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Embracing Nature's Catalysts: A Viewpoint on the Future of Biocatalysis

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Enzymes are nature's catalysts that are designed to accelerate specific reactions up to 10^6 times with high selectivity.[1] The enormous potential of enzymes as catalysts for organic synthesis has been recognized very early, as nicely described by Stanley Roberts in the late 90s.[2−4] Thus, these have become an increasingly attractive alternative to conventional chemical catalysts. The field experienced a real boom with the necessity to produce enantiopure compounds. Initially, mainly lipases and esterases were used for the synthesis of chiral compounds. However, the portfolio of available biocatalysts was soon expanded to include nitrilases, ketoreductases, and transaminases, etc.[5,6] These biocatalysts made essential building blocks for pharmaceutical syntheses such as amines and alcohols accessible. An important step forward was the implementation of directed evolution.[7−9] Directed evolution in the laboratory facilitated the adaption of enzymes to technical challenges such as substrate scope, selectivity, or process stability. While directed evolution showed remarkable results, a substantial amount of experimental efforts is needed for biocatalyst optimization. Many examples have also proved that integrative strategies of computational methods with directed evolution outperformed individuals. An increasing number of computational methodologies and tools have been developed to assist in the faster identification of suitable enzyme starting activities and the design of smaller and smarter enzyme mutant libraries.[10−12] Recently, Janssen and Wu computationally redesigned a highly selective aspartase, which they claim "is a notoriously difficult candidate as a starting point for evolution", for the hydroamination of different acrylates.[13]

Evolved enzymes are not only used for individual reactions but also for cascading several enzymes or incorporating newly developed enzymes into metabolic pathways (synthetic biology).[14−16] In the last 20 years, many companies realized the potential of applying selective enzymes to manufacture chemicals, active compounds, or generate entirely novel materials. In this respect, biotechnology/biocatalysis groups were implemented in industrial research departments, and companies were founded to push the technology even further. Simultaneously, in academia, new research centers with a cross-disciplinary structure have been established. Looking at this research field from this perhaps somewhat academic point of view, it looks very promising. Despite considerable progress, the scientific breakthroughs and possibilities to manufacture all kinds of molecules are currently not reflected in the market. In the chemical industry, there is pressure to achieve economic success and to live up to the expectation that had been placed on it. Interestingly enough, efficient, economic processes very often show also an improved overall eco-balance. From companies or even investors, some obstacles become apparent that dampen the current and noncurrent market success. Scientists in the industry are almost in a "depressed mood" about the question: What should we do to become more successful and with which products can we aim to have the right impact on the market?

In this viewpoint, I will discuss current problems and challenges of transferring biocatalysis into processes to manufacture chemical compounds. I will mainly focus on the chemical industry, but I will also address issues that apply to both the chemical and pharmaceutical industries. I would also like to point to recent reviews on the scientific and future developments of biocatalysis in the pharmaceutical industry.[17,18] I will further evaluate possible scientific and technical developments that indicate on how to overcome these challenges. Biocatalysis is regarded as a key technology for the coming years. With this in mind, I hope that you will find this viewpoint to be a practical and useful guide toward a prosperous future of applying enzymes as catalysts.

WHAT ARE THE TOP THREE CURRENT CHALLENGES OF BIOCATALYSIS?

Talking to many scientists in different industries as well as listening to plenary discussions at meetings it became evident that transferring reactions catalyzed by enzymes into industrial processes faces three major challenges to be successful (Figure 1).

Performance. When considering biocatalysis alongside heterogeneous catalysis, although some overlap in operating conditions such as pH, temperature, and pressure is possible between reactions, in general, with heterogeneous catalysis, space-time yields (STY) between 1 and 10 kg L^{-1} h^{-1} are obtained. In comparison, biocatalysis frequently reaches STYs in the range of 0.001−0.3 kg L^{-1} h^{-1}. Thus, the challenge in industrial biocatalysis is to identify and to engineer the perfect enzyme for the synthesis of chemical products and to implement

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Figure 1. Enzymes are considered to be little miracle substances for innovation, process advantages, and cost reductions. Whether in the food industry, pharmaceutical industry, textiles, detergents, biorefineries, fine and specialty chemicals, paper, polymers, and cosmetics, the application areas in which enzymes can be used sensibly and profitably are diverse and nowhere near exhausted. Despite this significant progress already achieved, there are various challenges that biocatalytic manufacturing has to face to be successful. The poor performance of the biocatalysts, time-consuming and costly process developments, along with little insights into market developments and intelligence are major issues that industry faces with biocatalysis. It is worth mentioning that already today solutions exist for these issues. They can be resolved with the increasingly rapid progress of science and technologies as well as a new way of thinking and learning necessary for a thriving and effective utilization of biocatalysts in industry.

it into novel pathways. There are numerous reports on novel enzymes that perform new and outstanding reactions. The chemical, medicinal, and pharmaceutical industries generally take full advantage of the high substrate specificity typically associated with enzyme catalysis to produce fine chemicals and pharmaceutical compounds of high enantiomeric purity. Despite significant productivity gains, large amounts of organic compounds are still produced by the chemical industry, mainly because of the large manufacturing volumes. For industrial applications, enzymes are required to have high product titers as well as space-time yields. In addition to high productivity these enzymes must often also exhibit excellent stability under the given process conditions. The production of acrylamide with nitrile hydratase (STYs between 53 and 93 g L^{-1} h^{-1}) on a 50 000 t a^{-1} scale is one of the most impressive industrial applications of enzymes.[19−21] It should also be said that the productivity of acrylamide production by nitrile hydratase reaches the levels of heterogeneous catalysts.

Unfortunately, a quick survey of available data demonstrates that the performance of exciting new enzymes and reactions has not been satisfactory for industrial processes. While this may at first seem like a pitfall, it highly depends on the value of the product being made. Much work has been focused on extending the substrate repertoire, altering the selectivity and expanding the enzyme portfolio. Nevertheless, many new forthcoming opportunities will be targeted on a modification to enable process applications. For industrial processes, the entire process chain, including the availability of the substrate, must be considered early on in process development. While selectivities of enzymes are often excellent, researchers are less concerned about the productivity and operational stability of enzymes. These indicators are typical of little interest to academic groups and, thus, should be pursued by industrial groups to achieve the needed high STYs. Very often the quantity of enzyme added is raised to improve the productivity or STY itself. With respect to this measure, we have to take the "trade-off" between the quantity of cells or enzymes and the productivity into consideration. Understanding this "trade-off" is key to what works and what does not. High productivities on the other hand can cause the limiting product titer to be reached too fast. Then *in situ* product removal must be invoked or the catalyst separated and recycled. Another related issue is that growing cells are simply too slow for large volume productions, but in a two-stage strategy they can be very successful. Consequently, if these parameters are not exploited in processes, reactions result in high enzyme costs per product, low productivities, or too elaborate downstream processing. It is worth mentioning that during the past 15 years, there has been a continuous flow of reports describing proteins stabilized by the introduction of mutations. A very recent article featured how the boiling point stability on an alcohol dehydrogenase was approached through computer-guided enzyme engineering.[22]

Cost and Timeline. Recent advances in bioinformatics, protein biochemistry, protein engineering, and modern biotechnology have revolutionized the "faster" identification and development of new enzymes for industrial processes. The computer-assisted engineering of enzymes will improve their performance and their integration and combination with further enzymes in reaction cascades to expand the reaction scope. Moreover, rapid DNA assembly, genome editing, pathway refactoring, and design along with high-throughput screening enable the automation of chemical production processes by microbes. A centerpiece to this development is the iterative application of the Design-Build-Test-Learn (DBTL) cycle that has been applied, e.g., in the synthesis of long-chain alcohols, flavonoids, alkaloids, and *N*-heterocycles.[23−25] Biofoundries provide an integrated infrastructure to enable the rapid design, construction, and testing of genetically reprogrammed organisms for biotechnology applications and research.[26] The microbial production of the antimalarial drug artemisinin and

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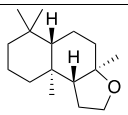
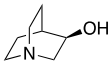
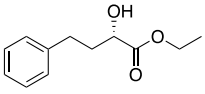
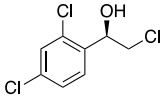
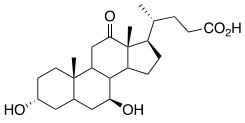
Table 1. Examples of Industrial Applications of Microbial Catalysts

Product	Application	Enzyme	Space-time yield	Amount of cells
 (-)-ambrox®	fragrance intermediate	squalene hopene cyclase	12 g L^{-1} h^{-1}	375 g L^{-1} [a]
 *R*-3-quinuclidinol	Building block of antimuscarinic agents	keto-reductase	63 g L^{-1} h^{-1}	8 g L^{-1} [b]
 *S*-2-hydroxy-4-phenylbutyrate	precursor to ACE inhibitors	β-ketoacyl-ACP reductase	103 g L^{-1} h^{-1}	30 g L^{-1} [c]
 *R*-2-chloro-1-(2,4-dichlorophenyl)ethanol	intermediate for antifungal agents	keto-reductase	11 g L^{-1} h^{-1}	9.5 g L^{-1} [b]
 12-oxocheno-deoxycholic acid	key precursor for ursodeoxy-cholic acid	12α-hydroxysteroid dehydrogenase	68 g L^{-1} h^{-1}	5 g L^{-1} [d]

[a]Wet-weight cells. [b]Freeze-dried cells. [c]Dry cells. [d]Lyophilized *E. coli* cells harboring *Rr*12α-HSDH and LDH genes, respectively.

of the platform chemical 1,3-propanediol are two examples of how engineering biology can enable more effective and sustainable manufacturing processes. However, engineering biology is still time-consuming, unpredictable, and expensive. To engineer the microbe *Escherichia coli* (*E. coli*) to produce 1,3-propanediol took 15 years and >$200 million in research and development. To produce artemisinin, 10 years of research and development (roughly 150 person-years of work) and >$150 million were needed.[27,28] These data conclude that there is a clear market need for technologies that reduce the development time and costs of cell factories, which also provide access to new chemical space. There is another aspect to be noted: the limited portfolio of products accessible. Smaller and more suitable chemical products cannot be tackled as their market volume is limited. We will not recover at least a part of the massive investments needed for research and development within a reasonable time frame. As a consequence, we have to concentrate on products with a substantial market value, and the stakes are usually very high. More importantly, research goals have to be achieved much faster and more cost-effectively. This will enable us to access a much broader product portfolio.

Market Intelligence. Keeping track of the competition and the state of industry is an integral part of operating any business. Traditionally, that information has been termed "market intelligence". In recent years, however, the practice of collecting market intelligence has expanded to include analysis and analytics. In this context, market intelligence uses multiple sources of information to create a broad picture of the company's existing market, customers, problems, competition (estimated guesses on technologies, raw materials used, synthesis routes, production line size, etc.), and growth potential for new products and services. Sources of raw data for that analysis include sales logs, surveys, and social media, among many others. The pharmaceutical industry is increasingly turning to the use of engineered biocatalysts for both lead generation of active compounds and the sustainable manufacture of active pharmaceutical ingredients (APIs). However, the key challenge lies in the fast identification of a suitable catalyst at an early stage in route development for a new drug. The linkage of biocatalysis with chemical catalysis, route scouting, and process development along with a balanced selection of biocatalysts at hand that enables to react immediately, are pivotal strategies for a successful application of the technology. In contrast to the pharmaceutical industry, other industries such as the chemical industry need to be more concerned about the targets, they can address with biocatalysis. While stand-alone products are much more challenging to establish, it might be challenging to get to the right scale of the economy with one specific product. At the same time, it should be stressed that such a product might also disturb a fully integrated value chain. As a product moves from raw material to the product of industrial interest, value is added at each step in the manufacturing process. Chemical product platforms often involve one chemical product becoming the starting material for many other products or the raw material for the next process that will produce the next chemical product on to the final product (e.g., multistage value-

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chain networks producing intermediate chemicals with different functionalities for polymers, fabrics, pharmaceuticals, cosmetics, dyes, etc.). The novel value chain faces the risk of being in competition with well-optimized, cost-effective synthesis routes from fossil resources to produce chemicals that already have a market.

Furthermore, researchers and scientists educated in biotechnology and biocatalysis have to learn how to think in chemical value chains, which include the safe management and use of chemicals throughout their entire life cycle, from production, transport, and use through eventual recycling or disposal. It should be taken into consideration that long timelines in getting the permit to bring the product to the market or getting the acceptance of your customers are often overlooked. There is quite a gap between having a process established and the product launched on the market. Depending on the product, for instance, a new polymer, that can take up to 10 years. The conclusions are that the necessary market intelligence for the development of a successful product seems to be a matter of course. However, it is an absolute weak point, especially for many small companies.

HOW CAN THESE CHALLENGES BE ADDRESSED?

The question that we now encounter concerning the previously discussed main top challenges is whether the current results of research reports indicate how to skip them and successfully expand the application of enzymes. The following section sets out problem-solving solutions covering the rapid identification and improvement of biocatalysts, the design, and the broader applicability of biocatalysts in processes.

Performance. *Biocatalysts.* As a result of the steady growth of the biocatalyst market, there is an increasing demand for enzymes that are active toward novel and often non-natural substrates. We thus need to identify biocatalysts with unique and desired properties for the production of new organic compounds through novel reaction pathways and their efficient application in established processes. Therefore, extended programs with combined different strategies, such as working with cultivable and noncultivable organisms, using sequence- and function-based bioinformatic approaches, performing high-throughput screenings, and constructing libraries in plasmids have been used to obtain a wide diversity of novel enzymes.[29−33] However, natural evolution has adjusted today's enzymes to perfectly fit into their respective physiological niches. In order to overcome these difficulties, a repertoire of tools for enzyme engineering was developed.[34] The implementation of directed evolution strategies, pioneered by Arnold, Stemmer, and Reetz around the turn of the 21st century, has been the most crucial of all.[35−39] In addition to enhancing existing enzymatic properties, directed evolution has empowered researchers to develop enzymes with novel capabilities. While the scoring in selectivity is often excellent, the activity and overall stability are often rather poor for the desired reaction. One of the most urgent and immediate challenges in industrial biocatalysis is the raising of the product titer, which is reflected in the activity as well as stability of an enzyme. Irrespective of the product, high STYs, and double-digit gram per liter concentrations should be realized. Besides, high product concentrations considerably simplify the processing of the product and generally also enable higher product purity and quality. In this sense, more considerable attention should be given to enzyme activity, which is also reflected in the cost of the applied biocatalyst as well as the price of the target product. Notably, some biocatalytic processes with impressive titers or STYs that have been published in recent years are highlighted in Table 1. Examples include the preparation of fragrance intermediate (−)-ambrox[40] and the synthesis of enantiopure pharmaceutical building blocks (*R*)-3-quinuclidinol[41] and (*S*)-2-hydroxy-4-phenylbutyrate.[42] Other compounds, such as the enantiopure (*R*)-2-chloro-1-(2,4-dichlorophenyl)ethanol is used as an intermediate for antifungal agents[43] or 12-oxochenodeoxycholic acid used as a key precursor for ursodeoxycholic acid.[44]

Expanding the Portfolio of Enzymes. Rapid access to enzymes with a variety of natural and even non-natural catalytic activities has expanded the synthetic organic chemist's toolbox. Nice collections of enzymes and variants are offered by several companies. These biocatalysts can now be assessed for the catalysis of synthetic organic reactions. The enormous progress in sequencing technology has led to an exponential increase in the number of sequence data that can be considered as a gold mine. These genes encode for a huge variety of novel and mostly unexplored enzymes useful for biocatalysis or metabolic engineering of new pathways that can be *in silico* screened and analyzed to identify prospective candidates for further data-driven explorations and optimizations by evolution.[11,45] Moreover, it has been shown by us and others that probing catalytic promiscuity reveals novel enzyme function that can be readily optimized by applying evolutionary enzyme engineering methods. This "chemomimetic" approach leads to new enzymes by engineering established reactions into promiscuous proteins.[46] These engineered enzymes catalyze chemical transformations that are new to biochemistry yet have been discovered in synthetic organic chemistry.[47] Besides, recent developments have brought into focus the transfer of retrosynthetic strategies into the field of biocatalysis ("biocatalytic retrosynthesis").[48,49] Turner and Humphreys proposed guidelines and rules for "biocatalytic retrosynthesis" and provided examples of current applications of biocatalysis using worked examples and case studies.[50]

Nonetheless, inspecting current synthesis routes or using scouting software to develop novel routes for product synthesis, we recognize that the chemical space is limited. Reaction classes such as reductive aminations, C−H functionalizations, and C−C bond formations are showing great promise; however, other classes are currently under-exploited and therefore warrant further investigation. From my point of view, it would be beneficial if we had access to these types of reactions shown in Table 2. Furthermore, improved generality and substrate-size tolerability for several already established transformations and efficient methodologies for ATP, SAM, CoA cofactor generation, and reuse are needed. In this light, "chemomimetic" strategies that draw from synthetic chemistry (mechanisms) and nonbiological reagents or strategies will guide the development of novel biocatalysts. In order to achieve these fundamental objectives, we shall endeavor to promote interactions between chemistry, biology, and engineering.

Timeline and Costs. *Fast Development Is Possible.* In recent years, some new and interesting enzymes have been developed. Imine reductases, for example, are particularly interesting biocatalysts that made it already into pilot-type production for the synthesis of *N*-cyclopropylcyclohexanamine[66] or the in LSD1 inhibitor GSK287955.[67] This development is impressive because it is an excellent example that we can develop and characterize enzymes for synthetic applications in a remarkably short time. In this context, it is worth adding that some of the European research projects

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Table 2. A Few Examples of Desired New Reaction Chemistries by Biocatalysis

new reaction chemistry	comments
halogenation	few good starting points; particular interest in fluorinations[51,52]
amide-bond formation	broadly applicable amide-bond forming reactions[53]
C−C bond formation and cleavage	beyond aldolases; larger synthons that typical 1−2 carbon fragments; novel activities from cyclases[54,55]
ether formation	mostly investigations on the degradation of ethers; first indications from cyclases[56,57]
carbonylation	Starting points for enzymatic carbonylation of C−H and C-X bonds[58,59]
functionalization of C═C bonds	enzymes that perform hydrations, hydroaminations etc.; broadly applicable hydratases[13,60−62]
isomerization	broadly applicable isomerizing biocatalysts[63]
reduction of isolated C═C bonds	first activities reported[64,65]

rendered outstanding services to this success story. Being a member of such a consortium or network facilitates not only access to the most recent findings from science but also a gain of expertise in the development of future solutions. The time pressures present in pharmaceutical process development are incompatible with the long lead times required for engineering a suitable biocatalyst for the synthesis of a target molecule. The ability to identify, obtain, and test biocatalysts for the synthesis of pharmaceutical intermediates in a timely fashion is crucial. In general, 10 to 12 recurring enzyme classes are essential that include typical master enzymes. They should cover the broadest possible substrate spectrum of the enzyme family and also serve as a starting point for further optimization. We select candidates for this portfolio from the biodiversity of enzyme superfamilies using bioinformatics and compile them as representative kits. Further, they can also be put together based on specific representative variants of enzyme engineering projects. Finally, the pressing question is how fast these starting enzymes can be engineered for better performance and whether these newly engineered catalysts can outcompete other chemical technologies?

Smart Libraries: Robotics vs Computational Design. The rapid generation of enzyme variants using new molecular biology techniques combined with high-throughput screening allowed for enzymes to be improved for desired properties much faster. The directed evolution approach to enzyme optimization has shortened development times by combining molecular biology, automated robotics, and bioinformatic analysis of mutational effects. At the same time, we should bear in mind that, despite the enormous investments in vast amounts of disposable materials and expensive equipment as well as specialized experts that operate the devices, emphasis should be given to major investments in knowledge to make future developments more efficient. A further option is the generation of small but smart enzyme mutant libraries and the advancement of inaccurate screening systems for the desired reaction or property. Analytics is demanding and time-consuming, especially for technical developments, and thus represents a particular bottleneck. Computational software tools, combined with a better understanding of the construction of enzymes, contribute to a more intelligent provision of enzyme variants.[11,68,69] These smaller and smarter libraries can then be screened with more sophisticated analytics. Future engineering should also include more "substrate-walking" strategies for screening to evaluate the novel or improved activity of an enzyme for the actual target reaction with more complex substrates.[70,71] In this respect, engineering the substrate specificity is applied to a series of substrates with sequential modifications that link the natural substrate through to the desired substrates. Experiences from our laboratory have shown that the construction and screening of mutant libraries is a matter of weeks instead of months

Process and Production. In biocatalysis, the focus is always very much centered around the development of the enzyme catalyst. The consequence is that a lot of exciting projects in the industry are killed because they did not consider the entire process early enough. Generally, product titers and suitable production lines affect the industrial application of biocatalysts. Early calculations might indicate that a few grams per liter are sufficient to meet the target costs. However, product isolation from low titers adds quite an unforeseen contribution to the manufacturing costs. Higher titers provide to a much simpler overall recovery process and help reach high product quality targets. Most projects will need a contract manufacturer to produce the first amounts of the product in multipurpose facilities. In this context, it should be noted that such production

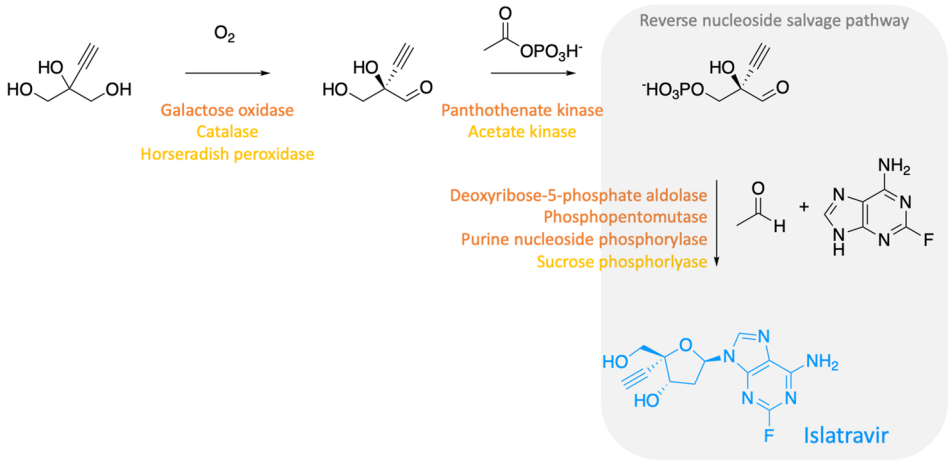

Figure 2. Summary of the biocatalytic cascade for the synthesis of the investigational drug islatravir. Evolved enzymes and auxiliary enzymes are highlighted in orange and yellow, respectively. Galactose oxidase and panthothenate kinase have been applied as immobilized enzymes.

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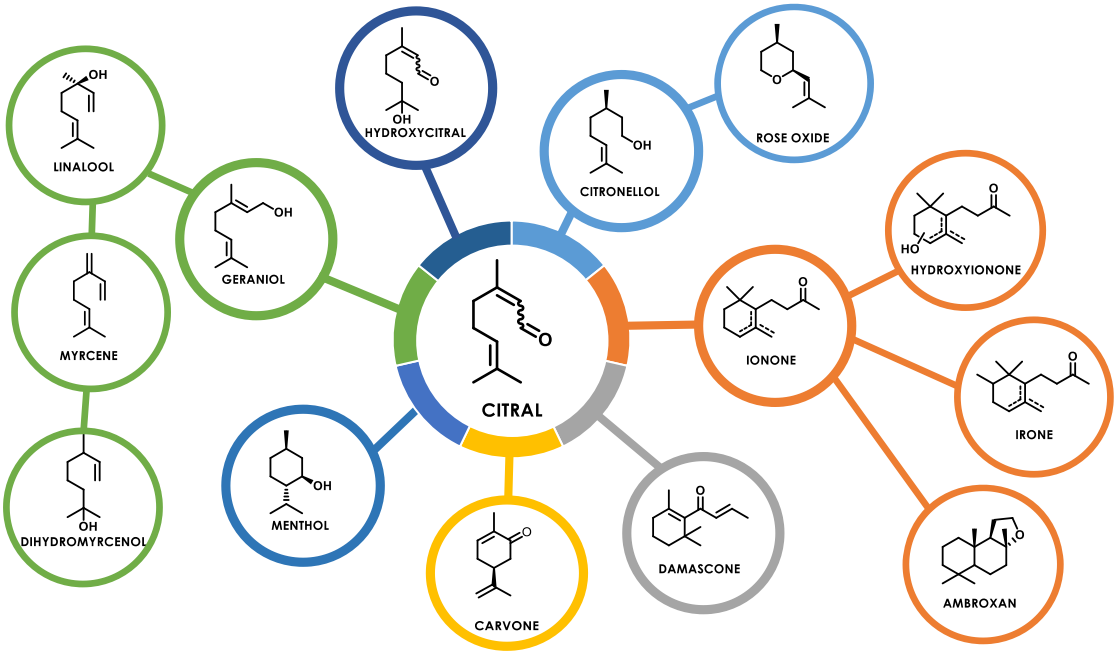

Figure 3. Citral-value chain offers new access to flavor and fragrance compounds.

lines will never be ideally suited for the target-product and, thus, costly improvisation and further experimentation are required. Especially for smaller products, it might be helpful to have several very similar products in line so that they can be produced using the same equipment. Such a multipurpose plant would benefit from the scale of the economy. Therefore, it is extremely wise to have a process engineer as early as possible around to assist and advise on designing the right process and on providing practical support on engineering targets for your catalysts. A very recent perspective by Woodley summarizes some of the recent developments in the field and the implications for reaction engineering, with a focus on sustainable chemical production.[72,73] Yet, entirely new concepts in process design are still missing. Inspirations are once more gained from nature that has developed systems to hold even toxic or reactive compounds in high concentrations in, for instance, glands of insects or plants.[74]

Moreover, biocatalytic cascade reactions have demonstrated a tremendous potential during the last years. As a result, various papers and reviews have been published that demonstrated *in vitro*/*in vivo* approaches,[15,75−78] *de novo* multienzyme pathways,[16,79] development of synthetic cell analogues,[80−82] or that discussed the challenges and strategies for assembling cascade reactions and increasing overall cascade productivity.[83−85] A very recent remarkable example is the development of a biocatalytic cascade for the synthesis of the investigational drug islatravir for the treatment of human HIV (Figure 2).[86] Previous reports for the synthesis of islatravir relied on multistep synthesis that required protecting/deprotecting as well as intermediate purification reaction steps. Researchers at Merck and Codexis re-engineered a bacterial biosynthesis pathway to run in reverse for biocatalytic islatravir manufacture. They have assembled a total of nine enzymes that included five evolved enzymes and four additional auxiliary enzymes to give islatravir in 51% overall yield as a single isomer in aqueous solution. This work truly shows the power of enzyme cascades for the synthesis of complex molecules.

Market Intelligence. When compared to synthetic methodologies that employ precious metals for catalysis, the costs, the excellent selectivity, the higher product quality, and sustainability benefits are apparent. In addition, the reduction of reaction steps in processes (also no protection/deprotection), the ability to use different starting materials and less energy consumption, waste, as well as side product formation are further advantages. Thanks to these assets, consideration could be given to the integration of biocatalytic products and processes into the "chemistry tree of products" representing the chemical space and relationships between chemical structures. This also includes the concept of platform and value chain that are closely related. For biotechnologists, a platform is the molecular biological tool kit comprised of promoters, plasmids, microorganisms, etc. The thinking is centered on how to use these tools for the development of new products and, thus, become faster and more efficient. It also fosters the synthesis of different products based on the same biological systems. In chemistry, this term is used to refer to a catalyst system or a specific apparatus technology. The platform gives access to very similar products based on a conventional catalyst type and a common multiproduction plant. Owing to their potentials as platform chemicals for many applications in the industry, such as for the synthesis of polymers and commodity chemicals, the microbial production of C2 to C6 alcohols, diamines, diols, and (di)carboxylic acids has received much interest in industrial biotechnology.[87−90]

Efficient execution confers a competitive edge in terms of manufacturing and development costs. In biocatalysis, however, these examples are rare. The BASF ChiPros technology, a pool of chiral amine products with one technology platform, one lipase catalyst, and one acylation agent, is one of the few examples of how this can be done.[91,92]

Value chains (chemistry tree of products) start with a specific starting material that is converted into as many downstream products as possible. This creates an entire tree of chemical products that, in turn, benefit from each other. Such chains can be repeatedly found in cost-effective chemical productions. Often a kind of "cherry-picking" takes place by selecting a supposedly valuable product and developing a biotechnological

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process for it. However, the fact that such a process can be implemented in production fails because the cost structure for other products in the value chain collapses. A biotechnological alternative must, therefore, aim to replace an entire value creation chain or to set up an entirely new value chain. This concept could be used for the development of value chains based on fatty acids or citral (see also Figure 3) by using only a few enzymes. Citral is mainly used in lemon essence, vitamin A, ionone, menthol, and others. Report data showed that 5.93% of the citral market demand in lemon essence, 43.86% in vitamin A, 9.80% in ionone, and 31.84% in menthol in 2015. Furthermore, the global citral market is valued at 762 million USD in 2020 is expected to reach 688.1 million USD by the end of 2026 (https://www.marketwatch.com/press-release/citral-market-2020-global-industry-brief-analysis-by-top-countries-data-market-size-definition-industry-trends-news-and-significant-growth-with-regional-trends-by-forecast-2026-2020-03-24). With the development of economy, these industries will need more citral. So, citral has a huge market potential in the future.

From an industrial biocatalysis perspective, I am continually amazed at how many companies and groups have the same ideas and focus on the same type of products. I think we should not limit ourselves to use enzymes that are already available or to products being obvious to many groups. There are numerous promising product opportunities, especially in the field of smaller and higher-value-added products. It should be emphasized, however, that most likely a market for these novel products has to be prepared. We might need to permits to sell the product ourselves from our starting materials or tailor them specifically to our customers' needs. New paint coats or plastics have to undergo performance tests, new fragrances might need a REACH registration, or a new milk peptide needs toxicological studies for registration. All of this could take several years, and it is, therefore, necessary to learn to speak the "language" of not only chemists but also process engineers, computer and software engineers, as well as maybe field experts, farmers, and registration experts in order to be successful. Also, a very good understanding of the various applications of manufactured products by the customers as well as competing technologies for manufacture is vital. The selection of the right products to apply biotechnology is a pivotal question to be successful in business. For products already on the market ("drop-in-solutions"), biocatalysis holds the promise of a shorter route, cheaper starting material, or better quality. Miscellaneous route scouting tools are available or being developed by different companies in order to design more efficient processes for certain types of molecules. A broad portfolio of mostly smaller but high value products ranging from flavor and fragrance compounds, e.g., ambrox,[40,93,94] biotin vitamins,[95,96] or pheromones[97,98] can be manufactured based on biocatalysis. In the future, emerging new and innovating products, like sweeteners, dyestuff, oligosaccharides, functional peptides, nutraceuticals, just to mention a few, will be manufactured using the technological advantage of biocatalytic routes.

The Future Remains Promising. Enzymes are vital in achieving the urgent needs of our society. There are exciting possibilities ahead which need money and support from all of us. However, it is important for the community that we achieve our promises and goals. My vision for the future is to fully exploit the mechanism of enzymes with high intrinsic activities toward novel reactions, the consideration of the value tree of chemistry to identify novel product opportunities, and increased and closer collaborations to gain speed but also insights into biocatalysts and scientific developments.

A significant topic of technical relevance will undoubtedly be the combination of enzymes into new, or especially, non-natural synthesis pathways. This enables to produce favorable compounds, active substances, or more complex building blocks in a single step. It will be interesting to see whether we will be able to include the full repertoire of chemical catalysts in this process as well. Interesting reports indicate that it is possible to mask chemical catalysts that are normally not compatible with water in such a way that they can also be used in biocatalytic routes.[99] Another aspect of our efforts to combine several enzymes and their variants is that we can synthesize entire drug families in the laboratory. The combination of enzyme and metabolic engineering enables for example the expansion of the terpenoid kingdom to access a plethora of novel, non-natural terpenoids.[100,101] We will then no longer have to search for new natural substances in nature but will be able to mimic natural product synthesis in the lab. We can imitate plant syntheses in the laboratory and provide compounds that are not found in nature. In combination with synthesis machines, we can succeed in integrating biocatalysis into the development of new compounds from the outset and then become a valuable component of new syntheses. The development of new smaller, artificial enzymes, even based on peptides, will be a further hot topic. Enzymes are large, and not all their functions might be essential for organic synthesis. We can combine our know-how and the concept of directed evolution and develop new biocatalysts. Hilvert has recently published groundbreaking work on the evolution of a highly active and enantiospecific metalloenzyme from peptides.[102] However, it is also possible to imagine that it is possible to develop small, robust enzymes that have bound metals and can be regenerated electrochemically. Such small biocatalysts, which achieve a high catalyst density on a surface, can also be used to tackle large products and important reactions such as butane to butanol or propene to acrylic acid. As already described above, it is, of course, a fundamental prerequisite that the portfolio of enzymes and accessible reactions is expanded. In addition to synthetic applications, there are, of course, other essential fields of work for which the development of new biocatalysts will be of great importance. These include numerous environmental challenges. Carbon dioxide is a molecule that is very difficult to grasp in organic chemistry. There have always been major projects to produce necessary basic chemicals from CO_2/CO but unfortunately with little success. Microorganisms and the enzymes involved could be used here. Clostridia, which can convert lower synthesis gas into ethanol, should be mentioned here.[103,104] LanzaTech has already demonstrated this on a larger scale. However, engineering Clostridia, by introducing novel enzymatic activities, it should be possible to establish a broad portfolio of compounds based on C1 building blocks. Another topic of urgent need is nitrogen management in our fields as well as in drinking water. Here we could succeed in defusing slurry-employing enzymes and thus avoid contamination of the groundwater with nitrate. Enzymes can also be of great importance in degrading microplastics and polymers. The first enzymes that cleave esters, so-called PET esterases, have been described in the literature and there are also starting points for the cleavage of amide and urethane bonds.[105−108] Microorganisms that are able to degrade PET are described, and international research projects are also underway for the other polymers. However, the development and synthesis of new degradable polymers is also a

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field in which enzymes can contribute.[109] This is just a small selection of ideas discussed in the community of enzyme engineers. Nevertheless, it demonstrates that they are quite several fields where enzymes will contribute to finding solutions for the benefits of humankind.

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Notes

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