Panel Discussion on Catalysis or Biocatalysis?

The Panel Discussion that characterize this Special Supplement to Chimica Oggi - Chemistry Today is dedicated to the Catalysis and Biocatalysis. Some of the main companies focused in these sectors seated around a virtual to express their opinion whether the future belongs to catalisys or biocatalysis.

The following players have joined the initiative: Codexis, Johnson Matthey, Solvias, Umicore and Zymtronix. You will find hereafter really interesting views and opinions regarding the continuous evolution of chemocatalysis, artificial enzymes, new technologies enabling the running of both catalysis and biocatalysis and tailored-processes.

Enjoy the reading.
TKS Publisher Editorial Board

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BIOCATALYSIS: WHEN AND HOW TO EMPLOY

Jim Lalonde - Codexis

The field of Biocatalysis for the manufacture of pharmaceutical APIs and intermediates has progressed rapidly in the last few years with many recent commercial applications for APIs such as sitagliptin, atorvastatin, simvastatin and crizotinib (1). This progression can be attributed to the convergence of several technology advancements (2). When contemplating when and how to incorporate a biocatalytic step in the synthesis of an API, there are several considerations. Certain transformations are so well established, that enzymes "off the shelf" may be available, while other types of transformations may require an enzyme engineering program to produce a customized enzyme.

First, the availability of enzymes that have been engineered to be process ready have been produced from enzyme engineering programs. In particular, libraries of ketone reductases (KREDs) for the asymmetric reduction of ketones to chiral alcohols and transaminases (TAs) for the asymmetric conversion of ketones to chiral primary amines have been produced with wide substrate acceptance. Enzymes for some hydrolytic transformations, including nitrile to carboxylic acid or carboxamide, ester to alcohol and acid, or amide to amine and acid are also often available off the shelf. Several enzyme companies such as Codexis supply panels of these enzymes for testing. When choosing a supplier, ensure that these enzymes are available in commercial quantities and requisite quality.

> Smart libraries" of mutational library variants can be produced using automated PCR methods

If the required biocatalyst is not available off the shelf, one can have a custom enzyme developed using enzyme engineering techniques. The state of the art of technologies for the reading and writing DNA sequences have dramatically decreased in cost and as a result publicly available databases of enzymes have exploded. Molecular modeling tools have been developed which allow for in silico testing of three dimensional structural models of enzymes. Thus, instead of random screening of enzyme, a new target substrate is first tested for a potential fit using molecular modeling and these enzyme models are then used to design targeted mutational libraries. Smart libraries of enzyme variants are expressed and screened in high throughput for attributes such as enantioselectivity and activity. In parallel, Next-Gen sequencing can be used to correlate the sequence of variants with test results. Advanced machine learning algorithms are used to deconvolute the individual contributions of mutations to the overall performance. Beneficial mutations are recombined and deleterious mutations are removed in subsequent libraries. This process can be done iteratively until the desired activity and selectivity is reached.

REFERENCES

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CHEMOCATALYSIS OR BIOCATALYSIS? THIS IS THE QUESTION

Beatriz Dominguez - Johnson Matthey

Chemocatalysis or biocatalysis? Both of these technologies can open new synthetic routes that are more economic and also more environmentally friendly. There is no optimal choice of one technology over the other. Instead both must be explored to identify all available options, tailored to the specific needs of an individual transformation.

Biocatalysis can be described as the application of nature's catalysts – enzymes – to industrial processes. The high degree of regio-, chemo- and stereo-selectivity that can be achieved under mild conditions makes biocatalysis an attractive, cost-effective industrial process. Novel and improved biocatalysts are continually being sought owing to the high level of enzyme substrate specificity. JM's approach to enzyme discovery is threefold: we look for novel enzymes in protein databases, searching for those sequences that will give us the desired activity; we explore metagenomic libraries, where genetic information is taken directly from soil samples, and we use enzyme engineering to improve the catalytic traits of active enzymes.

> To thrive in this industry, a flexible, expertise-driven approach to enzyme selection and reaction development is needed to maximise economic viability

The route from hit (active enzyme) to industrial catalyst requires know-how and expertise. For an enzyme to become an economically viable catalyst, the substrate concentration has to be increased and the enzyme loading reduced. Protein engineering has an important role during this intensification process. We approach protein engineering by relying on bioinformatics, where substrate binding in the protein's active site can be modelled in silico to identify those amino acids that are more likely to play a role in catalysing the reaction. This rational, knowledge-based approach to mutagenesis allows us to keep the size of our mutant libraries relatively small, i.e. tens of thousands rather than millions of variants. High throughput methods are then required to screen the resulting libraries. Nowadays, technological advances have enabled process automation, allowing whole libraries of enzymes to be tested rapidly. After a suitable catalyst (wild type or engineered enzyme) has been identified, further optimisation of the reaction can be done at small scale in the laboratory. This stage of development is difficult, requiring the provision of case-by-case process chemistry.

The reduction of activated olefins is one successful example where both biocatalysis and chemocatalysis are viable routes and highlights the variety of chemistries that catalysis enables. The stereochemistry of the reduction can be controlled by choosing chemo- or biocatalysis: biocatalysis yields a formal trans addition of hydrogen while chemocatalysis yields the cis addition.

Biocatalysis is an exciting, enabling field that is already revolutionising industrial processes by providing alternative, efficient synthetic routes. To thrive in this industry, a flexible, expertise-driven approach to enzyme selection and reaction development and intensification is needed to maximise economic viability. The choice of whether to use a chemo- or biocatalyst for industrial applications depends on a number of factors specific to the transformation, including finding the most cost-efficient use of time, money and resources. Ultimately, the biggest advantage for industry is being able to make that choice through examining all options.

COMPLEMENTARY TECHNOLOGIES

Jürgen Rotzler - Solvias

Catalysis plays a key role in gaining the economic as well as ecological advantages of modern sustainable chemistry. Consequently, catalysts are currently involved in more than 80% of all manufacturing processes in the chemical industry. Especially in the area of fine chemicals, homogeneous catalysis has become an essential and powerful tool for synthetic organic chemists. Offering high activities and superior selectivities under mild reaction conditions, homogeneous catalysis has outperformed many established stoichiometric reaction systems. Thus, modern homogeneous catalysts find a plethora of applications i.e. hydroformylation, (asymmetric) hydrogenation, oxidation, metathesis, as well as carbonylation and cross-coupling reactions.

> Providing ligands reliably on scale enables customers to implement new, more efficient synthetic pathways and benefit from sustained cost savings

In a similar fashion, biocatalysts have evolved from isolated purely wild type enzymes with often very narrow substrate scope, narrow optimal operating temperature range, pH range, etc. and low volume efficiency to highly engineered and widely applicable catalytic systems. Methods like immobilization on resins, formation of enzyme agglomerates or whole cell systems in combination with new reactor designs allow for efficient use of enzymes as biocatalysts in large scale production. While (dynamic) kinetic resolutions using lipases, asymmetric reduction using ketoreductases or asymmetric reductive amination using transaminases are nowadays well established to form chiral centers on large scale, more and more other enzyme classes find their way towards industrial application. The possibility to tailor the biocatalysts for the substrate of choice is one of the major driving forces.

The usefulness of new catalytic methodologies for larger-scale production is closely associated not only with the streamlining of the process itself (catalyst loading, accessibility of key starting materials, temperature, safety, volume efficiency, etc.) but also with the commercial availability of biocatalysts or chemocatalysts. While the successful development of biocatalysts relies on enzyme evolution, chemocatalyst systems consisting of metal precursors and ligands rely on the availability of a large diversity of ligands which allows evaluating the specific demands made on the catalyst system. In turn, modularity in ligand design is an important key for a successful and fast lead finding and subsequent optimization. The resulting large variety of modular (chiral) ligands has led to significant progress in substrate scope, reaction conditions as well as catalyst costs and availability. Providing these ligands reliably on scale enables customers to implement new, more efficient synthetic pathways and benefit from sustained cost savings in the competitive fine chemical market.

Overall, both, chemocatalysis and biocatalysis, are well established technologies for large scale production. The huge variety of commercially available ligands and the chance to tailor enzymes to specific needs by evolution provides chemists with complementary technologies to tackle daily occurring challenges in organic chemistry.

CHEMOCATALYSIS: AN EVER EVOLVING FIELD

Christophe Le Ret - Umicore

Chemocatalysis is a vital industrial process. In fact, most industrial chemical syntheses now involve at least one catalytic step. Specifically considering homogeneous catalysis, involving metal complex mediated reactions, the large pre-existing catalyst portfolio makes chemocatalysis applicable to virtually any chemical reaction. This versatility is reflected in industrial demand for chemocatalysts, which continues to grow.

> Putting together innovation and academic research, the field will only continue to evolve to deliver even better chemistry

It is important that any chemocatalyst provider works with customers to overcome their unique process challenges. The user's needs are typically twofold. First, they require a catalyst able to perform their given reaction at peak efficiency. Second, the catalyst must offer selectivity, targeting the desired transformation while leaving other functional groups unaffected. It is therefore important to work together with customers, to co-create a bespoke catalytic process that works for their given reaction.

As a given reaction advances from laboratory to industrial scales, catalyst suppliers can also provide expertise for the best manner to maintain both reaction and cost effectivity, while also complying with environmental regulations. These considerations often go hand-in-hand as more efficient catalysts engenders reduced loadings and improved sustainability and cost effectivity. For homogeneous catalysis - and indeed any reaction involving precious metals - the separation and recycling of waste metals is essential from regulatory, environmental or cost-benefit perspectives. One of the process challenges the customer faces is understanding the different methods of metal separation and recycling at the various stages of production. This is an area that catalyst providers can assist any industrial partner in overcoming. At the laboratory scale, waste metals can be separated from the final product using metal scavengers, molecules which bind selectively and strongly to metal catalysts to remove them from the reaction media. While this approach works at a small scale, it might not be commercially viable or cost-effective in larger industrial production. Before reaching this scale,

alternative separation strategies must be designed. These either remove the metal from the reaction media still containing the final product, for instance using activated carbon, or to remove the product from the reaction media containing the metal, for instance by precipitating the product. Either option is viable and only by working with experts can the process from lab to industry be streamlined.

The field of chemocatalysis is ever-evolving. Driven by our improved understanding of chemical processes, innovative chemocatalysts are continually being developed. Cross-coupling and metathesis reactions – respectively subjects of the 2010 and 2005 Nobel Prize in Chemistry - are two examples of innovative chemocatalysis-driven processes. These catalysts are essential for a wide variety of chemistry-enabled fields, spanning from API synthesis to electronic materials. Research is ongoing in this field. Previously metathesis reactions could only form trans-stereoisomers. Now, cis-stereoisomers can reliably be formed, achievable through newly developed catalyst ligands. Offering this synthetic versatility is essential to the field of oleochemical synthesis.

Homogeneous catalysis presents a vital route of industrial chemical synthesis, used by many companies. With ongoing innovation and further academic research, we are confident the field will only continue to evolve to deliver even better chemistry.

ARTIFICIAL ENZYMES FOLD AND THE FUTURE UNFOLDS FOR DESIGNER BIOCATALYSTS

Stéphane Corgié - Zymtronix

We are entering a technological age in which, if you can think it, then you can compute it and make it. 3D printers are appearing in homes and on manufacturing lines to produce objects that are only limited by one's imagination. Similarly, supercomputers have enabled a precise understanding of how proteins fold and how their geometries infer catalytic properties. A field of protein engineering is emerging where computer-generated proteins can be mass produced, sparking the era of de-novo biocatalysts.

A good catalyst has to increase the speed, specificity and productivity of a chemical reaction, it does so by controlling the geometry of the protein scaffold around the to-be converted chemical. Nature has achieved this over billions of years, selecting evolutionary paths for enzymes to be optimally structured such that the folding of their amino acid sequence orientates chemicals in the right configuration, controlling the specificity and chirality of the catalytic reactions. All the industrial enzymes currently used in production are variants of those naturally occurring enzymes.

> The technology of de novo enzymes holds the key to advancing new industrial chemistries

Currently, enzyme engineering requires a natural protein as a blueprint and a deep mechanistic understanding to efficiently tweak its structure. For example, Frances Arnold's team from Caltech has been pioneering the evolution of unique monooxygenases to catalyze valuable oxidative reactions. Some of these reactions, like C-Si coupling, do not even exist in nature. But engineering enzymes with the desired activity, efficiency and stability for the industrial production of chemicals is like finding a needle in a haystack: it requires the screening of tens of thousands of enzyme mutants derived from the natural sequence blueprint. Heavy automation by robotic platforms that transform, clone and assay enzymes, combined with big data analyses, are required to artificially evolve natural enzymes for peak industrial performance in a timely fashion.

What if you could quickly find the proverbial needle in the haystack and produce the right industrial biocatalyst at scale without the need for a natural protein blueprint? This is the approach taken with de-novo enzymes – biocatalysts designed and selected in computers and to be synthesized from scratch. This revolution arises from the science of predicting via computation how the sequence of a protein folds and understanding how that fold provides stability and catalytic activity in different environmental conditions, such as variations in temperature and pH.

David Baker's team at the University of Washington is leading the field by finding the rules of protein folding and using those rules to design proteins with computer algorithms. In parallel, Kendall Houk's team at UCLA, with the help of supercomputers, is exploring the chemical reactions these digital enzymes can perform. Their collaboration is now imagining, drawing, computing, and synthesizing active purely man-made biocatalysts. These artificial enzymes work as well as the natural ones. Michael Hecht's group at Princeton University recently designed a de-novo hydrolase and incorporated it in a bacterium to replace a key enzyme in iron metabolism.

In combination with innovations in industrial processes, the technology of de-novo enzymes holds the key to advancing new industrial chemistries driven by the rapid and rational-design of biocatalysts.