Photoswitchable Nanoparticles for Triggered Tissue Penetration and Drug Delivery

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Photoswitchable Nanoparticles for Triggered Tissue Penetration and Drug Delivery

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Supporting Information

ABSTRACT: We report a novel nanoparticulate drug delivery system that undergoes reversible volume change from 150 to 40 nm upon phototriggering with UV light. The volume change of these monodisperse nanoparticles comprising spiropyran, which undergoes reversible photoisomerization, and PEGylated lipid enables repetitive dosing from a single administration and enhances tissue penetration. The photoswitching allows particles to fluoresce and release drugs inside cells when illuminated with UV light. The mechanism of the light-induced size switching and triggered-release is studied. These particles provide spatiotemporal control of drug release and enhanced tissue penetration, useful properties in many disease states including cancer.

INTRODUCTION

Controlled release technology is expected to have a profound impact in many medical fields including oncology. The incorporation of chemotherapeutic agents in nanoparticle (NP) delivery vehicles has improved drug solubility, reduced clearance, reduced drug resistance, and enhanced therapeutic effectiveness. With controlled release NP systems, a single dose can sustain drug levels within the desired therapeutic range for long periods in various diseases (e.g., diabetes or cancer). Several nanoparticulate therapeutics, for example, Doxil (~100 nm PEGylated liposome loaded with doxorubicin) and Abraxane (~130 nm albumin-bound paclitaxel nanoparticles), have been approved by the FDA, and have shown improved pharmacokinetics and reduced adverse effects compared to their parent drugs. However, currently approved nanomedicines provide modest survival benefits for patients, perhaps in part because of poor tumor penetration.

Nanoparticle size is one crucial determinant of accumulation and penetration into tumor tissue. Nanoparticles with sub-100 nm sizes are optimal for the enhanced permeation and retention (EPR) effect for passive tumor targeting. However, physiological barriers, such as the dense interstitial matrix—a complex assembly of collagen, glycosaminoglycans, and proteoglycans—hinder the delivery of drugs throughout the entire tumor. For example, Doxil (~100 nm) is found trapped near the tumor vasculature. Although the small size (molecular weight = 544 Da) of doxorubicin released from Doxil allows rapid diffusion, doxorubicin cannot migrate far from the particles due to rapid uptake of doxorubicin by perivascular cells, which results in heterogeneous therapeutic effects. Deep penetration of nanoparticles in tumors is necessary to enhance their therapeutic effect.

Another significant drawback of commercially available drug delivery NPs is that drugs are released at a predetermined rate irrespective of patient needs or changing physiological circumstances. A triggerable drug delivery system would allow repeated on-demand dosing that would be adaptable to the patients’ regimen and allow multiple dosages from a single administration. It might also help address the potential importance of timing on therapeutic effect (“chrono-administration”) in the treatment of cancer, a concept that is receiving burgeoning recognition, for example, the periodicity of VEGF expression in breast cancer regulates tumor cancer vascular permeability. Another clinical example of the importance of timing is that periodic infusion of angiotensin II via the tail vein can enhance macromolecular delivery into tumors by overcoming the barrier of elevated interstitial fluid pressure within tumors; no such increase of macromolecular uptake occurs either by an acute or chronic increase in blood pressure induced by angiotensin II. Furthermore, the permeability of many tumor models varies with time and in response to treatment, so that vascular pore sizes vary greatly, resulting in heterogeneous NP extravasation and drug delivery efficacy. On-demand drug release from NPs accumulated in tumors could allow in situ chrono-administration, potentially increasing drug retention in cancers, maximizing tumor killing and minimizing metastatic spread.

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Here, we have developed a photoswitching nanoparticulate system that uses light as the remote means of triggering both on-demand drug release and reversible changes in particle volume to enhance tissue penetration.

RESULTS AND DISCUSSION

Photochromic properties are controllable light-induced changes in color or reversible photoexcited transformations between two isomers. There has been intensive investigation of photochromic materials for applications from sunglasses to optically rewritable data storage, optical switching and chemical sensing. The photoswitchable NPs developed here were composed of spiropyran (SP, a family of photochromic molecules, Figure 1a,b) and lipids. SP consists of a nitrobenzopyran and an indoline moiety with orthogonal orientation (Figure 1a). Both moieties absorb in the ultraviolet spectrum independently. Ultraviolet light (UV, 365 nm) induces ring-opening in the pyran to form merocyanine (MC, Figure 1a). The nitrophenol and indoline chromophores are merged to form one large planar π-system, leading to intense absorption in the visible (Vis) spectral region (500–600 nm). The zwitterionic MC form is less stable than the hydrophobic SP form and undergoes spontaneous ring-closing back to SP in the dark that is accelerated by visible light (500–600 nm).

Figure 1. (a) Structure and photoisomerization reaction between spiropyran (SP) and merocyanine (MC). (b) Abbreviations for SP and MC derivatives. (c) Scheme of photoswitching SP NP18 composed of SP-C9 and DSPE-PEG. Yellow oval, SP molecule; blue line, the alkyl chain (R) in SP; red, lipid part; green line, PEG. SP NP C9 are converted to MC NP18 (purple sphere: MC molecule) by UV light irradiation; the reversible photoisomerization from MC NP18 to SP NP18 happens in dark but is accelerated by visible light (500–600 nm).

Formulation of Photoswitchable NPs with Light-Triggered Size Changes. SP derivatives bearing hydrophobic alkyl chains (Figure 1a,b) were synthesized by coupling 2-hydroxy-S-nitrobenzaldehyde with substituted 2,3,3-trimethyl-3H-indolium iodide (Figure S1a). NPs were initially prepared by direct nanoprecipitation of SP alone (an extensively used simple method for the preparation of NPs with therapeutic agents embedded in the hydrophobic matrices). An acetonitrile solution of SP-C9 (10 mg/mL) was nanoprecipitated into water (final acetonitrile/water = 1/40, v/v), resulting in NP sizes of 198.1 ± 2.5 nm with a polydispersity of 0.09 ± 0.02, determined by dynamic light scattering (DLS, N = 5, Table S1).

Irradiation of the SP-C9 NPs with UV light (365 nm, intensity ∼1 W/cm², ∼ 3.1 × 10⁻⁶ einstein) led to photoisomerization and the subsequent conversion of hydrophobic SP-C9 to amphiphilic MC-C9, and a change in the sizes of the NPs. The irradiated NPs had a bimodal size distribution (Figure S1b), with one peak at 39.6 ± 3.0 nm (N = 5, 99.1% of number population, determined by DLS; attributable to NPs assembled by MC-C9), and another at 202.1 nm (0.9% of number population; attributable to NPs formed with SP-C9). After irradiation, the colorless NP solution became purple, with a strong Vis absorption band characteristic of MC-C9 (maximum absorption wavelength λmax = 560 nm; Figure S1c,d). Nanoprecipitation of a SP analogue with a shorter alkyl chain, SP-C7, produced NPs that did not undergo a significant size change upon UV irradiation (Table S1).

SP-C9 NPs formed in aqueous solution aggregated when introduced into PBS (Table S1), presumably due to salt-induced screening of electrostatic repulsive forces between particles. In addition, the NPs had low actual drug loadings wt % (loading wt % < 1%) and efficiencies (<13%; Table S2). The loading efficiency did not increase in NPs made of SPs with a longer alkyl chain (SP-C18, Table S2). Higher drug loading of delivery vehicles is desirable for optimal therapeutic effect, to enhance the potency of NPs that reach the tumors.

To improve the stability and loading efficiencies of NPs while maintaining the NPs’ photoswitching properties, we produced hybrid SP/lipid-polyethylene glycol (PEG) NPs (termed NP18; Figure 1c) using a rapid ultrasonication method. An acetonitrile solution of SP-C9 (1 mg/mL) was slowly added into a 4 wt % ethanolic aqueous solution containing lecithin and 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-N-carboxy(polyethylene glycol)-5000 (DSPE-PEG, [SP-C9]/[DSPE-PEG]/[lecithin] = 32/16/1), followed by addition of water to adjust the organic/aqueous solution volume ratio to 1/10. After sonication for 8 min and filtration of the organic solvent, SP NP18 were obtained with an average hydrodynamic diameter of 143.2 ± 2.1 nm and a polydispersity of 0.03 ± 0.01 (N = 5, Figure 2a). SP-C9 was not detected by HPLC in the filtrate after repetitive washing of the NP18 by ultracentrifugation, indicating that SP-C9 was completely incoroporated into NPs (Figure S2a,b). After UV illumination (30s, ~100% conversion to MC), the absorption band of the NP18 moved to a λmax at 551 nm (Figure 2b). As with the nonhybrid NPs, UV irradiation of NP18 induced a size change (to 47.1 ± 1.3 nm, polydispersity of 0.05 ± 0.02, N = 5). These results confirmed that both the photochromic properties of SP-C9 and light-triggered size change were maintained in the SP NP18.

MC NP18 reverted to SP NP18 in darkness or by Vis light, with an accompanying increase in volume (Figure 2a). Consequently, there could be inaccuracies in measuring MC NP18 size by relatively slow techniques such as DLS. To confirm particle shrinkage after irradiation (Figure 2a), we produced NP18 containing MC–CN, a similar but relatively stable MC.
results indicated the similarly neutral charges of NPH before and after irradiation. No aggregation was observed for over 4 h.

For eventual clinical translation, it has to be possible for NPs to be stable during manufacturing, storage, and transportation. SP NPs were lyophilized for 48 h with bovine serum albumin (BSA, NP/BSA = 1/15, w/w), a known lyoprotectant reagent, then stored at −20 °C for over one month. The subsequent reconstitution of lyophilized SP NPs in PBS did not significantly change the NP sizes and photochromic properties (Figure S4). Lyophilization of SP NPs in water (without albumin) led to micrometer-sized, nondispersible aggregates upon reconstitution in PBS. Since albumin is used clinically, this lyoprotection strategy may be useful for potential translational of SP NPs.

To examine whether this formulation could be used to form NPs containing a broad range of compounds, we tested the ability to encapsulate rhodamine B, coumarin 6, cyanine 5 (Cy5), paclitaxel, docetaxel, proparacaine, and doxorubicin. NPs with adjustable loadings up to 10 wt % (with relatively high loading efficiencies) and low polydispersities were readily obtained for all of the therapeutics (docetaxel, doxorubicin, proparacaine) and dyes (Cy5, rhodamine B, coumarin 6) tested (Table 1).

HeLa (cervical cancer cell), PC-3 (human prostate carcinoma), and human umbilical vein endothelial cells (HUVEC) were used to assess the cytotoxicity of SP NPs. Following 72 h of exposure to NPs, cell viability was determined by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The SP NPs did not cause significant cytotoxicity in either cell line except at extremely high concentrations (Figure S5a). The EC_{50} values (the concentrations at which cell viability was reduced by 50%, determined by interpolation from the data in Figure S5a) for the SP NPs in those NP samples were 9.53 mM for HUVEC (6.33 mg/mL NPs), 7.01 mM for HeLa cells (4.66 mg/mL NPs), and 7.41 mM for PC-3 cells (4.92 mg/mL NPs). In a 70 kg adult, these EC_{50} values are approximately equivalent to 70 g/dose (~1 g/kg) assuming NPs are restricted to the 14 L extracellular fluid, or 25 g/dose (~350 mg/kg) if the NPs are restricted to the 5 L bloodstream, extremely high doses compared to those used clinically with Doxil (dosage: 50 mg/week).

Table 1. Characteristics of Photoswitching SP NPs

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<th>initial LD %</th>
<th>actual LD %</th>
<th>LD efficiency %</th>
<th>size (nm)</th>
<th>polydispersity</th>
<th>size-UV (nm)</th>
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* Determined by DLS and HPLC. Abbreviations: LD, loading; size-UV, sizes of NPs treated by UV irradiation (N = 5). Data are means ± SD (N = 5).
Repetitive Photoswitching and Light-Triggered Drug Release Profiles of NPs. The repeatability of the photoswitching property of NP$_{H}$ was evaluated by alternating cycles of UV and Vis light. This modulation was fully reversible for at least 4 continuous cycles (UV irradiation for 30 s and Vis light for 3 min, Figure 3). However, the absorbance at the MC-C9 peak maximum decreased 43% after 4 cycles, and was accompanied by a reduction in size from 143.2 to 98.7 nm in the SP state (Figure 3). The decrease of absorbance after repetitive irradiation could be due to photofatigue (the loss of performance in photoisomerization) — a common property of organic photochromic compounds. The absorption intensity of MC in NP$_{H}$ at 511 nm faded at a rate dependent on the UV and Vis light. This modulation was fully reversible for at least 4 cycles. Data are means ± SD, $N = 4$.

We hypothesized that the phototriggered shrinkage of NP$_{H}$ might induce drug release. In the absence of UV phototriggering, drugs (e.g., doxorubicin) and dyes (e.g., rhodamine 6B) loaded in SP NP$_{H}$ showed slow release in PBS that was complete within 48−72 h (Figure 4, Figure S7). Upon UV irradiation (30 s), NP$_{H}$ encapsulating rhodamine 6B (loading wt% = 4.3%) released 29.3% of the loaded dye within 1 h as determined by HPLC, while 7.2% was released in the same period without UV irradiation. Of note, the release kinetics of NP$_{H}$s that had been triggered (Figure 4, blue line) eventually slowed to a rate similar to that of NP$_{H}$s that were not irradiated (Figure 4, black line). This decrease in the release rate could be explained by the majority of the MC-C9 in NPs spontaneously converting back to SP-C9, resulting in NPs reassembled in their original structure. In a separate group, UV triggering (30 s irradiation) was conducted every 3 h for three cycles (Figure 4, green line), with an increase in release at each event.

UV-triggered release was demonstrated in cells by fluorescence imaging of SP NP$_{H}$ loaded with calcein. Calcein was selected because its fluorescence self-quenches while it is entrapped inside particles, whereas calcein released from particles will become diluted and fluoresces. SP NP$_{H}$ loaded with calcein (2.7 wt %) were incubated with HeLa cells. After 4 h, the media containing NP$_{H}$s was removed and the cells were washed with PBS. Cells in medium were then illuminated by UV (365 nm) for 2 s, left in darkness for 5 min, then imaged (Figure S8). Strong fluorescence intensity with an emission maximum at 510 nm was noted in the cells, indicating that the calcein was released from NPs that had been taken up. Illumination followed by imaging was repeated 5 times, during which the fluorescence intensity gradually increased to saturation (Figure S8a,b). Cells treated with same NPs but without UV irradiation did not fluoresce, suggesting that the UV triggered rapid calcein release and intracellular dispersal from SP NP$_{H}$. These results were validated by flow cytometry, which showed a 24.7-fold increase in fluorescence intensity after a 10 s UV treatment (Figure S8c).

Surface Functionalization of NPs. Nanoparticle therapeutic effect can be enhanced and toxicity reduced by surface modification with moieties that allow intracellular penetration and/or targeting of specific tissues. To examine the potential suitability of the NP$_{H}$ for targeted drug delivery, we formulated NPs (NP$_{M}$) composed of SP-C9 and a mixture of DSPE-PEG$_{3400}$-maleimido (DSPE-PEG-MAL) and DSPE-PEG in a 4/1 ratio (w/w), 153.1 nm in diameter and with a polydispersity of 0.09. A cell penetration peptide (Cpp) Cys-Tat (47−57) (sequence: CYGRKKRQRQR-NH$_2$) was introduced onto SP NP$_{M}$ by reaction of the carboxyl-terminal Cys of the peptide with the MAL on the NP$_{M}$ surface (NP$_{M}$/Cpp = 100/1, w/w). The fluorescence intensity of HeLa cells incubated with the resulting NPs (SP NP$_{M}$/Cpp) for 30 min, measured by flow cytometry, was 7.1 times higher than that of cells treated with SP NP$_{M}$ lacking Cpp (NP$_{M}$/Cpp = 100/1, w/w). The fluorescence intensities of 1940 ± 215 and 273 ± 197, respectively; Figure 5a).

We compared the cytotoxicity of doxorubicin-loaded SP NP$_{M}$/Cpp (doxorubicin/SP NP$_{M}$/Cpp) to that of SP NP$_{M}$ without Cpp (doxorubicin/SP NP$_{M}$). HeLa cells were incubated with doxorubicin/SP NP$_{M}$/Cpp or doxorubicin/SP NP$_{M}$ for 2 h, then incubated in medium without NPs for a total of 48 h; cell viability was measured by MTT assay. The doxorubicin/SP NP$_{M}$/Cpp were significantly more cytotoxic...
suggest that the SP NPs M\textsuperscript{−}\textsubscript{M} infiltration into the collagen (Figure 6). Free ICG penetrated aptamers\textsuperscript{40} or other peptides\textsuperscript{41}) to enhance drug delivery. Functionalized by a broad range of biomolecules (e.g., Cy5/SP NPM-Cpp for 30 min; (b) MTT assay to determine the (0.74%; 7.4 mg/mL\textsuperscript{12}) similar to the 9.0 transport through a dense collagen gel at a concentration could have a bearing on therapeutic effectiveness. We evaluated whether the light- triggered size change could enhance diffusive penetration through tissue matrices. As discussed above, the ability to penetrate tissue tumors (e.g., LS174T) and murine tumors (e.g., MCalV).\textsuperscript{10b,42}

As discussed above, the ability to penetrate tissue\textsuperscript{95 wt % of dense collagens, rendering the delivery of drugs through the 4.0 ± 0.21 mm into the collagen gels, ICG/SP NP\textsubscript{H} penetrated 8.3 ± 0.10 mm without UV triggering, and ICG/SP NP\textsubscript{H} triggered by UV for 10 s penetrated 12.1 ± 0.02 mm (N = 4, \(P < 0.005\) for irradiated ICG/SP NP\textsubscript{H} compared to free ICG and unirradiated ICG/SP NP\textsubscript{H}). (The mechanical properties of collagen barely change after 1 h irradiation at 254 nm UV light, ~1.7 \(\times\) 10\textsuperscript{−6} einstein.\textsuperscript{43}) By fitting the fluorescence intensity of ICG/SP NP\textsubscript{H} to a one-dimensional diffusion model, we obtained an average diffusion coefficient of 2.24 \(\pm\) 0.42 \(\times\) 10\textsuperscript{−6} cm\textsuperscript{2}s\textsuperscript{−1} for UV-triggered ICG/SP NP\textsubscript{H} (\(N = 4, P < 0.005\) compared to free ICG), while the diffusion coefficient for unirradiated ICG/SP NP\textsubscript{H} (7.65 \(\pm\) 1.63 \(\times\) 10\textsuperscript{−7} cm\textsuperscript{2}s\textsuperscript{−1}; \(N = 4\)) was not statistically significantly different from that of free ICG.\textsuperscript{44} Gel penetration was further enhanced by increasing irradiation: ICG/SP NP\textsubscript{H} irradiated twice (for 10 s each, separated by 3 h), \(3.59 \pm 19.4 \times 10^{-12}\) cm\textsuperscript{2}s\textsuperscript{−1} for nonirradiated ICG/SP NP\textsubscript{H} (Figure 6). The relatively low diffusion rate of free ICG in collagen gels compared to NP\textsubscript{H}s might be partly due to the lipophilicity of ICG.\textsuperscript{44} Gel penetration is a horizontal capillary tube, then incubated for a further 12 h at 37 °C. A NIR imaging system was used to track particle infiltration into the collagen (Figure 6). Free ICG penetrated than the doxorubicin/SP NP\textsubscript{M} (Figure 5b). These results suggest that the SP NP\textsubscript{M}s have the capacity to be functionalized by a broad range of biomolecules (e.g., aptamers\textsuperscript{40} or other peptides\textsuperscript{41}) to enhance drug delivery.

**Light-Triggering Enhances Diffusion in Collagen Matrices.** As discussed above, the ability to penetrate tissue could have a bearing on therapeutic effectiveness. We evaluated whether the light- triggered size change could enhance diffusive transport through a dense collagen gel at a concentration (0.74%; 7.4 mg/mL\textsuperscript{15}) similar to the 9.0 ± 2.5 mg/mL of interstitial matrix estimated for interstitial collagen in human tumors (e.g., LS174T) and murine tumors (e.g., MCalV).\textsuperscript{10b,42} SP NP\textsubscript{H}s (1 mg/mL) loaded with 5 wt % indocyanine green (ICG), a NIR dye, were placed in contact with collagen gels in a horizontal capillary tube, then incubated for a further 12 h at 37 °C. A NIR imaging system was used to track particle infiltration into the collagen (Figure 6). Free ICG penetrated
cornea to the anterior chamber difficult. Particles containing Cy5 (Cy5/SP NPH) were applied to fresh cadaveric porcine corneas with or without UV light triggering for 1 min, and incubated for 8 h. Gross examination of the corneas and NIR scanning of Cy5 in corneal cross section demonstrated that the diffusion of Cy5/SP NPH was markedly enhanced by UV light triggering (Figure 7). Histologically, corneas treated with Cy5/SP NPH and UV light were indistinguishable from untreated controls under light microscopy, showing no tissue injury (Figure S9). Since collagen is one of the major components of the interstitial matrix, these results suggest the potential usefulness of SP NPH for light-triggered drug delivery to targeted tissues, for example, eyes and tumors. These results are consistent with a recent report that polymeric micelles targeting tissues, for example, eyes and tumors.45The histological findings, together with the benign cytotoxicity (Figure S5) are consistent with a favorable safety profile, but this remains to be validated by in vivo studies.

The wavelengths of the UV light we used for SP NPH triggering might limit the application of this technology to areas of the body that can be illuminated directly, for example, the eyes and ears. Of note, the photochromic conversion of SP could be potentially triggered at depths up to several centimeters by near-infrared lasers using two-photon technology.46 Since MC NPH (emission max = 672 nm) is autofluorescent,46c,47 in fact, NPs surface-modified with SP would be sufficient for use in microscopic imaging, unlike free MC.

The fluorescent photochromic properties of NPs could be used to track them in biological studies (e.g., intracellular drug delivery) with greater reliability than with simple fluorescence, which can be confounded by interfering fluorophores or in vivo autofluorescence.46g Here, we evaluated whether fluorescence switching of SP NPH could be achieved in living cells in vitro in a HeLa cell line (Figure 8a). We loaded Cy5 (emission max = 690 nm) into SP NPH since its emission spectrum would have little overlap with that of MC NPH (emission max = 672 nm). Cy5-containing SP NPH was incubated with HeLa cells for 2 h in darkness then exposed to UV illumination for 2 s, causing immediate fluorescence attributable to MC NPH (Figure 8b). Fluorescence microscopy (Figure 8) indicated that Cy5 (red color) and MC-containing hybrid NPs (green color) were colocalized in HeLa cells (orange color in Figure 8d). The fluorescent photochromic properties of NPs could be used to track them in biological studies (e.g., intracellular drug delivery) with greater reliability than with simple fluorescence, which can be confounded by interfering fluorophores or in vivo autofluorescence.46g

**Figure 7.** Ex vivo study of Cy5/SP NPH penetration in porcine corneas. (a) Fresh corneas after an 8-h treatment with Cy5/SP NPH (with or without UV irradiation for 1 min) or Cy5. The green color indicates the presence of Cy5 (a blue dye that becomes greenish in the slightly yellow tissue of the eye); (b) near-infrared images of cross sections of corneas tissues treated as in panel (a). The scale bar = 1 cm.

**Figure 8.** Fluorescence images of the internalization of Cy5-containing NPH by HeLa cells after 2 h incubation. (a) Nuclear staining with DAPI (blue color). (b) NPHs were illuminated by UV for 2 s then imaged with 560 nm emission filters (green color); NPHs were seen to be internalized. (c) The red color (emission at 700 nm) shows the Cy5 loaded in the SP NPH. (d) The overlay of panels a–c. The orange color demonstrated colocalization of SP NPH with Cy5. The scale bar = 50 μm.
to produce, and tolerated lyophilization, which may facilitate potential clinical translation.28 The NP_{PEG} could achieve high loadings with various drugs (chemotherapeutic, local anesthetics). The NP_{PEG} developed here could be adapted for a range of applications, as they could be modified with functional ligands. The phototriggering system could also be used to enhance NP_{Polar} tissue penetration, which might improve antitumor efficacy, penetration into ocular tissue and across the tympanic membrane. This is quite different from conventional approaches, where external energy sources enhance penetration by disrupting tissues.40

The photoswitchability is an attractive feature in that it can allow fine spatiotemporal control of drug release: drug is released at the irradiated site, during irradiation. This approach also obviates the need for developing a specific ligand to the tissue of interest. We have previously developed an analogous approach to the same problem by decorating nanoparticles with nonspecific ligands caged with photosensitive chemical protecting groups; upon irradiation, the caging groups would come off, allowing the nanoparticles to bind.41 These two approaches and others42 could prove synergistic.

**ASSOCIATED CONTENT**

Supporting Information

Experimental details, characterization of SP NP_{Polar}, in vitro characterization, ex vivo histology, mechanism study. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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