Electronic Supplementary Information

Magnetic field activated drug delivery using thermodegradable azo-functionalised PEG-coated core-shell mesoporous silica nanoparticles

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Materials and Methods

Materials. 4,4'-azobis(4-cyanovaleric acid) (ACA) was purchased from Pfaltz & Bauer. 1-Hydroxybenzotriazole (HOBt), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC.HCl) were obtained from CreoSalus Inc. (Louisville, KY, USA). *N*,*N*-diisopropylethylamine (DIEA) was purchased from Alfa Aesar. NH_2-PEG-NH_2 (M_n = 1500 g.mol^{-1}), tetraethoxysilane (TEOS), 3-aminopropyltriethoxysilane (APTES), l-arginine and cetyltriethylammonium bromide (CTAB) were purchased from Sigma-Aldrich. HOOC-PEG-COOH abbreviated as PEG-COOH (M_n = 2000 g.mol^{-1}) was purchased from Iris Biotech. Superparamagnetic iron oxide nanoparticles (SPION, Fe_3O_4) were purchased from Ocean Nanothech LLC (San Diego, CA, USA).

Instruments.

Proton nuclear magnetic resonance spectroscopy (^{1}H-NMR). ^{1}H-NMR analysis was carried out with a 300 MHz Bruker-UltraShield spectrometer. The samples were dissolved either in $CDCl_3$ or D_2O (10 mg/mL).

Gel permeation chromatography (GPC). GPC analysis was performed using a JASCO chromatography system equipped with a Waters column (Ultrahydrogel 120, 7.8 x 300 mm) [eluent: PBS buffer 10 mM pH 7.4 containing 100 mM NaCl; flow rate: 1 mL/min; UV detector (214 nm); temperature: 25 °C]. All samples were filtered with 0.20 μm polyethersulfone syringe filters before injection.

Fourier transform infrared spectroscopy (FTIR). FTIR spectra were recorded using a JASCO FT/IR-420 spectrometer averaging 16 scans in the range of 4000-400 cm^{-1} at a resolution of 4 cm^{-1} with samples mixed in KBr pellets.

Matrix-assisted laser desorption/ionisation time-of-flight (MALDI- TOF) mass spectrometry. MALDI-TOF mass spectra were recorded in positive mode with an Applied Biosystems Voyager-DE STR mass spectrometer (UCLA Molecular Instrumentation Center) equipped with a nitrogen laser (337 nm, 3 ns pulse width). 1 μl of Azo-PEG solution (1 mg/mL) was mixed with 1 μl of 2,5-dihydroxybenzoic acid (10 mg/mL) in acetonitrile/water solution (1:1) containing 0.1% TFA on a stainless steel target plate and allowed to dry in a vacuum chamber. Data were collected in reflection mode using an accelerating voltage of 25 kV and a delay time of 400 ns. The accumulated spectra shown were obtained by 500 laser shots.

Differential scanning calorimetry (DSC). The thermal analysis was carried out with a Perkin-Elmer Pyris Diamond DSC equipped with internal coolant and nitrogen purge gas. The samples (8 mg) were heated in hermetically sealed aluminium pans from 0 to 300 °C and cooled to 50 °C at a flow rate of 10 °C/min. An empty aluminium pan was used as a reference.

Thermogravimetric analysis (TGA). Thermogravimetric analysis was performed using a Perkin-Elmer Pyris Diamond TG/DTA under argon. Approximately 4-10 mg of samples were loaded in platinum pans. The curves were recorded from 50 to 500 °C at a scan rate of 10 °C/min. An empty platinum pan was used as a reference.

Transmission electron microscopy (TEM). TEM analysis was performed using a JEM1200-EX (JEOL) at an accelerating voltage of 80 kV. A suspension of nanoparticles (5 μL) was dropped on a microgrid followed by the removal of excess solution with filter paper and then air-drying.

Nitrogen adsorption and desorption isotherms were obtained at 77 K using an Autosorb-iQ (Quantachrome Instruments).

Fluorescence. The fluorescence release profiles were recorded by an Acton Spectra Pro 2300i CCD and excited by a CUBE 445-40C (Coherent Inc., Santa Clara, CA, USA) laser.

Zeta-potentials were measured by ZetaSizer Nano (Malvern Instruments Ltd., Worcestershire, U.K.).

Synthesis of Azo-PEG. ACA (4.0 g, 14.29 mmol) was dissolved in 60 mL of dichloromethane. The solution was cooled to 0 °C in an ice bath to prevent cleavage of the azo bond. The carboxylic acid group was pre-activated with DIEA (3.7 mL, 21.42 mmol), HOBt (2.37 g, 17.85 mmol) and EDC.HCl (3.42 g, 17.85 mmol) for 30 min under argon atmosphere. Afterwards, NH_2-PEG-NH_2 (5.36 g, 3.57 mmol) was added. The mixture was stirred for another 4 h at room temperature under argon atmosphere. The resultant mixture was diluted in dichloromethane and washed with brine (3 x 200 mL). The combined organic layers were dried with magnesium sulfate, and then concentrated by rotary evaporation. The polymer was precipitated in cold diethylether and collected by filtration. Finally, the polymer was dialyzed overnight against deionized water (MWCO = 1000 Da) at 4 °C and lyophilized. Azo-PEG was characterized by ^{1}H-NMR, GPC, MALDI-TOF, DSC and TGA (Figure S1). The DSC heating curve (heating rate 10 °C/min) of Azo-PEG shows a sharp melting endothermic peak at 30 °C and an exothermic peak at 120 °C, which is ascribed to the decomposition of the azo bond (C–N bond breaking and N_2 gas release) (Figure S1D). The DSC trace confirms that the grafting does not affect the thermal behavior of the azo moiety as the observed decomposition temperature for Azo-PEG is closed to that for common azo compounds.[1] From the TGA curve of Azo-PEG (Figure S1E), a barely noticeable loss of mass is observed at 120 °C confirming the azo decomposition temperature. During thermal decomposition of the azo bond there is only the release of N_2, which constitutes less than 2.8 wt% of the Azo-PEG. In contrast, the TGA curve shows a full polymer decomposition at about 414 °C, that takes place after the azo decomposition.

Thermal degradation study. Azo-PEG was dissolved in D_2O (10 mg/mL) and filtered using 0.20 μm polyethersulfone syringe filter. The solution was placed in a water bath at either 37 °C or 65 °C, and analyzed by ^{1}H-NMR and GPC at different time points over 24 h.

Synthesis of Core-shell Fe_3O_4@SiO_2 mesoporous nanoparticles and functionalisation of the surface with amine groups. Core-shell Fe_3O_4@SiO_2 mesoporous nanoparticles were obtained through a two-step process. First 1 g of a chloroform dispersion of SPION (2.5 mg L^{-1}) was added to a 10 mL water solution containing 30 mg of CTAB. The mixture was then sonicated for 10 min to allow an homogeneous dispersion of the organic solvent in the water phase after which the resulting solution was stirred at

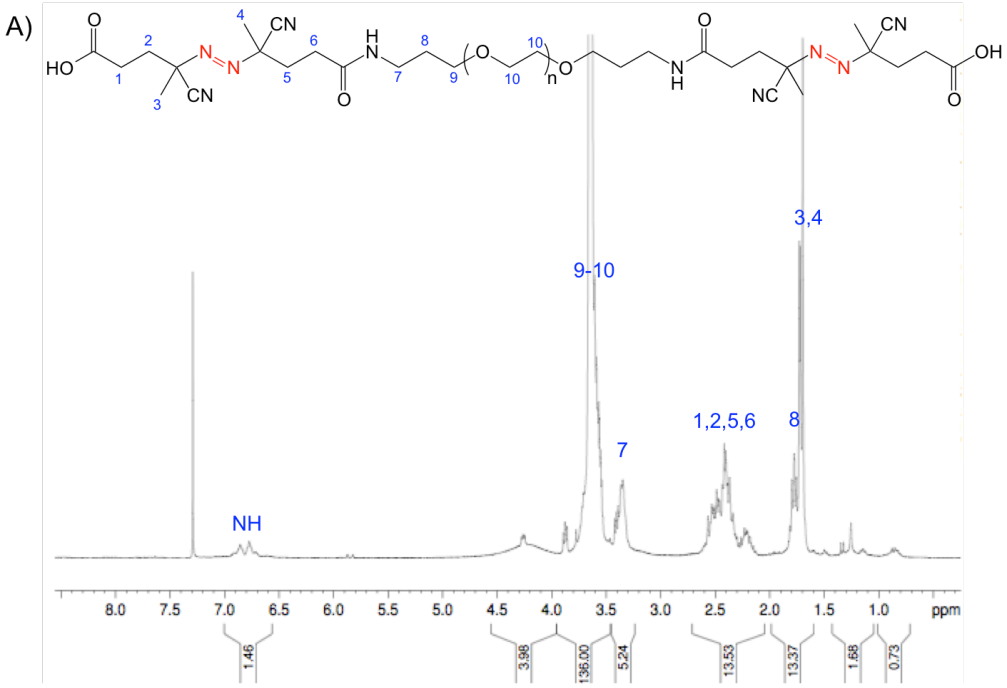
85 °C to allow the chloroform to evaporate. Once the solution became clear the flask was sonicated for another minute to ensure a good dispersion of the SPION. After another 10 minutes at 85 °C, 12.5 mg of arginine was added and finally 100 μL of TEOS were added rapidly. In order to obtain functionalised core-shell nanoparticles, APTES was added to the mixture after 3 h leading to nanoparticles with amine group on the surface. The surfactant was extracting by mixing an ethanolic dispersion of the nanoparticle with ethanol and ammonium nitrate (6 g L^{-1}).

Polymer grafting to amine-modified core-shell Fe_3O_4@SiO_2 mesoporous nanoparticles (MSN). The polymers (Azo-PEG and PEG-COOH) were attached to the surface of the particles by standard coupling reaction between the carboxylic groups of the polymers and the amines at the surface of the particles. First the polymers (50 mg) were activated in water (1 mL) after mixing sequentially with DIEA (12 μL), HOBt (6 mg) and EDC.HCl (7 mg). Then the activated polymer solution was mixed with the nanoparticles (10 mg in 0.75 mL water) and let to react overnight at room temperature. During the first 15 min the reaction took place in an ice bath to avoid any local heating that may cause the degradation of the polymer. The grafting of the polymer to the nanoparticles was confirmed by FTIR. The grafting density of polymer was determined by TGA (Figure S2). To account for the decomposition of the APTES modification, the final weight loss was determined by subtracting the weight loss of amine-modified nanoparticles (MSN) from the weight loss of the polymer-grafted nanoparticles (MSN-PEG or MSN-Azo-PEG). It was assumed the difference in weight loss was only from the attached polymer. The grafting density was estimated to be 7% and 13% for MSN-PEG and MSN-Azo-PEG respectively.

Live/Dead® Viability Assay. The cytotoxicity of polymer-functionalised nanoparticles and degradation products and the effect of magnetic field exposure were investigated on NIH/3T3 cells using the Live/Dead® Viability/Cytotoxicity Kit (Molecular Probes). NIH/3T3 cells were grown at 37 °C, 5% CO_2 up to confluence. After trypsinization, cells were washed with DMEM containing 10% FBS and 1% Pen/Strep by centrifugation. 150 μL of the cell suspension containing 1×10^3 cells were inoculated into each well of 16-well microscope slide, and cells were seeded for 24 h. Nanoparticles (MSN-PEG or MSN-Azo-PEG) were then added at different concentrations (0, 25, 50, 100 and 200 μg/mL) to each well. The cells were then

exposed to an oscillating magnetic field (15 cycles of 90 seconds heating and 110 seconds cooling). In duplicate plates, cells were not exposed to magnetic field and served as control to check the toxicity of nanoparticles. The cells were incubated with nanoparticles for 24 h at 37 °C before washing with 100 μL of PBS. Then, the cells were stained with 100 μL of the live/Dead assay reagents (2 μM calcein and 4 μM ethidium homodimer), and incubated at 37 °C for 25 min. The labeled cells were visualized using a Zeiss Axiovert Observer Z1 inverted fluorescent microscope (green fluorescence: live cells, red fluorescence: dead cells) and counted using ImageJ software (at least 150 cells were counted for each treatment). The assay was performed in triplicates.

Supplementary Figures



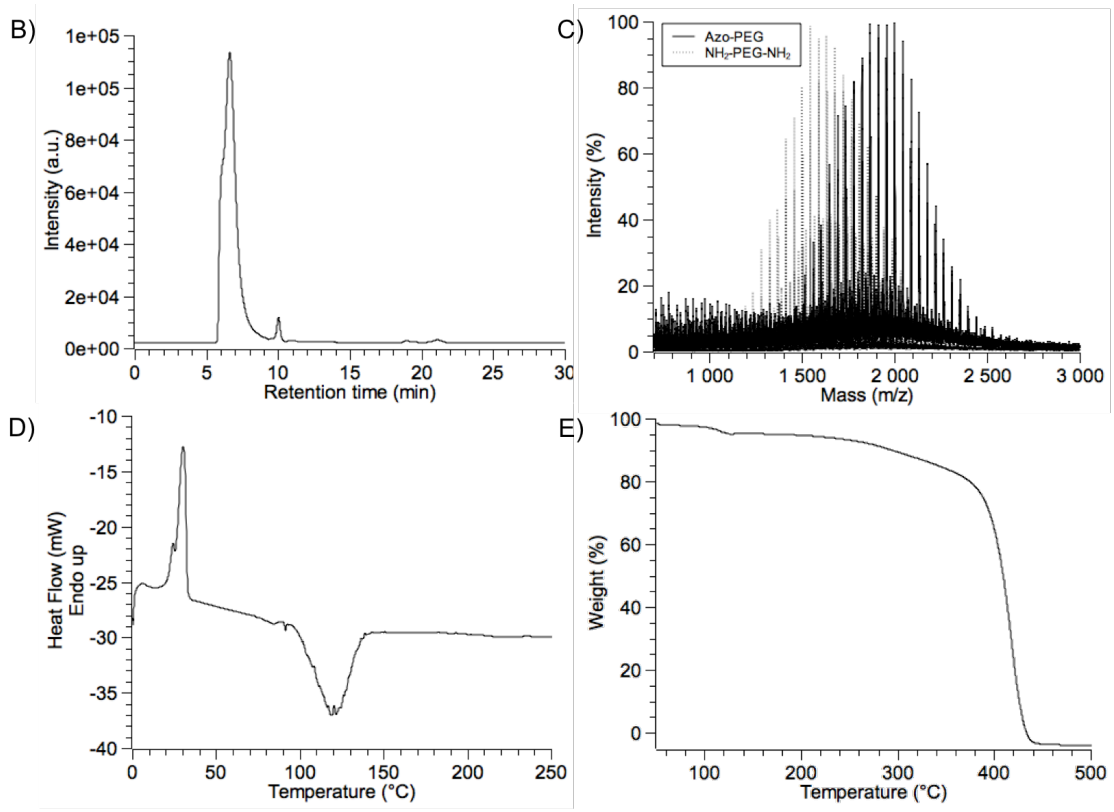


Figure S1. Characterization of Azo-PEG. A) NMR spectrum in $CDCl_3$; B) GPC chromatogram; C) MALDI-TOF spectra of Azo-PEG and NH_2-PEG-NH_2; D) DSC heating curve; and E) TGA curve.

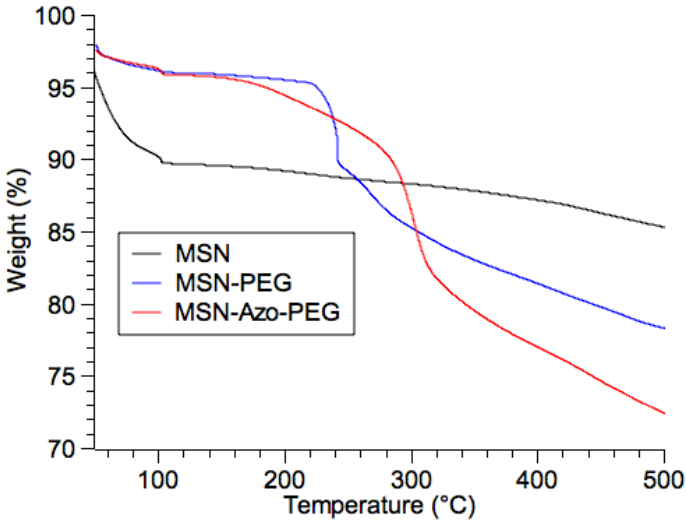


Figure S2. TGA curves of core-shell Fe_3O_4@SiO_2 mesoporous nanoparticles functionalised with amine groups (MSN), grafted with PEG-COOH (MSN-PEG) and Azo-PEG (MSN-Azo-PEG).

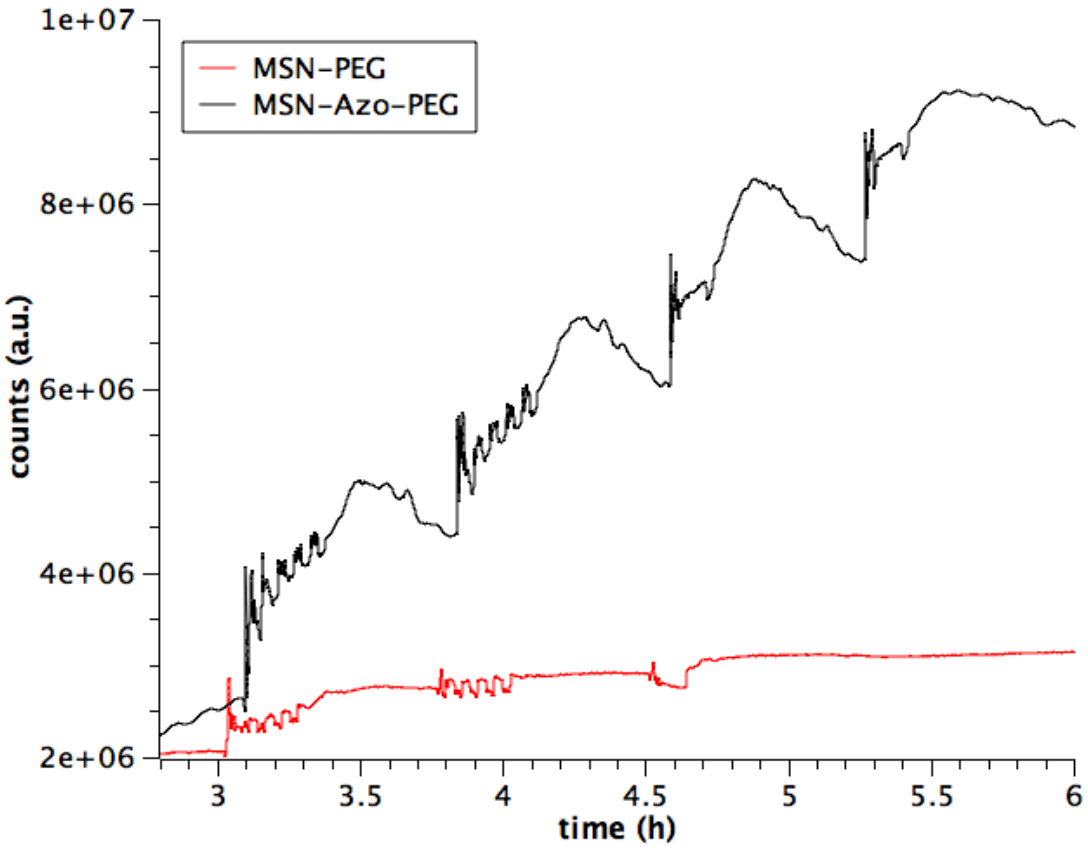


Figure S3. Real time fluorescence measurement, 2 series of 5 cycles (90 seconds on followed by 110 seconds cooling down) were applied to both type of particles, then 20 min straight of magnetic heating was applied to the MSN-PEG and 2 series of 10 min straight were applied to the MSN-Azo-PEG. For obvious reasons no magnetic stirring was used during the experiment, which might explain why the fluorescence signal increases after every series (the dye is diffusing out and crossing the laser path) and then slowly decrease (the dye is distributing more evenly in the solution with time).

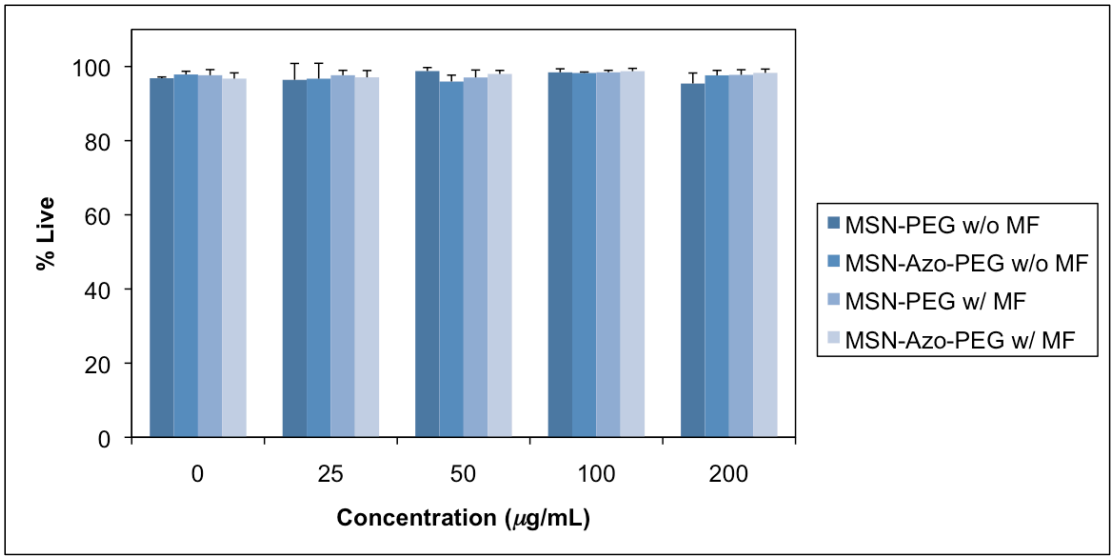
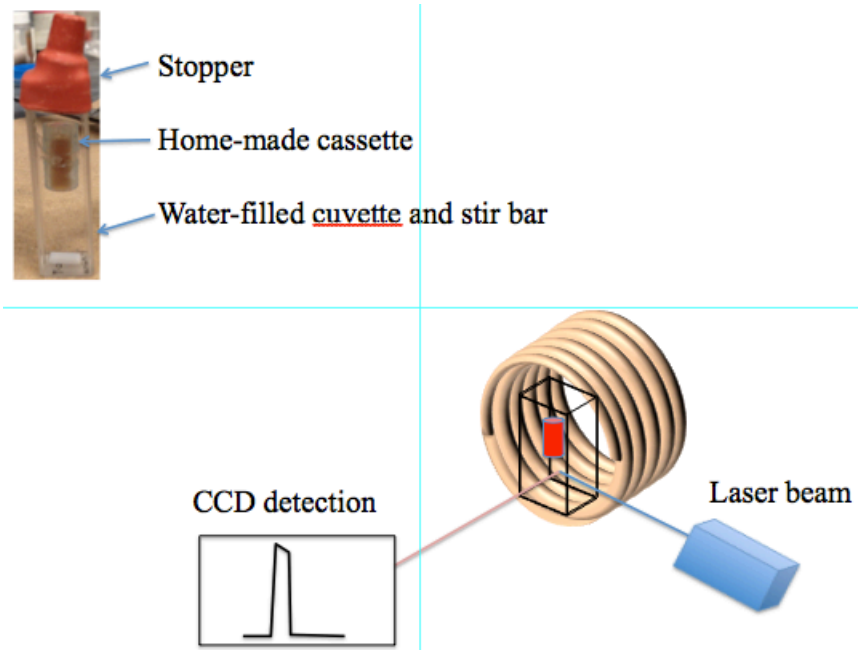


Figure S4. Live/Dead® cytotoxicity assay performed on NIH/3T3 cells treated with MSN-PEG and MSN-Azo-PEG and exposed to an oscillating magnetic field (MF) (15 cycles of 90 seconds heating and 110 seconds cooling). The cells were incubated 24 h with nanoparticles before staining with Live/Dead assay reagents (green fluorescence: live cells, red fluorescence: dead cells). The cell viability was quantified by counting the percentage of green stained cells (at least 150 cells were counted for each treatment and n = 3).



Scheme S1. Real-time experiment set-up.

Reference:

1. T. Cheikhalard, L. Tighzert, and J. P. Pascault, *Angew. Makromol. Chem.*, 1998, **256**, 49–59.