Chemical Doping of Iron Oxide Inside Apoferritin to Form an MRI Contrast Agent with High r_1 and Low r_2

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Introduction: There is a strong need for molecular MRI contrast agents that are detectable in sub-µM concentrations (1,2). Current techniques to improve contrast agent detection include the use of nanoparticles in order to increase the transverse relaxivity by either increasing the size of the superparamagnetic particle or by doping the nanoparticle crystal in order to enhance the magnetic moment (3). Although T_2-shortening agents often have high relaxivities, agent accumulation is often less detectable because of the short intrinsic T_2 in tissue. With the same relaxivity, a T_1 shortening particle can be detected *in vivo* in roughly 30X lower concentrations. T_1-shortening nanoparticles have been developed by either loading with paramagnetic ions (4, 5), or by shrinking the particle to increase the volume fraction of spin-canted layer (6). These techniques result in high r_1 but often also high r_2, placing a limit in concentration of agent detected with T_1-weighting. Here we propose to use chemical doping with tungsten to disrupt exchange coupling in a crystal to localize the net magnetic moment, creating a paramagnetic crystal. We tested this concept by adding tungstate to the iron oxide crystal to form an amorphous composite with iron oxide and tungsten inside the apoferritin protein shell.

Figure 1.

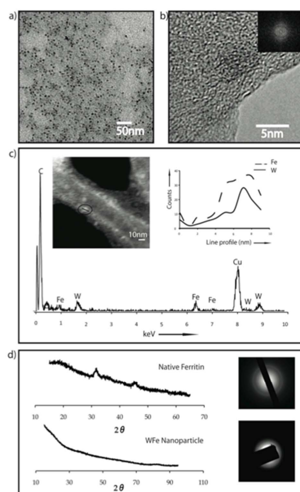

Figure 2.

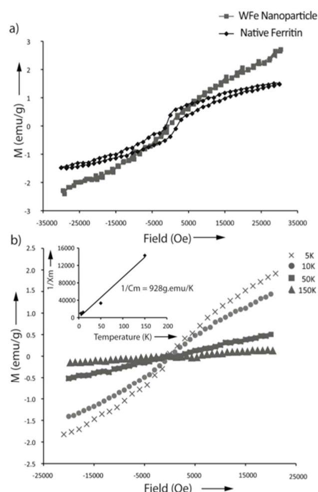

Figure 3.

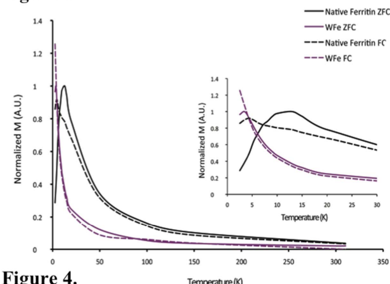

Figure 4.

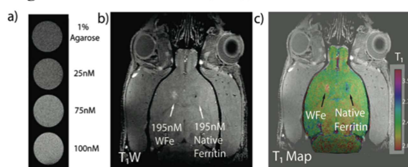

Figure 1. a) TEM of WFe-apoferritin nanoparticles. b) HREM showing no lattice patterns. c) STEM and EDX showing Fe and W evenly distributed within the apoferritin core. d) XRD and SAED of WFe-apoferritin nanoparticles showing clear lack of structure and diffraction.

Figure 2. a) Magnetic reversals showing the paramagnetic behavior of the WFe-apoferritin particles. Native ferritin is ferromagnetic at 5K. b) Temperature dependent measurements indicate the Curie constant.

Figure 3. ZFC and FC for WFe-apoferritin and native ferritin. The blocking temeperature is reduced compared to Native Ferritin and the coupling below the curie temperature is ferromagnetic.

Figure 4. a) Nanoparticles suspended in 1% agarose were detected at concentrations as low as 25nM. b) Hyperintensity was found at the site of WFe injection. c) T_1-map indicated that the T_1 at the site of WFe injection was 22% shorter than that of Native Ferritin at the same concentration.

Methods: *Particle Synthesis:* To synthesize the tungsten and iron oxide filled apoferritin, 2µM apoferritin was buffered in 0.05M MES at pH 8.5. Separately, we used 48mM $FeCl_2$, and 48mM Na_2WO_6. All solutions were de-aerated with N_2 and kept at 55 to 60°C under vacuum. We added the 48mM $FeCl_2$ at 12.5µl/min to the stirring apoferritin solution using a syringe pump for a total of 140mins. Fifty minutes into $FeCl_2$ delivery, the 48mM Na_2WO_6 was added simultaneously at 12.5µl/min for a total of 40 minutes. 200µl of 300mM sodium citrate was then added. The solution was sonicated for 10 min, and spun for 10 min. at 957 · *g*. The supernatant was collected and dialyzed (8kDa cut-off) overnight against de-ionized water. The protein solution was filtered using 0.8µm and 0.2µm syringe filters. Protein and metal concentrations were obtained with a Bradford assay and ICP-OES. *Relaxometry:* Samples containing 1% agarose/sample were used to measure relaxivity at 37°C using a MQ60 1.5T Bruker Minispec. T_2 values were measured with a CPMG pulse sequence (Inter pulse τ = 4ms, TR = 15s, 75 points) and T_1 with an IR pulse sequence (First TI = 5ms, last TI = 20,000ms, TR = 15s, 8 averages, 10 points). *Electron Microscopy and Diffraction:* Samples were adsorbed on Cu-C grids and transmission electron microscopy (TEM) images were obtained with a Philips CM12S Scanning TEM. High Resolution Electron Microscopy (HREM) and Energy dispersive X-Ray (EDX) were measured with a Philips FEI CM-200 TEM. Selected Area Electron Diffraction (SAED) samples were adsorbed on a holey-copper grid and loaded in a JEOLARM200F. X-Ray Diffraction (XRD) powder samples were loaded on a SIEMENS D5000 powder X-Ray Diffractometer with a CuKα1 source. *SQUID Magnetometry:* A Quantum Design MPMS-5S Superconducting Quantum Interference Device (SQUID) was used. Reversal curves were obtained at 5K from 3T to -3T. *In vivo Imaging:* Adult Sprague Dawley rats were secured to a stereotactic frame. A 10µL Hamilton syringe needle was used to inject into the caudate/putamen. 8µL of WFe and Native Ferritin were injected into the left and right hemispheres, respectively. Rats were imaged on a 7T Bruker scanner using a surface RF coil. A FLASH sequence was used (TE/TR= 3.8ms/55.9ms, NEX 4) and a T_1-map was obtained with a RARE sequence, TE/TR = 10.88ms/233.3ms, 500ms, 1200ms, 2500ms, 5000ms, NEX 1.

Results and Conclusions: The addition of a strong ligand, tungstate, in the iron oxide crystal resulted in a paramagnetic particle. Relaxometry experiments demonstrated a particle r_1 of 4870$mM^{-1}s^{-1}$, and r_2/r_1 of 1.86. Electron microscopy indicated that the particles were monodisperse, with fully filled apoferritin cores, and with no apparent lattice spacings (Figure 1a and b). Further studies on the characteristics of the crystal with EDX, STEM, XRD and SAED indicated that 1) tungsten was incorporated into the iron oxide crystal evenly, as seen in Figure 1c. 2) The particles were amorphous with a lack of diffraction patterns as compared to native ferritin (Figure 1d). SQUID magnetometry suggested that the crystal was a Curie-Weiss paramagnet with an overall magnetic moment of 64μ_B. (Figure 2a, b). This resulted in a per- Fe^{3+} relaxivity of ~ 386$mM^{-1}s^{-1}$. ZFC an FC results showed a reduced blocking temperature compared to native ferritin (Figure 3). The particles were detected *in vitro* at concentrations as low as 25nM. The tungsten-iron apoferritin particles were detected *in vivo*, with a T_1 22% shorter than the control (Figure 4a-c). The chemical disorder in the amorphous composite allows individual paramagnetic moments to remain de-coupled. Since dipolar interactions between paramagnetic moments and proton spins are local, only surface un-coupled moments are available to contribute to bulk relaxation. We conclude that chemical doping may be a novel route towards the rational design of sensitive T_1-shortening nanoparticle contrast agents.

References: (1)Fumita, M. et al., Neuropsychopharmacology(2001). (2) Kyrtsonis, MC., Eur J Haematol (2004) (3)Jun, YW et al., Acc. Chem. Res. (2008). 4) Kalman, FC. et al., Angew. Chem (2010). (5) Aime, S. et al., Agew. CHem (2002) (6) Kim, BH. JACS (2011)