Electronic Supplementary Material (ESI) for Materials Chemistry Frontiers.


# Supporting Information

## Organic molecules with propeller-structure for enhanced photoacoustic imaging and photothermal therapy

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**Real-time PA imaging of SLN**

Male Wistar rats weighing 250-300 g (InVivos Pte Ltd, Singapore) were used for in vivo PA imaging study. All experimental procedures used in this study were approved by the Institutional Animal Care and Use Committee of the National University of Singapore. The animals were housed at an animal care facility with constant temperature and humidity and were provided free access to food and water. For the PA imaging, the animals were anesthetized with pentobarbital (50 mg/kg bolus and 15 mg/kg/h maintenance, intraperitoneal) throughout the experiments. The body temperature was measured via a rectal probe and maintained at 37 ± 0.5°C by a self-regulating thermal plate (TCAT-2 Temperature Controller, Physitemp Instruments, Inc., New Jersey, USA). The hair in the axillary region was removed with a shaver and the left SLN was exposed. For each experiment, we obtained a baseline photoacoustic image of the region of interest (ROI) before BTPETTQ NPs injection, followed by intradermal injection of BTPETTQ NPs on the left forepaw pad and post-injection PA imaging for duration of 90 mins using laser wavelength 800 nm.

**Cell culture and cytotoxicity**

HeLa cancer cells were pre-cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% FBS and 1% penicillin–streptomycin to 80% confluence. Methylthiazolyldiphenyl-tetrazolium (MTT) assays were applied to assess the metabolic activity of HeLa cells. The cells were subcultured in 96-well plates (Costar, IL, USA) to an intensity of $5 \times 10^4$ cells/mL. The medium was replaced by fresh culture medium with

different concentrations of BTPETTQ NPs and BTPETTQ NPs-Tat. After 4 h incubation, the cells were washed three times with 1 × PBS buffer and fresh culture medium was added into the wells. A group of selected wells were irradiated by 808 nm laser at 0.8 W $cm^2$ for 10 min while the other group were untreated. 100 μL of freshly prepared MTT solution (0.5 mg/mL in 1 × PBS) was added into each well after 24 h incubation. After 3 h incubation, the MTT solution was removed and dimethyl sulfoxide (DMSO, 100 μL) was then added into each well. The plate was gently shaken for 5 min to dissolve all the precipitates. The absorbance of MTT at 570 nm was measured by the microplate reader (Genios Tecan). Cell viability was calculated by the ratio of absolute absorbance of the cells incubated with NPs suspension to that of the cells incubated with culture medium only.

**Calculation of photothermal conversion efficiency**

According to Roper's report, [1, 2] the energy balance of the system can be listed as:

$$\sum_i m_i C_{p,i} \frac{dT}{dt} = Q_{N4} + Q_{Dis} - Q_{Surr} \quad (1)$$

Where $m_i$ and $C_{p,i}$ are the mass and heat capacity of water or DMSO. T is the solution temperature, $Q_T$ is the energy inputted by the samples. $Q_{Dis}$ is the energy input by the sample cells, $Q_{Surr}$ is heat conduction away to the air.

$Q_T$ equals to the heat dissipated by electron-phonon relaxation induced by the laser irradiation.

$$Q_T = I(1 - 10^{-A_{808}})\eta \quad (2)$$

Where I is laser power, which is 0.8 W, η is photothermal conversion efficiency. $A_{808}$ is the absorbance of the samples at 808 nm when the mass concentration is 0.1 mg/mL. In addition, the heat dissipated from the solvent and container, $Q_{Dis}$ is measured to be 7.2 mW and 2.8 mW for pure water and pure DMSO, respectively.

The energy transfer to air is:

$$Q_{Surr} = hS(T - T_{surr}) \quad (3)$$

Where h is heat transfer coefficient, S is the surface area. $T_{surr}$ is the ambient temperature, which is 22.7 °C.

As the heat input $Q_T$ and $Q_{Dis}$ is based on laser input, the $Q_{Surr}$ increase with temperature increase. Under laser irradiation, the temperature of the system increases to equilibrium, where the temperature is defined as $T_{max}$. When temperature reach $T_{max}$, the input is equal to heat output, as a result,

$$Q_T + Q_{Dis} = hS(T_{max} - T_{surr}) \quad (4)$$

To calculate hS, a dimensionless drive force temperature is defined as

$$\theta = \frac{T - T_{surr}}{T_{max} - T_{surr}} \quad (5)$$

And a sample system time constant is defined as

$$\tau_s = \frac{\sum m_i C_{p,i}}{hS} \quad (6)$$

Where $m_i$ is 0.35 g for water and 0.385 g for DMSO and $C_{p,i}$ is 4.2 J/g °C for water and 2 J/g °C for DMSO.

Combine Eq (1) and (6)

$$\frac{d\theta}{dt} = \frac{1}{\tau_s}\left(\frac{Q_T + Q_{Dis}}{hS(T_{max} - T_{surr})} - \theta\right) \quad (7)$$

During the cooling stage as shown in Figure S3A, laser input is shut off, and $Q_T + Q_{Dis} = 0$, and hence Eq (7) becomes

$$t = -\tau_s(\ln \theta) \quad (8)$$

From Figure S5 plot, $\tau_s$ is calculated to be 201 s, 239 s and 254 s for BTPETTQ solution, NPs and Au nanorods, respectively. Then hS is calculated to be 3.85, 6.15, 5.79 mW/ °C, respectively. Finally, through Eq (4) and Eq (2), the photothermal conversion efficiency is calculated to be ~46%, 40% and 29% for BTPETTQ solution, NPs and Au nanorods, respectively.

**Calculation of Tat conjugation efficiency**

After HIV-1 Tat conjugation, the Tat-functionalized NPs were dialyzed against Milli-Q water using a 12 kDa molecular weight cutoff dialysis membrane for three days to remove the unreacted Tat. The dialysis water were collected and freeze-dried. The obtained solid was dissolved in water and quantified by Quant-iT™ Protein Assay Kit. The amount of Tat conjugated to the surface of NPs were calculated and the conjugation efficiency of Tat were calculated to be 90% mol Tat/mol DSPE-PEG.

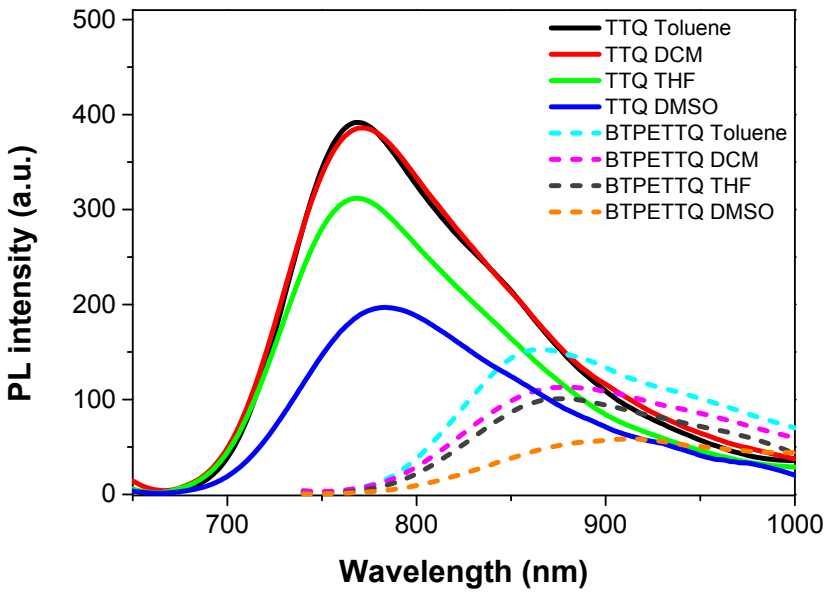


**Figure S1.** PL intensity of TTQ and BTPETTQ in different solvents with different polarities.

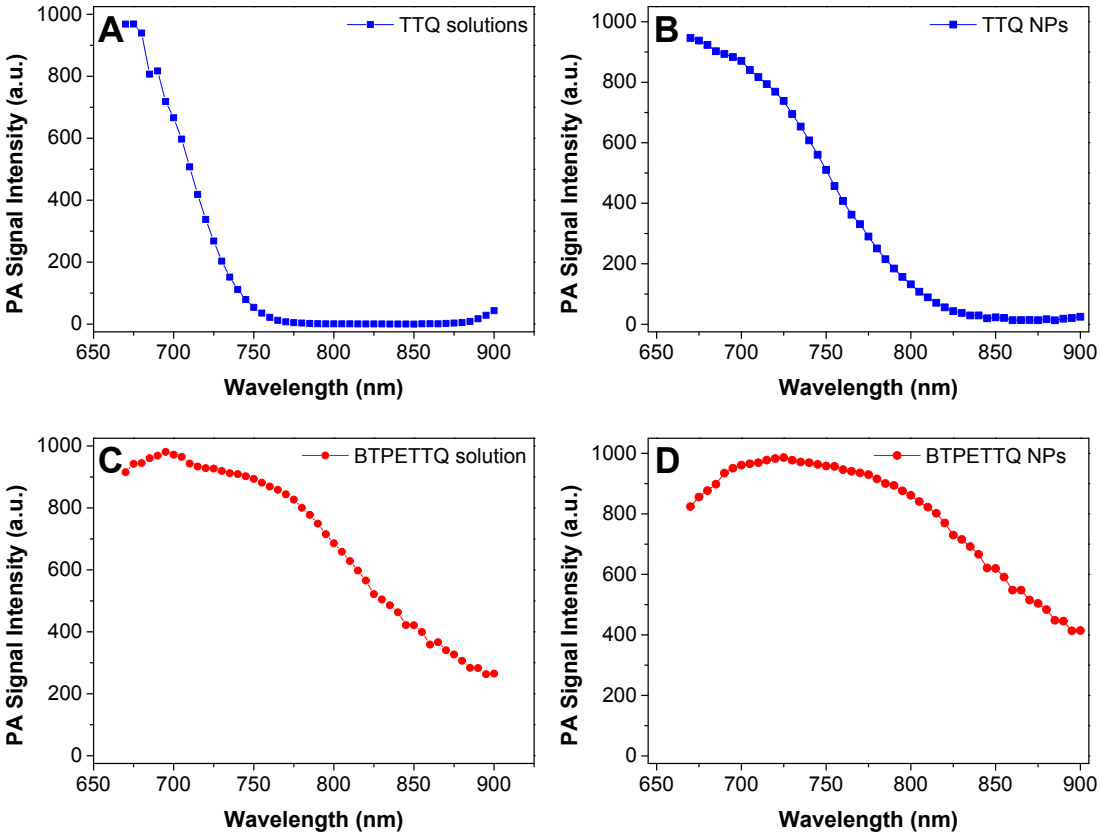


**Figure S2.** PA signal intensity of TTQ solution (A), NPs (B), BTPETTQ solution (C) and NPs (D) upon laser excitation at different wavelengths from 670 nm to 900 nm.

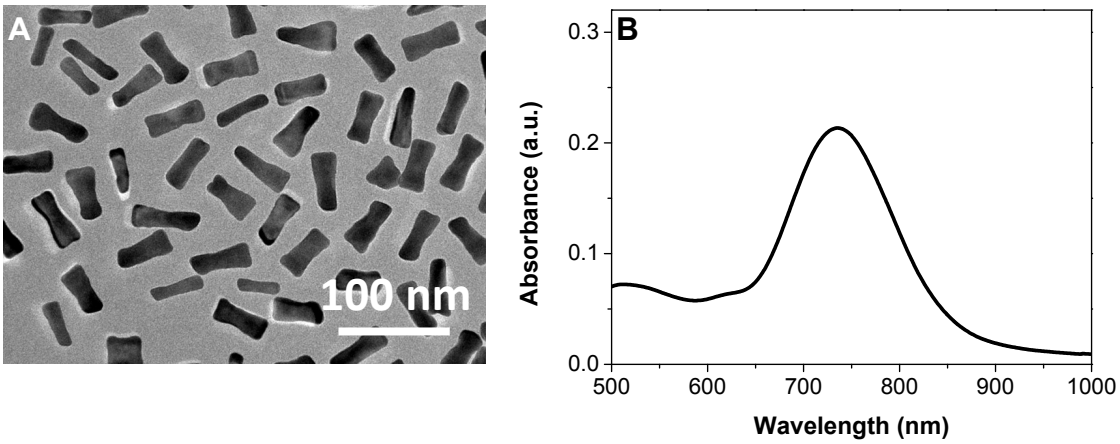


**Figure S3.** (A) TEM image of Au nanorods. (B) UV-vis absorption spectrum of Au nanorods.

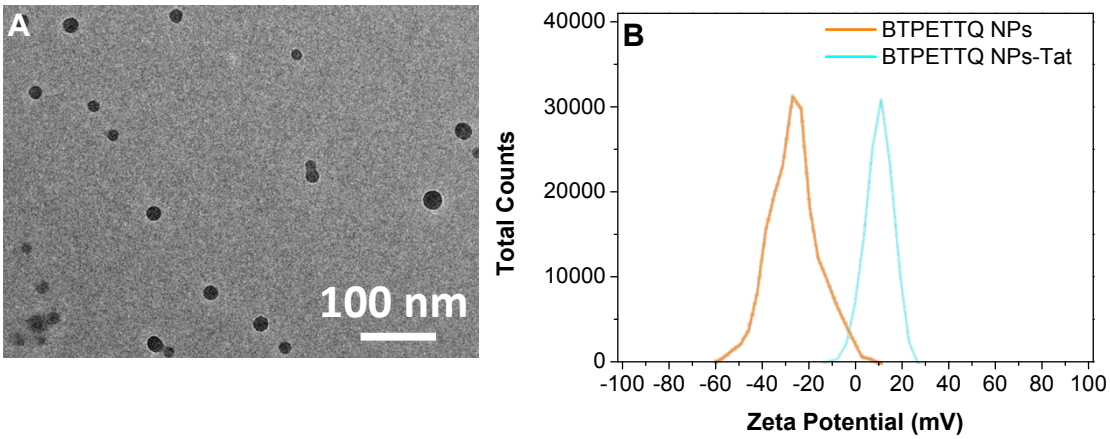


**Figure S4.** (A) TEM image of BTPETTQ NPs-Tat. (B) Zeta potential of BTPETTQ NPs BTPETTQ NPs-Tat.

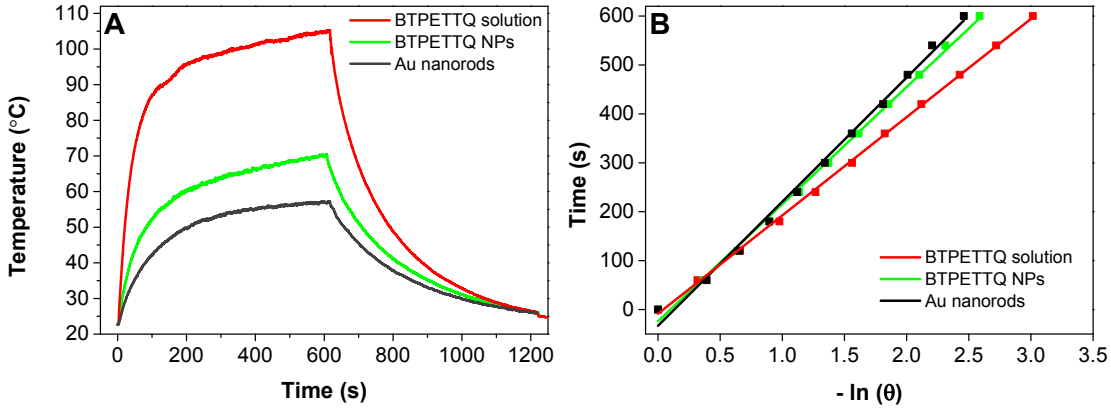


**Figure S5**. (A) Temperature changes of BTEPTTQ solution, NPs and Au nanorods with a concentration of 100 μg/mL, in which the laser is switched off after 10 min irradiation. (B) Plot of time starting from the cooling stage versus negative natural logarithm of drive force temperature. The slop represents the sample system time constant $\tau_s$.

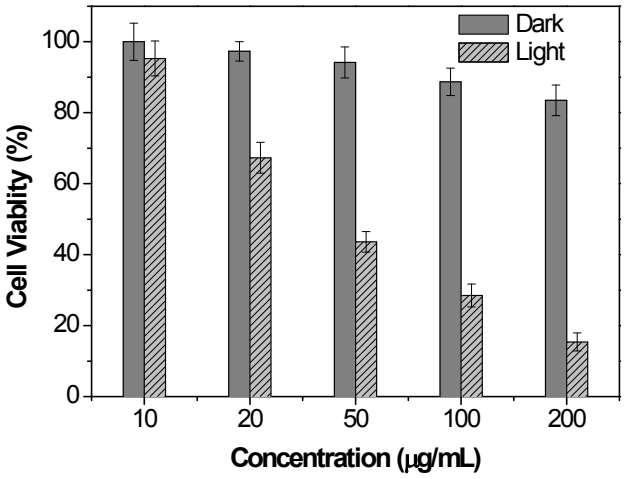


**Figure S6.** Cell viabilities of HeLa cells after incubation with different concentrations of BTPETTQ NPs under dark or with 808 nm NIR laser irradiation for 10 min with power density of 0.8 W/cm$^2$.

## References

[1] D. K. Roper, W. Ahn, M. Hoepfner, *J. Phys. Chem. C*, **2007**, 111, 3636.
[2] Q. Tian, F. Jiang, R. Zou, Q. Liu, Z. Chen, M. Zhu, S. Yang, J. Wang, J. Wang, J. Hu, *ACS Nano* **2011**, 5, 9761.