Controlled aggregation of ferritin for MRI of actin polymerization

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Introduction: Several diseases lead to the accumulation of iron in the cell cytoplasm (1-2). There is clinical interest in measuring the concentration of iron in various tissues noninvasively. However, iron is deposited in the form of ferritin, and the spatial distribution of ferritin is known to vary between and within tissue types. MRI has been used to assess the iron content, and the aggregation of ferritin in vivo has been suggested as the cause for the differences between estimated liver ferritin iron relaxivities in vivo and those measured in vitro (3). Models have been developed to account for aggregation of ferritin (4). However, aggregation is likely to take on a distribution of geometries in tissue, and it is important to understand how the MRI signal is affected by this distribution. Modulating the distribution of iron oxide particles has been suggested as a way to make molecular imaging agents (6). Additionally, the overexpression of ferritin has been proposed as a reporter protein strategy for MRI (4-5). This if the aggregation state of ferritin in a cell can be modulated this would be a strategy for having cells make there own molecular imaging agents. In this work, we simulated the effects of aggregate size and ferritin spacing within the aggregate on T_2 (or $1/R_2$). To test the effects of ferritin spacing in a biologically relevant system, we conjugated actin to ferritin and allowed the polymerization of the actin dictate the spacing of the ferritin molecules and measured changes in T_2.

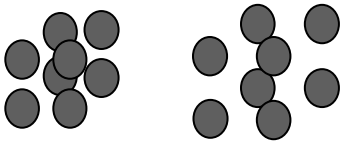

Figure 1: Illustration of the aggregation process in simulations of 12 nm perturbers with two different inter-particle spacings in 2x2x2 aggregates (left to right).

Methods: A computer program was written to determine optimal spacing of ferritin aggregates similar to previous models (7). Water diffused through a 10 μm^3 volume of 6.1 nM ferritin-sized perturber aggregates of varying sizes and spacing (Fig 1), and the MRI signal was computed. A CPMG experiment was simulated, where the phase of each water was reversed at the specified diffusion time fo 4 msec. The effective diffusion coefficient was 4.24×10^{-4} mm^2/sec. To modulate ferritin spacing in vitro, ferritin was conjugated to actin, which was polymerized by ATP. Avidin-ferritin, composed of horse-spleen ferritin conjugated to avidin (EY Labs, Inc) was conjugated to biotinylated actin, and the actin was polymerized using 10 mM ATP. Electron microscopy was done to confirm polymerization with a Jeol (Peabody, MA) JEM-200CX. MRI was done on a Bruker 11.7 T, 31 cm bore magnet with a CPMG pulse sequence (TE/TR = 6/3000 ms).

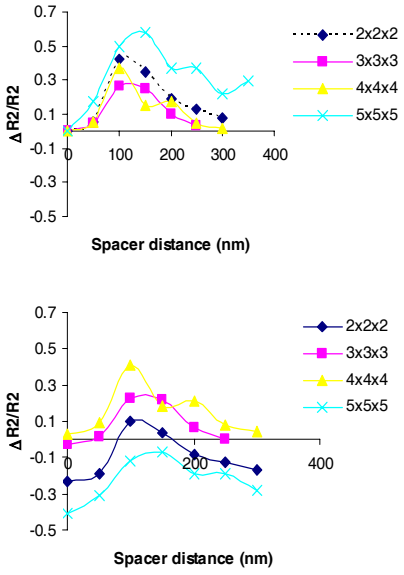

Figure 2: Simulations of the effect of ferritin aggregation and inter-ferritin spacing on the MRI signal. Plots show the R_2 change as a function of inter-ferritin spacing when compared to (top) zero-distance aggregation and (bottom) randomly distributed ferritins in a 10 μm^3 voxel. The legend shows the number of perturbers in each of the three dimensions of the aggregate.

Results and Discussion: The model shows that increasing the distance between ferritins in varying size aggregates can cause an increase in R_2 by up to 70% as compared to an aggregate with no space between ferrtins (Fig. 2,top). In comparison to randomly distributed ferritin, aggregation can either increase or decrease R2 depending on the ferritin spacing (Fig2,bottom). In vitro, actin-ferritin polymerization was observed in TEM, and the amount of negative stain by uranyl acetate could be modulated to show the ferritin cores (Fig 3). Polymerization of actin-ferritin caused T_2 to decrease significantly (*, $p<0.05$), and this decrease could be modulated by increasing the amount of actin to lead to different ferritin spacing (Fig. 4). Taken together, these results demonstrate the complexity of the relaxation effects of ferritin aggregation, and it is likely that this modulation of the relaxivity occurs in vivo as well. It should be possible to construct a reporter strategy in vivo that can modulate R_2 with the polymerization of actin. Since binding of bundling proteins to actin is sensitive to cellular metabolite concentrations and cell cycle, this may lead to a functional reporter gene that can modulate relaxation in response to cellular processes.

References: 1. Roualt TA, Nat Chem Biol. 2(8):406-14; 2006. 2. Stark DD et al Radiology 148:743-751,1983. 3. Gossuin et al. J Magn Reson Imag. Wood et al.Magn Reson Med. 51(3):607-11 (2004). 20;690-696, (2004) 4. Cohen B. et al. Neoplasia 7:2, 109-117, (2005). 5. Genove et al. Nat Med. 11(4): 450-4.5; 2005. 6. Wunderbaldinger et al. Acad Radiol. 2002 Aug;9 Suppl 2:S304-6. 7. Weisskoff RM et al. Magn Reson Med. 31:60-610 (1994).

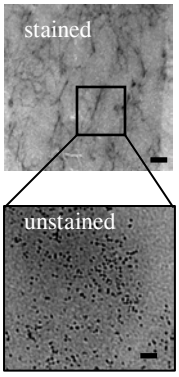

Figure 3: Transmission electron micrographs of polymerized Actin-ferritin in vitro in agar. (Top) 0.5% uranyl acetate, 100 nm scale bar and (Bottom) unstained samples, 20 nm scale bar, showing ferritin attached to the actin filaments. G- actin was polymerized to f-actin in the presence of actin-bound ferritin. The form of f-actin was similar to that observed when ferritin was not present. Polymerization took place in about 45 minutes in vitro, and was stable for up to several hours, as observed with EM.

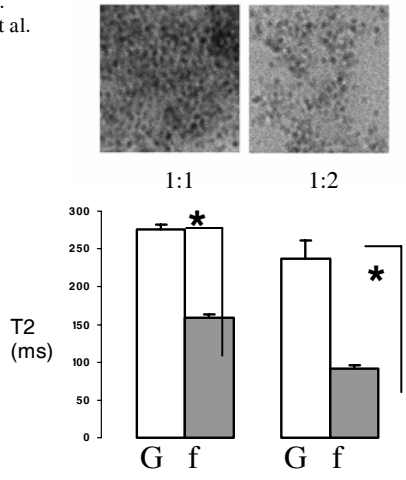

Figure 4: Modulating T_2 by changing the space between ferritin molecules in aggregates. This was accomplished by modulating the amount of avidin-ferritin attached to actin. (Top) Transmission electron micrographs of f-actin-ferritin conjugates adsorbed to a carbon grid with (left) 1:1 biotinilated: unbiotinylated actin and (right) 1:2 ratio. Total ferritin concentration was constant. Note decreased density of electron-dense ferritin in 1:2 aggregates. (Bottom) T_2 values for the actin-ferritin solution during polymerization from G- to f-actin. T_2 decreased with actin-ferritin polymerization, and also with increased spacing between ferritins in the aggregates.