Synthetic biology

Synthetic biology has been subject to numerous definitions, however all of the available ones agree that synthetic biology is the study of applying the principles of engineering to biology for productive purposes: the use of synthetic biology tools to deliberately design and construct biological systems or their parts to serve a particular purpose. A key area in synthetic biology is the construction of living “production plants” for the manufacture of complex chemical compounds from simpler starting materials, other research areas include bioinformatics (using synthetic gene networks), synthetic genomics and genome minimisation as well as the biosafety and societal aspects of synthetic biology among others. [1]

1. B. Wiltschi and A. Glieder, Synthetic Biology for Organic Syntheses, in *Green and Sustainable Medicinal Chemistry: Methods, Tools and Strategies for the 21st Century Pharmaceutical Industry*, The Royal Society of Chemistry, 2016, ch. 14, pp. 165-179.

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What is synthetic biology?

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In synthetic biology, “chassis” cells –cells within which the desired metabolic pathways can be expressed– are considered living cells that possess a basic metabolic pathway to produce primary metabolites and are capable of essential chemical transformations such as the production of energy and co-factor regeneration.

The chassis can thus be furnished with a desired function such as the ability to produce a chosen chemical compound; whether the compound is natural or non-natural does not hold a bearing on the process as long as the genetic information for driving its synthesis is available or can be designed. The chassis is endowed with the appropriate genetic system that consists of devices (e.g. different DNA vectors), made of different parts (e.g. promoters, terminators, ribosome binding sites), **Figure 1** below.[1]

The concept of a chassis cell and its genetic system given in Figure 1 is an overly simplified representation; even in the simplest unicellular life form, genetic and metabolic processes do not work independently but have complex interactions in the cellular environment. However, the modular approach for the systematic assembly of a more complex genetic system from the well-defined essential parts is useful and essential for a systematic approach, as such powerful strategies that link the constituent parts together in an ordered, parallel or combinatorial manner have been developed.[2]

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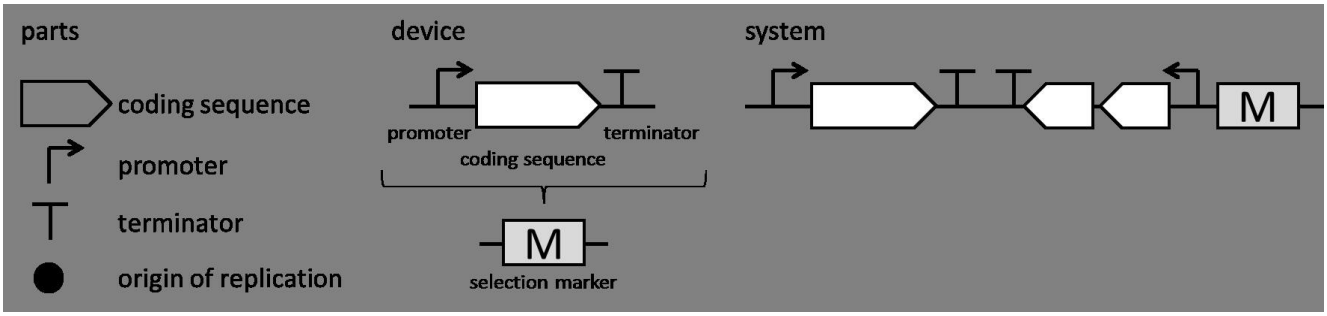

Figure 1: ***Parts, devices and systems - the make of a chassis cell (reproduced directly from Glieder,[1] with permission from the Royal Society of Chemistry)***

1. B. Wiltschi and A. Glieder, Synthetic Biology for Organic Syntheses, in *Green and Sustainable Medicinal Chemistry: Methods, Tools and Strategies for the 21st Century Pharmaceutical Industry*, The Royal Society of Chemistry, 2016, ch. 14, pp. 165-179.
2. B. M. Nestl, S. C. Hammer, B. A. Nebel and B. Hauer, New Generation of Biocatalysts for Organic Synthesis, *Angew. Chem. Int. Ed.*, 2014, **53**, 3070-3095.

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Benefits of biosynthetic approaches

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The biggest challenges to the production of fine chemicals on large scale include environmental pollution, toxic waste, and the increasing cost of prevention and clean-up of environmental waste. Compared with chemical synthesis, biosynthetic approaches can offer several advantages; enzymes generally operate under biologically relevant conditions such as ambient temperatures and pressures, aqueous media and neutral pHs. This enables an array of diverse enzymes to work concurrently in the same environment e.g. a cell, as such it becomes possible to assemble a multistep synthetic pathway for the *in vivo* production of a complex molecule from cheap and abundant materials. This is in great contrast to traditional chemical multistep syntheses which would require purification, recovery and further processing of intermediates *en route* to the final product. [1]

These characteristics of biotransformation facilitate “one-pot synthesis”, which is a challenging approach in chemotransfomations. The use of biosynthesis would thus circumvent the need for energy intensive and harsh reaction conditions, toxic or caustic chemicals and would enable the reactions to be carried out in greener solvents.[1] Bioderived chemicals would also limit the greenhouse gas emissions and the cost through use of cheaper simpler starting materials such as CO_2,CO, hydrogen and salts.[2]

Although the repertoire of products made by naturally occurring organisms is broad, it is still predefined by evolution as the organisms have adapted their biochemistries for survival in particular environments. Synthetic biology can be used to modify the biochemistries of these organisms and tailor their metabolic pathways towards the

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production of pharmaceutically relevant products or intermediates from simple molecules.[3]

1. D. R. Nielsen and T. Seok Moon, From promise to practice: The role of synthetic biology in green chemistry, *EMBO Rep.*, 2013, 14, 1034-1038.
2. B. Wiltschi and A. Glieder, Synthetic Biology for Organic Syntheses, in *Green and Sustainable Medicinal Chemistry: Methods, Tools and Strategies for the 21st Century Pharmaceutical Industry*, The Royal Society of Chemistry, 2016, ch. 14, pp. 165-179.
3. B. M. Nestl, S. C. Hammer, B. A. Nebel and B. Hauer, New Generation of Biocatalysts for Organic Synthesis, *Angew. Chem. Int. Ed.*, 2014, **53**, 3070-3095.

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Applications

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This approach has already found its way into industrial scale production of a variety of important molecules such as the production of insulin from *E. coli* and more recently in the production of naturally occurring bio-products such as the plastic monomers succinic acid and lactic acid, and *n*-butanol as a solvent and next generation biofuel.[1] This area is still in its nascence, but as it begins to mature it has the potential to carry significant impact on the green credentials of the pharmaceutical and fine chemical industry. A review on recent developments in the field of synthetic biology in the pharmaceutical industry has been published by Breitling and Takano.[2]

1. B. Wiltschi and A. Glieder, Synthetic Biology for Organic Syntheses, in *Green and Sustainable Medicinal Chemistry: Methods, Tools and Strategies for the 21st Century Pharmaceutical Industry*, The Royal Society of Chemistry, 2016, ch. 14, pp. 165-179.
2. R. Breitling and E. Takano, Synthetic biology advances for pharmaceutical production, *Curr. Opin. Biotechnol.*, 2015, **35**, 46-51.

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Case studies

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XCase study 2

Stereoselective C-H amination is a very attractive strategy for the conversion of simple low cost chemical starting materials to high value chiral amine building blocks, and has therefore attracted much interest in organic chemistry. α-Methylbenzylamine is a substructure of many complex organic molecules and can therefore be a valuable intermediate in their synthesis. [1][2][3]

The most successful chemical strategies reported so far have involved intramolecular and transition-metal catalysed reactions[4] with a number of methods using both chemical[5] and enzymatic[6][7] catalysts. A cascade of two enzymes was reported recently for the terminal amination of fatty acid methyl esters[8] employing a recombinant biosynthetic cascade in a whole cell system. Previous work had already shown that whole-cell biotransformation is able to produce chiral amine from secondary alcohol, albeit in low conversion of 3%.[9]

Given that natural biosynthetic cascades often suffer from limited substrate ranges, CHEM21 researchers were interested to generate a *de novo* biosynthetic multi-enzyme cascade that is guided in design and build by retrosynthetic considerations.[10] The target enzymes for the cascade were carefully chosen to be complementary in substrate recognition. By introducing the pathway into *Escherichia coli* CHEM21 researchers generated a biocatalyst which converts ethylbenzenes **1a-e** to predominantly (R)-1-phenylethanamines **4a-e** respectively (Figure 1) with conversions of up to 26% and ee values of 97.5%. Under the present reaction conditions, no additional co-factor except for the amine donor IPA and molecular oxygen was required. The CHEM21 approach is the first fully enzymatic approach to generate chiral amines at the benzylic position from alkyls.[11]

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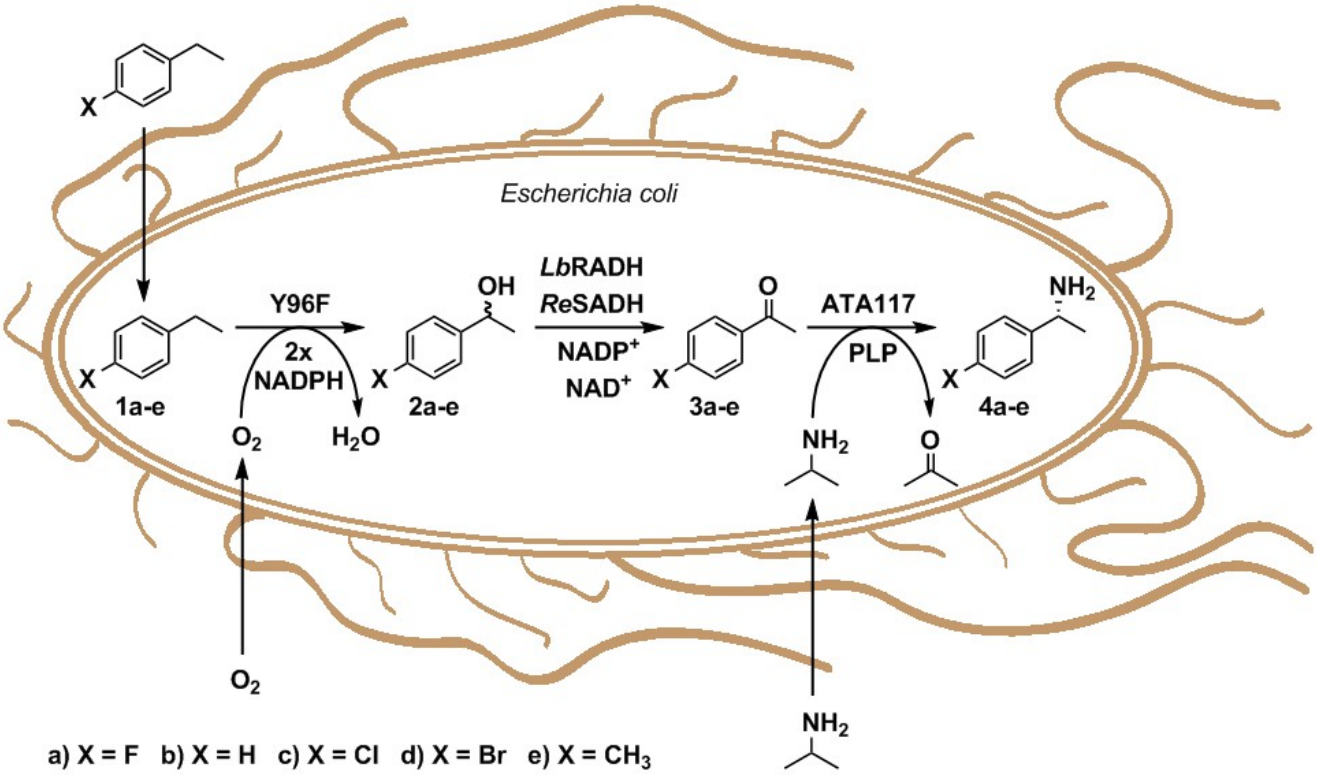

Figure 1: ***E.coli BL21(DE3) cells harbouring a self-sufficient P450 monooxygenase (Y96F)[12][13][14] which produces scalemic mixtures of alcohols with a general preference for the (R)-enantiomer,[15] (R)- and (S)-selective alcohol dehydrogenases (LbRADH using $NADP^+$ [16][17][18][19][20] and ReSADH using NAD^+ as co-factor[21][22][23]), and an w-transaminase (ATA117 requiring an amine donor and pyridoxal 5'-phosphate (PLP)[24][25][26]) capable to convert 4-substituted ethylbenzenes 1a-e into alcohols 2a-e, ketones 3a-e andfinally amines 4a-e (Flitsch et al., 2016 [11]).***

The flexible generic nature of the design of the cascade will allow for the easy substitution of individual enzymes with homologues or mutants and thereby provide a cassette based modular approach for the design and construction of alternative cascades for the enantioselective C-H amination of other substrates.

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XCase study 3

The stereo- and regioselective oxidative functionalisation of olefins is amongst the most challenging reactions in organic chemistry and much effort has been made to develop selective methodologies that cover a broad range of substrates. The asymmetric dihydroxylation is ideally suited for the preparation of chiral building blocks for asymmetric synthesis including the synthesis of pharmaceuticals, fine chemicals, agrochemicals, polymers and natural products.

The most widely applied technique for the preparation of enantiomerically pure cis-diols is the Sharpless dihydroxylation. Using catalytic amounts of osmium(VIII)-oxide in combination with a secondary oxidant and a chiral cinchona alkaloid ligand, various functionalised and non-functionalised olefins of different classes can be converted into their corresponding diols yielding good to excellent stereoselectivities for five of the six alkenes (*mono*-, *gem*-di-, *trans*-di-, tri- and tetra-substituted alkenes). However, in addition to their toxicity, these metal catalysts can also lead to by-product formation due to over-oxidation and cleavage of the diol products.[1][2]

Analogously to chemical strategies, nature has evolved biocatalysts that are highly selective and have been shown to catalyse a wide variety of challenging oxidation reactions. Nevertheless, the diversity of accessible *cis*-diols is limited by enzyme availability. Despite the vast number of characterised hydroxylating enzymes, only Rieske non-heme iron oxygenases (ROs) display the remarkable ability to stereoselectively introduce two hydroxyl groups in one enzymatic step.[3] Like the well-known P450 monooxygenases, these versatile biocatalysts display a relaxed substrate specificity and furthermore can catalyse various other oxidation reactions including monohydroxylations, dealkylations, desaturations, epoxidations and oxidative cyclizations. [3][4][5][6][7][8]

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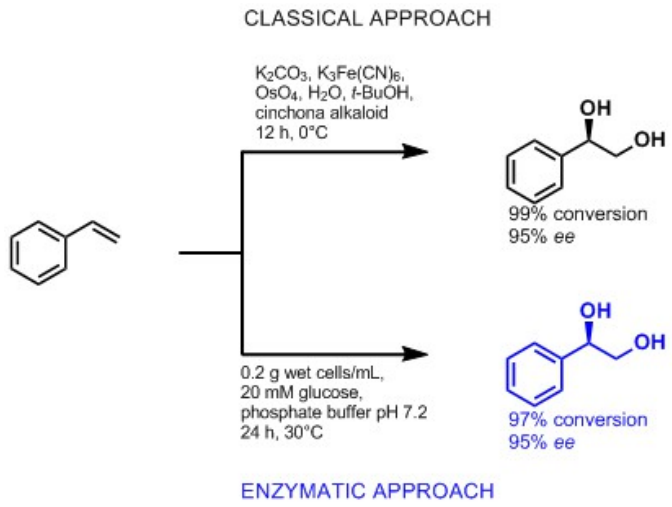

Scheme 1: ***Classical and novel asymmetric dihydroxylation approach to the oxyfunctionalization of alkenes(Hauer et al, 2015; Kumar et al., 2004 [7][8])***

Rieske non-heme iron oxygenases (ROs) represent promising biocatalysts for oxyfunctionalization as they can be engineered to efficiently catalyse the selective mono- and dihydroxylation of various olefins. The introduction of a single point mutation improved selectivities (≥ 95%) and conversions (> 99%) towards selected alkenes. By modifying the size of the amino acid side chain, CHEM21 researchers were able to modulate the regio- and stereoselectivity of these enzymes. For distinct substrates, mutants displayed altered regioselectivities or even favoured opposite enantiomers compared to the wild type ROs, offering a sustainable approach for the oxyfunctionalisation of a wide variety of structurally different olefins.[8]

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Summary and further reading

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