Distinguishing magnetic nanoparticles by r2/r1

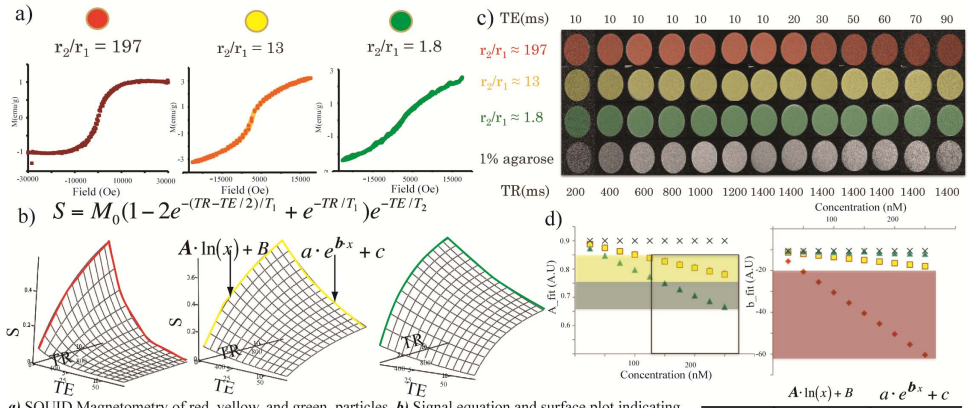
Veronica Clavijo Jordan[1,2] and Kevin M Bennett[3]

[1]*University of Hawaii at Manoa, Honolulu, HI, United States,* [2]*Arizona State University, Tempe, AZ, United States,* [3]*University of Hawaii at Manoa, HI, United States*

Introduction: Targeted nanoparticles have been developed as contrast agents for MRI to detect molecules and cells in vivo. A fundamental constraint in nanoparticle agents and, indeed, in many MRI contrast agents, is that they are not readily distinguished from each other after delivery. This constraint has been recently overcome in specific, fabricated agents by tuning off-resonance and exchange through geometry (1). Other groups have developed chemical exchange saturation transfer agents and took advantage of their off-resonance effects from their ligands (2,3). Here we demonstrate that typical superparamagnetic nanoparticles of similar size can be distinguished from eachother based on the ability to modify the crystal structure in the apoferritin crystal core. By creating crystal structures with distinct r_2/r_1 inside the protein cavity it is possible to synthesize "colored particles". The r_2/r_1 creates a unique profile in a three-dimensional space with axes of TE and TR. The nanoparticles are detected by traversing this surface with the MRI parameters TE and TR, and reconstructing the unique profiles to distinguish the different particles. This may represent a unique method to differentiate multiple particles within the same image with ~nM sensitivity non-invasively with MRI.

Methods: *Particle Synthesis "red particle":* To synthesize magnetoferritin with manganese ions adsorbed to surface hydrophilic channels, we used our published protocol where apoferritin was filled by making a total of 12 $MnCl_2$ and 8 $FeCl_2$ additions to a apoferritin solution, ensuring that manganese was added first in the sequence (4). *Particle synthesis "green particle":* To synthesize a tungsten and iron oxide filled apoferritin, 2μM apoferritin was buffered in 0.05M Morpholino Ethanesulfoxide (MES) at pH 8.5, 48mM $FeCl_2$ and 48mM Na_2WO_6 were de-aerated for at least 15 minutes with N_2, and kept in a water bath at 55- 60°C. We added the de-aerated 48mM $FeCl_2$ at a rate of 12.5μl/min, to the apoferritin solution using a syringe pump for a total of 140 minutes. Fifty minutes into $FeCl_2$ delivery, the de-aerated 48mM Na_2WO_6 was added at a rate of 12.5μl/min for a total of 40 minutes and 200μl of 300mM sodium citrate was added. The solution was then sonicated for 10 minutes and spun for 10 minutes at 957 · *g*. The supernatant was collected and dialyzed overnight against de-ionized water using 8,000 MW cut off dialysis tubing. The protein solution was filtered using 0.8 and 0.2μm syringe filters. Total protein concentration was obtained with a Bradford Assay Kit and metal concentation with ICP-OES. *Particle synthesis "yellow particle":* To synthesize a tungsten and iron filled apoferritin with Mn^{2+} ions adsorbed on the pores, both previous synthesis schemes were combined. Firstly, a previously characterized WFe nanoparticle solution was obtained as described herein. The concentration of $MnCl_2$ and $FeCl_2$ solutions were tailored to a ratio of 2400 Mn and 24000 Fe ions per protein. The solution was suspended in 0.05M MES buffer at pH 8.5, de- aerated for at least 15 minutes and heated 0.05M MES buffer. $MnCl_2$ and $FeCl_2$ were pumped at a rate of 12.5μl/min in alternate intervals of 10 minutes each for a total of 50 minutes and purified in the same manner as for the "yellow particle". *Relaxometry:* Relaxivity was measured using a MQ60 1.5T Bruker Minispec relaxometer (Bruker Optics, The Woodlands, TX). Bruker's curve-fitting tool was used to find the corresponding T_2 values using a Carr-Purcell-Meiboom-Gill pulse sequence (CPMG) (Inter pulse τ = 4ms, gain = 62, TR = 15s, 75 points). T_1 values were obtained using the curve- fitting and the relaxation times were obtained with an Inversion Recovery pulse sequence (First TI = 5ms, last TI = 20,000ms, duration factor = 2.513, TR = 15s, 8 averages, 10 points). *Superconducting Quantum Interference Device (SQUID Magnetometry):* Samples were freeze-dried using a lyophilizer after freezing at -80°C overnight. Each sample was weighed before measurements. The sample was loaded into a SQUID magnetometer sample holder. A Quantum Design MPMS- 5S SQUID was used for all measurements. Hysteresis curves were obtained at 5K from +30000Oe to - 30000Oe. *In vitro MR Imaging:* Samples were suspended in 1% agarose gels and imaged in a 7T Bruker small animal scanner using a quadrature volume coil. A Multi-Shot Multi-Echo pulse sequence was used with the following parameters: TE/TR=10ms/200,400,600,800,1000,1200,1400ms, and TE/TR=10,20,30,40,60,70,90ms/1400ms and NEX=2. *Image Processing:* Images were analyzed with the ImageJ software. ROIs were selected for each particle-containing phantom, as a control a 1% agarose gel without agent was used to account for the background.

Results and Conclusion: The ability to tune the magnetic properties of the crystal within apoferritin resulted in a set of three different particles, the "red" particles with the highest r_2/r_1 of 197, "yellow" with r_2/r_1 of 13, and the "green" with the lowest r_2/r_1 of 1.8. These particles were the result of adsorbing and doping the iron oxide crystal with Mn^{2+} and $(WO_4)^{-2}$, respectively. The strategic placement of these ions allowed us to tune the magnetization and result in a set of particles that range from paramagnetism (tungsten doped) to superparamagnetism (Mn^{2+} adsorbed, and Mn^{2+}adsorbed on a tungsten doped crystal). SQUID magnetometry in Figure 1a demonstrated their magnetic differences, where the most superparamagnetic particles showed slight coercivity, and the paramagnetic crystal showed almost linear field dependence within -3T to 3T. We simulated the effect that these particles would have on the surrounding water using the signal equation in Figure 1b, each point in the surface plot is the value of the signal of the surrounding water when scanned with their specific TE and TR combination. We see that each particle has a unique surface plot. By isolating the edge where TE is constant at a low value (10ms) we see that the profile with varying TR has the most prominent increase rate for the green particle followed by a less prominent rate for the yellow particle and a low rate for the red particle. Conversely, isolating the edge where TR is constant at a high value (1400ms) we see that the profile with varying TE has the most prominent decay rate for the red particle, followed by a less prominent decay for the yellow particle and a low rate for the green particle. These unique signatures are attributed to the fact that these three particles have significantly and uniquely different r_2/r_1 combinations. We tested this in vitro in a gel phantom (Figure 1c) and assigned each signal a color depending on their increase and decay rates. Table 1 includes the constants that were fit to a logarithmic function for the "increase" profile and an exponential function for the "decay" profile. These constants combinations are unique for each particle. Figure 1d also illustrates how this technique is robust for particle concentrations of at least 100nM. At lower concentrations it would be difficult to discriminate amongst particles. Further work is needed to distinguish nanoparticles within an imaging voxel. We conclude that magnetic nanoparticles can be distinguished on the basis of r_2/r_1.



a) SQUID Magnetometry of red, yellow, and green particles. ***b)*** Signal equation and surface plot indicating the profiles and equations for fitting. Coefficient "A" and "b" are unique for each particle. ***c)*** in vitro phantom of red, yellow, and green particles suspended in 1% agarose gel. The imaging technique scanned the surface plot profiles in order to determine the most significant differences amongst particles. ***d)*** Concentration limits where the particles can still be significantly different from each other. Table indicatingthe unique coefficients for each color in vitro.

	$A \cdot \ln(x) + B$	$a \cdot e^{bx} + c$
Red	6.50E+03	-2.37E-02
Yellow	6.43E+03	-2.24E-03
Green	7.18E+03	-6.96E-04
1%	6.23E+03	-1.58E-03

References: (1) G. Zabow et al. *Nature Methods* 5, 668 (2008) (2)M. McMahon *Magn Reson Med, 60*(4), 803-812 (3)S. Viswanathan et al. *Angew. Chem. Int. Ed* 2009, 48, 9330-9333 (4) V. Clavijo Jordan et al. Mag.Res.Med 64, 5(2010).