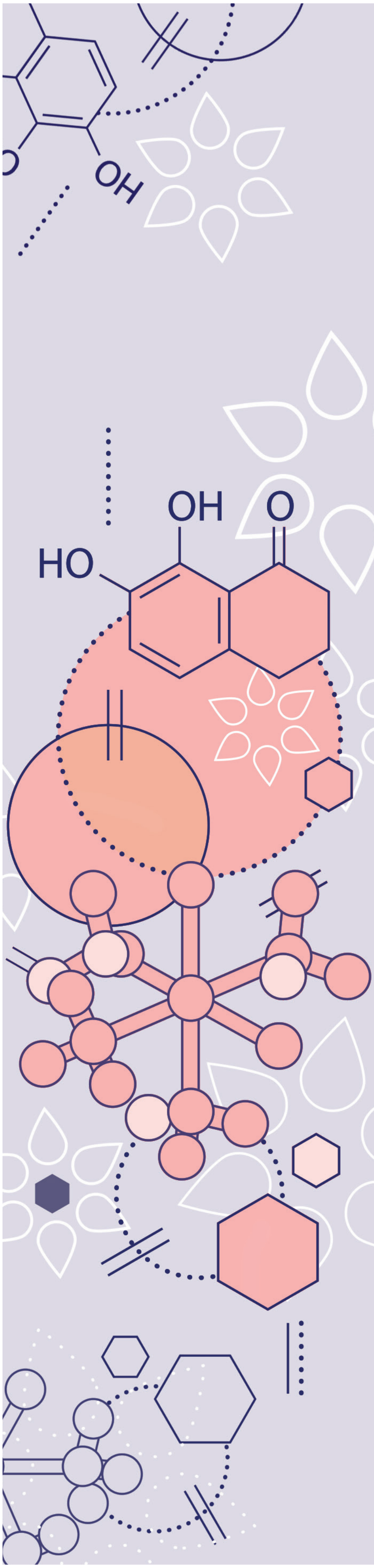


Metals are the elements of life

Expert in the cell biology of metals, **Professor Nigel Robinson**, is applying his knowledge to biotechnology and is acting as an example for others who aim to do the same. Here, he provides details of an innovative network he is leading, and elaborates on the huge importance metals play in catalysis

Can you introduce the Metals in Biology Network?

The UK Biotechnology and Biological Sciences Research Council (BBSRC), with support from the Engineering and Physical Sciences Research Council (EPSRC), is funding 13 BBSRC Networks in Industrial Biotechnology and Bioenergy (BBSRC NIBB) to the tune of £18 million. The Metals in Biology Network is one of them.

The aim of the BBSRC NIBB is to foster collaborations between academia, industry, policy makers and NGOs in order to identify new approaches to tackle scientific challenges, translate research and deliver key benefits in industrial biotechnology and bioenergy. These multidisciplinary networks will drive new ideas to harness the potential of biological resources for producing and processing materials, biopharmaceuticals, chemicals and energy.

What are the goals of the Network?

It has three overarching goals. The first is to generate industry-led, industry-linked funding applications. The second is to disseminate opportunities arising from advances in the metals in biology subdiscipline. Finally, we are aiming to assemble a metals in biology innovation community.

Why did the Network decide to focus on the role of metals in biology?

Nearly half of enzymes are estimated to require metals. The prevalence of metalloenzymes means that success in synthetic biology may pivot upon a community having pioneered the engineering of metal supply in microorganisms, plants and animal cells. Crucially, most metalloproteins bind to one or more incorrect metals (including metal pollutants) more tightly than the correct ones. Thus, the metal handling systems of cells are vital to sustain adequate metal-protein speciation *in vivo*.

Cells hold the abundance of each metal in check by sensors that detect metal surplus or deficiency. These sensors control metal import, export, trafficking and storage systems, and switch metabolism to take advantage of more available metals and minimise demand for those in deficiency. Specialised delivery proteins called metallochaperones also directly target some metals and metal-containing prosthetic groups to a subset of apoenzymes. Under these carefully controlled conditions, metalloenzymes acquire the correct metals.

Recent years have seen advances in understanding how cells help proteins acquire metals. A network such as this one, which is exploiting this knowledge to engineer metal supply and the metal sites of enzymes to drive new industrial reactions, is timely.

Does collaboration play a part in this Network?

Absolutely! Collaboration is crucial to the success of the Network – it is bringing together academics with interests in subjects from across the board, including enzymology, chemistry, synthetic biology and computational chemistry. It is also fostering collaboration through a close working relationship with emerging and established companies.

Is the Network holding any upcoming events or workshops?

This year, we saw great success with scoping workshops for each of the seven themes within the Metals in Biology Network. Consequently, in 2015, we intend to run a second set of these workshops and are planning a joint event in conjunction with the bioprocessing and crossing biological membranes BBSRC NIBB in March, plus a larger scale collaborative event with other BBSRC NIBB on 'antimicrobials' towards the end of the year. In terms of funding, we have regular calls for £5,000 Business Interaction Vouchers and another round of Proof of Concept grants in March 2015. We are also holding additional events – several of which we have planned with other organisations – that will appear on the website throughout 2015.

Seven strands

The UK-based **Metals in Biology Network** has assembled teams in seven key areas. With help from members of these groups, Professor Nigel Robinson elaborates on the goals, progress and applications of each theme

METALS IN BIO-PROCESSING

The next generation of drugs is being dominated by biologics manufactured in mammalian cell cultures, typically Chinese hamster ovary (CHO) cells. For health and safety reasons, blood plasma products are excluded from the culture media. Unlike microbial cells, which acquire metals from inorganic sources, animal cells have their metals delivered by proteins circulating in the blood, but the metal control circuits will not work if key plasma metal delivery proteins are missing. This group is coming up with solutions for this challenge.

"Microbialising the metal control of the CHO cell chassis has the potential to yield new mammalian expression technologies with novel methods of delivering trace metals to improve cell growth, product synthesis and product quality."

Professor Mark Smales,
University of Kent

METAL-RELATED ANTIMICROBIALS

There are opportunities to produce new antimicrobials that subvert metal handling in fungi and bacteria for use in consumer goods, industrial processes, food production and packaging, agriculture and therapeutics. Network members have worked with Syngenta to uncover the mode of action of metal-related antifungals. They are currently working with Procter and Gamble (P&G) to develop new antimicrobials that interfere with microbial metal homeostasis.

"An ongoing collaboration, funded outside of the BBSRC NIBB between P&G and Professor Robinson on cell metal regulation in Salmonella, aims to build a fundamental understanding of how microbes detect metals to guide the design of novel antimicrobial treatments."

Dr Elena Lurie-Luke,
P&G

METALS IN THE ENVIRONMENT

Network members are working with the petrochemical industry on the biological acquisition of and microbial resistance to metals in industrial residues, and to bioaccumulate and biodetect metals. Metal nanoparticles represent one option for metal sequestration. There is also scope to manipulate metal circuits to engineer microbes or plants that hyperaccumulate metals as biocatalysts, which would add value to bioremediation of toxic metals from industrial waste streams.

"The Metals in Biology Network is developing new functional materials using microbial systems that can tap into waste materials. Here, there are clear synergies between Metals in Biology, other BBSRC NIBB, and related UK and European Union funding initiatives in the environmental sector."

Professor Jonathan Lloyd,
University of Manchester

METAL-RELATED NUTRITION AND SUPPLEMENTS

The absorption of metals from food has implications for health, food security and environmental sustainability. There are biotechnological approaches to optimise metal absorption from mixed supplements, including manipulation of capsule formulation. Such advances require interactions between manufacturers, biologists, chemists, and safety and legislative authorities.

"Humans need vitamin B12 – a cobalt-containing vitamin – in their diet, but plants do not make it. Work, therefore, is underway to enhance the biomanufacture of vitamin B12 by engineering the enhanced incorporation of cobalt."

Professor Martin Warren,
University of Kent

METALLOENZYME ENGINEERING FOR BIO-ENERGY AND INDUSTRIAL BIOTECHNOLOGY

This encapsulates a skilled community of bioinorganic chemists who understand the intimate details of the coordination spheres of metals in proteins; environments that tune the properties of the metals to drive catalysis. It is possible to tailor these environments to drive new reactions or optimise activity under conditions suited to an industrial process. Network members are currently engineering metalloenzymes that make alkanes and alkenes, in collaboration with petrochemical companies.

"We have identified the first bacterial lignin peroxidase, which has the potential to be used to produce renewable chemicals from lignin."

Professor Tim Bugg,
University of Warwick

METAL CIRCUITS FOR SYNTHETIC BIOLOGY, BIO-ENERGY AND INDUSTRIAL BIOTECHNOLOGY

Metal sensors and transporters are known for a wide range of activities in a huge spectrum of organisms. There is scope to enhance metal supply and optimise metalloenzyme activity for biotransformations. An example is the sustainable manufacture of isobutanol – a substitute fossil fuel. The patented process includes microorganisms engineered to express the enzyme dihydroxyacid dehydratase, which contains iron sulphur clusters. By overexpressing the sensors Aft1 and Aft2, it is possible to engineer a bypass to normal regulation. Network members discovered Aft2, as well as other genes that are exploited in engineering the metal circuitry of isobutanol-producing organisms.

"Only now are we beginning to understand the complexities of metallocofactor assembly and insertion. This knowledge will open up an array of metalloenzymes to synthetic biology approaches that can harness the power of these natural catalysts for novel biotransformations."

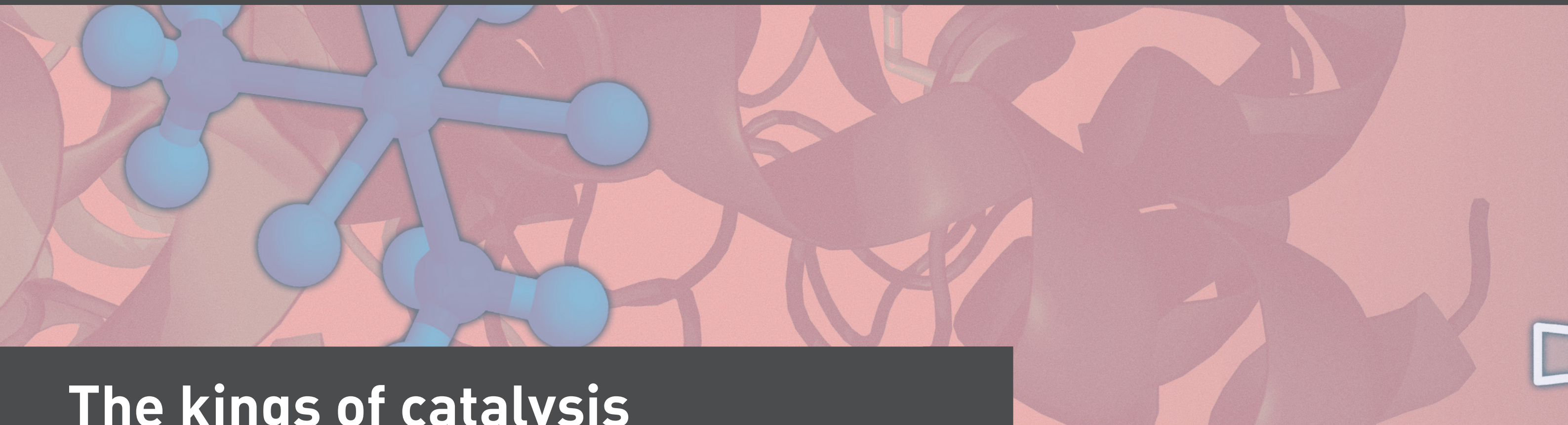
Professor Nick Le Brun,
University of East Anglia

TOOLS AND TECHNOLOGIES FOR METALS IN BIOLOGY

There is potential to develop bespoke, high-resolution, solution-state nuclear magnetic resonance (NMR) spectroscopy dedicated to metals in biology. Recent electronic and sensitivity improvements in NMR make it possible to expand the so-called X-nuclei toolbox. Both NMR and chemical probes can detect a range of elements and provide a unique technological advantage in the study of cellular and metabolic metals.

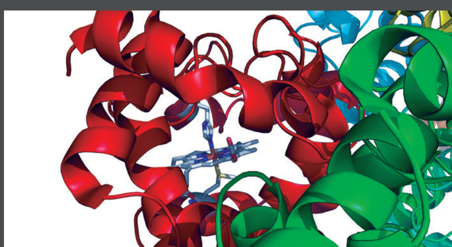
"Synthetic chemists have developed cell-permeable complexes that exploit the intense and 'tuneable' light emission of lanthanides and transition metals. Such probes can be adapted to detect metals in cells. We are designing new emissive molecules that will respond to other metal ions with tuneable affinities and that can be guided to different parts of the cell."

Professor Gareth Williams,
Durham University

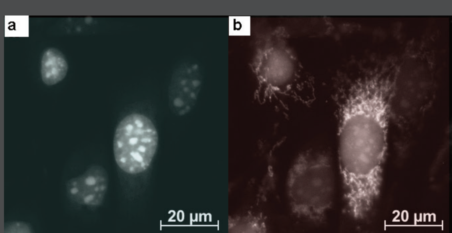


The kings of catalysis

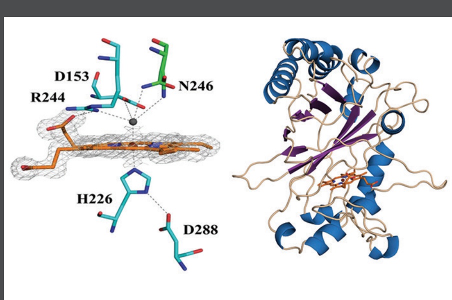
The **Metals in Biology BBSRC Networks in Industrial Biotechnology and Bioenergy** is applying recent scientific advances to novel industrial applications. As half of all enzymes and up to a third of all proteins require metals, the impact of this collaborative effort looks set to be extensive



Iron-containing haem, as illustrated here at the centre of the red domain, is one of the most common and versatile of catalytic protein metallocofactors.



A luminescent platinum complex emits different colours of light according to its localisation within mammalian cells.



In 2011, the first lignin – Peroxidase DypB from Rhodococcus jostii RHA1 – was discovered. Bacterial lignin-oxidising enzymes will be valuable biocatalysts for the conversion of lignin in plant biomass to renewable chemicals.

METALS ARE CRUCIAL elements of life on Earth and important for countless biological processes. Many proteins contain metal atoms, which are essential for the performance of their cellular functions: iron in haemoglobin and zinc in transcription factors, for instance.

Where metals are perhaps most important in biology is in the process of catalysis. Astoundingly, 47 per cent of enzymes require metals for their activity. Enzymes often have multiple metal ions in their active site, using their chemical properties to complement the amino acids. As amino acids only account for a small proportion of possible chemical functionality, metal cofactors essentially enable the huge functional diversity of catalysts.

Considering the prevalence and significance of metals in biology, and the importance of catalysis to industry, understanding how proteins bind metals is crucial to industrial progress. Furthermore, the success of synthetic biology approaches, which endeavour to engineer cells for new purposes, may hinge on the ability to rewire the circuits that supply the correct metals to enzymes.

A CROSS-CUTTING NETWORK

Nigel Robinson, Professor of Biomolecular Sciences at Durham University, UK, has studied the cell biology of metals for three decades, and is particularly interested in how the correct metals locate to the correct proteins. Having witnessed advances in the field and sensing the time was right to capitalise on progress, Robinson proposed the Metals in Biology Network.

Primarily funded by the Biotechnology and Biological Sciences Research Council (BBSRC), Metals in Biology is one of 13 UK-based BBSRC Networks in Industrial Biotechnology and Bioenergy (NIBB) and is targeted to establish collaborations between industry and academia, and translate cutting-edge research to society. As metalloproteins contribute to diverse areas, from the production of bioenergy and industrial feedstock to bioremediation and medicine, the impact of this work will be widely felt.

At the outset of the network, Robinson envisaged supporting research in seven key areas of significant potential and importance. To facilitate this, he organised a series of scoping workshops to put together collaborating teams of researchers. "Ideas have often emerged after the workshops when individuals had a chance to cogitate," Robinson elucidates. Subsequently, follow-up ad hoc workshops have allowed defined grant teams to put together applications.

Some of the very earliest discoveries about metals, reclassifying them as important parts of proteins, not just contaminants, were made in the UK. This legacy is still felt today, as the UK remains home to leaders in the field, working to show how cells handle metals and the processes within metal-dependent enzymes. Hence, in the long run, the Metals in Biology BBSRC NIBB aims to make the UK a world leader in this field of R&D.

BUILDING LINKS

While the UK metals research community is strong, it is also geographically dispersed. The Network will therefore consolidate and coordinate the activities of these different communities, bringing together the efforts of chemists, biologists, mathematicians and engineers to enable the efficient transfer of knowledge. Furthermore, by connecting researchers with industry, the Metals in Biology BBSRC NIBB will accelerate the translation of its findings.

Indeed, the major challenge for Robinson and the Network is to publicise the significance of the discipline outside the confines of academia, to make industry aware of the opportunities to innovate in this field. To ensure this dissemination takes place, and that

scientific advances reach society, each team contains representatives from both industry and academia.

> Despite only being in existence for just over a year, the Metals in Biology BBSRC NIBB has made remarkable progress

The Network provides more tangible support to its members in the form of funding, awarded to projects in each of the seven areas. Grant applications are constantly being reviewed and there are regular calls for Business Interaction Vouchers to the tune of £5,000 each, which encourage and support collaboration between academic and industrial partners. Furthermore, up to 30 Proof of Concept awards are available to members, which can reach £100,000. Similar to the Business Interaction Vouchers, these help fund collaborative research projects of industrial relevance.

NEW PRODUCTS AND PROCESSES

One particularly promising area for innovation is the optimisation of metal-supply circuits. A specific example is the commercially viable manufacture of isobutanol involving engineered iron circuits. Isobutanol is an in-demand chemical and a fossil fuel substitute, and the ability to engineer modifications that enable its sustainable production illustrates the relevance of this field to industry.

Additional opportunities lie in microbe inhibition; the metal handling circuits in microorganisms can be subverted to inhibit their growth. Using a technique that mimics the immune system's ability to manipulate metal supply, there are opportunities to produce new antimicrobials, which could find application in consumer goods, food production, agriculture and medicine.

While metals are in many cases beneficial elements in biochemistry, they can also be toxic. In this context, an important application the Network is exploring is bioremediation. Current work involves using microbes as a more efficient means of recovering metals from waste streams, while simultaneously exploiting them to synthesise high value metal-biocatalysts. In the future, the Network hopes to work with industry to optimise in situ approaches for the bioremediation of radionuclides.

A SENSE OF COMMUNITY

Despite only being in existence for just over a year, the Metals in Biology BBSRC NIBB has made remarkable progress. With over 250 registered members, the Network has already funded more than a dozen individual projects, which are currently springing up across the UK. Their first round of Proof of Concept applications equated to twice the Network's budget. As a testament to the number of good ideas, some projects were held back for the second round, which is due to start soon.

Using insights from real-life metal handling circuits, the Network's members are starting to design exciting new technologies. By connecting people and ideas, and the research and industry communities, the Network is increasing the competitiveness of the UK in this exciting and emerging field.

Formed at an opportune time – just as enzyme metallation is starting to become important for industrial biotechnology – the Metals in Biology BBSRC NIBB is developing and coordinating a cross-disciplinary community in the UK. Through regular workshops and meetings, and with a large conference planned for 2016, this community is growing stronger, and developing integrative and world-leading research geared towards industry.

METALS IN BIOLOGY BBSRC NIBB

OBJECTIVES

- To generate industry-led and industry-linked funding applications
- To disseminate the opportunities arising from advances in the metals in biology subdiscipline
- To assemble a Metals in Biology innovation community

KEY COLLABORATORS

Professor Martin Warren, University of Kent, UK

PARTNERS

The two collaborating Universities already have more than 250 partners with about a third of these coming from outside academia. A few partners have contributed to, and are named within, this article.

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