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Development of Semiconducting Polymer Nanoparticles for Photoacoustic Imaging

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Abstract: Semiconducting polymer nanoparticles (SPNs) have evolved into a new class of photonic materials with a great potential for biomedical applications. Depending on the polymer structures, SPNs can be developed into optical agents for fluorescence and chemiluminescence imaging, photosensitizers for photodynamic therapy, and heat converter for photothermal therapy. In this Feature article, we summarize our recent work on the development of SPNs for in vivo photoacoustic (PA) imaging, a state-of-art imaging modality that converts light energy into mechanical acoustic waves to provide deeper tissue penetration. The structure-property relationship and doping approaches are discussed to reveal the importance of promoting nonradiative decay in amplifying the PA brightness of SPNs. Moreover, their imaging applications including lymph node mapping, tumor imaging and monitoring of pathological indexes are highlighted. Our studies demonstrate that SPNs can serve as versatile PA agents for advanced molecular imaging applications.

Keywords: Polymer nanoparticles; Photoacoustic imaging; Tumor imaging; Semiconducting polymers

1. Introduction

Photoacoustic (PA) imaging based on the PA effect that converts light energy into mechanical acoustic waves, which minimizes the light scattering issue and thus provides deeper tissue imaging penetration and higher spatial resolution as compared with traditional optical imaging methods.^[1] Compared to other high-resolution optical imaging modalities which suffer from the limitation of tissue-penetration depth less than 1 mm, PA imaging technology can be detected up to a few centimeters deep in tissues. In addition, PA imaging possess multichannel detection capabilities by using mutiple wavelengths, along with its low cost, portability, lack of ionizing radiation and high resolution^[2] Because only a few exogenous agents are able to emit PA signals such as melanin and hemoglobin, development of imaging agents for PA imaging has been a focus in the area of biomaterials. The suitability of many optical materials for PA imaging have been tested, which include near-infrared (NIR) organic dyes,^[3] fluorescent proteins,^[4] porphysomes,^[5] inorganic metallic nanoparticles,^[6] up-conversion nanoparticles,^[7] carbon nanomaterials^[8] and 2D materials.^[9] While these materials have their own merits, each has the limitation, for instance generally poor photostability for organic dye and fluorescent proteins,^[10] potential phototoxic for porphysomes due to the strong ability of generating singlet oxygen,^[11] and flat PA spectral for carbon nanomaterials.^[12] Thus, it is necessary to continuously develop alternative imaging agents with improved properties to explore the potential of PA imaging in fundamental biology and medicine.

Semiconducting polymer nanoparticles (SPNs) have gained increasing attention as a new type of optical nanomaterials because of their excellent optical properties and relatively good biocompatibility.^[13] SPNs contain the key component: optoelectronically active semiconducting polymers (SPs) that have been used for electronic applications such as solar cells,^[14] light-emitting diodes, field-effect transistors^[15] and tissue engineering.^[16] The optical properties of SPNs are

mainly determined by SPs with π -electron delocalized backbones, and thus selection of SPs is a crucial step to fulfill specific imaging applications.^[17] SPs originally used for light-emitting diodes applications are suitable for fluorescence imaging applications because of their intrinsically high photoluminescence quantum yields. These corresponding light-emitting SPNs have been validated for fluorescence cell tracking,^[18] tumor imaging^[19] and ultrafast hemodynamic imaging.^[20] Also, we have demonstrated their ability to participate in the reaction with reactive oxygen species to generate chemiluminescence, allowing for imaging of drug-induced hepatotoxicity^[21] and neuroinflammation.^[22]

Recently, we transformed SPs formerly designed for photovoltaics for PA imaging because these SPs generally have NIR absorption. Moreover, because of their high photothermal conversion efficiency, these SPNs can also be used for photothermal cancer therapy^[23] and real-time activation of neurons^[24] under NIR irradiation. Our and others groups have shown that SPNs can serve as versatile nanoplatform for *in vivo* PA imaging.^[25]

In this feature article, we summarize our recent work on the development of SPNs for PA imaging. Poly(cyclopentadithiophene-*alt*-benzothiadiazole) (SP1), poly(acenaphthothienopyrazine-*alt*-benzodithiophene) (SP2) and five diketopyrrolopyrrole (DPP)-derivatives (SP3-SP8) (Scheme 1) have been respectively transformed into SPN1-8 and used for different PA imaging applications including lymph node imaging, tumor imaging, and disease microenvironment imaging. In the following, we first discuss the potential advantages of SPNs in PA imaging, followed by the general design consideration and approaches to achieve high PA brightness. Then, we will highlight the versatility of SPNs to evolve into smart activatable probes that changes their acoustic signals responding to the target of interest in living animals. At last, the

summary and perspectives are given.

Scheme 1. (a) Chemical structures of SP1-8 used for PA imaging. (b) Chemical structures of amphiphilic lipids and polymers used for the preparation of SPNs (c) Chemical structures of PC70BM, F-DTS and pH-BDP.

2. Advantages in PA Imaging

To evaluate the potential of SPNs for PA imaging, we initially evaluated SP1 and SP2. Nanoprecipitation is a general way to prepare water-soluble SPNs. A water-miscible organic solvent such as tetrahydrofuran (THF) is usually used to dissolve both SP and the amphiphilic matrix, and then the mixture is rapidly injected to water under ultrasonification. Due to sudden decrease in solvent hydrophobicity, strong interaction between SP and the hydrophobic part of the amphiphilic polymer occurs, leading to water-soluble spherical nanoparticles.^[26]

Dipalmitoylphosphatidylcholine (DPPC) was used as the amphiphilic component to respectively co-precipitate with SP1 and SP2, resulting in the high stability for SPNs under physiologically conditions (Figure 1a).^[13b] As shown in Figure 1b, SPN1 and SPN2 had NIR absorptions with the maxima at 660 and 700 nm, respectively. Both SPNs efficiently generated PA signals under NIR pulsed laser irradiation with strong absorption in the NIR region. Because SPN1 had 4.65-fold higher mass extinction coefficients as compared to that of SPN2 (Figure 1b), the peak PA signals of SPN1 at 690 nm is 4.85-fold of that for SPN2 at 705 nm. Furthermore, at the same mass concentration, the PA amplitude of SPN1 was 5.2 and 7.1-times higher than SWNTs and GNR, respectively (Figure 1c), which was consistent with the rank order of their mass extinction coefficients (93, 50 and 45 cm⁻¹ mg⁻¹ ml for SPN1, SWNTs and GNRs, respectively). Different from GNR, the PA signal of SPN1 remained unchanged after exposure to 2.4×10^4 pulses laser at 9 mJ cm⁻² fluence (Figure 1d), indicating that SPN1 had higher photostability. Due to the high PA brightness, SPN1 was administered intravenously to the healthy mice for lymph nodes (LN) imaging at a relatively low dosage (50 µg per mouse). At 24 h post-injection, strong PA signal was detected in the lymphatic networks of living mice, showing effective accumulation in brachial lymph nodes (BLNs), inguinal lymph nodes (ILNs) and superficial cervical lymph nodes (SCLNs) (Figure 1e). These data show that SPNs generally have a high mass extinction coefficient, strong PA brightness and excellent photostability, and thus are promising for long-term PA molecular imaging.

Figure 1. (a) Schematic of the preparation of SPNs through nanoprecipitation. (b) UV-visible absorption (dashed lines) and photoacoustic spectra (solid lines) of SPNs. (c) PA amplitudes of the nanoparticles based on the same mass ($25 \mu\text{g mL}^{-1}$) (top) and molar (48 nM) (bottom) concentrations in an agar phantom. (d) PA amplitudes of indicated nanoparticles in agar phantoms versus number of laser pulses. (e) Ultrasound (upper) and PA/ultrasound co-registered (lower) images of mouse lymph nodes following tail vein injection of SPN1 ($50 \mu\text{g}/\text{mouse}$). Images represent transverse slices through the lymph nodes. BLN, brachial lymph node; ILN, inguinal lymph node; SCLN, superficial cervical lymph node as indicated by white dashed circles and arrows. Reprinted with permission from ref 13a, Copyright 2014, Nature Publishing Group.

3. Structure-Property Relationship

To reveal the guidelines for the design of efficient SPNs, the relationship between polymer structures and PA properties was studied using DPP-based SPs as the model system. Three DPP-based copolymers (SP3-5) were respectively encapsulated by 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy (poly (ethylene glycol))-2000] (DSPE-mPEG2000) to form SPN3-SPN5.^[24a] Furthermore, their absorption, fluorescence, PA and photothermal properties of these SPNs were studied and compared with SPN1. As shown in Figure 2a, the maximum absorption peak of SPN1, SPN3, SPN4 and SPN5 were at 660, 635, 712, and 748 nm,

respectively. At the same mass concentration, SPN5 exhibited the highest PA signal at 710 nm among these SPNs (Figure 2b), which was ~ 3.70, 5.40 and 3.20 times brighter than those of SPN1, SPN3 and SPN4, respectively. The order of PA amplitudes for SPNs was opposite to that for the fluorescent intensities under the same mass extinction coefficients (Figure 2c). As these SPNs were not phosphorescence, the radiative decay and thermal deactivation competed with each other upon light illumination to determine the fluorescence and PA signals, respectively. Among these SPNs, SPN5 favors nonradiative deactivation more because of its strongest electron donor–acceptor backbone structure and narrowest band gap, which leads to the highest ability of transforming photon energy into heat and in turn the brightest PA signals.^[27]

With the highest PA brightness, SPN5 was exemplified for the *in vivo* imaging using the HeLa xenograft tumor mouse model. After systemic administration of SPN5, the PA signal in the tumor area gradually increased and reached maximum at 2 h. At this timepoint, the PA signal was 5.3-fold of the background signal. Furthermore, the 3D PA images at 2 h post-injection clearly indicated that PA signals resulted from the areas within and outside the blood vessels in the tumor (Figure 2e). The real-time *in vivo* PA spectrum extracted from the tumor area of SPN5-injected mice completely differed from that of saline-treated mice (Figure 2d) but closely resembled its *in vitro* spectrum (Figure 2b). This validated that the enhanced PA signal in tumor was due to the accumulation of SPN5 in tumor.

Figure 2. UV-vis absorption (a) and PA (b) spectra of SPNs in $1 \times$ PBS at pH = 7.4 (c) Normalized fluorescence and PA intensities under same mass extinction coefficients of SPNs at 710 nm. (d) *In vivo* real-time PA spectra extracted from the tumors in living mice after systemic administration of SPN5 or saline for 2 h. (e) 3D images of subcutaneous tumors of a living mouse 2 h post-injection of SPN5 ($30 \mu\text{g mouse}^{-1}$). PA images were acquired at 750 nm. Reprinted with permission from ref 25a, Copyright 2015, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

4. Signal Amplification

The structural-property studies reveal that the non-radiative deactivation plays a key role in promoting the conversion of photon energy into heat and subsequently high PA signals. Thus, photoinduced electron transfer (PET) as a non-radiative process can be used to quench fluorescence and enhance heat generation of SPNs. We thus proposed an intraparticle doping approach to induced PET within SPNs to amplify PA brightness.^[19b]

We designed and prepared five SP1-based nanoparticles with different doping amount of PC70BM (0, 4, 10, 20 and 40 w/w% percentage of PC70BM, respectively) and encapsulated them into PEG-b-PPG-b-PEG through nanoprecipitation (Figure 3a).^[19b] The PL spectrum of SPN1-F0 has a maximum emission at 840 nm, which is gradually quenched with increasing doping amount

of PC70BM (Figure 3b). All the SPNs showed noticeable PA signals from 680 to 825 nm (Figure 3c). The PA intensities of SPN1-F20 and SPN1-F40 were almost the same, which indicated that the optimum doping amount of PC70BM was 20 w/w%. The PA intensity of SPN1-F20 was 2.6-fold higher as compared to that of SPN1-F0. Meanwhile, after laser irradiation for 300 s, the maximum solution temperature of SPN-F20 could reach was 62 °C, which was 1.3-fold higher than that of SPN1-F0 (Figure 3d). The trend of PA brightness, fluorescence intensity and photothermal efficiency of SPNs coincided very well upon increasing the doping amount of PC70BM. With the best PA and photothermal efficiency, SPN1-F20 was chosen for the *in vivo* experiments with SPN1-F0 as the control, the PA imaging and therapeutic capability of the SPNs were then evaluated by 4T1 xenograft tumor model. As the PA images of SPNs shown, the PA brightness of SPN1-F20 in the tumor area was much stronger than that of SPN-F0 (Figure 3e). The 3D reconstruction of PA signal from the tumor of SPN1-F20-injected mice clearly showed that the PA signals were observable both inside and outside of the tumor blood vessels, demonstrating the extravasation ability of SPN1-F20. The therapeutic results also indicated that SPN-F20 have a superior PTT effect than that of SPN1-F0. After systemic administration of SPN1-F20, SPN1-F0, or saline for 6 h, the tumor temperature of the SPN1-F20-injected 4T1-tumor bearing mice was 1.3 and 1.7-fold of that for SPN1-F0-injected and saline-injected mice after laser irradiation for 2 min, respectively (Figure 3f). These results indicated that SPN-F20 was an outstanding theranostic agent for PA imaging and PTT.

Figure 3. (a) Schematic illustration of PET-induced amplified theranostic SPNs. (b) PL, (c) PA spectra of SPNs ($2 \mu\text{g mL}^{-1}$) in PBS solution ($1 \times \text{PBS}$, $\text{pH} = 7.4$). Excitation for PL: 650 nm. The arrows indicate the increase (b) or decrease (c) of the intensities with the increased doping amount of PC70BM in SPNs. (d) The temperature of SPN ($15 \mu\text{g mL}^{-1}$) solutions ($1 \times \text{PBS}$, $\text{pH} = 7.4$) as a function of laser irradiation time at the power intensity of 0.5 W cm^{-2} . (e) PA images of tumor after systemic administration of SPN1-F0 and SPN1-F20 ($200 \mu\text{L}$, $100 \mu\text{g mL}^{-1}$) for 6 h. (f) IR thermal images of 4T1 tumor-bearing mice under 808 nm laser irradiation (0.3 W/cm^2) after systemic administration of saline, SPN1-F0, or SPN1-F20 ($200 \mu\text{L}$, $100 \mu\text{g mL}^{-1}$) for 6 h. Reprinted with permission from ref 19b, Copyright 2016, American Chemical Society.

However, the intraparticle doping approach may encounter the issue of leakage. To solve this

problem, we directly introduced PET into the SP backbone. A series of self-quenched SPs were developed using poly {3-(5-(9-hexyl-9-octyl-9H-fluoren-2-yl)thiophen-2-yl)-2,5-bis(2-hexyldecyl)-6-(thiophen-2-yl)pyrrolo(3,4-c)pyrrole-1,4(2H,5H)-dione} (PDPPF, SP6) as the backbone framework and benzothiadiazole (BT) as the electron-deficient doping unit.^[25c] By controlling the doping amount of BT during polymerization, self-quenched SPs termed as SP7 and SP8 were synthesized, which had 5% and 10% BT in the backbone, respectively. To endow SPs with good water solubility and biocompatibility, PEG-*b*-PPG-*b*-PEG was used to encapsulate SPs, getting water-soluble SPNs (Figure 4a). The PA spectrum of SPN8 was close to its extinction, which showed the BT-related band in the range of 700-800 nm. Also, the PA spectral profile of SPN8 was distinct from that of animal blood, making it easy to be detected from blood. (Figure 4b). The PA amplitudes of SPN8 at 680 nm were then determined at a series of concentrations from 5 to 1000 mg mL⁻¹ (Figure 4c), showing a linear relationship between the PA intensities and the concentrations. Doping BT into SP6 backbones substantially promoted the non-radiative channels, lead to quenched fluorescence and enhanced thermal deactivation of SPN7 and SPN8. SPN8 was further utilized to develop nanoprobe (SPN8-RGD) for *in vivo* PA imaging of tumor by conjugating cyclic-RGD to the surface of SPN8 by virtue of its high PA imaging capability. After systemic administration of SPN8-RGD into tumor-bearing mice for 4 h, the PA signal in the tumor area reached maximum, which was 4.7-fold higher than that of the tumor background (Figure 4d). Moreover, the PA signal remained relatively high in tumor area even after systemic administration for 24 h, demonstrating the excellent tumor imaging capability of SPN8-RGD.

Figure 4. (a) Schematic illustration for preparation of self-quenched SPN. (b) PA spectrum of SPN8 (0.5 mg mL^{-1}) in PBS ($\text{pH} = 7.4$) and PA spectrum of mouse blood (c) The PA amplitudes at 680 nm as a function of concentrations of SPN8. Inset: PA images of SPN8 at different concentrations (from left to right: 62.5, 125, 250, 500, 1000 mg mL^{-1}). (d) PA images of tumor after systemic administration of SPN8-RGD ($30 \text{ } \mu\text{g mouse}^{-1}$) for 0, 4 and 24 h. Reprinted with permission from ref 25c, Copyright 2016, Elsevier Ltd.

5. Design of Activatable Molecular Probes

Unlike conventional contrast agents, activatable probes can send out specific signals in response to biomolecular targets or events of interest. Compared with other PA contrast agents relied on simple accumulation through the enhanced permeability and retention effect or active targeting to sites of interest, activatable imaging probes have a lot of advantages, such as low background noise, real-time correlation between signals and diseases status, and concentration independent contrast.^[28] SPNs-based activatable PA imaging agents have been developed to monitor different kinds of biological mediators such as reactive oxygen species (ROS)^[13b] and pH.^[25] Detection of ROS *in vivo* is a crucial but challenging task because most PA imaging agents

are unstable in the presence of ROS. SPN1 was developed into a ratiometric PA nanoprobe (RSPN) by doping a ROS-sensitive NIR dye (IR775S) (Figure 5a).^[13b] By virtue of the high tolerance of SPN1 to ROS, PA peak of RSPN at 700 nm remained almost the same in the presence of ONOO⁻ or ClO⁻, while the peaks at 735 and 820 nm decreased significantly due to the oxidation of IR775S (Figure 5b), which makes the ratiometric PA imaging of ONOO⁻ or ClO⁻ (PA₇₀₀/PA₈₂₀) available. Murine macrophage RAW264.7 cells were then utilized to evaluate the capacity of detecting ROS *in vitro* for RSPN. Strong PA signals can be observed at both 700 and 820 nm for RSPN incubated RAW264.7 cells in the resting state. After pretreating cells with lipopolysaccharide (LPS) and interferon- γ (IFN- γ) which can stimulate cells to generate ROS, PA signal at 820 nm decreased remarkably because of the oxidation of IR775S by ROS. When N-acetylcysteine (NAC) was utilized to treat the cells together with LPS and IFN- γ , PA signal at 820 nm partially recovered as NAC was a ROS scavenger (Figure 5c). The significant difference of ratiometric PA signals between resting, stimulated and NAC-protected cells can also be observed from overlaid pseudocolor PA images, demonstrating the feasibility of monitoring ROS *in vitro* by RSPN. Quantification of ratiometric PA signals showed LPS and INF- γ treated cells had the highest PA₇₀₀/PA₈₂₀ value of 7.3 $\pm$ 0.96, which followed by NAC-protected cells (3.3 $\pm$ 0.78) and resting cells (1.4 $\pm$ 0.43) (Figure 5d). Furthermore, the *in vivo* ROS imaging capability of RSPN was tested by utilizing a murine model of zymosan-induced acute edema. At t = 120 min post-injection, PA₇₀₀/PA₈₂₀ for zymosan-treated mice reached 2.7 $\pm$ 0.31 which was approximately 2-fold higher than that of saline-treated mice, indicating the excellent capability of detecting ROS *in vivo* for RSPN (Figure 5e).

Figure 5. (a) Schematic illustration of RSPN nanoprobe for ratiometric PA imaging of ROS. (b) Representative PA spectra of RSPN ($5 \mu\text{g mL}^{-1}$) in the absence and presence of H_2O_2 or ONOO^- ($5 \mu\text{M}$). (c) PA images of RAW264.7 cell pellets without any treatment (top), treated with LPS/IFN- γ (middle) or LPS/IFN- γ /NAC (bottom). (d) Quantification of PA ratio ($\text{PA}_{700}/\text{PA}_{820}$) for RAW264.7 cell pellets in figure (c). Error bars represents standard deviations from four measurements. *Statistically significant difference in $\text{PA}_{700}/\text{PA}_{820}$ between LPS/IFN- γ treated and untreated or LPS/IFN- γ /NAC treated cell pellets (* $p < 0.05$). (e) Quantification of PA ratio ($\text{PA}_{700}/\text{PA}_{820}$) as a function of time post-injection of RSPN in the thigh of mice treated with saline (-Zymosan) and zymosan (+Zymosan), respectively. *Statistically significant difference in $\text{PA}_{700}/\text{PA}_{820}$ between saline-treated and zymosan-treated mice start from 10 min post-injection at all time points (* $p < 0.05$). Reprinted with permission from ref 13a, Copyright 2014, Nature Publisher Group.

pH has been proved to be a crucial physiological parameter that plays a critical role in cellular and tissue homeostasis. Abnormal pH is related to many diseases such as cancer,^[29] inflammation,^[30] cardiac ischemia,^[31] and Alzheimer's disease.^[32] For *in vivo* PA imaging of pH, an activatable PA semiconducting oligomer nanoprobe (SON) which consisted of a semiconducting

oligomer (F-DTS) and a boron-dipyrromethene dye (pH-BDP) has been developed (Figure 7a).^[24b] Under acidic condition, the hydroxyl group of pH-BDP will be protonated, which causes the absorption change of pH-BDP, thus inducing the absorption change of SONs. Taking SON₅₀ which had optimized 50_{w/w}% amount of pH-BDP as an example, the absorption peak at 750 nm assigned to pH-BDP decreased with the decreasing of pH, while almost no change was observed for the absorption peak at 680 nm (Figure 6b). As a consequence, the PA amplitude at 680 nm remained almost the same, while the PA amplitude at 750 nm decreased significantly upon the change of pH from 7.4 to 5.5 (Figure 6c), showing the feasibility of ratiometric PA imaging of pH. Furthermore, Good linear correlation between ratiometric PA signal (PA_{680}/PA_{750}) and pH can be found from pH = 7.4 to 5.5 (Figure 6d), which permitted the quantification of pH *in vivo* by the fitted line. The SON₅₀ was then utilized for *in vivo* ratiometric PA imaging of tumor. After systemic administration of SON₅₀ for 6 h, the ratiometric PA signal ($\Delta PA_{680}/\Delta PA_{750}$) in tumor area reached 6.9 ± 0.6 , which was significantly higher than the signal before injection (Figure 6e). Based on the calibration curve in Figure 6d, the pH value of tumor area for living mice could be estimated as 6.3.

Figure 6. (a) Schematic illustration of doping-induced PA amplification and pH responsive capability. (b) UV spectra of SON₅₀ (1.2 $\mu\text{g mL}^{-1}$) under different pH values. (c) PA images of SON₅₀ solutions at pH = 7.4, 6.4 and 5.5. The pulse laser was tuned to 680 nm and 750 nm for ratiometric PA imaging. (d) The ratiometric PA signal (PA₆₈₀/PA₇₅₀) of SON₅₀ as a function of pH value. The linear fitting was obtained from pH = 7.4 to 5.5. (e) Ratiometric PA images ($\Delta\text{PA}_{680}/\Delta\text{PA}_{750}$) of subcutaneous HeLa tumor in nude mice before and 6 h after systemic administration of SON₅₀ (25 $\mu\text{g mouse}^{-1}$). The error bars represent standard deviations of three separate measurements. Reprinted with permission from ref 25b, Copyright 2016, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

6. Conclusion

SPNs have many intrinsic advantages including good biocompatibility, excellent photostability and oxidative tolerance for *in vivo* applications. By changing the chemical structures of SPs and encapsulating them into different amphiphilic polymers, different imaging applications can be fulfilled. In generally, SPNs can show better photostability and generate higher PA signal as

compared to concentration-match SWNTs and GNRs. The structure-property studies have further revealed the feasibility to improve the PA brightness by choosing low-emissive SPs with high photothermal conversion efficiency. Through intraparticle or intrapolymer doping with electron-deficient components, PET, a nonradiative heat decay process, can be promoted within SPNs, leading to amplified PA signals.

Due to the easy control over the dimension of SPNs, they can be tailored for various applications. Coating with DPPC, a lipid, facilitates the lymph node uptake, permitting effective PA mapping of lymphatic systems in living mice. Because the principle of PA imaging aligns well with that of PTT, SPNs are also ideal for PA imaging guided PTT of tumor in living mice. The simple preparation through nanoprecipitation also provides the versatility to develop SPNs into smart activatable probes. Until now, SPN-based activatable PA probes have been designed and used for imaging of chemical mediators and indexes such as ROS and pH, which are closely related to the pathological conditions of many diseases such as cancer, inflammation, cardiac ischemia, and Alzheimer's disease.

In summary, our recent studies have shown that SPNs can serve as a versatile platform for PA imaging applications. The large library of SPs allows us to develop different kinds of SPNs with desirable properties for different imaging tasks, which include lymph node imaging, tumor imaging, detection of disease biomarkers and light theranostics. Although challenges such as long-term in vivo toxicity are present to further advance their applications in life science, we believe that SPNs have the potential for pre-clinical and even clinical applications.

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SPNs have many intrinsic advantages including good biocompatibility, excellent photostability and oxidative tolerance for *in vivo* applications. By changing the chemical structures of SPs and encapsulating them into different amphiphilic polymers, different imaging applications can be fulfilled. Through intraparticle or intrapolymer doping with electron-deficient components, PET, a nonradiative heat decay process, can be promoted within SPNs, leading to amplified PA signals. SPNs are also ideal for PA imaging guided PTT of tumor in living mice. The simple preparation through nanoprecipitation also provides the versatility to develop SPNs into smart activatable and used for imaging of chemical mediators and indexes such as ROS and pH.

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