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Supporting Information

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Two-Photon-Triggered Drug Delivery in Cancer Cells Using Nanoimpellers**

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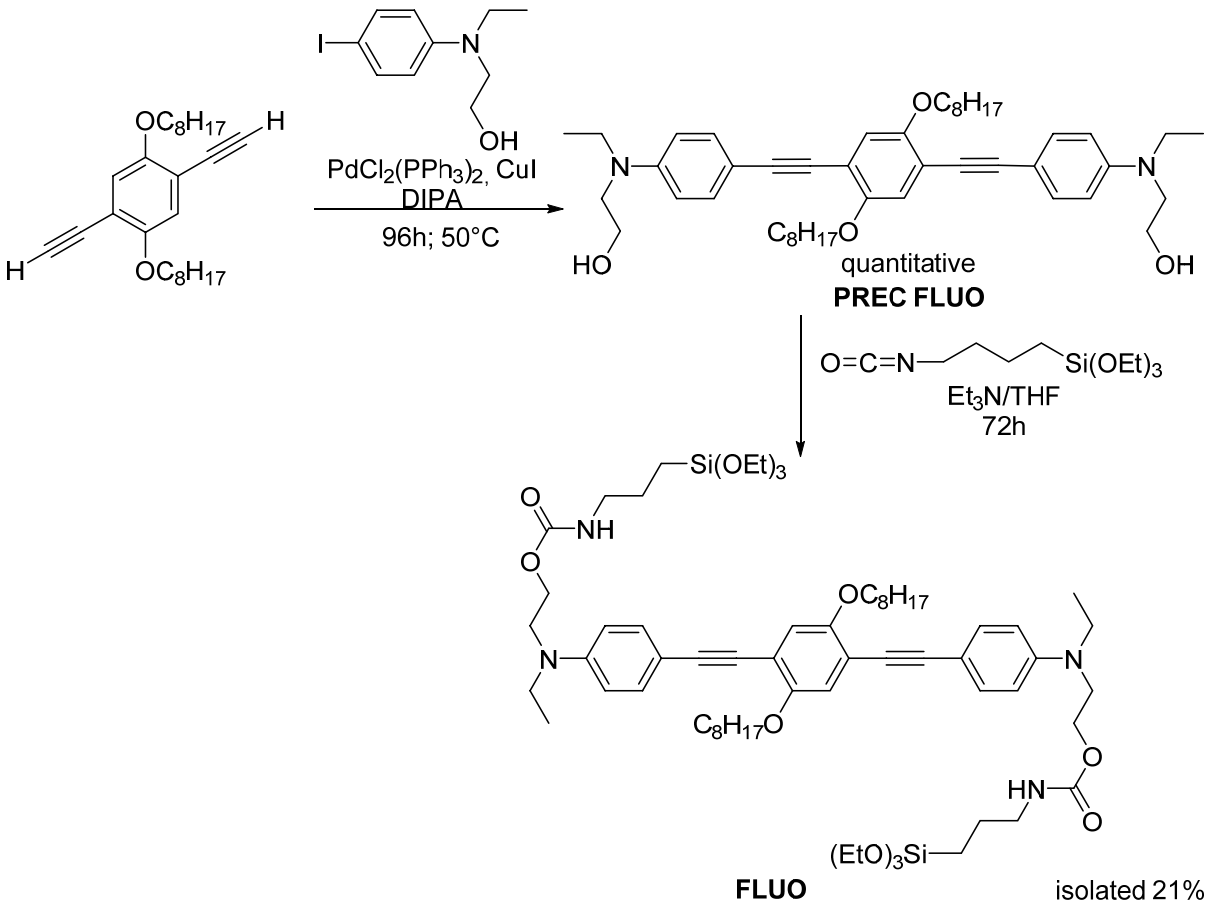
EXPERIMENTAL SECTION

General Procedures. THF and diisopropylamine were dried over sodium/benzophenone or CaH_2 respectively. Triethylamine, triethoxy(3-isocyanatopropyl)silane, bis(triphenylphosphine) dichloro-palladium, copper iodide were purchased (Alfa and Aldrich) and used without further purification. 1,4-Diethynyl-2,5-bis(octoxy)benzene[i] and 2-[ethyl(4-iodophenyl)amino]ethanol [ii] were prepared according to the literature. Flash chromatography purifications were carried out on an Armen Spot II Ultimate instrument. ^{1}H and ^{13}C NMR spectra were recorded with a Brucker AC 400 spectrometer. Chemical shifts (in δ units, ppm) are referenced to TMS using $CHCl_3$ (δ = 7.26 ppm) and $CDCl_3$ (δ = 77.0 ppm) as the internal standards, respectively, for ^{1}H and ^{13}C NMR spectra. IR spectra were recorded on a Perkin-Elmer 100 FT spectrophotometer. Absorption spectra were recorded on a Hewlett-Packard 8453 spectrophotometer and fluorescence data were collected on a Perkin-Elmer LS55 fluorimeter. Mass spectrometry was carried out at the Laboratoire de Spectrometrie de Masse (Lyon, France) with a Thermo-Finnigan MAT95 apparatus in electronic impact ionization mode.

SYNTHESIS AND CHARACTERIZATION OF ORGANIC COMPOUNDS

AZO precursor. The aminobenzene precursor (2.1 g) was placed in a 100 mL two necks round bottom flask with absolute ethanol (50 mL) and stirred under nitrogen flow at 54°C. Then, isocyanatopropyltriethoxysilane (2800 µL) was added through micropipette. Finally, the mixture was stirred overnight. The compound was stored in the fridge. The concentration was 4.2 mg of AZO per 100 µL EtOH (50 mL EtOH for 2.1 g of AZO).

FLUO precursor. **FLUO** was prepared according to a Pd(0) catalyzed double Sonogashira cross-coupling between 1,4-diethynyl-2,5-bis(octyloxy)benzene with two equivalents of 2-[ethyl(4-iodophenyl)amino]ethanol, giving in quantitative yield crude bis(ethanolamine) FLUO precursor (**FLUO PREC**) after 96 h reaction at 50°C in diisopropylamine. Crude **FLUO PREC** was then reacted in triethylamine with triethoxy(3-isocyanatopropyl)silane in refluxing THF during 72h. **FLUO** was obtained in 21% yield after a flash chromatography purification over a 15µm spherical silica column with CH_2Cl_2-AcOEt solvent mixture according to the (1:0 v:v) to (1:1 v:v) elution gradient (Scheme 1)



Scheme 1 : Synthesis of **FLUO**

PREC FLUO. In a 25 mL round-bottomed two-necked flask, 500 mg (1.307 mmol) of 1,4-diethynyl-2,5-bis(octoxy)benzene, 0.836 mg (2.875 mmol of 2-[ethyl(4-iodophenyl)amino]ethanol, 36 mg (0.052 mmol) of bis(triphenylphosphine)dichloropalladium and 9 mg (0.052 mmoles) of copper iodide were introduced under nitrogen and dissolved into 6 mL of freshly distilled and degassed diisopropylamine. The mixture was stirred at 50 °C for 96 hours and filtered after cooling to room temperature over a silica gel plug eluted with dichloromethane-ethylacetate solvent mixture according to the (1:0 v:v) to (1:1 v:v) gradient. The filtrate was concentrated *in vacuo* and 970 mg of **PREC FLUO** was obtained in 99% yield as a black solid and will be used without further purification in the following step.^{1}H NMR (400 MHz, $CDCl_3$) : δ = 7.38 (d, *J* = 9Hz, 4H, H_{Ph}), 6.96 (s, 2H, $H_{Ph\ (central)}$), 6.67 (d, *J* = 9Hz, 4H, H_{Ph}), 4.01 (t, *J* = 6.5 Hz, 4H, O$\underline{CH_2}$), 3.79 (t, *J* = 6.0 Hz, 4H, N-$\underline{CH_2}$), 3.48 (t, *J* = 6.0 Hz, 4H, $\underline{CH_2}$-OH), 3.43 (d, *J* = 7.1 Hz, 4H, $\underline{CH_2}$ of Et), 1.83 (dt, *J* = 14.6, 6.5 Hz, 4H, O-CH_2-$\underline{CH_2}$), 1.28-1.25 (m, 20H, CH_2 alkyl), 1.16 (t, *J* = 7.1 Hz, 6H, CH_3 of Et), 0.87(t, *J* = 6.9 Hz, 6H, CH_3) ; ^{13}C NMR (101 MHz, $CDCl_3$) : δ =153.7, 148.3, 133.3, 117.1, 114.4, 112.25 (4C) 110.8, 96.2, 85.5, 70.0, 60.5, 52.6, 45.9, 32.2, 29.85 (4 C), 29.7; 26.5 23.1, 19.6, 14.54 (2C). IR (neat KBr) ν_{max}/cm^{-1} = 3064, 2921, 2869, 2850, 2154, 2026, 1704, 1596, 1538, 1503, 1488, 1466, 1409, 1387, 1274, 1260, 1248, 1213, 1192, 1164, 1127, 1102, 1064, 1042, 1003, 889,

849, 790, 753, 725; UV/Vis λ_{max} ($CHCl_3$): 385 nm ; Emission ($CHCl_3$): λ_{max}=428 nm ($\lambda_{excitation}$=385 nm) ; MS (EI) *m/z* (%) : 709 (50) [M^+], 355 (100) ; HRMS (EI) : *m/z* calcd for $C_{46}H_{64}N_2O_4$: 709.4939, found 709.4929.

FLUO. In a 50 mL round-bottomed two-necked flask equipped with a condenser and a magnetic stirrer were introduced, under nitrogen, 500 mg (0.712 mmol) of **PREC FLUO** , 10 mL of THF and 1 mL of triethylamine, both being freshly distilled and degassed. The mixture was refluxed for 72 hours under stirring. After cooling, solvents were removed *in vacuo* and the product was purified under flash chromatography over a 15 µm spherical silica column eluted with CH_2Cl_2-AcOEt solvent mixture according to the (1:0 v:v) to (1:1 v:v) gradient. **FLUO** was obtained in 21% yield (180 mg) (purification not optimized). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37 (d, *J* = 8.9 Hz, 4H, H_{Ph}), 6.95 (s, 2H, $H_{Ph\ (central)}$), 6.65 (d, *J* = 8.9 Hz, 4H, H_{Ph}), 4.96- 4.93 (m, 2H, NH), 4.19 (t, *J*= 6.4 Hz, 4H, N-CH_2-CH_2-O), 4.00 (t, *J* = 6.4 Hz, 4H, OCH_2), 3.81 (q, *J* = 7.0 Hz, 12H, Si-O-CH_2), 3.54 (t, *J* = 6.4 Hz, 4H, N-CH_2-CH_2-O), 3.41 (q, *J* = 6.9 Hz, 4H, N-CH_2-CH_3), 3.17 (t, *J*= 6.5 Hz, 4H, NH-CH_2), 1.85-1.79 (m, 4H, O-CH_2-CH_2), 1.65-1.57 (m, 4H, NH-CH_2-CH_2-CH_2-Si), 1.57-1.49 (m, 4H, O-CH_2-CH_2-CH_2-), 1.40-1.25 (m, 16H, CH_2), 1.22 (t, *J* = 7.0 Hz, 18H, Si-O-CH_2-CH_3), 1.17 (t, *J* = 7.1 Hz, 6H, N-CH_2-CH_3), 0.86 (t, *J* = 6.9 Hz, 6H, CH_3 alkyle), 0.62 (t, *J*=8.1Hz, 4H, Si-CH_2) ; UV/Vis λ_{max} ($CHCl_3$) : 388 nm ; Emission ($CHCl_3$): λ_{max}= 426 nm ($\lambda_{excitation}$=388 nm) ; MS (EI) *m/z* (%) : 1203 (100) [M^+], 602 (35) ; HRMS (EI) : *m/z* calcd for $C_{66}H_{106}N_4O_{12}Si_2$: 1203.7419, found 1203.7446.

SYNTHESIS AND CHARACTERIZATION OF THE NANOMATERIALS

MA NPs. A mixture of cetyltrimethylammonium bromide (690 mg, CTAB), and sodium hydroxide (40 ml, 0.2 M) was stirred at room temperature during 50 minutes at 700 Rpm in a 500 mL three necks round bottom flask. Then, an alcoholic solution of the alkoxysilylated AZO (n_0 = 3.7 10^{-4} mol, in 1.8 mL EtOH) was added to elaborate MA NPs, and the stirring speed was changed to 1000 Rpm. One minute later, tetraethoxysilane (3.6 mL, TEOS) was added to the aforementioned solution, and after 40 seconds, an aqueous solution (260 mL) was poured out. The solution was then heated through a hair drier (T_0=25°C, T'=28-30°C in 1-2 min), in order to trigger the condensation process. After 5 minutes 30 seconds of reaction, a solution of hydrochloric acid (36 mL + aliquots of HCl 0.2 M) was added to quench the reaction by reaching a pH of 6.9. Fractions were gathered in propylene tubes and collected by centrifugation during 15 minutes at 21 kRpm. The sample was then extracted twice with an alcoholic solution of ammonium nitrate (6 $g.L^{-1}$, NH_4NO_3), and washed three time with ethanol, water, and ethanol. Each extraction involved a sonication step of 30 minutes at 50°C in order to remove the CTAB surfactant;

the collection was carried out in the same manner. The as-prepared material was dried under air flow few hours.

MF NPs. A mixture of cetyltrimethylammonium bromide (690 mg, CTAB), and sodium hydroxide (40 ml, 0.2 M) was stirred at room temperature during 50 minutes at 700 Rpm in a 500 mL three necks round bottom flask. Then, an alcoholic solution of the alkoxysilylated FLUO (n_0 = 1.0 10^{-5} mol, in 0.4 mL THF), and the stirring speed was changed to 1000 Rpm. One minute later, tetraethoxysilane (3.6 ml, TEOS) was added to the aforementioned solution, and after 40 seconds, an aqueous solution (260 mL) was poured out. The solution was then heated through a hair drier (T_0=25°C, T'=28-30°C in 1-2 min), in order to trigger the condensation process. After 5 minutes 30 seconds of reaction, a solution of hydrochloric acid (36 mL + aliquots of HCl 0.2 M) was added to quench the reaction by reaching a pH of 6.9. Fractions were gathered in propylene tubes and collected by centrifugation during 15 minutes at 21 kRpm. The sample was then extracted twice with an alcoholic solution of ammonium nitrate (6 $g.L^{-1}$, NH_4NO_3), and washed three time with ethanol, water, and ethanol. Each extraction involved a sonication step of 30 minutes at 50°C in order to remove the CTAB surfactant; the collection was carried out in the same manner. The as-prepared material was dried under air flow few hours.

MAF NPs. A mixture of cetyltrimethylammonium bromide (690 mg, CTAB), and sodium hydroxide (40 ml, 0.2 M) was stirred at room temperature during 50 minutes at 700 Rpm in a 500 mL three necks round bottom flask. Then, the alcoholic solution of the alkoxysilylated AZO (n_0 mol of AZO, see Table 1 page 2, in 0.8 mL EtOH), as well as the tetrahydrofurane solution of the alkoxysilylated FLUO (n_0 mol of Fluo, see Table 1 page 2) were added, and the stirring speed was changed to 1000 Rpm. One minute later, tetraethoxysilane (3.6 mL, TEOS) was added to the aforementioned solution, and after 40 seconds, an aqueous solution (260 mL) was poured out. The solution was then heated through a hair drier (T_0=25°C, T'=28-30°C in 1-2 min), in order to trigger the condensation process. After 5 minutes 30 seconds of reaction, a solution of hydrochloric acid (36 mL + aliquots of HCl 0.2 M) was added to quench the reaction by reaching a pH of 6.9. Fractions were gathered in propylene tubes and collected by centrifugation during 15 minutes at 21 kRpm. The sample was then extracted twice with an alcoholic solution of ammonium nitrate (6 $g.L^{-1}$, NH_4NO_3), and washed three time with ethanol, water, and ethanol. Each extraction involved a sonication step of 30 minutes at 50°C in order to remove the CTAB surfactant; the collection was carried out in the same manner. The as-prepared material was dried under air flow few hours.

RHODAMINE B LOADING OF MA and MAF NPs. A mixture of surfactant free MA or MAF NPS (40 mg), rhodamine B (12 mg), and deionized water (12 mL) was sonicated during 30 minutes and then

stirred two days at room temperature. Afterwards, the sample was collected by centrifugation during 15 minutes at 21 kRpm. Several aqueous washings were performed (30 mL each), and the sample was centrifugated to remove the unloaded rhodamine cargos. Eventually, the NPs were dried under vaccum for few hours. Note that, the various steps following the dye loading were all done in the absence of light.

CAMPTOTHECIN LOADING OF MA and MAF NPs. A mixture of surfactant free MA or MAF NPS (24 mg), camptothecin (3 mg), and dimethyl sulfoxide (1.5 mL) was sonicated during 30 minutes and then stirred two days at 30°C in a 5 mL round bottom flask with a ½ cm stir bare. Afterwards, the sample was collected by centrifugation during 15 minutes at 21 kRpm. One washing was performed with dimethylsulfoxide (5 mL), and two aqueous washings were performed as well (30 mL each), and the sample was centrifugated. Eventually, the NPs were dried under vaccum for few hours. Note that, the various steps following the drug loading were all done in the absence of light.

UV-VISIBLE SPECTROSCOPY OF MA, MF, AND MAF-x NANOMATERIALS

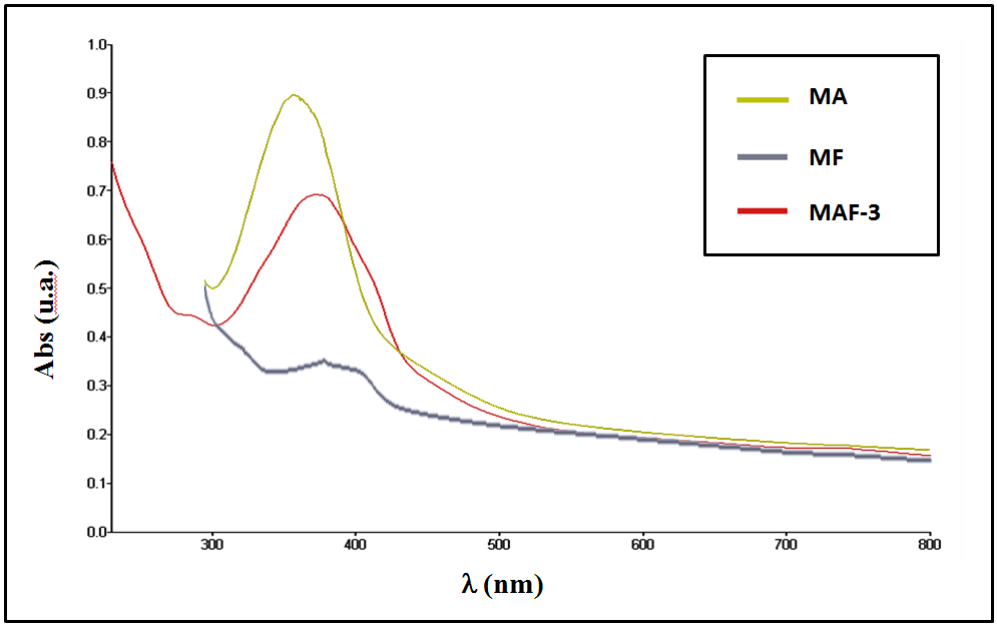


Figure S1. UV-Visible spectra of **MA, MF**, and **MAF-3** nanoimpellers, demonstrating the incorporation of both the azobenzene and the fluorophore species.

MA, MF, AND MAF-x NANOMATERIALS FULL CHARACTERIZATION

For each nanomaterials (**MA MF MAF-1** to **MAF-4**), Transmission Electron Microscopy (TEM), UV-Visible spectroscopy (UV-Vis), Dynamic Light Scattering (DLS), X-Ray Diffraction (XRD), and Nitrogen Adsorption-Desorption (N_2 Ads) characterizations are presented in the following pattern:

TEM	TEM
UV-Vis	DLS
XRD	N_2 Ads

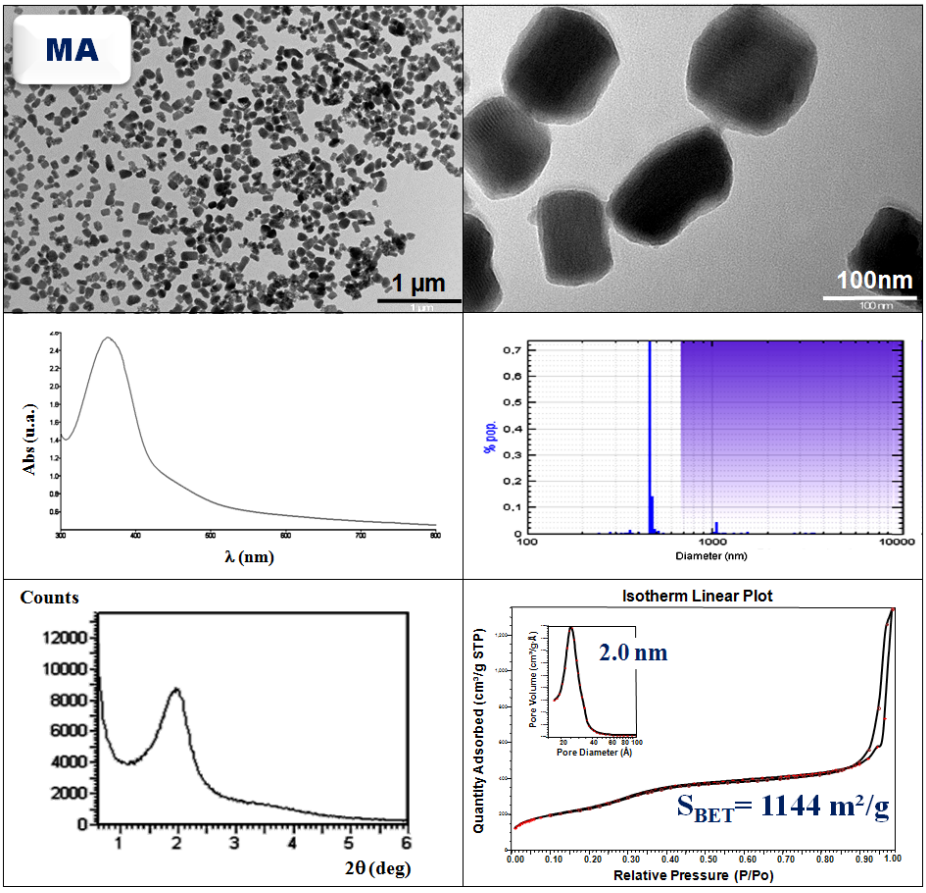


Figure S2. TEM, UV-Vis, DLS, XRD, and N_2 Ads characterizations of MA NPs.

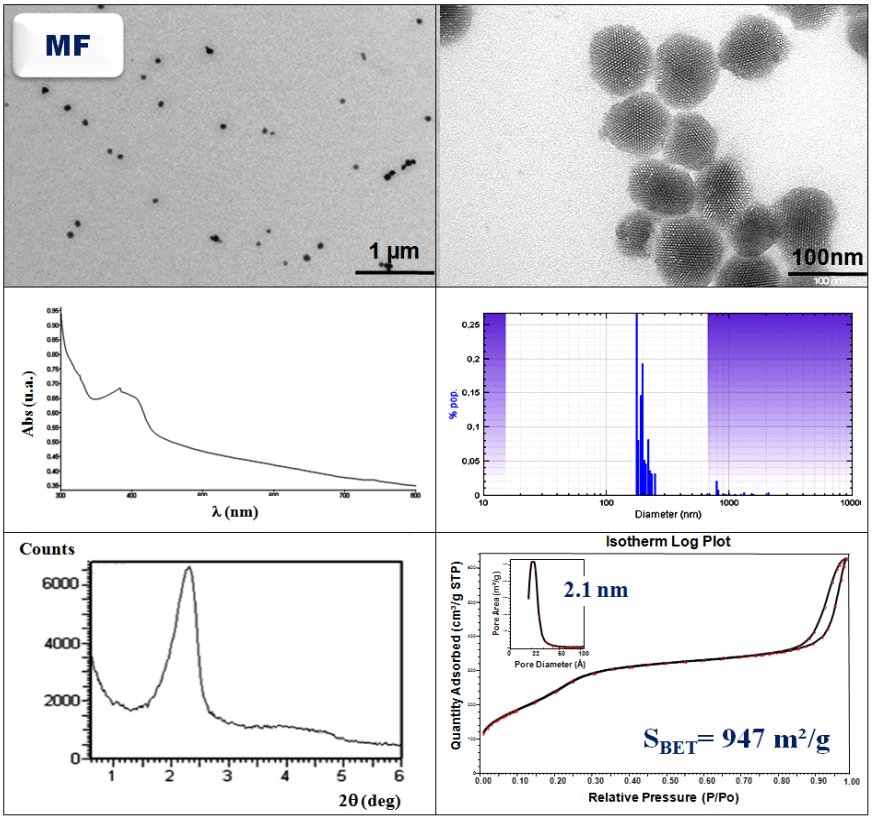


Figure S3. TEM, UV-Vis, DLS, XRD, and N_2 Ads characterizations of MF NPs.

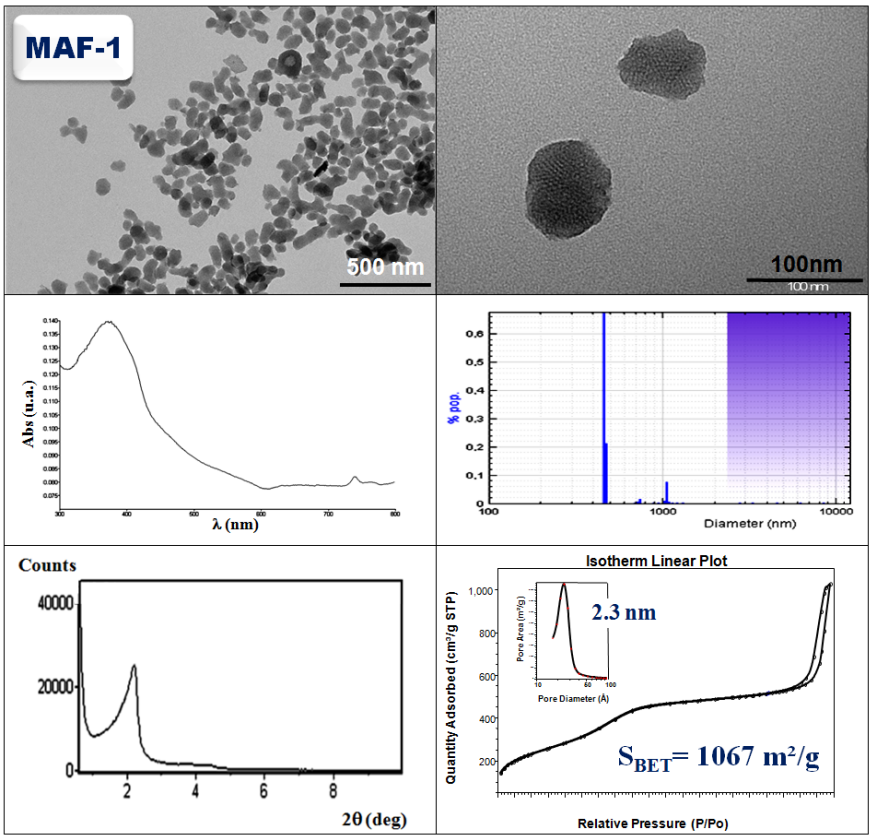


Figure S4. TEM, UV-Vis, DLS, XRD, and N_2 Ads characterizations of MAF-1 NPs.

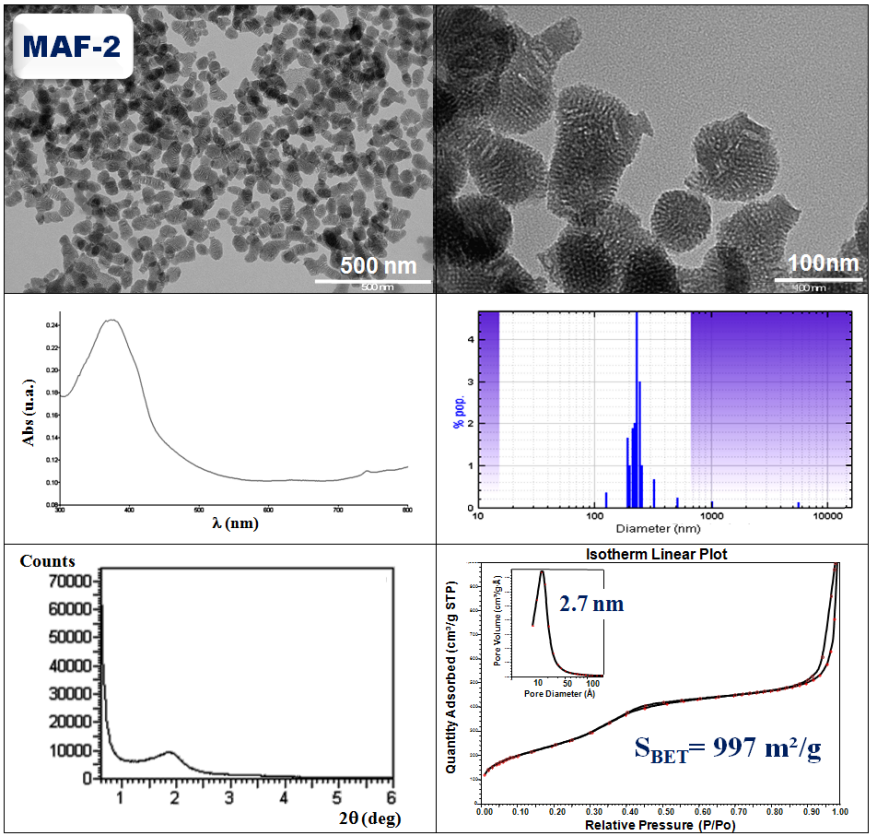


Figure S5. TEM, UV-Vis, DLS, XRD, and N_2 Ads characterizations of MAF-2 NPs.

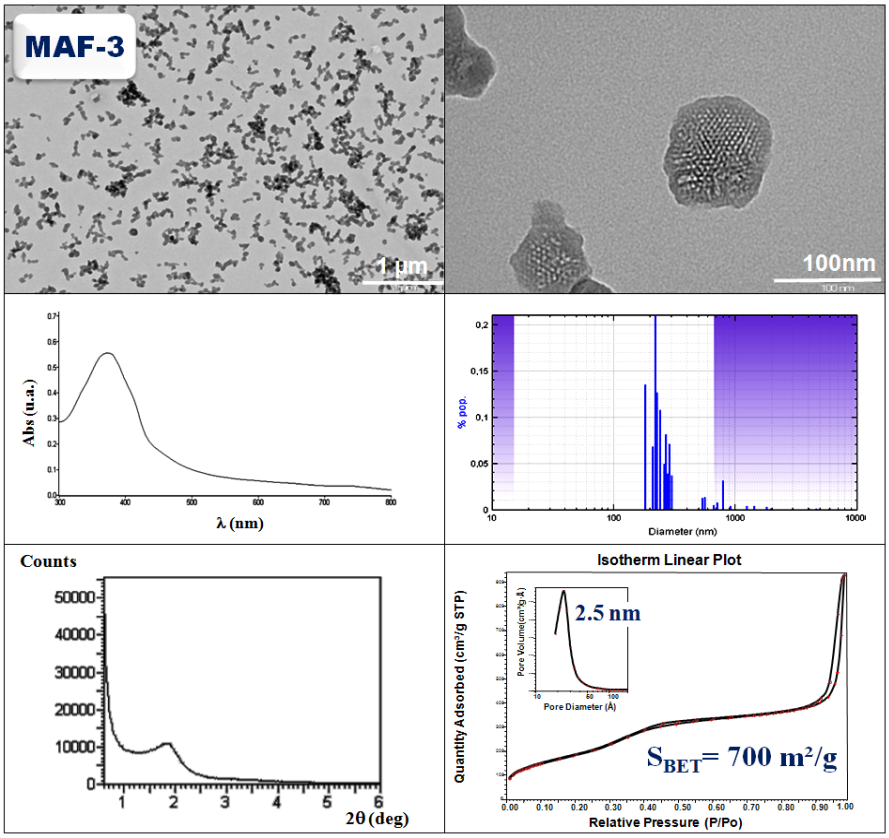


Figure S6. TEM, UV-Vis, DLS, XRD, and N_2 Ads characterizations of MAF-3 NPs.

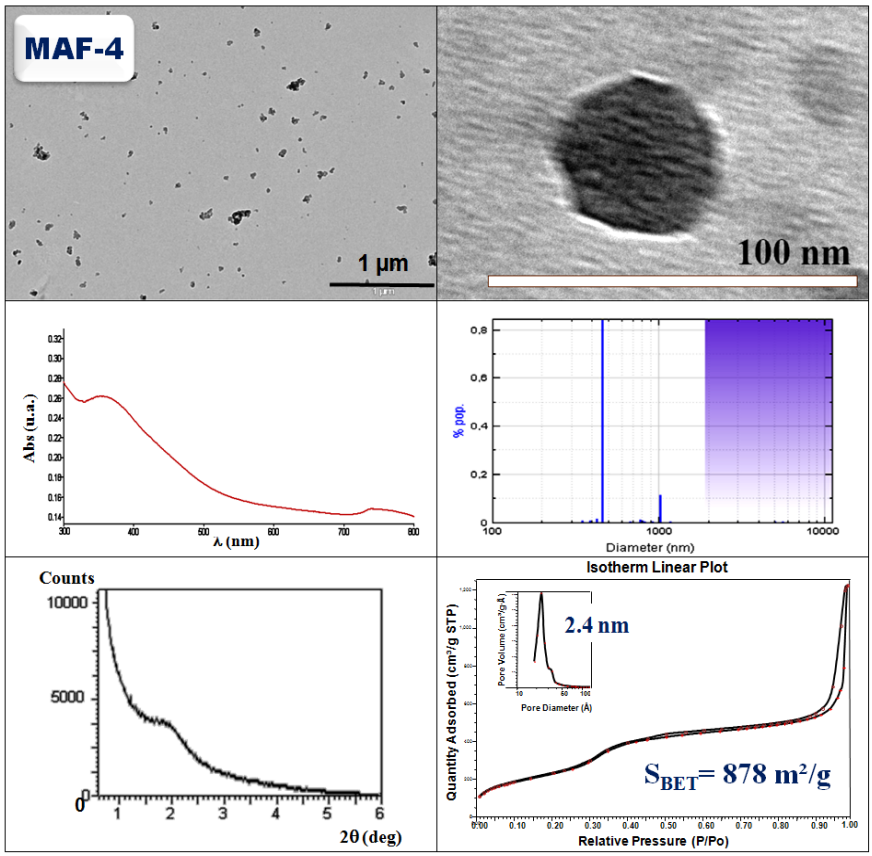


Figure S7. TEM, UV-Vis, DLS, XRD, and N_2 Ads characterizations of MAF-4 NPs.

ONE-PHOTON RELEASE OF RHODAMINE B LOADED MA AND MAF NPS.

The 1-photon triggered release of rhodamine B was performed according to the following scheme:

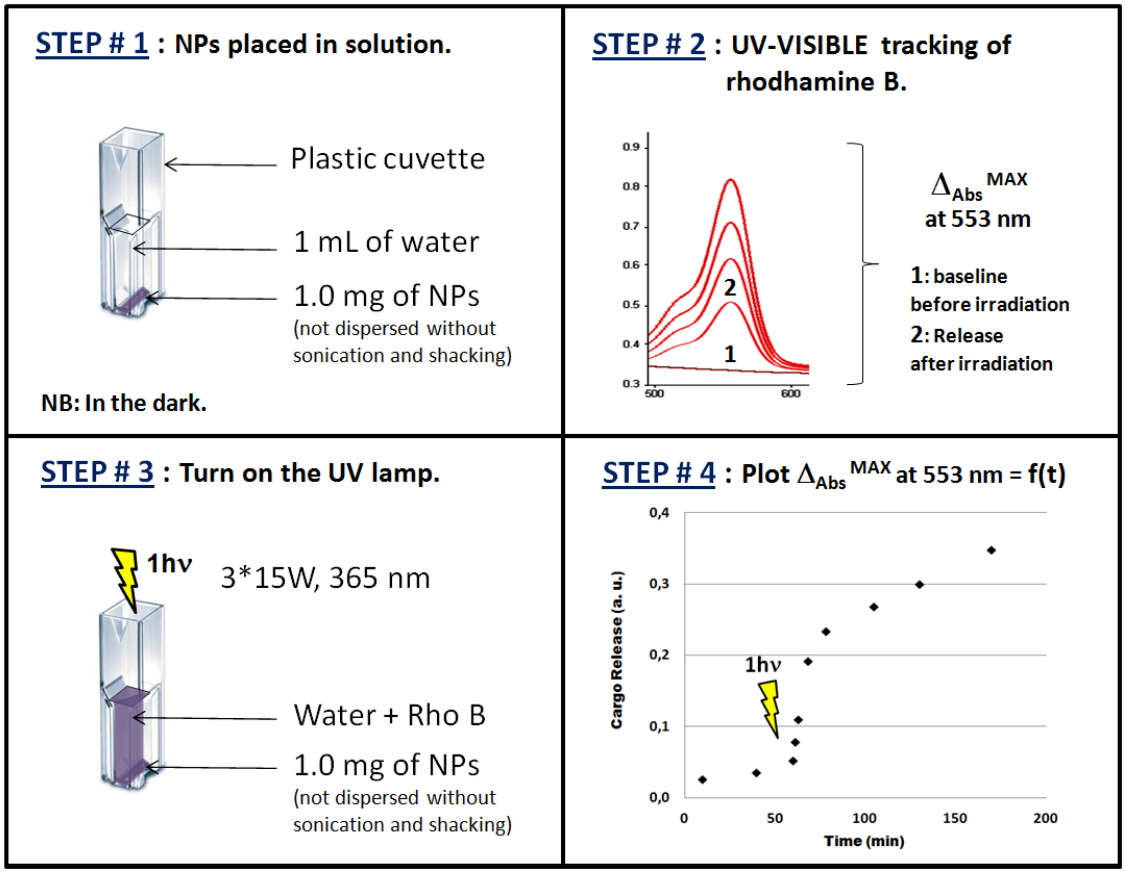


Figure S8. 1-photon triggered release of rhodamine B on MA and MAF NPS.

TWO-PHOTON FLUORESCENCE IMAGING: experimental.

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 cells.cm^{-2}. Adherent cells were then washed once and incubated in 1 mL medium containing nanoimpellers at a concentration of 40 µg.mL^{-1} for 20 h. 15 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 µg.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 µm.

TWO-PHOTON TRIGGERED DRUG DELIVERY

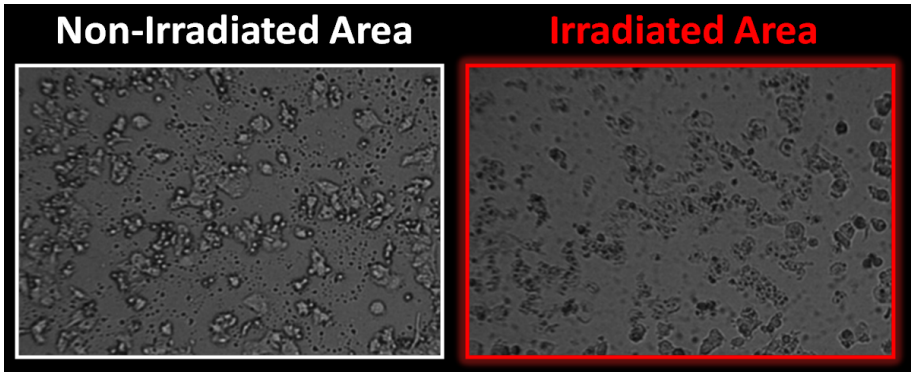


Figure S9 Two-photon triggered drug delivery with **MAF-4+C** on MCF-7 cells before and after laser irradiation

IN-VITRO CONTROL OF UNLOADED NANOCARRIERS : TPE with the Carl Zeiss microscope

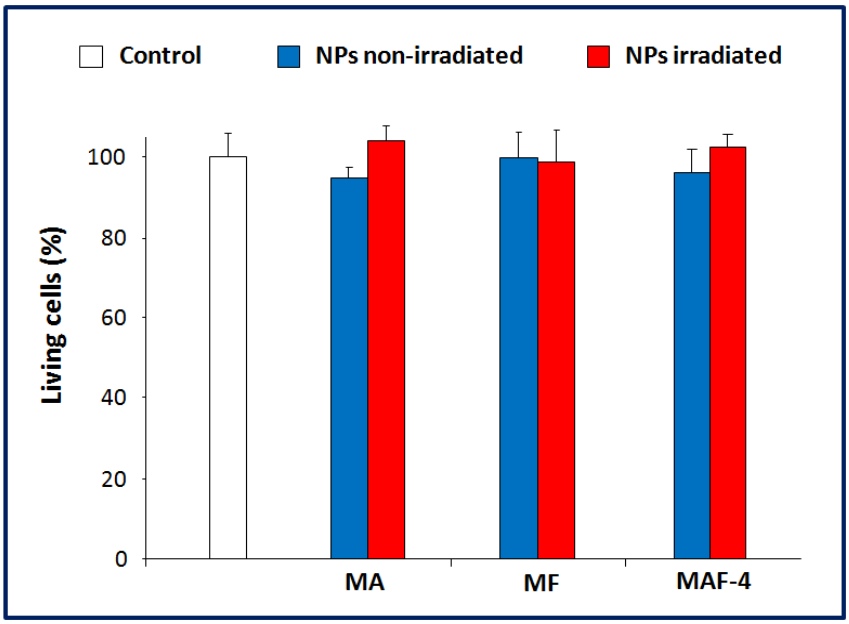


Figure S10. 2-Photon *in-vitro* control with **MA**, **MF**, and **MAF-4** nanoimpellers (40 µg.mL^{-1}) not loaded with camptothecin. The irradiation did not produce any cell death. Conditions: with a focused laser beam and at maximum laser power (laser power input 3 W, laser power outpout before the objective 900 mW.cm^{-2}). The well was irradiated with three scans of 1.57 s each per irradiated area, in four different areas, without overlaps between irradiated areas, with an objective: Carl Zeiss NA 0.3, 10x.

IN-VITRO CONTROL OF UNLOADED NANOCARRIERS : CYTOTOXICITY FOR 4 DAYS

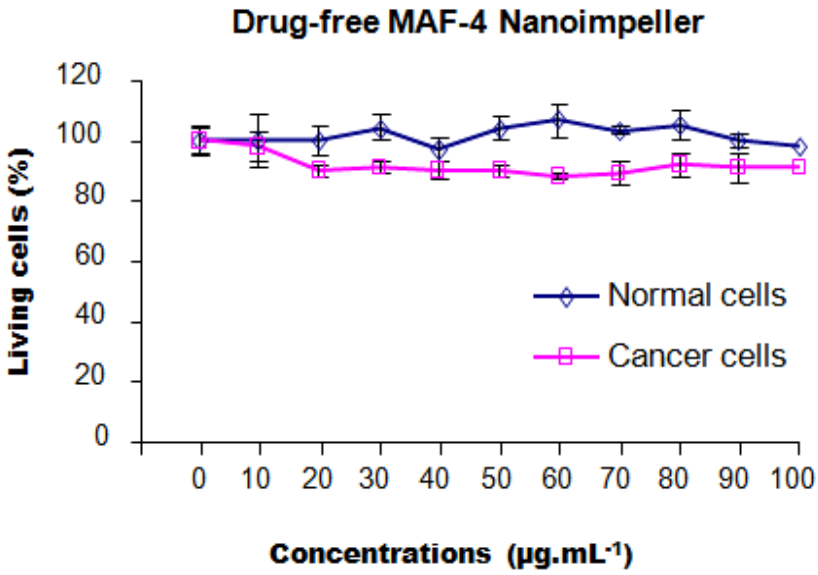


Figure S11. Absence of toxicity of free drug nanocarrier. Cancer (MCF-7) and normal (fibroblasts) cells were incubated with increasing concentrations of **MAF-4** (from 10 to 100 µg.mL-1). After 4 days treatment, a MTT assay was performed and data are mean ± SD of 3 experiments.

IN-VITRO CONTROL OF DRUG-LOADED NANOIMPELLERS: TPE with the Leica microscope

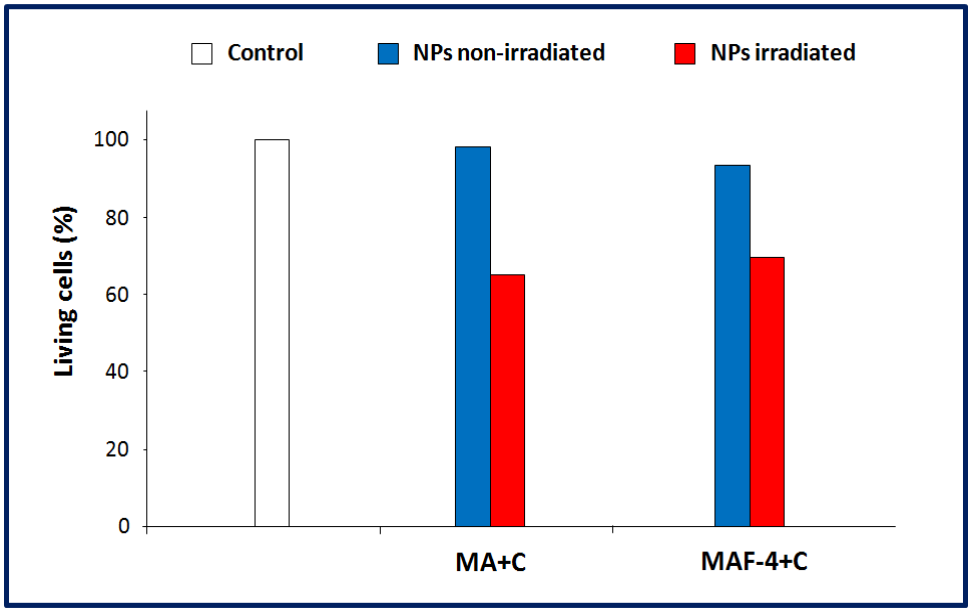


Figure S12. 2-Photon triggered *in-vitro* delivery of camptothecin, comparison between nanoimpellers **MA+C** and **MAF-4+C** , incubated at 80 µg.mL^{-1}. MCF-7 cells were submitted (or not) to laser irradiation; with a Leica Microscope (laser power input 1.5 W, objective lens 10x, NA 0.4). 4 different areas were irradiated for 3 min each, leading to 90% of the well surface irradiated at 760 nm. 35% of cancer cell death was observed with **MA+C** in these conditions (long irradiation time, lower power, less focused laser beam than with the Carl Zeiss microscope), which is probably due to the photothermal isomerization of the azobenzene moiety under these conditions.

IN-VITRO CONTROL OF UNLOADED NANOIMPELLERS : TPE with the Leica microscope

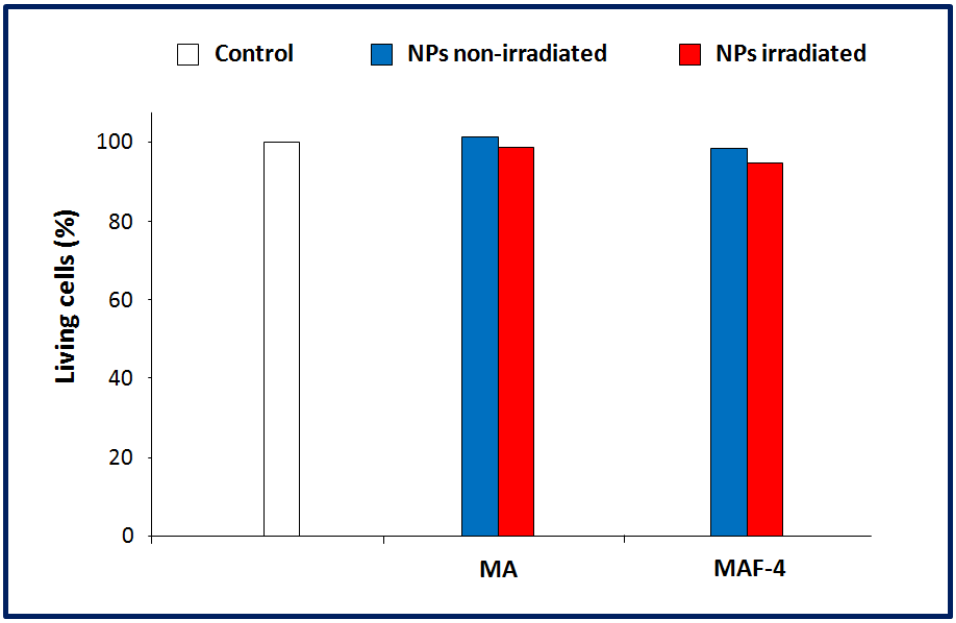


Figure S13. 2-Photon *in-vitro* control with **MA**, and **MAF-4** nanoimpellers (80 µg.mL^{-1}) not loaded with camptothecin. The irradiation (same conditions as Figure S10) did not produce any cell death.

PREMATURE RELEASE CONTROLS OF THE NANOIMPELLERS IN SOLUTION

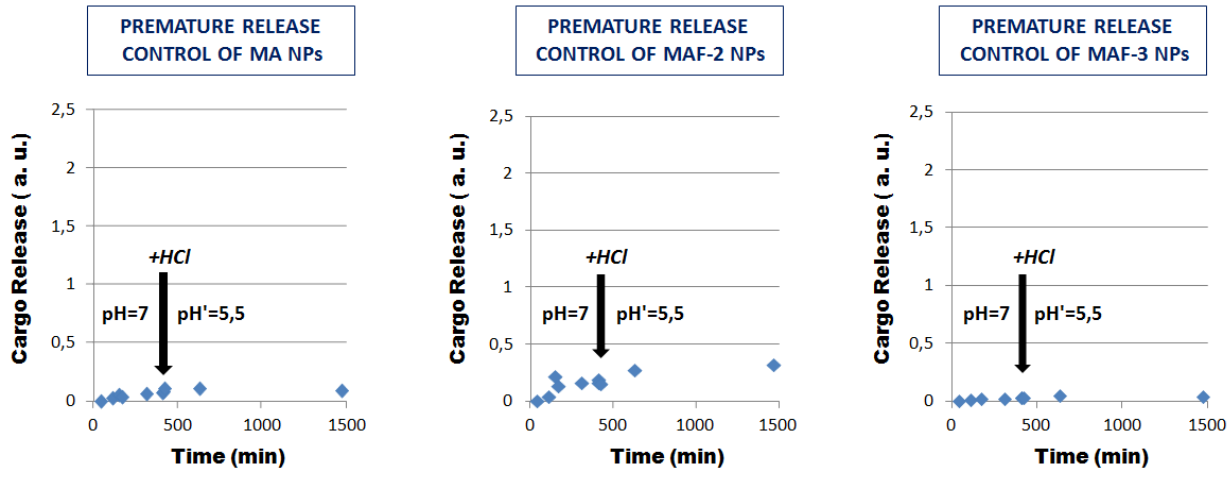


Figure S14. Control of the premature release of camptothecin-loaded nanoimpellers in aqueous media at pH 7 and 5.5.

[i] C. Weder, M. S. Wrighton, *Macromolecules* **1996**, *29*, 5157-5165.
[ii] C. Monnereau, E. Blart, F. Odobel, *Tetrahedron Lett*. **2006**, *46*, 5421-5423.