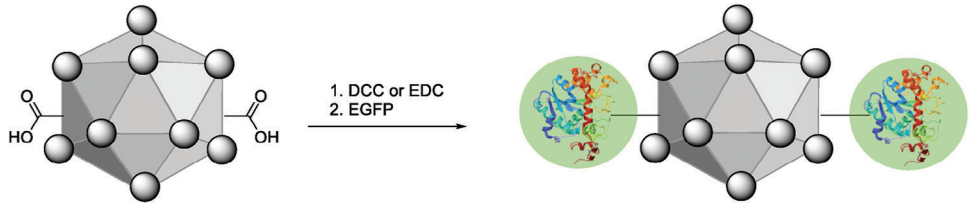
In contrast to *Inside@MOF*, the enzyme stability might be lower as the unfolding of the enzyme is not suppressed. Though, as the attachment is not reversible compared to surface-attached enzymes, less leaching of enzymes is observed.[46] If reaction conditions are not selected wisely, a leaching of enzymes can be observed.[46] As the enzyme is presented on the surface for both, surface-attached and covalent bound MOFs, mass transfer becomes less of an issue compared to *Inside@MOFs*. In case of covalent bound CAL-B@3D-IRMOF-3, an increase of >1000-fold of activity in the transesterification was observed (**Scheme 7**). The MOF-scaffold could provide confined spaces nearby resided enzyme for substrates to enhance the efficiency.[30]



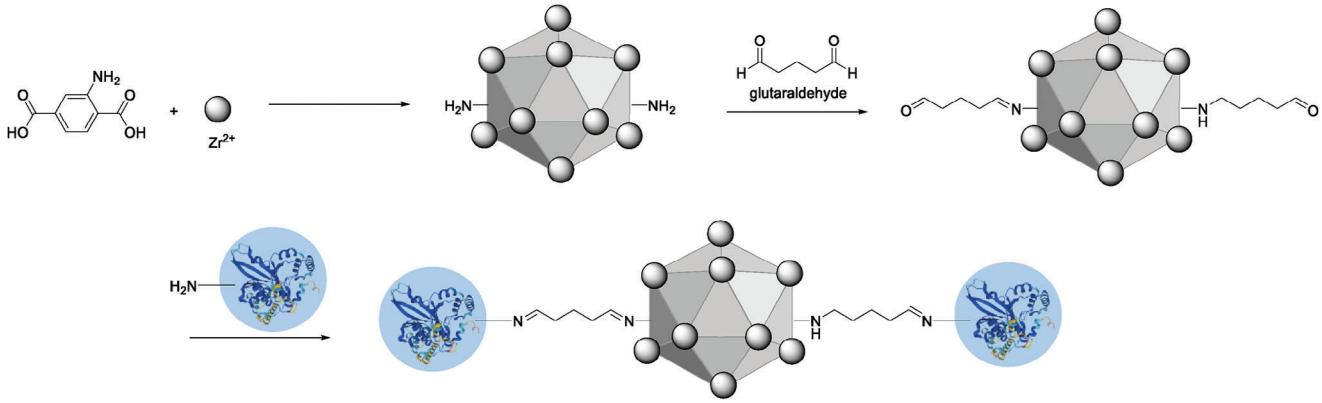
Scheme 4. Sheltering of catalase (1TGU, PDB) by *Enzyme@MOF*-formation from proteinase K (1CNM, PDB) by Tsung et al.[37]

Simpler modulation of the enzyme is possible with the *Inside@MOF* strategy, however, since no modification of the enzymes is required. By using this strategy, a modulation of a variety of enzymes is possible.[83]

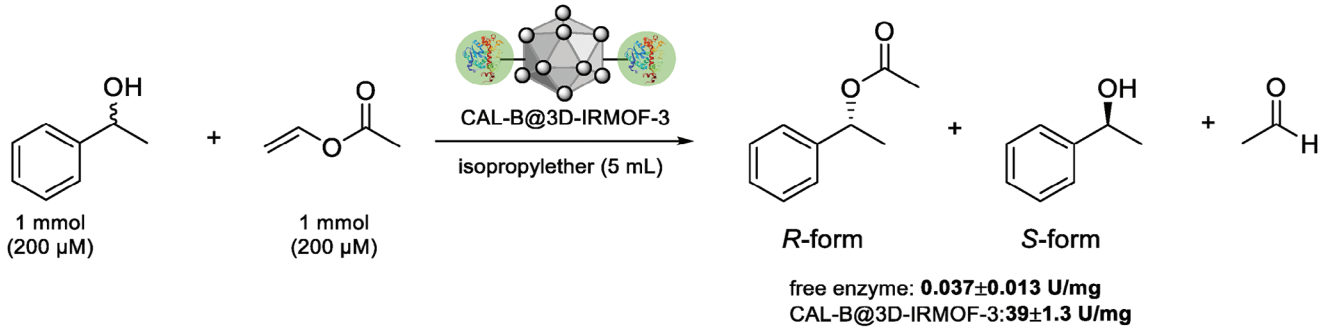
As MOFs can suffer from mass transfer limitations,[84] the embedding of enzymes further increases its limitations. However, by the in situ-strategy the pores size can be further enlarged. In case of the Enzyme@Mg-MOF-74, β-glucosidase increased the pore size by 44%.[85] In another example the post-infiltration of proteases to MIL-101(Al)-NH_2 did not lead to any change of the pore-size or morphology.[86] Similar results are obtained when enzymes are immobilized by surface attachment[87] or covalent binding. In case of the encapsulation of cytochrome c (Cyt c) and horse radish peroxidase (HRP), the infiltration caused the pore



Scheme 5. Covalent-binding of CAL-B (4K6G, PDB) on MOFs by DCC or EDC from Park et. al.[30]



Scheme 6. Covalent binding of SHE (A0A6G0UAH3, UniProt) on MOFs by glutaraldehyde.



Scheme 7. Kinetic resolution of *rac*-1-phenylethanol using CAL-B@3D-IRMOF-3 (4K6G, PDB) by Park et al.

size to decrease, to ⅓ or ⅙, respectively. It is however very likely that the mean pore size decreases due to the infiltration of the enzyme as it occupies the free pore volume.[22] Therefore, in case of encapsulated enzymes it is not easy to predict the change of the pore-size.

Nevertheless, mass transfer issues can be tackled by, e.g., increase of pore-size by carefully choosing parameters or change of reaction set-up. Often the change of batch-type to flow reactions can tackle mass transfer issues. Whole cell reactions are also tackled by mass transfer issues whereby the transfer to flow systems can be impactful for higher conversion rates. For example, in case of imine reductases (IREDs), the relative conversion was increased by 61% using non-optimized flow conditions.[88]

Since in case of *Outside@MOFs* the enzymes are presented on the surface, change of pH can have a higher impact on them compared to *Inside@MOFs*. Nevertheless, in case of covalently bound SEH@UiO66-NH_2 a slight increase of stability was gained at more basic pH values and higher temperatures up to 35 °C. At 40 °C and 45 °C significant higher activities were obtained when SEH@UiO66-NH_2 were used.[33]

Interestingly, Chen *et al.* observed that when encapsulated enzymes (Cyt c@MHNiO and HRP@MHNiO) were incubated at 100°C for an hour, residual activities of the enzymes were still detectable (≈80%) while the free enzyme did not show any activities at all (**Scheme 8**).[22] Yet, when the enzymes were incubated at 90 °C for three hours, the residual activities decreased to ≈10% or 30% for Cyt c@MHNiO and HRP@MHNiO, respectively.[22]

In another study, Marti-Gastaldo et al. showed that a protease@MIL101(Al)-NH_2 (1IBQ) was still active up to 105 °C, while the free enzyme did not show any activity >55 °C. Protease@MIL101(Al)-NH_2 showed an activity of 78% at 95 °C, which decreased significantly at 105 °C to < 20%. Also, while the free enzyme did not show any activities at pH 9 – 12, the encapsulated enzyme still showed 20 –30% with little to no change.[86]

The examples from Chen et al.[22] and Marti-Gastaldo et al.[86] are very exciting as enzymes are shown to have high activities in the range of 80 – 105 °C while the normal range for free enzymes are in most cases well below these temperatures. Most likely the folding of encapsulated enzymes is highly suppressed, wherefore residues activities are still observable at harsher conditions such as temperatures up to 100 – 105°C and pH > 9. In contrast, under such conditions typically free enzymes show no or very low activities. These results also open up perspectives towards synthetic and technically feasible applications of encapsulated enzymes at elevated reaction temperatures.

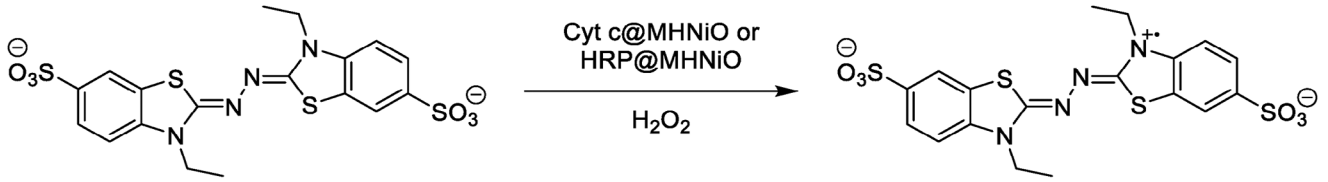
Furthermore, post-synthetic encapsulated Cyt c (Cyt c@NU-1000) showed enhanced resilience against organic media as the TON did not significantly decrease in hexane, acetone, THF, MeOH or dioxane while the TON of free enzyme decreased in that order.[89]

The different advantages of the presented strategies are summarized in **Table 2**.

4.4. Advantages & Drawbacks When Using MOF-Immobilized Enzymes in Comparison to Processes with Free Enzymes, Cells or Other, "Standard" Immobilization Methods for Enzymes

Attaching enzymes to MOFs offers several advantages beyond immobilization, such as ease of handling under different reaction conditions, facilitating reaction work-up, and allowing for catalyst recyclability.[47,50] Furthermore, encapsulating enzymes in MOFs also provides other benefits, which can be classified into various categories that often overlap. Numerous studies have demonstrated that encapsulation significantly improves enzyme stability, with enhancements observed in areas such as temperature and pH value, as well as resistance to harmful solvents and inhibitors.[90]

For example, Li et al.[91] successfully improved the enzyme stability by encapsulating phospholipase B (PLB) as biocatalyst in MOFs. Moreover, they were able to enhance the relative activity of PLB by nearly 15% in a temperature range of 20°C to 60°C and



Scheme 8. Enzymatic oxidation of ABTS by Cyt c@MHNiO and HRP@MHNiO by Chen et al.

Table 2. Properties of different immobilization strategies for enzyme immobilization on MOFs.

criterium	free enzyme	surface attachment	covalent linkage	encapsulation	In situ synthesis
mass transfer	high	medium	medium	limited	limited
pH-stability	low	low	medium	high	high
solvent stability	low	low	medium	high	high
temperature stability	low	low	medium	high	high
protease stability	low	low	low	medium	high
denaturation	low	low	high	low	low
leaching		high	low	medium	low
modification enzyme		not necessary	activation	no	no

a pH range of 5.0 to 8.0.[91] Additionally, they demonstrated that the integrated PLB in MOF had almost no activity loss after 30 days of storage, whereas the free PLB showed a loss of more than 70% activity.[91]

Sha et al.[92] reported that MOFs significantly enhance the stability of cytochrome c against different denaturing organic solvents. This enables to conduct the synthesis in organic solvents, which can be beneficial due to their high substrate solubility, ease of removal, and range of polarity and protic or aprotic properties.[92]

However, the advantages of MOFs for enzyme immobilization goes beyond improvement in terms of stability. Several studies have demonstrated that MOFs can lead to synergistic effects when enzymes are encapsulated. For example, Gkaniatsou et al.[93] showed that their combined system of MP-8@MIL-101(Cr) exhibited not only enhanced resistance against acidic conditions and long-term stability, but also an improved selectivity for the oxidation of methyl orange.

Another major advantage of MOFs against other encapsulation methods has been investigated by Shen et al.[24] They observed that the enzymes often show partial or even complete loss of activity after encapsulation due to confinement effects, competing coordination or negative environmental effects. To overcome these issues, they developed a new synthetic strategy for *Enzyme@MOF* complexes that utilizes biomacromolecules to create the desired microenvironment.[24]

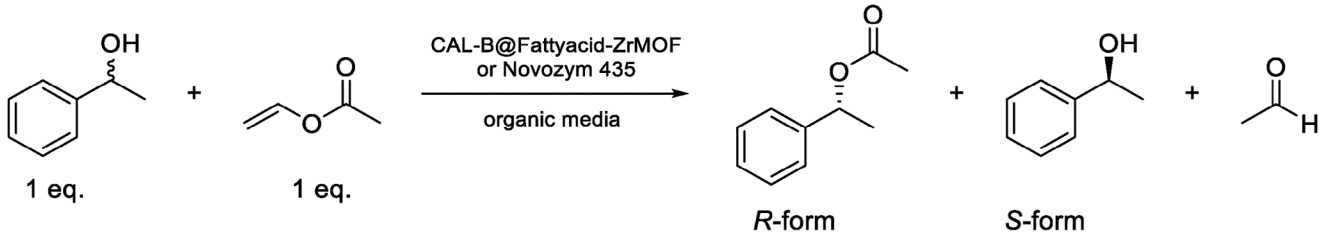
From a practical point of view, there are even more advantages. Zhang et al.[94] demonstrated how MOFs can combine target enzymes, such as lactate dehydrogenase (LDH), with corresponding coenzyme regeneration systems within one framework. This not only ensures that the enzymes are in close proximity to each other, but also achieves a 1.5-fold higher turnover rate. Such enzyme cascade systems have been developed by Chen et al.[95] and Liang et al.[96] as well.

Park et al. have shown that by covalent immobilization of CAL-B using fatty acids as crosslinker an enhancement of its stability in organic media can be achieved. While the free enzyme did not show any conversion in hexane, MTBE, THF, *t*-BuOH and acetonitrile, a moderate to high enantioselective conversion was observed when using such a MOF-entrapped lipase, especially in hexane.[31] However, immobilized CAL-B is commercially available and the esterification is also normally carried out in organic media. Therefore, this type of immobilized CAL-B catalyst serves as a better benchmark for comparison. Typically, this acrylamide supported CAL-B (Novozym 435) is used in organic solvent. For example, when Fukuda et al. used Novozym 435 in hexane similar conversions were obtained (**Scheme 9**).[97]

4.5. Selected Examples of Organic Synthetic Biotransformations with MOF-Immobilized Enzymes

While applications of "*Enzyme@MOF*" composites as catalysts for preparative and in particular large-scale industrial processes represent an ultimate goal of this concept, up to now many examples of such heterogenized "*Enzyme@MOF*"-type biocatalysts have not been applied in preparative synthetic processes and most available data are obtained from enzyme activity tests rather than from synthetic processes. However, some attempts to use such "*Enzyme@MOF*"-type catalysts in organic-synthetic applications have been also made, and in the following such examples are summarized.

Lipases, characterized as triacylglycerol hydrolases (EC 3.1.1.3), are widely employed enzymes in organic synthesis. Due to the importance of this enzyme class for such applications and their high operational stability in organic media (as the typical type of solvent being used in organic synthesis), accordingly from an early stage on there also have been various investigations into the immobilization of lipases using metal-organic frameworks (MOFs) as carriers. The use of MOFs as immobilization platforms for lipases has gained considerable attention and has also been subject of a very recent extensive study.[98] *Candida antarctica* lipase B (CAL-B), which is a widely employed enzyme in organic synthesis, has been successfully immobilized on MOFs by Jung et al.[31] by means of post-synthetic surface modification of MOFs. It was demonstrated that local environments of specific biocatalysts can be efficiently modified, thus resulting in improved activity, particularly also in polar organic media.[31] In general, a series of fatty acids (C12-C22) were conjugated with the amino groups of 2-amino-1,4-benzene dicarboxylate (NH2-BDC) in UiO-66-NH_2, which is a Zr-based MOF.[31] As shown in **Figure 4**, CAL-B was then covalently bound to carboxylate groups on the surface of the ZrMOF. This surface modification was anticipated to significantly improve the enzyme's activity in acetonitrile. In addition, transesterification reactions catalyzed by CAL-B-behenate conjugated ZrMOF were reported to exhibit significant conversions such as 46.0% conversion in hexane (Figure 4).[31] In contrast, in the absence such fatty acid-based conjugation, less satisfactory results were



Scheme 9. Esterification of *rac*-1-phenylethanol with vinyl acetate using CAL-B@Fattyacid-ZrMOF[31] or Novozym 435.[97]

obtained, which underlines the crucial role of the fatty acid conjugation for enhancing the catalytic performance of CAL-B.[31] These results indicate that surface modification of MOFs is a versatile strategy to improve the performance of biocatalysts in organic reaction environments.[30,31]

In addition, Cao and Wu et al.[99] successfully immobilized *Bacillus subtilis* lipase (BSL2) on HKUST-1 as the MOF material. In the presence of BSL2@Cu-BTC as a catalyst, high conversions of over 90% even after 10 cycles as well as 99.6% as initial conversion was achieved (as shown in **Scheme 10**). Furthermore, the immobilization showed a significant positive effect on the specific activity of the enzyme at higher reaction temperatures compared to the free enzyme BSL2.[26]

An alternative method was developed by Cai et al. by encapsulating lipase CAL-B in a first step in the zeolitic imidazole framework-8 (ZIF-8) as a MOF structure, and subsequent binding of the resulting composites to a microporous resin through physical adsorption (**Figure 5**).[41]

In this study, a range of properties of the immobilized lipase were investigated, including solvent tolerance and surface characterization.[41] While the activity of the immobilized lipase was assessed in a 2 mL tube and quantified using GC,[41] however, specific conversions or yields resulting from preparative biotransformations were not reported. Up to now only activity measurement of these MOF-immobilized enzymes were conducted, which have been presented by units (U mg^{-1}). Thus, data from preparative biotransformations are currently not available and would have to be determined in future work in order to enable an assessment of the efficiency in synthetic applications on preparative scale. Nonetheless, the findings of this study demonstrate that this MOF-based immobilization technique is able to significantly improve properties of the lipase.

In contrast, applications of alternative enzyme immobilisates based on "classic" absorption techniques, e.g., utilizing synthetic resins, are much more advanced at the current stage and can serve as a benchmark for evaluating such MOF-lipase-immobilisates in the future. An example for such a process with a "standard" enzyme immobilisate is given in the following. In a study conducted by Rodrigues et al., a comparative analysis was performed using two commercially available resins, namely Accurel MP 1000 and Lewatit VP OP 1600, for lipase from *Candida parapsilosis* (CpLIP2) immobilization.[100] Their objective was to produce biodiesel (fatty acid methyl esters, FAME) through the transesterification of jatropha oil with methanol in a lipid/aqueous batch-reaction.[100] The oil was dispersed in a buffer solution containing an excess of methanol. A 10 w/w%

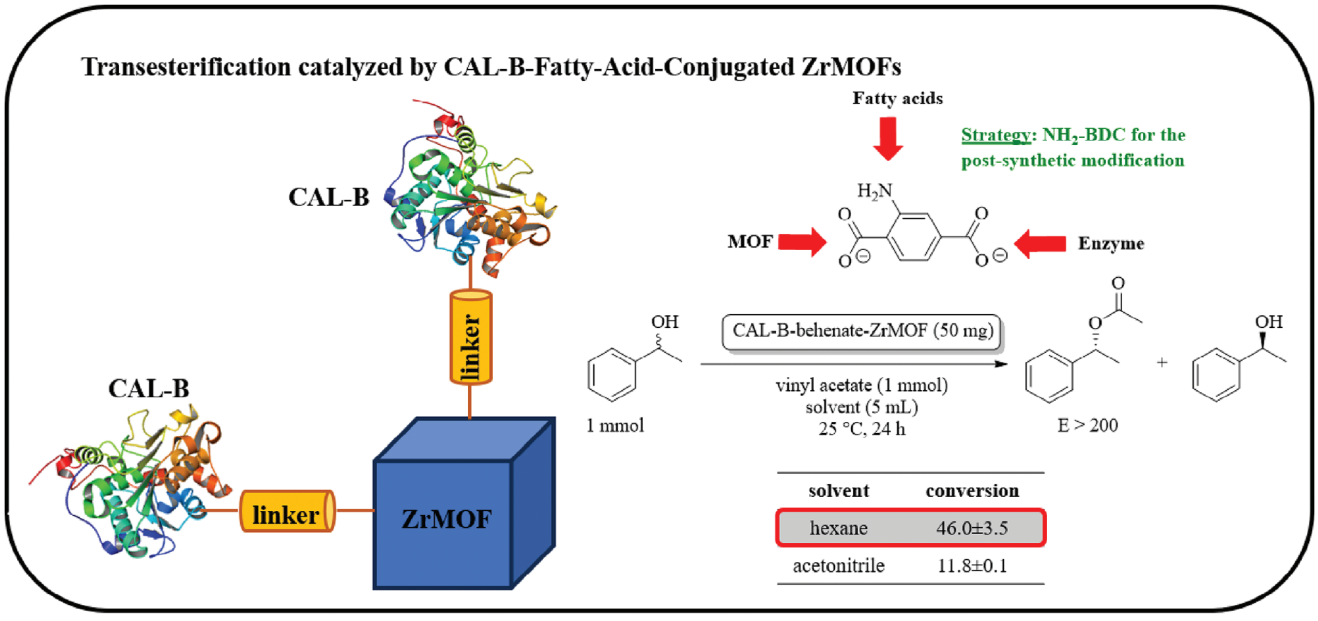
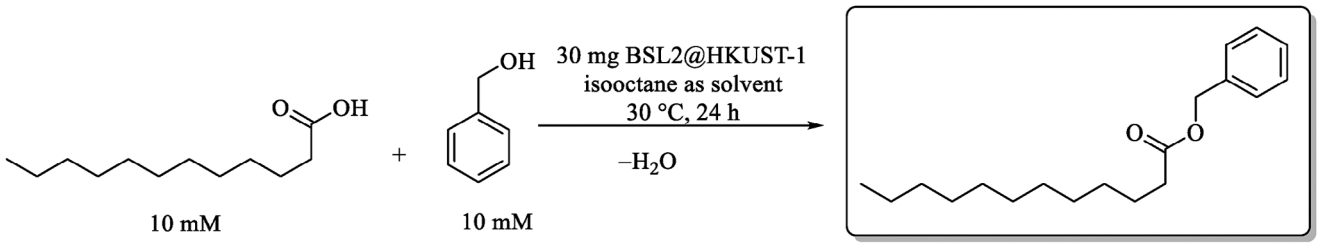


Figure 4. General concept of the design of CAL-B-fatty acid-conjugated MOF (4K6G, PDB) composites and their use as a biocatalyst in transesterification reactions.



Scheme 10. Esterification of lauric acid and benzyl alcohol with an "*Enzyme@MOF*" composite as catalyst.[26]

loading of immobilized biocatalyst relative to the amount of jatropha oil was used.[100] The results showed that lipase immobilized on these resins achieved maximum FAME yields of around 80% after an 8-hour reaction time, as determined by GC analysis.[100] Both resins exhibited high operational stability over five consecutive 8-hour batches.[100] These findings highlight the potential of synthetic resins as effective carriers for lipase immobilization in biodiesel production processes, and also serve as a benchmark for future analogous processes with "*Enzyme@MOF*"-type catalysts.

In the field of "*Enzyme@MOF*" composites, in recent years enzyme encapsulation in MOFs has emerged as an increasingly studied research area, also with respect to process development.[21] Two alternative processes, one operating in batch mode and the other as a continuous flow process, highlight the potential applications of such an enzyme encapsulation. A general comparison of these processes is illustrated below in **Figure 6**.

Previous studies have demonstrated that MOF-hosted enzymes can be utilized in continuous flow processes[101] with packed-bed reactors, as shown by Greifenstein et al.[21] In their study, they presented the fabrication of suitable biocatalysts for continuous-flow reactions in aqueous and organic solvents by embedding the esterase EST2 obtained from the thermophilic organism *Alicyclobacillus acidocaldarius* (AsEST2) into the pores of NU-1000 as the MOF material.[21] The flow reactor was integrated into an HPLC system for online analysis. They reported that the enzyme stability of the esterase under aqueous condition was increased 30-fold and the reactor proved excellent enzyme stability and long-term performance in aqueous solution with a remarkable productivity of 0.54 g $g^{-1}h^{-1}$ (**Scheme 11**).[21]

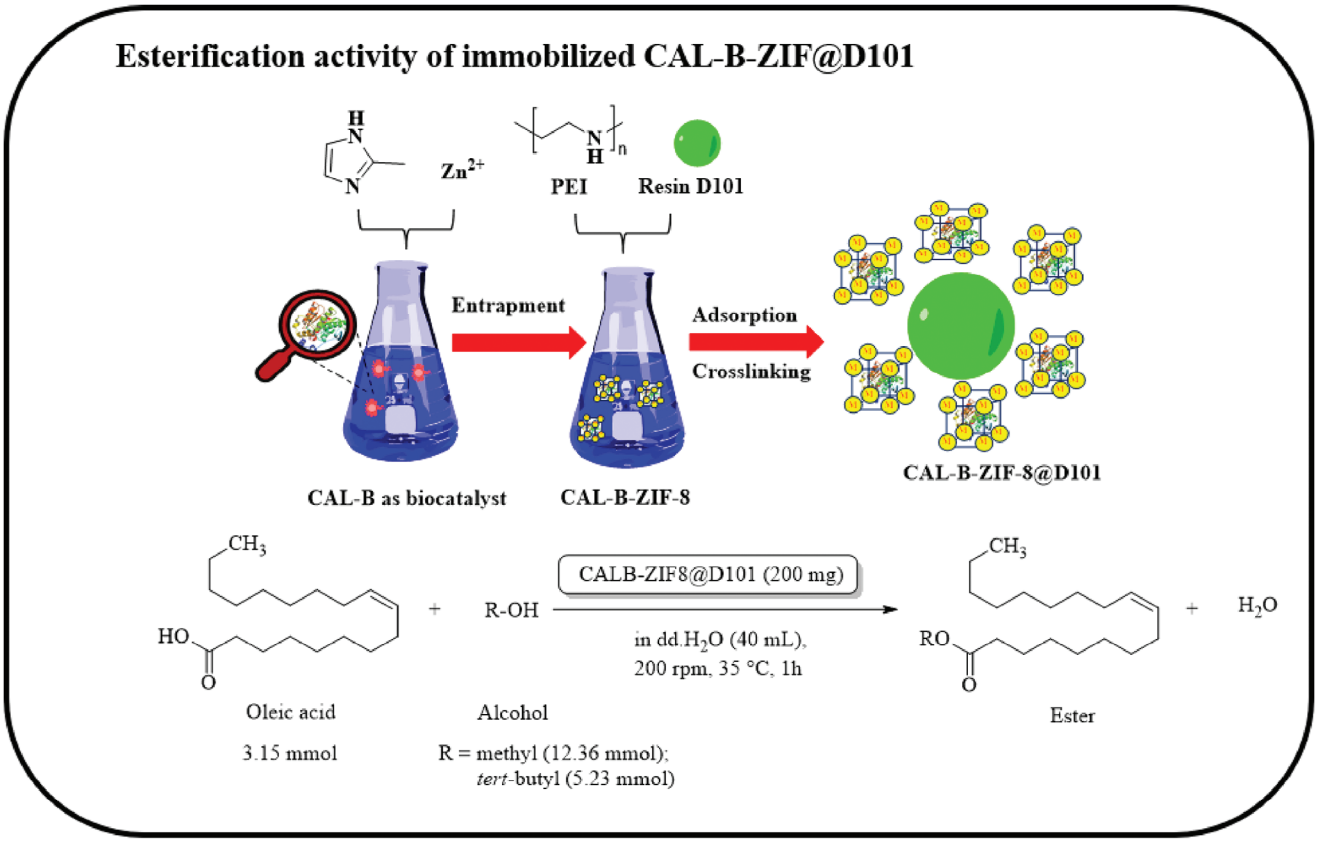


Figure 5. Concept of the preparation of CAL-B-ZIF@D101(4K6G, PDB) and its use as a biocatalyst in esterification reactions.[41]

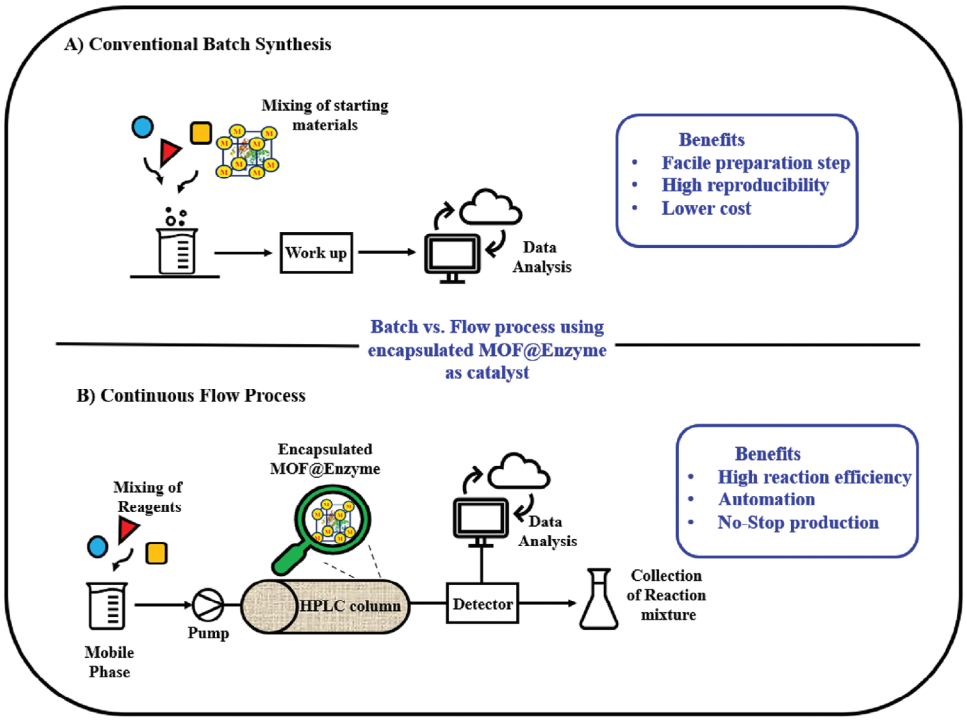


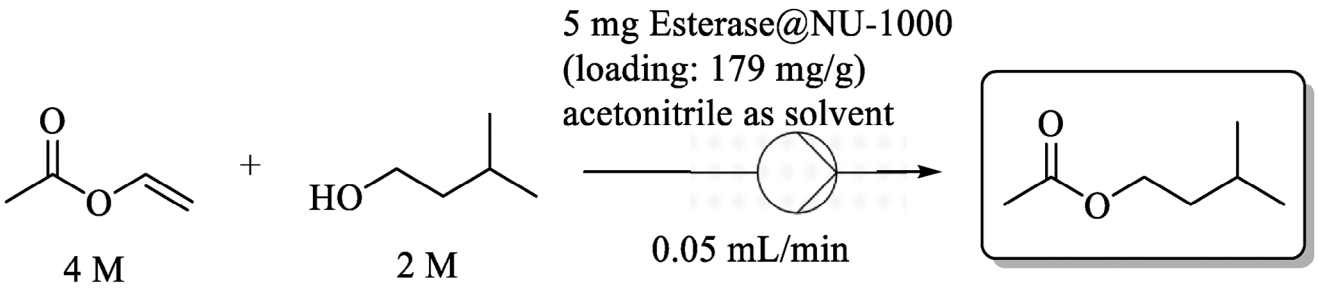
Figure 6. General description of different reaction processes using immobilized "*Enzyme@MOF*" composites as a biocatalyst.

Recently, Tian et. al published an innovative approach based on multi-compartmental MOF-74 microreactors derived from Pickering double emulsions.[40] Some chemoenzymatic cascade reactions, such as those driven by Grubb's catalyst/ CAL-B lipase for olefin metathesis/transesterification were provided in a multicompartmental micro-reactor and showed 2.24 – 5.58-folds improvement of conversion to the product in comparison to the homogeneous counterparts by using GC as analytical methodsy.[40] A general overview as example is presented in **Figure 7**.

Chen *et al*. reported an alternative carrier for enzyme encapsulation using HOF-101, a hydrogen-bonded organic framework, with Cyt c (cytochrome c) as the biocatalyst.[102] The determination of the enzyme activity was performed through a spectrometric absorption test, allowing for the identification and measurement of enzymatic activity within the framework. While the results are promising in terms of enzyme activity and stability, in analogy to most of the "*Enzyme@MOF*" approaches, further investigations and optimizations would be necessary to evaluate the feasibility and efficiency of this type of catalyst on a larger, preparative scale. In order to explore the scope and limitations of this carrier class, other enzymes should also be verified. Overall, the design of "*Enzyme@MOF*" composites and their use for process development have already shown significant progress and MOF-type materials can be considered as a new platform for enzyme catalyst preparation. In general, however, the overall number of organic-synthetic biotransformations with "*Enzyme@MOF*"-type composites is still limited, thus representing a young and at the same time highly emerging research field.[103]

5. "*Enzyme@MOF*" Composites: Conclusions about the State-of-the-Art, Considerations Related to Research Tasks for "Enzyme&MOF" in Efficient Processes & a Future Outlook

The demonstrated proof-of-concepts for enzyme-containing MOFs as catalysts in organic syntheses revealed remarkable properties of the resulting enzyme immobilisates and, thus, an



Scheme 11. Synthesis of isoamyl acetate using continuous flow with "*Enzyme@MOF*" as catalyst.[21]

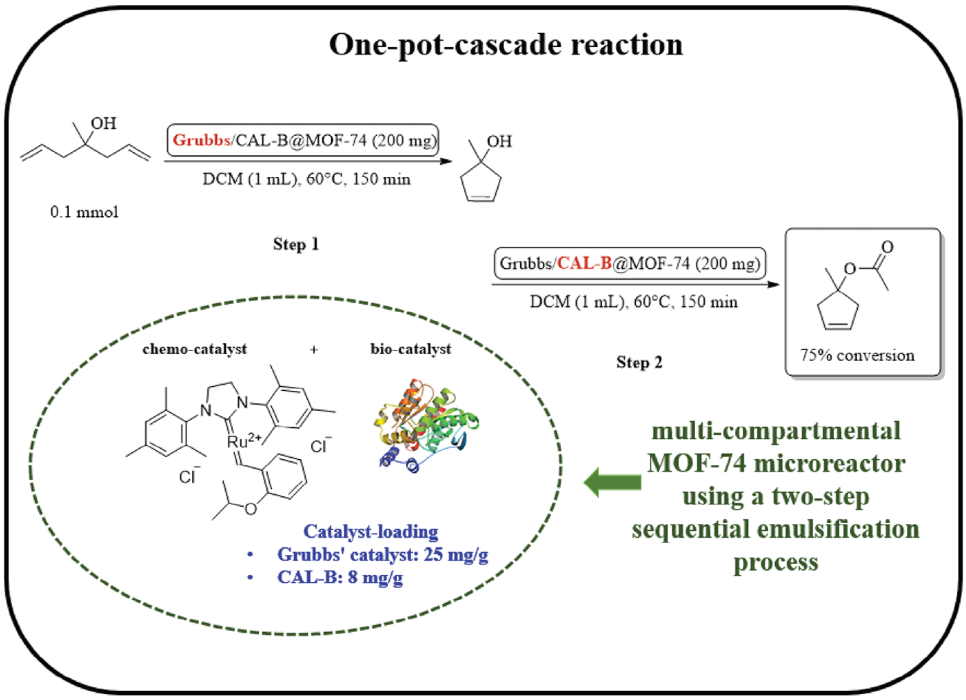


Figure 7. Schematic illustration for the reaction networks of chemoenzymatic cascade reaction using Grubbs' catalyst/CAL-B @MOF-74.[40]

enormous potential of this new generation of biocatalysts for applications in organic synthesis and also on industrial scale. In terms of application range, it is promising for future progress in this field that already a broad range of "*Enzyme@MOF*" composites have been prepared and biochemically characterized. The identified properties (at least for some of the prepared "*Enzyme@MOF*" composites), such as substantially improved stability at an elevated temperature, open up a perspective for applications of enzymes in new "process windows" being not suitable for current enzyme systems.

On the other hand, however, what is needed is an increased number of organic synthetic applications of such "*Enzyme@MOF*" composites in biotransformations under process conditions that are also attractive for preparative and even technical purposes. In general, the number of such synthetic examples is very limited at the current stage. In most cases the unique properties of "*Enzyme@MOF*" composites have been demonstrated only in "activity tests" (e.g., measuring consumption of a cofactor by spectroscopy) but not under preparative conditions (see, e.g., Table 1 with the various examples). Since it makes a substantial difference in terms of applicability if, e.g., a high temperature stability is observed under "assay conditions" or in an organic-synthetic process running, e.g., at high substrate loading, for future work it will be important to demonstrate the utility of such "*Enzyme@MOF*" with beneficial properties also in preparative scale applications with additional downstream-processing, product isolation and study of the recyclability of an "*Enzyme@MOF*" immobilisate under such conditions.

A further current limitation is related to the number of enzymes, which have been studied up to now in the field of "*Enzyme@MOF*" composites as new types of heterogeneous catalysts for biotransformations. So far, most examples are related to enzymes types such as hydrolases, in particular lipases, and oxidoreductases, specifically from the enzyme classes of alcohol dehydrogenases, peroxidases and oxidases. From an enzyme catalysis perspective, it should be added that these enzyme classes, at least in part, are known to represent very robust catalysts, which are already known to be tolerant to "enzyme stress conditions" such as high temperature or organic media when applying them in heterogeneous form with "standard" immobilization methods. As a representative example, lipases (in particular the immobilized lipase from *Candida antarctica* B, e.g., in form of the commercially available sample CAL-B Novozyme 435) should be mentioned here, which are known already today as biocatalysts with high operational stability at elevated temperature and even when being used in pure organic medium. Therefore, the suitability of this concept of "*Enzymes@MOF*" composites needs still to be demonstrated for a broader number of enzymes and enzyme classes, e.g., for enzyme representatives from the organic-synthetically relevant enzyme classes of Baeyer-Villiger monooxygenases, P450-type monooxygenases, ene reductases, transaminases, aldolases, oxynitrilases and many more. What, for example, is lacking so far, according to best of our knowledge, are the preparation and synthetic-organic application of "*Enzymes@MOF*" composites with lyases, which are important enzymes in the field of carbon-carbon bond formation. In addition, with respect to the currently already applied "*Enzymes@MOF*"-biocatalysts, it appears to be desirable to have more studies, which compare those "*Enzymes@MOF*" catalysts with other heterogeneous biocatalysts under identical or at least comparable process conditions and at process conditions, which are also attractive for preparative purpose (such as, e.g., high substrate loading).

Last not least, from a process perspective, operational stability as well as recyclability of "*Enzyme@MOF*" composites have to be demonstrated and insight into stability issues related to enzymes as well as MOF (e.g., abrasion effects during the reaction) needs to be broader studied.

On the other hand and in spite of the many challenges being ahead of us in this still relatively young research field of "*Enzyme@MOF*", novel unique biotransformation processes and substantial improvements of biocatalysis can be expected by applying such "*Enzyme@MOF*" composites due to the unique properties MOF offer. For example, embedding enzymes within MOF structures should have a dramatic effect on folding and unfolding issues, and, thus, a strong impact on stability. It can be expected that in the future we will see biotransformations, which reach completely novel process conditions being not applicable so far for currently available enzyme systems such as unusual temperature ranges for a certain enzyme or improved solvent tolerance. Therefore, such "*Enzyme@MOF*" composites, which also enable an efficient separation from the reaction mixture and simplified downstream-processing as well as the option of re-use of this catalyst, will also contribute to a new generation of biotransformation processes with improved efficiency, economy and sustainability.

One may raise the question how such valuable processes can be approached and what types of research tasks have to be addressed when developing such "*Enzyme@MOF*" composites for efficient organic syntheses. Accordingly, in the following some personal considerations are given, which have been made from the perspective and "the eyes" of an organic chemist and process development research:

- To which extent can the "*Enzyme@MOF*" immobilization retain the enzymatic activity? This is an often neglected issue when it comes to enzyme immobilization. While typically recycling numbers are regarded and described as the key criteria when it comes to the discussion of immobilization efficiency, the initial loss of activity also can play a major role. Thus, experimental conditions for the preparation of "*Enzyme@MOF*" composites have to be found, which lead ideally to both, efficient MOF formation as well as high remaining enzyme activity. In terms of experimental characterization of the resulting "*Enzyme@MOF*" composite, it has to be studied how much (amount) of the enzyme is entrapped in the MOF and to which extent this enzyme is still active in the MOF. It should be added that also potential mass transfer limitations can contribute to such data (when comparing an immobilized with a "free" enzyme).
- To which extent can the "*Enzyme@MOF*" composite be used for a synthetic process running at attractive synthetic conditions? When writing this review, we became aware that up to now organic synthetic processes in the presence of such "*Enzyme@MOF*" catalysts have rarely been carried out. However, for an evaluation for process efficiency and comparison with analogous processes based on the use of the same enzymes but with different heterogeneous materials as solid support, such studies would be of importance. When designing such organic-synthetic processes, the impact of substrate concentration (and, thus, the potential impact of the substrate in terms of enzyme inhibition and deactivation, respectively) has to be investigated as well as studies on the impact of organic solvents, which can be beneficial when developing an efficient organic-synthetic process.
- To which extent can the "*Enzyme@MOF*" composite be recycled and what is the loss of catalytic activity per reaction cycle? For getting an insight into this issue, determining the loss of enzyme activity is of utmost importance as a research task. It also should be added that the outcome of such a study has also an impact on the decision about the preferred reactor. If a substantial loss of enzyme activity is observed, tedious preparation of fixed-bed reactors with immobilized "*Enzyme@MOF*" catalysts therein would be less attractive, while being highly attractive in terms of both achieving high overall turnover number as well as simplified downstream-processing (due to elegant separation of immobilized enzyme from the reaction mixture) in case that a high remaining activity per reaction cycle can be achieved.

Furthermore, such "*Enzyme@MOF*" composites represent a unique opportunity for compartmentalization of catalysts, which is an exciting option to combine "non-compatible" catalytic reactions by spatial catalyst separation, thus leading to chemoenzymatic multi-step one-pot synthesis[23] without the need for work-up of intermediates. Compared to "standard" immobilization methods, tailor-made MOFs with defined pore size also are suitable to fine-tune such processes by allowing only the desired substrate(s) to reach the MOF-embedded enzyme in the pore while avoiding a direct contact with undesired components in the reaction mixture.

Requiring complementary competencies from different fields, e.g., molecular biology and microbiology for enzyme design and preparation, material science and physics for MOF preparation and characterization, organic chemistry for synthetic applications, it also becomes evident that interdisciplinary research is required to exploit the high potential of such an "*Enzyme@MOF*" composites technology, hence realizing applications even on technical scale.

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Conflict of Interest

The authors declare no conflict of interest.

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biocatalysis, chemoenzymatic synthesis, heterogeneous catalysis, immobilization, industrial synthesis, metal organic frameworks, MOF

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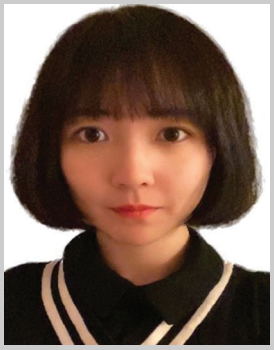
Harald Gröger studied chemistry at the Universities of Erlangen-Nürnberg and Oldenburg and completed his doctoral thesis at the University of Oldenburg in 1997. After postdoctoral research from 1997 to 1998 at the University of Tokyo, he joined the research department "Chemische Forschung" of SKW Trostberg AG. In 2001, after the merger of SKW Trostberg AG with Degussa-Hüls AG to Degussa AG, he became Project Manager in the Project House Biotechnology of Degussa AG, and from 2004 to 2006, he worked as a Senior Project Manager in the research unit Service Center Biocatalysis of Degussa AG. In 2006, he was appointed as an associate professor of organic chemistry at the University of Erlangen-Nürnberg, and since April 2011, he is full professor at the Faculty of Chemistry of Bielefeld University. Heading the Chair of Industrial Organic Chemistry and Biotechnology, his research interests comprise the development of biocatalytic processes, the combination of chemo- and biocatalysis toward multi-step one-pot processes and the development of sustainable and technically feasible processes for the production of industrial chemicals. Harald Gröger and his teams were awarded the Degussa Innovation Award 2003 (category: new products) and the Degussa Innovation Award 2005 (category: new or improved processes). In addition, he was awarded the Carl-Duisberg-Memorial-Prize 2008 of the German Chemical Society (GDCh), the Otto Roelen Medal 2014 of the DECHEMA and the German Catalysis Society (GeCatS), and the Jörg Schwarzbich Inventor Award 2019 of the Bielefeld University Society (UGBi). He also served as visiting professor at Osaka University in 2014 and Toyama Prefectural University in 2019 as well as a "Specially Appointed Professor" at Osaka University in 2022 and 2023.

Adil Allahverdiyev obtained his B.Sc. (2017) and M.Sc. (2021) in biochemistry as well as his B.Sc. (2020) in chemistry from Bielefeld University, specializing in (metal)organic- and enzyme catalysis. He subsequently started his doctoral thesis in the group of Prof. Dr. Gröger focusing on organic and enzymatic synthesis of renewable fuel as well as high pressure reactions for polymer modification. He came into contact with MOF chemistry as part of a collaboration with the group of Prof. Dr. Stefan Wuttke (BC Materials) and has been studying "enzymes@MOF compounds" since then.



Jianing Yang received her B.Sc. (2019) and M.Sc. (2021) in chemistry from Bielefeld University, focusing on organic synthesis, bioorthogonal chemistry and enzyme catalysis. Currently, she is a doctoral student at Bielefeld University under the guidance of Prof. Dr. Gröger. Her primary research revolves around the experimental and computational exploration of biocatalysts, enzyme encapsulation utilizing various MOF/COF carriers, and optimizing biocatalytic processes on a preparative scale.

Johannes Stiehm received his B.Sc. (2019) and M.Sc. (2023) in chemistry from Bielefeld University, focusing on the organic synthesis of model compounds and the conversion of biorenewables using recyclable metal catalysts. He is currently a doctoral student under the supervision of Prof. Dr. Gröger at Bielefeld University. His research focuses on the investigation of catalytic systems in high pressure reactions and the utilization of MOF-type catalysts, which is done collaboration with the group of Prof. Dr. Stefan Wuttke (BC Materials).