Contrast Agents

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What We Can Really Do with Bioresponsive MRI Contrast Agents

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contrast agents · magnetic resonance imaging · molecular sensors · nuclear magnetic resonance · paramagnetic complexes

Dedicated to Professor Nikos K. Logothetis on the occasion of his 65th birthday

***B**ioresponsive MRI contrast agents hold great promise for monitoring major physiological and pathological processes in a non-invasive manner. They are capable of altering the acquired MRI signal as a consequence of changes in their microenvironment, thus allowing real-time functional reporting in living organisms. Importantly, chemistry offers diverse solutions for the design of agents which respond to a great number of specific targets. However, the path to the successful utilization of these biomarkers in the desired functional MRI studies involves careful consideration of multiple scientific, technical, and practical issues across various research disciplines. This Minireview highlights the critical steps for planning and executing such multidisciplinary projects with an aim to substantially improve our knowledge of essential biological processes.*

1. The Role of Bioresponsive MRI Contrast Agents in Molecular and Functional Imaging

Monitoring and visualization of biological processes is the ultimate goal of all bio-imaging techniques. Amongst these, magnetic resonance imaging (MRI) is probably the most preferred choice, because of its superb spatio-temporal resolution and unlimited depth of tissue penetration. The specificity of MRI was dramatically improved through the use of contrast agents, which has led to extensive expansion of this research area over the last three decades. Recently, great efforts have been made to develop and use bioresponsive MRI contrast agents.[1–3] These are molecular probes that respond to changes in their microenvironment by altering the MRI signal they produce. Consequently, they are capable of reporting changes in various biological parameters in their vicinity, thus providing valuable information on the existence and execution of numerous and essential physiological and pathological processes at the cellular and molecular level.

Bioresponsive MRI contrast agents possess enormous potential to monitor biological processes: every biological target or event can be associated with alterations in a specific parameter in the tissue, for example, the concentration of a particular endogenous ion or molecule, change in activity of an enzyme, or the temperature. This accordingly allows the design of a tailor-made reporter molecule which becomes activated when the desired event or change in concentration of the target takes place. Finally, the existence of different modalities for the generation, alteration, and recording of the MRI signal results in unprecedented diversity in the information that can be acquired using these agents.

This potential was identified roughly two decades ago, when the first so-called "smart" MRI contrast agent was prepared and used to visualize gene expression, in that case the common gene marker β-galactosidase.[4,5] Since then, hundreds of these sensor molecules that were tailor-made for different purposes have been reported. The vast majority of the existing agents were only shown to be active in vitro, with follow-up in vivo success still pending. In fact, only a few of them have reached the final phase, namely, a clear demonstration of their applicability in vivo.[5–7] Apparently, the complexity of the whole approach is much greater than initially anticipated, and requires proficient expertise across many scientific disciplines. The purpose of this Minireview is not to extensively list bioresponsive MRI contrast agents that have been reported so far, as there are many reviews which cover the major research directions and the most prominent examples. Instead, the intention is to familiarize readers with the main principles involved in the preparation of bioresponsive MRI contrast agents, as well as the major challenges and obstacles which prevent their wider in vivo application. In

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fact, the key to ultimate success lies in addressing these complex issues in a multidisciplinary manner, through chemistry, biology, physics, and engineering, and in managing seamless communication amongst them. These aspects will be critically discussed, with the author's personal view on the most reasonable directions that should lead to the desired results and wider implementation of this approach in the establishment of novel functional MRI methods to study different biological processes.

2. Design Principles and Pitfalls

There are several ways to classify bioresponsive contrast agents. The most commonly used is based on the type of desired target or event to be monitored (such as pH value, ion or molecule concentration, enzyme activity). Less frequently, bioresponsive contrast agents are grouped on the basis of their chemical structure (for example, acyclic or macrocyclic paramagnetic complexes, proteins, or fluorinated organic molecules). However, these classification strategies may sometimes be misleading when the practical applications of bioresponsive agents are discussed. Different contrast agents that respond to a particular type of target or event can be made, but their monitoring by MRI can fail for many reasons, for example, lack of signal sensitivity in vivo (see Section 3). In fact, once their practical characteristics are analyzed, the best strategy is to evaluate the origin of the MRI signal that they produce.

All MRI techniques rely on various nuclear magnetic resonance (NMR) phenomena, where the magnetization of the nuclei studied is assessed under different conditions and converted into the resulting NMR signal. Subsequently, if a change in this signal can be caused by external stimuli (for example, a change in the concentration of the ion or molecule, or enzyme activity), then the same event could in principle be followed by MRI. Naturally, many practical aspects limit the use of these numerous NMR phenomena.

A weak sensitivity is probably one of the decisive factors for the use of only a couple of NMR-active nuclei with a large natural abundance in MRI (^{1}H or ^{19}F). Alternatively, hyperpolarization techniques may temporarily increase the abundance of a particular NMR-active nucleus with spin $^{1}/_{2}$ (see Section 2.4), thus allowing specific NMR and MRI studies to be performed.[8] In any case, it is crucial that scientists are familiar with the fundamental principles of each NMR method as well as their major advantages and limitations. Only in such cases will the follow-up steps be successful beyond the preparation of the potent bioresponsive MRI contrast agent, and lead to their use in functional MRI.

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2.1. Paramagnetic T_1 and Superparamagnetic T_2 Contrast Agents for ^{1}H MRI

These agents affect the longitudinal (T_1) or transverse (T_2) component of nucleus magnetization by enhancing the T_1 or T_2 relaxation of water protons.[9,10] Subsequently, shorter relaxations times result in a stronger alteration of the MRI signal in regions where the contrast agent is present compared to regions where protons relax at the standard rate.

Typical T_1 contrast agents are based on paramagnetic complexes of gadolinium(III) and manganese(II) with chelators that serve to reduce the toxicity of these ions. However, these complexes possess water molecules incorporated into their inner sphere, which is the major source of rapidly relaxing protons. Depending on several parameters, amongst which the number of directly coordinated water molecules, their exchange with bulk water (water exchange rate), and the tumbling rate (rotational correlation time) are the most influential, the paramagnetic complex is more or less potent in affecting proton relaxation. Quantitatively, this potency is expressed through the term longitudinal relaxivity (r_1), which indicates the enhancement of the relaxation rate per 1 mM concentration of the paramagnetic ion. Nevertheless, the final contrast obtained in the MRI experiment is the sum of the signals of the paramagnetic contrast agent (major component), and the bulk water that is not influenced by the contrast agent, the protons of which still relax at a slower rate, depending on the nature of their environment (for example, a solution or a tissue).

Similarly, the effect of T_2 contrast agents is expressed through the change in transverse relaxivity r_2. In this case, higher r_2 relaxivity results in signal loss. Typical T_2 agents are based on superparamagnetic iron oxide nanoparticles, although paramagnetic metals such as dysprosium(III) or thulium(III) have also been used recently to improve the performance of these particles. These agents strongly affect the local magnetic field and hence the magnetization of nearby water protons. Therefore, as a consequence of changes in water diffusion near the particle, the size of the particle is very important for the final effect on the MRI signal.

Bioresponsive T_1 and T_2 agents are capable of altering their relaxivity because of changes in their microenvironment (Figure 1). The changes can be irreversible (for example, as a result of enzyme activity which causes permanent structural changes of the complex), thereby allowing functional imaging in only one direction, or reversible (for example, because of a changed concentration of a target ion or molecule), principally allowing the visualization of dynamic biological processes. Furthermore, depending on the direction of the relaxivity change, one can follow the signal enhancement

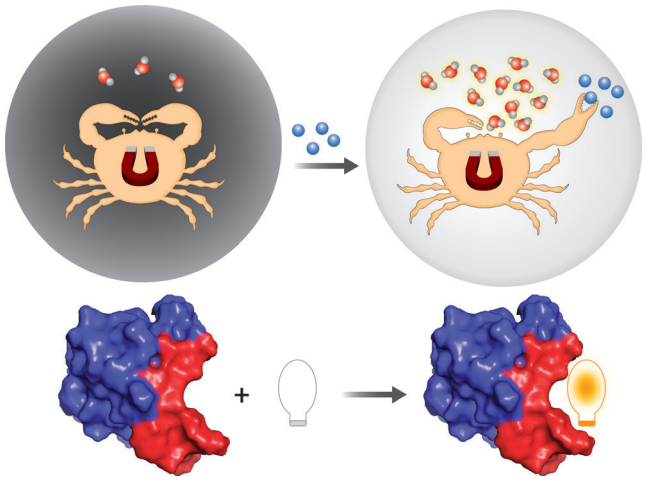

Figure 1. The mechanisms exploited to cause changes in the T_1 or T_2 relaxation times. Top: The paramagnetic metal ion is entrapped in a chelator to reduce its toxicity; however, certain functional groups are weakly coordinated to the metal ion and they flip towards the target ion.[11,12] Displacement of the functional group gives rise to an increase in inner-sphere hydration, thus a larger number of water molecules are influenced by the paramagnetic metal ion, its relaxivity increases, as well as the MRI signal. Bottom: A smaller paramagnetic agent specifically interacts with the high-molecular-weight macromolecule (e.g. with an active site of the protein), thereby slowing down the rotation of the whole system and thus changing the r_1 of the paramagnetic agent.[13] Alternatively, a target ion or molecule may selectively bind to a protein, thus changing the r_1 or r_2 value of the paramagnetic or superparamagnetic agent, respectively.[14,15]

during the particular event (by the so-called "turn-on" mechanism) or its attenuation (by the "turn-off" mechanism).

Despite these efforts, the practical utilization of bioresponsive T_1 and T_2 agents in vivo has been limited to date, even though a large number of contrast agents have been reported to be active in vitro. There are several reasons for this situation. First and foremost, the sensitivity of MRI is lower than many other imaging approaches which use contrast agents, such as positron emission tomography (PET), single photon emission computed tomography (SPECT), and optical imaging methods, whose sensitivities range from pM to nM for PET or SPECT and optical methods, respectively.[16] Consequently, higher concentrations of contrast agents are required to produce the MRI signal, especially when the detectable changes originate from bioresponsive T_1 agents. As a result, not every ionic or molecular target can be used as a trigger for bioresponsive T_1 agents. In fact, only those with an abundance comparable to the detection limit of T_1 agents (μM concentrations and above) can generate sufficient signal changes. Accordingly, many cations and anions from various physiological fluids[17] or highly abundant neurotransmitters are suitable targets.[18] Similarly, only proteins reaching μM levels or enzymes with fast kinetics can be suitable targets.[19] However, it should be noted that intracellular targets also require appropriate strategies for internalization of bioresponsive agents in the cells to deliver sufficient and detectable amounts.

2.2. *CEST Agents*

Signal generation for chemical exchange saturation transfer (CEST) agents is entirely different from that of T_1 or T_2 relaxing agents.[20,21] In this case, the exchangeable protons on the CEST agent transfer the magnetization to the bulk water molecules. When the radiofrequency pulse is selectively applied to these protons, part of the energy is delivered to bulk water, thus reducing their magnetization and the MR signal.

The CEST effect depends to a great extent on the chemical exchange rate of protons in amide, amine, hydroxy, or thiol functional groups. This phenomenon is commonly exploited for the design of bioresponsive agents, because a wide range of "stimuli" can be used to affect the exchange rates (e.g. pH or temperature change).[22] Furthermore, their frequency (chemical shift) plays an important role, especially if it is sufficiently distant from the frequency of bulk water. A very elegant solution has been found by developing paramagnetic CEST (paraCEST) agents, where the use of a paramagnetic metal center causes the shift of the frequency, thereby enabling better specificity and greater exchange rates because of larger differences in frequencies (Figure 2).

The great advantage of this method is the ability to activate the CEST signal on demand. Unlike T_1 and T_2 agents, there is no background signal for CEST agents, which automatically facilitates the analysis of the results obtained.

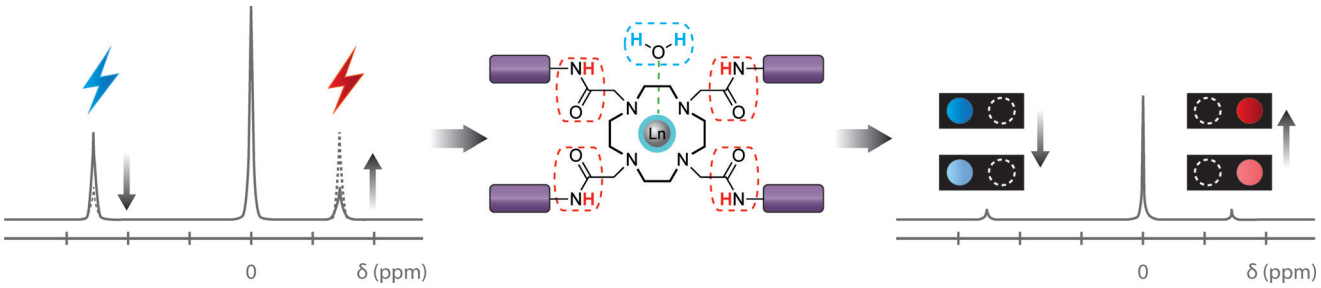

Figure 2. Bioresponsive agents based on the paraCEST effect: The frequency of specific exchangeable protons is distant from that of bulk water. The applied radiofrequency pulse at this frequency (red or blue flashes) causes the loss of magnetization of these protons and also of the bulk water, thus transforming this negative signal into MRI contrast. The nature of the groups incorporated in the structure of the contrast agent (violet rounded rectangles) influences the intensity of the CEST effect (—— and •••••, left spectrum). These changes in the properties of exchangeable protons (colored on the structure inside the dashed rounded rectangles) caused by enzyme activity, ions, or a change in redox state alter the chemical exchange rate and consequently the CEST effect and the CEST MRI signal.[23–28]

This feature is certainly very useful for bioresponsive CEST agents—there is no interference between different types of signals, so the results are very reliable. However, a limited number of these agents can be used in practice. First of all, a large number of exchangeable protons is needed to induce the effect, which therefore necessitates that high concentrations of bioresponsive agents are applied (>mM). Consequently, targets present in lower quantities should not be considered for monitoring by this method. Useful advances have been made in mapping pH in vivo by using bioresponsive agents because this parameter strongly affects proton exchange rates.[6] Potentially, enzymatic reactions with fast kinetics could also be monitored by an irreversibly changed bioresponsive CEST agent. Here, the formation of a sufficient quantity of CEST active species can be observed in a reasonable time window, before the agent washes out. Finally, it must be noted that temperature can greatly affect the CEST effect. Namely, the high-power radiofrequency pulses (used for excitation of exchangeable protons) may increase tissue temperature, thereby changing the exchange rates of protons in the bioresponsive agent. Consequently, the final results obtained in the CEST experiment can be ambiguous.

2.3. Bioresponsive Agents Suitable for ^{19}F MRI

The ^{19}F isotope is a rare example of a nucleus other than ^{1}H that is suitable for MRI applications. It has a few favorable NMR properties, among which is the high natural abundance of this isotope and the nuclear spin of $^{1}/_{2}$, which results in this isotope having the second highest NMR sensitivity after ^{1}H. On the other hand, its low abundance in living systems means that exogenous fluorinated contrast agents must be used at concentrations higher than is common for ^{1}H MRI probes when performing ^{19}F MRI.

However, signal manipulation appears easier with ^{19}F MRI—hence several bioresponsive agents have been reported to date. A commonly applied strategy exploits the paramagnetic relaxation enhancement (PRE) effect, which affects the ^{19}F relaxation processes.[29] Here, the ^{19}F atoms are brought into proximity to paramagnetic species, such as lanthanide ions, often integrated in the same contrast agent. The paramagnetism of ions results in changes in the ^{19}F T_1 and T_2 times, which can be detected using appropriate imaging methods, because of the effect of these relaxation times on the intensity of the MRI signal (Figure 3).

The second strategy used for bioresponsive ^{19}F contrast agents takes advantage of the large range of chemical shifts that this nucleus exhibits ($\delta \approx 300$ ppm). Its electronic microenvironment is exceptionally sensitive, thereby leading to substantial changes in the signal intensity or frequency shift upon an ionic, dipole, or hydrophobic interaction with the target ion or molecule. Consequently, a number of ^{19}F-based reporter molecules have been developed for a variety of physiological phenomena, including measuring changes in the temperature, pH value, as well as O_2 or metal ion concentrations, or specific gene reporters.[36] Finally, the substantial difference in the frequencies between two resonances combined with the existence of exchange between them also gives rise to exploration of the CEST approach for ^{19}F-based agents, which can be conveniently used for the simultaneous detection of multiple endogenous metal ions.[37]

A few advantages and drawbacks must be considered prior to the utilization of these probes. Since the gyromagnetic ratios between ^{1}H and ^{19}F are similar, the major hardware requirements for performing the MRI scans are essentially the same and only minor customizations (e.g. the use of ^{19}F only or tunable ^{1}H/^{19}F coils) must be performed prior to ^{19}F MRI studies. The low abundance of ^{19}F atoms in living organisms results in an absence of background signals and allows quantitative measurements. Concurrently, superimposition of ^{19}F with ^{1}H MR images is required to obtain the appropriate anatomical information. However, the major obstacle for the practical use of these agents is the detection

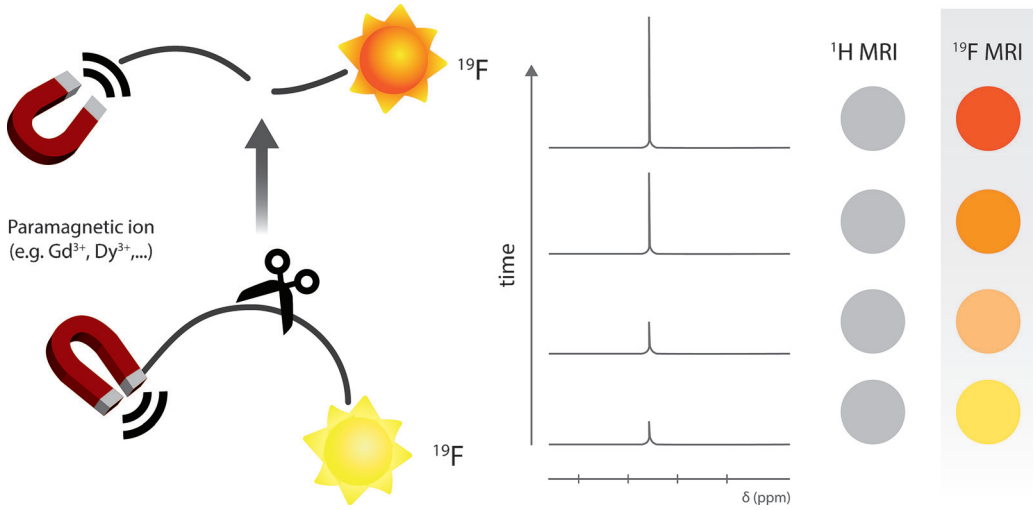

Figure 3. Visualization of the enzyme activity using the PRE effect by means of ^{19}F MRI. The paramagnetic ion is proximate to the ^{19}F reporter, thereby causing elimination of the ^{19}F MRI signal as a result of a strong T_2* relaxation. The signal recovery is achieved by the enzyme activity, which separates the ^{19}F moiety from the paramagnetic center.[30,31] Analogous reversible changes in the ^{19}F MRI signal can be caused through the ion-triggered PRE or a paraSHIFT mechanism (paraSHIFT: paramagnetically shifted NMR resonance),[32,33] or through assembly/disassembly processes.[34,35]

threshold. Millimolar concentrations of chemically equivalent ^{19}F are required for reliable studies, while acquisition times can still be very long (ca. 1 h), thereby only allowing biological processes with slow kinetics to be monitored.[38] Although the higher load of equivalent ^{19}F atoms per contrast agent molecule may appear to be a straightforward solution, their use in large amounts results in poor water solubility and poor in vivo applicability, while improving the interactions with potential targets (e.g. O_2, CO_2).[39]

2.4. Hyperpolarized Bioresponsive Contrast Agents

Several NMR-active nuclei have low isotope abundance, low γ values, and consequently longer relaxation times. The acquisition times are, therefore, also very long, thus making these agents less practical for monitoring biologically relevant processes. Nevertheless, recent advances in various hyperpolarization techniques hold great promise for the development of bioresponsive agents which may robustly report changes in their environment. Basically, the hyperpolarization alters the nuclear spin populations with respect to the equilibrium state, which leads to a several-fold increase in the NMR signal intensity and rapid acquisition of different nuclei, such as ^{13}C, ^{15}N, ^{89}Y, or ^{129}Xe.[40,41] Once the sensitivity of the heteronuclear MRI/MR measurement is significantly improved, versatile studies with high spatial and especially temporal resolution can be performed, including of a number of potential targets, especially metabolites.[42–46]

A major practical drawback of hyperpolarized agents is the rapid loss of polarization and, therefore, their short half-life. The polarization method differs depending on the nucleus of choice, and the relaxation time of the nucleus also determines the rate of return to the equilibrium state. Although the existence of several active nuclei offers great versatility in terms of the structures and properties of bioresponsive agents as well as their interaction with desired targets, the use of hyperpolarized agents for this purpose is still challenging due to specific technical demands related to this method.

3. Practical Issues Beyond Chemistry

The preparation and successful testing in vitro by means of NMR and MRI may be a very encouraging phase in the development of bioresponsive MRI agents, but a number of challenges still remain on the road to their final utilization in vivo.

As already mentioned in Section 2, the sensitivity of NMR/MRI-based techniques is amongst the lowest of all imaging methods. Consequently, the applied concentrations of MRI contrast agents must be much higher than their analogues used in for example, PET, SPECT, or optical techniques. A further specific issue related to bioresponsive agents is the nature of the processes that underlie signal changes. It should be noted that, in the majority of cases, the changes in the MR signal cannot vary greatly (e.g. increase several-fold) as a result of the interaction of the bioresponsive agent with its target. This is simply a consequence of the physical phenomena related to the specific contrast mechanism, that is, T_1 or T_2 ^1H MRI agents—a change of the signal by a factor of two can be considered very large for these sensors. Comparison with, and expectations to achieve the same degree of signal changes as, in fluorescent sensors used in vivo, is wrong, because the physics of the signal origin and its changes is completely different.

Although very useful for common clinical MRI studies, the presence of water in tissue is at the same time the major hurdle for all bioresponsive ^1H MRI contrast agents (T_1/T_2/CEST). Water enables high-resolution anatomical images to be recorded, but is involved in several exchange phenomena and generates a large background signal. This greatly complicates the interpretation of results achieved using bioresponsive agents. In turn, the recorded MRI signal is the sum of the signals from the background and the contrast agent. However, differentiating between them is often impossible because the latter signal is dependent on the probe concentration and its efficacy under a certain set of conditions (r_1 or r_2 values at the "turn off/on" state). Similar problems also arise with other heteronuclear bioresponsive agents, the frequency of which remains the same, while only the signal intensity changes. In this case, the signal intensity changes upon the interaction of the agent with its target, and also proportionally with the concentration of the contrast agent. Therefore, control or quantification of its concentration is necessary during the analysis of results to make appropriate conclusions regarding the target's behavior and/or concentration.

There is one more practical issue related to bioresponsive agents which affects their ultimate in vivo application. Once prepared, novel agents usually undergo initial characterization, which aims to explore their potential to produce strong changes in the MRI signal related to the type of triggering event. Most commonly, this includes assessment of T_1 or T_2 relaxation times (i.e. r_1 or r_2 relaxivities for ^1H agents), the CEST effect, or frequency changes generated in simple buffered aqueous solutions in the presence of the target. However, these in vitro studies actually provide only preliminary information on the activity of a potential agent in vivo, and results obtained in this fashion are in most cases nontransferable to follow-up MRI experiments. The main factors that lead to the mismatch between in vitro and in vivo results are:

a) The determined amplitudes of the T_1/r_1, T_2/r_2, or the CEST effect values do not indicate the same level of MRI signal/contrast changes; indeed, they are usually higher than those finally obtained in MRI experiments.
b) The background signals are different in aqueous solution and tissue, that is, the diamagnetic contributions of water protons are different for T_1 or T_2 agents.
c) Complex environments may reduce signal changes or generate parallel signals that mask the signal generated by bioresponsive agents, such as formation of ternary complexes of Gd-based complexes with anions, various tissue-exchange phenomena for CEST agents, additional T_1 signals from blood in the case of dynamic, functional MRI studies.

d) Differences in the composition of investigated in vitro and in vivo systems (e.g. buffered medium versus tissue, respectively) lead to different (bio)distributions of the agent, thus causing concentration inhomogeneities and serious ambiguities in results because of the great varieties in local concentrations of the agent.

As a consequence, the results obtained in simple environments (buffered media) can, in the best case, provide a very good estimate of the potential behavior of the bioresponsive agent in vivo; however, no final conclusions can be drawn prior to the execution and evaluation of the actual in vivo experiments.

Finally, the main imaging procedures (pulse sequences, acquisition procedures, data analysis) are also strongly influenced in this specific context. In many cases, the standard imaging procedures used for common MRI contrast agents are not adjusted to the demands of bioresponsive agents and, therefore, cannot deliver the desired results. The existence of a number of contrast agents that use various contrast mechanisms, at different frequencies and with a wide range of relaxation times (from very short to very long), requires specific procedures, often tailored to the nature of the agent. As the temporal aspect is essential for studying biological processes with these agents, custom-made imaging procedures must be implemented to change and adjust the common MRI pulse sequences for acquisition on a rapid temporal scale. Additionally, they must assume elimination or filtering of the MRI effects not originating from the bioresponsive agent itself. Probably the best example is the functional MRI used in neuroimaging which relies on so-called blood oxygen level dependent (BOLD) signals originating from the paramagnetic iron present in the blood of living organisms.[47] The BOLD signal seriously interferes with the signal changes originating from any potentially active bioresponsive agent that operates at the ^{1}H frequency,[48,49] and the required fine adjustments of imaging acquisition parameters to avoid it can play a crucial role in determining the outcome.

4. The Way To Go

Whereas the previous sections summarized the major obstacles related to the in vivo use of bioresponsive MRI agents, this section will try to indicate the best strategies for improving the current situation and to point to directions which may result in valuable improvements.

It is clear that the development of a potent bioresponsive agent already begins with its careful design and preparation. Over the past decade, we have learned a great deal about the main parameters responsible for the activity of these agents and how these are correlated with the structure of the agent. Novel approaches to signal generation, such as paraSHIFT probes for ^{1}H MRI, are also enriching this field and may inspire further improvements.[50] However, developing the chemistry of these agents can certainly be focused on attempts to make the changes between the turn-off and turn-on state more pronounced, through stronger changes in the signal intensity or larger differences in the frequencies. As a consequence of the requirement to enhance both the MR signal and the changes at two different states, a reasonable solution for bioresponsive agents is the use of nanosized carriers, such as dendrimers, nanoparticles, or liposomes (Figure 4).[51–53] Primarily, they deliver a large payload of the active moieties and hence achieve a high local concentration in the tissue of interest. Furthermore, they may improve the biocompatibility of the original agents and overcome some of their initial drawbacks (cytotoxicity, fast diffusion, nonspecific accumulation). Finally, these carriers are suitable for additional combination and loading with different functional molecules, which can lead to further improvement of the bioresponsive nanosystem. To this end, targeting vectors can improve the delivery of the agent in the desired region, or additional functional molecules can serve as the source of a second, constant, and potentially quantifiable signal (e.g. ^{19}F MRI or PET/SPECT tracers). This can rule out the concentration-dependence of the signal produced by contrast agents (see Section 3), and allow unambiguous conclusions about the response and activity of the contrast agent. Substantial progress in the preparation of concentration-independent probes has been made recently,[54] and their incorporation into nanosystems can be very advantageous.

Methods to characterize and validate the potential of new bioresponsive agents for successful in vivo application also require considerable improvement. The current characterization procedure is mainly focused on standard physicochemical and biochemical methods, which include relaxometry (titrations with the targets, analysis of NMR dispersion profiles) or cell toxicity assays. However, this is insufficient, because there is a crucial requirement for experiments executed in complex environments, which would lead to a much better characterization of the agents, possibly already anticipating their in vivo potential. Specific tissue models with preserved functionality may substantially facilitate the validation of new agents ex vivo,[55] as well as the appropriate

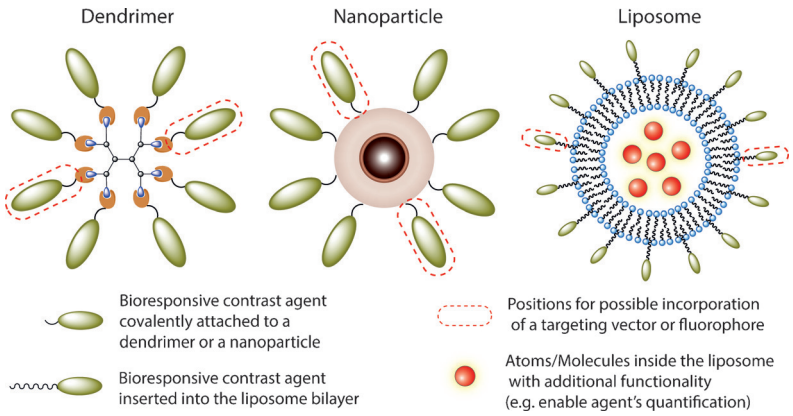

Figure 4. Bioresponsive and multifunctional contrast agents for MRI based on dendrimer, nanoparticle, or liposome carriers. The additional functionalities improve the in vivo performance of these nanosystems and can facilitate their utilization in functional MRI studies.

animal models.[56] Importantly, the selection of tissue or animal models and the appropriate ex vivo/in vivo experiments should be carefully performed to avoid any ambiguities in results. While some of the potential problems in this direction might be eliminated with the intelligent design of agents (see Section 2), the chosen model and experimental procedure must also ensure measurement of the analyte of interest to obtain results that clearly reflect its biological role.

On the other hand, the preparation and handling of these sophisticated in vitro, ex vivo, or in vivo animal models must become simpler and more accessible to a greater number of end users to achieve wider usage. Accordingly, recent advances in tissue engineering which create substitutes that mimic various types of tissues might be very useful.[57] If the number of such more-sophisticated, robust, and usable methods increases, they will become an indispensable tool for the preliminary, but very reliable, validation of the potency of bioresponsive probes prior to any in vivo studies.

Finally, the contribution of MRI physics and engineering can greatly improve the chance of achieving the desired application of bioresponsive agents. As a consequence of the specific needs of types of contrast agent, the target, or the system under investigation (cell or tissue cultures, animal models), technical support with the appropriate hardware (sensitive receiver coils for different frequencies, animal holders) is necessary for obtaining the best results. Without this, all the previous efforts related to the preparation of the agent could fail. Furthermore, the development and implementation of suitable MRI procedures tailored for specific purposes and for the nature of the bioresponsive agent is also essential. In particular, the imaging methods must account for the high signal specificity and achieve the highest possible contrast-to-noise ratio, but with the required accuracy and temporal resolution. The suppression of unwanted signals or their avoidance (e.g. the BOLD signal in functional MRI, see Section 3) should be of primary interest. For experiments with high temporal resolution (up to a few seconds), appropriate selection of sequences which allow shorter acquisition times should be made. Specialized data analysis techniques must be applied when performing the dynamic MRI recordings to unambiguously extract the signal originating from the bioresponsive agent. It is clear that data recorded in such a manner are complex and can only be analyzed by applying custom-made algorithms developed by experts with specific knowledge and experience.

Along these lines, it is clear that a high-quality interaction between scientists with diverse backgrounds is necessary to result in the successful characterization and utilization of bioresponsive agents. So far, this was an additional hurdle for many researchers, especially chemists, interested in this topic but lacking large instrumental facilities and specialized expertise. Therefore, laboratories with appropriate in-house facilities have mainly ventured into this direction, while a large number of researchers did not dare to proceed, despite being interested or excited in this research field. Nevertheless, easier availability of MRI scanners more recently and widely increased interest in following biological processes with functional MRI could dramatically change the current situation and encourage the interaction between research groups with different expertise for the preparation and in vivo utilization of bioresponsive agents.

5. Conclusions

The development of bioresponsive agents has entered a phase in which their wider utilization is expected to meet diverse biomedical research and clinical demands. This Minireview highlights the richness of approaches that can be used to address these demands, but also emphasizes a need

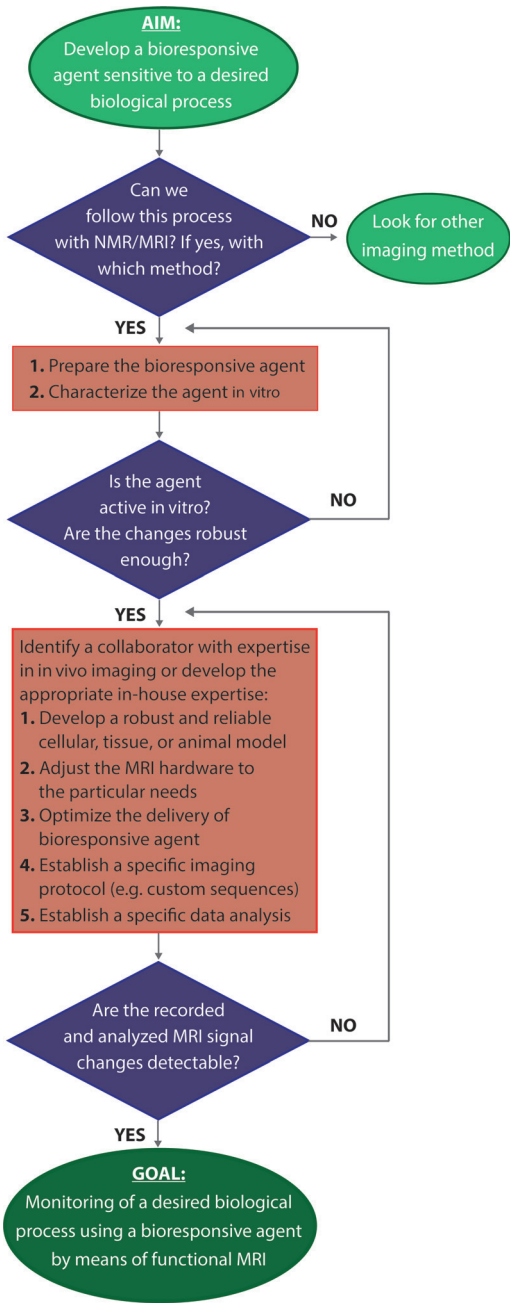

Figure 5. Not every bioresponsive contrast agent enables the monitoring of biological processes by means of MRI. As a consequence of the challenging and demanding nature of this research, serious planning is required prior to the initial work, while many iterative steps follow once the initial set of agents have been prepared and analyzed.

for every attempt during the development of novel agents to be carefully planned, with diverse inputs from science and technology. Therefore, although chemistry principally allows the design and preparation of bioresponsive agents to study any type of process, a number of aspects across the research disciplines must be considered prior to or during the practical study (Figure 5). As an example, an intelligent combination of approaches that merged protein engineering, physical chemistry, and neuroscience ultimately resulted in a valuable tool to map dopamine and study brain function.[7] It is clear that such attempts are highly multidisciplinary, requiring a deep understanding of different topics and effective communication between scientists and other experts involved. If these demands are met, the outcome of this unique research enterprise may result in unprecedented success, with great benefits for science and society.

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