Magnetic resonance for \textit{in vitro} medical diagnostics: superparamagnetic nanoparticle-based magnetic relaxation switches

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Abstract. Advances in magnetic resonance (MR) miniaturization, along with nanoparticles and biotechnology, are extending MR applications in diagnostics to beyond the medical imaging regime. The principles behind magnetic resonance switch (MRSw) biosensors, as well as a summary of rapidly developing fields including MR miniaturization and MRSw demonstrations, are presented here. Due to the range of applications of MRSw biosensor tests and the breakthroughs in downsized instruments, continued development will enable the deployment of MRSw biosensors in a wide variety of settings and with potentially unlimited targets.

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1. Introduction

Nuclear magnetic resonance (NMR) spectroscopy and magnetic resonance imaging (MRI) have been used since their inception for non-destructive sample evaluation and characterization. Accordingly, medical applications have been a major focus since the conception of magnetic resonance (MR), primarily due to its usefulness for the non-invasive investigation of patient health.

Most medical employment of MR has taken place in the conventional NMR/MRI laboratory, where large, superconducting magnets produce high magnetic fields of remarkable uniformity to provide a wide variety of clinically relevant information via sophisticated signal acquisition sequences. Innovations in MR have enabled new applications outside of the conventional high-field NMR/MRI laboratory. Examples include the following: MR imaging and relaxation measurements obtained using unilateral and portable sensors [1]–[7]; spectral resolution measurements on single-sided, low-field systems obtained using ex situ methodologies and hardware shimming [8]–[12]; high-resolution spectra obtained using hand-portable magnets and microcoils [9], [13]–[16]; and open MRI systems used for the imaging of animals, plants and human limbs and joints [17]–[20]. Although many of these innovations have occurred over the past two decades, non-conventional MRI systems for animal imaging have been commercially available for longer [21]–[28]. For in-depth information on these breakthroughs, see the above cited articles and reviews.

Recently, portable MR technology has been combined with superparamagnetic nanoparticles to produce a new approach for in vitro diagnostics. This new technology, termed magnetic relaxation switch (MRSw) technology, created a new class of MR diagnostic sensors that have the potential to provide sensitive, selective, immuno- and molecular-diagnostics in point of care settings.

MRSw assays use polymer-coated superparamagnetic iron oxide nanoparticles, which are also used as MRI contrast agents and for biochemical separations. In MRSw assays, the presence of a target substance, or an analyte, causes the particles to transition between dispersed and clustered states [25] and affect the $T_2$ relaxation times of surrounding water molecules. Because of the non-optical nature of MR, the measurement of MRSw can take place directly in the biological specimen of interest. Analyte sensitivity and selectivity can be achieved by functionalizing the nanoparticles with a binding agent, such as an antibody or oligonucleotides. In the literature to date, primarily benchtop relaxometers and high-field MRI scanners have been used for MRSw measurements of a wide variety of analytes and specimen types. These include viruses [26], bacterial cells [27], proteins [28], nucleic acids [28] and small molecules [29] in solutions of buffer, blood [27], serum, plasma, urine and crude cell lysate [28]. Sensitivities as low as picomolar concentrations for proteins or single virus particles have been demonstrated. Due to the broad range of possible target analytes and the capability to obtain measurements without sample preparation, MRSw technology has a remarkable potential to impact several fields of applications, from medical diagnostics, environmental sensing and homeland security.

This paper provides a mini-review and introduction to this emerging field, including a brief overview of relevant nanomaterials, an introduction to the underlying physics of MRSw, a summary of the literature and a description of portable and miniaturized MR instrumentation.
Figure 1. Superparamagnetic versus ferromagnetic particles in (a) the absence or (b) the presence of an external magnetic field. The superparamagnetic nanoparticles are single domain ferro- or ferrimagnets. The magnetic moment of superparamagnetic particles in the absence of an external field is time averaged to zero, similarly to the net magnetic moment of a superparamagnetic particle assembly.

2. Background

2.1. Superparamagnetic particles and $T_2$ relaxation

MRSw biosensing is based on the properties of superparamagnetic particles. Superparamagnetism is a form of magnetism observed in small ferro- and ferrimagnetic nanomaterials that are composed of single-domain magnetic crystals. Due to their small size, there is no long-range order in these materials and they exhibit novel magnetic properties (figure 1). One of these novel properties is that at low applied fields the materials saturate with a relatively high magnetization. When held below the material’s Curie temperature, the magnetic moments inside a magnetic domain align parallel to an applied field to produce the high magnetization. Another novel property is that after an applied field has been removed there is no net remanent magnetization. The lack of remanent magnetization is due to the fact that the magnetization of each superparamagnetic domain readily jumps between two stable orientations that are separated by a small energy difference. In fact, the energy difference is so small that thermal energy cancels the total particle magnetization. Hence, superparamagnetic materials act as paramagnets; however, their magnetic susceptibility and magnetization in low to moderate external fields are much larger [30]. This is an important property that allows superparamagnetic particles to function such that they dominate the $T_2$ relaxation of samples in which they are suspended, eliminating the background effects of a large amount of paramagnetic material (such as heme in blood).
Iron, cobalt, and nickel compounds and their alloys, rare earth elements such as gadolinium and certain intermetallics such as gold and vanadium are ferromagnets that can be used to produce superparamagnetic particles when the magnetic domain size is on the nanoscale. Other materials can be used including ferrites composed of iron oxides or containing other elements such as aluminum, copper, cobalt, nickel, manganese, magnesium, iron and zinc. A general formula for these ferrites is \( \text{MO} \cdot \text{Fe}_2\text{O}_3 \). Additionally, hexagonal ferrites and magnetic garnets, which are cubic insulators composed of iron, can be used. Magnetite (\( \text{Fe}_3\text{O}_4 \)) with cubic crystalline structure is a commonly used ferrite due to its favorable magnetic moment.

The majority of published MRSw work has historically focused on superparamagnetic iron oxide (SPIO) nanoparticles. SPIO nanoparticles contain single to several superparamagnetic iron oxide (magnetite) cores suspended in a non-magnetic matrix. Superparamagnetic particle cores consist of either a single or an ensemble of superparamagnetic grains; in the presence of a magnetic field they are magnetized in the direction of the external field, because on average more grains will align their magnetization with the external field. Once the external field is removed, the magnetic moments randomize, so that the net magnetization of the ensemble returns to zero. This loss of magnetic order is termed the Néel relaxation, and can be described by the equation

\[
\tau_{\text{Néel}} = \tau_0 e^{K V / k_b T},
\]

where \( K \) is the anisotropy constant of the material, \( V \) the particle volume, \( k_b T \) the thermal energy (\( k_b \) is the Boltzmann constant and \( T \) is the temperature in kelvins) and \( \tau_0 \) the attempt time, which is characteristic of a material. There is no dependence on \( B_0 \) for \( \tau_{\text{Néel}} \). Because the transition time depends on the diameter of the grain, a critical diameter number exists for superparamagnetism. For magnetite, the diameter is 21 nm, set by the fact that the relaxation time should be less than 1 s [30]. If the ensemble is composed of monodisperse superparamagnetic grains that all have the same relaxation times, the relaxation of the ensemble is described by the same equation. Particles comprised of a polydisperse ensemble of grains have a relaxation time that consists of a superposition of exponential decays of each grain size. Note that the Néel relaxation is in the absence of a magnetic field and describes timescales determined by the magnetic anisotropy energy of the material. The magnetic anisotropy energy is a measure of the difficulty of overcoming the energy barrier for changing the magnetization orientation from the original easy axis. See [35], where a nice summary of superparamagnetic particle properties is provided, for additional information on superparamagnetism.

SPIOs are typically categorized based on their overall diameter, ranging from 2 nm to micrometers. Their overall diameter encompasses their metal core(s), any polymeric scaffold and organic surface coating to endow water solubility [49]. SPIO particles between 300 nm and 3.5 \( \mu \)m have been called oral-SPIO because they were first used for in vivo imaging via oral delivery, such as the silane-coated contrast agent Gastromor, also known as ferumoxsil [41]. Like most particles larger than 50 nm, oral-SPIOs contain multiple ferromagnetic grains in a non-magnetic matrix. Over a matter of minutes, a solution of oral-SPIO particles can settle due to their large size [50]. Settling complicates their use for MRSw; however, surface treatments have been used to provide adequate buoyancy to the particles such that they do not settle, and timed mixing steps with rapid measurements have been used to ensure reproducible suspension of the particles during measurement [50, 51].

Standard SPIO (SSPIO) nanoparticles have hydrated diameters of 60–150 nm. Like oral-SPIO, these particles contain more than one iron core per particle. A solution of these particles does not settle, but under certain conditions these particles can aggregate when placed in a

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magnetic field [18]. This property is used for magnetic separations by SSPIO such as those manufactured by companies like Milteny Biotech and Invitrogen. Similar field-dependent aggregation will be observed for larger particles [53]–[55] and can be used for sensitivity enhancement of MRSw biosensors [50]. However, utilization of these particles for MRSw biosensors must be accompanied by proper controls to ensure that the observed change in signal is due to specific binding and not field-induced clustering.

Particles of single-crystal core or hydrodynamic diameter less than ~50 nm are known as ultrasmall SPIO (USSPIO), some of which have been developed to produce the in vivo contrast agents Feridex and Combidex, also known as ferumoxide and ferumoxtran [41, 42], [50]–[52]. Although their density is higher than the density of water, their size is small enough that Brownian motion keeps them suspended in solution [51, 52]. These particles do not separate from the solution in the presence of a magnetic field gradient [50, 51, 56, 57], but rather the suspended particle solution behaves like a ferrofluid [50].

A variety of SPIO particle sizes and particle materials have been used for MRSw assays, ranging from tens of nanometers to micron sizes. MRSw technology was pioneered using a variant of monocryllalline iron oxide nanoparticles (MION), single-crystal USPIO particles [24, 30] known as cross-linked iron oxide (CLIO) nanoparticles, named after their cross-linked dextran polysaccharide coating [25]. Like MION, CLIO nanoparticles have iron oxide cores between 3 and 5 nm in size with a cubic close-packed structure of \((\text{Fe}_2\text{O}_3)_n(\text{Fe}_2\text{O}_4)_m\). Efforts to achieve optimal particle properties, such as increased magnetic moment, are ongoing [39, 40].

Many different methods have been used for synthesizing superparamagnetic nanoparticles [32]–[40]; several groups have worked on nanoparticle characterization and detailed reports exist in the literature [38], [41]–[48]. See the above citations for a detailed review of these methods.

In designing MRSw biosensors, it is important to understand the relaxation mechanisms of the superparamagnetic particles. Due to their extensive use as contrast agents for MR imaging, the theory underlying both transverse and longitudinal relaxation mechanisms has been well developed for superparamagnetic particles. It has been shown that relaxation mechanisms depend on the characteristics of the particles, including whether they are ferro-, ferri- or antiferromagnetic [31]. The theoretical foundation for how SPIO nanoparticles affect the measured \(T_2\) relaxation rates began about two decades ago [58]. This theoretical work was based on early experimental observations that the solvent longitudinal and transverse relaxivities (defined as \(R_1 = 1/T_1\) and \(R_2 = 1/T_2\), respectively) were a function of particle size [50]. Subsequent theoretical work on magnetic particles demonstrated that Monte Carlo simulations of a distribution of magnetic particles in a matrix of hydrogen nuclei could accurately reproduce the dependence of \(R_2\) on the size of iron oxide micro- and nanoparticles [58, 59]. Simulations and experimental data showed that \(R_2\) and \(R_2^*\) (where \(R_2^* = 1/T_2^*\)) increased with particle diameter until about 100 nm; then \(R_2\) decreased with increasing particle size and \(R_2^*\) reached a plateau (figure 2). Further experimental work and computer simulations explored the dependence of \(R_2\) on the concentration of dissolved iron, magnetic susceptibility and temperature [59]. This early work laid the foundation for the development of a set of analytical models that accurately reproduced the dependence of transverse relaxivity on particle size, magnetization, iron concentration, temperature and inter-echo delay [60]–[63].

\(^2\) \(T_2^*\) is a relaxation constant that in the case of a perfectly homogeneous external field is equal to \(T_2\). However \(T_2^*\) is typically dominated by field inhomogeneities in portable systems. In these systems \(T_2^*\) is related to \(T_2\) by the following equation: \(R_2^* = R_2 + \Delta\phi\), where \(\Delta\phi\) is dephasing due to field inhomogeneities.

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Figure 2. Dependence of transverse relaxivity ($R_2$) on nanoparticle diameter as evaluated from equations found in [65]–[67]. (a) In the motional averaging regime (red line) the $R_2(1/T_2)$ measured by a CPMG sequence increases as particle size increases because the refocusing pulses are ineffective in counteracting the dephasing effects of the nanoparticles. As the system transitions to the visit-limited regime (blue), the refocusing pulses begin to become effective and the $R_2$ decreases as particle size increases. For homogeneous magnetic fields, the $R_2^*$ in the motional averaging regime matches the $R_2$ and the $R_2^*$ reaches a constant value in the visit-limited regime, as calculated in Brooks [65]. When the $R_2^*$ is less than the $R_2$ of either the motional averaging regime or the visit-limited regime, the system is in the static dephasing regime. (b) The same red and blue curves plotted in terms of $T_2$ and diameter, on a linear scale. In this figure, the black dashed line represents the $T_2^*$ measured in a non-uniform magnetic field where $T_2^*$ is always lower than $T_2$ and does not reflect the particle size. The data points are the same as well. This format is more intuitive to the MRSw assay designer.

As there are several theoretical regimes for nanoparticle-induced relaxation, it is important to understand the limits of the regimes and the characteristics of the system of interest to ensure the proper application of the analytical models. Superparamagnetic nanoparticles are typically divided into categories of strongly magnetized and weakly magnetized particles, based on the relative magnitude of the precession frequency difference between nuclei at the surface of the nanoparticle and nuclei distant from the nanoparticle, $\Delta \omega$, and the inter-echo delay of the CPMG detection sequence, $\tau_{CP}$. $\Delta \omega$ is essentially a relative measure of the effect of the dipolar magnetic field generated by a superparamagnetic particle on the resonant frequency of hydrogen nuclei in adjacent water molecules. When the product $\Delta \omega \tau_{CP} > 1$, the particles are classified as strongly magnetized, and when $\Delta \omega \tau_{CP} < 1$, the particles are classified as weakly magnetized. For typical relaxometers, $\tau_{CP}$ is no shorter than tens of microseconds, so $\Delta \omega$ must be less
than $10^5$ for the particles to be within the weakly magnetized regime. Most superparamagnetic nanoparticles used for MRSw assays have a surface dephasing $\Delta \omega$ of approximately $1 \times 10^7$; therefore they are classified as strongly magnetized. This means that the inter-echo delay is always longer than the amount of dephasing that occurs at the surface of a particle.

Another characteristic of superparamagnetic nanoparticle solutions that is used to differentiate physical behavior is the diffusion time, or travel time, of water ($\tau_D$) relative to the inter-echo time of the pulse sequence, $\tau_{CP}$. Nanoparticle solutions are in the long echo limit when the $\tau_D$ is significantly less than $\tau_{CP}$. $\tau_D$ can be determined from the relationship

$$\tau_D = \frac{R^2}{D},$$

where $\tau_D$ is the time it takes for a water molecule to diffuse the distance of the radius of a nanoparticle, $R$, and $D$ is the diffusion constant of water, $10^{-9}$ m$^2$ s$^{-1}$. $\tau_D$ can be thought of as the time it takes for a water molecule to pass a hemisphere of a nanoparticle, or a flyby time. When $\tau_D$ is much larger than $\tau_{CP}$, the nanoparticle system is within the ‘short echo limit’. Typical CPMG sequences have echo times of the order of hundreds of microseconds to several milliseconds. Therefore, the ‘short echo limit’ cannot be approached unless the nanoparticle diameter approaches 1000 nm. The most common MRSw biosensors are within the ‘long echo limit’ because the length of the inter-echo delays ($\tau_{CP} > 0.25$ ms) is longer than the time it takes for a water molecule to diffuse past the hemisphere of a nanoparticle (0.2–100 $\mu$s).

As the particle size of a solution of superparamagnetic particles at fixed iron concentration is increased, there is an initial increase in $R_2$, then a plateau and later a decrease (figure 2(a)). The regime on the left-hand side of the curve has been termed the motional averaging regime, the regime in the middle has been termed the static dephasing regime, and the regime on the right has been termed the visit-limited or slow motion regime [57]. The boundaries between the motional averaging and visit-limited regimes can be determined by generating plots such as those shown in figure 2(a) or they can be determined by the relationship between $\Delta \omega$ and $\tau_D$. If $\Delta \omega \tau_D < 1$ then the system is in the motional averaging regime; if $\Delta \omega \tau_D > 1$ then the system is in the visit-limited regime. As the diameter of the particles increases in the motional averaging regime, the refocusing echoes in the CPMG pulse sequence cannot efficiently refocus the magnetization that has been dephased by the nanoparticles, hence the increase in $R_2$ (or decrease in $T_2$). In other words, the refocusing pulses cannot compensate for increased dephasing by larger nanoparticles. The flat region of the static dephasing regime is due to $R_2$ being limited by $R_2^*$. The decrease of $R_2$ with increasing diameter in the visit-limited regime results in the refocusing pulses being able to refocus the dephasing caused by the nanoparticles. Also apparent in figure 2 is that the $R_2$ in the slow motion regime exhibits a dependence on the inter-echo delay of the spin-echo sequence [58].

In a homogeneous magnetic field, one can determine which regime applies to a sample by comparing $R_2$ to $R_2^*$; the two values are identical in the motional averaging or static dephasing regime and they are different in the visit-limited regime [62, 63]. This approach has been employed for determining the physical characteristics of MRSw biosensor systems [68]–[70]. However, in the cases of inhomogeneous fields, such as those present on benchtop and portable MR devices, $T_2^*$ is dominated by the field gradient. In fact, the measured $T_2^*$ value is not indicative of the particle or particle cluster size state (figure 2(b)).

The analytical models that explain the dependence of $R_2$ on particle size are instructive in understanding the $T_2$ sensitivity to the particle clustering of MRSw assays. In these models, the concentration of iron was held constant, while $R_2$ was monitored as a function of nanoparticle size.
diameter, which is analogous to what occurs in an MRSw assay. Additionally, these models were extended to explain the behavior of a system of clustering superparamagnetic particles [67]. In this study, the experimental system consisted of superparamagnetic nanoparticles that clustered due to a change in the pH of the solution. After an initial phase that was attributed to the stabilization of dispersed particles, $R_2$ increased with agglomeration until a plateau was reached prior to a decrease in $R_2$ with agglomeration. The shape of the $R_2$ response as particles agglomerated generally matched the expected trend for the increase in average nanoparticle size, which was similar to the shape of the dashed line in figure 2. Additionally, Roch et al [71] showed general quantitative agreement between the measured and expected $R_2$ values. Similar exercises have since been carried out by subsequent authors to validate the qualitative nature of the $T_2$ response they were observing and determine which regime their nanoparticle assays fell within [51, 64, 66]. The similarity between the $R_2$ of particle agglomerates and that of spherical nanoparticles suggests that one can equate nanoparticle aggregates and spherical shapes. Even though this assumption may seem to be in contradiction with the fractal nature of nanoparticle agglomerates, the shape of the nanoparticle aggregates observed by the MR measurement is determined by the ensemble of diffusing water molecules in solution, which can be approximated by the radius of hydration measured by light scattering [66]. The lack of dependence on shape was confirmed by a recent study [68].

Work by Jasanoff’s group at MIT demonstrated the extension of the original outer sphere theory to nanoparticle aggregates by outlining the relationship between the parameters of the outer sphere theory and the fractal nature of nanoparticle aggregates [64]. That work was extended by researchers at Massachusetts General Hospital (MGH) to show that nanoparticle cluster size was inversely related to the $T_2$ of clusters in the motional averaging regime and linearly related to the $T_2$ of clusters in the visit-limited regime [39, 51, 66], validating the application of the outer sphere theory to MRSw. Additionally, because the fractal dimension of nanoparticle clusters is approximately 2, the number of nanoparticles in an aggregate has been shown to be linearly related to the measured $T_2$ value for particles and clusters in the visit-limited regime. These observations indicate that the application of the outer-sphere theory can provide useful insights, on at least a semi-quantitative level, into the understanding and designing of MRSw biosensors. More recent work by Jasanoff’s group has confirmed that from a theoretical view, compact clusters induce equivalent $T_2$ relaxation as single particles of similar size. Additionally, their Monte Carlo simulations showed that there is a dependence on the shape of clusters comprising particles with diameters of $\sim 20$ nm but little dependence on the shape of clusters comprising larger particles with diameters of $\sim 200$ nm. In fact, for the larger particles, there was little difference between the $T_2$ relaxation of isotropic clusters and linear rods of particles. This has favorable implications for MRSw biosensor design in that the cluster geometry does not need to be controlled to provide reproducible and precise measurements [68].

The analytical models for $R_2$ can be applied to magnetic relaxation biosensors to aid in the design of biosensor assays. Conveniently, these models accurately predict the dependence of $R_2$ on parameters that a biosensor designer can control—iron concentration, temperature, magnetic susceptibility and particle size [59]. Additionally, these analytical models allow for predictive modeling of the dependence of $T_2$ relaxation on these parameters. Results are not entirely quantitative, but the general trends and response curves predicted by these models can be instructive.

Perhaps the most useful analytical model for the conditions of most magnetic relaxation biosensor assays reported to date is the chemical exchange model for strongly magnetized
Figure 3. Description of how the different models for nanoparticle agglomeration map onto the \( T_2 \) versus diameter curves. (a) For the monodisperse model the \( T_2 \) will follow the curve as analyte is added because the average diameter will cover all intermediate diameters between the initial and final states. (b) For the polydisperse model the \( T_2 \) will transition between the two points on this curve. The response curve will be linear with regard to analyte addition because particles transition between state 1 and state 2. The slope of the response curve is directly proportional to the sensitivity of the assay.

spheres developed by Brooks \[65\]

\[
1/T_2 = \frac{(4/9)V\tau_D(\Delta\omega_r)^2}{1 + (4/9)^2(\tau_D/\tau_{CP})^2\alpha^3},
\]

(3)

\[
\alpha = \left[ \frac{\Delta\omega\tau_{CP}}{a + b\Delta\omega\tau_{CP}V} \right]^{1/3},
\]

(4)

where \( 1/T_2 \) is the transverse relaxivity, \( V \) the volume fraction of iron in solution, \( \tau_D \) the diffusion, or flyby time, \( \Delta\omega_r \) the frequency shift at the surface of a particle relative to bulk solution, \( \tau_{CP} \) one half of the inter-echo delay in a CPMG sequence and \( a \) and \( b \) are derived constants (\( a = 1.34 \) and \( b = 0.99 \)) \[61\]. Equations (3) and (4) can be used to generate a curve that describes the dependence of \( R_2 \) on particle sizes, as shown by the red and blue lines in figure 2. The black line in figure 2(a) comes from a formula for the static dephasing regime. This theory is not fully discussed here because the static dephasing equation applies only when the \( R_2^* \), which is dictated by the homogeneity magnetic field, is smaller than \( R_2 \), which is not the case for portable MRSw detectors.

A simple modification of equation (3) can be used to generate a plot that is more intuitive to an assay developer. This plot is in terms of \( T_2 \) and particle diameter with linear units rather than logarithmic units (figure 3). As discussed above, magnetic relaxation biosensor assays function due to a transition between dispersed and clustered states. For a given agglomerative assay, the measured \( T_2 \) can follow one of two pathways over the course of an analyte titration.

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The population of dispersed particles can cluster in a uniform manner leading to an increase in average particle size that is proportional to the amount of analyte that has been added. This type of agglomeration is termed the monodisperse model because it would lead to a monodisperse intermediate population of nanoparticles. In this case, $T_2$ would be expected to decrease as particle size increases as long as the system is within the motional averaging regime. As the system approaches and enters the visit-limited regime, $T_2$ would increase with particle size (figure 3(a)). This is the type of behavior observed by Roch et al when a change in pH resulted in the flocculation of nanoparticles [67].

A different type of agglomeration that may occur is one where the addition of an analyte seeds the self-assembly of clusters, a process with energetics similar to crystal formation or fractal aggregation. For this model, one would expect a preferred size for nanoparticle clusters that depended on the conditions of the solution. Systems that followed this model would exhibit polydisperse intermediate populations; one would find a mixture of nanoparticles with discrete sizes. Given two discrete populations, dispersed particles and clustered particles, the system would transition between the $T_2$ value of the starting monodisperse population of unclustered particles and the final $T_2$ value of the fully clustered particles (figure 3). For both models, full titration may lead to a monodisperse solution of clustered nanoparticles. Although the exact energetics, kinetics and thermodynamics of particle agglomeration will depend on the characteristics of the assay system such as valency and binding affinities, these two models are instructive in understanding the dependences and possible scenarios one may encounter during MRSw biosensor design.

2.2. Magnetic resonance switch (MRSw) biosensors

2.2.1. Biosensor fundamentals. MRSw biosensors require the measurement of the bulk $T_2$ relaxation of the medium (e.g. water in blood, sputum and urine). MR signals arise from the nuclei of water hydrogen atoms. According to the classical description of NMR, these nuclei can be thought of as having tiny spins that precess in the presence of an external magnetic field, such as that provided by a permanent magnet inside an NMR instrument. The rate of precession of the nuclear spins is directly proportional to the strength of the magnetic field. Local microscopic variations or non-uniformities in $B_0$, not refocused by the CPMG pulse sequence, are dominated by the agglomeration state of the particles. Since the extent of clustering depends on the amount of analyte present, the $T_2$ relaxation measurement can be used to determine the amount of analyte present in the sample.

When exposed to an applied field, superparamagnetic particles create a local magnetic field dipole, leading to a local field ‘gradient’, or spatially changing magnetic field directly around each particle. The field experienced by a given spin is the sum of the applied field and the local field generated by a particle. Spins that are near the particle precess at different frequencies than spins further away. During the timescale of a $T_2$ measurement, water molecules are constantly diffusing, experiencing field variations; hence, spins precession frequency varies, leading to the loss of coherence or synchronicity between the spins within an ensemble. In addition the field of the SPIO is constantly fluctuating based on the Néel relaxation, and in some cases proton exchange is also possible.

MRSw biosensors achieve selective binding sensitivity of a nanoparticle for a desired molecular target by attachment of binding moieties such as antibodies or oligonucleotides to the nanoparticle. If the binding group is multivalent, i.e. can bind to more than one site on a given...
MRSw biosensors are based on the MR detection of the transition of dispersed and clustered populations of functionalized superparamagnetic nanoparticles. The nanoparticles consist of a superparamagnetic iron oxide core (orange), a polymer coating (gray) and selective binding agents (blue). For one biosensor configuration, the addition of analyte (red) leads to nanoparticle–analyte aggregates.

analyte, and there are multiple binding sites per nanoparticle, the addition of an analyte to target-sensitized nanoparticles leads to the formation of nanoaggregates (figure 4). In this manner, nanoparticles can be configured to switch from a dispersed state to an aggregated state due to the presence of an analyte. The basis of this transition is the tailored affinity of nanoparticle surface groups for a specific analyte. Similarly, nanoparticles can be configured to switch from aggregated to dispersed states due to the presence of an analyte. A wide range of strategies have been employed by researchers to effect a clustering state transition of nanoparticles.

For MRSw biosensors, the rate of the transition between dispersed and clustered nanoparticles depends on various parameters that can be controlled for a specific set of conditions. $T_2$ measurements can be carried out in real time during the analyte-induced response or at the end point of the clustering reaction. For the former, $T_2$ changes as a function of measurement time and the rate of $T_2$ change can be correlated to a quantitative amount of analyte. For the latter, after the incubation time, $T_2$ remains constant as a function of measurement time, and the magnitude of $T_2$ can be correlated to a quantitative amount of analyte. Both measurement approaches have been used for MRSw biosensors to date.

Sample mixing and loading, as well as $T_2$ measurements, can be completed in tens of seconds, making sample incubation the rate-limiting step for MRSw measurements. To date, incubation times have ranged between zero and several hundreds of minutes. Numerous studies have demonstrated real-time $T_2$ measurement immediately after sample mixing [28], [71]–[73], but most quantitative data acquired have used end-point readings. In some cases, faster kinetics can be achieved by using systems that transition from clustered nanoparticles to dispersed nanoparticles [29, 72, 73]. However, for both types of assay designs, significant variation in reaction rates has been observed.

A theoretical study from the laboratory of Jasanoff suggested how several parameters, such as particle concentration, functional group density and ratio of particle types, can be
optimized to achieve reaction rates of the order of seconds [64]. Their simulations predicted that reaction rates can vary over three orders of magnitude within reasonable activation kinetics, bio-molecular on and off rates, particle concentrations and functionalization levels. Koh in the laboratories of Weissleder and Josephson performed a series of experiments where parameters such as target valency and size were altered, leading to a change in the system sensitivity and EC_{50} values [74].

Shapiro et al [68] proposed a two-step model for MRSw agglomeration. The first step consisted of the activation of both species of nanoparticles due to the presence of an analyte [64]. Such an activation results from the analyte binding to or analyte-induced modification of the particle surface. The second step consisted of agglomeration of the activated nanoparticles. They assumed that the rate of the first step was much higher than that for the second step, causing agglomeration to be the rate-limiting step. Interestingly, for sensors that are based on nanoparticle dispersion, deactivation and dispersion are likely both fast steps, explaining why for some sensors much higher rates are observed for nanoparticle dispersion. This model has been used to predict how changes in particle concentration, the number of functional groups per particle and the ratio between particle types influence the observed binding kinetics and particle size serving as general guidelines for navigating the parameter space of MRSw biosensor design. The work by Shapiro et al indicates that a measurement time of less than 10 min can be achieved for most nanoparticle preparations and that optimized sensors may allow for single-second measurement times.

One must remember that the accuracy of these predictions depend on the assumption that the agglomeration step is rate limiting, which may not be a valid assumption in some cases. However, these simulations are valuable tools for navigating the complex parameter space of magnetic relaxation biosensors. These and previous results indicate that a measurement time of less than 10 min should be feasible for typical nanoparticle preparations and that optimized sensors may allow for measurement times as short as tens of seconds.

2.2.2. Demonstrations of MRSw biosensors. Two distinct properties of MRSw are their breadth of application and their capability to detect target analytes in opaque samples. As described in more detail in table 1, MRSw have been used to detect DNA, RNA, proteins, enzymes, small molecules, hormones, bacterial cells, ions, eukaryotic cells, viruses and antibodies in a variety of sample matrices including whole cell lysates, whole blood, serum, plasma and urine. The high tolerance for opaque samples and large amounts of background substances stems from the non-optical nature of the MR measurement and the properties of superparamagnetic nanoparticles. For most other biosensor methods, non-specific binding and other surface-mediated effects lead to background interference and necessitate a wash step. The breadth of applications and high background tolerance of MRSw are unprecedented among biosensor technologies. While most examples in table 1 are proof of concept experiments, work over the past couple of years at T2 Biosystems has demonstrated that MRSw switches can be used to detect target analytes at limits of detection and dynamic ranges appropriate for clinical applications [75, 76].

Interestingly, particle agglutination is not the only approach that can be used with MRSw. Several groups have used the method of tagging analytes with superparamagnetic nanoparticles and measuring the presence of analyte via a change in T_2 or T^*_2. As mentioned above, when non-uniform magnets are in use for detection, T_2 must be used as a readout sensitive to the
Table 1. A table summarizing previous works that have demonstrated the usefulness of MRSw.

| Analyte                  | Limit of detection                                  | Assay format and methods                                    | References |
|--------------------------|-----------------------------------------------------|----------------------------------------------------------------|------------|
| Nucleic acids            |                                                     |                                                                |            |
| 24 base pair synthetic   | Femtomoles of DNA in 1 ml                           | Tight correlation ($r^2 = 0.99$) and equivalent sensitivity   | [71]       |
| oligonucleotide sequence  |                                                     | between MRSw and a PCR-dependent photometric assay.          |            |
| 54-mer telomeric repeat  | Attomoles of DNA oligonucleotides in 50 µl          |                                                                | [77]       |
| test sequence            |                                                     |                                                                |            |
| Extracted green          |                                                     |                                                                |            |
| fluorescent protein      |                                                     |                                                                |            |
| (GFP) mRNA and GFP       |                                                     |                                                                |            |
| GFP mRNA directly in cell|                                                     |                                                                |            |
| lysate                   |                                                     |                                                                |            |
| Gene delivery            |                                                     |                                                                |            |
| Proteins                 |                                                     |                                                                |            |
| GFP                      | Single nanomolar                                    | Agglomerative immunoassay.                                   | [28]       |
| The avidin system        |                                                     |                                                                |            |
| Hormone human            | 3.6 nM hCG or 0.1 mol of analyte per nps            | Antibody-conjugated nps.                                     | [66]       |
| chorionic gonadotropin   |                                                     |                                                                |            |
| (hCG)                    |                                                     |                                                                | [79]       |
| Multivalent protein A    | 1 µg ml$^{-1}$                                     | Agglomerative immunoassay.                                   | [79]       |
| hCG                      | LOD improved 3000 fold of the work reported in      |                                                                |            |
|                          | [68]                                                |                                                                | [80]       |
| Analyte                     | Limit of detection | Assay format and methods                                                                 | References |
|----------------------------|--------------------|----------------------------------------------------------------------------------------|------------|
| Human serum albumin (HSA)  | 1 pM–1 µM yielded a linear $T_2$ response | HSA functionalized nps (competing with free HSA in solution to bind to anti-HAS).            | [81]       |
| Enzymes                    |                    | Two types of nps with one of two complimentary 18 base pair strands of DNA mixed in an equimolar ratio with their oligonucleotides hybridized creating np agglomerates cross-linked via double-stranded DNA. By design, the cross-linking double-stranded DNA contained a sequence that could be selectively cleaved by the enzyme. | [73]       |
| Dam methylase              |                    | Similar to [64], methylated-DNA selective GATC endonuclease, Dpn1, was used to change the clustering. Nps decorated with biotinylated peptide containing the DEVD amino acid sequence or with the protein avidin. | [28]       |
| Renin protease             | LOD of 31 nM h$^{-1}$ substrate hydrolyzed, biosensor benchmarked against fluorescence resonance energy transfer (FRET) | Added the protease of interest to a bi-biotinylated peptide that contained a cleavable amino acid sequence. After incubation monodisperse avidin-coated nps were added. | [82]       |
| Analyte                                                                 | Limit of detection                                      | Assay format and methods                                      | References |
|------------------------------------------------------------------------|--------------------------------------------------------|---------------------------------------------------------------|------------|
| Trypsin and matrix metalloproteinase 2 (MMP-2) proteases in unpurified fibrosarcoma cell growth supernatant | 0.05 µg ml\(^{-1}\) for trypsin                        | Ligand-mediated agglomerative or dispersive assay.            | [82]       |
| Telomerase, the activity of which can be elevated in tumor malignancies |                                                        | Nps functionalized with oligonucleotides that hybridize to the telomeric repeats. | [77]       |
| Horseradish peroxidase (HRP), and myeloperoxidase (MPO)               | LOD for HRP was 0.1 U ml\(^{-1}\), and for MPO 0.003 U ul\(^{-1}\) | Nps functionalized with either dopamine (for HRP) or serotonin (for MPO) were acting as electron donors for enzyme-catalyzed reduction of hydrogen peroxide. | [83]       |
| MMP-2 activity                                                        | LOD of 170 ng ml\(^{-1}\) or 9.4 U ml\(^{-1}\), which is within the concentration range of MMP-2 typically found in tumor cells | Two types of nps coated with biotin with a cleavable peptide attached to polyethylene glycol (PEG). The bulky PEG groups inhibited the binding between biotin and streptavidin. Addition of MMP-2 led to the cleavage of the peptide linker that attached PEG to the nps exposing the biotin and avidin such that the nps self assembled into clusters. | [84]       |
| Virus                                                                  |                                                        | Nps with monoclonal antibodies by means of a protein G coupling method. The monovalent antibodies bound selectively to coat proteins on the surface of either HSV-1 or ADV. | [26]       |
| Herpes simplex virus (HSV-1) and adenovirus (ADV)                     | LOD: five viral particles in 10 µl                      |                                                               |            |
| Analyte                              | Limit of detection                                           | Assay format and methods                                                                 | References |
|-------------------------------------|-------------------------------------------------------------|-----------------------------------------------------------------------------------------|------------|
| Small Molecules                     |                                                             | A bi- or multi-valent binding agent is used to cluster nps that have been conjugated with a derivative of the target. | [72]       |
| D phenylalanine (D-Phe)             |                                                             | For glucose the nps were decorated with two glucosamine hydrochloride and pre-complexed with the glucose binding protein concanavalin A (Con A). For detection of the influenza HA peptide, the nps were functionalized with HA peptide and pre-clustered with anti-HA antibody. The folic acid biosensor consisted of nps decorated with folic acid and an anti-folic acid antibody that pre-clustered the nps. | [29]       |
| Glucose, hemagglutinin (HA) peptide and folic acid | LOD below 50 mg dl$^{-1}$ for glucose |                                                                                       |            |
|                                     | The HA assay was 80 times more sensitive than the glucose assay and the folic acid assay was 1000 times more sensitive than the glucose assay |                                                                                       |            |
| Ions                                |                                                             | Calmodulin protein and a calmodulin-binding peptide to endow calcium-dependent agglomeration upon superparamagnetic iron oxide nps. The surface chemistry used for ion-selective electrodes was adapted for use with MRSw biosensors. | [65, 85]   |
| Ca$^{2+}$                           | The response range for wild-type calmodulin was 0.1–1.0 $\mu$M, and for the variant of calmodulin 1–100 $\mu$M |                                                                                       |            |
| Ca$^{2+}$                           | The surface chemistry used for ion-selective electrodes was adapted for use with MRSw biosensors. Functionalization with diglycolic anhydride, which complexes Ca$^{2+}$ at molar ratios of 2 : 1 and 3 : 1. Because calcium can induce the self-assembly of multiple diglycolic anhydride moieties, addition of Ca$^{2+}$ to the targeted nps led to cluster formation. | [86]       |
| Analyte | Limit of detection | Assay format and methods | References |
|---------|--------------------|--------------------------|------------|
| Cells: Mycobacterium avium paratuberculosis (MAP) | 40 colony forming units (CFU) in 10 µl of milk and 40 CFU in 20 µl of blood | Anti-MAP conjugated nps. | [27] |
| Staphylococcus aureus (S. aureus) | LOD of 10 CFU in 10 µl | Particles decorated with vancomycin, which binds to peptide moieties on the cell wall of S. aureus. | [23] |
| Con A | Con A concentrations of 1 µg µl⁻¹ were detected | Dextran-coated SPIOs in a competitive assay with Con A. The dextran and the starch produced by bacterial metabolic activity compete for binding of Con A. | [87] |
| Mammalian cells (mouse macrophages) | Single mouse macrophage cell in 10 µl (calibration with hemacytometer) | Fluorescein-conjugated dextran-coated particles. Macrophages uptake the particles, and unbound nps were separated by washing. | [55] |
| Cancer cells | Two cells in a 1 µl sample | Antibody-coated nps. | [55] |
| Cancer cells from a fine needle aspirate of a mouse xenograft tumor | | Comparison of the MRSw to flow cytometry and Western blot analysis showed good agreement and required much fewer cells. | [69] |
| Bacillus Calmette-Gurin (BCG) as a surrogate for Mycobacterium tuberculosis | | | [39] |
presence of superparamagnetic particles, whereas when uniform magnetic fields are used, $T_2^*$ may be used as a readout.

An example of the use of non-uniform magnets and the detection of analytes by means of tagging them with superparamagnetic particles is the work of Weissleder at MGH. In that work, they used a non-uniform permanent magnet (50 ppm) and $T_2$ measurements to measure the presence of and biomarker profiles of different types of mammalian cells—mouse macrophages and breast cancer cells. Mouse macrophages were detected by first incubating the cells with fluorescein-decorated superparamagnetic nanoparticles. After incubation to allow the macrophages to uptake the dextran-coated nanoparticles, the tagged cells were washed to remove unbound nanoparticles and the $T_2$ of the resultant solution was measured. These $T_2$ measurements were calibrated with a hemacytometer reference method to determine the limit of detection, which was a single cell in 10 $\mu$l or 100 cells ml$^{-1}$. A similar approach was used to measure the types of biomarker expressions on breast cancer cells by using antibody-coated nanoparticles targeted to specific cell-surface biomarkers [43]. That work was recently extended to the detection of cancer cells in fine needle aspirates [69].

An analogous cell-tagging and detection example but with a uniform magnet is the work by a group at the University of New Mexico and Sandia National Laboratories led by Sillerud. They demonstrated the use of a microfabricated detection coil in combination with a highly uniform (0.06 ppm) permanent magnet to detect the presence of cells tagged by micron-sized superparamagnetic nanoparticles. The presence of the particles was detected by measuring a change in $T_2^*$ [70]. One drawback of using $T_2^*$ is that it requires a highly uniform magnet, providing an inherent limit to system miniaturization and cost.

2.3. NMR hardware for MRSw

A key component for enabling the successful application of MRSw biosensors is tailoring the processing and detection platform to the setting in which it will be used. A variety of fields would greatly benefit from a universal detection technology such as MRSw biosensors, for instance bio-warfare first responders, ambulances and home diagnostic testing. Such applications require mobile, robust, compact instrumentation. Other applications, such as biomarker discovery and clinical testing, require automation and high throughput, while applications such as health clinics and doctor’s offices require a compact, simple to use benchtop unit. Furthermore, based on the reversible nature of MRSw [29], the potential for monitoring of target analyte levels with implantable MRSw is also possible. This concept was introduced by the laboratories of Cima and Langer at MIT using the protein biosensor for hCG and a polydimethylsiloxane (PDMS) device. This device was designed to contain the nanoparticle reagents between two polycarbonate membranes that had 10 nm pores allowing for the passage of the hCG target while containing the nanoparticle reagents. The feasibility of this device for real-time sensing was confirmed by observing changes in $T_2$ inside the reservoirs due to changes in hCG levels in the surrounding solution by means of MRI [88, 89]. Subsequent work with this device will demonstrate its usefulness for detecting in vivo circulating biomarkers. Given the appropriate instrumentation, one can envision applications where implanted MRSw devices are measured in a simple, portable format. Such instrumentation and applications are under development at T2 Biosystems and in the Cima and Langer laboratories.

Over the last couple of decades, advances in the field of portable and micro-NMR have provided the technological breakthroughs in miniaturized MR detectors for developing truly portable and integrated diagnostic measurement devices. These breakthroughs include new
types of detection coil fabrication methods [105]–[107] that allow for submicroliter detection volumes and submillimeter sizes in solenoidal coils that are either hand wound [90]–[92] or machine wound [93, 94]; or planar detection coils that allow straightforward integration with silicon microchannels [95, 96] and microfluidic systems [97, 98]. Additional breakthroughs have demonstrated that nanoliter volumes can be detected with high sensitivity using microfabricated coils [13, 99]–[102]. These advances in miniaturized detection coil design and fabrication have been applied to MR instruments with permanent magnets [13]–[16], [70]. See the above cited articles for more details.

MRSw biosensors that use $T_2$ relaxation as a readout allow for measurements in relatively non-uniform magnets, which can be very compact and low cost. The application of pulse sequences such as the CPMG sequence that enable accurate measurements in highly inhomogeneous magnetic fields has been critical to enabling MR to enter applications that require low cost and portability, such as MRSw. An instructive parallel example is the improvement and optimization of the CPMG sequence specifically for the most extreme example of portable MR relaxation measurements in inhomogeneous fields—oil well logging. This application consists of obtaining relaxation measurements external to a single-sided magnet and planar detection coil inserted deep within an oil well [103].

Several groups are developing portable and/or single-sided systems for various MR applications using novel permanent magnet configurations [104, 108]. The technical advances that result from efforts to achieve portable MR spectroscopy and imaging will directly benefit MRSw. Some of the other applications benefiting from these advances include oil well logging [7, 103], art preservation and on-site materials characterization [1, 4, 5], [109]–[113], food analyses [110, 111], imaging [5], [112]–[114] and MR spectroscopy [8]–[10], [13] and relaxometry. Closed magnet designs used for higher resolution applications are more relevant to most MRSw biosensor applications. One of the most notable designs that has been refined over the years is the Halbach magnet design [115]. Several adaptations of the Halbach ring have been implemented, from wedge elements, to square blocks, cylinders and hexagons, in a wide variety of elements and rings. An extension of the Halbach dipole ring is the NMR-cuff (Cut open, Uniform, Force Free) designed to open and close the ring around a test object and the NMR Mandhalas with two concentric Halbach rings and thus a variable field depending on alignment of the two rings [116]. The mini Hallbach with the addition of magnetic block elements [12] generates a sweet spot for imaging and high homogeneity for spectroscopy. The Halbach Box is another great example of a homogeneous magnet for small volume spectroscopy [15, 16]. These small magnets produce fields between 0.5 and 2 T, weighing from a few grams to a few kilograms and when combined with microcoils yield impressive homogeneity.

A team in the Weissleder group has demonstrated the use of a downsized magnet, downsized detection coils and downsized spectrometer components for the detection of MRSw. This team, lead by Lee, built a 0.49 tesla $T_2$ relaxometer from a palm-sized magnet and planar microcoils [55]. A few of the spectrometer components were integrated onto a printed circuit board. With this system they report an 80-fold increase in mass sensitivity for an MRSw model system. While the performance of the system could be further optimized, it was a powerful demonstration of the portability and ease of use available to MRSw applications.

An additional benefit of miniaturizing the MR detection system is greater multiplexing capability. Because the homogeneous region of a magnet is limited, the smaller volume occupied by microcoils can allow for multiple detection coils within a single magnet. Lee et al utilized this approach by constructing an array of eight microfabricated planar detection coils for
their miniaturized MR instrument [59]. The eight coils were connected to a single-channel spectrometer by means of a multiplexer switch. This system was used to detect eight biomarkers from single samples in parallel for both diabetes and cancer biomarker panels [117]. This work was a powerful demonstration of how MRSw can provide users with readings for a panel of biomarkers relevant to a specific diagnostic condition, such as cancer. Because of the breadth of demonstrated target analytes for MRSw, such panels will be able to span an unprecedented range of analyte classes (e.g. cellular, protein, molecular, protein, enzymatic and therapeutic) and yield a virtually limitless set of test menus for specific applications. Lee et al used a microcoil, flow through system for the detection of bacterial cells [43]. The system also contained an embedded filter and allowed for the integration of wash steps for removal on unbound particles to enhance the sensitivity of the system [39].

Several advances have happened in miniaturization of the NMR spectrometer. Single board spectrometers have been demonstrated [121]–[127], and there are already commercial options of systems by companies such as Margritek and Tecmag. The laboratory of Ham has demonstrated the capability to significantly downsize the spectrometer via the use of silicon radiofrequency integrated circuits [118, 119]. The miniaturization of the NMR RF transceiver and several successful demonstrations of its use for MRSw biosensors have been presented [118, 119]. The ‘palm NMR’ system described in [123, 124] combined the Ham laboratory’s miniaturized spectrometer with T2 Biosystems’ miniaturized magnet and detection coils to provide a miniature system with sufficient performance for MRSw switch detection. While the SNR performance of the system can be further improved, the results were indicative of the potential for miniaturization of the MRSw switch hardware.

The breakthroughs in MR detection instrumentation indicate that MR detection can be tailored to biosensor applications that require downsized and portable readers. Efforts are currently under way at T2 Biosystems to produce completely integrated, portable MRSw biosensor instruments. Any such system will likely consist of an MR reader (magnet, detection coil, transceiver electronics and control board), as well as a user interface, power source and connection to external data networks. Depending on the application, the MRSw biosensor instrument may also provide disposable cartridge reading and handling, fluidics actuation, sample processing and temperature control. A shoebox-sized, fully integrated NMR reader that weighs less than 8 lbs was developed by a team at T2 Biosystems [75, 76]. This device consisted of a 0.5 T magnet, a spectrometer, an operating system, a touch-screen user interface and dc power input. An improved MR reader has also been developed, consisting of a custom single board spectrometer, board, RF probe and transceiver electronics; a single scan SNR of 100 s for single microliter samples [76], demonstrating that a highly compact and inexpensive MR system can be fabricated.

Due to the range of applications of MRSw biosensor tests and the breakthroughs in downsized instruments, continued development will enable the deployment of MRSw biosensors in a broad variety of settings and with potentially unlimited targets.

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