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Advantages of macromolecular to nanosized chemical-exchange saturation transfer agents for MRI applications

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Abstract

Chemical-exchange saturation transfer (CEST) agents are a new class of MRI contrast agents that offer a number of advantages over conventional Gd^{3+} agents. Over the past few years, a variety of small-molecule CEST agents responsive to physiological conditions, such as pH and temperature, have been designed and their imaging applications have been reported. One of the major drawbacks of current small-molecule CEST agents is their relatively low sensitivity. The advantages of using macromolecular and nanosized systems with large numbers of exchangeable groups to improve contrast sensitivity are highlighted in this brief review. Although this approach has been shown to amplify contrast sensitivity, other limitations, including relatively small chemical-shift differences between the exchanging species and bulk water and less than optimal proton exchange rates, still exist. By addressing these issues, it is anticipated that CEST agents will find useful applications in the detection of specific biomarkers of disease.

MRI

MRI is one of the most powerful imaging modalities in clinical medicine and an active area of continuing research. MRI is noninvasive, delivers no radiation burden and, most importantly, yields excellent spatial resolution of soft tissue [1]. Although an overwhelming majority of contrast-enhanced clinical exams are performed with paramagnetic Gd^{3+} complexes [2–4], this family of contrast agents provides limited physiological or biochemical information. For this reason, considerable effort has been devoted toward the development of new contrast agents that alter image intensity in response to physiological and metabolic indicators [5].

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Chemical-exchange saturation transfer agents

Chemical-exchange saturation transfer (CEST) agents represent a new class of MR contrast agents that operate by altering the total water signal intensity rather than by altering the T_1 or T_2 relaxation times of water protons [6]. A CEST agent by definition contains labile protons (-NH, -OH, -SH or H_2O) that exchange with the protons of bulk water. Provided that the **chemical shift** of these labile protons differ sufficiently from bulk water protons, so that they may be selectively tagged or saturated by use of a frequency-selective radio frequency (RF) pulse, the net effect of **chemical exchange** then is to partially transfer those saturated spins into the bulk water pool and reduce the intensity of the bulk water signal (Figure 1). This decreased signal intensity can be observed with the aid of an NMR spectrometer by selectively presaturating the CEST agent in incremental steps over a chosen frequency range and then plotting the residual bulk water signal intensity as a function of **saturation** frequency. The curve generated is typically referred to as a CEST spectrum. In an MR image, the decreased signal intensity translates to a negative contrast similar to that produced by T_2 agents [7].

The major advantage of CEST agents over Gd^{3+} -based agents is that one can switch the image contrast 'on' or 'off' at will by gating the RF presaturation pulse. This unique feature eliminates the need to acquire pre- and post-injection images to determine signal changes and, therefore, minimizes possible errors due to time delays and motion-induced artifacts [6]. Furthermore, multiple CEST agents can, in principle, be administered simultaneously and each agent can be activated separately by a RF pulse specific for the chemical shift of its mobile protons, therefore offering the intriguing possibility of multiplexing.

For CEST to occur, the exchange rate between the labile protons and the protons of the bulk water must be slow on the NMR timescale, a condition met when ω is greater than or equal to k_{ex} , where ω represents the chemical shift difference between the spins and k_{ex} represents the exchange rate. This relationship indicates that, for larger ω values, faster exchanging systems will still meet this requirement. This expands the number and type of chemical systems available as potential CEST agents. The first few exogenous CEST agents proposed by Balaban and co-workers were low-molecular weight diamagnetic molecules [8,9]. Many of these molecules showed a significant ability to alter the total water signal intensity but only at relatively high concentrations (10–100 mM). The CEST spectrum of barbituric acid, one of the earliest studied CEST agents, is shown in Figure 2. The ω in this system is approximately 5 ppm. This small ω can lead to direct saturation of the bulk water and also limits the maximum k_{ex} to the order of 650 s^{-1} (for a 3T clinical magnet) to meet the slow exchange requirement ($\omega \gg k_{ex}$) [10]. This limitation can be addressed by utilizing CEST agents that possess exchangeable protons, having a much wider range of chemical shifts. Paramagnetic complexes are ideal for this purpose [6,11].

Paramagnetic CEST agents

Paramagnetic lanthanide ions, when chelated by a **DOTA-tetraamide** ligand, often exhibit a single, highly-shifted metal ion-bound water resonance in their 1H NMR spectra [12]. The chemical shift of this bound water molecule can vary from +500 ppm to -702 ppm

depending upon which lanthanide ion is used [13]. This unique feature can be very beneficial for CEST since ω is much larger, thus the probability of directly saturating bulk water protons is largely eliminated and the maximum permissible exchange rate is amplified. These paramagnetic lanthanide-based CEST agents are now referred to as **paramagnetic (PARA)CEST** agents. The first example of a PARACEST agent was EuDOTA-(glycine ethyl ester)₄³⁺, a complex with a bound-water exchange peak at approximately 50 ppm, which represents the residual fraction of bulk water intensity at the resonance frequency of the exchanging bound-water molecule (Figure 3) [14].

The CEST spectrum of this agent also showed a significant reduction in the bulk water intensity after pre-irradiation at 50 ppm, the magnitude of which depends upon the concentration of the agent. CEST spectra of this sort have now been reported for many other lanthanide ion complexes, but the Eu³⁺ complexes continue to dominate the literature because of the unusually slow water-exchange kinetics displayed by these complexes [15]. Paramagnetic lanthanide ions also have the ability to induce shifts in the resonance frequencies of other proton-exchangeable groups such as amide and alcohol protons, which happen to be in close proximity to the paramagnetic center. Although these shifts are not as large as those of the metal ion-bound water molecules, they do offer some advantages for CEST because they typically exchange protons more slowly with bulk water protons than do entire water molecules from an inner-sphere position on the lanthanide ion to water in the bulk. PARACEST has been observed in exchangeable alcohol protons [16,17], coordinated amide protons [18] and proximal amide protons [19]. One nice feature of lanthanide-based PARACEST agents is that isostructural complexes can be prepared for most of the lanthanide ions with any given ligand system. This means that more than one PARACEST agent can potentially be administered simultaneously, and each could be activated separately by an appropriate choice of frequency-selective RF pulse [20]. This concept was nicely illustrated by using Eu³⁺ and Tb³⁺ complexes of the same ligand for cell-labeling experiments [21], thereby demonstrating the multiplexing potential of PARACEST MRI.

Responsive CEST & PARACEST agents

One emerging area of **MRI contrast-agent** development is the design of responsive contrast agents that respond to changes in the concentration of biologically important species, such as an enzyme, or a physiological parameter, such as pH, temperature or tissue redox. ‘Smart agents’ such as these are particularly relevant in making MRI an important molecular imaging modality. One advantage of the CEST-type platform for developing responsive imaging agents is the possibility of creating contrast that is independent of agent concentration, a feature not available with Gd³⁺-based agents. For this reason, a large effort has been focused on designing PARACEST agents that are responsive to a variety of physiological parameters in biological systems.

The CEST efficiency is highly dependent on the chemical exchange rate of the mobile protons on the agent. Since k_{ex} is sensitive to the chemical environment and other physico-chemical variables, such as pH and temperature, the possibility of using PARACEST agents to monitor temperature [22,23] and pH [24,25] is very promising. Furthermore, the ligand structure of a paramagnetic complex can be altered to make it sensitive to the presence of a

variety of metabolites, such as lactate [26] and glucose [27,28], metal ions [29] and enzyme activity [30]. PARACEST agents responsive to nitric oxide [31] and phosphate ester interactions [32] have also been reported. These advantages illustrate the promise of using PARACEST for MRI at the cellular and molecular level.

Limitation of small-molecule CEST agents

One of the major limitations of small-molecule CEST agents is their relatively low contrast sensitivity, usually requiring concentrations in the range of 1–10 mM for a typical agent [6]. Several approaches have been taken to amplify the CEST signal. Unlike Gd^{3+} -based contrast agents where optimization involves complicated design features, such as number and residence time of coordinated water molecules, second-sphere parameters and rotation correlation times to improve relaxivities [33], the CEST signal can be amplified by simply increasing the number of exchangeable groups on an agent. One of the more exciting new areas for CEST is macro molecular and nanosized systems that can enhance contrast sensitivity and potentially reduce toxic effects by reducing the amount of required agent [34].

Towards macromolecular & nanosized CEST agents

Macromolecular and nanosized MRI bioimaging probes have a number of advantages, including a potential reduction in toxicity, increased blood circulation times, improved contrast sensitivity and the possibility of attaching various functionalities for targeting. These materials can be derived from various materials, such as metal oxides, metals, quantum dots, liposomes, polymers, dendrimers and biomacromolecules, such as proteins and viruses. The various versions of macromolecular and nanosized materials share the advantages of small size, but their suitabilities for different applications depend on their chemistries and pharmacological tolerance. The combination of nanosized materials with CEST agents has significant potential in molecular imaging [35].

Polymer, dendrimer & biomacromolecule-based diamagnetic CEST agents

Building on the observation that polymers have the capacity to deliver higher payloads of exchangeable groups per mole, the CEST signal from polymeric compounds have been shown to enhance contrast sensitivity to a significant degree by simultaneous saturation of multiple exchangeable protons. Goffeney *et al.* reported contrast sensitivity increases by a factor as high as 500,000 using cationic polymers containing multiple amide protons [36]. A comparison of the CEST properties of poly-L-lysine (PLL), poly-L-glutamate (PLE), polyallylamine (PAA), polyethylenimine (PEI) and generation-5 star-burst PAMAM dendrimer (SPD-5) showed some interesting basic trends (Figure 4). CEST was not detected for PAA and PEI, presumably due to the fast exchange of the protons in primary amino groups, while PLL, PLE and SPD-5 showed a noticeable CEST effect, due to slower exchange of the amide protons. When CEST was measured on samples of PLL (100 μ M), PLE (500 μ M) and SPD-5 (1000 μ M), where the fractional concentrations of exchangeable protons were comparable, a 43% change in bulk water intensity was seen for PLL, while

SPD-5 showed a 51% change even though there was a significant difference in proton exchange rates (~ 140 vs ~ 77 s⁻¹, respectively) in these two polymers.

Snoussi *et al.* further improved on the enhancement factor determined for PLL by mixing a positively charged polymer with polyuridylic acid (poly[rU]), a single-stranded RNA consisting of uridine repeat units connected by phosphodiester, to impart negative charge to the polymer mix [37]. The poly(rU) amino protons and hydroxyl protons with chemical shifts at 10.8 and 6.4 ppm, respectively, are capable of exchange. The CEST effect for the imino protons of 15 μ M poly(rU) ranged from 10 to 70% using different powers. Poly(rU) is a good vehicle for conjugation with a counter-charged polymer for drug/gene delivery purposes, hence, titration of a fifth generation dendrimer (G-5) with poly(rU) revealed that a single molecule of poly(rU) binds approximately ten dendrimers. The CEST profiles (Figure 5) for the poly(rU), dendrimer and complex (poly[rU]: dendrimers) showed a decrease in CEST efficiency when poly(rU) was conjugated to the dendrimers. There was, however, a notably better CEST response for the imino protons of the complex compared with the amide protons of the dendrimer.

Chemical exchange saturation transfer was also extended to probing endogenous metabolites as gene reporters, as well as monitoring changes in metabolite levels *in vivo*. Exchange between the hydroxyl protons of glycogen and water by CEST spectroscopy and imaging (glycoCEST) was first reported by van Zijl *et al.* [38]. Glycogen, a high-molecular weight polymer composed of glucose units linked together by 1,4-glycosidic bonds, plays a fundamentally important role in glucose storage and homeostasis in human liver. Alterations in glycogen metabolism are thought to be important in common diseases such as Type 2 diabetes, obesity and insulin resistance, therefore, having an imaging tool available to routinely monitor glycogen levels in humans could have a substantial impact in clinical medicine. Although the -OH protons of glycogen are thought to undergo fast chemical exchange ($k_{ex} > 10^3$ s⁻¹) with bulk water protons and have only a small chemical shift difference (~ 1 – 1.2 ppm) with water, CEST was detected in both phantoms (Figure 6) and perfused mouse livers (Figure 7).

GlycoCEST imaging was performed on mouse livers perfused with Krebs–Henseleit buffer before and after addition of glucagon. The initial glycoCEST image showed that glycogen was abundant and distributed throughout the liver. Upon addition of glucagon, a hormone known to stimulate glycogenolysis, glucose was immediately detected in the buffer solution surrounding the perfused organ, consistent with glycogen degradation (not shown) and the gradual disappearance of the glycoCEST signal with time (Figure 7). These data suggest it may be possible to use this method to image glycogen in the human liver at high magnetic fields.

More recently, glycosaminoglycans (GAGs) were detected *ex vivo* and *in vivo* by probing the -NH (+3.2 ppm relative to bulk water) and -OH (+0.90–1.90 ppm relative to bulk water) protons in the disaccharide repeating units of the molecule [39]. CEST spectra were recorded before and after treatment of cartilage with trypsin (Figure 8). The -OH protons, although closer to water, were found to be more suitable for GAGCEST, compared with the -NH protons. The CEST images of a bovine patella and patellofemoral human knee joint

showed distinct darkening at the site of GAG depletion (Figure 9), illustrating MRI applications in GAG assessment.

Polymer-based PARACEST agents

Like **diamagnetic CEST** agents, the contrast sensitivity of PARACEST agents can also be improved by using polymeric systems. The signal amplification offered by polymer-based systems will be essential for the detection of targets that are present in concentrations too low to be detected by a single paramagnetic center. The first example based on this strategy was presented by Aime and co-workers in 2003 by exploiting the formation of a noncovalent supramolecular adduct between a para magnetic shift reagent [TmHdotp]⁴⁻ and a macro molecular substrate polyarginine [40]. As a source of exchangeable protons, poly-arginine contains two different pools of potential CEST proton activation sites: one from the amide protons of the backbone and another from the guanidine protons of the side chains. The CEST spectrum of free polyarginine did not show a CEST peak from either pool of protons. However, after adding an 18 molar excess of [TmHdotp]⁴⁻ to poly arginine, the resulting supramolecular adduct showed a CEST peak at approximately 30 ppm (Figure 10). Apparently, [Tm(Hdotp)]⁴⁻ chelates form ion pairs with the positively charged guanidino groups along the polypeptide surface and this results in an overall hyperfine shift of the guanidine protons to low field that, in turn, changes the guanidine protons from a k_{ex} greater than ω system (no CEST) to a ω greater than k_{ex} system where CEST is observed. Quantitative comparisons of CEST containing different absolute concentrations of supramolecular adduct showed that the detection limit of this system, based on the assumption that a 5% change in water signal is easily detected, was quite low, approximately 1.7 μ M based on the polyarginine concentration and 30 μ M based upon [TmHdotp]⁴⁻.

A second example of the considerable enhancement on gains by polymerizing a CEST agent was recently reported by Wu *et al.* [41]. A DOTA-tetraamide with an acrylamide moiety on one extended amide side-chain was prepared and a series of homopolymers were prepared by using classical free radical chain polymerization conditions, in which the degree of polymerization varied from ten to 17, depending on the amount of initiator added (2, 5 and 10% w/w) (Figure 11). Both the high-resolution ¹H NMR spectra of the Eu³⁺-monomers and the CEST spectra of the Eu³⁺-polymers were consistent with the square antiprism coordination (SAP) geometry. The CEST effect on a per Eu³⁺ basis was found to be identical for each polymer and the corresponding monomer. A fit of the CEST spectra to a three-pool exchange model demonstrated that water exchange remained largely unaffected by formation of a polymer with both monomer and polymer exhibiting similar intermediate-to-slow water exchange rates. The polymeric agents showed a much lower detection limit (defined as a 5% decrease in total water signal intensity) in the range of 60–130 μ M, compared with 1300–1700 μ M for the monomer. The advantage of using a low-molecular weight polymeric PARACEST agent was even more evident in CEST images of phantoms containing low concentrations of monomer versus polymer.

Dendritic PARACEST agents

Dendrimers are multivalent macromolecules with regular, highly branched structures of nanoscopic dimensions (2–10 nm) resembling those of proteins. The well-controlled sizes and well-characterized purities of dendritic architectures in addition to their multivalent properties have encouraged researchers to conjugate them to MRI contrast agents [42]. The first example of a dendritic-based PARACEST agent was reported by Pikkemaat *et al.* for pH mapping [43]. In this system, CEST resulting from proton exchange between amide protons on the appended Yb-DOTAM ligands and bulk water was shown to be sensitive to pH and dependent on the size of the resulting dendrimer. Coupling of Yb-DOTAM to first- and third-generation poly(propylene imine) dendrimers resulted in pH sensitive PARACEST agents with four and 16 agents per dendrimer, respectively (Figure 12). The Yb-DOTAM complexes attached to the dendrimers via one side-chain group remained in the same square antiprismatic coordination geometry as seen for the free complex in solution. Proton-exchange peaks representing the seven exchangeable amide protons of each Yb-DOTAM moiety were observed between -12 and -16 ppm, compared with the distinct two amide exchange peaks for free Yb-DOTAM at -13.5 and -16.1 ppm. Interestingly, the maximum CEST effect for these systems were observed at different pH values, from pH 7.5 for free Yb-DOTAM complex to 6.5 and 6.2 for the first- and third-generation dendritic PARACEST agents, respectively. The CEST detection limits were 300 μM for Yb-DOTAM and 20 μM for the third generation dendritic agent, consistent with the expected 16-fold increase in contrast sensitivity.

Ali *et al.* recently reported the use of dendritic PARACEST agents for simultaneous tracking of the relative *in vivo* pharmacokinetics of two different sized dendrimer nanoparticles [44]. YbDOTA-(gly)₄⁻ and EuDOTA-(gly)₄⁻ were conjugated to second-generation (Yb-G2) and to fifth-generation (Eu-G5) polyamidoamine (PAMAM) dendrimers, respectively. A mouse model of mammary carcinoma was selected for the pharmacokinetic studies. Although the *in vivo* CEST effects could not be used to determine the absolute concentrations of each agent within the tumor, the concentration ratios of the two CEST agents within the tumor were determined by evaluating the ratio of their CEST effects for each agent. This information was used to assess the relative pharmacokinetics of the two nanoparticles within the tumor tissue (Figure 13).

The pharmacokinetics reflected the expected lower tissue permeability of the larger dendrimer, Eu-G5, compared with the smaller dendrimer, Yb-G2 and the slower elimination rate of the larger dendrimer also resulted in retention of more Eu-G5 in the tumor at prolonged times. This study illustrates a unique and powerful feature of CEST, simultaneous monitoring of two different imaging agents in the same study. This cannot be easily envisioned when using other paramagnetic imaging agents.

Perfluorocarbon nanoparticle-based PARACEST agent

Flacke *et al.* used lipid encapsulated liquid perfluorocarbon nanoparticles modified with surface targeting ligands to carry high payloads of conjugated GdDTPA to biological targets [45]. More recently, this same group prepared a functionalized lipid-conjugated EuDOTA-

(gly)₄[−] derivative and incorporated equally high payloads of this PARACEST agent into the nanoparticle surface (Figure 14) [46].

Imaging of control nanoparticles showed no appreciable contrast, while the PARACEST nanoparticles produced a greater than 10% decrease in water signal intensity upon activation by a frequency-selective pulse. PARACEST nanoparticles coated with antifibrin antibodies were readily detected when bound to the surface of blood clots, despite the presence of substantial magnetization transfer from proteins and macromolecules in the clot.

Virus nanoparticle-based PARACEST agent

The architecture of viral particles consists of interior and exterior regions that can be chemically or genetically modified, offering potentially highly differentiated nanoparticles for attachment of specific targeting groups [35]. The natural cell-targeting capability of virus particles can be altered biologically to carry a payload of imaging and therapeutic agents to other targets. This could become an interesting platform for molecular imaging [47,48]. Recombinant adeno-virus type 5 particles were labeled with two different bifunctional ligands capable of forming stable CEST-type complexes with paramagnetic lanthanide ions (Figure 15) [49].

The number of ligands that could be attached to a single virus particle varied from 630 to 1960 and was dependent on the chemical reactivity, the amount of the bifunctional ligand added and the reaction time. The bioactivity of each labeled adenovirus PARACEST agent was found to be highly dependent upon the number of conjugated paramagnetic complexes. The potential of such conjugates to act as PARACEST imaging agents was evaluated by using an on-resonance WALTZ sequence for CEST activation. A 12% decrease in bulk water signal intensity relative to controls demonstrated that these viral-based PARACEST agents could potentially serve as targeted agents for molecular imaging.

Liposome-based CEST agents

Another way of increasing the contrast sensitivity of CEST agents is by encapsulating paramagnetic complexes in the inner core of liposomes to act as water shift reagents (SR). Liposomes present an ideal platform for this purpose as the aqueous core contains a large amount of water that can exchange with extra-liposomal water in the bulk, the rate of which is determined in this case by the properties of the liposomal semi-permeable membrane. To induce a shift in the intraliposomal water, a SR with fast-exchanging bound water (e.g., LnDOTMA, LnDOTA or LnHPDO3A) can be encapsulated inside the liposomes [7]. Such **liposome-based (LIPO) CEST agents** are considerably more sensitive for CEST, because the number of exchangeable protons is determined by the amount of water encapsulated inside the liposomes, not by the concentration of the shift reagent alone.

The first generation of LIPOCEST systems used TmDOTMA as the encapsulated SR, a fast water-exchange system that yields a downfield shift in the resonance frequency of the encapsulated water molecules [50]. The chemical shift of the intraliposomal water protons was, in this case, limited by the concentration of the encapsulated SR that, in turn, was dictated by the ionic strength of the liposomal suspension. At the maximum concentration of

encapsulated SR, the resonance frequency of the internal water proton was shifted only approximately 4 ppm away from the bulk water (Figure 16A). The sign of the shift was dependent on the dipolar constants (C_D) of the Ln^{3+} used as shift reagent ($C_D > 0$ for Tm and $C_D < 0$ for Dy). Nevertheless, the contrast sensitivity for CEST detection was incredibly low, approximately 90 pM. The second generation of LIPOCEST agents aimed at increasing the chemical shift difference between the internal and bulk water. A larger chemical shift difference would clearly be more advantageous as it would reduce artifacts due to field inhomogeneity in the bulk water signal, overcome conventional magnetization transfer effects arising from endogenous proteins and exploit a faster exchange rate resulting in a more efficient CEST.

The magnitude of the hyperfine shifted water inside a liposome is given by:

$$\delta_{\text{wat}} = \delta^{\text{DIP}} + \delta^{\text{BMS}}$$

where the dipolar (δ^{DIP}) and the bulk magnetic susceptibility (δ^{BMS}) contributions are both proportional to the SR concentration and magnetic moment of the Ln^{3+} ion. The δ^{BMS} is zero for spherical liposomes but has a marked contribution if the Ln^{3+} ion is confined to a nonspherical compartment [51]. Terreno *et al.* changed the shape of liposomes by first encapsulating a hypo-tonic solution of a neutral, paramagnetic SR (LnHPDO3A) and then dialyzing the liposomes against an isotonic solution [52]. The osmotic pressure difference between the inside and outside of the phospholipid bilayer caused the liposomes to shrink, leak water and change shape. Thus, by changing the liposomal shape, the δ^{BMS} contribution becomes nonzero and adds (or subtracts) to the dipolar shift component. Since water leaves the inner compartment, the SR becomes more concentrated in the intra-liposomal compartment and this also enhances the dipolar contribution, δ^{DIP} . It was found that shrinking the liposomes increased the chemical shift difference between the internal and external water protons from 3.5 to 6.1 ppm for encapsulated TmHPDO3A (Figure 16B). Finally, the shifts were further increased by orienting the liposomes in a magnetic field with the use of an amphiphilic Ln^{3+} complex incorporated into the phospholipid bilayer (Figure 16C). Two Ln^{3+} ions, Tm^{3+} and Dy^{3+} , with different dipolar constants were used. The incorporation of an amphiphilic Ln^{3+} complex in the bilayer not only increased the chemical shift of the intraliposomal water proton (from ± 4 to +30 ppm for the Tm^{3+} complex and to -45 ppm for the Dy^{3+} complex), but also affected the direction of the shift. Incorporating an amphiphilic SR in the phospholipid bilayer provided the added benefit of increasing the number of Ln^{3+} centers inside the liposomes, since half of the incorporated SR pointed towards the inside of the liposomes, which then enhanced both the dipolar and BMS contribution.

The amount of SR that can be encapsulated is dictated by osmotic pressure. Charged complexes inevitably have counter-ions that also contribute to an increase in osmolarity, thereby limiting the amount of SR that can be encapsulated. Thus, encapsulating a neutral agent will allow entrapment of more SR for a given solution osmolarity. Aime *et al.* showed that entrapment of neutral multimeric Tm^{3+} complexes inside nonspherical liposomes with

an amphiphilic Tm^{3+} complex in the phospholipid bilayer yielded substantially larger shifts in the intraliposomal water resonance, compared with monomer agents (Figure 17) [53].

This same team also demonstrated that both the spherical and the osmotically shrunken liposomes, which had markedly different resonance frequencies of the intraliposomal water protons, allowed multiple visualization of two agents in the same image voxel [54].

The use of liposomes as responsive CEST agents was later on extended by encapsulating TmHPDO3A , together with a highly fluorinated compound (NH_4PF_6) inside a temperature-sensitive nonspherical liposome (Figure 18) [55].

Both the Tm^{3+} complex and the fluorinated compound served as an indicator of the liposome integrity as a function of temperature. At temperatures below the liposomal melting temperature, both agents remained encapsulated and a CEST signal from the intraliposomal water was observed while the ^{19}F signal was not observed, due to paramagnetic relaxation by the Tm^{3+} complex. However, at temperatures near the phase-transition temperature of the liposome membrane, both agents began leaking out and this resulted in the disappearance of the CEST effect and appearance of the ^{19}F signal. This nicely illustrated the principle of using a liposomal reporter system for delivery and release of drugs.

Future perspective: detecting & imaging specific disease biomarkers

In this brief review, we introduced the basic principles of CEST methods for introducing contrast in MRI, described the sensitivity advantages of amplifying a CEST signal by using polymers of both diamagnetic and paramagnetic CEST agents and reviewed the potential uses of endogenous -NH and -OH exchanges for imaging physiology. The potential advantages of using CEST methods for detecting and imaging endogenous biomarkers are numerous, yet many technical obstacles remain. One of the biggest limitations of imaging endogenous biomarkers is the chemical shift of exchangeable protons in biology is relatively small, while the number of different exchanging -OH and -NH groups are innumerable. Nevertheless, CEST is highly sensitive to the rates of proton exchange in these groups and these may be more sensitive to biology than we even realize at this stage. Thus, despite these inherent limitations, we anticipate that endogenous CEST will find useful applications in clinical medicine to help characterize specific disease states. We also reviewed the principles of exogenous PARACEST agents and their many potential applications as biosensors. One can easily predict that chemists will continue to find ways to optimize exchange rates in these systems and demonstrate exchange systems with much larger chemical shifts than those currently available. The possibility of imaging specific biomarkers of disease using PARACEST agents makes this an especially attractive area of research for young chemists.

Glossary

Chemical - exchange saturation transfer

Contrast mechanism based on the chemical exchange of spins between two chemically distinct sites, one of which can be saturated by using a frequency selective radio frequency pulse

Chemical shift

Refers to a frequency difference between any nucleus and a reference compound such as tetramethylsilane. Chemical shifts are reported in units of ppm; frequency difference/operating frequency of magnet

Chemical exchange

Dynamic process involving the exchange of labile protons of a compound in solution

Saturation

Condition in which nuclear spins aligned with an external magnetic field are promoted to a higher energy state where the spins become aligned against the magnetic field due to the application of a radio frequency pulse, leading to a decrease in net bulk spin magnetization

MRI contrast agents

Typically, paramagnetic complexes that highlight differences between normal and abnormal tissue in MRI

Paramagnetic chemical exchange saturation transfer

Chemical exchange saturation transfer arising from presaturation of exchangeable protons on a paramagnetic molecule or complex

DOTA-tetraamide

Octadentate ligand derived from 1,4,7,10-tetraazacyclo dodecane –1,4,7,10-tetraacetate (DOTA) by forming four amide bonds with the four appended carboxyl groups

Diamagnetic chemical-exchange saturation transfer

Chemical exchange saturation transfer arising from presaturation of exchangeable -NH and -OH protons on a diamagnetic molecule

Liposome-based chemical exchange saturation transfer

Chemical exchange saturation transfer arising from a liposomal system in which the resonances of the entrapped water protons are shifted away from the external water with the aid of an encapsulated paramagnetic shift reagent

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Executive summary

- Chemical exchange saturation transfer (CEST) agents are a new class of MRI contrast agents that produce contrast by decreasing the total water signal intensity observed in an magnetic resonance image.
- The structure of CEST agents can be altered to make them responsive to a variety of metabolites and physiological indicators.
- Polymeric, dendritic and macromolecular diamagnetic (DIA)CEST agents increase contrast agent sensitivity by allowing simultaneous saturation of numerous labile protons undergoing exchange with bulk water protons.
- Endogenous -NH and -OH protons from glycogen and glycosaminoglycans offer opportunities for imaging physiology.
- Similar to DIACEST agents, the contrast sensitivity of paramagnetic (PARA)CEST agents can be enhanced by employing the polymeric or dendritic platform to significantly lower the detection limit compared with monomeric PARACEST agents.
- Due to the multivalent properties of perfluorocarbon nanoparticles and the natural cell targeting capability of viruses, one can envision that more nanoparticle-based PARACEST agents will be used to detect low concentrations of biological targets, such as cell receptors.
- Liposome-based CEST is an innovative approach for lowering the detection limits of CEST agents into the sub-nanomolar range.
- Distorting liposome structure from spherical to nonspherical can further increase the shift difference between the internal and external water protons, making it more useful for MRI.

Figure 1. Chemical exchange saturation transfer mechanism between two distinct proton pools undergoing chemical exchange.

The application of a radio frequency pulse at the resonance frequency of the labile protons increases the number of spins aligned against the external magnetic field B_0 and this results in the saturation of the labile proton resonance. Since the two proton pools are in chemical exchange, the saturation is transferred to the bulk water resonance leading to a decrease in the bulk water signal intensity.

B_0 : Magnetic field; CEST: Chemical-exchange saturation transfer; RF: Radiofrequency.

Figure 2. The chemical exchange saturation transfer spectrum of 125 mM solutions of barbituric acid recorded at 300 MHz, pH 7.0 and 37°C.

The y-axis M_s/M_0 corresponds to the ratio of the bulk water signal intensity with saturation at the exchanging site (M_s) to the intensity without saturation (M_0) and is expressed as a percentage. The x-axis (saturation offset) refers to the chemical shift or frequency of the exchanging proton(s) at which the presaturation pulse is applied relative to the frequency of the bulk water protons set arbitrarily to 0 ppm. M_s : Exchanging site; M_0 : Saturation. Figure reproduced from data presented in [6].

Figure 3. The chemical exchange saturation transfer spectrum of a 30 mM solution EuDOTA-(glycine ethyl ester) 3+4 recorded at 270 MHz and 25°C.

For comparative purposes, the chemical exchange saturation transfer spectrum of barbituric acid is shown on the same scale as that of the Eu³⁺ complex.

M_s : Exchanging site; M_0 : Saturation.

Figure reproduced from data presented in [6].

Figure 4. Poly-L-lysine, poly-L-glutamate, polyethylenimine and generation-5 starburst polyamidoamine dendrimer and their corresponding chemical exchange saturation transfer spectra recorded on a 500-MHz VARIAN spectrometer using a saturation time of 10 s, saturation power of 100 Hz and an interscan delay of 17 s.

The y-axis S (normalized water intensity) is the ratio of the bulk water signal intensity with saturation at the exchanging site to the intensity without saturation.

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Figure 5. (A) Fragment of poly(rU), chemical exchange saturation transfer spectra of poly(rU) (5 μM), G-5 dendrimer (100 μM) and dendrimer-poly(rU) (100 μM /10 μM) and (B) asymmetry analysis removing water saturation effect using monochromatic irradiation power of 1 mW, temperature of 37°C and pH of 7.3.

The y-axis S_{sat}/S_0 is the ratio of the bulk water signal intensity with saturation at the exchanging site (S_{sat}) to the intensity without saturation (S_0).

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Figure 6. Fragment of glycogen showing exchangeable -OH protons (A), GlycoCEST spectra and MTR_{asymp} plots (B) for samples of glycogen in PBS buffer (pH 7.4) acquired at 9.4 T, 10 s presaturation pulse with saturation power of 1.9 μ T and temperature of 37°C.

The y-axis S/S_0 is the ratio of the bulk water signal intensity with saturation at the exchanging site (S) to the intensity without saturation (S_0). Figure reproduced with permission from [38]. © National Academy of Sciences, USA (2007).

Figure 7. Perfused fed mouse liver images acquired at 4.7 T and 37°C showing the depletion of glycogen upon perfusion of glucose-free media containing 500 pg/ml glucagon.

The first image (gray scale) shows chemical exchange saturation transfer (CEST) effect in the liver from presaturation at 1.0 ppm for 1 s at 3.0 μ T. The colorized glycoCEST images show CEST effect [MTR_{asym} (1 ppm)] as a function of perfusion time. Perfusion of glucagon causes the liver signal to increase, which corresponds to a decrease in CEST effect. Continuous perfusion of glucagon causes the disappearance of glycogen and the liver becomes more uniformly blue. Figure reproduced with permission from [38]. © National Academy of Sciences, USA (2007).

Figure 8.

(A) Fragment of glycosaminoglycans showing exchangeable -OH and -NH protons and (B) chemical exchange saturation transfer spectra of a cartilage obtained after trypsinization highlighting amide and hydroxyl proton regions and corresponding asymmetry plots.

The y-axis M_{sat} is the ratio of the bulk water signal intensity with saturation at the exchanging site to the intensity without saturation

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Figure 9. *Ex vivo* chemical exchange saturation transfer images taken from a trypsinization series on one bovine patella.

One side (marked depleted) was trypsinized for 60 min (A) and twice for 60 min (B), while the control side stayed immersed in PBS. (A) The difference image represents the subtraction of the image at $\delta = +1.0$ ppm from the image at $\delta = -1.0$ ppm. (B) *In vivo* chemical exchange saturation transfer images of a human patella and the difference image. The total duration of the presaturation pulse sequence was 320 ms at an average radio frequency power of 42 Hz.

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Figure 10.
Supramolecular adduct formed between polyarginine and $[Tm(Hdotp)]^{4-}$.

Figure 11. (A) Eu-monomer, a plot of water intensity (M_s/M_0) as a function of agent concentration (Eu-monomer, Eu-poly2 [2, 5 and 10%]; middle) and (B) HyperChem model of a polymeric paramagnetic chemical exchange saturation transfer agent.

M_s : Exchanging site; M_0 : Intensity without saturation.

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Figure 12. (A) Third-generation (G3) Yb-DOTAM-terminated poly(propylene imine) dendrimer, (B) Yb-monomer and (C) the corresponding proton-density weighted turbo spin-echo images at different pH. Reproduced with permission from [43]. © John Wiley & Sons.

Figure 13.

(A) Axial MR images of a MCF-7 mammary carcinoma grown in the flank mouse after injection of Eu-G5 and Yb-G2. (B) Decrease in MR signal of the tumor after injection relative to the average magnetic resonance signal before injection was used to measure the temporal changes of each chemical exchange saturation transfer effect.

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Figure 14. Perfluorocarbon-filled nanoparticle functionalized with numerous EuDOTA-(gly)₄[−] derivative on its surface.

Figure 15. Virus nanoparticle with a large number of paramagnetic chemical exchange saturation transfer agents conjugated on its surface.
Data from [49].

Figure 16. Liposome-based chemical exchange saturation transfer particles entrapping a hydrophilic Tm^{3+} complex in the cavity that results in a shift in the internal water resonance. Altering the liposomal shape showed different internal water shifts. **(A)** Spherical liposome gave an internal water shift of approximately +4 ppm and **(B)** nonspherical liposome gave an internal water shift of approximately +6.1 ppm and **(C)** nonspherical liposome incorporating an amphiphilic Tm^{3+} complex in the liposomal bilayer gave a much shifted internal water peak at approximately +30 ppm.

Figure 17. ^1H NMR spectra (14.1 T, 298 K) of shrunken liposomes incorporating a Tm^{3+} amphiphilic complex in the bilayer and encapsulating a mononuclear, binuclear and trinuclear Tm^{3+} complex.

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Figure 18. ^1H chemical exchange saturation transfer effect and the ^{19}F signal intensity as a function of temperature.

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