Electronic Supplementary Information

A thieno-isoindigo derivative-based conjugated polymer nanoparticle for photothermal therapy at NIR-II bio-window

Zuwu Wei, # [a,b,c] Fangqing Xue, # [d] Fuli Xin, [e] Ming Wu, [b,c] Bingxi Wang, [a] Xiaolong Zhang, [b, c] Sen Yang, [b,c] Zhiyong Guo, [a] Xiaolong Liu [b,c] **

a. College of Materials Science and Engineering, Fuzhou University, Fuzhou 350108, P. R. China
b. The United Innovation of Mengchao Hepatobiliary Technology Key Laboratory of Fujian Province, Mengchao Hepatobiliary Hospital of Fujian Medical University, Fuzhou 350025, P.R. China
c. Mengchao Med-X Center, Fuzhou University, Fuzhou 350116, P. R. China
d. Shengli Clinical Medical College of Fujian Medical University, Fuzhou 350001, P. R. China
e. Liver Disease Center, the First Affiliated Hospital of Fujian Medical University, Fuzhou 350005, P.R. China
# These authors contributed equally to this work.

Experiments

Materials

Methanol (CH_3OH), methylbenzene, tetrahydrofuran (THF), N,N-dimethylformamide (DMF), trimethylamine (TEA) and 1-hexadecanol were provided by Sinopharm Chemical Reagent Co., Ltd (China). 4,7-dibromobenzo[1,2-c:4,5-c']bis([1,2,5]thiadiazole) was obtained from Wuxi Senior Material Co. Ltd. 3-(2-octyl-dodecyl)-thiophene was obtained from Derthon OPV Co Ltd. Tris(dibenzylideneacetone)dipalladium ($Pd_2(DBA)_3$) and Tri(o-tolyl)phosphine ($P(tolyl)_3$) were provided by Sigma Aldrich Co., Ltd. 4',6- Counting Kit-8 (CCK-8), Diamidino-2-Phenylindole (DAPI) Cell and Annexin V, FITC Apoptosis Detection Kit were purchased from Dojindo Molecular Technologies. LIVE/DEAD® Viability/Cytotoxicity Kit was bought from Invitrogen. All the reactions were carried out under argon atmosphere.

Cell Culture

The human Cervical cancer cells (Hela) were cultured with RPMI-1640 (Gibco) containing 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin–streptomycin) (Corning) in a humidified environment with 5% CO_2 and 95% air at 37 °C.

Synthesis of conjugated polymer BTPBFDTS

(3Z,7Z)-3,7-bis(2-bromo-4-(2-decyltetradecyl)-5-oxo-4,5-dihydro-6H-thieno[3,2-b]pyrrol-6-ylidene)-3,7-dihydrobenzo[1,2-b:4,5-b']difuran-2,6-dione (129 mg), 4,4'-Bis(octyl)-5,5'-bis(trimethyltin)-dithieno[3,2-b:2',3'-d]silole (75 mg), $Pd_2(DBA)_3$ (7.5 mg), and $P(tolyl)_3$ (30 mg) were dissolved in 8 mL toluene and then refluxed for 48 h. When cooling down to 25˚C, the mixture was added in the 50 mL cold methanol under vigorous stirring, and the precipitate was enriched through filtration, then purified by Sohxlet extraction using CH_3OH, CH_2Cl_2, sequentially. The CH_2Cl_2 fraction was concentrated and reprecipitated with CH_3OH. The dark green solid was collected and dried under vacuum.

Preparation of polymer nanoparticles ($NPs_{BTPBFDTS}$)

The $NPs_{BTPBFDTS}$ nanoparticles were prepared by microemulsion method. 1 mg of conjugated polymer BTPBFDTS, 20 mg PEG-PCL and 5 mg DOTAP were completely dissolved in 2 mL CH_2Cl_2. Subsequently, the solution was rapidly injected into 10 mL PVA aqueous solution (10 mg·mL^{-1}) to obtain the primary W/O emulsion after being sonicated in an ice bath for 3 min using an ultrasonic cell disruptor (Scientz-IID, Ningbo, China). Then, the system was further vibrated under R.T for 12 h to evaporate the CH_2Cl_2, and then ultra-filtered again at a speed of 4000 rpm for 0.5 h to remove free PEG-PCL, PVA and DOTAP to obtain the $NPs_{BTPBFDTS}$ NPs.

Preparation of $NPs_{BTPBFDTS}$@HA nanoparticles

$NPs_{BTPBFDTS}$@HA NPs were obtained by an extrusion method. First, sodium hyaluronate (1 mL, 1 mg·mL) and $NPs_{BTPBFDTS}$ (1 mL, 10 mg·mL^{-1}) were mixed. Then, the mixture was extruded using an Avanti mini-syringe (Avanti, USA) through 200 nm and 100 nm polycarbonate porous membranes 11 times to obtain $NPs_{BTPBFDTS}$@HA NPs.

Characterization

HNMR spectra were detected by Bruker Ultrashield 400 Plus NMR spectrometer. UV-Vis-NIR spectra was ascertained by Cary 5000 UV-Vis-NIR spectrophotometer.

Dynamic light scattering (DLS) was performed by Zetasizer Nano ZS. Transmission electron microscopy image was obtained through transmission electron microscopy with an accelerating voltage at 200 kV. Laser-confocal scanning imaging of Hela cells was measured by confocal microscope Zeiss LSM780. The photoacoustic properties of $NPs_{BTPBFDTS}$@HA NPs were evaluated by a home-made multi-spectral photoacoustic microscopy system.[1, 2]

Photothermal effect and photostability of $NPs_{BTPBFDTS}$@HA NPs under 1064 nm laser irradiation

The aqueous dispersion of $NPs_{BTPBFDTS}$@HA NPs (1 mL) with different concentrations (0, 6.25, 12.5, 25, 50, 75 and 100 $\mu g \cdot mL^{-1}$) in cuvette was irradiated by a 1064 nm laser (1 $W \cdot cm^{-2}$) for 10 min. The changes of temperature were measured by a thermocouple microprobe (ϕ = 0.5 mm). Then, the photostability of $NPs_{BTPBFDTS}$@HA NPs was studied under the photothermal heating and nature cooling cycles by the 1064 nm laser irradiation at the power density of 1.0 $W \cdot cm^{-2}$. The photothermal conversion efficiency was evaluated and calculated using the reported method.[3]

Photoacoustic properties of $NPs_{BTPBFDTS}$@HA NPs under 1064 nm laser irradiation

The photoacoustic properties of $NPs_{BTPBFDTS}$@HA NPs were evaluated by a home-made multi-spectral photoacoustic microscopy system. The main experimental process was performed and calculated using the reported method.[1]

Cellular internalization analysis

To survey the intracellular uptake efficiency of $NPs_{BTPBFDTS}$@HA nanoparticles, the Hela cells were seeded in the laser-confocal scanning imaging dishes and allowed to adher for 12 h. Then, the experiments were stochastically divided into following four groups: (1) Control, (2) $NPs_{BTPBFDTS}$@HA, (3) $NPs_{BTPBFDTS}$ and (4) $NPs_{BTPBFDTS}$@HA with HA pre-treated Hela cells. After 3 or 6 hours, the Hela cells were washed with fresh PBS for three times, and then fixed by 4% formaldehyde for 10 min. Afterwards, the cell nuclei were stained by DAPI (5 μM) for 10 min. Finally, the cells were imaged by confocal laser scanning microscopy.

***In Vitro* PTT Therapy**

$NPs_{BTPBFDTS}$@HA NPs in PBS were diluted to different concentrations using

RPMI-1640 medium. Then, the Hela cells were seeded in 96-well plates and divided into four groups: (1) $NPs_{BTPBFDTS}$@HA, (2) $NPs_{BTPBFDTS}$@HA+1064 nm, (3) $NPs_{BTPBFDTS}$+1064 nm and (4) $NPs_{BTPBFDTS}$@HA+Hela pre-treated by HA+1064 nm. After 6 hours, the cell culture medium was replaced by 30 μL fresh RPMI-1640 medium. Then, the group (1) was maintained in darkness, while other groups were irradiated for 5 min using 1064 nm laser with 0.8 $W \cdot cm^{-2}$ power. Afterwards, the cells were further cultured at 37 ºC with 5% CO_2 and 95% air for 24 h. Finally, the fresh medium was replaced by 100 μL 10% CCK-8 medium and then further incubated the cells at 37 °C for 2 h. After that, the absorbance (450 nm) was measured using plate reader of Molecular Devices. The cell viability was evaluated using the reported method.[4]

Live/Dead Cell Staining Assay

Hela cells were seeded in 96-well plates (5×10^4 cells per well) and divided into six groups: (1) Control, (2) $NPs_{BTPBFDTS}$@HA NPs, (3) 1064 nm laser only, (4) $NPs_{BTPBFDTS}$ +1064 nm; (5) $NPs_{BTPBFDTS}$+Hela pre-treated by free HA+1064 nm and (6) $NPs_{BTPBFDTS}$ +1064 nm. After 1064 nm irradiation, the cells were incubated at 37 ºC with 5% CO_2 and 95% air for 24 hours. Finally, the fresh RPMI-1640 medium containing LIVE/DEAD® Viability/Cytotoxicity Kit was added into plates and further incubated for 15 min. Eventually, the cells were detected and analyzed through fluorescence microscope.

Apoptosis Evaluation by Flow Cytometry

Hela cells were cultured in 6-well plates (3×10^5 cells per well). After 24 h culture, the cells were divided into six groups: (1) Control, (2) $NPs_{BTPBFDTS}$@HA, (3) 1064 nm, (4) $NPs_{BTPBFDTS}$ +1064 nm; (5) $NPs_{BTPBFDTS}$@HA +Hela pre-treated by HA+1064 nm and (6) $NPs_{BTPBFDTS}$@HA +1064 nm. After 1064 nm irradiation, the cells were further cultured for 24 hours in cell incubator. Afterwards, all the cells were washed by fresh PBS (3 times), digested and then collected by centrifugation. After being further washed with PBS (3 times), the cells were dispersed in 500 μL of annexin binding buffer (1X). After that, all cells were stained by PI or Annexin-V, FITC about 20 min and finally analyzed by flow cytometry.[5]

Live Subject Statement

All animal experiments were performed strictly under the guidelines of the National animal management regulations of China and the animal study guidelines of Fujian Medical University, as well as approved by the Animal Ethics Committee of Mengchao Hepatobiliary Hospital of Fujian Medical University.

***In Vivo* PTT Therapy**

The balb/c nude mice bearing Hela tumors were stochastically divided into 5 groups with five mice per group as follows: (1) Control (mice were only intratumorally injected with saline), (2) $NPs_{BTPBFDTS}$@HA NPs, (3) Saline+1064 nm laser, (4) $NPs_{BTPBFDTS}$ + 1064 nm laser and (5) $NPs_{BTPBFDTS}$@HA+1064 nm laser. Mice bearing Hela tumors were intravenously injected with 100 μL (4.0 mg/mL) of nanoparticles, and mice treated with the same volume of saline were used as control. After 12 h of NPs injection, the mice were irradiated 10 min by 1064 nm laser with 1 W cm^{-2} power. Meanwhile, the temperature changes at the tumor sites in group 3-5 were performed using a thermal camera during the irradiation. The body weight and the tumor volume of the mice were detected every 2 days. In addition, the hematoxylin and eosin (H&E) and the immunofluorescence staining (Ki-67) were performed to investigate the tissue destruction and cell apoptosis after therapy.

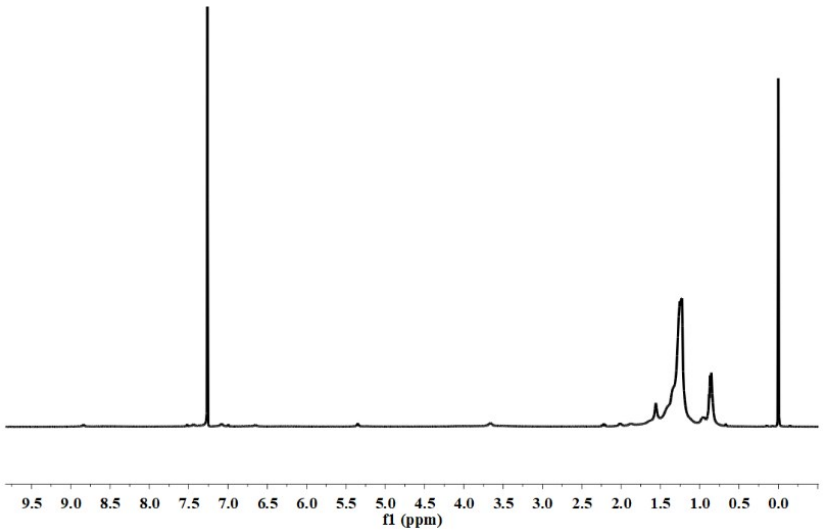


Fig. S1 ^{1}H NMR spectrum of conjugated polymer BTPBFDTS.

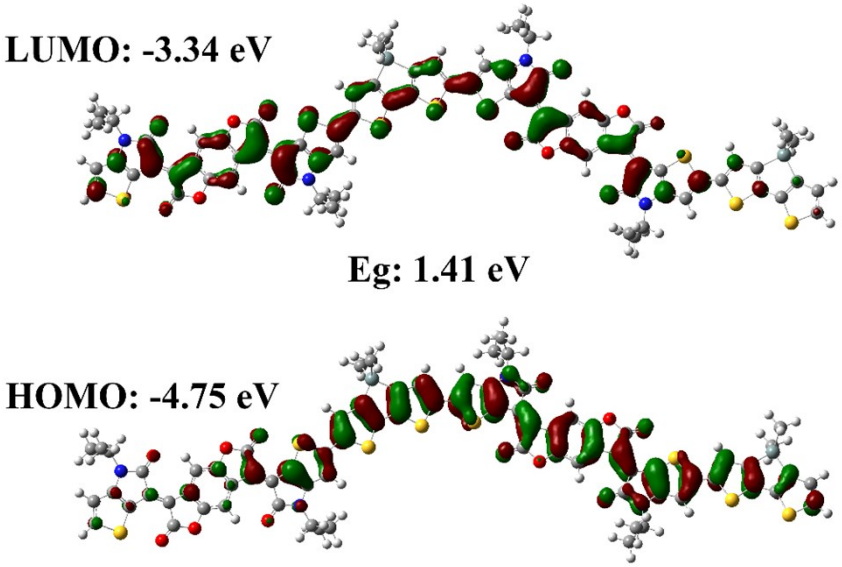


Fig. S2 Density functional theory (DFT) calculation of the HOMO and LUMO of BTPBFDTS dimer. DFT calculations were performed on the dimer to get insight on the frontier orbital distribution and level of the polymer. All the calculations were performed using Gaussian09 program. Eg = E_{LUMO}−E_{HOMO}.

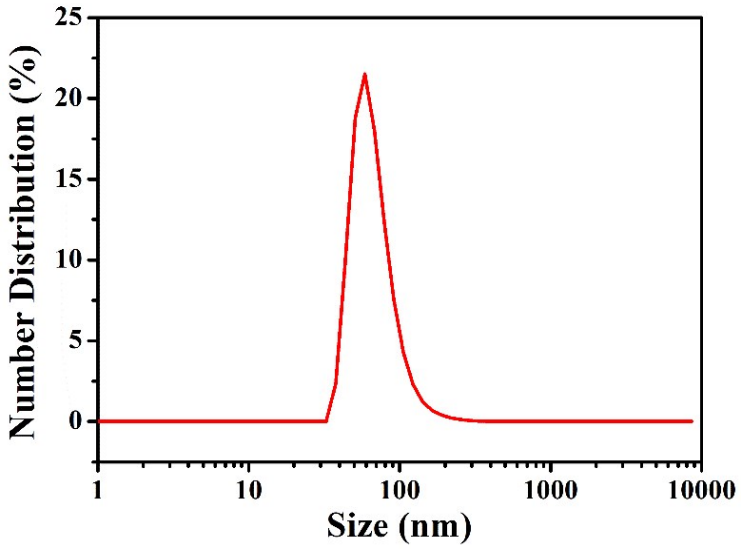


Fig. S3 Size distributions of $NPs_{BTPBFDTS}$@HA nanoparticles in PBS measured by DLS.

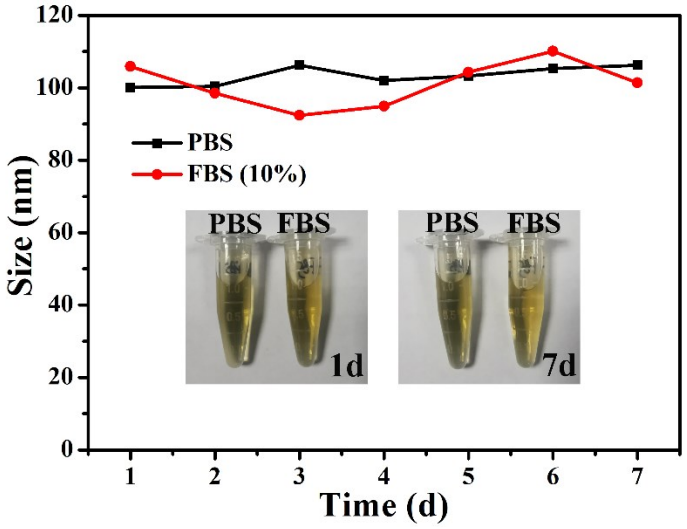
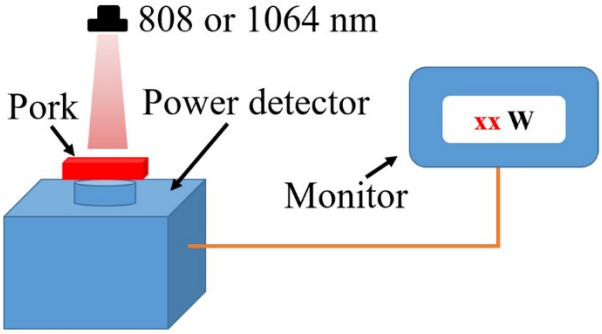


Fig. S4 Size distributions of $NPs_{BTPBFDTS}$@HA nanoparticles in PBS or FBS (10%) measured by DLS.



wavelength	808 nm	1064 nm
Before	1 W	1 W
After	0.238 W	0.524 W

Fig. S5 Representative procedure to schematically show the experimental design of the light attenuation assay.

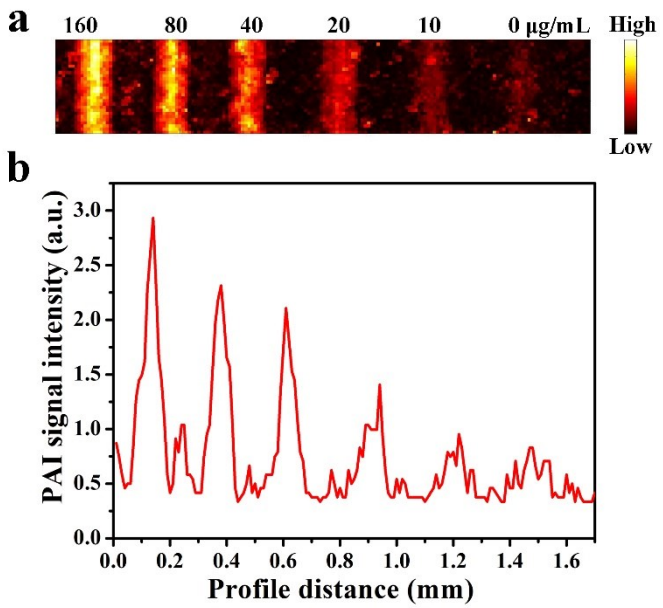


Fig. S6 a) PA images of $NPs_{BTPBFDTS}$@HA NPs in PBS with different concentrations. PBS was applied as a control. b) Intensity profiles from (a).

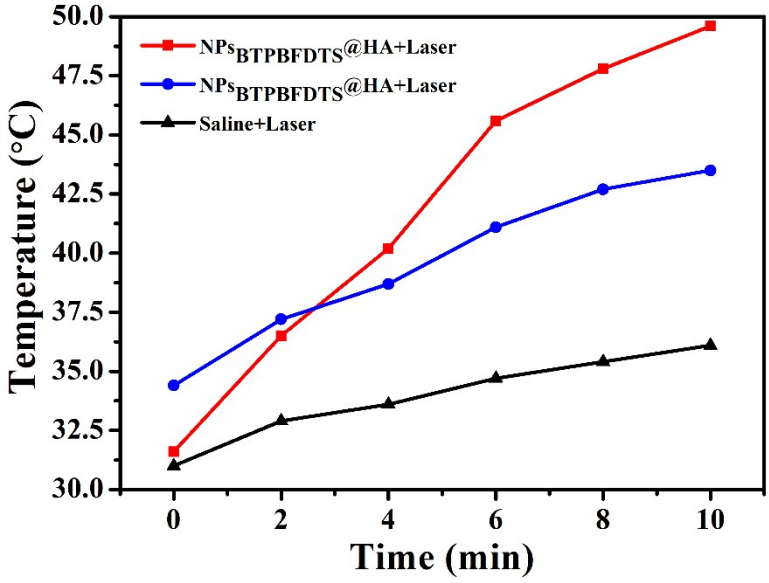


Fig. S7 Temperature elevation curve at the tumor site upon laser irradiation during the indicated treatment.

References

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