Dynamic imaging with MRI contrast agents: quantitative considerations
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Received 2 December 2005; accepted 2 December 2005

Abstract

Time-resolved MRI has had enormous impact in cognitive science and may become a significant tool in basic biological research with the application of new molecular imaging agents. In this paper, we examine the temporal characteristics of MRI contrast agents that could be used in dynamic studies. We consider “smart” T\textsubscript{1} contrast agents, T\textsubscript{2} agents based on reversible aggregation of superparamagnetic nanoparticles and sensors that produce changes in saturation transfer effects (chemical exchange saturation transfer, CEST). We discuss response properties of several agents with reference to available experimental data, and we develop a new theoretical model that predicts the response rates and relaxivity changes of aggregation-based sensors. We also perform calculations to define the extent to which constraints on temporal resolution are imposed by the imaging methods themselves. Our analysis confirms that some small T\textsubscript{1} agents may be compatible with MRI temporal resolution on the order of 100 ms. Nanoparticle aggregation T\textsubscript{2} sensors are applicable at much lower concentrations, but are likely to respond on a single second or slower timescale. CEST agents work at high concentrations and temporal resolutions of 1–10 s, limited by a requirement for long presaturation periods in the MRI pulse sequence.

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Keywords: Contrast agent; Molecular imaging; Sensor; Temporal resolution; Superparamagnetic nanoparticle

1. Introduction

Many of life’s most important processes occur within a fraction of a second. With temporal and spatial resolution orders of magnitude cruder than optical imaging, MRI seems a deeply insufficient tool for investigation of this “short-timescale physiology.” But with opaque specimens, noninvasive imaging with MRI may still be a uniquely valuable approach. Echo planar pulse sequences allow images to be collected in tens of milliseconds — fast enough to freeze-frame the beating heart or record rapid fluctuations of blood flow in the brain \cite{1}. In many other contexts, the limitation on MRI has not been its intrinsic acquisition rate, but rather the absence of contrast mechanisms that can translate biological events of interest into MRI-detectable signal changes.

Newly developed contrast agents, products of the growing field of molecular imaging, are beginning to change this situation \cite{2,3}. Several groups have introduced agents useful for mapping static or slowly varying patterns of gene expression and epitope distribution \cite{4–7}. Contrast agents have also been developed for sensing more dynamic variables such as pH \cite{8–10}, oxygen tension (p\textsubscript{O\textsubscript{2}}) \cite{11,12}, ion and metabolite concentrations \cite{13–15}, and enzyme-catalyzed reactions \cite{5,16}. These agents have been referred to as “smart” contrast agents and function as sensors in MRI, analogous to fluorescent probes used in optical imaging and microscopy. Some MRI sensors could, in principle, be applied to study fast (generally cellular) physiological events, for example: endocytic pH changes during vesicle recycling on the 1-s timescale, calcium signaling transients lasting 10–100 ms, p\textsubscript{O\textsubscript{2}} fluctuations within 2 s of modulations to oxidative metabolism and changes in protein phosphorylation in seconds to minutes during neuronal synaptic plasticity. In each case, however, the practical time resolution attainable with contrast agent-enhanced MRI may be limited by the agent’s molecular properties and by the physical requirements of contrast generation.
Characteristics of contrast agents and methods for their detection vary widely. Historically, the most commonly used are small paramagnetic metal chelates which act by shortening T1 (longitudinal) relaxation times [17]. MRI sensors (Fig. 1) have been constructed from these agents by coupling determinants of their T1 relaxivity—solvent accessibility, rotational correlation time, or the spin and redox state of the metal—to the presence of specific molecular targets. Sensors based on T2 (transverse) relaxation rate changes have also been produced, most recently by coupling the aggregation state of superparamagnetic iron oxide nanoparticles (SPIOs) to physiological signals of interest. Aggregation of SPIOs even in concentrations as low as 10 mg/L can change observed T2s quite dramatically [18]. A third family of MRI sensors has been based on chemical exchange saturation transfer (CEST) effects [19]. Labile protons or water molecules bound to a CEST agent can be selectively saturated and will exchange with bulk solvent protons to decrease the local MRI signal. In CEST-based sensors, the exchange rate or chemical shift of bound protons is modulated by interaction with the sensor’s target [20,21].

To be useful for dynamic imaging studies, an MRI sensor must produce a fast and large enough signal change to be detected on the desired timescale. Although the steady-state detection thresholds for various contrast agents have been discussed extensively in the literature (e.g., Refs. [22–25]), the interaction between sensitivity and dynamics has been less thoroughly examined. At the same time, a systematic analysis of advantages and disadvantages of available contrast mechanisms is essential to the development of effective strategies for fast-timescale molecular imaging. In this paper, we review the molecular mechanisms of some paradigmatic smart T1-, T2- and CEST-based contrast agents, and consider the agents’ response rates using a combination of simulations and reference to existing experimental data. Throughout the paper, we estimate the temporal resolution of each molecular imaging strategy as the inverse of the duration of the impulse response that relates an underlying stimulus or change in conditions, via the contrast agent and MRI pulse sequence, to a detectable signal change. Because of the importance of the MRI acquisition method in determining this temporal resolution, we include a discussion of detection methods themselves along with consideration of the different classes of molecular imaging agents.

2. Materials and methods

2.1. Bloch equation simulations

Magnetization trajectories were estimated by using Euler integration of the Bloch equations to simulate one or two spin-pool evolution in the presence of relaxation and chemical exchange. Radiofrequency (RF) excitation pulses were modeled as instantaneous rotations, and Larmor precession was omitted from the calculations. Presaturation used in CEST simulations was modeled as a π pulse train delivered at a fixed frequency (generally 2 kHz) during a presaturation period TP. Parameter settings are noted where appropriate in the text. Time steps used in the calculations were chosen to be a factor of 10 below the smallest characteristic time relevant to the simulation. Macroscopic susceptibility effects (i.e., T2* relaxation components) were omitted. All simulations were performed using custom routines running under Matlab (Mathworks, Natick, MA, USA) on a Linux-based workstation.

2.2. SPIO aggregation simulations

SPIO aggregation and disaggregation were simulated using the Euler method, applied to the Smoluchowski equation [see Eq. (16) below], which describes the transition rates among single particles and aggregates of all allowable sizes. The aggregation kernel \( K_{agg} \), adapted from Odriozola et al. [26], describes the rate constant for binding of two aggregates of compositions \((i, j)\) and \((k, m)\) as a function of their diffusion-limited collision rate and their probability of sticking upon collision:

\[
K_{agg}(i,j,k,m) = k_d(i,j,k,m) \\
\times \frac{P_{agg}(i,j,k,m)[(i+j)(k+m)]^b}{1 + P_{agg}(i,j,k,m)[a(i+j)(k+m)]^b - 1} \quad (1)
\]
where ε and b correspond to Odriozola's constants \( N_{11} \) and \( b \), respectively, with respective values of 6.1 and 0.35. \( k_d(i, j, k, m) \) is the diffusion-limited collision rate due to Brownian motion, given by:

\[
k_d(i, j, k, m) = \frac{2k_B T}{3\eta} \left[ \left( \frac{i+j}{2} \right)^{1/\alpha} + \left( \frac{k+m}{2} \right)^{1/\alpha} \right] \left[ \left( \frac{i+j}{2} \right)^{-1/\alpha} + \left( \frac{k+m}{2} \right)^{-1/\alpha} \right]
\]

(2)

where \( k_B \) is the Boltzmann constant, \( T \) is the absolute temperature, \( \eta \) is the medium viscosity and \( df \) is the fractal dimension of the aggregates (assumed to be constant throughout aggregation). \( P_{\text{stick}}(i, j, k, m) \) is the sticking probability of the two aggregates, given by:

\[
P_{\text{stick}}(i, j, k, m) = P_{11} \left[ N_i \left( \frac{i}{i+j} \right) N_j \left( \frac{m}{m+k} \right) + N_j \left( \frac{k}{k+m} \right) N_i \left( \frac{j}{i+j} \right) \right]
\]

(3)

where \( N_x \) for \( x = i, j, k, m \) represents the fraction of functional groups of the corresponding type available for binding, e.g.:

\[
N_i = \frac{(f - 1)i - j + 1}{fj}
\]

(4)

where \( f \) is the number of functional groups per particle and \( (i, j) \) is the aggregate composition. \( P_{11} \) is the sticking probability for a collision of two oppositely functionalized monomers, which we estimated by assuming cubical geometry for the two types of functional groups, with sides of length \( d_a \) and \( d_b \), and calculating the fraction of the particle surface that is covered by the functional group (for given functionalization ratios \( f \) and \( g \) and particle radius \( r \)). The product of these surface fractions is then multiplied by the bimolecular sticking probability \( P_{\text{bin}} \) for free functional groups in solution, which may be roughly estimated as the ratio of their known binding on-rate \( k_{\text{on}} \) and their theoretical diffusion-limited collision rate. We include an additional factor of 6 to account for an assumption that the cubic functional groups are optimally oriented on the particle surface. The resulting expression is:

\[
P_{11} = P_{\text{bin}} \left( \frac{6d_a^2}{4\pi r^2} \right) \left( \frac{6d_b^2}{4\pi r^2} \right)
\]

(5)

The fragmentation kernel \( K_{\text{frag}} \), adapted from Laurenzi and Diamond [27], describes the rate constant of fragmentation of an aggregate of composition \((i+k, j+m)\) into two aggregates \((i, j)\) and \((k, m)\):

\[
K_{\text{frag}}(i, j, k, m) = k_{\text{off}} \left[ A_t(i, j) A_g(k, m) + A_t(k, m) A_g(i, j) \right] \times \frac{N_{\text{on}}(i, j) N_{\text{on}}(k, m)}{N_{\text{on}}(i + k, j + m)}
\]

(6)

Here, \( k_{\text{off}} \) is the bimolecular dissociation rate for the complimentary functional groups. \( A_t \) and \( A_g \) are the number of free functional groups of the two types in aggregates of a given composition, i.e.:

\[
A_t(i, j) = (f - 1)i - j + 1; \quad A_g(i, j) = (g - 1)j - i + 1
\]

(7)

\( N_{\text{iso}} \) is a combinatorial factor describing the number of possible arrangements of particles in an aggregate of a given composition:

\[
N_{\text{iso}}(i, j) = \frac{f^i g^j (f - i)! (g - j)!}{[A_t(i, j)] [A_g(i, j)] i! j!}
\]

(8)

In order to minimize the computational cost of our simulation, we imposed an upper limit on the allowed aggregate size, \( C(z, z) \), with \( z = 45 \). This limit allowed the kernels to be predefined, greatly speeding up the simulations. Under simulation conditions that favor extensive aggregation, this limit causes an underestimation of average particle size and an accumulation, or “bunching”, of the aggregate population at sizes near the maximum. However, the parameters used in most of our runs produced aggregates with average sizes well below this upper limit and resulting size distributions that did not exhibit significant bunching. When noticeable bunching did occur, it produced only a minimal effect on simulation time courses (below 5% for \( T_{\text{obs}} \)), with “saturating” effects on steady-state size evident only when this size approached 2z.

2.2.1. Additional calculations and data presentation

Additional computations, including calculations of percent signal change for \( T_1 \) and CEST imaging, were performed using Matlab. Kaleidagraph (Synergy Software, Reading, PA, USA) and Adobe Illustrator (Adobe Systems, San Jose, CA, USA) were used in the preparation of figures.

3. Results and discussion

3.1. Dynamic imaging with smart \( T_1 \) contrast agents

3.1.1. Temporal resolution of \( T_1 \)-weighted imaging

Several of the first MRI sensors were \( T_1 \) agents synthesized by Meade and colleagues, which respond to their targets with roughly twofold changes in \( T_1 \) relaxivity [5]. Changes in \( T_1 \) induced by these agents are usually detected using gradient-recalled echo (GRE), spin-echo (SE) or inversion-recovery (IR) imaging pulse sequences [28]. The \( T_1 \) observed in the presence of a smart \( T_1 \) contrast agent is given by the approximation, generally valid for low agent concentrations ([M]):

\[
\frac{1}{T_1(i)} = \frac{1}{T_{10}} + R_1(t)[M]
\]

(9)

where \( T_{10} \) is the background \( T_1 \) in the absence of contrast agent and \( R_1(t) \) is the time-dependent relaxivity of the
is dependent on the previous acquisition, and the new equilibrium signal is 1.0 s TR. As with Ernst angle GRE imaging, the signal at each acquisition increase in signal after T1 decreases is slower. In the bottom panel, an Ernst angle excitation. Although the overall signal level is higher, the place sharply after the change in T1. The middle panel shows the effect of contrast agent; R1(t) could also be rewritten as R10 + ΔR1(t), where R10 is the “resting” relaxivity and ΔR1(t) is the temporally varying component of relaxivity, typically linked to a phenomenon of interest.

The sampling rate of a time varying T1-weighted signal is determined by 1/TR, where TR is the repetition time of the pulse sequence; entire three-dimensional volumes may be acquired with each repetition if echo planar readout (EPI) is used. The contrast-to-noise ratio (CNR) of a GRE or SE imaging method designed to detect changes in T1 is maximized when TR equals <T1(t)>, the average value of T1 over time, and when Ernst angle RF excitation is used [in a GRE pulse sequence, this means that the flip angle $\pi$ would be set equal to $\cos^{-1}(\exp(-TR/T1))$; in contrast, the temporal resolution of a T1-weighting scheme is maximized when the RF flip angle is set to $\pi/2$ — only under this condition is the signal observed at each time point independent of the T1 observed in the previous acquisition. For the same reason, GRE or SE imaging with $\pi/2$ excitation achieves better temporal resolution than IR imaging, despite the advantages of IR for measurements of absolute T1. Fig. 2 demonstrates these effects in simulated T1-weighted signals acquired with $\pi/2$ and Ernst angle GRE, and with standard IR pulse sequences. Practical consequences of the sensitivity of dynamic T1-weighted imaging to flip angle are that SNR sacrifices may be required to optimize temporal resolution, particularly for short TR values, and that spatially varying RF excitation (e.g., produced by a surface coil) is likely to produce inhomogeneous temporal dependence.

With T1-weighted GRE or SE acquisition (echo time $\ll$ T2, TR) and ideal $\pi/2$ pulse excitation, the relative signal ($I_n$) at the end of the $n$th acquisition period is given by:

$$L \propto 1 - \exp\left\{-\int_{t_{n-1}}^{t_n+TR} \frac{1}{T1(t)} dt\right\}$$

(10)

where $t_{n-1}$ and $t_n (= t_{n-1} + TR)$ are the RF excitation times preceding the ($n-1$)th and $n$th acquisitions, respectively. The recorded T1-weighted MRI signal therefore reflects the history of T1 between each RF excitation and the next, so contributions from instantaneous events cannot be recognized with temporal resolution better than that defined by TR. Assuming ideal flip angles are used, the time resolution of a T1-weighted molecular imaging experiment will therefore be limited by the smallest TR consistent acceptable MRI contrast changes given the amount of T1 change produced by the contrast agent and the noise level of the scanner.

3.1.2. Response rates of T1-based MRI sensors

T1 contrast agents are typically organic complexes containing a paramagnetic metal ion such as gadolinium (Gd), which strongly promote the relaxation of the water protons due to the influence of unpaired electron spins of the metal ion. Factors that are important in determining the relaxivity of a contrast agent (reviewed in Refs. [17,29]) are contained in the Solomon–Bloembergen–Morgan (SBM) equations; these include timescales for various molecular motions, structural features of the contrast agent and spin properties of the paramagnetic metal ion. Some of these properties can be manipulated in such a way as to produce a change in relaxivity coupled to specific events. Among them are the coordination number of the water molecules, $q$; the lifetime of the water molecule in the complex, $\tau_m$; and the rotational correlation time of the whole complex, $\tau_R$. If one of these parameters differs significantly between two “states” or conformations of a contrast agent, the intensity of the signal observed with MRI is also changed. The timescale on which the change occurs is strongly dependent on the type of the contrast agent and on which property has been affected.

The best explored class of T1 contrast agents relies on the change in solvent exposure to the paramagnetic metal ion (Fig. 1A) [30]. The resting state of the contrast agent usually has relatively low relaxivity, with limited water access to the metal ion. The contrast agent is switched into a state of higher relaxivity by a biochemical trigger, such as the activity of an enzyme or a spike in concentration of an intracellular messenger. Several agents that work by this type of mechanism have been reported in the literature. Louie et al. [5] synthesized a caged contrast agent called EgadMe, which is a substrate for the enzyme...
\(\beta\)-galactosidase. The activity of \(\beta\)-galactosidase uncages the agent by cleaving a galactopyranosyl group, exposing a Gd coordination site to raise the agent’s effective \(q\) from 0.65 to 1.02; this structural change in turn gives rise to an increase in T1 relaxivity from 0.903 to 2.72 mM (measured at 11.7 T). Like many enzymes, \(\beta\)-galactosidase obeys so-called Michaelis–Menten kinetics, meaning that the rate of product formation depends on parameters \(k_{cat}\) and \(K_m\) in the following equation:

\[
V = \frac{k_{cat} [E][S]}{K_m + [S]}
\]

where \([E]\) is the total enzyme concentration, \([S]\) is the substrate (caged contrast agent) concentration and \(V\) is the rate of uncaged product formation in moles per second. The cleavage of EgadMe takes place with a \(k_{cat}\) of \(2.4 \times 10^{-3}\) nmol U\(^{-1}\) s\(^{-1}\) and a \(K_m\) of 0.0182 mM; this means for example that a 100-\(\mu\)M concentration of the agent would require on the order of several minutes to be cleaved by 100 U/ml (~1 \(\mu\)M) of \(\beta\)-galactosidase. Enzymes most likely to be useful for activating contrast agents are those that cleave carbon–carbon, carbon–nitrogen or carbon–oxygen bonds; these typically have a \(k_{cat}\) significantly smaller than 1 s\(^{-1}\) and are therefore likely to process their substrates over timescales much longer than the TR of a typical T1-weighted imaging sequence. In these cases, the temporal resolution of the method would be limited by the enzyme activity level, although with large enough enzyme and substrate concentrations the onset of enzyme activity might be recognized with TR-limited precision.

Faster relaxivity changes are likely to be produced by ion-sensing MRI contrast agents. Sensors for calcium and for zinc have been produced [13,14]; as with EgadMe, they respond to their targets with conformational changes that expose a bound gadolinium atom to water. T1 relaxivity changes from 3.26 to 5.76 mM\(^{-1}\) s\(^{-1}\) (11.7 T), and from 4 to 6 mM\(^{-1}\) s\(^{-1}\) (7 T), for the calcium and zinc sensors, respectively. No kinetic data are available for either of these contrast agents, but because their mechanisms are somewhat similar to small fluorescent ion sensors, the response rates of these sensors may be relevant. In particular, the calcium-sensitive agent synthesized by Li et al. [13] has similar affinity and calcium-liganding groups to Fura-2 and related BAPTA-based “fast” calcium sensors. These dyes have measured \(k_{on}\) for calcium on the order of \(10^9\) \(M^{-1}\) s\(^{-1}\) [31]. Assuming an MRI calcium sensor had an equivalent \(k_{on}\) and a \(K_d\) of 1 \(\mu\)M, and if it was used at a concentration of 100 \(\mu\)M to detect (buffered) calcium concentration fluctuations between 0.1 and 2 \(\mu\)M, its overall response rate would be limited by dissociation and would likely be close to a millisecond. This rate is two orders of magnitude below practical TR values. Time resolution in imaging experiments using MRI ion sensors would therefore be determined by the repetition rate of the imaging procedure.

Another method shown to enhance the relaxivity of a T1 contrast agent is to increase its rotational correlation time, \(\tau_R\). For small molecules, \(\tau_R\) dominates the total correlation time (\(\tau_C\)), a parameter that enters into the SBM equations and strongly influences the magnetic field dependence of T1 relaxivity. Since

\[
\tau_R = 4\pi a^3 \eta/3k_B T
\]

where \(k_B\) is the Boltzmann constant; changes in \(\tau_R\) could be effected either by manipulating the viscosity (\(\eta\)) or temperature (\(T\)) of the system, or by changing the effective radius (\(a\)) of the contrast agent. MRI contrast changes due to changes in the \(\tau_R\) of a T1 agent are sometimes dramatic, and sensors involving changes in \(\tau_R\) (Fig. 1B) may be particularly valuable for dynamic imaging at today’s most common clinical scanner field strengths (\(\leq 3\) T).

De Leon-Rodriguez et al. [32] followed these principles and designed a paramagnetic peptide (Gd\(^{3+}\)-G80BP) that senses its protein target (Gal80) by binding to it; a related, but enzyme-dependent approach was taken in a study by Aime et al. [11]. In the Gal80/Gd\(^{3+}\)-G80BP case, association produces a roughly 25-fold increase in \(\tau_R\), corresponding to the ratio of Gal80 complex to free peptide molecular sizes (here assuming equal density). At 0.5 T, the binding reaction produced a T1 relaxivity change from 8.3 to 44.8 mM\(^{-1}\) s\(^{-1}\). Protein–protein association rates tend to be on the order of \(10^6\) M\(^{-1}\) s\(^{-1}\) [33,34], meaning that a high-affinity binding reaction initiated by 10 \(\mu\)M concentrations of dissociated species might approach its equilibrium with a characteristic time on the order of 0.1–1 s. Dissociation rates for this type of interaction vary widely, but will be slower for complexes with \(K_d \ll 10^{-5}\). Because these timescales are on the order of typical T1-weighted MRI TR values, sensors based on protein binding-induced changes in \(\tau_R\) may often, but not always, respond with rates that are limiting for the temporal resolution of a dynamic MRI experiment.

3.2. Dynamic imaging with SPIO aggregation-based T2 agents

3.2.1. Temporal resolution of T2-weighted imaging

An endogenous T2 contrast agent (deoxyhemoglobin) and an exogenous blood-pool contrast agent (mononuclear SPIO or MION) have been heavily used in dynamic functional brain imaging experiments (fMRI), where contrast and temporal resolution are governed by hemodynamic effects [35,36]. Smart iron oxide agents have also been introduced and applied in cell labeling, cell tracking and molecular sensing [18,37]. Although they are physically large (10–100 nm), an advantage of SPIO agents is that they have high relaxivities (10–100 mM\(^{-1}\) Fe s\(^{-1}\)) and are detectable at very low particle concentrations — several orders of magnitude below concentrations required for most T1 agents [38,39]. SPIO agents also produce magnetic susceptibility variations that may be visualized even at fairly low spatial resolution by T2*-weighted imaging.
T2-weighted imaging is usually performed using SE pulse sequences, sometimes in conjunction with Carr–Purcell–Meiboom–Gill (CPMG) readout [28]. GRE sequences can be used to produce related T2*-weighted images. Contrast is established during the echo time (TE) of these sequences or across multiple echo times with CPMG. Assuming T1 and TR are static or that TR > T1, the relative T2-weighted signal in a single-echo (GRE) sequence is given by:

\[
S_1 = \frac{1}{T_2(1)} \exp \left(-\frac{1}{T_2(1)} \int_{t_0}^{1+\text{TE}} dt \right) - \frac{1}{T_2(2)} \exp \left(-\frac{1}{T_2(2)} \int_{t_0}^{1+\text{TE}} dt \right)
\]

where T2 is affected by the T2 contrast agent’s relaxivity (R2) according to:

\[
\frac{1}{T_2(t)} = \frac{1}{T_2(0)} + [R_2(t) + \Delta R_2(t)] |M|
\]

where T20 is the background T2, and R20 and \(\Delta R_2(t)\) are the static and time-dependent components of T2 relaxivity, respectively. Because echo times are usually short (10–100 ms), and necessarily less than TR, the temporal “precision” of T2-weighted imaging can be considered to be higher than that of T1-weighted imaging. On the other hand, unless CPMG or phase-locking strategies are used, the image sampling rate is still limited by TR, and very short T2 changes may be missed entirely with probability (1–TE/TR), even if they would be likely to produce a signal change when sampled optimally. A clear advantage of scan series data obtained with T2-weighted imaging sequences, however, is that their time dependence does not depend on the RF flip angle.

### 3.2.2. SPIO aggregation sensors

Several types of T2 relaxation-promoting smart contrast agents have been produced. Of these, agents based on the aggregation of biocompatible SPIO nanoparticles demonstrate the largest relaxivity changes and appear to be easily tailored to a variety of applications [18]. Super-paramagnetic sensors are formed by functionalizing the surfaces of SPIOs with molecules (usually biomolecules, i.e., proteins or nucleic acids) that associate with complementary groups on other SPIOs, either when activated in some way or when bridged by a particular target molecule of interest. Because the particles are multivalent, this binding or bridging induces formation of aggregates, somewhat similar to the process of immunoprecipitation. Aggregation can dramatically amplify the transverse relaxivity of SPIOs, significantly reducing the T2 relaxivity even of very dilute SPIO suspensions (<10 mg/L Fe). Perez et al. [16,40,41] pioneered this approach and have applied it to make MRI sensors for specific oligonucleotides, protease and nuclease activity, and protein–protein binding, including antibody/epitope recognition. Our group has adapted this approach to construct an SPIO bioconjugate sensor for calcium [42].

The strong T2 effects produced by SPIO sensors make these contrast agents potentially attractive tools for dynamic MRI studies in vivo. Little is currently known, however, about the response dynamics of aggregation-based sensors. Most of the existing SPIO sensors appear to respond to their targets on a several-minute timescale, although some enzyme-dependent variants are slower. It is currently unknown to what extent design characteristics of the sensors affect rates of aggregation and concomitant relaxivity changes. Given the relative absence of experimental data related to biofunctionalized nanoparticle aggregation kinetics, we here explore theoretical determinants of SPIO aggregation dynamics in some detail through computational modeling, with the goal of identifying key parameters affecting the time courses and extents of aggregate formation. In addition, we apply a rudimentary approach to approximating changes in T2 relaxivity produced by SPIO aggregates; this allows us to convert calculated aggregation times into estimated MRI signal changes.

#### 3.2.3. Model of SPIO aggregation behavior

To model physiologically induced SPIO aggregation behavior, we considered the following reaction scheme:

\[
A + B \xrightleftharpoons{\text{activation}} A^* + B^* \xrightleftharpoons{\text{aggregation}} \sum_i C(i,j)A_i^*B_j^*
\]

where A and B denote two types of bioconjugated SPIO nanoparticles with different functional groups. Under resting or baseline conditions, the groups on A and B particles have minimal binding affinity for one another. When one or both of the two types of functional groups are activated (converting A to A* and B to B*), their binding affinity increases, initiating the process of particle aggregation. The end-product of the aggregation is a distribution of clusters [denoted by the summation ratio on the right-hand side of Eq. (15)] with compositions A_i^*B_j^* and concentrations C(i, j), where i is the number of A-type particles and j is the number of B-type particles in a given aggregate of total size i+j. Disaggregation can be induced by deactivating the functional groups, causing dissociation at the molecular level; here we assume that dissociation need not precede deactivation. For simplicity, we also assume that the activation and deactivation steps happen much faster than aggregation and disaggregation. In many cases, this is a reasonable approximation, given the relatively slow timescales for aggregate (dis)assembly (1–1000 s) compared to the timescales of potential (de)activation events (e.g., milliseconds for Ca^{2+}-induced protein conformational changes). Some triggering events, like enzymatic proteolysis, might be much slower, however, in which case the fast-activation condition would be violated and the model we develop would have to be modified.

Aggregation phenomena are commonly formulated using the Smoluchowski equation [43], a general differential equation describing the formation and fragmentation of various size aggregates. The Smoluchowski model assumes...
that every transition in the system involves either the breakup of an aggregate into two smaller species or the agglomeration of two aggregates into a larger cluster:

$$\frac{d[C(u,v)]}{dt} = \frac{1}{N_0} \sum_{i=0}^{u} \sum_{j=0}^{v} K_{\text{agg}}(i,j,u-i,v-j)C(i,j)C(u-i,v-j)$$

$$- \frac{1}{N_0} \sum_{i=0}^{u} \sum_{j=0}^{v} K_{\text{frag}}(i,j,u-i,v-j)C(u,v)$$

$$- \frac{i}{N_0} \sum_{j=0}^{v} K_{\text{agg}}(i,j,u,v)C(i,j)C(u,v)$$

$$+ \frac{i}{N_0} \sum_{j=0}^{v} K_{\text{frag}}(i,j,u,v)C(u+i,v+j)$$

Here, $C(u,v)$ is the concentration of aggregates that contain $u$ particles of type A and $v$ particles of type B. The coefficients $K_{\text{agg}}$ and $K_{\text{frag}}$ are “kernels” for aggregation and fragmentation, respectively — they function like rate constants and reflect the probability that aggregates of size $(u,v)$ will be formed by or broken into clusters of all other allowed sizes. The first term of Eq. (16) is the instantaneous rate at which aggregates of composition $(u,v)$ are formed through the binding of all possible pairs of smaller aggregates. Similarly, the second term accounts for the breakup of $(u,v)$ clusters into all possible smaller fragment pairs. The third term is the rate at which aggregates of composition $(u,v)$ bind to other clusters. The final term is the rate at which $(u,v)$ clusters break off from larger aggregates of size $(u+i,v+j)$. $K_{\text{agg}}$ and $K_{\text{frag}}$ were adapted from the literature [26,27] in order to reflect the scenario in Eq. (15). As is common in other models, our approach assumed that the aggregates are fractal (irregular at all length scales) and noncircular (any two particles are connected through only one chain). These assumptions are valid for rigid particles with small (protein-sized) functional groups tightly anchored to the particle surface. See Materials and Methods for further details.

In order to simulate the aggregation of SPIOs, we used Euler integration to obtain mass-action solutions to Eq. (16). Fig. 3A shows representative aggregation and disaggregation time courses produced by our simulations. We depict both the mass-averaged mean particle radius $<r>$ (relative to an individual particle) and the mass-averaged mean aggregate size $<n>$ as functions of time. The corresponding steady-state distribution (limit at infinite time) of aggregate sizes and compositions is shown in Fig. 3B. These time courses and distributions are consistent with results from previous Monte Carlo simulations and experiments involving general reversible aggregation phenomena [27,44]. To facilitate the comparison of time courses for varying sets of parameters, we defined an “apparent” aggregation time constant $T_{\text{obs}}$ as the value for which $<n>(T_{\text{obs}})-<n>(0)$
equals \((1-1/e)[<n>(\infty)-<n>(0)]\), where \(<n>(\infty)\) is the steady-state value of \(<n>\) (as \(t\) goes to infinity), for an aggregation process initiated at \(t=0\).

Our simulations investigated the dependence of \(T_{\text{obs}}\) and \(<n>(\infty)\) on the total nanoparticle concentration, the number of functional groups per particle and the concentration ratio of complementary particles. The results, shown in Fig. 3C, demonstrate a generally nonlinear dependence of \(T_{\text{obs}}\) and \(<n>(\infty)\) on these parameters. As concentration increases, both the rate of approach to equilibrium and the steady-state size rise in a supralinear fashion (panel C-i), as would be expected for a multibody process. For increasing number of functional groups per particle, \(T_{\text{obs}}\) approximates an inverse exponential dependence, while \(<n>(\infty)\) increases roughly linearly (panel C-ii). As shown in panel C-iii, the maximal aggregation rate and size are found when the concentrations of complementary particles are equal. Overall, these results suggest that the kinetics of aggregation are highly sensitive to the particulars of their design and deployment, with aggregation times varying over three orders of magnitude within a feasible concentration and functionalization range. At the higher end of this range, theoretically achievable aggregation times are as short as several seconds.

As shown in the sample time courses of Fig. 3A (gray regions), aggregation upon the removal of the activating physiological stimulus can occur on timescales considerably faster than those seen for aggregation. However, the rate of disaggregation can vary widely depending on the specific deactivation mechanism. For example, given an allosteric mechanism where activation and deactivation of particle functional group(s) can occur in both bound and unbound states, deactivation can be expected to increase dramatically the bimolecular dissociation rate \(k_{\text{off}}\) (in addition to reducing \(k_{\text{on}}\), leading to fast disaggregation. By contrast, if the functional group(s) can only be deactivated in the unbound state, \(k_{\text{off}}\) remains unchanged for bound particles, resulting in a much slower disaggregation process. In general, at low nanoparticle concentrations, we expect disaggregation to occur faster than aggregation, making aggregation the limiting factor in the reversible response rate of an aggregation-based sensor.

It should also be noted that the rates of aggregation and disaggregation in biological tissues (e.g., cell cytoplasms) may be significantly slower than the rate observed in solution [45]. Experiments have shown that molecular crowding in the cellular milieu reduces the effective diffusivity of solutes in a size-dependent manner, decreasing it by roughly a factor of 4 for most biological molecules and by a factor of 40 or more for particles with diameters above a 50- to 80-nm threshold. Single SPIOs in the 30-nm size range should fall below this threshold, but aggregates larger than a certain size will be above it. Since under many conditions monomers are the single most common species in the aggregation reaction, it may be possible to roughly approximate the slowing of aggregation in the cellular environment by dividing the Brownian diffusivity constant by around a factor of 4 (here we assumed that diffusion of each aggregate was determined by the Stokes–Einstein equation only).

3.2.4. SPIO aggregation-induced changes in relaxivity

The model developed above makes predictions about the dependence of SPIO aggregation rate on various parameters of MRI sensors; aggregation time constants are likely to vary from several seconds to many minutes, depending on concentration, functionalization, monomer ratio and, in biological environments, size. But how is the aggregation rate of these particles likely to translate into MRI-observable signal changes? Although strong changes in T2 relaxivity upon aggregation of SPIOs have been reported, little work has been done to explain the mechanism of aggregation-induced contrast change. Previous theoretical analyses, Monte Carlo simulations and experimental work have shown that the T2 relaxivity of iron oxide nanoparticles is well described by the motional averaging approximation when mean Larmor frequency shift at the particle surface \((\Delta\omega)\) and the time constant for water diffusion around the particle \((\tau_d)\) satisfy the condition \(\tau_d\Delta\omega<1\) [46,47]. This condition is fulfilled for uncoated iron oxide crystals smaller than approximately 30 nm. For coated SPIOs such as MION-46 [48], which contains a crystalline core of around 5 nm diameter surrounded by an organic shell of roughly 10 nm thickness, this maximum size for motional averaging is relaxed to around 350 nm, due to a lower field strength at the particles’ water-accessible outer surfaces.

In the motional averaging regime, outer sphere theory predicts the component of T2 relaxation due to a suspension of field-perturbing SPIO particles to be given by:

\[
1/T2_{\text{SPIO}} = (4/9)V(\Delta\omega)^2\tau_d
\]  

(17)

where \(V\) is the volume fraction of solution occupied by the particles, \(\tau_d\) is defined as \(\tau_d=r^2/D\), where \(r\) is the particle radius and \(D\) is the self-diffusion coefficient of water, and \(\Delta\omega\) is given by \(\Delta\omega=(1/3)\mu_0\gamma M\), where \(M\) is the particle magnetization, \(\mu_0\) is the free space magnetic permeability and \(\gamma\) is the proton gyromagnetic ratio.

For aggregate sizes below the motional averaging cutoff, Eq. (17) and the definition of \(\tau_d\) predict an increase in relaxivity proportional to the square of aggregate radius. For fractal aggregates, the relationship between the number of particles in the aggregate \((n)\) and its radius of gyration \((r)\) is:

\[
r \propto n^{1/df}
\]

(18)

where \(df\) is the aggregate’s fractal dimension. Typically, \(df\) is between 1.75 and 2.3 in fractal aggregates [49,50], with reaction-limited aggregation (see above) resulting in larger \(df\).

In addition, Eq. (17) and the definition of \(\Delta\omega\) predict a quadratic dependence of relaxivity on particle magnetization, \(M\). At field strengths above 1 T, SPIO magnetic moments are known to be saturated [48]. Under these
Fig. 4. Relaxivity changes during SPIO aggregation and disaggregation. (A) The temporal evolution of percent MRI signal change (top) and ratio of net T2 relaxivity, relative to unaggregated monomer solution (bottom), corresponding to the aggregation time course shown in Fig. 3A. (B) Apparent signal change time constant T_{obs} (filled circles), time to reach a 5% signal change T_{5%} (filled squares) and the steady-state percent signal decrease (hollow diamonds), as a function of initial monomer concentration.

conditions, M is field-independent and roughly proportional to \( r \) of a given aggregate:

\[
M \propto \rho \frac{n}{r^3} \propto n^{n-3} \propto n^{1/3} \tag{19}
\]

Because \( V \) and \( D \) are independent of aggregation state, we can combine Eqs. (17)–(19) to obtain a set of proportionality:

\[
1/T_{2SPIO} \propto \rho^{2/3} \propto n^{2/3} \propto n^{1/3} \tag{20}
\]

Since at (typical) low SPIO volume fractions, 1/T2_{SPIO} is approximately equal to R2 times the particle concentration [cf. Eq. (14)], Eq. (20) predicts an increase in T2 relaxivity with increasing aggregate size when the fractal dimension is greater than 2. In the limiting case where aggregates are composed of fully packed spherical particles (df=3) the proportionality reduces to an \( r^2 \) dependence.

We simulated fractal aggregates with df=2.2, which is on the high end of the reaction-limited aggregation regime. This choice of df is somewhat arbitrary, and one would expect the fractal dimension to vary considerably depending on the details of particle geometry and functionalization. Although variation of df within the typical range would have a large effect on predicted relaxivity, our simulations demonstrated that it had a negligible effect on kinetics (data not shown). For aggregates with df=2.2, relation (20) predicts a relaxation proportional to \( n^{2/11} \). In order to characterize the relaxivity of a given population of aggregates, we therefore calculated the mass average of \( n^{2/11} \) and scaled it by the known single-particle relaxivity.

Fig. 4A shows the temporal evolution of relative relaxivity (normalized by the unaggregated particle relaxivity) and corresponding percent change in MRI signal intensity during the SPIO aggregation and disaggregation time courses shown in Fig. 3A. Signal intensity was calculated by assuming a baseline T2 for the medium of 100 ms and setting the TE to the T2 observed in the presence of nonaggregated SPIOs. Fig. 4B shows the predicted apparent time constant (T_{obs}, defined as above) of the change in MRI signal intensity, along with the maximal steady-state signal change, as a function of particle concentration. These results suggest that single-second temporal resolution may be possible for aggregation sensors with particle concentrations in the 20- to 100-nM range, but that lower SPIO concentrations will lead to considerably slower response rates. From a practical standpoint, it is also useful to know how long it takes the aggregation-induced signal change to reach a minimal detection threshold. We defined T_{5%} to be the time it takes to obtain a 5% change in signal, during aggregation of initially fully disaggregated particles. Fig. 4B shows that at high concentrations, T_{5%} is predicted to be of order 0.1 s, but that at lower concentrations, where 5% is close to the maximal signal change, T_{5%} is close to 100 s and actually longer than T_{obs}.

3.3. Time-resolved imaging with CEST agents

3.3.1. Temporal resolution of CEST-based imaging

The CEST effect [19] has recently been explored as a basis for MRI contrast agents because of its potential independence of paramagnetic metal complexes and possible advantages in sensitivity compared with some T1 contrast agents. CEST contrast enhancement is achieved by selectively saturating the proton magnetization of a small pool of labile protons associated with a CEST agent; these protons exchange with bulk solvent, producing a decrease in the bulk magnetization that gives rise to the detected MRI signal. CEST techniques have been applied to produce contrast changes in vitro, using a variety of agents including paramagnetic lanthanide complexes (PARACEST agents) [51]. CEST contrast changes have also been created in vivo using saturation of amide protons of endogenous proteins or exogenous species [52,53]. With CEST-based sensors, the efficiency of saturation transfer from the agent depends on select features of the agent’s chemical environment, giving rise to modulations in detected MRI intensity that could, in principle, be followed in a dynamic imaging experiment.

In CEST MRI, saturation transfer to longitudinal bulk magnetization takes place during a presaturation period TP, after which RF excitation is delivered and any CEST-related changes in bulk magnetization are measured with a standard readout module (GRE, SE, EPI, etc.). Usually, TP is set to
be almost as long as TR, in order to maximize CEST contrast for the given repetition rate. Observed CEST-dependent magnetization is influenced by the inherent spin-lattice relaxation rate of bulk water protons (1/T1w), by the time constant (τm) for chemical exchange between CEST and bulk proton pools during the presaturation time, and by the efficiency and dynamics of the saturation itself. With a smart CEST contrast agent, τm and the saturation efficiency could depend on the configuration of the agent and would become time-dependent variables.

Analytical expressions for CEST-dependent MRI signal changes can be obtained under various simplifying assumptions [21,24]. A reasonable assumption is that the magnetization of the CEST pool reaches a steady state very rapidly during saturation (i.e., much faster than TP). A further approximation is that RF irradiation of the CEST pool does not directly saturate the bulk proton pool — this is true when the RF offset (Δω) between the bulk water frequency (ω1) and CEST pool frequency satisfies: Δω/ω1 >> 1. If these two conditions are met, and the steady-state partially saturated magnetization of the CEST proton pool is given by (1 – x)M0s (with M0s being the equilibrium magnetization of the saturated CEST pool and x the fractional saturation of this pool), the following differential equation describes the evolution of bulk proton magnetization during TP:

\[
\frac{dM_{\text{cw}}(t)}{dt} = \left( \frac{M_{\text{0a}} - M_{\text{cw}}}{T1w} \right) - \frac{x M_{\text{cw}}}{\tau w}
\]

\[
\tau w = 111 \tau m / c, \text{ where } c \text{ is the concentration of exchange sites on the CEST agent and given a bulk proton concentration of 111 M. Assuming perfect RF flip angles (see } T1 \text{ discussion above) and ideal CEST weighting (TE<T2<TR-TP), a formula for relative signal observed in a serial imaging experiment can be obtained by integrating Eq. (21) over TP, with the initial condition that bulk magnetization is transverse (M_{zw}=0) before saturation begins:

\[
I \propto \frac{\tau w}{\tau w + xT1w} - \frac{\tau w}{\tau w} \exp \left( - \frac{\tau w + xT1w}{\tau w} TP \right)
\]

If a CEST-based MRI sensor was being used, terms in Eq. (22) would be replaced with integrals over TP, similar to Eqs. (10) and (13) (omitted here for notational simplicity). Because CEST contrast develops during the entire saturation period, the temporal resolution of a CEST experiment cannot exceed 1/TP. In analogy to the role of TR in T1-weighted molecular imaging, the requirement that TP be long enough to obtain sufficient CNR therefore sets a practical limit on the repetition time for MRI time series using CEST-based sensors.

Fig. 5 shows a contour plot of ideal CEST signal changes as a function of TP and τm, assuming a CEST agent is “turned on and off” with each image acquisition. The plot was calculated using Eq. (22) and overestimates signal change for cases where the RF offset (Δω) between the bulk water frequency (ω1) and the saturated pool fails to satisfy Δω/ω1 >> 1, when Δω and the proton exchange time fail to obey τmΔω >> 1, or when x << 1 (as often expected under practical saturation powers). For any given noise level, the signal changes reported in the figure suggest realistic limitations on scan rate. The well-known dependence of the CEST effect on τm translates into faster possible time resolution for agents where saturation transfer changes occur around smaller τm values. The plot extends to τm’s less than 10 μs, which have been reported for exchanging sites on some lanthanide complexes; at field strengths below 10 T, however, application of these complexes is likely to be compromised by the requirements on τmΔω. As with T1-weighted imaging, the use of nonideal flip angles could degrade the temporal resolution below the CNR-dependent limit, by compromising the independence of each MRI acquisition. Temporal resolution could also be degraded if the CEST pool magnetization were not maintained near its steady-state value at all times, but we found no evidence of this in magnetization evolution simulations covering a broad range of conditions.

3.3.2. Response rates of CEST-based sensors

CEST agents have been used as MRI sensors for pH, lactate and glucose [9,10,15,20,53]. In all cases, the response rates of the sensors are likely to be extremely fast compared with the demands of the imaging method itself. CEST-based pH sensing is simplest to appreciate, given the well-known dependence of proton exchange rates on pH. Amide protons of endogenous proteins, and of two synthetic lanthanide complexes, have been used for pH-sensitive CEST [9,52,53]. Amide τm ranges from roughly 10^{-3} s^{-1} at pH 3 to 10^{3} s^{-1} above pH 9 [54], creating a change in
saturation transfer efficiency. The response rates to pH are likely to be extremely fast, although they have not been measured directly on the contrast agents. Rate constants for proton association reactions in general are thought to be on the order of $10^{10}$ to $10^{11}$ M$^{-1}$ s$^{-1}$ [55]. Base-catalyzed exchange is most common in the physiological pH range (pH 5–8) [52]; under base catalysis, the association and dissociation rate constants for glycine amide proton exchange (similar to the CEST pH sensor DOTAM-Gly) are roughly $1.4 \times 10^{11}$ and $8 \times 10^{5}$ s$^{-1}$, respectively [55]. Given these rates, it is likely that the response of CEST agents to pH changes takes place within a few microseconds. This timescale is negligible compared with the duration of presaturation pulses used to measure pH changes from agent concentrations even over 10 mM.

A related mechanism underlies low affinity glucose detection by a complex synthesized by Zhang et al. [20]. The unliganded form of the sensor functions efficiently as a CEST agent, due to exchange of water molecules at a free europium coordination site. In the presence of glucose ($K_d=2.6$ mM), the exchange site is partially blocked (cf. Fig. 1A), leading to a noticeable reduction in CEST efficiency when saturation is delivered at a ~28.5 ppm. When lactate is added at relatively high concentrations (the dissociation constant is over 100 μM), the degenerate resonance is resolved into discrete peaks in a lower field frequency range, ~14 to ~20 ppm. If saturation is selectively directed either at the free MBDO3AM amide peaks, or at the complexed-form amides, a change in CEST saturation efficiency results from binding of the lactate to the contrast agent. With neither the glucose nor the lactate sensor being a kinetic parameter describing the metabolite/sensor binding interaction known, but by analogy with other bimolecular binding pairs, the association rates are likely to be well over $10^5$ M$^{-1}$ s$^{-1}$, implying that the dissociation rates are at least 10 s$^{-1}$. Even given these conservative estimates, the kinetics of intermolecular interactions that underlie CEST-based lactate and glucose sensing are still significantly faster than the timescale used to build up CEST contrast, which was on the order of several seconds in these studies.

3.4. Comparison of dynamic molecular MRI methods

Fig. 6 presents a comparison of temporal resolutions for dynamic imaging approaches using smart T1 contrast agents, SPIO aggregation-based T2 sensors and CEST agents. For each of the three groups of molecular imaging agents we considered, a colored area denotes approximate conditions under which acceptable signal changes may be achieved, assuming realistic MRI acquisition conditions. This representation is a guideline only; it is by necessity highly reductionist, because the full range of relaxivities and interconnected trade-offs between CNR, repetition rate, spatial resolution, partial volume effects and signal averaging are impossible to address simultaneously in graphical form. Roughly speaking, we assume that a 5% signal change is sufficient for detection of an agent’s response in a time-resolved experiment. This requirement is lower than the standard generally required in contrast agent-enhanced anatomical (static) imaging, but reflects the important possibility, specific to dynamic imaging, of performing statistically rigorous within-subject comparisons to recognize small image intensity fluctuations. In human fMRI, the dominant form of time-resolved MRI today, signal changes lower than 1% are often robustly detected at the single-voxel level.

The “fast” T1 agent area in Fig. 6 (saturated red) was estimated assuming GRE imaging with $\pi/2$ excitation pulses, and a sensor with relaxivity changes on the millisecond or faster timescale, from 5 to 10 mM$^{-1}$ s$^{-1}$. The region is bounded at the right by concentration levels at which toxicity and T2 effects may interfere with applications; at low concentrations, signal changes are likely to be below the detection limit. In principle, TRs close to 10 ms could be used to obtain even higher temporal resolution than shown, but this would result in severe attenuation of the overall signal and under typical imaging conditions would be impractical. Very short TRs would also be incompatible with EPI volume acquisition. The “slow” T1 area in light red refers to T1 agents like the Gal80-binding agent and the β-galactosidase substrate discussed above, for which the molecular rate of response is the limiting factor on potential temporal resolution. The relaxivity changes produced by these sensors are on the same order of
magnitude as the “fast” agents, but signal averaging becomes a possibility when time resolution is no longer limiting, so it is likely that somewhat lower concentrations could be used.

The aggregation-based SPIO agents are represented by the green-shaded area. For these agents, the temporal resolution in T2-weighted imaging is also limited by the sensors’ rates of response, rather than by the imaging itself. The region plotted in Fig. 6 is estimated based on the theoretical analysis we have performed here; our modeling was consistent with data from the literature, but covered a wider variety of conditions than has yet been experimentally explored. The response times of aggregation-based sensors are predicted to vary approximately linearly from about 100 s at 1 nM SPIO concentration to 1 s at 0.1 μM, for particles with ~50 attached proteins; if the functionalization level is lowered, the rates would be decreased. At SPIO concentrations into the micromolar range, the aggregation effect would be yet faster, but concentrations significantly above 0.1 μM (10 mg/L Fe, assuming 2000 iron atoms per particle) would begin to attenuate the MRI signal too severely and would be inconsistent with physical echo times.

The blue area in Fig. 6 denotes conditions under which dynamic MRI with smart CEST agents could be performed. As our earlier discussion emphasized, the temporal resolutions and concentrations compatible with use of existing CEST-based sensors are completely constrained by the physical requirements of performing imaging with acceptable signal changes. To estimate the region in Fig. 6, we performed pulse sequence and magnetization simulations for a 300-μs τ_m CEST agent, under a range of repetition rates and concentrations, with TP–TR. Signal changes between exchanging (“on”) and nonexchanging (“off”) conditions were greater than 5% for concentrations above 1 mM and for 1- to 10-s saturation times. If agents with lower τ_m were used at very high field, it is likely that lower saturation times or concentrations could be used.

A conclusion from this comparison is that the fastest MRI molecular imaging temporal resolution (~100 ms) is likely to be obtained with T1 agents, while the lowest concentrations (and greatest signal changes) may be reached with SPIO aggregation sensors. Several caveats apply: First, most of our arguments have relied on simulations or calculations performed under idealizing assumptions; some of the findings (especially the aggregation simulations) await empirical validation and may incompletely account for realistic experimental conditions. Second, the discussion has focused on time dependence largely to the exclusion of other factors related to the contrast agents. In an actual dynamic MRI experiment, some agents with inferior “theoretical” time resolution may in fact be preferable for unrelated reasons. Third and last, we have not attempted a comprehensive discussion of all existing or putative smart contrast agents and mechanisms — the specific agents we have addressed were chosen because they are representative or of particular interest.

The guide we present in Fig. 6, and the more detailed analyses of the previous sections, gives an overview of considerations important to dynamic molecular imaging experiments. Although few such experiments have been reported to date, time-resolved molecular imaging of biologically significant targets (particularly in neuroscience and developmental biology) is clearly an urgent direction in MRI methods development. Quantitative description of contrast agent properties will help direct both the selection of viable experimental strategies and the design of new smart contrast agents with improved characteristics. Our discussion suggests that high CNR is the most important requirement for optimizing temporal resolution with T1 agents, where the sensitivity of the MRI methods currently limits acquisition rates. The development of T2 SPIO agents with enhanced kinetic properties is also clearly recommended, given the low concentrations at which these agents can be used; improved SPIO-based sensors could be produced by maximizing nanoparticle functionalization levels, or by loosely tethering particles to one another in order to speed aggregation.

Acknowledgments

The authors gratefully acknowledge comments from G. Odriozola and P. Gillis about the proposed models for nanoparticle aggregation and SPIO relaxivity changes, respectively. David Cory is thanked for helpful remarks on the manuscript. MGS was supported by a Hertz Fellowship and a Soros Fellowship, HF was supported by award DAMD17-03-1-0413 from the Department of the Army, and GGW and AJ were supported by a grant from the Raymond and Beverley Sackler Foundation.

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