Photochemistry

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Remote-Control Photorelease of Caged Compounds Using Near-Infrared Light and Upconverting Nanoparticles**

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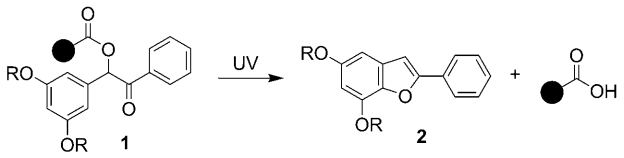
The light-triggered release of molecules from "caged" forms offers the potential to deliver innocuous agents to cells, tissues, and organisms, where they can be unmasked to their active states.[1] Because light can be readily tuned and focused, it can be spatially and temporally controlled to provide "on-command" drug delivery, unmasking of biochemical agents for enzyme and protein activation, and other biochemical and physiological studies. Several classes of small-molecule systems have been developed for use in these applications but all suffer from a serious drawback: they require high-energy ultraviolet or visible light as the trigger. Until methods that use less detrimental, lower-energy light that penetrates deeper into tissue without causing unwanted side reactions are developed, this technology will not find the widespread acceptance that it deserves.[1,2]

Multiphoton excitation with near-infrared (NIR) light has been presented as a practical solution to the issues associated with UV or visible light, given that it is less damaging and penetrates deeper into tissue.[3,4] What prevents the general use of the multiphoton excitation technique is that many "cages" do not have large enough multiphoton cross sections in order to be susceptible to NIR light, or that their susceptibility is linked to wavelengths of light that lie outside of the tuning range of pulsed lasers.[5,6] One illustrative example is the 3′,5′-dialkoxybenzoin cage **1** (Scheme 1), which together with 2-nitrobenzyl, coumarin-4-yl-methyl, *p*-hydroxyphenacyl, and 7-nitroindoline derivatives, is one of the most commonly employed classes of "photocages".[1] This versatile compound exhibits many appealing features. Its photochemistry is relatively universal, and esters, carbonates, carbamates, and phosphates can all be employed for photorelease.[7–13] The high quantum yield (>0.6) and fast rate (10^{10}–$10^{12}\ s^{-1}$) of photolysis allows efficient release ($>95\%$) using short laser pulses.[12,13] The photorelease does not generally lead to side reactions, and 5,7-dialkoxy-2-phenylbenzo[*b*]furan (**2**) is the only generated species (other than the released acid, alcohol, amine, or phosphate) in organic solvents.[8,14]



Scheme 1. Photolysis and release of caged compounds from the generalized 3′,5′-dialkoxybenzoin structure **1** using UV light to produce 2-phenylbenzo[*b*]furan **2** and a carboxylic acid. Alcohols, amines, and phosphates can also be released using this approach. R = alkyl.

The need for unsuitable UV light in the photolysis can be overcome by coupling the 3′,5′-dialkoxybenzoin to NIR-absorbing species that act as antennae, harvest the light, and convert it into the necessary UV light through a multiphoton process. Monodispersed core–shell upconverting nanoparticles (UCNPs) composed of $NaYF_4$ nanocrystals doped with lanthanides such as Tm^{3+} and Yb^{3+} ($NaYF_4$:TmYb) are excellent candidates for this task.[15–18] These UCNPs convert continuous-wave 980 nm laser light into a range of different wavelengths of light throughout the UV, visible, and NIR regions, many of which can be harnessed to drive the photoreactions of compounds anchored to their surfaces.[15–21] This "remote-control" photorelease is illustrated in Scheme 2 and is the focus of the studies described herein. Although we demonstrate the success of this strategy using a model compound that releases a carboxylic acid, this approach is expected to be very general and be able to perform equally well in the release of other caged benzoin compounds.

The appeal of UCNPs over multiphoton absorbing molecules can be justified on many levels. Most importantly, the Tm^{3+} and Yb^{3+} energy levels involved in the photophysics of the $NaYF_4$:TmYb nanoparticles are real and the multiple absorptions are sequential, in contrast to the simultaneous absorptions needed for typical two-photon excitation.[22] The sequential absorptions mean that the power density of the excitation source is reduced to about 10^4–10^7 orders of magnitude lower than that required for multiphoton photolysis, and allows for the employment of more economical, continuous-wave diode laser instead of a pulsed laser.[22] In biological applications, the use of a nanoparticle as a delivery vehicle offers additional benefits compared to molecular photorelease, namely: 1) UCNPs can be coated with biocompatible polymers that render them water-soluble in order to

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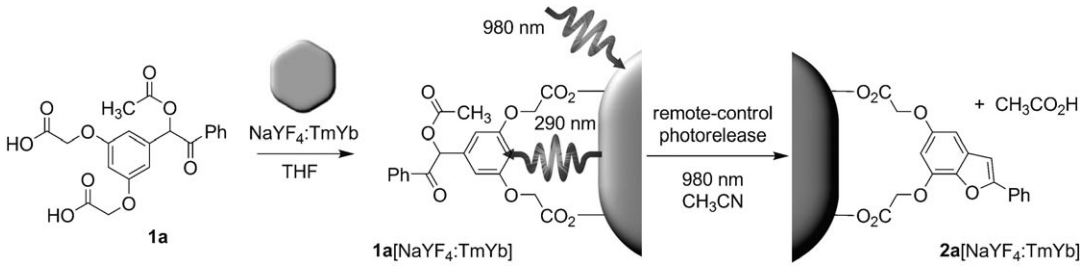

Scheme 2. Decoration of upconverting nanoparticles with 3′,5′-di(carboxymethoxy)benzoin cage **1a** produces the remote-control release system (**1a**[$NaYF_4$:TmYb]), which can be triggered by indirect irradiation with NIR light to generate **2a**[$NaYF_4$:TmYb] and release a carboxylic acid.

lengthen blood circulation time and attenuate uptake by the reticuloendothelial system;[23,24] 2) the UCNP surfaces can be loaded with multiple therapeutic agents to offer synergistic effects,[25,26] and with antibodies or other ligands to provide targeting and selectivity;[21] 3) as water solubility and biocompatibility are both provided by the decorated UCNPs, the cage itself can be lipophilic, therefore overcoming one of the most significant problems associated with the benzoin class of compounds;[8] and 4) the luminescent nature of the UCNPs make them ideal for providing information about where and when the release event has taken place ("release-and-report").[27,28] UCNPs based on lanthanide-doped $NaYF_4$ nanoparticles have already been shown to be biocompatible and nontoxic,[29] and have been used for in vitro photodynamic therapy,[23–25] in vitro and in vivo fluorescence bioimaging,[30–32] and other biological applications.[33–35]

The core–shell, lanthanide-doped $NaYF_4$:TmYb nanoparticles (core = $NaYF_4$:0.5 mol% Tm^{3+}:30 mol% Yb^{3+}; shell = $NaYF_4$) were synthesized by following the protocol of Qian and Zhang[35] with some minor modifications.[36] TEM images of the UCNPs demonstrate their nearly monodisperse particle size of height (44.7 ± 1.8) nm and diameter (41.4 ± 1.4) nm.[36] Powder X-ray diffraction[36] confirmed that the nanoparticles are highly crystalline and are hexagonal in phase, with no significant impurity phases present. The synthesis results in nanoparticles coated in oleate ions, which we anticipated could be displaced by a compound bearing two carboxylic acid groups. The previously described 3′,5′-di(carboxymethoxy)benzoin acetate **1a** (Scheme 2) suits this requirement well and was synthesized according to a reported procedure.[8]

Figure 1a illustrates how the absorption spectrum of 3′,5′-di(carboxymethoxy)benzoin acetate **1a** partially overlaps ($\lambda = 282$ nm) with the emission spectrum of the $NaYF_4$:TmYb nanoparticles ($\lambda = 290$ nm), and illustrates the rationale behind our choice of UCNPs and photocage. When a solution of benzoin **1a** in CD_3CN (8.87×10^{-3} M) is exposed to UV light (313 nm), **1a** is converted within 100 min[38] into its 5,7-di(carboxymethoxy)-2-phenylbenzo[*b*]furan (**2a**) counterpart and acetic acid in high yield (> 95%), as determined by ^{1}H NMR spectroscopy.[39] As expected, no hydrolyzed benzoin derivative was observed. The $^1D_2 \rightarrow {}^3H_6$ transition of the $NaYF_4$:TmYb nanoparticles also provides the UV light indirectly when irradiated with 980 nm light and should be effective to trigger the uncaging in a remote-control process.

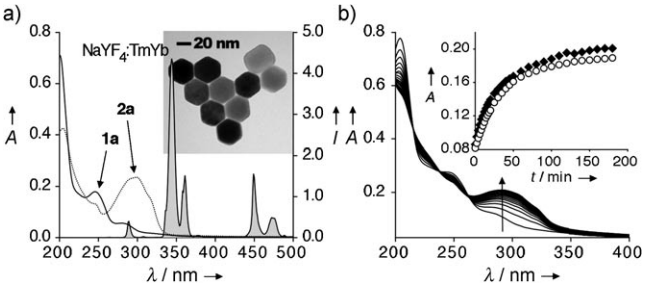

Figure 1. a) UV/Vis absorption spectra of a solution of 3′,5′-di(carboxymethoxy)benzoin **1a** (8.54×10^{-6} M) in CH_3CN before (solid line) and after irradiation with 365 nm light (dashed line) for 35 min, and the emission spectrum of a THF solution (1 wt%, $\lambda_{ex} = 980$ nm) of the $NaYF_4$:TmYb core–shell UCNPs (shaded). The inset shows the TEM photograph of **1a**[$NaYF_4$:TmYb]. The uniform size and hexagonal prism shape can be seen. b) Changes in the UV/Vis absorption spectra of a solution of **1a**[$NaYF_4$:TmYb] in CH_3CN when it is irradiated with 980 nm light (power = 4.4 W, power density = 550 W cm^{-2}).[36,37] The inset shows the changes in the absorbance at 300 nm when samples of the same solution are irradiated with 290 nm (circles) and 980 nm light (diamonds).

The $NaYF_4$:TmYb nanoparticles were loaded with 3′,5′-di(carboxymethoxy)benzoin acetate **1a** by simply stirring a colloidal dispersion of the two components overnight in THF.[36] After a number of centrifuging–resuspending purification cycles, the decorated nanoparticles (**1a**[$NaYF_4$:TmYb]) were dispersed in CH_3CN to provide a stock solution that is stable at room temperature for more than four weeks if stored in the dark (see the inset in Figure 1a for a TEM image of the decorated UCNPs). Sample solutions for subsequent photolysis experiments were prepared by further dilution with CH_3CN. The loading of 3′,5′-di(carboxymethoxy)benzoin **1a** onto the $NaYF_4$:TmYb nanoparticles was analyzed by UV/Vis absorption spectroscopy and was estimated by calculating the number of particles in an aliquot of the CH_3CN stock solution and the amount of chromophore **1a** removed from the UCNPs surfaces when a similar aliquot was treated with excess methylphosphonic acid to induce ligand exchange. 3′,5′-Di(carboxymethoxy)benzoin **1a** was loaded onto the $NaYF_4$:TmYb nanoparticles with an average amount of approximately 2 wt%, which corresponds to approximately 6800 molecules of cage per nanoparticle.[36]

Exposure of a solution of the free benzoin cage **1a** in CH_3CN to 980 nm continuous wave laser light (power = 4.37 W, power density = 556 W cm^{-2}) resulted in no observ-

able changes in the UV/Vis absorption spectrum even after one hour of irradiation,[36] thus demonstrating that the photocage is, as expected, not susceptible to direct multiphoton release in the absence of the UCNPs. On the other hand, the remote-control strategy employing **1a**[$NaYF_4$:TmYb] is very effective, as shown by the spectral changes when a dispersion of the decorated nanoparticles in CH_3CN was exposed to NIR light. As illustrated in Figure 1b, NIR light (980 nm) triggers a decrease in the absorptions in the UV region ($\lambda_{max} = 203$ nm and 248 nm) and a concomitant increase in longer wavelength absorptions ($\lambda_{max} = 283$–292 nm) corresponding to 5,7-di(carboxymethoxy)-2-phenylbenzo[*b*]furan attached to the nanoparticles (**2a**[$NaYF_4$:TmYb]), which have a spectral profile that are the same as those recorded when a dispersion of the **1a**[$NaYF_4$:TmYb] nanoparticles are exposed to UV light (290 nm) to trigger the direct photorelease.[36,40] It can be assumed that the photorelease arises from the 290 nm light generated by the multiphoton process. The changes in the absorptions at 300 nm, which correspond to the formation of benzofuran **2a**[$NaYF_4$:TmYb] induced using 980 nm (remote-control) excitation are almost identical to those produced using direct excitation (290 nm), thus suggesting that the two photolysis reactions are the same (Figure 1b, inset).[41] These experiments also imply that both direct and indirect irradiation drive the photolysis reaction yield to the same extent.

The NIR-activated organic–nanoparticle hybrid system has the potential to increase selectivity in photodynamic therapy and biochemical studies. The harnessing of low-energy NIR light and conversion into high-energy UV light overcomes the drawback that typical organic reactions cannot be triggered by NIR light, even though this type of light is necessary for biological applications. Our next generation of "cage-decorated" UCNPs will take advantage of the fact that the $NaYF_4$:TmYb nanoparticles have their most intense emission bands at 333–355 nm when excited by 980 nm light (Figure 1a). The design of 3′,5′-dialkoxybenzoin derivatives with red-shifted absorptions will better match the chromophore's absorption with the nanoparticles' emission profile, and enhance the efficiency of the remote-control photorelease. If properly designed, the improved system should also have red-shifted bands only in its benzoin form, thus eliminating any self-filtering effects that arise as the benzoin photoproduct absorbs more effectively at 290 nm. The results of these studies will be reported in due course.

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[37] Given that the absorption intensity is similar to that of the free cage **1a**, the concentration of decorated UCNPs is estimated to

be 10^{-5} M. It is assumed that the photochemistry of the free and anchored benzoin remains the same.
[38] The relatively long reaction time is not a reflection of the efficiency of the photochemistry, but of the fact that the ^{1}H NMR measurements require high concentrations (10^{-3} M) of the chromophore. At more realistic concentrations for practical applications (10^{-5} M), the release is five times as fast.
[39] All solution-state photoreactions of cage **1a** were carried out using the light source from a lamp used for visualizing TLC plates at 313 nm (Spectroline ENF-260C, 1.3 mW cm^{-2}).
[40] Monochromatic light centred at 290 nm was generated using a fluorescence spectrometer (PTI Quantamaster) with a 3 nm slit width.
[41] The small spot size of the light sources mean that it is critical to stir the two identical solutions of **1a**[$NaYF_4$:TmYb] in CH_3CN during the irradiation to ensure that representative UV/Vis spectra are measured. Spectral changes were recorded over time until no further changes were observed, at which time the photorelease was judged to be complete.

