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Multimodal Biophotonics of Semiconducting Polymer

Nanoparticles

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CONSPECTUS: Biophotonics as an interdisciplinary frontier plays an increasingly important role in modern biomedical science. Optical agents are generally involved in biophotonics to interpret biomolecular events into readable optical signals for imaging and diagnosis, or to convert photons into other forms of energy (such as heat, mechanical force or chemical radicals) for therapeutic intervention and biological stimulation. Development of new optical agents including metallic nanoparticles, quantum dots, up-conversion nanoparticles, carbon dots, and silica nanoparticles have contributed to the advancement of this field. However, most of these agents have their own merits and demerits, making them less effective as multimodal biophotonic platform.

In this Account, we summarize our recent work on the development of near-infrared (NIR) semiconducting polymer nanoparticles (SPNs) as multimodal light converters for advanced biophotonics. SPNs are composed of π -electron delocalized semiconducting polymers (SPs) and often possess the advantages of good biocompatibility, high photostability and large absorption coefficients. Because the photophysical properties of SPNs are mainly determined by the molecular structures of the precursor polymers, molecular engineering allows to finetune their photophysical process to obtain different optical responses, even to the light in the second NIR window (1000-1700 nm). Meanwhile, the facile nano-formulation methods of SPNs enable alteration of their outer and inner structures for diverse biological interactions.

The unique photophysical properties of SPNs have brought about ultrasensitive deep-tissue molecular imaging. NIR-absorbing SPNs with strong charge-transfer backbones can convert photoenergy into mechanical acoustic waves, permitting photoacoustic imaging that bypasses the issue of light scattering and reaches the centimetre tissue penetration depth. Differently, phenylenevinylene-containing SPNs can store photon energy *via* chemical defects and emit long-NIR afterglow luminescence with a half-life of ~ 6 min after cessation of light excitation. Such

afterglow process avoids tissue autofluorescence, giving rise to ultra-high signal-to-background ratios. So far, SPN-based molecular photoacoustic or afterglow probes have been developed to image disease tissues (tumors), biomarkers (biothiols and reactive oxygen species) and physiological indexes (pH and temperature) in different preclinical animal models.

The synthetic flexibility of SPNs further permits light-modulated biological and therapeutic interventions. Till now, SPNs with high photothermal conversion efficiencies have been shaped into the photothermal transducers to remotely regulate the biological events including protein ion channels, enzymatic activity, and gene expression. Meanwhile, in conjunction with the desired biodistribution and tumor-homing ability, SPNs have been doped or coated with other inorganic agents for amplified photothermal or self-regulated photodynamic cancer therapy.

This Account thus demonstrates that SPNs serve as a multimodal biophotonic nanoplatform to provide unprecedented opportunities for molecular imaging, noninvasive bioactivation and advanced therapy.

Keywords: Molecular imaging, biophotonics, photoacoustic imaging, polymer nanoparticles

1. INTRODUCTION

Biophotonics that deals with interactions between photons and biological matters plays a pivotal role in fundamental biology and medicine. As a multidisciplinary paradigm encompassing life sciences, optics, and engineering, biophotonics provides unique capabilities for noninvasive regulation of biological activities, ultrasensitive detection of biomarkers with spatial resolution ranging from nanometer to centimeter, and real-time tracking of pathological events with a broad temporal scale from femtoseconds to days.¹ As a result, biophotonics has been serving as an

indispensable tool in diverse biomedical applications including molecular imaging, biosensors, phototherapy, laser surgery, and optogenetics.²⁻⁵

Prosperity in nanotechnology over recent years has led to profound improvement in biophotonics.⁶ Coalescence of both broadens the vision of existing photonic modalities and imparts future biomedical techniques related to optical stimuli. Inorganic nanoparticles including semiconductor quantum dots, plasmonic nanostructures, silica nanoparticles, carbon dots and upconversion nanoparticles have been involved in biophotonics.⁷ In these applications, they often serve as the intermediators to transduce biomolecular interactions into optical signals, augment signal readout, or convert the light energy into heat or electric current to simulate the biological activities.⁸ However, they have their own merits and demerits including toxicity issue for quantum dots and upconversion nanoparticles, poor photostability for plasmonic nanostructures, and short excitation and emission wavelengths for carbon dots and silica nanoparticles, making them less versatile and effective in biophotonics. In contrast to inorganic nanoparticles, organic nanoparticles have been less exploited for biophotonics, although they have the advantages of easy functionalization, relatively low cost, and potentially high biosafety.⁹

Semiconducting polymer nanoparticles (SPNs), which are made of π -conjugated semiconducting polymers (SPs) originally used in organic electronics, have emerged as one of the most competent organic agents for biophotonics.¹⁰ Because the bandgaps of SPNs are mainly determined by the chemical structures of the precursor SPs, their photophysical properties are less dependent on their sizes, which is different from metallic nanoparticles. Thus, through molecular engineering, SPNs can possess versatile light-responsive properties to meet the specific requirements for different biophotonic applications. Until now, SPNs have been used as fluorescent agents for imaging of

biomolecules and living organisms,¹¹⁻¹³ phototherapeutic agents for cancer therapy,¹⁴ and optical regulators for bioactivation.¹⁵

Figure 1. Schematic illustration of SPNs for advanced biophotonic applications published from our group. PTT: photothermal therapy; PDT: photodynamic therapy.

In this Account, we summarize our work on development of SPNs for advanced biophotonic applications ranging from molecular imaging, to photothermal bioactivation and to cancer phototherapy (Figure 1). In the following, we first discuss the molecular design guidelines to modulate the photophysical processes of SPNs and also the nanoparticle functionalization to diversify their applications. Next, we highlight the applications of SPNs in molecular imaging with the emphasis on a newly discovered ultrasensitive optical imaging method—afterglow imaging and a deep-tissue hybrid imaging—photoacoustic (PA) imaging. We also discuss the examples of SPNs as photothermal regulators and phototheranostic agents for biological control and cancer therapy, respectively. At last, we share our opinions on the existing challenges and untapped potential of SPNs in biophotonics.

2. MOLECULAR DESIGNS, PHOTOPHYSICS, AND FUNCTIONALIZATION

Molecular engineering of SPs plays a crucial role in the modulation of photophysical processes of SPNs and ultimately determines their biophotonic applications.¹¹ Donor-acceptor (D-A) approach can be used to adjust the energy levels of SPs, wherein alternating electron-rich (donor) and electron-deficient (acceptor) moieties are incorporated into the π -conjugated backbones (Figure 2a).¹⁶ In general, D-A copolymers are synthesized *via* palladium-catalyzed polycondensation (e.g. Stille or Suzuki cross-coupling reactions) from donor and acceptor monomers. Some typical donors (e.g. thiophene, cyclopentadithiophene, and dithienosilole) and acceptors (e.g. benzothiadiazole, diketopyrrolopyrrole, and thiadiazoloquinoxaline) discussed in this Account are exemplified in Figure 2a. After polymerization, the highest and lowest occupied molecular orbitals (HOMO and LUMO, respectively) of the donor unit fuse with the respective orbitals of the acceptor unit to afford a new set of hybridized orbitals with narrowed bandgaps. The result is the red-shifted absorption for the synthesized copolymer relative to its monomers.

Photophysical processes of SPs upon light excitation or irradiation along with the corresponding biophotonic functions are depicted in the form of Jablonski diagram (Figure 2b).¹⁷ Upon light excitation, SP is excited from the ground state to the excited states of higher energy (S_n). The excited state of SP is unstable and subsequently undergoes non-radiative vibrational relaxation, also termed as internal conversion (IC), to the lowest vibrational level of excited state (S_1). Following this, there are two different pathways: either returning to the ground state *via* relaxation or going through intersystem crossing (ISC) to the triplet state (T_n). As for the direct relaxation to the ground state, one way is to the radiative decay with the emission of photons defined as fluorescence; while the other way is the non-radiative IC process that leads to heat generation. Thus, in addition to fluorescence agents, SP can be used as photothermal agents for PA imaging, photothermal therapy (PTT), and thermal regulation of biological functions. On the other hand,

once SP is transferred to triplet state, it might also relax *via* the emission of photons (phosphorescence); or it can transfer the energy to the surrounding molecules such as oxygen to generate singlet oxygen ($^1\text{O}_2$). $^1\text{O}_2$ not only can be used as the cytotoxic therapeutic molecule for photodynamic therapy (PDT), but also can react with the vinylene bonds in the π -conjugated backbone of polyphenylenevinylene (PPV)-based SPs to form unstable chemical defects (dioxetane units), which can spontaneously and slowly break down to release photons for afterglow imaging.

Nano-formulation is used to convert intrinsically hydrophobic SPs into water-soluble nanoparticles (SPNs).¹⁴ Two approaches are commonly employed including mini-emulsion and nano-precipitation (Figure 2c). Amphiphilic agents (e.g. surfactants, amphiphilic polymers) are generally used to facilitate the formation of stable SPNs in aqueous solution. Of these two approaches, nano-precipitation produces smaller nanoparticles (10-50 nm) than mini-emulsion because it utilizes water-miscible solvents (e.g. tetrahydrofuran) rather than water-immiscible solvents (e.g. dichloromethane). Optical properties of SPNs in aqueous solution are often slightly different from the precursor SPs in organic solvent (hypochromic shift of absorption and reduced fluorescent quantum yields), because of the changes of SPs from the initial extended state into a condensed state within nanoparticles.

The facile nano-formulation methods enable SPNs to be further functionalized through intra-particle doping or surface modification for a variety of biological applications (Figure 2d). Hydrophobic components can be incorporated into SPNs during nano-precipitation. For instance, doping of sensing molecules (e.g. bis(2,4,6-trichlorophenyl) oxalate, TCPO), inorganic nanostructures (e.g. nanoceria), electron acceptors, and therapeutic agents (e.g. anti-cancer drug) for smart chemiluminescence imaging,¹⁸ self-regulative PDT,¹⁹ amplified PTT,²⁰ and

combinational therapy,²¹ respectively. Meanwhile, surface engineering of SPNs can be conducted through chemical reactions such as click chemistry or carboxyl/amine coupling reaction. For example, targeting moieties (peptide and antibody) and reactive components (dyes and enzymes) can be linked to the SP backbones so as to direct nanoparticles to specific sites (e.g. cancer cells,²² organelles,²³ and ion channels,²⁴ etc.) and make them responsive to specific biomolecule of interest (e.g. protein sulfonic acid),²⁵ respectively.

Figure 2. Molecular designs, photophysics and functionalization. (a) Simplified mechanism of donor-acceptor engineering to reduce bandgap. Right: representative chemical structures of donors (red) and acceptors (blue). (b) Jablonski diagram of the photophysical processes of SPNs. Abs., absorption; IC., internal conversion; Fluo., fluorescence; ISC., intersystem crossing; phos., phosphorescence; PT, photothermal. (c) Scheme of preparation of SPNs. (d) Schematic illustration of components and surface modification of SPNs.

3. MOLECULAR AFTERGLOW IMAGING

Conventional photoluminescence imaging relies on fluorescent signals acquired upon real-time light excitation to delineate biological tissues, wherein auto-fluorescence from endogenous

fluorophores is inevitable. Thereby, conventional photoluminescence imaging has relatively low signal-to-background ratios (SBRs), resulting in limited sensitivity. Even though SPNs doped with chemiluminescent substrates have been exploited for self-illuminating imaging, endogenous reactive oxygen species (ROS) are required to trigger luminescence.¹⁸ In contrast, persistent or afterglow luminescence is an emerging paradigm of self-illuminating imaging. However, persistent luminescence behavior is limited to a few inorganic nanomaterials that have the rare-earth or heavy metal ingredients. In addition to the toxic concern, their luminescence half-lives are relatively short (< 200 s) in aqueous solution.²⁶

Recently, our group revealed that PPV-based SPNs can be used for NIR afterglow imaging.²⁷ The afterglow mechanism is different from that of persistent luminescent inorganic nanoparticles, wherein the photon energy is absorbed and stored in intrinsic defect lattices. Instead, light irradiation of PPVs leads to the generation of $^1\text{O}_2$, which subsequently reacts with the vinylene bond of PPVs *via* [2+2] cycloaddition to afford a high-energy intermediate PPV-dioxetane. Such light-induced chemical defects spontaneously undergo decomposition, slowly releasing photons as the product with a half-life of ~ 6 min. According to this mechanism, the afterglow of PPV-based SPNs was remarkably amplified by simply doping with a NIR-absorbing photosensitizer (NCBS) that had higher ability to generate $^1\text{O}_2$ than PPV (Figure 3b). In addition, due to the intraparticle energy transfer, the afterglow emission was red-shifted from 580 to 780 nm (Figure 3c). Such NIR afterglow allowed for deep tissue penetration at the thickness up to 4 cm, in sharp comparison with merely 2 cm by NIR fluorescence (Figure 3d). Similarly, the SBR of NIR afterglow captured through a living mouse (~ 1.7 cm) was 120 times higher than that of NIR fluorescence (Figure 3e). Moreover, the low-background afterglow imaging enabled clear visualization of tumor on a living mouse which was undiscernible by fluorescence shortly after

administration of nanoparticles (Figure 3f). Such superior detection sensitivity was later further improved by transforming MEHPPV into an amphiphilic derivative (PEG-PPVL).²⁸ By virtue of the higher density of poly(ethylene glycol) (PEG) and brighter afterglow, the PEG-PPVL-based nanoparticles had better accumulation in tumors relative to MEHPPV-based counterpart, affording ultrasensitive detection of nearly invisible peritoneal metastatic tumors.

Afterglow SPNs can be tailored for smart activatable molecular imaging such as detection of drug-induced hepatotoxicity. As illustrated in Figure 3g, an activatable afterglow probe (SPN-thiol) was designed to be initially in “off” state due to the electron transfer between the electron-withdrawing quencher (2,4-dinitrophenylsulfonyl, DNBS) and MEHPPV. In the presence of biothiols, the sulfonamide bond of DNBS was cleaved and thus led to the recovery of afterglow luminescence (“on” state). SPN-thiol was used to detect the hepatotoxicity generated by acetaminophen (APAP) overdose in mouse model (Figure 3h). Compared with saline treated mice, APAP treated mice demonstrated significantly attenuated (1.99-times lower) afterglow in liver after administration of SPN-thiol. While the APAP treated mice protected by antioxidant *N*-acetyl-*L*-cysteine (NAC) retained afterglow comparable to the control mice. The utility of SPN for ultra-sensitive afterglow imaging of biothiols was able to detect early hepatotoxicity within 30 min, even before noticeable histological changes.

Because the generation of PPV-based afterglow luminescence relies on oxygen, such afterglow can be used to evaluate oxygen levels: for instance, differentiating hypoxia (tumor) from normoxia (skin) environment in living mice.²⁸ Furthermore, because afterglow is a chemical reaction that is thermodynamically controlled, it is sensitive to temperature. Thus, afterglow can be used to monitor the temperature during PTT. A nanoparticle cocktail (SPN_{CT}) was synthesized to comprised PPV-PEG as temperature sensor and PCSD as photothermal agent for afterglow

imaging guided PTT, which represented the first organic nanoagent for optical imaging guided PTT with no need of light excitation.²⁹

Figure 3. Afterglow imaging. (a) Chemical structures of representative SPs and amphiphilic polymer for afterglow imaging. PPV-PEG: $x = 0.5$, $y = 0.5$; PPV-PEGL: $x = 0.11$, $y = 0.88$. (b) Proposed mechanism of amplified afterglow luminescence. (c) Afterglow spectrum of SPN-MEHPPV and SPN-NCBS5 after irradiation by 808 nm laser at 1 W cm⁻² for 1 min. (d) SBRs of afterglow and fluorescence imaging of SPN-NCBS5 as a function of tissue penetration depth. *Statistically significant difference between SBRs of afterglow and fluorescence intensities at 4 cm of tissue ($n = 3$, * $P < 0.0001$). (e) Fluorescence and afterglow luminescence images of SPN-NCBS5 solution (62.5 μ g mL⁻¹, 50 μ L) treated with pre-irradiation (808 nm, 1 W cm⁻², 1 min)

embedded beneath a living mouse. (f) Fluorescence and afterglow images of a tumor (indicated by white dashed circles) on a living mouse at 1 h after intravenous injection of SPN-NCBS5 (0.25 mg/mL, 0.2 mL). (g) Schematic of “turn-on” mechanism of SPN-thiol in the presence of biothiol. (h) Afterglow imaging of APAP-induced hepatotoxicity using SPN-thiol. Representative afterglow images of mice intraperitoneally injected with APAP, saline, and NAC together with APAP. Injection dosage: APAP, 300 mg kg⁻¹; NAC, 200 mg kg⁻¹; SPN-thiol, 0.25 mg mL⁻¹, 0.2 mL. Afterglow images were captured after irradiation by 808 nm at 1 W cm⁻² for 1 min. Error bars indicated standard deviation of three separate experiments (n = 3). Reprinted with permission from ref 27. Copyright 2017 Nature Publishing Group.

4. PHOTOACOUSTIC (PA) IMAGING

PA imaging is a hybrid imaging modality that takes advantage of PA effect to convert absorbed photons into acoustic waves.³⁰⁻³¹ Thus, PA imaging offers higher contrast and spatial resolution than traditional optical imaging in biological tissues.³² Because PA effect relies on thermoelastic expansion, photothermal property of a contrast agent plays a crucial role in PA intensities.³³ Because SPNs often possess excellent photo-stability and high mass absorption coefficient, they often have higher photothermal conversion efficiencies than most inorganic agents (gold nanorods, carbon nanotubes, etc.),³⁴ making them superior in long-term PA imaging.³⁵⁻³⁷

SPNs have been exploited for *in vivo* activatable PA imaging of biomarkers. A pioneer study was given to *in vivo* ROS sensing (ONOO⁻ and ClO⁻) by taking advantage of a ROS-inert SP and a ROS-responsive dye (IR775S).³⁸ Similarly, we developed another activatable nanoprobe for *in vivo* ratiometric PA imaging of pH.³⁹ Such nanoprobe (SON₅₀) contained a pH-inert semiconducting oligomer (SO: F-DTS) and a pH-sensitive boron-dipyrromethene (BODIPY) dye (pH-BDP) (Figure 4a). When pH decreased from 7.4 to 5.5, PA amplitude of SON₅₀ at 680 nm remained nearly unchanged; whereas, that at 750 nm assigned to pH-BDP remarkably decreased due to the protonation of pH-BDP (Figure 4b, c). Such a sensitive pH response of SON₅₀ permitted

rationetric PA (PA_{680}/PA_{750}) imaging of pH in the tumor of living mice (Figure 4d). In addition to utilization of biomarker-responsive dopant, the semiconducting matrix can be designed as the sensing component. For instance, we incorporated the ROS-oxidizable phenothiazine unit into a semiconducting oligomer amphiphile (SOA) and utilized it as the matrix to encapsulate the ROS-inert dye (NCBS) for PA imaging of ClO^- in the tumor of living mice.⁴⁰

To further improve the imaging performance, SPNs were synthesized to afford PA imaging in a more biologically transparent window (1000-1700 nm), termed as the second NIR window (NIR-II).⁴¹ Because of the reduced tissue scattering and higher safety limit, NIR-II light is expected to probe deeper into biological tissues than NIR-I light (650-950 nm).⁴²⁻⁴⁴ Incorporation of highly electron-deficient thiadiazoloquinoxaline into the backbone endowed SP-II with an intense absorption in the NIR-II window (Figure 4f).⁴⁵ Particularly, the corresponding nanoparticles (SPN-II) generated nearly identical PA amplitudes at 750 and 1064 nm (Figure 4e), enabling a fair comparison of PA penetration depths in both NIR windows. Utilization of SPN-II as the NIR-II PA agents led to the detection of signals through a 5-cm depth in biological tissues (Figure 4g), which was not possible for NIR-I PA imaging. In addition, NIR-II PA imaging allowed to image brain vasculature with the SBR that was 1.5-times higher than that for NIR-I PA imaging (Figure 4h).

Figure 4. PA imaging in the NIR-I and NIR-II windows. (a) Chemical structures of representative SPs used for PA imaging. (b) Scheme of ratiometric pH-sensing mechanism of SON₅₀. (c) PA spectra of SON₅₀ under different pH conditions. (d) Representative ratiometric PA (PA_{680}/PA_{750}) images (axial view) of one HeLa xenograft tumor bearing mouse at 0 and 6 h post-injection of SON₅₀. Yellow arrows indicated one blood vessel. Scale bar: 2 mm. (e) Schematic illustration of SPN-II with PA signals in both NIR windows. (f) UV-Vis-NIR absorption spectrum of SPN-II. (g) NIR-II (1064 nm) PA images of SPN-II solution embedded at 1 and 5 cm depth in chicken breast tissue. Energy density: 20 mJ cm⁻². (h) Representative *in vivo* PA images of rat brain vasculature in both NIR windows (750 and 1064 nm, respectively) at 70 min after intravenous administration of SPN-II. (b, c, and d) Reprint with permission from ref 39. Copyright 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim; (e, f, g, and h) Reprint with permission from ref 45. Copyright 2017 American Chemical Society.

5. PHOTOTHERMAK BIOACTIVATION

By taking advantage of the high photothermal conversion efficiencies, we have exploited a new research direction of SPNs in biophotonics: remote control of heat-related biomolecular activities. For example, we developed SPNs into photothermal modulator for remote activation of thermosensitive ion channel (transient receptor potential cation channel subfamily V member 1, TRPV1) located on the membranes of neurons.²⁴ To target TRPV1 channels, an anti-TRPV1 antibody was conjugated to the surface of a SPN (Figure 5a), leading to the final nanomodulator (SPNbc). SPNbc was able to specifically bind to TRPV1 channels, rapidly induce local temperature rise above the threshold (43 °C) upon laser irradiation at 808 nm, and instantly activate TRPV1 channels (Figure 5b). Such transient photoactivation was witnessed by the rapidly increased fluorescence from a Ca^{2+} indicator (Fluo-8) in TRPV1-positive cells (mouse neuroblastoma/DRG neuron hybrid ND7/23) but not for TRPV1-negative cells (Hela) within milliseconds after laser irradiation (Figure 5c). The TRPV1 ion channels could be reversibly activated and silenced by switching the laser on and off at the intervals of 0.5 s. Moreover, a higher mass extinction coefficient at the laser wavelength was essential for such a rapid response, because only SPN2 with a higher extinction coefficient at 808 nm relative to SPN1 (PCB-based SPN) could do so (Figure 5c).

Acting as photothermal transducers, SPNs can also be leveraged to remotely control enzymatic activity for enhanced cancer therapy.⁴⁶ We developed a thermo-sensitive nanoenzyme (PCB-Bro) by conjugating bromelain (Bro) to a semiconducting amphiphile PCB-PEG (Figure 5a). Bro is a protease that digests collagen, and its optimal activity is at around 45 °C. Upon laser irradiation, the SP core of PCB-Bro rapidly transformed photon energy into heat, leading to local temperature rise and consequently activating the proteolytic activity of Bro on the nanoparticle surface. After passive delivery of PCB-Bro into tumor, remote photothermal activation of PCB-Bro favored

efficient digestion of collagen in tumor extracellular matrix (ECM). Such *in situ* ECM degradation consequently facilitated accumulation of the nanoenzyme in tumor, resulting in elevated photothermal temperature and thus enhanced therapeutic efficacy relative to the precursor SPN without Bro conjugation.

Additional functionality such as gene delivery can be incorporated to SPNs to transform them into photothermal nanocarriers for remote activation of gene expression at designated locations.⁴⁷ The gene delivery capability was endowed by linking a cationic dendrimer gene vector (polyamidoamine, PAMAM) to the side chain of a SP *via* click chemistry (Figure 5a). The resulted dendronized SP (DSP) could form nanocomplex with plasmids through electrostatic attraction. To convert the photothermal signal into the cue for gene expression, the gene of interest cloned downstream of a heat shock promoter (HSP70) was incorporated into the plasmid. Upon laser irradiation, the photothermal heat generated from DSP stimulated the transformation of heat shock factor (HSF) from monomers into trimers (Figure 5d). HSF trimers subsequently translocated to the nucleus and bound to heat shock element (HSE) of HSP70, eventually activating gene transcription. Such transient activation of gene expression was confirmed by real-time optical monitoring of luciferase (Luc) expression. After short laser irradiation, luminescence signal was increased by 25-fold in HeLa cells transfected with DSP/pHSP70-*Luc* nanocomplex. Such activation was also observed in a subcutaneous mouse model, showing a 4.5-fold increase of bioluminescence (BL) after laser irradiation (Figure 5e, f). Serving as the first polymeric nanoplatform that integrates photothermal transducer with gene vector, DSP holds great promise in the development of nanomedicines for personalized gene therapy.

Figure 5. Photothermal bioactivation. (a) Chemical structures of representative SPs for photothermal regulation. (b) Schematic illustration of the photothermal activation of Calcium ion channel TRPV1 by SPN_{bc}. Real-time influx of Ca²⁺ was monitored by Fluo-8 with amplified fluorescence upon capturing Ca²⁺. (c) Quantification of fluorescence intensity from Fluo-8 in Figure 5b as a function of laser irradiation time. (d) DSP-regulated gene delivery and photothermal activation of gene expression. PS-PAA: poly(styrene)-*block*-poly(acrylic acid). (e) Representative BL images of a living mouse subcutaneously planted with DSP/pHSP70-Luc nanocomplexes transfected HeLa cells pellets (dashed circles) treated with (red circle) or without (white circle) 808 nm laser irradiation (0.42 W cm⁻²). (f) Quantification of BL changes in Figure 5e as a function of laser irradiation time. (b, c) Reprinted with permission from ref 24. Copyright 2016 American

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6. CANCER PHOTOTHERANOSTICS

Surface PEGylation of SPNs can lead to the good biodistribution and passive tumor targeting ability.⁴⁸ Thus, in association with their excellent photothermal properties, SPNs have been applied for cancer phototheranostics. Dependent on the molecular structures of SPs, the photothermal conversion efficiencies of SPNs range from 6% to 71%.^{34, 49} In addition to polymer screening, modulation of internal photophysical processes of SPNs can be used to amplify photothermal conversion. For instance, PCB-based SPNs were doped with fullerene to favor photo-induced electron transfer (PET) (Figure 6a).²⁰ Such doping quenched the fluorescence of PCB and promoted non-radiative relaxation to augment heat generation (Figure 6b, c), consequently affording improved anti-tumor PTT efficacy. To improve the tissue penetration, we further developed SPNs into NIR-II absorbing phototheranostics (Figure 6a).⁵⁰ After D-A molecular engineering, the SPN (SPN_{I-II}) with identical absorbance in both NIR windows was obtained (Figure 6d). Comparison studies proved that NIR-II light possessed superior deep tissue photothermal heating capability over NIR-I light (Figure 6e, f). Thus, SPN_{I-II} was able to effectively ablate the tumor artificially placed beneath 5-mm-thick biological tissue, validating the high potential of SPNs for deep-tissue PTT.

The synthetic flexibility of SPNs also enabled regulation of photodynamic process through hybridization with inorganic nanoparticles for enhanced PDT. For instance, a hybrid nanocomposite consisting of PCB as the photosensitizer and nanoceria as the ROS regulator were developed for self-regulated PDT (Figure 6a).¹⁹ Serving as ROS converter at pH 6.5 whereas ROS scavenger at pH 7.4, nanoceria induced enhanced ROS generation in the acidic tumor microenvironment but minimized ROS generation in neutral normal tissues (Figure 6g). Such

disease-correlated self-regulating theranostic nanocomposite not only reduced nonspecific side effects, but also contributed to the enhanced anti-tumor PDT efficacy (Figure 6h). Furthermore, a multilayered SPN (SPN-M1) coated with manganese dioxide (MnO_2) was devised to overcome the hypoxia limitation of tumor for amplified PDT.⁵¹ In hypoxic and acidic environment of solid tumor, MnO_2 nanosheets on the surface of SPN-M1 reacted with and transformed H_2O_2 into O_2 , enabling 2.68-fold higher yield of $^1\text{O}_2$ relative to the uncoated SPN (SPN-0) upon laser irradiation at 808 nm. As such, SPN-M1 demonstrated improved PDT efficacy than SPN-0 in mouse model.

Figure 6. Cancer Phototheranostics. (a) Chemical structures of representative SPs and dopant utilized for phototheranostics. (b) Schematic illustration of molecular mechanism of SPN-F20 with amplified PA signal and photothermal efficacy. (c) IR thermal images of SPN solutions with different doping amount of PCB70BM after laser irradiation for 5 min. SPN-F0, SPN-F4, SPN-F10, SPN-20, and SPN-F40 correspond to 0, 4, 10, 20, 40 w/w% of PC70BM, respectively. (d) Schematic of SPN_{I-II}. (e) Scheme of deeper tissue penetration of NIR-II light compared with NIR-I light at their respective exposure limit to skin (1 W cm^{-2} for 1064 nm while 0.3 W cm^{-2} for 808 nm). (f) Temperature change of SPN_{I-II} solution (50 $\mu\text{g mL}^{-1}$) as a function of tissue depth after

laser irradiation. Solid lines indicate fitted exponential decay curves. (g) Schematic of mechanism of self-regulated PDT by nanoceria-doped SPNs. (h) Hematoxylin and eosin staining of mouse muscle and tumor on living mice treated with SPN-C23 (nanoceria doped SPN), SPN-0, and saline after 808 nm laser irradiation (except for tumor on saline injected mouse). Scale bar: 200 μm . (b, c) Reprinted with permission from ref 20. Copyright 2016 American Chemical Society; (d, e, and f) Reprinted with permission from ref 50. Copyright 2018 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim; (g, h) Reprinted with permission from ref 19. Copyright 2017 American Chemical Society.

7. SUMMARY AND OUTLOOK

In this Account, we have summarized our recent work on the design of SPNs for advanced biophotonics. Molecular engineering of SPNs allows us to manipulate their bandgaps and regulate their internal photophysical processes to diversify their biophotonic responses. Meanwhile, nano-functionalization of SPNs provide the feasibility to modify their outer structures and inner compositions, enabling them as a multifunctional biophotonic nanoplatform. Such structural and functional versatility of SPNs in conjunction with their other intrinsic merits have helped advance the field of biophotonics, represented by their unprecedented applications ranging from ultrasensitive afterglow imaging and deep-tissue activatable PA imaging, to photothermal regulation of biomolecular activities and self-regulated phototheranostics.

Despite the considerable progress, development of SPNs in biophotonics is still in its infancy. Challenges and opportunities coexist, waiting to be addressed in the near future. First, the long-term biosafety of SPNs is still a concern. For rapid *in vivo* clearance, SPNs can be designed to be smaller than 5 nm to enable them pass through glomerular capillary wall for urinary excretion. Although we and others have recently discovered that imine bonds,⁵² imidazole units,⁵³ thiophene moieties,⁴⁰ and vinylene bonds can serve as the enzyme-cleavable linker in the conjugated backbone of SPs to facilitate the oxidation-induced degradation of SPNs,³⁴ more biodegradable

strategies are desired. Second, NIR-II light responsive SPNs are highly demanded to further improve the imaging performance of afterglow luminescence and PA signals. Third, future efforts can be made to leverage SPNs for the diseases other than cancer such as neurodegenerative diseases, cardiovascular diseases and autoimmune diseases.

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The authors declare no competing financial interest.

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Yuyan Jiang received her B.E. degree from Sun Yat-Sen University in 2016. She is currently a Ph.D. student under the supervision of Prof Kanyi Pu in School of Chemical and Biomedical Engineering at Nanyang Technological University. Her research focuses on development of SPN-based biophotonic nanagents for cancer theranostics.

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