An orthogonally regulatable DNA nanodevice for spatiotemporally controlled biorecognition and tumor treatment

Zhenghan Di1,2*, Bei Liu1*, Jian Zhao1,2, Zhanjun Gu3, Yuliang Zhao1,2,4†, Lele Li1,2,4†

Despite the potential of nanodevices for intelligent drug delivery, it remains challenging to develop controllable therapeutic devices with high spatial-temporal selectivity. Here, we report a DNA nanodevice that can achieve tumor recognition and treatment with improved spatiotemporal precision under the regulation of orthogonal near-infrared (NIR) light. The nanodevice is built by combining an ultraviolet (UV) light-activatable aptamer module and a photosensitizer (PS) with up-conversion nanoparticle (UCNP) that enables the operation of the nanodevice with deep tissue-penetrable NIR light. The UCNPs can convert two distinct NIR excitations into orthogonal UV and green emissions for programmable photoactivation of the aptamer modules and PSs, respectively, allowing spatiotemporally controlled target recognition and photodynamic antitumor effect. Furthermore, when combined with immune checkpoint blockade therapy, the nanodevice results in regression of untreated distant tumors. This work provides a new approach for regulation of diagnostic and therapeutic activity at the right time and place.

INTRODUCTION

DNA is emerging as a highly programmable building block for the design of molecular nanodevices with specific functions, including sensors, motors, and circuits (1–3). Upon interfacing with biology, such DNA nanodevices can carry out versatile tasks such as biosensing and imaging (4–7), molecular information computing (8–10), and controllable cargo transport and release (11–21). In particular, recent work has demonstrated that DNA nanodevices could work as smart drug delivery systems (17–21). A key feature of such devices is that they can perform cell subtype–targeted delivery in response to specific biological cues. Because of their high affinity and specificity to specific targets, aptamers that can selectively bind diseased cell–associated receptors have been incorporated into DNA nanodevices for intelligent delivery (18–21). For example, an aptamer-based DNA nanorobot permitted specific transport of molecular payloads to cancer cells in response to membrane epitopes (19). An aptamer-tethered DNA nanotrain was explored for tumor-selective drug delivery (20). Recently, we designed an aptamer-driven nanorobot for controlled delivery of therapeutic protein to tumor blood vessels in response to specific receptors on the surface of tumor vascular endothelial cells (21). Despite the progress made, the cell-surface receptors that these nanodevices recognize are not only exclusive to diseased cells but also expressed at a low level in normal cells (22). Therefore, such aptamer-based nanodevices can also bind with the same target on normal cells, leading to on-target off-tumor effects. The engineering of aptamer-based nanodevices with high spatial-temporal selectivity remains a challenge because of the lack of a design methodology.

Light has proven to be an appealing tool for precise regulation of chemical and biological activities both spatially and temporally (23, 24). For example, optogenetics has had a transformative impact in neurobiology and cell biology (25, 26). The engineering of biomolecules with photolabile groups allows controlled manipulation of gene expression (27), cell signaling (28), and therapeutic activity (29, 30). For example, a phototriggerable strategy was demonstrated for the spatiotemporal regulation of aptamer activity (31). However, most of these light-controlled systems are propelled with either ultraviolet (UV) or visible light and, thus, have shallow depth of tissue penetration and insufficient photoactivation efficiency, limiting their application in vivo. Recently, lanthanide-doped up-conversion nanoparticles (UCNPs) have enabled the use of near-infrared (NIR) photons for regulation of photosensitive moieties (32–37) owing to their unique conversion ability to absorb NIR light and emit tunable UV and visible light (38, 39). Most recently, UCNPs were leveraged as optogenetic actuators of NIR light for the regulation of deep brain neurons (34).

Inspired by the achievements, herein we present a conceptual approach for the design of NIR light–controlled DNA nanodevice that imparts biorecognition and tumor treatment with improved spatial-temporal selectivity. As a clinically approved treatment modality, photodynamic therapy (PDT) relies on the generation of cytotoxic reactive oxygen species (ROS) under irradiation of photosensitizers (PSs) (40). To date, its applications are still limited by low tissue penetration of UV/visible light and lack of spatial control over PSs (41). In our design, an orthogonal up-conversion nanotechnology was used to achieve intended regulation of DNA nanodevice with deep tissue-penetrable NIR light, allowing efficient PDT with improved tumor specificity. Furthermore, the combination of the nanodevice with immune checkpoint blockade therapy elicited both synergistic and abscopal effects in tumor-bearing mice by facilitating tumor infiltration of cytotoxic T cells.
Di et al., Sci. Adv. 2020; 6 : eaba9381     17 June 2020

RESULTS
Programming of the DNA nanodevice

We choose an aptamer that recognizes nucleolin (NCL) overexpressed on the surface of various cancer cells (31, 42) to demonstrate our design. As shown in Fig. 1A, the nanodevice (PT–UN) was constructed by controlled organization of UV light–activatable aptamer modules (L–Apt) and PSs on the surface of an orthogonal UCNPs. L–Apt is designed by hybridizing the aptamer with a complementary strand (L–HD) containing a commercially available photocleavable 2-nitrobenzyl linker (PC linker; fig. S1). This duplex state, the recognition capability of the aptamer to NCL is inhibited. The UCNPs act as a photoregulator to locally convert two NIR lights of different wavelengths (808 and 980 nm) into orthogonal UV and green up-conversion luminescence (UCL), respectively. UV UCL obtained under 808-nm irradiation enables photolysis of the PC bonds and liberation of the aptamer at desired time and site, while green UCL achieved upon 980-nm irradiation allows excitation of PSs for the production of cytotoxic ROS. After intravenous injection, PT–UN could specifically bind the NCL on tumor cell surface upon 808-nm NIR light excitation, visible green (522 and 541 nm) and red (656 nm) emissions were observed (Fig. 2D). In contrast, upon 980-nm NIR light excitation, visible green (522 and 541 nm) and red (656 nm) emissions of Er$^{3+}$ dominate the spectrum (Fig. 2E).

To achieve orthogonal UCL in response to two distinct NIR light (808 and 980 nm), we designed and synthesized the core-multishell–structured UCNPs (NaGdF$_4$:Yb,Er@NaYF$_4$:Yb,Tm@NaYbF$_4$:Nd@NaYF$_4$) with lanthanide activators (Er$^{3+}$ and Tm$^{3+}$) and sensitizers (Yb$^{3+}$ and Nd$^{3+}$) doped in separate layers (fig. S2A). Er$^{3+}$/Yb$^{3+}$ were codoped in the core for producing green emission under 980-nm excitation, while Tm$^{3+}$/Yb$^{3+}$ and Yb$^{3+}$/Nd$^{3+}$ were codoped in the second and third shell layers, respectively, to enable UV emission upon 808-nm excitation (32). To prevent the energy migration between the two luminescent regions, an inert interlayer of NaYF$_4$ without any dopant was introduced as the first shell layer. In addition, an NaYF$_4$ shell layer was grown outermost to minimize surface quenching effect. Transmission electron microscopy (TEM) showed that the as-synthesized UCNPs displayed uniform hexagonal plate–like shape with a size of ∼50 nm by 25 nm (Fig. 2A and fig. S2, B to H). The interplanar lattice spacing of 0.52 nm revealed by the high-resolution TEM image was identified as (100) crystal plane of the hexagonal phase (fig. S2I) (43). The multishelled structure could be identified via image contrast in the high-angle annular dark-field scanning TEM (HAADF-STEM) image (Fig. 2B). The four-shelled UCNPs with as-designed distribution of lanthanide ions in different layers were further confirmed by linear energy-dispersive spectroscopy scanning (fig. S2I). Then, the UCNPs were coated with mesoporous silica shell for the modification of L–Apt on their external surface and loading of Rose Bengal (RB) PSs in the mesopores (fig. S3A). Each step of the construction was verified by the measurement of zeta potential and UV-visible absorbance of the resulted NPs (fig. S3, B and C). The average number of L–Apt conjugated on each NP was calculated to be 93. The resulting PT–UN showed an average size of 60 nm by 35 nm (with a mesoporous silica layer of ∼5 nm), standing on the TEM grid on either the bottom face or the side face (Fig. 2C). Figure 2 (D and E) shows the UCL spectra of the UCNPs upon irradiation with two NIR light of different wavelengths. Under 808-nm NIR light irradiation, characteristic Tm$^{3+}$-dominated UV (347 and 363 nm) and visible blue (452 and 475 nm) emissions were observed (Fig. 2D). In contrast, upon 980-nm NIR light excitation, visible green (522 and 541 nm) and red (656 nm) emissions of Er$^{3+}$ dominate the spectrum (Fig. 2E).

We then evaluated the 808-nm NIR light–triggered activation of L–Apt. The AS1411 strand and L–HD strand were modified with fluorophore Cy5 and black hole quencher (BHQ2), respectively, yielding fluorescence resonance energy transfer (FRET) pair–labeled L–Apt with a low fluorescence background. Upon 808-nm NIR light irradiation (0.5 W cm$^{-2}$), PT–UN showed a notable increase in the Cy5 fluorescence intensity (Fig. 2F), indicating the liberation of the aptamer strand from the hybrid due to the photolysis of L–HD. As a control, when the nanoconstruct was modified with a DNA probe that had the same sequence as L–Apt but without a PC linker (nPT–UN), no obvious fluorescence change of Cy5 was observed under 808-nm NIR light irradiation, indicating that the introduction of the PC bond was crucial for the design. Because the green UCL band of UCNPs under 980-nm irradiation overlaps well with the absorbance spectra of RB PSs (Fig. 2G), the green UCL of the nanostructure was obviously quenched after loading of RB in the mesopores, while

---

**Fig. 1. Schematic showing the orthogonal regulation of DNA nanodevice for programmed tumor cell recognition and treatment.** (A) The orthogonal phot.activation behavior of the DNA nanodevice in response to two NIR light of different wavelengths. (B) Sequential activation of the nanodevice with orthogonal UCL for programmed tumor recognition and PDT.
the red UCL was barely influenced (Fig. 2H). The energy transfer from the UCNP to the RB was further confirmed by the time-resolved photoluminescence measurement, which reveals a significantly decreased lifetime of Er$^{3+}$ emission (from 487 to 317 µs) upon the loading of RB in the nanostructure (fig. S4, A and B). Next, the capability of PT-UN to produce single oxygen ($^{1}$O$_{2}$) under 980-nm NIR light irradiation was examined using the dye 1,3-diphenylisobenzofuran (DPBF) as an indicator of $^{1}$O$_{2}$. As expected, the absorption intensity of DPBF in PT-UN dispersion decreased upon 980-nm irradiation, but exhibited negligible change with 808-nm irradiation or without any irradiation (Fig. 2I), confirming that the photon energy absorbed by UCNP under 980-nm laser irradiation was transferred to RB for $^{1}$O$_{2}$ generation. In addition, no obvious leakage of RB from PT-UN was observed (fig. S4C), which was important for the application of such system for PDT.

Orthogonally regulated target recognition and photodynamic effect in vitro

Next, we examined the NIR light–triggered recognition of PT-UN to 4T1 breast cancer cells with NCL on their surface. The nanostructures were loaded with Cy5 dye instead of RB for the studies by confocal laser scanning microscopy (CLSM) and flow cytometry. As shown in Fig. 3A, CLSM images revealed much lower fluorescence signals in the cells treated with PT-UN compared with cells exposed to T-UN (wherein the system was modified with the aptamer strand only), indicating that formation of L-Apt blocked the recognition ability of the aptamer. Irradiation of PT-UN with 808-nm laser significantly increased their cellular uptake to a level comparable to that observed with T-UN, demonstrating that 808-nm NIR light irradiation liberated aptamer on the PT-UN surface for cell targeting. In contrast, 4T1 cells whose surface NCL was blocked by anti-NCL antibody exhibited no response to the photoactivated system, confirming that the NIR-responsive cellular uptake enhancement of PT-UN is directed by NCL-mediated binding. As a control, 808-nm NIR light irradiation of inactive nPT-UN led to no obvious change of their intracellular uptake, confirming the indispensable role of the PC element for the photoregulation design. The NIR light–controlled biorecognition was further confirmed by flow cytometry (Fig. 3B and fig. S5A). The cells treated with PT-UN plus 808-nm NIR light activation exhibited 2.3-fold higher fluorescence than those without
light irradiation, that is, to a level similar to that of cells incubated with T-UN. The colocalization study revealed that the photoactivated PT-UN mainly accumulated inside endo/lysosomes (fig. S5B).

Afterward, we investigated the orthogonal UCL–regulated photodynamic effect. Intracellular $^{1}$O$_2$ generation was first examined by using a fluorescent ROS probe, 2,7-dichlorodihydrofluorescein diacetate (H$_2$DCFDA). Cells treated with PT-UN displayed relatively dim ROS signal compared with cells exposed to T-UN under 980-nm irradiation because of their inhibited cell-targeting ability (fig. S6A). In contrast, sequential irradiation with 808- and 980-nm NIR light notably enhanced ROS signal of cells incubated with PT-UN (Fig. 3C), suggesting that the 808-nm NIR light–mediated recovery of the recognition capability enhanced intracellular ROS production. Quantitatively, ROS signal in PT-UN–treated cells with sequential...
irradiation of dual NIR light was 2.1-fold higher relative to those with 980-nm irradiation only (fig. S6B). As a control, cells incubated with inactive nPT-UN plus dual NIR light irradiation exhibited negligible change of intracellular ROS signal in comparison to that irradiated with 980-nm light only (fig. S6, A and B). Next, the photodynamic efficacy of PT-UN was examined by Cell Counting Kit-8 (CCK-8) assay. As shown in Fig. 3D, PT-UN successively treated with 808- and 980-nm NIR light showed stronger cytotoxicity than that irradiated by 980-nm NIR light only, indicating phototargeting-mediated enhancement of therapeutic efficiency. After sequential irradiation of the dual NIR light, viability of PT-UN–treated cells (32.3%) was comparable to that of cells exposed to T-UN (28.4%) and was 2.0-fold lower than that of cells incubated with PT-UN plus 980-nm light irradiation (63.2%) (Fig. 3E). For cells incubated with T-UN or nPT-UN, dual NIR light irradiation could not decrease cell viability in comparison to that irradiated with only 980-nm light (Fig. 3E). These results were further supported by calcein AM/propidium iodide (PI) costaining assay and annexin V and PI double staining analysis. PT-UN exposed to dual NIR light irradiation evoked the highest levels of dead cells represented by red fluorescence (Fig. 3F and fig. S6C) and the lowest level of healthy cells indicated by annexin V–APC/PI apoptosis assay (Fig. 3G and fig. S6D).

**Orthogonally regulated tumor targeting and therapy in vivo**

To investigate the NIR light-mediated regulation of the nanodevice in vivo, BALB/c mice bearing subcutaneous tumors were administered intravenously with T-UN, PT-UN, or nPT-UN followed by 808-nm NIR light irradiation at the tumor region. The whole-body...
fluorescence imaging was then performed at indicated time points. The fluorescence intensity at the tumor sites showed a time-dependent increase before reaching a maximum at 8 hours after injection in all groups (Fig. 4, A and B). Mice treated with PT-UN displayed relatively weak intratumoral fluorescence compared with those receiving T-UN. Irradiation of the tumor site in the PT-UN group with 808-nm NIR light led to enhanced intratumoral fluorescence (Fig. 4, A and B), which was 1.7-fold higher than the nonirradiated PT-UN group at 4 hours after injection (Fig. 4C). As a control, the phototriggering did not enhance the intratumoral accumulation of nPT-UN (Fig. 4, A to C). These results indicated that enhanced accumulation of PT-UN upon NIR triggering was due to photoactivation of the aptamer modules on their surface. Biodistribution of NPs was evaluated by ex vivo imaging of harvested organs and tumors 24 hours after administration (Fig. 4D). In the liver, the PT-UN + 808 nm group showed fluorescence intensity similar to that in the nonirradiated PT-UN group, but lower than that in the T-UN group. These data suggest that PT-UN could be locally activated to specifically recognize its target in tumor, while the off-target binding in liver was reduced, resulting in a higher specificity to tumor compared with the “always on” targeting system (T-UN). The fluorescence ratio of tumor to liver in the PT-UN + 808 nm group was 2.0-fold higher than that in the T-UN group (Fig. 4E), confirming that NIR irradiation enhanced accumulation of PT-UN in tumor relative to the level in liver.

We then assessed the PDT potency of the nanodevice. 4T1 tumor-bearing mice were intravenously injected with PT-UN or nPT-UN, followed by dual NIR light activation. The 980-nm laser irradiation was applied with an interval between short-time irradiation cycles to alleviate potential overheating effect (fig. S7). As shown in Fig. 5A, tumors in mice treated with phosphate-buffered saline (PBS) followed by sequential irradiation with two NIR light grew rapidly with the average volume similar to that in the PBS group, excluding the effect of irradiation on tumor regression. Treatment with PT-UN or nPT-UN had no antitumor effect. After irradiation with the 980-nm laser only, moderate tumor inhibition was observed in mice treated with PT-UN (48.6%) or nPT-UN (51.2%) compared with the PBS group. PT-UN with sequential irradiation of 808- and 980-nm NIR light exhibited enhanced tumor eradication (77.8%) compared with that irradiated with 980-nm NIR light alone, whereas tumor growth in mice treated with nPT-UN plus dual NIR light irradiation was similar to that irradiated by 980-nm laser alone, confirming that the 808-nm NIR light–mediated targeting enhanced the anticancer efficiency of PT-UN. As expected, PT-UN plus dual NIR light irradiation resulted in the least average tumor weight among all groups (Fig. 5B). The hematoxylin and eosin (H&E) and terminal deoxynucleotidyl 

![Fig. 5. Orthogonally regulated tumor treatment.](image-url)

**Fig. 5. Orthogonally regulated tumor treatment.** (A) Tumor growth curves of mice with different treatments. Group 1, phosphate-buffered saline (PBS); group 2, PBS + 808 nm + 980 nm; group 3, nPT-UN; group 4, PT-UN; group 5, nPT-UN + 980 nm; group 6, PT-UN + 980 nm; group 7, nPT-UN + 808 nm + 980 nm; and group 8, PT-UN + 808 nm + 980 nm. Data are means ± SD (n = 5). Statistical significance was determined by one-way analysis of variance (ANOVA). **P < 0.01. (B) Final weight of tumors collected from mice after various treatments. Data are means ± SD (n = 5). Statistical significance was determined by two-tailed Student’s t test. ns, not significant; **P < 0.01. (C and D) Hematoxylin and eosin (H&E) (C) and terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick end labeling (TUNEL) (D) staining of tumor sections collected from mice with indicated treatments. Scale bars, 100 (C) and 50 μm (D).
transferase–mediated deoxyuridine triphosphate nick end labeling (TUNEL) staining showed that the group treated with PT-UN plus dual NIR light activation had the highest level of necrosis in tumor tissues in comparison with the other groups (Fig. 5, C and D, and fig. S8). No abnormal change in body weight was observed in all groups (fig. S9A). In addition, PT-UN induced neither serum biochemistry defects nor organ damage (fig. S9, B to D), demonstrating that the platform is devoid of systemic toxicity.

**Combinational therapy with immune checkpoint blockade**

We further examined whether antitumor immunity triggered by the nanodevice could be combined with checkpoint blockade therapy to realize systemic cancer management. It has been reported that PDT could cause calreticulin (CRT) exposure, thus inducing immunogenic cell death (ICD) and activating the engulfment of apoptotic tumor cells by antigen-presenting cells (dendritic cells and macrophages) (44). Therefore, we first evaluated the ability of the nanodevice to stimulate ICD by detecting cell-surface CRT expression. As expected, PT-UN incubation followed by dual NIR light irradiation induced membrane CRT expression in vitro (fig. S10, A and B), suggesting phototriggered immunogenicity. The analysis of CRT expression in tumor tissues verified the immunogenic property of the platform in vivo (Fig. S10C). A bilateral tumor model of breast cancer was then established with the left tumors defined as primary tumors for NIR light irradiation and the right tumors designated as distant tumors without direct irradiation. When the primary tumors reached ~100 mm³, mice were randomly divided into four groups for the treatment with (i) PBS, (ii) α-programmed death-ligand 1 (α-PD-L1), (iii) PT-UN with dual NIR light irradiation [donated PT-UN(+)], and (iv) PT-UN(+) plus α-PD-L1. PT-UN was intravenously injected every 4 days for a total of two injections followed by sequential irradiation with dual NIR light, while α-PD-L1 was intravenously injected at indicated time after the irradiation (Fig. 6A). As shown in Fig. 6 (B to D), α-PD-L1 alone exhibited slight inhibition effect on growth of the primary and the distant tumors. PT-UN(+) led to extraordinary primary tumor inhibition but exhibited slight inhibition on the distant tumors. In contrast, the combination of PT-UN(+) with checkpoint blockade resulted in obvious growth inhibition of primary tumors (synergistic effect) and distant tumors (abscopal effect).

The antitumor performance on distant tumors implied a successful activation of systemic immune response. As anticipated, the...
percentage of infiltrating CD45<sup>+</sup> leukocytes in distant tumors increased significantly in the group of PT-UN(+) plus α-PD-L1 (35.80 ± 9.07%), compared with the PBS group (21.82 ± 6.61%) (Fig. 6E). Specifically, the proportion of CD8<sup>+</sup> T cells (1.46 ± 0.75%) and CD4<sup>+</sup> T cells (0.94 ± 0.40%) significantly increased in the PT-UN(+) plus α-PD-L1 group compared with the PBS group (CD8<sup>+</sup> T cells, 0.52 ± 0.32%; CD4<sup>+</sup> T cells, 0.34 ± 0.18%) (Fig. 6, F and G). Meanwhile, these positive responses led to the robust generation of immunostimulated cytokines including interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α), which play important roles in cytotoxic activities of cytotoxic T lymphocytes (Fig. 6H) (45). Together, PT-UN–mediated PDT coupled with α-PD-L1 treatment elicited potent systemic antitumor immune responses by promoting the infiltration of effector T cells.

**DISCUSSION**

Although nanotechnology holds great promise for controlled drug delivery since its demonstration for passive and active targeting, smarter systems with precise control over the anticancer response are urgently needed. In this work, we present a DNA-based nanodevice that permits tumor recognition and treatment with high spatial-temporal selectivity under the control of NIR light. UCNPs used here act as transducers to generate orthogonal UCL for the controlled operation of the nanodevice in the deep tissue–penetrable NIR window. We demonstrated that orthogonal regulation endows the nanodevice with spatiotemporal-controlled biorecognition, enhanced tumor specificity, and robust antitumor efficacy. Furthermore, the nanodevice generated an immunogenic tumor microenvironment and significantly improved the efficacy of immune checkpoint blockade therapy. We envision that the design illustrated here would open up an avenue for cancer precision diagnosis and therapy.

On the other hand, UCNPs have recently been used for NIR light–induced, deeper-penetrating PDT, in which the UCL is absorbed by a PS that then converts tissue oxygen into ROS to eradicate cancer cells (32, 33). Despite the progress made, the lack of precise spatial control over such nanophotosensitizers is accompanied with off-tumor phototoxicity. Our design allows conferring NIR light sensitivity to the biorecognition of the UCNP-based nanophotosensitizer to enable remote control of tumor targeting with high spatiotemporal precision and is, therefore, amenable to triggering of ROS generation at the right time and place. The present study highlights the potential of the integration of UCNPs with DNA nanotechnology for precision PDT.

**MATERIALS AND METHODS**

**Preparation of PT-UN**

Oleate-capped UCNPs were synthesized with a thermal decomposition approach (32, 43). The as-synthesized core-multishell UCNPs were first coated with mesoporous silica layer. Typically, 400 μl of the as-prepared UCNPs (25 nM) was slowly added to 20 ml of cetyltrimethylammonium bromide (CTAB) aqueous solution (5 mg ml<sup>−1</sup>), and the solution was stirred overnight. After adding ethanol (6 ml) and NaOH solution (2 M, 100 μl), the mixture was heated to 60°C under stirring. Then, 80 μl of TEOS (tetraethyl orthosilicate) was added to the mixture, and the resultant solution was stirred for 1 hour. One milliliter of ethanol solution containing 20 μl of 3-aminopropyltriethoxysilane was added dropwise. The mixture was stirred for 30 min, and the resulting particles were collected by centrifugation and washed three times with ethanol. The CTAB within the particles was removed by refluxing in ammonium chloride alcohol solution (2 mg ml<sup>−1</sup>) at 60°C for 2 hours. Next, the obtained UCNP@mSiO<sub>2</sub> (0.05 nmol) was dispersed in the mixture of HEPES buffer (20 mM and 150 mM NaCl, pH 7.2) and dimethylformamide (DMF; v/v, 7:3) containing sulfoosuccinimidyl 4-(Nmaleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC) (4 mg). After shaking for 6 hours, the maleimide-activated UCNP@mSiO<sub>2</sub> was collected by centrifugation and washed with DMF and the HEPES buffer. Subsequently, thiol-modified aptamer strands (10 nmol) activated by 1.5 equivalents of Tris (2-carboxyethyl) phosphine (TCEP) were added to the HEPES buffer containing the maleimide-modified UCNP@mSiO<sub>2</sub>. After shaking for 24 hours, the aptamer-modified UCNPs were collected by centrifugation, washed, and redispersed in 1 ml of HEPES buffer containing RB (1 mg ml<sup>−1</sup>) for loading of RB in the mesopores. After shaking for 24 hours, the RB-loaded particles were collected by centrifugation, washed, and added in 0.5 ml of hybridization buffer (20 mM HEPES, 150 mM NaCl, and 5 mM MgCl<sub>2</sub>) containing L-HD (6 nmol). After incubation for 4 hours, the resulting PT-UN was centrifuged, washed with the buffer three times, and redispersed in the buffer.

**Singlet oxygen detection**

Typically, PT-UN (2 × 10<sup>−4</sup> nmol) was added to a cuvette containing water solution of DPBF (1 ml, 10 μM). The mixture was then irradiated with 808-nm (0.5 W cm<sup>−2</sup>) or 980-nm (1.0 W cm<sup>−2</sup>) NIR laser for different times, and the corresponding absorption spectra were measured immediately. The change in absorbance of DPBF at 415 nm as a function of irradiation time was used to evaluate the O<sub>2</sub> generation ability.

**Cell culture**

4T1 cells were purchased from the American Type Culture Collection (Manassas, VA) and cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), penicillin (100 U), and streptomycin (0.1 mg ml<sup>−1</sup>) in a 37°C humidified incubator containing 5% CO<sub>2</sub>.

**Cellular uptake analysis**

For CLSM imaging, 4T1 cells were seeded in 35-mm confocal dishes at a density of ~1 × 10<sup>5</sup> cells per well overnight. Afterward, cells were washed twice with PBS and supplemented fresh Opti–minimum essential medium (MEM) containing 0.2 nM Cy5-loaded PT-UN, T-UN, or nPT-UN, followed with or without 808-nm laser irradiation (0.5 W cm<sup>−2</sup>, 10 min). After incubation for another 1 hour, the cells were washed with PBS twice, stained with Hoechst 33342, and subjected for CLSM imaging. For flow cytometry analysis, cells were cultured in a six-well plate at a density of ~2 × 10<sup>5</sup> cells per well, followed by the treatments with materials and irradiation as the same as those for CLSM imaging. The cells were washed with PBS twice, detached with 200 μl of 0.25% trypsin-EDTA solution, and resuspended in 300 μl of PBS containing 1% FBS for flow cytometry assay.

**Intracellular O<sub>2</sub> detection**

Intracellular O<sub>2</sub> was detected by both CLSM imaging and flow cytometry analysis. 4T1 cells were plated onto 35-mm confocal dishes (~1 × 10<sup>3</sup> cells per well, for CLSM imaging) or six-well plates (~2 × 10<sup>3</sup> cells per well, for flow cytometry analysis) 24 hours in advance. Then, the growth medium was replaced with Opti-MEM containing PT-UN, T-UN, or nPT-UN (0.2 nM). For sequential irradiation, the cells were first irradiated by 808-nm laser (0.5 W cm<sup>−2</sup>,
In vivo imaging

4T1 cells (~2 × 10^4 cells per well, 100 μl) were seeded in a 96-well plate beforehand. When the confluence reached 70 to 80%, cells were exposed to PT-UN, T-UN, or nPT-UN (0.2 nM), followed by the irradiation of 808-nm laser (0.5 W cm⁻², 10 min). After incubation for 2 hours, the cells were washed with PBS twice to remove uninternalized materials and then irradiated with 980-nm laser (1.0 W cm⁻², 3-min break after 1-min irradiation) for 20 min. Thereafter, cells were maintained at 37°C for another 24 hours. Last, cells were supplemented with 100 μl of FBS-free RPMI 1640 containing 10% CCK-8 1 hour after incubation, and absorbance at 450 nm was measured with the microplate reader.

Statistical analysis

Data were expressed as means ± SD. Student’s t-test was used for the comparison of difference between two groups, while one-way analysis of variance (ANOVA) was applied to evaluate the statistically significant difference of multiple groups. A P value of <0.05 was regarded statistically different. Both tests were carried out by SPSS 19.0 (SPSS Inc., Chicago, IL).

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/6/25/eaba9381/DC1

REFERENCES AND NOTES

1. N. C. Seeman, DNA in a material world. *Nature* **421**, 427–431 (2003).
2. K. Lund, A. J. Manzo, N. Dabby, N. Michelotti, A. Johnson-Buck, J. Nangreave, S. Taylor, R. Pei, M. N. Stojanovic, N. G. Walter, E. Winfree, H. Yan, Molecular robots guided by prescriptive landscapes. *Nature* **465**, 206–210 (2010).
3. H. Ramezani, H. Dietz, Building machines with DNA molecules. *Nat. Rev. Genet.** **21**, 5–26 (2019).
4. Y. J. Chen, B. Groves, R. A. Muscat, G. Seelig, DNA nanotechnology from the test tube to the cell. *Nat. Nanotechnol.* **10**, 748–760 (2015).
5. S. Modi, M. G. Swithin, D. Goswami, G. D. Gupta, S. Mayor, Y. Krishnan, A DNA nanorobot that maps spatial and temporal pH changes inside living cells. *Nat. Nanotechnol.* **4**, 325–330 (2009).
6. T. T. Chen, X. Tian, C. L. Liu, J. Ge, X. Chu, Y. Li, Fluorescence activation imaging of cytochrome c released from mitochondria using aptamer nanosensor. *J. Am. Chem. Soc.* **137**, 982–989 (2015).
7. K. Leung, K. Chakraborty, A. Saninathan, Y. Krishnan, A DNA nanomachine chemically resolves lysosomes in live cells. *Nat. Nanotechnol.* **14**, 176–183 (2019).
9. B. Groves, Y. J. Chen, C. Zurla, S. Pochekailov, P. J. Santangelo, G. Seelig, Computing in mammalian cells with nucleic acid strand exchange. Nat. Nanotechnol. 11, 287–294 (2016).

10. M. Rudchenko, S. Taylor, P. Pallavi, A. Dechkovskaya, S. Khan, V. P. Butler III, S. Rudchenko, M. N. Stojanovic, Autonomous molecular cascades for evaluation of cell surfaces. Nat. Nanotechnol. 8, 580–586 (2013).

11. Q. Hu, H. Li, L. Wang, H. Gu, C. Fan, DNA nanotechnology-enabled drug delivery systems. Chem. Rev. 119, 6459–6506 (2019).

12. J. D. Brodin, A. J. Sprangers, J. R. McMillan, C. A. Mirkin, DNA-mediated cellular delivery of functional enzymes. J. Am. Chem. Soc. 137, 14838–14841 (2015).

13. J. R. Burns, A. Sefert, N. Fertig, S. Howorka, A biomimetic DNA-based channel for the ligand-controlled transport of charged molecular cargo across a biological membrane. Nat. Nanotechnol. 11, 152–156 (2016).

14. A. T. Veetil, K. Chakrabarty, K. Xiao, M. R. Minter, S. S. Sisodia, Y. Krishnan, Cell-targetable DNA nanocapsules for spatiotemporal release of caged bioactive small molecules. Nat. Nanotechnol. 12, 1183–1189 (2017).

15. S. Ranaldo, C. Prévost-Tremblay, A. Idili, A. Vallée-Bélisle, F. Ricci, Antibody-powered nucleic acid release using a DNA-based nanomachine. Nat. Commun. 8, 15150 (2017).

16. H. Lee, A. K. Lytton-Jean, Y. Chen, K. T. Love, A. I. Park, E. D. Karagiannis, A. Sehgal, M. N. Stojanovic, Autonomous molecular cascades for evaluation of cell surfaces. Nat. Nanotechnol. 11, 389–393 (2016).

17. R. Mo, T. Jiang, R. DiSanto, W. Tai, Z. Gu, ATP-triggered anticancer drug delivery. Nat. Commun. 7, 389–393 (2012).

18. W. J. Wang, S. Yu, S. Huang, S. Bi, H. Y. Han, J.-R. Zhang, Y. Lu, J.-J. Zhu, Bioapplications of DNA nanotechnology at the solid-liquid interface. Chem. Soc. Rev. 48, 4982–4920 (2019).

19. S. M. Douglass, I. Bachelet, G. M. Church, A logic-gated nanorobot for targeted transport of molecular payloads. Science 335, 831–834 (2012).

20. G. Zhu, J. Zheng, E. Song, M. Donovan, K. Zhang, C. Liu, W. Tan, Self-assembled, aptamer-tethered DNA nanotransports for targeted transport of molecular drugs in cancer theranostics. Proc. Natl. Acad. Sci. U.S.A. 110, 7999–8003 (2013).

21. S. Li, Q. Jiang, S. Liu, Y. Zhang, Y. Tian, C. Song, J. Wang, Y. Zou, G. J. Anderson, J.-Y. Han, Y. Chang, Y. Liu, C. Zhang, L. Chen, G. Zhou, G. Nie, H. Yan, B. Ding, Y. Zhao, A DNA nanorobot functions as a cancer therapeutic in response to a molecular trigger in vivo. Nat. Biotechnol. 36, 258–264 (2018).

22. A. D. Keefe, S. Pai, A. Ellington, Aptamers as therapeutics. Nat. Rev. Drug Discov. 9, 537–550 (2010).

23. L. Kowalk, J. K. Chen, Illuminating developmental biology through photochemistry. Nat. Chem. Biol. 13, 587–598 (2017).

24. N. Ankenbruck, T. Courtey, Y. Naro, A. Deiters, Optochemical control of biological processes in cells and animals. Angew. Chem. Int. Ed. Engl. 57, 2768–2798 (2018).

25. R. L. Fork, Laser stimulation of nerve cells in Aplysia. Science 171, 907–908 (1971).

26. A. M. Packer, B. Roska, M. Häusser, Targeting neurons and photons for optogenetics. Nat. Neurosci. 16, 805–813 (2013).

27. S. A. Reis, B. Ghosh, J. A. Hendricks, D. M. Szantai-Kis, L. Tork, K. N. Ross, J. Lamb, W. Read-Button, B. Zheng, H. Wang, C. Salthouse, S. J. Haggarty, R. Mazitschek, Light-controlled modulation of gene expression by chemical optoepigenetic probes. Nat. Chem. Biol. 12, 317–323 (2016).

28. Z. Liu, Y. Liu, Y. Chang, H. R. Seyf, A. Henry, A. L. Mattheyes, K. Yehl, Y. Zhang, Z. Huang, K. Salata, Nanoscale optomechanical actuators for controlling mechanotransduction in living cells. Nat. Methods 13, 143–146 (2016).

29. W. A. Velena, J. P. van der Berg, M. J. Hansen, W. Szymanski, A. J. M. Driessen, B. L. Feringa, Optical control of antibacterial activity. Nat. Chem. 5, 924–928 (2013).

30. Y. F. Wang, D. S. Kohane, External triggering and triggered targeting strategies for drug delivery. Nat. Rev. Mater. 2, 17020 (2017).

31. L. Li, R. Tong, H. Chu, W. Wang, R. Langer, D. S. Kohane, Aptamer photoregulation in vivo. Proc. Natl. Acad. Sci. U.S.A. 111, 17099–17103 (2014).