BBSRC
Polaris House, North Star Avenue, Swindon, Wiltshire,
United Kingdom SN2 1UH
Telephone +44 (0) 1793 413200
Web http://www.bbsrc.ac.uk/

Standard PROPOSAL

Document Status: In Approver Pool
BBSRC Reference:

Networks in Industrial Biotechnology and Bioenergy (NIBB)
Committee D

Organisation where the Grant would be held

Organisation	Durham University	Research Organisation Reference:	MetalsNIBB
Division or Department	Biological and Biomedical Sciences		

Project Title [up to 150 chars]

Metals in Biology: The elements of Biotechnology and Bioenergy

Start Date and Duration

a. Proposed start date	20 January 2014	b. Duration of the grant (months)	36

Applicants

Role	Name	Organisation	Division or Department	How many hours a week will the investigator work on the project?
Principal Investigator	Professor Nigel Robinson	Durham University	Biological and Biomedical Sciences	4
Co-Investigator	Professor Martin Warren	University of Kent	Sch of Biosciences	2

Implications

Are there ethical implications arising from the proposed research? No

Provide details of what they are and how they would be addressed [up to 4000 characters]

Summary of Resources Required for Project

Financial resources

Summary fund heading	Fund heading	Full economic Cost	BBSRC contribution	% BBSRC contribution
Directly Incurred	Staff	0.00	0.00	80
	Travel & Subsistence	0.00	0.00	80
	Other Costs	500000.00	400000.00	80
	Sub-total	**500000.00**	**400000.00**	
Directly Allocated	Investigators	54852.80	43882.24	80
	Estates Costs	0.00	0.00	80
	Other Directly Allocated	0.00	0.00	80
	Sub-total	**54852.80**	**43882.24**	
Indirect Costs	Indirect Costs	0.00	0.00	80
Exceptions	Travel & Subsistence	0.00	0.00	100
	Other Costs	276313.00	276313.00	100
	Sub-total	**276313.00**	**276313.00**	
	Total	**831165.80**	**720195.24**	

Summary of staff effort requested

	Months
Investigator	5.75
Researcher	0
Technician	0
Other	0
Visiting Researcher	0
Student	0
Total	5.75

Additional Resources (Awarded During the Project)

£200k Business Innovation Vouchers

£103k Seeding Catalysts

Table of Contents

The sections of this report are:

Outcome Summary

Relative to the initial BBSRC investment, an Industry-Academia collaboration has been funded for every £17k, a job has been created or safeguarded for every £12k, and £1 million has been generated for every £100k. Products have been launched, patents, papers and books published.

Case studies have been created for funded project outlining diverse discoveries and outcomes. These are available from the Metals in Biology BBSRC NIBB website.

Over the past four years the Metals in Biology BBSRC NIBB (accumulated value ≈ £1 million, £700k for awards) has funded 43 Industry-Academia joint research programmes (12 Proof of Concept Projects including 4 Seeding Catalyst Awards and 31 Business Interaction Vouchers).

The large majority, 79%, of these programmes are new Academic-Industry collaborations and most, >90%, have expressed an intent to continue to higher technology readiness levels.

Our funding rates for Proof of Concept applications have remained at ≈ 28% throughout, including for the most recent seeding catalyst round (October 2017), exemplifying a high and ongoing level of demand.

A further £723k of matched funding has been leveraged from Industry partners.

“At least” £13.75 million (audit still in progress) of additional funding has been won from Industry, RCUK (NERC, EPSRC BBSRC including the National Productivity Investment fund), Innovate UK and RCUK IB-catalyst, Small Business Research Initiative from Business Wales, Microbiology Society, Royal Society, BBSRC GCRF and the Newton fund.

“At least” 86 jobs have been safeguarded or created. At this stage, many of these jobs are in research and development, but we would expect the fraction engaged in manufacturing to increase as projects progress along the TRLs.

The figure for number of jobs created / safeguarded was identified as follows:

- Each funded project was assumed to have either created, or safeguarded, one person per funded project = 43.
- OEFs were evaluated, based on their value, for jobs created / safeguarded = 37
- Feedback from members, via the survey, was also taken into account = 6
- Total number of jobs created / safeguarded = 43 + 37 + 6 = 86
- A number of additional jobs have been notified to us (via our member survey), but these figures have not been quantified at the time of writing.

At this stage, many of these jobs are in R&D and fixed term. An aspiration, as these projects advance along the TRLs, is that more become outside R&D and longer term.

We have engaged in 136 meetings, and organised events for 840 participants from 539 members (total over all time).

Membership has grown to its current level of 488 with a third from outside academia, 9% from overseas, 53% Biologists, 27% Chemists, 13% Engineers, 4% Environmental Sciences and 3% Social Sciences. The proportion of female members is 32 % and although there have been fewer funding applications from female PI's success rates are double compared to male PI's.

Funded projects span the bio-remediation, bio-manufacturing and bio-energy sectors.

Quotes and Testimonials

During the life of the Metals in Biology BBSRC NIBB we have received a range of quotes and testimonials from our members. This section includes a selection of these quotes, both from industry and academia.

Testimonials

Testimonials from Industry

“The BIV provided a quick, convenient and effective route for us to bring together the Manchester group’s expertise in bio-production of metal particles with Johnson Matthey’s catalysis know-how. We have begun to determine the potential of this technology for the production of novel catalysts and look forward to ongoing fruitful collaboration as the work develops further.”

Nigel Powell, Johnson Matthey

“This award provided a quick, convenient and effective route to bring together the Manchester group’s expertise in bioproduction of metal particles with Johnson Matthey’s catalysis know-how. We have begun to determine the potential of this technology for the production of novel catalysts.”

Nigel Powell, Johnson Matthey

“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

“The BIV has allowed P&G to establish a good relationship with academic experts in applied biofilm research and imaging, which we hope to take forward via future collaborations.”

P&G

“The learnings stemming from the BBSRC NIBB have helped to elucidate the importance of metal management for product development and formulation. We are applying these learnings to both current products and new product initiatives that will provide enhanced consumer experience.”

P&G

“For Biocatalysts Ltd, this study highlighted the importance of metal supplementation in commercial fermentation processes to maximize enzyme activity and yield.”

Mark Blight, Biocatalysts Ltd

“Your work provides us with a very useful number; the percentage of heme incorporated in the P450. In our customer project they ask us to measure total heme in homogenised fermenter biomass with a CO assay. This, of course hides the protein:heme ratio and the final number needs to be between about 10-20 µM. Again, if we make a lot of protein then this helps this to increase. Equally, if we get good heme incorporation then this value will also rise. So, 2 factors determine the product specification in terms of heme content; the protein yield and the % heme loading. We had no measure of the heme loading to date and so did not know if there was room for improvement. Your data suggests that there is. Thanks, this is a rewarding outcome of the BiV for Biocatalysts.”

Biocatalysts Ltd

“This work showed that the P450 enzyme was sub-optimally loaded with heme. Further enhancement of heme incorporation could increase the commercial yields of this enzyme, so we support further investigations into this and are pleased to continue our collaboration with Professor Le Brun’s laboratory.”

Biocatalysts Ltd

“The project worked a lot better than even we expected. This BIV research program contributed to the scale-up of nootkatone (grapefruit flavour) synthesis leading to commercial launches in Europe and Asia”.

Oxford Biotrans Ltd

“We were pleased to see such encouraging results from the project, both in terms of the whole-cell bioconversions and the subsequent derivatisation of the metabolites.”

Jason King, Oxford Biotrans

“The collaboration generated many ideas for further development of the proposed system and also for other photonics-based solutions to problems in biomolecular imaging. We are therefore discussing a variety of future projects and exploring funding for a longer and more in-depth collaboration. Dr Joanna Coote, who worked on this project for ZiNIR said “This has been a fascinating project where we have learned a great deal about fluorescence imaging techniques, both in biomedical science and in industrial biotechnology. We have generated a great many ideas for further development of the proposed system using ZiNIR´s spectrometer chips, and also for other photonics-based solutions to problems in biomedical and molecular imaging, and I look forward to a further collaboration with Po-Wah on a longer and more in-depth project.”

ZiNIR Ltd

“Our BIV is helping to understand the fundamentals of recombinant protein expression in E. coli.”

UCB-New Medicines

“The BBSRC NIBB has helped us to build a substantial collaboration with Chris Schofield and co- workers with respect to the late stage functionalisation of drug like molecules by enzyme and non- enzyme catalysed methods.”

UCB-Celltech

“Through the BIV, the network has allowed us to better evaluate the readiness level of Louise Horsfall’s developing technology. This project assessed the potential to recover copper(II) from DRAM® media filters and its bioconversion to metallic nanoparticles harnessing the ability of M. psychrotolerans to reduce Cu(II) to Cu(0). Approximately 12% of Cu(II) ions are reduced by M. psychrotolerans to metallic Cu(0) nanoparticles after overnight incubation in LB medium containing 5 mM CuSO4. It is expected that the gradual release of copper from the DRAM® media may alleviate copper toxicity to live cells and thereby increase productivity. It may be possible to scale up for commercial metal recovery.”

Epona Technologies Ltd

“We are very pleased that this BBSRC NIBB-funded Business Interaction Voucher has made it possible for us to establish contact with the University of York and support their research towards the development of additional uses for UK-grown Miscanthus.”

Mike Cooper, Miscanthus Nursery Ltd

“Without the BBSRC Metals in Biology grant we would have found it much more difficult to collaborate with the University of Southampton on investigations in to anti-microbial copper nanoparticles.”

Copper Clothing Ltd

“Production of enzymes in quality and quantity sufficient for biophysical and structural analysis has consistently been a major bottleneck for drug discovery efforts. This scheme is hugely welcomed, to help overcome these bottlenecks, not just for my company but for the entire pharmaceutical sector.”

Andreas Kuglstatter, Roche Innovation Centre

“The results from this collaboration have enabled us to develop techniques and gain experience which will help towards the development of alternative plant-based remediation practices for sweeper wastes.”

Yorwaste Ltd

“The learnings stemming from the BBSRC NIBB have helped to elucidate the importance of metal management for product development and formulation. We are applying these learnings to both current products and new product initiatives that will provide enhanced experience. We are very pleased that this BBSRC NIBB-funded Business Interaction Voucher has made it possible for us to establish contact with the University of York and support their research.”

Hans de Bie, WeissBioTech GmbH

“We are pleased to be part of this research project as we believe that LPMO’s will play a significant role in the future in starch hydrolysis and starch modification. Not only will LPMO’s help with making current processes more efficient but they will also assist in the development of new starch derived products. So far the development of the LPMO’s has progressed very swiftly and we are excited to see the next steps in this project as it has a great potential. We fully support the work and methods employed by the team. We highly appreciate this work and progress.”

Hans de Bie, WeissBioTech GmbH

“Collaboration through the metals in biology NIBB has opened up an area of research we had not previously considered. Preliminary data has given us an insight into the role of particular metal ions throughout the growth of our industrial microbes and has the potential to aid us in further targeted optimisation of our microbes and fermentation process.”

Dr Liz Jenkinson, Green Biologics Ltd

“The BBSRC NIBB has helped us substantially build on our collaboration with Keith Lindsey at Durham University School of Biological & Biomedical Sciences and to further our work on the synthesis of copper nanoparticles from waste water.”

Andrew Moore, Northumbrian Water

“This study has helped us see the potential future use of plants, plant cell culture or specific plant-produced compounds to remove contaminating copper and other trace metals from, for example, waste water in order to synthesize commercially valuable metal nanoparticles.”

Andrew Moore, Northumbrian Water

“The collaboration has allowed us to apply expertise in mass spectrometry to an industrially relevant area in seed enhancement, springboarding further research into the area of nutrient delivery.”

Croda Europe

“Through the input of science and support by John Innes Centre in this project, the speed to commercial establishment of the new start-up business and product development has been considerably accelerated.”

AgriTopics Ltd

“The growth of Escherichia coli in the novel cell culture system in a defined minimal medium is comparable with growth in traditional glass vessels; this opens up a new market opportunity for this system as cell culture chambers for microbiology.”

Kelly Davidge, Kirkstall

“With the expertise of the University of Manchester we have been able to add visible dyes to particles while retaining fluorescent signaling conjugated to the particle surfaces.”

Stephen Kilfeather, Aeirtec Ltd

“The project has demonstrated to us the range of avenues for incorporation of metal-enhanced fluorescence in enhancement of biomarker measuring platform sensitivity.”

Stephen Kilfeather, Aeirtec Ltd

“Through the BIV, the network has allowed us to better evaluate the readiness level of our collaborator’s developing technology. It may be possible to scale up this technology for commercial metal recovery.”

Epona Technologies Ltd

“With this funding, we were able to kick start a new collaboration, bringing technologies together that wouldn’t have been possible from any other funding source.”

Michal Mos, Terrevesta Ltd / Chris Chuck, University of Bath

“The work has been an eye-opener for the company in terms of the potential for optimisation of the plant, and a huge benefit in terms of skills transfer to our staff."

Michael Mason, Tropical Power Ltd

Testimonials from Academia

“An application submitted to BBSRC (DRINC) resulted from the BBSRC NIBB Metals and Nutrition meeting at Canterbury Cathedral lodge last December. This application would not have proceeded if not for this BBSRC NIBB workshop (where I meet Dora Periera). So, big thanks from me for all your efforts with this BBSRC NIBB! The application got through the pre-application stage and full application is now being considered on The Relationship between Dietary Iron and the Gut Microbiome. Can Dietary Iron Regime be Exploited to Improve Health.”

Simon Andrews, University of Reading

“Our demonstration of H2-driven NADPH recycling on the BBSRC NIBB project has led to a new collaboration within Oxford with Prof Luet Wong, on H2 driven NADPH-dependent cytochrome P450 reactions. We are currently preparing this work for publication. The BBSRC NIBB project helped provide proof-of-concept for some of the research that formed the basis of our successful bid for Translation funding under the IB Catalyst, round 3 (EP/N013514/1, £2.9m). It also helped in strengthening our link with project-partner GSK – they agreed to be on the Industrial Advisory Board for our IB Catalyst project, thus greatly supporting our bid. This Translation funding has allowed us to retain Dr Holly Reeve, who worked on the BBSRC NIBB project, as Project Manager, and another postdoc in my group, and so far we have recruited an additional 2 postdocs for the IB Catalyst project, bringing in a network of researchers from different backgrounds.”

Kylie Vincent, University of Oxford

“A Business Interaction Voucher from the Metals in Biology BBSRC NIBB, to test whether we could produce enough NADPH to drive some NADPH-dependent enzymes that GlaxoSmithKline are interested in provided real proof of the versatility of the HydRegen technology.”

Kylie Vincent, University of Oxford

“The BBSRC NIBB has helped in the preparation of a joint proposal with UCB on chemical methods for late stage functionalisation (EOI now with EPSRC). We are planning a BBSRC bid on enzymes for late stage functionalisation.”

Chris Schofield, University of Oxford

“The project has revealed the substrate selectivity of oxygenases is much wider than we had expected, further highlighting their potential in biocatalysts for medicinal chemistry.”

Chris Schofield, University of Oxford

“This BIV has sparked new collaborations to investigate the potential of our disulphide-folding machinery to assemble a range of protein targets of biotechnological importance.”

Mark Shepherd, University of Kent

“NIBB meetings have brought me into contact with a range of industrial contacts. This BIV sparked a new project with DiosynthBiotechnologies to investigate the potential of bacterial copper-tolerance machinery to facilitate assembly of protein targets of biotechnological importance.”

Mark Shepherd, University of Kent

“The PoC has made a very fruitful industrial collaboration possible by means of a very simple and timely funding system. Will launch a whole new industrial research area for us.”

Sarah Staniland, University of Sheffield

“By working with industrialists to address a specific question us academics have gained unique insights from those with first-hand experience of running a commercial process. Particularly valuable has been increased awareness of the complexities and practicalities of working ‘in-the field’ and the range of scientific challenges that are available to be addressed. Typically such insights are difficult to access and in large part this knowledge exchange was facilitated by the inter-disciplinary nature of the team that partnered industry-based chemists and biochemistry- facing academics. This is evidenced in feedback on our research in which the industrialists highlight wider ranging impact than had been identified in the original proposal.”

Julea Butt, University of East Anglia

“The award for this scoping project provided a mechanism for in-depth discussions and a

better understanding of each other's disciplines. Our discussions generated many innovative ideas and identified key resources needed to develop them. We have continued to communicate and discuss possible funding streams."

Po-Wah So, King's College London

"The BBSRC NIBB has brought me into contact with diverse researchers in the industrial sector, people who I would probably have never encountered through other channels. I was able to initiate a small-scale collaborative study with one such industrial contact, to determine whether metal- supply to their protein products was sub-optimal."

Kevin Waldron, Newcastle University

"The BBSRC NIBB has facilitated new academic and industrial collaborations. These collaborations have directly led to grant applications. The BBSRC NIBB has facilitated new academic collaborations in the EU and enabled us to identify new, valuable waste sources."

Neil Bruce, University of York

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

Professor Mark Smales, University of Kent

"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

"This BIV allowed us to build links with a world leading industrial partner via a simple funding scheme. The project has led to successful development of novel nanomaterials, an avenue which we are excited to continue to explore. It helped underpin a successful responsive mode grant application with the company as a formal project partner, which is an excellent outcome."

Jon Lloyd and Rick Kimber, University of Manchester

“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

“We showed that a novel compartmentalisation strategy can be used to produce a biotechnologically important enzyme in bacteria. Our results are being used to help acquire further strategic investment in the partner company.”

Professor Martin Warren, University of Kent

“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

“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

“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

“Our project provides a robust basis for the use of molecules inspired by pulcherrimic acid as ligands for the development of novel light-activated photoprotective compounds that could be used in sunscreen.”

Charareh Pourzand, University of Bath

“The on-going collaboration with Novozymes into the chemistry and activities of biomass-degrading enzymes continues to provide very fruitful research, not only in the discovery of the fundamental chemical processes exhibited by these enzymes but also their potential in biomass processing.”

Paul Walton, University of York

"The new form of LPMO enzymes is intriguing as it does not contain the usual amino acids at its active site, suggesting that it could be active on a new range of biomass components."

Paul Walton, University of York

"A BBSRC Metals in Biology NIBB scoping workshop highlighted advances in the understanding of metal-handling systems of microbes and hosts, with the aim of improving collaboration to tackle antimicrobial resistance."

Robert Poole, University of Sheffield

"This project laid the foundation for an efficient high-throughput workflow towards understanding and engineering one of nature's most challenging enzymes biotin synthase. A better and faster approach with much broader scope than ever before will allow us improving the synthesis of the important vitamin B7 more efficiently."

Christof Jäger, University of Nottingham

"Finding an easier and cheaper way to reclaim of platinum which would otherwise be lost to the environment is important for global resource management."

Helen Carney, Teesside University

"Increasing the stability of the enzyme catalyst in biocatalytic oxidation technology will improve the catalyst lifetime, leading to improved process productivity and broadening the range of products accessible to this technology platform."

Luet Wong, University of Oxford

"This collaboration has allowed us to initiate a collaboration with Piramal that will hopefully lead to many other useful interactions."

Gary Black, Northumbria University

BIV and PoC Public Summaries

The following pages provide an overview of the Business Innovation Vouchers (BIV) and Proof of Concept (PoC) funds that were awarded during this project. Success rates for PoCs were maintained at 28% throughout the lifetime of the network.

Business Interaction Vouchers – Projects funded by Metals in Biology Network

ID number	**BIVMiB001**
Title	Metal demands during protein overexpression in bacteria
Academic (lead) Partner	Peter Chivers, Durham University
Industrial Partner	Mark Blight, Biocatalysts Ltd
Public summary	Protein and enzyme overexpression is a major facet of industrial biotechnology. The capacity of host organisms for protein overexpression is not naturally optimized. Transition metals are key components of the cellular machinery required for protein synthesis. The effects of protein overexpression on metal allocation within the cell have not been examined. This project will explore the effects of protein overexpression on metal utilization in *Escherichia coli*, a widely used platform for biologics production. The objective is to gain insight into the cellular response to the metal demands of protein overexpression. Metal allocation and utilization will be studied using RNASeq to monitor changes in gene expression that are metal-regulated or encode metal-requiring enzymes and proteins related to protein synthesis.
Start date	16 February 2015

ID number	**BIVMiB002**
Title	Optimising metal acquisition by commercial metalloenzymes
Academic (lead) Partner	Kevin Waldron, Newcastle University
Industrial Partner	Stuart West, Biocatalysts Ltd
Public summary	Biocatalysts Ltd produce a number of metalloenzymes of commercial value through expression in bacteria and fungi. However, metal supply to these 'foreign' enzymes may not be optimal in these protein production hosts, so that a proportion of the commercial product is either bound to the 'wrong' metal, or lacks a bound metal ion altogether. Increasing the proportion of the enzyme that is correctly metal-loaded can directly increase profitability of metalloenzyme products. We will analyse the metal content of samples of the metalloenzymes produced by Biocatalysts. Where metal occupancy is found to be sub-optimal, we will work with the Biocatalysts technical team to test the effect of altering the bacterial/fungal growth conditions during enzyme production in order to increase the proportion of the target protein bound to the 'correct' metal ion. We will analyse the resulting enzyme samples to determine the effect of these changes on metal occupancy.
Start date	5 January 2015

ID number	**BIVMiB003**
Title	Optimisation of heme incorporation into a commercially important enzyme
Academic (lead) Partner	Nick Le Brun, University of East Anglia
Industrial Partner	Jon Wood, Biocatalysts Ltd
Public summary	Metals as cofactors of proteins and enzymes are essential for all of life. Many metalloproteins have properties that are useful outside of the cell, for example in the synthesis of useful materials or medicines. However, to exploit this the metalloprotein must be purified with the metallo- cofactor fully incorporated, requiring a matching of protein and cofactor synthesis/insertion. In this project, the academic partners, who are experts in metalloprotein chemistry, will provide analyses of commercially generated metalloprotein samples to assist the commercial partner in solving a problem of highly variable cofactor insertion.
Start date	1 February 2015

ID number	BIVMiB004
Title	Metalloenzyme system for Hydrogen-driven NADPH recycling in pharmaceutical synthesis
Academic (lead) Partner	Kylie Vincent, University of Oxford
Industrial Partner	David Tew, GSK
Public summary	Bacterial cells are miniature chemical factories, with enzymes as the key machinery for making molecules. Pharmaceutical companies have recognised the benefits of using enzymes instead of traditional chemical routes to make drug molecules. Enzymes generate less waste, make purer chemical products, and allow chemistry to be carried out in water rather than in toxic, polluting solvents. However, there are challenges to overcome before enzyme approaches can be widely adopted. Many enzymes only work in the presence of special helper-molecules called cofactors, which are used up during the production of chemicals. The cofactors are expensive and for enzyme processes to be economically viable, it is essential to have some way of recycling the cofactors. We have demonstrated a novel approach for recycling a common cofactor called NADH. In collaboration with GSK, this project extends the approach to a related cofactor, NADPH, and demonstrates NADPH recycling for enzyme synthesis of pharmaceutically-relevant molecules.
Start date	1 November 2014

ID number	BIVMiB007
Title	Developing an ultra-compact integrated hyperspectral monolithic fluorescence biosensing system
Academic (lead) Partner	Po-Wah So, King's College London
Industrial Partner	Joanna Coote, Zinir Ltd
Public summary	We are using magnetic resonance imaging (MRI) to look into the distribution of iron in the brain without surgery or biopsy, to learn whether iron accelerates/promotes ageing or disease. However, changes in the MRI signal can also be due to changes in cell structure and/or biological molecules in the brain. We aim to determine the specifications for a new ultra-compact, easily transportable device for simultaneously visualising a number of cells/biological molecules, individually labelled to emanate a characteristic type of light. We will assess the feasibility of incorporating such a device within a MRI system such that we can see cells/molecules using the new device but in a 3D anatomical/structural context provided by MRI. This readily transportable and unique device will monitor specific biological processes in living systems used in industrial biotechnology, enhancing efficiency (production) and improving manufacturing methods, and able to perform in environments that other devices cannot operate.
Start date	8 December 2014

ID number	BIVMiB008
Title	Metallo-enzymes for production of nootkatol, a potential new citrus flavour
Academic (lead) Partner	Luet Wong, University of Oxford
Industrial Partner	Jason King, Oxford Biotrans Ltd
Public summary	The sought after grapefruit flavour compound nootkatone is biosynthesised by air oxidation of valencene catalysed by a haem enzyme, firstly to nootkatol and then nootkatone. Haem enzymes are involved in the biosynthesis of numerous natural products, including flavours such as menthol and nootkatone but also medicinal compounds such as antibiotics, the antimalarial artemisinin and the anticancer drug taxol. The industry partner is developing commercial scale biocatalytic synthesis of nootkatone from valencene. The academic partner will modify the haem enzyme used in this process to produce nootkatol, which is found in minute quantities in grapefruit, to explore it's potential as a novel flavour. This primer-project and the nootkatone process will underpin future collaborative work within the metals-in -biology community (1) to test strains optimised (by others) in haem production to further enhance nootkatone/nootkatol synthesis, (2) to manipulate

	the primary and secondary coordination sphere of the haem moiety to further enhance nootkatol synthesis.
Start date	1 January 2015

ID number	**BIVMiB009**
Title	Adding Value to Galactomannan Polysaccharides with Cu Enzymes
Academic (lead) Partner	Julea Butt, University of East Anglia
Industrial Partner	Seth Hartshorne, Schlumberger Gould Research
Public summary	Concerns over fuel security are frequent news headlines and the rising costs of fuel are a daily reminder of the challenges faced by a global society with ever increasing energy demands. Medium- to long-term solutions to these challenges will require effective access to renewables alongside the development of infra-structures that enable such energy to be delivered to the point of need with the ease that is presently enjoyed with our use of fossil fuels. Improved access to the oil and gas reserves that are found in shale present an attractive option for the short- to medium-term. Here we aim to investigate opportunities to improve the recoveries of shale oil/gas through the use of copper-containing enzymes that can modify the properties of a natural biopolymer, guar gum, that plays a key role in the shale extraction process.
Start date	1 January 2015

ID number	**BIVMiB011**
Title	Assessing the bioavailability of metal ions accumulated by DRAM® filters
Academic (lead) Partner	Louise Horsfall, University of Edinburgh
Industrial Partner	Leigh Cassidy, Epona Technologies Ltd
Public summary	The environmental quality standard for copper in groundwater in the UK is set at 1 - 28 µg/l, due to its toxic nature, but copper compounds are one of the few pesticides allowed for organic food production. They are also used to control fungal diseases in vineyards and coffee plantations; as molluscicides and insecticides; as a disinfectant in the farming and fishery industries and to prevent algal blooms. Whilst the spirits industry produce whisky, vodka and gin in a process which involves a universal step of distillation in copper pot stills, producing waste contaminated with solvated copper ions. Consequently this has led to increasing concern over environmental copper levels, their toxicity and the adverse effects on humans and wildlife. Epona Technologies Ltd has developed DRAM® filters, which are able to accumulate polluting copper ions from industry and agriculture but, without steps to allow its reuse, the problem is just being transferred elsewhere. With the finite supply of copper and its ever increasing cost, employing bacteria for the recycling of such contaminants to allow their reuse may provide a long-term sustainable solution. The Horsfall Lab is currently examining the process of copper nanoparticle production by bacteria. We have already determined that *Morganella* sp. can transform copper ions from whisky waste into nanoparticles of solid, zero-valent copper in addition to the reported biotransformation of model solutions and we would like to determine whether the copper ions accumulated by DRAM® filters could be transformed into copper nanoparticles too. This would be dependent upon the bioavailability of the copper ions accumulated and whether the conditions of bioavailability coincide with the conditions under which transformation occurs. It would also be of significant importance and impact if the nanoparticles were retained by the DRAM® filters, allowing for a separate elution step.
Start date	1 January 2015

ID number	**BIVMiB012**
Title	Chelation Therapy in the Washing Machine
Academic (lead) Partner	Nick Jakubovics, Newcastle University
Industrial Partner	Adam Hayward, Procter and Gamble

Public summary	Biofilms are a major problem in all sorts of industrial settings, including bioprocessing facilities. Mechanical biofilm removal is not always possible due to the chemical and physical properties of the contaminated surface and the use of chemical agents is the most appropriate approach for biofilm control. This proposal involves working with an industrial partner (P&G) to develop novel chemical technologies for biofilm removal at low temperatures on a complex surface (laundry). We envisage that successful outcomes can be translated to biofilm control in many different settings including bioprocessing plants.
Start date	1 March 2016

ID number	**BIVMiB013**
Title	Development of new refolding methodologies for expression of heme protein targets
Academic (lead) Partner	Emma Raven, University of Leicester
Industrial Partner	Andreas Kuglstatter, Roche Innovation Center, Switzerland
Public summary	Heme (iron)-containing enzymes are a mainstay of industrial biotechnology, and the industry depends on fundamental improvements in methodology emerging from academic groups around the world to harness the potential of their investments in biopharmaceuticals, bioenergy, biocatalysis and drug design. For a number of complex reasons, the interactions between industry/biotechnology and academic laboratories are often less facile and less extensive than they could be, so that new (often Specialist and/or unpublished) information is not transferred fluently to industrial partners. Our overall objective is to use this project to develop new refolding methodologies for expression of difficult (insoluble) heme protein targets, and to set up an on-going dialogue between industrial and academic partners with mutual cognate interests in specific heme enzymes targets. The methodologies that we develop will open up new avenues for industry partners in cases where they have intractable (insoluble) protein targets.
Start date	1 February 2016

ID number	**BIVMiB014**
Title	Metal interactions with a novel disulphide folding catalyst: a new strategy to improve antibody fragment production
Academic (lead) Partner	Mark Shepherd, University of Kent
Industrial Partner	David Humphreys, UCB- New Medicines
Public summary	The production of biotherapeutics has a total market value of around £100 Billion per year. This project has the potential to develop a new system to improve the production of antibody fragments in *E. coli*, a product with wide ranging applications in diagnostics (*e.g.* pregnancy tests), human therapeutics and as fundamental research tools. In addition, future consumer applications might include the use of antibodies in shampoos to prevent the formation of dandruff or in toothpaste to protect against tooth decay. This project explores the impact of copper upon a novel protein folding catalyst (ScsC), with the overall aim of improving the quality of antibodies produced in *E. coli*.
Start date	1 October 2015

ID number	**BIVMiB015**
Title	Analysis of ferritin iron in pea flour
Academic (lead) Partner	Janneke Balk, John Innes Centre
Industrial Partner	Patrick Mitton, Agritopics Ltd
Public summary	Peas provide a rich source of proteins and nutrients for human diets. They also contain relatively high levels of iron in a special form which is very easy for the body to absorb. To extract this high value component or use pea flour directly in food products, the first step is to mill the dry peas. Together with the industrial partner AgriTopics, we will evaluate different milling procedures, milling fractions and particle sizes to optimize this first step for producing nutritional flour and iron

	extraction. The project will provide the basis for the development of new iron supplements, which could also be used clinically to treat iron deficiency anaemia, and specialist flours for the food industry. Our application fits within the remit of the Metals in Biology BBSRC NIBB as it investigates the industrial processes for refining peas to produce a bio-available iron nanoparticle for the health (and food) industries.
Start date	1 October 2015

ID number	**BIVMiB017**
Title	Extracting mercury from industrial waste using microalgae
Academic (lead) Partner	Mark van der Giezen, University of Exeter
Industrial Partner	Tonnie Schuijl, Reym
Public summary	Affordable and sustainable energy is an important global challenge. Biofuels are seen as possible solutions; however, currently they have limited environmental benefits and put pressure on land and water. Algal biofuels could overcome many drawbacks of terrestrial plant-based biofuels but are currently more energy-intensive and expensive. Water pollution is another global problem and causes over 14,000 deaths each day. By 2025, 1.7 billion people are faced with absolute water scarcity and two-thirds will have drinking water shortages. Unfortunately, many essential industrial activities contribute greatly to water pollution and more sustainable production methods or waste management practices are required to support the global demand for products but protect water sources. With relevance to both challenges, we will grow algae on metal contaminated industrial waste streams followed by hydrothermal liquefaction. This process will separate waste into four fractions: water, gas, oil and solids, the latter containing the metal waste, thereby valorising waste.
Start date	1 July 2016

ID number	**BIVMiB018**
Title	Investigating platinum group metals in wastes from roadside verges
Academic (lead) Partner	Neil Bruce, University of York
Industrial Partner	Richard Bate, Yorwaste Ltd
Public summary	Platinum group metals (PGMs) are rare elements that are essential components in a widening number of industrial applications and particularly used in catalytic converters on road traffic vehicles. Over time, these metals are lost from the converters via exhaust fumes, with significant levels being deposited onto roads and verges. This increasing dispersal and dilution into the environment is of growing concern. Plants can take up PGMs, and our work at the University of York has demonstrated that the subsequently harvested, PGM-rich plant biomass has catalytic activity. This project aims to measure the levels of palladium and platinum in roadside verge wastes from rural, inner city and highway road stretches. Plants will be grown on the wastes to see if they can take up PGMs. This study will enable us to understand if significant levels of PGMs exist in road sweeping waste and whether a phytoextraction process is a viable technology to recover it.
Start date	1 July 2016

ID number	**BIVMiB019**
Title	Studies into the Uptake and Distribution of Metal Oxide Nanoparticles in Plants
Academic (lead) Partner	Neil Bricklebank, Sheffield Hallam University
Industrial Partner	Hannah Griffiths, Croda Europe Ltd
Public summary	The uptake of metals is essential for the growth and development of healthy plants. Plants obtain the metals they need from soil or from fertilizers applied to the growing plant. One of the most important metals is zinc which is found in many metalloenzymes. Zinc is also essential for humans who gain it from the grains and vegetables which they eat. In this project we will study the effect of zinc, in the form of a formulation containing zinc oxide, on the growth of plants

	and use a new analytical tool, known as Laser Ablation-Inductively Coupled Plasma-mass spectrometry (LA-ICP-MS), to study the uptake and distribution of zinc in plants. This project fits within the remit of the BBSRC-NIB because it will investigate metal availability, uptake and assimilation into biomolecules required for bio-energy production. It will also enable us to develop new tools and technologies for studying metals in biological systems.
Start date	18 May 2016

ID number	**BIVMiB020**
Title	Novel disposable cell culture systems for microbial growth in metal-regulated environments
Academic (lead) Partner	Robert Poole, University of Sheffield
Industrial Partner	Kelly Davidge, Kirkstall Ltd
Public summary	Growing cells and tissues in biotechnology requires a well-defined growth environment. Important factors include design of the culture apparatus and growth medium, which must provide all nutrients including metals in biologically accessible forms, but not in excess. Most growth vessels are metal or glass, but these can leach or adsorb metal ions. Synthetic materials, however, may be biologically inert and interact little with dissolved metals. This project will test the suitability for microbial growth of miniaturised growth chambers (Quasi-Vivo® by Kirkstall) that were developed for culturing mammalian cells. These chambers are made from biocompatible materials and, under flow conditions, mimic conditions in the body. We will grow bacteria in such chambers and test their ability to provide environments in which the metal concentrations available for growth will be varied from 'trace' to toxic. The work has potential for developing improved methods of cell culture in industrial biotechnology.
Start date	1 January 2016

ID number	**BIVMiB022**
Title	Cloning and metal analysis of recombinant aldehyde ferredoxin Oxidoreductase (AOR)
Academic (lead) Partner	Martin Warren, University of Kent
Industrial Partner	Michelle Gradley, ZuvaSyntha Ltd
Public summary	The aim of the project is to enhance the recombinant production of a key enzyme of biotechnological importance with a view to the production of metal-cofactor complete protein. Specifically, the aim is to find optimal conditions that allow for overproduction of recombinant aldehyde ferredoxin oxidoreductase (AOR), an enzyme that allows the transformation of carboxylic acids into aldehydes. AOR has an unusual metal requirement in that it contains an oxo-tungsten centre bound to a pair of molybdopterin cofactors and a 4Fe-4S cluster. We will clone two mesophilic forms of AOR from *Clostridium ljungdahlii* and another thermophilic form of the enzyme from *Pyrococcus furiosus*. The enzyme will be produced with a His-tag to allow for easy purification. Moreover, the protein will also be produced with a tag to allow for the protein to be targeted to a bacterial microcompartment (BMC). BMCs are utilised by nature to help accommodate enzymes that produce aldehydes as pathway intermediates.
Start date	1 January 2016

ID number	**BIVMiB023**
Title	Maximising Biomarker Detection Sensitivity through Metal Enhanced Fluorescence
Academic (lead) Partner	Lu Shin Wong, University of Manchester
Industrial Partner	Stephen Kilfeather, Aeirtec Ltd
Public summary	Fluorescence-based immunosorbent assays have become a key technology for detection and quantification of microbial contamination (in water, chemical, food and drug production), as well as for molecular biomarkers in agriculture, drug

	discovery, industrial biotechnology, medical diagnostics and cellular imaging. Here, the selectivity of antibody binding combined with fluorescence spectroscopy, has led to huge advances in sensitivity, selectivity and speed. Typically, these assays employ Fluorescence-based immunosorbent assays have become a key technology for detection and quantification of microbial contamination (in water, chemical, food and drug production), as well as for molecular biomarkers in agriculture, drug discovery, industrial biotechnology, medical diagnostics and cellular imaging. Here, the selectivity of antibody binding combined with fluorescence spectroscopy, has led to huge advances in sensitivity, selectivity and speed. Typically, these assays employ an immobilised antibody to capture the target molecule from the test sample, followed by the immobilisation of a second antibody bearing a fluorescent label. To maximise the fluorescence output from the label, many researchers have started to harness metal enhanced fluorescence (MEF) as a means to improve diagnostic sensitivity. Here, the co-location of the antibody in the vicinity of a metallic nanoparticle results in a large enhancement of fluorescence output (in the order of 100-fold). This project aims at improving the sensitivity of these MEF-based assay systems by the application of state-of-the-art bioconjugate methods to control the orientation of the immobilised antibodies with respect to the nanoparticle.
Start date	1 July 2016

ID number	**BIVMiB024**
Title	Investigating uptake and catalytic potential of miscanthus grown on palladium mine wastes
Academic (lead) Partner	Neil Bruce, University of York
Industrial Partners	David Stone, AgriKinetics Ltd; Mike Cooper, Miscanthus Nursery Ltd
Public summary	We have shown that Arabidopsis plants grown in liquid culture can take up palladium, depositing it as nanoparticles. Following a processing step, the nanoparticle-containing biomass can be used directly as a catalyst for industrially important reactions. Palladium mining wastes contain significant levels of palladium which are uneconomical to recover using conventional methods; mining areas also need to be re-vegetated. Phytoextraction using field-relevant species could be a viable and environmentally sustainable method to re-green these sites while generating a catalytic product of higher value than the bulk metal. Our preliminary studies at the Centre for Novel Agricultural Products (CNAP) have demonstrated that the grass, miscanthus, can take up palladium from synthetic mine wastes, but not yet at levels that confer catalytic activity. A major limitation to uptake is the insolubility of palladium in the wastes. This project will enhance palladium availability, measure accumulation in miscanthus and provide biomass for catalysis testing.
Start date	1 April 2016

ID number	**BIVMiB026**
Title	Microbial recovery of metals from contaminated Miscanthus used in the industrial remediation of degraded landscapes
Academic (lead) Partner	Chris Chuck, University of Bath
Industrial Partner	Michal Mos, Terravesta Ltd
Public summary	Metal leaching from mining and further industrial activity has the potential to degrade landscapes across the globe. However, recently a range of techniques have been trialled and brought to market to restore the natural capital of these areas. One of the most promising is growing *Miscanthus x giganteus*, an energy crop that can remove metal contamination while being used as a biofuel feedstock. However, the processing of the contaminated *Miscanthus x giganteus* remains an issue. In this BIV we will explore using the oleaginous yeast *M. pulcherrima*, which produces metal chelators such as pulcherriminic acid, as a method of valorising the Miscanthus biomass into a range of products including a palm oil substitute and removing the metal waste into a smaller containable

	volume. This method will then be compared to hydrothermal processing of the Miscanthus waste.
Start date	1 February 2016

ID number	**BIVMiB030**
Title	Investigating the antimicrobial properties of copper infused fabrics
Academic (lead) Partner	Bill Keevil, University of Southampton
Industrial Partner	Rory Donnelly, Copper Clothing Ltd
Public summary	The University of Southampton have been testing the antimicrobial effects of copper for many years, and have shown that copper can kill bacterial cells in several ways, including rupturing the cell, and destroying the DNA. Copper Clothing Ltd has begun investigating new processes for incorporating copper into their antimicrobial fabrics. Initial work will investigate the antimicrobial properties of fabrics infused with copper using novel technologies. The new fabrics developed by Copper Clothing Ltd. will be tested for levels of antimicrobial kill in a range of bacteria, both by culture and by using fluorescence microscopy to determine the presence of living but non culturable organisms – bacterial that are not killed by the copper, but survive in a stressed form. Future work plans to use bio-produced nano-copper for incorporation into the fabrics and determine their improved efficacy against superbugs compared to the current novel fabric chemistries.
Start date	1 August 2016

ID number	**BIVMiB031**
Title	Bioaccumulation of platinum from waste
Academic (lead) Partner	Helen Carney, Teesside University
Industrial Partner	Pattanathu Rahman, TeeGene Biotech Ltd
Public summary	Platinum is a scarce metal, being one of the least abundant elements in the earth's crust and as such has a high material value. This research will focus on the recovery of platinum from wastewaters where it is in its soluble, ionic form. Platinum is present in wastewater from a diverse range of sources such as metal refining, chemical industries and hospital waste where it can be found as a component of chemotherapy drugs. Bacteria have been found to take up and accumulate platinum using both active and passive methods, often referred to as biosorption and bioaccumulation. The proposed research, a collaboration between TeeGene, Teesside University, and University of York, will investigate the potential of bacteria to recover platinum from industrial wastewater with the aim of re-using the recovered metal. The project aims to identify a suitable bacterium to be used in a waste refining process and identify any physicochemical factors that influence platinum recovery.
Start date	1 September 2016

ID number	**BIVMiB032**
Title	Embedding technical expertise in the optimisation of trace metal supplementation strategies for successful biomethane production
Academic (lead) Partner	Yue Zhang, University of Southampton
Industrial Partner	Michael Mason, Tropical Power Ltd
Public summary	Transformation of waste biomass into bioenergy and useful resources is a key component in 21st century industrial biotechnology. It is increasingly clear that successful biomethanisation of mixed biomass requires complex enzyme systems that are produced by both natural and engineered synthetic microbial communities. Trace quantities of metals play a role in certain essential metallo-enzymes, and thus in ensuring that microbial communities function in the most effective and productive way. There is a growing commercial market in trace metal supplements, but these are often generic rather than based on specific requirements. The current project involves transfer of knowledge and expertise in determining trace metal requirements to a UK company using novel waste

	feedstocks in Africa for renewable biomethane production. This will enable the company to formulate specific trace metal mixtures for optimum plant performance, while the scientific knowledge gained will contribute to creating future markets for UK suppliers of tailored supplements.
Start date	16 January 2017

ID number	**BIVMiB035**
Title	Metal utilisation in *Clostridium* microbial biocatalysts
Academic (lead) Partner	Peter Chivers, Durham University
Industrial Partner	Li enkinson, Green Biologics Ltd
Public summary	*Clostridium* is an exemplar for microbial fermentations that convert biomass to renewable chemicals, such as butanol (with diverse uses including consumer fuels, paints and coatings and food additives) and acetone (used in cosmetics, plastics and numerous other markets). These fermentations depend upon metalloenzymes to convert starting material to product. The optimisation of metal supply is therefore critical for cost-efficiency. Currently, little is known about the metal requirements of industrial *Clostridium* species, or the metal circuitry important for maintaining metal supply to these pathways. We will identify the metal requirements during different fermentative processes and in different environments (batch vs. continuous; lab vs. industrial). Results will be applied to commercial operations for immediate outcomes including reducing waste and water use impacts. To further the development of *Clostridium* in a variety of IB processes, the metal sensor components of the metal circuitry will be identified to enable fine-tuning of metal supply pathways.
Start date	1 April 2017

ID number	**BIVMiB036**
Title	Improving biocatalytic processes by enzyme stability enhancement
Academic (lead) Partner	Stuart Ferguson, University of Oxford
Industrial Partner	Jason King, Oxford Biotrans Ltd
Public summary	The industry partner has licensed biocatalytic oxidation technology from the University of Oxford for the production of fine chemicals such as flavours, fragrances, agrochemicals and active pharmaceutical ingredients. The first product is nootkatone, the high-value grapefruit flavour compound. This industrial biotechnology approach not only replaces classical, more energy- demanding and polluting chemical processes, it also enables non-fossil fuel, sustainable feedstocks to be used. A key improvement to the process is enhancement of the stability of the enzyme catalyst that lies at the heart of the technology, making it applicable to a wider range of products.
Start date	1 July 2017

ID number	**BIVMiB037**
Title	Exploiting the commercial potential of novel biometallic catalysts
Academic (lead) Partner	Jon Lloyd, University of Manchester
Industrial Partner	Nigel Powell, Johnson Matthey plc
Public summary	This project brings together biotechnologists from the University of Manchester and experts in industrial catalysis at Johnson Matthey, a leading multinational specialty chemicals and sustainable technologies company headquartered in the United Kingdom. This Business Interaction project will facilitate collaborative discussions required to underpin the development and exploitation of a new generation of “biometallic” industrial catalysts. They are based on naturally occurring metal- reducing bacteria that are able to accumulate metals from process environments, as catalytically active nanoparticles, while also expressing enzymes that are able to extend the range and complexity of industrial reactions that can be produced from these novel microorganisms. This novel extension of “synthetic biology” has the potential to transform several sectors of UK industry including those of industrial biotechnology and makers and

	users of catalysts, simplifying current processes, underpinning novel reactions and extending the range of available products.
Start date	1 June 2017

ID number	**BIVMiB041**
Title	Exploiting a copper-dependent chaperone system to improve bioprocessing of therapeutic antibodies
Academic (lead) Partner	Mark Shepherd, University of Kent
Industrial Partner	FUJIFILM Diosynth Biotechnologies
Public summary	The production of biotherapeutics has a total market value of around £100 Billion per year. This project expands the repertoire of a novel system that has been shown to improve the production of antibody fragments in E. coli, a product with wide ranging applications in diagnostics (e.g. pregnancy tests), human therapeutics and as fundamental research tools. In addition, future consumer applications might include the use of antibodies in shampoos to prevent the formation of dandruff or in toothpaste to protect against tooth decay. This project focusses upon copper-dependent protein folding catalysts (Scs proteins), with the overall aim of improving the yield and quality of antibody fragments produced in E. coli. Previous antibody targets include the cancer therapeutic heceptin, whereas the current work has a particular focus on Lucentis, a therapeutic antibody used to treat macular degeneration.
Start date	1 September 2017

ID number	**BIVMiB040**
Title	A pilot study to characterise plant-derived compounds that promote the synthesis of copper nanoparticles from contaminating copper ions in waste water
Academic (lead) Partner	Keith Lindsey, Durham University
Industrial Partner	Andrew Moore, Northumbrian Water
Public summary	Contamination of land and waterways by toxic metals is a serious environmental problem particularly in areas of the UK where mineral mining was once widespread. However, if the polluting metal can be sequestered into bioactive metal nanoparticles (NPs) then these NPs have important commercial values e.g. Copper NPs have diverse uses ranging from industrial catalysts to antimicrobials in food packaging. Previous work has established that crude plant extracts when mixed with solutions of metal ions extracts stimulate the synthesis of metal nanoparticles. The aim of this project is to determine the identity of the bio-active molecules within the plant extracts that are required for Copper NPs synthesis and their mode of action. The results from this pilot study will inform future use of plants, plant cell cultures or specific plant-produced compounds to remove contaminating copper, and other trace metal ions from waste water in order to synthesize commercially valuable metal nanoparticles for further exploitation in a specific collaboration with our industrial partners.
Start date	1 August 2017

ID number	**BIVMiB042**
Title	Creating new starch active copper LPMOs through the generation of loop libraries
Academic (lead) Partner	Jonathan Worrall, University of Essex
Industrial Partner	Johannes de Bie, WeissBioTech
Public summary	The efficient deconstruction of plant biomass into biofuels and other chemicals is a key challenge to secure a low carbon economy. In nature, many microorganisms secrete enzymes that can break down recalcitrant biomass that is composed mostly of lignocellulose into soluble substrates. Harnessing the catalytic power of these enzymes to treat biomass outside of their natural habitats is challenging and a major goal of industrial biotechnology. Recently, a new class of enzyme that drastically increases the efficiency of biomass conversion has been identified. These enzymes contain a copper ion and are called lytic polysaccharide monooxygenases (LPMOs). The aim of this project is to assess

	whether second generation LPMOs with enhanced substrate activities can be created. As a proof of principle, we will use a starch degrading LPMO as a template to design and synthesize DNA libraries that will then be screened for substrate activity.
Start date	23 October 2017

ID number	**BIVMiB043**
Title	Investigating the link between metal homeostasis, sporulation, and solvent production in the Clostridial ABE fermentation process
Academic (lead) Partner	Peter Chivers, Durham University
Industrial Partner	Liz Jenkinson, Green Biologics Ltd
Public summary	Clostridia are exemplars of fermentative microbes that convert biomass to renewable chemicals. Green Biologics Limited use this process commercially to produce the platform solvent chemicals butanol (with diverse uses including consumer fuels, paints and coatings and food additives) and acetone (used in cosmetics, plastics and numerous other markets). Solvent yield is limited by the physiology of the microbes under production conditions, including the onset of sporulation. Endospore formation diverts energy to generate biomass, which does not contribute to solvent production. A detailed understanding of the link between metal ions and Clostridium metabolism and physiology during solvent production will provide the means to improve strains for greater solvent yields, an important factor in the economic viability of the process.
Start date	1 November 2017

ID number	**BIVMiB044**
Title	Evaluation of the potential of the molybdenum-containing enzyme DMSO reductase as an oxygenation catalyst
Academic (lead) Partner	Gary Black, Northumbria University
Industrial Partner	Robert Holt, Piramal Healthcare UK Ltd
Public summary	Over the past 30 years science has made huge advances in understanding how biological systems work and this understanding is now being translated into valuable tools for the manufacture of products. This new technology, referred to as biotechnology, can bring many advantages over more traditional methodology. For example, pharmaceuticals are usually very complex molecules that have traditionally been manufactured using conventional chemistry techniques that rely on reactive and sometimes difficult to handle materials; in contrast, biotechnological approaches make use of Nature's own catalysts (called enzymes) to carry out reactions under very benign conditions and unlike conventional catalysts enzymes are biodegradable and non-toxic. This project is exploring the potential of enzymes to carry out complex oxidation chemistry which can be applied in the manufacture of pharmaceuticals and other important products. Emphasis will be placed on getting the enzymes to work effectively in the non-natural environment of a chemical reactor.
Start date	1 November 2017

Proof of Concept Awards– Projects funded by the Metals in Biology Network

ID number	**POCMiB002**
Title	Enhancing *E. coli* for optimal cofactor insertion into heme and iron-sulfur cluster proteins
Academic (lead) Partner	Nick Le Brun, University of East Anglia
Industrial Partner	Mark Blight, Biocatalysts Ltd
Public summary	Metals as cofactors of proteins and enzymes are essential for all of life. Many metalloproteins have properties that are useful outside of the cell, for example in the synthesis of useful materials or medicines. However, to exploit this, the metalloprotein must be purified with the metallo-cofactor fully incorporated; incomplete incorporation results in major inefficiencies in the production process. A strategy to improve cofactor insertion is to more carefully match protein synthesis with cofactor synthesis/insertion. While in some cases this can be achieved by manipulation of the growth conditions, for example by slowing down protein synthesis, for commercial processes where protein yields are key, important economic and production benefits should be achievable through the engineering of cell factories to increase their capacity to incorporate the cofactor. In this project, the academic partners, who are experts in iron metabolism and iron-protein chemistry, propose to generate strains of E. coli that have significantly increased capacity to incorporate iron sulfur clusters and heme into a range of protein targets. These strains, together with growth protocols developed to use them to best effect, will be tested for the production of examples of commercially relevant iron-sulfur and heme proteins by the commercial partner.
Start date	1 August 2015

ID number	**POCMiB011**
Title	Mag-Tag: magnetite nanoparticle affinity tags for industrial biotechnology protein purification
Academic (lead) Partner	Sarah Staniland, University of Sheffield
Industrial Partner	Mark Blight, Biocatalysts Ltd
Public summary	Enzymes are protein catalysts which can perform highly specific biotransformations to convert starting materials into desired (often) complex products using mild, aqueous reaction conditions. These key capabilities are difficult to achieve with conventional catalysts, making enzymes ideally suited to the industrial manufacture of foodstuffs, biofuels, pharmaceuticals and a range of other industrial biotechnology. The current challenge to the widening the use of enzymes is the expense of producing them. Large scale industrial purification of enzymes is often prohibitively costly due to the need for expensive, highly functionalised purification resins. This cost barrier limits the use of enzymes to industrial applications where unpurified, crude enzymes are suitable, or where the end product is sufficiently prized to enable the cost of purification to be recouped. We propose a revolutionary, cheap, universally applicable, enzyme purification method to widen the use of purified enzymes in industry. We will use protein fusion-tag technology to purify enzymes directly from crude preparations using cheap, unfunctionalised magnetic iron-oxide nanoparticles, which can then be bulk purified through magnetic separation. By substantially reducing the costs of purification we seek to make

	enzymes an affordable, green and sustainable method of producing a wide range of products.
Start date	1 October 2015

ID number	**POCMiB016**
Title	Proline Hydroxylases for Biocatalysis
Academic (lead) Partner	Chris Schofield, University of Oxford
Industrial Partner	Daniel Brookings, UCB Celltech
Public summary	Biocatalysis is a successful method for the industrial production of small- or macro-molecules and can enable production of molecules that are difficult, expensive or often impossible to synthesise. Metallo-enzymes (biological molecules that use metals to catalyse chemical reactions) are abundant in nature and are used by microbes for biosynthesis of small-molecules such as antibiotics or for the modification of biological macromolecules. The long term objective of this project is to develop and provide access to metallo-enzymes useful for biocatalysis in cells on an industrial scale. In collaboration with UCB Celltech we will focus on enabling discoveries that will support useful bio- catalysis and bio-transformations for the generation of end products or intermediates involved in the manufacture of biofuels, pharmaceuticals, fragrances, or flavours and also for the late stage diversification of tools for drug discovery (i.e. compound libraries) in a sustainable manner (i.e. Green Chemistry). There is a current lack of accessible libraries of oxygenase enzymes suitable for use in biocatalysis and little information on how their activity is limited by metal binding in cells. Our wide range of resources will allow us to efficiently explore and engineer novel methods for accessing industrial production of new chemical entities.
Start date	12 November 2015

ID number	**POCMiB019**
Title	Light-activated caged-iron chelator for skin photoprotection based on the natural product pulcherrimic acid
Academic (lead) Partner	Charareh Pourzand, University of Bath
Industrial Partner	Timothy Miller, Croda Europe Ltd
Public summary	At present, there is a significant need to counteract the cellular mechanisms that cause skin damage upon prolonged exposure to the UV component of sunlight. Exposure of skin cells to UVA, the oxidizing component of sunlight promotes the generation of harmful reactive oxygen species and leads to an immediate release of labile iron and susceptibility to both oxidative membrane damage and necrotic cell death. Research at Bath since 2006 has resulted in the synthesis and biological validation of light-activated protective compounds (i.e. light-activated caged-iron chelators, CICs) that respond to the UVA-component of sunlight. Upon activation by sunlight these ‘intelligent’ compounds release an active iron trapping agent (iron chelator) to remove the potentially harmful free labile iron released in skin cell and thereby to protect against iron-catalysed oxidative damage and cell death. A critical requirement for CIC technology is readily available, chemically tractable iron chelators, in which the iron-binding motif can be reversibly modified (caged). In this context, we plan to isolate and modify (cage) the pulcherrimic acid, a natural product from the yeast *M. pulcherrima* with iron chelating activity and subsequently evaluate its photoprotective

	activity against UVA-induced iron damage in cultured skin cells.
Start date	28 September 2015

ID number	**POCMiB022**
Title	New routes for expression of heme protein targets
Academic (lead) Partner	Emma Raven, University of Leicester
Industrial Partner	Andreas Kuglstatter, Roche Innovation Center
Public summary	Heme-containing enzymes are a mainstay of industrial biotechnology, and the industry depends on fundamental improvements in methodology emerging from academic groups around the world to harness the potential of their investments in biopharmaceuticals, bioenergy, biocatalysis and drug design. For a number of complex reasons, the interactions between industry/biotechnology and academic laboratories are often less facile and less extensive than they could be, so that new (often specialist and/or unpublished) information is not transferred fluently to industrial partners. Our overall objective is to use this project to develop new refolding methodologies for expression of difficult (insoluble) heme protein targets, and to set up an on-going dialogue between industrial and academic partners with mutual cognate interests in specific heme enzyme targets. The methodologies that we develop will open up new avenues for industry partners in cases where they have intractable (insoluble) protein targets.
Start date	27 November 2015

ID number	**POCMiB024**
Title	Tailoring the in planta synthesis of metal nanoparticles for production of high-value catalysts
Academic (lead) Partner	Neil Bruce, University of York
Industrial Partner	Richard Bate, Yorwaste Ltd
Public summary	Platinum group metals (PMGs) are used in an ever-expanding arrange of technologies and demand is spiralling upwards. PGMs are rare, exist in low concentrations and expensive to mine. It is essential that these metal reserves are utilised and recycled responsibly, not dispersed and lost into the environment. Plants can take up metals from their environment, and, in the case of PGMs, can deposit them as nanoparticles within their tissues. Nanoparticles have remarkable properties, when compared to the bulk of the same metal, which have been exploited. For example palladium nanoparticles are important catalysts for many pharmaceutical applications. Although currently synthesised chemically, we have shown that plants containing palladium nanoparticles can also be used to make efficient biocatalysts. These biocatalysts utilise carbon-neutral plant biomass, reduce processing steps by using the nanoparticles together with the plant material and concentrate valuable metals from waste sources. The addition of specific peptides (very small proteins) to solutions of metals increases nanoparticle formation, and alter size and shape; factors that can be used to optimise catalysts for different processes. This proposal is to investigate if expression of peptides in plants can be used to increase the formation, and control the size of, plant-derived biocatalysts.
Start date	1 November 2015

ID number	**POCMiB028**

Title	Arginine-terminated LPMOs: a new face in biomass breakdown?
Academic (lead) Partner	Paul Walton, University of York
Industrial Partner	Novozymes A/S
Public summary	The efficient conversion of abundant biomass into liquid biofuel is of vital importance in meeting the world's energy demands. Until recently, however, and despite its unrivalled calorific potential it has not been possible to take abundant biomass, which is composed mostly of lignocellulose, and industrially convert it through to bioethanol. The reason for this is the chemical recalcitrance of the cellulosic biomass. Of the available methods, the use of enzymes to perform the breakdown looks promising, especially enzymes called, lytic polysaccharide monooxygenases (LPMOs). LPMOs have overturned our understanding of biomass conversion as they boost significantly the conversion of biomass to ethanol. This particular project aims to study a whole new exciting class of metal- containing LPMOs which do not contain the usual active site amino acids (histidine), thereby offering new insight into how biology performs the conversion of biomass, and consequently humankind's ability to use biomass as a sustainable fuel source.
Start date	1 July 2017

ID number	**POCMiB029**
Title	Engineering metal dependent biotin synthase for the biotechnological production of biotin
Academic (lead) Partner	Christof Jaeger, University of Nottingham
Industrial Partner	Adriana Botes, VideraBio Ltd
Public summary	Biotin (vitamin B7) is an essential cofactor in bacteria, fungi and plants. It is used in vitamin food supplements and primarily for the enhancement of animal feed. Its production is challenging and particularly expensive with sales prices of ~$1600/kg. In this project we aim to demonstrate a potential way towards a sustainable and cost effective biotechnological production of biotin. We will initiate engineering the key bottleneck enzyme biotin synthase which is responsible for an iron sulfur cluster-induced sulfur insertion reaction. We are going to investigate the first steps into rational computational enzyme design in alignment with the development of automated high throughput, multidimensional, in vivo assays for this enzyme for use in our on-site robotics suite. Our approach will act as starting point for not only rational informed directed evolution strategies, but also will integrate regulatory elements for the repair mechanisms of the host cells.
Start date	15 July 2017

ID number	**ISCFPOCMiB035**
Title	Adding value to biocatalytic hydroxylation products for synthesis and drug discovery
Academic (lead) Partner	Jeremy Robertson, University of Oxford
Industrial Partner	Jason King, Oxford Biotrans Ltd
Public summary	This project exploits the special chemical properties of a set of complex biological catalysts (enzymes) that contain iron. The enzymes will be produced in growing engineered E. coli cells, and the iron centre, when bound to oxygen from the air, is uniquely able to introduce a polar 'handle' onto organic

	molecules. We intend to use these iron-containing enzymes to produce compounds of value to the pharmaceutical industry whose drug discovery campaigns usually begin by screening compound collections for 'hits' or, in fragment-based approaches, the features of weakly-active 'fragment' compounds are combined to identify promising 'leads'. This depends crucially on access to small organic molecules, whose nature dictates the trajectory of the whole drug discovery campaign. The highest chance of identifying leads that develop successfully into successful drugs arises when the initial compound/fragment collection is structurally diverse; therefore, we aim to diversify compound collections in a two-stage process that mimics the biosynthesis of known medicines such as the anti-cancer drug taxol. Stage one is the above-mentioned enzymatic introduction of the 'handle'; in stage two, the chemical properties of this handle will be exploited to introduce features that promote favourable interactions with drug targets.
Start date	1 November 2017

ID number	**ISCFPOCMiB038**
Title	Arginine terminated LPMOs—a new face in biomass breakdown? Follow-on funding
Academic (lead) Partner	Paul Walton, University of York
Industrial Partner	Jens Nielsen, Novozymes
Public summary	The generation of fuels/commodity chemicals from sustainable biomass hinges on a single key issue. This issue is that biomass (e.g. wood, plant matter) is very hard indeed to break down in a controlled manner, severely hindering its sustainable conversion into useful materials such as bioethanol and other chemicals. It is an issue which has bedevilled the bio-based industry. However, in a major breakthrough in 2010/11 we discovered copper-containing *lytic polysaccharide monooxygenases* (LPMOs) which are natural enzymes that can break down cellulose (a plant based polysaccharide) in a highly efficient manner. LPMOs are now used in biorefineries to generate bioethanol and have transformed the industry. In this project, we seek to maximise the ability of a new type of LPMO to break down woody biomass. If the ability of this enzyme to convert lignin could be harnessed then it would be a significant addition to the biomass industry.
Start date	1 December 2017

ID number	**ISCFPOCMiB032**
Title	Site-specific bioconjugate chemistry for antibody-nanoparticle conjugates
Academic (lead) Partner	Lu Shin Wong, University of Manchester
Industrial Partner	Stephen Kilfeather, Aeirtec Ltd
Public summary	Fluorescence-based immunosorbent assays have become a key technology for the detection and quantification of biomolecules, and have found application in a range of fields from the testing of microbial contamination (in water, chemical, food and drug production), biomarkers (in medical diagnostics and drug discovery) and in biomedical imaging. This project will develop production methods for metal nanoparticle-antibody conjugates that are robust and scalable, which would be needed for commercial implementation. These hybrid metal-biomolecule materials offer advantageous

	spectroscopic properties that could greatly increase detection sensitivity. It is envisaged that such nanoparticle-antibody conjugates will themselves be highly sought-after industrial biotechnology products for the applications noted above. In addition, they will be utilised in the development of new biologics and peptides. Indeed, the development of such products will be greatly enhanced and accelerated by incorporation of very sensitive and faster monitoring assays that these conjugates will enable.
Start date	1 November 2017

ID number	**ISCFPOCMiB041**
Title	Biosynthesis of bimetallic nanoparticles for fine and speciality chemical production
Academic (lead) Partner	Jon Lloyd, University of Manchester
Industrial Partner	Nigel Powell, Johnson Matthey plc
Public summary	This project combines biotechnologists from the University of Manchester and industrial catalysis experts at Johnson Matthey, a multinational speciality chemicals and sustainable technologies company. This “Proof of Concept” award will exploit the ability of microorganisms to produce bimetallic nanocatalysts for fine and speciality chemical production. Metal-reducing bacteria are able to recover a wide range of metals from process environments as catalytically active nanoparticles. By taking advantage of the unique biochemistry of metal-reducing bacteria, we will produce highly reactive and tunable metallic nanoparticles for speciality chemical production. These will include bimetallic nanoparticles that offer advantages over monometallic catalysts due to the combination of properties from the presence of two metals (rather than one) and from additional properties due to the synergy of the two metals, offering increased efficiency and specificity for speciality chemical production. This novel biotechnological process also offers a simple, cost-effective, environmentally friendly synthesis route for bimetallic catalyst production.
Start date	1 November 2017

Case Studies

There are currently 39 case studies available from the funded Metals in Biology BBSRC NIBB projects and are complimented by 2 further case studies which are related to events run by this network.

BIVMiB003 BB/L013711/1 1 February – 31 July 2015
POCMiB002 BB/L013711/1 1 August 2015 – 31 May 2016
Funded by a Metals in Biology BBSRC NIBB Business Interaction Voucher and Proof of Concept Funding
metals.bbsrcnibb@durham.ac.uk @METALSBBSRCNIBB http://community.dur.ac.uk/MiB_NIBB/

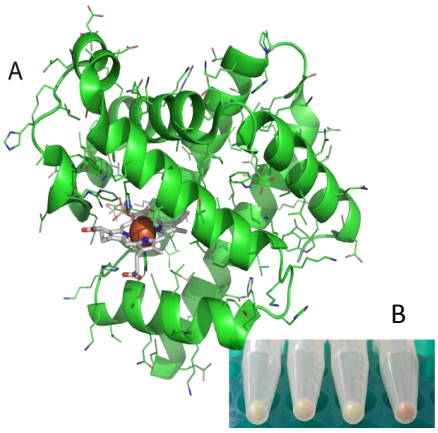
Enhancing *E. coli* for optimal cofactor insertion into heme-containing proteins

"This work showed that the P450 enzyme was sub-optimally loaded with heme. Further enhancement of heme incorporation could increase the commercial yields of this enzyme, so we support further investigations into this and are pleased to continue our collaboration with Professor Le Brun's laboratory." **Biocatalysts Ltd**

Nick Le Brun, Myles Cheesman, Jonathon Todd & Jason Crack, University of East Anglia; Mark Blight & Jon Wood, Biocatalysts Ltd

OUTCOMES: Our initial set of studies showed that the amount of cytochrome $P450_{BM3}$ recovered from *E. coli* cell lysates was low and that only 56% of the P450 contained heme. Moreover, $P450_{BM3}$ existed as truncated protein; most likely due to proteolysis in the cell lysates. We then explored several different strategies to improve heme incorporation into several heme-containing proteins, including over-expression of parts of the heme biosynthetic pathway and the expression of membrane-bound importers capable of taking up intact heme from the growth medium. Using *E. coli* (a workhorse for protein production), we generated a system that gives ~3-fold enhancement of heme incorporation into two very different 'test' heme-containing proteins (*E. coli* bacterioferritin and human myoglobin). Preliminary results with a third 'test' protein indicated that the effect is reproducible for other heme-containing proteins. To date, we have not seen any negative effects on yield of protein. These are all important properties for a process that could be commercially useful.

A) Cartoon structure of human myoglobin showing the heme group with an iron atom at its centre. **B**) *E. coli* cell pellets. The two tubes on the left show pellets of cultures without the heme enhancement system; the two on the right with it. Each pair shows before and after induction of heme protein production.



INITIAL AIMS: Many metalloproteins have the potential to be used as biocatalysts in the synthesis of useful materials or medicines. However, to exploit this the metalloprotein must be purified with its metallo-cofactor fully incorporated to avoid major inefficiencies in the production process. A strategy to improve cofactor insertion is to more carefully match protein synthesis with cofactor synthesis/insertion. This should be achievable by engineering cell factories to increase their capacity to produce and incorporate the cofactor. In these projects, we will first analyse the level of heme insertion in a commercially relevant metalloprotein, cytochrome P450. We will then generate *E. coli* strains of that have increased capacity to incorporate heme into cytochrome P450, which, together with optimised growth protocols, will be tested for the production by the commercial partner.

- Awarded funding (£12k) from UEA to continue the project
- Aim to submit application to BBSRC Follow on Funding Pathfinder scheme
- Aim to test increased heme incorporation into wider range of proteins

BIVMiB008 BB/L013711/1
1 July – 30 April 2015
Funded by a Metals in Biology BBSRC NIBB Business Interaction Voucher
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Metallo-enzymes for production of nootkatol a potential new citrus flavour

"The project worked a lot better than even we expected. This BIV research program contributed to the scale-up and commercialisation of nootkatone (grapefruit flavour) production". Oxford Biotrans Ltd

OUTCOMES: Samples of the pure nootkatol isomers were produced and delivered to the industry partner. The potential market of these novel flavours is being assessed. Enzyme variants that gave increased proportions of either nootkatol isomer were generated. Process optimisation also led to improved yields of the nootkatone production process. New strains from the NIBB MiB network can be applied to the systems and processes developed in this BiV project to benefit the UK industrial biotechnology sector.

INITIAL AIMS: The sought after grapefruit flavour compound nootkatone is biosynthesised by air oxidation of valencene catalysed by a haem enzyme, firstly to nootkatol and then nootkatone. Haem enzymes are involved in the biosynthesis of numerous natural products, including flavours such as menthol and nootkatone but also medicinal compounds such as antibiotics, the antimalarial artemisinin and the anticancer drug taxol. The industry partner is developing commercial scale biocatalytic synthesis of nootkatone from valencene. The academic partner will modify the haem enzyme used in this process to produce nootkatol, which is found in minute quantities in grapefruit, to explore its potential as a novel flavour. This primer-project and the nootkatone process will underpin future collaborative work within the metals-in-biology community (1) to test strains optimised (by others) in haem production to further enhance nootkatone/nootkatol synthesis, (2) to manipulate the primary and secondary coordination sphere of the haem moiety to further enhance nootkatol synthesis.

Luet Wong ***University of Oxford***
Jason King ***Oxford Biotrans Ltd***

- Nootkatol and nootkatone produced
- Products introduced in Europe & Asia
- Relevant to GCRF-related initiatives on antimicrobial natural products

BIVMiB036 BB/L013711/1
1 July – 31 December 2017
Funded by Metals in Biology BBSRC NIBB Business Interaction Voucher
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Improving biocatalytic processes by enzyme stability enhancement

"Increasing the stability of the enzyme catalyst in biocatalytic oxidation technology will improve the catalyst lifetime, leading to improved process productivity and broadening the range of products accessible to this technology platform," Luet Wong, University of Oxford

Stuart Ferguson and Luet Wong, University of Oxford; Jason King, Oxford Biotrans Ltd

RESULTS: To try to produce a P450BM3 enzyme containing a covalently bound heme, we first introduced a nucleophilic side chain close to the heme vinyl groups. Cysteine and histidine substitutions were introduced by site-directed mutagenesis to give a total of 20 single and double mutants. Expression trials showed that several mutants either did not produce holoprotein (the peptide and non-peptide parts of the enzyme) or that protein was produced at a low level. The nine mutants that formed holoprotein and the wild-type enzyme were tested for their ability to oxidise lauric acid (a natural substrate of the enzyme) and propylbenzene (an unnatural substrate). The wild type was the most active of the enzymes at oxidising lauric acid and each of the mutants had a similar profile to the wild type (63% to 90% of the activity). The activity profiles were also virtually identical for propylbenzene oxidation. Next, the wild type and mutant P450BM3 enzymes were treated with dithinoite, in expectation of form a covalently bound heme group. Encouragingly, the P450BM3 enzymes treated with dithinoite had a similar pattern of activity as the untreated enzymes in lauric acid and propylbenzene oxidation assays. Unexpectedly however, further tests showed that there was no covalent attachment of the heme vinyl groups to the introduced cysteine and histidine residues under the reaction conditions we used.

INITIAL AIMS: The industry partner has licensed biocatalytic oxidation technology from the University of Oxford for the production of fine chemicals such as flavours, fragrances, agrochemicals and active pharmaceutical ingredients. This approach not only replaces classical, more energy-demanding and polluting chemical processes, it also enables the use of sustainable feedstocks that are not fossil fuels. Enhancing of the stability of the enzyme catalyst that lies at the heart of the technology will be a key improvement to the process, making it applicable to a wider range of products. In this project we aim to increase the stability of the cytochrome P450BM3 by introducing a covalently bound heme into the enzyme.

- **We were surprised not to be able to covalently attach heme onto the P450BM3 protein**
- **Systematically altering the conditions (time, concentration) for dithionite treatment should be explored as there is precedent for the attachment procedure being successful**

ISCFPOCMiB035 BB/L013711/1 BB/SCA/MetalsinBiology/17
1 November 2017 – 28 February 2018
Funded by a Metals in Biology BBSRC NIBB Seeding Catalyst grant
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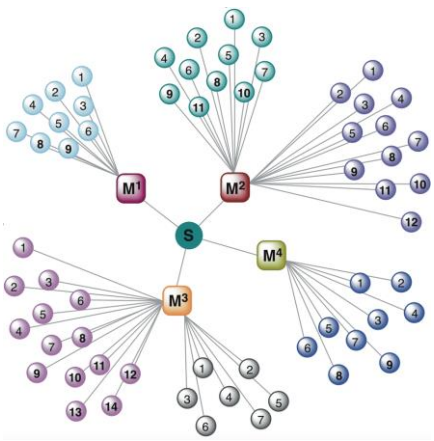
Adding value to biocatalytic hydroxylation products for synthesis and drug discovery

"We were pleased to see such encouraging results from the project, both in terms of the whole-cell bioconversions and the subsequent derivatisation of the metabolites." Jason King, Oxford Biotrans

Jeremy Robertson and Luet Wong, University of Oxford; Jason King, Oxford Biotrans



Engineered $P450_{BM3}$-mediated hydroxylation of fragment-sized unfunctionalised substrates (S) provides a series of metabolites (M) that may then be elaborated into a diversified set of new fragment molecules.

RESULTS: Following preliminary optimisation studies, engineered *E. coli* cells expressing cytochrome P450 mutants from *Bacillus megaterium* ($P450_{BM3}$) were used to produce two hydroxylated derivatives (termed **A** and **B**) of a nitrogen heterocyclic fragment molecule (*N*-Boc-8-azaspiro[4,5]decane). For **A**, 91% conversion of the starting material was achieved after 43 h; for **B**, 85% conversion was achieved after just 3 h and the reaction was complete within 20 h; the cells were still active and were re-used in an identical reaction. Both reactions scaled well to 340 mg of substrate. These proof-of-concept reactions provided synthetically useful outcomes, with reactions processing 1.70–3.4 g of substrate per litre of culture. The second phase of the project scoped a proposed method for elaborating the hydroxylated fragments into a variety of derivatives, based on tethered C–H insertion reactions. Two Rh(II)-catalysed variants were studied: (1) Du Bois' conditions for nitrenoid insertion; (2) Doyle–Lee carbenoid insertion. These reactions generated several derivatives that included three C–H amination products, currently assigned as two stereoisomers of the five-membered cyclic sulfamate (β-insertion) and the product of γ-insertion, a six-membered bridged cyclic sulfamate. This work provided insight into the likely outcomes of C–H insertion reactions in conformationally-biased molecules.

INITIAL AIMS: We intend to use iron-containing enzymes to produce compounds of value to the pharmaceutical industry. Drug discovery campaigns based on fragment-based screening combine the features of a number of 'fragment' compounds that are weakly-active at the desired target to identify promising 'lead' compounds. The highest chance of identifying leads that develop into successful drugs arises when the initial fragment collection is structurally diverse. Therefore, we aim to diversify compound collections in a two-stage process that mimics the biosynthesis of known medicines such as the anti-cancer drug paclitaxel (Taxol). Stage one employs engineered *E. coli* cells to enzymatically introduce a 'handle' (a hydroxyl group) onto a nitrogen heterocyclic fragment. In stage two, the chemical properties of this handle will be exploited to introduce features that promote favourable interactions with drug targets.

- Collaboration to continue to identify parameters that lead to shorter reaction times and higher conversions and substrate concentrations, and to improve product isolation
- Literature survey undertaken to identify alternatives to the Rh(II)-catalysed reactions
- Manuscript published: Syntrivanis et al. (2018) EUR J ORG CHEM.: https://onlinelibrary.wiley.com/doi/full/10.1002/ejoc.201801206

BIVMiB013 BB/L013711/1
1 February 2016 – 31 July 2016
POCMiB022 BB/L013711/1
27 November 2016 – 26 May 2016
Funded by a Metals in Biology BBSRC NIBB Business Interaction Voucher and Proof of Concept funding
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New routes for the expression of heme protein targets

"Production of enzymes in quality and quantity sufficient for biophysical and structural analysis has consistently been a major bottleneck for drug discovery efforts. This scheme is hugely welcomed, to help overcome these bottlenecks, not just for my company but for the entire pharmaceutical sector." Andreas Kuglstatter, Roche Innovation Centre

Emma Raven, University of Leicester
Andreas Kuglstatter, Roche Innovation Centre

OUTCOMES: In this project, new expression methods were developed for a range of different heme enzymes that are not readily expressed using conventional methodologies in *E. coli.* Targets from Emma Raven's laboratory were used as a 'test bed' for other heme systems. Human CLOCK protein, which is important in the control of circadian rhythm and thus an important drug target, was included amongst the targets.

INITIAL AIMS: Heme-containing enzymes are a mainstay of industrial biotechnology, and the industry depends on fundamental improvements in methodology emerging from academic groups to harness the potential of their investments in biopharmaceuticals, bioenergy, biocatalysis and drug design. For a number of complex reasons, the interactions between industry/biotechnology and academic laboratories are often less facile and less extensive than they could be, so that new (often specialist and/or unpublished) information is not transferred fluently to industrial partners.

Our overall objective is to use this project to develop new refolding methodologies for expression of difficult (insoluble) heme protein targets, and to set up an on-going dialogue between industrial and academic partners with mutual cognate interests in specific heme enzyme targets. The methodologies that we develop will open up new avenues for industry partners in cases where they have intractable (insoluble) protein targets.

- New expression methods may overcome bottlenecks in drug discovery

BIVMiB011 BB/L013711/1
1 January 2015 – 28 February 2015
Funded by a Metals in Biology BBSRC NIBB Business Interaction Voucher
metals.bbsrcnibb@durham.ac.uk @METALSBBSRCNIBB http://prospect.rsc.org/MiB_NIBB/

Assessing the bioavailability of metal ions accumulated by DRAM® filters

"Through the BIV, the network has allowed us to better evaluate the readiness level of our collaborator's developing technology. It may be possible to scale up this technology for commercial metal recovery." Epona Technologies Ltd

Louise Horsfall, University of Edinburgh
Leigh Cassidy, Epona Technologies Ltd

A DRAM filter

OUTCOMES: This project assessed the potential to recover Cu(II) from DRAM® media filters and convert it to metallic copper nanoparticles using *M.psychrotolerans*. Using a $CuSO_4$ solution, Cu(II) was accumulated on DRAM filters. Approximately 20% of this bound Cu(II) could be made available for biotransformation into metallic copper nanoparticles. These particles were visualised and positively identified by electron microscopy. Thus *M.psychrotolerans* can form nanoparticles from Cu(II) accumulated on DRAM® filters. Approximately 12% of Cu(II) was reduced by *M.psychrotolerans* to metallic copper nanoparticles. Although the proof of principle study was successful with model solutions and controlled copper solutions, the industrially used DRAM® media — with its unknown contaminants —is currently beyond our methods of nanoparticle isolation and analysis, so further work is needed to identify nanoparticles from this source.

INITIAL AIMS: There is increasing concern over environmental copper levels, their toxicity and the adverse effects on humans and wildlife. Epona Technologies Ltd has developed DRAM® (device for the remediation and attenuation of multiple pollutants) filters, which can accumulate polluting copper ions from industry and agriculture. We would like to determine whether the copper ions accumulated by DRAM® filters can be transformed into copper nanoparticles by *Morganella* sp., so offering a way to recycle copper.

- ***M.psychrotolerans* can form copper nanoparticles from Cu(II) accumulated on DRAM® filters**
- **Paper: *Cueva & Horsfall (2017) Microb Biotechnol. 10: 1212-1215***

BIVMiB026 BB/L013711/1
01 February 2016 – 31 July 2016
Funded by a Metals in Biology BBSRC NIBB Business Interaction Voucher
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Microbial recovery of metals from contaminated *Miscanthus* used in the industrial remediation of degraded landscapes

"With this funding, we were able to kick start a new collaboration, bringing technologies together that wouldn't have been possible from any other funding source."

Chris Chuck, University of Bath; **Michal Mos**, Terravesta Ltd

OUTCOMES: The project demonstrated that the most suitable technique for metal recovery from *Miscanthus* grown on contaminated land was hydrothermal liquefaction. The hydrothermal liquefaction of the *Miscanthus* produced a reasonable bio-oil yield, and in addition the majority of metals from the *MIscanthus* partitioned in the aqueous phase or the solid residue and could be recovered and/or recycled easily. Further work to increase the bio-oil content needs to be conducted, as well as further optimisation to partition the metals into the solid residue while decreasing the carbon content.

Chris and Michal discuss their collaborative work

INITIAL AIMS: Metal leaching from mining and other industrial activity has the potential to degrade landscapes across the globe. However, several techniques have recently been trialled and brought to market to restore the natural capital of such areas. One of the most promising is growing *Miscanthus x giganteus*, an energy crop that can remove metal contamination while being used as a biofuel feedstock. However, the processing of the contaminated *M. x giganteus* remains an issue. In this study we explored the use of the oleaginous yeast *M. pulcherrima* — which produces metal chelators such as pulcherriminic acid — as a method of valorising the *Miscanthus* biomass into a range of products including a palm oil substitute, and removing the metal waste into a smaller containable volume. This method was compared to hydrothermal processing of the *Miscanthus* waste.

- **Hydrothermal liquefaction was the most suitable technique for metal recovery**
- **Further work is underway to assess the applicability of the technique**

BIVMiB019 BB/L013711/1
18 May – 17 November 2016
Funded by a Metals in Biology BBSRC NIBB Business Interaction Voucher
metals.bbsrcnibb@durham.ac.uk @METALSBBSRCNIBB http://prospect.rsc.org/MiB_NIBB/

Studies into the uptake and distribution of metal oxide nanoparticles in plants

"The collaboration has allowed us to apply expertise in mass spectrometry to an industrially relevant area in seed enhancement, springboarding further research into the area of nutrient delivery." Croda Europe

Neil Bricklebank, Malcolm Clench & Catherine Duckett, Sheffield Hallam University
Kathryn Knight & Marta Dobrowolska, Croda Europe

OUTCOMES: Two samples of barley seeds coated with zinc oxide particles and different seed treatments were prepared by Croda. Seeds from each sample were germinated at Sheffield Hallam University and harvested at different growth points. The selected germinated seeds were embedded in gelatin, cryosectioned and then analysed by LA-ICP-MS. Untreated, germinated seeds were used as the control. The results provide a two-dimensional 'map' (figure) showing the location of the zinc within the seed at different time points throughout its germination. The results clearly show that as the seed is germinated the zinc is transported from the coating applied to the surface and into the shoot of the seedling. The results are being used by Croda to assess the effectiveness of their products that are used in commercial seed coatings, including surfactants, adjuvants and formulation aids, on the uptake of metals by plants

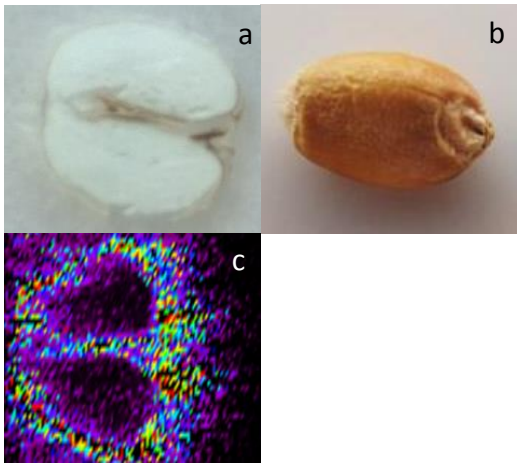

Image of (a) un-germinated seed, (b) section of treated seed tissue, and (c) LA-ICP-MS image showing distribution of zinc in seed tissue.

INITIAL AIMS: The uptake of metals is essential for the growth and development of healthy plants. Plants obtain the metals they need from soil or from fertilizers applied to the growing plant. One of the most important metals is zinc, which is found in many metalloenzymes. Zinc is also essential for humans who gain it from dietary grains and vegetables. In this project we will study the effect of zinc, in the form of a formulation containing zinc oxide, on the growth of plants and use a new analytical tool — known as laser ablation-Inductively coupled plasma-mass spectrometry (LA-ICP-MS) — to study the uptake and distribution of zinc in plants.

- **Results used by Croda to assess the effectiveness of products used in commercial seed coatings on the uptake of metals by plants**
- **Innovate UK funding won to advance the work**
- **Croda awarded a BBSRC iCase studentship to continue the work**

BIVMiB0031 BB/L013711/1
01 October 2015 – 31 July 2016
Funded by Metals in Biology BBSRC NIBB Business Interaction Voucher
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Bioaccumulation of platinum from waste

"Finding an easier and cheaper way to reclaim of platinum which would otherwise be lost to the environment is important for global resource management." Helen Carney, Teesside University

Helen Carney and Caroline Orr, Teesside University; Frans Maathuis, University of York; Pattanathu Rahman, TeeGene Biotech

OUTCOMES: We tested several microbial species — *Shewanella algae, Pseudomonas aeruginosa, Bacillus megatherium*— that were suggested by the literature to have biosorption properties and so have the ability remove platinum from solution. Our studies showed that under the conditions tested, *S. algae* removed more platinum from a solution (hexachloroplatinic (IV) acid, see figure) than *P. aeruginosa* or *B. megaterium*. The optical density of the platinum solution was not reduced by *B. megaterium* and a reduction of only between 5.7% – 6.5% was observed with *P. aeruginosa.* However, a a 55% reduction was shown with *S. algae*. This work confirmed the findings of other studies that showed that *S.algae* could take-up platinum ions from solution. The results add to knowledge in an important area for waste management, since finding an easier and cheaper way to reclaim platinum that would otherwise be lost to the environment is important for global resource management. A logical next step to this work would be to determine the optimal conditions (salt concentrations, temperature, time) required by biosorbants to remove platinum from the two broad categories of waste – high volume, low concentration (e.g. sewage waste or mining waters) and low volume, high concentration (e.g. electroplating discharge). Additional work could consider the role of contaminants and whether uptake is passive or involves hydrogenase enzymes.

Dilutions of hexachloroplatinic (IV) acid prior to analysis. Darker solutions contain more platinum.

INITIAL AIMS: Platinum is a scarce metal, being one of the least abundant elements in the earth's crust and as such has a high material value. This research will focus on the recovery of platinum from wastewaters, where it is present as a soluble, ionic form. Platinum enters wastewater from a range of sources such as metal refining and chemical industries as well as hospital waste, where it can be found as a component of chemotherapy drugs. Bacteria can take-up and accumulate platinum using both active and passive methods, often referred to as biosorption and bioaccumulation respectively. This project, which is a collaboration between TeeGene Biotech, Teesside University and University of York, will investigate the potential of microbes to recover platinum from solutions, with the aim of recycling the recovered metal. The project aims to identify a suitable microbe that can be used in a waste refining process and identify any physicochemical factors that influence platinum recovery.

- **Ongoing business–academia relationship sustained via dissertation research**

BIVMiB032 BB/L013711/1
16 January – 14 July 2017
Funded by Metals in Biology BBSRC NIBB Business Interaction Voucher
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Embedding technical expertise in the optimisation of trace metal supplementation strategies for successful biomethane production

"The work has been an eye-opener for the company in terms of the potential for optimisation of the plant, and a huge benefit in terms of skills transfer to our staff" Michael Mason, Tropical Power Ltd

Yue Zhang, University of Southampton and Michael Mason, Tropical Power Ltd

RESULTS: The project developed methods that are helping a UK company, which has built and is currently operating Africa's first grid-connected anaerobic digester, to determine more precisely the trace element requirements for optimum digestion of their novel agricultural waste feedstocks. We developed a method suitable for use in Africa that uses simple multi-purpose apparatus to test which trace elements are actually required. The methodology was made available to the industrial partner in the form of a training video and a detailed description of the procedures. In addition we helped our partner company interpret historical data from the digestion plant and provided them with a simple spreadsheet-based calculator to allow them to maintain steady state concentrations of essential elements in the digester in proportion to the feed added. The work also added tantalisingly to growing evidence that minor trace elements such as tungsten may play a critical role in the function of these microbial systems.

Tropical Power's grid-connected anaerobic digester in Kenya

INITIAL AIMS: Transformation of waste biomass into bioenergy is a key component in 21st century industrial biotechnology. It is increasingly clear that successful biomethanisation of mixed biomass requires complex enzyme systems that are produced by both natural and engineered synthetic microbial communities. Trace quantities of metals, which are required by certain essential metallo-enzymes, are needed to ensure that these microbial systems function in the most effective and productive way. There is a growing commercial market in trace metal supplements, but formulations of these are often generic rather than based on specific requirements. The current project will transfer knowledge and expertise in determining trace metal requirements to a UK company that uses novel waste feedstocks in Africa for renewable biomethane production. This will enable the company to formulate specific trace metal mixtures for optimum plant performance, and the scientific knowledge gained will contribute to the creation of future markets for UK suppliers of tailored supplements.

- **Collaboration broadened into new scientific areas help meet bioenergy needs of lower income countries**
- **Academic and industrial collaborators partnering on stage 1 GCRF application**

BIVMiB009 BB/L013711/1
1 January 2015 – 25 October 2015
Funded by a Metals in Biology BBSRC NIBB Business Interaction Voucher
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Adding value to galactomannan polysaccharides with copper enzymes

"By working with industrialists to address a specific question, we have gained unique insights from those with first-hand experience of running a commercial process." Julea Butt

Julea Butt, University of East Anglia
Seth Hartshorne, Schlumberger Gould Research

Guar beans, from which guar gum is made

OUTCOMES: Guar gum is a galactomannan that is comprised of covalently linked sugar molecules, namely mannose and galactose. In this project, polymer hydrolysis resulting in chain shortening was demonstrated using a commercially available enzyme, cellulase. Oxidation of the galactose sidechains within the polymer in aqueous solution was shown using another commercially available enzyme, galactose oxidase. In addition, electrochemical oxidation of the galactose and mannose sugars was demonstrated by oxidation of aqueous guar suspensions at a graphite electrode. The results demonstrate that commercially available enzymes offer routes to controlled modification of guar in aqueous solution and this offers prospects for the development of more sustainable routes to industrial-scale galactomannan modification.

INITIAL AIMS: Concerns over fuel security are frequent headline news and the rising costs of fuel are a daily reminder of the challenges faced by a global society with ever- increasing energy demands. Medium- to long-term solutions to these challenges will require effective access to renewable energy alongside the development of infrastructures that enable such energy to be delivered to the point of need with the same ease as fossil fuels. Improved technologies to increase the recovery of natural gas presents an attractive option for the short to medium term. Here we aim to investigate opportunities to develop improved stimulation technologies through the use of copper-containing enzymes that can modify the rheological properties of a natural biopolymer guar.

- **Commercially available enzymes might provide a more sustainable route to industrial-scale guar gum modification**
- **Modified polysaccharides such as guar could improve hydrocarbon recovery technologies**

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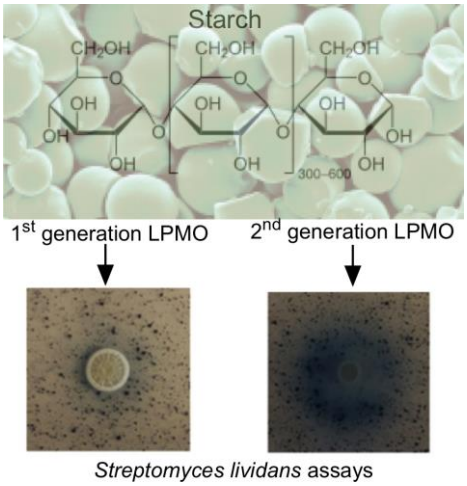
Creating new starch-active copper LPMOs through the generation of loop libraries

"We are pleased to be part of this research project as we believe that LPMOs will have significant role in starch hydrolysis and starch modification. So far the development of the LPMOs has progressed very swiftly and we are excited to see the next steps in this project." Johannes de Bie, WeissBioTech

Jonathan Worrall, University of Essex; Johannes de Bie, WeissBioTech

OUTCOMES: To create second-generation starch-active lytic polysaccharide monooxygenases (LPMOs), loops that form the active surface surrounding the catalytic copper ion were targeted. In total, there are five active surface loops that may be considered important for interaction and specificity with starch. Active surface loop libraries have been designed *in silico* with saturating mutations in selected amino acid positions in each loop based on sequence variability within the starch-active LPMO family. This business interaction voucher allowed for the synthesis — by combining site evaluation library and combinatorial library technology — of two out of the five loop libraries in a starch-active LPMO. These two loop libraries will be screened in *Streptomyces lividans* for enhanced activity relative to the wild-type LPMO. Following this, we will conduct *in vitro* characterisation of recombinantly produced proteins and then test the activity of the selected variants under industrial conditions.



Improving the degradation efficiency of insoluble starch granules through the creation of 2nd generation LPMOs.

INITIAL AIMS: The efficient deconstruction of plant biomass into biofuels and other chemicals is a key challenge to secure a low carbon economy. In nature, many microorganisms secrete enzymes that can break down recalcitrant biomass that is composed mostly of lignocellulose into soluble substrates. Harnessing the catalytic power of these enzymes to treat biomass outside of their natural habitats is challenging and a major goal of industrial biotechnology. Recently, a new class of enzyme that drastically increases the efficiency of biomass conversion has been identified. These enzymes contain a copper ion and are called lytic polysaccharide monooxygenases (LPMOs). The aim of this project is to assess whether second-generation LPMOs with enhanced substrate activities can be created. As a proof of principle, we will use a starch-degrading LPMO as a template to design and synthesize DNA libraries that will then be screened for substrate activity.

- Two loop libraries from a starch-active LPMO were synthesised
- Further project work between the academic and industrial partners is ongoing

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LPMOs: a new face in biomass breakdown?

"The on-going collaboration with Novozymes into the chemistry and activities of biomass-degrading enzymes continues to provide very fruitful research, not only in the discovery of the fundamental chemical processes exhibited by these enzymes but also their potential in biomass processing." Paul Walton, University of York

Paul Walton and Gideon Davies, University of York;
Jens Erik Nielsen Novozymes, Denmark

OUTCOMES: This project examined the structure and reactivity of a new class of *lytic polysaccharide monooxyegenase* (LPMO) enzyme, which were expressed in eukaryotic systems to high yield by Novozymes. The objectives of the work were to establish whether the enzyme required metal ions for maximal activity, and if so, how those metal ions interacted with the enzyme. We also aimed to establish whether the enzyme was active on lignocellulosic substrates with an oxidoreductase type action. A series of metal-binding studies was performed using isothermal calorimetry experiments, from which it was determined that metal binding was weak and non-specific, unlike the canonical class of other LMPOs. Electron paramagnetic resonance studies showed that the enzyme bound copper. Structures of the enzyme showed that the new class of LPMOs forms interactions with lignocellulosic-type substrates near the active site. This is now an area of active investigation.

INITIAL AIMS: The efficient conversion of abundant biomass into liquid biofuel is of vital importance in meeting the world's energy demands. Despite the unrivalled calorific potential of biomass, which is composed mostly of lignocellulose, it has not been possible until recently to convert it through to bioethanol. The reason for this is the chemical recalcitrance of the cellulosic biomass. One promising way to breakdown lignocellulose involves the use of enzymes, especially lytic polysaccharide monooxygenases (LPMOs). LPMOs have overturned our understanding of biomass conversion as they boost significantly the conversion of biomass to ethanol. This project aims to study a new exciting class of metal-containing LPMOs which do not contain the usual active-site amino acids, thereby offering new insight into how biology performs the conversion of biomass and consequently our ability to use biomass as a sustainable fuel source.

- This work is continuing with BBSRC NIBB follow-on funding to examine the reaction of lignin components with the enzyme

ISCFPOCMiB038 BB/L013711/1 BB/SCA/MetalsinBiology/17
1 December 2018 – 31 January 2018
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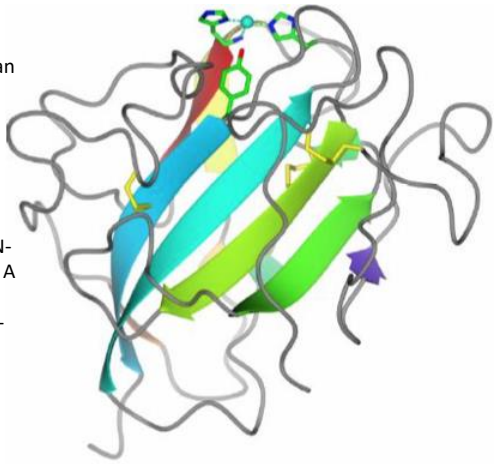
Arginine-terminated LPMOs: a new face in biomass breakdown? Follow-on studies

"The new form of LPMO enzymes is intriguing as it does not contain the usual amino acids at its active site, suggesting that it could be active on a new range of biomass components." **Paul Walton, University of York**

Paul Walton and Gideon Davies, University of York;
Jens Erik Nielsen, Novozymes, Denmark



The overall structure of a typical LPMO has an extended flat face in the middle of which lies the enzyme's active site, containing copper ion coordinated by a N-terminal histidine. A new type of LPMO has a conserved N-terminal arginine instead of an N-terminal histidine.

OUTCOMES: In this project we examined the activity of this new LPMO using ultraviolet–visible spectroscopy and electron paramagnetic resonance. We also determined the structure of the enzyme, gaining particular insight into how it interacts with its substrates. This finding opens up the possibility of tailoring the enzyme to carry out new types of reactions which are of importance to biomass degradation. While we cannot report the details of the findings due to IP reasons, the project was successful in that we developed a new type of assay for this class of enzymes and we also demonstrated that the LMPOs had a new type of enzymatic activity on a range of substrates.

INITIAL AIMS: The generation of fuels and commodity chemicals from sustainable biomass hinges on a single key issue: that biomass (e.g. wood, plant matter) is very hard to break down in a controlled manner. The use of copper-containing lytic polysaccharide monooxygenases (LPMOs) — natural enzymes that are highly efficient at breaking down cellulose — could help circumvent this. In this project, we seek to maximise the ability of a new type of LPMO to break down woody biomass. Our previous Metals in Biology-funded studies showed that this new LPMO can bind metal ions, and we have also obtained a full molecular structure of the enzyme using X-ray diffraction. The structure of this new class of LPMOs suggests that they could be active on lignin and/or lignin components and that, indeed, these LPMOs use metal ions as part of their catalytic cycle. If true, this would represent a wholly new activity for LPMOs and be an exciting addition to the field of biomass degradation.

- **The partners are in discussions on how to take the project forward**

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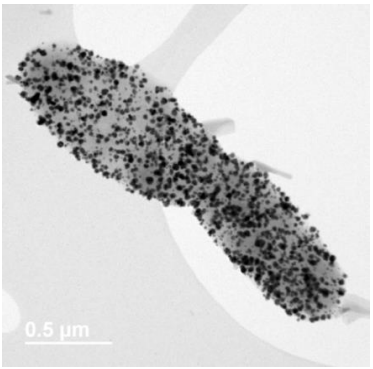
Exploiting the commercial potential of novel biometallic catalysts

"The BIV provided a quick, convenient and effective route for us to bring together the Manchester group's expertise in bioproduction of metal particles with Johnson Matthey's catalysis know-how. We have begun to determine the potential of this technology for the production of novel catalysts." Nigel Powell, Johnson Matthey.

Jon Lloyd, Nick Turner and Richard Kimber, University of Manchester; Nigel Powell, Johnson Matthey

OUTCOMES: Metal-reducing bacteria can accumulate metals from process environments in the form of catalytically active nanoparticles, offering a simple and green method for high-value nanoparticle production. These nanoparticles have many applications, including in the production of fine and speciality chemicals such as pharmaceutical intermediates, fats and oils, and upgrading of fuels and biorenewables. Bimetallic nanoparticles offer a number of advantages over their monometallic counterparts due to the combined properties of the two metals present, and through new properties created from the synergy between these metals.

We investigated the potential for a metal-reducing bacterium to produce bimetallic nanoparticles from metal solutions containing a range of metals supplied in combination. Metallic nanoparticles were biosynthesised at the University of Manchester and then Johnson Matthey's scanning transmission electron microscopy facilities were used to characterise the products. We found that the pattern of distribution of the metallic nanoparticles was highly dependent on the combination of metals supplied to the cells. Evidence was provided for the formation of bimetallic nanoparticles for some examples of metal combinations, and these nanomaterials are the focus of future work.



Bimetallic nanoparticles produced during the project

INITIAL AIMS: This project brings together biotechnologists from the University of Manchester and experts in industrial catalysis at Johnson Matthey, a leading multinational specialty chemicals and sustainable technologies company headquartered in the UK. This project will facilitate collaborative discussions required to underpin the development and exploitation of a new generation of 'biometallic' industrial catalysts. These are based on naturally occurring metal-reducing bacteria that are able to accumulate metals from process environments (as catalytically active nanoparticles), while also expressing enzymes that are able to extend the range and complexity of industrial reactions that can be produced from these novel microorganisms. This novel extension of synthetic biology has the potential to transform several sectors of UK industry, including those of industrial biotechnology and makers and users of catalysts, simplifying current processes, underpinning novel reactions and extending the range of available products.

- Obtained further funding through a BBSRC NIBB Proof of Concept award
- Industrial partner supported successful BBSRC Responsive Mode grant awarded to academic partner

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1 November 2017 – 28 February 2018
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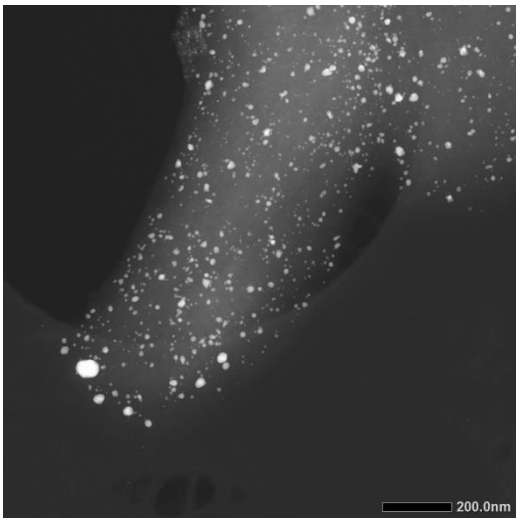
Biosynthesis of bimetallic nanoparticles for fine and specialty chemical production

"This award provided a quick, convenient and effective route to bring together the Manchester group's expertise in bioproduction of metal particles with Johnson Matthey's catalysis know-how. We have begun to determine the potential of this technology for the production of novel catalysts." Nigel Powell, Johnson Matthey

Jon Lloyd and Richard Kimber, University of Manchester; Nigel Powell, Johnson Matthey plc

RESULTS: Building on from a successful Business Interaction Voucher project with Johnson Matthey, we continued to investigate the biosynthesis of novel bimetallic nanoparticles for fine and specialty chemical production. Electron microscopy revealed that different metals have varying affinities for forming bimetallic nanoparticles and that the bimetallic nature is also affected by the order the metals are supplied to the bacteria. In addition, we found that the pH buffer used during synthesis can exert some control over the formation of the bimetallic nanoparticles. This knowledge will help us tailor these products going forward. Several of the biosynthesised nanoparticles showed promising catalytic activity. Although they did not perform to the same level as a commercial catalyst, this project has provided us with valuable insights into the optimisation of these bionanocatalysts which we are continuing to explore.



Electron microscope image of bacteria that contain bimetallic nanoparticles. Image provided by G. Goodlet, Johnson Matthey Technology Centre.

INITIAL AIMS: Metal-reducing bacteria are able to recover a wide range of metals from process environments as catalytically active nanoparticles. This project will produce bimetallic nanocatalysts for use in fine and speciality chemical production. Bimetallic nanoparticles offer advantages over monometallic catalysts due to the properties that arise from the presence and synergy of the two metals, offering increased efficiency and specificity for speciality chemical production. This novel biotechnological process offers a simple, cost-effective, environmentally friendly synthesis route for bimetallic catalyst production.

- **The partners will continue to work together to optimise and tailor the catalytic activity of these materials**
- **We will then seek to identify potential avenues for further funding**

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14 August – 13 October 2017
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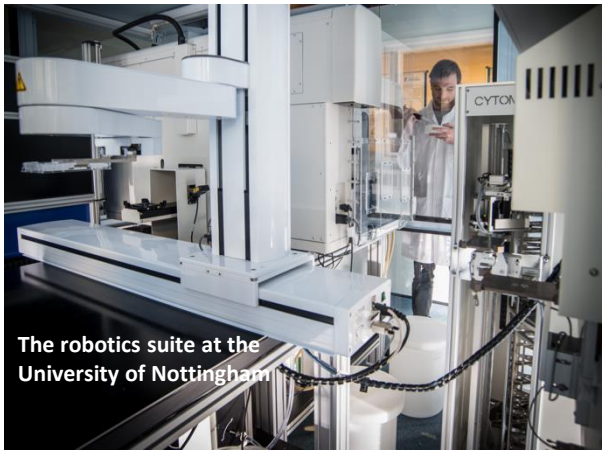
Engineering metal-dependent biotin synthase for the biotechnological production of biotin

"This project laid the foundation for an efficient high-throughput workflow towards understanding and engineering one of nature's most challenging enzymes biotin synthase. A better and faster approach with much broader scope than ever before will allow us improving the synthesis of the important vitamin B7 more efficiently." Christof Jäger, University of Nottingham

Christof Jäger, Anna Croft and Katalin Kovacs
University of Nottingham; Adriana Botes, VideraBio



The robotics suite at the University of Nottingham

In order to set the stage for an effective high-throughput enzyme screening approach for the enzyme biotin synthase we have developed and tested two biotin assays. One was based on a previously described *Corynebacterium glutamicum* indicator strain and the second made use of a biotin analogue bound to avidin (HABA/avidin). Both assays have the potential to be adapted for automated screening of large libraries in multi-well plate format. We showed that the *C. glutamicum*-based bioassay measured cell growth using biotin prepared from stock or produced in *E. coli*. Assays in which multi-well plates were manually loaded or prepared using the liquid handling unit of the robotic suite both showed that the biotin concentration was directly proportional to the ability to support *C. glutamicum* growth. In the second affinity-based assay, the amount of free HABA reflects the amount of biotin that is present once it is displaced from avidin. The assay was optimised for use in96-well plate format. The computational approaches focused on targeting potential mutation sites of biotin synthase that influence the redox reactivity of one or both iron–sulfur clusters and thus potentially influence sulfur insertion and cluster repair kinetics. For that reason we developed scripts to analyse the electrostatic effect of the protein environment (due to directional polarity) on the cluster. This type of analysis makes it possible to spot individual amino acids that influence the reactivity significantly, without being directly in contact with the active site. These amino acids will be taken forward to mutation studies.

INITIAL AIMS: Biotin (vitamin B7) is used primarily for the enhancement of animal feed and also in vitamin food supplements. Its production is challenging and hence the product is expensive (around $1600/kg). This project aims to demonstrate a potential way towards a sustainable and cost-effective production of biotin. The enzyme biotin synthase, which contains a delicate iron-sulfur cluster, is a key bottleneck in the biosynthetic pathway of biotin production. We will investigate the first steps into the rational computational design of this enzyme, together with the development of automated high-throughput, multidimensional *in vivo* assays. Our approach will act as starting point for not only for rational informed directed evolution strategies, but also will integrate regulatory elements for the repair mechanisms of the host cells.

- Project continued during doctoral training programme rotations
- BBSRC responsive mode grant application in preparation

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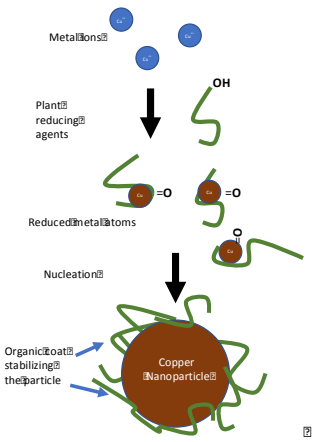
A pilot study to characterize plant-derived compounds that promote the synthesis of copper nanoparticles from contaminating copper ions in waste water

"This study has helped us see the potential future use of plants, plant cell culture or specific plant-produced compounds to remove contaminating copper and other trace metals from, for example, waste water in order to synthesize commercially valuable metal nanoparticles." Andrew Moore, Northumbrian Water Ltd

Ahmed Mohamed, Keith Lindsey and Jennifer Topping, Durham University; Andrew Moore, Northumbrian Water Ltd



Schematic of metal nanoparticle formation in a plant extract (taken from Makarov *et al*., 2014).

RESULTS: Leaf extracts from either coriander or mint were able to facilitate the formation of copper nanoparticles (CuNPs) from copper sulphate solution. Characterization of the CuNPs showed their size ranged from 28-36 nm, they were surrounded by proteins and a proportion of them existed as CuO. The crucial role of plant proteins or protein-containing moieties in CuNP formation was shown by removal of the protein fraction from the plant extracts; CuNPs were not formed in the protein-free fraction. Proteomic analysis revealed that although there was variability between the plant species studied, 105 proteins were associated with the CuNPs formed by both the mint and coriander extracts. Further work, and evidence from the literature, suggested that CuNP formation may be dependent upon protein mixture composition, rather than individual proteins. The second part of our work focused on the potential applications of the CuNPs. The bioactivity of the bio-synthesized CuNPs was compared with commercially available CuNPs in several biological assays. No difference between the two types of CuNPs was observed, confirming that the bio-CuNPs could be used successfully in biotechnological applications.

INITIAL AIMS: Contamination of land and waterways by toxic metals is a serious environmental problem, particularly in areas where mineral mining was once widespread. If the polluting metal can be sequestered into bioactive metal nanoparticles then the nanoparticles could have value in various applications, and the land would be decontaminated using an eco-friendly approach. Plant compounds are thought to be able to precipitate metal ions from dilute solutions to form metal nanoparticles through reduction of the metal ions into metal atoms that coalesce into nanoparticles. There are several possible plant compounds that can act as bio-reductants including flavonoids, terpenoids, sugars and proteins. In this project we studied the formation of copper nanoparticles (CuNPs) from a copper sulphate solution following the addition of plant leaf extract from either mint or coriander, with the aim of gaining a better understanding of how this process occurs, as well as characterising the CuNPs and the bioactive constituents within the plant extracts.

- Plant proteins can be used to reclaim copper in solution in the form of CuNPs
- Bio-CuNPs have the same bioactive properties as commercial (chemically produced) CuNPs
- Further work needed to identify which (if any) of the identified proteins in isolation are sufficient to form CuNPs

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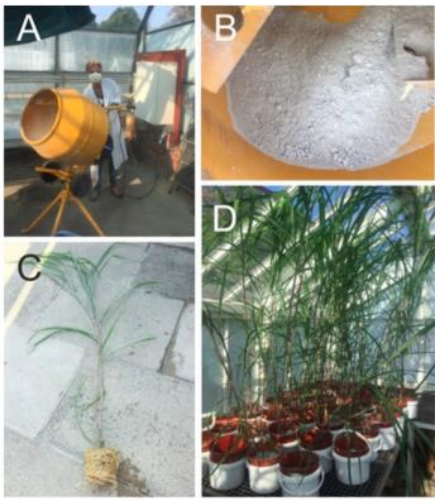
Investigating uptake and catalytic potential of *Miscanthus* grown on palladium mine wastes

"We are very pleased that this BBSRC NIBB-funded Business Interaction Voucher has made it possible for us to establish contact with the University of York and support their research towards the development of additional uses for UK-grown Miscanthus." Mike Cooper, Miscanthus Nursery Ltd

Neil Bruce & Elizabeth Rylott, University of York
David Stone, AgriKinetics Ltd
Mike Cooper, Miscanthus Nursery Ltd

OUTCOMES: To simulate mine waste, *Miscanthus* plants were grown on synthetic mine tailings (containing kaolinite, gravel and palladium). The plants were dosed fortnightly with potassium cyanide (KCN) to solubilise palladium in the tailings. We achieved our objective to test the effect of multiple KCN treatments on palladium uptake by *Miscanthus.* Although the application of KCN significantly increased the concentration of palladium in the aerial tissues, additional KCN applications did not enhance palladium uptake. There was also an increase in the appearance of necrotic tissues with increasing number of KCN applications. These results suggest that palladium, or other metals in the tailings, were accumulating in the plants to phytotoxic levels. Our studies indicate that achieving palladium levels required for use as a commercially-comparable catalyst is difficult. Our further studies are investigating if lower levels of palladium in plant biomass can be used in alternative catalysis methods (controlled, low-energy pyrolysis), as well as whether synthetic biology methods can be used as an improved alternative to KCN solubilising treatments.



Mixing (A) and appearance (B) of synthetic mine tailings. Four-month old *Miscanthus* plants (C) and plants one week after planting (D) in synthetic mine tailings.

INITIAL AIMS: Following palladium mining and extraction, mined areas and waste tailings need to be re-vegetated. Tailings still contain significant levels of palladium but recovery using conventional methods is currently uneconomical. Plants can be used to re-green mined areas and have the potential to 'phytoextract' residual levels of precious metals, which could be used as catalysts. Because the insolubility of palladium in the waste is a major limitation to uptake, this project will determine the effects of solubilising treatments on palladium uptake and accumulation in *Miscanthus*.

- **Sequential KCN applications did not enhance *Miscanthus* palladium uptake**
- **Lower palladium levels and other solubilising methods are under investigation**

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P CMiB024 BB/L013711/1
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Funded by a Metals in Biology BBSRC NIBB Business Interaction Voucher and Proof of Concept funding metals.bbsrcnibb@durham.ac.uk @METALSBBSRCNIBB
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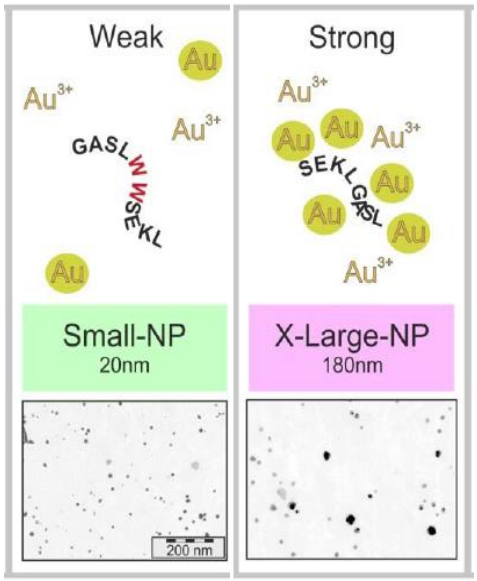
The use of platinum group metal nanoparticles in wastes from roadside verges for the production of high-value catalysts

"The results from this collaboration have enabled us to develop techniques and gain experience which will help towards the development of alternative plant-based remediation practices for sweeper wastes." Yorwaste Ltd

Neil Bruce and Elizabeth Rylott, University of York;
Richard Bate, Yorwaste Ltd

OUTCOMES: We have achieved our objectives to investigate nanoparticle formation using synthetic peptides and analyse catalytic activity in the subsequently pyrolysed nanoparticle-containing plant biomass. Our promising results demonstrate that the expression of synthetic peptides in plants can be used to alter nanoparticle size and subsequent catalytic activity in plants. As part of our third objective, to determine if plants could be used to selectively take up platinum group metals from sweeper wastes, we have shown that sweeper wastes contain detectable levels of valuable metals. However, our studies show that further work is needed to understand the phytotoxicity behind these wastes so that they can be optimised to allow plant growth. Our wider studies indicate that synthetic biology could be used to develop plants that can selectively take up platinum group metals from metal-rich wastes.



Peptide sequences control the size of nanoparticles (NP)

INITIAL AIMS: Platinum group metals are rare elements that are particularly used in catalytic converters on road traffic vehicles. Over time, palladium and other valuable metals are lost via exhaust fumes and deposited onto roads and verges. Waste collected from road sweepers contains detectable levels of palladium. Plants can take up platinum group metals as nanoparticles in their tissues. With the ultimate goal of recycling these rare metals, the aims of this project were to:

1. Investigate if the expression of synthetic peptides in plants can control nanoparticle size
2. Analyse catalytic activity in plant biomass that contains pyrolysed nanoparticles
3. Determine if plants can be used to selectively take up platinum group metals from sweeper wastes

- **Results from this project are being investigated further as part of funding from the New Zealand Ministry of Business, Innovation and Employment Global Strategic Partnership**

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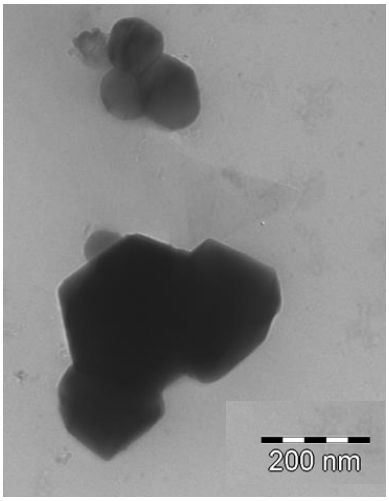
Investigating the antimicrobial properties of copper-infused fabrics

"Without the BBSRC Metals in Biology grant we would have found it much more difficult to collaborate with the University of Southampton on investigations into anti-microbial copper nanoparticles." **Copper Clothing Limited**

Bill Keevil & Susanna Sherwin, University of Southampton; Rory Donnelly, Copper Clothing Ltd

Of the fabric samples tested with three different bacteria, the thin, single-layered bamboo viscose and nylon fabrics impregnated with copper showed a greater than 99.9% reduction of bacteria at 24h. In contrast, the more absorbent and thicker tea towel fabrics impregnated with silver or copper showed no reduction of bacteria after 24h. It is suggested that the main difference between the two types of fabrics were the thickness and absorbency. To investigate a sustainable way of producing copper nanoparticles, the bacterium *Morganella psychrotolerans* was trained to grow on $CuSO_4$ agar. The industrial partner was able to select for variants that could survive in the presence of this usually bactericidal chemical. When $CuSO_4$ was added to the growth media, the bacterial pellet took on a brown colour, suggesting that copper nanoparticles were present inside the bacteria. Although we were unable to visualise these, we managed to image nanoparticles of various sizes present in the supernatant of the growth media, the largest of which are well defined hexagonal nanoparticles (Figure).



Nanoparticles of copper present in the supernatant of *Morganella psychrotolerans* after overnight growth in $CuSO_4$. Hexagonal particles layered together to form a multi-sided aggregate.

INITIAL AIMS: Copper ions can not only kill bacteria, but also destroy DNA, reducing the potential for horizontal transfer of resistance genes. The Industrial partner is currently using industrially made copper for the manufacture of antimicrobial fabrics, and is investigating renewable processes for incorporating copper into their fabrics. In order to determine the efficacy of producing and using nanoparticle copper as part of antimicrobial fabric manufacture, it is necessary to set a base-line of the levels of the antimicrobial capability of industrially sourced copper in copper clothing. The academic partner will evaluate fabrics using culture and advanced microscopy methods to determine their antimicrobial properties. They will also work to harness *Morganella spp* bacteria that are able to extract copper from their environment and contain it as nanoparticle-copper within their cells.

- **Thin, single-layered fabrics impregnated with copper showed a greater than 99.9% reduction in bacteria**
- ***Morganella spp* can extract copper from the environment and contain it as nanoparticle copper**
- **Copper Clothing Ltd used follow-on funding to show that copper impregnated wound dressings can reduce time for wounds to heal by 80% and is seeking approval to go to market**

POCMiB011 BB/L013711/1
01 October 2015 - 30 November 2016
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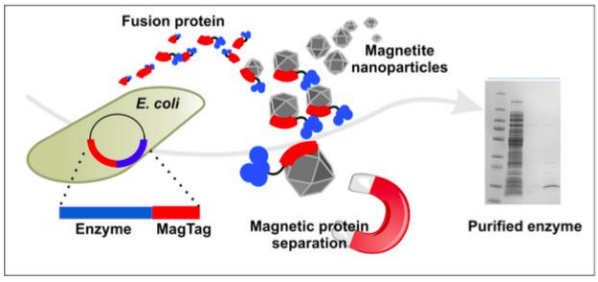
Mag-Tag: magnetite nanoparticle affinity tags for industrial biotechnology protein purification

"This PoC project has made a very fruitful industrial collaboration possible by means of a very simple and timely funding system. It will launch a whole new industrial research area for us." Sarah Staniland, University of Sheffield."

Sarah Staniland & Andrea Rawlings, University of Sheffield; Mark Blight, Biocatalysts Ltd

OUTCOMES: In this study, we used a protein that had high binding affinity and selectivity for certain magnetic materials that we previously identified. We used our affinity protein as a fusion tag (MagTag) to a test protein, GFP (green fluorescent protein), as this allowed us to track the binding and release of the target (the GFP fusion protein) via simple fluorescence measurements. During the course of the project we made the GFP–MagTag fusion construct and showed that the presence of the magnetic material binding tag had no detrimental impact on production of the GFP.



Schematic overview of the principle of the MagTag fusion protein purification system.

We optimised a simple synthetic route to the fabrication of cheap magnetic nanoparticles and demonstrated that the fusion protein could bind these under industrially relevant conditions, namely using crude cell lysate with a high optical density. Fluorescence measurements showed that we could successfully capture the GFP fusion protein from the lysate, out-competing other proteins within the sample. Importantly, we were able to show that it was possible to recover the GFP from the nanoparticles after binding and clean-up.

INITIAL AIMS: Enzyme catalysts are ideally suited to the industrial manufacture of foodstuffs, biofuels and pharmaceuticals, yet the current challenge to the widening the use of enzymes is the expense of producing them on a large scales due to the need for expensive, highly functionalised purification resins. We propose a revolutionary, cheap, universally applicable, enzyme purification method to widen the use of purified enzymes in industry. We will use protein fusion-tag technology to purify enzymes directly from crude preparations using cheap, unfunctionalised magnetic iron-oxide nanoparticles, meaning that the enzymes can then be bulk purified through magnetic separation. By substantially reducing the costs of purification we seek to make the use of enzymes an affordable, green and sustainable method of producing a wide range of products.

- **University funding awarded for further studies**
- **Awarded BBSRC follow-on funding for further development**
- **Seeking intellectual property protection**
- **Manuscript: Rawlings (2016) Biochem. Soc. Trans. 44: 790-795**

Kent and UCB partner on new strategy to boost antibody production

A new partnership between the University of Kent and biopharmaceutical company UCB — funded by a business interaction voucher from Metals in Biology BBSRC NIBB — has identified a potential new way to improve the production of protein-based drugs.

Many drugs that are used to treat cancer and autoimmune disease, as well as insulin, are proteins, and as such need to be made in cells, for example bacteria, mammalian cells or yeast. To function as drugs, proteins must be correctly folded, a process that depends on the formation of disulphide bonds.

Mark Shepherd from the University of Kent, who was the principle investigator on this project, has previously characterised a protein called ScsC (survival of *Salmonella* under copper stress protein, see figure). This enzyme catalyses the formation of disuphide bridges and its activity is influenced by copper.

"The ability of ScsC to facilitate disulphide folding in the *E. coli* periplasm has huge potential for the production of proteins of therapeutic importance."

In this project with UCB, he tested whether the the Scs system and copper could used in *E. coli* to improve the yield of a therapeutically relevant antibody fragment through its effects on disulphide bridge formation.

"Improving the yield of correctly-folded antibody fragments (and other high-value proteins) is of clear benefit to UCB," says David Humphreys, industrial partner on this project.

The study used Fab (fragment antigen-binding region) fragments of the breast cancer drug trastuzumab (Herceptin). Compared to full length antibodies, Fab fragments have advantages that include improved specificity, increased delivery options and more economical production systems.

Results from the study showed that Fab fragments of trastuzumab were successfully expressed in *E. coli* periplasm.

"This project has sparked a new relationship with UCB to investigate the potential of our disulphide-folding machinery to assemble a range of protein targets of biotechnological importance," says Mark. Indeed, the partners hope to continue their work to investigate the interaction of ScsC with other protein targets.

"The ability of ScsC to facilitate disulphide folding in the *E. coli* periplasm has huge potential for the production of proteins of therapeutic importance," concludes Mark.

BIVMiB014 BB/L013711/1

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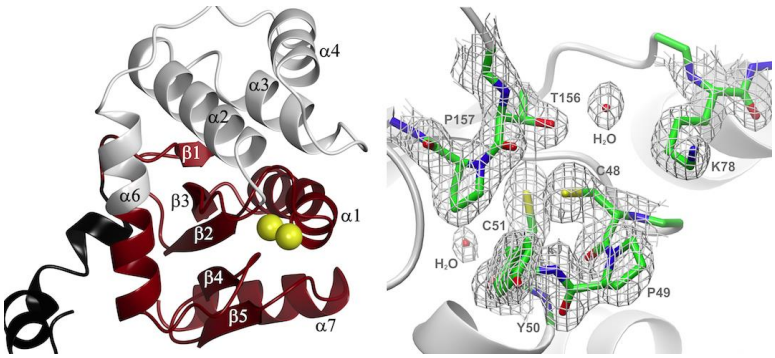
For more information please contact m.shepherd@kent.ac.uk



Structure of ScsC, a thioredoxin-like protein of *Salmonella* with a potential role in disulphide folding of therapeutic proteins. Overall protein fold and active site S atoms (yellow spheres) are shown on the left, and the local environment of the CPYC active site is depicted on the right.

BIVMiB041 BB/L013711/1
1 September – 31 October 2017
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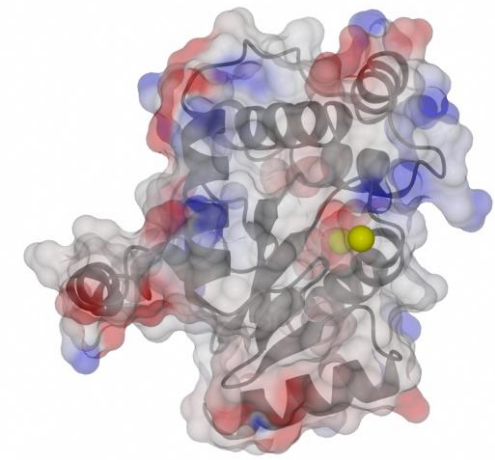
Exploiting a copper-dependent chaperone system to improve bioprocessing of therapeutic antibodies

"NIBB meetings have brought me into contact with a range of industrial contacts. This BIV sparked a new project with Diosynth Biotechnologies to investigate the potential of bacterial copper-tolerance machinery to facilitate assembly of protein targets of biotechnological importance." Mark Shepherd, University of Kent

Mark Shepherd, University of Kent; Christopher Lennon, FujiFilm Diosynth Biotechnologies



Structure of ScsC, a thioredoxin-like protein of Salmonella with a potential role in disulphide folding of therapeutic proteins. Negative and positive surface charge are shown in red and blue, respectively. Sulphur atoms of active site cysteines are shown as yellow spheres.

This project explored the impact of copper and the Salmonella Scs proteins upon the assembly of Herceptin and Lucentis, therapeutic antibodies used to treat breast cancer and macular degeneration respectively. First we developed systems for expression of Herceptin and Lucentis in *E. coli*. After this we used state-of-the-art mass spectrometry approaches to perform quantitative proteomics measurements on *E. coli* strains grown in the presence and absence of copper to assess total protein and antibody abundance. We showed that copper elevates protein levels in *E. coli*. Copper diminished the expression of Herceptin, an effect that was reversed by expression of ScsABCD. We also showed that the native disulphide-folding machinery in *E. coli* is essential for Herceptin production.

INITIAL AIMS: The production of biotherapeutics has a total market value of around £100 billion per year. As well as therapeutic uses, antibodies have applications as research tools, in diagnostics and in consumer healthcare products. We have previously investigated the potential of copper-dependent protein folding catalysts (Scs proteins) to improve the production of antibody fragments of Herceptin (Trastuzumab) in *E. coli (*BIVMiB014). This project expands the repertoire of that system; we will build upon work done so far with Herceptin and also study Lucentis (Ranibizumab).

- Future studies may focus on the effects of copper and ScsABCD on Lucentis yield

BIVMiB001 BB/L013711/1
16 February – 15 August 2015
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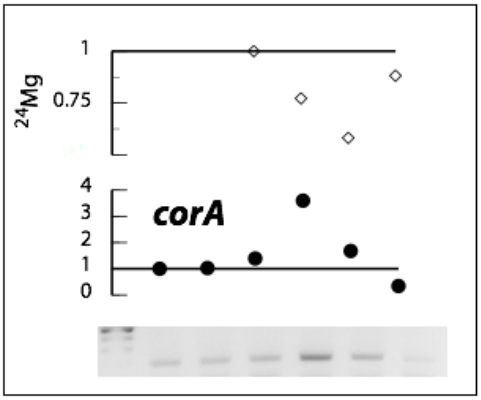
Metal demands during protein overexpression in bacteria

"For Biocatalysts Ltd, this study highlighted the importance of metal supplementation in commercial fermentation processes to maximise enzyme activity and yield."

Peter Chivers, Durham University; Mark Blight, Biocatalysts Ltd



Cellular Mg content is inversely correlated with changes in transcript levels of the *corA* gene, which is associated with Mg uptake.

OUTCOMES: Transcript levels and metal content of *E. coli* cells were measured at different time points post-induction during a representative fermenter run. Increased transcript levels of genes important for Mg, Fe, Mn, and Ni acquisition were observed in the latter half of the protein overexpression time course (≥ 9 h post-induction). These increases correlated with decreases in total cellular metal content for each metal, consistent with metal deficiency sensed by metal-responsive transcriptional regulators. These deficiencies have potential effects on translational efficiency (Mg), synthesis of non-natural amino acids that affect the fidelity of tRNA charging (Ni), and posttranslational processing of newly synthesized polypeptides (Fe and Mn). No evidence for Zn-deficiency or Cu-stress was detected based on transcript levels and metal content. These results suggest straightforward strategies — namely metal supplementation — to ensure metal supply is maintained during the protein overexpression time course.

INITIAL AIMS: Protein overexpression is a major facet of industrial biotechnology, yet the capacity of host organisms to overexpress proteins is not naturally optimized. Transition metals are key components of the cellular protein synthesis machinery. This project will explore the changes in metal demands brought about by protein overexpression in *Escherichia coli*, a widely used platform for biologics production. Metal allocation and use will be determined by transcript analysis to monitor changes in the expression of metal-regulated genes and measurement of cellular metal content to establish links between changes in gene expression and metal supply.

- Supplementing growth media with metals could improve the quality and/or quantity of protein synthesis

BIVMiB035 BB/L013711/1
1 April – 30 September 2017
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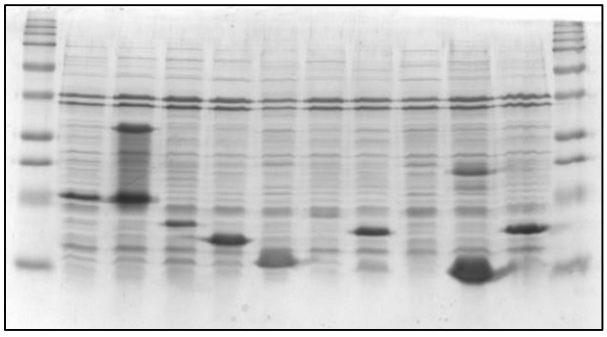
Metal utilisation in *Clostridium* microbial biocatalysts

"For GBL, this study highlighted the importance of metal requirements for commercial and research and development processes."

Peter Chivers, Durham University; Liz Jenkinson, GBL

OUTCOMES: Metal contents were measured at different times during a GBL fermentation protocol used for Research and Development. The ICP-MS analysis revealed a large increase in metal content in *Clostridium,* coincident with the onset of butanol production. To complement the experimental data, bioinformatics analyses identified ten candidate metal sensor genes. These genes encode regulator proteins responsible for sensing and responding to changes in metal content. In several cases, their regulatory targets have also been identified. The proteins encoded by these genes have been overexpressed in *E. coli* to facilitate future studies of their metal selective transcriptional responses. These results will enable more detailed analysis of metal homeostasis networks in *Clostridium* to understand their link with butanol production or competing processes .



SDS-PAGE analysis of overexpression of *Clostridium* metal sensor proteins in *E. coli*

INITIAL AIMS: Little is known about the metal-demands of *Clostridium* strains during solvent production. This project will explore the metal requirements of solventogenic *Clostridium* during commercial and research and development processes used by GBL. Metal content will be analysed by ICP-MS. A complementary aim is to identify the metal sensor genes responsible for maintaining metal homeostasis so that they may be overexpressed in *E. coli* to define the metal selective response of each protein.

- **Understanding metal demands could lead to improved control of fermentation processes**

BIVMiB043 BB/L013711/1
1 November 2017 – 4 January 2018
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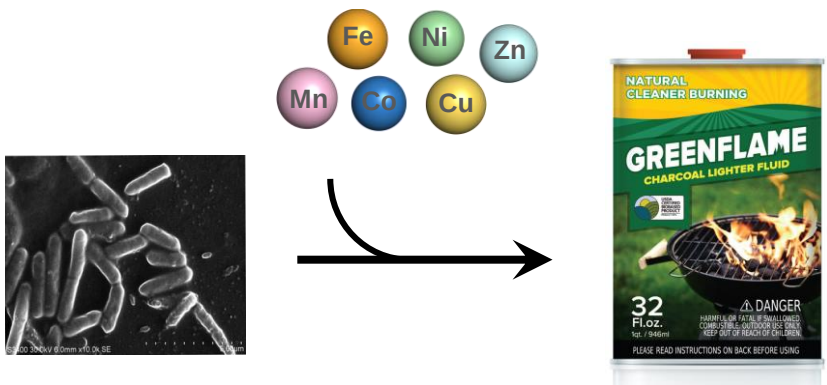
Investigating the link between metal homeostasis, sporulation, and solvent production in the *Clostridial* ABE fermentation process

"This study advanced understanding of metal requirements for commercial and research and development processes."

Peter Chivers, Durham University; Liz Jenkinson, GBL

OUTCOMES: Biomass samples were collected by GBL at 3-h intervals for metal content analysis and RNA isolation. An asporogenic mutant that did not show changes in metal content was sampled in parallel to understand the genetic requirements for changes in metal homeostasis. The project also provided key knowledge transfer – the metal content and RNA sample analysis were carried out at Durham by a GBL scientist. The informal communications during the 3-week visit will enhance future work at both sites.



AIMS: Solventogenic Clostridia were first used for fermentative acetone-butanol-ethanol (ABE) production roughly 100 years ago. The project focused on establishing a detailed picture of the changes in metal utilisation and homeostasis at the onset of sporulation using a combination of RNASeq and ICP-MS to correlate initiation of the sporulation genetic program and the role of metalloenzymes and proteins.

- **Understanding metal utilisation during sporulation could lead to improved control of fermentation processes**

BIVMiB002 BB/L013711/1
5 January – 4 July 2015
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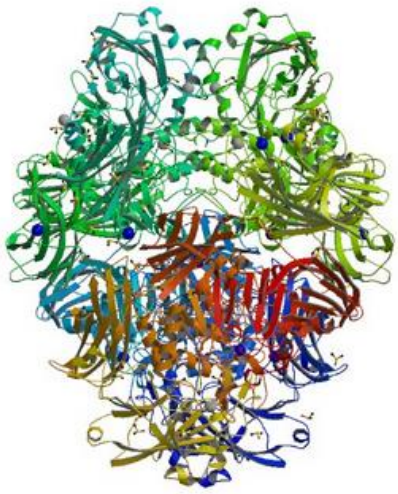
Optimizing metal acquisition by commercial metalloenzymes

"The BBSRC NIBB has brought me into contact with diverse researchers in the industrial sector, people who I would probably have never encountered through other channels." Kevin Waldron, Newcastle University

Kevin Waldron, Newcastle University
Stuart West, Biocatalysts Ltd

OUTCOMES: Three variants of magnesium-dependent galactosidase were selected for analysis. Because of the relative instability of complexes formed by magnesium compared to other transition metals, we hypothesised that it was likely that these enzymes could wrongly acquire a non-native, more stable complex-forming metal ion, either during heterologous expression or during sample preparation. Such association with the non-native metal cofactor is likely to lead to inhibition of the enzyme activity.
Our studies showed that small amounts of copper, zinc and nickel (but not iron) were present on the target enzyme in the preparations. The quantity of contaminating metals varied slightly between the longitudinal samples, and interestingly also varied between the three enzyme variants, suggesting possible differences in their metal-binding properties. Importantly, although the absolute quantity of these contaminating metals was low when expressed as a percentage of the total enzyme present (ranging from 3.8 — 12.8% occupancy), suggesting they would make only a minor diminution of the total enzyme activity of the enzyme preparations; a potential increase in enzyme activity of 4-12% would impact on profitability of commercial products.



Crystal structure of beta-galactosidase, PDB ID: 3SEP

INITIAL AIMS: Biocatalysts Ltd produce a number of metalloenzymes of commercial value through expression in bacteria and fungi. However, metal supply to these 'foreign' enzymes may not be optimal in these protein production hosts, so that a proportion of the commercial product is either bound to the 'wrong' metal, or lacks a bound metal ion altogether. Increasing the proportion of the enzyme that is correctly metal-loaded can directly increase profitability of metalloenzyme products. We will analyse the metal content of samples of the metalloenzymes produced by Biocatalysts.

- Although only a small amount of contaminating metals were detected, they reduce enzymatic activity – eliminating these contaminants would increase activity and thus profitability of these commercial enzyme preparations.

BIVMiB020 BB/L013711/1
1 January – 30 September 2016
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Novel disposable cell culture systems for microbial growth in metal-regulated environments

"The growth of Escherichia coli in the novel cell culture system in a defined minimal medium is comparable with growth in traditional glass vessels; this opens up a new market opportunity for this system as cell culture chambers for microbiology." **Kelly Davidge, Kirkstall**

Robert Poole, University of Sheffield; Kelly Davidge, Kirkstall

OUTCOMES: First, we showed that a standard laboratory strain of *E. coli* was able to grow in three different types of novel cell culture vessels; this is the first demonstration of miniaturised bacterial growth capability in the novel cell culture chambers. Second, we showed that the chambers do not leach significant amounts of metals into the bacterial growth medium. This applies even when the culture medium has been treated to deplete selected metals, thus providing a potential concentration gradient between the culture chamber materials (silicone and acrylic) and the medium. We conclude that these vessels are suitable for bacterial growths involving metal-controlled conditions.

The Quasi Vivo® QV500 chamber is made from polydimethylsiloxane and allows for submerged cell culture

INITIAL AIMS: Growing cells and tissues for biotechnology uses requires a well-defined growth environment, which must provide all nutrient — including metals — in biologically accessible forms, but not in excess. Most growth vessels are metal or glass, but these can leach or adsorb metal ions. Synthetic materials, however, may be biologically inert and interact little with dissolved metals. This project will test the suitability of miniaturised growth chambers (known as Quasi Vivo®, developed by Kirkstall, originally for culturing mammalian cells) for microbial growth. These chambers are made from biocompatible materials and, under flow conditions, mimic conditions in the body. We will grow bacteria in such chambers and test their ability to provide environments in which the metal concentrations available for growth will be varied from trace levels to toxic levels. The work has potential for developing improved methods of cell culture in industrial biotechnology.

- Relevant to future development of a gut model incorporating both mammalian and microbial cells in metal-controlled conditions
- Potential new market opportunities for the Kirkstall cell culture chambers

BIVMiB022 BB/L013711/1
01 September 2016 – 28 February 2017
Funded by a Metals in Biology BBSRC NIBB Business Interaction Voucher
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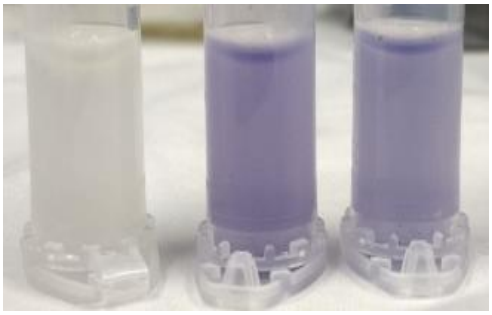
Cloning and metal analysis of recombinant aldehyde ferredoxin oxidoreductase

"We showed that a novel compartmentalisation strategy can be used to produce a biotechnologically important enzyme in bacteria. Our results are being used to help acquire further strategic investment in the partner company." Martin Warren, University of Kent

Martin Warren and Stephanie Frank, University of Kent; Michelle Gradley, ZuvaSyntha Ltd.



The activity of recombinant AOR in *E. coli*, as shown by an increase in the violet colour.

OUTCOMES: We first cloned two genes encoding for aldehyde ferredoxin oxidoreductase (AOR) from *Clostridium ljungdahlii* into *E. coli*. The purified proteins, termed AOR1 and AOR2, contained the predicted Fe-S centre and and a tungsten-pterin cofactor, which was produced by the *E.coli*. We next showed that the enzymes were active (but only when produced under strictly anaerobic conditions) in a whole cell assay (figure), with AOR2 displaying more activity than AOR1. We then investigated the targeting of AOR proteins to a bacterial microcompartment (BMC), by expressing the enzymes with a tag that directs them to the BMC. When the enzymes were co-produced with BMC shell-proteins, the AORs were indeed targeted to the BMC. Our work with the expression of AOR from the thermophile *Pyrococcus furiosus* proved more problematic, and is still under investigation. Our results demonstrate that recombinant AORs are active in *E. coli* if grown and kept under anaerobic conditions, and can be targeted to BMC. Our next steps are to see if the activity of AOR can be enhanced in acteogens, since this provides a powerful way in which acetate can be redirected for the production of commodity chemicals.

INITIAL AIMS: The aim of the project is to enhance the recombinant production of a key enzyme of biotechnological importance: aldehyde ferredoxin oxidoreductase (AOR). This enzyme allows the transformation of carboxylic acids into aldehydes, which could have use in the sustainable production of 1,3-butadiene – a key commodity chemical for the rubber and tyre market. We intend to explore the recombinant production of AORs in *E. coli* and to determine conditions that allow for the successful incorporation of its unusual metal complement, which includes a tungsten-molybdopterin cofactor and a 4Fe-4S centre. Moreover, we also want to see if this protein can be targeted to bacterial microcompartments (BMC) — proteinaceous organelles that can be used to encase metabolic process to protect a cell from toxic products — and to determine if AORs retain their metal complement once inside the BMC.

- The results this project have secured two further grants: Early Stage Catalyst Funding from BBSRC and Proof of Concept Funding from C1Net
- ZuvaSyntha and the University of Kent are seeking IP projection for aspects of this work

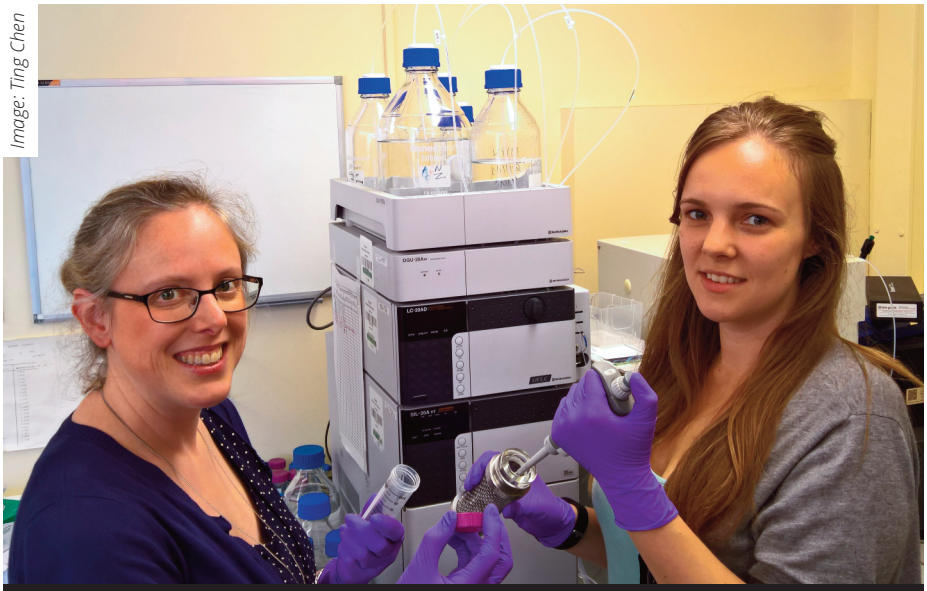
A collaborative journey to greener chemicals

Kylie Vincent, Holly Reeve and colleagues at Oxford University are working on ways to make greener chemicals. With government funding, the technology could soon be used in industry...

We are working on greener ways to make chemicals that are used in medicines, food and cosmetics by using enzymes that are found in nature – an area of research known as industrial biotechnology. Our group came to work in industrial biocatalysis in a roundabout way. We were studying types of bacteria that use the very small amounts of hydrogen gas found in the environment as 'food' or energy. To do so, these bacteria contain enzymes – hydrogenases – in their cells which use metal ions to split apart the hydrogen molecule. We were interested in how nature uses metal ions to enable, or catalyse, the split of the hydrogen molecule to better understand how we could make use of this reaction in the lab.

In the course of this work, we carried out collaborative experiments with Dr Oliver Lenz's group at the Technical University of Berlin into a special hydrogenase that uses hydrogen as a fuel to drive the production of a biological molecule called nicotinamide adenine dinucleotide hydride (NADH) [1]. NADH is an important energy currency inside cells, and is required for the function of many types of enzymes. In particular, NADH is essential to the types of enzymes that are gaining attention for their ability to make complicated chemicals such as pharmaceuticals, or flavour or fragrance chemicals in greener ways than in the past.

Image: Ting Chen



Kylie Vincent and Holly Reeve

Although the use of enzymes as biocatalysts in chemical synthesis is really taking off, a key challenge is developing an efficient supply of NADH. We quickly realised that we could make use of components from bacterial cells to assemble a system for recycling NADH that could be used outside of the bacterial cell by incorporating some of the cell components onto cheap carbon beads. The use of hydrogen gas as the energy source to drive the chemistry gives a much cleaner way of supplying the NADH for enzyme-catalysed chemical synthesis [2].

We developed this into a technology that we call HydRegen (short for hydrogen-driven regeneration). We were able to show quite quickly that our HydRegen beads work with a wide range of NADH-dependent enzyme reactions. This was a key finding, as it increases the potential applications of the technology. We filed a patent application with support from Oxford University's Technology Transfer company, Oxford University Innovation, and began talking to some of the companies that are interested in using NADH-dependent enzymes to make complex chemicals.

One of the questions we were frequently asked was whether we could extend to the related biological cofactor NADPH (nicotinamide adenine dinucleotide phosphate), as this is essential to many other industrially relevant enzymes. However, NADPH presents even more of a headache in industrial applications of enzymes as it is more expensive and less stable than NADH. After winning the prestigious Royal Society of Chemistry's Emerging Technologies Competition

in 2013, we were mentored by Dr Ian Churcher from the pharmaceutical company GlaxoSmithKline, who helped to advise us on development of the technology.

GlaxoSmithKline were natural partners as we started to explore the scope for extending our HydRegen beads to NADPH in an industrial setting. Together, we secured short-term funding in the form of a Business Interaction Voucher from the Metals in Biology BBSRC NIBB, to test whether we could produce enough NADPH to drive some NADPH-dependent enzymes that GlaxoSmithKline are interested in. The project was successful, and provided real proof of the versatility of the HydRegen technology.

However, major research challenges remain. The two enzymes we use are excellent biocatalysts – the hydrogenase enzyme splits the hydrogen molecule, causing electron transfer through the electronically conductive carbon beads to support the recycling of NADH (or NADPH) at the other enzyme. But they are complex metalloenzymes that can only be produced in bacterial cells.

Although making these enzymes for use in the lab is now relatively straightforward, making them on an industrial scale remains unchartered territory. We still need to find the best ways to make the bacteria produce these enzymes in high quantities, the minimal steps needed to isolate the enzymes, and the best ways to handle them once they are separated from the cells. To address these research challenges, we applied to a government funding scheme called the Industrial Biotechnology Catalyst scheme (run through the BBSRC/EPSRC/Innovate UK), and in January 2016 we were awarded £2.9 million of funding for a major research programme to explore and de-risk the scalability of the HydRegen technology.

We have assembled an industrial advisory board, taking advantage of industry contacts we have made along the way at Oxford University-led meetings, at Metals in Biology BBSRC NIBB meetings, and with help from Oxford's technology transfer team. The advisory board is helping to keep the project focussed on addressing real industry challenges in biotechnology.

"A Business Interaction Voucher from the Metals in Biology BBSRC NIBB, to test whether we could produce enough NADPH to drive some NADPH-dependent enzymes that GlaxoSmithKline are interested in provided real proof of the versatility of the HydRegen technology."

The Industrial Biotechnology Catalyst-funded project is now up and running successfully, and one of the most exciting parts of the first six months has been seeing the project generate new fundamental insight into the enzyme systems and new patentable discoveries, as well as advances in the core HydRegen technology itself. We have also continued to make industrial contacts and develop our commercialisation strategy in conjunction with Oxford University Innovation.

We have always enjoyed the interplay between detailed studies of enzyme function and development of applications of enzyme biocatalysis. In parallel to the Industrial Biotechnology Catalyst project, we continue to work on fundamental studies into hydrogenase enzymes, and to develop new tools for studying metalloenzymes, supported by BBSRC responsive mode grants on which we are co-investigators [3]. Our strengthened understanding of how hydrogenase enzymes work, and the accumulation of related know-how in the group continues to feed very productively into our development of the HydRegen technology.

Indeed, the Industrial Biotechnology Catalyst funding should help this technology to cross the so-called 'valley of death' that often impedes early-stage technologies. With this funding, and our additional grants, we hope to bring the HydRegen system to market in the next 3-4 years.

1 Lauterbach, L., Idris, Z., Vincent, K.A., Lenz, O. 'Catalytic properties of the isolated diaphorase fragment of the NAD^+-reducing [NiFe]-hydrogenase from *Ralstonia eutropha*' PLoS ONE, 2011, 6, (10): e25939.

2 Reeve, H.A., Lauterbach, L., Lenz, O., Vincent, K.A. 'Enzyme-modified particles for selective bio-catalytic hydrogenation via H_2-driven NADH recycling' ChemCatChem, 2015, 7, 3480-3487.

3 Hidalgo, R., Ash, P.A., Healy, A.J., Vincent, K.A. 'Infrared spectroscopy during electrocatalytic turnover reveals the Ni-L active site state during H_2 oxidation by a NiFe hydrogenase' Angew. Chemie. Int. Ed. 2015, 54, 7110-7113.

Kylie Vincent
Holly Reeve
Department of Chemistry
University of Oxford
Kylie.vincent@chem.ox.ac.uk
Holly.reeve@chem.ox.ac.uk
Tel: 01865 272678
http://vincent.chem.ox.ac.uk/index.htm
http://vincent.chem.ox.ac.uk/hydregen.htm
BBSRC reference B MiB00
BB L013711 1

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GlaxoSmithKline were natural partners as we started to explore the scope for extending our HydRegen beads to NADPH in an industrial setting. Together, we secured short-term funding in the form of a Business Interaction Voucher from the Metals in Biology BBSRC NIBB, to test whether we could produce enough NADPH to drive some NADPH-dependent enzymes that GlaxoSmithKline are interested in. The project was successful, and provided real proof of the versatility of the HydRegen technology.

However, major research challenges remain. The two enzymes we use are excellent biocatalysts – the hydrogenase enzyme splits the hydrogen molecule, causing electron transfer through the electronically conductive carbon beads to support the recycling of NADH (or NADPH) at the other enzyme. But they are complex metalloenzymes that can only be produced in bacterial cells.

Although making these enzymes for use in the lab is now relatively straightforward, making them on an industrial scale remains unchartered territory. We still need to find the best ways to make the bacteria produce these enzymes in high quantities, the minimal steps needed to isolate the enzymes, and the best ways to handle them once they are separated from the cells. To address these research challenges, we applied to a government funding scheme called the Industrial Biotechnology Catalyst scheme (run through the BBSRC/EPSRC/Innovate UK), and in January 2016 we were awarded £2.9 million of funding for a major research programme to explore and de-risk the scalability of the HydRegen technology.

We have assembled an industrial advisory board, taking advantage of industry contacts we have made along the way at Oxford University-led meetings, at Metals in Biology BBSRC NIBB meetings, and with help from Oxford's technology transfer team. The advisory board is helping to keep the project focussed on addressing real industry challenges in biotechnology.

"A Business Interaction Voucher from the Metals in Biology BBSRC NIBB, to test whether we could produce enough NADPH to drive some NADPH-dependent enzymes that GlaxoSmithKline are interested in provided real proof of the versatility of the HydRegen technology."

The Industrial Biotechnology Catalyst-funded project is now up and running successfully, and one of the most exciting parts of the first six months has been seeing the project generate new fundamental insight into the enzyme systems and new patentable discoveries, as well as advances in the core HydRegen technology itself. We have also continued to make industrial contacts and develop our commercialisation strategy in conjunction with Oxford University Innovation.

We have always enjoyed the interplay between detailed studies of enzyme function and development of applications of enzyme biocatalysis. In parallel to the Industrial Biotechnology Catalyst project, we continue to work on fundamental studies into hydrogenase enzymes, and to develop new tools for studying metalloenzymes, supported by BBSRC responsive mode grants on which we are co-investigators [3]. Our strengthened understanding of how hydrogenase enzymes work, and the accumulation of related know-how in the group continues to feed very productively into our development of the HydRegen technology.

Indeed, the Industrial Biotechnology Catalyst funding should help this technology to cross the so-called 'valley of death' that often impedes early-stage technologies. With this funding, and our additional grants, we hope to bring the HydRegen system to market in the next 3-4 years.

1 Lauterbach, L., Idris, Z., Vincent, K.A., Lenz, O. 'Catalytic properties of the isolated diaphorase fragment of the NAD^+-reducing [NiFe]-hydrogenase from *Ralstonia eutropha*' PLoS ONE, 2011, 6, (10): e25939.

2 Reeve, H.A., Lauterbach, L., Lenz, O., Vincent, K.A. 'Enzyme-modified particles for selective bio-catalytic hydrogenation via H_2-driven NADH recycling' ChemCatChem, 2015, 7, 3480-3487.

3 Hidalgo, R., Ash, P.A., Healy, A.J., Vincent, K.A. 'Infrared spectroscopy during electrocatalytic turnover reveals the Ni-L active site state during H_2 oxidation by a NiFe hydrogenase' Angew. Chemie. Int. Ed. 2015, 54, 7110-7113.

Kylie Vincent
Holly Reeve
Department of Chemistry
University of Oxford
Kylie.vincent@chem.ox.ac.uk
Holly.reeve@chem.ox.ac.uk
Tel: 01865 272678
http://vincent.chem.ox.ac.uk/index.htm
http://vincent.chem.ox.ac.uk/hydregen.htm
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POCMiB016 BB/L013711/1
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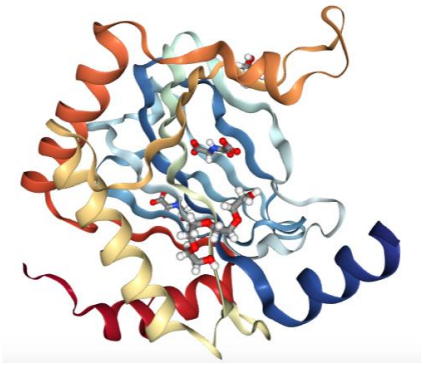
Proline hydroxylases for use in biocatalysis

"The project has revealed the substrate selectivity of oxygenases is much wider than we had expected, further highlighting their potential in biocatalysis for medicinal chemistry." Christopher Schofield, University of Oxford

Christopher Schofield & Michael McDonough, University of Oxford; Daniel Brookings, UCB Celltech

OUTCOMES: Our work concerned studies on a family of metal-dependent enzymes that add oxygen (or sometimes chlorine or bromine) atoms to drug-like small molecules and proteins. We focused on proline hydrolases, which in nature catalyse hydroxylation of the 5-membered ring amino acid proline. When we investigated the selectivity of the proline hydroxylases for different ring sizes and substitutions, we found that they can catalyses the hydroxylation of an unexpectedly wide range of rings, including bicyclic ring structures; some of these products are precursors for conversion into potential antibiotics. We also explored how these hydroxylases bind iron, using both assays for product formation and by X-ray crystallographic analyses of 'mutant' enzyme structures.



Cartoon of HIF prolyl hydroxylase 2 in complex with N-oxalylglycine (PDB-ID 5L9R)

Interestingly, we found that the proline hydroxylases can work with only two — rather than the normal three — points of attachment (ligand) of iron to the protein. These results inspired us to study metal binding by enzymes in cells (using mass spectrometry) and to study variations on iron binding by other types of hydroxylases. In one case we found the hydroxylase can work with only one protein ligand.

INITIAL AIMS: Metal-dependent enzymes are incredibly powerful biological catalysts. In nature, biocatalysts can modify a common chemical scaffold to give several other products, which may have very different biological functions. Harnessing the power of such late-stage modification for drug discovery has potential to generate many molecules from a single drug candidate and could enable the efficient discovery of optimised molecules. There is a current lack of accessible libraries of oxygenase enzymes that are suitable for use in biocatalysts, and little information on how their activity is limited by metal binding in cells. We aim to develop proof of concept for the use of engineered proline hydroxylases for the stereoselective oxidation of substrates of choice.

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- Manuscript published: Zhang et al. (2017) PNAS 114: 4667- 4672
- Symposium on late-stage modification of pharmaceuticals
- Project expanded to include another potentially useful enzymes

POCMiB019 BB/L013711/1
01 October 2015 – 31 July 2016
Funded by Metals in Biology BBSRC NIBB Proof of Concept funding
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Light-activated caged-iron chelator for skin photoprotection based on the natural product pulcherrimic acid

"Our project provides a robust basis for the use of molecules inspired by pulcherrimic acid as ligands for the development of novel light-activated photoprotective compounds that could be used in sunscreen."
Charareh Pourzand, University of Bath

Charareh Pourzand, Ian M. Eggleston, Daniel Henk and Chris Chuck, University of Bath; Timothy Miller, Croda Europe

OUTCOMES: First, we produced pulcherrimin and purified pulcherrimic acid (PA) from non-sterile culture. Around 150 mg/L of pulcherrimin was produced on a 10L-scale culture of an over-producing strain of *M. pulcherrima*. In addition, synthetic authentic samples of PA were successfully prepared in the chemistry laboratory. The synthetic approach was robust and scalable, and should be suitable for the preparation of a range of amino-acid derived PA analogues and caged compounds. Although the natural PA extracted from the *Mp* yeast was indistinguishable from the authentic synthetic material, biological studies used the highly pure synthetic PA, due to lack of optimum purity of PA isolated from yeast. Our results showed that PA is not cytotoxic *per se* when exposed to cultured primary skin fibroblasts overnight up to a concentration of 50 uM. PA (20-30 uM) conferred significant photoprotection against UVA-induced damage and cell death, and was was superior to the clinically used bidentate iron chelator deferiprone at an equimolar concentration. Further chemical development work is required to build on the promising results obtained so far in order to obtain light-activatable caged compounds. Nevertheless, these promising results provide proof of concept for the potential development of photolabile caged PA as topical sunscreen ingredients.

INITIAL AIMS: There is a significant need to counteract the cellular mechanisms that cause skin damage upon prolonged exposure to the UV component of sunlight. Exposure of skin cells to UVA promotes the generation of harmful reactive oxygen species and leads to an immediate release of labile iron and susceptibility to oxidative membrane damage and necrotic cell death. We have previously synthesised and validated light-activated protective compounds (i.e. light-activated caged-iron chelators, CICs) that release an active iron chelator upon sunlight exposure, which could protect against iron-catalysed oxidative damage and cell death. A critical requirement for CIC technology is readily available, chemically tractable iron chelators, in which the iron-binding motif can be reversibly modified (caged). In this context, we plan to isolate and modify (cage) pulcherrimic acid, a natural product from the yeast *M. pulcherrima* with iron chelating activity, and subsequently evaluate its photoprotective activity against UVA-induced iron damage in cultured skin cells.

- Our chemical synthesis and biological validation of these compounds was essential to maximise impact for near future collaboration with Croda Europe
- We are considering medium-term collaborations with Croda Europe via BBSRC responsive mode or a BBSRC stand-alone LINK application

BIVMiB015 BB/L013711/1
01 October 2015 – 31 March 2016
Funded by a Metals in Biology BBSRC NIBB Business Interaction Voucher
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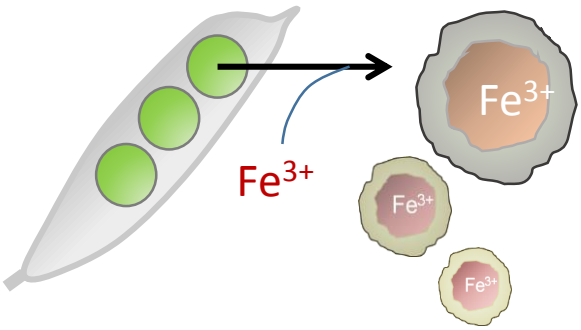
Analysis of ferritin iron in pea flour

"Through the input of science and support by John Innes Centre in this project, the speed to commercial establishment of the new start-up business and product development has been considerably accelerated." AgriTopics Ltd

Janneke Balk, John Innes Centre; **Patrick Mitton**, AgriTopics Ltd



Iron-rich ferritin nanoparticles can be extracted from peas

OUTCOMES: Peas are rich in nutrients and protein, but highly undervalued as a healthy part of our diet. In this project we tested the effect of different milling techniques on the extraction of ferritin, an iron-rich protein nanoparticle, from dried peas. We also investigated the nutritional profile of pea flour before and after ferritin extraction. We found that a specialised milling process, developed and optimised by the industry partner AgriTopics, is as good, or even better than a range of alternative milling processes for the extraction of ferritin. We demonstrated that while the extraction procedure removes 3% of the sugars from the pea flour, fatty acids, protein and micronutrients remained in sufficient quantities For the flour to be used for food products.

INITIAL AIMS: Peas provide a rich source of proteins and nutrients for human diets. They also contain relatively high levels of iron in a form that is very easy for the body to absorb. To extract this high value component and/or use pea flour directly in food products, the first step is to mill the dry peas. Together with the industrial partner AgriTopics, we will evaluate different milling procedures, milling fractions and particle sizes to optimize this first step for producing nutritional flour and iron extraction. The project will provide the basis for the development of new iron supplements, which could also be used clinically to treat anaemia, and specialist flours for the food industry.

- **This work enabled data-led food recipe trials to be conducted**
- **Academic group is now able isolate the larger amounts of pea ferritin required to run human bioavailability trials**
- **Industrial partner will develop the pea flour for food/nutrition products**

BIVMiB012 BB/L013711/1
1 March 2016 – 31 August 2016
Funded by a Metals in Biology BBSRC NIBB Business Interaction Voucher
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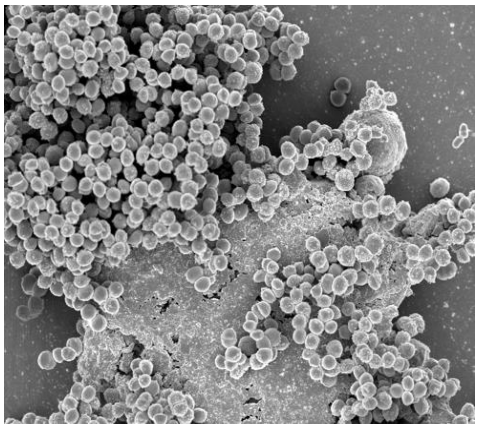
Household biofilm chelation therapy

"The BIV has allowed P&G to establish a good relationship with academic experts in applied biofilm research and imaging, which we hope to take forward via future collaborations." P&G

Nicholas Jakubovics, Newcastle University; Peter Chivers, Durham University; Adam Hayward, P&G



Field emission scanning electron micrograph of an *M. luteus* biofilm

OUTCOMES: Following initial screening, three strains of bacteria were selected as representatives of household-relevant microbes. Several chelating agents were applied to biofilms of these microbes under conditions found in typical household cleaning regimes. Despite efforts to optimize biofilm formation, *C. propinquum* and *M. luteus* biofilms were very weak and chelating agents did not reduce the biofilm biomass. *S. aureus* biofilms were stronger, but were not significantly reduced by chelating agentsImages of *M. luteus* biofilms cultured under typical domestic conditions showed that biofilms were relatively thin and patchy across the surface. The biofilm architecture did not appear to be affected by treatment with chelating agents. Higher resolution field emission scanning electron microscopy images showed that microbial cells were present in patches, and that there were other areas where cells were absent, but the residue from previous biofilm growth was clearly apparent.

INITIAL AIMS: Effective removal of biofilms is important for household hygiene and disinfection. Many surfaces around the home are known to harbor biofilm, including food-contact surfaces in the kitchen, bathroom surfaces and kitchen appliances. These surfaces are usually difficult to clean with conventional detergents which can create issues such as visual fouling, undesirable odours or even transfer of pathogenic bacteria. Consequently there is a need to find new, broad-spectrum and fast-acting technologies that can remove household biofilms. This study explored the value of chelating agents for household biofilm dispersal.

- **Chelators do not have intrinsic anti-biofilm activity against selected model bacteria**
- **Imaging methods gave important detailed insights into biofilm architecture**

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01 November – 31 December 2017
Funded by Metals in Biology BBSRC NIBB Business Interaction Voucher
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Evaluation of the potential of the molybdenum-containing enzyme DMSO reductase as an oxygenation catalyst

"This collaboration has allowed us to initiate a collaboration with Piramal that will hopefully lead to many other useful interactions," Gary Black, Northumbria University

Gary W Black and Justin J Perry, Northumbria University; Robert A Holt, Piramal Healthcare UK

Picture: pixabay.com

A chiral drug has a spatial arrangement of atoms that cannot be superimposed on its mirror image – rather like a pair of mittens.

OUTCOMES: In total six DMSO reductase enzyme preparations were produced from several bacterial strains from *Rhodobacter capsulatus, Rhodobacter sphaeroides, Cupriavidus metallidurans, Aeromonas hydrophila* and *Oceanithermus profundus,* and their capacity to perform complex oxidation chemistry was determined.

INITIAL AIMS: The enantiomers of a chiral drug — one that has a spatial arrangement of atoms that cannot be superimposed on its mirror image — can have different properties with respect to pharmacology, metabolism, immune response and so on. There has been significant effort to develop cost-effective and scalable methodology for the synthesis of enantiomerically pure compounds, but the development of processes involving oxidative reactions has lagged behind. Dimethylsulphoxide reductase (DMSOR) is a molybdenum-containing enzyme that can reduce sulphoxides to the corresponding sulphides. When the sulphoxide is a mixture of both enantiomers, DMSOR reduces one enantiomer much more rapidly, which leads to enantiomeric enrichment of the slower reacting enantiomer. This project will explore the potential of DMSOR to carry out complex oxidation chemistry

- Depending further results, the industrial and academic partners are considering an application to Innovate UK

King's College and ZiNIR chip in to design novel imaging system

Business interaction voucher funding from the Metals in Biology BBSRC NIBB has enabled King's College London and technology development company ZiNIR to collaborate on the design of a novel ultra-compact imaging system. Such an imaging modality might be used to assess the vitality of biofuel crops in the field as well as other industrial biotechnology applications.

The project brought together the expertise of Po-Wah So, from Kings College London, who researches the MRI-based detection of iron in biological and pathophysiological processes, with ZiNIR's capabilities in photonics research.

The aim of this collaboration was to identify the technical specifications of an ultra-compact, hyperspectral fluorescence (that is, fluorescence across the electromagnetic spectrum from ultraviolet to long-infrared) imaging instrument that is capable of simultaneously sensing the fluorescence signature of a range of biomolecules.

Ian Goodyer from ZiNIR explains, "imaging of biological molecules that contain metal ions has a number of uses, ranging from industrial biotechnology through to biomedical applications such as the diagnosis of Alzheimer´s disease."

One potential application of such imaging in industrial biotechnology is the monitoring of chlorophyll fluorescence — which is highly sensitive to changes in the efficiency of photosynthesis — as an indicator of the vitality and growth rate of biofuel crops.

To identify the technical specifications, the collaborators conducted a literature review of the current status of MRI-optical dual imaging instruments, their applications and associated challenges, together with a patent search for fluorescence imaging systems.

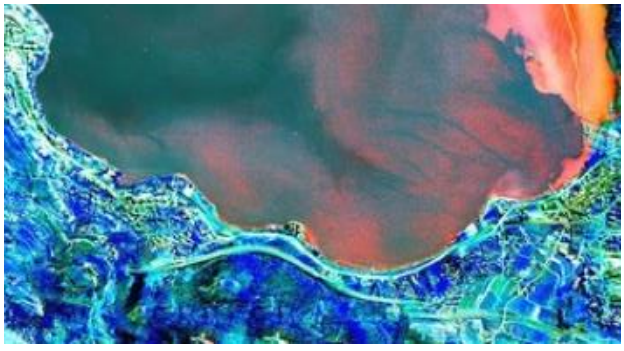
"The literature and patent search identified number of parallels between the requirements for fluorescence imaging in plants and in preclinical experimental studies," highlights Po-Wah. "A set of technical specifications for the imaging system was defined to satisfy the requirements of both application areas," she says.

Since ZiNIR has designed a novel semiconductor chip-based spectrometer that can detect multiple wavelengths simultaneously, an ultimate aim would be to develop such a device using use ZiNIR's chip technology that could be incorporated into an MRI imaging system. This would have the advantages of being small and easily transportable.

Joanna Coote, who worked project for ZiNIR said "This has been a fascinating project that generated a great many ideas for further development of the proposed system. I look forward to a further collaboration with Po-Wah on a longer and more in-depth project."

Manuscript: Walker et al (2016) Aging 8: 2488-2508

For more information please contact po-wah.so@kcl.ac.uk



"Imaging of biological molecules that contain metal ions has a number of uses, ranging from industrial biotechnology through to biomedical applications including identification of novel therapies to treat Alzheimer´s disease."

BIVMiB023 BB/LO13711/1
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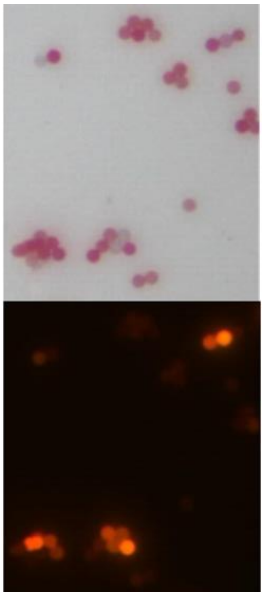
Maximising biomarker detection sensitivity through metal-enhanced fluorescence

"With the expertise of the University of Manchester we have been able to add visible dyes to particles while retaining fluorescent signaling conjugated to the particle surfaces." Aeirtec Ltd

Lu Shin Wong, University of Manchester
Stephen Kilfeather, Aeirtec Ltd



OUTCOMES: This business interaction voucher was used to develop a collaborative relationship related to the production of protein—metallic nanoparticle conjugates that could be used in Aeirtec's multiplex immunoassay assay platform. The BIV was used to part-fund research by an MSc students and two undergraduate summer internships working in Lu Shin Wong's lab. The study focused on the bioconjugate chemistries for linking dyes, proteins and metallic nanoparticles to each other. Comparative analyses were conducted with several diamine linker molecules using a variety of protecting groups. Robust and quantifiable bioconjugate chemistries were developed and delivered to Aeirtec.

Visible dyes (top image) can be added to particles while retaining fluorescent signaling (bottom image)

INITIAL AIMS: Fluorescence-based immunosorbent assays are a key technology for measuring microbial contamination and molecular biomarkers. Typically, these assays use an immobilised antibody to capture the target molecule from the test sample, followed by the immobilisation of a second antibody bearing a fluorescent label. Metal-enhanced fluorescence (MEF) — where the second antibody is co-localised with a metallic nanoparticle — could improve diagnostic sensitivity. This project aims to improve the sensitivity of MEF-based assay systems by applying tailored bioconjugation methods to control the orientation of the immobilized antibodies with respect to the nanoparticle.

- **Robust and quantifiable bioconjugation methods delivered to industrial partner**
- **BBSRC iCASE PhD studentship awarded**

ISCFPOCMiB032 BB/L013711/1 BB/SCA/MetalsinBiology/17
1 November 2017 – 28 February 2018
Funded by Metals in Biology BBSRC NIBB Seeding Catlayst grant
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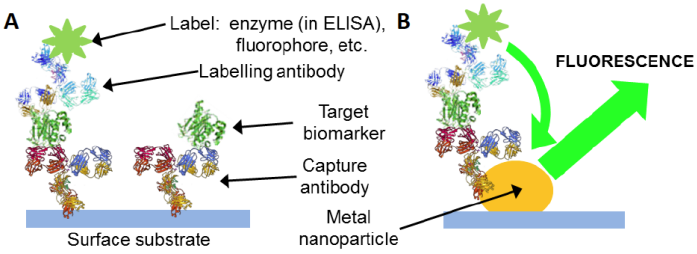
Site-specific bioconjugate chemistry for antibody-nanoparticle conjugates

"The project has demonstrated to us the range of avenues for incorporation of metal-enhanced fluorescence in enhancement of biomarker measuring platform sensitivity." Stephen Kilfeather, Aeirtec Ltd.

Lu Shin Wong, University of Manchester; Stephen Kilfeather, Aeirtec Ltd.



a) Schematic diagram if immunosorbent assay and b) assay employing a metal nanoparticle that results in metal-enhanced fluorescence.

RESULTS: We developed a collaborative relationship related to the production of protein–metallic nanoparticle conjugate materials for use in diagnostics platforms and that could potentially be incorporated into Aeirtec's existing platform. The project part-funded a postdoctoral researcher working on the analysis of gold nanoparticle aggregation and a PhD student involved in the chemical synthesis of linker molecules that will enable the attachment of protein molecules to the nanoparticles. The partner company benefited from discussing chemistry in relation to our capacity to generate a metallic–protein microparticle surface. The interaction has now set a direction for incorporation of metals alongside proteins, and forms the basis of the continued research by the PhD student.

INITIAL AIMS: Fluorescence-based immunosorbent assays have become a key technology for the detection and quantification of biomolecules in a range of fields such as the testing of microbial contamination (in water, chemical and during food and drug production), to measure biomarkers (in medical diagnostics and drug discovery) and in biomedical imaging. The use of metal-enhanced fluorescence is an active area of research that is being studied to improve the fluorescence output of these assays. This project will develop production methods for metal nanoparticle–antibody conjugates that are robust and scalable, which would be needed for commercial implementation. These hybrid metal-biomolecule materials offer advantageous spectroscopic properties that could greatly increase detection sensitivity of the assays.

- **Aeirtec will contribute to a BBSRC iCASE PhD application**
- **Together with the University of Manchester, Aeirtec is exploring routes toward a larger collaboration to take forward the diagnostics applications of the project**

Workshops kickstart a *Rhodococcus* molecular toolkit

Workshops funded by Metals in Biology BBSRC NIBB have built a new community of scientists involved in *Rhodococcus* research and initiated a project to develop a community resource of molecular biology tools to enable the wider use of *Rhodococcus* bacteria in industrial biotechnology.

Rhodococcus species — the majority of which are non-pathogenic soil inhabitants — contain useful enzymes, pathways and systems that can be harnessed for industrial biotechnology applications. For example, they are able to breakdown environmental pollutants and explosives, aid the recycling of rubber tyres, be used in biofuel production systems, turn metal contaminants into useful products and synthesise complex chemical compounds.

A scoping workshop on "Metalloproteins in Biocatalysis and Bioenergy" was held at Durham University in July 2014; not long after the start of the Metals in Biology Network. At this meeting, Alison Parkin from the University of York and Colin Murrell from University of East Anglia, advocated the idea of holding a focused workshop on *Rhodococcus*. "We realised that there were a number of research groups in the UK who were working on *Rhodococcus* and related genera of actinomycetes, and that there was common interest in the biology, molecular genetics and the potential for biotechnological applications of this group of bacteria," says Colin.

Alison highlights that that *Rhodococcus* research is an area where the UK has both strength and breadth, "UK science in this area spans industrial applications, environmental science and understanding disease". A key consideration was the format of the focused meeting to help maximize potential output. "I wanted to have a meeting where attendees could have chance to get to know the main players in this field in a very focused, friendly and open manner that would let us discuss future directions and find new ways of working as a strong team," says Alison.

The meeting, held at the University of York in November 2015, had about 20 attendees (a summary of the meeting is available here). As well as allowing academic researchers and industry scientists to mix, the meeting initiated new projects. For example, as a result of the meeting, Alison's lab will now be able to start studying new bacteria and enzymes to find new biocatalysts that activate challenging chemical reactions.

One key theme that emerged at this meeting was the paucity of molecular biology tools (such as plasmids, vectors and mutagenesis protocols) to extract and manipulate *Rhodococcus* enzymes to enable their optimization for research and industrial biotech applications. To address this challenge, the attendees proposed the establishment of a molecular tool kit for *Rhodococcus*.

This idea was championed by Jon Marles-Wright and Louise Horsfall from the University of Edinburgh. "Building a molecular biology toolkit, based on established synthetic biology standards and containing well characterised parts for the control of gene expression and genome editing, will enable us to use *Rhodococcus* species more widely as an industrial host," says Jon. "It will also speed up research by making the tools available freely to anyone interested in working with these strains".

To initiate this project, Jon collated information related to *Rhodococcus* strains, genomes and genetic tools used in the attendees' research groups. The next phase of the project is moving into the lab; during the summer of 2016, Jon and Louise have a group of Masters students who are competing in the iGEM competition – an international synthetic biology completion for early career scientists.

"The iGEM competition has an ethos of openness and collaboration, so the idea of developing a set of community tools to enable *Rhodococcus* species to be easily engineered and used in research and industrial settings was something that really appealed to our students", says Jon. The plan is for part of the team to focus on a *Rhodococcus* toolkit, with the *Rhodococcus jostii* RHA1 strain as their model system. The students will also be involved in maintaining the community of *Rhodococcus* researchers.

So as a result of the workshops, the community-building that researchers hoped to achieve has indeed worked and they are now starting to construct a shared resource for molecular biology tools in *Rhodococcus*. "This has been a brilliant result", concludes Alison.

Attendees at the *Rhodococcus*-focused workshop

For more information please contact
metals.bbsrcnibb@durham.ac.uk
http://community.dur.ac.uk/MiB_NIBB/
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Metals in Biology Workshop
26-27 November 2015
Funded by a Metals in Biology BBSRC NIBB workshop funding
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Metal-related antimicrobials: targeting the Achilles heel of bad bugs

A BBSRC Metals in Biology NIBB scoping workshop highlighted advances in the understanding of metal-handling systems of microbes and hosts, with the aim of improving collaboration to tackle antimicrobial resistance.

Robert Poole
University of Sheffield

There is a long history of using metals to fight microbes[1]. Historically, some unpleasantly hazardous metals have been used to treat infections, such as mercury for syphilis, as well as arsenic and antimony for Leishmania. In agriculture, copper sulphate in Bordeaux mixture — identified in the 1880s — is an effective fungicide for treating diseased vines. More recently, steel fixtures and fittings in hospitals have been replaced with copper ones, since copper surfaces (unlike those containing iron) are antimicrobial barriers.

A range of products with antimicrobial properties currently on the market contain metal chelants such as ethylene diamine tetra acetic acid (EDTA). A well-known anti-dandruff shampoo, which generates multiple billions of dollars of revenue each year, contains zinc pyrithione (ZPT). This compound treats dandruff that is triggered by the fungal microflora of the scalp by interfering with the iron-handling circuitry of fungi through an intricate sequence of biochemical interactions (which also involve copper)[2].

Metals can act as antimicrobials because broadly speaking, host immune systems have evolved to exploit metal availability to combat infections. Hosts protect against infection through the sequestration of nutrient metals (that are essential to microbes – a concept called nutritional immunity[3] – that has garnered renewed attention in recent years**.** In turn Microbial pathogens fight to obtain valuable elements such as iron from hosts, often releasing iron-scavenging siderophores.

This triggers an evolutionary arms race fought on a battle ground of iron, with hosts producing defensive siderocalins to bind microbial siderophores, the microbes in turn selecting for stealth siderophores that are not recognised by siderocalins, combatted by stealth siderophores or enterochelin-like molecules released from adapted hosts.

Host immune cells such as macrophages engulf microbes whereupon a specialised protein, natural resistance associated with macrophage protein 1 (NRAMP1), helps to kill the entrapped invader. Some years after its discovery, NRAMP1 was found to pump vital metals such as iron from the microbe-containing compartment, presumably to starve it of essential elements. The compartment subsequently fills with a toxic dose of copper. Neurophils release calprotectin to scavenge zinc and manganese, starving microbes of these essential elements.

As details of the cell biology of metal availability are uncovered, it becomes possible to tailor more precise antimicrobial treatments by design, not just stumbled upon empirically or by evolution. Metals, and by implication chelants, ionophores, and agents that interfere with the metal-handling systems of microbes and hosts, are increasingly recognized among the promising candidates for new antimicrobials[4].

At the BBSRC Metals in Biology NIBB scoping workshop we highlighted new knowledge of microbe and host metal- handling systems and explored why metal availability is the microbial Achilles heel. This event brought together multiple research communities to encourage innovation at this academia-business interface. and revealed opportunities to collaborate to help tackle the scourge of antimicrobial resistance.

1. *The Physicochemical Basis of Therapy (1979)* 385-442
2. Antimicrob. Agents Chemother. (2011) 55, 5753-5760
3. *Nature Rev. Micro.* (2012) 10, 525-537
4. *Nature* (2015) 521, 402

OUTCOME: The workshop highlighted opportunities arising from increased communication between the diverse communities exploiting and developing metal-related antimicrobials, investigating the cell biology of metals and nutritional immunity. Robert Poole, University of Sheffield, edited a dedicated volume of *Advances in Microbial Physiology,* volume 70 'Microbiology of Metal ions' https://www.elsevier.com/books/microbiology-of-metal-ions/author/978-0-12-812386-7

Project Outcomes

This section includes the project outcome information, where no case study is currently available (these case studies are being produced under the auspices of the impact plan under BB/R00218/1).

Business Interaction Vouchers Public Summaries of Project outcomes, case studies in progress – Projects funded by Metals in Biology Network

ID number	BIVMiB017
Title	Extracting mercury from industrial waste using microalgae
Academic (lead) Partner	Mark van der Giezen, University of Exeter
Industrial Partner	Tonnie Schuijl, Reym
Public summary	Affordable and sustainable energy is an important global challenge. Biofuels are seen as possible solutions; however, currently they have limited environmental benefits and put pressure on land and water. Algal biofuels could overcome many drawbacks of terrestrial plant-based biofuels but are currently more energy-intensive and expensive. Water pollution is another global problem and causes over 14,000 deaths each day. By 2025, 1.7 billion people are faced with absolute water scarcity and two-thirds will have drinking water shortages. Unfortunately, many essential industrial activities contribute greatly to water pollution and more sustainable production methods or waste management practices are required to support the global demand for products but protect water sources. With relevance to both challenges, we will grow algae on metal contaminated industrial waste streams followed by hydrothermal liquefaction. This process will separate waste into four fractions: water, gas, oil and solids, the latter containing the metal waste, thereby valorising waste.
Start date	1 July 2016
Public summary of project outcomes	Mercury contaminated petrochemical waste is routinely stored in abandoned mineshafts. Considering the volume of these wastes (several metric tonnes/day in the Netherlands alone), compaction of the toxic mercury alone would save the industry money and space. Mercury contaminated sludge from 6 different locations in the Netherlands was processed. To assess the microbial diversity associated with this material, sludge was diluted and incubated under various growth conditions. DNA was isolated from individual morphologically distinct colonies. In addition, total environmental DNA was isolated directly from sludge to assess the metagenome of this material. Several complete genomes were assembled from these experiments. Analysis indicated a metabolic potential to deal with heavy metals, in particular mercury. Another strand of research investigated the possibility to remove metal contaminants using hydrothermal liquefaction (HTL) with or without biomass in the form of algae or alginate beads. Different HTL temperatures were tested. Our results show that algae alone are not capable of removing the high mercury load from the sludge. Alginate beads shows better removal capabilities that algae but the combination of alginate beads loaded with algae shows excellent mercury removal capabilities with all mercury removed from the water phase. HTL under different conditions indicated that algae alone with the sludge were least effective with removing 96.9% of mercury. HTL with sludge alone was most effective with 100% of the mercury ended up in the solid fraction. The alginate beads conditions resulted in 99.9% of mercury removed. It is clear that we have demonstrated the ability of HTL to massively reduce the volume of the mercury containing sludge. We have successfully demonstrated the potential of HTL to remove and concentrate metal contaminants from industrial waste streams. Similar to our successful removal of metal contaminants from mine waste, we have now also clearly demonstrated that our approach can be successfully applied to petrochemical waste as well.

Facts and Figures

This section summarises outputs generated by the Metals in Biology BBSRC NIBB, supplementing the Outcome Summary section. The full sets of data will be made available when complete (as two Excel spreadsheets).

Membership Information

The following membership information is taken for the whole of the membership (including past and present members).

Male / Female ratio

Gender	Count	%
Female	170	32%
Male	369	68%
Grand Total	539	100%

Disciplines

Category	Count	%
Biology	285	52.9%
Chemistry	145	26.9%
Engineering	70	13.0%
Environmental Science	19	3.5%
Social Science	17	3.2%
Computer Science	1	0.2%
Mathematics	1	0.2%
Physics	1	0.2%
Grand Total	539	100%

Location

Region	Count	%
North East	115	21%
South East	85	16%
North West	57	11%
Yorkshire and the Humber	47	9%
East	33	6%
Scotland	33	6%
London	31	6%
East Midlands	30	6%
South West	26	5%
Europe	24	4%
West Midlands	23	4%
International	21	4%
Wales	10	2%
Northern Ireland	4	1%
Grand Total	539	100%

Affiliation

Affiliation	Count	%
HEI	362	67%
Industry	140	26%
Research Organisation	16	3%
Governmental Department	11	2%
Charity	9	2%
NGOs	1	0%
Grand Total	539	100%

Other Funding Won (OEF)

OEFs related to this network has been recorded on the extranet, totalling £12.002 million (details in table at end of report)

Follow on Funding

This has been challenging to audit but where the information is available it is included under other funding won or match funding.

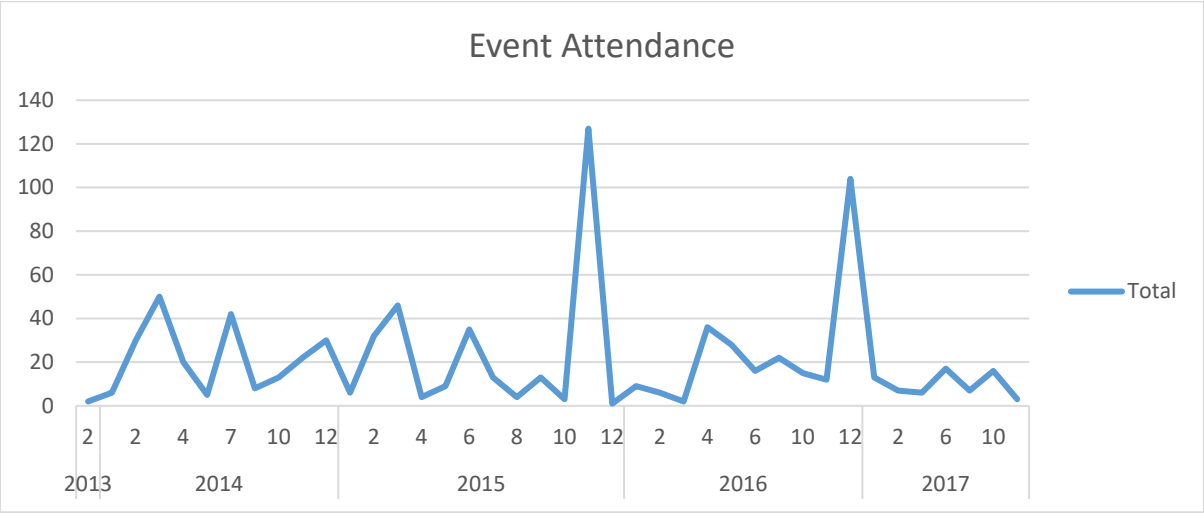
Match Funding

The match funding reported for this network is £723k. This amount is composed of:

- £623k industry match funding.
- £100k HEI contribution (for projects supported at 80% fEC).

Workshops

Attendance at the events during the life of the network was a total of 840. There was an ongoing series of activities, as shown in the graph below. November 2015 contained 6 different events, including the 'Metal-Related Antimicrobials Event'. The community event took place in December 2016.



Desire to Proceed

A review of the final reports, and case studies, identifies a desire to proceed with the research (to higher TRLs) by 93.8% of grant awardees, to date.

Publications

We have validated outputs in discovery journals (PNAS, Nature Communications, Nature Chemical Biology) plus an entire journal volume inspired by one of our workshops (see individual case study). Members have also informed us that papers will arise in the future from on-going work which was instigated by this Network; for example *"PoC work is being continued by a BBSRC DTP student so there will be papers credited to the Metals in Biology BBSRC NIBB in due course."*

See the table overleaf for the full breakdown.

Metals in Biology BBSRC NIBB - Publications

No	Year	Authors	Title	Publication	Volume / Edition / Page
1.	2017	Zhang Z, Smart TJ, Choi H, Hardy F, Lohans CT, Melodie MIA, Richardson SW, Paton RS, McDonough MA, Schofield CJ.	Structural and stereoelectronic insights into oxygenase-catalyzed formation of ethylene from 2-oxoglutarate.	Proceedings of the National Academy of Sciences	114 / 4667 - 4672
2.	2017	Moore SJ, Sowa ST, Schuchardt C, Deery E, Lawrence AD, Ramos JV, Billig S, Birkemeyer C, Chivers PT, Howard MJ, Rigby SE, Layer G, Warren MJ.	Elucidation of the biosynthesis of the methane catalyst coenzyme F(430).	Nature	543 / 78 - 72
3.	2017	Poole RK.	Preface.	Advances in Microbial Physiology	70 / 1st / xi -xii
4.	2017	Poole RK.	Microbiology of Metal Ions.	Advances in Microbial Physiology	70 / 1st / 1 - 379
5.	2017	Malavia D, Crawford A, Wilson D.	Nutritional Immunity and Fungal Pathogenesis: The Struggle for Micronutrients at the Host-Pathogen Interface.	Advances in Microbial Physiology	70 / 1st / 85 - 103
6.	2017	Vallières C, Avery SV.	Metal-Based Combinations That Target Protein Synthesis by Fungi.	Advances in Microbial Physiology	70 / 1st / 105 - 121

No	Year	Authors	Title	Publication	Volume / Edition / Page
7.	2017	Turner AG, Ong CY, Walker MJ, Djoko KY, McEwan AG.	Transition Metal Homeostasis in Streptococcus pyogenes and Streptococcus pneumoniae.	Advances in Microbial Physiology	70 / 1st / 123 - 191
8.	2017	Dalecki AG, Crawford CL, Wolschendorf F.	Copper and Antibiotics: Discovery, Modes of Action, and Opportunities for Medicinal Applications.	Advances in Microbial Physiology	70 / 1st / 193 - 260
9.	2017	Pal C, Asiani K, Arya S, Rensing C, Stekel DJ, Larsson DGJ, Hobman JL.	Metal Resistance and Its Association With Antibiotic Resistance.	Advances in Microbial Physiology	70 / 1st / 261 - 313
10.	2017	Barwinska-Sendra A, Waldron KJ.	The Role of Intermetal Competition and Mis-Metalation in Metal Toxicity.	Advances in Microbial Physiology	70 / 1st / 315 - 379
11.	2017	Osman D, Foster AW, Chen J, Svedaite K, Steed JW, Lurie-Luke E, Huggins TG, Robinson NJ.	Fine control of metal concentrations is necessary for cells to discern zinc from cobalt.	Nature Communications	8 :1884 / 1 - 12
12.	2017	Foster AW, Pernil R, Patterson CJ, Scott AJP, Pålsson LO, Pal R, Cummins I, Chivers PT, Pohl E, Robinson NJ.	A tight tunable range for Ni(II) sensing and buffering in cells.	Nature Chemical Biology	13 / 409 - 414
13.	2017	Hobbs, C; Reid, JD.,Shepherd, M.	The coproporphyrin ferrochelatase of Staphylococcus aureus: mechanistic	Biochemical Journal	474 / 3513 - 3522

No	Year	Authors	Title	Publication	Volume / Edition / Page
			insights into a regulatory iron-binding site.		
14.	2017	Reeve HA, Ash PA, Park H, Huang A, Posidias M, Tomlinson C, Lenz O, Vincent KA.	Enzymes as modular catalysts for redox half-reactions in H2-powered chemical synthesis: from biology to technology.	Biochemical Journal	474 / 215 -230.
15.	2017	Cueva ME, Horsfall LE.	The contribution of microbially produced nanoparticles to sustainable development goals.	Microbial Biotechnology	10 / 1212-1215
16.	2016	Osman D, Piergentili C, Chen J, Sayer LN, Usón I, Huggins TG, Robinson NJ, Pohl E.	The Effectors and Sensory Sites of Formaldehyde-responsive Regulator FrmR and Metal-sensing Variant.	Journal of Biological Chemistry	291 / 19502 - 19516
17.	2016	Denby KJ, Iwig J, Bisson C, Westwood J, Rolfe MD, Sedelnikova SE, Higgins K, Maroney MJ, Baker PJ, Chivers PT, Green J.	The mechanism of a formaldehyde-sensing transcriptional regulator.	Scientific Reports	6 / 38879
18.	2016	Rawlings A	Membrane proteins: always an insoluble problem?	Biochemical Society Transactions	44 / 790 - 795
19.	2016	Walker T, Michaelides C, Ekonomou A, Geraki K, Parkes HG, Suessmilch M, Herlihy	Dissociation between iron accumulation and ferritin upregulation in the aged substantia	Aging	8 / 2488 -2508

No	Year	Authors	Title	Publication	Volume / Edition / Page
		AH, Crum WR, So PW.	nigra: Attenuation by dietary restriction		
20.	2015	Osman D, Piergentili C, Chen J, Chakrabarti B, Foster AW, Lurie-Luke E, Huggins TG, Robinson NJ.	Generating a Metal-responsive Transcriptional Regulator to Test What Confers Metal Sensing in Cells.	Journal of Biological Chemistry	290 / 19806 - 19822
21.	2018	Syntrivanis L, Wong LL, Robertson J.	Hydroxylation of Eleuthoside Synthetic Intermediates by P450BM3 (CYP102A1)	European Journal of Organic Chemistry	https://doi.org/10.1002/ejoc.201801206
22.	2019	Osman D, Martini MA, Foster AW, Chen J, Scott AJP, Morton RJ, Steed JW, Lurie-Luke E, Huggins TG, Lawrence AD, Deery E, Warren MJ, Chivers PT, Robinson NJ.	Bacterial sensors define intracellular free energies for correct enzyme metalation.	Nature Chemical Biology	https://www.nature.com/articles/s41589-018-0211-4

Metals in Biology BBSRC NIBB – Other external funding (OEF) received

Reference	Title	PI	Institution	Value (£K)	Funder
OEFMiB001	A new generation of E. coli expression hosts and tools for recombinant protein production	Robinson, Colin	University of Kent	2024.903	Innovate UK
OEFMiB002	The geology, geometallurgy and geomicrobiology of cobalt resources leading to new product streams	Lloyd, Jonathan	University of Manchester	459.258	NERC
OEFMiB003	Allay Remediation – ALgae-cLAY composite beads for passive water	Greenwell, Christopher	Durham University	23.522	Small Business Research Initiative
OEFMiB004	Development of versatile cell-metal regulation model (multiple metals)	Robinson, Nigel	Durham University	161.501	P&G
OEFMiB005	New Routes to Driving Enzyme-Catalysed Chemical Synthesis Using H2 Gas	Vincent, Kylie	University of Oxford	2911.05	Innovate UK
OEFMiB006	Enzyme co-localisation and aggregation for enhanced metabolic activity for commodity chemicals	Warren, Martin	University of Kent	187.394	Innovate UK
OEFMiB007	The Relationship between Dietary Iron and the Gut Microbiome. Can Dietary Iron Regime be Exploited to Improve Health?	Andrews, Simon	University of Reading	633.02	BBSRC
OEFMiB008	Valorising industrial waste streams and reducing pollution using microalgae	van der Giezen, Mark	University of Exeter	100	NERC (GW4+ DTP
OEFMiB009	Physics of Life Network 2 (PoLNet2)	McLeish, Tom	Durham University	254.064	EPSRC
OEFMiB010	Developing ways of implementing biocatalytic hydrogenation reactions in flow	Vincent, Kylie	University of Oxford	100	BBSRC
OEFMiB011	How do plants sense iron?	Balk, Janneke	John Innes Centre	100	BBSRC DTP

OEFMiB012	UK-India Newton Fund Biotechnology Solutions for Waste Scoping Visit to India	Lloyd, Jonathan	University of Manchester	2	Newton fund
OEFMiB013	Industrial Applications of Metal-Microbe Interactions	Lloyd, Jonathan	University of Manchester	2	BBSRC
OEFMiB014	Novel antimicrobial agents for bacterial pathogens of livestock: light-activated CO-releasing molecules	Poole, Robert	University of Sheffield	674.947	BBSRC
OEFMiB015	Biotechnology-Nanotechnology Hybrid Materials for Diagnostic Devices in Health and Agriculture	Wong, Lu Shin	University of Manchester	94.992	BBSRC NPIF
OEFMiB016	Enhancing Metal Uptake in Soya Crops	Bricklebank, Neil	Sheffield Hallam University	106.638	Innovate UK
OEFMiB017	Molecular engineering of high activity multifunctional biometallic catalysts for clickable chemistries	Lloyd, Jonathan	University of Manchester	892	BBSRC
OEFMiB018	Towards improved bioprocess kinetics for C1 feedstock fermentation in acetogens	Warren, Martin	University of Kent	49.55	C1Net Proof of Concept Funding
OEFMiB019	ZPT mechanism of action	Schroeder, Martin	Durham University	318.464	P&G
OEFMiB020	Exploiting a novel chelant database to probe cellular metal-handling	Sharples, Gary	Durham University	55	P&G
OEFMiB021	Identifying chelant combinations to enhance antibacterial efficiency	Sharples, Gary	Durham University	112.5	P&G
OEFMiB022	METALLOCHAPERONES: The partitioning of metals to delivery pathways	Robinson, Nigel	Durham University	405.226	BBSRC
OEFMiB023	Genetic and molecular basis of organic-arsenic-microbe interactions in arsenic prone aquifers (GOAM)	Lloyd, Jonathan	University of Manchester	585.040	NERC
OEFMiB024	Biologically Upcycling Metals	Horsfall, Louise	University of Edinburgh	1020.945	EPSRC
OEFMiB025	Understanding and exploiting biological metal-nanoparticle synthesis for metal recovery	Horsfall, Louise	University of Edinburgh	327.153	BBSRC

OEFMiB026	CobW: A Cobalt Shuttle Service for Vitamin B12	Young, Tessa	University of Melbourne	100.0	EU
OEFMiB027	The development of a novel sustainable bioactive gel for application to new and deployed waste water pipes to inhibit damaging plant root ingress	Lindsey, Keith	University of Durham	104.0	ERDF
OEFMiB028	Development of LA-ICPMS and associated techniques for the analysis of crop enhancement products	Bricklebank, Neil	Sheffield Hallam University	197.0	BBSRC Croda