

Supplementary Information for

Thiophene-Derived Polymer Dots for Imaging Endocytic Compartments in Live Cells and Broad-Spectrum Bacteria Killing

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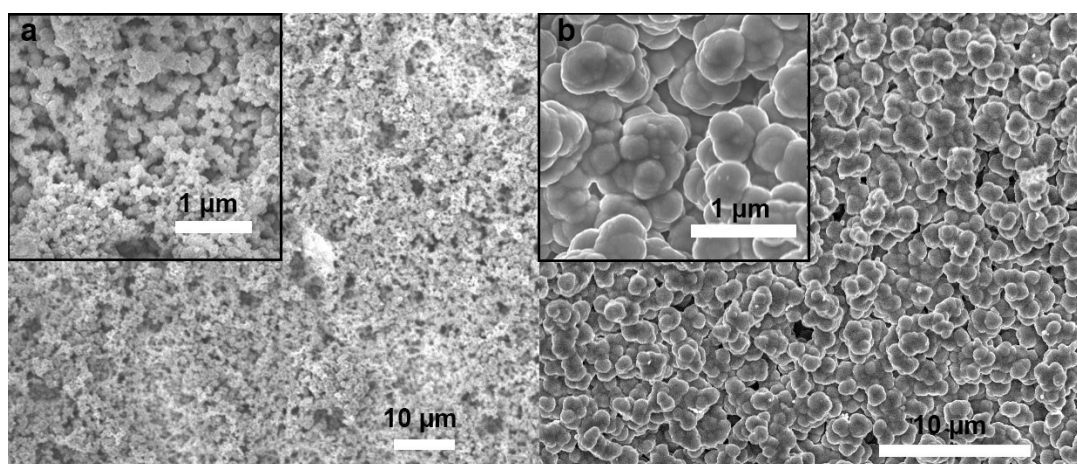


Figure S1: Field-effect scanning electron microscopy (FESEM) of pTh films obtained after (a) 30 min and (b) 60 min electropolymerization of 2,2'-bithiophene monomers. Insets show the magnified views.

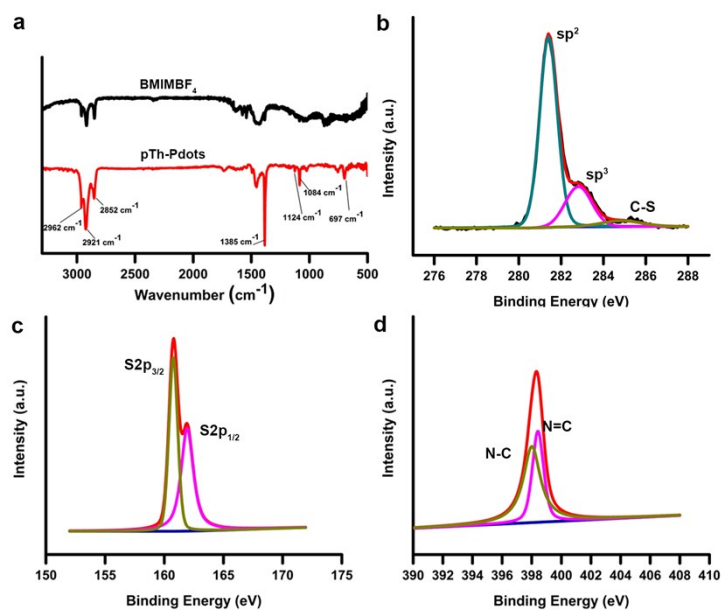


Figure S2: (a) Spectra of Fourier transform infrared spectroscopy (FTIR) for pTh-Pdots and BMIMBF₄. (b – d) High-resolution C1s (b), S2p (c), and N1s (d) X-ray photoelectron spectroscopy (XPS) spectra of pTh-Pdots.

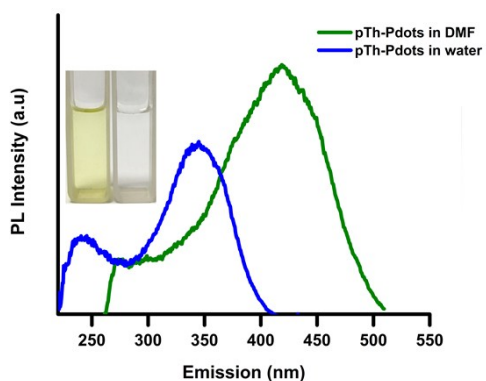


Figure S3: PL excitation spectra of pTh-Pdots in DMF (emission at 530 nm) and pTh-Pdots in water (emission at 435 nm), respectively. Inset shows the brightfield images of pTh-Pdots in DMF and water, respectively.

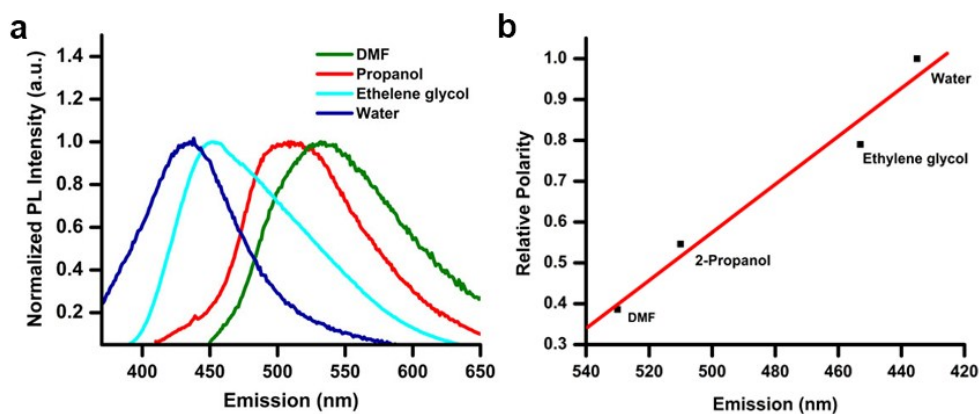


Figure S4: (a) PL intensity of pTh-Pdots suspended in solvents with varying polarity. (b) Linear relationship between PL emission peak and relative polarity.¹

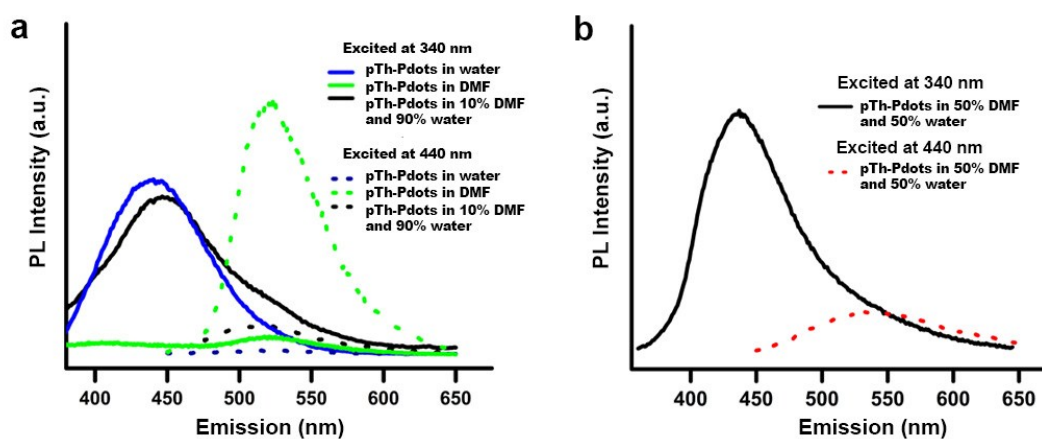


Figure S5: (a) PL of pTh-Pdots in water, pTh-Pdots in DMF, and DMF suspension of pTh-Pdots (10%) diluted in water (90%). (b) PL of DMF suspension of pTh-Pdots (50%) diluted in water (50%).

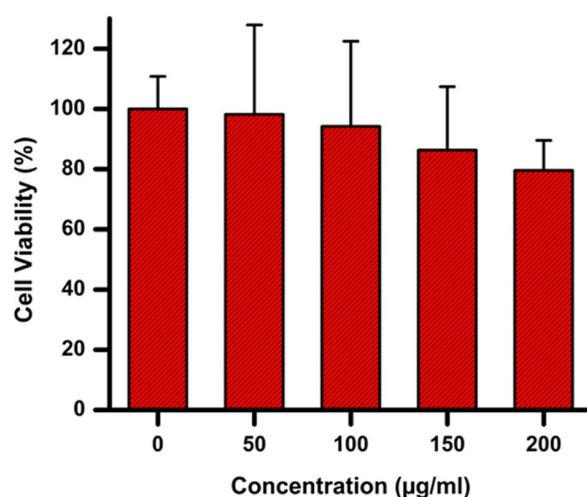


Figure S6: MTT assay of HeLa cells after overnight incubation with pTh-Pdots at various concentrations.

The cell viability was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasodium bromide (MTT) based cell growth determination kit (Sigma-Aldrich). Briefly, after washing with phosphate buffer saline (PBS), the cells were incubated in the culture medium containing MTT reagent solution (5 mg/ml MTT in DMEM without phenol red) (10% v/v) for 4 h at 37 °C. MTT solvent (0.1 N HCl in anhydrous isopropanol) is then added to the culturing well (50% v/v), followed by measuring the absorbance of MTT formazan crystals at 570 nm using a Victor3 plate reader (PerkinElmer).

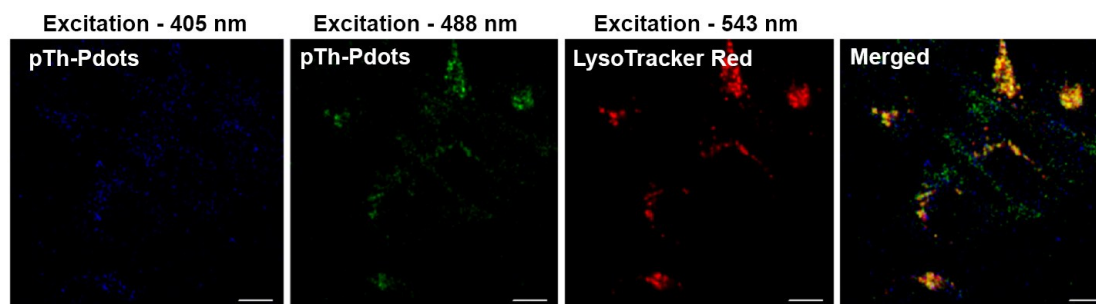


Figure S7: Confocal imaging of HeLa cells incubated with pTh-Pdots (water stock diluted in cell medium to 50 µg/ml) for 3 h, and co-localization study with lysosome marker (LysoTracker Red).

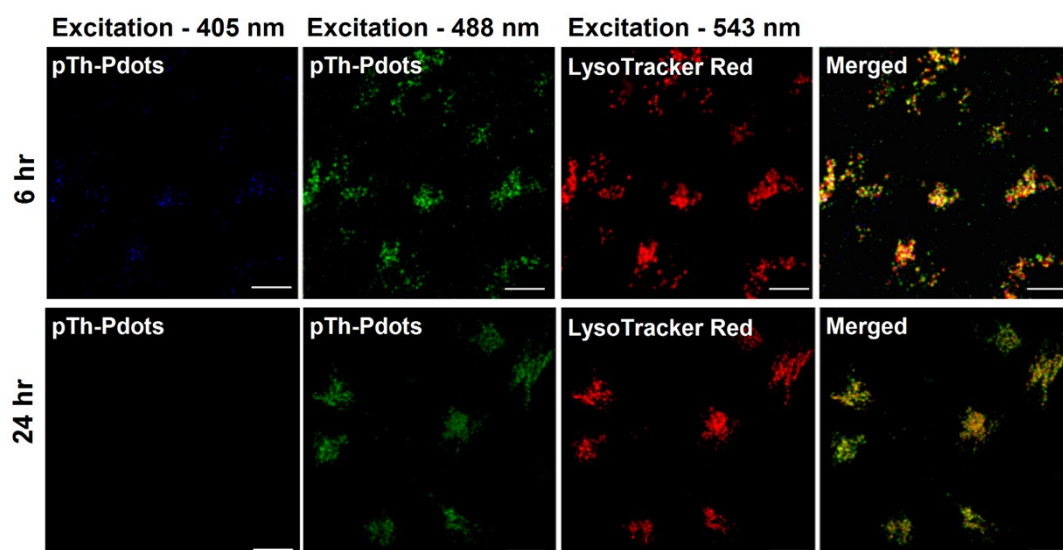


Figure S8: Confocal imaging of HeLa cells incubated with pTh-Pdots (DMF stock diluted in cell medium to 5 $\mu\text{g/ml}$) for 6 h and 24 h, and co-localization study with lysosome marker (LysoTracker Red).

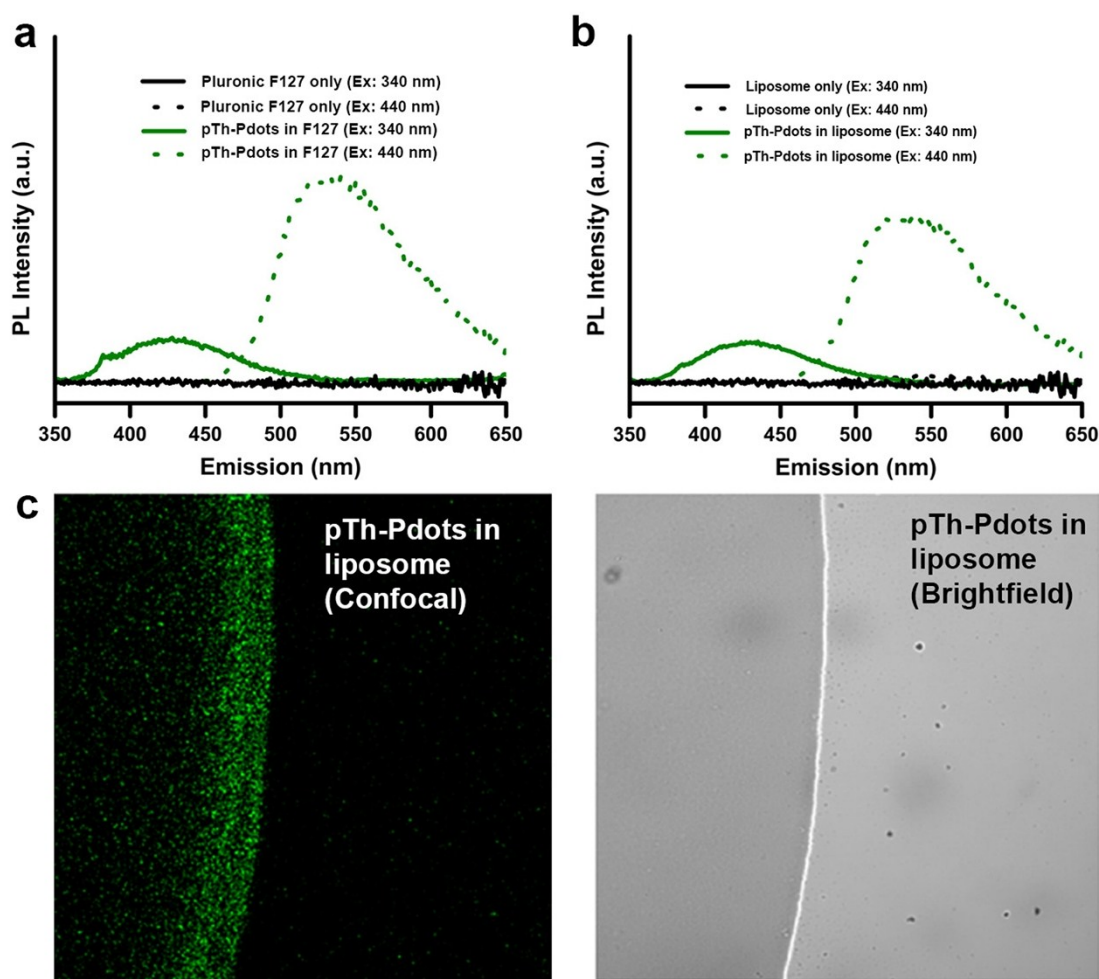


Figure S9: (a) PL spectra of Pluronic F127 polymer and pTh-Pdots encapsulated by Pluronic F127, excited at 340 or 440 nm. (b) PL spectra of DPPC liposomes and liposomes incorporated with pTh-Pdots (originally stocked in DMF). (c) Confocal fluorescence image (left) and bright-field image (right) of a water droplet containing pTh-Pdots incorporated liposomes. The borderline between the droplet and the dry glass coverslip can be clearly seen in the bright-field image (bright line). In the fluorescence image, it is seen that liposomes are more concentrated at the edge.

The pure liposomes and pTh-Pdots incorporated liposomes are synthesized using the thin film hydration method.² Briefly, DMF from the DMF suspension of pTh-Pdots was extracted in a rotary evaporator at 80 °C under a reduced pressure (11 mbar). 5 mg of the phospholipid 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC, Sigma Aldrich) was dissolved in chloroform and mixed with the dried pTh-Pdots. Subsequently, chloroform was extracted at 37 °C under a reduced pressure (474 mbar) in the rotary evaporator to give a thin lipid film. The thin film was subsequently hydrated at 45 °C, by adding 2 mL DI water. Finally, the free pTh-Pdots and free lipids were removed by ultracentrifugation (molecular weight cut-off 50 KDa).

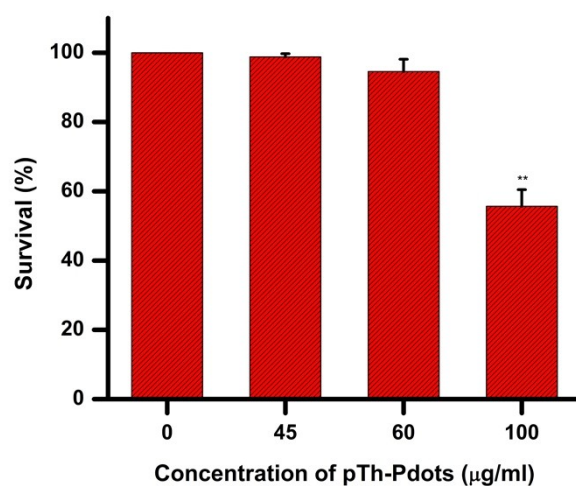


Figure S10: Dose dependent studies for *P. aeruginosa*. Optical density readings taken at 600 nm (OD600).

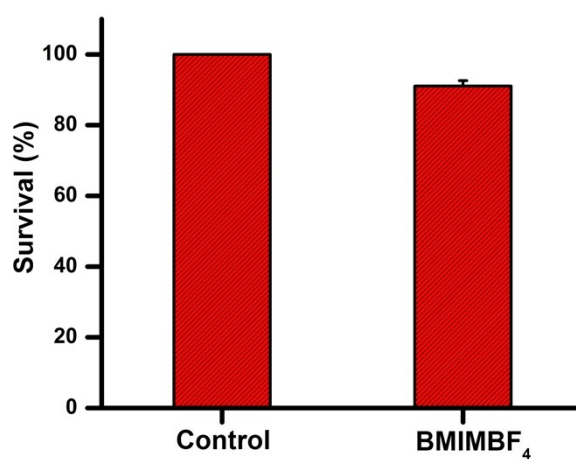


Figure S11: The influence of BMIMBF₄ (0.5 mg/mL) to *E. coli* growth. The average and standard deviation are obtained from 3 independent experiments.

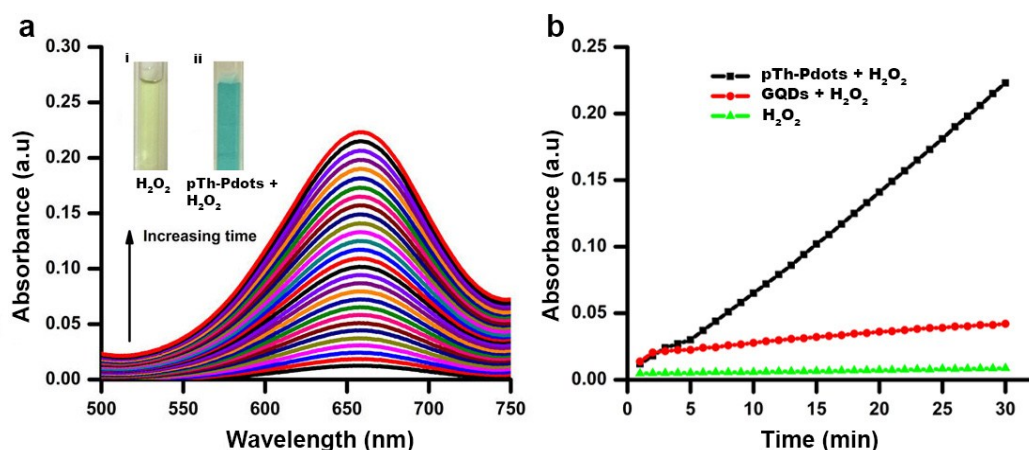


Figure S12: (a) Absorption spectra of the mixture of 10 µg/ml pTh-Pdots, 0.25 mM H_2O_2 and 0.6 mM 3,3',5,5'-Tetramethylbenzidine (TMB) with various reaction durations (0 – 30 min). TMB is a commonly used reporter for peroxidase activity (upon oxidation by hydroxyl radicals, it turns blue and increases absorbance at 650 nm). The photographs in the inset shows that TMB turns into blue in the presence of both H_2O_2 and TMB (30-min reaction). (b) Absorbance (at 650 nm) of 0.6 mM TMB (as the indicator of peroxidase activity) at various reaction durations, in the presence of 0.25 mM H_2O_2 , or 10 µg/ml GQD + 0.25 mM H_2O_2 , or 10 µg/ml GQD + 0.25 mM H_2O_2 .

Reference:

1. C. Reichardt, in *Solvents and Solvent Effects in Organic Chemistry*, Wiley-VCH Verlag GmbH & Co. KGaA, 2002, pp. 389-469.
2. M. Mathiyazhakan, Y. X. Yang, Y. B. Liu, C. G. Zhu, Q. Liu, C. D. Ohi, K. C. Tam, Y. Gao and C. J. Xu, *Colloid Surf. B*, 2015, **126**, 569-574.