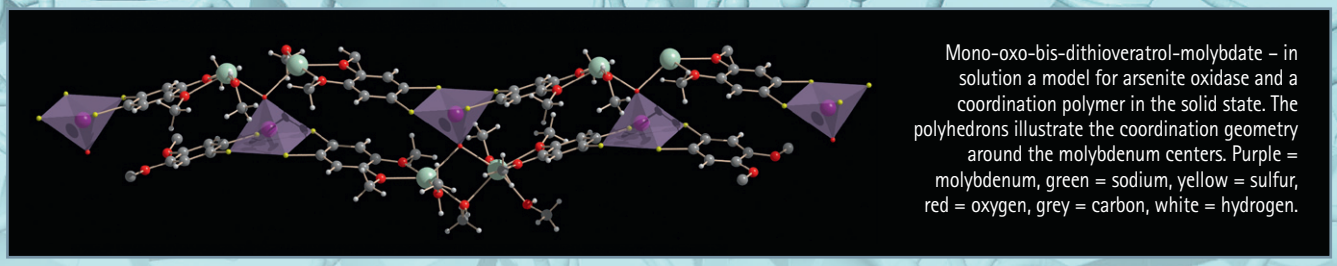


Mono-oxo-bis-dithioveratrol-molybdate – in solution a model for arsenite oxidase and a coordination polymer in the solid state. The polyhedrons illustrate the coordination geometry around the molybdenum centers. Purple = molybdenum, green = sodium, yellow = sulfur, red = oxygen, grey = carbon, white = hydrogen.

Researchers aim to get to the heart of enzyme activity

Molybdenum cofactor-dependent enzymes play a central role in many important biological processes, and severe health problems result when they fail to develop properly. **Professor Carola Schulzke** tells us about the work of the MOCOMODELS project in synthesising complexes that mimic the natural molybdenum cofactor of the respective enzymes

A failure in the process of generating the three main molybdenum-dependent enzymes, sulfite oxidase, xanthine oxidoreductase and aldehyde oxidase, is the root cause of both molybdenum cofactor deficiency (MocoD) and sulfite oxidase deficiency (iSOD), two rare diseases which cause neurological damage. Based at the University of Greifswald in Germany, Professor Carola Schulzke is the coordinator of Mocomodels, an ERC-backed initiative investigating these enzymes, which are common to all kingdoms of life. "We want to understand how these enzymes work. In the long run this could help us synthetically generate drugs that could counter the effects of these diseases," she outlines. This is very much a long term goal however, and the project's immediate focus is more on fundamental research into how these enzymes' active sites operate. "The project aims to understand what components are needed in order to make comparatively small active model compounds which can interact with specific proteins in order to restore activity," says Professor Schulzke.

The project aims to synthesise a large variety of molybdenum complexes that mimic the natural molybdenum co-factor of the respective enzymes, the most important of which is sulfite oxidase. The natural co-factor itself is quite complex. "It has a unique ligand system, which is biologically made from guanosine triphosphate (GTP), so from a nucleoside triphosphate. It has properties which are absolutely unique for this type of enzyme," explains Professor Schulzke. Researchers are building on knowledge of the structure of the molybdenum co-factor to develop these complexes, and are also considering their suitability to bind in the active site of the protein. "We basically broke the structure down, and are trying to model certain aspects of the complex. We will leave some parts out, in order to see if they are really crucial for activity or stability, or if we can just ignore them, which will make our work much easier," continues Professor Schulzke. "We aim to find the compound that has the required level of activity, while at the same time being as simple as possible."

We want to understand how these **molybdenum-dependent enzymes** work. In the long run this could help us **synthetically generate drugs** that could **counter the effects** of these diseases

Molybdenum complexes

A number of different approaches are being used to synthesise the molybdenum complexes, building on fundamental knowledge of the co-factor and its structure. The project is comprised of four main sub-projects, with researchers looking at the common features of these enzymes, one of which is the presence of a metal. "The metal in the middle is most often molybdenum, but we also sometimes see tungsten and we will test rhenium as a substitute," says Professor Schulzke. The project is looking at alternative metals, investigating the potential benefits they offer in terms of stability and activity. "The human organism only uses molybdenum in this kind of enzyme, but tungsten is used in nature, in particular in organisms which have been on the earth for a very long time," outlines Professor Schulzke. "Tungsten is known to work in exactly the same way as molybdenum, with the same coordination in the enzymes. There is slightly different reactivity of course, and there are also organisms which can use both, depending on the conditions."

There has been a gradual evolution over time from tungsten towards molybdenum, with more modern organisms typically using molybdenum in these enzymes. Nevertheless, tungsten is known to behave very similarly to molybdenum, while Professor Schulzke says it also offers other benefits.

"It's easier to oxidise than molybdenum, which can have an impact on stability. We hope that replacing molybdenum with tungsten will represent another means of increasing stability," she explains. The third metal is rhenium, which is to the right of tungsten in the periodic table, so it has a diagonal relationship with molybdenum. "Rhenium has been shown to catalyse oxygen-atom transfer, so we can use it for oxygen-atom transfer with dithiolene ligands," continues Professor Schulzke. "We are very curious about the impact on proteins of going from molybdenum to rhenium. It might be that we can reach the perfect balance between activity and stability."

Researchers have made tungsten and rhenium complexes and tested them for catalytic activity, with results so far showing that tungsten is very often a slower catalyst than molybdenum, while rhenium is fairly similar. The project is also investigating whether these complexes could be incorporated within biotechnologically generated apo-enzymes, and are assessing their suitability and effectiveness as a treatment for iSOD. "Our partner in Postdam is producing the enzymes with the proteins. We incubate our complexes together with the enzymes, and then we basically take the proteins and enzymes out of this incubation solution, and test them to see whether the metals are present in the protein or not," explains Professor Schulzke. While the initial results were disappointing, Professor Schulzke says researchers are learning

Claudia working with the stills.

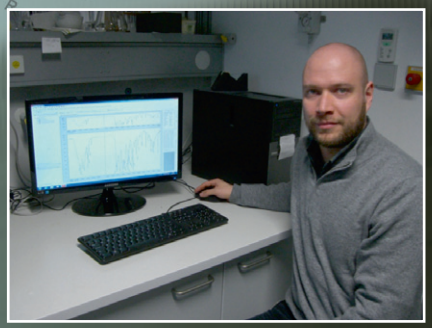
about the underlying factors which result in better binding and better activity. "What seems to be really important is that our models have the potential to induce hydrogen bonding," she outlines.

A variety of sophisticated analytical methods have been used in the testing process. Most of the complexes have been found to be catalytically active, but the more active they are, the more sensitive they are. "The more sensitive they are the less suitable they are for testing in a biological environment," says Professor Schulzke. There is a clear relationship between the stability of the active site and the ability to catalyse oxygen atom transfer, which is an important consideration in terms of the potential clinical applications of these complexes. "The protein has an important role to play in terms of stabilising the active site. Recent tests have shown that we can combine our active sites with the protein," continues Professor Schulzke. "If we want to look towards using these complexes in a hospital setting then I think we need to prioritise stability."

Treatment

This work holds real importance to the treatment of both iSOD and MocoD. Both are extremely rare diseases, with only around 100 cases reported last year, yet Professor Schulzke believes it is likely that this figure understates the true picture. "It's not so easy to diagnose, as there are different types of deficiency related to the molybdenum enzymes. To diagnose MocoD, you need to first have the idea that this might be behind the symptoms," she says. Early diagnosis is crucial to patients' prospects, and recent years have also seen advances in treatment, with a patient in Australia becoming the first to be successfully treated for MocoD in 2009; new treatment methods are being

Mohsen at his fume hood.



Ivan working with the IR spectrophotometer.

Yulia working in the Dry-/Glove-box.

At a glance

Full Project Title

Synthesis of mono-dithiolene molybdenum complexes and their evaluation as potential drugs for the treatment of human isolated sulfite oxidase deficiency (MOCOMODELS)

Project Objectives

Two symptomatically indistinguishable fatal diseases (molybdenum cofactor deficiency (MocoD with subtypes A and B) and isolated sulfite-oxidase-deficiency (iSOD)) are caused by a failure at different levels of the expression and assembly of a certain group of enzymes. While some MocoD patients have been successfully treated by injection of an organic molecule that takes part in the proteins' development there is no cure for iSOD. This project aims to develop a substitute for the very sensitive metal based part of the enzyme, to test its potential to generate an active enzyme together with the biotechnologically produced enzyme- peptide and to evaluate the semi-synthetic enzyme's applicability as a treatment. By developing distinct synthetic model compounds with various functional groups present we hope to find the best compromise between synthetic feasibility, stability and reactivity. Our findings will also provide a deep insight into the structure-function relationships of the molybdenum dependent enzymes.

Project Partners

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Professor Carola Schulzke

Carola Schulzke studied chemistry at the Universität Hamburg, Germany and obtained her PhD 2000 with Dieter Rehder in the field of bioinorganic vanadium chemistry. After postdocing with Sandro Gambarotta in Ottawa she started to work on her own project with Felix Tuczek in Kiel, Germany. 2002-2009 she worked as juniorprofessor at the Georg-August-Universität Göttingen and 2009-2012 as assistant professor at Trinity College Dublin. 2012 she became a professor (chair for bioinorganic chemistry) at the Ernst-Moritz-Arndt-Universität Greifswald taking the ERC project MocoModels with her. Her main research interests are synthetic bioinorganic chemistry and crystallography.

ERNST MORITZ ARNDT
UNIVERSITÄT GREIFSWALD

Nicolas working on the X-ray diffractometer.

developed. "A group in Cologne is using biotechnology methods to producing a precursor of the ligand. Children who are diagnosed soon after birth, or possibly even pre-natally, and then treated immediately, can develop quite normally," continues Professor Schulzke.

The project's long-term goal is to develop a compound which contains all the indispensable functional groups while remaining stable and active, which will provide a new treatment option. More immediately, Professor Schulzke says her research will help build a deeper understanding of structure-function relationships in the active site. "We hope that we can determine which of the functions are really necessary in order to maintain activity," she outlines. From this, researchers can then analyse the complexes and work to improve their stability, to a point where they could be used in treatment; this is very much a long-term goal however, and Professor Schulzke plans to pursue further research into the co-factors. "I will keep working on refining and designing co-factor models, and hopefully they can be used as treatment for this disease in the future. Within the timeframe of this ERC project we will understand exactly what we have to do in order to get there," she says.

The current ERC team (left to right: Mohsen, Nicolas, Claudia, Carola, Yulia, Ivan).