Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Intracellular Nitric Oxide Delivery From Stable NO-Polymeric Nanoparticle Carriers

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1. Experimental details:

Materials:

OEGMA were purchased from Sigma-Aldrich and passed through column of basic alumina prior to use to remove inhibitor. 2,2-Azobisisobutyronitrile (AIBN) was purchased from Sigma-Aldrich and crystallised from methanol and stored at 0 ^{o}C before use. The RAFT agent 4-Cyanopentanoic Acid Dithiobenzoate (CPADB) was prepared according to Mistsukami and co-workers method. All other chemicals are purchased from Sigma-Aldrich, supplied at the highest purity available unless mentioned.

Equipment:

^{1}H NMR

All NMR spectra were recorded using a Bruker 300 MHz spectrometer. All chemical shifts were reported in ppm (δ) relative to tetramethylsilane, referenced to the chemical shifts of residual solvent resonances.

Gel Permeation Chromatography (GPC)

GPC was used to determine the molecular weight and polydispersity index of the prepared polymers. The eluent was *N,N*-dimethylacetamide [DMAc; 0.03% w/v LiBr, 0.05% w/v 2, 6-dibutyl-4-methylphenol (BHT)] at 50 ^{o}C (flow rate of 1 mL min-1) with a Simadzu modular system comprising an SIL-10AD autoinjector, a Polymer Laboratories 5.0 μL bead-size guard column (50 × 7.8 mm) followed by four linear PL (Styragel) columns (105, 104, 103 and 500Å) and an RID-10A differential refractive-index detector. The GPC calibration was performed with narrow-polydispersity polystyrene standards ranging from 104 to 106 000 g/mol. A total of 50 μL of polymer solution (2 mg mL^{-1} in DMAc) was injected for every analysis.

Attentuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR)

ATR-FTIR measurements were performed using a Bruker IFS66\S Fourier transform spectrometer by averaging 128 scans with a resolution of 4 cm^{-1}.

Dynamic Light Scattering (DLS)

The average diameters and size distributions of the prepared micelles were measured by using a Malvern Zetasizer Nano Series running DTS software (laser, 4 mW, λ = 633 nm; angle 173^{o}). Samples were filtered to remove dust using microfilter 0.45 μm prior to measurements.

UV-vis. spectroscopy

UV-Vis measurements were recorded in a quartz cuvette using a CARY 300 spectrophotometer from Bruker.

Raman analysis.

Raman spectra were acquired using the Raman-Station 400 coupled with Raman Micro 300 with an excitation wave length of 785 nm from PerkinElmer LAS GmbH. Raman spectra were recorded at room temperature in the spectral region from 200 to 1000 cm^{-1} covering the S-S and C-S vibrational modes.

Transmission Electron Microscopy (TEM)

TEM micrographs were obtained using a JEOL 1400 transmission electron microscope. It was operated at an acceleration voltage of 80 kV. The samples were prepared by casting the micellar solution (1 mg mL^{-1}) onto a formvar-coated copper grid. No staining was applied.

Mass Analysis.

Electrospray-ionization mass spectrometry (ESI-MS) experiments were carried out using a Thermo Finnigan LCQ Deca ion trap mass spectrometer (Thermo Finnigan, San Jose, CA). The instrument was calibrated with caffeine, MRFA, and Ultramark 1621 (all from Aldrich) in the mass range 195-1822 Da. All spectra were acquired in positive ion mode over the mass to charge range, *m*/*z*, 100-2000 with a spray voltage of 5 kV, a capillary voltage of 44 V, and a capillary temperature of 275 °C. Nitrogen was used as sheath gas while helium was used as auxiliary gas. The sample (1mg/ml) was prepared by dissolving in a 60:40 v/v mixture of

tetrahydrofurane (THF): methanol with an acetic acid concentration of 0.4 mM. 56 Spectra were recorded in positive ion mode with an instrumental resolution of 0.1 Da. All reported molecular weights were calculated via the program package CS ChemDraw 12.0 and monoisotopic. The theoretical molecular weight over charge ratios (*m*/*z*, assuming *z*+1) were calculated using the exact molecular mass of the predominant isotope within the structure.

Methods

Synthesis of 4-Cyanopentanoic Acid Dithiobenzoate

Synthesis of Dithiobenzoic Acid (DTBA). Sodium methoxide (30% solution in methanol, 180.0 g, 1.0 mol) was added to a thoroughly dried 1 L, three-necked round-bottomed flask equipped with a magnetic stir bar, addition funnel (250 mL), thermometer, and rubber septum for liquid transfers. Anhydrous methanol (250 mL) was added to the flask via a cannula, followed by rapid addition of elemental sulfur (32 g, 1.0 mol). Benzyl chloride (63 g, 0.5 mol) was then added dropwise via the addition funnel over a period of 1 h, at room temperature under a dry nitrogen atmosphere. The reaction mixture was heated in an oil bath at 67 °C for 10 h. After this time, the reaction mixture was cooled to 0 °C using an ice bath. The precipitated salt was removed by filtration and the solvent was removed in vacuum. Deionized water (500 mL) was added to the residue. The solution was filtered a second time and then transferred to a 2 L extraction funnel. The crude sodium dithiobenzoate solution was washed with diethyl ether (3 × 200 mL). Diethyl ether (200 mL) and 1.0 N HCl (500 mL) were added, and dithiobenzoic acid was extracted into the ethereal layer. Deionized water (300 mL) and 1.0 N NaOH (600 mL) were added, and sodium dithiobenzoate was extracted to the aqueous layer. This washing process was repeated two more times to finally yield a solution of sodium dithiobenzoate.

Synthesis of Di(thiobenzoyl) Disulfide. Potassium ferricyanide(III) (32.93 g, 0.1 mol) was dissolved in deionized water (500 mL). Sodium dithiobenzoate solution (350 mL) was transferred to a 1 L conical flask equipped with a magnetic stir bar. Potassium ferricyanide solution was added dropwise to the sodium dithiobenzoate *via* an addition funnel over a period of 1 h under vigorous stirring. The red precipitate was filtered and washed with deionized water until the washings became colorless. The solid was dried in vacuum at room temperature overnight.

Synthesis of 4-Cyanopentanoic Acid Dithiobenzoate. Distilled ethyl acetate (80 mL) was added to a 250 mL round-bottomed flask. Dry 4,4-azobis(4-cyanopentanoic acid) (5.84 g,

21.0 mmol) and di(thiobenzoyl) disulfide (4.25 g, 14.0 mmol) were added to the flask. The reaction solution was heated at 75 for 14 h. The ethyl acetate was removed in vacuum. The crude product was isolated by column chromatography (silicagel 60 Å, 70-230 mesh) using ethyl acetate:hexane (2:3) as eluent. Fractions that were red in color were combined and dried over anhydrous sodium sulfate overnight. The solvent mixture was removed in vacuum, and the red oily residue placed in a freezer at -20 °C, whereupon it crystallized. The target compound was recrystallized from benzene.

^{1}H-NMR confirmed the structure expected. ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm from TMS): 2.1 (3H, s, C$\underline{H_3}$), 2.5 (2H, dt, -C$\underline{H_2}$-CH_2CO_2H), 2.7 (2H, t, -C$\underline{H_2}$-COOH), 7.2 (2H, aromatic group), 7.6 (1H, aromatic group), 7.8 ppm (2H, aromatic group).

^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm from TMS): 224, 175, 145, 135, 130, 128, 126, 120, 45, 35, 32, 25.

Synthesis of 2-vinyl-4,4-dimethylazlactone (VDM)

VDM was synthesized according to Fontaine, Pascual and co-workers.[1] A brief description is given.

Synthesis of *N*-acryloyl-2-methylalanine. To a solution of 1.768 g (4.42×10^{-2} mol) of sodium hydroxide in 4.4 mL of water, cooled to 0 °C using an ice bath, are slowly added 2 g (1.94×10^{-2} mol) of 2-methylalanine and 2.0 mg (9.07×10^{-6} mol) of 2,6-di-*tert*-butyl-*p*-cresol. When the solution is homogeneous, 2 g (2.21×10^{-2} mol) of acryloyl chloride were added dropwise under stirring and keeping the temperature at 0 °C using an ice bath. After complete addition of acryloyl chloride, the stirring is continued for 3 h. To this solution was slowly added 2.3 mL of concentrated hydrochloric acid. During the addition a white solid is formed. The reaction solution was stirred for 30 min. The solid was filtered and was recrystallized from a mixture of ethanol and water (1/1 in volume). The white solid was filtered and dried under vacuum. Yield: 60%. mp 201–202 °C. ^{1}H NMR (DMSO d_6), δ in ppm: 1.36 (s, $C(CH_3)_2$); 5.56 (dd, H_{trans}, $J_{Htrans–Hcis}$ = 2.1 Hz, $J_{Htrans–Hgem}$ = 10.1 Hz); 6.08 (dd, H_{cis}, $J_{Hcis–Htrans}$ = 2.1 Hz, $J_{Hcis–Hgem}$ = 17.1 Hz); 6.25 (dd, H_{gem}, $J_{Hgem–Htrans}$ = 10.1 Hz, $J_{Hgem–Hcis}$ = 17.1 Hz); 8.29 (s, NH–); 12.20 (s, COOH).

Synthesis of VDM. 0.4 g (2.54×10^{-3} mol) of *N*-acryloyl-2-methylalanine and 0.38 g (3.75×10^{-3} mol) of triethylamine were added to 10 mL of acetone. The resulting solution was cooled at 0 °C using an ice bath and 0.277 g (2.5×10^{-3} mol) of ethyl chloroformate was added dropwise. The solution was stirred for 3 h at 0 °C. The solution was then filtered and

the white solid was washed with acetone. The filtrate was concentrated under vacuum and the filtrate residue was distilled under vacuum (bp 47 °C at 4 mmHg) to give a colourless oil. Yield: 49%. FTIR , ν in cm^{-1}: 1820; 1670; 1651; 1070. ^{1}H NMR ($CDCl_3$), δ in ppm: 1.47 (s, $C(CH_3)_2$); 5.96 (dd, H_{trans}, $J_{Htrans–Hcis}$ = 2.0 Hz, $J_{Htrans–Hgem}$ = 9.9 Hz); 6.23 (dd, H_{cis}, $J_{Hcis–Htrans}$ = 2.0 Hz, $J_{Hcis–Hgem}$ = 17.6 Hz); 6.27 (dd, H_{gem}, $J_{Hgem–Htrans}$ = 9.9 Hz, $J_{Hgem–Hcis}$ = 17.6 Hz).^{13}C NMR ($CDCl_3$), δ in ppm: 24.50 ($C(CH_3)_2$); 64.61 ($C(CH_3)_2$); 123.88 (CH_2=CH); 128.81 (CH_2=CH); 158.92 (C=N); 180.53 (C=O).

Synthesis of POEGMA MacroCTA

$[OEG\text{-}MA]_0$:$[CPADB]_0$:$[AIBN]_0$ = 100.0:1.0:0.1. $OEG\text{-}MA_{300}$ (2.3 g, 7.66×10^{-3} mol), CPADB (0.0214 g, 7.66×10^{-5} mol), AIBN (1.35 mg, 7.62×10^{-6} mol) and toluene (20 mL) were prepared in a round bottom flask (50 mL), equipped with a magnetic stirrer bar. The reaction mixture was degassed with nitrogen for 30 minutes. The degassed solution was immersed in a pre-heated oil bath at 70 ^{o}C for 17 h. The reaction was then placed in an ice bath for about 15 min to terminate polymerisation and two aliquot were sampled for GPC and ^{1}H NMR analyses. The monomer conversion was determined by ^{1}H NMR analysis to be 67%. The reaction medium was precipitated in cold petroleum spirit (boiling range of 40-60 ^{o}C) and centrifuged (7000 rpm for 5 mins). The precipitation and centrifugation steps were repeated three times to remove any traces of unreacted monomer and then the reaction medium was dried in vacuum oven. POEGMA was analysed by ^{1}H NMR and GPC. The molecular weight determined by GPC is 21,000 g/mol (M_n (theoretical) = 20,000 g/mol at 67% OEGMA conversion) and PDI = 1.12.

Synthesis of POEGMA-b-PVDM

$[POEG\text{-}MA]_0$:$[VDM]_0$:$[AIBN]_0$ = 1:144:0.25. POEG-MA (M_n = 20,000 g/mol) (1.0 g, 5×10^{-5} mol), VDM (1.0 g, 7.3×10^{-3} mol) and AIBN (2 mg, 1.25×10^{-5} mol) were dissolved in 5 ml of toluene in a round bottom flask (10 mL), equipped with a magnetic stirrer bar. The reaction mixture was degassed with nitrogen for 45 minutes. The degassed solution was immersed in a pre-heated oil bath at 70 ^{o}C for 14 hours. The reaction was then placed in an ice bath for about 15 minutes to terminate the polymerisation and two aliquot were collected for GPC and ^{1}H NMR analyses. VDM conversion was determined by ^{1}H NMR analysis and is equal 60%. The reaction medium was precipitated in diethyl ether and centrifuged (7000 rpm for 5 mins). The purification process was repeated three times and the reaction medium was dried in vacuum oven. The reaction was followed by ATR-FTIR to check the presence of azlactone functionality in the block copolymer (signal at 1820 cm^{-1}). The copolymers were

analysed by GPC, UV-vis and ^{1}H NMR spectroscopy. The molecular weight (determined by GPC) is 33,000 g/mol (M_n (theoretical) = 32,000 g/mol at 60% VDM conversion) and PDI = 1.28.

Removal of thiocarbonylthio end group from the polymeric chain

The removal of RAFT end group was performed according to Perrier and co-workers' method.[2] POEGMA-*b*-PVDM (1.0 g, 3.3×10^{-5} mol), AIBN (0.112 g, 6.9×10^{-4} mol L-1) and toluene (6 mL) were added to a round bottom flask, equipped with a magnetic stirrer bar ([POEGMA-*b*-PVDM]:[AIBN] = 1:20). The reaction mixture was degassed with nitrogen for 20 minutes. The degassed solution was immersed in a pre-heated oil bath at 80 ^{o}C for 3 hours. The reaction was then precipitated in diethyl ether and centrifuged. The purification process was repeated three times and the copolymer was dried in oven under vacuum. The samples were analysed by ATR-FTIR to confirm the presence of azlactone groups after reaction at 1820 cm^{-1}. Aliquots were sampled for GPC, ^{1}H NMR and UV-Vis analyses. UV-Vis confirms the disappearance of RAFT agent.

Synthesis of GSNO

GSH (308 mg, 1×10^{-3} mol), HCl (0.1 mL, 32%) and water (3 mL) were prepared in a vial. The glass vial was put in an ice bath and covered with aluminium foil because GSNO is sensitive to light. Then, $NaNO_2$ (70 mg) was slowly poured into the bottle and the reaction was left for 30 minutes. The reaction was purified by precipitation twice in acetone: water mixture (4:1 v:v), twice in acetone and finally, in diethyl ether. Then, GSNO was dried in vacuum oven. Approximately 200 mg of pink solid is obtained (yield = 60%). Mass spectroscopy was performed to confirm the synthesis of GSNO. Signal at 347.15 Da (attributed to GSNO/H^+) and at 369.13 (attributed GSNO/Na^+) are observed.

Conjugation of GSNO to VDM

All the reactions with GSNO were performed in the dark to avoid the degradation of GSNO by the light. The amount of triethylamine should be relatively low to avoid the degradation of GSNO compound.

2.5 mg of VDM (1.8×10^{-5} mol) was diluted in DMSO (50 µL). 10 mg of GSNO (2.98×10^{-5} mol) was also dissolved in DMSO (50 µL) in the presence of triethylamine (5 µL, 5×10^{-5} mol). GSNO solution was added to the block copolymer solution. Then, the reaction mixture was covered with aluminium foil and was left reacted at 30 ^{o}C for 5 hours. The resulting

reaction was monitored by mass spectroscopy, ATR-FTIR spectroscopy and ^{1}H NMR analysis. Mass spectroscopy shows the presence of new signal at 475.15 Da (GSNO-VDM/H^{+}) and at 497.13 (GSNO-VDM/Na^{+}).

Conjugation of GSNO to block copolymer

100 mg of block copolymer was dissolved in DMSO (500 µL). 50 mg of GSNO (14.9×10^{-5} mol) was also dissolved in DMSO (250 µL) in the presence of triethylamine (5 µL, 5×10^{-5} mol)GSNO solution was added to the block copolymer solution. Then, the reaction mixture was covered with aluminium foil and was left reacted at 30°C for 5 hours. The resulting reaction was examined with ATR-FTIR spectroscopy to check the successful conjugation.

Synthesis of nitric oxide encapsulated micelles

Equipped with a magnetic stirrer bar, the conjugated block copolymer with GSNO (100 mg) was dissolved in DMSO (500 µL) under moderate stirring at room temperature. Water (5 mL) was added at a very slow rate, and then the reaction was dialysed for 24 hours protected by aluminium foil to avoid the degradation of GSNO by the light. The water was changed every 3 hours for 12 hours. The reaction was then sampled for dynamic light scattering (DLS), transmission electron microscopy (TEM), and UV-VIS spectrometry analysis.

Cell culture.

The human neuroblastoma cell line BE(2)-C and normal fetal lung fibroblasts MRC5 were grown in Dulbecco's Modified Eagle's Medium: Nutrient Mix F-12 (DMEM) supplemented with 10% (v/v) Fetal Calf Serum (FCS) in a ventilated tissue culture flask T-75. The cells were incubated at 37 °C in a 5% CO_2 humidified atmosphere and passaged every 2-3 days when monolayers at around 80% confluence were formed. Cell density was determined by counting the number of viable cells using a trypan blue dye (Sigma-Aldrich) exclusion test. For passaging and plating, cells were detached using 0.05% trypsin-EDTA (Invitrogen), stained using trypan blue dye, and loaded on the hemocytometer.

Cell viability.

The cytotoxicity of cisplatin was tested *in-vitro* by a standard cell viability Alamar Blue Assay. The assay is based on the ability of living cells to convert blue redox dye (resazurin) into bright red resorufin which can be read in a spectrophometric reader.[3] The intensity of the color is proportional to the cell viability. The cells were seeded at 2500 cells/well for

BE(2)-C and 5,000 cells/well for MRC5 in 96 well tissue culture plates and incubated for 24 h. Prior to exposure to cells with cisplatin, BE(2)-C and MRC5 cells were pre-treated with nanoparticles containing GSNO (final concentration 0.5 mM) for 8 h at 37 ^{o}C. Following the treatment of cells with NO donor, cells were rinsed and fresh medium containing cisplatin with concentration range of 0.1 – 300 μM was added. At 72 h post drug incubation, treatments were removed and fresh media was added (100 μL) followed by the addition of Alamar Blue dye (20 μL) to each well and the cells were incubated for 6 h and followed by spectrophotometric analysis. Cell viability was determined as a percentage of untreated control cells, and IC_{50} values were calculated via regression analysis using OriginPro 8 software.

The toxicity of NO functionalized nanoparticles was studied using MRC-5 and BE(2)-C cells. BE(2)-C and MRC-5 cells were incubated with NO functionalized nanoparticles (0.5 mM of NO) for 8 h at 37 ^{o}C, and then, the cells were rinsed with phosphate buffer. Finally, the cells were incubated for 72 h. The cell viability was determined by Alamar Blue Assay using the same method described with the treatment with cisplatin. The results are presented in **Table S2**.

Determination of NO release using Griess reagent kit

NO released from the nanoparticles at predetermined time intervals was quantified by the standard Griess reagent kit (G7921), which is widely used for nitrite determination. As NO is readily oxidized to nitrite and nitrate, nitrates that have been reduced to nitrites using nitrate reductase. Briefly, Griess reagent (100 μl) was mixed with 300 μL of the nitrite-containing sample and 2.6 mL of deionized water and incubated for 30 minutes at room temperature. Nitrite concentrations in the samples should fall within the linear range of the assay (approximately 1–100 μM). UV-vis. absorbance of the resulting solution was determined at 540 nm, and the total nitrite concentration in the sample solution was calculated from a standard curve and converted to cumulative NO release.

DAF-FM detection of nitric oxide in neuroblastoma cells

For this purpose, BE(2)-C neuroblastoma cells (5000 cells/well) were plated into 4 well Lab Tek^{TM} chamber slides pre-coated with poly-D-lysine hydrobromide (Sigma-Aldrich) for 10 minutes. After 4 days, cells were loaded with the probe by replacing media with DMEM containing the 5 μM DAF-FM DA and incubated at 37 ^{o}C for 20 mins. The cells were then

washed three times with media to remove excess probe and then incubated with fresh media for 15 mins to complete de-esterification of the intracellular diacetates. The GSNO nanoparticles (250 μM) were added to BE(2)-C loaded with DAF-FM for 2 h in the presence of ascorbic acid (5 mM) for the more efficient NO release. Upon the completion of NO donor treatment, cells were then rinsed with PBS and fixed in 4% paraformaldehyde for 10 min at room temperature. Following fixation, cell plasma membrane was stained with Alexa Fluor 594 wheat germ agglutinin (5.0 μg/mL) for 10 minutes at room temperature. When labelling is complete, the labelling solution was removed and cells were washed twice in PBS solution. The slides were then left to air dry briefly, prior to adding ProLong® Gold Antifade Reagent with DAPI mounting media and covering with a coverslip. The slide was stored in the dark overnight and then sealed with nail polish. Slides were stored at 4 ^{o}C until required for imaging using confocal laser scanning microscopy (CLSM). CLSM images of cells were captured using a confocal microscope (Zeiss LSM 780) with a 40× glycerol immersion objective (1.2 numerical apertures). Released nitric oxide upon reaction with DAF-FM, Alexa Fluor® 594 wheat germ agglutinin, DAPI was detected at an emission/excitation maxima of 495/515nm, 591/618 nm, 350/461 nm respectively. All the experiments were done in triplicate.

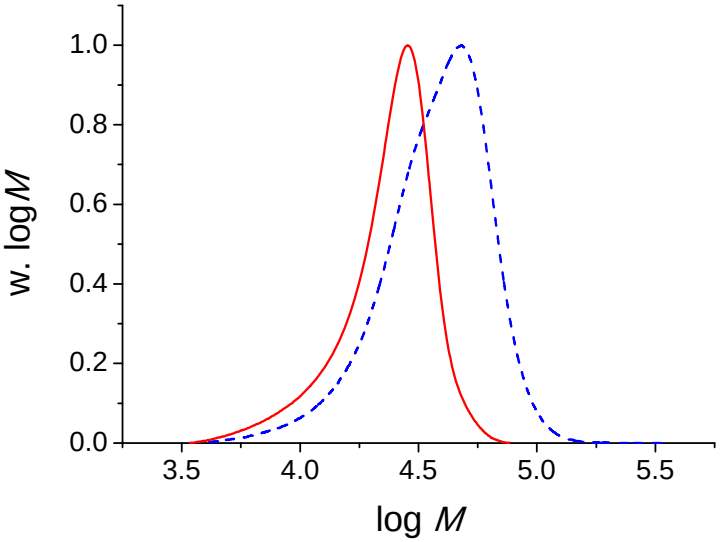


Figure S1. GPC chromatograms of POEGMA (red line) and POEGMA-*b*-VDM copolymers (blue dash line). DMAC was used as eluent.

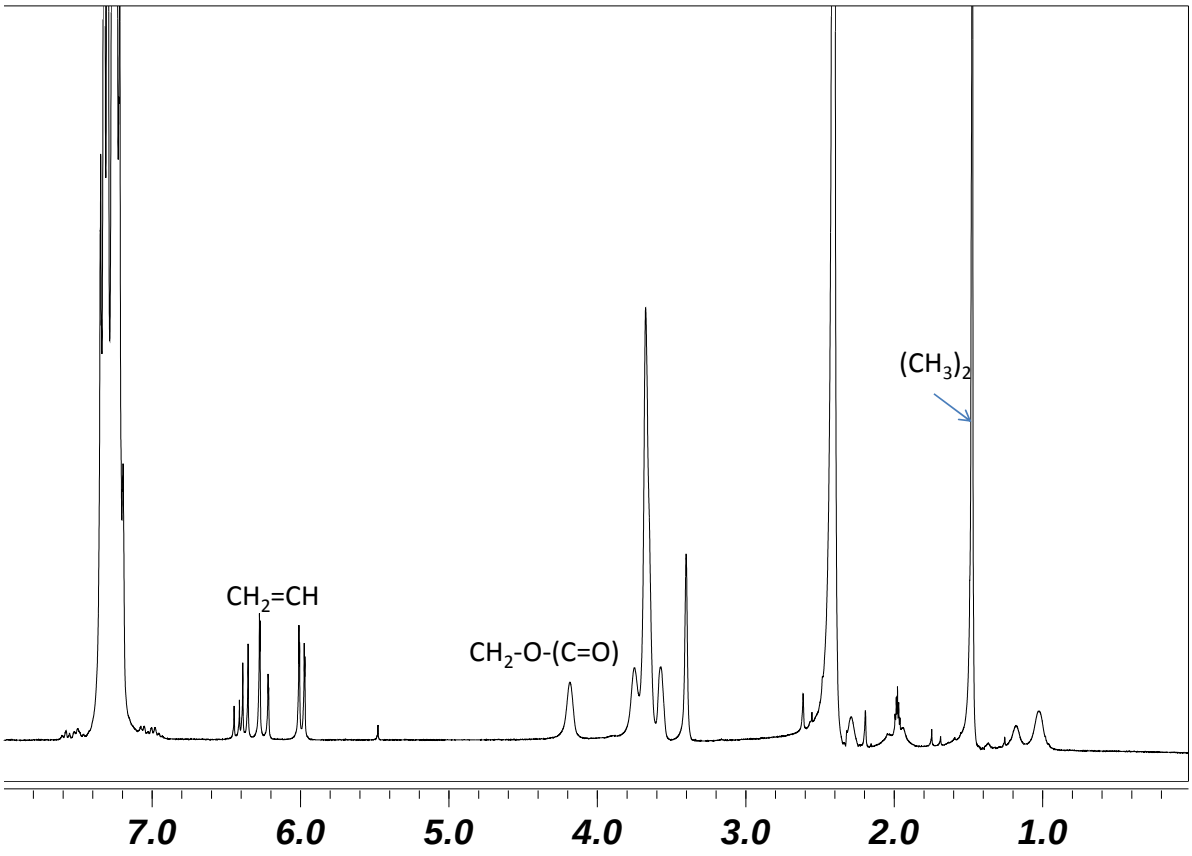


Figure S2. ^{1}H NMR spectrum of crude POEGMA-*b*-VDM copolymers, recorded in DMSO deuterated (300 MHz).

Comment: ^{1}H NMR spectroscopy was used to determine the VDM conversion using the following equation:

$$\textit{VDM conversion (\%)} = [1 - (I^{6.0\text{-}6.6ppm}/3)/(I^{1.4ppm}/6)] \times 100$$

Where, $I^{6.0\text{-}6.6ppm}$ and $I^{1.4ppm}$ correspond to intensity of vinylic bond between 6.0-6.6 ppm and of methyl of VDM at 1.4 ppm, respectively.

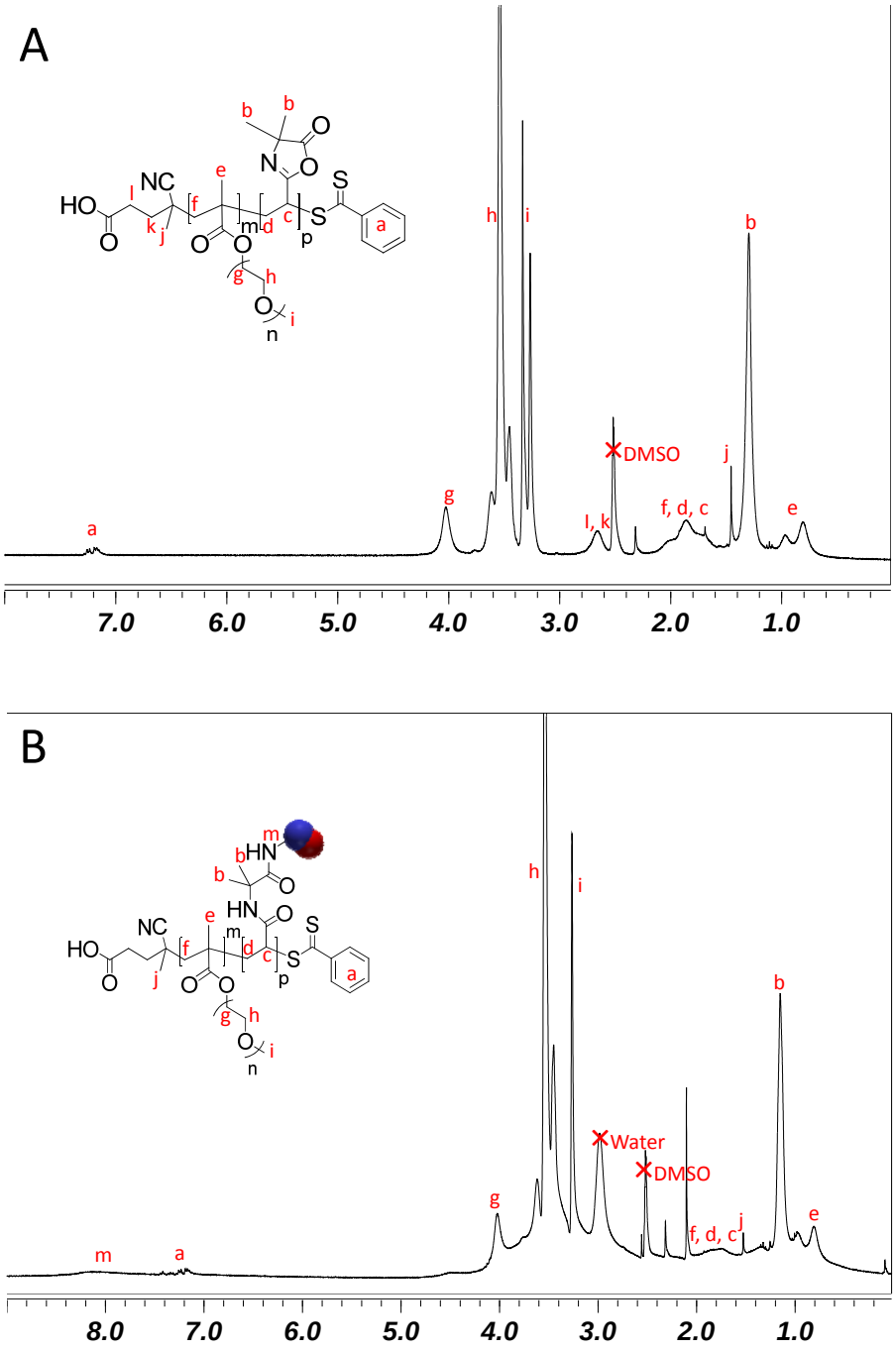


Figure S3. ^{1}H NMR spectra of (A) POEGMA-*b*-VDM copolymers and (B) POEGMA-*b*-VDM after attachment of GSNO. Recorded in DMSO (300 MHz).

Note: Molecular weight of POEGMA-*b*-VDM calculated by ^{1}H NMR spectroscopy according to the following:

$$M_n^{POEGMA} = [(I^{4.1ppm}/2)/(I^{7.2ppm}/5)] \times MW^{OEGMA} + [(I^{1.4ppm}/6)/(I^{7.2ppm}/5)] \times MW^{VDM} + MW^{RAFT\ agent}$$

Where, $I^{1.4ppm}$, $I^{4.1ppm,}$ and $I^{7.2ppm}$ correspond to intensity of methyl of VDM at 1.4 ppm, ester bond of POEGMA and aromatic group at 7.2 ppm, respectively.

Comment: the successful conjugation of GSNO to the polymer was confirmed by the shift of VDM signal from 1.5 to 1.4 ppm, and by the apparition of signals at 8.0 ppm and 2.1 ppm attributed to N-H amide and CH_2 of glutathione, respectively.

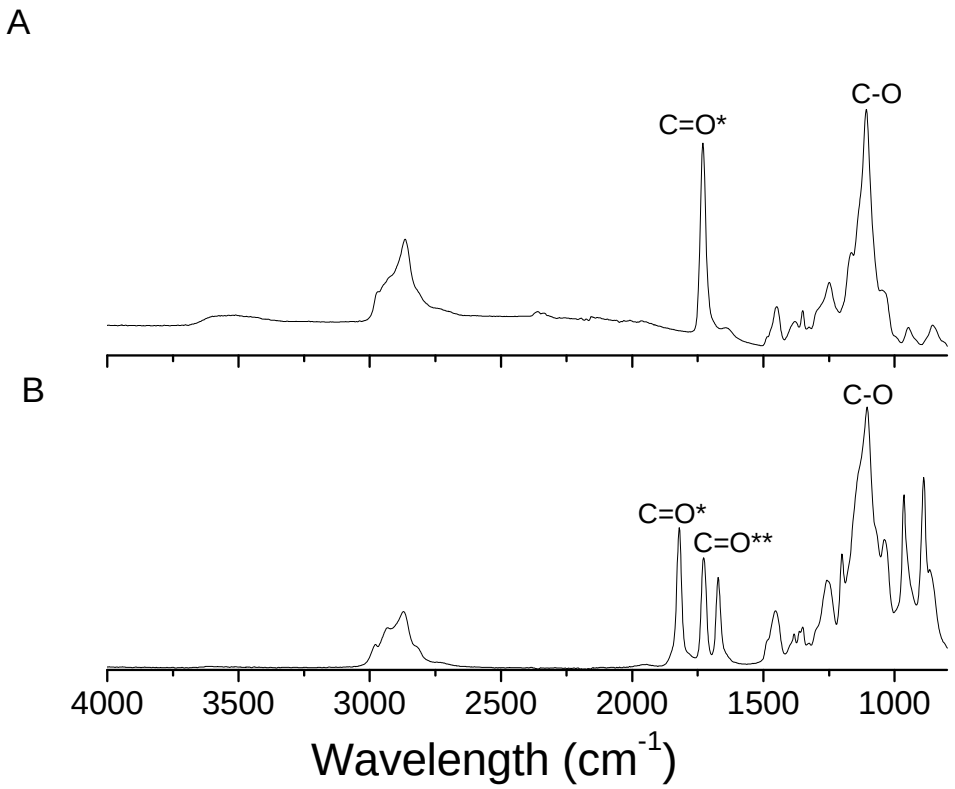


Figure S4. ATR-FTIR spectra of A- purified POEGMA homopolymers and B- purified POEGMA-*b*-VDM copolymers.

Note: C=O* and C=O** correspond to C=O ester bond of OEGMA (at 1730 cm^{-1}) and C=O bond of VDM (at 1820 cm^{-1}), respectively. C-O corresponds to ether C-O of OEGMA.

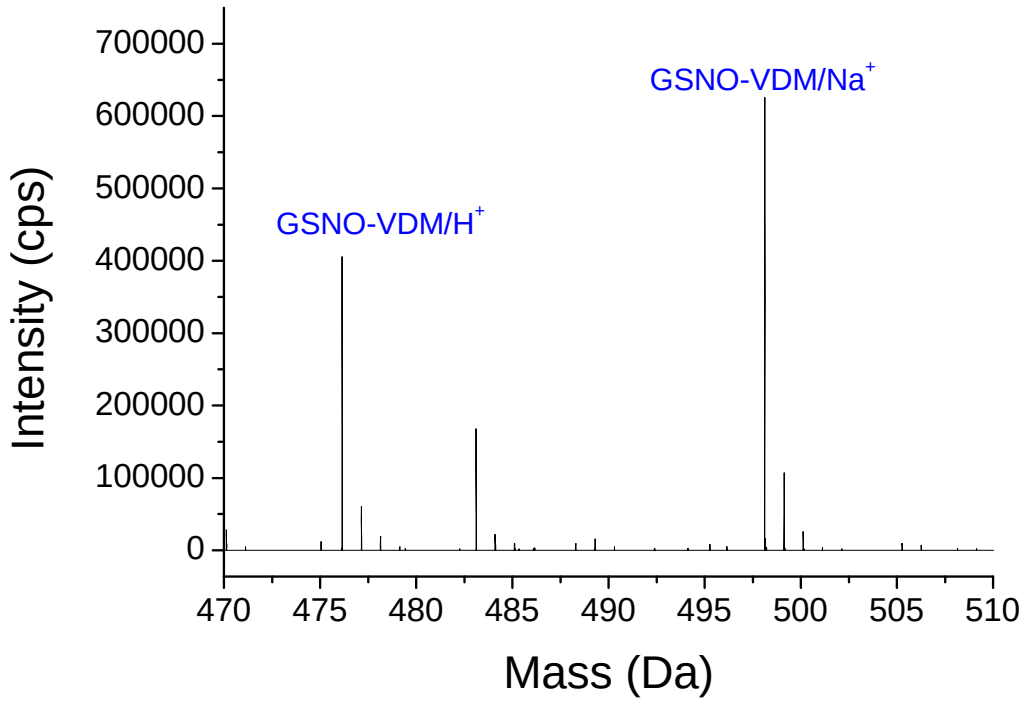


Figure S5. ESI spectrum of GSNO conjugated to VDM.

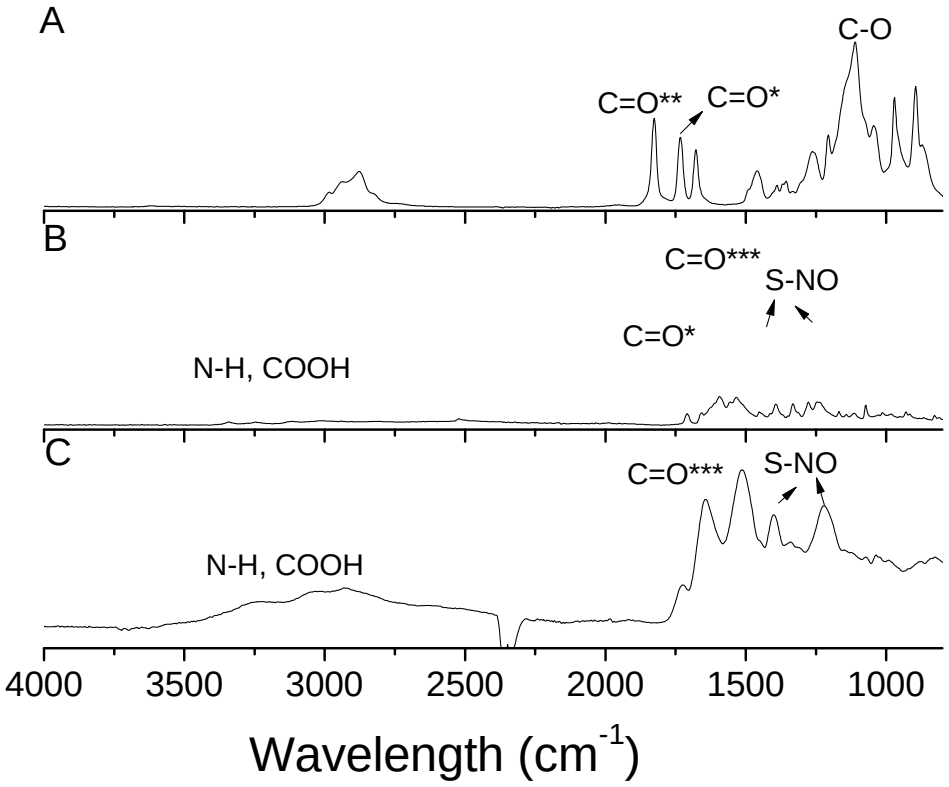


Figure S6. ATR-FTIR spectra of A- purified POEGMA-*b*-VDM copolymers, B- purified POEGMA-*b*-VDM copolymers after reaction with GSNO and C- GSNO.

Note: C=O*, C=O** and C=O*** correspond to C=O ester bond of OEGMA, C=O bond of VDM and C=O amide bond (after reaction of VDM with GSNO), respectively. C-O corresponds to ether C-O of OEGMA.

Comment: The successful conjugation of GSNO to polymeric nanoparticles is indicated by the presence of S-NO absorption signals at 1250 and 1460 cm^{-1}. In addition, the absence of azlactone absorption peak at 1820 cm^{-1} and the presence of strong signal at 1650 cm^{-1} attributed to C=O amide also confirm the attachment of GSNO to the polymers.

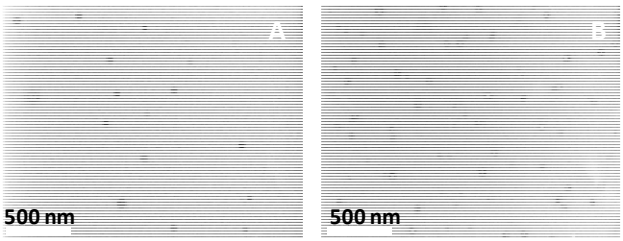


Figure S7. TEM pictures NO nanoparticles: (A) before and (B) after attachment of NO-donors to the polymeric nanoparticles.

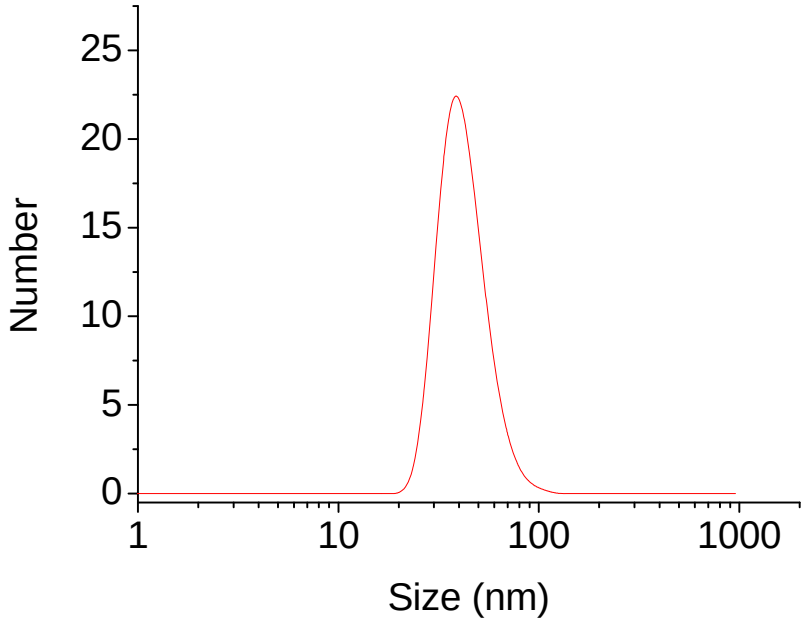


Figure S8. Number distribution of purified POEGMA-*b*-VDM copolymers in aqueous solution determined by dynamic light scattering.

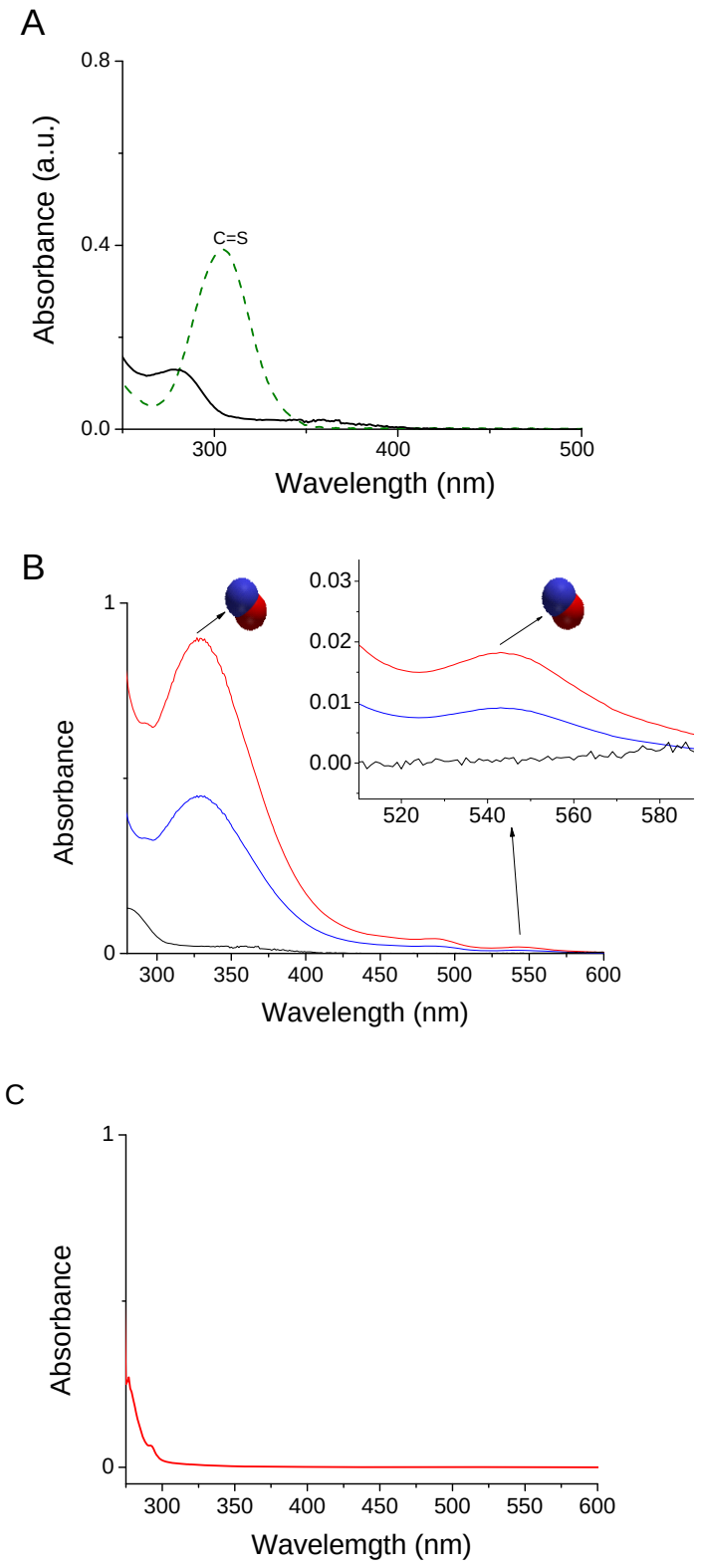
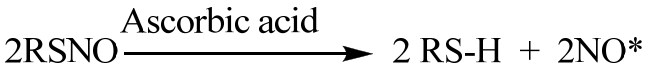


Figure S9. UV-vis. spectra: (A) of (green dash line) purified POEGMA-*b*-VDM copolymers before removal of RAFT end group and (full line) purified POEGMA-*b*-VDM copolymers after removal of RAFT agent using Perrier and co-workers' method;[2] (B) of (red line) of

GSNO in water, (blue line) GSNO conjugated with copolymers and (black line) no functional polymers; (C) GSH functionalized nanoparticles.

Comment: GSNO concentration has been calculated according the following equation: $[GSNO] = (Abs^{340} / (\varepsilon^{340} \times l)) \times 100$, *with* Abs^{340}, ε^{340} *and l correspond to the absorbance at 544 nm, extension coefficient of GSNO (850.0* $M^{-1}cm^{-1}$*) and pathlength of the cuvette.*[4]



Scheme S1. Mechanism of NO release of nitrosothio- compounds in the presence of ascorbic acid.

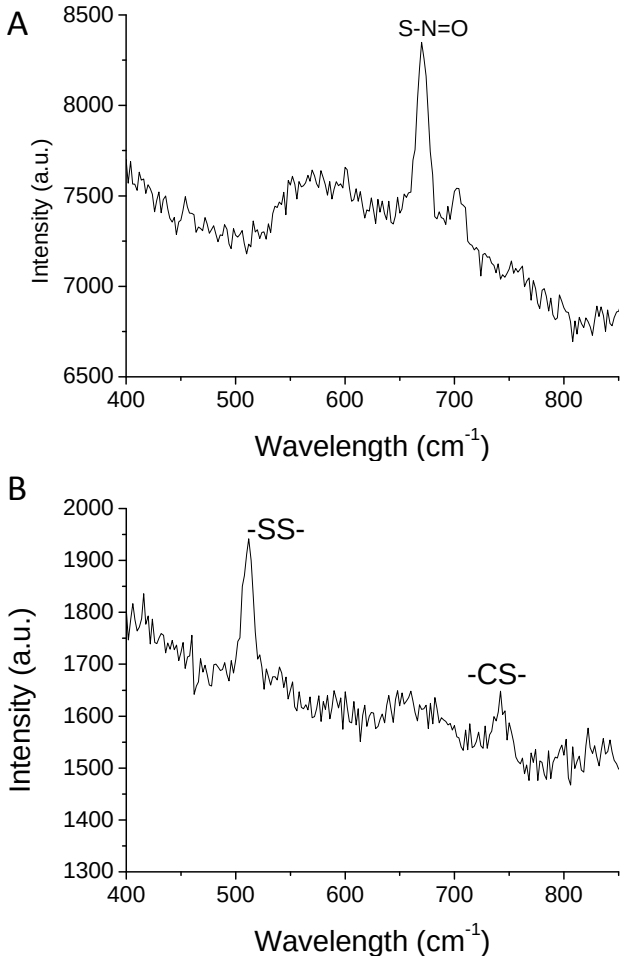


Figure S10. RAMAN spectrum of GSNO functionalized nanoparticles A) before and B) after release of NO.

Comment: RAMAN spectroscopy confirms the formation of disulfide by the presence of signal at 500 cm^{-1} and the absence of S-NO signal at 3800 cm^{-1} after treatment of NO functionalized nanoparticles with ascorbic acid. The formation of disulfide is attributed to the oxidation of thiol by the oxygen. These results are in perfect agreement with the data reported in the literature.[5]

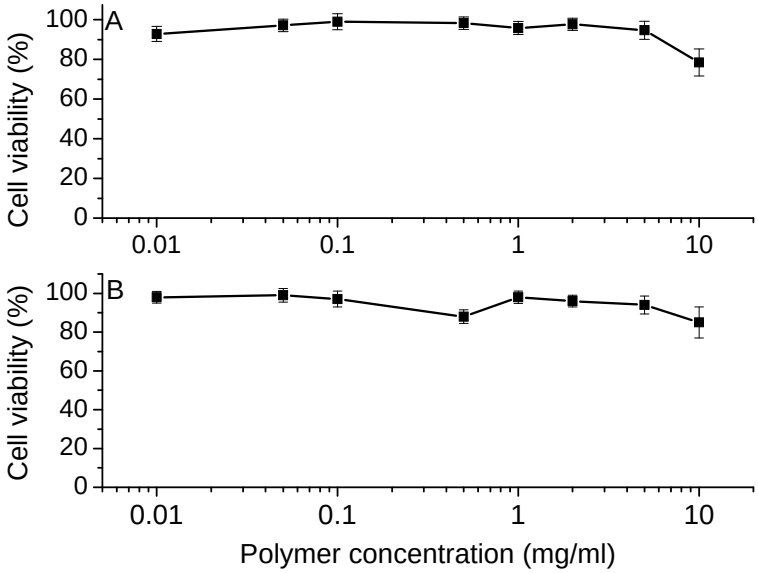
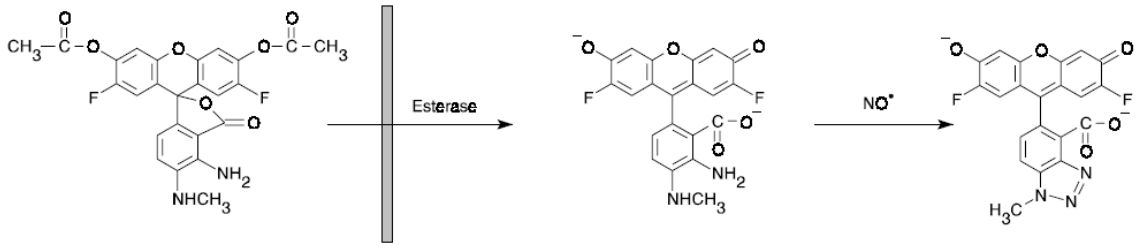


Figure S11. Cell viability of (A) MRC-5 and (B) BE(2)-C cells in the presence of different concentrations of polymer.



Scheme S2. Mechanism of DAF-FM DA in the cells. First, esterase cleaved the methyl groups to release DAF-FM in the cells, and then, NO reacts with DAF-FM to yield a green fluorescence emission.

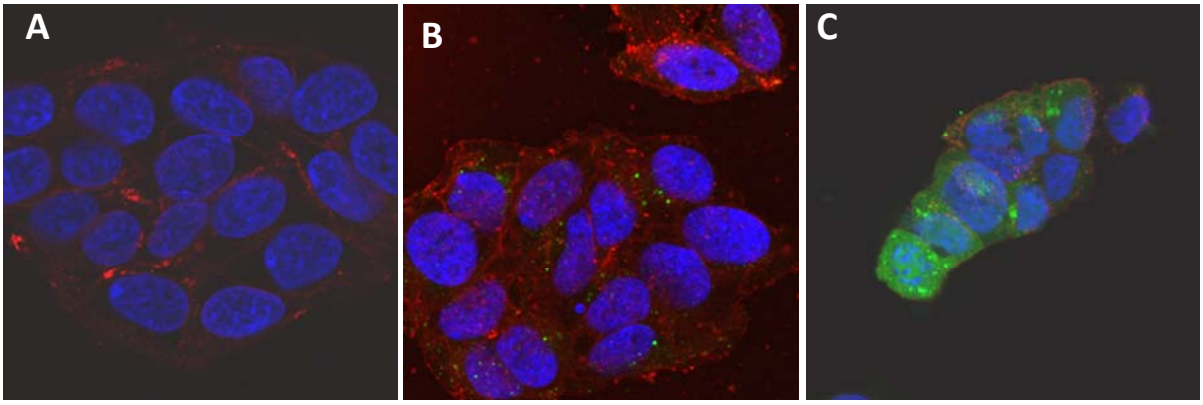


Figure S12. Additional confocal microscopy pictures of BE(C)-2 cells: (A) non treated cells, (B) treated cells with DAF-FM DA (without addition of NO nanoparticles), and (C) cells treated with NO functionalized nanoparticles and DAF-FM DA.

*Comment: the green fluorescence shows the release of NO in the cells due to the reaction of NO with DAF-FM (**Scheme S2**). The cells were treated with DAPI and Alexa Fluor 594 wheat germ agglutinin to stain the nucleus and the cell plasma membrane, respectively. The cells are incubated with DAF-FM DA for 20 min at 37 ^{o}C, and then, incubated with NO functionalized nanoparticles for 2 h.*

Confocal microscopy confirms the absence of green fluorescence when these cells are not treated (Picture A). The presence of weak green fluorescence in the picture B is attributed to the reaction of DAF-FM with NO endogenously produced by the cells. Picture C shows the cells treated with NO functionalized nanoparticles in the presence of DAF-FM. The green fluorescence is due to the reaction between the dye and NO released from the nanoparticles.

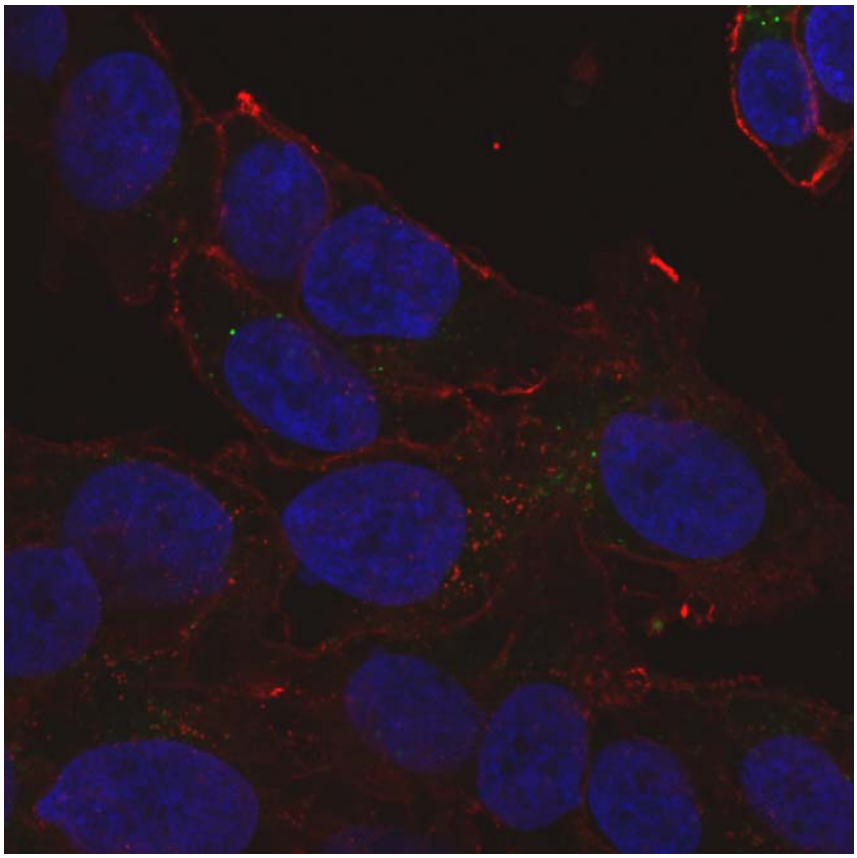


Figure S13. *Confocal microscopy picture of BE(C)-2 cells treated with DAF-FM DA with glutathione functionalized nanoparticles.*

Comment: The presence of weak green fluorescence is attributed to the reaction of DAF-FM with NO endogenously produced by the cells. GSH functionalized nanoparticles do not enhanced the production of iNOS.

Table S1. Cell viability of MRC-5 and BE(C)-2 cells in the presence of NO functionalized nanoparticles and nanoparticles without cisplatin. [NO donor concentration = 0.4 mM].

Cells	Nanoparticles with NO	Nanoparticles without NO
MRC-5	95 (+/- 3.0)	97 (+/- 4.0)
BE(2)-C	97 (+/-3.5)	98 (+/- 2.5)

Note: All toxicity studies have been repeated in 3 distinct experiments (in triplicate).

Table S2. Molecular weights and composition of the polymers used in this study.

Polymers	$\alpha^{M\,(a)}$	M_n (b) (g/mol)	PDI(b)	M_n (c) (g/mol)	$N^{VDM\,(d)}$
POEGMA	67%	21 000	1.12	20 000	-
POEGMA-*b*-VDM	60%	33 000	1.28	32 000	85
POEGMA-*b*-VDM/GSNO*		70 000	1.61	nd	

Note: (a) monomer conversion determined by ^{1}H NMR spectroscopy, (b) determined by GPC, (c) determined by ^{1}H NMR spectroscopy, (d) Number of VDM per polymer chain, (e) POEGMA-b-VDM/GSNO correspond to copolymer after attachment of GSNO.*

Table S3. IC_{50} values of cisplatin for MRC-5 and BE(C)-2 cells in the presence of GSH functionalized nanoparticles. [GSH concentration = 0.4 mM].

Cells	IC_{50} values (µM)
MRC-5	10.15 (+/- 1.15)
BE(2)-C	7.05 (+/-1.01)

Note: All toxicity studies have been repeated in 3 distinct experiments (in triplicate).

Additional references:

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