BIVMiB040 BB/L013711/1
1 August – 31 October 2017
Funded by Metals in Biology BBSRC NIBB Business Interaction Voucher
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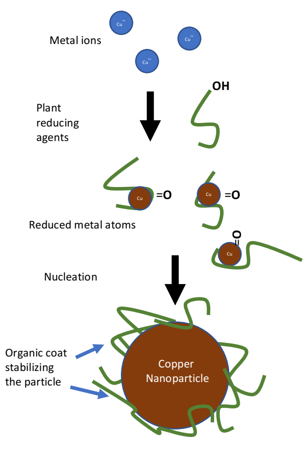
Metals IN BIOLOGY

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

Durham University NORTHUMBRIAN WATER living water

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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BIVMiB011 BB/L013711/1
1 January 2015 – 28 February 2015
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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*

BIVMiB018
01 July 2016 – 31 December 2016
POCMiB024
01 November 2015 – 31 December 2016
Funded by a Metals in Biology BBSRC NIBB Business Interaction Voucher and Proof of Concept funding
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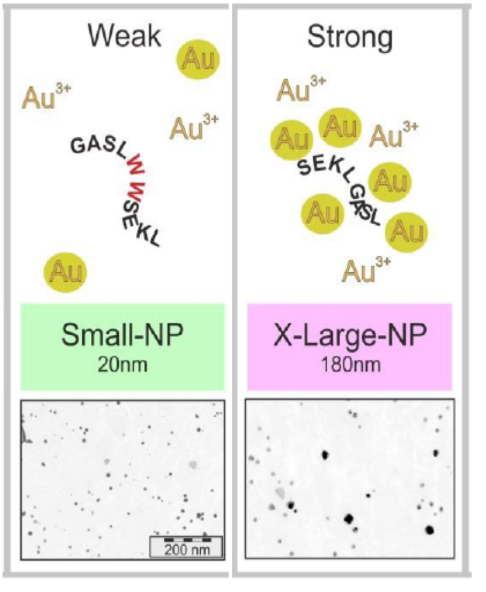
The use of platinum group metal nanoparticles in wastes from roadside verges for the production of high-value catalysts

"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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BIVMiB019 BB/L013711/1
18 May – 17 November 2016
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Metals IN BIOLOGY

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

Sheffield Hallam University CRODA

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

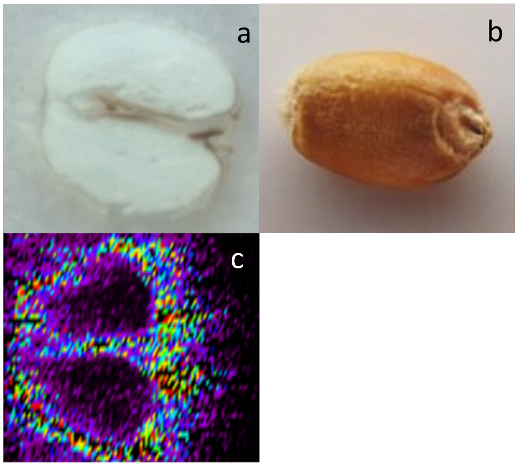

Image of (a) un-germinated seed, (b) section of treated seed tissue, and (c) LA-ICP-MS image showing distribution of zinc in seed tissue.

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**

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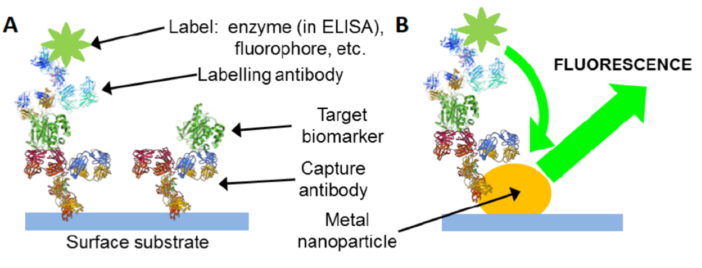
Site-specific bioconjugate chemistry for antibody-nanoparticle conjugates

"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**

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1 July – 31 December 2016
Funded by a Metals in Biology BBSRC NIBB Business Interaction Voucher
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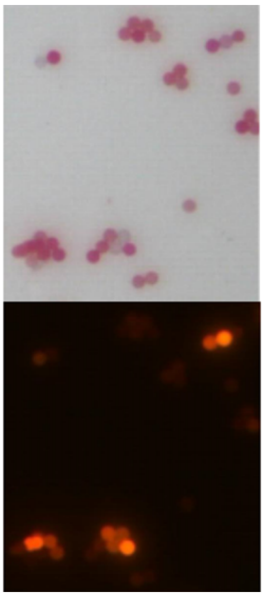
Metals IN BIOLOGY

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**

POCMiB011 BB/L013711/1
01 October 2015 - 30 November 2016
Funded by a Metals in Biology BBSRC NIBB Proof of Concept funding
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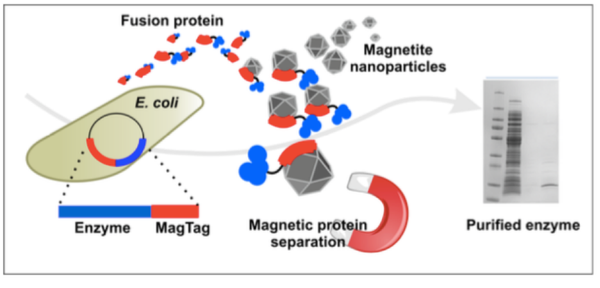
Mag-Tag: magnetite nanoparticle affinity tags for industrial biotechnology protein purification

"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**

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01 August 2016 – 31 January 2017
Funded by a Metals in Biology BBSRC NIBB Business Interaction Voucher
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Metals IN BIOLOGY

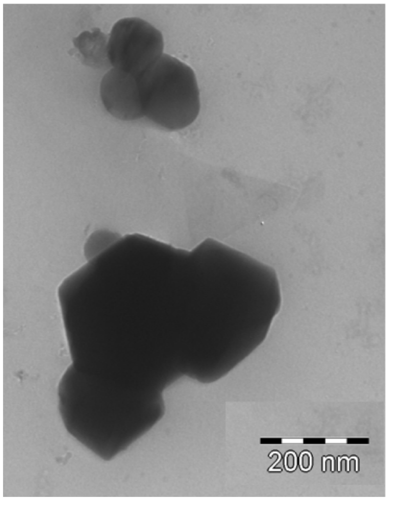
Investigating the antimicrobial properties of copper-infused fabrics

"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

University of Southampton | Copper Clothing Everyone's talking about it ...

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**

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Metals IN BIOLOGY

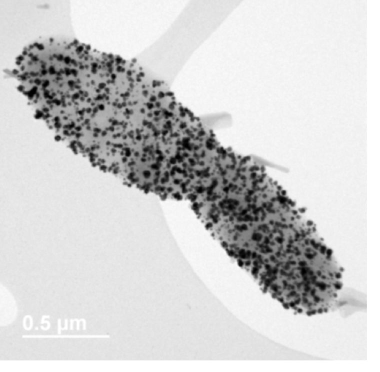
Exploiting the commercial potential of novel biometallic catalysts

"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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Metals IN BIOLOGY

Biosynthesis of bimetallic nanoparticles for fine and specialty chemical production

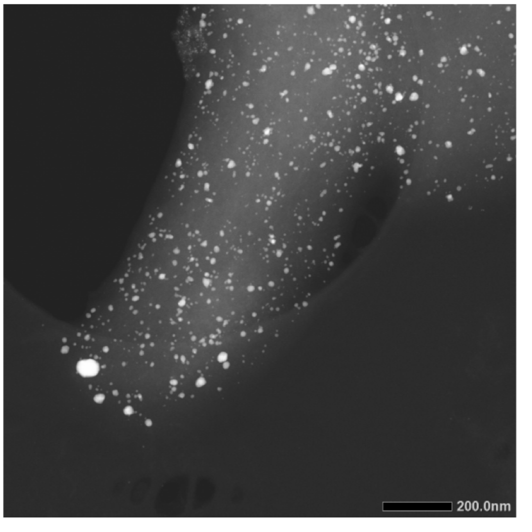
"This award provided a quick, convenient and effective route to bring together the Manchester group's expertise in bioproduction of metal particles with Johnson Matthey's catalysis know-how. We have begun to determine the potential of this technology for the production of novel catalysts." Nigel Powell,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

MANCHESTER 1824 The University of Manchester

JM Johnson Matthey Inspiring science, enhancing life

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**

Durham University

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