Designed on the computer, produced in cells

Artificial enzymes open up completely new possibilities

Chemical reactions accelerated by a catalyst play a fundamental role in our lives. Professor Thomas Ward, who heads the NCCR Molecular Systems Engineering and has been an active SNI member for many years, leads a team that combines different types of catalysis. The researchers' goal is to find new synthetic pathways that ensure effective, safe production of a wide variety of compounds, both inside and outside cells, that can also be applied in diagnostics and therapy.

Nobel Prize for specific type of catalysis

This year, the Nobel Prize in Chemistry went to the two professors Benjamin List and David W.C. MacMillan. Both focus their research on catalysis – chemical reactions that are accelerated by a catalyst. The catalyst enables and accelerates the reactions, but is itself not consumed.

Catalysis plays a major role in many areas of our lives. All living organisms depend on catalytic processes in order to survive with as little energy input as possible. Numerous chemical syntheses in industrial processes are also only possible and economical thanks to catalysts.

The two researchers received the 2021 Nobel Prize for the development of organocatalysis, a process in which relatively simple organic compounds without metals are used as catalysts.

Combined form of catalysis

In the SNI network, the team under long-time SNI member Professor Thomas Ward of the University of Basel's Department of Chemistry is working on catalytic conversions.

His approach is based on combining two types of catalysis (see box page 7) and producing catalysts with the advantages of both natural enzymes and catalytic metal complexes. To do this, Ward's team integrates metal complexes into natural proteins. The protein acts as a host and creates a propitious environment that enables a catalytic transformation to convert substrates into products with minimal energy input.

The resulting hybrid catalysts ideally display novel properties and are characterized by high activity and selectivity. Importantly, they are fully compatible with a cellular environment, including natural enzymes. Accordingly, artificial enzymes can be integrated into new-to-nature metabolic pathways to produce high added-value products within a living cell, as demonstrated by Ward's team at the NCCR Molecular Systems Engineering.

Optimized, highly complex compounds

While the process of integrating a metal complex into a protein may sound simple to a layperson, it is anything but. Natural proteins exhibit highly complex architectures, and are made up of amino acid chains that fold according to a specific blueprint. Their three-dimensional structure affects the functionality of proteins – it is only when the proteins are folded correctly that they can fulfill their task as a biocatalyst (enzyme) in cells.

In nature, enzymes have evolved in living organisms over millions of years. They are elementary components of a well-rehearsed process, ensuring that the multitude of chemical processes in cells run smoothly. Producing new artificial enzymes in the laboratory that are superior to natural ones in terms of their properties and able to catalyze new-to-nature reactions requires a great deal of know-how, as well as a portion of luck.

Effective solution

The Ward team has succeeded in producing such artificial enzymes, with characteristics found neither in the individual components, nor in nature at large.

The researchers often rely on what is known as biotin-streptavidin technology for this purpose. Streptavidin is a bacterial protein that displays an exceptionally strong binding affinity to the vitamin biotin. Linking a metal complex with (modest) catalytic activity to biotin ensures that in the presence of streptavidin the metal is incorporated into streptavidin, thereby creating an artificial metalloenzyme.

Thomas Ward and his group are developing artificial metalloenzymes that have new properties.
(Image: M. Wegmann, SNI)

A versatile artificial metalloenzyme

Almost ten years ago, scientists from the Ward lab succeeded in creating an artificial metalloenzyme that catalyzes one of the most challenging reactions in chemistry: the functionalization of an inert C–H bond. To this end, they integrated a catalytic rhodium metal complex into the streptavidin host.

This new combination initially accelerated the targeted chemical reaction with only a low yield. Guided by a detailed understanding of the catalytic mechanism, two close-lying amino acid residues of streptavidin were mutated. This led to a hundredfold acceleration of the reaction, as these mutations enabled a critical deprotonation step, essential for the reaction to proceed smoothly.

Host structure is crucial

As part of an SNI PhD school project, the Ward team has developed an artificial hydrogenase that supports the splitting of water into its components oxygen and hydrogen. “Hydrogenases are of great interest because they are suitable for the production of hydrogen as an energy storage medium,” explains Thomas Ward.

In the project, former SNI PhD student Dr. Sascha Keller used a cobalt metal complex that he integrated into different variants of the streptavidin complex. The work showed that the streptavidin amino acids located close to the incorporated metal complex have a major influence on the hydrolytic activity of the artificial enzyme. Presumably, protons are efficiently shuttled via these amino acids during the catalytic reaction.

Sources:

Science (2012): **Biotinylated Rh(III) Complexes in Engineered Streptavidin for Accelerated Asymmetric C-H Activation**
doi: 10.1126/science.1226132)

Helvetica Chimica Acta 2018, 101 (4): **Photo-Driven Hydrogen Evolution by an Artificial Hydrogenase Utilizing the Biotin-Streptavidin Technology**
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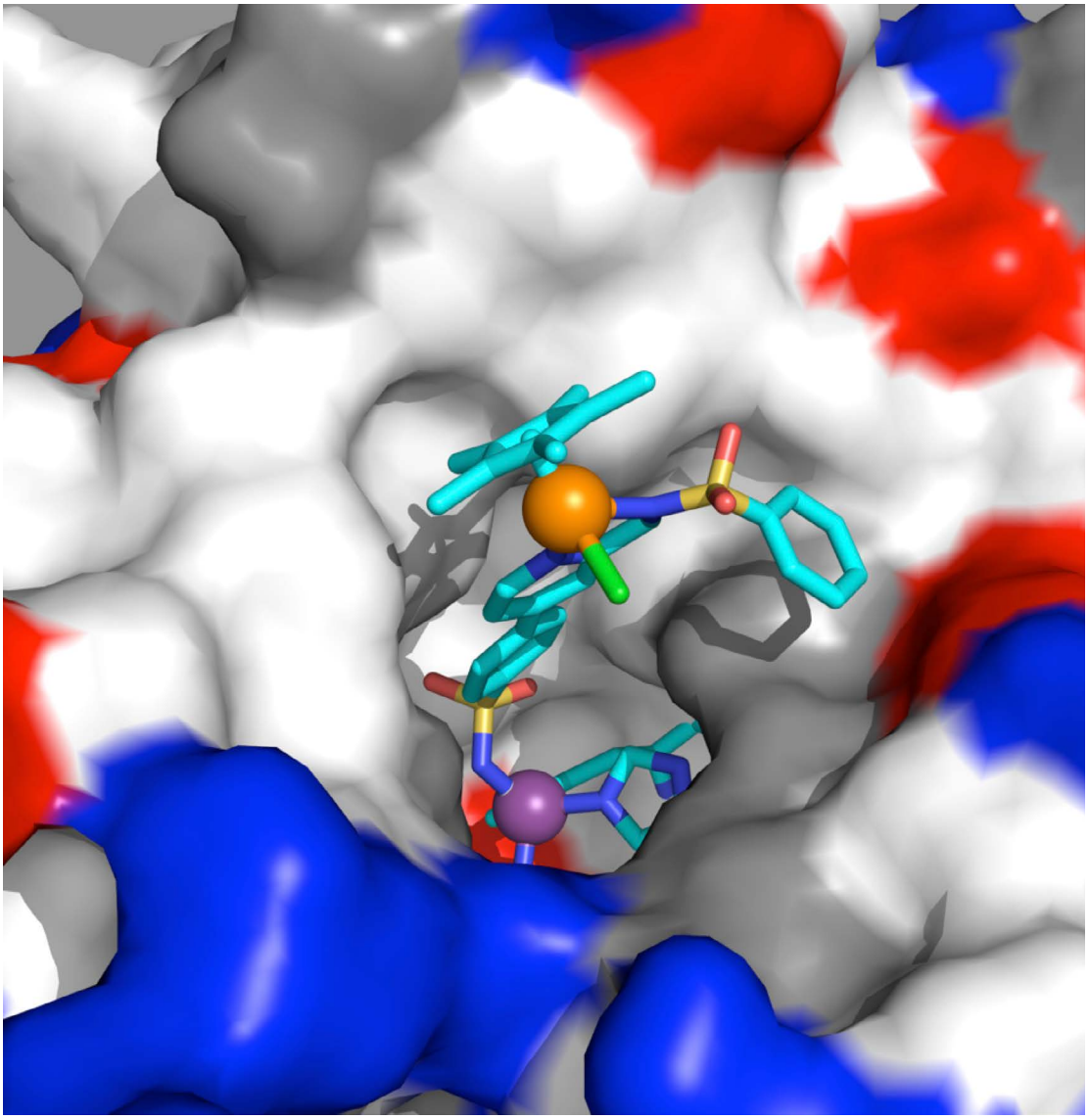

High-resolution X-ray structure of a metal catalyst. (Image: Department of Chemistry, University of Basel)

Sources:

DrEAM_ERC:
https://erc.europa.eu/projects-figures/erc-funded-projects/

***Nature* (2016):**
Directed evolution of artificial metalloenzymes for in vivo metathesis
doi:10.1038/nature19114

Living "factories" also work

Meanwhile, researchers have also succeeded in producing similar artificial metalloenzymes in living cells (*in vivo*). This work is supported primarily by an ERC advanced grant (DrEAM_ERC).

Before the artificial enzymes can be used in a cell, researchers must ensure that all components can be taken up by the cells. In addition, it is important to know which compartments of a cell offer suitable conditions for the reaction to be catalyzed.

In the development of a metalloenzyme that catalyzes the formation of carbon-carbon double bonds (i.e. alkene metathesis), this was the space between the inner membrane of the cytoplasm and the outer membrane in gram negative bacteria. The researchers were able to create conditions that met the requirements so that the artificial metalloenzyme could be assembled by the bacteria in this reaction compartment, known as the periplasm.

They used specially developed strains of the intestinal bacterium *Escherichia coli*, which produce streptavidin in the periplasm, to produce the targeted artificial metalloenzyme Biot-Ru-SAV. The enzyme contains a catalytic ruthenium metal complex. It accelerates the desired chemical reaction with a ring-shaped molecule as the product, which the researchers can easily detect thanks to its fluorescence.

Catalysts – Effective chemical reactions

Chemists distinguish between heterogeneous, homogeneous and enzymatic catalysis.

In **heterogeneous catalysis**, the substrate and catalyst are in different physical states. This group includes, for example, exhaust gas catalysts, in which the solid metals platinum and rhodium catalyze the conversion of the gases carbon monoxide and nitrogen monoxide to carbon dioxide and nitrogen.

In **homogeneous catalysts**, the substrate and catalyst are in the same physical state. An example of this is the lead chamber process, which was already known in the Middle Ages and was used to produce sulfuric acid. In a reaction optimized in the 19th century, nitrogen oxides are used as catalysts that oxidize sulfur dioxide.

In numerous other homogeneous catalytic chemical reactions, metal complexes are used as catalysts.

Enzymatic catalysis uses special natural proteins (enzymes) as catalysts. These naturally occurring enzymes have evolved over millions of years and make life as we know it possible. Roughly four thousand enzymes are used by the human body to maintain its activities. In many cases, they work far more specifically and effectively than synthetic, organometallic catalysts.

Chemically speaking, enzymes are macromolecules consisting of a large number of amino acids, and often have a metal ion in the active site. By contrast, the catalysts used in synthetic chemistry are usually much less complex chemical compounds.

By combining chemical and natural building blocks, it is possible to produce artificial enzymes that do not occur in nature but combine the benefits of both enzymes and homogeneous catalysts. Ideally, they possess properties not found in the individual components. Perhaps the most appealing feature of artificial metalloenzymes is the fact that their catalytic activity can be optimized by introducing mutations in the gene encoding the host protein. In a Darwinian spirit, it is thereby possible to apply evolutionary pressure to "force" an artificial metalloenzyme to adapt to the stringent conditions imposed by the experiment.

Optimized outcome

The researchers were able to further increase the yield of the artificial enzyme and the end product by a technique known as directed evolution. Similar to natural selection, selection pressure through the composition of the culture medium ensures that only bacterial strains containing the artificial enzyme can survive. To this end, the researchers developed a simple and robust screening method allowing them to test thousands of bacterial strains and select the best producers.

"With the system we applied, we can develop entirely new synthetic pathways. We can catalyze new individual reactions, but also entire reaction cascades, turning cells into molecular factories," remarked Thomas Ward of this approach.

Active in mammalian cells

As well as using the artificial metalloenzymes in bacterial strains, the researchers are also able to initiate a reaction cascade in mammalian cells.

For example, they developed a ruthenium metalloenzyme that can enter a mammalian cell. Inside the cell, the artificial enzyme catalyzes the production of a specific thyroid hormone. This activates a synthetic gene switch that leads to the production of the enzyme luciferase. Luciferase catalyzes a chemical conversion that is accompanied by the emission of light – which the researchers can follow and quantify microscopically.

"This finding highlights the potential to integrate artificial enzymes into mammalian cells, thus expanding the available toolbox to reprogram cells for therapeutic purposes," Thomas Ward explained.

New SNI project planned

In addition to new synthetic routes, the artificial metalloenzymes also offer opportunities in diagnostics and therapy.

As part of a new project in the SNI PhD School, the Ward team, in collaboration with Professor Melpomeni Fani of the University Hospital, will investigate how metal complexes can be specifically incorporated into receptor proteins found primarily on the cell surface of cancer cells. Linking a highly specific inhibitor of such receptor proteins to the metal catalyst allows the latter to accumulate where its therapeutic action is required: in the proximity of the tumor.

The purpose of these artificial metalloenzymes is to catalyze the uncaging of a chemotherapeutic drug. The active ingredient is not toxic to cells as long as it is caged. The uncaging of the active drug only occurs in the presence of the artificial metalloenzyme, which is mostly present on the surface of cancer cells. This catalytic strategy will thus minimize the undesirable side-effects of many chemotherapeutic drugs.

In collaboration with Professor Fani, researchers will test this innovative strategy both for diagnostic and therapeutic purposes for various types of cancers.

Numerous possible applications

Thus far, the Ward group has engineered fourteen catalytic transformations with artificial metalloenzymes. Importantly, no natural enzyme is known for any of these transformations.

Sources and further information:

Nat. Commun., 2018, 9, 1943:
A Cell-Penetrating Artificial Metalloenzyme Regulates a Gene Switch in a Designer Mammalian Cell
https://doi.org/10.1038/s41467-018-04440-0

"The prospect of combining both artificial and natural enzymes in a cell opens fascinating perspectives on the development of cellular factories for the production of biofuels and high added-value chemicals. We can use artificial metalloenzymes not only to produce new chemicals with high added value, but also to support the development of diagnostic methods and effective therapies."

Professor Thomas Ward, Department of Chemistry and Director NCCR Molecular Systems Engineering, University of Basel

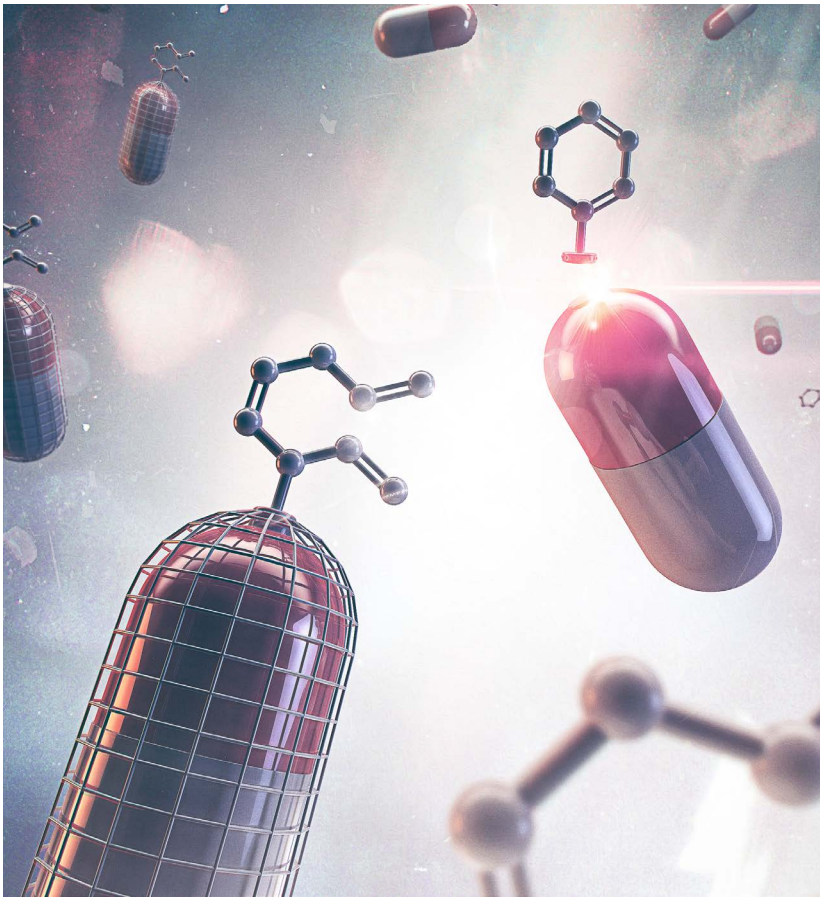

Illustration for the title page (J. Am. Chem. Soc 2019) showing the concept of releasing a caged drug. (Image: Department of Chemistry, University of Basel)