Electronic Supplementary Material (ESI) for Photochemical & Photobiological Sciences.


# A pyrene-based two-photon excitable fluorescent probe to visualize nucleus in live cells

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## Supporting Information

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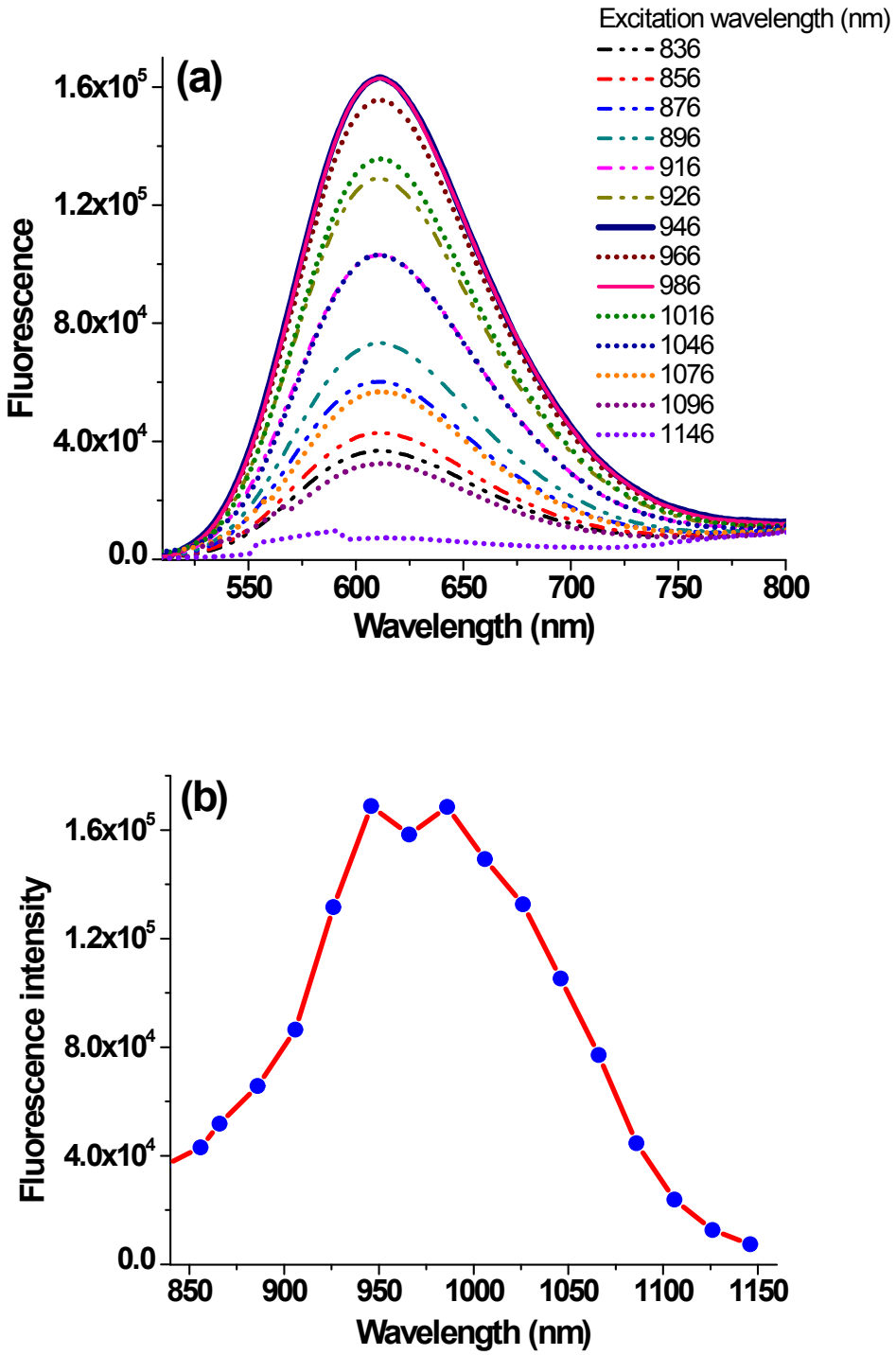



**Figure S1.** (a) The fluorescence spectra recorded at 610 nm for probe **1** (1 x $10^{-5}$ M, in DCM) by exciting at 850 nm – 1150 nm wavelength range at room temperature. Figure (b) represents the plot of recorded fluorescence intensity at 610 nm as a function of excitation wavelength for Probe **1**.

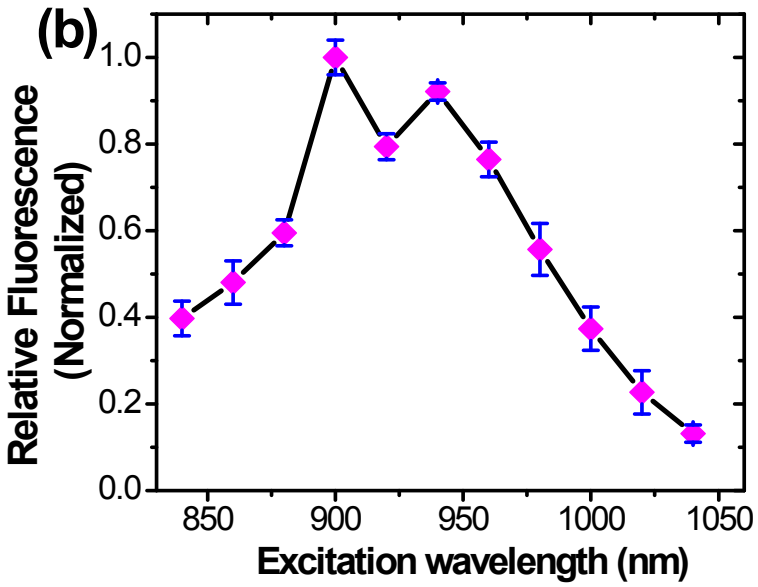



**Figure S2.1** The normalized fluorescence emission recorded at 610 nm by exciting the probe **1** (1 x $10^{-5}$ M, in ethanol) in the wavelength range 850 – 1050 nm.

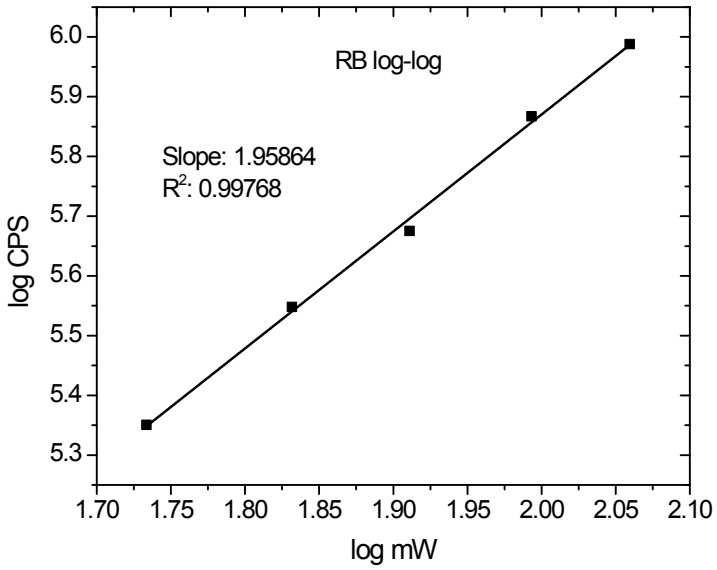



**Figure S2.2** Logarithmic plots of the power dependence of relative two-photon induced luminescence intensity of Rhodamine B.

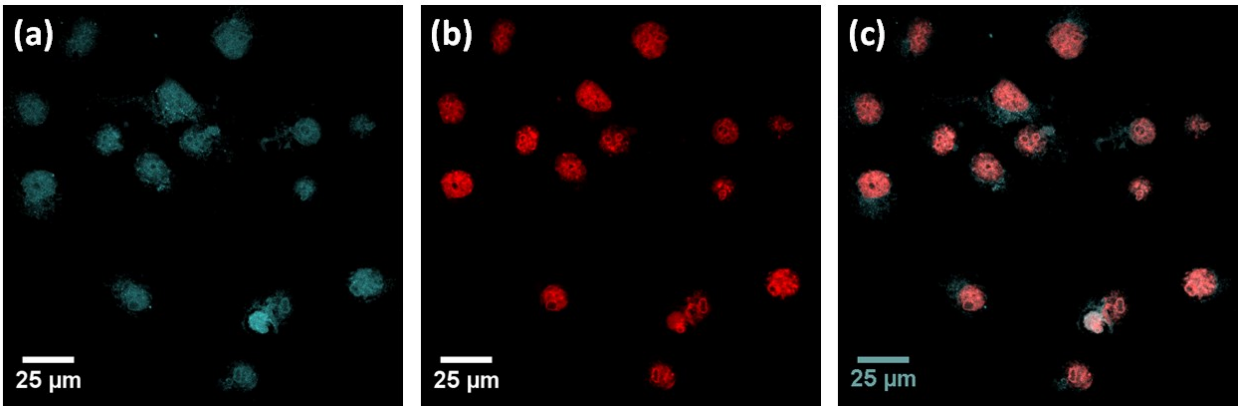



**Figure S3.** Fluorescent confocal microscopy images of COS-7 cells expressed with pmTurquoise-H2A (14 hours) and treated with probe **1** (500 nM) for 30 minutes. Images show the fluorescence of pmTurquoise-H2A expression (a), **1** staining (b) and their co-localization (c). pm Turquoise-H2A was excited with 454 nm laser line and emissions were collected from 465 nm to 525 nm. Probe 1 was excited with 488 nm lase line and emission were collected from 570 nm to 700 nm.

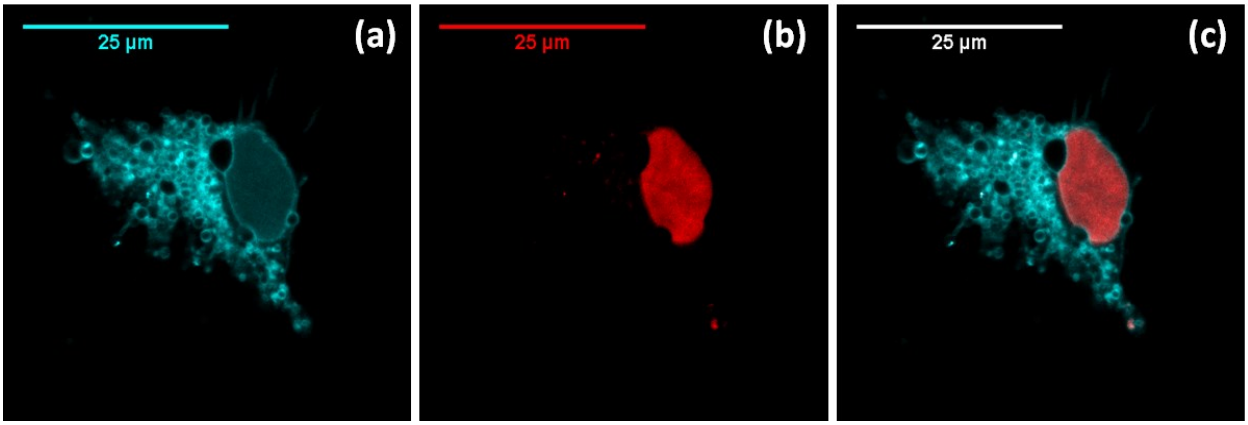



**Figure S4.** Fluorescent confocal microscopy images of COS-7 cells expressed with pmTurquoise-H2A (nucleus), pPalmitoyl-m-Turquoise2 (membranes) for 14 hours and treated with probe **1** (500 nM) for 30 minutes. Images show the fluorescence of pmTurquoise-H2A expression / pPalmitoyl-m-Turquoise2 expression (a), probe **1** staining (b) and their co-localization (c). pm Turquoise-H2A and pPalmitoyl-m-Turquoise2 were excited with 454 nm laser line and emissions were collected from 465 nm to 525 nm. Probe **1** was excited with 488 nm laser line and emission were collected from 570 nm to 700 nm.

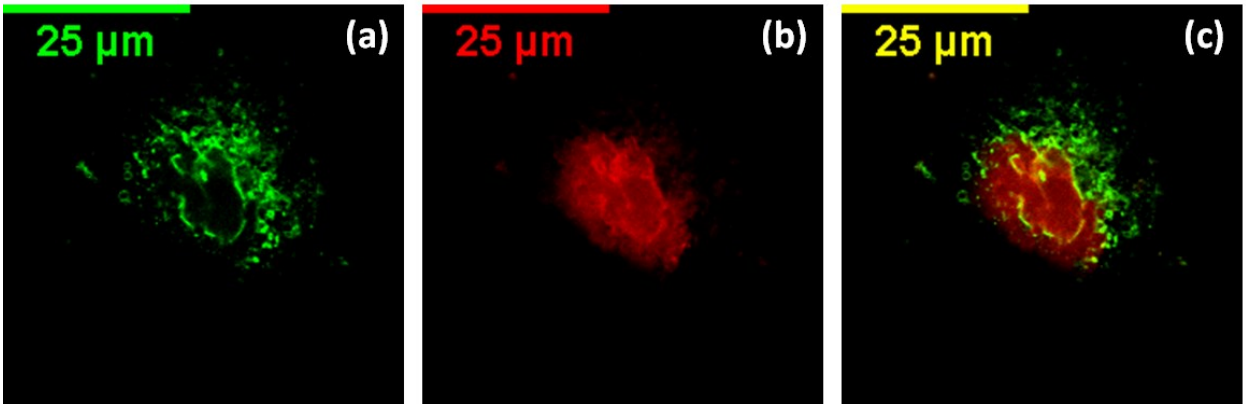



**Figure S5.** Fluorescent confocal microscopy images of COS-7 cells stained with MitoTracker® Green DND-26 (100 nM) and probe **1** (500 nM) for 30 minutes. Images show the fluorescence of MitoTracker® Green (a), probe **1** staining (b) and their overlapped image (c). pm MitoTracker® green was excited with 488 nm laser line and emissions were collected from 495 nm to 530 nm. Probe **1** was excited with 488 nm laser line and emission were collected from 595 nm to 700 nm.

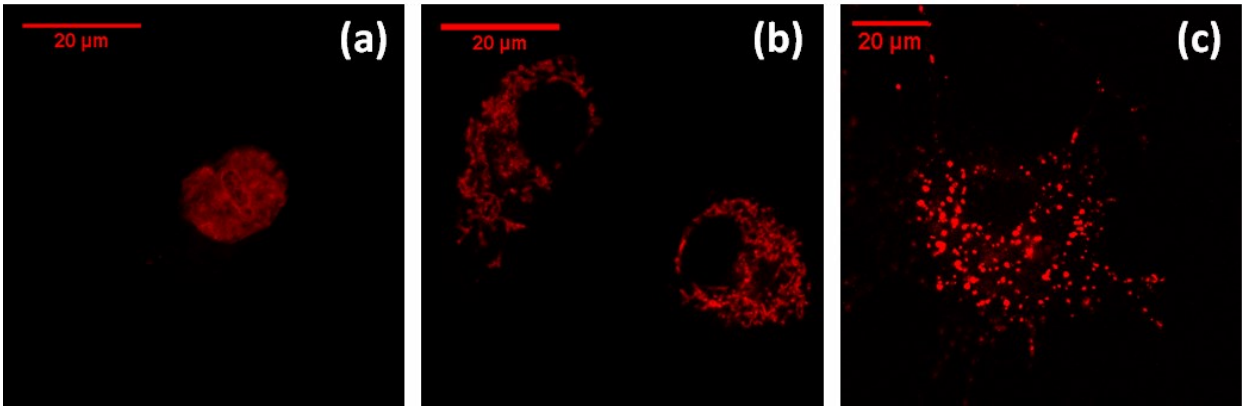



**Figure S6.** Fluorescent confocal microscopy images of COS-7 cells stained with Probe **1** (a) (a), **2** (b), and **3** (c) with 500 nM concentration for 30 minutes. Images Probes **1** and **2** were excited with 488 nm laser line and emissions were collected from 570 nm to 700 nm. Probe **3** was excited with 561 nm laser line and emission were collected from 595 nm to 700 nm.

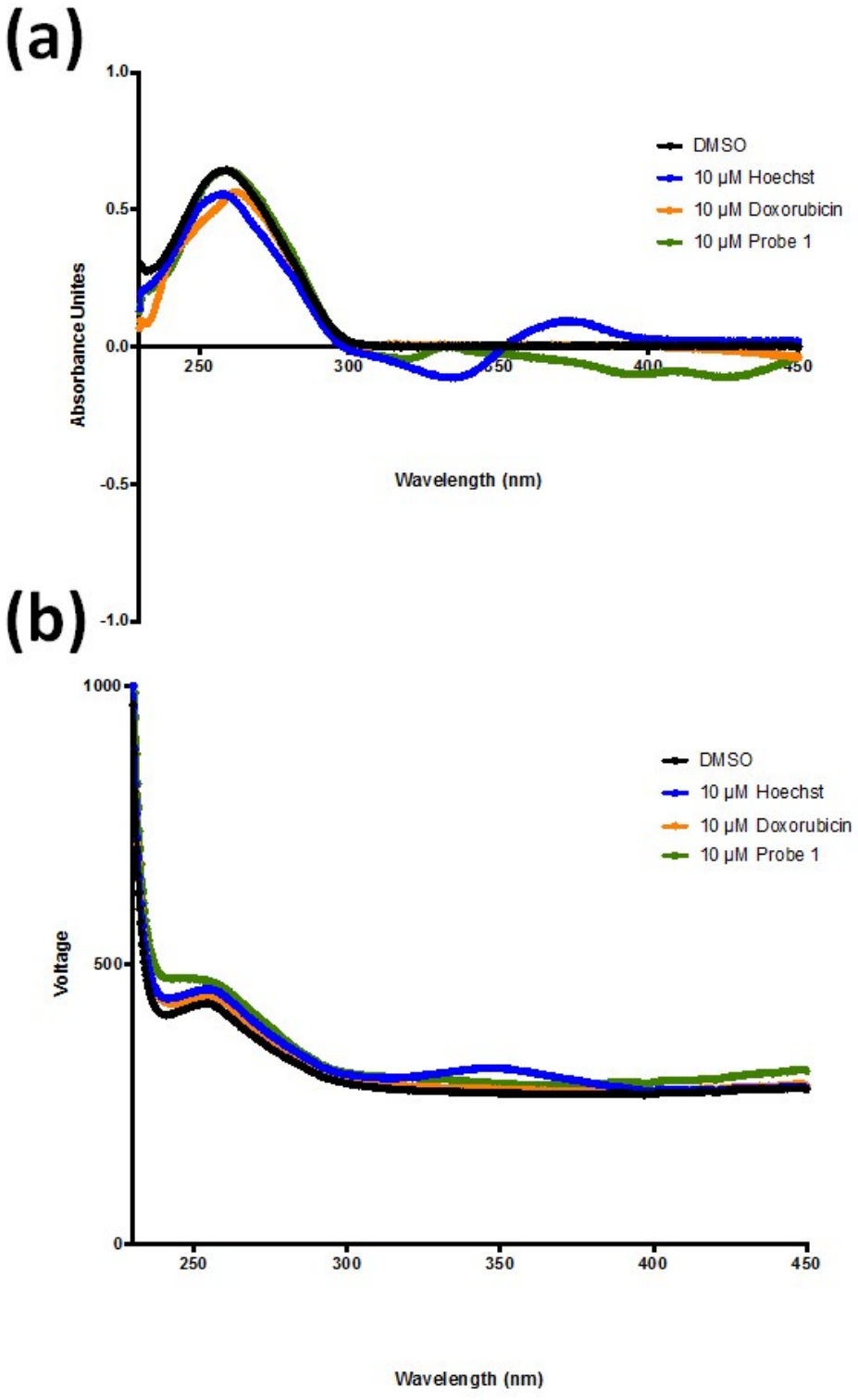
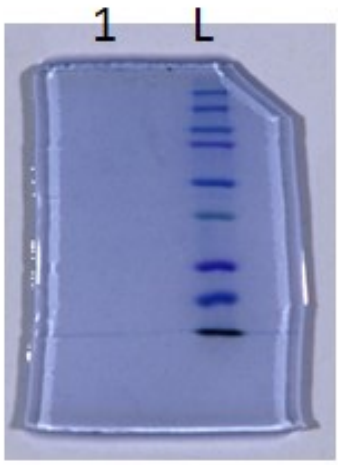



**Figure S7** Fluorescent Absorbance (a) and voltage (b) spectra obtained obtained for Hoechst, Doxorubicin, and **1** dissolved in DMSO (10 µM) upon addition of calf thymus DNA at room temperature.




L = Kaleidoscope protein ladder
1= DNA sample used for CD.
Coomassie staining shows no protein contaminants

**Figure S8** Agarose gel electrophoresis result of the calf thymus DNA sample used for CD spectral analysis to confirm no protein contaminations in the sample.