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Triplet-Triplet Annihilation Upconversion-Based Photolysis: Applications in Photopharmacology

Maxime Klimezak, Juliane Chaud, Anaïs Brion, Frédéric Bolze, Benoit Frisch, Béatrice Heurtault, Antoine Kichler, and Alexandre Specht*

The emerging field of photopharmacology is a promising chemobiological methodology for optical control of drug activities that could ultimately solve the off-target toxicity outside the disease location of many drugs for the treatment of a given pathology. The use of photolytic reactions looks very attractive for a light-activated drug release but requires to develop photolytic reactions sensitive to red or near-infrared light excitation for better tissue penetration. This review will present the concepts of triplet-triplet annihilation upconversion-based photolysis and their recent in vivo applications for light-induced drug delivery using photoactivatable nanoparticles.

The emerging field of photopharmacology is a promising chemobiological methodology for optical control of drug activities. It is based on the incorporation of light-responsive moieties into the structure of drugs allowing reversible or irreversible modulation of their activity and could ultimately solve the off-target toxicity outside the disease location of many drugs for the treatment of a given pathology.

The reversible strategy has already been extensively reviewed and is based on the uses of molecular photoswitches.^[10,11,16] In this process, light triggers the reversible isomerization between two forms and allows

the modulation of biological activities if the two isomers of the photoactivatable drug possess different potencies against the desired target.

Conversely, irreversible modulation is based on the incorporation of photocleavable protecting groups (PPGs),^[17,18] also called photocages, in order to mask the pharmacophoric features of a drug. The so-called “uncaging” process irreversibly removes the photolabile moiety, leading to a local concentration jump of the parent drug. A second and irreversible strategy based on the concept of ligand-directed photocatalysts for the release of a biological effector has recently been developed.^[19–21] A detailed description of this strategy called catalytic bioorthogonal uncaging is however outside the scope of this review.

For biomedical applications, the “uncaging” method looks extremely attractive since it allows for a spatio-temporal controlled drug release. The most popular PPGs, which were first introduced to control different biological processes in vitro, are the *o*-nitrobenzyl-based systems and their derivatives.^[22] Other PPGs (like *o*-nitrophenethyl, coumarinyl, or phenacyl-based PPGs) have also been developed in particular to increase the efficiency of the photolytic reactions.^[18] The major drawback of many of those PPGs relies on the use of UV irradiation for the uncaging process, which is characterized by low penetration depth in living tissues due to light absorption of native biological chromophores, high diffusion by scattering tissues, leading to background cellular phototoxicity. Therefore, far red and Near Infra Red (NIR)-sensitive PPGs are highly sought after in order to reach a few cm for light penetration. Indeed, in the so-called photo-therapeutical window (between 600 and 1000 nm) light scattering is moderate and there is little competing absorption from cells or tissues endogenous biomolecules.^[23] Three different strategies have been explored to overcome the difficulty that only high-energy light can induce photolytic reactions.

1. Introduction

Chemical-based delivery systems that allow for a non-invasive control of the release of drugs are highly sought after since they could enhance the control over drug concentration at a specific site of action.^[1–4] Therefore, various strategies have been developed for triggering drug release by internal stimuli (based on endogenous metabolic conversions, pH, enzymatic activation, temperature, etc.) or external stimuli (e.g., ultrasound and light). Light^[5–15] is a particularly attractive source of external stimulation due to its high spatio-temporal precision and bio-orthogonality.

M. Klimezak, J. Chaud, F. Bolze, A. Specht
Laboratoire de Chémo-Biologie Synthétique et Thérapeutique (CBST)
Équipe Nanoparticules Intelligentes
Université de Strasbourg
CNRS, CBST UMR 7199, Illkirch Cedex F-67401, France
E-mail: specht@unistra.fr

J. Chaud, A. Brion, B. Frisch, B. Heurtault, A. Kichler
Inserm UMR_S 1121
EMR 7003 CNRS
Université de Strasbourg
Biomaterials and Bioengineering
Centre de Recherche en Biomédecine de Strasbourg
1 rue Eugène Boeckel, Strasbourg F-67000, France

The ORCID identification number(s) for the author(s) of this article can be found under <https://doi.org/10.1002/adhm.202400354>

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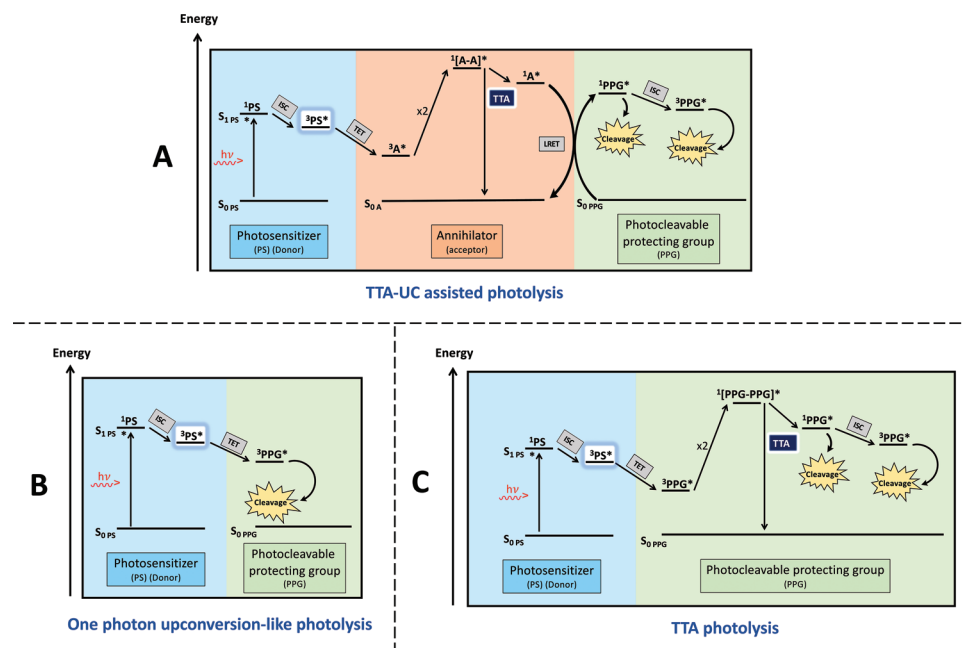


Figure 1. Principle and simplified Jablonski diagrams for TTA-UC assisted photolysis (A), one photon upconversion-like photolysis (B), and TTA photolysis (C).

The first strategy is based on the recent development of red and NIR-sensitive PPGs, in particular, based on mesoBODIPY^[24] and Xanthenium^[25] moieties.

The second strategy, which has been extensively reviewed by us and others,^[26–30] is related to the use of two-photon excitation (TPE) to perform the photolytic reaction. In TPE, a chromophore will not reach an excited state by absorption of one photon with the energy $E = h\nu$, but by the simultaneous absorption (within <1 fs) of two photons of half the energy $E' = h\nu/2$. Due to the quadratic dependency of this non-linear process, focalized (for spatial compression of photons) and pulsed excitation light (for temporal compression of photons) is required (with pulses in the fs-ns range with peak power in the range of 10^8 W cm⁻²). This leads to high control of the photoactivation zone in a very small 3D volume (few fL) surrounding the focal point of the optical system. However, for biomedical applications, two-photon absorption is a time-consuming spot-by-spot scanning process to expose macroscopic materials, like tissues or organs, and requires expensive equipment.

Those two strategies have already been used for the development of efficient optical probes to dissect virtually any type of cellular process in cell physiology.^[29] For a detailed overview of the history of PPG development, we refer readers to two recent reviews.^[29,31]

Molecular engineering of far red to NIR sensitive PPGs using one-photon or TPEs usually leads to PPGs with extended π -systems and low aqueous solubilities which in turn restricts their use in photopharmacology. One strategy to overcome this hurdle consists of using upconversion nanoparticles (UC-NPs). Therefore, light UC-NPs have been recently introduced as a third strategy to perform a photolytic reaction using far red or NIR excitation.^[15] The UC process has been established since the

1960s to generate anti-Stokes shift luminescence.^[32] This process relies on the existence of multiple intermediate states to accumulate low-energy excitation photons. Those systems are able to absorb energies from two or more photons and to generate subsequently one emission photon with higher energy. UC process can take place in organic (mostly using triplet-triplet annihilation [TTA] of organics dyes) and inorganic (mostly using Lanthanide-based NPs) materials and many high brightness upconversion systems have been reported recently using nanomaterials.^[33] Lanthanide (Ln) doped materials were first investigated in the context of UC-assisted uncaging. For this purpose, inorganic lanthanide-based upconverting nanoparticles (L-UCNPs) have been widely studied.^[34–38] However, they still suffer mostly from a high-power excitation requirement (10^1 – 10^4 W cm⁻²) and inherently modest UC quantum yields owing to low absorptions and emission cross-sections. Therefore, alternative and more biocompatible UC approaches have been recently developed, particularly using another type of UC system based on TTA upconversion (TTA-UC) of organic chromophores.^[39,40] The UC-NPs based on TTA of organic chromophores have been used to assist a photolytic reaction using low-power far red or NIR excitations. **Figure 1** summarizes the 3 TTA-UC-based strategies from a photophysical point of view that will be discussed in this review. Those strategies have been recently used for the preparation of nanoparticles for a light-assisted drug release. Notably, the uses of nanoparticles offer additional advantages such as the possibility to add targeting motifs at the surface of the particles.

In this review, we will focus on the recent development of TTA-UC NPs able to perform TTA-UC-based uncaging using excitation in the photo-therapeutic window, with particular attention on the in vivo applications of such drug delivery systems (DDS).

2. TTA-UC-Based Photolysis: Toward In Vivo Applications of the Uncaging Concept

2.1. TTA-UC: Principle

TTA-UC was first reported by Parker and Hatchard in 1962^[41] at low temperatures and by Balushev and co-workers in 2003 in solution.^[42] In the past 20 years, this UC process has been applied to various fields, such as solar energy conversion,^[43] photocatalysis,^[44,45] organic light-emitting diode (OLED),^[46] bioimaging,^[47] and biomedicine,^[48] using TTA-UC based materials including polymeric solids, micro and nanocapsules, nanofibers, and lipidic nanoparticles.^[49]

TTA-UC is achieved through several photochemical processes using a chromophore pair (e.g., a sensitizer and an acceptor) leading to the conversion of low-energy photons, typically in the far red or near-infrared (NIR) range, into higher-energy photons, usually in the visible or ultraviolet range. This anti-Stokes phenomenon involves the following key concepts: first, the sensitizer (also called donor) absorbs a photon leading to the population of a triplet state form through an intersystem crossing (ISC) from a singlet excited state. Second, the sensitizer with an appropriate triplet energy level is able to transfer energy to an acceptor (also called annihilator) through a dexter type triplet-triplet energy transfer (TTET). Third, two acceptors in a triplet excitation state will undergo a TTA to an acceptor in its ground state together with an acceptor in a singlet high-energy excitation state. Finally, if this latter state is emissive, an anti-Stokes delayed luminescence is observed upon a radiative relaxation.

To evaluate the efficiency of TTA-UC, the quantum yield (Φ_{UC}) which is defined as the incident photon-to-luminescence electron ratio (UC emission divided by the number of absorbed photons) is a key parameter and can be theoretically calculated by multiplying the quantum yields of each individual process described above.

$$\phi_{UC} = \phi_{ISC} \times \phi_{TTET} \times \phi_{TTA} \times \phi_f \quad (1)$$

where Φ_{ISC} , Φ_{TTET} , Φ_{TTA} , and Φ_f are the quantum yields of respectively the ISC of the sensitizer, the donor-to-acceptor TTET, the acceptor-acceptor TTA, and the fluorescence of the acceptor.

However, it is practically very difficult to determine TTA-UC quantum yield by measuring the quantum yields of each individual step and two practical ways are used: by actinometry or using an integrating sphere. The first method requires a quantum counter or an internal standard as a reference material and the Φ_{UC} value can be calculated as follows:

$$\Phi_{UC} = \Phi_{std} \times (A_{std}/A_{UC}) \times (I_{UC}/I_{std}) \times (\eta_{UC}/\eta_{std})^2 \quad (2)$$

where Φ_{std} is the quantum yield of the luminescent reference material, A_{std} is the absorbance of the reference material at the excitation wavelength, A_{UC} is the absorbance of the UC material at the excitation wavelength, I_{UC} is the photoluminescence of the UC material, I_{std} is the photoluminescence intensity of the reference material, η_{UC} is the refractive index of the matrix of the UC material, and η_{std} is the refractive index of the matrix of the reference material.

The second method is based on the direct use of an integrating sphere to simply evaluate the Φ_{UC} . The interior of this device can collect and gather in one outlet all emitting light of a sample. Therefore, using a shortpass filter, through which only wavelengths shorter than that the one of the incident light passes, the upconverted fluorescence quantum yield can be directly evaluated. The conversion of low-energy photons to higher-energy photons using TTA-UC materials requires several essential prerequisites. The energy of the triplet state of the sensitizer must be higher than the energy of the triplet state of the acceptor. Furthermore, a high ISC yield for the sensitizer is preferred in order to have an efficient UC. A long triplet lifetime (more than microseconds) for both chromophores is preferred to provide enough time for both the TTET and the TTA processes to occur.

Since both TTET and TTA are Dexter-type energy transfer processes, both chromophores should be within 1 nm, and efficient TTA-UC processes are observed if a good chromophore diffusivity is present in the matrix.^[50] Finally, the concentration of UC chromophores, the choice of the media or materials where the UC process occurs, the presence of oxygen,^[51,40] the excitation wavelength, and the operating temperature^[52] also have an influence on the efficiency of the UC.

2.2. TTA-UC Nanomaterials Developed for Green to Blue, Red to Blue, or NIR to Green Light UC

To address most of the limitations of inorganic UCNPs (e.g., requirements of a high power density excitation source, narrow absorption band, relatively low quantum yield, and possible toxicity of lanthanide ions in the target sites), organic TTA-UC systems have been recently introduced for applications in bioimaging^[47] and phototherapy.^[48] A series of TTA sensitizers, such as green-absorbing platinum/palladium octaethylporphyrin (PtOEP, 1/PdOEP, 2), red-absorbing meso-tetraphenyl-tetrabenzoporphyrin platinum (II)/palladium (II) (PtTPBP, 3/PdTPBP, 4), near-red absorbing palladium (II) octobutoxyphthalocyanin (PdPc(OBu)₈) 5 and the B,N-heteroarene-based sensitizer (BNS) 7, metal-free BODIPY-based photosensitizers like 2,6-diodo-BODIPY 6 and BDP-F 8 (Figure 2) and emitters like perylene 9, *t*-butylperylene 10, 9,10-bis((triisopropylsilyl)ethynyl)anthracene (TIPS-BEA) 11, Rubene 12, 9, 10-diphenylanthracene (DPA) 13, and 9-phenylacetyleneanthracene (PEA) 14, have been used to prepare TTA-UC NPs which are able to convert green, red, or NIR light to blue or green light. In particular, liposomes^[53] and polymeric micelles^[54] composed of the platinum (II) octaethylporphyrin 1/9,10-diphenylanthracene DPA 13 couple have been developed for green to blue light upconversion. Other systems have also been reported, such as liposomes^[53-55] and lipidic nanocapsules,^[56] composed of PdTPBP 4/perylene 9 or *t*-butylperylene 10 for red to blue light upconversion and mesoporous silica nanoparticles (MSN)^[57] composed of BDP-F 8 and PEA 14 for far red to blue light upconversion.

All the spectral properties and the photochemical properties of those TTA-UC chromophores and TTA-UC NPs are summarized in Table 1. Noteworthy, the applications of those TTA-UC NPs in the field of drug delivery will be discussed in Section 2.6.

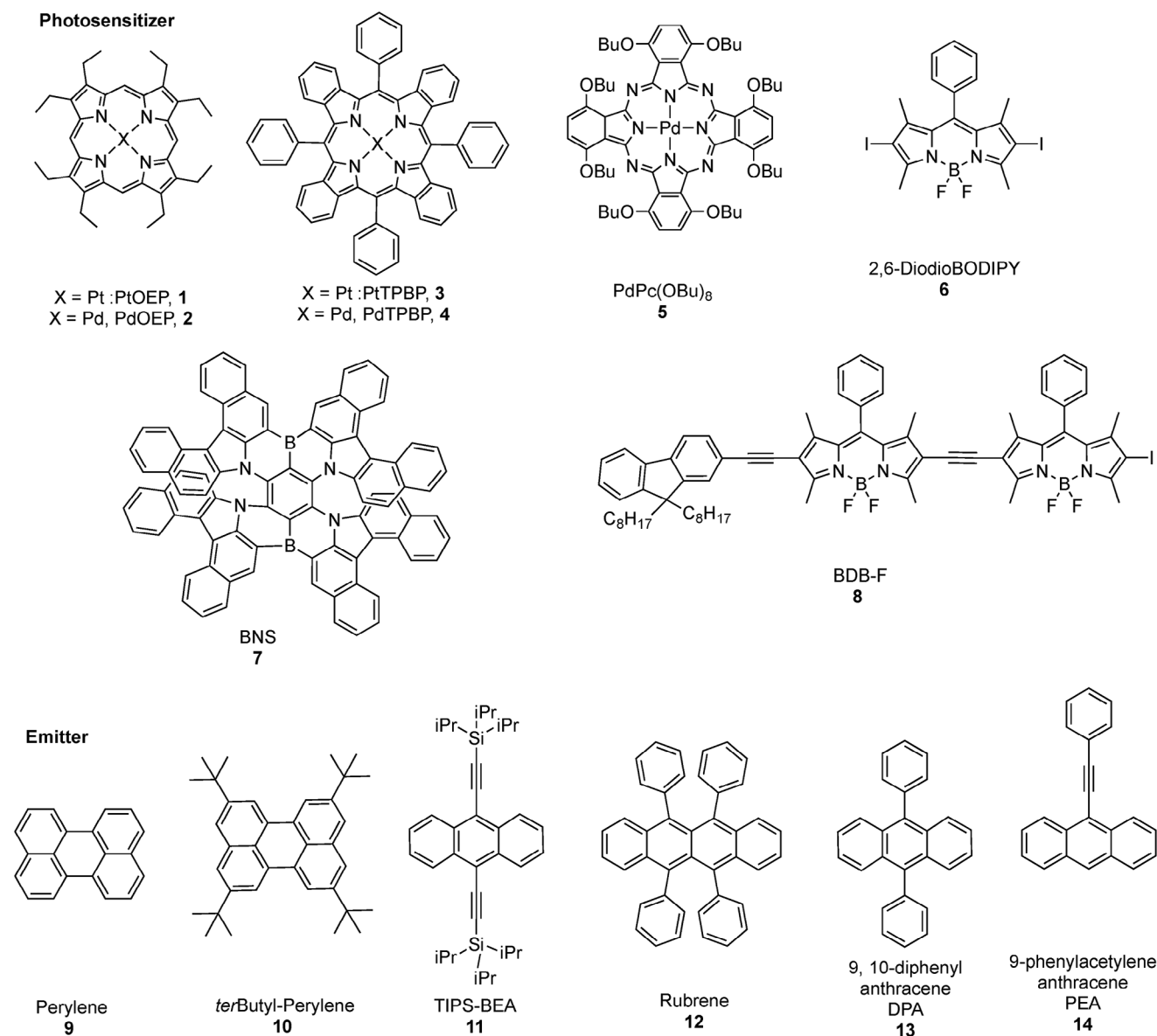


Figure 2. Chemical structures of common photosensitizers and emitters used for the preparation of TTA-UC NPs for NIR/red to blue or green to blue light upconversion.

2.3. Uncaging: Principle and Molecular Engineering for the Development of Efficient Blue and Green Light Sensitive PPGs

For biomedical applications, the use of photolytic reactions looks very attractive for light-activated drug release. Photoactivatable pro-drugs using organic PPGs should allow to implement the uncaging concept for various therapeutical applications. The efficiency of a PPG to undergo a specific chemical bond cleavage using classical one-photon excitation (also called uncaging cross-section) is defined as the product of the molar extinction coefficient (ϵ) and the quantum yield of photolytic reaction (ϕ_{li}). In the last decade, various PPGs have been described for bond cleavage using visible light. Coumarinyl, meso-BODIPY, and perylen-3-ylmethyl PPG have been described during the last decade^[10,15,17,18,24,25,63] for their blue or green light sensitiv-

ity which nicely overlaps with the emission properties of TTA-UC NPs (see Section 2.2). Coumarinyl PPGs have also been extensively optimized leading to interesting PPGs for blue to green light excitation.^[64–71] The mechanism of photocleavage of coumarin PPGs is based on a heterolytic bond cleavage from the first singlet excited state (S_1) of this chromophore leading to the formation of contact ion pair of a methylcoumarinyl carbocation and a payload anion. Starting from the 385 nm sensitive 7-diethylaminocoumarin (DEAC), Ellis-Davies in 2013, reported an acrylamide extended coumarinyl PPG in position 3 leading to a blue light sensitive chromophore with an increase of the quantum yield value.^[64]

The same year, Jullien reported respectively a sulfur and a dicyanomethylene substitution in position 2 leading to the blue and green light-sensitive PPGs **15** and **16** (Figure 3).^[66] Zhu's group

Table 1. Photophysical and photochemical properties of several sensitizer/annihilator couples for green to blue, red to blue, or NIR to green light UC.

Sensitizer/annihilator couple	UC range [nm]	ΔE_{uc} [eV]	Φ_{uc} [%]	Ref.
PtOEP 1/DPA 13	532 to 430	0.50	4.5 for silica NPs in water 2.3 for liposome in water 1.9 for polymeric micelles in water	[58] [53] [54]
PdTPBP 4/Perylene 9	635 to 470	0.69	7.2 in a toluene solution 0.5 for liposome in water	[59] [53]
PdTPBP 4/terButyl-Perylene 10	635 to 470	0.69	0.5 for liposome in water 5.4 for lipidic nanocapsules in water	[55] [56]
PdPc(OBu) ₈ 5/Rubrene 12	730 to 570	0.48	ND	[60]
2,6 Diiodo BODIPY 6/Perylene 9	532 to 470	0.30	8.77 in a Toluene solution	[61]
BDP-F 8/PEA 14	650 to 432	0.96	2 for MSN in water	[57]
BNS 7/TIPS-BEA 11	721 to 470	1.03	2.9 in a Toluene solution	[62]

ND = not determined.

reported several 3 and 2 position modifications of the DEAC chromophore too.^[67] In particular, the electron-rich styryl appendages at the 3-position and the incorporation of julolidine moieties significantly increased the photolytic reaction quantum yield of blue light and green light-sensitive PPG (see DEACAS and compounds 17 and 18). The Kele group introduced in 2019 various donor systems at the 3-position of the DEAC PPGs leading in

particular to a water-soluble and green light-sensitive benzothiazolium derivative 19.^[69] However, this latter compound showed a substantial decrease in the uncaging quantum yield. In 2021, our group was able to develop rigid and electron-rich coumarinyl derivatives obtained by domino reactions initiated by a 5-*exo*-Dig cyclocarbopalladation (see PPG 20) leading to a 2.8 times increase of the uncaging efficiency for a blue light excitation.^[70]

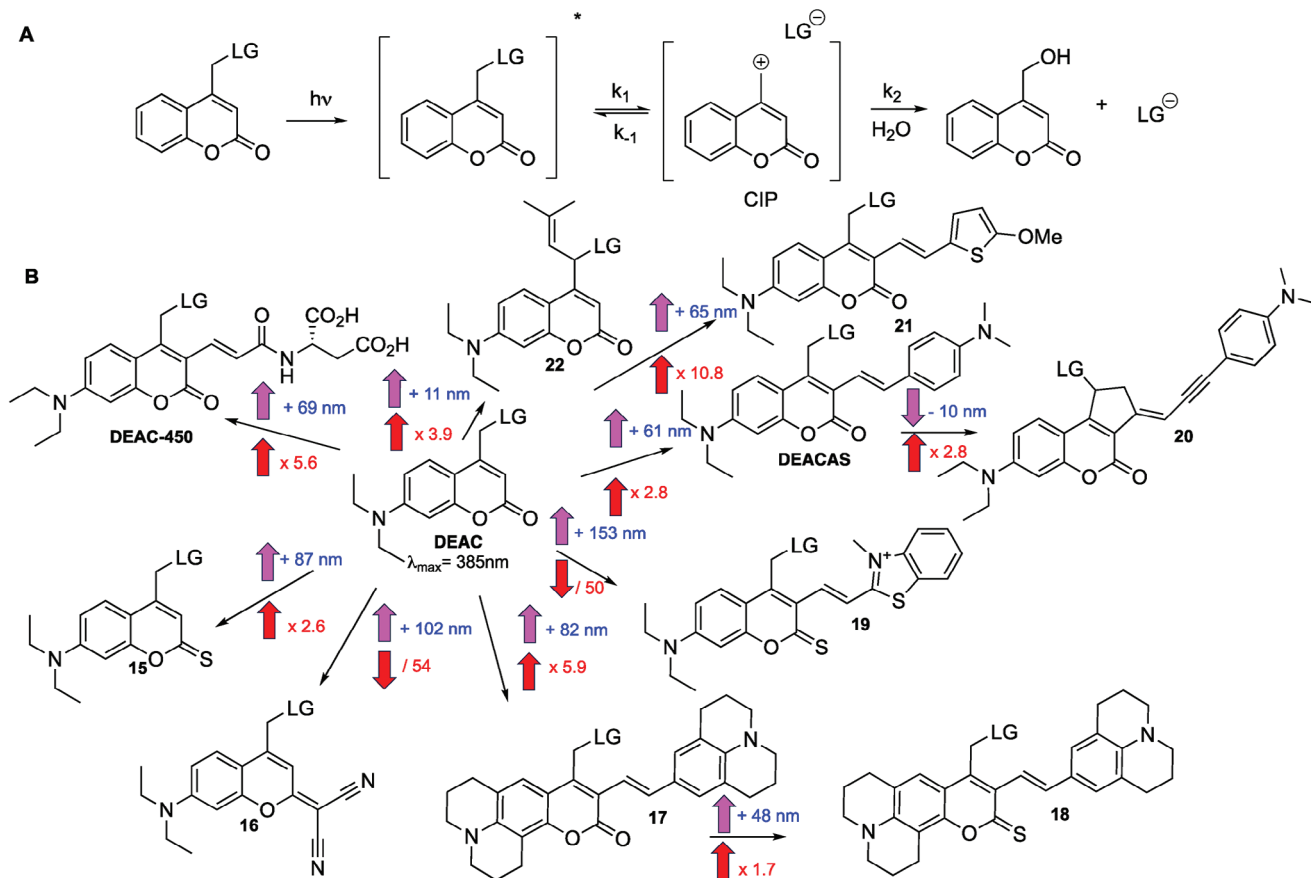


Figure 3. A) Coumarinyl-based PPG photocleavage mechanism. B) Molecular engineering of the DEAC PPG and influence on the absorption maximum (pink arrows; top number) and the quantum yield for the release of a benzoic acid payload (red arrows).

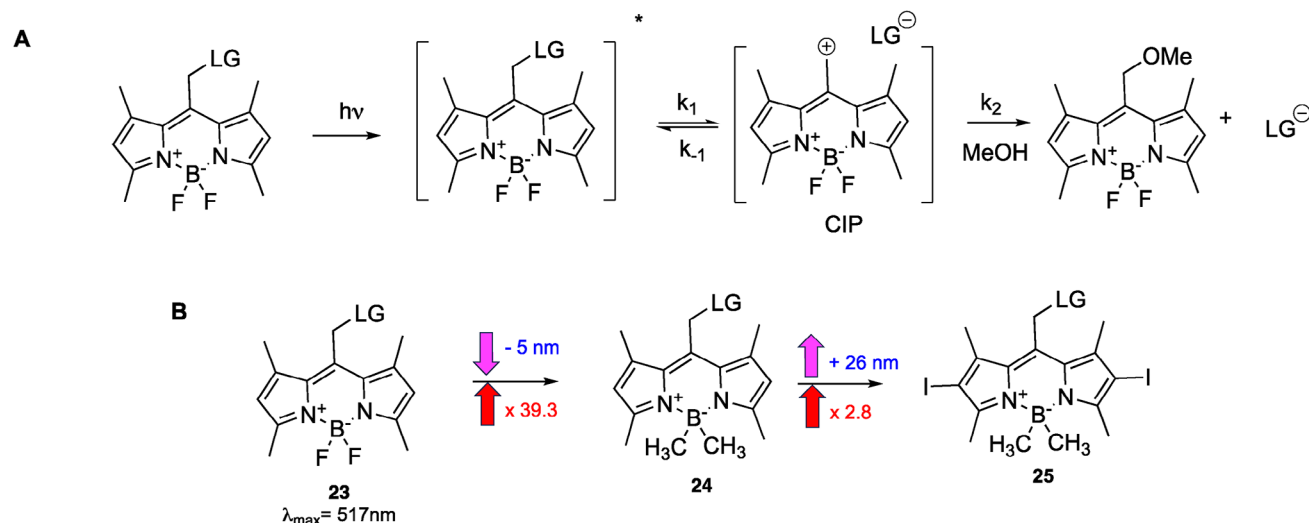


Figure 4. A) BODIPY-based PPG photocleavage postulated mechanism in MeOH. B) Molecular engineering of green light sensitive BODIPY-based PPGs and influence on the absorption maximum (pink arrows; top number) and the quantum yield for the release of acetic acid (red arrows).

In 2023, Zhu and co-workers reported the introduction of electron-rich thiophene at position 3 of the DEAC chromophore leading to the PPG 21 with a 0.76 uncaging quantum yield.^[71] In addition, the rational design of DEAC analogs substituted at the benzylic position also led to a significant increase in the uncaging quantum yield of the DEAC PPGs, in particular, the stabilization of an intermediate chromophore cation using an allylic substituent at the benzylic position (see compound 22) led almost to a four times increase of the uncaging quantum yield.^[72] This latter strategy should be explored in the future to increase the uncaging efficiency of blue or green light-sensitive coumarinyl chromophores and should lead to even more efficient PPGs.

In 2005 *meso*-methyl BODIPY PPGs were independently introduced by Weinstain and Winter.^[73,74] They reported BODIPY-based PPGs capable of direct photoheterolytic release of various payloads using a green light excitation (Figure 4A for the postulated photolytic mechanism). However, this first *meso*-methyl BODIPY PPG 23 showed a relatively low uncaging quantum yield. The same groups were able to dramatically increase *meso*-methyl BODIPY's uncaging efficiencies by systematic structure-activity relationship studies.^[75] In particular, dialkylborono analogs exhibited up to a 40-fold increase in the uncaging quantum yield leading to compound 24 (Figure 4B). In addition, BODIPYs substituted by electron-withdrawing groups, like iodine, are also able to increase the uncaging quantum yield (see compound 25). All the spectral properties and the photochemical properties of those PPGs are summarized in Table 2. In summary, synthetic chemists have developed during the last decade visible and NIR light-sensitive PPGs leading especially to highly efficient PPGs for green or blue light photoactivation. However, most of those chromophores are highly hydrophobic and/or are not suitable for red light or NIR light excitation. Nevertheless, they look very attractive for the development of NPs able to release a payload using light, in particular by combining them with TTA-UC NPs.

Table 2. Photophysical and photochemical properties of perylen-3-ylmethyl, coumarinyl, and *meso*-BODIPY PPGs for the release of a *p*-methoxybenzoate payload.

Compound	Abs. λ _{max} [nm]/ε [M ⁻¹ cm ⁻¹]	Φ _u	ε Φ _u [M ⁻¹ cm ⁻¹] at λ _{max}	Ref.
Perylen-3-ylmethyl	439/62 000	0.093	5770	[63]
DEAC	385/20 000	0.07	1400	[67]
DEAC-450	454/43 000	0.39	16 770	[64]
15	472/31 000	0.18	5580	[65]
16	487/33 000	0.0013	43	[65]
17	467/35 000	0.41	14 350	[69]
18	515/25 000	0.7	17 500	[67]
19	538/40 500	0.0014	57	[71]
DEACAS	446/41 000	0.2	8200	[67]
20	437/42 500	0.57	24 225	[70]
21	450/45 000	0.76	34 200	[71]
22	396/13 800 ^{b)}	0.27 ^{b)}	3726	[72]
23	517/71 000	0.0014 ^{b)}	99	[24]
24	512/69 000	0.055 ^{b)}	3795	[24]
25	538/61 000	0.156 ^{b)}	9516	[24]

^{a)} Based on the release of a glutamate payload ^{b)} Based on the release of an acetate payload.

2.4. TTA-UC Assisted Photolysis: Principle and Application to the Design of Light Activatable DDS

Since TTA-UC NPs can efficiently convert two or more low-energy continuous wave NIR or red photons into a higher energy photon, TTA-UC-based nanoparticles can serve as photon-upconverting transducers to assist the uncaging process (Figure 1A). The first example of the use of such TTA-UC NPs for the activation of a prodrug was reported by Bonnet et al. for the activation of ruthenium (II) polypyridyl compounds

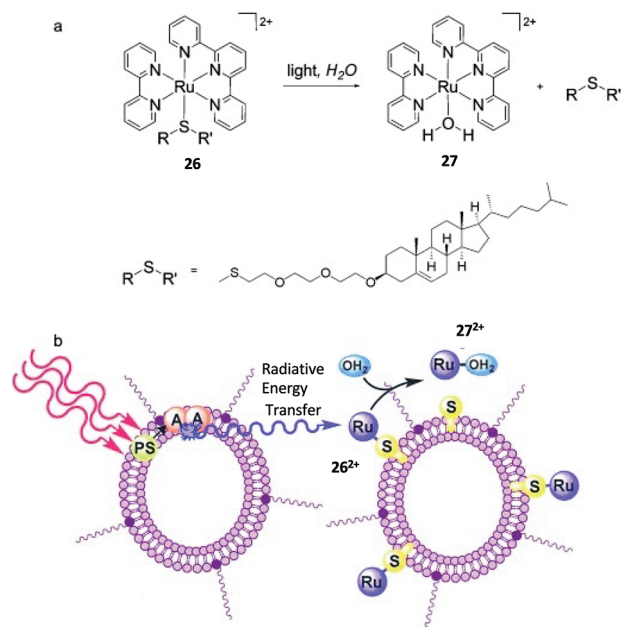


Figure 5. A) Chemical structures of $[\text{Ru}(\text{tpy})(\text{bpy})(\text{SRR}')]^{2+}$ **26** and $[\text{Ru}(\text{tpy})(\text{bpy})(\text{H}_2\text{O})]^{2+}$ complex **27** and the conversion of **26** into **27**. b) The TTA-UC process in the lipid bilayer for activating localized ruthenium complexes using a photosensitizer (PS) and an annihilator (acceptor A). Adapted with permission.^[53] 2014, Wiley-VCH GmbH.

using red light.^[53] In this case, light-sensitive liposomes bearing ruthenium (II) polypyridyl compounds have been photoactivated by radiative energy transfer using TTA-UC liposomes (**Figure 5**). Therefore, blue light-sensitive ruthenium (II) polypyridyl-functionalized liposomes (e.g., $[\text{Ru}(\text{tpy})(\text{bpy})(\text{SRR}')]^{2+}$ **26** was mixed with TTA-UC liposomes obtained using the PdTPBP 4/perylene **9** TTA-UC couple) were used to trigger the hydrolysis of the Ru-S bond under a 630 nm irradiation and this, in turn, led to the release of the cytotoxic $[\text{Ru}(\text{tpy})(\text{bpy})(\text{H}_2\text{O})]^{2+}$ complex **27** (**Figure 5**). The same group further studied a more efficient procedure for the activation of the ruthenium prodrug by using the TTA-UC NPs taking advantage of Förster resonance energy transfer (FRET)-induced photolysis.

They used PEGylated liposomes with three photoactive components (e.g., the PdTPBP **4**, perylene **9**, and prodrug **26**) in the same liposome and were able to demonstrate that the efficiency of the photolysis was increased by this non-radiative energy transfer.^[76] In addition, Bonnet and co-worker were also able to demonstrate that the addition of antioxidants in the liposome formulation can overcome the problem of sensitivity of such TTA-UC liposomes to dioxigen.^[77]

In such an uncaging strategy the efficiency can be calculated as

$$Q_E = \epsilon_s \times \Phi_{\text{UC}} \times L_{\text{FRET}} \times \Phi_u \quad (3)$$

where Q_E is the photolytic efficiency, ϵ_s is the molar extinction coefficient of sensitizers, Φ_{UC} is TTA-UC quantum yield, L_{FRET} is the FRET efficiency, and Φ_u is the photolytic quantum yield of PPGs.

In 2005, Kohane and co-workers were able to perform a TTA-UC-assisted photolysis of the DEAC caged cell-binding peptide cyclo-Arg-Gly-Asp-Lys (cyclo-RGDfK).^[54] Therefore, they used the PtOEP (**1**) photosensitizer and DPA (**13**) annihilator into PLA-PEG polymeric micelles functionalized with DEAC-caged cyclo-RGDfK in order to perform a photo-controlled binding to the cell surface (by a TTA-UC followed by FRET assisted photolysis) with green light LED irradiation at low irradiance for short periods.^[54] Using the same type of polymeric micelles this group was able to demonstrate that photo-targeting can improve the targeting of nanoparticles to tumors.^[78] In order to improve the anti-stokes shifting from far-red to deep-blue light and to optimize this type of light activatable DDS, Han et al. introduced a new TTA system.^[57] A TTA-UC core-shell structured MSN was developed in two steps. First, metal-free BDP-F molecule **19** (a highly far-red-sensitive photosensitizer) and PEA **25** (a deep-blue emitter) were used. Second, a layer containing the prodrug DEAC-chlorambucil was added to the TTA-MSN. This TTA-UC nanoplatform was stimulated by a 650 nm LED light leading to the release of the anticancer prodrug chlorambucil in vivo by a TTA-UC-assisted photolysis.

Our group was also able to develop such a type of photoactivatable DDS.^[79] First, we were able to use lipid nanocapsules^[56] (LNC) in order to increase the UC quantum yield since this type of lipidic nanoparticle can accommodate PdTPBP 4/*t*-butylperylene **10** in its hydrophobic lipid core. Second, we were able to covalently graft a prodrug (melphalan) to the surface of the NP by using a stable carbamate linkage leading to a totally inactive DDS in the absence of light but being activated upon light irradiation. Third, for the preparation of our photocleavable linker, defined as bifunctional PPG derivatives that can be linked to our LNCs and release a drug, we decided to use a DEACAS PPG in order to have a perfect match between the emission of the *t*-butylperylene **10** and the absorption of the PPG and to increase the FRET efficiency. This TTA-UC-based approach allowed us to quantitatively release the anticancer drug melphalan (prodrug **28**) using a red-light LED (**Figure 6**). To note, we were also able to develop a liposome-based DDS using the same strategy.^[55]

2.5. Upconversion-Like Photolysis: Principle and Application to the Design of Light Activatable DDS

The TTA-UC is based on the development of a long-living triplet state sensitizer and a Dexter-type energy transfer to a luminescence emitter, in order to use a triplet state sensitizer to trigger a photolytic reaction (**Figure 1B**). Wang and co-workers verified that during the TTET process, the energy can be directly transferred to a PPG without the participation of emitter molecules.^[80] Therefore, uncaging with PPGs that require a triplet-activated state for their uncaging mechanism could be activated by a photosensitizer with a higher Triplet excited state energy level (T_1) and a lower first excited singlet state level (S_1) than the PPG (of course if the PPG mechanism requires a T_1 intermediate). This first upconversion-based photolysis was achieved using PdTPBP **3** as a photosensitizer and a chlorambucil prodrug using the BODIPY PPG **25**.^[80]

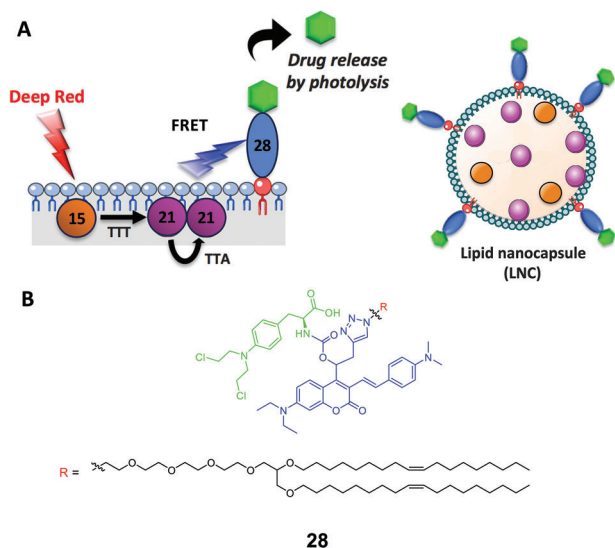


Figure 6. A) Design of light-activable NP using TTA-UC assisted drug-delivery system: upconversion couple photosensitizer (15) and Acceptor (21) are integrated into the core of the LNC and the caged drug (28) is anchored at its surface allowing efficient deep-red light TTA-UC assisted photolysis. b) Structure of the photoactivatable melphalan prodrug 28. Adapted with permission.^[56] 2022, Wiley-VCH GmbH.

In such a strategy the uncaging efficiency can be calculated as

$$Q_E = \epsilon_s \times \phi_{ISC} \times \Phi_{TTET} \times \Phi_u \quad (4)$$

where Q_E is the photolytic efficiency, ϵ_s is the molar extinction coefficient of sensitizers, Φ_{TTET} is the TTET quantum yield between the sensitizer and the PPG, and Φ_u is the photolytic quantum yield of PPGs.

This very interesting way to release a drug using a one-photon upconversion-like photolysis strategy enables to cleave green light-activatable prodrugs with red light in the presence of a red light-excitable photosensitizer. The concept was extended to the preparation of photoactivatable DDS by loading the two components (e.g., the PtTPBP photosensitizer and the BODIPY-based chlorambucil prodrug) in biocompatible and biodegradable polymeric micelles. The therapeutic effect of this red-light photoactivatable DDS has been demonstrated through *in vitro* and *in vivo* studies, indicating promising potential in cancer therapy.^[81]

Very recently this concept led to the development of NIR (690 nm) sensitive DDS. Therefore, the group of Wang developed several prodrugs of chlorambucil, vadimezan, indomethacin, naproxen, ibuprofen, benzyloxycinnamic acid, tetracaine, dopamine, tyramine, and homoveratrylamine using the iodoBODIPY PPG 25 in combination with the Os(btpy)₂²⁺ photosensitizer 29 in PLA/PEG polymeric nanoparticles (Figure 7).^[82]

2.6. TTA Photolysis: Principle and Application to the Design of Light Activatable DDS

In a second and probably a more general process, developed by Han^[83] and co-workers called triplet-triplet annihilation photolysis (TTAP) (also called triplet fusion driven photolysis more re-

cently), the photosensitizers absorb low-energy long-wavelength photons and reach their singlet excited state, subsequently, they populate their long-lived triplet excited state through rapid ISC, then they undergo collisions to a PPG and transfer their energy by TTET to this latter compound. Then two PPGs in a triplet excitation state can undergo a TTA to form a PPG in its ground state together with a PPG in a singlet high-energy excitation state (Figure 1C). Consequently, if this latter excited state of the PPG drives a photolytic reaction the uncaging of the biological effectors occurs.

In such a strategy the uncaging efficiency can be calculated as

$$Q_E = \epsilon_s \times \phi_{ISC} \times \Phi_{TTET} \times \Phi_{TTA} \times \Phi_u \quad (5)$$

where Q_E is the photolytic efficiency, ϵ_s is the molar extinction coefficient of sensitizers, Φ_{ISC} is the ISC quantum yield of the sensitizer, Φ_{TTET} is the TTET quantum yield between the sensitizer and the PPG, and Φ_u is the photolytic quantum yield of PPGs.

Han and co-workers first tested the ability of several photosensitizers and PPG couples to undergo a TTAP and selected the PdTPBP/perylene-3-ylmethyl PPG couple due to their excellent photolytic yield to prepare a so-called TTAP NP using an oleylamine-substituted amphiphilic polymer (e.g., PSMA-PEG-OAm).^[83] Noteworthy, the hydrophobic perylene-3-ylmethyl PPG was used to develop a caged chlorambucil analog (see compound 30 in Figure 8) in order to enable the *in vivo* inhibition of cancer cell growth after red light excitation of the TTAP NP.^[83] More recently, Han and co-workers combined the same concept, using a metal-free far-red sensitizer (e.g., BDB-F 8) and prodrug 30 couple, for immunotherapy for the treatment of local and abscopal tumors.^[73] However, this latter strategy requires to select the right PPG in order to obtain a high photolytic yield.

2.7. TTA-UC-Based Photolysis: In Vivo Application in the Field of Photopharmacology

As described above UC-NPs based on TTA of organic chromophores showed interesting properties to assist a photolytic reaction using low-power far red or NIR excitations. Therefore, several groups asked whether such a strategy can also work *in vivo* in mice. Figure 9 summarizes the 3 TTA-UC-based strategies and their applications for light-induced anticancer drug release.

The first *in vivo* demonstration of TTA-UC-induced drug release using red light activation was made by Han and co-workers in 2017.^[57] To perform the so-called photoactivated chemotherapy (PACT) the authors used MSN containing a dye pair, methyl oleate oil to prevent oxygen quenching, and polymer F-127 loaded with a coumarin–chlorambucil prodrug. After having shown that photolysis indeed induced the release of the alkylating drug chlorambucil—which in turn led *in vitro* to tumor cell killing—they went for *in vivo* experiments using breast cancer cell line (4T₁) tumor-bearing mice. 1 h after intra-tumoral injection of the NPs loaded with the coumarin–chlorambucil prodrug, tumor sites were irradiated with a 650 nm far-red LED (180 J cm⁻²; 100 mW cm⁻², 30 min). They found that in the group of irradiated mice, the tumor size significantly decreased while it was not the case in the non-irradiated group.

Following this pioneering work which demonstrated that organic TTA-UC delivery systems can be used *in vivo* to induce

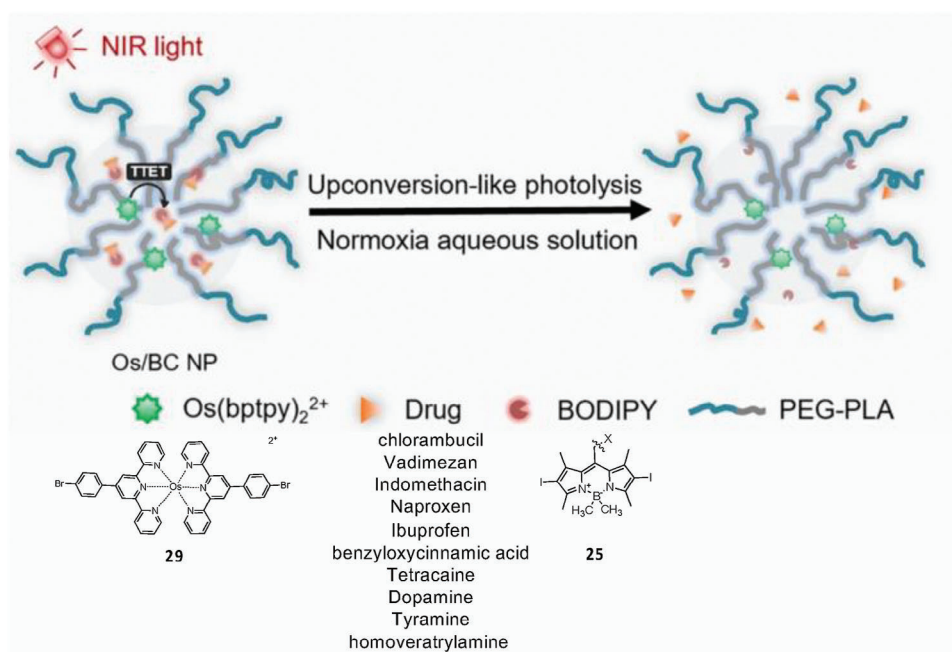


Figure 7. Schematic illustration of NPs for one-photon upconversion-like photolysis for NIR light-induced drug release on PEG-PLA nanomicelles. Adapted with permission.^[82] 2023, Springer Nature.

photocleavage with the subsequent release of an active drug, we developed another TTA-UC delivery system: TBPe/Pd-TBPB loaded LNC of 50 nm were modified at their surface with a coumarinyl PPG conjugated to the antitumoral drug melphalan (compound **28**, Figure 6). After having shown that the LNCs can photorelease melphalan through meat slices with up to 8 mm thickness using a 630 nm excitation LED system we went for in vivo studies using human breast adenocarcinoma (MDA-MB-231) subcutaneous tumor-bearing nude mice. Irradiation at 641 nm (540 J cm^{-2} ; 200 mW cm^{-2} , $3 \times 15 \text{ min}$) was performed directly after the intra-tumoral injection of the LNCs. We found that tumor growth was inhibited in the mice treated

with LNC-UC-Prodrug + irradiation while for the non-irradiated group, the tumor volume quickly increased.^[56]

In parallel, other strategies were developed for inducing drug release following photocleavage. Han et al. reported in 2021 a strategy that avoids the LRET step via directly sensitizing the prodrug with a photosensitizer.^[83] TTA-mediated photolysis (TTAP) was performed using a perylene-chlorambucil conjugate (= pro-chlorambucil) encapsulated into 30 nm NPs composed of oleylamine-substituted amphiphilic polymer (PSMA-PEG-OAm) loaded with the NIR photosensitizer PdTPBP. As the authors found that pro-chlorambucil could be photolyzed and released from TTAP NPs into cancer cells, inducing cell death, they tested their new DDS in vivo. The efficiency of the system was evaluated using 4T₁ tumor-bearing mice 4 h after the injection of TTAP NPs into the tumors with irradiation using a 650 nm NIR light (36 J cm^{-2} ; 20 mW cm^{-2} , 30 min). The results showed that TTAP NPs + irradiation treatment decreased the growth rate of the tumor but only a combination with the checkpoint blockade programmed death-ligand 1 antibody (α -PD-L1) led to complete eradication of the tumor. Han et al. went further by adding to their delivery system a tumor-targeting motif (cyclical Arg-Gly-Asp (cRGD)). The cRGD-TTAP NPs were injected intravenously into mice bearing a subcutaneous 4T₁ tumor. 1 day after, 650 nm far-red light LED irradiation was done (36 J cm^{-2} ; 20 mW cm^{-2} , 30 min). The size of cRGD-TTAP NP-treated tumors was significantly reduced 14 days post-treatment, indicating that their nanoparticles could be effectively targeted to a localized subcutaneous tumor.

Finally, in 2023, Han et al. used 24 nm nanoparticles generated with their unsaturated alkene-substituted amphiphilic polymer PSMA-OAm-PEG loaded with the chlorambucil-erylene conjugate together with a BODIPY-based photosensitizer.^[84] After cell culture validation of the release of the FDA-approved drug

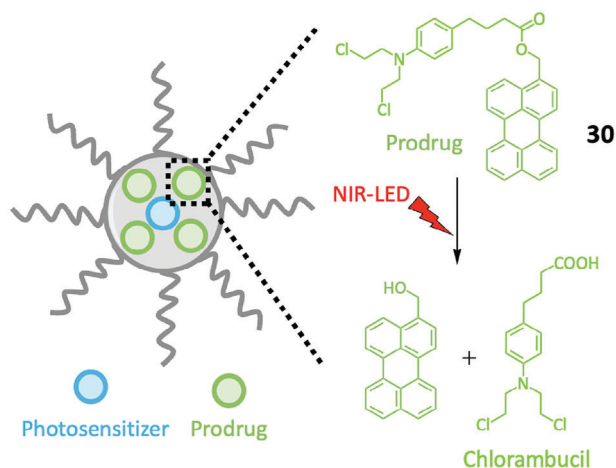


Figure 8. Schematic illustration of TTAP NPs and the related photolytic reaction of a photoactivatable chlorambucil prodrug **30**. Adapted with permission.^[40] 2022, American Chemical Society.

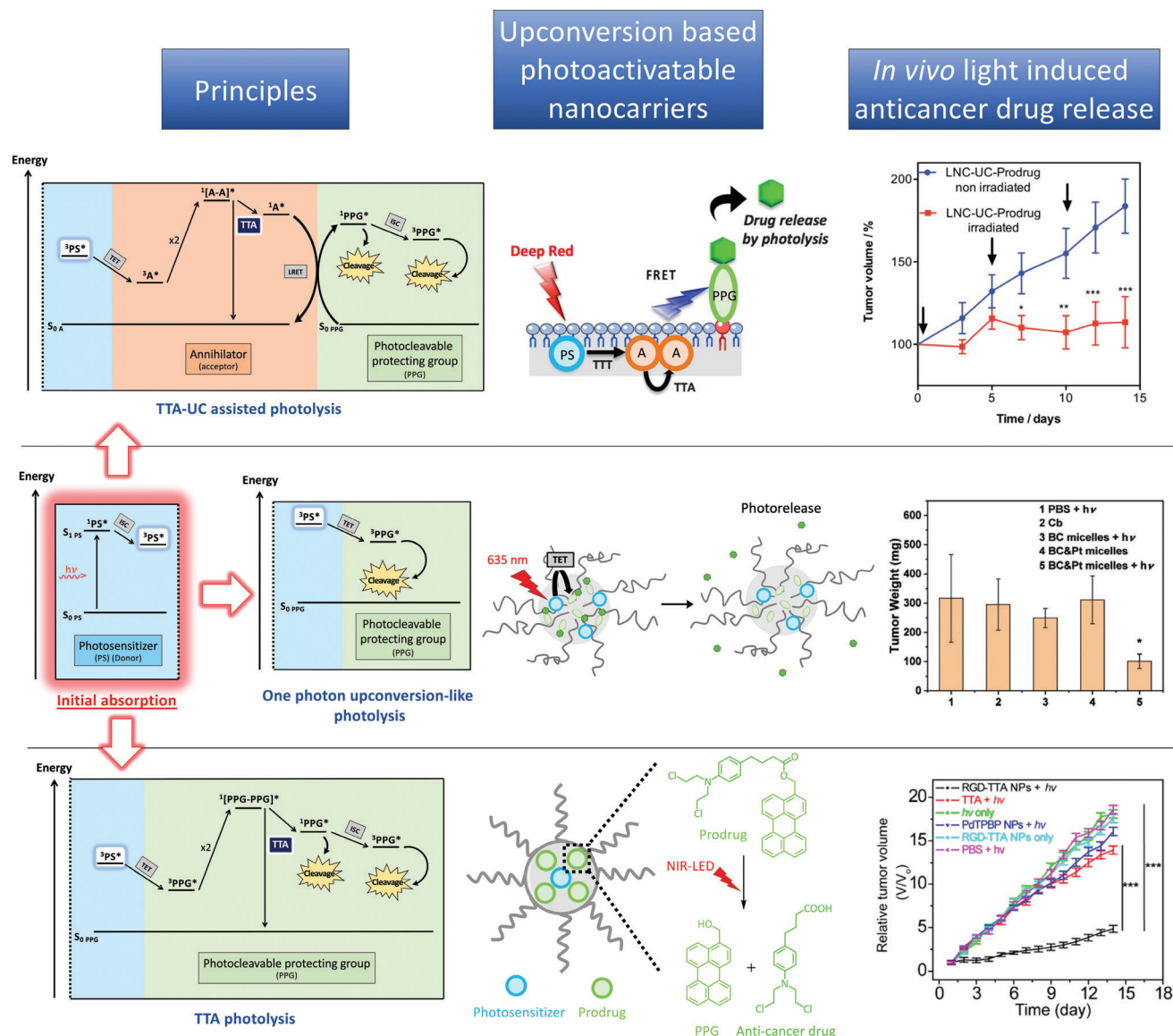


Figure 9. Principle and in vivo applications of TTA-UC-assisted photolysis, one photon upconversion-like photolysis, and TTA photolysis. Adapted with permission.^[40,56,81] 2022, American Chemical Society, 2022 and 2020, Wiley-VCH GmbH.

chlorambucil, they conducted experiments in CT26-tumor-bearing mice (Table 3). The irradiation was performed 1 h after intra-tumoral injection of the NPs with 656 nm far red-light LED for 30 min at 20 mW cm⁻². 11 Days post-treatment, the tumor growth was almost completely stopped when using their NPs + irradiation while it was not the case for the incomplete or non-irradiated formulations. To further boost the anti-tumoral efficiency of their system, Han and co-workers loaded into the NPs an indoleamine 2,3- dioxygenase inhibitor (IDOi; INCB024360 analog) which can reverse the immune suppression caused by IDO. Using a bilateral CT26 tumor mouse model (tumor present in the left and right sides of the mouse's back) they tested whether they can suppress the tumor growth in the non-injected tumor. Therefore, the IDOi-NPs were only injected into one of the tumors which was then irradiated. Very interestingly,

not only the growth of the injected tumor was suppressed but they also observed tumor shrinkage in the non-injected tumor, indicating that their DDS allowed for inhibition of the injected tumor but also for a distant tumor.

Interestingly in parallel to the Han team, the group of Wang developed another delivery system based on one-photon upconversion-like photolysis.^[81] They used 40 nm sized polymeric micelles generated with an amphiphilic block copolymer composed of poly(lactic acid)-poly(ethylene glycol) (PLA5000-mPEG3400) in which they encapsulated a prodrug and a photosensitizer. As a prodrug, they used a BODIPY-based photocleavable chlorambucil and photosensitization was done with platinum (II) tetraphenyltetrabenzoporphyrin (PtTPBP) through a triplet-triplet energy transfer (TTET) process. After in vitro validation of the system, they evaluated the efficiency of the photolysis

Table 3. In vivo application of TTA-UC photolysis.

NPs	Sensitizer/annihilator ^{a)}	Released drug	In vivo model	Irradiation conditions	NP Administration	Ref.
Mesoporous silica NP + amphiphilic polymer F-127	BDP-F (8)/PEA (14)	Chlorambucil	Breast cancer cell line 4T ₁ -tumor-bearing mice	180 J cm ⁻² 100 mW cm ⁻² , 30 min	Intra-tumoral injection	[57]
Lipid nanocapsules (LNC)	PdTPBP (4)/TBPe (10)	Melphalan	Human breast adenocarcinoma (MDA-MB-231) subcutaneous tumor-bearing nude mice	540 J cm ⁻² 200 mW cm ⁻² , 3 × 15 min	Intra-tumoral injection	[56]
Oleylamine-substituted amphiphilic polymer (PSMA-PEG-OAm)	PdTPBP (4)/prodrug perylene chlorambucil (30)	Chlorambucil	Subcutaneous 4T1 tumor xenograft in mice	36 J cm ⁻² 20 mW cm ⁻² , 30 min	Intra-tumoral injection	[83]
Targeted cRGD PSMA-PEG-OAm nanoparticles	PdTPBP (4)/prodrug perylene chlorambucil (30)	Chlorambucil	Subcutaneous Breast cancer cell line 4T1-tumor-bearing mice	36 J cm ⁻² 20 mW cm ⁻² , 30 min	Intravenous injection	[83]
PSMA-OAm-PEG nanoparticles	BODIPY-based photosensitizer BDB-F (8)/prodrug perylene chlorambucil (30)	Chlorambucil	Subcutaneous murine colorectal carcinoma CT26-tumor-bearing mice	36 J cm ⁻² 20 mW cm ⁻² , 30 min	Intra-tumoral injection	[84]
Polymeric micelles poly(lactic acid)-poly(ethylene glycol) (PLA5000-mPEG3400)	PtTPBP (3)	Chlorambucil	Subcutaneous HeLa tumor (cervical carcinoma cells) in nude mice	60 J cm ⁻² 200 mW cm ⁻² , 5 min	Intra-tumoral injection	[81]
Polymeric micelles poly(lactic acid)-poly(ethylene glycol) (PLA5000-mPEG5000)	Os(bptpy) ₂ ²⁺ (29)	Chlorambucil	Subcutaneous HeLa tumor (cervical carcinoma cells) in nude mice	180 J cm ⁻² 300 mW cm ⁻² , 10 min	Intravenous injection	[82]
Self-assembled BODIPY ₃ -tris(2-aminoethyl)amine (BTAEA) + lipid DSPE-mPEG2000	PtTPBP (3)	Paclitaxel	Subcutaneous 4T1 tumor-bearing BALB/c mice	120 J cm ⁻² 200 mW cm ⁻² , 10 min	Intravenous injection	[85]
Polymeric micelles (polyD,L-lactic acid-polyethylene oxide)	PdOEP (1)/DPA (13)	Uncaging of a targeting motif	Subcutaneous tumors (U87 glioblastoma) in nude mice	60 J cm ⁻² 200 mW cm ⁻² , 5 min	Intravenous injection	[78]

^{a)} Annihilators are only used in the case of TTA-UC photolysis and TTA-photolysis.

in vivo using nude mice bearing tumors obtained after subcutaneous injection of HeLa cells (cervical carcinoma cells). Irradiation with a 635 nm laser (60 J cm⁻²; 200 mW cm⁻², 5 min) was done 1 h after intra-tumoral injection of the NPs (done on days 1, 4, 7, and 9). On day 11, it was observed that the tumor growth was inhibited in the group of mice that received the NPs + light irradiation while in the control group (NPs without irradiation) the tumor volume increased.

In 2023, the Wang group reported even more promising results obtained with a similar system (polymeric micelles and a BODIPY-chlorambucil prodrug) but using this time an osmium-containing PS (compound 29). For evaluating the in vivo therapeutic efficacy, the formulations were intravenously injected in HeLa tumor-bearing mice on days 1 and 6, and light irradiation was done on days 2 and 7 at 690 nm, 300 mW cm⁻², 10 min. They found that the group treated with [Os(bptpy)₂²⁺/BODIPY-chlorambucil prodrug NPs + light] exhibited the greatest suppression effect on tumor growth as compared to all the other groups.^[83]

In order to be able to extend easily their concept to a variety of other drugs (i.e., without the need for complicated prodrug synthesis), the same group developed an interesting concept: the active drugs were encapsulated into nanoparticles that

can dissociate upon irradiation.^[85] To achieve such a DDS, a (BODIPY)₃-tris(2-aminoethyl)amine (BTAEA) was used which was shown to be 1) photocleavable and 2) able to self-assemble into NPs. The authors further found that it was possible to encapsulate into those NPs the photosensitizer PtTPBP (platinum (II) tetraphenyltetraazaporphyrin), as well as the anti-tumoral drug paclitaxel. Moreover, they could modify the NPs by adding a PEGylated lipid and the resulting DDS had an average size of 95 nm and negative zeta potential. Drug release after irradiation and subsequent paclitaxel-mediated cell death were confirmed in vitro. Next, they used subcutaneous 4T₁ tumor-bearing BALB/c mice for in vivo evaluation. The NPs were injected intravenously and one day later, light irradiation was performed with a 635 nm laser (120 J cm⁻²; 200 mW cm⁻², 10 min). The treatments were repeated every 3 days and on day 14, the tumor volume was measured. The results, which showed a nice reduction in tumor growth with the complete formulation + light, demonstrate the great potential of their photoresponsive carrier.

To finish, it is worth mentioning that upconversion in vivo has also been used in another context than the photorelease of an anti-tumoral drug, namely for the phototargeting of nanoparticles.^[78] To do this, Kohane's group generated polymeric micelles (polyD,L-lactic acid-polyethylene oxide) NPs

using PdOEP as a photosensitizer, DPA annihilator, and a NIR dye (DiR) for in vivo imaging. Then, a photocleavable caging group [7-(diethylamino) coumarin-4-yl]methyl (DEACM) was conjugated to the targeting ligand cRGDFK (conjugated at the end of the PEG chain) to block its binding to the receptors. To evaluate their system in vivo, the authors used nude mice bearing subcutaneous tumors (U87 glioblastoma). After intravenous injection of the NPs, the tumor sites were irradiated at 530 nm (60 J cm⁻²; 200 mW cm⁻², 5 min) and whole-body fluorescence imaging was done at different time points. Tumor accumulation of their NPs was observed indicating a successful targeting strategy.

3. Summary and Outlook

TTA is able to combine the energy of two triplet-excited molecules onto one molecule to populate a higher excited state. This process has been used to generate an anti-stokes light emission and was applied in the field of photopharmacology to induce a photolytic reaction. This allowed to use low power far red or NIR excitations in order to trigger the controlled release of a drug or a biological effector using light since visible light has major drawbacks, including low penetration depth and phototoxicity, especially for the most energetic range of the spectrum (e.g., violet light). Time-consuming molecular engineering of PPGs has recently led to the development of red-sensitive PPGs, however, the uses of TTA-UC-based nanoparticles combined with more “classical” visible light-sensitive PPGs (e.g., blue or green light-sensitive PPGs) has already allowed to trigger the release of payloads in vivo using light. TTA-UC assisted photolysis was first developed, this latter process is based on a non-radiative energy transfer between a TTA-UC NP and a PPG leading to NPs in order to be able to apply those photoactivatable NPs in the theragnostic field (e.g., those latter NPs are able to emit photon and to release a payload using light excitation). To increase the efficiency of the uncaging process by directly using either a TTET or a TTET followed by a TTAT process in order to activate a PPG without the need for a luminescence emitter; two TTA-UC-based strategies have also been developed. Those two latter strategies called respectively one photon upconversion-like photolysis and TTA photolysis allowed to prepare photoactivatable NPs with higher light sensitivity but they required to select the appropriate PPG for a high-yield payload release. Noteworthy, those two strategies are not necessarily compatible with the majority of the PPGs already described in the literature. In the future, all those photoactivatable NPs should allow versatile uses with modifiable properties, like size and excitation/emission wavelengths, in particular, to perform a light-induced drug release using NIR-I or maybe NIR-II excitations. However, to further enhance their therapeutic efficacy these responsive NPs should be modified with ligands able to target the diseased tissues. In addition, the pharmacodynamic and the long-term toxicity of those NPs have to be evaluated before any translation to clinical applications. Finally, it is worth mentioning that all those nanoplatforms are not only restricted to anticancer drug release but could be used for a wide range of in vitro or in vivo applications requiring programmed release of drugs or biological effectors by photoactivation. Ultimately, this type of strategy could solve the off-target toxicity outside the disease location of many drugs for the treatment of several pathologies.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

drug delivery, nanomedicine, photopharmacology, triplet-triplet annihilation upconversion, uncaging

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Maxime Klimezak is a third-year Ph.D. student under the supervision of Dr. Alexandre Specht at the Laboratoire de Chemo-Biologie Synthétique et Thérapeutique (UMR 7199, University of Strasbourg / CNRS). His thesis is based on the synthesis of various photocleavable protecting groups, photosensitizers, and self-immolative linkers to trigger the controlled release of drugs through assisted photolysis on nanoparticles.



Juliane Chaud received her Ph.D. in Biological and Therapeutic Chemistry from the University of Strasbourg in 2021. Her thesis was based on the synthesis and application of photolabile protecting groups for in vivo drug release. After a post-doctoral position working on the synthesis and modification of functionalized lipids for transfection applications, she joined the biotech industry to continue working on new delivery systems.



Anaïs Brion is an engineer in Biology. Since her Ph.D. in Molecular Biology at the University of Strasbourg, she has worked on developing in vitro and in vivo models to evaluate new pharmacological treatments including nanovectorization of drugs.



Frédéric Bolze received his Ph.D. from the University of Strasbourg and Dr. from the University of Burgundy. After a post-doctoral position at UT Austin, he was appointed at the University of Strasbourg in 2002. His research interests mainly focus on the biological application of non-linear optical chromophores and fluorophores for biological applications.



Benoît Frisch is director of research UMR7199, CNRS – University de Strasbourg, Faculty de Pharmacy. He is the co-leader of the 3Bio Team (Biovectorization, Bioconjugation, Biomaterial, 20 persons). He has expertise in the formulation, design, functionalization, characterization, and in vitro and in vivo evaluation of drug delivery systems (DDS) such as liposomes, lipids, polymer nanoparticles, or implants. He works in bioconjugate chemistry, immunology, pharmaceutical techniques, and the optimization and characterization of drug delivery systems in the form of macro(micro,nano) particles.



Béatrice Heurtault is a pharmacist and full professor of pharmaceuticals at the Faculty of Pharmacy of the University of Strasbourg. She worked for 20 years at the Centre National de la Recherche Scientifique at Strasbourg and recently joined the Biomaterials and Bioengineering research unit UMR_S1121 of INSERM and the University of Strasbourg. She focuses her research activities on the development and characterization of innovative drug delivery systems mainly based on nano and microparticles for various applications such as vaccine delivery and treatment of tumors and inflammation.



Antoine Kichler is a research director at the CNRS. He received his Ph.D. in pharmaco-chemistry from the University of Strasbourg, France. After two post-doctoral positions, he moved to Genethon in Evry (France) to head a group working on non-viral gene delivery before joining the Faculté de Pharmacie of Strasbourg in 2012. His research interests are in the field of vectorization of drugs, proteins, and nucleic acids using a variety of delivery systems including cell-penetrating peptides, polymers, and lipids.



Alexandre Specht received his Ph.D. in Chemical Biology from Strasbourg University. After post-doctoral research at the Howard Hughes Medical Institute at UCLA (USA), he joined the Centre National de la Recherche Scientifique at Strasbourg in 2004 as a researcher (CR CNRS) and was promoted to research director (DR CNRS) in 2015. Since 2011, he has been the group leader at the Faculty of Pharmacy of Strasbourg (UMR 7199, University of Strasbourg/CNRS). His group develops photochemical tools for the regulation of various biological activities and nanoparticles for light-induced drug release.