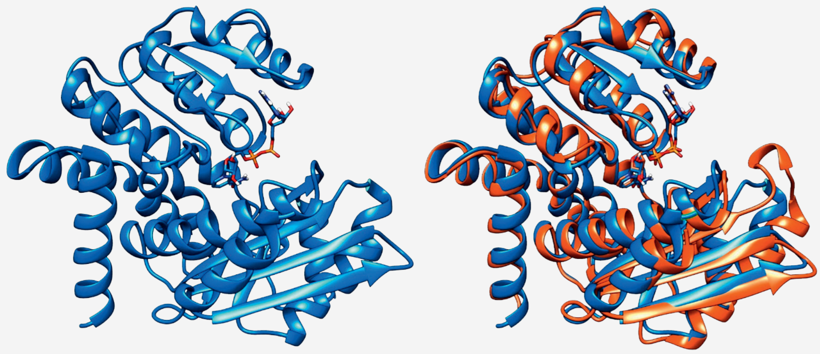
Developing new pathways for biocatalytic cascades

Sustainability is an increasingly prominent issue in the chemical industry, as researchers seek to develop new methods of producing key compounds that don't depend on the use of fossil-based resources. **Dr Francesco Mutti** tells us about the Biosusamin project's work in designing and developing biocatalytic cascades for producing amines, in particular chiral amines

A type of organic compound, amines are commonly applied in the synthesis of a number of chemical products and materials, including certain drugs, polymers and dyes, with chiral amines particularly important for pharmaceutical manufacturing. Current methods of producing amines are mainly based on the use of ketones as the starting material, yet with concern growing over the sustainability of these methods, researchers in the Biosusamin project are looking to develop alternative methods. "We are going to run out of oil at some point, and then we will have to change the way we make these amines," stresses Dr Francesco Mutti, the project's Principal Investigator. While not many ketones are available via renewable resources, this is not the case with bio-based molecules that often contain alcohol functional groups, a major area of interest to Dr Mutti. "This is one important reason to develop methods to convert alcohols into amines," he says. "Another important reason centres on how amines are currently made in industry, which is relatively inefficient. Several steps are involved, leading to waste."



Analysis and comparison of the 3D structure of amine dehydrogenases.

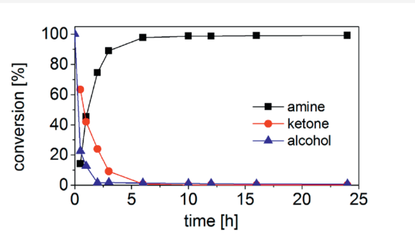
Intermediate steps

This complexity does not however guarantee that the final product will be suitable for use in industry, and it may be necessary to include further steps, to improve the purity of the compound for example. There is not a direct relationship between the number of steps and the amount of waste, as one step may be less efficient than another, but in general, additional waste is generated with each step in a multi-step chemical process. "This is especially the case if, after a single step, you need to isolate and purify your intermediate before starting the next step as it is unavoidable in traditional chemical production," stresses Dr Mutti. One key goal in the project is therefore to minimise the number of biochemical steps involved in the conversion of starting material. "The aim is to minimise the number of steps, and to avoid intermediate chemical work-ups, such as purification and isolation. Using the strategies developed in Biosusamin, based on biocatalytic cascade chemistry, we aim to emulate what nature does in a normal metabolic pathway," says Dr Mutti.

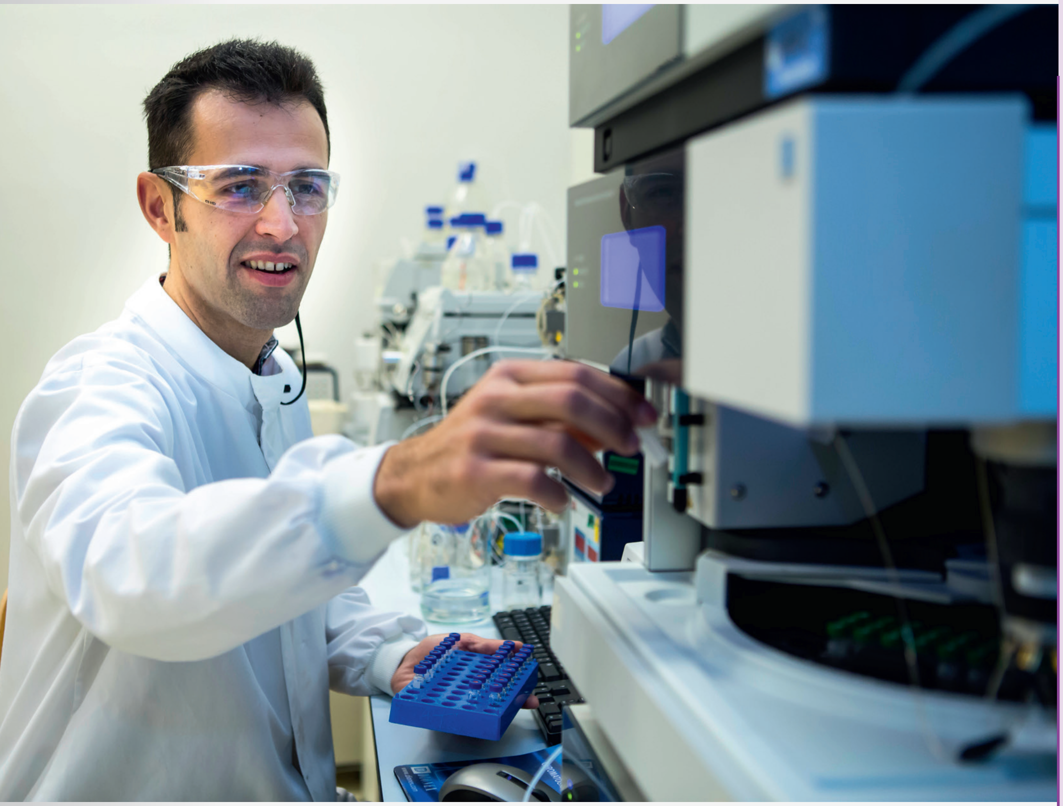
Many steps are typically involved in the natural metabolic pathways that researchers are seeking to emulate in the project. The main difference with the traditional synthetic chemistry approach is that Dr Mutti and his colleagues are designing cascades in which the steps run sequentially and concurrently. "The overall process runs from one step to the other without stopping," he says.

Using the strategies developed in **Biosusamin**, based on **cascade chemistry**, we aim to emulate what nature does in a **normal metabolic pathway**

Researchers are using amine dehydrogenases (AmDHs) as biocatalysts in the key steps of these pathways. "This is a new class of enzymes that we are creating in the laboratory by inducing mutations in other enzymes to create amination activity," explains Dr Mutti. "We study the crystal structure of available enzymes – we do computational studies of enzymes, analyse them and do molecular simulations. In this way we can gain an understanding of where we have to induce mutations in order to obtain the final catalyst that we need for a specific transformation."



The amine dehydrogenases generated so far through the current approach have been relatively limited in scope however, capable of acting effectively on some molecules, while failing with others. Researchers are working to improve existing methodologies, which it is hoped will prove effective on a wider range of molecules, providing a strong foundation for continued development in this area. "This will allow us to obtain a toolbox of AmDHs, to transform different types of molecules," says Dr Mutti. While the initial aims of the project were relatively limited, it has since become apparent that quite a wide range of products could potentially be obtained with this new family of enzymes, reinforcing the wider relevance of this research. "With this type of methodology, we do not need to use any toxic intermediate, compound or reagent," explains Dr Mutti. "With this multi-step cascade, molecular oxygen is used as an oxidant and everything runs at room temperature. So it's safer, and a lot of energy is saved."

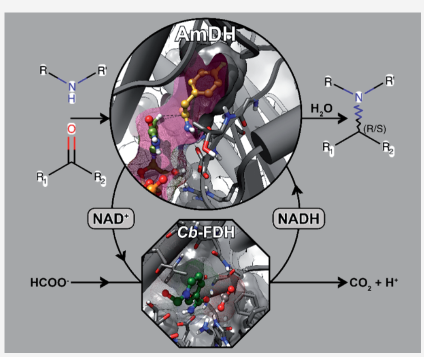


Atom efficiency

This is an important issue in terms of resource efficiency, while Dr Mutti is keen to stress that the atom efficiency is also greatly improved. This relates to the conversion efficiency of a chemical process. "If all the atoms used along the route are incorporated into the final product, then you have the maximum efficiency of 100 percent. However, with traditional chemistry, some waste is typically generated," outlines Dr Mutti. By contrast, Dr Mutti says that efficiency with this new methodology developed in the project approaches 100 percent, which will help in meeting ever-more stringent environmental standards. "This is an increasingly important issue," he points out. "A second important point is that there is also an advantage from an economic perspective. If you can optimise the utilisation of resources by reducing waste, then that also helps maximise profits."

These are important issues for many companies, so there is a corresponding level of interest in the project's research in the commercial sector. However, while one patent has already been filed around the exploitation of the alcohols to amines transformation, Dr Mutti says that more research is required before wider exploitation can be considered. "We need to improve the efficiency of the catalysts, for example the AmDHs, and also other enzymes. This is lab-scale work, that we can do in my group. At the moment we are developing these processes, which are running very effectively on the lab-scale. Then, later on we will look towards exploitation," he says. The project's research could also be integrated with findings from other initiatives working in similar areas. "By combining the results of our project with results from other projects, I think that we can help to build a new generation of biochemical processes for use over the next decade," continues Dr Mutti.

This points to a wider change in the chemical industry, as sustainability becomes an ever more prominent issue. While work in Biosusamin has centered around the production of chiral and achiral amines, Dr Mutti says that his research in this area will continue beyond the term of the project. "We aim to generate a new type of bioorganic chemistry, in which we can start with material from renewable sources, and use it to produce a wide variety of the compounds which we need in our daily lives. This will reduce our dependence on fossil-based resources," he outlines.



Schematic representation of the biocatalytic reductive amination.

BIOSUSAMIN

The design and development of efficient biocatalytic cascades and biosynthetic pathways for the sustainable production of amines

Project Objectives

In general, research in Mutti's lab aims at the development of novel atom-efficient and sustainable biocatalytic routes for the manufacture of high value chemical products and materials. This work involves the creation of enzymes with improved or unprecedented activities (i.e. not known in nature). The research line includes bioorganic chemistry, enzyme engineering, biochemical characterisation of enzymatic reaction and mechanisms as well as computational studies.

Project 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).

Project Partners

- Professor Tom Grossmann, Department of Chemistry, VU University Amsterdam, The Netherlands
- Dr Frank Hollmann, Department of Biotechnology, Technical University Delft, The Netherlands
- Dr Karim Cassimjee, EnginZyme AB, Stockholm, Sweden
- Dr Michael Breuer, BASF SE, White Biotechnology Research, Ludwigshafen, Germany

Contact Details

Dr Francesco Mutti, Associate Professor & Head of Biocatalysis, University Amsterdam
Van't Hoff Institute for Molecular Sciences (HIMS)
Faculty of Sciences
University of Amsterdam
Science Park 904
1098 XH Amsterdam
The Netherlands
T: +31 (0)205 258 264
E: f.mutti@uva.nl
W: www.hims-biocat.eu

Dr Francesco Mutti

Dr Francesco Mutti obtained his master's and PhD in chemistry at the University of Milan (2008). He was postdoctoral researcher at the KF University of Graz (2009-2012) and at Manchester Institute of Biotechnology (MIB), The University of Manchester (2013-2014). After a short period as PI at the MIB, he was appointed tenure-track chair of the Biocatalysis group at the University Amsterdam (2015) where he is currently associate professor.