Lanthanides in magnetic resonance imaging

Melanie Bottrill, Lilian Kwok and Nicholas J. Long

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Magnetic Resonance Imaging is perhaps the most important and prominent technique in diagnostic clinical medicine and biomedical research. Its success and development as an imaging technique has been aided by the characteristics of contrast agents that enhance signal intensities and improve specificity. Gadolinium(III) remains the dominant starting material for contrast agent design but other lanthanide ions (and other oxidation states *i.e.* +2) are also being increasingly investigated as alternatives to gadolinium(III) within laboratory conditions. This *critical review* provides a concise summary of the MRI-active gadolinium(III) complexes to date – their pros and cons, an outline of contrast agents based on other lanthanide ions (*e.g.* europium, dysprosium), and directs the reader to newer, more speculative areas of lanthanide-containing contrast agent design.

Department of Chemistry, Imperial College London, Exhibition Road, South Kensington, London, UK SW7 2AZ.
E-mail: n.long@imperial.ac.uk; Tel: 020 75945781

Melanie Bottrill

Melanie Bottrill, born in Birmingham in 1982, obtained an MSci from Imperial College London in 2004. She is currently undertaking a PhD under the supervision of Dr Nicholas Long on nanoparticulate materials for biomedical imaging, focusing on biocompatible quantum dots for cell labelling studies.

1 Introduction and scope

Molecular imaging is one of the most exciting and rapidly growing areas in science. Biological processes can be studied *in vivo* and non-invasively at the cellular and molecular level by a range of imaging modalities. Of these, Magnetic Resonance Imaging (MRI) has become one of the most important and evolved as a prominent technique in diagnostic clinical medicine and biomedical research. The use of MRI eliminates the need for invasive diagnostic procedures, and it has been shown to provide physiological information earlier in clinical investigation. The technique also offers fast scan times, the capacity to produce excellent quality and high-resolution images and the avoidance of radiochemicals. Amongst the common modalities, MRI has the greatest spatial resolution and clinical potential, and can consistently image structures in the millimetre range without the use of ionising radiation such as that used in X-ray and CT scanning. Despite these attractive

Nick Long

Nick Long, born in Bristol in 1965, obtained a BSc from the University of Durham and a PhD from the University of Exeter in 1989 under the supervision of Eddie Abel and Tony Osborne. He was a Demonstrator in Inorganic Chemistry in Exeter before moving to the University of Cambridge to become the Adrian Research Fellow with Darwin College, and also a Temporary Lecturer in Inorganic Chemistry. In January 1995, he was appointed to a Lectureship in Inorganic Chemistry at Imperial College London and he is now a Reader in Inorganic Chemistry. His expertise lies in applied synthetic chemistry, particularly transition metal and lanthanide coordination and organometallic chemistry, and he has a number of areas of interest within catalysis and materials science. Recently, he has commenced research into the synthesis of biomedical imaging agents for magnetic resonance imaging (MRI) and positron emission tomography (PET). New transition metal (Gd, Fe, Mn) and lanthanide-containing materials are a focus, along with the production of ^{11}C-labelled species, via homogeneous catalysis, coordination of ^{11}CO and the application of these species on a nano-scale via microfluidics.

Lilian Kwok

Lilian Kwok, born in Hong Kong in 1983, is currently in her final year of MSci in Chemistry undergraduate studies at Imperial College London. Her research project is on the catalytic asymmetric synthesis of antitumour agent Halomon under the supervision of Dr Christopher Braddock.

parameters, the introduction of MRI as a molecular imaging modality has been hampered by its low sensitivity compared to radionuclear methods such as PET and SPECT. However, with recent developments in chemistry and the synthesis of powerful, innovative, specific and multimodal contrast agents, MRI is at the forefront of molecular imaging.

MRI relies upon the enhancement of local water proton relaxation in the presence of a contrast agent. The most commonly used contrast agents nowadays are thermodynamic and kinetically stable low molecular weight gadolinium compounds. The unique magnetic properties of the gadolinium(III) ion are instrumental in enhancing the relaxation rate of water protons in tissues.

Although some iron- and manganese-containing materials are commercially important, the predominant contrast agents in commercial use today, Magnevist® and Dotarem®, are comprised of a Gd^{III} atom within chelating ligands based on a polyaminocarboxylate motif (linear DTPA and cyclic DOTA respectively). Along with the continued dominance of gadolinium, in the last few years there has been increasing interest in the utilisation of other lanthanide ions (in the +2 and +3 oxidation states) in MRI contrast agents. New challenges now are to design systems endowed with improved relaxivity, responsiveness and specific targeting abilities. The scope of this review is to illustrate attempts to solve some of the problems found with MRI-active gadolinium(III) complexes to date, to outline contrast agents based on other lanthanide ions (*e.g.* europium, dysprosium) and to direct the reader towards the newer, more speculative areas of lanthanide-containing contrast agent design. There are a number of excellent reviews[1–8] already published that cover the development and properties of first and second generation contrast agents, particularly focusing on gadolinium, and hence this material will not be covered in detail but will be referenced accordingly to direct the reader.

1.1 What is MRI?

In the world of medicine, MRI is a non-invasive procedure based on the magnetic fields of protons within the body, producing two-dimensional views of internal organs or tissues.[9] The technique is based upon the principles of nuclear magnetic resonance, discovered independently by Bloch and Purcell in 1946, for which they were awarded the 1952 Nobel Prize.[10,11] In 1973, Lauterbur used the principles of NMR with a strong and weak magnetic fields to identify the position of a particular nucleus, as the strength of the field is proportional to the radiofrequency.[12] Lauterbur did not make any postulations as to the potential application for this research, although the comment was made that there was a signal difference between cancerous tissue and normal tissue. Nevertheless, clear potential for the use in clinical imaging was realized, and as soon as eight years after this idea was reported, prototype MRI machines were invented. In 1980, medical MRI scans commenced and since then MRI has become an essential tool in forming diagnoses in medicine.

1.2 What makes a good contrast agent?

MRI contrast agents are chemical compounds that are able to markedly alter the relaxation times of water protons in tissues where they are distributed.[3] This in turn leads to remarkable improvements in medical diagnoses, as they facilitate higher sensitivity and specificity and better tissue characterisation.[2] Contrast agents can be divided into two groups depending on whether they cause changes in either T_1 (longitudinal relaxation – in simple terms, the time taken for the protons to realign with the external magnetic field) or T_2 (transverse relaxation – in simple terms, the time taken for the protons to exchange energy with other nuclei) relaxation rates of the water protons, these being known as positive or negative agents respectively.[3] (For a full description of relaxivity and the physical processes associated with MRI please refer to one of the books published on MRI.[8,13]) The ability of an agent to affect T_1 and T_2 is characterised by the concentration-normalised relaxivities r_1 and r_2 respectively. These parameters refer to the amount of increase in $1/T_1$ and $1/T_2$ respectively, per millimole of agent, and are normally quoted as a rate ($mM^{-1}\ s^{-1}$).[4] The values are used to determine the efficiency of a contrast agent, and they consist of contributions from both inner sphere and outer sphere relaxation mechanisms.[14] The signal observed in MRI tends to increase with an increase in $1/T_1$ and decrease with an increase in $1/T_2$, and it is usual for contrast agents to affect both $1/T_1$ and $1/T_2$ to varying degrees.[4] (For further detailed analysis of T_1 and T_2, consult refs. 13 and 14.) Positive contrast agents are commonly made up of paramagnetic materials, mainly those based on metal ions with large numbers of unpaired electrons, such as Mn^{2+} (5 unpaired electrons), and Gd^{3+} (7 unpaired electrons). With positive contrast agents, a similar effect is seen on both T_1 and T_2, but because T_1 is larger than T_2, shortening of T_1 is observed. This results in the image being brighter within areas where the agents are taken up, due to brightness being a function of T_1. Thus species with high T_1 values lend themselves to darker images. Negative contrast agents influence the signal intensity by shortening transverse relaxation (T_2), thereby producing darker images as high T_2 results in increased brightness of the images. Negative contrast agents are commonly formed of superparamagnetic materials such as iron oxide nanoparticles.[15]

Some 35% of MRI exams occur with contrast agents, and gadolinium(III) reagents are commonly focused on due to the coupling of a large magnetic moment with a long electron spin relaxation time of 10^{-9}s at the magnetic field strengths used in MRI techniques.[2] Considerations need to take into account the toxicity of this heavy metal, as its radial size is approximately equal to that of calcium(II), and as a result it can disrupt calcium(II) mediated signalling, forming strong complexes that can accumulate within the body.[4] The correct choice of ligand can prevent this *in vivo* transmetallation occurring, and this has enabled gadolinium complexes to be developed for MRI applications. Contrast agents used in the body clearly should be biocompatible, but there are also other issues to be addressed.[2] These include the requirements of rapid renal excretion, water solubility, stability in aqueous conditions, and a low osmotic potential when in solution for clinical work.[2,3] In addition, at least one water molecule must be bound to the gadolinium centre (*i.e.* within the coordination sphere) and this will undergo rapid exchange with the water molecules of the surrounding solution to affect the relaxation

time of all the solvent protons.[2] The images obtained are representations of the relaxation times of these protons, and so exchange has to be rapid.[2]

1.3 Factors controlling relaxivity

Relaxivity is controlled by parameters that will be outlined and briefly discussed in this section as these points are essential to the understanding and design of contrast agents. The Solomon–Bloembergen–Morgan equations bring these important parameters together and these equations have been outlined and discussed in many previous reviews and further details can be obtained from for example, Kowalewski *et al.*,[16] Caravan *et al.*,[4] and Aime *et al.*[3] The inner sphere relaxation mechanism utilises gadolinium and directly bound water interactions, and the outer sphere mechanism is based upon interactions between the second sphere and closely diffusing water molecules.[14] Fig. 1 demonstrates the different parameters that need optimisation for high relaxivity. These include the hydration of the metal ion, the mean residence time of the water molecule in the first coordination sphere (τ_M), and the tumbling rate of the species, which is characterised by the rotation correlation time (τ_R).[7]

(a) Inner sphere relaxivity

The relaxivity of a contrast agent at commonly used imaging fields (0.5–1.5 T) can most effectively be controlled when the complexes are designed with the inner sphere relaxation mechanisms in mind. These mechanisms are governed by the following equation:[14]

$$R_{1P}^{IS} = \frac{Cq}{55.6} \frac{1}{T_{1M} + \tau_M}$$

This equation illustrates the inner sphere contribution to relaxivity, where C = molar concentration of paramagnetic compound, q = number of bound water molecules, and T_{1M} = longitudinal relaxation time of the bound water protons.[14] T_{1M}, is controlled by the molecular rotational correlation time, τ_R, whereby slower tumbling of the contrast agents leads to faster relaxation rates, and hence relaxivity.[14]

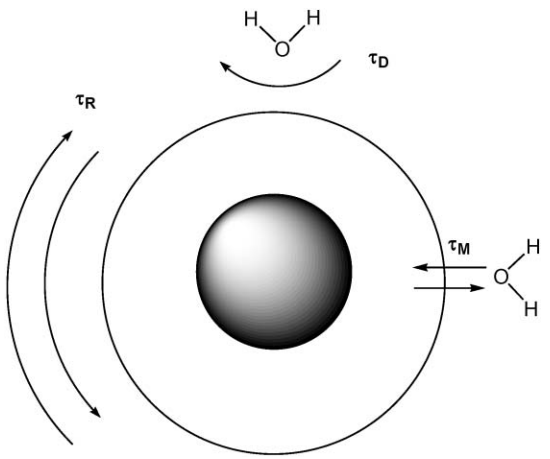

Fig. 1 Illustration of the different parameters that require optimisation for maximum relaxivity of gadolinium contrast agents.

The number of bound water molecules, q, is usually 1 (especially in current commercially available contrast agents) and this is due to the octadentate ligands used to prevent dissociation of the metal.[7] These are very stable complexes, and prevent the release of the metal within the body. However there have been recent examples of stable contrast agents containing two water molecules bound to the gadolinium (see section **4.3**).[3] This is an attractive development as higher numbers of water molecules present enhance the relaxivity of the complex. As mentioned previously, T_{1M} is dominated by molecular reorientation τ_R.[14] It also depends on the residence time of the bound water, τ_M, and electron paramagnetic relaxation, T_{1e} and T_{2e}. Aime *et al.* provide a full and detailed explanation of these factors in their recent review article, and hence a full explanation is not provided here.[3] In basic terms, T_{1e} and T_{2e} are frequency dependent, which means that at usual field strengths (0.5–1.5 T) τ_M, T_{1e} and T_{2e} are insignificant.[3] Hence at these fields consideration is mainly given to the molecular rotation correlation time (examples are discussed in sections **4** and **5**).

(b) Outer sphere relaxivity

Contrast agents can display relaxivity even when $q = 0$. Thus, as there is no water in the inner coordination sphere the relaxivity must come from outer sphere contributions.[17] These can take two forms; (i) second sphere relaxation where water molecules hydrogen bonded to the carboxylate oxygen atoms are relaxed *via* dipolar mechanisms and (ii) outer sphere relaxation which arises due to diffusion of water molecules in the bulk near to the Gd(III) complex.[4,18] The parameters focused on are the electronic relaxation time of the metal, the distance of the closest approach of solvent and solute and the sum of their diffusion coefficients.[2] The outer sphere relaxivity is usually estimated by equations proposed by Freed.[19] These equations are not covered in this review as they are discussed in detail elsewhere[3,4] It is important to note that this model is only an approximation for the polyaminocarboxylate ligands used in contrast agents because it does not take into account interactions of water with the complex, which for these ligands are important.[3] On the whole it seems that outer sphere contributions to relaxivity are not very well understood, and this phenomenon is overlooked when contrast agents are being developed.[4]

1.4 The impact of high field instrumentation

One interesting aspect of MR imaging is the rise in use of high field instruments. These new systems now operate in the 3 tesla field range and as a result new contrast agents need to be designed with different criteria in mind.[20] The requirements for these contrast agents are that they combine a fast water exchange rate with effective coupling of the gadolinium–water vector and the tumbling of the complex as outlined by Parker *et al.* recently.[20,21] In addition, a greater contribution from the water molecules in the second coordination sphere is desirable.[20] In these reports, the strategy was to engineer a complex featuring the Gd ion at the barycentre of a macromolecular structure, so that it resides upon any axis of rotational motion. Dendritic systems are attractive in this

regard, and examples of *C*-4 symmetric, medium MW conjugates incorporating 12 glucose or galactose groups linked *via* four dendritic wedges to a central Gd complex have been characterised. The enhanced relaxivity was interpreted in terms of effective motional coupling and large contributions from second sphere water molecules.

MR imaging at these high magnetic fields is now becoming commonplace and the design of contrast agents must also advance with new ideas being sought to maintain the pace of the last 20 years worth of research. The advantages of high field instrumentation are the increase in signal to noise ratio, reduced scan times and improved resolution. Further research into these areas will increase the maximum relaxivity attainable and maintain progress in this rapidly developing area.

2 Clinically approved gadolinium chelates

Of the six clinically approved contrast agents used worldwide for intravenous administration, four of them are gadolinium based with a polyaminocarboxylate ligand forming highly stable complexes (the other two are mangafodipir trisodium,[22] also known as Telescan®, and ferumoxides[23,24] which are superparamagnetic iron oxide nanoparticles often coated with dextrans). The anionic complexes of $Gd(DTPA)^{2-}$ (Magnevist®) and $Gd(DOTA)^{-}$ (Dotarem®) were the first complexes used in clinical practice, and Gd(DTPA-BMA) (Omniscan®) and Gd(HPDO3A) (Prohance®) are neutral compounds based on the structures of the anionic complexes (Fig. 2). $Gd(DTPA)^{2-}$ (C) forms a stable octacoordinated chelate, similarly to $Gd(DOTA)^{-}$ (A), but their formation is very different. DTPA is commercially available, but DOTA requires the preparation of 1,4,7,10-tetra-azacyclododecane, which is time consuming.[25] The DTPA ligand forms stable chelates based on a distorted tricapped trigonal prism with the three nitrogens, five carboxylic oxygens, and the necessary water molecule (omitted from Fig. 2) coordinating to the gadolinium centre.[26] DOTA forms very stable lanthanide chelates because the tetra-aza cycle is able to adopt its most stable conformation. The solid state X-ray structure of $Gd(DOTA)^{-}$ indicates that the Gd^{3+} is situated in the centre of a capped square antiprismatic cage, with the water molecule in an axial position.[27] When in solution, it exists as a pair of interchangeable isomers; the major square antiprismatic and the minor twisted square antiprismatic (Fig. 3).[28] The neutral complexes of Gd(DTPA-BMA) and Gd(HPDO3A) share a similar stability in thermodynamic terms.[29] HPDO3A complexes are kinetically stronger because the rigid ring structure of the macrocycle prevents release of the metal, as at least five coordination sites would have to break simultaneously, whereas in DTPA-BMA, it is possible to see sequential breaking of the coordination sites.[30] The potential for release of Gd^{3+} from the DTPA-BMA ligand is minimised by formulating the complex with 5% excess calcium ligand to scavenge competing biometals.[31]

These first generation contrast agents are very useful, and have accounted for the rise in MRI scans being used in diagnosis. They distribute mainly into the intravascular and interstitial space, and although they are deemed to be non-specific, they can accumulate within the kidneys due to glomerular filtration.[32] The next generation of contrast agents are designed to be more specific and more effective, with an unusually high relaxivity,[33] greater thermodynamic stability and a more favourable rate of excretion. As will be seen in some examples discussed later, this can be achieved by attaching the contrast agent to larger structures such as dendrimers[34] or micelles.[35] In efficiency terms, the new reagents are usually compared to the DOTA and DTPA complexes due to their prior clinical approval.[36]

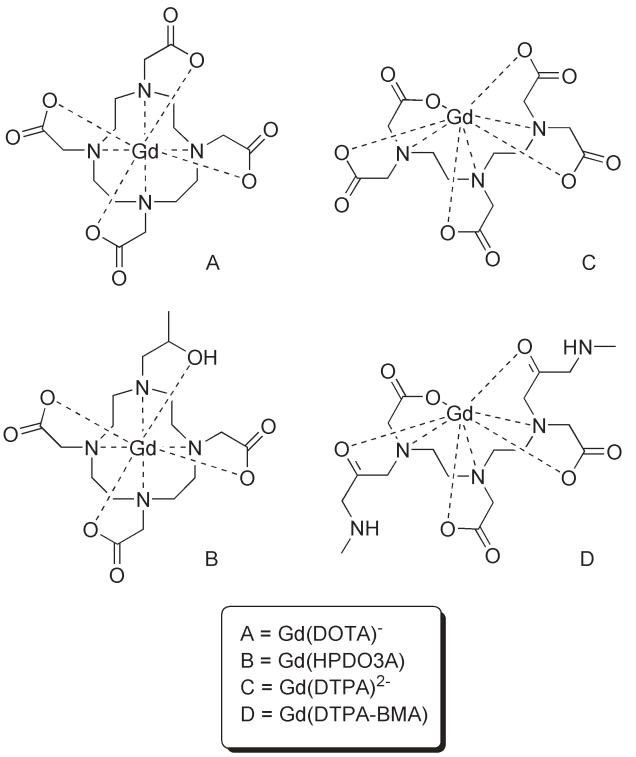

Fig. 2 Structures of the commercially available gadolinium(III)-based contrast agents.

3 Lanthanide(III)-based complexes for MRI

The review articles cited in the introduction cover the major developments in paramagnetic contrast agents based on Gd(III) chelates over the last 10–15 years. More recently there has been an increase in the number of reports published on

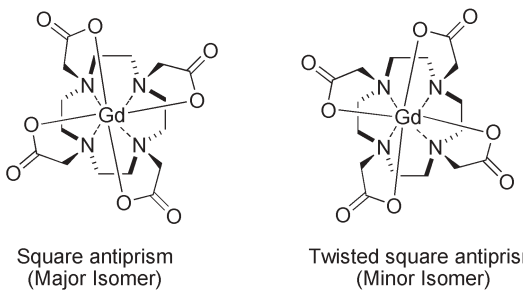

Fig. 3 The minor and major isomers of DOTA-based MRI contrast agents. The minor isomer exhibits faster water exchange than the major isomer, suggesting that compound should be of a TSA structure.

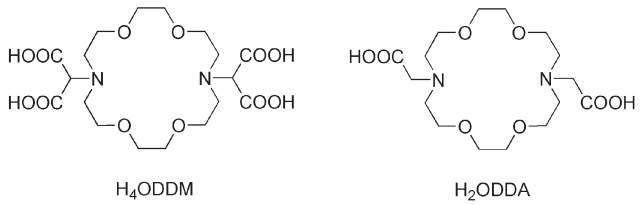

Fig. 4 The structures of the ligands $ODDMH_4$ (left) and $ODDAH_2$ (right) used by Merbach *et al.* in the design of redox responsive Eu(II)-based contrast agents.

paramagnetic contrast agents utilising other lanthanide ions with chelating ligands. This is due to the potential use of Eu(II) as a redox responsive contrast agent,[37] and Dy(III) as a high field contrast agent.[38]

3.1 Europium(II) complexes as redox responsive contrast agents

Europium(II) analogues have been proposed as alternatives to Gd(III) because they are isoelectronic—each having seven unpaired electrons.[37] In addition, europium(II) was thought to be a good choice as a spectroscopic probe for calcium(II) in biological systems because its ionic radius falls between those of Ca(II) and Sr(II) (125 pm, compared with 112 and 126 pm respectively), and its chemical properties are similar to those of the alkaline earth metal ions.[6,37,39]

Merbach *et al.* reported the water exchange kinetics and electronic relaxation of the following Eu(II) complexes: $[Eu(DTPA)(H_2O)]^{3-}$, $[Eu(ODDM)]^{2-}$ and $[Eu(ODDA)(H_2O)]$, where the ligands $ODDMH_4$ and $ODDAH_2$ are shown in Fig. 4:[37] Initially the DTPA analogue was investigated, but it was found that the redox stability of the Eu(II) poly(aminocarboxylate) compound was too low to be useful, and other analogues were sought.[37] The search for these ligands led to the azacrown ethers depicted in Fig. 4 because these macrocycles form relatively stable complexes with divalent and trivalent ions of large ionic radius. In addition, $ODDM^{4-}$ shows a large selectivity for Sr(II) against Ca(II), and due to the radii of the ions in question this indicated a viable ligand for the Eu(II) ion.[37] It was found that the redox potentials of these complexes were $E_{½} = -1.35$ V for Eu^{III}/Eu^{II}(DTPA), $E_{½} = -0.92$ V for Eu^{III}/Eu^{II}(ODDM) and $E_{½} = -0.82$ V for Eu^{III}/Eu^{II}(ODDA), indicating that the azacrown ether complexes are more redox stable than the DTPA complex.[37] At physiological pH, the macrocyclic complexes did not display any proton catalysed dissociation, and so it was postulated that these compounds could be useful redox responsive MRI agents, even if they were still some way from being commercially viable.[14,37] Redox responsive MRI contrast agents are useful as they are responsive to the partial pressures of oxygen, and this is significant in a variety of diseases and conditions, such as strokes and tumours, as well as blood flow investigations into arterial and venous blood.[6] The concern was that the *in vivo* applicability was questionable, due to difficulties in controlling the stability of the Eu(II) state.[6] Hence it was proposed that a macrocyclic ligand was required that had the optimum cavity size in order to render the complex thermodynamically and redox stable.

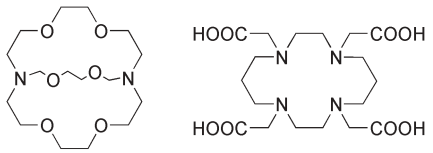

Fig. 5 The structures of cryptand 2.2.2 (left) and TETA (right) used by Merbach *et al.* in the development of redox responsive Eu(II)-based contrast agents.

Further work in obtaining a stable redox active europium-based MRI active chelate has been undertaken with a 10-coordinate complex formed with the cryptand 2.2.2 (4,7,13,1,6,21,24-hexaoxo-1,10-diazabicyclo[8.8.8]hexacosane) found to have high redox stability (Fig. 5). In addition, it has two inner sphere water molecules and rapid water exchange rates.[40] This was considered a useful structure to build a contrast agent around as it had promising characteristics for increased proton relaxivity: stable against oxidation; two inner sphere coordinated water molecules; water exchange rates within the optimal rate for good relaxivity; and a macromolecular structure to allow for slow tumbling of the complex.[40] The only problem with this complex was that the stability of the Eu(II) chelate was 10^7 times higher than that of the Eu(III) chelate, which meant that the Eu(III) complex could be susceptible to dissociation, possibly releasing toxic Eu(III) into the body.[41] The 2.2.2 cryptand was found to be useful and required little modification, which led to the replication of the experiment with ligands such as TETA (Fig. 5) and DOTA.[41] However, the formal redox potentials for these compounds indicated a lower stability compared to the 2.2.2 cryptand, and lay between those of the aqueous ion and the ODDM and ODDA ligands discussed previously. This was assumed to be due to the size of the ligand, and the presence of the carboxylate groups, both of which are unfavourable in the reduced state.[41] Nevertheless, the proton relaxivity was also analysed for these compounds. From this data it was found that the TETA complex had low relaxivity (2.60 $mM^{-1}s^{-1}$, 298 K, 20 MHz), due to the mechanism consisting of outer sphere contributions only. This indicated that no inner sphere water molecules were present and this was confirmed by the crystal structure of the strontium(II) analogue with no sign of the crucial inner sphere water molecule.[41] (Strontium(II) complexes are generally isomorphic to Eu(II),[42] and are accepted as an alternative to Eu(II) crystal structures where they cannot be obtained.[41]) The DOTA complex demonstrated a relaxivity rate typical to that shown for low molecular weight complexes with one exchangeable water molecule (4.32 $mM^{-1}s^{-1}$, 298 K, 20 MHz).[41] This value is close to that shown for the analogous gadolinium complex, and is thought to be due to the relaxivity in both analogues being limited only by rotation due to slow electron spin relaxation.[41] Merbach *et al.* propose that the next step in the development of these redox responsive contrast agents is to form large macromolecules to slow down rotation, and hence optimise relaxivity. This has been used in the past for gadolinium complexes to good effect.

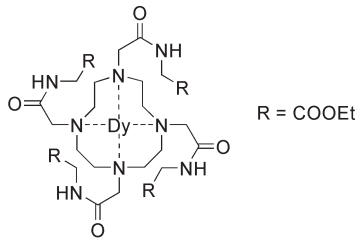

Fig. 6 The structure of Dy-DOTA-4AmCE used as a paramagnetic negative contrast agent.

3.2 Dysprosium(III) complexes as high-field contrast agents

Dysprosium(III) is another lanthanide ion that has been used in MRI, being classed as a negative contrast agent. Clinical MRI is moving towards the use of high magnetic fields, and in these cases commercial Gd(III) based contrast agents exhibit poor water relaxivity. As a result, the interest in dysprosium-based complexes is increasing as they display slow water exchange, due to the need to lengthen the residence time in order to optimise the r_2 relaxivity. Dy(III) has a large magnetic susceptibility which induces local field gradients resulting in a lowering of T_2.[38] The magnetic field dependence of $1/T_1$ and $1/T_2$ of solvent protons in water has been examined in numerous accounts using some Dy(III) analogues of clinically used Gd(III) agents.[43–45] Thus in general, dysprosium complexes where the water molecules have a long residence time, possess potential application as negative contrast agents at high magnetic fields due to their efficient transverse relaxivity.[38]

However, Muller *et al.* have demonstrated *via* Dy-DOTA-4AmCE[46] (Fig. 6), that lengthening the residence time of water can actually be detrimental, because the transverse relaxivity can then be limiting.[46] Experiments have shown that the transverse relaxivity of complexes with fast exchange of water protons increases with the square of the magnetic field and the residence time (τ_M).[47] At high magnetic fields, a residence time of greater than 1 μs restricts the relaxivity for dysprosium complexes, whereas residence times of less than 100 ns limits the relaxivity at both low and high magnetic fields. Therefore, to have optimum r_2 at the high magnetic fields required, the residence time needs to be optimised between 0.1 and 1 μs.[46]

The overall conclusion from the use of dysprosium(III) complexes as contrast agents is that due to the balancing of factors required to optimise r_2, design of a suitable molecular structure is crucial. Fine tuning these residence times of the water protons, and hence the relaxivity, may lead to promising contrast agents for high field magnetic resonance imaging.[46] The use of dysprosium(III) compounds as negative contrast agents is still fairly new, and it will be interesting to look at their development over the next 5–10 years.

3.3 Ln(III) compounds as PARACEST contrast agents

There are alternatives to using T_1 shortening contrast agents as contrast can originate from altering proton density or the total water signal that is detected. This can be done by using a technique known as Magnetisation Transfer (MT). MT is based upon magnetisation interactions that occur between two different regions of protons, for instance bulk water and macromolecular protons found within the body.[48] Balaban *et al.* demonstrated how this can occur when they used a presaturation pulse to saturate the broad water signal that is present behind the bulk water signal in tissue.[49] In simpler terms, applying a selective RF pulse to one set of protons transfers saturation to the other, which results in a decrease in the signal that is dependant on the magnitude of the magnetisation transfer between the protons.[50] Gadolinium(III) cannot be used in this technique because the T_1 of water would be too short, but other lanthanides, such as Eu(III), can be used because they have smaller magnetic moments, relaxing bulk water much less efficiently.[51] The use of MT techniques led to the development of CEST (*C*hemical *E*xchange dependent *S*aturation *T*ransfer) contrast agents by Balaban *et al.*[52] This was based on macromolecules found within the body, such as amino acids, nucleotides and carbohydrates, that contain OH and NH groups.[52] These groups can exchange protons with bulk water and it was demonstrated that contrast can be switched on and off by the application of a saturating pulse a few ppm away from the resonance of bulk water.[53] In other words, when the irradiating pulse is switched on, the pulse sequence illuminates the tissue where water is in exchange with the CEST agent.[52] Contrast agents that belong in this group are classed as negative contrast agents because they decrease the intensity of the water signal.[54]

The use of paramagnetic lanthanide complexes as CEST reagents is favourable as the paramagnetic ion induces a large shift in the resonance of the nuclei surrounding it, which then causes a more efficient transfer of the magnetisation (hence the name PARACEST).[54] Aime *et al.* reported that the Eu[DOTAM]$^{3+}$ complex (Fig. 7), which again is found as two isomers similar to the parent analogue of Ln[DOTA], exhibits an NMR signal for the water bound to the metal centre at low temperatures in deuterated acetonitrile.[55] Based on these results, Zhang *et al.* demonstrated that the resonance of the bound water of a similar DOTA-tetra(amide) derivative (1,4,7,10-tetraazacyclodecane tetrakis(ethyl-acetamidoacetate) – Fig. 7) could be observed at temperatures up to 40 °C.[53] Utilising this and the fact that the NMR shift between bound and bulk water in the system was large (49 ppm), they postulated that the reagent might be a useful PARACEST contrast agent, as diamagnetic CEST agents have effects that are difficult to determine from the tissue.[53]

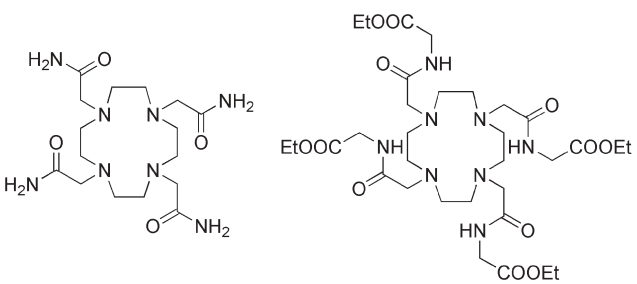

Fig. 7 The structures of DOTAM (left) and DOTA-tetra(amide) (right) analogues used in the development of PARACEST reagents.

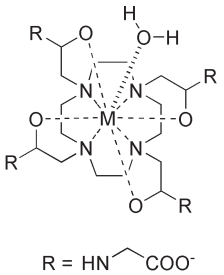

Fig. 8 The structure of the DotamGLY complex used as a PARACEST reagent where M = Eu(III), Tb(III).

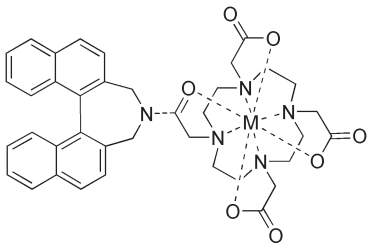

Fig. 9 The structure of a DOTA analogue functionalised with a rigid moiety in an attempt to decrease the tumbling of the molecule and hence increase the relaxivity.

Aime *et al.* also used the compound depicted in Fig. 8 as a PARACEST agent, using the same DotamGLY ligand around a metal centre of either europium(III) or terbium(III).[54] The ^{1}H NMR behaviour of these two agents is very different, as predicted by their different magnetic characteristics.[56] Both of the agents have two pools of exchangeable protons—the coordinated water protons and the amide protons.[57] Irradiation at 50 ppm from the bulk water resonance detects a response from the europium agent, whilst switching to irradiation at 600 ppm from bulk water detects the terbium agent.[54] The agents were then incubated with rat heptoma (HTC) tumour cells to generate uptake of the complexes, and the same irradiation process was repeated. Irradiation at 600 ppm caused exclusive detection of the terbium complex containing cells, and at 50 ppm the europium containing cells.[54] This study demonstrated that PARACEST reagents can be optimised to respond to a specific parameter of their environment, and these results expand MR imaging to the field of cell tracking *in vivo*.[54]

3.4 Luminescent Ln(III) compounds to aid the design of MRI contrast agents

Europium(III) is a poor relaxation agent compared to Gd(III) and Eu(II), but it is an efficient luminescent species, and consequently can be used as a structural probe for corresponding Gd(III) analogues.[58] This aids the development of new contrast agents, as ligand systems can be developed and evaluated using europium(III). The binding of contrast agents to biomolecules can be analysed using luminescence studies to analyse where binding may occur and to what degree, thus allowing for modifications to take place before a final ligand system can be proposed. A good review covering luminescent lanthanide chemistry has been previously published,[59] consequently only a couple of recent examples will be outlined here.

The introduction of hydrophobic functionality onto the periphery of gadolinium-based chelates has generated contrast agents that are directed towards vascular imaging.[60,61] This causes binding to human serum albumin (HSA), which results in compartmentalisation, and enhancement of relaxation rate due to the decrease in the rate of tumbling.[58] However, the full relaxation rate is rarely achieved, due to independent rotations of the bound complex. Lowe *et al.* designed a DOTA ligand with a rigid moiety in a bid to prevent this rotation (Fig. 9).[58] The europium(III) analogue of this ligand was used to determine the binding affinities of the complex with HSA from luminescent enhancement of the bound species. In the presence of HSA the luminescence spectrum changes by an identifiable increase in the intensity of the Eu(III) emission—thought to be due to more efficient energy transfer from the binapthyl chromophore to the europium(III) in the presence of the protein.[58] Luminescent lifetime measurements also indicated the presence of a bound, inner sphere water molecule both before and after binding to HSA, essential for MRI use. The luminescence enhancement was used to calculate the binding affinity for HSA, which was found to be relatively high.[58] This work is currently ongoing, and further updates are expected on the relaxation rate measurements for the gadolinium complex and on structural changes to the ligand to enhance the binding affinity for the protein.

In addition to europium(III), other trivalent lanthanide ions have been used for luminescence studies. Europium(III) and terbium(III) are the most common ions used because they emit in the visible spectrum, in the red and green regions respectively. In addition, they possess long luminescent lifetimes and this means that background fluorescence from tissues can be rejected by temporal gating.[62] Li *et al.* designed a range of lipophilic chelates for MRI and fluorescence imaging based on the ligand DTPA-PDA shown in Fig. 10.[62] The lipophilicity of these ligands can be altered by adjusting the chain length of the alkyl groups. The idea behind creating a lipophilic chelate was to tag the cell membrane with the contrast agent.[62] This is in contrast to the more common hydrophilic chelates used in aqueous conditions, as the aim of the research was to use the tagged cells for repetitive imaging.[62] This is not possible in human studies as maintaining a gadolinium complex within the body, even if well encapsulated would not be recommended based on the toxicity of free gadolinium ions.[63] (However, it must be noted that toxicity studies undertaken in the course of this study found no evidence of gross toxicity.) The addition of these lipophilic gadolinium complexes to Hela cells resulted in rapid uptake

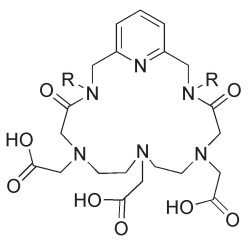

Fig. 10 The structure of the ligand DTPA-PDA (where R = alkyl) used as a lipophilic chelating ligand for tagging cell membranes.

into the cell membrane and increases in the intensity of T_1 weighted images.[62] The mechanism of uptake was studied using diffusion enhanced fluorescence resonance energy transfer (DEFRET) with the terbium(III) analogue of the complex. Trivalent terbium and europium are efficient energy donors in DEFRET imaging, thus they can be used to identify localisation of the complexes within biological systems.[62] It was postulated that the hydrophobic alkyl groups insert into the cell membranes, enabling the hydrophilic section to face the extracellular fluid for water exchange.[62]

4 Further developments in contrast agents

4.1 Smart contrast agents

One of the most rapidly developing areas in MRI is the design and use of responsive or 'smart' contrast agents. These are contrast agents whose relaxivity is responsive to changes in physiological surroundings.[14] This can be through changes in pH, partial pressure of oxygen (as seen for Eu(II)/Eu(III) redox pairs previously), metal ion concentration or enzyme activity.[14] An excellent overview was published by Lowe in 2002,[14] and hence this area will not be covered in great detail, other than to provide recent examples of these types of contrast agents. Lowe mentions that the drive behind this area of smart contrast agents is based on the fact that healthy tissue has a pH of 7.4, whereas tumour tissue is more acidic (6.8).[14] This could be used to generate contrast agents that can be used to map tumours.

In 2005, Tóth *et al.* published reports of a pH responsive contrast agent based on fullerenes.[64] Metallofullerenes encapsulate metal atoms into their interior space,[65] which provides a potential medicinal application because the fullerene cage protects the metal ion from release into the body, potentially allowing longer residence times *in vivo*.[64] Tóth *et al.* were not the first to propose gadofullerene derivatives as MRI agents however, and relaxivities for some compounds are available in the literature.[66,67] The general trend is that the values are high, up to 81 $mM^{-1}s^{-1}$, but also very varied. The availability of water-soluble gadofullerenes enabled further developments in this area,[68] but the relaxivity of these compounds was found to be lower, in the region of commercially available contrast agents.[64] In an attempt to understand the mechanism behind the relaxivity of these compounds, Tóth *et al.* fully characterised the water soluble gadofullerene derivatives $Gd@C_{60}(OH)_x$ and $Gd@C_{60}[C(COOH)_2]_{10}$.[64] The relaxivities measured for $Gd@C_{60}(OH)_x$ were approximately 10 times those of the commercially available contrast agents, with their high field maxima at approximately 40 MHz.[64] The relaxivities at 60 MHz were also measured as a function of pH, and it was observed that for both compounds the relaxivity increased considerably (a factor of 2.6 for $Gd@C_{60}(OH)_x$ and 3.8 for $Gd@C_{60}[C(COOH)_2]_{10}$) with decreasing pH.[64] The explanations offered for this are centred on the pH influencing the proton exchange rate or the molecular rotation rate—but it is unlikely to be solely limited by proton exchange as there is a temperature dependence seen. Static and dynamic light scattering (SLS and DLS) studies were carried out, which indicated aggregation—this is known to affect rotational correlation time and hence proton relaxivity.[64] Hence gadofullerene compounds of this type are ideal candidates for pH responsive MRI contrast agents, especially as they are able to cross cell membranes for intercellular MRI applications.[64]

4.2 Site-specific contrast agents

Commonly used blood-pool contrast agents are not specific to a type of tissue, even though they are not distributed evenly throughout the body, preferring to be distributed within the blood stream due to their hydrophilicity.[7] There has been a review recently on MRI contrast agents that specifically target different types of tissue, and hence the parameters involved will not be discussed here.[7] As this is a rapidly developing area, some recent examples will be given to demonstrate the applicability of these types of contrast agents.

In addition to the DOTA analogues extensively used in MRI, other derivatives of this basic cyclen macrocycle have been studied including phosphonate esters,[69] and methylene phosphonates.[70–72] Lukes *et al.* proposed that a bisphosphonate monoamide analogue of DOTA could be used as a potential bone imaging contrast agent.[73] Geminal bisphosphonates have been utilised in the treatment of bone diseases for around 20 years,[74] due to their high attraction for the surface of bone.[73] The presence of phosphorus acid moieties on cyclen macrocycles has been shown to lead to faster water exchange, and enhanced relaxivities for the gadolinium(III) chelates, obviously an attractive property of the ligands.[75,76] Previous attempts at the creation of a contrast agent featuring bisphosphonates resulted in a strong affinity between the contrast agent and the bone surface,[77] but the stability of these complexes was found not to be suitable for *in vivo* applications.[78] Lukes *et al.* reported the properties and synthesis of trivalent lanthanide complexes of BPAMD (Fig. 11) in an attempt to apply this complex to bone imaging.[73] The relaxivity was found to be higher than expected (5.34 $mM^{-1}s^{-1}$), higher than that of Gd-DOTA, which was considered to be surprising as the residence time of the water protons was longer than average (1.2 μs).[73] This is thought to be overcome by a larger rotational correlation time (88 ps), giving the larger relaxivity.[73] The potential for bone targeting was measured using a sorption experiment with hydroxyapatite used as the model for bone, and a ^{160}Tb-BPAMD analogue. The uptake was found to be 95% within an hour and reversible within about three days. In addition, after binding to the apatite, the rotational correlation time lengthens further, thus increasing the relaxivity again to 24.0 $mM^{-1}s^{-1}$ at 20 MHz.[73] All the evidence points to this complex being a suitable ligand for the MRI of bone tissue.

Another example of specific contrast agents recently published features a steroid conjugated contrast agent developed

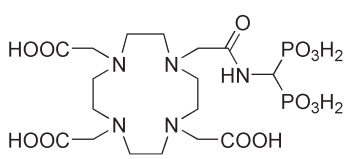

Fig. 11 The structure of BPAMD used in the synthesis of bone targeting MRI contrast agents.

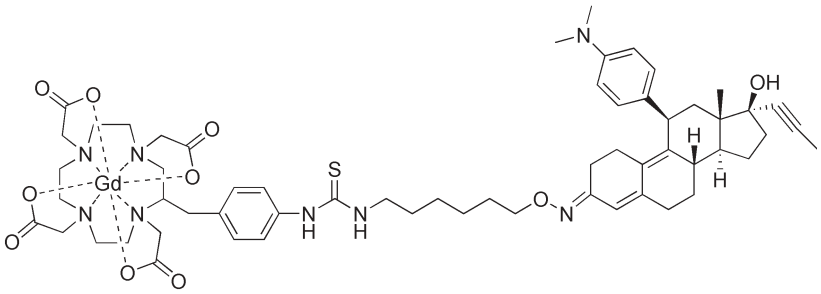

Fig. 12 The structure of Gd-DOTA conjugated to RU-486 as demonstrated by Meade *et al.*[79]

to selectively bind to a receptor that causes a gene expression pathway.[79] Meade *et al.* covalently attached the steroid RU-486 using an aminooxy-functionalised linker to a Gd-DOTA analogue. (Fig. 12):[79] This was found to bind selectively to the progesterone receptor causing transcription of a gene, which can be used to track the cell signalling pathway. The relaxivity of this compound was 8.5 $mM^{-1}s^{-1}$, higher than commercial Gd(III) analogues and assumed to be due to the hydrophobic nature of the steroid which creates a compound amphiphilic enough to aggregate, thus increasing the rotational correlation time and hence relaxivity.[79] This study is currently under further development to optimise the construction of the linker and chelate to provide a useful MRI contrast agent that can specifically track a cell line through the body.

4.3 Enhancement of relaxivity

The ability of an agent to affect T_1 and T_2 is characterised by the concentration-normalised relaxivities r_1 and r_2 respectively. The aim of a contrast agent is to maximise this value, and there are a number of parameters that can be changed in order to do this, as illustrated in section **1.3**. The majority of Gd(III) based contrast agents only have one water molecule attached, which hampers the ability to demonstrate good relaxivity. In fact, the image enhancing capabilities of the commercial poly(amino carboxylate) based chelates are only a few percent of what is predicted by the Solomon–Bloembergen–Morgan theory.[2,4,80] It was thought that with more water molecules in the inner sphere of the complex, relaxivity would be higher but the overall complex less stable, with the gadolinium less shielded from the environment and more likely to transmetallate. Hydroxypyridonate (HOPO)-based chelates (Fig. 13) offered higher relaxivity in addition to high stability, yet two water molecules (attributed to the high relaxivity) were found to be coordinated to the metal centre.[81] Deceleration of molecular tumbling of the contrast agent will also increase the relaxivity,[82] and this can be achieved by attaching the chelate to a rigid macromolecule, such as a dendrimer.[83] Dendrimers are solubilised according to the terminal group functionality,[84] and hence for a biological application water solubilising groups are required, such as hydroxyl groups.[83] Raymond *et al.* reported a Gd(HOPO)-based chelate attached to a dendron containing 12 hydroxyl functional groups to impart aqueous solubility to the molecule.[83] They reported a relaxivity three times that of the commercially available Gd(DO3A). It was also found that the dendritic contrast agent is optimal at 90 MHz, making it one of the first published reagents with a fast water exchange and a high relaxivity at the high magnetic fields used in the new generation of scanners.[83]

Another method of enhancing relaxivity is the use of larger complexes to reduce the rotational tumbling time of the molecule, as this will result in a higher contribution to the inner-sphere element of relaxivity.[85] An example of this was reported in 2004 by Wong *et al.* using a variation on the commonly used polyaminocarboxylate macrocycles.[85] They reported the synthesis and relaxivity of the contrast agents represented in Fig. 14. These compounds demonstrated relaxivity values of 5.87 $mM^{-1}s^{-1}$ and 6.14 $mM^{-1}s^{-1}$ at 20 MHz and 25 °C, significantly higher than Gd-DOTA (4.74 $mM^{-1}s^{-1}$) which was attributed to their larger size, causing lengthening of τ_R.[85] However, the water exchange rate was found to be slow, which causes a limitation upon the relaxivity, indicating that the compounds need to be optimised further before application.

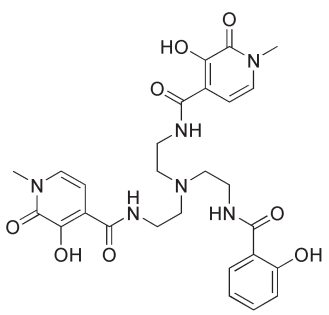

Fig. 13 The structure of a HOPO-based ligand (TREN-Me-3, 2-HOPOSAM) used in the design of MRI contrast agents with high relaxivity. This ligand structure was also used in the design of dendrimer-based contrast agents.

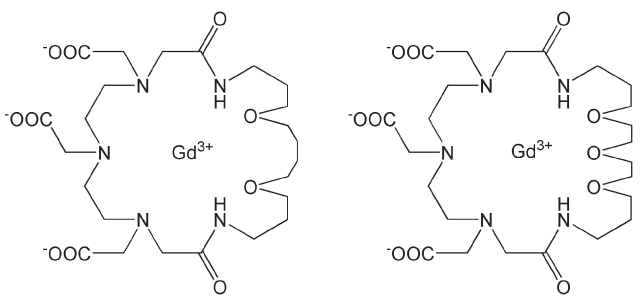

Fig. 14 The structure of the complexes synthesised by Wong *et al.*[85] which demonstrated relaxivities of 5.87 $mM^{-1}s^{-1}$ (left) and 6.14 $mM^{-1}s^{-1}$ (right) at 20 MHz and 25 °C.

The attachment of gadolinium chelates to larger molecules or carriers to reduce the tumbling time has been exploited in a variety of publications, and currently there is a gamut of contrast agents being developed based on nanoparticles where this effect is observed.

5 Nanoparticle-based MRI contrast agents

Nanoparticulate MRI contrast agents have the potential for application in targeted diagnostic studies, but also for image-monitored site-specific drug delivery, opening the door to new clinical approaches to disease treatment.[86] Superparamagnetic nanoparticles of iron oxide are already frequently used as negative MRI contrast agents. They disturb the magnetic field independent of environment, and hence can be used in any matrix, as water exchange is not required for contrast enhancement.[86] Ferumoxide is the clinically approved iron oxide contrast agent. Recently, accounts have been published of paramagnetic contrast agents that are based on nanoparticles, where the nanoparticles are labelled or loaded with Gd(III) using a variety of different binding techniques. The incorporation of gadolinium(III) moieties into a nanoparticle appears to be a very active area of development. This is one method that is considered for the specific targeting of contrast agents to different tissues and organs. It is a viable option because the amount of paramagnetic material at the site is very high due to the loading, thus enabling higher relaxivity rates and better signal contrast. As well as the loading, the size of the nanoparticulate contrast agent also slows down the tumbling rate, which in turn increases the relaxivity. This follows on from the use of macromolecules to slow down the tumbling rate, and essentially has the same effect.

In 2000, there was a report published on the synthesis of gadolinium loaded nanoparticles and their applicability as MRI contrast agents.[87] The polymeric core of the nanoparticle consisted of acidic methylacrylic acid that forms strong complexes with Gd(III), and this core was coated with a polymeric shell consisting mainly of ethylacrylate monomers (see Fig. 15). This allowed water to pass through, enabling the required rapid exchange, but also ensured their biocompatibility within the body.[87] The nanoparticles were analysed for their ability to modify the relaxation rate of water *in vitro*, and it was found that compared to the free polymer or free water, the relaxation rate was significantly reduced.[87] The relaxivity is not quoted, and there are no comparisons made to other contrast agents, commercial or otherwise so a direct comparison with other MRI contrast agents cannot be made.

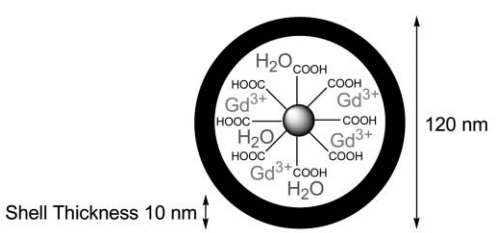

Fig. 15 Schematic representation of the Gd(III)-loaded core-shell nanoparticle, showing the metal loaded core in the presence of water molecules required for an MRI active contrast agent.

However, it is a good starting point for the discussion of nanoparticulate based reagents, and if further developed and tested, there would be a possibility for their use as a standard carrier or delivery agent as they specifically target the GI tract.

One of the major developments in this area was based upon a lipid encapsulated, perfluorocarbon nanoparticle, which contains paramagnetic moieties. This was used for the specific and sensitive detection of fibrin,[88,89] and for the detection of the molecular signature of angiogenesis.[90] These nanoparticles feature a Gd-DTPA-BOA complex which is incorporated into the surfactant layer, enabling the exchange of water molecules into the coordination sphere.[91] It was found that the relaxivity of these nanoparticulate contrast agents was much higher for each gadolinium ion than free Gd-DTPA contrast agents, due to a slower tumbling rate.[92] It is important that when analysing the relaxivities consideration needs to be given to the loading of these nanoparticles—each nanoparticle will contain a large number of paramagnetic contrast agents, giving a very high relaxivity overall. The payload is often in excess of 50,000 gadolinium ions per nanoparticle, which gives particle based relaxivities of around 1,000,000 mM^{-1} s^{-1}.[91]

The main issue with site-specific contrast agents is that unlike the blood-pool contrast agents, they accumulate and provide persistent signal. This also means that there is more potential for toxicity. The nanoparticulate system discussed previously was found to transmetallate rapidly upon addition of $ZnCl_2$, as demonstrated by the decrease in relaxivity.[91] This was thought to be due to the linearity of the DTPA chelate, and the loss of two coordination bonds to the gadolinium(III) ion for lipid attachment.[93] This idea was further developed by exchanging the paramagnetic chelate for Gd-DTPA-PE (phosphatidylethanolamine), which compared with Gd-DTPA-BOA, was found to have a relaxivity twice as large as the previously reported compound (33.7 $mM^{-1}s^{-1}$).[92] This was postulated as being due to faster water exchange at the metal centre. The report does not consider any of the transmetallation effects experienced by the previous compound, but it is thought that it may experience similar effects upon addition of $ZnCl_2$, because the linearity of the chelating ligand has not changed. Another development in this area was the incorporation of Gd-MeO-DOTA and Gd-MeO-DOTA-triglycine-PE and comparison with the previously reported Gd-DTPA-BOA in terms of relaxivity and transmetallation (Fig. 16).[91] These contrast agents demonstrated high relaxivity (29.8 mM^{-1} s^{-1} and 33.0 mM^{-1} s^{-1} for the Gd-MeO-DOTA and Gd-MeO-DOTA-triglycine-PE respectively). The increase in relaxivity of the triglycine analogue is thought to be due to the extended position of the chelate past the lipid surface, but this has not been tested and an optimal distance is yet to be proposed.[91] The transmetallation of these contrast agents was also considered because in the first generation of these nanoparticulate materials this process occurred rapidly. For these nanoparticulate contrast agents there was an improved retention of gadolinium within the nanoparticle,[91] which is also the case in DOTA analogues of commercial contrast agents. In addition, the use of the MeO-DOTA chelates permitted coupling to the lipid linker without the loss of coordination sites to the gadolinium ion, helping to reduce transmetallation.[91]

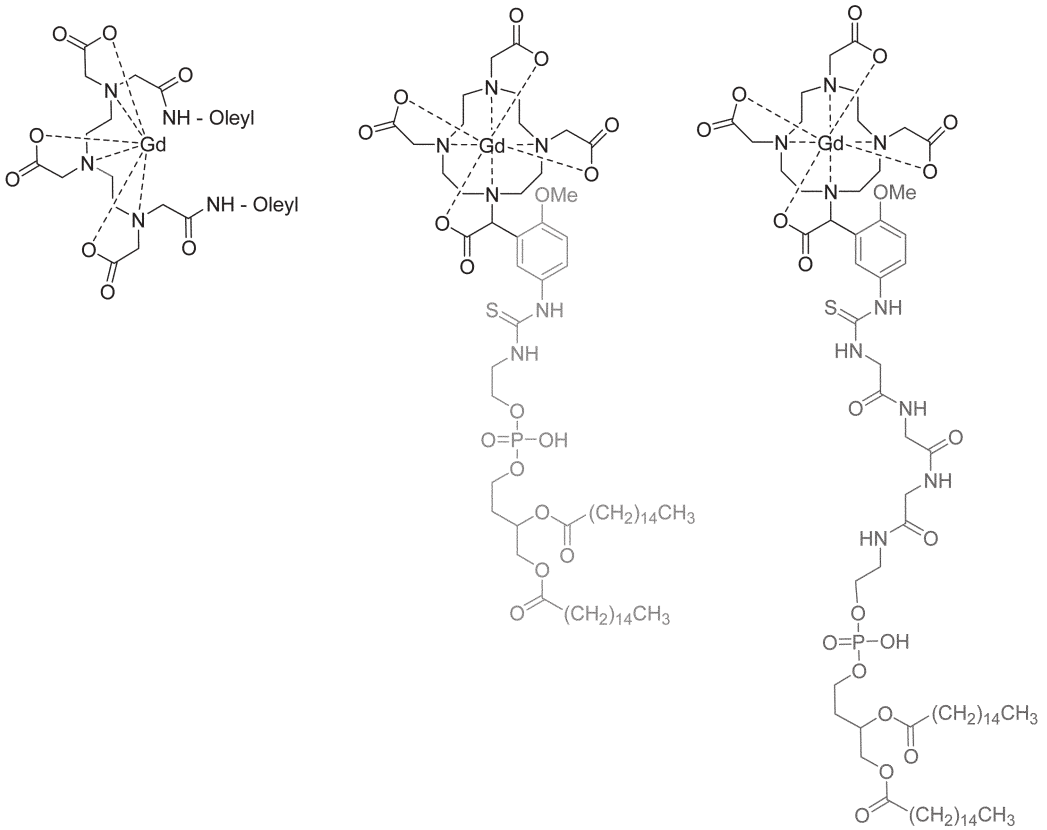

Fig. 16 The structure of Gd-DTPA-BOA (left), Gd-MeO-DOTA-PE (middle) and Gd- MeO-DOTA-triglycine-PE (right).

Bünzli *et al.* reported the synthesis of a podate ligand system (Fig. 17) complexed with luminescent lanthanide(III) ions and gadolinium(III).[94] The ligand was designed to encapsulate the metal ions by supramolecular interactions in solution and was found to form spherical nanoparticles.[94] The relaxivity of this species was 53 mM^{-1} s^{-1}, a magnitude higher than clinically approved contrast agents.[94] This was attributed to the porous structure of the self-aggregates allowing for water to freely circulate around the contrast agent.[94] Bünzli *et al.* propose that control over the coagulation process is possible; indicating that mass, size and shape of the particles could be controlled leading to further improvements in this system.[94] This could enable the design of contrast agents where relaxivity could be controlled depending on the physical properties of the nanoparticles.

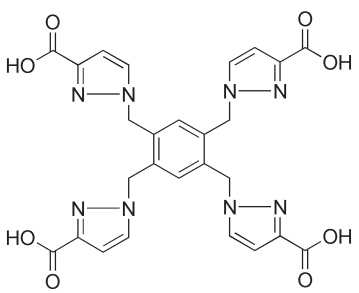

Fig. 17 The structure of the podate ligand used by Bünzli *et al.*[94] in the design of a nanoparticulate contrast agent.

Fayad *et al.* published a report into a high density lipoprotein-like nanoparticulate contrast agent, designed to target atherosclerotic plaques.[95] As they are based on an endogenous material, they are non-immunogenic. The HDL proteins were extracted from their source, and reconstructed with phospholipids, but with the inclusion of a phospholipid based contrast agent—Gd(DTPA-DMPE) (Fig. 18).[95] The relaxivity (10.4 mM^{-1} s^{-1}) was found to be independent of gadolinium concentration, and accumulation was observed locally to the plaque (in genetically engineered mice, 24 hours after injection), which was not observed when a control of Gd(DTPA-DMPE) was used in the absence of the HDL-like nanoparticle.[95] The specificity of this nanoparticulate contrast agent may allow the diagnosis and characterisation of atherosclerosis using non-invasive techniques,[95] which is of considerable value considering that heart disease, and in

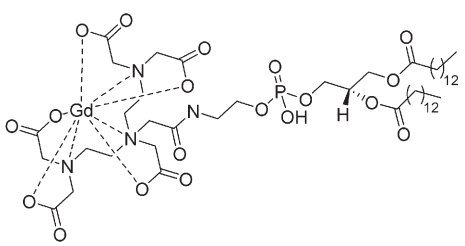

Fig. 18 The structure of Gd(DTPA-DMPE)—the phospholipid contrast agent used by Fayad *et al.*[95] in the specific targeting of atherosclerotic plaques.

particular atherosclerosis, is a prominent killer in the western world.

Wooley *et al.* derivatised non-immunogenic, and non-toxic shell-crosslinked knedel-like nanoparticles (SCKs) with gadolinium chelates for studies as MRI active structures.[96,97] SCK nanoparticles self assemble from amphiphilic block co-polymer micelles (in this case, poly(acrylic acid)), which are crosslinked to stabilise the structures (by carbodiimide-mediated condensation between the acid of the polymer and diamino terminated ethylene glycol).[96] The outer shell layer of SCK nanoparticles can be functionalised, and derivisation with folic acid has been carried out previously.[98] Characterisation of these particles indicated that the shell is heavily hydrated and this was thought to act as a reservoir for water molecules, essentially providing readily exchangeable water molecules for their potential application as MRI agents.[99] The shell layer consists of amines, amides and ether functional groups, which could be used to coordinate to the gadolinium centre, but this is not viable due to the toxicity in biological systems of the gadolinium ion.[100] Hence, a DTPA analogue was synthesised from the reaction of DTPA isothiocyanate[101] with ethylenediamine, and coordination of a gadolinium salt to produce the compound shown in Fig. 19: This was attached to the SCK nanoparticle *via* a carbodiimide mediated coupling reaction to form a peptide bond. The structure was found to have high molecular relaxivity (39 $mM^{-1}s^{-1}$), due to a large rotational correlation time and ease of water exchange.[96] The particles also have a large loading capacity similar to those outlined by Lanza *et al.*,[91] which potentially should serve to maximise contrast and shorten scan time.[96] Further biological assessments of these compounds are in progress, and are not yet reported.

Balkus *et al.* developed Gadolite, which is a NaY zeolite where the Na^+ is partially exchanged for Gd^{3+}, for imaging of the digestive tract.[102,103] Zeolites are chemically and thermally stable aluminosilicates that contain well-defined pore structures. These frameworks can serve, in this case, as carriers that contain encapsulated gadolinium(III) ions.[104] Peters *et al.* have carried out studies on the efficiency of the relaxation rates of water protons,[105] and the relationships between longitudinal and transversal relaxivities of these types of zeolites with different parameters of zeolites.[104] They found that destruction of the zeolite structure enlarges the cages, and these zeolites are more efficient at increasing the longitudinal relaxation of water protons—this was attributed to the larger numbers of water molecules within the zeolites cages.[104] There is a dramatic decrease in diffusion rate of water in zeolites that have smaller pore sizes. The report covers the effects of dealumination, calcination and pore size on the relaxivity of the zeolite, and makes the claim that these materials have potential as T_1 contrast agents at low field, and as T_2 contrast agents at high fields.[104]

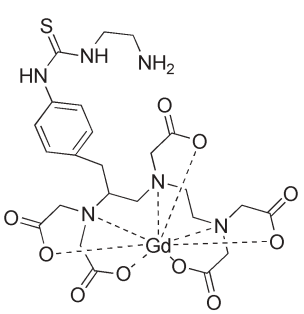

Fig. 19 The structure of the DTPA-analogue used to couple chelated Gd(III) contrast agents to SCK nanoparticles.

Two interesting examples have been recently published, which demonstrate the phenomenon of MRI detectable quantum dots.[106,107] Quantum dots are nanoparticulate clusters of semiconductor material (smaller than the Bohr exciton radius) that show quantum confinement effects. The quantum confinement effect means that the optical properties of these nanoparticles are controlled by their size, rather than their composition, which makes them useful optical imaging agents. The size of the band gap of these materials dictates the energy of the photon emitted, and according to Plancks equation, (where energy is inversely proportional to the wavelength) also the wavelength of emitted light. For instance, a cadmium selenide (CdSe) quantum dot of 2.3 nm diameter irradiated with ultraviolet radiation emits turquoise light, and a 5.5 nm particle emits orange light.[108] Quantum dots have been the focus of great interest recently based on their biological imaging capabilities,[109] *via* their bright fluorescence, photostability, and their narrow and size tuneable emission spectrum.[110] Quantum dots are generally synthesised in organic solvents, and are stabilised by a layer of organic passivating ligands that prevent surface oxidation, but this layer requires exchange for hydrophilic passivating ligands for the compounds to be used for *in vivo* and *in vitro* studies.[109,111–113] In one example of multifunctional imaging agents, the synthesis of paramagnetic, pegylated quantum dots was outlined.[106] The quantum dots had a high relaxivity, and were detectable by both fluorescence and magnetic resonance imaging.[106] The CdSe/ZnS core/shell quantum dots were synthesised using injection of precursors into a hot trioctylphosphine/hexadecylamine (TOPO/HDA) solvent mixture following literature procedures developed since the mid 1990's.[113–115] (There is a wealth of information on the synthesis of quantum dots and some useful reviews have been written by Weiss *et al.*,[109] O' Brien and Green,[116] Trindade *et al.*,[117] and Nie *et al.*[118]) A micellar and paramagnetic coating, composed of pegylated phospholipid (PEG-DPSE) and a paramagnetic lipid (Gd-DTPA-bis(stearylamide)), was applied to make them detectable and water-soluble.[106] The method used to solubilise the nanoparticles in aqueous media has been reported previously,[119] but it was adapted by the incorporation of the paramagnetic components. The relaxivities and proton relaxation times were measured and compared with those of a non-paramagnetically coated quantum dot.[106] It was found that the relaxivity per mM of paramagnetic quantum dots was approximately 2000 $mM^{-1}s^{-1}$, due to the loading of the nanoparticle with more than one paramagnetic lipid. This high value shows their potential as magnetic resonance imaging agents.[106] Successful bioconjugation was observed with cyclic RGD (arginine-glycine-aspartate) peptides—a useful drug and gene delivery linker.[120,121] It enabled targeting of this moiety to human endothelial cells *in vitro*.[106] The high relaxivity of the nanoparticle in

combination with the capability for fluorescence imaging means that a potential dual imaging agent has been successfully constructed. This is advantageous in that it has potential for guided surgery applications where the fluorescence imaging can guide incisions in surgical procedures, but in addition, MR imaging can be used to ensure that surgery is complete, for instance in removal of tumours.[122]

Another example was reported on the synthesis of biocompatible chitosan nanobeads incorporating quantum dots and Gd(DTPA).[107] These can be used as multifunctional biomarkers, paramagnetic and fluorescent, for cell labelling. Chitosan is a biopolymer, functionalised on the surface with amino and hydroxyl groups, and has been used in the delivery of therapeutics. The biodistribution and delivery mechanism is unknown, but the use of these multifunctional probes can benefit the study of biodistribution and delivery mechanisms of drugs and other therapeutics within cells.[107] The nanobeads were synthesised simply by adding 3-mercaptopropionic acid capped CdSe/ZnS quantum dots and Gd(DTPA) added to a solution of chitosan in acetic acid. The quantum dots and gadolinium chelate are electrostatically attracted to the positively charged chitosan backbone, which forms a three-dimensional mesh.[107] Previous results by Zhang *et al.*[107] and Fukumori *et al.*[123] demonstrated that the encapsulated gadolinium chelate will not be released from this mesh-like structure, although the only explanation for this occurring are interactions between the two moieties.[107] The magnetic resonance imaging capabilities of the nanobeads were studied, and it was found that as the concentration of the gadolinium chelate increased, the T_1 values dropped, which brings about an increase in signal intensity in images.[107] In terms of relaxivity, it is known that attaching heavy polymers to gadolinium chelates effects their relaxivity—it reduces the tumbling rate of the chelate, which causes an increase in the relaxivity.[124] It was found that r_1 values for these nanobeads were lower than that for 'free' Gd(DTPA), but this was only slight, and thought to be statistically insignificant.[107]

6 Conclusions and outlook

It is clear from the increasing number of publications that the area of gadolinium(III)-labelled nanoparticles for use as MRI contrast agents is rapidly increasing. There are three reasons for this development: firstly, the use of nanoparticles enables specificity to a target area according to the construction of the nanoparticle; secondly, the size of the nanoparticle slows down the tumbling rate of the contrast agent, which improves the relaxivity; and thirdly, the loading of the nanoparticles enables a large amount of paramagnetic reagent to reach the target site, generating a much larger relaxivity per millimolar amount of nanoparticle used when compared to a 'lone' paramagnetic chelate.

The introduction of dual imaging agents is also of importance, and in our opinion will become an increasingly important contribution to the field of biological imaging. The use of dual imaging agents has exciting potential as they can be used in surgery to guide the scalpel, to ensure all cancerous material has been removed, and to track and identify tumour cells. Multimodality imaging agents have the potential to provide more than one signal from a biological sample and in this way, can enhance the visualisation of biological material. Hence, there are exciting developments in the synthesis of quantum dots (QDs) with a water-soluble and paramagnetic micellular coating as molecular imaging probes for both fluorescence microscopy and MRI, and this is where our research efforts lie. The quantum dots preserve their optical properties and have a very high relaxivity. Targeting ligands can be coupled to these p-QDs *via* maleimide or other functional groups. It is predicted that nanoparticulate bimodal contrast agents may be of great use for the detection of (tumour) angiogenesis. The only concern regarding the use of these contrast agents would be the inherent toxicity of the constituent materials (Gd, Cd, Se *etc.*).[125–127] The toxicity question has yet to be conclusively answered but, as in the development of gadolinium(III) chelates, it is possible that this will be addressed with different capping agents and methods of encapsulation.

In addition to providing an improved relaxivity over small molecules, the use of macromolecular species in MRI applications has been shown recently to have other remarkable advantages. For example, Gd(III)-labelled macromolecules serve well in blood-pool imaging as a result of long circulation times, in contrast to their small-molecule counterparts, in which rapid clearance rates from the blood and passage into tissues effectively prohibits blood-pool imaging. Additionally, macromolecular structures often passively target tumourous tisues, by a mechanism known as the enhanced permeability and retention effect. It seems that contrast-enhancing nanoscopic materials will have a role to play, with the possibility of increased loading capacity of these materials. The high molecular relaxivity of the nanoscopic structure, which is a result of both a large rotational correlation time and loading capacity should also serve to maximise contrast enhancement and decrease scan time *in vivo*. The other aspect of the use of macromolecular species is in the development of new compounds possessing high intrinsic relaxivity in the 3 Tesla field range, now increasingly utilised in new clinical MRI instruments. Dendritic systems have been shown to possess the correct properties to be effective at these high magnetic fields. There is sure to be sustained interest in dendrimer technology and other macromolecular systems with regard to the next generation of MRI scanners.

Much innovative work has been performed by exploiting the magnetic properties of paramagnetic lanthanide complexes. A range of potential contrast agents have been identified but for many of them there is still a long way to go before they could even be considered for clinical trials and commercial application. Despite the promising preliminary results of these compounds, much remains to be done. Comprehensive *in vivo* as well as *in vitro* pharmacological and toxicological analyses must be performed to assess safety and efficacy of such potential candidates. In order to be approved for clinical use, further in-depth study, iteration and modification is required. But the prize that awaits is so attractive, the desire and motivation for this hard work is undoubtedly there. With the current efforts devoted to the development of structure–activity relationships, enhanced targeting systems and the delivery of large amounts of agents to target tissues, it will be

fascinating to see how much more chemists and their collaborators can achieve in the near future.

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