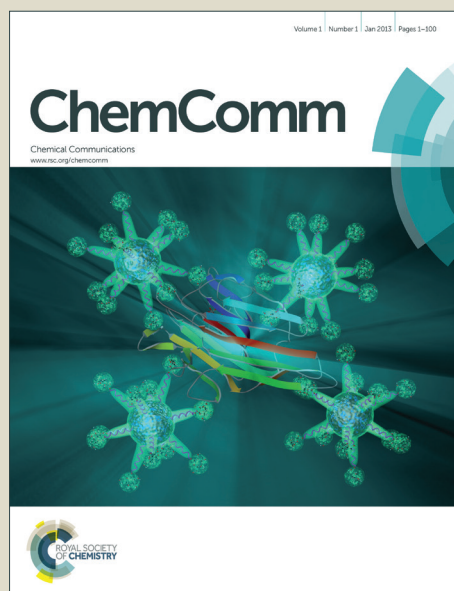


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COMMUNICATION

Near Infrared Activation of an Anticancer Pt^{IV} Complex by Tm-Doped Upconverting Nanoparticles

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The Pt^{IV} complex *cis,cis,trans*-[Pt(NH₃)₂(Cl)₂(O₂CCH₂CH₂CO₂H)₂] is photoactivated by near infrared light (980 nm) using NaYF₄:Yb³⁺/Tm³⁺@NaYF₄ core-shell upconversion nanoparticles. Coupling of this cisplatin precursor with the biocompatible PEGylated phospholipid DSPE-PEG(2000)-NH₂ affords a valuable approach to decorate the surface of the nanoparticles, providing a novel photoactivatable nanomaterials capable of releasing Pt^{II} species upon NIR light excitation.

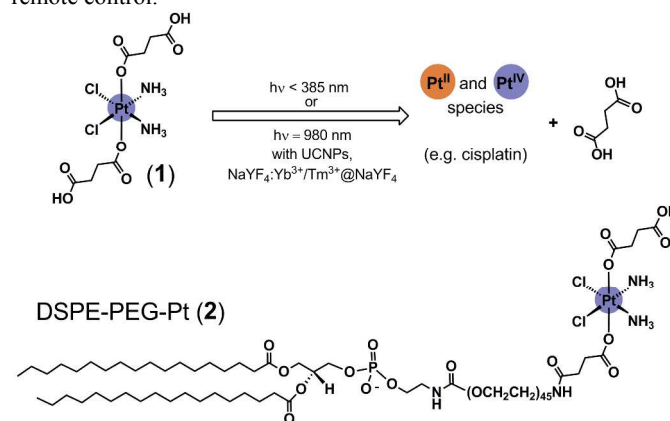
Light-induced activation of transition metal complexes acting as anticancer prodrugs has been proven a promising approach to induce novel modes of actions and to localize toxicity to irradiated tissue areas, hence potentially limiting unwanted side effects associated with cancer treatment.^{1,2} Although encouraging *in vivo* activity has been recently demonstrated in the case of photoactivatable Pt and Rh complexes,^{3,4} a major limitation for the progression of this class of anticancer agents towards preclinical and clinical stages is related to their poor absorption properties in the therapeutic window, i.e. 600–1000 nm, where tissue penetration is higher and direct damaging of cellular components lower.⁵

Upconversion nanoparticles (UCNPs) are innovative materials which have the capability to overcome such fundamental drawback of transition metal complexes. They have unique optical features allowing efficient conversion of near infrared (NIR) photons into visible and UV light *via* multiphotonic energy transfer processes.^{6,7} Potentially, light emitted by UCNPs can be used to excite metal complexes and prompt their photochemistry upon 980-nm excitation. In addition to upconverted luminescence, magnetic resonance relaxivity (Gd-containing UCNPs) and ease of radiolabeling (e. g. ¹⁸F) have also made UCNPs excellent new probes for multimodal (optical/MRI/PET-SPECT) imaging.⁶ Such promising features combined with the low toxicity of UCNPs *in vitro* and *in vivo*⁶ suggest these materials are ideal candidates for applications in nanomedicine and photochemotherapy.

Despite a number of reports has shown how UCNPs can be integrated to porphyrin-like photosensitizers or caged compounds to afford novel NIR-activatable nanomaterials for therapy and imaging,^{8,9,10} very little has been done to develop UCNPs as

phototriggers capable of promoting functional structural changes in metal complexes (or chromophores^{11,12} in general). Following the pioneering work by the groups of Fuyou Li and Branda on photoswitchable UCNP-systems based on organic dyes (e.g. dithienylethene),^{13,14,15} Ford and collaborators showed 980-nm photorelease of NO from the Roussin's Black Salt embedded in UCNP composites,^{16,17} and we recently reported on the NIR-activated pyridine release in the Ru polypyridyl complex [Ru(bpy)₂(pyridine)₂]²⁺.¹⁸

In this communication, we expand the potentially broad application of these nanomaterials by demonstrating UCNPs can mediate the NIR-photoactivation of the Pt^{IV} complex *cis,cis,trans*-[Pt(NH₃)₂(Cl)₂(O₂CCH₂CH₂CO₂H)₂] (**1**, Scheme 1). This compound is representative of a class of anticancer prodrug candidates¹⁹ that has shown significant promise and reached advanced stages in clinical trials.²⁰ Notably, complex **1** has an high reduction potential compared to other Pt(IV) analogues,²¹ a fundamental requirement to exhibit dark stability (i.e. low toxicity) in the cellular environment. Moreover, we show that functionalization of **1** with the biocompatible PEGylated phospholipid DSPE-PEG(2000)-NH₂²² to afford **2** (Scheme 1) offers a viable strategy to decorate UCNPs and obtain nanomaterials capable of releasing Pt^{II} species upon NIR remote control.



Scheme 1

Using a two step procedure previously reported,^{14,18} we prepared core-shell NaYF₄:Yb³⁺/Tm³⁺@NaYF₄ UCNPs. Firstly, NaYF₄:Yb³⁺/Tm³⁺ (69.5/30/0.5 mol%) nanocrystals with an average size of ca. 30 nm (Fig. 1a, ESI,† Fig. S1) were synthesized by thermal decomposition in presence of oleic acid and octadecene and next used as seeds for growing a protective NaYF₄ shell (ESI,† page S3). TEM shows that resulting core-shell NaYF₄:Yb³⁺/Tm³⁺@NaYF₄ UCNPs are uniform and have an average diameter of ca. 37 nm (Fig. 1b, ESI,† Fig. S1). Characterization of both types of UCNPs was completed by IR, XPS and emission spectroscopy (ESI,† Fig. S2–S5). As previously demonstrated,²³ UCNP passivation with un-doped NaYF₄ increases considerably the upconversion efficiency compared to corresponding core-only nanocrystals.

In THF ($\lambda_{\text{exc}} = 980$ nm, Fig. 1c), NaYF₄:Yb³⁺/Tm³⁺@NaYF₄ UCNPs display multiple emission maxima which are typical of Tm³⁺ electronic transitions and fall at 345 and 360 nm (³P₀→³F₄ and ¹D₂→³H₆), 450 and 475 nm (¹D₂→³F₄ and ¹G₄→³H₆), 645, 690 and 720 nm (¹G₄→³F₄ and ³F₃→³H₆) and at 800 nm (³H₄→³H₆).²⁴ Upconversion of NIR light occurs through a non-linear multiphoton process and, expectedly, changes in the excitation power density alter the relative intensities of the emission bands (ESI,† Fig. S4–S8).²⁴ Crucially, bands at 345 nm, 360 nm and 450 nm significantly rise with respect to the 475-nm emission in the case of Yb³⁺/Tm³⁺-doped core-shell UCNPs. Indeed, such improvement in the UCNPs emission properties is key since complex **1** only absorbs in the UV region of the spectrum, up to 390 nm (Fig. 1c).

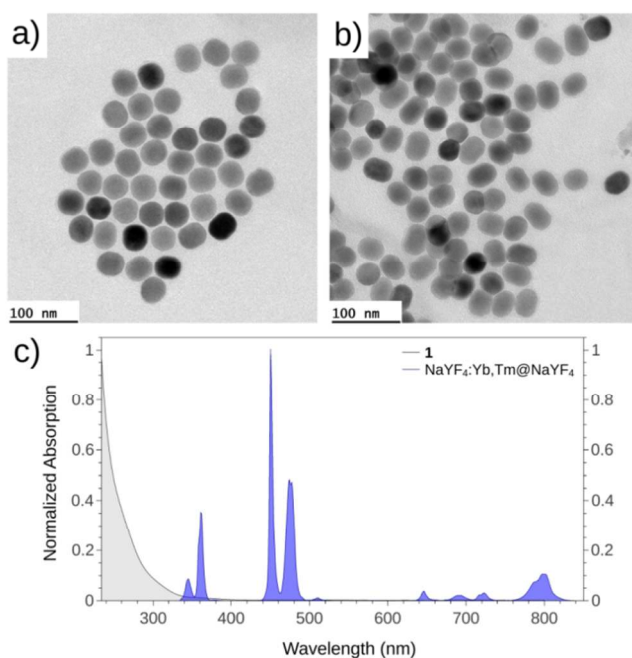


Fig. 1 TEM images of oleate-capped (a) core NaYF₄:Yb³⁺/Tm³⁺ and (b) core-shell NaYF₄:Yb³⁺/Tm³⁺@NaYF₄ UCNPs; (c) overlap between the UV-Vis spectrum (normalized, gray) of **1** and the upconverted emission spectrum (normalized, blue) of core-shell NaYF₄:Yb³⁺/Tm³⁺@NaYF₄ UCNPs (THF, $\lambda_{\text{exc}} = 980$ nm).

To test whether such overlap between the absorption of **1** and the emission of NaYF₄:Yb³⁺/Tm³⁺@NaYF₄ UCNPs is suitable for NIR-light photoactivation of the complex, we

prepared a suspension containing oleate-capped core-shell UCNPs (5 mg·mL⁻¹) and **1** (150 μ M) in buffer (PBS/D₂O, pH = 7.3, 0.4 mL) and investigated the photolysis of the complex upon 980-nm excitation by NMR. For each time point, the sample was centrifuged and the supernatant used for collecting NMR spectra, a necessary procedure to minimize signal broadening due to the paramagnetic nanoparticles and obtain good quality data.

The ¹H NMR spectrum of **1** in the dark displays two diagnostic pseudo-triplets between 2.30 and 2.60 ppm, corresponding to the two non-equivalent CH₂ protons of the coordinated succinates. NIR irradiation of the reaction mixture with a 980-nm continuous-wave laser (4.9 W·cm⁻²) causes succinate dissociation, as clearly observed by the appearance of the singlet at 2.33 ppm (Fig. 2a, red circle). The photoreaction is practically complete after 4 h under the chosen conditions and control experiments confirm that direct excitation of **1** at 385 nm (40 mW cm⁻², 10 min) similarly promotes the release of the axial ligands, whereas in the absence of UCNPs no changes in the ¹H NMR of **1** are observed after 7 h of irradiation at 980 nm (ESI,† Fig. S9 and S10).

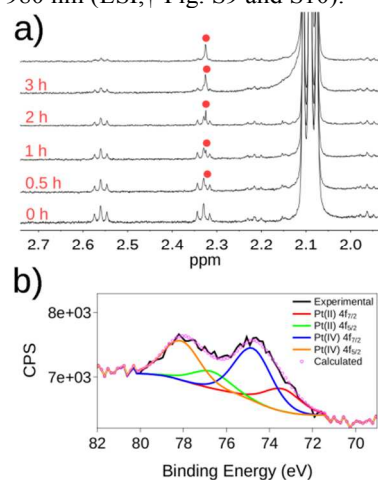


Fig. 2 (a) Near infrared ($\lambda_{\text{exc}} = 980$ nm, 4.9 W·cm⁻²) photolysis of **1** in the presence of core-shell NaYF₄:Yb³⁺/Tm³⁺@NaYF₄ UCNPs followed by ¹H NMR (PBS/D₂O, pH = 7.3, 0.4 mL); the signal relative to photoreleased succinate is labelled with a red circle (●); (b) Pt 4f_{7/2} and 4f_{5/2} XPS spectrum of **1** (supernatant) after irradiation at 980 nm (4.9 W·cm⁻², 4h) in the presence of core-shell NaYF₄:Yb³⁺/Tm³⁺@NaYF₄ UCNPs.

Besides free succinate, **1** is known to simultaneously generate Pt^{IV} and Pt^{II} photoproducts upon light activation.^{25,26} Recently, we showed how high concentration of **1** and low UVA light dose (ca. 4.8 J·cm⁻²) resulted prevalently in the formation of a range of Pt^{IV} species, among which the cytotoxic dihydroxido complex *cis,cis,trans*-[Pt^{IV}(NH₃)₂(Cl)₂(OH)₂].²⁶ Some of these Pt^{IV} photoproducts are reasonably evolving to cytotoxic species in the cellular environment, however it is desired that Pt^{IV} prodrugs such as **1** could undergo efficient Pt^{IV} → Pt^{II} photoreduction (in irradiated areas) and generate cisplatin-like species, since these have typically superior and better understood antiproliferative activity.

For this reason, we employed X-ray photoelectron spectroscopy (XPS) to assess the oxidation state of the Pt-containing photoproducts obtained from the various photoreactions. XPS allowed determining the Pt^{II}/Pt^{IV} ratio of samples, although partial X-ray-induced reduction should

always be taken into account as previously reported for other systems.²⁷

Results indicate partial formation of Pt^{II} species (Pt^{II}:Pt^{IV} = 30:70) in the Pt 4f_{7/2} and 4f_{5/2} XPS spectrum of the supernatant solution obtained by NIR excitation of **1** in the presence of UCNPs (Fig. 2b). A similar finding is obtained for the complex under direct UVA irradiation (ca. 36 J·cm⁻², Pt^{II}/Pt^{IV} = 56/44). On the contrary, no trace of Pt is obtained for the UCNP pellet precipitated from the reaction mixture, suggesting that **1** and its photoproducts do not adsorb on the UCNPs surface after photoactivation.¹⁸ Consistently, **1** retains the Pt^{IV} oxidation state in the dark (Pt^{IV} = 100%) and after 7 h irradiation at 980 nm in the absence of UCNPs (ESI,† Fig. S11 and S12).

After demonstrating NaYF₄:Yb³⁺/Tm³⁺@NaYF₄ UCNPs have the capability of functioning as NIR phototriggers for the activation of Pt^{IV} complexes, we explored the surface functionalization of the nanoparticles for the loading of **1**. The carboxylic groups of the complex were exploited for its coupling with the PEGylated-phospholipid DSPE-PEG(2000)-NH₂ via an EDC-based conjugation method (ESI,† page S3).²⁸ The resulting water-dispersible DSPE-PEG-Pt^{IV} adduct (**2**, Scheme 1) can interact with the oleate chains covering the UCNPs by means of the phospholipidic tails, while the hydrophilic PEG units of the polymer are exposed towards the solvent, further stabilizing the nanoparticles and improving their water solubility.²⁹

Characterization of NaYF₄:Yb³⁺/Tm³⁺@NaYF₄@**2** nanoparticles was achieved by different techniques (ESI,† Fig. S2, S13–S17). XPS measurements unequivocally confirm the presence of Pt^{IV} (100%) on the UCNPs surface, while TEM indicates nanoparticles are rather uniform and monodisperse and that no significant changes in the morphology occurred upon functionalization. Under the preparation conditions chosen for NaYF₄:Yb³⁺/Tm³⁺@NaYF₄@**2**, some degree of aggregation is possibly occurring as DLS provides a hydrodynamic radius for the system in water of ca. 157 nm. Nevertheless, such dimensions are appropriate for passive targeting via the enhanced permeation effect (EPR).³⁰ Besides the typical oleic acid stretching bands at 2930 and 2850 cm⁻¹, IR displays a new band characteristic of the PEG C–O–C stretching mode at 1110 cm⁻¹.³¹ Furthermore, the absorption spectrum of NaYF₄:Yb³⁺/Tm³⁺@NaYF₄@**2** in aqueous solution shows the Yb³⁺ band at ca. 980 nm together with features in the UV region which are consistent with the presence of oleate and **2** on the UCNPs surface (ESI,† Fig. S15). Importantly, decoration of UCNPs with **2** does not seem to affect their upconversion properties, as also confirmed in the case of analogue NH₂-free PEGylated-phospholipid UCNPs (ESI,† Fig. S16). Indeed, no significant quenching of the UCNP emission bands at 345 and 360 nm is apparent in both cases. However, occurrence of a FRET process may still be plausible in the case of the Pt^{IV} loaded system, considering the limited spectral overlap between **1** and UCNPs.

¹H NMR photolysis experiments on NaYF₄:Yb³⁺/Tm³⁺@NaYF₄@**2** were performed in order to establish if the material was capable of photoreleasing biologically active Pt^{II}/Pt^{IV} upon NIR light excitation. Fig. 3a compares the photochemical behaviour of **2** alone upon 385-nm excitation (40 mW·cm⁻², grey panel) and NaYF₄:Yb³⁺/Tm³⁺@NaYF₄@**2** (5 mg·mL⁻¹) under NIR irradiation (λ_{exc} = 980 nm, 7.3 W·cm⁻²).

The ¹H NMR spectrum of **2** in buffer shows four pseudo-triplets between 2.30 and 2.65 ppm (grey panel), corresponding to its four non-equivalent protons. Two of these signals

disappear upon UV irradiation (10 min), while the singlet relative to free succinate and two pseudo-triplets relative to the PEG-anchored succinate appear alongside (ESI,† Fig. S18 and S19). Differently, when NaYF₄:Yb³⁺/Tm³⁺@NaYF₄@**2** is suspended in PBS/D₂O buffer and the solution is then centrifuged to extract the supernatant, signals corresponding to **2** cannot practically be detected in the ¹H NMR spectrum of such fraction. The result is in agreement with the absence of Pt in the supernatant and demonstrates the good stability of the adduct.

Yet, NIR irradiation of the NaYF₄:Yb³⁺/Tm³⁺@NaYF₄@**2** solution clearly leads to the appearance of free succinate in the ¹H NMR spectrum (Fig. 3a, red circle). The photoreaction is complete in 3.5 h and control experiments at 980 nm in the absence of UCNPs show that **2** cannot be photoactivated at such wavelength (ESI,† Fig. S20).

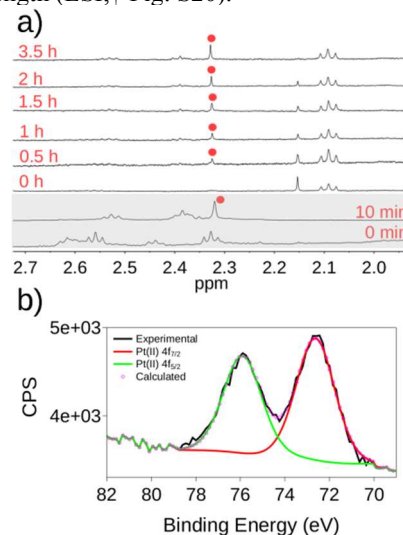


Fig. 3 (a) Near infrared (λ_{exc} = 980 nm, 7.3 W·cm⁻²) photolysis of NaYF₄:Yb³⁺/Tm³⁺@NaYF₄@**2** UCNPs followed by ¹H NMR (PBS/D₂O buffer, pH = 7.3, 0.4 mL); the signal relative to photoreleased succinate is labeled with the red ●; the grey panel shows the photolysis of **2** upon UVA excitation (λ_{exc} = 385 nm, 40 mW·cm⁻², 10 min) followed by ¹H NMR (PBS/D₂O buffer, pH = 7.3); (b) Pt 4f_{7/2} and 4f_{5/2} XPS spectrum of **2** after irradiation at 980 nm (7.3 W·cm⁻², 3.5 h) in the presence of core-shell NaYF₄:Yb³⁺/Tm³⁺@NaYF₄ UCNPs.

XPS was also employed in case of NaYF₄:Yb³⁺/Tm³⁺@NaYF₄@**2** to understand the nature of the Pt^{II}/Pt^{IV} photoproducts generated by its photolysis at 980 nm (Fig. 3b). The supernatant solution analysed by XPS indicates that Pt^{IV} is fully converted in Pt^{II} at the end of the photoreaction. Also in this case, no trace of Pt species is found by XPS in the pellet separated from the photolysis reaction of the nanomaterials (ESI,† Fig. S12) and control experiments (dark and **2** at 980 nm without UCNPs, ESI,† Fig. S21) confirm that platinum is in the Pt^{IV} oxidation state.

In summary, we provided in this work clear spectroscopic evidences that UCNPs can be employed to photoactivate Pt^{IV} anticancer complexes with NIR light, a remarkable result considering the poor absorption properties of this class of derivatives in the visible and near infrared region of the spectrum. Furthermore, decoration of nanoparticles with a biocompatible (FDA-approved²²) PEGylated phospholipid functionalized with **1** appears to be a valuable strategy to develop photoactivatable nanomaterials capable of releasing

active Pt^{II} species upon NIR light excitation. More studies are needed to evaluate thoroughly the effect of the excitation power over cellular environments and to improve the efficacy of UCNPs as phototriggers by increasing their upconversion yield and optimize surface functionalization. However, activating Pt^{IV} precursors of anticancer agents, such as cisplatin, carboplatin or oxaliplatin, with NIR light is indeed an attractive prospect as these Pt^{IV} complexes have extensively been studied in the last years and some of them have even reached clinical trials (e.g. iproplatin).²⁰ In particular, the low toxicity (both *in vitro* and *in vivo*) of UCNPs with respect to other nanomaterials and their multimodal imaging capability are key advantages to exploit for novel applications in nanomedicine.

Notes and references

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† Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

Electronic Supplementary Information (ESI) available: Full experimental details and additional characterization data. See DOI: 10.1039/c000000x/

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