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# A cell viability-reporting photothermal theragnostic nanoprobe for intraoperative optical ablation and tracking of tumors

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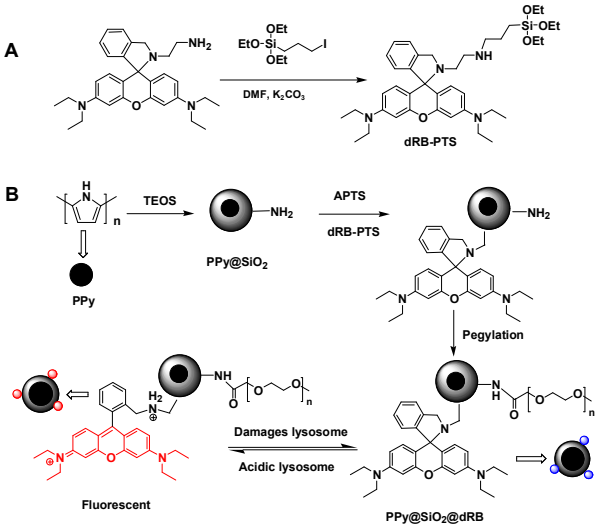
## Experimental

### Material and methods

LysoTracker Green DND-26 was purchased from Invitrogen. Bafilomycin A, and methoxypolyethylene glycol 5,000 acetic acid were purchased from Simga. DAPI (4'6-diamino-2-phenylindole) was obtained from Bioluminor. PPy nanoparticles were synthesized following a reported method.[1] dRB-EDA was prepared as previously described.[2] Unless specified, all other reagents were obtained from Alfa Aesar. Fluorescence emission spectra and UV-vis absorption spectra were recorded on a spectrofluorometer (SpectraMax M5, Molecular Device). Dynamic light scattering and surface potential analysis of the nanoparticles were performed on Zetasizer Nano ZS (ZEN3500, Malvern), NIR irradition was carried out by using an optical fiber coupled 808 nm high power laser diode (BWT Beijing Co., Ltd.). HepG2, 4T1 and HeLa cells were obtained from the American Type Culture Collection (ATCC). Confocal fluorescence microscopy images were obtained on Leica SP5 using the following filters: $\lambda ex$@488 nm and $\lambda em$@500-530 nm for LysoTracker Green DND-26 signal, and $\lambda ex$@543 nm and $\lambda em$@580-620 nm for the rhodamine signal. The fluorescence signals of LysoTracker green and rhodamine inside cells were merged using Photoshop CS 5.0.

### Preparation of PPy@$SiO_2$@dRB




**Scheme 1**. Synthesis of PPy@$SiO_2$@dRB

**Synthesis of dRB-PTS**: dRB-ethylenediamine (50 mg) was incubated with 3-iodopropyltrietoxysilane (50 µl) in DMF (500 µl) containing $K_2CO_3$ (100 mg). The mixture was incubated with gentle stirring at rt for 30 min and then directly used for coating of the silica nanoparticles.

**PPy@$SiO_2$**: PPy nanoparticles (90 mg) were dispersed in an aqueous solution containing ethanol (210 ml), ultrapure water (30 ml) and 25% ammonia solution (9.4 ml). The mixture was sonicated for 10 min followed by dropwise addition of TEOS (1.6 ml) in ethanol (12 ml). The solution was stirred vigorously at rt for 8 h. The resultant particles was collected by centrifuging at 15000 rpm for 20min, washed with ethanol and then re-dispersed in ultrapure water (360 ml) containing CTAB (1 g). After ultrasonication for 30 min, tetraethoxysilane (TEOS) (480 µl) was added dropwisely into the solution which was stirred vigorously at 45 °C for 24 h. The resultant particles was harvested by centrifugation, washed with ethanol and then dispersed in of ethanol (100 ml) containing $NH_4NO_3$ (2 g). The mixture was stirred at 45 °C for 24 h to remove CTAB, and then centrifuged to give PPy@$SiO_2$ which was washed with methanol before use.

**PPy@$SiO_2$@dRB:** PPy@$SiO_2$ (500 mg) was dispersed in tolulene (50 ml) containing of the aforementioned dRB-PTS solution and APTS (100 µl). The mixture was kept for 20 h at 80°C. The resultant particles were collected by centrifugation, amd then washed with ethanol-acetic acid solution. The formentioned particles (500 mg) were aded to anhydrous DMF (20 mL) containing NHS ester of polyethylene glycol (MW 5000, 0.145g) and triethylamine (200 µl). The mixture was sonicated for 90 min followed by addition of saturated $NaHCO_3$ solution (30 mL). The mixture was further sonicated for 90 min, and then centrifuged. The nanoparticles were collected, extensively washed with deionized water to remove residual reagents, and then stored in water (0.1mg $ml^{-1}$) for subsequent characterization.

**$SiO_2$@dRB:** TEOS (2.3 ml) was added into a clean falsk containing deionized water (240 ml), cetyltreimethylammonium bromide (0.5 g) and aqueous sodium hydroxide solution (2 M, 1.8 ml) at 80 0C. the mixtyre was stirred vigorously for 1 h and then the product was collected by centrifugation. The resulatnt nanoparticles was then coated with dRB-PTS and APTS and then pegylated following the aforementioned precoedure to give $SiO_2$@dRB,

### Dynamic size and zeta potential measurements

PPy@$SiO_2$@dRB, PPy@$SiO_2$, and PPy were dispersed in distilled water (100 µg $ml^{-1}$), sonicated and then analyzed with a

Zetasizer NaNO ZS (ZEN3500) to determine their statistical size distributions and zeta potentials.

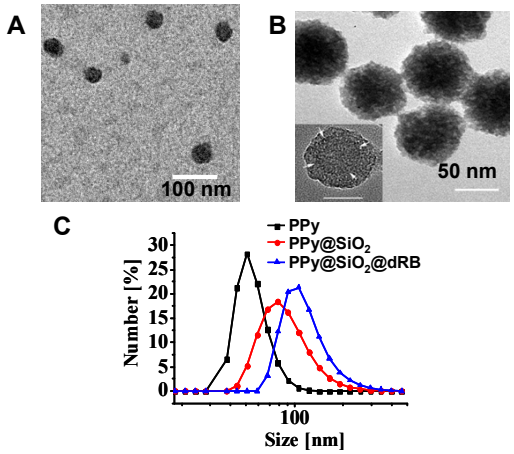



**Fig. S1** SEM images of PPy (A) and PPy@$SiO_2$@dRB (B); Dynamic light scattering analysis of PPy, PPy @$SiO_2$, and PPy@$SiO_2$@dRB (C).

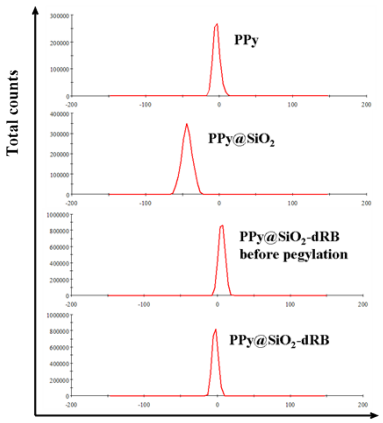



**Fig. S2** Zeta potentials of PPy, PPy@$SiO_2$ and PPy@$SiO_2$@dRB before and after pegylation.

**pH titration of PPy@$SiO_2$@dRB**

Aliquots of stock solution of PPy@$SiO_2$@dRB (10 µl, 5 mg $ml^{-1}$ in water) were respectively added to sodium phosphate buffers (100 mM, 1 ml) of various pH. The fluorescence emission@590 nm or UV-vis absorption of the solutions was recorded as a function of pH using *λex*@560 nm. The titration curves were plotted by fluorescence emission intensities@590 nm over pH.

**PPy@$SiO_2$@dRB mediated conversion of NIR light into heat**

Ultrapure water (2 ml) containing various amounts of PPy@$SiO_2$@dRB (0, 0.1, 0.2, or 0.5 mg $ml^{-1}$) were respectively illuminated with NIR light for 10 min (2 w $cm^{-2}$). The temperature of the solutions was recorded as a function of illumination time.

**Fluorescent reporting of lysosomes with PPy@$SiO_2$@dRB**

HeLa, HepG2 and 4T1 cells were seeded on 35 mm glass-bottom dishes (NEST) and incubated for 24 h in DMEM supplemented with 10% FBS. The cells were cultured for 2 h in DMEM spiked with PPy@$SiO_2$@dRB (50 µg $ml^{-1}$) and DAPI (1 µM) . The cells were washed with PBS (1 ml) and further incubated with Lysotracker green (1 µM) for 30 min in DMEM. The resultant cells were placed in fresh medium and then analyzed by confocal fluorescence microscopy.

For reversal staining of lysosomes, the three cell lines were pre-incubated for 4 h at 37 °C in the absence or presence of BFA (50 nM), and then cultured for 2 h in DMEM containing PPy@$SiO_2$@dRB (50 µg $ml^{-1}$) and DAPI (1 µM). The cells were washed with PBS (1 ml), incubated with Lysotracker green (1 µM) in DMEM for 30 min, and then analyzed by confocal fluorescence microscopy.

**NIR mediated photothermal effects in PPy@$SiO_2$@dRB treated cells**

HepG2, HeLa cells and 4T1 were respectively cultured for 6 h in DMEM supplemented with or without PPy@$SiO_2$@dRB (50 µg $ml^{-1}$) and $SiO_2$@dRB (50 µg $ml^{-1}$), and then illuminated under NIR laser for 10 min (2 w $cm^{-2}$). The cells were collected by centrifugation, washed with PBS and then analyzed by fluorescence microscopy and MTT assay. The fluorescence values were analyzed by the software of ImageJ.

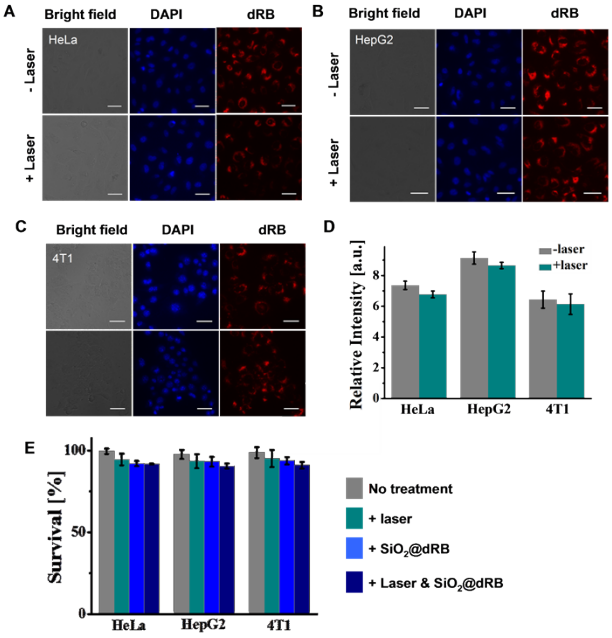



**Fig. S3** Effects of NIR irradiation on fluorescence of $SiO_2$@dRB within cells. HeLa (A), HepG2 (B) and 4T1 cells (C) were respectively cultured for 6 h in DMEM supplemented with $SiO_2$@dRB (50 µg $ml^{-1}$) and DAPI (1 µM) and then illuminated with or without NIR light in DMEM for 10 min. The cells were probed by confocal fluorescence microscopy. (D) Intracellualr fluroescence emission@575-590 nm was collected using *λex*@510-560 nm; (E) cell viability was determined by MTT assay.

**NIR triggered photothermal effects on subcutaneous tumors from mice treated with PPy@$SiO_2$@dRB**

Female Balb/c mice were obtained from Xiamen University Laboratory Animal Center. The 4T1 murine breast tumor models were generated by injection of the cells onto the flanks of each mouse. At 5-10 days after transplantation, the mice were injected intravenously via the tail vein with PPy@$SiO_2$@dRB (30 mg $kg^{-1}$ in mice) or $SiO_2$@dRB (30 mg $kg^{-1}$ in mice). After 12 h, the mice were anesthetized, and subcutaneous tumors was excised, washed with phosphate buffered saline (PBS) and then subjected to *ex vivo* analysis for the fluorescence intensity. The tumors were then illuminated with NIR light for 5 min (2 w $cm^{-2}$) and then analyzed for fluorescence intensity.

**Cytotoxicity of PPy@$SiO_2$@dRB**

HepG2 cells, 4T1 cells and HeLa cells were seeded in 96-well plates at a density of $1x10^4$ cells/well and then incubated

overnight in DMEM containing 10% fetal bovine serum (FBS). The cells were washed with PBS and then incubated in fresh medium containing various amounts of PPy@$SiO_2$@dRB (0, 0.05, 0.1, 0.2 mg $ml^{-1}$) for 48 h. The cells were incubated in DMEM medium containing tetrazonium dye (0.25 mg $ml^{-1}$) and then cultured for 4 h at 37°C. After removal of the supernatants, DMSO (100 µl) was added to each well to dissolve the formazan. The plate was shaken for 5 min and then analyzed by SpectraMax M5 to record the absorbance @490nm.

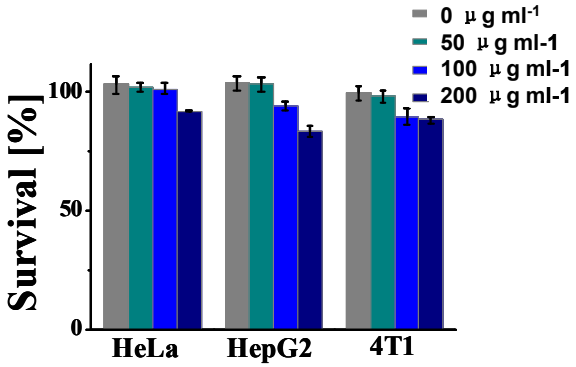



**Fig. S4** Cytotoxicity of PPy@$SiO_2$@dRB.

**References**

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2 X. Wu, Z. Wu, Y. Yang, S. Han, *Chem. Comm.,* 2012, **48**, 1895.