Porous Mn^{2+} - Fe_3O_4 Nanoparticles with High T_1 and T_2 Relaxivity

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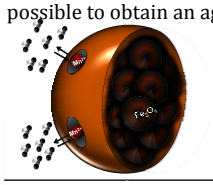
Introduction: Targeted and smart molecular contrast agents have made it possible to detect events in the molecular scale *in vivo* (1). While many iron oxide based contrast agents with per ion T_2 relaxivity of ~100$mM^{-1}s^{-1}$ allow the detection of nM concentrations, common lanthanide chelates such as Gd-DTPA, with T_1 relaxivity ~5$mM^{-1}s^{-1}$, require µM-mM concentrations, making the study of molecular events difficult with T_1 weighting. For a given T_1 and T_2 relaxivity, T_1 changes are more readily detected in vivo due to longer tissue T_1 than T_2. Recently, graphitic carbon shells of Fe-Co alloys have been developed with high T_2 and T_1 relaxivities to detect sub-nanomolar concentrations (5,6). Ferritin has been used as a MRI contrast agent (2-4) that is small (13 nm), and easily functionalized. Because it is partially filled in its native form, several groups have developed methods to synthesize magnetoferritin to increase its transverse relaxivity (2,3) but only few have demonstrated enhanced longitudinal relaxivity (~80$mM^{-1}s^{-1}$ per ion), by using the apoferritin protein as a cage for paramagnetic ions (4). Here we synthesized apoferritin with Fe_3O_4 crystal cores and Mn^{2+} ions bound at metal binding sites located in the hydrophilic channels of the protein (7). This newly synthesized particle has an unusually high per-ion T_1 relaxivity, likely due to a cooperative relationship between the magnetite crystal core and the Mn^{2+} ions bound to the protein channels. While the particles have a T_2 relaxivity of 133$mM^{-1}s^{-1}$, the T_1 effect can nonetheless be detected *in vivo* in tens of nM concentrations. It may be possible to further exploit cooperative effects between different metal species in porous nanoparticles to increase R_1.

Methods: Nanoparticle Synthesis: 2µM Apoferritin (Sigma Aldrich, St Louis) buffered in 0.05M MES at pH 8.5, 48mM Fe(II)Chloride (Sigma Aldrich, St. Louis) and 4.8mM Mn(II)Chloride (Sigma Aldrich, St. Louis) were de-aerated for 30 minutes with N_2 (50psi). The apoferritin solution was kept in a water bath at 55 to 60°C. Every ten minutes, 125µl of Mn(II)Chloride were added to the apoferritin solution, for a total of 4 times. Every ten minutes 125µl of Fe(II)Chloride were added. This was repeated for 12 and 8 additions of Mn(II)Chloride and Fe(II)Chloride. The solution turned turquoise upon by the 10th addition of metal and dark brown color by the 20th addition. Samples were dialyzed against 0.15M NaCl, and were filtered using a magnetic column (Miltenyi Biotec, Gladbach, Germany) and washed with 0.15 NaCl buffer. The resulting protein concentration was obtained with a Bradford assay, and inductively coupled plasma – optical emission spectroscopy (ICP-OES) was used to measure total iron and manganese concentration. Electron Microscopy: Samples were adsorbed on Cu-C grids and transmission electron microscopy (TEM) images were obtained using a Philips CM12 electron microscope, also high-resolution transmission electron microscopy (HREM) images were obtained using a Philips CM200-FEG TEM/STEM. Fast Fourier Transform patterns of the HRTEM images were obtained from 4 different particles using Imagej (NIH). Relaxometry: Relaxivity measurements were performed utilizing a 1.5T Bruker relaxometer. Bruker's minispec software and exponential curve-fitting were utilized on several different dilutions of particle suspended in a 1% agarose gel to find the corresponding T_2 values (Inter pulse τ = 20ms, 200 points) and T1 values (pulse separations ranging from 5 to 20000ms, 4 scans, 7 points). In order to compare across agents of different size we introduced a volumetric relaxivity that takes into account the per-particle relaxivity per unit volume of particle. In vivo Imaging: Adult male Sprague Dawley rats were scanned in a 7T Bruker and a surface RF coil after stereotactic injection of control (native ferritin or magnetoferritin) and our agent into the striatum. An IRTruFISP sequence (TE.TR= 2.2/4.4ms, flip angle= 60°) and a MSME sequence (TE/TR= 11/2500ms, flip angle= 180°) were performed to obtain the T_1 and the T_2 maps.

Results and Conclusions: A schematic of the interaction of the particle with the surrounding water is depicted in Figure 1 (top). We suspect a cooperative effect between the Mn^{2+} ions located in the channels and the ferromagnetic crystal core while exchanging with the bulk water. Notably, we were only able to increase T_1 relaxivity if both manganese and iron were incorporated in a 1:10 concentration ratio respectively, and then only if Manganese was added first. Electron microscopy showed high electron dense cores ranging from 3nm to 6nm in diameter (Figure 2). Based on the inverse FFT, we measured an average fringe spacing of 2.5Å, consistent with magnetite (Figure 3). Following our protocol ICP-OES provided with a total of approximately 7 Mn^{2+} ions per particle which corresponds to previous reported values for ferritin binding (8), and confirming that no alloy was formed as all manganese ions were bound to the channels. Based on volumetric relaxivities the particles had high T_1 and T_2 relaxivity values as reported in Table 1. Furthermore, to confirm the effectiveness *in vivo* we injected this agent into a rat striatum. As a control we used native ferritin and magnetoferritin at the same concentrations. Because the R_2/R_1 of this agent is considerably high it was difficult to null the T_2* effect and obtain a clear hyperintense region caused by the agent with a routine T_1-Weighted sequence. However, T_1 maps obtained from the IR-TrueFISP sequence show a prominent decrease in T_1 (Figure 4). We conclude that the proposed synthesis configuration creates a ferritin crystal core composed of Fe_3O_4. By taking advantage of the specific Mn^{2+} binding sites located in the hydrophilic channels it is possible to obtain an agent with unusually high T_1 and T_2 relaxivities due to a cooperative effect of the ions and the crystal.



		R_2 $mM^{-1}s^{-1}$	R_1 $mM^{-1}s^{-1}$
Manganese doped particles	Fe	133	1.449
	Mn	31112	338.6
	Particle	222481	2421
	Volumetric /nm³	193.40	2.10
Magnetoferritin particles	Fe	78	0.07
	Particle	404,045	407
	Volumetric /nm³	351.23	0.35

Figure 1. Representation of the synthesized particle depicting a magnetite crystal core and Mn^{2+} ions bound to protein channels.
Table 1. Relaxometry results reported in a per-ion, per particle and in a volumetric basis (per-particle relaxivity/particle volume).

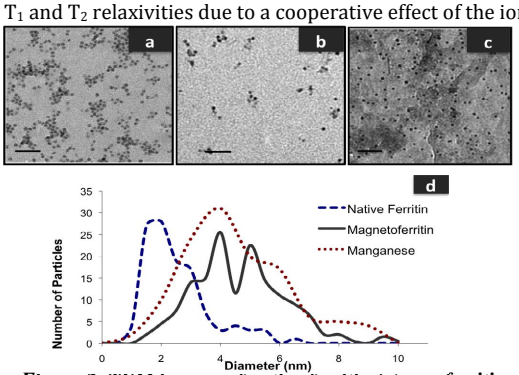


Figure 2. TEM images of native ferritin (a), apoferritin with Mn^{2+} and magnetite cores (b), and magnetoferritin (c). Particle size distribution based on the metal cores (d).

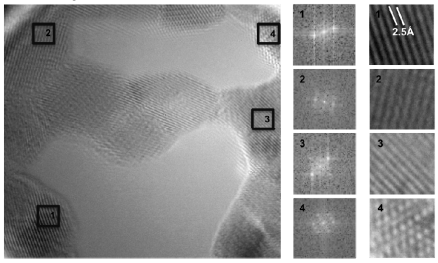


Figure 3. (top right) HRTEM showing lattice frindges. FFT and Inverse FFT of ROIs confirm the presence of magnetite in the apoferritin cores (line spacing = 2.5Å).

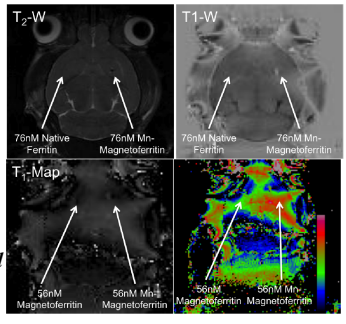


Figure 4. (bottom right) In vivo MRI of rat striatum injected with control (either native ferritin or magnetoferritin) for T_2 and T_1-weighted (retro phase corrected) as well as a T_1 map of the injection site.

References: **1.** Merchant et al. IJRI 14(3).2004 **2.** Bulte JW et al. JMRI. 4(3).1994 **3.** Uchida M, et al. Magn Reson Med 2008;60(5):1073-1081 **4.** Aime S, et al. Agew. chem. Int. Ed2202, 41, No.6. **5.** Seo WS et al, Nat. Mater. 2006;5:971–976 **6.** Lee J et al., MRM 62:000-000(2009) **7.** Wardeska J, et al. J. of Biol. Chem. Vol 261. No. 15. 6677-668 (1986).