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Grand Challenges in Biocatalysis

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INTRODUCTION

Biocatalysis Is an Enabling Technology for Chemical Synthesis

Biocatalysis comprises the use of nature's catalytic repertoire to facilitate chemical reactions (Sheldon and Woodley 2018; Sheldon and Brady 2019).

Enzymes catalyze a broad range of chemical transformations, generally under very mild reaction conditions and with high selectivity. These features make enzymes attractive catalysts for industrial chemical transformations, enabling less resource-consuming and waste-generating synthesis routes. Therefore, biocatalysis is already today an important pillar of chemistry and continues gaining relevance in academic research and in industrial application.

The last two decades have seen an exponential expansion of biocatalytic tools ranging from new catalysts with tailored properties to new reaction engineering concepts. As a result, the relevance of biocatalysis in the chemical industry has been also increasing steadily.

Today's grand challenge in biocatalysis is to keep this impetus up and further consolidate and expand the toolbox of biocatalysis.

A few aspects will be highlighted in the following:

Tailored Enzymes for Precision Chemistry

Although we already have access to a broad range of biocatalysts, there is still an urgent need for more enzymes/biocatalysts to cover an even larger space of chemical reactions and reaction conditions. Natural diversity represents a rich source of new enzymes obtained, for example, from metagenomic libraries and from sequence libraries (Lorenz and Eck 2005). Naturally occurring (wild-type) enzymes, however, have evolved to suit their host organisms' needs and not the needs of the organic chemist. Fortunately, we now have tools of enzyme engineering at hand with which the properties of wild-type enzymes can be modified to suit the needs of chemical transformations (Bornscheuer et al. 2012; Arnold 2018; Qu et al. 2020). In principle, enzyme variants with tailored selectivity, substrate specificity, activity, and stability under "unnatural" reaction conditions can be generated using the tools of enzyme engineering (directed evolution, semi-rational design, and in silico design).

Nevertheless, enzyme engineering can be a tedious, time- and resource-consuming process, especially screening large libraries. Therefore, further improvements are urgently needed not only to generate new, tailored catalysts but also to expand our understanding of structure–property relationships. New high-throughput screening methods to assay huge libraries will allow for covering a larger sequence space and, maybe, also discovering "needles in the haystack" such as enzymes with unforeseen properties. Machine learning algorithms may also play a central role here. Likewise, new algorithms for enzyme modeling will enable us to more efficiently design enzyme variants.

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New Chemical Transformations Using Enzymes

While wild-type enzymes catalyze a broad range of useful chemical transformations, there are still many "white spots" in the reactivity landscape of biocatalysis. In the past years, we have seen how chemistry-inspired enzyme engineering resulted in new enzymes catalyzing bond formation reactions such as C-Si-, C-B-, C-C-, or C-N-bonds previously not conceivable in biocatalysis (Arnold 2018). Likewise, utilizing chemical concepts such as photochemical activation have resulted in new, "nonnatural" transformations using enzymes.

Chemically modified protein scaffolds, so-called hybrid enzymes, continue receiving considerable attention. Hybrid enzymes combine the chemical versatility of transition metal catalysis with the selectivity and specificity of enzymes exceeded by the well-defined three-dimensional structure of protein active sites (Steinreiber and Ward 2008).

Also, so-called Plurizymes, i.e., engineered enzymes exhibiting multiple active sites (Santiago et al. 2018) and catalyzing several transformations, represent an interesting next-generation approach for cascade reactions (vide infra) (Alonso et al. 2020).

We believe that we have just scratched the surface here and expect that further smart applications of chemical principles to enzyme reactions will result in more exciting transformations. But also nature still has a lot of surprises for us in the chest. New cofactors are constantly being discovered, enabling new chemical reactivities; but also the existing natural cofactor repertoire is waiting to be fully elucidated.

Reaction/Reactor Concepts for Efficient Application

Today, biocatalysis research too often is stuck at the proof-of-concept level. Biocatalysis publications rarely surpass millimolar substrate concentrations. Chemical reactions, in contrast, generally entail reagent concentrations orders of magnitude higher or even neat (i.e., solvent-free) conditions. Bridging the "concentration gap" will be of utmost importance to further increase the acceptance of biocatalysis among organic chemists.

En route to this goal, we should reconsider the role of water as solvent of choice for biocatalytic reactions. For a large part of the reagents of interest, water is a suboptimal solvent and does not support the high reagent concentrations required. Water-soluble cosolvents can only partially compensate. Promising concepts comprise nonaqueous reaction media or two-phase reaction setups. Also new, tailored solvents may play a significant role here (Lozano et al. 2015; Lozano et al. 2017).

Enzyme Immobilization

For a range of industrial applications, immobilized, recyclable biocatalysts are imperative (Sheldon and van Pelt 2013). Next to heterogenization and recovery of biocatalysts, enzyme immobilization offers further possibilities to tailor the properties of given enzymes. Multipoint and multisubunit immobilization can result in more robust enzymes; engineering of the microenvironment of the immobilized enzyme offers the possibility to tune enzyme activity (Mateo et al. 2007; Garcia-Galan et al. 2011). Co-immobilization of several enzymes enables exciting possibilities for multistep cascade reactions, but the pros and cons must be carefully considered (Arana-Peña et al. 2020). Tailoring of supports and enzymes may enable the one-step purification–immobilization of the target enzyme (Barbosa et al. 2015). Despite the progress and impressive examples of enzyme immobilization, this process remains largely trial and error. Rational approaches to design enzyme immobilization and maximize the residual activity and stability of the immobilisate are urgently needed.

Is Biocatalysis Green?

"Environmental benignity" is a reoccurring claim in biocatalysis publications. Arguments used to substantiate this are mild reaction conditions, water as solvent, and the biobased-ness and biodegradability of enzymes. While we certainly agree that these features are likely to contribute to a low environmental impact of biocatalysis, we also emphasize that biocatalysis also has an environmental impact. Resources are consumed and wastes are generated during the preparation of the catalysts, during the reaction, and in downstream processing, overall placing a burden on the environment. We therefore urge all authors to use "green claims" very cautiously, ideally quantifying the resources consumed and wastes generated and comparing the environmental impact with existing reaction procedures.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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