Development of organic semiconducting materials for deep-tissue optical imaging, phototherapy and photoactivation

Li, Jingchao; Pu, Kanyi

2019

Li, J., & Pu, K. (2019). Development of organic semiconducting materials for deep-tissue optical imaging, phototherapy and photoactivation. Chemical Society Reviews.
doi:10.1039/C8CS00001H

<https://hdl.handle.net/10356/87449>

<https://doi.org/10.1039/C8CS00001H>

© 2018 The Author(s) (Royal Society of Chemistry). This is the author created version of a work that has been peer reviewed and accepted for publication by Chemical Society Reviews, The Author(s) (Royal Society of Chemistry). It incorporates referee's comments but changes resulting from the publishing process, such as copyediting, structural formatting, may not be reflected in this document. The published version is available at: [<http://dx.doi.org/10.1039/C8CS00001H>].

Downloaded on 26 Apr 2023 22:39:11 SGT

Development of organic semiconducting materials for deep-tissue optical imaging, phototherapy and photoactivation

Jingchao Li, and Kanyi Pu*

Biophotonics as a highly interdisciplinary frontier often requires the assistance of optical agents to control the light pathways in cells, tissues and living organisms for specific biomedical applications. Organic semiconducting materials (OSMs) composed of π -conjugated building blocks as the optically active components have recently emerged as a promising category of biophotonic agents. OSMs possess the common features including excellent optical properties, good photostability and biologically benign composition. This review summarizes the recent progress in the development of OSMs based on small-molecule fluorophores, aggregation-induced emission (AIE) dyes and semiconducting oligomer/polymer nanoparticles (SONs/SPNs) for advanced biophotonic applications. OSMs have been exploited as imaging agents to transduce biomolecular interactions into second near-infrared fluorescence, afterglow or photoacoustic signals, enabling deep-tissue ultrasensitive imaging of biological tissues, disease biomarkers and physiological indexes. By finetuning the molecular structures, OSMs can also convert light energy into cytotoxic free radicals or heat, allowing for effective cancer phototherapy. Due to their instance light response and efficient light-harvesting properties, precise regulation of biological activities using OSMs as the remote transducers has been demonstrated for protein ion channels, gene transcription and protein activation. In addition to highlighting OSMs as a multifunctional platform for a wide range of biomedical applications, current challenges and perspectives of OSMs in biophotonics are discussed.

1. Introduction

Biophotonics that involves the study of light-activated biological processes at the molecular, cellular or tissue levels is an interdisciplinary research frontier encompassing life science, optics and engineering.¹ Significant progress in the application of biophotonics in modern medicine has been achieved in the past decade owing to the improvements of photonic technologies.² For example, biophotonics has allowed us to accurately distinguish early-stage lesions with high spatial resolution ranging from nanometer to centimetre scale, longitudinally observe pathological events with an extremely broad temporal range from femtoseconds to days, sensitively detect disease biomarkers at a limit of detection (LOD) down to nanomole, and precisely control therapeutic process and biological activities.^{3,4}

Biophotonic applications often rely on optical materials that have the light absorption and conversion capabilities. Advancement of chemistry and nanotechnology has led to many biophotonic agents, which include inorganic optical nanomaterials including semiconducting quantum dots (QDs),⁵ silica nanoparticles,⁶ gold nanoparticles,⁷ carbon nanotubes,⁸ 2D transition metal dichalcogenide nanosheets,⁹ nanographene oxide,¹⁰ and upconversion nanocomposites.¹¹ They have shown the capabilities

to transduce biomolecular interactions into optical signals to allow noninvasive disease diagnosis,¹² as well as convert light energy into free radicals or thermal effect for localized cancer phototherapy,¹³ controlled cargo delivery,¹⁴ biological stimulation,¹⁵ and photochemical tissue binding.¹⁶ However, inorganic nanoparticles have been excreted slowly after administration and largely retained in major organs of the reticuloendothelial system, such as the liver and spleen, potentially resulting in long-term toxicity.¹⁷ In sharp contrast, organic optical agents that are made from completely benign and biologically inert components have the potential to circumvent the toxic concerns while possessing the optical advantages equal or even superior to inorganic optical nanomaterials.¹⁸

As an emerging class of organic optical agents, organic semiconducting materials (OSMs) have been proven effective in biophotonics. OSMs can be characterized by small molecules, polymers or nanoparticles composed of electron-delocalized π -conjugated components. Typical OSMs include small molecular fluorophores, aggregation-induced emission (AIE) molecules/nanoparticles, and semiconducting oligomer/polymer nanoparticles (SONs/SPNs). The photophysical properties of OSMs are mainly determined by the chemical structures or aggregate states of electron-delocalized π -conjugated components, and thus can be adjusted through rational molecular engineering. Until now, OSMs have been exploited as contrast agents for cell imaging and tracking,¹⁹ targeting tumor imaging,²⁰ vascular imaging,²¹ lymph node imaging,²² drug release tracking,²³ real-time monitoring of

School of Chemical and Biomedical Engineering, Nanyang Technological University,
70 Nanyang Drive, 637457, Singapore.
Email: kypu@ntu.edu.sg

physiological indexes and biomarkers,^{24, 25} and phototherapeutic agents for cancer and bacteria killing.^{26, 27}

In this review, we summarize the recent progress of OSMs as a class of optical agents for biophotonic applications: (i) deep-tissue molecular optical imaging including second near-infrared (NIR-II) fluorescence, chemiluminescence, afterglow and photoacoustic (PA) imaging, (ii) cancer phototherapy, and (iii) biological photoactivation (Fig. 1). The molecular or nanoparticle design principles of OSMs are discussed along with their key applications in optical molecular imaging with the emphasis on the strategies to amplify brightness, improve tissue penetration depth and enhance signal-to noise ratios. Next, rational designs and examples of OSMs as therapeutic nanoagents for amplified or combinational cancer phototherapy are discussed. Then, the potentials of OSMs as light transducers are revealed using the examples of regulating different biological activities. At last, a brief summary is given together with the discussion of current challenges and perspectives in this field.

2. NIR-II fluorescence imaging

Fluorescence imaging is a convenient and cost-effective method for biological analysis, clinical diagnosis, therapeutic monitoring and drug discovery.^{28, 29} In vivo fluorescence imaging have been performed with light excitation in different regions, which include the visible region (400-700 nm), the conventional first NIR region

(NIR-I, 700-1000 nm) and the second NIR region (NIR-II, 1000-1800 nm).³⁰ Because visible light has shallow tissue penetration depth (~1 mm) and can be significantly distributed by tissue autofluorescence,³¹ the general research interests of fluorescence imaging had been focused in the NIR-I region, defined as “biological transparency NIR window”,³² allowing for light penetration depth of 1-3.5 mm.³³ However, NIR-I fluorescence imaging still has the intrinsic issues of relatively high tissue autofluorescence and unsatisfactory imaging depth. By contrast, NIR-II fluorescence imaging has significantly diminished background noise and improved tissue penetration depth (5-20 mm) due to the minimal photon scattering and low tissue absorption of NIR-II light.³⁴ With these merits, NIR-II fluorescence imaging is able to visualize biological molecules and tissue structures in deep tissue that are otherwise difficult to achieve for the NIR-I imaging.³⁵

Because not many optical agents can emit light in the NIR-II window,³⁶ Dai's and other groups have made efforts to design and develop NIR-II fluorescent agents, including inorganic nanoparticles such as single-walled carbon nanotubes (SWCNTs),³⁷⁻³⁹ QDs,⁴⁰⁻⁴⁴ and rare-earth doped nanoparticles,⁴⁵ and OSMs composed of organic semiconducting nanoparticles and small molecular fluorophores (Table 1).^{36, 46, 47} In contrast to inorganic nanoparticles, OSMs are more desirable and optimal candidates for NIR-II fluorescence imaging mainly because their optical properties, pharmacokinetics, and biodistribution can be easily tuned to fulfill diverse applications.

Fig. 1 Summary of the applications of OSMs for deep-tissue optical imaging (NIR-II fluorescence, chemiluminescence, afterglow and photoacoustic), phototherapy and biological photoactivation.

2.1. NIR-II fluorescent nanoparticles

To generate NIR-II fluorescent organic nanoparticles, hydrophobic fluorophores or molecules have been transformed into water-soluble SONs through nanoprecipitation (Fig. 2a). For instance, Dai and colleagues embedded a commercially-available water-insoluble IR-1061 dye (Scheme 1) into a poly(acrylic acid) (PAA) matrix with surface coating of an amphiphilic polymer phospholipid-conjugated polyethylene glycol (DSPE-*m*PEG) to generate IR-1061-PEG

nanoparticles that displayed a major fluorescence emission peak at 920 nm and a shoulder peak at 1064 nm.⁴⁸ This simple embedding process afforded a relatively high fluorescence quantum yield (QY) of 1.8% for IR-1061-PEG nanoparticles. Intravenous injection of IR-1061-PEG nanoparticles allowed for NIR-II fluorescence imaging of different inner organs including lung and kidney, and subcutaneous arteries in hind-limb femoral and abdomen of living mice. In another study, Cheng and colleagues subsequently synthesized a NIR-II absorbing small molecule, 5,5'-(1H,5H-benzo[1,2-c:4,5-

c']bis[1,2,5]thiadiazole-4,8-diyl)bis(N,N-bis(4-(3-((tert-butyl)dimethylsilyl)oxy)propyl)phenyl)thiophen-2-amine) (CQ1, Fig. 2b) and encapsulated it into phospholipids to yield CQS1000 nanoparticles as imaging probes.⁴⁹ With a NIR-II fluorescence emission at 1000 nm, CQS1000 nanoparticles allowed for noninvasive and dynamical visualization and monitoring of circulatory systems, including hind limb and cerebral blood vessels, angiogenesis of tumors, arterial thrombus formation and incomplete hind limb ischemia, and lymphatic drainage and routing, as well as imaging-guided precise resection of tumors and sentinel lymph node mapping and biopsy.

AIE nanoparticles, a class of organic luminophores showing enhanced brightness upon aggregation,⁵⁰ have been another candidates for NIR-II fluorescence imaging. Tang's group recently described the use of AIE nanoparticles assembled from a small

molecular TQ-BPN (Fig. 2a–b) as contrast agents for angiography in mouse brain and tumor.⁵¹ The formed TQ-BPN AIE nanoparticles showed an absorption peak at 630 nm and emitted light in a broad range of 700–1200 nm (Fig. 2c). Owing to the long-wavelength emission and high fluorescence brightness (QY = 2.8%), TQ-BPN AIE nanoparticles allowed to directly visualize the brain vasculature in a high temporal resolution (25 frames per second) and spatial resolution (2.6 μm) and deep tissue penetration up to 800 μm (Fig. 2d). *In vivo* real-time microscopic angiography using TQ-BPN AIE nanoparticles as the NIR-II fluorescent probes enabled monitoring of the photothrombotic ischemia (PTI) induced blood-brain barrier (BBB) damage in a mouse brain (Fig. 2e), and visualization of the enhanced permeability and retention (EPR) effect in tumor sites (Fig. 2f).

Scheme 1. Chemical structures of organic small molecules, fluorophores and semiconducting oligomers (SOs) used for preparation of OSMs.

Fig. 2 (a) Schematic illustration of the preparation of organic nanoparticles via nanoprecipitation. (b) Chemical structures of CQ1, TQ-BPN and SP1 used for preparation of SONS, AIE nanoparticles and SPNs for NIR-II fluorescence imaging. (c) UV-Vis absorption and photoluminescence (PL) spectra of TQ-BPN AIE nanoparticles dispersed in water. The inset shows the photographs of the bright-field (left) and NIR-II (right) images of the nanoparticles in aqueous dispersion. (d) In vivo real-time fluorescence microscopic imaging of mouse brain vasculature at different depths (200 and 800 μm) after the intravenous injection of TQ-BPN AIE nanoparticles. (e) NIR-II fluorescence microscopic images and corresponding heat maps of brain blood vessels before and after PTI induction using TQ-BPN AIE nanoparticles. (f) NIR-II fluorescence microscopic imaging for visualizing EPR effect in old (left) and new (right) tumors at different time points after intravenous injection of TQ-BPN AIE nanoparticles. (g) UV-Vis absorption and fluorescence emission spectra of SPN1. (h) A time course of NIR-II fluorescence images of a mouse hindlimb immediately after intravenous injection of SPN1, showing the blood flow front moving inside the femoral artery (indicated by red arrows). (i) Time course NIR-II fluorescence images of a mouse femoral artery after subtracting background. (c–f) were reprinted with permission from ref. 51. Copyright 2018 John Wiley & Sons Ltd. (g–i) were reprinted with permission from ref. 52. Copyright 2017 John Wiley & Sons Ltd.

In addition to SONS and AIE nanoparticles, SPNs made from hydrophobic π -conjugated semiconducting polymers (SPs) through nanoprecipitation have been used as NIR-II fluorescence imaging agents (Fig. 2a). Utilizing a donor–acceptor (D–A) alternating copolymerization reaction, Dai and co-workers synthesized a poly(benzo[1,2-b:3,4-b']difuran-alt-fluorothieno-[3,4-b]thiophene (SP1, Fig. 2b) and formulated it into SPN1 with the assistance of DSPE-mPEG.⁵² SPN1 had a high QY ($\approx 1.7\%$) and showed an absorption peak at 654 nm and an emission peak at 1048 nm with a large Stokes shift (≈ 400 nm) (Fig. 2g). The use of SPN1 allowed fluorescence imaging of living cells at > 1000 nm window for the first time, and enabled real-time tracking of arterial blood flow in a mouse hind-limb with an exposure time as short as 20 ms and an ultrafast frame rate of 25 frames per second in the NIR-II window (Fig. 2h), as well as facilitated spatiotemporally resolved imaging of blood flow pattern in cardiogram waveform over a single cardiac cycle (~ 200 ms) of a mouse (Fig. 2i).

2.2. NIR-II fluorescent small molecular fluorophores

To achieve rapid renal clearance of imaging agents, a significant amount of efforts have been made to synthesize small molecular fluorophores with the size below the renal filtration threshold of

~ 40 kD (5.5 nm).^{53–55} The first NIR-II fluorescent small molecular fluorophore was developed by Dai and co-workers and utilized for NIR-II fluorescence imaging guided surgery.⁵⁶ A donor–acceptor–donor (D–A–D) structured CH1055 composed of benzobisthiadiazole (BBTD) as the acceptor and triphenylamine as the donor was PEGylated to yield water soluble CH1055-PEG (Fig. 3a), showing the maximum excitation at ~ 750 nm and the maximum emission at 1055 nm with a tail extending into the NIR-II region. With a relatively small size (8.9 kD), CH1055-PEG displayed a rapid renal clearance with $\sim 90\%$ removal through urine within 24 h after intravenous injection, while nearly no accumulation in the liver tissue (Fig. 3b). In striking contrast, the injected SWCNTs had a slower pharmacokinetics and were almost retained in the liver and spleen. In addition, CH1055-PEG showed a high signal contrast in mapping sentinel lymph node (SLN), which enabled selective removal of SLN to alleviate lymphoedema and prevent cancer metastasis (Fig. 3c). NIR-II fluorescence imaging of an orthotopic glioblastoma brain tumor at a depth of ~ 4 mm was also performed using CH1055-PEG (Fig. 3d). After 72 h of intravenous injection, the tumor was clearly visualized with a tumor-to-normal tissue (T/NT) ratio of 5.5. Imaging of brain vasculature in living mice was also carried out, showing that the imaging quality of CH1055-PEG in the

NIR-II window was obviously higher than that of indocyanine green (ICG) in the NIR-I window (Fig. 3e). CH1055 was attached with a small protein anti-epidermal growth factor receptor (anti-EGFR) affibody for passive tumor accumulation (Fig. 3a). Such a chemical bioconjugation not only facilitated targeting NIR-II fluorescence imaging of EGFR-overexpressed squamous cell carcinoma (SAS) tumors with an unprecedentedly high T/NT ratio up to ~15 (Fig. 3f), but also permitted the first proof-of-concept NIR-II imaging guided tumor excision surgery (Fig. 3g).

Despite the promising example of CH1055, the applications of small molecular fluorophores in NIR-II fluorescence imaging are still limited mainly due to their low fluorescence QYs (0.2–0.31%) in aqueous solutions, attributed to the strong interactions between the π -conjugated segments and environmental water molecules.^{46, 47, 56} Thus, rational design of molecular structures have been performed to develop new fluorophores with improved fluorescence brightness. Replacing the triphenylamine in CH1055 with bulky 3,4-ethoxylene dioxythiophene (EDOT) as the donor generated a novel NIR-II fluorophore (IR-E1, Scheme 1).⁵⁷ Such replacement reduced the inter- and intra-molecular interactions between the π -conjugated backbones, increasing the QY to 0.7% at

1071 nm in aqueous solution, more than doubly higher relative to CH1055 (0.3%). However, this improvement is still insufficient to provide high-quality imaging performances.

To significantly improve the fluorescence brightness, shielding units have been introduced to construct shielding unit–donor–acceptor–donor–shielding unit (S–D–A–D–S) type NIR-II small molecular fluorophores. For instance, Dai and co-workers synthesized a S–D–A–D–S structured fluorophore (IR-FE, Scheme 1) with BBTD, EDOT and dialkyl-fluorene serving as the acceptor, donor and shielding unit, respectively.^{58, 59} The introduction of dialkyl-fluorene as a shielding unit protected the π -conjugated backbone from intermolecular interactions and reduced aggregation, resulting in an improved QY of 2.0–2.6% in aqueous solution. In addition to the introduction of dialkyl-fluorene as the shielding unit, incorporating of tert(ethylene glycol) (TEG)-substituted thiophene as the bridging donor unit afforded another S–D–A–D–S structured NIR-II fluorophore (IR-FG, Scheme 1).⁶⁰ In such a molecule, TEG substitution could distort the conjugated backbone and increase the dihedral angle between acceptor and donor, reducing the molecular interactions and increasing the QY to 1.9%.

Fig. 3 (a) Chemical structure of CH1055 and schematic illustration of the preparation of CH1055-PEG and CH1055-affibody. (b) NIR-II fluorescence imaging (1200 nm long-pass filter, 100 ms) of a mouse in the supine position at 15 and 870 s after the intravenous injection of CH1055-PEG or SWCNTs. (c) NIR-II fluorescence imaging (1200 nm long-pass filter, 200 ms) of a nude mouse with a 4T1 xenograft tumor at 10 min, 5 h and 24 h after the intradermal injection of CH1055-PEG near the base of the tail. White arrow points to the tumor location. (d) NIR-II fluorescence imaging (1200 nm long-pass filter, 800 ms) of glioblastoma brain tumor at 24 h post intravenous injection of CH1055-PEG. (e) NIR-II fluorescence imaging of brain vasculature through the scalp and skull in C57BL/6 mice with shaved heads using either ICG or CH1055-PEG. The greyscale bar corresponds to all NIR-II images. (f) NIR-II fluorescence imaging of SAS tumor at 1 and 6 h after intravenous injection of CH1055-affibody (60 μ g). The upper panel shows clear tumor targeting and the bottom panel shows mice injected with a blocking dose consisting of CH1055-affibody plus free affibody concomitantly intravenously injected. Inset shows the SAS tumor *ex vivo* after excision in both a targeted and blocked mouse. The red circle corresponds to the normal tissue region used to calculate the T/NT ratio. (g) Representative images of a mouse at 24 h post-injection before (upper) and after (bottom) performing NIR-II fluorescence image-guided surgery to excise the tumors. Reproduced from ref. 56. By permission of Nature Publishing Group.

The QYs of small molecular fluorophores could be further increased by introducing two donor units in the π -conjugated backbones to have a S–D2–D1–A–D1–D2–S structure.⁶¹ This molecular engineering on the donor units has allowed the generation of a novel NIR-II molecular fluorophore IR-FTAP (Scheme 1), in which the alkyl thiophene and thiophene were employed as the first and second donors to connect the acceptor unit (BBTD) and the shielding unit (dialkyl-fluorene), respectively. Such a structure afforded a larger distortion of the π -conjugated backbone and further reduced intermolecular interactions, resulting in an enhanced QY of 5.3% for IR-FTAP in aqueous solution.

In addition to these chemical strategies, simple binding of small molecular fluorophores with proteins can improve the QYs.⁴⁷ This should be due to the fact that the aggregates of fluorophores are hindered by the protein bindings and therefore a rigid fluorophore conformation can be maintained to minimize torsional and consequently minimize non-radiative decay.⁴⁷ For example, the QYs of FD-1080 (Scheme 1) could be increased from 0.31% to 5.94% after binding with fetal bovine serum (FBS).⁴⁷ Besides the high-resolution deep-tissue NIR-II fluorescence imaging of hindlimb vasculature and brain vessel, the FD-1080-FBS complexes could be utilized for NIR-II fluorescence dynamic imaging of liver in living mice to quantify the respiratory rate. In another study, FBS binding afforded an around 6-fold increment in the QYs for a cyanine dye that displayed tail emission in the 1000–1300 nm range.⁶² The NIR-II fluorescence imaging of this cyanine dye was used to image liver and intestine clearly in a CD-1 mouse after administration, while was not possible for NIR-I imaging.

2.3. NIR-II fluorescent activatable probes

NIR-II fluorescence imaging has exhibited unprecedented advantages over the traditional NIR-I fluorescence imaging, but the aforementioned agents fail to provide specific and sensitive signals toward targets of interest, leading to poor signal-to-background ratios (SBRs).⁶³ Thus, activatable NIR-II fluorescent probes that can produce signals only in response to specific disease biomarkers or biological events have been recently proposed.⁶³ As an example, Tian and co-workers devised an activatable organic nanoprobe that displayed hydrogen sulphide (H_2S)-activated ratiometric fluorescence and NIR-II emission for the deep-tissue imaging of H_2S -rich colorectal cancers.⁶⁴ Such a nanoprobe (termed as NIR-II@Si) was fabricated by trapping a H_2S -responsive boron-dipyrromethene (ZX-NIR) dye and a H_2S inert aza-BODIPY (aza-BOD) dye into hydrophobic interior of silica coated micelles (Fig. 4a–b). Upon reaction with H_2S , the fluorescence emission at 600 nm was quenched, while that at 700 nm showed no appreciable change under excitation at 520 nm, allowing for ratiometric fluorescence detection (I_{700}/I_{600}) of H_2S with a LOD of 37 nM. Moreover, this H_2S specific response triggered a NIR-II fluorescence “turn-on” under NIR laser irradiation (Fig. 4c–d). The H_2S -activated NIR-II fluorescence signals were clearly detected even at a depth of 10 mm, while the NIR-I fluorescence signals were only visible below 3 mm. By using NIR-II@Si as the activatable and target specific nanoprobe, deep-tissue NIR-II fluorescence imaging of H_2S -rich colorectal HCT116 tumors in animal models was achieved with a NIR-II signal ratio between the tumor and normal site (T/N) as high as 5.7 (Fig. 4e–g). In sharp contrast, no NIR-II fluorescence signals could be detected in the HepG2 tumors because of the low level of H_2S , even though NIR-I fluorescence signals were observed (Fig. 4f).

Fig. 4 (a) Chemical structure of aza-BOD. (b) Chemical structures of ZX-NIR and NIRII-HS. (c) Mechanism of H_2S activated NIR-II fluorescence signals. (d) Time-dependent NIR-II fluorescence spectra of NIR-II@Si in the presence of H_2S with excitation at 780 nm. Inset shows the photograph of the H_2S -activated NIR-II emission. NIR-I and NIR-II fluorescence imaging of HCT116 tumors (e), HepG2 tumors (f) and normal tissues (g) after subcutaneous injection of NIR-II@Si. Reprinted with permission from ref. 64. Copyright 2018 John Wiley & Sons Ltd.

Table 1 Summary of organic and inorganic agents for NIR-II fluorescence imaging.

Probes	Excitation/emission	QY	Imaging applications	Refs
SWCNTs	785 nm/1100-1400 nm	0.4%	Vascular, deep-tissue anatomical and cancer metastasis	37-39
Ag ₂ S QDs	808 nm/1100-1400 nm	15.5%	Tumor, lymphatic drainage and vascular networks	40, 41
Ag ₂ Se QDs	785 nm/1300 nm	29.4%	Deep organs and vascular structures	42
PbS QDs	450 nm/1000-1200 nm	6-16%	Breast tumor and lymph node	43, 44
LaF3:Nd nanoparticles	808 nm/910, 1050 and 1340 nm	50%	Internal tissue	45
IR-1061-PEG nanoparticles	808 or 980 nm/1000-1400 nm	1.8%	Inner organs and blood vessels	48
LbL-IR1061 nanoparticles	808 nm/1100 nm	1.8%	Evaluation of biodistribution, pharmacokinetics, and toxicities	30
CQS1000 nanoparticles	808 nm/1000 nm	/	Circulatory systems and imaging-guided tumor resection and sentinel lymph node biopsy	49
TQ-BPN AIE nanoparticles	635 nm/900-1200 nm	2.8%	Brain capillaries, blood-brain barrier damage and EPR effect in tumor	51
SPN1	808 nm/1050-1350 nm	1.7%	Cancer cells and arterial blood flow	52
SCH1100	808 nm/1100 nm	0.2%	Prostate cancers	46
FD-1080	1064 nm/1080 nm	0.31%	Hindlimb vasculature, brain vessel, respiratory craniocaudal motion of liver	47
CH1055	808 nm/1055 nm	0.3%	SLN, orthotopic glioblastoma brain tumor, brain vasculature and imaging guided tumor surgery	56
IR-E1	808 nm/1071 nm	0.7%	TBI-induced hemodynamic abnormalities and cerebrovascular damages	57
IR-FEP	808 nm/910 and 1100 nm	2.0%	Blood flow in hindlimb and tumors	58
IR-FEPC	808 nm/1100 nm	2.6%	Adult female ovary in living mice	59
IR-FG	808 nm/1050 nm	1.9%	Histological tissue staining	60
IR-FTAP	808 nm/1048 nm	5.3%	Blood vessels of a mouse hindlimb	61
NIR-II@Si	780 nm/900-1300 nm	0.37%	H ₂ S in tumor	64
HISSNPs	808 nm/900-1500 nm	/	Tumor microenvironment	65

Because most biomarkers are often expressed in multiple types of cells, activatable probes may encounter the issue of nonspecific activation and are unable to accurately identify the complex and dynamic pathological environment.⁶⁶ To improve the diagnostic specificity and accuracy, Fan and colleagues developed a dual-pathological-parameter cooperatively activatable organic nanoprobe by using a “dual lock-and-key” strategy.⁶⁵ IR-1061 was conjugated with hyaluronic acid (HA) to generate amphiphilic IR-1061-pendent HA polymers that consequently self-assembled into nanoparticles (“single lock-and-key”-controlled HINPs) in aqueous solution. Further crosslinking of the HA on the surface of HINPs using disulfide formed the final nanoprobe (“dual lock-and-key”-controlled HISSNPs). Both HINPs and HISSNPs had very weak fluorescence owing to the fluorescence-quenched aggregation state

of IR-1061. Overexpressed hyaluronidase and thiols in tumor microenvironments specifically cleaved HA chains and disulfide bonds, respectively, resulting in the dissociation of nanoparticles and the decrease of the aggregation degree of IR-1061, leading to fluorescence “turn-on”. With HA-mediated targeting and EPR effect, HISSNPs effectively accumulated into tumor tissues, and allowed such a cooperatively activatable fluorescence resonance property for NIR-II fluorescence imaging of specific tumors. As compared to HINPs, HISSNPs not only significantly reduced the nonspecific activation in vivo, but also achieved a higher T/NT ratio up to 15.4 (3.3 for HINPs) and higher tumor-to-liver ratio of 5.87 (1.1 for HINPs) at 24 h post-injection.

3. Chemiluminescence imaging

Chemiluminescence imaging that relies on the energy release from a chemical reaction has appeared to be an ideal technology for different biological analysis and disease diagnosis.⁶⁷⁻⁶⁹ The major advantages of chemiluminescence imaging is the negligible photo-damage and no background noise from biological tissues attributed to the elimination of light excitation, permitting deep-tissue imaging with a relatively high SBR.⁷⁰⁻⁷² At present, a great number of OSMs including organic semiconducting nanoparticles and dioxetane-based small molecules have been utilized as probes for chemiluminescence imaging.

3.1. Chemiluminescent nanoprobos

The chemiluminescence imaging of targets of interest with organic semiconducting nanoprobos is generally achieved through a two-step process (Fig. 5a). First, the targets of interest react with the responsive substrates in the nanoprobos to afford high-energy intermediates. Second, intermolecular electron transfer from the fluorophores or SPs to the high-energy intermediates occurred through the chemically initiated electron-exchange luminescence (CIEEL) mechanism, leading to the generation of chemiluminescence signals.⁷² Since reactive oxygen species (ROS) have high activities to react with responsive substrates,⁷³⁻⁷⁷ chemiluminescent organic semiconducting nanoprobos have been widely utilized to detect ROS in living systems.

Murthy and co-workers reported the utilization of fluorescent dye (pentacene) encapsulated peroxalate nanoparticles as contrast agents for *in vivo* chemiluminescence imaging of hydrogen peroxide (H_2O_2),⁷² a major ROS in biological systems that modulated redox-dependent cellular signaling transductions.⁷⁸⁻⁸⁰ On interaction with H_2O_2 , the peroxalate ester groups within nanoparticles were specifically oxidized to generate high-energy dioxetanediones that could chemically excite the nearby fluorescent dye, resulting in chemiluminescence at an emission wavelength of 630 nm. Such peroxalate nanoparticles were able to detect H_2O_2 at a concentration as low as 250 nM, while this LOD was far above the *in vivo* concentration (~100 nM) in the normal conditions.⁸¹ Soon afterwards, several other chemiluminescent organic semiconducting nanoprobos with the encapsulation of peroxalate compounds have been designed and developed for H_2O_2 imaging (Table 2), but failed to provide a high detection sensitivity in living subjects, largely because their LOD is still a little high (10–100 nM).⁸²⁻⁸⁶

For ultrasensitive imaging of H_2O_2 , Rao and co-workers contributed a SPN-based sensor that was able to simultaneously but differentially detect drug-induced ROS and reactive nitrogen species (RNS) using chemiluminescence and fluorescence independent optical channels.⁸⁷ In such a system, poly(2,7-(9,9-dioctylfluorene)-*alt*-4,7-bis(thiophen-2-yl)benzo-2,1,3-thiadiazole) (SP2, Fig. 5a) was used as both the chemiluminescence resonance energy transfer (CRET) energy acceptor and the fluorescence resonance energy transfer (FRET) energy donor. A cyanine dye IR775S (Scheme 1) and bis-(2,4,5-trichloro-6-(pentylloxycarbonyl)phenyl)oxalate (CPPO, Scheme 1) were encapsulated into SP2 matrix, acting as the fluorescence-based responsive sensor and H_2O_2 reactive chemiluminescent substrate, respectively. Combining CRET and

FRET into a signal SPN2 afforded CRET-FRET-SPN2 (CF-SPN2). To endow the ability to target hepatocytes for hepatotoxic evaluation, an amphiphilic PEG-grafted poly(styrene) copolymer with a conjugation of galactose (PS-g-PEG-Gal) was used to prepare CF-SPN2. As the FRET acceptor, IR775S within CF-SPN2 decomposed after being oxidized by both peroxynitrite ($ONOO^-$) and hypochlorite (ClO^-), leading to FRET abolishment and thereby ratiometric fluorescence signals. Whereas, only a chemiluminescence response of CF-SPN2 to H_2O_2 occurred without external light excitation, allowing for highly selective and sensitive imaging of H_2O_2 with a LOD as low as 5 nM (Fig. 5b). The improved detection sensitivity of this approach enabled early-stage *in vivo* detection of anti-pyretic acetaminophen (APAP) and isoniazid (INH) induced hepatotoxicity (Fig. 5c–d).

Pu's group have reported another optimized approach, intraparticle energy level alignment, to amplify the chemiluminescence signals of SPNs for ultrasensitive detection of H_2O_2 .⁸⁸ Five polyfluorene derived SPs with different molecular orbitals were chosen as the chemiluminescence reporters to respectively pair with a chemiluminescent substrate peroxalate bis(2,4,6-trichlorophenyl) oxalate (TCPO, Scheme 1), affording a series of SPNs that selectively emitted chemiluminescence signals in response to H_2O_2 . Among these SPNs, the energy interval between the highest occupied molecular orbital (HOMO) of poly[(9,9'-dioctyl-2,7-divinylene-fluorenylene)-*alt*-(2-methoxy-5-(2-ethylhexyloxy)-1,4-phenylene)] (SP3, Scheme 2) and the lowest unoccupied molecular orbital (LUMO) of 1,2-dioxetanedione was the smallest, and thereby the intermolecular electron transfer between them was most facilitated. As a result, SPN3 had the highest chemiluminescence efficiency and enabled detection of H_2O_2 with a LOD down to 5 nM. Further doping of a naphthalocyanine NIR dye (NCBS, Scheme 1) created an intraparticle CRET and endowed SPN3 with the ability to emit luminescence NIR light, allowing for *in vivo* ultrasensitive imaging of H_2O_2 in the mouse models of lipopolysaccharide (LPS) induced peritonitis and neuroinflammation.

To improve the NIR chemiluminescence efficacy of SPNs for H_2O_2 detection, Kim and co-workers subsequently proposed a simple nanophotonic energy relay way.⁸⁹ SPN4 was constructed by nanoprecipitation of a low-bandgap conjugated polymer (SP4, Scheme 2), CPPO and BODIPY dye (Scheme 1). As compared to other probes used for H_2O_2 detection, the only difference relied in the use of BODIPY dye as an energy gap-bridging photonic molecule to effectively accept the energy from chemically excited intermediate of peroxalates and then relay it to SP4 in the same nanoscopic space through the consecutive intra-particle energy transfer. Such an energy-relayed effect boosted the chemiluminescence emission by 50-fold and thus enabled reliable detection of H_2O_2 with a LOD down to 1 nM and presented a fairly high tissue penetration depth (>12 mm). Due to these merits, SPN4 facilitated sensitive deep-tissue chemiluminescence imaging of inflammation in the mouse models of arthritis and peritonitis.

In addition to SPNs, AIE nanoparticles have been utilized for chemiluminescence imaging of H_2O_2 .^{82, 90} In one example, a specially designed small molecule TBD (Scheme 1), CPPO and soybean oil were co-precipitated with an amphiphilic triblock copolymer (PEG-b-PPG-b-PEG) to obtain TBD AIE nanoparticles.⁹⁰

Chemiluminescence of TBD AIE nanoparticles in the presence of H_2O_2 was around 900-fold higher than that with other ROS, allowing detection of H_2O_2 at an ultralow concentration of 2 nM. In addition, the encapsulated soybean oil served as a retarder to slow down the reaction rate between H_2O_2 and CPPO, prolonging the half-life of chemiluminescence to 2.3 h for *in vivo* imaging applications. Because the H_2O_2 concentration in tumors was obviously elevated relative to normal tissues,⁹¹ TBD AIE nanoparticles was able to accurately distinguish primary and peritoneal metastatic tumors.

In addition to the imaging of H_2O_2 , chemiluminescent OSMs were used to detect superoxide anions ($\text{O}_2^{\bullet-}$), one of the most common ROS generated from the reduction of oxygen molecules.^{92, 93} Tang and colleagues recently synthesized a chemiluminescent probe (SPN5) through nano-precipitation of SP5 comprising two covalently connected segments.⁹⁴ In such a nanoparticle, imidazopyrazinone moiety on the side chain of SP5 acted as both the $\text{O}_2^{\bullet-}$ responsive

group and CRET donor, while SP5 was utilized as both the CRET acceptor and signal amplification matrix. The specific reaction between imidazopyrazinone moiety and environmental $\text{O}_2^{\bullet-}$ produced high energy (Fig. 5e), which was then transferred to SP5 through CRET, leading to intense luminescence at 560 nm (Fig. 5f). The LOD of SPN5 was estimated to be 19.3 pM (Fig. 5g). Additionally, the covalently linked molecular structure of SP5 contributed to a prolonged chemiluminescence time more than 25 min, favoring a practical application of *in vivo* molecular imaging. With these advantages over other existing small molecule compounds, SPN5 was utilized for chemiluminescence imaging of $\text{O}_2^{\bullet-}$ in mouse models of inflammation and tumor. After the local injection of SPN5, chemiluminescence signal in tumor tissues was 3-fold higher than that for normal tissues, while the signal intensity in the tumor tissues reduced after the intratumoral injection of ROS scavenger (Tiron) (Fig. 5h).

Fig. 5 (a) Chemical structure of SP2 and schematic illustration of mechanism of chemiluminescent nanoprobe for imaging of target of interest. (b) Chemiluminescence response of CF-SPN2 to H_2O_2 at different concentrations (Inset, representative chemiluminescence images of CF-SPN2 response to indicated concentrations of H_2O_2). (c) Representative chemiluminescence images of mice receiving intraperitoneal injection of APAP or saline (control), followed by intravenous injection of CF-SPN2 at 18 min. (d) Representative chemiluminescence images of mice receiving intraperitoneal injection of INH or saline (control), followed by intravenous injection of CF-SPN2 at 10 min. (e) Schematic illustration of the molecular structure of SP5 and $\text{O}_2^{\bullet-}$ sensing using SPN5. (f) Excitation/emission spectra (black lines) and chemiluminescence spectrum (red line) of SPN5 in the presence of 10 μM $\text{O}_2^{\bullet-}$. (g) Linear relationship between chemiluminescence intensity of SPN5 and $\text{O}_2^{\bullet-}$ concentration. (h) Representative chemiluminescence images of tumor (I), tumor + Tiron (II) and normal tissue (III) in mice followed by the local injection of SPN5. (b–d) were reprinted with permission from ref. 87. By permission of Nature Publishing Group. (f–h) were reprinted with permission from ref. 94. Copyright 2016, American Chemical Society.

Scheme 2. Chemical structures of some SPs used for preparation of SPNs.

3.2. Chemiluminescent small-molecular probes

A class of chemiluminescent small molecular probes based on dioxetane have been developed for imaging of different disease biomarkers.⁹⁵⁻⁹⁹ These small-molecular probes do not require any prior oxidation steps to trigger chemiexcitation because the dioxetane compounds are pre-oxidized in a thermally stable form, which is different from most of other chemiluminescent nanoprobes.¹⁰⁰⁻¹⁰² Such chemiluminescence is excited upon removal of the protecting groups (PGs), which results in an electron transfer from a phenolate to the peroxide bond of dioxetane (Fig. 6a).^{96, 103} To optimize the optical properties for *in vivo* imaging applications, various electron-withdrawing groups (EWGs) have been conjugated at the ortho position of phenol, leading to a 1000-fold increase in the chemiluminescence quantum efficiency and red-shifted emission in the NIR region.^{98, 104}

Chemiluminescent small molecular probes using a 2-aza-Cope reactive trigger as the PG were developed for activatable detection of formaldehyde (FA) in living animals.¹⁰⁵ Specific reaction between FA and the 2-aza-Cope reactive trigger within probes resulted in an immolation of the two-carbon linker, generating free phenoxy-dioxetane that subsequently decomposed to produce chemiexcitation. Two different EWGs were conjugated to the dioxetane scaffolds to allow the visible (CFAP540 probe) and NIR (CFAP700 probe) emission, respectively (Fig. 6b). These two probes displayed a robust response to FA, with maximum light emission increased 500-fold for CFAP540 and 33-fold for CFAP700 (Fig. 6c-d). Due to the superior signal-to-noise response, CFAP540 detected FA at the concentration as low as 10 μM , while CFAP700 could detect 25–50 μM levels of FA. Meanwhile, the probes showed a high selectivity for FA with minimal interference from other biologically relevant aldehydes and molecules. In addition, CFAP540 was cell-trappable because of the exist of methyl ester substituent, making it much suitable for FA detection in living cells (Fig. 6e). Whereas, the NIR emission profile of CFAP700 was exploited for imaging of exogenously injected FA (Fig. 6f) and endogenous FA released from

folate metabolism in living mice (Fig. 6g). Beyond this example, various other responsive PGs have been applied to trigger the chemiexcitation, allowing for imaging of different targets of interest in living biological systems (Fig. 6h), including H_2O_2 ,⁹⁸ H_2S ,¹⁰⁶ hypoxia,¹⁰⁷ β -galactosidase,^{104, 108, 109} and Cathepsin B.⁹⁵ (Table 2).

4. Molecular afterglow imaging

Afterglow imaging is a luminescent process that occurs after the removal of illumination source, providing a promising imaging technique for *in vivo* disease diagnosis owing to its elimination of real-time excitation and minimum tissue autofluorescence.¹¹⁰⁻¹¹² Thus afterglow imaging has the advantages of relatively high SBRs and high imaging sensitivity. The current afterglow luminescence in experimental animals has been largely relied on inorganic nanoparticles. However, these inorganic contrast agents have the concerns of low brightness, short luminescence time and underlying toxicity.¹¹³⁻¹¹⁵ As alternative candidates, a category of biocompatible organic nanoparticles with an ultralong luminescence lifetime and relatively high brightness have been developed, permitting high quantify *in vivo* afterglow imaging applications.¹¹⁶

To provide ideal organic afterglow probes, Pu's group used a top-down approach to greatly preserve the aggregates of phosphorescent molecules within the nanoparticles (SONs-T).¹¹⁷ As compared to the nanoparticles made from a bottom-up approach (SONs-B), SONs-T facilitated the formation of H-aggregates more due to their stronger molecular packing. Since H-aggregation provided lower energy levels that served as additional energy traps to effectively stabilize the triplet excited states, SONs-T had 1.75-fold longer phosphorescence lifetime (861 ms) relative to SONs-B (492 ms). Such an ultralong phosphorescence could be repeatedly activated with nearly no loss in the total intensity, allowing for longitudinal afterglow imaging of lymph nodes in living mice with a SBR as high as 40.

Fig. 6 (a) Chemiluminescent activation pathway of dioxetane probes for imaging of target of interest (PG, protecting groups; ET, electron transfer; EWG, electron-withdrawing groups). (b) Chemical structures of CFAP540 and CFAP700. Chemiluminescence kinetic profiles of (c) CFAP540 and (d) CFAP700 to 0 mM FA (blue) and 10 mM FA (green or red). (e) Representative chemiluminescence cell images of HEK293 cells after treatment with CFAP540 and FA at different concentrations. (f) Chemiluminescence imaging of CFAP700 in response to exogenously injected FA at different doses. (g) Chemiluminescence imaging of mice after an intraperitoneal injection of tetrahydrofolate (THF), then aqueous solution of sodium bisulfite (NaHSO₃) or water, then CFAP700. (h) Other responsive PG used for chemiluminescence imaging of different targets of interest. (c–g) were reprinted with permission from ref. 105. Copyright 2018 John Wiley & Sons Ltd.

Despite the feasibility to use SONs-T for *in vivo* afterglow imaging, their brightness was low and their emission was in the visible region. To overcome these limitations, Pu's group have further developed a class of biodegradable SPNs showing a more than 100-fold higher brightness than inorganic probes and emitting long NIR luminescence for molecular afterglow imaging in living mice.¹¹⁸ We demonstrated that the chemical structures of SPs, rather than the nanoparticle structures played an indispensable role in producing the afterglow luminescence, and only phenylenevinylene (PPV)-based SPs with strong electron-donating substituents could afford detectable afterglow signals. The mechanism of afterglow luminescence was described as follow (Fig. 7a): light irradiation of PPVs generated ¹O₂ that reacted with the vinylene bond (C=C) in PPVs to form a dioxetane intermediate; the unstable intermediate then spontaneously degraded into a PPV-aldehyde to produce luminescence. Among all PPV-based SPs, MEHPPV (SP6) displayed the highest afterglow intensity and was thus transformed into the nanoparticles doped with NCBS at a weight percentage of 5% (SPN6-NCBS5). NCBS doping contributed to an amplified afterglow owing to the increased generation of ¹O₂ by NCBS, and modulation of the emission into the NIR optical window through the energy transfer (Fig. 7b–c). Such an amplified SPN6-NCBS5 allowed an afterglow imaging penetration depth of 4 cm *in vitro* and 1.7 cm

through a body of mouse, as well as permitted fast and high-contrast afterglow imaging of lymph nodes and tumors in living mice with a SBR more than 127-fold higher than that obtained by NIR fluorescence imaging (Fig. 7d–e).

SPN6-NCBS5 could be developed into a smart activatable afterglow probe (termed as SPN6-thiol) after a covalent conjugation with an electron-withdrawing quencher (2,4-dinitrophenylsulfonyl, DNBS) through sulfonamide bonds for specific and sensitive imaging of biothiols. Biothiols including glutathione (GSH), homocysteine (Hcy) and cysteine (Cys) are the major portions of antioxidants that defend against oxidative stress in living body. The luminescence of SPN6-thiol was substantially quenched at its initial afterglow “off” state due to the intra-particle efficient electron transfer, while the sulfonamide bonds on the surface of SPN6-thiol, when activated by biothiols, could be cleaved to release DNBS from nanoparticles (Fig. 7f). As a result, the electron transfer was abolished to turn on afterglow signals (Fig. 7g), allowing for high-sensitive early-stage imaging of APAP-induced hepatotoxicity (Fig. 7h). Because the APAP treatment induced oxidative and nitrosative stress and thus consumed biothiols, the afterglow signals for the APAP-injected mice were 1.99-fold lower than that for the control mice without APAP injection at t = 2 h post-injection of SPN6-thiol.

Fig. 7 (a) Proposed mechanism for the afterglow luminescence of PPV-based SPs. R_1 and R_2 are alkyl chains. (b) Schematic illustration of the proposed mechanism for 1O_2 -sensitizer-amplified NIR afterglow of SPN6-NCBS5. (c) NIR-induced afterglow luminescence spectra of SPN6 without the NCBS doping and SPN6-NCBS5 dispersed in 1x PBS buffer. (d) Fluorescence (left) and afterglow luminescence (right) imaging of a lymph node in a living mouse at $t = 65$ min after intradermal injection of SPN6-NCBS5 to the forepaw of mouse. (e) Representative afterglow images (upper panel) and fluorescence images (lower panel) of a tumor in a living mouse at representative time points after systemic administration of SPN6-NCBS5 via tail vein injection. The tumor was on the right shoulder as indicated by the white dashed circles and arrows. (f) Schematic illustration of the design and turn-on mechanism of biothiol activatable afterglow probe (SPN6-thiol). (g) Afterglow luminescence spectra of SPN6-thiol in the absence or presence of Cys. (h) Representative afterglow luminescence images of mice with or without intraperitoneal injection of APAP, followed by the administration of SPN6-thiol via tail vein at $t = 20$ min later. Afterglow luminescence images were acquired for 180 s after irradiation at 808 nm (1 W/cm^2) for 1 min. Reprinted with permission from ref. 118. By permission of Nature Publishing Group.

To optimize the biodistribution of afterglow probes, Pu's group subsequently synthesized an amphiphilic polymer composed of a hydrophobic PPV backbone and hydrophilic PEG brushes (Fig. 8a).¹¹⁹ This PPV-derived amphiphile was able to self-assemble into nanoparticles (SPPVN) that had a smaller size (24 nm), higher energy transfer efficiency and brighter afterglow luminescence as compared with the counterpart SPN6 (34 nm) prepared from nanoprecipitation. Due to the smaller size and higher surface PEG density, SPPVN showed a faster and better accumulation into tumors than SPN6, allowing for afterglow imaging of xenograft tumors ($\approx 5 \text{ mm}^3$) as early as 40 min post intravenous injection (Fig. 8b). In sharp contrast, the tumors were only detectable with fluorescence imaging at 4 h post-injection for SPPVN, and at 4 h and 8 h post-injection with afterglow imaging and fluorescence imaging for SPN6, respectively. Such enhanced afterglow imaging

performance of SPPVN also enabled detection of early-stage tumors with the size as small as $\approx 1 \text{ mm}^3$ and tiny peritoneal metastatic tumors that were almost invisible to naked eyes (Fig. 8c).

Because afterglow luminescence from PPVs is dependent on the generation of 1O_2 , it shows an oxygen-sensitive nature that can be utilized for *in vivo* imaging of tissue oxygen levels.¹¹⁹ In addition, the temperature-dependent luminescence is another intrinsic characteristic for afterglow of PPVs, because the photo-releasing decomposition of dioxetane intermediate is thermodynamically controlled.¹¹⁸ Therefore, Pu's group recently developed a semiconducting polymer nanococktail (SPN_{CT}) that had a temperature-correlated afterglow luminescence for cancer photothermal therapy (PTT).¹²⁰ This SPN_{CT} was made of the amphiphilic PEG grafted PPV and a NIR absorbing poly(silolodithiophene-alt-diketopyrrolopyrrole) (PCSD, SP7, Fig. 8a),

which served as the temperature-monitoring sensor and the photothermal agent, respectively (Fig. 8d). NIR laser irradiation of SPN_{CT} elevated the temperature, which in turn accelerated decomposition of the PPV-dioxetane, affording increased afterglow signal. Such an afterglow imaging of SPN_{CT} facilitated real-time temperature monitoring during PTT without light excitation (Fig. 8e).

5. PA imaging

PA imaging has emerged as a promising imaging modality for providing three-dimensional (3D) images with real-time correlation, deep imaging penetration depth up to 5–7 cm and relatively high spatial resolution.^{121–124} During PA imaging, the optical absorbers (imaging agents) in biological tissues of interest are irradiated by a short-pulsed laser and convert the photon energy into heat, leading to the generation of transient thermo-elastic expansion and thus wideband acoustic waves. These ultrasonic waves can be recorded by ultrasonic transducers to produce PA images. Therefore, PA imaging possesses the merits of optical and acoustic methods including sensitive optical absorption contrast and minimized acoustic scattering.¹²⁵

5.1. “Always on” PA probes

To provide detectable variations for PA imaging in deep tissue, a broad range of exogenous imaging contrast agents have been designed and developed.^{126–128} Owing to the good photo-stability, high mass absorption coefficient and excellent photothermal

conversion efficiency, OSMs have been exploited as a class of “always on” PA probes, allowing for *in vivo* imaging of blood vessels,¹²⁹ lymph nodes,^{130, 131} protein sulfenic acids⁷⁵ thrombus,¹³² tumors^{133–135} and so on.

PA imaging of blood vessels has emerged as a challenging undertaking on the molecular imaging front, in which blood generates interferential PA signal because of its strong light-absorption property, leading to poor imaging performance, sensitivity and SBR.¹²⁹ Thereby, there is a demand to develop PA imaging probes exhibiting higher PA signals relative to the surrounding vasculatures. In this regard, an organic PA contrast agent with a broad and strong optical absorption in the visible-light region has been developed through the nanoprecipitation of a newly designed PIID-DTBT (SP8, Scheme 2) with functional poly(styrene-co-maleic anhydride) (PSMA).¹²⁹ The higher PA intensity than vasculatures under 700 nm illumination made SPN8 be a highly effective contrast agent for PA imaging of blood vessels. In another study, Liu and co-workers synthesized a SP with strong absorption in the NIR window, poly[9,9-bis(4-(2-ethylhexyl)phenyl)fluorene-alt-co-6,7-bis(4-(hexyloxy)phenyl)-4,9-di(thiophen-2-yl)-thiadiazoloquinoxaline] (PFTTQ, SP9, Fig. 9a), and formulated it into SPN9, which displayed a high PA signal benefited from its large absorption coefficient and high non-radiative quantum yield of almost 100%.²¹ The authors demonstrated the feasibility to utilize SPN9 as a PA probe for brain vasculature imaging with a ~3-fold signal enhancing.

Fig. 8 (a) Molecular structures of PPV-PEG and SP7 used for preparation of nanoparticles for afterglow imaging. (b) Representative *in vivo* fluorescence and afterglow luminescence images of tumors in living mice at 40 min after intravenous injection of SPPVN or SPN6. (c) Fluorescence and afterglow images of mice after skin resection to expose the abdominal cavity at 1.5 h post-injection of SPPVN or SPN6. Tumor regions are marked by white circles. (d) Schematic illustration of the mechanism for photothermally enhanced afterglow luminescence of SPNCT. (e) Representative thermal and afterglow images of tumor in living mice at 24 h after intravenous injection of SPNCT under laser irradiation at 808 nm for different times. (b–c) were reprinted with permission from ref. 119. Copyright 2018 John Wiley & Sons Ltd. (d–e) were reprinted with permission from ref. 120. Copyright 2018 John Wiley & Sons Ltd.

Noninvasive PA imaging of lymph nodes is highly desirable for the detection of cancer metastasis and lymph node biopsy.¹³⁰ To fully utilize the potential of SPNs for PA imaging of lymph nodes, SP10 (Fig. 9b) with strong absorption in the NIR window was transformed into SPN10, which produced a higher PA signal and showed better photo-stability relative to the most commonly used SWNTs and gold nanorods on a per mass basis.¹³¹ Systemic administration of SPN10 permitted whole-body PA imaging of lymph nodes in living mice with a high SBR of 13.3, at a low injection mass (50 μ g) (Fig. 9c). Similarly, the utilization of newly designed SONs that show strong NIR absorption, excellent imaging contrast and quick body clearance for effective PA imaging of sentinel lymph nodes has also been reported.¹³⁰

Pu's group recently reported a reaction-based nanoprobe (rSPN10) for imaging of protein sulfenic acids,⁷⁵ a class of proteins whose cysteines were oxidized into sulfenic acid (SOH) by ROS.^{136, 137} The surface of rSPN10 was modified with 1,3-cyclohexanedione groups that could covalently bind to SOH sites (Fig. 9d).⁷⁵ Such a nanoparticle reaction enabled monitoring of the dynamic changes in the concentration of protein sulfenic acids under elevated oxidative stress in cancer cells, and real-time PA imaging of protein sulfenic acids in the tumor microenvironment of living mice (Fig. 9e).

In addition, the feasibility to utilize NIR absorbed nanoprobes, self-assembled from amphiphilic perylene-3,4,9,10-tetracarboxylic

diimide (PDI, Fig. 9f) derivatives for selective PA imaging of early thrombus in living mice was demonstrated.¹³² Surface modification of these nanoparticles with RGD peptides enabled their specific bindings to Glycoprotein IIb/IIIa overexpressed in early thrombus. In combination with other superiorities, such as relatively high PA intensity, good colloidal stability and long blood-circulating half-life (around 22 h), RGD-PDI nanoparticles enabled the visualization of FeCl₃-induced mouse model of jugular vein thrombus, affording nearly 4-fold higher PA signal relative to that in the control and old thrombus groups (Fig. 9g).

Beyond the abovementioned imaging applications, a large variety of OSMs have been developed and used for PA imaging of tumors.¹³⁸⁻¹⁴⁰ In particularly, a series of SPNs composed of low-bandgap diketopyrrolopyrrole (DPP)-based SPs were synthesized to systematically study and compare their PA properties.¹³³ SPNs self-assembled from amphiphilic SPs attached with PEG side chains often have a small size and PEG-passivated surface, and thus can efficiently accumulate into tumors of living mice, permitting PA imaging of tumors with high SBR.^{134, 135} Moreover, rational designs of molecular or nanoparticle structures contributed to the generation of OSMs exhibiting amplified PA brightness, leading to sensitive tumor imaging.

Fig. 9 (a) Chemical structure of SP9 used for preparation of SPN9 for PA imaging of blood vessels. (b) Chemical structure of SP10 used for preparation of SPN10. (c) PA images of mouse lymph nodes following tail vein injection of SPN10. Images represent transverse slices through the lymph nodes. BLN, brachial lymph node; ILN, inguinal lymph node; SCLN, superficial cervical lymph node. (d) Illustration of the mechanism of the specific reaction between rSPN10 and BSA-SOH. (e) PA images with axial view of tumors at 24 h time-points after systemic administration of rSPN10 or SPN10 via tail vein injection. (f) Chemical structure of PDI used for preparation of RGD-PDI nanoparticles. (g) PA images of the mouse jugular veins with early or old thrombus after an intravenous injection with RGD-PDI nanoparticles, RAD-PDI (non-targeting) nanoparticles, blocking group, and PBS (Control) at 24 h post-injection treatment time. (c) was reprinted with permission from ref. 131. By permission of Nature Publishing Group. (d–e) were reprinted with permission from ref. 75. Copyright 2016, American Chemical Society. (g) was reprinted with permission from ref. 132. Copyright 2017, American Chemical Society.

Fig. 10 (a) Chemical structures of SP11-14 used for preparation of SPNs for amplified PA imaging of tumors. (b) Schematic illustration of intra-particle molecular orbital engineering design approach for amplified tumor PA imaging. (c) Schematic illustration of self-quenched molecular engineering approach to synthesize self-quenched SPN12 via nanoprecipitation for enhanced tumor PA imaging. (d) Schematic illustration of molecular structure engineering to synthesize SPN13 from vinylene bond incorporated SP13 for enhanced tumor PA imaging. (e) Schematic illustration of surface engineering approach to synthesize SPN14-SiO₂ for amplified tumor PA imaging.

For instance, intra-particle molecular orbital engineering that favored photo-induced electron transfer (PET) resulted in quenched fluorescence and enhanced heat generation for nanoparticles upon laser irradiation, leading to amplified PA signal.¹⁴¹ This approach was demonstrated by doping (6,6)-phenyl-C71-butyric acid methyl ester (PC70BM) that acted as an electron acceptor into SPN10, wherein SP10 served as the electron donor through nanoprecipitation (Fig. 10b).¹⁴¹ The HOMO and LUMO of SP10 and PC70BM were ideally aligned to facilitate intra-particle PET. The PA brightness of PC70BM doped SPN10 was increased by 2.6-fold relative to the nondoped SPN10 at the same concentration, and thus afforded 1.8-fold PA signal amplification to clearly delineate tumors.

As an alternative to intra-particle engineering, a self-quenched molecular engineering approach that incorporated an electron-deficient structure unit into polymer backbones has been proposed to amplify PA brightness of SPNs.¹⁴² This was feasible because the electron-deficient units promoted the nonradiative decay, leading to fluorescence quenching and thus enhanced thermal deactivation. Doping of benzothiadiazole (BT) as an electron-deficient structure unit with an amount of 10% into the backbone of PDPF (SP11) yielded SP12 (Fig. 10a), and its corresponding SPN12 after nanoprecipitation with amphiphilic triblock copolymer (PEG-b-PPG-b-PEG) showed a 1.7-fold PA signal amplification as compared with the control SPN11 (Fig. 10c). Therefore, SPN12 after surface conjugations of cyclic-RGD was highly promising to effectively delineate the xenografted 4T1 tumors in living mice with the PA intensity increased by 4.7-fold after systemic administration.

Another strategy to obtain SPNs with amplified PA signals is to incorporate vinylene bond as a structural unit into the backbone of SPs.¹⁴³ Because of the low molecular weight and properly planar conformation, vinylene bonds facilitated the electron delocalization and increased the mass absorption coefficients, improving the photothermal conversion efficiencies and amplifying PA signals. As demonstrated in a recent study of Pu's group, SP13 (Fig. 10a) with the incorporation of vinylene bond was nanoprecipitated with an amphiphilic polymer (PLGA-PEG) to generate SPN13, showing a 2.2-fold higher PA signal relative to its nanoparticle counterpart without vinylene bonds (Fig. 10d), which allowed tumor PA imaging in living mice in a more sensitive and effective way.¹⁴³ Moreover, SPN13 could be efficiently digested in the presence of peroxidase owing to the oxidizable nature of vinylene bonds, representing a class of biodegradable agents for cancer theranostics.

In addition to these approaches relying on the molecular engineering of SPs, Pu et al. have found that surface coating of SPNs with silica layers amplified PA brightness, largely due to the higher heat interfacial conductance for the interface between silica layer and water relative to that between the SP core and water.¹⁴⁴ Both poly(cyclopentadithiophene-alt-diketopyrrolopyrrole) (SP14, Fig. 10a) and SP10 were developed into silica-coated SPNs through nanoprecipitation and surface coating of a thin silica shell. Silica-coated SPNs had a ~1.4 higher PA amplitudes than that of the corresponding uncoated SPNs (Fig. 10e).

5.2. Activatable PA probes

Rational design of molecular structure leads to the generation of a number of organic optical PA contrast agents that have shown promising applications in the visualization of biological structures and disease biomarkers. However, these “always on” probes often suffer from a strong background signal and thus low SBR, even if with conjugations of a specific targeting ligand.¹²¹ On the contrary, activatable PA probes that can only be activated by specific biomolecular recognition or interaction serve as exciting candidates for detection of disease-related biomarkers and physiological indexes with improved imaging sensitivity and specificity (Table 2).

An activatable PA probe (SPN10) was developed through nanoprecipitation of SP10 and ROS-responsive IR775S (Fig. 11a) for real-time imaging of ONOO⁻ and ClO⁻ in drug-induced mice.¹³¹ Upon activation with ONOO⁻ or ClO⁻, the PA peak of SPN10 at 700 nm corresponded to SP10 remained nearly the same, while the peak at 735 nm decreased significantly and that at 820 nm almost disappeared owing to the ROS-mediated rapid oxidative decomposition of IR775S (Fig. 11b), resulting in ratiometric PA imaging (PA₇₀₀/PA₈₂₀) of ONOO⁻/ClO⁻. With a high selectivity towards ONOO⁻/ClO⁻ and low LOD of ~50 nM, SPN10 was used as an activatable PA contrast agent for *in vivo* real-time imaging of ROS in the zymosan-induced mouse models of acute edema (Fig. 11c). The ratiometric PA signal (PA₇₀₀/PA₈₂₀) gradually increased to 2.7 at 120 min post-injection of SPN10 for zymosan-treated mice, about 2-fold higher relative to the control mice (Fig. 11d).

To detect drug-induced ROS in tumor modes, Pu's group recently developed a semiconducting macromolecular activatable probe based on the nanoparticle regrowth enhanced PA signals.¹⁴⁵ This macromolecular probe (PCBP) was comprised of a NIR absorbing phthalocyanine (Pc, scheme 1) as the hydrophobic core and four PEG as the hydrophilic arms. These two components were covalently linked by responsive phenylboronic acid pinacol ester groups that could be specifically cleaved by ROS including H₂O₂ and ONOO⁻. Such a ROS response allowed it to undergo a self-immolative process to release PEG and regrowth of remaining Pc segments into large nanoparticles *via* increased hydrophobic interaction and π - π stacking. Larger nanoparticles were beneficial to heat transfer, thus displaying an enhanced PA signal for imaging of ROS. After an intravenous injection of PCBP into tumor-bearing mice, PA signal in the tumors pre-treated with D,L-Buthionine-(S,R)-sulphoximine (BSO) to have an elevated ROS level was 2.03 times higher than that of untreated tumors. It should be noted that the small size of PCBP contributed to its effective penetration into the tumors of living mice, while the ROS-activated nanoparticle regrowth could extend the retention time along with an amplified PA signal, allowing for imaging of ROS during chemotherapy.

To increase the specificity of activatable PA probes toward a certain ROS, Pu's group synthesized an activatable NIR-absorbing SON for real-time ratiometric PA imaging of ONOO⁻ in the tumor tissues of living mice.¹⁴⁶ Such a probe (termed as SON-BT) was composed of a boronate-caged boron-dipyrromethene dye (BBD, Scheme 1) as the primary component for ONOO⁻ sensing and a tris(pentafluorophenyl)borane (TPFB, Scheme 1) as the secondary component. BBD responded to both ONOO⁻ and H₂O₂, but the doping of large excess bulky boranes inhibited the reaction between BBD and H₂O₂, probably owing to the weaker oxidative capability of H₂O₂ relative to ONOO⁻, affording the specific response

of SON-BT only toward ONOO⁻. Upon addition of ONOO⁻, PA signal at 680 nm (PA₆₈₀) remained nearly unchanged, while that at 750 nm (PA₇₅₀) significantly increased due to the rapid oxidative cleavage of borate ester moiety of BBD by ONOO⁻, generating an anionic phenoxide product with the characteristic maximum absorption at 745 nm. The ratiometric PA signal (PA₇₅₀/PA₆₈₀) of SON-BT increased linearly as the concentration of ONOO⁻, with a LOD of ~100 nM, and thus potentially be used to monitor the variation of ONOO⁻ levels in xenograft tumor modes after drug treatment.

Pu's group have also proposed a self-assembly approach to synthesize an activatable and degradable PA probe for *in vivo* specific imaging of ClO⁻.¹⁴⁷ In this approach, a semiconducting oligomer (SO1, scheme 1) with the integration of a π -conjugated but ClO⁻ degradable backbone (phenothiazine) was grafted with PEG chains to allow its spontaneous encapsulation of ROS-inert NCBS dye, providing a SON (SON-N) through π - π stacking and hydrophobic interactions. The presence of ClO⁻ induced the degradation of SON-N, whose PA signal at 680 nm was hence declined, while its PA signal at 780 nm from NCBS remained nearly unchanged. These spectral changes allowed ratiometric PA imaging (PA₇₈₀/PA₆₈₀) specifically toward ClO⁻ with a LOD of 0.70 μ M. The feasibility of SON-N to be used as an activatable probe for *in vivo* imaging of ClO⁻ was demonstrated on subcutaneous xenograft tumor models.

In addition to ROS detection, activatable organic PA nanoprobe can be used for *in vivo* ratiometric imaging of pH. As a very critical physiological parameter, pH participates in the cellular and tissue homeostasis and its aberrance has distinct influence on biomolecular structure, protein activity and metabolic rate.¹⁴⁸⁻¹⁵¹ Pu's group recently developed an activatable nanoprobe (termed as SON-BDP) with an amplified PA signal for pH imaging in the tumor tissues.¹⁵² SON-BDP displayed a quenched fluorescence and in turn enhanced PA brightness, because of the PET between encapsulated SO2 acting as the inert PA matrix and BODIPY dye (pH-BDP) serving as both the PA enhancer and pH indicator (Fig. 11e). Hydroxyl group on the backbone of pH-BDP underwent protonation in an acidic environment to generate signal alteration. In the pH range from 7.4 to 5.5, absorbance of pH-BDP at 750 nm decreased as a function with pH, while that of SO2 at 680 nm barely changed. As a result, the PA signals at 750 and 680 nm changed accordingly (Fig. 11f), permitting ratiometric PA imaging (PA₆₈₀/PA₇₅₀) of pH in tumors after an intravenous injection of SON-BDP (Fig. 11g-h), as well as pH estimation of tumor at ~6.3 according to the calibration curve.

In another work, Liu and his colleagues reported another activatable pH probe *via* self-assembly of benzo[]phenoxazine (BPOx) and IR825 (scheme 1) with human serum albumin (HSA).¹²² Within this probe (HAS-BPOx-IR825), IR825 was inert to pH change, acting as an internal reference, while BPOx responded to pH, because of its protonation and intramolecular charge-transfer process with pH change, serving as a pH indicator. As the decrease of pH values, the PA signal at 680 nm for BPOx displayed an obvious increase, while that at 825 nm for IR825 remained unchanged, affording a good pH-dependent ratiometric PA imaging (PA₆₈₀/PA₈₂₅) in the range of 5.0–7.0. This ratiometric response combining with quantitative analysis using HAS-BOPx-IR825 as an activatable probe was used for PA imaging of pH in tumor microenvironment, as well as pH variation after injection of buffer solution.

Fig. 11 (a) Proposed mechanism for ROS PA imaging. (b) Representative PA spectra of SPN10 in the absence and presence of ROS. (c) Representative PA/US overlaid images of saline-treated (-zymosan) and zymosan-treated (+zymosan) regions in the thigh of living mice. SPN10 was intramuscularly injected into the thigh 20 min after zymosan treatment. (d) Ratio of PA amplitude at 700 nm to that at 820 nm (PA700/PA820) as a function of time post-injection of SPN10. (e) chemical structures of SO₂ and pH-BDP used for pH sensing. (f) PA images of the SON-BDP solution at pH = 7.4, 6.4, or 5.5. A pulsed laser was turned to 680 or 750 nm for ratiometric imaging. (g) Schematic illustration of the amplification and sensing of SON-BDP-based activatable probe for pH. (h) Representative ratiometric PA images ($\Delta PA_{680}/\Delta PA_{750}$) of tumors in living mice at before (0 h) and 6 h after the intravenous administration of SON-BDP. (b–d) were reprinted with permission from ref. 131. By permission of Nature Publishing Group. (f–h) were reprinted with permission from ref. 152. Copyright 2016 John Wiley & Sons Ltd.

Some activatable organic PA probes have been utilized for imaging of enzyme activity in living animals, which is a meaningful but challenging task to decipher enzyme function and detect related diseases.^{153, 154} The smart PA response for enzymes is relied on their specific enzymatic cleavage of targeted substrates.^{155, 156} Such enzymatic cleavage can initiate the self-assembly or induce the separation of fluorophores so as to afford PA signal variations (Fig. 12a). Matrix metalloproteinases (MMPs) are the most prominent family of proteinases associated with tumors and can degrade the extracellular matrix (ECM) components.¹⁵⁵ Wang and co-workers developed an activatable PA probe targeted for MMPs (MMP-2 and MMP-9), which consisted of purpurin 18 as the NIR fluorophore, Pro-Leu-Gly-Val-Arg-Gly (PLGVRG) peptide as the enzyme-responsive linker, and RGD peptide as the cancer targeting ligand.¹⁵⁷ MMP-2/-9 triggered a specific cleavage of PLGVRG peptide and thereby self-assembly of residual purpurin 18 into nanoparticles, resulting in enhanced PA signals for imaging of overexpressed MMPs in xenografted mouse models. To specifically detect the MMP-2 activity in a follicular thyroid carcinoma, Gambhir and co-workers developed an activatable organic PA probe by conjugating an Alexa750-fluorophore and a black hole quencher

(BHQ3) on each side of a MMP-2 specific cleavable cell-penetrating peptide, CXEEEXPLGLAGRRRRRXK.¹⁵⁶ Due to the presence of BHQ3 and Alexa750, this probe showed two PA peaks at 675 and 750 nm. MMP-2 induced a specific cleavage of peptide, leading to separation of these two fluorophores. Subsequently, BHQ3 conjugated to the cell-penetrating peptide effectively accumulated into tumors, while the Alexa750 fragment diffused away, resulting in enhanced subtraction PA signals. β -Galactosidase is an important enzyme for living organisms as it break down lactose into galactose and glucose, providing energy and carbon source for metabolism.¹⁵⁸ Pu's group developed an activatable PA probe composed of a D-galactose caged NIR hemicyanine dye (CyOH, Fig. 12b) and linked PEG chains for PA imaging of β -Galactosidase.¹⁵⁹ The designed probe, termed as CyGal-P, was initially in its PA "turn-off" state owing to the diminished electron-donating ability of oxygen atoms in the caged CyOH. While β -Galactosidase-mediated specific cleavage of D-galactose moiety activated PA signal for imaging of β -Galactosidase in associated tumor nodes (Fig. 12c).

Apart from the detection of ROS, pH and enzyme, an activatable PA probe (termed as HyP-1) was developed for imaging of hypoxic environment.¹⁶⁰ Hypoxia refers to a lower tissue oxygen level (<5

mmHg, 0.7%) than the physiological level (30–52 mmHg, 3.9–6.8%) in normal tissues, which is a pathogenic indicator of solid tumors.^{161,}

¹⁶² HyP-1 was made of unsymmetric aza-BODIPY containing a hypoxia-responsive trigger on one side and a methoxy substituent on the other side (Fig. 12d). The PA response of HyP-1 to hypoxia relied on the conversion of N-oxide to aniline, eliciting a red-shift of its absorbance from 670 to 760 nm for PA signal enhancing. Rapid and highly selective hypoxia-mediated activation of HyP-1 could be observed in cancer cells cultured under hypoxic conditions, even at an oxygen content as low as < 0.1%. Thereby, activatable HyP-1 was a promising probe for PA imaging of hypoxia in a tumor-bearing mouse model and a mouse hindlimb ischemia model (Fig. 12e–f).

5.3. NIR-II PA probes

In view of the advantages of NIR-II light, it is envisioned that PA imaging in the NIR-II window can give a further improved tissue penetration depth and enhanced imaging contrast, regardless of the slightly higher endogenous absorption in this window.¹⁶³ A desired imaging depth of 11.6 cm in chicken breast tissues has been reached through PA imaging at 1064 nm using a phosphorus phthalocyanine-based organic probe.¹⁶⁴ However, a very small amount of NIR-II PA imaging applications have been demonstrated, which is largely attributed to the lack of available contrast agents.

Pu's group synthesized an organic imaging probe (SPN15) with a broadband absorption in both the NIR-I and NIR-II windows and applied it for NIR-II PA imaging (Fig. 13a–b).¹⁶⁵ Rational molecular engineering afforded a D–A1–D–A2 structured SP15 (Fig. 13a), which exhibited a lower bandgap and subsequently strong absorption in the NIR-II window. After nanoprecipitation of SP15 with an amphiphilic PEG-b-PPG-b-PEG, the formed SPN15 endowed PA imaging at 1064 nm a 1.4-times higher signal-to-noise ratio (SNR) than that at 750 nm at the tissue depth of 3 cm. Such an excellent NIR-II PA imaging probe was applied for *in vivo* brain vasculature

imaging on living rats. At 70 min post-injection of SPN15 *via* tail vein, NIR-II PA imaging at 1064 nm provided a higher contrast and clearer branch vessels relative to NIR-I PA imaging at 750 nm, showing a 1.5-times increase in SNR (Fig. 13c–d).

Using suitable contrast agents, NIR-II PA imaging has been extended to visualize a mouse brain tumor model, as demonstrated by Liu and co-workers.¹⁶⁶ In their work, a well-known electron donor benzodithiophene was copolymerized with a strongly electron-deficient acceptor benzobisthiadiazole to yield SP16 (Fig. 13a) that exhibited a red-shifted absorption in the NIR-II window with a relatively high extinction coefficient (22.6 L/g/cm). The corresponding SPN16 generated strong PA signals at 1064 nm that could be detectable even at a very low concentration of 6 µg/mL. In addition, PA imaging of orthotopic brain tumors in living mice was successfully performed at 1064 nm using intravenously injected SPN16 as a contrast agent. The tumors started to be clearly delineated at 3 h post-injection and the tumor regions as deep as 3.4 mm were clearly imaged at 24 h with a SBR of 59.

In another study, Mei and co-worker synthesized a completely new thienoisindigo (TII)-based SP17 (Fig. 13a) with a strong absorption in 1000–1350 nm and transformed it into SPN17 for tumor PA imaging in the NIR-II region.¹⁶⁷ Benefiting from the electron deficiency and high planarity of TII in SP17, the corresponding nanoparticles (SPN17) generated a strong PA signal in the NIR-II optical window, affording an imaging depth up to 5.3 cm with a SBR of 82 in chicken-breast tissue under 1064 nm excitation (Fig. 13e). With a local injection of SPN17, both the skin tissue of rats and tumors in living mice could be clearly depicted by PA imaging conducted at multiple wavelengths ranging from 800 to 1300 nm. As compared to the images from the control tumors, the PA signals in the tumor regions were enhanced by approximately 7.0- and 13.3-fold at 1100 and 1300 nm after an intratumor injection of SPN17, respectively (Fig. 13f).

Fig. 12 (a) Mechanisms of activatable PA probes for imaging of enzymes. (b) Schematic illustration of activation mechanism of CyGal-P for β-Galactosidase PA imaging. (c) Representative PA images of SKOV3 tumor-bearing mice at before (0 min) and 60 min post-injection time point after an intravenous injection of CyGal-P or CyGal. (d) Chemical structures of HyP-1 and red-HyP-1 and the activation mechanism for hypoxia PA imaging. (e) PA images (770 nm) of the tumor-bearing and control flank at before (0 h) and 5 h after intratumoral or subcutaneous injection of HyP-1. (f) PA images (770 nm) of the ischemic and control leg at before (0 h) and 1 h after intramuscular injection of HyP-1. (b–c) were reprinted with permission from ref. 159. Copyright 2018 John Wiley & Sons Ltd. (d–f) were reprinted with permission from ref. 160. By permission of Nature Publishing Group.

Table 2 Representative OSMs used for imaging of disease-related biomarkers.

Probes	Imaging mode	Biomarker	LOD	Imaging applications	Refs
Dye@peroxalate nanoparticles	Chemiluminescence	H ₂ O ₂	250 nM	Exogenous H ₂ O ₂ and LPS-induced acute inflammation	72
CPPO@AIE nanoparticles	Chemiluminescence	H ₂ O ₂	80 nM	LPS-induced arthritis	82
CPPO@DSA nanoparticles	Chemiluminescence	H ₂ O ₂	100 nM	LPS-induced arthritis and Duox2-derived viral infection in a nasal mucosa	83, 84
HPOX nanoparticles	Chemiluminescence	H ₂ O ₂	100 nM	LPS-induced inflammation	85
CPPO@Cy5 nanoparticles	Chemiluminescence	H ₂ O ₂	10 nM	Exogenous H ₂ O ₂ , LPS-induced acute inflammation and blood sugar level	86
CF-SPN	Chemiluminescence/ fluorescence	H ₂ O ₂ / ONOO ⁻	5 nM/10 nM	APAP and INH induced hepatotoxicity	87
SPN3	Chemiluminescence	H ₂ O ₂	5 nM	LPS induced peritonitis and neuroinflammation	88
SPN4	Chemiluminescence	H ₂ O ₂	1 nM	Inflammation in murine models of arthritis and peritonitis	89
TBD-AIE nanoparticles	Chemiluminescence	H ₂ O ₂	2 nM	Primary and peritoneal metastatic tumors	90
SPN5	Chemiluminescence	O ₂ ^{•-}	19.3 pM	Visualization of O ₂ ^{•-} level differences between normal and tumor tissues	94
NIR-II@Si	NIR-II fluorescence	H ₂ S	37 nM	H ₂ S-rich colorectal tumors	64
CHS-3	Chemiluminescence	H ₂ S	5.4 μM	H ₂ S in living animals	106
HyCL-1, HyCL-2	Chemiluminescence	Hypoxia	\	Nitroreductase, tissue oxygenation and hypoxia in tumors	107
Probe 3	Chemiluminescence	β-Gal	\	β-Galactosidase activity in tumor models	108
Probe 7	Chemiluminescence	H ₂ O ₂	30 nM	Endogenous H ₂ O ₂ in LPS induced inflammatory mice	98
CFAP540, CFAP700	Chemiluminescence	Formaldehyde	10 μM	Endogenous formaldehyde released from folate metabolism in living mice	105
SPN6-thiol	Afterglow	biothiols	0.6 μM	Drug-induced hepatotoxicity	118
SPN8	Photoacoustic	ONOO ⁻ /ClO ⁻	~50 nM	ROS in zymosan-induced mouse models of acute edema	131
PCBP	Photoacoustic	ONOO ⁻ / H ₂ O ₂	0.15 mM	Drug-induced ROS in tumor tissues	145
SON-BT	Photoacoustic	ONOO ⁻	100 nM	ONOO ⁻ levels in xenograft tumor modes after drug treatment	146
SON-N	Photoacoustic	ClO ⁻	0.70 μM	ClO ⁻ in tumor models	147
SON-BDP	Photoacoustic	pH	7.4–5.5	Tumor pH	152
HAS-BOPx-IR825	Photoacoustic	pH	5.0–7.0	Tumor pH and pH variation	122
CyGal-P	Photoacoustic	β-Gal	/	β-Galactosidase activated imaging and PTT of tumors	159
HyP-1	Photoacoustic	Hypoxic	/	Hypoxic in a mouse tumor and hindlimb ischemia model.	160

Fig 13. (a) Chemical structures of SP15, SP16 and SP17 used for PA imaging in the NIR-II window. (b) Schematic illustration of SPN15 for NIR-I and NIR-II PA imaging. (c) Representative PA images of rat cortex at 70 min post-injection of SPN15 at 750 (NIR-I) and 1064 nm (NIR-II). (d) SNR in decibels of brain cortex at 70 min post-injection of SPN15 at 750 and 1064 nm. (e) SNR of SPN17 at the concentration of 40 $\mu\text{g/mL}$ contained in a transparent plastic tube as a function of depth from illuminated tissue surface with a laser wavelength at 1064 nm. (f) In vivo PA/US imaging of a mouse tumor (dashed circles) with or without the intratumor injection of SPN17. (b–d) were reprinted with permission from ref. 165. Copyright 2017, American Chemical Society. (e–f) were reprinted with permission from ref. 167. Copyright 2017 John Wiley & Sons Ltd.

6. Phototherapy

Phototherapy has been considered as an emerging cancer therapeutic modality and attracted considerable attention, which is much different from conventional chemotherapy, radiotherapy, and gene therapy owing to its features including noninvasiveness, simple operation, negligible drug resistance, high temporal-spatial resolution and minimized adverse side effects.^{168–170} In general, phototherapy relies on the conversion of light into hyperthermia for photothermal therapy (PTT) or ROS for photodynamic therapy (PDT), thereby requiring light-absorbing materials as therapeutic agents.^{171–174} Nowadays, a large amount of nanomaterials have been extensively exploited as agents for cancer phototherapy, in particular, OSMs featured by strong light absorbance, tunable optical property and good photostability have become a recent addition to this class of therapeutic agents.¹⁷⁵

6.1. PTT

A broad range of OSMs with diverse optical cores, particle architectures and surface properties have been designed and used as photothermal therapeutic agents for cancer ablation.^{176–181} Their photothermal conversion efficiencies have been reported in the range of 33.3–65.0% (Table 3), which are equivalent to or even higher than those of the most commonly used inorganic nanoparticles, such as SWCNTs (38.3%),¹⁸² UCNPs (38.1%),¹⁸³ black phosphorus QDs (28.4%),¹⁸⁴ CuS nanocrystals (25.7%),¹⁸⁵ Au nanorods (21%),¹⁸⁶ and so on. Moreover, the PTT performance of OSMs can be further improved by modulating their chemical structures or components.¹⁸⁷

Quenching the fluorescence emission of OSMs is an effective way to improve the photothermal conversion efficiency. For example, a

facile intraparticle molecular orbital engineering approach was used to quench the fluorescence of SPN10 and ultimately enhance the PTT efficiency.¹⁴¹ SPN10 was doped with PC70BM at different amounts to favor the intra-particle PET. With increasing the doping amount of PC70BM, the fluorescence intensities of SPN10 decreased rapidly and the temperatures of nanoparticle solution under laser irradiation increased gradually. As a result, SPN10 with a 20% doping amount (SPN10-F20) showed a 1.3-fold maximum temperature increase as compared to the nondoped nanoparticles (SPN10-F0), affording a better efficiency for cancer PTT.

In addition to the intraparticle molecular orbital engineering approach, self-quenched molecular engineering approach was used to improve the photothermal properties of SPNs.¹³³ Pu and Rao et al. synthesized a series of D–A structured SPs with DPP as the electron acceptor copolymerized with three different electron-donating monomers and then transformed them into water-soluble SPNs.¹³³ Among these SPNs, SPN18 showed the highest fluorescence quenched efficiency, displaying the best photothermal property. Later on, this approach was used to develop other D–A structured molecules and SPs for enhanced PTT performance.^{188–191} For instance, Dong and colleagues recently synthesized a DPP derivative (termed as DPPCN-Fc, Scheme 1), wherein two ferrocene (Fc) moieties acted as the electron-donating unites to quench the fluorescence through a PET process.¹⁹⁰ After self-assembly into nanoparticles through π – π stacking interaction and hydrophobic interaction, DPPCN-Fc SONs exposed under an 730 nm laser irradiation resulted in a temperature elevation of 33.4 $^{\circ}\text{C}$ within 10 min and showed an amplified photothermal conversion efficiency reached up to 59.1%. In another study, Liu and co-workers conjugated a planar electron donor with the DPP electron acceptor

to quench the fluorescence of SP19 (Scheme 2) and thus increased the photothermal heating efficiency of SPN19 for cancer PTT.¹⁹¹

Since the light-harvesting property is directly related to the extinction coefficient that greatly modulates PTT efficiencies of SPNs, integration of light-harvesting units into D–A structured SPs has been recently exploited for further enhancing light-absorbing ability and in turn improving the photothermal conversion efficiency.¹⁹² Lee and co-authors synthesized a D–A structured SP20 (Scheme 2) contained a porphyrin-pyrene pendant acting as the light-harvesting unit.¹⁹² In addition, fluorescence quenching effect occurred during transformation of SP20 into nanoparticles through nanoprecipitation. The fluorescence quenching effect and enhanced light-absorbing ability synergistically improved the photothermal conversion efficiency of SPN20 to 62.3%.

In addition, the PTT performance of SPNs can be significantly improved by incorporating vinylene bond into the backbone of SPs.¹⁴³ In a recent study of Pu's group, incorporation of vinylene bond into SPN13 increased the maximum photothermal temperature and photothermal conversion efficiency by 1.2- and 2.4-fold, respectively, relative to the control SPN18 without vinylene bond (Fig. 14a–b).¹⁴³ Such an amplified PTT performance of SPN13 allowed effective ablation of tumors in living mice, while the tumors of SPN18-treated mice grew as fast as those of saline-treated mice (Fig. 14c).

Despite great efforts have been devoted to increase the photothermal conversion efficiencies of therapeutic agents, current PTT applications are only suited for superficial tumors due to the limited penetration depth of laser.¹⁹³ To achieve effective ablation of deep-seated tumors, NIR-II PTT has been recently used because of the intrinsic advantages of NIR-II light, including deeper tissue penetration and larger maximum permissible exposure (MPE) for skin as compared to the NIR-I light.^{193–195} To reveal the practical superiority of NIR-II over NIR-I light in term of cancer PTT, Pu's group have recently synthesized a semiconducting copolymer SP21 (Fig. 14d) that showed dual-peak absorption in both the NIR-I and NIR-II windows and formulated it into a nanoparticle (SPN21) through nanoprecipitation with PEG-b-PPG-b-PEG.¹⁹⁶ The deeper tissue penetration in combination with a higher MPE limit of 1064 nm laser (1 W/cm²) relative to that of 808 nm laser (0.3 W/cm²) allowed higher heat generation for SPN21 solution covered with chicken breast tissue of different thicknesses (Fig. 14e–g), even though the photothermal conversion efficiency of SPN21 at 1064 nm (43.4%) was nearly the same to that at 808 nm (44.9%). In a mimic deep-tissue tumor model at a tissue depth of 5 mm, intratumoral injected SPN21 with 1064 nm laser irradiation generated more heat and totally ablated tumors, while 808 nm laser failed to do so (Fig. 14h–i). Such a deep-tissue heating capability also allowed effective PPT of xenograft tumors after systemic administration of SPN21.

Fig. 14 (a) Photothermal heating and natural cooling cycles of SPN13 and SPN18 under a 808 nm laser irradiation with power density of 0.3 W/cm². (b) Thermal images of PBS, SPN13 and SPN18 at their respective maximum temperatures (both the corresponding maximum temperature values and photothermal conversion efficacies are indicated below the respective images). (c) Tumor growth curves of mice after systemic injection of saline, SPN13 or SPN18 with 808 nm laser irradiation. (d) Chemical structure of SP21 used for deep-tissue NIR-II PPT. (e) Scheme illustrates the deeper tissue penetration of 1064 nm laser relative to that of 808 nm. (f) Schematic illustration of photothermal study of the SPN21 solution at different tissue penetration depths. (g) Fitted exponential decay of temperature change of the SPN21 solution at different depths of chicken breast tissue under different laser conditions. (h) Mean tumor temperature as a function of laser irradiation time after intratumoral administration of SPN21. (i) Tumor growth curves of mice after intratumoral injection of SPN21 or saline with and without irradiation of 808 or 1064 nm laser. (a–c) were reprinted with permission from ref. 143. Copyright 2018, American Chemical Society. (e–i) were reprinted with permission from ref. 196. Copyright 2018 John Wiley & Sons Ltd.

In addition to SPN21, some other SPNs have been subsequently developed and used for NIR-II PTT of cancers so far.^{197, 198} For instance, a thieno-isoidindigo derivative-based D-A structured polymer (SP22, Scheme 2) with a maximum absorption peak at approximately 1107 nm was synthesized and formulated into nanoparticles.¹⁹⁷ The obtained SPN22 showed a superior photothermal conversion efficiency (66.4%) under laser irradiation at 1064 nm. Similarly, another SP (termed as TBDOPV-DT) possessing a same polymer backbone as SP22 was transformed into nanoparticles to have a narrow and strong absorbance in the NIR-II window and a photothermal conversion efficiency of 50%.¹⁹⁸

6.2. PDT

Due to the characteristic absorbance that can be utilized to produce ROS under laser irradiation, a number of organic semiconducting components have been formulated into nanoparticles for cancer PDT.^{199, 200} The nanoparticle forming can well overcome the drawbacks of hydrophobicity and poor photostability, as well as afford targeting delivery into tumors through surface modifications, leading to effective PDT performance.²⁰¹ Singlet oxygen ($^1\text{O}_2$) is the major ROS type generated during PDT with OSMs as agents.²⁰¹ However, PDT inherently requires O_2 to generate $^1\text{O}_2$ and thus its therapeutic efficacy is generally limited due to the hypoxic microenvironment of solid tumors.¹⁶¹ Therefore, there is an urgent demand to develop effective strategies to amplify or modulate the PDT performances.

Incorporation of photosensitizers into SPNs has been considered as a good method to enhance the PDT efficiency (Table 3).²⁰² Wu and colleagues have synthesized a class of photodynamic agents with amplified generation of $^1\text{O}_2$ by doping tetraphenylporphyrin (TPP) and Chlorin e6 (Ce6) into SPNs.^{203, 204} These designs allowed efficient resonance energy transfer (RET) from SPs to photosensitizer molecules, resulting in a 3.5–5-fold improvement in $^1\text{O}_2$ quantum yield, which led to better outcomes of cancer PDT in living mice. By utilizing the same RET mechanism, Han et al. subsequently constructed a novel dyad small molecule (termed as RET-BDP, Scheme 1) by connecting a fluorophore (distyryl-BODIPY) as the energy donor moiety to a photosensitizer (diiodo-distyryl-BODIPY) as the energy acceptor.²⁰⁵ After being encapsulated with folic acid modified PEG-b-PPG-b-PEG, RET-BDP molecules were formulated into SOMs that exhibited a 1.8-fold higher $^1\text{O}_2$ quantum yield relative to the nanoparticle counterparts of photosensitizer alone under NIR laser irradiation, which enabled a better therapeutic PDT effect against cancer cells both *in vitro* and *in vivo*. In addition to the RET mechanism, photosensitizer incorporation has induced a synergistic effect that can be utilized to enhance PDT efficiency.²⁰⁶ For instance, Dong and colleagues synthesized a novel D-A-D structured organic photosensitizer (termed as DPPBDPI, Scheme 1) by linking DPP and BODIPY with a benzene ring as a π bridge, wherein the synergistic effect of DPP and BODIPY elevated the $^1\text{O}_2$ quantum yield to 80%.²⁰⁶

The fact that some AIE photosensitizers display efficient $^1\text{O}_2$ generation in aggregate state makes AIE based nanoparticles be an alternative candidates for cancer PDT, but most of these photosensitizers often suffer from the drawbacks of absorption and

emission at short wavelengths, and low efficiency of $^1\text{O}_2$ generation.²⁰⁷ To address these issues, Liu's group used a new molecular design strategy to synthesize a novel AIE photosensitizer (termed as TPETCAQ, Scheme 1).²⁰⁸ Introduction of a phenyl ring between the dicyanovinyl (DC) group and methoxy-substituted tetraphenylethylene (TPE) within TPETCAQ led to increased $^1\text{O}_2$ generation efficiency while addition of another DC group to the acceptor red-shifted the absorption and emission to the NIR region. As such, the corresponding AIE nanoparticles after surface modification with a cell membrane penetration peptide enabled image-guided PDT of tumors.

In addition to the molecular engineering approaches, O_2 -evolving nanoparticles that can continuously generate O_2 have been developed and used to overcome the hypoxic microenvironment of tumor, representing a class of promising candidates for amplified PDT.²⁰⁹ Pu's group utilized an one-pot straightforward method to in-situ grow manganese dioxide (MnO_2) nanosheets on the surface of SiO_2 -coated SPN10, yielding a hybrid core-shell nanoparticle (SPN10-M1).²¹⁰ The surface coated MnO_2 nanosheets acted as a sacrificing component to convert H_2O_2 to O_2 under hypoxic and acidic tumor microenvironment, resulting in a 2.68-fold higher $^1\text{O}_2$ generation under NIR laser irradiation at 808 nm (Fig. 15a–c). With such an oxygen-evolution capability, SPN10-M1 displayed a better efficiency in eradicating cancer cells both *in vitro* and *in vivo* as compared with the uncoated counterpart (SPN10-M0).

To increase the therapeutic efficiency and selectivity for cancers but reduce the risk of damage to normal tissues of PDT, Pu's group developed a nanoceria doped hybrid SPN10 that could self-regulate the PDT properties according to tumor microenvironment for optimized therapy.²¹¹ The nanoceria functioned as a smart intraparticle modulator to decrease the ROS generation from SPN10 under a 808 laser irradiation at physiologically neutral environments, but increase the ROS generation at pathologically acidic conditions, respectively (Fig. 15d). Under laser irradiation, the nanoceria doped hybrid SPN10 (SPN10-C) showed a 2.9-fold improvement in killing cancer cells relative to that for SPN10 without doping nanoceria (SPN10-C0). Moreover, the self-regulated properties of SPN10-C not only afforded a better PDT performance for cancer ablation under NIR laser irradiation, but also resulted in reduced nonspecific damage to normal tissue (Fig. 15e).

In addition, a novel strategy called enzyme-enhanced phototherapy (EEPT) has been proposed for cancer treatment, showing a significantly enhanced therapeutic efficiency as compared to typical PDT.²¹² This nanoparticle platform was constructed by covalently conjugating glucose oxidase (GOx) onto the surface of SPN2 to afford SPN2-GOx, which efficiently catalyzed the conversion of glucose in tumor tissues into gluconic acid and H_2O_2 . Under laser irradiation, the in situ generated H_2O_2 was photolyzed to produce hydroxyl radical ($\bullet\text{OH}$), the most reactive ROS, to kill cancer cells (Fig. 15f). In view of the persistent immobilization of SPN2-GOx in tumors, a conspicuous inhibition of tumor growth was realized in xenograft-bearing mice (Fig. 15g), suggesting the potential for effective cancer treatment.

Fig 15. (a) Schematic illustration for the H_2O_2 -responsive mechanisms of SPN10-M1 and (b) the detailed mechanism of SPN10-M1 for amplified PDT in tumor. (c) The generation of $^1\text{O}_2$ determined by the increased fluorescence of SOSG for SPN10-M0 and SPN10-M1 with or without addition of H_2O_2 under hypoxic environment under NIR laser irradiation at 808 nm. (d) Schematic illustration of the synthesis of SPN10-C and the self-regulated photodynamic properties at physiologically neutral and pathologically acidic conditions. (e) Histological H&E staining for mouse tumor and muscle at 24 h after the treatment with saline, SPN10-C or SPN10-C0 under a 808 nm laser irradiation. (f) Schematic illustration of SPN2-GOx for generation and photolysis of H_2O_2 in tumor for therapy. (g) Tumor growth curves in the xenograft-bearing mice after different treatments. (a–c) were reprinted with permission from ref. 210. (d–e) were reprinted with permission from ref. 211. (f–g) were reprinted with permission from ref. 212. Copyright 2017, American Chemical Society.

6.3. Combinational phototherapy

Owing to the limitations of monotherapy, cancer recurrence often occurs after treatment.²¹³ To further improve the therapeutic efficiency, combinational cancer therapy has been widely used.^{214, 215} The combination of PTT and PDT can induce a synergistic effect to amplify the PDT efficacy, as photothermal effects accelerate the blood flow to allow more oxygen transported into tumors.^{216, 217} To realize such a combinational phototherapy of cancer, Liu and colleagues co-encapsulated PFVBT (SP23, Scheme 2) with efficient ROS generating capability and PIDTTQ (SP24) with excellent photothermal effect into one single SPN, which displayed a high $^1\text{O}_2$ quantum yield of 60.4% and photothermal conversion efficiency of 47.6%.²¹⁸ The co-loaded SPNs with conjugation of anti-HER2 affibody allowed for targeted PTT and PDT of HER2 over-expressing tumors with ~53% of tumor suppressive efficiency. In addition to the co-doping of two different SPs, rational molecular design of D–A structured SPs contributed to generate light-triggered photoconversion behaviors with both photothermal and photodynamic performance. As demonstrated in a recent study of Chen's group, a low-bandgap D–A type SP25 (Fig. 16a) with BT as the electron donor and BIBDF as the electron acceptor has been synthesized and transformed into SPN25.²¹⁹ Laser irradiation of SPN25 allowed singlet-to-triplet transition triggered by the charge transfer in excited D–A system to produce $^1\text{O}_2$, as well as nonradiative decay induced by the high electron-deficiency of electron acceptor to generate thermal effect (Fig. 16b).

Furthermore, SPN25 exhibited an enhanced cellular uptake efficiency and effective tumor accumulation, accelerating the synergistic PDT/PTT effects for severe cell damage and tumor ablation.

In addition to SPNs, molecular engineering of organic small molecules has led to the generation of SONS for synergistic PTT/PDT. For instance, triphenylamine (TPA) was conjugated to the DPP backbone for absorption red-shift and charge transport capacity enhancement, obtaining a D–A–D structured DPP-TPA (Scheme 1).²²⁰ After self-assembly into SONS, the absorption of DPP-TPA further red-shifted from 630 nm to 660 nm and the D–A–D structure was further enhanced, which facilitated the charge transport to generate more heat under laser irradiation. The obtained DPP-TPA nanoparticles exhibited a high photothermal conversion efficiency of 34.5% and $^1\text{O}_2$ quantum yield of 33.6%, and thus effectively ablated tumors under laser irradiation at 660 nm. In another study, conjugated coupling of BDP monomers into trimeric BDP (tri-BDP) could trigger photoconversions from fluorescence to singlet-to-triplet or nonradiative transitions and red-shift the absorption into the NIR region.²²¹ Therefore, tri-BDP based nanoparticles not only had a 2-fold enhanced singlet oxygen generation efficiency (18%), but also showed a preferable photothermal conversion efficiency (45.2%) under a NIR light exposure, realizing complete tumor ablation through their cooperative anticancer outcome.

Photothermal-chemotherapy that combines thermal effect and drug-induced toxicity has been considered as a promising treatment for solid tumor to overcome the drug resistance of conventional chemotherapy.²²² In such a therapeutic system, photothermal effect can induce cancer cell death and also facilitate the drug release, and thus enhance chemotherapeutic efficiency.^{9, 223, 224} Pu's group recently utilized amphiphilic SP10 attached with PEG as both the photothermal agents and nanocarriers to encapsulate an anti-cancer drug doxorubicin (DOX), obtaining multifunctional DSPN10 for imaging guided chemo-photothermal therapy.²²⁵ After intravenous injection into tumor-bearing living mice, DSPN10 exhibited a superior antitumor efficacy over SPN10-PEG and free DOX because of the synergistic effect of PTT and chemotherapy. Similarly, Yang and Li et al. contributed another two related studies, in which anticancer drugs were loaded into SPNs with high photothermal conversion efficiency to allow light-triggered enhanced drug release for synergistic photothermal-chemotherapy of xenograft tumor models in living subjects.^{226, 227} In addition, polyaniline (PANI)-based SPNs in combination with rapamycin (RAPA), an antiangiogenic drug that can destroy the tumor vessels and inhibit the tumor growth have been developed for cancer therapy.²²⁸ NIR laser irradiation of these PANI SPNs resulted in thermal generation and bursting release of RAPA, which enabled enhanced photothermal and antiangiogenic combination therapy.

The combination of chemotherapy and PDT has also been used to achieve high therapeutic outcomes, in particular for the ROS-responsive drug delivery systems.^{229, 230} Liu's group reported a novel therapeutic SPN based on a conjugated-polyelectrolyte (CPE) polyprodrug for cancer targeted PDT and chemotherapy.²³¹ In this platform, PEGylated CPE serving as both the photosensitizer and nanocarrier was covalently conjugated with DOX through a ROS-cleavable linker (thioether). Upon laser irradiation, the generated ROS from photosensitizer not only could be used for PDT, but also caused on-demand drug release due to the cleavage of the linker, allowing for combinational and enhanced cancer therapeutic efficiency. In another study, Gu and colleagues reported the combination of PDT with a hypoxia-responsive drug-release system based on SPNs for enhanced cancer therapy.²³² ROS-generating and hypoxia-sensitive 2-nitroimidazole-grafted polymer (SP26) was utilized to encapsulate DOX through a double-emulsion method to generate DSPN26. Laser irradiation of DSPN26 produced $^1\text{O}_2$ for PDT and rapidly consumed the dissolved oxygen, leading to a local hypoxic environment. Therefore, the hydrophobic 2-nitroimidazole (NI) groups were reduced to hydrophilic 2-aminoimidazoles (AI) under the bioreductive conditions of cells, resulting in the dissociation of DSPN26 for drug release (Fig. 16c). Such a light-activated hypoxia-responsive drug delivery system allowed for combinational photodynamic-chemotherapy of tumors with an enhanced therapeutic efficiency.

Fig. 16 (a) Chemical structure of SP25 used for preparation of SPN25 for combinational cancer PTT and PDT. (b) Photophysical mechanism of SPN25 for photoactive cancer therapy (Ex., excitation; NRR, nonradiative recombination; Phos., phosphorescence; ISC, intersystem crossing). (c) Schematic of the preparation and light-activated hypoxia-responsive drug delivery system of DSPN26. (a–b) were reprinted with permission from ref. 219. Copyright 2017 John Wiley & Sons Ltd. (c) was reprinted with permission from ref. 232. Copyright 2016 John Wiley & Sons Ltd.

Table 3 Summary of OSMs for cancer PTT and PDT (ΔT = temperature increase; η = photothermal conversion efficiency; $\Phi\Delta$ = $^1\text{O}_2$ generation efficiency).

OSMs	Surface property	Laser irradiation	PTT/PDT effect	Refs
PFTTQ SPNs	DSPE-PEG ₂₀₀₀	808 nm (0.75 W/cm ² , 10 min)	ΔT = 30 °C (0.5 mg/mL)	176
Polypyrrole nanoparticles	Poly(vinyl alcohol)	808 nm (0.5 W/cm ² , 5 min)	ΔT = ~34 °C (1 mg/mL)	177
BDT-IID SPNs	PSMA	660 nm (0.2 W/cm ² , 10 min)	ΔT = 30 °C (0.1 mg/mL), η = ~45%	178
TDI nanoparticles	Poly(acrylic acid)	660 nm (1 W/cm ² , 10 min)	ΔT = 36.8 °C (50 μM), η = 41%	179
DPP SPNs	PSMA	808 nm (0.5 W/cm ² , 5 min)	ΔT = 36.4 °C (50 $\mu\text{g/mL}$), η = ~65%	180
Porphyrin conjugates	Peptide	635 nm (1.2 W/cm ² , 10 min)	ΔT = 35.3 °C (0.8 mg/mL), η = 54.2%	181
PDPP-TT SPNs	<i>m</i> PEG-b-PHEP	808 nm (2 W/cm ² , 15 min)	ΔT = 29.3 °C (30 $\mu\text{g/mL}$),	187
Porphyrin SPNs	DSPE-PEG, cell penetrating peptide	808 nm (0.75 W/cm ² , 4 min)	ΔT = 42 °C (50 $\mu\text{g/mL}$), η = 63.8%	188
TPA-T-TQ SONS	DSPE-PEG ₂₀₀₀	808 nm (0.8 W/cm ² , 5 min)	ΔT = ~53 °C (100 μM)	189
SPN19	DSPE-PEG ₂₀₀₀	808 nm (0.8 W/cm ² , 3 min)	ΔT = 44 °C (12.5 $\mu\text{g/mL}$)	191
SPN20	PMHC18- <i>m</i> PEG, HOOC-PEG-COOH	635 nm (1.4 W/cm ² , 5 min)	ΔT = 33 °C (400 $\mu\text{g/mL}$), η = 62.3%	192
DPPCN-Fc nanoparticles	\	730 nm (1.0 W/cm ² , 10 min)	ΔT = 33.4 °C (100 $\mu\text{g/mL}$), η = 59.1%	190
SPN10-F20	PEG-b-PPG-b-PEG	808 nm (0.5 W/cm ² , 5 min)	ΔT = ~34 °C (15 $\mu\text{g/mL}$)	141
SPN13	PLGA-PEG	808 nm (0.3 W/cm ² , 5 min)	ΔT = ~30 °C (60 $\mu\text{g/mL}$), η = 71%	143
SPN21	PEG-b-PPG-b-PEG	1064 nm (1.0 W/cm ² , 6 min)	ΔT = ~50 °C (20 $\mu\text{g/mL}$), η = 43.4%	196
SPN22	<i>m</i> PEG-b-PHEP	1064 nm (2.0 W/cm ² , 5 min)	ΔT = 31.4 °C (30 $\mu\text{g/mL}$), η = 66.4%	197
TBDOPV-DT SPNs	<i>m</i> PEG-PDLLA	1064 nm (0.9 W/cm ² , 10 min)	ΔT = 25.7 °C (10 $\mu\text{g/mL}$), η = 50%	198
PT2 SPNs	DSPE-PEG	808 nm (5.0 W/cm ² , 20 min)	$\Phi\Delta$ = 0.61	200

TTD AIE nanoparticles	DSPE- <i>m</i> PEG, DSPE-PEG-cRGD	530 nm (0.25 W/cm ² , 15 min)	$\Phi\Delta = 0.51$ (1 μ g/mL)	201
PFBT-TPP SPNs	PSMA	460 nm (100 mW/cm ² , 20 min)	$\Phi\Delta = 0.35$ (20 μ g/mL)	202
Ce6-PFDTBT SPNs	PSMA	520 nm (0.1 W/cm ² , 10 min)	$\Phi\Delta = \sim 0.4$ (5 μ g/mL)	203
TPP-PFBT SPNs	PSMA	460 nm (50 mW/cm ² , 8 min)	$\Phi\Delta = 0.5$ (20 μ g/mL)	204
RET-BDP SPNs	PEG-b-PPG-b-PEG	645 nm (10 mW/cm ² , 20 min)	$\Phi\Delta = 0.53$ (5 μ M)	205
DPPBDPI SPNs	\	Xenon lamp (8 min)	$\Phi\Delta = \sim 0.8$ (5 μ M)	206
TPETCAQ AIE nanoparticles	DSPE-PEG, HIV-1 Tat	400-1000 nm (60 mW/cm ² , 6 min)	$\Phi\Delta = 0.81$	208
SPN10	PS-b-PAA, silica, MnO ₂ , PEG-b-PPG-b-PEG	808 nm (0.44 W/cm ² , 6 min)	$\Phi\Delta = 0.073$ (10 μ g/mL)	210, 211
SPN23-24	DSPE-PEG, anti-HER2	808 nm (1 W/cm ² , 10 min)	$\Delta T = 40$ °C (100 nM), $\eta = 47.6\%$, $\Phi\Delta = 0.604$	218
SPN25	PEG-PCL	785 nm (1.5 W/cm ² , 10 min)	$\Delta T = 65.2$ °C (20 μ g/mL), $\eta = 34.7\%$, $\Phi\Delta = 0.1$	219
DPP-TPA nanoparticles	\	660 nm (1.0 W/cm ² , 10 min)	$\Delta T = \sim 34$ °C (80 μ g/mL), $\eta = \sim 34.5\%$, $\Phi\Delta = 0.336$	220
Tri-BDP nanoparticles	PEG-PCL	780 nm (0.5 W/cm ² , 5 min)	$\Delta T = 38$ °C (80 μ g/mL), $\eta = 45.2\%$, $\Phi\Delta = 0.18$	221

7. Photoactivation

Remote regulation of biomolecular activities in living systems at designated locations and times is of fundamental necessity for better understanding of physiological processes and pathogenesis, and potentially leads to innovative therapeutic medicine.²³³ On this front, magnetic fields,²³⁴ genetic engineering,²³⁵ chemical modification,²³⁶ ultrasounds,²³⁷ electric fields,²³⁸ radio waves²³⁹ and light irradiation²⁴⁰ are mainly used approaches that can control cellular behaviors, protein activities, transgene systems and so on. Among these approaches, photoactivation that utilizes light stimulus to trigger biological activation has gained increasing popularity and interests owing to its advantages, such as easy production, good controllability, simple operability, high spatiotemporal resolution and noninvasive nature.^{241, 242}

7.1. Photoactivation of ion channels

Rapid thermal stimulation in innocuous temperature range can open the Ca²⁺ channels in plasma membranes, eliciting the activation of neuron.²⁴³ Gold nanorods and carbon nanomaterials have been

used to convert photon energy into local heat for remote photoactivation of temperature-sensitive transient receptor potential cation channel subfamily V member 1 (TRPV1, a typical Ca²⁺ channel protein) intrinsically overexpressed on plasma membrane of neurons.²⁴⁴⁻²⁴⁶ However, these agents are short of the specificity and have the risk of damaging cellular membrane. To overcome these limitations, Pu's group synthesized a TRPV1 targeted SPN14 for specific photothermal activation of neurons.²⁴⁷ In this study, SP14 was transformed into SPN14 through nanocoprecipitation to have a faster heating capability under NIR laser irradiation at 808 nm as compared to both SPN10 and gold nanorods. Anti-TRPV1 antibodies were conjugated onto SPN14 to generate semiconducting polymer nanobioconjugates (SPN14_{bc}) that could precisely target TRPV1, ensuring quick diffusion of locally generated heat from SPN14_{bc} to the TRPV1 ion channels under the NIR laser irradiation (Fig. 17a). Thereby, SPN14_{bc} worked as a wireless remote nanomodulator to rapidly and specifically activate the TRPV1 ion channels, inducing intracellular Ca²⁺ influx of neurons within milliseconds in a safe and reversible manner (Fig. 17b).

Fig. 17 (a) Schematic illustration of SPN_{bc} controlled photothermal activation of Ca²⁺ channels in neurons. The intracellular concentration of Ca²⁺ was monitored in real-time by using Fluo-8 as the indicator, which turned on its fluorescence upon binding with Ca²⁺. (b) Representative confocal fluorescence images of mouse neuroblastoma/DRG neuron hybrid ND7/23 or HeLa cells treated with SPN14_{bc} or SPN10_{bc} before and after laser irradiation time at 808 nm for 2 s. (c) Schematic illustration of the synthesis of SPN7-C. (d) Schematic illustration of proposed apoptosis mechanism induced by SPN7-C under NIR laser irradiation. (a–b) were reprinted with permission from ref. 247. Copyright 2016, American Chemical Society. (c–d) were reprinted with permission from ref. 250. Copyright 2018, American Chemical Society.

TRPV1 is also highly over-expressed in many types of cancer cells,²⁴⁸ and thereby activation of TRPV1 ion channels represents a promising approach for cell-specific therapy.²⁴⁹ For instance, use of a temperature-responsive semiconducting photothermal nanoagonist enabled the remote and specific apoptosis of cancer cells from cell membrane.²⁵⁰ To make the nanoagonist responsive to NIR light, SP7 with silicon in the backbone to red-shift the absorption was designed. Self-assembly of SP7, capsaicin (Cap, a TRPV1 agonist) and lipid layers led to the generation of semiconducting photothermal nanoagonist (SPN7-C, Fig. 17c). Under the NIR laser irradiation at the time scale of seconds, photothermal conversion of SPN7 increased the temperature and melted the lipid layers, allowing for the controlled release of Cap to active TRPV1 Ca²⁺ channels on cellular membrane. As a cumulative consequence of multiple photoactivation of TRPV1 ion channels, overinflux of Ca²⁺ into mitochondria occurred, inducing cell apoptosis specifically for TRPV1-positive cancer cells (Fig. 17d).

7.2. Photoactivation of gene expression

Photoactivation can also be used to remotely switch on gene expression for treating inherited genetic diseases and cancers.^{251,252} A proof-of-concept application of photothermal semiconducting polymer nanocarriers for remote photoactivation of gene expression in living cells and animals was recently demonstrated by Pu's group.²⁵³ A NIR light absorbing dendronized semiconducting polymer (DSP, Fig. 18a) was synthesized by grafting polyamidoamine (PAMAM) side chains and PEG blocks to SP10, serving as a nanocarrier to load plasmids with the gene of interest cloned downstream of a heat shock promoter (HSP70). After cellular internalization, the plasmids were released from DSP and entered into cellular nucleus, while DSP retained in the cytoplasm to transform photon into heat under NIR laser irradiation. This mild photothermal effect did not cause obvious cytotoxicity, but effectively triggered a heat-inducible promoter to active the

intracellular downstream targeting gene transcription and protein expression (Fig. 18b–c). Using luciferase gene (pSV40-Luc) as a model gene, such DSP-mediated photoactivation could rapidly and safely resulted in 25- and 4.5-fold increases in the luciferase expression in living cells and mice, respectively (Fig. 18d–e).

Wang and colleagues subsequently reported a similar strategy for remote photoactivation of intracellular gene expression using SPNs as the photothermal nanotransducers under NIR laser irradiation.²⁵⁴ To improve the specificity and efficiency, they conjugated a cell-penetrating peptide (Tat) onto SPN27 to allow its location on the surface of cell membrane after incubation. Upon irradiation with 808 nm laser, SPN27-Tat generated localized heat in plasmids transfected cancer cells. As a result, the heat-inducible HSP-70 promoter could start transcription of any downstream gene of interest (for example, enhanced green fluorescence protein, EGFP) inside cells.

7.3. Photoactivation of enzyme activity

Remote controlling of enzyme activity is no longer just a method to regulate cell fates, but also provides new opportunities for monitoring health and treating diseases.^{255–257} Because the activity of most enzymes is temperature dependent, photothermal effect mediated photoactivation can be used to dynamically regulate enzyme activity.^{258,259} In this regards, Pu's group recently described the design of a semiconducting polymer nanoenzyme, a SPN/enzyme complex, that could be activated by NIR laser for enhanced cancer PTT.²⁶⁰ To improve the coupling efficiency of enzyme, SP10 grafted with short-chain methoxy-PEG (Mw = 1000) and long-chain carboxyl-PEG (Mw = 2000) was designed and synthesized (Fig. 19a). Bromelain (Bro), a temperature-sensitive enzyme that could proficiently digest collagen, was covalently conjugated to SPN10 *via* a carbodiimide coupling reaction, affording SPN10-Bro. SPN10-Bro could convert photon energy into heat under NIR laser irradiation at 808 nm, leading to increased local

temperature. As the optimal activity of Bro was at $\sim 45^\circ\text{C}$, the local heat resulted in more than 2-fold enhancement in the enzymatic activity of SPN10-Bro (Fig. 19b–c). Such a photothermally triggered remote activation of enzyme enabled an improved penetration depth of SPN10-Bro in 3D tumor spheroids and an enhanced nanoparticle accumulation in tumors (Fig. 19d), which should be

attributed to the in-situ digestion of collagen type I (Fig. 19e), the most abundant ECM proteins in tumor microenvironment. Therefore, SPN10-Bro induced higher heat generation in the tumor regions under 808 nm laser irradiation and eventually afforded a better anticancer efficiency relative to control SPN10.

Fig. 18 (a) Chemical structure of DSP. (b) Illustration of the DSP-mediated gene delivery and remote phototactivation of intracellular gene expression under 808 nm laser irradiation. (c) Representative confocal fluorescence images of HeLa cells transfected with DSP/pHSP70-EGFP nanocomplexes with a N/P ratio of 7.5:1 with or without laser irradiation at 808 nm (0.75 W/cm^2) for 30 min. Red indicates the DSP, blue indicates the cell nucleus and green indicates the expressed EGFP. (d) Illustration of laser irradiation in living nude mice and (e) whole-animal BL images at representative times with (left side of animal) and without (right side of animal) laser irradiation at 808 nm (0.42 W/cm^2). The red/white dashed circles indicates the respective regions subcutaneously implanted with HeLa cells pellets transfected with DSP/pHSP70-Luc nanocomplexes and treated with/without laser irradiation. (b–e) were reprinted with permission from ref. 253. Copyright 2017 John Wiley & Sons Ltd.

Fig. 19 (a) Chemical structure of SP10-PEG. (b) Illustration of the photothermally triggered enzyme activation of SPN10-Bro towards collagen I digestion for enhanced accumulation of nanoparticles in tumors. (c) Enzymatic activity of SPN10-Bro with or without NIR laser irradiation using gelatin as substrate, the enzymatic activity was defined as gelatin digestion unit (GDU). (d) Fluorescence intensities of 3D tumor spheroids after treatments of SPN10 or SPN10-Bro with or without 808 nm laser irradiation at different depths. (e) Immunofluorescence collagen I staining images of 4T1 tumors after intratumoral injection of saline, SPN10 or SPN10-Bro with or without NIR laser irradiation. Cell nucleus (blue color) were stained by 4',6-diamidino-2-phenylindole (DAPI) and collagen I (green color) was stained by Alexa Fluor 488 conjugated anti-collagen I antibody. (b–e) were reprinted with permission from ref. 260. Copyright 2018 John Wiley & Sons Ltd.

Fig. 20 (a) Illustration of the strategy for augmenting light reaction of isolated chloroplasts utilizing SPNs. The chemical structures of SP28 and SP29 are shown. PS = photosystem, PQ = plastoquinone, DCPIP = 2,6-dichlorophenolindophenol dye. (b) The absorption changes of DCPIP at 600 nm for chloroplast (black line), SPN28 (red line), and chloroplast/SPN28 (blue line) upon irradiation with a intensity of 4 mW/cm² for 5 min. (c) ATP production of SPN28, chloroplast, and chloroplast/SPN28 complex under light irradiation. (a–c) were reprinted with permission from ref. 261. Copyright 2018 John Wiley & Sons Ltd.

7.4. Photoactivation of chloroplast photosynthesis

In addition to the photothermal effect of OSMs, their unique light harvesting ability can allow activation of chloroplast photosynthesis (Fig. 20a).²⁶¹ Two typical light-harvesting SPs (SP28 and SP29) with backbone structure containing fluorene units were utilized to nanoprecipitate into SPN28 and SPN29, respectively, which were then coated onto the surface of chloroplasts. In such a chloroplast/SPNs system, SPNs could strongly absorb the irradiated light, especially UV light, and emitted visible light for chloroplast absorption. As a result, photosystem II (PS II), a critical protein complex in chloroplasts, captured more visible light to oxidize water, which produced more electrons in the electron-transport chain, leading to augmented photosynthetic activity (Fig. 20b) and more ATP generation (Fig. 20c). This work presented the potential to utilize light-harvesting OSMs for reforming chloroplasts to increase solar energy conversion.

8. Summary and outlook

The convergence of chemistry, materials science, photology and medicine has facilitated the development of a large number of OSMs with tunable optical properties, excellent photo-stability and good cytocompatibility for versatile biophotonic applications. The emergence of OSMs has provided new opportunities for *in vivo* deep-tissue optical imaging with improved tissue penetration depths, spatial resolutions, detection sensitivities and SBRs as compared with the traditional NIR-I fluorescence imaging. Moreover, by incorporating molecular sensors or target-responsive

components, OSMs have been developed into smart activatable optical probes to correlate signals with the level or activity of disease-specific biomarkers. NIR-II fluorescent OSMs have enabled deep-tissue visualization of vascular circulation, cardiac cycle, tumors and brain tissues, imaging-guided precise resection of tumors and sentinel lymph node biopsy, as well as molecular detection of H₂S, GSH and enzymes in tumor microenvironment. Chemiluminescent OSMs in both nanoparticle and small-molecular forms have been used to detect biomarkers, such as ROS, hypoxia and enzymes. OSM-based afterglow agents have been utilized to delineate lymph nodes, xenograft/metastatic tumors, tissue oxygen levels and drug-induced hepatotoxicity, as well as real-time temperature monitoring during PTT. PA OSMs have permitted *in vivo* imaging of blood vessels, lymph nodes, tumors, thrombus and protein sulfenic acids and detection of ROS, pH, enzyme activities and hypoxic conditions.

Owing to their highly efficient light conversion capabilities under NIR laser irradiation, OSMs can serve as phototherapeutic agents for cancer PTT and PDT. Rational alternation of molecular structures or nanoparticle components have been an effective way to improve the photothermal conversion efficiencies and ¹O₂ generation efficiency, optimize phototherapeutic properties responding to tumor microenvironment, and combine phototherapy with other therapeutic modes, enabling high treatment outcomes. Taking advantage of the photothermal effects, OSMs are able to be used as heat transducers to remotely regulate ion channels, enzyme activity and gene transcription.

With these promising successes, OSMs have the potential to be translated from basic research in experimental animal models to

daily clinical trials, but several critical concerns need to be addressed. First, OSMs possess relatively good biocompatibility and high biosafety, as well as desirable blood circulation time after administration in living animals. However, they often encounter the main problems of high accumulation in livers and slow excretion from the body because of their large sizes. To address this concern, other designs of OSMs are needed to enhance their biodegradability or to reduce their dimension to the level below the renal filtration threshold (~5 nm) for rapid clearance via urine excretion. In this regard, we and others have recently shown that imine bonds,²⁶² imidazole units,²⁶³ thiophene moieties,¹⁴⁷ and vinylene bonds¹⁴³ can serve as the cleavable linker in the conjugated backbone of SPs to facilitate the bio-degradation of SPNs, and these molecular designs should be applicable to other OSMs. Alternatively, nanoprecipitation of semiconducting oligomers should be a potential method to prepare ultrasmall nanoparticles with the diameter smaller than 5 nm. Second, there are still only a few activatable agents that have been reported for NIR-II fluorescence/PA imaging and phototherapy, which is largely due to the challenges in chemical modifications of NIR-II light responsive agents. With the emergence of new NIR-II dyes or polymers, it is expected that more and more activatable NIR-II OSMs will be developed for disease detection and treatment in photomedicine. Third, although the tissue penetration depth of molecular optical imaging in living subjects has been improved, it is still shallower than clinically available computed tomography (CT), positron emission tomography (PET) and magnetic resonance (MR) imaging that are able to 'see through' the entire human body with almost limitless depth in soft tissues. Integration of OSMs with endoscopic diagnostic technologies should be a solution.

By virtue of the facile preparation, OSMs can be further multi-functionalized to broaden their biomedical applications. For example, encapsulating of different semiconducting components potentially can lead to both deep-tissue imaging property and phototherapeutic effect. Moreover, other imaging components such as radioisotopes and iron oxide nanoparticles can be incorporated into OSMs, allowing for multimodal molecular imaging of diverse biological systems. The versatile surface and structure characteristics of OSMs enable the attachment of genes or antigens or loading of drugs to produce new-generation of therapeutic agents with gene therapy, immunotherapy or chemotherapy capabilities. All the merits of OSMs will allow them to be used as the multi-functional platform to investigate, detect and treat diseases other than cancer, such as autoimmune diseases, cardiovascular diseases and neurodegenerative diseases.

Conflicts of interest

There are no conflicts to declare

Acknowledgements

K.P. thanks Nanyang Technological University (Start-up grant: NTU-SUG: M4081627.120) and Singapore Ministry of Education, Academic Research Fund Tier 1 (RG133/15 M4011559 and 2017-T1-002-134-RG147/17) and Academic

Research Fund Tier 2 (MOE2016-T2-1-098) for the financial support.

Notes and references

- G. Xu, S. Zeng, B. Zhang, M. T. Swihart, K.-T. Yong and P. N. Prasad, *Chem. Rev.*, 2016, **116**, 12234-12327.
- K. K. Ng and G. Zheng, *Chem. Rev.*, 2015, **115**, 11012-11042.
- S.-m. Park, A. Aalipour, O. Vermesh, J. H. Yu and S. S. Gambhir, *Nat. Rev. Mater.*, 2017, **2**, 17014.
- J. Lee, D. G. Udugamasooriya, H.-S. Lim and T. Kodadek, *Nat. Chem. Biol.*, 2010, **6**, 258.
- O. T. Bruns, T. S. Bischof, D. K. Harris, D. Franke, Y. Shi, L. Riedemann, A. Bartelt, F. B. Jaworski, J. A. Carr and C. J. Rowlands, *Nat. Biomedical Engineering*, 2017, **1**, 0056.
- A. Burns, H. Ow and U. Wiesner, *Chem. Soc. Rev.*, 2006, **35**, 1028-1042.
- J. Song, X. Yang, Z. Yang, L. Lin, Y. Liu, Z. Zhou, Z. Shen, G. Yu, Y. Dai and O. Jacobson, *ACS Nano*, 2017, **11**, 6102-6113.
- C. F. Chiu, W. A. Saidi, V. E. Kagan and A. Star, *J. Am. Chem. Soc.*, 2017, **139**, 4859-4865.
- S. Wang, Y. Chen, X. Li, W. Gao, L. Zhang, J. Liu, Y. Zheng, H. Chen and J. Shi, *Adv. Mater.*, 2015, **27**, 7117-7122.
- Z. Sheng, L. Song, J. Zheng, D. Hu, M. He, M. Zheng, G. Gao, P. Gong, P. Zhang, Y. Ma and L. Cai, *Biomaterials*, 2013, **34**, 5236-5243.
- L. Rao, L. L. Bu, B. Cai, J. H. Xu, A. Li, W. F. Zhang, Z. J. Sun, S. S. Guo, W. Liu and T. H. Wang, *Adv. Mater.*, 2016, **28**, 3460-3466.
- S. K. Maji, S. Sreejith, J. Joseph, M. Lin, T. He, Y. Tong, H. Sun, S. W. K. Yu and Y. Zhao, *Adv. Mater.*, 2014, **26**, 5633-5638.
- H. Moon, D. Kumar, H. Kim, C. Sim, J.-H. Chang, J.-M. Kim, H. Kim and D.-K. Lim, *ACS Nano*, 2015, **9**, 2711-2719.
- J. Shi, L. Wang, J. Zhang, R. Ma, J. Gao, Y. Liu, C. Zhang and Z. Zhang, *Biomaterials*, 2014, **35**, 5847-5861.
- J. Li, J. Liu and C. Chen, *ACS Nano*, 2017, **11**, 2403-2409.
- S. Han, B. W. Hwang, E. Y. Jeon, D. Jung, G. H. Lee, D. H. Keum, K. S. Kim, S. H. Yun, H. J. Cha and S. K. Hahn, *ACS Nano*, 2017, **11**, 9979-9988.
- S. Sharifi, S. Behzadi, S. Laurent, M. L. Forrest, P. Stroeve and M. Mahmoudi, *Chem. Soc. Rev.*, 2012, **41**, 2323-2343.
- K. Pu, N. Chattopadhyay and J. Rao, *J. Controlled Release*, 2016, **240**, 312-322.
- C.-S. Ke, C.-C. Fang, J.-Y. Yan, P.-J. Tseng, J. R. Pyle, C.-P. Chen, S.-Y. Lin, J. Chen, X. Zhang and Y.-H. Chan, *ACS Nano*, 2017, **11**, 3166-3177.
- E. Ahmed, S. W. Morton, P. T. Hammond and T. M. Swager, *Adv. Mater.*, 2013, **25**, 4504-4510.
- J. Liu, J. Geng, L.-D. Liao, N. Thakor, X. Gao and B. Liu, *Poly. Chem.*, 2014, **5**, 2854-2862.
- H. Zhu, Y. Fang, X. Zhen, N. Wei, Y. Gao, K. Q. Luo, C. Xu, H. Duan, D. Ding, P. Chen and K. Pu, *Chem. Sci.*, 2016, **7**, 5118-5125.
- J.-C. Yu, Y.-L. Chen, Y.-Q. Zhang, X.-K. Yao, C.-G. Qian, J. Huang, S. Zhu, X.-Q. Jiang, Q.-D. Shen and Z. Gu, *Chem. Commun.*, 2014, **50**, 4699-4702.
- K. Pu, A. J. Shuhendler and J. Rao, *Angew. Chem. Int. Ed.*, 2013, **52**, 10325-10329.

25. K. Sun, Y. Tang, Q. Li, S. Yin, W. Qin, J. Yu, D. T. Chiu, Y. Liu, Z. Yuan, X. Zhang and C. Wu, *ACS Nano*, 2016, **10**, 6769-6781.
26. L. Cheng, W. He, H. Gong, C. Wang, Q. Chen, Z. Cheng and Z. Liu, *Adv. Funct. Mater.*, 2013, **23**, 5893-5902.
27. H. Yuan, B. Wang, F. Lv, L. Liu and S. Wang, *Adv. Mater.*, 2014, **26**, 6978-6982.
28. L. Yuan, W. Lin, K. Zheng, L. He and W. Huang, *Chem. Soc. Rev.*, 2013, **42**, 622-661.
29. T. Kowada, H. Maeda and K. Kikuchi, *Chem. Soc. Rev.*, 2015, **44**, 4953-4972.
30. X. Dang, L. Gu, J. Qi, S. Correa, G. Zhang, A. M. Belcher and P. T. Hammond, *Proc. Natl. Acad. Sci. U. S. A.*, 2016, **113**, 5179-5184.
31. D. Kobat, N. G. Horton and C. Xu, *J. Biomed. Opt.*, 2011, **16**, 106014.
32. M.-F. Tsai, S.-H. G. Chang, F.-Y. Cheng, V. Shanmugam, Y.-S. Cheng, C.-H. Su and C.-S. Yeh, *ACS Nano*, 2013, **7**, 5330-5342.
33. C. E. Tedford, S. DeLapp, S. Jacques and J. Anders, *Lasers Surg. Med.*, 2015, **47**, 312-322.
34. R. Wang, X. Li, L. Zhou and F. Zhang, *Angew. Chem., Int. Ed.*, 2014, **53**, 12086-12090.
35. Y. Tsukasaki, M. Morimatsu, G. Nishimura, T. Sakata, H. Yasuda, A. Komatsuzaki, T. M. Watanabe and T. Jin, *RSC Adv.*, 2014, **4**, 41164-41171.
36. G. Hong, A. L. Antaris and H. Dai, *Nat. Biomedical Engineering*, 2017, **1**, 0010.
37. C. Liang, S. Diao, C. Wang, H. Gong, T. Liu, G. Hong, X. Shi, H. Dai and Z. Liu, *Adv. Mater.*, 2014, **26**, 5646-5652.
38. G. Hong, J. C. Lee, J. T. Robinson, U. Raaz, L. Xie, N. F. Huang, J. P. Cooke and H. Dai, *Nat. Med.*, 2012, **18**, 1841-1846.
39. K. Welsher, S. P. Sherlock and H. Dai, *Proc. Natl. Acad. Sci.*, 2011, **108**, 8943-8948.
40. C. Li, Y. Zhang, M. Wang, Y. Zhang, G. Chen, L. Li, D. Wu and Q. Wang, *Biomaterials*, 2014, **35**, 393-400.
41. G. Hong, J. T. Robinson, Y. Zhang, S. Diao, A. L. Antaris, Q. Wang and H. Dai, *Angew. Chem., Int. Ed.*, 2012, **124**, 9956-9959.
42. B. Dong, C. Li, G. Chen, Y. Zhang, Y. Zhang, M. Deng and Q. Wang, *Chem. Mater.*, 2013, **25**, 2503-2509.
43. A. Sasaki, Y. Tsukasaki, A. Komatsuzaki, T. Sakata, H. Yasuda and T. Jin, *Nanoscale*, 2015, **7**, 5115-5119.
44. Y. Nakane, Y. Tsukasaki, T. Sakata, H. Yasuda and T. Jin, *Chem. Commun.*, 2013, **49**, 7584-7586.
45. U. Rocha, K. U. Kumar, C. Jacinto, I. Villa, F. Sanz-Rodriguez, A. Juarranz, E. Carrasco, F. C. van Veggel, E. Bovero, J. G. Sole and J. Daniel, *Small*, 2014, **10**, 1141-1154.
46. Y. Sun, C. Qu, H. Chen, M. He, C. Tang, K. Shou, S. Hong, M. Yang, Y. Jiang and B. Ding, *Chem. Sci.*, 2016, **7**, 6203-6207.
47. B. Li, L. Lu, M. Zhao, Z. Lei and F. Zhang, *Angew. Chem., Int. Ed.*, 2018, **130**, 7605-7609.
48. Z. Tao, G. Hong, C. Shinji, C. Chen, S. Diao, A. L. Antaris, B. Zhang, Y. Zou and H. Dai, *Angew. Chem., Int. Ed.*, 2013, **52**, 13002-13006.
49. K. Shou, C. Qu, Y. Sun, H. Chen, S. Chen, L. Zhang, H. Xu, X. Hong, A. Yu and Z. Cheng, *Adv. Funct. Mater.*, 2017, **27**, 1700995.
50. Y. Hong, J. W. Lam and B. Z. Tang, *Chem. Soc. Rev.*, 2011, **40**, 5361-5388.
51. J. Qi, C. Sun, A. Zebibula, H. Zhang, R. T. Kwok, X. Zhao, W. Xi, J. W. Lam, J. Qian and B. Z. Tang, *Adv. Mater.*, 2018, **30**, 1706856.
52. G. Hong, Y. Zou, A. L. Antaris, S. Diao, D. Wu, K. Cheng, X. Zhang, C. Chen, B. Liu, Y. He, J. Z. Wu, J. Yuan, B. Zhang, Z. Tao, C. Fukunaga and H. Dai, *Nat. Commun.*, 2014, 4206.
53. H. S. Choi, W. Liu, P. Misra, E. Tanaka, J. P. Zimmer, B. I. Ipe, M. G. Bawendi and J. V. Frangioni, *Nat. Biotechnol.*, 2007, **25**, 1165.
54. J.-H. Park, L. Gu, G. Von Maltzahn, E. Ruoslahti, S. N. Bhatia and M. J. Sailor, *Nat. Mater.*, 2009, **8**, 331-336.
55. H. Kang, J. Gravier, K. Bao, H. Wada, J. H. Lee, Y. Baek, G. El Fakhri, S. Gioux, B. P. Rubin, J. L. Coll and H. S. Choi, *Adv. Mater.*, 2016, **28**, 8162-8168.
56. A. L. Antaris, H. Chen, K. Cheng, Y. Sun, G. Hong, C. Qu, S. Diao, Z. Deng, X. Hu, B. Zhang and H. Dai, *Nat. Mater.*, 2016, **15**, 235.
57. X. D. Zhang, H. Wang, A. L. Antaris, L. Li, S. Diao, R. Ma, A. Nguyen, G. Hong, Z. Ma, J. Wang, S. Zhu, J. M. Castellano, T. Wyss-Coray, Y. Liang, J. Luo and H. Dai, *Adv. Mater.*, 2016, **28**, 6872-6879.
58. Q. Yang, Z. Ma, H. Wang, B. Zhou, S. Zhu, Y. Zhong, J. Wang, H. Wan, A. Antaris, R. Ma, X. Zhang, J. Yang, X. Zhang, H. Sun, W. Liu, Y. Liang and H. Dai, *Adv. Mater.*, 2017, **29**, 1605497.
59. S. Zhu, S. Herraiz, J. Yue, M. Zhang, H. Wan, Q. Yang, Z. Ma, Y. Wang, J. He, A. L. Antaris, Y. Zhong, S. Diao, Y. Feng, Y. Zhou, Y. Kuai, G. Hong, Y. Liang, A. J. Hsueh and H. Dai, *Adv. Mater.*, 2018, **30**, 1705799.
60. S. Zhu, Q. Yang, A. L. Antaris, J. Yue, Z. Ma, H. Wang, W. Huang, H. Wan, J. Wang and S. Diao, *Proc. Natl. Acad. Sci.*, 2017, **114**, 962-967.
61. Q. Yang, Z. Hu, S. Zhu, R. Ma, H. Ma, Z. Ma, H. Wan, T. Zhu, Z. Jiang and W. Liu, *J. Am. Chem. Soc.*, 2018, **140**, 1715-1724.
62. S. Zhu, Z. Hu, R. Tian, B. C. Yung, Q. Yang, S. Zhao, D. O. Kiesewetter, G. Niu, H. Sun, A. L. Antaris and X. Chen, *Adv. Mater.*, 2018, **30**, 1802546.
63. S. Jeong, J. Song, W. Lee, Y. M. Ryu, Y. Jung, S.-Y. Kim, K. Kim, S. C. Hong, S. J. Myung and S. Kim, *Nano Lett.*, 2017, **17**, 1378-1386.
64. G. Xu, Q. Yan, X. Lv, Y. Zhu, K. Xin, B. Shi, R. Wang, J. Chen, W. Gao, P. Shi, C. Fan, C. Zhao and H. Tian, *Angew. Chem., Int. Ed.*, 2018, **130**, 3688-3692.
65. Y. Tang, Y. Li, X. Hu, H. Zhao, Y. Ji, L. Chen, W. Hu, W. Zhang, X. Li, X. Lu, W. Huang and Q. Fan, *Adv. Mater.*, 2018, 1801140.
66. I. Takashima, R. Kawagoe, I. Hamachi and A. Ojida, *Chem. Eur. J.*, 2015, **21**, 1382-2044.
67. L. J. Kricka, *Clin. Chem.*, 1991, **37**, 1472-1481.
68. E. S. Lee, V. Deepagan, D. G. You, J. Jeon, G.-R. Yi, J. Y. Lee, D. S. Lee, Y. D. Suh and J. H. Park, *Chem. Commun.*, 2016, **52**, 4132-4135.
69. A. Liu, F. Zhao, Y. Zhao, L. Shangguan and S. Liu, *Biosens. Bioelectron.*, 2016, **81**, 97-102.
70. S. Bi, B. Ji, Z. Zhang and S. Zhang, *Chem. Commun.*, 2013, **49**, 3452-3454.
71. Z. Wang, J. Li, B. Liu, J. Hu, X. Yao and J. Li, *J. Phys. Chem. B*, 2005, **109**, 23304-23311.
72. D. Lee, S. Khaja, J. C. Velasquez-Castano, M. Dasari, C. Sun, J. Petros, W. R. Taylor and N. Murthy, *Nat. Mater.*, 2007, **6**, 765.

73. J. Li, Y. Chen, Y. Yang, N. Kawazoe and G. Chen, *J. Mater. Chem. B*, 2017, **5**, 1353-1362.
74. C. R. Reczek and N. S. Chandel, *Curr. Opin. Cell Biol.*, 2015, **33**, 8-13.
75. Y. Lyu, X. Zhen, Y. Miao and K. Pu, *ACS Nano*, 2016, **11**, 358-367.
76. Z. Chen, Z. Liu, Z. Li, E. Ju, N. Gao, L. Zhou, J. Ren and X. Qu, *Biomaterials*, 2015, **39**, 15-22.
77. X. Chen, F. Wang, J. Y. Hyun, T. Wei, J. Qiang, X. Ren, I. Shin and J. Yoon, *Chem. Soc. Rev.*, 2016, **45**, 2976-3016.
78. B. C. Dickinson and C. J. Chang, *Nat. Chem. Biol.*, 2011, **7**, 504-511.
79. J. Li, J. Zhang, Y. Chen, N. Kawazoe and G. Chen, *ACS Appl. Mater. Interfaces*, 2017, **9**, 35683-35692.
80. Y. Zhou, W. Pei, X. Zhang, W. Chen, J. Wu, C. Yao, L. Huang, H. Zhang, W. Huang and J. S. C. Loo, *Biomaterials*, 2015, **54**, 34-43.
81. M. Giorgio, M. Trinei, E. Migliaccio and P. G. Pelicci, *Nat. Rev. Mol. Cell Biol.*, 2007, **8**, 722-728.
82. J. Geng, K. Li, W. Qin, B. Z. Tang and B. Liu, *Part. Part. Syst. Charact.*, 2014, **31**, 1238-1243.
83. Y.-D. Lee, C.-K. Lim, A. Singh, J. Koh, J. Kim, I. C. Kwon and S. Kim, *ACS Nano*, 2012, **6**, 6759-6766.
84. H. J. Kim, Y. H. Seo, S. An, A. Jo, I. C. Kwon and S. Kim, *Theranostics*, 2018, **8**, 1798-1807.
85. S. Cho, O. Hwang, I. Lee, G. Lee, D. Yoo, G. Khang, P. M. Kang and D. Lee, *Adv. Funct. Mater.*, 2012, **22**, 4038-4043.
86. C. K. Lim, Y. D. Lee, J. Na, J. M. Oh, S. Her, K. Kim, K. Choi, S. Kim and I. C. Kwon, *Adv. Funct. Mater.*, 2010, **20**, 2644-2648.
87. A. J. Shuhendler, K. Pu, L. Cui, J. P. Uetrecht and J. Rao, *Nat. Biotechnol.*, 2014, **32**, 373-380.
88. X. Zhen, C. Zhang, C. Xie, Q. Miao, K. L. Lim and K. Pu, *ACS Nano*, 2016, **10**, 6400-6409.
89. 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.
90. D. Mao, W. Wu, S. Ji, C. Chen, F. Hu, D. Kong, D. Ding and B. Liu, *Chem*, 2017, **3**, 991-1007.
91. J. Liu, Q. Chen, W. Zhu, X. Yi, Y. Yang, Z. Dong and Z. Liu, *Adv. Funct. Mater.*, 2017, **27**, 1605926.
92. K. Teranishi and T. Nishiguchi, *Anal. Biochem.*, 2004, **325**, 185-195.
93. M. Sekiya, K. Umezawa, A. Sato, D. Citterio and K. Suzuki, *Chem. Commun.*, 2009, **0**, 3047-3049.
94. P. Li, L. Liu, H. Xiao, W. Zhang, L. Wang and B. Tang, *J. Am. Chem. Soc.*, 2016, **138**, 2893-2896.
95. M. E. Roth - Konforti, C. R. Bauer and D. Shabat, *Angew. Chem., Int. Ed.*, 2017, **129**, 15839-15844.
96. L. S. Ryan and A. R. Lippert, *Angew. Chem., Int. Ed.*, 2018, **57**, 622-624.
97. J. Cao, W. An, A. G. Reeves and A. R. Lippert, *Chem. Sci.*, 2018, **9**, 2552-2558.
98. O. Green, T. Eilon, N. Hananya, S. Gutkin, C. R. Bauer and D. Shabat, *ACS Cent. Sci.*, 2017, **3**, 349-358.
99. D. Shabat, N. Hananya, O. Green, R. Blau and R. Satchi-Fainaro, *Angew. Chem., Int. Ed.*, 2017, **56**, 11793-11796.
100. S. Gnaïm, O. Green and D. Shabat, *Chem. Commun.*, 2018, **54**, 2073-2085.
101. A. P. Schaap, R. S. Handley and B. P. Giri, *Tetrahedron Lett.*, 1987, **28**, 935-938.
102. N. Hananya and D. Shabat, *Angew. Chem., Int. Ed.*, 2017, **56**, 16454-16463.
103. O. Seven, F. Sozmen and I. S. Turan, *Sens. Actuators, B*, 2017, **239**, 1318-1324.
104. O. Green, S. Gnaïm, R. Blau, A. Eldar-Boock, R. Satchi-Fainaro and D. Shabat, *J. Am. Chem. Soc.*, 2017, **139**, 13243-13248.
105. K. J. Bruemmer, O. Green, T. A. Su, D. Shabat and C. J. Chang, *Angew. Chem., Int. Ed.*, 2018, **130**, 7630-7634.
106. J. Cao, R. Lopez, J. Thacker, J. Moon, C. Jiang, S. Morris, J. Bauer, P. Tao, R. Mason and A. Lippert, *Chem. Sci.*, 2015, **6**, 1979-1985.
107. J. Cao, J. Campbell, L. Liu, R. P. Mason and A. R. Lippert, *Anal. Chem.*, 2016, **88**, 4995-5002.
108. N. Hananya, A. Eldar Boock, C. R. Bauer, R. Satchi-Fainaro and D. Shabat, *J. Am. Chem. Soc.*, 2016, **138**, 13438-13446.
109. T. Eilon-Shaffer, M. Roth-Konforti, A. Eldar-Boock, R. Satchi-Fainaro and D. Shabat, *Org. Biomol. Chem.*, 2018, **16**, 1708-1712.
110. J. Shi, X. Sun, J. Li, H. Man, J. Shen, Y. Yu and H. Zhang, *Biomaterials*, 2015, **37**, 260-270.
111. L. Zhan-Jun, Z. Hong-Wu, S. Meng, S. Jiang-Shan and F. Hai-Xia, *J. Mater. Chem.*, 2012, **22**, 24713-24720.
112. T. Maldiney, G. Sraiki, B. Viana, D. Gourier, C. Richard, D. Scherman, M. Bessodes, K. Van den Eeckhout, D. Poelman and P. Smet, *Opt. Mater. Express*, 2012, **2**, 261-268.
113. Z. Li, Y. Zhang, X. Wu, L. Huang, D. Li, W. Fan and G. Han, *J. Am. Chem. Soc.*, 2015, **137**, 5304-5307.
114. T. Maldiney, A. Bessière, J. Seguin, E. Teston, S. K. Sharma, B. Viana, A. J. Bos, P. Dorenbos, M. Bessodes and D. Gourier, *Nat. Mater.*, 2014, **13**, 418-426.
115. A. Abdulkayum, J.-T. Chen, Q. Zhao and X.-P. Yan, *J. Am. Chem. Soc.*, 2013, **135**, 14125-14133.
116. S. Xu, R. Chen, C. Zheng and W. Huang, *Adv. Mater.*, 2016, **28**, 9920-9940.
117. X. Zhen, Y. Tao, Z. An, P. Chen, C. Xu, R. Chen, W. Huang and K. Pu, *Adv. Mater.*, 2017, **29**, 1606665.
118. Q. Miao, C. Xie, X. Zhen, Y. Lyu, H. Duan, X. Liu, J. V. Jokerst and K. Pu, *Nat. Biotechnol.*, 2017, **35**, 1102.
119. C. Xie, X. Zhen, Q. Miao, Y. Lyu and K. Pu, *Adv. Mater.*, 2018, **30**, 1801331.
120. X. Zhen, C. Xie and K. Pu, *Angew. Chem., Int. Ed.*, 2018, **130**, 4002-4006.
121. Q. Miao and K. Pu, *Bioconjugate Chem.*, 2016, **27**, 2808-2823.
122. Q. Chen, X. Liu, J. Chen, J. Zeng, Z. Cheng and Z. Liu, *Adv. Mater.*, 2015, **27**, 6820-6827.
123. C. Kim, T. N. Erpelding, L. Jankovic, M. D. Pashley and L. V. Wang, *Biomed. Opt. Express*, 2010, **1**, 278-284.
124. J. Li, F. Cheng, H. Huang, L. Li and J.-J. Zhu, *Chem. Soc. Rev.*, 2015, **44**, 7855-7880.
125. Y. Jiang and K. Pu, *Small*, 2017, **13**, 1700710.
126. J. Weber, P. C. Beard and S. E. Bohndiek, *Nat. Methods*, 2016, **13**, 639-650.
127. W. Li and X. Chen, *Nanomedicine*, 2015, **10**, 299-320.
128. L. Nie and X. Chen, *Chem. Soc. Rev.*, 2014, **43**, 7132-7170.
129. J. Zhang, H. Chen, T. Zhou, L. Wang, D. Gao, X. Zhang, Y. Liu, C. Wu and Z. Yuan, *Nano Res.*, 2017, **10**, 64-76.
130. X. Cai, X. Liu, L. D. Liao, A. Bandla, J. M. Ling, Y. H. Liu, N. Thakor, G. C. Bazan and B. Liu, *Small*, 2016, **12**, 4873-4880.
131. K. Pu, A. J. Shuhendler, J. V. Jokerst, J. Mei, S. S. Gambhir, Z. Bao and J. Rao, *Nat. Nanotechnol.*, 2014, **9**, 233-239.

132. C. Cui, Z. Yang, X. Hu, J. Wu, K. Shou, H. Ma, C. Jian, Y. Zhao, B. Qi and X. Hu, *ACS Nano*, 2017, **11**, 3298-3310.
133. K. Pu, J. Mei, J. V. Jokerst, G. Hong, A. L. Antaris, N. Chattopadhyay, A. J. Shuhendler, T. Kurosawa, Y. Zhou, S. S. Gambhir, Z. Bao and J. Rao, *Adv. Mater.*, 2015, **27**, 5184-5190.
134. C. Xie, X. Zhen, Q. Lei, R. Ni and K. Pu, *Adv. Funct. Mater.*, 2017, **27**, 1605397.
135. C. Yin, X. Zhen, H. Zhao, Y. Tang, Y. Ji, Y. Lyu, Q. Fan, W. Huang and K. Pu, *ACS Appl. Mater. Interfaces*, 2017, **9**, 12332-12339.
136. C. M. Furdul and L. B. Poole, *Mass Spectrom. Rev.*, 2014, **33**, 126-146.
137. Y. H. Seo and K. S. Carroll, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 16163-16168.
138. X. Liang, Y. Li, X. Li, L. Jing, Z. Deng, X. Yue, C. Li and Z. Dai, *Adv. Funct. Mater.*, 2015, **25**, 1451-1462.
139. Q. Fan, K. Cheng, Z. Yang, R. Zhang, M. Yang, X. Hu, X. Ma, L. Bu, X. Lu and X. Xiong, *Adv. Mater.*, 2015, **27**, 843-847.
140. H. Chen, J. Zhang, K. Chang, X. Men, X. Fang, L. Zhou, D. Li, D. Gao, S. Yin, X. Zhang, Z. Yuan and C. Wu, *Biomaterials*, 2017, **144**, 42-52.
141. Y. Lyu, Y. Fang, Q. Miao, X. Zhen, D. Ding and K. Pu, *ACS Nano*, 2016, **10**, 4472-4481.
142. C. Xie, P. K. Upputuri, X. Zhen, M. Pramanik and K. Pu, *Biomaterials*, 2017, **119**, 1-8.
143. Y. Lyu, J. Zeng, Y. Jiang, X. Zhen, T. Wang, S. Qiu, X. Lou, M. Gao and K. Pu, *ACS Nano*, 2018, **12**, 1801-1810.
144. X. Zhen, X. Feng, C. Xie, Y. Zheng and K. Pu, *Biomaterials*, 2017, **127**, 97-106.
145. C. Xie, X. Zhen, Y. Lyu and K. Pu, *Adv. Mater.*, 2017, **29**, 1703693.
146. J. Zhang, X. Zhen, P. K. Upputuri, M. Pramanik, P. Chen and K. Pu, *Adv. Mater.*, 2017, **29**, 1604764.
147. C. Yin, X. Zhen, Q. Fan, W. Huang and K. Pu, *ACS Nano*, 2017, **11**, 4174-4182.
148. L. Frullano, C. Catana, T. Benner, A. D. Sherry and P. Caravan, *Angew. Chem., Int. Ed.*, 2010, **122**, 2432-2434.
149. S. A. Hilderbrand, K. A. Kelly, M. Niedre and R. Weissleder, *Bioconjugate Chem.*, 2008, **19**, 1635-1639.
150. S. Schreml, R. J. Meier, O. S. Wolfbeis, M. Landthaler, R.-M. Szeimies and P. Babilas, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 2432-2437.
151. J. Y. Ko, S. Park, H. Lee, H. Koo, M. S. Kim, K. Choi, I. C. Kwon, S. Y. Jeong, K. Kim and D. S. Lee, *Small*, 2010, **6**, 2539-2544.
152. Q. Miao, Y. Lyu, D. Ding and K. Pu, *Adv. Mater.*, 2016, **28**, 3662-3668.
153. A. Dragulescu-Andrasi, S.-R. Kothapalli, G. A. Tikhomirov, J. Rao and S. S. Gambhir, *J. Am. Chem. Soc.*, 2013, **135**, 11015-11022.
154. K. Yang, L. Zhu, L. Nie, X. Sun, L. Cheng, C. Wu, G. Niu, X. Chen and Z. Liu, *Theranostics*, 2014, **4**, 134.
155. K. Kessenbrock, V. Plaks and Z. Werb, *Cell*, 2010, **141**, 52-67.
156. J. Levi, S.-R. Kothapalli, S. Bohndiek, J.-K. Yoon, A. Dragulescu-Andrasi, C. Nielsen, A. Tisma, S. Bodapati, G. Gowrishankar and X. Yan, *Clin. Cancer Res.*, 2013, **19**, 1494-1502.
157. D. Zhang, G. B. Qi, Y. X. Zhao, S. L. Qiao, C. Yang and H. Wang, *Adv. Mater.*, 2015, **27**, 6125-6130.
158. N. Albayrak and S. T. Yang, *Biotechnol. Bioeng.*, 2002, **77**, 8-19.
159. X. Zhen, J. Zhang, J. Huang, C. Xie, Q. Miao and K. Pu, *Angew. Chem., Int. Ed.*, 2018, **130**, 7930-7934.
160. H. J. Knox, J. Hedhli, T. W. Kim, K. Khalili, L. W. Dobrucki and J. Chan, *Nat. Commun.*, 2017, **8**, 1794.
161. Y. Dai, C. Xu, X. Sun and X. Chen, *Chem. Soc. Rev.*, 2017, **46**, 3830-3852.
162. J. M. Brown and W. R. Wilson, *Nat. Rev. Cancer*, 2004, **4**, 437-447.
163. Y. Jiang and K. Pu, *Adv. Biosys.*, 2018, 1700262.
164. Y. Zhou, D. Wang, Y. Zhang, U. Chitgupi, J. Geng, Y. Wang, Y. Zhang, T. R. Cook, J. Xia and J. F. Lovell, *Theranostics*, 2016, **6**, 688.
165. Y. Jiang, P. K. Upputuri, C. Xie, Y. Lyu, L. Zhang, Q. Xiong, M. Pramanik and K. Pu, *Nano Lett.*, 2017, **17**, 4964-4969.
166. B. Guo, Z. Sheng, D. Hu, X. Lin, S. Xu, C. Liu, H. Zheng and B. Liu, *Mater. Horiz.*, 2017, **4**, 1151-1156.
167. J. Wu, L. You, L. Lan, H. J. Lee, S. T. Chaudhry, R. Li, J. X. Cheng and J. Mei, *Adv. Mater.*, 2017, **29**, 1703403.
168. J. Li, Y. Hu, J. Yang, P. Wei, W. Sun, M. Shen, G. Zhang and X. Shi, *Biomaterials*, 2015, **38**, 10-21.
169. C. Liang, L. Xu, G. Song and Z. Liu, *Chem. Soc. Rev.*, 2016, **45**, 6250-6269.
170. S. S. Lucky, K. C. Soo and Y. Zhang, *Chem. Rev.*, 2015, **115**, 1990-2042.
171. J. Li, R. Cai, N. Kawazoe and G. Chen, *J. Mater. Chem. B*, 2015, **3**, 5806-5814.
172. P. Wang, X. Li, C. Yao, W. Wang, M. Zhao, A. M. El-Toni and F. Zhang, *Biomaterials*, 2017, **125**, 90-100.
173. C. S. Jin, J. F. Lovell, J. Chen and G. Zheng, *ACS Nano*, 2013, **7**, 2541-2550.
174. S. Wang, J. Zhao, H. Yang, C. Wu, F. Hu, H. Chang, G. Li, D. Ma, D. Zou and M. Huang, *Acta Biomater.*, 2017, **58**, 442-454.
175. J. Li, J. Rao and K. Pu, *Biomaterials*, 2018, **155**, 217-235.
176. J. Geng, C. Sun, J. Liu, L. D. Liao, Y. Yuan, N. Thakor, J. Wang and B. Liu, *Small*, 2015, **11**, 1603-1610.
177. K. Yang, H. Xu, L. Cheng, C. Sun, J. Wang and Z. Liu, *Adv. Mater.*, 2012, **24**, 5586-5592.
178. K. Chang, Y. Liu, D. Hu, Q. Qi, D. Gao, Y. Wang, D. Li, X. Zhang, H. Zheng, Z. Sheng and Z. Yuan, *ACS Appl. Mater. Interfaces*, 2018, **10**, 7012-7021.
179. S. Zhang, W. Guo, J. Wei, C. Li, X.-J. Liang and M. Yin, *ACS Nano*, 2017, **11**, 3797-3805.
180. S. Li, X. Wang, R. Hu, H. Chen, M. Li, J. Wang, Y. Wang, L. Liu, F. Lv and X.-J. Liang, *Chem. Mater.*, 2016, **28**, 8669-8675.
181. Q. Zou, M. Abbas, L. Zhao, S. Li, G. Shen and X. Yan, *J. Am. Chem. Soc.*, 2017, **139**, 1921-1927.
182. P. Zhang, H. Huang, J. Huang, H. Chen, J. Wang, K. Qiu, D. Zhao, L. Ji and H. Chao, *ACS Appl. Mater. Interfaces*, 2015, **7**, 23278-23290.
183. X. Zhu, W. Feng, J. Chang, Y.-W. Tan, J. Li, M. Chen, Y. Sun and F. Li, *Nat. Commun.*, 2016, **7**, 10437.
184. Z. Sun, H. Xie, S. Tang, X. F. Yu, Z. Guo, J. Shao, H. Zhang, H. Huang, H. Wang and P. K. Chu, *Angew. Chem., Int. Ed.*, 2015, **127**, 11688-11692.
185. Q. Tian, F. Jiang, R. Zou, Q. Liu, Z. Chen, M. Zhu, S. Yang, J. Wang, J. Wang and J. Hu, *ACS nano*, 2011, **5**, 9761-9771.
186. D. K. Roper, W. Ahn and M. Hoepfner, *J. Phys. Chem. C*, 2007, **111**, 3636-3641.

187. D. Li, G. Zhang, W. Xu, J. Wang, Y. Wang, L. Qiu, J. Ding and X. Yang, *Theranostics*, 2017, **7**, 4029-4040.
188. B. Guo, G. Feng, P. N. Manghnani, X. Cai, J. Liu, W. Wu, S. Xu, X. Cheng, C. Teh and B. Liu, *Small*, 2016, **12**, 6243-6254.
189. J. Qi, Y. Fang, R. T. Kwok, X. Zhang, X. Hu, J. W. Lam, D. Ding and B. Z. Tang, *ACS Nano*, 2017, **11**, 7177-7188.
190. P. Liang, Q. Tang, Y. Cai, G. Liu, W. Si, J. Shao, W. Huang, Q. Zhang and X. Dong, *Chem. Sci.*, 2017, **8**, 7457-7463.
191. B. Guo, Z. Sheng, D. Hu, A. Li, S. Xu, P. N. Manghnani, C. Liu, L. Guo, H. Zheng and B. Liu, *ACS Nano*, 2017, **11**, 10124-10134.
192. J. Zhang, C. Yang, R. Zhang, R. Chen, Z. Zhang, W. Zhang, S. H. Peng, X. Chen, G. Liu, C. S. Hsu and C.-S. Lee, *Adv. Funct. Mater.*, 2017, **27**, 1605094.
193. H. Lin, S. Gao, C. Dai, Y. Chen and J. Shi, *J. Am. Chem. Soc.*, 2017, **139**, 16235-16247.
194. X. Ding, C. H. Liow, M. Zhang, R. Huang, C. Li, H. Shen, M. Liu, Y. Zou, N. Gao and Z. Zhang, *J. Am. Chem. Soc.*, 2014, **136**, 15684-15693.
195. J. Zhou, Y. Jiang, S. Hou, P. K. Upputuri, D. Wu, J. Li, P. Wang, X. Zhen, M. Pramanik, K. Pu and H. Duan, *ACS Nano*, 2018, **12**, 2643-2651.
196. Y. Jiang, J. Li, X. Zhen, C. Xie and K. Pu, *Adv. Mater.*, 2018, **30**, 1705980.
197. Z. Cao, L. Feng, G. Zhang, J. Wang, S. Shen, D. Li and X. Yang, *Biomaterials*, 2018, **155**, 103-111.
198. T. Sun, J.-H. Dou, S. Liu, X. Wang, X. Zheng, Y. Wang, J. Pei and Z. Xie, *ACS Appl. Mater. Interfaces*, 2018, **10**, 7919-7926.
199. L. Cheng, D. Jiang, A. Kamkaew, H. F. Valdovinos, H. J. Im, L. Feng, C. G. England, S. Goel, T. E. Barnhart, Z. Liu and W. Cai, *Adv. Funct. Mater.*, 2017, **27**, 1702928.
200. L. Guo, J. Ge, Q. Liu, Q. Jia, H. Zhang, W. Liu, G. Niu, S. Liu, J. Gong, S. Hackbarth and P. Wang, *Adv. Healthcare Mater.*, 2017, **6**, 1601431.
201. M. Li, Y. Gao, Y. Yuan, Y. Wu, Z. Song, B. Z. Tang, B. Liu and Q. C. Zheng, *ACS Nano*, 2017, **11**, 3922-3932.
202. K. Chang, Y. Tang, X. Fang, S. Yin, H. Xu and C. Wu, *Biomacromolecules*, 2016, **17**, 2128-2136.
203. Y. Tang, H. Chen, K. Chang, Z. Liu, Y. Wang, S. Qu, H. Xu and C. Wu, *ACS Appl. Mater. Interfaces*, 2017, **9**, 3419-3431.
204. S. Li, K. Chang, K. Sun, Y. Tang, N. Cui, Y. Wang, W. Qin, H. Xu and C. Wu, *ACS Appl. Mater. Interfaces*, 2015, **8**, 3624-3634.
205. L. Huang, Z. Li, Y. Zhao, J. Yang, Y. Yang, A. I. Pendharkar, Y. Zhang, S. Kelmar, L. Chen, W. Wu, J. Zhao and G. Han, *Adv. Mater.*, 2017, **29**, 1604789.
206. J. Zou, Z. Yin, P. Wang, D. Chen, J. Shao, Q. Zhang, L. Sun, W. Huang and X. Dong, *Chem. Sci.*, 2018, **9**, 2188-2194.
207. G. Feng, W. Wu, S. Xu and B. Liu, *ACS Appl. Mater. Interfaces*, 2016, **8**, 21193-21200.
208. W. Wu, D. Mao, F. Hu, S. Xu, C. Chen, C. J. Zhang, X. Cheng, Y. Yuan, D. Ding and D. Kong, *Adv. Mater.*, 2017, **29**, 1700548.
209. J. Kim, H. R. Cho, H. Jeon, D. Kim, C. Song, N. Lee, S. H. Choi and T. Hyeon, *J. Am. Chem. Soc.*, 2017, **139**, 10992-10995.
210. H. Zhu, J. Li, X. Qi, P. Chen and K. Pu, *Nano Lett.*, 2018, **18**, 586-594.
211. H. Zhu, Y. Fang, Q. Miao, X. Qi, D. Ding, P. Chen and K. Pu, *ACS Nano*, 2017, **11**, 8998-9009.
212. K. Chang, Z. Liu, X. Fang, H. Chen, X. Men, Y. Yuan, K. Sun, X. Zhang, Z. Yuan and C. Wu, *Nano Lett.*, 2017, **17**, 4323-4329.
213. C. He, D. Liu and W. Lin, *ACS Nano*, 2015, **9**, 991-1003.
214. J. Xu, L. Xu, C. Wang, R. Yang, Q. Zhuang, X. Han, Z. Dong, W. Zhu, R. Peng and Z. Liu, *ACS Nano*, 2017, **11**, 4463-4474.
215. Z. Guo, Y. Zou, H. He, J. Rao, S. Ji, X. Cui, H. Ke, Y. Deng, H. Yang, C. Chen, Y. Zhao and H. Chen, *Adv. Mater.*, 2016, **28**, 10155-10164.
216. Q. Chen, L. Feng, J. Liu, W. Zhu, Z. Dong, Y. Wu and Z. Liu, *Adv. Mater.*, 2016, **28**, 7129-7136.
217. C. Chu, H. Lin, H. Liu, X. Wang, J. Wang, P. Zhang, H. Gao, C. Huang, Y. Zeng, Y. Tan, G. Liu and X. Chen, *Adv. Mater.*, 2017, **29**, 1605928.
218. G. Feng, Y. Fang, J. Liu, J. Geng, D. Ding and B. Liu, *Small*, 2017, **13**, 1602807.
219. T. Yang, L. Liu, Y. Deng, Z. Guo, G. Zhang, Z. Ge, H. Ke and H. Chen, *Adv. Mater.*, 2017, **29**, 1700487.
220. Y. Cai, P. Liang, Q. Tang, X. Yang, W. Si, W. Huang, Q. Zhang and X. Dong, *ACS Nano*, 2017, **11**, 1054-1063.
221. S. Ye, J. Rao, S. Qiu, J. Zhao, H. He, Z. Yan, T. Yang, Y. Deng, H. Ke, H. Yang, Y. Zhao, Z. Guo and H. Chen, *Adv. Mater.*, 2018, 1801216.
222. T. Liu, C. Wang, X. Gu, H. Gong, L. Cheng, X. Shi, L. Feng, B. Sun and Z. Liu, *Adv. Mater.*, 2014, **26**, 3433-3440.
223. X. Liu, F. Fu, K. Xu, R. Zou, J. Yang, Q. Wang, Q. Liu, Z. Xiao and J. Hu, *J. Mater. Chem. B*, 2014, **2**, 5358-5367.
224. X. Liu, Q. Wang, C. Li, R. Zou, B. Li, G. Song, K. Xu, Y. Zheng and J. Hu, *Nanoscale*, 2014, **6**, 4361-4370.
225. Y. Jiang, D. Cui, Y. Fang, X. Zhen, P. K. Upputuri, M. Pramanik, D. Ding and K. Pu, *Biomaterials*, 2017, **145**, 168-177.
226. Y. Cao, J. Yi, X. Yang, L. Liu, C. Yu, Y. Huang, L. Sun, Y. Bao and Y. Li, *Biomacromolecules*, 2017, **18**, 2306-2314.
227. D.-D. Li, J.-X. Wang, Y. Ma, H.-S. Qian, D. Wang, L. Wang, G. Zhang, L. Qiu, Y.-C. Wang and X.-Z. Yang, *ACS Appl. Mater. Interfaces*, 2016, **8**, 19312-19320.
228. J. Wang, F. Guo, M. Yu, L. Liu, F. Tan, R. Yan and N. Li, *J. Controlled Release*, 2016, **237**, 23-34.
229. Y. Wang, Y. Xie, J. Li, Z.-H. Peng, Y. Sheinin, J. Zhou and D. Oupický, *ACS Nano*, 2017, **11**, 2227-2238.
230. L. Feng, L. Cheng, Z. Dong, D. Tao, T. E. Barnhart, W. Cai, M. Chen and Z. Liu, *ACS Nano*, 2016, **11**, 927-937.
231. Y. Yuan, J. Liu and B. Liu, *Angew. Chem., Int. Ed.*, 2014, **53**, 7163-7168.
232. C. Qian, J. Yu, Y. Chen, Q. Hu, X. Xiao, W. Sun, C. Wang, P. Feng, Q. D. Shen and Z. Gu, *Adv. Mater.*, 2016, **28**, 3313-3320.
233. G. G. Genchi, A. Marino, A. Grillone, I. Pezzini and G. Ciofani, *Adv. Healthcare Mater.*, 2017, **6**, 1700002.
234. H.-M. Yun, S.-J. Ahn, K.-R. Park, M.-J. Kim, J.-J. Kim, G.-Z. Jin, H.-W. Kim and E.-C. Kim, *Biomaterials*, 2016, **85**, 88-98.
235. E. Arbely, J. Torres-Kolbus, A. Deiters and J. W. Chin, *J. Am. Chem. Soc.*, 2012, **134**, 11912-11915.
236. M. Wang, S. Sun, C. I. Neufeld, B. Perez - Ramirez and Q. Xu, *Angew. Chem., Int. Ed.*, 2014, **53**, 13444-13448.
237. B. Hu, Y. Zhang, J. Zhou, J. Li, F. Deng, Z. Wang and J. Song, *PLoS One*, 2014, **9**, e95168.

238. E. D. Kirson, V. Dbalý, F. Tovaryš, J. Vymazal, J. F. Soustiel, A. Itzhaki, D. Mordechovich, S. Steinberg-Shapira, Z. Gurvich and R. Schneiderman, *Proc. Natl. Acad. Sci.*, 2007, **104**, 10152-10157.
239. I. B. Leibiger and P.-O. Berggren, *Nat. Med.*, 2015, **21**, 14-16.
240. D. Lee, J. H. Hyun, K. Jung, P. Hannan and H.-B. Kwon, *Nat. Biotechnol.*, 2017, **35**, 858-863.
241. L. Lin, L. Liu, B. Zhao, R. Xie, W. Lin, H. Li, Y. Li, M. Shi, Y.-G. Chen and T. A. Springer, *Nat. Nanotechnol.*, 2015, **10**, 465-471.
242. M. Karimi, P. Sahandi Zangabad, S. Baghaee-Ravari, M. Ghazadeh, H. Mirshekari and M. R. Hamblin, *J. Am. Chem. Soc.*, 2017, **139**, 4584-4610.
243. A. Tay and D. Di Carlo, *Nano Lett.*, 2017, **17**, 886-892.
244. H. Huang, S. Delikanli, H. Zeng, D. M. Ferkey and A. Pralle, *Nat. Nanotechnol.*, 2010, **5**, 602-606.
245. E. Miyako, J. Russier, M. Mauro, C. Cebrian, H. Yawo, C. Menard-Moyon, J. A. Hutchison, M. Yudasaka, S. Iijima and L. De Cola, *Angew. Chem., Int. Ed.*, 2014, **53**, 13121-13125.
246. H. Nakatsuji, T. Numata, N. Morone, S. Kaneko, Y. Mori, H. Imahori and T. Murakami, *Angew. Chem., Int. Ed.*, 2015, **54**, 11725-11729.
247. Y. Lyu, C. Xie, S. A. Chechetka, E. Miyako and K. Pu, *J. Am. Chem. Soc.*, 2016, **138**, 9049-9052.
248. T. T. Wu, A. A. Peters, P. T. Tan, S. J. Roberts-Thomson and G. R. Monteith, *Cell Calcium*, 2014, **56**, 59-67.
249. S. Orrenius, B. Zhivotovsky and P. Nicotera, *Nat. Rev. Mol. Cell Biol.*, 2003, **4**, 552-565.
250. X. Zhen, C. Xie, Y. Jiang, X. Ai, B. Xing and K. Pu, *Nano Lett.*, 2018, **18**, 1498-1505.
251. X. Wu, Y. Zhang, K. Takle, O. Bilsel, Z. Li, H. Lee, Z. Zhang, D. Li, W. Fan, C. Duan, E. M. Chan, C. Lois, Y. Xiang and G. Han, *ACS Nano*, 2016, **10**, 1060-1066.
252. E. Miyako, T. Deguchi, Y. Nakajima, M. Yudasaka, Y. Hagihara, M. Horie, M. Shichiri, Y. Higuchi, F. Yamashita, M. Hashida, Y. Shigeri, Y. Yoshida and S. Iijima, *Proc. Natl. Acad. Sci. USA*, 2012, **109**, 7523-7528.
253. Y. Lyu, D. Cui, H. Sun, Y. Miao, H. Duan and K. Pu, *Angew. Chem., Int. Ed.*, 2017, **129**, 9283-9287.
254. Y. Wang, S. Li, P. Zhang, H. Bai, L. Feng, F. Lv, L. Liu and S. Wang, *Adv. Mater.*, 2018, **30**, 1705418.
255. S. Tadepalli, J. Yim, S. Cao, Z. Wang, R. R. Naik and S. Singamaneni, *Small*, 2018, **14**, 1702382.
256. H. Link, K. Kochanowski and U. Sauer, *Nat. Biotechnol.*, 2013, **31**, 357.
257. S. H. Kim, K.-R. Kim, D.-R. Ahn, J. E. Lee, E. G. Yang and S. Y. Kim, *ACS Nano*, 2017, **11**, 9352-9359.
258. C. Wang, Q. Zhang, X. Wang, H. Chang, S. Zhang, Y. Tang, J. Xu, R. Qi and Y. Cheng, *Angew. Chem., Int. Ed.*, 2017, **56**, 6767-6772.
259. M. D. Blankschien, L. A. Pretzer, R. Huschka, N. J. Halas, R. Gonzalez and M. S. Wong, *ACS Nano*, 2012, **7**, 654-663.
260. J. Li, C. Xie, J. Huang, Y. Jiang, Q. Miao and K. Pu, *Angew. Chem., Int. Ed.*, 2018, **57**, 3995-3998.
261. Y. Wang, S. Li, L. Liu, F. Lv and S. Wang, *Angew. Chem., Int. Ed.*, 2017, **129**, 5392-5395.
262. T. Lei, M. Guan, J. Liu, H.-C. Lin, R. Pfattner, L. Shaw, A. F. McGuire, T.-C. Huang, L. Shao, K.-T. Cheng, J. B. H. Tok and Z. Bao, *Proc. Natl. Acad. Sci. USA*, 2017, **114**, 5107-5112.
263. T. Repenko, A. Rix, S. Ludwanowski, D. Go, F. Kiessling, W. Lederle and A. J. Kuehne, *Nat. Commun.*, 2017, **8**, 470.

Table of Contents

Recent progress in developing organic semiconducting materials (OSMs) for deep-tissue optical imaging, cancer phototherapy and biological photoactivation is summarized.

Biography

Jingchao Li received his PhD degree in Materials Science and Engineering from the University of Tsukuba (Japan) in 2017. Then he worked as a Postdoctoral Research Fellow at Prof. Kanyi Pu's group in the School of Chemical and Biomedical Engineering (SCBE), Nanyang Technological University (NTU). His current research focuses on the development of advanced agents for cancer theranostics.

Kanyi Pu received his PhD from the National University of Singapore in 2011 followed by a postdoctoral study at Stanford University School of Medicine. He joined the School of Chemical and Biomedical Engineering (SCBE) at Nanyang Technological University (NTU) as an Associate Professor in 2015. His research lies at the intersection of chemistry, nanotechnology, biophotonics and medicine, aiming to develop smart molecular probes and advanced imaging technologies to understand, detect and treat life-threatening diseases.