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Photoactivatable Organic Semiconducting Pro-nanoenzymes

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ABSTRACT: Therapeutic enzymes hold great promise for cancer therapy; however, *in vivo* remote control of enzymatic activity to improve their therapeutic specificity remains challenging. This study reports the development of an organic semiconducting pro-nanoenzyme (OSPE) with a photoactivatable feature for metastasis-inhibited cancer therapy. Upon near-infrared (NIR) light irradiation, this pro-nanoenzyme not only generates cytotoxic singlet oxygen ($^1\text{O}_2$) for photodynamic therapy (PDT), but also triggers a spontaneous cascade reaction to induce the degradation of ribonucleic acid (RNA) specifically in tumor microenvironment. More importantly, OSPE-mediated RNA degradation is found to down-regulate the expression of metastasis-related proteins, contributing to the inhibition of metastasis after treatment. Such a photoactivated and cancer-specific synergistic therapeutic action of OSPE enables complete inhibition of tumor growth and lung metastasis in mouse xenograft model, which is not possible for the counterpart PDT nanoagent. Thus, our study proposes a phototherapeutic-proenzyme approach towards complete-remission cancer therapy.

INTRODUCTION

Drug therapeutics in the form of small molecules and proteins is promising for treatments of solid tumors and metastasis.¹⁻⁴ However, most existing drugs often pose serious threats to normal tissues owing to their “always-on” pharmacological effect. To overcome this issue, pro-drugs that only become cytotoxic in response to exogenous stimuli or endogenous cancer-specific biomarkers have been proposed.⁵⁻⁶ For example, banoxantrone- and camptothecin-based prodrugs were developed for cancer therapy, which were active in the presence of hypoxic environment and biothiols, respectively.⁷⁻⁸ In addition, a dendrimer-protected protease was used as the proenzyme with pH responsive activity for specific induction of cancer cell apoptosis.⁹ All the reported systems have shown the improved therapeutic specificity towards diseases cells/tissues.

Several approaches have been adapted to regulate the activity of enzymes in living systems, including reversible chemical engineering,¹⁰⁻¹¹ conditional protein splicing¹² and alternating magnetic field.¹³ In comparison, photoactivation has the advantages of non-invasiveness, simple maneuverability, flexible controllability, high spatiotemporal resolution and minimal side-effects.¹⁴⁻¹⁷ In general, the enzymatic activity is inhibited by conjugation with certain photo-cleavable caging groups.¹⁸ However, because majority of photo-cleavable reactions are only responsible to ultraviolet (UV) and visible light, this strategy has poor tissue penetration.¹⁹ In contrast, near-infrared (NIR) light with deep-tissue penetrating ability can be

used to control the enzymatic activity.²⁰ However, it exerts the photothermal effect of optical agents, and thus only works for thermosensitive enzymes, which often do not have therapeutic effect.²⁰⁻²² As a result, NIR light regulation of the activity of therapeutic proenzymes has been barely exploited for cancer therapy.

In this study, we report the synthesis of an organic semiconducting pro-nanoenzyme (OSPE) that can be remotely activated with NIR photo-irradiation in tumor microenvironment for metastasis-inhibited cancer therapy. OSPE contains a semiconducting polymer nanoparticle (SPN) core, which is conjugated with an inactive proenzyme *via* a singlet oxygen ($^1\text{O}_2$) cleavable linker (Figure 1a). SPNs are a new class of optical nanomaterials applicable for molecular imaging,²³⁻²⁵ phototherapy²⁶⁻²⁸ and biological activation.^{29, 30} The proenzyme (EBAP) is derived from cytotoxic ribonuclease A (RNase A) that degrades intracellular ribonucleic acid (RNA) to induce cell death and its activity is inhibited by caging with a hydrogen peroxide (H_2O_2) responsive phenylboronic acid pinacol (BAP) group. Upon NIR laser irradiation, the SPN core generates $^1\text{O}_2$ that not only permits photodynamic therapy (PDT) but also initiates the cleavage of the $^1\text{O}_2$ -responsible linker to release EBAP; the released EBAP then undergoes a deprotection process triggered by H_2O_2 in tumor microenvironment, restoring the enzyme activity for cancer-specific RNA degradation. As such, OSPE represents a smart pro-nanoenzyme, whose full activation requires both NIR laser irradiation and reaction with chemical mediator in tumor microenvironment. In association with PDT, OSPE-mediated RNA degradation can downregulate

the expression of metastasis-related proteins, contributing to the inhibition of metastasis after treatment. Thereby, OSPE-mediated phototherapy possesses a unique synergistic pharmacological effect, demonstrating the potential for complete-remission cancer therapy.

RESULTS AND DISCUSSION

The organic semiconducting pro-nanoenzyme (OSPE) was constructed through the following three main steps (Figure 1b,c): (i) synthesis of the BAP-caged proenzyme (EBAP); (ii) synthesis of the organic nanoparticle (SPNB) with the $^1\text{O}_2$ -cleavable linker; and (iii) conjugation of EBAP onto the surface of SPNB. The H_2O_2 -responsive compound was synthesized (Figure S1, Supporting Information) and used to cage the native RNase A enzyme at the lysine residues, affording EBAP. MALDI-TOF-MASS assay confirmed the conjugation, showing approximately 7 BAP molecules per enzyme (Figure S2, Supporting Information). The $^1\text{O}_2$ -cleavable linker, (Z)-2,2'-(ethene-1,2-

diylbis(sulfanediyl))diethanamine (BSDA), was synthesized (Figures S3 and S4, Supporting Information) and utilized to functionalize poly(ethylene glycol) (PEG) chains to yield PEG-BSDA (Figure S5, Supporting Information). The photodynamic polymer (PCB) was synthesized *via* Suzuki polycondensation reaction and then grafted with methoxy-PEG (Mw = 1000) and PEG-BSDA (Mw = 2000) at a molar ratio of 4:1, resulting in PCB-PEG-BSDA (Figure S6, Supporting Information). This polymer spontaneously assembled into the nanoparticle (termed as SPNB) in aqueous solution. SPNB was subsequently conjugated with EBAP to afford OSPE. To synthesize the control nanoparticles without enzyme modification, PCB was grafted with methoxy-PEG (Mw = 1000) and carboxyl-PEG (Mw = 2000) at a molar ratio of 4:1 to afford PCB-PEG. PCB-PEG had the structure similar to the previously polymer used for photothermal therapy,²⁰ which could also assemble into the nanoparticle in aqueous solution, termed as SPNC (Figure 1d).

Figure 1. Design and synthesis of OSPE for cancer therapy. (a) Illustration of the proposed mechanism for the photoactivated synergistic therapeutic action of OSPE including PDT and intracellular RNA degradation. (b) Synthesis of $^1\text{O}_2$ -responsive polymer PCB-PEG-BSDA. (c) Synthesis of OSPE. (d) Structure of PCB-PEG and the control nanoparticle (SPNC) without enzyme modification. (i) 4-Nitrophenyl chloroformate, triethylamine, tetrahydrofuran (THF), 25 °C, 2 h. (ii) RNase A, 0.1 M NaHCO_3 buffer (pH 8.5), dimethyl sulfoxide (DMSO), 25 °C, 12 h. (iii) $\text{Pd}(\text{PPh}_3)_4$, K_2CO_3 , methyltriethylammonium chloride, toluene/ H_2O , 100 °C, 24 h. (iv) NaN_3 , THF/ N,N -dimethylformamide (DMF), 25 °C, 12 h. (v) Tert-butyl (2-mercaptoethyl)carbamate, NaOH , ethanol, 25 °C, 24 h. (vi) Dichloromethane (CH_2Cl_2)/trifluoroacetic acid (TFA), 25 °C, 1 h. (vii) Carboxyl-PEG-alkyne, EDC/NHS, DMSO, 25 °C, 48 h. (viii) CuBr , N,N,N',N',N'' -pentamethyldiethylenetriamine (PMDETA), methoxy-PEG-alkyne, THF, 25 °C, 48 h. (ix) EBAP, EDC/NHS, 1x PBS buffer (pH 7.4).

The hydrodynamic size of OSPE was measured to be 28 nm (Figure 2a), which was larger than SPNC (17 nm) and SPNB (18 nm). The polydispersity indexes (PDIs) of SPNC, SPNB and OSPE were measured to be ~0.2, indicating the good uniformity of these nanoparticles (Figure S7a, Supporting Information). SPNB showed a positive surface charge (11 mV) due to the modification of BSDA, while further conjugation of EBAP reduced the zeta potential to

-12 mV for OSPE (Figure S7b, Supporting Information). The hydrodynamic sizes of SPNC, SPNB and OSPE did not significantly change after storage in PBS buffer (Figure S7c, Supporting Information), suggesting their good colloidal stability. In agarose gel electrophoresis, OSPE migrated a much shorter distance than SPNC and SPNB (Figure 2b), further implying the successful conjugation of EBAP with SPNB. The weight ratio of enzyme to the SPN

core was 84% determined by Bradford protein assay. As indicated by transmission electron microscopy (TEM) image, OSPE was spherical and had a homogenous size distribution (Figure 2c).

Both absorption and fluorescence spectra of OSPE were almost identical to those of SPNC (Figure 2d), suggesting the surface modification had negligible influence on their optical properties. The absorption of SPNC and OSPE ranged from 600 to 900 nm, enabling the generation of $^1\text{O}_2$ under NIR laser irradiation as confirmed using singlet oxygen sensor green (SOSG) as an indicator (Figure 2e), which was similar with our previous studies.^{31, 32} The generated $^1\text{O}_2$ was able to cleave the BSDA linker to release

the proenzyme (EBAP) from OSPE (Figure 2f). After 30 min laser irradiation, the proenzyme release efficiency reached to 59% regardless of H_2O_2 treatment. To confirm the NIR laser irradiation triggered activation of OSPE, a RNaseAlert® Kit was used to measure the enzymatic activity. In the presence of H_2O_2 , the enzymatic activity of OSPE was elevated by 3.7-, 4.6- and 6.2-fold after 10, 20 and 30 min of NIR laser irradiation, respectively (Figure 2g). While, only slight increase in enzymatic activity was observed for OSPE after treatments with H_2O_2 or laser irradiation alone. These results verified that the enzymatic activity of OSPE could be specifically restored by photo-irradiation-caused release along with H_2O_2 induced deprotection.

Figure 2. *In vitro* characterization of OSPE along with the control SPNC. (a) Dynamic light scattering (DLS) profiles and (b) agarose gel electrophoresis of SPNC, SPNB and OSPE. (c) Representative TEM image of OSPE. (d) UV-Vis absorption and fluorescence spectra of SPNC and OSPE. (e) Generation of $^1\text{O}_2$ for SPNC and OSPE under NIR laser irradiation after different irradiating time. (f) Release of proenzyme from OSPE as a function of laser irradiating time. (g) Relative enzymatic activity of OSPE upon NIR laser irradiation for different time in the absence and presence of H_2O_2 . [SPN] = 40 $\mu\text{g/mL}$ for SPNC, SPNB and OSPE in PBS buffer (pH = 7.4), [H_2O_2] = 50 μM . The laser irradiation wavelength was at 808 nm with a power density of 0.3 W/cm^2 . Error bars represented standard deviations of three separate measurements ($n = 3$).

The cellular uptake of OSPE was investigated against 4T1 cancer cells. After treatment for 24 h, the fluorescence intensity of OSPE-treated cells was almost identical to that of SPNC-treated cells (Figure 3a,b), suggesting surface enzyme conjugation did not have any obvious influence on the internalization of nanoparticles. This should be due to the similar negative zeta potential for SPNC and OSPE (Figure S7b, Supporting Information). Next, the ability of OSPE for cancer therapy was evaluated. Note that SPNC and OSPE alone caused nearly no toxicity to 4T1 cancer cells even at a high concentration up to 50 $\mu\text{g/mL}$ (Figure 3c). After treatment with SPNC or OSPE for 24 h, 4T1 cancer cells were irradiated by NIR laser at 808 nm with a power density of 0.3 W/cm^2 . The laser irradiation was controlled in a discontinuous manner to

maintain the temperature below the apoptotic threshold (43 $^{\circ}\text{C}$) (Figure S8, Supporting Information). The laser irradiation alone without nanoparticle treatment showed negligible effect on the cell viability, while the cell viabilities decreased with increased concentrations for both SPNC and OSPE upon laser irradiation. At the concentration of 40 $\mu\text{g/mL}$, the cell viability of OSPE-treated cells was 17.9%, which was 3.4-fold lower than that of SPNC-treated cancer cells (61.6%). These data verified the better therapeutic efficiency of OSPE over SPNC.

To study the therapeutic mechanism of OSPE, intracellular $^1\text{O}_2$ generation and RNA degradation were examined. Cell permeant 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA), a fluorescent turn-on indicator for ROS, was used to detect the intracellular generation of $^1\text{O}_2$. Upon

laser irradiation, similar fluorescence signal was observed for SPNC- and OSPE-treated cancer cells (Figure 3d,e), while negligible fluorescence signal was detected in the cells from other groups. Furthermore, RNA in the treated cells was extracted and assessed using agarose gel electrophoresis. Obvious RNA degradation was only observed for OSPE-treated 4T1 cancer cells with laser irradiation (Figure 3f), but RNA was intact for the cells from other treated groups. In addition, no obvious RNA degradation occurred in OSPE-treated normal fibroblast cells, regardless of laser irradiation. This is because the RNase A

(EBAP) released from OSPE was in its caged and inactive form due to the relatively low H_2O_2 level in the normal cells (Figure S9a, Supporting Information). This was again validated by the observation that free RNase A could induce severe RNA degradation in both 4T1 cancer cells and normal fibroblasts with or without laser irradiation (Figure S9b, Supporting Information). Therefore, the synergistic therapeutic action of OSPE was fully activated only when light irradiation and H_2O_2 coexisted as proposed in Figure 1a, leading to its high specificity to cancer cells and superior anticancer efficacy over SPNC.

Figure 3. *In vitro* OSPE-mediated cancer therapy. (a) Mean fluorescence intensity (MFI) of 4T1 cancer cells without treatment (control) or after treatment with SPNC or OSPE ([SPN] = 20 $\mu\text{g/mL}$) for 24 h (N.S., no significant difference, $n = 3$). (b) Representative confocal fluorescence images of 4T1 cancer cells after treatment with SPNC or OSPE ([SPN] = 20 $\mu\text{g/mL}$) for 24 h. Blue fluorescence was from cell nucleus stained with 4',6-diamidino-2-phenylindole (DAPI) and red fluorescence indicated the signals from nanoparticles. (c) Cell viabilities of 4T1 cancer cells after treatments with SPNC or OSPE at different concentrations ([SPN] = 0, 10, 20, 30, 40 and 50 $\mu\text{g/mL}$) with or without 808 nm laser irradiation (0.3 W/cm^2) for 10 min ($*p < 0.05$, $n = 3$). (d) Representative confocal fluorescence images and (e) flow cytometry analysis of 4T1 cancer cells treated with SPNC or OSPE ([SPN] = 40 $\mu\text{g/mL}$) with or without 808 nm laser irradiation (0.3 W/cm^2) for 10 min. The cells after different treatments were stained with H_2DCFDA . (f) Agarose gel electrophoresis of RNA extracted from 4T1 cancer cells treated with SPNC or OSPE ([SPN] = 40 $\mu\text{g/mL}$) with or without 808 nm laser irradiation (0.3 W/cm^2) for 10 min.

In vivo cancer therapy was conducted by intravenously injecting OSPE or SPNC into 4T1 tumor-bearing mice, followed by local NIR photoactivation (Figure 4a). Tumor growth and metastasis were monitored after treatment. Whole-body NIR fluorescence imaging was used to evaluate the accumulation of nanoparticles in tumors. The fluorescence signals in tumor areas gradually increased and reached the similar maximum (17 times higher than the background) at 12 h post-injection for SPNC and OSPE (Figure 4b,c). This indicated both nanoparticles could accumulate into tumors *via* the enhanced permeability and retention (EPR) effect. Such ideal tumor accumulation of nanoparticles should be attributed to their high surface PEG density (0.9 PEG/nm^2) and small sizes (17 nm for SPNC and 28 nm for OSPE). This was similarly observed in the previous reports for the nanoparticles as-

sembled from PEG-grafted polymers.^{33, 34} In addition, similar biodistribution was observed for them (Figure S10, Supporting Information), showing the highest accumulation in liver followed by tumor and lung.

According to the imaging results, the tumors of mice were locally irradiated with NIR laser at 808 nm at a power density of 0.3 W/cm^2 (the maximum permissible exposure for skin) for 10 min at 12 h post-injection of saline, SPNC or OSPE for cancer therapy. Note that the laser irradiation was controlled in a discontinuous manner to maintain the tumor temperature below the apoptotic threshold (Figure S11, Supporting Information). Such a low laser power density and discontinuous short irradiation time ensured the biosafety of phototherapy, allowing for its potential use in clinics. No obvious change in the body weights of mice for all groups was observed (Figure

S12, Supporting Information). Histological hematoxylin and eosin (H&E) staining (Figure 4d) and immunofluorescence caspase-3 staining (Figure S13, Supporting Information) revealed that much severer cell apoptosis and necrosis were observed for the tumors in OSPE-injected mice as compared to SPNC-injected mice after 10 min laser irradiation, but the tumors in other groups did not show any damages. Thereby, the tumor growth was completely suppressed only for OSPE-injected mice with laser irradiation (Figure 4e), while causing no histopathological damages to major organs including heart, liver, spleen and kidney of living mice (Figure S14, Supporting Infor-

mation). More importantly, OSPE-mediated cancer therapy effectively inhibited the lung metastasis as indicated by the histological staining (Figure 4f), but obvious tumor metastasis was detected for other treatment groups 14 days post-treatment. Moreover, the number of pulmonary metastatic nodules was counted and the results showed that the number of metastatic nodules in OSPE-injected and laser-irradiated group was reduced by nearly 100%, as compared to the control groups (Figure 4g). Such a complete-remission cancer therapy has been barely reported for other photodynamic agents.³⁵

Figure 4. *In vivo* OSPE-mediated cancer therapy. (a) Schematic illustration of OSPE-mediated complete inhibition of tumor growth and metastasis. (b) Fluorescence imaging of 4T1 tumor-bearing mice at representative timepoints after intravenous injection of SPNC or OSPE (200 μ L, [SPN] = 200 μ g/mL). Tumors were indicated by the white dashed circles. (c) Quantification of tumor fluorescence intensity as a function of post-injection time of SPNC or OSPE (n = 6). (d) Histological H&E staining of tumor sections from 4T1 tumor-bearing mice after different treatments. (e) The tumor growth curves in 4T1 tumor-bearing mice (* p < 0.05, n = 6). (f) Histological H&E staining of pulmonary metastatic tumors in 4T1 tumor-bearing mice. Tumors were indicated by the black dashed curves. (g) Quantitative analysis of pulmonary metastatic nodules in different treatment group (** p < 0.001, n = 6). In (d)-(g), 4T1 tumor-bearing mice were intravenously injected with saline, SPNC or OSPE (200 μ L, [SPN] = 200 μ g/mL) and then treated with or without 808 nm laser irradiation (0.3 W/cm²) for 10 min.

Mechanism of OSPE-mediated complete-remission cancer therapy was studied. Generation of $^1\text{O}_2$ in tumors was examined by measuring the fluorescence signal of SOSG. Almost no green fluorescence could be detected for the tumors after treatments with nanoparticles or laser irradiation alone (Figure 5a). In contrast, after laser

irradiation, obvious green fluorescence signals were observed for the tumors of both SPNC and OSPE-injected mice, showing ~30-fold signal increase relative to those without laser irradiation (Figure 5b). In addition, RNA was extracted from the tumor lysates to assess RNA degradation. Obvious RNA degradation only occurred in the

tumors of OSPE-injected mice after NIR laser irradiation (Figure 5c). Fluorescence quantification further revealed that RNA integrity dropped to ~60% in OSPE-injected

and laser-irradiated group, while other groups were still close to 100 % (Figure 5d).

Figure 5. Mechanism study of OSPE-mediated complete-remission cancer therapy. (a) Representative confocal fluorescence images of tumor sections from 4T1 tumor-bearing mice after different treatment. Blue fluorescence was from cell nucleus stained with DAPI and green fluorescence indicated the signals from SOSG. (b) Quantitative analysis of SOSG MFI in tumor sections from 4T1 tumor-bearing mice after intravenous injection of saline, SPNC or OSPE with or without an 808 nm laser irradiation (** $p < 0.001$, $n = 6$). (c) Agarose gel electrophoresis of RNA exacted from tumor lysates of 4T1 tumor-bearing mice. (d) RNA integrity in the tumor tissues from 4T1 tumor-bearing mice after intravenous injection of saline, SPNC or OSPE with or without an 808 nm laser irradiation (** $p < 0.01$, $n = 6$). (e) Representative confocal fluorescence images of VCAM-1, HGF and MTA2 expression in the tumors from 4T1 tumor-bearing mice after different treatments. Blue fluorescence was from cell nucleus stained with DAPI and green fluorescence indicated the signals from the VCAM-1, HGF or MTA2 staining. Relative expression of VCAM-1 (f), HGF (g) and MTA2 (h) in the tumor tissues from 4T1 tumor-bearing mice after intravenous injection of saline, SPNC or OSPE with or without an 808 nm laser irradiation (** $p < 0.001$, $n = 6$). (i) Schematic illustration of the proposed mechanism of OSPE-mediated inhibition of lung metastasis. In (a)–(h), 4T1 tumor-bearing mice were intravenously injected with saline, SPNC or OSPE (200 μ L, [SPN] = 200 μ g/mL) and then treated with or without 808 nm laser irradiation (0.3 W/cm²) for 10 min.

To further gain insight into the underlying mechanism of metastasis inhibition by OSPE-mediated cancer therapy, the expression of proteins associated with metastasis, including vascular cell adhesion molecule-1 (VCAM-1), hepatocyte growth factor (HGF) and metastasis-associated protein 2 (MTA2) in the tumor tissues after different treatments was studied with immunofluorescence staining. VCAM-1, a cell-cell adhesion protein expressed on the surface of tumor cells, facilitates the lung metastases by binding with monocytes through α_4 -integrin mediated adhesion.³⁶ HGF is a scatter factor that induces tumor cells to lose their contacts with neighbors and become highly mobile, initiating tumor invasion and metastasis.³⁷ MTA2 inhibits the cell adhesion molecule epithelial transmembrane glycoprotein (Ep-CAM) and E-cadherin, and thus promotes the cell migration and metastasis.³⁸ Confocal fluorescence imaging revealed that

very weak green fluorescence signals from these protein staining were observed in the tumor slides from OSPE-injected and laser-irradiated group (Figure 5e); in contrast, obvious green fluorescence signals were detected in all other groups. Signal quantification (Figure 5f–h) indicated that only OSPE-mediated phototherapy dramatically reduced the expression of VCAM-1 (to 22.4%), HGF (to 27.5%) and MTA2 (to 16.7%) relative to the saline-treated control groups. As summarized in Figure 5i, these results thereby validated that RNA degradation induced by photoactivation of OSPE impeded the translation of messenger RNA and thus downregulated the expression of metastasis-related proteins, ultimately contributing to the complete inhibition of lung metastasis.^{39, 40} To deconvolve the individual role of the OSPE core and EBAP in inhibiting tumor growth and metastasis, additional *in vivo* experiments need to be conducted in the future study.

These can involve the evaluation of other control nanoparticles, such as EBAP conjugated SPNs via a $^1\text{O}_2$ -inert linker and EBAP conjugated nanoparticles based on different polymer cores that cannot generate $^1\text{O}_2$ under NIR laser irradiation.

CONCLUSION

In conclusion, we have synthesized an organic semi-conducting pro-nanoenzyme (OSPE) that can be remotely activated by NIR light and validated its potential for complete-remission cancer therapy. NIR photoactivation not only enabled the generation of cytotoxic $^1\text{O}_2$ for PDT, but also specifically initiated the cascade activation of proenzymes starting from $^1\text{O}_2$ -induced release of the proenzymes from OSPE to the final restoration of enzymatic activity stimulated by the upregulated chemical mediator (H_2O_2) in the tumor microenvironment. Such a cancer-specific photoactivation of OSPE induced RNA degradation, downregulated the expression of metastasis-related proteins, and eventually contributed to the inhibition of metastasis after treatment. Thus, by virtue of such a photoactivated synergistic therapeutic action, OSPE was able to completely suppress tumor growth and prevent lung metastasis, which was proved otherwise impossible.

To the best of our knowledge, this study presents the first photoactivatable organic pro-nanoenzyme that has the potential for complete-remission cancer therapy. Although NIR laser certainly improved the tissue penetration depth as compared to visible light, it still has limited tissue penetration. Thereby, the current pro-nanoenzyme design is more suitable for treatment of superficial tumors such as melanoma. However, by further red-shifting the light response into the second NIR window or by integrating with endoscopic light delivery system, it is promising to use such pro-nanoenzymes to treat deep-seated tumors.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Detailed experiment procedures and supporting figures (PDF)

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Notes

The authors declare no competing financial interests.

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