Supporting Information

pH-Responsive and Near-infrared-emissive Polymer Nanoparticles for Simultaneous Delivery, Release, and Fluorescence Tracking of Doxorubicin *in vivo*

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Experimental

Materials and Animals

Tetrakis(triphenylphosphine)palladium (0) was purchased from Sigma-Aldrich. Pyridinium p-toluenesulfonate was purchased from J&K Chemical Ltd. (Shanghai, China). Dextran and doxorubicin hydrochloride were purchased from Sangon Biotech Co. Ltd. (Shanghai, China), and doxorubicin was obtained through neutralization with triethylamine. 2,7-Bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9,9-bis[3'-(dimethylamino) propyl] fluorene was prepared by the method described elsewhere.[1] 5,7-Bis(5-bromo-2-thienyl)-2,3-dimethyl-thieno[3,4-b]pyrazine was purchased from Beijing Allmers Chemical S&T Co. Ltd. Male ICR mice were supplied by the Experimental Animal Center of Nanjing Medical University (Nanjing, China). Murine hepatic cancer cell line H22 was purchased from Shanghai Institute of Cell Biology (Shanghai, China).

All animal studies were performed in compliance with guidelines set by the Animal Care Committee at Drum Tower Hospital.

Synthesis of Poly{9,9-bis(N,N-dimethylpropan-1-amino)-2,7-fluorene-alt-5,7-bis(thiophen-2-yl)-2,3-dimethylthieno[3,4-b] pyrazine} (BTTPF)

2,7-Bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9,9-bis[3'-(dimethylamino) propyl] fluorene (100 mg), 5,7-bis(5-bromo-2-thienyl)-2,3-dimethyl-thieno[3,4-b]pyrazine (82.6 mg), and $Pd(PPh_3)_4$ (55 mg) were dissolved in a degassed mixture of toluene (60 mL) and K_2CO_3 (2 mol/L, 20 mL). The mixture was stirred at 85~90 ^oC for 48 h under argon atmosphere. After cooling down to room temperature, the mixture was extracted with chloroform, washed with brine and distilled water, and then recrystallized from acetone to afford a black solid 69 mg (yield 61.6%). ^1H NMR (300 MHz, $CDCl_3$, δ): 7.40-7.70 (m, Ar-**H**), 2.76 (s, pyrazine-C**H**$_3$), 2.07-2.15 (m, Fluorene-C**H**$_2$, NC**H**$_2$, NC**H**$_3$), 0.88 (m, $CH_2C\mathbf{H}_2CH_2$). The molecular weight of BTTPF was determined by GPC (calibrated by polystyrene standard). The weight-average molecular weight of BTTPF was 11600, and the polydispersity was 1.47.

Synthesis of m-Dextran.

M-dextran was synthesized by aldolization using pyridinium p-toluenesulfonate as a catalyst. Briefly, 1.0 g of dextran (Mn~9-11 kDa) was added to a flame-dried flask and purged with argon. Anhydrous DMSO (10 mL) was added and stirred until complete dissolution of the dextran. Pyridinium p-toluenesulfonate (PPTS, 15.6 mg, 0.062 mmol) was added to the solution followed by 2-ethoxypropene (4.16 mL, 37 mmol). The flask was purged with argon and then sealed with parafilm to prevent evaporation of 2-ethoxypropene. The reaction was stirred at room temperature for 1 h, and then was quenched with triethylamine (1 mL, 7 mmol). The mixture was precipitated and washed three times in basic water (pH ~8) to prevent undesired degradation and collected by centrifugation (6000 rpm, 15min). Residual water was moved by lyophilization, yielding m-dextran (1.43 g) as a white solid. ^1H NMR (300 MHz, DMSO-d_6, δ): 4.88 (br, dextran C_1-**H**), 3.55-3.85 (br, dextran C_2-**H** ~ C_6-**H**), 3.40 (m, OC**H**$_2$CH$_3$), 1.30 (m, C(C**H**$_3$)$_2$), 1.10 (m, OCH$_2$C**H**$_3$).

Preparation of DOX/BTTPF Encapsulated NPs

The NPs encapsulating DOX and BTTPF were prepared via a single emulsion (oil-in-water) solvent evaporation/extraction method. Briefly, m-dextran (50 mg), DOX (0.08 mg), and BTTPF (0.08 mg) were dissolved in dichloromethane (DCM) (0.6 mL). This solution was added to 2 mL of polyvinyl alcohol (PVA) solution (3%) and sonicated for 45 cycles (1 s each with a duty cycle of 60%). The resulting emulsion was poured into 10 mL of the second PVA solution (0.3%) and stirred at room temperature for 2 h allowing the organic solvent to evaporate. The NPs were collected by centrifugation (13000 rpm, 15 min), and the supernatant was conserved for calculating the encapsulation efficiency (EE) of the NPs. The collected NPs were washed with distilled water three times, and then dried by lyophilization and stored at 4 ^{o}C.

The concentration of DOX and BTTPF in supernatant conversed was determined by measurement of UV-Vis absorption at 480 and 570 nm compared to the standard curves using a MAPADA UV-1800 UV-Vis spectrophotometer, and then the mass of DOX and BTTPF encapsulated was calculated.

NPs Size Analysis by Dynamic Light Scattering

NPs size distributions and average particle diameters were determined by dynamic light scattering using a Malvern Mastersizer 2000 particle size analyzer. The NPs were suspended in basic water (pH ~8), and three measurements were taken of the resulting solution.

Scanning Electron Microscopy

The NPs were characterized by scanning electron microscopy using a Hitachi S-4800 FE-SEM. The NPs were suspended in basic water (pH ~8), and the resulting solution was dripped onto silicon wafers. The sample was allowed to air dry for 1 d. The NPs were then sputter coated with a 10 nm layer of chrome and imaged.

Fluorescence Spectra and Fluorescence Lifetime

Fluorescence spectra of all samples were obtained using a steady-state spectrofluorometer (HORIBA JOBIN YVON FM-4NIR). The decay curves of DOX in DCM and before or after degradation of the NPs were measured using a time-resolved spectrofluorometer (HORIBA JOBIN YVON TemPro-01) with a 452 nm pulsed excitation source. The fluorescence lifetimes were then determined by fitting with a secondary exponential as a function of time.

NPs Degradation

The NPs were suspended in either a 0.2 M PBS (pH 7.4) or 0.2 M acetate buffer (pH 5.0), and incubated at 37 ^{o}C. At set time points, the fluorescence spectra of the samples were measured using a steady-state spectrofluorometer (HORIBA JOBIN YVON FM-4NIR).

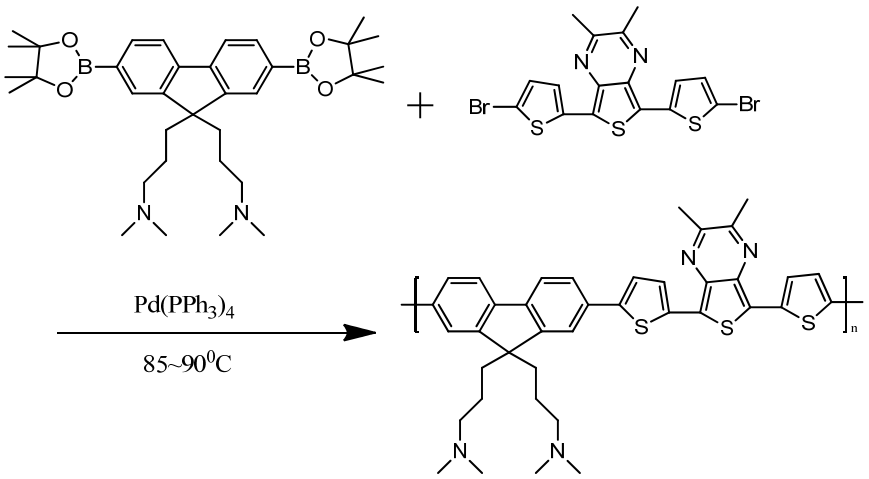
***In vitro* Drug Release**

In vitro release profiles of DOX from the NPs were investigated in 0.2 M acetate buffer (pH 5.0). The NPs were dispersed in 0.2 M acetate buffer (pH 5.0), and transferred to a dialysis membrane bag (MWCO=1000 Da). The release experiment was initiated by placing the dialysis bag in 150 mL of acetate buffer (pH 5.0). The release medium was stirred at 37 ^{o}C. At set time points, the solution in the dialysis bag were taken out and analyzed for the fluorescence intensity of BTTPF at 690 nm using excitation wavelength of 480 nm, and after analysis the solution were put back. Meanwhile, 3 mL of the releasing medium were taken out, and the concentration of released DOX in the release medium was determined by measurement of UV-Vis absorption at 480 nm compared to the standard curve.

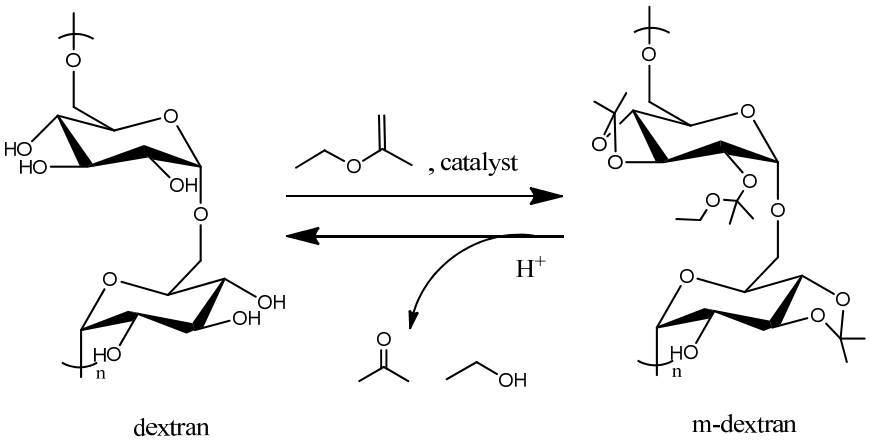
***In vivo* Fluorescence Imaging**

The mouse was injected with 0.4 mL of NPs (25mg/mL) solution via a tail vein. The real-time NIR fluorescence imaging was performed using a Maestro^{TM} EX fluorescence imaging system (Cambridge Research & Instrumentation, Cri, USA). The light with a central wavelength of 595 nm was selected as excitation source, and *in vivo* fluorescence imaging was conducted from 680 to 780 nm (with 10 nm step). The mouse autofluorescence was removed using spectral unmixing software.

To build a subcutaneous tumor model, H22 tumor cells (5-6 × 10^6 cells for per mouse) were inoculated subcutaneously to mice (6-8 weeks, 20-30 g) at the left axilla. The H22 tumor bearing mouse was injected subcutaneously at the tumor site with 0.1 mL of NPs (25mg/mL) solution. At set time points, the mouse was observed using the Maestro^{TM} EX fluorescence imaging system. The light with a central wavelength of 455 nm was selected as excitation source, and *in vivo* fluorescence imaging was conducted from 680 to 780 nm (with 10 nm step). Thereafter, tumor bearing mouse was sacrificed at 16 d post-injection. Tumor, heart, brain, liver, spleen, kidneys, stomach, intestines, and lungs were harvested, and isolated organ NIR fluorescence from 680 to 780 nm was collected to estimate the tissue distribution (ex: 455 or 595 nm).



Scheme S1. Synthetic route to NIR fluorescence probe BTTPF.



Scheme S2. Synthesis route and acid-degradation of m-dextran.

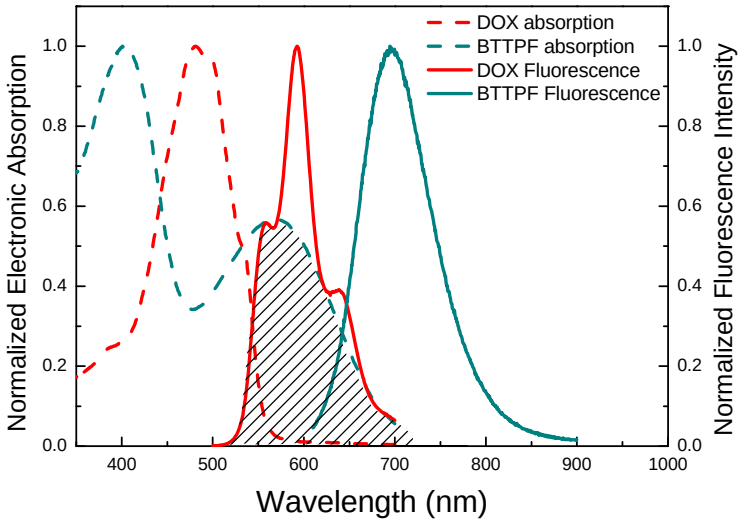


Figure S1. Normalized electronic absorption and fluorescence emission spectra of DOX and BTTPF in DCM.

Table S1. Fluorescence lifetime of DOX.

Fluorescence lifetime	τ_1	τ_2
Free DOX	1.34 ns (85.9%)	2.56 ns (14.1%)
DOX in integrate NPs	0.09 ns (86.8%)	1.68 ns (13.2%)
DOX after degradation of the NPs	1.03 ns (98.9%)	2.25 ns (1.1%)

References

1. H. Wang, P. Lu, B. Wang, S. Qiu, M. Liu, M. Hanif, G. Cheng, S. Liu and Y. Ma, *Macromol Rapid Commun*, 2007, **28**, 1645-1650.