Aggregation of an Endogenous MR Reporter Protein to Increase Relaxivity

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Introduction: Manipulating iron metabolism pathways to increase cellular ferritin and ferritin overexpression have been proposed as methods for detection of gene expression using MRI (1-3). Studies to date have shown that overexpression of ferritin can increase transverse relaxation rates in tissue. If it is assumed that the R_2 relaxivity of ferritin (per iron) at 11.7T is approximately 10/mM/s, and the background R_2 of the brain is approximately 33 /sec, it follows that, to achieve a 10% change in R_2, ferritin subunits must be overexpressed to concentrations on the order of 10 μM in a single pixel. Normal iron levels in brain tissue suggest ferritin concentrations of approximately 10 nM in a cell. To bypass the large levels of ferritin expression necessary it is important to find ways to improve the relaxivity of ferritin. One way to improve relaxivity of iron oxide particles is to make them aggregate, shifting relaxivity into the "static-dephasing" regime (4). In this work, we show that making ferritin aggregate can increase its relaxivity *in vitro*. An advantage of aggregation is that it could be controlled inside cells (5), and could thereby be used as a reporter of molecular events occurring on the order of seconds or less.

Methods: *Sample Preparation:* Cationized horse spleen ferritin (CFer) was obtained from Sigma (St. Louis). We used 3,3'-dithiobis(sulfosuccinimidylpropionate) (DTSSP, Pierce), to cross-link primary amines on the CFer in the presence of TRIS (for controls) and PBS (for cross-link samples) with a 12-nm linker. After one hour, PBS was added to the controls and TRIS was added to the cross-link samples. In half of each sample, DTT was added to cleave the disulfide bond forming the DTSSP linker to equalize volumes of each buffer between samples. The DTSSP-molecule is shown in Fig. 1. Aggregates were visualized with electron microscopy in the cross-link samples, and sizes of the aggregates were estimated. *Magnetic Resonance Imaging:* Samples were imaged in a Bruker 11.7T, 31 cm bore scanner. A multi-echo spin-echo sequence (TE/TR = 6-60/2000, 10 echoes) was used to map T_2 by fitting (Paravision).

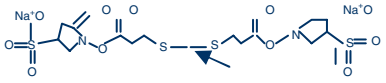


Figure 1: Structure of DTSSP and site of cleavage (arrow).Ferritin was cross-linked on either end.

Results and Conclusions: Cross-linking of ferritin increased R_2 over the control (unaggregated) sample (Fig 2), and the signal intensity was decreased by 30% in T_2-weighted images (inset). Addition of DTT to the sample to disaggregate ferritin decreased R_2 to the control value. R_2 relaxivity was increased significantly ($p<0.05$, t-test) in aggregates over controls (Fig. 3). Based on EM (Figure 4), aggregates contained 211 ± 107 ferritins. Ferritin aggregation should be straightforward *in vivo*, making the aggregation quickly switchable and detectable by MRI.

References: 1.Koretsky AP et al. Proc Intl Soc Magn Reson Med. 1: 69, 1996. 2.Cohen B. et al. Neoplasia 7:2, 109-117, (2005). 3. Genove G et al. Nature Med. 11:4,450-4, (2005). 4. Weisskoff RM et al. Magn Reson Med. 31:60-610 (1994). 5. Bogdanov A Jr et al. Mol Imag. (1):16-23, 2002.

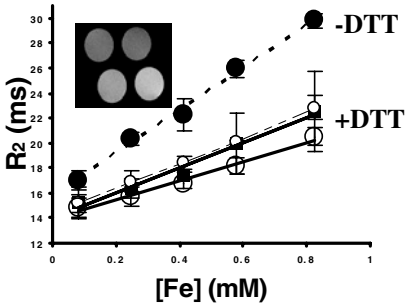


Figure 2: (inset) T_2-weighted images of aggregate (top samples) and control (bottom samples)in the two highest [Fe]. The T_2 relaxivities of aggregates (filled circles) were significantly higher than in controls (open circles). Addition of DTT to the aggregate sample decreased relaxation to the level of controls, indicating cleavage of the cross-linker. (Mean± 1SD, between 3 experiments).

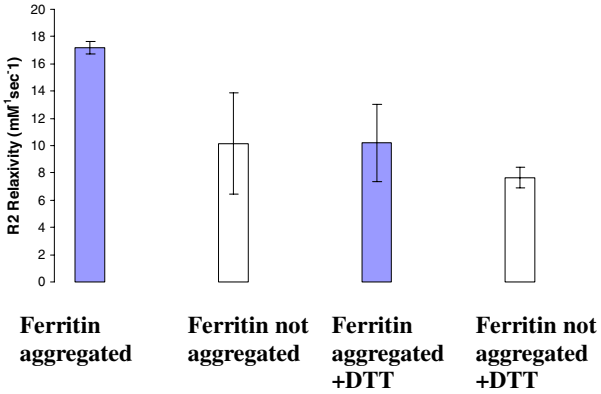
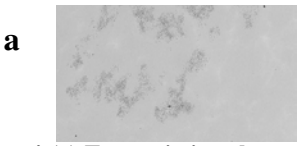


Figure 3: R_2 relaxivity (in mM^{-1} Sec^{-1} in cationized ferritin aggregates (filled) and controls (unfilled) with (+D) and without (-D) DTT. (Mean± 1SD, between 3 experiments).



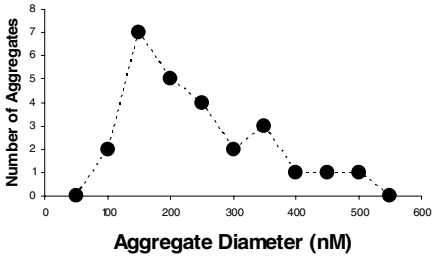


Figure 4:(a) Transmission electron micrograph of aggregated cationized ferritin. Individual ferritin cages are visible as dots due to the electron-dense iron core. (b) Histogram of the size of the aggregates as measured with EM.