Electronic Supplementary Information

Aggregation-induced near-infrared emitting platinum(II) terpyridyl complex: cellular characterisation and lysosome-specific localisation

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Experimental Section

Materials and Reagents: 1-(4-Bromophenyl)-1,2,2-triphenylethylene, 4'-(4-bromophenyl)-2,2':6',2"-terpyridine, 4-iodobenzoyl chloride, bis(pinacolato)diboron, trimethylsilylacetylene and 3,6,9,12-tetraoxatridecan-1-ol were purchased from Aladdin Chemical Co. (Shanghai, China) and used as received. [1,1'-Bis(diphenylphosphino)ferrocene]dichloropalladium(II), potassium acetate, sodium carbonate, tetrakis(triphenylphosphine)palladium, *cis*-dichlorobis platinum, *trans*-dichlorobis(triphenylphosphine)palladium(II), ammonium hexafluorophosphate and cuprous iodide were purchased from JK Chemical (Beijing, China) and used without further

purification. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), LysoTracker Green and Pluronic (F127) were purchased from Sigma-Aldrich (Shanghai, China). Different organic solvents of acetonitrile (ACN), methanol (MeOH), ethanol (EtOH), 1,4-dioxane (DOA), diethyl ether (DE), hexane (HA), ethyl acetate (EA), tetrahydrofuran (THF), *N,N*-dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) were of analytical grade and were used without further purification. The reactions were performed under an argon atmosphere using standard Schlenk techniques unless otherwise specified. The water used in all experiments was prepared with a Milli-Q water purification system; it had a resistivity of > 18.2 M W cm^{-1}.

Characterisation: The ^{1}H and ^{13}C NMR spectra were obtained at room temperature on a Bruker 400 MHz spectrometer with tetramethylsilane (δ = 0) used as an internal reference. The high-resolution mass spectra were obtained with a GCT Premier CAB048 mass spectrometer operated in matrix-assisted laser desorption ionisation time-of-flight mode. Fluorescence spectra were recorded on a SPEX Fluorolog-3-TCSPC spectrometer (Horiba), while absorption spectra were recorded on a Beckman DU 800 spectrophotometer. Infrared spectra (IR) spectra were obtained as KBr disks by using a Bio-Rad FTS-7 FTIR spectrometer (4000-400 cm^{-1}).The nanoparticle (NP) size and distribution of the NPs were measured by dynamic light scattering (DLS), using a Zetasizer Nano ZS (Malvern Panalytical). Olympus IX71 was used for confocal laser scanning microscopy imaging. The morphology of the NPs was investigated with an FEI Tecnai G2 F30.

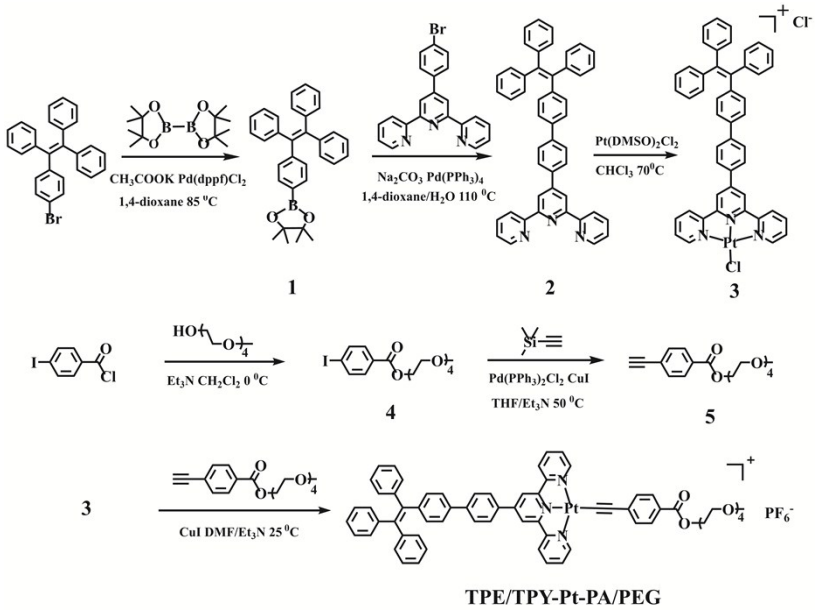
Fabrication of TPE/TPY-Pt-PA/PEG@F127 NPs: TPE/TPY-Pt-PA/PEG (0.3 mg) and F127 (2 mg) were first dissolved in 200 μL of dimethyl sulfoxide (DMSO). The mixture of TPE/TPY-Pt-PA/PEG and F127 was then added dropwise into 5 mL of deionised water at a rate of 0.02 mL min^{-1} via a syringe pump. The colloidal dispersion was further stirred for another 4 h, and the temperature was fixed at 25 °C during the self-assembly process. The organic solvent was removed by dialysis (molecular weight cutoff, 3000 Da) against deionised water for 3 days.

MTT Assays: HeLa cells were plated into 96-well tissue culture plates (10^4 cells per well) in growth medium (100 μL) and incubated at 37 °C under a 5% CO_2 atmosphere for 24 h. TPE/TPY-Pt-PA/PEG@F127 NPs and cisplatin (positive control) were then added to the wells to concentrations ranging from 1 to 100 μM. Wells containing growth medium without cells were used as blank controls. The microplate was incubated at 37 °C under a 5% CO_2 atmosphere for 24 h, and MTT in phosphate buffered saline (PBS) (5 mg mL^{-1}, 10 μL) was then added to each

well, after which incubation was continued for another 2.5 h. The culture medium was removed to reduce interference with the spectrometer reading. DMSO (100 μL) was added to each well and mixed thoroughly by pipetting 10−20 times to dissolve the blue formazan. Absorption was measured at 570 nm by using a microplate reader (PowerWave XS, BioTek). The half maximal inhibitory concentration (IC_{50}) value of the complex was determined from the dose dependence of surviving cells after exposure to the complex for 24 h relative to the controls.

Live-Cell Confocal Microscopy: HeLa cells in growth medium were incubated with the TPE/TPY-Pt-PA/PEG@F127 NPs (20 μM) at 37 °C for 4 h, followed by incubation with LysoTracker Green (100 nM) for 20 min at 37 °C. The culture medium was then removed and washed thoroughly with PBS (1 mL × 5). The treated coverslips were then mounted in standard mounting media and imaged using a fluorescence microscope (Olympus IX71). The excitation wavelength was 488 nm for both TPE/TPY-Pt-PA/PEG@F127 NPs and LysoTracker Green at 60% laser power. For TPE/TPY-Pt-PA/PEG NPs, a 600–750 nm emission filter was used. For LysoTracker Green, a 500–580 nm emission filter was used. The overlap coefficient of TPE/TPY-Pt-PA/PEG@F127 NPs with LysoTracker Green was determined with an Olympus Fluoview ver. 4.2a viewer.

Synthesis



Scheme S1. Synthetic route to TPE/TPY-Pt-PA/PEG.

4,4,5,5-Tetramethyl-2-[4-(1,2,2-triphenylvinyl)phenyl]-1,3,2-dioxaborolane (1). This was synthesised according to the reported literature method.[1] A Schlenk tube was charged with 1-(4-bromophenyl)-1,2,2-triphenylethylene (2.00 g, 4.87 mmol), potassium acetate (1.91 g, 19.48 mmol), bis(pinacolato)diboron (1.49 g, 5.89 mmol) and Pd(dppf)Cl_2 (177 mg, 0.24 mmol) in anhydrous 1,4-dioxane (20 mL). The system was degassed by applying three freeze–pump–thaw cycles to replace air with argon. The reaction was performed at 85 °C for 15 h. After the mixture was cooled to room temperature, it was diluted with dichloromethane, washed with water and dried over $MgSO_4$. After solvent removal under reduced pressure, the residue was purified by silica gel column chromatography (hexane/dichloromethane, 7:3) to afford **1** (2.11 g, yield: 95%) as a white solid. ^{1}H NMR (400 MHz, chloroform-d), δ (ppm): 7.11–7.05 (d, J = 7.4 Hz, 2H), 7.13–6.90 (m, 17H), 1.31 (s, 12H).

4'-[4'-(1,2,2-Triphenylvinyl)-(1,1'-biphenyl)-4-yl]-2,2':6',2''-terpyridine (2). Compound **1** (550 mg, 1.20 mmol) and 4,4,5,5-tetramethyl-2-(4-(1,2,2-triphenylvinyl)phenyl)-1,3,2-dioxaborolane (458 mg, 1 mmol) were dissolved in 1,4-dioxane (30 mL), and then 2 M aqueous Na_2CO_3 solution (3.0 mL) was added. The mixture was stirred for 40 min under an argon atmosphere at room temperature. Pd(PPh_3)$_4$ (34 mg, 0.03 mmol) was then added, and the reaction mixture was stirred at 110 °C for 22 h. After the product was cooled to room temperature, it was concentrated and purified by column chromatography (dichloromethane/*n*-hexane, 1:2) to afford **2** (429 mg, yield: 67%). ^{1}H NMR (400 MHz, chloroform-d), δ (ppm): 8.77 (s, 2H), 8.74 (d, J = 4.6 Hz, 2H), 8.68 (d, J = 7.8 Hz, 2H), 7.96 (d, J = 8.2 Hz, 2H), 7.89 (t, J = 7.8 Hz, 2H), 7.70 (d, J = 8.2 Hz, 2H), 7.43 (d, J = 8.2 Hz, 2H), 7.39–7.33 (m, 2H), 7.20–6.97 (m, 17H).

Compound 3. A stirred mixture of Pt(DMSO)$_2$$Cl_2$ (0.20 g, 0.48 mmol) and **2** (307 mg, 0.48 mmol) in $CHCl_3$ (30 mL) was heated at 70 °C for 24 h to give a yellow precipitate. The resultant precipitate was collected and used as a crude product without further purification for the next step.

2,5,8,11-Tetraoxatridecan-13-yl-4-iodobenzoate (4). 2,5,8,11-Tetraoxatridecan-13-ol (208 mg, 1.0 mmol) was dissolved in 10 mL of anhydrous CH_2Cl_2/Et_3N solution (1:1 v/v) at 0 °C. 4-Iodobenzoyl chloride (399 mg, 1.5 mmol) was then dissolved in 10 mL of anhydrous CH_2Cl_2 and was added to the reagent. The reaction mixture was stirred at 0 °C for 2 h, and then the reaction

solvent was removed *in vacuo*. The crude product was purified by column chromatography (petroleum ether/ethyl acetate, 1:1) to afford **4** (367 mg, yield: 84%). ^{1}H NMR (400 MHz, chloroform-d), δ (ppm): 7.78 (d, J = 6.2 Hz, 2H), 7.35 (d, J = 5.6 Hz, 2H), 4.51–4.41 (m, 2H), 3.86–3.77 (m, 2H), 3.72–3.59 (m, 10H), 3.54 (d, J = 3.8 Hz, 2H), 3.37 (s, 3H).

2,5,8,11-Tetraoxatridecan-13-yl-4-ethynylbenzoate (5). Compound **4** (438 mg, 1 mmol) was dissolved in 25 mL of dry THF/Et_3N solution (3:1, v/v) in a Schlenk flask and degassed with argon for 15 min. Subsequently, 21 mg of $Pd(PPh_3)_2Cl_2$ (30 μmol) and 12 mg of CuI (63 μmol) were added, followed by the addition of 0.7 mL of trimethylsilylacetylene (5 mmol). The reaction was stirred at 50 °C for 22 h. After filtration through a bed of Celite, the solvent was removed *in vacuo*. The crude product was used for the next step without further purification. The crude product obtained was dissolved in 10 mL of THF. Tetrabutylammonium fluoride (5 mL of a 1 M solution in THF) was added, and the resulting mixture was stirred at room temperature for 2 h. The solution was then diluted with 20 mL of ethyl ether, poured into a separatory funnel, and washed with water (30 mL × 1). The organic layer was collected, and the solvent was removed *in vacuo*. The crude product was purified by column chromatography (petroleum ether/ethyl acetate, 1:1) to afford **5** (151 mg, yield: 45%). ^{1}H NMR (400 MHz, chloroform-d), δ (ppm): 8.02 (d, J = 10.2 Hz, 2H), 7.55 (d, J = 8.6 Hz, 2H), 4.54–4.43 (m, 2H), 3.87–3.79 (m, 2H), 3.75–3.60 (m, 10H), 3.54 (d, J = 3.6 Hz, 2H), 3.37 (s, 3H), 3.24 (s, 1H).

TPE/TPY-Pt-PA/PEG. Compounds **5** (67.2 mg, 0.20 mmol) and **3** (104 mg, 0.12 mmol) were dissolved in degassed dimethylformamide (10 mL). Triethylamine (1 mL) and a catalytic amount of CuI were added to the reaction mixture. After the reaction mixture was stirred for one day, the resulting mixture was evaporated to dryness and the residue was purified by recrystallisation through slow diffusion of diethyl ether into methanol solution to give a dark-red solid (60.2 mg, yield: 43%). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 9.07–8.96 (m, 4H), 8.87 (d, J = 7.8 Hz, 2H), 8.50 (t, J = 7.8 Hz, 2H), 8.19 (d, J = 8.2 Hz, 2H), 7.89 (d, J = 8.4 Hz, 2H), 7.84 (t, J = 8.0 Hz, 4H), 7.65 (d, J = 8.4 Hz, 2H), 7.54 (d, J = 8.2 Hz, 2H), 7.22–6.98 (m, 17H), 4.30 (s, 2H), 3.71 (s, 2H), 3.60–3.56 (m, 2H), 3.55–3.45 (m, 8H), 3.41–3.38 (m, 2H), 3.21 (s, 3H); ^{13}C NMR (400 MHz, DMSO-d_6), δ (ppm): 165.82, 159.10, 154.46, 143.63, 142.46, 141.59, 140.49, 136.90, 133.95, 132.28, 131.92, 131.22, 131.13, 130.15, 129.57, 128.84, 128.51, 128.44, 128.35, 127.48, 127.30, 127.17, 126.72, 121.29, 79.80, 79.47, 79.14, 71.77, 70.39, 70.29, 70.09, 68.86, 64.47,

58.55; IR peaks (KBr disk, ν/cm^{-1}): 2118 *ν*(C≡C), 1714, 1601, 1558, 1477, 1463, 1444, 1411, 1359, 1272, 1173, 1105, 1031, 1003, 844, 785, 767, 736, 701. FAB-MS: *m*/*z* = 1169.3794 $[M]^+$.

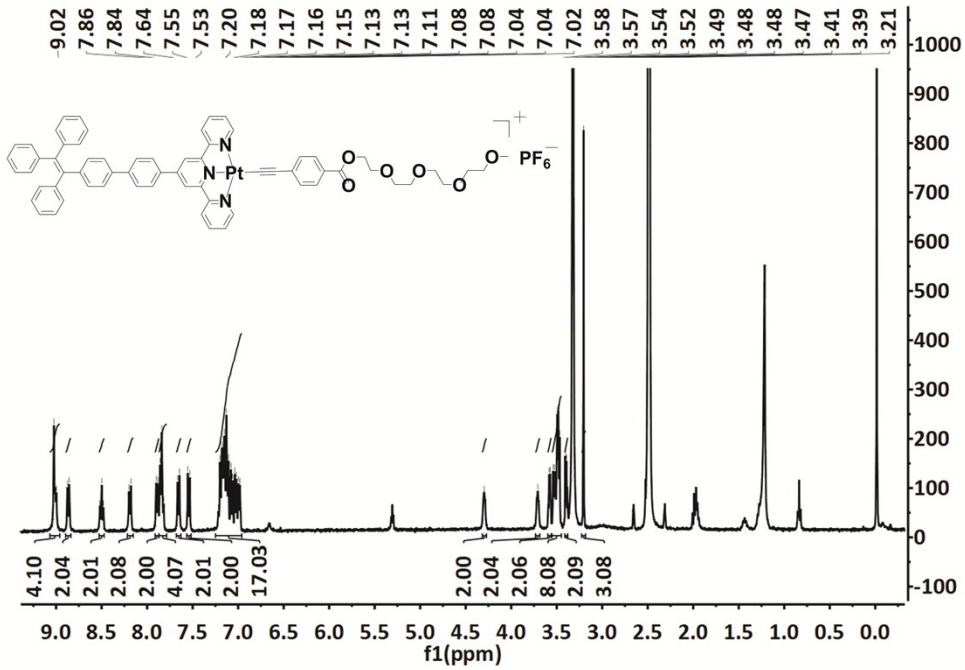


Fig. S1. ^{1}H NMR spectrum of TPE/TPY-Pt-PA/PEG (400 MHz, DMSO-d_6)

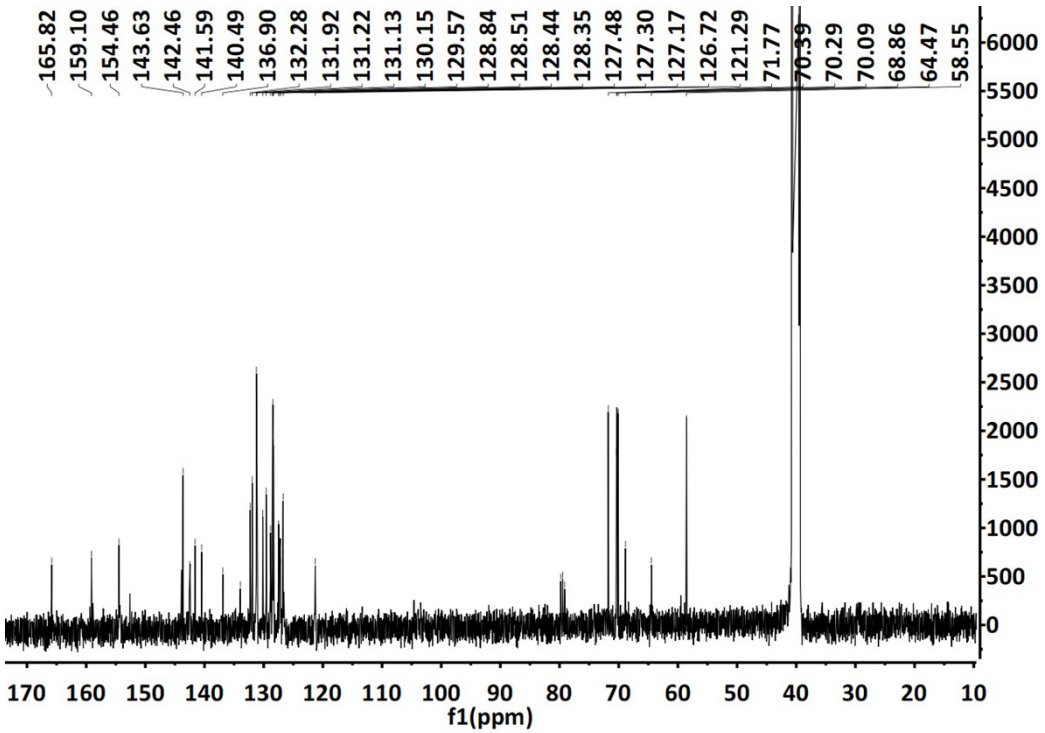


Fig. S2. ^{13}C NMR spectrum of TPE/TPY-Pt-PA/PEG (400 MHz, DMSO-d_6)

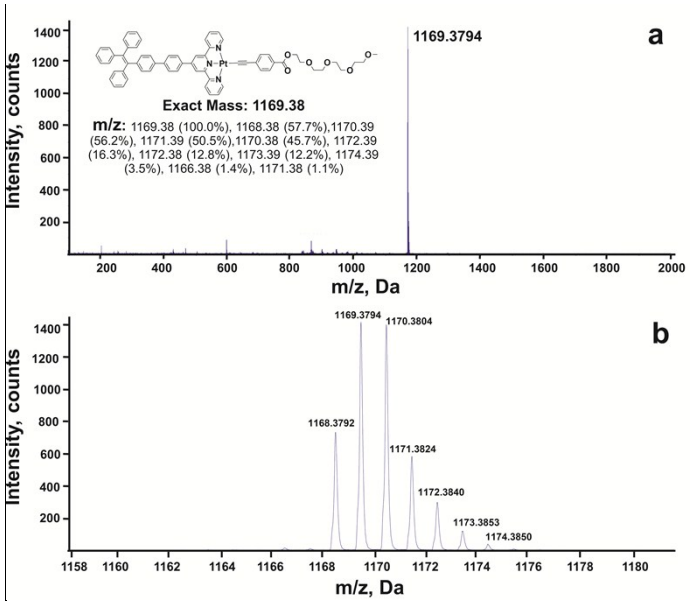


Fig. S3. High-resolution mass spectrum of TPE/TPY-Pt-PA/PEG: (a) full spectrum and (b) expansion from 1158 to 1180 Da. The peaks are labelled by mass. The m/z of TPE/TPY-Pt-PA-PEG predicted by ChemBioDraw Ultra 13.0 are 1169.38 (100.0%), 1168.38 (57.7%), 1170.39 (56.2%), 1171.39 (50.5%), 1170.38 (45.7%), 1172.39 (16.3%), 1172.38 (12.8%), 1173.39 (12.2%), 1174.39 (3.5%), 1166.38 (1.4%) and 1171.38 (1.1%).

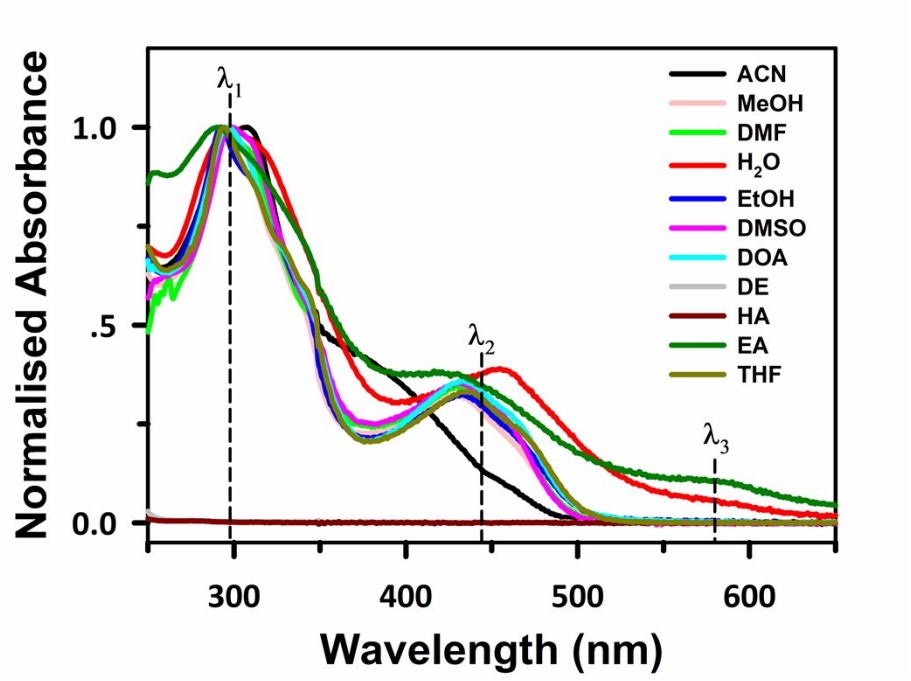


Fig. S4. Normalised UV–vis absorption spectra of TPE/TPY-Pt-PA/PEG in different solvents.

Table S1. UV-vis data of TPE/TPY-Pt-PA/PEG in different solvents at 298 K.

TPE/TPY-Pt-PA/PEG	λ_1[a] / nm	λ_2[a] / nm	λ_3[a] / nm
ACN	300	400	-
MeOH	292	431	-
DMF	297	430	-
H_2O	299	455	581
EtOH	293	432	-
DMSO	298	430	-
DOA	296	433	-
DE	-	-	-
HA	-	-	-
EA	290	435	587
THF	294	436	-

[a]The maximum absorption peak.

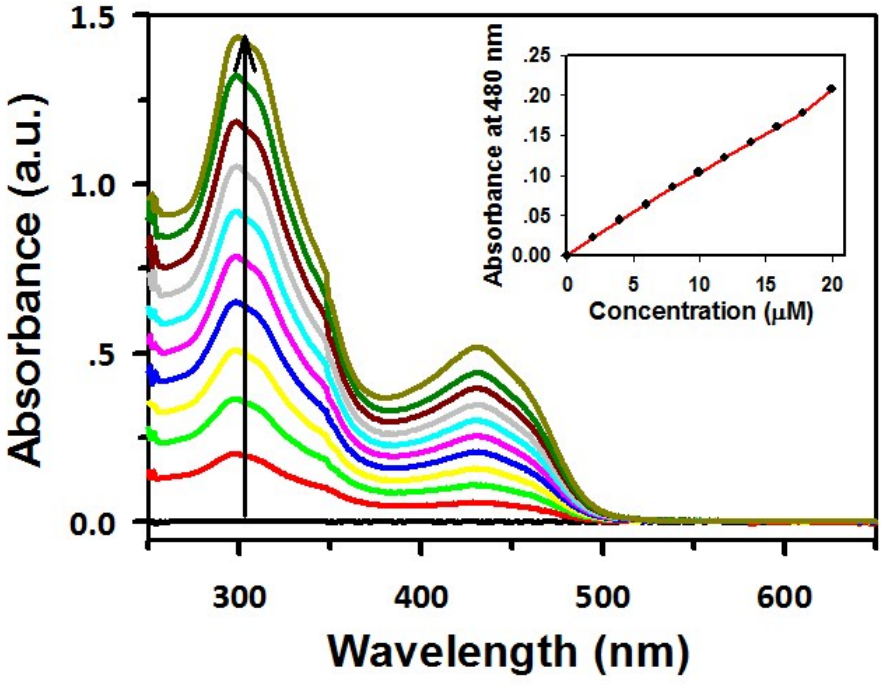


Fig. S5. Concentration-dependent UV–vis absorption spectra of TPE/TPY-Pt-PA/PEG in acetonitrile (from 0 μM to 20 μM) at 298 K. The inset shows the absorbance at 480 nm at different concentrations of TPE/TPY-Pt-PA/PEG.

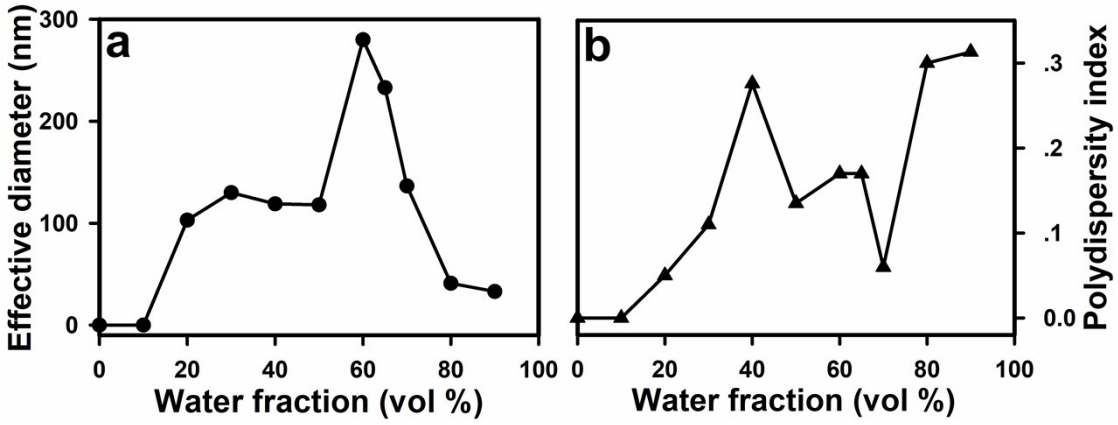


Fig. S6. (a) Effective diameter (nm) of TPE/TPY-Pt-PA/PEG nanoaggregates in acetonitrile with increasing water content from 0% to 90%. (d) Polydispersity indices of TPE/TPY-Pt-PA/PEG nanoaggregates in acetonitrile with increasing water content from 0% to 90%.

Table S2. Photophysical data for TPE/TPY-Pt-PA/PEG at different aggregate states.

	Aggregate[a]	**Solid state**[b]	**NPs**[c]
Quantum yield (Φ,%)[d]	3.41	1.31	0.51
Lifetime (τ, ns)[e]	19.06	3.16	14.16
Emission max (λ_{em}, nm)[f]	730	728	736

[a]Aggregates in CH_3CN/water solution (3:7, v/v), 100 μM; [b]thin film on glass cover slide; [c]TPE/TPY-Pt-PA/PEG@F127 NPs in water, 100 μM; [d]quantum yield measured using an integrating sphere; [e]time-domain method; [f]emission maximum.

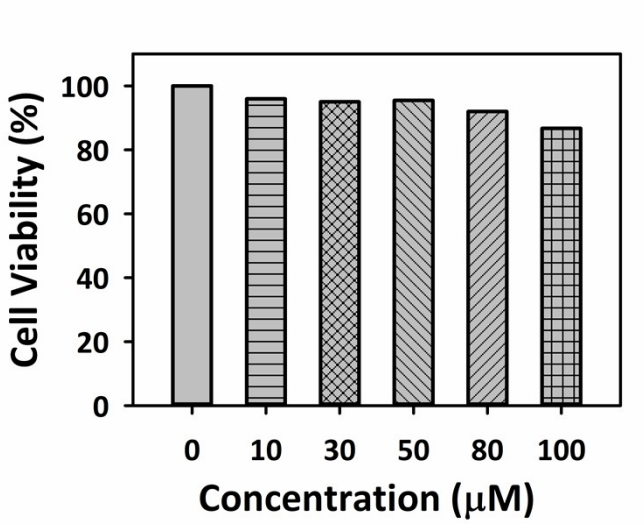


Fig. S7. Metabolic viability of HeLa cancer cells after incubation with TPE/TPY-Pt-PA/PEG@F127 NPs at different TPE/TPY-Pt-PA/PEG concentrations for 24 h

1. D. Ding, C. C. Goh, G. Feng, Z. Zhao, J. Liu, R. Liu, N. Tomczak, J. Geng, B. Z. Tang, L. G. Ng and B. Liu, *Adv. Mater.*, 2013, **25**, 6083–6088.