Biocatalysis

As introduced in Topic 1 the tools of modern molecular biology have led to an explosion in the number and range of biocatalysts available for use. Bespoke recombinant mutant enzymes for organic synthesis can now be rapidly developed and biocatalysis is now an underpinning part of the green chemistry tool box to enable safe and low environmental impact synthesis. Many classes of enzyme of interest to the synthetic organic chemist are now available commercially. Biocatalysis can provide unique catalytic opportunities not accessible with conventional chemistry and can also facilitate efficient shorter routes to complex chiral intermediates.

Whilst biocatalysts may offer various advantages over conventional routes, one should also be aware that significant lifecycle burdens are often generated during the downstream processes for bio-reactions. [1] The product streams from bio-reactions are typically dilute aqueous solutions, which can bring specific problems that need to be addressed from both an environmental and process optimisation standpoint. For example large amounts of organic solvents may be used for extraction. [2] All new methodologies developed should be assessed from a holistic perspective, in order to ensure that potential environmental 'hotspots' are not missed downstream from the actual synthetic step: see Topic 2: Metrics for further study on holistic assessment of methodologies.

Learning Objectives

By the end of this module you should:

- Be aware of the concept of biocatalytic retrosynthesis and the potential of enzyme catalysts to offer assistance in synthetic chemistry;

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- Understand how enzyme catalysts can be as applied to the synthesis of certain target molecules and functional groups;
- Be able to consider biocatalysts within your retrosynthetic analysis.

1. S. Kim, C. Jimenez-Gonzalez and B. E. Dale, Enzymes for pharmaceutical applications—a cradle-to-gate life cycle assessment, *Int. J. LCA*, 2009, **14**, 392-400.
2. C. Jimenez-Gonzalez and J. M. Woodley, Bioprocesses: Modeling needs for process evaluation and sustainability assessment, *Comput. Chem. Eng.*, 2010, **34**, 1009-1017.

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Introduction to enzyme catalysis and biocatalytic retrosynthesis

Biocatalysis can present a number of advantages including reduced cost of goods, improved environmental impact, better safety profiles, improved step and atom economy and reduced solvent usage, all of which can contribute to more sustainable manufacturing processes. A major development in the field of biocatalysis is the impact that protein engineering and directed evolution have made on the properties of biocatalysts. Another, relatively new concept is that of designing new synthetic routes to targte molecules using 'biocatalytic retrosynthesis' . With biocatalytic retrosynthesis, molecules are disconnected in a different way than if solely chemical reagents are relied upon leading to the development of new pathways and new ways of making molecules.

What is biocatalytic retrosynthesis?

E. J. Corey pioneered retrosynthetic analysis in organic molecules: the process of 'deconstructing' a target molecule into readily available starting materials. [1] Now, with biocatalysis surging and rightly staking a claim to be a viable option for chemical synthesis, we need to rethink the way in which target molecules can be constructed with the assistance of enzymes. 'Biocatalytic retrosynthesis' has potential as a construction and optimisation methodology and is becoming increasingly important for biochemical pathway design and generation.

Very few organic textbooks include enzyme-catalyzed reactions as options for synthesis during retrosynthetic analysis. The potential impact of enzymes in the synthesis of complex molecules has not been fully realised

The following reference provides an excellent overview of biocatalytic retrosynthesis:

N. J. Turner and E. O'Reilly, Biocatalytic retrosynthesis, *Nat Chem Biol*, 2013, **9**, 285-288.

1. E. James Corey, The Logic of Chemical Synthesis: Multistep Synthesis of Complex Carbogenic Molecules (Nobel Lecture), *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 455-

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465.

Categorisation of enzymes

In this video Bettina Nestl introduces the six major enzyme classes and the types of reactions that can be performed by them.

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Transcript

So what are the six big classes of enzymes or bio-catalysts we talked about? And here, i have summarized the six major enzyme classes and some of the selected reactions which can be performed by using these enzyme catalysts.

So some of these enzymes catalysts have a vibrant range of activities and activity is based on the nature.

And therefore it's like the enzyme Commission has ordered units by combining like these certain reactions and into like enzyme classes or EC numbers.

And the first enzyme class which has the EC number 1 are the oxidoreductases.

And the oxidoreductases, the name says it already, they do perform oxidation and reduction reactions.

So some of the selected reactions of oxidoreductases are the reduction of carbonyls, imines and C=C bonds.

Also on oxidoreductases are able to perform reductive amination of carbonyls.

And the oxidation of alkenes, alkanes, and C-N and C-O bonds.

Oxidoreductases mostly need co-factors, therefore we have to also consider the regeneration of the co-factor which is applied in the catalysis using oxidoreductases.

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So it's like the co-factor is essential for activity in order to perform the reduction and oxidation chemistry.

The second class are the transferases and the name already says it.

Transferases catalyze the functional group; like they transfer functional group from one molecule to another.

And the transfer of functional groups include amino groups, acyl, phosphoryl, methyl glycosyl, nitro and sulfur containing groups.

These are just a few examples of the functional groups which can be converted or transformed from one molecule to another and which are catalyzed by these big class of transferases.

The third class are the hydrolases.Tthe hydrolases are the biggest class of enzymes which are applied in industry nowadays, and hydrolases catalyze the hydrolysis of esters, amides, lactones, lactams, epoxides and nitriles.

You see that a lot of certain compounds of substrates can be targeted by using this hydrolases.

But hydrolases can not only perform the hydrolysis of those compounds, they can also catalyse the reverse reaction.

They can also form esters, or amides, or lactones and for the reverse reaction you can use it like this hydrolases which is also remarkable to this class of enzymes.

You can do it like this reaction in organic solvents, and purely organic solvents.

The fourth class of enzymes are the lyases.

And the lyases are enzymes which catalyze the cleavage and formation of our bonds.

So that we can add small molecules to double bonds, such as C=C double bond C=N double bond and C=O bonds.

A few examples that we give on these lyases so that you can see what is the chemistry behind them.

And at the moment there's a big interest in increasing the toolbox of lyases, because it's

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like we need more enzymes for the formation of new molecules or products.

The fifth class are the isomerases; the isomerases are a rather small class of enzymes, and they catalyse the interconversion of isomers.

The name says it already, so they are involved in isomerisations.

And these isomerisations include racemisations, epimerisations and rearrangement reactions.

The last, and the sixth class, are the ligases; the ligases are a rather small class of enzymes.

And the ligases are also called sintertases and they are involved in the formation of complex compounds and usually they need ATP for doing this.

So this is like a small overview of the enzyme classes and some of the reactions which can be addressed by using those enzymes..

Mechanism of enzyme catalysis

In this video Bettina Nestl explains the four major mechanisms of enzyme catalysis.

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Transcript

I just want to summarise the four major mechanisms of enzyme catalysis.

Enzymes can use a variety of mechanisms in order to catalyze the reaction or in order to convert one substrate into the corresponding product.

Here you see the four major mechanisms of enzyme catalysis which are called the proximity effects, the acid/base catalysis, the nucleophilic catalysis and the metal ion

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catalysis.

The proximity effect is also called catalysis by approximation and it means that it includes direction of two substrates and you have to bring these two substrates closely to each other in order to reach this enhancement in the rate, in order to perform this reaction.

So the substrates have to be taken in a proper orientation in order to be coupled and also in order to be transformed into the product.

One of the biggest mechanisms in catalysis is still acid base catalysis and therefore amino acids, in the active sites of these enzymes, take over the role of water and therefore either donate protons or accept protons.

Furthermore we have some amino acids which can be acting as nucleophiles and therefore can undergo covalent catalysis.

For instance, serine/cysteine is a good example of a nucleophilic amino acid in the active side which can act as a nucleophile and therefore can perform a certain chemical reaction.

And then, some of the cofactors we were mentioning before are metal ions.

Most of the enzymes contain metal ions as cofactors and therefore it's a huge impact of metal ion catalysis in enzyme catalysis and these metal ions can serve as electrophilic catalysts; they can stabilize negative charges on reaction intermediates so they are very useful for the enzyme catalysed reaction..

Individual enzyme classes

In this video Bettina Nestl goes through the individual enzymes classes in turn and looks in more detail at some of the reactions that can be catalysed by them.

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Enzymes from a retrosynthetic perspective

Following on from the previous lesson where the different enzyme classes and their mechanisms were discussed, this lesson looks at the use of these enzymes in the synthesis of certain target molecules and functional groups. The enzymes have been systematically analysed and classified as to which functionalities can be produced from a retrosynthetic perspective. For some functionalities a number of suitable enzymes are available that are capable of performing the transformation to produce a given functionality, in other cases only a small number of enzymes exist.

Details of enzymes

Click each reaction below to listen to Bettina Nestl talk about enzymes from a retrosynthetic perspective.

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Case studies

In this lesson we will look at some examples where biocatalysis has been employed for the synthesis of Active Pharmaceutical Ingredients (APIs). This will demonstrate the potential impact of biocatalysis in the pharmaceutical industry, in terms of a move towards lower environmental impact manufacturing processes, as well as making important medicines on the required scale at an affordable price.

CHEM21 Case Study: Reductive Amination

Chiral amines are an important class of organic compounds that serve as key intermediates in the synthesis of a variety of biologically active molecules.[1] The formation of C=N bonds by the condensation of carbonyls and amines, followed by reduction of the *in situ* formed imine represents the most straightforward strategy for the generation of chiral amines.

Most work has focused on the transition metal-catalyzed asymmetric hydrogenation of imines and particular attention was paid to the reductive amination of carbonyl compounds with primary amines. Several reagents and combinations have been reported through the years with a predominant role being played by modified borohydride reagents.[2] Inspired by the biological system NAD(P)H, Hantzsch ester has been employed as a hydrogen donor in combination with chiral phosphoric acid for the enantioselective reductive amination of aliphatic ketones to furnish amines with excellent enantioselectivities.[3][4][5]

The application of biocatalysis is an important complement to chemical catalysis for chiral amine synthesis in water. Two main types of enzymes were investigated so far for the transformation of carbonyl groups into amines: amino acid dehydrogenases and w-transaminases. Researchers within CHEM21 have identified imine reductases[6] as biocatalyst for chemo- and stereoselective intermolecular reductive aminations. This novel imine reductase-catalyzed approach constitutes a direct method for the synthesis of valuable amines under mild reaction conditions.[7]

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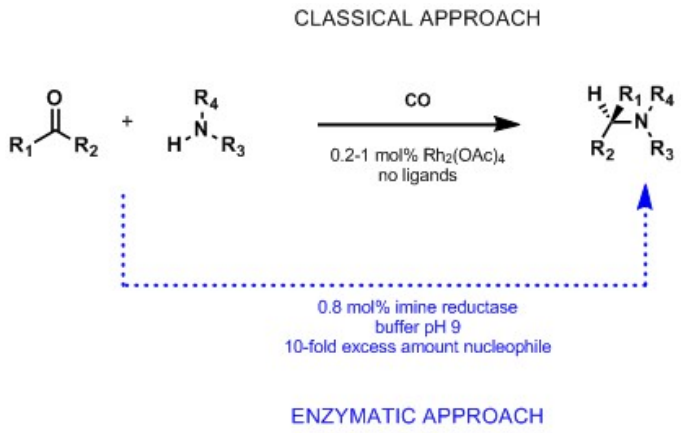

Scheme 1: ***Classical and novel reductive amination (Hauer et al, 2015; Chusov and List, 2014 [7][8])***

This approach represents a simple and complementary strategy for the generation of novel carbon nitrogen bonds in aqueous reaction media. These reactions proceed efficiently for a variety of carbonyl compounds and amine nucleophiles. Low amounts of the imine intermediate led to the formation of valuable amines in good yields and high selectivities. A highly chemo- and stereoselective imine reductase was applied in reductive aminations of a panel of carbonyls and amine nucleophiles to afford primary and secondary amine products in moderate to high yield as well as enantiomeric excess.

1. D. Ghislieri and N. J. Turner, Biocatalytic Approaches to the Synthesis of Enantiomerically Pure Chiral Amines, *Top Catal*, 2014, 57, 284-300.
2. T. C. Nugent and M. El-Shazly, Chiral Amine Synthesis – Recent Developments and Trends for Enamide Reduction, Reductive Amination, and Imine Reduction, *Advanced Synthesis & Catalysis*, 2010, **352**, 753-819.
3. V. N. Wakchaure, J. Zhou, S. Hoffmann and B. List, Catalytic Asymmetric Reductive Amination of α-Branched Ketones, *Angewandte Chemie International Edition*, 2010, **49**, 4612-4614.
4. W. Li and X. Zhang, *Formation of Amines*, Springer, Berlin, 2014.
5. S. Hoffmann, A. Majeed Seayad and B. List, A Powerful Brønsted Acid Catalyst for the Organocatalytic Asymmetric Transfer Hydrogenation of Imines, *Angewandte Chemie International Edition*, 2005, **44**, 7424-7427.

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6. P. N. Scheller, S. Fademrecht, S. Hofelzer, J. Pleiss, N. J. Leipold Friedemann and Turner, B. M. Nestl and B. Hauer, Enzyme toolbox: novel enantiocomplementary imine reductases, *Chembiochem*, 2014, **15**, 2201–2204.
7. P. N. Scheller, M. Lenz, B. Hammer Stephan C and Hauer and B. M. Nestl, Imine Reductase-Catalyzed Intermolecular Reductive Amination of Aldehydes and Ketones, *ChemCatChem*, 2015, **7**, 3239–3242.
8. D. Chusov and B. List, Reductive Amination without an External Hydrogen Source, *Angewandte Chemie International Edition*, 2014, 53, 5199-5201.

CHEM21 Case Study: Whole-cell Imine Reductases for Asymmetric Reduction of Cyclic Amines

As important structural motifs, chiral amines are found in a variety of industrially relevant compounds such as pharmaceuticals, agrochemicals and fine chemicals.[1] A variety of methods are available for their synthesis that include metal catalysed and organocatalysed asymmetric synthesis; despite this the most commonly used method in industry to obtain chiral amines is through recrystallization (**Scheme 1**) or kinetic resolution,[2][3] with chiral cyclic amines more commonly obtained through recrystallisation. As such the maximum theoretical yield for the desired enantiomer is capped at 50%. The use of co-crystallising agent also means that the process uses up a stoichiometric amount of reagent that is destined for waste, essentially making the process mass intensive.

Scheme 1: ***Chiral amines are obtained in industry by co-crystallisation with chiral agent***

The alternative is to consider the use of biocatalysis for the synthesis of chiral cyclic amines, in particular those biocatalysts that can be modified and enhanced through

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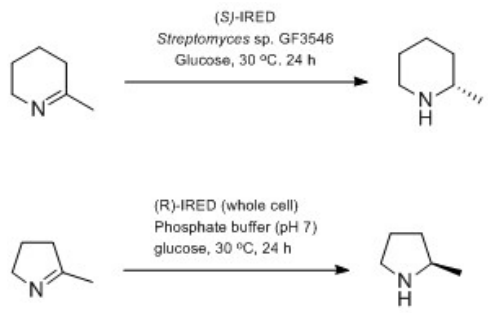
protein engineering and directed evolution. These include monoamine oxidases (MAO-N), phenyl alanine ammonia lyases and aminotransferases however, the majority of these biocatalysts are limited to the synthesis of primary amines. On the other hand, the asymmetric reduction of imines offers an alternative route for the generation of these molecules; this approach has been examined in chemocatalytic approaches as well as in tandem with biocatalysts, however studies into the enzymatic approach have been limited.[4]

Imine reductases (IREDs) catalyse the reduction of imines to amines by using NADH or NADPH as cofactors. These enzymes from different strains of *Streptomyces* sp. have been previously shown to reduce prochiral cyclic imines to their corresponding chiral amines, and the sequences for the two enantiocomplimentary IREDs from *Streptomyces* sp. GF3587 and *Streptomyces* sp. GF3546 have been reported.[5] Researchers in the CHEM21 consortium have investigated the applicability and scope in substrate of both *R*- and *S*-imine reductases by expressing them in *Escherichia coli*. and found that the (*S*)-IRED can mediate the enantioselective reduction of five-, six- and seven-membered imines as well as dihydroquinolines, β-carbolines and iminium ions in high enantiomeric excess (*ee*).[6] The (*R*)-IRED, overexpressed in *E. coli* also displayed a broad substrate scope, allowing the highly enantioselective preparation of chiral cyclic secondary amines that carry broad structural features, moreover the enzyme showed higher activity than has been previously reported, demonstrating its potential for applicability on industrial scale (**Scheme 2**).[4]



Scheme 2: ***Enantiocomplementary IREDs investigated by the CHEM21 researchers (Turner et al. 2013, 2015 [4][5])***

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1. M. E. Welsch, S. A. Snyder and B. R. Stockwell, Privileged scaffolds for library design and drug discovery, *Current Opinion in Chemical Biology*, 2010, **14**, 347-361.
2. D. L. Silverio, S. Torker, T. Pilyugina, E. M. Vieira, M. L. Snapper, F. Haeffner and A. H. Hoveyda, Simple organic molecules as catalysts for enantioselective synthesis of amines and alcohols, *Nature*, 2013, **494**, 216-221.
3. M. Binanzer, S. - Y. Hsieh and J. W. Bode, Catalytic Kinetic Resolution of Cyclic Secondary Amines, *Journal of the American Chemical Society* , 2011, 133, 19698-19701.
4. S. Hussain, F. Leipold, E. Man Henry and Wells, S. P. France, G. Mulholland Keith R and Grogan and N. J. Turner, An (R)-Imine Reductase Biocatalyst for the Asymmetric Reduction of Cyclic Imines, *ChemCatChem*, 2015, **7**, 579–583.
5. Patent EP2330190, 2011.
6. F. Leipold, S. Hussain, D. Ghislieri and N. J. Turner, Asymmetric Reduction of Cyclic Imines Catalyzed by a Whole-Cell Biocatalyst Containing an (S)-Imine Reductase, *ChemCatChem*, 2013, **5**, 3505-3508.

CHEM21 Case Study: Biocatalysis in Bio-derived Solvents

To study this area in more depth, see Biocatalysis

This case study was provided by Giulia Paggiola during her time at the Green Chemistry Centre of Excellence, University of York

One of the major obstacles to industrial uptake of biocatalysis is the difficulty in achieving similar reaction conditions to those of organic chemical reactions.[1] In biotransformations, water is traditionally used as the reaction solvent. However, there are many associated issues with the use of water as the reaction medium. These include issues with the solubility of the organic substrates (which generally require the addition of an organic water-soluble co-solvent), purification and enzyme recovery, as well as the incurred cost and environmental impact of contaminated aqueous waste treatment.[2]

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These issues can be overcome by the use of organic solvents as the reaction media, an approach that is well established in academic research but is not common in industrial applications.[3][4] However the approach to the use of organic solvents in enzymatic reactions has been somewhat incidental. There have been limited studies into the use of "green" solvents as reaction media for enzymatic transformations and include the use of ionic liquids, supercritical CO_2 and some bio-derived solvent.[5][6] The CHEM21 researchers investigated a more systematic approach in assessing the behaviour of supported *Candida Antarctica* lipase B (Novozyme 435) in 24 organic solvents including classical as well as bio-derived solvents (**Figure 1**); this was investigated through carrying out a kinetic study of the reaction of hexanol with dodecanoic acid to produce the industrially relevant hexyl laurate, used in the personal care and cosmetics industries.[2]

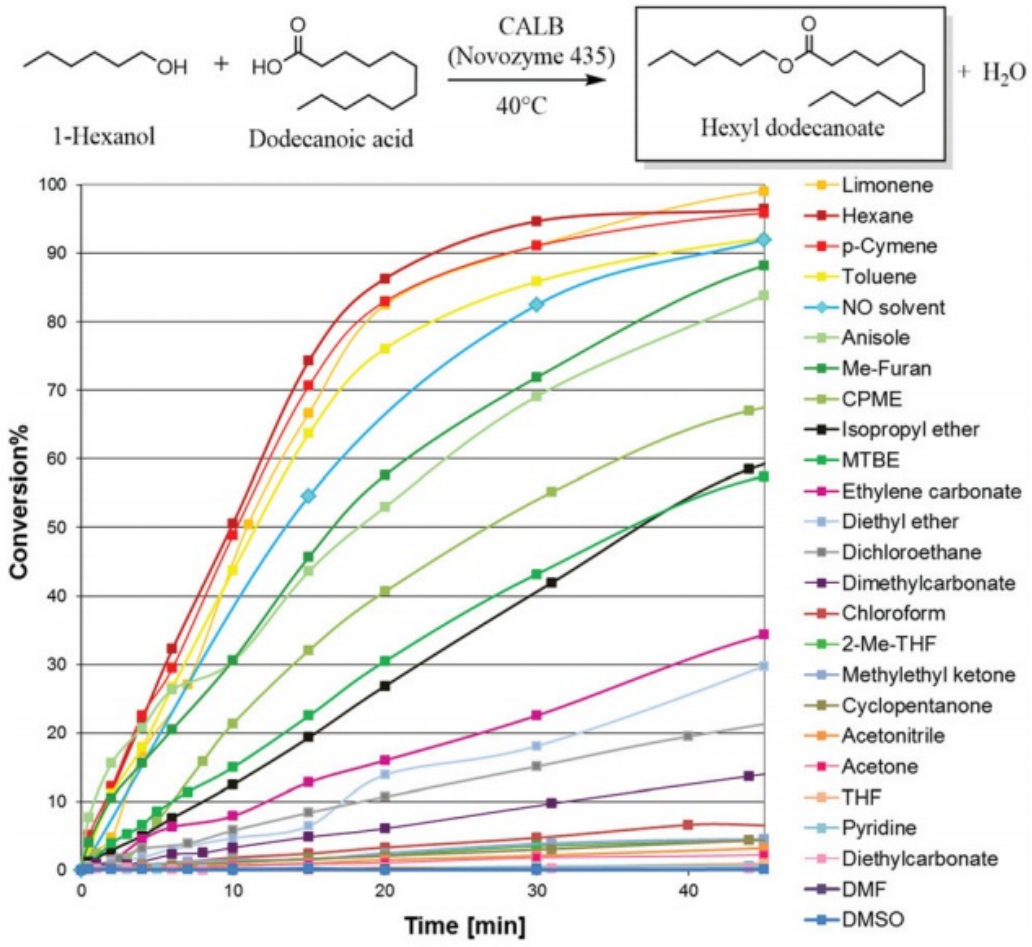

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Figure 1: ***Kinetic Screening for Novozyme 435 mediated esterification reaction of hexanol with dodecanoic acid, in a variety of organic solvents (Reproduced from G. Paggiola et al [2] with permission of The Royal Society of Chemistry)***

The researchers observed a correlation between high activity of Novozyme 435 and solvent properties, specifically hydrogen-bond accepting ability and molar volume. In comparison to previous studies, the study found that molar concentration was an important factor; it is hypothesised that this is related to the partition coefficient (log P) and its ability to remove structural water from the enzyme active site. The bioderived solvents that performed best were limonene and *p*-cymene with the former outperforming the classically used hexane, offering an effective and sustainable approach for industrial fatty acid ester synthesis.[2]

1. S. Wenda, S. Illner, A. Mell and U. Kragl, Industrial biotechnology-the future of green chemistry?, *Green Chem.*, 2011, **13**, 3007-3047.
2. G. Paggiola, A. J. Hunt, J. McElroy Con R and Sherwood and J. H. Clark, Biocatalysis in bio-derived solvents: an improved approach for medium optimisation, *Green Chem.*, 2014, **16**, 2107–2110.
3. *Enzyme Catalysis in Organic Synthesis*, (ed. H. Gröger and O. May) Wiley-VCH Verlag GmbH & Co. KGaA, 2012.
4. C. Jimenez-Gonzalez, P. Poechlauer, Q. B. Broxterman, B. - S. Yang, Dam Ende, J. Baird, C. Bertsch, R. E. Hannah, P. Dell'Orco, H. Noorman, S. Yee, R. Reintjens, A. Wells, V. Massonneau and J. Manley, Key Green Engineering Research Areas for Sustainable Manufacturing: A Perspective from Pharmaceutical and Fine Chemicals Manufacturers, *Org. Process Res. Dev.*, 2011, **15**, 900-911.
5. M. J. Hernáiz, A. R. Alcántara, J. I. García and J. V. Sinisterra, Applied Biotransformations in Green Solvents, *Chem. Eur. J.*, 2010, **16**, 9422-9437.
6. Z. Yang and W. Pan, Ionic liquids: Green solvents for nonaqueous biocatalysis, *Enzyme Microb. Technol.*, 2005, **37**, 19-28.

API case studies

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Content coming soon.

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Study exercises

Once you have worked through the below exercises by completing the gaps highlighted in blue, you can compare your answers to these worked examples.

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Summary and further reading

In this module we have seen that many enzymes can be applied in biocatalytic retrosynthetic analysis, and it is important that this is applied more often in route design, combining bio and chemo-catalysts to design new routes to target molecules. To achieve this there are some remaining challenges in the field, including the identification of reactions not available in the enzyme tool box and the design of new biocatalysts with broad substrate scope, that are highly active and stable under chemical process conditions, via the combination of enzyme engineering with bioinformatic methods.

Recommended reading:

A. S. Wells, Biocatalysis for Medicinal Chemistry, in *Green and Sustainable Medicinal Chemistry: Methods, Tools and Strategies for the 21st Century Pharmaceutical Industry*, The Royal Society of Chemistry, 2016, ch. 15, pp. 180-191.

C. M. Clouthier and J. N. Pelletier, Expanding the organic toolbox: a guide to integrating biocatalysis in synthesis, *Chem. Soc. Rev.*, 2012, **41**, 1585-1605.

G. W. Huisman and S. J. Collier, On the development of new biocatalytic processes for practical pharmaceutical synthesis, *Curr. Opin. Chem. Biol.*, 2013, **17**, 284-292.

Enzyme Catalysis in Organic Synthesis, (ed. H. Gröger and O. May) Wiley-VCH Verlag GmbH & Co. KGaA, 2012.

Biocatalysis in Organic Synthesis, *Science of Synthesis* (ed. W. - D. Fessner and N. J. Turner) vol. 1, 2015.

K. Faber, *Biotransformation in Organic Chemistry*, Springer Verlag, Sixth., 2011.

S. Warren and P. Wyatt, *Organic Synthesis: The Disconnection Approach*, John Wiley and Sons, 2008.

N. J. Turner and E. O'Reilly, Biocatalytic retrosynthesis, *Nat Chem Biol*, 2013, **9**, 285-288.

M. Breuer, K. Ditrich, T. Habicher, B. Hauer, M. Keßeler, R. Stürmer and T. Zelinski,

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Industrial Methods for the Production of Optically Active Intermediates, *Angew. Chem. Int. Ed.*, 2004, **43**, 788-824.

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