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Supplementary Information

Promoting high T_2 contrast in Dy-doped MSNs through Curie effects

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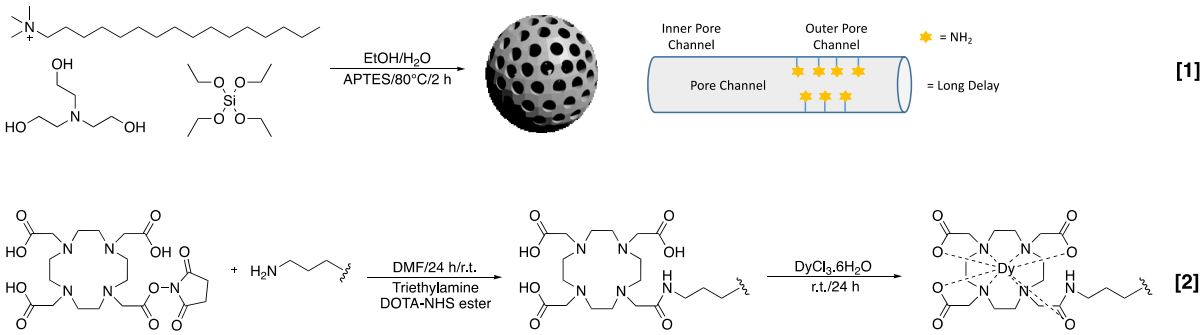
Experimental Section

Chemicals:

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) was purchased from CheMatech. All other chemicals were purchased from Sigma-Aldrich and used as received. Ultrapure RO water (Millipore) with a resistivity of 18.2 MΩ·cm was used throughout.

Synthetic Scheme:



ESI 1. Synthetic procedure to produce Dy-MSNs. The MSNs are synthesised by a brief modification of the Stöber method, using a long delay co-condensation reaction which introduces amino anchor points in the outer pore channel of the particles [1]. These are then employed to tether DOTA at the periphery of the pore, with subsequent Dy^{3+} chelation resulting in the generation of the desired nanoparticles [2].

Preparation of 10% aminated MSNs by a modified Stöber method [1]:

Cetyl trimethylammonium bromide (CTAB, 1.77 mmol) and triethanol amine (TEA, 6.9 mmol) were dissolved in a water/ethanol mixture (1.88:16.2 mL EtOH:H_2O), and the solution vigorously stirred at 80 °C for 20 minutes. Next tetraethylorthosilicate (TEOS, 5.18 mmol) was added dropwise (1 mL/min), with the solution stirred at 80 °C for a further 60 minutes. TEOS (0.65 mmol) and 3-aminopropyltriethoxysilane (APTES, 0.65 mmol) were added to the reaction and the mixture stirred at 80 °C for a total reaction time of 2 hours. The reaction was cooled to room temperature and the silica nanoparticles collected by centrifugation at 13,500 rpm for 20 minutes. The purification of the particles was conducted by resuspension of the particles in EtOH and subsequent centrifugation at 13,500 rpm for 20 min, with the process then repeated. Next the particles were suspended in acidic EtOH (10 vol%) and then sonicated for 30 min, to ensure complete removal of the surfactant template, before centrifuging again (13,500 rpm, 20 min). Finally, the nanoparticles were resuspended in EtOH and collected by centrifugation (13,500 rpm, 20 min) twice more and left to dry under vacuum.

Dy loading to produce Dy-MSNs [2]:

Aminated MSNs (50 mg) were dispersed in anhydrous dimethylformamide (DMF, 15 mL) by sonication and stirred vigorously at room temperature for 24 hours with 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, varied, 0.413 μmol - 9.85 μmol) and triethylamine (1.08 mmol) added to the reaction mixture. The resulting silica nanoparticles (DOTA-MSNs) were purified by centrifuging three times with EtOH as the solvent (13,500 rpm, 20 min). Dysprosium (III) chloride hexahydrate ($DyCl_3.6H_2O$, varied, 0.825 μmol - 19.7 μmol) was added and the reaction stirred at room temperature for 24 h. The quantities of DOTA-NHS-ester and $DyCl_3.6H_2O$ were chosen to give a range of Dy-loadings within the MSNs. The obtained Dy-MSNs were purified by centrifuging three times with EtOH as the solvent (13,500 rpm, 20 min).

Characterisation:

Dynamic Light Scattering (DLS) analysis was performed on a Malvern Zetasizer Nano with a 532 nm laser as the light source. The samples for DLS were prepared by dispersing nanoparticles (*ca.* 1 mg/mL) in ultrapure water. The corresponding Dy^{3+} concentrations were calculated and verified using inductively coupled plasma mass spectrometry (ICP-MS) analysis (Perkin Elmer NexION 2000B). The samples were prepared by hydrolysing the nanoparticles in 3 mL HNO_3 (70 %) overnight. The samples were then adjusted to a final dilution of 2 % HNO_3 using 18.2 MΩ deionised water. The calibration curve was obtained using an external calibration analysis (a series of standards of known concentrations were prepared to form a linear for calibration, diluted from a 10 ppm standard). A control reaction was also conducted to determine the specificity of the Dy-loading where MSNs without DOTA were doped with Dy^{3+}. This was done to confirm the absence of any physisorbed Dy^{3+} on the MSN surface. A control reaction using MSNs without DOTA was done to check the specificity of the chelation for low, medium and high levels of Dy-loading. All three samples showed good specificity with > 98.5 % of Dy^{3+} ions chelated (1.40 %, 0.68 % and 0.91 % Dy^{3+} loading for the low, medium and high Dy-loading control reactions respectively). A Quantum Design Magnetic Property Measurement System (MPMS)-XL was used to investigate spin-spin interactions within the Dy-doped MSNs, through investigation into the divergence of individual frequency profiles with changes in temperature. The temperature was decreased to 2 K with the AC susceptibility recorded for four

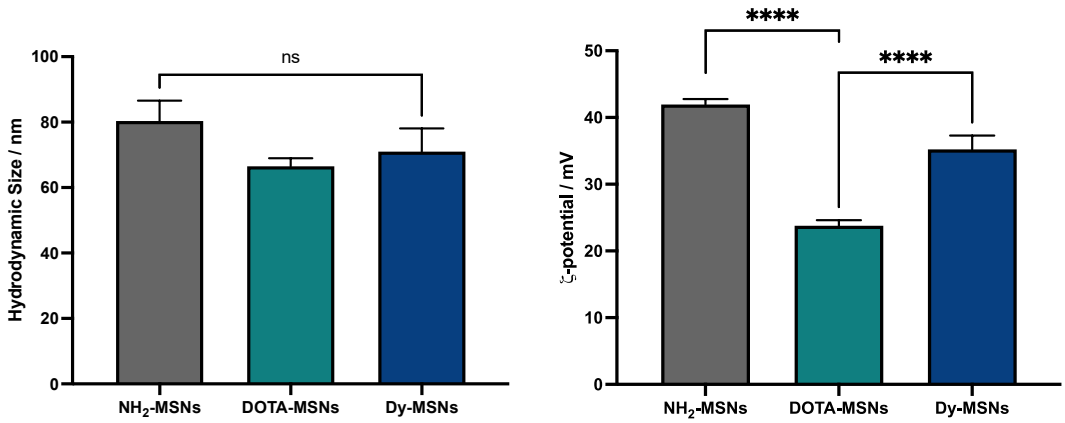
frequencies (1 Hz, 10 Hz, 100 Hz and 1000 Hz). The measurement was repeated every 0.5 K as the temperature was increased to 26 K. After this the AC susceptibility was recorded in increments of 1 K up to a temperature of 50 K. Finally, the measurements were collected every 5 K from 50 K to 270 K. For proton relaxation time measurements, the nanoparticles were dispersed in ultrapure water with desired Dy^{3+} concentrations, and the measurements were performed with a Bruker AVIIIHD 500 (11.7 T). A Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence was used for T_2 measurements. The relaxation rate ($1/T_2$) was plotted versus Dy^{3+} concentration (mM) and relaxivities were obtained from the slope of a subsequent linear fit.

T_2 maps and T_2-weighted images were acquired on a Magnex/Varian DDR2 preclinical MRI system operating at 7 T, with a volume transmit/receive 72 mm proton coil. T_2-weighted images were acquired via a fast spin echo sequence with echo times chosen to provide a representative contrast difference of the particles by effectively highlighting intrinsic differences in T_2. Specifically, the T_R was 5 s, echo chain length 2, effective T_E 250 ms, matrix 128 × 128, in a 60 × 60 × 2.8 mm^3 FOV with a readout bandwidth of 100 kHz following a 1400 ms sinc 90° excitation and a 3.2 ms mao refocussing pulse. Quantitative T_2 maps were obtained by a series of gradient-echo images obtained after the application of an MLEV "T_2 prep" pulse train (consisting of an iterated series of ($90º_x$ $180º_y$ $90º_x$) pulses played after a $90º_x$ excitation and before a $-90º_x$ refocussing pulse; 128x128 matrix size, in a 45 × 45 × 2.8 mm^3) with 9 echo times (T_E = 15, 27, 39, 51, 64, 76, 88, 145 ms). The data was regridded, reconstructed, and the multiple-echo data fitted to an appropriate signal model in xrecon (https://github.com/tesch1/Xrecon) by weighted nonlinear least squares. Complex images were zero-filled by a factor of 4 and the resulting k-space hanning-weighted to reduce Gibbs ringing prior to Fourier transformation and the subsequent determination of T_2. Diffusion data was obtained by a spin-echo EPI sequence with the same FOV as above; a 50 kHz bandwidth, 9 s T_R, but a constant 43 ms T_E and with the addition of isotropic diffusion gradients after excitation with 22 b-values between

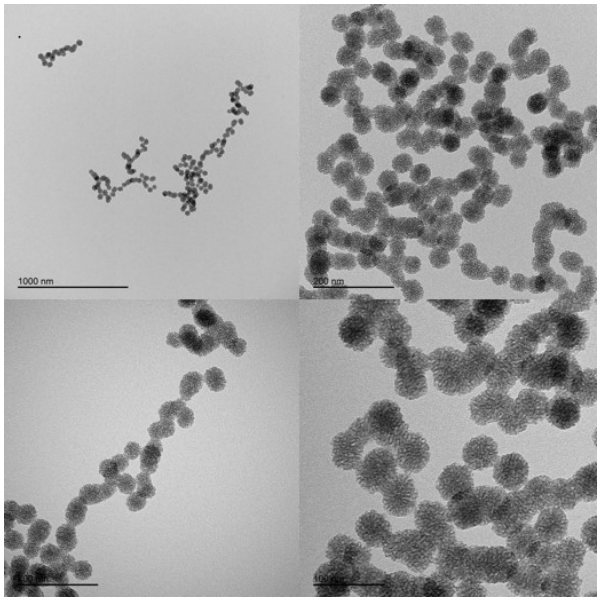
0 and 1114 s mm^{-2}. An apparent diffusion coefficient was computed by fitting to an isotropic monoexponential signal model. Transmission electron microscopy (TEM) images were acquired using a JEM-2100 (JEOL, Japan) operated at 200 kV. The samples for TEM were prepared by depositing a drop of the aqueous colloidal suspension onto a copper grid. Fourier transform infrared (ATR-IR) analysis was conducted on an IRTracer-100 (Shimadzu) spectrometer.

Sample	**Hydrodynamic Size (nm)**	**ζ-potential (mV)**
NH_2-MSNs (10 % amination)	74.4 ± 0.4	41.4 ± 0.8
DOTA-MSNs	66.5 ± 2	23.8 ± 0.9
Dy-MSNs	70.3 ± 7	35.5 ± 3

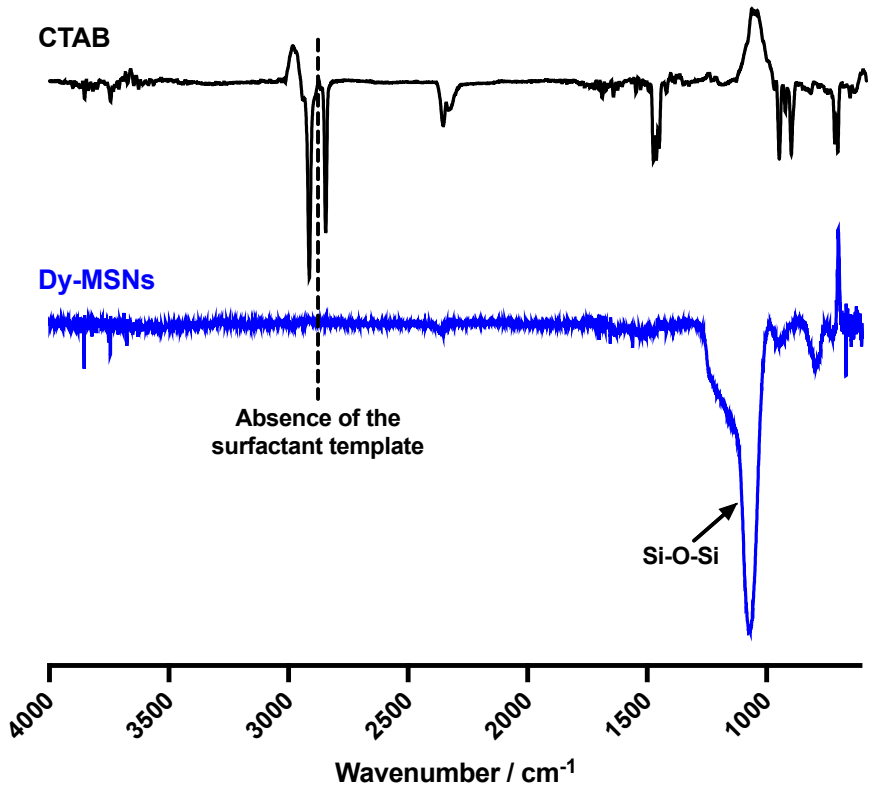
ESI 2. A table summarising the average hydrodynamic size and ζ–potential of aminated MSNs (10 % amination, NH_2-MSNs), DOTA-MSNs and Dy-MSNs. Both average hydrodynamic size and average ζ–potential was averaged using a minimum of three independently synthesised samples. The values for the Dy-MSNs were calculated from the average of all the samples used in this work.



ESI 3. The hydrodynamic size (n = 3, two-tailed t-test, ns, $P > 0.05$) and ζ-potential (n = 3, two-tailed t-test, ****, $P < 0.0001$), measured by DLS, of aqueous 1 mg/mL solutions of NH_2-MSNs, DOTA-MSNs and Dy-MSNs.



ESI 4. TEM images of Dy-MSNs with the scale bars indicating (a) 1000 nm, (b) 200 nm, (c) 200 nm and (d) 100 nm.



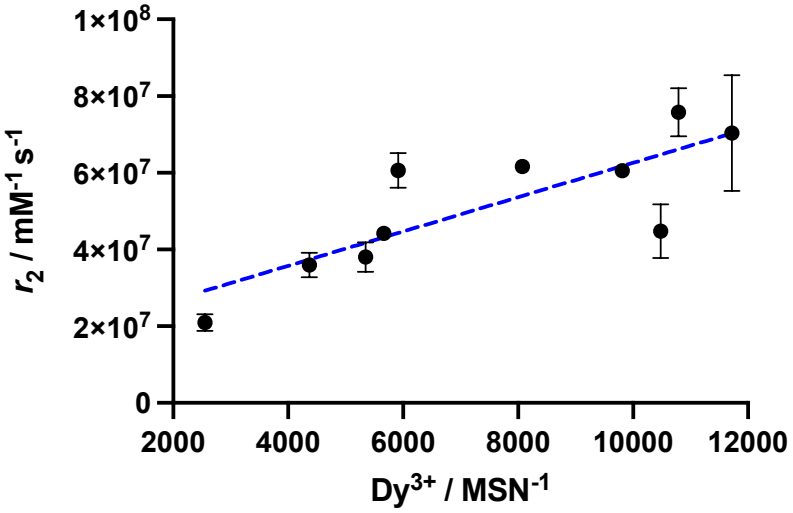
ESI 5. ATR-IR data for CTAB (black) and Dy-doped MSNs (blue). Both the Si-O-Si architecture and the removal of the surfactant template can be clearly observed.

Sample	Dy^{3+} / MSN
1	774
2	2553
3	4370
4	4949
5	5347
6	5665
7	5912
8	8075
9	9813
10	10480
11	10792
12	11720
13	12244

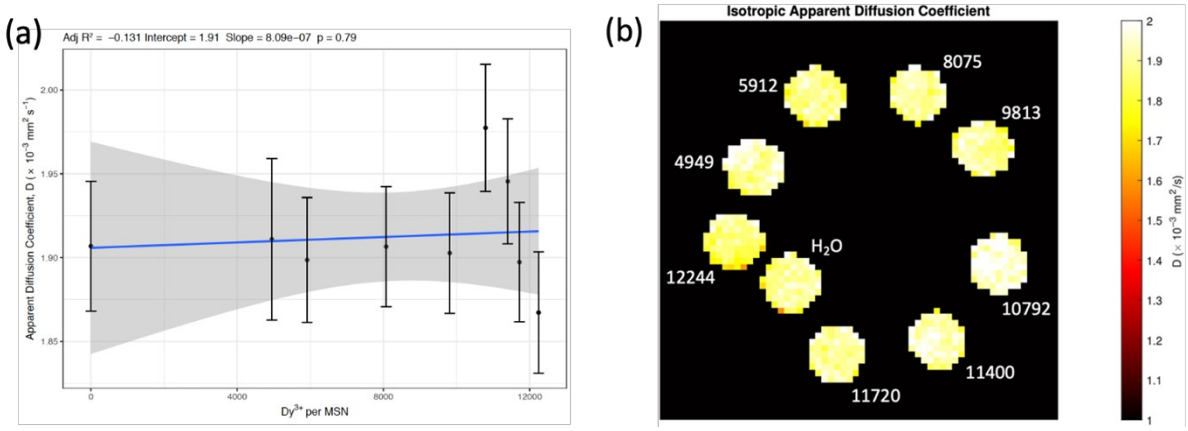
ESI 6. A table summarising the number of Dy^{3+} atoms (estimated) per nanoparticle. As can be observed in the table there is a clear increase in loading density for all the samples (calculated from ICP data).

Element	wt% ([Dy^{3+}] = 0.10 mM)	wt% ([Dy^{3+}] = 0.17 mM)
Oxygen	65.96 % ± 2.68	66.96 % ± 0.80
Silicon	32.03 % ± 2.46	30.24 % ± 0.80
Dysprosium	2.01 % ± 0.23	2.80 % ± 0.01

ESI 7. EDX measurements showing the composition of the nanoparticle. The results are consistent with a SiO_2 architecture with Dy-doped within the structure. The values obtained by EDX are consistent with those obtained by ICP.



ESI 8. The transversal relaxivity rate ($^{r}{}_{2}$) per particle recorded at 11.7 T for Dy-doped MSNs with a range of different loading densities.



ESI 9. The isotropic apparent diffusion coefficient ($^{D}{}_{eff}$) recorded at 7 T by MRI. (a) Recorded mean $^{D}{}_{eff}$ for each sample, with error bars representing the standard deviation of the measured value. (b) Map of $^{D}{}_{eff}$. The estimated number of Dy^{3+} atoms per MSN can be seen highlighted in ESI 9 (b). As can be seen in (a) and (b) there both are no significant differences between $^{D}{}_{eff}$ for any of the samples by pairwise t-testing; and additionally, there is no significant trend for altered $^{D}{}_{eff}$ with increasing Dy loading (p = 0.79 via a linear model fit), indicating that there are no differences to the diffusive water access between samples.