Magnetogenic Probes

DOI: 10.1002/anie.201305662

Magnetogenesis in Water Induced by a Chemical Analyte

Jens Hasserodt, Jacek Lukasz Kolanowski, and Faycal Touti*

chemical imaging · iron · magnetization · molecular sensors · water

This Minireview aims to shed light on the emergent field of inducing a change in the magnetic properties of a solution-phase sample by exposing it to a chemical analyte. A considerable body of knowledge exists on materials that alter their magnetic characteristics after a change in the surrounding physical conditions and a number of cases even exist of solution-phase samples that do so under these same circumstances. However, examples of dissolved molecules or particles that react in this fashion under constant conditions and in response to an analyte are limited. Although some cases in organic solvents are discussed, the emphasis of this Minireview is on water. Our aim is to provide the reader with guidelines for designing new magnetogenic probes for the detection of the desired chemical analyte.

1. Introduction

In the field of chemical imaging,[1] magnetic modes of physical detection should be considered attractive alternatives to more established ones such as those based on electromagnetic waves (absorbance, fluorescence, phosphorescence, interferometry), radioactivity, or electric currents (conductometry). There are several advantageous features of detecting paramagnetic molecules in the solution phase:

1) In contrast to radioactive compounds, the signal is not constantly emitted, but only when an external magnetic field is applied;
2) the external magnetic field interacts only slightly with other components of the sample;
3) neither the signal nor the molecule from which it originates experience any fatigue, as is commonplace with fluorescent or radioactive molecules;
4) a magnetic detection process is environmentally harmless so that no colateral degradation of the sample needs to be feared;
5) the signal emitted by the magnetic molecule is not depleted during its passage through the sample;
6) highly specific detection is possible for many sample environments, since they contain no (other) paramagnetic component.

Newly established paramagnetism may also influence other properties of the sample (optical and relaxatory ones (NMR)), and thus lead to the opportunity of a multimodal readout. Possible weaknesses are 1) a limited detection sensitivity and 2) the need for complex instrumentation. In principle, two types of instruments are used to detect paramagnetism in the liquid phase, namely an electron spin resonance (ESR) spectrometer for direct detection or a nuclear magnetic resonance (NMR) spectrometer for indirect detection. The development of small and inexpensive portable NMR devices consisting of a permanent magnet of low field strength is a welcome drive towards instrumentation of significantly reduced complexity.[2a,b] An opposite trend consists of the development of NMR spectrometers and magnetic resonance imaging (MRI) scanners equipped with superconducting magnets of ever higher field strength. As this Minireview addresses magnetic properties of molecules in solution, tribute has to be paid to the chemists in the field of MRI contrast agents, who have devised an extraordinary number of paramagnetic molecules that operate in aqueous solution. The magnetic properties of these molecules arise from their permanent electron spin, which is detected through its influence on the relaxation time of nuclear spins of neighboring water molecules. This electron spin/nuclear spin interaction is at the origin of the concentration-dependent

[*] Prof.Dr. J. Hasserodt, Dr. J. L. Kolanowski, Dr. F. Touti
Laboratoire de Chimie, UMR CNRS UCBL 5182
Université de Lyon-ENS de Lyon
46 allée d'Italie, 69364 Lyon Cedex 07 (France)
E-mail: jens.hasserodt@ens-lyon.fr
Homepage: http://www.ens-lyon.fr/CHIMIE

shortening of the nuclear relaxation times, defined as relaxivity, and can be detected by a variation in the NMR signal. In 1946, Bloch et al. were the first to suggest the use of paramagnetic metal ions for the modification of NMR signals.[3]

For the 1990s alone, it was estimated that more than 30 tons of gadolinium ions were injected into the veins of patients worldwide[4] to enhance MRI-based clinical diagnostics. The whereabouts of the agent can be shown in an MR image by translating the variation in the shortening of the relaxation times of hydrogen nuclei of surrounding water molecules into different gray-scale levels. Importantly, these MRI hypersignals are caused by the recruitment of permanently paramagnetic agents to a particular location/tissue through passive differential diffusion and distribution processes or by specific delivery strategies.

In the past 15 years, new contrast agents have surfaced that have the added value of responding reversibly or irreversibly to a chemical analyte of interest ($p(O_2)$,[5] enzymes,[6] metals,[7] pH value)[8] by changing their signaling power. Truly sophisticated concepts have been reported[7c,9] where the agent's relaxivity is modified by the presence of the analyte. Without any exception, these probes are based on modifying their relaxatory powers; the magnitude of their electronic spin remains untouched. They thus operate by a change in the efficiency of the electron spin/nuclear spin interaction and not a change in magnetization. With this important distinction in mind, we address in this Minireview the de novo creation (off-on mode) of an electronic spin in a sample and not simply its increase from a measurable base value, and we call it magnetogenesis. This newly created paramagnetic property may be detected by simple methods that do not require any spatial resolution, or be exploited in advanced chemical and medical imaging which detects the whereabouts of the probe in a complex, heterogeneous, and spatially structured sample. The off-on response of magnetogenic probes has advantages for a wide range of applications, including a matchless signal-to-background ratio, whereby the background is the signal in the presence of the molecule (the probe) and the absence of the chemical analyte. The expression "chemical analyte" implies a chemical or biochemical of interest that interacts with the probe either in a supramolecular fashion or modifies it permanently under constant conditions of the medium (solvent identity, pH value, temperature, ionic force, concentration etc.).

In Section 2 we will address with the aid of selected examples the current perception of molecules that respond in solution to a change in the chemical composition by altering their magnetic properties. They do so almost exclusively by an incomplete, gradual, and reversible shift of the spin equilibrium, and we thus refer to it as "magneto-modulation".

2. Reversible Magneto-Modulation

There are numerous reports on the modulation of the magnetic properties of a sample by applying an external stimulus as this approach is attractive for the design of molecular devices, switches, probes, and other applications.[10]

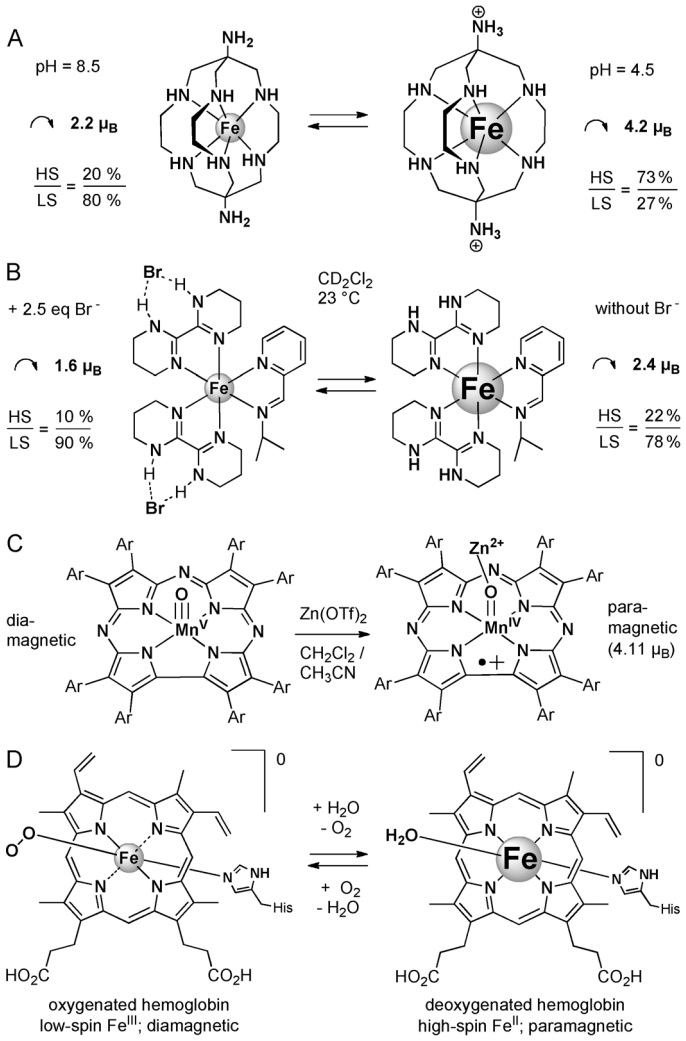
Jens Hasserodt completed his PhD at Heidelberg University and spent 7 years at The Scripps Research Institute with Profs. R. Lerner and K. Janda, first as a postdoctoral student and from 1998 as an Assistant Professor. In 2001 he became professor at the ENS Lyon. He has dedicated the past 12 years to enzyme inhibition and detection, particularly in molecular imaging. He was the recipient of the 2009 CLARA trophy for innovation.

Jacek Kolanowski simultaneously studied biotechnology (BSc in 2007) and chemistry (MSc in 2009 with Prof. Koroniak) at Adam Mickiewicz University (Poznan, Poland). He then spent one year as a research assistant with Prof. Röschenthaler (Bremen, Germany). In October 2013, he obtained his PhD in chemistry (2013) from the ENS Lyon (supervisor Prof. Hasserodt). His interests focus on devising chemical tools for solving biological problems.

Faycal Touti studied chemistry (MSc 2008, ENS Lyon) and pharmacy (MSc 2010, UPS Toulouse). In september 2013, he obtained a PharmD and a PhD from the ENS Lyon, after having prepared his dissertation in the group of Prof. Hasserodt on the development of magnetogenic probes. His research interests lie in the fields of chemical biology, biotechnology, and chemical probing.

The substantial number of reports on spin equilibria, and in particular magnetically bistable compounds (spin crossover, SCO) has been reviewed thoroughly.[11] Certain subfields were also reviewed elsewhere, including polynuclear metal complexes,[12] valence tautomeric compounds (VT),[13] and other charge-transfer systems, such as cyanide-bridged Prussian Blue analogues,[14a,b] which comprise the "molecular squares".[15] However, the field has principally focused on solid-state materials, such as crystalline polymorphs, coordination polymers,[16] and metal-organic frameworks (MOFs) to explore cooperative effects. The field has concentrated on the use of physical stimuli to cause a magneto-modulating effect with the aim of creating new memory devices. The influence of noncoordinative chemical components on the magnetic properties of materials has received increased consideration.[17] The fact that the change in magnetization is often associated with a modification of optical[18] and electrical properties[19] has led to multisignaling applications.[11]

In contrast, what are the documented cases of discrete molecular entities in solution that undergo an alteration of their electronic spin state upon interaction with an external stimulus? In fact, only examples of reversible spin equilibria have so far been reported and were reviewed up to 2008.[20] These reactions involve principally mono- or dinuclear coordination compounds of transition metals. Excess quanti-



Scheme 1. Influence of constitutional equilibria on spin equilibria for two Fe^{II} complexes (A and B); internal electron transfer caused by peripheral analyte interaction (C); mechanism exploited by BOLD fMRI (D).

ties of analyte are required to show a gradual modulation (Scheme 1 A,B). Apart from the fact that cooperative effects are generally absent for dissolved mononuclear chelates, a new concern arises when moving to the solution phase, namely probe stability—a challenge that has been declared of prime concern.[21] A good number of coordination compounds have been reported that mainly respond to temperature changes, some to irradiation, while those that react to chemical stimuli are limited and were summarized this year.[21,22] These are often not investigated as responsive, diagnostic probes, as they consist mostly of low-denticity ligands that result in poor stability in competitive solvents. However, the analysis of these cases provides a welcome overview of the different mechanisms that allow the magnetic properties to be controlled in solution. Interactions between discrete coordination compounds capable of altering their spin state and other chemicals in solution are either limited to the periphery of the complexes or affect the constitution of the first coordination sphere.

Table 1: Relative performance of reversible magneto-modulation.

Nature of probe–analyte interaction	Complex strength versus solvent competition	Specificity	Effect on magnetic properties	Verdict
electrostatic[a]	very weak (−−)	very low (−−)	very weak (−−)	6−
H bonding	weak (−)	medium (+)	weak (−)	1−
coordinative displacement	medium (+)	medium (+)	medium (+)	3+
protonation	strong (++)	high (++)	medium (+)	5+

[a] In polar solvents.

2.1. Interaction with the Periphery

The cases of an interaction with the periphery (Table 1) are principally based on electrostatic interactions (nonspecific interactions, ion pairing, or hydrogen bonding). These phenomena are usually limited in their impact (for an exception, see Ref. [23]). A change in the solvent polarity may influence the paramagnetism of a sample, without any hydrogen-bonding phenomena being involved.[23,24] More polar solvents tend to favor the low-spin (LS) state because of the significantly smaller volume it adopts[25] (see also the difference in the size of the central atoms in the schemes). It has been suggested that a switch to the high-spin (HS) state has to compensate for the work required to separate the solvent molecules and so provide a suitably sized and shaped enclosure in which the solute can be accommodated.[24b] Positively charged complexes may interact with anionic analytes, but the phenomenon is only observed in apolar media and at higher concentrations.[26]

Magneto-modulation caused by hydrogen bonding usually requires electronic communication between the hydrogen-bond donor/acceptor site and the metal center. Partial proton transfer from a hydrogen-bond donor site on the periphery increases its electron density, which can be transmitted to the coordinating atom. This in return shortens the coordination bond and consequently favors the LS state. It was shown early on in examples of Fe^{III} complexes with triethylenetetramine-like (trien-like) ligands that solvents with a higher hydrogen-bond acceptor capacity (higher Gutmann donor number DN) promote the LS state.[27] A similar but much less pronounced effect has been observed for these systems in acetonitrile when exchanging a BPh_4 counterion by a halide ion (stabilization by 0.2–0.3 Bohr magnetons μ_B in acetonitrile; μ_B are the units for paramagnetism and the μ_B value is roughly proportional to the spin value, see Section 3).[27a] In a very recent example, a ternary iron(II) complex with NH groups in its ligands was shown to be of low intermediate spin at room temperature in water (1.2 μ_B), but of high intermediate spin in nitromethane (3.3 μ_B).[28] An extreme case of hydrogen bonding is the total transfer of a proton from the donor to the acceptor. In the case of ternary complex $[Fe(bzimpy)_2]^{2+}$ (bzimpy = 2,6-bis(benzimidazol-2'-yl)pyridine), deprotonation of its NH donor site is caused by a solvent of exceptionally high donor number (hexamethylphosphoramide (HMPA), DN = 30), which thus makes the complex diamag-

netic. The same complex is already more than 50% HS in solvents of lower DN (MeOH, DN = 19, 3.0 μ_B, 56% HS).[29] For the opposite case, protonation, the binary Fe^{II} complexes of a class of bicyclic and totally aliphatic hexaamines, the sarcophagines, are particularly illustrative examples (Scheme 1A).[30] Although no electronic communication between the protonated nitrogen atom and the metal center can be claimed here, the newly generated electrostatic repulsion between the metal center and the charged ammonium group likely causes lengthening of the coordination bonds and thus favors adoption of a largely HS state (4.2 μ_B), starting from an initially intermediate spin (2.2 μ_B). Compared to most examples above, these complexes are exceptional in that they are stable in water. However, the already paramagnetic quality of the initial complex ("on") as well as the susceptibility to oxidation under air limits its interest for further development in a probe.

In addition to the above examples, cases of more specific analyte response have also been reported. Ni and Shores proposed a quaternary homoleptic ferrous complex comprised of three bidentate bis(amidine) ligands as a magneto-responsive anion sensor.[31] The magnetic moment of the initial complex (4.7 μ_B, estimated to correspond to 92% HS) drops to 2.7 μ_B (estimated to be 30% HS) when two equivalents of a bromide salt are added at −40 °C in dichloromethane. To observe this effect at ambient temperature, one bis(amidine) ligand was replaced with a pyridylimine ligand, thus obtaining a response to 2.5 equiv of bromide of 1.6 μ_B (ca. 10% HS) versus an initial 2.4 μ_B (ca. 20% HS, Scheme 1B, hypothetical structure for the bromide effect).[32] The instability of these complexes, especially in more competitive solvents, the limited magnitude of their response, and their inactivation rather than activation by the analyte prompted the authors to explore binary complexes with tren-based (tren = tris(2-aminoethyl)amine) hexadentate ligands for the same sensing application.[33] Although anion binding in acetonitrile was observed, a magneto-modulating response has not yet been demonstrated.

A remarkable case of off-on magnetogenesis through peripheral interaction is the Lewis acid/base interaction (Zn^{2+}) with a high-valent manganese-oxo porhyrinoid complex (Scheme 1C).[34] The magneto-modulation mechanism in this system is similar to that based on hydrogen bonding, as discussed earlier, but with the opposite effect: the zinc-oxo interaction withdraws electron density from the initially diamagnetic metal center, thus promoting the adoption of a lower oxidation state and concomitant establishment of a radical cationic character in the ligand. In view of the highly reactive high-valency metal center, it remains to be seen to what extent this strongly magneto-modulating system can be adapted to more realistic sample environments. In conclusion, a pronounced and selective magnetogenic response as a result of weak probe–analyte interactions remains a challenge.

2.2. Change of the First Coordination Sphere

Interactions that affect the constitution of the first coordination sphere promise a much more noticeable change in paramagnetism. Decoordination, if finely controlled, can serve as a mechanism for sensor design, but should avoid the replacement of more than one coordinating site on the metal center during activation, because this would increase the risk of nonselective activation of the probe.[35] Displacement of one coordinating site by another can significantly change the ligand field and give rise to dramatic changes in the electronic spin. The switch of hemoglobin from a paramagnetic to a diamagnetic state during oxygenation[36] is an inspiring biological example (Scheme 1D). This fortuitously responsive probe has been exploited since the early 1990s in blood oxygen level dependent functional MRI (BOLD-fMRI) and has revolutionized the neurosciences.[37] Several synthetic examples have been reported, too. They may be divided into those where a) monodentate ligands are displaced on ternary complexes (or higher) or where b) a tethered arm of a hexadentate ligand is decoordinated, thus amounting to cleavage of a chelate ring. These strategies are reminiscent of the fluorogenic indicator displacement assays.[38] Although case (b) is energetically much more challenging, it offers the prospect of a significantly more stable initial probe and higher analyte selectivity. Examples in category (a)[39] are mostly based on Fe^{II} and mainly take advantage of the coulombic attraction between anionic analytes and the cationic metal center, which may result in LS→HS activation (or deactivation in the case of CN^-). Certain neutral interaction partners, such as MeCN, displace their monodentate competitor by causing an opposite direction of activation (HS→LS) because of their high ligand field.

It was our research group that reported the first controlled example for category (b).[40] The ferrous complex (**12**/**12′**, Scheme 6A) showed a large magnetic modulation through decoordination of a pendent arm; this case will be discussed in Section 4 because it is part of a larger story on a probe that shows irreversible magnetogenesis. Another case of pendent-arm displacement on a HS Fe^{II} complex by an anion did in fact not lead to magneto-modulation.[41] A very recent example concerns a binary Fe^{II} complex, the pendent arm of which exhibits a limited ligand field/basicity and is thus partially displaced by chloride in MeCN.[42] The potential of this system for the design of magneto-responsive probes was, however, not discussed.

A variant of the mechanism involving changing the first coordination sphere consists of the additional coordination of a ligand/analyte;[20a,22] this alters, of course, the coordination number. The arguably most widely studied examples are diamagnetic square-planar Ni^{II} complexes, which may welcome nucleophilic ligands into their axial position and thus lead to octahedral paramagnetic complexes. Numerous attempts have been made to control this process and the associated magnetogenesis.[43] However, square-planar Ni^{II} complexes are intrinsically prone to competitive addition of other nucleophiles such as water or N donors (see also Section 3).[44] Thus, the recognition specificity in complex media and the stability of the square-planar diamagnetic forms in aqueous samples are compromised.

What all the above examples have in common is the reversible nature of their interaction.[21] As we shall see in Section 4, a probe that suffers irreversible chemical conver-

sion by its target analyte offers the prospect of much higher specificity and higher initial probe stability. To target analytes that act as chemical reactants, stoichiometrically or catalytically, the putative probe should comprise a trigger moiety separated from a magnetogenic core by an "intelligent" spacer, thus giving rise to a three-component construct of high modularity.[45]

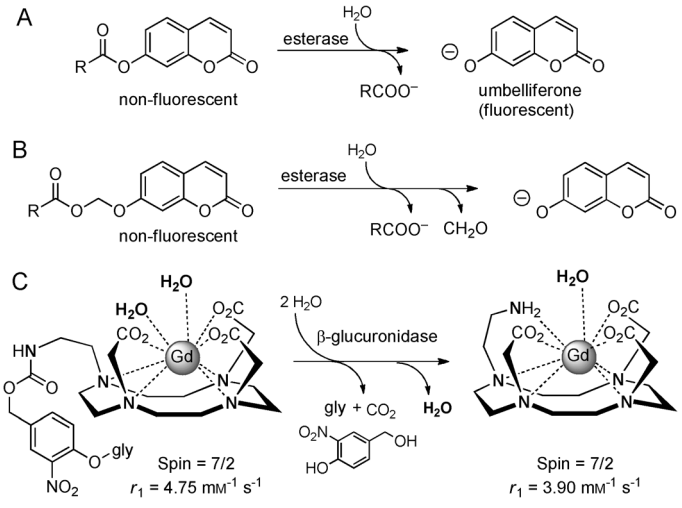
3. Choice of the Optimal Molecular Platform for the Design of Magnetogenic Probes

3.1. Hallmarks

At the beginning of the design of any tool, molecular or otherwise, one needs to set the requirements that it needs to satisfy. We propose six principal characteristics (hallmarks) that a responsive probe should have for effective analyte detection:

1) robustness,
2) fast response kinetics,
3) maximum signal difference,
4) initially a silent probe (off-on),
5) a decidedly binary response,
6) low toxicity (if considering in vivo applications)

A responsive probe must necessarily show an intrinsic reactivity to be susceptible to the target analyte. This requirement can become a serious hurdle in its design, in that the molecule may show residual spontaneous degradation in the absence of the analyte. This in turn likely results in the formation of the signaling molecule and thus in a false-positive signal, a well-recognized challenge in the design of responsive probes (for two examples, see Scheme 2 A,B).[46] It is often difficult for the reader of scientific reports to assess the robustness of a given probe design from the furnished response data. If data are only given for the favorable case of high analyte activity or a large analyte-to-probe ratio, then the active transformation reaction may be much faster than the spontaneous degradation reaction so that no increase of a background signal needs to be feared during the monitoring period. In a more realistic setting of a heterogeneous sample with a spatially complex structure, the probe requires time to reach all sites (diffusion), and it may be in this very time period that it starts to generate a false-positive signal that seriously puts the active response result in doubt.



Scheme 2. Examples of nonmagnetogenic probes discussed in the light of hallmark satisfaction (gly = glucuronyl; r_1 = relaxivity).

A fast probe response is an obvious requirement. A responsive probe may show high, medium, or low reactivity toward the target analyte. Its signal may arise after one chemical step or after two or more. The consequences of slow response are dire: higher concentrations of the intact probe and/or the target analyte need to be established at the site where the analyte resides to observe the same signal intensity as for the case of a fast-responding probe at the same delay time. Simply increasing the delay time does not alleviate the situation: the diffusion rate of the activated probe remains roughly the same, and the signal from that particular site does not increase because of loss to other parts. A slow response does not exactly improve this situation when probes are considered that react through two steps or more: in structurally complex samples, the analyte residing in a particular place may chemically modify the probe as planned. However, the slow translation of this modification into a legible signal in the second step, if it is the rate-determining one (for an example, see Scheme 2 B),[46a] allows the intermediate to diffuse away from the site before "lighting up". All these considerations can be summarized by the phenomenon of signal dilution that amounts to a decrease in detection sensitivity.

It is clear that no matter what the identity of the physical signal that serves to detect the analyte (the detection modality), a maximum emission intensity of the activated probe is always desirable. This ensures the highest difference between the "before" and "after" state of the responsive probe (for an opposite example, see Scheme 1 C).[47] In the particular case of a magnetogenic probe this would imply the generation of a high number of unpaired electrons at the nucleus.

It is highly desirable to design a probe that is initially silent (Scheme 2 A,B).[48] The absence of any signal in the absence of analyte simplifies the interpretation of the image or detection result enormously. It also makes the dream of achieving analyte quantitation much more realistic. It has been repeatedly stressed in the case of responsive probes for MRI that already emit a sizeable signal before they encounter the target that unambiguous image interpretation would require knowing the concentration of converted and unconverted probe at the site of interest.[8a,49] Without this knowledge, it cannot be ruled out that the observed signal is simply the result of an accumulation of the untouched probe for physicochemical reasons (biodistribution, diffusion, lipophilicity, charge, etc) without the presence of any analyte at all. For the same reasons outlined above, the opposite scenario of an on→off probe[31,50] is less attractive (for an example showing this tendency,[47] even though the process does not reach an off state, see Scheme 2 C).

Depending on the nature of the activation reaction (reversible versus irreversible reaction), the choice for one side or the other of the associated chemical equilibrium may

be more or less decided. In other words, it should be avoided that only part of the population of intact probes is converted by the analyte, or that only part of the population of activated probes emits a signal (for comparison see Scheme 1 A,B). The majority of detection schemes covered by Table 1 enter into this category. In contrast: a switch of the entire population from 0 to 100% should be the goal (true for all examples in Scheme 2). Otherwise, the same complications arise that were already mentioned for the previous hallmark of probe design.

3.2. Choice of Magnetogenic Core

Magnetization is the density of magnetic dipole moments induced by the presence of an external magnetic field generated by a permanent magnet surrounding the sample. Such magnetic moments are the result of the spin of electrons and nuclei as well as electron orbital movements. One speaks of an electronic and a nuclear angular momentum, and the former is composed of a spin angular momentum and an orbital angular momentum. The nuclear angular momentum can be neglected because it is approximately 1000 times smaller than the electronic one. A magnetic moment can be detected when unpaired electrons are present in the atom. Compounds that possess such unpaired electrons are called paramagnetic and are attracted by the external magnetic field, while those that are diamagnetic are repelled. The overall spin S generated by these electrons is the major contribution to the magnetic moment for elements of lower atomic numbers Z. The contribution by the electron orbital movement becomes increasingly important for elements of higher atomic numbers. Finally, the electronic spin and the orbital spin can interact (spin-orbit coupling), and this also contributes to the magnetic moment; its level depends on the element and the external magnetic field. Spin-orbit coupling can be quenched to different degrees by changing the electronic configuration and symmetry through the choice of the surrounding molecular scaffold. While it is very small for first-row transition-metal complexes, it contributes more significantly in heavier d-block metal and lanthanide complexes, and can exceed the spin-only contribution in actinide complexes.

A probe must possess a section that is capable of emitting a detectable signal. For a magnetically responsive probe this unit cannot simply possess unpaired electrons, and thus a spin and a magnetic moment, but rather it has to be capable of adopting two different magnetic states depending on its interaction with the analyte (or stimulus). Three types of structurally analogous pairs may be envisaged where this is possible: a) a duo where one compound is an organic radical, b) an internal redox duo experiencing electron transfer, and c) a duo that consists of two spin states (generally a low-spin and a high-spin state).

a) Radicals: Although paramagnetic organic radicals may be generated from a diamagnetic precursor and thus fulfill this requirement, they are usually rather unstable. A few exceptions are fairly stable in solution (e.g. spin labels) and have occasionally been considered for the design of responsive organic compounds that become paramagnetic radicals or lose this quality.[51] Examples of organic radicals that change their level of paramagnetism are also common in solid-state paramagnetic/diamagnetic switches that respond to physical stimuli.[10,52] Upon heating or irradiation, the paramagnetism of the radical becomes quenched because of a change in its relative position in the crystal lattice. These switches are based on a pronounced cooperative effect and their mode of action can thus not be transferred to the solution phase unless some sort of self-assembly can be achieved.[53] On the other hand, the quenching of two radicals contained in one isolated molecule in solution has been reported on a number of occasions, but only as a result of physical stimuli (summarized in Ref. [10]) or a pH change,[51a] but not for other chemical stimuli. Some examples were reported where a photoswitchable moiety caused a change in the intramolecular communication between two radicals, thereby leading to magneto-modulation.[54]

b) Intramolecular redox reactions: A redox-active analyte may change the magnetic properties of a coordination compound by exchanging electrons directly with the magnetogenic core in an intermolecular redox reaction. Such a process makes it rather difficult to confer any detection specificity for a particular analyte onto the probe, but should rather serve to characterize a general redox potential, which is of great interest for biological research. Any intermolecular electron flow may also cause undesired side reactions. Another way of modifying the magnetic quality of a molecular entity would be an intramolecular electron-transfer process, namely, a redox reaction caused by an external stimulus that is not redox-active. Most transition-metal ions that can adopt at least two stable oxidation states are suitable candidates, and even radicals may be considered. An intramolecular redox-driven change in magnetism has been widely reported for both the solution and the solid state, but almost exclusively as a result of a physical stimulus such as light, temperature, or pressure.[10,13,14a] Numerous examples from two types of electron transfer have been reported: 1) ligand-to-metal transfer, also referred to as valence tautomerism (VT), including the classic semiquinone-catechol cobalt complexes, where a Co^{III} LS center becomes a Co^{II} HS one ($\Delta e_{unpaired} = 3$); and 2) metal-to-metal transfer in polynuclear complexes, where Fe-Co, Fe-Fe, Fe-Ni, and other couples are bridged by ligands such as cyanide, including the classic example of prussian blue $[Fe_4[Fe(CN)_6]_3]$. Molecules that operate in this fashion must ensure the possibility of electron transfer between the two redox-active portions of the molecule, that is, between the highest occupied molecular orbital (HOMO) of the donor portion and the lowest unoccupied molecular orbital (LUMO) of the receptor unit. The opportunity thus arises of making compounds that respond to the presence of a chemical analyte should they interact in such a fashion so as to invert the HOMO–LUMO relationship, thereby leading to electron transfer and a change in the oxidation state of the metal center. If it is coupled with a change of the spin state of a central metal ion, then the change in magnetization can be very high. Only a few examples exist where the intramolecular electron transfer is caused by a purely non-redox-active stimulus.[34,51a,55]

c) Low-spin/high-spin switching: Significant changes in magnetization can be obtained with a probe that switches from a low-spin (LS) to a high-spin (HS) state as a response to ligand modification. Ligand field theory stipulates that initially degenerate d orbitals experience a splitting into different energy levels as a result of the approach of the ligand(s) (Figure 1 A). Some d orbitals are more affected than others because the approach of the ligand is directional, that is, metal and ligand orbitals of the same symmetry interact more strongly. By modulating the field of these ligands, one may induce the complex to adopt either a LS or HS state. Field splitting depends as much on the σ-donating as on the π-accepting capacity of the ligand(s). The presence of a low-lying antibonding π orbital (π*) is necessary for a given ligand to be a good π acceptor; the resulting increase in the ligand field is referred to as π back-bonding. As we shall see in Section 3.3 (Table 2), imine-type ligands (containing sp^2-configured nitrogen atoms) cause a particularly strong splitting of the ligand field because of their high σ-donor and π-acceptor qualities.

The nature of the metal ion also plays a role in field splitting: the higher the oxidation number, the higher the energy splitting, which favors the LS state. Only octahedral (or pseudo-octahedral) complexes with a d^4 to d^7 configuration can effectively adopt either a LS or a HS state (Figure 1 B–E), with the exception of a d^8 configuration (Figure 1 F), should the corresponding complex change its coordination chemistry from square planar (LS and diamagnetic) to tetrahedral or octahedral (both HS and paramagnetic) in the process.[11a,22] A tetrahedral geometry normally favors the HS state for the first-row transition metals because the ligand-induced splitting energy is only 4/9 of that observed for an octahedral coordination geometry, and thus too small to overcome the spin-pairing energy. On the other hand, second- and third-row transition metals are mostly found in the LS state because of strong field splitting. Lanthanides are not suitable either, as they are always found in only one spin state. Their paramagnetism is also as much dependent on spin-orbit coupling as on their number of unpaired electrons. No matter if the strategy of internal electron transfer (b) or that of LS-HS switching (c) is pursued, the choice of the right metal is of pivotal importance.

The d^4 to d^8 metal ions have advantages and drawbacks when viewed from the hallmarks of probe design listed above. In fact, the electronic configuration not only has a critical influence on the maximal signal difference attainable ($e_{HS\text{-}LS}$, difference in unpaired electrons before and after activation), it also determines whether a true off-on activation mode is feasible. In the best case, the LS state should thus show no unpaired electrons at all (diamagnetic, off, spin = 0), which is only possible for an octahedral d^6 and a square-planar d^8 configuration. On the other hand, a maximal signal difference

Table 2: Hexadentate ligands giving rise to binary Fe^{II} complexes.

Podand	Base system	N hybridization	Spin state
branched	Py3tame (C-branched)	6 N-sp^2	LS[59]
	tptMetame (C-branched)	3 N-sp^2, 3 N-sp^3	SCO[60]
	Py3tren (N-branched)	6 N-sp^2	LS[61]
	trimethylenediamine	4 N-sp^2, 2 N-sp^3	LS[62]
	2,5,8-triazanon-1-ene	4 N-sp^2, 2 N-sp^3	LS[63]
	cis,*cis*-1,3,5-cyclohexane	3 N-sp^2, 3 N-sp^3	LS[64,41]
macrocyclic	triazacyclononane	3 N-sp^2, 3 N-sp^3	LS[65a,b]
bicyclic	bicyclo[7.5.5]nonadecane	3 N-sp^2, 3 N-sp^3	LS[66a,b]
	bispidine	4 N-sp^2, 2 N-sp^3	LS[67a,b]

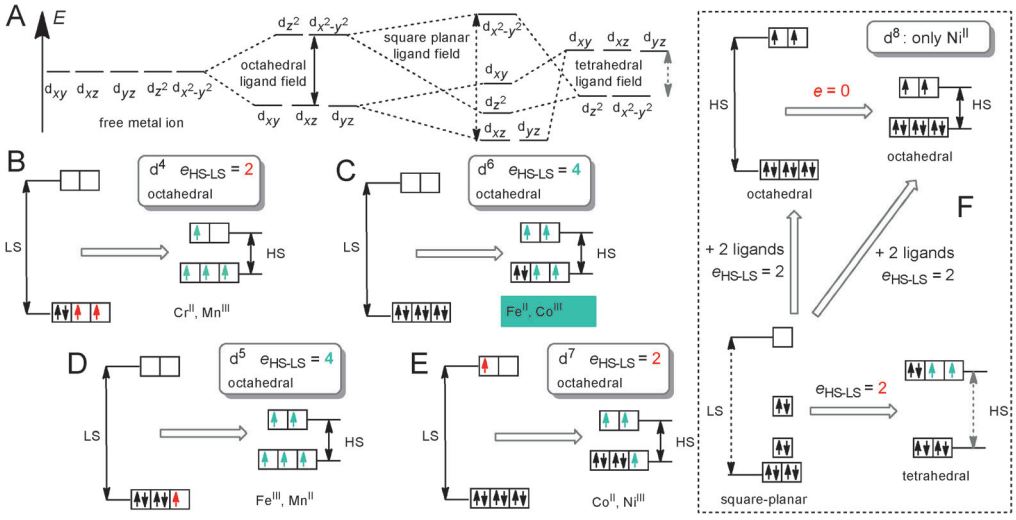


Figure 1. Field splitting (Δ*E*, length of double arrows) caused by ligands of different strength and resulting d electron configurations for low-spin and high-spin complexes of first-row transition metals in terms of the presence or absence of an off-on relationship, and the magnitude of the signal difference ($e_{HS\text{-}LS}$); dashed arrow: 1.23 of Δ*E*(octahedral); gray dashed arrow: 4/9 of Δ*E*(octahedral).

is only ensured for d^5 and d^6 configurations ($e_{HS\text{-}LS} = 4$ e); others give only half this difference (2 e). In theory, the d^6 ions Fe^{II} and Co^{III} can be considered optimal, with the d^5 ions Fe^{III} and Mn^{II}, as well as d^8 square-planar Ni^{II} as possible alternatives. In practice, however, Mn^{II} suffers from such a high pairing energy that it is very difficult to attain its LS state,[56] and Co^{III} is found in the LS state in the vast majority of cases; ligands producing a particularly weak ligand field (fluoride) are required to turn it into its HS state (see CoF_6^{3-}). Thus, no practical ligand system can be found that may cause Co^{III} to adopt both of the spin states as a result of slight structural modifications.

Another hallmark of probe design is robustness, and the remaining contenders (Fe^{III}, Fe^{II}, and Ni^{II}) show various characteristics in this regard. Ferric complexes (Fe^{III}) of both spin states can be quite stable. However, although a good number of ligand systems is known that leads to either LS or HS complexes, this selection is more limited than that known for ferrous complexes (Fe^{II}).[11a] This results in the difficulty of identifying a duo of structurally related ligands where the corresponding ferric complex adopts a decidedly LS state while the other leads to a fully HS one. The hope of observing the maximum signal difference (hallmark) promised theoretically by a ferric system is thus diminished. On the other hand, ferrous complexes in the HS state may or may not show a tendency towards oxidation, and the ligand system has thus to be designed so as to minimize it. Importantly, a large range of different ligands are at our disposal that cause Fe^{II} to adopt one of the two spin states.[11a,57] We thus have within our grasp a sharp magnetogenic response caused by ligand modification under the influence of a chemical analyte. Furthermore, LS ferrous complexes are highly stable and show a certain level of kinetic inertness.[58]

Finally, Ni^{II} complexes, although having a true "off status" for the initial probe and suffering from a mediocre signal difference ($e_{LS\text{-}HS} = 2$), adopt a square-planar geometry that is not only unstable in water but also in organic solvents that contain a complex mixture of nucleophiles.[44a,c] So far, no convincing design has been reported where the LS/off state of Ni^{II} chelates was explored for probing/analysis in aqueous or complex samples. In addition, only a limited range of ligand systems are available to modulate the spin state of Ni^{II} complexes. In the following we, therefore, direct our attention towards Fe^{II} systems.

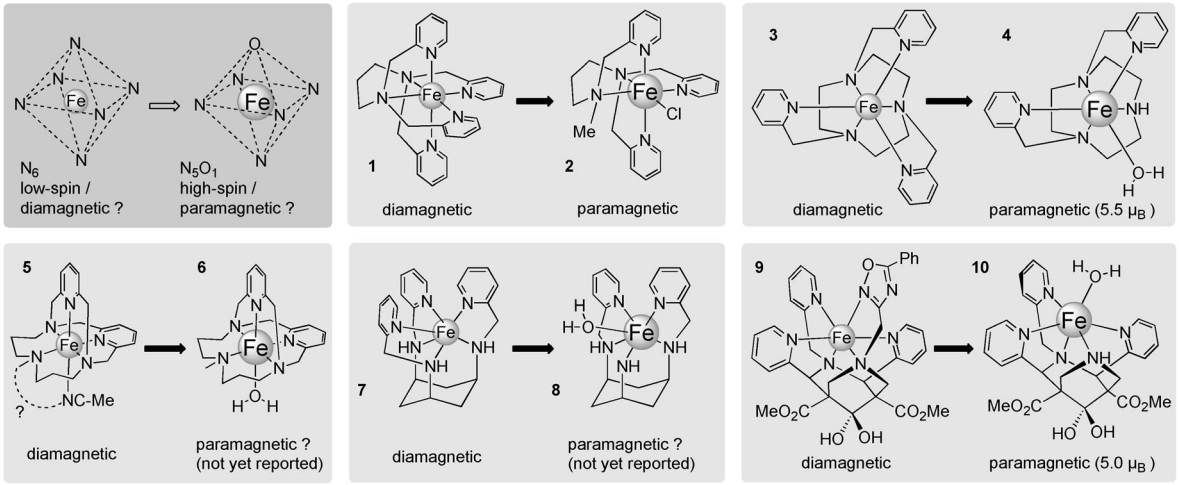
3.3. Fe^{II} LS/HS Duos

Well-established methods for tuning the magnetic state of iron(II) in its complexes comprise:

1) variation of the nature of the coordinating atom (mainly, N, O, S),
2) variation of their nucleophilicity/basicity/σ-donating capacity by, for example, decoration with electron-withdrawing or -donating groups,
3) variation of the hybridization of the N atom (imine versus amine = aromatic versus aliphatic = π back-bonding or not),
4) impeding optimal orbital overlap by introducing steric clash,
5) presence or absence of the macrocyclic effect, and
6) switching between five- and six-membered chelate rings.

In applying these criteria, we will not overlook the primordial requirement of solution-phase stability. Indeed, Fe^{II} complexes with hexadentate ligands "are known to have stability constants of the order of 10^{25}",[20b] and "most Fe^{II} SCO systems based on multidentate ligands are so stable that ligand dissociation does not interfere with the spin equilibrium even in polar solvents".[20d] However, "ligand dissociation and replacement reactions are more likely to occur for complexes of mono- and bidentate ligands, but even for multidentate ligands replacement of a single chelate arm has been observed."[20c] These thermodynamic considerations should not hide the fact that for solution-phase applications in complex samples, especially biological ones, kinetic factors will almost always override them:[68] in fact it is the exchange equilibria with other, abundant metal ions and the rates at which they are established that will decide whether slow or rapid probe degradation occurs.[69] What is thus expected to work in aqueous media is a ligand structure for which an N6 and an N5 ligand can be constructed, because even changing the coordination motif from N_6 to N_5O_1 during probe response (Scheme 3) will make a switch to a HS system highly likely, provided the oxygen atom is neither sp^2-configured (part of a carbonyl group) nor part of triplet oxygen (see BOLD fMRI). From this it can be concluded that if an octahedral ferrous chelate can be identified that is fully low spin at room temperature and in aqueous solution, it is almost guaranteed that a high-spin version of it can be obtained under the same conditions if one arm becomes decoordinated or cleaved off.

These considerations prompted us to examine the literature for cases of binary ferrous complexes that are fully LS in solution, preferably in water (Table 2). By studying the broad review by Halcrow on ferrous complexes with multidentate N ligands one may identify several LS-HS duos; for some of them both derivatives were already characterized magnetically, for other duos only one has so far been characterized.[57] However, that review also illustrates that rather few hexa-/pentadentate systems do in fact ensure a robust low-spin state and so might be attractive for the present task. Multidentate ligands that give rise to binary low-spin ferrous complexes comprise 1) branched podands, 2) macrocyclic podands, and 3) multicyclic rigid podands (Table 2). Branched podand-based N_6-Fe^{II} chelates can be subdivided into a) tripodal ligands branched by a single atom, b) alkyl-diamine-based podands, and c) two unique cases with special branching units. Examination of examples entering into category 1a shows that a simple branched N_6 ligand (tptMetame) cannot force the iron center to adopt the LS state if "only" three nitrogen atoms are sp^2-configured.[60] Only ligands with six imine-type nitrogen atoms have, currently, yielded LS complexes: While Py3tame[59] suffers strain (its LS state can thus be considered weak), Py3tren[61] exhibits a tendency for decoordination of the pendent arm,[70] and so despite the presence of six



Scheme 3. Reported duos of structurally related fully LS and HS ferrous complexes.

coordination sites of high ligand field, their suitability for the design of a magnetogenic unit remains limited.

Alkyl-1,2-diamines (category 1b) lead to intermediate paramagnetism in Fe^{II} complexes despite them generally being equipped with four sp^2-configured nitrogen atoms;[71] they have also been observed to show decoordination of the pendent arm.[62] Expansion of the ethylene bridge by one further methylene group reliably leads to low-spin complexes (see Scheme 3, **1**[62] and its HS analogue **2**).[72] Three ligands were found to belong to category 1c (unique branched ligands). Only two of them (2,5,8-triazanonene and r-1,c-3,c-5-triaminocyclohexane (tach); **7**) caused the corresponding Fe^{II} complexes to adopt a LS state, but have the advantage of doing so not only in the solid state but also in solution, including water.[41,63,64] They exhibit four and three sp^2-configured nitrogen atoms, respectively, out of six. For category 2 (macrocyclic podands), it should be mentioned that many phthalocyanine Fe^{II} complexes were obtained in the LS state, but as the ligands are tetradentate the octahedral complexes cannot be binary. The rigid planar nature of these N_4 macrocyclic ligands precludes the design of N_6 derivatives that lead to hexacoordinate, binary Fe^{II} complexes. It has been shown that the required chelate ring sizes are simply too large to form. Accordingly, it is not apparent how these types of ligands may be useful in the design of robust magnetogenic probes for use in the solution phase, apart maybe from their synthetically challenging incorporation into a multicyclic system. On the other hand, the tripyridylmethyl derivative of the N_3 macrocycle triazanonane (tacn) has led to the preparation of a fully LS Fe^{II} complex (three imines/three amines, Scheme 3, **3**) in the solid state.[65] This LS state is maintained in various aqueous media,[73] while the corresponding complex lacking one pyridylmethyl arm[74] is of course HS (**4**).[73] The HS and LS versions were shown to be visible and invisible, respectively, in MR images of a live mouse.[75] As we shall see in Section 4.3, this system can be effectively transformed into two types of magnetogenic probes that respond to various chemical stimuli. A reported bicyclic N_5 ligand can be assigned to category C. Its dark-red ternary ferrous complex (**5**) was recrystallized from acetonitrile and proved to be LS in the solid state.[66] The sixth coordination site was occupied by an acetonitrile ligand. No hexadentate ligand or its corresponding complex has yet been reported, but there is no reason why this system may not also serve as a promising target for the design of a robust magnetogenic probe by the introduction of another pendant arm (**5**). We recently reported new N_6 members for category C, namely two bicyclic, rigid, and hexadentate ligands of the unique class of the bispidines (Scheme 3).[67b] Multidentate bispidines have previously been reported to form highly stable complexes.[76] We prepared new bispidine ligands that led to the discovery of the first two binary LS Fe^{II} chelates (**9**) for this large class of bicyclic structures. Their LS nature and high stability was confirmed in water and organic solvents at ambient temperature, and their magnetism was explored exhaustively.[67b] Simple removal of one coordinating arm leads to a fully HS system in water (**10**; 5.0 μB).[67] A new robust off-on duo of ferrous complexes in aqueous media at room temperature has thus become available. While macrocyclic as well as bicyclic ligands (categories 2 and 3) may generally require significantly higher synthetic efforts, it is important to note that the reported hexadentate bispidine ligands can be efficiently prepared on a 10 gram scale.[67b]

In conclusion, this literature survey reveals that binary LS Fe^{II} complexes are not that numerous after all. It also teaches us that simple branched N_6 ligands do not appear to impose a LS state if they do not exhibit more than three imine-type coordination sites. For this reason, and for reasons of solution stability, macrocyclic or bicyclic N_6 ligands should be preferred. Some of the promising LS-HS duos for the design of a putative magnetogenic probe are presented in Scheme 3.

4. Irreversible Magnetogenic Response

4.1. Irreversibility

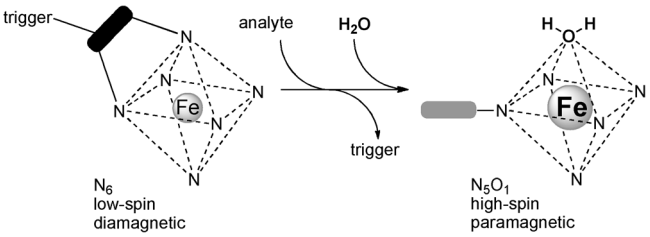
Duos of constitutionally distinct Fe^{II} complexes can be interconverted by either a reversible or an irreversible interaction with the analyte. A reversible mode is charac-

terized by a lower energy difference between the starting material and product, and thus a weak driving force and a less decided thermodynamic equilibrium. This may have seriously unwelcome consequences if aiming at more competitive media (water) or more complex samples containing a variety of nucleophiles[21] where nonspecific interactions cause the generation of various degrees of false signal (hallmark: robustness). Furthermore, while a reversible mode of action has the advantage of likely being instantaneous (hallmark kinetics), the total stability of both states may not easily be achieved. So even if the analyte/probe ratio significantly exceeds 1:1, it usually does not manage to switch the probe entirely to the opposite state, and one is confronted by the presence of subtle spin equilibria (see hallmark "binary response", and Scheme 1 A,B). On the other hand, an irreversible activation process, where the probe suffers permanent covalent modification by its target analyte, theoretically offers total transformation, a decided shift of the reaction equilibrium, and thus a perfectly binary response (hallmark). This also opens up the opportunity to render the initial probe structure much more stable towards its environment (hallmark, Scheme 2 C).

However, it is by no means clear how to achieve this practically. The initial structure would have to store a high energy content that is only unleashed when the two specific reaction partners meet, thus ensuring a sufficient driving force for total transformation. Even if an instantaneous response is often impossible to realize in irreversibly responding probes, the chemist can at least work toward conversion kinetics that correspond favorably with the physical detection process and may even achieve some degree of temporal resolution. What also sets the detection of a chemical reactivity apart from that of simple analyte presence is the characterization of molecular function in a sample, whether it is a biological one or a technological one. Should this reactivity be of a catalytic nature, then the detection process may need to be highly sensitive in view of the low to very low analyte/probe ratios that may be reached ("catalytic signal amplification").

4.2. Three-Component Design

An irreversible response can arguably be achieved only by exploiting the intrinsic reactivity of an analyte and not by its mere presence, as is the case in the magneto-modulating examples of Section 2. If the analyte shows a chemical reactivity, then one needs to identify a moiety (a trigger) that is susceptible to this reactivity for incorporation into the probe. Once the moiety is transformed by the analyte, then this chemical event needs to be transduced to the coordination unit of the probe, that is, the "signal-emitting device". Indeed, irreversibly responding probes targeting chemically reactive analytes are widely used for optical detection (fluorescence). They are often constructed as three-component probes (Schemes 2 B and 4) where the central unit is an auto-immolative spacer; this concept ensures maximum adaptability to the chemical reactivity of the target analyte and a generally modular design that aids in adaptation to specific needs (solubility, pharmacokinetics, biocompatibility, bioconjugation) as well as more freedom in achieving the maximum thermodynamic and kinetic stability of the initial chelate. However, the incorporation of the spacer also increases the complexity of the construct, and its immolation during a response event constitutes an extra chemical reaction associated with its own kinetics; in the best cases, they should not be slower than those of probe conversion by the analyte.



Scheme 4. Irreversible magnetogenic response to an analyte by a three-component probe.

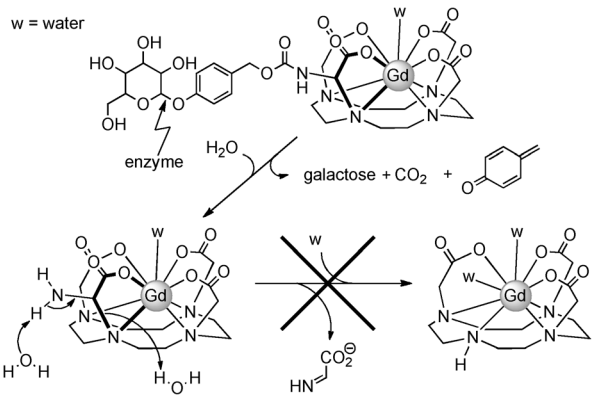
4.3. Irreversible Activation Strategies

We have now seen the possibilities for the construction of an iron(II)-based magnetogenic core. The remaining task consists of identifying an irreversible chemical reaction that decisively modifies the first coordination sphere to satisfy the hallmark features in Section 3. This may be achieved by either the decoordination of a pendent arm or its entire loss by fragmentation. Triggering decoordination of a pendent arm requires the unmasking of a property that favors it, for example, competition between coordination of the pendent arm and protonation. On the other hand, causing its entire loss by fragmentation, calls for the cleavage of a covalent bond within a chelate ring comprising the multidentate ligand. Cleaving a five- or six-membered ring system is, in general, energetically a highly demanding task. Two unsuccessful cases of auto-immolative schemes involving a coordination compound may serve as examples:

1) a pendent arm containing a *para*-hydroxybenzyl-like spacer did not immolate because it was a constitutive part of a five-membered chelate ring;[77] however, it was shown to fragment willingly if the same hexadentate ligand was not complexed by a central iron atom;
2) an aminal (an N,N-acetal) did not immolate to result in departure of a coordinating arm because it was an integral part of a chelate ring involving a gadolinium ion[50,78] (see bold ring in Scheme 5), even though it is well known that simple aminals with N–H bonds are not stable in aqueous media.

These insights allow two new strategies[40,79] to be identified to defeat the imposing strength of the chelate effect (a thermodynamic hurdle) and to observe suitably fast response kinetics despite the rigid, multicyclic chelate structure (an activation barrier).

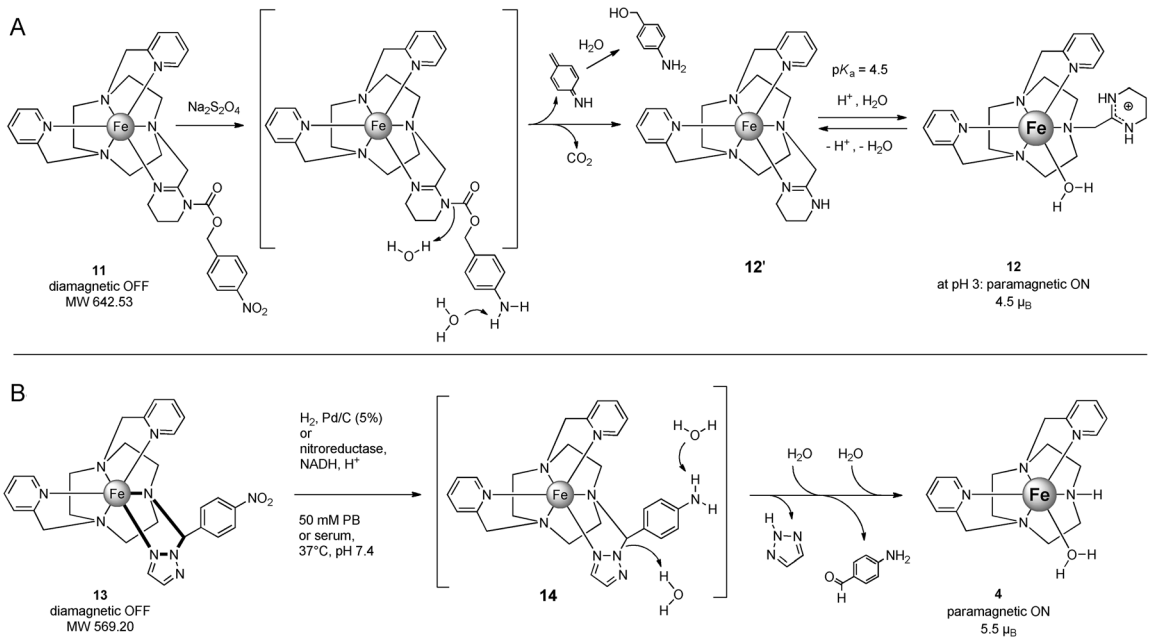
In the first strategy (Scheme 6 A),[40] the activation cascade comprises three subsequent events: 1) transformation of the trigger by the analyte, 2) immolation of the spacer,



Scheme 5. Stabilization of an aminal by incorporation into a chelate ring.

and 3) decoordination of the pendent arm through protonation. In fact, the action of the analyte causes the deacylation (or more precisely: decarbamylation) of a coordinated amidine moiety and thus the unmasking of its particularly high basicity. While uncoordinated amidinium ions exhibit a pK_a value of 12.6, their aminoacyl counterparts have a value of only 7.6, in other words a basicity that is five orders of magnitude lower. Not surprisingly, these pKa values become greatly perturbed if the amidine is engaged in a coordination bond. The value for the amidine unit in the activated iron complex (**12**/**12′**) was found to be 4.5, and thus is eight orders of magnitude lower than for an uncoordinated amidine. Extrapolating this difference to the acylated version should give an even lower pK_a value for the initial probe **11**. This would make it largely immune to decoordination through protonation and would thus contribute to its robustness. Equally important for probe robustness is the established high stability of the aliphatic carbamyl link towards spontaneous hydrolysis and the decidedly low-spin nature of the coordination motif. As a result, probe **11** is remarkably stable in aqueous media (pH 3.5): it shows no signs of degradation over two days at room temperature or 1.5 h at 100 °C. The chelate effect is defeated here, and the ring opened, as a consequence of the high basicity of a pendent coordinated arm, but only at a pH lower than 4.5. While this effect has been exploited with other molecular moieties to open chelate rings,[80] its masking in the design of a responsive probe and the use of an amidine to benefit from the presence of an imine of high ligand field are new to this field. The separation of the trigger unit from the coordination motif (modular design) will likely allow for future adaptation to a variety of other chemical analytes. It remains to be seen whether this promising irreversible probe technology can be made to operate at a neutral pH value and in physiological media.

The second strategy (Scheme 6B)[79] gave rise to a magnetogenic probe (**13**) that indeed functions at a neutral pH value in physiological media, including blood serum. Probe activation does not comprise separate immolation of the spacer but, rather, an initial transformation of the trigger by the chemical analyte results directly in the opening of a chelate ring (bold in Scheme 6B). This became possible only by screening the structure space for the optimal combination of iron chelation and the right mixed aminal with a 1,2,3-triazole unit and a macrocyclic secondary amine. This unit is only stable if it is engaged in a five-membered chelate ring with the iron center and bears a phenyl substituent. In the absence of iron chelation, it instantaneously hydrolyzes if exposed to traces of water. Probe **13** responds when electronic communication between the analyte-susceptible trigger (a nitro group) and the aminal triazole is reversed by reducing the nitro group to an electron-donating amine. The resulting construct (**14**) may be considered a phenylogous orthoamide, with a free NH unit and a reasonably good leaving group



Scheme 6. Two independent concepts for magnetogenic probes.[40, 79] The five-membered chelate ring to be cleaved is highlighted in bold (B).

(triazole). Therefore, cleavage of the chelate ring can occur by simple elimination, and not by a nucleophilic attack; this may explain the good response kinetics with a half-time of roughly 20 min in 50 mM phosphate buffer at 37 °C (Figure 2, top). The chemical analyte may be molecular hydrogen in the presence of a catalyst (palladium on carbon) or even the enzyme nitroreductase that operates with the cofactor NADH. The hexadentate ligand on which **13** is based can be synthesized in a convergent three-component condensation in benzene from the corresponding pentadentate ligand, *para*-nitrobenzaldehyde, and 1,2,3-triazole. The general reaction was introduced and explored in the 1990s by Katritzky et al.[81] It is a condensation reaction that stores considerable energy in the forming aminal. We demonstrated that this highly unstable species can be trapped by titration with an iron salt. Substitution with a nitro group makes the product perfectly stable in physiological media. The complex, therefore, can be regarded as "spring-loaded", an all-important aspect in defeating the chelate effect once the nitro group is reduced. Magnetogenesis in aqueous samples of probe **13** can not only be easily monitored by NMR spectroscopy (determination of the longitudinal relaxation time T_1 of the water hydrogen resonance, Figure 2, top), these T_1 values can also be measured and translated into gray-scale images (phantom images) by MRI (Figure 2, bottom). Both monitoring modes also illustrate the total stability of the probe in the absence of the analyte (hallmark robustness).

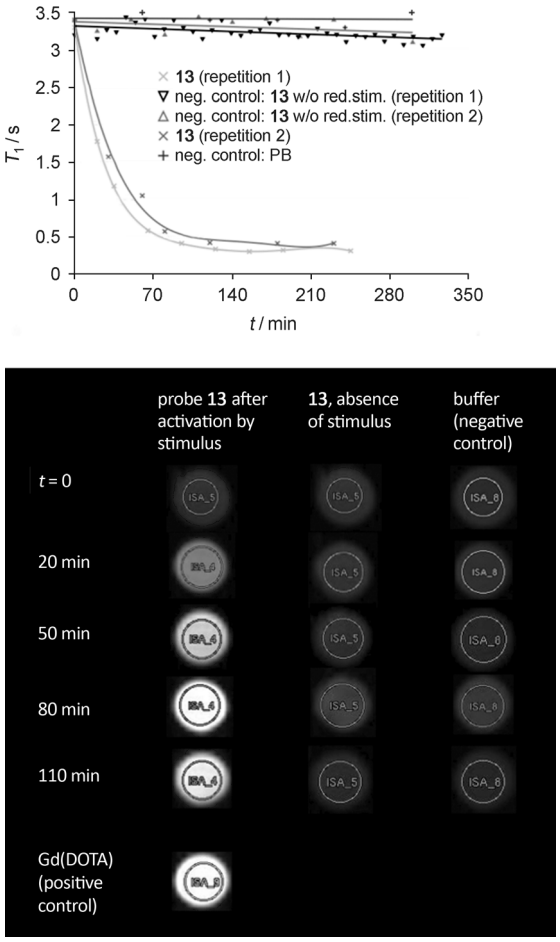


Figure 2. Water-T_1(NMR) monitoring (top) and T_1-weighted MRI monitoring (bottom) of a magnetogenic response by probe **13**. w/o red. stim. = without reductive stimulus, PB = phosphate buffer.

5. Conclusions and Future Directions

New enabling technologies are in urgent need to advance the field of chemical imaging.[1] A magnetic mode may prove a welcome alternative to current optical ones in the detection by responsive molecular probes, whether in samples of technological interest, of biological origin, or in vivo. A range of mechanisms have been reported that can modulate the magnetic properties of discrete molecules in solution in response to an analyte. The weakness of this response, its lack of specificity, and the instability of the systems in competitive solvents calls for new design initiatives. In contrast to their reversible mode of response, probes that react irreversibly should offer high specificity, robustness, and a decisive response. In the biomedical arena, the field of the anatomical imaging modality MRI still seeks probes to render the dream of routine molecular MRI a reality. Strategies have been suggested that give rise to significant signal differences upon activation, but they are not based on magneto-modulation.

Probes that can be activated and that give a non-negligible signal before they encounter their target or do not show total transition in the presence of it may prove useful if they are detected by an additional, orthogonal detection mode followed by careful analysis. However, MRI probes that respond to a (bio-)chemical analyte in an off-on mode promise simple and robust detection, and thus have been called for on numerous occasions.[48,49b,82] A magnetogenic concept,[73,83] as described here, appears to be a viable answer. It is based on iron in its oxidation state II and thus benefits from its environmentally benign nature (green chemistry) and especially the principal possibility of eradicating its spin altogether ($S = 0$, off) as well as its metabolic recognition and homeostatic management in all living organisms.

Future challenges in its further development depend on the application. For analysis of complex biological samples the activated probe should not be hampered in developing its full signal by other sample components. Response kinetics should approach an instantaneous quality, especially for in vivo use. Toxicity issues arising from the high-spin nature of the activated probe should be minimized. Selectivity for a given chemical analyte will require a series of adaptation efforts that go beyond the chemical mechanisms covered by this Minireview. Biologists call for reversibly responsive probes that detect the presence of dynamically expressed proteins of interest. In view of their presence in only tiny concentrations, the design of such a probe remains a formidable task. In this Minireview we hope to have provided the reader with a source of inspiration for his own design endeavors.

J.L.K. and F.T. thank the Ligue Contre le Cancer and the Ministere de la Recherche (both France) for a PhD fellowship, respectively.

Received: July 1, 2013
Published online: November 26, 2013

[1] Nat. Res. Council USA, *Visualizing Chemistry: the Progress and Promise of Advanced Chemical Imaging*, National Academies Press, Washington (DC), **2006**.
[2] a) H. J. Chung, T. Reiner, G. Budin, C. Min, M. Liong, D. Issadore, H. Lee, R. Weissleder, *ACS Nano* **2011**, *5*, 8834 – 8841; b) M. Liong, M. Fernandez-Suarez, D. Issadore, C. Min, C. Tassa, T. Reiner, S. M. Fortune, M. Toner, H. Lee, R. Weissleder, *Bioconjugate Chem.* **2011**, *22*, 2390 – 2394.
[3] F. Bloch, W. Hansen, M. Packard, *Phys. Rev.* **1946**, *69*, 127.
[4] P. Caravan, J. J. Ellison, T. J. McMurry, R. B. Lauffer, *Chem. Rev.* **1999**, *99*, 2293 – 2352.
[5] S. Aime, M. Botta, E. Gianolio, E. Terreno, *Angew. Chem.* **2000**, *112*, 763 – 766; *Angew. Chem. Int. Ed.* **2000**, *39*, 747 – 750.
[6] a) J. W. Chen, M. Q. Sans, A. Bogdanov, R. Weissleder, *Radiology* **2006**, *240*, 473 – 481; b) Y. Li, V. R. Sheth, G. Liu, M. D. Pagel, *Contrast Media Mol. Imaging* **2010**, 219 – 228.
[7] a) A. Jasanoff, *Curr. Opin. Neurobiol.* **2007**, *17*, 593 – 600; b) J. L. Major, G. Parigi, C. Luchinat, T. J. Meade, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 13881 – 13886; c) E. L. Que, C. J. Chang, *Chem. Soc. Rev.* **2010**, *39*, 51 – 60.
[8] a) S. Aime, F. Fedeli, A. Sanino, E. Terreno, *J. Am. Chem. Soc.* **2006**, *128*, 11326 – 11327; b) Y. Wu, T. C. Soesbe, G. E. Kiefer, P. Zhao, A. D. Sherry, *J. Am. Chem. Soc.* **2010**, *132*, 14002 – 14003.
[9] a) B. Yoo, M. D. Pagel, *Front. Biosci.* **2008**, *13*, 1733 – 1752; b) C. Shen, E. J. New, *Curr. Opin. Chem. Biol.* **2013**, *17*, 158 – 166.
[10] O. Sato, J. Tao, Y.-Z. Zhang, *Angew. Chem.* **2007**, *119*, 2200 – 2236; *Angew. Chem. Int. Ed.* **2007**, *46*, 2152 – 2187.
[11] a) *Spin Crossover in Transition Metal Compounds I*: *Vol. 1* of *Topics in Current Chemistry* (Eds.: P. Gütlich, H. A. Goodwin), Springer, Berlin, Heidelberg, New York, **2004**; b) *Spin-Crossover Materials* (Ed.: M. A. Halcrow), Wiley, Oxford, **2013**.
[12] O. Kahn, *Angew. Chem.* **1985**, *97*, 837 – 853; *Angew. Chem. Int. Ed. Engl.* **1985**, *24*, 834 – 850.
[13] C. G. Pierpont, *Coord. Chem. Rev.* **2001**, *216 – 217*, 99 – 125.
[14] a) C. G. Pierpont, *Coord. Chem. Rev.* **2001**, *219*, 415 – 433; b) N. Shimamoto, S.-I. Ohkoshi, O. Sato, K. Hashimoto, *Inorg. Chem.* **2002**, *41*, 678 – 684.
[15] G. N. Newton, M. Nihei, H. Oshio, *Eur. J. Inorg. Chem.* **2011**, 3031 – 3042.
[16] O. Kahn, C. J. Martinez, *Science* **1998**, *279*, 44 – 48.
[17] a) G. J. Halder, C. J. Kepert, B. Moubaraki, K. S. Murray, J. D. Cashion, *Science* **2002**, *298*, 1762 – 1765; b) O. Cador, A. Dei, C. Sangregorio, *Chem. Commun.* **2004**, 652 – 653; c) D. Pinkowicz, R. Podgajny, B. Gaweł, W. Nitek, W. Łasocha, M. Oszajca, M. Czapla, M. Makarewicz, M. Bałanda, B. Sieklucka, *Angew. Chem.* **2011**, *123*, 4059 – 4063; *Angew. Chem. Int. Ed.* **2011**, *50*, 3973 – 3977.
[18] a) M. Engeser, L. Fabbrizzi, M. Licchelli, D. Sacchi, *Chem. Commun.* **1999**, 1191 – 1192; b) C. Edder, C. Piguet, J.-C. G. Bünzli, G. Hopfgartner, *Chem. Eur. J.* **2001**, *7*, 3014 – 3024.
[19] a) M. E. Itkis, X. Chi, A. W. Cordes, R. C. Haddon, *Science* **2002**, *296*, 1443 – 1445; b) N. Hoshino, F. Iijima, G. N. Newton, N. Yoshida, T. Shiga, H. Nojiri, A. Nakao, R. Kumai, Y. Murakami, H. Oshio, *Nat. Chem.* **2012**, *4*, 921 – 926.
[20] a) J. K. Beattie, *Adv. Inorg. Chem.* **1988**, *32*, 1 – 53; b) H. Toftlund, *Coord. Chem. Rev.* **1989**, *94*, 67 – 108; c) H. Toftlund, *Monatsh. Chem.* **2001**, *132*, 1269 – 1277; d) H. Toftlund, J. J. McGarvey, *Spin Crossover in Transition Metal Compounds I*: *Vol. 1* of *Topics in Current Chemistry*, Springer, Berlin/Heidelberg, **2004**, pp. 151 – 166; e) N. Hassan, A. B. Koudriavtsev, W. Linert, *Pure Appl. Chem.* **2008**, *80*, 1281 – 1292.
[21] M. P. Shores, C. M. Klug, S. R. Fiedler, *Spin-Crossover Materials* (Ed.: M. A. Halcrow), Wiley, Oxford, **2013**, pp. 281 – 301.
[22] B. Weber in *Spin-Crossover Materials* (Ed.: M. A. Halcrow), Wiley, Oxford, **2013**, pp. 55 – 76.
[23] D. Siretanu, D. Li, L. Buisson, D. M. Bassani, S. M. Holmes, C. Mathonière, R. Clérac, *Chem. Eur. J.* **2011**, *17*, 11704 – 11708.
[24] a) J. Rall, M. Wanner, M. Albrecht, F. M. Hornung, W. Kaim, *Chem. Eur. J.* **1999**, *5*, 2802 – 2809; b) E. Evangelio, C. Rodriguez-Blanco, Y. Coppel, D. N. Hendrickson, J. P. Sutter, J. Campo, D. Ruiz-Molina, *Solid State Sci.* **2009**, *11*, 793 – 800.
[25] a) R. A. Binstead, J. K. Beattie, T. G. Dewey, D. H. Turner, *J. Am. Chem. Soc.* **1980**, *102*, 6442 – 6451; b) P. Gutlich, H. A. Goodwin, *Spin Crossover in Transition Metal Compounds I*, *Topics in Current Chemistry*, Springer, Berlin, **2004**, pp. 1 – 47.
[26] A. J. Conti, C. L. Xie, D. N. Hendrickson, *J. Am. Chem. Soc.* **1989**, *111*, 1171 – 1180.
[27] a) M. F. Tweedle, L. J. Wilson, *J. Am. Chem. Soc.* **1976**, *98*, 4824 – 4834; b) R. H. Petty, E. V. Dose, M. F. Tweedle, L. J. Wilson, *Inorg. Chem.* **1978**, *17*, 1064 – 1071.
[28] S. A. Barrett, C. A. Kilner, M. A. Halcrow, *Dalton Trans.* **2011**, *40*, 12021 – 12024.
[29] a) W. Linert, M. Enamullah, V. Gutmann, R. Jameson, *Monatsh. Chem.* **1994**, *125*, 661 – 670; b) M. Enamullah, W. Linert, *J. Coord. Chem.* **1996**, *38*, 337 – 346.
[30] a) L. L. Martin, K. S. Hagen, A. Hauser, R. L. Martin, A. M. Sargeson, *Chem. Commun.* **1988**, 1313 – 1315; b) L. L. Martin, R. L. Martin, A. M. Sargeson, *Polyhedron* **1994**, *13*, 1969 – 1980.
[31] Z. Ni, M. P. Shores, *J. Am. Chem. Soc.* **2009**, *131*, 32 – 33.
[32] Z. Ni, A. M. McDaniel, M. P. Shores, *Chem. Sci.* **2010**, *1*, 615 – 621.
[33] A. M. McDaniel, C. M. Klug, M. P. Shores, *Eur. J. Inorg. Chem.* **2013**, 943 – 950.
[34] P. Leeladee, R. A. Baglia, K. A. Prokop, R. Latifi, S. P. de Visser, D. P. Goldberg, *J. Am. Chem. Soc.* **2012**, *134*, 10397 – 10400.
[35] a) L. Fabbrizzi, M. Licchelli, G. Rabaioli, A. Taglietti, *Coord. Chem. Rev.* **2000**, *205*, 85 – 108; b) D. W. Blakesley, S. C. Payne, K. S. Hagen, *Inorg. Chem.* **2000**, *39*, 1979 – 1989; c) K. P. Bryliakov, E. A. Duban, E. P. Talsi, *Eur. J. Inorg. Chem.* **2005**, 72 – 76; d) J. England, G. J. P. Britovsek, N. Rabadia, A. J. P. White, *Inorg. Chem.* **2007**, *46*, 3752 – 3767.
[36] L. Pauling, C. D. Coryell, *Proc. Natl. Acad. Sci. USA* **1936**, *22*, 210 – 216.
[37] S. Ogawa, T. M. Lee, A. R. Kay, D. W. Tank, *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 9868 – 9872.
[38] a) L. Fabbrizzi, A. Leone, A. Taglietti, *Angew. Chem.* **2001**, *113*, 3156 – 3159; *Angew. Chem. Int. Ed.* **2001**, *40*, 3066 – 3069; b) B. T. Nguyen, E. V. Anslyn, *Coord. Chem. Rev.* **2006**, *250*, 3118 – 3127.
[39] a) C. A. Reed, F. Guiset, *J. Am. Chem. Soc.* **1996**, *118*, 3281 – 3282; b) C. R. Goldsmith, R. T. Jonas, A. P. Cole, T. D. P. Stack, *Inorg. Chem.* **2002**, *41*, 4642 – 4652; c) J. P. López, F. W. Heinemann, R. Prakash, B. A. Hess, O. Horner, C. Jeandey, J.-L. Oddou, J.-M. Latour, A. Grohmann, *Chem. Eur. J.* **2002**, *8*, 5709 – 5722; d) N. Ortega-Villar, V. M. Ugalde-Saldívar, M. C. Muñoz, L. A. Ortiz-Frade, J. G. Alvarado-Rodríguez, J. A. Real, R. Moreno-Esparza, *Inorg. Chem.* **2007**, *46*, 7285 – 7293; e) A. Draksharapu, Q. Li, H. Logtenberg, T. A. van den Berg, A. Meetsma, J. S. Killeen, B. L. Feringa, R. Hage, G. Roelfes, W. R. Browne, *Inorg. Chem.* **2012**, *51*, 900 – 913.
[40] F. Touti, P. Maurin, L. Canaple, O. Beuf, J. Hasserodt, *Inorg. Chem.* **2012**, *51*, 31 – 33.
[41] M. L. Childers, J. Cho, C. A. S. Regino, M. W. Brechbiel, A. G. DiPasquale, A. L. Rheingold, S. V. Torti, F. M. Torti, R. P. Planalp, *J. Inorg. Biochem.* **2008**, *102*, 150 – 156.

[42] N. Ségaud, J. N. Rebilly, K. Sénéchal-David, R. Guillot, L. Billon, J.-P. Baltaze, J. Farjon, O. Reinaud, F. Banse, *Inorg. Chem.* **2013**, *52*, 691 – 700.
[43] a) L. Sacconi, P. Nannelli, U. Campigli, *Inorg. Chem.* **1965**, *4*, 818 – 822; b) M. Boiocchi, L. Fabbrizzi, F. Foti, M. Vazquez, *Dalton Trans.* **2004**, 2616 – 2620; c) H. Ohtsu, K. Tanaka, *Inorg. Chem.* **2004**, *43*, 3024 – 3030; d) S. Thies, C. Bornholdt, F. Köhler, F. D. Sönnichsen, C. Näther, F. Tuczek, R. Herges, *Chem. Eur. J.* **2010**, *16*, 10074 – 10083.
[44] a) R. F. Pasternack, E. G. Spiro, M. Teach, *J. Inorg. Nucl. Chem.* **1974**, *36*, 599 – 606; b) J. K. Beattie, M. T. Kelso, W. E. Moody, P. A. Tregloan, *Inorg. Chem.* **1985**, *24*, 415 – 418; c) T. La, R. A. Richards, R. S. Lu, R. Bau, G. M. Miskelly, *Inorg. Chem.* **1995**, *34*, 5632 – 5640.
[45] F. Kratz, I. A. Muller, C. Ryppa, A. Warnecke, *ChemMedChem* **2008**, *3*, 20 – 53.
[46] a) E. Leroy, N. Bensel, J. L. Reymond, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2105 – 2108; b) J. A. Richard, M. Massonneau, P. Y. Renard, A. Romieu, *Org. Lett.* **2008**, *10*, 4175 – 4178; c) L. D. Lavis, T.-Y. Chao, R. T. Raines, *Chem. Sci.* **2011**, *2*, 521 – 530.
[47] J. A. Duimstra, F. J. Femia, T. J. Meade, *J. Am. Chem. Soc.* **2005**, *127*, 12847 – 12855.
[48] M. Baker, *Nature* **2010**, *463*, 977 – 980.
[49] a) M. L. Garcia-Martin, G. V. Martinez, N. Raghunand, A. D. Sherry, S. Zhang, R. J. Gillies, *Magn. Reson. Med.* **2006**, *55*, 309 – 315; b) E. Terreno, D. D. Castelli, A. Viale, S. Aime, *Chem. Rev.* **2010**, *110*, 3019 – 3042; c) V. Catanzaro, C. V. Gringeri, V. Menchise, S. Padovan, C. Boffa, W. Dastrù, L. Chaabane, G. Digilio, S. Aime, *Angew. Chem.* **2013**, *125*, 4018 – 4022; *Angew. Chem. Int. Ed.* **2013**, *52*, 3926 – 3930.
[50] T. Chauvin, P. Durand, M. Bernier, H. Meudal, B. T. Doan, F. Noury, B. Badet, J. C. Beloeil, E. Toth, *Angew. Chem.* **2008**, *120*, 4442 – 4444; *Angew. Chem. Int. Ed.* **2008**, *47*, 4370 – 4372.
[51] a) A. Dei, L. Sorace, *Dalton Trans.* **2003**, 3382 – 3386; b) F. Hyodo, K. I. Matsumoto, A. Matsumoto, J. B. Mitchell, M. C. Krishna, *Cancer Res.* **2006**, *66*, 9921 – 9928.
[52] a) J. L. Brusso, O. P. Clements, R. C. Haddon, M. E. Itkis, A. A. Leitch, R. T. Oakley, R. W. Reed, J. F. Richardson, *J. Am. Chem. Soc.* **2004**, *126*, 14692 – 14693; b) C. S. Clarke, J. Jornet-Somoza, F. Mota, J. J. Novoa, M. Deumal, *J. Am. Chem. Soc.* **2010**, *132*, 17817 – 17830.
[53] I. Ratera, D. Ruiz-Molina, J. V. Gancedo, K. Wurst, N. Daro, J. F. Létard, C. Rovira, J. Veciana, *Angew. Chem.* **2001**, *113*, 933 – 936; *Angew. Chem. Int. Ed.* **2001**, *40*, 919 – 922.
[54] a) K. Hamachi, K. Matsuda, T. Itoh, H. Iwamura, *Bull. Chem. Soc. Jpn.* **1998**, *71*, 2937 – 2943; b) K. Matsuda, M. Matsuo, M. Irie, *J. Org. Chem.* **2001**, *66*, 8799 – 8803.
[55] a) D. W. Low, J. R. Winkler, H. B. Gray, *J. Am. Chem. Soc.* **1996**, *118*, 117 – 120; b) T. Kurahashi, A. Kikuchi, T. Tosha, Y. Shiro, T. Kitagawa, H. Fujii, *Inorg. Chem.* **2008**, *47*, 1674 – 1686; c) M. Nihei, Y. Sekine, N. Suganami, K. Nakazawa, A. Nakao, H. Nakao, Y. Murakami, H. Oshio, *J. Am. Chem. Soc.* **2011**, *133*, 3592 – 3600.
[56] B. Trzaskowski, A. Les, L. Adamowicz, *Int. J. Mol. Sci.* **2003**, *4*, 503 – 511.
[57] M. A. Halcrow, *Polyhedron* **2007**, *26*, 3523 – 3576.
[58] A. E. Martell, R. D. Hancock, *Metal Complexes in Aqueous Solutions*, Plenum, New York, **1996**.
[59] F. L. Urbach, S. O. Wandiga, *J. Chem. Soc. D* **1970**, 1572a.
[60] A. H. Al-Obaidi, K. B. Jensen, J. J. McGarvey, H. Toftlund, B. Jensen, S. E. Bell, J. G. Carroll, *Inorg. Chem.* **1996**, *35*, 5055 – 5060.
[61] M. A. Hoselton, L. J. Wilson, R. S. Drago, *J. Am. Chem. Soc.* **1975**, *97*, 1722 – 1729.
[62] M. Tamura, Y. Urano, K. Kikuchi, T. Higuchi, M. Hirobe, T. Nagano, *J. Organomet. Chem.* **2000**, *611*, 586 – 592.
[63] V. M. Ugalde-Saldívar, M. E. Sosa-Torres, L. Ortiz-Frade, S. Bernès, H. Höpfl, *J. Chem. Soc. Dalton Trans.* **2001**, 3099 – 3107.
[64] M. L. Childers, F. Su, A. M. Przyborowska, B. Bishwokarma, G. Park, M. W. Brechbiel, S. V. Torti, F. M. Torti, G. Broker, J. S. Alexander, R. D. Rogers, K. Ruhlandt-Senge, R. P. Planalp, *Eur. J. Inorg. Chem.* **2005**, 3971 – 3982.
[65] a) L. Christiansen, D. N. Hendrickson, H. Toftlund, S. R. Wilson, C. L. Xie, *Inorg. Chem.* **1986**, *25*, 2813 – 2818; b) K. Wieghardt, E. Schoffmann, B. Nuber, J. Weiss, *Inorg. Chem.* **1986**, *25*, 4877 – 4883.
[66] a) T. J. Hubin, J. M. McCormick, S. R. Collinson, M. Buchalova, C. M. Perkins, N. W. Alcock, P. K. Kahol, A. Raghunathan, D. H. Busch, *J. Am. Chem. Soc.* **2000**, *122*, 2512 – 2522; b) S. Collinson, N. W. Alcock, A. Raghunathan, P. K. Kahol, D. H. Busch, *Inorg. Chem.* **2000**, *39*, 757 – 764.
[67] a) H. Börzel, P. Comba, K. Hagen, Y. Lampeka, A. Lienke, G. Linti, M. Merz, H. Pritzkow, L. Tsymbal, *Inorg. Chim. Acta* **2002**, *337*, 407 – 419; b) J. L. Kolanowski, E. Jeanneau, R. Steinhoff, J. Hasserodt, *Chem. Eur. J.* **2013**, *19*, 8839 – 8849.
[68] A. D. Sherry, P. Caravan, R. E. Lenkinski, *J. Magn. Reson. Imaging* **2009**, *30*, 1240 – 1248.
[69] a) P. Wedeking, K. Kumar, M. F. Tweedle, *Magn. Reson. Imaging* **1992**, *10*, 641 – 648; b) M. Port, J.-M. Idee, C. Medina, C. Robic, M. Sabatou, C. Corot, *Biometals* **2008**, *21*, 469 – 490.
[70] C. M. Klug, A. M. McDaniel, S. R. Fiedler, K. A. Schulte, B. S. Newell, M. P. Shores, *Dalton Trans.* **2012**, *41*, 12577 – 12585.
[71] a) J. J. McGarvey, I. Lawthers, K. Heremans, H. Toftlund, *Inorg. Chem.* **1990**, *29*, 252 – 256; b) J. K. McCusker, H. Toftlund, A. L. Rheingold, D. N. Hendrickson, *J. Am. Chem. Soc.* **1993**, *115*, 1797 – 1804.
[72] V. Balland, F. Banse, E. Anxolabehere-Mallart, M. Ghiladi, T. A. Mattioli, C. Philouze, G. Blondin, J. J. Girerd, *Inorg. Chem.* **2003**, *42*, 2470 – 2477.
[73] V. Stavila, M. Allali, L. Canaple, Y. Stortz, C. Franc, P. Maurin, O. Beuf, O. Dufay, J. Samarut, M. Janier, J. Hasserodt, *New J. Chem.* **2008**, *32*, 428 – 435.
[74] A. H. R. Al-Obaidi, J. J. McGarvey, K. P. Taylor, S. E. J. Bell, K. B. Jensen, H. Toftlund, *J. Chem. Soc. Chem. Commun.* **1993**, 536 – 538.
[75] F. Touti, A. K. Singh, P. Maurin, L. Canaple, O. Beuf, J. Samarut, J. Hasserodt, *J. Med. Chem.* **2011**, *54*, 4274 – 4278.
[76] a) G. D. Hosken, R. D. Hancock, *J. Chem. Soc. Chem. Commun.* **1994**, 1363 – 1364; b) G. D. Hosken, C. C. Allan, J. C. A. Boeyens, R. D. Hancock, *J. Chem. Soc. Dalton Trans.* **1995**, 3705 – 3708.
[77] V. Stavila, Y. Stortz, C. Franc, D. Pitrat, P. Maurin, J. Hasserodt, *Eur. J. Inorg. Chem.* **2008**, 3943 – 3947.
[78] T. Chauvin, S. Torres, R. Rosseto, J. Kotek, B. Badet, P. Durand, E. Toth, *Chem. Eur. J.* **2012**, *18*, 1408 – 1418.
[79] F. Touti, P. Maurin, J. Hasserodt, *Angew. Chem.* **2013**, *125*, 4752 – 4756; *Angew. Chem. Int. Ed.* **2013**, *52*, 4654 – 4658.
[80] M. P. Lowe, D. Parker, O. Reany, S. Aime, M. Botta, G. Castellano, E. Gianolio, R. Pagliarin, *J. Am. Chem. Soc.* **2001**, *123*, 7601 – 7609.
[81] A. R. Katritzky, X. F. Lan, J. Z. Yang, O. V. Denisko, *Chem. Rev.* **1998**, *98*, 409 – 548.
[82] *Molecular Imaging: Principles and Practice*, R. Weissleder, B. D. Ross, A. Rehemtulla, S. S. Gambhir (Eds.) Pmph USA Ltd, **2010**.
[83] a) J. Hasserodt, WO2005094903; b) J. Hasserodt, P. Maurin, F. Touti, FR201257413; c) J. Hasserodt, *New J. Chem.* **2012**, *36*, 1707 – 1712.