BIVMiB011 BB/L013711/1
1 January 2015 – 28 February 2015
Funded by a Metals in Biology BBSRC NIBB Business Interaction Voucher
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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***

Biotechnology and Biological Sciences Research Council

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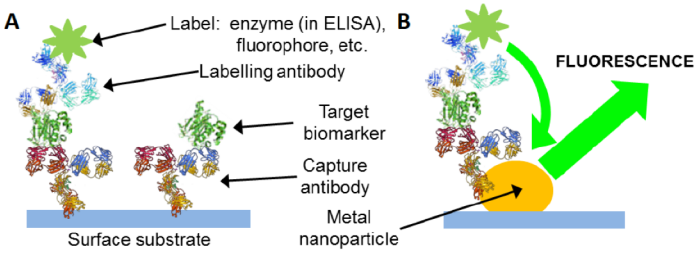
Site-specific bioconjugate chemistry for antibody-nanoparticle conjugates

"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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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

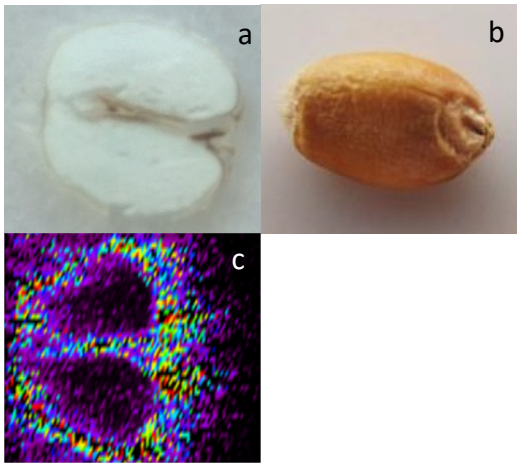

Image of (a) un-germinated seed, (b) section of treated seed tissue, and (c) LA-ICP-MS image showing distribution of zinc in seed tissue.

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**

Biotechnology and Biological Sciences Research Council

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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

Biotechnology and Biological Sciences Research Council

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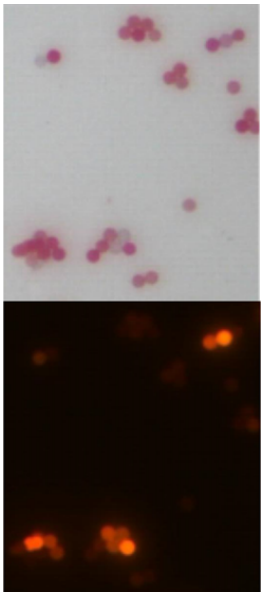
Metals IN BIOLOGY

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**

Durham University · University of Kent · UKRI Biotechnology and Biological Sciences Research Council

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.

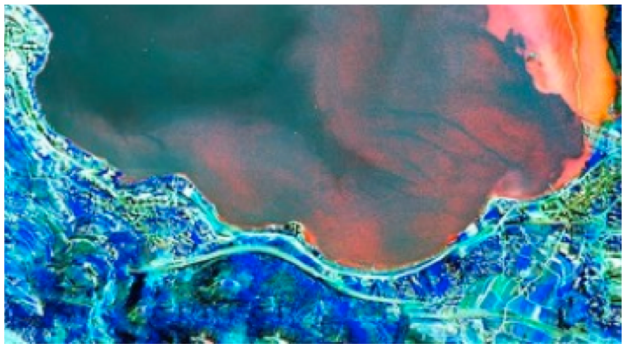
"The literature and patent search identified number of parallels between the requirements for fluorescence imaging in plants and in preclinical experimental studies," highlights Po-Wah. "A set of technical specifications for the imaging system was defined to satisfy the requirements of both application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

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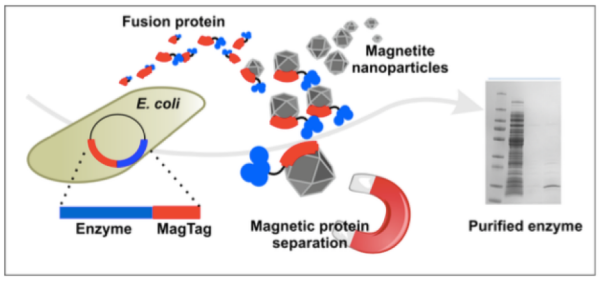
Mag-Tag: magnetite nanoparticle affinity tags for industrial biotechnology protein purification

"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**