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MRI Biosensors: A Short Primer

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Abstract

Interest in Magnetic Resonance Imaging (MRI) contrast agents for molecular imaging of biological function experienced a surge of excitement approximately 20 years ago with the development of the first activatable contrast agents that could act as biosensors and turn “on” in response to a specific biological activity. This brief tutorial, based on a short course lecture from the 2011 ISMRM meeting, provides an overview of underlying principles governing the design of biosensing contrast agents. We describe mechanisms by which a magnetic resonance imaging (MRI) contrast agent can be made into a sensor for both T1 and T2 types contrast agents. Examples of biological activities that can interact with a contrast agent are discussed using specific examples from the recent literature to illustrate the primary mechanisms of action that have been utilized to achieve activation. MRI sensors for pH, ion binding, enzyme cleavage, and oxidation-reduction are presented. This article is not meant to be an exhaustive review, but an illustrative primer to explain how activation can be achieved for an MRI contrast agent. Chemical exchange saturation transfer (CEST) is not covered as these agents were covered in a separate lecture.

Keywords

Activatable probes; relaxation agents; tutorial

Activatable agents: The Dream

The Holy Grail for molecular imaging is to be able to noninvasively diagnose disease earlier and with greater accuracy by examining molecular signatures in tissues. “Traditional” imaging focuses primarily on anatomical structure, looking for abnormal structure as a diagnostic marker, and contrast is provided by the differential ability of tissues to absorb and transmit energy emitted by the imaging modality utilized. However, structural changes tend to happen very late in disease progression and it would be far preferable to detect disease in its early stages when intervention could be more effective. Before structural changes take place, there are subtle biochemical changes happening in the early stages of disease—the dream of molecular imaging is to be able to detect those small changes. While targeted contrast agents can be used to label and report on the position of specific molecules, there is great interest in the ability to perform functional imaging with MRI, to report not just on molecule location but functional activity. Functional imaging has theoretical advantages over imaging with targeted contrast agents including the ability to amplify signals--a single molecule of interest can activate several contrast agent biosensors. Furthermore, theoretically there is no requirement to wash out background contrast agent as inactive agents would not contribute signal. In the ideal case only agents in the vicinity of their activating molecule of interest will turn “on” and produce signal, thus acting as biosensors.

Fluorescent probes that respond to biological signals are widely available commercially, primarily for *in vitro* work. These probes respond to an external trigger by a change in emission wavelength, ratio of emission wavelengths, or in intensity. MRI biosensors

similarly can shift in “color” (CEST agents) or in intensity (nonCEST agents); we will focus on the latter in this review. To date, biosensing MRI has been achieved mostly by relaxation agents. To understand how these function, we must first discuss mechanisms for contrast enhancement (i.e. affecting relaxation).

How to modulate MRI contrast enhancement efficiency

Relaxivity is a concentration independent measure of a contrast agent’s ability to affect contrast (values given in $mM^{-1}s^{-1}$) (1,2). Biosensors are typically designed to be effective only when activated by the target of interest and in the ideal case relaxivity is high when the agent is turned “on” and close to zero when the agent is “off”. The mechanism for modulating relaxivity depends upon the type of MRI agent under consideration; here, we will focus primarily on T1 and T2 contrast agents.

T2 agents affect nearby water protons through a local magnetic field effect. To alter a T2 agent’s ability to affect nearby protons, one needs to modify the strength of the local field it produces. Iron oxide nanoparticles are the most well-known agents in this class. The most common method to modulate relaxivity is to alter the size of the contrast agent, and thus the strength of the magnetic field, such as through a controllable aggregation of the agents. Another approach is to genetically program cells of interest to produce “natural” contrast agents, such as iron ferrying molecules, in response to specific cues (3–6).

T1 agents, on the contrary, require direct interaction with water protons and gadolinium chelates are the most widely used example of standard T1 agents. Chelates are molecules that can bind and hold gadolinium in a “cage” very tightly to avoid the possibility for free gadolinium to be released in the body. There are a number of routes to affect a T1 agent’s ability to interact with water protons, these generally approach modulating three parameters that have the largest effect on relaxivity:

1. hydration (q) (number of water molecules bound to the contrast agent),
2. water exchange rate (τ_m), (how quickly the water molecules can bind and release from the contrast agent), and
3. the rotational tumbling rate for the agent (τ_R) (how rapidly the contrast agent can rotate in solution).

The relaxation of bulk water protons in a paramagnetic solution has been described by the modified Solomon-Bloembergen-Morgan (SBM) model, a series of equations that describe the relationship between relaxivity and a number of other factors present in this type of system (1,2). In greatly simplified terms, in the SBM equations, factors appearing in the numerators will scale relaxivity proportionately, while factors in the denominator will have inverse relationship to relaxivity. Factors such as hydration (q) are directly proportional to relaxation and exhibit a simple linear relationship such that relaxivity increases linearly with (q). Faster water exchange rates result in increased relaxivity because faster exchange means shorter Gd-water bonds, and relaxation has an inverse sixth power dependence on this bond length. Tumbling time has a bit more complicated relationship to relaxivity, but it is an inverse relationship so that slower tumbling times mean higher relaxivity. These are, of course, theoretical predictions under ideal conditions, and as we will see, actual results may vary. The descriptions above are highly simplified, and make certain assumptions in the applications of the SBM equations, thus, we direct the ambitious and curious reader to the excellent literature that more thoroughly describes the underlying physics behind paramagnetic relaxation enhancement (1,2,7).

We will examine a number of examples of MRI biosensors for different biological processes and for each, illustrate the achievement of activation by different mechanisms.

pH Sensors

There is a great deal of interest in sensors for pH because of the pH decrease that is observed for hypoxic tissues such as in cancer and metabolic disease. The goal for these types of agents is to be able to report on actual pH values for tissues *in vivo*. One approach to the design of pH sensors is to include a moiety that undergoes pH dependent protonation and deprotonation, which subsequently alters the manner in which that moiety can interact with the rest of the contrast agent's components, and thus. affects relaxivity. Below are three examples illustrating how pH dependent protonation can affect hydration, molecule size, or combinations of these.

pH Sensors: Activation by changing degree of hydration

Gadolinium is nine coordinate, meaning it has 9 sites to bind to other atoms. One way to affect the relaxivity for a contrast agent is to manipulate the number of waters that are bound to gadolinium through the introduction of protonatable groups that bind reversibly with pH to the gadolinium ion. For example, the generalized agent shown in Figure 1 is a derivative of Gd-DOTA with a protonatable R-OH group (R = organic molecule). The underlying principle is that the (de)protonation of the R-OH affects its ability to coordinate to gadolinium. When the group is deprotonated (R-O) the oxygen can coordinate to gadolinium and displace water, so that the hydration number (q = number of waters bound) is equal to one. When the group is protonated (R-OH), it no longer binds to gadolinium, thus freeing up an additional site for a water molecule to bind (q = 2). Relaxivity increases with the number of waters coordinated to gadolinium so this agent is turned on by protonation at lower pHs (roughly stated: lower pH = more H+ = protonation). Most gadolinium chelates work with a maximum of q = 2, in order to not upset the stability of chelation. The gadolinium must be stably bound to prevent the toxicity that can result from free ions.

A specific example of this mechanism is represented by work from Sherry and colleagues. in which the R group is a nitrophenol (8). Figure 2a shows GdNP-DO3A (1-methlyene-(p-**N**itro**P**henol)-1,4,7,10-tetraazacyclo**DO**decane- 4,7,10-tri**A**cetate). This contrast agent works by a similar mechanism to that described for the generic agent above. As the nitrophenol group protonates it dissociates from the gadolinium and allows water access, and, as shown in the profile for relaxivity versus pH (Figure 2b), the effectiveness of the agent is higher at lower pHs.

pH Sensors: Activation by changing rotational tumbling

Another mechanism to use pH to alter relaxivity is given in this example from work by Aime and colleagues. In this contrast agent (Figure 3a) the gadolinium chelate is attached to a polymer molecule, polyornithine, that protonates and deprotonates with pH (9). When the pH is greater than 9, the amino groups on the polyornithine are positively charged NH_3^+ groups. As the pH increases these deprotonate and as the neutral charge of the molecule increases it tends to form alpha helical structures, which are more rigid and tumble more slowly in solution. This work reports the effectiveness of the contrast agent in terms of the ratio between the transverse and longitudinal components of the relaxation rate (R_{2p}/R_{1p}). This touches upon a key concern with the use of MR biosensors, that the effect of the contrast agent is measured by observing proton relaxation rates, but this is a function of the contrast agent's concentration as well as relaxivity value. By using the ratio, the authors maintain that the dependence on concentration is eliminated. As illustrated in Figure 3b, as pH increases from 7 to 10 there is a nearly linear increase in the ratio.

pH sensor: Activation by changes in size

Wilson and colleagues have reported water soluble Gd@C60 metallofullerenes that undergo a pH dependent aggregation (10). Gd@C60[C(COOH)2]10 and Gd@C60-(OH)**x** are shown in Figure 4a. These are novel structures that have extraordinarily large relaxivities (up to 80 $mM^{-1}s^{-1}$ has been reported). These large values may be explained by recent work with mesoporous silica scaffolds, suggesting that locating gadolinium inside scaffolds, where water may experience restrictions in motion akin to “organization”, results in decreased rates of water exchange and increases in relaxivity (11). The sizes for these metallofullerenes were found to be strongly dependent on pH, increasing from 50–70 nm at pH 9 up to 700–1200 nm as pH decreased to 4, suggesting aggregation, as shown in Figure 4b. Below pH 3, the authors note that the aggregation is irreversible. In a related report from Toth et al (12), the relaxivities for both metallofullerenes was found to concomittantly increase with decreasing pH, increasing 2.6 × for Gd@C60-(OH)**x** and 3.8× for Gd@C60[C(COOH)2]10 ad shown in Figure 4c. The authors attribute the relaxivities for these agents primarily to outer sphere protons, which are those on water molecules relatively far from the gadolinium (not bound).

pH sensor: Activation by more complicated mechanisms

Contributions to relaxivity changes are not always so clearcut as in the previous examples, and a combination of factors may be at play. In the pH sensitive agent shown in Figure 5a, a gadolinium chelate is coupled to a dendrimer particle (13). Coupling of the chelate to the dendrimer slows the rotation for the gadolinium agent, which increases relaxivity. In this system both phosphonates on the gadolinium macrocycle and amine groups on the dendrimer surface are sensitive to pH. As these amines on the dendrimers (de)proprotonate the rigidity of the dendrimer changes, which alters rotation tumbling rate. As the phosphonates (de)prononate this alters the exchange rate with water. The authors note a slow exchange of bound water, and a fast outer sphere exchange. All of these factors contribute towards the observed increase in relaxivity at lower pHs (Figure 5b).

pH sensor: Unknown mechanism

At times, pronounced pH effects for contrast agents may be observed before the mechanism for the effect can be understood. For example, Wilson and colleagues have also shown that single walled carbon nanotube with sidewall defects can be loaded with Gd^{3+} and these loaded nanotubes are superparamagnetic with very large relaxivities of 180 $mM^{-1}s^{-1}$ per gadolinium ion at 1.5 T at pH 6.5 (14). The Gd^{3+} ions appear to exist as clusters in the nanotubes. The large relaxivity values are attributed to geometric confinement in the nanotubes, which is believed to alter a number of the factors influencing relaxivity including slowing tumbling and decreasing water mobility (15). It was assumed that the nanotubes might show a pH dependent aggregation similar to the behavior of gadofullerene. Their pH dependence increased with lower pH, as for observed for gadofullerenes, with a sharp transition near pH 7. But no aggregation was observed, nor was any loss of gadolinium ions from the tubes detected. Changes in exchange kinetics may be at work in this confined geometry; for example, it has been suggested for gadofullerenes that there is water proton exchange between bulk water and protonated sites on the fullerene cage; one might envision pH dependent protonation altering such exchange in the nanotubes. At this time the mechanism of the pH response remains a mystery.

Ion sensors

MRI sensors of ions have focused primarily on biologically important ions such as calcium and zinc that play key roles as components of protein complexes as well as in signaling processes. Due to MRI’s relatively low sensitivity, ions in greater endogenous concentration

have received the most attention. Approaches to achieve activation in the presence of the ion of interest are similar to those for pH sensing above, where alterations to hydration or size are achieved by changes to contrast agent structure as a function of ion concentration.

Zinc sensor: Activation by changing degree of hydration

Zinc is a major component of a number of important protein complexes and enzymes and also plays a regulatory role in gene expression. Similar to the pH agents, one method to achieve zinc sensing is to include moieties that change in their ability to coordinate to gadolinium in the presence or absence of zinc. The Zn sensing agent in Figure 6, Gd-daa3 (daa3, diaminoacetate with three methylenes), contains 2 N-acetic acid groups with selective binding for zinc--in the absence of zinc these bind to the gadolinium (16). In a mechanism common to many activatable agents that modulate hydration, when the agent binds its target (zinc ion) the 2 N acetic acid groups bind zinc, thus release from gadolinium, and open up 2 binding sites for water. It was observed that binding to zinc doubled the relaxivity for this contrast agent (60MHz, 37°C). Measurements for hydration number q, which consisted of D_2O studies using the terbium (Tb) analog, yielded q values that changed from 0.3 ± 0.1 (no zinc) to 1 ± 0.1 (zinc present). The theory does not completely explain this q value, which would be expected to be near 2.0 in the presence of zinc, but is supportive for the proposed mechanism of action. The Kd (dissociation constant) for Zn-Gd-daa3 was measured to be 2.4×10^{-4} M, the Tb D_2O experiments were done at 300 microM $ZnCl_2$ so it is likely that zinc was dissociating during the experiments allowing them to observe a mixed population of contrast with bound Zn and no Zn giving them q = 1 instead of 2 (16).

Calcium sensor: Activation by changing degree of hydration

Figure 7a gives another example of a similar agent that senses calcium (17). In the same theme as the zinc agent the gadolinium chelate is attached to an aminobisphosphonate calcium chelator. In practice, however, the change in relaxivity in the presence of calcium is in the opposite direction of the agent above. In the presence of calcium the q value decreased from 0.83 to 0.36 and relaxivity decreased with increasing calcium concentration (steep decrease from zero to two equivalents of calcium, Fig 7b). It was proposed that the bulky calcium chelator group sterically blocks water access in a calcium-dependent manner.

Copper sensor: Activation by changing degree of hydration

The same mechanism for controlling relaxivity can be applied to any ion binding contrast agent, and an example for copper is shown in Figure 8a (18). The principle is the same, that an ion binding moiety attached to the gadolinium chelate hinders the binding of water to the chelate unless it is in the presence of its favored ion, in this case copper. Several derivatives of the agent were synthesized, as shown in Figure 8b, to try to optimize the response to copper. Relaxivity increases with copper concentration as shown in Figure 8c for one of the derivatives with greatest sensitivity to copper.

Enzyme sensors

Sensors of enzymatic activity were one of the first activatable MRI probes reported. Conceptually, the mechanism of activation for MRI sensors of enzyme activity are much like optical reporters—the enzyme cleaves some portion of the probe that resembles its substrate, and that cleavage results in a change in properties of the probe. This strategy lends itself well to probes based on modulation of q.

Enzyme sensor: Activation by changing degree of hydration

For example, the beta-glucuronidase sensor reported by Duimstra et al, shown in Figure 9a, consists of GdDOTA coupled to a β-glucuronic acid moiety (19). The β-glucuronic acid

coordinates to gadolinium through an oxygen, thus occupying a binding site that otherwise could be available to water. This moiety is released in the presence of the enzyme β-glucuronidase, thus providing additional binding sites for water to the probe. Studies in a number of different types of buffer demonstrated how the solution environment can influence the behavior of the probe and in fact, for some of the buffers tested, the relaxivity was actually higher in the uncleaved probe, counter to expectations. Phosphate and acetate buffers, for example may coordinate to the probe and compete for binding, and in these buffers the relaxivities were generally lower that in pyridine buffers (pyridine is a poor binding ligand for gadolinium). Increasing T1 relaxation with exposure to enzyme was observed in human serum, which bodes well for in vivo application of this probe (Fig 9b)

Enzyme sensor: Activation by changing size

Protease specific probes have long been a goal for cancer imaging and matrix metalloproteins are a popular target given their believed role in tumor growth and metastasis. In the example presented here, very small (<8nm) citrate coated iron oxide nanoparticles with both positive and negatively charged surface domains are electrostatically stabilized by coupling them to PEG (the polymer, polyethylene glycol). This configuration is referred to as their “low-relaxivity stealth state”(20). The conjugation is through a linker that includes a peptide sequence that is recognized and cleaved by MMP-9. The PEG chains are released after MMP-9 cleavage and the loss of these stabilizing groups results in aggregation of the iron oxide particles through interactions between the charged domains at their surfaces resulting in an increase in relaxivity (Figure 10). The authors note that PEG 5000 was required to achieve stabilization and that probes made with PEG 2000 were not stable and precipitated from solution. Relaxivity for the intact probe was 41 mM $Fe^{-1}s^{-1}$ at 0.94T but post cleavage relaxivities were not reported. MRI studies of the probes in the presence of MMP-9 showed that over time, the particle sizes increased, attributed to aggregation, and that signal intensity decreased, attributed to increases T2* relaxivity.

Other novel approaches

Most of the probes we have discussed so far achieve their sensing actions through interactions that result in modifications to the structure of the probe. Often this change is irreversible, as is the case for cleavage by enzymes, or very difficult to reverse, as is the case for ion binding, where the ions, once bound, can be hard to release. A new class of agents was recently introduced that are probes that can sense activity in a reversible fashion.

These probes can respond reversibly to redox or light activation through isomerization of an attached spiropyran or spiroxazine molecule. As shown for a redox probe in Figure 11a, the familiar gadolinium chelate is coupled to a spironapthoxazine (SO) group that isomerizes between different configurations upon oxidation or reduction (21). In the SO configuration shown on the right there is an additional site of access for water. In the presence of NADH, for example, the probe shifts to the SO form and displays a decrease in fluorescence (Fig 11b) and increase in q, resulting in an increase in MRI signal (Fig 11c 3). Upon oxidation with hydrogen peroxide, the fluorescence can be regained and the relaxivity decreased, confirming a return of the probe to the MC (merocyanine) configuration.

A T2 probe system based on these principles was developed by attaching spiropyran groups to the nanoparticles of iron oxide (Fig 12a) (22). The spiropyran group reversibly isomerized in response to irradiation by different wavelengths of light. With visible irradiation, the spiropyran adopts a structure that is more hydrophobic thus causing the nanoparticles to aggregate in aqueous solution with concomitant increase in relaxivity as illustrated in Figure 12b.

Other mechanisms to note

It is beyond the scope of this review, but the past 5 years have seen an increase in research geared to developing contrast enhancement schemes for MRI that are not solely dependent on exogenous contrast agents. Some interesting mechanisms are outlined below with references to sources for additional information.

1. Increase iron content in cells/tissues of interest by introducing gene for ferritin (23). In this work a chimeric ferritin was developed, in which the feedback regulation was turned off, so that transfected cells would sequeseter iron, thus increasing MRI contrast.
2. Introduce gene for an enzyme that will act upon introduced probes (24). This is a somewhat complicated, multistep system in which cells are engineered to express secreted alkaline phosphatase. The enzyme cleaves an engineered substrate to generate adenosine. The adenosine binds aptamers on the iron oxide particles, which leads to aggregation, and thus, increased relaxivity.
3. Introduce gene from magnetotactic bacteria (25). Magnetotactic bacteria contain magnetosomes, which are magnetic structures unique to each species of magnetotactic bacteria. While many genes are likely to be required in the formation of magnetosomes, when a gene known to be involved with magnetosome production, *magA*, is introduced to cells, these cells produce intracellular iron oxide particles.
4. Use GFP for MRI contrast (26). This is an interesting development in which magnetization transfer contrast (MTC) is used to detect the presence of green fluorescent protein. The macromolecular proton pool associated with GFP interacts with to bulk water in a manner that can be exploited to produce MRI contrast.
5. Multimodality approaches. A particular challenge for activatable probes is a method to determine the concentration of the probes, so that activation can be distinguished from changes in concentration. Multimodality probes may assist in this last challenge, as complementary imaging methods, such as positron emission tomography, can be used for quantitation simultaneous with the molecular imaging by MRI (27).

Summary

The examples provided here demonstrate that activatable agents for T1 and T2 are possible and that a number of different mechanisms to achieve activation have been successfully employed. The most common mechanism utilized by contrast agents has been modulation to hydration, but changes to size, rigidity and exchange rate have also been pursued. Some strategies yield reversible activation-deactivation pathways, as was illustrated by the redox and light sensing probes.

As successful as activatable probes have been *in vitro*, some challenges remain that have hampered their translation to the clinic. Sensitivity, of course, is always a concern, and the probes must show significant change that they can be detected against the background that might be found *in vivo*. Access of the probes to the target molecule is also a challenge and *in vivo* distribution of the probes requires that sufficient probe accumulate at sites of interest, even in the presence of clearance and filtration. But the ability to noninvasively image biochemical processes *in vivo* is a significant problem that continues to be of great interest, and the search for the clinically relevant MRI sensor is an ongoing effort. For the interested reader we direct you to these other recent reviews for more information on activatable contrast agents (28–33).

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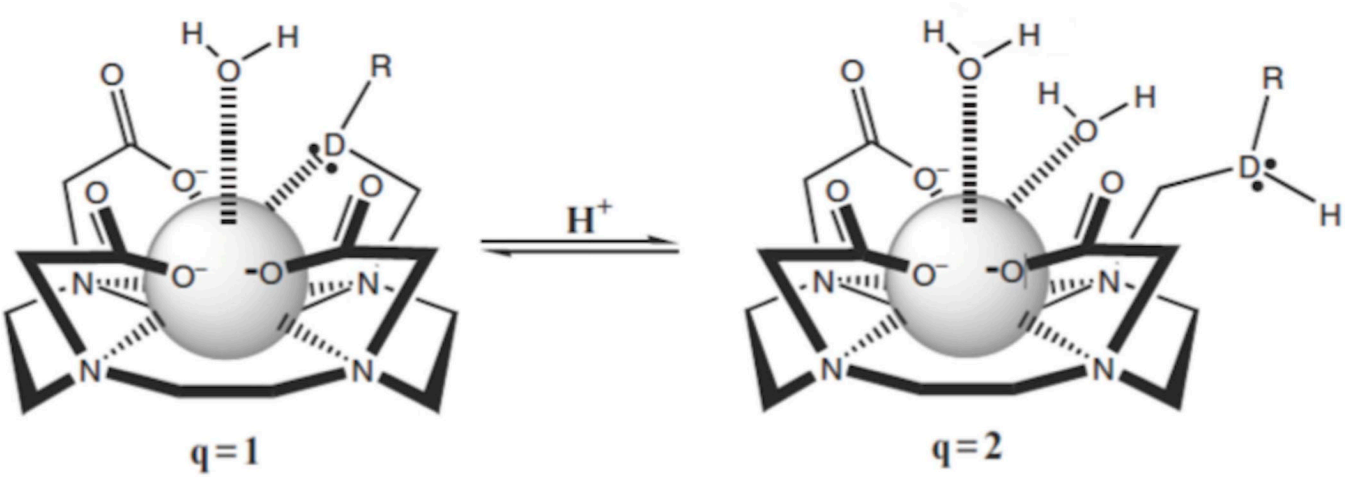

Figure 1.
General structure and mechanism for T1 pH sensing agent. A protonatable group on the contrast agent, that is pH responsive, binds reversibly to gadolinium thus blocking access to water. This results in decreased relaxivity when the protonatable group is bound to gadolinium and an increase in relaxivity when the group is not bound.

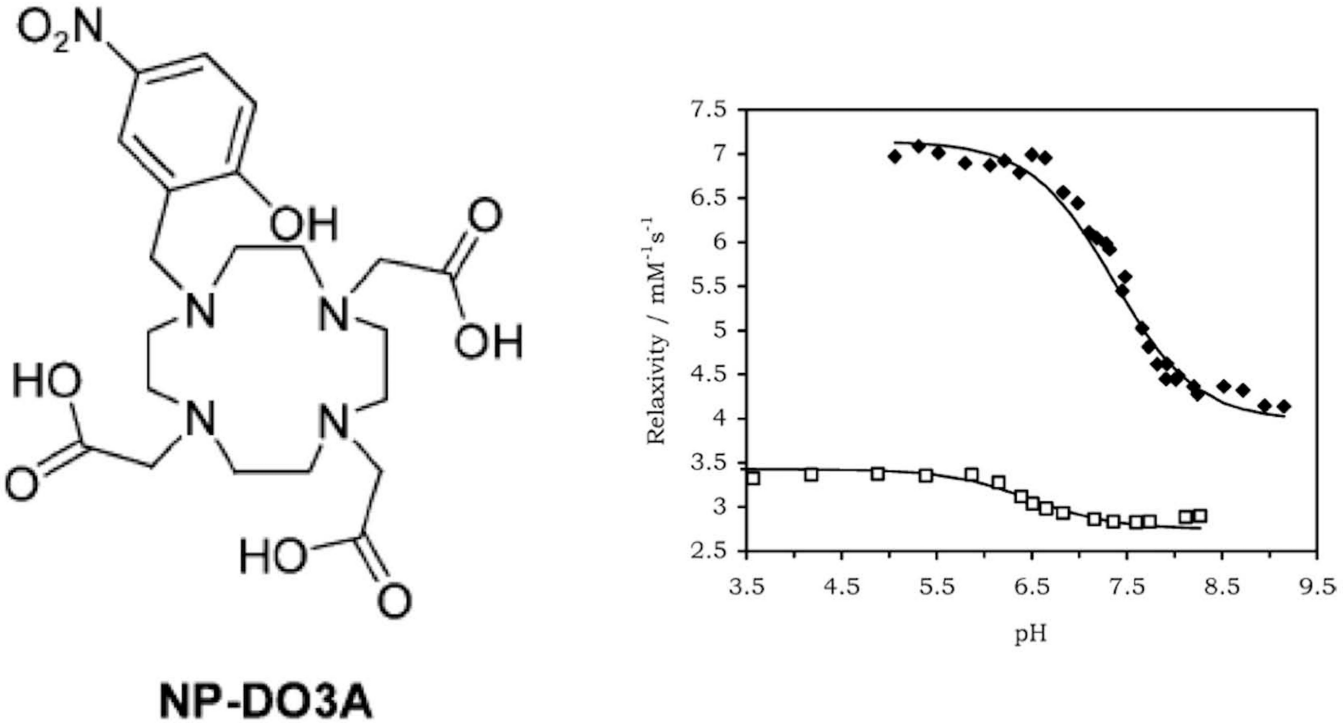

Figure 2.

a. Structure of pH sensing MRI contrast agent NP-DO3A. A phenol group is attached which dissociates from gadolinium when protonated. b. Relaxivity pH profile for NP-DO3A. Filled diamonds show increasing relaxivity with decreasing pH. Reprinted with permission from Woods et al (8). Copyright (2004) American Chemical Society.

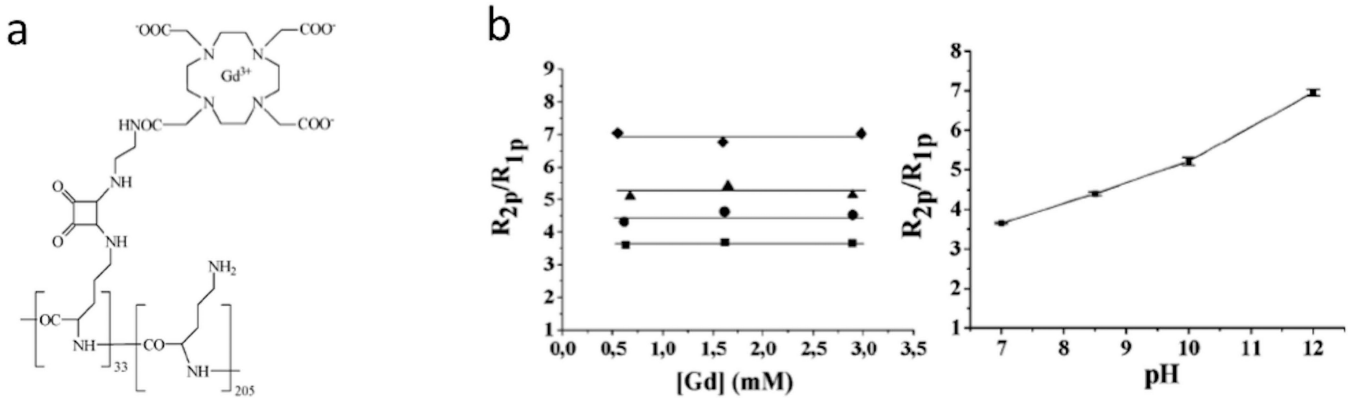

Figure 3.

a. Structure of ratiometric pH-responsive probe. GdDOTA is attached to a polymer that adopts a more rigid alpha-helical conformation when deprotonated. b. Ratio between the transverse and longitudinal components of the relaxation rate (R_{2p}/R_{1p}). This ratio increases nearly linearly with pH. Reprinted with permission from Aime (9). Copyright (2006) American Chemical Society.

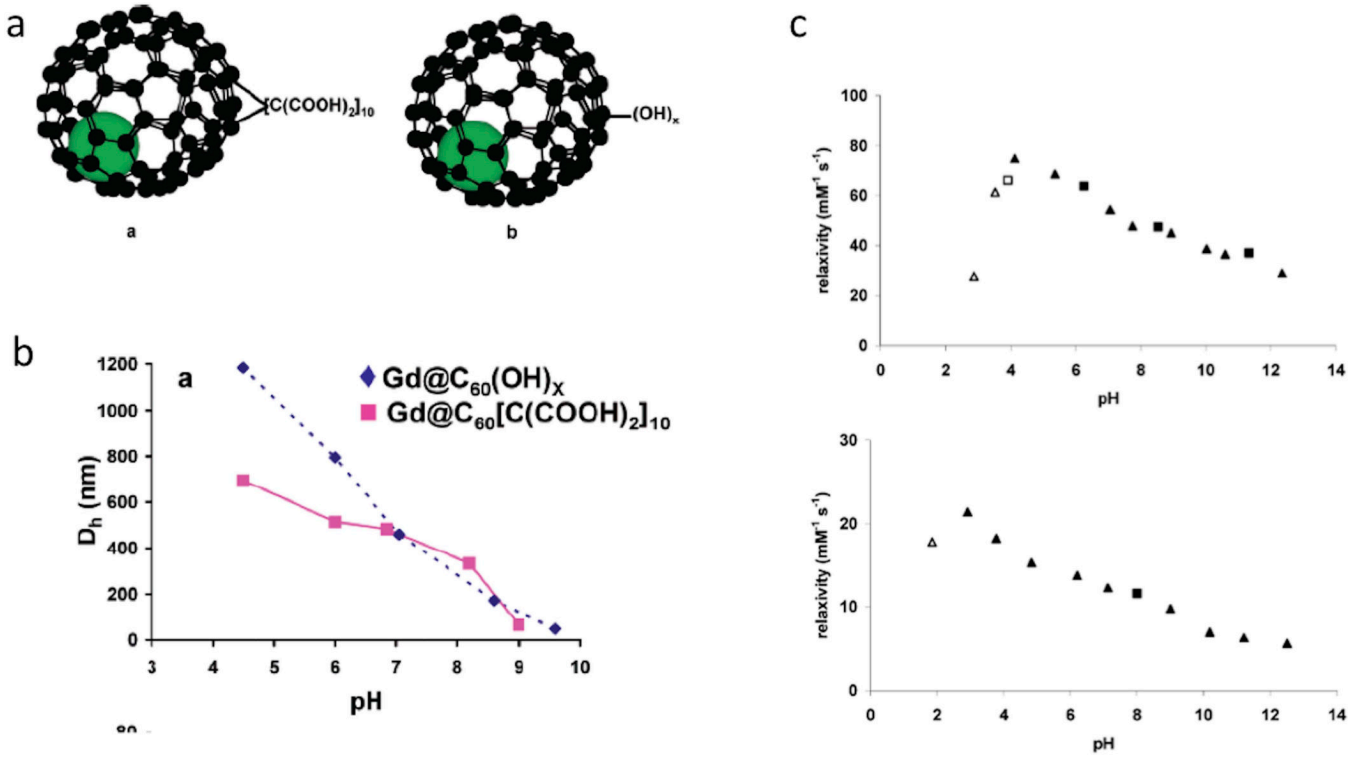

Figure 4.

a. Gadolinium based metallofullerene that responds to pH. Two different fullerenes are shown. Reprinted with permission from Sitharaman et al (11). Copyright (2004) American Chemical Society. b. Size of the aggregates formed by the agents in panel a, as a function of pH. Aggregation increases with lower pH. c. Relaxivities for both species increases with decreasing pH down to pH 4. Then relaxivity falls off as precipitation occurs. Reprinted with permission from Toth et al (12). Copyright (2005) American Chemical Society.

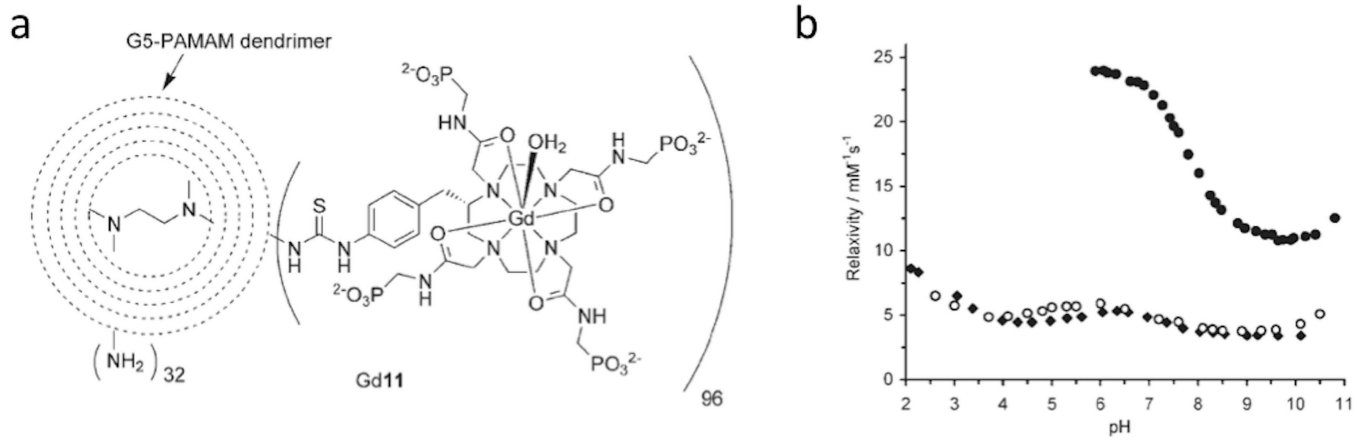

Figure 5.

a. Dendrimer based pH sensitive probe. Gadlinium chelate is attached to a G-5 PAMAM dendrimer. The additional group slows rotation of the GdDO3Aincreasing relaxivity. Both the phosphonates on the macrocycle and the amine groups on the dendrimer can (de)protonate with pH. b. Relaxivity as a function of pH. As pH decreases the combined effect of the protonation of the amines and phosphonates lead to increased relaxivity at lower pH. Reprinted with permission from Ali (13). Copyright (2008) Wiley-VSH Verlag.

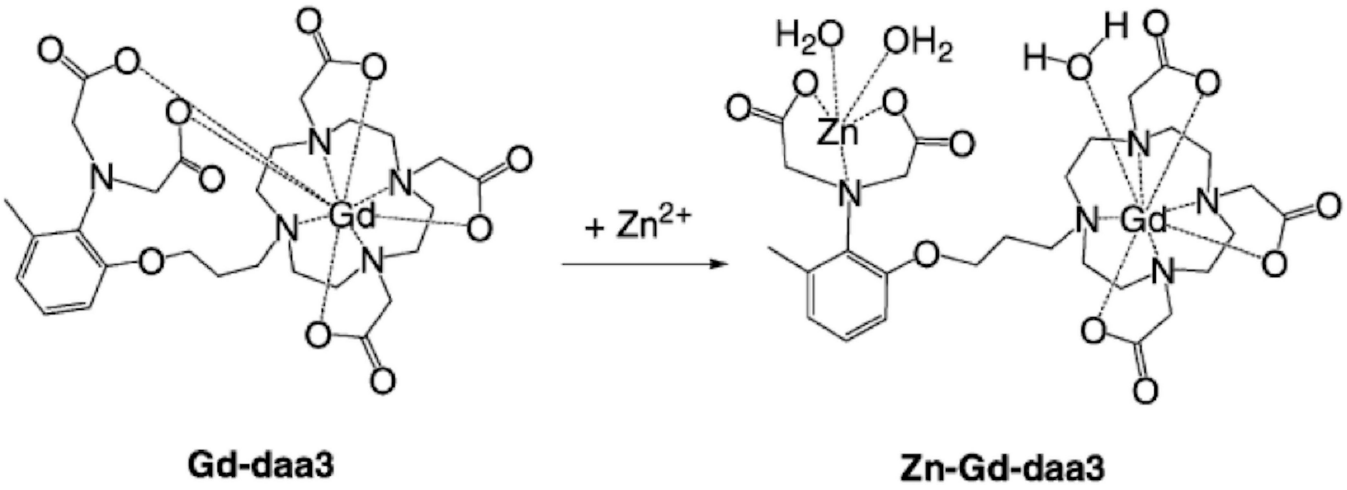

Figure 6.
Structure of a Zn sensing contrast agent. Binding of zinc pulls an arm away from gadolinium, allowing access to water and increased relaxivity. Reprinted with permission from Major et al (16). Copyright (2008) American Chemical Society.

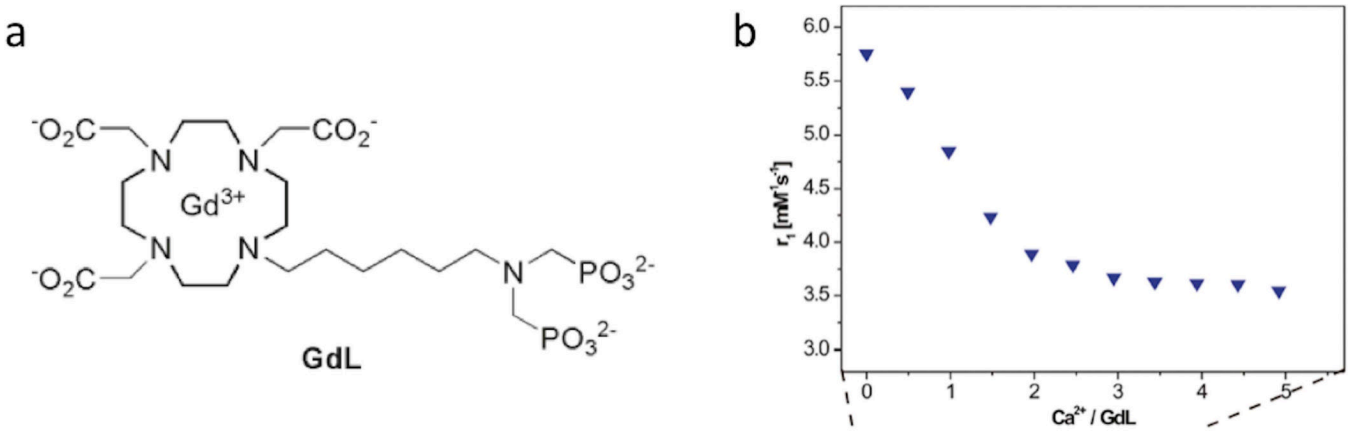

Figure 7.
Structure of a calcium sensing contrast agent. The aminobis (methylenephosphonate) attached to GdDO3A has affinity for calcium. The binding of calcium appears to create steric hindrance and blocks water access to gadolinium. Reprinted with permission from Mamedov et al (17). Copyright (2010) American Chemical Society.

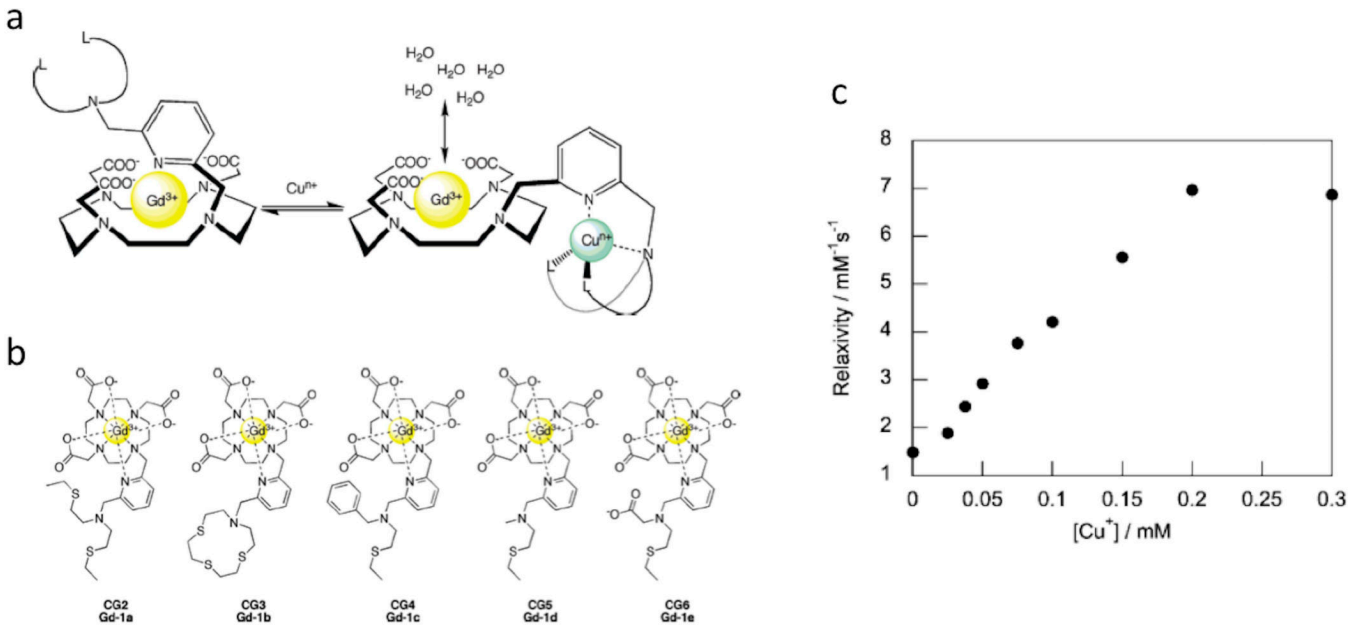

Figure 8.

Mechanism of action for copper sensing probe. As seen in other examples, binding of the ion opens accessibility for water. b. Derivatives of the copper sensing agent designed to alter copper binding selectivity. c. Nearly linear increase of relaxivity with copper concentration for derivative CG-2. Reprinted with permission from Que et al (18). Copyright (2009) American Chemical Society.

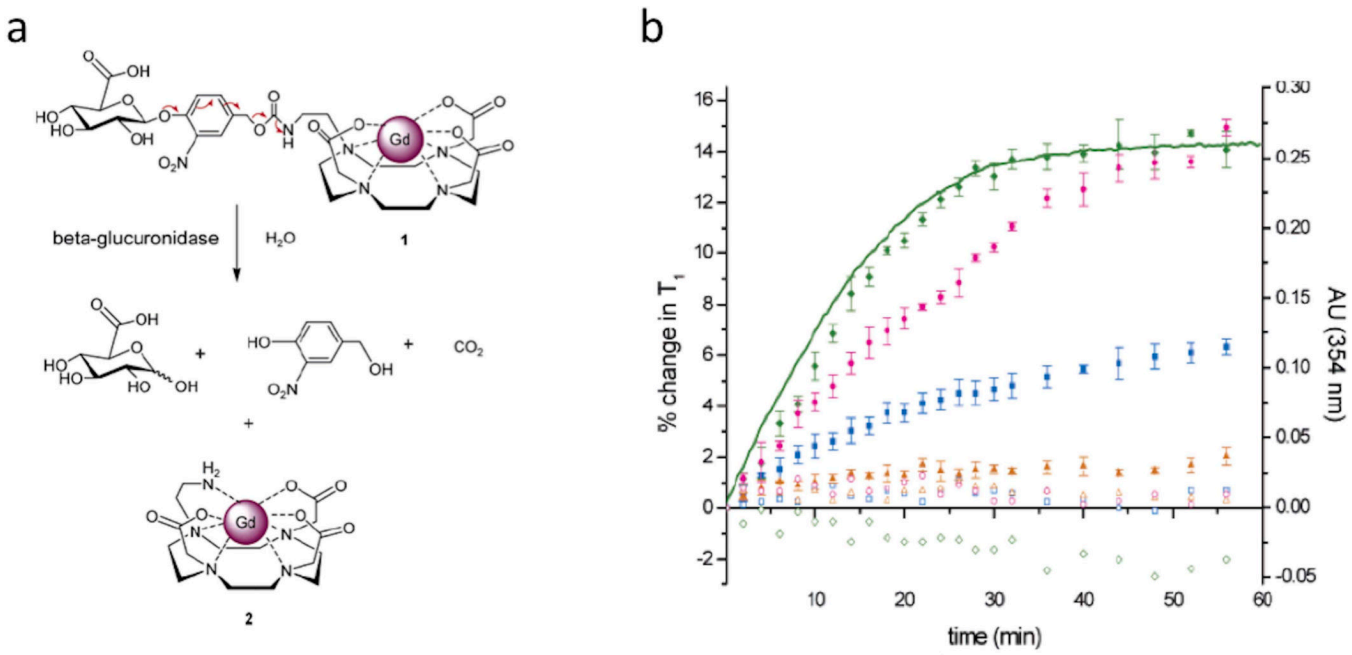

Figure 9.

a. Enzyme sensing by gadolinium based agent. b. Kinetics for enzyme catalyzed hydrolysis of the agent in different buffers. **Green** = acetate buffer pH 5.0; **Pink** = male human blood serum; **Blue** = phosphate buffer with 0.01% (w/v) bovine serum albumin (BSA), pH) 7.4;, **orange** = phosphate buffer with 0.01% (w/v) bovine serum albumin (BSA), 24 mM NaHCO3, pH) 7.4.,**unfilled** symbols = no enzyme. Observed effect at ending timepoint is largest for human serum (pink). Green line is reaction monitored at absorption at 354nm wavelength. Reprinted with permission from Duimstra et al (19). Copyright (2005) American Chemical Society.

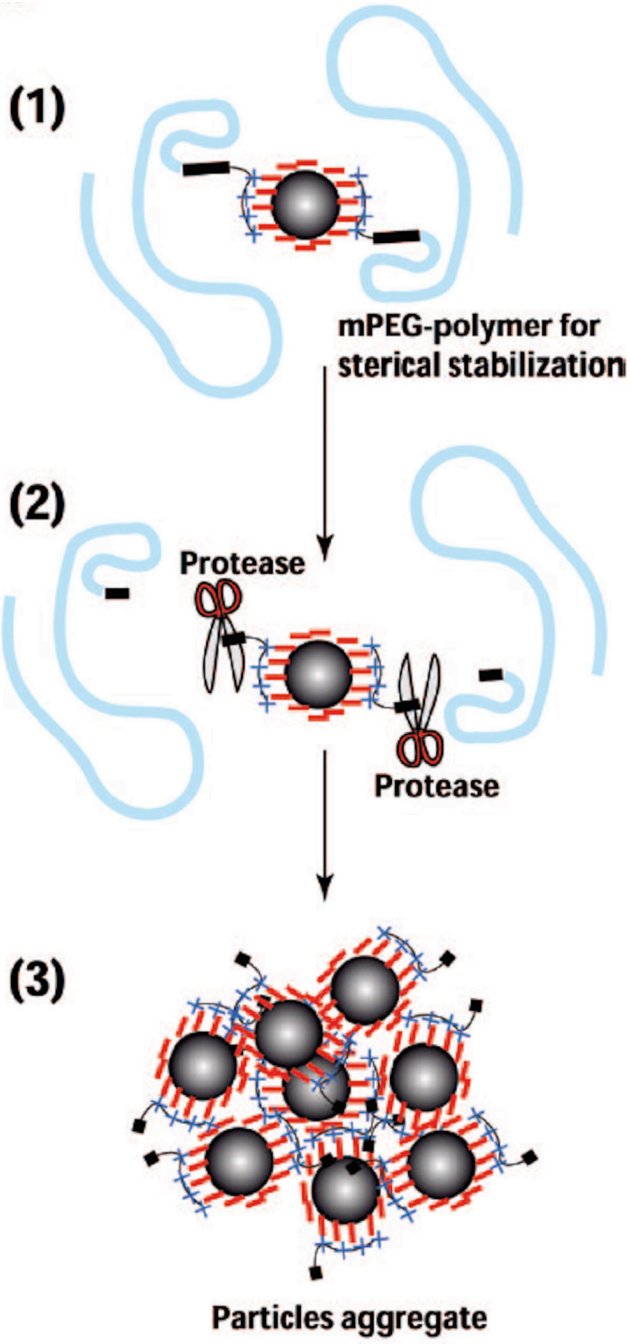

Figure 10.
System for sensing protease activity. Very small iron oxide nanoparticles are attached to PEG polymers through linkers containing peptide sequences that can be cleaved by protease. Upon cleavage the PEG polymers are released, exposing positive and negatively charged domains on the nanoparticle surfaces that interact to form aggregates. Reprinted with permission from Schellenberger et al (20). Copyright (2008) American Chemical Society.

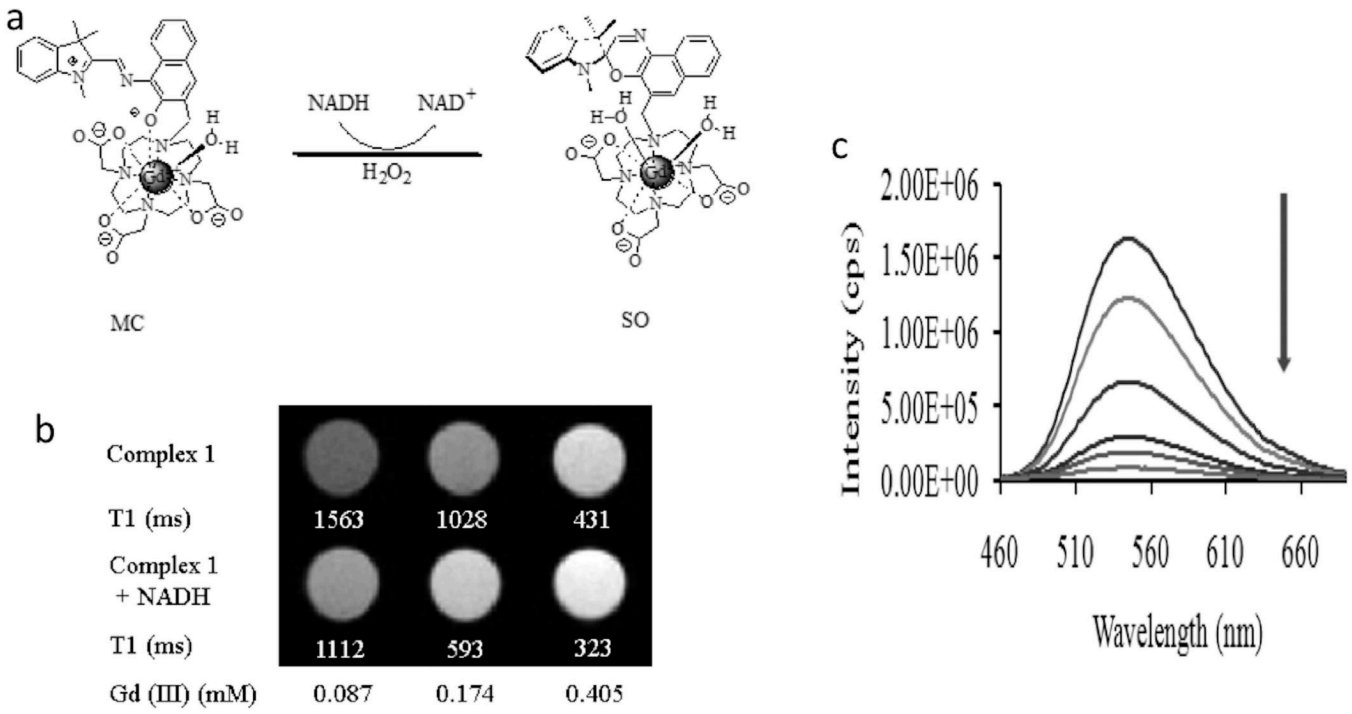

Figure 11.

a. Redox responsive agent. In presence of NADH the MC form of the agent switches to a closed ring SO form of the agent. The closed ring does not bind gadolinium and a site for water access is exposed. Increased water interaction with gadolinium increases relaxivity. This process is reversed by exposure to hydrogen peroxide wherein the ring opens, and an oxygen can bind to gadolinium blocking water access. b. Fluorescence changes in the agent upon exposure to NADH. Fluorescence decreases that the agent switches to SO conformation, as expected with the loss of the conjugated double bond system (chain of alternating double-single bonds between carbon atoms, these systems often with fluoresce). c. Solutions of agent in the presence and absence of NADH. An increase in T1 contrast upon exposure to NADH is observed. Reprinted with permission from Tu et al (21). Copyright (2009) American Chemical Society.

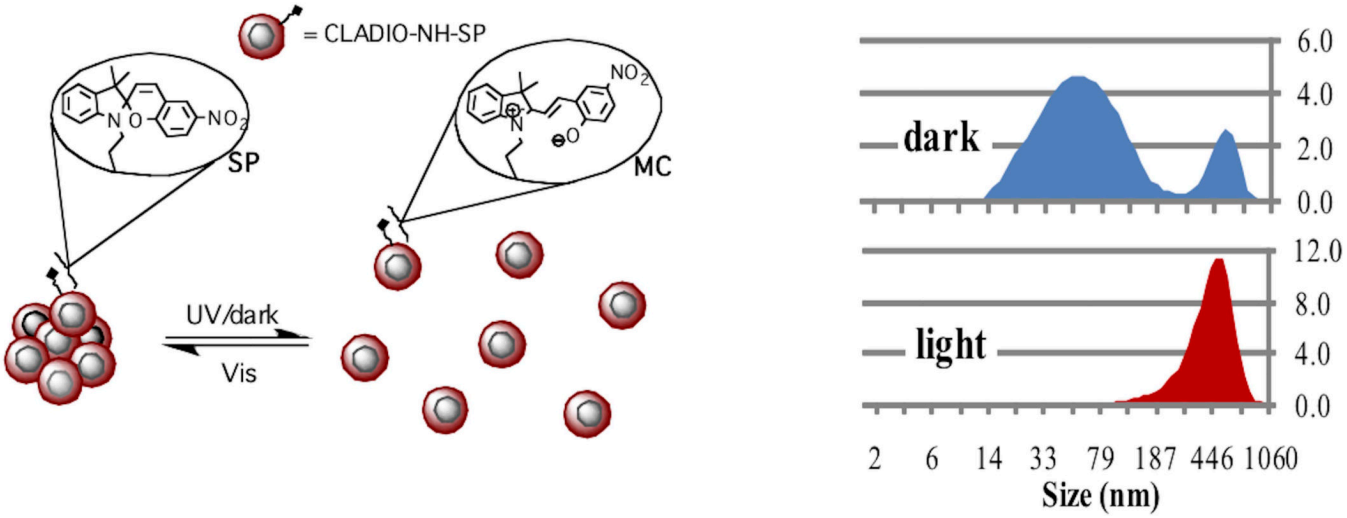

Figure 12.

a. T2 agent responsive to light activation. Spiropyran groups, which reversibly respond to wavelengths of light, are attached to the surface of iron oxide nanoparticles. Upon visible light exposure the spiropyran converts to a closed ring form that is more hydrophobic, resulting in aggregation of the nanoparticles. With UV irradiation, the spiropyran switches to an open conformation (ring opened), which is more hydrophilic and the particles exist as monomers. b. Exposure to visible light affects size. DLS measures show confirm an increase in size as the particles are exposed to visible light. Reprinted with permission from Osborne et al (22). Copyright (2010) American Chemical Society.