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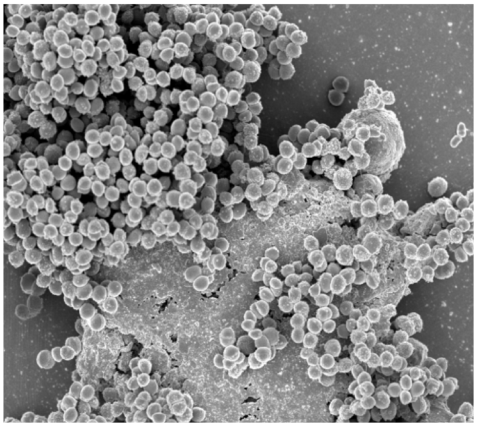
# Household biofilm chelation therapy

**"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**

# 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**

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Metals IN BIOLOGY

# 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**

UNIVERSITY OF BATH CRODA

**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

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

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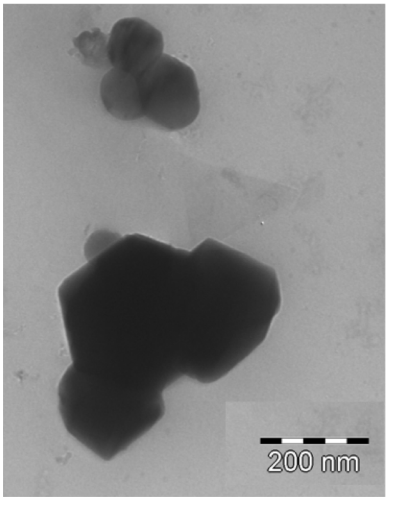
# Investigating the antimicrobial properties of copper-infused fabrics

*"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**