

## Review

# Why not just switch on the light?: light and its versatile applications in the field of nanomedicine

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## Abstract

Over the last decade, the emerging field of nanomedicine has undergone rapid progresses. Different internal and external stimuli like pH, temperature, radiation, ultrasound or light have been introduced to expand the diagnostic and therapeutic options of various applications within the field. This review focuses on the novel application of light in the field of nanomedicine as a mechanism to control drug delivery, release and biochemical and genetic functionality at the target. The field of functional nanomaterials for medicine, and in particular of light responsive nanocarriers, polymers and biomolecules offer new therapeutic options but also requires substantial further research to render this approach broadly applicable in clinical practice.

**Keywords:** light; nanoparticles; photodynamic therapy; photothermal therapy.

## Introduction

A variety of new nanomaterials such as polymers, liposomes, micelles, dendrimers or metallic nanoparticles have shaped the constant and rapid progressing field of nanomedicine within the last decade. Today, nanoparticles have found their way into the clinical domain as drug delivery systems, for imaging, sensing and therapy. To provide specific characteristics, nanoparticles can be tailored into “intelligent” nanoparticles through stimulus responsiveness. Stimuli responsive nanoparticle for medicine can be classified based on the type of stimuli into locally/internal triggered systems responding to their close environment, and externally triggered stimuli-responsive nanoparticles that can be remote-controlled even from outside the body. Various internal stimuli such as pH, redox potential, enzymatic activity, temperature and external stimuli like ultrasound, magnetic field, temperature and light

are being intensively investigated. Out of all these stimuli, light shows particularly attractive features such as high sensitivity, ease of controllability and a range of physical properties (e.g., light intensity, wavelength, exposure time) that allow in principle to design selective and multiplexed activities to be programmed into a material. Therefore, it is not surprising that a significant effort is currently invested into the development of light responsive nanoparticles, oligonucleotides or peptides.

This review presents a brief overview on light and its applications within the field of nanomedicine. It will describe mechanisms of light-controlled drug delivery, controlled drug release, light-controlled activity switching for biochemical mechanisms, gene expression and gene silencing at the target. The aim of this paper is to identify opportunities, describe gaps, and thus to stimulate further research, such that light-controlled nanomedical therapies develop into well tolerated, highly effective interventions to the benefit of the patient.

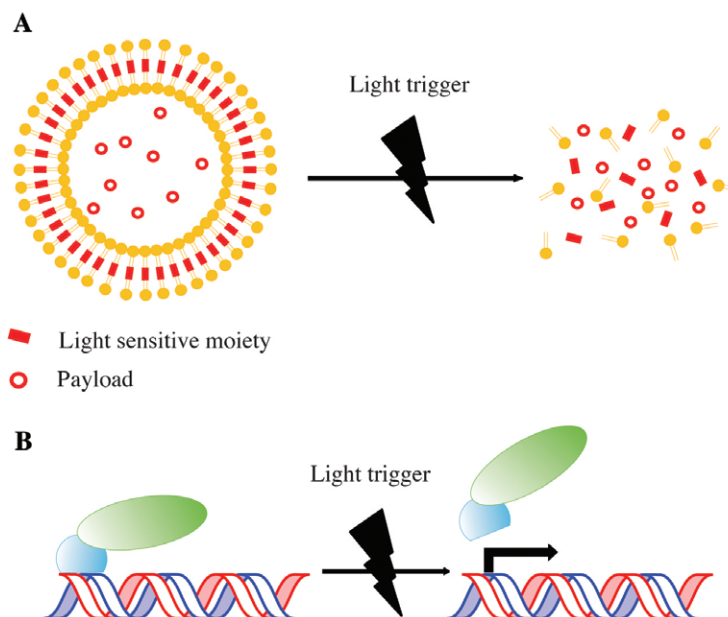
## Application of light in nanomedicine

### Light for triggered release and activation of drugs and biomolecules

Despite the efforts in drug delivery design and developments, major obstacles such as endosomal escape and efficient payload release within the diseased tissue and cell have to be overcome for efficient clinical application. Light can be used to enhance drug delivery and payload release by applying light sensitive moieties to drug delivery platforms and of photolabile protecting groups to biologically active molecules by a strategy called caging (Figure 1).

Caging is an attractive way of turning biological molecules e.g., nucleic acids (DNA, RNA), proteins or peptides light sensitive for the investigation of biological processes. Caged biomolecules incorporate a light-removable protecting group, so-called “caging group”, which aborts its native biological or biochemical activity. Since caging of ATP was first reported in 1978 by Kaplan et al., several different photolabile groups have been introduced to turn biomolecules temporarily inactive (1–3). Examples of caged biomolecules are neurotransmitters (4), nucleotides (4), peptides (5, 6), siRNA (7) or DNA (8). The most widely used caged neurotransmitter so far is glutamate for which different protecting groups have been applied (9). RNA interference is a mechanism able to inhibit protein translation by gene silencing. Nguyen et al. caged a 1-(2-nitrophenyl)ethyl (NPE) group to the 5′ terminal phosphate of the siRNA antisense strand, which inactivates

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**Figure 1** (A) Light responsive drug delivery system built through covalent incorporation of specific light-sensitive chemical groups (red rectangles) with the aim to locally release cargo (red circles) by illumination. (B) Site-specific caging of DNA can be used for light-activated gene expression.

the siRNA activity (10). They could demonstrate an approximately 70% efficient light induced RNA interference using wavelengths between 345 and 385 nm. An alternative form to siRNA mediated control of gene silencing has been reported by Young et al. (11). They introduced a caging group to DNAzymes to inhibit hybridization with mRNA. DNAzymes are enzymatically active desoxyoligonucleotides, which can cleave RNA in a site-specific manner. Translation of mRNA can be aborted upon illumination with UV-light to photo-release the caging group. Caging of DNA has widely been studied as seen from several publications (8, 12, 13). To render those approaches suitable for future clinical application, extension of the work towards longer wavelengths and therefore reduced toxicity should be accompanied by identification of suitable in vitro and in vivo disease models of human disease.

Light-responsive materials for drug delivery can be constructed by the covalent incorporation of specific light-sensitive chemical groups with the aim to locally release cargo by illumination. The synthesis of a photocleavable amphiphilic block copolymer has been demonstrated by Cabane et al. (14). As photosensitive molecule they introduced an o-nitrobenzyl linker between the hydrophobic and hydrophilic blocks, which form vesicles or micelles upon self-assembly in aqueous solution. Successful disruption of the vesicles could be demonstrated after irradiation with UV-light by electron microscopy and dynamic light scattering data. The design of photocleavable liposomes for drug delivery using different photolabile groups has been reported in several publications (15, 16). Dvir et al. presented a simple proof of concept by carboxylated polystyrene nanoparticles labeled with the unspecific amino acid sequence YIGSR, which

adheres to  $\beta 1$  integrins present on most cell surfaces (4, 17). The peptide was caged with a nitrobenzyl group, which could be removed via illumination, leading to nanoparticle binding to the cells. Another approach of light sensitive nanoparticles currently being investigated uses nano-impellers. Nano-impellers are nanomechanical systems allowing the spatiotemporal drug release upon illumination, turning them into an attractive application for clinical trials (5, 6, 18, 19). A clear disadvantage of many published systems is the requirement for light energy in the UV range, limiting their application due to phototoxicity and the very limited penetration range of short wavelength light in biological tissues.

#### Light induced gene expression and control of gene silencing

Light-mediated control of gene expression and silencing is a powerful and fast growing field in the areas of systems biology, functional genomics and biotechnology. Spatiotemporal and precise gene expression represents the most fundamental level of further complex biological processes such as the control of thousand of proteins and the associated control of metabolic processes. Therefore, light represents a suitable stimulus for in vitro as well as in vivo studies as it is non-invasive, sensitive and allows the spatiotemporal and precise application without interfering with metabolic conditions. Light-induced gene expression can either be achieved using caged biomolecules such as plasmid DNA (12, 13), transcription factors (8, 20, 21) or via photoreceptors harboring a chromophore (9, 22, 23). Several reports focused on caged plasmid DNA's have been published, whereas effective gene

expression remained a major challenge due to ineffective random backbone modifications (8, 10). In addition, successful uncaging and activation of gene expression required high levels of light that can cause phototoxicity (24). A more effective approach for light controlled activation of gene expression was shown by Yamaguchi et al. using a site-specific labeling of the promoter region with a biotinylated photolabile group, leading to effective activation of gene expression in HeLa cells even under low levels of light (12). A successful gene regulation system combining light-sensitive proteins and programmable zinc finger transcription factors has been published by Polstein et al. (14, 20). The system is based on two light-inducible fusion proteins from *Arabidopsis thaliana*, GIGANTEA (GI) fused to a Zinc finger protein leading the complex to the target DNA sequence and the LOV domain of FKF1 fused to the transcriptional activation domain VP16. Illumination with light leads to fusion of the GI and LOV domain, which guides the LOV-VP16 domain to the target gene and enables gene expression.

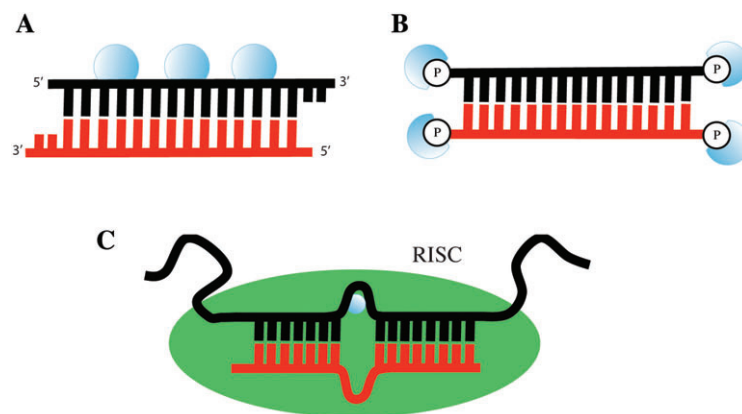
Beside light induced gene expression, the focus of photochemical control of gene function has been directed to RNA interference. RNA interference represents one of the major approaches leading to gene silencing/such as that occurring in embryogenesis) and is being extensively explored as a therapeutic strategy for different kind of diseases, including cancer. Two primary approaches for photochemical regulation have been developed. The caging groups are either covalently attached to the phosphate backbone or terminal phosphates or on the nucleotide bases to inhibit the further process of RNA induced silencing (Figure 2). The first report of caged siRNA has been described by Shah et al. using 1-(4, 5-dimethoxy-2-nitrophenyl)ethyl (DMNPE) attached to the phosphate backbone which only showed a 3% caging efficiency (15, 16, 24). Caging of guanosine and thymidin bases by attaching 2-(2-nitrophenyl)propyl (NPP) groups has been reported by Mikat and Heckel (25). The modifications have shown knockdown efficiency of about 75% after light irradiation. Jain et al. designed a siRNA caged at the terminal phosphates with a

cyclo-dodecyl DMNPE, which is more bulky and therefore shows higher steric hindrance (11, 26). In contrast to the DMNPE, which has been introduced to the phosphate backbone, siRNA terminally caged with cyclo-dodecyl DMNPE showed an efficiency of 89%.

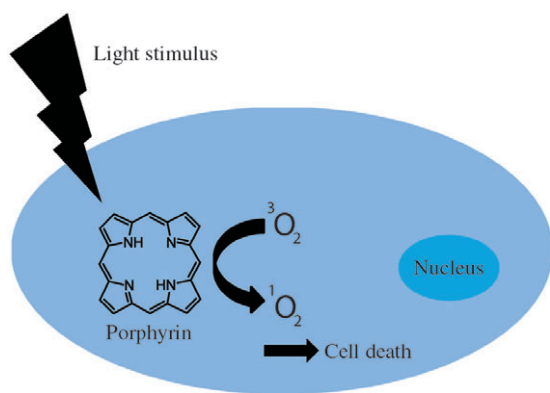
### Photodynamic therapy

The therapeutic effect of light has been known for thousands of years and was applied by the Egyptians, Indians and Chinese (8, 12, 13, 27). Its therapeutic relevance to cancer treatment and further development into the photodynamic therapy (PDT) was reported at the beginning of the last century by Oscar Raab, a German medical student and his professor Hermann von Tappeiner (28). The principle of photodynamic therapy involves the administration of a photosensitizer, which will form highly reactive singlet oxygen radical (ROS) from molecular oxygen after illumination with light (Figure 3). Singlet oxygen radicals are known to cause severe damage to biological macromolecules such as membrane lipids and proteins (29). After absorption of light, photosensitizers will change from a ground state into a relatively long-lived excited triple state and a short-lived excited single state. The excited single state can return to the ground state by emitting fluorescence that can be used for clinical detection. In the excited triple state, the photosensitizer molecule can transfer its energy via a type-I or -II reaction. In the type-I reaction, the photosensitizer can react directly with a surrounding substrate to form radicals, which then can further interact with oxygen to produce oxygenated products. In the type-II reaction the energy of the excited photosensitizer can be directly transferred to oxygen to form highly reactive singlet oxygen (30).

Photodynamic therapy has found its way into clinical applications using nanocarrier platforms as delivery system such as photodynamic eye therapy for the treatment of neovascularization, abnormal endothelial proliferation or for different cancer treatments (bladder, skin, head and neck, esophageal, or endobronchial cancer) (31, 32). A number of nanoparticle-



**Figure 2** Schematic illustration of caged siRNA strategies. (A) Caged phosphate backbone and (B) caged terminal phosphates of siRNA. (C) Introduction of a caged base into a siRNA antisense strand to inhibit RNA interference.



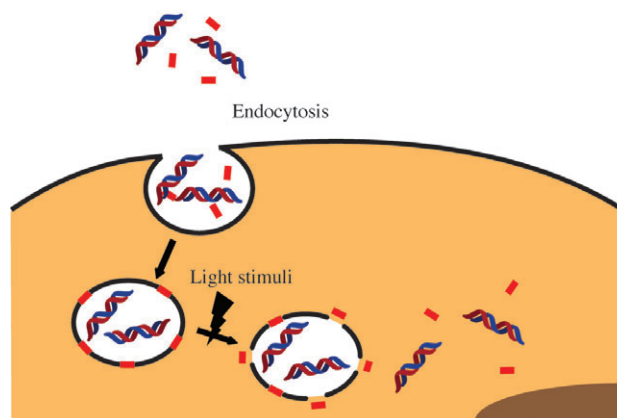
**Figure 3** Light activation of a photosensitizer leads to the formation of highly reactive singlet oxygen for selective cell killing.

based photodynamic therapies have been approved by the U.S. Food and Drug Administration (FDA) such as e.g., Visudyne<sup>®</sup>, Photofrin<sup>®</sup>, Levulan<sup>®</sup> Kerastick<sup>®</sup> opening doors for future applications and new possible approaches for future therapies (31). There are several advantages of PDT as a clinical application including a single dose requirement for treatment followed by illumination compared to radiotherapy and chemotherapy, which both depend on a treatment over several weeks or months. Further, it is a local treatment without interfering with the whole organism and retreatment can be simply done in the case of recurrence of a tumor without severe healthy tissue damage. However, further development in the direction of controlled drug release, as well as improved payload capacity of nanoparticle-based delivery systems is warranted.

### Photochemical internalization (PCI)

One of the key challenges that still needs to be overcome in order to enable the clinical application of therapeutic delivery of different payloads is endosomal escape. Various strategies have been developed to achieve endosomal escape and these are either based on the characteristic endosomal property of a lower intracellular pH compared to the cytoplasm, incorporation of fusogenic peptides into the endosomal membrane or a strategy called photochemical internalization (PCI). PCI is a site-specific method for intracellular drug delivery by induced endolysosomal escape based on photostimulation. The principle behind PCI relies on photodynamic therapy targeted to endosomes or lysosomes, whereas the vesicular membrane bursts after coming into contact with highly reactive singlet oxygen after illumination of the photosensitizer (Figure 4). In comparison to conventional photodynamic therapy, where the intracellular localization of the photosensitizer does not play an important role because of its complete cellular destruction, PCI is based on the specific accumulation of the photosensitizer in the endolysosomal compartment to achieve endosomal escape without harming the rest of the cell (33).

A fate that may be a consequence to nanocarriers after endocytotic uptake, is the accumulation in the endolysosome, whereas PCI offers a good solution. Lai et al. have demon-



**Figure 4** Intracellular drug delivery induced by endolysosomal escape based on photostimulation. Light-irradiation after endocytotic uptake leads to endolysosomal membrane burst upon highly reactive singlet oxygen.

strated the effective delivery of doxorubicin and saporin by photochemical internalization using a polyamidoamine (PAMAM) dendrimer (34, 35). Recently, Lu et al. reported the overcoming of doxorubicin drug resistance in vivo by applying dendrimer phthalocyanine-encapsulated polymeric micelles combined with doxorubicin into doxorubicin-resistant bearing mice (36). It has also been shown by Nishiyama et al. that PCI can mediate gene transfection, using a combinational system including polymeric micelles incorporating pDNA and a dendrimer-based photosensitizer (37). Both polymeric micelles are assumed to be taken up by the cells at the same time. After illumination, a remarkable enhancement of transgene expression could be detected while retaining cell viability. Beside enhancement of gene expression, PCI can also be used for siRNA mediated gene knockdown studies. The first application of PCI to facilitate endosomal escape of siRNA was reported in 2007 by Oliveira et al. (38). They used TPPS<sub>2a</sub> as photosensitizer together with a siRNA able to knock-down epidermal growth factor receptor (EGFR) expression. Complexes of EGFR siRNA and Lipofectamine were applied to the cells. A 10-fold increased efficiency in EGFR knock-down could be detected after illumination compared to siRNA treatment alone. A recently published study by Varkouhi et al. presents PCI mediated enhancement of gene silencing using a polymer-based nanocarrier platform consisting out of cationic polymethacrylates and N,N,N-trimethylated chitosan (39). Furthermore, PCI can enhance the effect of targeted protein toxins that have reached the tumor cells (40). Targeted protein toxins consist of a protein toxin moiety, initiating cytotoxicity and a cell binding moiety, which targets the protein actively to the cell. Denileukin diftitox is the first FDA approved protein toxin for treatment of cutaneous T-cell lymphoma.

### Photothermal therapy

Hyperthermia is a non-invasive approach for cancer treatment based on the principle of spatiotemporally increasing the temperature to promote selective destruction of cancer cells, which



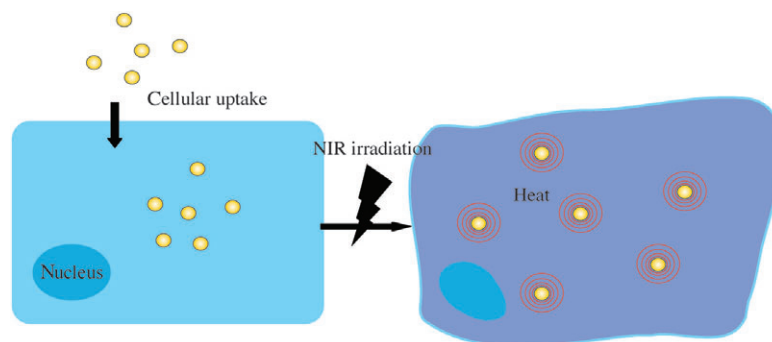
are more sensitive to hyperthermia than normal cells due to their higher metabolic rates. Several different approaches have already been applied for delivery of thermal energy such as ultrasound, microwaves or radiofrequency pulses (41–43). A disadvantage is their dispersive property with the result that high fluences (high amount of particles that intersect an area at a specific timepoint) are needed, which lead to undesirable hyperthermic effects on surrounding tissues. Within the last few years, gold nanoparticles have received increasing attention due to their versatile applications such as imaging, cancer therapy, drug delivery and especially because of their unique surface plasmon resonance (SPR) absorption at visible or Near-infrared (NIR) wavelengths (44). The use of NIR is desirable due to its deep penetrating capacity and minimal interference with water and biomolecules in tissues. The principle of photothermal therapy is the combination of light and gold nanoparticles (e.g., gold nanospheres, nanorods, nanoshells, nanocages) for clinical treatment. Illumination of gold nanoparticles leads to conversion of absorbed light into thermal energy, the resulting heat causes cell and tissue destruction (Figure 5). El-Sayed et al. have shown the use of gold nanorods labeled with an anti-EGFR antibody for selective photothermal treatment of cancer cells (45). A dual-modality approach for photodynamic and photothermal therapy has been recently published by Kuo et al. (46). They used gold nanomaterials conjugated with the hydrophilic photosensitizer, indocyanine green, to achieve photothermal therapy (PTT) and photodynamic therapy (PDT). The combination of PTT and PDT showed enhanced destruction of cancer cells in contrast to their single application effectiveness. Photothermal tumor ablation in mice could be proven by O’Neal et al. using gold nanoshells (47). They subcutaneously injected murine colon carcinoma cells into immune-competent mice, followed by injection of gold nanoshells. After 6 h of circulation, tumors were illuminated with NIR. All treated mice looked healthy and tumor free after more than 90 days post-treatment.

### Photoswitchable fluorescent nanoparticles

Over the past decades a huge number of nanoparticles made of different materials have been developed and these have biological and medical applications. Whereas many of those

platforms have been developed for the purpose of improved drug delivery and therapy another promising direction, which has attracted considerable interest is molecular imaging. Nanoparticle-based imaging offers a non-invasive and quantitative detection method of biomolecules, while at the same time improves sensitivity and specificity of diagnostic imaging as a tool for e.g., early cancer detection. Fluorescence spectroscopy is a powerful method used for molecular imaging of living cells, allowing very sensitive measurements at high resolution. Fluorescence imaging is based on the principle of the absorption of light by a fluorescent dye (e.g., fluorophore or fluorochrome), which emits fluorescent light at a longer wavelength than that absorbed. Fluorescent nanoparticles such as polymer NPs, silica NPs, gold NPs or quantum dots (QD) gained intensive interest during the last years. They can be produced by doping the material with suitable fluorescent dyes or luminescent metals while quantum dots can directly be applied due to their intrinsic fluorescence properties (48). The advantages of fluorescent nanoparticles compared to normal organic dyes are higher brightness due to the fact that a nanoparticle can carry several dye molecules, increase in photostability because the dyes are entrapped within the nanoparticles, higher specificity upon their functionalization properties and their long-term-tracking ability.

Understanding cellular networks is the essential key factor to understand the complex structure of certain diseases. To achieve this goal, significant progress has been made in the development of quantum dots for cellular sensing which have been recently reviewed (49). Sensing quantum dots are based on the principle of the recognition of an analyte, which acts as a fluorescence quencher, by a receptor or chemosensor causing changes upon emission of the fluorophore. Various quantum dots based on overcoating of the core with ZnS or CdSe to improve their fluorescence quantum yield and additional modification of the surface properties to increase their emission have been reported (50–52). Furthermore, this concept can be used to prepare glucose or maltose sensing systems, whereas a photoinduced electron transfer (PET) from the coating molecules to the valence band of an excited quantum dot results in emission quenching as shown by Cordes and Sandros et al. (53, 54).



**Figure 5** Photothermal therapy is based on intracellular uptake of gold nanoparticles, which after irradiation with near-infrared light convert absorbed light into thermal energy for specific destruction of cancer cells.

Beside the mentioned applications, the most largely exploited photoswitchable fluorescent sensing mechanism is by fluorescence resonance energy transfer (FRET) by which an energy transfer from a QD to a fluorophore will be determined. Until now, several strategies allowing generic on/off photoswitching based on FRET have been reported (55–57). Another more complex approach for photoswitching has been explored by dye doped nanoparticles allowing coactive triggering of multiple processes. These dye doped sensing nanoparticles were first introduced by Kopelman in the late 1990s and are called photonic explorers for bioanalysis with biologically localized embedding (PEBBLEs) (58). The insertion of chemosensors into nanoparticles shows several advantages as minimization of interaction with other biomolecules within the cells or the introduction of multifunctional sensing schemes as for example by pH sensitivity. A wide variety of PEBBLEs have been reported since their development, whereas further literature can be found here (59–61).

However, the ability of QDs to combine molecular imaging and therapy can open new doors for clinical application, but the toxicity of especially heavy metals used in QD synthesis such as cadmium is an important concern (62).

### Limiting factors of light

Light as external stimuli for enhanced drug delivery, cargo release, imaging and therapy offers some attractive features such as high sensitivity and spatiotemporal control. However, the major drawback of light is tissue penetration depth, restricting its applications. Solutions to overcome this problem have been made by development of near infrared (NIR) light sensitive photochemical compounds. Near-infrared light at wavelengths of 700–1000 nm can penetrate up to several centimeters deep into tissues without causing any damage (63). This renders NIR much more attractive than the often used UV-light regarding its potential for severe tissue damage. The use of two-photon excitation systems as well as application of upconverting nanoparticles, both provide possible solutions of how to overcome the problem of tissue penetration depth. Two-photon excitation depends on the principle of exciting a caged group by absorption of two photons induced via a pulsed laser. This method allows the usage of caged groups, which absorb light in the UV range but can be excited via pulsed NIR. Upconverted nanomaterials are able to convert NIR into UV light, which generates the same benefit as seen for the two-photon excitation. Practical application of such systems into clinical trials might still need some time due to the fact that most published data using NIR, show irradiation times of hours, which might interfere with clinical practice (64).

### Conclusion

The use of light as an external stimulus is a promising approach for a wide range of applications within the field of nanomedicine based on its attractive properties such as sensitivity

and biocompatibility, in particular for wavelengths longer than UV. Furthermore, it has shown advantages regarding its high spatial and temporal precision. However, the major drawback of light is tissue penetration depth, which severely restricts the applications of caged compounds, light sensitive drug delivery systems and light-based therapies into clinical application. Thus, approaches like the usage of NIR linked to two-photon uncaging and up-converting systems seem to be promising but further optimization of these methods is needed to increase the chance of further application in clinical trials.

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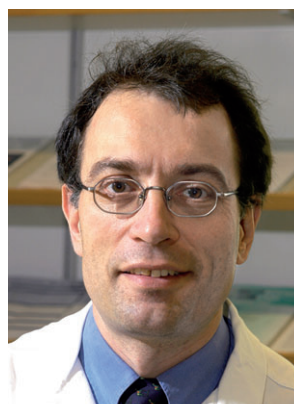
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