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Review

Tandem Reactions Combining Biocatalysts and Chemical Catalysts for Asymmetric Synthesis

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Abstract: The application of biocatalysts in the synthesis of fine chemicals and medicinal compounds has grown significantly in recent years. Particularly, there is a growing interest in the development of one-pot tandem catalytic systems combining the reactivity of a chemical catalyst with the selectivity engendered by the active site of an enzyme. Such tandem catalytic systems can achieve levels of chemo-, regio-, and stereo-selectivities that are unattainable with a small molecule catalyst. In addition, artificial metalloenzymes widen the range of reactivities and catalyzed reactions that are potentially employable. This review highlights some of the recent examples in the past three years that combined transition metal catalysis with enzymatic catalysis. This field is still in its infancy. However, with recent advances in protein engineering, catalyst synthesis, artificial metalloenzymes and supramolecular assembly, there is great potential to develop more sophisticated tandem chemoenzymatic processes for the synthesis of structurally complex chemicals.

Keywords: tandem catalysis; chemoenzymatic; biocatalysis; dynamic kinetic resolution; artificial metalloenzyme; asymmetric synthesis

1. Introduction

Living beings do not use enzymes in isolation. However, they build up the living system by applying multi-step synthesis strategies catalyzed by enzymes acting cooperatively. In that way, complex molecules are built from simple elements through multi-step biosynthetic routes. The cooperative action of a sequence of enzymatic reactions unveils the mysteries of "perfect" reaction systems with maximized energy utilization efficiency and minimal waste generation. Such synergy inspires biochemists to mimic nature to develop multi-step catalysis for selective synthesis, termed tandem catalysis. Compared with stepwise synthesis, one-pot tandem reactions offer an attractive approach to improve the overall synthetic efficiency by eliminating the purification steps of intermediates, suppressing the side reactions and enhancing selectivity of the product by building dynamic equilibrium in each step (which improves productivity by allowing equilibrium reactions to proceed to nearly full conversion). Thus, it is not surprising that from synthetic and industrial standpoints, there is an increasing interest in eco-friendly tandem processes.

Tandem catalysis employing the same type of catalyst, such as multi-step chemocatalysis or biocatalysis, has been extensively studied [1,2]. Recently, there is a new trend to combine chemocatalysis and biocatalysis in one-pot to obtain synergistic synthetic abilities that cannot be achieved by either separately [3]. Chemocatalysis and biocatalysis are generally considered two different fields, each with their unique considerations. The catalysts from these fields either catalyze completely different reactions, or similar reactions with different rates, selectivity and substrate scopes. Importantly, the catalysts from each of these fields have distinct advantages and limitations. Organometallic catalysts play a key role in the manufacturing of chemicals. They have wide substrate

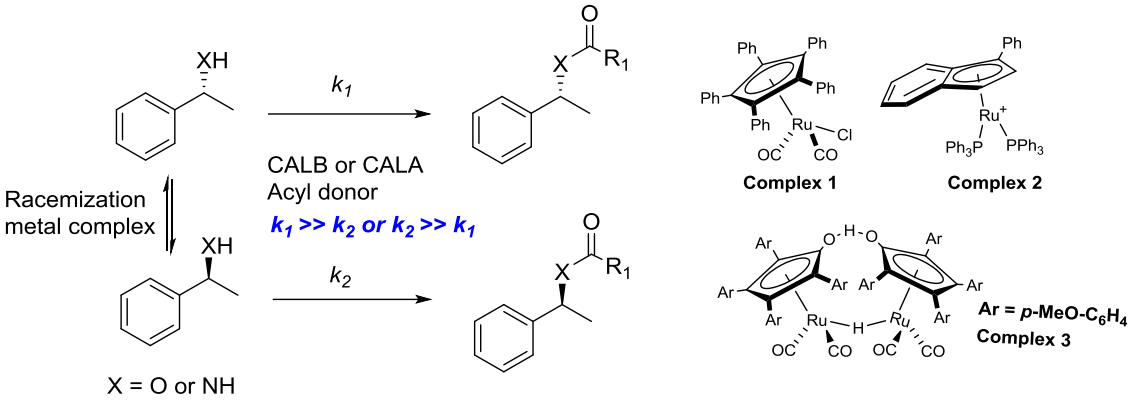
scopes and high productivity, but they usually show poor regio-, stereo- and enantio-selectivity that have to be overcome by tedious ligand design. Most of the reactions catalyzed by transition-metal complexes are performed under harsh conditions, such as high temperature and pressure. Biocatalysis is becoming more widely used in the pharmaceutical industry due to significant advances in enzyme discovery, supply and improvements. There has also been an increase in applications of these biocatalysts for chiral catalysis and green chemistry [4–7]. Biocatalysts typically have high regio-, stereo- and enantio-selectivity, but low productivity. Considering those factors, combining the two technologies into a tandem one-pot reaction would allow access to more enantiopure compounds.

Unlike developing tandem catalysis with the same type of catalyst, it is more challenging to combine chemocatalysis and biocatalysis in one-pot due to mutual inactivation. With the exception of lipases and serine proteases, the majority of enzymes are not able to maintain high catalytic activity in organic solvents and at high temperatures. Similarly, most transition-metal complexes are inhibited in aqueous solution with or without cellular components. Several strategies have been developed to overcome these obstacles, including using biphasic systems, the development of supramolecular hosts and the development of artificial metalloenzymes to compartmentalize the chemical catalysts. Both metal catalysts and biocatalysts have been engineered to show higher activity in aqueous solutions or organic solvents with the utilization of catalyst immobilization and protein engineering respectively. In this review, we will cover major accomplishments in one-pot chemoenzymatic reactions within the last three years, including dynamic kinetic resolution, one-pot concurrent transformations in aqueous solutions, and interfacing transition-metal complexes with living cells.

2. Dynamic Kinetic Resolution

Chiral molecules with non-superimposable mirror images can have striking differences in biological activities, such as pharmacology, toxicology, pharmacokinetics, and metabolism [8]. In fact, more than half of the drugs currently in use are chiral compounds. In order to fulfill the increasing demand for enantiopure compounds, significant advances in asymmetric synthesis and catalysis have been achieved [9,10]. Dynamic kinetic resolution (DKR) catalyzed by transition-metal racemization complexes and kinetic resolution enzymes, have been employed as efficient methods to prepare chiral alcohols and amines that constitute important synthetic building blocks of various chemical products, such as agrochemicals, food additives, fragrances and pharmaceuticals [11]. Enzymatic kinetic resolution (KR) of racemic mixtures is the most common approach to access enantiomerically pure alcohols and amines on an industrial scale due to the high activity and selectivity of enzymes [12]. The resolution of racemic alcohols or amines is generally accomplished through (*R*)- or (*S*)- selective acylation of their enantiomers by using a lipase or a serine protease as the resolving enzyme. However, enzymatic KR suffers from the limitation that only 50% of the theoretical yield could be obtained for the desired enantiomer. Integrating a racemization catalyst to continuously replenish the consumed enantiomer could theoretically drive the resolution up to 100% (Scheme 1). The compatibility between the enzyme and the isomerization catalyst is essential for a successful DKR system. The KR enzyme must have sufficient enantioselectivity ($k_{fast}/k_{slow} \geq 20$) and the rate of isomerization (k_{rac}) must be at least 10 times faster than the enzyme-catalyzed reaction of the slow reacting enantiomer (k_{slow}).

Since the pioneering work of William [13], Bäckvall [14] and Kim [15] on developing practical systems that combined metal catalysts with lipases or serine proteases for DKR of alcohols and amines, a variety of studies have been performed to improve these systems, such as discovering catalysts that could efficiently racemize alcohols and amines at mild conditions, improving the stability and catalytic efficiency of enzymes, and expanding the substrate scopes [9,10]. To date, immobilized *Candida antarctica* lipase B (CALB) [16] and *C. antarctica* lipase A (CALA) [3] have been the most common enzymes of choice to prepare *R*- or *S*- enantiomers of alcohols respectively, owing to their robustness and activity in organic solvents at temperatures up to 100 °C.



Scheme 1. An example of selective chemoenzymatic dynamic kinetic resolution (DKR) of secondary alcohols or primary amines by recently developed transition-metal complexes. CALB, *Candida antarctica* lipase B; CALA, *C. antarctica* lipase A.

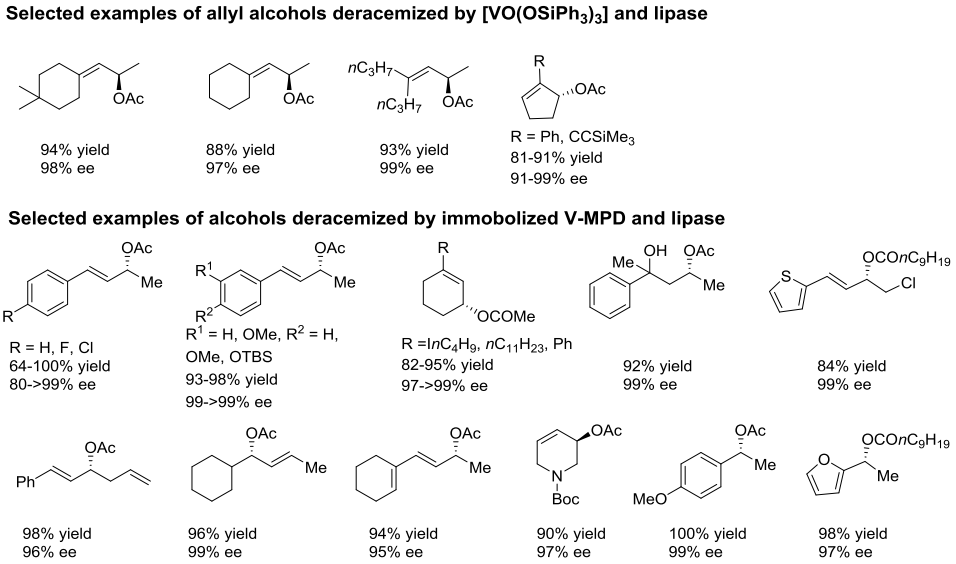
2.1. Dynamic Kinetic Resolution of Secondary Alcohols and Derivatives

For the racemization of alcohols, the most commonly employed chemical catalysts are ruthenium complexes. Monomeric ruthenium pentamethylcyclopentadiene complex **1** developed by Bäckvall and co-workers [17] has been widely used in tandem with different enzymes to deracemize a wide range of functionalized secondary alcohols, including aliphatic alcohols [18,19], allylic alcohols [20–22], chlorohydins [23], diols [24,25], homoallylic alcohols [26], and *N*-heterocyclic 1,2-aminos alcohols [27] with excellent yields and enantiomeric excess (*ee*). Recently, complex **1** has been employed to synthesize biologically active 5,6-dihydropyran-2-ones and the corresponding δ-lactones [26]. Several new ruthenium complexes were developed to be active at room temperature for pairing with thermolabile enzymes. Nolan and co-workers recently reported cationic ruthenium indenyl complex **2** that could catalyze racemization of secondary alcohols without a strong base [28]. By coupling **2** with Novozyme® 435 (Strem, Boston, MA, USA) the DKR of various secondary alcohols was achieved in high yield and *ee* at room temperature. At the same time, the group of Martín-Matute found that a commercially available $[Ru(p\text{-cymene})Cl_2]_2$ with the ligand 1,4-bis-(diphenylphosphino)butane could be coupled with a lipase from *Pseudomonas stutzeri* for the efficient DKR of α-hydroxyl ketones at ambient temperature [29]. The resulting enantiopure compounds provided straightforward access to a variety of diols and amino alcohols in a diastereo- and enatioselective manner.

Other more cost-effective and readily accessible metal complexes, such as iridium, aluminum and vanadium complexes, have also been investigated for DKR of secondary alcohols [9]. Akai and co-workers have demonstrated that the oxyvanadium (V) complex $[VO(OSiPh_3)_3]$ in tandem with various lipases allowed for DKR of a wide range of linear and cyclic allylic secondary alcohols [30,31] (Scheme 2). Recently, they prepared a novel oxyvanadium catalyst (V-MPD), immobilized inside mesoporous silica (MPS) [32]. This heterogeneous catalyst could be recycled six times without any loss in activity, and it could mediate the racemization of benzylic, heteroaromatic and propargylic alcohols (Scheme 2).

The application of transition-metal catalysts in terms of DKR has been mainly limited to academic research due to their high cost and low stability. In fact, several heterogeneous and immobilized racemization catalysts have been made to improve the total turnover number (TON) of catalysts [10,32,33]. In addition, some heterogeneous acid catalysts, such as zeolite and nanozeolite microspheres, combined with immobilized lipases have been developed for efficient DKR of benzylic alcohols [10]. Very recently, Tang and co-workers reported a core-shell nanozeolite@enzyme bi-functional catalyst consisting of CALB immobilized on H-β zeolite microspheres coated with polydiallydimethylammonium chloride (PDDA) [34,35]. This core-shell structure modulated the optimum rate of racemization and KR to achieve the best catalytic performance. PDDA also protected

the interaction between the products and the acidic core to minimize side products. However, this system was limited to kinetic resolution of benzylic alcohols.



Scheme 2. Scope of DKR systems involving vanadium complexes and various enzymes. V-MPD, novel oxyvanadium catalyst.

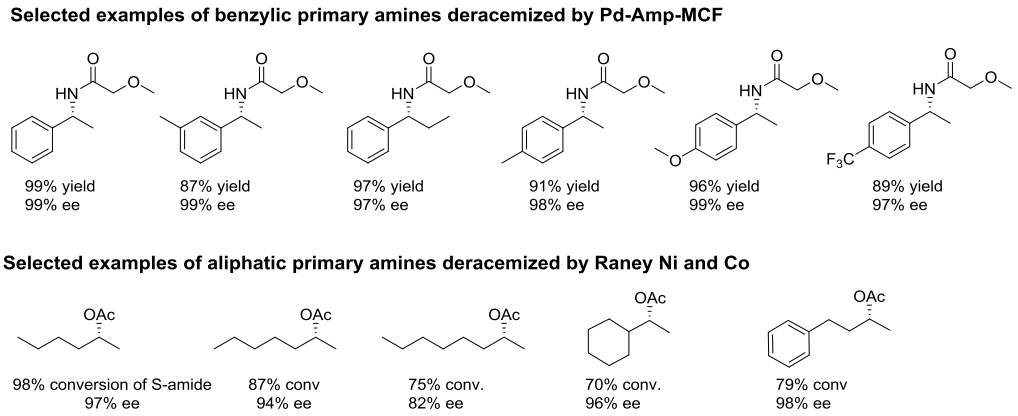
In addition to improving the catalytic efficiency of the racemization catalysts, a considerable amount of work has been carried out to improve the catalytic performance of lipases as biocatalysts through immobilization, cross-linking, surfactant stabilization or enzyme engineering. For example, Kim and co-workers recently coated lipoprotein lipase (LPL) from *Burkholderia* species with dextrin and ionic surfactant to produce activated lipoprotein lipase (LPL-D1) [36]. LPL-D1 was 3000-fold more active than its native protein in organic solvent and can facilitate DKR of diarylmethanols that had sub-optimal yields and enantiopurities. Bäckvall and co-workers applied a focused combinatorial gene mutagenesis technique to discover a mutant of *Candida antarctica* lipase A-Y93L/L367I with more than 30 times improvement on enantioselectivity of *sec*-alcohols in organic solvent [37]. More examples published before 2015 can be found in several reviews [9,10].

2.2. Dynamic Kinetic Resolution of Amines

The DKR of amines is more challenging due to a lack of efficient amine racemization catalysts. Amines are strong metal ligands; and high temperatures are utilized to prevent complexation of metals to the amines. In addition, highly active imine intermediates are likely to take part in several side reactions, which are more favored at elevated temperatures [9,10]. To date, a ruthenium complex Shvo analogue **3** coupled with CALB is the most practical method for DKR of aliphatic and benzylic primary amines at 90 °C [38–40].

In recent years, several palladium-based heterogeneous racemization catalysts have also been developed for DKR of benzylic primary amines. Bäckvall and co-workers developed a DKR system by using a catalyst consisting of palladium nanoparticles supported on amino-functionalized siliceous mesocellular foam (Pd(0)-Amp-MCF) to convert 1-phenylethylamine to an amide at 50 °C with sensitive Amano Lipase PS-C1 (*Burkholderia cepacia* lipase immobilized on ceramic beads) (Scheme 3). Pd-Amp-MCF is more efficient due to shorter reaction times and high TON compared with Pd nanoparticles alone [41]. Similarly, Li and co-workers investigated the effect of alkali salts on the catalytic activity of Pd nanoparticles on micro/mesoporous silica or activated carbon, and discovered that alkali salts greatly enhanced the selectivity of Pd catalysts [42]. Liang and coworkers used a similar system to synthesize rasagiline [43]. Moreover, a modified solvent extraction system and a

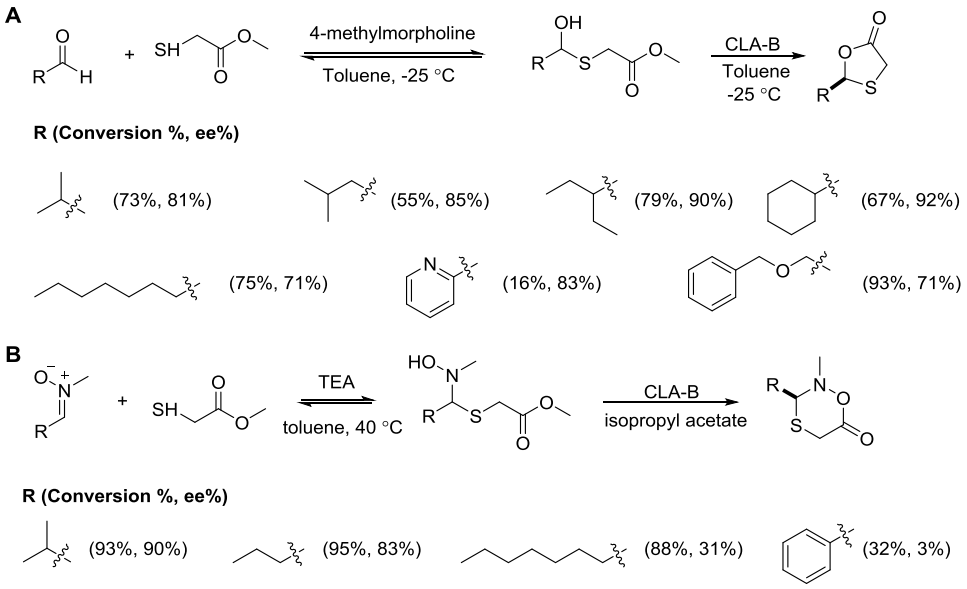
continuous flow reactor were developed to compartmentalize the Pd-based nanoparticles and lipases to overcome the incompatibility of reaction conditions required for the racemization and enzymatic steps [44–46]. However, Pd nanocatalysts did not work well for aliphatic amines. Raney Ni and Co displayed preferences for aliphatic primary amine racemization (Scheme 3), but they have inhibitory effects on the enzyme, resulting in a slow DKR system [47].



Scheme 3. Scope of DKR systems involving Pd(0)-Amp-MCF and Raney Ni or Co. Pd(0)-Amp-MCF, Pd(0)-aminopropyl-mesocellular foam.

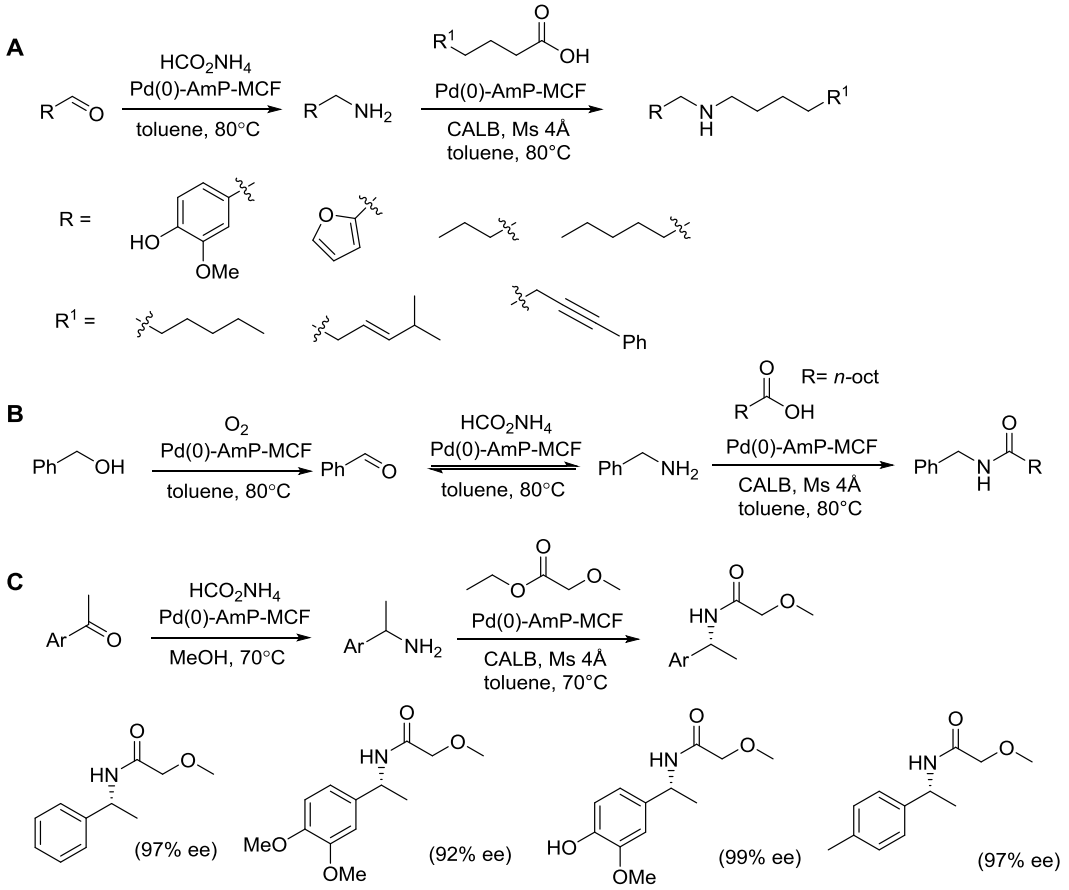
2.3. Other Tandem Reactions by Transition Metal Catalysts and Lipases

Except for DKR of alcohols and amines, lipases have also been coupled with base catalysts or transition metal catalysts for synthesis of more complex compounds. Ramström and co-workers recently reported asymmetric synthesis of 1,3-oxanthiolan-5-one derivatives [48] and oxathiazinanones [49] through dynamic covalent kinetic resolution. In the first case, dynamic hemithioacetal formation combined with intramolecular, CALB-catalyzed lactonization resulted in the final product in good conversion with moderate to good enantiomeric excess (*ee*) (Scheme 4A). In the second case, CALB catalysis was coupled with a dynamic dominonitrone addition-cyclization pathway to synthesize new, six-membered N,O,S-containing heterocycles (Scheme 4B).



Scheme 4. Asymmetric synthesis of (**A**) 1,3-oxaathilan-5-one and (**B**) oxathiazinanones through dynamic covalent kinetic resolution. TEA, triethanolamine.

Most recently, Berglund and co-workers integrated heterogeneous Pd(0)-aminopropyl-mesocellular foam (Pd(0)-AmP-MCF) or Pd(0)-aminopropyl-conrolled pore glass (Pd(0)-AmP-CPG) and CALB catalysts in one-pot for eco-friendly and asymmetric synthesis of valuable molecules such as amines and amides from an aldehyde, ketone or an alcohol respectively in good to high overall yields [50]. In this work, they developed several novel cocatalytic relay sequences, including reductive amination/amidation (Scheme 5A), aerobic oxidation/reductive amination/amidation (Scheme 5B), and reductive amination/dynamic kinetic resolution (Scheme 5C).



Scheme 5. Examples of integrated heterogeneous metal/enzymatic multiple relay catalysis for asymmetric synthesis: (**A**) reductive amination/amidation; (**B**) aerobic oxidation/reductive amination/amidation; (**C**) reductive amination/dynamic kinetic resolution.

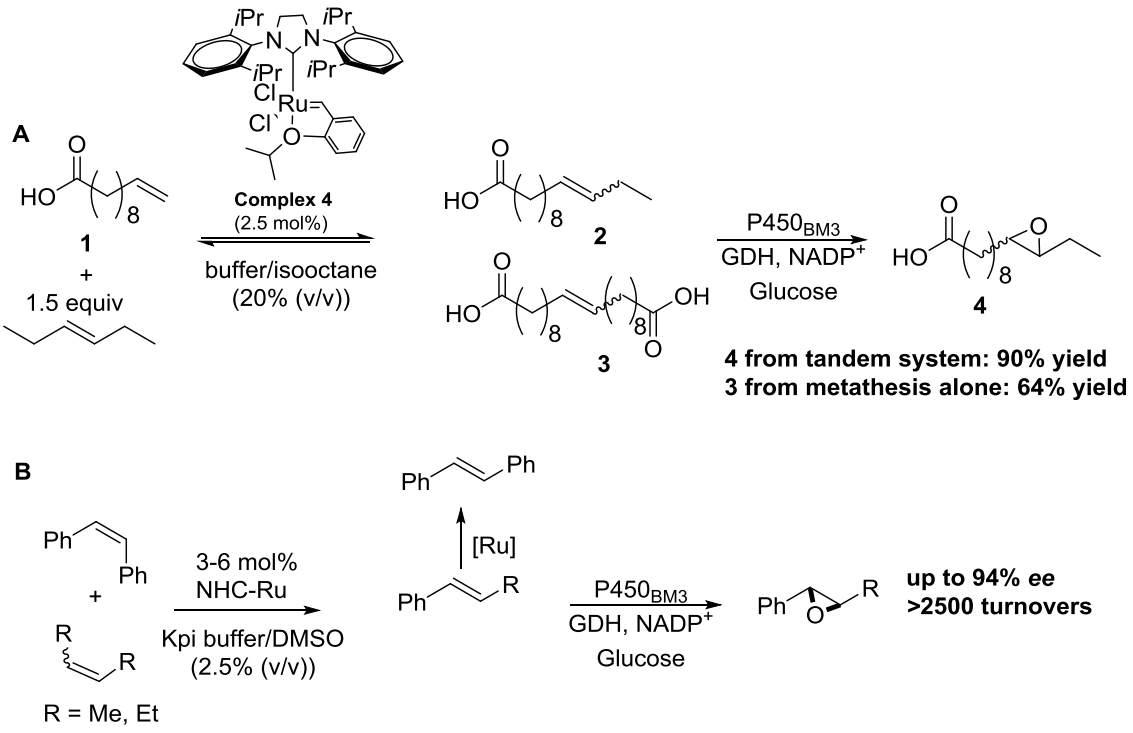
3. One-Pot Chemoenzymatic Transformations

Except for the above-mentioned immobilized lipases and serine proteases, the majority of biocatalysts have poor stability in organic solvents and at high temperatures. To address the synthetic challenges of both chemistry and biology, there is an ongoing interest to combine chemocatalytic and biocatalytic reactions in a one-pot process in aqueous solution and at ambient temperatures. Use of water as a solvent is ecofriendly and reduces organic solvent usage and waste generation. It also provides accessibility to all enzymes in nature. Currently one-pot cascade or concurrent reactions avoid purification of intermediates and thus save time and cost. However, the development of such a system is generally hampered by incompatible reaction conditions, catalyst inhibition, undesired side reactions and poor solubility of substrates. Several approaches have been implemented to improve the one-pot operation of chemocatalysts and biocatalysts, including partitioning in a biphasic system, incorporation into a supramolecular cage, catalysts compartmentation, and development of artificial metalloenzymes [51,52].

3.1. Concurrent Tandem Reactions by Transition-Metal Complexes and Enzymes

Transition-metal catalysis and biocatalysis are two different disciplines in terms of synthesis. Some metal-catalyzed transformations cannot be accessed by enzymes, such as Pd-catalyzed cross coupling reactions [53], Wacker-oxidation [54], and Ru-catalyzed metathesis [55,56]. On the other hand, biocatalysis has strong selectivity on both substrates and products, enabling much cleaner reactions. The motivation to combine them in one-pot is to exploit the synthetic power that cannot be achieved by either of them separately.

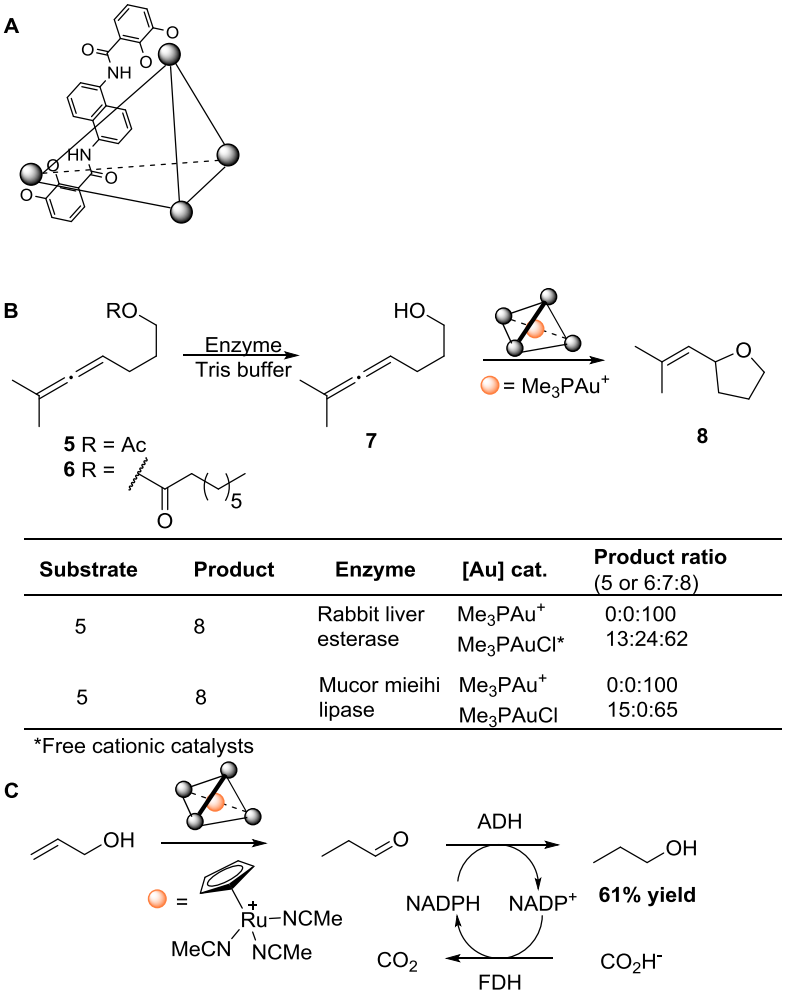
There are a few successful examples of coupling Pd-catalyzed Suzuki cross-coupling reactions and Heck reactions in tandem with alcohol dehydrogenase in an aqueous buffer by using either a biphasic system [57] or additive tagged Pd-nanoparticles [58,59]. Recently, Zhao and coworkers successfully combined Ru-catalyzed olefin metathesis with a P450-monooxygenase catalyzed oxidation in a biphasic system for the synthesis of various epoxides [60]. Importantly, this work demonstrated the power of chemoenzymatic one-pot processes to achieve higher yields compared with the sequential two-step reactions. In this case, the cooperation of ruthenium metathesis and P450-monooxygenase led to a dynamic equilibrium of alkenes and a selective epoxidation of the cross-metathesis products **3** (Scheme 6A). The yield of **4** was 1.5 times higher than the hypothetical yield (≤64%) resulting from stepwise reactions. Later, they developed a similar system by using an engineered P450 to convert a mixture of alkenes into a single aryl epoxide in high enantiomeric excess and moderate yield (Scheme 6B) [61].



Scheme 6. Combination of an olefin metathesis with a P450 catalyzed epoxidation in a tandem-type one-pot process for the synthesis of (**A**) 10-undecenoic acid epoxide and (**B**) aryl epoxides. GDH, glucose dehydrogenase; NADP, nicotinamide adenine dinucleotide phosphate. Adapted from [61]. Copyright American Chemical Society, 2015.

The coordination of transition-metal complex to the enzyme is one of the major factors contributing to decreased catalytic abilities of both catalysts, thus hampering the progress of coupled tandem reactions [62,63]. Toste and co-workers addressed this limitation by encapsulating Au(I) or Ru(II) complexes in a Ga_4L_6 tetrahedral supramolecular cluster [62]. By doing so, they successfully

coupled Au(I)-Ga_4L_6 host-guest with lipases and esterases for cascade hydrolysis, followed by a hydroalkoxylation of alkenes (Scheme 7). They also achieved a Ru(II)-mediated olefin isomerization of 2-propen-1-ol to give propanal, followed by reduction to propanol by an alcohol dehydrogenase (ADH). In both cases, the yields were improved relative to applying free cationic catalysts directly.



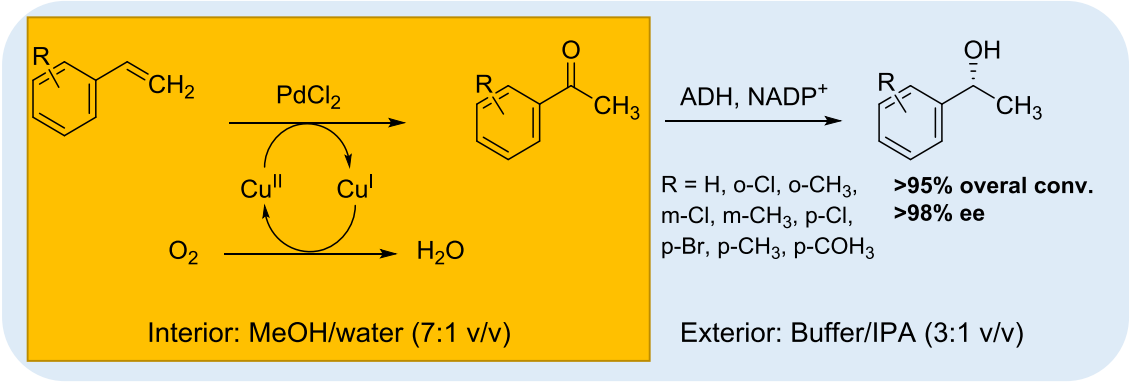
Substrate	Product	Enzyme	[Au] cat.	Product ratio (5 or 6:7:8)
5	8	Rabbit liver esterase	Me_3PAu^+	0:0:100
			Me_3PAuCl*	13:24:62
5	8	Mucor mieihi lipase	Me_3PAu^+	0:0:100
			Me_3PAuCl	15:0:65

*Free cationic catalysts

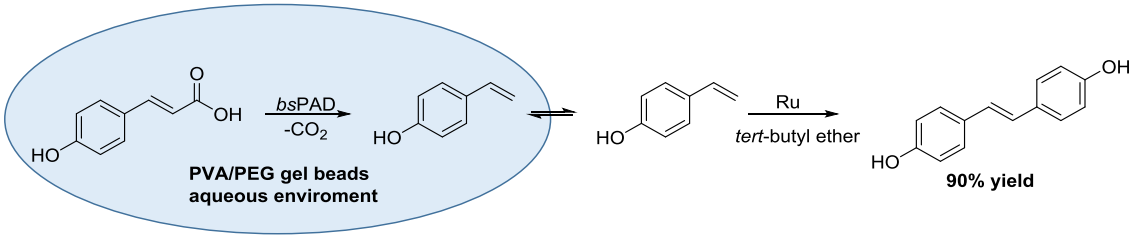
Scheme 7. A schematic view of the Ga_4L_6 tetrahedral supramolecular assembly (**A**) in which each edge of the tetrahedron represents a bisbidentate ligand and each vertex represents a gallium center (grey balls); (**B**,**C**) are metal catalysts encapsulated within a supramolecular complex in tandem with biocatalysts. ADH, alcohol dehydrogenase; FDH, formaldehyde dehydrogenase. Reprinted by permission from Macmillan Publishers Ltd.: Nature Chemistry [62]. Copyright 2013.

Catalysts compartmentation is another useful strategy to solve several incompatibility problems when combining heterogeneous organic or inorganic catalysis with enzyme catalysis. Careful reaction design led to efficient chemoenzymatic transformations catalyzed by immobilized chemical catalysts and biocatalysts, which are utilized in different compartments. Those multi-pot reactions were generally ecofriendly by avoiding intermediate isolation steps [64–66]. Recently, one-pot compartmentations also have been achieved by using membrane or encapsulation techniques. Gröger and co-workers combined noncompatible CuCl/$PdCl_2$-catalyzed Wacker oxidation with alcohol dehydrogenase-catalyzed ketone reduction to convert styrene enantioselectively into 1-phenyl-ethanol in one-pot with good conversion and *ee* (Scheme 8) [67]. To overcome mutual inactivation of both catalysts, Wacker oxidation was conducted in the interior of a polydimethylsiloxane (PDMS) thimble that enabled the diffusion of only the organic substrate and product into the exterior where the

biotransformation takes place (Scheme 8). In another example, they developed a one-pot cascade reaction combining a co-factor free decarboxylase from *Bacillus subtilis* named *bs*PAD with a Ru metathesis catalyst to produce high-value antioxidants in good yield from bio-based precursors [68]. Encapsulation of *bs*PAD in an aqueous environment created by poly(vinyl alcohol)/poly(ethylene glycol) (PAV/PEG) cryogels enabled the enzyme functionalize in pure organic solvent (Scheme 9). Compartmentation not only overcame the catalytic incompatibility issue, but also realized the recycling of valuable catalysts in a more convenient manner.



Scheme 8. Combination of Wacker oxidation and enzymatic reduction in one-pot aqueous media through compartmentalization. Adapted from [67]. Copyright WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, 2015.



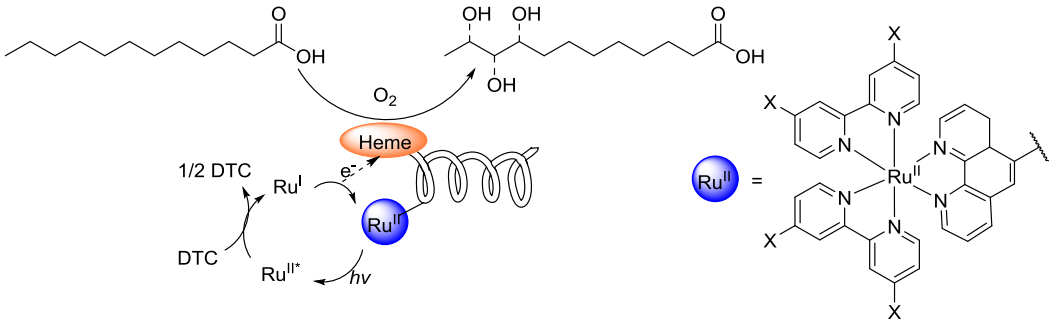
Scheme 9. One-pot cascade reaction combining an encapsulated decarboxylase with a metathesis catalyst for the synthesis of bio-based antioxidants. PVA, poly(vinyl alcohol); PEG, poly(ethylene glycol); *bs*PAD, co-factor free decarboxylase from *Bacillus subtilis*.

3.2. Coupling Visible-Light Photoredox Catalysis with Biocatalysts

Visible-light photoredox catalysis has been considered a highly desirable process in response to the interest in renewable energy and green chemistry. Organic transformations are afforded by combining photoredox with transition-metal complexes, or electrocatalysis [69]. Recently, photoredox has entered the realm of biocatalysis to form a more synergistic framework for catalysis of challenging reactions under mild conditions. In these examples, biotransformations of organic molecules are driven by the energy from the photoredox-catalyzed, light-dependent process.

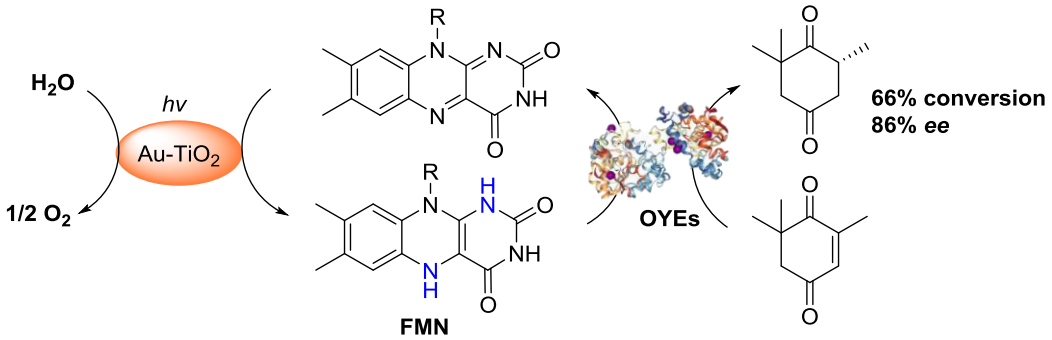
Cheruzel and co-workers developed hybrid P450 BM3 heme domains containing a covalently attached Ru(II) photosensitizer to afford light-driven hydroxylation of lauric acid with improved TON and initial reaction rate compared with normal P450 systems (Scheme 10) [70]. This process also circumvented the use of reductases and NAD(P)H cofactors by employing Ru(II) mediated electron transfer processes to reduce Fe(III) to Fe(II) in the presence of dithiocarbamate (DTC). In a follow-up study, Park and co-workers created a cofactor-free light-driven whole cell cytochrome P450 catalysis for the bioconversion of various substrates, including marketed drugs simvastatin® (Merck & Co., Inc., Kenilworth, NJ, USA), lovastatin and omeprazole [71]. Instead of transition-metal photoredox,

an organic dye, eosin Y that can easily enter the cytoplasm of *Escherichia coli* (*E. coli*) and specifically bind to the heme domain of P450 was used. Under visible-light irradiation, the reductive quenching of excited eosin Y resulted in electron transfer to Fe(III) in the P450 heme domain. The activated P450 conducted selective organic transformations that were controlled by the catalytic cycle of P450. This work demonstrated a whole cell platform for co-factor free, reductase-independent P450 photocatalysis.



Scheme 10. Cooperative Ru(II) (X = H or OMe) photoredox catalysis and P450 BM3 biocatalysis. Adapted from [70]. Copyright American Chemical Society, 2013.

Instead of coupling with oxidation enzymes, photoredox catalysts, working as hydride transfer catalysts, have also been incorporated with reductases such as alcohol dehydrogenase [72] and glucose dehydrogenase [73] to prepare chiral alcohols and L-glutamate respectively. Unlike the aforementioned studies in which organic sacrificial electron donors were used, Corma and co-workers used light-driven and titanium dioxide-promoted water oxidation to drive redox reactions catalyzed by flavin-based old yellow enzyme (OYE) (Scheme 11) [74]. The protons and electrons were generated by Au/TiO_2 photoredox catalyst via the oxidation of water under UV irradiation, and then were supplied to the flavin of old yellow enzymes for asymmetrically reducing conjugated C=C bonds. As the oxidation of water to oxygen is the rate-limiting step of the current process, there should be room for improvement with some optimization. Most recently, Ru(II) and Ir(II) complexes were successfully applied as photosensitizers for regeneration of nicotinamide adenine dinucleotide phosphate (NADPH) in OYE catalytic cycles. However, those systems required an extra sacrificial electron donor, triethanolamine [75].



Scheme 11. TiO_2 and OYE-based photoenzymatic reduction of ketoisophorone. FMN, flavin mononucleotide, OYE, old yellow enzyme. Reprinted with permission from Macmillan Publishers Ltd.: Nature Chemistry [74]. Copyright 2014.

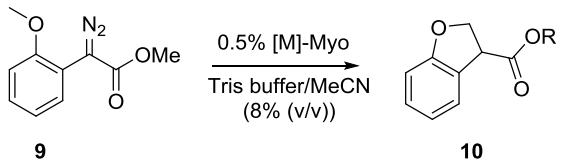
3.3. Artificial Metalloenzymes for Selective Transformations

An intense area of research has been the generation of artificial metalloenzymes by incorporating metal catalysts into protein scaffolds such as streptavidin [76], bovine serum albumin, and

apo-myoglobin [77]. With the aid of computational, molecular and structural biology, functional artificial metalloenzymes have been developed by de novo design or protein redesign processes [78]. Not limited to mimicking naturally occurring transformations, biochemists are currently trying to generate artificial metalloenzymes with catalytic abilities that have no equivalents in either chemical catalysis or biocatalysis. Additionally, similar to the incorporation of transition-metal complexes within supramolecular host-guest complexes, the use of artificial metalloenzymes enables the catalytic activity of transition-metal catalysts in biological environments, allowing synthetic catalysts to work collaboratively with other enzymes. There are several recent reviews discussing the newest techniques and examples of various artificial metalloenzymes [51,76,78–84]. To avoid duplication, we will only highlight the major accomplishments since 2015 in terms of tandem catalysis.

One of the biggest motivations to develop artificial metalloenzymes is to access both the reactivity and substrate scope of metal complexes together with the regio-, stereo- and enantio-selectivities afforded by the protein scaffolds. Hartwig and co-workers reported a concise method of replacing iron in Fe-porphyrin IX (Fe-PIX) proteins with abiological, noble metals to create enzymes that can catalyze reactions not catalyzed by native Fe-enzymes or other metalloenzymes [85]. They conducted directed evolution of a modified myoglobin containing an Ir(Me) site. Impressively, the resulting mutants could catalyze enantioselective C–C bond formation through carbene insertion and also the enantio-diastereoselective cyclopropanation of unactivated olefins (Scheme 12). This method sets the stage for the generation of artificial enzymes from innumerable combinations of PIX—proteins scaffolds and unnatural metal cofactors for various abiological transformations.

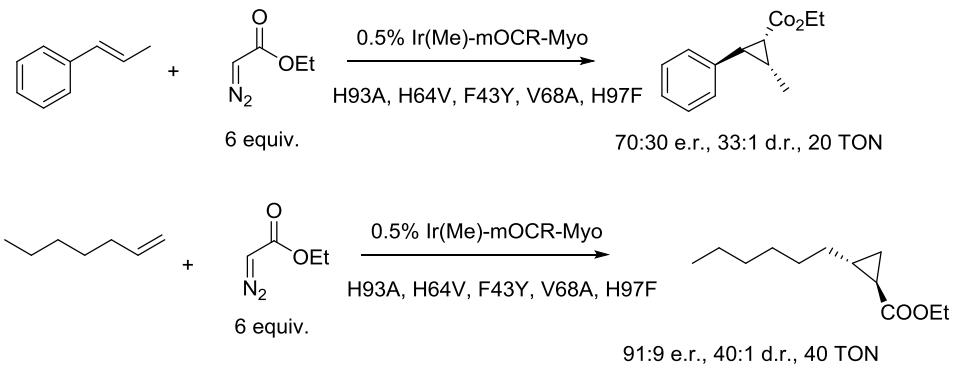
A



9 **10**

M	Myo-Mutant	e.r. (TON)
Fe(Cl)-PIX	93A	-- (0)
Ir(Me)-PIX	93A	-- (~60)
Ir(Me)-PIX	93A, 64L, 43L, 33V	85:15 (134) (-) enantiomer
Ir(Me)-PIX	93G, 64L, 68A, 33F, 99V	16:84 (62) (+) enantiomer

B



Scheme 12. Artificial Ir(Me)-based myoglobin-catalyzed C–H insertion for C–C bond formation and carbene addition to internal or aliphatic olefins: (**A**) insertion carbenes into C–H bonds; (**B**) carbene addition to internal or aliphatic olefins. Reprinted with permission from Macmillan Publishers Ltd.: Nature [85]. Copyright 2016.

Instead of performing directed evolution in vitro, Ward and coworkers utilized compartmentalization and in vivo evolution of a streptavidin (SAV)-biotin based artificial metalloenzyme for olefin metathesis (Figure 1) [86]. They created an *E. coli* strain for periplasmic expression of SAV with a biotinylated Hoveyda-Grubbs catalyst. The periplasm offered an auspicious environment for artificial metalloenzymes and facilitated artificial metathase-catalyzed metathesis in vivo and its directed evolution in a high throughput manner. This strategy not only created an artificial metalloenzyme comparable with commercial catalysts, showing activity for different metathesis substrates, but also represented the systematic implementation and evolution of an artificial metalloenzyme that catalyzes an abiotic reaction in vivo with other potential applications, such as non-natural metabolism.

Similar to the supramolecular complex, the protein scaffolds compartmentalize the chemical catalysts and avoid mutual inactivation of transition metal complexes and enzymes. By embedding a biotinylated d^6-Ir pianostood complex within SAV, Ward and co-workers enabled Ir-based transfer hydrogenation in the presence of *E. coli* cell free extracts and cell lysates [87]. In addition, they applied a similar strategy to create an artificial transfer hydrogenase (ATHase) that was successfully coupled with various NADH-, FAD- and heme-dependent enzymes for orthogonal redox cascade reactions that could not have been generated when free Ir-complex was used [88]. Significantly, by coupling ATHase with monoamine oxidase (MAO-N), NADPH regeneration was achieved and L-pipecolic acid was prepared with 99% *ee* (Scheme 13).

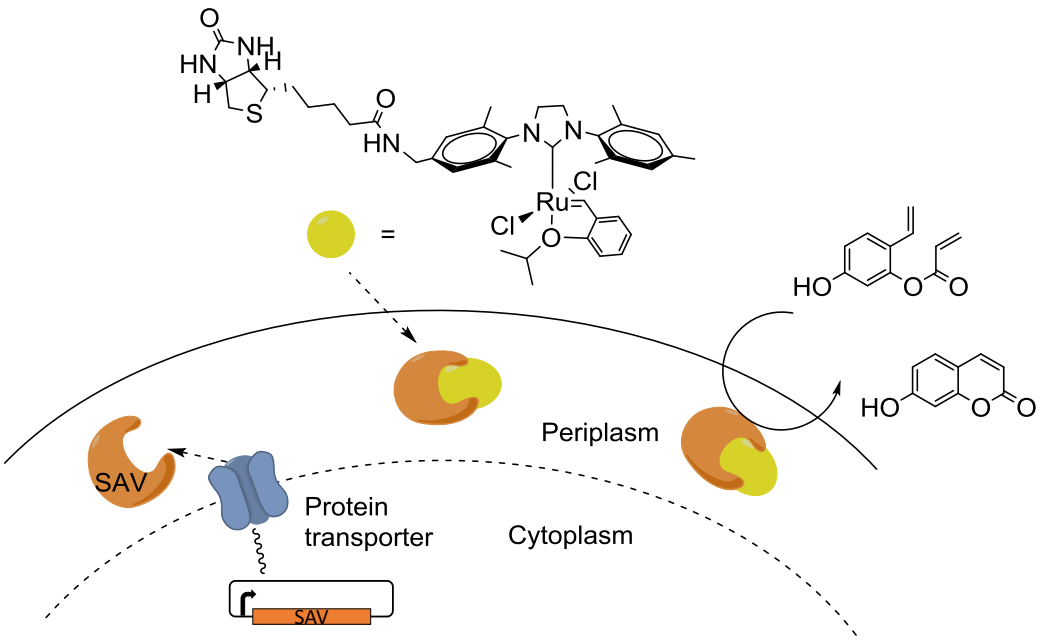
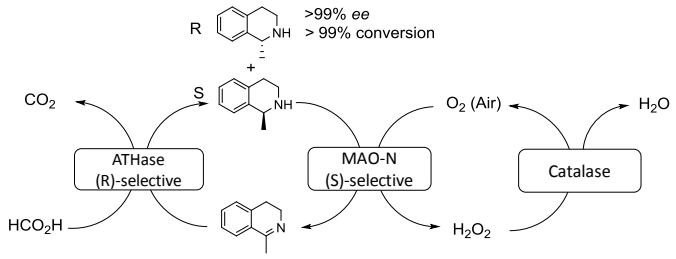
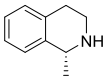
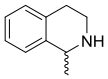
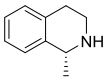
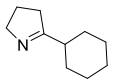
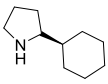
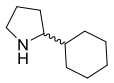
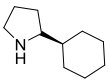
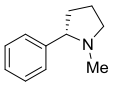
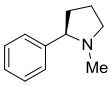
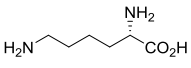
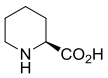


Figure 1. Streptavidin (SAV)-based artificial metalloenzymes for in vivo metathesis. The dashed arrows indicate the transportation of the chemical catalyst and streptavidin into the periplasm. Reprinted with permission from Macmillan Publishers Ltd.: Nature [86]. Copyright 2016.



Selected examples of orthogonal redox cascades combining ATHase with oxidase or oxygenase

Substrate	Prodcut	Conv. (%)	ee (%)
		>99%	>99%
		98%	99%
		98%	99%
		99%	>99%
		65%	99%
		88%	86%

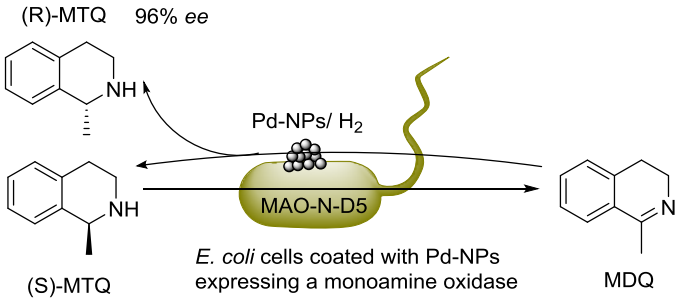
Scheme 13. Enzyme cascade for the double stereoselective deracemization of amines. Reprinted with permission from Macmillan Publishers Ltd.: Nature Chemistry [88]. Copyright 2013.

4. Interfacing the Transition-Metal Catalysis with Living Cells

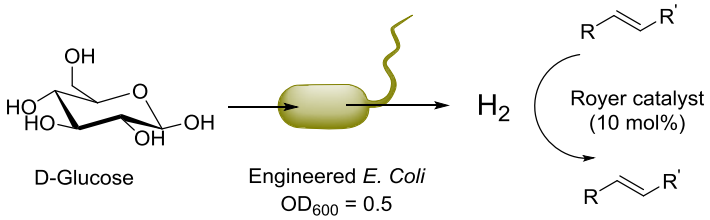
The use of whole cells as a catalytic "factory" to synthesize fine chemicals, pharmaceuticals, and steroids is an emerging area due to several advantages. The cell could provide natural protection for the proteins and thus improve their catalytic turnover. The compartmentalization also enables concurrent chemoenzymatic reactions to occur without inactivation due to incompatibility of the biocatalysts and other transition-metal complexes [3,89,90]. There has been much progress in engineering multi-enzymatic steps in cells for chemical production by applying metabolic engineering strategies [91]. However, the adaption of metal catalysis to the whole cell catalytic system remains widely unstudied mainly due to the deactivation of metal complexes under biological conditions. We will highlight recent examples in this area and the progress towards bio-orthogonal catalysis with organometallic compounds.

Other than the example discussed in Section 3.2 about cofactor-free light-driven whole cell cytochrome P450 catalysis, a biometallic whole cell catalyst for enantioselective deracemization of secondary amines was engineered by Lloyd and co-workers [92]. The engineered aerobic cultures of *E. coli*, overproducing a recombinant monoamine oxidase (MAO-N-D5) possessing high enantioselectivity against chiral amines, were coated with nanoscale Pd(0) precipitated via bioreduction reactions (Scheme 14). The whole cell catalyst was prepared (*R*)-1-methyltetrahydroisoquinoline (MTQ) with 96% *ee* by a dynamic process, in which MAO selectively oxidized *S* enantiomer of racemic mixture. The resulting 1-methyl-3,4-dihydroisoquinoline (MDQ) was then reduced back to the racemic amine by nonselective Pd/H_2 reduction. This work is important for the preparation of chiral secondary amines that are hard to obtain via normal metal-lipase DKR system. More recently, Balskus and co-workers reported a method for alkene hydrogenation that utilized the Royer Pd catalyst [93] and hydrogen

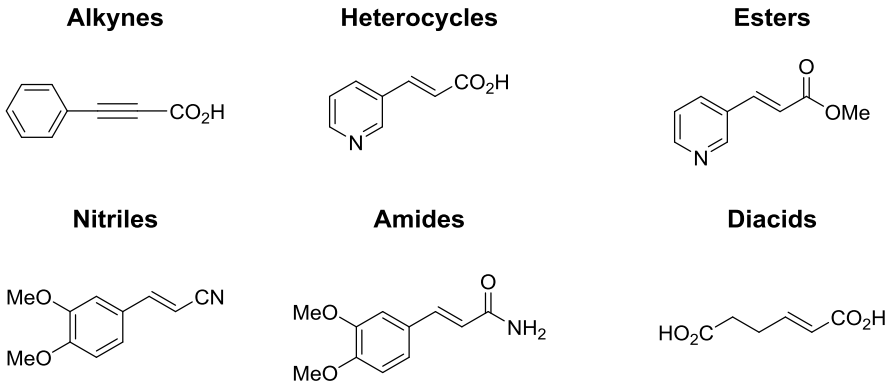
gas generated directly by an engineered *E. coli* (Scheme 15) [94]. This work first demonstrated that the metabolic output of living microbes and a biocompatible non-enzymatic transformation could be combined to enable preparative scale chemical synthesis.



Scheme 14. Deracemization of a cyclic secondary amine by engineered biometallic whole cell catalyst. MTQ, methyltetrahydroisoquinoline; MDQ, 1-methyl-3,4-dihydroisoquinoline; MAO-N-D5, monoamine oxidase. Adapted from [92]. Copyright American Chemical Society, 2011.



Selected substrates and the yields of the corresponding hydrogenation product



Scheme 15. A biocompatible alkene hydrogenation combines organic synthesis with microbial metabolism. Adapted from [94]. Copyright WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, 2014.

Considerable attention has been focused on improving the biocompatibility of transition-metal complex-catalyzed bio-orthogonal reactions in living cells. Currently, bio-orthogonal reactions have many applications for selective labeling and modification of biomolecules in living systems [95]. Except for the work by Ward and coworkers mentioned in Section 3.3, there are few examples of using such techniques for chemical production. However those results made researchers more aware of the need to prepare more biocompatible transition-metal complexes that are able to function in living cells. For instance, Eric and co-workers published a set of organometallic Ru complexes for the catalytic uncaging of allylcarbamate (alloc)-protected amines within mammalian cells. They applied this method in activating a caged anticancer drug, which efficiently induced apoptosis in HeLa cells [96]. Recently Mascareñas and co-workers improved this scenario further by using a designed Ru complex that accumulated preferentially inside the mitochondria of mammalian cells while keeping its ability to

uncage alloc-protected amines [97]. Except for the Ru catalysts, Pd complexes have also been applied in living cells for activation of intracellularly lysine-based proteins by decaging a propargyloxycarbonyl (Proc)-caged lysine analogue [98].

5. Future Prospects and Conclusions

The combination of chemocatalysis and biocatalysis in a one-pot for concurrent transformations is still in its infancy. This is particularly true for chemoenzymatic transformations in aqueous media. DKR catalyzed by transition-metal complexes and lipases or serine proteases have been fully developed to efficiently prepare most enantiopure primary and secondary alcohols. However, more advanced racemization catalysts should be developed for more challenging substrates such as chlorohydrins, alcohols with distant olefin groups and tertiary alcohols. In contrast to alcohols, there are significantly fewer available DKR systems for amines, especially aliphatic amines and secondary amines, due to the lack of an efficient racemization strategy. The examples of one-pot tandem chemoenzymatic reactions involving other enzymes in aqueous media are even sparser, and development of this field has been slow, mainly due to the mutual inactivation of metal complexes and enzymes. Unlike multistep enzymatic transformations occurring in cells, resulting in complex molecules, the tandem processes developed so far generally consist of two or three catalytic steps for simple molecule synthesis. To address those issues, it is essential to take advantage of several research areas including, but not limited to, protein engineering, chemical catalyst synthesis, supramolecular assembly, artificial metalloenzymes and whole cell catalysis.

Synthetic chemists have been focusing on developing water-soluble transition-metal catalysts that are effective in aqueous solutions. The progress in this area has enabled several metal-catalyzed reactions in buffer solutions (e.g., olefin metathesis [99–101], Pd-catalyzed hydrogenation [102], and C–C coupling reactions [103]). Several methods have also been applied for improving enzymes as biocatalysts. Immobilization of enzymes on heterogeneous catalysts [104–106] and preparation of cross-linked enzyme aggregates [107] are well-developed methods to improve the activity and stability of enzymes at extreme conditions, and to facilitate enzyme recycling. In addition, engineering enzymes by rational design [108,109] and directed evolution [110] are alternative ways to obtain enzyme mutants with improved properties and new functions. The combination of these techniques will enable integration of chemocatalysis and biocatalysis for advanced synthesis.

Inspired by naturally occurring reactions processed in different organelles, compartmentalization is becoming an attractive strategy to avoid mutual inactivation between catalysts from different disciplines. Creation of supramolecular hosts with hydrophobic cavities and a transition metal complex is a very appealing strategy for controlling the metal complex properties in competitive water solvents containing proteins and other cellular components [111]. Similarly, artificial metalloenzymes have been further developed to integrate transition-metal catalysts into cascade reactions with other biocatalysts and foster bioorthogonal transformations catalyzed by metal complexes in living cells. Importantly, generating artificial metalloenzymes is an advanced way to create catalysts with novel activities to address more challenging transformations. Protein engineering strategies can be further applied to artificial metalloenzyme development to improve its catalytic performance.

Finally, whole cell fermentation is a well-known technique to produce metabolite-related chemicals. However, there are very few known processes that integrate bio-orthogonal reactions catalyzed by transition-metal catalysts into multi-step biocatalytic systems in cells for complex transformations. The progress of developing biocompatible transition-metal complexes that function in cells, together with strategies of engineering enzymatic multi-step catalysis in vivo [112,113], will open new windows for creating new cell factories for chemical production.

In conclusion, interest in combining biocatalysts and chemical catalysts continues to grow. With recent advances in protein engineering, catalyst synthesis, artificial metalloenzymes and supramolecular assembly, there is a great potential to develop sophisticated tandem chemoenzymatic

processes for synthesis of complex chemicals in an ecofriendly manner. Therefore, more accomplishments in this area are expected in the near future.

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Author Contributions: Yajie Wang and Huimin Zhao wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

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