Supporting Information

Cellular metabolism of fluorescent nanoprobes formed by self-assembly of amphiphiles: dynamic trafficking from Golgi apparatus to Lysosome

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1. Chemical structure and morphology of self-assembled structures of TPE-11

Due to the amphiphilic feature (the hydrophilic pyridinium head and aromatic and aliphatic joint), TPE-11 self-assembles in aqueous solution and forms flake-like structures. The average diameter is 40 ± 11 nm, and the thickness indicated by small angle X-ray scattering is 6.3×0.2 nm, corresponding to the extended length of TPE-11 molecule. The zeta potential of the self-assembled nanoparticles is 21.7 mV, which is determined by Zeta Sizer at 37 °C. This result indicate that the nanoparticles should be positively charged.

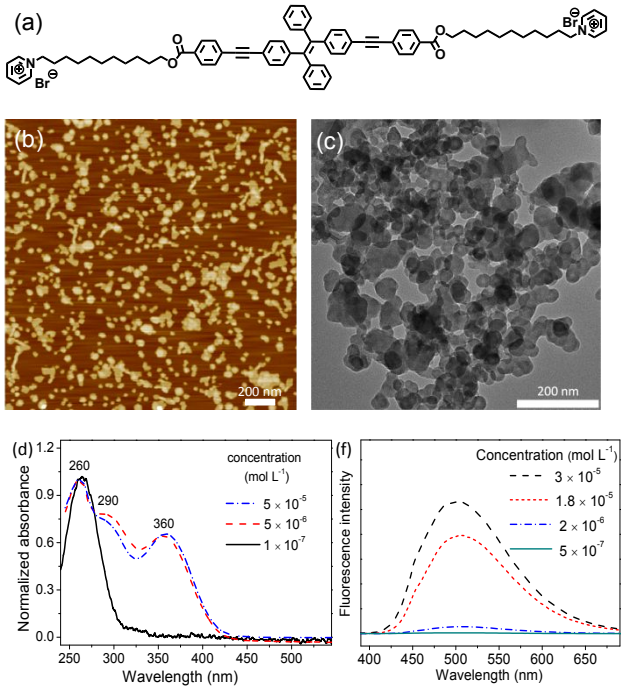

Figure S1 (a) molecular structure of TPE-11; (b) AFM and (c) TEM images of the self-assembled structure of TPE-11 in aqueous solution. The (d) UV-vis and (f) fluorescence spectra of TPE-11 at different concentrations.

2. The detailed parameters for CLSM images and spectra of TPE-11 in cells

The instrument for CLSM images was TCS-SP8 (LEICA, Germany). The detailed parameters for CLSM were listed as following Table S1 and Table S2. The fluorescence spectra of TPE-11 in HeLa cells were shown in Figure S2 (excitation wavelength, 405 nm).

Table S1 The parameters of CLSM Settings

Name	Value
Scan Mode	xyz
Scan Direction X	Bidirectional
Scan Speed	400 Hz
StagePosX	77601.5 µm
StagePosY	40303.1 µm
ZPosition	157.3 µm
IsSuperZ	0
Magnification	63
Objective Name	HC PL APO CS2 63x/1.40 OIL
Numerical Aperture	1.4
RefractionIndex	1.518
Pinhole	114.7 µm
Pinhole Airy	1.20 AU
Emission Wavelength for PinholeAiry Calculation	580.0 nm

Table S2 The parameters of CLSM detectors

Name	Channel	Type			Location	Active	Gain	Offset
PMT 1	Channel 1	PMT	(497nm - 553nm)		Internal	Inactive	845.2	-0.13
HyD SMD 2	Channel 2	HyD mode	(558nm - 563nm)	Standard	Internal	Inactive	10	-0.01
PMT 3	Channel 3	PMT	(563nm - 569nm)		Internal	Inactive	830.6	0
HyD SMD 1	Channel 4	HyD mode	(569nm - 574nm)	Standard	Internal	Inactive	100	-0.01
PMT 5	Channel 5	PMT	(574nm - 686nm)		Internal	Active	771.2	-1.6
PMT Trans	Transmission Channel	PMT			TLD	Active	420.6	-3.31

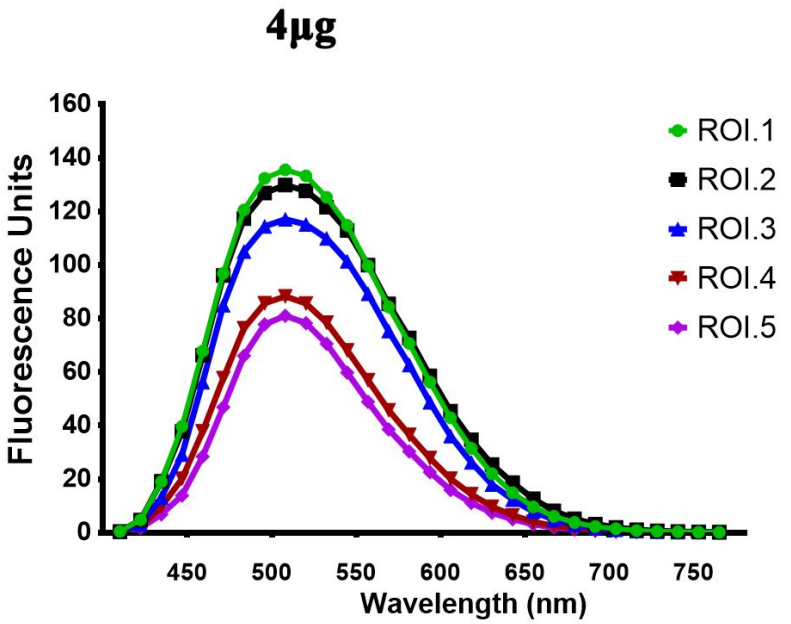

Figure S2 The spectra of TPE-11 in HeLa cells. HeLa cells were treated TPE-11 with 4ug mL^{-1}. ROI means “Region of interest”.

3. The cell lines were tested for TPE-11 treatment

We have tested A549 (human lung adenocarcinoma) and MCF7 (human breast adenocarcinoma) cell lines besides HeLa cells. Fluorescent signals in these two cells are shown the similar results with HeLa cells. The typical CLSM images after TPE-11 treatment with MCF7 and A549 cells are shown in Figure S3. We also tested TPE-11 treatment in human embryonic stem cells (hESCs) and their differentiation into neuron-like cells [1].

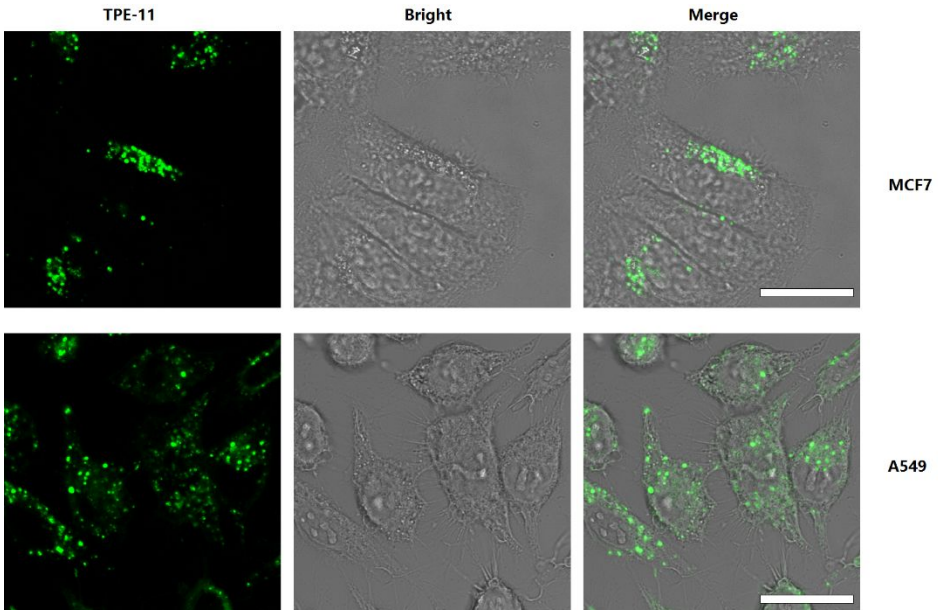

Figure S3 The CLSM images of MCF7 and A549 cells labeled with TPE-11. Scale Bar: 25 μm

4. The localization analysis for TPE-11 and Golgi apparatus (GOL) or Endoplasmic reticulum (ER) proteins on HeLa cells

The co-localization of TPE-11 with GOL and ER was realized by labeling them with GM130 and Calnexin, respectively. As shown in 2D images, the fluorescent signal of TPE-11 (blue) overlapped, or located near with GM130 (red) rather than with Calnexin (green) after being treated with TPE-11 for 4 h (Figure S4a) and 6 h (Figure S4b).

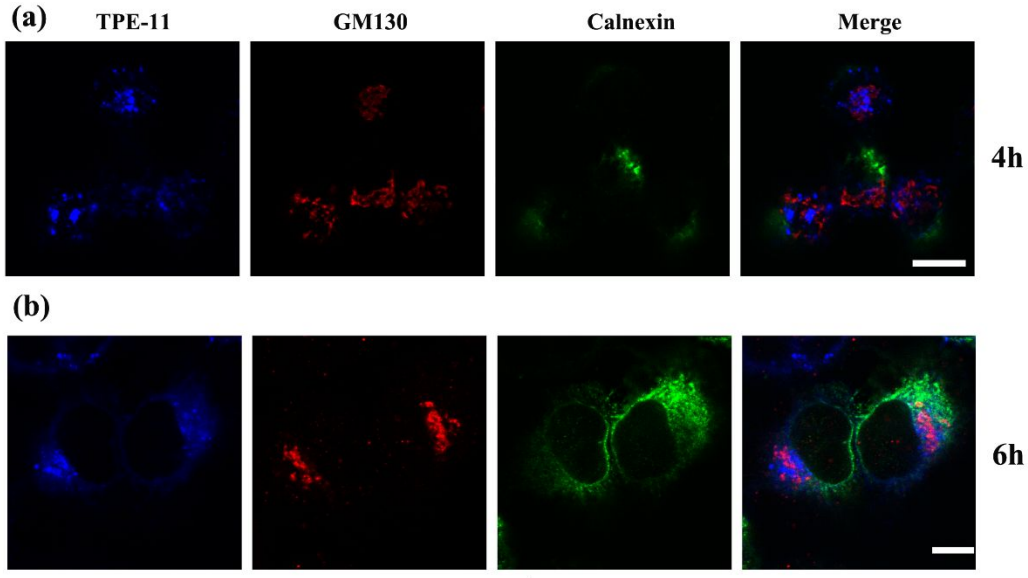

Figure S4 The co-localization analysis of TPE-11 (blue) with Calnexin (green) and GM130 (red) in HeLa cells. The cells were treated by TPE-11 for (a) 4 h and (b) 6 h. Scale bars: 10 μm.

5. The co-localization analysis from multiple viewpoints and more cell types

We expanded the number of cells for co-localization analysis. We also used other cell line A549 and made local magnification besides HeLa cells. The result was shown that TPE-11 particles surrounded GM130 (Golgi apparatus) closely and far away from Calnexin (ER) in A549 cells (Figure S5b, S5c), like in HeLa cells (Figure S5a).

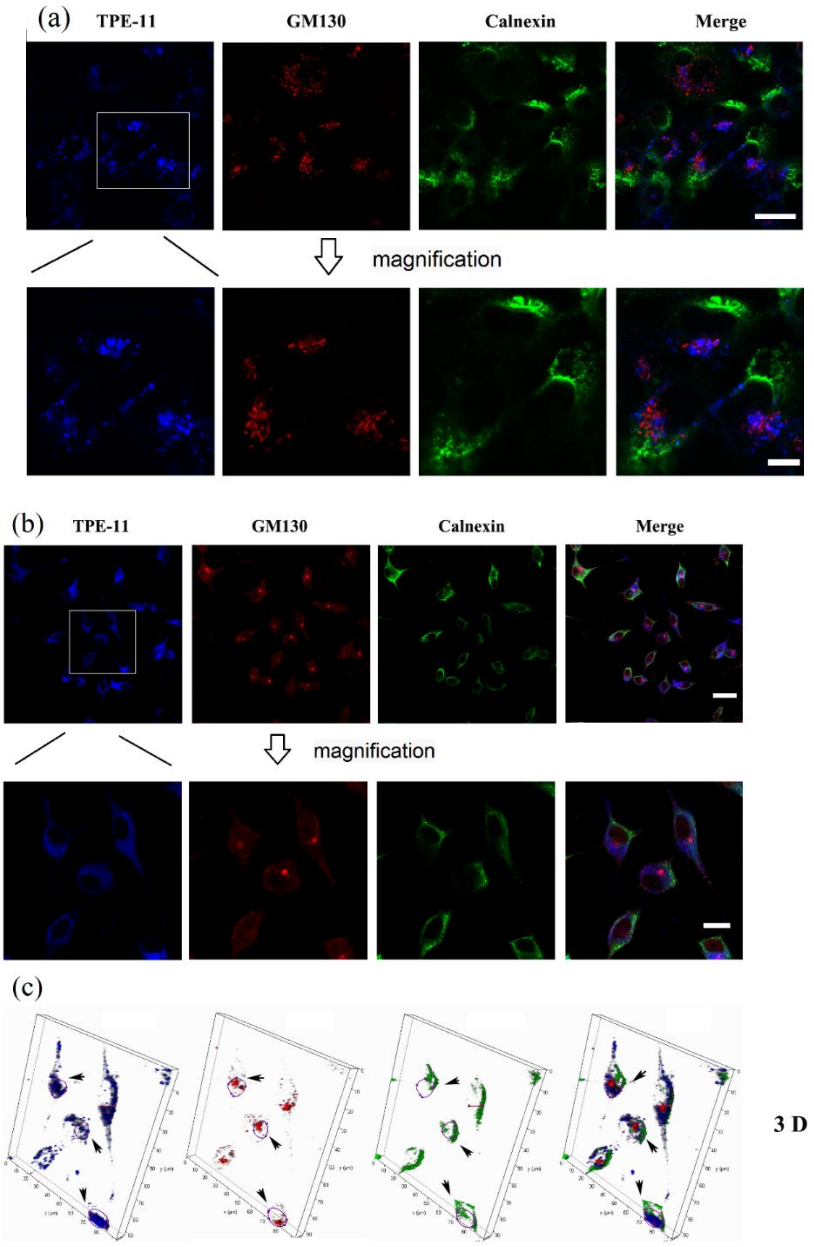

Figure S5 (a) Co-localization analysis of TPE-11 6 h-treatment to A549 cells. Scale bar 20 μm (up),7.5 μm (down). (b) TPE-11 6h treatment in A549 cells lization analysis. Scale bar 30 μm (up), 15 μm (down). (c) The 3D images of localization analysis. The circles indicate the aggregated TPE-11 particles in cells. The co-localizations of TPE-11 (blue) and GM130 (red) were indicated by arrows (black).

The protein GM130 represents for Golgi apparatus, or protein Calnexin for ER. We use “Leica Application Suite X” tool to analyze the co-localization of TPE-11 with Calnexin and GM130. The

typical co-localization analysis for TPE-11 and GM130 (or Calnexin) was shown in Table S3 and S4.

Table S3 Co-localization analysis for TPE-11 and GM130, Calnexin in HeLa cells

TPE-11 (blue) and GM130 (red)	Colocalization Unit	
ROI: regions of interest	Overlap Coefficient	0.7278
	Colocalization Rate	65.57%
	Colocalization Area	6.31 μm^2
	Area ROI	9.69 μm^2
	Area Foreground	9.62 μm^2
	Area Background	0.07 μm^2
TPE-11 (blue) and Calnexin (green)	Overlap Coefficient	0.6304
	Colocalization Rate	39.01%
	Colocalization Area	6.40 μm^2
	Area ROI	42.38 μm^2
	Area Foreground	16.40 μm^2
	Area Background	25.98 μm^2

Table S4 Co-localization analysis for TPE-11 and GM130, Calnexin in A549 cells

TPE-11 (blue) and GM130 (red)	Colocalization Unit	
ROI: regions of interest	Overlap Coefficient	0.6704
	Colocalization Rate	55.42%
	Colocalization Area	10.42 μm^2
	Area ROI	22.33 μm^2
	Area Foreground	20.27 μm^2
	Area Background	2.06 μm^2
TPE-11 (blue) and Calnexin (green)	Overlap Coefficient	0.5916
	Colocalization Rate	42.26%
	Colocalization Area	16.85 μm^2
	Area ROI	41.24 μm^2
	Area Foreground	39.88 μm^2
	Area Background	1.36 μm^2

We compared 30 groups of CLSM images with co-localization rates in HeLa and A549 cells. The result was shown in Figure S6. There were significant difference for TPE-11 and GM130 with TPE-11 and Calnexin by variance analysis. The Statistical p value is less than 0.01 (**) for HeLa cells and less than 0.05(*) for A549 cells.

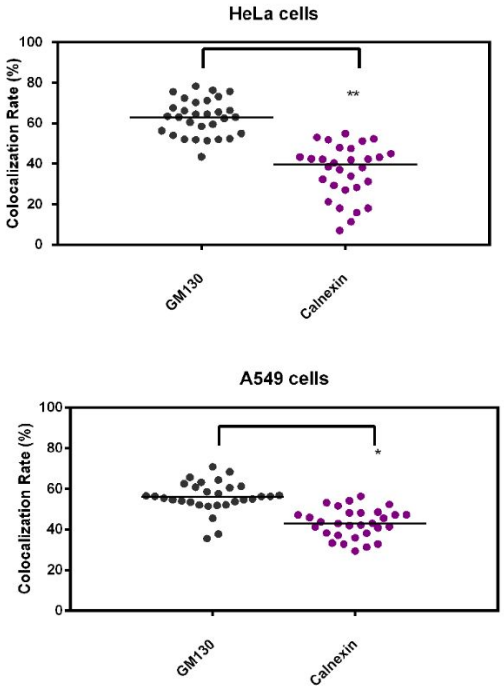

Figure S6 The analysis on co-localization rate in HeLa and A549 cells.

6. The aggregated TPE-11 was partly co-localized with Lysosome in cells for long time culture

The co-localization of TPE-11 and Lysosome was analyzed on the cells cultured for 12 h. The CLSM images show that TPE-11 and the Lyso-tracker were largely co-localized in 2D images (Figure S7a) or 3D (Figure S7b).

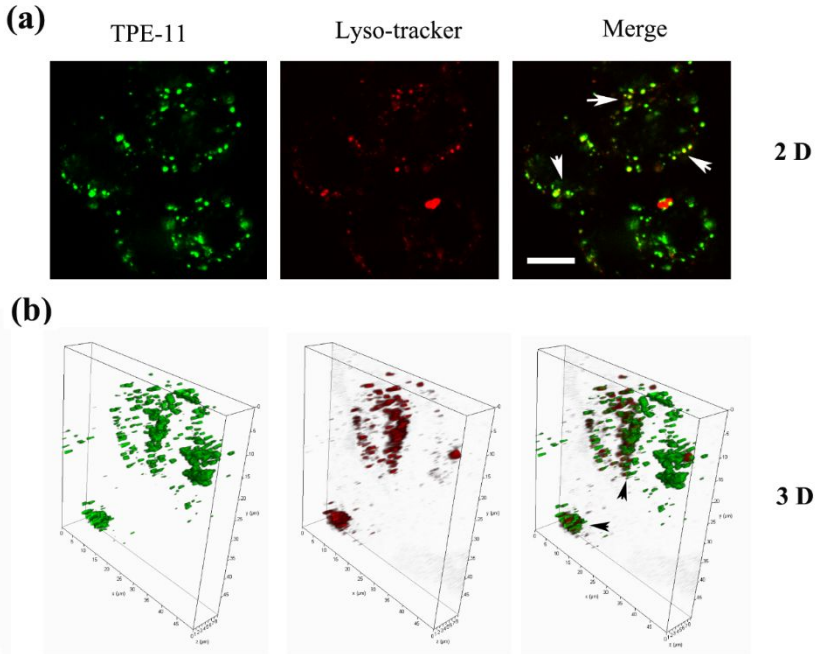

Figure S7 CLSM images of the HeLa cells treated with TPE-11 (green) and Lyso-tracker (red) for 12 h. (a) 2D images, Scale bar: 10 μm. (b) 3D images. The colocalizations of TPE-11 (blue) and lyso-tracker (red) were indicated by arrows.

7. CLSM analysis on the cells treated with different concentrations of TPE-11

Two typical concentrations were selected to investigate the concentration effect on the labeling of the HeLa cells. The Labeling followed the procedures described in the experimental section. The culturing time was 3 h. The concentrations were 16.0 and 4.0μg mL^{-1}, noted as high and low concentrations, respectively. At 16.0 μg mL^{-1}, TPE-11 has the highest quantum yield. As shown in Figure S8, the two groups of cells were labeled with TPE-11, and the fluorescence of the cells labeled with 4.0 μg mL^{-1} TPE-11 is slightly weaker, but still good enough for imaging of the cells.

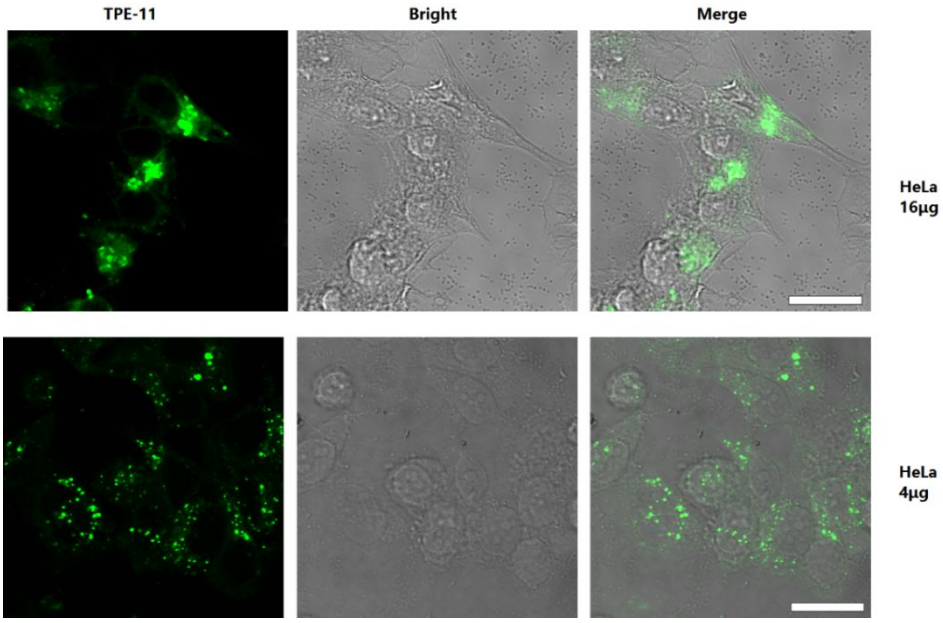

Figure S8 The CLSM images of HeLa cells labeled with 16 and 4 μg mL^{-1} TPE-11. Scale Bar: 25 μm.

Reference

1. Zhou S, Zhao H, Feng R, Ding L, Li Z, Deng C, He Q, Liu Y, Song B and Li Y. Application of amphiphilic fluorophore-derived nanoparticles to provide contrast to human embryonic stem cells without affecting their pluripotency and to monitor their differentiation into neuron-like cells. Acta Biomater. 2018:78:274-284.