(Chemo)enzymatic cascades – Nature's synthetic strategy transferred to the laboratory

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Abstract: The astonishing efficiency with which living organisms build complex molecules from abundant starting materials has inspired chemists for centuries. Among the synthetic strategies that nature uses to achieve this efficiency, the combination of several enzymatic transformations in cascading sequences is of outstanding importance. With the rise of biocatalysis, researchers now have the tools at hand to mimic this strategy and develop artificial enzyme cascades of impressive complexity. This editorial review aims to introduce the reader to some key aspects of *(Chemo)enzymatic Cascades*, as well as to put the submissions to the present Special Issue into a broader perspective.

Keywords: Cascade reaction, tandem reaction, domino reaction, biocatalysis, synthetic efficiency

Highlights:

- Cascade sequences of biotransformations are a key aspect of nature's synthetic strategy.
- Multi-enzymatic and chemo-enzymatic cascades attract increasing interest in biocatalysis.
- The term 'cascade' is used in a very broad sense for a variety of biocatalytic one-pot systems, which can, however, be classified into a limited number of general designs.
- The contributions to this Special Issue cover many aspects of the topic, and illustrate many of the advantages of (Chemo)enzymatic Cascades.

1 Introduction: Nature's synthetic strategy

The most efficient chemical factories known are the living organisms. Their exquisite efficiency is due to the *synthetic strategy* — in the sense that Nicolaou gave to this expression[1] — that evolution has optimised over time. This *natural synthetic strategy* is based on three key aspects: (*i*) the use of enzymes as catalysts; (*ii*) compartmentalisation of enzymes or even entire biosynthesis routes in cellular organelles or compartments; and (*iii*) the sequential use of these catalysts in cascade reactions (biosynthetic pathways). Biocatalysis, to reach its objective of transferring the exquisite synthetic efficacy that enzymes show in nature to the laboratory, had to mimic this approach.

Much has already been said about the advantages offered by enzymes as catalysts and about the role of recombinant DNA techniques, especially PCR, in facilitating the production of many enzymes that were previously difficult to access, and that are capable of catalysing a myriad of chemical reactions.[2-4] In addition, these techniques have allowed the development of strategies to engineer aspects of enzymatic catalysis that are of main importance, such as substrate specificity, regio- and enantioselectivity, stability, *etc*.[4-9] Compartmentalisation into different organelles as observed in living beings is mimicked in the field of biocatalysis by different immobilisation strategies (physical confinement of an enzyme or several enzymes in a given region of space), such as microencapsulation, or entrapment in liposomes or hollow fibers, among other examples.[10-11]

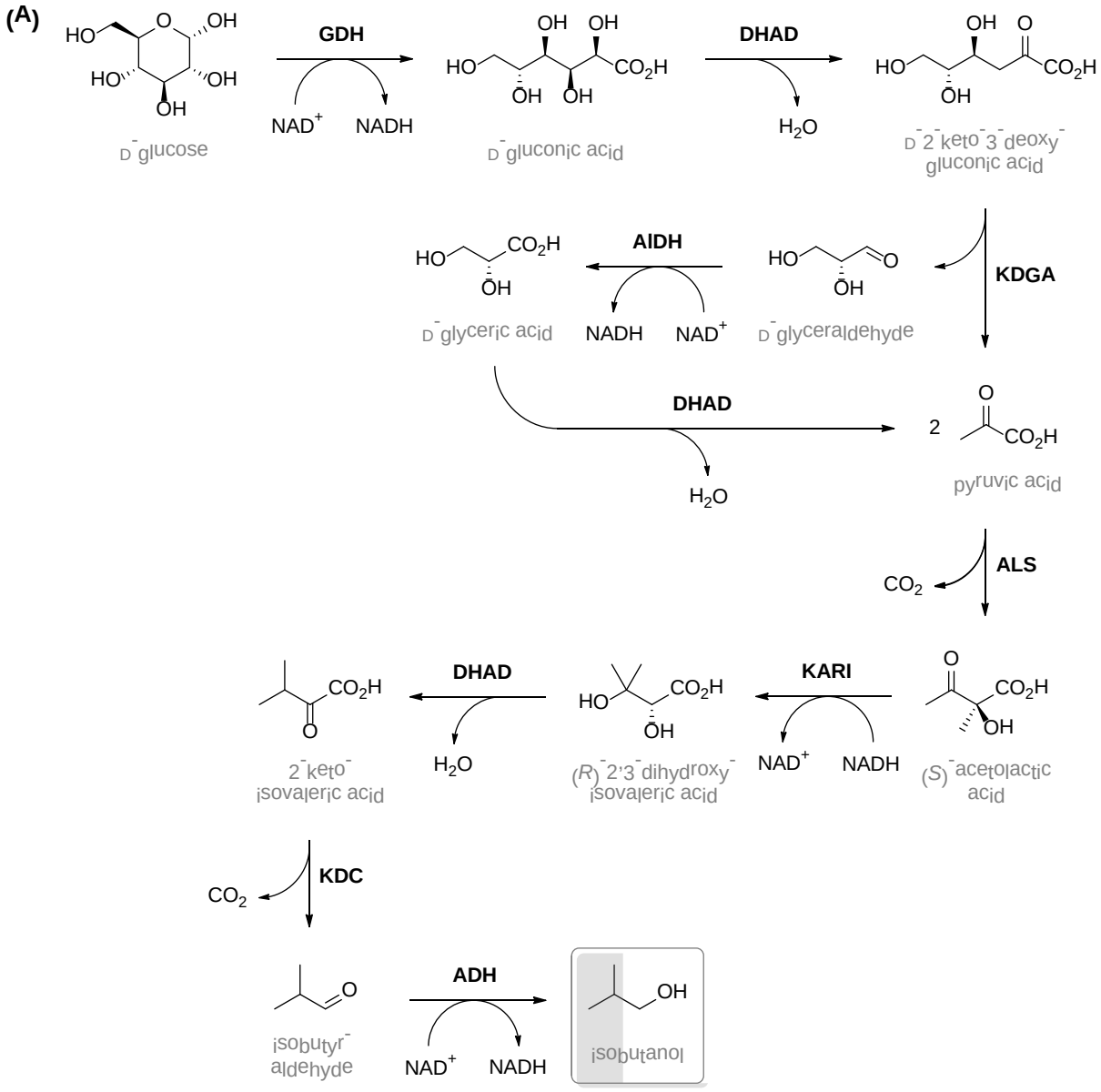
The third cornerstone of the *natural synthetic strategy* is the use of enzymes acting sequentially in (sometimes very complicated) biosynthetic routes. This joint, cascading action of a sequence of enzymes allows living organisms to build complex structures from simple starting materials, to obtain and to store energy, and to explore and to communicate with their environment. During the course of evolution, complex metabolic networks to perform thousands of different chemical reactions that take place inside a cell have been developed. In these processes the product of one enzyme is the substrate for the next one, allowing rapid complexity generation, the shifting of reaction equilibria, the elimination of inhibition problems caused by product excess, or the channelling of substrates from one active site to another. In the field of biocatalysis, multi-enzyme cascades may be implemented *via* the use of isolated multi-enzyme systems in homogeneous phase to create artificial pathways (also called *artificial metabolisms*),[12] which offer essentially the same advantages. In nature, some multi-enzymatic routes have been optimised throughout evolution by joining two or more enzymes in a single protein, giving rise to the appearance of multifunctional enzymes or enzyme complexes. This approach can also be mimicked by biocatalysis, for instance by crosslinking several proteins using bifunctional reagents, by fusion of genes encoding different enzymes or by modular assembling of proteins on synthetic scaffolds.[13-17]

Over the course of the last decade, biocatalytic cascade reactions have grown into an intensively investigated research area, as evidenced by more than 100 articles published on this topic in the last five years,[18-23] and by the organisation of a series of international conferences dedicated to this subject. The *Multistep Enzyme-Catalyzed Processes* (MECP) conference series was organised under the auspices of the European Section on Applied Biocatalysis (ESAB) and promoted, among others, by Professor Herfried Griengl, who chaired the first MECP conference held in Graz in April 2006. It was necessary to wait six years, until April 2012, to celebrate the second MECP conference, which was also held in Graz, co-organized by the University of Graz, the Graz University of Technology, and the Austrian Centre of Industrial Biotechnology (ACIB). However, due to rapid growth in this field it was only two years later that the most recent edition of the series, MECP14, was held in Madrid, co-organized by the Spanish National Research Council (CSIC) and the Spanish Society of Biotechnology (SEBiot).

It was during the organisation phase of MECP14 when the idea to edit this special issue of the *Journal of Molecular Catalysis B: Enzymatic* devoted to **(Chemo)Enzymatic Cascade Reactions** emerged and crystallised and therefore the Guest Editors are indebted to this conference.

2 Biocatalytic cascades: definitions and classifications

The rapid growth of biocatalytic cascade reactions as a research topic has led to the development of a broad variety of cascade systems that adopt different key aspects of nature's synthetic strategy. The majority of the reported reaction systems comprise several enzymes working together concurrently in the same reaction vessel. However, multi-enzyme systems to which the involved biocatalysts are added sequentially at different time points, and sequences of spontaneous reactions that are merely initiated by an enzymatic transformation are commonly referred to as cascades as well. Indeed, recent literature examples of biocatalytic cascades (as categorised by the authors) range from complex multi-enzyme networks that combine ten or more individual biotransformations,[24-25] to simple transamination reactions that are rendered irreversible *via* the spontaneous aromatisation of the reaction co-product (Scheme 1).[26]



Scheme 1. Two literature examples illustrating the diverse use of the term 'biocatalytic cascade': **(A)** Conversion of D-glucose into isobutanol by an eight-enzyme, ten-step reaction sequence; **(B)** conversion of acetophenone into (*S*)-1-phenylethylamine using an amino donor that shifts the reaction equilibrium by spontaneous aromatisation. Both systems have been described as 'biocatalytic cascade' by the authors in their respective publications. Enzyme acronyms: GDH, glucose dehydrogenase; DHAD, dihydroxy acid dehydratase; KDGA, 2-keto-3-deoxygluconate aldolase; AlDH, glyceraldehyde dehydrogenase; ALS, acetolactate synthase; KARI, ketol-acid reductoisomerase; KDC, 2-ketoacid decarboxylase; ADH, alcohol dehydrogenase; ω-TA, ω-transaminase.

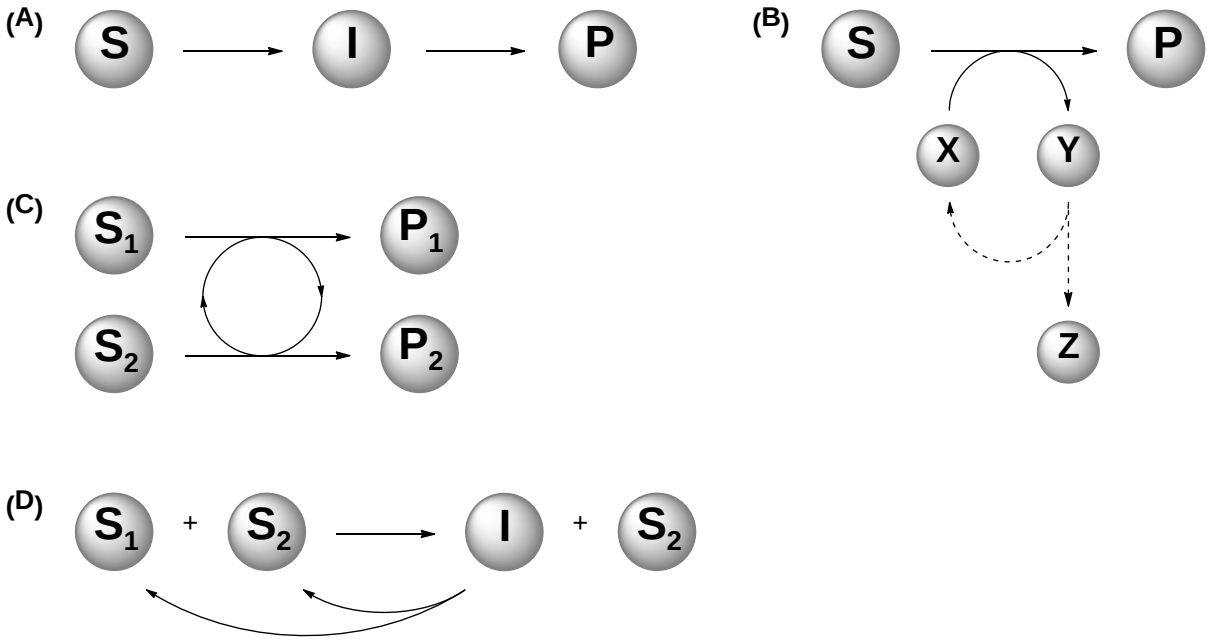
This tendency towards terminological generalisation has a simple reason: There is just no commonly accepted definition of the term 'cascade reaction', or of related descriptors such as 'domino' or 'tandem' – neither in the context of the chemical sciences in general, nor within the particular field of biocatalysis. Still, some authors have tried to bring order to this area of reaction terminology by proposing clearly distinct meanings for the three above-mentioned terms. For instance, in a review article published in 2001, Faber and coworkers distinguish between biocatalytic 'domino' (or 'cascade') systems as spontaneous sequences that proceed *via* highly reactive intermediates and are only initiated by an enzyme, and 'tandem' systems, which combine several enzymatic transformations that in principle could also be carried out separately.[27] This is a sensible differentiation, especially in view of the classical definition of domino reactions put forth by Tietze,[28] and of the now widely accepted term 'tandem catalysis' for one-pot combinations of several catalysts.[29-30] However, many authors still use the three terms rather interchangeably, and particularly within the biocatalysis research community, 'cascade' seems to have become the descriptor of choice for almost any type of one-pot process.

For the purpose of this Special Issue, we have chosen to follow the trend apparent in the recent literature and adopt a fairly broad conception of (chemo)enzymatic cascades – one that includes all concurrent biocatalytic one-pot systems, irrespective of whether they combine several enzymes, enzymes and chemocatalysts, or enzymes and spontaneous (non-catalysed) transformations. We believe that such a broad use of the term is warranted not only by the according example of other researchers in the field, but also by the general characteristics that these systems have in common.

All one-pot systems, irrespective of whether the involved reactions take place concurrently or in temporally separated fashion, share the obvious advantages arising from the elimination of intermediate work-up and purification steps: operational simplicity combined with a reduced consumption of chemicals (particularly solvents), energy, space, and time.[31] Cascades as concurrent one-pot sequences offer additional benefits that result from the immediate succession of the individual transformations. For instance, toxic or unstable intermediates may be consumed in the same instant as they appear, which leads to safer processes and prevents undesired side reactions. Likewise, reversible reactions may be driven to completion by a thermodynamically favourable follow-up reaction.

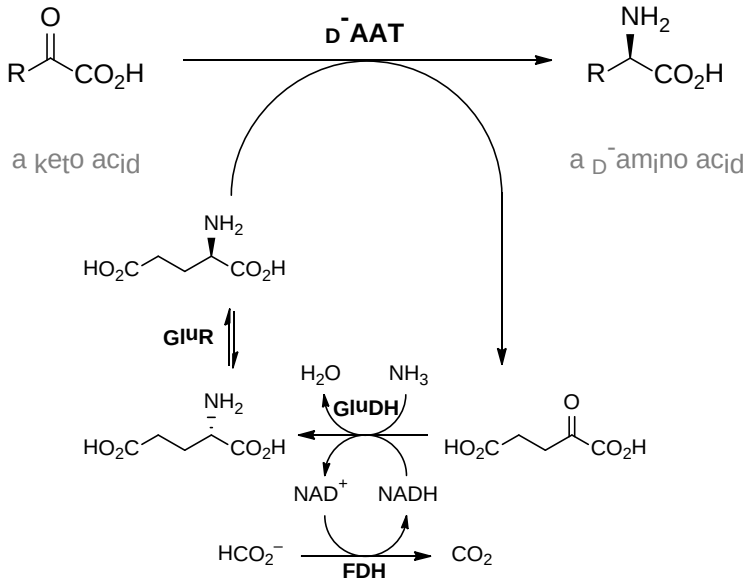
Creative cascade design is crucial for capitalising on these potential benefits, and biocatalysis offers ample possibilities in this regard: In nature, enzymes operate under a rather narrow range of conditions (compared to man-made catalysts), which often results in overlapping reaction optima and hence high compatibility. Moreover, many enzymatic transformations depend on the presence of a cofactor – a circumstance that is traditionally seen as a challenge for application, but may also represent an additional opportunity for coupling several reactions in a cascade system.[32] Consequently, biocatalytic reactions have been combined in many different ways, and for many different purposes. Nonetheless, most published cascade systems can be assigned to one out of four designs with their distinct characteristics and advantages.[21]

The most straightforward design is a linear sequence of transformations, which convert a single substrate into a single product *via* one or more intermediates (Scheme 2, A). Enzyme-initiated spontaneous domino reactions fall into this category, as do many multi-enzymatic and chemo-enzymatic reaction systems. Indeed, even dynamic kinetic resolution (DKR) processes can be viewed as a special type of linear cascade, in which the (reversible) interconversion of substrate enantiomers is coupled to the (irreversible) transformation of preferably only one enantiomer into a product that is stereochemically stable under the reaction conditions. The displacement of unfavourable equilibria and the *in situ* generation of toxic or unstable intermediates are common benefits of linear designs.



Scheme 2. General cascade designs: **(A)** Linear cascade, **(B)** orthogonal cascade, **(C)** parallel cascade, and **(D)** cyclic cascade.

Orthogonal designs, in contrast, comprise only a single synthetically relevant transformation, which is coupled to 'auxiliary' reactions that promote the formation of the desired product; for instance, by the regeneration of cofactors or cosubstrates, or by the removal of troublesome coproducts (Scheme 2, B). Whether straightforward cofactor regeneration systems do already constitute biocatalytic cascades may be questionable, but there are literature examples of auxiliary reaction setups that do undoubtedly deserve to be termed orthogonal cascades. A classic case is Soda's system for the production of D-amino acids from α-keto acids catalysed by a D-amino acid transaminase in combination with glutamate racemase, L-glutamate dehydrogenase and formate dehydrogenase for regeneration of the cosubstrate D-glutamate (Scheme 3).[33]



Scheme 3. A classic example of an orthogonal cascade design: A D-amino acid transaminase is combined with three auxiliary enzymes to realise a formal D-selective reductive amination of keto acids using ammonium formate as sole stoichiometric reagent. Enzyme acronyms: D-AAT, D-amino acid transaminase; GluR, glutamate racemase; GluDH, L-glutamate dehydrogenase; FDH, formate dehydrogenase.

In parallel cascade designs, the problem of cofactor regeneration is addressed in a different way: Instead of coupling the desired transformation to a synthetically irrelevant auxiliary reaction, it is combined with a second reaction of synthetic interest and matching cofactor demand (Scheme 2, C). For instance, the kinetic resolution of a chiral alcohol catalysed by an alcohol dehydrogenase (ADH) may be coupled to the asymmetric reduction of a prochiral ketone by the same enzyme, resulting in a closed redox cycle.[34] While parallel designs ensure efficient use of the involved cofactors or cosubstrates, they suffer from the disadvantage of affording several products, which require separation. The benefits of the simple reaction setup might hence be outweighed by issues of downstream processing.

Cyclic cascade designs are also possible, but are limited to special applications. The most common examples are deracemisation systems for amines, α-amino acids, *sec*-alcohols, or α-hydroxy acids that combine enantioselective oxidation with non-stereoselective reduction. In these systems, one enantiomer of the racemic substrate is selectively oxidised to a prochiral analogue (an imine, α-imino acid, ketone, or α-keto acid, respectively), which in turn is reduced back to the racemic starting material (Scheme 2, D). Over several cycles of this redox cascade, the more slowly oxidised enantiomer accumulates. Until recently, cyclic cascades of this type have been limited to chemo-enzymatic systems, usually combining an oxidase with ammonia-borane as reducing agent. Only in 2013, Ward, Turner, Hollmann, and co-workers have demonstrated that the combination of a monoamine oxidase and an artificial transfer hydrogenase (*i.e.*, a biotinylated iridium complex bound to streptavidin) can effect the same transformations with high efficiency and minimal interference of the catalysts.[35]

Overall, the four main types of cascade designs provide only a loose framework for the classification of enzymatic cascade processes. Combinations of these approaches are possible and may further increase the efficiency of a multi-enzyme system. For instance, the main ideas of linear and parallel designs (implementation of a one-pot sequence of reactions, and internal recycling of cofactors, respectively) can be combined in redox-neutral linear cascades, where the first transformation provides the cofactor for the second, and *vice versa*.[36] Likewise, a cyclic element can be introduced into a linear sequence if a coproduct of the second step is at the same time a substrate for the first (an approach called 'regeneration cascade' by Rother and co-workers).[37] Moreover, any type of biocatalytic cascade can be practically implemented in a variety of ways: the involved enzymes may be simply combined in solution, they may be coexpressed in the same microbial cell (or expressed separately in different hosts, which are later combined), they may be co-localised *via* immobilisation or encapsulation, or kept at distance from each other *via* compartmentalisation, depending on what suits the investigated reaction system best. The possibilities for the creative design of biocatalytic cascades are therefore virtually limitless, and we hope that the examples collected in this Special Issue will help to illustrate the broad scope of options.

3 Examples of cascades in this Special Issue

In the present issue we have tried to cover some areas related to (chemo)enzymatic cascade protocols through two review and ten article contributions made by leading authors in the field. Thus, due to the relevance of enantiomerically pure 2-hydroxycarboxylic acid derivatives, **van Rantwijk & Stolz** [38] summarise the recent development of enzymatic cascades to obtain these compounds by combination of hydroxynitrile lyases together with nonselective nitrile hydratases or nitrilases to obtain the corresponding 2-hydroxyamides and 2-hydroxycarboxylic acids, respectively. The authors focus especially on the effects of different enzyme preparations (*e.g.*, using whole cell systems or cross-linked enzyme aggregates), and on the use of directed mutagenesis of the nitrilase,

providing a selective biocatalyst applied to these processes. **Sehl, Maugeri & Rother** [39] cover recent examples of asymmetric multi-step synthetic routes to obtain vicinal amino alcohols, molecules with wide applications in chemical industry as precursors of various drugs. They highlight some recently developed chemoenzymatic protocols especially focusing on phenylpropanolamines such as norpseudoephedrine and norephedrine. Among the different enzymatic strategies developed, the combination of transaminases with carboligases or alcohol dehydrogenases has gained more relevance in the last years to synthesise these compounds.

As already mentioned above, one of the most prominent advantages related to the implementation of multi-step one-pot transformations is their step economy. Carrying out several reactions in direct succession without purification of intermediates helps to maximise the overall yield and minimise the use of reagents, catalysts and solvents. Various contributions to this issue demonstrate this point: **Romano *et al.*** [40] have designed a new chemoenzymatic synthesis of precursors to Bimatoprost and Latanoprost, two prostaglandin analogues employed for glaucoma treatment, by using *Pichia anomala* yeast containing three reacting enzymes (an esterase, an alcohol dehydrogenase and an enoate reductase). Thus, these biocatalysts are responsible of an ester hydrolysis, a stereoselective ketone reduction and a carbon-carbon double bond reduction, respectively. Moreover, by modifying the reaction medium it is possible to suppress the C=C reduction activity, giving access to an unsaturated building block. **Kurina-Sanz *et al.*** [41] have shown a one-pot two-step protocol to get access to several epoxides and diols starting from the corresponding α-chloro or α-bromoacetophenone derivatives. After stereoselective bioreduction employing resting or lyophilised *Rhodotorula* sp. LSL yeast cells in plain water without the addition of an external cofactor or a hydrogen donor, the cyclisation of the corresponding enantioenriched (*R*)-halohydrins is achieved under alkaline conditions. **Brenna *et al.*** [42] have demonstrated the application of two isolated enzymes, an enoate reductase from *Saccharomyces cerevisiae* and an alcohol dehydrogenase from *Rhodococcus erythropolis* or from *Parvibaculum lavamentivorans*, to obtain the most odorous stereoisomers of the commercial fragrance Muguesia®. Thus, starting from a prochiral α,β-unsaturated ketone precursor, both diastereomers are attained in high yields and selectivities. In a similar scheme, **Gatti *et al.*** [43] show the application of an enoate reductase from *Saccharomyces cerevisiae* and an alcohol dehydrogenase from *Rhodococcus erythropolis* or from a commercial source, in order to give access to the four possible stereoisomers of a β-substituted γ-hydroxy ester precursor of *Nicotiana tabacum* lactone, a compound with great potential in the fragrance field. After biotransformation using both enzymes, a final cyclisation step in acidic conditions is performed to access all diastereomers in high yields and excellent enantio- and diastereoselectivities.

Multi-step protocols can also be useful for *in situ* generation of chemical species that are unstable or that can be harmful to the (bio)catalyst, thus being continuously provided at low concentrations to immediately react within a second substrate. **Gotor, Gotor-Fernández and co-workers** [44] describe a one-pot chemoenzymatic methodology to synthesise a series of γ-butyrolactone derivatives from the corresponding 3-substituted cyclobutanones *via* Baeyer–Villiger oxidation. Peracetic acid, which acts as the oxidising species, is formed *in situ* by lipase-catalysed perhydrolysis of ethyl acetate in the presence of the urea–hydrogen peroxide complex, thereby minimising its poisoning effect on the biocatalyst. After reaction optimisation, lipase B from *Candida antarctica* was selected as the best candidate, and the target lactones were isolated in high yields after a simple extraction protocol. Using a similar approach, **Wiemann *et al.*** [45] demonstrate the epoxidation of limonene in good yield through perhydrolysis of ethyl acetate catalysed by lipase B from *Candida antarctica*. In this case the formation of hydrogen peroxide is attained by a two-step cascade process: reduction of anthraquinone into anthrahydroquinone using hydrogen and a palladium catalyst, followed by auto-oxidation yielding equimolar amounts of H_2O_2.

Another advantage derived from cascade methods can be the generation in the reaction medium of chemical species that are expensive and that can be (enzymatically) obtained from a cheaper source. **Lemaire *et al.*** [46] describe different one-pot multi-step stereoselective cascade reactions applied to the straightforward synthesis of various nitrocyclitols. In these systems, two kinases (acetate kinase and dihydroxyacetone kinase) are used to obtain dihydroxyacetone phosphate from dihydroxyacetone, which subsequently reacts with a nitroaldehyde in the presence of an aldolase (L-fuculose-1-phosphate aldolase or L-rhamnulose-1-phosphate aldolase), responsible for the selective C–C bond formation. Then, a spontaneous intramolecular Henry reaction provides the nitrocyclitol moiety with high diastereoselectivity. Finally, a phosphatase (acid phosphatase from wheat germ) is employed to dephosphorylate the final compounds. The stereoselectivities displayed by the aldolases vary depending on the aldehyde used, and the configuration of the aldol adduct has a profound influence on the stereochemical outcome of the Henry reaction. **Lewkowicz *et al.*** [47] propose several multi-step enzymatic systems to synthesise pyrimidine nucleoside 5'-diphosphates starting from readily accessible reagents. To access uridine 5'-diphosphate, a simple methodology is used involving commercial yeast extract as biocatalyst and uridine 5'-monophosphate, prepared employing whole cells from *Corynebacterium ammoniagenes* in the presence of uracil and orotic acid, or *Raoultella planticola* from uridine. For thymidine 5'-diphosphate synthesis, two one-pot multi-step systems are used sequentially. 2'-Deoxyribose-5-phosphate is formed from glucose by *Erwinia carotovora* whole cells, followed by the action of phosphopentomutase and thymidine phosphorylase, affording thymidine in high yield. Then, this nucleoside is converted into the final product combining *E. coli* BL21 *pET22b-phoRp* (a strain expressing an acid phosphatase from *R. planticola*) and *S. cerevisiae*.

Dynamic protocols are widely employed in recent years as an efficient way to obtain enantiomerically pure products at high extent from easily available racemates. In these concurrent processes, the combination of several catalysts can improve the yield of the desired compound by recycling the non-reacting enantiomer *via* racemisation. **Ansorge-Schumacher, Kara and co-workers** [48] have studied the dynamic kinetic resolution of benzoin combining a lipase from *Pseudomonas stutzeri* with a heterogenous chemocatalyst (Zr-TUD-1) used as the racemising agent. Especial attention is set on the effect of water activity and the reaction solvent employed. Hence, different co-solvents are studied, including deep eutectic solvents. After optimisation, cyclopentyl methyl ether is selected as the best candidate, and the process is run in a continuous fashion in a stirred tank reactor to synthesise the (*S*)-benzoin ester in enantiopure form.

In the last years, multi-step systems are also being used as sensitive assays for determining a specific target. **Charmantray *et al.*** [49] have developed a fluorometric assay for the determination of dihydroxyacetone phosphate (DHAP). This compound is reduced to L-glycerol-3-phosphate with α-glycerophosphate dehydrogenase, and is recycled *via* the oxidation reaction catalysed by α-glycerophosphate oxidase, releasing hydrogen peroxide. Then, the reaction of H_2O_2 with Amplex® Red reagent in the presence of horseradish peroxidase leads to the fluorescent product resorufin. The limit of quantification of DHAP has been estimated at 1 pmol (5 nM), roughly 2250-fold more sensitive than the usual assay based on spectrophotometric detection.

4 Conclusions

Inspired by nature's biosynthetic pathways, scientists have developed a vast variety of reaction systems in which enzymatic transformations are coupled to each other, or to chemo-catalytic or even spontaneous reactions. As diverse as these systems can be, they are usually all subsumed under the term "cascade", which therefore has acquired a very generic meaning. In the present Special Issue of

the *Journal of Molecular Catalysis B: Enzymatic* we have tried to cover the topic in all its breadth: from the simple biocatalytic formation of a reactive species to complex multi-enzyme systems, from continuously operated preparative transformations to a fast and highly sensitive small-scale assay. The examples collected in this issue show the enormous potential offered by (chemo)enzymatic cascade reactions, demonstrating many advantages over conventional sequential reaction schemes in terms of productivity, selectivity, and cost as well as environmental efficiency. We are convinced that the ongoing efforts in this research area will bring us closer to achieving the goal of transferring nature's synthetic efficiency to the laboratory.

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References

[1] K. C. Nicolaou, J. S. Chen, Chem. Soc. Rev. 38 (2009), 2993–3009.
[2] A. S. Bommarius, B. R. Riebel-Bommarius, Biocatalysis: Fundamentals and Applications, Wiley-VCH, Weinheim, 2004.
[3] K. Faber, Biotransformations in Organic Chemistry – A Textbook, 6th ed., Springer, Heidelberg, 2011.
[4] M. T. Reetz, J. Am. Chem. Soc. 135 (2013), 12480–12496.
[5] E. García-Junceda, J. F. García-García, A. Bastida, A. Fernández-Mayoralas, Bioorg. Med. Chem. 12 (2004), 1817–1834.
[6] D. Böttcher, U. T. Bornscheuer, Current Opinion in Microbiology 13 (2010), 274–282.
[7] H. Kries, R. Blomberg, D. Hilvert, Curr. Opin. Chem. Biol. 17 (2013), 221–228.
[8] Y. Li, P. C. Cirino, Biotechnol. Bioeng. 111 (2014), 1273–1287.
[9] U. T. Bornscheuer, G. W. Huisman, R. J. Kazlauskas, S. Lutz, J. C. Moore, K. Robins, Nature 485 (2012), 185–194.
[10] R. A. Sheldon, S. van Pelt, Chem. Soc. Rev. 42 (2013), 6223–6235.
[11] D. P. Patterson, B. Schwarz, R. S. Waters, T. Gedeon, T. Douglas, ACS Chem. Biol. 9 (2013), 359–365.
[12] W.-D. Fessner, C. Walter, Angew. Chem. Int. Ed. 31 (1992), 614–616.
[13] D. E. Torres Pazmiño, R. Snajdrova, B.-J. Baas, M. Ghobrial, M. D. Mihovilovic, M. W. Fraaije, Angew. Chem. Int. Ed. 47 (2008), 2275–2278.
[14] L. Iturrate, I. Sánchez-Moreno, I. Oroz-Guinea, J. Pérez-Gil, E. García-Junceda, Chem. Eur. J. 16 (2010), 4018–4030.
[15] J. E. Hudak, R. M. Barfield, G. W. de Hart, P. Grob, E. Nogales, C. R. Bertozzi, D. Rabuka, Angew. Chem. Int. Ed. 51 (2012), 4161–4165.
[16] R. Chen, Q. Chen, H. Kim, K.-H. Siu, Q. Sun, S.-L. Tsai, W. Chen, Current Opinion in Biotechnology 28 (2014), 59–68.
[17] S. Schoffelen, J. C. M. van Hest, Soft Matter 8 (2012), 1736–1746.
[18] S. Riva, W.-D. Fessner (Eds.), Cascade Biocatalysis: Integrating Stereoselective and Environmentally Friendly Reactions, Wiley-VCH, Weinheim, 2014.
[19] I. Oroz-Guinea, E. García-Junceda, Curr. Opin. Chem. Biol. 17 (2013), 236-249.
[20] I. Sánchez-Moreno, I. Oroz-Guinea, L. Iturrate, E. García-Junceda, in: E. M. Carreira, H. Yamamoto (Eds.), Comprehensive Chirality, Elsevier, Amsterdam, 2012, pp. 430–453.
[21] E. Ricca, B. Brucher, J. H. Schrittwieser, Adv. Synth. Catal. 353 (2011), 2239–2262.

[22] P. A. Santacoloma, G. Sin, K. V. Gernaey, J. M. Woodley, Org. Process Res. Dev. 15 (2011), 203-212.
[23] R. A. Sheldon, in: E. García-Junceda (Ed.), Multi-Step Enzyme Catalysis, Wiley-VCH, Weinheim, 2008, pp. 109-135.
[24] J.-K. Guterl, D. Garbe, J. Carsten, F. Steffler, B. Sommer, S. Reiße, A. Philipp, M. Haack, B. Rühmann, A. Koltermann, U. Kettling, T. Brück, V. Sieber, ChemSusChem 5 (2012), 2165-2172.
[25] J. S. Martín del Campo, J. Rollin, S. Myung, Y. Chun, S. Chandrayan, R. Patiño, M. W. W. Adams, Y. H. P. Zhang, Angew. Chem. Int. Ed. 52 (2013), 4587-4590.
[26] B. Wang, H. Land, P. Berglund, Chem. Commun. 49 (2013), 161-163.
[27] S. F. Mayer, W. Kroutil, K. Faber, Chem. Soc. Rev. 30 (2001), 332-339.
[28] L. F. Tietze, Chem. Rev. 96 (1996), 115-136.
[29] D. E. Fogg, E. N. dos Santos, Coord. Chem. Rev. 248 (2004), 2365-2379.
[30] J.-C. Wasilke, S. J. Obrey, R. T. Baker, G. C. Bazan, Chem. Rev. 105 (2005), 1001-1020.
[31] A. Bruggink, R. Schoevaart, T. Kieboom, Org. Process Res. Dev. 7 (2003), 622–640.
[32] S. Kara, J. Schrittwieser, F. Hollmann, M. Ansorge-Schumacher, Appl. Microbiol. Biotechnol. 98 (2014), 1517–1529.
[33] N. Nakajima, K. Tanizawa, H. Tanaka, K. Soda, J. Biotechnol. 8 (1988), 243-248.
[34] F. R. Bisogno, I. Lavandera, W. Kroutil, V. Gotor, J. Org. Chem. 74 (2009), 1730-1732.
[35] V. Köhler, Y. M. Wilson, M. Dürrenberger, D. Ghislieri, E. Churakova, T. Quinto, L. Knörr, D. Häussinger, F. Hollmann, N. J. Turner, T. R. Ward, Nat. Chem. 5 (2013), 93-99.
[36] J. H. Schrittwieser, J. Sattler, V. Resch, F. G. Mutti, W. Kroutil, Curr. Opin. Chem. Biol. 15 (2011), 249–256.
[37] T. Sehl, J. Kulig, R. Westphal, D. Rother, in: P. Grunwald (Ed.), Industrial Biocatalysis, Pan Stanford Publishing, Singapore, 2014.
[38] F. van Rantwijk, A. Stolz, J. Mol. Catal. B: Enzym., doi: 10.1016/j.molcatb.2014.08.012.
[39] T. Sehl, Z. Maugeri, D. Rother, J. Mol. Catal. B: Enzym.
[40] M. L. Contente, P. Zambelli, S. Galafassi, L. Tamborini, A. Pinto, P. Conti, F. Molinari, D. Romano, J. Mol. Catal. B: Enzym., doi: 10.1016/j.molcatb.2014.05.022.
[41] C. Aguirre-Pranzoni, F. R. Bisogno, A. A. Orden, M. Kurina-Sanz, J. Mol. Catal. B: Enzym., doi: 10.1016/j.molcatb.2014.07.011.
[42] E. Brenna, M. Crotti, F. G. Gatti, D. Monti, F. Parmeggiani, A. Pugliese, S. Santangelo, J. Mol. Catal. B: Enzym., doi: 10.1016/j.molcatb.2014.10.006.
[43] E. Brenna, F. G. Gatti, D. Monti, F. Parmeggiani, A. Sacchetti, J. Valoti, J. Mol. Catal. B: Enzym.
[44] D. González-Martínez, M. Rodríguez-Mata, D. Méndez-Sánchez, V. Gotor, V. Gotor-Fernández, J. Mol. Catal. B: Enzym., doi: 10.1016/j.molcatb.2014.09.002.
[45] S. Ranganathan, T. Gärtner, L. O. Wiemann, V. Sieber, J. Mol. Catal. B: Enzym.
[46] F. Camps Bres, C. Guérard-Hélaine, V. Hélaine, C. Fernandes, I. Sánchez-Moreno, M. Traïkia, E. García-Junceda, M. Lemaire, J. Mol. Catal. B: Enzym., doi: 10.1016/j.molcatb.2014.10.016.
[47] A. L. Valino, A. M. Iribarren, E. Lewkowicz, J. Mol. Catal. B: Enzym.
[48] A. Petrenz, P. D. d. María, A. Ramanathan, U. Hanefeld, M. B. Ansorge-Schumacher, S. Kara, J. Mol. Catal. B: Enzym., doi: 10.1016/j.molcatb.2014.10.011.
[49] B. Légeret, L. Hecquet, F. Charmantray, J. Mol. Catal. B: Enzym., doi: 10.1016/j.molcatb.2014.07.005.