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A Novel Tumor and Mitochondria Dual-targeted Photosensitizer Showing Ultra-efficient Photodynamic Anticancer Therapy Activities

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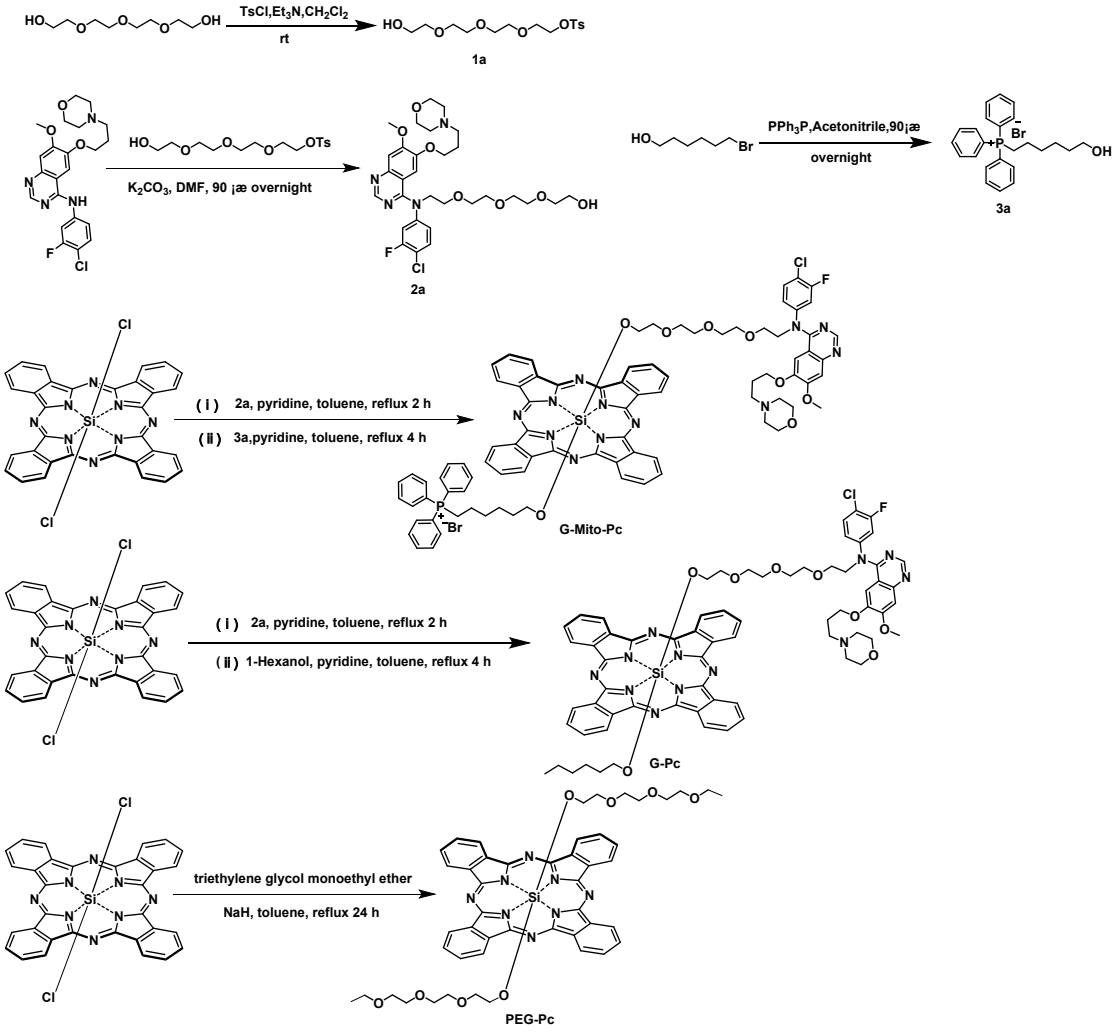
Ⅰ. Experimental Section

1. Materials and general methods:

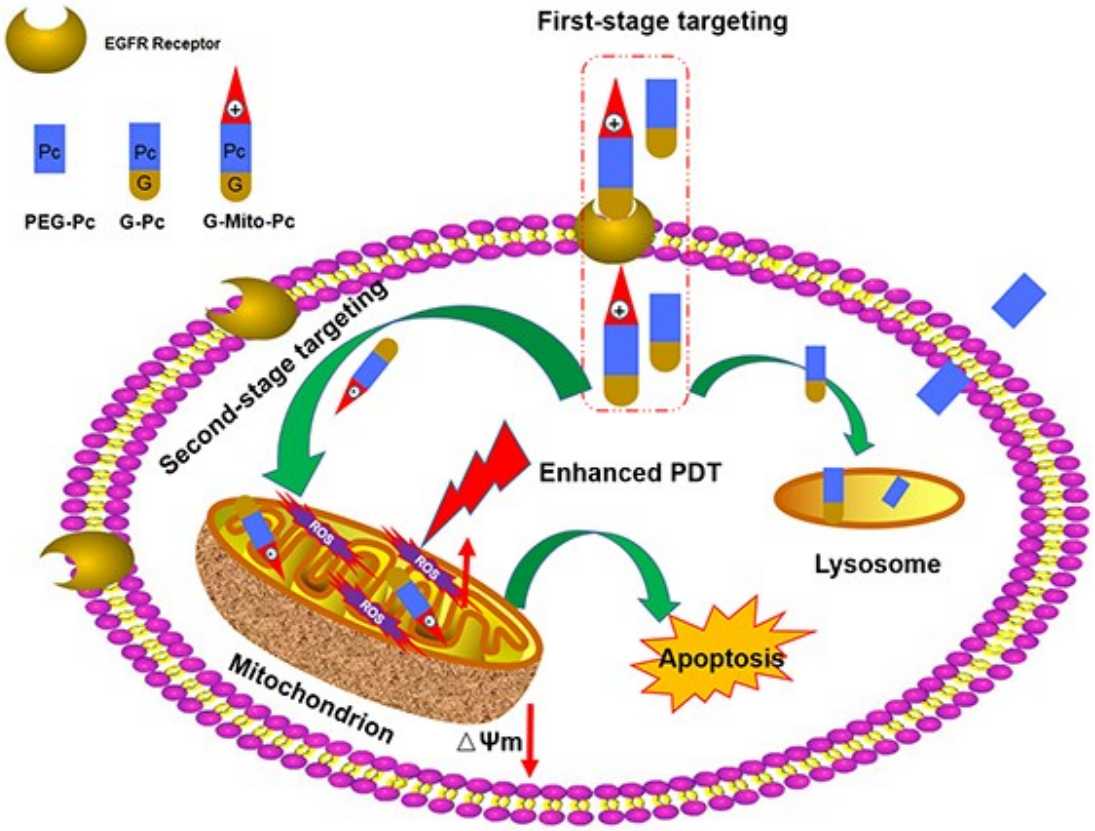
Materials: Methylbenzene was distilled over sodium, and all other solvents and chemical reagents were purchased from commercial sources and without further purification. All reactions were carried out under an atmosphere of nitrogen.

Instruments: The ^{1}H NMR spectra were recorded on a Bruker AVANCE III 400 MHz NMR instrument at 298 K in $CDCl_3$ or DMSO-d_6. Chemical shifts are expressed in ppm relative to TMS (0 ppm). HRMS analyses were carried out on LC-QTOF-MS(G6520B/G6520B). Electronic absorption spectra were measured on a Beijing PuXi Tu-1901 spectrometer. Fluorescence spectra were recorded on a Varian carye clipse spectrometer with Xe lamp as the excitation source at room temperature. Confocal laser scanning microscopy (CLSM) images were performed on an Olympus FV1000-IX81 CLSM and a Leica TCS SP confocal system (Leica, Germany). Flow cytometry experiments were conducted by BD FACSAriaIII (BD Biosciences).

2. Synthesis and characterization of G-Mito-Pc, G-Pc, and PEG-Pc:



Scheme S1. Synthetic routes of G-Mito-Pc, G-Pc, and PEG-Pc



Scheme S2. Schematic illustration of the tumor and mitochondria dual-targeted PDT.

Synthesis and Characterization of 1a:

The trimethylene glycol monomethyl ether (9.8 mL, 61 mmol) was dissolved in dichloromethane (100 mL). Triethylamine (10.2 mL,73mmol) was added to the reaction mixture. Then the 4-toluenesulfonyl chloride (2.387g，12.5 mmol) was added and the reaction mixture was stirred at room temperature overnight. After the reaction was complete the solvent was removed under reduced pressure *in vacuo* and the resulting oily mixture was washed with hydrochloric acid and dichloromethane three times and dried over Na_2SO_4. The solvent was subsequently evaporated to dryness under reduced pressure. This oil was purified *via* silica gel chromatography using 75% EtOAc in Petroleum ether as eluent resulting in a colorless oil (3.310 g, 76%).^{1}H NMR (400 MHz, $CDCl_3$) δ

7.80 (d, *J* = 7.2 Hz, 2 H), 7.35 (d, *J* = 7.6 Hz, 2 H), 3.80 – 3.60 (m, 16H), 2.45 (s, 3H).

Synthesis and Characterization of 2a:

The mixture of 1a (1.046 g, 3.0 mmol) and gefitinib (1.341 g, 3.0 mmol) was dissolved in anhydrous DMF (10 ml). Then the anhydrous K_2CO_3 (1.242 g, 9 mmol) was added to the reaction mixture. The reaction was stirred under nitrogen atmosphere at 90 ℃ 24 h. When the reaction was completed, the solvent was removed under reduced pressure and the resulting yellow oily mixture was washed water and dichloromethane three times and dried over Na_2SO_4. The solvent was subsequently evaporated to dryness under reduced pressure. This oil was purified *via* silica gel chromatography using 10% MeOH in CH_2Cl_2 as eluent resulting in a yellow oil (0.785 g, 65%).^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (s, 1H, Ar-H), 7.75 (s, 1H, Ar-H), 7.17-7.15 (m, 1 H, Ar-H), 7.05-7.00 (m, 1 H, Ar-H), 6.95-6.93 (m, 1 H, Ar-H), 6.61 (s, 1 H, Ar-H), 4.18(t, *J*=12.0 Hz, 2 H, CH_2), 4.09(t, *J*=8.0 Hz, 2 H, CH_2), 3.92(s, 3 H, CH_3), 3.75(t, *J*=8.0 Hz, 2 H, CH_2), 3.67(t, *J*=8.0 Hz, 4 H, CH_2), 3.59-3.57 (m, 2 H, CH_2), 3.53-3.50(m, 8H, CH_2), 3.49-3.46(m, 2 H, CH_2), 3.21(s, 1H, OH), 2.52-2.48 (m, 2H, CH_2), 2.43(s, 4H, CH_2), 2.04-2.00 (m, 2 H, CH_2).

Synthesis and Characterization of G-Mito-Pc:

A mixture of silicon(IV) phthalocyanine dichloride (122 mg, 0.20 mmol), alcohol *2a* (125 mg, 0.20 mmol), and pyridine (1 mL, 12.36 mmol) in toluene (15 mL) was kept stirring under nitrogen atmosphere at 115 ℃ for 2 h. To this reaction mixture, a solution of 3a (177 mg, 0.40 mmol) and pyridine (0.5 mL, 6.18 mmol) in toluene (10 mL) was added, and the mixture was kept stirring under reflux for a further 4 h. After the reaction was completed, the solvents was removed under reduced pressure. Then the residue was redissolved in CH_2Cl_2. Then it was purified *via* silica gel chromatography using CH_2Cl_2:MeOH (25:1) as eluent resulting in a blackish blue mixture (35 mg, 11%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.59-9.56 (m, 8 H), 8.45-8.41 (m, 8 H), 7.91 (t, *J* = 7.6 Hz, 4 H),

7.75-7.49 (m, 15 H), 7.19-7.13(m, 4 H), 4.37 (s, 2 H), 4.22 (s, 2 H), 3.91(s, 3 H), 3.57-3.51 (m, 3 H), 3.10 (t, *J* = 4.8 Hz, 2 H), 2.75(t, *J* = 4.8 Hz, 2 H), 2.71-2.61(m, 2 H), 2.21 (t, *J* = 4.8 Hz, 2 H), 1.97 (s, 2 H), 1.53 (t, *J* = 5.2 Hz, 2 H), 1.35 (d, *J* = 6.4 Hz, 2 H), 0.36(s, 2 H), 0.28 (t, *J* = 5.2 Hz, 2 H), -0.41 (t, *J* = 8.0 Hz, 2 H), -1.58 (t, *J* = 8.0 Hz, 2 H), -1.72 (t, *J* = 6.8 Hz, 2 H), -2.08 (t, *J* = 5.2 Hz, 2 H), -2.21 (t, *J* = 5.2 Hz, 2 H). HRMS (ESI) m/z calcd for $C_{86}H_{82}ClFN_{12}O_8Si$ $[M-Br]^+$: 1523.5558, found: 1523.5543.

Synthesis and Characterization of G-Pc:

A mixture of silicon(IV) phthalocyanine dichloride (122 mg, 0.20 mmol), alcohol *2a* (125 mg, 0.20 mmol), and pyridine (1 mL, 12.36 mmol) in toluene (15 mL) was kept stirring under nitrogen atmosphere at 115 ℃ for 2 h. To this reaction mixture, a solution of 1-hexanol (177 mg, 0.40 mmol) and pyridine (0.5 mL, 6.18 mmol) in toluene (10 mL) was added, and the mixture was kept stirring under reflux for a further 4 h. After the reaction was completed, the solvents was removed under reduced pressure. Then the residue was redissolved in CH_2Cl_2. Then it was purified *via* silica gel chromatography using CH_2Cl_2:MeOH(10:1) as eluent resulting in a blackish blue mixture (38 mg, 15%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.64-9.61 (m, 8 H), 8.48-8.45 (m, 8 H), 7.80 (s, 1 H), 7.25 (s, 1 H), 7.18 (s, 1 H), 7.12 (t, *J* = 8.8 Hz, 1 H), 6.99 (s, 2 H), 4.21 (s, 2 H), 4.08 (s, 2 H), 3.84 (s, 3 H), 3.52 (d, *J* = 9.6 Hz, 8 H), 3.10 (s, 2 H), 2.75 (s, 2 H), 2.40 (s, 3 H), 2.34 (s, 4 H), 2.21 (t, *J* = 4.8 Hz, 2 H), 1.90 (s, 2 H), 1.52 (t, *J* = 4.8 Hz, 3 H), 0.28 (t, *J* = 5.6 Hz, 2 H), 0.10 (s, 4 H), -0.68 (t, *J* = 8.0 Hz, 2 H), -1.61 (d, *J* = 8.0 Hz, 2 H), -1.70 (t, *J* = 5.6 Hz, 2 H), -2.06 (t, *J* = 4.8 Hz, 2 H), -2.18 (t, *J* =5.6 Hz, 2 H). HRMS (ESI) m/z calcd for $C_{68}H_{68}ClFN_{12}O_8Si$ $[M+H]^+$: 1263.4803, found: 1263.4836.

Synthesis and Characterization of PEG-Pc:

A mixture of silicon(IV) phthalocyanine dichloride (122 mg, 0.20 mmol), triethylene glycol monoethyl ether (178 mg, 1.00 mmol), and NaH (23 mg, 1.00 mmol) in toluene (15 mL) was kept stirring under nitrogen atmosphere at 120

℃ for 24 h. After the reaction was completed, the solvents was removed under reduced pressure. And the residue was redissolved in CH_2Cl_2. Then it was purified *via* silica gel chromatography using CH_2Cl_2:MeOH (15:1) as eluent to give PEG-Pc as a a blackish blue solid (55 mg , 31.0%). ^{1}H NMR (400 MHz, DMSO-d_6 contain a trace amount of pypridine-d_5) δ (ppm) 9.70-9.67(m, 8 H), 8.54-8.50 (m, 8 H), 3.17 (q, *J* = 7.2 Hz, 4 H), 3.05 (t, *J* = 4.8 Hz, 4 H), 2.83 (t, *J* = 4.8 Hz, 4 H), 2.32 (t, *J* = 4.8 Hz, 4 H), 1.60 (t, *J* = 4.8 Hz, 4 H), 0.92 (t, *J* = 7.2 Hz, 6 H), 0.33 (t, *J* = 5.2 Hz, 4 H), -2.03 (t, *J* = 5.6 Hz, 4 H). HRMS (ESI) m/z calcd for $C_{48}H_{50}N_8O_8Si$ $[M+Na]^+$: 917.3442, found: 917.3419.

3. Cells culture and measurements

Cells culture:

The cell lines Hela cells (human cervical cancer), A549 cells (human lung adenocarcinoma), MDA-MB-468 cells（breast cancer）and HELF cells (human embryonic lung fibroblast) were incubated with DMEM (Dulbecco·s modified Eagle·s medium) with 10% fetal bovine serum (FBS). The cell line A549 (human pulmonary carcinoma) was incubated with F12K medium with 10% fetal bovine serum (FBS). All cells were cultured at 37 °C with 5% CO_2.

Dark cytotoxicity assay:

Cells were seeded into a 96-well cell culture plate with the density of 5.0×10^4 cells /ml and cultured at 37 °C with 5% CO_2 for 12 h. G-Mito-Pc, G-Pc, and PEG-Pc were diluted into DMSO (contain 5% CEL). After that, the compounds were further diluted into different concentrations (1-0.50 μM) with fresh medium. The Per well was added in 100 μL medium containing drug. After a 24 h incubation, the old medium was replaced by fresh medium. Then per well was added in 10 μL MTT solution (5 mg/mL^{-1} in PBS) and the cells were further

incubated for 4 h. The old medium was removed carefully and 100 μL DMSO was added in per well. The absorbance at 570 nm was monitored by a Microplate reader. The survival curves were plotted as a concentration dependence curve of the drug.

Colocalization experiments

The cells were seeded in 20 mm confocal dishes and incubated at 37 °C with 5% CO_2 for 12 h. Then the cells were incubated with G-Mito-P, G-Pc, and PEG-Pc respectively for 24 h. After washing out the excess compound with PBS three times, the cells were stained with Mito Tracker green (2 μM) for 30 minutes, Lyso Tracker Red (2 μM) for 60 minutes or DAPI Tracker (2 μM) for 10 minutes at 37 °C respectively. After that, the cells were rinsed with PBS three times and taken by Olympus FV1000-IX81 CLSM and a Leica TCS SP confocal system (Leica, Germany).

Analysis of mitochondrial membrane potential (MMP):

Approximately 1.0 $\times 10^5$ Hela cells were seeded in 20 mm confocal culture dishes and cultured to adhere for 12 h. Then the cells were incubated with different concentration of G-Mito-Pc (0, 3 , 5, 10 nM) for 24 h with 5% CO_2, followed with illumination for 2 minutes by LED light (λ=670 nm, 12.5 $mWcm^{-2}$ for 2 min, 1.5 $J \cdot cm^{-2}$). After 24 h of treatment, the cells were washed three times with PBS and stained with a JC-1 dye (6 μM) to measure the mitochondrial membrane potential according to the directions. After 70 minutes later, the fluorescence intensity of the cells was measured by confocal laser scanning microscopy with a 488 nm laser and a 543 laser.

The MTT assay of Photodynamic Cytotoxicity In Vitro

Cells were seeded into a 96-well cell culture plate with the density of 5.0×10^4cells /ml and cultured at 37 °C with 5% CO_2 for 12 h. G-Mito-Pc, G-Pc and PEG-Pc were diluted into DMSO (contain 5% CEL). After that, the compounds were further diluted into different concentrations (0.1-10 nM for G-Mito-Pc,1-500 nM for G-Pc or PEG-Pc) with fresh medium. Per well was added in 100 μL medium containing drug. After a 24 h incubation, the old medium was replaced by fresh medium and the cells were exposed to red light (λ=670 nm, 12.5 $mWcm^{-2}$ for 2 min, 1.5 $J\cdot cm^{-2}$). The cells after irradiation were cultured again for 24 h. Then per well was added in 10 μL MTT solution (4 mg/mL in PBS) and the cells were further incubated for 4 h. The old medium was removed carefully and 100 μL DMSO was added in per well . The absorbance at 570 nm was recorded by a Microplate reader. The survival curves were plotted as a concentration dependence curve of the drug, and IC_{50} values were calculated.

Chromatin condensation assay using Hoechst 33258 staining

To study the change about the chromatin morphology, approximately 1.0×10^5 Hela cells were seeded in 20 mm confocal dishes and incubated for 12 h. Then the cells were cultured with different concentrations of G-Mito-Pc (0, 3, 8 nM) for 24 h, followed with illumination for 2 minutes by LED light (λ=670 nm, 12.5 $mWcm^{-2}$ for 2 min, 1.5 $J\cdot cm^{-2}$). The cells were cultured with G-Mito-Pc (0 and 0.5 μM) in the dark as the parallel control. After 24 h, the cells were washed three times with PBS, fixed with paraformaldehyde for 10 minutes, and stained with Hoechst 33258 dye (5 μg/ml) for 30 minutes . The fluorescence intensity of the cells was measured by confocal microscopy with a 405 nm laser.

Cell morphology research experiment

The Hela cells suspension of 10 ml were seeded into culture bottles with the density of 10.0×10^4 cells /ml and cultured at 37 °C with 5% CO_2 for 12 h. G-Mito-Pc, G-Pc, and PEG-Pc were diluted into DMSO (contain 5% CEL). After that, the compounds were further diluted into 3 nM with fresh medium. After a 24 h incubation, the old medium were replaced by fresh medium and the cells were exposed to red light (λ=670 nm, 1.5 $J\cdot cm^{-2}$*).* The cells after irradiation were cultured again for 24 h. Then the images were taken by the Ordinary Inverted Microscopy.

Molecular dynamics simulations

The membrane models were constructed of 54 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 36 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) and 10 cholesterol molecules in each of the leaflet to resemble the mitochondrion membranes[1] using CHARMM-GUI web server[2]. Subsequently, the models were immerged into a TIP3P water box of size 7.8 × 7.8 × 10.9 nm^3 and further subjected to molecular dynamics (MD) simulation using AMBER16 software package[3] with the Lipid14[4] and AMBER ff99SB force field[5]. Appropriate sodium and chloride ions were added into the system for charge neutralization at 0.15 M ionic strength. Each photosensitizer (G-Pc and G-Mito-Pc) was positioned in the bulk solution with a center-of-mass distance of 4 nm away from the center of the membrane in the z direction (see Figure S19 for the initial structure of the model). The geometries of G-Pc and G-Mito-Pc were optimized at the B3LYP/6-31G*[6] using Gaussian 09[78], and the generalized AMBER force field (GAFF) parameters of the molecules were generated using Antechamber implemented in AmberTools[9, 10]. Periodic boundary conditions were applied in all three directions. The cutoff value for the noncovalent interactions was 1.0 nm. Electrostatic interactions were calculated by Particle-Mesh Ewald (PME) method[11]. All covalent bonds were constrained using the SHAKE algorithm[12]. For each system, energy minimization was performed

using steepest descent method for 5000 steps, followed by conjugated gradient algorithm for 5000 steps[13]. The minimized structure was first gradually heated to 303 K for 0.5 ns, and then equilibrated for 2 ns with force constants of 10.0 and 2.5 kcal·mol^{-1}·Å^{-2}, respectively. G-Pc and G-Mito-Pc were pulled into the center of membrane with a force constant of 10 kcal·mol^{-1}·Å^{-2}. Finally, a 100 ns-long productive MD simulation was carried out for each system. All of the simulations were carried out at constant pressure (1 bar) and 303 K in the isothermal-isobaric (NPT) ensemble in control of the Berendsen (semi-isotropical coupling) barostat[14] and the Langevin algorithm[15], respectively. The equilibrated MD trajectories (the last 80 ns) were used for the analysis of free energy and membrane integrity.

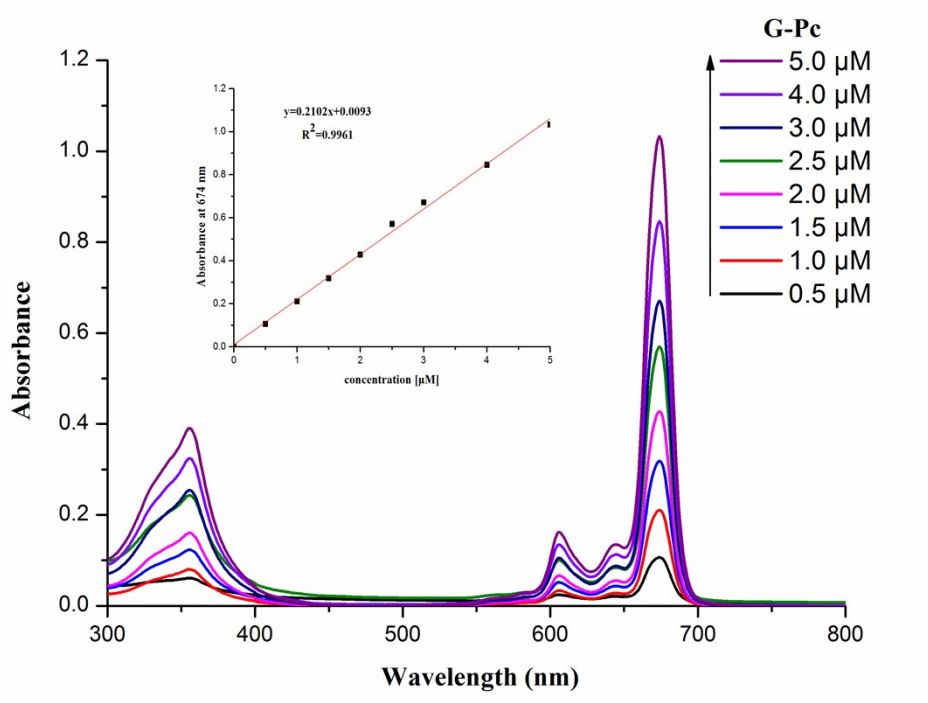
The free energy was dependent on the distance between the Pc silicon atom of the photosensitizer and the mass center of the bilayer in z direction (*d*), and the angle between the normal vectors of the planar Pc moiety and the bilayer surface (*α*) (see Figure S20). The free energy difference (Δ*G*) was calculated using following equation:

$$\Delta G = -RT \ln[\rho(d,\alpha)/\rho_0(d,\alpha)]$$

where *R* is the ideal gas constant, *T* is the temperature, *ρ* is the Boltzmann probability of configurational state, and the ρ_0 means the most likely state in the simulations. The integrity of cell membrane were evaluated by the lipid order parameter, which is experimentally measured by nuclear magnetic resonance. We calculated the so-called deuterium order parameter (S_{CD}) using following equation [16]:

$$S_{CD}=\frac{1}{2}\langle 3\cos^2\theta - 1\rangle$$

where *θ* is the instantaneous angle between carbon-deuterium bond and the direction of bilayer normal, and the angular brackets denote time average



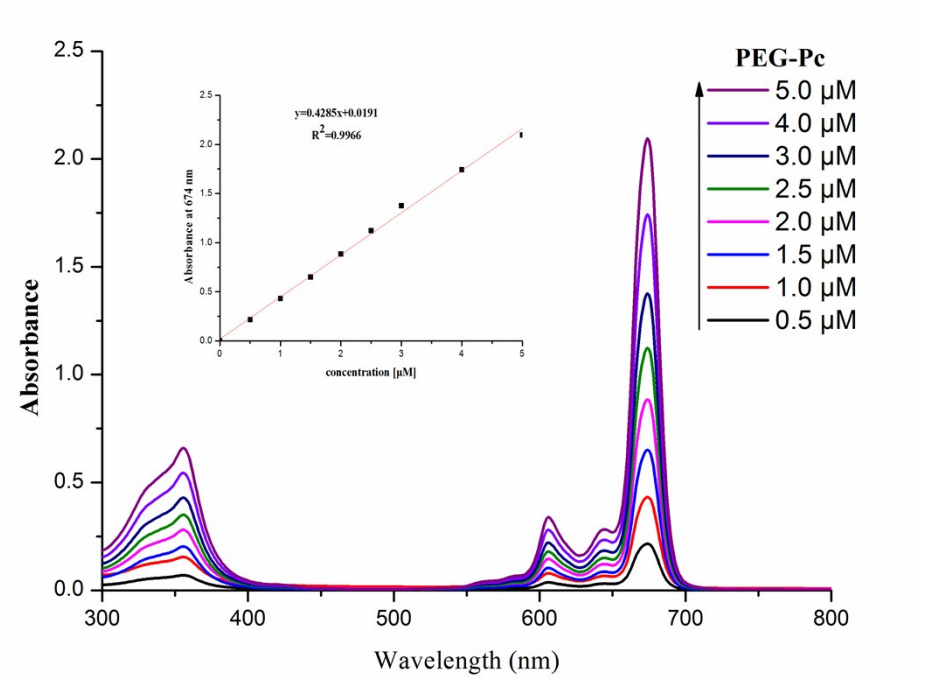
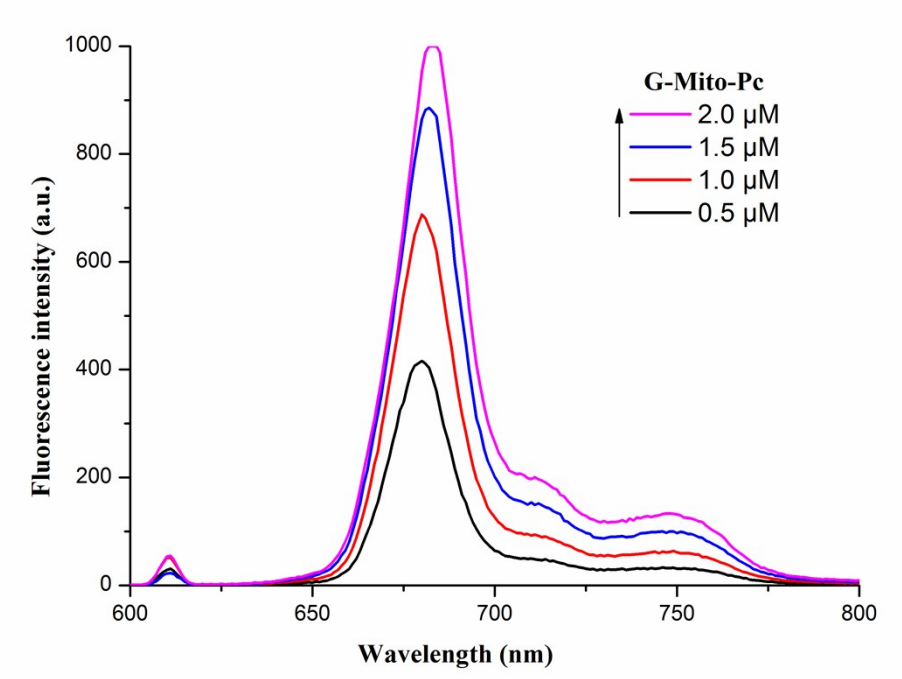
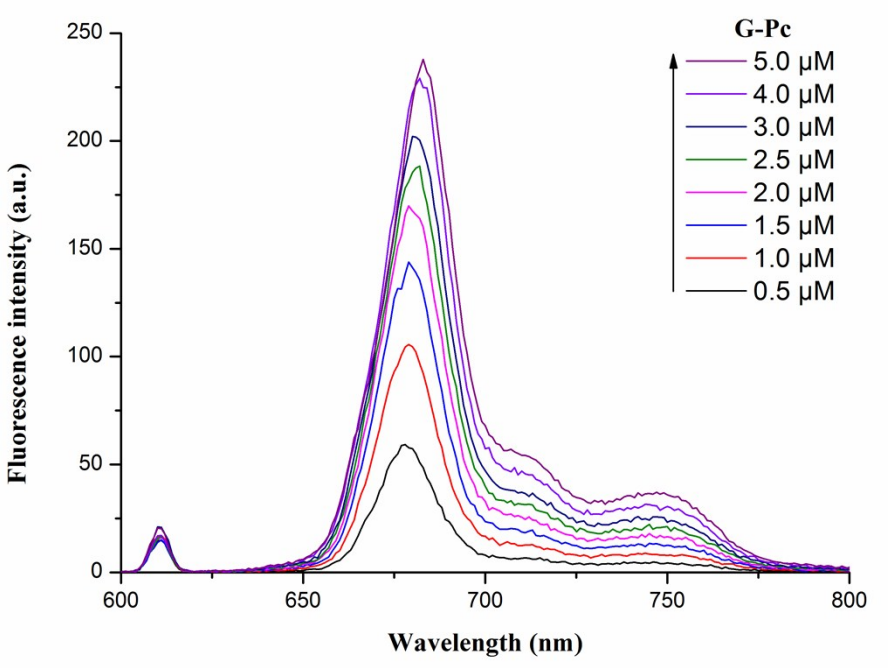


Figure S1. UV/vis absorption spectra of **G-Pc** and **PEG-Pc** in DMF at di fferent concentrations. The inset plots absorbance absorption of the maxi mum absorption wavelength versus the concentration of the conjugate.


G-Mito-Pc
2.0 μM
1.5 μM
1.0 μM
0.5 μM
Fluorescence intensity (a.u.)
Wavelength (nm)
0
200
400
600
800
1000
600
650
700
750
800


G-Pc
5.0 μM
4.0 μM
3.0 μM
2.5 μM
2.0 μM
1.5 μM
1.0 μM
0.5 μM
Fluorescence intensity (a.u.)
Wavelength (nm)
0
50
100
150
200
250
600
650
700
750
800

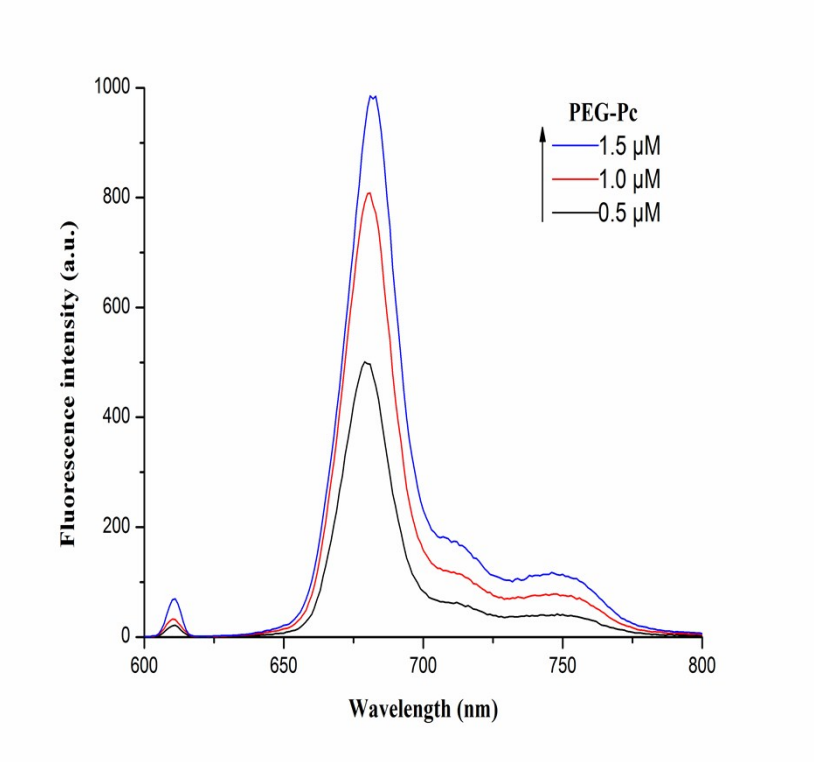


Figure S2. Fluorescence spectra (λ_{ex}=610 nm) of G-Mito-Pc, G-Pc and **PEG-Pc** in DMF at different concentrations.

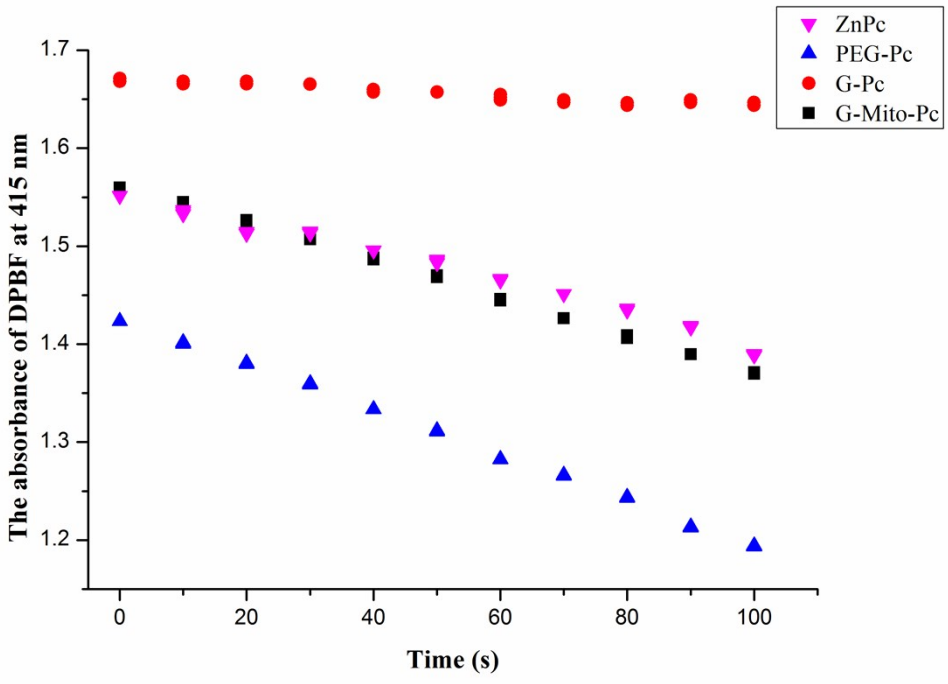
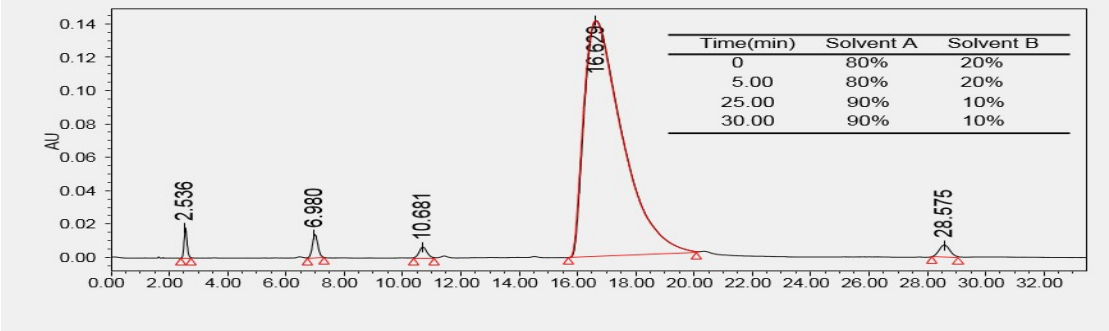
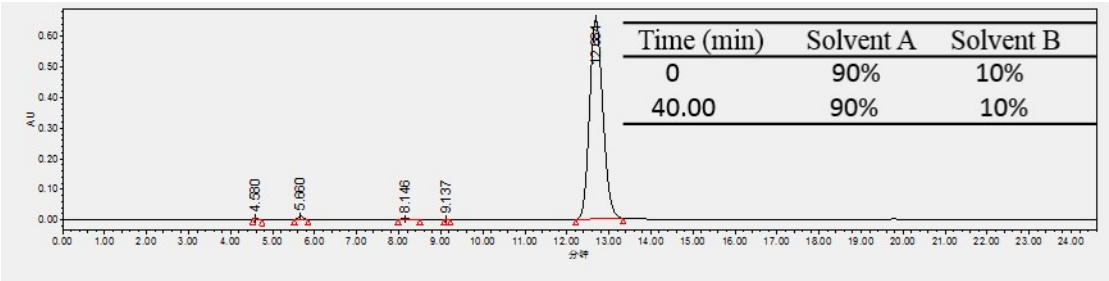


Figure S3. The absorbance spectra of DPBF at 415 nm in different DMF solutions (contain **G-Mito-Pc**, **G-Pc** or **PEG-Pc**)



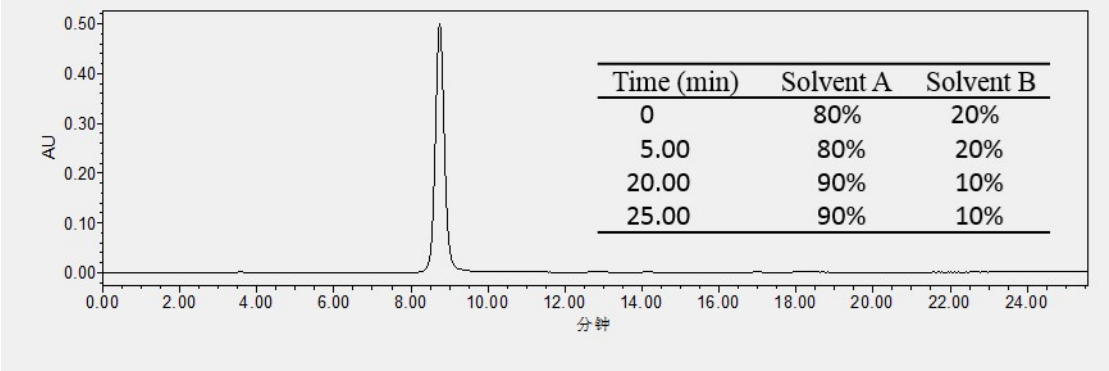
Peak No	Retention Time / min	High	Area Percentage / %
1	2.563	18060	1.33
2	6.980	13899	0.93
3	10.681	6385	0.84
4	16.629	141717	95.59
5	28.575	705	1.30

Figure S4. HPLC spectrum and table of **G-Mito-Pc** (solvent A: DMF; solvent B: 10 mM TEA, adjusted to pH=5.0 with phosphatea). Detection at 680 nm.



Peak No	Retention Time / min	High	Area Percentage / %
1	4.580	3527	0.13
2	5.660	11249	0.73
3	8.146	3105	0.26
4	9.137	308	0.01
5	12.684	653611	98.87

Figure S5. HPLC spectrum and table of **G-Pc** (solvent A: DMF; solvent B: 10 mM TEA, adjusted to pH=5.0 with phosphatea). Detection at 680 nm.



Time (min)	Solvent A	Solvent B
0	80%	20%
5.00	80%	20%
20.00	90%	10%
25.00	90%	10%

Figure S6. HPLC spectrum and table of **PEG-Pc** (solvent A: DMF; solvent B: dH_2O). Detection at 680 nm.

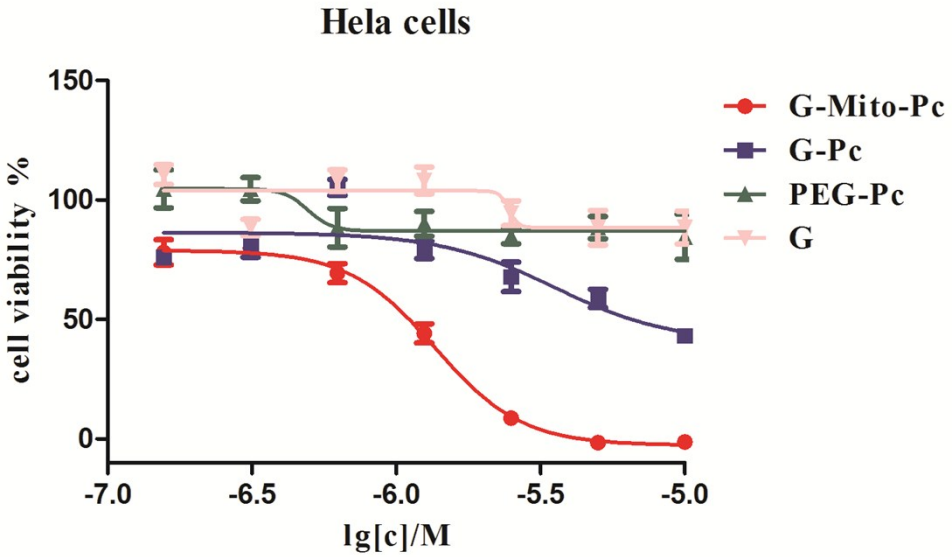


Figure S7. In vitro dark cytoxicity towards Hela cells. Data are expressed as the mean ± SD of three independent experiments.

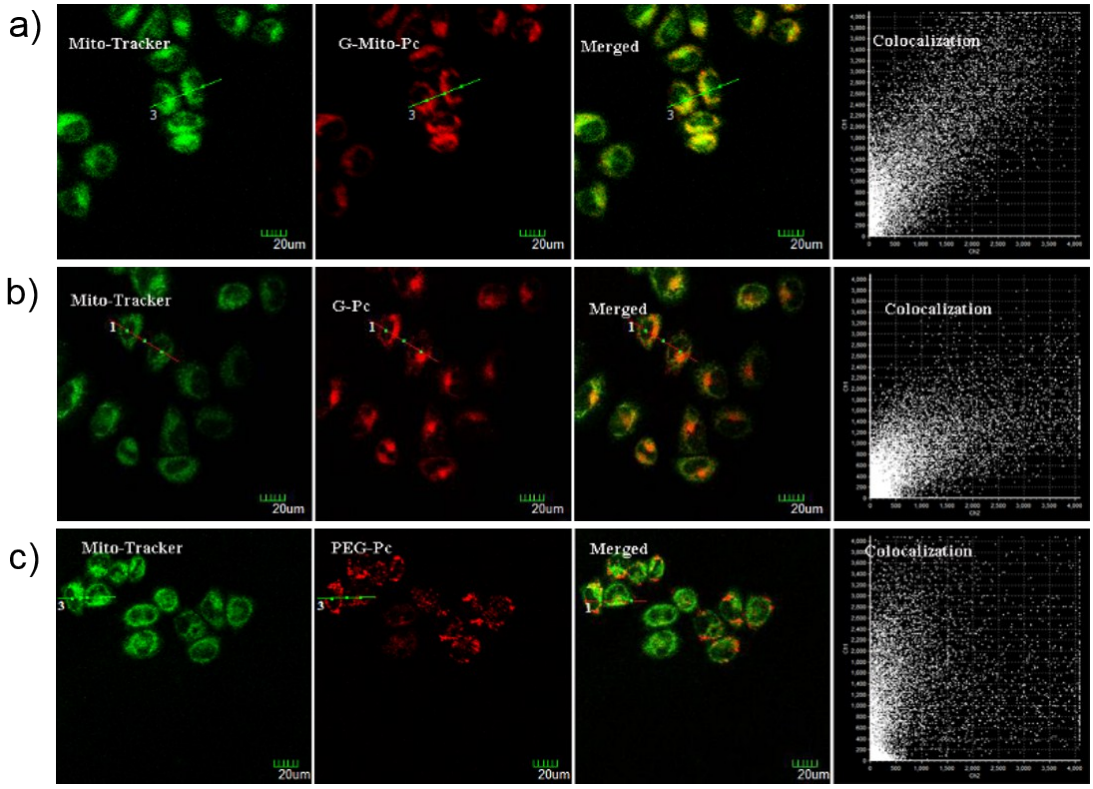


Figure S8. Confocal microscopy images of Hela cells. The cells were incubated with a) **G-Mito-Pc** (0.25 μM, up row), b) **G-Pc** (0.50 μM, middle row) and c) **PEG-Pc** (0.50 μM, down row) at 37 °C for 24 h and then incubated with MitoTracker Green (2.00 μM) at 37 °C for 30 min. Cells were viewed in green channel for Mito Tracker Green (λ_{ex} = 488 nm, λ_{em} = 510-570 nm) and red channel for **G-Mito-Pc**、**G-Pc** and **PEG-Pc** (λ_{ex} = 633 nm, λ_{em} = 690 nm), respectively

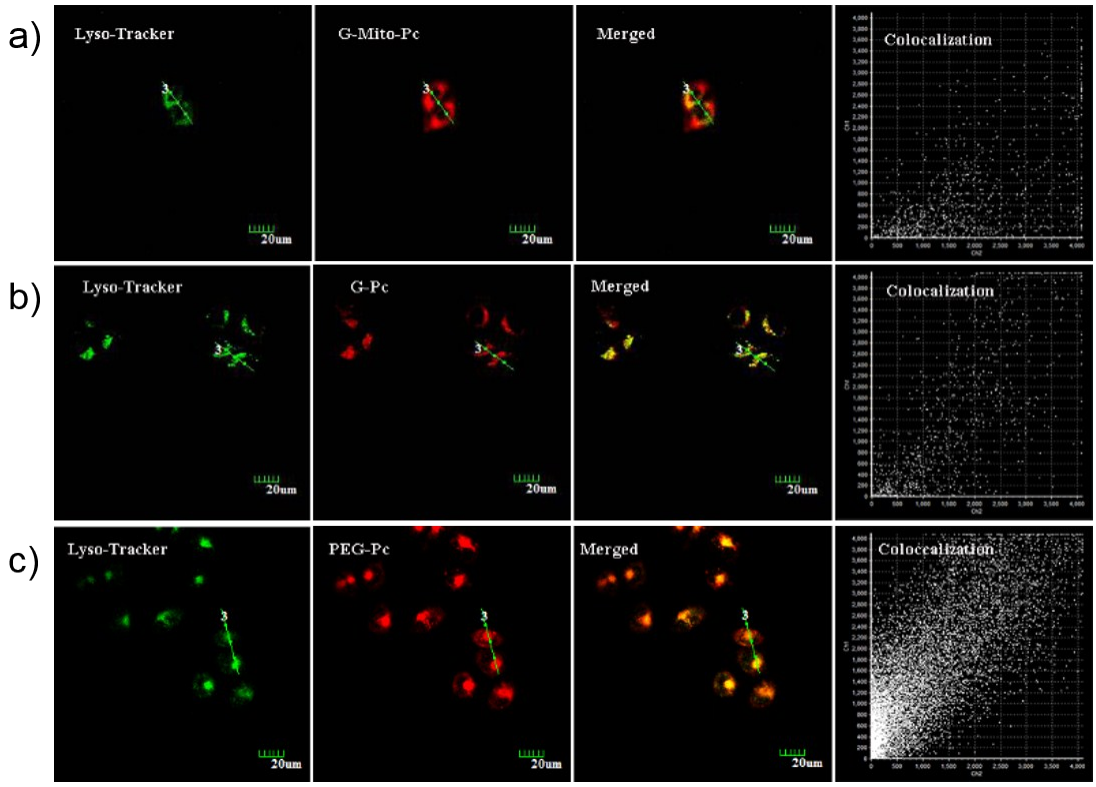


Figure S9. Confocal microscopy images of Hela cells. The cells were incubated with a) **G-Mito-Pc** (0.25 μM, up row), b) **G-Pc** (0.50 μM, middle row) and c) **PEG-Pc** (0.50 μM, down row) at 37 °C with 5% CO_2 for 24 h and then incubated with LysoTracker Red (2.00 μM) for 60 min. Cells were viewed in green channel for Lyso Tracker Red (λ_{ex} = 568 nm, λ_{em} = 575-595 nm) and red channel for **G-Mito-Pc**、**G-Pc** and **PEG-Pc** (λ_{ex} = 633 nm, λ_{em} = 690 nm), respectively.

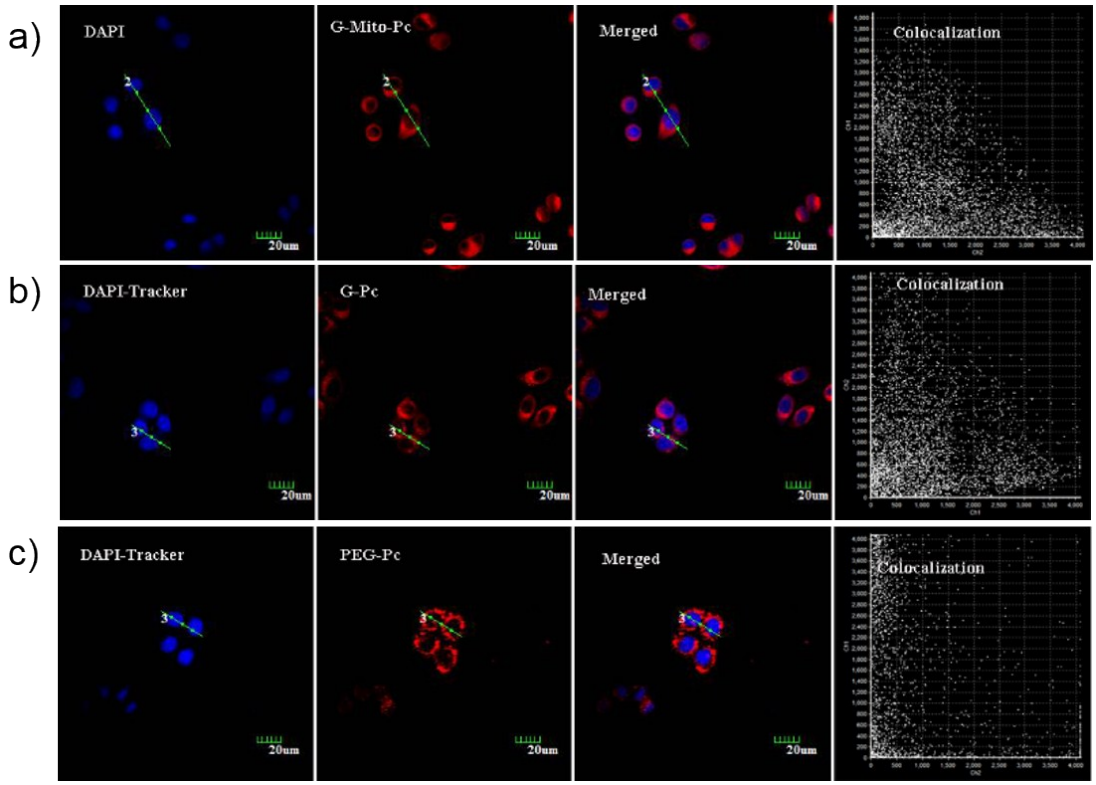


Figure S10. Confocal microscopy images of Hela cells. The cells were incubated with a) **G-Mito-Pc** (0.25 μM, up row), b) **G-Pc** (0.50 μM, middle row) and c) **PEG-Pc** (0.50 μM, down row) at 37 °C for 24 h and then incubated with DAPI Tracker (2.00 μM) for 10 min. Cells were viewed in blue channel for DAPI Tracker (λ_{ex} = 405 nm, λ_{em} = 425-475 nm) and red channel for **G-Mito-Pc**、**G-Pc** and **PEG-Pc** (λ_{ex} = 633 nm, λ_{em} = 690 nm), respectively.

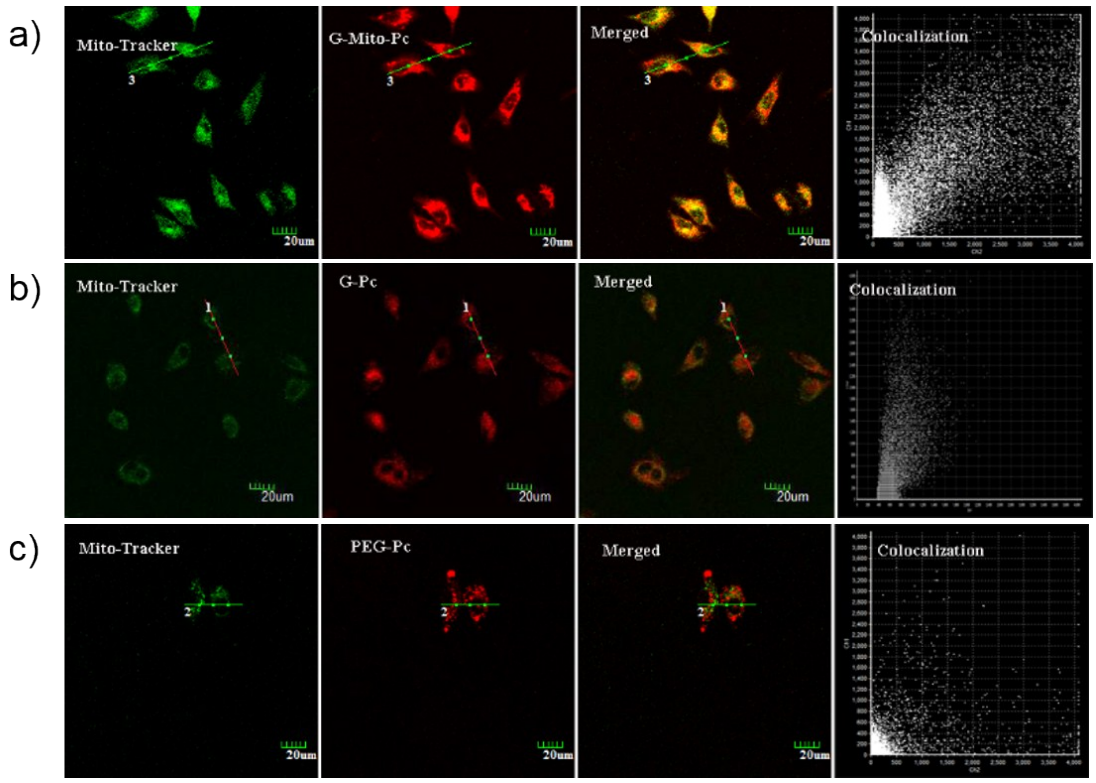


Figure S11. Confocal microscopy images of A549 cells. The cells were incubated with a) **G-Mito-Pc** (0.25 μM, up row), b) **G-Pc** (0.50 μM, middle row) and c) **PEG-Pc** (0.50 μM, down row) at 37 °C for 24 h and then incubated with MitoTracker Green (2.00 μM) at 37 °C for 30 minutes. Cells were viewed in green channel for Mito Tracker Green (λ_{ex} = 488 nm, λ_{em} = 520-560 nm) and red channel for **G-Mito-Pc**、**G-Pc** or **PEG-Pc** (λ_{ex} = 633 nm, λ_{em} = 690 nm), respectively.

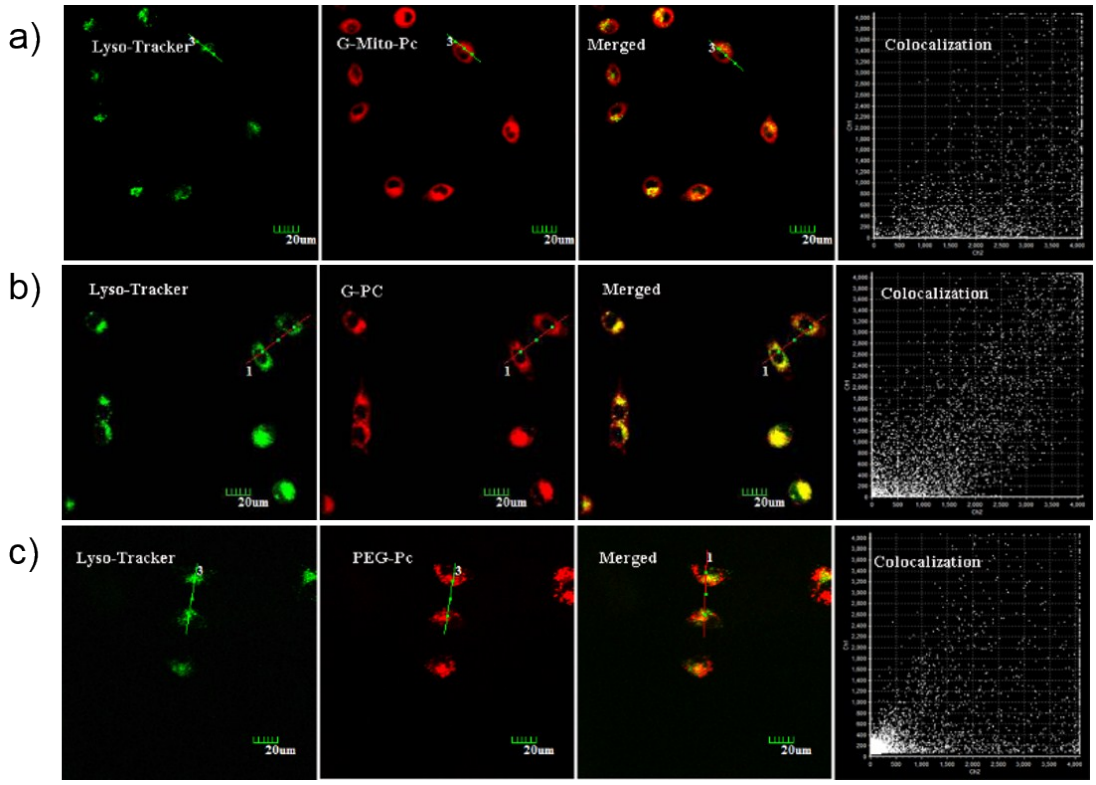


Figure S12. Confocal microscopy images of A549 cells. The cells were incubated with a) **G-Mito-Pc** (0.25 μM, left row), b) **G-Pc** (0.50 μM, middle row) and c) **PEG-Pc** (0.50 μM, right row) at 37 °C with 5% CO_2 for 24 h and then incubated with LysoTracker Red (2.00 μM) for 60 minutes. Cells were viewed in green channel for Lyso Red (λ_{ex} = 568 nm, λ_{em} = 575-595 nm) and red channel for **G-Mito-Pc**、**G-Pc** or **PEG-Pc** (λ_{ex} = 633 nm, λ_{em} = 690 nm), respectively.

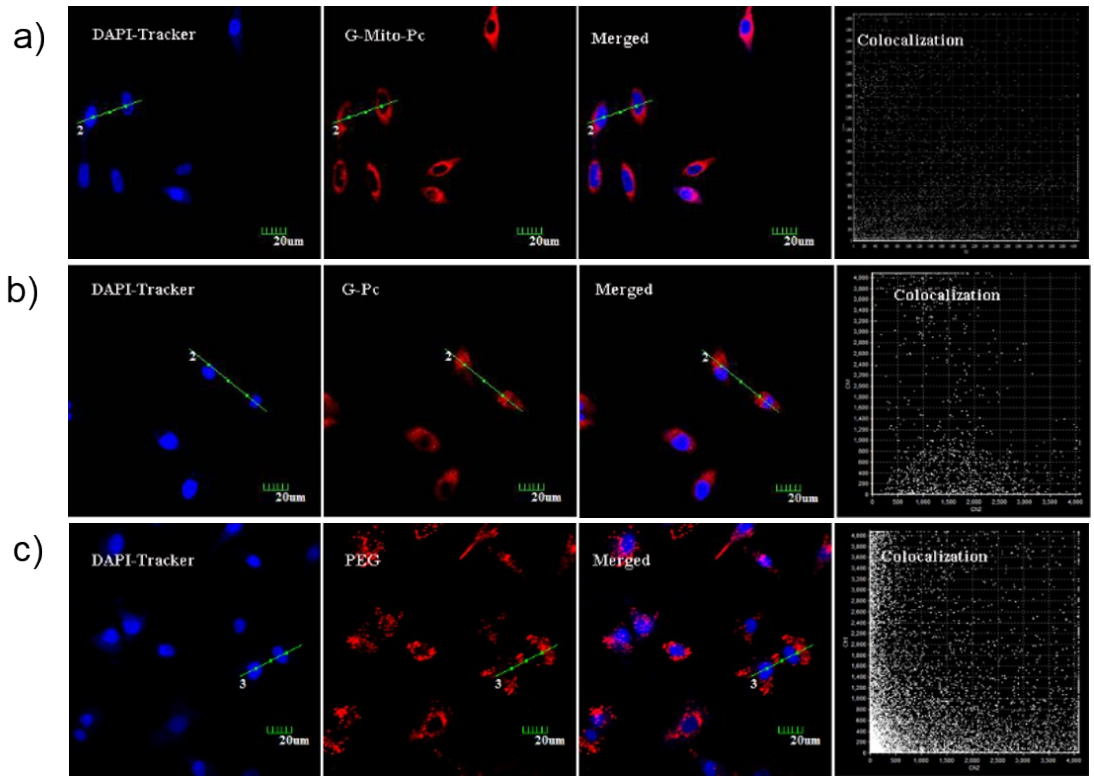


Figure S13. Confocal microscopy images of A549 cells. The cells were incubated with a) G-Mito-Pc (0.25 μM, up row), b) G-Pc (0.5 μM, middle row) and c) PEG-Pc (0.50 μM, down row) at 37 °C for 24 h and then incubated with DAPI Tracker (2.00 μM) for 10 minutes. Cells were viewed in blue channel for DAPI Tracker (λex = 405 nm, λem = 425-475 nm) and red channel for G-Mito-Pc、G-Pc and PEG-Pc (λex = 633 nm, λem = 690 nm), respectively.

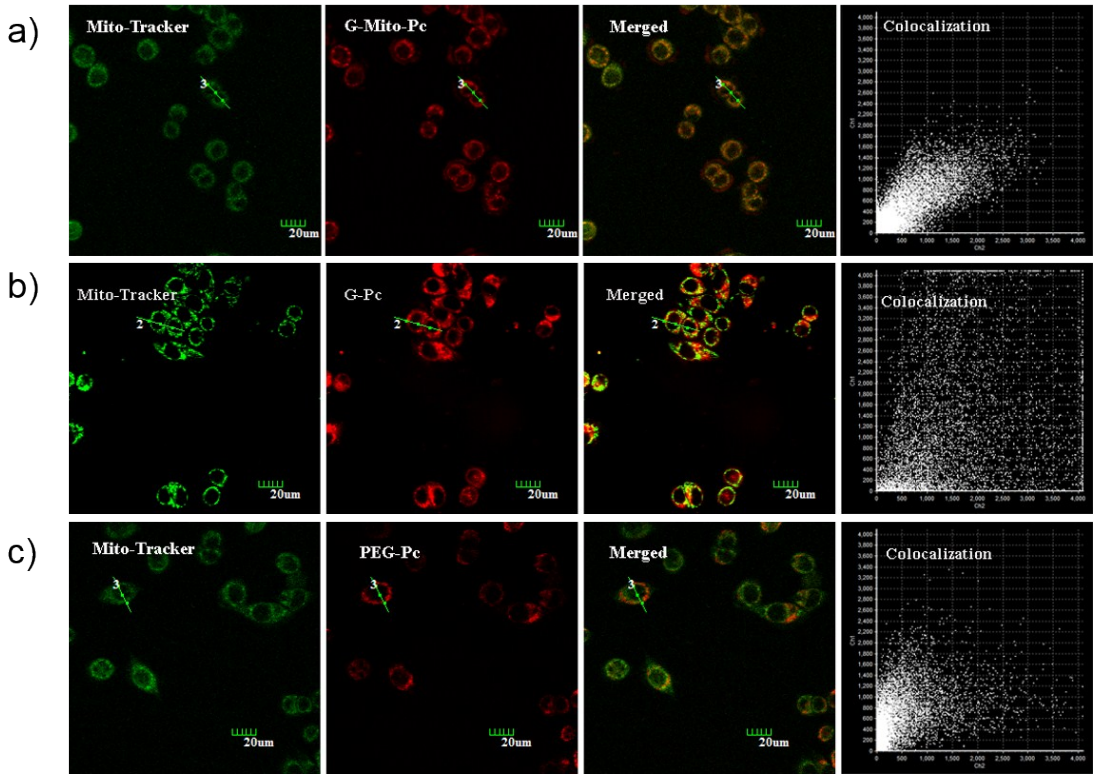


Figure S14. Confocal microscopy images of MDA-MB-468 cells. The cells were incubated with a) G-Mito-Pc (0.25 μM, up row), b) G-Pc (0.50 μM, middle row) and c) PEG-Pc (0.50 μM, down row) at 37 °C for 24 h and then incubated with MitoTracker Green (2.00 μM) at 37 °C for 30 min. Cells were viewed in green channel for Mito Tracker Green (λex = 488 nm, λem = 510-570 nm) and red channel for G-Mito-Pc、G-Pc or PEG-Pc (λex = 633 nm, λem = 690 nm), respectively.

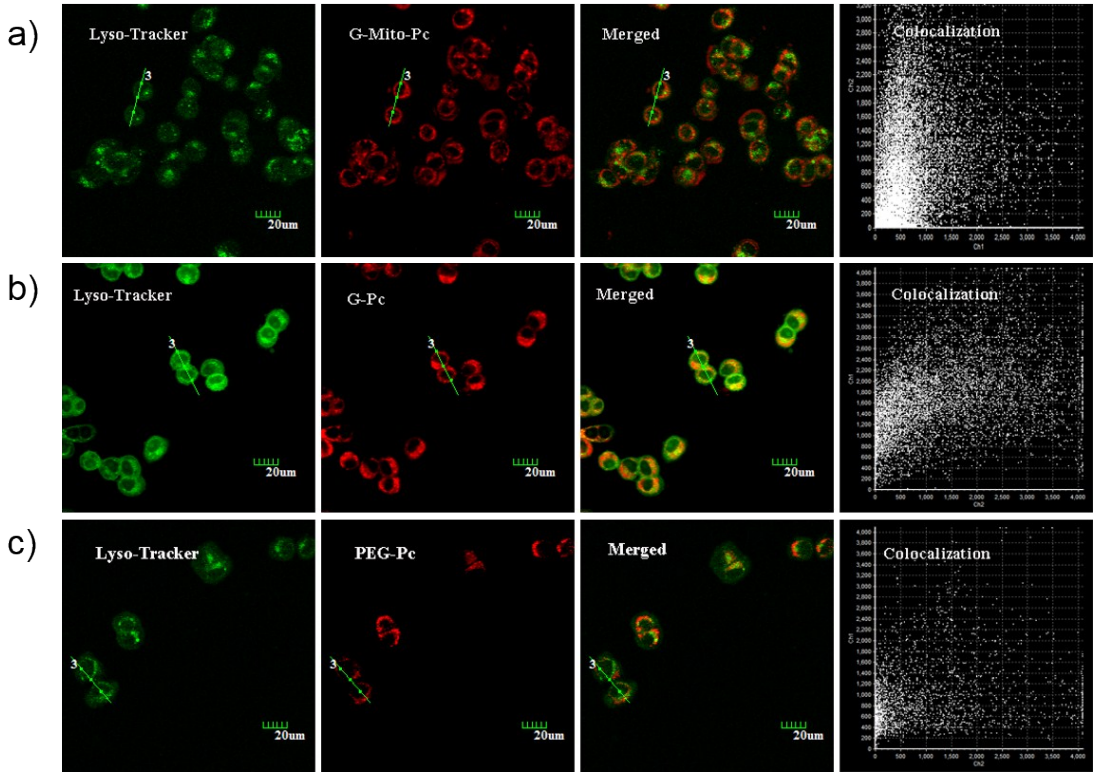


Figure S15. Confocal microscopy images of MDA-MB-468 cells. The cells were incubated with a) G-Mito-Pc (0.25 μM, left row), b) G-Pc (0.50 μM, middle row) and c) PEG-Pc (0.50 μM, right row) at 37 °C with 5% CO2 for 24 h and then incubated with LysoTracker Red (2.00 μM) for 60 minutes. Cells were viewed in green channel for Lyso Red (*λ*ex = 568 nm, *λ*em = 575-595 nm) and red channel for G-Mito-Pc、G-Pc or PEG-Pc (*λ*ex = 633 nm, *λ*em = 690 nm), respectively.

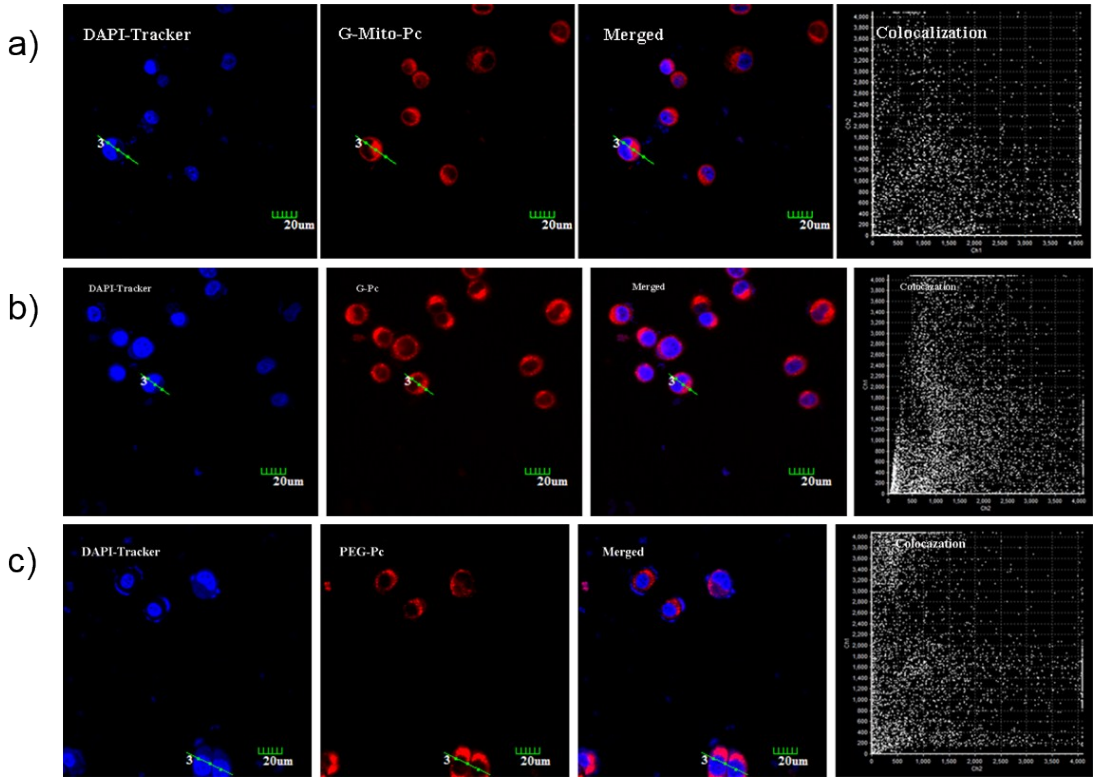
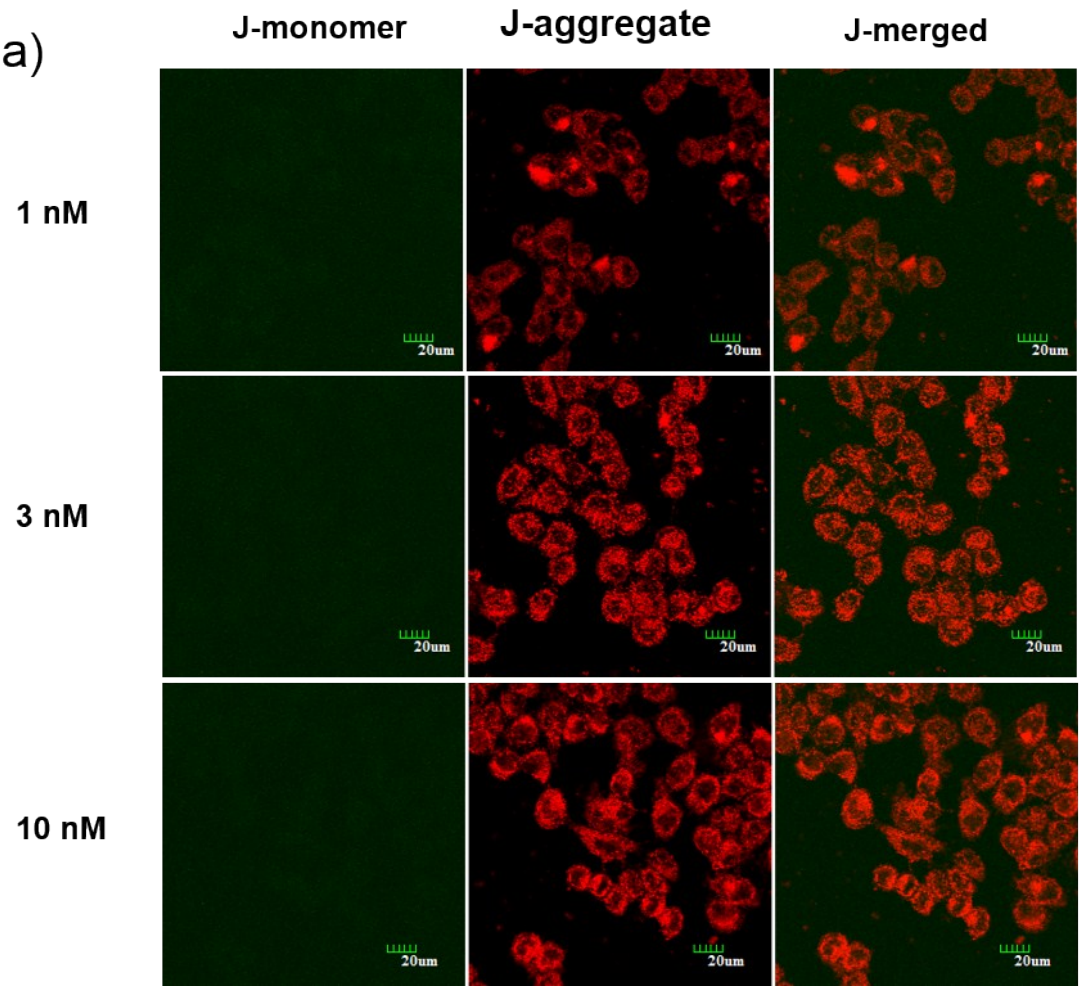


Figure S16. The confocal microscopy images of MDA-MB 468 cells. The cells were incubated with a) **G-Mito-Pc** (0.25 μM, up row), b) **G-Pc** (0.50 μM, middle row) and c) **PEG-Pc** (0.50 μM, down row) at 37 °C for 24 h and then incubated with DAPI Tracker (2.00 μM) for 10 minutes. Cells were viewed in blue channel for DAPI Tracker (λ_{ex} = 405 nm, λ_{em} = 425-475 nm) and red channel for **G-Mito-Pc**、**G-Pc** or **PEG-Pc** (λ_{ex} = 633 nm, λ_{em} = 690 nm), respectively.

a)



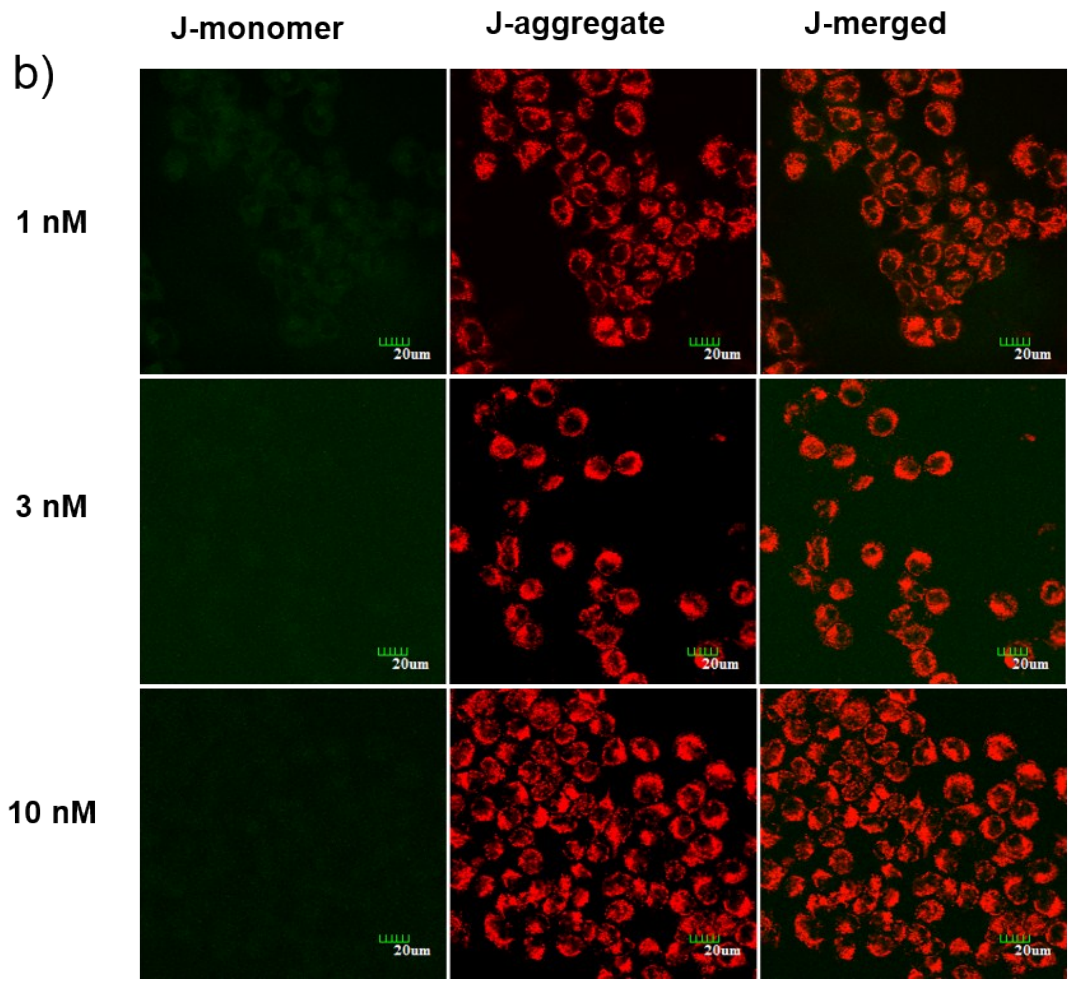


Figure S17. The confocal microscopy images of MMP for HeLa cells after PDT (treated with **G-Pc** or **PEG-Pc**).

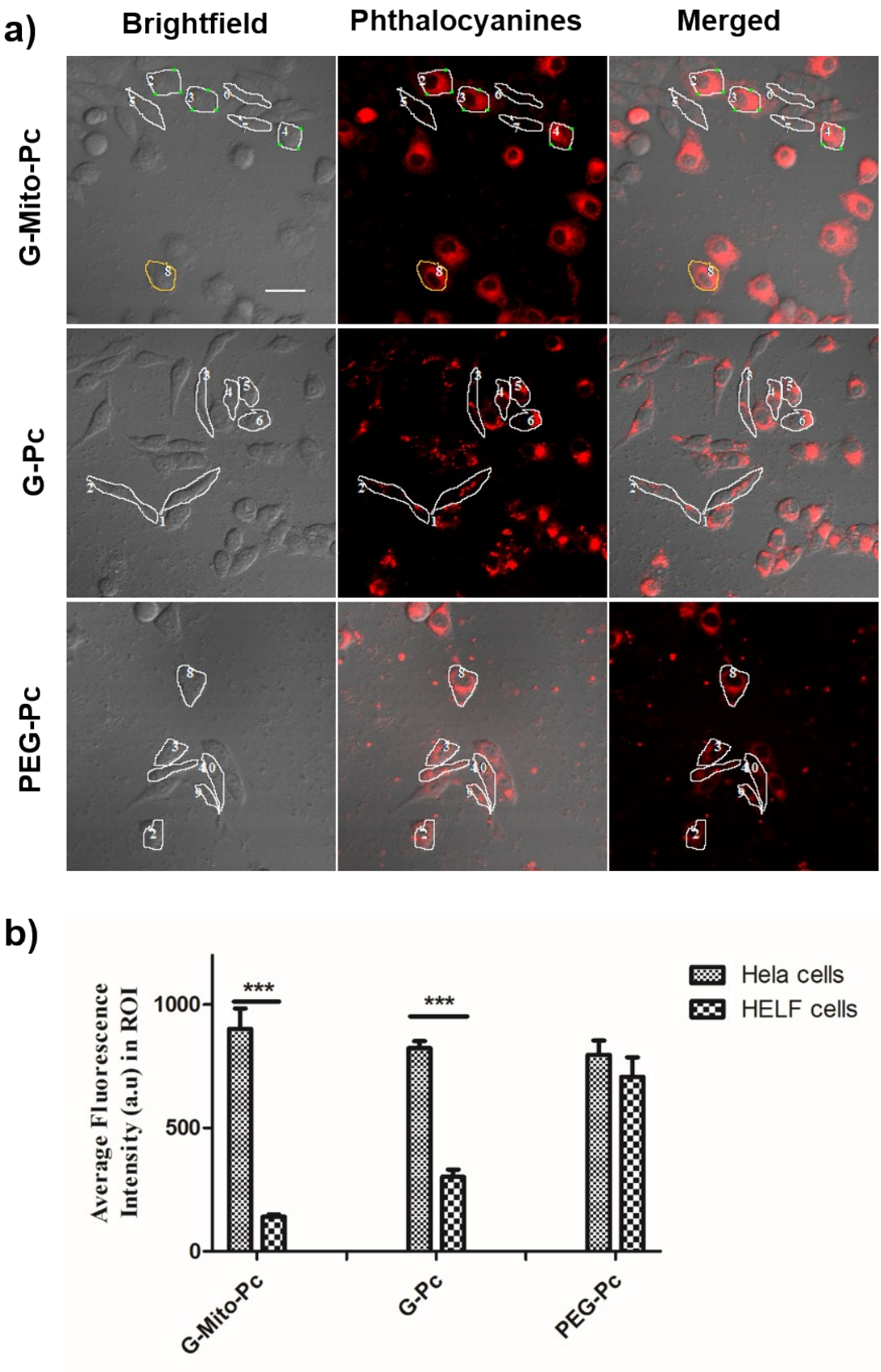


Figure S18 a) Confocal fluorescence images of mixed Hela (round) and HEIF cells (strip) after incubation with G-Mito-Pc, G-Pc, and PEG-

Pc for 24 h (all at 0.5 μM). b) Average fluorescence intensity of G-Mito-Pc, G-Pc, and PEG-Pc in Hela and HELF cells. All the images share the same scale bar of 30 μm. (Data are expressed as the mean ± SD of three independent experiments. Statistical Data: *** p< 0.001, ** p< 0.01, *p < 0.05).

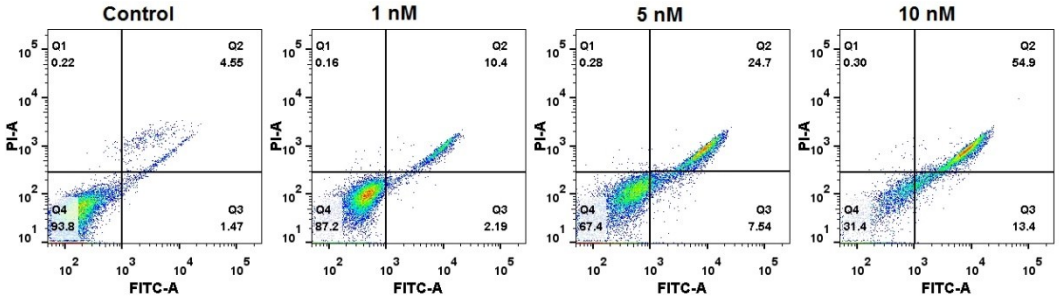


Figure S19. Flow cytometry quantification of annexin V-FITC and PI double labeled HeLa cells. The cells were treated with the G-Mito-Pc (1 nM, 5 nM, and 10 nM) at 37 °C for 12 h. After that, the cells were treated by LED light irradiation for 2 min and measured 30 min later.

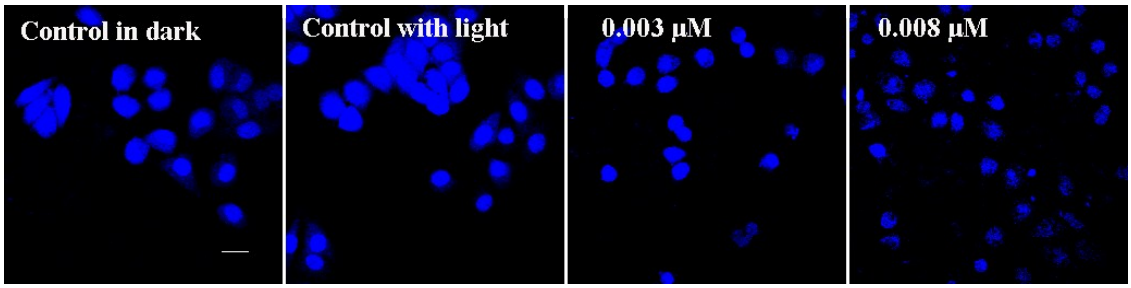


Figure S20 The fluorescent images of Hela cells treated with **G-Mito-Pc** and stained with Hoechst 33258. All the images share the same scale bar of 30 μm.

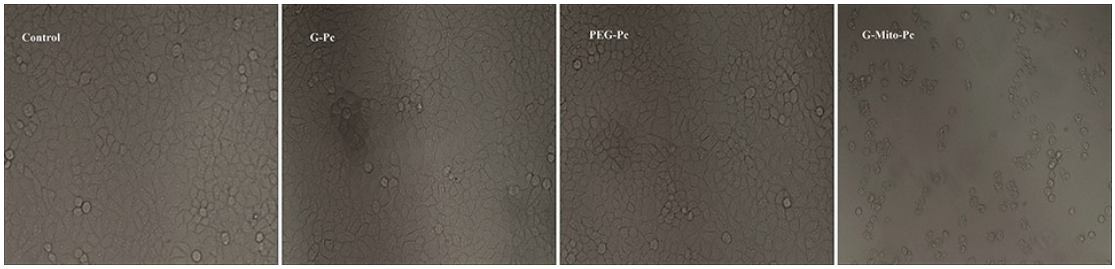


Figure S21. Phase contrast images of Hela cells treated with G-Mito-Pc, G-Pc or PEG-Pc at the consistent concentrations (3 nM). Note that images were taken after 24 h of PDT treatment.

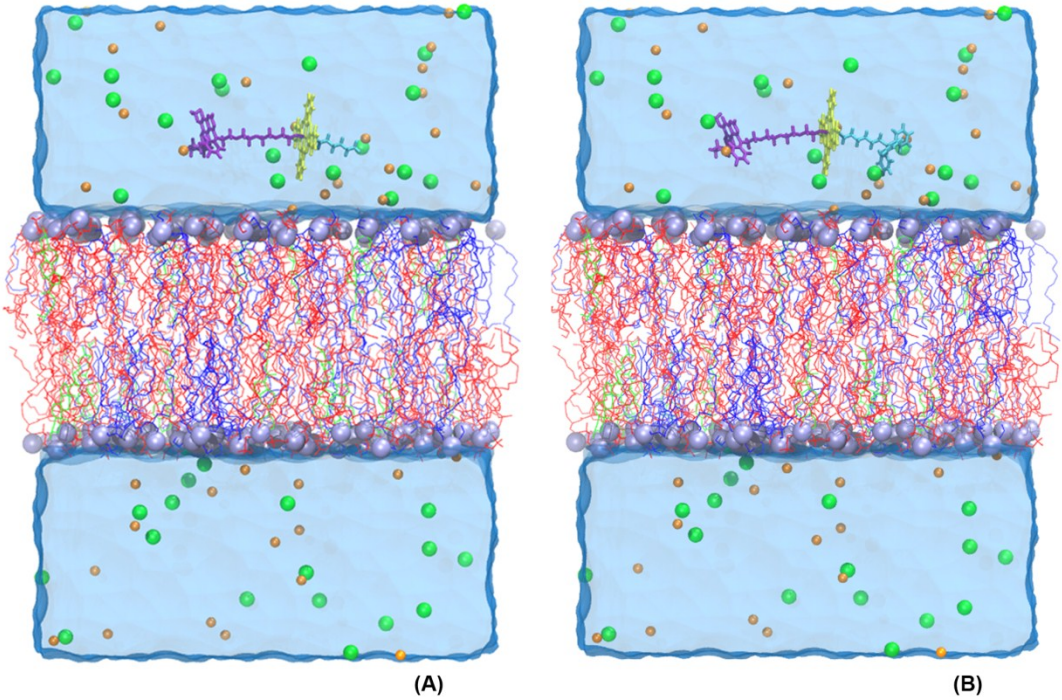


Figure S22. Initial structure of the G-Pc model (A) and G-Mito-Pc model (B). G-Pc and G-Mito-Pc contain three moieties with different colors. Pc and Gefitinib moieties are shown in yellow and purple, respectively. TPP and hexyloxy moieties are all shown in cyan. DOPC, DOPE and cholesterol molecules are showed with line in red, blue and green, respectively. The phosphorous atoms, sodium and chloride ions are shown as ice blue, orange and green van de Waals spheres, respectively.

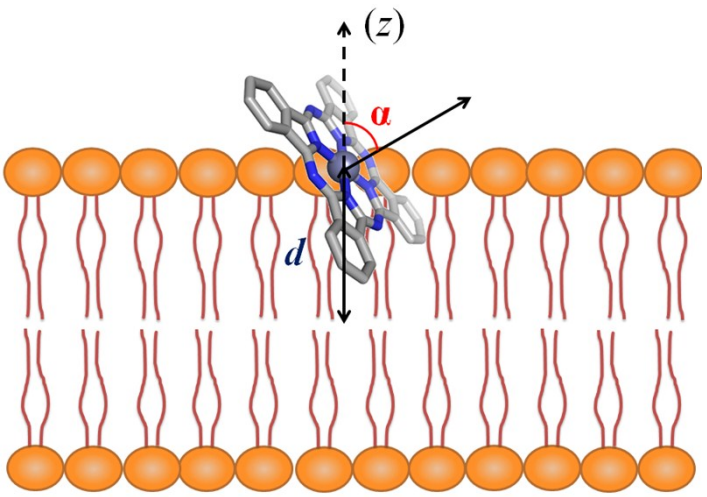


Figure S23. Distance between silicon and the mass center of the bilayer in z direction (*d*) and the angle between the normal vectors of the planar Pc moiety and the bilayer surface (*α*).

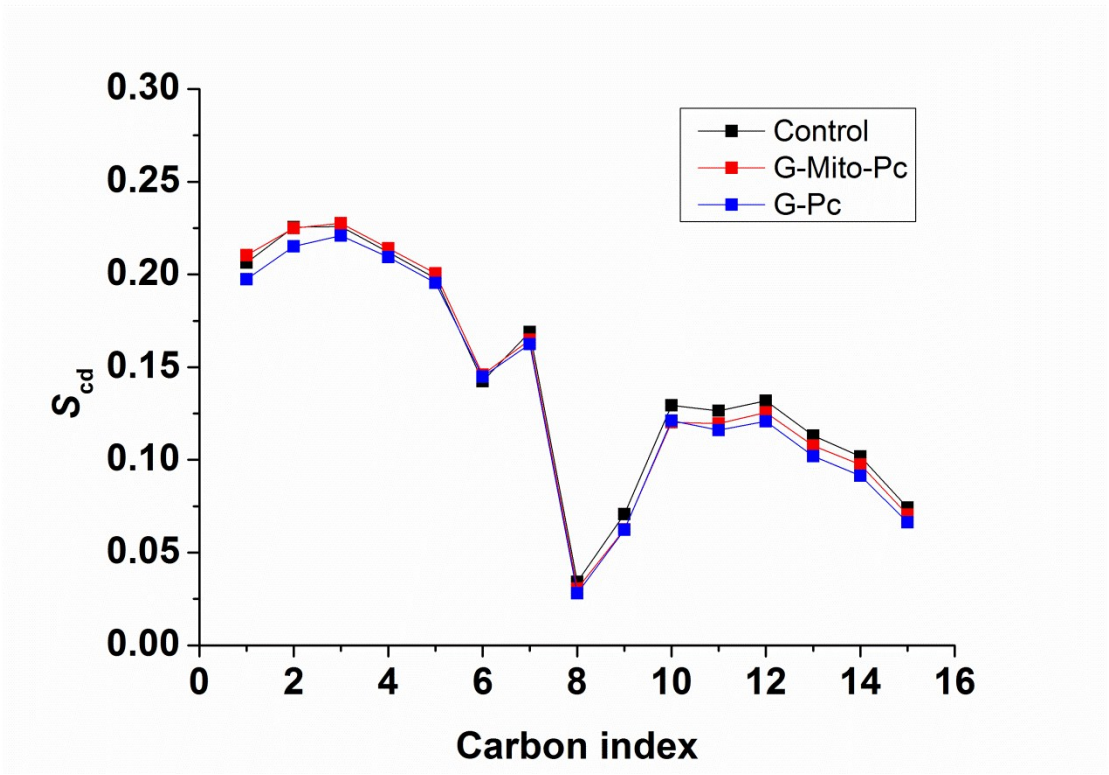


Figure S24. Deuterium order parameters S_{cd} of the oleic chain. The carbon index started from the carboxyl group of the fatty acid chain. The legend "control" indicates that there was no membrane insertion of other molecules.

Table S1. Photophysical and Photochemical Data for G-Mito-Pc, G-Pc, and PEG-Pc in DMF.

Compd	λ_{max}^{abs} (nm) (log ε)	λ_{max}^{ex} (nm)	Φ_F[b]	Φ_Δ[c]
G-Mito-Pc	**675 (5.57)**	**680**	**0.44**	**0.40**
G-Pc	**674 (5.32)**	**679**	**0.10**	**0.10**
PEG-Pc	**674 (5.63)**	**680**	**0.43**	**0.30**

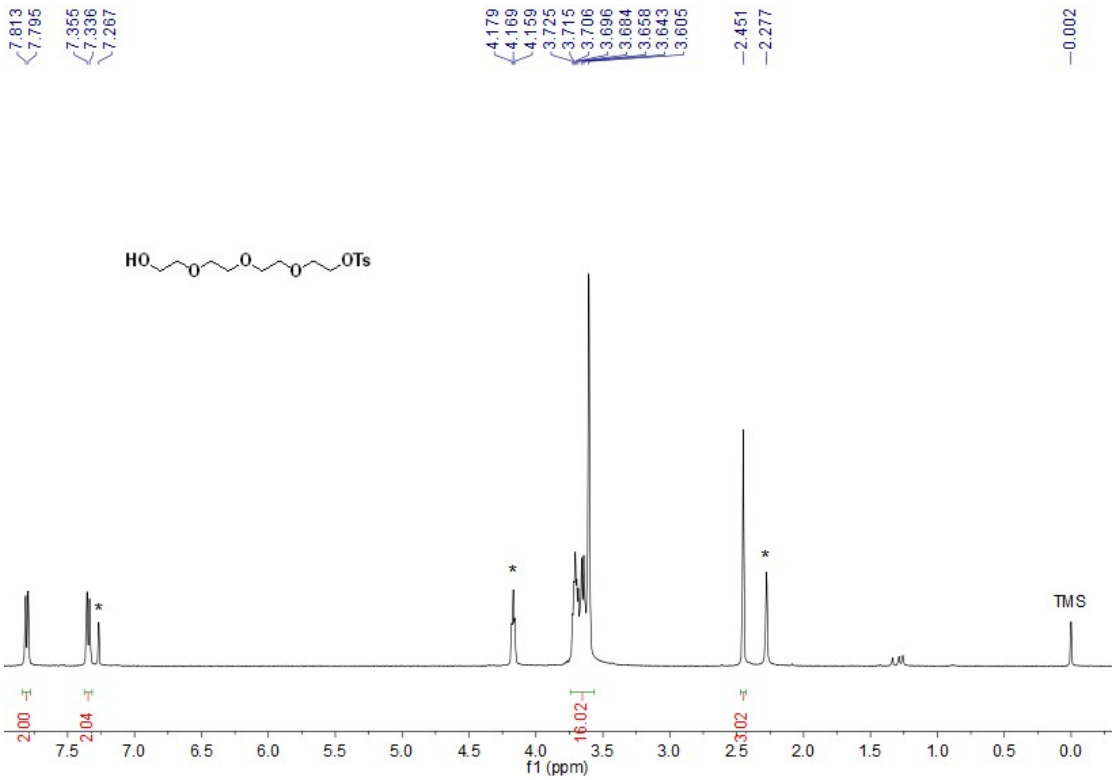
[a]Excited at 610 nm. [b]Using ZnPc in DMF as the reference (Φ_F = 0.28 and $\Phi\Delta$ = 0.56 in DMF).

Table S2. IC_{50} values for G-Mito-Pc, G-Pc, and PEG-Pc in vitro anticancer activity assays.

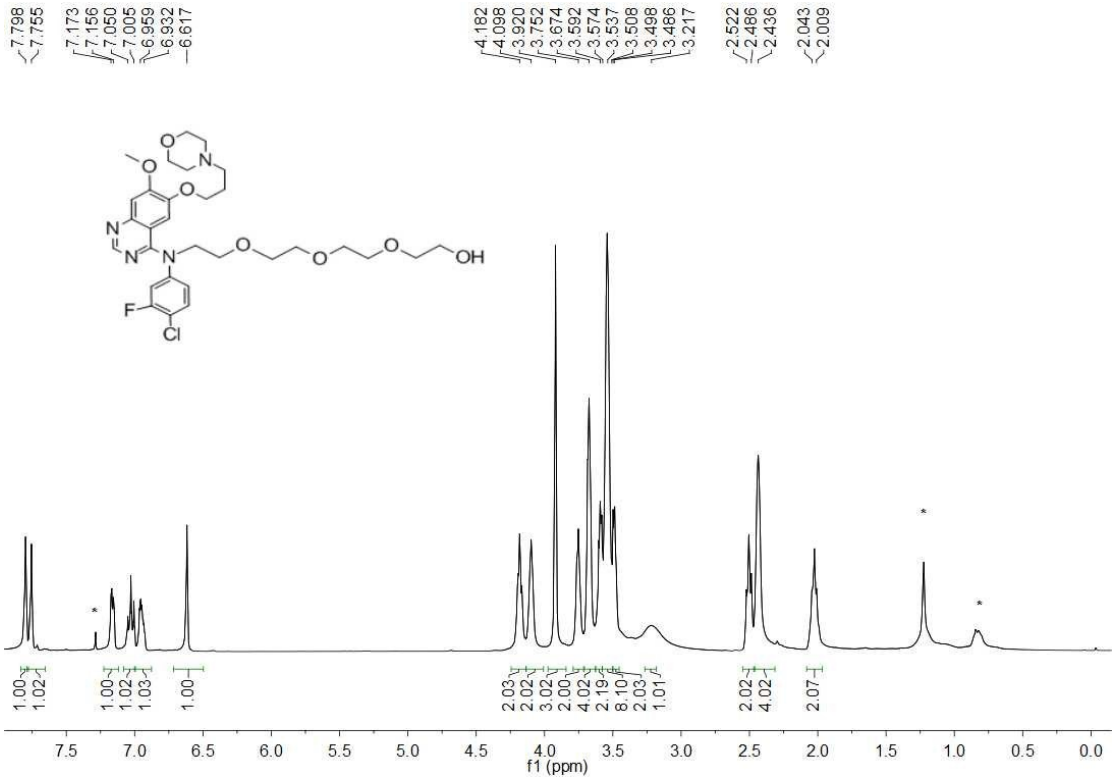
	IC_{50} (nM)		
Compd	**Hela cells**	**A549 cells**	**MDA-MB 468 cells**
G-Mito-Pc	**1.57±0.88**	**5.03±0.81**	**0.96±0.89**
G-Pc	**35±0.26**	**55±0.11**	**32±0.32**
PEG-Pc	**119±1.27**	**157±1.96**	**40±0.18**

In the presence of light (λ= 670 nm, 12.5 mWcm^{-2} for 2 min, 1.5 J·cm^{-2}). Data are expressed as mean values ± standard error of mean value (SEM) of three independent experiments.

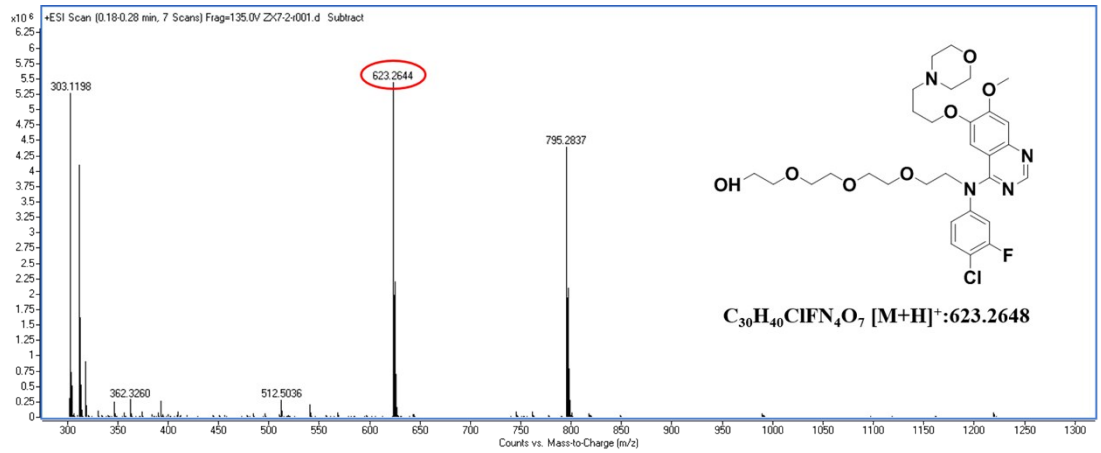
NMR and HRMS spectrum



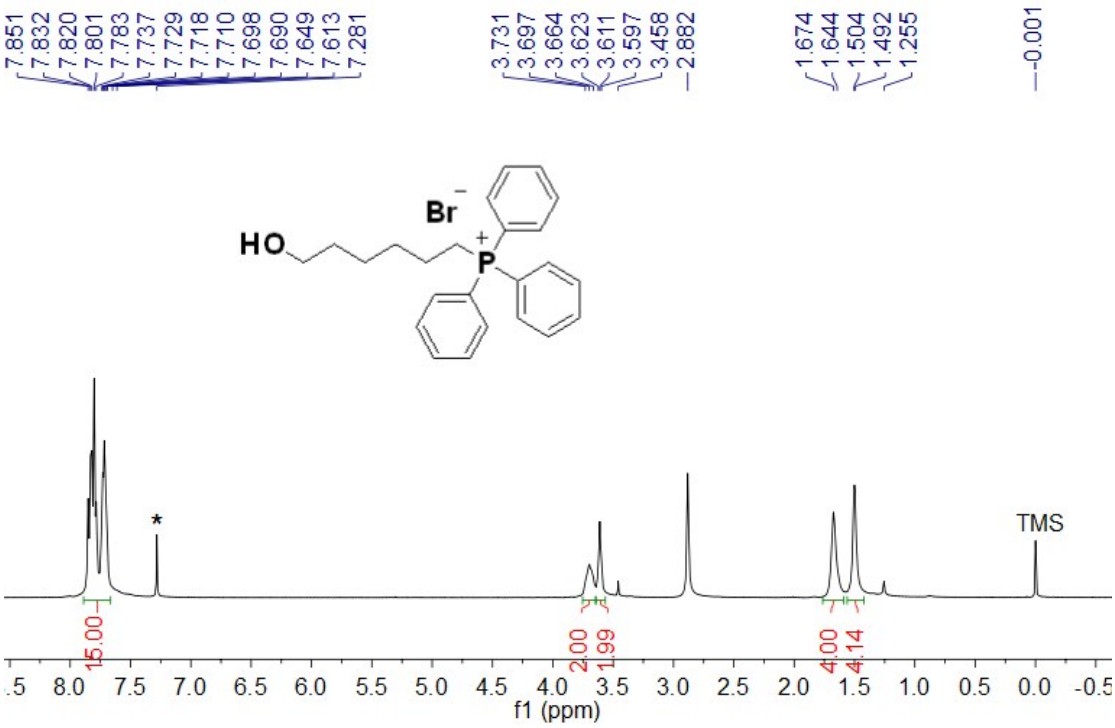
^{1}H NMR spectrum of 1a



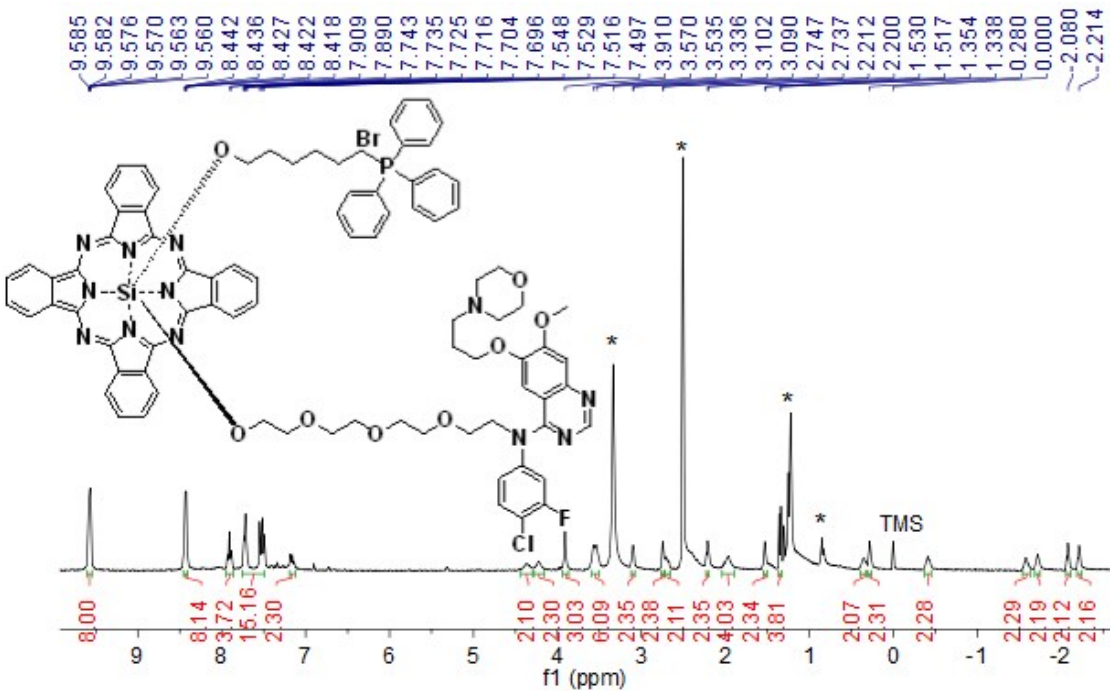
^{1}H NMR spectrum of 2a



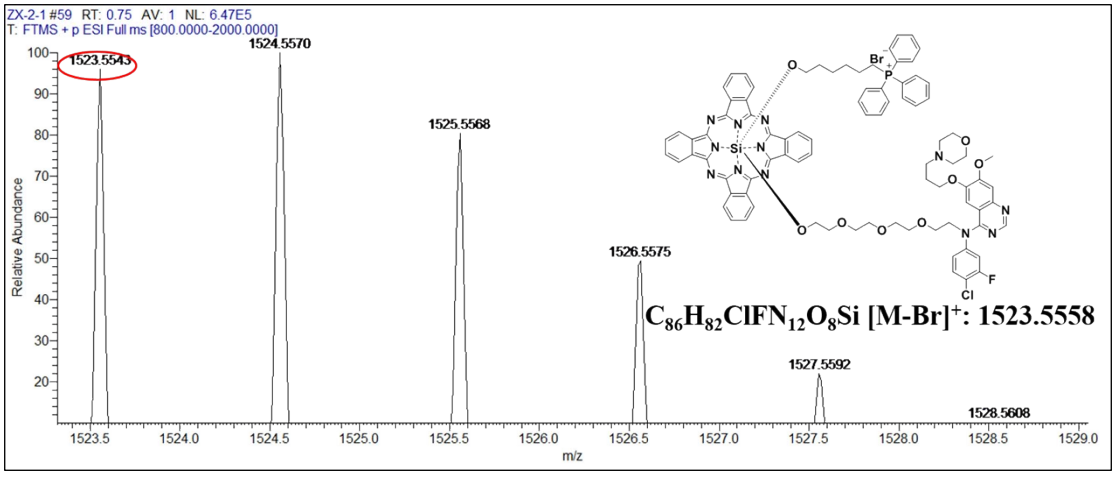
HRMS spectrum of 2a



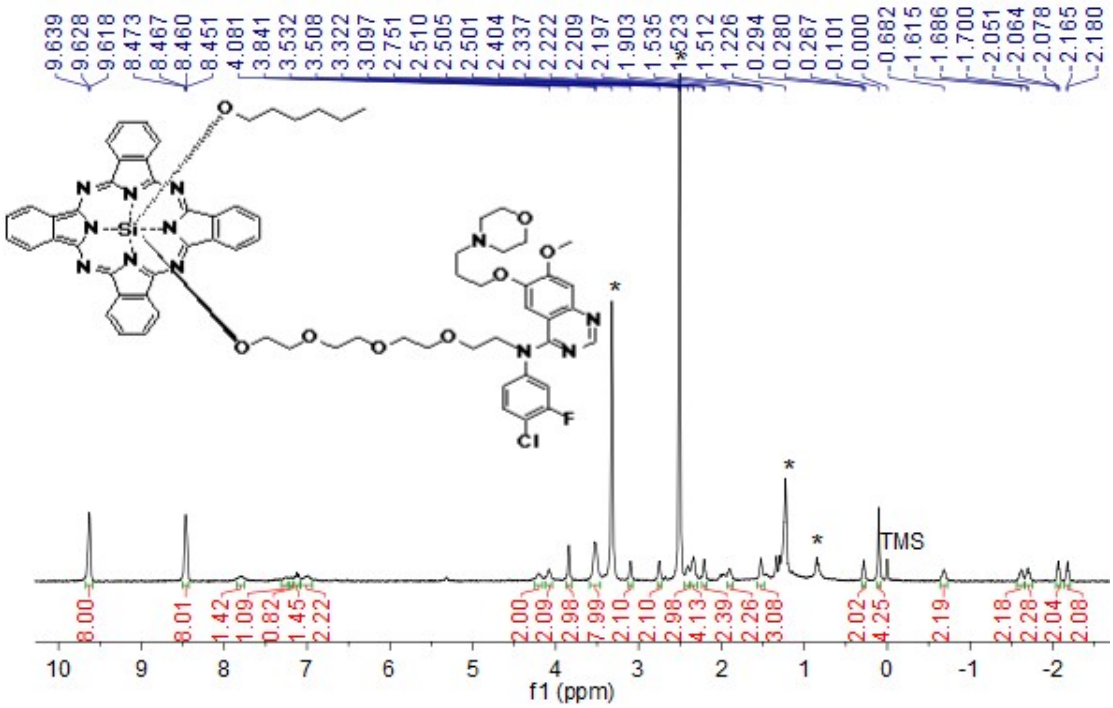
^{1}H NMR of 3a



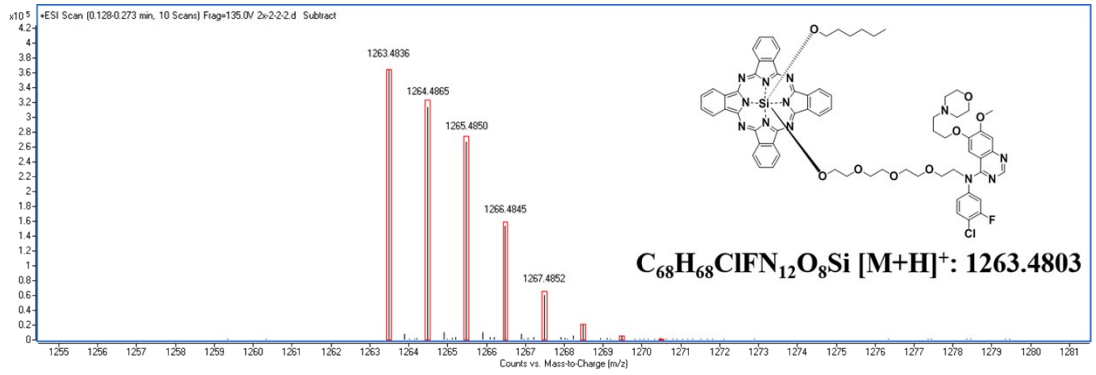
^{1}H NMR of G-Mito-Pc



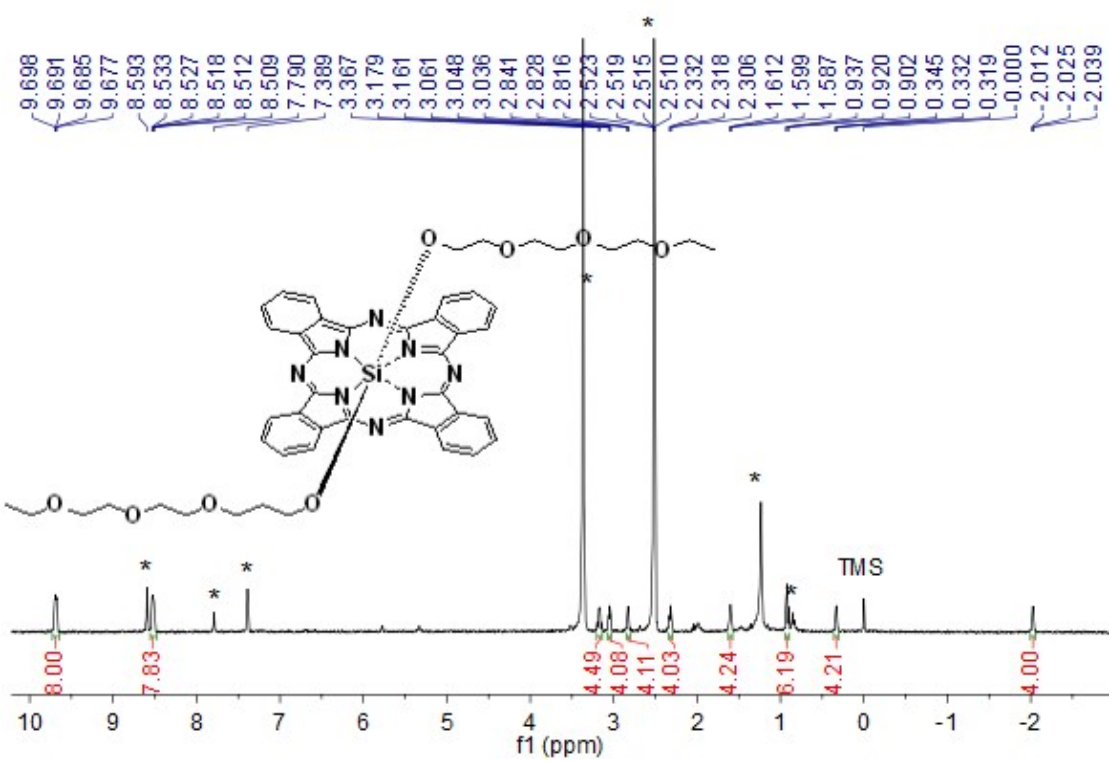
HRMS spectrum of G-Mito-Pc.



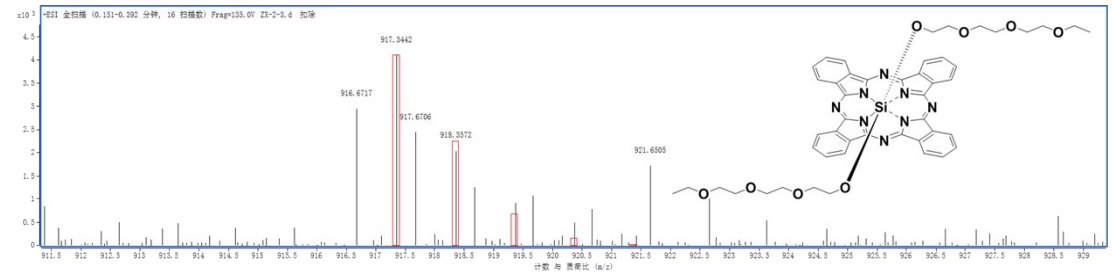
^{1}H NMR of G-Pc



HRMS spectrum of G-Pc.



^{1}H NMR of PEG-Pc.



HRMS spectrum of PEG-Pc.

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