Electronic Supplementary Material (ESI) for Nanoscale.

Breakable mesoporous silica nanoparticles for targeted drug delivery

Laura Maggini,[*,§] Ingrid Cabrera-Puig,[§] Amparo Ruiz-Carretero,[§] Eko A. Prasetyanto,[§] Eric Robinet,[‡] and Luisa De Cola**[§,¥]

[§] Laboratoire de Chimie et des Biomatériaux Supramoléculaires, Institut de Science et d'Ingénierie Supramoléculaires (ISIS), Universitè de Strasbourg, 8 allée Gaspard Monge, 67083 Strasbourg, France.

[¥] Institut für Nanotechnologie (INT) - Building 640, Karlsruhe Institute of Technology (KIT) - Campus Nord, Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Deutschland.

[‡] Institut Hospitalo-Universitaire de Strasbourg (IHU), 1 place de l'Hôpital, 67091 Strasbourg, France.

1. Materials

All commercial solvents and reagents were used as received from, without further purification. Tetraethyl orthosilicate (TEOS), (3-Aminopropyl)triethoxysilane (APTES), Amino propyl dimethyl methoxy silane (APDMMS), bis(triethoxysilyl-propyl)disulfide (BTSPD), Nile red (NR), Temozolomide (TMZ), Dithiothreitol (DTT), Glutathione (GSH), Rhodamine B isothiocyanate (RITC), Folic Acid, RGD, Phosphate Buffered Saline tablets, were purchased from Sigma Aldrich. BTDSP and DAPI were purchased from Santa Cruz Biotechnology. Erbitux® antibody was kindly provided by Dr. E. Robinet, IHU, University Hospital of Strasbourg. Dulbecco's Modified Eagle's Medium (DMEM), Phosphate Buffered Saline (PBS), Fetal Bovine Serum (FBS), Penicillin Streptomycin and L-Glutamine were purchased from Gibco (Life Technologies). Lysotracker Green DND-26 and Phalloidin Alexa Fluor® 647 were purchased from Invitrogen. Glioma C6 cells were obtained from ATCC/LGC Standards GmbH (Wesel, Germany) and cultivated according to the provider's protocol.

2. Synthesis

2.1 Synthesis of Rhodamine B doped disulfide nanoparticles (*ss*-**NPs**)*:* 2.5 mg of RITC were dissolved in EtOH (5 mL) before adding APTES (6 μL). In another flask CTAB (250 mg) was dissolved in a solution of distilled water (110 mL), EtOH (10 mL) and NaOH (2M, 0.875 mL) that was heated to 80 °C and stirred vigorously. The RITC/APTES solution was stirred for 30 minutes before adding TEOS (0.875 mL) and BTSPD (0.390 mL). Once the temperature of the CTAB solution had stabilized, the solution containing the silane sources was added. After 6 h the solution was cooled to r.t. and the particles, recovered by centrifugation (20 min at 20 krpm), were then purified through a sequence of sonication/centrifugation cycles in EtOH. To remove the surfactants from the pores, the particles were dissolved in acidic EtOH (100 mL, 30 μL of HCl) and refluxed o.n. The particles were hence purified by a sequence of sonication/centrifugation cycles in EtOH and finally dried under vacuum. The material was finally characterized by means of XPS, TGA, HR-TEM, SEM, SAXS and N_2 adsorption techniques.

2.2 Encapsulation of Nile Red in ss-**NPs** (**NR⊙***ss*-**NPs**)*:* encapsulation of nile red (NR) within the pores of the *ss*-**NPs** was performed by wet extraction methodology.[50] Specifically 10 mg of *ss*-**NPs** were dispersed in 9 mL of EtOH by sonication whilst 1 mg of NR was dissolved in 1 mL of EtOH. The latter solution was added to the dispersion and the whole introduced in a rotary shaker for 3 days. The mixture was then centrifuged (20 min at 20 krpm) and the supernatant removed, before

adding to the pelleted particles fresh EtOH (10 mL). The material was again purified by a sequence of sonication/centrifugation cycles in EtOH until no more emission of NR was detected in the washings (monitored by PL), and finally dried under vacuum. The material was finally characterized by means of XPS, TGA, and SEM techniques.

*2.3 Encapsulation of temozolomide (TMZ) in ss-***NPs** (**TMZ⊙***ss-***NPs**)*:* encapsulation of temozolomide (TMZ) within the pores of the *ss-***NPs** was performed by wet extraction methodology.[1] Specifically, 30 mg of *ss-***NPs** were dispersed in 9 mL of EtOH by sonication whilst 5 mg of TMZ were dissolved in 1 mL of EtOH. The latter solution was added to the dispersion and the whole introduced in a rotary shaker for 1 day. The mixture was then centrifuged (20 min at 20 krpm) and the supernatant removed, before adding to the pelleted particles fresh EtOH (10 mL). The material was again purified by a sequence of sonication/centrifugation cycles in EtOH until no more absorption of TMZ was detected in the washings (monitored by UV-Vis), and finally dried under vacuum. The material was finally characterized by means of XPS, TGA, and SEM techniques.

2.4 Synthesis of Rhodamine B doped NPs (**NPs**)**:** 2.5 mg of RITC were dissolved in EtOH (1.5 mL) before adding APTES (6 μL). In another flask CTAB (250 mg) was dissolved in a solution of distilled water (120 mL) and NaOH (2M, 0.875 mL) that was heated to 80 °C and stirred vigorously. The RITC/APTES solution was stirred for 30 minutes before adding TEOS (1.25 mL). Once the temperature of the CTAB solution had stabilized, the solution containing the silanes was added. After 2 h the solution was cooled to r.t. and the particles, recovered by centrifugation (20 min at 20 krpm), were then purified through a sequence of sonication/centrifugation cycles in EtOH. To remove the surfactants from the pores, the particles were dissolved in acidic EtOH (100 mL, 30 μL of HCl) and refluxed o.n. The particles were hence purified by a sequence of sonication/centrifugation cycles in EtOH and finally dried under vacuum. The material was finally characterized by means of XPS, TGA, HR-TEM, SEM, SAXS and N_2 adsorption techniques.

2.5 Encapsulation of TMZ in NPs (**TMZ⊙NPs**): Encapsulation of TMZ within the pores of the **NPs** was performed by wet extraction methodology.[1] Specifically 30 mg of **NPs** were dispersed in 9 mL of EtOH by sonication whilst 5 mg of TMZ were dissolved in 1 mL of EtOH. The latter solution was added to the dispersion and the whole introduced in a rotary shaker for 1 day. The mixture was then centrifuged (20 min at 20 krpm) and the supernatant removed, before adding to the pelleted particles fresh EtOH (10 mL). The material was again purified by a sequence of sonication/centrifugation cycles in EtOH until no more absorption of TMZ was detected in the

washings (monitored by UV-Vis), and finally dried under vacuum. The material was finally characterized by means of XPS and TGA analysis techniques.

2.6 Synthesis of Rhodamine B doped ss-NPs functionalized with RGD peptide (**RGD-*ss*-NPs**): 20 mg of *ss*-**NPs** were dispersed by sonication in DMSO (1 mL) and the solution diluted in toluene (4 mL). In a separate flask, RGD (0.08 mg) and 3-aminopropyldimethylmethoxysilane (3 μL) were mixed in DMSO (1 mL).[2] *N*-hydroxysuccinimmide (0.03 mg) and (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.05 mg) were added into the mixture and stirred for 2 h. This solution was hence added to the particles suspension and the whole kept stirring at r.t. for further 20 h. The mixture was then centrifuged (20 min at 20 krpm) and the supernatant removed, before adding to the pelleted particles fresh EtOH (10 mL). The particles were hence purified by a sequence of sonication/centrifugation cycles in EtOH and finally dried under vacuum. The material was finally characterized by means of XPS, TGA, and SEM analysis techniques.

2.7 Encapsulation of temozolomide (TMZ) in **RGD**-*ss*-**NPs** (**TMZ⊙RGD**-*ss*-**NPs**)*:* encapsulation of temozolomide (TMZ) within the pores of the **RGD**-*ss*-**NPs** was performed by wet extraction methodology.[1] Specifically, 30 mg of **RGD**-*ss*-**NPs** were dispersed in 9 mL of EtOH by sonication whilst 5 mg of TMZ were dissolved in 1 mL of EtOH. The latter solution was added to the dispersion and the whole introduced in a rotary shaker for 1 day. The mixture was then centrifuged (20 min at 20 krpm) and the supernatant removed, before adding to the pelleted particles fresh EtOH (10 mL). The material was again purified by a sequence of sonication/centrifugation cycles in EtOH until no more absorption of TMZ was detected in the washings (monitored by UV-Vis), and finally dried under vacuum. The material was finally characterized by means of XPS, TGA and SEM techniques.

3. Instruments

3.1 Scanning Electron Microscopy (SEM): SEM images were recorded with a FEI Quanta FEG 250 instrument (FEI corporate, Hillsboro, Oregon, USA) with an acceleration voltage of 20 kV. The sample is prepared by drop-casting a dispersion of particles in EtOH onto a glass cover slip, subsequently sputter coated with Au (Emitech K575X peltier cooled) for 60 s at 60 mA prior to fixation on an Al support.

3.2 Transmission Electron Microscopy (TEM): TEM samples were analyzed on a Hitachi 7500 instrument at an acceleration voltage of 80 kV. Ethanolic suspensions of the materials were drop-casted onto Formvar coated Cu grids (400 mesh) and allowed to dry overnight prior to

visualization. Cell samples were prepared according to the standard procedure.[3] The epoxy resin was finally cut by ultramicrotome in slices 50-70 nm thick. For EDX mapping analysis a FEI Titan instrument operating at 300 kV was employed.

3.3 X-ray Photoelectron Spectroscopy (XPS): XPS analysis was performed using a K-Alpha™+ X-ray Photoelectron Spectrometer (XPS) System (Thermo Scientific). Monochromatic Al K alpha X-rays were used (15 keV, 72 W, 200 mm spot diameter). Spectra were measured using a pass energy of 200 eV for survey spectra and 50 eV for core level spectra. The analyzed samples were prepared by drop-casting an ethanolic dispersion (0.1 mg/mL) of the particles onto a glass coverslip pre-coated with Au (Emitech K575X peltier cooled) for 3 min at 60 mA.

3.4 Thermogravimetric Analysis (TGA): TGA analyses were conducted on a Perkin Elmer TGA4000 Instrument machine under nitrogen atmosphere. The samples (0.1 – 2 mg) were kept at 100 °C for 30 minutes for stabilization, then heated from 100 to 750 °C at a speed of 10 °C/min, before being held at this temperature for further 30 minutes before cooling. The analyses were performed under a gas flow of N_2 at 60 mL/min.

3.5 Dynamic Light Scattering (DLS) and Zeta Potential (ZP): DLS and ZP measurements were conducted on a Delsa Nano C Particle Analyzer (Beckman Coulter, Brea, CA, USA; operative wavelength 655 nm). All DLS measurements of the nanoparticles were conducted in water (≈ 0.1 mg/mL), and the Contin algorithm was used to supply the hydrodynamic diameters as intensity and volume distributions. ZP analyses were performed in PBS buffer (pH 7.4; ≈ 0.1 mg/mL).

3.6 Attenuated Total Reflectance (ATR-FTIR): ATR measurements were performed on an ATR IRAffinity-1 instrument (Shimadzu Scientific Instruments). The analyzed samples were prepared by drop-casting an ethanolic dispersion (0.1 mg/mL) of the particles directly onto the sample plate surface.

3.7 Nitrogen Adsorption: Porosimetry analyses of the samples were performed using a Micromeritics porosimeter (model ASAP-2020). The samples were degassed at 150 °C for 3h and N_2 adsorption/ desorption measurement was done at -196 °C. The surface areas and pore volume were calculated by BET method and the pore size distributions were calculated by DFT methods.

3.8 Small Angle X-ray Scattering (SAXS): SAXS measurements of samples were performed with SAXSess Small-angle X-Ray Scattering instrument (Anton Paar GmbH, Austria). The Kratky type camera is attached to a laboratory X-Ray generator (PW3830, PANalytical), and was operated with a fine focus glass sealed X-Ray tube at 40 kV and 50 mA (Cu Kα, α = 0.1542 nm). Detection was

performed with the 2D imaging plate and analyzed by an imaging plate reader Cyclone® (Perkin Elmer). Measurements were performed with standard solid sample holder for 30 min. The two-dimensional intensity data were converted to one-dimensional data with SAXSQuant software (Anton Paar GmbH, Austria).

3.9 Photophysical measurements: Absorption spectra were measured on a Shimadzu UV-3600 spectrophotometer double-beam UV–VIS–NIR spectrometer and baseline corrected. Steady-state emission spectra were recorded on a Horiba Jobin–Yvon IBH FL-322 Fluorolog 3 spectrometer equipped with a 450 W xenon arc lamp, double-grating excitation, and emission monochromators (2.1 nm mm^{-1} of dispersion; 1200 grooves mm^{-1}) and a TBX-04 single photon-counting detector. Emission spectra were corrected for source intensity (lamp and grating) and emission spectral response (detector and grating) by standard correction curves.

3.10 ICP-AES equipment: Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) analyses were performed with a Thermo Scientific instrument, ICAP 6500. Samples (1 ml) were diluted to a total volume of 10 ml using aqueous 2% nitric acid. All solutions were freshly prepared from UV sterilized deionized (about 18 MΩ) water. Standard Si solutions (1ppm, 2 ppm, 3 ppm) were used for calibration.. Reported measurements in each sample represent the average of 3 measurements, and are back-calculated to offset the initial dilution. The individual measurements are typically obtained with a relative standard deviation of approximately 0.1 ppm per sample.

3.11 Confocal Microscope: Confocal imaging was performed with a Zeiss LSM 710 confocal microscope system equipped with a 63x magnification, numerical aperture 1.3 of Zeiss LCI Plan-NEOFLUAR water immersion objective lens (Zeiss GmbH, Germany).

3.12 Flow Cytometer: Samples were acquired on a LSRII cytometer (Becton Dickinson Biosciences, San Diego, CA, USA). Quality control was performed regularly using Cytometer Setup & Tracking beads (BD Biosciences) to ensure consistency of fluorescence intensity measurements throughout all experiments. Cell debris and dead cells were excluded using Forward Scatter Area and Side Scatter Area and cell aggregates were excluded using Side Scatter Width and Side Scatter Area. Rhodamine fluorescence of 5,000 living single cells was analyzed after monoparametric acquisition using the FL 2 Area parameter (excitation with a 488 nm Blue Laser, 575/26 nm emission filter). FACSDiva™ software version 6.1.2 (BD Biosciences) was used for data analysis and graphical output. Data are expressed as the percentage of positive cells, normalized by the relative emission intensities of the particles.

4. Methods

*4.1 Breakability tests performed on ss-***NPs.** The reduction of the disulfide bonds by dithiothreitol (DTT) was monitored (from 0 to 192 hours) using UV-Vis spectroscopy following the absorbance at 290 nm of the cyclic compound resulting from the oxidation of DTT. *ss*-**NPs** were suspended in PBS (0.1 mg/mL) and subsequently DTT at a concentration of 5 mM was added to the *ss*-**NPs** suspension while flowing nitrogen to avoid oxidation of DTT by oxygen. The solution was kept at 37 °C in oxygen free conditions. 1.5 mL aliquots were taken at different time points and absorbance was recorded. Control experiments using standard **NPs** (0.1 mg/mL) with DTT (5 mM) and DTT (5 mM) alone were performed under the same conditions. For the reduction of *ss*-**NPs** with reduced glutathione (GSH) the particles were suspended in a PBS solution of reduced GSH (2 μM and 10 mM) with a concentration of 0.1 mg/mL (final volume of 15 mL). The solution was initially sonicated for 10 minutes and then kept at 37 °C while stirring. Aliquots of 1.5 mL were taken at different time points (from 0 to 168 hours) for PL, DLS and TEM analysis. Specifically, a drop of the suspension was used to prepare a TEM sample of the degraded material, then the suspension was centrifuged for 30 minutes at 14.5 krpm to allow sedimentation of the intact particles, hence the supernatant fraction was collected and its emission and scattering measured. For the PL analysis, the samples were excited at λ_{exc} = 500 nm keeping the same slits values for all the measurements. Control experiments using *ss*-**NPs** in pristine PBS without GSH and **NPs** in presence of 10 mM of GSH were also performed under the same conditions. To evaluate the effects of the reduction of *ss*-**NPs** on guest release, Nile red (NR) loaded *ss*-**NPs** were suspended (0.1 mg/mL) in a PBS solution of GSH (2 μM, 5 mM and 10 mM). An equal volume of EtOAc was added to extract the NR dye released from *ss*-**NPs**. The emission signal of the dye in the organic phase was followed in time (from 0 to 168 h). The samples were excited at λ_{exc} = 520 nm keeping the same slits values for all the measurements. A similar suspension of *ss*-**NPs** in PBS without GSH was also prepared and studied under the same conditions.

4.2 Cell experiments: For cell culture, Glioma C6 cells were cultured inside a culture media containing 88% Dulbecco's Modified Eagle Medium (D-MEM), 10% Fetal Bovine Serum (FBS), 1% penicillin–streptomycin and 1% L-glutamine 200 mM under 37 ^{0}C and 5% of CO_2 atmosphere. For nanoparticle uptake experiments Glioma C6 cells were seeded onto glass cover slips in a 24-well cell culture plate at a density of 1.5×10^4 cells per well and allowed

to grow 24 h. Afterwards the media was removed and fresh media containing *ss*-**NPs** at a concentration of 50 µg/mL was added to the cells and incubated for 2 h at 37 ºC in a humidified atmosphere with 5% CO_2. Afterwards cells were then washed 5 times with PBS and fixed with 4 % PFA. In order to visualize the nuclear region, the nucleus was stained with DAPI and washed three times with PBS. The glass cover slips were mounted onto glass slides for confocal microscopy measurements. The excitation wavelength for DAPI and RITC (contained in the framework of the particles) were 405 and 488 nm respectively. For quantitative nanoparticle uptake analysis determined *via* flow cytometry, C6 Glioma cells were seeded in a 24-well cell culture plate at a density of 5.0×10^4 cells per well and allow to grow for 24 h. After this time, the cells were treated with 20 and 50 µg/mL of nanoparticles and incubated for 2 h. Control cells were treated with equivalent volumes of culture media. After incubation with the particles cells were trypsinated, centrifuged for 3 min at 1 krpm and the resultant pellet was re-suspended in 500 µL of PBS. Cells were centrifuged one more time and finally the pellet was re-suspended in 500 µL with PBS (4% PFA) for FACS measurements. For the Z-stacking experiment cells were prepared as previously explained for the cellular uptake and incubated with *ss*-**NPs** under the same conditions. After 24 h of incubation cells were washed 5 times with PBS and fixed with 4% PFA. Cells were then washed with PBS and kept in Triton X-100 (0.1 % in PBS) for 10 minutes and afterwards in 1% bovine serum albumin (BSA) in PBS for 20 min. The cell layer on glass cover slip was stained with Phalloidin Alexa Fluor® 647 for F-actin/membrane staining, for 20 min in the dark at room temperature, and washed twice with PBS. The nuclear region was stained with DAPI. The cover slips were mounted onto glass slides for confocal microscopy measurements. The excitation wavelength for DAPI and RITC (contained in the framework of the particles) were 405 and 488 nm respectively, while with Phalloidin Alexa Fluor® 647 was excited at 650 nm. For co-localization experiments Glioma C6 cells (1.5×10^4) were seeded onto glass bottom dishes and allowed to grow for 24 h. After this time the culture media was removed and fresh media containing *ss*-**NPs** at a concentration of 50 µg/mL was added to the cells and incubated for 24 h at 37 ºC in a humidified atmosphere with 5% CO_2. Cells were then washed 5 times with PBS and 2 mL of pre-warmed culture media containing Lysotracker Green at a concentration of 60 nM were added. After an incubation period of 1 h, live cells were washed with PBS and observed with the confocal microscope. The excitation wavelength for Lysotracker Green was 504

nm. For the preparation of biological TEM samples Glioma C6 cells were seeded in a 24-well cell culture plate at a density of 1.5×10^4 cells per well and allowed to grow for 24 h. After this time the media was removed and fresh media containing *ss*-**NPs** at a concentration of 50 μg/mL was added to the cells and incubated for 2 h at 37 ºC in a humidified atmosphere with 5% CO_2. Subsequently, cells were washed with PBS five times and fresh media was added to each well. Cells were then incubated for 3, 24, and 48 h. After this time, cells were again washed with PBS and fixed with glutaraldehyde (2.5%) for 2h. Cells were washed again with PBS and stained with OsO_4, before undergoing dehydration and resin embedding, according to the standard procedure.[3] For the determination of the cytotoxicity of *ss*-**NPs**, Glioma C6 cells were seeded in a 24-well cell culture plate at a density of 1.5×10^4 cells per well and allowed to grow for 24 h. After this time, the cells were treated with 5, 10, 20, 60 and 80 μg/mL of nanoparticles, and control cells were treated with equivalent volumes of culture media. Cells were incubated for 48 h, after which they were trypsinated, centrifuged for 3 min at 1 krpm and the resultant pellet was re-suspended in 1 mL of culture media. Cell viability was measured using CASY® Cell Counter and Analyzer. For the determination of the cytotoxicity of temozolomide loaded *ss*-**NPs,** Glioma C6 cells were seeded in glass cover slips in a 24-well cell culture plate at a density of 1.5×10^4 cells per well and allowed to grow 24 h. Afterwards the media was removed and fresh media containing **TMZ⊙***ss*-**NPs** at different concentrations (20 and 50 μg/mL) was added to the cells and incubated for 3h at 37 ºC in a humidified atmosphere with 5% CO_2. **TMZ⊙NPs** and TMZ in PBS were also incubated with the cells under the same conditions for comparison purposes. Control cells were treated with equivalent volumes of culture media. After 3 h the cells were trypsinated, centrifuged for 3 min at 1 krpm and the resultant pellet re-suspended in 500 μL of culture media. Cell viability was measured using CASY® Cell Counter and Analyzer. To study the cellular excretion of *ss*-**NPs** Glioma cells were seeded in glass cover slips in a 24-well cell culture plate at a density of 1.5×10^4 cells per well and allowed to grow 24 h. After this time the media was removed and fresh media containing *ss*-**NPs** at a concentration of 50 μg/mL was added to the cells and incubated for 2 h at 37 ºC in a humidified atmosphere with 5% CO_2. **NPs** were also incubated with the cells under the same conditions for comparison purposes. Subsequently, cells were washed with PBS five times and fresh media was added to each well. Cells were then incubated for 3, 24, and 48 h. After this time the culture media was collected for further Si quantification

using ICP-AES and the cells were fixed with PFA (4%). In order to visualize the nuclear region, the nucleus was stained with DAPI and the glass cover slips were mounted onto glass slides for confocal microscopy measurements.

Supporting Images and Tables

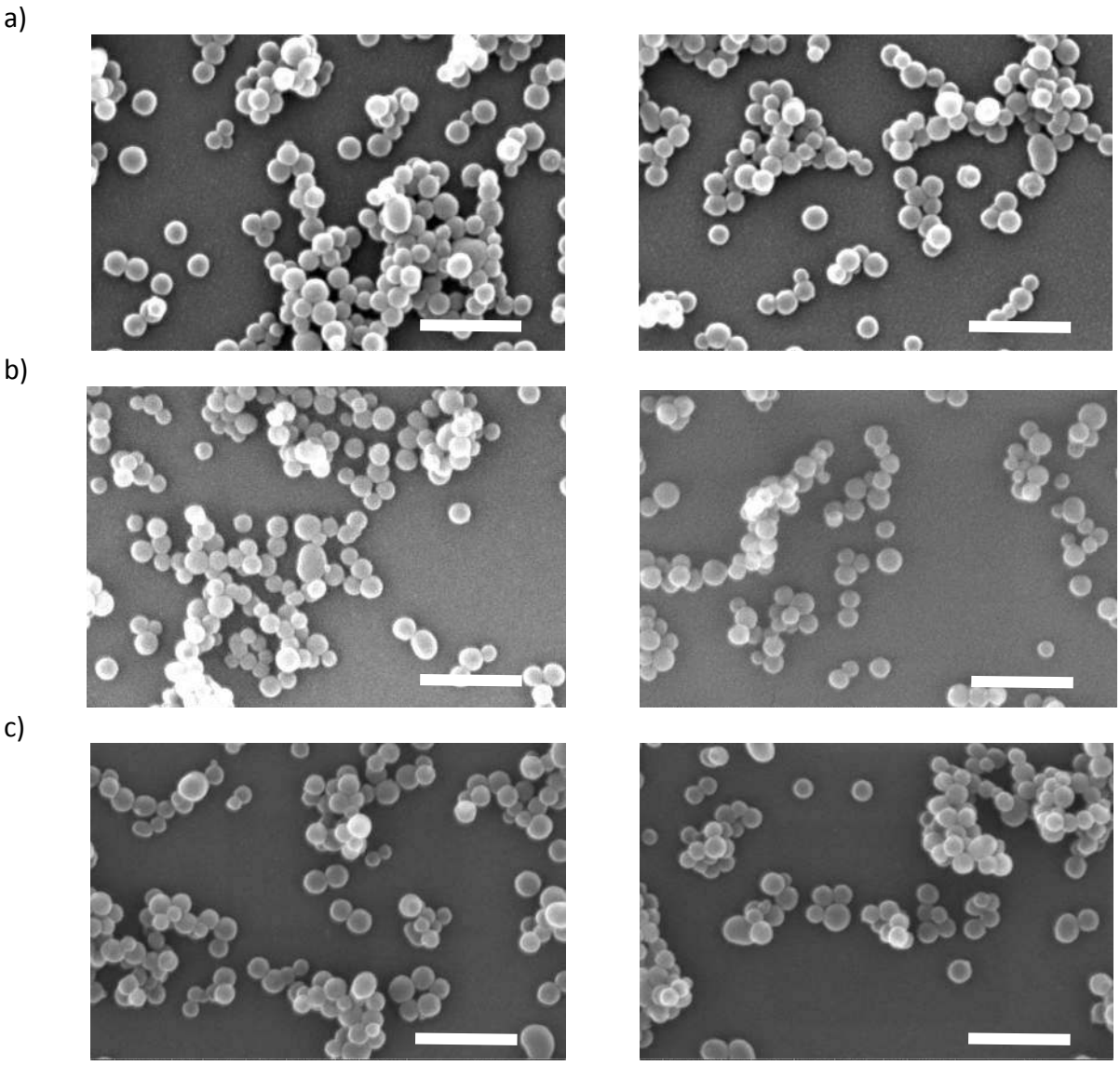


Image S1. SEM analysis of: a) *ss*-**NPs**; b) **NR⊙***ss*-**NPs**; and c) **TMZ⊙***ss*-**NPs**. Scale bar = 500 nm.

a)

b)

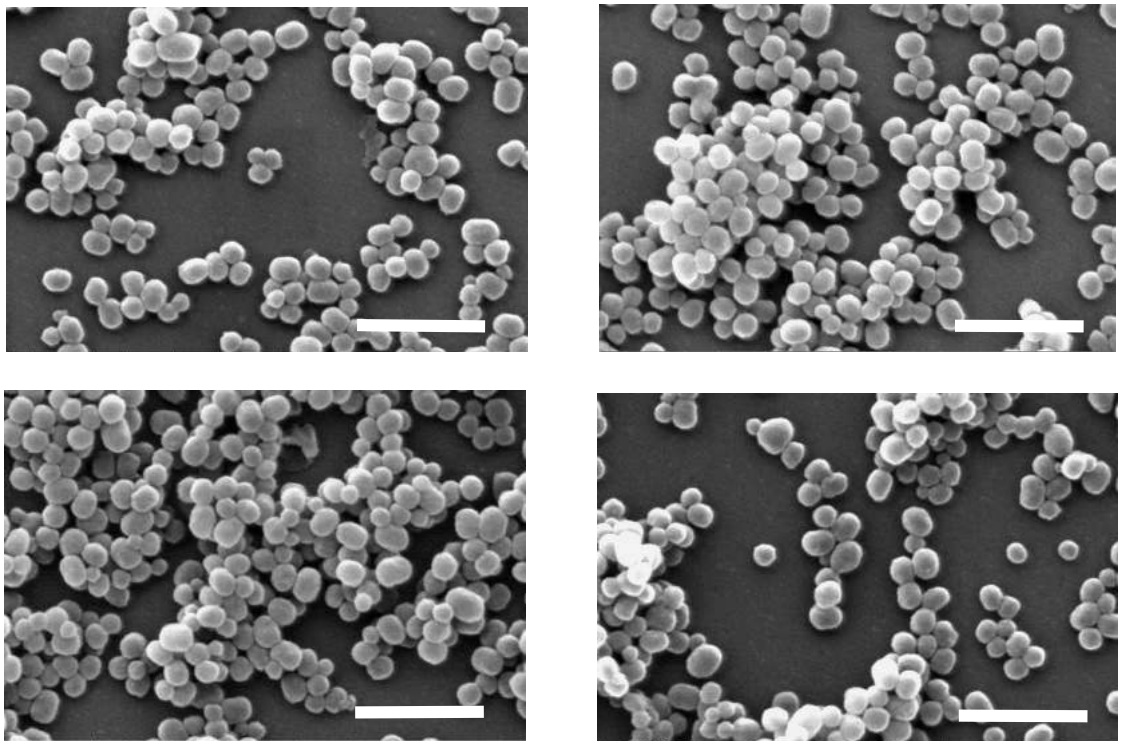


Image S2. SEM analysis of: a) **NPs**; and b) **TMZ⊙NPs**. Scale bar = 500 nm.

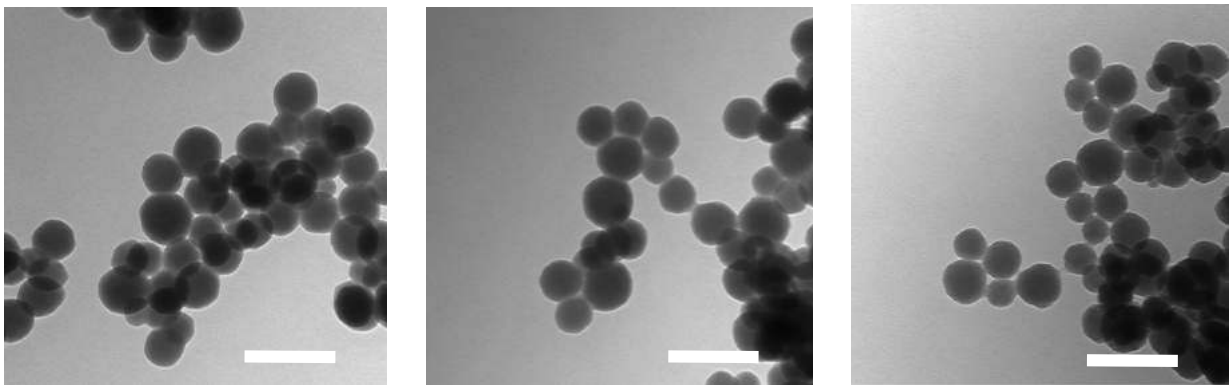


Image S3. TEM images of *ss*-**NPs** (scale bar = 200 nm).

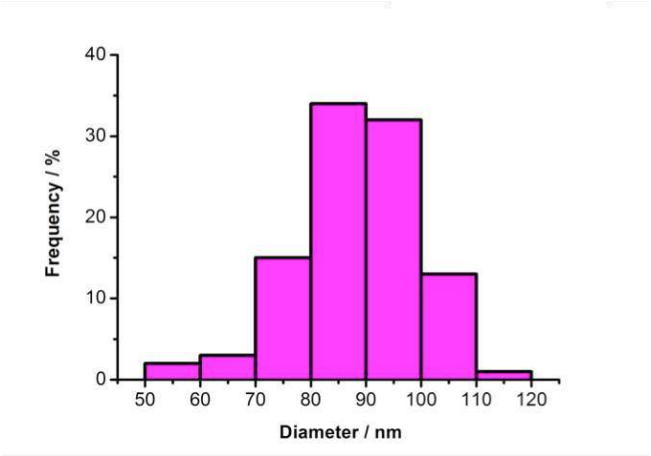


Image S4. TEM size distribution for *ss*-**NPs** (count performed on 300 nanoparticles; average diameter 88.9 ± 10.9 nm).

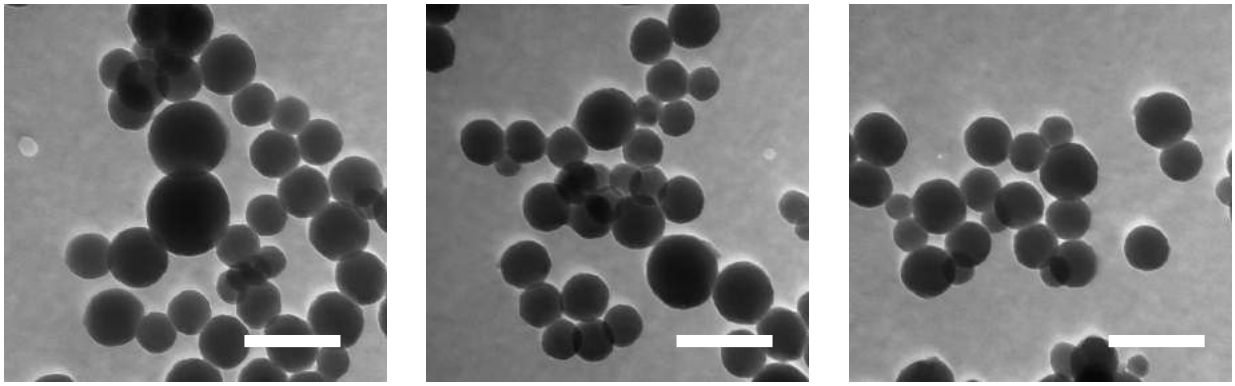


Image S5. TEM images of **NPs** (scale bar = 200 nm).

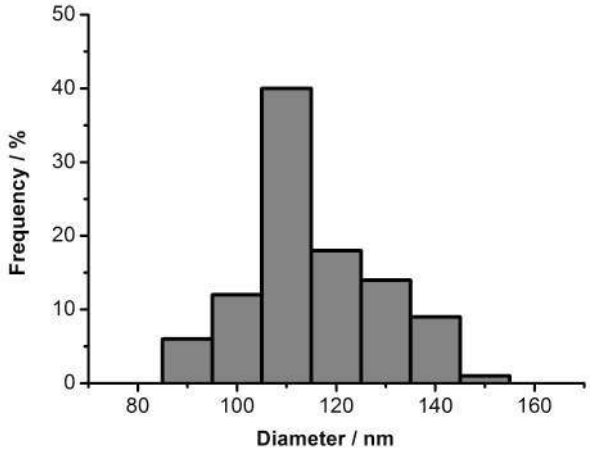


Image S6. TEM size distribution for **NPs** (count performed on 300 nanoparticles; average diameter 110.1 ± 13.2 nm).

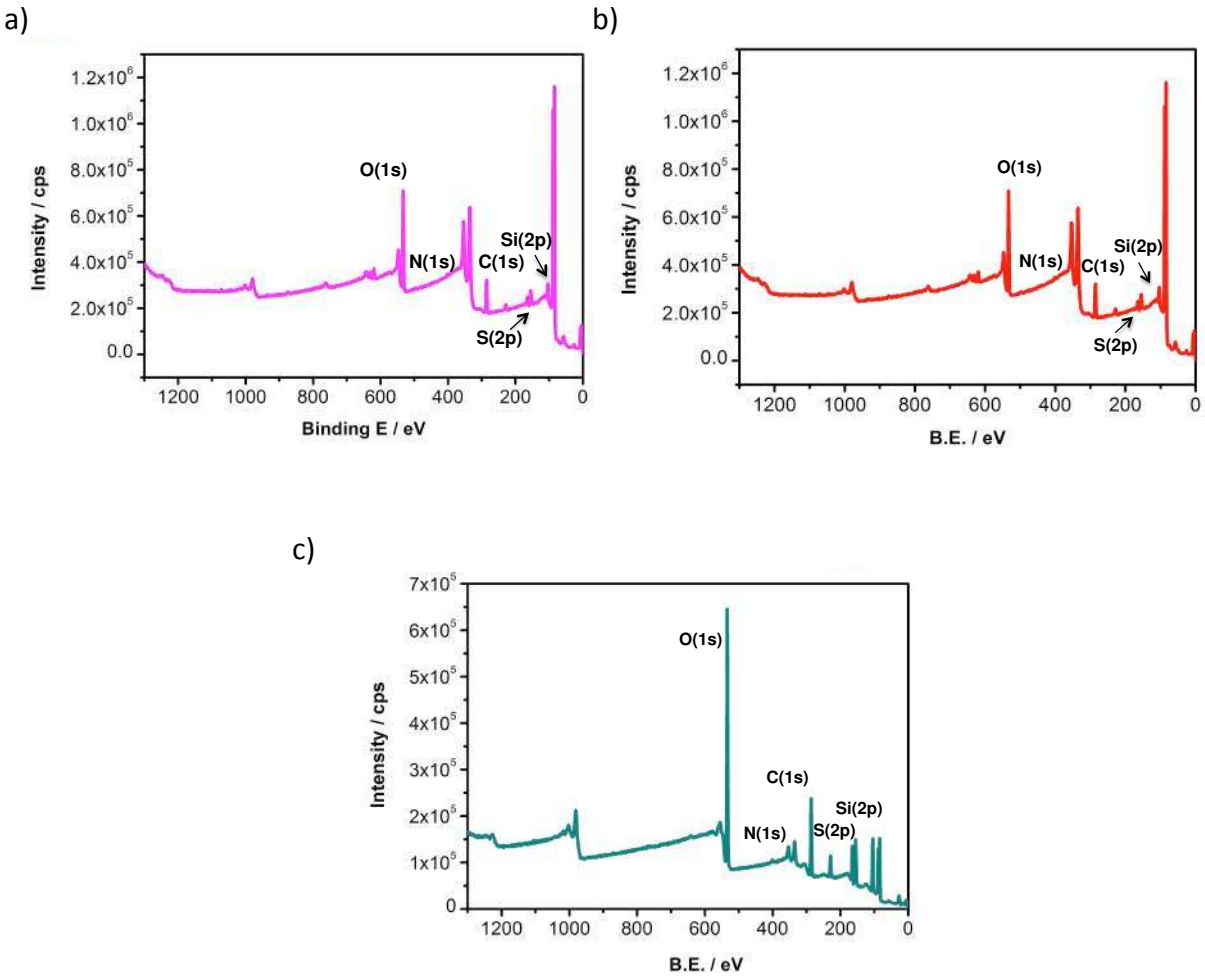


Image S7. XPS surevey spectra of: a) *ss*-**NPs**; b) **NR⊙***ss*-**NPs**; and c) **TMZ⊙***ss*-**NPs**.

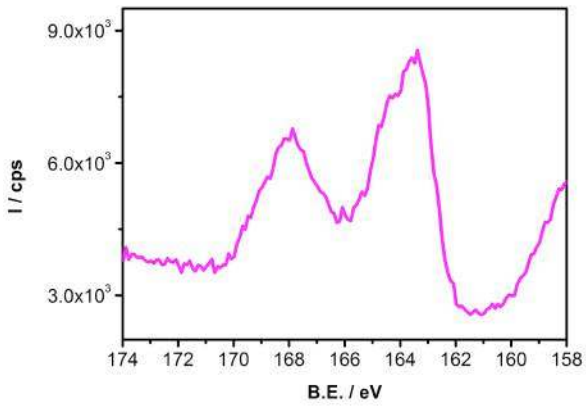


Image S8. High resolution XPS analysis of the signal of S(2p) in *ss*-**NPs**.

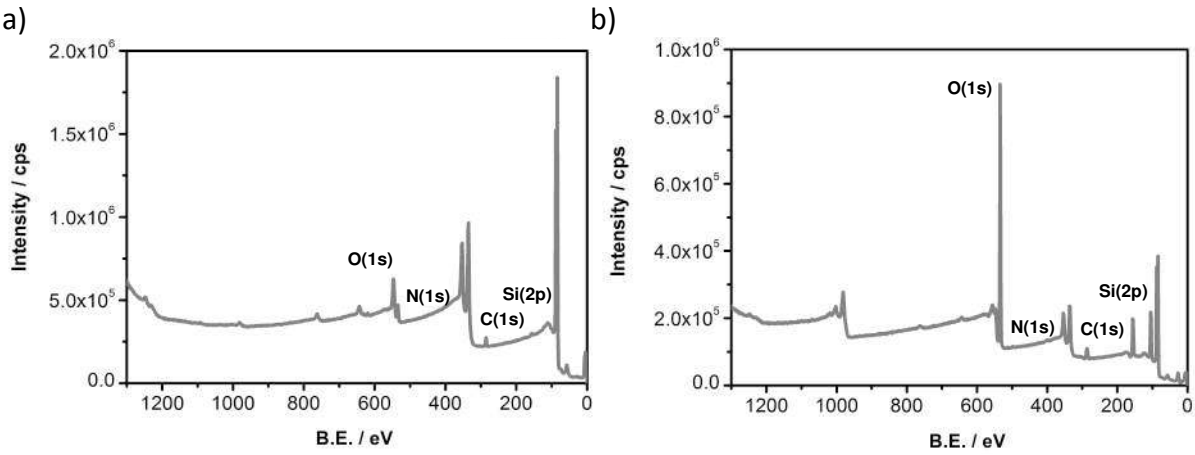


Image S9. XPS survey spectra of: a) **NPs**; and b) **TMZ⊙NPs**.

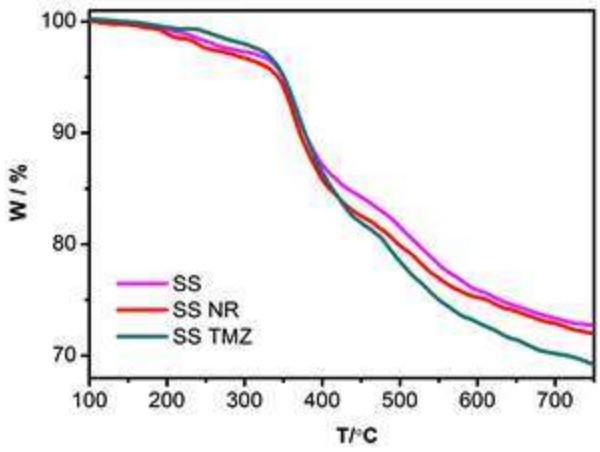


Image S10. TGA curves of: ***ss*-NPs**; **NR⊙*ss*-NPs**, and **TMZ⊙*ss*-NPs**.

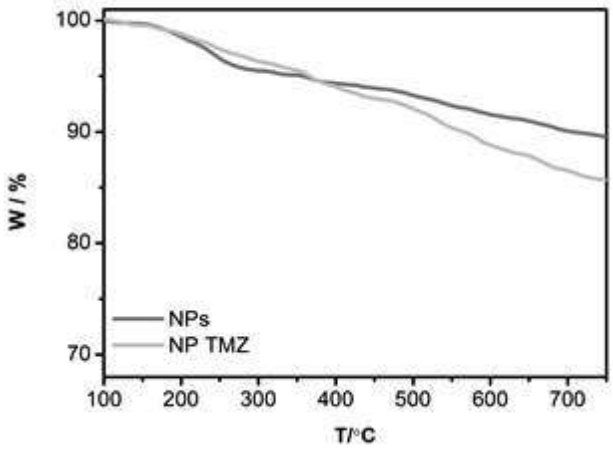


Image S11. TGA curves of: **NPs**; and **TMZ⊙NPs**.

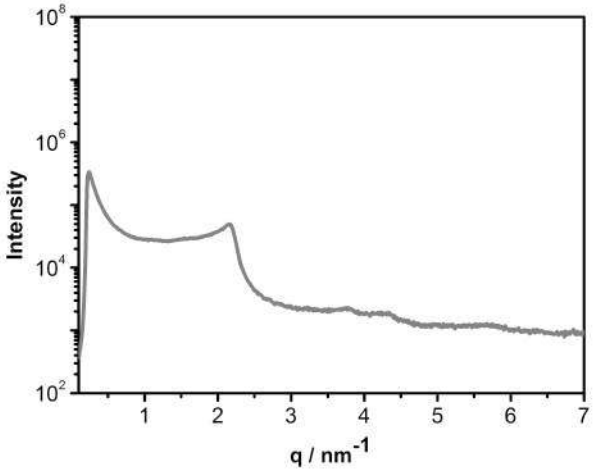


Image S12. SAXS profile of **NPs**.

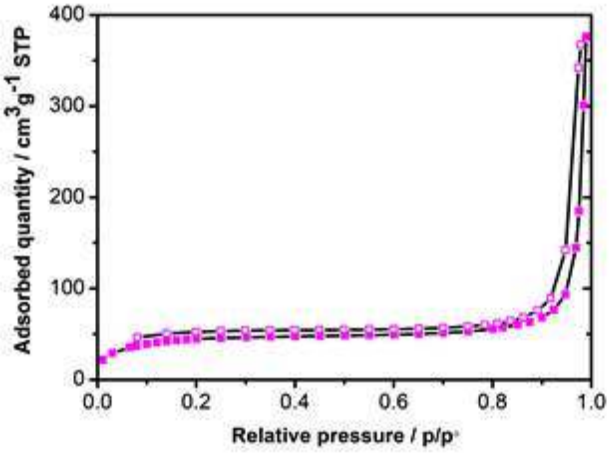


Image S13. N_2 adsorption/desorption curve of *ss*-**NPs**.

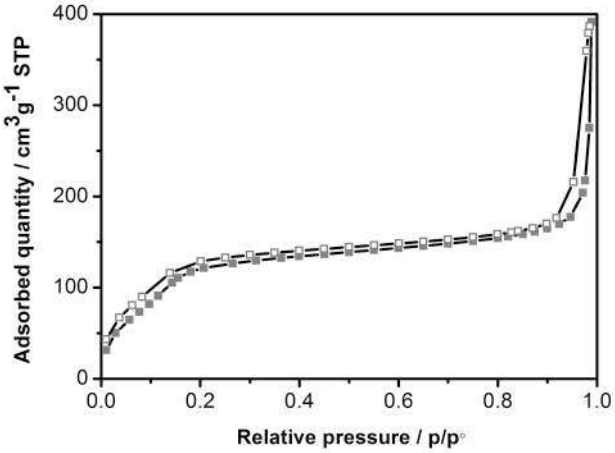
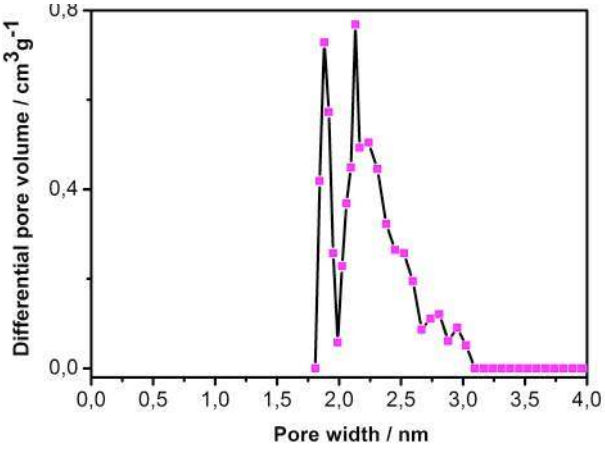


Image S14. N_2 adsorption/desorption curve of **NPs**.

a)



b)

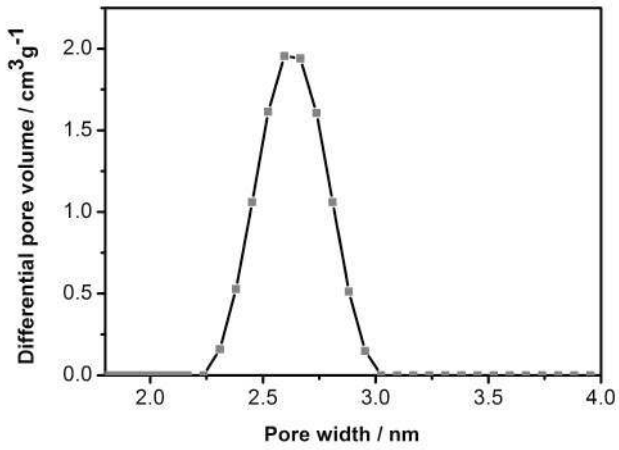
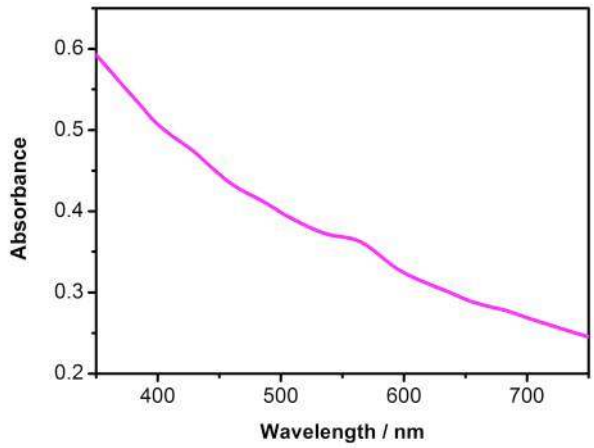


Image S15. Pore size distribution of: a) *ss*-**NPs**; and b) **NPs** obtained from N_2 adsorption analysis.

a)



b)

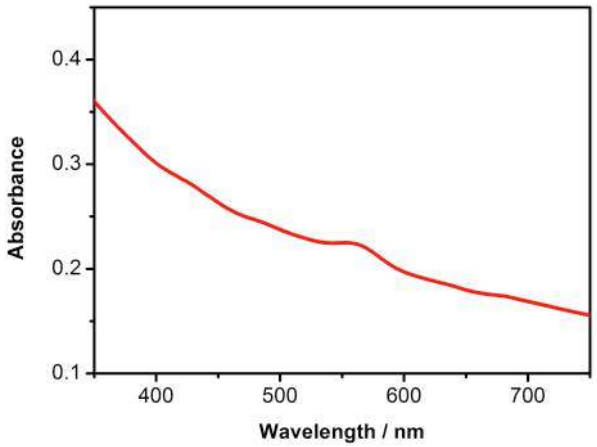
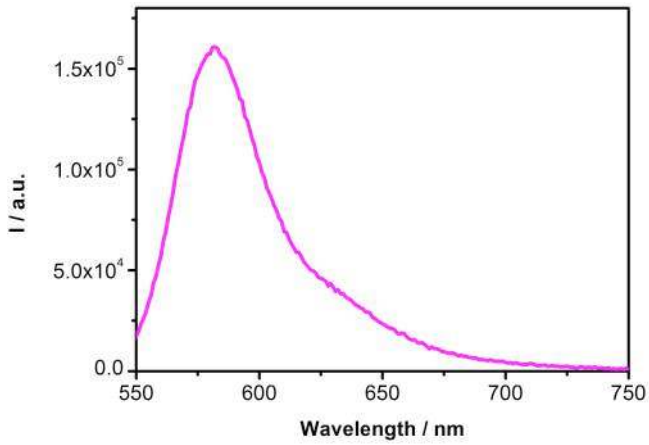


Image S16. UV-Vis spectra of: a) *ss*-**NPs**; and b) **NR⊙***ss*-**NPs** performed in DI H_2O (0.1 mg/mL).

a)



b)

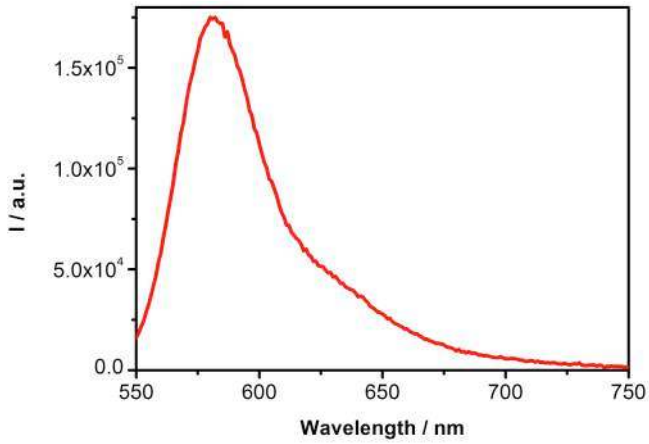
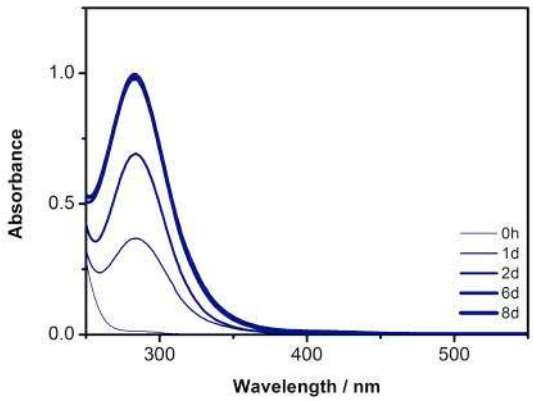
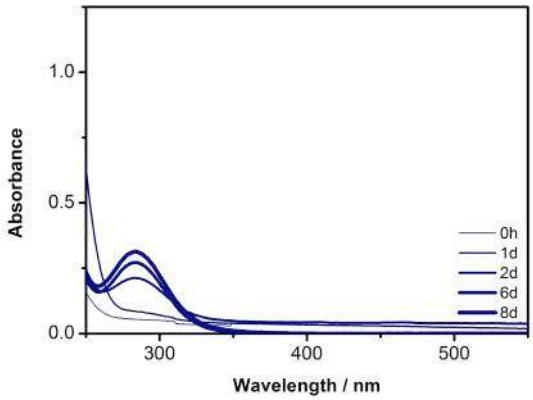


Image S17. PL spectra (l_{exc} = 500 nm) of: a) *ss*-**NPs**; and b) **NR⊙***ss*-**NPs** performed in DI H_2O (0.1 mg/mL).

a)



b)



c)

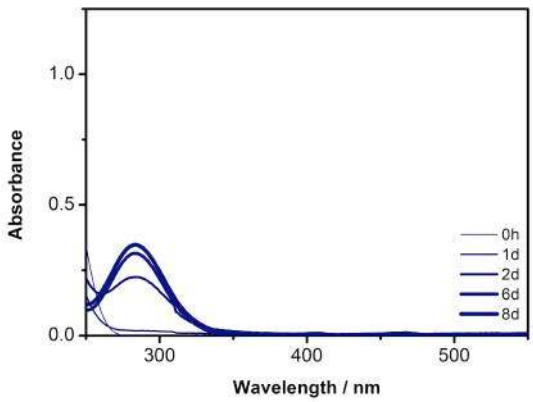


Figure S18. DTT (5 mM) titration curves for: a) *ss*-**NPs** (0.1 mg/mL, PBS); b) **NPs** (0.1 mg/mL, PBS); c) DTT.

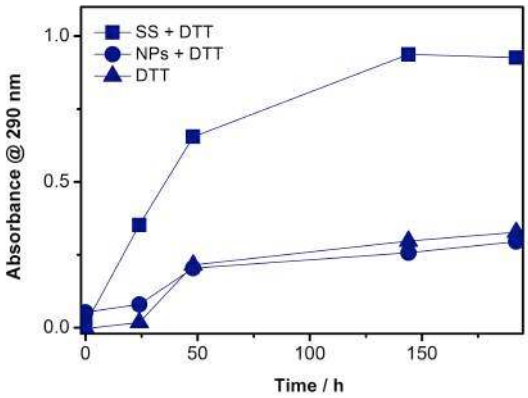


Image S19. Plot of the absorption at 290 nm taken at different time points (0- 8 d) of the cyclic oxidation product of DTT for: ***ss*-NPs** in presence of DTT 5 mM (square); **NPs** in the presence of DTT 5 mM (circle); DTT 5 mM (triangle).

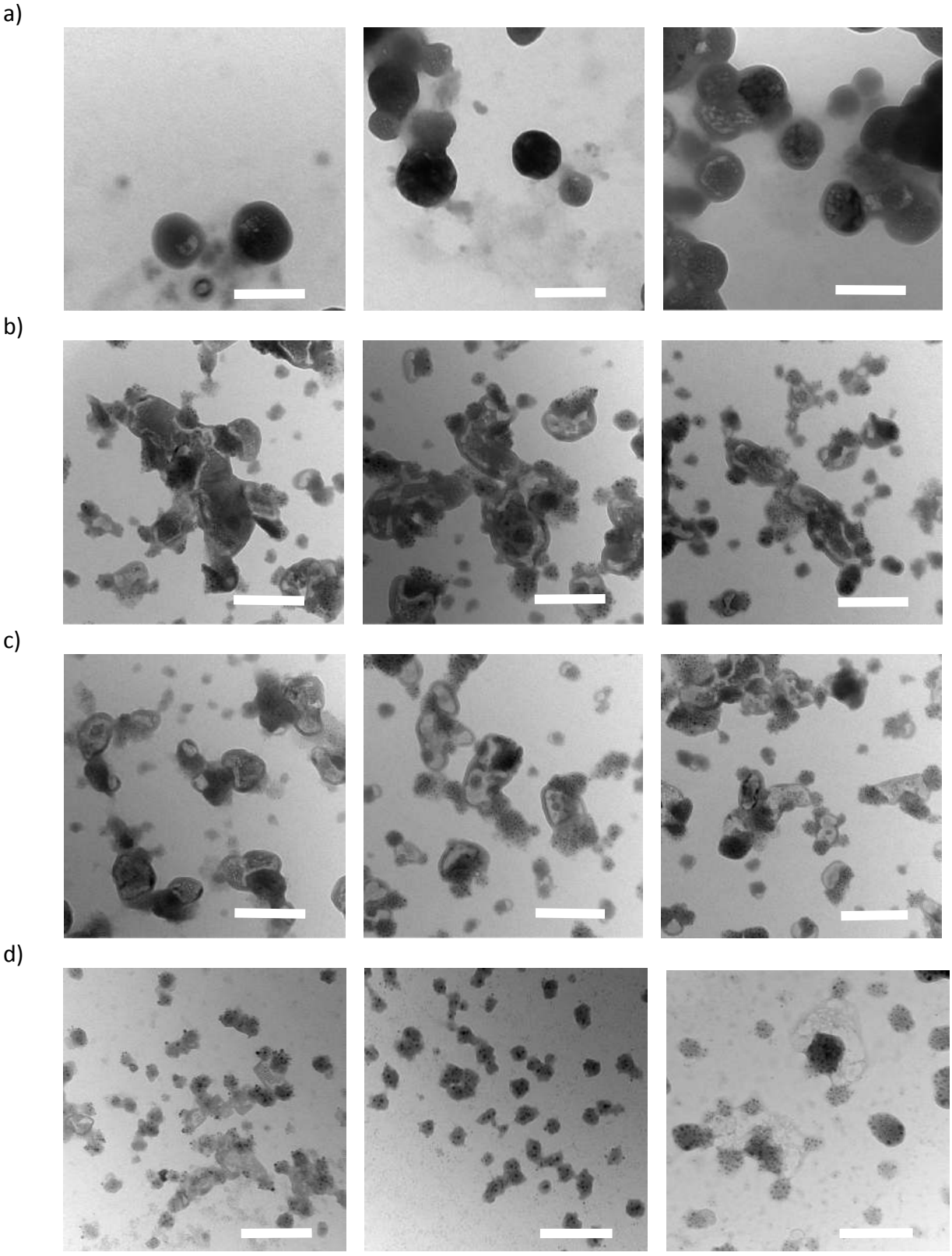


Image S20. TEM analysis of the *ss*-**NPs** stirred at 37 °C in presence of GSH 10 mM in PBS for: a) 1 d; b) 3 d; c) 5 d; and d) 7 d. Scale bar = 200 nm.

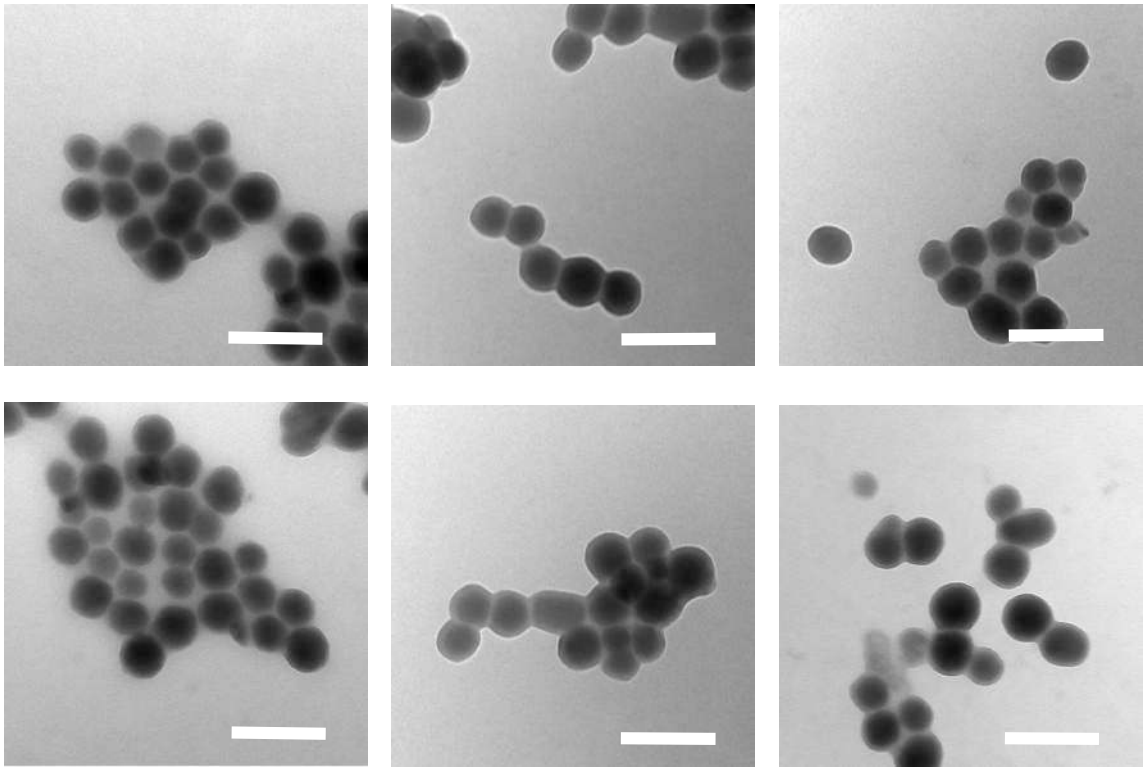


Image S21. TEM analysis of the *ss*-**NPs** stirred at 37 °C in PBS for 7 d. Scale bar = 200 nm.

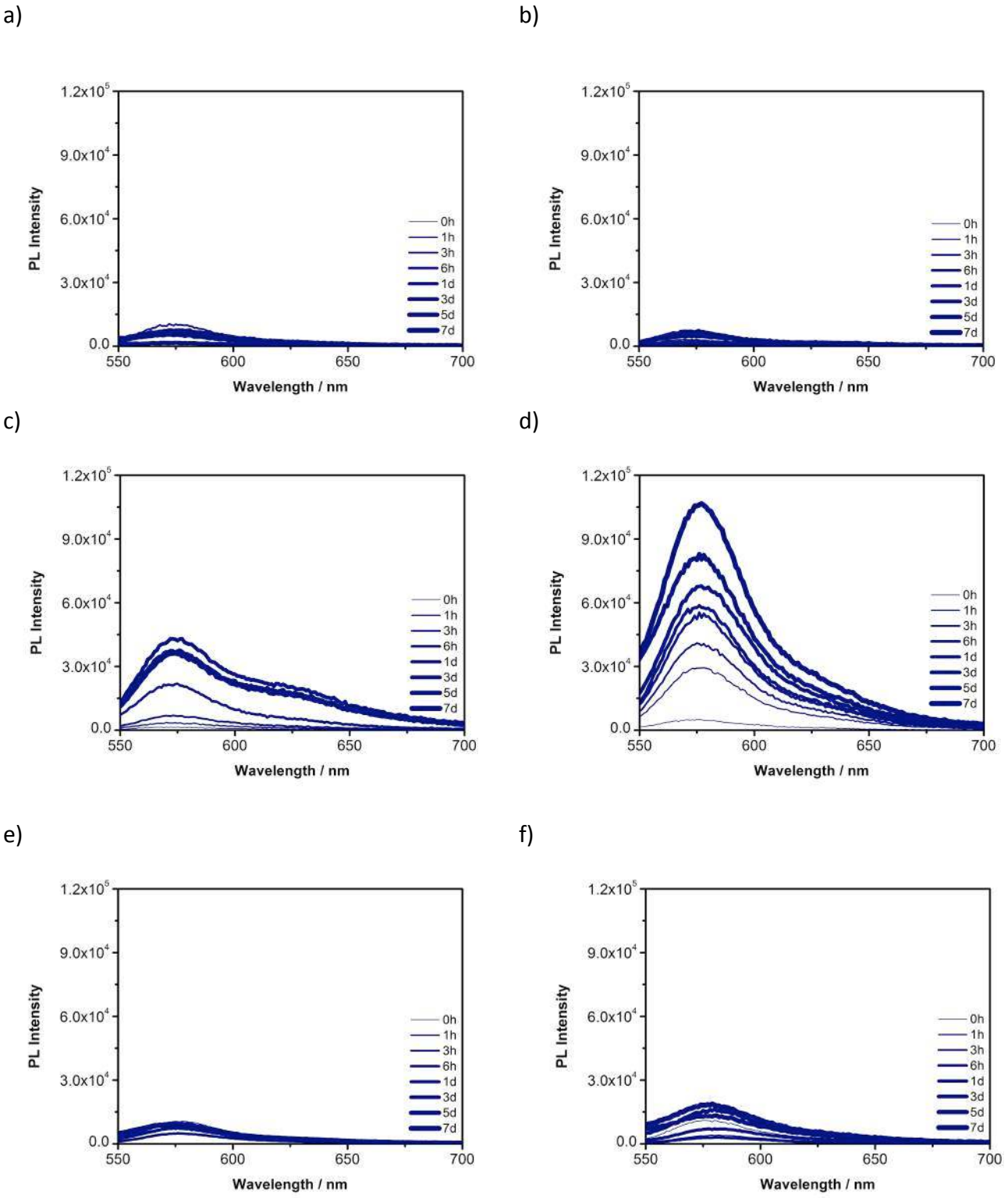
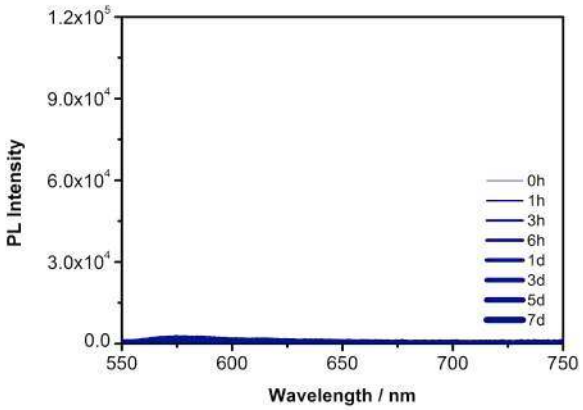
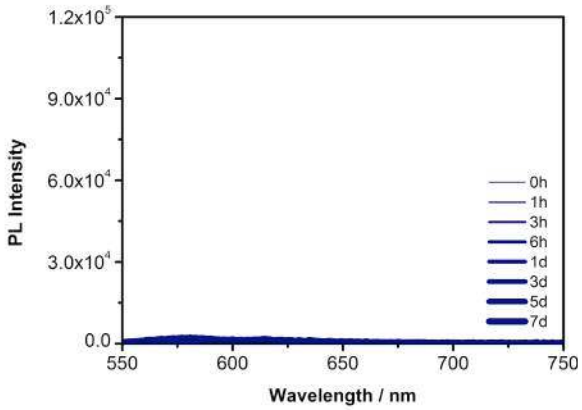


Image S22. PL emission taken at different time points of the supernatant phase of centrifuged suspensions of *ss*-**NPs** (0.1 mg/mL) stirred at 37 °C in: a) PBS, b) 2 mM, c) 5 mM and d) 10 mM PBS solution of GSH. The experiment was performed also with a suspension of **NPs** (0.1 mg/mL) dispersed in e) PBS, and f) 10 mM PBS solution of GSH. Scale bars are kept the same in all the grpahs for comparative purposes.

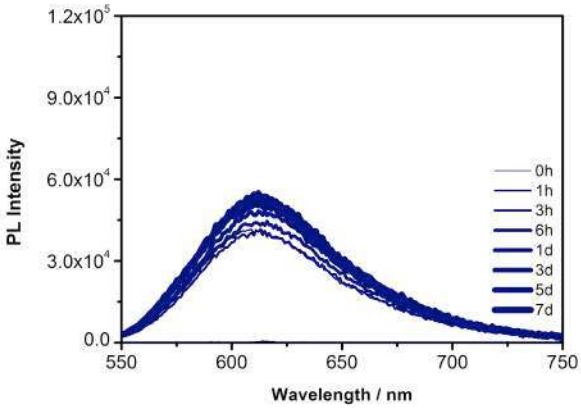
a)



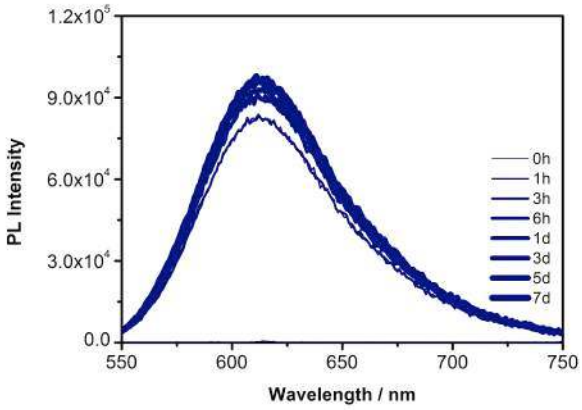
b)



c)



d)



e)

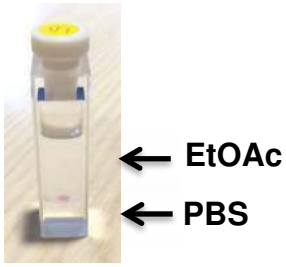


Image S23. PL emission taken at different time points of the EtOAc phase of the dye release experiment at 37 °C of **NR⊙*ss*-NPs** (0.1 mg/mL) dispersed in: a) PBS; b) 2 μM, c) 5 mM and d) 10 mM PBS solution of GSH; d) Image of a cuvette containing the PBS dispersion of **NR⊙*ss*-NPs** and the top EtOAc organic layer. After the beginning of the reaction particles tend to accumulate at the interface of the two liquid layers.

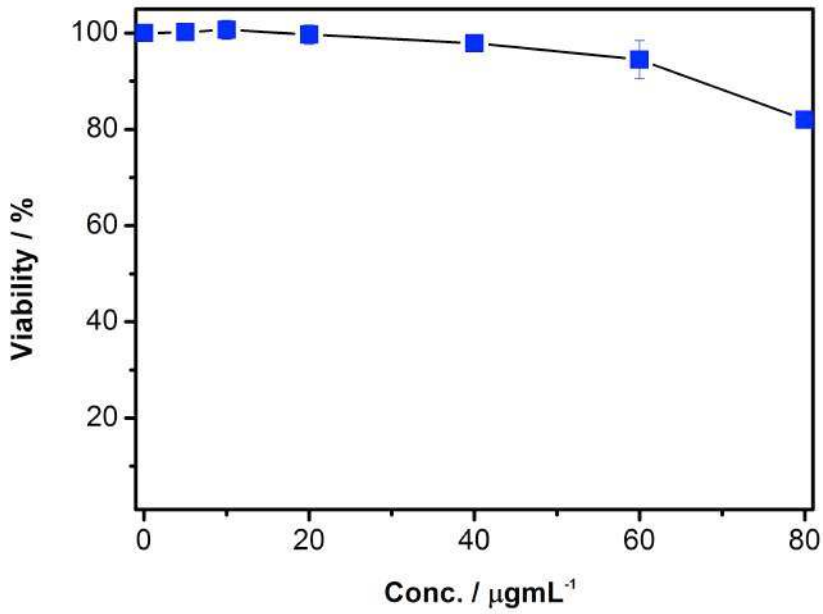


Figure S24. Cell viability of C6 Glioma cells at 48 h treated with different concentrations of *ss*-**NPs**.

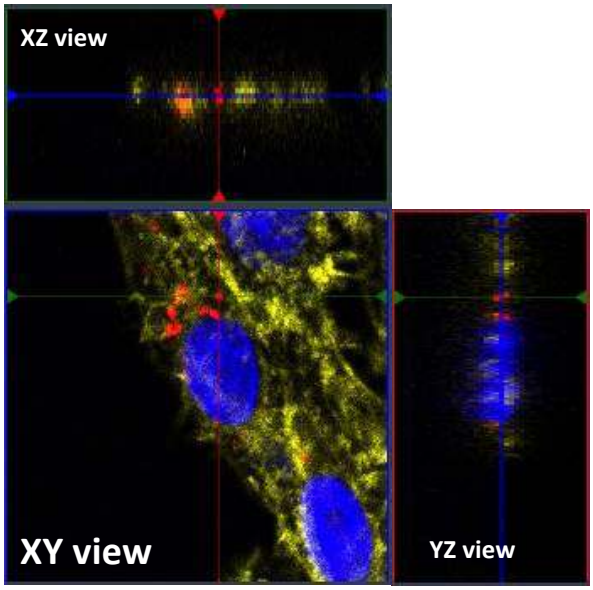


Figure S25. Orthogonal projection of the Z-stack reconstruction of Glioma C6 cells showing the presence of uptaken **NPs** (in red). The membrane of the cells was stained with Phalloidin Alexa Fluor® 647 (yellow) and the nuclei with DAPI (blue). The excitation wavelength for DAPI and RITC (contained in the framework of the particles) were 405 and 488 nm, while Phalloidin Alexa Fluor® 647 was excited at 650 nm.

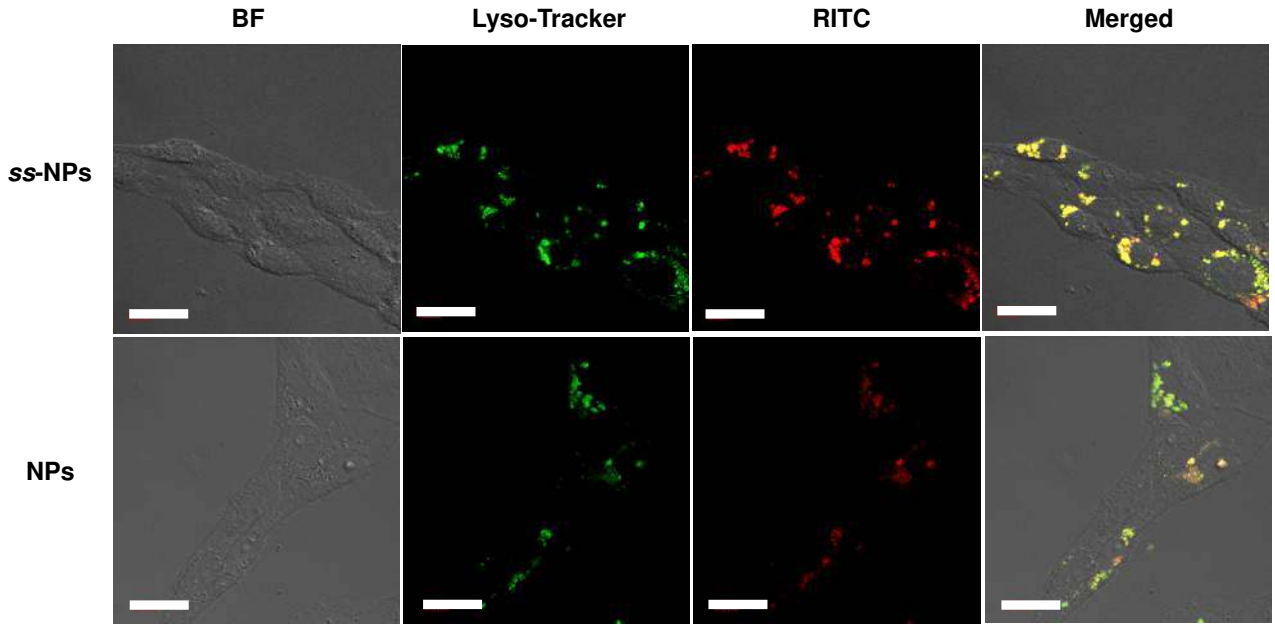


Figure S26. Co-localization of *ss*-**NPs** and **NPs** with lysosomes. The lysosomes were labeled with LysoTracker® Green (green) and the particles were followed thanks to the signal of RITC (red). The excitation wavelength for DAPI and RITC (contained in the framework of the particles) were 405 and 488 nm, while LysoTracker® Green was excited at 504 nm. The yellow areas correspond to the co-localization of the particles with the lysosomes, indicating the presence of the material in this acidic organelle. Scale bar = 20 μm.

a)

b)

c)

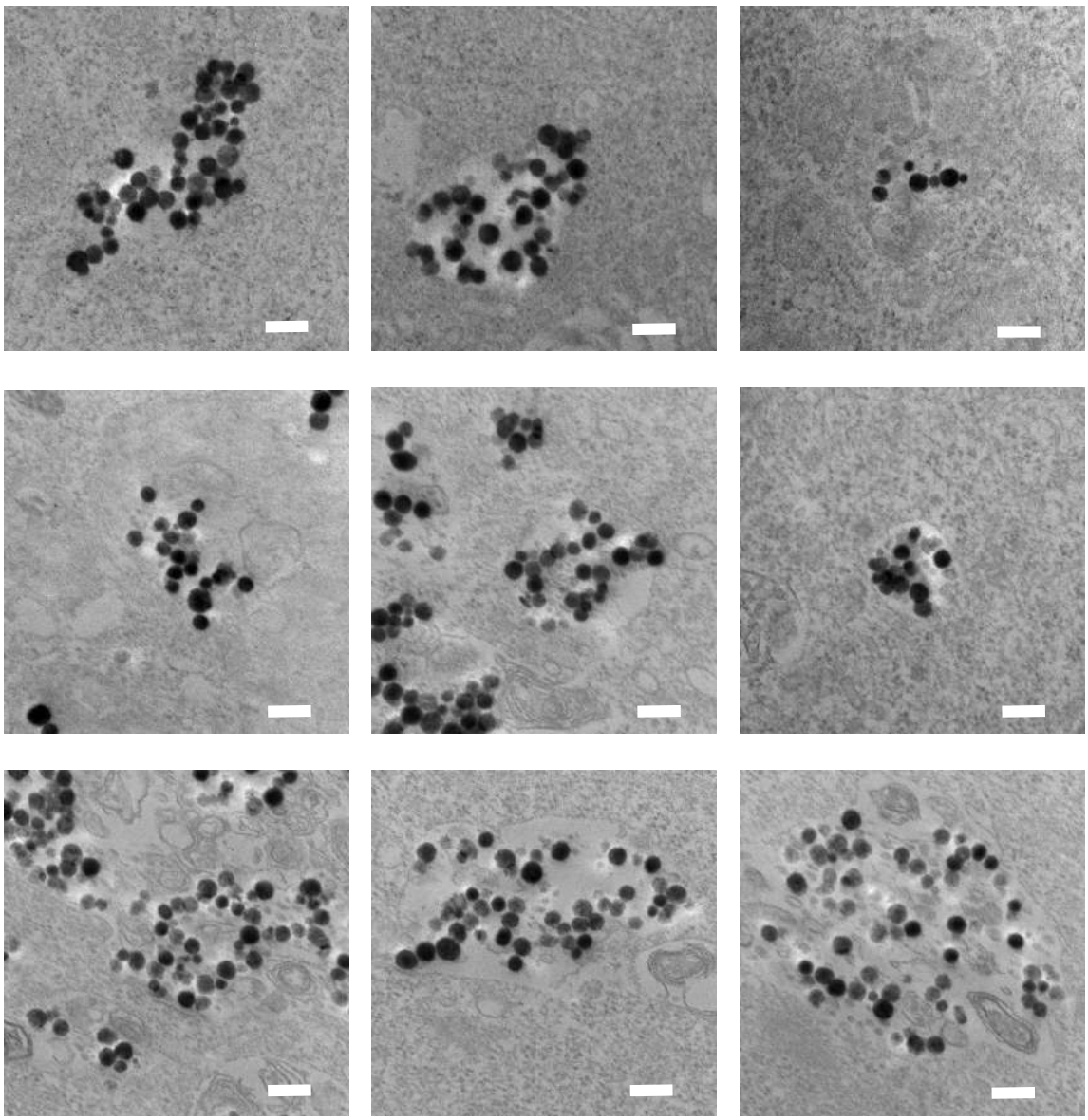


Figure S27. TEM imaging of *ss*-**NPs** in Glioma C6 cells taken: a) 3 h, b) 24 h, and c) 48 h after incubation. Scale bar = 200 nm.

a)

b)

c)

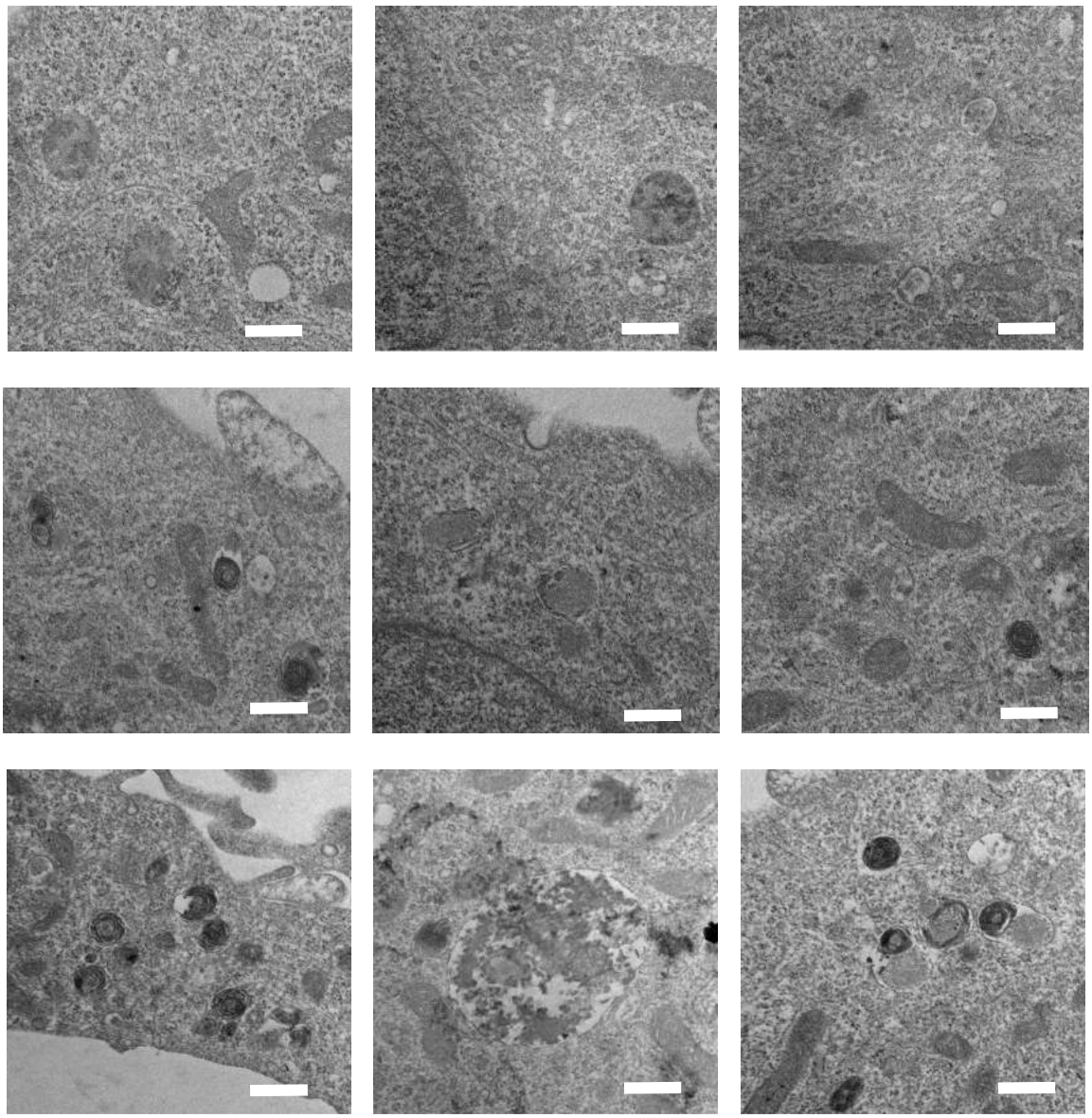
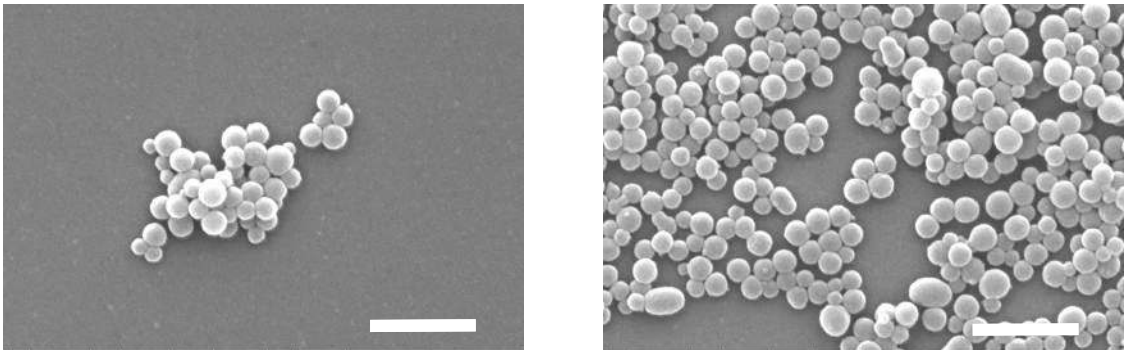


Figure S28. TEM imaging of Glioma C6 cells taken at the same time points of experiment S27: a) 3 h, b) 24 h, and c) 48 h after the adhesion and CM change. Scale bar = 500 nm.

a)



b)

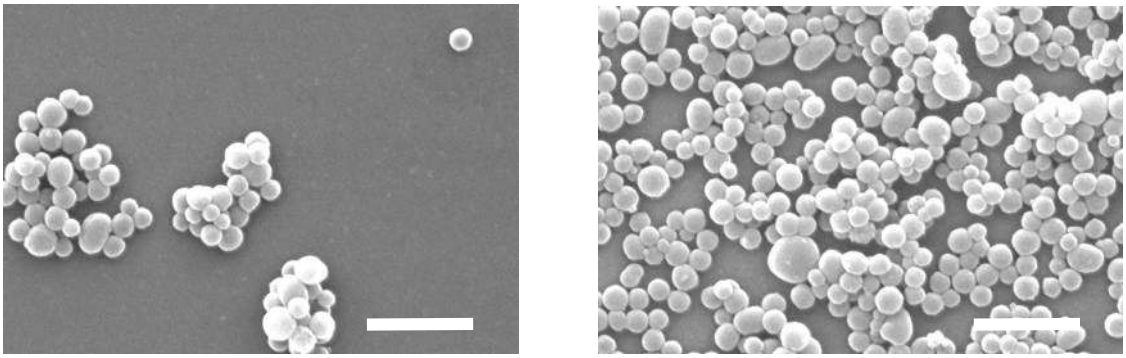


Figure S29. SEM analysis of: a) **RGD**-*ss*-**NPs**, and b) **TMZ⊙RGD**-*ss*-**NPs**. Scale bar = 500 nm.

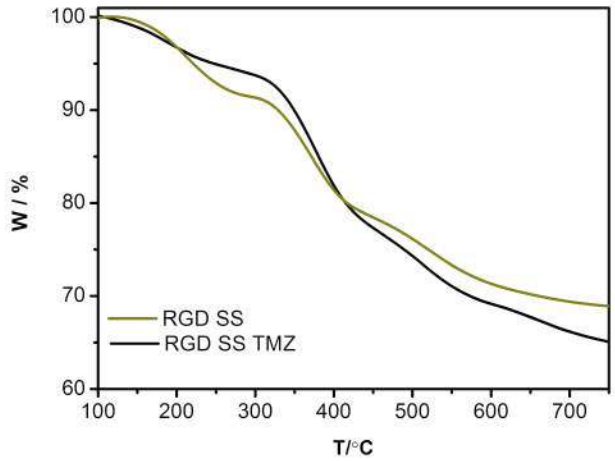


Figure S30. TGA curves of: **RGD**-*ss*-**NPs** and **TMZ⊙RGD**-*ss*-**NPs**.

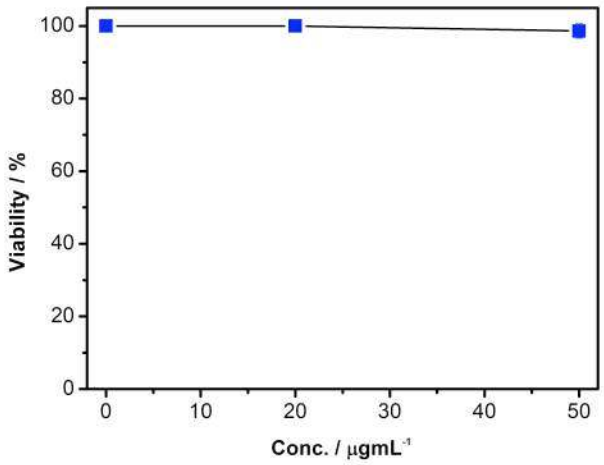


Figure S31. Cell viability of C6 Glioma cells at 48 h treated with different concentrations of **RGD**-*ss*-**NPs**.

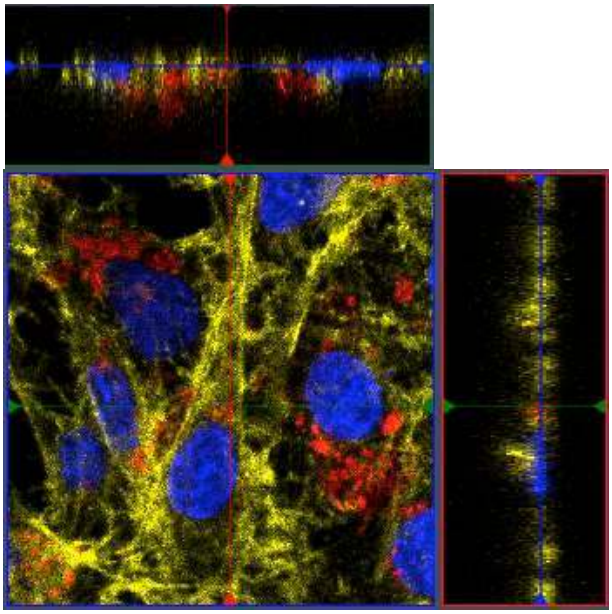


Figure S32. Orthogonal projection of the Z-stack reconstruction of Glioma C6 cells showing the presence of uptaken **RGD**-*ss*-**NPs** (in red). The membrane of the cells was stained with Phalloidin Alexa Fluor® 647 (yellow) and the nuclei with DAPI (blue). The excitation wavelength for DAPI and RITC (contained in the framework of the particles) were 405 and 488 nm, while Phalloidin Alexa Fluor® 647 was excited at 650 nm.

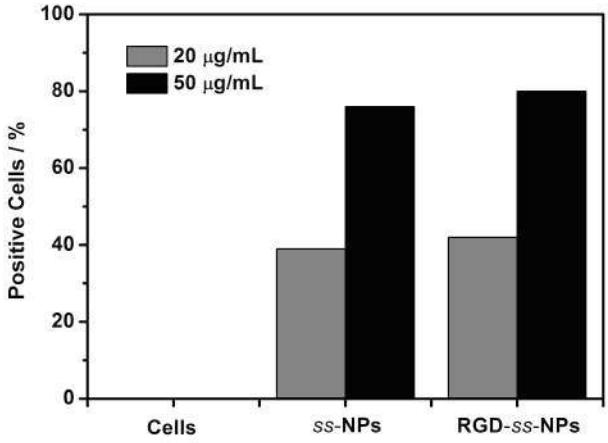


Figure S33. Flow cytometry quantification of the *ss*-**NPs** and the **RGD**-*ss*-**NPs** uptaken by the Glioma C6 cells upon 2 h contact, expressed as % of positive cells.

Table S1. Dynamic light scattering analysis results.

	DLS d_{hyd} [nm] in PBS (pH = 7.4)*	**Polydispersity index**
***ss*-NPs**	104.6 ± 26.4	0.2
NPs	118.6 ± 31.5	0.2

*The values reported are derived from the volume distribution.

Table S2. XPS results.

	XPS Atomic percentage [%]*				
	C(1s)	N(1s)	O(1s)	S(2p)	Si(2p)
***ss*-NPs**	47.9 ± 2.9	3.7 ± 0.4	10.5 ± 2.2	1.9 ± 1.3	36.0 ± 1.4
NR⊙*ss*-NPs	53.9 ± 0.9	8.3 ± 1.1	14.9 ± 1.3	2.0 ± 0.2	20.9 ± 1.9
TMZ⊙*ss*-NPs	61.7 ± 0.8	7.8 ± 1.5	16.4 ± 3.5	2.2 ± 0.8	11.9 ± 2.9
NPs	18.1 ± 2.4	1.3 ± 0.1	56.9 ± 0.8	--	23.7 ± 2.0
TMZ⊙NPs	37.5 ± 0.2	7.0 ± 0.7	38.8 ± 2.1	--	16.7 ± 1.2

*The values reported are the resulting average of three experiments.

Table S3. TGA results ***ss*-NPs** and derivatives.

	TGA weight loss [%]
***ss*-NPs**	27.4
NR⊙*ss*-NPs	28.1
TMZ⊙*ss*-NPs	30.9

Table S4. TGA results **NPs** and derivatives.

	TGA weight loss [%]
NPs	10.5
TMZ⊙NPs	14.5

Table S5. N_2 adsorption analysis results for *ss*-**NPs**.

	BET surface area [m^2 g^{-1}]	**Average pore diameter [nm]**	**Average pore volume [cm^3g^{-1}]**
ss-**NPs**	161.2	2.2	0.6

Table S6. N_2 adsorption analysis results for **NPs**.

	BET surface area [m^2 g^{-1}]	**Average pore diameter [nm]**	**Average pore volume [cm^3g^{-1}]**
NPs	479.2	2.6	0.6

Table S7. XPS results of the functionalized *ss*-**NPs**.

	XPS Atomic percentage [%]*				
	C(1s)	N(1s)	O(1s)	S(2p)	Si(2p)
RGD-*ss*-**NPs**	54.4 ± 5.9	5.9 ± 1.7	9.6 ± 1.8	2.5 ± 0.7	27.6 ± 2.9
TMZ⊙RGD-*ss*-**NPs**	65.5 ± 4.6	12.5 ± 1.6	12.9 ± 0.6	4.3 ± 1.4	4.8 ± 1.0

*The values reported are the resulting average of three experiments.

Table S8. TGA results of the functionalized **RGD**-*ss*-**NPs** and **TMZ⊙RGD**-*ss*-**NPs**.

	TGA weight loss [%]
RGD-*ss*-**NPs**	30.9
TMZ⊙RGD-*ss*-**NPs**	35.0

References

1. M. Vallet-Regi, A. Rámila, R. P. del Real and J. Pérez-Pariente, *Chem. Mater*., 2001**, 13**, 308.
2. J. Lu, M. Liong, Z. Li, J. I. Zink and F. Tamanoi, *Small,* 2010**, 6**, 1794.
3. M. B. Daniel Hora, P. Jendelova, V. Herynek, M. Trchova, Z. Pientka, E. Pollert, M. Hajek and E. Sykova, *Bioconj. Chem.,* 2007**, 18**, 635.