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Expanding the Boundary of Biocatalysis: Design and Optimization of *in vitro* Tandem Catalytic Reactions for Biochemical Production

Yajie Wang^{#1}, Hengqian Ren^{#1}, and Huimin Zhao^{1,2,*}

¹Department of Chemical and Biomolecular Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 6180

²Departments of Chemistry, Biochemistry, and Bioengineering, Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801

[#] These authors contributed equally to this work.

Abstract

Biocatalysts have been increasingly used in the synthesis of fine chemicals and medicinal compounds due to significant advances in enzyme discovery and engineering. To mimic the synergistic effects of cascade reactions catalyzed by multiple enzymes in nature, researchers have been developing artificial tandem enzymatic reactions *in vivo* by harnessing synthetic biology and metabolic engineering tools. There is also growing interest in the development of one-pot tandem enzymatic or chemo-enzymatic processes *in vitro* due to their neat and concise catalytic systems and product purification procedures. In this review, we will briefly summarize the strategies of designing and optimizing *in vitro* tandem catalytic reactions, highlight a few representative examples, and discuss the future trend in this field.

Keywords

enzymatic; chemoenzymatic; multistep; tandem; *in vitro*

1. Introduction

In living organisms, an individual enzyme catalyzes limited simple reactions, but the cooperative action of a series of enzymes build up complex molecules from simple molecules to support the living functions such as generation or storage of energy, as well as environmental sensing and communication. The cascade or concurrent reactions catalyzed by multiple enzymes demonstrate the strongest synthetic power in nature, which makes an irreversible process reversible, eliminates inhibition problems caused by excess product, or circumvents the lack of substrate scattered in bulk solution. The synergistic effect of natural synthetic networks inspires chemists to develop artificial multi-step tandem reactions for selective synthesis of complex molecules (Clomburg et al., 2017). To date, tandem reactions

*To whom correspondence should be addressed, Phone: (217) 333-2631. Fax: (217) 333-5052. zhao5@illinois.edu.

by using whole microorganisms (*in vivo*) have been extensively studied in the synthetic biology and metabolic engineering fields (Nielsen et al., 2016). There are growing interests to build cooperative tandem reactions by coupling multiple enzymes or combining organometallic catalysts with biocatalysts *in vitro* since the resulting catalytic systems are simpler for optimization, generate less side products and waste, and allow for easy product purification (Kohler et al., 2015).

To date, *in vitro* tandem enzymatic reactions have been well developed for the preparation of alcohols, acids, acid derivatives, amino acids, amines, nucleosides/nucleotides, nucleosides/nucleotides derivatives, oligosaccharides and glycoconjugates (Bornscheuer et al., 2012). The field of tandem chemo-enzymatic reaction is still in its infancy (Wang et al., 2016). Recently, lipase has been successfully coupled with various transition metal complexes for readily deracemizing secondary alcohols and primary amines (Verho et al., 2015). This accomplishment clearly shows the advantages of multistep one-pot processes, so-called tandem reactions. Compared with step-wise synthesis, one-pot tandem reactions improve overall synthetic efficiency and reduce waste generation by avoiding intermediate purification steps. Additionally, tandem reactions improve the overall asymmetric synthesis efficiency by converting achiral substrates to single enantiopure product containing a single enantiomer. Last but not least, the cooperative effect among different catalytic steps enhances the selectivity and activity by an equilibrium process.

However, combining multiple enzymes or combining enzymes with chemical catalysts in a tandem process is not simply replicating the catalytic behavior of each catalyst. For multi-enzymatic reactions, several strategies have been developed to balance the reaction rate of each step and maintain enzymatic activity under reaction conditions, including cell free metabolic engineering (CFME) and modeling (Dudley et al., 2015). Protein engineering methods have been applied, such as protein immobilization and fusion protein construction, to improve the overall efficiency of multi-enzymatic reactions (Ji et al., 2016; Jia et al., 2014). Specifically, it is more challenging to find conditions to enable catalytic activity of organometallic catalysts and biocatalysts in one-pot due to incompatibility and mutual inactivation. To overcome those difficulties, several novel strategies have been developed, including supramolecular assembly and compartmentalization. In this review, we will summarize the major tandem enzymatic and chemo-enzymatic reactions developed so far for selective synthesis and discuss the design and engineering strategies for system optimization.

2. Tandem enzymatic reactions

2.1 Tandem enzymatic reactions: simple systems

From aerobic respiration to photosynthesis, millions of compounds are synthesized through enzymatic reactions during the lifespan of all living organisms. These compounds, even those with very complex structures, are converted from simple chemical resources such as glucose, through the metabolic network in the cell. Tandem enzymatic reactions, or pathways in other words, can be considered as subunits of the whole metabolic network. Although enzymatic reactions have long been used for chemical synthesis due to many of their excellent features, particularly the high selectivity and specificity, multiple tandem

enzymatic reaction systems developed by chemists recently are not simple combinations of enzymatic reactions catalyzed by individual enzymes. Besides all the advantageous properties of enzymatic reactions, tandem enzymatic reactions can be much more cost-effective by using inexpensive substrates, potentially making them economically feasible. Moreover, one-pot tandem enzymatic reactions simply avoid the purification of the intermediates, which makes the process more efficient than step-wise enzymatic reactions.

While such tandem enzymatic reactions can be accomplished *in vivo*, as widely used in today's fermentation industry, there are many advantages to use *in vitro* systems. First, as no competing pathways exist in the *in vitro* system and no metabolic burden or toxicity issues need to be considered, the yield can be much closer to the theoretical yield than the *in vivo* system. Second, due to its simplicity, it would be more practical and effective to optimize the system through modeling and adjusting the enzymes' concentrations. Moreover, artificial tandem enzymatic reactions which can produce non-natural compounds are readily set up without potential toxicity issues brought by the product. In the past decade, many tandem enzymatic reaction systems have been developed to produce chemicals which were comprehensively reviewed elsewhere (Oroz-Guinea et al., 2013). Here we will focus on a few recent examples.

Although wild type enzymes are used in most of the cases, variants with novel activities created by protein engineering can also be used in the tandem reaction systems (Birmingham et al., 2014; Tavanti et al., 2017). For example, through computational design, researchers have engineered the promiscuous aldolase RA95.5–8 to catalyze asymmetric Michael additions and Knoevenagel reactions via Schiff base intermediates. Recently Hilvert's group discovered novel activities of those aldolase variants. The variant RA95.5–8F can catalyze the synthesis of (*R*)-methanol from acetone and 6-methoxy-2-naphthaldehyde, while the variant T53L/K210H RA95.5–8 can catalyze the dehydrated (*R*)-methanol to γ -nitroketones (Garrabou et al., 2017; Obexer et al., 2017). By combination of those two aldolase variants, they achieved similar yield and stereoselectivity in one-pot as the reaction using the purified intermediate (Figure 1).

2.2 Tandem enzymatic reactions: complex system

Although *in vitro* tandem enzymatic reactions have a number of advantages over *in vivo* tandem enzymatic reactions, their weaknesses are also obvious, with the main weakness being the decreased flexibility. For example, most of the tandem reactions only include two or three enzymes. As a result, the substrates that can be used as well as the products that can be generated are largely limited. However, an ideal *in vitro* tandem reaction system should be sufficiently flexible to generate a broader product scope while using the low-cost substrates. Therefore, *in vitro* tandem reaction systems with higher complexity for synthesizing chemicals, such as carbohydrates, fuels, and fine chemicals are desired (Fessner, 2015; Guterl et al., 2012; Khattak et al., 2014; Schoevaart et al., 2000; Wagner et al., 2015). In recent years, great efforts were spent in the emerging CFME field to address such a challenge (Dudley et al., 2015). A CFME system usually involves four or more enzymes and uses inexpensive substrates if possible, as the cost-effectiveness is a required design criteria (Caschera et al., 2015; Zhang, 2015). Ideally, it should combine catabolic

pathways and anabolic pathways, so that both the building blocks and the energy for synthesizing the target molecules can be generated from simple resources, such as glucose. However, the design and test of such a system would be very challenging as tens of enzymes might be involved (Karzbrun et al., 2011). Recently, Bowie and coworkers successfully combined the Embden-Meyerhof-Parnas glycolytic pathway and the mevalonate pathway to produce monoterpenes (Korman et al., 2017). A super-complex system with 27 enzymes was reconstituted in one-pot which enabled the system to synthesize monoterpenes by using glucose as the only substrate. While specific designs, such as the enzymatic purge valve nodes, helped the catabolic pathway and the anabolic pathway work harmonically in the system (Opgenorth et al., 2014). By switching the monoterpene synthases in the system, different types of monoterpenes such as limonene, pinene and sabinene can be produced. This system can produce monoterpenes with titers an order of magnitude higher than the cellular toxicity limit (15 g/L) with an almost theoretical yield (>95%), which demonstrates the great advantage of using enzymatic tandem reaction *in vitro*.

In addition to transferring the existing pathways from *in vivo* to *in vitro*, scientists are also able to design *de novo* complex pathways, which can accomplish difficult biosynthetic tasks. In 2017, the crotonyl-coenzyme A (CoA)/ethylmalonyl-CoA/hydroxybutyryl-CoA (CETCH) cycle was constructed by Erb and coworkers, which provides a seventh CO₂ fixation pathway besides the six naturally evolved ones (Schwander et al., 2016) (Figure 2). Instead of being restricted to known enzymes, the researchers sought for all reactions that are biochemically feasible. Multiple cycles were designed from scratch and their thermodynamic feasibility was evaluated. Then, enzymes that can catalyze the reactions in the CETCH cycle were identified from bioinformatics databases and characterized individually *in vitro*. After combining the auxiliary proofreading and cofactor regeneration processes, the CETCH cycle involves 17 enzymes in total which came from nine different organisms from all three domains of life. Through enzyme engineering and metabolic proofreading, the CETCH cycle can convert CO₂ into organic molecules at a rate of 5 nanomoles of CO₂ per minute per milligram of protein.

2.3 Optimization of the tandem enzymatic reactions

In order to optimize tandem enzymatic reaction systems, especially systems with high complexity, modeling is an indispensable strategy (Chen et al., 2013; Rollin et al., 2015). Although the modeling of stoichiometry seems to be easy, to set up a dynamic model is much harder due to not only the non-linear behavior of enzymes, but also the unpredictable interactions between chemicals and enzymes in the system. Even though plenty of studies have shown the possibility to set up dynamic models for the *in vitro* systems, most of them dealt with relatively simple systems (Ardao et al., 2013; Dvorak et al., 2014; Findrik et al., 2007; Ishii et al., 2007; Van Hecke et al., 2009). Moreover, as the dynamic parameters of enzymes vary a lot under different conditions, such as pH and buffer components, the modeling strategies depend on the parameters which are obtained from specific condition and can hardly be accurate. To overcome this limitation, Panke and coworkers applied an approach based on classical engineering systems theory for optimization of their dihydroxyacetone phosphate (DHAP) producing system (Hold et al., 2016). By characterizing their 10-enzyme system in a continuous stirred tank reactor (CSTR), assisted

by an online electrospray ionization mass spectrometry, a sufficiently detailed response of the system to the dynamic challenges was recorded. The successful parameterization of this system enabled the researchers to perform forward design. Although this work was done in CSTR, the obtained model should also be readily used to design batch reactions.

In addition to modeling, inspired by nature where tandem reactions are catalyzed by multi-enzyme complexes (MECs), the immobilization of the enzymes involved in a tandem reaction system serve as another promising way to improve the reaction efficiency (Ji et al., 2016; Jia et al., 2014). While the enzymes form an artificial complex, the intermediates involved in the tandem reaction can be transported between enzymes more efficiently. As high local concentrations of the intermediates are maintained, the formation of the final product should be more dynamically favorable. Moreover, diffusion losses, especially of the highly reactive intermediates, can be largely avoided (Ogawa et al., 2002). The immobilization of enzymes can offer other advantages, such as the improvement of the stability of enzymes, though the conformation of the enzyme might get affected, resulting in activity loss to some extent. To date, multiple methods for the attachment of enzymes to inorganic surfaces have been developed. However, in most cases, only 3 or fewer enzymes can be co-localized due to various challenges, such as the different responses of enzymes to different attachment methods (You et al., 2013; 2014). But recently Travis and coworkers demonstrated the possibility to create a 10-step enzymatic reaction on nanoparticles (Mukai et al., 2017). Although the activity of individual enzyme was higher in solution, the conversion of glucose to lactate was much higher when all the enzymes were tethered. In addition to the inorganic surfaces, DNA as a programmable polymer offers chemists another option for enzyme immobilization (Rajendran et al., 2017). The ability of DNA to form various nanostructures makes it possible to control the spatial organization of the enzymes. Besides the assembly of enzymes involved in the tandem reaction, the cofactors involved in the tandem reaction could also be readily controlled (Fu et al., 2014).

As an alternative to the material assisted enzyme co-localization strategy, another approach is to directly engineer the enzymes to either form fusion proteins or induce self-assembly. In order to form fused proteins, linkers between enzymes involved in the tandem reaction were frequently used (Yu et al., 2015). Similar to the material assisted enzyme immobilization methods, the conformation of enzyme might be affected by the linkers or its fusion partners, which may lower or even completely abolish the activity. However, as the fusion protein can be prepared as a single protein, and no further chemical modification is needed, such an approach provides ease to researchers to prepare enzyme complexes for tandem reactions. Besides the application for the assembly of enzymes that are directly involved in the product synthesis, another application is to fuse the enzymes with their redox partners, such as the widely used fusion protein of P450 with the NAD(P)H reductase (Andre et al., 2013; Peters et al., 2017). In addition to using linkers to form enzyme complex, adding peptide/protein tags to the enzymes can serve as an alternative approach (Han et al., 2017; Liu et al., 2017; Proschel et al., 2015).

3. Tandem Chemo-enzymatic Reactions

Organometallic catalysis and biocatalysis are two different disciplines in terms of reaction categories, catalytic conditions, substrates scopes and selectivities. Organometallic catalysts have wide substrate scopes and high productivity, and are still the key for bulk chemical manufacturing. Biocatalysis is becoming more widely used in the pharmaceutical industry due to its high regio-, stereo- and enantioselectivity, and growing advances in enzyme discovery and engineering. To access the advantages of both catalytic disciplines, there is a growing interest to combine organometallic catalysts in tandem with enzymes to realize synthetic power that cannot be achieved by either of them alone (Denard et al., 2013; Wang et al., 2016).

Unlike developing tandem enzymatic reactions, it is more challenging to combine organometallic catalysts and biocatalysts due to incompatibility and mutual inactivation of metal complexes and enzymes. Up to date, only lipases and serine proteases could maintain high catalytic activity in organic solvents and at high temperature. Similarly, most transition-metal complexes are inhibited in aqueous solution with or without cellular components. In this section, we will summarize the major tandem chemo-enzymatic reactions developed in organic solvent and aqueous buffer solutions, and discuss the novel strategies used to combine organometallic catalysts with biocatalysts in one-pot.

3.1. One-pot tandem chemo-enzymatic reactions in organic solvent

Dynamic kinetic resolution (DKR) of alcohols and amines based on transition metal-catalyzed racemization and lipase-catalyzed resolution in pure organic solvent has been developed towards a mature technology for preparation of enantiopure secondary alcohols and primary amines (Scheme 1). Lipase deracemizes a mixture of enantiomers by selectively acylating desired enantiomer. The drawback of such enzymatic kinetic resolution is that a maximum yield of only 50% can be obtained. However, it can be resolved by using a racemization catalyst to replenish the consumed enantiomer and drive the resolution up to 100% yield of desired enantiomer. Since the pioneering demonstration of the DKR concept by the group of Williams (Allen et al., 1996), Bäckvall (Pamies et al., 2003) and Kim (Kim et al., 2003), intense studies were conducted to develop efficient racemization catalysts, improve enzyme stability and expand substrate scope (Verho et al., 2015; Wang et al., 2016). To date, immobilized *Candida antarctica* lipase B (CALB) and *C. antarctica* lipase A (CALA) are the best enzymes of choice due to their robustness and activities in pure organic solvent at elevated temperature up to 100 °C (Martin-Matute et al., 2007).

Ruthenium, iridium, alumina and vanadium complexes are the most well-developed catalysts for alcohol racemization. They were used in tandem with various lipases to deracemize a wide range of functionalized secondary alcohols, including aliphatic, allylic alcohols, chlorohydrins, diols, homoallylic alcohols, and *N*-heterocyclic 1,2-amino alcohols, α -hydroxyl ketones with excellent yields and enantiomeric excess (*ee*) (Verho et al., 2015). Besides the above-mentioned homogeneous catalysts, heterogeneous acids, zeolites (Magadum et al., 2017; Xu et al., 2017) and vanadium based mesoporous silica (Sugiyama et al., 2016) were also reported as racemization catalysts of secondary alcohols. The DKR of amines is more challenging due to a lack of efficient racemization catalysts. To date,

coupling ruthenium complexes, analogues of Shvo catalyst, with CALB is the most practical approach for DKR of aliphatic or benzylic primary amines at 90 °C (Verho et al., 2015). In recent years, Pd nanoparticles-based heterogeneous catalysts were employed for racemization of benzylic primary amines under mild conditions. However, they did not work well for aliphatic amines (Zhang et al., 2017).

3.2. One-pot tandem chemo-enzymatic reactions in aqueous solution

Except for lipases and serine proteases, most biocatalysts have poor stability in organic solvents and at elevated temperature. In the past 20 years, there has been a growing interest to develop tandem reactions in aqueous solutions to combine unique transformations catalyzed by organometallic catalysts and biocatalysts (Wang et al., 2016). The major motivation to associate biocatalysts with organometal catalysts in one pot is to utilize the synthetic power from each discipline for specific transformations that could not be realized by either of them alone. In nature, enzymes have specific substrate binding site for highly regio- stereo- and enantioselective synthesis. Accordingly, organometallic catalysts could access some reactions that enzymatic processes do not exist or work only on limited substrate scope, for example, hydrogenation, cross-coupling reaction, Wacker-oxidation, olefin metathesis, etc.

Unlike the DKR system in organic solvents, the development of tandem chemo-enzymatic reactions in aqueous solution is still in its infancy. The number of transformations amenable to these processes are small portion of catalytic reactions reported in organometallic chemistry or synthetic biology. Table 1 summarizes the representative work of one-pot chemo-enzymatic reactions that can occur in aqueous-based solution. They are metal-catalyzed hydrogenation in tandem with isomerase or hydrolase catalyzed reactions; organometallic-catalyzed racemization in tandem with enantioselective hydrolysis; Pd/Cu catalyzed Wacker oxidation in tandem with enzymatic catalyzed asymmetric reduction or reductive amination; metal-catalyzed metathesis in tandem with enzymatic decarboxylation, ester hydrolysis or P450-catalyzed epoxidation; metal-catalyzed cross coupling in tandem with enzymatic reduction, oxidation and hydrolysis; Au catalyzed isomerization in tandem with enzymatic hydrolysis.

Those reactions were either performed in one-pot sequential manner by avoiding the purification of intermediates when different reaction conditions are required for organometallic and enzymatic step, or in concurrent manner when two catalytic steps are compatible with each other. Enlighteningly, a few cooperative concurrent reactions were reported to reveal the significance of tandem reactions compared with the sequential transformations. For example, Zhao and Hartwig groups jointly reported an example of Ru-catalyzed olefin metathesis coupled with a P450-monooxygenase that selectively oxidized one of the cross-metathesis product against others. The overall yield of concurrent reactions is 1.5 times higher than the sequential one (Figure 3) (Denard et al., 2014).

3.3 Novel Strategies to combine transition metals and enzymes in one-pot tandem reactions

Incompatibility of the individual reaction steps with one another is the major challenge to combining transition metal catalysts with biocatalysts in one-pot. Besides immobilized lipases and serine proteases, the majority of biocatalysts have poor stability in organic solvents. Most of the transition metal catalysts are not active in aqueous solution. Mutual inactivation also occurs frequently due to the coordination of the transition metal complex to the protein catalysts. In this section, we will discuss some novel strategies developed so-far to combine transition metals and enzymes in one-pot, including supramolecular assemblies, artificial metalloenzymes, compartmentalization, whole cells, and co-immobilization (Table 2).

Incorporation of transition metal complexes within supramolecular hosts is a valuable way to solve those problems. A long-term goal of supramolecular chemistry is construction of assemblies that mimic the desirable qualities of protein catalysts (Wiester et al., 2011). It has been found that supramolecular assemblies could stabilize reactive metal complexes and increase their turn over numbers (Brown et al., 2011). Additionally, supramolecular complexes enable the catalytic activity of several transition metal catalysts in water by providing a bio-inspired hydrophobic cavity (Bistri et al., 2015; Kaphan et al., 2015; Pluth et al., 2007). Importantly, supramolecular cage prevents the direct interaction of transition metal with enzymes, thereby averting their mutual inactivation (Kohler et al., 2013; Wang et al., 2013). Lastly, supramolecular assemblies provide extra stereo-, regio- and enantio-selectivity by providing a well-defined cavity for the reaction compared with the reaction performed in bulk solvent (Brown et al., 2009; Yoshizawa et al., 2006). Using a supramolecular approach, Toste and coworkers created various tandem reactions employing esterases, lipases or alcohol dehydrogenase with gold (I) or ruthenium (II) complexes encapsulated in a Ga₄L₆ tetrahedral supramolecular cluster without mutual inactivation (Figure 4) (Wang et al., 2013). Their findings suggested that supramolecular assemblies may provide a general strategy for carrying out classic organic reactions in tandem with enzymatic reactions in aqueous solution.

Artificial metalloenzyme resulting from encapsulation of transition metal catalyst within a protein scaffold is a special form of supramolecular host-assemblies. Ward and coworkers developed an artificial (*R*)-selective transfer-hydrogenase (ATHase) by incorporation of a biotinylated [Cp*Ir(Biot-*p*-L)Cl] complex within streptavidin (Sav). Sav worked as a neutral shield for the Ir complex to keep it from interacting with other biocatalysts. In that way, they successfully combined ATHase with various NADH-, FAD- and haem-dependent enzymes resulting in orthogonal redox cascades that could not be achieved when the free Ir-complex was used (Kohler et al., 2013), including a double stereoselective amine deracemization, production of L-pipecolic acid from L-lysine (Figure 5).

Compartmentalization is another strategy to couple chemical catalysts with biocatalysts in one-pot by protecting the enzymes from the organic solvent or metal catalysts from cellular components; as well as shielding catalytic centers from one another to avoid mutual inactivation. Besides the traditional biphasic system (Marr et al., 2011), more novel methods have been developed for compartmentalization purpose including hydrogel immobilization,

membrane filtration and whole-cell isolation. Gröger and coworkers realized enzymatic decarboxylation (Gómez Baraibar et al., 2016) and alcohol dehydrogenation (Heidlindemann et al., 2014) in organic solvent by encapsulating the enzymes in poly(vinyl alcohol)/poly(ethylene glycol) cryogels or acrylate-based superabsorbent polymer respectively. They coupled these two enzymes with ruthenium-catalyzed metathesis and a proline-derivative-catalyzed aldol reaction for efficient preparation of antioxidants 4,4'-dihydroxystilbene and 1,3-diols separately. In another study, they combined Wacker oxidation with enzymatic reduction or hydroamination in a one-pot process in aqueous media by withholding the detrimental effect of *Cu* ions from biotransformation in the interior of a polydimethylsiloxane thimble (PDMS) that only enabled the free exchange of organic chemical (Figure 6) (Sato et al., 2015; Uthoff et al., 2017).

Although supramolecular strategies have enabled the catalytic activity of several transition metal catalysts in aqueous environment, many metal cofactors are still inhibited by the cellular components and therefore require purified protein for tandem reactions in most cases. Using cellular membrane as a barrier to protect metal catalysts from cellular inhibitors is an attractive strategy. Recently, Ward and coworkers created an *E. coli* strain for periplasmic expression of Sav with a biotinylated Hoveyda-Grubbs catalyst for olefin metathesis *in vivo* (Jeschek et al., 2016). The periplasm provided an environment with low concentrations of inhibitors for metathesis and thus enabled the high-throughput directed evolution of artificial metalloenzymes *in vivo*. Additionally, the use of whole cell provides a natural protection for biocatalysts. Lloyd and coworkers prepared (*R*)-1-methyltetrahydroisoquinoline (MTQ) with 96% *ee* by a cooperative tandem reaction consisting of enantioselective monoamine oxidation catalyzed by an engineered *E. coli* strain and nonselective reduction catalyzed by nanoscale Pd (0) coated on the cell surface (Foulkes et al., 2011) (Figure 7).

Another interesting strategy to enable chemo-enzymatic reactions in one pot is to create hybrid enzyme-metal nanoparticles that bear orthogonal but cooperative catalytic centers. This design straddles the disciplines of supramolecular and compartmentalization. The peptide is a robust biomaterial and can stabilize the performance of inorganic catalysts (San et al., 2011). The resulting enzyme-metal complex could work as a metalloenzyme with the catalytic activity originated from the introduced metal. Significantly, the surrounding proteins not only serve as protecting cage but also work as catalysts for distinct bioactivity (Engström et al., 2013; Filice et al., 2013; San et al., 2011). In one example, Bäckvall and coworkers created a hybrid catalyst consisting of CALB and Pd nanoparticles co-immobilized into the compartments of mesoporous silica for DKR of 1-phenylethylamine (Engström et al., 2013). The co-immobilization resulted in a more efficient cooperation between two catalysts and recyclability of both Pd nanoparticles and CALB at the same time (Figure 8).

4. Future perspectives and conclusions

Unlike multistep enzymatic transformations occurring in cells that generate complex molecules, tandem reactions *in vitro* generally consist of two to three steps and the reaction categories only represent a small portion of bio- or chemo-transformation available in

synthetic biology or organometallic chemistry. The development of *in vitro* tandem processes is challenged by the limited number of biocatalysts and strategies to balance the activity of each step. The combination of chemocatalysts and biocatalysts in one-pot further illustrates the issues of incompatibility and mutual inactivation between individual catalysts. To address these problems, it is essential to take advantages of several research areas including but not limited to protein engineering, artificial metalloenzyme, system modeling, chemical catalysts design and development, supramolecular assembly, and compartmentalization.

Protein engineering strategies can be used to broaden substrate scopes and enhance regio-, stereo-, enantio-selectivities and stability of current biocatalysts, and these properties are important for developing both multi-enzymatic and tandem chemo-enzymatic reactions. It is especially important to enable enzymatic function in organic solvents or at high temperature to realize more possibilities of cooperative metal-enzymatic transformations. There are some well-developed methods to improve enzyme performance in harsh condition, such as immobilization of enzymes on heterogeneous support (Sheldon et al., 2013); preparation of cross linked enzyme aggregates (Velasco-Lozano et al., 2016), surfactant treatment (Kim et al., 2011), the creation of protein mutants via rational design and directed evolution (Bornscheuer et al., 2012).

Artificial metalloenzyme are a growing field to expand the natural enzymatic repertoire with abiotic reactions (Hyster et al., 2016). It combines the versatile reaction scope of transition metal catalysts with the beneficial effects of enzymes, such as high selectivity over both substrates and products. So far, a few well-developed strategies are available for developing new artificial metalloenzyme, for example, natural metalloenzymes could be engineered for abiotic transformations (Kan et al., 2016); computational redesign (Khare et al., 2012), incorporating noble-metal complexes with protein scaffold (Srivastava et al., 2015) and replacing the native metal of a metalloenzyme with a noble metal complex (Key et al., 2016). Importantly, artificial metalloenzyme improved the performance of metal catalysts in aqueous solution and prevented the mutual inactivation of metal catalysts with other biocomponents (Cangelosi et al., 2014), which enables the coupling of more metal catalysts in tandem with multi-step enzymatic reactions for selective synthesis. Last but not least, advanced protein engineering tools such as directed evolution can be directly applied to artificial metalloenzyme to improve its catalytic performance. Artificial metalloenzyme undoubtedly would contribute more to *in vitro* tandem reactions in near future.

Although there are countless enzymatic reactions in nature, only few of them can serve as useful catalysts for either tandem enzymatic or chemoenzymatic reaction systems. One of the major challenges is to obtain functional enzymes in reasonable amount and purity. *Escherichia coli* has been widely used for the heterologous expression of proteins, but most of the enzymes, especially those from eukaryotes, can get misfolded when expressed in *E. coli*, which abolishes their activities. Moreover, the expression of enzymes which require post-transcriptional or post-translational modifications, or enzymes which have a very high molecular weight can be even more challenging. Therefore, the development of hosts and processes for more efficient protein expression and purification is always desirable. In addition, as the tandem enzymatic reaction systems tend to be more complex, dynamic

modeling of such systems can not only be an effective way for optimization, but also one of the main approaches for the system design. As the activities of enzymes can vary significantly from one system to another, how to model the reaction system with high accuracy can be a major problem. Besides making more detailed dynamic dataset for each enzyme, developing methods for real time monitoring of the components in the reaction system is another possible solution.

The combination of chemocatalysis and biocatalysis in one-pot for concurrent transformations is still in its infancy. The development of this field is relatively slow mainly due to the incompatibility and mutual inactivation of metal complexes and enzymes. To expand the spectrum of chemical catalysts in a buffer system, chemists have made efforts on developing aqueously effective transition metal catalysts. The achievements made so far have enabled several metal-catalyzed reactions in aqueous solutions, including olefin (Vougioukalakis, 2016), hydrogenation, and C-C coupling reactions (Wang et al., 2016). Importantly, a few metal catalysts such as Cu, Pd and Ru have been integrated into living cell for biorthogonal protein chemistry (Li et al., 2014; Yang et al., 2014). Although there are very few examples to integrate bio-orthogonal reactions into chemical synthetic pathways in living cells, there is an opportunity to create new cell factories for distinct chemical production by harnessing the synthetic power of transition metal catalysts. Additionally, as discussed in the previous session, supramolecular assemblies and compartmentalization could provide alternative strategies to enable the function of water-insoluble metal complexes in buffer solutions and avoid mutual inactivation of chemical catalysts and biocatalysts.

In summary, the development of *in vitro* tandem enzymatic and chemo-enzymatic processes continues to grow. With the development of protein engineering, artificial metalloenzyme and system design and modeling, there is an immense opportunity to develop sophisticated tandem processes mimicking biosynthetic pathways for complex synthesis. Additionally, with the aid of organic synthesis, supramolecular assemblies and compartmentalization, more organometallic catalysis can be utilized in aqueous solutions and integrated with enzymatic steps for selective synthesis. Based on these achievements, the field will continue to expand in the future.

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References

1. Allen JV, Williams MJ. 1996 Dynamic kinetic resolution with enzyme and palladium combinations. *Tetrahedron Lett*, 37, 1859–62
2. Andre C, Kim SW, Yu XH, Shanklin J. 2013 Fusing catalase to an alkane-producing enzyme maintains enzymatic activity by converting the inhibitory byproduct H₂O₂ to the cosubstrate O₂. *Proc Natl Acad Sci U S A*, 110, 3191–96 [PubMed: 23391732]
3. Ardao I, Zeng AP. 2013 In silico evaluation of a complex multi-enzymatic system using one-pot and modular approaches: Application to the high-yield production of hydrogen from a synthetic metabolic pathway. *Chem Eng Sci*, 87, 183–93

4. Asikainen M, Krause N. 2009 Tandem Enzyme/Gold-Catalysis: From Racemic α -Allenic Acetates to Enantiomerically Enriched 2,5-Dihydrofurans in One Pot. *Adv Synth Catal*, 351, 2305–09
5. Birmingham WR, Starbird CA, Panosian TD, Nannemann DP, Iverson TM, Bachmann BO. 2014 Bioretrosynthetic construction of a didanosine biosynthetic pathway. *Nat Chem Biol*, 10, 392–9 [PubMed: 24657930]
6. Bistri O, Reinaud O. 2015 Supramolecular control of transition metal complexes in water by a hydrophobic cavity: a bio-inspired strategy. *Org Biomol Chem*, 13, 2849–65 [PubMed: 25608497]
7. Boffi A, Cacchi S, Ceci P, Cirilli R, Fabrizi G, Prastaro A, Niembro S, Shafir A, Vallribera A. 2011 The Heck reaction of allylic alcohols catalyzed by palladium nanoparticles in water: chemoenzymatic synthesis of (R)-(-)-rhododendrol. *Chemcatchem*, 3, 347–53
8. Bornscheuer UT, Huisman GW, Kazlauskas RJ, Lutz S, Moore JC, Robins K. 2012 Engineering the third wave of biocatalysis. *Nature*, 485, 185–94 [PubMed: 22575958]
9. Brown CJ, Bergman RG, Raymond KN. 2009 Enantioselective Catalysis of the Aza-Cope Rearrangement by a Chiral Supramolecular Assembly. *J Am Chem Soc*, 131, 17530–31 [PubMed: 19950985]
10. Brown CJ, Miller GM, Johnson MW, Bergman RG, Raymond KN. 2011 High-Turnover Supramolecular Catalysis by a Protected Ruthenium(II) Complex in Aqueous Solution. *J Am Chem Soc*, 133, 11964–66 [PubMed: 21736357]
11. Burda E, Hummel W, Gröger H. 2008 Modular Chemoenzymatic One-Pot Syntheses in Aqueous Media: Combination of a Palladium-Catalyzed Cross-Coupling with an Asymmetric Biotransformation. *Angew Chem Int Ed*, 47, 9551–54
12. Cangelosi VM, Deb A, Penner-Hahn JE, Pecoraro VL. 2014 A de novo designed metalloenzyme for the hydration of CO₂. *Angew Chem Int Ed Engl*, 53, 7900–3 [PubMed: 24943466]
13. Caschera F, Noireaux V. 2015 A cost-effective polyphosphate-based metabolism fuels an all E. coli cell-free expression system. *Metab Eng*, 27, 29–37 [PubMed: 25446973]
14. Chen S-T, Huang W-H, Wang K-T. 1994 Resolution of Amino Acids in a Mixture of 2-Methyl-2-propanol/water (19:1) Catalyzed by Alcalase via in Situ Racemization of One Antipode Mediated by Pyridoxal 5-Phosphate. *JOC*, 59, 7580–81
15. Chen X, Zhang C, Zou R, Zhou K, Stephanopoulos G, Too HP. 2013 Statistical experimental design guided optimization of a one-pot biphasic multienzyme total synthesis of amorpho-4,11-diene. *PLoS One*, 8
16. Clomburg JM, Crumbley AM, Gonzalez R. 2017 Industrial biomanufacturing: The future of chemical production. *Science*, 355, eaag0804
17. Denard CA, Bartlett MJ, Wang Y, Lu L, Hartwig JF, Zhao H. 2015 Development of a One-Pot Tandem Reaction Combining Ruthenium-Catalyzed Alkene Metathesis and Enantioselective Enzymatic Oxidation To Produce Aryl Epoxides. *ACS Catal*, 5, 3817–22
18. Denard CA, Hartwig JF, Zhao H. 2013 Multistep One-Pot Reactions Combining Biocatalysts and Chemical Catalysts for Asymmetric Synthesis. *ACS Catal*, 3, 2856–64
19. Denard CA, Huang H, Bartlett MJ, Lu L, Tan Y, Zhao H, Hartwig JF. 2014 Cooperative Tandem Catalysis by an Organometallic Complex and a Metalloenzyme. *Angew Chem Int Ed*, 53, 465–69
20. Dudley QM, Karim AS, Jewett MC. 2015 Cell-free metabolic engineering: Biomanufacturing beyond the cell. *Biotechnol J*, 10, 69–82 [PubMed: 25319678]
21. Dvorak P, Kurumbang NP, Bendl J, Brezovsky J, Prokop Z, Damborsky J. 2014 Maximizing the Efficiency of Multienzyme Process by Stoichiometry Optimization. *Chembiochem*, 15, 1891–95 [PubMed: 25099170]
22. El-Sepelgy O, Brzozowska A, Rueping M. 2017 Asymmetric Chemoenzymatic Reductive Acylation of Ketones by a Combined Iron-Catalyzed Hydrogenation–Racemization and Enzymatic Resolution Cascade. *Chemsuschem*, 10, 1664–68 [PubMed: 28244251]
23. Engström K, Johnston EV, Verho O, Gustafson KPJ, Shakeri M, Tai C-W, Bäckvall J-E. 2013 Co-immobilization of an Enzyme and a Metal into the Compartments of Mesoporous Silica for Cooperative Tandem Catalysis: An Artificial Metalloenzyme. *Angew Chem Int Ed*, 52, 14006–10
24. Erdmann V, Lichman BR, Zhao J, Simon RC, Kroutil W, Ward JM, Hailes HC, Rother D. 2017 Enzymatic and Chemoenzymatic Three-Step Cascades for the Synthesis of Stereochemically Complementary Trisubstituted Tetrahydroisoquinolines. *Angew Chem Int Ed*, 56, 12503–07

25. Fessner W-D. 2015 Systems Biocatalysis: Development and engineering of cell-free “artificial metabolisms” for preparative multi-enzymatic synthesis. *N Biotechnol*, 32, 658–64 [PubMed: 25534674]
26. Filice M, Marciello M, Morales MdP, Palomo JM. 2013 Synthesis of heterogeneous enzyme–metal nanoparticle biohybrids in aqueous media and their applications in C–C bond formation and tandem catalysis. *Chem Commun*, 49, 6876
27. Findrik Z, Vasic-Racki D. 2007 Biotransformation of D-Methionine into L-methionine in the cascade of four enzymes. *Biotechnol Bioeng*, 98, 956–67 [PubMed: 17534960]
28. Foulkes JM, Malone KJ, Coker VS, Turner NJ, Lloyd JR. 2011 Engineering a Biometallic Whole Cell Catalyst for Enantioselective Deracemization Reactions. *ACS Catal*, 1, 1589–94
29. Fu JL, Yang YR, Johnson-Buck A, Liu MH, Liu Y, Walter NG, Woodbury NW, Yan H. 2014 Multi-enzyme complexes on DNA scaffolds capable of substrate channelling with an artificial swinging arm. *Nat Nanotechnol*, 9, 531–36 [PubMed: 24859813]
30. Fuchs M, Schober M, Pfeffer J, Kroutil W, Birner-Gruenberger R, Faber K. 2011 Homoallylic Alcohols via a Chemo-Enzymatic One-Pot Oxidation–Allylation Cascade. *Adv Synth Catal*, 353, 2354–58
31. Garrabou X, Verez R, Hilvert D. 2017 Enantiocomplementary Synthesis of γ -Nitroketones Using Designed and Evolved Carboligases. *J Am Chem Soc*, 139, 103–06 [PubMed: 27992715]
32. Gauchot V, Kroutil W, Schmitzer AR. 2010 Highly recyclable chemo-/biocatalyzed cascade reactions with ionic liquids: one-pot synthesis of chiral biaryl alcohols. *Chemistry (Easton)*, 16, 6748–51
33. Gómez Baraibar Á, Reichert D, Mügge C, Seger S, Gröger H, Kourist R. 2016 A One-Pot Cascade Reaction Combining an Encapsulated Decarboxylase with a Metathesis Catalyst for the Synthesis of Bio-Based Antioxidants. *Angew Chem Int Ed*, 55, 14823–27
34. Guterl J-K, Garbe D, Carsten J, Steffler F, Sommer B, Reiß S, Philipp A, Haack M, Rühmann B, Koltermann A, Ketting U, Brück T, Sieber V. 2012 Cell-Free Metabolic Engineering: Production of Chemicals by Minimized Reaction Cascades. *Chemsuschem*, 5, 2165–72 [PubMed: 23086730]
35. Han GH, Seong W, Fu Y, Yoon PK, Kim SK, Yeom SJ, Lee DH, Lee SG. 2017 Leucine zipper-mediated targeting of multi-enzyme cascade reactions to inclusion bodies in *Escherichia coli* for enhanced production of 1-butanol. *Metab Eng*, 40, 41–49 [PubMed: 28038953]
36. Heidlindemann M, Rulli G, Berkessel A, Hummel W, Gröger H. 2014 Combination of Asymmetric Organo- and Biocatalytic Reactions in Organic Media Using Immobilized Catalysts in Different Compartments. *ACS Catal*, 4, 1099–103
37. Hold C, Billerbeck S, Panke S. 2016 Forward design of a complex enzyme cascade reaction. *Nat Commun*, 7, 12971 [PubMed: 27677244]
38. Hyster TK, Ward TR. 2016 Genetic Optimization of Metalloenzymes: Enhancing Enzymes for Non-Natural Reactions. *Angew Chem Int Ed*, 55, 7344–57
39. Ishii N, Suga Y, Hagiya A, Watanabe H, Mori H, Yoshino M, Tomita M. 2007 Dynamic simulation of an in vitro multi-enzyme system. *FEBS Lett*, 581, 413–20 [PubMed: 17239859]
40. Jeschek M, Reuter R, Heinisch T, Trindler C, Klehr J, Panke S, Ward TR. 2016 Directed evolution of artificial metalloenzymes for in vivo metathesis. *Nature*, 537, 661–65 [PubMed: 27571282]
41. Ji QZ, Wang BC, Tan J, Zhu LC, Li LY. 2016 Immobilized multienzymatic systems for catalysis of cascade reactions. *Process Biochem* 51, 1193–203
42. Jia F, Narasimhan B, Mallapragada S. 2014 Materials-Based Strategies for Multi-Enzyme Immobilization and Co-Localization: A Review. *Biotechnol Bioeng*, 111, 209–22 [PubMed: 24142707]
43. Kan SBJ, Lewis RD, Chen K, Arnold FH. 2016 Directed evolution of cytochrome c for carbon–silicon bond formation: Bringing silicon to life. *Science*, 350, 1048–51
44. Kaphan DM, Levin MD, Bergman RG, Raymond KN, Toste FD. 2015 A supramolecular microenvironment strategy for transition metal catalysis. *Science*, 350, 1235–38 [PubMed: 26785485]
45. Karzbrun E, Shin J, Bar-Ziv RH, Noireaux V. 2011 Coarse-Grained Dynamics of Protein Synthesis in a Cell-Free System. *Phys Rev Lett*, 106, 048104 [PubMed: 21405367]

46. Key HM, Dydio P, Clark DS, Hartwig JF. 2016 Abiological catalysis by artificial haem proteins containing noble metals in place of iron. *Nature*, 534, 534–7 [PubMed: 27296224]
47. Khare SD, Kipnis Y, Greisen P, Jr., Takeuchi R, Ashani Y, Goldsmith M, Song Y, Gallaher JL, Silman I, Leader H, Sussman JL, Stoddard BL, Tawfik DS, Baker D. 2012 Computational redesign of a mononuclear zinc metalloenzyme for organophosphate hydrolysis. *Nat Chem Biol*, 8, 294 [PubMed: 22306579]
48. Khattak WA, Ul-Islam M, Ullah MW, Yu B, Khan S, Park JK. 2014 Yeast cell-free enzyme system for bio-ethanol production at elevated temperatures. *Process Biochem*, 49, 357–64
49. Kim H, Choi YK, Lee J, Lee E, Park J, Kim M-J. 2011 Ionic-Surfactant-Coated Burkholderia cepacia Lipase as a Highly Active and Enantioselective Catalyst for the Dynamic Kinetic Resolution of Secondary Alcohols. *Angew Chem Int Ed*, 50, 10944–48
50. Kim MJ, Chung YI, Choi YK, Lee HK, Kim D, Park J. 2003 (S)-selective dynamic kinetic resolution of secondary alcohols by the combination of subtilisin and an aminocyclopentadienylruthenium complex as the catalysts. *J Am Chem Soc*, 125, 11494–5 [PubMed: 13129341]
51. Kohler V, Turner NJ. 2015 Artificial concurrent catalytic processes involving enzymes. *Chem Commun (Camb)*, 51, 450–64 [PubMed: 25350691]
52. Kohler V, Wilson YM, Durrenberger M, Ghislieri D, Churakova E, Quinto T, Knorr L, Haussinger D, Hollmann F, Turner NJ, Ward TR. 2013 Synthetic cascades are enabled by combining biocatalysts with artificial metalloenzymes. *Nat Chem*, 5, 93–9 [PubMed: 23344429]
53. Korman TP, Opgenorth PH, Bowie JU. 2017 A synthetic biochemistry platform for cell free production of monoterpenes from glucose. *Nature Commun*, 8, 15526 [PubMed: 28537253]
54. Li J, Yu J, Zhao J, Wang J, Zheng S, Lin S, Chen L, Yang M, Jia S, Zhang X, Chen PR. 2014 Palladium-triggered deprotection chemistry for protein activation in living cells. *Nat Chem*, 6, 352–61 [PubMed: 24651204]
55. Liu YC, Hickey DP, Guo JY, Earl E, Abdellaoui S, Milton RD, Sigman MS, Minter SD, Barton SC. 2017 Substrate Channeling in an Artificial Metabolon: A Molecular Dynamics Blueprint for an Experimental Peptide Bridge. *Acs Catal*, 7, 2486–93
56. Magadam DB, Yadav GD. 2017 One-pot synthesis of (R)-1-(pyridin-4-yl)ethyl acetate using tandem catalyst prepared by co-immobilization of palladium and lipase on mesoporous foam: Optimization and kinetic modeling. *Chirality*, 29, 811–23 [PubMed: 28960471]
57. Makkee M, Kieboom APG, van Bekkum H. 1985 Combined action of an enzyme and a metal catalyst on the conversion of D-glucose/D-fructose mixtures into D-mannitol. *Carbohydr Res*, 138, 237–45
58. Makkee M, Kieboom APG, Van Bekkum H, Roels JA. 1980 Combined action of enzyme and metal catalyst, applied to the preparation of D-mannitol. *JCS Chem Comm*, 930–31
59. Marr AC, Liu S. 2011 Combining bio- and chemo-catalysis: from enzymes to cells, from petroleum to biomass. *Trends Biotechnol*, 29, 199–204 [PubMed: 21324540]
60. Martin-Matute B, Backvall JE. 2007 Dynamic kinetic resolution catalyzed by enzymes and metals. *Curr Opin Chem Biol*, 11, 226–32 [PubMed: 17349815]
61. Mukai C, Gao LZ, Nelson JL, Lata JP, Cohen R, Wu LR, Hinchman MM, Bergkvist M, Sherwood RW, Zhang S, Travis AJ. 2017 Biomimicry Promotes the Efficiency of a 10-Step Sequential Enzymatic Reaction on Nanoparticles, Converting Glucose to Lactate. *Angew Chem Int Edit*, 56, 235–38
62. Nielsen J, Keasling Jay D. 2016 Engineering Cellular Metabolism. *Cell*, 164, 1185–97 [PubMed: 26967285]
63. Obexer R, Godina A, Garrabou X, Mittl PR, Baker D, Griffiths AD, Hilvert D. 2017 Emergence of a catalytic tetrad during evolution of a highly active artificial aldolase. *Nat Chem*, 9, 50–56 [PubMed: 27995916]
64. Ogawa K, Nakajima-Kambe T, Nakahara T, Kokufuta E. 2002 Coimmobilization of Gluconolactonase with Glucose Oxidase for Improvement in Kinetic Property of Enzymatically Induced Volume Collapse in Ionic Gels. *Biomacromolecules*, 3, 625–31 [PubMed: 12005536]
65. Opgenorth PH, Korman TP, Bowie JU. 2014 A synthetic biochemistry molecular purge valve module that maintains redox balance. *Nature Commun*, 5, 4113 [PubMed: 24936528]

66. Oroz-Guinea I, Garcia-Junceda E. 2013 Enzyme catalysed tandem reactions. *Curr Opin Chem Biol*, 17, 1039–39
67. Pamies O, Backvall JE. 2003 Combination of enzymes and metal catalysts. A powerful approach in asymmetric catalysis. *Chem Rev*, 103, 3247–62 [PubMed: 12914497]
68. Peters C, Rudroff F, Mihovilovic MD, Bornscheuer UT. 2017 Fusion proteins of an enoate reductase and a Baeyer-Villiger monooxygenase facilitate the synthesis of chiral lactones. *Biol Chem*, 398, 31–37 [PubMed: 27289001]
69. Pluth MD, Bergman RG, Raymond KN. 2007 Acid Catalysis in Basic Solution: A Supramolecular Host Promotes Orthoformate Hydrolysis. *Science*, 316, 85–88 [PubMed: 17412953]
70. Proschel M, Detsch R, Boccaccini AR, Sonnewald U. 2015 Engineering of Metabolic Pathways by Artificial Enzyme Channels. *Front Bioeng Biotechnol*, 3, 168 [PubMed: 26557643]
71. Rajendran A, Nakata E, Nakano S, Morii T. 2017 Nucleic-Acid-Templated Enzyme Cascades. *Chembiochem*, 18, 696–716 [PubMed: 28150909]
72. Rollin JA, Martin del Campo J, Myung S, Sun F, You C, Bakovic A, Castro R, Chandrayan SK, Wu CH, Adams MW, Senger RS, Zhang YH. 2015 High-yield hydrogen production from biomass by in vitro metabolic engineering: Mixed sugars coutilization and kinetic modeling. *Proc Natl Acad Sci U S A*, 112, 4964–69 [PubMed: 25848015]
73. Rulli G, Duangdee N, Hummel W, Berkessel A, Gröger H. 2017 First Tandem-Type One-Pot Process Combining Asymmetric Organo- and Biocatalytic Reactions in Aqueous Media Exemplified for the Enantioselective and Diastereoselective Synthesis of 1,3-Diols. *European J Org Chem*, 2017, 812–17
74. San BH, Kim S, Moh SH, Lee H, Jung D-Y, Kim KK. 2011 Platinum Nanoparticles Encapsulated by Aminopeptidase: A Multifunctional Bioinorganic Nanohybrid Catalyst. *Angew Chem Int Ed*, 50, 11924–29
75. Sato H, Hummel W, Gröger H. 2015 Cooperative Catalysis of Noncompatible Catalysts through Compartmentalization: Wacker Oxidation and Enzymatic Reduction in a One-Pot Process in Aqueous Media. *Angew Chem Int Ed*, 54, 4488–92
76. Scalacci N, Black GW, Mattedi G, Brown NL, Turner NJ, Castagnolo D. 2017 Unveiling the Biocatalytic Aromatizing Activity of Monoamine Oxidases MAO-N and 6-HDNO: Development of Chemoenzymatic Cascades for the Synthesis of Pyrroles. *ACS Catal*, 7, 1295–300
77. Schnapperelle I, Hummel W, Groger H. 2012 Formal asymmetric hydration of non-activated alkenes in aqueous medium through a “chemoenzymatic catalytic system”. *Chemistry (Easton)*, 18, 1073–6
78. Schoevaart R, van Rantwijk F, Sheldon RA. 2000 A Four-Step Enzymatic Cascade for the One-Pot Synthesis of Non-natural Carbohydrates from Glycerol. *JOC*, 65, 6940–43
79. Schwander T, Schada von Borzyskowski L, Burgener S, Cortina NS, Erb TJ. 2016 A synthetic pathway for the fixation of carbon dioxide in vitro. *Science*, 354, 900–04 [PubMed: 27856910]
80. Sheldon RA, van Pelt S. 2013 Enzyme immobilisation in biocatalysis: why, what and how. *Chem Soc Rev*, 42, 6223–35 [PubMed: 23532151]
81. Simons C, Hanefeld U, Arends IWCE, Maschmeyer T, Sheldon RA. 2007 Towards catalytic cascade reactions: asymmetric synthesis using combined chemo-enzymatic catalysts. *Topics in Catalysis*, 40, 35–44
82. Srivastava P, Yang H, Ellis-Guardiola K, Lewis JC. 2015 Engineering a dirhodium artificial metalloenzyme for selective olefin cyclopropanation. *Nat Commun*, 6, 7789 [PubMed: 26206238]
83. Sugiyama K, Oki Y, Kawanishi S, Kato K, Ikawa T, Egi M, Akai S. 2016 Spatial effects of oxovanadium-immobilized mesoporous silica on racemization of alcohols and application in lipase-catalyzed dynamic kinetic resolution. *Catal Sci Technol*, 6, 5023–30
84. Tavanti M, Parmeggiani F, Castellanos JRG, Mattevi A, Turner NJ. 2017 One-Pot Biocatalytic Double Oxidation of α -Isophorone for the Synthesis of Ketoisophorone. *Chemcatchem*, 9, 3338–48
85. Tenbrink K, Seßler M, Schatz J, Gröger H. 2011 Combination of Olefin Metathesis and Enzymatic Ester Hydrolysis in Aqueous Media in a One-Pot Synthesis. *Adv Synth Catal*, 353, 2363–67

86. Uthoff F, Sato H, Gröger H. 2017 Formal Enantioselective Hydroamination of Non-Activated Alkenes: Transformation of Styrenes into Enantiomerically Pure 1-Phenylethylamines in Chemoenzymatic One-Pot Synthesis. *Chemcatchem*, 9, 555–58
87. Van Hecke W, Bhagwat A, Ludwig R, Dewulf J, Haltrich D, Van Langehove H. 2009 Kinetic Modeling of a Bi-Enzymatic System for Efficient Conversion of Lactose to Lactobionic Acid. *Biotechnol Bioeng*, 102, 1475–82 [PubMed: 18988269]
88. Velasco-Lozano S, López-Gallego F, Mateos-Díaz JC, Favela-Torres E. 2015 Cross-linked enzyme aggregates (CLEA) in enzyme improvement – a review. *Biocatalysis*, 166–177
89. Verho O, Bäckvall J-E. 2015 Chemoenzymatic Dynamic Kinetic Resolution: A Powerful Tool for the Preparation of Enantiomerically Pure Alcohols and Amines. *J Am Chem Soc*, 137, 3996–4009 [PubMed: 25730714]
90. Vougioukalakis G 2016 Recent Advances in Olefin Metathesis. *Molecules (Basel, Switzerland)*, 21, 1751
91. Wagner N, Bosshart A, Failmezger J, Bechtold M, Panke S. 2015 A Separation-Integrated Cascade Reaction to Overcome Thermodynamic Limitations in Rare-Sugar Synthesis. *Angew Chem Int Ed*, 54, 4182–86
92. Wang Y, Bartlett MJ, Denard CA, Hartwig JF, Zhao H. 2017 Combining Rh-Catalyzed Diazocoupling and Enzymatic Reduction To Efficiently Synthesize Enantioenriched 2-Substituted Succinate Derivatives. *ACS Catal*, 2548–52
93. Wang Y, Zhao H. 2016 Tandem Reactions Combining Biocatalysts and Chemical Catalysts for Asymmetric Synthesis. *Catalysts*, 6, 194
94. Wang ZJ, Clary KN, Bergman RG, Raymond KN, Toste FD. 2013 A supramolecular approach to combining enzymatic and transition metal catalysed. *Nat Chem*, 100, 3
95. Wiestner MJ, Ulmann PA, Mirkin CA. 2011 Enzyme Mimics Based Upon Supramolecular Coordination Chemistry. *Angew Chem Int Ed*, 50, 114–37
96. Xu Y, Wang M, Feng B, Li Z, Li Y, Li H, Li H. 2017 Dynamic kinetic resolution of aromatic sec-alcohols by using a heterogeneous palladium racemization catalyst and lipase. *Catal Sci Technol*, DOI: 10.1039/c7cy01954h
97. Yang M, Li J, Chen PR. 2014 Transition metal-mediated bioorthogonal protein chemistry in living cells. *Chem Soc Rev*, 43, 6511–26 [PubMed: 24867400]
98. Yoshizawa M, Tamura M, Fujita M, Fujita M. 2006 Diels-alder in aqueous molecular hosts: unusual regioselectivity and efficient catalysis. *Science*, 312, 251–54 [PubMed: 16614218]
99. You C, Zhang YHP. 2013 Self-Assembly of Synthetic Metabolons through Synthetic Protein Scaffolds: One-Step Purification, Co-immobilization, and Substrate Channeling. *ACS Synth Biol*, 2, 102–10 [PubMed: 23656373]
100. You C, Zhang YHP. 2014 Annexation of a High-Activity Enzyme in a Synthetic Three-Enzyme Complex Greatly Decreases the Degree of Substrate Channeling. *ACS Synth Biol*, 3, 380–86 [PubMed: 24283966]
101. Yu K, Liu CC, Kim BG, Lee DY. 2015 Synthetic fusion protein design and applications. *Biotechnol Adv*, 33, 155–64 [PubMed: 25450191]
102. Zhang X, Jing L, Chang F, Chen S, Yang H, Yang Q. 2017 Positional immobilization of Pd nanoparticles and enzymes in hierarchical yolk-shell@shell nanoreactors for tandem catalysis. *Chem Commun*, 53, 7780–83
103. Zhang YH. 2015 Production of biofuels and biochemicals by in vitro synthetic biosystems: Opportunities and challenges. *Biotechnol Adv*, 33, 1467–83 [PubMed: 25447781]

Figure 1:
Synthesis of γ -nitroketones by aldolase variants (Garrahou et al., 2017).

Figure 2:

The CETCH cycle contains 17 enzymes from 9 different organisms, which can convert CO₂ into organic molecules at a rate of 5 nanomoles of CO₂ per minute per milligram of protein (Schwander et al., 2016). Ccr: crotonyl-CoA carboxylase/reductase; Ecm: ethylmalonyl-CoA mutase; Epi: ethylmalonyl-CoA/methylmalonyl-CoA epimerase; Fdh: formate dehydrogenase; Hbd: 4-hydroxybutyryl-CoA dehydratase; Hbs: 4-hydroxybutyryl-CoA synthetase; Kat: katalase; Mas: malate synthase; Mch: mesaconyl-CoA hydratase; Mcl: β-methylmalyl-CoA lyase; Mcm: methylmalonyl-CoA mutase; Mco: methylsuccinyl-CoA oxidase; Mct: malyl-CoA thioesterase; Pco: propionyl-CoA oxidase; Pkk: polyphosphate kinase; Scr: succinyl-CoA reductase; Ssr: succinic semialdehyde reductase.

Reaction	2 [mM]	3 [mM]	4 [mM]	4 Yield %	Yield increase ^b
tandem	9.5	50.1	27.3	27.3	1.5
metathesis ^a	18.0	77	-	-	-

^ametathesis without P450, ^b4/2 in the metathesis reaction alone

Figure 3.

Concurrent olefin cross-metathesis/epoxidation of 10-undecenoic acid with *trans*-3-hexene.

Figure 4.

A, Esterase- or lipase-mediated acetate hydrolysis followed by Au (I)-catalyzed hydroalkoxylation. B, Ru(II)-mediated olefin isomerization followed by ADH-catalyzed reduction (Wang et al., 2013).

Figure 5.

A, (*S*)-selective monoamine oxidase (MAO-N) was coupled with (*R*)-selective ATHase for double stereoselective deracemization of amines. B, Production of L-pipecolic acid from L-lysine by combining (*S*)-selective ATHase with an L-amino acid oxidase (LAAO) and a D-selective amino acid oxidase (DAAO). Catalase was used to decompose H₂O₂ generated in both case (Kohler et al., 2013).

Figure 6. Site-isolation of catalysts using a PDMS thimble for the combination of a Wacker oxidation and an enzymatic reduction catalyzed by ADH or transamination catalyzed by transaminase (Sato et al., 2015).

Figure 7. The cyclic deracemization of MTO through MDQ using palladized biocatalysts with the monoamine oxidase-N insert (Foulkes et al., 2011).

Figure 8.
DKR of amine with a bifunctional catalyst in which Pd nanoparticles and CALB are co-immobilized in the same pore of siliceous mesocellular foams (Engström et al., 2013).

Scheme 1.

Dynamic kinetic resolution of amines or alcohols by coupling CALB or CALA in tandem with racemization catalysts

Table 1.

Typical one-pot tandem chemo-enzymatic reactions in aqueous solution

Organometal Catalysts	Comments	Enzymatic Reaction	Reaction Type	Product	Citation
Pt (hydrogenation)	HE/Whole cell	D-glucose isomerase/ monoamine oxidase	concurrent/ cooperative	D-mannitol/ Chiral quinoline	(Foulkes et al., 2011; Makkee et al., 1985; Makkee et al., 1980)
Rh (hydrogenation)	HE	hydrolase	sequential	L-alanine	(Simons et al., 2007)
Pt (racemization)	HO	hydrolase	DKR	Enantiopure allyl alcohol	(Allen et al., 1996)
Pyridoxal 5-phosphate (racemization)	HO	hydrolase	DKR	L-amino acid	(Chen et al., 1994)
Fe (Hydrogenation- racemization)	HO	lipase (acylation)	cooperative	Enantiopure secondary alcohol	(El-Sepelgy et al., 2017)
Pd/Cu (Wacker oxidation)	HO/Compat.	(R) - ADH (ketone reduction)	sequential/ concurrent	Chiral alcohols	(Sato et al., 2015; Schnapperelle et al., 2012)
Pd/Cu (Wacker oxidation)	HO/Compat.	transaminase (asymmetric reductive amination)	concurrent	Chiral amines	(Uthoff et al., 2017)
Ru (metathesis)	HO/Compat.	decarboxylase (Decarboxylation)	concurrent	Antioxidant 4,4'- dihydroxylstilbene derivatives	(Gómez Baraibar et al., 2016)
Ru (metathesis)	HO	esterase (Hydrolysis)	sequential	Cyclic malonic acid monoesters	(Tenbrink et al., 2011)
Ru (metathesis)	HO/biphasic	P450 (epoxidation)	cooperative /sequential	Epoxide	(Denard et al., 2015; Denard et al., 2014)
Ru (metathesis)	HC	MAO-N (Aromatizing)	concurrent	Pyrrolines	(Sealacci et al., 2017)
Pd (Suzuki-coupling)	HO	ADH (Ketone reduction)	Concurrent /sequential	Chiral biaryl alcohol	(Burda et al., 2008; Gauchot et al., 2010)
Ir (Barbier-type coupling)	HO	galactose (alcohol oxidation)	sequential	Homoallylic sec- alcohols	(Fuchs et al., 2011)
Pd (Heck coupling)	HE	(R)-ADH (Ketone reduction)	sequential	4-(4-Methoxyphenyl)- butan-2-one	(Boffi et al., 2011)
Rh (diazo-coupling)	HO	ene-reductase (reduction)	sequential	Chiral 2-aryl-substitued succinate derivatives	(Wang et al., 2017)
Au (cycloisomerization)	HO	lipase (hydrolysis)	concurrent	2,5-dihydrofurans	(Asikainen et al., 2009)
Au (hydroalkyloxylation) Ru (olefin isomerization)	Supramol.	esterase/lipase/ADH	concurrent	Substituted tetrahydrofuran /propanal	(Wang et al., 2013)
Organocatalytic aldol reaction	HO	ADH	Concurrent	1,3-Diols	(Rulli et al., 2017)
Potassium phosphate (cyclization)	HO	carboligase/transaminase	Sequential	Trisubstituted tetrahydroisoquinolines	(Erdmann et al., 2017)

Abbreviation: HO: homogeneous catalysts, HE: heterogeneous catalysts, Compat.: compartmentalization, Supramol. Supramolecular encapsulation, HC: whole cell.

Table 2.

The novel strategies to couple transition metals (or chemical catalysts) with enzymes in one-pot tandem reactions.

Strategy	Advantages	Tandem Reactions
Supramolecular Assembly (Artificial metalloenzyme)	<ul style="list-style-type: none"> • realize catalytic activity of metal catalysts in aqueous solution • stabilize metal complexes and improve turn over number (TON) • prevent interaction of metal complexes with enzymes • provide extra selectivity of metal catalysts 	<ul style="list-style-type: none"> • Au or Ru in tandem with esterase, lipase, ADH; • Ir in tandem with oxidoreductases
Compartmentalization	<ul style="list-style-type: none"> • protect enzyme from organic solvent • protect metal catalysts from aqueous solution • prevent mutual inactivation 	<ul style="list-style-type: none"> • Ru in tandem with decarboxylase; • proline in tandem with ADH; • Wacker oxidation in tandem with enzymatic reduction or hydroamination
Whole-cell	<ul style="list-style-type: none"> • protect biocatalysts from metal catalysts • prevent interaction of metal with cellular components • avoid protein purification 	<ul style="list-style-type: none"> • Pd(0)-catalyzed reduction in tandem with monoamine oxidation
Co-immobilization	<ul style="list-style-type: none"> • stabilize metal catalysts • improved synergic effect • catalyst regeneration 	<ul style="list-style-type: none"> • CALB and Pd nanoparticles co-immobilized into the mesoporous silica for DKR of amine