Summary

Exploring transition metal catalysis in water for *in vivo* applications

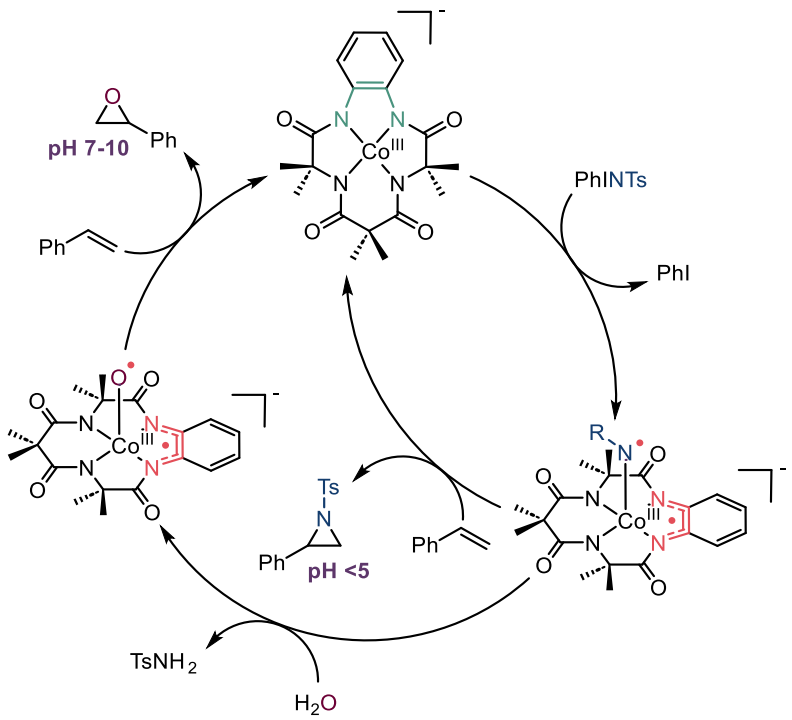
Transition metal catalysis proves a powerful tool to achieve otherwise synthetically challenging, or even impossible, transformations with (high) selectivity and is therefore employed in various areas of chemistry. Recently, transition metal-catalysed reactions have also been successfully performed in cells (*in vitro*) and living systems (*in vivo*). This subfield of bioorthogonal chemistry is devoted to complementing Nature's repertoire of reactions with catalysts that are not naturally present in Nature: new-to-nature catalysts.

As outlined in **Chapter 1**, these new-to-nature catalysts have been employed to mimic reactions commonly encountered in biosynthetic and metabolic pathways, such as redox reactions and transfer hydrogenations, but also to perform new-to-nature reactions like functional group modifications, deprotection, cycloaddition, cyclisation, and cross-coupling reactions with new-to-nature substrates. These transition metal-catalysed reactions have led to various applications ranging from induced cell death by disrupting cellular equilibria to the controlled release and synthesis of fluorophores, drugs, and other biologically active compounds. When further developed, such applications may become useful in biological and medicinal chemistries as they present alternative routes for, e.g., chemical labelling of biomolecules and cancer treatment.

The achievements made thus far reveal the potential of transition metal catalysis in biological settings. Interestingly, the scope is limited compared to the breadth of transition metal-catalysed reactions that have been unlocked for synthetic applications. Translating transition metal-catalysed reactions from flasks to cells is non-trivial as the conditions in cells are fairly different compared to the highly controlled and adaptable conditions achieved in a flask. The development of catalytic systems for future applications *in vivo* therefore proceeds through many steps, starting with evaluating their reactivity, selectivity, and stability in water and under biologically relevant and

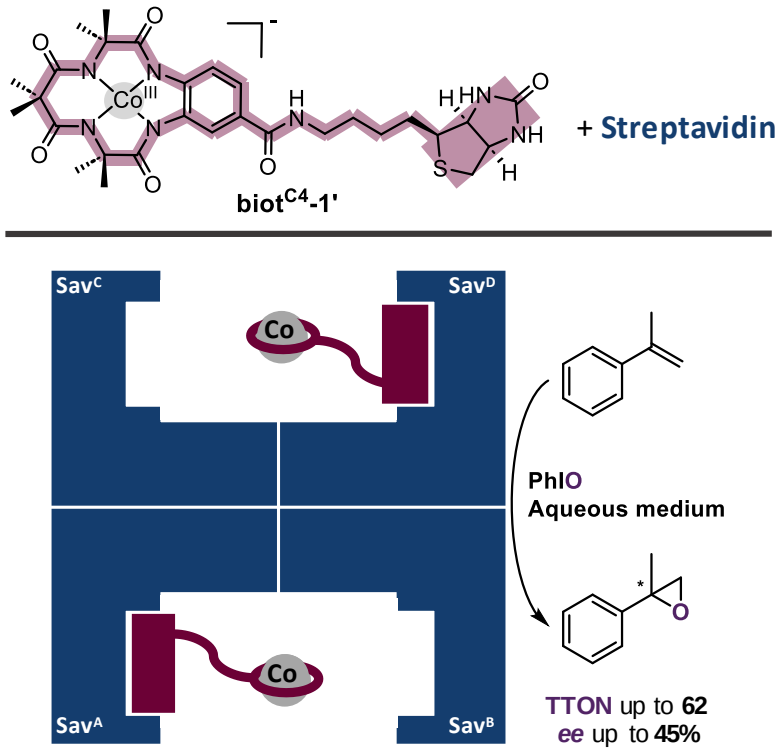
biomimetic conditions. Hence, this dissertation aims to explore transition metal catalysed reactions that are of interest for future *in vivo* applications in aqueous media.

In **Chapter 2**, we set out to perform styrene aziridination in water and under biologically relevant conditions using a water-soluble Li[Co^{III}($TAML^{red}$)] complex. Performing nitrene transfer reactions in water is non-trivial as it is typically associated with the formation of oxygen-containing side products. Indeed, we observed that the cobalt-catalysed nitrene transfer reaction to styrene afforded styrene oxide as the major product, next to minor amounts of aziridine product. In a combined experimental and theoretical approach, we reveal the role of water in epoxide formation, which was found to occur via the hydrolysis of the cobalt nitrene radical intermediates, affording oxyl radical intermediates that are active in oxygen atom transfer to styrene. Based on the insights derived from computational and experimental mechanistic studies, we discovered that the hydrolysis of nitrene radical complexes can be prevented or stimulated by changing the pH, yielding either the desired aziridine product (pH = 4) or styrene oxide (pH between 7 and 10) selectively.



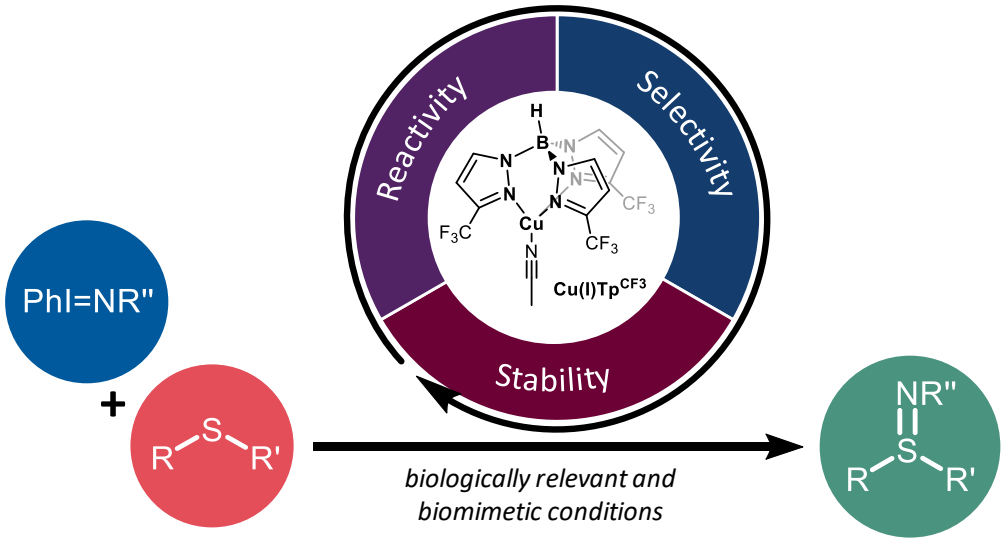
Scheme 1. Styrene aziridination with [Co^{III}($TAML^{red}$)]$^-$ in water: understanding and preventing epoxidation via nitrene hydrolysis (Chapter 2).

Through Co(TAML)-catalysed nitrene and oxygen atom transfer catalysis, aziridines and epoxides can be (selectively) accessed in water, as outlined in Chapter 2. Since most drugs bear (multiple) stereogenic centres, the asymmetric synthesis of these moieties is of considerable interest. In **Chapter 3**, we set out to explore asymmetric radical-type oxygen atom transfer with the use of a Co(TAML)-based artificial metalloenzyme generated through anchoring of an achiral, biotinylated Co(TAML) catalyst within streptavidin. In the presence of iodosylbenzene and α-methylstyrene, the Co(TAML)-based artificial metalloenzyme affords the corresponding enantioenriched epoxide with increased TTON's, representing an improvement in terms of enantioselectivity and activity compared to enantiopure Co(TAML) analogues. X-ray structures of two different Co(TAML) · Sav artificial metalloenzymes reveal the importance of noncovalent interactions within the secondary coordination sphere provided by the protein. These interactions tend to affect the precise localisation of the catalyst inside the protein, which is reflected in the formation of the enantioenriched epoxide.



Scheme 2. A Co(TAML)-based artificial metalloenzyme for asymmetric radical-type oxygen atom transfer catalysis (Chapter 3).

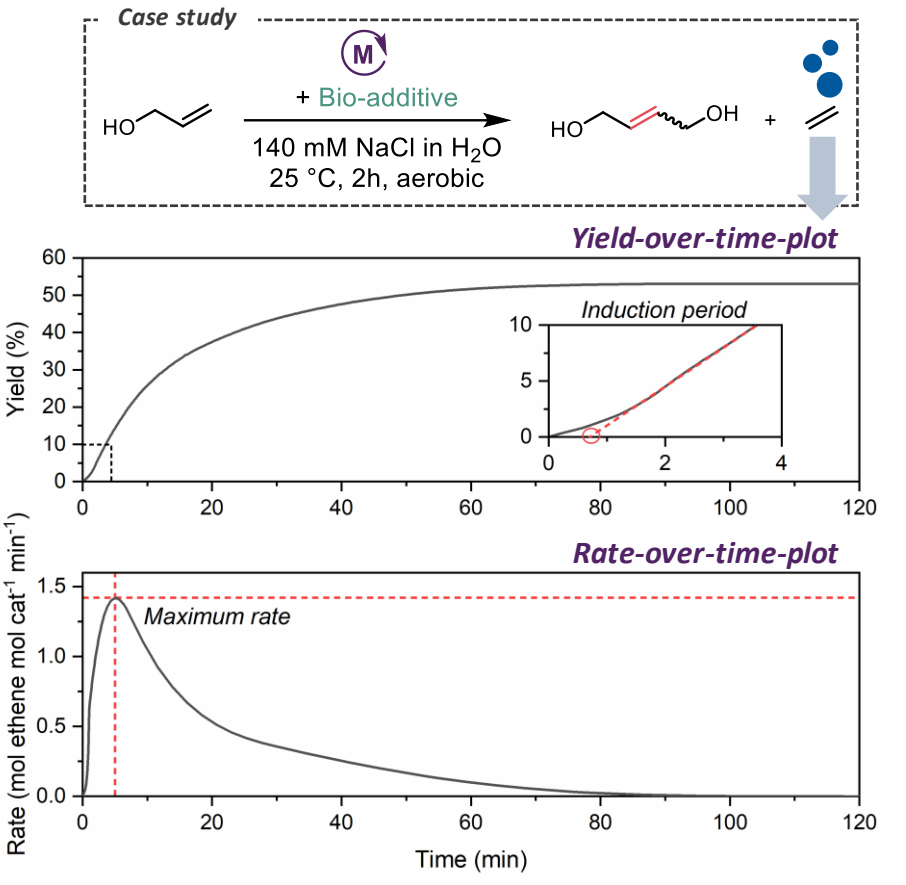
With the Li[Co^{III}(TAML^{red})] complex demanding acidic conditions for selective nitrene transfer catalysis in water, we sought a catalytic system that is more compatible with biologically relevant and biomimetic conditions. In **Chapter 4**, we explore copper-catalysed nitrene transfer in aqueous media for future application *in vivo*. The Cu(I)Tp^{x} catalysts employed in this study display excellent nitrene transfer reactivity, high chemoselectivity, and good biomolecule compatibility, enabling the sulfimidation reaction of thioethers under biologically relevant and biomimetic conditions. Late stage sulfimidation of a prodrug afforded a reported drug molecule with anticancer activity in good yields, providing proof of principle for potential intracellular drug synthesis applications. Finally, we show how catalyst solubility issues in buffered media can be addressed by encapsulating the catalyst in liposomes. The protective function of the liposomes was also observed to have a positive effect on the biomolecule compatibility of the encapsulated catalyst.



Scheme 3. Copper-catalysed sulfimidation in aqueous media: a fast, chemoselective and biomolecule-compatible reaction (Chapter 4).

Throughout our studies, we noticed that obtaining kinetic data under biologically relevant and biomimetic conditions is rather challenging. Substantial concentrations of salts and biomolecules hamper the application of typically employed solution-phase analytical techniques, and there are only limited alternative protocols available. Given that detailed mechanistic data is crucial for iterative catalyst development for *in vivo* applications, we set out to develop a protocol to measure reaction kinetics under

biomimetic conditions, which is presented in **Chapter 5**. This protocol is highly compatible with complex media, as it is based on gas evolution as a probe. By producing hundreds of data points through 'bubble counting,' reaction rates can be obtained as a function of reaction progress, leading to easy assessment of critical catalyst parameters such as induction and deactivation processes. Moreover, we show that the progress of two transition metal-catalysed bioorthogonal chemical reactions, among which the ruthenium-catalysed olefin cross-metathesis reaction, can be accurately monitored in the presence of various bio-additives, cell-culture media, and cell lysates. As many transition metal-catalysed bioorthogonal chemical reactions currently developed and employed for various applications in cells and living systems evolve one or more equivalent(s) of gas per product, the developed protocol is highly compatible.



Scheme 4. Gas evolution as a tool to study reaction kinetics under biomimetic conditions (Chapter 5).

By exploring transition metal-catalysed reactions in water for *in vivo* applications, this dissertation has contributed to the subfield of bioorthogonal chemistry devoted to complementing Nature's repertoire of reactions. Our studies have revealed the

challenges associated with the performance of transition metal catalysis in aqueous media and how they can be addressed by a detailed understanding of a catalytic system or with the use of host systems such as liposomes. Apart from these fundamental studies, we have performed explorative studies under biologically relevant and biomimetic conditions in the context of intracellular drug synthesis. We have employed a cobalt-based artificial metalloenzyme to afford an enantioenriched epoxide via asymmetric oxygen atom transfer and discrete copper-based catalysts to synthesise a drug molecule via nitrene transfer. Moreover, we have developed a new and compatible protocol that enables detailed kinetic studies in complex reaction media, comparable to the cellular environment, to facilitate the translation of transition metal catalysis from flasks to cells. Hence, we envision that our findings can support further development of the new to-nature catalysts and reactions investigated in this dissertation and those that have yet to be explored in aqueous media for future application in cells and living systems.