Coordination-Responsive Longitudinal Relaxation Tuning as a Versatile MRI Sensing Protocol for Malignancy Targets

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Biomarkers (e.g., acidity, H$_2$O$_2$, hypoxia, and specific molecules) as one primary component of tumor microenvironments are closely associated with occurrence, invasion, and metastasis of malignancy, thus can act as biological targets. However, their monitoring remains a challenging task. Herein, a coordination-dependent longitudinal relaxation tuning (CLRT) that occurs between a Mn$^{2+}$ “donor” and a Mn$^{2+}$ “acceptor” is established to enable biological target sensing. Relying on the differences of coordination ability and spatial structure between donors and acceptors, the biological targets as Mn$^{2+}$ acceptor can take Mn$^{2+}$ away from the donors (i.e., modified ligands) in nanoscale probes, which consequently varies T1-weighted (T1W) magnetic resonance imaging (MRI) signal. The coordination ability and spatial structure of the modified Mn$^{2+}$ “donor” and the pore diameter of donor carrier are demonstrated to determine the feasibility, specificity, and generality of CLRT. With CLRT, this MRI-based ruler is demonstrated for the successful specific detection of biological targets (i.e., hyaluronic acid and glutathione) of malignancy, and its potential in quantitative measurement of hyaluronic acid is further demonstrated. CLRT can serve as a novel and general sensing principle to augment the exploration of a wide range of biological systems.

1. Introduction

It has been extensively documented that tumor microenvironments (TMEs) play a predominant role in development, invasion, and metastasis of malignancy. A comprehensive understanding of TME is a prerequisite of establishing high-efficient treatment approaches. Therefore, monitoring TMEs’ biomarkers using many clinically noninvasive medical imaging technologies, e.g., ultrasound (US), photoacoustic (PA), magnetic resonance imaging (MRI), has aroused considerable attention with the progress of bio-nanotechnology. In an attempt to explore biomarker sensing and tumor grading, a large variety of imaging principles have been developed to realize specific detection of some certain biomarker, whereas the versatility of these principles is poor. Typically, the rule of fenton or fenton-like principle was only confined to H$_2$O$_2$ sensing, and the principle of pH-responsive Mn$^{2+}$ dissolution-enhanced T1 MRI signals is limited to pH sensing. New sensing protocol featuring high specificity and maximum versatility is still desirable but challenging.

Coordination-responsive phenomena such as ligands exchange-mediated hydrophobicity–hydrophilicity transition have been a reliable tool in biomedical research and gains increasing interest in scientific community. As a paradigm, ligands exchange resulting from competitive coordination can augment the application window via switching hydrophobic nanoparticles into hydrophilic ones preferably applicable for biological applications. In this study, a coordination-responsive magnetic resonance tuning phenomenon, i.e., coordination-responsive longitudinal relaxation tuning (CLRT), has been established to detect biological targets. CLRT originates from the competitive coordination, where the biological targets as...
Mn$^{2+}$ acceptor seize Mn$^{2+}$ from the donors (modified ligands) grafted in mesoporous carriers due to its stronger coordination ability toward Mn$^{2+}$. Similar to distance-dependent magnetic resonance tuning (MRET),[8] this novel sensing principle is also basing on the variation of T1 magnetic resonance imaging (MRI) signals, since the more complex spatial structure of biological target can impair H$_2$O longitudinal relaxation when the target is detected. Thus, CLRT also features a high versatility after rationally designing Mn$^{2+}$ donor and mesoporous carriers aiming at a given biological target (designated as Mn$^{2+}$ acceptor). This T1 modality was the preferable one in comparison to T2 modality in MRI.[8]

To explore its potential, two tailor-made model systems was designed to detect two different tumor biological targets using the principle of CLRT, i.e., hyaluronic acid (HA) and glutathione (GSH), respectively.[9] More significantly, we explored the determining factors of CLRT, and evaluated influences of carriers’ pore diameter, coordination ability, and spatial structure of Mn$^{2+}$ donor (modification ligands) on the specificity and feasibility of CLRT. Furthermore, the CLRT probe (i.e., HA) was employed to quantitatively detect HA in both in vitro and in vivo levels, consequently reflecting the potential of CLRT in quantitative analysis of various biomolecules in living systems.

2. Results and Discussion

2.1. CLRT Principle

The CLRT principle involves three components, a modified ligands (Mn$^{2+}$ donor, A), a biological target (Mn$^{2+}$ acceptor, B), and a mesoporous carrier (C). Mesopore size of C and the differences of coordination ability and spatial structure between A and B determine the CLRT phenomenon (Figure 1a). In detail, depending on its less size than the mesopore size of C and larger coordination ability than A, Mn$^{2+}$ acceptor (B) is allowed to enter large mesopore of C, snatch Mn$^{2+}$ from C–A–M (M presenting Mn$^{2+}$) and generate B–M via the coordination exchange, realizing CLRT. The variation of longitudinal relaxation rate (1/T1) of Mn$^{2+}$ can be employed to account for the CLRT, and 1/T1 is obtained according to the inner-sphere relaxation formula (Equation 1).[10]

$$\frac{1}{T_1} = \frac{q P_m}{T_{1m} + \tau_m}$$

where $P_m$ is the mole fraction of Mn$^{2+}$, $T_{1m}$ is the relaxation time of water proton spin, $q$ is the number of bound water molecules per Mn$^{2+}$ ion, $\tau_m$ is the lifetime of the solvent molecule in the complex ($\tau_m$ is the reciprocal of the solvent exchange rate, $k_e$). Among them, $T_{1m}$, $q$, and $\tau_m$ are highlighted, because they influence the degree of CLRT phenomenon in this model (Figure 1a).

C–A can be regarded as a whole chelator of Mn$^{2+}$, and it features larger spherical radius and higher rigidity than B chelator. Therefore, Mn$^{2+}$ in B–M produced after coordination exchange shares shorter rotational correlation time ($\tau$) and longer $T_{1m}$ than that in C–A–M, consequently slowing relaxation of water proton spin in B–M (Figure 1b), since larger molecule or size of Mn$^{2+}$ chelator is beneficial for improving $\tau$ and decreasing $T_{1m}$.[10] This result eventually decreases longitudinal relaxation rate of Mn$^{2+}$ (1/T1), resulting in a weaker T1-weighted (T1W) MRI signal. With neglecting the limitation of C mesopore toward the water exchange, the more dense spatial structure of B than A also increases $\tau_m$ (Figure 1c), which further contributes to the decreased 1/T1 and T1W MRI. Therefore, the T1W can be expected to be tuned via the CLRT principle, and the biological target B can be detected when C–A is rationally designed. In particular, the decreased MRI signal as ruler via CLRT is more preferable than previous protocols, since it is difficult to discern whether the intensified MRI signal is resulted from particle accumulation or biological target triggering in previous protocols when using increased T1W MRI signal to reflect biological target.[6a,8]

2.2. CLRT Exploration in Hyaluronic Acid (HA) Detection

The CLRT allows the variation of the T1 MRI signal to be utilized as a nanoscale ruler for detection of malignancy targets or biomarkers. To demonstrate its potential in detail, a model system with three components is designed, wherein C and A are mesoporous organosilica nanoparticles (MONs) (i.e., mesoporous carrier) and modified folic acid (FA) (i.e., Mn$^{2+}$ donor), respectively, and a biomarker of malignancy, i.e., HA,[9a,b] is used as the Mn$^{2+}$ acceptor (B). The C–A–M structure consisting of MON, FA, and Mn$^{2+}$ (abbreviated into MON–FA–Mn) was obtained (Figure 2a), wherein MONs platform composed of organic framework (R = –S–S–S–S–) were first prepared via a well-established method,[11] followed by –NH$_2$ and FA modifications and Mn$^{2+}$ chelation. The monodispersed MON–FA–Mn shares a maximum pore diameter of 13 nm (Figure 2; Figure S1, Supporting Information) that can allow most biological targets to enter mesopores and simultaneously ward off ultralarge metal transport proteins (MTPs). The chelated Mn$^{2+}$ ions are uniformly distributed in mesoporous channels of MON platform (Figure 2f–i). The twice variations of zeta potential and two emerging stretching vibration peaks of –NH$_2$ from 1027 to 1005 cm$^{-1}$ and C–O at 1730 cm$^{-1}$ from 16.1 mV. X-ray photoelectron spectroscopy (XPS) spectrum of MON–FA–Mn indicates the valence of Mn in MON–FA–Mn is +2 (Figure S4, Supporting Information).

The tumor biomarker associated with tumor metastasis,[9b] i.e., HA, was attempted to explore the potential of CLRT using its tailor-made and specific model probe (MON–FA–Mn). The
detailed process is indicated in Figure 3a, wherein the biological target, i.e., HA (B) molecules, can capture Mn$^{2+}$ from MON–FA–Mn (C–A–M) due to its stronger coordination than FA (A). This coordination exchange of Mn$^{2+}$ from C–A–M to B–M alters the $\tau_R$, $T_{1m}$, and $\tau_m$, because the more complex spatial structure of HA than FA intensify the stereo-hindrance effect against H$_2$O and the smaller size and poor rigid of HA chelator than MON–FA chelator reduce $\tau_m$.[10] This phenomenon consequently results in a longitudinal relaxation variation that can be reflected by in vitro T1W MRI. It is clearly found that the T1W MRI performance (i.e., contrast and signal intensity) of MON–FA–Mn evidently drops when incubating with HA.
via comparing labeled number 1 and number 4 (Figure 3b,c), indicating Mn$^{2+}$ capture by HA from MON–FA–Mn results in coordination-responsive MRI. The electron spin resonance (ESR) spectra of MON–FA–Mn before and after incubating with HA and HA solution before and after incubating with MON–FA–Mn demonstrate Mn$^{2+}$ ions departure from MON–FA–Mn (Figure 3d) and capture by HA (Figure 3e), consequently equipping HA solution with T1W MRI (Figure 3b,c) and resulting in an emerging UV–vis peak of HA–Mn at 310 nm in the UV–vis spectra (Figure 3g).\[14\] Although Mn$^{2+}$ entrapped in HA (labeled number 5) is more than that in labeled number 4 (74\% vs 26\%) (Figure 3f), the poorer T1W-MRI outcome is observed. This result sufficiently demonstrates the reduced rigidity and radius of HA in comparison to MON–FA indeed diminish $\tau_R$ and increase $T_1m$, and larger stereo-hindrance in HA–Mn$^{2+}$ severely hampers the exchange of H$_2$O molecules bounded in HA–Mn and increases $\tau_m$.\[10\] Therefore, T1W-MRI performance of the incubated mixture of MON–FA–Mn and HA (labeled number 2) is lower than that in labeled number 1, demonstrating the potential of this CLRT.

Figure 2. Syntheis and characterizations of CLRT probe (i.e., MON–FA–Mn). a) Preparation and composition schematic of MON–FA–Mn. b,c) TEM images of MON–FA–Mn. d) SEM and e) dark-field STEM images of MON–FA–Mn. f–i) Atom mapping images of MON–FA–Mn including O, Si, S, Mn. j) Zeta potential of the intermediate products (e.g., MON, MON–NH$_2$, and MON–FA) and the ultimate MON–FA–Mn. k) FTIR spectra of MON, MON–NH$_2$, and MON–FA. l) Raman spectra of MON–FA and MON–FA–Mn.
Figure 3. CLRT explorations via using MON–FA–Mn as the probe for HA detection. a) Operation schematic of CLRT for HA detection using MON–FA–Mn in which LRT represents longitudinal relaxation time. b–c) T1W MRI images (c) and d) corresponding T1W signal intensities of labeled number 1–6, wherein labeled number 1–6 represent MON–FA–Mn, the mixture of MON–FA–Mn and HA, HA solution, the centrifugal MON–FA–Mn after incubation with HA for 1.0 h, HA solution after incubation with MON–FA–Mn for 1.0 h, and water, respectively. e) ESR spectra of labeled number 1 (Sample A) and labeled number 4 (Sample B). f) Normalized Mn content of labeled number 1–6 represent P < 0.01, 0.005, and 0.001, respectively. Data are presented as the mean ± SEM. g) UV-vis spectra of labeled number 3 (HA solution) and labeled number 5 (supernatant). Note ***, ***, and * represent P < 0.01, 0.005, and 0.001, respectively. EDTA features stronger coordination ability than FA, which results in no evident variation of T1W MRI. Therefore, three concerns, i.e., pore diameter of mesoporous carrier (C), spatial structure, and coordination ability of modified ligands (Mn2+ donor, A), should be taken seriously when using CLRT as a MRI ruler for detection of biological targets, because they decide \( \tau_{LRT} \), \( T_{1m} \), and \( \tau_{m} \) of CLRT and further influence its feasibility, generality, and specificity. To comprehensively understand it, we also examine whether CLRT operates with other Mn2+ acceptors (e.g., GSH, fetal bovine serum (FBS), and ethylene diamine tetraacetic acid (EDTA)) using the HA-specific model probe (MON–FA–Mn) and evaluate the influences of three concerns on CLRT involving feasibility and specificity. Despite featuring low molecular weight, GSH fails to capture Mn2+ due to its weaker coordination ability toward Mn2+ than FA, which results in no evident variation of T1W MRI (Figure S7, Supporting Information). This phenomenon declares the coordination ability of initial modification ligands in probe manipulates the coordination-responsive MRI. Especially for FBS, the maximum 13 nm mesopore in MON–FA–Mn prevented overlarge-molecular-weight (OMW) proteins from entering mesoporous channels, resulting in less than 7% Mn2+ release from MON–FA–Mn within 24 h (Figure S8, Supporting Information) and no evident variation of T1W MRI (Figure S9, Supporting Information). This phenomenon not only demonstrates the robust stability of MON–FA–Mn, but also suggests that the pore diameter of mesoporous carrier also governs the coordination-responsive MRI. As a universally accepted strong chelator toward metal ions, EDTA features stronger coordination ability than FA and lower molecular weight than HA, which determines EDTA can enter mesoporous channels of MON–FA–Mn and capture Mn2+. Depending on the decreased stereo-hindrance of EDTA due to its simpler spatial structure than FA, EDTA can accelerate the exchange rate of water, resulting in shortened LRT and intensified T1W MRI (Figure S10, Supporting Information). Results indeed show Mn2+ captured by EDTA and intensified T1W MRI performance when MON–FA–Mn
incubates with EDTA (Figures S11 and S12, Supporting Information), realizing the CLRT with a intensified T1W MRI ruler. Unfortunately, akin to previous strategies, it is also difficult to discern the intensified T1W MRI behavior is resulted from continuous accumulation of particles or CLRT.

2.4. Generalization Exploration of CLRT

Further generalization of CLRT with another class of mesoporous carriers (C), Mn\(^{2+}\) donors (modified agents, A), and Mn\(^{2+}\) acceptors (biological target, B) is examined. For instance, hollow mesoporous silica nanoparticles (HMSN) and –NH\(_2\)-contained side chains acted as carriers and Mn\(^{2+}\) donors, respectively, and the biological target of tumor, i.e., GSH, \(^9\) acts as the Mn\(^{2+}\) acceptor. Akin to MON–FA–Mn, the GSH-specific HMSN–NH\(_2\)–Mn was also obtained via a successive process, wherein HMSN platform with a diameter of 400 nm was first fabricated via our well-established method,\(^{17}\) and then amino-propyltriethoxysilane (APTES) modification was carried out for grafting –NH\(_2\), followed by coordination with Mn\(^{2+}\) due to the excellent coordination ability of –NH\(_2\).\(^{18}\) The maximum pore diameter remains less than 3 nm (Figure S13, Supporting Information) and Mn\(^{2+}\) ions are also uniformly distributed in mesoporous channels (Figure S14, Supporting Information). The chelation amount of Mn\(^{2+}\) is around 4.5% via inductively coupled plasma-atomic emission spectrometry (ICP-AES) method.

The 3 nm mesopores of HMSNs carriers and modification ligands determine the specific detection of GSH, e.g., the modification of –NH\(_2\)-carrying APTES indirectly determines the weaker coordination ability and reduced stereo-hindrance of –NH\(_2\) than GSH, and the maximum 3 nm mesopore merely allows low-molecular-weight GSH to enter mesoporous channels and capture Mn\(^{2+}\), as depicted in Figure 4a. Results definitely indicate Mn\(^{2+}\) in HMSN–NH\(_2\)–Mn is captured by GSH via the competitive coordination (Figure 4b) and bring about a decreased MRI performance (Figure 4c,d). In contrast, the mesopore size (less than 3 nm) can prevent HA from entering mesoporous channels of HMSN–NH\(_2\)–Mn and capturing Mn\(^{2+}\) (Figure 4h), ultimately resulting in no variation of T1W MRI (Figure 4f,g), as displayed in the schematic (Figure 4e). Therefore, CLRT can be considered as a general concept to provide coordination-responsive MRI contrast effects that can be potentially utilized as a nanoscale ruler for specific detection of biological targets after rationally designing model system.

2.5. In Vitro and In Vivo CLRT Exploration for Quantitative HA Detection

We further examine the CLRT sensing concept in in vitro and in vivo systems for HA detection. T\(_1\) relaxation coefficient (r1) of CLRT probe (MON–FA–Mn) before and after incubation with HA was further evaluated. Relying on the principle
of CLRT, the r1 value of MON–FA–Mn considerably drops when mixing with HA (Figure 5a,b), indicating the occurrence of Mn2+ captured by HA to MON–FA–Mn. An approximately linear relationship between longitudinal time and HA concentration is directly established within 5 × 10⁻³ m (Figure 5c). As a paradigm, the corresponding longitudinal time upon incubation with 0.3 mg mL⁻¹ of HA is ≈513 ms according to the fitted liner equation, and this value is approximately identical to the actually measured value (511 ms), suggesting the detection limit of HA via CLRT using MON–FA–Mn as probe approaches 0.3 mg mL⁻¹. More significantly, ∆R₁, which is defined as the difference in longitudinal relaxation rate (R₁ = 1/T₁) between preincubation and postincubation with HA solution, shows a consistent decrease in the T₁W MRI signal.[8] Results manifest another linear correlation between HA concentration and ∆R₁ is observed over the whole monitoring window, and more HA results in larger ∆R₁ and decreased T₁W MRI signal, as evidenced in Figure 5d. This linear correlation offers the possibility for the quantitative measurement of HA.

Encouraged by the successful results of in vitro HA detection, in vivo HA detection using the CLRT sensing ruler was implemented on VX2 tumor subcutaneously implanted in New Zealand rabbits. MON–FA–Mn particles share an excellent biocompatibility, since they display a neglectable cytotoxicity and in vivo blood toxicity (Figures S15 and S16, Supporting Information). The blood half-life of MON–FA–Mn that was used to investigate the in vivo biostability of MON–FA–Mn was determined from time-dependent quantifications of Mn and Si, respectively. It is clearly found that the blood half-life of MON–FA–Mn according to Si quantification is approximately equal to that obtained according to Mn quantification (Figure S17, Supporting Information), accounting for no Mn2⁺ leaching or capture by MTPs during blood circulation, which demonstrates the robust biostability of MON–FA–Mn. The excellent biostability is ascribed to the fact that the appropriate mesopores in MON can prohibit OMW MTPs from entering mesoporous channels and capturing Mn2⁺, displaying a spatial confinement effect. As well, the time-dependent distributions of MON–FA–Mn in main organs were obtained (Figure S18, Supporting Information).

T₁W MRI results exhibit that the contrast and signal intensity of VX2 tumor continuously increase within 8 h, but once the intravenous (i.v.) postinjection time exceeds 8 h, the contrast and signal intensity reversely decrease, as shown in Figure 6a,b. In contrast, Mn accumulation in VX2 tumors fails to decrease until the postinjection time exceeds 12 h, indicating the turning point of Mn accumulation is 4 h later than that of T₁W MRI performance. This intriguing phenomenon suggests that the impairing effect toward MRI performance that is resulted from Mn2⁺ capture by HA in VX2 tumor defeats the positive effect of continuous MON–FA–Mn accumulation for intensifying MRI after 8 h. Therefore, the in vivo decreased MRI after Mn2⁺ capture by HA can realize HA detection and concurrently overcome the interference of particles’ accumulation-enhanced MRI in previous strategies. Moreover, time-dependent accumulations of Mn and Si in tumor exhibit an inconsistent variation pace (Figure 6c), which further demonstrates Mn2⁺ detachment from MON–FA–Mn. The results sufficiently reflect the potential of CLRT for the detection of biological targets in living systems.

In general, i.v. injection disables the quantitative detection of specific targets due to unknown accumulation of...
probes in lesions and no constant component as the ratio-
metric reference,[19] in contrast, intratumoral (i.t.) injection
was universally accepted to address it.[8] Furthermore, i.t.
injection was carried out to quantitatively monitor HA and
its distribution in xenografted VX2 tumor subcutaneously
implanted in nude mice. It is clearly found that the T1W
MRI signal is significantly intensified after i.t. injection
of CLRT probes, while after 1 h postinjection, the signal drops
a lot due to the presence of Mn2+ capture by HA (Figure S19,
Supporting Information). We further change the HA levels
in the mice using a different amount of hyaluronidase that
can accelerate HA degradation.[20] Comparing postinjection
and 1 h postinjection of the CLRT probe, the ∆R1 values at
the region of interest (ROI) in tumor are quantified to be
0.15, 0.36, and 0.61 s⁻¹ (Figure 6d), and the amount of HA
in each tumor is determined to be 0.8, 3.3, and 5.6 mg mL⁻¹,
respectively, using high-performance liquid chromatography
(HPLC).[21] The plot of ∆R1 versus HA amount indicates that
the HA level in the tumors is well represented by the T1W
MRI signal (Figure 6e). The results manifest the potential of
using CLRT for the quantitative analysis of various biomol-
ecules in living systems.

3. Conclusion

In summary, a new MRI-based sensing approach basing on
CLRT has been established as a proof-of-concept to realize
detection of biological targets. Two model systems, i.e., MON–
FA–Mn and HMSN–NH2–Mn, were designed to explore the
potential of CLRT, and both allow the MRI as ruler to detect two
biomarkers (HA and GSH) of malignancy. Moreover, three con-
cerns, i.e., pore diameter of mesoporous carrier and the coor-
dination ability and spatial structure of Mn2+ donor (modified
ligands) on CLRT probes, have been demonstrated to govern
the feasibility, specificity, and generality of the CLRT sensing
protocol. Therefore, rationally designing CLRT probe aiming at
a certain biological target should be taken into account when
using CLRT as a MRI sensing. In particular, in vitro and in vivo
experiments validated the exploration of CLRT in quantitatively
detecting tumor biomarkers (i.e., HA) via the decreased T1W
MRI signal using the CLRT probe (i.e., MON–FA–Mn). The
T1W MRI-based CLRT can potentially provide an alternative
and/or complementary approach for monitoring a variety of
biological targets, because MRI can offer high-resolution ano-
tomical information and soft tissue contrast. However, the

Figure 6. In vivo evaluation of CLRT on VX2-bearing New Zealand rabbit. a) In vivo T1W MRI images of VX2 tumors implanted on the liver of New
Zealand rabbit before and after the i.v. injection of MON–FA–Mn for different time intervals (Dose: 2 mg Mn/Kg), wherein yellow arrows indicate the
VX2 tumor. b) Time-dependent Mn accumulation in VX2 tumor and T1W signal value obtained from inset (a) c) Accumulation of Si and Mn in VX2
tumor as a function of time the i.v. injection of MON–FA–Mn for different time intervals. Data are presented as the mean ± SEM. d) In vivo T1W MRI
images of VX2 tumors implanted on the subcutaneous site of nude mice before and after the i.t. injection of MON–FA–Mn and 1 h postinjection
(Dose: 2 mg Mn/Kg), wherein 1/T1 in the region of interest (ROI) circled by red dotted line was determined, and ∆R1 was the difference value between
postinjection and 1 h postinjection.
CLRT method is still at its infancy, and they need to be further improved for a wide window of in vivo applications involving imaging and detection of biomarkers. Also, quantitative detection represented by variations of relaxation coefficient or relaxation time needs to be further explored.

4. Experimental Section

**In Vivo T1W MRI of MON–FA–Mn:** The in vivo MR imaging experiment was performed on UNITED IMAGING (uMR 570, 1.5 T), and the pulse sequence used was FSE-T1WI with the following parameters: TR = 1000 ms, TE = 20, slice thickness = 2.00 mm, matrix = 180 × 180, Acq (NEX) = 1. When using EDTA and HA as the Mn2+ acceptors, six groups were set, i.e., labeled number 1: MON–FA–Mn alone, labeled number 2: coexisting mixture of HA (or EDTA or GSH) or complete FBS + MON–FA–Mn, labeled number 3: HA (or EDTA or GSH) solution or complete FBS alone, labeled number 4: redispersed centrifugal precipitate after MON–FA–MN incubation with HA (or EDTA or GSH) or complete FBS, labeled number 5: centrifugal supernatant after MON–FA–MN incubation with HA (or EDTA or GSH) or complete FBS, labeled number 6: water. The concentration of MON–FA–Mn was fixed to 3 mg mL−1 in all corresponding groups. The concentrations of HA were 2.0 mg mL−1, and the concentration of GSH was 5 × 10−3 M. The incubation durations were 1 and 8 h when HA (or EDTA or GSH) or FBS were added dropwise in case of VX2 cells’ redispersion. These cells were then in vivo T1W MRI was carried out. Hyaluronidase with different concentrations (i.e., 0.3, 1.2, 2.5 U mL−1, respectively) was injected into the tumor, after 8 h, CLRT probes of the same concentration (Mn Dose: 2 mg Mn/Kg) was intratumorally injected into the VX2 tumors. At three time points, i.e., preinjection, postinjection, and 1 h postinjection, T1W MRI images were acquired. The identical MRI sequences and parameters to those in aforementioned in vivo experiments were employed. HA content was detected via HPLC technology using a size exclusion column, 0.05 u phosphate buffer (pH, 5.0) mobile phase at a flow rate of 1.0 mL min−1 and ultraviolet absorbance detection at 200 nm.

**Statistical Analysis:** All the experiments were performed in triplicate. The obtained data were expressed as the mean value ± standard deviation (SD) and the statistical significance between two groups was analyzed by the Student’s two-tailed t-test through SPSS 22.0. Single, double, and triple asterisks represent p ≤ 0.01, 0.005, and 0.001, respectively and “p < 0.01” was considered statistically significant.

**Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

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**Conflict of Interest**

The authors declare no conflict of interest.

**Keywords**

biological targets, coordination-dependent longitudinal relaxation tuning, pore diameters, spatial structures, T1-weighted MRI

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