Enzymes as the building blocks of knowledge

Dr Wolfgang Hütter heads a team in the process of creating a diversity of hydroxylated building blocks through catalysis with carbon-hydrogen activating enzymes. Below, he explains the benefits of interdisciplinary approaches to his work and the broad applications the team’s findings can have in the future.

Can you begin by introducing the research you are currently performing?

Roughly speaking, organic molecules consist of a hydrocarbon backbone, equipped with functional groups that constitute the reactive part of the molecule. Consequently, classical organic synthesis is mainly based on reactions at these moieties. However, it would be favourable to modify molecules directly at their carbohydrate backbone. This type of reaction is called carbon-hydrogen (C-H) activation, as it addresses non-reactive C-H bonds. Without special catalysts, very harsh conditions are required to initiate C-H activations that would, in most cases, destroy the molecule. Moreover, it is particularly difficult to address a targeted C-H bond selectively. In the last decade, there has been much progress in C-H activation in mild conditions, especially in the field of metal catalysis. However, it remains a great challenge in organic chemistry.

How are you endeavouring to solve the challenges involved in the chemical synthesis of C-H activation?

In contrast to synthetic chemistry, C-H activations are very common in biosynthesis. The secret to nature’s success are highly specialised enzymes: oxygenases. They contain a metal ion in their active site – typically iron – which is oxidised by molecular oxygen so that an extremely reactive iron-oxo complex is formed. This is located in the active site ‘pocket’ of the enzyme. There, a specific substrate molecule is precisely adjusted, so that only the targeted C-H bond is in close contact to the iron-oxo complex. More generally, in these enzymes, maximal chemical reactivity is combined with perfect control of the selectivity. Thus, in biocatalysis, the key issues occurring in organic chemistry are principally solved.

Why has your team decided to produce alpha-ketoglutarate dependent enzymes and create variants via rational design and focused libraries?

There are two major groups of C-H activating enzymes, cytochrome P450 monoxygenases and alpha-ketoglutarate dependent dioxygenases. Although less established, we found that the alpha-ketoglutarate dependent enzymes are more convenient for our purposes. For example, in contrast to many P450 enzymes, they do not require a reductase as a co-enzyme and, in most cases, they can be easily produced in Escherichia coli. The 3D structures are often available and they catalyse a broad variety of CH activations with perfect selectivity.

Can you explain how an interdisciplinary approach is supporting your research activities?

Our approach aims at a detailed knowledge of the catalytic process in the enzymes. Therefore, we need information about their catalytic activity, their 3D structure, catalytic intermediates and the amino acid residues involved in the catalytic process. This requires a number of complex analytical techniques, which can only be performed by specialised groups from the fields of organic chemistry, biochemistry and biophysical chemistry. In the current project, our group is mainly dealing with enzyme production and catalysis. Our partners perform spectroscopic methods (such as electron paramagnetic resonance to characterise radical intermediates), crystal structure analysis and protein modelling.

How do you plan to develop this research in the next five years?

Our project is still in its infancy, but the interdisciplinary approach we have adopted is ideally suited to investigate other alpha-ketoglutarate dependent enzymes too. For example, we have just started a project with two hydroxylases from carnitine biosynthesis that we want to convert into useful biocatalysts via enzyme design. We are also interested in the role of alpha-ketoglutarate dependent enzymes in antibiotic biosynthesis. Currently, we are dealing with the biosynthesis of echinocandins, which are complex cyclic peptides with antifungal properties, where we discovered a novel type of proline hydroxylase.
The complexity of catalysis

An interdisciplinary team based at the University of Freiburg, Germany, is attempting to better understand catalytic mechanisms. By comparing different selectivities, the project may ultimately lead to enhanced usability of particular enzymatic systems for a variety of biotechnological applications.

**BIOSYNTHESIS DENOTES A** process whereby simple structures are turned into more complex ones. Sometimes, it is sufficient to assemble simple building blocks to obtain a complex macromolecule, while at other times, the original substance needs to go through a chemical modification before it can be installed. It is not fully understood how natural processes are able to create such remarkable reactions through biosynthesis and, although science has uncovered a wealth of information, there is still much to discover.

Researchers at the University of Freiburg are, therefore, investigating these mechanisms by analysing enzymes to identify what makes them such efficient and selective catalysts.

**ALPHA-KEToglutarate DEPENDENT ENZYMES**

One particular challenge for chemical synthesis is that of carbon-hydrogen (C-H) activation. While scientists have been able to make progress in C-H activation, especially through the use of transition metal catalysts, it is still extremely difficult to address a specific C-H bond of a molecule while all others remain intact.

While scientists have been able to make progress in C-H activation, it is still a challenge to address C-H bonds selectively; C-H activating enzymes are ideal models to learn more about this type of reaction.

Led by Dr Wolfgang Hützel, the team has focused on uncovering more about enzymes, specifically alpha-ketoacid and alpha-ketoglutarate dependent enzymes. "We want to find out how these enzymes manage to attack only one specific C-H bond of their substrate," says Hützel. "This will help us specifically alter this selectivity, which is of great interest to academic research and biotechnological applications."

**PROLINE HYDROXYLASES MODEL SYSTEM**

In order to further their understanding of how alpha-ketoglutarate dependent enzymes direct their substrates to form a single product selectively, the team has chosen a specific model system. The amino acid proline has four different non-activated C-H bonds, three of which can be converted selectively to hydroxyl groups with proline hydroxylases. For this reason, and the simplicity of the substrate molecule, proline hydroxylases constitute a unique system to study selective hydroxylation with enzymes.

An important consideration in Hützel’s work is that of creating a diversity of hydroxylated building blocks. The greater the number of different selectivities that can be compared, the more knowledge of catalytic mechanisms that can be generated. Additionally, while a single enzyme often produces a very limited number of products, designing many enzymes with slightly different catalytic properties enables the creation of several products. Usability is another consideration: "In general, the ability to produce a broad diversity of products is an important criterion for how useful enzymatic systems are for biotechnological applications," explains Hützel.

**OVERCOMING LIMITATIONS THROUGH COLLABORATION**

Given the nature and scope of all that is involved with a project such as this, Hützel has adopted a highly interdisciplinary collaborative approach. To uncover the secrets of biocatalysts is no small endeavour. Indeed, the potential applications of their research are extremely broad, so involving many branches of science offers the necessary breadth of considerations.

However, catalysis with nonheme hydroxylases has some limitations; as the isolated enzymes are very sensitive, whole cells biotransformation has to be used for technical application, which involves other challenges. "The enzyme is located in the cell, meaning the substrate must enter the cell to be converted, which can be a serious problem," elaborates Hützel. "Fortunately, for our amino acid substrates, the transport into the cell is not a major barrier." The fact that the products Hützel’s team is creating are very polar generates other issues, as it is difficult to isolate them from a fermentation broth, which usually contains large amounts of compounds with similar properties. Despite these challenges, the interdisciplinary approach the researchers have adopted provides an abundance of knowledge and resources to overcome them. Although there is still much to be uncovered about the processes nature seems to perform so easily, these are truly exciting times for the advancement of chemical synthesis.

**SYNTHESIS OF CHEMICAL BUILDING BLOCKS WITH ALPHA-KETOACID AND ALPHA-KEToglutarate DEPENDENT ENZYMES**

**OBJECTIVE**

To discover how alpha-ketoacid and alpha-ketoglutarate dependent enzymes selectively attack specific carbon-hydrogen bonds of their substrate and to specifically alter their selectivity, which is relevant for academia and biotechnology applications.

**KEY COLLABORATORS**

Dr Erik Schleicher, Professor Oliver Einsle. Junior Professor Stefan Günther, Professor Michael Müller, University of Freiburg, Germany

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WOLFGANG HÜTTEL studied chemistry in Bonn, Germany, and graduated under Professor Christian Wandrey at the Institute of Biotechnology in the Research Centre Jülich, Germany. In 2006, he was a DAAD Postdoctoral Fellow under Professor Peter F. Leadlay in the Department of Biochemistry at University of Cambridge, UK. Subsequently, he joined the group of Professor Michael Müller at the Institute of Pharmaceutical Chemistry, University of Freiburg, Germany, where he became junior group leader in 2008 and has remained since.

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