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Engineering Persistent Luminescence Nanoparticles for Biological Applications: From Biosensing/Bioimaging to Theranostics

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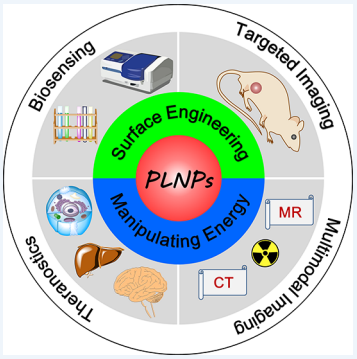
CONSPECTUS: Persistent luminescence nanoparticles (PLNPs) are unique optical materials emitting long-lasting luminescence after ceasing excitation. Such a unique optical feature allows luminescence detection without constant external illumination to avoid the interferences of autofluorescence and scattering light from biological fluids and tissues. Besides, near-infrared (NIR) PLNPs have advantages of deep penetration and the reactivation of the persistent luminescence (PL) by red or NIR light. These features make the application of NIR-emitting PLNPs in long-term bioimaging no longer limited by the lifetime of PL. To take full advantage of PLNPs for biological applications, the versatile strategies for bridging PLNPs and biological system become increasingly significant for the design of PLNPs-based nanoprobes.



In this Account, we summarize our systematic achievements in the biological applications of PLNPs from biosensing/bioimaging to theranostics with emphasizing the engineering strategies for fabricating specific PLNPs-based nanoprobes. We take surface engineering and manipulating energy transfer as the major principles to design various PLNPs-based nanoprobes based on the nature of interactions between nanoprobes and targets. We have developed target-induced formation or interruption of fluorescence resonance energy transfer systems for autofluorescence-free biosensing and imaging of cancer biomarkers. We have decorated single or dual targeting ligands on PLNPs for tumor-targeted imaging, and integrated other modal imaging agents into PLNPs for multimodal imaging. We have also employed specific functionalization for various biomedical applications including chemotherapy, photodynamic therapy, photothermal therapy, stem cells tracking and PL imaging-guided gene therapy. Besides, we have modified PLNPs with multiple functional units to achieve challenging metastatic tumor theranostics. The proposed design principle and comprehensive strategies show great potential in guiding the design of PLNPs nanoprobes and promoting further development of PLNPs in the fields of biological science and medicine.

We conclude this Account by outlining the future directions to further promote the practical application of PLNPs. The novel protocols for the synthesis of small-size, monodisperse, and water-soluble PLNPs with high NIR PL intensity and superlong afterglow are the vibrant directions for the biomedical applications of PLNPs. In-depth theories and evidence on luminescence mechanism of PLNPs are highly desired for further improvement of their luminescence performance. Furthermore, other irradiations without tissue penetrating depth limit, such as X-ray, are encouraged for use in energy storage and re-excitation of PLNPs, enabling imaging in deep tissue in vivo and integrating other X-ray sensitized theranostic techniques such as computed tomography imaging and radiotherapy. Last but not least, PLNPs-based nanoprobes and the brand new hybrids of PLNPs with other nanomaterials show a bright prospect for accurate diagnosis and efficient treatment of diseases besides tumors.

1. INTRODUCTION

Persistent luminescence (PL), also called long afterglow, is an optical phenomenon where a material stores various radiations quickly and slowly emits luminescence for minutes, hours, or even days after ceasing excitation.[1−4] The early bulk persistent phosphors were produced by solid reaction,[5] and mainly applied in civil uses, such as security signs and emergency route signs.[2] The unparalleled long afterglow of persistent phosphors enables avoiding interference of autofluorescence and scattering light from biological fluids and tissues, making them attractive for excitation-free biosensing,[6−9] bioimaging,[10−26] and theranostics.[27−36] However, bulky persistent phosphors prepared via traditional high-temperature solid reaction are unsuitable for biomedical applications due to poor biocompatibility and large irregular sizes. Therefore, new synthesis approaches for preparing PL nanoparticles (PLNPs), including sol−gel process, solvothermal, and template methods, have been proposed to control their morphology in nanoscale and manage luminescent features.[1,2,4]

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The fabrication of PLNPs-based nanoprobes for biological applications is guided by following criteria: (i) intense and stable initial luminescence, long afterglow, and repeated renewability (afterglow can be activated again by red light); (ii) appropriate size (<200 nm) for in vivo application; (iii) target-triggered signal change for biosensing; (iv) passive or active targeting ability for bioimaging; (v) specific multifunctionality for theranostics. The significant progress in PL mechanism, size and morphological control, operational long afterglow, and light renewability of PLNPs has highly promoted their biological applications.[1−4] The versatile strategies to bridging PLNPs and biological application are urgently needed for wide applications of PLNPs.

In this Account, we present an overview of our efforts on the engineering of PLNPs for biosensing,[6,8] bioimaging,[13,18,20,22−24] and theranostics,[30−35] emphasizing versatile strategies for engineering PLNPs. As the interactions between nanoprobes and targets are related to surface functionalization and energy transfer, the design of PLNPs-based nanoprobes is generalized to surface engineering and manipulating energy transfer as the major design principles. We systematically introduce fluorescence resonance energy transfer (FRET) systems, single or dual targeting ligands, multimodal imaging techniques, liposome, cell-permeable peptide, gene delivery carrier, and diverse therapeutic strategies to PLNPs for fabricating diverse nanoprobes for autofluorescence-free biosensing, targeted bioimaging, cell tracking, and theranostics (Figure 1).

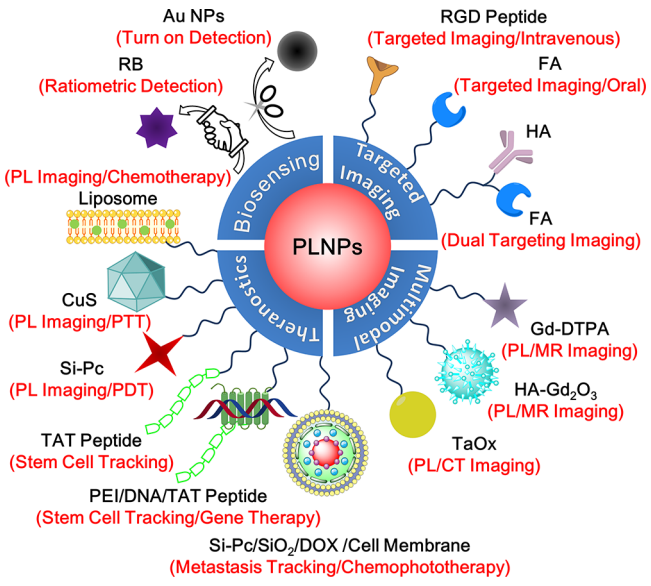

Figure 1. Engineering strategies of PLNPs for biological applications in our group.

2. ENGINEERING PLNPs FOR BIOSENSING

Fluorescent detection with the merits of high sensitivity and simple operation is increasingly used in fundamental research and clinic. However, traditional fluorescent probes suffer from poor signal−noise ratio (SNR) due to interferences from autofluorescence and scattering light in biological matrixes under constant external excitation. PLNPs with long afterglow enable eliminating interferences from autofluorescence and scattering light by pre-excitation before detection to solve the problem of high background signal in conventional fluorescent biosensing.[6,8]

Considering the chemical inertness and excellent photostability of PLNPs, designing FRET systems is the most suitable strategy for fabricating PLNPs-based nanoprobes. In 2011, we reported the first PLNPs nanoprobe for turn-on PL detection of α-fetoprotein (AFP) in biological fluids based on target-induced interruption of FRET system without in situ excitation (Figure 2A).[6] The FRET system was fabricated using polyethylenimine capped $Ca_{1.86}Mg_{0.14}ZnSi_2O_7$:Eu,Dy PLNPs (PLNPs-PEI) as donor and AFP-antibody-decorated gold nanoparticles (Ab-Au NPs) as acceptor via electrostatic interaction. The PL of the PLNPs lasted 6 h, and was stable in the duration of 30−180 min after stopping excitation, offering a reliable time window for quantitative detection without in situ excitation (Figure 2B). AFP addition destroyed the FRET system, resulting in PL turn-on due to the competition of AFP with the PLNPs for Ab-Au NPs. The nanoprobe enables highly sensitive, selective and autofluorescence-free detection of AFP with the detection limit of 0.41 μg L^{-1}. We explored the nanoprobe to monitor the excreted AFP during cell growth, and demonstrated its robust biosensing capability in serum and cellular level. The developed nanoprobe represents the pioneering biosensing application of PLNPs and breaks a new path for designing highly sensitive nanoprobes.

The integration of PLNPs-based FRET system and ratiometric fluorescent detection shows great potential in synergistic improvement of accuracy, selectivity, and sensitivity of biosensors. We used the same PLNPs to build a FRET probe for ratiometric luminescence detection of prostate-specific antigen (PSA) (Figure 3).[8] PSA antibody PS6 bioconjugated PLNPs (PLNPs-PS6) and PSA antibody 8A6-modified rhodamine B (RhB-8A6) were employed as donor and acceptor, respectively. PSA triggered the formation of FRET system via sandwich assembly of PLNPs-PSA-RhB, resulting in ratiometric luminescence change. The FRET strategy, PL nature and ratiometric luminescence approach synergistically ensure the outstanding sensitivity and selectivity, enabling autofluorscence-free detection of PSA in biological fluids with the detection limit of 0.09 μg L^{-1}. The successful building of FRET-based ratiometric luminescent nanoprobes paves the way to designing PLNPs-based biosensors via surface engineering and manipulating energy transfer.

3. ENGINEERING PLNPs FOR BIOIMAGING

Conventional fluorescence imaging under constant in situ excitation often suffers from interference from tissue autofluorescence and light scattering, and phototoxicity. Near infrared (NIR)-emitting PLNPs offer great opportunities to overcome the above obstacles for *in vivo* bioimaging due to the absence of constant in situ excitation, high SNR, red light renewability, and superior in vivo imaging depth, showing a bright prospect in bioimaging in vivo.

3.1. Tumor-Targeted Imaging via Intravenous Administration

Recently, we prepared NIR-emitting Cr(III) and Pr(III) doped zinc gallogermanate PLNPs (ZGGO:Cr,Pr) via a citrate sol−gel method along with a subsequent reducing atmosphere-free heating at 1000 °C. c(RGDyK) peptide with tumor targeting ability was grafted to the PLNPs for tumor-targeted imaging (Figure 4).[13] Codoping Pr(III)/Cr(III) and creating Zn deficiency improved PL intensity and afterglow. The doped

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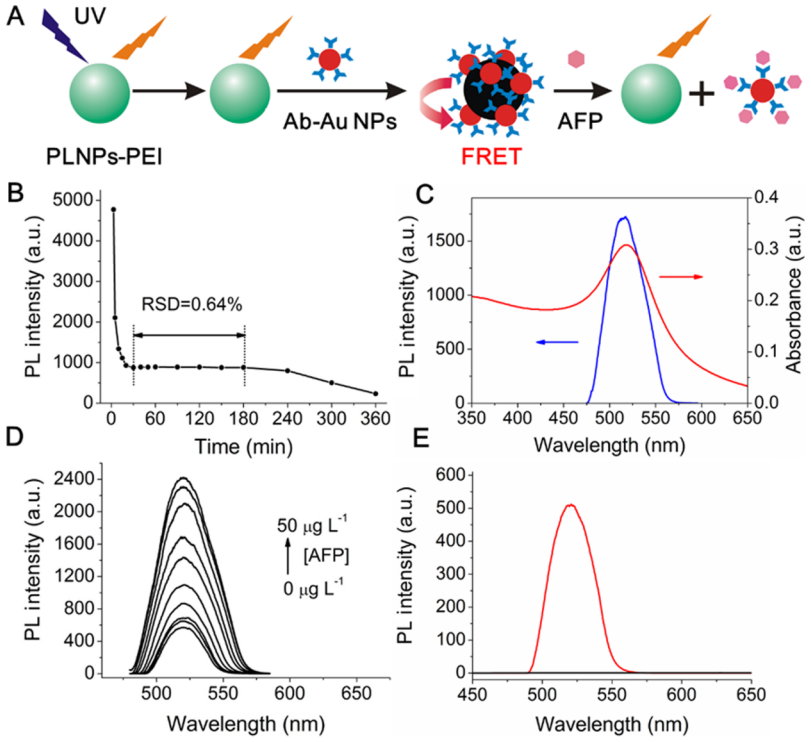

Figure 2. (A) Illustration for FRET inhibition assay of AFP with PLNPs-PEI as donor and Ab-Au NPs as acceptor. (B) Time-dependent PL intensity of PLNPs-PEI after 10 min exposure to a UV lamp. (C) Photoluminescent spectra of PLNPs-PEI (blue) and absorption spectra of Ab-Au NPs (red). (D) AFP dependent PL turn-on of PLNPs-PEI/Ab-Au NPs. (E) PL spectra of a serum sample with detectable AFP in the absence (black) or presence (red) of PLNPs-PEI/Ab-AuNPs after stopping excitation, showing no PL background without in situ excitation.[6] Reprinted with permission from ref 6. Copyright 2011 American Chemical Society.

Pr(III) likely increased trap density and depth to prolong the long afterglow duration. The PLNPs were coated with 3-aminopropyltriethoxysilane (APTES) for subsequent PEGylation. Further conjugation with PEG and targeting peptide endows PLNPs with favorable aqueous dispersity and tumor-targeted ability, respectively. Good aqueous dispersity ensures homogeneous distribution of PLNPs in aqueous solution for good reproducibility and biocompatibility. The functionalized NIR-emitting PLNPs with bright PL, long afterglow (15 days), and low toxicity allow successful tumor-targeted imaging in vivo via intravenous injection.

3.2. Tumor-Targeted Imaging via Oral Administration

Small size of PLNPs benefits their blood circulation and accumulation in tumors while high PL intensity guarantees sensitive imaging at a low dosage of agents. So, various methods have been employed to tune the morphology and size of PLNPs, and improve the afterglow intensity and duration.[1−4] However, it is challenging to synthesize monodisperse nanosized PL materials with bright luminescence and long afterglow. Recently, we developed a surfactant-assisted hydrothermal approach followed by a short-time sintering and post hydrothermal treatment to prepare ultrabright triple-doped zinc gallogermanate PLNPs (ZGGO:Cr,Yb,Er) with good monodispersity and superlong NIR PL (Figure 5).[18] Codoping Yb(III) and Er(III) (instead of Pr(III)[13]) adjusts the trap density and depth, enabling brighter initial NIR luminescence and longer afterglow (20 days). Folic acid (FA) was grafted on the surface of PLNPs to offer tumor-targeting ability. We also demonstrated that oral administration of FA-functionalized ZGGO:Cr,Yb,Er gave better targeting performance than intravenous injection.

3.3. Dual-Targeting Imaging

Introducing dual-targeting strategy to PLNPs nanoprobes is attractive for further improving specificity in bioimaging due to dual specific ligand−receptor interactions on the surface of tumor cells. Recently, we integrated the outstanding advantages of NIR-emitting PLNPs and dual-targeting strategy to fabricate PLNPs-based dual-targeting nanoprobe for tumor imaging (Figure 6).[20] Two targeting ligands, hyaluronic acid (HA) and FA, were conjugated on the surface of Cr(III) and B(III) codoped zinc gallate PLNPs (ZGO:Cr,B) to give dual targeting probe PLNPs-HA/FA. This work provides a new strategy for the fabrication of PLNPs-based nanoprobes with more specific tumor-targeting ability.

3.4. Multimodal Imaging

Multimodal imaging as a promising approach that combines the advantages of different imaging modalities enables the diagnosis with high sensitivity and high resolution simultaneously.[37] Therefore, it is appealing to integrate the unique merits of PLNPs and the superiorities of multimodal imaging for designing high-performance PLNPs imaging nanoprobes.[22,26] As magnetic resonance (MR) imaging simultaneously provides physiological and anatomical information with high spatial resolution,[37] we decorated a clinic MR contrast agent, Gd-DTPA, on the PLNPs to create PLNPs-based dual-modal imaging nanoprobe (Figure 7A).[22] The prepared PLNPs-Gd-DTPA nanoprobe not only kept excellent long afterglow, but also enhanced the relaxivity of Gd-DTPA. The PLNPs-based dual-modal imaging nanoprobe allows in vivo PL and MR imaging with high sensitivity and preferable spatial resolution. The successful fabrication of PLNPs-based dual modal imaging

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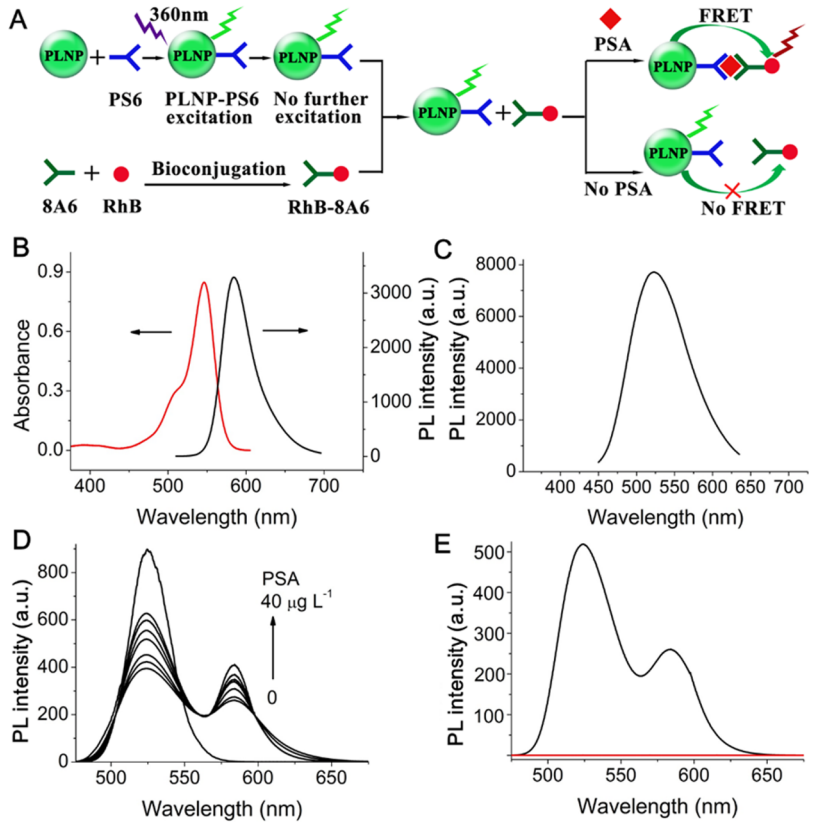

Figure 3. (A) Illustration of PLNP-based FRET immunoassay for ratiometric luminescent detection of PSA. (B) UV−vis spectra (red) and PL spectra (black) of RhB-8A6. (C) PL spectra of PLNP−PS6. (D) PSA dependent luminescence of PLNP-PS6/RhB-8A6. (E) PL spectra of 10 μg L^{-1} PSA spiked serum in the absence (red) or presence (black) of PLNP−PS6 and RhB-8A6 without in situ excitation.[8] Reprinted with permission from ref 8. Copyright 2015 Royal Society of Chemistry.

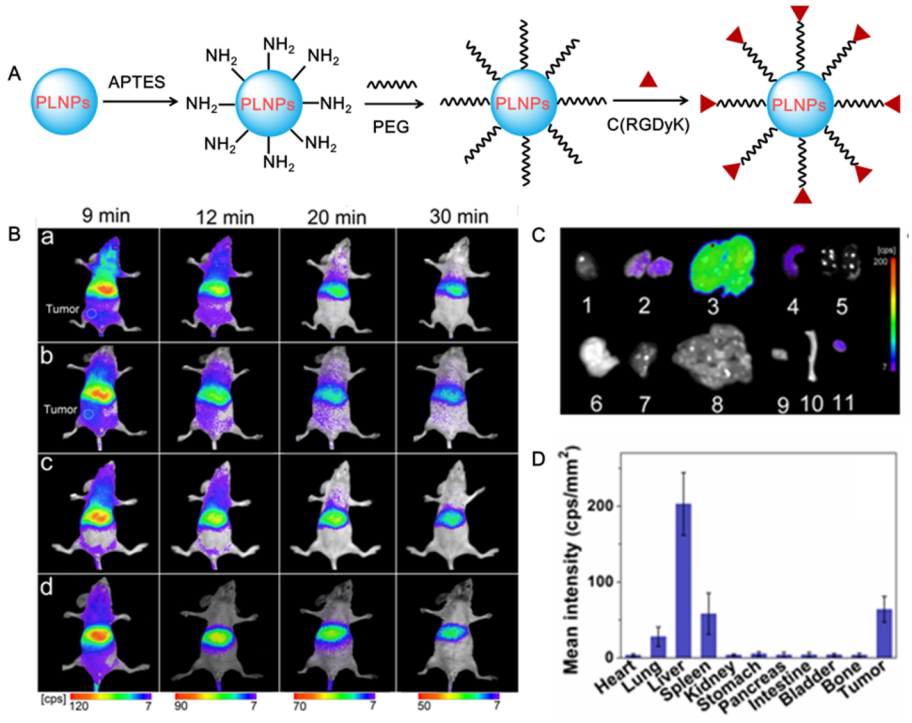

Figure 4. (A) Preparation and surface modification of PLNPs for tumor-targeted imaging. (B) In vivo PL images of tumor-bearing mice (a, b) and normal mice (c, d) after intravenous injection of PLNPs-PEG (a, c) and PLNPs-RGD (b, d). cps, counts per second, unit for luminescence intensity. (C) Typical ex vivo luminescence images of isolated organs and tumor from a tumor-bearing mouse. (D) Semiquantification of PLNPs-RGD in isolated organs and tumor of the mice.[13] Reprinted with permission from ref 13. Copyright 2013 American Chemical Society.

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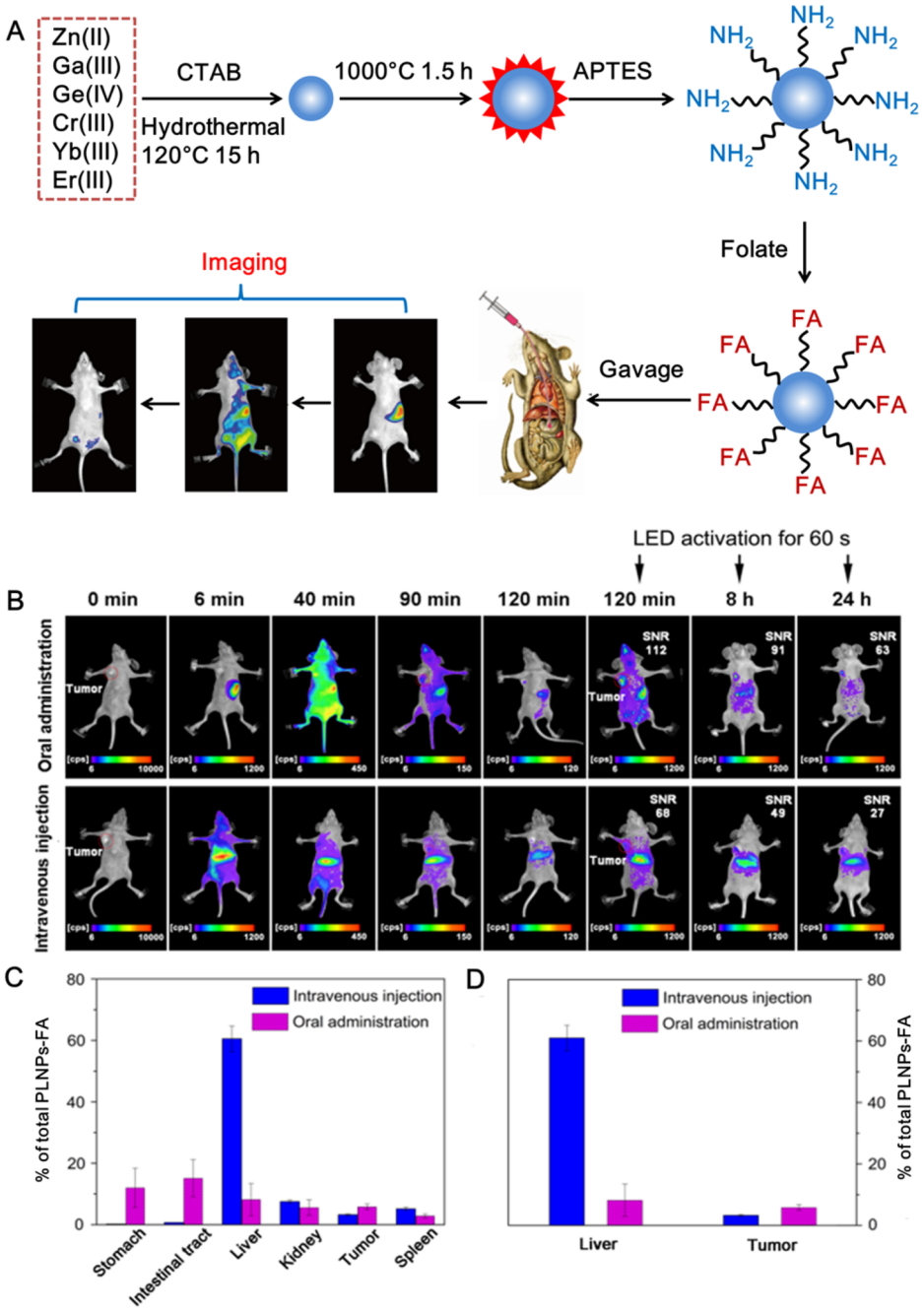

Figure 5. (A) Synthesis and surface functionalization of PLNPs-FA for oral administrated in vivo bioimaging. (B) Change of in vivo PL images of tumor bearing mouse with time after oral administration and intravenous injection of PLNPs-FA (5 min irradiation with a 254 nm UV lamp before injection). The reactivation of postinjected mice was performed with a red LED at 2 h, 8 and 24 h after UV lamp irradiation. cps as shown in Figure 4. (C, D) Quantification of PLNPs-FA in isolated organs of mice.[18] Reprinted with permission from ref 18. Copyright 2016 Royal Society of Chemistry.

nanoprobe provides a promising addition to bimodal luminescent nanoparticles.

To simplify synthesis procedures and improve the MR imaging ability, we developed a method for the preparation of the dual-modal nanoprobe for NIR PL and MR imaging (Figure 7B).[23] Nanosized and monodisperse NIR-emitting ZGO:Cr were synthesized by a hydrothermal method (i.e., from high-temperature aqueous solution at high vapor pressure). HA-Gd_2O_3 nanoparticles were prepared with HA as template via a biomolecule-guided growth to endow the nanoparticles with good biocompatibility and tumor-targeting ability. The dual-modal imaging nanoprobe was fabricated by conjugating PLNPs and HA-Gd_2O_3. The prepared nanoprobe exhibited renewable NIR PL and high T_1 relaxivity. The unique advantages make the nanoprobe successful in the application of tumor-targeting NIR PL and MR dual modal imaging, providing a convenient way for building targeted PL/MR dual modal nanoprobes.

Computed tomography (CT) imaging is one of the most widely used diagnostic imaging techniques besides MR imaging in clinic, which possesses outstanding merits of high spatial resolution, no tissue penetrating depth limit and ease of

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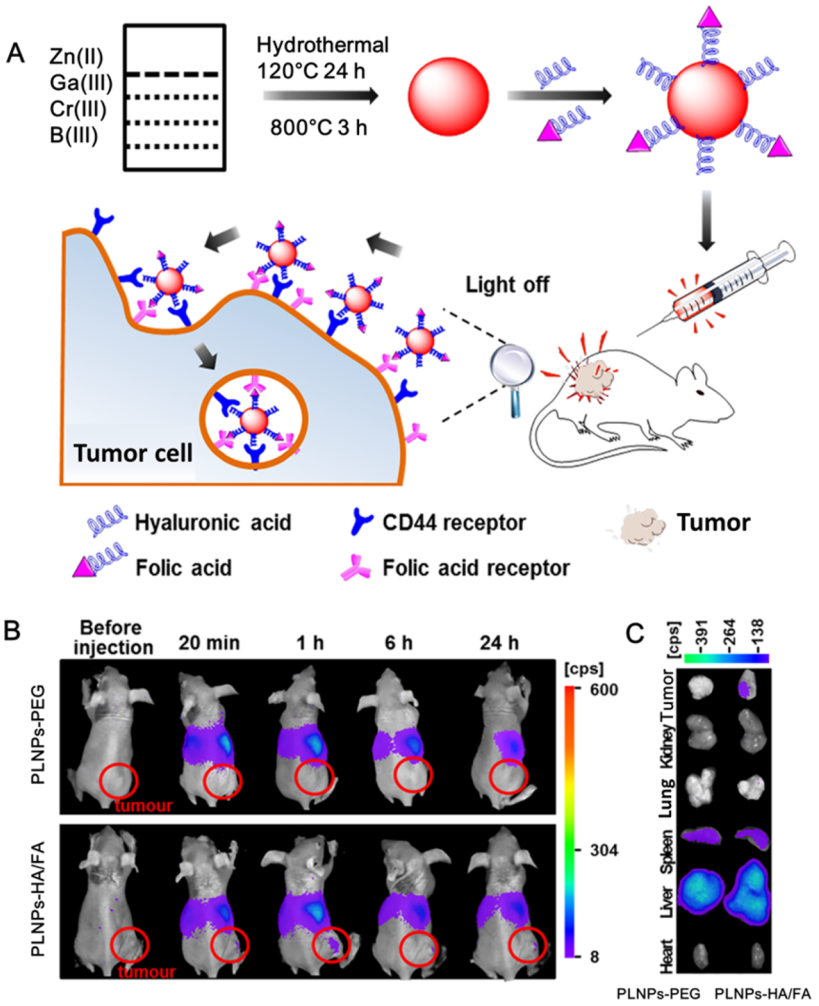

Figure 6. (A) Preparation of dual-targeting PLNPs-HA/FA for bioimaging. (B) PL imaging of tumor-bearing mice after intravenous injection of PLNPs-PEG and PLNPs-HA/FA. cps as shown in Figure 4. (C) Representative ex vivo NIR luminescence images of isolated organs from a tumor-bearing mouse at 24 h postinjection of PLNPs-PEG (left) and PLNPs-HA/FA (right).[20] Reprinted with permission from ref 20. Copyright 2016 Royal Society of Chemistry.

forming 3D visual image reconstruction of disease sites.[37] The combination of CT and PL imaging shows great potential in acquiring comprehensive and reliable diagnosis information. Low-cost and bioinert TaO_x (tantalum oxide) with strong X-ray absorption capacity and excellent biocompatibility is an excellent candidate as CT contrast agent. Recently, we engineered PLNPs/TaO_x core−shell nanostructure for PL and CT dual-modal bioimaging in vivo (Figure 8).[24] In situ growth of TaO_x on PLNPs provides excellent CT imaging capability, and further PEGylation and modification with cyclic-NGR peptide endows long blood circulation time and tumor targeting ability. The work further shows the universality of PLNPs-based multimodal imaging nanoprobes.

4. ENGINEERING PLNPs FOR THERANOSTICS

Theranostics, integrating the modalities of imaging and therapy, provides great opportunities to diagnose diseases early and to real-time monitor therapeutic effect.[38] Various nanoprobes with diverse imaging and therapy abilities have been explored for tumor theranostics so far.[38] Even so, autofluorescence-free optical imaging is urgently needed to design the probes for improving theranostic efficacy. Thus, novel strategies for integrating PLNPs and therapeutic techniques are highly desired for the fabrication of PLNPs-based theranostic nanoprobes.

4.1. PLNPs-Based Chemotherapy

Chemotherapy is widely used in clinic for tumor treatment, but suffers from poor targeting ability, serious side effects and troublesome administration of many hydrophobic drugs.[39] Nanotechnology-based imaging-guided drug delivery enables tumor-targeted delivery of hydrophilic and hydrophobic drugs, monitoring the pharmacokinetics of drugs and real-time supervising the therapeutic response of tumors, showing bright future for overcoming the above-mentioned limitations.[39] PLNPs with unique merits are emerging as a type of star materials for sensitive luminescent imaging-guided drug delivery. New PLNPs-based nanocarriers with facile synthesis procedures, good biocompatibility and high drug loading efficiency are urgently needed for sensitive imaging guided drug delivery.

Liposome with the merits of excellent biocompatibility, biodegradability and low toxicity is a classic and extensively used nanocarrier.[39] The combination of PLNPs and liposome offers great opportunities for the fabrication of smart PLNPs-based drug delivery systems. Recently, we reported liposome-coated PLNPs (PLNPs-Liposome) as a drug carrier for PL

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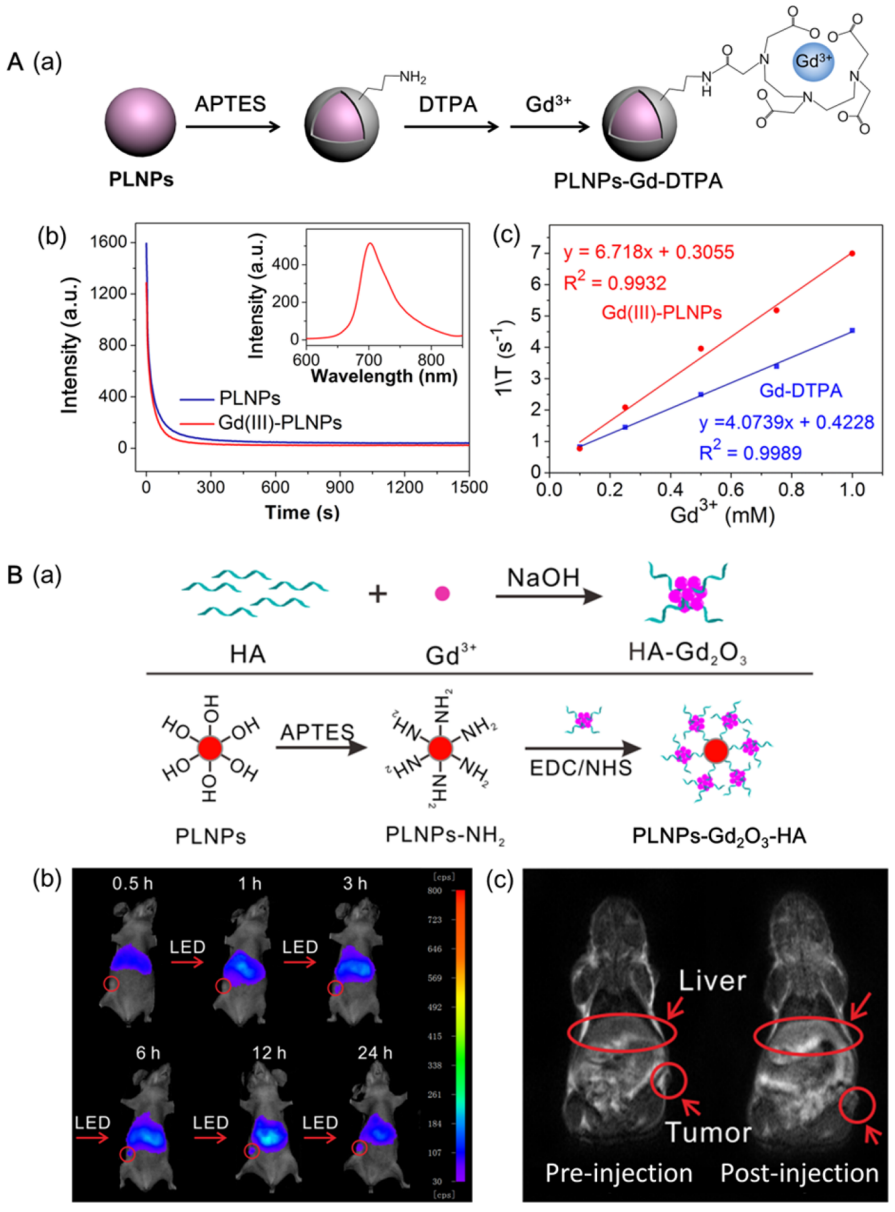

Figure 7. (A) Grafting Gd-DTPA on PLNPs for dual-modal bioimaging: (a) synthesis of the dual-modal nanoprobe; (b) afterglow decay curves for PLNPs and PLNPs-Gd-DTPA aqueous dispersion after 5 min irradiation with 254 nm UV lamp. Inset: PL spectra of PLNPs-Gd-DTPA aqueous dispersion at 20 s after stopping excitation; (c) T_1-relaxation rate as a function of Gd concentration.[22] Reprinted with permission from ref 22. Copyright 2014 American Chemical Society. (B) PLNPs-Gd_2O_3-HA for PL and MR imaging: (a) preparation protocol; (b) in vivo PL images of HepG 2 tumor balb/c nude mice after intravenous injection of PLNPs-Gd_2O_3-HA. cps as shown in Figure 4; (c) in vivo T_1-weighted MR images of the mouse treated with PLNPs-Gd_2O_3-HA before and 6 h after intravenous injection.[23] Reprinted with permission from ref 23. Copyright 2017 Royal Society of Chemistry.

imaging guided chemotherapy (Figure 9A).[33] ZGGO:Cr PLNPs were coated with liposome via a simple thin layer evaporation method. The prepared PLNPs-Liposome exhibited PL, red LED light-renewability, excellent biocompatibility, and high drug encapsulation efficiency. The PLNPs-Liposome enabled in vivo PL imaging of cells, while paclitaxel-loaded liposome-PLNPs (PLNPs-PTX) gave a remarkable therapeutic effect in vitro. The PLNPs-PTX was applied in passively targeted imaging tumor, monitoring drug delivery, and dramatically inhibited tumor growth without autofluorescence interference. The integration of liposome and PLNPs provides a promising way for the fabrication of sensitive PL imaging guided-drug carriers with high drug loading and theranostic efficacy.

4.2. PLNPs-Based Photodynamic Therapy (PDT)

The long afterglow nature of PLNPs also offers an internal light source for PDT.[29,30] PDT employs photosensitizer to generate singlet oxygen ($^1O_2$) or reactive oxygen species under light exposure to eradicate tumors, and has been approved in clinic for the treatment of various tumors.[40] Nevertheless, external light source possesses intrinsically limited tissue penetrating depth, which makes PDT fail in the application of tumor therapy in deep tissues. In addition, continuous laser illumination suffers from the risk of overheating induced tissue damage, hindering extensive PDT application. PLNPs with long afterglow and light renewability show great potential as an internal light source for PDT without the need for constant

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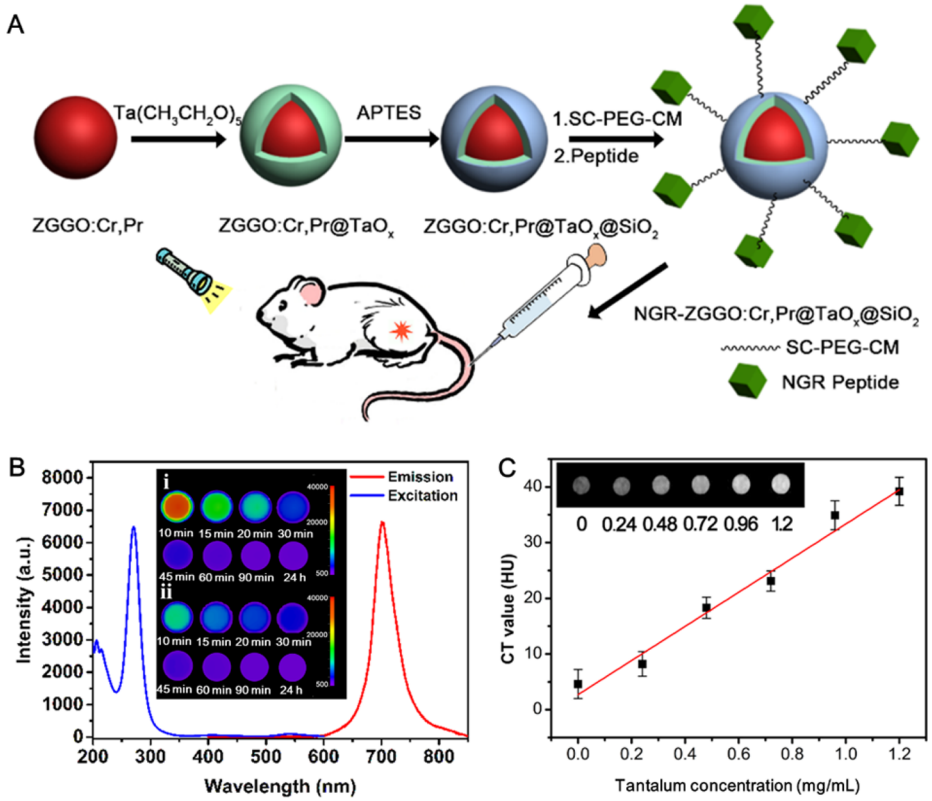

Figure 8. (A) Synthesis of NGR-ZGGO:Cr,Pr@TaO_x@SiO_2 for PL and CT imaging. (B) Excitation (blue) and emission (red) spectra of aqueous ZGGO:Cr,Pr@TaO_x@SiO_2 dispersion. Inset: NIR afterglow decay images at different times after 10 min irradiation with 254 nm UV lamp (i), and reactivation with LED excitation after 24 h (ii), cps as shown in Figure 4. (C) CT images (inset) and CT values of PEG-ZGGO:Cr,Pr@TaO_x@SiO_2 with different mass concentrations.[24] Reprinted with permission from ref 24. Copyright 2015 Royal Society of Chemistry.

external irradiation. Recently, we conjugated photosensitizer to PLNPs for PL-sensitized PDT (Figure 9B).[30] Silicon phthalocyanine (Si-Pc) as photosensitizer was grafted on PLNPs to generate PLNPs-Si-Pc nanoprobe. The overlap of long afterglow spectrum of PLNPs and absorption spectrum of Si-Pc enables continuous generation of $^1O_2$ induced by the renewable PL. PLNPs-Si-Pc nanoprobe owns favorable cellular biocompatibility in dark. The cellular viability was only 10% after the exposure of 10 min activation with 808 nm NIR light of the pre-excited Si-Pc-PLNPs due to favorable PDT. Moreover, pre-excited Si-Pc-PLNPs in combination with 10 min re-excitation of 808 nm light remarkably suppressed tumor growth. No obvious histophysiological damage in organs of mice was found after the administration of pre-excited PLNPs-Si-Pc nanoprobe and re-excitation of laser irradiation. The design of PLNPs-sensitized PDT system opens a new way to next generation of PDT with no need for constant external light irradiation.

4.3. PLNPs-Based Photothermal Therapy (PTT)

Activatable optical imaging plays a significant role in highly sensitive tumor-targeted imaging. Introducing PLNPs to activatable nanoprobes enables further improving their sensitivity. For tumor therapy, PTT, which employs PTT agents to generate heat from light for tumor ablation, owns the unique advantages of high selectivity and minimal invasiveness.[40] Moreover, PTT agents with strong NIR absorbance are excellent candidates as luminescent quenchers in activatable luminescent nanoprobes. Therefore, it is significant to fabricate PLNPs-based activated imaging-guided PTT nanoprobes for theranostics. Recently, we developed an activatable multifunctional PLNPs-CuS nanoprobe for in vivo turn-on PL imaging-guided PTT (Figure 10).[31] We employed CuS nanoparticles as biocompatible PTT agent with impressing luminescent quenching ability due to their strong NIR absorption, and used matrix metalloproteinases (MMPs)-specific peptide substrate to link PLNPs and CuS to generate a MMP-activatable theranostics system. The PLNP-CuS nanoagent with good biocompatibility was activated by MMP-2 for sensitive turn-on PL imaging of MMP-2 excreted by SCC-7 tumor cells. In addition, we also demonstrated its robust PTT of tumors in cellular level and in vivo. The successful combination of PLNPs-based activatable imaging and PTT provides a new thought for the design of highly sensitive and efficient theranostic nanoprobes for the diagnosis and treatment of tumors in vivo.

4.4. PLNPs for Stem Cell Tracking and Therapy

Stem cells not only serve as drug and gene carriers for tumor-targeted treatment due to their natural high tumor affinity, but also play a vital role in regeneration medicine because of good self-renewability and low immunogenicity.[41,42] As stem-cells-based therapy usually takes a long time, noninvasive and sensitive nanoprobes are important for tracking their long-term fate and migration.[41] To overcome the drawbacks of genetic transfection of reporter genes, such as tedious manufacture and clinical safety concerns, various imaging agents have been employed to track stem cells in vivo via direct labeling of stem cells in the past decades.[41] Nevertheless, traditional down-conversion fluorescent nanoprobes suffer from the interferences of autofluorescence and scattering light of tissues, while upconversion nanoparticles with low quantum yield exhibit

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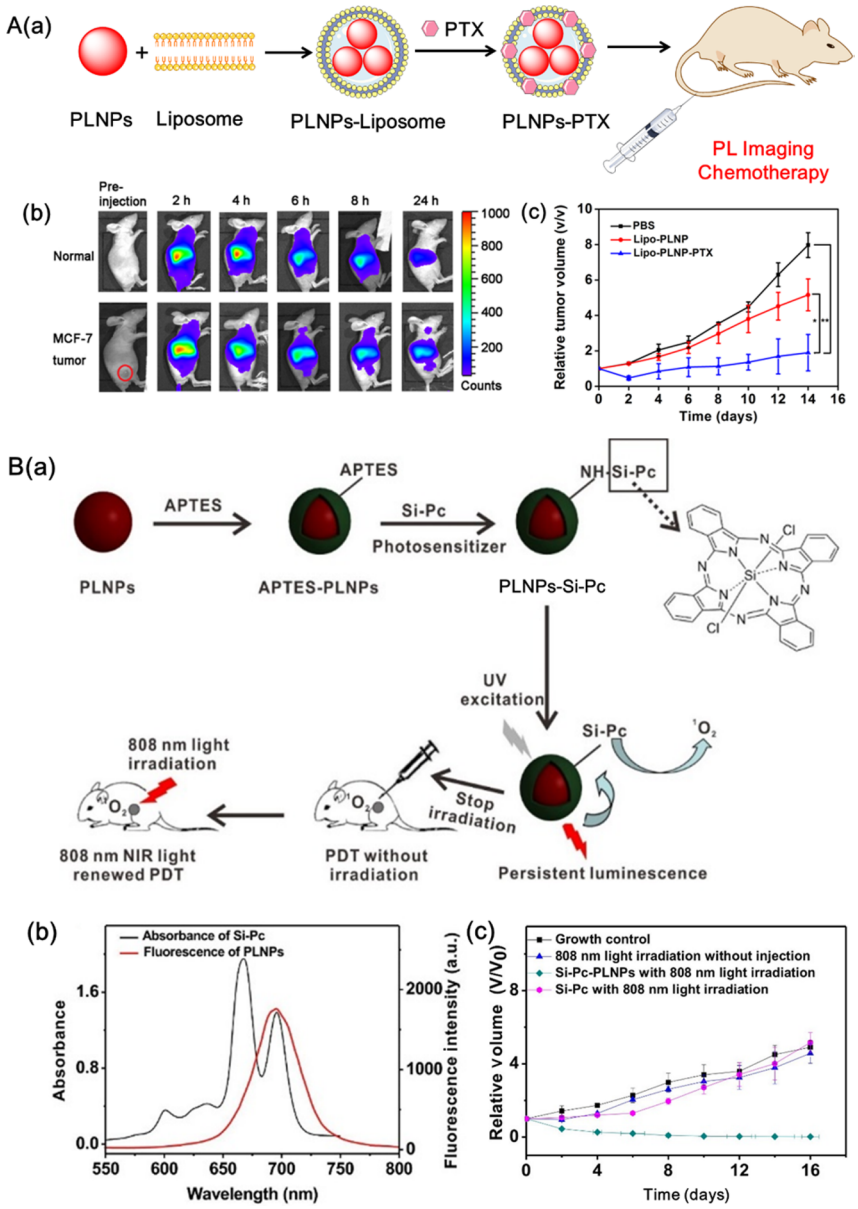

Figure 9. (A) Preparation of drug-loaded liposome-coated PLNPs (PLNPs-PTX) for imaging-guided chemotherapy: (a) synthesis protocol; (b) in vivo PL images of normal mice and tumor-bearing mice after intravenous injection of PLNPs-PTX; (c) tumor growth curves of three mice groups after various treatments.[33] Reprinted with permission from ref 33. Copyright 2017 American Chemical Society. (B) 808 nm NIR light renewable PL sensitized PDT: (a) conjugation of Si-Pc and PLNPs for PDT; (b) absorbance spectra of Si-Pc and fluorescence spectra of the PLNPs; (c) PL sensitized in vivo PDT.[30] Reprinted with permission from ref 30. Copyright 2016 Royal Society of Chemistry.

the risk of tissue damage caused by constant high-energy laser excitation. Therefore, novel sensitive and safe labeling techniques are highly desired for stem cell tracking and therapy.

PLNPs with high SNR also show great potential in reliable and sensitive stem cells tracking. Very recently, we engineered PLNPs with transactivator of transcription (TAT) penetration peptide for long-term tracking of adipose-derived stem cells (ASC) with no need for constant external excitation (Figure 11).[32] TAT peptide was conjugated on the surface of PLNPs to enhance their biocompatibility, and cellular uptake. In vitro labeling and imaging investigation reveal effective labeling ASC with TAT-PLNPs for at least 3 days without obvious leakage from labeled cells. In vivo tracking of stem cells shows successful detection of even only 10 PLNPs-labeled ASCs with SNR = 2.9 due to low background and red light renewability. In contrast, both quantum dots (QDs) and dye labeling ASC exhibited poor SNR due to strong autofluorescence.

To further demonstrate the long-term tracking capacity of PLNPs in stem-cells-based therapy in vivo, we employed TAT-PLNPs to track ASC during wound healing and tumor-homing processes. PLNPs-labeled ASC promoted wound healing effectively as free stem cells without impairing proliferation and differentiation of stem cells. The obvious PL signal of PLNPs could be observed as long as scar shedding at 7 day, and residual PLNPs-labeled stem cells could be detected even after 21 days. In the tumor models, PLNPs-labeled stem cells not only exhibited negligible adverse effect on tumor-homing ability of stem cells, but also sensitively tracked the migration of stem cells during the tumor-homing process. In contrast, free PLNPs mainly accumulated in reticuloendothelial system organs. Our

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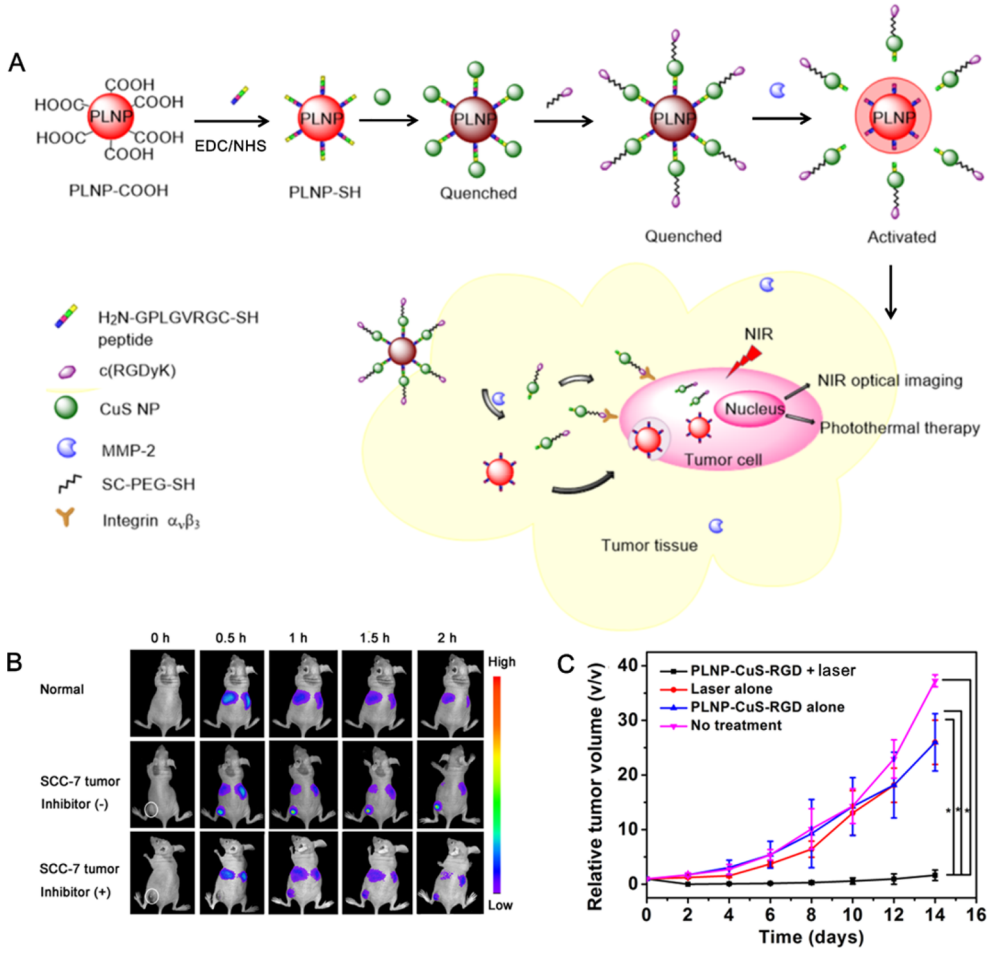

Figure 10. (A) Scheme for synthesis of the activatable nanoprobe PLNP-CuS-RGD for in vivo imaging-guided PTT. (B) In vivo PL images of tumor-bearing mice after intravenous injection of the nanoprobe with or without inhibitor. (C) Tumor growth curves in each mice group after different treatments.[31] Reprinted with permission from ref 31. Copyright 2016 American Chemical Society.

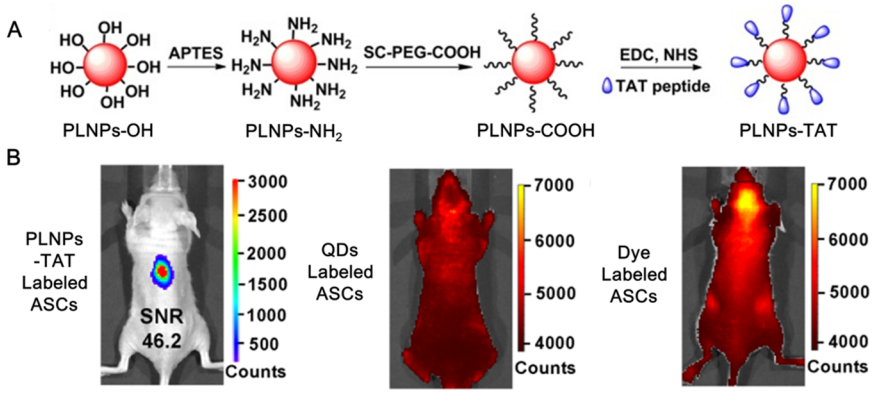

Figure 11. (A) Fabrication of PLNP-TAT for stem cell tracking and therapy. (B) Comparison of in vivo images obtained with LPLNPs-TAT, QDs, and dye labeled ASCs.[32] Reprinted with permission from ref 32. Copyright 2016 American Chemical Society.

study demonstrates that PLNPs are an excellent candidate for sensitive stem cells tracking in both wound healing and tumor-homing processes without in situ excitation.

4.5. PLNPs for Cell Tracking and Gene Therapy

The outstanding stem-cells-tracking capability motivates us to design stem-cells-based therapeutic agents for tumor treatment using PLNPs as highly sensitive tracking nanoprobe. Mesenchymal stem cells (MSC)-based gene therapy is a highly efficient therapeutic approach against gliobastoma,[42] and reliable, noninvasive, and sensitive imaging techniques are essential to track the fate, migration and distribution of therapeutic stem cells in brain tissues. Recently, we developed dual-functional PLNPs-engineered MSC for sensitive PL-guided gene therapy of glioblastoma (Figure 12A).[34] PEI, PEG, and TAT penetration peptide were sequentially decorated on PLNPs to give loading ability of EGFP-TRAIL (enhanced green fluorescence protein-human tumor necrosis factor-related apoptosis inducing ligand) plasmid DNA, excellent aqueous

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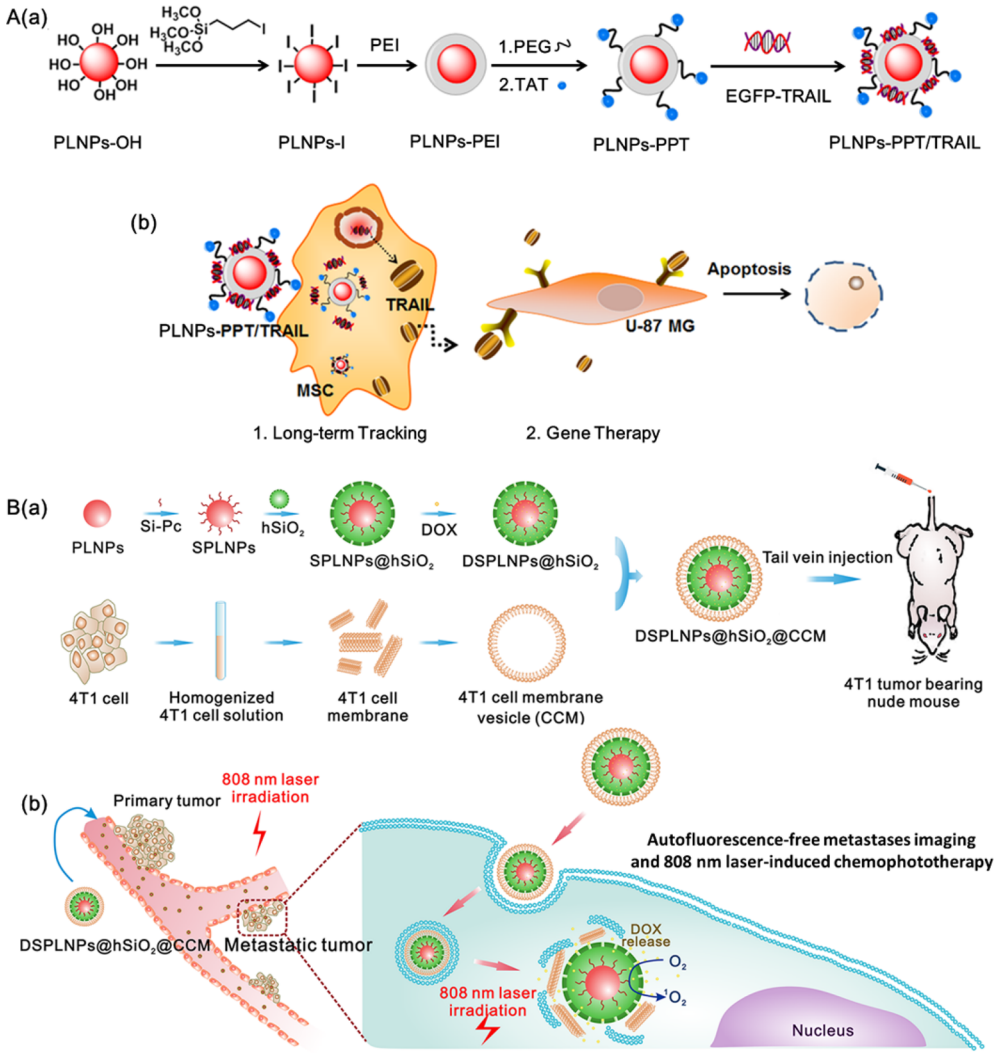

Figure 12. (A) Dual-functional PLNPs-engineered MSC for stem cell tracking and gene therapy: (a) preparation protocol; (b) cell tracking and gene therapy.[34] Reprinted from ref 34. Copyright 2017 John Wiley and Sons. (B) DSPLNPs@hSiO$_2$@CCM for in vivo autofluorescence-free metastases imaging and chemophototherapy: (a) synthesis scheme; (b) in vivo autofluorescence-free imaging and chemophototherapy of metastases.[35] Adapted from ref 35. Copyright 2018 American Chemical Society.

dispersity, and effortless stem cell internalization. The positively charged PLNPs-PEI-PEG-TAT (PLNPs-PPT) nanoprobe was further conjugated with EGFP-TRAIL plasmid DNA via electrostatic adsorption. The formed PLNPs-PPT and PLNPs-PPT/TRAIL both exhibited good biocompatibility, and their labeled stem cells exhibited negligible adverse effects on the differentiation capacity of MSC. In vitro gene transfection investigation reveals that PLNPs-PPT could not only deliver therapeutic genes, but also track stem cells for a long time. We also found that PLNPs-PPT labeled MSC could across the corpus callosum and migrate to tumors after administration in U87 MG tumor-bearing mice, but could not migrate in the brain tissue of normal mice. These results demonstrate that PLNPs-PPT labeled MSC possess tumor-targeting ability and enabled sensitive tracking of the migration of stem cells in vivo. Additionally, the PLNPs-PPT/TRAIL engineered MSC exhibited remarkable tumor inhibiting effect in vitro and in vivo. The successful fabrication of PLNPs-PPT/TRAIL engineered MSC with fascinating therapeutics performance paves the way for designing multifunctional PLNPs-involved stem-cells-based therapeutic nanoprobes.

4.6. PLNPs-Based Tracking and Combined Therapy of Metastatic Tumor

Metastasis is directly or indirectly responsible for the death of patients with cancer, so highly sensitive detection and efficient treatment of metastases is of great significance for curing cancer.[43] However, small size, dispersed distribution and unvascularized anatomy make it difficult to detect and treat metastasis in vivo. Very recently, we reported biomimetic engineering of PLNPs for long-term autofluorescence-free metastasis tracking and 808 nm laser controlled chemo-PDT.[35] The nanoplatform (DSPLNPs@hSiO$_2$@CCM) consists of PLNPs core modified with Si-Pc photosensitizer (SPLNPs), doxorubicin (DOX) loaded hollow silica interlayer (hSiO$_2$), and cancer cell membrane (CCM)-based protecting shell for tracking and cascade therapy of metastatic tumors (Figure 12B). The PLNPs provide autofluorescence-free tracking signal and serve as an internal light to trigger PDT. CCM coating endows the nanoplatform with targeting ability to metastasis and prevents drug leakage. Moreover, the PL induced PDT can destroy CCM and trigger the release of drugs in silica to treat metastasis. The NIR light reactivatable PLNPs-

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based nanoplatform enables not only long-term autofluorescence-free tracing of metastases, but also 808 nm laser controlled cascade chemophotodynamic therapy in vivo, allowing accurate diagnosis and controllable therapy for metastasis. This work clearly illustrates that surface engineering and manipulating energy transfer can take full advantage of the unique PL properties of PLNPs, and enable providing nanoplatforms to solve significant science concerns in biomedical fields.

5. CONCLUSIONS AND OUTLOOK

We have summarized our recent achievements on the engineering of PLNPs for biomedical applications based on the design principle of surface modification and manipulating energy transfer. Our research attempts to take advantage of the fascinating features of PLNPs, such as mysterious long afterglow and red light renewability, to improve sensitivity and selectivity of nanoprobes in biosensing and bioimaging and theranostics. Various strategies for engineering PLNPs have been developed to solve the key issues covering most important aspects of modern biomedical applications, including highly sensitive and selective biosensors, tumor-targeted imaging, reliable and accurate stem cells tracking and stem-cells-based therapy, high-performance multimodal imaging nanoprobes and smart theranostic nanoagents.

Despite substantial progress made by our and other groups, it is fair to admit that the development of PLNPs in biomedical applications is still in the early stage. First, high-quality PLNPs with small and uniform size, bright initial PL and long PL duration are extremely deficient. Novel general synthesis methods are highly desired to control the shape, morphology, homogeneity, and PL performance of PLNPs. Second, other irradiations without tissue penetrating depth limit, such as X-ray, are highly promoted to reactivate PLNPs for tumor imaging in deep tissue, and they could also endow the PLNPs nanoprobes with other theranostic functions such as CT imaging and radiotherapy. Third, the luminescent mechanism of PLNPs should be further and in depth investigated, which strongly influence the sustainable development of PLNPs with much more fascinating features. Last but not least, current PLNPs-based nanoprobes mainly focus on the diagnosis and treatment of tumors. Enormous efforts should be taken to explore the application of PLNPs in diagnosis and treatment of other serious diseases such as cardiovascular and cerebrovascular diseases, digestive system disorders, and orthopedic diseases via versatile strategies.

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Notes

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