Electronic Supplementary Information

Photosensitizer Loaded Hemoglobin-Polymer Conjugate as a Nanocarrier for Enhanced Photodynamic Therapy

Xin Xu[1], Yuecheng Cui[1], Huixuan Bu[1], Jiaming Chen[1], Yang Li[2], Guping Tang[2], Li-Qun Wang[1]*

[1] MOE Key Laboratory of Macromolecular Synthesis and Functionalization, Department of Polymer Science and Engineering, Zhejiang University, Hangzhou 310027, P. R. China.

[2] Department of Chemistry, Zhejiang University, Hangzhou 310028, China

** E-mail: lqwang@zju.edu.cn*

Synthesis of macroinitiator mPEG-Br

Typically, mPEG (5g, 1mmol) was dissolved in 30ml toluene refluxing for 3h at 100℃, followed by reduced pressure distillation to remove the traces of water. Then, the obtained mPEG was added to 30ml anhydrous THF with 0.55ml (4mmol) trimethylamine and sealed by rubber stopper. BIBB (0.5ml,4mmol) in 5ml anhydrous THF was added dropwise, then the mixture was stirred at 35℃ for 24h. After the reaction, the mixture was filtered and the solvent was removed. The product was dissolved in dichloromethane and extracted with saturated $NaHCO_3$ and NaCl for several times. The organic phase was acquired and dried with anhydrous Na_2SO_4 overnight, then precipitated into cold diethyl ether and dried in vacuum. Finally, white powder was collected.

HPLC analysis

HPLC was used to determine the modified Hb. We measured pure Hb and Hb-TCPP conjugate using analytical reverse-phase HPLC with a 330 Å C-4 column (4.6×250 mm). Conjugated Hb and unconjugated Hb were separated using a gradient of 20%-60% acetonitrile in water containing 0.1% trifluoroacetic acid (TFA) as eluent solvents over 90 min at the flow rate of 1mL/min, and was measured at 220 nm.

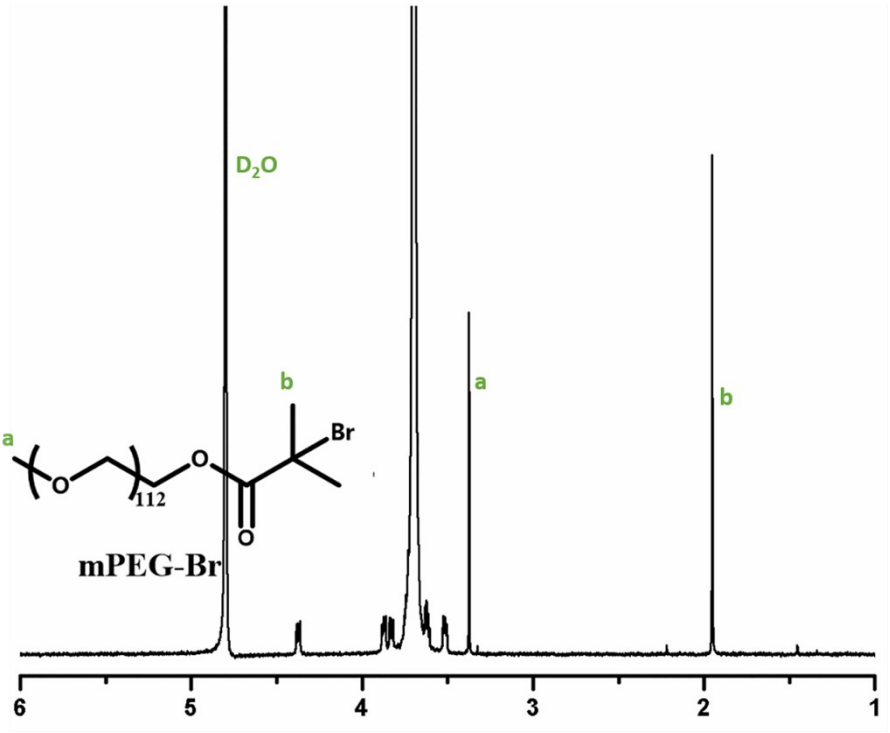

Fig. S1 ^{1}H NMR of mPEG-Br in D_2O

The conversion rate of the reaction was calculated by the areas of peak *a* and peak *b*, followed by the equation 1:

$$Convertion\ Rate\ \% = \frac{I_c}{2I_a} 100\ \%$$

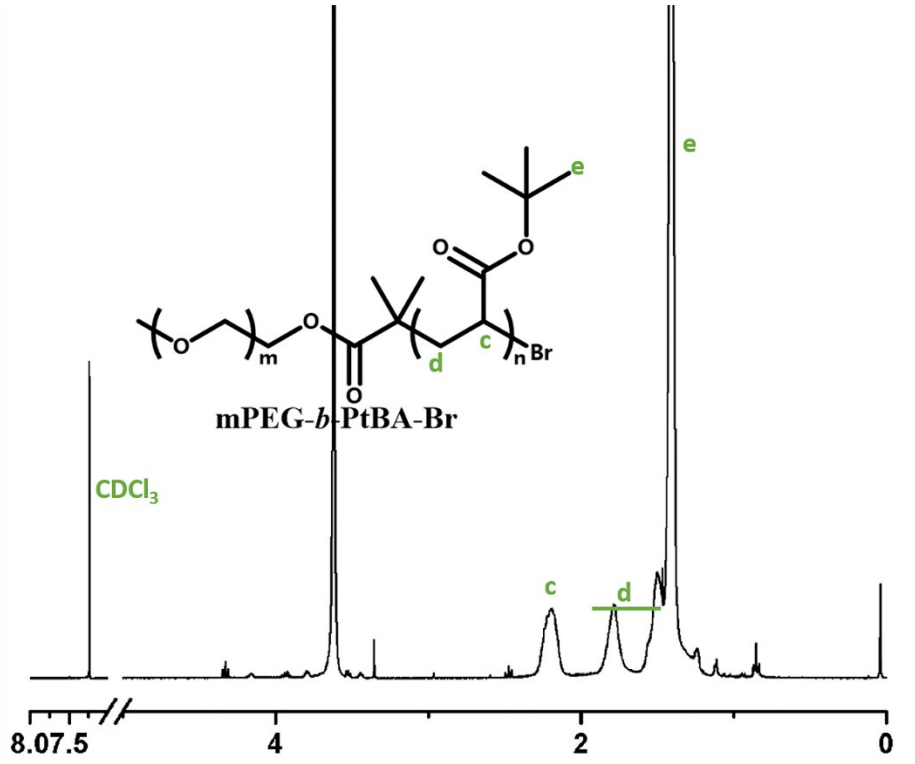

Fig. S2 ^{1}H NMR of mPEG-*b*-PtBA-Br in $CDCl_3$

The polymerization degree (n) of mPEG-*b*-PtBA-Br was calculated according to the areas of peak e and peak a, and the calculation formula is shown in Equation 2:

$$DP = \frac{I_e}{3I_a}$$

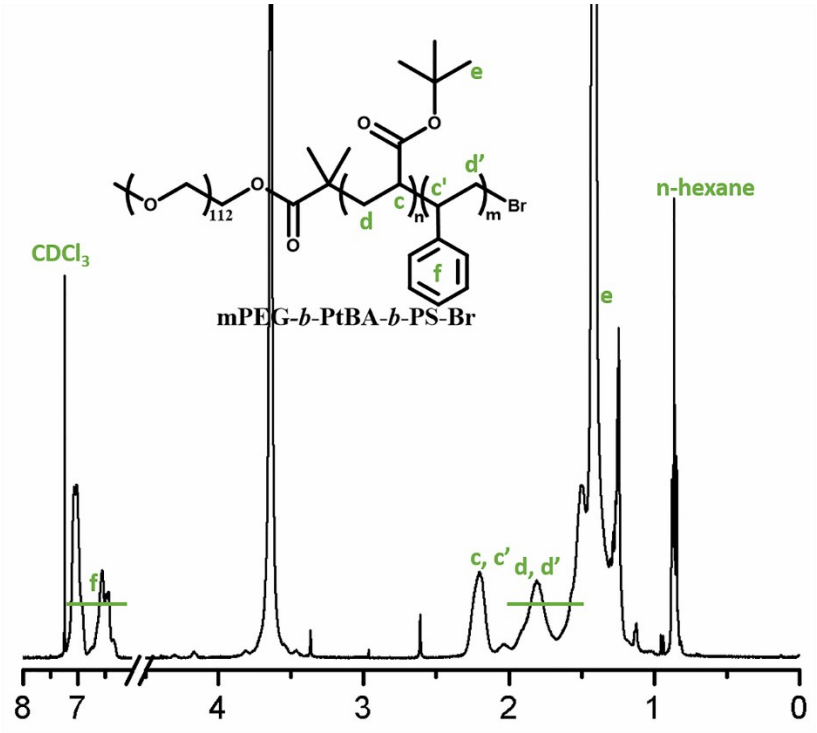

Fig. S3 ^{1}H NMR of mPEG-*b*-PtBA-*b*-PS-Br in $CDCl_3$

The polymerization degree (n) of mPEG-*b*-PtBA-*b*-PS-Br was calculated according to the areas of the peak f and peak a, and the calculation formula is shown in Equation 3:

$$DP = \frac{3I_f}{5I_a}$$

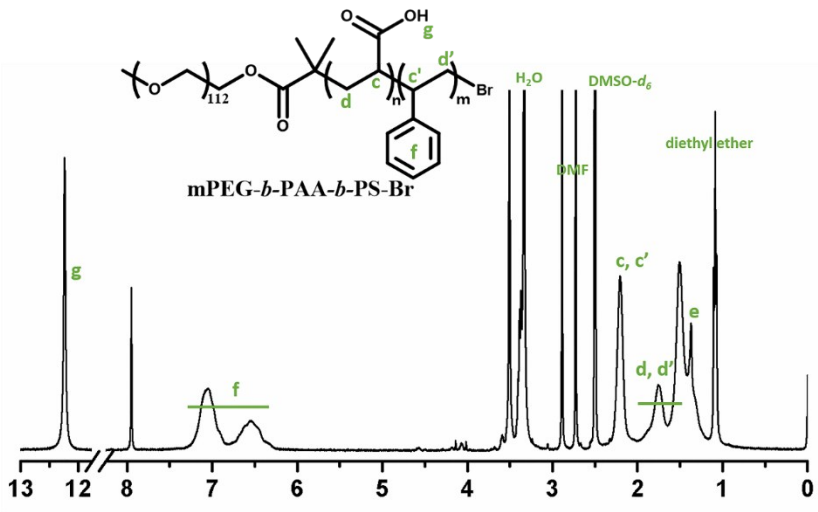

Fig. 4 ^{1}H NMR of mPEG-*b*-PAA-*b*-PS-Br in DMSO-d_6

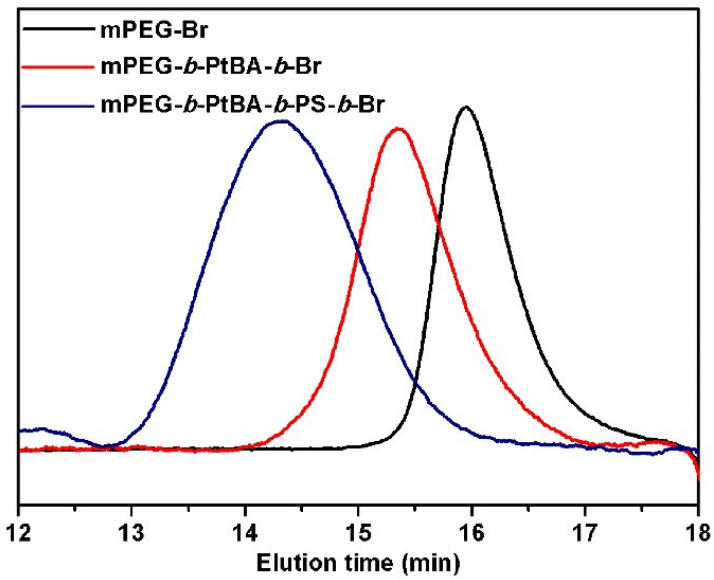

Fig.S5 GPC of mPEG-Br，mPEG-*b*-PtBA-Br，mPEG-*b*-PtBA-*b*-PS-Br using DMF as eluent

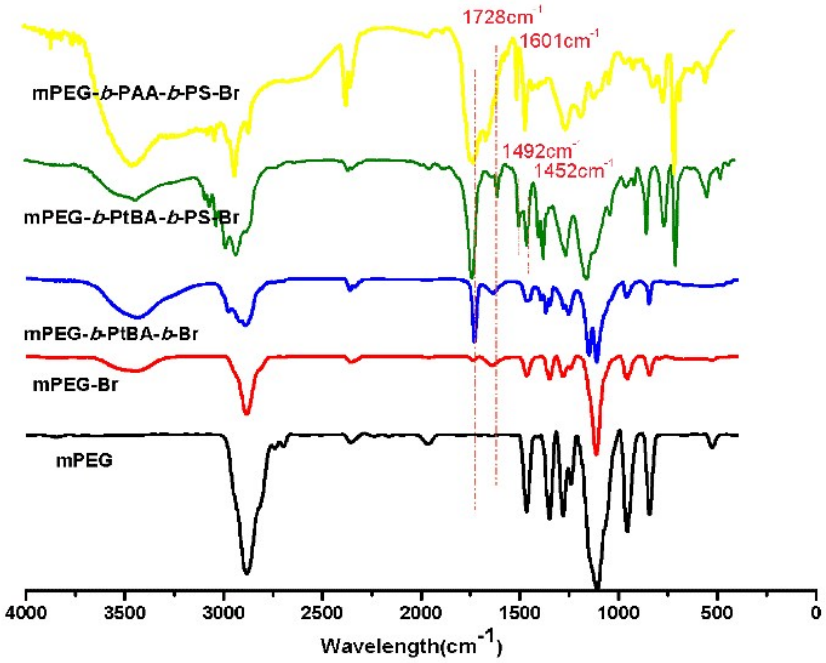

Fig. S6 FT-IR of mPEG, mPEG-Br, mPEG-*b*-PtBA-Br, mPEG-*b*-PtBA-*b*-PS-Br and mPEG-*b*-PAA-*b*-PS-Br.

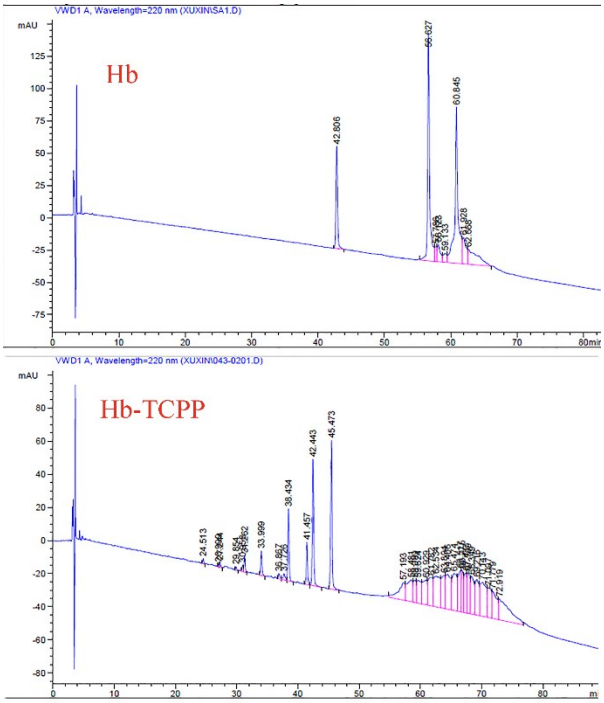

Fig. S7 C-4 reverse-phase HPLC of Hb and Hb-TCPP. Peaks are as follows: heme (42 min.); α-subunits (61 min.); β subunits (61 min.), α+n and β+n (55 min-80 min), n represents the number of TCPP.

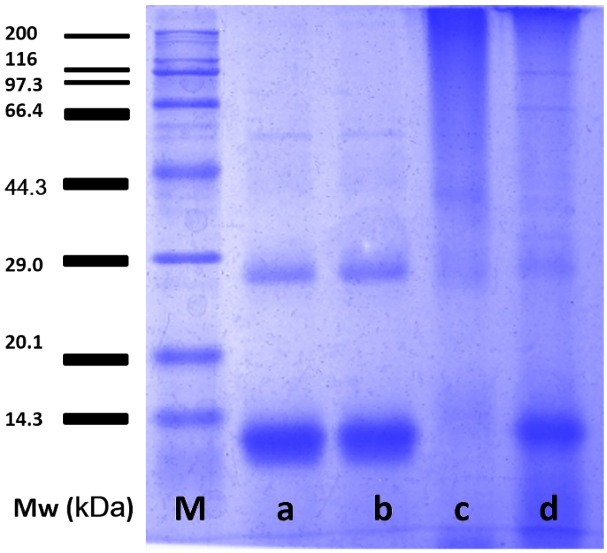

Fig. S8 SDS-PAGE of Hb (Lane a), physical blends of TCPP-Hb and mPEG-*b*-PAA-*b*-PS micelles (Lane b)、 TCPP-Hb-mPEG-*b*-PAA-*b*-PS micelles conjugates (Lane c) and TCPP-Hb-mPEG-*b*-PAA-*b*-PS micelles conjugates with Hb residual (Lane d)

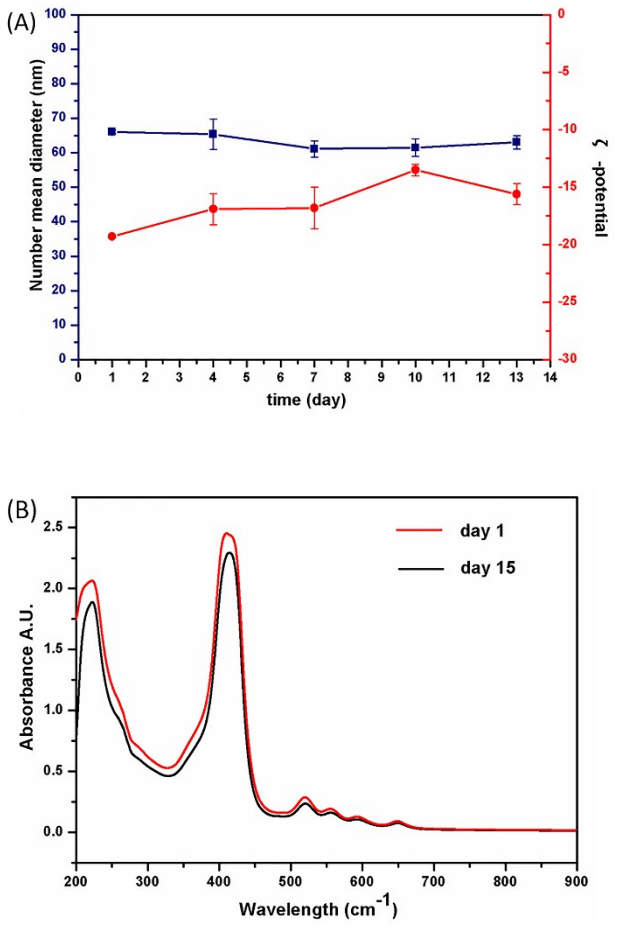

Fig. S9 Stability of HbTcMs conjugate in phosphate buffer (pH 7.4, 10 mM) at room temperature for 15 days detected by (A) DLS and (B) UV-vis.

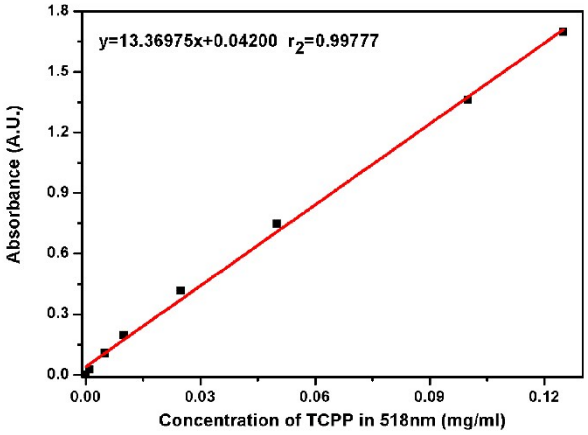

Fig. S10 The standard curve of TCPP in phosphate buffer solution (PBS, pH7.4) at 518nm via UV-vis.

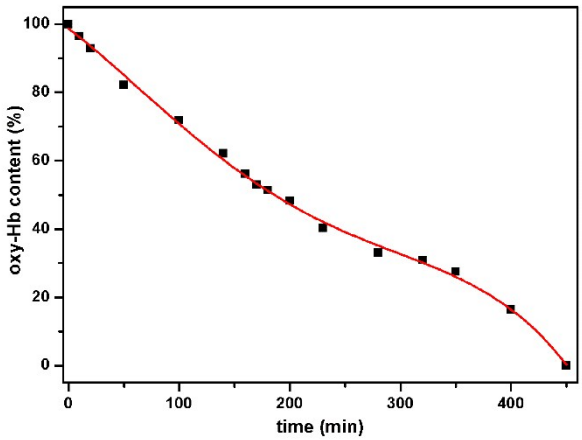

Fig. S11 Stability of Oxy-Hb in free Hb at room temperature.

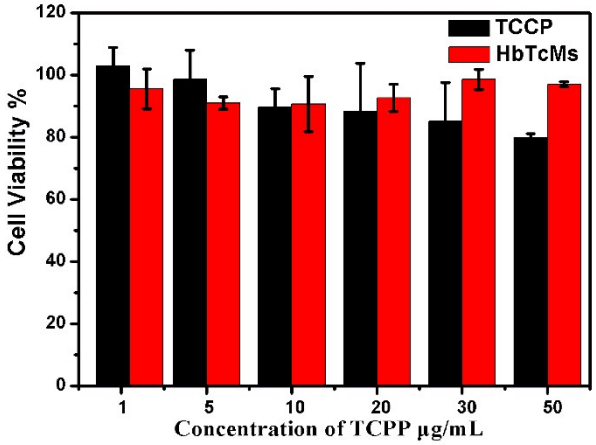

Fig. S12 Relative cell viabilities of $4T_1$ cells after incubated with TCPP、HbTcMs conjugates with different concentrations in darkness.

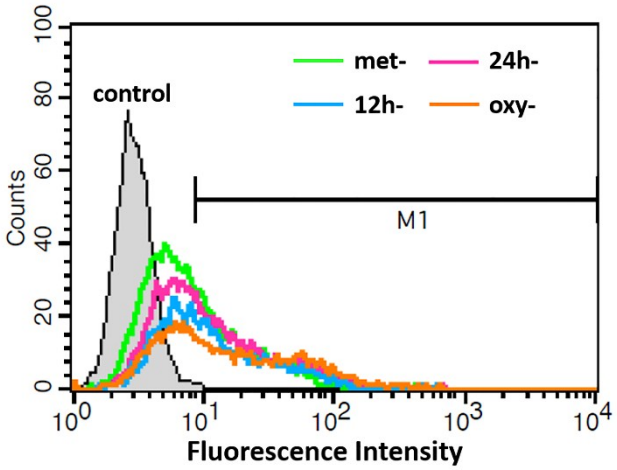

Figure S13 Flow cytometry analysis of $4T_1$ cells after treatment with HbTcMs of met-, 24h-, 12h- and oxy-, stained with a ROS probe, DCFH-DA (4 μM, Ex/Em: 488/ 515 nm). The cells were irradiated with a 660 nm laser for 2 min (70 mw/cm^2)