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Ultrahigh magnetic resonance contrast switching with water gated polymer–silica nanoparticles†

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Very high T_1 magnetic resonance imaging (MRI) switches can be obtained with pH-responsive polymer-coated paramagnetic mesoporous silica nanoparticles (MSNs), as the local environment traverses the pK_a of the polymer coat ($\Delta r_1 \sim 50 \text{ mM}^{-1} \text{ s}^{-1}$ at 1.5 T and $\Delta r_1 \sim 22 \text{ mM}^{-1} \text{ s}^{-1}$ at 3 T). We assign these characteristics to a strong peripheral hydration capping at the mesopores, impacting channel-confined water mobility such that outer sphere contributions to contrast are greatly enhanced.

Magnetic resonance imaging (MRI) is a powerful non-invasive diagnostic technique with micron spatial resolution and deep tissue penetration that empowers clinicians to resolve and monitor a wide variety of potentially fatal internal pathological conditions.^{1,2} Frequent low contrast-to-noise problems can be alleviated through the use of contrast agents (CAs), most notably chelated paramagnetic ions, such as gadolinium(Gd^{3+}).³ The contrast generating efficiency for a Gd^{3+} -based CA can be defined by its longitudinal relaxivity, denoted r_1 ($r_1 = \Delta(1/T_1)/[\text{CA}]$; where T_1 is the longitudinal relaxation time and $[\text{CA}]$ is the concentration of the CA), with a high r_1 correlating to a lower required Gd^{3+} dose, which is, of course, clinically desirable. To improve the natively low molecular r_1 values a broad range of paramagnetically doped nanomaterials, with reduced tumbling rates (τ_R), have been reported.⁴ Nanoparticulate CAs additionally present a route to controlled blood circulation times, additional imaging modality incorporation, and tumour accumulation.⁵ In prior work, we, and other researchers, have shown that Gd-chelate modified mesoporous silica nanoparticles (Gd-MSNs) offer a synthetically tuneable, and biocompatible, platform with high associated image contrast.^{6–12} It has been prior noted that restricted water mobility (elongated diffusional correlation times, τ_D)

within the nanoconfinement of a porous reservoir significantly boosts r_1 , in large part by increasing the role played by outer sphere effects.^{13,14}

Stimuli-responsive “smart” nanoparticulate CAs, where contrast can be switched by an endogenous or exogenous stimulus, such as light, enzyme activity, redox environment, or local pH,¹⁵ can provide specific information on the local physiological environment, facilitating the ability to distinguish between healthy tissues and lesions, for example.^{16,17} In reference to proton relaxation theory,^{18,19} tuneable contrast generation can be achieved by the modulation of the diffusive mobility of either inner-sphere (those bound to the metal ions at $\sim 3.1 \text{ \AA}$) or outer-sphere (with water-to-Gd distances $\geq 4.0 \text{ \AA}$) water molecules.²⁰ For example, a number of switchable nanoparticulate CAs have been designed where the inner-sphere (IS) water exchange rate (τ_M) can be modulated by the local environment.^{21–24} Prior reported smart CAs possess, however, only moderate relaxometric switches (*i.e.* $\Delta r_1 < 15 \text{ mM}^{-1} \text{ s}^{-1}$ and often much less).^{25,26} It is also known that relaxivity unhelpfully decreases for typical Gd-based CAs at the higher imaging field strengths that are becoming increasingly common.^{20,27} There is, therefore, substantial room to design a high field effective CA that exhibits a significant environmentally triggered switch. To date, no stimuli-responsive CA characteristics have been reported that operate through a modulation of OS contributions.

Herein, we report a versatile surface-initiated reversible addition-fragmentation chain-transfer (SI-RAFT) polymerisation approach to modify Gd-chelate doped MSNs with a stimuli-responsive, externally grafted, polymer shell (Fig. 1a). Poly(methacrylic acid) (PMAA) has been widely reported within pH-responsive drug delivery applications,^{28–30} possessing an associated pK_a of 5.2, clinically relevant, for example, to mapping deviations in pH associated with chronic inflammation.^{31,32} It is also known that in its charged state PMAA has a particularly strong association with water.³³ It was envisaged that such a capping would impact the mobility of particle internalised water, and hence optimise OS relaxivities (r_1^{OS}).

Initially, paramagnetic Gd-MSNs (with gadolinium (iii) 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid, Gd-DO TA, modified pore channels) were synthesised according to

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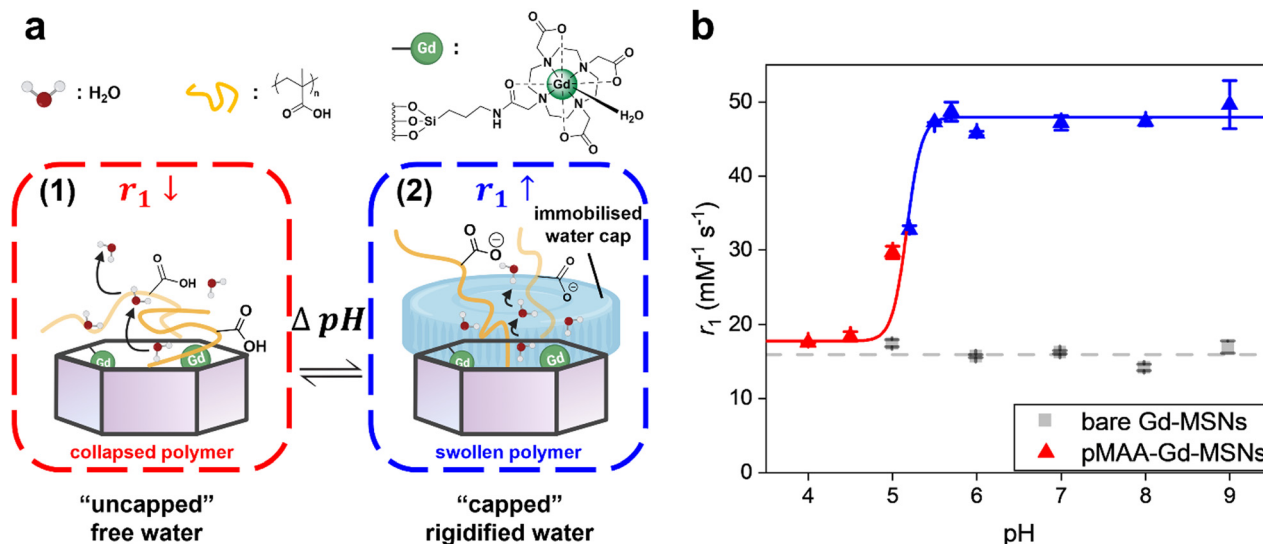


Fig. 1 (a) An illustration highlighting the pH-responsive T_1 switch for pMAA-Gd-MSNs. For an uncharged/collapsed conformation (1), the peripheral pMAA shell possesses limited hydration, and moderate r_1 values. As the polymer shell charges and swells, its hydration increases dramatically, and a “water cap” is formed (2). (b) A plot showing the longitudinal relaxivity values (at 1.41 T) for the bare Gd-MSNs and pMAA-Gd-MSNs. The latter exhibits a $\Delta r_1\%$ > 182%, with the r_1 trend fitted using a Boltzmann equation to give an associated estimated $pK_a = 5.2$ ($R^2 = 0.99$) as expected.

prior reports.^{34,35} The particles exhibited high colloidal uniformity with an associated size of 49.5 ± 4.2 nm (resolved by transmission electron microscopy, TEM, ESI 1a†) and a corresponding pore diameter of 3.2 ± 0.2 nm (Barrett-Joyner-Halenda pore size analysis, ESI 2†). A time delayed co-condensation method (with 0.15 mol% of an aminated silane added), biases the localisation of amino anchor groups at either the internal or external pore channel, as prior reported.³⁶ Chemical modification with an activated DOTA-NHS ester and subsequent metalation leads to the generation of the desired paramagnetic MSNs. The outer surface of Gd-MSNs was exclusively modified with a 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid modified silane (DDMAT-silane) chain transfer agent (CTA), (~ 2.9 nm in size, too large to enter the mesopores, ESI 3†). Particle modification was confirmed by ultraviolet-visible spectroscopy (UV-Vis, ESI 4†), thermogravimetric analysis (TGA, ESI 5†) and attenuated total reflectance infrared spectroscopy (ATR-IR, ESI 6†), with a calculated CTA grafting density of *ca.* 4.5 groups nm^{-2} (ESI 7†). This density facilitates the formation of a dense, thickness tuneable (based on RAFT conditions, ESI 8†), polymer brush coating from a wide variety of potential polymerizable monomers.^{37–39} The generated polymer coated Gd-MSNs show an observed (and expected) increase in particle diameter after the SI-RAFT polymerisation (TEM, ESI 1b, c, and DLS ESI 8†). The living-character of the polymerisation process was confirmed by proton nuclear magnetic resonance spectroscopy (^1H NMR, ESI 9†), where monomer consumption was shown to follow pseudo-first-order kinetics (ESI 9 insets†).^{40,41} The generated pMAA-Gd-MSNs possess high colloidal stability, across a broad pH range (in 10 mM Britton-Robinson buffer solution, pH 4.0–9.0), with extremely low polydispersity variation over 30 days (ESI 10,† polydispersity indices < 0.08). An expected pH-responsive switch in hydrodynamic diameter as the pK_a of pMAA⁴² is traversed (ESI 8a†). The particles

exhibit no toxic effects during a 48 h exposure to HeLa cells or HEK-293T cells (ESI 11†).

Relaxivities of pMAA-Gd-MSNs were first assessed by NMR (1.4 T), where dramatic enhancements in r_1 were observed as the pK_a of pMAA is traversed (Fig. 1b, $\Delta r_1 = 30.3 \pm 3.2 \text{ mM}^{-1} \text{ s}^{-1}$ across 1.0 pH-unit, from pH 4.0 to pH 7.0). This “switch-on” response is not observed in the absence of a pMAA polymer coating; native Gd-MSNs exhibit constant relaxivities across the full pH range. In analogous poly(dimethylaminoethyl methacrylate) (pDMAEMA) brush coated particles, where a much weaker H-bond association with bulk water is expected,^{43,44} (ESI 12†) the determined relaxivities overlap with those of bare Gd-MSNs and are non-responsive (ESI 13†). For the pMAA particles, associated image contrast enhancements were confirmed through spatially-resolved T_1 -mapping experiments on clinical imaging scanners (1.5 T and 3 T; Fig. 2a), with associated T_1 and R_1 values shown in ESI 14.† These switches in r_1 are the highest reported at both magnetic field strengths (Fig. 2b and c, $\Delta r_1 = 50.5 \text{ mM}^{-1} \text{ s}^{-1}$ at 1.5 T and at $\Delta r_1 = 21.8 \text{ mM}^{-1} \text{ s}^{-1}$ at 3 T).^{25,26} It is also notable that the relaxivities, at both fields, exceed the theoretical maxima for solely IS contributions (optimised $r_1^{\text{IS}} \sim 40 \text{ mM}^{-1} \text{ s}^{-1}$ at 1.5 T and $\sim 20 \text{ mM}^{-1} \text{ s}^{-1}$ at 3 T),^{20,27} suggesting the presence of a composite IS/OS contribution. In fact,^{6,45} IS contributions are expected to be much lower than that here (*ca.* $16 \text{ mM}^{-1} \text{ s}^{-1}$ at 1.5 T, seen ESI 16†). OS contributions are, then, both substantial and responsible for the observed Δr_1 switch (more analyses detailed below).

In examining the effect of polymer thickness and Gd-localisation within the particle mesopores, we note that a larger magnitude switch is observed with thicker polymer shells (at 1.4 T, ESI 17a†) but that switching magnitudes are largely



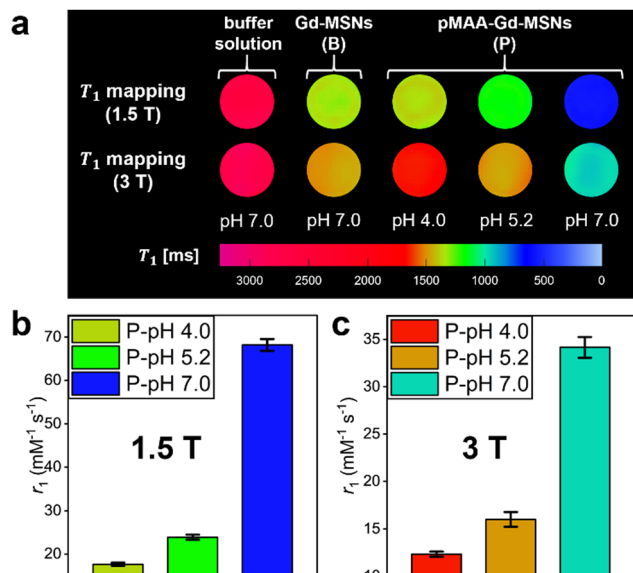


Fig. 2 (a) T_1 maps, recoded at pH 4.0, pH 5.2 and pH 7.0 (1.5 T and 3 T clinical MRI scanners), for the pMAA-Gd-MSNs (denoted as "P-"). The T_1 maps for the bare-Gd-MSNs are reported at pH 7.0 (denoted as "B-"). The MR derived relaxivities for the associated nanoparticles, across a range of pH, are included at both 1.5 T (b) and 3 T (c).

insensitive to Gd-depth (at 1.4 T, ESI 17b†). This is further confirmatory of a polymer-mediated origin.

To further examine the origin of this large magnitude relaxivity switch we refer to Solomon-Bloembergen-Morgan (SBM) theory (ESI 15†). Contributions from polymer-swelling induced changes in global particle rotation are negligible (ESI 16a†). An analysis of Eu-analogues confirms metal hydration to

be the same (ESI 18†) in polymer modified particles at both pH 4.0 and pH 7.0. Additionally, the modulation of the water exchange rate (τ_M) through changes in the conformational state of the polymer cannot fully account for such a high observed switch in the acquired r_1 value. Specifically, the modelled nuclear magnetic relaxation dispersion (NMRD) profile (ESI 16b†) for the IS contribution to r_1 highlights that the role of τ_M is much less significant than the expected influence of τ_D , accounting only for (at best) ~20% of the switch in relaxivity. In recent work the presence of an MSN peripheral immobile water layer has been reported to have a substantial effect on the diffusion coefficient (D) of pore-internal water.⁴⁶ It is known that a charged polyacid brush has an unusually strong association with water, dramatically reducing its mobility.^{47,48} Within a modified SBM model (ESI 15†), an entirely realistic (30–90 fold) reduction in internal water diffusion, D , can account for the enhancement in relaxivity (Fig. 3). We propose, then, that the triggered polymer charging generates a peripheral particle water "cap" that dramatically increases OS relaxivity by virtue of its impact on particle internalised water.

To summarise, we present here paramagnetic inorganic-organic hybrid nanoparticles that exhibit a pH-mediated contrast switch that is sharp (across < 1.0 pH-unit), of an unprecedented magnitude, clinically relevant, and mechanistically new.

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Conflicts of interest

There are no conflicts of interest to declare.

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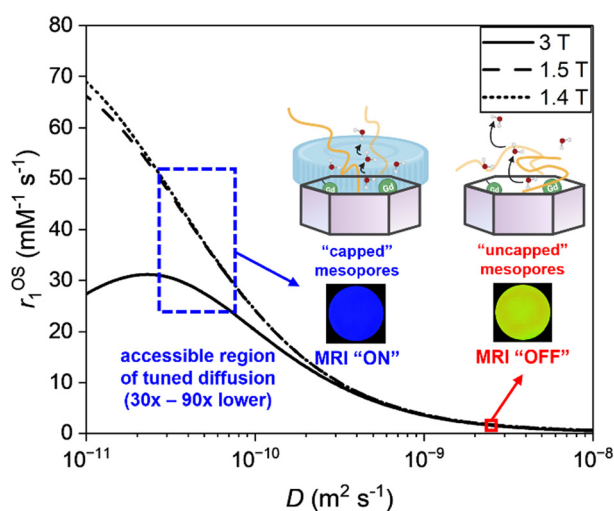


Fig. 3 The effect of the local diffusion coefficient of water on the outer-sphere relaxivity. This analytical treatment employs a modified Freed equation (at 1.4, 1.5 and 3 T), and considers the diffusion of water at $r_{GdH} = 4.0$ Å from the paramagnetic centres (ref. 49). The diffusion coefficient of confined H $_2$ O with "uncapped" mesopores is taken from ref. 46. The diffusion coefficient of confined H $_2$ O with capped mesopores is deduced from the experimental relaxivity data, where D is observed to be reduced by 30–90x.



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