Supplementary Information

Water Gated Contrast Switching with Polymer-Silica Hybrid Nanoparticles

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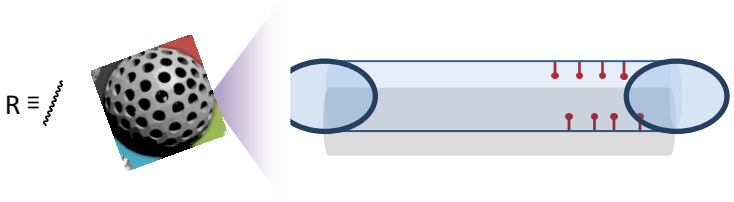
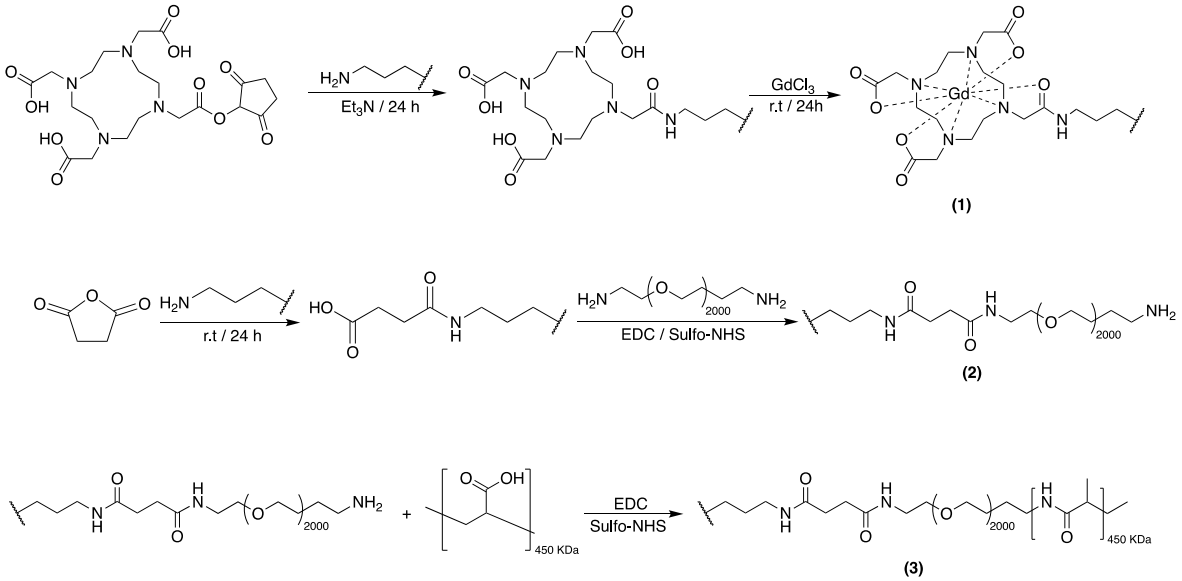
Experimental Section

Chemicals:

Triethanol amine (TEA) was purchased from Scientific Laboratory Supplies, 37% hydrochloric acid (HCl) and 70% nitric acid (HNO_3) were purchased from Fischer Scientific and poly(ethylene glycol) (NH_2-PEG_{2000}-NH_2) was purchased from Rapp Polymere. All other chemicals were purchased from Sigma-Aldrich and used as received. Ultrapure water (Millipore) with a resistivity of 18.2 MΩ·cm was used throughout.



Synthetic scheme:



Synthesis of Gd functionalised MSNs (Gd-DOTA-MSNs) [1]:

A long delay co-condensation synthesis was performed to produce NH_2-MSNs with 10% amination by a subtle modification of the standard Stöber method. Cetyl trimethylammonium bromide (CTAB, 1.77 mmol) and triethanol amine (TEA, 6.9 mmol) were dissolved in a mixture of ethanol (0.03 mol) and ultrapure water (0.89 mol) under vigorous stirring. The solution was brought to 80°C and left stirring for 20 min. Tetraethylorthosilicate (TEOS, 5.18 mmol) was added dropwise (1 mL/min) and the solution stirred for a further 60 min at 80°C. 3-aminopropyltriethoxysilane (APTES, 0.650 mmol) and TEOS (0.65 mmol) were added and the solution vigorously stirred at 80°C for a total reaction time of 2 h. The reaction was then cooled to room temperature before the particles were collected using centrifugation (13,500 rpm, 20 min) and washed twice with ethanol. The particles were dispersed in acidic ethanol (10 vol%), sonicated for 30 minutes and then collected by centrifugation to remove the surfactant template. Two further washings in ethanol were conducted before the particles were left to dry under vacuum. The obtained NH_2-MSNs (200 mg) were dispersed in DMF (15 mL) before the addition of 2,2',2''-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (DOTA-NHS ester, 5 μmol) and triethylamine (1.08 mmol). The resultant solution was stirred at room temperature for 24 h after which they were collected via centrifugation and washed three times with ethanol to yield DOTA-MSNs. The particles

were dispersed in ethanol (10 mL) before the addition of $GdCl_3$ (10 µmol). This was left under vigorous stirring at room temperature for 24 h before collecting the particles via centrifugation and washing three times with ethanol. The resulting Gd-DOTA-MSNs were left to dry under vacuum.

Functionalisation of Gd-DOTA-MSNs with poly(ethylene glycol) (Gd-MSNs-Pg) [2]:

Gd-MSNs (100 mg) were dispersed in DMF (15 mL) under sonication. Succinic anhydride (1.25 mmol) was added and vigorous stirring was carried out at room temperature for 24 h. The nanoparticles were washed three times with ethanol and dried under vacuum to yield carboxylic acid functionalised particles. These particles (20 mg) were dispersed in ultrapure water (5 mL) before the addition of N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC, 0.068 mmol) and N-hydroxysulfosuccinimide (sulfo-NHS, 0.069 mmol). The solution was stirred at room temperature for 30 min before adding NH_2-PEG_{2000}-NH_2 (5 mg, MW = 2 KDa) in phosphate buffer (2 mL, pH 8). This was left stirring overnight then washed three times with a mix of ultrapure water and ethanol to yield Gd-MSNs-Pg.

Synthesis of poly(acrylic acid) functionalised nanoparticles (Gd-MSNs-PgPAA) [3]:

Poly(acrylic acid) (40 mg, MW = 450 KDa) was dissolved in water (5 mL) and the pH adjusted to pH 7.0 using sodium hydroxide (1 M). EDC (0.125 mol) and sulfo-NHS (0.138 mol) were added and the solution left stirring for 30 min at room temperature. Gd-MSNs-Pg (20 mg) were dispersed in phosphate buffer (2 mL, pH 8.0) and added to the reaction mixture, which was left stirring for 24 h at room temperature. Finally, the nanoparticles were collected by centrifugation at 13,500 rpm for 20 min, washed five times with a H_2O/EtOH mixture and dried under vacuum.

Synthesis of fluorescein loaded Gd-MSNs-PgPAA:

20 mg of Gd-MSNs-Pg were dispersed in 3 mL of distilled H_2O under sonication. Then, 500 µL of a fluorescein solution in PBS 1X (28.8 µM) was added. The mixture was stirred at r.t for 24 h. Afterwards, 40 mg of PAA (450 KDa) was dissolved in 6 mL of water and the pH adjusted to pH = 7 with NaOH (1 M). 24 mg of EDC and 30 mg of Sulfo-NHS were added and the reaction stirred at r.t for 30 min. This

solution was then added into the first solution and stirred at r.t for 24 h. Finally, the nanoparticles were collected by centrifugation and washed several times with a H_2O/EtOH mixture and dried under vacuum.

Characterisation:

A Malvern Zetasizer Nano with a 532 nm laser as the light source was used for the Dynamic Light Scattering (DLS) analysis. The samples for DLS were prepared by dispersing the nanoparticles (*ca.* 1 mg/mL) in ultrapure water. To assess colloidal stability at different pH, Gd-MSNs and Gd-MSNs-PgPAA nanoparticles were incubated for 1 h between pH = 3.0 and pH = 11.0 in a physiological solution of saline 0.9 %. TEM images were obtained by JEM-2100 (JEOL, Japan) operated at 200 kV. Samples for TEM were prepared by depositing and drying of a drop of an aqueous colloidal suspension of nanoparticles onto a copper grid. The average diameter was calculated by counting 50 particles. Nitrogen adsorption-desorption analysis was performed with a Micromeritics 3Flex surface characterization analyser. Gd concentrations were obtained using ICP-OES (Optima 8000, PerkinElmer). Samples for ICP-OES were hydrolysed into 3 mL of HNO_3 at 150 °C for 3 h. Then, the samples were adjusted to a final volume of 10 mL and filtered using 0.45 µm filters. [Gd^{3+}] calibration curve was prepared using a Gd^{3+} ICP standard solution (Sigma-Aldrich). 8 solutions with Gd^{3+} concentrations ranging from 0.2 to 50 ppm were measured obtaining the calibration curve. Controls using MSNs without DOTA resolved that > 95% of the Gd^{3+} ions are chelated (Gd^{3+} weight percentage of 2.45 ± 0.42 wt% vs 0.12 ± 0.02 wt%). Fourier transform infrared (FT-IR) analysis was carried out using an IRTracer-100 (Shimadzu) spectrometer. Fluorescence measurements were carried out with the use of a Fluorolog® (Horiba Scientific). Aqueous solutions of fluorescein loaded Gd-MSNs-PgPAA (NP) were incubated at pH = 4.0 and 9.0, to evaluate both conformational states, for 6 hours at r.t., precipitated by centrifugation, and the supernatant collected to measure the fluorescence recovery. Proton relaxation time measurements were acquired at 1.5 T with a Spinsolve Benchtop NMR (Magitrek) at room temperature. The nanoparticles were dispersed in ultrapure water at the desired

Gd^{3+} concentration. The longitudinal relaxation rate ($1/T_1$) was plotted versus the Gd^{3+} concentration (mM) and relaxivities obtained from the slope of the subsequent linear fit. T_1-weighted images were acquired using Varian (7.0 T) equipment with a routine spin-echo sequence. 1 mg/mL aqueous solutions of Gd-MSNs-PgPAA at pH = 4.0, pH = 6.0 and pH = 7.0 were imaged with H_2O as a reference. ImageJ was used to measure the signal intensities in 5 consecutives axial slices along each sample. Relaxivity values at 7 T were calculated using a look-Locker inversion recovery (8 FA 15º readouts, T_I = [0.1, 0.17, 0.3, 0.5, 0.8, 1.4, 2.4, 4] s) for three different concentrations of Gd-MSNs-PgPAA at pH = 4 and pH = 7.

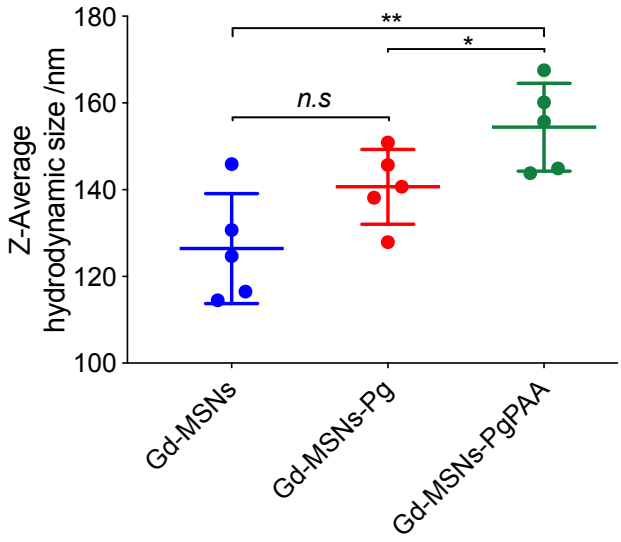


Figure S1. *Z*-Average hydrodynamic size of 5 independent syntheses, measured by DLS, of aqueous solutions at 1 mg/mL of Gd-MSNs, Gd-MSNs-Pg and Gd-MSNs-PgPAA.

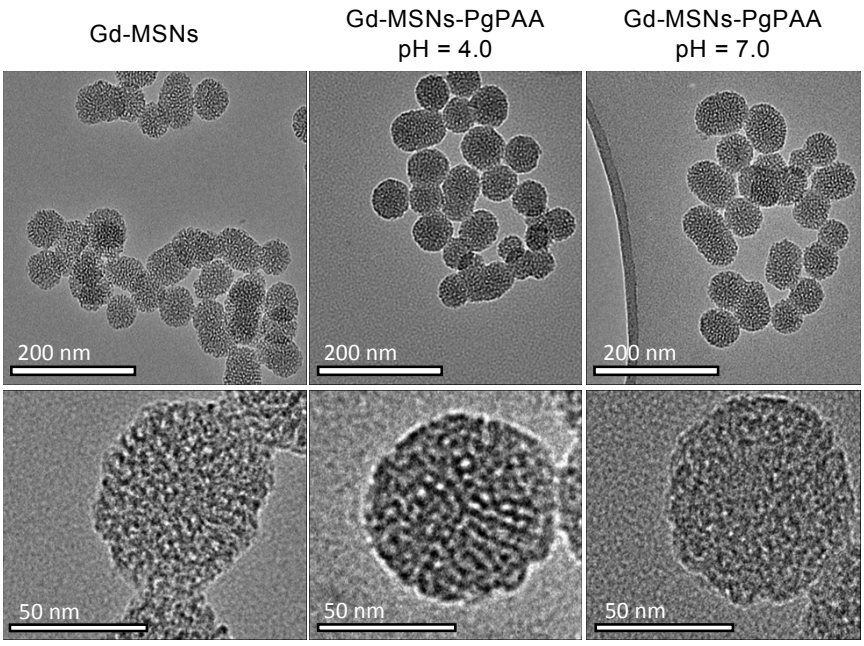


Fig S2. TEM images of Gd-MSNs and Gd-MSNs-PgPAA at pH = 4.0 and pH = 7.0 at two magnifications. The spherical particles show a slight difference in core size from 55.3 ± 6.9 nm for Gd-MSNs to 60.9 ± 8.3 nm for Gd-MSNs-PgPAA.

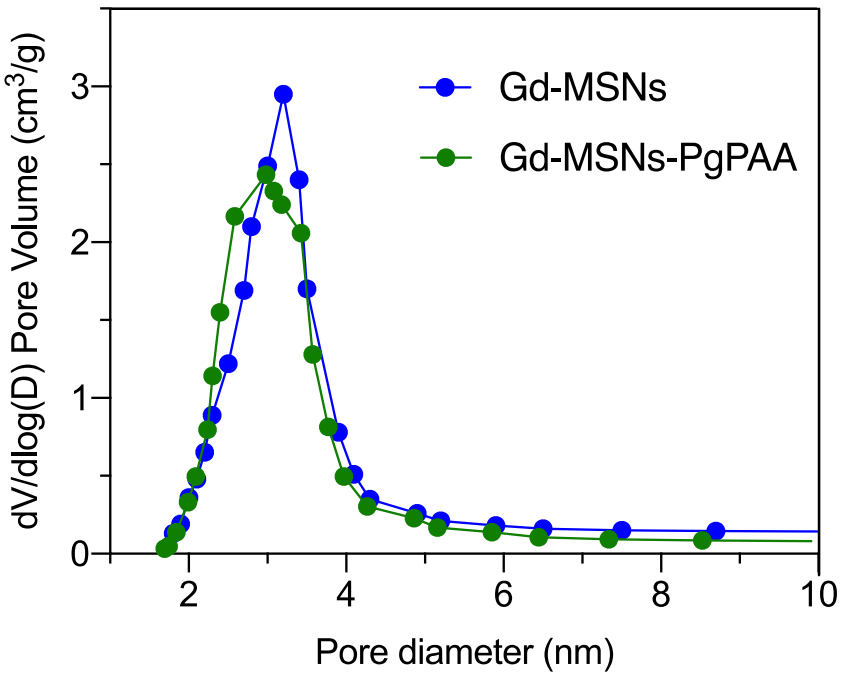


Fig S3. Pore size distribution of Gd-MSNs and Gd-MSNs-PgPAA. An average pore size of 3.2 nm for Gd-MSNs and 3.0 nm for Gd-MSNs-PgPAA was calculated from the adsorption using the Barrett-Joyner-Halenda (BJH) method.

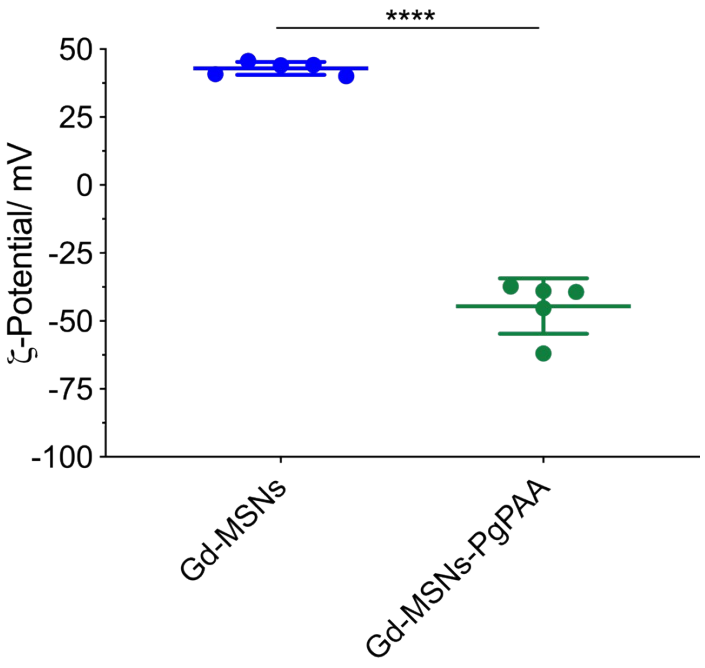


Fig S4. ζ-Potential values aqueous solutions at 1 mg/mL of Gd-MSNs and Gd-MSNs-PgPAA of 5 independent synthesis (two-tailed t-test, ****, P < 0.0001).

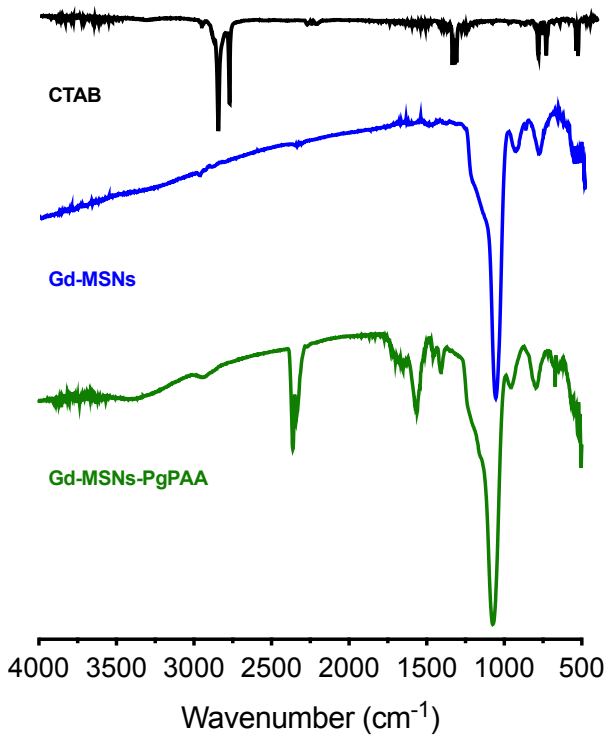


Fig S5. FTIR spectra of Cetyl trimethylammonium bromide (CTAB, black), Gd-MSNs (blue) and Gd-MSNs-PgPAA (green).

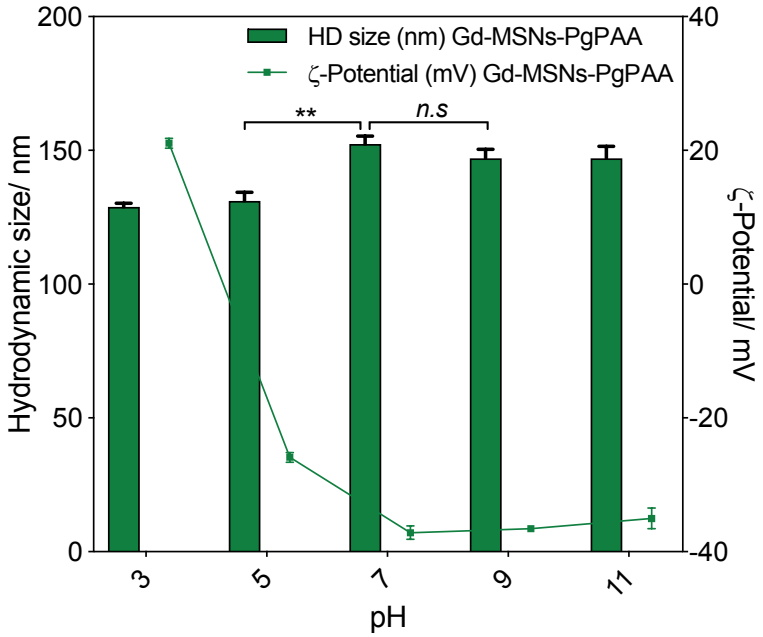


Fig S6. Resolved stability of Gd-MSNs-PgPAA at different pH values. Hydrodynamic size (bar, n = 3, mean ± sd) and ζ-Potential (line, n = 3, mean ± sd) were measured at pH range between 3.0 – 11.0 (two-tailed t-test, **, P = 0.0011; two-tailed t-test, n.s, P = 0.1583).