Electronic Supplementary Material (ESI) for ChemComm.

Supplementary information for

Supramolecular core-glycoshell polythiophene nanodots for targeted imaging and photodynamic therapy

Hai-Hao Han,[ab] Chang-Zheng Wang,[a] Yi Zang,[b] Jia Li,[b]* Tony D James[c] and Xiao-Peng He[a]*

[a]*Key Laboratory for Advanced Materials & Institute of Fine Chemicals, East China University of Science and Technology, 130 Meilong Rd., Shanghai 200237, PR China, E-mail: xphe@ecust.edu.cn (X.-P. He)*

[b]*National Center for Drug Screening, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 189 Guo Shoujing Rd., Shanghai 201203, PR China, E-mail: jli@simm.ac.cn (J. Li)*

[c]*Department of Chemistry, University of Bath, Bath, BA2 7AY, UK*

Content list:

S1. Additional figures

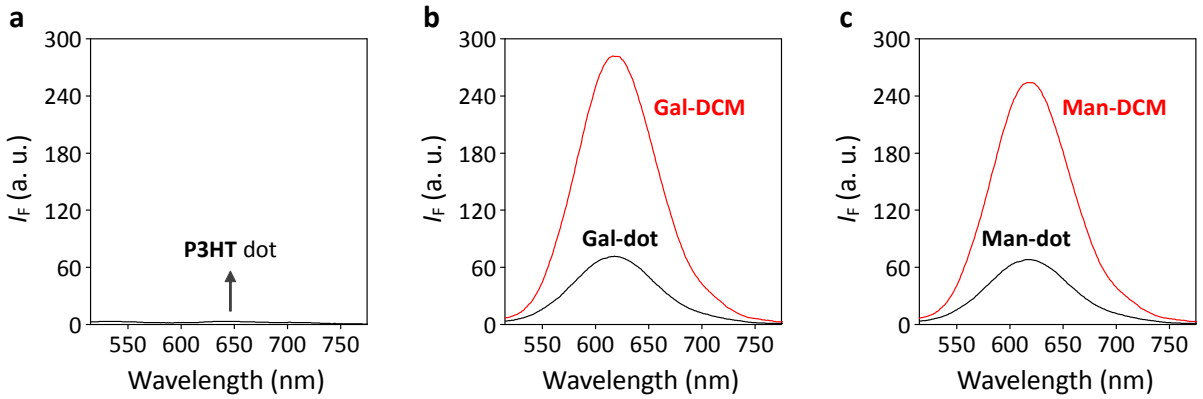

Fig. S1. Fluorescence spectra of (a) **P3HT** dot (16 ppm), (b) **Gal-DCM** (5 μM) and **Gal-dot** (**Gal-DCM**/**P3HT** = 16 ppm/5 μM) and (c) **Man-DCM** (5 μM) and **Man-dot** (**Man-DCM**/**P3HT** = 16 ppm/5 μM) in Tris-HCl (0.01 M, pH 7.4). The fluorescence was measured on a Varian Cary Eclipse fluorescence spectrophotometer with an excitation wavelength of 480 nm.

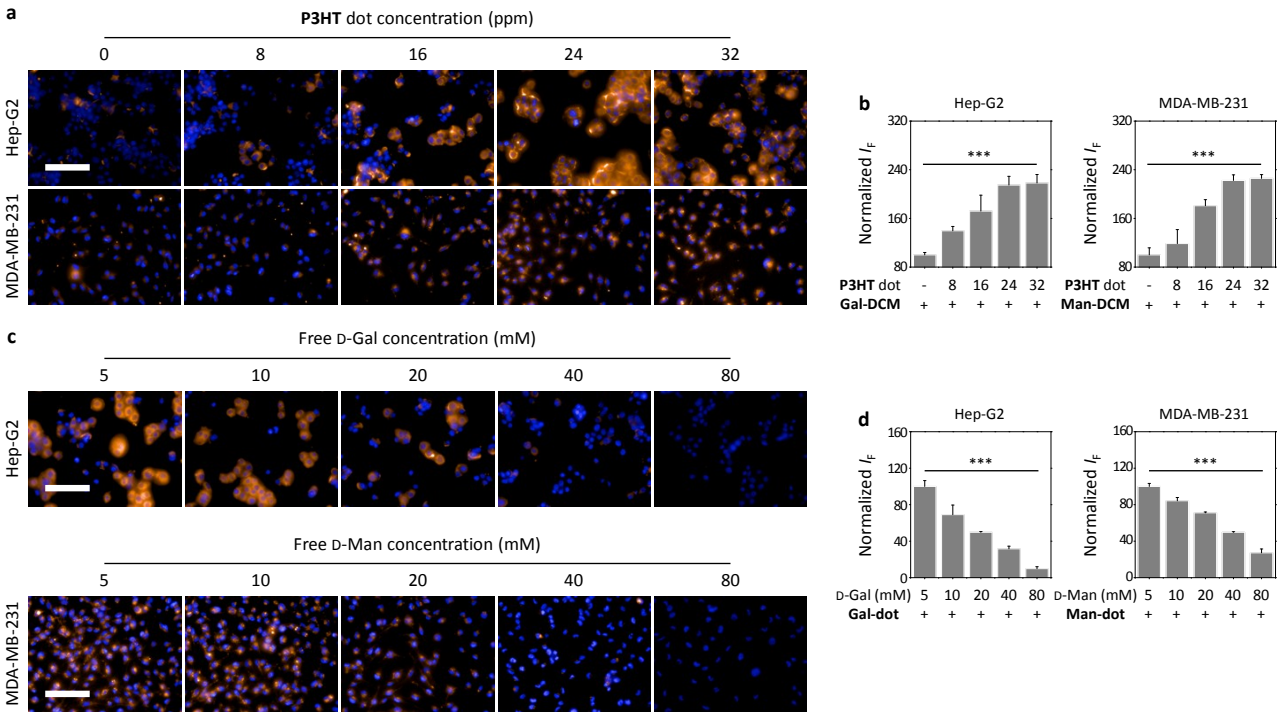

Fig. S2. Fluorescence imaging (a) and quantification (b) of Hep-G2 and MDA-MB-231 cells with **Gal-DCM** (5 μM) and **Man-DCM** (5 μM), respectively, in the presence of increasing **P3HT** nanodot (0-32 ppm). Fluorescence imaging (c) and quantification (d) of Hep-G2 and MDA-MB-231 cells with **Gal-dot** (**Gal-DCM**/**P3HT** nanodots = 5 μM/16 ppm) and **Man-dot** (**Man-DCM**/**P3HT** nanodots = 5 μM/16 ppm) pretreated with increasing D-galactose and D-mannose, respectively. For fluorescence imaging, the excitation and emission wavelengths for DCM are 460-490 nm and 560-630 nm, respectively (scale bar = 100 μm; ****P*<0.005). Cell nuclei were stained by Hoechst 33342.

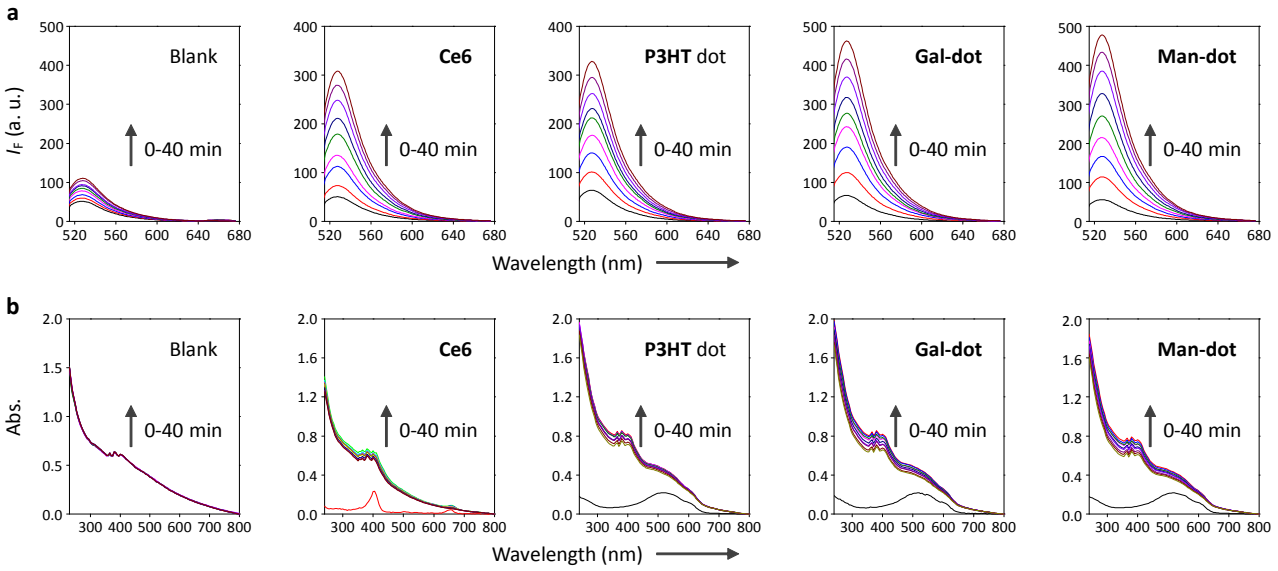

Fig. S3. (a) Fluorescence titration of rhodamine 123 (1.5 μM, a known fluorogenic probe for reactive oxygen species) in the absence (blank) and presence of **Ce6** (1 μM, a commercially available photosensitizer), **P3HT** dot (8 ppm), **Gal-dot** (**P3HT** dot/**Gal-DCM** = 8 ppm/1μM) and **Man-dot** (**P3HT** dot/**Man-DCM** = 8 ppm/1 μM) with increasing light irradiation time (a broadband light of 40 mW cm^{-2}) in (Tris-HCl buffer (0.01 M, pH 7.4). The fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer with an excitation wavelength of 485 nm. (b) UV-vis absorbance titration of 9,10-diphenylanthracene (**DPA**) (20 μM, a widely used $^1O_2$ trapper) in the absence (blank) and presence of **Ce6** (1 μM; the bottom curve is the absorbance of **Ce6** alone), **P3HT** dot (8 ppm; the bottom curve is the absorbance of **P3HT** dot alone), **Gal-dot** (**P3HT** dot/**Gal-DCM** = 8 ppm/1μM; 8 ppm; the bottom curve is the absorbance of **Gal-dot** alone) and **Man-dot** (**P3HT** dot/**Man-DCM** = 8 ppm/1 μM; the bottom curve is the absorbance of **Man-dot** alone) with increasing light irradiation time (a broadband light of 40 mW cm^{-2}) in (Tris-HCl buffer (0.01 M, pH 7.4).

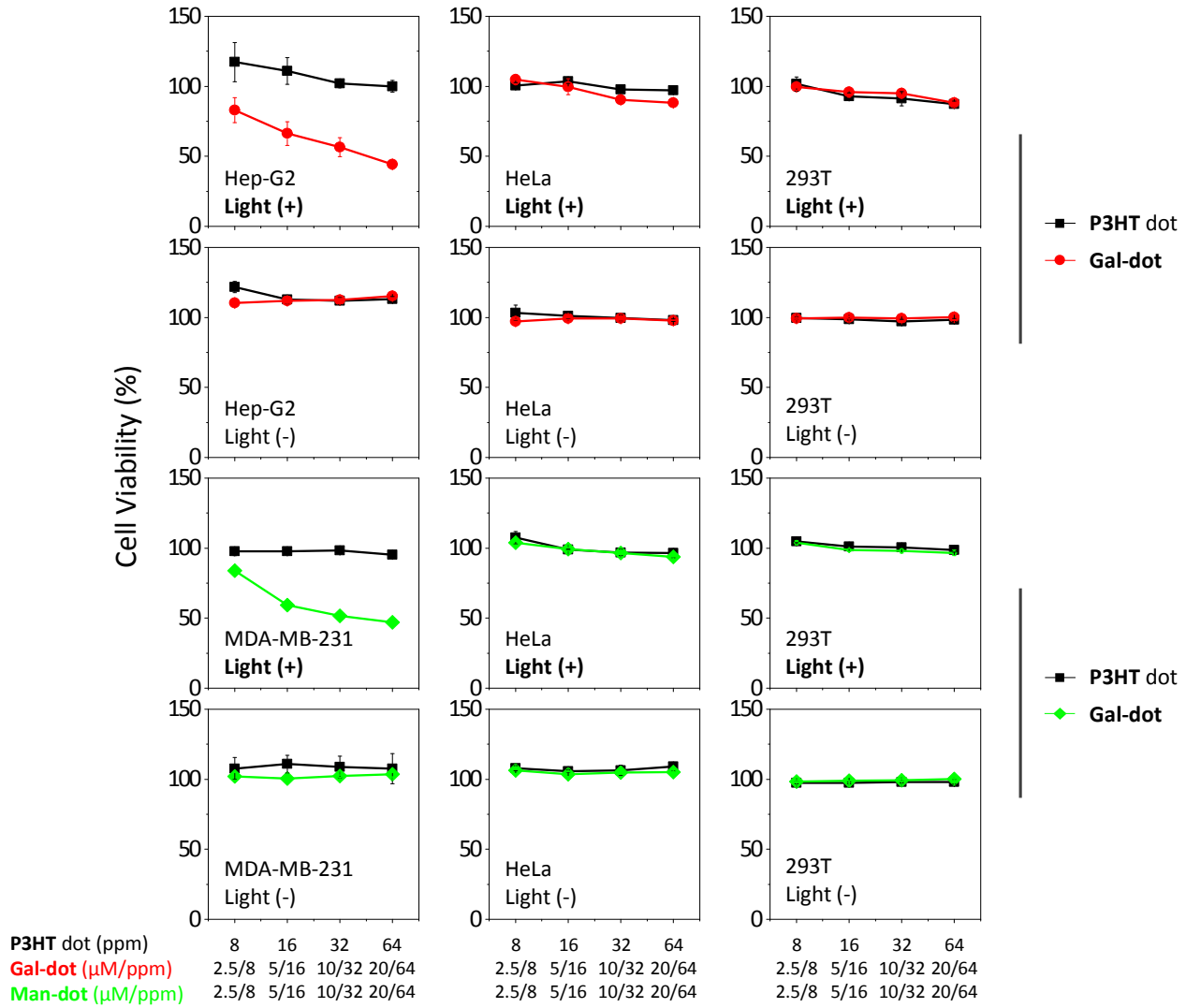

Fig. S4. Concentration-dependent cytotoxicity of **P3HT dot**, **Gal-dot** and **Man-dot** with different cells including Hep-G2 (human liver cancer), MDA-MB-231 (human triple-negative breast cancer), HeLa (human cervical cancer) and 293T (human embryonic kidney) with (light (+)) or without (light (−)) broadband light irradiation.

S2. Experimental section

General remarks. All purchased chemicals and reagents are of analytical grade. Poly(3-hexylthiophene-2,5-diyl) (**P3HT**) was purchased from TCI Chemicals. Poly(styrene-*co*-maleic anhydride) (**PSMA**) and tetrahydrofuran (THF, anhydrous, 99.9%) were purchased from Sigma-Aldrich. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM 400 MHz spectrometer with tetramethylsilane (TMS) as an internal reference. Fluorescence spectra were recorded by a fluorescence spectrometer (Varian Cary Eclipse). Absorption spectra were measured on a Varian Cary 500 UV-Vis spectraphotometer. High resolution mass spectra (HRMS) were recorded with a Waters Micromass LCT mass spectrometer. The morphology of the nanodots was measured by transmission electron microscopy (TEM, Hitachi H-600). Dynamic light scattering (DLS) was carried out on a Horiba LB-550 Dynamic Light Scattering Nano-Analyzer. High Performance Liquid Chromatography (HPLC) was carried out on a Shimadzu Prominence Series equipment.

Preparation of P3HT nanodots. P3HT was dissolved in THF at 50 °C and diluted to a 0.2 mg mL^{-1} stock solution. **PSMA** was dissolved in THF and diluted to a 1 mg mL^{-1} stock solution. To prepare the nanodots, the **P3HT** solution was mixed with 0.1 mL of **PSMA** solution in THF (2.9 mL), and the resulting mixture was quickly injected to a deionized water (25 mL) with sonication in an ultrasonic bath, followed by an additional sonication for 2 min. Then, THF was removed by nitrogen bubbling, and the nanodot solution was concentrated by heating up to 100-120 °C. The resulting residue was immediately filtered through a 0.22 μm membrane and the resulting **P3HT** solution was used as is.

Preparation of glycoprobes. Gal-DCM and **Man-DCM** were synthesized according to our previously developed protocol (Ji *et al. Adv. Funct. Mater.* 2015, **25**, 3483). To test structural homogeneity and purity of the glycoprobes, HRMS and HPLC were carried out.

Gal-DCM. HRMS (ESI, *m/z*): $[M+Na]^+$ calcd for $C_{32}H_{38}N_6O_7Na^+$ 641.2700, found 641.2689. HPLC: t_R = 13.0 min of mobile phase ($MeOH/H_2O$ = 95%/5%, *v/v*, 0.2 mL min^{-1}), purity 99%.

Man-DCM. HRMS (ESI, *m/z*): $[M+Na]^+$ calcd for $C_{32}H_{38}N_6O_7Na^+$ 641.2700, found: 641.2696. HPLC: t_R = 12.8 min of mobile phase ($MeOH/H_2O$ = 95%/5%, *v/v*, 0.2 mL min^{-1}), purity >99%.

Supramolecular preparation of glyco-dots. To a glycoprobe (**Gal-DCM** or **Man-DCM**) solution in Tris-HCl buffer (0.01 M, pH 7.4) was added **P3HT** nanodot. Then, the resulting mixture was incubated for 5 min and then used as is for characterization and cellular experiments.

Cell culture. Hep-G2 and Hela cells were maintained in a Dulbecco's Modified Eagle's Medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Gibco, Gland Island, NY,

USA) and MDA-MB-231 cells were cultured in RPMI 1640 (Gibco Laborato- ties, Grand Island, NY) supplemented with 5% fetal bovine serum (Gibco, Gland Island, NY, USA) in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C and split when the cells reached 90% confluency.

Real-time quantitative PCR. Total RNA was isolated from cells and tissues using TRIzol Reagent (Invitrogen) according to the manufacturer's protocol. Complementary DNA generated using a PrimeScript® RT reagent kit (TaKaRa, Dalian, China) was analyzed by quantitative PCR using SYBR® Premix Ex TaqTM. Real-time PCR was performed using a 7300 Real-Time PCR system (Applied Biosystems, CA, USA). GAPDH was detected as the housekeeping gene. Primers for qPCR were as follows:

GAPDH forward, 5'-ATCACTGCCACCCAGAAGAC-3'
and reverse, 5'-ATGAGGTCCACCACCCTGTT-3'
ASGPR1 forward, 5'-CTGGACAATGAGGAGAGTGAC-3'
and reverse, 5'-TTGAAGCCCGTCTCGTAGTC-3'
Mannose Receptor forward, 5'-GCAGCTCTGGGAACTTGGAT-3'
and reverse, 5'-TTGCCTGGTGTCCAGTAGGA-3'

Fluorescence imaging of cells. Cells (Hep-G2: 25000 per well; HeLa: 12000 per well; MDA-MB-231: 25000 per well) were seeded on a black 96-well microplate with optically clear bottom (Greiner bio-one, Germany) overnight. The cells were incubated with glyco-dots of different concentrations for 15 min. For the competition assay, Hep-G2 and MDA-MB-231 cells were preincubated with free D-galactose and D-Mannose, respectively, for 2 h, followed by incubation with glyco-dots for 15 min. The cells nuclei were stained with Hoechst 33342 (5 μg mL^{-1}) at 37 °C in a humidified atmosphere of 5% CO_2 in air for 5 min. Then, cells were washed with PBS (phosphate buffered saline) three times. The fluorescence images were recorded using an Operetta high-content imaging system (Perkinelmer, US) with an excitation wavelength of 460-490 nm and emission wavelength of 560-630 nm, and was quantified and plotted by columbus analysis system (Perkinelmer, US).

Cell viability assay upon light treatment. Cells were plated overnight on 96-well plates at 8000 cells per well in growth medium. After seeding, cells were treated with glyco-dots of different concentrations for 15 min. Then, cells were gently washed with PBS once. Samples (n = 3) with a total volume of 100 μL in 96-well plates were irradiated by a broadband light source of broadband light (40 mW cm^{-2}) for 60 min. Then, an MTS/PMS (20:1, Promega Corp) solution (10 μL per well) was added to each well containing 100 μL of serum-free culture medium, and the microplate was shaken gently. After incubation at 37 °C under 5% CO_2 for 2 h, the mixture (80 μL) was transferred

to another 96-well plate to minimalize the optical density produced by glycoprobes. The absorbance of the mixture solutions was measured at 490 nm with 650 nm as a reference with an M5 microplate reader (Molecular Device, USA). The optical density of the result in MTS assay was directly proportional to the number of viable cells.

Double-staining cell death assay. Cells (Hep-G2: 25000 per well; MDA-MB-231: 25000 per well; HeLa: 12000 per well) were seeded on a black 96-well microplate with optically clear bottom (Greiner bio-one, Germany) overnight. The cells were incubated with glyco-dots of different concentrations for 15 min. Then, cells were gently washed with PBS once. Samples (n = 3) with a total volume of 100 µL in 96-well plates were irradiated by a broadband light source of broadband light (40 mW cm^{-2}) for 60 min. Then, the cells were stained with a mixture of Hoechst 33342 (5 µg mL^{-1}) and Sytox Green (SYTOX® Green Nucleic Acid Stain reagent, 2.5 µg mL^{-1}) at 37 °C in a humidified atmosphere of 5% CO_2 in air for 10 min. Then, cells were washed with PBS three times. The fluorescence images were recorded using an Operetta high content imaging system (Perkinelmer, US) with excitations of 360-400 nm (emission channel: 410-480 nm) and 490-510 nm (emission channel: 530-590 nm) for Hoechst 33342 and Sytox Green, respectively, and was quantified and plotted by columbus analysis system (Perkinelmer, US).