Acid-catalyzed proton exchange as a novel approach for relaxivity enhancement in Gd-HPDO3A-like complexes†

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A current challenge in medical diagnostics is how to obtain high MRI relaxation enhancement using GdIII-based contrast agents (CAs) containing the minimum concentration of GdIII ions. We report that in GdHPDO3A-like complexes a primary amide group located in close proximity to the coordinated hydroxyl group can provide a strong relaxivity enhancement at slightly acidic pH. A maximum relaxivity of \( r_1 = 9.8 \text{ mM}^{-1} \text{s}^{-1} \) at acidic pH was achieved, which is more than double that of clinically approved MRI contrast agents under identical conditions. This effect was found to strongly depend on the number of amide protons, i.e. it decreases with a secondary amide group and almost completely vanishes with a tertiary amide. This relaxivity enhancement is attributed to an acid-catalyzed proton exchange process between the metal-coordinated OH group, the amide protons and second sphere water molecules.

Introduction

GdIII based MRI contrast agents (CAs) are used in about 40–50% of clinical scans to increase the tissue contrast in relaxation weighted images, by shortening the relaxation times of the water molecules in their proximity. Although some of these CAs have successfully been on the market for more than 30 years, strategies to obtain high MRI relaxation enhancement with the minimum concentration of GdIII are still a challenge in the field of medical diagnostics.† The exchange of the mobile protons from a GdIII complex with the bulk water is one of the processes able to enhance the nuclear relaxation rate of solvent water protons.† Among the clinically approved GdIII CAs, Gd(HPDO3A) [HPDO3A = 10-(2-hydroxypropyl)-1,4,7,10-tetra-azacyclododecane-1,4,7-triacetic acid; Scheme 1] is a neutral complex featuring a coordinated hydroxyl group and a water molecule, both of which contain protons responsible for transferring the paramagnetism of the GdIII ion to bulk water. Typically, the hydroxyl proton of Gd(HPDO3A) is involved in slow exchange with the bulk water at physiological pH.

Scheme 1 The GdIII complexes synthesized and discussed in the present work.
Thus, Ln(HPDO3A) complexes have been used for Chemical Exchange Saturation Transfer (CEST) MRI applications, exploiting the –OH group as a saturable pool of protons. The relatively short Gd–OH bond distance (Gd–OH: 2.32 Å, Gd–OH2: 2.51 Å) results in a stronger contribution to the relaxivity ($r_1$) than that of the coordinated water molecule. At pH > 10, the interplay of the short exchange lifetime of the hydroxyl proton ($t_{ex} = 1/k_{ex} = 1/k_{OH}[OH^-]$), where $k_{OH} = 5.2 \times 10^9 \text{M}^{-1} \text{s}^{-1}$) with the short Gd–OH distance leads to a substantial base-catalyzed proton exchange contribution to $r_1$ ($\Delta r_1 = 1.2 \text{mM}^{-1} \text{s}^{-1}$). Moreover, it has recently been shown that the proton exchange of the hydroxyl group in Gd(CF$_3$-HPDO3A) (Scheme 1) can be accelerated by the basic component of buffers (e.g. phosphate, carbonate and HEPES) allowing a slight increase in $r_1$ even at neutral pH. Also, the introduction of an appropriate group in close proximity to the metal ion has been shown to modulate $r_1$ by restricting rotation about the Gd–O$_w$ bond or by accelerating proton exchange. Acid-catalysed proton exchange has also been observed in the case of Gd(DOTA-tetrais(N-methylacetamide), a tripotent Gd$^{3+}$ complex with a very slow water exchange rate ($k_{ex} = 4.5 \times 10^5 \text{s}^{-1}$). The value of $r_1$ is doubled at pH ≈ 1 compared to neutral pH, while the rate constant of the acid-catalyzed exchange of the inner-sphere water protons, $k_{ac}$, is 2.4 × 10$^6 \text{M}^{-1} \text{s}^{-1}$. Also, in the case of Gd(DOTA), lowering the temperature to 273 K, and thus reducing $k_{ex}$, resulted in a slight increase of $r_1$ due to acid-catalysed proton exchange.

In this work, we expanded the group of HPDO3A-like ligands by replacing the methyl group in the ordinary hydroxypropyl arm of HPDO3A with electron-withdrawing amide groups. Our ultimate goal was to investigate the effect of labile amide protons in the proximity of the coordinated hydroxyl group on the proton exchange relaxation enhancement. Thus, three new GdHPDO3A-like complexes containing primary, secondary and tertiary amides (Gd(HPADO3A), Gd(BzHPADO3A) and Gd(PipHPADO3A), Scheme 1) were synthesized and investigated by $^1$H and $^{17}$O NMR relaxometry as well as CEST, and solution thermodynamic and kinetic studies.

## Results and discussion

### Synthesis

The ligand HPADO3A was obtained from the ring opening of glycidamide with the secondary amine of DO3A(tBuO)$_3$, followed by deprotection of the t-butyl esters with trifluoroacetic acid (TFA) and dichloromethane (DCM). The ligands containing secondary and tertiary amides were prepared by the reaction of DO3A(tBuO)$_3$ and methyl (2R)-glycinate, followed by aminolysis with benzyllamine (BzHPADO3A) or piperidine (PipHPADO3A). Then, the final ligands were obtained by deprotection of the t-butyl esters using TFA/DCM (1 : 1) and the Gd$^{3+}$ complexes by the reaction of the ligand with GdCl$_3$ at pH = 7.0 in aqueous solution.

### pH dependence of relaxivity

The relaxivity vs. pH profiles of the three Gd-HPADO3A complexes, measured at 20 MHz and 298 K (Fig. 1), show a significant change in the $r_1$ values at pH 6–7. At pH > 7, the $r_1$ values for all three Gd$^{3+}$-complexes are similar and comparable to those measured for Gd(HPDO3A) and related systems. However, significant differences were found in the $r_1$ values at acidic pH; the relaxivity is very high for Gd(HPADO3A) ($r_1 = 9.8 \text{mM}^{-1} \text{s}^{-1}$) and it gradually decreases by reducing the number of amide protons on the ligands. Thus, a remarkable enhancement is detectable for Gd(HPADO3A) in the physiologically relevant 7.4–5.5 pH range ($\Delta r_1 = 5.5 \text{mM}^{-1} \text{s}^{-1}$ from pH 5 to 7.4). This $\Delta r_1$ is similar to those found for the well-known pH-responsive systems such as Gd$^{3+}$-complexes of DO3A-sulfonamides ($\Delta r_1 = 5.5$) or DO3A-aminoethyl-derivatives ($\Delta r_1 = 3.4$). In these examples, the variation of $r_1$ was attributed to a change of hydration state of the Gd$^{3+}$ complex from $q = 2$ to $q = 0$, under acidic and basic conditions, respectively. In contrast, the large $\Delta r_1$ of Gd(HPADO3A) cannot be explained by the change of the hydration state, because the inner-sphere water molecule remains coordinated in the entire pH range as confirmed by $^{17}$O NMR studies (see below and the ESI†).

### Analysis of the proton exchange mechanism

Under acidic conditions, the higher relaxivity is presumably due to the proton exchange between the –OH group of Gd(HPDO3A) and the bulk. The overall relaxivity, $r_1$, is given by eqn (1):

$$ r_1 = r_1^{ex} + r_1^{ac} + r_1^{ac} $$

(1)
where $r^{rel}_{i}$ and $r^{os}_{i}$ are the relaxivity components due to the inner and outer sphere water molecules and the $\text{\textendash}\text{OH}$ group, respectively. $r^{pr}_{i}$ can be expressed as follows:

$$r^{pr}_{i} = \frac{c}{111.1} \frac{1}{T^{H}_{ip} + \tau_{p}}$$

where $c$ is the concentration of the complex, and $T^{H}_{ip}$ and $\tau_{p}$ are the longitudinal relaxation time and the lifetime of the $\text{\textendash}\text{OH}$ proton, respectively. The characteristic pH dependence of the proton transfer process via Gd(L)H\textsuperscript{+} complex cannot be operative in the enhancement of the magnetic activity is proportional to the number of protons of the Gd(HPADO\textsubscript{3}A), to the tertiary amide, Gd(PipHPADO\textsubscript{3}A). An interpretation is also supported by the CEST spectra of the Eu\textsuperscript{III} complexes with HPADO\textsubscript{3}A-derivatives (Fig. 2, S15 and S16\textsuperscript{+}). In this case, fast proton exchange between the OH and amide protons results in a broad coalesced signal at $\text{\textasciitilde}26$ ppm under acidic conditions. The interaction between hydroxyl and amide groups vanishes upon deprotonation of the OH group and the corresponding CEST signal disappears.

The mechanism proposed is also consistent with earlier studies on proton exchange processes of several amides with water.\textsuperscript{14} The acid and base catalysis in such processes was interpreted by considering the (de)protonation of the amide group via the formation of H-bonded complexes between the proton donors and acceptors.\textsuperscript{15} This mechanism requires the diffusion-controlled formation of a H-bonded complex and subsequently the rapid separation of the corresponding conjugate acid and base.\textsuperscript{16}

In accordance with Scheme 2, the rate of proton exchange is $r_{i} = k_{H}[\text{H}^{+}][\text{GdL}]$. Because of the fast internal rearrangement, the alcoholic $\text{\textendash}\text{OH}$ and amide $\text{\textendash}\text{CONH}_{2}$ protons cannot be distinguished and their lifetime is $\tau_{p} = (k_{H}[\text{H}^{+}])^{-1}$. Thus, eqn (2) can be rewritten as follows:

$$r_{i} = \frac{1}{1 + K_{Gd(L)H^{+}}[H^{+}]^{2}} \left[ \frac{N}{T^{H}_{ip} + (k_{H}[H^{+}])^{-1}} \right] + \frac{cK_{Gd(L)H^{+}}[H^{+}]}{111.1} \left[ \frac{Gd(L)H^{+}+Gd(L)H^{+}\text{\textendash}\text{CONH}_{2}}{Gd(L)H^{+}+Gd(L)H^{+}\text{\textendash}\text{CONH}_{2}} \right]$$

where $N$ is the number of labile protons ($N = 1\text{--}3$), and $Gd(L)H_{1}^{\text{\textendash}CONH_{2}}$ and $Gd(L)H_{1}^{\text{\textendash}CONH_{2}}$ are the sum of $r^{rel}_{i}$ and $r^{os}_{i}$, for GdL and Gd(L)H\textsubscript{1} respectively. The experimental data (Fig. 1) were fitted to eqn (3) and the results are listed in Table 1.

The H\textsuperscript{+} assisted exchange of the labile protons of GdHPADO\textsubscript{3}A-derivatives is characterized by very similar $k_{H}$ and $T^{H}_{ip}$ values (Table 1), confirming analogous behaviour of these complexes. In each system, $k_{H}$ is about an order of magnitude larger than the typical rate constants for diffusion-controlled proton exchange processes between conjugate acid--base pairs. This lends strong support to the simultaneous double-site
exchange mechanism proposed in Scheme 2. The results also verify that the proton relaxation enhancement of GdHPADO3A-derivatives, under acidic conditions, is exchange controlled \((T_1^p \ll 1/k_\text{ex}([H^+]))\) at pH > 5.0 and relaxation controlled \((T_1^p \gg 1/k_\text{ex}([H^+]))\) at pH < 5.0.

In order to obtain further information on the mechanism of the acid–base catalysed proton-exchange processes of GdHPADO3A-derivatives, the relaxivity of GdIII-complexes was measured in the absence and in the presence of NH₄Cl at pH = 6.0, 298 K and 20 MHz (Fig. S6†). The \([\text{NH}_4\text{Cl}]\) dependent relaxation enhancement can readily be interpreted by considering that NH₄⁺ as a Bronsted base, catalyzes the exchange of the –OH proton. The corresponding rate constant is \(k_\text{OH}^\text{B} \) These are practically the same for the three GdHPADO3A-derivatives and very similar to \(k_\text{OH}^\text{A} \), which characterizes the OH⁻ ion assisted, diffusion-controlled proton-exchange process of the hydroxyl proton in Gd(HPADO3A) (Table 1). This result clearly confirms that the exchange between NH₄⁺ and the labile protons of Gd(HPADO3A) derivatives proceeds via the general base catalysed proton transfer mechanism.†

**CEST experiments**

Further insight into the mechanism of the acid–base catalyzed proton-exchange process was obtained by Chemical Exchange Saturation Transfer Magnetic Resonance Imaging (CEST-MRI) experiments, on EuIII complexes of HPADO3A-derivatives at variable pH (Fig. 2 and S13–S16†). A clear decrease of ST% effect was observed by moving from acidic to basic pH. This seems to be consistent with deprotonation of the coordinated hydroxyl group. In particular, for a 20 mM water solution of Eu(HPADO3A), one CEST peak is present at 26 ppm, with an ST% that decreases from 14.9% at pH = 4.6 to ca. 2% at pH = 7.5 (\(B_1 = 12 \mu T\)). In order to evaluate the presence of two distinguishable peaks, the Z-spectrum of EuHPADO3A was acquired at low temperature (278 ± 2 K) and pH 4.5 by using a pre-saturation pulse of 6 µT. As reported in Fig. S15,† two peaks are clearly detectable, belonging to the two exchangeable pools of protons. Upon increasing the temperature, there is an increase of exchange rate and coalescence of the two peaks. Analogously, upon using higher \(B_1\) fields (e.g. 12 µT) there is a broadening of the signals that are no more distinguishable. This behaviour was also confirmed by Z- and ST-spectral simulation of the Eu(HPADO3A) complex by using Bloch equations modified for chemical exchange† considering a three-pool exchange system with bulk water at zero –OH at 30 and –NH₄ at 25 ppm.

The same pH dependence as for Eu(HPADO3A) was observed for Eu(BzHPADO3A), but the ST% measured under the same conditions as those for Eu(HPADO3A) (pH 4.6 and 20 mM) was lower (8.2%) due to the presence of two exchangeable protons instead of three (Fig. S15†). On the other hand, for Eu(PipHPADO3A) the CEST signal was too weak to be observed (Fig. S16†). This pH dependent behaviour is also shown clearly in Fig. 2C through the CEST-MR image of phantoms containing Eu(HPADO3A) at different pH values, as previously highlighted in the case of a dimeric Eu₃(HPADO3A)₂ complex.†

**Relaxometric analysis**

A detailed \(^1\)H and \(^17\)O NMR relaxometric study (including NMRD profiles at 298 and 310 K, temperature dependence of the \(^17\)O NMR transverse relaxation rate, \(R_3\), and paramagnetic shift, \(\Delta\omega_p\) of the solvent water, Fig. 3 and S7–S12†) was carried out under both acidic and neutral conditions. A least-square fit of the data was carried out using the established theory of paramagnetic relaxation. This is expressed by the Solomon-

### Table 1 Kinetic and relaxation parameters for the proton exchange reactions of the GdIII complexes of HPADO3A, BzHPADO3A and PipHPADO3A compared to Gd(HPADO3A) (20 MHz, 0.15 M NaCl, 298 K)

|        | \(k_\text{ex}^\text{B} \times 10^{-11}\) | \(k_\text{ex}^\text{A} \times 10^{-10}\) | \(k_\text{ex}^\text{B} \times 10^{-9}\) |
|--------|--------------------------------------|--------------------------------------|--------------------------------------|
| HPA-D03A | 4.57 ± 0.07                          | 4.32 ± 0.03                          | 5.6 ± 0.2                            |
| BzHPA-D03A | 4.57 ± 0.04                          | 4.32 ± 0.03                          | 5.6 ± 0.4                            |
| PipHPA-D03A | 4.57 ± 0.04                          | 4.32 ± 0.03                          | 5.6 ± 0.4                            |
| HP-D03A | 4.57 ± 0.07                          | 4.32 ± 0.03                          | 5.6 ± 0.2                            |

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Bloomer–Morgan and Freed equations,\textsuperscript{19} for the inner sphere (IS) and outer sphere (OS) proton relaxation mechanisms, respectively, and the Swift–Connick textsuperscript{20} theory for \textsuperscript{17}O relaxation. The NMRD profiles at acidic pH were fitted adding a fixed proton exchange contribution of $r_1 (q_{IS}^0)$ to the equations (Table 2). Moreover, the contribution of second sphere (SS) water molecules, H-bonded to the amide group, was also considered ($r_{Gd(SS)}^0 = 3.5 \AA, q_{SS}^0 = 3-5$). Such a mechanism was also previously reported for the dimeric Gd\textsubscript{2}(HPADO\textsubscript{3}A)\textsubscript{2} complex where the increase of $r_1$ could be explained only by taking into account the contribution of 4 SS water molecules.\textsuperscript{18}

In all complexes, the coordinated water molecule is in fast exchange with the bulk ($r_M$ in the range 21.6–45.0 ns, Table 2), with values comparable to the dimeric complex Gd\textsubscript{2}(HPADO\textsubscript{3}A)\textsubscript{2} (ref. 18) and the recently reported Gd(HPDO\textsubscript{3}MA) (Scheme 1),\textsuperscript{23} but almost one order of magnitude faster than in the case of Gd(HPDO\textsubscript{3}A).\textsuperscript{4} Furthermore, the presence of an important second sphere contribution supports the proton exchange mechanism shown in Scheme 2. In this mechanism, SS water molecules have a strong interaction with the amide groups and are involved in the proton exchange between the hydroxyl and amide groups. The rotational correlation time of these SS water molecules – $\tau_{SS}^0 \approx 10-30$ ps – is in line with the values reported in the literature.\textsuperscript{22}

The relaxivity was also measured (pH 7.4, 298 K and 20 MHz) by dissolving the three complexes in reconstituted human serum (Seronorm). The $r_1$ values reported in Table 2 are higher than those measured in pure water; in particular, a 56% increase is observed for Gd(HPADO\textsubscript{3}A), 71% for Gd(BzHPADO\textsubscript{3}A) and 85% for Gd(PipHPADO\textsubscript{3}A). The significant $r_1$ increase observed in

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
 & \textbf{Gd(HPADO\textsubscript{3}A)} & \textbf{Gd(BzHPADO\textsubscript{3}A)} & \textbf{Gd(PipHPADO\textsubscript{3}A)} \\
\hline
\textbf{Parameter}\textsuperscript{a} & \textbf{pH 4.2} & \textbf{pH 7.4} & \textbf{pH 4.2} & \textbf{pH 7.4} & \textbf{pH 4.2} & \textbf{pH 7.4} \\
\hline
$\tau_{m}^{20}$ (mM$^{-1}$ s$^{-1}$) & 9.8 (2) & 4.3 (1) & 7.2 (1) & 4.5 (1) & 5.3 (2) & 4.6 (1) \\
$\tau_{m}^{20,298}$ (mM$^{-1}$ s$^{-1}$) & 4.99 (2) & 6.7 (1) & 2.87 (2) & 7.7 (1) & 8.5 (1) \\
$\tau_{m}^{298}$ (mM$^{-1}$ s$^{-1}$) & 12.0 (7) & 8.5 (1.1) & 6.8 (1.6) & 10.2 (1.4) & 10.1 (1.0) & 3.5 (9) \\
$\tau_{m}^{298}$ (ns) & 4.5 (6) & 13.8 (8) & 5.0 (9) & 13.1 (9) & 10.4 (7) & 25.2 (9) \\
$\tau_{m}^{29}$ (ps) & 24.5 (5) & 20.2 (2.4) & 21.6$^b$ & 24.2 (2.1) & 21.6$^b$ & 45.0 (1.4) \\
$\tau_{m}^{23}$ (ps) & 62.0 (1.2) & 62.0 (1.3) & 70.8 (2.1) & 70.8 (1.5) & 82.2 (1.5) & 82.2 (2.0) \\
$\Delta H_m$ (kJ mol$^{-1}$) & 15.3 (7) & 14.9 (9) & 13.0 (5) & 7.3 (3) \\
$A/m$ (10$^6$ rad s$^{-1}$) & $-3.4$ (1) & $-3.4$ (1) & $-3.4$ (1) & $-3.4$ (1) \\
$s^0$ & 5 & 5 & 4 & 4 \\
$\tau_{m}^0$ (ps) & 30.0 (2.5) & 12.7 (9) & 24.5 (2.1) & 23.2 (1.0) & 24.0 (1.3) & 10.1 (9) \\
\hline
\textsuperscript{a} The parameters fixed in the fitting procedure are $q = 1$, $r_{GdO} = 2.5$ Å, $r_{GdH} = 3.0$ Å, $r_{GdH} = 4.0$ Å, $298^{0}$D$_{GdH} = 2.25 \times 10^{-5}$ cm$^{-2}$ s$^{-1}$, $E_h = 18$ kJ mol$^{-1}$, $E_r = 1$ kJ mol$^{-1}$, and $r_{Gd(SS)} = 3.5$ Å$^{*}$.$^b$ These values were fixed considering the value obtained for Gd(HPADO\textsubscript{3}A) at pH 4.2.
\end{tabular}
\caption{Selected best-fit parameters obtained from the analysis of the 1/T$_1$ $^1$H NMRD profiles (298 and 310 K) and $^{17}$O NMR data for Gd(HPADO\textsubscript{3}A), Gd(BzHPADO\textsubscript{3}A), and Gd(PipHPADO\textsubscript{3}A)\textsuperscript{25}}
\end{table}

Fig. 3 $^1$H NMRD profiles of Gd(HPADO\textsubscript{3}A) (black circles), Gd(BzHPADO\textsubscript{3}A) (red squares) and Gd(PipHPADO\textsubscript{3}A) (blue triangles) at pH 4.2 (top) and 7.4 (below).
Conclusions

In conclusion, we have shown that acid-catalyzed proton exchange processes can strongly enhance the relaxivity and saturation transfer of LnIII complexes, when amide protons are present in the proximity of the metal ion coordinated hydroxyl group. A mechanism to explain such behaviour was proposed, which involves protonation, hydrogen-bonding with second sphere water molecules, and a concerted rearrangement of the hydroxyl bonds. Moreover, fitting the \( t_1 \) vs. pH curves allowed the kinetic parameters for the simultaneous double-site proton exchange process to be obtained. This resulted in a \( k_H \) value ca. 10 times larger than the typical rate constants for diffusion-controlled proton-exchange, between conjugate acid–base pairs.

Both relaxation and saturation transfer enhancements are pH dependent and they disappear when the hydroxyl group deprotonates at pH 6–7. However, a \(^{1}H\) and \(^{17}O\) NMR relaxometric study, at both acidic and neutral pH, demonstrated that no evident differences exist in the relaxation parameters such as \( \tau_M, \Delta H_M \) and \( t_M \). The water exchange rate is fast (\( \tau_M \approx 20 \text{ ns} \)) in

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Table 4  Rate constants (\( k_i \)) and half-lives (\( t_{1/2} = \ln 2/k_i \)) characterising the dissociation reactions of Gd(HPADO3A), Gd(DOTA), Gd(HPDO3A) and Gd(BT-DO3A) complexes at 298 K

|           | Gd(HPADO3A) | Gd(DOTA) | Gd(HPDO3A) | Gd(BT-DO3A) |
|-----------|-------------|----------|------------|-------------|
| \( k_1 \) (M\(^{-1} \) s\(^{-1} \)) at pH = 7.4 | (1.6 ± 0.1) \times 10^{-4} | 1.8 \times 10^{-6} | 2.9 \times 10^{-4} | 2.8 \times 10^{-5} |
| \( k_2 \) (s\(^{-1} \)) at pH = 7.4 | 6.41 \times 10^{-12} | 7.28 \times 10^{-14} | 1.15 \times 10^{-11} | 1.35 \times 10^{-12} |
| \( t_{1/2} \) (hour) at pH = 7.4 | 3.00 \times 10^{-7} | 2.64 \times 10^{-9} | 1.67 \times 10^{-7} | 1.42 \times 10^{-8} |

\( a \) Ref. 30b, 0.15 M NaCl, 298 K. \( b \) Ref. 27, 0.1 M NaCl, 298 K.
all complexes and a second sphere contribution was considered, to fit the NMRD data.

Furthermore, Gd(HPADO3A) was shown to maintain, and even improve, the thermodynamic stability and kinetic inertness compared to the clinically approved Gd(HPADO3A). This indicates that the presence of the amide substituents attached to the hydroxy-propyl side chain does not alter the thermodynamic and kinetic properties of the resulting Gd(III)-complexes.

Finally, it must be emphasised that the acid-catalysed proton exchange is an important approach for relaxivity enhancement. It opens the way for the design of several innovative chemical structures, not only in Ln(III)-based agents, but also for other paramagnetic metal complexes that can improve their MRI efficiency via this mechanism. Moreover, the modulation of these proton exchange processes may allow the determination of important physico-chemical parameters (e.g. pH, temperature, concentration of important anions and cations, etc.) in vivo, by classical $T_1$, CEST-MRI or a combination of these techniques.

Conflicts of interest
There are no conflicts to declare.

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