Supporting Information

Pyrene-Based Water Dispersible Orange Emitter for One and Two-Photon Fluorescence Cellular Imaging

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1.^{1}HNMR spectra for Pyrene4BTF, Pyrene4BTF-N3, Pyrene4BTF-PEGCOOH, Pyrene4BTF-PEG-TAT and MALDI-TOF spectrum of Pyrene4BTF, Pyrene4BTF-PEGCOOH

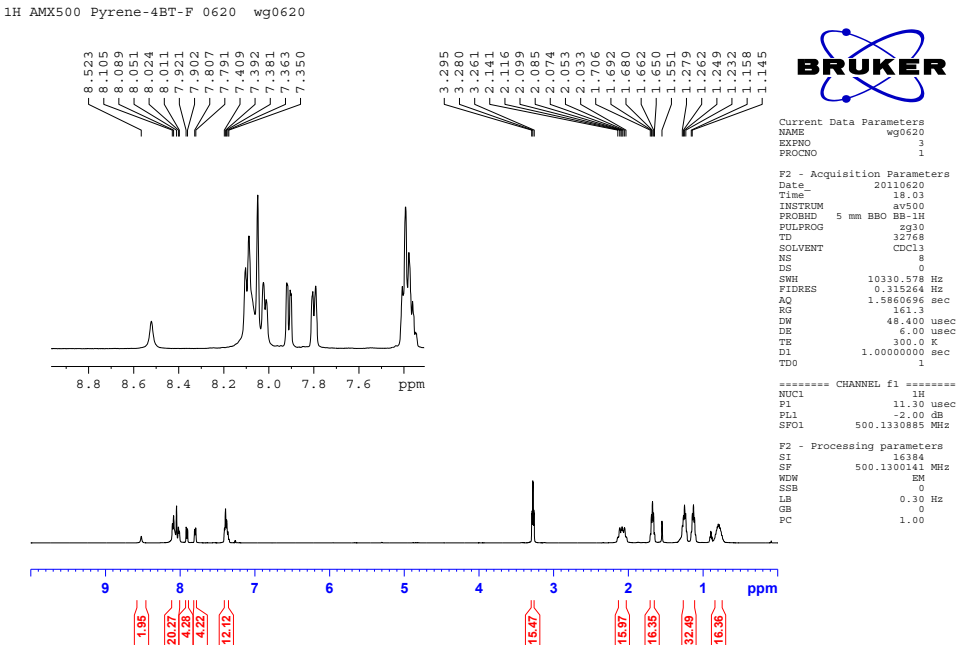

Figure S1. ^{1}H NMR spectrum of Pyrene4BTF in $CDCl_3$.

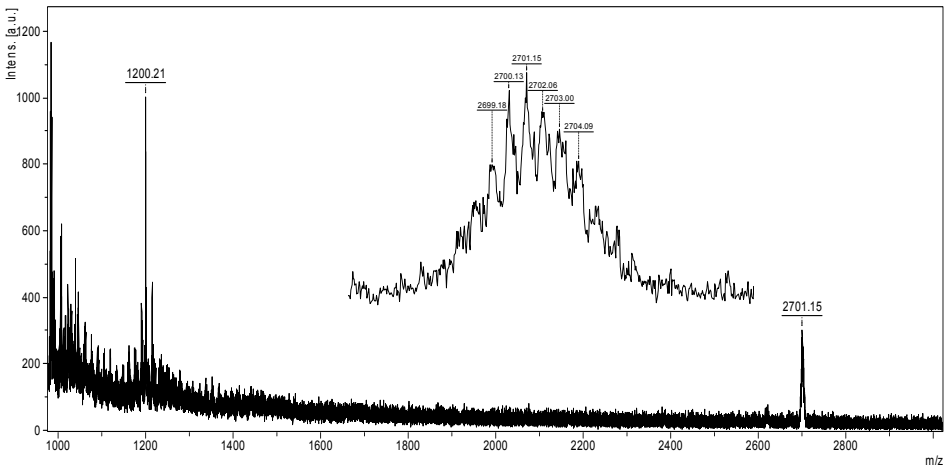

Figure S2. MALDI-TOF mass spectrum of Pyrene4BTF.

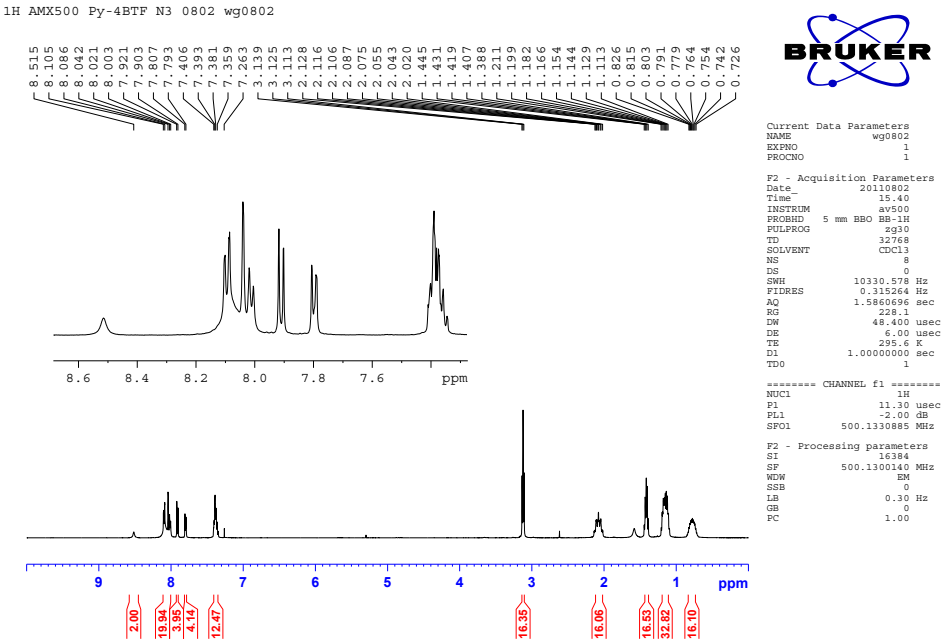

Figure S3. ^{1}H NMR spectrum of Pyrene4BTF-N3 in $CDCl_3$.

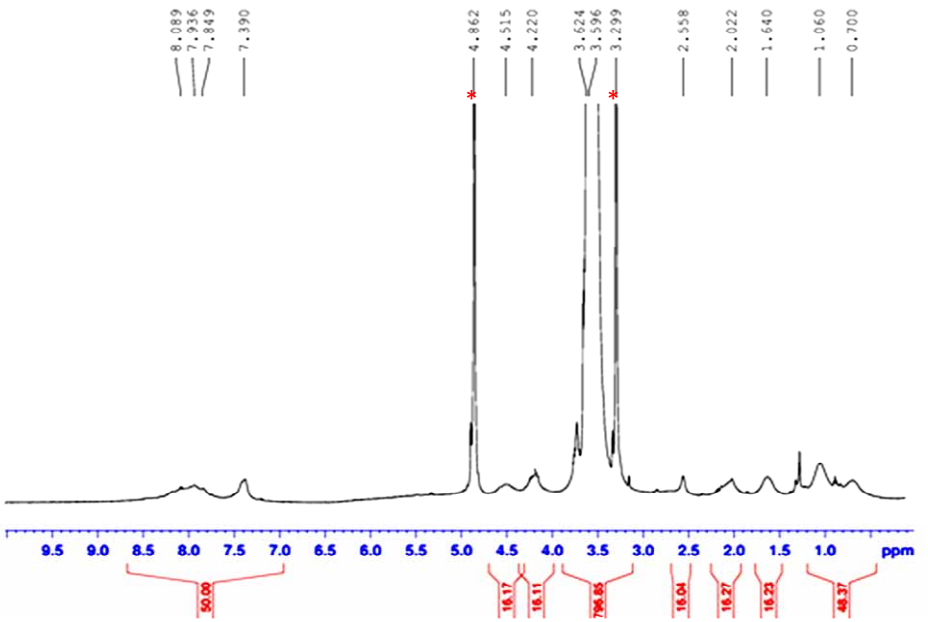

Figure S4.^{1}H NMR spectrum of Pyrene4BTF-PEGCOOH in CD_3OD (* indicate solvent peaks).

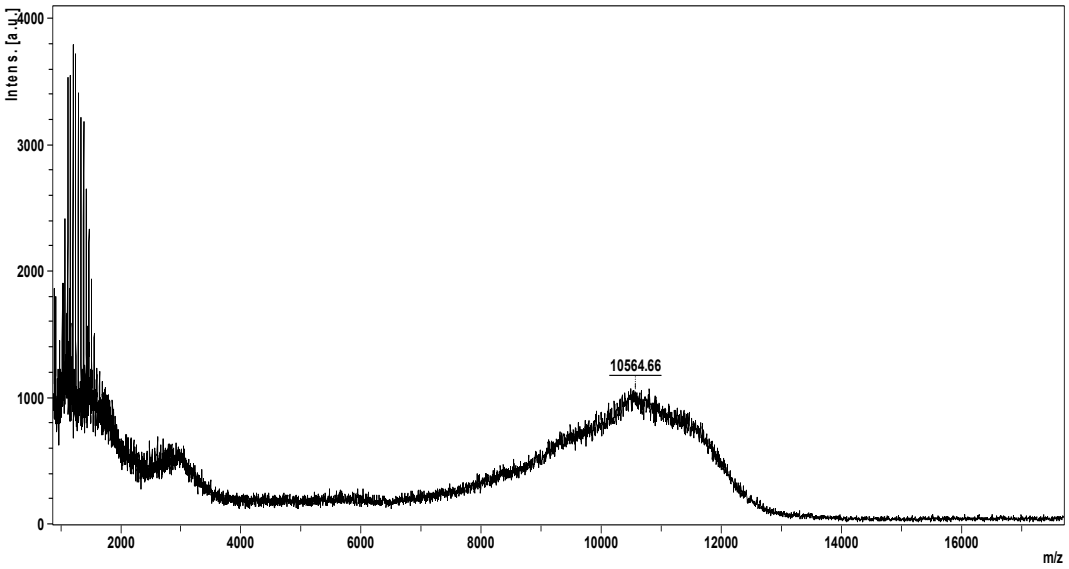

Figure S5. MALDI-TOF mass spectrum of Pyrene4BTF-PEGCOOH.

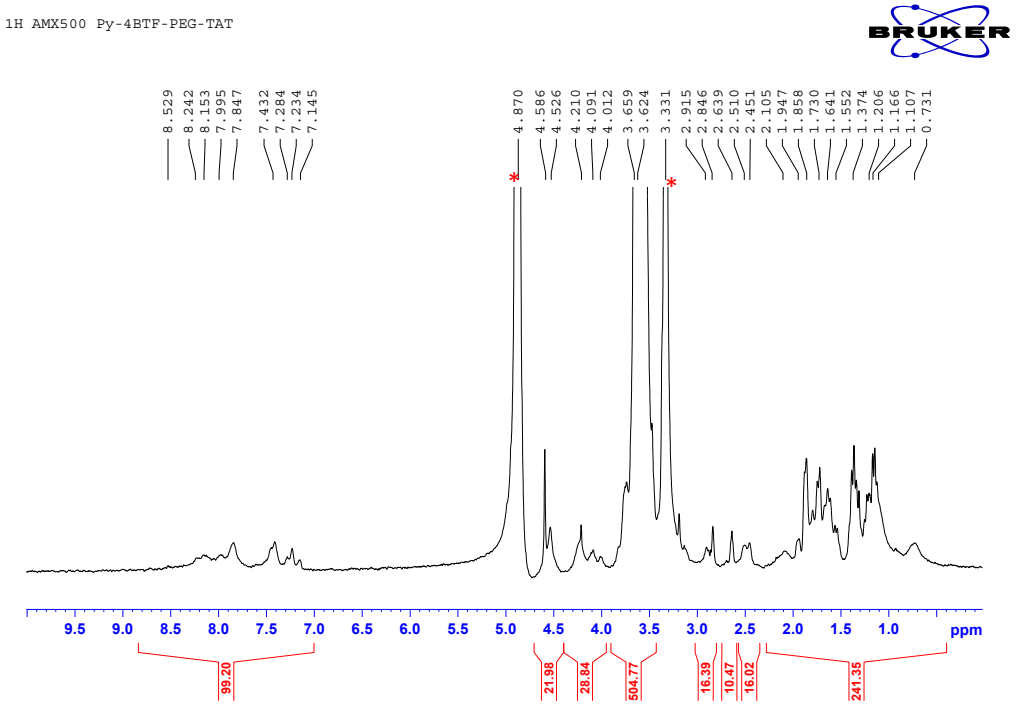

Figure S6. ^{1}H NMR spectrum of Pyrene4BTF-PEG-TAT in CD_3OD (* indicate solvent peaks).

2. DLS Measurement and TEM result

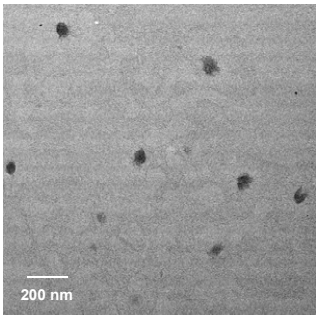

Figure S7. TEM image for Pyrene4BTF-PEG-TAT nanoparticles.

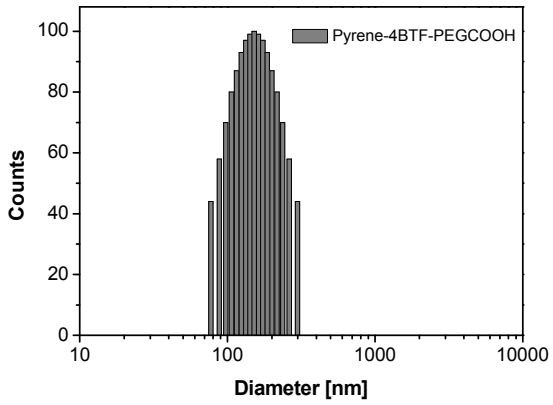

Figure S8. DLS spectrum of Pyrene-4BTF-PEGCOOH in water.

3. TPA Cross Sections Measurement

Two-photon absorption (TPA) spectra were measured using two-photon excited fluorescence (TPEF) spectroscopy.[1] The measurements were conducted with the excitation of 1 kHz pulse train having a typical pulse duration of 120 fs and energy of 0.6 μJ/pulse from an optical parametric amplifier, which was driven by a Ti:sapphire regenerative amplifier. TPEF was collected in a conventional back-scattering geometry, dispersed in a 50 cm monochromator and detected with a photomultiplier using standard lock-in amplification.

The concentrations of solutions were about 10 μM for Pyrene4BTF in toluene and Pyrene4BTF-PEG-TAT in water, respectively. Rhodamine 6G in methanol was used as the references. TPA cross sections were calculated from equation:

$$\frac{\delta_2}{\delta_1} = \frac{F_2 \eta_1 c_1 n_1}{F_1 \eta_2 c_2 n_2}$$

Where δ_1 and δ_2 are the TPA cross sections, F_1 and F_2 are the TPEF intensities, η_1 and η_2 are the fluorescence quantum yields, c_1 and c_2 are the concentrations, n_1 and n_2 are the refractive indexes of solvents (1 corresponds to reference, 2 is sample).

4. Cellular Imaging of Hela Cells Incubated with Pyrene4BTF-PEGCOOH with/without Free tat Peptide

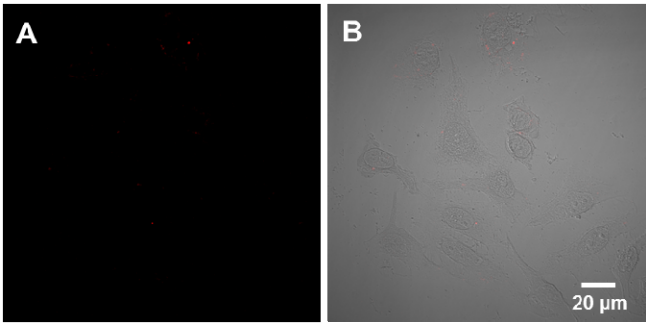

Figure S9. (A) CLSM, (B) CLSM/transmission overlap images of Hela cells incubated with Pyrene4BTF-PEGCOOH for 2 h.

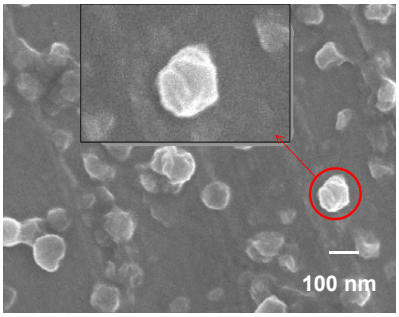

Figure S10. SEM image for Pyrene4BTF-PEGCOOH nanoparticles.

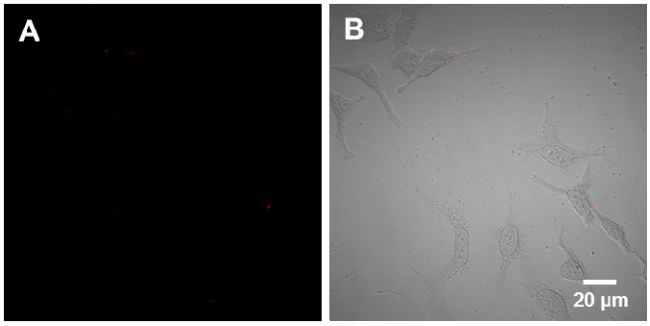

Figure S11. (A) CLSM, (B) CLSM/transmission overlap images of Hela cells incubated with Pyrene4BTF-PEGCOOH in the presence of free tat peptide for 2 h.

Reference:

(1) C. Xu, W. W. Webb, *J. Opt. Soc. Am. B* **1996**, *13*, 481.