Electronic Supplementary Information (ESI)

Tetraphenylethene-Substituted Pyridinium Salt with Multiple Functionalities: Synthesis, Stimulus-Responsive Emission, Optical Waveguide and Specific Mitochondrion Imaging

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Experimental section

Materials and Instrumentations

Tetrahydrofuran (THF), toluene and ethanol were distilled from sodium benzophenone ketyl, calcium hydride and magnesium, respectively, under nitrogen immediately prior to use. MitoTracker Red CMXRos and other chemicals were purchased from Aldrich and used as received without further purification. ^{1}H and ^{13}C NMR spectra were measured on a Bruker AV 400 spectrometer in deuterated chloroform, dimethylsulfoxide (DMSO) or methanol using tetramethylsilane (TMS; $\delta = 0$) as internal reference. High resolution mass spectra (HRMS) were recorded on a GCT premier CAB048 mass spectrometer operated in MALDI-TOF mode. UV spectra were measured on a Milton Roy Spectronic 3000 Array spectrophotometer. Photoluminescence (PL) spectra were recorded on a Perkin-Elmer LS 55 spectrofluorometer. The PL quantum yield of TPE-Py in THF solution was measured using coumarin 153 (Φ_F = 58% in ethanol) as standard. Solid-state fluorescence quantum yields were determined using a calibrated integrating sphere. The morphologies of the TPE-Py nanoaggregates were investigated on a JOEL 2010 transmission electron microscope (TEM) at an accelerating voltage of 100 kV. Thermogravimetric analysis (TGA) was carried on a TGA Q5000 under air at a heating rate of 10 ^{o}C/min. Thermal transitions of the luminogen before and after mechanical, thermal and solvent perturbations were investigated by differential scanning calorimetry (DSC) using a TA DSC Q1000 under air at a heating rate of 10 ^{o}C/min. Powder X-ray diffraction (XRD) patterns were recorded on a X'pert PRO, PANalytical diffractometer using Cu-Kα radiation at 40 kV and 40 mA.

Preparation of Nanoaggregates

Stock THF solution of TPE-Py with a concentration of 2×10^{-3} M was prepared. Aliquots of the stock solution were transferred to 10 mL volumetric flasks. After appropriate amounts of THF were added, water was added dropwise under vigorous stirring to furnish 2 × 10^{-5} M solutions with different water contents (0–99 vol %). The PL measurements of the resulting solutions were then performed immediately.

Optical waveguide measurement

To measure the microarea PL spectra of a single TPE-Py microrod, the microrods dispersed on a glass cover-slip were excited with a UV laser (λ = 351 nm, Beamlok, Spectra-physics). The excitation laser was filtered with a band-pass filter (330–380 nm) and then focused to excite the microrod with an objective (50x, N.A. = 0.80). The spot size was less than 2 μm. The collected PL emission was coupled to a grating spectrometer (Acton, SP-2358) with matched ProEm: 512B EMCCD camera (Princeton Instruments).

Cell Imaging

HeLa cells were grown overnight on a plasma-treated 25 mm round coverslip mounted onto a 35 mm Petri dish with an observation window. The living cells were stained with either 5 μM of TPE-Py (by adding 2 mL of a 5 mM stock solution of TPE-Py in DMSO to 2 mL culture) or 100 nm of MitoTracker Red for 15 min. The cells were imaged under an inverted fluorescent microscope (Nikon Eclipse TE2000-U) or confocal laser scanning microscope (LSM7 DUO, Carl Zeizz).

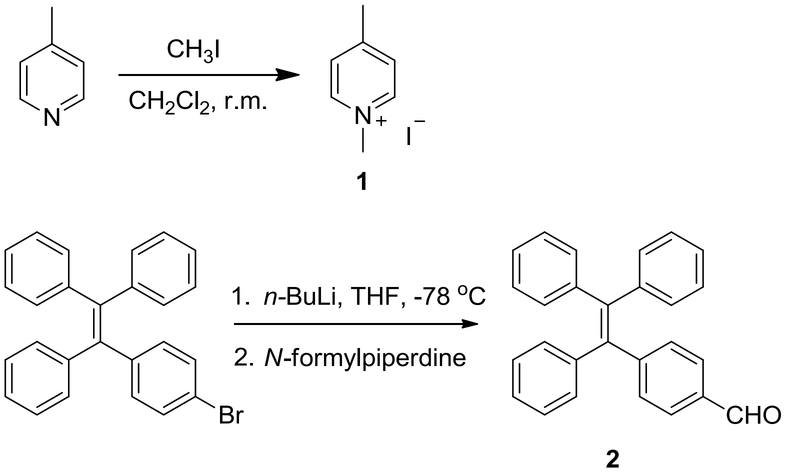
Synthesis

Compound **1** and **2** were prepared according to the synthetic route shown in Scheme S1. Details can be found in the previous publications.[1,2] Their characterization data are given below.

1,4-dimethylpyridinium iodide (1). ^{1}H NMR (400 MHz, $CDCl_3$), δ (TMS, ppm): 8.73 (d, J = 6.4 Hz, 2H), 7.92 (d, J = 6.0 Hz, 2H), 4.36 (s, 3H), 2.68 (s, 3H). HRMS (MALDI-TOF): m/z 107.7845 $[(M–I)^+$, calcd 108.0813]

4-(1,2,2-Triphenylvinyl)benzaldehyde (2). ^{1}H NMR (400 MHz, $CDCl_3$), δ (TMS, ppm): 9.90 (s, 1H), 7.61 (d, J = 8.2 Hz, 2H), 7.19 (d, J = 8.2 Hz, 2H), 7.11 (m, 9 H), 7.02 (m, 6H). HRMS (MALDI-TOF): m/z 361.1588 $[(M+1)^+$, calcd 360.1514]

4-{2-[4-(1,2,2-triphenylvinly)phenyl]vinyl}-1-methylpyridinium hexafluoro phosphate (TPE-Py). A solution of **2** (200 mg, 0.55 mmol) and iodide salt of **1** (130 mg, 0.55 mmol) in dry ethanol (15 mL) was refluxed under nitrogen for 48 h. After the reaction mixture was cooled to ambient temperature, the solvent was evaporated under reduced pressure. The solid was dissolved in acetone (5 mL) and a saturated aqueous solution of KPF_6 (5 mL) was then added. After stirring for 30 min, the solution was evaporated to dryness. The residue was purified by a silica gel column chromatography using dichloromethane/acetone mixture (5:1 v/v) as eluent to give a yellow product in 53% yield. ^{1}H NMR (400 MHz, $CDCl_3$), δ (TMS, ppm): 8.40 (d, J = 6.4 Hz, 2H), 7.80 (d, J = 5.6 Hz, 2H), 7.51 (d, J = 16 Hz, 1H), 7.31 (d, J = 8 Hz, 2H), 7.01–7.13 (m, 19H), 6.95 (d, J = 16 Hz, 1H), 4.27 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 152.36, 145.45, 145.01, 142.84, 142.68, 141.55, 140.06, 139.87, 133.22, 131.37, 130.65, 130.55, 127.91, 127.79, 127.58, 126.80, 126.73, 123.37, 123.11, 46.83. HRMS (MALDI-TOF): m/z 450.2123 [$(M\text{–}PF_6)^+$, calcd 450.2222].



Scheme S1. Synthetic route to intermediate **1** and **2**.

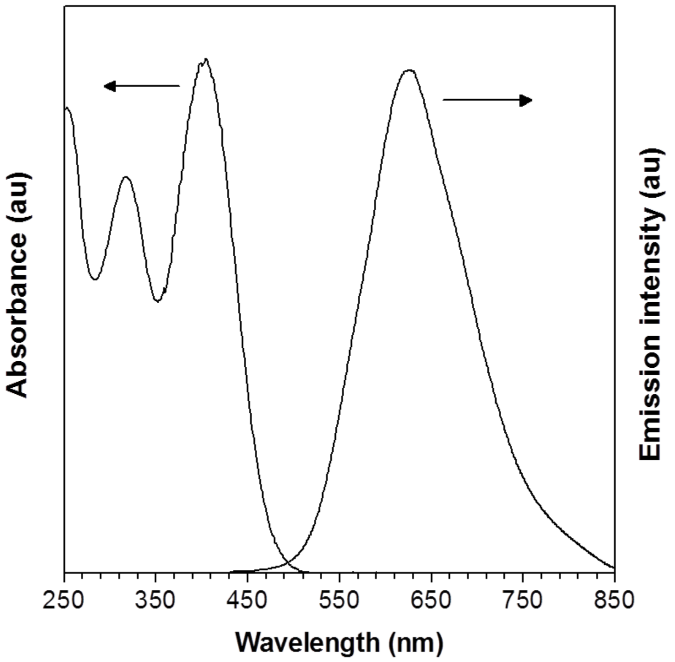


Fig. S1 Absorption and emission spectra of TPE-Py in THF solutions. Solution concentration: 20 μM; excitation wavelength: 403 nm.

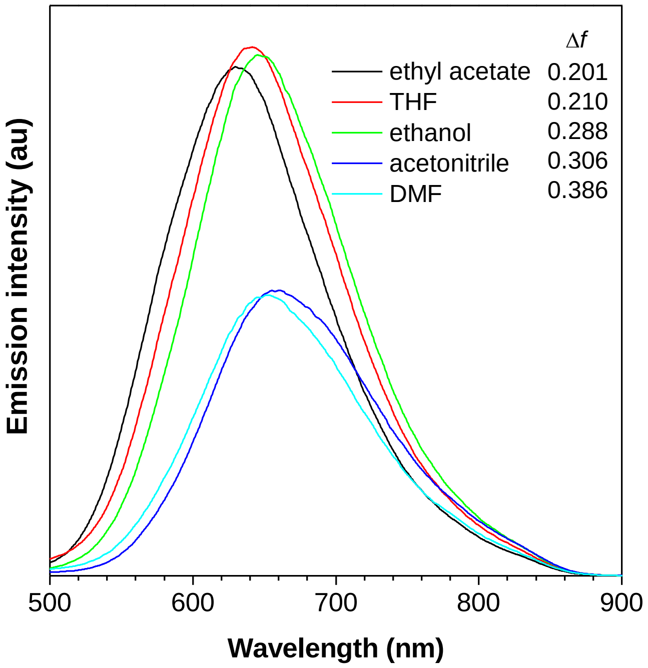


Fig. S2 Emission spectra of TPE-Py in different solvents. Solution concentration: 20 μM. Abbreviation: THF = tetrahydrofuran, DMF = dimethylformamide, Δ*f* = solvent polarity parameters.

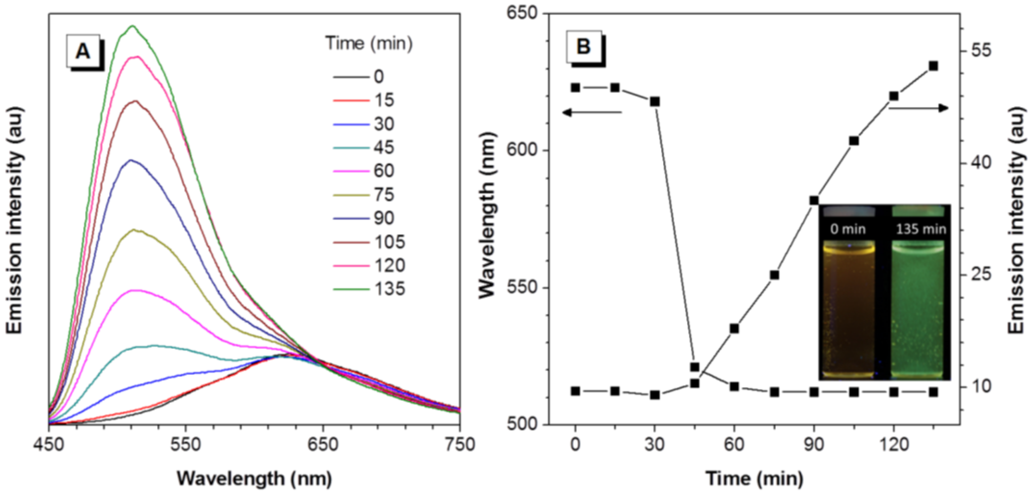


Fig. S3 Change in the emission spectra of TPE-Py in 90% aqueous mixture with standing time at room temperature. (B) Plot of wavelength and emission intensity versus the standing time from 0 to 135 min. Inset in (B): Photographs of TPE-Py in 90% aqueous mixture taken at 0 and 135 min under 365 nm UV illumination. Solution concentration: 20 μM; excitation wavelength: 386 nm.

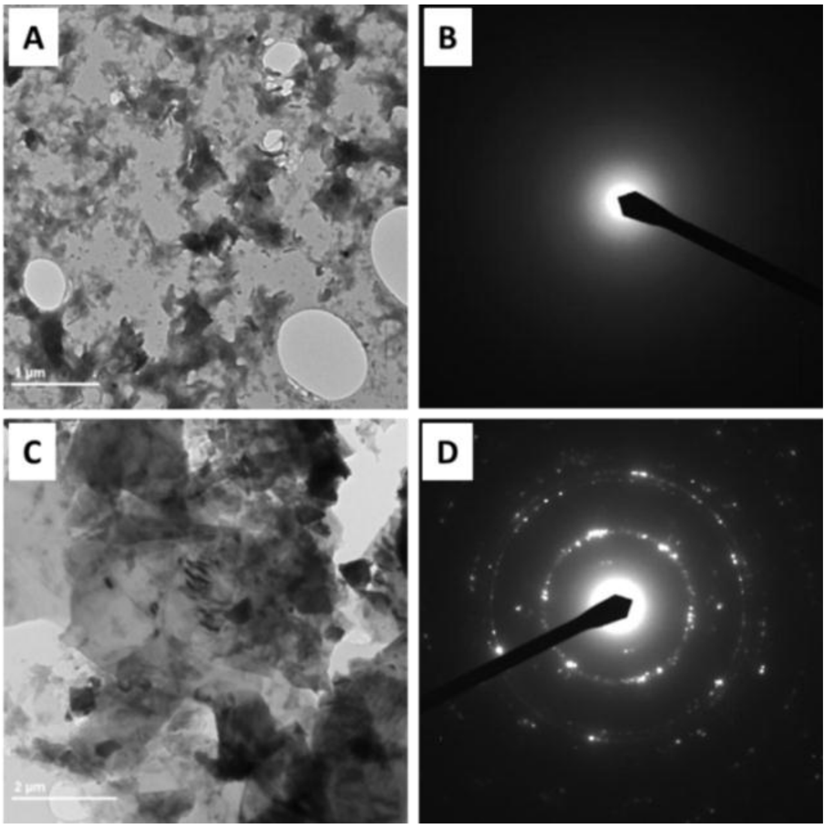


Fig. S4 (A and C) TEM images and (B and D) ED patterns of (A and B) amorphous and (C and D) crystalline aggregates of TPE-Py formed in 90% aqueous mixture (A and C) before and (B and D) after standing at room temperature for 135 min.

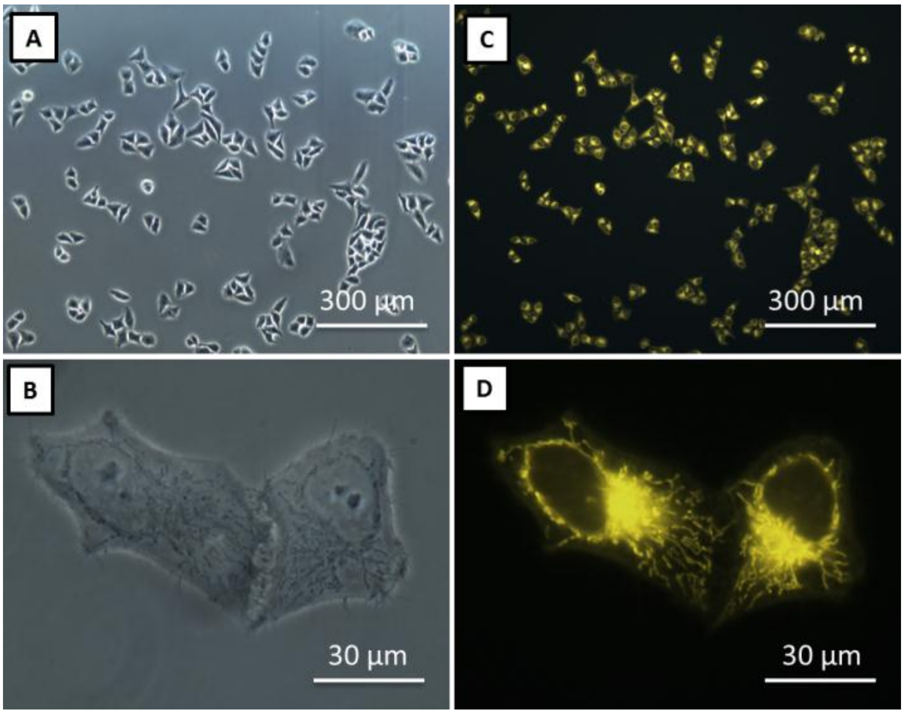


Fig. S5 (A and B) Bright-field and (C and D) fluorescent images of HeLa cells stained with TPE-Py (5 μM) for 15 min.

References

1. M. Busi, B. Cantadori, F. Boccini, R. De Zorzi, S. Geremia and E. Dalcanale, *Eur. J. Org. Chem.*, 2011, 2629.
2. (a) R. Hu, J. L. Maldonado, M. Rodriguez, C. Deng, C. K. W. Jim, J. W. Y. Lam, M. M. F. Yuen, G. Ramos-Ortiz and B. Z. Tang, *J. Mater. Chem.*, 2011, **22**, 232. (b) M. Busi, B. Cantadori, F. Boccini, R. De Zorzi, S. Geremia and E. Dalcanale, *Eur. J. Org. Chem.*, 2011, **2011**, 2629.