Summary

Photocatalysis for Applications in Living Cells

Bioorthogonal catalysis has emerged as a potent tool for introducing new-to-nature reactions in living organisms, facilitating diverse biological applications such as fluorescence imaging, drug synthesis and protein labeling. While transition metal catalysts have been employed for catalyzing reactions in living cells, achieving precise control over reactions remains a challenge. Photocatalysis provides a controllable approach to catalyzing reactions in both temporal and spatial dimensions within living organisms, constituting a burgeoning research field. However, this approach faces numerous challenges, including the development of new photocatalytic reactions that operate under physiological conditions, implementing photocatalysis for targeting cancer cells, and utilizing red or near-infrared light (NIR) for catalysis. In response to these challenges, our research involves designing diverse transition metal catalysts to enable efficient and controlled catalysis targeting cancer cells, thereby holding promise for potential disease treatment.

In **Chapter 1**, we review the current progress and outline existing issues and challenges in the field of bioorthogonal catalysis in living organisms. The primary concern revolves around addressing the imperative need for achieving efficient, controllable and targeted catalysis within cancer cells. Transition metal catalysts facilitate the introduction of various novel reactions, but the challenge is to ensure a high efficiency of these reactions. Photocatalysis, through the utilization of different wavelength light, offers temporal and spatial control over reaction rate, yield and selectivity, enabling the controllable synthesis and release of fluorophores and drugs in living cells. Polymer hyaluronic acid provides an alternative way to achieve targeted selectivity for chemistry in cancer cells. This targeted chemistry minimizes off-target effects, reduces toxicity to normal cells and enhances reagent utilization efficiency (Figure 1). Our objective is to design catalytic systems mainly use of these effects and catalyze reactions with external light stimuli for optimal control.

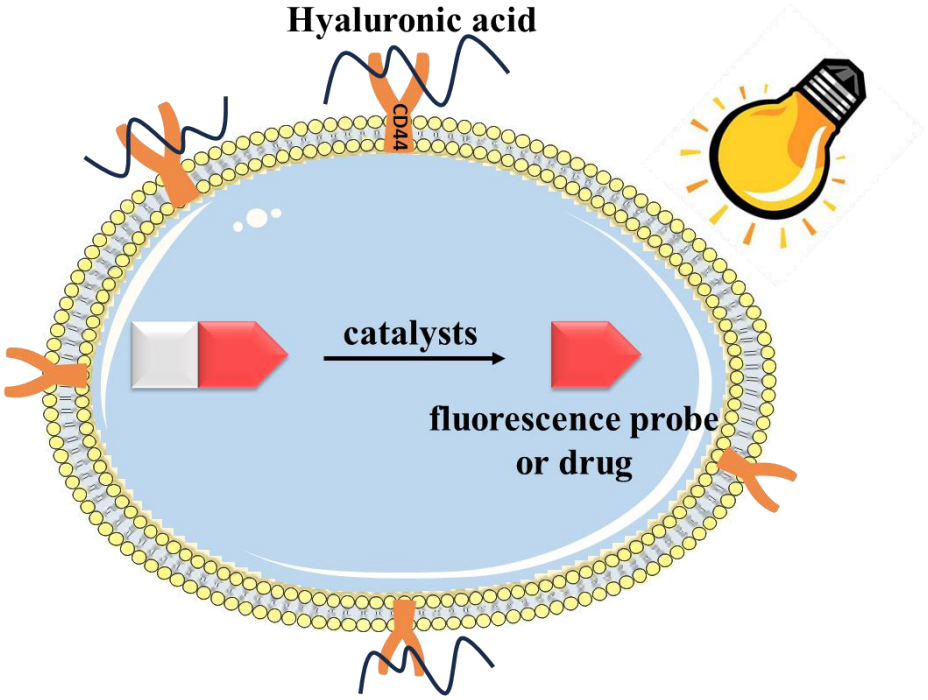

Figure 1. *Photocatalysis targeting cancer cells for fluorescence imaging and drug synthesis reviewed in Chapter 1.*

In **Chapter 2**, we report a photoswitchable catalyst designed for regulating the reactivity of cyclization reactions under mild conditions. An azobenzene-bearing N-heterocyclic carbene-based gold catalyst was designed and synthesized. Our findings demonstrate that the catalyst can be reversibly switched between its *trans* and *cis* isomers by utilizing UV and visible light and these configurations remain stable during the reaction process (Figure 2). Notably, the *trans*-Cat exhibits almost two times the catalytic activity compared to the *cis*-Cat. DFT calculations suggest that the *trans*-Cat is more favorable for substrate binding, as the *cis*-Cat features more crowding around the vacant site. Photoswitchable metal catalysts enable the spatial and temporal control of catalytic reactions, presenting significant implications for various applications, including smart drugs and other biological processes.

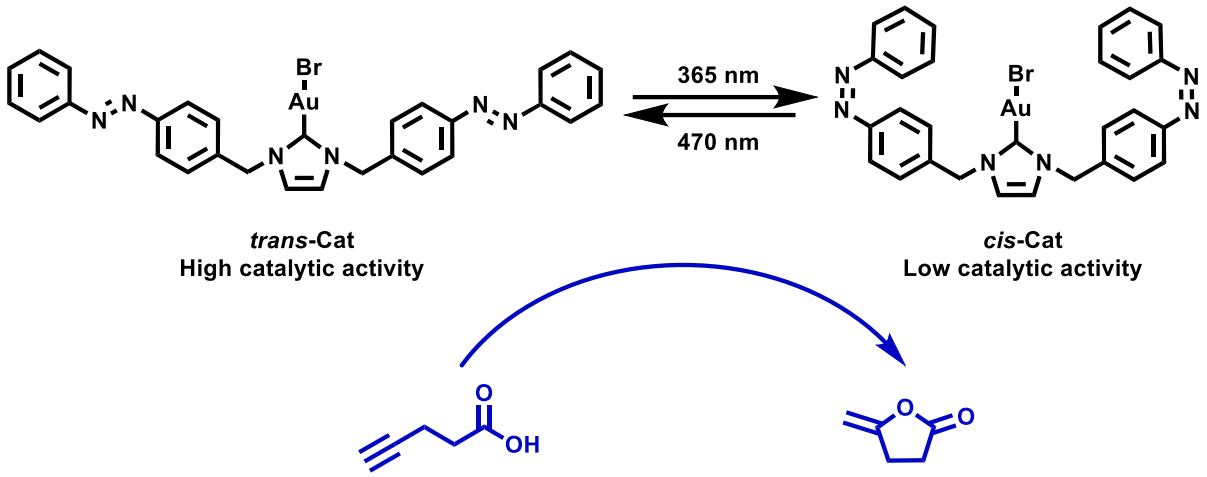

Figure 2. *Photoswitchable catalyst for regulating the reactivity of cyclization reactions in Chapter 2.*

The azide reduction reaction is an important reaction for the application of photocatalysis in living cells due to the low toxicity and biological inertness of azide substrates. In **Chapter 3**, we focused on the activation of pro-probe or prodrug by visible light, targeting cancer cells using a ruthenium photocatalyst delivered *via* a polysaccharide assembly. The photocatalyst assembly was constructed through supramolecular interactions, involving hyaluronic acid functionalized with β-cyclodextrin and an adamantane-bearing tris(bipyridine)ruthenium(II) catalyst. The polysaccharide hyaluronic acid selectively targets cancer cells, and the modified β-cyclodextrin provides a binding site for the adamantane functionalized catalyst through supramolecular interactions. Under visible light irradiation, the ruthenium photocatalyst effectively reduces the azide pro-probe to produce rhodamine as the fluorescent probe. In a similar fashion, an anticancer drug can be generated by deprotection of the amine group, also under physiological relevant conditions. Catalytic experiments conducted in living cells demonstrate that the photocatalyst assembly efficiently targets cancer cells and catalyzes the generation of fluorescent probe through visible light irradiation, relevant for cancer cell imaging. A similar conversion of prodrug to drug induces cancer cell death (Figure 3). The photocatalytic activation of prodrug provides spatial and temporal control over the synthesis of anticancer drug, and the catalyst assembly targeting cancer cells enables more precise control of reactions occurring within cancer cells.

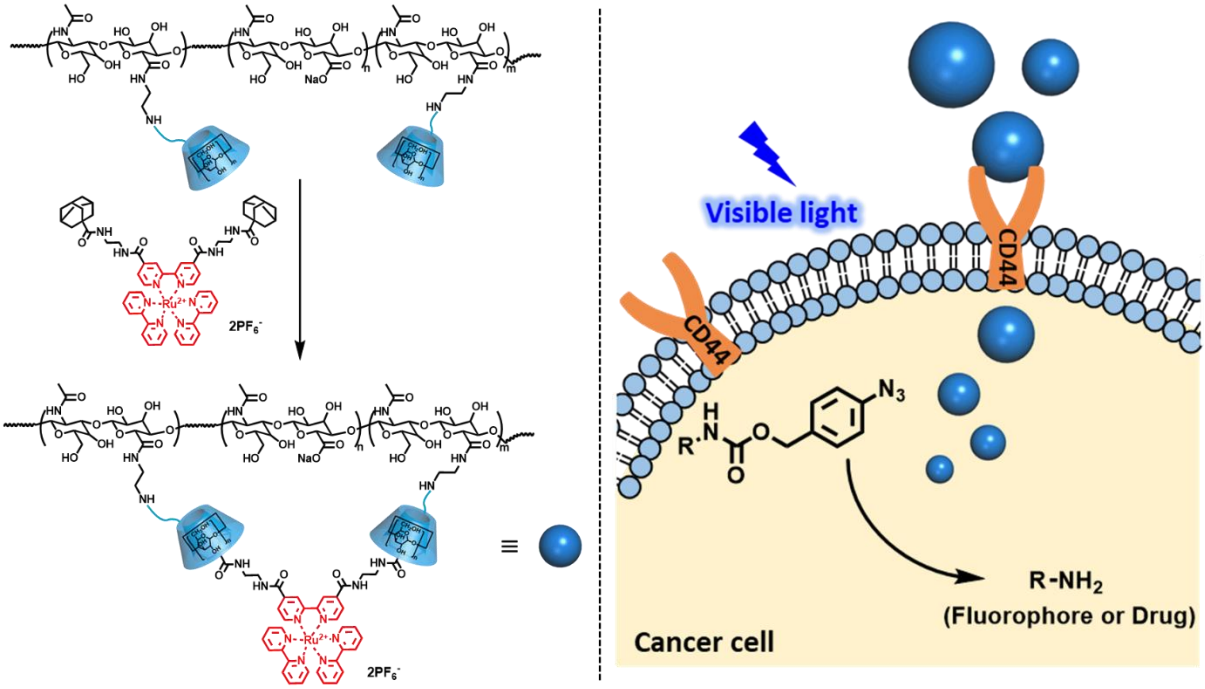

Figure 3. *Visible-light-induced pro-probe and prodrug activation targeting cancer cells using Ru(bpyAda)(bpy)$_2$⊂HACD supramolecular photocatalyst in Chapter 3.*

Visible-light-induced photocatalysis in living cells has been studied in **Chapter 3**. Subsequently, we aim to utilize longer-wavelength red light or NIR light for catalyzing azide reduction reactions within living cells, capitalizing on the advantages of red light such as low biological toxicity and deeper tissue or tumor penetration. The primary challenge of red light-induced photocatalysis is to develop efficient photocatalyst systems which can be achieved in two ways: 1) direct red light photocatalysis and 2) indirect red light photocatalysis using upconversion. Direct red light photocatalysis employs photocatalysts such as transition metal complexes and organic dyes that can directly absorb red light to reach the excited state and participate in photocatalytic reduction reactions, and approaches along these lines will be discussed in **Chapter 4**. Indirect red light photocatalysis involves the upconversion of low-energy NIR light photons into high-energy visible light photons, which can be used for photoreduction catalysis. This approach will be discussed in **Chapter 5** in which lanthanide upconversion nanoparticles are combined with ruthenium photocatalysts.

In **Chapter 4**, we report photocatalysts that are directly activated by red light to produce the rhodamine probe under physiological relevant conditions. These photocatalysts exhibit good absorption of red light and display varying catalytic activities for the conversion of the rhodamine azide substrate when using different reductants, such as NaAsc, GSH and NADH under 640 nm red light irradiation. Notably, photocatalysts PPIX-2CH$_3$, Ce6-3CH$_3$, ZnPc, MgPc, CoPc and SnTPP(OH)$_2$, SnPc(OH)$_2$, Sn(PPIX-2CH$_3$)(OH)$_2$ and Sn(Ce6-3CH$_3$)(OH)$_2$ exhibit high catalytic activity for rhodamine azide conversion (Figure 4). In several reactions the presence of air (oxygen) decreases the conversion, most likely as it acted as a quencher of the excited state. To facilitate the application of these photocatalysts within living cells, a supramolecular strategy was employed to bind photocatalysts functionalized with adamantane to hyaluronic acid polymers that contain β-cyclodextrin units, to enhance water solubility and

reduce air quenching. Unfortunately, this strategy has not resulted in sufficient conversions under physiological conditions, nonetheless, the results lay a foundation for future biological applications involving red light-induced catalysis in living cells.

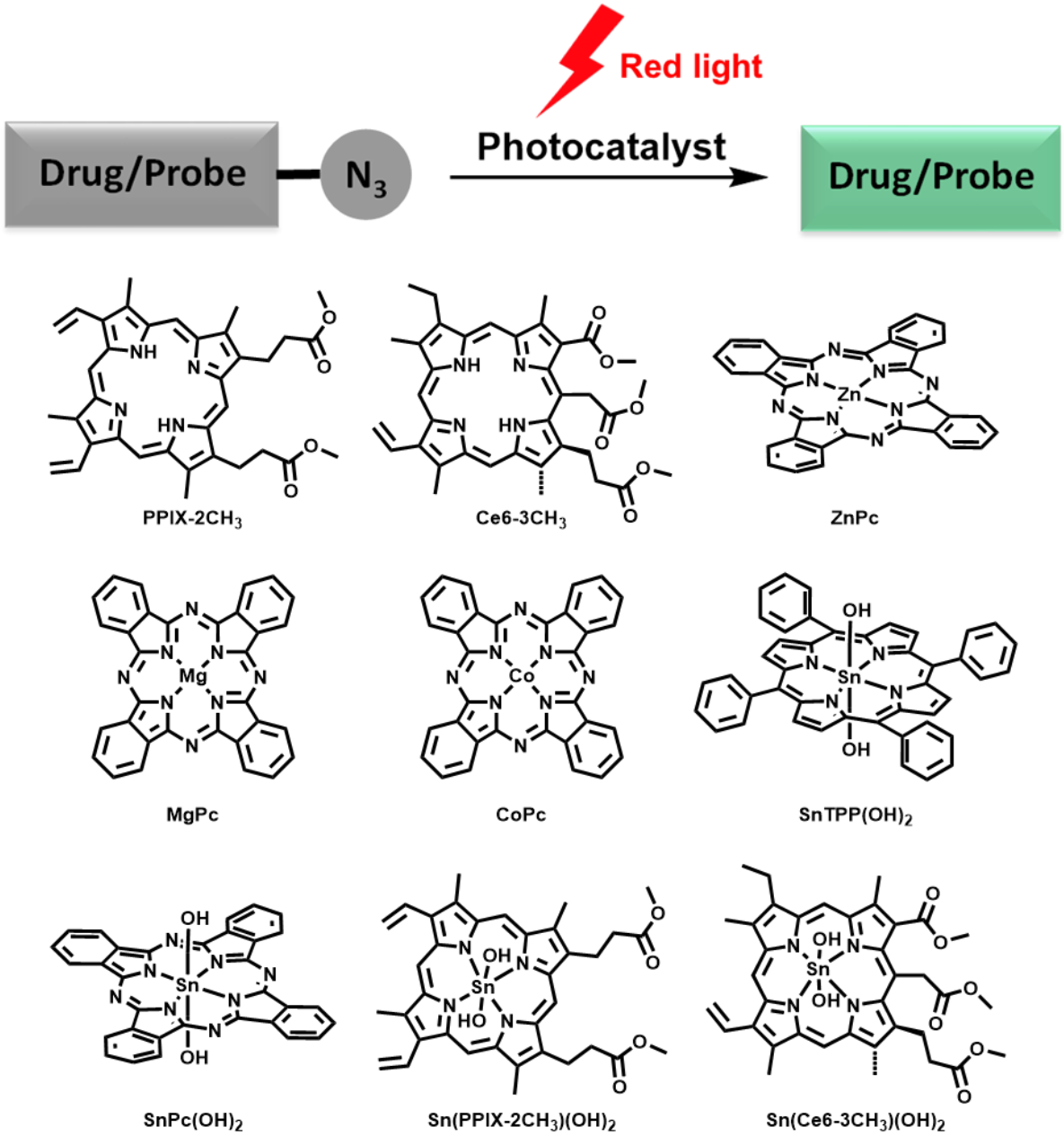

Figure 4. *Photocatalysis of azide reduction reactions using red light and the structures of catalytically active photocatalysts reported in Chapter 4.*

Indirect NIR light photocatalysis employing an upconversion strategy for the azide reduction reaction was explored in **Chapter 5**. A visible light photocatalyst was covalently linked to lanthanide upconversion nanoparticles through a condensation reaction to the poly(allylamine) that covered the nanoparticles, forming a NIR-induced catalytic system. Importantly, the absorption of the ruthenium photocatalyst and emission of upconversion nanoparticles exhibit a significant spectral overlap. Effective energy transfer from upconversion nanoparticles to photocatalysts occurrs due to the favorable spectral overlap and close spatial proximity,

providing promising opportunities for subsequent photoreduction catalysis (Figure 5). The rhodamine azide reduction reaction was carried out using 980 nm NIR light. While significant conversion was obtained, showing proof of concept, the observed catalytic efficiency is relatively low, potentially due to the the low energy transfer efficiency. Although the catalytic efficiency needs to be improved for application in living cells, this pioneering strategy holds promise for advancing NIR-induced organic synthesis of fluorescent probe or drug within living cells.

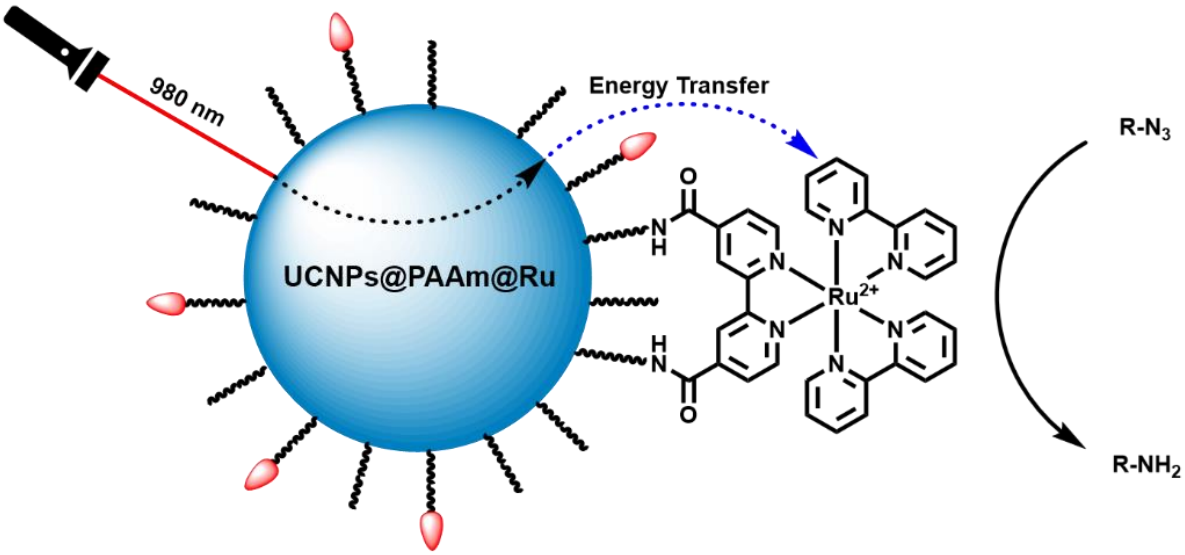

Figure 5. *Indirect NIR photocatalysis by the connection between lanthanide upconversion nanoparticles and ruthenium photocatalyst, active in azide reduction reactions under 980 nm NIR light irradiation, reported in Chapter 5.*

The central focus in this thesis is on photocatalysis in living cells. We comprehensively explore the expansion of novel photocatalytic reactions to physiological conditions, targeting photocatalysis in cancer cells and the utilization of long-wavelength red light and NIR light photocatalysis. The research described in this thesis contributes to the development of efficient, targeted and controllable catalytic systems, which provides tools to deepen our understanding of biological processes and contributing to the treatment of disease.