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Upconverting Nanoparticles For The Near Infrared Photoactivation Of Transition Metal Complexes: New Opportunities And Challenges In Medicinal Inorganic Photochemistry

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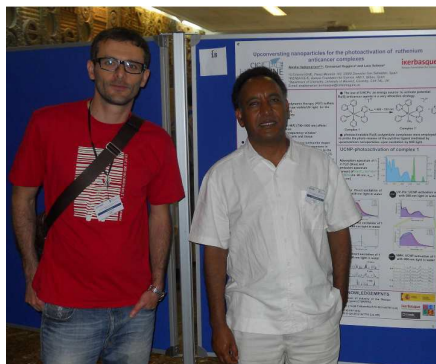
The article highlights the emergent use of upconverting nanoparticles as tools for the near infrared photoactivation of transition metal complexes, identifying opportunities and challenges of this approach in the context of medicinal inorganic chemistry.



Emmanuel Ruggiero (left) was born in Naples (Italy) in 1987. He received his BSc (2009) and MSc (2011) degrees in Chemistry from the University of Naples Federico II. He then moved to Pisa as research fellow of NEST-Scuola Normale Superiore where he worked on the synthesis of non-natural peptides. In 2013, he started his PhD under the supervision of Dr. Luca Salassa at CIC biomaGUNE (Spain), where he is exploring the use of upconverting nanoparticles as tools for the near infrared photoactivation of metal-based prodrugs.

Silvia Alonso de Castro (right) obtained her BSc (2011) and MSc (2013) degrees in Applied Chemistry and Pharmacology at the University Jaume I (Castellón de la Plana,

Spain). During her undergraduate thesis, she developed new tetrapeptidic hydrogels under supervision of Dr. Juan F. Miravet and studied their rheology in a joint project with P&G. Silvia joined the Salassa group in mid 2014 for her PhD on the development of upconverting nanomaterials for theranostics.



Dr. Abraha Habtemariam (right) obtained his BSc degree from the University of East Anglia and his PhD from the University of Sussex. After a post-doctoral fellowship at the University of Toronto (Canada), he joined Peter Sadler's group at Birkbeck College, the University of London and in 1996 moved to the University of Edinburgh. In 2007 he moved to the University of Warwick as a Senior Research Fellow. He was Ikerbasque Visiting Professor at CIC biomaGUNE, San Sebastián, Spain (2013-2014).

Dr. Luca Salassa (left) obtained his MSc degree and PhD at the University of Turin (Italy). After a postdoctoral experience in Missoula (USA) at the University of Montana, he was awarded a Marie Curie IEF fellowship (2008) and joined the Sadler group at the University of Warwick (UK). In 2012, he moved to CIC biomaGUNE in San Sebastián (Spain) where he established his research group. Luca has been awarded an Ikerbasque Professorship recently.

Introduction

The portion of the electromagnetic spectrum that spans from UV to near infrared (NIR) has wavelength-dependent effects on biomolecules and displays different tissue penetration capabilities, as a result of the distinct absorption properties that tissue constituents have.¹ As shown in Fig. 1 for skin, NIR light is the most appropriate for maximum tissue penetration² and for limiting photodamage. In fact, prolonged exposure to short-wavelength light (e.g. UVC-A) can seriously harm cells by inducing photochemical reactions. On the other hand, water absorption is

predominant at wavelengths higher than 1000 nm, reducing penetration of light and causing overheating of biological components.

For this reason, the so-called “phototherapeutic window” is considered to be in the range 650–1000 nm. Light of approx. 800 nm is ideal for use in biological systems since it does minimize excitation of biomolecules and water heating.¹

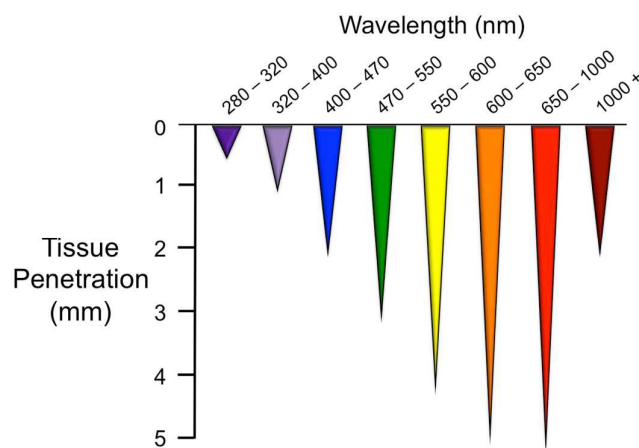


Fig. 1. Penetration depth of light of different wavelengths in skin tissue. Image adapted from reference 1.

As well documented by several review articles,³ the success of photodynamic therapy (PDT)⁴ has prompted medicinal inorganic chemists to look at the unique photophysics and photochemistry of metal complexes and to devise new light-activatable prodrugs which improve the selectivity of metal-based anticancer agents. Various design concepts have been adopted in the development of photoactivatable metal complexes. Apart from their use as alternative PDT photosensitizers, metal-based prodrugs have also been designed to exert their antineoplastic action through mechanisms that are different from PDT, i.e. oxygen independent, as for example DNA-photobinding or photouncaging of bioactive molecules. However, oxygen sensitization is often concurrent with photochemical transformation of the prodrug agent.

The great majority of light-activatable metal-based prodrugs are inactive upon NIR light excitation, since they typically require high-energy UV-visible light to trigger a photochemical response. Such intrinsic feature of metal complexes hampers somewhat their current progress towards more advanced preclinical and clinical

stages. In principle, NIR light sources would favour the use of photoactivatable metal-based prodrugs in a wider range of cancers and allow tuning light doses during treatment. For example, the maximum permissible exposure (MPE) of human skin for visible light (400–700 nm) is $0.20 \text{ W}\cdot\text{cm}^{-2}$, while MPE for NIR light can reach $0.73 \text{ W}\cdot\text{cm}^{-2}$.⁵ Furthermore, the higher penetration depth of NIR light would enable the treatment of thicker tumours.⁶

Nevertheless, it is fundamental to point out that visible light can be effective in the appliance of metal complexes in photochemotherapy of more superficial tumours, as recently demonstrated by the approval for clinical trials of a PDT ruthenium-polypyridyl photosensitizer.⁷

Excluding from the discussion inert metal-based systems that work as PDT photosensitizers, the synthetic design of metal complexes that can be light-activated in the phototherapeutic window has been extremely challenging. Very few exceptions have been reported due to the trade-off that exists between achieving good absorption in the red part of the spectrum and maintaining high photoreactivity.⁸ This requires an appropriate choice of ligands (e.g. dye-like) and optimal tuning of ligand and metal orbital energies, which in practice is not always feasible. Indeed, photoactivation of metal complexes in the phototherapeutic window or nearby has been reported, however generally such systems exploit weak absorption bands, whose intensity is hard to improve.⁹

As an alternative photoactivation approach, inorganic nanomaterials offer outstanding optical features which can serve to shift excitation wavelengths for metal complexes into the red and near infrared regions. Moreover, the versatile surface chemistry of nanomaterials provides a myriad of strategies to develop innovative metal-based prodrug carriers for photochemotherapy.

In this Frontier article we discuss recent research efforts aimed at combining a specific class of nanomaterials, that is upconverting nanoparticles, with photoactivatable metal complexes of interest in medicinal inorganic chemistry and carry out near infrared photoactivation. For the sake of brevity, we have omitted from this summary the early pioneering work on quantum dots and the promising results

obtained by triplet-triplet annihilation upconversion approaches, which have been reviewed elsewhere.¹⁰

Upconverting nanoparticles

In recent years, upconverting nanoparticles (UCNPs) have been intensely investigated for application as imaging probes and drug carriers in biology and medicine, due to their exceptional optical and chemical properties. UCNPs are lanthanide-doped crystals which absorb and convert low-energy NIR photons (980 or 808 nm) into high energy UV-visible light.¹¹ In UCNPs, upconversion is a non-linear optical luminescence phenomenon where two or more photons are absorbed by a material and a single photon of shorter wavelength (Anti-Stokes shift) is subsequently emitted. Various mechanisms can contribute to upconversion emissions, including excited state absorption (ESA), energy transfer upconversion (ETU) and photon avalanche (PA), ETU being the dominating pathway in UCNPs.¹² Typically, the brightest UCNPs are composed of a fluorine inert matrix (NaREF_4 , RE= Y, Gd, Lu) which offers doping sites for optically active lanthanides ions. Ions employed as dopants are divided into two classes: donors (typically Yb^{3+} ca. mol 20%) and acceptors (Er^{3+} , Tm^{3+} , Ho^{3+} , Eu^{3+} , Tb^{3+} ca. mol <5%). Fig. 2 shows the emission spectra and energy level diagrams for typical $\text{NaYF}_4:\text{Yb,Tm}$ and $\text{NaYF}_4:\text{Yb,Er}$ UCNPs. Upconversion emissions in these UCNPs profit from the good absorption cross-sections of Yb^{3+} at 980 nm and can be obtained by excitation with relatively low power ($0.1\text{--}10^2 \text{ W}\cdot\text{cm}^{-2}$).

NIR activation of metal complexes by UCNPs

The interest of inorganic medicinal chemistry for UCNPs began very recently (around 2012), motivated by the prospect that upconverted UV-visible emissions might activate transition metal complexes adsorbed or anchored onto the surface of nanoparticles by means of NIR-light excitation. Few, yet encouraging, systems have demonstrated Er- and Tm-doped UCNPs indeed provide a novel route for near infrared photochemistry in metal complexes. These early examples involve so far four transition metals, Mn, Fe, Ru and Pt, all of which undergo chemical transformation upon UV-visible light excitation. The primary and basic objectives of these studies were to explore different anchoring strategies for loading photoactivatable metal complexes onto UCNPs and evaluate the photochemical behaviour of the resulting nanomaterials under NIR light irradiation. Evidently, several of the systems reported here should be improved both in terms of photoreactivity and applicability in biological environments for use in photochemotherapy. However, they represent a benchmark which may serve to develop new light-activation approaches and to improve the design of UCNP-based nanomaterials for more advanced applications in biology and medicine.

In 2012 Ford and Zhang first reported in a joint article the NIR-light activation of the photoactive Roussin's black salt anion, $\text{Na}[\text{Fe}_4\text{S}_3(\text{NO})_7]$ (RBS).¹⁵ This inorganic salt has a broad absorption band in the visible region (400–600 nm) and has been extensively investigated as nitric oxide (NO) photoreleasing moiety (Fig. 3a). NO plays a key role in vasodilatation, immune response, tumour growth and suppression.¹⁶ Core@shell $\text{NaYF}_4:\text{Yb},\text{Er}@\text{NaYF}_4$ UCNPs (ca. 60 nm) were selected for attempting NIR photo-stimulation of the RBS anion. Silica coating of UCNPs served to load RBS via impregnation of the mesoporous SiO_2 shell, whose pores were eventually covered with polyallylamine hydrochloride (PAA HCl) to afford nanoconstructs capable of photoreleasing NO. The use of an NO sensitive electrode confirmed that the nanoparticles successfully generated NO upon NIR-light remote activation in a power-dependent manner (1–4.5 W) and using sequential excitation times of 30, 60, 120, and 240 s.

Shortly after, the Ford group encapsulated UCNP and the RBS anion in a NIR transparent biocompatible polymer disk, as implant model for the controlled therapeutic release of NO.¹⁷ The disk was made of a poly(dimethylsiloxane) matrix where RBS and Er- or Tm-doped core@shell NaGdF₄@NaYF₄ particles were successfully trapped due to their affinity for the hydrophobic polymer. UCNP with different acceptor ions (Er³⁺, Tm³⁺) were capable of photo-stimulating NO release from RBS upon 980-nm irradiation (2.5 W·cm⁻²). When different porcine tissue samples (skin, muscle, fat) were employed as barrier in front of the polymer disk, irradiation experiments demonstrated that NIR light could penetrate the tissue protective layer, and generate NO from the RBS precursor, even if in lower amounts. An oscillating laser beam photolysis apparatus was designed to deliver heterogeneously high laser power densities (300 W·cm⁻²) to the polymeric disk in these experiments and avoid tissue damage.

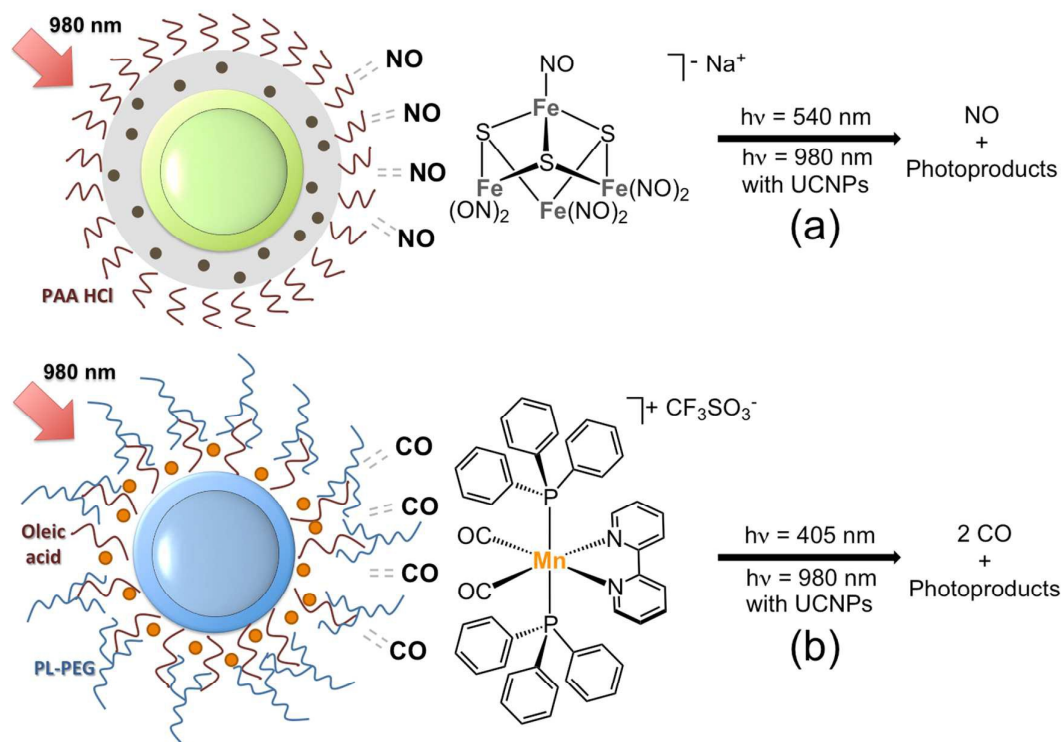


Fig. 3. Schematic representation of UCNPs nanocarriers for the NIR-light photorelease of (a) NO from RBS (•) and (b) CO from the complex *cis,trans*-[Mn(bpy)(CO)₂(PPh₃)₂][CF₃SO₃] (•). Adapted from references 15 and 18.

In 2015, Ford, Zheng and their co-workers have directed the use of UCNP towards the phototriggered release of carbon monoxide (CO) from the complex *cis,trans*-[Mn(bpy)(CO)₂(PPh₃)₂][CF₃SO₃] (where bpy = 2,2'-bipyridine and PPh₃ = triphenylphosphine, Fig. 2b).¹⁸ CO is an attractive small molecule for phototherapeutic applications due to its cytoprotective and anti-inflammatory properties.¹⁹ The Mn complex was loaded on core@shell NaGdF₄:Yb,Tm@NaGdF₄ UCNP (20–30 nm) functionalized with an amphiphilic PEG polymer, which provided the appropriate hydrophobic environment to entrap securely the water insoluble *cis,trans*-[Mn(bpy)(CO)₂(PPh₃)₂]⁺. Loading efficiency was determined by ICP-AES indicating that at best 80% of the Mn complex used in the preparation protocol could be incorporated onto the UCNP. In addition, this nanocarrier could elicit photochemical uncaging of CO under NIR-light irradiation (2 W, t = 0–5 min) as evidenced by gas chromatography and myoglobin binding studies.

Since 2013, our laboratory started a research program on upconverting nanomaterials for NIR-light photoactivation of coordination and organometallic compounds. Our first study was concerned with the UCNP-mediated photoactivation of *cis*-[Ru(bpy)₂(py)₂][Cl]₂ (where py = pyridine),²⁰ chosen as model for biologically active ruthenium polypyridyl complexes, which have found application in neuronal stimulation,²¹ protein inhibition²² and cancer therapy.^{8,23} *Cis*-[Ru(bpy)₂(py)₂][Cl]₂ has a well-understood photochemical behaviour²⁴ and photodissociates one pyridine ligand with high efficiency (Fig. 4a) upon excitation of its metal-to-ligand charge transfer band. In this study, 980-nm photoactivation (25 W·cm⁻², t = 0.5–4 h) of the complex was achieved by employing large NaYF₄:Yb,Er nanoparticles (80 nm), selected for their brightness. Photocleavage of one coordinated pyridine ligand and subsequent formation of the *cis*-[Ru(bpy)₂(py)(H₂O)]²⁺ complex was monitored overtime by UV-visible (red-shift of the lowest-energy absorption band) and by ¹H NMR (appearance of diagnostic signals for the photoproducts), analyzing aliquots of the supernatant solution obtained upon centrifugation of the reaction mixture. After 4 h of NIR light irradiation, 70% of *cis*-[Ru(bpy)₂(py)₂][Cl]₂ underwent photoconversion as determined by integration of ¹H NMR signals.

UCNPs were previously treated with HCl to remove oleic acid ligands from their surface, improve their aqueous solubility and facilitate surface adsorption of the charged Ru complexes. XPS analysis revealed that a fraction of *cis*-[Ru(bpy)₂(py)₂][Cl]₂ was still adsorbed onto the surface of UCNPs even after extensive washing, indicating electrostatic interactions between the negatively charged naked UCNPs and the positively charged complexes were sufficient to provide enough confinement for the NIR photochemistry to occur.

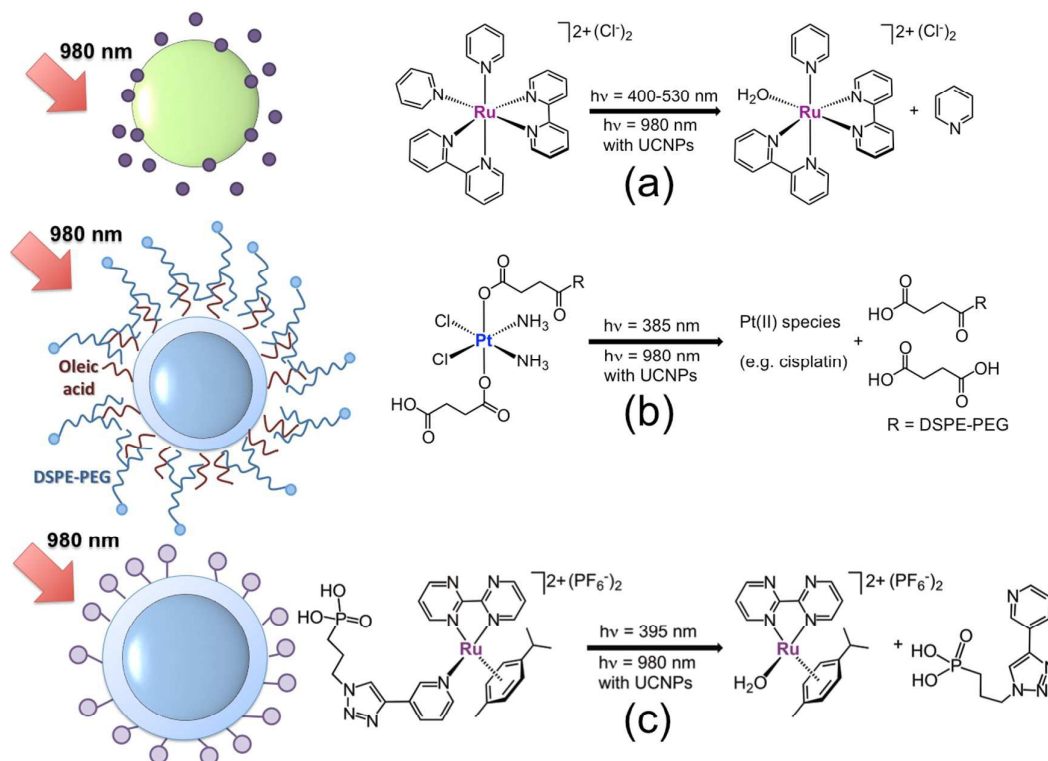


Fig. 4. Schematic representation of UCNP systems for the NIR-light activation of (a) *cis*-[Ru(bpy)₂(py)₂][Cl]₂ (•), (b) *cis,cis,trans*-[Pt(NH₃)₂Cl]₂(O₂CCH₂CH₂CO₂H)₂ functionalized with DSPE-PEG (•) and (c) [(η⁶-p-cym)Ru(bpm)(P-Trz-Py)][PF₆]₂ (•). Adapted from references 20, 26 and 28.

Inert Pt^{IV} complexes have attracted a lot of attention in the last decade as light-activatable prodrug candidates. They often combine little or no dark toxicity in cells and high stability in cellular environments with the capacity of being photoconverted into the anticancer-active square planar Pt^{II} species under UVA-visible light irradiation.²⁵ However, Pt^{IV} complexes suffer from extremely poor absorptions in the visible region, which rarely extend above 400 nm. In order to demonstrate this

limitation could be overcome, we employed NIR light ($7.3 \text{ W}\cdot\text{cm}^{-2}$, $t = 0.5\text{--}3.5 \text{ h}$) and Tm-doped $\text{NaYF}_4\text{:Yb,Tm@NaYF}_4$ UCNP (40 nm) to promote the photoreduction of *cis,cis,trans*-[Pt(NH₃)₂(Cl)₂(O₂CCH₂CH₂CO₂H)₂] to cisplatin-like Pt^{II} species (Fig. 4b).²⁶ A biocompatible (FDA-approved) PEGylated phospholipid was coupled with the Pt^{IV} complex to decorate UCNP with prodrug molecules. In the nanocarrier, the oleate chains capping UCNP hydrophobically interact with the tails of the PEGylated phospholipid, while the hydrophilic PEG units of the polymer are exposed towards the solvent, conferring further stabilization and water solubility to the system. After 3.5 h of NIR-light irradiation, the Pt^{IV} prodrug agent loaded onto the nanocarrier was fully converted into Pt^{II} species, as determined by XPS and NMR analysis of the supernatant solution (separated from the UCNP-containing pellet by centrifugation).

Earlier this year we showed that UCNP were also suitable for NIR-light photoactivation of pyridinato Ru^{II}-arene complexes. Previous studies²⁷ showed such class of compounds is stable in the dark and selectively dissociates the monodentate pyridinato ligand coordinated to the Ru centre upon visible light excitation; hence generating highly reactive aqua species with a potential to exert anticancer activity. In order to control by NIR light this key reaction in the mechanism of action of anticancer Ru-arene piano-stool complexes, we prepared two novel pyridinato Ru^{II}-arene complexes and functionalized via click chemistry the photolabile pyridinato ligand with a phosphonate moiety. The derivative $[(\eta^6\text{-p-cym})\text{Ru}(\text{bpm})(\text{P-Trz-Py})][\text{PF}_6]_2$ (where bpm = 2,2'-bipyrimidine and P-Trz-Py = [3-(1-pyridin-3-yl-[1,2,3]triazol-4-yl)-propyl]phosphonic acid diethyl ester, Fig. 4c) was directly loaded onto $\text{NaYF}_4\text{:Yb,Tm@NaYF}_4$ UCNP (40 nm) exploiting the high affinity of phosphonates for UCNP surface. A rough estimate by UV-visible provided a complex/UCNP ratio of approximately 3000:1 (3.5% wt). Once conjugated to UCNP, the Ru complex selectively generated the aqua derivative $[(\eta^6\text{-p-cym})\text{Ru}(\text{bpm})(\text{H}_2\text{O})]^{2+}$ upon 980-nm excitation. Approximately 55% of the starting Ru complex was photoconverted after 4.5 h at a power density of $8.1 \text{ W}\cdot\text{cm}^{-2}$. NMR and UPLC-MS demonstrated that the aqua photoproduct was able to bind the DNA model base guanosine 5'-monophosphate when the two were co-incubated.²⁸

Later on in 2016, the group of Wang used Tm-doped UCNPs to activate another Ru polypyridyl complex by NIR light (Fig. 5). $[\text{Ru}(\text{bpy})_2(\text{C}_{18}\text{H}_{37}\text{CN})_2][\text{Cl}]_2$ was loaded onto diamond-shaped $\text{LiYF}_4:\text{Yb},\text{Tm}$ UCNPs (70 nm along the long axis), taking advantage of the long aliphatic chain of the $\text{C}_{18}\text{H}_{37}\text{CN}$ ligands to promote strong hydrophobic interactions with the oleic acid ligands of UCNPs. Afterward, UCNPs were capped with PEG to improve aqueous solubility. ICP-AES indicated that a loading capability of 0.01 g of complex per gram of particles was achieved. The resulting nanocarriers released the two nitrile monodentate ligands upon 980-nm excitation ($3 \text{ W}\cdot\text{cm}^{-2}$, $t = 10\text{--}70 \text{ min}$), generating the bio-active species $[\text{Ru}(\text{bpy})_2(\text{H}_2\text{O})_2]^{2+}$. UV-visible measurements of supernatant solutions after centrifugation showed that ca. 80 % of photoconversion is reached upon 40 minutes of NIR-light irradiation. As reported earlier,²⁹ such aqua product was able to bind plasmid DNA whilst no change in electrophoresis mobility was noted under dark conditions.³⁰

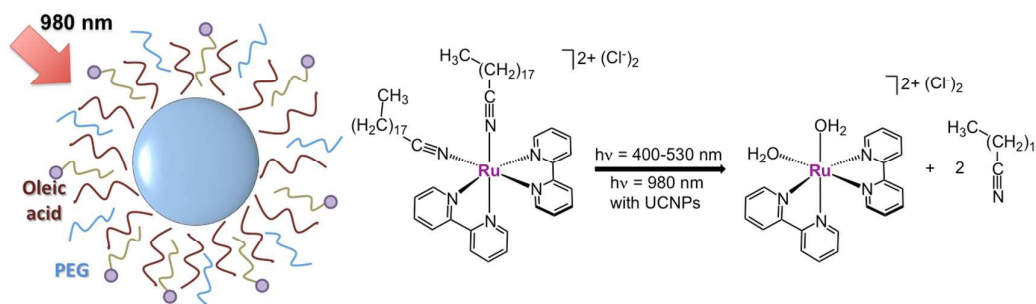


Fig. 5. Schematic representation of the NIR-light activation of the prodrug candidate $[\text{Ru}(\text{bpy})_2(\text{C}_{18}\text{H}_{37}\text{CN})_2][\text{Cl}]_2$ (*) mediated by UCNPs. Adapted from references 30.

The group of Wu has exploited Ru-polypyridyl photochemistry to achieve NIR-light controlled release of clinically approved anticancer drug doxorubicin. They prepared $\text{NaYF}_4:\text{Yb},\text{Tm}@\text{NaYF}_4$ particles coated with mesoporous silica to encapsulate doxorubicin and the complex $[\text{Ru}(\text{bpy})_2(\text{PMe}_3)((3\text{-aminopropyl})(\text{EtO})_3\text{Si})][\text{PF}_6]_2$. The silyl group of the aminopropyl ligand was key for covalently anchoring of the Ru compound onto UCNPs. As schematized in Fig. 6, the Ru complex acted as photocleavable molecular valve, which trapped doxorubicin molecules inside silica pores.³¹ Under NIR-light irradiation as low as $0.35 \text{ W}\cdot\text{cm}^{-2}$, the complex photodissociated the silyl ligand and liberated doxorubicin from the UCNPs. Authors performed control experiments on nanoparticles grafted with azobenzene, which was selected as a photoactive but non

cleavable valve. Doxorubicin release profiles of this nanomaterials proved the delivery of the drug was efficiently controlled by the photochemistry of the ruthenium complex. Laser power density of $0.64 \text{ W}\cdot\text{cm}^{-2}$ could induce doxorubicin release from the system through a thick pork tissue. Upon 10–30 min of NIR-light exposure ($0.35 \text{ W}\cdot\text{cm}^{-2}$), the nanocarrier ($300 \mu\text{g}/\text{ml}$) incubated in HeLa cells decreased cell viability to 30–40%, while the system displayed no toxicity under dark conditions. Control experiments under NIR light irradiation also demonstrate that UCNP loaded with the Ru complex without doxorubicin did not affect cell viability. Remarkably, the maximum light intensity used to photoactivate the Wu nanoconstruct was lower than the maximum permissible exposure of skin.

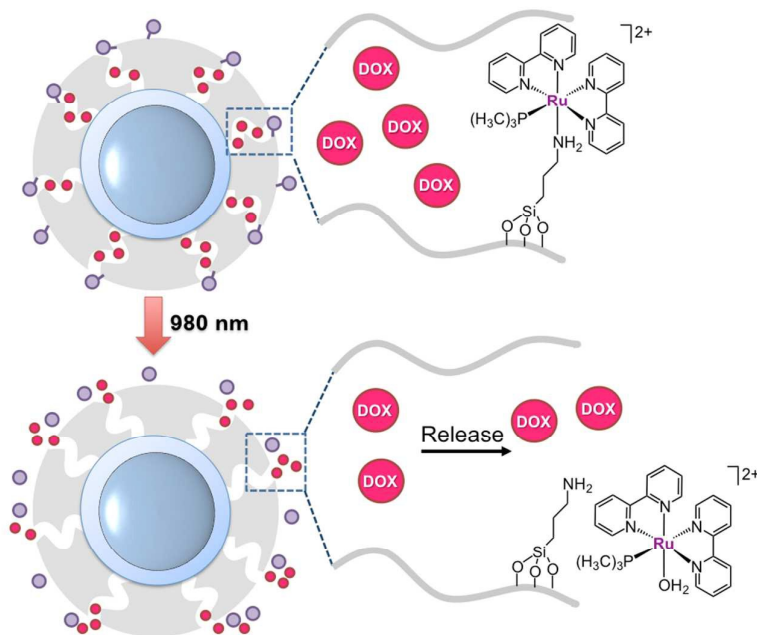


Fig. 6. Schematic representation of the nanocarrier developed by Wu and coworkers. Doxorubicin (DOX, \bullet) is release by NIR-light activation of a Ru polypyridyl complex (\bullet) acting as valve. The figure is adapted from reference 31

Conclusions and future outlook

NIR-light photochemical activation of biologically relevant metal complexes is still in its infancy. UCNP hold great promise as NIR-light sensitizers and the examples reported indeed show UCNP can shift activation wavelengths of metal-based prodrugs into the so-called phototherapeutic window. However, systems based on UCNP and metal

complexes work so far at a proof-of-concept level and many more obstacles need to be overcome to advance forward. In first instance, lowering the laser power density employed during light administration and light exposure time are fundamental requirements. In this context, it will be crucial to improve upconversion efficiency of UCNPs and optimize the photochemical response of nanoconstructs for maximal efficiency. The ultimate goal is to achieve excitation intensities that are lower than $0.73 \text{ W}\cdot\text{cm}^{-2}$. Although much needs to be improved, the Wu group has shown that the choice of the photosensitive compounds is critical and that activation of ruthenium metal complexes can be achieved with such intensities or lower and through tissues.^{31,32}

In addition, NIR-sensitive hybrid materials based on UCNP and metal complexes have to face and tackle challenges that are typically encountered in the development of nanodelivery systems and nanodrugs. These include aspects of chemical design such as optimizing solubility of nanoplateforms and their loading with metal-based drugs. Moreover, systematic biological studies are fundamental to obtain a thorough knowledge on the interaction between UCNPs and the healthy and tumour cell microenvironments, understanding how to improve materials circulation and distribution in organisms, and tailoring their toxicity mechanism of action to specific tumours.³³

Therefore, many more years of investigation are waiting ahead to eventually translate the use of UCNPs in a real clinical application. Yet, NIR-activated photochemistry of metal complexes may lead in the process to new discoveries and also provide innovative solutions for other applications.

Further to their use as phototriggers and luminescence probes, UCNPs nanomaterials also offer outstanding opportunities for others imaging modalities. Their composition and structural features make UCNPs suitable for magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET) and single-photon emission computed tomography (SPECT). Often these modalities have successfully been exploited simultaneously in one UCNP.^{11a}

Together with the good biocompatibility and relatively low toxicity profile *in vitro* and *in vivo*,³⁴ these features make UCNPs worth investigating for application in theranostics.

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References

- 1 D. Barolet, *Semin. Cutan. Med. Surg.*, 2008, **27**, 227.
- 2 S. Stolik, J. A. Delgado, A. Perez and L. Anasagasti, *J. Photoch. Photobio. B*, 2000, **57**, 90.
- 3 (a) N. J. Farrer, L. Salassa and P. J. Sadler, *Dalton Trans.*, 2009, 10690; (b) U. Schatzschneider, *Eur. J. Inorg. Chem.*, 2010, 1451; (c) E. C. Glazer, *Isr. J. Chem.*, 2013, **53**, 391.
- 4 J. M. Dabrowski and L. G. Arnaut, *Photoch. Photobiol. Sci.*, 2015, **14**, 1765.
- 5 Laser safety handbook, Northwestern University, Evanston, 2011.
- 6 K. Plaetzer, B. Krammer, J. Berlanda, F. Berr and T. Kiesslich, *Lasers Med. Sci.*, 2009, **24**, 259.
- 7 (a) G. Shi, S. Monro, R. Hennigar, J. Colpitts, J. Fong, K. Kasimova, H. Yin, R. DeCoste, C. Spencer, L. Chamberlain, A. Mandel, L. Lilge and S. McFarland, *Coord. Chem. Rev.*, 2015, **282-283**, 127; (b) <http://mcfarland.acadiau.ca/news-reader/items/our-licensed-pdt-agent-nears-human-clinical-trials.html>
- 8 (a) E. Wachter, D. K. Heidary, B. S. Howerton, S. Parkin and E. C. Glazer, *Chem. Commun.*, 2012, **48**, 9649; (b) M. J. Rose and P. K. Mascharak, *Inorg. Chem.*, 2009, **48**, 6904; (c) M. J. Rose, N. L. Fry, L. Hinck and P. K. Mascharak, *J. Am. Chem. Soc.*, 2008, **130**, 8834.
- 9 (a) B. Banik, K. Somyajit, G. Nagaraju and A. R. Chakravarty, *RSC Adv.*, 2014, **4**, 40120; (b) S. L. H. Higgins and K. J. Brewer, *Angew. Chem. Int. Ed.*, 2012, **51**, 11420; (c) M. A. Sgambellone, A. David, R. N. Garner, K. R. Dunbar and C. Turro, *J. Am. Chem. Soc.*, 2013, **135**, 11274; (d) J. Wang, J. Newman Jr., S. L. H. Higgins, K. M. Brewer, B. S. J.

Winkel and K. J. Brewer, *Angew. Chem. Int. Ed.*, 2013, **52**, 1262; (e) B. Maity, M. Roy and A. R. Chakravarty, *J. Organom. Chem.*, 2008, **693**, 1395; (f) J. D. Knoll, B. A. Albani, and C. Turro, *Acc. Chem. Res.*, 2015, **48**, 2280; (g) U. Basu, I. Pant, P. Kondaiah and A. R. Chakravarty, *Eur. J. Inorg. Chem.*, 2016, 1002; (h) Y. Sun, L. E. Joyce, N. M. Dickson and C. Turro, *Chem. Commun.*, 2010, **46**, 6759; (i) T. Gianferrara, C. Spagnul, R. Alberto, G. Gasser, S. Ferrari, V. Pierroz, A. Bergamo and E. Alessio, *ChemMedChem* 2014, **9**, 1231; (j) H. Yin, M. Stephenson, J. Gibson, E. Sampson, G. Shi, T. Sainuddin, S. Monro and S. A. McFarland, *Inorg. Chem.* 2014, **53**, 4548.

10 (a) C. R. Maldonado, L. Salassa, N. Gomez-Blanco and J. C. Mareque-Rivas, *Coord. Chem. Rev.*, 2013, **257**, 2668; (b) P. T. Burks and P. C. Ford, *Dalton Trans.* 2012, **41**, 13030; (c) S. Bonnet, *Comment. Inorg. Chem.*, 2015, **35**, 179.

11 (a) J. Zhou, Z. Liu and F. Li, *Chem. Soc. Rev.*, 2012, **41**, 1323; (b) M. Haase and H. Schäfer, *Angew. Chem. Int. Ed.*, 2011, **50**, 5808; (c) J. Shen, G. Chen, A.-M. Vu, W. Fan, O. S. Bilsel, C.-C. Chang and G. Han, *Adv. Opt. Mater.*, 2013, **1**, 644.

12 L. Tu, X. Liu, F. Wu and H. Zhang, *Chem. Soc. Rev.*, 2015, **44**, 1331.

13 (a) F. Vetrone, R. Naccache, V. Mahalingam, C. G. Morgan and J. A. Capobianco, *Adv. Funct. Mater.*, 2009, **19**, 2924; (b) D. Chen and P. Huang, *Dalton Trans.*, 2014, **43**, 11299.

14 (a) R. Kompan, J. P. Klare, B. Voss, J. Nordmann, H. J. Steinhoff and M. Haase, *Angew. Chem. Int. Ed.*, 2012, **51**, 6506; (b) B. Voss and M. Haase, *ACS Nano*, 2013, **7**, 11242; (c) T. Rinkel, J. Nordmann, A. N. Raj and M. Haase, *Nanoscale*, 2014, **6**, 14523.

15 J. V. Garcia, J. P. Yang, D. K. Shen, C. Yao, X. M. Li, R. Wang, G. D. Stucky, D. Y. Zhao, P. C. Ford and F. Zhang, *Small*, 2012, **8**, 3800.

16 (a) L. J. Ignarro, *Nitric Oxide: Biology and Pathobiology*, Elsevier Inc., Burlington, 2nd edn., 2010; (b) K. D. Bloch, F. Ichinose, J. D. Jr Roberts and W. M. Zapol, *Cardiovasc. Res.*, 2007, **75**, 339.

17 P. T. Burks, J. V. Garcia, R. Gonzalez-Irias, J. T. Tillman, M. T. Niu, A. A. Mikhailovsky, J. P. Zhang, F. Zhang and P. C. Ford, *J. Am. Chem. Soc.*, 2013, **135**, 18145.

18 A. E. Pierri, P. J. Huang, J. V. Garcia, J. G. Stanfill, M. Chui, G. Wu, N. Zheng and P. C. Ford, *Chem. Commun.*, 2015, **51**, 2072.

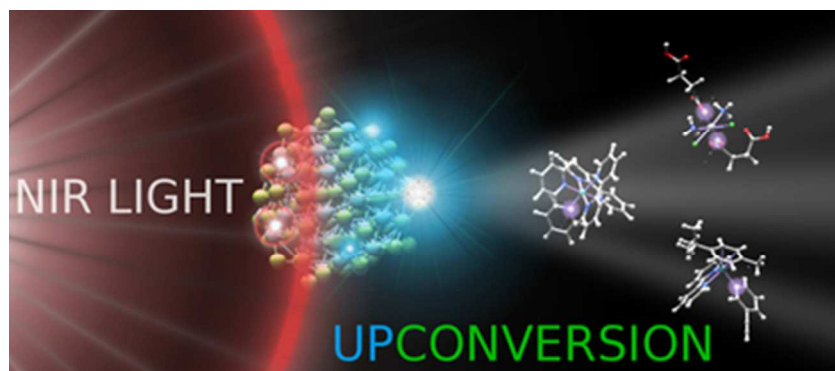
19 R. Motterlini and L. E. Otterbein, *Nat. Rev. Drug. Discov.*, 2010, **9**, 728.

20 E. Ruggiero, A. Habtemariam, L. Yate, J. C. Mareque-Rivas and L. Salassa, *Chem. Commun.*, 2014, **50**, 1715.

- 21 (a) E. Fino, R. Araya, D. S. Peterka, M. Salierno, R. Etchenique and R. Yuste, *Front. Neural Circuits*, 2009, **3**, 1; (b) R. Araya, V. Andino-Pavlovsky, R. Yuste and R. Etchenique, *ACS Chem. Neurosci.*, 2013, **4**, 1163.
- 22 (a) T. Respondek, R. N. Garner, M. K. Herroon, I. Podgorski, C. Turro and J. J. Kodanko, *J. Am. Chem. Soc.*, 2011, **133**, 17164; (b) R. Sharma, J. D. Knoll, P. D. Martin, I. Podgorski, C. Turro and J. J. Kodanko, *Inorg. Chem.*, 2014, **53**, 3272; (c) T. Respondek, R. Sharma, M. K. Herroon, R. N. Garner, J. D. Knoll, E. Cueny, C. Turro, I. Podgorski and J. J. Kodanko, *ChemMedChem*, 2014, **9**, 1306; (d) S. D. Ramalho, R. Sharma, J. K. White, N. Aggarwal, A. Chalasani, M. Sameni, K. Moin, P. C. Vieira, C. Turro, J. J. Kodanko and B. F. Sloane, *PLoS ONE*, 2015, **10**, e0142527.
- 23 (a) B. S. Howerton, D. K. Heidary and E. C. Glazer, *J. Am. Chem. Soc.*, 2012, **134**, 8324; (b) M. Dickerson, B. Howerton, Y. Bae and E. C. Glazer, *J. Mater. Chem. B*, 2016, **4**, 394.
- 24 (a) D. V. Pinnick and B. Durham, *Inorg. Chem.*, 1984, **23**, 1440; (b) E. Borfecchia, C. Garino, L. Salassa, T. Ruiu, D. Gianolio, X. Zhang, K. Attenkofer, L. X. Chen, R. Gobetto, P. J. Sadler and C. Lamberti, *Dalton Trans.*, 2013, **42**, 6564.
- 25 E. Ruggiero, S. Alonso-de Castro, A. Habtemariam and Luca Salassa, *Struct. Bond.*, 2014, **165**, 69.
- 26 E. Ruggiero, J. Hernandez-Gil, J. C. Mareque-Rivas and L. Salassa, *Chem. Commun.*, 2015, **51**, 2091.
- 27 (a) S. Betanzos-Lara, L. Salassa, A. Habtemariam, O. Novakova, A. M. Pizarro, G. J. Clarkson, B. Liskova, V. Brabec and P. J. Sadler, *Organometallics*, 2012, **31**, 3466; (b) S. Betanzos-Lara, L. Salassa, A. Habtemariam, P. J. Sadler, *Chem. Commun.* 2009, 6622.
- 28 E. Ruggiero, C. Garino, J. C. Mareque-Rivas, A. Habtemariam and L. Salassa, *Chem. Eur. J.*, 2016, **22**, 2801.
- 29 T. N. Singh and C. Turro, *Inorg. Chem.*, 2004, **43**, 7260.
- 30 Y. Chen, G. Jiang, Q. Zhou, Y. Zhang, K. Li, Y. Zheng, B. Zhang and X. Wang, *RSC Adv.*, 2016, **6**, 23804.
- 31 S. He, K. Krippes, S. Ritz, Z. Chen, A. Best, H.-J. Butt, V. Mailänder and S. Wu, *Chem. Commun.*, 2015, **51**, 431.
- 32 Z. Chen, W. Sun, H.-J. Butt and S. Wu, *Chem. Eur. J.*, 2015, **21**, 9165.
- 33 (a) Z. Cheng, A. Al Zaki, J. Z. Hui, V. R. Muzykantov, and A. Tsourka, *Science*, 2012, **338**, 903; (b) V. P. Chauhan and R. K. Jain, *Nat. Mat.*, 2012, **12**, 958; (c) V. R. Devadasu, V. Bhardwaj and M. N. V. R. Kumar, *Chem. Rev.*, 2013, **113**, 1686; (d) A. Gnach, T.

Lipinski, A. Bednarkiewicz, J. Rybka and J. A. Capobianco, *Chem. Soc. Rev.*, 2015, **44**, 1561.

34 Y. Sun, W. Feng, P. Yang, C. Huang and F. Li, *Chem. Soc. Rev.*, 2015, **44**, 1509.



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