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Near infrared light mediated release of doxorubicin using upconversion nanoparticles†

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Lanthanide doped upconversion nanoparticles grafted with a photo-caged analog of doxorubicin allow near IR-release of doxorubicin.

The low efficacy of current cancer chemotherapy is attributed to accumulation of drugs in non-cancerous tissues, eventually causing side effects. To address this serious issue, current research has focused on designing stimulus-responsive drug delivery agents.[1] An array of delivery vehicles were designed involving components that are responsive to different stimuli including pH,[2] intra-cellular enzymes,[3] heat[4] or light.[5] Recently, we and other groups have reported photosensitive drug delivery systems, which are comprised of a nitrobenzyl-type 'photocaged' anticancer drug conjugated to another small or macromolecule.[6,7] In each of these cases drug release from the conjugate was mediated by UV light. A major limitation of this approach for eventual *in vivo* drug release is the low tissue penetrating ability of UV light and inevitable DNA damage.[8–11] Unfortunately, the lower energy of more tissue-penetrating photons in the near IR region has precluded photochemical decaging at those wavelengths (although singlet oxygen dependent decaging has been achieved[12,13]).

A potential solution to this problem is afforded by lanthanide doped upconverting nanoparticles (Ln-UCNPs). Ln-UCNPs have emerged as a novel class of luminescent probes with applications in many fields such as bio-imaging, diagnosis, drug delivery, and therapeutics due to the unique optical property of lanthanides known as upconversion.[14,15] These nanoparticles, upon near IR excitation (typically 980 nm) can emit UV, visible and/or near infrared (NIR) light. In this work, we have focused on $LiYF_4$:Tm^{3+}/Yb^{3+}-UCNPs because of their strong UV emission upon 980 nm excitation.[16] Two strong emission bands centered at 353 and 368 nm, which are assigned to $^3P_0 \rightarrow {}^3H_6$ and $^3P_0 \rightarrow {}^3F_4$ respectively, can be used as an internal source for UV light to facilitate photochemical reactions that require high energy UV light.

Recently Chien *et al.*,[15] demonstrated release of anti-cancer drug doxorubicin (Dox) from SiO_2 coated Ln-UCNPs tagged with caged folic acid. Upon irradiation and decaging, the Ln-UCNPs were internalized *via* the folate receptor followed by release of thiolated Dox by intracellular disulphide reduction. While an interesting design, folate receptor expression levels and inefficient endocytosis limit the total amount of Dox released by this approach. In a report by Yang *et al.*,[17] mesoporous silica coated Ln-UCNPs were used for the purpose of Dox release from a photosensitive outer capsule. Although this design was innovative, the release of Dox from the light-opened capsule was slow.

We reasoned that this slow release and the dependence on endocytosis could both be avoided by loading a photocaged Dox directly onto the surface of the Ln-UCNP *via* a photocleavable linkage. This simple design would allow for direct and rapid diffusion of the free drug from the Ln-UCNP whether or not the Ln-UCNP has been internalized. We envisioned such a system could be constructed by attaching Dox onto a Ln-UCNP by binding a Dox conjugate directly to the surface exposed lanthanide ions (Fig. 1). We have shown previously that bis-carboxylated ligands form a tight coordinative complex with Ln-UCNPs,[18,19]

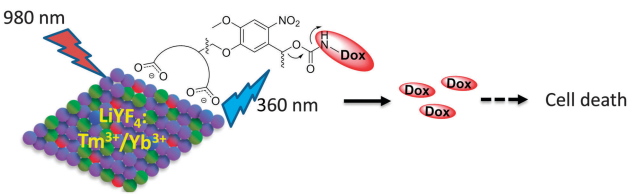

Fig. 1 Schematic representation of the drug releasing system synthesized from $LiYF_4$:Tm^{3+}/Yb^{3+} nanoparticles and Dox conjugates. The incoming NIR light excites the Ln-UCNPs and emits upconverted UV light at 365 nm. The UV light then induces the photocleavage and leads to the release of Dox, unmasking its cytotoxicity.

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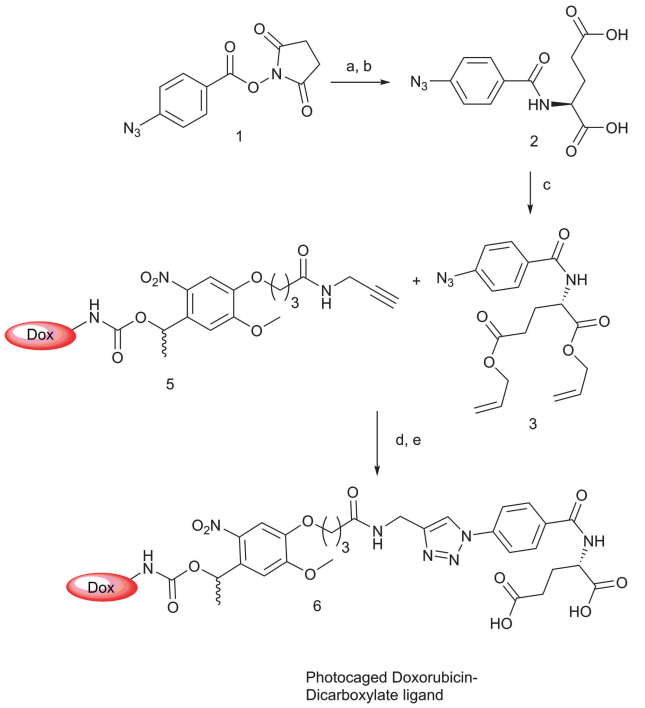

Scheme 1 Synthesis of Dox(COOH)$_2$. *Reagents and conditions*: (a) Glu(OtBu)$_2$, DMF (b) trifluoroacetic acid in DCM, 59% 2 steps, (c) allyl bromide, K_2CO_3 in DMF, 92%, (d) $CuSO_4$, sodium ascorbate, TBTA in H_2O and DMSO (1 : 1) ratio, 72%, (e) Pd(PPh$_3$)$_4$, morpholine in DMSO, 46%.

and so we first prepared a conjugate of Dox containing glutamic acid as the source of the two carboxylates (Scheme 1).

We first used the NHS ester of *p*-azidobenzoic acid to amidate Glu(OtBu)$_2$ We later found that the acidic conditions required for deprotection of the OtBu groups caused degradation of Dox, so we removed them giving free carboxylate compound **2** followed by conversion to the bis-allyl compound **3**. Then, we coupled the allyl azide **3** and Dox-nitroveratryl-alkyne **5**[6] *via* a Cu(II) mediated click reaction. Finally, we deprotected the dicarboxylate unit using Pd(PPh$_3$)$_4$ in presence of morpholine to yield the final product **6** (Dox-(COOH)$_2$).

The standard LiYF$_4$:Tm^{3+}/Yb^{3+}-UCNPs (ESI,† Fig. S1A) are capped with oleate, which blocks access to coordination by our Dox conjugate. To remove the oleate cap we treated the Ln-UCNPs with HCl at pH 4 followed by ether precipitation to prepare the OA free UCNPs (ESI,† Fig. S1B).[18] Incubation of the Dox-(COOH)$_2$ and the Ln-UCNPs lead to efficient loading of Dox onto the surface of the Ln-UCNP as evidenced by the deep purple color of the UCNPs. The loading induced a red-shift of Dox absorption as shown in Fig. 2. This red shift was surprising; however, this type of red shift has been observed in complexes of Dox with copper.[20] Since the red-shift in the copper complex is due to copper coordination *via* the anthraquinone group in Dox, we were concerned that our Dox conjugates were coordinated to the Ln-UCNPs improperly. The OA-free-Ln-UCNPs contain many Y^{3+} ions on the surface, which could coordinate with the anthraquinone moiety. Indeed, the highly similar spectrum was observed when we incubated Dox with YCl$_3$ (ESI,† Fig. S2)

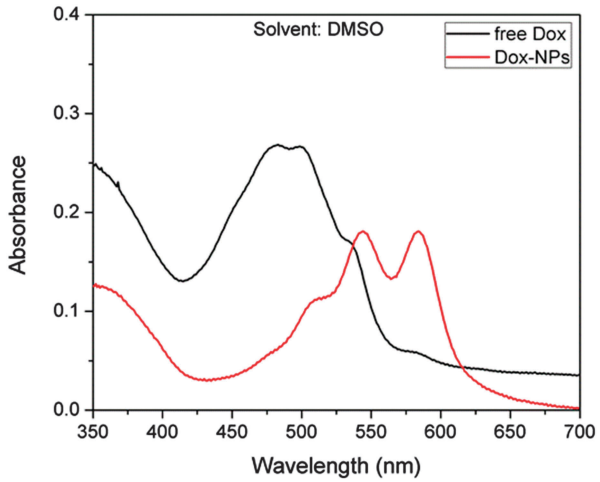

Fig. 2 Absorption spectra of free Dox and Dox-UCNPs in DMSO. A red-shift was observed due to the formation of complex between the surface Y^{3+} ions and Dox.

suggests this was the case. Moreover, after multiple attempts, we were unable to release any free Dox from these Ln-UCNPs with light—further evidence that the Dox-(COOH)$_2$ was coordinated to the UCNP, but not with its carboxylate ligands.

To circumvent this problem, we decided to form a metal ion complex with Dox prior to loading onto the Ln-UCNPs. We reasoned that a pre-formed a Dox-metal ion complex would force coordination with the Ln-UCNPs *via* the carboxylates. Based on the ability to pull Dox into liposomes *via* coordination,[21] and the moderate stability constant of (Dox)$_2$-Mn^{2+},[22] Mn^{2+} was chosen as the coordinative ion. We first formed a saturated complex between Dox-(COOH)$_2$ and Mn^{2+} (ESI,† Fig. S3) and then incubated the (Dox-(COOH)$_2$)$_2$–Mn^{2+} complex with our Ln-UCNPs in DMSO/PBS. We could empirically monitor the

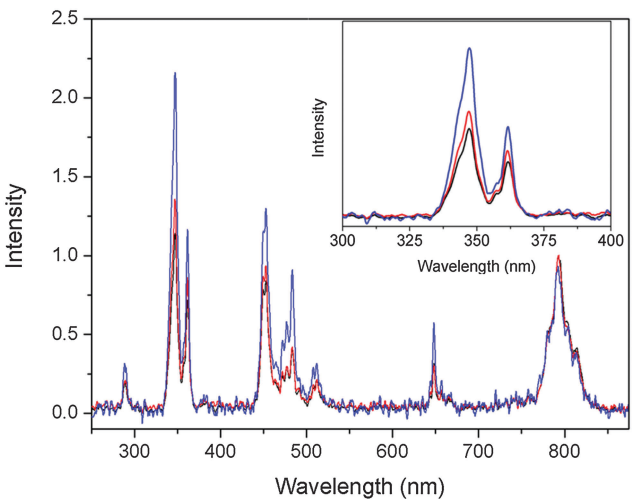

Fig. 3 Evidence of loading of Dox onto the Ln-UCNP surface. Luminescence emission spectra of OA-free-Ln-UCNPs (blue), Dox-Ln-UCNPs before irradiation (black) and Dox-UCNPs after 60 min irradiation (red). Inset: enlarged luminescent emission from upconverted UV light. All three spectra were normalized to the NIR emission centered at 800 nm for comparison.

loading due to the characteristic purple color of the Ln-UCNPs formed. As expected, loading the Ln-UCNPs with $(Dox\text{-}(COOH)_2)_2\text{-}Mn^{2+}$ led to a substantial quenching of Ln-UCNP luminescence at 365 nm as compared to free Ln-UCNPs due to the presence of the nitroveratryl group (Fig. 3). Based on this decrease we could calculate the energy transfer efficiency as 41.7%. In addition, using the absorbance of Mn^{2+}-complexed Dox-$(COOH)_2$, we were able to calculate the surface coverage of Dox to be approximately 1500 molecules of Dox per nanoparticle (ESI,† Fig. S4).

We then irradiated our Dox-loaded Ln-UCNPs with near- IR light to see if Dox could be released. As expected, we observed a decrease in 365 nm fluorescence quenching after irradiation (Fig. 3). In parallel, we observed a time-dependent release of Dox from the Ln-UCNPs and into solution (Fig. 4). After 60 minutes ~40% of the Dox was released based on comparison of the absorbance values of the supernatant to the starting Ln-UCNPs loaded with Dox (ESI,† Fig. S5). Further irradiation did not lead to additional release. A control test in the dark was also carried out to confirm that sonication and centrifugation do not contribute to the release of Dox from the Dox-Ln-UCNPs. After 60 minutes, little Dox release was observed (Fig. 4B). The release of free Dox could also be confirmed by fluorescence, which showed a time-dependent increase, presumably due to a reduction in self-quenching and release from the complex with Y^{3+} (ESI,† Fig. S6).

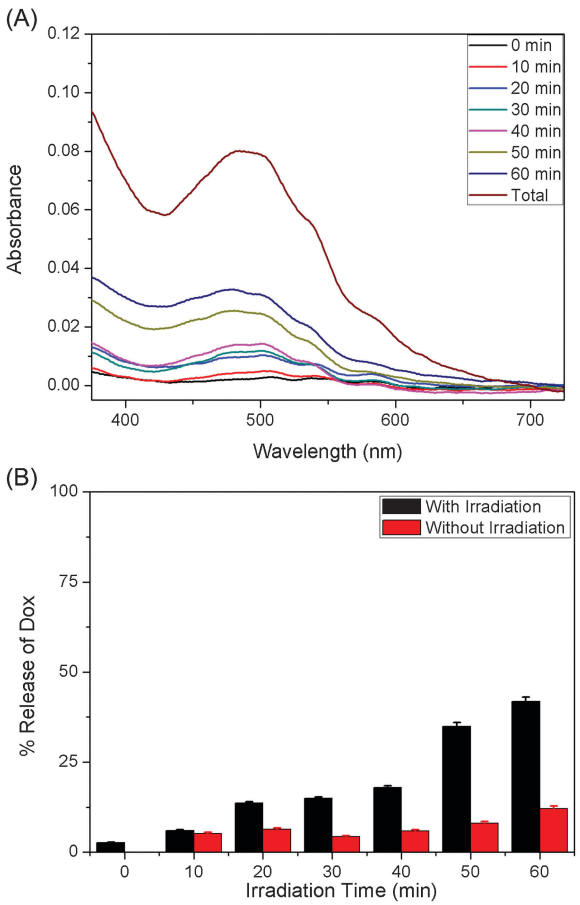

Fig. 4 Time dependent release of Dox from Ln-UCNPs with near IR light. (A) Absorption spectra of the supernatant from Dox-Ln-UCNPs solution upon 980 nm irradiation at different times were measured at the time shown. At each time point the sample was sonicated and centrifuged prior to measuring absorbance. The value for total Dox was determined by dissolving an equivalent amount of Dox-$(COOH)_2$ in water and measuring its absorbance (B) comparison of the release of Dox with and without irradiation. Each data point is an average of duplicate experiments. Error bars denote the range.

It is interesting that the Dox released absorbs at a wavelength characteristic of free Dox, not the $(Dox)_2Mn$ complex. This is advantageous because it is the release of free Dox, not the complex, which will ultimately be important for drug delivery. The release of non-coordinated Dox also supports our use of the moderately stable Mn^{2+} complex. However, the moderate affinity could also be responsible for our inability to release all of the Dox from the UCNPs. We speculate that that some of the free Dox is re-binding to the UCNPs *via* the anthraquinone group after it is released, a problem that could potentially be solved by using a stronger coordinating metal. Ultimately, studies in more complex media will allow us to optimize these two competing factors.

In summary, we have developed a simple system for near-IR controlled release of Dox into solution from nanoparticles. In contrast to other systems, Dox is loaded directly onto the nanoparticle surface at high density and is released efficiently with 980 nm light. This system or derivatives thereof should be compatible with release of Dox into cells in response to tissue penetrating near-IR light.

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