

Journal of Materials Chemistry B

Materials for biology and medicine

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: Y. Huang, F. Qiu, R. Chen, D. Yan and X. Zhu, *J. Mater. Chem. B*, 2020, DOI: 10.1039/D0TB00262C.



This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.

The table of contents entry

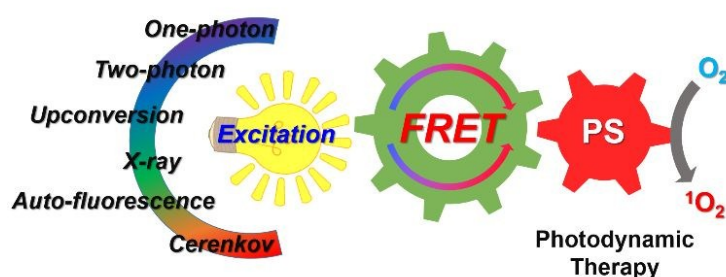
View Article Online
DOI: 10.1039/D0TB00262C

Fluorescence resonance energy transfer-based drug delivery systems for enhanced photodynamic therapy

Yu Huang, Feng Qiu, Rongjun Chen, Deyue Yan and Xinyuan Zhu

In this Review, recent advances of fluorescence resonance energy transfer-based drug delivery systems for enhanced photodynamic therapy are described, and the current challenges and perspectives in this emerging field are also discussed.

ToC figure



REVIEW

Fluorescence resonance energy transfer-based drug delivery systems for enhanced photodynamic therapy

Yu Huang,^{†ab} Feng Qiu,^{†cd} Rongjun Chen,^{*b} Deyue Yan^a and Xinyuan Zhu^{*a}Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

Abstract Photodynamic therapy (PDT) has received an increasing attention in disease treatment due to its minimally-invasive, selective destruction with combination of a photosensitizer (PS), light, and oxygen. However, the limited cytotoxic singlet oxygen (¹O₂) generation and thin tissue penetrability have been two major barriers in the conventional PDT, hindering its further development and clinical use. Recently, fluorescence resonance energy transfer-based drug delivery systems (FRET-DDSs), indirectly activating PS drugs by a donor fluorophore, have been successfully applied to alleviate these issues. The transfer of excitation energy from donors to PS drugs can significantly boost its light harvesting and extend the field of light source, which dramatically improves its production efficiency of singlet oxygen, thus leading to highly efficient and deep-tissue-penetrable PDT for the treatment of bacteria, cancer and other diseases. In this Review, we give the first-known overview of recent advances in FRET-DDSs for the enhanced PDT. In particular, dependent on the excitation energy mechanism in the FRET process, six major types of FRET-DDSs, including one-photon, two-photon, upconversion, auto-fluorescence, X-ray, and Cerenkov excited FRET-DDSs in PDT applications are summarized in detail. Furthermore, future research directions and perspectives in this emerging field are also discussed.

1. Introduction

Photodynamic therapy (PDT) has been considered as a potential therapeutic intervention against diseases due to its minimally-invasive nature, localized therapy with minimal or no damage to healthy tissues, and fast healing process.¹⁻³ Three elements are required simultaneously in PDT: a light-activatable photosensitizer (PS) drug, a light source with an appropriate wavelength, and surrounding oxygen.⁴ When illuminated by light at a specific wavelength, a PS absorbs the light energy and can be promoted into an excited singlet state. The energy of the excited singlet state can be dissipated by thermal decay, or the emission of fluorescence, or moving to a lower energy excited triplet state *via* intersystem crossing. At the excited triplet state, the PS can undergo a photochemical reaction with the surrounding molecules to generate reactive species through two PDT mechanisms, namely Type I and Type II PDT (Fig. 1a).⁵⁻⁷ A PS in Type I PDT can transfer an electron to various receptor biomolecules (e.g. proteins and nucleic acids), leading to generation of reactive free radicals and radical ions (e.g.

superoxide anion, hydroxyl radical and hydrogen peroxide). In Type II PDT, a PS can interact directly with molecular oxygen, leading to generation of abundant highly reactive and cytotoxic singlet oxygen, which is considered the principal mechanism responsible for the destruction of target cells.⁷⁻¹⁰

Although PDT has enormous potential, several main challenges remain to be addressed: (1) most PS drugs have poor water solubility and easily aggregate after administration due to their hydrophobic nature; (2) PS drugs have poor selectivity to target cells; (3) small-molecule PS drugs have short half-life, and are easy to be removed by the body; (4) most PS drugs are usually activated by UV/visible light, which holds a short light penetration depth, thus restricting PDT to superficial diseases; (5) PDT is an oxygen-consuming modality, however, the hypoxia microenvironment in some diseases, like solid tumors, can severely hinder singlet oxygen generation.¹¹⁻¹⁷ These issues above can significantly decrease PDT efficacy.¹⁸⁻²⁰ Therefore, in the past two decades, continuous efforts have been made to develop some new PS drug systems with improved properties, but there still exist some disadvantages, including difficulty in synthesis, limited penetration depth and low singlet oxygen production.²¹⁻²⁵ Recently, fluorescence resonance energy transfer-based drug delivery systems (FRET-DDSs) have been developed for various biological applications, including bio-imaging, bio-sensing, disease diagnosis and therapy.²⁶⁻³⁰ These DDSs can improve water solubility of hydrophobic drugs and protect them in the blood stream after administration.³¹⁻³³ Moreover, targeting ligands can be incorporated onto these nanoscale DDSs to enhance drug accumulation at target sites through both the enhanced permeability and retention (EPR) effect and active targeting mechanisms.³⁴ Thus, design of FRET-DDSs

^a School of Chemistry and Chemical Engineering, Shanghai Key Laboratory for Molecular Engineering of Chiral Drugs, Shanghai Jiao Tong University, 800 Dong Chuan Road, Shanghai 200240, P. R. China.

^b Department of Chemical Engineering, Imperial College London, South Kensington Campus, London, SW7 2AZ, United Kingdom.

^c Department of Oral & Maxillofacial-Head & Neck Oncology, Department of Laser and Aesthetic Medicine, Shanghai Ninth People's Hospital, National Clinical Research Centre for Oral Diseases, Shanghai Jiao Tong University School of Medicine, 639 Zhizaoju Road, Shanghai 200011, P. R. China.

^d School of Chemical and Environmental Engineering, Shanghai Institute of Technology, 100 Haiquan Road, Shanghai 201418, P. R. China.

* Corresponding authors: xyzhu@sjtu.edu.cn and rongjun.chen@imperial.ac.uk.

† These authors contributed equally to this work.

with high singlet oxygen generation in aqueous solution is much desirable to enhance PDT.

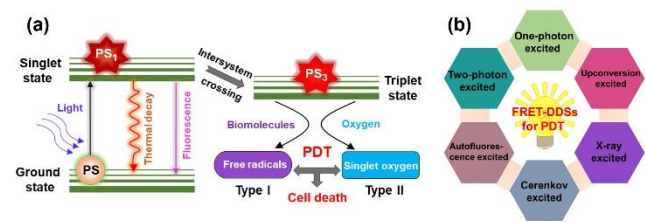


Fig. 1 (a) Schematic illustration of the PDT process through type I and type II mechanisms, respectively; (b) six different types of FRET-DDSs, including one-photon, two-photon, upconversion, auto-fluorescence, X-ray, and Cerenkov excited FRET-DDSs in PDT applications.

It has been well reported that the transfer of excitation energy from donor materials with robust excitation to PS drugs can significantly boost light harvesting, markedly red-shift excitation wavelength, and dramatically improve production of singlet oxygen, thus leading to highly efficient and deep-tissue-penetrable PDT.³⁵⁻⁴⁰ Besides, FRET-DDSs can endow multifunctional features, including simultaneous diagnostic imaging and therapy, and oxygen self-sufficiency to overcome hypoxia.⁴¹⁻⁴⁵ Therefore, FRET-DDSs have attracted wide attention in PDT for disease treatment.^{46,47} Apart from the mentioned FRET-DDSs, there are many other DDSs reported for enhanced PDT. For example, the stimuli responsive DDSs with controlled drug release performance (including pH-, redox-, enzyme-, temperature-, and ultrasound-responsive mechanisms) are well reported to enhance PDT.⁴⁸⁻⁵¹ Herein, we focus on using the FRET-DDSs mechanisms for enhanced PDT. In this Review, dependent on the excitation energy mechanism of donor materials in the FRET process, we particularly highlight six different types of FRET-DDSs for PDT, including one-photon, two-photon, upconversion, auto-fluorescence, X-ray, and Cerenkov excited FRET-DDSs (Fig. 1b). Besides, the mechanisms, advantages, shortages and applications of each DDS for the enhanced PDT applications were summarized in Table 1. As far as we know, this systematic Review would be the first-known overview of recent advances in FRET-DDSs for the enhanced PDT, which would offer some forward-looking perspectives on the creation of effective PDT platforms for clinical translation.

Table 1. The mechanisms, advantages, shortages and applications of different kinds of DDSs for enhanced PDT applications.

Mechanisms	Advantages and shortages	Applications
One-photon excited FRET mechanism	<i>Advantages:</i> large light harvesting and high ¹ O ₂ production. ^{24,52-55} <i>Shortages:</i> limited laser excitation wavelength and thin tissue penetrability. ²⁴	Antibacterial PDT, ^{35,56} and antitumor PDT. ^{57,58,64-66}

Two-photon excited FRET mechanism	<i>Advantages:</i> large laser excitation wavelength, high ¹ O ₂ production and deep tissue penetration. ^{24,67-80,85,86} <i>Shortages:</i> small focal area and expensive laser source. ^{24,71}	Antitumor PDT. ^{67-80,85,86}
Upconversion excited FRET mechanism	<i>Advantages:</i> large laser excitation wavelength, high ¹ O ₂ production and deep tissue penetration. ^{24,49,90,98} <i>Shortages:</i> small focal area, expensive laser source, potential toxicity to normal cells by upconversion material. ^{24,49}	Antitumor PDT. ⁹⁰⁻¹⁰⁵
Auto-fluorescence excited FRET mechanism	<i>Advantages:</i> high ¹ O ₂ production and deep tissue penetration. ^{107-114,115} <i>Shortages:</i> auto-fluorescence is highly dependent on the catalytic reaction. ^{107,115}	Antibacterial PDT, ^{35,118} and antitumor PDT. ^{116,117,119-121}
X-ray excited FRET mechanism	<i>Advantages:</i> large laser excitation wavelength and deep tissue penetration. ^{122,123-125} <i>Shortages:</i> potential toxicity to normal cells by X-ray, and expensive laser source. ¹²³⁻¹²⁵	Antitumor PDT. ¹²⁶⁻¹³⁶
Cerenkov excited FRET mechanism	<i>Advantages:</i> large laser excitation wavelength and deep tissue penetration. ¹³⁷⁻¹⁴⁸ <i>Shortages:</i> potential toxicity to normal cells by Cerenkov, and expensive laser source. ¹³⁷⁻¹³⁹	Antitumor PDT. ¹⁴⁹⁻¹⁵³

Other DDSs	Advantages: multi-stimuli responsive mechanisms (including pH-, redox-, enzyme-, temperature-, and ultrasound-responsive mechanisms) and controlled drug release performance. 10,11,24,48-51,174	Antitumor PDT. 10,11,15,17,24,28,48-51,104,176
	Shortages: highly dependent on the external and internal stimuli, limited laser excitation wavelength and thin tissue penetrability. 24,48	

2. One-photon excited FRET-DDSs for PDT

In the past few years, numerous fluorescent materials have been developed as donors to convert energy to PSs for improving singlet oxygen generation *via* a one-photon excited FRET process. The one-photon excited FRET-DDSs hold a great promise for PDT applications.

Thanks to the excellent light-harvesting activity of conjugated polymers, the transfer of excitation energy along their backbone to an acceptor PS drug can result in a highly amplified signal to the PS.^{35, 52-55} Based on this, Wang and co-workers reported a novel one-photon excited FRET-DDS for antibacterial PDT *via* complexation of an anionic conjugated polythiophene (PTP) and a cationic PS drug of 5,10,15,20-tetrakis[4-(6-*N,N,N*-trimethylammomiumhexyloxy)phenyl]porphyrin bromide (TPPN) (Fig. 2a).⁵⁶ Under white light irradiation, the energy transfer from PTP to TPPN took place and the indirectly activated TPPN reacted with oxygen molecules to enhance the efficiency of singlet oxygen production as compared to TPPN alone. At a white light dose of 27 J · cm⁻², an about 70 % reduction of bacterial viability was observed after treatment with this PTP/TPPN complex. Besides, Wu and co-workers reported another one-photon excited FRET-DDS for antitumor PDT,⁵⁷ whereby a PS drug of tetraphenylporphyrin (TPP) was covalently incorporated into the π -conjugated backbone of poly[(9,9-dioctylfluorenyl-2,7-diyl)-*alt*-co-(1,4-benzo-2,1,3-thiadiazole)] (PFBT) to overcome the potential issue of PS drug leakage (Fig. 2b). This PFBT-TPP system had excellent stability and showed a high energy transfer efficiency from PFBT to TPP, thus leading to a high-yield of singlet oxygen production (30 %) to kill MCF-7 cancer cells efficiently. The *in vivo* anticancer study showed that tumors were significantly inhibited or eradicated after treatment with PFBT-TPP and light irradiation. Additionally, Huang and co-workers linked Pt(II) porphyrin (a PS drug that is highly sensitive to molecular oxygen) into a water-soluble, hyperbranched, conjugated polyelectrolyte (HCPE) to improve the singlet oxygen production (Fig. 2c, d).⁵⁸ This complex of HCPE-Pt(II) porphyrin could self-assemble into a nanoparticle due to its amphiphilic structure. Under excitation at 532 nm, this nanoparticle exhibited an excellent anticancer effect owing to its high singlet oxygen quantum yield (80%) *via* the FRET process from HCPE components to Pt(II) porphyrin.

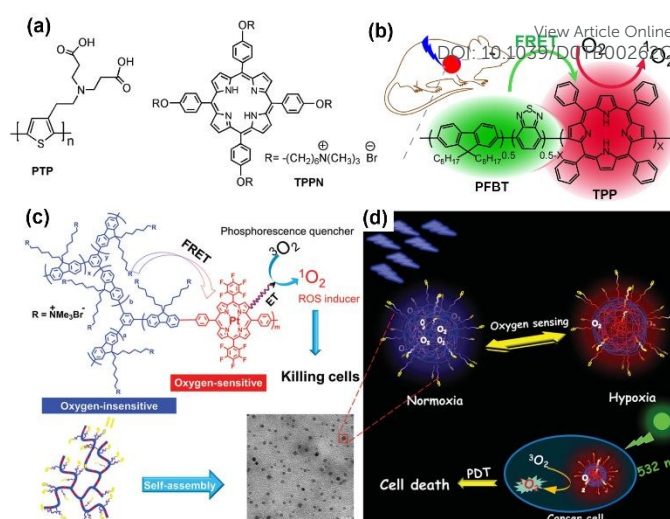


Fig. 2 (a) Chemical structures of PTP and TPPN, reproduced from ref. 56 with permission from the American Chemical Society. (b) Schematic illustration of the FRET process from PFBT to TPP for singlet oxygen generation, reproduced from ref. 57 with permission from the American Chemical Society. (c) Chemical structure, self-assembly and TEM image of the complex of HCPE-Pt(II) porphyrin and (d) schematic illustration of its PDT application, reproduced from ref. 58 with permission from John Wiley and Sons.

Fluorescent quantum dots (QDs) have a strong light absorbing capacity due to the large transition dipole moment, and long-range emission from the UV to the infrared region.⁵⁹⁻⁶³ These make QDs ideal energy donors for indirect activation of PSs to enhance singlet oxygen generation to kill target cells. In 2003, Samia and colleagues reported the first QD-based FRET-DDS to facilitate excitation of a PS for PDT application.⁶⁴ The PS drug, silicon phthalocyanines Pc4, was linked non-specifically to cadmium selenide (CdSe) QDs *via* the alkyl amino groups. They found that the conjugation of QDs offered an indirect excitation of Pc4 at 488 nm through an efficient FRET process (with an efficiency of around 77 %), enabling the use of an excitation wavelength where the PS alone does not absorb in a normal scenario. Furthermore, Charron and co-workers covalently coupled Chlorin e6 (Ce6) to less toxic InP/ZnS QDs *via* NHS-EDC chemistry (Fig. 3a).⁶⁵ Under 350 nm laser illumination, the cytotoxic singlet oxygen was produced through a FRET process from QDs to Ce6. *In vitro* evaluation of this QD-Ce6 complex in MDA-MB-231 breast cancer cells showed a decrease of 40 % in cell viability compared to the controls (Fig. 3b). Unfortunately, these conjugates had a poor water-solubility, which hindered their biological applications. To alleviate this problem, Tsay and co-workers reported a water-soluble, peptide-coated QD-PS conjugate for effective singlet oxygen production.⁶⁶ In this work, two PS drugs of Rose Bengal (RB) and Ce6 were covalently attached onto phytochelatin-related peptides, and the resulting PS-peptide conjugates were subsequently employed to overcoat QDs of CdSe/CdS/ZnS. The final QD-PS-peptide complex could offer a high-yield singlet oxygen generation (31 %) for PDT through a FRET mechanism from the QD to the PS upon an excitation at 532 nm.

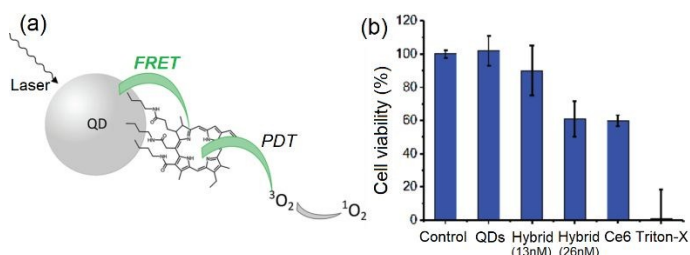


Fig. 3 (a) Schematic illustration of a QD-Ce6 complex for singlet oxygen production through the FRET process. (b) Cell viability after treatment with QDs, Ce6, and the QD-Ce6 complex following UV irradiation. Reproduced from ref. 65 with permission from the American Chemical Society.

Conventionally, commercial porphyrin-based PSs have a strong absorption band around 400 nm (Soret band) and a satellite band at about 650 nm (Q band), these PSs could be used to produce reactive oxygen species (ROS) directly *via* visible light irradiation.²⁴ Thus, the PDT based on one-photon excited FRET-DDSs with short wavelength laser (*e.g.* UV light) illumination is limiting, due to its low penetration ability and unwanted phototoxicity to the normal cells. In contrast, the one-photon excited DDSs with drug triggered release activity is highly desirable and novel through a synergy effect of chemotherapy and PDT treatment. For example, our group reported the construction of Ce6-conjugated amphiphilic hyperbranched polyphosphoester (HBPTK) with many thioketal units (HBPTK-Ce6), which could wrap Camptothecin (CPT, a hypoxia-inducible factor-1 α (HIF-1 α) inhibitor) to form nanoparticles in water (Fig. 4a).²⁸ Upon 660 nm laser irradiation, HBPTK-Ce6 could generate singlet oxygen to kill cancer cells in the presence of oxygen. Meanwhile, singlet oxygen produced by energy transfer also could trigger the degradation of the polyphosphoester containing thioketal units, leading to an efficient CPT release and a fast diffusion of nanoparticles into the tumor (Fig. 4b, c). Due to the synergy therapy, this DDS of HBPTK-Ce6@CPT exhibited the higher tumor inhibition rate both *in vitro* and *in vivo*, in comparison with other control administrations (Fig. 4d). The hematoxylin and eosin (H&E) staining of major organs and serum biochemistry study showed low side toxicity of the HBPTK-Ce6@CPT nanoparticles in mouse.

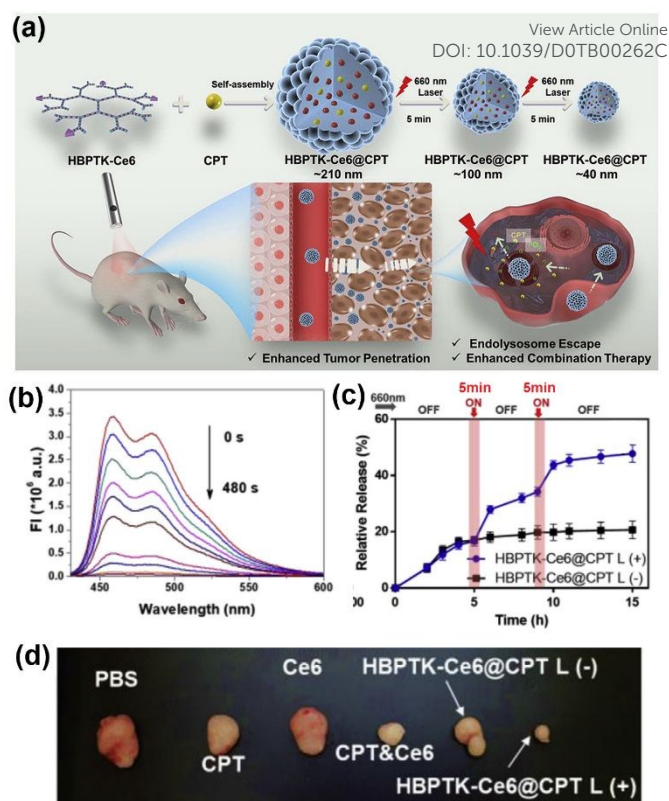


Fig. 4 (a) Schematic illustration of the HBPTK-Ce6@CPT for synergy effect of chemotherapy and PDT *via* an energy transfer process. (b) Fluorescent spectra of DPBF with HBPTK-Ce6 under the 660 nm laser irradiation for different time; (c) CPT release curves of HBPTK-Ce6@CPT under laser irradiation; (d) Photos of the size of tumors with various treatments. Reproduced from ref. 28 with permission from Elsevier.

3. Two-photon excited FRET-DDSs for PDT

In spite of the above-mentioned advantages of one-photon excited FRET-DDSs for PDT, the short light penetration into tissues (< 3 mm) has been a major issue limiting their use.⁵⁵ Therefore, two-photon excited FRET-DDSs have been developed to improve the tissue penetration.⁶⁷⁻⁷⁰ In the two-photon excited FRET-DDSs, energy donors with a large two-photon cross-section can absorb two low-energy photons simultaneously, and in turn, emit higher-energy photons to activate PS drugs for singlet oxygen production. Because the two-photon absorbing donors can be excited by near Infrared (NIR) light in this process, a much deeper tissue penetrability would be achieved as compared to traditional PDT.⁷¹

Recently, our group developed a PS-conjugated core-shell unimolecular micelle (HCP@HPE-Ce6) with a large, two-photon absorbing, hyperbranched conjugated polymer (HCP) as the inner core and a thermo-responsive hyperbranched polyether (HPE) as the outer shell (Fig. 5a).⁷² Upon irradiation with NIR light at 800 nm, the photothermal effect of the HCP core increased local temperature, and then induce the collapse of the HPE shell, which readily enhanced the efficiency of FRET by shortening the distance between HCP and Ce6. It was found that the concentration of cytotoxic singlet

oxygen produced by HCP@HPE-Ce6 micelles under irradiation at 800 nm *via* the two-photon excited FRET strategy was much higher than that of free Ce6 treated with traditional 650 nm laser excitation. Compared with free Ce6, animal fluorescence imaging showed that the HCP@HPE-Ce6 micelles could provide a relatively long retention time and a high tumor accumulation *via* the EPR effect. The *in vivo* anticancer study showed that most cancer cells were effectively killed at the tumor site after treatment with the HCP@HPE-Ce6 micelles under NIR light irradiation at 800 nm, but only part or a small part of tumor was destructed in the free Ce6 groups, suggesting a significant enhancement of antitumor PDT by this two-photon excited FRET-DDS (Fig. 5b). Besides, H&E staining of major organs in HCP@HPE-Ce6 micelles pre-treated group showed low side toxicity of HCP@HPE-Ce6 nanoparticles in mouse after PDT treatment.

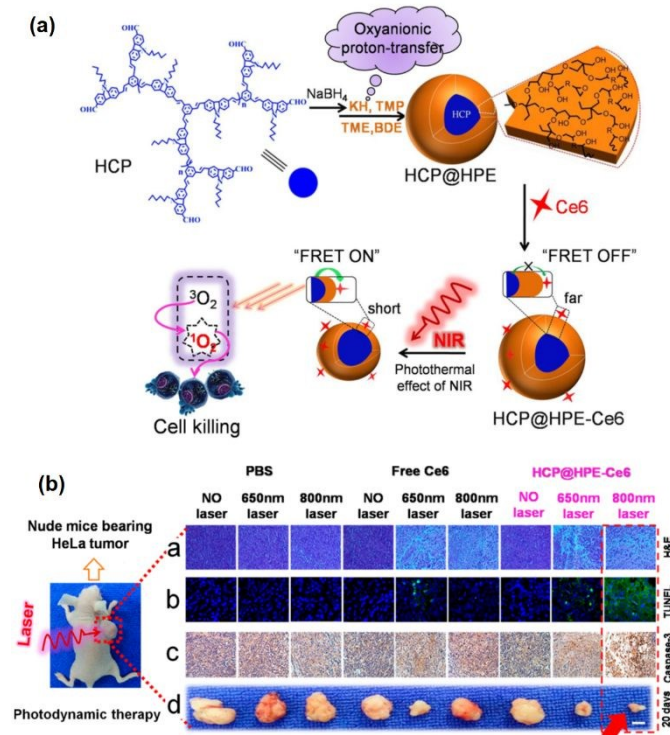


Fig. 5 (a) Synthesis of thermo-responsive HCP@HPE unimolecular micelles and illustration of the combination of two-photon excited FRET and photothermal effect of NIR for PDT. (b) *In vivo* study of the PDT efficacy in HeLa tumor-bearing mice after various treatments *via* intravenous injection of different agents, followed by laser irradiation at 800 nm. Reproduced from ref. 72 with permission from the American Chemical Society.

Some two-photon absorbing fluorescent dyes can also be utilized to construct two-photon excited FRET-DDSs for enhanced PDT. Kim and co-workers used the fluorescent dye of 9,10-bis(40-(400-amino-styryl)styryl)anthracene (BDSA) as a two-photon absorbing donor and the PS drug of 2-devinyl-2-(1-hexyloxyethyl) pyropheophorbide (HPPH) as an acceptor, which were both encapsulated into an organically modified silica (ORMOSIL) nanoparticle.⁷³ Under NIR light irradiation at 850 nm, a FRET efficiency of about 73 % was observed and a high-yield singlet oxygen production was achieved for HeLa cell killing. Cheng and co-workers co-encapsulated the two-photon

absorbing fluorescein isothiocyanate (FITC) and the PS drug of Pd-meso-tetra(4-carboxyphenyl)porphyrin (PdTPP) into different topological domains of mesoporous silica nanoparticles (MSNs).⁷⁴ The well-ordered mesoporous structure of MSNs could facilitate the rate of energy transfer (up to 93 %) from FITC to PdTPP, resulting in high singlet oxygen production and efficient PDT efficacy both *in vitro* and *in vivo* under NIR light illumination at 900 nm. In addition, due to the large two-photon absorption cross-sections ($10^3 \sim 10^5$ Goeppert-Mayer), some well-designed QDs were developed as two-photon absorbing donors to activate PS drugs for PDT.⁷⁵⁻⁷⁷ In 2013, Chou and co-workers reported a core-shell CdSe/CdS/ZnS QD for electrostatically absorbing the PS of sulfonated aluminium phthalocyanine (AlPcS) (Fig. 6a).⁷⁸ Upon excitation at 800 nm, the FRET efficiency between QDs and AlPcS reached around 90 %, and thus singlet oxygen was efficiently generated. As a result, more than 90 % of HeLa cells were killed by this two-photon excited FRET-DDS after laser irradiation for 60 min (Fig. 6b). Fowley and co-workers conjugated the PS drug of RB onto the surface of CdSe/ZnS QDs through an amide linkage.⁷⁹ With a two-photon laser irradiation at 800 nm, a FRET efficiency of as high as 99.5 % between the QDs and RB and a consequent high-yield cytotoxic singlet oxygen production caused considerable tumor cell death. The same research group further reported a novel two-photon excited FRET-DDS based on carbon quantum dots (CQDs) in combination with the PS of protoporphyrin IX (PTIX) for anticancer PDT (Fig. 6c).⁸⁰ These CQDs showed a maximum emission at 430 nm, which overlapped well with the absorption of PTIX, ensuring an efficient FRET process under two-photon excitation at 800 nm, leading to a large quantity of singlet oxygen, and as a result inducing a reduction of 82 % in HeLa cell viability. Moreover, in the fibrosarcoma tumor-bearing mouse model (Fig. 6d), the mice treated with CQDs-PTIX conjugates *via* an intra-tumoral injection showed a tumor suppression of 60 % after laser irradiation at 800 nm. By contrast, a significant increase of 65% in tumor size was observed in the control mice that only pre-treated with either CQDs-PTIX or laser alone.

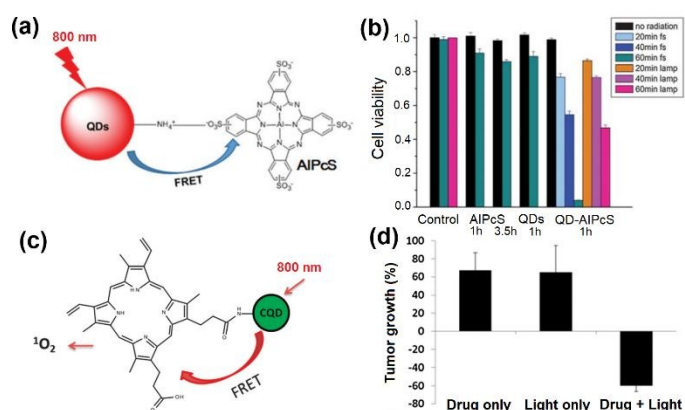


Fig. 6 (a) Schematic illustration of the QD-AlPcS conjugate for anticancer PDT under two-photon excitation at 800 nm. (b) HeLa cell viability after treatment under different conditions to evaluate the PDT efficacy. Reproduced from ref. 78 with permission from Royal Society of Chemistry. (c) Schematic illustration of the CQD-PTIX conjugate for anticancer PDT under two-photon excitation at 800 nm. (d) PDT efficacy in tumor-bearing mice after treatment with CQD-PTIX alone (drug only), 800 nm laser alone (light only), and CQD-

PTIX together with 800 nm laser (drug + light). Reproduced from ref. 80 with permission from Royal Society of Chemistry.

Gold nanorods (AuNRs) are well known for the large two-photon absorption cross-section and the tunable longitudinal plasmon band.^{24,81-84} In view of these advantages, Zhao and co-workers developed a core-shell composite, AuNR/SiO₂-T790, which consists of AuNR as a core and the silica shell covalently bound with the PS of porphyrin (T790) (Fig. 7a).⁸⁵ Under irradiation with a two-photon laser at 800 nm, the fluorescence of T790 was enhanced significantly through the energy transfer from AuNR, resulting in a highly efficient singlet oxygen production. A silica shell thickness of 20 nm was determined to be the ideal distance between AuNR and T790 molecules to generate optimum two-photon excited fluorescence. The *in vitro* PDT study showed that this AuNR-T790 composite could induce 50 % HepG2 cell death upon laser irradiation for 8 min (Fig. 7b). To evaluate the *in vivo* anticancer PDT efficacy of the AuNR-based two-photon excited FRET-DDS, Chen and co-workers developed a mesoporous silica-coated AuNR (MS-AuNR) encapsulated with the PS drug of PdTPP (Fig. 7c).⁸⁶ They found that the silica shell could enhance the two-photon fluorescence of AuNR and the mesoporous structure of silica could afford high PdTPP loading. Under two-photon excitation at 800 nm, activation of PdTPP via an intra-particle energy transfer from AuNR led to generation of cytotoxic singlet oxygen. In a breast cancer mouse model, the mice treated with MS-AuNR-PdTPP and 800 nm laser irradiation showed a remarkable enhancement of tumor inhibition as compared with the control mice, suggesting an effective *in vivo* anticancer PDT by using the two-photon excited FRET approach (Fig. 7d).

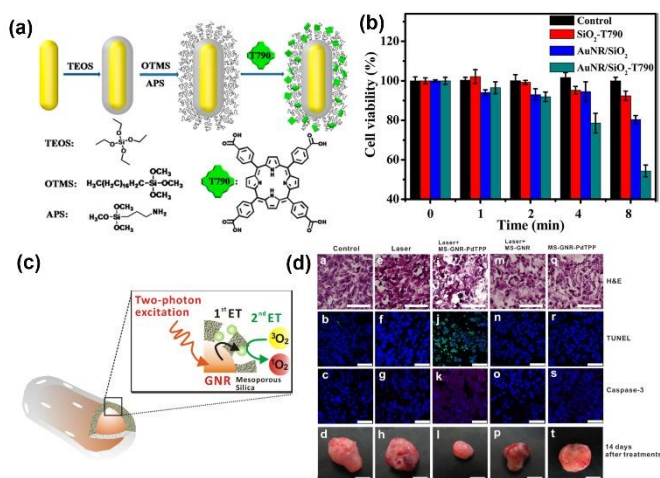


Fig. 7 (a) Schematic preparation of AuNR/SiO₂-T790. (b) Time-dependent viability of HepG2 cancer cells after incubation with AuNR/SiO₂-T790 and SiO₂-T790 nanoparticles, respectively under femtosecond (fs) laser illumination at 800 nm. Reproduced from ref. 85 with permission from the American Chemical Society. (c) Schematic illustration of MS-AuNR encapsulated with PdTPP for anticancer PDT. (d) Evaluation of *in vivo* PDT efficacy in breast tumor-bearing mice through intra-tumoral injection of different agents, followed by irradiation using an 800 nm fs pulsed laser. Reproduced from ref. 86 with permission from Ivyspring International Publisher Theranostics.

4. Upconversion excited FRET-DDSs for PDT

DOI: 10.1039/D0TB00262C

Upconversion fluorescence is a process whereby upconversion nanoparticles (UCNPs) absorb low-energy light and emit high-energy light through sequential excitation with multiple photons.^{24, 87-89} Typically, the upconversion emission needs sequential absorption of multiple photons using a real metastable state with a microsecond laser, while two-photon fluorescence demands simultaneous absorption of two photons using a virtual state with a femtosecond laser. Furthermore, the laser power density required in upconversion is usually lower than that in the two-photon process.²⁴ Thus, the emission efficiency of upconversion is higher than that in the two-photon process when the same power density is used.¹⁹ This is why UCNPs are useful energy donors to activate PS drugs for enhanced PDT through an upconversion excited FRET process.^{47, 90-98}

Zhang and co-workers first reported the utilization of UCNPs for PDT application. In their work, NaYF₄:Yb/Er UCNPs were coated by a mesoporous silica shell, which acted as a drug carrier for efficient encapsulation of PS, Zinc(II)-phthalocyanine (ZnPc).⁹⁹ They found that these ZnPc could be activated through the FRET process from UCNPs, and then singlet oxygen could be continuously produced for cancer cell killing under NIR laser excitation at 980 nm. Liu and coworkers non-covalently incorporated Ce6 onto PEGylated UCNPs to construct a nanocomplex of UCNP-Ce6 for enhanced PDT (Fig. 8a).¹⁰⁰ Upon irradiation with NIR laser at 980 nm, Ce6 could be activated efficiently via the energy transfer from UCNPs to generate cytotoxic singlet oxygen. It was found that 70 % of tumors were completely eliminated after UCNP-based PDT treatment. Importantly, the NIR light-triggered PDT using UCNP-Ce6 showed a much deeper tissue penetration depth than that of direct excitation by visible light, which was evidenced by the PDT treatment to the tumor blocked with a 8 mm pork tissue (Fig. 8b,c). Besides, Cui and co-workers developed NaYF₄:Yb/Er UCNPs which were coated with FA-modified chitosan for a high encapsulation of ZnPc (10 wt%), denoted as FASOC-UCNP-ZnPc (Fig. 8d).¹⁰¹ In the S180 sarcoma-bearing mice model, it was found that the tumor was inhibited efficiently in the mice after simultaneous pretreatment with the ZnPc-loaded UCNPs and 980 nm laser exposure for 30 min. Interestingly, this work was further extended to investigate the depth of tissue penetration during PDT by using this the UCNPs-based FRET-DDS (Fig. 8e). A 1 cm pork tissue was placed over the tumor during PDT treatment, and a higher inhibition of tumor growth was demonstrated in mice irradiated with a 980 nm laser as compared to those treated with a 660 nm laser, suggesting that the deep tissue penetration could be achieved by this upconversion excited FRET-DDS. They also found that the survival rates of mice in the deep penetrating PDT group induced by 980 nm light irradiation reach to 88 %, which was much higher than the control mice group. These results indicated that the nanoparticles of FASOC-UCNP-ZnPc had low side effect in mice and the PDT treatment based on FASOC-UCNP-ZnPc could effectively improve the survival quality of mice and prolong their lifetime.

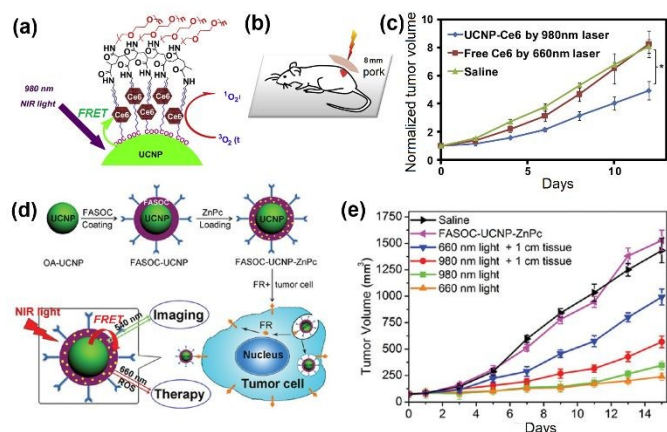


Fig. 8 (a) Schematic illustration of the UCN-Ce6 nanocomplex containing Ce6 and PEGylated UCNPs for PDT under excitation by NIR laser at 980 nm. (b) Schematic of experiment design in vivo experiment. Tumors were hidden below a pork slice (8 mm) during light treatment. (c) The growth of tumors on different groups of mice after various treatments. The relative tumor volumes were normalized to their initial sizes. Reproduced from ref. 100 with permission from Elsevier. (d) Schematic illustration of the synthesis of FASOC-UCNP-ZnPc nanoconstruct and FA-mediated binding of tumor cells with FA receptor expression. (e) Comparison of the therapeutic efficacy of deep-tissue PDT triggered by 980 and 660 nm light after placed a 1 cm pork tissue over the tumor. Reproduced from ref. 101 with permission from the American Chemical Society.

Upconversion excited FRET-DDSs have also been used for enhanced photo-chemo therapy. It is noted that solid tumors have a hypoxic microenvironment, which is remarkably resistant to PDT.¹⁰²⁻¹⁰⁴ To address this problem, our group developed a novel, NIR light-triggered and oxygen/Pt(II) self-generating nano-prodrug (UCPP) based on UCNPs for cancer therapy (Fig. 9a).¹⁰⁵ In this system, an anticancer complex *cis,trans,cis*-[Pt(N₃)₂(OH)₂(NH₃)₂] (denoted as Pt(IV)) acted as an oxygen self-generating drug and was linked to the terminal of oligomer Ce6-PEG. The resulting amphiphilic oligomer Ce6-PEG-Pt(IV) (denoted as CPP) could be self-assembled on the UCNPs surface of NaYbF₄:Tm@CaF₂ (denoted as UCPP) to form a nano-prodrug. At the meantime, the UCNPs-embedded PEG-Ce6 nanoparticles (denoted as UPC) and UCNPs-loaded PEG-Pt(IV) nanoparticles (denoted as UPP) were used as control. Toxicity study showed a negligible toxicity to normal cells after treated with UCPP at a high concentration of 20 μM in dark. Upon NIR laser irradiation at 980 nm, the UCNPs could convert the NIR light into visible light, and then transfer energy for triggering the decomposition of Pt(IV) to release the active Pt(II) for chemotherapy, and activating the Ce6 for PDT. Through this upconversion excited FRET-DDS of UCPP, sustained generation of molecular oxygen could compensate the oxygen consumption during PDT and overcome the hypoxia-induced PDT resistance, resulting in the significant improvement of anticancer efficacy in the HeLa-tumor bearing mouse model (Fig. 9b, c).

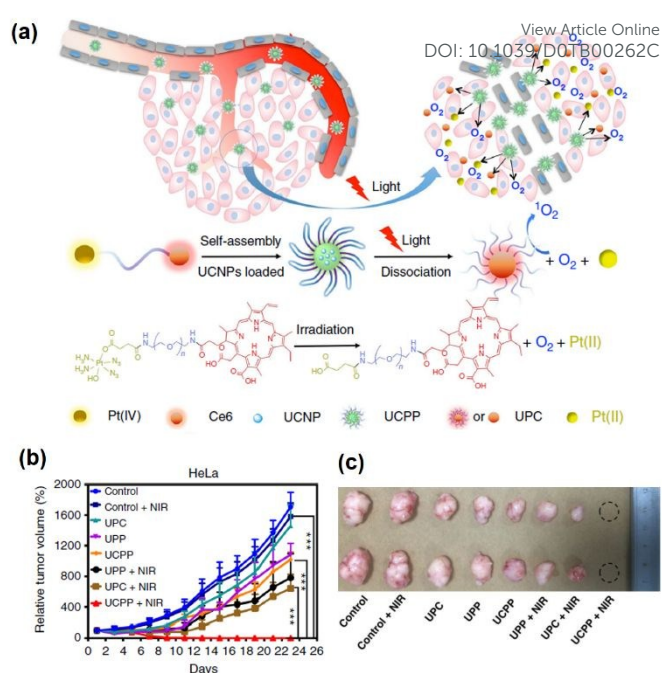


Fig. 9 (a) Schematic illustration of the UCPP for PDT. (b) Tumor volume of HeLa tumor-bearing mice after treatment under various conditions. (c) Representative images of striped HeLa tumors after treatment under various conditions. Reproduced from ref. 105 with permission from Nature Publishing Group.

5. Auto-fluorescence excited FRET-DDSs for PDT

The NIR light offering deep tissue penetration has been well developed for improving PDT *via* two-photon and upconversion excited FRET strategies. However, the penetration depth of NIR light could only reach several centimeters, which still limits the PDT applications of two-photon and upconversion excited FRET-DDSs in superficial disease therapies.¹⁰⁶ To enable treatment of intraperitoneal metastasis-tumor, a novel auto-fluorescence excited DDS without outer light source is highly desirable.¹⁰⁷ Inspired by the light-generating reactions used by fireflies and jellyfish, many auto-fluorescent, FRET-based nanomaterials have been constructed for various bioapplications, including immunoassay analysis,¹⁰⁸ inflammation detection,^{109,110} drug delivery,¹¹¹ tumor imaging,¹¹² DNA detection,¹¹³ and clinical disease diagnostics.¹¹⁴

Bioluminescence is an important auto-fluorescence generated from a chromophore substrate catalysed by an enzyme.¹¹⁵ This bioluminescence can be used to excite PSs to efficiently produce ROS *via* a bioluminescence resonance energy transfer (BRET) approach. Lai and colleagues reported a luciferase-immobilized QD system (QD-RLuc8), which exhibited the red emission at 655 nm after addition of coelenterazine (Fig. 10a, b).¹¹⁶ Such bioluminescence could activate porphyrin to generate ROS in the presence of oxygen *in vitro*. After 20-day treatment of QD-RLuc8 and coelenterazine *via* intravenous injection, the tumor growth profile was significantly inhibited compared to the other groups (Fig. 10c, d). The immunohistochemical analysis also confirmed the obvious cell

proliferation in tumor *via* treatment with BRET-mediated PDT. Furthermore, no significant body weight loss in all of pre-treated groups in the *in vivo* study demonstrated the safety treatment of this BRET-mediated PDT. Besides, Li and co-workers reported the production of ROS from the carbon dot-protoporphyrin IX conjugate (CDs-PIX) by D-luciferin catalysis in the presence of magnesium ion and adenosine triphosphate (ATP) *via* BRET.¹¹⁷

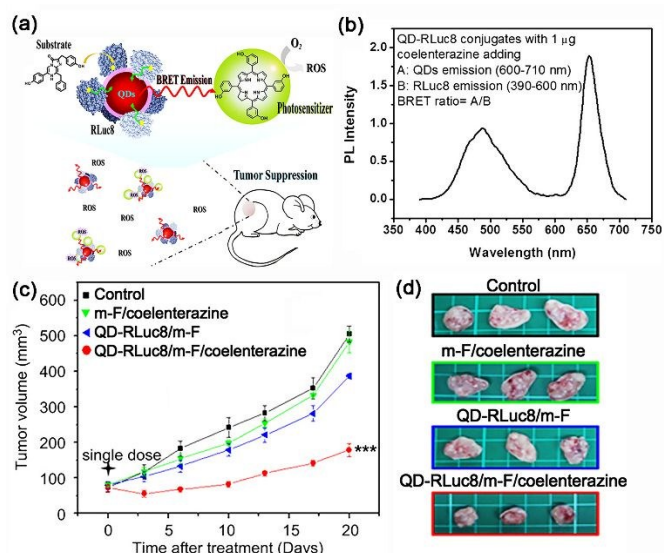


Fig. 10 (a) Schematic representation of QD-RLuc8 for BRET-based PDT. (b) Luminescence emission spectrum of QD-RLuc8 with addition of coelenterazine. (c) A549 tumor volume as a function of treatment time. (d) Photo of tumors after 20-day treatment with QD-RLuc8 and coelenterazine. Reproduced from ref. 116 with permission from Elsevier.

Compared with traditional PDT, luciferase-based BRET-DDSs show the low selectivity due to the lack of active targeting effect. Usually, hydrogen peroxide (H_2O_2), a fundamental mediator in various cell signalling pathways, is overproduced abnormally in inflammation. Thus, H_2O_2 -responsive luminescent materials have been proved highly selective systems for early diagnosis of many bacteria and cancers.²⁹ In 2012, Wang and co-workers reported a H_2O_2 -triggered BRET system containing luminol as a substrate, horseradish peroxidase (HRP) as an enzyme and cationic oligo (*p*-phenylene vinylene) (OPV) as an energy acceptor (Fig. 11a, b).¹¹⁸ Upon addition of H_2O_2 , luminol emitted blue luminescence, well overlapped with the absorption of OPV for BRET. When oxygen was in the surrounding of OPV, ROS was produced from the excited OPV. With increasing the OPV concentration, the luminescence intensity of OPV was increased, leading to the enhanced ROS generation. The *in vivo* experiment indicated that the luminol-based BRET-DDS exhibited a good inhibition of HeLa tumor growth after 20-day treatment, in comparison with other control groups (Fig. 11c). Moreover, BRET-based PDT treatment showed the low toxic side effect, because growth trends of body-weight in all of mice were similar. This BRET system also had the excellent antimicrobial activity toward *Candida albicans*, reaching a high killing

efficiency of 98 % (Fig. 11d). It has been reported that chlorin is a good photosensitizer for highly efficient ROS generation. However, the emission of luminol cannot meet the requirement for excitation of chlorin. Thus, Wang and co-workers reported the FA and HRP-bifunctionalized, conjugated polymer dots (FH-Pdots) by self-assembly with amphiphilic Janus dendrimers (Fig. 11e, f).¹¹⁹ After treatment with H_2O_2 , the red emission of chlorin at 590 nm could be obtained to generate enhanced ROS with existence of Pdots *via* the BRET-FRET approach. Because the higher concentration of H_2O_2 in tumor cells than that of normal cells, the FH-Pdot system can be used for selective PDT against tumor. Imaging results confirmed that red fluorescence from FH-Pdots was observed in the cytoplasm of MCF-7 cells, while the low emission was shown in NIH 3T3 cells (Fig. 11g). Correspondingly, the cell viability of MCF-7 cells (17 %) was much lower than that of NIH 3T3 cells (72 %) after PDT treatment. These results suggest this that BRET-DDS hold great promise in the tumor-selective PDT.

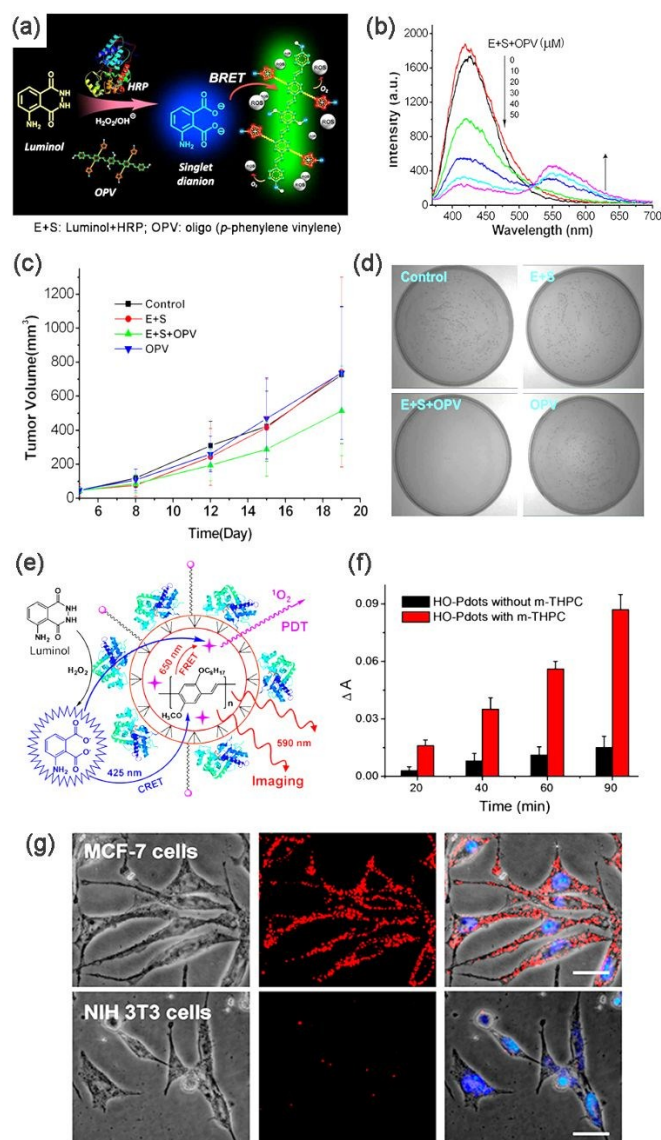


Fig. 11 (a) Schematic illustration of the H_2O_2 -triggered BRET system containing luminol as a substrate, HRP as an enzyme and OPV as an

energy acceptor for PDT. (b) Luminescence spectra of luminol in the presence of various concentrations of OPV. (c) The change of tumors volume during 19-day treatment with the H_2O_2 -triggered BRET system. (d) Photographs of *Candida albicans* plates treated with the luminol-based DDS with and without OPV. Reproduced from ref. 118 with permission from the American Chemical Society. (e) Schematic illustration of FH-Pdots and their ROS generation mechanism. (f) ROS generation with or without m-THPC doped as a function of irradiation time. (g) CLSM images of MCF-7 and NIH 3T3 cells incubated with FH-Pdots. Reproduced from ref. 119 with permission from the American Chemical Society.

To date, many chemiluminescence (non-enzymatic autofluorescence) systems have been reported for various bioapplications, including tumor and tissues imaging, drug delivery, *etc.*^{107,111,112} Among these, the H_2O_2 -triggered, peroxalate-based chemiluminescence system is a powerful diagnostic tool for biosensing and bioimaging.¹¹² Kim and co-workers prepared a chemiluminescence nanoscale system containing bis[3,4,6-trichloro-2(pentyloxy carbonyl) phenyl] oxalate (CPPO), BODIPY and diphenylamino-substituted poly(cyano-phenylenevinylene) (DPA-CN-PPV), which could emit NIR luminescence *via* chemiluminescence resonance energy transfer (CRET) upon addition of H_2O_2 .¹²⁰ The tissue penetration depth of this CRET system was deeper than that of the NIR emission. This enabled the peritonitic inflammation to be real-time detected in a hair-covered mouse model. Liu and co-workers reported another novel CPPO-based CRET-DDS for PDT (Fig. 12a-c).¹²¹ They synthesized a new NIR photosensitizer TBD with aggregation-induced emission (AIE) property, which was co-encapsulated with CPPO by soybean oil and Pluronic F127 to form C-TBD nanoparticles (C-TBD NPs). C-TBD NPs produced effective singlet oxygen when exposed to H_2O_2 *via* the CRET approach. Here, the soybean oil used as a retarder could slow down the consumption of CPPO and lengthen the time of chemiluminescence, favorable for *in vivo* applications of C-TBD NPs. The *in vivo* study revealed that C-TBD NPs exhibited the luminescence with a high signal-to-background ratio, where the metastasis tumor in the intraperitoneal region was specifically observed after 1.5 h post-intravenous injection. Combination with a H_2O_2 enhancer agent of β -phenylethyl isothiocyanate (FEITC), the growth of tumor volume was tremendously inhibited after 15-day treatment in comparison with other administration groups (Fig. 12d). Histological analysis, immunofluorescence staining and Western blot analysis further confirmed the effectiveness of the CRET-mediated PDT with C-TBD NPs (Fig. 12e). There was less weight loss after C-TBD NPs administration in comparison of normal mice. In addition, no obvious damages were found in the H&E stained images of kidney, spleen, and liver slices from mice pre-treated with C-TBD NP administration, indicating negligible toxicity of C-TBD NPs.

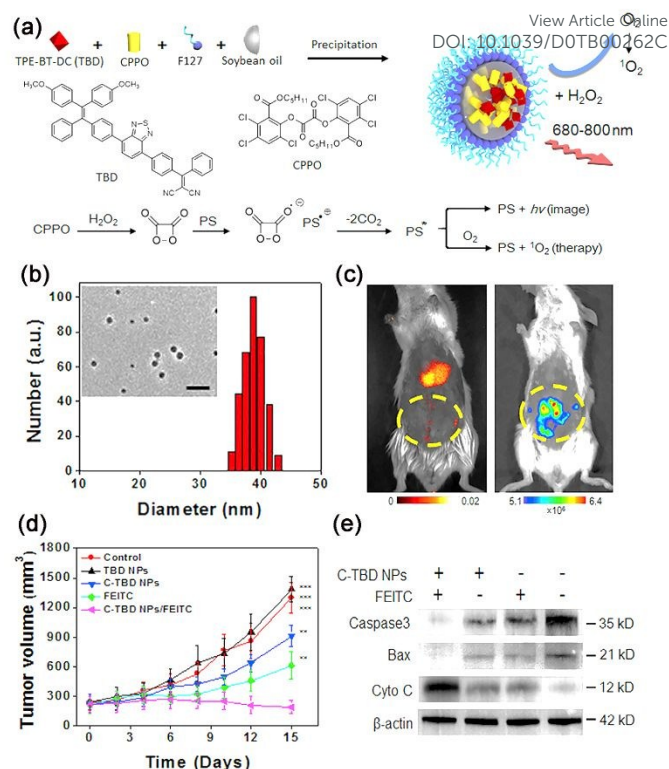


Fig. 12 (a) Schematic illustration of C-TBD NPs for $^1\text{O}_2$ production via the CRET strategy. (b) Size of C-TBD NPs; (c) *In vivo* fluorescence (left) and chemiluminescence (right) images of breast tumor in the abdomen of mouse after 1.5 h post-intravenous injection of C-TBD NPs. (d) Tumor growth curves under different conditions. (e) Western blot analysis of the cytosolic level of caspase-3, bax, and cytochrome c (Cyto C) of tumor cells with different treatments. Reproduced from ref. 121 with permission from Cell Press.

6. X-ray excited FRET-DDSs for PDT

Currently, X-ray technology has been widely used in clinical disease diagnosis, which is capable of deeper penetration into tissues than visible and NIR light. When a proper scintillator is irradiated by X-ray, the luminescence can be emitted *via* recombination of electron-hole pairs at the activated centre of scintillator. Foulger and co-workers reported that lutetium oxyorthosilicate doped with cerium phosphors (LSO:Ce) could be coated with organic fluorophores, and luminescent properties of the resulting system could be tuned from blue to green and red through a FRET process under X-ray excitation, which could be used as drug delivery system with the concentration of around 0.3 mg mL^{-1} .¹²² Thus, these scintillators have been used as luminescent probes in various biological applications including bioimaging and biosensor.¹²³ X-ray, as the external light source, excited PDT can not only have deep tissue penetration for intraperitoneal tumor therapy, but also show the high selective treatment.^{124,125}

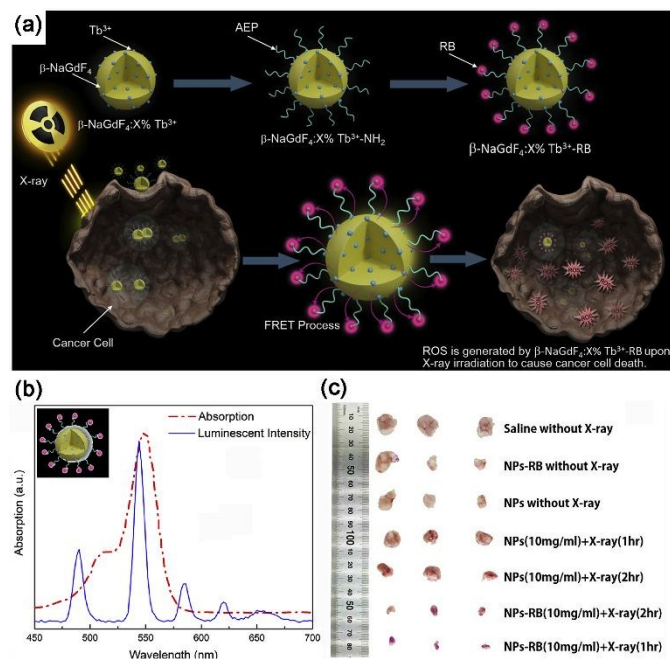


Fig. 13 (a) Schematic illustration of the synthesis route of the NaGdF₄:Tb³⁺-RB nanoparticle and its X-PDT mechanism. (b) The absorption spectrum of RB and the luminescence spectrum of NaGdF₄:Tb³⁺. (c) Size of tumors from HepG2 cell-bearing nude mice pre-treated with different treatments. Reproduced from ref. 127 with permission from Elsevier.

Lanthanide-doped fluoride/oxide nanoparticles have been confirmed as an important kind of scintillating nanoparticles. Dujardin and colleagues reported the synthesis of polysiloxane-coated Tb₂O₃ conjugated with porphyrin as a PS (Tb₂O₃@SiO₂-porphyrin).¹²⁶ The radioluminescence spectrum of Tb₂O₃ was overlapped with the absorption spectrum of porphyrin, showing an efficient X-ray-excited resonance energy transfer (XeRET). The singlet oxygen was generated from X-ray irradiation of Tb₂O₃@SiO₂-porphyrin under an aerobic environment. In 2018, Lu and co-workers prepared novel β -NaGdF₄:Tb³⁺ nanoparticles. RB as a PS was covalently grafted on the surface of scintillating NaGdF₄:Tb³⁺@SiO₂-RB nanoparticles (Fig. 13a, b).¹²⁷ The efficient singlet oxygen generation was attributed to their ultra-high XeRET efficiency of 99.63%, resulting from their overlapped spectra of β -NaGdF₄:Tb³⁺ with RB and the singlet oxygen quantum yield of RB (75%). Tb₂O₃@SiO₂ nanoparticles showed low cytotoxicity under X-ray irradiation in the *in vitro* study (Fig. 13c). However, the HepG2 cell viability was lower than 20% after pre-treatment of NaGdF₄:Tb³⁺@SiO₂-RB at a concentration of 1 mg mL⁻¹ and X-ray irradiation for 90 min. The antitumor efficacy of NaGdF₄:Tb³⁺@SiO₂-RB was also evaluated in the HepG2-bearing xenograft mouse model (Fig. 13d, e). After intratumoral injection, a significant inhibition of tumor growth was achieved for NaGdF₄:Tb³⁺@SiO₂-RB with X-ray radiation (1.17 Gy h⁻¹), as compared to the other pre-treated groups. These results demonstrated the effective PDT with singlet oxygen production triggered *via* XeRET. They also found that mice weight was slightly decrease under the irradiation of X-ray compared with

other groups without irradiation, due to the systemic toxicity from the X-ray. H&E staining of major organs in all pre-treated groups showed the similar damage to that of X-ray only groups, demonstrating that low toxicity of NaGdF₄:Tb³⁺@SiO₂-RB nanoparticles. To date, many other lanthanide elements (La, Lu) coupled with Methylene Blue (MB) and protoporphyrin IX, as well as the core-shell-shell structure have been explored for potential X-ray excited PDT applications.¹²⁸⁻¹³²

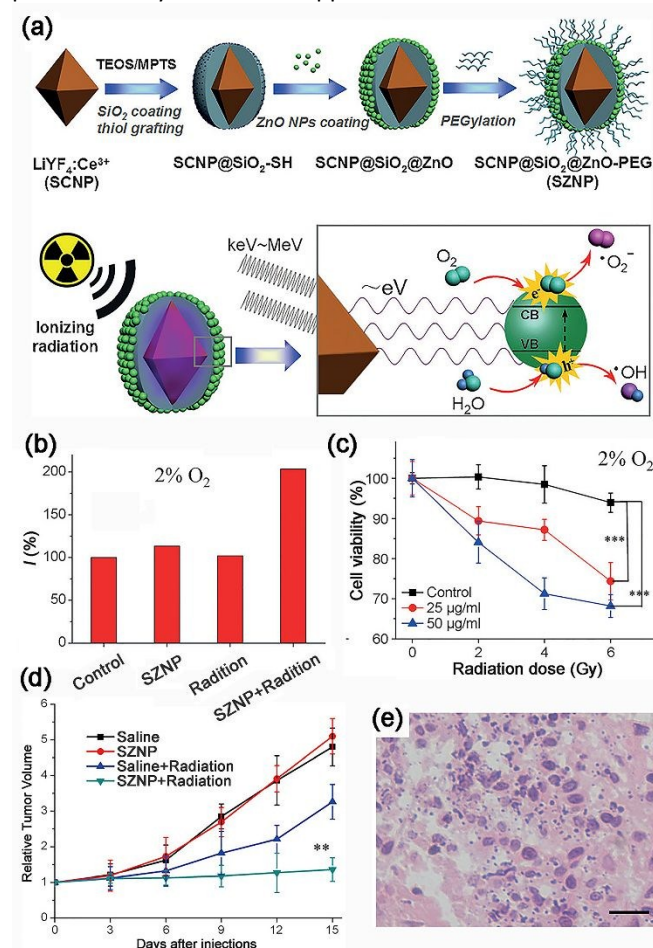


Fig. 14 (a) Schematic illustration of the synthesis route of monodisperse Ce^{III}-doped LiYF₄@SiO₂@ZnO and the mechanism of ionizing radiation-induced PDT. (b) Relative intensities of ROS-induced DCF fluorescence after incubation of HeLa cells with LiYF₄@SiO₂@ZnO in a hypoxic environment; the control group denotes to the cells not exposed to radiation. (c) Viabilities of hypoxic HeLa cells incubated with LiYF₄@SiO₂@ZnO under various doses of X-ray radiation. (d) The change in relative tumor volume with time after exposure of the tissues to different administrations. (e) H&E staining of tumor tissue sections from mice 48 hours after treatment with LiYF₄@SiO₂@ZnO and 8 Gy X-ray radiation, Scale bar = 20 µm. Reproduced from ref. 133 with permission from John Wiley and Sons.

Conventional PDT involving O₂ consumption usually shows low therapeutic efficacy against hypoxic tumor. Shi and co-workers constructed core-shell Ce^{III}-doped LiYF₄@SiO₂@ZnO, in which luminescence generated from Ce^{III}-doped LiYF₄ (denoted as SZNP) under X-ray irradiation could produce electron-hole pairs in ZnO *via* FRET, leading to formation of highly reactive

hydroxyl radicals ($\cdot\text{OH}$) from the reaction of electron-hole pairs with water (Fig. 14).¹³³ Such toxic $\cdot\text{OH}$ exhibited an efficient tumor inhibition both *in vitro* and *in vivo*. The staining of H&E from major organs showed the slight damage for 30 days after the intravenous injection. However, tumors pre-treated with Ce^{III} -doped $\text{LiYF}_4@/\text{SiO}_2@/\text{ZnO}$ under irradiation exhibited the shrinkage and fragmentation of nucleus in HeLa cells, indicative of the enhanced antitumor therapeutic efficacy of this X-ray excited FRET-DDS.

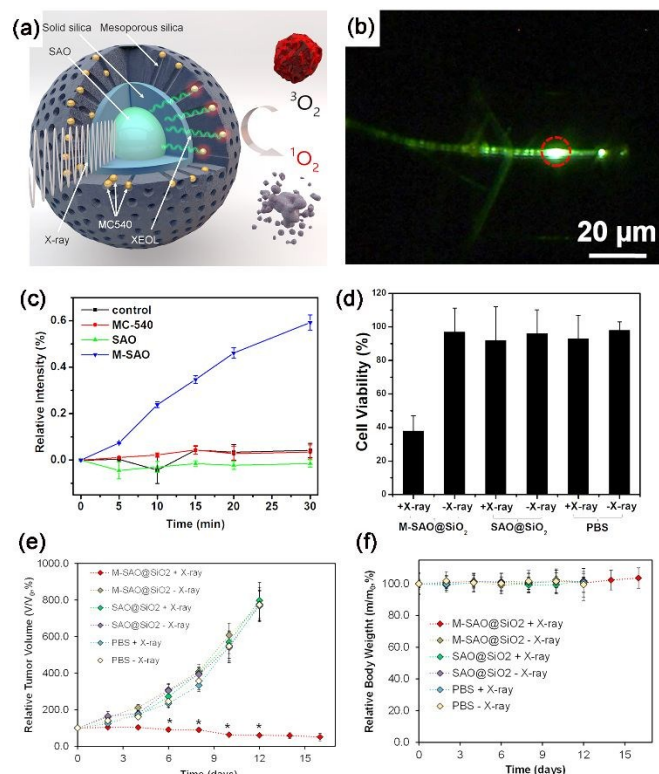


Fig. 15 (a) Structure of silica-coated $\text{SrAl}_2\text{O}_4:\text{Eu}^{2+}$ and the mechanism of $^1\text{O}_2$ generation via X-ray excited resonance energy transfer. (b) Luminescence image of the SAO wire by X-ray. (c) $^1\text{O}_2$ production from M-SAO@SiO₂ and the other comparison groups by using singlet oxygen sensor green (SOSG) as an indicator. (d) U87MG cell viability after pre-treatment with M-SAO@SiO₂ and the other comparison groups with and without X-ray radiation. (e) Relative tumor volume and (f) relative body weight with time after exposure of the tissues to different administrations. Reproduced from ref. 134 with permission from the American Chemical Society.

IIIA elements in the periodic table are another important resource for preparation of scintillating nanoparticles. Xie and co-workers reported the synthesis of silica-coated $\text{SrAl}_2\text{O}_4:\text{Eu}^{2+}$ (SAO@SiO₂), which exhibited the strong green luminescence under X-ray irradiation (Fig. 15a, b).¹³⁴ After loading MC540 as a PS into the SAO@SiO₂ to form MC540-SAO@SiO₂ (M-SAO@SiO₂), the singlet oxygen was efficiently generated by the XERET approach. The significant damage to U87MG cells was observed both *in vitro* and *in vivo* by using a low X-ray dose of 0.5 Gy (Fig. 15c-e). Simultaneously, the organs and body weight of xenograft mice showed few changes during 16-day treatment

with M-SAO@SiO₂ (Fig. 15f). Later, they confirmed that the efficient tumor inhibition with this X-PDT was caused by effective combination of PDT and radiotherapy.¹³⁵ Shen and co-workers prepared novel $\text{LiGa}_5\text{O}_8:\text{Cr}$ scintillating nanoparticles which could emit the NIR luminescence under X-ray irradiation.¹³⁶ After encapsulating $\text{LiGa}_5\text{O}_8:\text{Cr}$ and 2,3-naphthalocyanine as PS drugs into mesoporous silica, the nanoparticles suppressed the growth of H1299 orthotopic tumor efficiently *via* the XERET-mediated PDT.

7. Cerenkov excited FRET-DDSs for PDT

Cerenkov luminescence (CL) has received increasing attention on biological applications, due to its high signal-to-noise, deep tissue penetration, no excitation light and low dose of radionuclide, which combines the advantages of both chemiluminescence and X-ray excited luminescence.¹³⁷ To date, many radioisotope-containing nanoparticles have been developed to emit luminescence in the UV region.^{138,139} Moreover, the NIR light could be achieved from these nanomaterials *via* Cerenkov luminescence resonance energy transfer (CLRET).¹⁴⁰ Such unique optical properties has emerged as a new optical technology for wide biological applications including imaging of tumor,¹⁴¹⁻¹⁴⁴ sentinel lymph nodes,¹⁴⁵ A β plaques,¹⁴⁶ and glioma,¹⁴⁷ as well as phototherapy.¹⁴⁸

Recently, Ngwa and co-workers investigated the Cerenkov-excited PDT (CePDT) on the HT1080 tumor model by using an ^{18}F -fluorodeoxyglucose (^{18}F -FDG) with titanium dioxide (TiO₂), which generated cytotoxic hydroxyl radicals from the reaction between electron-hole pairs and water through CLRET.¹⁴⁹ The *in vitro* experiment proved that the significantly higher activity of ^{18}F -FDG (> 15 MBq) was required for inhibition of tumor growth with TiO₂. However, Cerenkov photons of ^{18}F as a source was much lower than other radionuclides. In addition, Liu and co-workers reported the use of ^{68}Ga as the CL source for CePDT (Fig. 16a, b).¹⁵⁰ Compared with ^{18}F -FDG, ^{68}Ga -labeled bovine serum albumin (^{68}Ga -BSA) showed a higher inhibition of 4T1 cell growth with the existence of dextran-modified TiO₂ nanoparticles (D-TiO₂), which was in agreement with luminescent intensity of Cerenkov accumulation in tumor tissues (Fig. 16c-e). *In vivo* results also revealed that the mixture of ^{68}Ga -BSA and D-TiO₂ displayed significantly better tumor suppression than other administrations (Fig. 16f). At the same time, no significant body weight loss and no obvious morphology change of major organs were observed in all groups, suggesting the minimal side effect of CePDT. Taking the advantage of ^{68}Ga radionuclide, this is a new PET-guided CePDT for precision treatment of cancer.

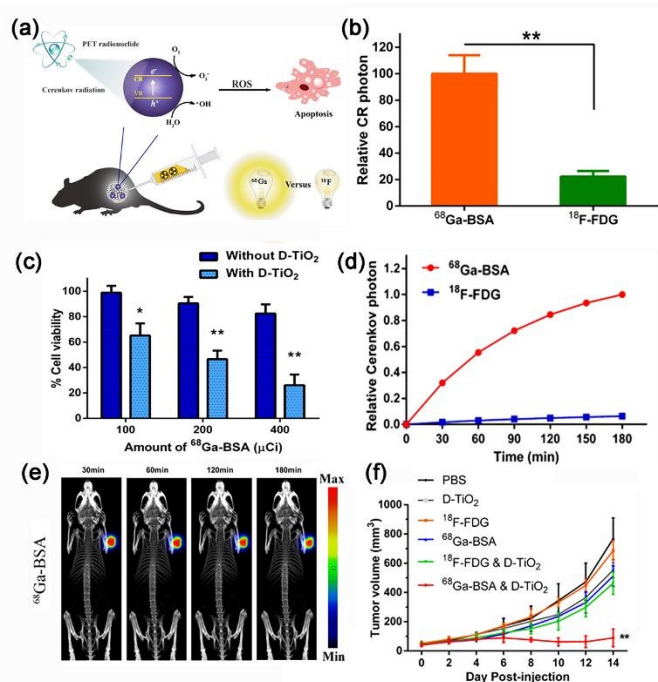


Fig. 16 (a) Schematic illustration of Cerenkov-excited PDT via Cerenkov luminescence resonance energy transfer by combining the ^{18}F radionuclide and TiO_2 . (b) Cerenkov photon accumulation from intracellular ^{68}Ga -BSA and ^{18}F -FDG. (c) 4T1 cell viability after treatment with D- TiO_2 and ^{68}Ga -BSA. (d) Time-dependent Cerenkov photon accumulation of ^{68}Ga -BSA and ^{18}F -FDG. (e) Dynamic PET images of 4T1 tumor-bearing mice pre-treated with ^{68}Ga -BSA for 30 min, 1 h, 2 h and 3 h. (f) Time-dependent relative tumor volume after pre-treatment with different administrations. Reproduced from ref. 150 with permission from the American Chemical Society.

Other radionuclides (e.g. ^{90}Y , ^{89}Zr) have been explored for PDT applications through CLRET.^{151–153} Cai and co-workers prepared hollow mesoporous silica nanoparticles (HMSNs) followed by loading Ce6 and ^{89}Zr oxalate (^{89}Zr HMSN-Ce6) (Fig. 17a, b).¹⁵² Owing to its high-energy β emitters and long half-life, the ^{89}Zr oxalate served as good Cerenkov luminescence for excitation of Ce6 to generate singlet oxygen. The luminescence of Ce6 was observed clearly in the ^{89}Zr HMSN-Ce6 via CLRET. This emission was retained and detected for 14 days, leading to continuous generation of singlet oxygen (Fig. 16c) and effective damage to tumor *in vitro* and *in vivo* (Fig. 17d, e). H&E staining was used to evaluate the morphologies of tumor and tissues with 7 days post-treatment. The results demonstrated that most of the tumor tissue with ^{89}Zr HMSN-Ce6 was destroyed, while the tissues in the other control groups retained their normal morphology, suggesting the low toxicity of ^{89}Zr HMSN-Ce6.

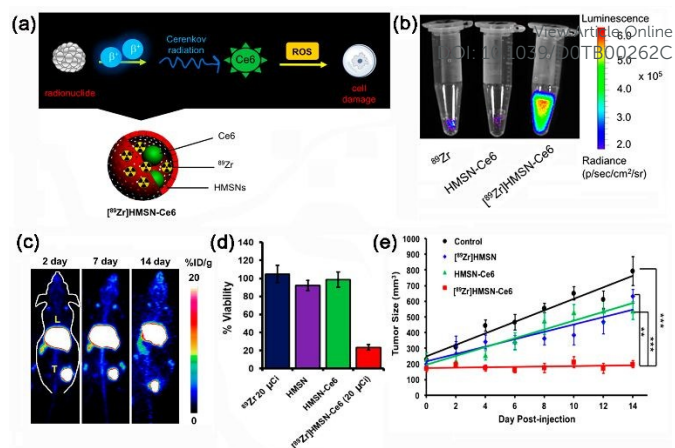


Fig. 17 (a) Schematic illustration of ^{89}Zr HMSN-Ce6 and its mechanism for CePDT via CLRET. (b) Luminescence imaging of the PBS solution containing ^{89}Zr , HMSN-Ce6 and ^{89}Zr HMSN-Ce6, respectively (emission filter of 690–710 nm). (c) In vivo PET imaging of ^{89}Zr HMSN-Ce6 at different time points. (d) 4T1 cell viability after pre-treatment with different administrations. (e) Time-dependent tumor growth after different administrations in mice. Reproduced from ref. 152 with permission from the American Chemical Society.

Recently, Cai's group reported magnetic targeting of ^{89}Zr -MNP/TCPP for CePDT (Fig. 18a).¹⁵³ In this work, magnetic ($\text{Zn}_{0.4}\text{Mn}_{0.6}$) Fe_2O_4 nanoparticles (MNPs) as a drug carrier showed the higher active targeting ability than HMSNs. After surface conjugation of meso-tetrakis(4-carboxyphenyl) porphyrin (TCPP) and chelator-free labelling of ^{89}Zr , ^{89}Zr -MNP/TCPP exhibited not only highly selective accumulation in tumor tissues, but also efficient Ce6 luminescence through CLRET (Fig. 18b–e). Thus, ^{89}Zr -MNP/TCPP represents an ideal DDS for CePDT. The volume of 4T1 tumor growth was inhibited remarkably by ^{89}Zr -MNP/TCPP with magnetic targeting (Fig. 18f, g), while no significant weight loss was observed in all therapeutic groups, implying the low side effect of the treatment. Moreover, no noticeable organ damage was found over 60 days except liver, which was damaged at 7 days, but self-recovered within two months.

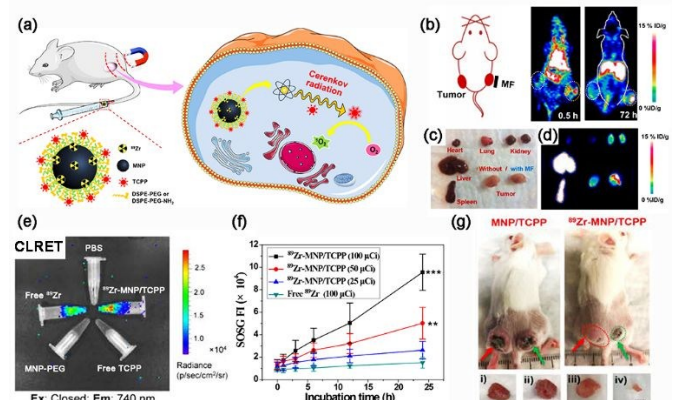


Fig. 18 (a) Schematic illustration of magnetism-guided CePDT via CLRET. (b) PET imaging of 4T1 tumor-bearing mice with an external magnetic field attached onto the right tumor side. (c) Photograph and (d) PET imaging of main organs after pre-treatment with ^{89}Zr -

MNP/TCPP. (e) CLRET imaging of different solution samples. (f) Determination of singlet oxygen generation by measuring the fluorescence intensity changes of SOSG from different samples. (g) Photographs of mice and dissected tumors on Day 14 post-treatment. Red arrows: nonmagnetic targeting tumors, Green arrows: magnetic targeting tumors. Reproduced from ref. 153 with permission from the American Chemical Society.

8. Conclusions and perspectives

Significant breakthroughs have been made in the design of novel DDSs to overcome major limitations in traditional PDT including poor water-solubility, off-targeting and short half-life.^{154,155} Integration of the FRET technology in DDSs has opened up a highly promising avenue to enhance PDT through one-photon, two-photon, upconversion, auto-fluorescence, X-ray and Cerenkov excitation. The FRET-DDSs have several advantages in PDT applications: (i) the classic hydrophobic PS is used as a guest molecule by encapsulation or conjugation in this DDS, which can improve its water solubility; (ii) attaching targeting ligands onto the FRET-DDSs or using an enhanced permeability and retention (EPR) effect of nanotechnology can enhance the accumulation of PS to targeted site; (iii) FRET-DDSs can protect PS in the blood stream after administration; (iv) molecules as the energy donors with robust excitation by two-photon, upconversion, auto-fluorescence, X-ray and Cerenkov can enhance the depth of tissues penetration during PDT process; (v) FRET-DDSs can endow multifunctional features, including simultaneous diagnostic imaging and therapy, oxygen self-sufficiency property to overcome hypoxia and so on. These advantages pave the way for highly efficient and deep-tissue-penetrable PDT for the treatment of bacteria, cancer and other diseases by using the FRET-DDSs. However, it should also be mentioned that PDT using FRET-DDSs is still in its infancy with many challenges awaiting solutions. The following directions and perspectives deserve attention in future studies.

Firstly, avoiding or minimizing undesired toxicity to normal tissues is a critical for the use of FRET-DDSs for PDT. It has been reported that some nanoscale FRET-DDSs, such as AuNRs and silica nanoparticles, have high redox reactivity on their surface, which could generate unwanted ROS and oxidative stress to the normal cells.¹⁵⁶ Some QDs and UCNPs containing heavy metal ions (*e.g.* Cd²⁺, Hg²⁺, *etc.*) have been found toxic to normal cells owing to ROS production and DNA damage.¹⁵⁷⁻¹⁵⁹ Furthermore, these nanoparticles can be captured by the reticuloendothelial system after intravenous administration, leading to a quick removal from the blood circulation and lower cellular uptake at target sites.¹⁶⁰⁻¹⁶² Besides, the off-targeted nanoparticles mainly reach the liver and spleen, which can cause liver and spleen toxicity and generate inflammatory responses.¹⁶³⁻¹⁶⁵ Hence, it is important to construct FRET-DDSs with biocompatible materials for PDT to avoid unwanted side effects on healthy tissues.

Secondly, use of a suitable, inexpensive and safe laser is desired for applications of FRET-DDSs in PDT. In spite of significant red-shifting of excitation wavelength *via* two-photon and upconversion excited FRET technology during PDT, the focal

area of the laser beam is generally small.⁸⁴ The small focal area is not suitable for treatment of bulky tumors.¹⁶⁶ Also, the light source is usually costly. Furthermore, X-ray and Cerenkov excited FRET-DDSs can increase the tissue penetration depth from several millimetres to several centimeters, but the irradiation of X-ray and Cerenkov will cause unnecessary phototoxicity to normal cells.³⁹ Therefore, it is crucial to enlarge the laser beam diameter, reduce costs and improve treatment safety for translating FRET-DDSs to clinical PDT practice.

The last, but not the least, the challenge of tumor hypoxia remains to be addressed in future anticancer PDT since PDT is an oxygen-consuming treatment modality.¹⁰²⁻¹⁰⁵ The hypoxic microenvironment in solid tumors is caused by the presence of pre-existing hypoxic cells due to the exhaustion of blood supply during the rapid tumor growth; and also associated with the PDT process through rapid shutdown of tumor vasculature and fast consumption of local oxygen.^{24,167,168} To date, some interesting solutions have been proposed to overcome the PDT-induced hypoxia, for example through the use of a hyperbaric oxygen therapy which offers a pure oxygen environment to patients in a pressurized sealed chamber for promoting oxygen transportation to hypoxic tumors.¹⁰⁵ However, the side effects, including barotrauma and hyperoxic seizures, may seriously hinder its clinical applications.¹⁶⁹⁻¹⁷¹ Recently, some oxygen-generating materials including MnO₂ and catalase have been reported to overcome hypoxia.¹⁷²⁻¹⁷⁶ However, these materials are highly dependent on the catalytic reaction by endogenous enzymes. Therefore, new strategies that can facilitate the PDT independence of the oxygen concentration at a tumor site will be favorable.

In conclusion, recent years have witnessed growing research interest on development of novel FRET-DDSs for enhanced PDT applications. Although increasing efforts need to be made in the clinical translation of PDT technologies, it is believed that more novel and safer FRET-DDSs will be developed in the future thanks to the rapid development of both optical technology and nanotechnology.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (51690151, 81802694), National Key Research and Development Plan of China (2016YFA0201500), Shanghai Municipal Government (18JC1410800, 17ZR1441700), and China Postdoctoral Science Foundation (2019M651524). This work was also funded with UK aid from the UK government, and the U.K. Department of Health and Social Care is gratefully acknowledged for funding the Future Vaccine Manufacturing Research Hub through the Engineering and Physical Sciences Research Council (EPSRC, grant number: EP/R013764/1).

References

1. D. E. J. G. J. Dolmans, D. Fukumura and R. K. Jain, *Nat. Rev. Cancer*, 2003, **3**, 380-387.
2. J. F. Lovell, T. W. B. Liu, J. Chen and G. Zheng, *Chem. Rev.*, 2010, **110**, 2839-2857.
3. T. A. Theodossiou, A. R. Gonçalves, K. Yannakopoulou, E. Skarpen and K. Berg, *Angew. Chem., Int. Ed.*, 2015, **54**, 4885-4889.
4. X. Wang, J. Hu, P. Wang, S. Zhang, Y. Liu, W. Xiong and Q. Liu, *Theranostics*, 2015, **5**, 772-786.
5. W.M. Sharman, C.M. Allen and J. E. van Lier, *Drug Discov. Today*, 1999, **4**, 507-517.
6. M. Triesscheijn, P. Baas, J. H. M. Schellens and F. A. Stewart, *Oncologist*, 2006, **11**, 1034-1044.
7. I. J. Macdonald and T. J. Dougherty, *J. Porphyr. Phthalocyanines*, 2001, **5**, 105-129.
8. A. P. Castano, P. Mroz and M. R. Hamblin, *Nat. Rev. Cancer*, 2006, **6**, 535-545.
9. A. Ormond and H. Freeman, *Materials*, 2013, **6**, 817-840.
10. D. B. Guo, S. T. Xu, Y. Huang, H. Jiang, W. Yassen, N. Wang, Y. Su, J. Qian, J. Li, C. Zhang and X. Y. Zhu, *Biomaterials*, 2018, **177**, 67-77.
11. P. Sun, N. Wang, X. Jin and X. Y. Zhu, *ACS Appl. Mater. Interfaces*, 2017, **9**, 36675-36687.
12. S. Singh, A. Aggarwal, N. V. S. D. K. Bhupathiraju, G. Arianna, K. Tiwari and C. M. Drain, *Chem. Rev.*, 2015, **115**, 10261-10306.
13. J. Zhao, K. Xu, W. Yang, Z. Wang and F. Zhong, *Chem. Soc. Rev.*, 2015, **44**, 8904-8939.
14. H. Chen, J. Tian, W. He and Z. Guo, *J. Am. Chem. Soc.*, 2015, **137**, 1539-1547.
15. C. L. Tu, L. J. Zhu, P. P. Li, Y. Chen, Y. Su, D. Y. Yan, X. Y. Zhu and G. Y. Zhou, *Chem. Commun.*, 2011, **47**, 6063-6065.
16. M. Ethirajan, Y. Chen, P. Joshi and R. K. Pandey, *Chem. Soc. Rev.*, 2011, **40**, 340-362.
17. C. Ma, L. Shi, Y. Huang, L. Shen, H. Peng, X. Y. Zhu and G. Zhou, *Biomater. Sci.*, 2017, **5**, 494-501.
18. E. K. Lim, T. Kim, S. Paik, S. Haam, Y. M. Huh and K. Lee, *Chem. Rev.*, 2015, **115**, 327-394.
19. C. Wang, L. Cheng and Z. Liu, *Theranostics*, 2013, **3**, 317-330.
20. J. Hu, Y. A. Tang, A. H. Elmenoufy, H. Xu, Z. Cheng and X. Yang, *Small*, 2015, **11**, 5860-5887.
21. H. Dong, S. R. Du, X. Y. Zheng, G. M. Lyu, L. D. Sun, L. D. Li, P. Z. Zhang, C. Zhang and C. H. Yan, *Chem. Rev.*, 2015, **115**, 10725-10815.
22. G. Chen, I. Roy, C. Yang and P. N. Prasad, *Chem. Rev.*, 2016, **116**, 2826-2885.
23. L. Cheng, C. Wang, L. Feng, K. Yang and Z. Liu, *Chem. Rev.*, 2014, **114**, 10869-10939.
24. S. S. Lucky, K. C. Soo and Y. Zhang, *Chem. Rev.*, 2015, **115**, 1990-2042.
25. J. P. Taquet, C. Frochot, V. Manneville and M. Barberi-Heyob, *Curr. Med. Chem.* 2007, **14**, 1673-1687.
26. Y. Huang, L. Y. Shen, D. B. Guo, W. Yassen, Y. Wu, Y. Su, D. Chen, F. Qiu, D. Y. Yan, X. Y. Zhu, *Chem. Commun.*, 2019, **55**, 6735-6738.
27. N. T. Chen, S. H. Cheng, C. P. Liu, J. S. Souris, C. T. Chen, C. Y. Mou and L. W. Lo, *Int. J. Mol. Sci.*, 2012, **13**, 16598-16623.
28. H. Jin, T. Zhu, X. G. Huang, M. Sun, H. G. Li, X. Y. Zhu, M. L. Liu, Y. B. Xie, W. Huang and D. Y. Yan, *Biomaterials*, 2019, **211**, 68-80.
29. Y. Huang, F. Qiu, D. Chen, L. Shen, S. Xu, D. Guo, Y. Su, D. Yan and X. Y. Zhu, *Small*, 2017, **13**, 1604062.
30. F. Qiu, D. Wang, Q. Zhu, L. Zhu, G. Tong, Y. Lu, D. Yan and X. Y. Zhu, *Biomacromolecules*, 2014, **15**, 1355-1364.
31. Y. F. Wang, T. Zhang and X. J. Liang, *Small*, 2016, **12**, 6451-6477.
32. Y. Huang, L. Yu, J. Ren, B. Gu, C. Longstaff, A. D. Hughes, S. A. Thom, X. Y. Xu and R. J. Chen, *J. Control. Release*, 2019, **300**, 1-12.
33. C. Wang, Z. Wang, X. Zhao, F. Yu, Y. Quan, Y. Cheng and H. Yuan, *Acta Biomater.*, 2019, **85**, 218-228.
34. T. K. Chen, B. He, J. S. Tao, Y. He, H. Deng, X. Q. Wang and Y. Zheng, *Adv. Drug Deliv. Rev.*, 2019, **143**, 177-205.
35. H. Yuan, B. Wang, F. Lv, L. Liu and S. Wang, *Adv. Mater.*, 2014, **26**, 6978-6982.
36. X. Feng, Y. Tang, X. Duan, L. Liu and S. Wang, *J. Mater. Chem.*, 2010, **20**, 1312-1316.
37. Y. Huang, D. L. Wang, X. Y. Zhu, D. Y. Yan and R. J. Chen, *Polym. Chem.*, 2015, **6**, 2794-2812.
38. T. S. Corbitt, J. R. Sommer, S. Chemburu, K. Ogawa, L. K. Ista, G. P. Lopez, D. G. Whitten and K. S. Schanze, *ACS Appl. Mater. Interfaces* 2009, **1**, 48-52.
39. M. Sivasubramanian, Y. C. Chuang and L. W. Lo, *Molecules*, 2019, **24**, 520.
40. Y. Huang, R. Dong, X. Y. Zhu and D. Y. Yan, *Soft Matter*, 2014, **10**, 6121-6138.
41. M. Wu, Y. Ding and L. L. Li, *Nanoscale*, 2019, **11**, 19658-19683.
42. R. H. Müller, K. Mäder and S. Gohla, *Eur. J. Pharm. Biopharm.*, 2000, **50**, 161-177.
43. G. Calixto, J. Bernegossi, B. Fonseca-Santos and M. Chorilli, *Int. J. Nanomed.*, 2014, **9**, 3719-3735.
44. O. C. Farokhzad and R. Langer, *ACS Nano*, 2009, **3**, 16-20.
45. R. Q. Li, C. Zhang, B. R. Xie, W. Y. Yu, W. X. Qiu, H. Cheng and X. Z. Zhang, *Biomaterials*, 2019, **194**, 84-93.
46. J. Yao, M. Yang and Y. Duan, *Chem. Rev.*, 2014, **114**, 6130-6178.
47. N. M. Idris, M. K. G. Jayakumar, A. Bansal and Y. Zhang, *Chem. Soc. Rev.*, 2015, **44**, 1449-1478.
48. R. Zhou, S. Zhu, L. Gong, Y. Fu, Z. Gu and Y. Zhao, *J. Mater. Chem. B*, 2019, **7**, 2588-2607.
49. Y. Wang, G. Q. Wei, X. B. Zhang, F. N. Xu, X. Xiong and S. B. Zhou, *Adv. Mater.*, 2017, **29**, 1605357.
50. M. Wang, Y. Zhai, H. Ye, Q. Lv, B. Sun, C. Luo, Q. Jiang, H. Zhang, Y. Xu, Y. Jing, L. Huang, J. Sun and Z. He, *ACS Nano*, 2019, **13**, 7010-7023.
51. N. Zhang, F. Zhao, Q. Zou, Y. Li, G. Ma and X. Yan, *Small*, 2016, **12**, 5936-5943.

52. H. Y. Liu, P. J. Wu, S. Y. Kuo, C. P. Chen, E. H. Chang, C. Y. Wu and Y. H. Chan, *J. Am. Chem. Soc.*, 2015, **137**, 10420-10429.
53. X. Q. Shen, L. Li, H. Wu, S. Q. Yao and Q. H. Xu, *Nanoscale* 2011, **3**, 5140-5146.
54. S. Y. Li, K. W. Chang, K. Sun, Y. Tang, N. Cui, Y. Wang, W. P. Qin, H. Xu and C. F. Wu, *ACS Appl. Mater. Interfaces*, 2016, **8**, 3624-3634.
55. J. Li and K. Pu, *Chem. Soc. Rev.*, 2019, **48**, 38-71.
56. C. Xing, Q. Xu, H. Tang, L. Liu, S. Wang, *J. Am. Chem. Soc.*, 2009, **131**, 13117-13124.
57. K. Chang, Y. Tang, X. Fang, S. Yin, H. Xu, and C. Wu, *Biomacromolecules*, 2016, **17**, 2128-2136.
58. X. Zhou, H. Liang, P. Jiang, K. Y. Zhang, S. Liu, T. Yang, Q. Zhao, L. Yang, W. Lv, Q. Yu and W. Huang, *Adv. Sci.*, 2016, **3**, 1500155.
59. P. Alivisatos, *Pure Appl. Chem.*, 2000, **72**, 3-9.
60. B. Dubertret, P. Skourides, D. J. Norris, V. Noireaux, A. H. Brivanlou and A. Libchaber, *Science*, 2002, **298**, 1759-1762.
61. V. Biju, *Chem. Soc. Rev.*, 2014, **43**, 744-764.
62. J. Zhou, Y. Yang and C. Y. Zhang, *Chem. Rev.*, 2015, **115**, 11669-11717.
63. M. Montalti, A. Cantelli and G. Battistelli, *Chem. Soc. Rev.*, 2015, **44**, 4853-4921.
64. A. C. S. Samia, X. Chen and C. Burda, *J. Am. Chem. Soc.*, 2003, **125**, 15736-15737.
65. G. Charron, T. Stuchinskaya, D. R. Edwards, D. A. Russell, and T. Nann, *J. Phys. Chem. C*, 2012, **116**, 9334-9342.
66. J. M. Tsay, M. Trzoss, L. Shi, X. Kong, M. Selke, M. E. Jung and S. Weiss, *J. Am. Chem. Soc.* 2007, **129**, 6865- 6871.
67. J. D. Bhawalkar, N. D. Kumar, C. F. Zhao and P. N. Prasad, *J. Clin. Laser Med. Surg.*, 1997, **15**, 201-204.
68. M. A. Oar, J. M. Serin, W. R. Dichtel, J. M. J. Fréchet, T. Y. Ohulchanskyy and P. N. Prasad, *Chem. Mater.*, 2005, **17**, 2267-2275.
69. J. R. Starkey, A. K. Rebane, M. A. Drobizhev, F. Meng, A. Gong, A. Elliott, K. McInerney and C. W. Spangler, *Clin. Cancer Res.* 2008, **14**, 6564-6573.
70. X. Shen, F. He, J. Wu, G. Q. Xu, S. Q. Yao and Q. H. Xu, *Langmuir*, 2011, **27**, 1739-1744.
71. K. Ogawa and Y. Kobuke, *Anti-Cancer Agents Med. Chem.* 2008, **8**, 269-279.
72. Y. Huang, F. Qiu, L. Y. Shen, D. Chen, Y. Su, C. Yang, B. Li, D. Y. Yan and X. Y. Zhu, *ACS Nano*, 2016, **10**, 10489-10499.
73. S. Kim, T. Y. Ohulchanskyy, H. E. Pudavar, R. K. Pandey and P. N. Prasad, *J. Am. Chem. Soc.*, 2007, **129**, 2669-2675.
74. S. H. Cheng, C. C. Hsieh, N. T. Chen, C. H. Chu, C. M. Huang, P. T. Chou, F. G. Tseng, C. S. Yang, C. Y. Mou and L. W. Lo, *Nano Today*, 2011, **6**, 552-563.
75. S. C. Pu, M. J. Yang, C. C. Hsu, C. W. Lai, C. C. Hsieh, S. H. Lin, Y. M. Cheng and P. T. Chou, *Small*, 2006, **2**, 1308-1313.
76. D. R. Larson, W. R. Zipfel, R. M. Williams, S. W. Clark, M. P. Bruchez, F. W. Wise and W. W. Webb, *Science*, 2003, **300**, 1434-1436.
77. A. C. S. Samia, X. Chen and C. Burda, *J. Am. Chem. Soc.*, 2003, **125**, 15736-15737. DOI: 10.1039/D0TB00262C
78. K. L. Chou, N. Won, J. Kwag, S. Kim and J. Y. Chen, *J. Mater. Chem. B*, 2013, **1**, 4584-4592.
79. C. Fowley, N. Nomikou, A. P. McHale, P. A. McCarron, B. McCaughan and J. F. Callan, *J. Mater. Chem.*, 2012, **22**, 6456-6462.
80. C. Fowley, N. Nomikou, A. P. McHale, B. McCaughan and J. F. Callan, *Chem. Commun.*, 2013, **49**, 8934-8936.
81. R. Vankayala, A. Sagadevan, P. Vijayaraghavan, C. L. Kuo and K. C. Hwang, *Angew. Chem., Int. Ed.*, 2011, **50**, 10640-10644.
82. M. Camerin, M. Magaraggia, M. Soncin, G. Jori, M. Moreno, I. Chambrier, M. J. Cook and D. A. Russell, *Eur. J. Cancer*, 2010, **46**, 1910-1918.
83. N. Gao, Y. Chen, L. Li, Z. Guan, T. Zhao, N. Zhou, P. Yuan, S. Q. Yao and Q. H. Xu, *J. Phys. Chem. C*, 2014, **118**, 13904-13911.
84. Y. Shen, A. J. Shuhendler, D. Ye, J. J. Xu and H. Y. Chen, *Chem. Soc. Rev.*, 2016, **45**, 6725-6741.
85. T. Zhao, K. Yu, L. Li, T. Zhang, Z. Guan, N. Gao, P. Yuan, S. Li, S. Q. Yao, Q. H. Xu and G. Q. Xu, *ACS Appl. Mater. Interfaces*, 2014, **6**, 2700-2708.
86. N. T. Chen, K. C. Tang, M. F. Chung, S. H. Cheng, C. M. Huang, C. H. Chu, P. T. Chou, J. S. Souris, C. T. Chen, C. Y. Mou and L. W. Lo, *Theranostics*, 2014, **4**, 798-807.
87. F. Wang and X. Liu, *Chem. Soc. Rev.*, 2009, **38**, 976-989.
88. J. C. Boyer, F. Vetrone, L. A. Cuccia and J. A. Capobianco, *J. Am. Chem. Soc.*, 2006, **128**, 7444-7445.
89. F. Auzel, *Chem. Rev.*, 2004, **104**, 139-174.
90. G. S. Yi and G. M. Chow, *Chem. Mater.*, 2006, **19**, 341-343.
91. C. X. Huang, H. J. Chen, F. Li, W. N. Wang, D. D. Li, X. Z. Yang, Z. H. Miao, Z. B. Zha, Y. Lu and H. S. Qian, *J. Mater. Chem. B*, 2017, **5**, 9487-9496.
92. Y. Liu, X. Meng and W. Bu, *Coord. Chem. Rev.*, 2019, **379**, 82-98.
93. J. Wu, S. Du and Y. Wang, *J. Mater. Chem. B*, 2019, **7**, 7306-7313.
94. H. Qiu, M. Tan, T. Y. Ohulchanskyy, J. F. Lovell and G. Chen, *Nanomaterials*, 2018, **8**, 344.
95. H. Xiang and Y. Chen, *Small*, 2019, **15**, 1805339.
96. S. Wu and H. J. Butt, *Adv. Mater.*, 2016, **28**, 1208-1226.
97. B. Zheng, H. Wang, H. Pan, C. Liang, W. Ji, L. Zhao, H. Chen, X. Gong, X. Wu and J. Chang, *ACS Nano*, 2017, **11**, 11898-11907.
98. N. M. Idris, M. K. Gnanasammandhan, J. Zhang, P. C. Ho, R. Mahendran and Y. Zhang, *Nat. Med.*, 2012, **18**, 1580-1585.
99. H. S. Qian, H. C. Guo, P. C. L. Ho, R. Mahendran and Y. Zhang, *Small*, 2009, **5**, 2285-2290.
100. C. Wang, H. Q. Tao, L. Cheng and Z. Liu, *Biomaterials*, 2011, **32**, 6145-6154.
101. S. Cui, D. Yin, Y. Chen, Y. Di, H. Chen, Y. Ma, S. Achilefu and Y. Gu, *ACS Nano*, 2012, **7**, 676-688.
102. G. L. Semenza, *Nat. Rev. Cancer*, 2003, **3**, 721-732.
103. W. R. Wilson, and M. P. Hay, *Nat. Rev. Cancer*, 2011, **11**, 393-410.
104. D. B. Guo, S. T. Xu, N. Wang, H. Jiang, Y. Huang, X. Jin, B. Xue, C. Zhang and X. Y. Zhu, *Biomaterials*, 2017, **144**, 188-198

105. S. T. Xu, X. Y. Zhu, C. Zhang, W. Huang, Y. F. Zhou and D. Y. Yan, *Nat. Commun.*, 2018, **9**, 2053.
106. C. Ash, M. Dubec, K. Donne and T. Bashford, *Lasers Med. Sci.*, 2017, **32**, 1909-1918.
107. M.-K. So, C. Xu, A. M. Loening, S. S. Gambhir and J. Rao, *Nat. Biotechnol.*, 2006, **24**, 339-343.
108. J. S. Lee, H.-A. Joung, M.-G. Kim and C. B. Park, *ACS Nano*, 2012, **6**, 2978-2983.
109. A. J. Shuhendler, K. Pu, L. Cui, J. P. Uetrecht and J. Rao, *Nat. Biotechnol.*, 2014, **32**, 373.
110. R. Liu, J. Tang, Y. Xu and Z. Dai, *ACS Nano*, 2019, **13**, 5124-5132.
111. Y. Yan, P. Shi, W. Song and S. Bi, *Theranostics*, 2019, **9**, 4047-4065.
112. N. Komatsu, K. Terai, A. Imanishi, Y. Kamioka, K. Sumiyama, T. Jin, Y. Okada, T. Nagai and M. Matsuda, *Sci. Rep.*, 2018, **8**, 8984.
113. C. Chen and B. Li, *J. Mater. Chem. B*, 2013, **1**, 2476-2481.
114. Z. Chen, P. Li, Z. Zhang, X. Zhai, J. Liang, Q. Chen, K. Li, G. Lin, T. Liu and Y. Wu, *Anal. Chem.*, 2019, **91**, 5777-5785.
115. P. Carriba, G. Navarro, F. Ciruela, S. Ferré, V. Casadó, L. Agnati, A. Cortés, J. Mallol, K. Fuxe, E. I. Canela, C. Lluís and R. Franco, *Nat. Methods*, 2008, **5**, 727-733.
116. C.-Y. Hsu, C.-W. Chen, H.-P. Yu, Y.-F. Lin and P.-S. Lai, *Biomaterials*, 2013, **34**, 1204-1212.
117. K. Yang, C. Wang, C. Liu, S. Ding, F. Tian and F. Li, *J. Mater. Sci.*, 2019, **54**, 3383-3391.
118. H. Yuan, H. Chong, B. Wang, C. Zhu, L. Liu, Q. Yang, F. Lv and S. Wang, *J. Am. Chem. Soc.*, 2012, **134**, 13184-13187.
119. Y. Zhang, L. Pang, C. Ma, Q. Tu, R. Zhang, E. Saeed, A. E. Mahmoud and J. Wang, *Anal. Chem.*, 2014, **86**, 3092-3099.
120. Y. H. Seo, A. Singh, H.-J. Cho, Y. Kim, J. Heo, C.-K. Lim, S. Y. Park, W.-D. Jang and S. Kim, *Biomaterials*, 2016, **84**, 111-118.
121. D. Mao, W. Wu, S. Ji, C. Chen, F. Hu, D. Kong, D. Ding and B. Liu, *Chem*, 2017, **3**, 991-1007.
122. M. K. Burdette, Y. P. Bandera, E. Zhang, A. Trofimov, A. Dickey, I. Foulger, J. W. Kolis, K. E. Cannon, A. F. Bartley, L. E. Dobrunz, M. S. Bolding, L. McMahon and S. H. Foulger, *Langmuir*, 2019, **35**, 171-182.
123. X. Ou, Y. Chen, L. Xie, J. Chen, J. Zan, X. Chen, Z. Hong, Y. He, J. Li and H. Yang, *Anal. Chem.*, 2019, **91**, 10149-10155.
124. Y. Liu, W. Chen, S. Wang and A. G. Joly, *Appl. Phys. Lett.*, 2008, **92**, 043901.
125. L. Ma, X. Zou, B. Bui, W. Chen, K. H. Song and T. Solberg, *Appl. Phys. Lett.*, 2014, **105**, 013702.
126. A.-L. Bulin, C. Truillet, R. Chouikrat, F. Lux, C. Frochot, D. Amans, G. Ledoux, O. Tillement, P. Perriat, M. Barberi-Heyob and C. Dujardin, *J. Phys. Chem. C*, 2013, **117**, 21583-21589.
127. W. Zhang, X. Zhang, Y. Shen, F. Shi, C. Song, T. Liu, P. Gao, B. Lan, M. Liu, S. Wang, L. Fan and H. Lu, *Biomaterials*, 2018, **184**, 31-40.
128. A. H. Elmenoufy, Y. a. Tang, J. Hu, H. Xu and X. Yang, *Chem. Commun.*, 2015, **51**, 12247-12250.
129. Y. Tang, J. Hu, A. H. Elmenoufy and X. Yang, *ACS Appl. Mater. Interfaces*, 2015, **7**, 12261-12269.
130. C. C. Hsu, S. L. Lin and C. A. Chang, *ACS Appl. Mater. Interfaces*, 2018, **10**, 7859-7870. DOI: 10.1039/D0TB00262C
131. S. L. Yefimova, T. N. Tkacheva, P. O. Maksimchuk, I. I. Bepalova, K. O. Hubenko, V. K. Klochkov, A. V. Sorokin and Y. V. Malyukin, *J. Lumin.*, 2017, **192**, 975-981.
132. H. Homayoni, K. Jiang, X. Zou, M. Hossu, L. H. Rashidi and W. Chen, *Photodiagn. Photodyn. Ther.*, 2015, **12**, 258-266.
133. C. Zhang, K. Zhao, W. Bu, D. Ni, Y. Liu, J. Feng and J. Shi, *Angew. Chem., Int. Ed.*, 2015, **54**, 1770-1774.
134. H. Chen, G. D. Wang, Y.-J. Chuang, Z. Zhen, X. Chen, P. Biddinger, Z. Hao, F. Liu, B. Shen, Z. Pan and J. Xie, *Nano Lett.*, 2015, **15**, 2249-2256.
135. G. D. Wang, H. T. Nguyen, H. Chen, P. B. Cox, L. Wang, K. Nagata, Z. Hao, A. Wang, Z. Li and J. Xie, *Theranostics*, 2016, **6**, 2295-2305.
136. H. Chen, X. Sun, G. D. Wang, K. Nagata, Z. Hao, A. Wang, Z. Li, J. Xie and B. Shen, *Mater. Horiz.*, 2017, **4**, 1092-1101.
137. R. S. Dothager, R. J. Goiffon, E. Jackson, S. Harpstrite and D. Piwnica-Worms, *PLOS ONE*, 2010, **5**, e13300.
138. Y. Wang, Y. Liu, H. Luehmann, X. Xia, D. Wan, C. Cutler and Y. Xia, *Nano Lett.*, 2013, **13**, 581-585.
139. C. A. Ferreira, D. Ni, Z. T. Rosenkrans and W. Cai, *Angew. Chem., Int. Ed.*, 2019, **58**, 13232-13252.
140. W. Guo, X. Sun, O. Jacobson, X. Yan, K. Min, A. Srivatsan, G. Niu, D. O. Kiesewetter, J. Chang and X. Chen, *ACS Nano*, 2015, **9**, 488-495.
141. Z. Hu, Y. Qu, K. Wang, X. Zhang, J. Zha, T. Song, C. Bao, H. Liu, Z. Wang, J. Wang, Z. Liu, H. Liu and J. Tian, *Nat. Commun.*, 2015, **6**, 7560.
142. C. Kaittanis, T. M. Shaffer, A. Bolaender, Z. Appelbaum, J. Appelbaum, G. Chiosis and J. Grimm, *Nano Lett.*, 2015, **15**, 8032-8043.
143. K. C. L. Black, A. Ibricevic, S. P. Gunsten, J. A. Flores, T. P. Gustafson, J. E. Raymond, S. Samarajeewa, R. Shrestha, S. E. Felder, T. Cai, Y. Shen, A.-K. Löbs, N. Zhegalova, D. H. Sultan, M. Berezin, K. L. Wooley, Y. Liu and S. L. Brody, *Biomaterials*, 2016, **98**, 53-63.
144. X. Sun, X. Huang, J. Guo, W. Zhu, Y. Ding, G. Niu, A. Wang, D. O. Kiesewetter, Z. L. Wang, S. Sun and X. Chen, *J. Am. Chem. Soc.*, 2014, **136**, 1706-1709.
145. S. B. Lee, G. Yoon, S.-W. Lee, S. Y. Jeong, B.-C. Ahn, D.-K. Lim, J. Lee and Y. H. Jeon, *Small*, 2016, **12**, 4894-4901.
146. H. Fu, C. Peng, Z. Liang, J. Dai, B. Liu and M. Cui, *Chem. Commun.*, 2016, **52**, 12745-12748.
147. D. Lewis, R. Mair, A. Wright, K. Allinson, S. Lyons, T. Booth, J. Jones, R. Bielik, D. Soloviev and K. Brindle, *Theranostics*, 2018, **8**, 3991-4002.
148. G. Pratz and D. S. Kapp, *Nat. Nanotechnol.*, 2018, **13**, 354-354.
149. Z. Ouyang, B. Liu, S. Yasmin-Karim, E. Sajo and W. Ngwa, *Phys. Med.*, 2016, **32**, 944-947.
150. D. Duan, H. Liu, Y. Xu, Y. Han, M. Xu, Z. Zhang and Z. Liu, *ACS Appl. Mater. Interfaces*, 2018, **10**, 5278-5286.
151. B. A. Hartl, H. Hirschberg, L. Marcu and S. R. Cherry, 2016, **35**, 185-192.

152. A. Kamkaew, L. Cheng, S. Goel, H. F. Valdovinos, T. E. Barnhart, Z. Liu and W. Cai, *ACS Appl. Mater. Interfaces*, 2016, **8**, 26630-26637.
153. D. Ni, C. A. Ferreira, T. E. Barnhart, V. Quach, B. Yu, D. Jiang, W. Wei, H. Liu, J. W. Engle, P. Hu and W. Cai, *J. Am. Chem. Soc.*, 2018, **140**, 14971-14979.
154. R. Liu, M. N. Yu, X. T. Yang, C. S. Umeshappa, C. Hu, W. Q. Yu, L. Qin, Y. Huang and H. L. Gao, *Adv. Funct. Mater.*, 2019, **29**, 1808462.
155. X. L. Zheng, D. Y. Pan, M. Chen, X. H. Dai, H. Cai, H. Zhang, Q. Y. Gong, Z. W. Gu and K. Luo, *Adv. Mater.*, 2019, **31**, 1901586.
156. C. Guo, Y. Xia, P. Niu, L. Jiang, J. Duan, Y. Yu, X. Zhou, Y. Li and Z. Sun, *Int. J. Nanomed.*, 2015, **10**, 1463-1477.
157. J. Yu, Y. Rong, C. Kuo, X. Zhou and D. T. Chiu, *Anal. Chem.*, 2017, **89**, 42-56.
158. W. K. B. Khalil, E. Girgis, A. N. Emam, M. B. Mohamed and K. V. Rao, *Chem. Res. Toxicol.*, 2011, **24**, 640-650.
159. K. M. Tsoi, Q. Dai, B. A. Alman and W. C. Chan, *Acc. Chem. Res.*, 2013, **46**, 662-671.
160. S. D. Li and L. Huang, *Biochim. Biophys. Acta*, 2009, **1788**, 2259-2266.
161. T. Zhu, L. Shi, C. Yu, Y. Dong, F. Qiu, L. Y. Shen, Q. Qian, G. Y. Zhou and X. Y. Zhu, *Theranostics*, 2019, **9**, 3293-3307.
162. D. L. Wang, T. Zhao, X. Y. Zhu, D. Y. Yan and W. X. Wang, *Chem. Soc. Rev.*, 2015, **44**, 4023-4071.
163. C. Saraiva, C. Praca, R. Ferreira, T. Santos, L. Ferreira and L. Bernardino, *J. Control. Release*, 2016, **235**, 34-47.
164. S. N. Thomas and A. Schudel, *Curr. Opin. Chem. Eng.*, 2015, **7**, 65-74.
165. C. Tapeinos, M. Battaglini and G. Ciofani, *J. Control. Release*, 2017, **264**, 306-332.
166. A. Stratan, A. Zorila, L. Rusen and G. Nemes, *Opt. Eng.*, 2014, **53**, 122513.
167. Z. Huang, H. Xu, A. D. Meyers, A. I. Musani, L. Wang, R. Tagg, A. B. Barqawi and Y. K. Chen, *Technol. Cancer Res. Treat.*, 2008, **7**, 309-320.
168. A. Master, M. Livingston and A. Sen Gupta, *J. Control. Release*, 2013, **168**, 88-102.
169. P. M. Petre, F. A. Baciewicz, S. Tigan and J. R. Spears, *J. Thorac. Cardio. Sur.*, 2003, **125**, 85-95.
170. S. I. Gundersen and A. F. Palmer, *Biotechnol. Progr.*, 2008, **24**, 1353-1364.
171. B. Karagoz, S. Suleymanoglu, G. Uzun, O. Bilgi, S. Aydin, A. Haholu, O. Turken, Y. Onem and E. G. Kandemir, *Basic Clin. Pharmacol. Toxicol.*, 2008, **102**, 287-292.
172. W. W. Zhu, Z. L. Dong, T. T. Fu, J. J. Liu, Q. Chen, Y. G. Li, R. Zhu, L. G. Xu and Z. Liu, *Adv. Funct. Mater.*, 2016, **26**, 5490-5498.
173. Q. Chen, J. Chen, C. Liang, L. Feng, Z. Dong, X. Song, G. Song and Z. Liu, *J. Control. Release*, 2017, **263**, 79-89.
174. D. Hu, Z. Chen, Z. Sheng, D. Gao, F. Yan, T. Ma, H. Zheng and M. Hong, *Nanoscale*, 2018, **10**, 17283-17292.
175. H. Chen, J. Tian, W. He and Z. Guo, *J. Am. Chem. Soc.*, 2015, **137**, 1539-1547.
176. L. Y. Shen, Y. Huang, D. Chen, F. Qiu, C. Ma, X. Jin, X. Y. Zhu, G. Y. Zhou and Z. Y. Zhang, *Theranostics*, 2017, **7**, 4537-4550.