ACCOUNTS of chemical research

Article

pubs.acs.org/accounts

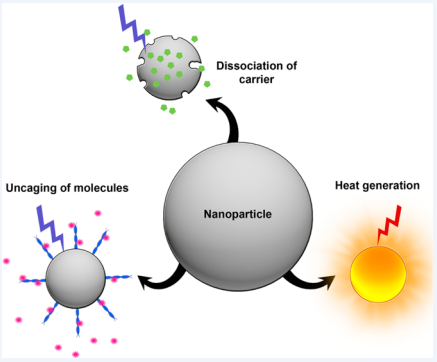
# Photocontrolled Nanoparticle Delivery Systems for Biomedical Applications

Akshaya Bansal[†,‡] and Yong Zhang*[,†,‡]

[†]Department of Biomedical Engineering, Faculty of Engineering, National University of Singapore, 117575 Singapore

[‡]NUS Graduate School for Integrative Sciences & Engineering, National University of Singapore, 117456 Singapore

**CONSPECTUS:** "Smart" stimuli-responsive nanomaterials are becoming popular as targeted delivery systems because they allow the use of internal or external stimuli to achieve spatial or temporal control over the delivery process. Among the stimuli that have been used, light is of special interest because it is not only noninvasive but also controllable both spatially and temporally, thus allowing unprecedented control over the delivery of bioactive molecules such as nucleic acids, proteins, drugs, etc. This is particularly advantageous for biomedical applications where specificity and selectivity are highly desired.




Several strategies have evolved under the umbrella of light based delivery systems and can be classified into three main groups. The first strategy involves "caging" of the bioactive molecule using photolabile groups, loading these caged molecules onto a carrier and then "uncaging" or activating them at the targeted site upon irradiation with light of a particular wavelength. The second strategy makes use of nanocarriers that themselves are made photoresponsive either through modification with photosensitive groups or through the attachment of photolinkers on the carrier surface. These nanoparticles upon irradiation dissociate, releasing the cargo encapsulated within, or the photolinkers attaching the cargo to the surface get cleaved, resulting in release. The third approach makes use of the surface plasmon resonance of noble metal based nanoparticles. Upon irradiation with light at the plasmon resonant frequency, the resulting thermal or nonthermal field enhancement effects facilitate the release of bioactive molecules loaded onto the nanoparticles. In addition, other materials, certain metal sulfides, graphene oxide, etc., also exhibit photothermal transduction that can be exploited for targeted delivery. These approaches, though effective, are constrained by their predominant use of UV or visible light to which most photolabile groups are sensitive. Near infrared (NIR) excitation is preferred because NIR light is safer and can penetrate deeper in biological tissues. However, most photolabile groups cannot be excited by NIR light directly. So light conversion from NIR to UV/visible is required. Nanomaterials that display upconversion or two-photon-excitation properties have been developed that can serve as nanotransducers, converting NIR to UV/visible light to which the aforementioned photoresponsive moieties are sensitive. This Account will review the existing light-based nanoparticle delivery systems, their applications, the limitations they face, and the technologies that have emerged in an effort to overcome these limitations.

## 1. INTRODUCTION

Nanomaterials owing to their high surface to volume ratio and ability to penetrate deeper into tissues and cross membrane barriers are a promising platform for applications, such as drug delivery and targeted cancer therapy.[1,2] Within the purview of nanotechnology, nanoparticle-based controlled-delivery systems are gaining enormous interest. Traditional systems based on biodegradable or bioerosive polymeric nanoparticles, though effective in terms of attaining temporal control over the release process, lacked the spatial control often desired for targeted therapy since the release itself was not cell or tissue specific. Besides, varying fragment sizes resulting from the degradation process made toxicity studies challenging.[3]

To attain greater control over the delivery process, that is, delivering the bioactive cargo to the right place and at the right time, smart or "on demand" release systems have come to the forefront, made possible due to the development of stimuli-responsive nanomaterials. These nanomaterials, as their name suggests, respond to specific stimuli, which can be chemical or physical and endogenous or exogenous. Some endogenous stimuli include pH and ionic concentration, while exogenous stimuli could be temperature, light, electric or magnetic fields,[4] or even mechanical stress. However, endogeneous triggers such as pH may vary from person to person, which makes systems based on these stimuli challenging in terms of applicability. Exogenous stimuli are thus more promising in this regard.

Of the exogenous stimuli-responsive systems, those based on light have garnered enormous interest since light as a stimulus is not only noninvasive but also controllable both spatially and temporally, allowing for greater safety, specificity, and therapeutic efficacy. Photocontrolled delivery can be achieved through several strategies. Bioactive cargo itself can be "caged" using a photolabile group that gets released when irradiated

Received: June 9, 2014
Published: August 19, 2014

   dx.doi.org/10.1021/ar500217w | Acc. Chem. Res. 2014, 47, 3052−3060

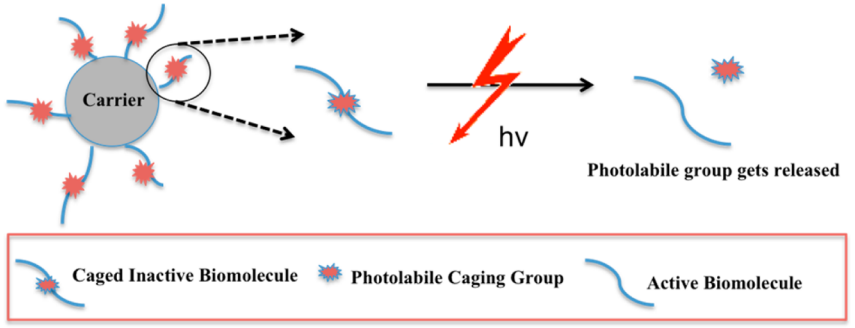



**Figure 1.** Schematic illustration of UV mediated photouncaging.

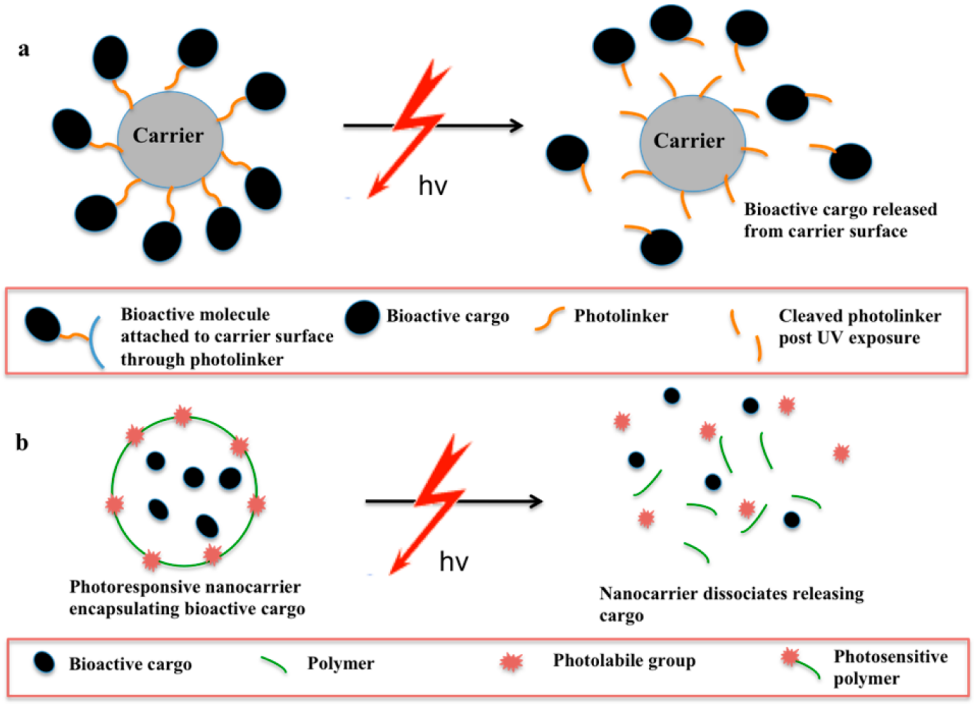



**Figure 2.** Schematic illustration of photocontrolled delivery using photoresponsive nanocarriers: (a) nanocarrier modified using photolinkers; (b) photoresponsive carriers encapsulating the bioactive cargo within.

with light of a suitable wavelength. Alternatively, photolinkers binding the cargo to the carrier surface can be used, which upon irradiation get cleaved,[5,6] or the carrier itself can be made photoresponsive and amenable to dissociation upon exposure to light.[7] In addition, materials capable of photothermal transduction can also be used for release of loaded cargo. Despite numerous benefits, light based controlled delivery systems are faced with certain issues that limit their clinical potential. The main roadblock is the widespread use of low penerating or toxic UV[8] and visible light to which most photosensitive compounds respond.[9] This issue can be resolved with the use of emerging technologies like upconversion and two-photon excitation that allow the use of deeper penetrating and biologically friendly NIR light instead of the conventional excitation wavelengths. This Account will describe the various approaches that have been adopted for light based delivery systems, their applications, their limitations, and how emerging technologies like upconversion have been used to overcome some of these roadblocks, easing the path from bench to bedside.

## 2. STRATEGIES FOR NANOPARTICLE-BASED PHOTOCONTROLLED DELIVERY

Photocontrolled nanoparticle-based delivery systems deploy three main strategies to achieve light based control over the delivery process. These are described in this section.

### 2.1. Photocaging of Bioactive Cargo

In this strategy, the nanocarrier itself is not photoresponsive and photocontrol is achieved through direct modification of the bioactive cargo with a photolabile "caging" moiety as illustrated in Figure 1. Bioactive molecules such as drugs, nucleic acids, and even small molecules can be temporarily "inactivated" by blocking key functional groups (carboxyl groups, amino groups, phosphate moieties, hydroxyl groups, etc.) using photolabile molecules.[10] Upon irradiation with light of a suitable wavelength (usually UV), this photolabile group gets released, rendering the biomolecule active again. This process of covalently linking the photolabile group to a biomolecule is termed "caging", and the biomolecule is said to be "caged".[11] Thus, nanocarriers loaded with photocaged biomolecules can be delivered systemically, and activation of the biomolecule can


dx.doi.org/10.1021/ar500217w | Acc. Chem. Res. 2014, 47, 3052−3060

be achieved in the region of interest by irradiating that region alone.

Numerous photolabile molecules have been developed for this purpose and can be divided in to different categories depending on the mechanism of photolysis. These include *o*-nitrobenzyl and related groups (for example, nitrophenyl ethyl (NPE), *o*-nitrobenzyl (NB), 1-(4,5-dimethoxy-2-nitrophenyl) diazoethane (DMNPE)), coumarin-4-ylmethyl and related groups (for example, 7-methoxycoumarin-4-ylmethyl (MCM)), *p*-hydroxyphenacyl (pHP) group (a promising alternative to the nitrobenzyl-based groups to cage biomolecules), and other miscellaneous groups, such as nitroindolinyl (NI) and 4-methoxyl-7-nitroindolinyl (MNI). A detailed review of how these photolabile groups work has been provided by Yu et al.[11]

### 2.2. Photoresponsive Nanocarriers

Photocontrol can also be achieved by using nanocarriers that are themselves photoresponsive. This can be realized in two different ways. The surface of the nanocarriers can be modified using photolinkers to which the bioactive cargo is then attached. These photolinkers cleave upon irradiation, releasing the cargo[5] (Figure 2a). Alternatively, for polymeric nanoparticles in particular, the constituent polymer can be modified using photosensitive moieties making the resulting particles photoresponsive (Figure 2b).[12,13] Depending on the type of photosensitive moiety used and the nature of the interaction of the constituent polymers, these particles can respond to light reversibly or irreversibly.

**2.2.1. Polymeric Nanoparticles with Reversible Light Response.** Most polymers responding to light in a reversible manner do so either by photoisomerization or photodimerization. Reversible photoisomerization involves change of chemical species from one isomeric form to another upon absorption of light of a particular wavelength. Reversal is achieved by absorption of light of another wavelength.[12] These isomers differ from each other in physical, chemical, and optical properties such as refractive index, dipole moment, and geometry, thus causing changes in the properties of the bulk material in which they are incorporated. A prominent example of a chromophore exhibiting such behavior is azobenzene. Upon absorption of UV light, it undergoes a *trans* to *cis* conversion, which can be reversed upon visible light irradiation. The planar *trans* form is stable and apolar, while the bent *cis* form is less stable and has a dipole moment of about 4.4 D, making it polar.[14] This behavior of azobenzene has been exploited for light induced reversible micellization.[15] Other compounds that exhibit such light dependent behavior include spiropyrans, spirooxazine, diarylethenes, fulgides, etc. In the case of spiropyrans and spiroxazines, irradiation with UV light results in the transformation from a cyclic ring structure to an open planar structure, which is reversible upon visible irradiation, while for diarylethenes and fulgides, the opposite holds true. Irradiation with UV results in the closure of six membered ring structures present within the core of these compounds while with visible light, the open structure reforms. Reversible light dependent behavior can also be attributed to photodimerization, particularly for coumarin-based polymers.[16] Coumarin moieties undergo dimerization via a photocycloaddition when irradiated with UV light between 300 and 350 nm. Photoscission occurs at wavelengths below 260 nm. Using this property of coumarin, polymer systems with reversible photocleavage and photo-cross-linking have been developed for applications such as controlled biomolecule encapsulation and delivery, where encapsulation can be achieved via dimerization upon exposure to light at one wavelength and release via photoscission upon irradiation at another.

**2.2.2. Polymeric Nanoparticles with Irreversible Light Response.** Polymers that respond to light irreversibly do so primarily via photocleavage of photolabile groups incorporated in their structure or through irreversible photo-cross-linking. Photocleavage has been used for irreversible micelle disruption. Block copolymers involved in micelle formation contain hydrophobic and hydrophilic parts that enable self-assembly in a given solvent. Photolabile groups are incorporated into the structure and, upon irradiation, get cleaved. This results in a hydrophobic−hydrophilic imbalance ultimately resulting in the disruption of the micelles, which can be used for targeted release of cargo encapsulated inside the micelle core.[17] Irreversible light response of some polymer systems can also be attributed to photo-cross-linking. An application of light induced photo-cross-linking is stabilization of micelles that are normally sensitive to the nature of solvent, ionic strength, pH, etc. One such method of achieving this has been through the incorporation of cynamoyl moieties in the block copolymer. Upon UV irradiation, these moieties participate in cross-linking, resulting in the stabilization of the micelles, making them impervious to some of the stimuli that may otherwise have caused their disruption.[18]

### 2.3. Photothermal Transduction and SPR-Based Field Enhancement

Nanoparticles made up of noble metals such as gold or silver, metal sulfides such as AuS and CuS, and more recently graphene based nanomaterials exhibit photothermal properties that have been exploited in controlled delivery systems. Noble metal nanoparticles exhibit localized surface plasmon resonance (LSPR), a collective oscillation of free electrons upon interaction with light. The SPR wavelength can be tuned across visible and NIR light ranges by changing nanoparticle size, shape, and composition.[19] These plasmon resonant particles have excellent light to heat conversion efficiencies and exhibit field enhancement effects, both of which have been used in controlled delivery systems.[20] One strategy involves attaching the therapeutic molecule covalently to the carrier surface. Upon irradiation with a pulsed laser, the nanoparticles undergo reshaping or break apart, destroying the covalent bonds attaching the cargo to the surface. Such a strategy has been reported for delivering the chemotherapeutic drug doxorubicin, using silver nanoparticles upon excitation with an 800 nm laser.[21] Another strategy involves attaching a "carrier" molecule, usually a nucleic acid, to the nanoparticle surface and loading the bioactive molecule of interest through weaker, typically noncovalent interactions onto the carrier molecule. Upon irradiation with light at SPR wavelength, the nanoparticles absorb energy that thermally or nonthermally modulates the interaction between the carrier molecule and the bioactive molecule to be delivered, resulting in its release.

The latter strategy though more complex has the advantage of using lower laser power densities and shorter irradiation times. It has been used for targeted nucleic acid delivery, wherein the nucleic acid to be delivered is loaded through complementary base pairing with the attached carrier molecule (Figure 3a). Upon irradiation with light at SPR wavelength, release occurs through either a thermal or nonthermal mechanism. For nanorods, the release is primarily through a


dx.doi.org/10.1021/ar500217w | Acc. Chem. Res. 2014, 47, 3052−3060

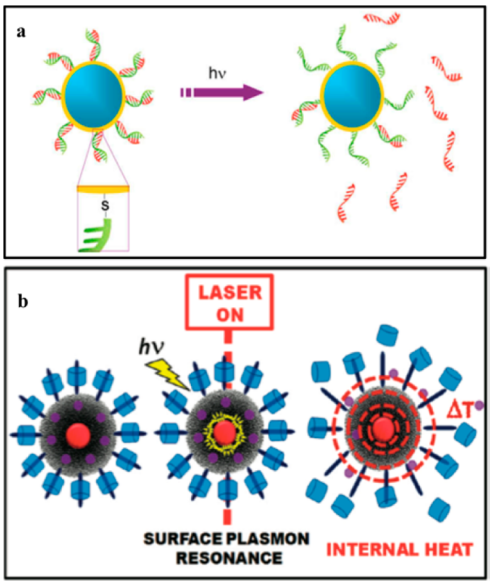



**Figure 3.** Schematic illustration of light-controlled release of ssDNA from Au nanoshells (a).[20] Release of Dye molecules due to photothermal-induced internal heating of Au@MSN@Valve through the surface plasmon effect (b).[24] Reprinted with permission from ref 20, copyright 2009 Elsevier, and from ref 24, copyright 2012 American Chemical Society, respectively.

thermal mechanism, while for nanoshells, owing to a greater absorption cross section, a nonthermal light induced release is prominent. This involves a transfer of "hot" or excited electrons from the metal to the "carrier" nucleic acid, increasing the electrostatic repulsion between the carrier and therapeutic nucleic acid, thereby resulting in dehybridization and release.[22]

Besides these strategies, the light to heat conversion of gold nanoparticles has also been used to achieve controlled delivery through the use of temperature-responsive polymers like poly(*N*-isopropylacrylamide) (PNIPAM). This polymer undergoes phase transition at its lower critical solution temperature (LCST), swelling at temperatures below the critical temperature and shrinking above it. With PNIPAM coated with gold nanorods, this transition can be achieved simply by irradiation with NIR light. Upon irradiation, heat generated by the nanorod causes the polymer to shrink, which in turn releases the trapped cargo.[23] In addition, the reversibility of shrinking and swelling behavior allows for a means of reversibly loading and releasing the desired cargo.

Recently, mesoporous silica nanoparticles (MSNs) encapsulating gold nanoparticles have been reported that use the light to heat conversion of these encapsulated nanoparticles or nanorods to unblock their mesopores and release the trapped cargo. For instance, Croissant and Zink synthesized MSNs encapsulating a gold nanoparticle and a guest dye molecule. The pores were blocked using a nanovalve mechanism consisting of cucurbit[6]uril rings encircling stalks attached to the pores (Figure 3b). Upon NIR irradiation, heat generated by gold nanoparticles resulted in a decrease in the ring−stalk binding constant and pore opening.[24] Mesopores have also been blocked using PNIPAM, which shrank when exposed to the heat generated by encapsulated gold nancages, thereby releasing the drug loaded in the pores.[25]

Certain metal sulfides, graphene based nanomaterials, etc. also show photothermal properties, albeit through a different mechanism. CuS nanoparticles absorb NIR due to the d−d transition of $Cu^{2+}$ ions and can generate heat for use in photothermal therapy,[26−28] controlled delivery, etc. For instance, Lu and co-workers used hollow CuS nanoparticles for transdermal delivery of drugs by focused thermal ablation of the skin via irradiation with a pulsed nanosecond NIR laser.[29]Another metal sulfide, AuS, has also been used for these applications.[30] Gold/gold sulfide composites have strong NIR absorption, attributed to a dielectric core, metal shell structure and typically have a smaller diameter than NIR-resonant particles.[31] In addition to these metal sulfides, graphene based nanomaterials, particularly graphene oxide (GO) are also becoming popular in photothermal-controlled delivery systems.[32] This is due to the high surface area of GO, which provides for efficient drug loading, excellent NIR absorption, and NIR to heat conversion, in addition to low cost of fabrication.[33] As such, graphene oxide composites conjugated with branched polyethylenimine and PEG have been reported for targeted gene delivery through photothermal disruption of endosomes upon irradation with NIR light.[34]

## 3. BIOMEDICAL APPLICATIONS OF PHOTOCONTROLLED DELIVERY SYSTEMS

### 3.1. Intracellular Study

Studying the underlying dynamics of cellular functions, such as energy production, neurotransmitter release, and muscle contraction, requires site and time specific control over activation of the bioactive moieties involved. To this end, photocaging has been used extensively for studying these processes by caging the implicated molecules and then using light to uncage them in a targeted manner.

Some of the small molecules and ions that have been caged include ATP, the energy currency of the cell, amino acids like glutamate, which are important neurotransmitters, and calcium ions ($Ca^{2+}$), an important secondary messenger. Caged ATPs have been used widely as on-demand sources of energy for cellular functions such as movement of motor proteins.[35] Calcium ions ($Ca^{2+}$) are important secondary messengers and control several physiological processes such as muscle contraction, neurotransmitter release, and ion channel gating. Use of caged calcium has enabled a greater understanding of the site, timing and mode of action of calcium in these cellular processes.[36] Glutamate activates glutamate receptors (GluRs) such as $\alpha$-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA). Using caged forms has elucidated specific mechanisms of neural signaling in which glutamate plays a major role such as studying the role of activation of postsynaptic AMPA receptors (a glutamate receptor) in the induction of NMDA receptor dependent long-term potentiation (a major cellular mechanism underlying learning and memory).[37]

Caging of proteins and peptides has also afforded a systematic way of studying and regulating biological processes, such as cell cycle regulation[38] and cell motility,[39] in which these biomacromolecules play key roles. In addition to the bioactive molecules mentioned above, biomolecules such as nucleic acids have also been caged. Several groups have utilized caged nucleic acids to manipulate gene expression either for studying gene function or as a therapy for diseases like cancer where specific genes may be aberrantly activated, playing a major role in tumor progression. Thus, knocking these down could potentially stop or retard tumor growth.[40]


dx.doi.org/10.1021/ar500217w | *Acc. Chem. Res.* 2014, 47, 3052−3060

Caging of bioactive molecules, though useful, suffers from certain limitations in that the design and synthesis of new caged compounds can be tedious in addition to the relative impermeability of these caged molecules in crossing the cell membrane on their own. As a result, nanocarriers that can carry these caged compounds or are light responsive themselves with the bioactive cargo encapsulated within are garnering enormous interest. For instance, lipid vesicles that could rupture when irradiated with red light have been developed. These were used to study apoptosis in targeted cells through light triggered release of a small molecule inhibitor of the kinase BCR-ABL.[42] Besides these lipid vesicles, gold nanoparticles that serve as bulky caging groups have also been reported. For example, using gold nanoparticles modified using a photocleavable succinimidyl ester (UV responsive), Nakanishi et al. delivered histamine to targeted HeLa cells, where it was used to study histamine receptor dependent intracellular $Ca^{2+}$ increase (Figure 4).[41]

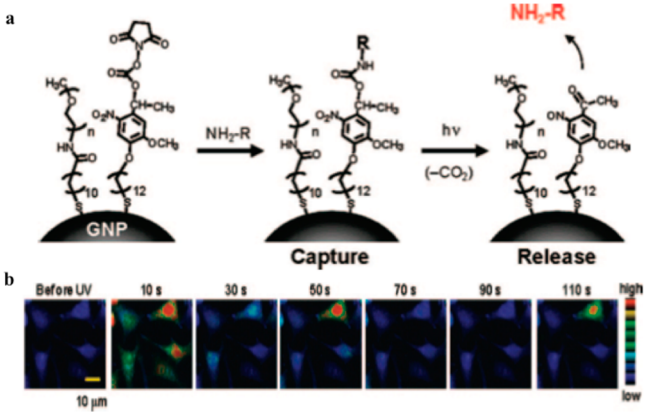



**Figure 4.** Schematic showing the capture and release of amines on gold nanoparticles having a photocleavable succinimidyl ester (a). Fluorescence (pseudocolor) images indicating intracellular $Ca^{2+}$ concentration in cells incubated with gold nanoparticles carrying histamine upon UV irradiation (b). Reprinted with permission from ref 41. Copyright 2009 American Chemical Society.

### 3.2. Therapeutic Applications

Photocontrolled release of drugs and nucleic acids allows one to potentially achieve such a targeted therapy using a noninvasive stimulus, light. To this end, nanoparticles modified with photocleavable linkers have been reported for delivery of chemotherapeutic drugs. For instance, UV mediated cleavage of a nitrobenzyl linker was reported for delivery of 5-fluorouracil in the treatment of breast cancer.[5] In another study, 5 nm sized gold nanoparticles were attached via photolinkers to the pores of MSNs, which contained the cargo to be delivered, thereby blocking the pores. When irradiated with UV light, the gold nanoparticles were released, unblocking the pores and releasing the trapped cargo (Figure 5).[6] Similar strategies using different types of "capping" or pore blocking moieties have also been reported.[43,44] Interestingly, the capping moiety itself can be toxic, and this has been exploited for a combination therapy by Knezevik and Lin.[45] The drug, camptothecin was loaded into the MSNs, and the pores were blocked using cadmium sulfide (CdS) nanoparticles through a photocleavable carbamate linkage. CdS nanoparticles, though toxic on their own, had reduced toxicity when bound to the MSNs. Upon irradiation with UV light, the photolinker was cleaved resulting in the release of both the drug and the toxic CdS nanoparticles, resulting in a synergistic cancer killing effect.[45]

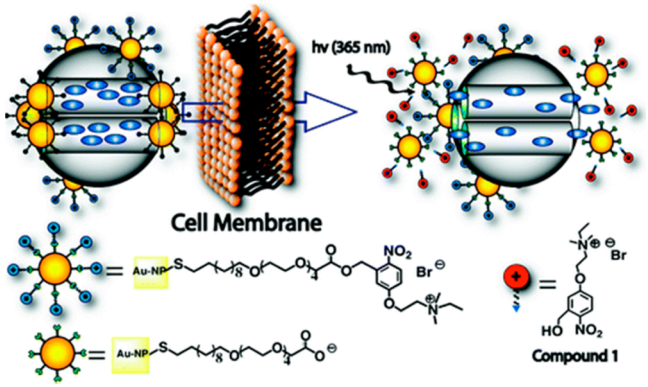



**Figure 5.** Schematic illustration of the photoinduced intracellular controlled release from mesoporous silica nanoparticles capped with organically derivatized gold nanoparticles (PR–AuNP–MSN). Reprinted with permission from ref 6. Copyright 2009 American Chemical Society.

Photoresponsive polymeric nanoparticles have also become increasingly popular in photocontrolled drug delivery systems. Photodegradable polymers made of polymers such as polyurethane and encapsulating water insoluble drugs have been used to trigger cell death in a targeted manner upon UV illumination.[46] Polymeric micelles have also come to the forefront[47] with the hydrophobic and hydrophilic balance of these particles being exploited for light based disruption of these micellar assemblies.[15] Furthermore, coumarin based photodimerization has also been used for light controlled micelle stabilization and disruption. The Zhao group incorporated coumarin into the hydrophobic block of their amphiphilic block copolymer.[48] Upon irradiation with UV > 310 nm, the coumarin moiety underwent dimerization resulting in the stabilization of the micelle. Irradiation with UV less than 260 nm resulted in de-cross-linking, micelle disruption, and cargo release.[49]

Lastly, noble metal based nanoparticles that exhibit SPR have also been used extensively in targeted drug and nucleic acid delivery systems. The light to heat conversion of silver nanoparticles was exploited for delivering doxorubicin covalently attached to iron oxide nanoparticles peppered with these Ag nanoparticles (Figure 6).[21] Similarly, nucleic acids or drugs loaded onto gold nanoparticles either covalently or via loading to attached carrier moieties have also been deployed for targeted delivery of these bioactive molecules. As a proof of concept, GFP expression in a lung cancer cell line, H1299, that stably expressed GFP was knocked down using this strategy. It resulted in a 47–49% knockdown.[22] Besides using the SPR property of these particles, photolinker modified gold and silver nanoparticles have also been reported as photocontrolled delivery systems. For instance, Han and co-workers synthesized positively charged gold nanoparticles with photocleavable *o*-nitrobenzyl ester linkages. DNA was loaded onto the nanoparticles via electrostatic interactions with the positively charged surface. Upon irradiation with UV light, the nitrobenzyl linkage was cleaved, resulting in a charge reversal at the nanoparticle surface and subsequent DNA release.[50]


dx.doi.org/10.1021/ar500217w | *Acc. Chem. Res.* 2014, 47, 3052–3060

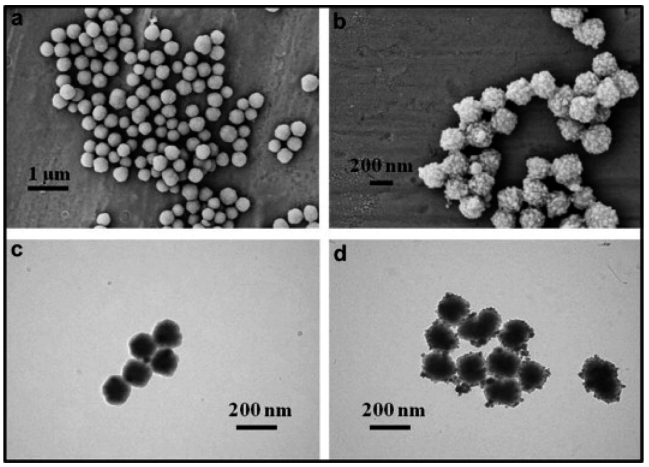



**Figure 6.** SEM (a, b) and TEM (c, d) images of (a, c) $Fe_3O_4$@C nanospheres and (b,c ) $Fe_3O_4$@C@Ag nanoparticles. Reprinted with permission from ref 21. Copyright 2013 Elsevier.

## 4. CURRENT CHALLENGES AND POTENTIAL SOLUTIONS

Most of the photosensitive groups used in the strategies mentioned above are sensitive to UV light, which besides having low tissue penetration is also associated with DNA damage and is unsuitable for clinical therapy. Thus, this field will benefit enormously if a longer wavelength of light, such as NIR, could be used instead. However, owing to the sensitivity of the photosensitive species to UV/visible light, transducers that can convert longer wavelength light to these shorter wavelengths are required. This can be achieved through technologies like two-photon excitation and upconversion. Both these technologies allow the use of the biologically transparent NIR light, which is converted into the lower wavelength UV and visible region by a nonlinear process involving simultaneous (two-photon excitation) or sequential absorption (upconversion) of two or more NIR photons. So instead of using UV or visible light directly, excitation can be achieved through NIR, which has deeper tissue penetration and biological transparency in comparison. Since the efficiency of the simultaneous two photon absorption is considerably lower than sequential absorption,[51] upconversion processes have a higher quantum yield compared with two-photon excitation and can be realized using inexpensive continuous wave lasers.

Capitalizing on this advantage, nanoparticles based on upconversion have become increasingly popular as nanotransducers for photocontrolled delivery systems. Upconversion nanoparticles (UCNs) typically consist of a rare-earth metal fluoride lattice doped with lanthanide ions, such as ytterbium ($Yb^{3+}$), thulium ($Tm^{3+}$), or erbium ($Er^{3+}$). They are excited in the NIR range and have tunable fluorescence emission depending on the dopant types and their concentrations. UCNs have been used as such for the delivery and photoactivation of caged bioactive cargo, as well as in phototriggered release systems as a part of micelles and hydrogels. Photocaged bioactive cargoes that have been delivered using UCNs include chemotherapeutic drugs, small interfering RNA (siRNA), and plasmids for applications such as targeted cancer therapy, site-specific gene knockdown, and gene expression. Typically, the bioactive caged cargo is loaded onto the UCNs and incubated with cells or injected into the body. Upon site-specific irradiation with NIR, the UCNs produce UV

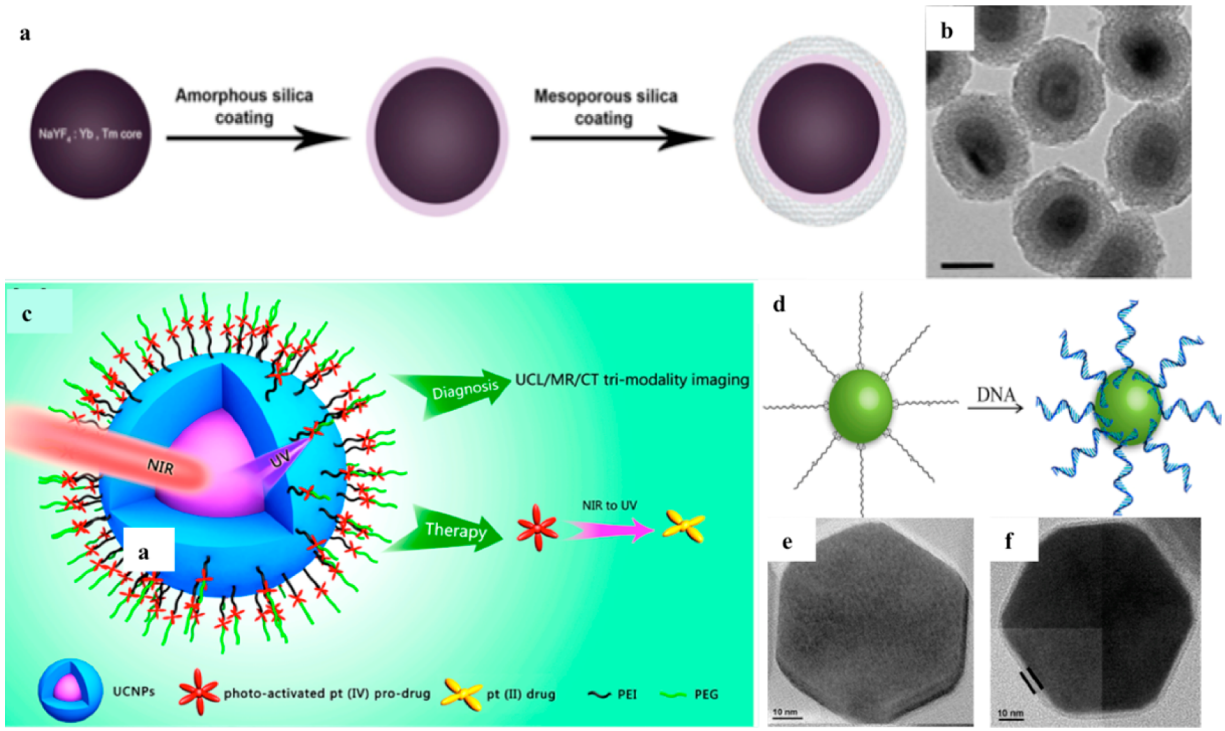



**Figure 7.** Surface coatings of UCNs. Schematic showing process of mesoporous silica coating (a) and TEM of mesoporous silica coated UCNs (b).[55] Schematic illustration of UCNP−DPP−PEG nanoparticles (c).[57] DNA-functionalized UCNs (d) and TEM images of the unmodified (e) and DNA-modified UCNs (f) (markings in part f indicate DNA layer).[58] Reprinted with permission from ref 55, copyright 2012 National Academy of Sciences, and from refs 57 and 58, copyright 2013 American Chemical Society.


dx.doi.org/10.1021/ar500217w | Acc. Chem. Res. 2014, 47, 3052−3060

or visible light required for uncaging, thereby activating the delivered molecules at the desired site.

A commonly used surface coating for UCNs is mesoporous silica (Figure 7a,b), owing to its low cytotoxicity and the presence of pores on the surface that facilitate loading of the cargo. Mesoporous silica coated UCNs have been reported for photocontrolled delivery of chemotherapeutic drugs and nucleic acids. For instance, using the *cis*/*trans* photoisomerization of the UV sensitive azo moiety (covering the mesopores) site specific release of a loaded drug[52] was achieved though upconverted UV light. Alternatively, photocontrol could also be attained by capping the pores using a cross-linked photosensitive linker.[53] Another strategy that greatly increased the amount of drug delivered was encapsulation of the caged drug along with a UCN yolk within a mesoporous silica shell. Upon irradiation, the upconverted UV light produced by the UCN uncaged the drug, which when released through the pores could kill cells at the irradiation site.[54] Photocontrolled gene delivery was demonstrated by Jayakumar and co-workers who loaded caged GFP plasmid into the pores of mesoporous silica coated $NaYF_4$ UCNs doped with $Yb^{3+}$ and $Tm^{3+}$ and showed that gene expression was restricted only to NIR irradiated cells.[55] The same group also demonstrated simultaneous delivery and photoactivation of photomorpholinos and TPPS2a, a photosensitizer used for photochemical internalization (PCI) using these particles. This resulted in endosomal escape enhanced gene knockdown in targeted cells *in vitro* and *in vivo* in a murine melanoma model.[56] Besides silica or mesoporous silica coating, UCNs can also be modified with polymers (Figure 7c)[57] and even nucleic acids (Figure 7d–f).[58]

UCNs have also been used solely as nanotransducers for phototriggered release systems as a part of light responsive micelles and hydrogels. When coencapsulated within light responsive micelles along with the cargo, they have been shown to trigger micelle disruption through the upconverted light produced upon NIR irradiation.[59] Similarly, hydrogels made with photosensitive group modified polymers that entrap UCNs have also been studied. For instance, Yan and co-workers[60] prepared a cross-linked hydrogel structure held together by photoresponsive o-nitrobenzyl groups. The UCNs and model bioactive cargo were entrapped in this hydrogel. Upon irradiation with NIR light at 980 nm, the UCNs emitted UV light, resulting in the cleavage of the o-nitrobenzyl groups and release of the entrapped biomolecules. It is important to note that both the above triggered release systems made use of very high laser power (3–5 W) for prolonged time periods (30 min and above).[60] This is a potential limiting factor for the use of these systems *in vivo* due to possibly deleterious heating effects.

UCNs though promising as nanotransducers for photocontrolled delivery systems are faced with certain limitations. Their quantum yield is low (usually <1%), requiring relatively high irradiation power densities for excitation. In addition, the most commonly used NIR excitation wavelength is 980 nm, very close to the absorption maximum of water (970 nm). This could result in harmful heating effects, particularly if high excitation power densities are used. Thus, this field would benefit from UCNs with higher quantum yields and use of excitation wavelengths where the absorption coefficient of water is low. Some efforts have already been made in this regard. Quantum yields of UCNs have been improved through coating these particles with undoped metal fluoride shells (to reduce surface quenching effects) (Figure 8a), using host

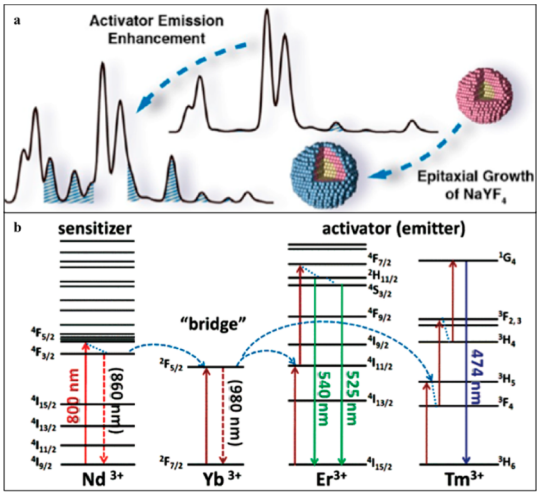



**Figure 8.** Epitaxial growth of an undoped $NaYF_4$ shell to enhance UCN fluorescence emission (a).[61] Upconversion process of $Nd^{3+} \rightarrow Yb^{3+} \rightarrow Er^{3+}$ ($Tm^{3+}$) tridopant system with 800 nm excitation (b).[62] Repinted with permission from ref 61, copyright 2012 American Chemical Society, and from ref 62, copyright 2013 John Wiley and Sons.

lattices different from the widely used $NaYF_4$ lattice, exploring different dopant types, varying concentrations of doping ions, etc.[61,63] In addition, UCNs with alternative excitation wavelengths have been also been reported. This has been achieved through introducing dopants like neodymium ions ($Nd^{3+}$) that allow these particles to be excited by 800 nm (Figure 8b) light where the absorption coefficient of water is low.[64] UCNs with excitation wavelength greater than 980 nm have also been synthesized by changing the host lattice.[65] Although these advances tackle the existing problems with UCNs by improving their optical properties, they have yet to undergo in vitro and in vivo testing.

## 5. CONCLUSIONS AND FUTURE OUTLOOK

Photocontrolled nanoparticles allow unprecedented control over the delivery process through their use of a noninvasive and spatially and temporally controllable external stimulus, light. Several different strategies have evolved that allow the use of specific wavelengths of light to achieve controlled release or activation of bioactive molecules such as nucleic acids, drugs, and even small molecules and ions. Their biological applications include studying cellular processes such as neurotransmitter release and cell motility and therapeutic applications such as targeted cancer therapy. Issues such as the predominant use of UV and visible light limit the clinical potential, but emerging technologies like upconversion that allow the use of deeper penetrating NIR light have come a long way in overcoming this issue, thereby bringing light based delivery systems a step closer to practical use.

## AUTHOR INFORMATION

### Corresponding Author

*Prof. Yong Zhang. Mailing address: Department of Biomedical Engineering Faculty of Engineering, Block EA #03-12 National University of Singapore 9 Engineering Drive 1, Singapore 117575. Phone: +65-65164871. Fax: +65-68723069. E-mail: biezy@nus.edu.sg.

## Funding

We acknowledge the funding support from Singapore A*STAR BMRC (wbs number R397000119305) and A*STAR SERC Biomedical Engineering Programme (wbs number R397000128305). We also acknowledge the support from the National Natural Science Foundation of China (Grant No. 31328009).

## Notes

The authors declare no competing financial interest.

## Biographies

**Akshaya Bansal** received her bachelor degree in bioengineering. Currently, she is a Ph.D. student at Graduate School for Integrative Sciences & Engineering, National University of Singapore (NUS).

**Yong Zhang** is a Professor at Department of Biomedical Engineering, National University of Singapore (NUS) and a senior member of NUS NanoCore Research Institute and NUS Graduate School for Integrative Sciences and Engineering (NGS). His current research interests include nanobiophotonics, nanomedicine, biomedical microdevices, and tissue engineering.

## REFERENCES

(1) Chanana, M.; Rivera_Gil, P.; Correa-Duarte, M. A.; Liz-Marzán, L. M.; Parak, W. J. Physicochemical Properties of Protein-Coated Gold Nanoparticles in Biological Fluids and Cells before and after Proteolytic Digestion. *Angew. Chem., Int. Ed.* **2013**, *52*, 4179−4183.

(2) Bae, K. H.; Chung, H. J.; Park, T. G. Nanomaterials for cancer therapy and imaging. *Mol. Cells* **2011**, *31*, 295−302.

(3) Liechty, W. B.; Kryscio, D. R.; Slaughter, B. V.; Peppas, N. A. Polymers for drug delivery systems. *Annu. Rev. Chem. Biomol. Eng.* **2010**, *1*, 149−173.

(4) Davis, M. E. The first targeted delivery of siRNA in humans via a self-assembling, cyclodextrin polymer-based nanoparticle: From concept to clinic. *Mol. Pharmaceutics* **2009**, *6*, 659−668.

(5) Agasti, S. S.; Chompoosor, A.; You, C.-C.; Ghosh, P.; Kim, C. K.; Rotello, V. M. Photoregulated release of caged anticancer drugs from gold nanoparticles. *J. Am. Chem. Soc.* **2009**, *131*, 5728−5729.

(6) Vivero-Escoto, J. L.; Slowing, I. I.; Wu, C.-W.; Lin, V. S. Y. Photoinduced intracellular controlled release drug delivery in human cells by gold-capped mesoporous silica nanosphere. *J. Am. Chem. Soc.* **2009**, *131*, 3462−3463.

(7) Tong, X.; Wang, G.; Soldera, A.; Zhao, Y. How can azobenzene block copolymer vesicles be dissociated and reformed by light? *J. Phys. Chem. B* **2005**, *109*, 20281−20287.

(8) Vrouwe, M. G.; Pines, A.; Overmeer, R. M.; Hanada, K.; Mullenders, L. H. UV-induced photolesions elicit ATR-kinase-dependent signaling in non-cycling cells through nucleotide excision repair-dependent and-independent pathways. *J. Cell Sci.* **2011**, *124*, 435−446.

(9) Katz, J. S.; Burdick, J. A. Light-responsive biomaterials: Development and applications. *Macromol. Biosci.* **2010**, *10*, 339−348.

(10) Sortino, S. Photoactivated nanomaterials for biomedical release applications. *J. Mater. Chem.* **2012**, *22*, 301−318.

(11) Yu, H.; Li, J.; Wu, D.; Qiu, Z.; Zhang, Y. Chemistry and biological applications of photo-labile organic molecules. *Chem. Soc. Rev.* **2010**, *39*, 464−473.

(12) Ercole, F.; Davis, T. P.; Evans, R. A. Photo-responsive systems and biomaterials: Photochromic polymers, light-triggered self-assembly, surface modification, fluorescence modulation and beyond. *Polym. Chem.* **2010**, *1*, 37−54.

(13) Pasparakis, G.; Manouras, T.; Vamvakaki, M.; Argitis, P. Harnessing photochemical internalization with dual degradable nanoparticles for combinatorial photo−chemotherapy. *Nat. Commun.* **2014**, *5*, No. 3623, DOI: 10.1038/ncomms4623.

(14) Schumers, J. M.; Fustin, C. A.; Gohy, J. F. Light-responsive block copolymers. *Macromol. Rapid Commun.* **2010**, *31*, 1588−1607.

(15) Jiang, J.; Tong, X.; Morris, D.; Zhao, Y. Toward photocontrolled release using light-dissociable block copolymer micelles. *Macromolecules* **2006**, *39*, 4633−4640.

(16) Trenor, S. R.; Shultz, A. R.; Love, B. J.; Long, T. E. Coumarins in polymers: From light harvesting to photo-cross-linkable tissue scaffolds. *Chem. Rev.* **2004**, *104*, 3059−3078.

(17) Jiang, J.; Tong, X.; Zhao, Y. A new design for light-breakable polymer micelles. *J. Am. Chem. Soc.* **2005**, *127*, 8290−8291.

(18) Sandholzer, M.; Bichler, S.; Stelzer, F.; Slugovc, C. UV-induced crosslinking of ring opening metathesis block copolymer micelles. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 2402−2413.

(19) Voliani, V.; Signore, G.; Vittorio, O.; Faraci, P.; Luin, S.; Peréz-Prieto, J.; Beltram, F. Cancer phototherapy in living cells by multiphoton release of doxorubicin from gold nanospheres. *J. Mater. Chem. B* **2013**, *1*, 4225−4230.

(20) Barhoumi, A.; Huschka, R.; Bardhan, R.; Knight, M. W.; Halas, N. J. Light-induced release of DNA from plasmon-resonant nanoparticles: Towards light-controlled gene therapy. *Chem. Phys. Lett.* **2009**, *482*, 171−179.

(21) Chen, J.; Guo, Z.; Wang, H. B.; Gong, M.; Kong, X. K.; Xia, P.; Chen, Q. W. Multifunctional Fe3O4@C@Ag hybrid nanoparticles as dual modal imaging probes and near-infrared light-responsive drug delivery platform. *Biomaterials* **2013**, *34*, 571−581.

(22) Huschka, R.; Barhoumi, A.; Liu, Q.; Roth, J. A.; Ji, L.; Halas, N. J. Gene silencing by gold nanoshell-mediated delivery and laser-triggered release of antisense oligonucleotide and siRNA. *ACS Nano* **2012**, *6*, 7681−7691.

(23) Kawano, T.; Niidome, Y.; Mori, T.; Katayama, Y.; Niidome, T. PNIPAM gel-coated gold nanorods for targeted delivery responding to a near-infrared laser. *Bioconjugate Chem.* **2009**, *20*, 209−212.

(24) Croissant, J.; Zink, J. I. Nanovalve-controlled cargo release activated by plasmonic heating. *J. Am. Chem. Soc.* **2012**, *134*, 7628−7631.

(25) Yang, J.; Shen, D.; Zhou, L.; Li, W.; Li, X.; Yao, C.; Wang, R.; El-Toni, A. M.; Zhang, F.; Zhao, D. Spatially confined fabrication of core−shell gold nanocages@ mesoporous silica for near-infrared controlled photothermal drug release. *Chem. Mater.* **2013**, *25*, 3030−3037.

(26) Zhou, M.; Zhang, R.; Huang, M.; Lu, W.; Song, S.; Melancon, M. P.; Tian, M.; Liang, D.; Li, C. A chelator-free multifunctional [64Cu] CuS nanoparticle platform for simultaneous micro-PET/CT imaging and photothermal ablation therapy. *J. Am. Chem. Soc.* **2010**, *132*, 15351−15358.

(27) Tian, Q.; Tang, M.; Sun, Y.; Zou, R.; Chen, Z.; Zhu, M.; Yang, S.; Wang, J.; Wang, J.; Hu, J. Hydrophilic flower-like CuS superstructures as an efficient 980 nm laser-driven photothermal agent for ablation of cancer cells. *Adv. Mater.* **2011**, *23*, 3542−3547.

(28) Guo, L.; Yan, D. D.; Yang, D.; Li, Y.; Wang, X.; Zalewski, O.; Yan, B.; Lu, W. Combinatorial photothermal and immuno cancer therapy using chitosan-coated hollow copper sulfide nanoparticles. *ACS Nano* **2014**, *8* (6), 5670−5681.

(29) Ramadan, S.; Guo, L.; Li, Y.; Yan, B.; Lu, W. Hollow copper sulfide nanoparticle-mediated transdermal drug delivery. *Small* **2012**, *8*, 3143−3150.

(30) Gobin, A. M.; Lee, M. H.; Halas, N. J.; James, W. D.; Drezek, R. A.; West, J. L. Near-infrared resonant nanoshells for combined optical imaging and photothermal cancer therapy. *Nano Lett.* **2007**, *7*, 1929−1934.

(31) Gobin, A. M.; Watkins, E. M.; Quevedo, E.; Colvin, V. L.; West, J. L. Near-infrared-resonant gold/gold sulfide nanoparticles as a photothermal cancer therapeutic agent. *Small* **2010**, *6*, 745−752.

(32) Matteini, P.; Tatini, F.; Cavigli, L.; Ottaviano, S.; Ghini, G.; Pini, R. Graphene as a photothermal switch for controlled drug release. *Nanoscale* **2014**, *6*, 7947−7953.

(33) Yang, K.; Feng, L.; Shi, X.; Liu, Z. Nano-graphene in biomedicine: theranostic applications. *Chem. Soc. Rev.* **2013**, *42*, 530−547.

(34) Kim, H.; Lee, D.; Kim, J.; Kim, T.-i.; Kim, W. J. Photothermally triggered cytosolic drug delivery via endosome disruption using a functionalized reduced graphene oxide. *ACS Nano* **2013**, *7*, 6735−6746.
(35) Hess, H.; Clemmens, J.; Qin, D.; Howard, J.; Vogel, V. Light-controlled molecular shuttles made from motor proteins carrying cargo on engineered surfaces. *Nano Lett.* **2001**, *1*, 235−239.
(36) Ellis-Davies, G. C. Neurobiology with caged calcium. *Chem. Rev.* **2008**, *108*, 1603−1613.
(37) Bagal, A. A.; Kao, J. P. Y.; Tang, C.-M.; Thompson, S. M. Long-term potentiation of exogenous glutamate responses at single dendritic spines. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 14434−14439.
(38) Nguyen, A.; Rothman, D. M.; Stehn, J.; Imperiali, B.; Yaffe, M. B. Caged phosphopeptides reveal a temporal role for 14−3-3 in G1 arrest and S-phase checkpoint function. *Nat. Biotechnol.* **2004**, *22*, 993−1000.
(39) Ghosh, M.; Song, X.; Mouneimne, G.; Sidani, M.; Lawrence, D. S.; Condeelis, J. S. Cofilin promotes actin polymerization and defines the direction of cell motility. *Science* **2004**, *304*, 743−746.
(40) Kala, A.; Friedman, S. H. Enhanced light-activated RNA interference using phosphorothioate-based dsRNA precursors of siRNA. *Pharm. Res.* **2011**, *28*, 3050−3057.
(41) Nakanishi, J.; Nakayama, H.; Shimizu, T.; Ishida, H.; Kikuchi, Y.; Yamaguchi, K.; Horiike, Y. Light-regulated activation of cellular signaling by gold nanoparticles that capture and release amines. *J. Am. Chem. Soc.* **2009**, *131*, 3822−3823.
(42) Gregersen, K. A.; Hill, Z. B.; Gadd, J. C.; Fujimoto, B. S.; Maly, D. J.; Chiu, D. T. Intracellular delivery of bioactive molecules using light-addressable nanocapsules. *ACS Nano* **2010**, *4*, 7603−7611.
(43) Knežević, N. Ž.; Trewyn, B. G.; Lin, V. S. Y. Functionalized mesoporous silica nanoparticle-based visible light responsive controlled release delivery system. *Chem. Commun.* **2011**, *47*, 2817−2819.
(44) He, D.; He, X.; Wang, K.; Cao, J.; Zhao, Y. A light-responsive reversible molecule-gated system using thymine-modified mesoporous silica nanoparticles. *Langmuir* **2012**, *28*, 4003−4008.
(45) Knezevic, N. Z.; Lin, V. S. A magnetic mesoporous silica nanoparticle-based drug delivery system for photosensitive cooperative treatment of cancer with a mesopore-capping agent and mesopore-loaded drug. *Nanoscale* **2013**, *5*, 1544−1551.
(46) Lv, C.; Wang, Z.; Wang, P.; Tang, X. Photodegradable polyurethane self-assembled nanoparticles for photocontrollable release. *Langmuir* **2012**, *28*, 9387−9394.
(47) Zhao, Y. Rational design of light-controllable polymer micelles. *Chem. Rec.* **2007**, *7*, 286−294.
(48) He, J.; Zhao, Y. Light-responsive polymer micelles, nano- and microgels based on the reversible photodimerization of coumarin. *Dyes Pigm.* **2011**, *89*, 278−283.
(49) Jiang, J.; Qi, B.; Lepage, M.; Zhao, Y. Polymer micelles stabilization on demand through reversible photo-cross-linking. *Macromolecules* **2007**, *40*, 790−792.
(50) Han, G.; You, C. C.; Kim, B. j.; Turingan, R. S.; Forbes, N. S.; Martin, C. T.; Rotello, V. M. Light-regulated release of DNA and its delivery to nuclei by means of photolabile gold nanoparticles. *Angew. Chem.* **2006**, *118*, 3237−3241.
(51) Dong, B.; Xu, S.; Sun, J.; Bi, S.; Li, D.; Bai, X.; Wang, Y.; Wang, L.; Song, H. Multifunctional NaYF4: Yb3+, Er3+@ Ag core/shell nanocomposites: Integration of upconversion imaging and photothermal therapy. *J. Mater. Chem.* **2011**, *21*, 6193−6200.
(52) Liu, J.; Bu, W.; Pan, L.; Shi, J. NIR-Triggered Anticancer Drug Delivery by Upconverting Nanoparticles with Integrated Azobenzene-Modified Mesoporous Silica. *Angew. Chem., Int. Ed.* **2013**, *52*, 4375−4379.
(53) Yang, Y.; Velmurugan, B.; Liu, X.; Xing, B. NIR photoresponsive crosslinked upconverting nanocarriers toward selective intracellular drug release. *Small* **2013**, *9*, 2937−2944.
(54) Zhao, L.; Peng, J.; Huang, Q.; Li, C.; Chen, M.; Sun, Y.; Lin, Q.; Zhu, L.; Li, F. Near-infrared photoregulated drug release in living tumor tissue via yolk-shell upconversion nanocages. *Adv. Funct. Mater.* **2013**, *24*, 363−371.
(55) Jayakumar, M. K.; Idris, N. M.; Zhang, Y. Remote activation of biomolecules in deep tissues using near-infrared-to-UV upconversion nanotransducers. *Proc. Natl. Acad. Sci. U.S.A* **2012**, *109*, 8483−8488.
(56) Jayakumar, M. K. G.; Bansal, A.; Huang, K.; Yao, R.; Li, B. N.; Zhang, Y. Near-infrared-light-based nano-platform boosts endosomal escape and controls gene knockdown in vivo. *ACS Nano* **2014**, *8*, 4848−4858.
(57) Dai, Y.; Xiao, H.; Liu, J.; Yuan, Q.; Ma, P. a.; Yang, D.; Li, C.; Cheng, Z.; Hou, Z.; Yang, P.; Lin, J. In vivo multimodality imaging and cancer therapy by near-infrared light-triggered trans-platinum pro-drug-conjugated upconverison nanoparticles. *J. Am. Chem. Soc.* **2013**, *135*, 18920−18929.
(58) Li, L.-L.; Wu, P.; Hwang, K.; Lu, Y. An exceptionally simple strategy for DNA-functionalized up-conversion nanoparticles as biocompatible agents for nanoassembly, DNA delivery, and imaging. *J. Am. Chem. Soc.* **2013**, *135*, 2411−2414.
(59) Yan, B.; Boyer, J.-C.; Branda, N. R.; Zhao, Y. Near-infrared light-triggered dissociation of block copolymer micelles using upconverting nanoparticles. *J. Am. Chem. Soc.* **2011**, *133*, 19714−19717.
(60) Yan, B.; Boyer, J.-C.; Habault, D.; Branda, N. R.; Zhao, Y. Near infrared light triggered release of biomacromolecules from hydrogels loaded with upconversion nanoparticles. *J. Am. Chem. Soc.* **2012**, *134*, 16558−16561.
(61) Su, Q.; Han, S.; Xie, X.; Zhu, H.; Chen, H.; Chen, C.-K.; Liu, R.-S.; Chen, X.; Wang, F.; Liu, X. The effect of surface coating on energy migration-mediated upconversion. *J. Am. Chem. Soc.* **2012**, *134*, 20849−20857.
(62) Shen, J.; Chen, G.; Vu, A. M.; Fan, W.; Bilsel, O. S.; Chang, C. C.; Han, G. Engineering the upconversion nanoparticle excitation wavelength: Cascade sensitization of tri-doped upconversion colloidal nanoparticles at 800 nm. *Adv. Opt. Mater.* **2013**, *1*, 644−650.
(63) Chen, G.; Ohulchanskyy, T. Y.; Kachynski, A.; Ågren, H.; Prasad, P. N. Intense visible and near-infrared upconversion photoluminescence in colloidal $LiYF_4:Er^{3+}$ nanocrystals under excitation at 1490 nm. *ACS Nano* **2011**, *5*, 4981−4986.
(64) Xie, X.; Gao, N.; Deng, R.; Sun, Q.; Xu, Q.-H.; Liu, X. Mechanistic investigation of photon upconversion in $Nd^{3+}$-sensitized core−shell nanoparticles. *J. Am. Chem. Soc.* **2013**, *135*, 12608−12611.
(65) Zheng, J.; Wang, X. F.; He, W. Y.; Bu, Y. Y.; Yan, X. H. Seven-photon ultraviolet upconversion emission of Er3+ induced by 1540-nm laser excitation. *Appl. Phys. B: Laser Opt.* **2013**, *115*, 443−449.