

## SUPPLEMENTARY INFORMATION

### Fluorescent Periodic Mesoporous Organosilica Nanoparticles Dual-Functionalized via Click Chemistry for Two-Photon Photodynamic Therapy in Cells

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#### I- EXPERIMENTAL SECTION

**Materials.** Toluene, CH<sub>2</sub>Cl<sub>2</sub>, *N,N*-dimethylformamide and triethylamine were freshly distilled over CaH<sub>2</sub>, and THF freshly distilled over sodium/benzophenone before being used. Extraction and column chromatography solvents were purchased in anhydrous form, and used as received. Cetyltrimethylammonium bromide (CTAB), sodium hydroxide, doxorubicin hydrochloride, azidopropyltrimethoxysilane, and ammonium nitrate were purchased from Sigma-Aldrich. Absolute ethanol was purchased from Fisher Chemicals. Hydrochloric acid was purchased from Anal. R. Norma Pure. 1,2-bis(triethoxysilyl)ethylene was purchased from ABCR. All other reagents were purchased from Acros, Aldrich or Alfa-Aesar, and used

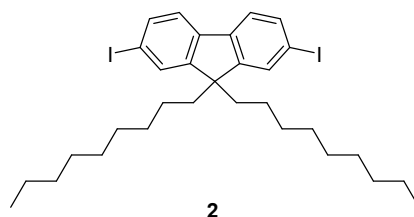
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<sup>1</sup> Authors J.C. and S.P. contributed equally to the work.

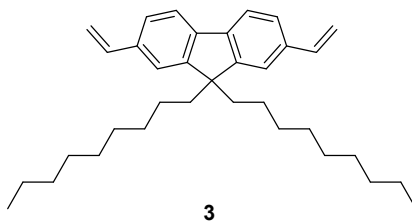
without further purification, unless indicated otherwise. (3-azidopropyl)trimethoxysilane was obtained from a reported procedure by M. Ortega-Muñoz *et al.*<sup>1</sup>

**Methods.** All air and moisture sensitive manipulations were carried out using standard techniques, with flame-dried reaction vessels, anhydrous solvents, and under argon atmosphere. Column chromatography was performed on Fluka silica gel 60 (40-63  $\mu\text{m}$ ).  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on a Bruker Advance III 200 and on a Bruker Advance 300 spectrometer. Shifts ( $\delta$ ) are given in parts per million with respect to solvent residual peak, and coupling constant ( $J$ ) are given in Hertz.  $^{29}\text{Si}$  and  $^{13}\text{C}$  CPMAS solid state NMR sequences were recorded with a VARIAN VNMRS300, using Q8MH8 and adamantane references respectively. Melting points were measured on Stuart SMP 10. LC/MS analyses were performed on a Shimadzu LCMS-2020. UV/Vis absorption spectra were recorded using a Hewlett-Packard 8453 spectrophotometer and a Jasco V-570 spectrophotometer, and fluorescence data were collected on a Perkin-Elmer LS55 fluorimeter or on a Horiba Jobin-Yvon Fluorolog. Dynamic light scattering analysis were performed using a Cordouan Technologies DL 135 Particle size analyzer instrument. TEM images were recorded with a JEOL instrument. SEM images were recorded with a FEI instrument. All photophysical studies have been performed with freshly-prepared air-equilibrated solutions at room temperature (298 K). UV/Vis absorption spectra of  $10^{-5}$  M solutions were recorded on a Jasco V-670 spectrophotometer. Steady-state and time-resolved fluorescence measurements were performed on dilute solutions (*ca.*  $10^{-6}$  M, optical density  $< 0.1$ ) contained in standard 1 cm quartz cuvettes using Fluorolog-3 (Horiba Jobin Yvon) and/or Edinburgh Instruments (FLS920) spectrofluorimeters.

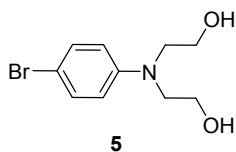
## II- TWO-PHOTON FLUOROPHORE SYNTHESIS AND CHARACTERIZATIONS



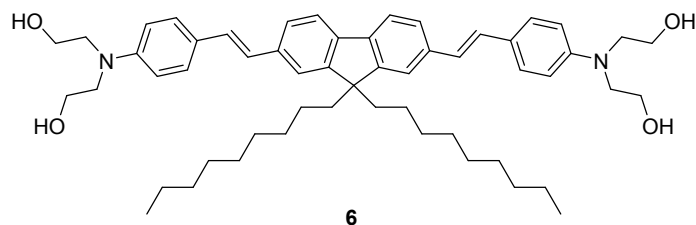
**2,7-diiodo-9,9-dinonyl-9H-fluorene<sup>2</sup> (2):** To a stirred mixture of 2,7-diiodo-9H-fluorene<sup>3</sup> (5.0 g, 12.0 mmol), in DMSO (20 mL) under argon atmosphere, were added successively KI (199 mg, 1.20 mmol), and powdered KOH (3.36 g, 59.8 mmol) at 0 °C. 1-bromononane (5 mL, 26.3 mmol) was then added dropwise, and the mixture was stirred at room temperature under inert atmosphere for 24 hours. The reaction was then quenched with a saturated NH<sub>4</sub>Cl solution (200 mL), and the mixture extracted with petroleum ether (5 x 80 mL). The combined organic layers were washed with water (50 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated under reduced pressure. The resulting crude orange oil was purified by a column chromatography of silicagel (eluent: petroleum ether) followed by a distillation under reduced pressure (70 °C, 0.106 mmHg) to eliminate the excess of 1-bromononane, to give (**x**) as a pale yellow powder (6.39 g, 79%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 200 MHz),  $\delta$  (ppm): 7.67 (d,  $J$  = 1.5 Hz, 2H), 7.63 (s, 2H), 7.40 (d,  $J$  = 8.6 Hz, 2H), 1.99 – 1.76 (m, 4H), 1.37 – 0.93 (m, 24H), 0.85 (t,  $J$  = 6.7 Hz, 6H), 0.57 (m, 4H).



**2,7-divinyl-9,9-dinonyl-9H-fluorene<sup>2</sup> (3):** In a Schlenk tube under argon atmosphere were introduced successively dried PdCl<sub>2</sub>(dppf) (31.1 mg, 0.381 mmol), and **2** (6.39 g, 9.53 mmol) in anhydrous degassed THF (12 mL). A vinylmagnesium bromide degassed solution (1M in THF, 0.895 mL, 0.895 mmol) was then added dropwise at 0 °C. The mixture was allowed to warm up to room temperature and stirred under inert atmosphere for 4 hours, then a saturated NH<sub>4</sub>Cl solution was added and the mixture was filtered on Celite<sup>®</sup>. The filtrate was washed with more NH<sub>4</sub>Cl solution (50 mL), then extracted with petroleum ether (4 x 40 mL). The combined organic layers were washed with water (50 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated under reduced pressure. The resulting crude oil was purified by a column chromatography of silicagel (eluent: petroleum ether, 100%) to give **3** as a pale yellow oil (4.20 g, 94%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 200 MHz),  $\delta$  (ppm): 7.62 (d,  $J$  = 7.8 Hz, 2H), 7.40 (d,  $J$  = 1.4 Hz, 2H), 7.35 (s, 2H), 6.80 (dd,  $J$  = 17.6, 10.9 Hz, 2H), 5.79 (dd,  $J$  = 17.6, 0.8 Hz, 2H), 5.25 (dd,  $J$  = 10.9, 0.8 Hz, 2H), 2.06 – 1.87 (m, 4H), 1.34 – 0.93 (m, 24H), 0.83 (t,  $J$  = 6.6 Hz, 6H), 0.63 (m, 4H).

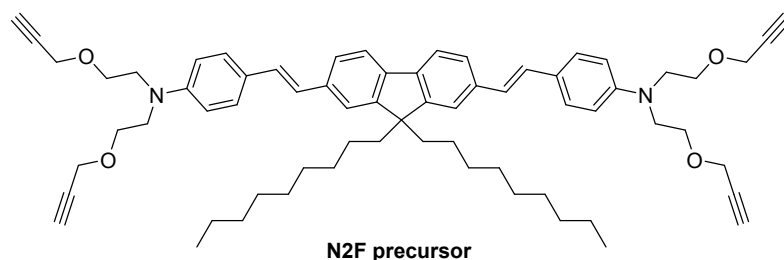


***N,N*-di(2-hydroxyethyl)-4-bromoaniline (5):** To a stirred solution of *N*-phenyldiethanolamine (2.0 g, 11.0 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) was added *N*-bromosuccinimide (2.16 g, 12.1 mmol). The mixture was stirred at room temperature and in the dark for 24 hours. A saturated Na<sub>2</sub>CO<sub>3</sub> solution was then added until discoloration, and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (4 x 50 mL). The combined organic layers were washed with water, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The resulting crude was purified by a column chromatography of silicagel (gradient eluent: CH<sub>2</sub>Cl<sub>2</sub>:EtOAc, 6:4 to 2:8), then washed with Et<sub>2</sub>O to give (**x**) as a white solid (2.55 g, 89%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 200 MHz), δ (ppm): 7.30 (d, *J* = 9.1 Hz, 2H), 6.59 (d, *J* = 9.0 Hz, 2H), 3.85 (t, *J* = 4.9 Hz, 4H), 3.56 (t, *J* = 4.9 Hz, 4H), 2.86 (s, 2H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 50 MHz): 146.94, 132.04, 114.32, 108.90, 60.57, 55.39.

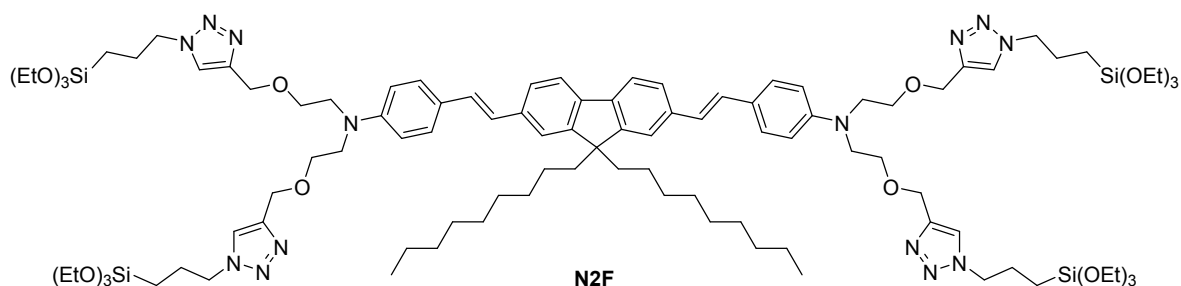


**2,2',2'',2'''-(((1*E*,1'*E*)-(9,9-dinonyl-9*H*-fluorene-2,7-diyl)bis(ethene-2,1-diyl))bis(4,1-phenylene))bis(azanetriyl))tetrakis(ethan-1-ol) (6):** In a Schlenk tube under argon atmosphere were introduced successively 2,7-divinyl-9,9-dinonyl-9*H*-fluorene (500 mg, 1.06 mmol), P(*o*-Tolyl)<sub>3</sub> (64.7 mg, 0.21 mmol), Pd(OAc)<sub>2</sub> (11.9 mg, 0.053 mmol), and *N,N*-di(2-hydroxyethyl)-4-bromoaniline (829 mg, 3.19 mmol). The mixture was dried under high vacuum, then DMF (5.5 mL) and Et<sub>3</sub>N (1.38 mL, 9.88 mmol) were added. This mixture was heated to 90 °C and stirred under inert atmosphere for 24 hours. A saturated NH<sub>4</sub>Cl solution (20 mL) was then added, and the mixture was filtered on Celite®. The filtrate was washed with more NH<sub>4</sub>Cl (50 mL) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (4 x 50 mL). The combined organic layers were washed with water (7 x 50 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated under

reduced pressure. The resulting dark green solid was used without further purification in next step.



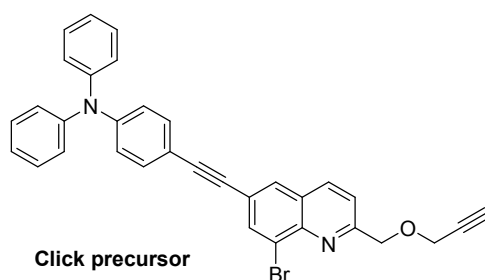
**4,4'-((1*E*,1'*E*)-(9,9-dinonyl-9*H*-fluorene-2,7-diyl)bis(ethene-2,1-diyl))bis(*N,N*-bis(2-(prop-2-yn-1-yloxy)ethyl)aniline) (N2F precursor):** To a stirred mixture of **6** (200 mg, 0.241 mmol) and KI (8.0 mg, 0.048 mmol) in dry DMF (3 mL) under argon atmosphere was added NaH (77.2 mg, 1.93 mmol, 60% weight in mineral oil). A propargyl bromide solution (0.215 mL, 1.93 mmol, 80% weight in toluene) was then added, and the mixture was stirred at room temperature under argon atmosphere for 24 hours. The reaction was then quenched with H<sub>2</sub>O (30 mL), and extracted with CH<sub>2</sub>Cl<sub>2</sub> (5 x 40 mL). The combined organic layers were washed with H<sub>2</sub>O (5 x 40 mL), dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The crude was purified by a column chromatography of silicagel (gradient eluent: toluene:CHCl<sub>3</sub>, 100:0 to 0:100), then further purified by a second column chromatography (eluent: toluene:CHCl<sub>3</sub>, 50:50) to afford the **N2F precursor** as a green solid (189 mg, 80%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 200 MHz),  $\delta$  (ppm): 7.63 (d, *J* = 8.0 Hz, 2H), 7.63 (d, *J* = 8.0 Hz, 4H), 7.49 (d, *J* = 8.0 Hz, 2H), 7.45 (d, *J* = 8.0 Hz, 2H), 7.09 (s, 2H), 6.97 (d, *J* = 10.0 Hz, 2H), 6.97 (d, *J* = 10.0 Hz, 4H), 4.66 (t, *J* = 7.0 Hz, 8H), 4.17 (d, *J* = 2.0 Hz, 8H), 3.30 (t, *J* = 7.0 Hz, 8H), 2.28 (s, 4H), 2.01 (m, 4H), 1.26-1.07 (m, 26H), 0.71 (t, *J* = 6.0 Hz, 6H).



**4,4'-((1*E*,1'*E*)-(9,9-dinonyl-9*H*-fluorene-2,7-diyl)bis(ethene-2,1-diyl))bis(*N,N*-bis(2-((1-(3-(triethoxysilyl)propyl)-1*H*-1,2,3-triazol-4-yl)methoxy)ethyl)aniline) (N2F):** The

tetrapropargyld **N2F precursor** (30 mg, 30.6  $\mu\text{mol}$ ) was mixed with  $[\text{CuBr}(\text{PPh}_3)_3]$  (1.5 mg, 1.6  $\mu\text{mol}$ ) in anhydrous THF (3 mL), placed in a 10 mL microwave sealable reactor, and (3-azidopropyl)triethoxysilane (3 mg, 122  $\mu\text{mol}$ ) was added. The tube was flushed with argon, then microwave irradiation (max power 200 W, 100  $^\circ\text{C}$ , 15 min) was applied. After evaporation of the solvents, extraction with pentane and concentration, compound **N2F** was obtained quantitatively (60 mg, 3.05  $10^{-2}$  mmol).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 200 MHz),  $\delta$  (ppm): 7.71 (d,  $J = 8.0$  Hz, 2H), 7.65 (d,  $J = 8.0$  Hz, 4H), 7.45 (d,  $J = 8.0$  Hz, 2H), 7.43 (d,  $J = 8.0$  Hz, 2H), 7.28 (s, 4H), 7.06 (s, 4H), 6.75 (d,  $J = 10.0$  Hz, 2H), 6.75 (d,  $J = 10.0$  Hz, 4H), 4.66 (t,  $J = 7.0$  Hz, 8H), 4.37 (t,  $J = 7.9$  Hz, 8H), 3.91 (s, 8H), 3.84 (q,  $J = 6.9$  Hz, 24H), 3.30 (t,  $J = 7.0$  Hz, 8H), 2.04 (t,  $J = 7.8$  Hz, 8H), 1.72 (m, 8H), 1.25 (t,  $J = 6.8$  Hz, 36H), 1.20-1.08 (m, 26H), 0.71 (t,  $J = 2.6$  Hz, 6H), 0.67 (t,  $J = 7.0$  Hz, 8H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ),  $\delta$  (ppm): 7.33, 14.32, 18.62, 22.49, 23.30, 29.02, 29.09, 29.25, 31.68, 52.01, 58.24, 64.16, 67.61, 112.15, 120.24, 124.19, 125.23, 128.03, 129.17, 129.28, 131.90, 131.99, 132.51, 144.32, 147.70, 147.83.  $^{29}\text{Si}$  NMR (80 MHz,  $\text{CDCl}_3$ ),  $\delta$  (ppm): -45.90 ppm. FTIR (neat KBr),  $\nu$  ( $\text{cm}^{-1}$ ): 2977, 2931, 1607, 1521, 1475, 1390, 1104, 1064, 956, 785, 545. UV/Vis  $\lambda_{\text{max}}$  (EtOH): 408 nm. HRMS (ESI $^{+}$ ):  $m/z$  1969.18, calcd for  $\text{C}_{64}\text{H}_{109}\text{N}_{14}\text{O}_{12}\text{Si}_4$ : 1970.1, found.

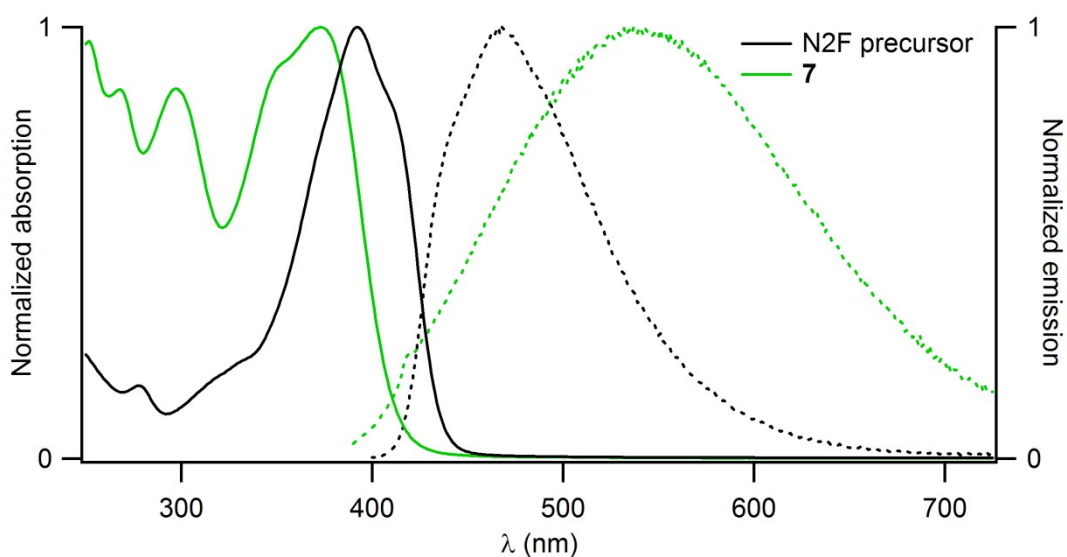
### III- SYNTHESIS OF THE PHOTOSENSITIZERS



#### **4-((8-bromo-2-((prop-2-ynyloxy)methyl)quinolin-6-yl)ethynyl)-N,N-diphenylaniline**

**(Click precursor):** To a stirred solution of (8-bromo-6-((4-(diphenylamino)phenyl)ethynyl)quinolin-2-yl)methanol<sup>[4]</sup> **7** (51 mg, 100  $\mu\text{mol}$ ), in anhydrous THF (1 mL), was added a cristal of KI, propargyl bromide (22.3 mg, 0.15 mmol, 80% weight in toluene) and NaH (8 mg, 0.2 mmol, 60% weight in mineral oil). The reaction mixture was stirred overnight at room temperature, quenched with a saturated  $\text{NaHCO}_3$  solution, and extracted with EtOAc. The combined organic layers were dried over  $\text{Na}_2\text{SO}_4$ , and concentrated under reduced pressure.

The crude was purified by a column chromatography of silicagel (eluent: toluene) to afford the **Click precursor** as a brown viscous oil (17.0 mg, 31%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 200 MHz)  $\delta$  (ppm): 2.50 (t,  $J = 2.3$  Hz, 1H), 4.38 (d,  $J = 2.3$  Hz, 2H), 4.97 (s, 2H), 6.97-7.18 (m, 8H), 7.27-7.42 (m, 6H), 7.68 (d,  $J = 8.5$  Hz, 1H), 7.92 (d,  $J = 1.4$  Hz, 1H), 8.08-8.14 (m, 2H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 50 MHz): 58.5, 73.2, 75.2, 87.1, 92.2, 115.2, 120.8, 122.1, 122.6, 123.9, 125.3, 128.5, 129.6, 130.3, 132.8, 135.7, 147.2, 148.6, 160.3. LC-MS:  $\text{C}_{33}\text{H}_{23}\text{BrN}_2\text{O}$  calcd for  $[\text{M} + \text{H}]^+$ : 543.11, found 542.90.



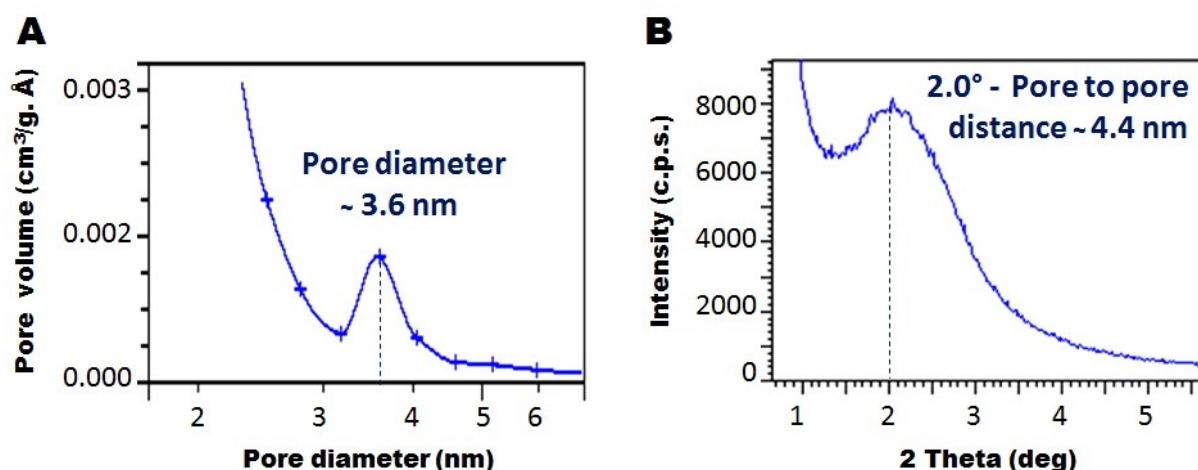
**Figure S1.** Absorption (solid lines) and emission (dashed lines) spectra of N2F precursor and click precursor models in ethanol.

#### IV- PMO NPs SYNTHESSES AND CHARACTERIZATIONS

**EN2C NPs.** A mixture of CTAB (125 mg, 0.343 mmol), distilled water (60 mL), and sodium hydroxide (437  $\mu\text{L}$ , 2 M) was stirred at 80°C for 2 h at 700 rpm in a 100 mL round bottom flask. Then, 1,4-bis(triethoxysilyl)ethylene (300  $\mu\text{L}$ , 2.63 mmol) was added along with the N2F (30 mg, 15  $\mu\text{mol}$ , in 900  $\mu\text{L}$  of anhydrous THF), and (3-azidopropyl)triethoxysilane (100 mg, 0.400 mmol), and the condensation process was conducted for 2 h.<sup>4</sup> Afterwards, the solution was cooled to room temperature while stirring; fractions were gathered in propylene tubes and the NPs were collected by centrifugation during 15 minutes at 21 krpm. The sample was then extracted twice with an alcoholic solution of ammonium nitrate (6  $\text{g.L}^{-1}$ ), and washed three times with ethanol, water, and ethanol. Each extraction involved a sonication

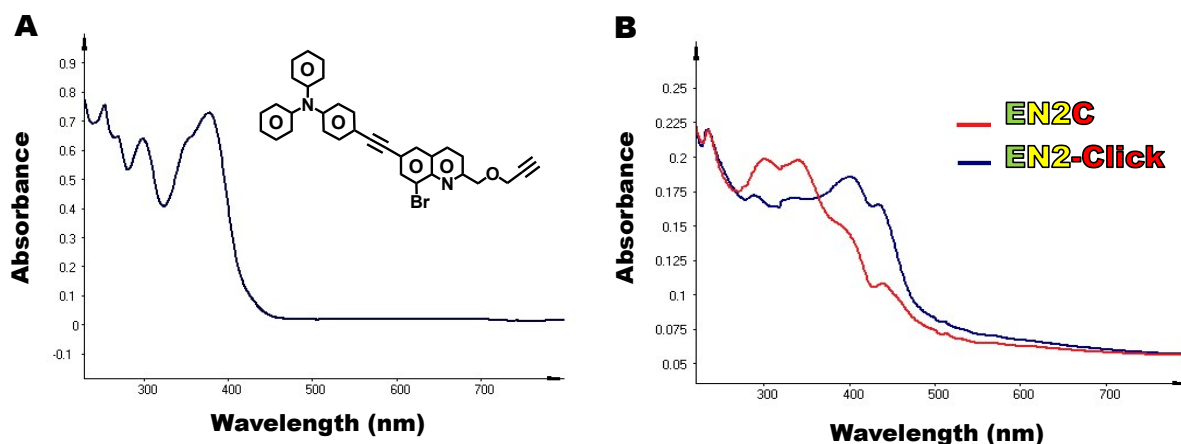
step of 30 minutes at 50 °C; the collection was carried out in the same manner.<sup>5</sup> The as-prepared material was dried for few hours under vacuum.

**Click chemistry on EN2C NPs.**<sup>6</sup> To a mixture of the EN2C NPs (5 mg) and [CuBr(PPh<sub>3</sub>)<sub>3</sub>] (5 mg, 5.4 μmol) in water (1.5 mL) and *tert*-butanol (1.5 mL) placed in a 10 mL microwave sealable reactor was added the **Click precursor** (3 mg, 5.5 μmol). The tube was flushed with argon, then microwave irradiation (max power 200 W, 100 °C, 15 min) was applied. After evaporation of the solvents, THF (3 mL) was added and a second microwave irradiation (max power 200 W, 100 °C, 15 min) was applied. The THF was evaporated and the NPs were washed four times with water, four times with THF, and two times with acetone. Each washing involved a sonication step of 20 seconds and the material collection was carried out by centrifugation during 8 minutes at 10 krpm. The as-prepared material was dried for few hours under vacuum.

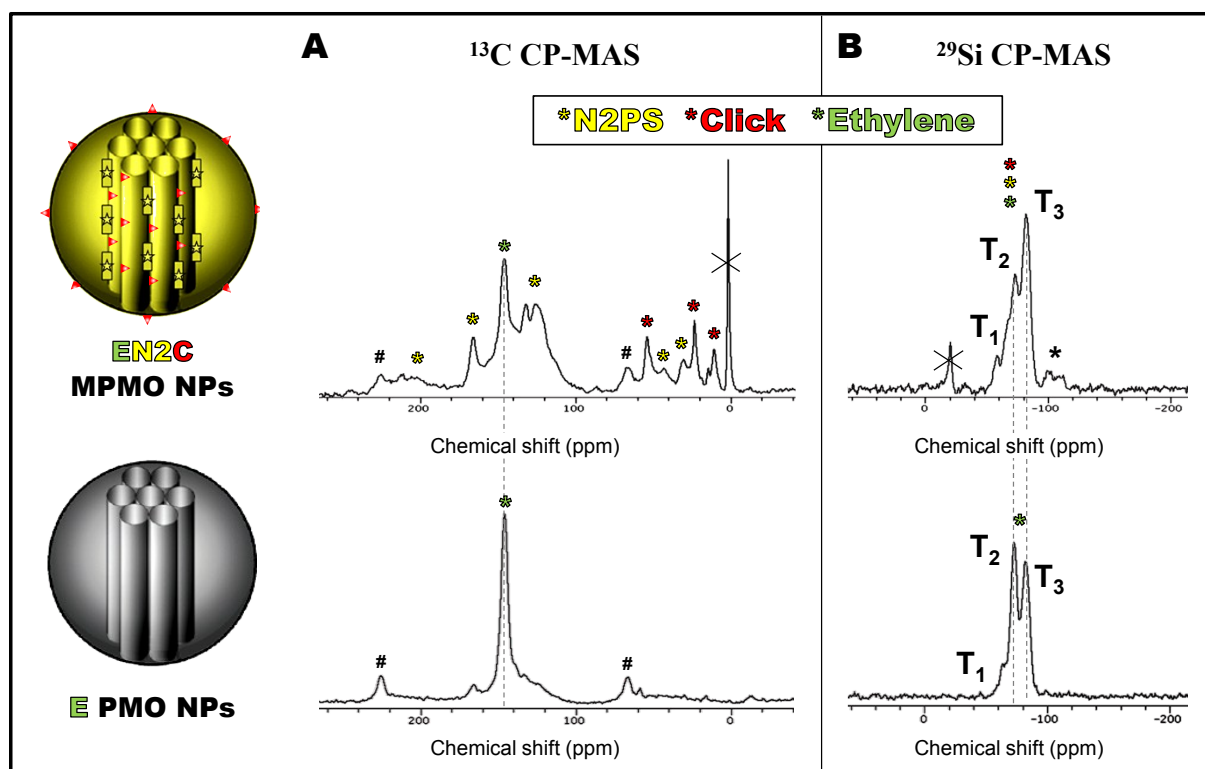


**Figure S2.** Pore size distribution from the Barrett-Joyner-Halenda (BJH) desorption data (A), and X-ray diffraction pattern of EN2C NPs (B), validating the mesoporosity of the NPs. The calculated BJH average pore size distribution from the adsorption data was of 35.62 Å.

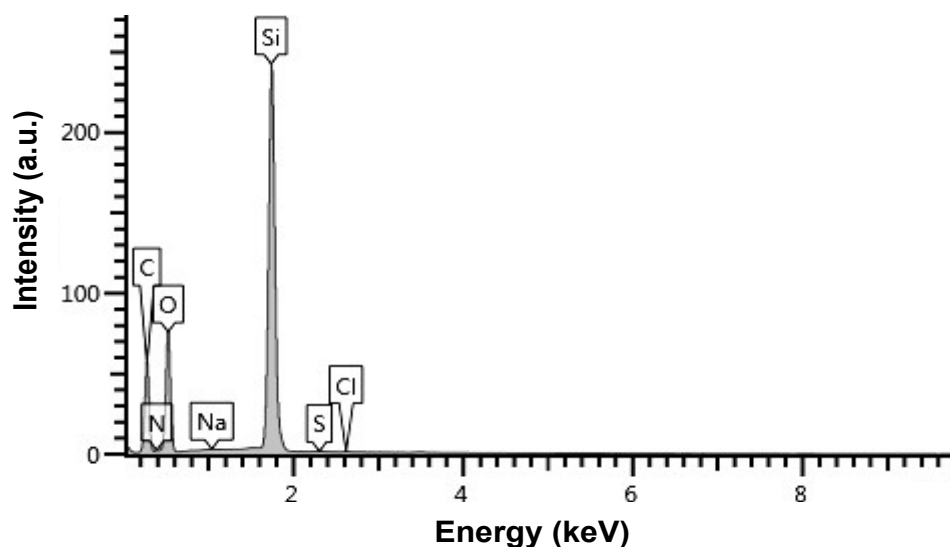




**Figure S3.** UV-visible spectra of the photosensitizer precursor for the click chemistry (A), EN2C NPs and the resulting EN2-Click NPs (B) in ethanol.



**Figure S4.** Solid state NMR  $^{13}\text{C}$  (A) and  $^{29}\text{Si}$  (B) CPMAS spectra of the EN2C MPMO NPs and of a control of E PMO NPs, confirming the presence of the N2PS, CLIK, and Ethylene moieties. # denotes the spinning side bands. The black asteriks (\*) in the -100 ppm region of the  $^{29}\text{Si}$  spectra of the EN2C corresponds to a partial cleavage of the Si-C bonds into Si-O ones ( $\text{Q}_3$  and  $\text{Q}_4$  environments).



**Figure S5.** EDS spectrum of the EN2C NPs.

**Table S1.** EDS elemental analysis of EN2C NPs.

Element	Weight %	Weight % Sigma	Atomic %
C	44.00	0.12	56.92
N	0.47	0.14	0.52
O	28.32	0.09	27.50
Na	0.04	0.01	0.03
Si	27.10	0.07	14.99
S	0.03	0.01	0.01
Cl	0.05	0.01	0.02
<b>Total:</b>	<b>100.00</b>	<b>/</b>	<b>100.00</b>

## **V- LOADING AND RELEASE EXPERIMENT**

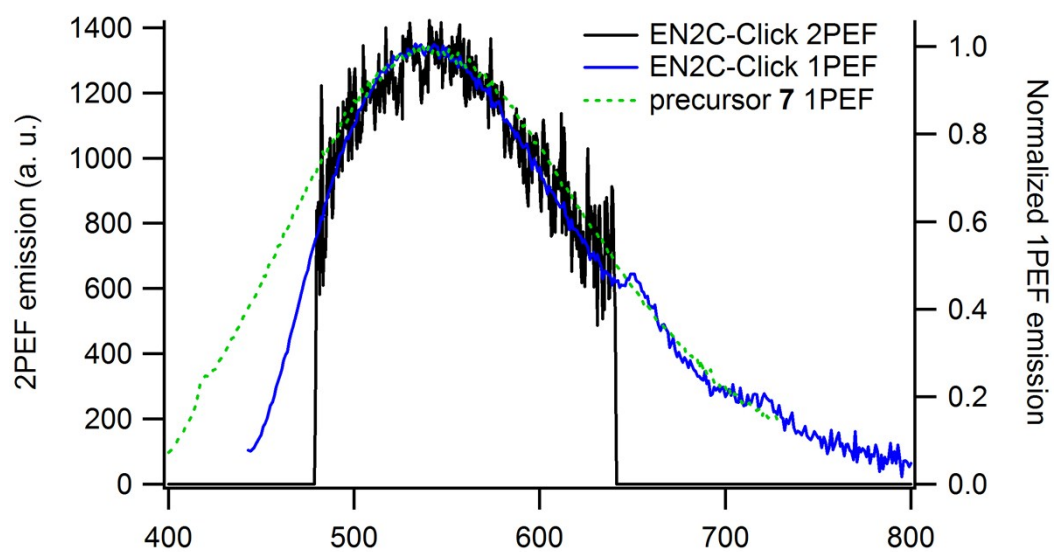
**Preparation of DOX-loaded PMO NPs.** A mixture of water (0.5 mL), DOX (1 mg), and EN2C NPs (10 mg) was prepared in an eppendorf tube, sonicated for 20 min, and stirred overnight at room temperature. The preparation was centrifuged at 10000 rpm in 40 mL propylene tubes, and the supernatant was removed. The nanomaterial was washed five times with water (10 mL), and dried under vacuum. The loading capacities (LC) were calculated, by the titration of doxorubicin in the supernatant fractions, using with the following equation:

$$LC = [m_{\text{DRUG LOADED}}/m_{\text{NPs}}]*100$$

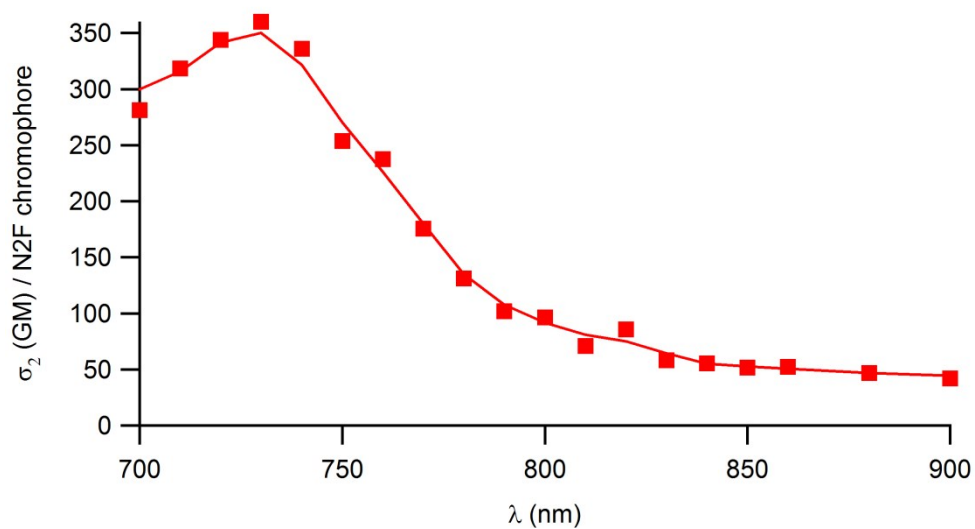
**Release of DOX from PMO NPs via pH trigger.** EN2C-DOX NPs (1 mg) were placed into a plastic cuvette and deionized water (1 mL) was then carefully added without dispersing the NPs. The absence of dispersed NPs was confirmed by the acquisition of a UV-Visible spectrum, and absorption measurements were then performed overtime to plot the baseline of the release profile at neutral pH. Aliquots of hydrochloric acid were then added to reach pH 5.5 and the release was monitored with UV-Visible spectroscopy. The Beer-Lambert law was used to quantify the percentage of DOX released.

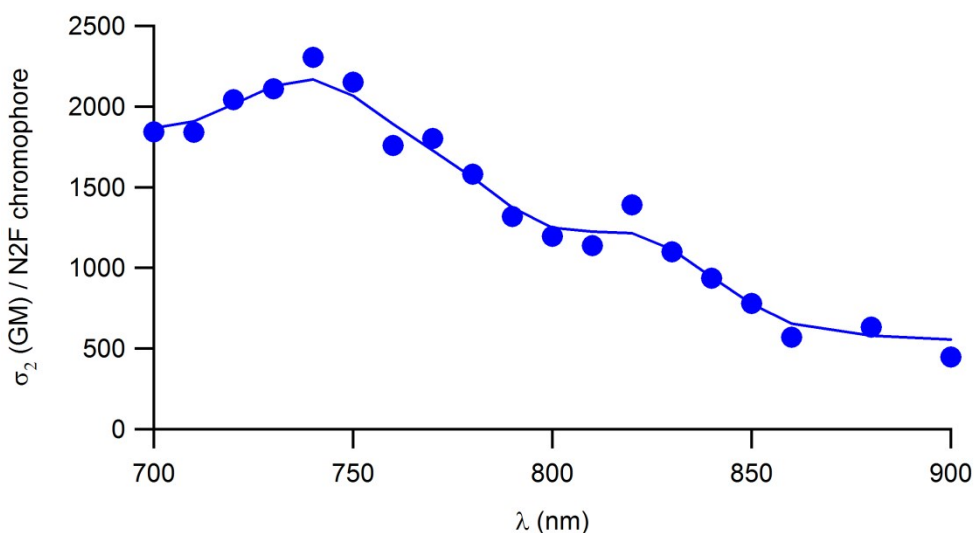
## VI- PHOTOPHYSICAL CHARACTERIZATIONS

2PA cross sections ( $\sigma_2$ ) were determined from the two-photon excited fluorescence (TPEF) cross sections ( $\sigma_2\Phi$ ) and the fluorescence emission quantum yield ( $\Phi$ ). TPEF cross sections of  $10^{-4}$  M solutions were measured relative to fluorescein in 0.01 M aqueous NaOH for 715-980 nm,<sup>7, 8</sup> using the well-established method described by Xu and Webb<sup>7</sup> and the appropriate solvent-related refractive index corrections.<sup>9</sup> Reference values between 700 and 715 nm for fluorescein were taken from literature.<sup>10</sup> The quadratic dependence of the fluorescence intensity on the excitation power was checked for each sample and all wavelengths. Measurements were conducted using an excitation source delivering fs pulses. To span the 700-980 nm range, a Nd:YLF-pumped Ti:sapphire laser was used generating 150 fs pulses at a 76 MHz rate. The excitation was focused into the cuvette through a microscope objective (10X, NA 0.25). The fluorescence was detected in epifluorescence mode via a dichroic mirror (Chroma 675dxcru) and a barrier filter (Chroma e650sp-2p) by a compact CCD spectrometer module BWTek BTC112E. Total fluorescence intensities were obtained by integrating the corrected emission.



**Figure S6.** Comparison between two-photon excited fluorescence (2PEF) upon excitation at 800 nm and one-photon excited fluorescence (1PEF) of EN2C-Click NPs and precursor **7** in ethanol.





**Figure S7.** 2PA spectra of EN2C (top, red) and EN2-Click (bottom, blue) NPs in ethanol.

## VII- IN-VITRO STUDIES

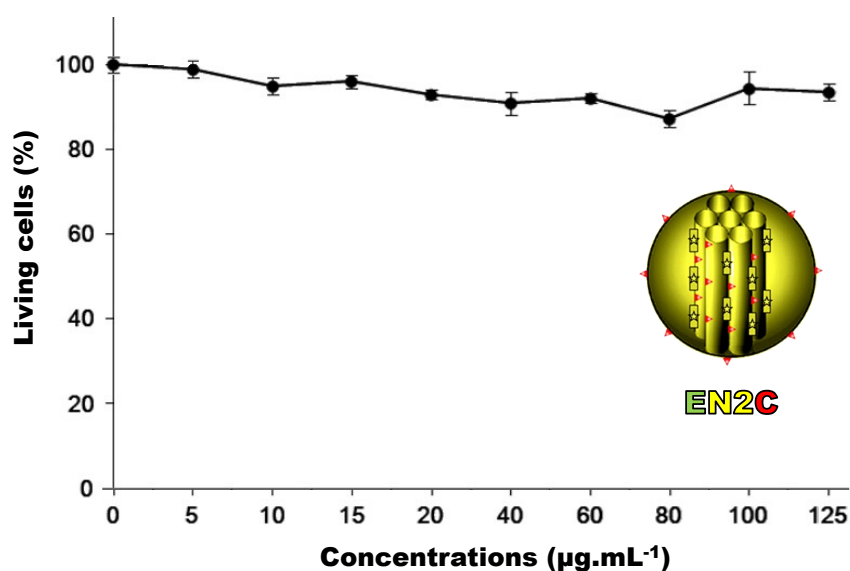
**Cell culture.** Human breast cancer cells MCF-7 (purchased from ATCC) were cultured in DMEM supplemented with 10% fetal bovine serum and 50  $\mu\text{g.mL}^{-1}$  gentamycin and allowed to grow in humidified atmosphere at 37 °C under 5 % CO<sub>2</sub>.

**TPE-PDT experiments.** For *in vitro* TPE-PDT, MCF-7 cells were seeded into a 384 multiwell glass-bottomed plate (thickness 0.17 mm), with a black polystyrene frame, 1000 cells per well in 50  $\mu\text{L}$  of culture medium, and allowed to grow for 24 h. Then cells were treated with 40  $\mu\text{g.mL}^{-1}$  PMO and 20 h after, cells were submitted (or not) to laser irradiation; with the Carl Zeiss Microscope (laser power input 3W). Half of the well was irradiated at 760 nm by three scans of 1.57 s duration in 4 different areas of the well. The laser beam was focused by a microscope objective lens (Carl Zeiss 10x/0.3 EC Plan-Neofluar). The scan size does not allow irradiating more areas without overlapping. After 2 days, the MTS assay was performed as previously described<sup>11</sup> and was corrected according to the following formula  $\text{Abs}_{\text{control}} - 2 \times (\text{Abs}_{\text{control}} - \text{Abs}_{\text{PMO}})$ .

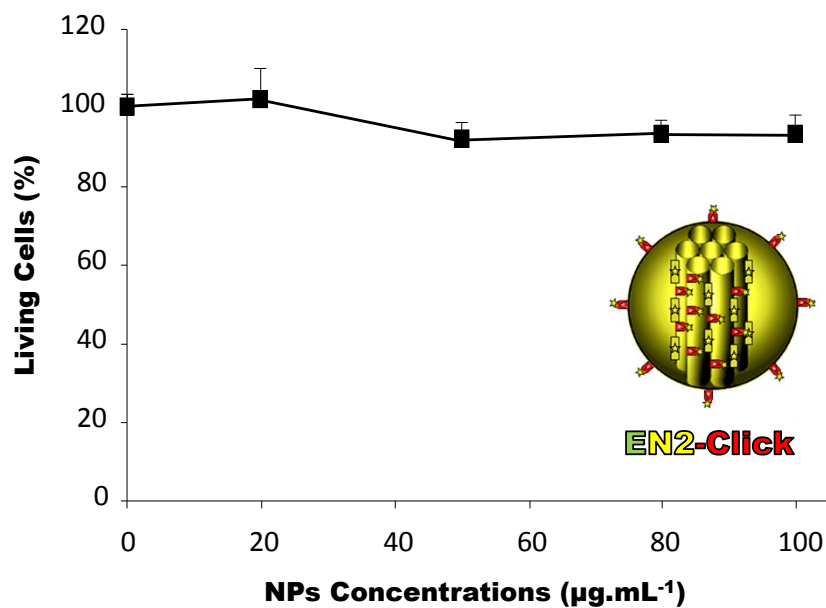
**Two-photon fluorescence Imaging.** The day prior to the experiment, MCF-7 human breast cancer cells (purchased from ATCC) were seeded onto bottom glass dishes (World Precision Instrument, Stevenage, UK) at a density of  $10^6 \text{ cells.cm}^{-2}$ . Adherent cells were then washed once and incubated in 1 mL medium containing nanoparticles at a concentration of 40  $\mu\text{g.mL}^{-1}$  for 20 h. Fifteen minutes before the end of incubation, cells were loaded with Cell Mask

(Invitrogen, Cergy Pontoise, France) for membrane staining at a final concentration of 5  $\mu\text{g.mL}^{-1}$ . Before visualization, cells were washed gently with phenol red-free Dulbecco's modified Eagle's medium (DMEM). Cells were then scanned with a LSM 780 LIVE confocal microscope (Carl Zeiss, Le Pecq, France), at 760 nm with a slice depth (Z stack) of 0.62  $\mu\text{m}$ .

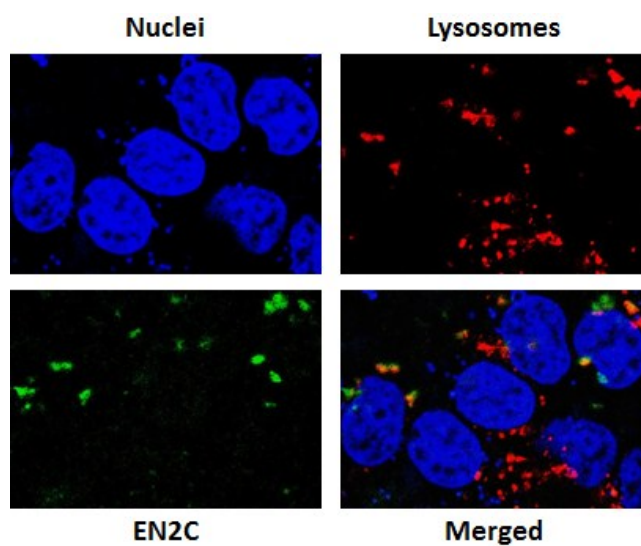
**Cytotoxicity measurement.** For *in vitro* cytotoxicity analysis, MCF-7 cells were seeded into a 96 well plate, 2000 cells per well in 200  $\mu\text{L}$  of culture medium, and allowed to grow for 24 h. Then cells were treated with increasing concentrations of PMO, and after 3 days, a MTT assay was performed as previously described.<sup>11</sup>



**Figure S8.** Cytotoxicity study of the EN2C NPs in MCF-7 cells 72 h after incubation, revealing the biocompatibility of the clickable nanomaterial.



**Figure S9.** Cytotoxicity study of the EN2-Click NPs in MCF-7 cells 72 h after incubation, revealing the biocompatibility of the clicked nanomaterials.



**Figure S10.** In-vitro two-photon fluorescence imaging of EN2C incubated for 20 h at  $40 \mu\text{g.mL}^{-1}$ . The nuclei and the lysosomes were stained by Hoechst 33342 and LysoTracker (Red DND-99), respectively.

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