Supporting information for:

Cell Penetrating Peptide Decorated-magnetic Porous Silicon Nanorods for Glioblastoma Therapy and Imaging

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Scanning Electron Microscope (SEM) images were obtained using high resolution SEM - JEOL JSM-7900 F. Transmission Electron Microscope (TEM) were performed using JEOL JEM 2100F. Dynamic Light Scattering (DLS) measurements of the formulations were performed on a Malvern nanozetasizer instrument in deionized water. Zeta potential (ZP) measurements of the nanoparticles were obtained using Malvern nanozetasizer instrument in deionized water. Infrared (FTIR) of the formulations were performed with Bruker FTIR VERTEX 70V (MESU 1424). The powder X-ray diffraction patterns were measured on a Bruker D8 discover.

I. Chemicals and materials

Silicon wafer was purchased from MEMC (Kuala Lumpur, Malaysia); (3-aminopropyl)triethoxysilane (APTES) and Silver Nitrate ($AgNO_3$) were purchased from Sigma Aldrich (Saint-Quentin-Fallavier, France); Superparamagnetic iron oxide nanoparticles (SPIONs) were purchased from US Research Nanomaterials Lab (Houston, USA), Inc; 1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine, 4-chlorobenzenesulfonate (DiD; D7757) were purchased from ThermoFisher Scientific (Waltham, Massachusetts, USA). Hydrofluoric acid (HF) was purchased from BASF; Nitric acid (HNO_3) and acetic acid (CH_3COOH) was purchased from Merck.

II. Preparation of porous silicon nanorods (pSiNRs)

Porous silicon nanorods (pSiNRs) were fabricated via electrochemical etching with highly doped p-type silicon wafers [0.01-0.02 Ω cm, (100) oriented - 6 inchs - Thick 493 - 523 nm] in aqueous hydrofluoric acid (HF - 5 wt.%) and acetic acid (CH_3COOH - 25 wt.%). Electrochemical etching was performed in a PVC cell with a platinum counter the electrode with *Source SP-150 Biologic*®. The etching waveform was composed of a lower current density of 0.6 A (~ 5 $mA.cm^{-2}$) applied for 40 seconds and higher current density pulse of 3.9 A (~ 26 $mA.cm^{-2}$) for 2 seconds. The waveform was repeated during 149 cycles generated porous silicon layer with stratified layer every 200 nm and with the higher porosity for the perforation.

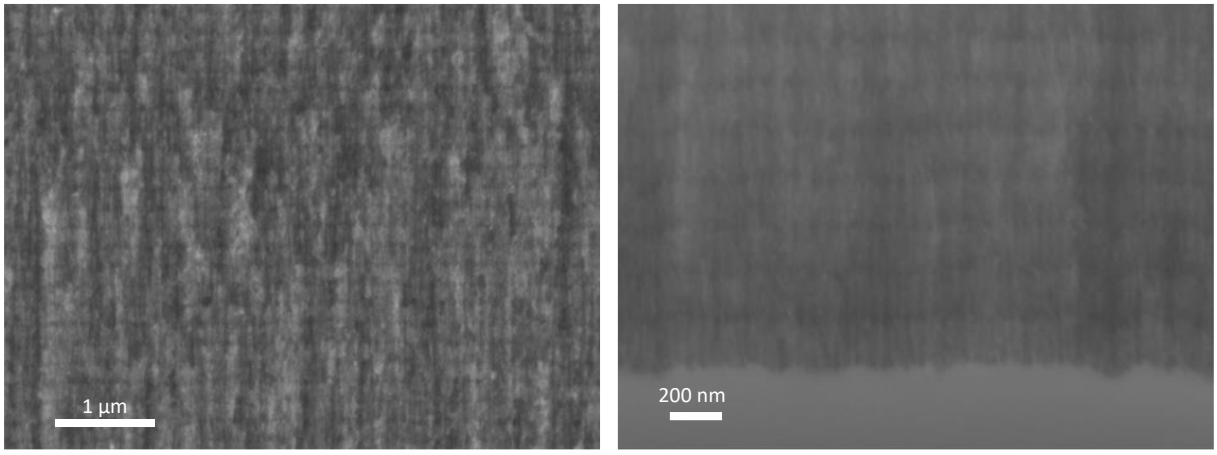


Figure S1. Scanning electron microscope images (SEM) of perforated porous silicon film. Scale bars: 1 µm (left image) and 200 nm (right image).

For the preparation of porous silicon nanowires, a solution of 366 mL of deionized water, 75 mL of HF and 9 mL of $AgNO_3$ was mixed. The perforated porous silicon wafer was dipped to the solution for 150 minutes at ambient temperature. After the MACE process, the silver nanoparticles were removed from the porous silicon nanowires by soaking during 30 minutes in 22 wt. % aqueous nitric acid solution. Then, the wafer was washed with a high amount of deionized water to remove the excess of nitric acid and the trace of silver nanoparticles. Afterward, the porous silicon nanowires (pSiNWs) were mechanically detached from the substrate.

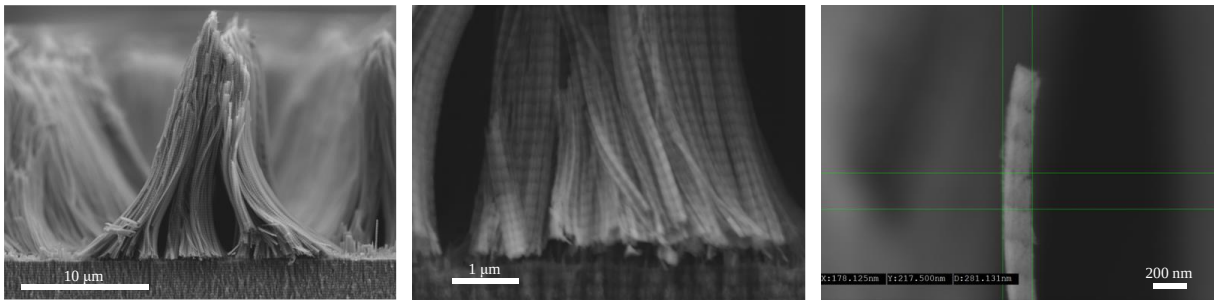


Figure S2. Scanning electron microscope (SEM) images of perforated porous silicon nanowires (pSiNWs). Scale bars: 10 µm (left image), 1 µm (center image) and 200 nm (right image).

The photography below represents the preparation steps of porous silicon nanowires:

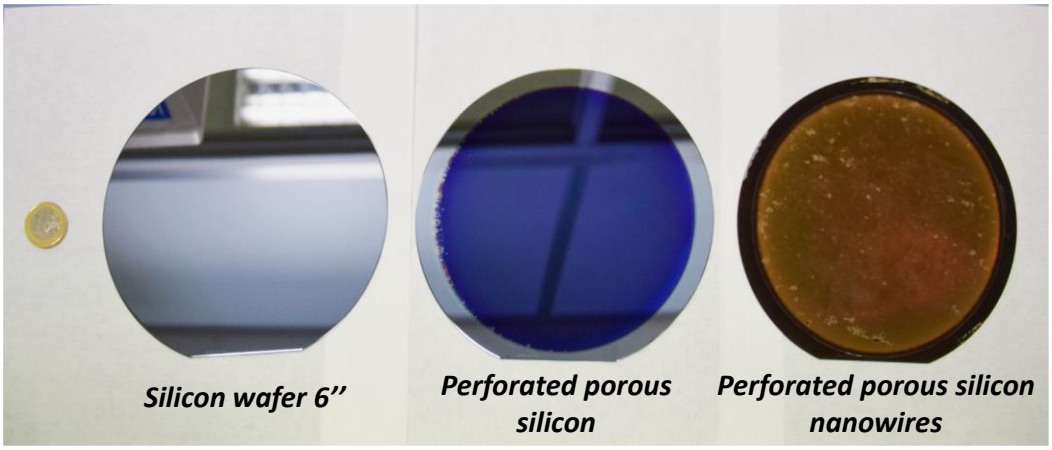


Figure S3. Photograph of 6" Si wafers before and after electrochemical etching, then after nanowire formation.

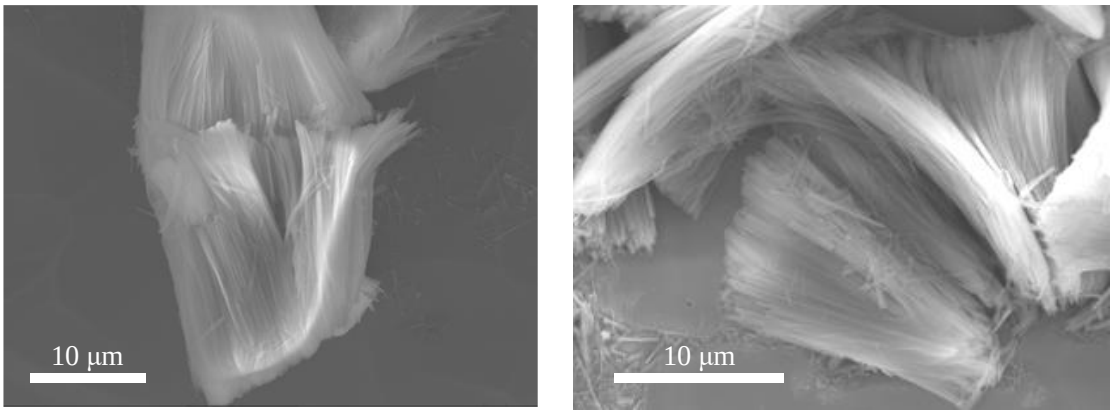


Figure S4. Scanning electron microscope (SEM) images of perforated pSiNWs after peeling-off. Scale bars: 10 μm (left image) and 10 μm (right image).

Once detached, the porous silicon nanowires (pSiNWs) were dispersed in water and fractured by ultrasonication for 24 hours to produce porous silicon nanorods (pSiNRs). The heaviest (biggest) particles were removed by spinning down at 4000 rpm for 10 minutes. Likewise, in order to eliminate the smallest particles, the nanoparticle solution was centrifuged at 9000 rpm for 10 minutes and the supernatant was removed. PSiNRs with desired dimension were suspended in absolute ethanol for the next step synthesis.

Figure S5. Colloidal solution of pSiNRs dispersed in water.

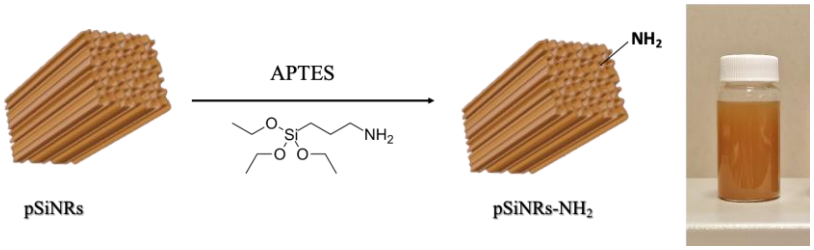
III. Chemical functionalization and decorations of pSiNRs

- Peptides

The NFL-TBS.40-63 peptide, or NFL-peptide (NH_2-YSSYSAPVSSSLSVRRSYSSSSGS-$CONH_2$) synthetized by PolyPeptide Group (Strasbourg, France) was used for this study. The NFL-peptide can be biotinylated (BIOT-NFL-peptide) or coupled to 5-carboxyfluorescein (FAM-NFL-peptide).

- Silanisation: functionalization of APTES onto pSiNRs

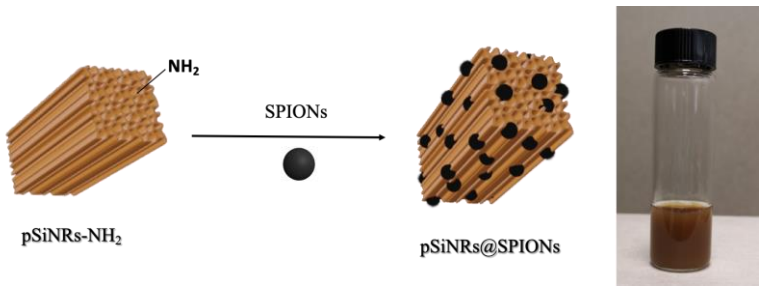
Freshly prepared pSiNRs were centrifuged at 9000 rpm for 15 minutes in absolute ethanol prior to add (3-aminopropyl)triethoxysilane (APTES). Then, 13 mg of pSiNRs were dispersed in 10 mL of absolute ethanol, 600 µL of APTES was added and reacted for 12h at 80 °C. The amine-functionalized nanoparticles were centrifuged at 9000 rpm and washed three times with absolute ethanol then deionized water. Functionalized pSiNRs with APTES are designated "pSiNRs-NH_2" in the following sections of this document.



Scheme S1. Schematic representing the grafting of APTES onto pSiNRs silicon surface and photograph of a vial filled with pSiNRs-NH_2 dispersed in deionized water.

- Decoration of pSiNRs-NH_2 with SPIONs

9 mg of superparamagnetic iron oxide nanoparticles (SPIONs) were added to 1 mL of deionized water and sonicated 12h to prepare non-aggregated SPIONs. The pSiNRs-NH_2 (7 mg) were dispersed in 2 mL of deionized water and mixed with SPIONs solution under 4 hours stirring. Afterward, the pSiNRs@SPIONs were rinsed several times with deionized water to discard the non-attached SPIONs.

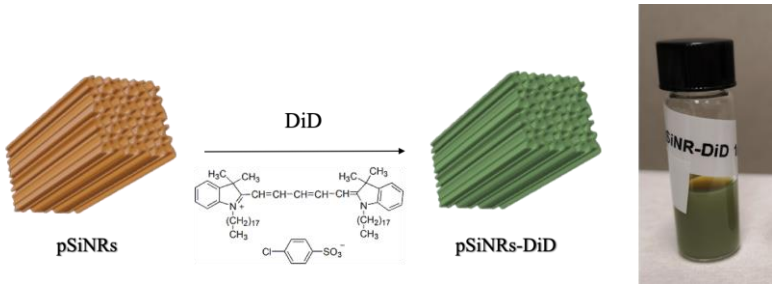


Scheme S2. Schematic represention the decoration of pSiNRs with SPIONs and photograph of a vial filled with pSiNRs@SPIONs dispersed in deionized water.

- DiD dye load in pSiNRs

1 mg of 1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindodicarbocyanine, 4-Chlorobenzenesulfonate Salt (DiD dye), was loaded with 20 mg of pSiNRs in absolute ethanol (5 wt. % of DiD compare

to the pSiNRs, DiD concentration: 1 mg/mL in absolute ethanol). The resultant loading suspension was stirred for 12h and washed several times with deionized water. Afterward, the pSiNRs-DiD were in suspended in deionized water with a concentration of 1 mg/mL.



Scheme S3. Schematic represention of DiD load into the mesoporous structure of pSiNRs and photograph of a vial filled with pSiNRs-DiD disperse in deionized water.

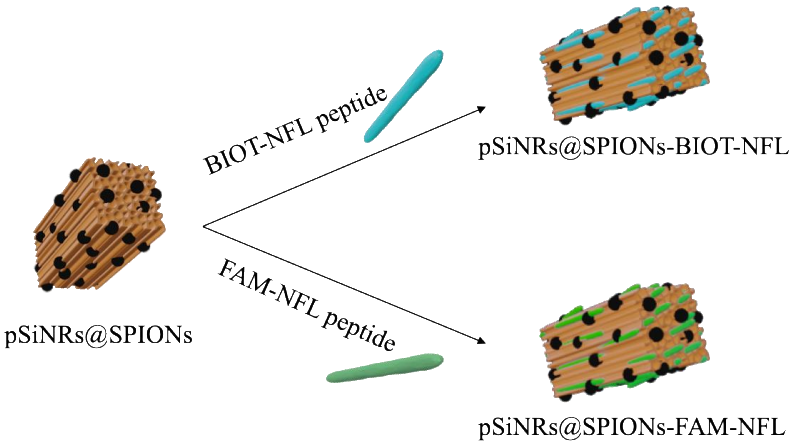
- <u>One pot synthesis: decoration of pSiNRs-NH_2 with Superparamagnetic Iron Oxide Nanoparticles (SPIONs) and DiD dye</u>

SPIONs: (9 mg) were added to 1 mL of deionized water and sonicated 12h to prepare non-aggregate nanoparticles. The pSiNRs-NH_2 (7 mg) were dispersed in 2 mL of deionized water, mixed with SPIONs, DiD solution (5 % W_{DiD}/W_{pSiNRs} – 1 mg/mL in absolute ethanol) and reacted for 12h as well. Afterward, the pSiNRs-DiD@SPIONs were rinsed several times with deionized water.

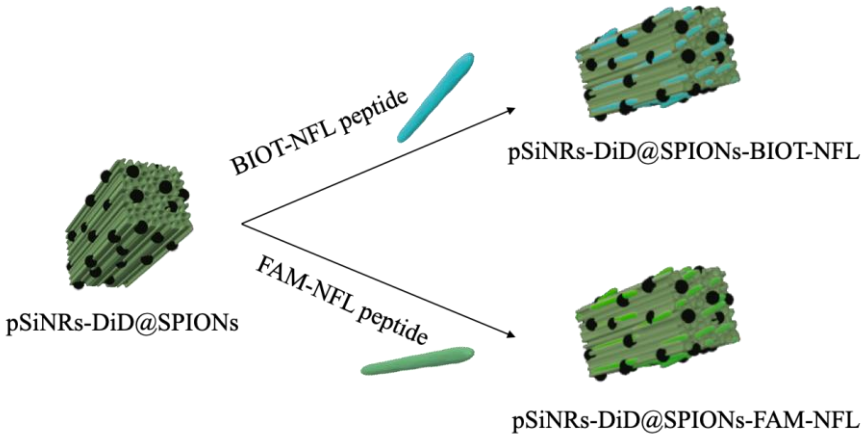
Scheme S4. Schematic representing simultaneous decoration and loading of SPIONs and DiD respectively, into the pSiNRs-NH_2 and photograph of a vial filled with pSiNRs-DiD@SPIONs dispersed in deionized water.

- <u>Decoration of pSiNRs@SPIONs or pSiNRs-DiD@SPIONs with NFL-peptides (BIOT or FAM)</u>

BIOT-NFL-peptide (1.5 mg) was mixed in 1 mL of deionized water and sonicated for 30 minutes. 3.5 mg of pSiNRs@SPIONs in 3 mL of deionized water were added to the peptide solution (1.5 mg/mL) and stirred for 12h at room temperature. The pSiNRs@SPIONs-BIOT-NFL were washed several times with deionized water. The finale concentration of the nanoparticle solution was 1 mg/mL. Same process was used for FAM-NFL-peptide decoration.



Scheme S5. Schematic representing the decoration of pSiNRs@SPIONs with BIOT-NFL or FAM-NFL.



Scheme S6. Schematic representing the decoration of pSiNRs-DiD@SPIONs with BIOT-NFL or FAM-NFL.

IV. Characterization of the formulations

4.1 Characterization of pSiNRs:

- TEM of pSiNRs

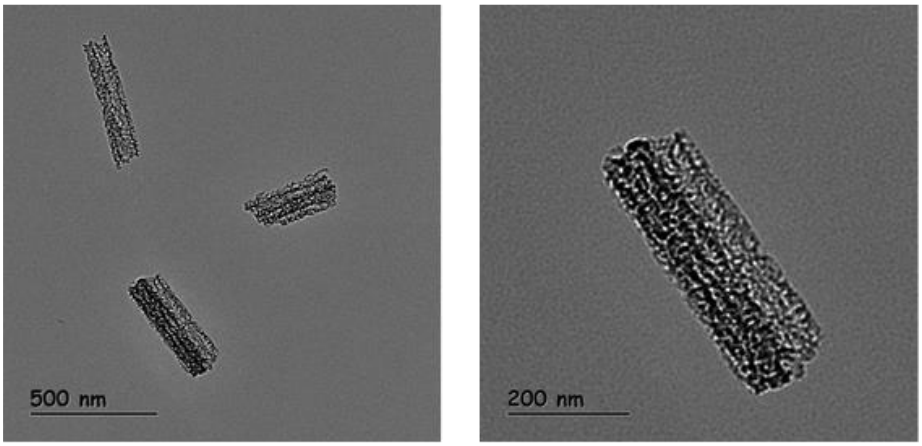


Figure S6. TEM images of pSiNRs. Scale bars: 500 nm (left image) and 200 nm (right image).

- SEM of pSiNRs

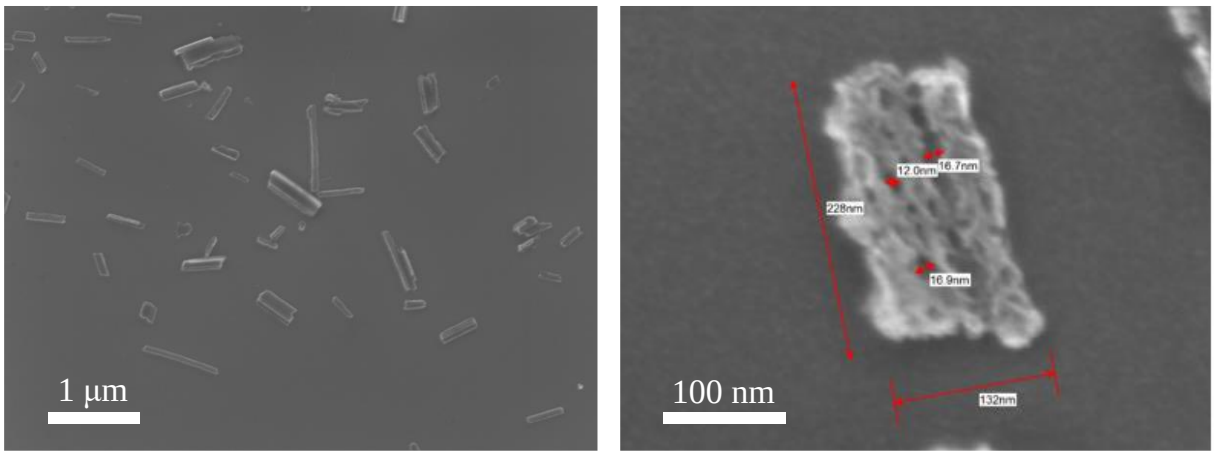


Figure S7. SEM images of pSiNRs.

- DLS, ZP, XRD, Adsorption/desorption of nitrogen and UV-vis spectroscopy characterizations of pSiNRs

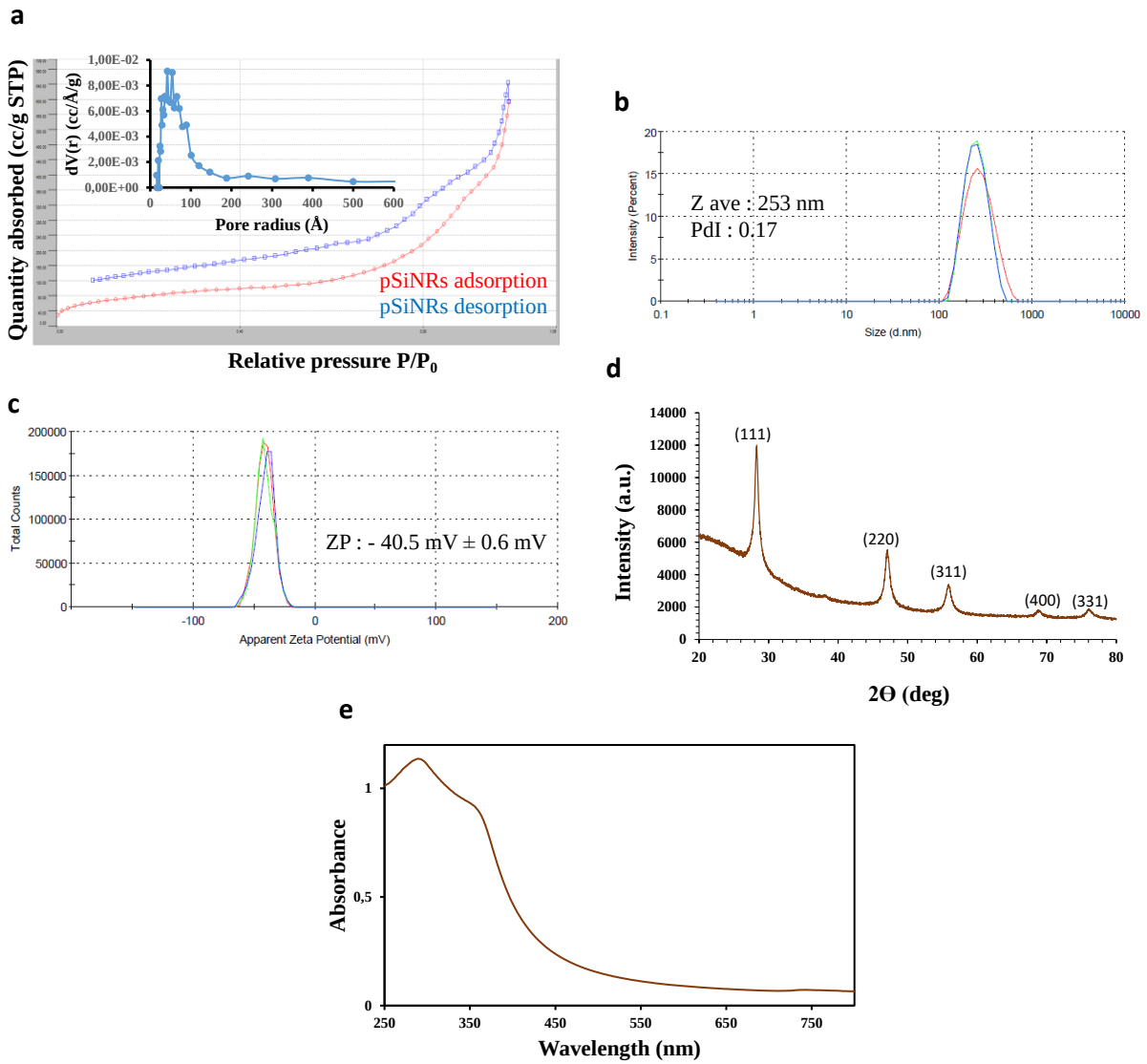


Figure S8. (a) Adsorption/desorption of nitrogen of pSiNRs and the BET pore size distribution (b) DLS intensity of pSiNRs (c) Zeta potential (ZP) of pSiNRs (d) X-ray diffraction of pSiNRs and (e) UV-vis spectroscopy of pSiNRs. Each measurement for DLS and ZP was an average of thirteen repetitions and repeated three times.

4.2 Characterization of aminated-pSiNRs

- DLS and ZP of aminated-pSiNRs

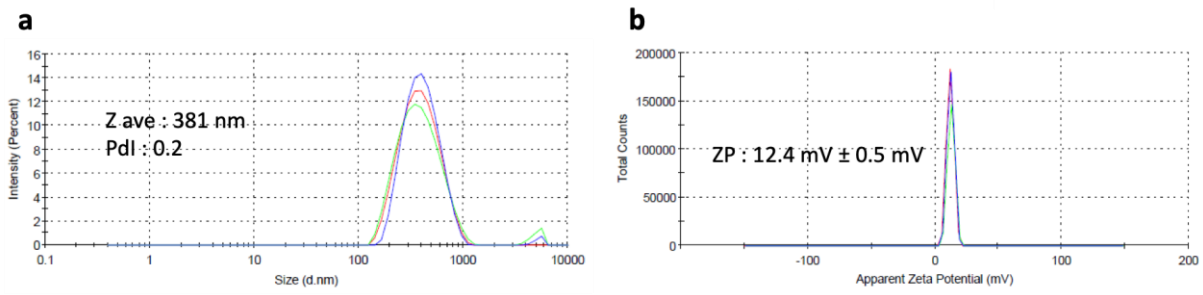


Figure S9. (a) DLS intensity of aminated-pSiNRs (b) ZP of aminated-pSiNRs. Each measurement was an average of thirteen repetitions and repeated three times.

- <u>(FTIR) Infrared of pSiNRs@SPIONs compared to fresh pSiNRs</u>

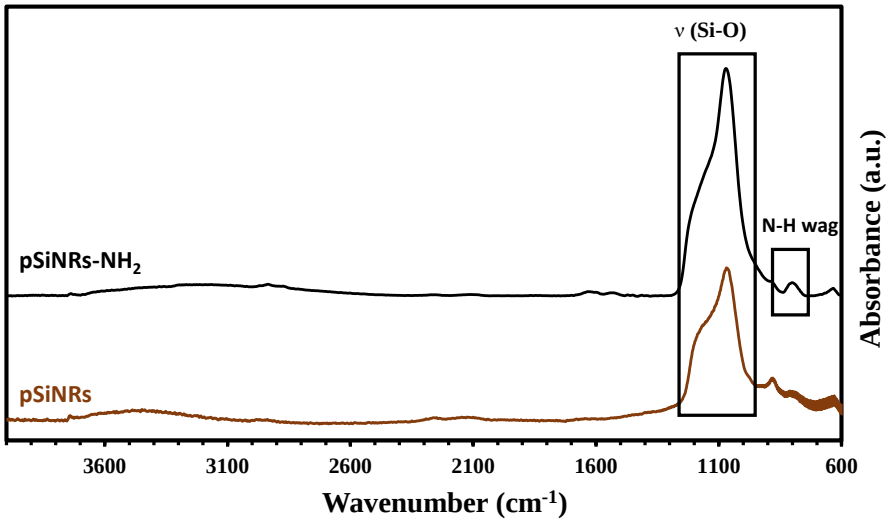


Figure S10. Attenuated Total Reflectance Fourier-Transform Infrared (ATR-FTIR) spectra of modified nanoparticles samples, from the bottom to the top: pSiNRs (brown trace) and pSiNRs-NH_2 (dark trace). Symbols: ν = stretching.

- <u>Thermogravimetric analysis (TGA) of aminated-pSiNRs compared to fresh pSiNRs</u>

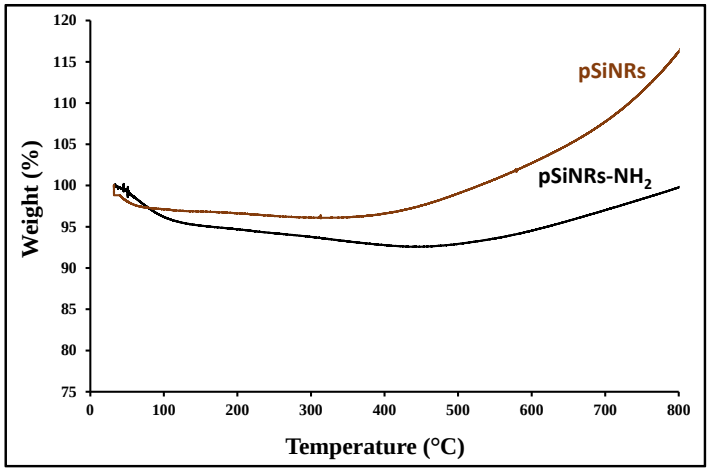


Figure S11. TGA of aminated-pSiNRs compared to fresh pSiNRs.

4.3 Characterization of pSiNRs@SPIONs

- TEM images of SPIONs

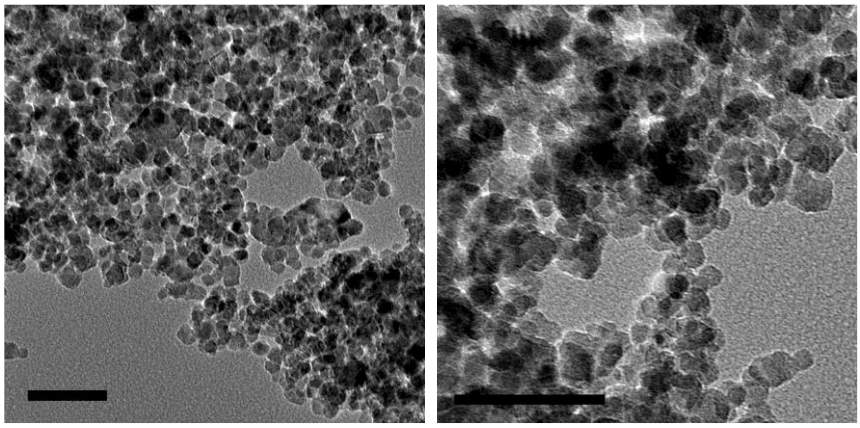


Figure S12. TEM images of SPIONs. Scale bars: 50 nm.

- Photograph of pSiNRs@SPIONs

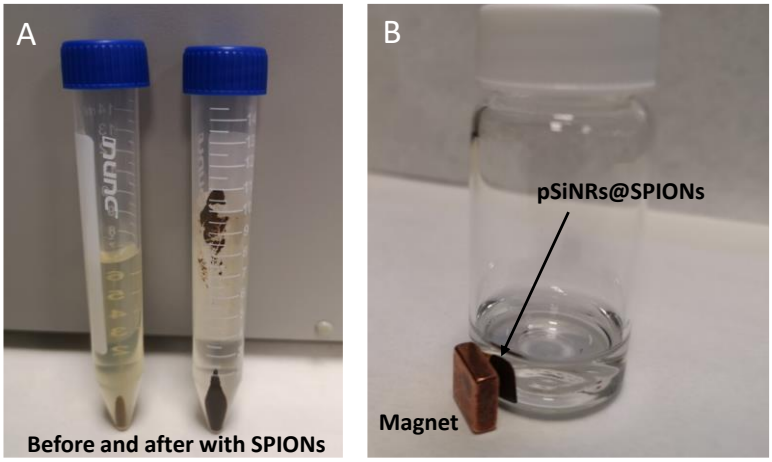


Figure S13. (a) Aminated-pSiNRs before and after adding SPIONs (b) Collect of pSiNRs@SPIONs under magnetic field.

- TEM images of pSiNRs@SPIONs

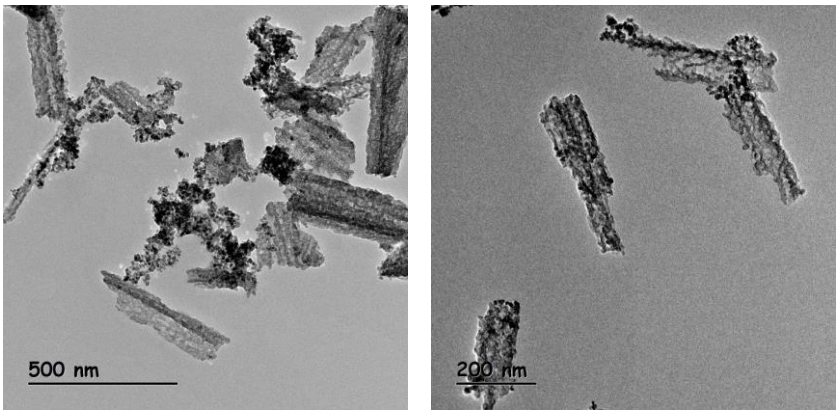


Figure S14. TEM images of pSiNRs@SPIONs. Scale bars: 500 nm (left image) and 200 nm (right image).

- SEM images of pSiNRs@SPIONs

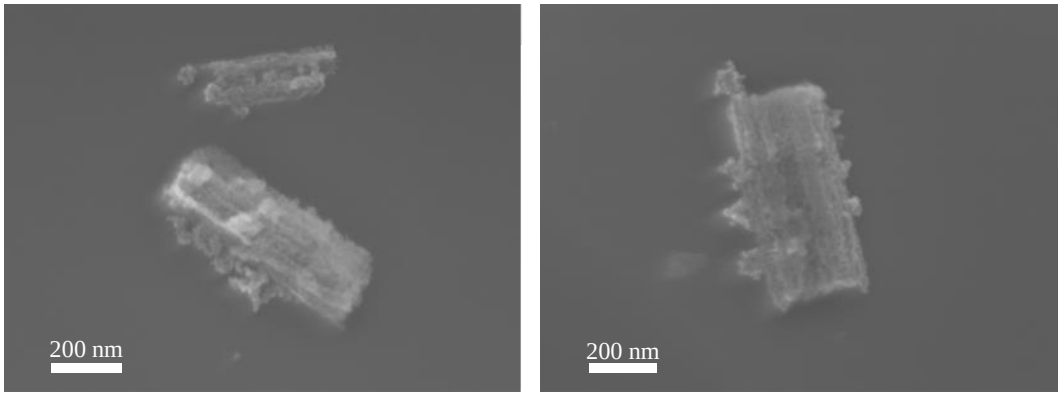


Figure S15. SEM images of pSiNRs@SPIONs. Scale bars: 200 nm (left image) and 200 nm (right image).

- EDX spectrum and mapping of pSiNRs@SPIONs: The SEM was coupled with an EDX detector and used with the AZTEC software to ensure the presence of SPIONs onto the pSiNRs using both spectrum and mapping functions.

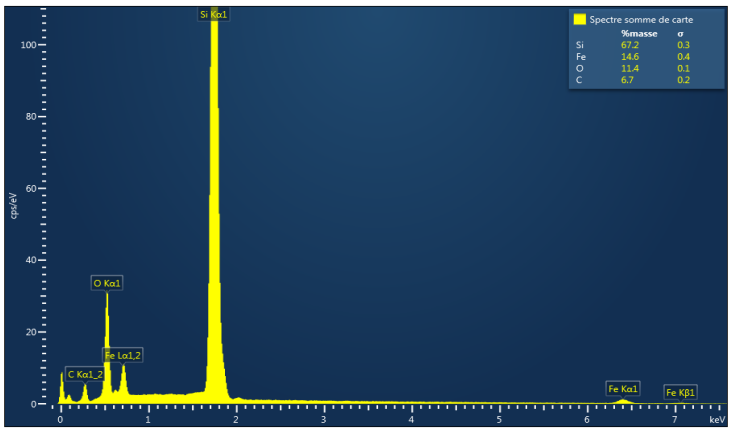


Figure S16. Energy Dispersive X-ray spectroscopy (EDX) spectrum of pSiNRs@SPIONs.

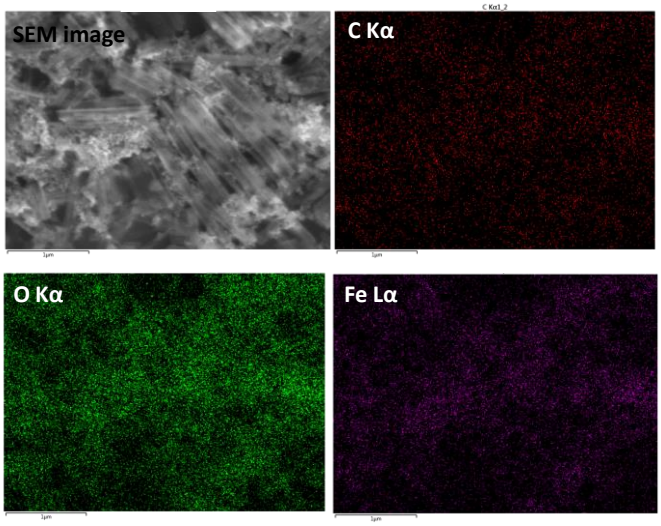


Figure S17. Energy Dispersive X-ray spectroscopy (EDX) mapping of pSiNRs@SPIONs. Scale bars: 1 µm.

- DLS and ZP of pSiNRs@SPIONs

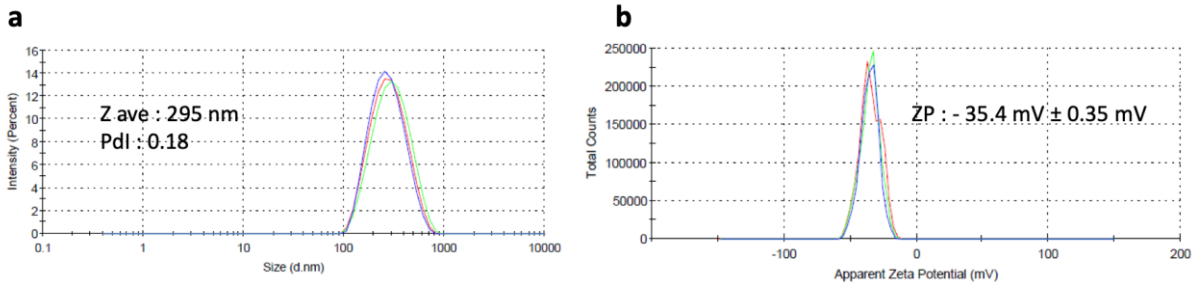


Figure S18. (a) DLS of pSiNRs@SPIONs and (b) ZP of pSiNRs@SPIONs. Each measurement was an average of thirteen repetitions and repeated three times.

- XRD of pSiNRs@SPONs compared to fresh pSiNRs

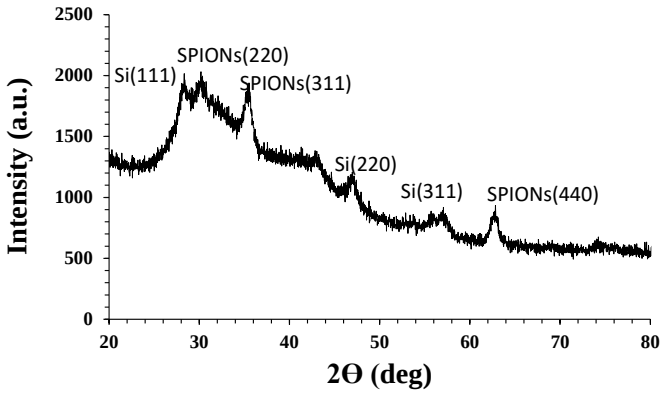


Figure S19. XRD spectrum of pSiNRs@SPIONs.

4.4 Characterization of nanoparticles formulations loaded with DiD dye

- DLS and ZP of pSiNRs-DiD and pSiNRs-DiD@SPIONs

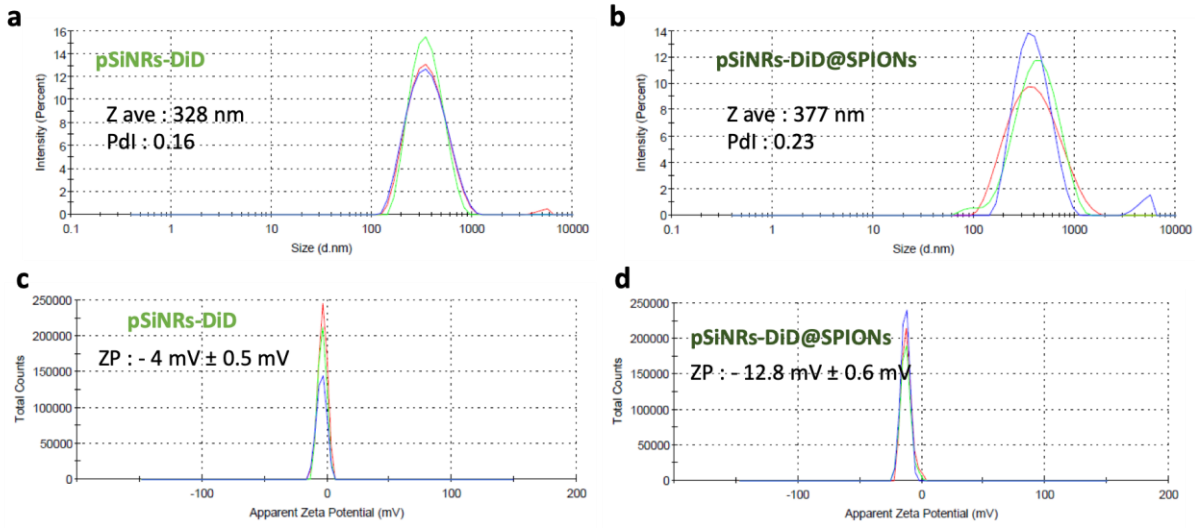


Figure S20. DLS in intensity of (a) pSiNRs-DiD and (b) pSiNRs-DiD@SPIONs. ZP of (c) pSiNRs-DiD and (d) pSiNRs-DiD@SPIONs. Each measurement was an average of thirteen repetitions and repeated three times.

- Infrared (FTIR) of pSiNRs-DiD@SPIONs

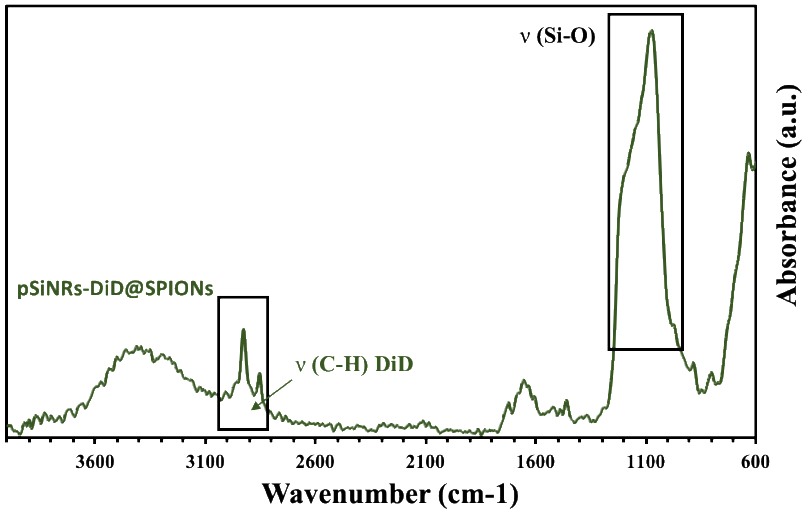


Figure S21. Attenuated Total Reflectance Fourier-Transform infrared (ATR-FTIR) spectra of pSiNRs-DiD@SPIONs (grey trace). Symbols: ν = stretching.

- UV-vis spectroscopy of pSiNRs-DiD and pSiNRs-DiD@SPIONs compared to fresh pSiNRs

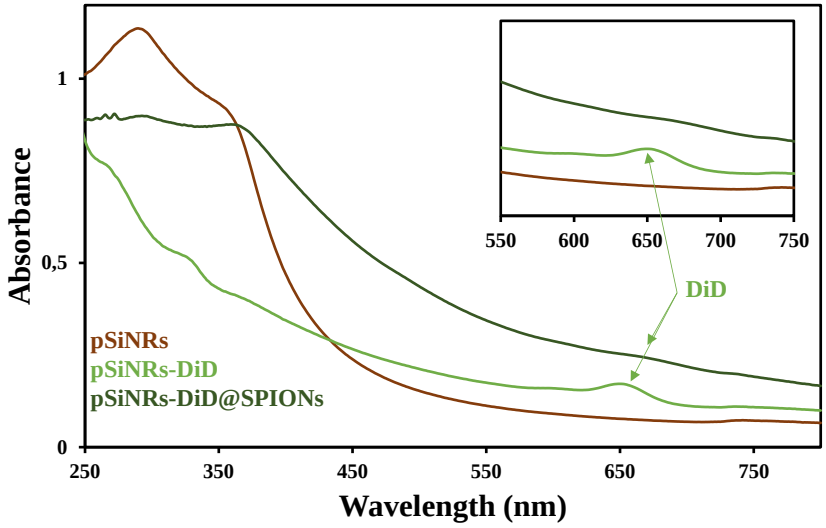


Figure S22. UV-vis absorbance spectra of pSiNRs (brown trace), pSiNRs-DiD (green trace), pSiNRs-DiD@SPIONs (dark green trace).

4.5 Characterization of pSiNRs@SPIONs-NFL-peptides (BIOT or FAM) and pSiNRs-DiD@SPIONs-NFL-peptides (BIOT or FAM)

- TEM images of pSiNRs@SPIONs-BIOT-NFL.

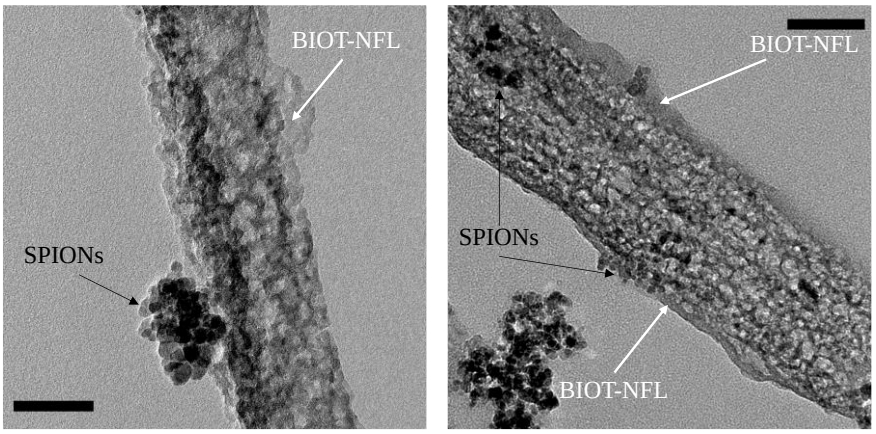


Figure S23. TEM images of pSiNRs@SPIONs-BIOT-NFL. Scale bars: 50 nm (left images) and 100 nm (right images).

- SEM images of pSiNRs@SPIONs-FAM-NFL and pSiNRs@SPIONs-BIOT-NFL.

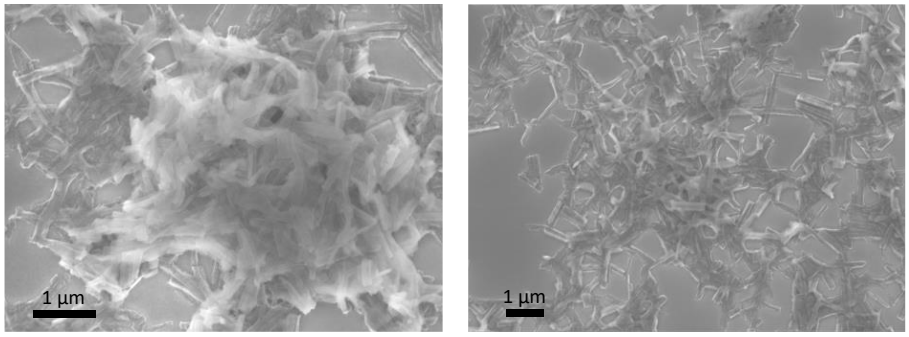


Figure S24. SEM images of pSiNRs@SPIONs-FAM-NFL.

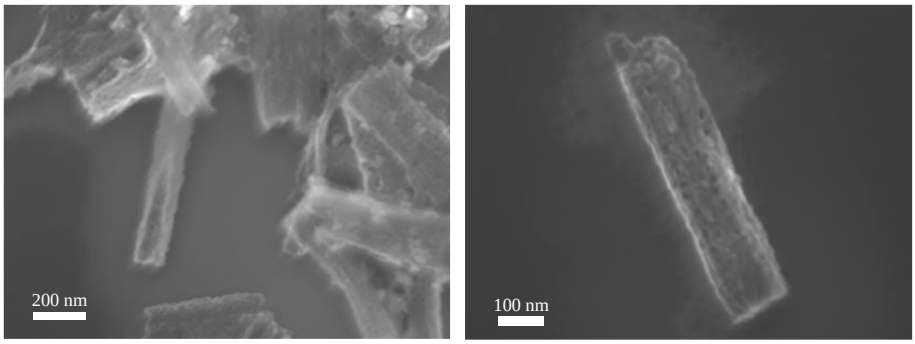


Figure S25. SEM images of pSiNRs@SPIONs-BIOT-NFL. Scale bars: 200 nm (left image) and 100 nm (right image).

- DLS of pSiNRs@SPIONs-NFL-peptides (BIOT or FAM) and pSiNRs-DiD@SPIONs-NFL-peptides (BIOT or FAM)

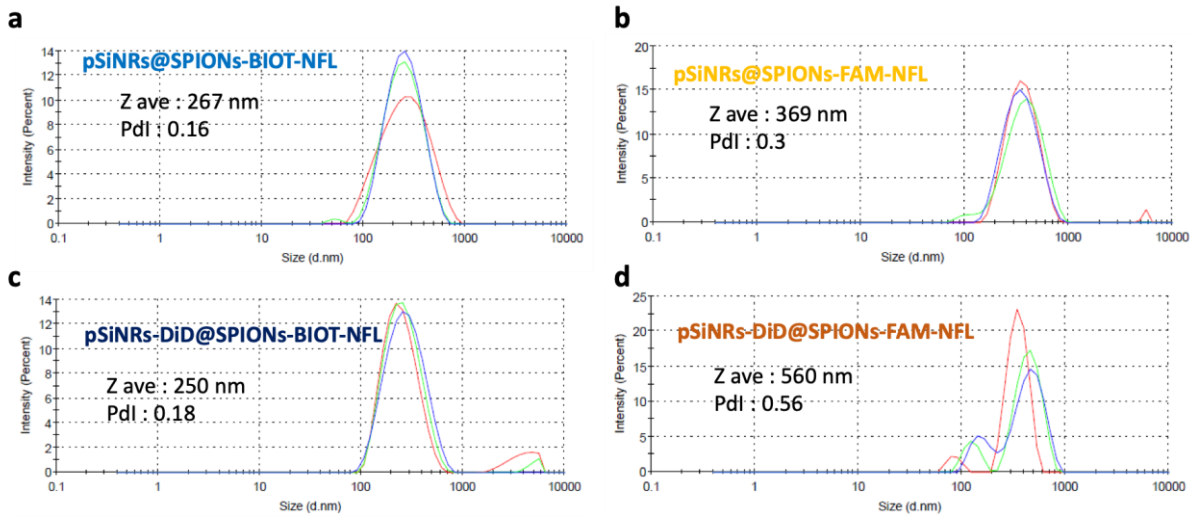


Figure S26. DLS measurements of (a) pSiNRs@SPIONs-BIOT-NFL, (b) pSiNRs@SPIONs-FAM-NFL, (c) pSiNRs-DiD@SPIONs-BIOT-NFL, and (d) pSiNRs-DiD@SPIONs-FAM-NFL. Each measurement was an average of thirteen repetitions and repeated three times.

- Zeta potential of pSiNRs@SPIONs-NFL-peptides (BIOT or FAM) and pSiNRs-DiD@SPIONs-NFL-peptides (BIOT or FAM)

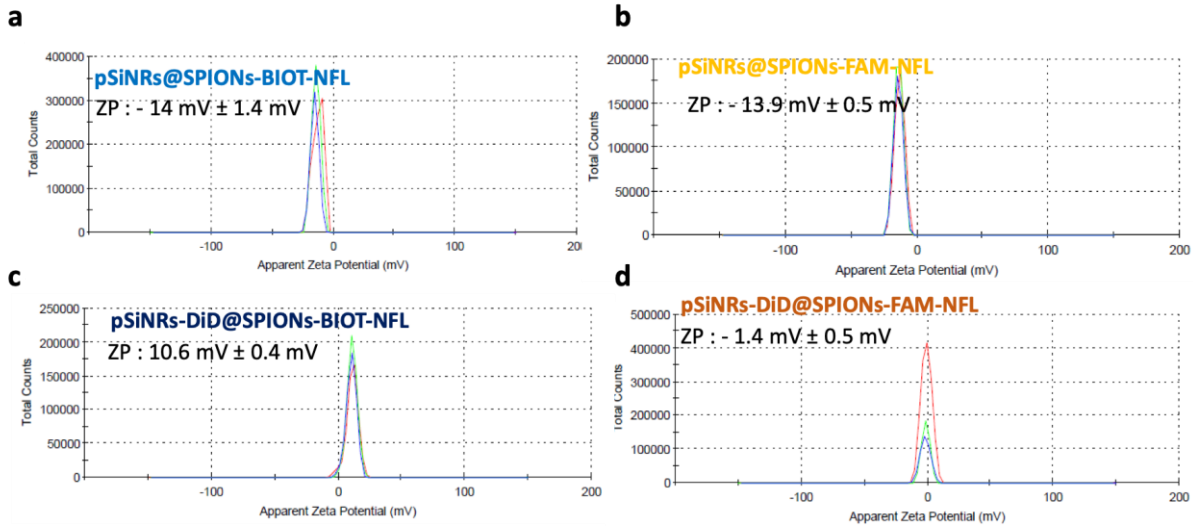


Figure S27. Zeta potential measurements of (a) pSiNRs@SPIONs-BIOT-NFL, (b) pSiNRs@SPIONs-FAM-NFL, (c) pSiNRs-DiD@SPIONs-BIOT-NFL, and (d) pSiNRs-DiD@SPIONs-FAM-NFL. Each measurement was an average of thirteen repetitions and repeated three times.

- Infrared (FTIR) of pSiNRs@SPIONs-BIOT-NFL and pSiNRs@SPIONs-FAM-NFL.

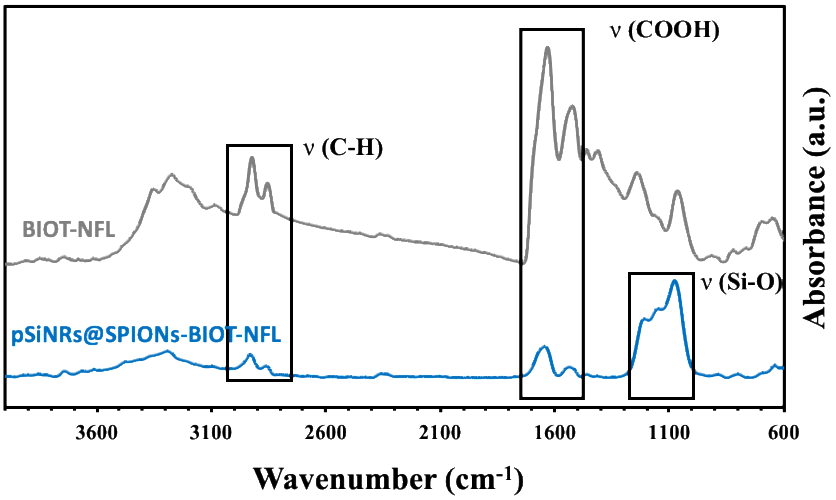


Figure S28. Attenuated Total Reflectance Fourier-Transform infrared (ATR-FTIR) spectra modified nanoparticles samples, from the bottom to the top: pSiNRs@SPIONs-BIOT-NFL (blue trace), BIOT-NFL: (grey trace). Symbols: ν = stretching.

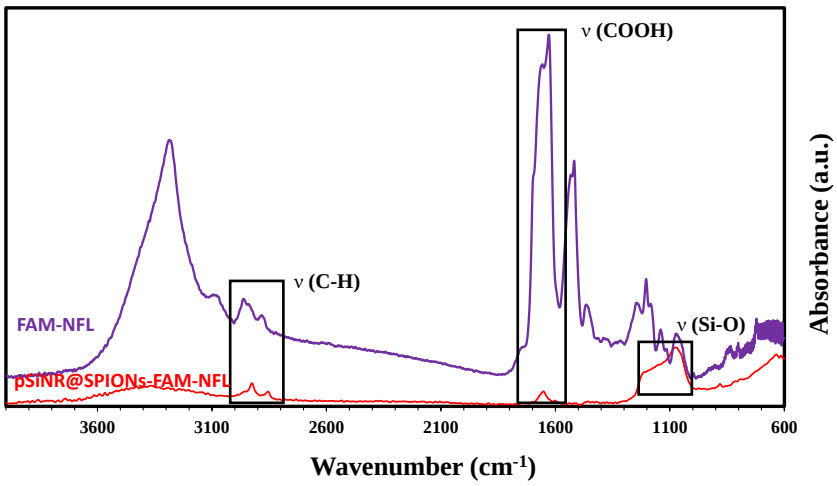


Figure S29. Attenuated Total Reflectance Fourier-Transform Infrared (ATR-FTIR) spectra modified nanoparticles samples, from the bottom to the top: pSiNRs@SPIONs-FAM-NFL (red trace), FAM-NFL-peptide (purple trace).

- Infrared (FTIR) of pSiNRs@SPIONs-NFL-peptides (BIOT or FAM) loaded with DiD dye

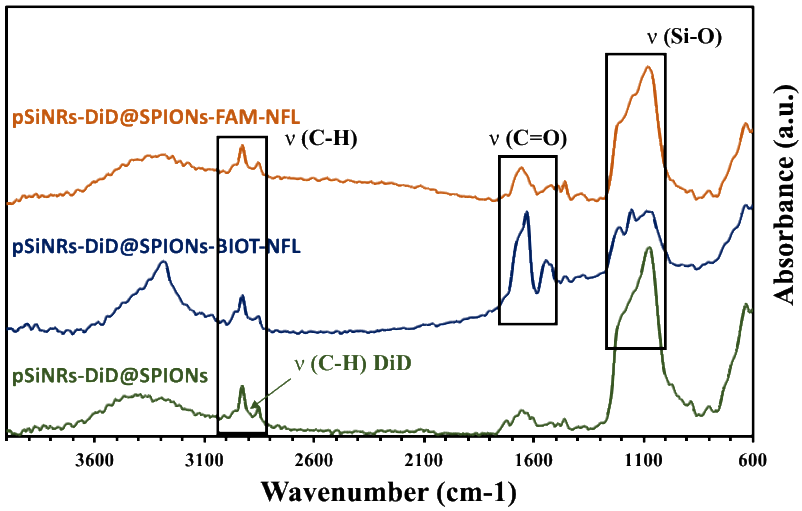


Figure S30. Attenuated Total Reflectance Fourier-Transform Infrared (ATR-FTIR) spectra modified nanoparticles samples, from the bottom to the top: pSiNRs-DiD@SPIONs (green dark trace), pSiNRs-DiD@SPIONs-BIOT-NFL (dark blue trace) and pSiNRs-DiD@SPIONs-FAM-NFL (orange trace).

- TGA of pSiNRs@SPIONs-BIOT-NFL

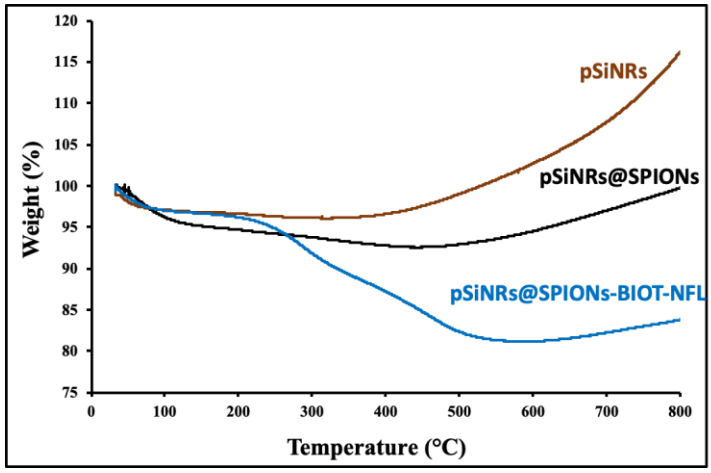


Figure S31. TGA of pSiNRs (brown trace), pSiNRs@SPIONs (dark trace) and pSiNRs@SPIONs-BIOT-NFL (blue trace).

- UV-vis spectroscopy of pSiNRs@SPIONs-NFL-peptides (BIOT or FAM) loaded with DiD dye

UV-vis spectroscopy was carried out in lambda 35 Perkin Elmer spectrometer. The nanoparticles formulations were dispersed in water.

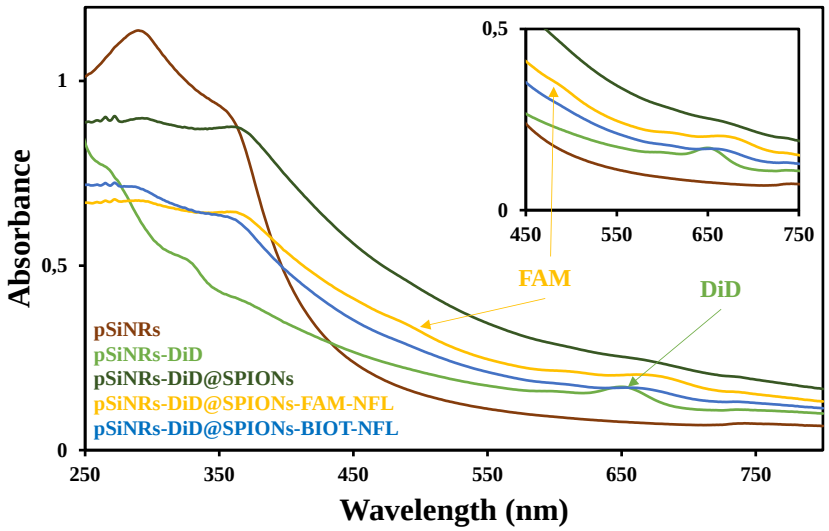


Figure S32. UV-vis absorbance spectra of pSiNRs (brown trace), pSiNRs-DiD (green trace), pSiNRs-DiD@SPIONs (dark green trace), pSiNRs-DiD@SPIONs-FAM-NFL (yellow trace) and pSiNRs-DiD@SPIONs-BIOT-NFL (blue trace).

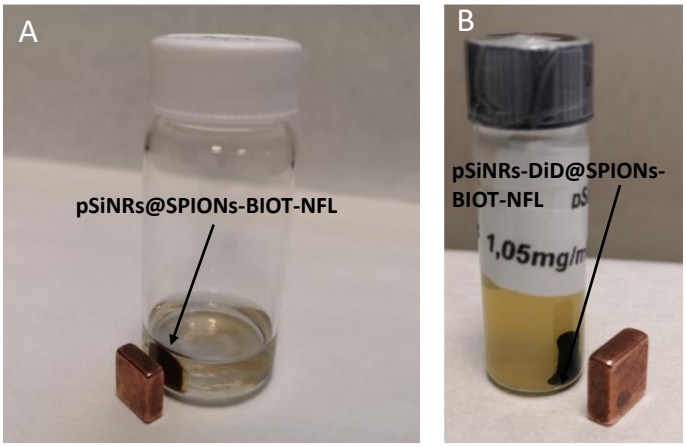


Figure S33. Magnetic properties of the final products (A) pSiNRs@SPIONs-BIOT-NFL and (B) pSiNRs-DiD@SPIONs-BIOT-NFL.

VI. Biology results

- Cells and reagent

F98 rat glioblastoma or SH-SY5Y neuroblastoma cells were cultured in Dulbecco′s modified Eagle′s medium (DMEM; Sigma-Aldrich) containing GlutaMax and supplemented with 10% of fetal bovine serum (Sigma-Aldrich), 1% of antibiotics (100X streptomycin/penicillin; BioWest, Nuaille, France) and 1% of non-essential amino acids (Sigma-Aldrich). Colchicine (C9754) have been purchased from Sigma-Aldrich.

- Mitochondrial activity

F98 rat GBM or SH-SY5Y neuroblastoma cells were seeded in 96-well plates at 1,000 cells per well and incubated for 24 hours at 37 °C and 5% CO_2. The culture media was removed, and Colchicine (1 µg/mL) or the formulations diluted in fresh media at different concentrations (25, 50, 100 or 200 µg/mL), were added for 72 hours at 37 °C and 5% CO_2. Cell mitochondrial activity was measured using the MTS survival assay (ab197010; Abcam, Paris, France). 20 µL of MTS reagent was added to each well for 4 hours. The number of living cells is directly proportional to the absorbance measured by the amount of light absorbance at 490 nm in a SpectraMax M2 multi-scanning spectrophotometer (Molecular Devices, San Jose, California, USA). Only formulations without DiD were tested with MTS assay, because an incompatibility between DiD probe and kit exist.

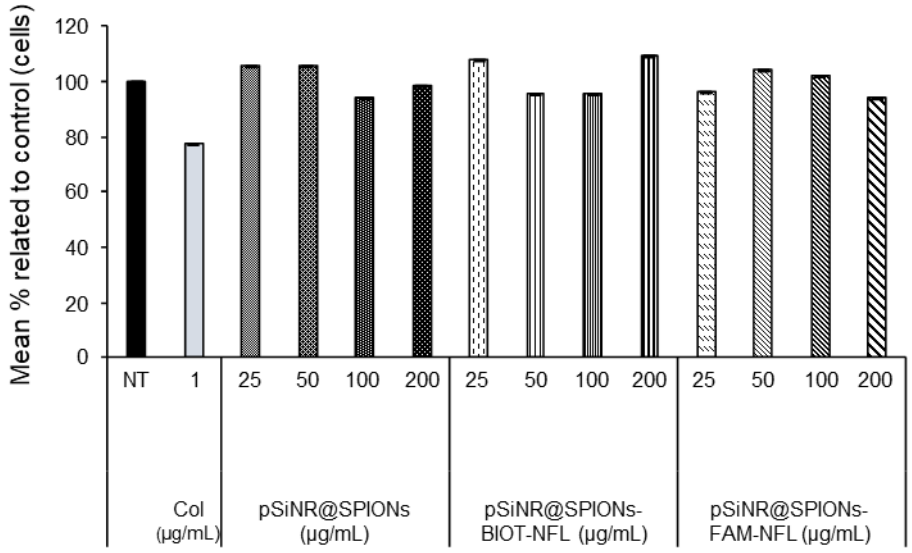


Figure S34. *In vitro* effects of the pSiNRs@SPIONs without or with NFL-peptides (BIOT or FAM) on mitochondrial activity of neuroblastoma cells (SH-SY5Y). Cells were treated with the formulations (25, 50, 100 or 200 µg/mL) or with the positive control Colchicine (Col, 1 µg/mL), for 72 hours, and mitochondrial activity was evaluated by MTS assay. Experiments were performed at least in triplicate. Data are represented as mean ± SEM.

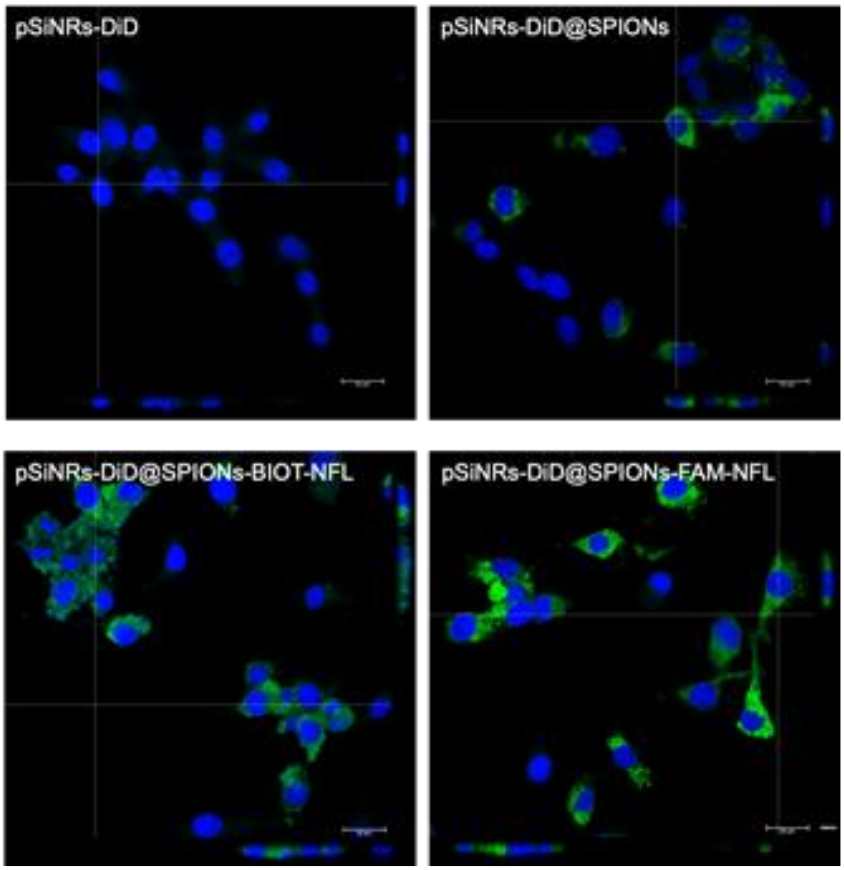


Figure S34. Confocal experiments show rat glioblastoma cells treated with pSiNRs, with pSiNRs@SPIONs, with pSiNRs@SPIONs-BIOT-NFL, or with pSiNRs@SPIONs-FAM-NFL. F98 cells were incubated 24 hours at 37 °C with nanoparticles at 10 µg/mL. Pictures illustrating the orthogonal projections obtained with a confocal microscope of cells treated. In blue, nucleus. Experiments were performed at least triplicate. Scale bars: 20 µm.

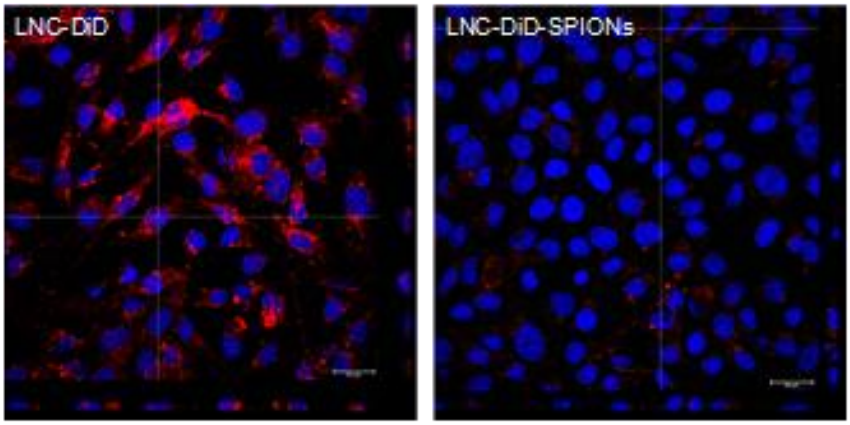


Figure S35. Confocal experiments show cellular uptake of LNC-DiD alone or functionalized with SPIONs in rat glioblastoma cells. F98 cells were incubated 24 hours at 37 °C with LNC-DiD without and with SPIONs at 2 mg/mL. Images were taken with a confocal microscope, nanoparticles loaded with DiD were visualized in red, and nucleus in blue. Pictures illustrating the orthogonal projections of cells. Experiments were performed at least triplicate. Scale bars: 20 µm.

- Analysis of cellular internalization by transmission electron microscopy (TEM)

F98 cells were seeded in 12-well plates at 100,000 cells per well and were incubated for 24 hours at 37 °C and 5% CO_2. Then, cells were treated with pSiNRs@SPIONs, pSiNRs@SPIONs-BIOT-NFL, or pSiNRs@SPIONs-FAM-NFL at 200 µg/mL for 72 hours. After incubation treatment, cells were washed (0.1 M phosphate buffer, pH 7.4) and fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer overnight at 4 °C. The next day, the fixator was removed, and cells were rinsed with 0.1 M phosphate buffer, and with distilled water, and post-fixed with 1% osmium tetroxide in water for 1 hour. Different water rinsing and ethanol incubation (15 minutes in 50° ethanol, 15 minutes in 70° ethanol, 15 minutes in 95° ethanol and 3 times 30 minutes in 100° ethanol) were realized. Then, cells were placed in a solution of 50% 100° ethanol and 50% Epon resin mixture overnight. Epon was removed and replaced by a pure Epon bath for 4 hours, then replaced by another bath 24 hours at 37 °C, 24 hours at 45 °C and 72 hours at 60 °C. The resin was polymerized at 60 °C, ultra-fine sections 60 nm thick were made with a UC7 ultramicrotome (Leica, Wetzlar, Germany) and deposited on 150 mesh copper grids. The sections were contrasted with 3% uranyl acetate in 50° ethanol for 15 minutes. Samples were observed using a 120 kV Jeol JEM-1400 electron microscope (Japan) with a SC1000 Orius model 832 (Gatan) 4k CCD camera.

- Analysis of DiD formulations cellular internalization by confocal microscopy

25,000 F98 cells were seeded in 24-well plates coated coverslips, for 48 hours at 37 °C. Then, the cells were treated for 24 hours with pSiNRs-DiD, pSiNRs-DiD@SPIONs, pSiNRs-DiD@SPIONs-BIOT-NFL, or pSiNRs-DiD@SPIONs-FAM-NFL at 10 µg/mL. After incubation, the cells were washed three times with 1X DPBS, fixed with 4% paraformaldehyde (15714; Delta microscopies, Mauressac, France) for 10 minutes, and incubated with DAPI (4′6-diaminido-2-phenylindole; Sigma-Aldrich) diluted in 1X DPBS (Dulbecco's Phosphate Buffered Saline; Gibco, Dardilly, France) at 1:300 for 10 minutes. The mounting with coverslips was realized with the Prolong Gold Antifade reagent (P36930; Thermo Fisher Scientific), and the observations were realized with a Leica TCS SP8 confocal microscope.

- Formulation of lipid nanocapsules loaded with DiD

Lipid nanocapsules (LNC) of 50 nm were prepared according to Heurtault *et al*, 2002[1]. Briefly, Kolliphor HS15 (0.846 g; BASF, Ludwigshafen, Germany), Labrafac WL 1349 (1.028 g; Gatefossé SA, Saint-Priest, France), Lipoïd S75-3 (0.075 g; Lipoïd Gmbh, Ludwigshafen, Germany), NaCl (0.075 g; Prolabo, Fontenay-sous-bois, France) and 2.962 mL of MilliQ water (Millipore, Bilerica, USA), were mixed and heated under magnetic stirring. The next step was to perform three cycles of heating and cooling between 60 °C and 90 °C. During the last cooling, 27.5 µL of DiD solution at 1 mg/mL in absolute ethanol at 80 °C and then 12.5 mL of cold water were added. The LNC were cooled under magnetic stirring for 5 minutes. A mixture between LNC-DiD and SPIONs (747319, Sigma-Aldrich) were realized to obtain LNC-DiD-SPIONs.

- Analysis of cellular internalization of lipid nanocapsules loaded with DiD probe by confocal microscopy

25,000 F98 cells were seeded in 24-well plates coated coverslips, for 48 hours at 37 °C. Then, the cells were treated for 24 hours with LNC-DiD or LNC-DiD-SPIONs at 2 mg/mL. After, the cells were washed three times with 1X DPBS, fixed with 4% paraformaldehyde for 10 minutes,

and incubated with DAPI diluted in 1X DPBS at 1:300 for 10 minutes. The mounting with coverslips was realized with the Prolong Gold Antifade reagent, and the observations were realized with a Leica TCS SP8 confocal microscope.

1 B. Heurtault, P. Saulnier, B. Pech, J. Proust and J. Benoit, *Pharmaceutical Research*, 2002, **19**, 875–880.