Sustainable Synthesis of Chiral Amines:

The HIMS-Biocat Way

Chirality and chiral amines

Chirality (derived from Greek "hand") is a geometric property observed in some molecules, particularly organic molecules. A molecule is chiral if it is not superposable with its mirror image in the same way as a right hand is not superposable with a left hand (Fig. 1). In chemistry and biology, these two "mirror images" of the same molecule are defined as enantiomers. Therefore, an enantiomer molecule can be identified either as S configured (from Latin sinister, meaning "left") or R configured (rectus, meaning "right") due to the spatial arrangement of the groups within the molecular structure.

Chirality of molecules has extremely important consequences, especially in the production of biologically active molecules such as pharmaceuticals and agrochemicals. In fact, every living system present on Earth comprises, for instance, chiral molecules such as proteins (made of only chiral, S-configured amino acids), nucleic acids (i.e., DNA, RNA: comprising chiral sugars) as well as utilises and produces various types of chiral metabolites for survival. As cells are essentially 'chiral biological architectures', cellular receptors are capable of interacting differently or selectively with one of the two enantiomeric forms of a chiral molecule. A tragic historical example is the molecule Thalidomide, commonly prescribed as a mixture of enantiomers between 1950 and 1960. One enantiomer possesses the desired anti-depression activity, while the other enantiomer is highly teratogenic. Thus, it is in many cases mandatory to produce biologically active chiral molecules in a single and pure enantiomeric form.

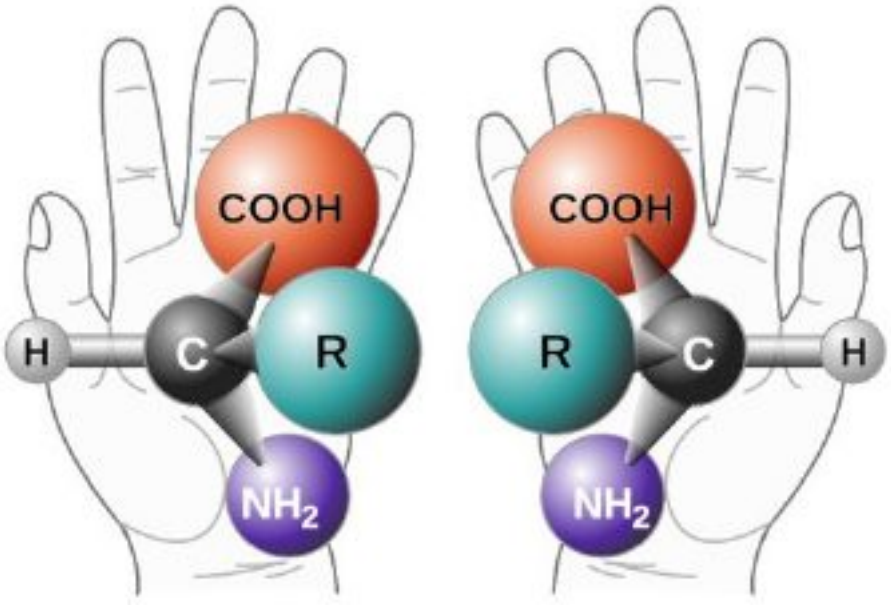

Fig.1

α-Chiral amines are organic compounds in which the core amine moiety (-NH2) is connected to a chiral carbon atom (C, similarly to Fig. 1). This class of compounds are the most widely used intermediates for the production of active pharmaceutical ingredients, fine chemicals and agrochemicals. Indeed, α-chiral amines constitute approximately 40% of the optically active drugs that are currently commercialised mainly as single enantiomers. Furthermore, sustainable synthesis of non-chiral amines is also of interest, particularly for the production of polymers, dyes and various types of bulk chemicals. In this scenario, the amine market size is expected to reach USD 14 billions by 2020. However, chiral and non-chiral amines are classically synthesised industrially through lengthy and inefficient chemical routes that produce copious amount of waste, consume considerable amount of energy, require harsh conditions and still involve mainly unsustainable transition metal catalysts. Furthermore, these methods for the synthesis of α-

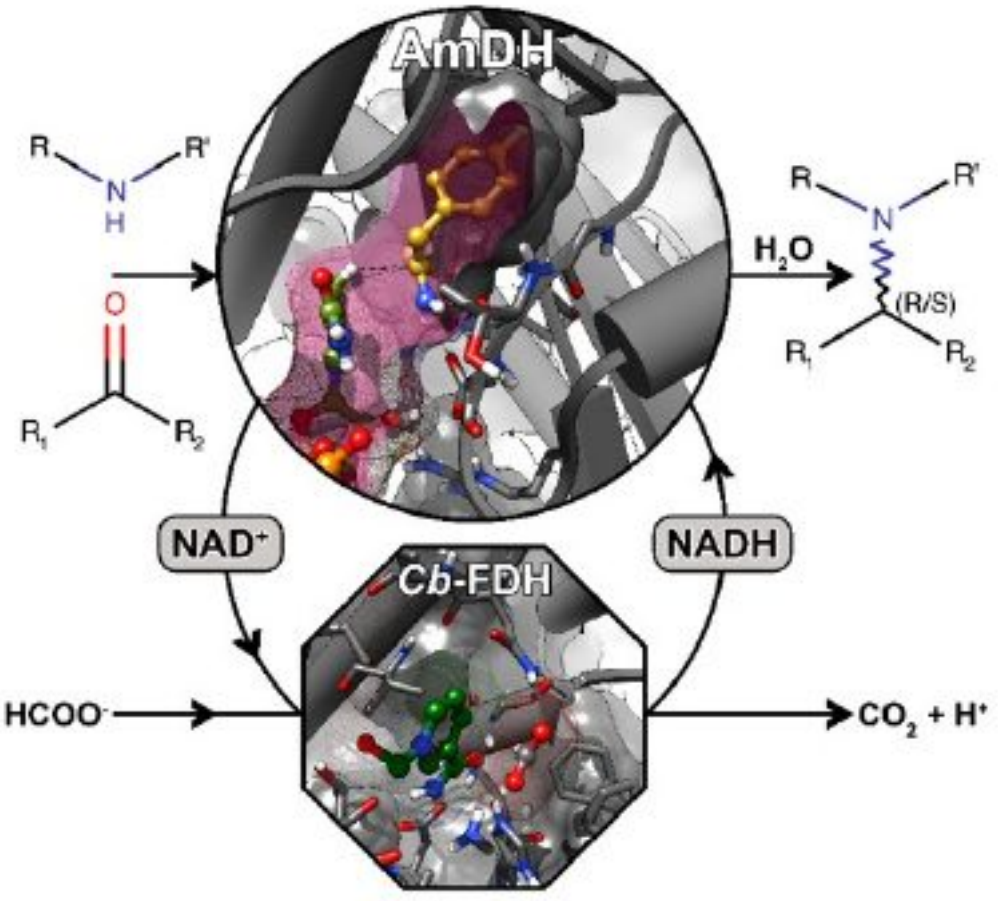

Fig. 2

chiral amines are often not completely selective; therefore, additional steps are required to upgrade the enantiomeric purity of the final product or intermediate.

Amine dehydrogenases as efficient biocatalysts for chiral amine synthesis

The Biocatalysis group at the University of Amsterdam (HIMS-Biocat), led by Prof. Francesco Mutti, has been working towards the development of sustainable routes for the manufacturing of amines, and in particular α-chiral amines. During the ERC-StG BioSusAmin project, the HIMS-Biocat researchers have characterised existing enzymes and created new ones — called amine dehydrogenases (AmDHs) — which can catalyse the synthesis of α-chiral amines with high chemical and enantiomeric purity, starting from inexpensive carbonyl compounds (Fig. 2).[1,2]

The work-flow for the generation of novel enzymes may start from the analysis of X-ray crystal structures of known enzyme scaffolds that possess defined structural features and catalytic activities. However, suitable scaffolds cannot be identified in many cases; therefore, HIMS-Biocat researchers apply computational methods in these cases in order to generate enzyme homology models in silico (i.e., computational models) by using diverse enzyme scaffolds as templates. Independently whether a 'real' enzyme scaffold or a homology model is considered, these enzyme structures are analysed and molecular docking as well as molecular dynamic simulations are performed. Thus, suitable sets of mutations are identified and rationalised. After the computational phase of the work, the envisaged mutations are introduced into a 'real' enzyme scaffold in the laboratory by molecular biology techniques. It is very common to generate a library of enzyme variants (i.e., mutated enzymes) and screen them for the desired properties (e.g., substrate scope, activity, chemical selectivity). Following this strategy, the HIMS-Biocat researchers are creating a tool-box of AmDHs that are capable of transforming structurally diverse molecules into amines of industrial interest. In particular, a new family of AmDHs was generated in the laboratory to have the specific property of converting carbonyl intermediates into important chiral methylbenzylamines, aminotetralines and aminochromanes, that are a recurrent structures in many drugs such as Rivastigmine, Calcimimetic, Bedoradrine, Setralin, Rotigotin, Terutroban, Etamicastat, Robalzotan, Alnespirone, Repaglinide, Dapoxetine, Levocetirizine, Garenoxacin, Solifenacin and others.[3,4] In general, the enzymatic amination reactions of carbonyl compounds catalysed by AmDHs operate under very mild conditions (aqueous buffer, room temperature, atmospheric

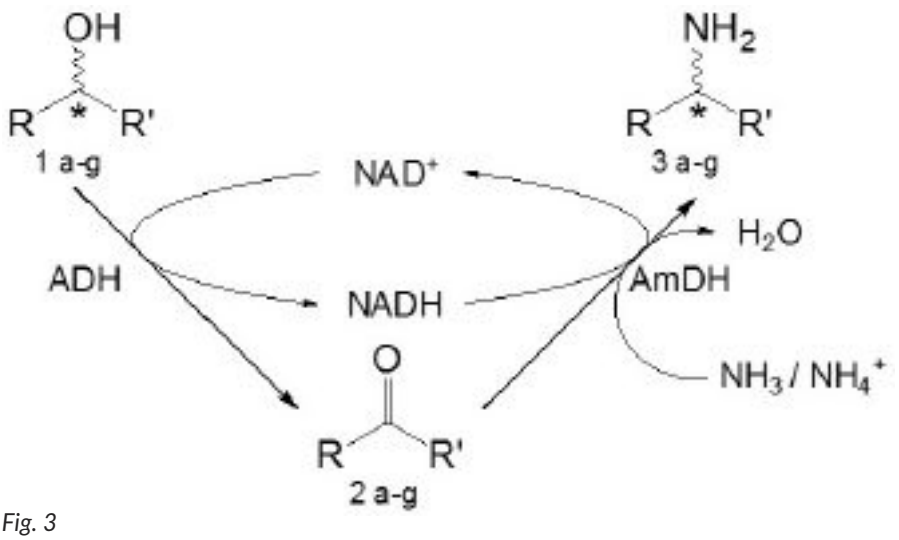

Fig. 3

Prof. Dr. Francesco Mutti

pressure) and generate a minimal amount of waste. Thus, the new chemical route and the biocatalysts are environmentally viable and economically profitable.

Accessing chiral amines from renewable feed-stock

A crucial aspect to enable truly sustainable manufacturing of amines is the availability of renewable material for their synthesis. On the one hand, amines are scarce in nature; the common chemical precursors for the synthesis of amines using either chemical or enzymatic methods are carbonyl compounds (i.e., ketones and aldehydes), which are mainly obtained from the processing and chemical transformations of petrol feedstock. On the other hand, bio-based molecules originated from renewable material contain a large number of alcohol groups. Therefore, the direct conversion of alcohol moieties into amine moieties, particularly α-chiral amine moieties, is another major objective of the ERC-StG BioSusAmin project. During the project, the team developed an innovative and highly efficient method to perform the challenging direct transformation of an alcohol group into a chiral amine group (Fig. 3).[5,6]

The method relies on two enzyme classes, namely alcohol dehydrogenases (ADHs) and the above mentioned amine dehydrogenases (AmDHs). The two enzymes work in tandem, thereby creating a biocatalytic network in which the required redox molecule (e.g. the coenzyme NAD) is internally recycled continuously. In other words, the coenzyme NAD acts as a 'shuttle' of electrons (e.g., hydride) from the first oxidative to the second reductive step. This process, called hydrogen-borrowing cascade, possesses the highest possible atom efficiency. Notably, the alcohol amination process depicted in Fig. 3 sources nitrogen from ammonia and generating water as the sole by-product. One other particularly important feature of the hydrogen-borrowing cascade is that it yields to highly valuable enantiomerically pure amines starting from mixtures of alcohol enantiomers via a single catalytic process. A follow-up study using co-immobilised enzymes demonstrated that the system is economically viable, as recycling of the enzymes for few cycles in the batch was accomplished.[7]

The HIMS-Biocat group demonstrated that the sustainable and atom-efficient amination of alcohols can be also achieved by the smart combination of two independent redox-neutral modules (Fig. 4).

The first module, catalysed by a NADP-dependent alcohol dehydrogenase and a NADP-oxidase, oxidises alcohols to carbonyl intermediates at the expense of innocuous molecular oxygen. The second module, catalysed by a NAD-dependent amine dehydrogenase and a formate dehydrogenase, performs the asymmetric amination of the carbonyl compound intermediate to yield the final α-chiral amine in enantiomerically pure form. As the two enzymatic modules have a divergent co-enzyme specificity (NAD vs NADP), multiple redox reactions can run simultaneously in the same vessel removing the need for a physical separation. This biocatalytic network profits of the exquisite selectivity of enzymes to use and recycle a particular source of 'nature hydride' (NADH or NADPH). Thus, α-chiral amines were obtained in >99% yield and >99% enantiomeric excess. Despite this excellent selectivity, the applicability of this artificial biocatalytic network is not limited to a particular type of alcohols as substrate, but the system is "substrate promiscuous" because diverse alcohols could be aminated (e.g., aliphatic, aromatic, aryl-aliphatic).

More in general, these biocatalytic net-

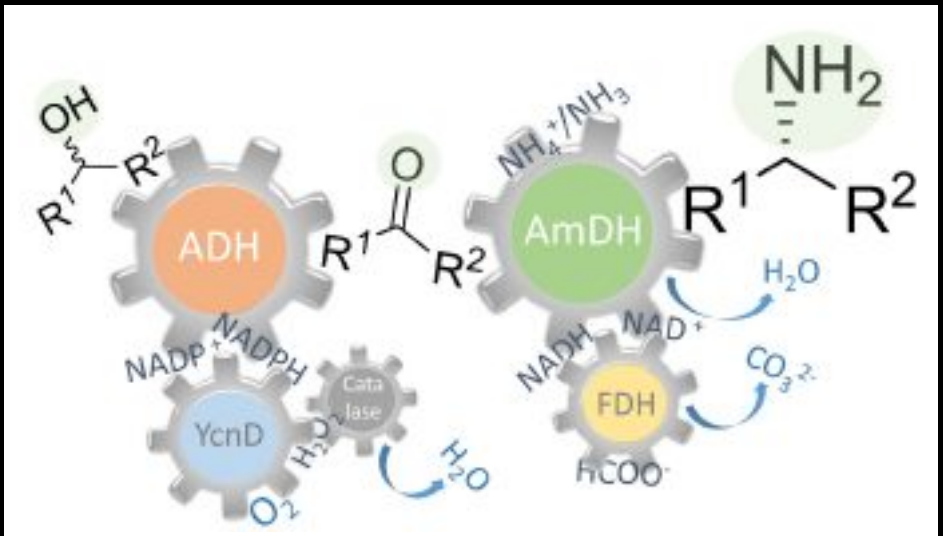

Fig. 4

works (Fig. 3 and 4) enable the minimisation of the number of chemical steps and avoid intermediate chemical work-ups such as purification and isolation steps. Thus, the main difference compared with the traditional synthetic chemistry approach is that Prof. Mutti and his coworkers are designing cascades in which the steps run sequentially and concurrently: the overall process runs from one step to the other without stopping. HIMS-Biocat researchers also expect that the implementation of their process in flow reactors will permit to meet the economic requirement for industrial applications as it was outlined in one their recent publications.[8]

Outlook

The BioSusAmin project is contributing to the development of a new generation of (bio)chemical processes that will reduce the impact of human activity on the environment and will make our society less dependent on non-renewable resources for chemical manufacturing. Increasing resource efficiency is also a fundamental aspect for securing growth and jobs as it will bring major economic opportunities, improve productivity, drive down costs and boost competitiveness. In summary, the BioSusAmin project underpins the quality of life in many areas, such as the sustainable manufacture of chemicals, healthcare, energy, environment and the creation of new job opportunities.

References

1 Knaus, T., Böhmer, W. & Mutti, F. G. Amine dehydrogenases: efficient biocatalysts for the reductive amination of carbonyl compounds. Green Chem. 19, 453-463, doi:10.1039/c6gc01987k (2017).

2 Tseliou, V., Masman, M. F., Bohmer, W., Knaus, T. & Mutti, F. G. Mechanistic Insight into the Catalytic Promiscuity of Amine Dehydrogenases: Asymmetric Synthesis of Secondary and Primary Amines. ChemBioChem, doi:10.1002/cbic.201800626 (2018).

3 Mutti, F., Tseliou, V., Knaus, T. & Masman, M. F. patent pending, No. EP19157237 (2019).

4 Tseliou, V., Knaus, T., Masman, M. F., Corrado, M. L. & Mutti, F. G. Creation of Amine Dehydrogenases with Unprecedented Catalytic Properties from a Cryptostereoselective Scaffold. manuscript under revision (2019).

5 Mutti, F. G., Knaus, T., Scrutton, N. S., Breuer, M. & Turner, N. J. Conversion of alcohols to enantiopure amines through dual-enzyme hydrogen-borrowing cascades. Science 349, 1525-1529, doi:10.1126/science.aac9283 (2015).

6 Baldenius, K.-U. et al. Redox self-sufficient bioamination of alcohols, WO 2016001362 (2016).

7 Bohmer, W., Knaus, T. & Mutti, F. G. Hydrogen-Borrowing Alcohol Bioamination with Coimmobilized Dehydrogenases. ChemCatChem 10, 731-735, doi:10.1002/cctc.201701366 (2018).

8 Bohmer, W. et al. Highly efficient production of chiral amines in batch and continuous flow by immobilized omega-transaminases on controlled porosity glass metal-ion affinity carrier. J. Biotechnol. 291, 52-60, doi:10.1016/j.jbiotec.2018.12.001 (2019).

BioSusAmin

PROJECT SUMMARY

The ERC Starting Grant project BioSusAmin aims at the development of atom-efficient and sustainable biocatalytic routes for the manufacturing of high value amine products and materials, through the creation of enzymes possessing unprecedented catalytic activities. The project entails bioorganic synthesis, enzyme engineering and characterisation as well as biochemical computational studies.

PROJECT PARTNERS

The BioSusAmin project is based at the University of Amsterdam's Science Park, which offers state-of-the art core facilities for chemical, biological and physical research. Mutti's lab is equipped with state-of-the-art instruments for molecular biology, biochemistry, analytical chemistry and chemical synthesis. Collaborations involve academic and industrial partners from Netherlands, UK, Sweden, Germany, Austria, Italy and Spain.

PROJECT LEAD PROFILE

Prof. Mutti, born in Bergamo (Italy), obtained Master's (2004) and PhD (2008) in Industrial Chemistry at the University of Milan. He was Post-Doc at the University of Graz (2009-2012) and at The University of Manchester (2013-2014). He is currently associate professor and chair of the Biocatalysis at the University of Amsterdam. Among the others, he received a Marie Curie fellowship and an ERC StG.

CONTACTS

Prof. Dr. Francesco Mutti
University of Amsterdam, HIMS institute
+31 (0)205 258 264
f.mutti@uva.nl

European Research Council
Established by the European Commission

FUNDING

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 638271, BioSusAmin).