The design of artificial enzymes for industrial purposes is developing quickly. Here, Dr Michèle Salmain explains her exciting investigations into developing new approaches to creating catalysts.

Can you introduce yourself and describe your background? How did you become interested in researching artificial enzymes – so called artzymes?

After receiving a chemical engineering degree from the Ecole Nationale Supérieure de Chimie de Paris in 1987, I completed a PhD at the Pierre et Marie Curie University under the supervision of Professor Gérard Jaouen in bioorganometallic chemistry, which was a rather new interface discipline at the time. As soon as I was hired by the French National Centre for Scientific Research (CNRS) as ‘chargée de recherche’ in 1992, I developed my own research project dealing with the organometallic chemistry of proteins. The general idea was to take advantage of the unique physicochemical properties of transition organometallic complexes to endow proteins with new functions. More recently, I have used my acquired background in protein chemistry to design artificial metalloenzymes by taking advantage of the unique catalytic properties of certain metal complexes.

What drives your scientific endeavours?

My general goal is to expand the scope of bioorganometallic chemistry in several directions. My research is mainly directed toward applications of designed transition organometallic complexes in bioanalysis and (bio)catalysis. For this aim, my team and I are developing methodologies for the bioconjugation of organometallics to proteins and protein-like compounds. These bioconjugates are then characterised by appropriate means and used for the targeted application.

You are currently working on a project to design artificial metalloenzymes using bovine beta-lactoglobulin as the protein host. What are metalloenzymes and why are they of catalytic interest?

Basically, artificial metalloenzymes are synthetic constructs comprising a biomolecular scaffold and a transition organometallic entity. Both species are assembled via a tight bond that can be of dative, covalent or supramolecular nature. Most of the time, the biomolecular scaffold is a protein with a well-defined 3D structure, generally including a pocket to host the metallic species and the substrates. Most of the artificial metalloenzymes have been specifically designed via orientation of the substrates to catalyse enantioselective transformations, the metal being responsible for the chemical activity and the protein scaffold inducing the selectivity.

Do your synthetic enzymes provide benefits over conventional ones?

From a general point of view, artificial metalloenzymes are aimed at bridging the gap between transition metal complexes and enzymes by combining the best characteristics of the two classes of catalysts to afford hybrid species with reactivities not found in nature nor readily accessible by directed evolution techniques.

Why have you decided to use bovine beta-lactoglobulin as the protein host?

It is an abundant protein found in the whey fraction of cow’s milk. It is commercially available at a reasonably good purity. Alternatively, its purification from milk (which is obviously an abundant source) can be achieved quite easily. Beta-lactoglobulin is well characterised from a structural point of view and has been shown to display an eight-stranded beta-barrel structural motif forming a hydrophobic calyx (a cup-like structure). As a result, this protein shows high affinity in the micromolar range for hydrophobic compounds such as fatty acids with the functional group pointing out of the calyx in contact with the solvent.

Can you describe the biggest difficulties you have encountered in your research to date? How have you overcome them and has the process of tackling them opened up any further areas for research?

The supramolecular anchoring approach of catalytically active metal complexes to beta-lactoglobulin has so far provided hybrid catalysts with comparatively modest enantioselectivities (enantiomeric excess of 32 per cent at best). X-ray crystallographic studies pointed out that the metal centre and its coordination sphere probably adopted several conformations when embedded within the protein scaffold that could account for the selectivity issue. Within the current year, we endeavoured a dative strategy to embed transition metal complexes to beta-lactoglobulin and found out that the resulting hybrid species displayed unexpectedly high activity and selectivity on our benchmark hydrogenation reaction. This certainly opens a wider field of research that we should now tackle, starting by properly understanding what the origin is of the unprecedented selectivity we observed with such hybrid catalysts.
The environmental impact of the industrial sector is an ever-growing problem that new, industrially relevant reaction schemes are required to tackle. A group in Chimie ParisTech is investigating the use of artificial enzymes for non-toxic chemistries as part of a solution to this issue.

A RECURRENT CONCERN in most industrial areas is the need for reducing carbon emissions and the environmental impacts of practices. Central to achieving this goal is the chemical sector, whereby attempts are being made to replace relevant chemical reaction methods with those that make the same products under much ‘greener’ conditions. Greener conditions could include lower temperatures and pressures, or the use of less toxic solvents during the reactions.

One solution proposed to this problem is the use of artificial enzymes or ‘artzymes’. These are natural proteins that scientists have functionalised to catalyse the target reaction. In this way, they can use the natural properties of the protein or DNA to accentuate the catalytic centre, which is artificially engineered into the biomolecule. Making a contribution to the development of artzymes for industrial purposes is researcher Dr Michèle Salmain of Chimie ParisTech. Salmain and her fellow researchers are specifically looking to create artificial metalloenzymes; broadly, the group is producing organometallic complexes to drive certain reactions and incorporating them into protein hosts to give the structures high enantioselectivity and chemoselectivity under environmentally friendly conditions.

GRAND DESIGNS
Salmain and her group took inspiration from pioneering work completed in 1978 in the artificial metalloenzyme field that used avidin or the bacterial homologue streptavidin. This protein has a high affinity for biotin, which thus serves as a linker to attach the catalytic metal complex to the protein.

The reaction that the researchers are focusing on is the reduction of ketones to produce secondary alcohols using a reaction known as ‘asymmetric transfer hydrogenation’. This reaction can produce two different enantiomers – non-superimposable molecules that are mirror images of each other and thus have different reaction schemes. Therefore, part of the challenge is to selectively produce the desired chiral product. For this reaction to proceed efficiently, ruthenium (II) and rhodium (III) organometallic complexes, developed by Salmain and her group, are used in water requiring formate to provide hydrogen. ”The wider aim of this work is to provide synthetic chemists with alternative routes to access chiral secondary alcohols and amines under mild conditions with high enantioselectivity,” Salmain expands.

While designing novel biocatalysts, she paid great attention to three parameters. The first was the protein scaffold, which she selected based on stability, availability and the presence of a binding pocket in which the reaction could proceed. Second, Salmain considered the method of incorporating the metallic complexes. Finally, she examined the catalytic activity that the transition metal complex would likely confer to the hybrid. These parameters are highly interdependent, as the surrounding protein environment affects catalysis and how well the metal complex is incorporated.
INTTELENCE

ARTZYMES

OBJECTIVE
To design artificial metalloenzymes to operate in environmentally friendly conditions.

KEY COLLABORATORS
Dr Alice Chevalley; Dr José de Jesus Cazares-Marinero, Chimie ParisTech, France
Dr Mickael Cherrier; Dr Juan Fontecilla-Camps, University Grenoble-Alpes, France

PARTNERS
French National Centre for Scientific Research • Chimie ParisTech (École Nationale Supérieure de Chimie de Paris) • CEA • Institute of Structural Biology (IBS)

FUNDING
French National Agency for Research (CNRS)

CONTACT
Dr Michèle Salmain
Sorbonne Universités
UPMC Université Paris 06
IPCM, UMR 8232
Chimie ParisTech
11 rue Pierre et Marie Curie
75005 Paris
France
T +33 01 44 27 67 32
E michele.salmain@chimie-paristech.fr
http://www.ipcm.fr/enzymesartificielles-689

MICHELE SALMAIN graduated from the École Nationale Supérieure de Chimie de Paris in 1987 and completed a PhD at the Université Pierre et Marie Curie in 1990, in the interface discipline coined bioorganometallic chemistry. As a researcher appointed by the CNRS, she develops research projects dealing with the organometallic chemistry of proteins.

PREFERENTIAL PROTEIN HOSTS
Initially, the group selected the protein papain as the scaffold for the complex. Salmain made use of bidentate ligands that could grab onto the catalytic metal complexes at one end and link them to the protein at the other. The researchers successfully produced a hybrid, but the enantiomeric excess – a measure of the amount of a specific enantiomer produced in comparison to the other – was only modest, and therefore not feasible for use on an industrial scale.

Latterly, the group used beta-lactoglobulin, a protein derived from cows’ milk that can bind fatty acids with high affinity via hydrophobic interactions. These fatty acids bind with the carboxylic, negative end at the entrance of the calyx to position the metal complex in the protein in a process known as supramolecular anchoring. From this, the group synthesised the hybrid protein-metallic complex, analysing the success of this using assembling studies. After successfully producing these hybrids, the group further characterised them using X-ray crystallographic studies to determine whether the structure of the complex was the desired one. “Through these structural studies, we confirmed that the complex was located at the entrance of the beta-lactoglobulin barrel in the hybrid with a rhodium(III) complex,” Salmain states.

After these steps, the group tested the catalytic activity of the synthesised hybrids using a standard hydrogenation reaction. Once each reaction was complete the researchers calculated the conversion rate of the artzyme and the enantiomeric excess using high pressure liquid chromatography to detect the species present in the reaction mixture at specific points in time.

STATE-OF-THE-ART-ZYMES
The researchers met with success in their studies incorporating the ruthenium and rhodium catalytic complexes into beta-lactoglobulin using a fatty acid linker. They trialled different lengths of fatty acids, but they found that the length had little effect on the enantiomeric excess and catalytic activity. Some of these hybrids catalysed the hydrogenation reaction with 94 per cent conversion rate and reached an enantiomeric excess of up to 77 per cent, compared to an enantiomeric excess of 41 per cent for the pioneering work with avidin. However, Salmain and her colleagues are looking to use genetic optimisation to adjust the protein host and improve the selectivity.

Using beta-lactoglobulin with an incorporated transition metal complex, the structure provides an advantageous reaction route that is more efficient to natural hydrogenation systems. “In nature, hydrogenation of ketones – and the reverse reaction of oxidation of alcohols – is catalysed by dehydrogenases, such as alcohol dehydrogenase, requiring low molecular weight co-factors,” Salmain elaborates. These co-factors have to be regenerated in another reaction step, reducing efficiency. The group’s novel transfer hydrogenases do not require a regeneration step, conferring a distinct advantage over natural hydrogenation.

ARTIFICIAL METALLOENZYME INTELLIGENCE
Despite her group’s great success with producing artificial metalloenzymes for hydrogenating ketones to alcohols, Salmain is looking to increase the number of reactions that can be carried out with the enzymes: “We plan to apply our approach to the design of artificial copper enzymes, so as to extend the activity of our beta-lactoglobulin based artzymes to Lewis acid catalysed reactions such as Diels-Alder cycloadditions, Michael additions and Friedel-Crafts reactions”. Overall, such work could transform the numbers of reactions that scientists can carry out using biocatalysts while increasing the reaction complexity possible. Further to this, the researchers are looking to understand the reasons behind why some of their artificial catalysts have good selectivities and reactivities while others do not, which should improve the artzyme design process. However, one thing is clear: with the added capabilities that researchers like Salmain create, the use of these greener catalysts will become routine, improving the cumulative environmental impact of the industrial sector.

ATTACHING THE METAL COMPLEX

- There are three main ways to create protein-metallic hybrid species:
  - **Covalent anchoring** – requires a chemical bond between the metal complex and an amino acid of the protein scaffold
  - **Supramolecular anchoring** – requires some affinity between the protein and the metal ligands that are part of the metal complex
  - **Dative anchoring** – involves direct coordination between a metal (not one of its ligands) and an amino acid