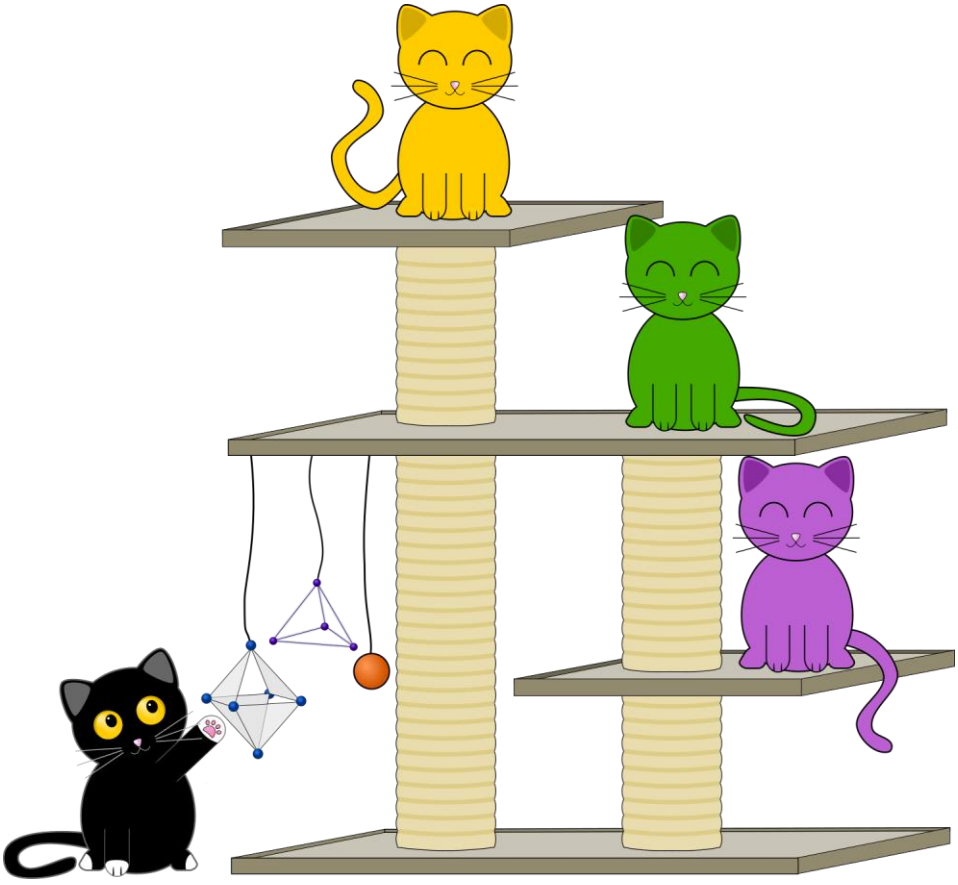
Summary



Transition metal catalysis is an invaluable tool to access a vast range of transformations which are otherwise difficult to achieve. While it is integral to synthetic chemistry in the lab, its power to control reactivity within living cells has only recently been discovered. Although nature has evolved enzymes which are able to carry out reactions extremely efficiently, their high levels of selectivity come at the cost of a limited substrate scope, and a limited range of transformations. Therefore transition metal catalysts are becoming an increasing popular method to accomplish new-to-nature reactions within living cells. Having access to a wide scope of efficient transition metal catalysed bioorthogonal reactions provides the opportunity to further develop new methods for biomolecular labelling and imaging, and new mechanisms for prodrug activation. This has the potential to further advance our understanding of biological systems and reduce unwanted side effects during drug treatments by improving the specificity and targeting with controlled metal-mediated activation. However, the environment of the cell is very different to the conditions which are typically required for transition metal catalysts to function, meaning that achieving *in vivo* transition metal catalysis is not a straightforward task.

In **Chapter 1** we explore the progress made in the field of *in vivo* transition metal catalysis over the last 20 years. A large range of catalytic systems have been applied in cells, covering a range of reactions and catalysts. The field is dominated by relatively simple deprotection reactions, where a small terminal group is cleaved to generate an active fluorophore or drug, but more complicated reactions involving bond-forming transformations and even cross-couplings have also been demonstrated. One common theme among all of these examples is poisoning of the catalyst by the biological components – high concentrations of metal-binding molecules in cells, in particular thiols, results in often irreversible binding of biomolecules to the metal centre, which deactivates the catalyst. This means that low yields and low catalytic efficiencies are found throughout the field. New strategies need to be developed in order to protect the metal catalysts from poisoning and improve their activity in cells and we propose that encapsulation of the catalyst within a supramolecular cage can fulfil this need. There are several examples of supramolecular cages which impart size selectivity upon the encapsulated catalyst, where small substrates can enter the cage and react with the catalyst, but large substrates are blocked by the windows of the cage. In addition, in many cases encapsulation improves the reactivity of the catalyst, with higher yields or selectivities being achieved as a result of pre-organising the substrate and catalyst, increasing the local concentration of the catalyst, and stabilising reactive catalytic intermediates. We therefore envision that by encapsulating the metal catalyst within a supramolecular cage, the catalyst may be protected from poisoning by blocking large biomolecules from reaching the metal centre, and in addition the overall performance of the catalyst may be improved due to confinement effects.

This hypothesis was validated in **Chapter 2**, were the effect of encapsulation on a gold catalyst under biological conditions was investigated. A small gold complex, Me_3PAuCl, which catalyses an intramolecular hydroarylation reaction to form a fluorescent dye, was encapsulated within a small anionic supramolecular cage, which has previously been demonstrated to undergo size selective reactions. It was demonstrated that the encapsulated gold complex was able to produce the dye in higher yields than the free complex under aqueous aerobic conditions and in the presence of various biological additives (Figure 1). The

cytotoxicity of the reaction components was determined, and it was found that the substrate for the reaction was highly cytotoxic. This means that low concentrations (1 μM) of substrate would be required to carry out the reaction inside living cells; however, catalysis carried out at this concentration in cell culture media was completely inhibited. In addition, cell permeability studies using confocal microscopy revealed that the cage itself was unable to cross the cell membrane. Although this catalytic system was unsuitable for application in living cells, the protective effect of the supramolecular cage was indeed demonstrated for reactions carried out *ex vivo*. Therefore encapsulation of a catalyst represents a viable strategy for improving catalysis under biological conditions.

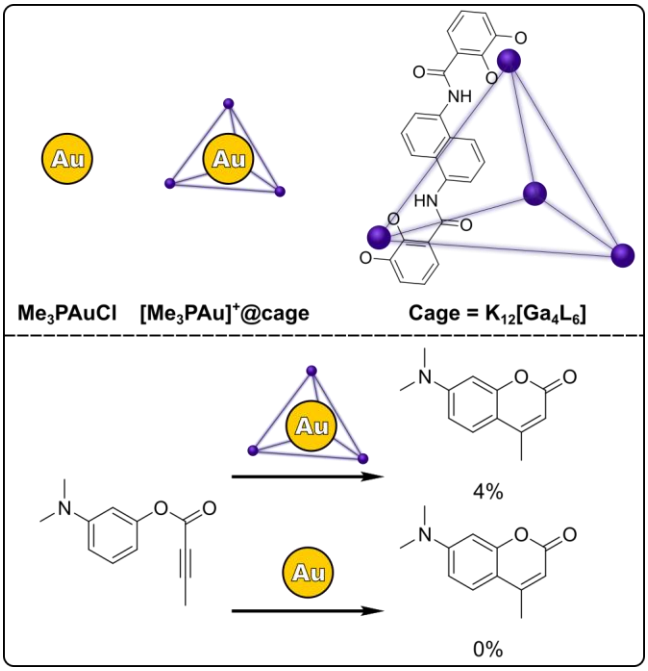


Figure 1. Above: Structures of the gold complex and the cage used in Chapter 2. Below: Yields of the gold catalysed intramolecular hydroarylation using free and encapsulated gold under biological conditions.

In **Chapter 3** we expanded this strategy to a palladium complex. Here a cationic supramolecular cage was selected, as cationic or neutral species are known to be more cell permeable than anionic species. Although palladium-based cages are unstable to biological conditions, we demonstrated that a platinum-based cage is stable to biological conditions, even at nanomolar concentrations. A small palladium complex, $[(Et_3P)_2PdCl_2]$, was successfully encapsulated within the cavity of this cage; however, purification of the complex was not possible, and a mixture of free palladium and encapsulated palladium was obtained. The reactivity of this encapsulated palladium complex was then investigated. We first looked at its reactivity towards the formation of a fluorescent dye via an intramolecular hydroarylation. Although the free palladium complex was completely inactive, initial experiments indicated that the encapsulated palladium complex and/or the cage itself were able to generate the dye. However, a lack of reproducibility meant that these results could not be confirmed. Further attempts to evaluate the reactivity of the encapsulated palladium complex were carried out using the Wacker-Tsuji oxidation of styrene and the double silylation of benzil as model systems (Figure 2). However, in all cases no reactivity was observed and it was not possible to determine if the encapsulated palladium complex is catalytically active.

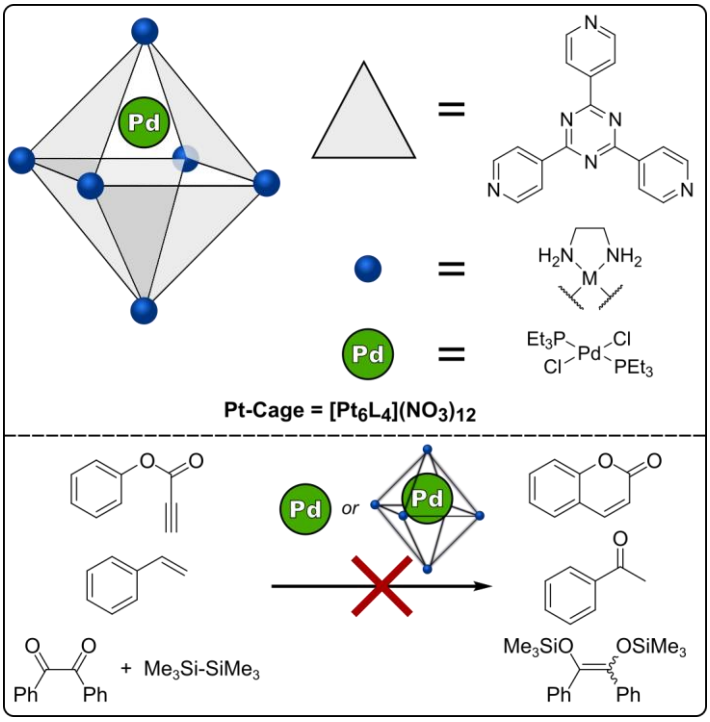


Figure 2. Above: Structures of the palladium complex and platinum cage used in Chapter 2. Below: Intramolecular hydroarylation, Wacker-Tsuji oxidation, and double silylation reactions attempted for the palladium complex.

We then set out to investigate if this same platinum cage could act as a protective host for a ruthenium olefin metathesis catalyst in **Chapter 4**. Encapsulation of standard apolar metathesis catalysts within this cage was not possible. However, the presence of the cage greatly improved the catalytic activity of a water soluble metathesis catalyst, AquaMet, compared to reactions carried out in the absence of cage. The presence of the cage greatly increased the rate of the reaction, which resulted in higher final yields of the product. Interestingly, it was found that the cause of this improvement was not due to any encapsulation effect, as NMR spectroscopy, GFN2-xTB, and UV/Vis spectroscopy studies revealed that there was no interaction between the catalyst and the cage. Instead, it was found that it was the nitrate counterions of the cage which were responsible for the improvement in activity, and that activity of the metathesis catalyst could be greatly improved by the presence of KNO_3 (Figure 3).

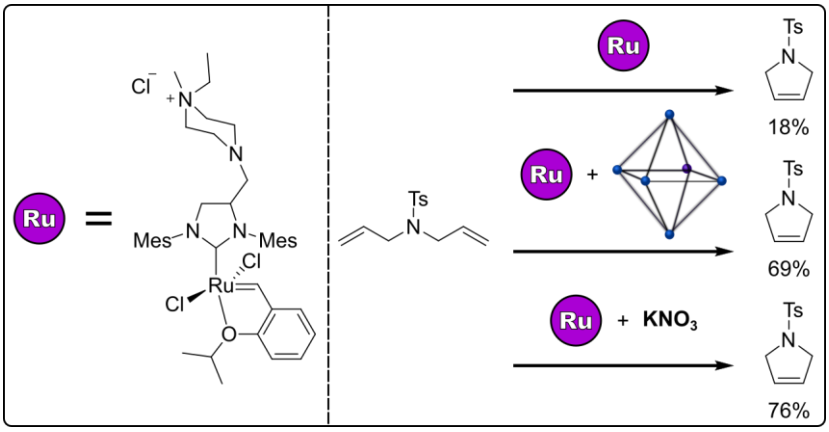


Figure 3. Left: Structure of AquaMet. Right: Yields of ruthenium catalysed ring closing metathesis with AquaMet, AquaMet in the presence of cage, and AquaMet in the presence of nitrate.

In **Chapter 5** we report out investigations on how to use these improved reaction kinetics in the presence of nitrate to improve the efficiency of olefin metathesis under biological conditions. Mass spectrometry studies show that the nitrate anions substitute the chloride ligand of the AquaMet to generate a new nitrate-AquaMet complex. It is this new nitrate complex which then exhibits faster initiation and faster catalysis than the standard chloride complex. These improved kinetics were also observed in the presence of biological additives (Figure 4). This means that the addition of nitrate to AquaMet provides kinetic protection for the metathesis reaction: the nitrate-complex was sufficiently fast that it was able to generate the product in good yields before becoming poisoned and deactivated by the biological additives; in contrast, the slower chloride-complex was poisoned before it was able to undergo catalysis.

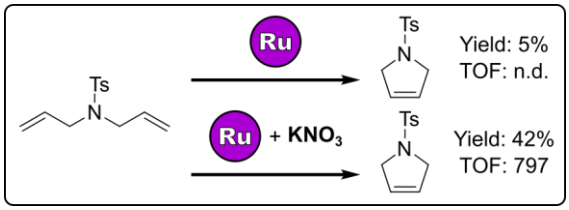


Figure 4. Yields and turnover frequencies (given in µmol ethene mol cat^{-1} min^{-1}) for ring closing metathesis in the presence of biological additives with AquaMet or AquaMet in the presence of nitrate.

In this thesis, we showed that new strategies are needed in the field of *in vivo* transition metal catalysis in order to overcome catalyst poisoning and improve catalytic activity. We have developed two new strategies for this purpose: physical protection, and kinetic protection. A supramolecular host can provide a physical barrier against biomolecules, and in this way poisoning can be reduced. However, the choice of host and the design of the catalytic system is highly important. First, the cage itself needs to be cell permeable in order to be able to deliver the catalyst to the inside of the cell. Secondly, a balance needs to be struck with respect to the size of the cage: very small windows will mean that the catalyst is well protected as this will make it difficult for biomolecules to enter the cage; however, this will also mean that the substrate scope will be limited to only very small molecules. For instances where a larger substrate is desired, a compromise needs to be made where larger windows allow for the larger substrate, but this also allow smaller biomolecules to reach the catalyst, leading to limited

protection only. Alternatively, kinetic protection can be used. Although catalyst poisoning is not prevented and will still occur, if a sufficiently fast reaction is selected then productive catalysis can take place before catalyst deactivation, and sufficient yield of the desired product can still be obtained. We applied these strategies to only three catalysts; however, we believe that these strategies show a lot of potential and in the future could also be expanded to other catalysts and other reactions.