Electronic supplementary information:

An NIR Dye Encapsulated Supramolecular Assembly for Imaging Mitochondria in Living Cells with Ultrastable Photostability

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Materials and methods

Reagents and instruments

All the reactions were carried out with dry, freshly distilled solvents under anhydrous conditions, and water used in test was distilled water. CB[8] was synthesized according to the literature.[1, 2] Other reagents were commercially available and used without further purification unless otherwise stated. Mito-Tracker Green FM, Mito-Tracker Deep Red FM were purchased from Invitrogen (USA).

Absorption spectra were recorded on an Agilent HP-8453 (Agilent, USA) absorption spectrometer. The steady-state fluorescence emission and excitation spectra were obtained by using a Cray Edipse fluorescence spectrophotometer (Varian, Australia). Fluorescence lifetimes were measured on a Horiba Jobin Yvon Fluoro Max-4 (TCSPC) instrument. Mass spectral studies were carried out usingLC/Q-Tof mass spectra(Micromass, England). NMR spectra were obtained with a Jasco FP-6500 spectrophotometer (Jasco, Japan). Atomic Force Microscopy (AFM) imagines was measured using Veeco Nanoscope IIIA+.

Photodegradation experiments

The photodegradation test was carried out in square cross-section quartz cells (l × 1 cm) and solutions of the samples were irradiated with a 500W iodine-tungsten lamp at room temperature. To eliminate the heat and absorb short wavelength light, a cold trap (5 L solution of 60 g/L $NaNO_2$ in 10 cm (width) × 30 cm (length) ×20 cm (height) was set up between the cells and the lamp. The distance between the cells and the lamp was 25 cm. The irreversible bleaching of the dyes at the absorption peak was monitored as a function of time. Samples were tightly sealed, but not deoxygenated with nitrogen, before the test.

Cell incubation and imaging

MCF-7 cells (human breast adenocarcinoma cells) were cultured in DEME medium supplemented with 10% fetal bovine serum at 37 ºC in an atmosphere containing 5% CO_2. For live cell imaging, compounds were added to cells grown in a confocal microscope dish for 10 min and washed with PBS (phosphate-buffered saline) three times. After replacement of the medium, cells were imaged using a OLYMPUS FV1000 confocal fluorescence microscope with a 200×objective lens (excited with the 515 nm channel). For subcellular localization analysis of dye staining, organelle specific fluorescent dyes were used. MCF-7 cells were first stained with 2 μM of PQ@CB[8] at 37 ºC in an atmosphere of 5% CO_2 for 10 min, and then washed with PBS three times. Cells were then incubated with MitoTracker Green FM (0.4 μM) at 37 ºC in an atmosphere of 5% CO_2 for 15 minutes, and then washed with PBS three times. After replacement of medium, cells were imaged using OLYMPUS FV1000 confocal fluorescence microscope with a 200×objective lens.

Synthesis

Synthesis of PQ was carried out according to a modification of literature procedures.[3] 1-Pyrenecarboxaldehyde (460.0 mg, 2.0 mmol) dissolved in dry THF (20 ml) was added to the MeOH solution containing N-ethyl-4-methylquinolinium chloride, (414.9 mg, 2.0 mmol) and piperidine (0.17 g, 2.0 mmol). The mixture was stirred at room temperature for 5 h under N_2 atmosphere, and the precipitation was collected by filtration, washed with 50 ml acetone and dried to give 671.3 mg product (the total yield was 80%).^{1}H NMR (400MHz, DMSO-d_6, 25℃) δ_H = 9.48 (1 H, d, J = 6.5 Hz), 9.28 (1 H, d, J = 15.6 Hz), 9.20 (1 H, d, J = 8.6 Hz), 9.12 – 9.00 (1 H, m), 8.98 (1 H, d, J = 6.5 Hz), 8.71 – 8.03 (12 H, m), 5.08 (2 H, d, J = 7.4 Hz), 1.64 (3 H, t, J = 7.1 Hz) ppm. ^{13}C NMR (400MHz, DMSO-d_6, 25℃) δ_C = 154.92, 152.92, 147.69, 135.95, 135.60, 135.47, 133.07, 131.58, 131.45, 131.36, 131.21, 131.17, 129.73, 126.85, 127.77, 127.41, 126.32, 125.92, 125.42, 124.06, 123.61, 123.07, 122.62, 122.38, 119.83, 119.56, 117.80, 52.69, 15.75 ppm. HRMS for $C_{29}H_{22}N^+$: *m/z*

(%)=384.1743 (100) (Calculated Mass: 384.1752).

Determination of binding constant

According to the literature[4], the binding constant value has been determined from the emission intensity data following the equation:

$$\frac{I_f}{I_0} = 1 + \left(\frac{I_\infty}{I_0} - 1\right)\frac{K_{eq} \times CB[8]}{1 + K_{eq} \times CB[8]}$$

where I_f/I_0 is the fluorescence enhancement when 100% of the dye has been included. I_0 is the fluorescence intensity of PQ in the absence of CB[8], while I_f is the observed fluorescence intensity at each host molecule concentration tested. K_{eq} is the equilibrium binding constant for the complex.

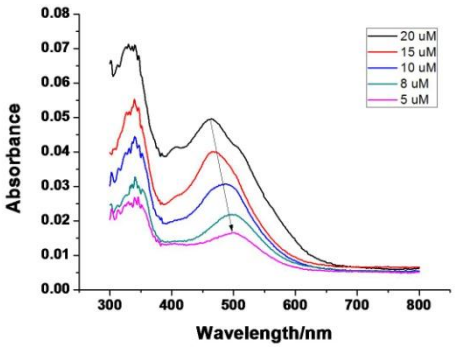

Fig. S1 Effect of concentration on the absorbance of dye PQ deaggregation at ca. 80 μM.

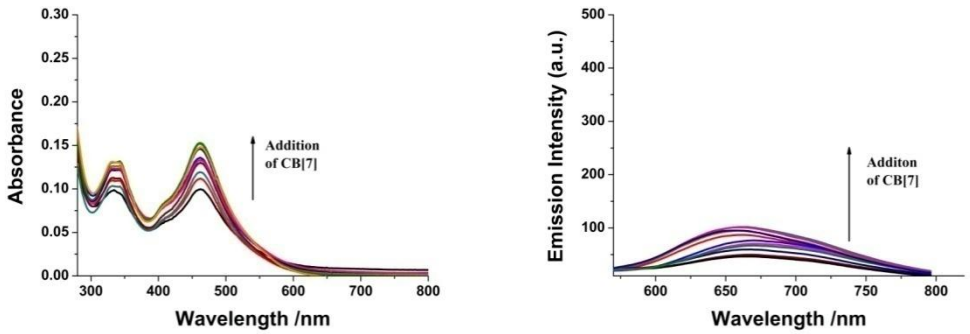

Fig. S2 a) Absorption spectra for PQ (10 μM in water) upon addition of CB[7] (0 to 1 equiv.); b) Fluorescence spectra for PQ (10 μM in water) upon addition of CB[7] (0 to 1 equiv.).

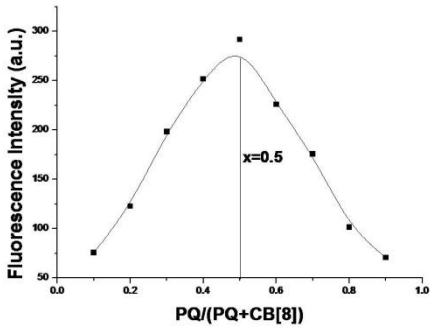

Fig. S3 Continuous variation Job's plot for PQ and CB[8] on the basis of F.L. spectra

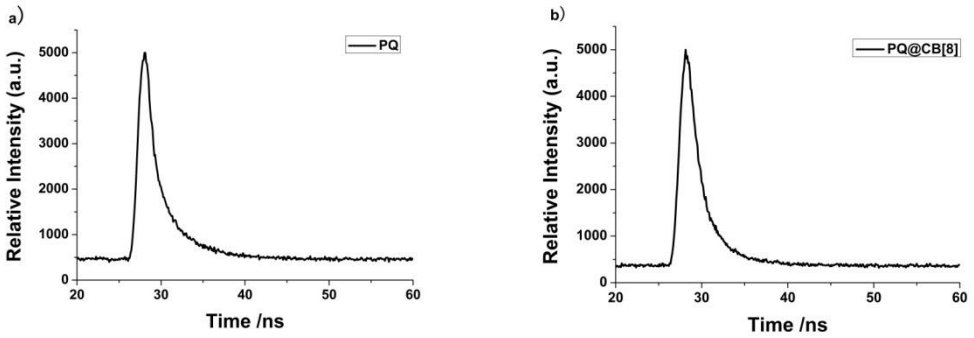

Fig. S4 Time resolved fluorescence decay traces (λ_{Ex} = 478 nm; λ_{Em} = 650 nm) of PQ in the absence (a) and presence (b) of CB[8] in aerated water.

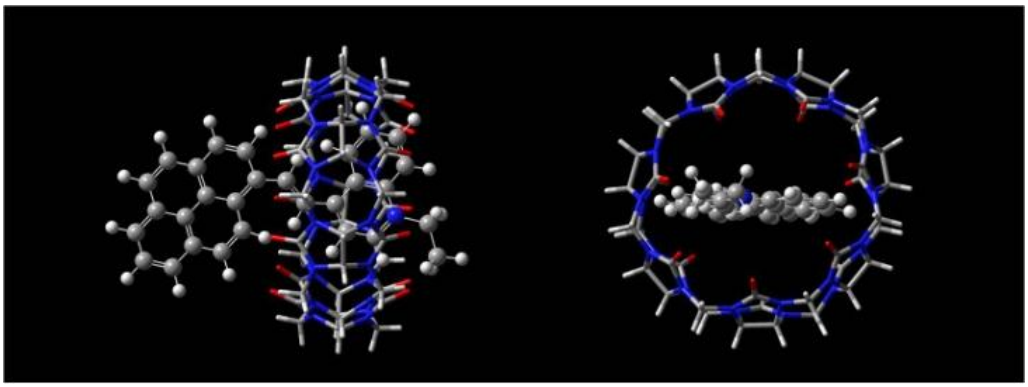

Fig. S5 Minimized structures of the inclusion complex PQ@CB[8] viewed from front and side, using DFT-B3LYP/6-31G* methods.

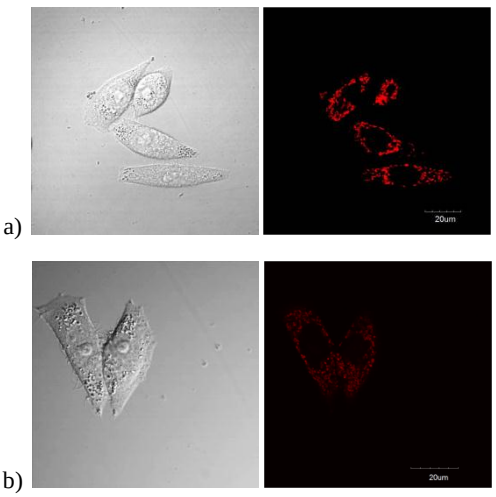

Fig. S6 Confocal microscopy images of MCF-7 cells incubated with 2 μM PQ and 20 μM CB[8] (a) or 2 μM PQ alone (b). Left: bright field images. Right: fluorescence images excited at 488nm. Both pictures were captured at same parameters.

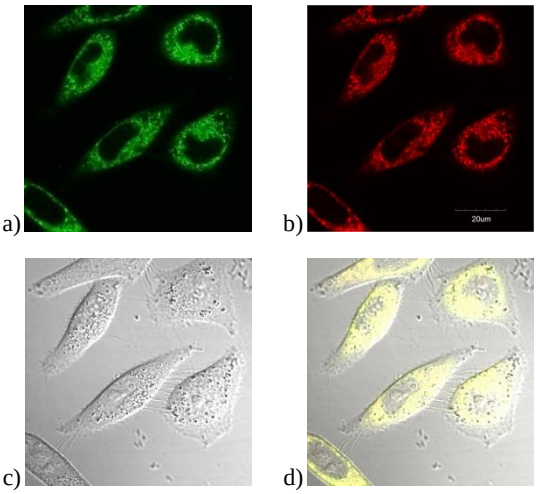

Fig. S7 Confocal images of PQ@CB[8] complex plus a mitochondrially specific dye in MCF-7 cells. (a) Fluorescence image of MCF-7 cells stained with Mito-Tracker Green FM; (b) fluorescence image of MCF-7 cells stained with PQ@CB[8] complex; (c) bright field; (d) merged image of (a), (b) and (c).

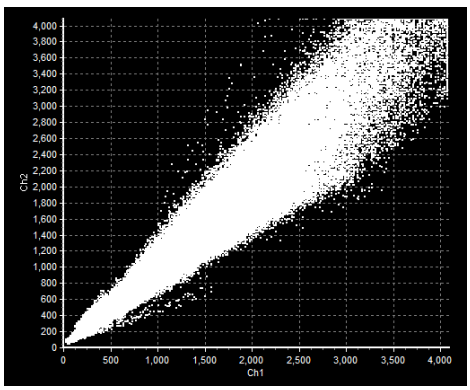

Fig. S8 The Pearson correlation coefficient to evaluate the colocalization between PQ@CB[8] and Mito-Tracker Green FM in the stain of MCF-7 cells.

References

1. J. Kim, I. S. Jung, S. Y. Kim, E. Lee, J. K. Kang, S. Sakamoto, K. Yamaguchi and K. Kim, *J. Am. Chem. Soc.*, 2000, **122**, 540-541.
2. A. Day, A. P. Arnold, R. J. Blanch and B. Snushall, *J. Org. Chem.*, 2001, **66**, 8094-8100.
3. K. N. Koh, K. Araki, A. Ikeda, H. Otsuka and S. Shinkai, *J. Am. Chem. Soc.*, 1996, **118**, 755-758.
4. Z. Li, S. Sun, F. Liu, Y. Pang, J. Fan, F. Song and X. Peng, *Dyes and Pigments*, 2012, **93**, 1401-1407.