Strategies for increasing the sensitivity of gadolinium based MRI contrast agents

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Gadolinium(III) complexes are often used in clinical MRI to increase contrast by selectively relaxing the water molecules near the complex. There is a desire to improve the sensitivity (relaxivity) of these contrast agents in order to detect molecular targets. This *tutorial review* describes the molecular factors that contribute to relaxivity and illustrates with recent examples how these can be optimized. It may be of interest to senior undergraduates and more advanced researchers interested in lanthanide chemistry, biophysics, and/or molecular imaging.

Introduction

Magnetic resonance imaging (MRI) is a routine diagnostic tool in modern clinical medicine. MRI has many advantages as a diagnostic imaging modality. It is noninvasive, delivers no radiation burden, and has excellent (submillimeter) spatial resolution. Soft tissue contrast is superb and MRI readily yields anatomical information. Moreover, there are many techniques that can provide contrast in MRI resulting in markedly different images from the same anatomical region. For instance, pulse sequences can be weighted to highlight differences among tissues that have different proton density, T_1 or T_2 relaxation times, different rates of water diffusion, or different chemical shifts (water *vs* lipids).

The overriding challenge with MRI is its relatively low sensitivity. Consider clinical imaging: what is primarily observed are hydrogen atoms from water that are present in tissue at $\sim$90 M. In order to induce additional contrast, a substance is required that will affect some property of the 90 M water protons to such an extent that an observable effect is achieved. Such substances are called MRI contrast agents. They are paramagnetic, superparamagnetic, or ferromagnetic compounds that catalytically shorten the relaxation times of bulk water protons.

All contrast agents shorten both T_1 and T_2. However it is useful to classify MRI contrast agents into two broad groups based on whether the substance increases the transverse relaxation rate ($1/T_2$) by roughly the same amount that it increases the longitudinal relaxation rate ($1/T_1$) or whether $1/T_2$ is altered to a much greater extent. The first category is referred to as T_1 agents because, on a percentage basis, these agents alter $1/T_1$ of tissue more than $1/T_2$ owing to the fast endogenous transverse relaxation in tissue. With most pulse sequences, this dominant T_1 lowering effect gives rise to increases in signal intensity; these are positive contrast agents. The T_2 agents largely increase the $1/T_2$ of tissue selectively and cause a reduction in signal intensity; these are negative contrast agents. Paramagnetic gadolinium based contrast agents are examples of T_1 agents, while ferromagnetic large iron oxide particles are examples of T_2 agents. Although there are some manganese- and iron-based contrast agents approved for clinical use, the overwhelming majority of contrast enhanced clinical exams are performed with gadolinium complexes. More than 10 million MRI studies are performed with gadolinium each year.

It is the action of the contrast agent on the relaxation properties of the water hydrogen nuclei that generates contrast; this is different from X-ray contrast media and nuclear imaging agents where the effect observed is more proportional to the concentration of iodine or the radionuclide. Since water is present at a much higher concentration than the contrast agent, the contrast agent must act catalytically to relax the water protons in order to observe an effect. The ability of a contrast agent to change a relaxation rate is represented quantitatively as relaxivity, r_1 or r_2, where the subscript refers to either the longitudinal ($1/T_1$) or the transverse rate ($1/T_2$). Relaxivity is simply the change in relaxation rate after the introduction of the contrast agent

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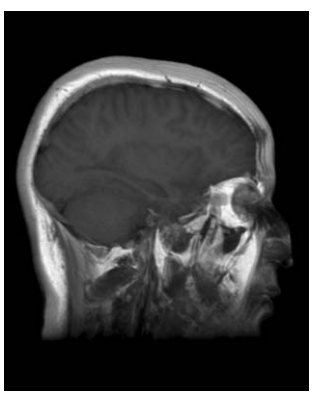

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($\Delta(1/T_1)$) normalized to the concentration of contrast agent or metal ion (M), eqn (1).

$$r_1 = \frac{\Delta(1/T_1)}{[\mathrm{M}]} \quad (1)$$

Some compounds are better magnetic catalysts than others (have higher relaxivity). Commercial contrast agents are only effective at high concentrations (>0.1 mM), and as a result of this there has been considerable effort to increase their sensitivity. Compounds with high relaxivity can be detected at lower doses, or provide greater contrast at equivalent doses to compounds with lower relaxivity.

Two approaches have been taken to increase molecular relaxivity: optimization of the molecular parameters that govern relaxivity and linking multiple gadolinium complexes together. This tutorial review will summarize these approaches and highlight successful design principles. Multimeric gadolinium complexes for MR imaging have been described for some time now. There are several approaches that rely on either covalent (*e.g.* polymers, dendrimers) or non-covalent (*e.g.* liposomes) assembly. Rational approaches are being applied that seek to optimize the relaxivity of these assemblies and also to target them to specific disease states. Relaxivity is dependent on external field, the electronic properties of the gadolinium, water exchange, rotational diffusion, first and second coordination sphere hydration, and the ion to water proton distance. Examples of the importance of each of these parameters will be described along with physical methods for the estimation of these parameters.

This review is not comprehensive and the number of references is limited by the journal. References are chosen as illustrative examples and in most instances there are several uncited references that could equally demonstrate the point. The goal of this tutorial is to explain some facets of MR contrast agent design and to provide the interested reader with the basis for a more critical reading of the literature.

New contrast agents and high relaxivity

Extracellular MRI contrast agents like Magnevist® ($[Gd(DTPA)(H_2O)]^{2-}$, see Chart for all complexes discussed) are cheap to produce and have an excellent safety profile. Doses as high as 0.3 mmol kg^{-1} are given clinically for angiography and cerebral perfusion applications. At these doses, T_1 in the presence of contrast agent is much shorter than T_1 in any other tissue and contrast is excellent. The drive for higher relaxivity doesn't come from making a better Magnevist, it comes from increasing the sensitivity of MRI to detect molecular targets.

In order to induce observable contrast in a robust clinical exam, a relaxation rate change of at least about 0.5 s^{-1} is required. For extracellular commercial contrast agents with a relaxivity of $\sim$4 mM^{-1} s^{-1}, this means a concentration of $\sim$125 μM. For targeted imaging and assuming a 1 : 1 binding stoichiometry (Gd : target molecule), this would require a biological target present with a concentration at least 125 μM. For absolute sensitivity, a more rigorous analysis by Wedeking *et al.*[1] gave a limit of detection of 30 μM in mouse skeletal muscle for the contrast agent $[Gd(HPDO3A)(H_2O)]$. This limits the number of potential biological targets for imaging using current technology.

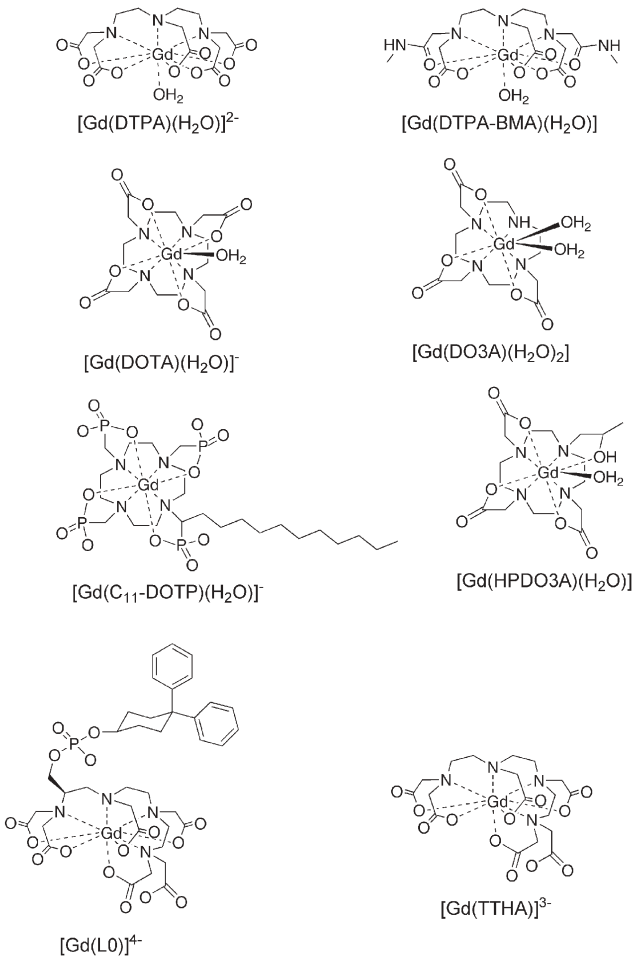

Chart 1

In order to achieve sufficient T_1 change, relaxivity and/or the number of Gd/molecule should be increased. Because relaxivity is so dependent on molecular motion, and because the mobility will be dependent on molecular size, rigidity, and possible protein binding, relaxivity must be optimized on a case by case basis. That is, there is no "high relaxivity" gadolinium complex that can be conjugated to a targeting vector that will necessarily give a targeted contrast agent with high relaxivity. The rotational dynamics of the final molecule are critical. With this caveat in mind, there are several strategies to achieve an effective molecular targeted MRI contrast agent.

Water proton relaxation by gadolinium(III) occurs *via* a dipolar mechanism and as such has a $1/r^6$ dependence on the distance between the ion and the nucleus. Because of this, it is critical to have one or more exchangeable waters in the inner-coordination sphere. However if the hydration number, q, is greater than one then the complex should be stable with respect to water displacement by endogenous ligands. Ultimately for *in vivo* use, the complex must be stable and kinetically inert with respect to gadolinium loss.

Water exchange in and out of the first coordination sphere should be fast. In order to increase the relaxation rate of bulk solvent two things must occur: the paramagnetic ion should

efficiently relax the water that comes into contact with it, and the relaxed water should exchange rapidly with the bulk water. For fast tumbling complexes such as $[Gd(DTPA)(H_2O)]^{2-}$, it is relaxation of the bound water that is the rate limiting process. Water exchange becomes important when rotational motion is slowed. The details are discussed more thoroughly later. To illustrate the impact of both water exchange and rotational motion, consider Fig. 1. Here the GdDTPA analog MS-325 and its bis(*N*-methyl)amido derivative[3] are shown and their relaxivities are given either in pH 7.4 phosphate buffered saline (PBS) or in a PBS solution containing excess human serum albumin (HSA). Gadolinium complexes with amido oxygen donor atoms are known to have slower water exchange rates.[2] The gadolinium complex of the bis(*N*-methyl)amide analog of DTPA, $[Gd(DTPA\text{-}BMA)(H_2O)]$, has a water exchange rate about 10 times slower than that of $[Gd(DTPA)(H_2O)]^{2-}$, and the analogs shown in Fig. 1 have similar water exchange rates to the parent complexes. In PBS, the relaxivity is similar because it is primarily the efficiency of relaxation of the bound water that limits the effect. This in turn is related to how fast the molecules tumble in solution and since they are of similar size the relaxivities are similar. In the presence of serum albumin, both complexes bind resulting in much slower tumbling and a more efficient relaxation mechanism of the bound water. Now the relative importance of water exchange becomes significant and the slow water exchange at the GdDTPA-BMA derivative limits its relaxivity. Increasing water exchange may improve relaxivity further, but only if the process for relaxing the bound water is very efficient.

For most biological targets of interest for imaging, multiple gadolinium ions are required to provide the necessary relaxation rate change. Linking multiple complexes together necessarily increases the effective correlation time for motion. However, understanding and controlling rotational flexibility has a large impact on the resultant relaxivity. Linking complexes in a linear fashion gives an oligomer with anisotropic rotation where rotation about the short axis of the molecule is fast and limits relaxivity. Dendrimers comprised of gadolinium complexes tend to have higher relaxivity because the dendritic structure imposes a more isotropic rotational dynamic and the effect of the increased molecular weight on relaxivity is more realized. The different types of motion are illustrated in Fig. 2. An example of a linear polymer is that described by Casali *et al.*[4] who reported a modified dextran polymer with DO3A-monoamide chelates with a total molecular weight of 52 kDa. The per gadolinium relaxivity of this multimeric compound was 10.6 mM^{-1} s^{-1} (37 °C, 0.47 tesla). The same type of gadolinium chelate appended 24 times to a polyamide dendrimer gives the compound Gadomer (also called Gadomer-17 because of its 17 kDa molecular weight). Under similar conditions (40 °C, 0.47 tesla), Gadomer has a relaxivity of 16.5 mM^{-1} s^{-1} per gadolinium.[5]

The gadolinium chelates in the dendrimer can also undergo internal motion about the linkages to the dendrimer core. Another approach to optimizing the effect of motion on relaxivity is to place the gadolinium at the barycenter of the molecule. The Guerbet group have a molecule called gadomelitol (also called P792 or Vistarem) that can be described as GdDOTA with large hydrophilic groups appended to each of the α-carbons on the acetate arms.[6] Although there is flexibility within the hydrophilic arms, the Gd-H_{water} vector cannot rotate independently of the entire molecule, Fig. 2. This lack of internal motion results in a remarkably high relaxivity, r_1 = 39.0 mM^{-1} s^{-1} (37 °C, 0.47 tesla) for a molecule of its size, 6.4 kDa. Parker and coworkers[7] have showed that this rotational effect can be modulated by adjusting the size of the hydrophilic "arms".

Another approach to controlling rotational flexibility is the metal templated self-assembly approach described by Jacques and Desreux.[8] They proposed appending gadolinium complexes to rigid bidentate or tridentate ligands, and then using a transition metal to assemble the gadolinium complexes in a rigid, compact space, Fig. 3 bottom. Livramento *et al.*[9] extended this by using q = 2 gadolinium complexes. Two complexes are covalently linked to a bipyridyl ligand. Three of these bipyridyl ligands coordinate Fe(II) in an octahedral fashion resulting in a high relaxivity per Gd and per molecule,

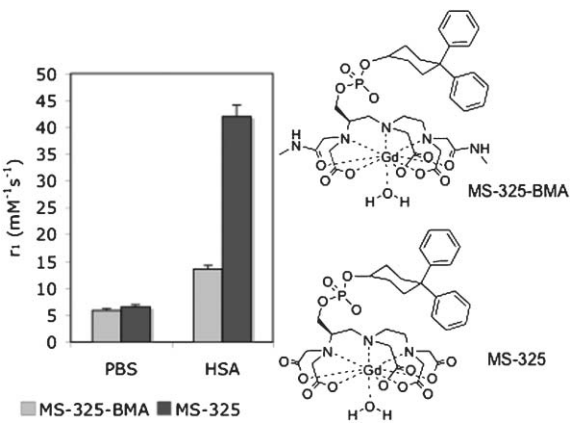

Fig. 1 Relaxivity of 0.1 mM MS-325 and MS-325-BMA in pH 7.4 phosphate buffered saline (PBS) or 0.67 mM human serum albumin (HSA) solution at 37 °C and 0.47 tesla. MS-325 is 88% bound to HSA under these conditions and MS-325-BMA is 83% bound.

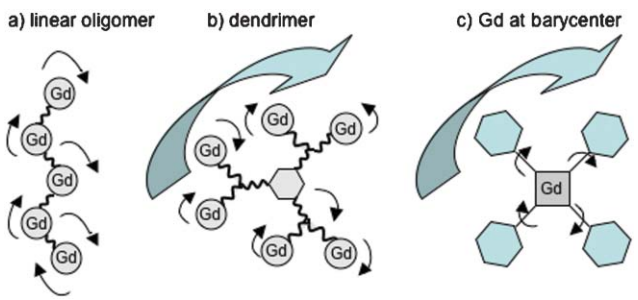

Fig. 2 Different constructs for increasing relaxivity. a) Linear polymer increases the number of Gd per molecule but low relaxivity because of flexibility/internal motion. b) Dendrimer increases the number of Gd per molecule and introduces more globular structure, slowing rotation and increasing relaxivity; internal motion still present. c) Monomer with Gd at barycenter of molecule; although the non-Gd containing arms are free to rotate, the Gd can only rotate at the rate of the entire molecule resulting in high relaxivity.

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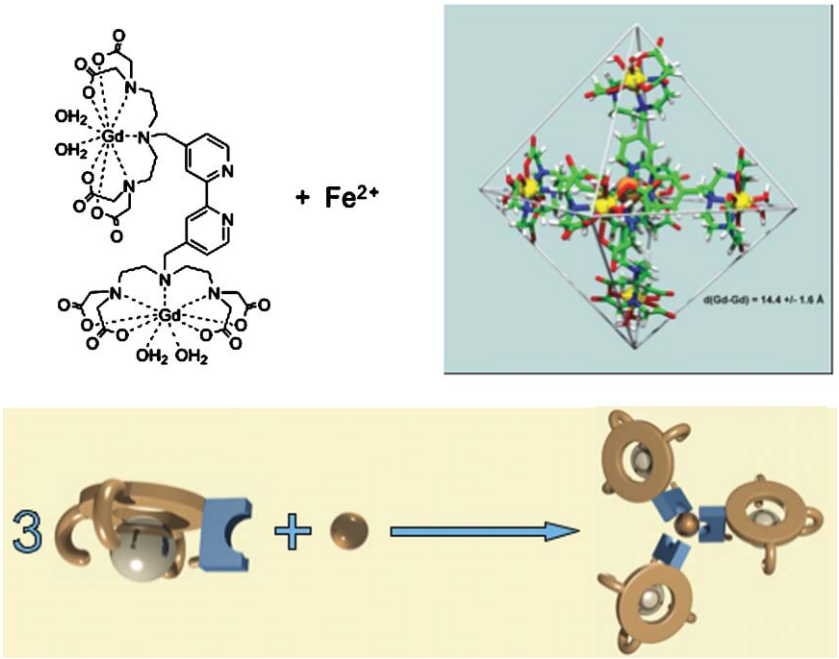

Fig. 3 Rigid self-assembly to increase relaxivity. Bottom: 3 Gd complexes with pendant bidentate ligands assemble around transition metal ion to increase molecular weight and increase relaxivity. Top: Example from Livramento *et al.*[9] where three bipy ligands with two $q = 2$ Gd complexes coordinate Fe(II) to yield a compact, high relaxivity assembly.

especially impressive given its high Gd content and relatively low molecular weight. The per Gd relaxivity of the starting material bipyridyl Gd dimer (Fig. 3, top left) is 12.5 mM^{-1} s^{-1} (40 MHz, 37 °C), which is quite good compared to 3.8 mM^{-1} s^{-1} for [Gd(DTPA)(H_2O)] under these conditions. The increased relaxivity stems from having two exchangeable waters and an increased molecular weight. In the presence of iron(II), the bipy ligands coordinate and the Gd(III) ions are now part of a rigid molecule with a much longer correlation time and the relaxivity doubles to 26.5 mM^{-1} s^{-1}. This is a very efficient relaxation agent from a synthetic perspective as well; it is a high relaxivity stable complex that is about 25% Gd by weight.

A different type of self-assembly was employed by the group at Washington University in St. Louis. This group used lipid–perfluorocarbon emulsions as a platform to build targeted contrast agents. This is shown schematically in Fig. 4a. Gadolinium chelates based on DTPA with long alkyl chains were used to co-localize into the lipid layer and provide thousands of Gd(III) per particle. Similarly, other functionalized lipids bearing targeting vectors could be introduced to the surface. In the cartoon in Fig. 4, there are lipids incorporated that contain biotin. The biotin moieties can bind to avidin that has been derivatized with an antibody. In this manner, they were able to synthesize contrast agents targeted to fibrin (present in clot).[10] Alternately the targeting vector could be directly linked to the particle *via* a lipid tail. This has been done using a small molecule that targets the integrin $\alpha_v\beta_3$ (for angiogenesis).[11] In addition to characterizing these particles *in vitro*, the *in vivo* MR imaging efficacy of these compounds has been demonstrated in appropriate disease state animal models. These are some of the few functional *in vivo* examples of bright spot molecular targeting with MRI.

The lipid–perfluorocarbon emulsion represents a platform technology for targeted imaging. The Washington University group have already made improvements in the per Gd

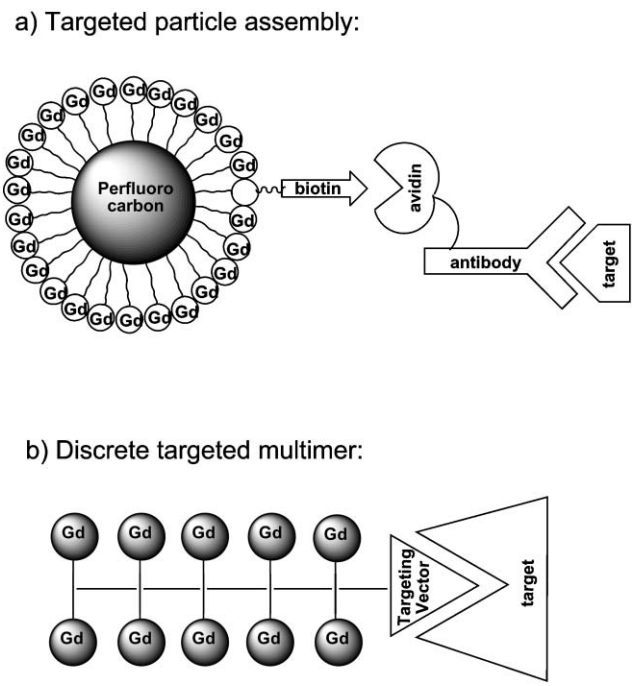

Fig. 4 Two approaches to targeted multimers. a) Self-assembly of Gd complexes containing lipophilic chains into a perfluorocarbon emulsion. Targeting achieved by co-assembly of lipid labeled biotin–avidin–antibody assembly, or direct targeting vector with lipid chain. b) Discrete multimer of gadolinium complexes with covalent linkage to targeting vector.

relaxivity of these particles[12] and further improvements could likely be made. Other particle-based approaches have been reported using, for example, polymerized vesicles[13] or targeted liposomes.[14] Because of their large size, the particle-based approach is limited to vascular targets. An alternate approach is to use discrete multimers of gadolinium complexes with some form of targeting, *e.g.* Fig. 4b. One example of this approach is EP-2104R, a molecule consisting of four gadolinium chelates linked to a fibrin targeted peptide[15] that is being developed for blood clot imaging.

Zhang *et al.*[16] showed that relaxivity of these types of multimeric agents could be improved if internal motion was restricted upon binding to the target. As a model system they used compounds containing four GdDTPA units that contained either one or two targeting vectors. Fig. 5 is a cartoon representing their approach. In the absence of protein, Fig. 5a, the compound was quite flexible and relaxivity was similar for each tetramer and limited to about 10 mM^{-1} s^{-1} per Gd at 20 MHz, 35 °C. When bound to serum albumin, the compound with one binding group (Fig. 5b) increased its relaxivity by a factor of three, but the gain in relaxivity was limited by the internal motion within the molecule. By adding a second binding group the molecule now becomes much more rigid upon binding to albumin with a concomitant increase in relaxivity.

An alternate approach to improving the sensitivity is to couple a change in the local environment to a change in the contrast agent. If motion, hydration number, or water exchange can be influenced by an ion, metabolite, enzyme, or a change in pH or chemical potential, then this change may be detected by a change in relaxivity. For example the Meade group showed that the enzyme β-galactosidase could alter q by cleaving a masking group and increase the relaxivity both *in vitro* and in an *in vivo* model of a developing frog embryo.[17] This is a powerful result because it demonstrates that MRI can

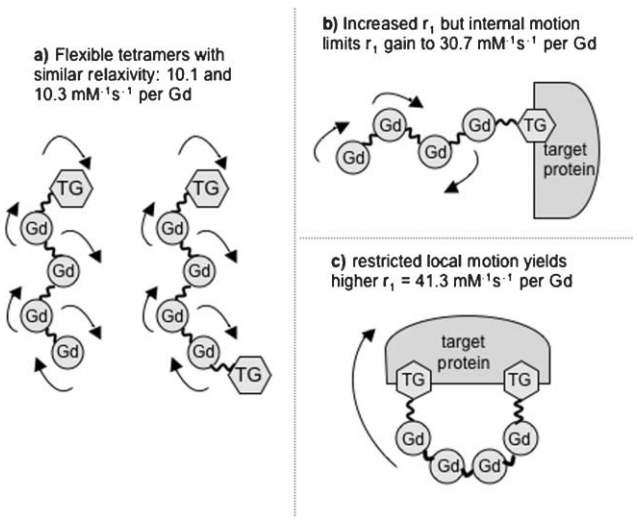

Fig. 5 Increasing relaxivity through multilocus binding and rigidification of the complex at the target. a) Flexible molecules have low relaxivity; b) binding to target slows rotation and increases relaxivity but compound still quite flexible; c) bind target *via* two binding groups limiting internal motion of multimer and increasing relaxivity.

be used to sense the presence of an enzyme that is only present at very low concentrations. The nanomolar enzyme acts catalytically on the contrast agent to change enough of the contrast agent at the 10^{-4} molar level, and this altered contrast agent can be detected *via* water at the molar level.

Nivorozhkin *et al.*[18] used a carboxypeptidase to modulate the protein binding properties of a Gd(III) complex. These workers used a GdDTPA derivative with a lipophilic amino acid (di-iodotyrosine) to target serum albumin. They found that albumin binding could be completely masked by coupling three positively charged lysine residues to the iodotyrosine. In HSA solution, only about 4% of the lysine complex was bound to the protein and relaxivity was 9.8 mM^{-1} s^{-1}. In the presence of the enzyme, the lysine residues were cleaved and the product was now greater than 70% bound to albumin and the relaxivity increased to 26.5 mM^{-1} s^{-1}. Lowe recently reviewed these types of activated contrast agents.[19]

These activatable contrast agents bring additional challenges. Several examples have been reported for *in vitro* systems, however there are far fewer successful examples *in vivo*. To illustrate the hurdles involved, consider as an example a hypothetical compound with pH dependent relaxivity. It is important to recall that the effect observed in the imaging experiment is T_1 change and not relaxivity. Ideally the signal change should be linearly related to relaxivity and hence pH change. However *in vivo*, T_1 change is typically nonlinear with contrast agent concentration because 1) of the pulse sequences used; 2) the compound is not uniformly distributed throughout the tissue but is typically extracellular and only has a significant relaxation effect on this extracellular water; 3) protein binding and viscosity differences may alter relaxivity. This makes it challenging to quantitatively relate signal change to pH. It is also very difficult to know the concentration of the contrast agent which leads to the ambiguity of whether the signal change is caused by a change in environment, *e.g.* pH, or whether there is a greater (or lesser) concentration of contrast agent in this tissue because of endothelial dysfunction. If one considers only a qualitative change in signal because of a binary event such as enzymatic cleavage of a substrate, one still has the problem of distinguishing concentration from relaxivity *in vivo*, and there is the additional problem of redistribution after the change in relaxivity. Nonetheless, the potential for sensing low concentration targets or monitoring key physiological parameters make these activatable contrast agents attractive.

These examples illustrate how relaxivity can be improved for stable gadolinium complexes by altering either the number of coordinated water molecules, the water exchange rate, or rotational dynamics of the molecule. Sometimes these parameters need to be altered in concert as Fig. 1 demonstrated where the relaxivity gain for MS-325 *versus* MS-325-BMA was only apparent when the complexes were bound to albumin. This example showed that water exchange alone was not enough to increase relaxivity, but without fast water exchange relaxivity is limited. It is instructive at this point to consider these and other molecular parameters in more detail. What is important for relaxivity? What can be ignored? How can the effect of these specific parameters be probed?

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Relaxivity

Relaxation of solvent water (bulk water) by a gadolinium complex is a multifaceted phenomenon. Water in close proximity to the ion is relaxed and then rapidly exchanges with the bulk. For this problem, water can be classified into three categories: inner-sphere water, where the water oxygen is directly coordinated to the Gd(III); 2nd-sphere water, describing water molecules that hydrate the complex and have a finite residency time that is longer than the translational diffusion time of pure water; outer-sphere water, where the interaction of the water with Gd(III) is governed solely by translational diffusion and a distance of closest approach. T_1 relaxation of water hydrogen by Gd(III) occurs *via* a dipolar mechanism. Relaxation will depend on the number of water molecules, their distance to the Gd(III), their rate of exchange with bulk solvent, and some correlation time, τ_c.

Fluctuating magnetic dipoles can induce spin transitions and cause spin relaxation. A correlation time is a time constant for characterizing these fluctuations; its reciprocal $1/\tau_c$ is the average rate constant for these fluctuating dipoles. There are several processes that lead to fluctuating magnetic dipoles. Electronic relaxation ($1/T_{1e}$) at the Gd(III) ion creates a fluctuating field. Rotational diffusion ($1/\tau_R$) of the complex creates a fluctuating field. Water exchange in and out of the first ($1/\tau_m$) or 2nd ($1/\tau_m'$) coordination sphere creates a fluctuating field for the hydrogen nucleus. It is the fastest rate (shortest time constant) that determines the extent of relaxation. For water in the second sphere, the relevant correlation time may be the lifetime of this water which may be on the order of 10's of picoseconds. Water in the inner-sphere typically has a much longer residency time (1 ns–10,000 ns), so the relevant correlation time is usually rotational diffusion or electronic relaxation.

Fig. 6 is an attempt to summarize this problem. The hydrogen nuclei are small magnetic dipoles denoted by the small vectors. There are q waters in the inner-sphere with a Gd–H distance r and a residency time τ_m, and q' waters in the second-sphere at a Gd–H distance r' and residency time τ_m'. The gadolinium ion is a much larger magnetic dipole denoted by the large vector and characterized by spin S; it undergoes relaxation that is described by T_{1e} and T_{2e}. Assuming the complex tumbles isotropically, rotational motion is described by a rotational correlation time, τ_R. Water in the outer-sphere is described by a translational diffusion correlation time τ_D and a distance of closest approach a. This gives 11 parameters to describe water relaxation at a given applied field B_0. Moreover, electronic relaxation itself is magnetic field dependent. It is obvious that the relative influence of all these parameters cannot be ascertained by a simple measurement of relaxivity. However some of these parameters can be determined independently and the effect of others can be simulated. A better understanding of the molecular basis of relaxivity has led to contrast agents with higher relaxivity.

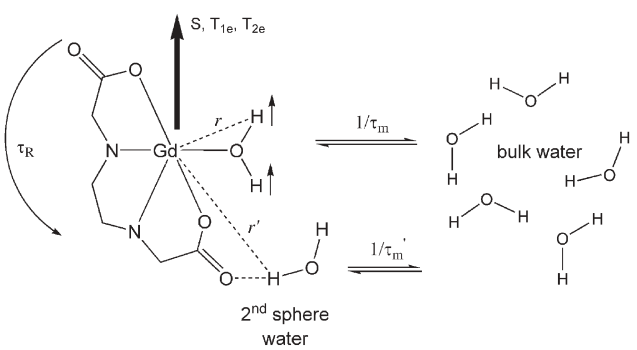

Fig. 6 Molecular parameters that influence inner- and 2nd-sphere relaxivity.

Inner-sphere relaxivity

Relaxivity arising from the inner-sphere water(s) is given by eqn (2), which incorporates 2-site exchange where T_{1m} is the T_1 of the water hydrogen in the inner-sphere and $[H_2O]$ is the water concentration in mM. In order to increase relaxivity, one can increase q or decrease T_{1m} or τ_m.

$$r_1^{IS} = \frac{q/[H_2O]}{(T_{1m} + \tau_m)} \quad (2)$$

Hydration number, *q*

It is of utmost importance that contrast agents administered for human use be safe. The co-ligand (*e.g.* DTPA) should form a sufficiently stable complex that the Gd(III) ion is not released into the body. This safety concern limits the ability to increase q to increase relaxivity. Typically as more coordination sites are opened up for water ligation, the thermodynamic stability of the complex decreases and the Gd(III) usually becomes more labile. A second trade-off with increasing the hydration number is that this often allows coordination of other ligands, such as endogenous phosphate or bicarbonate, which then displace the coordinated water molecules and reduce relaxivity. For example removing an acetate arm from $[Gd(DOTA)(H_2O)]^-$ to give $[Gd(DO3A)(H_2O)_2]$ doubles the hydration number but still results in a stable complex.[20] However the water molecules can be displaced by anion binding.[21] The hydroxypyridinone (HOPO) class of compounds described by Raymond and coworkers[22] appear to be stable $q = 2$ complexes that are resistant to anion coordination.

There are several physical methods to estimate the hydration number.[23,24] X-Ray crystallography is valuable but may not be reflective of the coordination sphere in aqueous solution. X-Ray crystallography is not useful for looking at the hydration number when the complex is bound to a protein. The Dy(III) paramagnetic induced shift of $H_2{}^{17}O$ is proportional to q, but it requires rather high concentrations (>10 mM) and the need to make a surrogate complex for the Gd(III) complex. Luminescence lifetime measurements on a Eu(III) or Tb(III) analog can be made in water and deuterium oxide to estimate q. Coordinated H_2O quenches fluorescence much more effectively than D_2O because of efficient energy transfer to the O–H oscillator. An empirical relationship is used to relate the decay constants in both solvents to the hydration number. The luminescence technique works well at low (μM) concentrations and can be used for protein bound complexes. Recently, Zech *et al.* applied pulsed

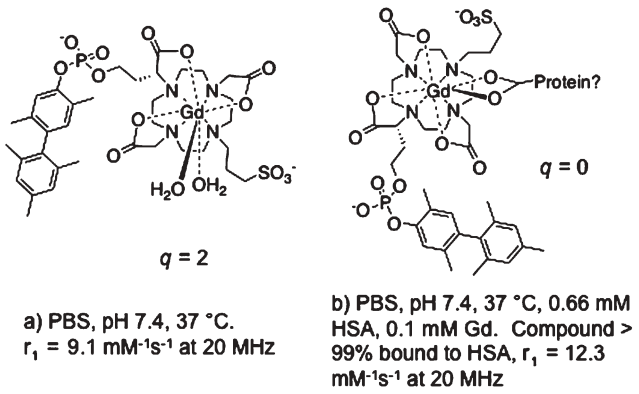

Fig. 7 Gadolinium complex that is $q = 2$ in buffered solution but $q = 0$ when bound to a protein. Hydration state suggested by relaxivity and confirmed by ^{1}H ENDOR.

electron–nuclear double resonance (ENDOR) spectroscopy to the determination of q.[25] ^{1}H ENDOR spectra were recorded in both H_2O and D_2O and the intensity of the difference ENDOR spectrum is reflective of the number of exchangeable protons. This technique does not require a surrogate lanthanide ion for Gd(III) and can also be used with protein bound complexes. For example, Zech *et al.* showed that the DO3A derivative shown in Fig. 7 has relatively high relaxivity in 10 mM phosphate buffer, consistent with the complex being $q = 2$. However in the presence of human serum albumin very little increase in relaxivity was observed. The ENDOR method clearly showed that the complex was $q = 2$ in buffer, but became $q = 0$ when bound to HSA, presumably because of coordination to the Gd by a protein side chain.

Inner-sphere water relaxation

The denominator of eqn (2) indicates that the relaxation time of the bound water, T_{1m}, should be as short as possible and likewise the water residency time should be short. For the first generation of MR contrast agents, $T_{1m} > \tau_m$ and it is T_{1m} that limits the relaxivity of these agents. This is the case illustrated by the compounds shown in Fig. 1 for their relaxivity in buffer. Dipolar relaxation arising from electron–nuclear spin coupling is given by eqn (3) and (4).

$$\frac{1}{T_{1m}} = \frac{2}{15}\frac{\gamma_H^2 g_e^2 \mu_B^2 S(S+1)}{r_{GdH}^6}\left[\frac{7\tau_{c2}}{1+\omega_S^2\tau_{c2}^2} + \frac{3\tau_{c1}}{1+\omega_H^2\tau_{c1}^2}\right] \quad (3)$$

$$\frac{1}{\tau_{ci}} = \frac{1}{\tau_m} + \frac{1}{\tau_R} + \frac{1}{T_{ie}}; i = 1,2 \quad (4)$$

Relaxation depends on the Gd–H distance, r_{GdH}, the proton Larmor frequency ω_H (in rad/s), the electron Larmor frequency ω_S ($\omega_S = 658\omega_H$), and correlation times τ_{c1} and τ_{c2}. The two terms in square brackets are sometimes referred to as dispersive terms because once $\omega^2\tau_c^2 > 1$, then the relaxation rate becomes slower and disperses with increasing frequency (field). Because the Larmor frequency of the electron is 658 times that of the proton, the first term in square brackets disperses at a lower field than the second. If the correlation time is not dependent on magnetic field, then the relaxation

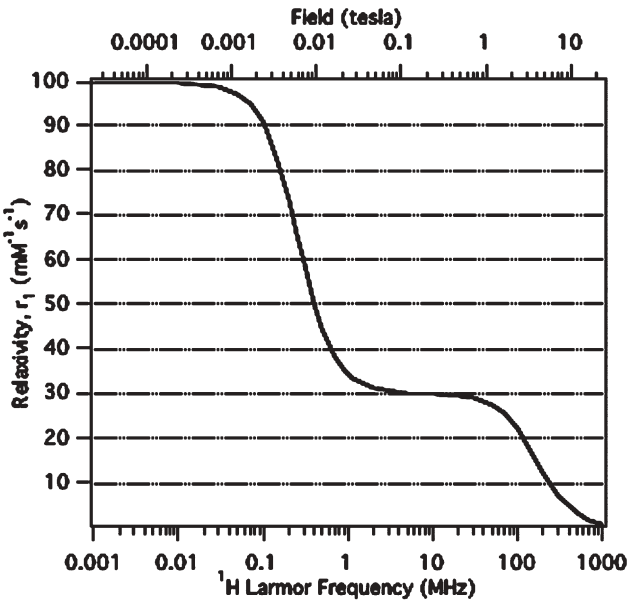

Fig. 8 NMRD profile for hypothetical complex where the correlation time is not field dependent. Note the two dispersions where relaxivity drops by 70 and 30% as indicated by eqn (3).

rate will depend on proton Larmor frequency as shown in Fig. 8 where at low fields the rate drops by 70% and then the final 30% disperses at higher fields. Fig. 8 is an example of a nuclear magnetic relaxation dispersion (NMRD) profile. For Gd(III) complexes, the correlation time is not independent of magnetic field and the NMRD behavior is more complex and will be discussed in more detail below.

Gadolinium–water distance

The term outside the square brackets contains some physical constants, the spin quantum number of Gd(III), $S = 7/2$, and Gd–H distance, r_{GdH}. Gd(III) is preferred as a relaxation agent because of its high spin number and relatively slow electronic relaxation. If the Gd–H distance could somehow be reduced, this would lead to significant increases in relaxivity. There are many values reported in the literature that range between 2.7 and 3.3 Å, but these numbers were obtained indirectly, typically from fitting NMRD data. Direct determination of the Gd–H ion–nuclear distance can be done using neutron diffraction on single crystals or using isotopic exchange methods in very concentrated solution.[26] ENDOR spectroscopy is another method for obtaining this critical parameter directly. ^{1}H ENDOR allows the determination of the hyperfine interactions between the Gd(III) ion and the water proton. 1D and 2D pulsed ENDOR studies[27] have demonstrated that this distance is about 3.1 Å for a range of 8- and 9-coordinate Gd(III) complexes and does not depend on co-ligand or total charge. It is unlikely that this distance can be shortened to benefit relaxivity.

Rotational diffusion

The correlation time is dominated by the shortest correlation time among rotation, electronic relaxation, or chemical

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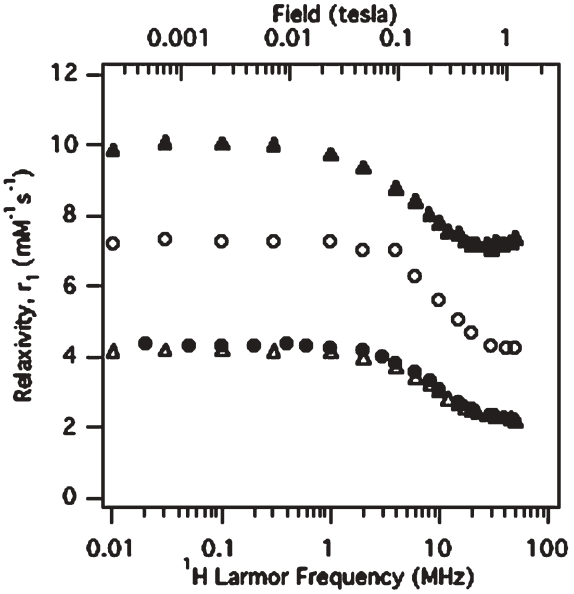

Fig. 9 NMRD of q = 1 MS-325 (▲), $[Gd(DTPA)(H_2O)]^{2-}$ ○) and their q = 0 analogs $[Gd(L0)]^{4-}$ (Δ), and $[Gd(TTHA)]^{3-}$ (●) at 25 °C, pH 7.4.

exchange and is given by eqn (4). By far, the most common field strength used for clinical imaging is 1.5 tesla (64 MHz proton frequency). At this field strength the dominant correlation time is almost always rotational diffusion. For simple monomeric gadolinium complexes τ_R is on the order of 0.1 ns. These small molecules have an average rotational rate constant on the order of a few gigahertz resulting in rather inefficient relaxation. Slowing down rotation results in an increase in relaxivity at 1.5 tesla. Fig. 9 shows the NMRD profiles for four complexes in water at 25 °C: $[Gd(DTPA)(H_2O)]^{2-}$, $[Gd(TTHA)]^{3-}$, MS-325, and the TTHA analog of MS-325 $[Gd(L0)]^{4-}$. MS-325 and GdDTPA have one inner-sphere water while the other two compounds are similar in size but are q = 0. The difference in relaxivity between $[Gd(DTPA)(H_2O)]^{2-}$ and $[Gd(TTHA)]^{3-}$ or between MS-325 and $[Gd(L0)]^{4-}$ is reflective of the contribution of the inner-sphere water to relaxivity. The inner-sphere contribution for MS-325 is greater than that of $[Gd(DTPA)(H_2O)]^{2-}$ because of the increased size and longer τ_R for MS-325.

However at higher fields, if rotation is slowed too much, then the inequality $\omega^2{\tau_c}^2 > 1$ holds and relaxivity decreases with increasing τ_R. This is illustrated in Fig. 10 where relaxivity is simulated for three values of τ_R, using values of other parameters as those reported for MS-325.[28] In Fig. 10, r_1 is simulated over a range of fields encountered in MRI for correlation times of 0.1 ns (typical of small molecule, extracellular agents), 1 ns (intermediate motion), and 10 ns (typical of albumin bound agents). Fig. 10 shows that increasing the rotational correlation time from 0.1 ns to larger values will increase relaxivity, but the benefits of very slow rotation are seen at lower field strengths. Note also that r_1 does not go to zero because there is also an outer-sphere component to relaxivity (see below) and the correlation times that govern outer-sphere relaxivity are quite short.

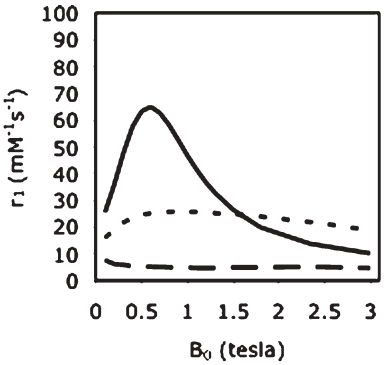

Fig. 10 Effect of rotational correlation time on relaxivity as a function of field strength. Long correlation time (τ_R = 10 ns, —) typical of albumin binding gives high r_1 that decreases with increasing field; intermediate correlation time (τ_R = 1 ns, - - -) shows relaxivity maximum pushed out to higher field; short correlation time (τ_R = 0.1 ns, ---) typical of ECF agents shows low, roughly field independent r_1. Simulations with other parameters typical of Gd-based agents.[28]

Rotational diffusion can be estimated from various physical methods such as NMR relaxation, EPR, fluorescence, or can be calculated from the Stokes–Einstein equation as described in recent reviews.[23,24] Rotation is often assumed to be isotropic. While this is often a reasonable assumption, for larger molecules this may not be valid. Anisotropic rotation or internal motion within a molecule will reduce the correlation time from that predicted by an isotropic model based on molecular weight. Anisotropy can be seen when relaxation is measured as a function of field strength. Lipari and Szabo[29] pointed out that for most complex models of motion the spectral density function can be approximated by using two correlation times—one for the global motion (τ_g) of the molecule and another for the local motion (τ_l) of the Gd–H_{water} vector.

$$\frac{1}{T_{1M}} = \frac{C}{r_{GdH}^6}\left[\left(\frac{7F\tau_{cgi}}{1+\omega_S^2\tau_{cgi}^2} + \frac{7(1-F)\tau_{cli}}{1+\omega_S^2\tau_{cli}^2}\right) + \left(\frac{3F\tau_{cgi}}{1+\omega_H^2\tau_{cgi}^2} + \frac{3(1-F)\tau_{cli}}{1+\omega_H^2\tau_{cli}^2}\right)\right] \quad (5)$$

$$\frac{1}{\tau_{cgi}} = \frac{1}{\tau_g} + \frac{1}{T_{ie}} + \frac{1}{\tau_m}; \frac{1}{\tau_{cli}} = \frac{1}{\tau_{cgi}} + \frac{1}{\tau_l}; i = 1,2 \quad (6)$$

Eqn (5) and (6) apply this to dipolar relaxation for the gadolinium case where C is a constant comprised of terms from eqn (3). The degree of isotropic motion is given by the order parameter S^2 (here denoted F to avoid confusion with the spin quantum number). At higher fields, $\omega_S\tau \gg 1$ and the spectral density is given by the second term in parentheses in eqn (5).

The effect of local motion is illustrated in Fig. 11 where relaxivity from 1–100 MHz is plotted for various order parameters for a system with a global correlation time of 10 ns and a local correlation time of 0.1 ns. In the extremes, motion is isotropic. When F = 1, the NMRD is that of a slow tumbling compound. When F = 0, the NMRD is typical of a small molecule. The effect of immobilization on even a very

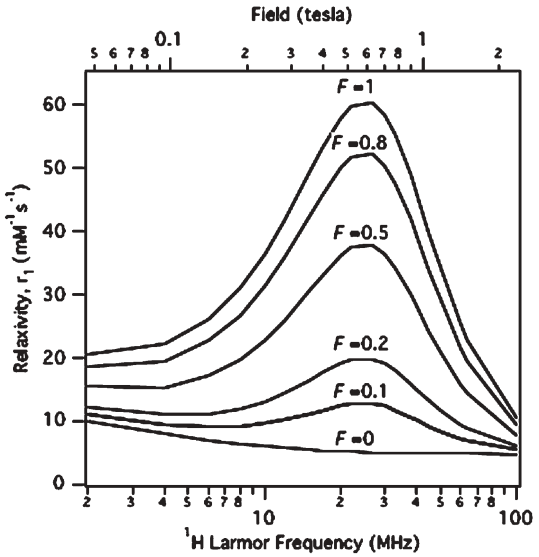

Fig. 11 Simulation showing the effect of anisotropy or internal motion on the field dependent relaxivity of a hypothetical Gd(III) compound. The complex has a slow motion component with t_{global} = 10 ns and a fast motion component with t_{local} = 0.1 ns. The degree of anisotropy is given by $0 \leqslant F \leqslant 1$.

flexible molecule has a marked impact on the NMRD curve. Even a small component of very slow motion will lead to this ''peaked'' feature that is observed at about 30 MHz.

It should be stressed that this is a phenomenological description. Motion is more complex and these correlation times do not represent discrete rotational processes. While it may be reasonable to compare τ_{global} and τ_{local} among similar molecules, this should be avoided for disparate molecules.

Electronic relaxation

Electronic relaxation for Gd(III) is a complex, magnetic field dependent phenomenon. At low fields (<0.1 tesla), electronic relaxation is very fast and becomes the dominant correlation time, eqn (7). However electronic relaxation decreases with increasing field strength and at some point becomes slower than rotational motion, eqn (8). The result is that the correlation time for nuclear relaxation changes as a function of field.

$$1/T_{1e},\ 1/T_{2e} \gg 1/\tau_R;\ T_{1e} \approx \tau_{c1},\ T_{2e} \approx \tau_{c2} \quad \text{at low field } (B_0 < 0.1 \text{ T}) \tag{7}$$

$$1/T_{1e},\ 1/T_{2e} \ll 1/\tau_R;\ \tau_R \approx \tau_{c1} \approx \tau_{c2} \quad \text{at high field } (B_0 > 1.5 \text{ T}) \tag{8}$$

The result of this crossover is seen in Fig. 10 for τ_R = 10 ns. At low fields, electronic relaxation is dominating τ_c and relaxivity increases as field increases because electronic relaxation slows and τ_c increases. By about 0.7 tesla relaxivity reaches a peak and starts to decline because $\tau_c\omega_H$ becomes larger than 1 and the denominator in eqn (3) becomes large.

Gd(III) complexes have a small zero field splitting (ZFS) on the order of 0.01–0.05 cm^{-1} ($D \sim$ 100–700 Gauss).[30] The electronic spin Hamiltonian is assumed to be comprised of Zeeman and ZFS terms

$$H_S(\beta,\gamma;t) = H_{Zeeman} + H_{ZFS}(\beta,\gamma;t) \tag{9}$$

The Hamiltonian, $H_{ZFS}(\beta,\gamma;t)$, of the permanent (static) ZFS depends on the polar angles (β,γ) specifying the orientation of the laboratory magnetic field in the ZFS principal axis system. In solution, it is also a stochastic function of time, modulated by rotational diffusion. There is also a time dependent part called the transient ZFS wherein rapid distortions (picosecond timescale) in the complex, caused by vibrations and solvent collisions, induce ZFS.

At high fields where the Zeeman energy is much greater than the ZFS and where the perturbation theory assumption (Redfield limit) is valid, proton relaxation can be described by the theory of Solomon, Bloembergen and Morgan (SBM).[24] Because S = 7/2, electronic relaxation is a multi-exponential process. However, Fries and Beloritzky[31] demonstrated that T_{1e}, which is important for nuclear relaxation at high fields, is effectively described by a monoexponential decay. In the high field limit, the field dependence of T_{1e} is described by a simple analytical expression. At high fields the discrete analytical expressions of SBM are valid.

The availability of the field cycling relaxometer has enabled collection of relaxation rate data over ^{1}H Larmor frequencies ranging from 0.01 MHz to 50 MHz and higher. These field dependent T_1 data are referred to as nuclear magnetic relaxation dispersions (NMRD). Field cycling relaxometry accesses very low fields (2 Gauss) where the Zeeman energy is much less than the ZFS energy. At these fields, the SBM theory is not valid. In practical terms, the static ZFS results in faster electronic relaxation (and lower relaxivity) than would be predicted by the SBM theory. These low field effects are shown in Fig. 12 where simulations are performed using computational approaches described by Schaefle and Sharp[32] for two hypothetical complexes with rotational correlation times of 0.1 ns and 10 ns. For the fast tumbling complex, Fig. 12a, the NMRD profiles are rather featureless. Omitting the static ZFS results in an increased relaxivity at low fields. From Fig. 12b, one sees that not only does the presence of ZFS decrease relaxivity, but the orientation of ZFS axes also plays a key role. When the water proton lies along the principal ZFS axis ($\theta = 0^\circ$, dashed line in Fig. 12), electronic relaxation is slower than when the water proton is perpendicular to this axis ($\theta = 90^\circ$, dotted line in Fig. 12). For the parameters used in Fig. 12b, low field relaxivity differs by over a factor of two. Fig. 12 also teaches that relaxivity is field independent (flat) at low fields, which means that obtaining the ZFS parameters from the NMRD profile is not possible since there are simply too many unknowns. Moreover, this discussion is probably too simplistic; for example Schaefle and Sharp have shown that higher order terms are also important determinants of relaxivity.[33] Note as well that the curves become coincident with the SBM (Zeeman only) curve at higher fields.

There are many reports in the literature that have listed molecular parameters derived from the fitting of NMRD curves to SBM equations, and these have been summarized in reviews.[23] SBM assumes no static ZFS, and that electronic

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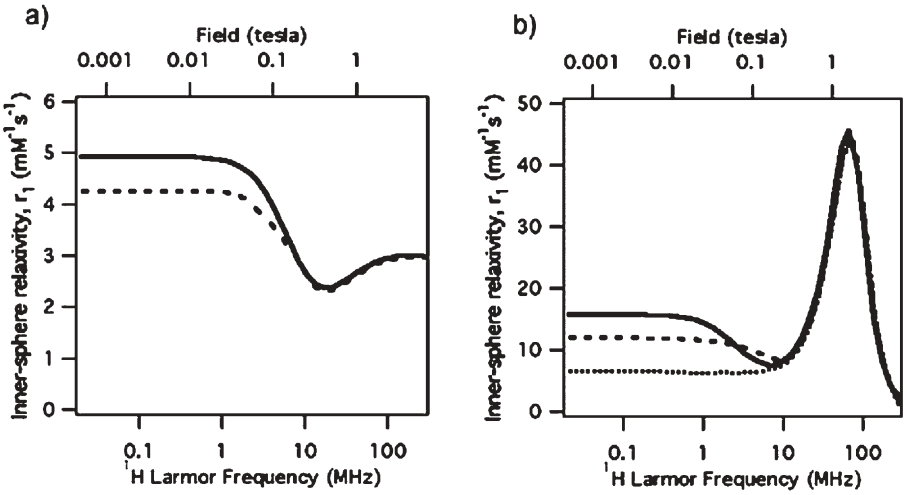

Fig. 12 Inner-sphere relaxivity calculated using the Zeeman only term (SBM theory, solid line) or taking into account an axial static ZFS, D = 0.05 cm^{-1} (dashed line where angle $\theta = 0^\circ$, dotted line where $\theta = 90^\circ$) for a) fast tumbling, τ_R = 0.1 ns or b) slow tumbling, τ_R = 10 ns molecule. Other parameters: Δ_v = 0.05 cm^{-1}, τ_v = 10 ps, τ_m = 100 ns.

relaxation only arises from transient distortions resulting in zero field splitting (this is referred to as the transient ZFS). Based on the discussion above, it is clear that these reports over-estimate the magnitude of this transient ZFS, because the transient ZFS parameters are forced by the curve fitting program to account for all the electronic relaxation seen at low field. Overestimation of the transient ZFS leads one to believe that electronic relaxation may limit relaxivity at 1.5 tesla, the field most commonly used in MRI. However, for the polyaminocarboxylates widely studied as ligands for Gd(III), electronic relaxation is likely inconsequential at 1.5 tesla.

Water exchange

Water exchange is of obvious importance for relaxivity. The coordinated water must be in rapid exchange with bulk solvent in order to transmit the relaxation effect to the solvent. There is a large body of work on water exchange in Gd(III) complexes. This is a readily accessible parameter *via* variable temperature ^{17}O NMR transverse relaxation rate measurements at high field. Some useful empirical relationships have emerged. Replacing an acetato oxygen donor with an amido oxygen donor will result in a slower water exchange rate. For complexes undergoing dissociative water exchange (the majority studied), increasing the steric bulk will increase the water exchange rate. For example replacing an acetato oxygen donor with the larger phosphonato oxygen donor increases the exchange rate.[34]

The water residency time τ_m ($k_{ex} = 1/\tau_m$) enters into the equation for relaxivity directly and also through T_{1m}. If water exchange is very fast, then the short τ_m can be the correlation time that governs T_{1m}. Relaxivity can be limited if water exchange is too slow because the relaxation effect is poorly transmitted to the bulk. Relaxivity can also be limited if water exchange is too fast because the water isn't coordinated to the Gd(III) long enough to be relaxed. This crossover dependence of relaxivity on τ_m is shown in Fig. 13 where inner-sphere relaxivity is simulated for a q = 1 system at 1.5 tesla for

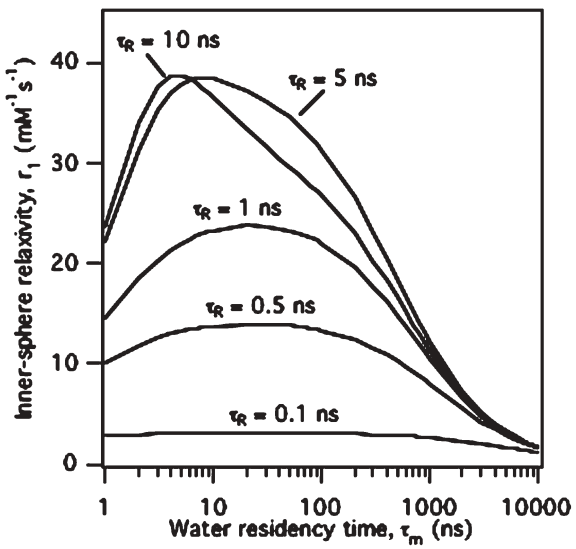

Fig. 13 Relationship between rotational diffusion and water exchange at a given field strength (1.5 tesla) for a q = 1 compound with a long (>10 ns) T_{1e}.

different values of τ_R. Fig. 13 teaches that relaxivity can be limited when water exchange is too fast or too slow, but this is true only if other parameters (*e.g.* τ_R) are optimized.

Fig. 13 also shows that having the "optimal" water exchange rate becomes more important when other parameters are optimized. For instance, when τ_R = 0.1 ns, any water residency time between 1–1000 ns will produce a relaxivity that is within 20% of the maximum at this value of τ_R. If τ_R is increased to 1 ns, then the range of acceptable τ_m values shrinks from 3–200 ns to give a relaxivity within 20% of the maximum. When τ_R is 10 ns, this range becomes 2–30 ns for τ_m. This indicates that depending on the application, the rotational dynamics may be sufficiently flexible that many possible gadolinium chelates will have good enough water exchange properties.

2nd-sphere and outer-sphere relaxivity

There is also a contribution to relaxivity from water diffusing nearby the gadolinium complex (outer-sphere relaxivity). Outer-sphere relaxivity can be predicted from the hard-sphere model of Hwang and Freed where relaxation is determined primarily by the diffusion coefficient of water and the distance of closest approach. Exchangeable hydrogens in the second coordination sphere also contribute to relaxivity. These exchangeable protons can come from water in the 2nd coordination sphere or protonation sites on the molecule. Fig. 9 clearly demonstrates that $q = 0$ compounds can have significant relaxivity. In the case of $[Gd(DTPA)(H_2O)]^{2-}$ only about half the effect is coming from the inner-sphere water.

There is ample empirical evidence to show that there is a 2nd-sphere effect. Consider the $q = 0$ complex $[Gd(TTHA)]^{3-}$ and the modified TTHA complex $[Gd(L0)]^{4-}$. The NMRD profiles of these complexes are plotted in Fig. 9. The relaxivity of both complexes is the same in water even though the larger $[Gd(L0)]^{4-}$ has a longer rotation correlation time. However $[Gd(L0)]^{4-}$ can bind to serum albumin. When bound to albumin, Fig. 14, the relaxivity of $[Gd(L0)]^{4-}$ is increased[28] as a result of a long lived proton(s), from either the complex or the protein, near the Gd(III). The outer-sphere model predicts that protein binding should have no effect on relaxivity. Another example of 2nd-sphere relaxation is given by $[Gd(C_{11}\text{-DOTP})]^{5-}$, another $q = 0$ complex.[35] Fig. 14 indicates that the relaxivity of $[Gd(C_{11}\text{-DOTP})]^{5-}$ is much greater than the $[Gd(L0)]^{4-}$, a molecule of similar size. In this case, the increased relaxivity likely results from exchangeable proton(s) associated with the negatively charged phosphonate surface. When rotation is slowed by binding to serum albumin, the relaxivity of $[Gd(C_{11}\text{-DOTP})]^{5-}$ is markedly increased, much more so than that of $[Gd(L0)]^{4-}$.

The magnitude of 2nd-sphere relaxivity is almost impossible to predict. For $q > 0$ compounds, relaxivity depends on all the parameters described above plus the number of exchangeable hydrogens in the 2nd sphere, the distance of these hydrogens to the Gd(III), and the lifetime of each of these hydrogens. The presence of a large 2nd-sphere effect is typically noticed when independently determined molecular parameters cannot account for the magnitude of the relaxivity observed. NMRD data have sometimes been modeled by adding additional parameters to account for 2nd-sphere relaxivity. However given the vast number of parameters involved, the physical meaning of fitted parameters must be questioned. Some parameters are heavily correlated.

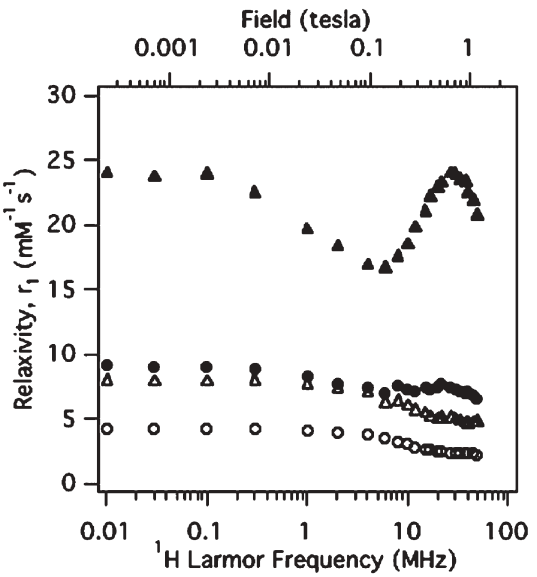

Fig. 14 Relaxivity of 0.1 mM $[Gd(L0)]^{4-}$ (circles) or $[Gd(C_{11}\text{-DOTP})]^{5-}$ (triangles) in the presence (solid symbols) or absence (open symbols) of 0.67 mM HSA at 25 °C, pH 7.4.

Effect of field strength and temperature

It is evident from Fig. 9–14 that relaxivity can be strongly dependent on applied field. Given that water exchange and rotational diffusion are both temperature dependent, it is obvious that relaxivity will be temperature dependent as well. Care should be taken when trying to compare data such that the temperature and field strength are the same. In the examples here, data are often chosen at 0.47 tesla (20 MHz) because these numbers are more often available. The ideal temperature at which to compare data is 37 °C, since this is physiological temperature and the majority of contrast agent applications are *in vivo* at 37 °C. Some examples were chosen for data at 25 °C because this was a temperature where the full complement of data were available.

The trend in NMR imaging, like spectroscopy, is toward higher fields. The vast majority of clinical MRI operate at 1.5 tesla (~64 MHz). There is a growing installed base of clinical scanners at 3 tesla, and for research purposes there are now whole body 7 tesla (300 MHz) instruments. Higher fields result in greater signal to noise ratio and increased resolution. Fig. 10 suggests that at high fields, the benefits of contrast agents should be less because relaxivity is lower. However one must keep in mind that relaxation times of tissue increase with increasing field strength, so less contrast agent of a fixed relaxivity is required to yield the same contrast. Long tissue relaxation times and high resolution scans result in long acquisition times. The presence of a relaxation agent significantly shortens the acquisition time, a fact that impacts whether the diagnostic test will be adopted clinically.

Fig. 10 suggests that correlation times on the order of 1 ns rather than 10 ns are preferred for 3 tesla. The metallostar (Fig. 3), with its combination of $q = 2$ and an intermediate rotational correlation time is a nice example of a high field agent. Its per gadolinium relaxivity at 37 °C is 23.8 and 15.6 mM^{-1} s^{-1} at 100 and 200 MHz. respectively.[9]

DOTA and DTPA

There are many possible complexes that can be used to create multimeric and targeted contrast agents. Novel complexes with very fast water exchange continue to be reported. High relaxivity $q = 2$ complexes that appear to be stable continue to appear. For the researcher looking for a gadolinium complex to create a multimeric and/or targeted contrast agent, gadolinium complexes derived from DOTA or DTPA ligands represent useful synthons. Because of their use in MRI and in radiotherapy, there is a vast literature on bifunctional

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lanthanide chelates based on either DOTA or DTPA. Numerous procedures exist for linking these complexes to targeting vectors. These are thermodynamically stable complexes. The parent complexes $[Gd(DTPA)(H_2O)]^{2-}$ and $[Gd(DOTA)(H_2O)]^{-}$ have been administered to millions of patients. GdDOTA derivatives are very inert to substitution making them very attractive for applications where the contrast agent has a long residency time in the body. GdDTPA derivatives are less inert and can undergo metal ion exchange at low pH. This property is useful in that radiolabeled complexes can be readily prepared by isotopic exchange at low pH. Both complexes are very soluble and hydrophilic. They possess water exchange rates that are very good ($k_{ex} = 10^7\ s^{-1}$ at 37 °C) and should not substantially limit relaxivity unless the rotational dynamics of the molecule are optimized. However, it should be stressed that these complexes should not be functionalized by converting a carboxylate group into an amide and using the amide nitrogen to link to the targeting group. The amido oxygen donor will result in a slower water exchange rate that can limit relaxivity as shown above in Fig. 1.

Conclusion

Much progress has been made in the design of new MRI contrast agents. Through a better understanding of the coordination chemistry and biophysics, improved contrast agents are making their way into clinical practice. Perhaps the most important parameter influencing relaxivity is rotational motion. Because relaxivity depends on rotational dynamics, new targeted contrast agents will necessarily have different relaxivities, and relaxivity will ultimately need to be optimized according to the application. There are several approaches that control the rotational dynamics in order to increase relaxivity. Novel strategies for linking multiple gadolinium complexes are also beginning to appear. There is a good and growing understanding of how to modulate the water exchange rate at the gadolinium and numerous complexes have exchange rates in a useful range. Stable $q = 2$ compounds continue to be prepared. It will be important to examine their *in vivo* stability to determine the utility of these compounds as synthons. At most imaging fields (1.5 tesla and up), the key parameters are rotation, hydration, and water exchange; electronic relaxation at the ion is not a factor. Additional relaxivity gains may be achieved from exchangeable hydrogens in the second coordination sphere, however at the moment, 2nd-sphere relaxivity is difficult to predict and to apply. MRI has become a routine clinical tool because of its resolution and excellent soft tissue contrast. The gains made in the study of the biophysics of gadolinium complexes promise to guide the design of future molecular MRI contrast agents.

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