Photocatalysis for applications in living cells

Liu, J.

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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.

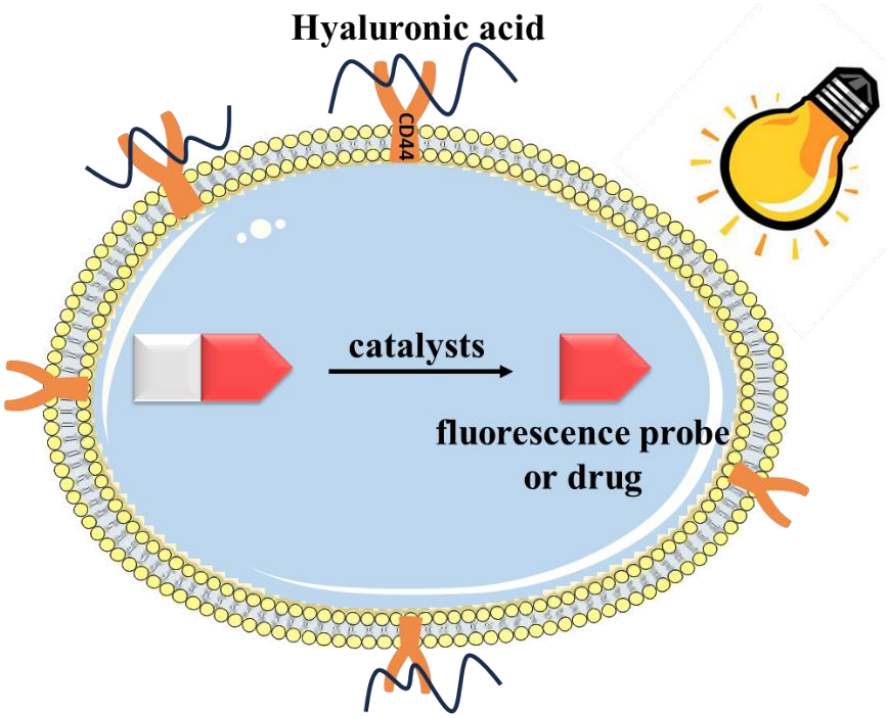


Figure 1. *Photocatalysis targeting cancer cells for fluorescence imaging and drug synthesis reviewed in Chapter 1.*

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.

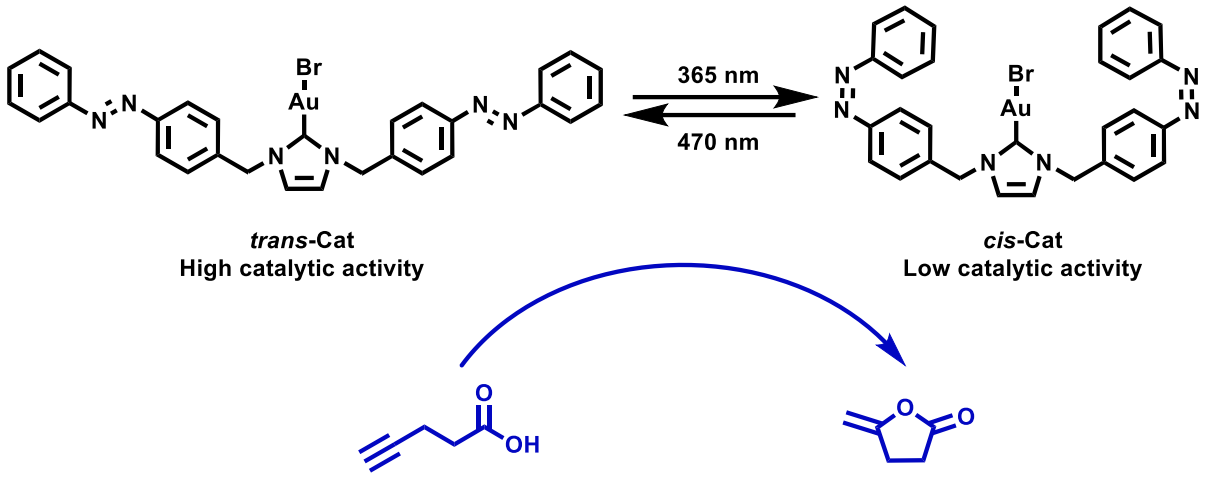


Figure 2. *Photoswitchable catalyst for regulating the reactivity of cyclization reactions in Chapter 2.*

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.

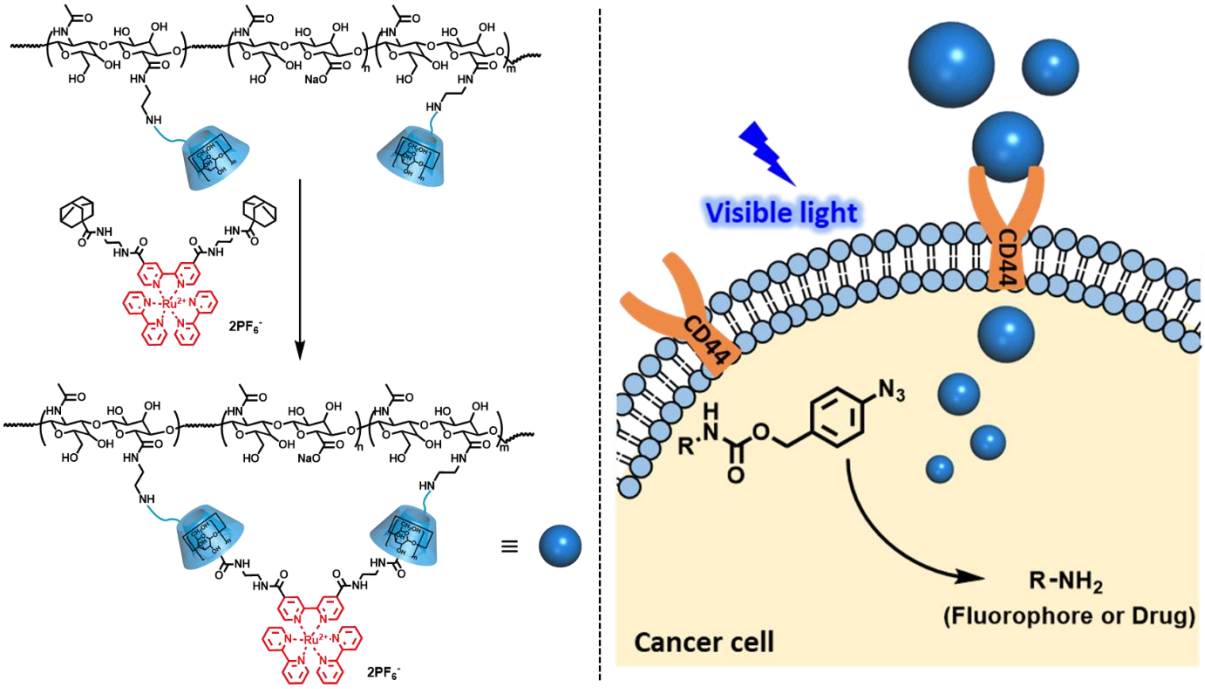


Figure 3. *Visible-light-induced pro-probe and prodrug activation targeting cancer cells using Ru(bpyAda)(bpy)$_2$⊂HACD supramolecular photocatalyst in Chapter 3.*

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.

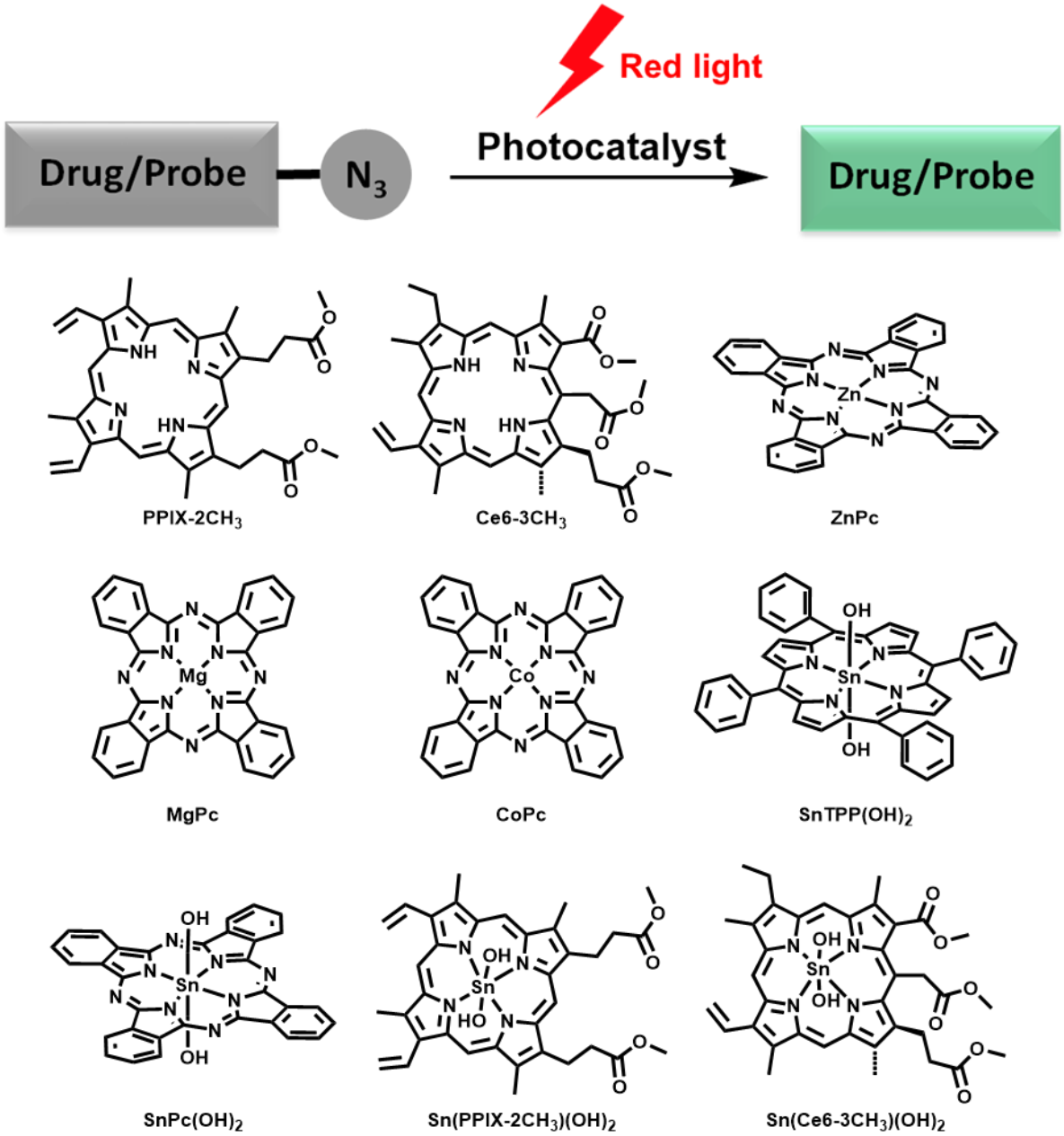


Figure 4. *Photocatalysis of azide reduction reactions using red light and the structures of catalytically active photocatalysts reported in Chapter 4.*

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.

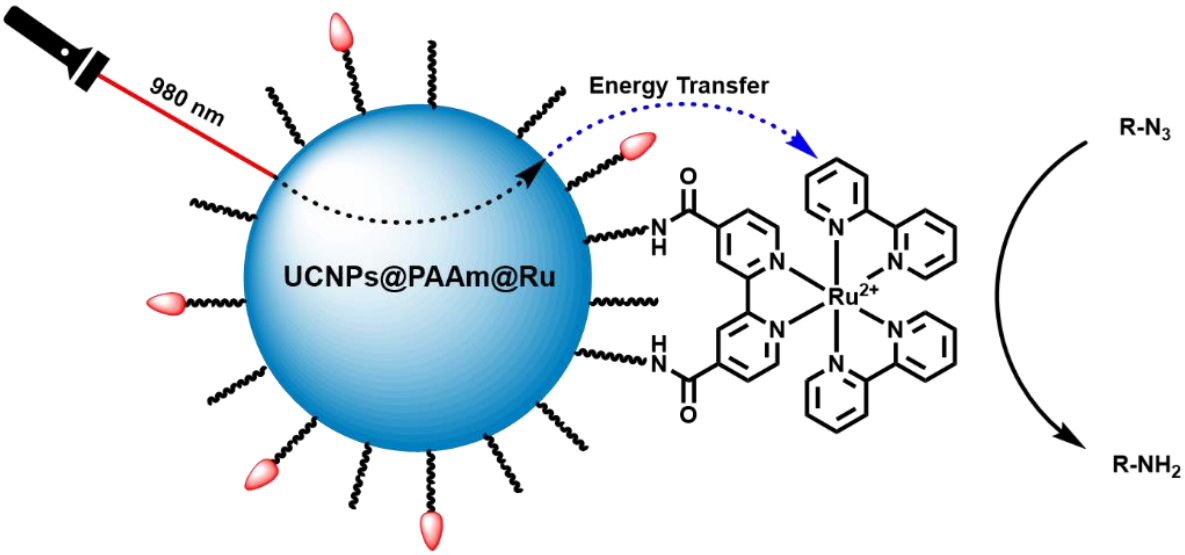


Figure 5. *Indirect NIR photocatalysis by the connection between lanthanide upconversion nanoparticles and ruthenium photocatalyst, active in azide reduction reactions under 980 nm NIR light irradiation, reported in Chapter 5.*

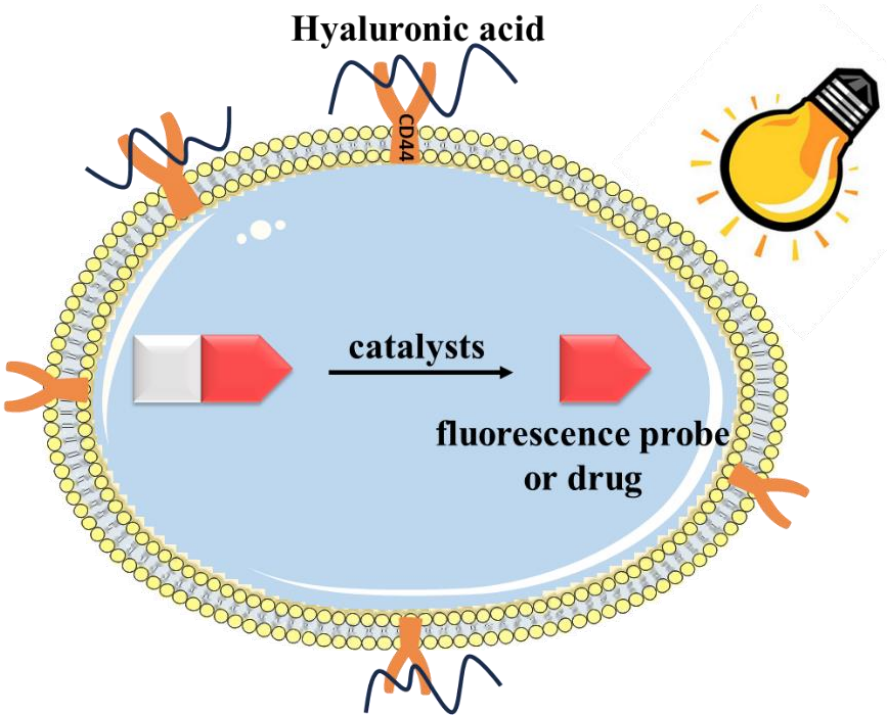
The central focus in this thesis is on photocatalysis in living cells. We comprehensively explore the expansion of novel photocatalytic reactions to physiological conditions, targeting photocatalysis in cancer cells and the utilization of long-wavelength red light and NIR light photocatalysis. The research 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.

Samenvatting

Fotokatalyse voor Toepassingen in Levende Cellen

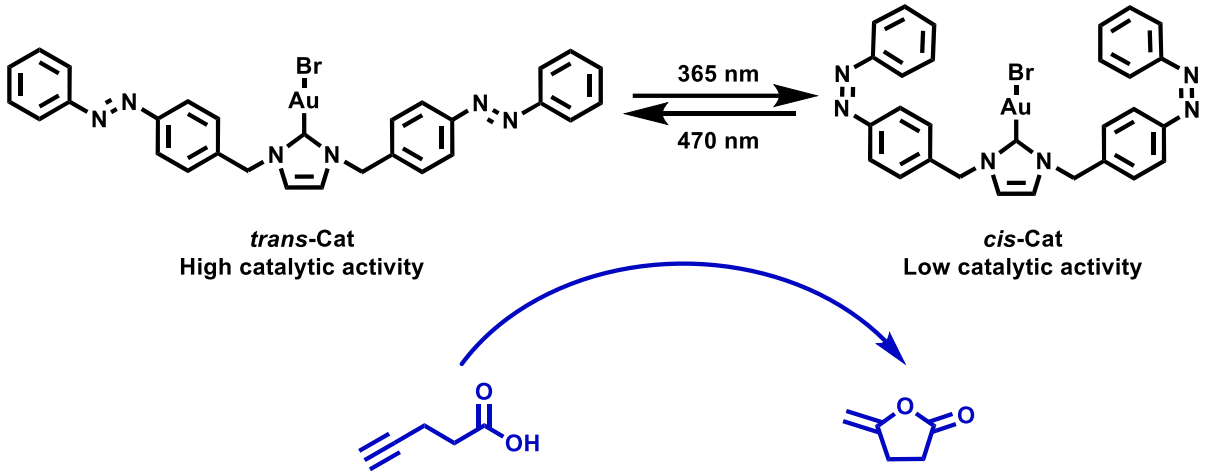
Bioorthogonale katalyse heeft zich ontwikkeld als een krachtig instrument om niet natuurlijke reacties in levende organismen uit te voeren. Hierdoor worden diverse biologische toepassingen mogelijk zoals beeldvorming via lokale fluorescentie, medicijnsynthese en het lokaal labelen van eiwitten. Hoewel er al overgangsmetaalkatalysatoren zijn gebruikt voor het katalyseren van reacties in levende cellen, zijn er nog verschillende uitdaging te overbruggen om chemische reacties met precieze controle in tijd en ruimte mogelijk te maken. Fotokatalyse biedt een strategie om reacties te katalyseren gecontroleerd in zowel tijd als ruimte ook binnen levende organismen, en is daarom een groeiend onderzoeksgebied. Voor deze strategie is de ontwikkeling van nieuwe fotokatalytische reacties die onder fysiologische omstandigheden werken nodig, alsmede de implementatie van fotokatalyse selectief gericht op kankercellen, en het gebruik van rood of nabij-infrarood licht (NIR) voor katalyse. Het onderzoek beschreven in dit proefschrift betreft het ontwerpen van diverse overgangsmetaalkatalysatoren om efficiënte en licht-gecontroleerde katalyse mogelijk te maken, met een oog op de toepassing in kankercellen, met als veelbelovende toepassing de mogelijke behandeling van ziektes.

In **Hoofdstuk 1** bespreken we de stand van zaken en de uitdagingen op het gebied van bioorthogonale katalyse in levende organismen. De grootste uitdaging is het ontwikkelen van efficiënte, controleerbare katalytische reacties gericht op kankercellen. Overgangsmetaalkatalysatoren faciliteren de introductie van verschillende nieuwe reacties die uitgevoerd kunnen worden in cellen, maar de uitdaging is ervoor te zorgen dat deze reacties een hoge efficiëntie hebben. Fotokatalyse, door het gebruik van licht met verschillende golflengtes, biedt temporale en ruimtelijke controle over de reactie in termen van snelheid, opbrengst en selectiviteit. Dit maakt de controleerbare synthese van fluoroforen en geneesmiddelen in levende cellen mogelijk is. Daarnaast biedt het gebruik van het polymeer hyaluronzuur een manier om selectief verbindingen te leveren aan kankercellen. Deze gerichte strategieën kunnen in principe off-target effecten minimaliseren, zoals de toxiciteit voor normale cellen en het leidt bovendien tot een meer efficiënt gebruik van reagentia (Figuur 1). Het doel van het onderzoek beschreven in dit proefschrift is om katalytische systemen te ontwerpen die gestuurd kunnen worden met externe lichtstimuli en selectief afgegeven kunnen worden aan kankercellen, om zo maximale controle van chemische reactie in cellen mogelijk te maken.



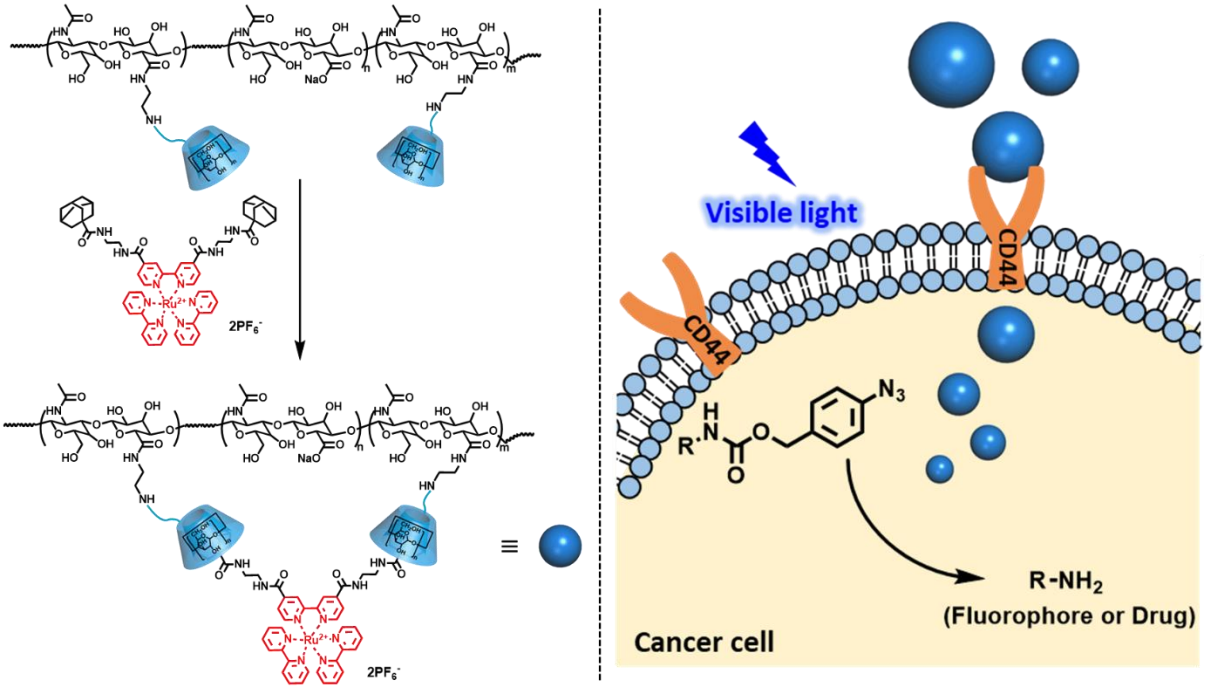
Figuur 1. *Fotokatalyse in kankercellen voor het lokaal maken van fluorescente moleculen en medicijnen, zoals besproken in Hoofdstuk 1.*

In **Hoofdstuk 2** rapporteren we een met licht schakelbare katalysator, ontworpen om ringsluitingsreacties onder milde omstandigheden te bewerkstelligen. Een goudkatalysator met een azobenzeen-bevattende N-heterocyclische carbeen eenheden werd ontworpen en gesynthetiseerd. De katalysator kan reversibel worden omgeschakeld tussen de *trans*- en *cis*-isomeren door gebruik te maken van UV en zichtbaar licht, en deze configuraties blijven stabiel tijdens het reactieproces (Figuur 2). Omdat de *trans*-Cat bijna twee keer de katalytische activiteit vertoont in vergelijking met de *cis*-Cat tonen we aan dat het een schakelbare katalysator is. DFT-berekeningen suggereren dat de *trans*-Cat gunstiger is voor substraatbinding, aangezien de *cis*-Cat minder ruimte heeft rondom het metaal centrum voor substraat coordinatie. Fotoschakelbare metaalkatalysatoren maken de ruimtelijke en temporele controle van katalytische reacties mogelijk, met aanzienlijke implicaties voor verschillende toepassingen, waaronder het ontwerp van slimme medicijnen en andere biologische processen.



Figuur 2. *Fotoschakelbare katalysator voor het reguleren van de reactieve eigenschappen van cyclisatiereacties in Hoofdstuk 2.*

De azide-reductiereactie is een belangrijke reactie voor de toepassing van fotokatalyse in levende cellen vanwege de lage toxiciteit en biologische inertie van azide-bevattende substraten. In **Hoofdstuk 3** richtten we ons op de activering van pro-probe en prodrug moleculen door een fotokatalytische reductiereactie gebruikmakend van zichtbaar licht. Hiertoe wordt een ruthenium gebaseerde fotokatalysator selectief afgeleverd aan kankercellen met behulp van een gefunctionaliseerde polysaccharide polymeer. Het hyaluronzuur van het polysaccaride polymeer is gefunctionaliseerd met β-cyclodextrine moleculen, die de fotokatalysator kan binden via de adamantaan groep van een analoog van de tris(bipyridine)ruthenium(II)-katalysator . Het polysaccharide hyaluronzuur richt zich selectief op kankercellen, en de gebonden adamantaan-gefunctionaliseerde katalysator kan zo selectief worden afgegeven. Onder bestraling met zichtbaar licht reduceert de ruthenium gebaseerde fotokatalysator de azide in het pro-probe molecuul om zo rhodamine als fluorescerende probe te produceren. Op een vergelijkbare manier kan onder fysiologisch relevante omstandigheden ook een anticancer-medicijn worden gegenereerd door conversie van de azidegroep naar een aminegroep. Katalytische experimenten uitgevoerd in levende cellen tonen aan dat het ruthenium complex selectief kankercellen binnengaat en dat na bestraling met zichtbaar licht rhodamine wordt gevormd. Een vergelijkbare omzetting van prodrug naar het medicijn doxorubicine veroorzaakt de dood van kankercellen (Figuur 3). Deze strategie van fotokatalytische activering van een prodrug biedt ruimtelijke en temporele controle over de synthese van antikankermedicijnen selectief in kankercellen, en opent de weg naar de ontwikkeling van zeer selectieve medicijnen.

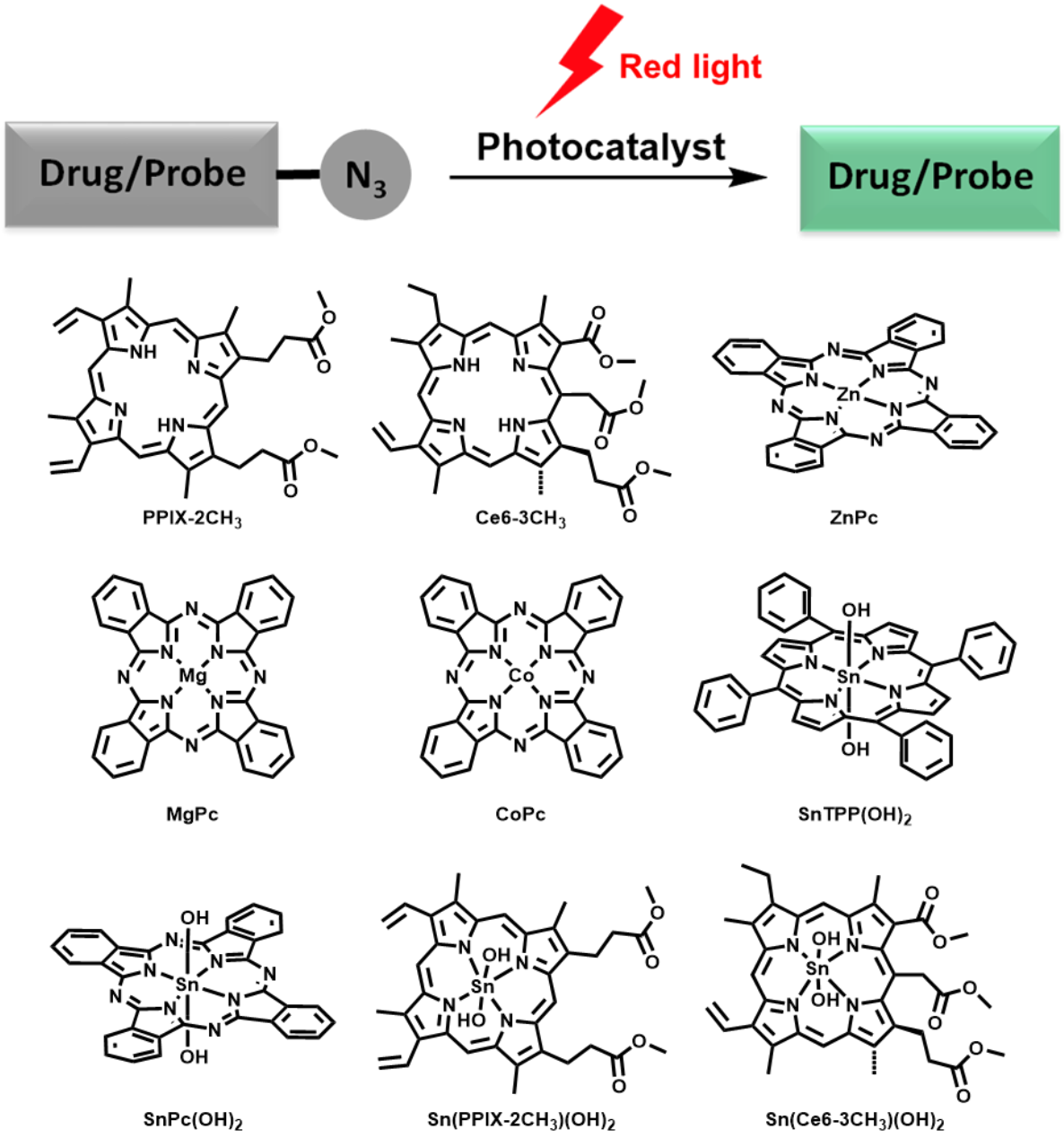


Figuur 3. *De conversie van een pro-probe en een prodrug, geïnduceerd door zichtbaar licht, met behulp van een Ru(bpyAda)(bpy)$_2$⊂HACD supramoleculaire fotokatalysator selectief afgeleverd aan kankercellen, zoals beschreven in Hoofdstuk 3.*

Het principe van fotokatalyse in levende cellen is beschreven in **Hoofdstuk 3**, waarbij gebruik werd gemaakt van blauw licht. In het vervolg van het onderzoek streven we ernaar om rood licht of NIR-licht te gebruiken voor het katalyseren van azide-reductiereacties in levende cellen. Het rode licht heeft grote voordelen ten aanzien van het gebruik van het blauwe licht, zoals lagere biologische toxiciteit en het vermogen om dieper door te dringen in weefsels of tumoren. De belangrijkste uitdaging van rood licht-geïnduceerde fotokatalyse is het ontwikkelen van efficiënte fotokatalysatorsystemen. Deze kunnen in principe op twee manieren worden gemaakt: 1) metaalcomplexen en kleurstof moleculen die direct rood licht absorberen voor fotokatalyse en 2) indirecte methoden waarbij rood met behulp van up-conversie wordt geconverteerd naar hoge energie blauw licht. In **Hoofdstuk 4** bespreken we de directe methode en rapporteren diverse overgangsmetaalcomplexen en organische kleurstoffen die rood licht direct kunnen absorberen. In **Hoofdstuk 5** wordt een indirecte methode besproken, waarbij de upconversie van laag-energetische NIR-lichtfotonen naar hoog-energetische zichtbare lichtfotonen wordt bewerkstelligd met lanthanide upconversion-nanodeeltjes, welke in combinatie met ruthenium gebaseerde fotokatalysatoren leiden to fotoreductie katalyse.

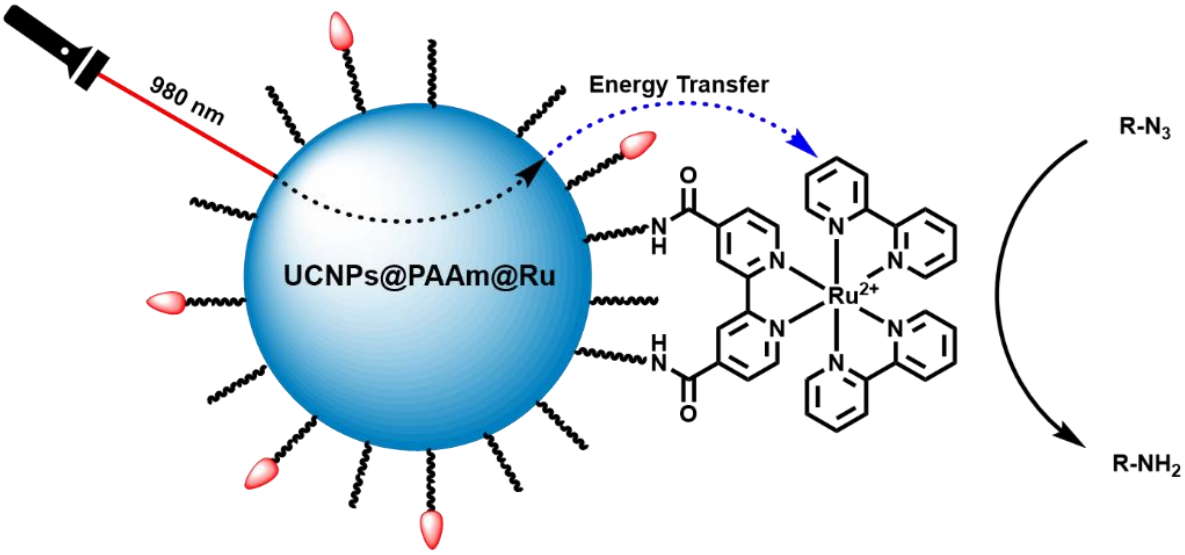
In **Hoofdstuk 4** rapporteren we het onderzoek naar fotokatalysatoren die direct worden geactiveerd door rood licht om de rhodamine probe te produceren onder fysiologisch relevante omstandigheden. Deze fotokatalysatoren vertonen een goede absorptie van rood licht en laten variërende katalytische activiteiten zien voor de omzetting van het rhodamine-azide substraat bij gebruik van verschillende reductiemiddelen, zoals NaAsc, GSH en NADH en onder het beschijnen met 640 nm rood licht. Fotokatalysatoren 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)$(OH)_2$ vertonen een hoge katalytische activiteit voor de omzetting van rhodamine azide (Figuur 4). In

verschillende reacties leidt de aanwezigheid van lucht (zuurstof) tot een afname van de omzetting, hoogstwaarschijnlijk omdat het fungeert als een dover van de geëxciteerde toestand. Om de toepassing van deze fotokatalysatoren binnen levende cellen te vergemakkelijken, werd een supramoleculaire strategie gebruikt om fotokatalysatoren, gefunctionaliseerd met adamantaan, te binden aan hyaluronzuur-polymeren die β-cyclodextrine-eenheden bevatten. Dit om de wateroplosbaarheid te verbeteren, de compatibiliteit met lucht te vergroten en de mogelijkheid te creëren om ze selectief aan kankercellen toe te dienen. Helaas heeft deze strategie nog niet geleid tot efficiënte omzettingen onder fysiologische omstandigheden. Desalniettemin leggen de resultaten een basis voor toekomstige biologische toepassingen met betrekking tot door rood licht geïnduceerde katalyse in levende cellen.



Figuur 4. *Fotokatalyse van azide-reductiereacties met rood licht en de structuren van katalytisch actieve fotokatalysatoren zoals gerapporteerd in Hoofdstuk 4.*

In **Hoofdstuk 5** werd indirecte fotokatalyse met NIR-licht voor de azide-reductiereactie onderzocht door gebruik te maken van upconversie nanodeeltjes. Een fotokatalysator voor zichtbaar licht werd covalent gekoppeld aan lanthanide upconversie nanodeeltjes *via* een condensatiereactie met het poly(allylamine) dat aan de buitenkant van het nanodeeltje zit. Hierdoor krijg je een NIR-geïnduceerd katalytisch systeem. Belangrijk is dat de absorptie van de ruthenium-gebaseerde fotokatalysator en de emissie van het upconversie nanodeeltje een aanzienlijke spectrale overlap vertonen. Effectieve energieoverdracht van de upconversie nanodeeltjes naar de fotokatalysatoren vindt plaats vanwege de gunstige spectrale overlap en de ruimtelijke nabijheid, wat mogelijkheden biedt voor het katalytisch fotoreductie proces (Figuur 5). De azide-reductie reactie om de rhodamine te genereren werd uitgevoerd met 980 nm NIR-licht. Zo werd een aanzienlijke omzetting verkregen, hetgeen de werking van het concept bewijst. De waargenomen katalytische efficiëntie is nog te laag voor toepassingen, mogelijk als gevolg van de lage efficiëntie van energieoverdracht. Als de katalytische efficiëntie beter is dan heeft deze strategie potentie voor toepassing voor NIR-licht geïnduceerde organische synthese van fluorescerende moleculen of medicijnen in levende cellen.



Figuur 5. *Indirecte NIR-fotokatalyse door de koppeling tussen lanthanide upconversie-nanodeeltjes en een rutheniumfotokatalysator, actief bij azide-reductiereacties onder 980 nm NIR-lichtbestraling, zoals gerapporteerd in Hoofdstuk 5.*

Het centrale thema in dit proefschrift is licht gecontroleerde katalyse voor toepassingen in levende cellen. We hebben nieuwe fotokatalytische reacties onderzocht en toegepast onder fysiologische omstandigheden, met zichtbaar blauw licht alsmede rood licht en NIR-licht. De resultaten van het in dit proefschrift beschreven onderzoek dragen bij aan de ontwikkeling van efficiënte, en met licht controleerbare katalytische systemen, die nieuwe methodes oplevert om ons begrip van biologische processen te verdiepen en bij te dragen aan de behandeling van ziektes.

List of Publications

Related to this thesis:

1. *A Photoresponsive Gold Catalyst Based on Azobenzene-Functionalized NHC Ligands*

   Jianghua Liu,[1-5] Eduard O. Bobylev,[2,5] Bas de Bruin,[1,3,4] Joost N. H. Reek.[1,3,8] *Chem. Commun.* **2023**, *59*, 8830-8833.

2. *Self-Assembled Ru-Polysaccharide Photocatalyst for Targeted Visible-Light-Induced Activation of Prodrugs*

   Jianghua Liu,[1-3,6] Dinghao Wu,[6] Sonja Pullen,[3,8] Alexander Kros,[1,6,8] Joost N. H. Reek.[1,3,6,8] *Manuscript in preparation.*

3. *Development of Red Light Photocatalysts for Azide Reduction Reactions*

   Jianghua Liu,[1-3] Sonja Pullen,[3,8] Joost N. H. Reek.[1,3,8] *Manuscript in preparation.*

4. *Near-Infrared Light Induced Azide Reduction Reactions Using a Photocatalyst Linked to an Upconversion Nanoparticle*

   Jianghua Liu,[1-3,7] Jun Yuan,[1,3,7] Sonja Pullen,[3,8] Hong Zhang,[7,8] Joost N. H. Reek.[1,3,8] *Manuscript in preparation.*

[1] Conceptual idea
[2] Experimental work
[3] Preparation of manuscript
[4] Computational study
[5] ESI-MS measurements
[6] In living cells application
[7] Nanoparticle preparation
[8] Project supervision

Other work:

5. *Targeted Polypeptide-Microtubule Aggregation with Cucurbit[8]uril for Enhanced Cell Apoptosis*

   Ying-Ming Zhang, **Jiang-Hua Liu**, Qilin Yu, Xin Wen, and Yu Liu. *Angew. Chem. Int. Ed.* **2019**, *58*,10553-10557.

6. *Photocleavable Supramolecular Polysaccharide Nanoparticles for Targeted Drug Release in Cancer Cells*

   Jiang-Hua Liu, Xianjing Wu, Ying-Ming Zhang and Yu Liu. *Asian J. Org. Chem.* **2018**, *7*, 2444-2447.

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