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A photoCORM nanocarrier for CO release using NIR light†

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A water-soluble nanocarrier for a photo-activated CO releasing moiety (photoCORM) that can be triggered with NIR excitation is described. This has an upconversion nanoparticle core encapsulated by an amphiphilic polymer imparting both water solubility and a hydrophobic interior containing the photoCORM *trans*-Mn(bpy)(PPh$_3$)$_2$(CO)$_2$. Such an ensemble offers a unique strategy for CO delivery to biological targets.

Carbon monoxide is an endogenously-produced small molecule in mammalian physiology[1] and has been shown to have cytoprotective and anti-inflammatory properties.[2] These beneficial effects have prompted development of various CO releasing moieties (CORMs) for the treatment (or prevention) of ischemia/reperfusion (I/R) injury and other therapeutic applications such as wound healing.[2–5] When photochemical excitation triggers CO uncaging from such a precursor,[4,5] this species can be termed a "photoCORM".[4a] This technique allows one greater precision in the timing and location of CO delivery.[3,5] A significant drawback, however, is that the UV and visible light used as the trigger has relatively poor transmission through biological fluids and tissues.[6] This issue leads to considerable interest in developing photochemical systems that can be activated with the tissue penetrating near-infrared (NIR) irradiations for the photo-uncaging of CO and other bioactive small molecules.[7–10] Described here is a new nanocarrier that incorporates, in a single unit, a NIR-to-visible upconverting nanoparticle, an active photoCORM and a biocompatible polymer that imparts water solubility to the system (Fig. 1).

Most photoCORMs are metal carbonyls, and CO photolability from such organometallic complexes typically results from populating metal-centered, ligand field (LF) excited states.[11] Since CO is a strong field ligand, the spin-allowed LF absorption bands are generally fairly high in energy, occurring in the near-UV region. For example, the water-soluble Re(I) carbonyl complex Re(bpy)(CO)$_3$(thp)$^+$ (**1**, thp = tris(hydroxymethyl)phosphine, bpy = bipyridine) reported from this laboratory,[4c] has the dual advantages that it displays a relatively high quantum yield for CO release (Φ_{CO}) and that both the photoCORM and the photoproduct are luminescent. Thus, the photoCORM location and reactions in cells can be imaged, although this required an excitation wavelength of 405 nm.

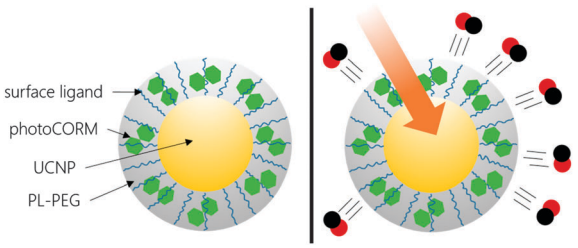

Fig. 1 Left: representation of a water-soluble nanocarrier used for CO uncaging. Right: upon 980 nm irradiation light (red arrow), the up-converted visible range emission from the UCNP gets re-absorbed by the photoCORM, leading to CO release.

A metal carbonyl such as **1** with unsaturated ligands will have other absorption bands such as metal-to-ligand charge transfer (MLCT) transitions that are stronger and more tunable by ligand modifications. While MLCT states display some photolability, perhaps owing to diminished metal-to-CO π-back-bonding,[12] complexes where the MLCT excited states are tuned to energies much lower than the LF states tend to have markedly decreased ligand lability.[11] Thus, it is difficult to modify such species sufficiently to facilitate photo-induced CO release at longer visible or NIR wavelengths.

Among the strategies used in this laboratory[7,8] and in others[9,10] to exploit NIR photolysis wavelengths for uncaging, lanthanide ion doped upconversion nanoparticles (UCNPs) have the advantages that they are activated by the sequential absorption of photons that can be effected by a simple,

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continuous-wave NIR laser.[8,13] These UCNPs emit visible range photons that can be absorbed by molecules in close proximity to uncage bioactive small molecules.[8,10] Additionally, the luminescent UCNPs can be used in bio-imaging and thus provide the opportunity for multi-modal drug delivery strategies and photodynamic therapy.[14]

The nanocarriers described here are composed of a NIR absorbing UCNP as the core, a photoCORM and a polymer matrix as the container. The UCNPs, synthesized from the oleate salts according to literature procedures,[10c,15] are $NaGdF_4$ nanoparticles doped with ytterbium (20%) as the NIR absorber and thulium (0.1%) as the visible emitter. These were coated with several monolayers of $NaGdF_4$ to minimize environmental quenching. TEM studies show that these core:shell $NaGdF_4$:Tm,Yb@$NaGdF_4$ UCNPs have high monodispersity with diameters ~12 nm (see Fig. S1, ESI†). The upconverted emission spectrum from these is shown in Fig. 2.

The photoCORM used here is the triflate salt of the manganese carbonyl $[Mn(bpy)(CO)_2(PPh_3)_2]^+$ (**2**), the synthesis of which is described in the ESI† and the crystal structure of which is shown in Fig. 3. The large hydrophobic triphenylphosphine ligands render this salt insoluble in aqueous solution, which is a desirable property in the present application. A key feature of **2** is that its electronic absorbance spectrum shows a strong band at λ_{max} 400 nm (ε = 5400 M^{-1} cm^{-1}) that tails into the visible (Fig. 1). Thus, this band overlaps with the emission bands seen upon 980 nm excitation of the Yb^{3+}/Tm^{3+} doped UCNPs used here. Additionally, aerated solutions of **2** in solvents ranging from acetonitrile to methylene chloride were found to be stable in the dark for at least 12 h.

Photolability toward visible excitation was demonstrated by irradiating an aerobic dichloromethane (DCM) solution of **2** with a 470 nm light emitting diode. The resulting changes in the optical and IR absorbance spectra are shown in Fig. S2 and S3 (ESI†). The characteristic MLCT band and the CO stretching bands (ν_{CO}) are completely bleached in these spectra upon exhaustive photolysis. Furthermore, analysis of the headspace by gas chromatography-thermal conductivity detection (GC-TD) indicated that ~1.85 CO equivalents were generated per equivalent of **2**, thus 470 nm photolysis in aerobic solutions leads to the release of both CO's (eqn (1)). The quantum yield

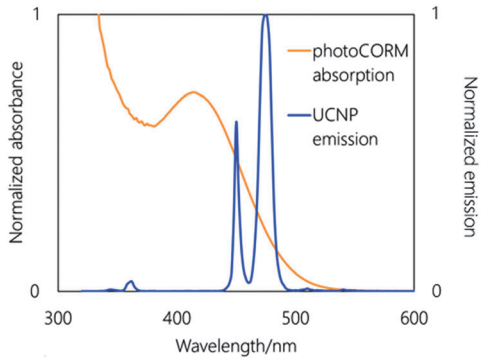


Fig. 2 Electronic absorbance spectrum of **2** (yellow) and emission spectrum (blue) of the $NaGdF_4$:Tm,Yb@$NaGdF_4$ UCNPs (λ_{irr} = 980 nm).

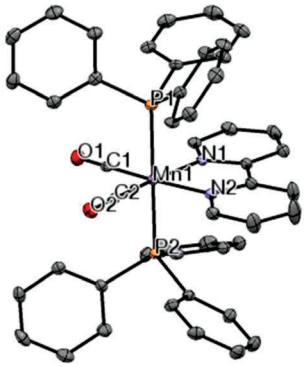
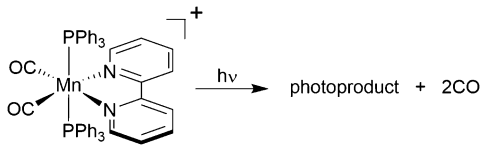


Fig. 3 Solid state molecular structure of $Mn(bpy)(CO)_2(PPh_3)_2^+$, shown with thermal ellipsoids at 50% probability, $CF_3SO_3^-$ counter ion and H atoms omitted for clarity. Selected bond lengths (Å) and angles (deg): Mn1–C1, 1.792(3); Mn1–C2, 1.794(3); Mn1–P1, 2.334(1); Mn1–P2, 2.326(1); P1–Mn1–P2, 177.62(3); P1–Mn1–C1, 89.31(8). The IR spectrum of **2** displayed two ν_{CO} bands at 1871 and 1940 cm^{-1} in dichloromethane (see ESI† for synthesis and crystallographic details).

for this process in DCM, calculated by monitoring changes in the optical spectra (see ESI†), was determined to be 0.26 ± 0.04.



(1)

The polymer matrix is prepared from an amphiphilic phospholipid-functionalized poly(ethylene glycol) (DSPE-PEG 2000). The particle encapsulation was carried out by adding 10 mg of cleaned core/shell UCNPs, 20 mg of the dried polymer powder (PL-PEG) and a 3 mL aliquot of chloroform to a clean flask. The resulting mixture was sonicated for one minute and allowed to stir for 2 h. The solvent was removed by evaporation to give a white powder that was easily dispersed in water. That this material represents the UCNPs coated with the DSPE-PEG 2000 polymer (UCNP@PL-PEG) was apparent by examining the emission from the aqueous suspensions in pH 7.4 phosphate buffered saline (PBS) solution, which displayed the visible emission spectra characteristic of the $NaGdF_4$:Tm,Yb@$NaGdF_4$ UCNPs (Fig. S4, ESI†). As initially prepared, the UCNPs have hydrophobic oleate surface ligands, thus are completely insoluble in aqueous media. In contrast, UCNP@PL-PEG conjugates have a hydrophilic surface that renders them water-soluble. Furthermore, the lipid-like interiors of the conjugates are well suited to accumulate the hydrophobic photoCORM **2**, thereby positioning that species close to the UCNP where it is most likely to capture the visible light resulting from upconverted NIR irradiation.

Incorporation of **2** into the nanocarrier was accomplished by dissolving the photoCORM in an aqueous acetonitrile solution where it has poor solubility, suspending the UCNP@PL-PEG in the same solution, and allowing the manganese complex to slowly infuse into the non-polar hydrophobic inner layers of UCNP@PL-PEG. Maximum loading of **2** was achieved using a mixed solvent system of 1% acetonitrile in water in which **2** is

marginally soluble but the UCNP@PL-PEG is fully suspended. A 2 mL solution containing 20 mg of UCNP@PL-PEG and 50 mg of **2** was stirred in the dark for 24 h to give a yellow solution. To remove the excess photoCORM, the particles were collected by centrifugation and then washed with acetonitrile to give an orange pellet that was easily dispersed in water to give a homogenously yellow colored solution. The presence of photoCORM in the dispersion was confirmed by UV-visible spectroscopy and by visual observation since the UCNP@PL-PEG is a white solid (colorless in solution). Using inductively coupled plasma/optical emission spectroscopy (ICP, see ESI†) analysis of the Mn in the resulting nanocarriers showed the loading efficiency to be $\sim$80%.

To test for leaching, an aqueous suspension (PBS) containing the photoCORM loaded UCNP@PL-PEG was allowed to stir at 25 °C for 7 days. This was then centrifuged, resulting in an orange pellet and colorless supernatant. ICP analysis of the latter indicated a manganese concentration of 33 ± 4 ppb, thus $<0.7\%$ of the available Mn leached from the nanocarriers into the supernatant. Therefore, the hydrophobic photoCORM can be infused into the lipid domains of the UCNP@PL-PEG nanocarriers, and the hydrophilic surface makes these constructs water-soluble while minimizing leaching.

The size distribution of the photoCORM nanocarriers was examined by dynamic light scattering. For a freshly suspended and filtered solution (see ESI†), the diameters of the vast majority of the particles fell into 16 to 30 nm range (Fig. 4). However, while the UCNP@PL-PEG nanocarriers suspended readily in phosphate buffer solution, settling was observed over long periods (>4 h). Brief sonication regenerated a homogenously yellow solution.

The efficacy with which visible light generated by NIR excitation of a UCNP activates a photoCORM through an emission/re-absorption mechanism will depend on donor/acceptor proximity, as well as on the upconversion efficiency and CO release quantum yield. Incorporating the hydrophobic photoCORM into the polymer also serves to position it close to the UCNP surface where it is more likely to absorb the emitted upconverted light. CO release upon NIR irradiation of an aqueous solution containing these nanocarriers was determined both *via* the myoglobin assay[16] and by headspace analysis with GC-TCD (see ESI†).

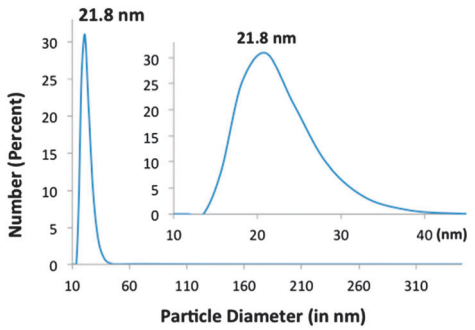


Fig. 4 Size distribution of UCNP@PL-PEG nanocarriers as determined by dynamic light scattering. Inset shows expanded graph.

A control experiment involving the direct excitation of the photoCORM was performed to establish whether DSPE-PEG 2000 is indeed permeable to CO, thereby allowing photochemically uncaged CO to escape. A pH 7.4 PBS solution containing myoglobin, sodium dithionite and the photoCORM-loaded nanocarriers was irradiated with 365 nm UV light. The photoreleased CO was detected *via* the characteristic spectral changes of carboxy-myoglobin, indicating that photo-labilized CO will migrate through the polymer matrix and release. When a similar solution was irradiated at 980 nm (2.0 W, 5 min), analogous spectral changes indicating carboxy-myoglobin formation was seen (Fig. S5, ESI†), thus, NIR irradiation of a solution containing photoCORM-loaded UCNP@PL-PEG releases CO. No CO release was observed in the dark.

This result was confirmed using GC-TCD. A 500 μL aqueous suspension of the photoCORM-loaded nanocarriers in pH 7.4 PBS (2 mg mL^{-1} based on UCNP mass) was sealed in a 2 mL vial equipped with a septum and was irradiated with 980 nm light (2.0 W, 5 min). After irradiation, a 500 μL aliquot of the headspace was analyzed by GC-TCD, and CO was identified by the chromatographic retention time (Fig. S6, ESI†).

In summary, we have developed a novel delivery strategy for the photochemical uncaging of CO using NIR light. Encapsulating upconverting nanoparticles in a phospholipid functionalized PEG serves two purposes. First, it renders them water soluble, an obvious advantage for use in biological systems. Secondly, it provides a hydrophobic region suitable for loading an organic soluble photoCORM. This positions the photoCORM in close proximity to the UCNP and minimizes leaching into the surrounding media. Irradiation of this construct with NIR light induces upconverted emission from the UCNP that is re-absorbed by the embedded photoCORM, leading, in turn, to photochemical uncaging of CO.

One can speculate that another advantage of this type of nanocarrier would be that modifications of the polymer surface could, in principle, be used to affect the circulation, targeting and elimination properties of the unit. Such constructs could prove to be important, light-triggered delivery vehicles for uncaging CO or other bioactive small molecules at specific physiological targets.

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