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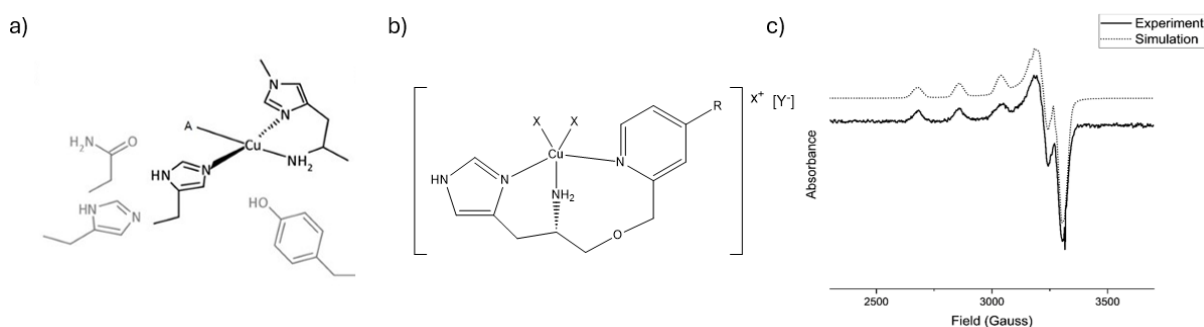
FRONTIERS

# Bio-inspired modelling of the copper-histidine brace active site of LPMOs using a novel N,N,N-ligand scaffold – towards artificial copper oxidases

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Lytic Polysaccharide Monooxygenases (LPMOs) are ubiquitous throughout nature<sup>1</sup> and are uniquely capable of breaking down biologically abundant, recalcitrant materials such as cellulose and chitin by C-H bond activation.<sup>2,3</sup> This chemistry occurs at the active site which contains a novel T-shaped 'histidine brace' motif (Figure 1a).<sup>4</sup> Previous work has focussed on elucidating the key structural features of the active site of the protein,<sup>3</sup> with particular focus on the subtle changes in the copper active site during the catalytic cycle, such as ligand exchange, copper oxidation state and the coordination geometry of the metal centre.<sup>5</sup> New protocols for the study and trapping of short-lived species have been developed utilizing EPR spectroscopy for the identification of the copper environment. Inspired by these findings, copper complexes (Figure 1b) of a novel tridentate N,N,N donor ligand have been developed which mimics the natural histidine brace found in LMPOs. Novel ligands (**L**<sub>1</sub>, where R is H and **L**<sub>2</sub>, where R incorporates a biotin moiety) and their corresponding copper complexes [Cu**L**<sub>x</sub>]**Y**<sub>2</sub> (where Y is Cl, NO<sub>3</sub>, BF<sub>4</sub>) have been synthesised and characterised (Figure 1b). EPR analysis of the complexes suggests a single species is present with spin Hamiltonian parameters consistent with a distorted square planar geometry with the unpaired electron in the d<sub>x<sup>2</sup>-y<sup>2</sup></sub> orbital.<sup>6</sup> Stopped flowed kinetic analysis of the system has been utilised to probe the redox processes taking place at the copper centre and to better understand the roles of oxidant and substrate in the catalytic cycle. These complexes offer further insight into the mode of action of LPMO proteins and their application in the production of bio-ethanol.



**Figure 1:** a) Representation of the active site of a typical LPMO (example from one of the AA9 class of the proteins),<sup>3</sup> showing the 'histidine brace' binding motif (where A is typically H<sub>2</sub>O or OH<sup>-</sup>); b) diagram of the model complexes produced in this research, c) EPR spectrum of the [Cu**L**<sub>2</sub>H<sub>2</sub>O]<sup>2+</sup> complex.

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# Multielectron-transfer Reactivity of End-on Dinitrogen Bridged Lanthanide Metallocenes

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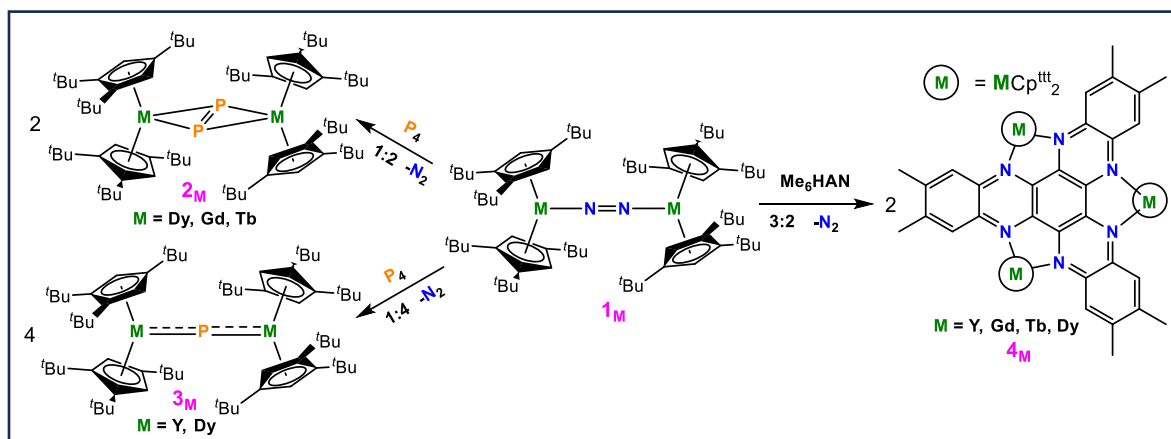
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In recent years, the divalent lanthanide metallocenes have attracted considerable attention due to their outstanding SMM behaviour, reactivity, and small-molecule activations.<sup>1,2</sup> The metallocenes of the ‘non-classical’ divalent lanthanides (Dy, Tb, Ho, Nd, Er) are of great interest here as they can also form radical-bridged complexes in reactions with N-heterocycles, leading to SMMs with strong magnetic hysteresis with coercivity, and high blocking temperatures ( $T_B$ ).<sup>3</sup> However, the use of non-classical divalent lanthanides for radical-bridged complexes, reactivity and small molecule activations is extremely rare as they are highly reactive and their isolation is also quite challenging.

Recently, we have reported that the end-on dinitrogen bridged lanthanide metallocene complexes (**1<sub>M</sub>**) behave as divalent synthons.<sup>4</sup> Herein, we have extended the chemistry of end-on dinitrogen bridged lanthanide metallocene towards the reactivity with small molecules and the making of rare trinuclear radical bridged complexes as shown in Scheme 1. The complex **2<sub>M</sub>** and **3<sub>M</sub>** represent the first example of side-on diphosphorus and phosphorus-bridged lanthanide metallocene respectively. Further, the magnetic study of **4<sub>Gd</sub>** reveals the presence of ferromagnetic exchange interactions for the first time in a radical bridged lanthanide metallocene complex. Magnetic measurements on **2<sub>M</sub>** are underway.



**Scheme 1.** Reactivity of end-on dinitrogen lanthanide metallocene with  $P_4$  and  $Me_6HAN$ .

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## Bio self-assembled molecular wires

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Miniaturization of wires has been one of the focal points of technological research. To develop a wire a metallic core is surrounded by a less conductive scaffold. Previous attempts have used carbon or DNA as an organic scaffold.<sup>1-2</sup> However, coiled coil peptides provide a promising avenue for nano-wire creation. Coiled coils are a self-assembling peptide structure made up of 2 or more  $\alpha$ -helices intertwined. Previous work has shown up to three metals binding within one coiled coil structure however more than this has never been demonstrated.<sup>3</sup> This project aims to explore multiple consecutive metal binding within a coiled coil. This will be done by combining computational methods to design peptide sequences, and experimental techniques to build and test each sequence (Figure). Once a sequence that can bind multiple metals successfully is developed, work into controlling the electron flow through modifying the metals and binding residues will be undertaken.

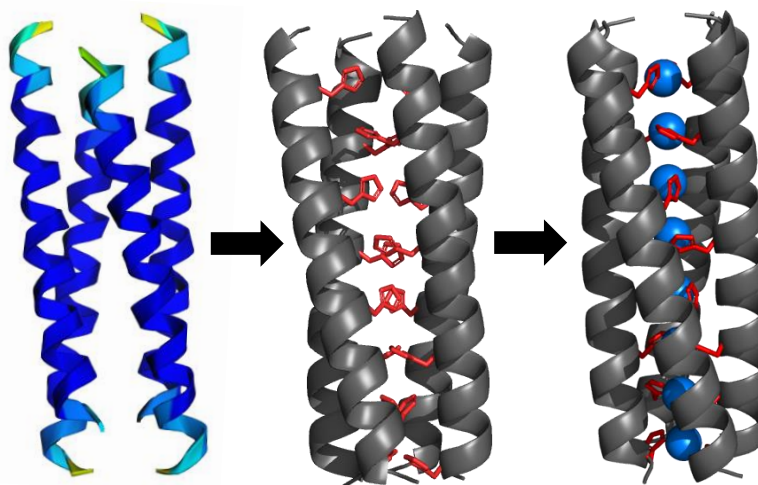


Figure. The design process of a de novo designed coiled coil. (Left) An AlphaFold predicted structure showing the sequence was predicted with high accuracy indicated by dark blue regions.<sup>4</sup> (Middle) The PyMOL model where the binding residues are highlighted in red. (Right) Dummy blue metals are placed into the hypothetical binding sites.

We thank the EPSRC and Dstl for an iCASE PhD studentship for A.L., and the Centre for Chemical and Materials Analysis in the School of Chemistry, for support of this research.

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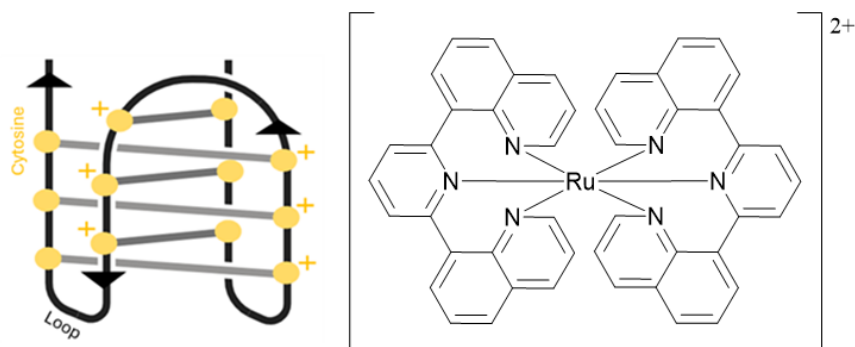
## Cis, fac-[Ru(bpq)<sub>2</sub>]<sup>2+</sup> derivatives for the switch on phosphorescent for the detection of i-Motif.

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i-Motifs are DNA secondary structures which are overrepresented in telomeric regions and the promoter regions of DNA. These form in cytosine-rich sequences in acidic or physiological conditions, forming four-stranded structures of intercalated hemi-protonated cytosine-cytosine base pairs.<sup>1</sup> The biological function of these i-motif structures is not currently properly well understood, with their existence only confirmed in 1993 and confirmed *in vivo* in 2018.<sup>2,3</sup> Therefore, the development of a luminescent probe will further aid in the understanding of its biological function.



(Left) Cartoon representation of i-Motif. (Right) Structure of [Ru(bpq)<sub>2</sub>]<sup>2+</sup>.

Previously, we showed that the *cis, fac* isomer of [Ru(bpq)<sub>2</sub>]<sup>2+</sup> (bpq = 2,6-bis-quinolinyl pyridine) binds iM more strongly than other DNA forms, and can detect its presence in mixtures of DNA through phosphorescence lifetime increase.<sup>4</sup> Here, we present work developing new *cis, fac*-[Ru(bqp)<sub>2</sub>]<sup>2+</sup> derivative as phosphorescent probes for i-motif detection using bqp analogues with adjusted  $\sigma$ -donor and  $\pi$ -acceptor properties. This aims to increase emission intensity and contrast and exploits a new synthetic method that strongly favours the formation of the desired facial isomers.

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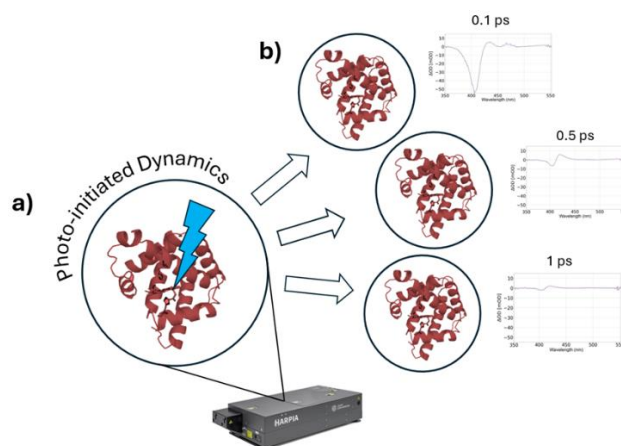


# Ultrafast Dynamics of Haem Enzymes

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Haem proteins serve diverse and crucial functions across a plethora of living organisms. The haem cofactor is central to the function of haem proteins, where the iron centre can access a range of oxidation states including high-valent Fe(IV) intermediates that allow the protein to perform powerful redox chemistry<sup>1,2</sup>. The specific redox properties are tuned by the surrounding protein matrix, which establishes a conduit for electron transfer (ET) and protein-coupled electron transfer (PCET) reactions between the active site and substrates<sup>3,4</sup>. Crucial to understanding differences in haem protein reactivity is the characterisation of transient intermediate states that form during the reaction on sub-ps timescales. Transient absorption spectroscopy (TA) and Femtosecond Stimulated Raman Spectroscopy (FSRS) are therefore ideally suited to provide new insight into the sequence of events that take place during haem protein reactions. Preliminary results from a series of TAS experiments probing the kinetics of excited state formation in a series of biologically relevant *Equus caballus* Myoglobin samples (Mb-Fe[III], Mb-Fe[II] and Mb-Fe[II]O<sub>2</sub>) clearly illustrate variation in the dynamics of excited state absorption (ESA) following excitation in the Soret and Q-bands. A typical scheme for an ultrafast (femtosecond) spectroscopy experiment to probe haem protein dynamics is outlined in **Figure 1**.



**Figure 1. General scheme for interrogating haem protein dynamics via Ultrafast (Femtosecond) Spectroscopy.** **a)** Haem protein dynamics are initiated by illuminating a protein sample with pulsed laser light tuned to the centre of an absorption feature from the samples static UV-VIS absorption spectrum. **b)** Dynamics are tracked spectroscopically by measuring successive absorption spectra at different time points following photo-excitation.

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# Investigating FGE-catalysed modifications of CAZymes; a new approach for monitoring protein-cellulose interactions

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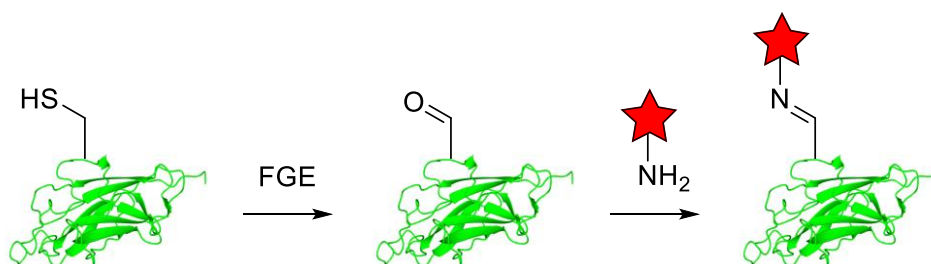
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Biofuels are a promising alternative to fossil fuels, due to the high abundance of lignocellulosic biomass. Many organisms are known to produce an array of carbohydrate active enzymes (CAZymes) which degrade polysaccharides, such as cellulose<sup>1</sup>. These enzymes are used in the industrial production of bioethanol. While their general mechanisms have been studied, little is known about their binding to extended cellulose surfaces. Expanding this knowledge and the understanding of synergism between classes of CAZymes may aid in their commercial exploitation.

Biophysical techniques, including NMR and X-ray crystallography, have been used to investigate these proteins, using short soluble oligosaccharides. However, these techniques cannot be applied to insoluble polysaccharides, which are often the natural substrates of these enzymes. Electron Paramagnetic Resonance (EPR) Spectroscopy has been used to gain dimensional information on lytic polysaccharide monooxygenase (LPMO) binding to cellulose fibers<sup>2</sup>. Unlike LPMOs, glycoside hydrolases and carbohydrate binding modules (CBMs) cannot naturally be studied using this technique, as they lack a paramagnetic species. Here we present methods to recombinantly modify a CBM using formylglycine generating enzyme (FGE), which inserts an aldehyde functionality (**Figure 1**). The aldehyde handle can be used to add a copper binding ligand, providing the paramagnetic species for EPR studies. Exploiting the binding of the protein to cellulose fibers, orientation-dependent spectra can provide information on interactions with polysaccharides, and how this differs between CAZymes.

Within this work, the reactivity of a synthetic probe is assessed. The methods for the mutation, expression and characterization of a mutant CBM containing the FGE recognition motif are presented.



**Figure 1.** An outline of the proposed method. A modified CBM including an LCTPSR FGE recognition motif is expressed with FGE, introducing an aldehyde functionality. The aldehyde is reacted with a hydrazone-functionalised EPR spin probe.

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# Synthetic magnesium tetrapyrrole radicals for mechanistic studies of photosystem II

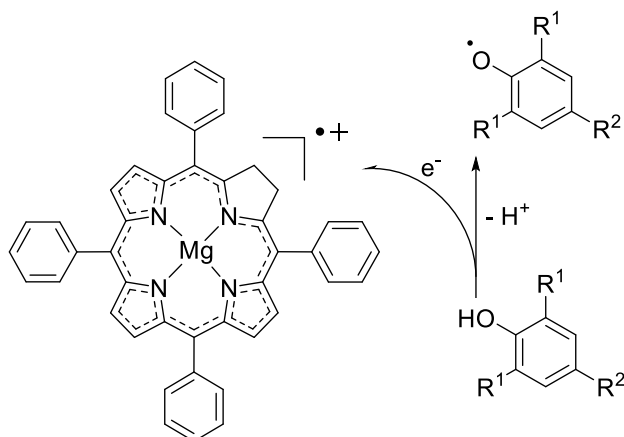
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Magnesium tetrapyrrole derivatives are fundamental to the reactivity of several photosynthetic pigments, most notably the chlorin complex chlorophyll-*a* in P680.<sup>1</sup> The P680 reaction centre consists primarily of 4 chlorophyll-*a* molecules. This gives an overall redox potential of 1.1-1.3 V (vs. SHE) whilst isolated chlorophyll-*a* *in vitro* has only shown potentials around 0.7-0.8 V.<sup>2</sup> Water oxidation reactions typically require extreme conditions and precious metal catalysts to work,<sup>3</sup> yet photosynthesis occurs under ambient conditions. Taking inspiration from biology, elucidating the conditions and the mechanisms which allow chlorophyll to generate such high redox potentials under mild conditions may lead to ground-breaking improvements in oxidation catalysis.

A series of magnesium porphyrin and chlorin surrogates for chlorophyll-*a* are synthesised. The oxidation of these to  $\pi$ -cation radicals is carried out via chemical and electrochemical methods. The radical species are then studied by EPR and UV-Vis spectroscopy to optimise the generation of these. After probing the  $\pi$ -cation radicals, their oxidation reactivity towards phenol substrates is investigated via time-resolved UV-Vis spectroscopy. Our promising initial results are presented here including the synthesis of previously uncharacterised  $\pi$ -cation radicals, optimisation of their synthesis and demonstration of their reactivity towards a range of substrates. Ultimately, we aim to develop our findings into a mechanistic understanding of the influence of the tetrapyrrole ligand on natural oxidation chemistry.



**Figure 1:** Target oxidation of a substituted phenol by a 1 electron oxidised magnesium tetraphenylchlorin  $\pi$ -cation radical.

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# The Synthesis of Gold Carbenes for Applications in Medicine

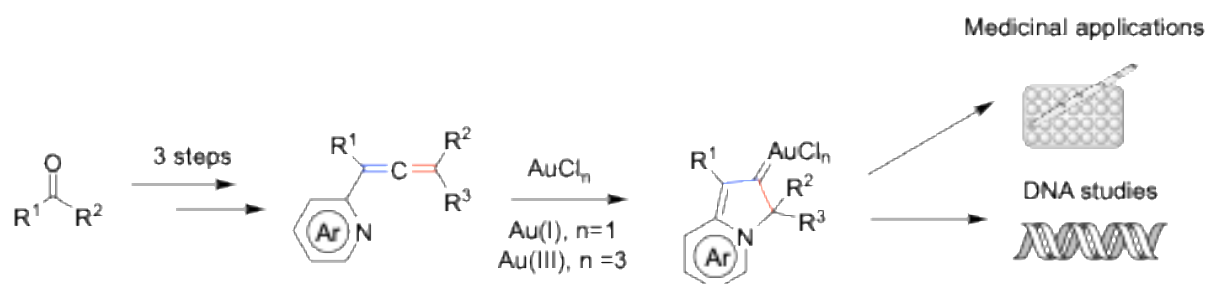
Imogen V. K. Turner,<sup>a</sup> María Paz Muñoz<sup>a</sup> and Zoë A. E. Waller<sup>b</sup>

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As the valuable properties of gold in biomedical applications are being unlocked<sup>1</sup>, promising results indicate that gold carbenes made from bis(pyridyl)allenes<sup>2</sup> have potential uses as anticancer, antifungal and antimicrobial agents.<sup>3-5</sup>



**Figure 1.** Summary of the synthesis of gold carbenes and the studies that will be performed.

In this poster, I will present the results that I have obtained so far during my PhD and future plans that I will develop during the project. The aim of this project is to synthesise and characterise novel  $\beta$ -N stabilised gold carbenes, made from allenes containing N-heterocycles (e.g. pyridyl) with different substituents and electronic properties. The potential medicinal properties of the gold complexes will be determined using antimicrobial and anticancer assays (through our collaborators), and their interactions with different DNA structures, such as i-motif secondary structures, will also be investigated. The substituents on the allene and the pyridine (or other N-heterocycles) will be varied to establish SAR (structure activity relationships). Information from these studies will allow the fine-tuning of the structure of the gold carbene to improve the effectiveness of the compounds and optimise their properties towards the desired applications.

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# Developing Lytic Polysaccharide Monooxygenase Inspired Artificial Metalloenzymes

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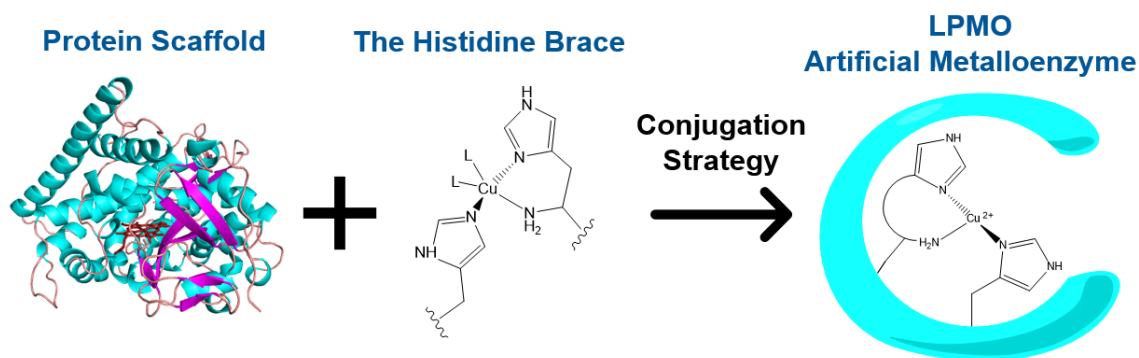
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The selective hydroxylation of activated and non-activated  $sp^3$  C-H bonds using molecular oxygen is a challenging yet highly desirable transformation in synthetic chemistry. Current methods often lack substrate scope and suffer from over-oxidation, whilst iron- and copper-dependent enzymes efficiently perform these hydroxylation reactions. Lytic polysaccharide monooxygenases (LPMOs) are one of such enzymatic families, able to selectively activate strong C-H bonds in polysaccharides (bond dissociation energy  $\approx$  100 kcal/mol). LPMOs are characterized by a conserved active site, where a copper ion is coordinated by two histidine residues, including the N-terminal amine, forming a distinctive “histidine brace” that is critical for their oxidative function.<sup>1</sup>

Despite displaying interesting chemistry, LPMOs are not well-suited for small molecule biocatalysis due to the positioning of their active site on the solvent-exposed surface, rather than within a pocket suitable for binding small molecules. To utilise the histidine brace’s oxidative power and create a tailored biocatalyst capable of carrying out selective oxidation on small molecules, we have designed a strategy to insert a histidine brace-like moiety into a binding pocket within a globular protein – to create an adaptable artificial metalloenzyme inspired by LPMOs.<sup>2-3</sup> In this work, we outline the modifications made to our chosen globular protein -the haem domain of a cytochrome P450 - to accommodate copper complexes, alongside detailing our conjugation strategies and activity assays of the ArM.



**Figure 1.** Strategy for LPMO inspired Artificial Metalloenzyme (ArM) Construction

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## Developing magnetic resonance imaging (MRI) contrast agents to report molecular biomarkers

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Pathological processes, such as chronic neuroinflammation, encompass a wide array of intricate molecular mechanisms. From aberrant cellular pathways to out of control immune responses, such interactions underlie numerous diseases.<sup>1,2</sup> Understanding these processes would enable early diagnosis, precise patient monitoring and improved treatment development. Despite its significance, current imaging approaches remain limited in their ability to track complex biochemical changes at the molecular level. Here, a crucial aspect is to be able to effectively detect molecular biomarkers in real time, *in vivo*.

This project aims to facilitate the multifactorial detection of molecular markers and enable the direct visualisation of pathological process by exploring the untapped potential of molecular MRI. Here, we report a library of 'direct detecting' class of PARASHIFT MRI contrast agent.<sup>3</sup> By harnessing the magnetic anisotropic properties of selected lanthanide and transition metals, this technique utilises paramagnetically shifted (PARASHIFT) proton resonances against a zero-noise background. These resonances are intrinsically temperature dependent, allowing simultaneous multiplex imaging of anatomy and temperature. Uniquely, it is feasible to report multiple biomarkers in a single acquisition with each PARASHIFT proton resonance as an independent image channel, revolutionising greyscale MR image into colour.

We synthesised and evaluated DOTA- and salen-based complexes, as well as multi-metal frameworks, to optimise the paramagnetic shift and relaxation properties of our reporter proton signal. Complexes were characterised by NMR spectroscopy, including temperature dependent NMR, measurement of longitudinal ( $T_1$ ) relaxation times and estimation of transverse ( $T_2$ ) relaxation. The ideal complexes provide sharp, highly shifted proton resonances (beyond +30 to -30 ppm) away from background biologically-relevant chemical shifts, while possessing fast longitudinal relaxation to improve acquisition sensitivity. Subsequent nanoparticle encapsulation of PARASHIFT contrast agent maximises localised concentration and theoretically improve sensitivity orders of magnitude for molecular imaging. Liposomes were chosen as the delivery approach due to their high versatility and biological compatibility. Properties including formulation, size and zeta potential were initially investigated. Complexes and systems with the best capabilities will provide the foundation for future *in vitro* and *in vivo* studies.

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## Novel MRI contrast agents for studying kidney pathophysiology

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Nephrons are the functional unit of the kidney, but current clinical quantitative methods mean that nephron number cannot be measured in an intact 3D kidney.<sup>1</sup> Magnetic resonance imaging is a non-invasive imaging technique with unlimited depth penetration that, particularly when combined with a contrast agent, allows high resolution visualisation of soft tissue.<sup>2</sup>

As opposed to gadolinium macrocycles – the gold standard for contrast agents – the salen ligand is a small, planar molecule whose properties can be functionalised in a variety of sites.<sup>3</sup> This functionalisation provides cell permeable MRI contrast agents that are able to permeate into cells for more detailed imaging of tissue structures.

This avenue of research has led to the production of a library of first row transition metal-based contrast agents that have been shown to affect  $T_1$  relaxation rate. We have further gone on to show that Mn-salen is an effective histological MRI tissue stain through soaking tissue in buffered solutions of complex and have confirmed distribution and complexation through atmospheric-pressure matrix assisted laser desorption ionisation (AP-MALDI) imaging.

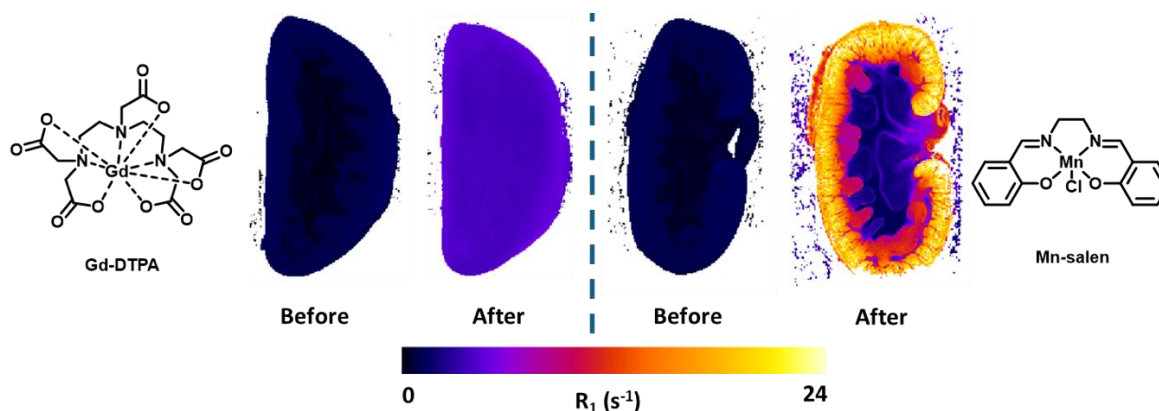


Figure 1 .  $R_1$  relaxation maps of ex vivo kidneys before and after soaking in contrast agent. Left: 2mM Gd-DTPA. Right: 4 mM Mn-salen.

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## Combined magnetic resonance imaging and mass spectrometry imaging workflow for visualising drug delivery

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Too many drugs fail clinical trials because we cannot measure whether the drug is delivered to the right tissue, and at the correct dose for the right duration. This project aims to address these challenges by developing an imaging workflow combining magnetic resonance imaging (MRI) for *in vivo* whole organ imaging and mass spectrometry imaging (MSI) for *in vitro* and *ex vivo* imaging of cell models and tissue sections. This approach will enable unbiased elucidation of drug distribution and endogenous metabolites in patient derived glioblastoma (GBM) samples.

We synthesised MR contrast agent-tagged compounds that exhibit theranostic properties. Toxicity was assessed via assays in patient derived GBM cell lines and retention of therapeutic effect confirmed. MRI experiments measured the relaxivity of the contrast agent-tagged chemotherapeutics, with similar values to the clinical standard (ProHance™). Initial mass spectrometry experiments show our theranostics can be ionised via atmospheric-pressure matrix-assisted laser desorption/ionisation (AP-MALDI) mass spectrometry. 3D spheroids have also been generated to better represent a tumour model for analysis by confocal microscopy, MRI and mass spectrometry following incubation with the complexes and free drug. A medium (1% agarose or 1% w/v alginate hydrogel) for spheroid suspension was optimised, and samples visualised via  $T_1$ -weighted MRI scans. Spheroids were analysed by AP-MALDI to determine drug delivery to the spheroid, and whether they remained conjugated.

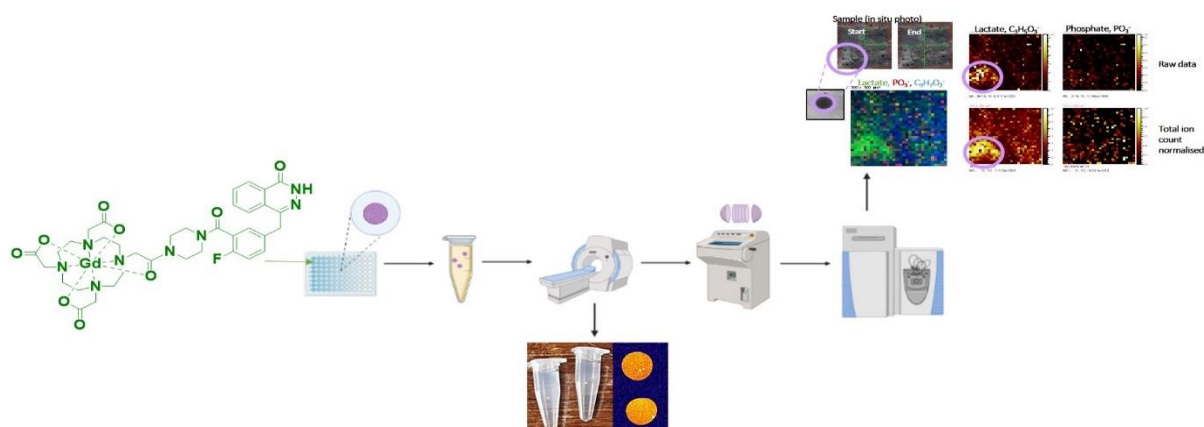


Figure 1. Visual representation of proposed workflow combining MRI and mass spectrometry imaging.



## High-Valent Iron Halides for Hydrocarbon Oxidation

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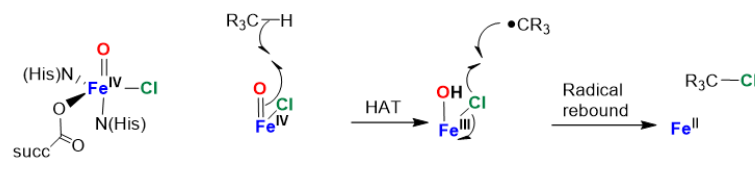
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Mild oxidative functionalisation of saturated hydrocarbons has remained a challenge in the chemical industry for many years because of their inherently strong C–H bonds.<sup>1</sup> Therefore, mild and selective methods to convert hydrocarbons into more useful, functionalised products such as halogenated organic products are highly sought after. Unfortunately, current halogenation techniques involve the use of harsh reaction conditions and the use of toxic reagents such as hydrohalic acid.<sup>2</sup>

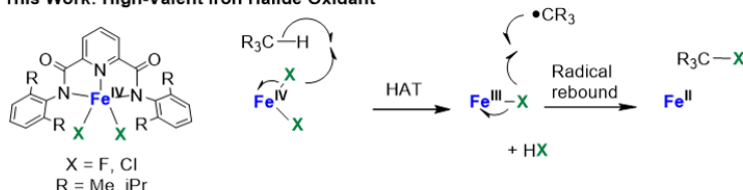
Inspiration for the functionalisation of inert hydrocarbons can be taken from naturally occurring metalloenzymes. Biological non-heme iron halogenase enzymes perform selective chlorination of hydrocarbons via a high-valent Fe<sup>IV</sup>-oxo reactive intermediate.<sup>3</sup> Efforts have been made to synthesise catalysts that mimic the reactivity of halogenases, with a high-valent metal-oxo moiety as the active oxidant.<sup>4</sup> Unfortunately, these systems are often unable to activate inert hydrocarbons and have poor reaction rates due to the properties of the metal-oxo active species.<sup>5</sup>

We have synthesised three novel Fe<sup>III</sup> bis-halide complexes and aim to chemically oxidise these complexes to their analogous high-valent Fe<sup>IV</sup> bis-halide derivatives. It is anticipated that these high valent complexes will react with hydrocarbon molecules, with a halide ligand performing both the hydrogen atom transfer (HAT) and radical rebound steps. Finally, we will investigate the reactivity of the high valent complexes towards hydrocarbon substrates with a variety of C–H bond strengths.

### Inspiration from Biology: Non-Heme Halogenase



### This Work: High-Valent Iron Halide Oxidant



Graphical representation of the difference in mechanism of HAT mediated halogenation of hydrocarbons in biology (halogenases) and biomimetic complexes (high valent iron bis-halides).

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## Enhancing the Performance of Metallo Coiled Coils for use as MRI Contrast Agents

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Magnetic resonance imaging (MRI) is a vital diagnostic technique, with contrast agents being used in over 30% of scans to enhance the contrast between different tissue types. Current contrast agents are based around Gd(III) macrocycles (such as Dotarem®). However, ongoing research has shown that metallated peptides, known as coiled coils (Figure 1a), act as more effective contrast agents compared to those current in clinical use, showing high relaxivities for both Gd(III) and Cu(II), (Figure 1b and 1c).<sup>1–3</sup> This is in part due to enhanced rotational correlation time, an important parameter which determines the effectiveness of MRI contrast agents.<sup>4</sup> These systems hold potential for heightened imaging sensitivity, reduced image acquisition time and reduced contrast agent dose. The potential increase in image sensitivity, could enable the detection of potential diseases which otherwise may have gone unnoticed, potentially providing early diagnosis and less time and money intensive treatment options. This poster will explore how structural modifications of these metallopeptides influence their binding affinities and overall MRI performance.

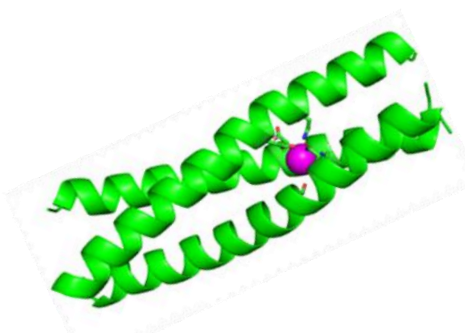


Fig. 1. Show a cartoon representation of a metalated coiled coil

We would like to thank the School of Physics and Astronomy and the CDT in topological design (EP/S02297X/1) for the Ph.D. studentship for K.A.H, and the Centre for Chemical and Materials Analysis in the School of Chemistry for support of this research.

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## Mapping Cisplatin Resistance: Investigating Metal Distribution in Ovarian Cancer Cells

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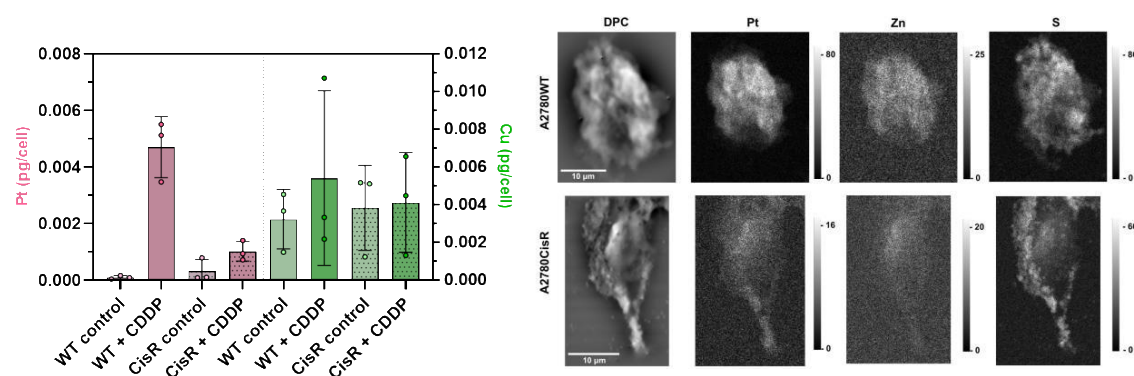
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Platinum-based drugs like cisplatin (CDDP) are standard treatments for ovarian cancer, but rising resistance presents a significant clinical challenge.<sup>1</sup> Recent studies in human biopsies show a correlation between resistance to platinum drugs and reduced platinum accumulation in ovarian cancer tissue. Correlation between Pt accumulation and endogenous copper trafficking has also been described *in vitro*.<sup>2,3</sup> This research aims to investigate whether CDDP resistance in ovarian cancer is associated with differences in platinum and copper accumulation using A2780WT (WT) and cisplatin-resistant A2780CisR (CisR) cell lines.

Cells were treated with 100  $\mu$ M CDDP prior to analysis. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was applied to measure bulk intracellular Pt and Cu concentrations (Fig. 1A). Nanofocussed X-ray Fluorescence (XRF) was used to analyse elemental distribution of Pt and correlate it with other biologically relevant elements. The I14 hard X-ray nanoprobe beamline facility at Diamond Light Source Synchrotron allowed for 2D elemental mapping at a 100 nm spatial resolution (Fig. 1B).

ICP-MS confirmed lower Pt accumulation in CisR cells, while basal Cu remained unchanged. XRF detected significant Pt distribution differences: in WT, Pt colocalized with nuclear Zn, whereas in CisR, Pt was more widely dispersed, suggesting altered drug trafficking. These findings confirm that reduced CDDP accumulation is a marker of resistance in this model. Future work will explore radioactive copper uptake to quantify Cu accumulation and its response to CDDP treatment.



**Figure 1. (A)** ICP-MS of WT and CisR cells untreated or treated with 100  $\mu$ M CDDP (Pt and Cu concentrations in pink and green respectively). **(B)** XRF analysis of WT and CisR cells treated with 100  $\mu$ M CDDP for 24 h -Differential Phase Contrast imaging (DPC), Pt, Zn, and S intensities (cps).

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# Reactions of Cobaloximes with Ni complexes relevant to Acetyl Coenzyme A Synthase

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Acetyl Coenzyme A Synthase (ACS) is a Ni-containing metalloenzyme responsible for the reversible synthesis of acetyl coenzyme A from coenzyme A, carbon monoxide and a cobalt-bound methyl group.<sup>1</sup> A mechanistic understanding of acetyl coenzyme A production is crucial given its ubiquity in cell metabolism,<sup>2</sup> and holds potential for more efficient and sustainable production of thioesters from CO.<sup>3</sup>

Studies of the native enzyme have been hampered by its air-sensitive nature and the convoluted spectroscopic data that arises from the presence of several metal centers in variable oxidation states.<sup>1,4</sup> As a result, molecular complexes are employed to replicate proposed intermediates of the A-cluster (the proposed substrate binding site of ACS). Recently published work on a Ni-Ni complex ([1], **Fig. 1B**) reminiscent of the A-cluster has successfully demonstrated thioester formation in the presence of CO, PhSNa and CH<sub>3</sub>I.<sup>4</sup> However, the product is obtained in low yield due to a proposed inefficient radical-based mechanism.<sup>4</sup>

As a result, we have been investigating an alternative methyl cation source. Cobaloxime compounds (**Fig. 1A**) have been extensively studied as models for coenzyme B<sub>12</sub> which is a crucial cofactor which serves as the methyl cation source in ACS.<sup>5</sup> These compounds have already demonstrated a capacity to act as methyl cation sources in similar systems<sup>6</sup> and we seek to investigate their reactivity with complex [1]. The synthesis and characterisation of various cobaloximes, as well as their reactivity with relevant Ni-containing compounds will be reported.

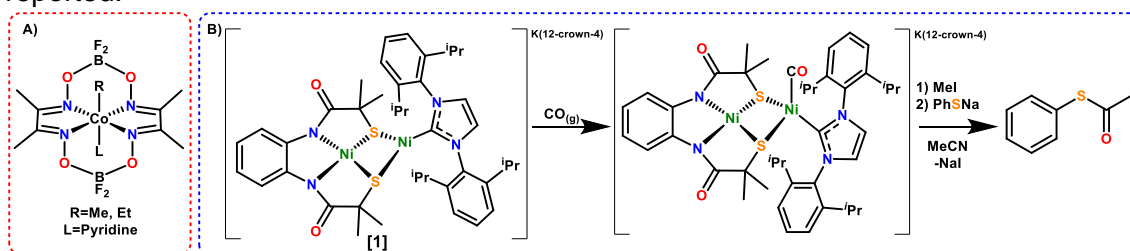


Figure 1 – A) the general structure of common alkyl cobaloximes B) A Ni-Ni bimetallic complex that will be further investigated in this work

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## Investigating the Function of the Bridgehead Group in the Active Site of [FeFe] Hydrogenase Enzymes

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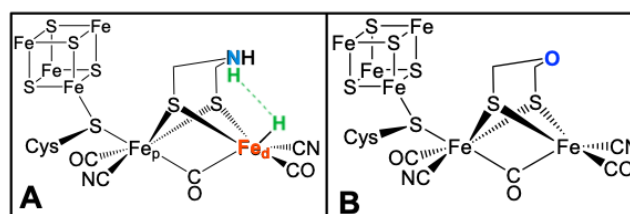
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The need for efficient catalysts in energy-converting reactions is becoming increasingly critical in light of the climate crisis. Hydrogen (H<sub>2</sub>) is proposed as a clean energy vector capable of storing renewable energy; however, there are currently no sustainable catalysts available for efficient H<sub>2</sub> production.<sup>1,2</sup> [FeFe] hydrogenase enzymes offer a potential solution, exclusively utilising an abundant metal in their active sites and exhibiting exceptionally high catalytic rates at thermodynamic equilibrium.<sup>3,4</sup> Understanding their mechanism may provide us with powerful design criteria for developing new, efficient, and affordable catalysts that enable renewable energy storage in the form of H<sub>2</sub>. The crucial mechanistic steps in the catalytic cycle of [FeFe] hydrogenases involve a Frustrated Lewis Pair (FLP) consisting of the -NH in the azadithiolate bridgehead and the Fe<sub>d</sub> centre, which polarises the H-H bond, facilitating heterolytic splitting of the H<sub>2</sub> molecule (Fig. A). Understanding the mechanism of the bridging ligand should illuminate the interactions essential for efficient catalysis and provide insights into the tunability of the enzyme, which will be important for optimisation in industry.

In this work, a combination of spectroscopic, electrochemical and structural techniques have been used to characterise an active-site variant of the [FeFe] hydrogenase containing an oxadithiolate (ODT) bridgehead, in which the -NH is replaced with an O (Fig. B). Our spectroscopic data suggest that the ODT variant exists in novel active-site states, which have unique structures as observed by X-ray crystallography as well as enhanced properties as observed by electrochemistry and spectroscopy. The structural changes to a nearby cysteine residue observed in the ODT variant underpin a potential mechanism for engineering artificial metalloenzymes with enhanced oxygen tolerance: a property of the native-like enzymes that greatly hinders industrial applicability.



A) Structure of the [FeFe] hydrogenase active site, highlighting the FLP mechanism. B) Structure of the modified active site in which the azadithiolate ligand has been substituted with an oxadithiolate (ODT).

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## Size-Controlled Synthesis of Monodisperse Manganese Oxide Nanoparticles

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There has been much attention in the recent years surrounding iron oxide nanoparticles (ION) in the biomedical imaging field<sup>1</sup>. Advances in the synthesis of uniform ION have led to developments in contrast agents for medical imaging.<sup>1</sup> However, ION contrast agents are limited due to their intrinsic dark signals observed in magnetic resonance imaging (MRI), making it difficult to distinguish between different tissues.<sup>2</sup> Manganese oxide nanoparticles (MON) are a promising alternative as they produce bright signals,<sup>2</sup> offering better contrast over ION.

Current methods for synthesising size-controlled, monodisperse MON involve harsh conditions, such as high temperatures.<sup>3,4</sup> We have adapted a method involving milder conditions to synthesis reproducible, monodisperse MON where nanoparticle size can be tailored. MON sizes and distributions were confirmed using transmission electron microscopy (TEM), dynamic light scattering (DLS) and energy dispersive x-ray spectroscopy (EDX). Future work would look to investigate the application of this synthesis to other transition metals in the hope to develop novel contrast agents for a range of medical imaging techniques.

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# Computational Discovery of Metallodrugs: Machine Learning Classifiers for Antimicrobial Activity Predictions

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In the last century, small organic molecules have dominated pharmaceutical research, sidelining metal-containing medicines (metallodrugs). Recent challenges to assumptions about metal toxicity have spurred renewed interest in metallodrug development, while diminishing returns in small molecule therapeutics have driven the exploration of 3D metal-based scaffolds.

Metal complexes offer unique properties, such as tuneable electronic behaviour and intricate 3D structures, enabling novel modes of drug action. However, these same properties complicate rational design, necessitating the development of computational tools to streamline discovery.

This work investigates the structural and electronic factors influencing bioactivity in transition metal-based antimicrobial agents. A library of 300 transition metal complexes from the Community for Open Antimicrobial Drug Discovery (CO-ADD)<sup>1,2</sup> was curated and analysed using density functional theory (DFT) methods to calculate geometric and electronic properties. Chemical descriptors were generated using revised autocorrelation functions (RACs) developed by MIT's Kulik group.<sup>3</sup> Machine learning classification models were trained to predict antimicrobial activity and toxicity against Gram-positive and Gram-negative bacteria, fungi, and human cells. Antifungal models were particularly promising, achieving prediction accuracy of up to 95% on the test set, with a ROC AUC value of 0.98.

These models were then applied to the tmQM dataset of 86,000 DFT modelled transition metal complexes, identifying promising antimicrobial agents for future synthesis and biological testing.<sup>4</sup>

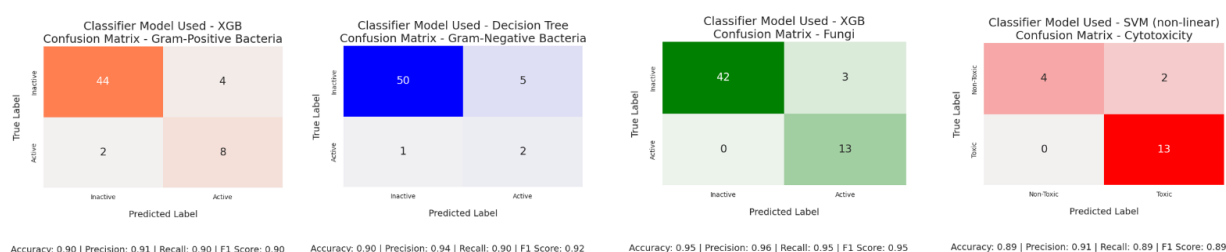


Figure 1 - Confusion matrices of the top-performing classification models for predicting metal complex bioactivity against different microorganism types (80:20 train:test split used)

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## Bismuth based antimicrobial agents to combat antibiotic resistance

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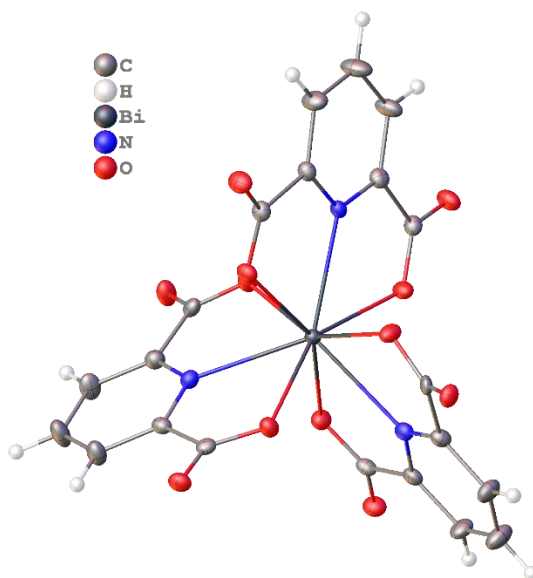
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There is an urgent need for the development of new metallo beta-lactamase (MBL) inhibitors. Beta-lactamase enzymes are a key mechanism of bacterial resistance against the widely used beta-lactam class of antibiotics. While serine beta-lactamase inhibitors such as clavulanic acid and sulbactam are available in the clinic, MBLs currently have no commercially available inhibitors.<sup>1</sup>

Bismuth is a generally non-toxic metal that may hold potential for developing MBL inhibitors. There is evidence that bismuth can displace zinc in the MBL active site, leaving it unable to hydrolyze beta lactam substrates.<sup>2</sup> However, bismuth compounds often struggle with poor solubility and/or bioavailability.

We have synthesized and characterized a library of ionic Bi(iii) complexes, using dipicolinic acid (DPA) and substituted DPA ligands via simple one-pot syntheses. These complexes have diverse solid state structures, determined using single crystal X-ray diffraction. They are water soluble, with some showing significantly greater MBL inhibition ability than colloidal bismuth subcitrate (CBS), an approved drug.



Crystal structure of one of the synthesized complexes

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## Design of bioactive transition metal prodrugs: a pair of isoelectronic Ru(II) and Rh(III) complexes

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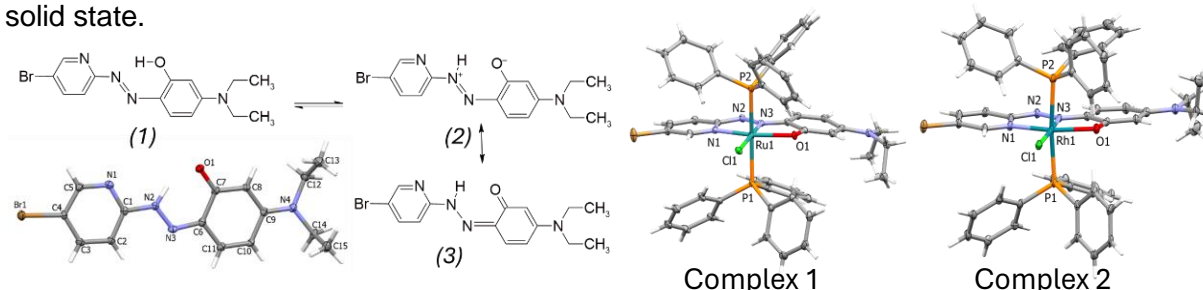
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Transition metal coordination complexes hold promise as novel drugs with new mechanisms of action that can combat resistance to existing drugs.<sup>1</sup> However they are often prodrugs which undergo substitution and/or redox reactions before they reach target sites and hence become multitargeting which presents challenges for understanding their mechanisms of action. We are focusing on the design of relatively inert metal complexes for which the activation mechanisms can be controlled in biological media such as cancer cell organelles.

Here, we report the synthesis and characterisation of isoelectronic 4d<sup>6</sup> octahedral Ru(II) and Rh(III) complexes, for which inertness is assisted by their low-spin configurations and by the strong  $\pi$ -acceptor properties of a tridentate azopyridine ligand. Features of the design will allow us to track the reactivity of these complexes in their transport to target sites in cells using e.g. ICP and X-ray fluorescence methods.<sup>2</sup> These include detection of the metal itself and the azopyridine via its Br label.

We have synthesized novel phosphinic Ru(II) and Rh(III) complexes with a 2-(5-bromo-2-pyridylazo)-5-(diethylamino)phenol (Br-Padap) ligand. The Br-Padap ligand can have tautomeric equilibria (1-2), as well as electron delocalization among the N2-N3-C6-C7-O1 atoms (2-3) (Figure), highlighting its structural versatility. In the solution, an N-H <sup>1</sup>H-NMR peak at 16 ppm suggests nitrogen protonation of the free ligand (2-3). This was confirmed by single-crystal X-ray diffraction. The bond distances suggest form 3 is preferred for Br-Padap in the solid state.



After the coordination of Br-Padap to Ru(II) and Rh(III), two new complexes **1** (82%) and **2** (79 %) were obtained with good yields, both exhibiting the same stereochemistry. In the equatorial position, each complex contains one chlorido and one Br-Padap negatively charged ligand. Also, two triphenylphosphine ligands (PPh<sub>3</sub>) in a *trans* configuration complete the distorted octahedral geometry, forming the complexes [Ru(Br-Padap)Cl(PPh<sub>3</sub>)<sub>2</sub>] (**1**) and [Rh(Br-Padap)Cl(PPh<sub>3</sub>)<sub>2</sub>]PF<sub>6</sub> (**2**). The Ru(II) compound is neutral, whereas the Rh(III) complex is positively charged. We are investigating their stability in solutions of biological relevance e.g. in the presence of the abundant intracellular thiol glutathione which might attack both the metal and the azo bond. The complexes exhibit interesting photochemistry, including intense MLCT transitions ( $\lambda_{\text{max}}$  553 and 590 nm for **1** and **2**, respectively).

**Acknowledgements:** We thank the Brazilian CNPq agency, EPSRC and Heraeus for their support for this work, and Warwick technical services and RTPs for their excellent assistance.

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## Ferrocenyl $\beta$ -Diketonate Compounds: A Versatile Platform for Increased Anti-Cancer Activity

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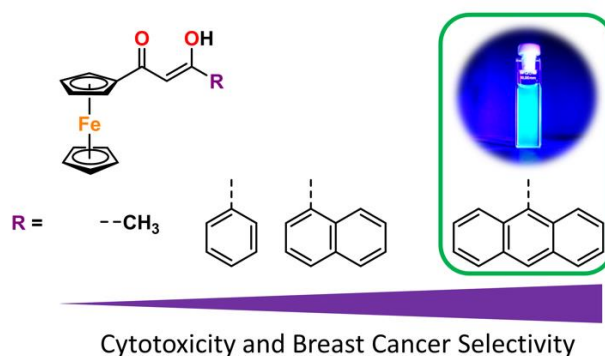
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Functionalising anti-cancer drugs with ferrocenyl moieties has proven to be an effective strategy to increase their activity and overcome drug resistance by dual mode activity.<sup>1</sup> Functionalising existing anti-cancer drugs can be challenging and results often in elaborate multi-step synthetic routes. A versatile alternative is the synthesis of ferrocenyl functionalised chelators to incorporate them in metallodrugs. For example,  $\beta$ -diketonates are easily functionalised with ferrocenyl moieties and yield highly active anti-cancer drugs by combining them with e.g. Ru(II) bipyridines or arenes,<sup>2, 3</sup> however, a systematic study of the ligands was missing to identify the most active motifs for future drug design.



**Figure:** General structure of the presented ferrocenyl  $\beta$ -diketonate derivatives, highlighting the fluorescence of the decomposed anthracenyl derivative.

To fill this gap, a series of aryl and benzyl functionalised ferrocenyl  $\beta$ -diketonates is presented.<sup>4</sup> The compounds were fully characterised and their cytotoxicity is presented against multiple cell lines. The compounds target the cells in a dual mode activity and form reactive oxygen species. Interestingly, the anthracenyl derivative forms a fluorescent decomposition product which is used to determine its intracellular distribution by confocal microscopy.

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# In a Flash: Carboxylate-Triazabutadienes For Light-Triggered Protein-to-Surface Bioconjugation

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Aryl diazoniums are well known for their ability to electrograft onto a wide variety of conducting and semi-conducting electrode surfaces to form a surface-carbon bond which has a large oxidation/reduction window and high stability.<sup>1</sup> However, due to their high reactivity and low stability, it is difficult to manipulate the properties of diazoniums for different applications, such as use with biomolecules. Triazabutadienes (TABDs) are promising targets for attachment of biomolecules to electrode surfaces due to their ability to release aryl diazoniums under mild conditions such as UV irradiation.<sup>2</sup> High water solubility has been achieved by inclusion of charged groups such as sulfonates into the TABD scaffold, increasing biocompatibility of bioconjugation reactions.<sup>3</sup>

The poster describes the variety of carboxylate-containing TABD scaffolds that have been synthesised to give access to several different bioconjugation strategies, including Cu-CAAC chemistry and biotin-streptavidin binding. The compatibility of these carboxylate-TABDs towards electrografting of redox active biomolecules onto electrode surfaces will be demonstrated. It is hoped that these carboxylate-TABDs will be a valuable tool for enzyme film electrochemistry and developing immobilised protein arrays for flow-catalysis, biosensing and spectroscopy.

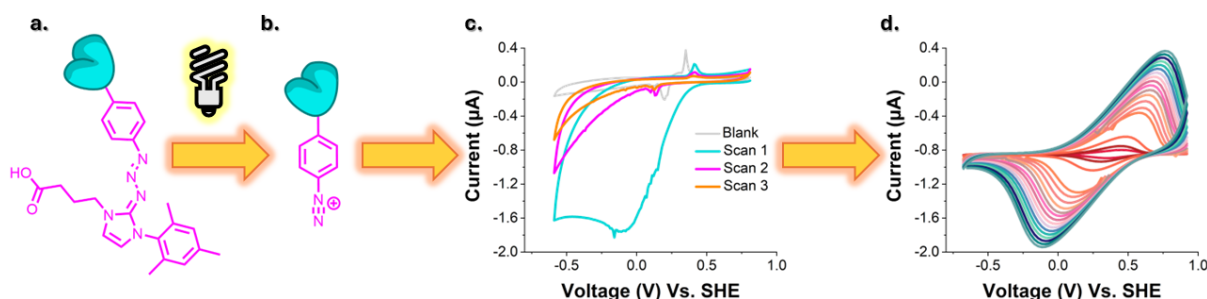


Figure 1. Illustrative representation of the light-triggered conversion of a bioconjugated carboxylate TABD to an aryl diazonium (a to b) which can undergo electrografting tracked by cyclic voltammetry (c), to enable electrochemical interrogation of the immobilized molecule (d).

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## New synthetic methods for key precursors of dimethylsulfoxide ruthenium drugs

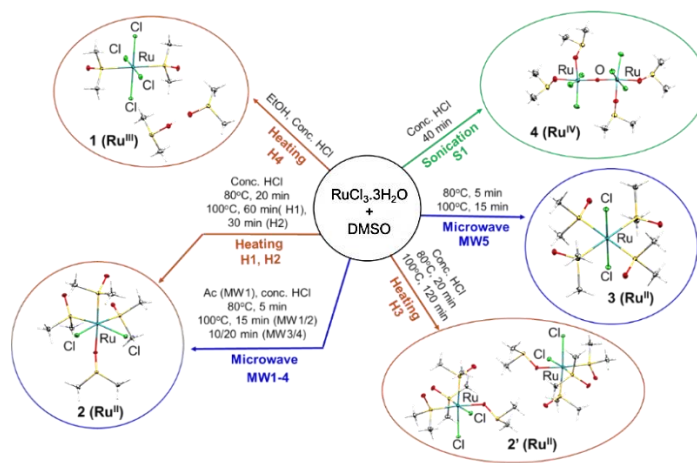
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Ruthenium-dimethylsulfoxide (DMSO) compounds are important precursors for synthesis of various useful materials with biological applications.<sup>1</sup> The octahedral complex Ru(III) complex [ImH][*trans*-RuCl<sub>4</sub>(DMSO)(Im)], where Im = imidazole, for instance has been on clinical trials as an anticancer agent.<sup>2</sup> However, efficient routes to their synthesis, commonly from RuCl<sub>3</sub>·3H<sub>2</sub>O, are challenging due to the need to control the extent and geometry of DMSO substitutions, *S* versus *O* DMSO coordination, and the Ru oxidation state (Ru(II/III/IV)). In this work we have compared conventional hotplate/stirrer methods with microwave- and sonication-assisted syntheses. The products [(DMSO)<sub>2</sub>H][*trans*-Ru(III)Cl<sub>4</sub>(DMSO-*S*)<sub>2</sub>] (**1**), *cis*-[Ru(II)Cl<sub>2</sub>(DMSO-*S*)<sub>3</sub>(DMSO-*O*)] (**2**), *trans*-[Ru(II)Cl<sub>2</sub>(DMSO-*S*)<sub>4</sub>] (**3**), and [(*cis*-Ru(IV)Cl<sub>3</sub>(DMSO-*O*)<sub>2</sub>)<sub>2</sub>(μ-O)] (**4**) were characterised by single crystal and powder x-ray diffraction, as well as UV-vis and NMR spectroscopy, MS, and cyclic voltammetry. For complexes **2** and **3**, microwave synthesis offers shorter times and higher yields. Additionally, complex **4** is the first example of an oxo-bridged binuclear Ru(IV) complex synthesized via sonication and its reaction with ferrocene as a reducing agent has been investigated. This study is a step towards optimising the synthesis of commonly used intermediates in metallodrug synthesis and diversifying alternative synthetic routes in coordination chemistry.



**Figure.** Conditions in conventional hotplate/stirrer (brown), microwave- (blue) and sonication-assisted (green) synthetic methods lead to different products from reactions of RuCl<sub>3</sub>·3H<sub>2</sub>O with DMSO.

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## RuCYP: Developing a functional ruthenium cytochrome P450

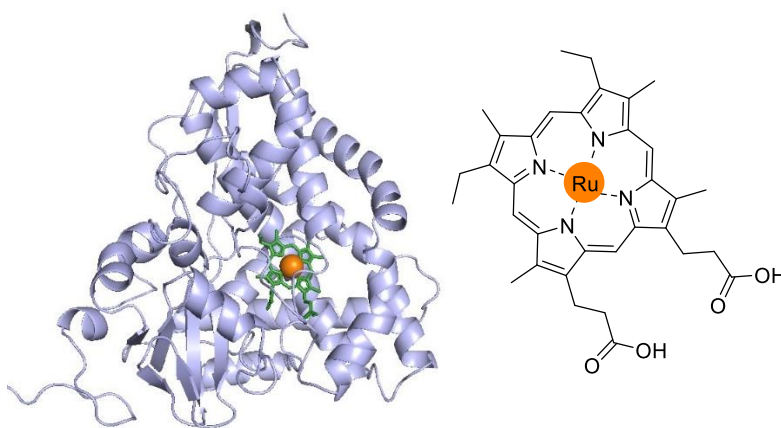
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Metal substitution in metalloenzymes provides a valuable approach to understanding their structure and reaction mechanisms. Replacing the native metal centre can alter the identity and lifetime of specific intermediates, offering deeper insights into the catalytic mechanism of the parent enzyme. Furthermore, the substitution of the metal centre may facilitate the discovery of novel chemistry or enhance the performance for biocatalytic applications.<sup>1</sup> Cytochromes P450 (CYPs) are Fe-centred hemoproteins found widely across Nature and biotechnology with the ability to perform selective C-H hydroxylation reactions on a broad range of compounds. The objective of our research is the preparation of CYPs, where the native heme cofactor is substituted by a precious metal analogue (such as ruthenium mesoporphyrin IX, RuMPIX), but still maintaining functionality (Figure 1).<sup>2</sup>

To achieve a RuCYP it was first necessary to express the parent protein lacking the native Fe-protoporphyrin IX (FePPIX) heme cofactor (*apo*-form) followed by incorporation of the desired metal complex (*holo*-form). To obtain the *apo*-CYP without FePPIX incorporation, it was necessary to carry out the expression in minimal media that has been rigorously deferrated. Concomitantly, RuMPIX-CO cofactor was synthesized and characterized, and strategies for decarbonylation were studied.<sup>3</sup> Different reconstitution methods to incorporate the RuMPIX into the *apo*-enzyme were also explored. The CYPs from *Bacillus Megaterium* (BM3), *Pseudomonas Putida* (P450cam) and *Thermoactinomyces daqus* (CYP119-TD) all showed the successful incorporation of RuMPIX, and their functionality as oxidative enzymes is currently being explored.



**Figure 1.** Structure of the heme domain of P450-AX (from *Meinhardsimonia xiamenensis*) with the native FePPIX cofactor incorporated (left). The targeted RuMPIX cofactor is also shown (right).

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