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A responsive particulate MRI contrast agent for copper (I): a cautionary tale

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Abstract

A responsive MION-based MRI contrast agent for the detection of copper(I) is presented. Induced agglomeration of azide and acetylene-functionalized magnetite nanoparticles via Cu(I) catalysed Huisgen cycloaddition leads to significant decrease in longitudinal relaxivity due to the slow exchange of water molecules trapped within the cluster with bulk solvent. Agglomeration leads to an initial two fold increase followed by a sharp and almost complete loss in transverse relaxivity for clusters larger than 200 nm in size. The decrease in r_2 for clusters reaching the static dephasing regime has two significant implications for particulate responsive MRI contrast agents. First, the maximum increase in r_2 is barely two fold, second, since r_2 does not increase continuously with increasing cluster size, the r_1/r_2 ratio cannot be used to determine the concentration of an analyte ratiometrically.

Introduction

Deregulation of copper homeostasis in the body leads to severe pathological disorders such as Wilson's disease (accumulation of copper) and Menkes' syndrome (copper deficiency). Neurodegenerative diseases such as Alzheimer's and Parkinson's, on the other hand, are characterized with miscompartmentalization of copper. A β plaques contain as much as 400 μ M of copper, two orders of magnitude higher than the normal brain extracellular level. Moreover, given the efficiency with which loosely bound Cu(I) catalyses the generation of hydroxyl radical, accumulation of Cu(I) induces oxidative stress with subsequent damage to cells, tissues and organs.¹ This reactivity is correlated to the pathogenicity of Parkinson's and Alzheimer's diseases as elevated copper concentrations in the cerebrospinal fluid² and A β amyloid plaques,³ respectively, are symptomatic of these disorders. Given the toxicity of loosely-bound (as opposed to protein-bound) Cu(I), a direct, selective and non-invasive probe capable of imaging its spatial distribution in the brain in-vivo is highly desirable. Magnetic Resonance Imaging (MRI), with its three-dimensional imaging capability and superior resolution remains the method of choice. Its application in copper imaging, however, first requires the development of responsive, or "smart" contrast agents.

Responsive MRI contrast-agents have received significant attention over the last decade. Their promulgation and application to biomedical imaging, however, has remained elusive,

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partly due to their negligible response under physiological conditions. The vast majority of responsive contrast agents, like their non-responsive predecessors, belong to either of two classes: those based on gadolinium complexes,⁴ and those incorporating Magnetic Iron Oxide Nanoparticles (MIONs).⁵ Gadolinium-based responsive probes rely on the dependence of their longitudinal relaxivity (r_1) on two parameters: the number of inner-sphere, or coordinated, water molecules (q-based contrast agents) and on the rotational correlation time of the complex (including Receptor Induced Magnetization Enhancement or RIME contrast agents). In terms of Cu(I) imaging, several q-based responsive MRI contrast agents have already been reported.⁶ These agents are plagued by two problems: their selectivity over Zn(II) has been brought into question,⁷ and their response in serum-like solutions is only a fraction of that observed in pure water.^{6b, 6c} This lower response results from displacement of inner-sphere water molecule(s) by coordinating endogenous anions, most notably carbonate and hydrogenphosphate.⁸ This significant drawback, common to most q-based gadolinium responsive contrast agents, limits their practical applications.

Responsive MION-based MRI contrast agents, on the other hand, rely on the effect of magnetic interactions between agglomerated magnetite or maghemite nanoparticles on both their longitudinal (r_1) and transverse (r_2) relaxivities.⁵ MIONs are separated into two classes based on their degree of aggregation: USPIOs refer to non-aggregated nanocrystals, whereas SPIOs refer to aggregated crystals, with cluster size typically > 50 nm.¹⁰ Dextran-coated USPIOs (ultra small particles of iron oxide) such as Sinerem® ($r_1 = 7.3 \text{ mM}^{-1} \text{Fe s}^{-1}$, $r_2 = 65 \text{ mM}^{-1} \text{Fe s}^{-1}$) are characterized by lower r_2 and higher r_1 than dextran-coated clusters of SPIOs (small particles of iron oxide) such as Endorem® (diameter = 200 nm, $r_1 = 4.7 \text{ mM}^{-1} \text{Fe s}^{-1}$, $r_2 = 120 \text{ mM}^{-1} \text{Fe s}^{-1}$ at 1.5 T and 37°C).⁹ Previous responsive particulate-based contrast agents were thus designed by functionalizing the dextran coating in such a way that interaction with a targeted biomarker selectively induces agglomeration of the nanoparticles. In all previous reports, this induced clustering increased r_2 and decreased r_1 . The ratio r_1/r_2 , which is independent on the concentration of contrast agent, directly enables detection of the biomarker even when the concentration of the probe is unknown. This method was previously employed to detect oligonucleotides,¹¹ proteins,¹² antibodies,^{11a} enzymes^{11a, 13} and viruses.¹⁴ Advantageously, as opposed to q-based gadolinium contrast agents, the response is independent of endogenous anions although the response observed in serum is always smaller than in buffer. A significant disadvantage of these MION probes, however, is the small (less than two-fold) change in relaxivity observed, thereby significantly limiting their practicality.

Importantly, in each of the above examples, the iron oxide cores (3–5 nm in diameter) were coated with a thick layer of dextran (20–30 nm) and further functionalized with proteins, antibodies, oligonucleotides or ligands. As a result, the cores remained significantly separated from each other (>50 nm) even in the agglomerated state. Previous work by Rotello, however, indicated that maximum magnetic interactions between MIONs occur when their interparticle distance decreased below 7 nm.¹⁵ We thus hypothesized that we could increase the response of this class of contrast agents by functionalizing the magnetite crystal with a thinner, more permeable and more flexible coating such as polyethylene glycol (PEG). Moreover, previous work by our group has indicated that PEG-functionalized nanoparticles, where the hydrophilic polymer is anchored to the magnetite core with a dopamide or other catechol-type anchors, are highly stable and demonstrate superior transverse and longitudinal relaxivity compared to dextran-coated USPIOs of similar size.¹⁶ Note that dopamine itself does decompose iron oxide nanoparticles upon direct functionalization on the crystal surface.¹⁷ However, conversion of the amine to an amide prevents the damaging redox reaction. The resulting dopamide-type anchors are thus highly stable.¹⁶

Selective agglomeration by copper(I) was achieved with Cu(I)-catalyzed Huisgen cycloaddition.¹⁸ This reaction occurs readily in water and advantageously is not affected by anions or large concentration of endogenous glutathione.¹⁹ Our responsive contrast agent for copper(I) thus consist of an equimolar mixture of two magnetite nanoparticles, both 11.6±0.7 nm in diameter, the first of which is functionalized by a dopamide-PEG terminated by an acetylene (Fe₃O₄@Dop-PEG-C≡CH, (**2**)), while the second bears a terminal azide (Fe₃O₄@Dop-PEG-N₃, (**1**), see Figure 1).

Results and Discussion

The two MIONs, Fe₃O₄@Dop-PEG-C≡CH (MION-1) and Fe₃O₄@Dop-PEG-N₃ (MION-2), were synthesized according to Scheme 1. Dop-PEG-BOC was synthesized from the commercial NHS-activated and BOC-protected polymer using standard coupling conditions. A mean molecular weight of 2000 g/mol was chosen as this PEG was previously demonstrated to effectively stabilize iron oxide nanoparticles and minimize aggregation, a necessity in the design of responsive MION-based contrast agents.²⁰ Deprotection of the amine yielded Dop-PEG-NH₂ that can be either modified to the acetylene, Dop-PEG-C≡CH (**1**), or the azide-terminated polymer, Dop-PEG-N₃ (**2**) in one step. Functionalization of MIONs with each ligand was performed according to a ligand-exchange protocol which was previously reported to be optimum for maintaining the high saturation magnetization and relaxivity of magnetite nanocrystals.¹⁶ Successful functionalization was assessed by infrared spectroscopy (Figure 2). TEM characterization indicated that the core size was maintained (Figure 3). Both MIONs are highly stable and remain well-dispersed in water over an extended period of time, with no change in longitudinal and transverse relaxivity and aggregation is observed over several months.

Cu(I) catalyzed Huisgen cycloaddition conjugates the nanoparticles into three-dimensional arrays that affect both their longitudinal (Figure 6) and transverse (Figure 7) relaxivities. As is apparent from the Dynamic Light Scattering (DLS) data, addition of Cu(I) conjugates the nanoparticles into arrays of significantly greater size, up to a mean of 790 nm upon addition of 100 μM Cu(I). These arrays are stable in water, and no precipitate is observed under these conditions.

Interestingly, even though Cu(I) is a catalyst and not a stoichiometric reagent for the Huisgen cycloaddition, both the r_1 and r_2 responses show a dependence on the concentration of the metal. Kinetics studies further demonstrate that regardless of the Cu(I) concentration, both r_1 and r_2 plateau after two hours (Figures 4 and 5). This behavior likely stems from the weak binding affinity of the PEG for copper.²¹ Sequestration of copper ions by the long PEG inside nanoparticles clusters likely prevents the metal from further catalyzing the expansion of the network, hence the dependency of the final relaxivity on the concentration of Cu(I).

As expected, the formation of the arrays decreases r_1 of the contrast agent from 11.9 mM_{Fe}⁻¹s⁻¹ to 6.2 mM_{Fe}⁻¹s⁻¹, corresponding to a change of 48% (Figure 6). This decrease is substantially greater than the 17% observed by Weissleder with his responsive contrast agent for oligonucleotides. These observations are in complete agreement with previous theoretical calculations by Gillis which involve the presence of two pools of water in equilibrium.²²

The relaxivity induced by superparamagnetic nanoparticles in aqueous colloidal suspension can be explained by the classical outer-sphere relaxation, or so-called Curie relaxation theory.²²⁻²³ In terms of aggregation, the Gillis group has previously calculated and observed a decrease in longitudinal relaxivity with increasing cluster size.²² This decrease was

attributed to a change in the accessibility of bulk water molecules to the magnetic nanocrystals. In essence, aggregation results in the formation of two pools of water molecules which are exchanging slower than they are relaxing. Although the water molecules inside the agglomerate relax rapidly, the bulk water (outside the aggregate) relaxes slowly such that the overall longitudinal relaxivity decreases. Theoretical calculations incorporating a virtual exchange between these two pools reproduce remarkably well the observed decrease in r_1 with increasing cluster size.²²

As opposed to previous studies on MION agglomeration,^{11–14} Cu(I) induced agglomeration does not result in a constant increase in transverse relaxivity upon cluster formation. Instead, at low Cu(I) concentrations r_2 nearly doubles, whereas at high concentrations it is mostly quenched (Figure 7). The initial increase and subsequent decrease in r_2 upon clustering of the nanoparticles is further apparent in the kinetic studies of cluster size and r_2 upon addition of 75 μM and 100 μM Cu(I) (Figures 5 and 8).

In terms of transverse relaxivity, the initial increase followed by a steep decrease in r_2 with increasing cluster size appears to vary considerably with previous reports of responsive particulate contrast agents. Our results, however, fully agree with theoretical calculations and experimental observations performed by the Gillis group.²² Unlike longitudinal relaxation, the effects observed for transverse relaxivity arise from changes in the global structure of the cluster and the magnetic field surrounding it. Initially, formation of small clusters falls within a domain where the motional averaging conditions $\Delta\omega\tau_D < 1$ is fulfilled.²⁴ The cluster itself behaves as a large magnetized sphere whose total magnetic moment increases according to Langevin's law. The relaxation is governed by the outer-sphere relaxation theory and is characterized by a long correlation time.

Eventually, the cluster becomes too large such that the motional averaging condition breaks down. T_2 is now governed by the Static Dephasing Regime (SDR), which refers to the dephasing of motionless magnetic moment in a non-uniform field created by randomly distributed dipoles. At this point, when the translational diffusion time of the cluster falls within $\tau_L > \tau_D > \tau_{\text{SDR}}$, a plateau is observed and r_2 remains constant with increasing cluster size. As the cluster size increases further, that is when $\tau_D > \tau_L$, the relaxation rate decreases with increasing cluster size.

In our system involving the aggregation of 11 nm wide magnetite nanoparticles, the plateau in transverse relaxivity is observed for cluster size ranging between 200–250 nm in diameter. The same plateau observed by Gillis in the decomposition-induced aggregation of AMI25 also occurred for cluster size between 200–250 nm in diameter.²² Although we observed a greater increase in r_2 (93% increase) than the AMI25 system (40%) comparison of our two systems show remarkable consistency as to the size of the cluster needed to reach the static dephasing regime.

T_1 -weighted gradient-echo and T_2 -weighted spin-echo MR images (phantoms) of solutions at equimolar iron concentrations of $\text{Fe}_3\text{O}_4@\text{Dop-PEG-C}\equiv\text{CH}$ (**1**) and $\text{Fe}_3\text{O}_4@\text{Dop-PEG-N}_3$ (**2**) in the absence and presence of copper confirms the relaxivity measurement (Figure 9). Colloidal suspensions of $\text{Fe}_3\text{O}_4@\text{Dop-PEG-C}\equiv\text{CH}$ (**1**) and $\text{Fe}_3\text{O}_4@\text{Dop-PEG-N}_3$ (**2**) as well as a sample containing a 1:1 ratio of the acetylene and azide-functionalized MION in the absence of Cu(I) each showed negative contrast enhancement in both a T_1 -weighted and a T_2 -weighted image by the same amount (Figure 9II, III and IV). It should be noted that due to the very short T_2 values of these samples, gradient-echo images are not purely T_1 -weighted, hence the negative contrast observed for the samples containing the MIONs versus pure water. Addition of 100 μM Cu(I) lengthens T_1 (decreases r_1) and shortens T_2 (increases r_2) which results in both darker gradient-echo and spin-echo images (Figure 9, V).

Indeed, T_1 agents results in positive contrast: gradient echo images are brighter the higher the longitudinal relaxivity; on the other hand T_2 agents produce negative contrast: spin echo images become darker the higher the transverse relaxivity. Addition of a higher concentration of Cu(I) (500 μ M, Figure 9VI) further decreases slightly r_1 but decreases significantly r_2 . As a result the spin-echo image becomes significantly brighter. Interestingly, the gradient-echo image also becomes brighter. This is likely due to the fact that T_2 is no longer short enough to affect gradient echo image so significantly, as a result the large clusters behave more as positive T_1 contrast agents.

These results have significant consequences in terms of both the design of SPIOs (cluster) as contrast agents for MRI, and more importantly, in the efficacy and practicality of responsive particulate MRI contrast agents. As our results clearly show, analyte-induced aggregation of MION – the basis for all responsive particulate MRI contrast agents – is doubly flawed. First, because the maximum increase in transverse relaxivity obtainable is barely two fold. It is questionable whether this response, which is significantly smaller than those observed for gadolinium-based contrast agents,⁶ is sufficient for in vivo imaging of targeted analytes in conditions where the local concentrations of the agent can vary greatly. Second, and perhaps most importantly, increased aggregation (increase in cluster size) does not result in a constant increase in transverse relaxivity. Rather, the small two-fold increase is followed by a sharp and almost complete loss in r_2 . The larger clusters relax water significantly worse than the corresponding non-agglomerated MIONs. As a result of the bell-shaped curve of r_2 versus cluster size, the ratio r_1/r_2 previously used to monitor the concentration of the analyte independently of the concentration of the contrast agent is inaccurate. Indeed, each value of r_1/r_2 corresponds to two different concentrations of analyte.

The selectivity of the responsive contrast agent towards physiological cations and neurologically relevant metal ions is shown in Figure 10. Neither the alkali nor the alkali earth ions influence the relaxivity of the nanoparticles, although their presence slightly decreases the response of the contrast agent to Cu(I). Coordination of these cations by the polyethylene glycol changes the conformation of the polymer, thereby affecting the accessibility of the solvent molecules to magnetite cores.²⁵ Addition of high concentrations of either Zn(II) or Cu(II) also increases r_2 , though significantly less than Cu(I). Although neither of these two metals is known to catalyze the 1,3-cycloaddition of alkynes and azides, both form stable complexes with azides.²⁶ In depth studies (Figures S1 and S2 in Supporting Information) indicate that it is indeed coordination to azides that result in the observed response. Addition of either Zn(II) or Cu(II) to azide-functionalized MIONs (without the acetylene-coated counterparts) result in consequential increase in r_2 .

Conclusions

We describe a novel responsive particulate MRI contrast agent for the detection of copper(I). Cu(I) catalysed Huisgen cycloaddition of acetylene and azide-functionalized PEGylated MIONs results in a significant decrease in longitudinal relaxivity and an initial two-fold increase followed by a plateau and a steep decrease in transverse relaxivity. In accordance with the calculations performed by Gillis,²² the decrease in r_1 is due to the formation of two pools of water molecules – those inside the clusters and those belonging to the bulk solvent – which exchange more slowly than they relax. The observed change in r_2 is due to MIONs reaching a static dephasing regime when the clusters reach a size above 200 nm. The presence of this “inversion point” has two significant implications in the applicability of responsive particulate MRI contrast agents. First, transverse relaxivity is limited to barely a two-fold increase, which is smaller than the change in r_1 observed for Gd-based systems.⁶ Other reported responsive MION systems have not surpassed this response.^{11–14} It is arguable whether this relatively small increase in r_2 can be used

clinically to detect biomarkers in vivo. Second, the ratio r_1/r_2 previously used to determine the concentration of an analyte independently of the concentration of the contrast agent is indeed inaccurate. The inflection of the r_2 vs [analyte] data, which mirrors that of the r_2 vs cluster size, indicate that each r_1/r_2 ratio corresponds to two widely different concentration of analyte, in this case, Cu(I). These two points bring into question the practicality of this class of responsive contrast agents in clinical MRI requiring the unequivocal detection and quantification of biomarkers.

Experimental Section

General considerations

Unless otherwise noted, starting materials were obtained from commercial suppliers and used without further purification. Water was distilled and further purified by a Millipore cartridge system (resistivity $18 \times 10^6 \Omega$). HPLC chromatograms were obtained from a Varian Prostar HPLC using acetonitrile/water gradients and a Varian Dynamax C18 semipreparative column. ^1H -NMR spectra were recorded on a Varian 500 at 500 MHz or on a Varian 300 at 300 MHz; the solvent residual peak was used as an internal reference. ^1H NMR data were recorded as follows: chemical shift (δ , ppm), multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet), integration, coupling constant (Hz). Mass spectra (LR = low resolution; HR = high resolution; ES MS = electrospray mass spectrometry) were recorded on a Bruker BioTOF II at the Mass Spectrometry Facility at the Department of Chemistry at the University of Minnesota, Twin-Cities. TEM images were collected on a JOEL JEM1210, FEI Tecnai T12, or on a JOEL 1200 EXII at 120 kV. Relaxivities were measured at 37°C and 1.5 T (60 MHz) on a Bruker Minispec mq60. The hydrodynamic size of the particle aggregates was measured by Dynamic Light Scattering (DLS) with a 90Plus/BI-MAS particle size analyzer (Brookhaven Instruments Corporation). Solid state infrared spectra were recorded on a Thermo Nicolet 6700 FTIR using an ATR adapter. Data was collected between 700 cm^{-1} and 3700 cm^{-1} .

NHS-PEG-Boc (3)—NHS-PEG-Boc was purchased from Jenkem and used without further purification. ^1H NMR (300 MHz, CDCl_3) δ = 5.12 (b, 190H), 4.14 (s, 2H), 3.68 (m, 167H), 2.70 (s, 4H), 1.43 (s, 9H). ESI-MS for main peak (m/z) = 1172 ($\text{M}+\text{H}^+$).

Dop-PEG-Boc (4)—NHS-PEG-Boc (3, 48 mg, 0.025 mmol) was dissolved in CHCl_3 under N_2 . Dopamine (8.0 mg, 0.37 mmol) was dissolved in dimethylacetamide (DMA) and added to the reaction mixture. N,N -Diisopropylethylamine (9.7 mg, 0.074 mmol) was added and the solution was stirred for 2 h at room temperature. The solvent was removed under reduced pressure and further purified by HPLC eluting with 0% water to 47% CH_3CN over 42 min. The product, Dop-PEG-Boc (4), was obtained as a white solid (36 mg, 68%). ^1H NMR (300 MHz, CDCl_3) δ = 6.81 (d, 1H, J = 8), 6.75 (s, 1H), 6.57 (d, 1H, J = 8), 3.94 (s, 2H), 3.61 (m, 167H, PEG), 2.71 (t, 2H, J = 7), 1.44 (s, 9H). ESI-MS for main peak (m/z) = 1191 ($\text{M}+\text{H}^+$), (Calcd. 1192).

Dop-PEG-NH₂ (5)—Dop-PEG-Boc (4, 35 mg, 0.015 mmol) was dissolved in CHCl_3 . Trifluoroacetic acid (2 mL) was added and the reaction mixture was stirred for 2 h at room temperature. The solvents were removed under reduced pressure to yield Dop-PEG-NH₂ (5, 33 mg, 94%) as a white solid. ^1H NMR (300 MHz, CDCl_3) δ = 6.82 (d, 1H, J = 8 Hz), 6.76 (s, 1H), 6.58 (d, 1H, J = 8), 3.96 (s, 2H), 3.60 (m, 153H, PEG), 2.70 (t, 2H, J = 6), 1.43 (s, 9H). ESI-MS for main peak (m/z) = 1163 ($\text{M}+\text{Na}^+$), (Calcd. 1140).

Dop-PEG-C $\equiv$ CH (1)—4-Pentynoic acid (3 mg, 0.03 mmol) was dissolved in DMA and HATU (12 mg, 0.032 mmol) was added to the solution. The reaction mixture was stirred for

15 min at room temperature. Dop-PEG-NH₂ (**5**, 70 mg, 0.03 mmol) was added followed by N,N-diisopropylethylamine (4 mg, 0.006 mmol). The solution was stirred overnight at room temperature. The solvent was removed *in vacuo* and the crude product purified by HPLC, eluting with 0% water to 47% CH₃CN over 42 min, yielding the product, Dop-PEG-C≡C (**4**) as an off-white solid (10 mg, 14%). ¹H NMR (300 MHz, CDCl₃) δ = 6.83 (d, 1H, *J* = 8), 6.76 (s, 1H), 6.59 (dd, 1H, *J*₁ = 8, *J*₂ = 2), 3.97 (s, 2H), 3.60 (m, 131H), 2.72 (t, 2H, *J* = 6), 2.65 (m, 2H), 2.45 (t, 2H, *J* = 3). ESI-MS for main peak (*m/z*) = 1222 (*M*+H⁺), (Calcd. 1221).

Dop-PEG-N₃ (2**)**—5-Azidopentanoic acid (4 mg, 0.03 mmol) was dissolved in DMA, and HATU (12 mg, 0.032 mmol) was added. The solution was stirred for 15 min. Dop-PEG-NH₂ (**5**, 70 mg, 0.030 mmol) added to the solution, followed by N,N-diisopropylethylamine (4 mg, 6 μmol). The solution was stirred overnight at room temperature. The solvent was removed *in vacuo* and the crude product purified by HPLC, eluting with 0% water to 47% CH₃CN over 42 min, yielding the product, Dop-PEG-N₃ (**2**) as an off-white solid (10 mg, 14%). ¹H NMR (300 MHz, CDCl₃) δ = 6.83 (d, 1H, *J* = 8), 6.77 (s, 1H), 6.59 (dd, 1H, *J*₁ = 8, *J*₂ = 2), 3.95 (s, 2H), 3.60 (m, 175H, PEG), 3.30 (t, 2H, *J* = 7), 2.73 (t, 2H, *J* = 6), 2.26 (t, 2H, *J* = 7), 1.68 (m, 4H). ESI-MS for main peak (*m/z*) = 1277 (*M*+H⁺), (Calcd. 1276).

Oleic acid-functionalized magnetite nanoparticles—Oleic-acid functionalized magnetite nanoparticles were synthesized from Fe(acac)₃, oleic acid and oleylamine according to the procedure developed by Wang and coworkers.²⁷

Surface functionalization of nanoparticles—Magnetite nanoparticles were refunctionalized with the Dop-PEG-N₃ ligand (**5**) or the Dop-PEG-C≡CH ligand (**4**) according to a biphasic protocol previously published.¹⁶

Relaxivity Data

Longitudinal (*T*₁) and transverse (*T*₂) relaxation times of the nanoparticle dispersions in mQ water were measured on a Bruker Minispec (60 MHz, 37°C) according to the inverse recovery sequence and the Carr-Purcell-Meiboom-Gill sequence, respectively. The concentration of iron for each probe was determined using the equation below. Briefly, 5 μL of each probe was suspended in 200 μL HNO₃ and 100 μL mQ water. Each sample was heated at 110°C overnight, after which *T*₁ of each solution was measured. The resulting concentration of iron was calculated from a calibration plot created from ICP-OES analysis of solutions of FeCl₃ in 2:1 HNO₃:mQ water. For each probe, the longitudinal (*r*₁) and transverse (*r*₂) relaxivities were fitted to the following equation.

$$r_i [\text{Fe}] = \frac{1}{T_{i,\text{obs}}} - \frac{1}{T_{i,\text{H}_2\text{O}}}, \text{ where } i=1, 2 \quad (1)$$

Kinetic Studies

Nanoparticle probes were suspended in a solution of mQ water ([Fe]_{total} = 120 μM, probe ratio acetylene:azide 1:1) and the relaxation times measured. Cu(Asc) was then added to bring the final concentration of Cu(I) to 0, 10, 25, 50 or 75 μM. In each run, [ascorbate] = 3 × [Cu(I)]. The excess ascorbate is necessary in order to maintain all copper in its +1 oxidation state. Relaxation times were then recorded over the next 60 min.

Selectivity Studies

Nanoparticle probes were suspended in a solution of mQ water ($[\text{Fe}]_{\text{total}} = 120 \mu\text{M}$, probe ratio acetylene:azide 1:1) and the relaxation times measured. The desired cation was then added and relaxation times measured after 20 min. The concentration of competing cations are 5.0 mM NaCl, 5.0 mM KCl, 0.50 mM MgCl_2 , 2.5 mM CaCl_2 , 0.50 mM ZnCl_2 , and 0.5 mM CuSO_4 . $\text{Cu}(\text{Asc})$ was then added to bring the final concentration of $\text{Cu}(\text{I})$ to 75 μM . The transverse relaxation time was then recorded over the next 20 min. Once the maximum T_2 reading was reached, T_1 was measured. Each experiment was repeated in triplicate.

MRI

MR images were acquired using a 9.4-T, 31-cm horizontal bore magnet (Magnex Scientific, Oxford, UK) interfaced with a Varian INOVA console (Varian, Palo Alto, CA, USA). The magnet was equipped with a gradient insert capable of reaching 450 mT/m in 200 μs (Resonance Research, Inc., Billerica, MA). The radiofrequency (r.f.) coil assembly consisted of a linear surface coil (12 mm diameter).

Gradient echo images were acquired using following parameters: repetition time (T_R) = 9.9 ms, echo time (T_E) = 4.97 ms, matrix = 256×256 , slice thickness = 1 mm, flip angle = 20° , FOV = 3 cm $\times$ 3 cm. Spin echo images were acquired using following parameters: T_R = 2 s, T_E = 8.47 ms, matrix = 256×128 , slice thickness = 1 mm, FOV = 1 cm $\times$ 2 cm. The images were reconstructed with in-plane two dimensional 4 times zero-padding.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Notes and references

- (a) Gaggelli E, Kozłowski H, Valensin D, Valensin G. Chem Rev. 2006; 106:1995–2044. [PubMed: 16771441] (b) Scott LE, Orvig C. Chem Rev. 2009; 109:4885–4910. [PubMed: 19637926]
- Pall HS, Blake DR, Gutteridge JM, Williams AC, Lunec J, Hall M, Taylor A. Lancet. 1987; 330:238–241. [PubMed: 2886715]
- (a) Brewer GJ, Kanzer SH, Zimmerman EA, Celmins DF, Heckman SM, Dick R. Am J Alzheimer Dis Other Dement. 2010; 25:490–497. (b) Zheng Z, White C, Lee J, Peterson TS, Bush AI, Sun GY, Weisman GA, Petris MJ. J Neurochem. 2010; 114:1630–1638. [PubMed: 20626553]
- Caravan P, Ellison JJ, McMurry TJ, Lauffer RB. Chem Rev. 1999; 99:2293–2352. [PubMed: 11749483]
- Laurent S, Forge D, Port M, Roch A, Robic C, Vander Elst L, Muller RN. Chem Rev. 2008; 108:2064–2110. [PubMed: 18543879]
- (a) Que EL, Gianolio E, Baker SL, Aime S, Chang CJ. J Chem Soc, Dalton Trans. 2010; 39:469–476. (b) Que EL, Chang CJ. J Am Chem Soc. 2006; 128:15942–15943. [PubMed: 17165700] (c) Que EL, Gianolio E, Baker SL, Wong AP, Aime S, Chang CJ. J Am Chem Soc. 2009; 131:8527–8536. [PubMed: 19489557]
- Major JL, Parigi G, Luchinat C, Meade TJ. Proc Natl Acad Sci. 2007; 104:13881–13886. [PubMed: 17724345]

8. (a) Bruce JI, Dickins RS, Govenlock LJ, Gunnlaugsson T, Lopinski S, Lowe MP, Parker D, Peacock RD, Perry JJB, Aime S, Botta M. *J Am Chem Soc.* 2000; 122:9674–9684.(b) Dickins RS, Aime S, Batsanov AS, Beeby A, Botta M, Bruce J, Howard JAK, Love CS, Parker D, Peacock RD, Puschmann H. *J Am Chem Soc.* 2002; 124:12697–12705. [PubMed: 12392417]
9. Rohrer M, Bauer H, Mintorovitch J, Requardt M, Weinmann HJ. *Invest Radiol.* 2005; 40:715–724. [PubMed: 16230904]
10. Geraldès CFGC, Laurent S. *Contrast Media Mol Imaging.* 2009; 4:1–23. [PubMed: 19156706]
11. (a) Perez JM, Josephson L, O'Loughlin T, Hogemann D, Weissleder R. *Nat Biotech.* 2002; 20:816–820.(b) Josephson L, Perez JM, Weissleder R. *Angew Chem, Int Ed Engl.* 2001; 40:3204–3206.
12. Yigit MV, Mazumdar D, Lu Y. *Bioconjugate Chem.* 2008; 19:412–417.
13. Zhao M, Josephson L, Tang Y, Weissleder R. *Angew Chem, Int Ed Engl.* 2003; 42:1375–1378. [PubMed: 12671972]
14. Perez JM, Simeone FJ, Saeki Y, Josephson L, Weissleder R. *J Am Chem Soc.* 2003; 125:10192–10193. [PubMed: 12926940]
15. Frankamp BL, Boal AK, Tuominen MT, Rotello VM. *J Am Chem Soc.* 2005; 127:9731–9735. [PubMed: 15998077]
16. Smolensky E, Park HYE, Berquo TS, Pierre VC. *Contrast Media Mol Imaging.* 2011; 6:189–199. [PubMed: 21861279]
17. Shultz MD, Reveles JU, Khanna SN, Carpenter EE. *J Am Chem Soc.* 2007; 129:2482–2487. [PubMed: 17290990]
18. Meldal M, Tornøe CW. *Chem Rev.* 2008; 108:2952–3015. [PubMed: 18698735]
19. Viguier RFH, Hulme AN. *J Am Chem Soc.* 2006; 128:11370–11371. [PubMed: 16939257]
20. LaConte LEW, Nitin N, Zurkiya O, Caruntu D, O'Connor CJ, Hu X, Bao G. *J Magn Reson Imaging.* 2007; 26:1634–1641. [PubMed: 17968941]
21. Healy JP, Pletcher D, Goodenough M. *J Electroanal Chem.* 1992; 338:155–165.
22. Roch A, Gossuin Y, Muller RN, Gillis P. *J Magn Magn Mater.* 2005; 293:532–539.
23. (a) Roch A, Muller RN, Gillis P. *J Chem Phys.* 1999; 110:5403–5411.(b) Roch A, Muller RN, Gillis P. *J Magn Reson Imaging.* 2001; 14:94–96. [PubMed: 11436221] (c) Gillis P, Moyny F, Brooks RA. *Magn Reson Med.* 2002; 47:257–263. [PubMed: 11810668] (d) Gossuin Y, Roch A, Muller RN, Gillis P. *J Magn Reson.* 2002; 158:36–42. [PubMed: 12419669] (e) Muller RN, Vander Elst L, Roch A, Peters JA, Csajbok E, Gillis P, Gossuin Y. *Adv Inorg Chem.* 2005; 57:239–292.
24. $\Delta\omega$ is the difference in angular frequency between the local field experienced by a proton at the equatorial line of the cluster surface; τ_D is the translational diffusion time around the cluster.
25. (a) Ono K, Konami H, Murakami K. *J Phys Chem.* 1979; 83:2665–2669.(b) Honda H, Ono K. *Macromolecules.* 1990; 23:4950–4953.(c) Hakem IF, Lal J, Bockstaller MR. *Macromolecules.* 2004; 37:8431–8440.
26. (a) Mautner FA, Kratky C. *Cryst Res Technol.* 1988; 23:1477–1481.(b) Lin W, Zhang X, He Z, Jin Y, Gong L, Mi A. *Synth Commun.* 2002; 32:3279–3284.(c) de Almeida Neves EF, Senise P. *J Inorg Nucl Chem.* 1972; 34:1915–1921.(d) Casella L, Gullotti M, Pallanza G, Buga M. *BioMetals.* 1990; 3:137–140.
27. Sun S, Zeng H, Robinson DB, Raoux S, Rice PM, Wang SX, Li G. *J Am Chem Soc.* 2003; 126:273–279. [PubMed: 14709092]

Fig. 1. Chemical structure and principle of our responsive contrast agent. Cu(I)-catalyzed Huisgen cycloaddition conjugates the MIONs in close proximity, resulting in changes both in r_1 and r_2 . Particles are fully coated with the Dop-PEG-N₃ (1) or Dop-PEG-C≡CH (2) ligands (simplified for clarity).

Fig. 2. Infrared spectra of a) Fe₃O₄@oleic acid, b) Fe₃O₄@Dop-PEG-C≡CH (**2**), c) Fe₃O₄@Dop-PEG-N₃ (**1**).

Fig. 3. Transmission Electron Micrographs and distribution of particle size of a) Fe₃O₄@Dop-PEG-C≡CH (2) and b) Fe₃O₄@Dop-PEG-N₃ (1). The particles have low polydispersity and monomodal size distributions with average diameters of 11.7 ± 0.7 nm and 11.6 ± 0.7 nm, respectively.

Fig. 4. Kinetics of the response of the longitudinal relaxivity (r_1) of the contrast agent to Cu(I). Experimental conditions: water, pH 7, $[\text{Fe}]_{\text{total}} = 120 \text{ }\mu\text{M}$, 1:1 azide:acetylene, $[\text{ascorbate}] = 3 \times [\text{Cu(I)}]$, 1.5 T, 37 °C.

Fig. 5.
Kinetics of the response of the transverse relaxivity (r_2) of the contrast agent to Cu(I).
Experimental conditions: water, pH 7, $[\text{Fe}]_{\text{total}} = 120 \text{ }\mu\text{M}$, 1:1 azide:acetylene, $[\text{ascorbate}] = 3 \times [\text{Cu(I)}]$, 1.5 T, 37 °C.

Fig. 6.

Decrease in longitudinal relaxivity (r_1) upon increase in cluster size of $\text{Fe}_3\text{O}_4@\text{Dop-PEG-N}_3$ (**1**) and $\text{Fe}_3\text{O}_4@\text{Dop-PEG-C}\equiv\text{CH}$ (**2**) induced by reaction with Cu(I). Cluster sizes were measured by DLS. Experimental conditions: water, pH 7, $[\text{Fe}]_{\text{total}} = 100 \mu\text{M}$, 1:1 azide:acetylene, $[\text{ascorbate}] = 3 \times [\text{Cu(I)}]$, 1.5 T, 37°C, reaction time 1 h.

Fig. 7.

Transverse relaxivity (r_2) as a function of agglomerate size. Increasingly large clusters were formed upon reaction with Cu(I). Array sizes were measured by DLS. Experimental conditions: water, pH 7, $[\text{Fe}]_{\text{total}} = 100 \mu\text{M}$, 1:1 azide:acetylene, $[\text{ascorbate}] = 3 \times [\text{Cu(I)}]$, 1.5 T, 37°C, reaction time 1 h.

Fig. 8. Kinetics of size of nanoparticles clusters (open triangles) and their effect on transverse relaxivity (filled circles) upon reaction with Cu(I). Experimental conditions: water, pH 7, $[\text{Fe}]_{\text{total}} = 100 \mu\text{M}$, 1:1 azide:acetylene, $[\text{Cu(I)}] = 75 \mu\text{M}$, $[\text{ascorbate}] = 225 \mu\text{M}$, 37°C , 1.5 T.

Fig. 9. MR images of samples in capillary tubes. (a) T_1 -weighted gradient echo image, (b) T_2 -weighted spin echo image. (I) mQ water, (II) $\text{Fe}_3\text{O}_4@\text{Dop-PEG-C}\equiv\text{CH}$ (**2**), (III) $\text{Fe}_3\text{O}_4@\text{Dop-PEG-N}_3$ (**1**), (IV) 1:1 ratio of (**1**): (**2**) no Cu(I)Asc, (V) 1:1 ratio of (**1**): (**2**) 100 μM Cu(I)Asc, (VI) 1:1 ratio of (**1**): (**2**) 500 μM Cu(I)Asc. In each case, $[\text{Fe}]_{\text{total}} = 1.05$ mM. scale bar = 1 mm. Reaction time: 16 hours at 25°C.

Fig. 10.

Selectivity of the responsive contrast agent to various physiological cations: (a) longitudinal relaxivity, (b) transverse relaxivity. White bars represent r_2 after addition of an excess of the appropriate cation (5 mM NaCl, 5 mM KCl, 0.5 mM MgCl₂, 2.5 mM CaCl₂, 0.5 mM ZnCl₂ and 0.5 mM CuSO₄). Black bars represent r_2 after subsequent addition of 75 μM CuCl and 0.225 mM Na ascorbate (20 min reaction time). Experimental conditions: water, pH 7, [Fe]_{total} = 100 μM, 1:1 azide:acetylene, 37°C, 1.5 T. Error bars represent s.d. ($n = 3$).

Scheme 1.

Synthesis of Fe_3O_4 @Dop-PEG- N_3 (**1**) and Fe_3O_4 @Dop-PEG- $\text{C}\equiv\text{CH}$ (**2**). Conditions: a) dopamine, DIPEA, RT, 2 h, b) TFA, CHCl_3 , RT, 2hr, c) pentynoic acid, HATU, DIPEA, DMA, RT, 15 h, d) 5-azidopentanoic acid, HATU, DIPEA, DMA, RT, 15 h, e) Fe_3O_4 @oleic acid, H_2O , RT, f) Fe_3O_4 @oleic acid, H_2O , RT.