Recent advances in engineering iron oxide nanoparticles for effective magnetic resonance imaging

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ABSTRACT

Iron oxide nanoparticle (IONP) with unique magnetic property and high biocompatibility have been widely used as magnetic resonance imaging (MRI) contrast agent (CA) for long time. However, a review which comprehensively summarizes the recent development of IONP as traditional T\textsubscript{2} CA and its new application for different modality of MRI, such as T\textsubscript{1} imaging, simultaneous T\textsubscript{2}/T\textsubscript{1} or MRI/other imaging modality, and as environment responsive CA is rare. This review starts with an investigation of direction on the development of high-performance MRI CA in both T\textsubscript{2} and T\textsubscript{1} modal based on quantum mechanical outer sphere and Solomon–Bloembergen–Morgan (SBM) theory. Recent rational attempts to increase the MRI contrast of IONP by adjusting the key parameters, including magnetization, size, effective radius, inhomogeneity of surrounding generated magnetic field, crystal phase, coordination number of water, electronic relaxation time, and surface modification are summarized. Besides the strategies to improve r\textsubscript{1} or r\textsubscript{2} values, strategies to increase the in vivo contrast efficiency of IONP have been reviewed from three different aspects, those are introducing second imaging modality to increase the imaging accuracy, endowing IONP with environment response capacity to elevate the signal difference between lesion and normal tissue, and optimizing the interface structure to improve the accumulation amount of IONP in lesion. This detailed review provides a deep understanding of recent researches on the development of high-performance IONP based MRI CAs. It is hoped to trigger deep thinking for design of next generation MRI CAs for early and accurate diagnosis.

1. Introduction

Various biomedical nanomaterials have been developed to act as agents to fulfill cell labeling [1–5] and separation [6–9], biological sensing [10–14], and disease diagnosis/therapy [15–18]. Among all biomedical nanomaterials, iron oxide nanoparticles (IONP) has been intensively investigated due to their unique magnetic property and high biocompatibility [19–23]. IONP based commercial MRI contrast agent (CA) (e.g. Resovist, Feridex), which shorten the transverse relaxation (T\textsubscript{2}) of proton, have been approved by the U.S. Food and Drug Administration (FDA) for clinical diagnosis. However, commercial available IONP based MRI CA show low MRI contrast ability due to low crystallinity or poor surface modification, which lead to reduced sensitivity and accuracy in MRI contrast imaging and limited application in early and accurate diagnosis [24–26]. Over the past years, tremendous efforts have been made to improve the relativity of IONP by engineering crystal and surface structure [27–29]. On the basis of relativity-structure assessment, IONP with high T\textsubscript{2} relativity were discovered to show several critical characteristics, including high saturated magnetization, large effective size, high inhomogeneous magnetic field, and suitable surface modification [30–32]. According to the classical outer-sphere theory, saturated magnetization and large effective size are
responsible for the field perturbation area for the outermost protons and proportional to the $T_2$ relaxation of MRI CA [33–35]. Thus, synthesis of IONP with high saturated magnetization and large size have firstly been investigated to increase the $T_2$ relaxivity of IONP. Additionally, high inhomogeneous magnetic field was founded to accelerate the dephasing process of protons around IONP, which significantly increased its $T_2$ relaxivity. With the development of nanoparticle synthesis, IONP with unique morphology has been developed through morphology controllable synthesis [36–38]. These IONP show large effective size and high inhomogeneous of surrounding magnetic field, causing the elevation of $T_2$ relaxivity. In addition, surface coating layer was discovered to affect the magnetic property of IONP and diffusion of surrounding proton [39–41]. Therefore, optimizing surface ligand of IONP have been conducted to form proper surface coating layer and increase $T_2$ relaxivity of IONP.

IONP based traditional $T_2$ CAs exert darken contrast in the region where they reside, which may result in false positive detection caused by the confusion between lesion and bleeding, calcification, or metal deposition. This limitation encourages researchers to develop IONP based longitudinal relaxation ($T_1$) CA, which exerts bright contrast to distinguish tissue [42–44]. However, traditional IONP exhibits high $r_2/r_1$ ratio, hindering its application as a $T_1$ CA. Based on the Solomon-Bloembergen-Morgan (SBM) theory, $T_1$ contrast ability is highly dependent on the chemical exchange efficiency between magnetic ions and protons. Currently, three key parameters have been unearthed to alter the chemical exchange efficiency and determine $T_1$ contrast capacity of IONP. First, high saturated magnetization causes high $r_2/r_1$ ratio and impair $T_1$ contrast imaging. Decreasing the size and crystallinity have been proved to successfully reduce saturated magnetization and $r_2/r_1$ ratio of IONP [45–47]. Second, coordination number of water ($q$) on the surface of IONP is another important parameter to tune. Since coordination number of iron ion is constant, the method to optimize the $q$ is directly increasing the number of iron ions on the surface or introducing other magnetic ions with large amounts of unpaired electrons. Thanks to the controllable synthesis and development of new generation of surface ligands, various IONP with high surface-to-volume ratio, specific composition, and optimal surface coating have been developed to show high $T_1$ contrast and exhibit potential to achieve accurate tumor contrast imaging [48–50]. Recently, an undesirable structure, that is the presence of undesirable Fe (II) ion on the surface of IONP, have been noticed. Due to its short electron relaxation time ($T_1$), Fe (II) ion is unfavorable to achieve efficient relaxation enhancement and severely reduces the $T_1$ contrast. Strategy to replace the Fe (II) on IONP surface with other magnetic ions with long $T_1$ have been proved to significantly increase $T_1$ contrast for high-performance $T_1$ contrast imaging [51].

Apart from fabricating IONP with high $T_2$ or $T_2$ contrast ability, high-performance IONP based MRI CA could also be fulfilled by increasing its contrast efficiency. Compared to the IONP in the form of single $T_2$ negative or $T_1$ positive, introducing $T_1$ moiety or other imaging contrast moiety into IONP could yield complementary diagnostic information to increase the contrast efficiency [52–54]. Recently, integration of IONP and gadolinium species have been developed as dual-modal CA (DMCA). A facile strategy is coating IONP with gadolinium based nanostructure or gadolinium complex [55–57]. However, the spin alignment on $T_1$ contrast shell is opposite to the local magnetic field generated by IONP core, which reduces its $T_1$ contrast and limits its application in accurate $T_1/T_2$ dual modal contrast imaging. Interestingly, this phenomenon is highly dependent on the distance between IONP and $T_1$ contrast moiety [58–59]. Introducing SiO$_2$ isolating layer to separate $T_1$ domain and IONP or forming Janus structure have been applied to increase the distance between IONP and $T_1$ moiety to develop high-performance IONP based $T_1/T_2$ DMCA [60]. Lately, doping manganese or gadolinium ions into IONP have been found to be another effective method to increase $T_1$ contrast of DMCA [61–63]. Apart from development of IONP with dual-modal MRI contrast, direct conjugation of secondary even tertiary imaging moiety with IONP is another effective method to improve its contrast efficiency. The main strategy to fabricate IONP based multi-modal imaging CA is surface conjugating IONP with secondary imaging components, such as single-photon emission computed tomography (SPECT) [64–65], positron emission tomography (PET) [66–68], computed tomography (CT) [69–71], fluorescence (FL) imaging [72–74], ultrasound (US) imaging [75–77], and photoacoustic (PA) imaging [78–81]. Based on the complementary signals, the regions of interest, including tumor, cardiovascular disease, and cell tracking could be rapidly and accurately detected.

For disease diagnosis, the contrast efficiency of MRI CA depends on the signal difference between lesion and adjacent healthy tissue. Therefore, there is a growth of interest on developing internal (lesion microenvironment) and external (specific physical condition) responsive MRI CAs [82–87]. Environment responsive MRI CA could respond to specific characters and exhibit triggered or switchable signals for highly specific and precise MRI images [88–94]. IONP based responsive MRI CA could be divided into three different manners, active, recovery, and switchable. Compared to the traditional MRI CA with fixed contrast capacity, IONP based responsive MRI CA with signal active or recovery capacity could enhance $T_1$ or $T_2$ signal in response to a specific stimulus of lesion. Recently, IONP based environment responsive MRI CA with modal switchable capacity have been developed to further increase the signal difference between lesion and normal tissue. Similar to the traditional MRI contrast imaging, there are two modals of switchable tumor microenvironment (TEM)-responsive MRI contrast imaging: $T_2$ contrast switch to $T_1$ contrast (mode I) and $T_1$ contrast switch to $T_2$ contrast (mode II). The strategy to develop modal switchable MRI CA is based on the fact that formation of magnetic nanocluster or nano-aggregation could improve $T_2$ relaxivity of ultrasmall IONP. Normally, ultrasmall IONP are considered as typical $T_1$ CA when it is monodisperse. With the specific surface modification, ultrasmall IONP could achieve the status change between cluster and monodisperse. These structural changes result in the decrease or increase of $T_2/T_1$ contrast, which endow CA to achieve modal switchable contrast imaging.

The signal difference between normal tissue and lesion is also directly influenced by the amount of IONP in lesion, which is mainly determined by its in vivo behavior. When IONP enter the physiological environment, serum proteins rapidly adsorb on its surface and form protein corona, resulting in the decrease of blood circulation time and accumulation in lesion [95–98]. IONP Surface modification of nanoparticles with specific ligands, such as polyethylene glycol (PEG) or zwiterionic small molecules, can effectively reduce the non-specific protein adsorption and elevate the accumulation amount of IONP in lesion [99–106]. In addition, the accumulation amount of IONP in lesion could be further increased by introducing targeting motif, e.g. antibodies, proteins, peptides, and aptamers to achieve targeted MR contrast imaging with enhanced contrast efficiency [27,107–111].

During the past decades, a number of reviews have been published to summarize the development of IONP as MRI CA. Yet a review comprehensively discussing high-performance IONP based MRI CAs with high $T_2$ relaxivity, high $T_1$ relaxivity, environment responsive contrast ability, multi-modal contrast capacity, and optimized in vivo behavior in recent progress is needed. This review discuss the parameters to affect IONP based MRI CA from contrast ability of IONP and contrast efficiency of IONP in vivo (Fig. 1). Detailed mechanism on development of IONP based MRI CA with high $T_2$ or $T_1$ relaxivity was firstly discussed according to classical quantum mechanical outer sphere and SBM theory. More importantly, some instance calculated $r_2$ and $r_1$ values of IONP through adjusting the theoretical related key parameters, including magnetization, size, effective radius, and inhomogeneity of surrounding generated magnetic field, crystal phase, coordination number of water, electronic relaxation time, and surface modification are presented to point out the direction of development of IONP with high contrast ability. After detailed discussion on contrast improvement, representative rational designs and advances of IONP to improve its contrast efficiency are reviewed. The enhancement of contrast efficiency could be
fulfilled by introducing other imaging modalities, endowing IONP with environment response capacity, and designing the surface ligand to optimize in vivo behavior. We hope this detailed review could build a bridge between the proof-of-concept to translate high-performance MRI CAs, further promoting rational design of magnetic nanoparticles for early and accurate lesion detection.

2. Key parameters enhancing T$_2$ relaxivities of IONPs

Based on its specific magnetic property, especially high saturated (M$_{s}$) value, IONP could accelerate the transverse relaxation of proton in surrounding tissue and decrease signal intensity in T$_2$-weighted MRI images. Thus, IONP has been used as T$_2$ CA for the past decades. Several commercial IONP based T$_2$ CAs have been approved by FDA in clinical application. However, some defects, such as low crystallinity and poor surface modification, result in the low contrast and reduce the quality of MRI contrast images. Over the past decades, many attempts and theoretical investigations have been conducted to improve the T$_2$ contrast of IONP.

2.1. Outer-sphere theory

Theoretically, T$_2$ relaxation of proton could be interpreted by three mechanisms, dipole-dipole coupling [112], curie spin relaxation [113, 114], and scalar or contact relaxation [115,116]. An accelerated relaxation phenomenon is observed in the existence of magnetized particles with weak magnetization [117], while the relaxation enhancement is limited. With the development of nanotechnology, IONP have been discovered to enhance the T$_2$ relaxation of proton [25,118–121]. With high magnetic moment, IONP could generate a local magnetic field under the external magnetic field which can effectively shorten the relaxation of surrounding proton [122]. According to the outer sphere theory, the proton dephasing process can be divided into three stages, motional average regime (MAR), static dephasing regime (SDR), and echo-limiting regime (ELR) based on the value of $\tau_0$ [118,123]. $\tau_0$ is the diffusion time and could be calculated by the following equation: $\tau_0 = d^2/4D$, where $d$ is efficient radius of particle and $D$ is the water diffusion coefficient. MAR condition is fulfilled when $\tau_0 < 1/(\gamma B_{eq})$, where $\gamma$ is the proton gyromagnetic ratio and $B_{eq}$ is the equatorial magnetic field. On the basis of that protons rapidly diffuse around IONP, protons experience quick magnetic field changing in MAR [124], which is effectively time-averaged. According to the quantum-mechanical outer-sphere theory, T$_2$ relaxivity of IONP is given by:

$$1/T_2 = r_2 = (256\pi/405)\frac{M_s^2}{r^2}$$

(1)

Where $M_s$, $r_s$, and $r$ are the saturation magnetization, thickness of an impermeable surface coating, and efficient radius of magnetic nanostructure. These key parameters are determined by the crystal and surface structure of IONP. Based on this equation, $T_2$ contrast of IONP is proportional to $M_s$ and $r$. Thus, one can develop IONP with high $T_2$ contrast ability by adjusting these key parameters. $T_2$ contrast of IONP can not uncontrolled increase with the increase of particles size. When the size of particle or cluster reaches a certain limit, the $T_2$ contrast IONP reaches the maximum value and fulfill the SDR [118,129]. In SDR, the $T_2$ relaxivity of IONP are given by

$$r_2 = \frac{8\gamma^2 A N_0}{81 \text{IO} Z} r M_s$$

(2)

where $A$ is the lattice parameter, $N_0$ is the Avogadro constant, $Z$ is number of formula units per unit cell, $r$ is the proton gyromagnetic ratio. Based on this equation, the $r_2$ value of IONP in SDR is highly dependent on its saturated magnetization. Therefore, one can further increase the $T_2$ contrast ability of IONP in SDR through elevating the saturated magnetization.

Along with the further increase of size, $T_2$ relaxivity of IONP reach to ELR [126]. In this regime, proton shows limited diffusion in a time interval. This undesired property leads to $T_2$ contrast reduction with their sizes increase, meaning that construction of high-performance $T_2$ MRI CAs cannot be achieved by endless increasing the radius of IONP.

2.2. Magnetic behavior

Based on the quantum-mechanical outer-sphere theory, improvement of $M_s$ can significantly elevate the $T_2$ contrast of IONP. Since magnetic properties of magnetic materials are highly dependent on their crystal structure, such as crystallinity, crystal composition, and crystal size, engineering the structure of IONP is an effective method to ameliorate magnetic behavior and $T_2$ MRI contrast. In the following sections, we will discuss the strategies to optimize crystal structure of IONP to improve $M_s$ value for enhanced $T_2$ contrast.

2.2.1. Crystallinity

The simplest attempt to improve magnetic moment of IONP is improving crystallinity. Structure order, determined by the crystallinity, affects the state of magnetic spin in IONP and determines its magnetic property. IONP could be mainly divided into three phases, those are magnetite, Wüstite, and Maghemite phase. Due to the growth kinetics of Wüstite phase, IONP exhibits typical mixed crystal phase and result in low crystallinity [36,127]. The existing undesired mixed phase in IONP would destroy the long-range-order of magnetic spin and reduce magnetic moment. With the development of nanosynthesis, IONP with high crystallinity could be obtained by tuning the synthesis parameter. For example, Hyeon and colleagues reported that synthesis of IONP via thermal decomposition of precursors in high boiling points solvent.
provided IONP with high crystallinity and magnetic moment [128,129], paving the way to fabricate high-performance T₂ MRI CA. In addition, IONP with high crystallinity could be obtained by other methods, such as polyol and microwave synthesis. Hachani et al. adapted polyol synthesis to get IONP at high temperature and pressure conditions. The as-prepared IONP show high crystallinity and Ms, resulting in high T₂ relaxivity [130].

2.2.2. Crystal composition

As an effective method, doping have been widely used to engineer the crystal composition of nanomaterial, which determines the magnetic property of magnetic material [131–133]. Magnetite phase show spinel or inverse spinel structure, which corresponds to the oxygen-packed face-centered cubic lattices with octahedral (O₈) and tetrahedral (T₄) site, respectively. Typically, Fe²⁺ ions occupies the O₈ and T₄ site, while Fe³⁺ occupies the O₈ site. Under an external magnetic field, the magnetic spin in O₈ site is aligned in parallel to the external magnetic field, while the magnetic spin in T₄ site aligns antiparallel to the external magnetic field. Therefore, increasing the magnetic spin in O₈ site or reducing the magnetic spin in T₄ site theoretically increases the magnetization of IONP. Cheon and co-author engineered the structure of Fe₃O₄ nanocrystal by introducing different divalent magnetic ions with different magnetic spin magnitudes, including Mn²⁺ (5 μB), Fe²⁺ (4 μB), Co²⁺ (3 μB), and Ni²⁺ (2 μB), to investigate the effect of dopant on its magnetic property and T₂ contrast (Fig. 2a and b) [27]. Interestingly, the magnetization and T₂ contrast gradually decreases with the order of Mn²⁺, Fe²⁺, Co²⁺, and Ni²⁺, which is highly consistent with the change of magnetic spin magnitude. It should note that Mn²⁺ doped Fe₃O₄ (MnₓFe₃₋ₙO₄) exhibits the highest saturation magnetization (110 emu/g = 110 A-m²/kg) and T₂ relaxivity (358 mM⁻¹ s⁻¹ at 1.5 T). MnₓFe₃₋ₙO₄ NP shows mixed spinel structure with Mn²⁺ occupied either T₄ or O₈ site. The rise of Mn²⁺ doping level may lead to two opposite effects: one is increasing magnetic spin in Fe₃O₄ nanocrystal and elevating the magnetic moment, the other one is disturbing the long-range order of magnetic spins and resulting in the decrease of magnetic moment. Recent research indicates that the saturation magnetization and T₂ contrast of MnₓFe₃₋ₙO₄ nanocrystal gradually increases with the Mn²⁺ doping level rising from x = 0 to 0.43, while decreases with the doping level further rise to x = 1.06 [134]. When x = 0.43, MnₓFe₃₋ₙO₄ nanocrystal shows highest saturation magnetization (89.5 emu/g = 89.5 A-m²/kg) and T₂ relaxivity (904.4 ± 11.1 mM⁻¹ s⁻¹ at 7 T). Apart from the magnetic ions, dopant non-magnetic ions into Fe₃O₄ nanocrystal have been proved to be an effective method to increase its magnetization and MRI contrast ability. Jang et al., constructed Zn²⁺ doped Fe₃O₄ nanocrystal [(ZnₓFe₃₋ₓ)O₄] with spinel structure (Fig. 2c-e) [135]. Extended X-ray absorption fine structure analysis indicates that Zn²⁺ mainly occupies the T₄ sites, which could effectively reduce the antiferromagnetic coupling interactions between T₂ and O₈ sites and increase the magnetic moment. The magnetic property investigation reveals that the saturation magnetization could be enhanced to 161 emu/g (161 A-m²/kg) when x = 0.4. This high saturation magnetization generate superior T₂ contrast with the r₂ value of 687 mM⁻¹ s⁻¹ at 4.5 T, which is approximately 2.5 and 1.6 times higher than that of Fe₃O₄ (276 mM⁻¹ s⁻¹) and MnFe₂O₄ (422 mM⁻¹ s⁻¹) nanocrystal.

2.2.3. Core-shell structure

Compared to the IONP, metallic iron nanoparticle exhibit significantly higher saturation magnetization at room temperature. Introducing metallic iron moiety into IONP could effectively increase its saturated magnetization and T₂ contrast [136–138]. To improve magnetic moment of IONP, Tilley and co-authors synthesized high crystaline iron/iron oxide core/shell nanocrystal by naturally oxidation iron...

Fig. 2. Magnetic behavior effect on T₂ relaxivity of IONP. (a) Mass magnetization values and schematics of spin alignments and (b) T₂ relaxivities of MnFe₂O₄ (MnMEIO), Fe₃O₄ (MEIO), CoFe₂O₄ (CoMEIO) and NiFe₂O₄ (NiMEIO). Reproduced with permission [27]. Copyright 2007, Nature Publishing Group. (c) Magnetic spin alignment diagram of (ZnₓFe₃₋ₓ)Fe₃O₄ nanoparticles with x = 0, 0.2, and 0.4 under applied magnetic field. (d) Mₐ and (e) r₂ values of (ZnₓMn₁₋ₓ)Fe₃O₄ and (ZnₓFe₁₋ₓ)Fe₂O₄ nanoparticles with different Zn doping level. Reproduced with permission [135]. Copyright 2009, John Wiley & Sons, Inc. (f) TEM image and (g) r₂ value of iron/iron oxide core/shell nanocrystal. Reproduced with permission [139]. Copyright 2011, John Wiley & Sons, Inc. (h) r₂ values of iron nanoparticle coated by various magnetic shell at the fixed larmor frequency. Reproduced with permission [141]. Copyright 2011, John Wiley & Sons, Inc. (i) Diagram of spin canting effect in various sized IONPs. (j) M – H curve of IONPs with sizes of 1.5, 2.2, and 3 nm at 300 K. Reproduced with permission [46]. Copyright 2011, American Chemical Society. (k–l) TEM images of cube and sphere nanoparticles. (m–n) Simulated magnetic spin state of cube and sphere, indicating the degree of spin canting against external magnetic field. Reproduced with permission [149]. Copyright 2012, American Chemical Society.
nanoparticle (Fig. 2f and g) [139]. Introducing iron core endows this core/shell nanostructure with high magnetization with the value of 150 emu/g (150 A-m^2/kg) (Fe), which is significantly higher than that of normal Fe₃O₄ nanocrystal. This far elevation of magnetization results in the obviously rising of T₂ relaxation from 145 mM⁻¹ s⁻¹ to 324 mM⁻¹ s⁻¹ at 9.4 T. It should note that the crystallinity of core/shell nanostructure is an important parameter to its T₂ relaxivity [140]. The amorph-Fe₃O₄ exhibits remarkably lower T₂ relaxivity (67 mM⁻¹ s⁻¹) than bcc-Fe-/Fe₃O₄ (220 mM⁻¹ s⁻¹) with high crystallinity, even much lower than Ferrixd (110 mM⁻¹ s⁻¹). The crystal structure of iron oxide shell is another parameter to affect its magnetization and T₂ relaxivity. Yoon et al. coated monometallic iron with MnFe₂O₄, Fe₂O₃, CoFe₂O₄, and FeO (Fig. 2h) [141]. These iron/iron oxide core/shell nanoshell structures exhibit increased magnetization and T₂ relaxivity with shell structures altered from FeO to CoFe₂O₄, Fe₂O₃, and MnFe₂O₄, consistent with the magnetization change of the shell structure. These results mean that coating monometallic iron with a magnetic shell with high magnetization could further improve its magnetization and T₂ relaxivity.

2.3. Effective radius

Previous researches indicate that IONP could be considered as a core/shell structure with the magnetic core and magnetically dead layer, where the magnetic spin is lack of full alignment. In the magnetically dead layer, the spin canting and other effects destroy the long-range-order of magnetic spins and result in the disorder of magnetic dipoles, which lead to the decrease of magnetic moment [142–147]. The thickness of spin-canting layer has been determined as about 0.9 nm. Thus, along with the increase of size, the ratio of spin-canting layer to whole magnetic nanoparticle decreases (Fig. 2i and j). It has been reported that the magnetization moment and r₂ value of IONP increases with the decrease of magnetically dead layer ratio. This phenomenon has been found in MnFe₂O₄ nanoparticle as well, which implies that one can develop IONP with high T₂ relaxivity through decreasing the magnetic dead layer ratio [132]. However, recent researches indicate that this elevation effect is connected to the size of IONP. Rinaldi and co-author synthesized IONP at the present of molecular oxygen to increase the magnetic diameter [148]. Interestingly, increasing the magnetic diameter for the IONP with physical diameter of 21 nm leads to slight improvement in the T₂ relaxivity. This result could be attributed to relatively low ratio of magnetically dead layer in IONP with large physical size, limiting the improvement on T₂ relaxivity by further decreasing the magnetically dead layer.

Morphology as a key factor to determine the surface structure of nanomaterials, has been proven to affect the canting spins on the surface of IONP as well. Cheon and co-authors compared the saturated magnetization of cubic and spherical (Zn₀.₆Fe₁₋₀.₄)Fe₂O₄ nanoparticle with the same magnetic volume (Fig. 2k-n) [149]. The orientations of the overall magnetic spin structure shows that the disordered spins are homogeneously distributed on the surface of spherical IONP with certain thickness. While, the disordered spins are mainly distributed at the corner of the cubic IONP. Compared to the spherical IONP without a certain exposed facet, cubic IONP shows the single crystal facet. This unique feature results in the similar spin state in the core and surface, which reduce surface anisotropy. The calculated disordered spins of cube are about 4%, which is significantly lower than that of sphere with the value of 8%. Therefore, the saturated magnetization of cube (165 emu/g = 165 A-m²/kg) is remarkably higher than that of sphere (145 emu/g = 145 A-m²/kg).

2.3. Effectual radius

Apart from Ms, size also determines the region, in which protons relaxation could be accelerated by IONP. Normally, the region could be simulated to a sphere covering the full IONP. The diameter of this region could be defined as the effective radius. Effective radii determined by the size is responsible for the field perturbation area for the outersphere protons and proportional to the T₂ relaxivity in MAR, which means improvement of T₂ relaxivity of IONP could be achieved by increasing the effective radius with maintained saturated magnetization. However, T₂ contrast of IONP are not uncontrolled increase with the increase of efficient radius. When the efficient radius of particle or cluster reach to a certain limit, the T₂ contrast of IONP reach to its maximum value and fulfill the SDR. In this section, we will focus on the strategy to develop high-performance T₂ CA by increasing effective radius of IONP through controllable synthesis.

2.3.1. Individual IONP

In MAR T₂ relaxivity of IONP is proportional to the square of effective radius according to outer sphere theory [124]. For spherical IONP, its effective radius is directly determined by its diameter, thus, increasing its diameter have been proven to be the simplest method to improve its T₂ relaxivity. Cheon and co-authors find that IONP shows a typical diameter-dependent T₂ relaxivity, that is, r₂ value increase with the rising of diameter [150]. This diameter-dependent T₂ relaxivity has been observed in other researches, which is highly consistent with the theoretical analysis [33,151–153]. Besides, sophisticated morphology of magnetic nanostructure can increase the effective radii of particle cores and improve intensity, direction, and gradient of the magnetic stray field, accelerating surrounding proton diffusion and dephasing. Zhao et al. developed a novel IONP with octapod morphology with the assistance of chloride ion (Fig. 3a–e) [154]. Due to its unique morphology, the calculated effective radius of IONP with octapod morphology is about 2.4 times higher than that of spherical IONP with the same solid volume and similar magnetization. Further MRI investigation indicates that the octapod IONP with edge length of 30 nm show an ultrahigh T₂ relaxivity with the value of 679.3 ± 30 mM⁻¹ s⁻¹ at 7 T, which is about 5.4 times larger than that of spherical IONP with the same geometric volume. Inspired by this research, a large number of IONPs with anisotropic morphologies and improved effective radii, such as plate [155,156], cube [157,158], and tripod [159], have been reported to show enhanced T₂ relaxivity. Recently, Yang et al., systematically investigate the morphology effect on the effective radius and T₂ relaxivity of IONP (Fig. 3f) [160]. They synthesized MnFe₂O₄ nanoparticles with the morphology of sphere, cube, plate, tetrahedron, rhombohedra, and octapod with the same solid volume and similar magnetization. The calculated effective radius shows a gradually decrease with the order of octapod, rhombohedra, tetrahedron, plate, cube, and sphere. The MRI analyses indicate that the T₂ relaxivities of all samples show descending tendency from octapod to sphere, which is the same to the tendency change of effective radius. The increased T₂ relaxivity along with the rising of effective radius demonstrate that effective radius can eventually determine T₂ relaxivity in MAR.

In contrast to the MAR, the large magnetic nanoparticles generate strong dipolar field around it in SDR, which significantly reduce the influence of proton diffusion on signal decay. Thus, the T₂ relaxivity of IONP can not increase with the rise of particle size and reach to a plateau [125,124]. Recently, Hyeon and co-workers investigate the T₂ relaxivity of cubic IONP with the size of 22, 28, 32, 42, and 49 nm (Fig. 3g and h) [156]. Due to the proper hydrodynamic diameter, cubic IONP with the size of 22 and 28 nm are in the SDR and show extremely high T₂ contrast with the similar r₂ values of approximately 800 mM⁻¹ s⁻¹. While, cubic IONP show reduced r₂ value when the size exceed 30 nm. These observations could be attributed to the large size of cubic IONP lead to them out of SDR and reach ELR. These results are highly consistent to the theoretical studies and point out that accurately controlling the particle size of IONP in SDR could obtain high-performance T₂ MRI CA.

2.3.2. Assembled IONP

Clustering of IONP is another effective method to increase their effective radius with increased T₂ relaxivity [126,161–165]. To generate assembled IONP, polymers have been chosen as candidates due to the finely controlled size and colloidal stability. Ai et al. encapsulate
hydrophobic IONP inside the core of polymeric micelle to form IO nanocluster (Fig. 4a–d) [164]. The $T_2$ relaxivity of IO nanocluster is approximately 6 times higher than individual IONP coated by PEG. Additionally, they observed that $T_2$ relaxivity increased with the increasing of the size and loading density of IONP. Compared to polymer, silica with high surface area and high-biocompatibility has been used as the matrix to construct IO nanocluster. For example, modifying dye doped silica with multiple IONP to improve the $T_2$ relaxivity of IONP has been reported (Fig. 4e–g) [165]. MRI contrast ability investigation indicates that $r_2$ value of silica based IO nanocluster is about 2.8 times higher than that of dispersed IONP. This clustering effect based $T_2$ relaxivity enhancement has also been observed in other researches, indicating its universality [21,166–168]. Besides, encapsulated IONP within silica matrix have been proved to develop high-performance IO nanocluster [162]. Weissleder and co-authors develop a strategy to encapsulate multicore IO nanocluster within the thin silica shell. With the increasing of size, IO nanocluster locates in MAR, SDR, and ELR, respectively. The IO nanocluster shows the highest $T_2$ relaxivity in SDR with the value of 695 mM $^{-1}$s$^{-1}$. These size-dependent $T_2$ relaxivity changing tendency of IONP has also been observed by Weller and co-authors. Moreover, Weller and co-authors further investigate the effect of size of clustered single IONP on the $T_2$ relaxivity of IO nanocluster [163]. They found that the $T_2$ relaxivity is regardless of the size of clustered single IONP in MAR. In contrast, $T_2$ relaxivity of IO nanocluster in the SDR seems to increase with size rising of the clustered single IONP. Since the size of IONP is proportional to its magnetic moment, these results may indicate that the $T_2$ relaxivity in SDR could be further increased by optimizing the magnetic property.

### 2.4. Synergistic effect of magnetic behavior and effective radius

Since individually increase the $M_s$ and effective radii of IONP could improve its $T_2$ relaxivity, one can hypothesize that simultaneous increase $M_s$ and effective radius of IONP could further increase its $T_2$ relaxivity. Unfortunately, the traditional synthetic method limits the simultaneous controlling the crystal structure and morphology. With the development of synthetic method, more and more researches on simultaneous improving $M_s$ and effective radius of IONP have been reported. Zhao et al. developed a novel strategy to improve the $M_s$ and effective radii of IONP through cation exchange reaction (Fig. 5a–e) [169]. They successfully synthesized cubical and octapod IONPs with high effective radii and magnetization moment. These engineered IONPs exhibit outstanding capacity to achieve $T_2$ contrast enhancement, especially zinc engineered octapod IONP ($r_2 = 754.2$ mM $^{-1}$s$^{-1}$ at 7 T). More recently, Gao and co-workers further elevate the dopant level of zinc in octapod IONP to further increase $M_s$ of IONP with high effective radius (Fig. 5f–h) [170]. The saturated magnetizations show the anti-V shape trend with the rising of zinc doping level. With the doping level of $x = 0.44$, the $M_s$ value reaches to the peak with the value of 88.9 emu/g (88.9 A-m$^2$/g). On the basis of the high effective radius and $M_s$ value, octapod $\text{Zn}_4\text{Fe}_3\text{O}_4$ nanoparticle ($x = 0.4$) exhibit the notably high $T_2$ relaxivity with the value of 989.1 mM $^{-1}$s$^{-1}$ at 7 T, which endow it as a sensitive $T_2$ CA to fulfill the sensitive detection on orthotopic and metastic tumor on mice model.
2.5. Inhomogeneous magnetic field

Theoretically, the diffusion of protons in the inhomogeneous magnetic field will lead to the dephasing of proton, which is the basis of the $T_2$ relaxation generation. The existence of $T_2$ MRI CA could generate an inhomogeneous magnetic field around it and accelerate the dephasing process of around protons, which is the basic goal of $T_2$ CA [119, 121, 171, 172]. The improvement of magnetic moment and size of IONP can enhance the local gradients of magnetic field around it and shorten the spin-spin relaxation time. Besides, IONP with anisotropic morphology, especially with sharp corner, could enhance the inhomogeneity of surrounding magnetic field. For instance, Gao and co-authors investigate the effect of the stray field gradient generated by Mn$_x$Fe$_{3-x}$O$_4$ nanoparticles with different anisotropic morphologies on their $T_2$ relaxivities (Fig. 6a–f) [160]. The Landau-Lifshitz-Gilbert (LLG) equation simulations indicate that different morphologies could effectively affect the shape, intensity, and gradient of the stray field. Moreover, the stray field generated by octapod morphology with eight sharp corners shows the highest inhomogeneity, resulting in the highest $T_2$ contrast among all samples. Recently, Zhou et al. investigate the inhomogeneity of stray magnetic field generated by IO based nano-cluster (Fig. 6g–s) [173]. They prepared a series of IO nano-clusters, including C1 (5 nm IONP only), C2 (15 nm IONP only), C3 (mixed 5 and 15 nm IONP), C6 (cubic IONP), C7 (plate IONP). The LLG simulation results indicate that magnetic field generated by C3 shows stronger inhomogeneity compared to C1 and C2. Therefore, C3 present significantly higher $T_2$ relaxivity (533.4 mM$^{-1}$s$^{-1}$) than C1 (231.6 mM$^{-1}$s$^{-1}$) and C2 (358.3 mM$^{-1}$s$^{-1}$). This magnetic field inhomogeneity based $T_2$ relaxivity enhancement is also observed in C6 and C7. These IO nano-clusters constructed by IONPs with anisotropic morphologies show enhanced magnetic field inhomogeneities and improved $T_2$ relaxivities compared to the traditional IO nano-clusters. Since electrons of atom surrounding the magnetic nanoparticles undergo circulation under external magnetic field, which could generate a small opposite magnetic field to the external magnetic field and further increase the inhomogeneity of local magnetic field, local magnetic field inhomogeneity could also be increased by the surface ligand of IONP as well. Gao and co-author investigated the influence of anchoring group on the local magnetic field [174]. They found that IONP modified by catechol and hydroxamate group generated stronger inhomogeneity than that modified by diphosphate group, due to the greatly contribution of π electron circulation on increasing the inhomogeneity of local magnetic field.

2.6. Surface coating structure

To act as a MRI CA for diagnosis, IONP need to be coated with hydrophilic layer, including small molecule, polymer, and protein, to disperse in aqueous solution [5, 175–178]. There are two main effects of surface coating on the $T_2$ relaxivity of IONP. Firstly, the coating layer require an anchoring moeity to chelate with Fe$^{2+}$ or Fe$^{3+}$ ions on surface, which may affect its magnetic property. Secondly, the coating layer can limit the diffusion of proton and hinder the interaction between proton and magnetic field induced by IONP.

2.6.1. Effect on magnetization moment

Due to the chelating with Fe$^{2+}$ and Fe$^{3+}$ ions, the coating layer may affect the arrangement of surface atoms and magnetization moment. Serna and co-authors report that the coordination of oleic acid to the
surface of IONP can effectively reduce the spin canting effect and increase the magnetic moment \[179\]. They observed that saturated magnetization of IONP coated with oleic acid (about 78 emu/g = 78 A⋅m²/kg) was higher than that without oleic acid. However, recent study indicate that chelating IONP with high affinity group may introduce structure defect and disturb the long-range-order of magnetic spin. Zeng et al. investigate the different surface coating ligand on the saturated magnetization of IONP (Fig. 7a–c) \[174\]. They found \(M_s\) value and \(T_2\) relaxivity of IONP decreased with the order of surface agent as hydroxamate > catechol > diphosphate, which was inversely correlated to their binding affinity to Fe\(^{3+}\) ions. This study suggests that coating IONP with moderate affinity group could optimize its magnetic property and improve its \(T_2\) contrast.

### 2.6.2. Proton diffusion

\(T_2\) relaxation of proton is highly determined by its diffusion behavior in the magnetic field gradients. The coating layer could affect the diffusion of proton by reducing the diffusion magnetic field strength, limiting proton diffusion, and forming hydrogen bond to proton. Previous research reported that the magnetic field generated by IONP decreased with the increase of the distance between IONP surface and proton. When the coating thickness increases to 10.8 nm, the magnetic field strength reduces to 2.3% of that on the surface \[183\]. This undesirable decrease may lead to the drop of \(T_2\) relaxivity of IONP. Dravid and co-authors found that with the increasing of silica shell thickness from 1 to 14 nm, the \(T_2\) relaxivity of IONP decreased from 94 to 32 mM\(^{-1}\)s\(^{-1}\) (Fig. 7d and e) \[180\]. Meanwhile, the coating layer reduces the proton diffusion efficiency within the layer, which benefits to its \(T_2\) relaxivity. Thus, one can optimize the coating layer structure to enhance the \(T_2\) relaxivity of IONP. Tong et al. investigated the effect of molecular weight of PEG on the \(T_2\) relaxivity of IONP (Fig. 7f–i) \[181\]. They modified 5 and 14 nm IONP by PEG chain with molecular weight of 550, 750, 1000, 2000, and 5000 Da, respectively. Regardless of the core size, \(T_2\) relaxivity of PEG modified IONP decreased from 94 to 32 mM\(^{-1}\)s\(^{-1}\) (Fig. 7f–i) \[181\]. Meanwhile, the coating layer reduces the proton diffusion efficiency within the layer, which benefits to its \(T_2\) relaxivity. Thus, one can optimize the coating layer structure to enhance the \(T_2\) relaxivity of IONP. Tong et al. investigated the effect of molecular weight of PEG on the \(T_2\) relaxivity of IONP (Fig. 7f–i) \[181\]. They modified 5 and 14 nm IONP by PEG chain with molecular weight of 550, 750, 1000, 2000, and 5000 Da, respectively. Regardless of the core size, \(T_2\) relaxivity of PEG modified IONP decreased from 94 to 32 mM\(^{-1}\)s\(^{-1}\) (Fig. 7f–i) \[181\].

IONP shows high biocompatibility, which is highly desirable for
biomedicine application. However, traditional IONP exhibits relatively low $T_1$ relaxivity and high $r_2/r_1$ ratio, hindering its application as the $T_1$ CA \[46, 155\]. Theoretically, $T_1$ relaxivity of IONP is determined by the chemical exchange efficiency between magnetic ions and proton, which is highly dependent on its crystal structure. Recently, a growing attention have focused on engineering crystal structure of IONP to improve its $T_1$ relaxivity and construction of IONP based $T_1$ CA.

3.1. SBM theory

$T_1$ CAs lead to the energy loss of spin through dipole-dipole interactions between water protons and magnetic ions, which causes the $T_1$ relaxation occur \[184, 185\]. There are three regions for interactions between water proton and magnetic ions, inner-sphere, secondary intermediate sphere, and outer sphere \[117\]. The inner-sphere describes direct interaction between water protons and magnetic ions and dominates the $T_1$ relaxation enhancement for the $T_1$ CAs. In secondary intermediate sphere and outer sphere, magnetic ions interact with the diffusing protons and exchangeable protons, which are not directly bind to the magnetic ions and accompany the exchange through hydrogen bond \[186, 187\]. However, the secondary intermediate sphere and outer sphere mediated $T_1$ relaxation enhancement are negligible compared to that caused by the inner sphere. Therefore, secondary intermediate sphere and outer sphere are often ignored due to the present of water proton in inner sphere in a real system. The $r_1$ value in inner sphere is given by \[188, 189\].

$$R_1 = qP_m\left[\frac{1}{(1/T_m + \tau_m)}\right]$$

$$\frac{1}{T_m} = \frac{2\gamma^2\gamma^2 S(S + 1)\mu^2}{15} \left[\frac{3\tau_{11}}{1 + 5\omega_0^2\tau_{11}^2} + \frac{7\tau_{12}}{1 + 10\omega_0^2\tau_{12}^2}\right]$$

$$\frac{1}{\tau_{11}} = \frac{1}{\tau_{1m}} + \frac{1}{\tau_{c1}} + \frac{1}{\tau_{c2}}$$

Where $P_m$ is the mole fraction of water coordinating to the metal center,
3.2. Reduction of magnetic moment

IONP exhibits high magnetization, meaning high $T_1$ contrast ability and high $r_2/r_1$ ratio. This defect highly hinders its application as a $T_1$ CA and should be overcome to develop IONP based $T_1$ CA. The magnetic property of IONP is highly determined by its crystal structure. One can optimize some key parameters, including size, crystallinity, and surface modification, to adjust the magnetic moment of IONP and develop $T_1$ CA. Recently, many strategies have been reported to reduce the magnetic moments of IONPs and optimize their $r_2/r_1$ ratio, which have been proved to be effective method to develop IONP based $T_1$ CA.

3.2.1. Spin disorder surface

Magnetic moment of IONP is highly dependent on its size. Normally, the magnetic moment decreases with the drop of its size, which could be ascribed to the increase of the spin disorders on the surface of IONP. This spin disorder surface of IONP are lack of full alignment and may destroy the long-range-order of magnetic spins, which could trigger the decrease of magnetic moment [144,146,190,191]. Since the thickness of this spin disorder surface is almost constant, the ratio of spin disorder surface to whole particles increase with the size decrease. For example, the proportion of spin disorder surface to entire particle is about 35% for 12 nm IONP, while increases to 48% when the size decreases to 5 nm [192]. Therefore, reducing the core size of IONP is the most straightforward method to reduce the magnetic moment of IONP and develop $T_1$ CA. Hyeon and co-authors synthesized IONP with size of 3 nm and investigated its $T_1$ contrast (Fig. 8a–e) [46]. Due to the remarkable spin canting...
effect, IONP with the size of 3 nm shows lower magnetic moment, higher $T_1$ relaxivity, and lower $r_2/r_1$ ratio compared to 12 nm IONP. However, the $T_1$ relaxivity of IONP does not always increase with the decrease of their sizes. Shen et al., reported that with the size increase, the $r_1$ value of IONP shows an anti-V shape curve and reach the plateau with the size of 3.6 nm (Fig. 8 f and g) [193]. Interestingly, the $r_2/r_1$ change exhibits a contrary tendency to $r_1$ change and reach the minimum at the size of 3.6 nm.

### 3.2.2. Crystal phase

Magnetite phase of IONP show the highest magnetic moment and is considered as the best candidate for $T_2$ contrast imaging among all phases. However, the high magnetic moment result in the high $r_2/r_1$ ratio and hinder its application as $T_1$ CA. Wüstite and Maghemite phase have been reported to show relative lower magnetic moment than magnetite phase, endowing them with the suitable magnetic property to reveal low $r_2/r_1$ ratio and be used as the $T_1$ CA [194, 195]. Compared to Wüstite phase composed with Fe$^{2+}$ cations (4 unpaired electrons), maghemite phase composed with Fe$^{3+}$ cations (5 unpaired electrons) presented higher $q$ values and $T_1$ relaxivity. Bawendi and co-authors fully oxidized the synthesized IONP from magnetite phase to maghemite by trimethylamine N-oxide [196]. The resultant product shows high $T_1$ relaxivity with the value of 5.2 mM$^{-1}$s$^{-1}$ and relatively low $r_2/r_1$ ratio with the value of 2.0 at 1.5 T, ensuring it to fulfill MRA and conventional $T_1$ MRI contrast in vivo.

### Table 1

| Regime | Optimized Parameter | Strategy | $r_2$ Value (mM$^{-1}$s$^{-1}$) |
|--------|---------------------|----------|-------------------------------|
| MAR    | $M_s$                | Dopant with Mn ions | 358 (1.5 T) [27] |
|        |                     | Dopant with Mn ions | 687 (4.5 T) [138] |
|        |                     | IONP coated iron   | 324 (9.4 T) [139] |
|        |                     | Coated monometallic iron with Mn doped IONP | 430 (0.47 T) [141] |
|        |                     | Reducing magnetically dead layer | 175 (1.7 T) [148] |
|        | Effective radii     | IONP with octapod morphology | 679 (7 T) [154] |
|        |                     | Clustering of IONP through polymer | 471 (1.5 T) [164] |
| SDR    | $M_s$                | Cubic IONP | 800 (3 T) [156] |
|        |                     | Increasing the size of clustered single IONP | 92 (3 T) [174] |

**Fig. 8.** Effect of spin disordered surface and crystal phase on $T_1$ relaxivity of IONP. TEM images of IONP with the sizes of (a) 1.5, (b) 2.2, (c) 3, and (d) 3.7 nm, respectively. (e) Plot of $T_1$ relaxation rate of IONP with different sizes. Reproduced with permission [46]. Copyright 2010, American Chemical Society. (f) $r_1$ value and $r_2/r_1$ ratio of exceedingly small IONP as a function of sizes. (g) Relative intensity of MR images for exceedingly small IONP with the sizes of 3.3, 3.6, and 4.2 nm, respectively. Reproduced with permission [193]. Copyright 2017, American Chemical Society.
3.3. Coordination number of water (q)

Theoretically, q is proportional to the \( T_1 \) relaxivity of \( T_1 \) CA. For IONP, its \( T_1 \) shorten effect could be attributed to existed iron ions on its surface. Since q of iron ion is constant, the straightforward method to optimize the q of IONP is increase the number of iron ions on surface \([155,197,198]\). The number of iron ions exposed on the surface of IONP is determined by two key factors: One is the surface to volume ratio, the other is the exposed crystal facet. Ordinarily, high surface to volume ratio and iron rich facet exposure are desirable to increase the number of iron ions on the surface of IONP. Thanks to the development of synthetic method, more and more IONPs with high surface to volume ratio and iron rich exposed surface have been reported to pursue IONP based high-performance \( T_1 \) CA \([44,199–202]\). In addition, q of \( T_1 \) CA is highly dependent on the number of unpaired electrons. Recently, introducing other magnetic ions with large amounts of unpaired electrons into crystal structure of IONP have been discovered to increase the q of IONP and elevate its \( T_1 \) relaxivity \([53,61,203–207]\). Here, strategies to increase q of IONP to raise its \( T_1 \) relaxivity have been reviewed in the following section.

3.3.1. Hollow structure

Surface iron ions of IONP, conducting chemical exchange with protons and accelerate their longitudinal relaxations, could be considered as the effective iron ions. Unfortunately, the chemical exchange between inner iron ions and protons have been blocked by the outershell, resulting in a reduction of the number of effective iron ions in IONP. Hollow structures with two interfaces between nanocrystal and surrounding environment can exceedingly rise the number of exposed magnetic ions, which is beneficial to elevate \( T_1 \) contrast of IONP \([208–211]\). Additionally, the hollow structure may disturb the long-range-order of magnetic spin and reduce magnetic moment, lowering the \( r_2/r_1 \) ratio. Inspired by these results, IONP with hollow structure have been synthesized to develop IONP based \( T_1 \) CA. Wei et al. synthesized hollow porous IO nanobox via the template-based method (Fig. 9a–e) \([210]\). The \( r_1 \) value of hollow IONP with the size of 14 nm is about 27.2 mM \(-1\) s \(^{-1}\), which is about 2.5 times higher than that of solid spherical IONP with similar size. More importantly, hollow IONP exhibits lower \( r_2/r_1 \) ratio than corresponding solid IONP with the value of 2.0 vs 10.4 due to its low magnetic moment. These results clearly demonstrate the promising potential of hollow IONP as a new \( T_1 \) CA with improved \( T_1 \) contrast. However, there are two key factors that should be paid attention to fabricating hollow IONP based \( T_1 \) CA, those are density and thickness of the shell. Since the elevation of \( T_1 \) relaxivity is mainly attributed to the addition of a new interface between IONP and proton environment, the efficiency of chemical exchange between iron ions in inner interface and proton determine the \( T_1 \) relaxivity of hollow IONP. The density and thickness determine the efficiency of water proton enter the cavity and achieve the chemical exchange. Thus, the desired hollow IONP based \( T_1 \) CAs should exhibit porous and thin shell.
3.3.2. Morphology

The $T_1$ relaxivity of spherical IONP have been comprehensively investigated. Changing the morphology of IONP from spherical to some specific shape can increase its surface-to-volume ratio and elevate the number of exposing magnetic ions. Consequently, the efficiency of chemical exchange reaction to surrounding proton and $T_1$ relaxivity of IONP improved. For example, the surface-to-volume ratio of cubic morphology is remarkably higher than that of spherical morphology. Demir and co-authors synthesized IO nanocubes with the size of ~9.7 nm [212]. The $T_1$ relaxivity assessment indicates that the $r_1$ value of this cubic IONP is about 8.3 mM$^{-1}$s$^{-1}$, which is higher than that of 3 nm spherical IONP. Author contribute this elevation effect on $T_1$ relaxivity to the increased surface-to-volume ratio from spherical to cube. Besides, $T_1$ contrast ability of IO nanowhisker with small diameter have been evaluated by Macher et al. (Fig. 9f-h) [201]. IO nanowhisker shows large aspect ratio with the length of 20 nm and diameter of 2 nm, which endows it with large surface-to-volume ratio to achieve efficient chemical exchange reaction to water. With high surface-to-volume ratio, this IO nanowhisker show high $T_1$ relaxivity with the $r_1$ value of 6.13 mM$^{-1}$s$^{-1}$, which is significantly higher than that of Magnetivit with the $r_1$ value of 3.3 mM$^{-1}$s$^{-1}$. It should note that this IO nanowhisker shows paramagnetic behavior due to the high surface-to-volume ratio, which result in forming iron-ligand complexes layer and reducing $T_2$ relaxivity. This effect ensure this IO nanowhisker with low $r_2/r_1$ ratio (1.83) to achieve sensitive $T_1$ contrast imaging.

Additionally, nanoparticles with anisotropic morphology can exhibit unique atomic package and show distinct interface to surrounding environment, which can affect the occupancy rate of magnetic ions on the surface and $T_1$ relaxivity of IONP. Zhou et al. obtained IO nanoplate with different thickness by controllable synthesis (Fig. 10a-h) [155]. This IO nanoplate exposes two Fe$_3$O$_4$ (111) facets, which are Fe$_{oct2-ter1}$ terminated. The exposed crystal facet could increase the interaction between surface iron ions and surrounding water proton. The $r_1$ value of IO nanoplate with the thickness of 2.8, 4.8, and 8.8 nm are 14.36 ± 1.24, 43.18 ± 3.33, and 38.11 ± 1.04 mM$^{-1}$s$^{-1}$, respectively, which are significantly higher than that of spherical IONP. It seems that $T_1$ relaxivities of IO nanoplates are highly determined by the ratio of (111) area to volume except for the 2.8 nm nanoplate, which could be attributed to the existence of spin-disorder at its corner. Further investigation on the effect of different exposed facet on $T_1$ relaxivity of IONP have been performed by Gao and co-authors (Fig. 10i-k) [160]. They studied $T_1$ relaxivity of Mn$_x$Fe$_{3-x}$O$_4$ nanoparticles with the morphologies of sphere, cube, plate, tetrahedron, and octapod with the exposed facet of (100), (111), (110), and (311), respectively. They found that different exposed facets of Mn$_x$Fe$_{3-x}$O$_4$ nanoparticles could provide different amounts of effective magnetic ions and result in the different increase degree on their $T_1$ relaxivities. The order of effective metals per $a^2$ on each facet is (110) > (111) ≈ (311) > (100). Unfortunately, the $r_1$ values of plate, tetrahedron, and octapod are almost the same. These results could be ascribed to the order of surface-to-volume ratios of these morphologies: sate octapod, plate, and tetrahedron, which partly offset the effect caused by the facet.

![Fig. 10. Effect of anisotropic morphology on $T_1$ relaxivity of IONP. TEM images of IO nanoplate with the thickness of (a) 8.8, (b) 4.8, and (c) 2.8 nm, respectively. (d) HRTEM image of IO nanoplate, indicating the (220) planes. (e) Perspective and (f) top views of Fe$_{oct2-ter1}$-terminated (111) planes of Fe$_3$O$_4$ structure, showing the iron iron-rich characteristics. (g) Relationships of $T_1$ relaxivity with the (111) surface of IO nanoplate compare to spherical IONP with equivalent whole surface area. (h) $T_1$ NMRD profiles of IO nanoplate with different thickness as the function of applied magnetic field. Reproduced with permission [155]. Copyright 2014, American Chemical Society. (i) The exposed faces of (100), (110), (111), and (311) of Mn doped IONP. (j) The relationship of surface-to-volume ratio and $T_1$ relaxivity. (k) The relationship of $r_1$ value and the number of effective magnetic metal ions on exposed facets. Reproduced with permission [160]. Copyright 2018, American Chemical Society.](image-url)
3.3.3. Unpaired electron

Some magnetic ions, such as manganese and lanthanide ions own large amounts of unpaired electrons, great potential to increase $q$ of IONP and regulate its $T_1$ relaxivity. Theoretically, the more unpaired electrons, the higher $q$ could be achieved. Compared to iron ions, those ions could be divided into two types, one exhibits longer $\tau_q$ and more electrons (including Mn, Gd, and Cu), the other only shows more electrons (such as Eu). In this section, we will discuss effect of single improvement of unpaired electrons on the $T_1$ relaxivity of IONP. Europium (III) ions with 6 unpaired electrons and $\tau_q$ with the value of $~10^{-14}$ s have been to be reported to improve the $T_1$ relaxivity of IONP. Yang et al. synthesized Eu doped iron oxide (EuIO) nanocube with the size of 14 nm [213]. $T_1$ relaxivity investigation indicates that $\tau_q$ value and $\tau_q/\tau_1$ ratio of this EuIO nanocube are $36.79 \pm 1.16$ mM$^{-1}$s$^{-1}$ and 2.65, which is more suitable to act as a $T_1$ CA compare to IONP. The elevation effect could be ascribed to higher chemical exchange of surface Eu (III) ions with nearby protons compare to iron ions.

3.4. Electronic relaxation time ($\tau_s$)

According to SBM theory, magnetic nanoparticle with long $\tau_s$ exhibits high $T_1$ relaxivity. Since Fe (II) ion exhibits fewer unpaired electrons and significantly shorter $\tau_s$ than Fe (III) ion ($10^{-9}$–$10^{-11}$ s and $10^{-12}$–$10^{-13}$ s for Fe (III) and Fe (II) ion) [214–216], this structure deficiency limits efficiency of relaxation enhancement of IONP in form of magnetite and limits its application as $T_1$ CA. Therefore, replacement of Fe (II) ions in magnetite by other magnetic ions with relative long $\tau_s$ may overcome the structure deficiency and increase the $T_1$ relaxivity of magnetite. In the following section, we will introduce some recent attempts to replace Fe (II) ions in magnetite and discuss the effect of different replaced magnetic ions on the $T_1$ relaxivity of magnetite.

3.4.1. Manganese

Manganese ions exhibit diverse valence state, such as $+2$, $+3$, $+4$, and $+7$. Among them, Mn (II) ion based probe has widely be used as a $T_1$ CA in MRI contrast imaging due to its paramagnetic behavior. Compared to Fe (II) ion, Mn (II) ion owns higher unpaired electron (5 unpaired electrons for Mn (II) ion and 4 unpaired electrons for Fe (II) ion) and exhibit longer $\tau_q$ ($10^{-8}$ s for Mn (II) ion and $10^{-12}$–$10^{-13}$ s for Fe (II) ion) [217,218]. Therefore replacement of Fe (II) ion in magnetite by Mn (II) ion may efficient improve the coordination number and $\tau_q$ optimizing its $T_1$ relaxivity. Huang et al. synthesized manganese engineered IONP (MnIONP) to investigate the effect of introducing Mn (II) ion on its $T_1$ contrast [153]. The $T_1$ relaxivity assessment indicates that engineered IONP with Mn (II) ion could efficiently improve its $T_1$ relaxivity. Gao and co-authors further engineered IONP with anisotropic morphologies by Mn (II) ion and investigated the influence of Mn (II) ion on their $\tau_q$ and $T_1$ relaxivity (Fig. 11a–g) [51]. The electron paramagnetic resonance spectrum analyses indicate that introducing of Mn (II) ion can remarkably increase the $\tau_q$ of pure IONP regardless of the morphologies. $\tau_q$ of MnIONP with the shape of cube, octapod, and plate are about 0.26, 0.19, and 0.23 ns, which are approximately 2.17, 2.38, and 1.44 times higher.
than those of corresponding pure IONP, respectively. Coupled with the abundantly exposed magnetic ions, this \( r_1 \) elevation effect remarkably increase the \( T_1 \) relaxivities of IO nanocube, octapod, and plate to 57.8, 62.1, and 22.4 mM\(^{-1}\)s\(^{-1}\) at 0.5 T.

### 3.4.2. Gadolinium

Due to its large amounts of unpaired electrons and long \( \tau_m \) paramagnetic gadolinium ions (Gd\(^{3+}\)) are widely used to construct \( T_1 \) CAs and exhibit high \( T_1 \) contrast through effective interacting with adjacent water protons. Compared to IONP, ultrasmall Gd\(_2\)O\(_3\) nanoparticles and Gd\(_2\)O\(_3\) nanoplate have been proved to exhibit more effectively \( T_1 \) contrast with lower \( r_2/r_1 \) ratio due to the low magnetic susceptibility. These results inspire researchers to embed Gd\(_2\)O\(_3\) nanocluster into IONP and investigate their \( T_1 \) contrast \([219]\). Small sized gadolinium-embddied IONP (GdIONP) have been reported to show high \( T_1 \) relaxivity, due to the spin-canting effects and introduction of Gd species \([220]\). GdIONP with the size of 4.8 nm shows \( T_1 \) relaxivity with the value of 7.85 mM\(^{-1}\)s\(^{-1}\) and \( r_2/r_1 \) ratio of 5.24 at 7 T. Gao and co-authors further engineered IO nanoplate with metal-rich exposed crystal surface by Gd ions to optimize its \( T_1 \) relaxivity \((\text{Fig. 11b-l})[221]\). Based on the synergistic effects of exposed metal-rich Fe\(_3\)O\(_4\) (100) facet and embedded Gd\(_2\)O\(_3\) clusters, this engineered IO nanoplate show an ultrahigh \( T_1 \) relaxivity with the value of 61.5 mM\(^{-1}\)s\(^{-1}\) and \( r_2/r_1 \) ratio of 2.4 at 0.5 T. The enhanced \( T_1 \) relaxivity could be ascribed to two main reasons, those are exposed Fe and Gd ions and terminated Fe\(_3\)O\(_4\) (100) basal plane. The exposed Fe and Gd ions could provide sufficient paramagnetic island and result in synergistic enhancement. In addition, the flatten surface is beneficial to the hoping of water proton on the surface of Gd engineered IO nanoplate and further improve the chemical exchange efficiency. It should be noted that its \( T_1 \) relaxivity is highly dependent on the amount of Gd ions. \( r_1 \) values increased from 46.7 ± 2.0 to 66.3 ± 3.1 mM\(^{-1}\)s\(^{-1}\) with the rising of Gd percentage from 11% to 26%. We speculate that this result may be a consequence of increased \( r_1 \) caused by the increase amount of Gd ions.

#### 3.4.3. Copper

Copper ions with the \( r_1 \) value of \( 10^{-9} \) have been reported to be a candidate \( T_1 \) MRI CA in the form of CuO and CuS nanoparticles \([223–226]\). In addition, the magnetization moment of copper based nanoparticles are significantly lower than that of IONP. Thus, engineering IONP by copper ions may improve its \( r_1 \) and reduce its magnetization moment, which may improve the \( T_1 \) relaxivity of IONP. One attempt have been carried out to improve the \( T_1 \) relaxivity of IONP by copper dopant \((\text{Fig. 11m-q})[222]\). Herranz and co-author developed a copper doped extremely small IONP (CuIONP). When the dopant percentage of copper is 4%, CuIONP exhibits high \( T_1 \) relaxivity with the \( r_1 \) value and \( r_2/r_1 \) ratio of 15.7 mM\(^{-1}\)s\(^{-1}\) and 2.1. The relative high \( r_1 \) value and low \( r_2/r_1 \) ratio endow these engineered IONP with good \( T_1 \) contrast to achieve MRI based angiography and tumor imaging.

#### 3.5. Chemical exchange

\( T_1 \) relaxivity of MRI CA is dependent on the chemical exchange efficiency between CAs and water protons at the interface. Surface ligand of CA could affect water diffusion, retention, and interaction with the magnetic centers, which determine \( \tau_1 \), \( \tau_0 \), \( \tau_{lm} \) and the chemical exchange efficiency between IONP and water proton. A deep investigation of the effect of surface ligand on the \( T_1 \) relaxivity of IONP can provide guidance in improving its \( T_1 \) contrast.

##### 3.5.1. Molecular weight

\( r_1 \) value of IONP is mainly determined by the exchange rate of water proton in its inner sphere. Theoretically, IONP stabilized by low molecular weight \((M_w)\) ligand would reduce the hydrodynamic volume, resulting in the accessibility reduction of water proton to the surface of IONP. Therefore, altering the \( M_w \) or chain length of the surface coating ligand seem to be the simplest way to adjust \( T_1 \) relaxivity of IONP. In previous study, the relationship between \( M_w \) of polyethylene glycol (PEG) and \( T_1 \) relaxivity have been investigated \((\text{Fig. 12a})[41]\). It seems that \( r_1 \) value increases with the rising of \( M_w \) of PEG. IONP coated by PEG with the \( M_w \) of 1000, 2000, 5000 Da show higher \( r_1 \) values than that with \( M_w \) of 550 and 750 Da. This \( M_w \) related \( T_1 \) relaxivity also been reported in other research. Tromsdorf et al. found that \( r_1 \) value of PEG coated IONP gradually increases with rising of \( M_w \) from 350 to 1100 Da, but decreases when \( M_w \) increases to 2000 Da \([199]\). The \( r_1 \) value decreasing at 2000 Da may be attributed to the aggregation of IONP.

#### 3.5.2. Hydrophilicity

Hydrophilicity increasing of surface area benefits the water access to the surface of IONP and elevates the chemical exchange efficiency. Bao et al. crosslinked tannic-acid coated IONP onto bovine serum albumin to form nanocluster \([227]\). The relaxivity measurement indicates that \( r_1 \) value of nanocluster is about 2 times higher than that of free nanoparticles. This \( r_1 \) value elevation could be ascribed to the high hydrophilicity around IONP and reduced mobility within the nanoclusters. In another research, Seo and co-authors modified Eu doped IONP (EuIONP) with citrate (Cit), alendronate (Ale), and PMAO/PEG (PP), which showed different hydrophilic \((\text{Fig. 12b-f})[228]\). The hydrophobicity of all samples were measured by the contact angle. The contact angles of Cit, Ale, and PP are immeasurable, 9.8°, and 112.4°, which means the hydrophobicity order is Cit « Ale « PP. This result is highly consistent with the changing of \( T_1 \) relaxivity with the order of EuIONP-Cit > EuIONP-Ale « EuIONP-PP.

#### 3.5.3. Electronic and magnetic interactions

It is well known that graphene oxide (GO) could interact with semiconducting oxide nanoparticles through excited-state electron transfer. The charge-transfer between electronic and magnetic interactions have been reported to exist between IONP and GO \([229–231]\). This unique phenomenon may affect the chemical exchange interaction between GO coated IONP and surrounding water proton. Recently, the effect of GO on the \( T_1 \) relaxivity of IONP have been investigated \((\text{Fig. 12g-i})[232]\). \( T_1 \) relaxivity assessment indicate that GO coated IONP show \( T_1 \) relaxivity with the value of 2.82 mM\(^{-1}\)s\(^{-1}\), which is about 6 times higher than that of free IONP with the \( r_1 \) value of 0.46 mM\(^{-1}\)s\(^{-1}\). The enhancement on \( T_1 \) relaxivity of IONP is believed to contribute to two mainly reasons. One is the energy exchange and charge transfer between GO and IONP, the other is the homogeneous dispersion of IONP. Notably, this research indicates that \( T_2 \) relaxivity of IONP could be suppressed by coating of GO, which could be ascribed to the limitation effect of GO on the spin of proton and local magnetic field. The decreasing effect on \( T_2 \) relaxivity could reduce \( r_2/r_1 \) ratio, further improving the availability of GO coated IONP as \( T_1 \) CA.

### 4. IONP based dual-modal imaging

Traditional strategies to achieve sensitive MRI contrast imaging by IONP is increasing its \( T_1 \) or \( T_2 \) relaxivity, which could improve the signal difference between normal tissue and lesion. Although \( T_1 \) image shows high tissue resolution and \( T_2 \) image exhibits high feasibility of detection under the assistant of single modal CA, such single mode CA are not yet perfect and facing huge challenges in accurate imaging of tiny lesion. To improve the sensitivity and accuracy, simultaneous acquisitions of \( T_1 \) and \( T_2 \) CA have attract considerable interest. Combination of \( T_1 \) and \( T_2 \) modal can provide complementary information for doctor to differentiate lesion from normal tissue with improved sensitivity and accuracy. In recent ten years, a number of IONP based \( T_1/T_2 \) dual-modal MRI CAs have been developed \([52,57,233–238]\). Besides to develop IONP with MRI based dual-modal contrast, direct conjugation secondary even tertiary imaging moiety with IONP is another effective method to improve its contrast efficiency. Currently, there are several representative imaging modalities have been used in clinical or preclinical research,
including MRI, SPECT, PET, CT, FL imaging, US imaging, and PA imaging. Introducing of these imaging modality to IONP can effectively overcome the instrumental limitation of MRI and increase its sensitivity to satisfy the requirement of accurate imaging. In the following section, we will focus on introducing recent method to fabricate IONP with dual-modality contrast to improve its diagnosis efficiency.

4.1. T<sub>1</sub>-T<sub>2</sub> dual-modality

For the purpose of dual-modal enhancement, the straightforward method to construct IONP based T<sub>1</sub>/T<sub>2</sub> dual-modal CA is introducing T<sub>1</sub> contrast domain into IONP. Since the direct chemical exchange to water proton is the basis of T<sub>1</sub> contrast imaging, this strategy often adopts as the T<sub>1</sub> contrast domain exposed on the outer shell [56,239–241]. Im et al. synthesized MnO coated IONP with core-shell, dumbbell-like, and flower structure and assessed their capacity on simultaneous enhancement on both T<sub>1</sub> and T<sub>2</sub> modal [Fig. 13a–d] [242]. The r<sub>1</sub> values and r<sub>2</sub>/r<sub>1</sub> ratios of Fe<sub>3</sub>O<sub>4</sub>@MnO, Fe<sub>3</sub>O<sub>4</sub>/MnO dumbbell, and Fe<sub>3</sub>O<sub>4</sub>/MnO flower are 1.3, 1.4, and 0.6 mM<sup>-1</sup>s<sup>-1</sup> and 28, 56, and 235, which are not suit applied as the T<sub>1</sub>/T<sub>2</sub> dual-modal CAs to early and accurate diagnosis. The relatively high r<sub>2</sub>/r<sub>1</sub> ratio of this kind of core-shell structure have been found in IONP coated by Gd<sub>2</sub>O<sub>3</sub> shell as well [Fig. 13e–h] [243]. Sun et al. constructed a yolk-like nanostructure using IONP as core and mesoporous Gd<sub>2</sub>O<sub>3</sub> layer as shell. The r<sub>2</sub>/r<sub>1</sub> ratio of this nanostructure is about 48.9, which is significantly higher than that of typical T<sub>1</sub>/T<sub>2</sub> dual-modal CA. These results could be ascribed to the fact that IONP could slow the T<sub>1</sub> spin fluctuation, which is ineffective for water proton relaxation and leads to a low T<sub>1</sub> MRI signal. Cheon and co-worker discovered that the speed of electron spin fluctuation of T<sub>1</sub> contrast domain is determined by the distance between T<sub>1</sub> and T<sub>2</sub> contrast domains [Fig. 13i–n] [58]. When the distance of the T<sub>1</sub> and T<sub>2</sub> domains is above a certain value, the electron spin fluctuation of T<sub>1</sub> domain accelerate water proton relaxation and result in a stronger T<sub>1</sub> MRI signal. Thus, tuning the distance between T<sub>1</sub> domain and IONP have been adopted to construct effective T<sub>1</sub>/T<sub>2</sub> dual-modal CAs [244]. Cheon and co-workers utilized SiO<sub>2</sub> as the isolation layer to separate T<sub>2</sub> domain (MnFe<sub>2</sub>O<sub>4</sub> nanoparticles) and T<sub>1</sub> domain (Gd<sub>2</sub>(CO<sub>3</sub>)<sub>3</sub>n2 shell) [Fig. 14a–c] [245]. Along with the thickness increase of SiO<sub>2</sub> layer in the
order 4, 8, 12, 16, and 20 nm, the $r_1$ value changes in the order 2.0, 4.0, 25.1, 33.1, and 32.5 mM$^{-1}$s$^{-1}$. On contrary, $T_2$ relaxivity decreases with the increasing of separating layer due to reduction effect on local magnetic field caused by SiO$_2$ layer. These results endow this core-shell nanostructure as a potential $T_1$/$T_2$ dual-modal CA. The generality of introduction of SiO$_2$ isolating layer to separate $T_1$ domain and $T_2$ domain have been demonstrated by the same group [60]. Various $T_2$ domains, such as Fe$_3$O$_4$ and CoFe$_2$O$_4$, and $T_1$ domains, including Eu$_2$O(CO$_3$)$_3$, Dy$_2$O(CO$_3$)$_3$, and [Im][Mn(BTC)(H$_2$O)] have been combined to construct artifact filtering imaging agent (AFIA). All of the AFIA exhibit simultaneous remarkable $T_1$ and $T_2$ signal enhancement. Except to SiO$_2$, polydopamine have been discovered to be another isolation shell to construct IONP based $T_1$/$T_2$ dual-modal CAs [233]. Another structure to regulate the distance between $T_1$ domain and $T_2$ domain is Janus structure. Cheng et al. synthesized dumbbell hybrid nanotrimers and utilized Pt nanocube to isolate Gd chelators attached Au nanoparticles and IONP (Fig. 14d–f) [236]. The linking distance between Au and IONP have been accurately regulated with the value of 5.4, 9.6, 10.7, and 12.9 nm. Consistent with the previous study, the dumbbell nanotrimers show increased $T_1$ relaxivity with the rising of distance between Au and IONP due to the reduced magnetic coupling between Gd and IONP. Based on their proper distance, dumbbell nanotrimers with the linking distance of 10.7 and 12.9 show obvious simultaneous $T_1$ and $T_2$ signal enhancement effect. Altering the structure of IONP have been proved to be effective strategy to adjust the contrast of IONP in $T_1$ and $T_2$ modal. The critical parameter, which determine a given IONP is $T_1$ or $T_2$ dominated MRI CA, is its $r_2/r_1$ ratio. Previous study indicate that engineering the structure of IONP, especially morphology, could increase its $T_1$ relaxivity and reduce its $T_2$ relaxivity, which endow it with favorable $r_2/r_1$ ratio to exhibit $T_1$/$T_2$ dual-modal contrast.

Recently, The $T_1$ and $T_2$ contrast ability of IO nanoplate with the thickness of 4.8 nm have been investigated [155]. Its exposed Fe$_3$O$_4$(111) facet provided sufficient iron ions to achieve chemical exchange to water proton, which increased its $T_1$ contrast ability. Besides, the thin thickness improved the spin canting effect and reduce its magnetization and $T_2$ contrast ability. Due to the lowering $T_2$ contribution and enhancing $T_1$ contribution, IO nanoplate with the thickness of 4.8 nm show significant signal enhancement in both $T_1$ and $T_2$ contrast imaging. This morphology dependent typical $T_1$ and $T_2$ dual-modal contrast have also been found in IONP with other morphology, such as nanocube [212].

In addition, dopant of paramagnetic ions, especially gadolinium ions into the crystal structure of IONP have been proved to be another effective method to construct IONP based $T_1$/$T_2$ dual-modal CA. Gao and co-authors embedded gadolinium cluster into IONP, which lead to a synergistic enhancement on $T_1$ and $T_2$ relaxivity of IONP (Fig. 15) [246]. The $r_1$ value of gadolinium doped IONP (GdIONP) is $\sim$69.5 mM$^{-1}$s$^{-1}$ in terms of Gd; and the $r_2$ value of GdIONP is about 146.5 mM$^{-1}$s$^{-1}$ in terms of Fe. Due to the dopant of gadolinium ions, the $r_1$ and $r_2$ values of GdIONP are both higher than Gd$_2$O$_3$ and IONP and show obvious signal enhancement in both $T_1$ and $T_2$ modal. Dopant other paramagnetic ions into IONP, such as manganese and europium, have been proved to achieve enhancement of $T_1$ and $T_2$ signal as well [213,234]. Xiao et al., have synthesized DSPE-PEG coated MnIONP as $T_1$/$T_2$ dual-modal CA.
They found that MnIONP coated with DSPE-PEG with the mass ratio of 1:20 showed harmonious $T_1$ and $T_2$ relaxivity and could be considered as an excellent candidate as $T_1$/$T_2$ dual-modal CA.

### 4.2. MRI-FL modality

Fluorescence imaging with high sensitivity is the earliest modality to be introduced into IONP to ameliorate its contrast sensitivity. Initially, the fluorescence dye or quantum dots were directly conjugated with IONP to form the MRI-fluorescence dual-modality CAs [248–250]. Xu and co-authors used IONP as seeds to grow CdSe QDs on its surface and obtained hybrid IONP with magnetic and fluorescence property [251]. This hybrid IONP could successfully achieve fluorescence imaging on cellular level, while the existed energy transfer between the fluorescent domain and IONP result in a fluorescence quenching and limit its application as a MRI-fluorescence dual-modality CA in vivo. Since this quench effect is highly dependent on the distance between the fluorescent domain and IONP, one can improve its fluorescent property by increasing this distance. Lee et al. attached IONP on dye-doped silica nanoparticles to form "core-satellite" nanostructure with MRI and fluorescence imaging capacity (Fig. 16 a–f) [73]. The fluorescence signal of this core-satellite nanostructure is enhanced by 1.7 times compare to the directly dye conjugated IONP with the assistance of separating effect of silica, which reduces quenching effect between IONP and fluorescent dye. Moreover, the core-satellite nanostructure with assembled IONP show increased $T_2$ relaxivity with the $r_2$ value of 397 mM$^{-1}$s$^{-1}$. The perfect MRI and fluorescent contrast ability endow this nanostructure to conduct sensitive imaging of sub-millimeter cellular clusters.

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Fig. 14. IONP based core-shell $T_1$/$T_2$ dual-modal CA. (a) Schematic and TEM image of core-shell type dual-modal CA. (b) TEM images of dual-modal CAs with different separating layer thickness. (c) $r_1$ and $r_2$ values of dual-modal CAs, MnFe$_2$O$_4$, Gd-DTPA, and Feridex. Reproduced with permission [245]. Copyright 2010, American Chemical Society. (d) Schematic illustrate the magnetic coupling of $T_1$ and $T_2$ CAs in core-shell and dumbbell structures. (e) Illustration of constructions of four different types of dumbbell-like or dumbbell heterostructures. (f) $T_1$- and $T_2$-weighted MRI images of dumbbell hybrid heterostructures. Reproduced with permission [236]. Copyright 2014, American Chemical Society.

Fig. 15. IONP based $T_1$/$T_2$ dual-modal CA. (a) TEM and (b) EDX mapping images of GdIONP. $T_1$- and $T_2$-weighted MR images of (c) GdIONP, (d) IONP, and (e) Gd$_2$O$_3$ nanoparticles, respectively. The analyses of (f) $T_2$ and (g) $T_1$ relaxation rate of GdIONP, IONP, and Gd$_2$O$_3$ nanoparticles. Reproduced with permission [246]. Copyright 2012, John Wiley & Sons, Inc.
4.3. MRI-PA modality

As a relatively new imaging technique, PA imaging exhibits high spatial resolution and fast real-time scan. Over the past decades, there are numerous materials, including gold [69,252-254], indocyanine green (ICG) [78,255], polypyrrole (PPy) [80], and diketopyrrolopyrrole (DPP) [79], have been applied to incorporating the advantages of PA imaging with the MRI to fabricate MRI-PA dual-modal CA. Interestingly, IONP was discovered to increase the signal of PA imaging due to the addition of IONP, which boosts the heat generation and heat dissipation pathways in the production of PA signal. Combining the high contrast imaging efficiency of IONP with retained MRI contrast property shows SPECT contrast imaging. Coupled with excellent stability, this CA provides sufficient signal to successfully achieve liver and spleen imaging on mice model. On the basis of long half-life, 125I have been investigated as the SPECT imaging moiety to construct IONP based MRI/SPECT dual-modal CAs.

4.4. MRI-PET/SPECT dual-modality

PET or SPECT, those signal is generated by gamma-rays emitted from decaying radioisotopes (e.g. 64Cu and 124I for PET and 99mTc and 131I for SPECT), show high sensitivity but relatively poor spatial resolution. Combination of PET or SPECT with MRI have been proved to improve the contrast imaging efficiency of IONP [256-258]. 124I have been successfully linked on MnIONP through chemical conjugation (Fig. 17a-c) [259]. The resultant hybrid CA maintain equivalent contrast effect corresponding to the CA of single MRI and PET modality. This unique feature endow this dual-modality CA with high spatial resolution and high sensitivity to perform lymph node imaging. On the assistant of a fused MRI-PET image, researchers can easily differentiate the tiny brachial lymph from axillary lymph node with the diameter of a few millimeters. Meanwhile, many attempts have been conducted to fabricate IONP based MRI-PET dual-modality CAs. IONP radiolabeled with 99mTc is reported as a MRI-PET dual-modality CA (Fig. 17d-k) [66]. The 99mTc labeled IONP with retained in vivo MRI contrast property shows SPECT contrast imaging. Coupled with excellent stability, this CA provides sufficient signal to successfully achieve liver and spleen imaging on mice model. On the basis of long half-life, 125I have been investigated as the SPECT imaging moiety to construct IONP based MRI/SPECT dual-modal CAs.

4.5. MRI-CT modality

Considering CT is advantageous in regard to its high resolution and ease of forming 3D visual reconstruction of tissue of interest, introducing CT contrast moiety onto IONP have been used to improve its imaging efficiency [260-263]. The common strategy is synthesis of Au nanoparticles hybridized IONP. Zhao et al. successfully synthesized strawberry-like Fe3O4-Au hybrid nanoparticle with enhanced X-ray attenuation and magnetic properties as an accurate MRI-CT dual-modal CA to distinguish the grade of live disease (Fig. 18a-e) [264]. Except to Au, the possibility of fabrication IONP based MRI-CT dual-modal CAs with other CT contrast moiety have been investigated as well. Multifunctional Fe3O4/TaOx core/shell nanoparticle have been synthesized by Hyeon and co-workers (Fig. 18F-I) [265]. On the basis of its low cost and high biocompatibility, TaOx is more promising as the CT contrast moiety to construct MRI-CT dual-modal CA compared to Au nanostructure. Fe3O4/TaOx core/shell nanostructure exhibits remarkable
5. IONP based responsive MRI CAs

The basis of MRI CA based disease diagnosis is the signal difference caused by the different accumulation amount of CA in lesion and surrounding tissue. Although surface modification could optimize the in vivo behavior of CA and improve their accumulation in lesion, the misaccumulation in normal tissue could not be avoided. Besides, the traditional CAs are “always on” systems that generate MR signal enhancement regardless of their location, which may result in poor target to background signal difference. Recently, responsive MRI CA have been designed to respond to specific changes in the surrounding physiological microenvironments and specific physical conditions [271]. Compared to the traditional MRI CA with fixed contrast capacity in both normal and lesion tissue, responsive MRI CAs are designed to switch “on” and “off” T2 or T1 signal change in response to a specific stimulus of lesion. Generally, responsive MRI CA could be divided into three types, those are active, recovery, and switchable. Discussion on the recent development of strategy to construct IONP based responsive MRI CA to assist doctors to achieve accurate and early lesion diagnosis is urgent and meaningful.

5.1. Active

Compared to single IONP, assembled architecture shows remarkably increased T2 contrast ability. Modifying nanoparticles with specific molecular have been proved to be an effective strategy to implement their self-assembly under stimuli [271]. This unique phenomenon provided opportunity for IONP to complete the conversion from single state to assembled state and achieve T2 active MRI imaging. Jasanoff and Co-worker engineered IONP with fused C2 domains of synaptotagmin 1 (C2AB), which could naturally respond to the concentration change of Ca\(^{2+}\) (Fig. 19a–e) [272]. Based on the two binding sites for Ca\(^{2+}\) in C2AB, this IONP could aggregate in the presence of Ca\(^{2+}\) and promote its T2 contrast. Along with the increasing of Ca\(^{2+}\) concentration from 0 to 1.2 mM, the r2 value elevates from 151 to 261 mM \(^{-1}\)s. [271]. Based on the calcium-dependent r2 change, this nanomaterial could be considered as a potential CA to monitor the dynamic change of Ca\(^{2+}\) concentration in brain. Additionally, IONP based nanostructure have been reported to respond to matrix metalloproteinase (MMP) enzymes. In this work, IONP bearing complementary azide and alkyne click moieties are individually prepared [273]. To achieve MMP response, a MMP responded peptide was conjugated on the surface of IONP to block the activity click reaction. In vitro study indicates that the two individual IONP successfully forms nanocluster at the present of MMP and result in a 160% increase in T2 activity. Recently, a cancer biomarker, glutathione (GSH), responsive IONP based active probe was developed (Fig. 19f–h) [274].
With the existence of GSH, the disulfide bond on the surface of IONP is reduced, inducing the aggregate of IONP. The status conversion from single state to aggregation state results in the interlocked responses of both $T_1$ and $T_2$ signals and is utilized to quantitatively map the GSH within brain gliomas.

5.2. Recovery

As a typical $T_2$ CA, IONP have been proved to quench the $T_1$ relaxivity of Gd chelate [275]. It has been reported that conjugating Gd chelates on IONP with bio-response linker could achieve $T_1$ relaxivity recovery (Fig. 20 a and b) [276]. In this study, the Gd chelate was conjugated on the silica coated IONP by MMP-2 responded peptides. To amplify the signal, dendrimers are introduced to increase the number of conjugated Gd chelate. MMP-2 successfully cleaves the peptide, resulting in release of Gd chelate from the local magnetic field generated by IONP and $T_1$ signal recovery. Based on the bio-response procedure, the detection limit of these systems to MMP-2 could be reduced to 0.5 nM achieving MMP sensing both at cellular level and xenograft tumors. $T_2$ relaxivity of IONP could be limited by a dense $T_1$ CAs shell as well. Introducing an environment responsive shell with $T_1$ contrast on the surface of IONP can initially shield its $T_1$ and $T_2$ relaxivity and achieve signal recovery under stimuli. Kim et al., developed a $T_1/T_2$ dual mode recovery imaging probe by coating IONP with redox-responsive paramagnetic Mn$_3$O$_4$ nano shell (Fig. 20c–g) [277]. Due to the strong silencing effect between Fe$_3$O$_4$ core and Mn$_3$O$_4$ shell, $T_1$ and $T_2$ contrast of this nanostructure are remarkably limited. Upon introduction to a tumor intracellular reducing environment, the Mn$_3$O$_4$ shell decomposed to release free Mn$^{2+}$ ions. This structure alteration resulted in exposure of the interior Fe$_3$O$_4$ core to aqueous environment and recovery of $T_1$ and $T_2$ relaxivity.

5.3. Switchable

Recently, IONP based environment responsive MRI CA with modal switchable capacity have been developed to further increase the signal difference between lesion and normal tissue. There are two modal of switchable responsive MRI contrast imaging: $T_2$ contrast switch to $T_1$ contrast (mode I) and $T_1$ contrast switch to $T_2$ contrast (mode II). The strategy to develop mode I MRI CAs is construction IONP based nano-cluster or nanoaggregation by responsive ligand. This kind of CA exist as nanocluster in blood circulation and normal tissue while collapse to dispersive nanoparticle, resulting in the $T_2$ contrast ability decrease and $T_1$ contrast capacity increase. On contrary, mode II CA is designed by in situ assemble of specific modified dispersive nanoparticles. Generally, ultrasmall IONP, typical with the size below 5 nm, have been considered as $T_1$ CAs. With specific surface modification, these nanoparticles form the nanoaggregation when it reach to the tumor tissue and can be readily enhance $T_2$ signal [88–90].

5.3.1. $T_2/T_1$ switchable

Lu et al. fabricated a pH-sensitive IO nanocluster through linking
IONP with i-motif DNA-derived pH-responsive linkers (Fig. 21a–g) [278]. Along with the decrease of pH value from 7.4 to 5.5, the state of IONP with i-motif DNA-derived pH-responsive linkers (Fig. 21a) drastically decrease in [278]. Along with the decrease of pH value from 7.4 to 5.5, the state of IONP with i-motif DNA-derived pH-responsive linkers (Fig. 21a) changes observed in HEPES buffer over multiple cycles of calcium or EDTA addition. Reproduced with permission [272]. Copyright 2018, Nature Publishing Group. (f) Schematic drawings to show molecular mechanism of GSH-induced agglomeration of intelligent probe. (g) Temporal evolution of \( \Delta R_1 \) and \( \Delta R_2 \) for intelligent probe and peptide modified IONP. Reproduced with permission [274]. Copyright 2021, John Wiley & Sons, Inc.

To further increase the controllability of this clustering process and MRI contrast modal conversion, light sensitive \( T_1/T_2 \) switchable CA has been developed recently (Fig. 21h–l) [279]. Li et al. modified ultrasmall IONP with light-addressable unit DA via PEG spacer. The generated \( \text{Fe}_3\text{O}_4\text{-PEG-DA} \) shows high \( T_2 \) relaxivity with the value of 3.83 mM \(^{-1}\)s\(^{-1}\) and could be used as a sensitive \( T_1 \) CA. Once laser irradiation with different duration time is applied, IO nanocluster with different aggregation degree can be formed. After 12 min irradiation, the size of IO cluster could reach 798.4 nm and result in the obviously increase of \( T_2 \) relaxivity (from 9.04 to 31.60 mM \(^{-1}\)s\(^{-1}\)) while reduction of \( T_1 \) relaxivity (from 3.83 to 1.61 mM \(^{-1}\)s\(^{-1}\)), showing a typical \( T_1/T_2 \) switchable process.

### 6. Optimization in vivo behavior of IONP

Apart from \( r_1 \) or \( r_2 \) value of MRI CA, the sensitivity and accuracy of MRI contrast imaging are also determined by the contrast efficiency of CA in vivo. Simply, the contrast efficiency of CA is dependent on the signal difference between normal tissue and lesion, which is most straightforward determined by the amount of CA in lesion. The surface ligand of IONP could affect its circulation behavior, accumulation amount in lesion, and uptake of tumor cells [96], thus IONP with optimized surface structure show perfect in vivo behavior and achieve high efficient contrast imaging.
6.1. Polymers

Polymers, including natural and synthetic polymer, functionalized IONP have drawn much attention due to the improvement on the structural stability, pharmacokinetics, and biodistribution of IONP.

6.1.1. Natural polymer

Natural polymeric ligands, such as polysaccharides and protein, commonly containing multiple active groups strongly binding with IONP for stability improvement in harsh biological environments. Dextran, a typical polysaccharide, has been extensively used for coating IONP with enhanced stability and functionality. The Dextran cross-linked IONP show negligible changes in size and morphology in the blood circulation [280]. Notably, the saccharides are natural signal molecular on the cell surface, modification IONP with polysaccharides exhibits targeting ability to some specific issue. Kamruzaman et al. observed that lactobionic acid (LA) modified IONP showed the capacity to target hepatocytes [281]. The uptake amount of LA modified IONP is significantly higher than unmodified and maltotrionic acid modified IONP. Further in vivo targeting capacity analyses indicate LA modified IONP only result in the signal changes in liver cells, revealing a obvious decrease of unspecific uptake into cells of RES and endowing IONP with long circulation time. In another study, PEG coated 3 nm IONP displayed lower toxicity but longer circulation time than clinically used GBCAs [46]. Additionally, these unique features endow PEGylated IONP with the ability to achieve dynamic time resolved MR angiography in rats.

6.1.2. Synthetic polymer

To improve the stability of IONP, coating IONP with synthetic polymer, such as polyethylene glycol (PEG), poly(lactic-co-glycolic acid) (PLGA) and Polyvinylpyrrolidone (PVP) have got more and more attention. Among all synthetic polymers, PEG as a US FDA approved ligand is extensively used to modify IONP due to its advantage on decrease the adsorption of protein in serum and prolonging its circulation time in the body [99, 284]. Weller and co-author investigated phagocytosis of PEG modified (PEGylated) IONP by J774 macrophages (Fig. 22a–d) [199]. They found that the uptake amount of PEGylated IONP was significantly lower than that of clinically used Resovist (commercial IONP based MRI CAs), revealing a obvious decrease of unspecific uptake into cells of RES and endowing IONP with long circulation time. In another study, PEG coated 3 nm IONP displayed lower toxicity but longer circulation time than clinically used GBCAs [46]. Additionally, these unique features endow PEGylated IONP with the ability to achieve dynamic time resolved MR angiography in rats. Because of its high stability, biodegradability, and biocompatibility, another synthetic polymer, PLGA, have been widely applied in construction IONP based MRI CA. Wang et al., developed IO loaded PLGA-mPEG nanoparticles as MRI CAs [285]. Due to their optimized
surface structure, this IO loaded PLGA-mPEG nanoparticle show higher contrast effect and longer half-life circulation time in comparison with Resovist. Modifying PLGA coated IONP with target motif can further improve contrast efficiency of IONP. Wang and co-workers functionalized PLGA-coated IONP by Arg-Gly-Asp (RGD) peptide [286]. Since RGD peptide has a tendency to bind activated platelets at the thrombus site, this MRI CA shows high affinity with thrombi and serves as a sensitive CA for early thrombi detection. Additionally, PVP attracted much interest in construction IONP based MRI CA because of non-toxicity, low cost, and antiviral properties. Chen and co-workers synthesized PVP coated IONP by a thermal decomposition method [287]. The PVP coated IONP exhibits high solubility and stability in various buffer and serum. They discover that macrophages take up greater amounts of large core PVP coated IONP than Feridex (clinical used MRI CAs), which results in the slightly higher contrast signal caused by PVP coated IONP than Feridex.

6.2. Target ligand

IONP modified with normal small molecular is easily uptake by macrophage and clear by RES, which decreases its contrast efficiency in vivo. To increase the accumulation of IONP in lesion, especially tumor, much attempt have been performed by coating IONP with targeted motif. Targeting ligands, including antibodies [109,288,289], peptides [290,291], aptamers [292,293], folic acids [294,295], and hyaluronic acid [296,297] have been used to modify IONP to achieve targeted MR contrast imaging. Jia et al. modified ultra-small IONP by c(RGDyK) molecular (Fig. 22e–j) [298]. Owing to the RGD modified surface could specifically recognize tumor angiogenesis, the RGD modified IONP shows high accumulation in tumor site, which results in remarkable MR signal enhancement at hepatic tumor and low detection limit with the size of 2.2 mm. Despite each targeting ligand enables IONP with targeting ability, the type of ligand can significantly affect its targeting capacities. For example, due to the lack of consistent covalent bonding site antibodies, which is difficult to controllable attach on the surface of IONP [299]. This defect limits the presentation of antibody binding sites and lower, even hinder, its binding activity, resulting the partial loss of target capacity of IONP.

6.3. Zwitterionic molecular

When IONP enters the physiological environment, serum proteins rapidly adsorb to its surface and form protein corona. The protein corona usually consists various proteins, including adhesion mediators, signaling and transport proteins, and apolipoproteins, which could improve the uptake efficiency of IONP by macrophages in MPS [95,97]. Additionally, the protein corona alters the surface interface between each particle and result in aggregation. These defects dramatically decrease the circulation time of IONP and its accumulation in lesion, especially tumor. Previous research indicated that zwitterionic molecular, that contain either zwitterionic group or a mixture of anionic and cationic groups, could effectively reduce the non-specific protein adsorption [105,301–303]. Bawendi and Co-author found that the size of zwitterionic dopamine sulfonate coated IONP (IO@ZDS) are similar when incubated with 1 PBS, 10% FBS, and 20% FBS. These results indicate the low nonspecific affinity of IO@ZDS towards to serum proteins due to its nearly neutral charge (Fig. 22k-m) [300]. Another study investigated the circulation fate of zwitterionic dopamine sulfonate
coated gadolinium doped IONP (GdIO@ZDS) in vivo [220]. The $T_1$-weighted signal change in heart is relative tiny after injection of GdIO@ZDS at 10, 30, and 60 min, indicating the slow elimination of GdIO@ZDS and long circulation time.

7. Improve biocompatibility

Although it is generally considered IONP are biocompatible in comparison to other metal oxide nanoparticles, there still remains concern on this aspect. Bare IONP have been found to generate reactive oxygen species and resulted in the in vitro cytotoxicity. Therefore, coating IONP with a more biocompatibility shell can effectively improve its biocompatibility. Since different surface coating layers show different effects on cell and different behavior in the body, various attempts have been performed to construct IONP with high biocompatibility through adjusting coating layer. Normally, the coating layer of IONP could be divided into two types, those are inorganic shell and organic shell.

7.1. Inorganic shell

Coating IONP with a biocompatible inorganic shell provides protection against release of iron ions and reactivity of IONP. After coating with the inorganic shell, the interaction activity to surrounding biological entities change from IONP to the inorganic shell. Consequently, the chemically inert shell prefer to develop IONP with high biocompatibility. Recently, gold and silica coated IONP have been discovered to be nontoxic due to their bio-inert property. Gold nanoparticle with excellent biocompatibility have been widely used in biomedical application. The cytotoxicity of gold nanoparticle have been investigated on retinal pigment epithelial (ARPE-19) cell line [304]. It appears that gold nanoparticle with small sizes shows high biocompatibility even the exposure concentration up to 5 mg/mL. Therefore, coating IONP by gold may increase its biocompatibility. Esparza and co-worker assessed the cytotoxic behavior of gold coated IONP [305]. Cytotoxicity tests indicate that the apoptosis rate of MDCK cells treated by gold coated IONP is about 0.71%, which is significantly lower than the control group of MDCK cells treated by IONP (40.33%). Gu and co-workers further assessed the in vivo biosafety of gold coated IONP using spleen-deficient rats. They found that coating IONP with gold can effectively increase the toxicity grade I concentration from 2.5 mg/mL of naked IONP to 5 mg/mL [306]. Further short-term genetic toxicity assessed by micronuclei and comet assay indicate that gold coated IONP show lower mutation and DNA
damage level compare to naked IONP.

Silica is generally recognized as safe by the US FDA. In recent years, various silica coated IONP with high biocompatibility have been used in biomedical application. However, it has been found that silica coated IONP show significant influence on the survival of mNSCs mouse stem cells, which may be ascribed to the release of free iron ions in cell cytoplasm after lysosomal degradation [307]. Further modification have been discovered to be an effective strategy to improve the biocompatibility of silica coated IONP. Injumpa et al. incubated PEG modified silica coated IONP, silica nanoparticles, and IONP with macrophage cells and investigated their cytotoxicity [308]. Compared to the IONP, PEG modified silica coated IONP produces less cytotoxicity. More importantly, PEG modified silica coated IONP show fewer effect on the secretion of pro-inflammatory cytokines (TNF-α and IL-6) than IONP. It should note that the cytotoxicity of silica coated IONP is highly dependent on its size. Lisi and co-workers find that sub 5 nm silica coated IONP do not alter stem cell characteristics and interfere with the commitment potential [309]. In vivo study over 7-week period reveal negligible acute and chronic toxicity after systemically administered this silica coated IONP in mice, showing excellent cytocompatibility.

7.2. Organic shell

Biocompatibility polymer coated IONP have been found to be relatively nontoxic and utilized to improve the biocompatibility of IONP. Natural polysaccharide, especially dextran, with superior biocompatibility has been considered as a promising candidate to improve biocompatibility of IONP. Muller et al. found that dextran coated IONP displayed no effect on cell viability and increasing in cytokines or superoxide production. No toxicity was observed after incubation human monocyte-macrophages with dextran coated IONP at concentration up to 1 mg/mL over 72 h [310]. Recently, another polysaccharide, hyaluronic acid (HA), with CD-44 target activity have been developed to confer biocompatibility. Atrei et al. observe a significant increase in cell viability of NIH3T3 cells exposed to the HA modified IONP respect to bare IONP. Moreover, morphometric analyses indicate that HA modified IONP show little effect on the morphology change than bare IONP, revealing an improved biocompatibility [311]. As another natural production, protein, have been used to stabilize IONP and improved biocompatibility. Roig and co-worker developed IONP outfitted with albumin (BSA) corona and assessed their cytotoxicity in adherent and suspension cells and model organism Caenorhabditis elegans [312]. In comparison to the IONP without coating, BSA coated IONP are efficiently protected in lysosome and lumen of C. elegans. Based on the high stability, BSA coated IONP are more biocompatible than the uncoated ones on cellular level and in C. elegans. Apart from natural polymer, some artificial biocompatible polymers are used to improve the biocompatibility of IONP. PVA coated IONP were found to promote BT-474 cell viability at concentrations up to 100 μg/mL. Besides, no significant ROS generation and morphology change was detected in BT-474 cells incubated with PVP coated IONP at the concentration of 50 μg/mL [313]. These results indicate that the artificial synthesized polymeric shell may also induce certain cytotoxicity in cells, although it shows high biocompatibility. In future, the long-term toxicity of IONP coated by various biocompatible shell should be systemic studied to figure out the relationship between the surface coating shell and biocompatibility and develop IONP with no toxicity in preclinical and clinical study.

8. Conclusion and perspective

IONP with unique magnetic property and high biocompatibility have been widely used as MRI CAs for a long time. To realize early and accurate lesion MRI detection, various attempts have been performed to optimize the structure of IONP. In this review, we summarize the recent progress to construct IONP based high-performance MRI CA through improve their contrast ability and efficiency. This review was started with a comprehensive discussion on the strategies to increase the contrast ability by rising $T_2$ or $r_1$ value of IONP. As a typical $T_2$ CA, we firstly generalized the parameters, which affect $T_2$ relaxivity of IONP. i) $M_s$. On the basis of classical theories, $T_2$ relaxivity of IONP is proportional to its $M_s$, which is highly dependent on its crystal structure. One can increase $M_s$ of IONP by improving the crystallinity, dopant, and forming unique structure to obtain IONP with high $T_2$ relaxivity. ii) Effective radius. The effective radius is responsible for the field perturbation area for the outersphere protons and proportional to the $T_2$ relaxivity in MAR. Therefore, $T_2$ relaxivity of IONP could be promoted by increasing its effective radius through rising crystal size, forming specific morphology, and assembling to nanocluster. It should note that when the particles or cluster size reach to a certain limit, $T_2$ contrast of IONP reaches to its maximum value and fulfills the SDR. iii) Proton diffusion behavior. Proton diffusion behavior in the magnetic field gradients have been discovered to influence the $T_2$ relaxivity of IONP. The anisotropic structure and proper coating layer could generate strong inhomogeneous magnetic field and suitable diffusion environment, which can increase proton diffusion efficiency and $T_2$ relaxivity of IONP. Compared to the systematical study on $T_2$ relaxivity of IONP, the research on its $T_1$ relaxivity is at the initial stage. Reduction the size of IONP, which can increase the $M_s$ value and increase the number of magnetic Fe ions on its surface, have been applied as the few exist methods to fabricate IONP based $T_1$ CAs decades ago. With the development of synthetic method, more and more novel attempts have been performed to optimize the structure of IONP to improve its $T_2$ relaxivity in the past ten years. By surveying the literature, we have covered a number of new structural optimization of IONP to ameliorate its $T_2$ relaxivity. i) Increasing $q$ value of IONP. IONPs with hollow structure and anisotropic morphology obtained by controllable synthesis could provide increased iron ion on the interfere surface between IONP and surrounding aqueous environment, which could rise its $q$ value and result in the $T_2$ relaxivity elevation. ii) Increasing unpaired electrons and improving $r_p$. The existence of ferrous ions with small amount of unpaired electrons and low $r_p$ in IONP limits its $T_1$ relaxivity. The unpaired electrons and $r_p$ of IONP could be increased by introducing other magnetic ions with sufficient unpaired electrons or long $r_p$, such as europium, gadolinium, and copper. iii) Optimizing surface structure. $T_1$ relaxivity of IONP is dependent on the chemical exchange efficient between IONP and water proton at the interface. Molecular weight, hydrophilic, and efficiency on charge-transfer between electronic and magnetic interactions of surface ligand have been considered as key parameters to optimize water diffusion, retention, and interaction with the magnetic centers. This beneficial effect could further improve chemical exchange efficiency between IONP and water proton and contribute to its $r_1$ value.

After detailed discussion on contrast ability improvement, this review then summarized the recent attempt to increase the contrast efficiency of IONP in vivo from three different directions. This part starts with the discussion on recent advances of fabricating IONP with dual-modal contrast. By precise controlling the crystal structure and complementary combination of various materials, IONP with dual-modality imaging capability are emerging as versatile platform to provide comprehensive diagnostic information in disease imaging. Then IONP with the capacity to response to specific changes in the surrounding physiological microenvironment have been discussed on three different manners, those are active, recovery, and switchable. Compared to the traditional MRI CA with fixed contrast capacity in both normal and lesion tissue, this microenvironment responsive MRI CA could switch “on” and “off” $T_1$ or $T_2$ signal change in response to a specific stimulus of lesion. Ultimately, comprehensive understanding of strategies to increase the accumulation of IONP based CA in the lesion site, especially tumor site are present. In our surveyed publications, modified with PEG, target motif, and zwitterionic molecular are most applied method to increase the positive or active target behavior of IONP. This surface functional motif can optimize the circulation behavior and increase
accumulation amount of IONP in lesion, which elevate the imaging efficiency of IONP.

Nevertheless, in spite of the remarkable progresses, there are still some obstacles ahead toward further clinical translation. Despite a number of studies have proved IONP exhibits low toxicity and high biocompatibility, biocompatibility study of IONP engineered by other cations is necessary. Additionally, all strategies are performed in the laboratory, which could not consider some practical consideration in translation to clinical, such as large-scale synthesis, long-term storage, and cost in time and money. However, we still believe that IONP based high-performance MRI CA hold great promise in further clinical early and accurate diagnosis. We hope that this comprehensive review could shed light on the development of next generation IONP based MRI CA.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] C. Tassa, S.Y. Shaw, R. Weisleder, Dextran-coated iron oxide nanoparticle: a versatile platform for targeted molecular imaging, molecular diagnostics, and therapy, Accounts Chem. Res. 44 (2011) 842-852.

[2] M. Janowski, J.W.M. Bulte, P. Walczak, Personalized nanomedicine advancements for stem cell tracking, Adv. Drug Deliv. Rev. 64 (2012) 1488–1507.

[3] L.A. Lane, X. Qian, S. Nie, SERS nanoparticles in medicine: from label-free detection to spectroscopic tagging, Chem. Rev. 115 (2015) 10489–10529.

[4] A. Taylor, K.M. Wilson, P. Murray, D.G. Feringa, H. Levy, Long-term tracking of cells using inorganic nanoparticles as contrast agents: are we there yet? Chem. Soc. Rev. 41 (2012) 2707–2717.

[5] M. Barrow, A. Taylor, P. Murray, M.J. Roseinsky, D.J. Adams, Design considerations for the synthesis of polymer coated iron oxide nanoparticles for stem cell labelling and tracking using MRI, Chem. Soc. Rev. 44 (2015) 6733–6748.

[6] J.E. Smith, C.D. Medley, Z. Tang, D. Shangguan, C. Lofton, W. Tan, Aptamer-conjugated nanoparticles for the collection and detection of multiple cancer cells, Anal. Chem. 79 (2007) 3075–3082.

[7] K.C.-F. Leung, S. Xuan, X. Zhu, D. Wang, C.-P. Chak, S.-F. Lee, et al., Gold and iron oxide hybrid nanocomposite materials, Chem. Soc. Rev. 41 (2012) 1911–1928.

[8] Y. Pan, X. Du, F. Zhao, B. Xu, Magnetic nanoparticles for the manipulation of proteins and cells, Chem. Soc. Rev. 41 (2012) 2912–2942.

[9] L. Yang, X. Zhang, M. Ye, J. Jiang, R. Yang, T. Fu, et al., Aptamer-conjugated nanomaterials and their applications, Adv. Drug Deliv. Rev. 63 (2011) 1361–1370.

[10] J.N. Anker, W.P. Hall, O. Lyandres, N.C. Shah, J. Zhao, R.P. Van Duyne, Biosensing with plasmonic nanosensors, Nat. Mater. 7 (2008) 442–453.

[11] K. Saha, S.S. Agasti, C. Kim, X. Li, V.M. Rotello, Gold nanoparticles in chemical and biological sensing, Chem. Rev. 112 (2012) 2739–2779.

[12] M.-Q. He, Y.-L. Yu, J.-H. Wang, Biomolecule-tailed assembly and morphology of gold nanoparticles for LSPR applications, Nano Today 35 (2020) 101005.

[13] Y. Song, W. Wei, X. Qu, Colorimetric biosensing using smart materials, Adv. Mater. 23 (2011) 4215–4236.

[14] R.E. Armstrong, M. Horakcek, P. Zijlstra, Plasmonic assemblies for real-time single-molecule biosensing, Small 16 (2020) 2003924.

[15] C. Sun, J.S.H. Lee, M. Zhang, Magnetic nanoparticles in MR imaging and Drug delivery, Adv. Drug Deliv. Rev. 60 (2008) 1252–1265.

[16] Z. Zhao, J. Bao, C. Fu, M. Lei, J. Cheng, Controllable synthesis of manganese oxide nanoparticles from 0-D to 3-D and mechanistic investigation of internal relation between structure and T1 relaxivity, Chem. Mater. 29 (2017) 10455–10468.

[17] L. Jia, X. Li, H. Liu, J. Xia, X. Shi, M. Shen, Ultrasound-enhanced precision tumor theranostics using cell membrane-coated and pH-responsive nanospheres assembled from ultrasmall iron oxide nanoparticles, Nano Today 36 (2021) 101022.

[18] D. Li, M. Shen, J. Xia, X. Shi, Recent developments of cancer nanomedicines based on ultrasmall iron oxide nanoparticles and nanoclusters, Nanomedicine 16 (2021) 609–612.
[38] C.-T. Yang, K.K. Ghosh, P. Padmanabhan, O. Langer, J. Liu, D.N.C. Eng, et al., X. Liu, H. Jiang, J. Ye, C. Zhao, S. Gao, C. Wu, et al., Nitrogen-doped carbon oxide nanoprobes as multifunctional bioimaging agent and Drug delivery system, Adv. Funct. Mater. 25 (2015) 6101–6111.

[42] X. Chen, G. Li, Q. Han, X. Li, L. Li, T. Wang, et al., Rational design of branched iron oxide nanoparticles for tumor-targeted magnetic resonance imaging, Adv. Funct. Mater. 27 (2017) 1604258.

[47] X. Chen, G. Li, Q. Han, X. Li, L. Li, T. Wang, et al., Rational design of branched iron oxide nanoparticles for tumor-targeted magnetic resonance imaging, Adv. Funct. Mater. 27 (2017) 1604258.

[52] Z. Zhou, R. Bai, J. Munasinghe, Z. Shen, L. Nie, X. Chen, T. Shin, et al., Engineering of inorganic magnetic hybrid nanoparticles with enhanced relaxivity for tumor imaging, Biomaterials 34 (2013) 225–232.

[54] Z. Zhao, C. Sun, J. Bao, L. Yang, R. Wei, J. Cheng, et al., Surface manganese substitution in magnetite nanocrystals enhances T1 contrast ability by increasing electron spin relaxation, J. Mater. Chem. B 6 (2018) 401–413.

[58] Z. Zhou, R. Bai, J. Munasinghe, Z. Shen, L. Nie, X. Chen, T. Shin, et al., Engineering of inorganic magnetic hybrid nanoparticles with enhanced relaxivity for tumor imaging, Biomaterials 34 (2013) 225–232.

[67] F. Chen, P.A. Ellison, C.M. Lewis, H. Hong, Y. Zhang, S. Shi, et al., Chelator-free iron oxide nanoparticles for reduced non-specific uptake by macrophage cells, Adv. Mater. 26 (2014) 6386–6390.

[72] J. Liu, K. Deng, S.T. Liu, C.J. Zhang, Y.-W. Ao, H. Wang, et al., Reduction-active Fe3O4@SiO2 magnetic nanoparticles as contrast agents for multimodal imaging, Wiley Interdiscip. Rev.-Nanomed. Nanobiotechnol. 8 (2016) 619–630.

[77] B.-Q. Lu, Y.-J. Zhu, H.-Y. Ao, C. Qi, F. Chen, Synthesis and characterization of magnetic iron oxide/calcium carbonate mesoporous nanocomposites as a promising vehicle for Drug delivery, ACS Appl. Mater. Interfaces 4 (2012) 6969–6974.

[89] L. Wang, J. Huang, H. Chen, H. Chen, H. Wu, Y. Xu, Y. Li, et al., Exerting enhanced permeability and retention effect driven delivery of ultrafine iron oxide nanoparticles with T1-T2 switchable magnetic resonance imaging contrast agent, ACS Appl. Mater. Interfaces 11 (2019) 4582–4592.

[91] L. Wang, J. Huang, H. Chen, H. Chen, H. Wu, Y. Xu, Y. Li, et al., Exerting enhanced permeability and retention effect driven delivery of ultrafine iron oxide nanoparticles with T1-T2 switchable magnetic resonance imaging contrast agent, ACS Appl. Mater. Interfaces 11 (2019) 4582–4592.

[93] C. Bai, Z. Liu, L. Song, W. Zhang, Y. Chen, F. Yang, et al., Time-dependent T1–T2 switchable magnetic resonance imaging realized by c(RGDyK) modified ultrasmall Fe3O4 switchable magnetic resonance imaging agent, Adv. Funct. Mater. 28 (2018) 1802821.

[94] C. Bai, Z. Liu, L. Song, W. Zhang, Y. Chen, F. Yang, et al., Time-dependent T1–T2 switchable magnetic resonance imaging realized by c(RGDyK) modified ultrasmall Fe3O4 nanoparticles, Adv. Funct. Mater. 28 (2018) 1802821.

[96] C. Bai, Z. Liu, L. Song, W. Zhang, Y. Chen, F. Yang, et al., Time-dependent T1–T2 switchable magnetic resonance imaging realized by c(RGDyK) modified ultrasmall Fe3O4 nanoparticles, Adv. Funct. Mater. 28 (2018) 1802821.

[97] C. Bai, Z. Liu, L. Song, W. Zhang, Y. Chen, F. Yang, et al., Time-dependent T1–T2 switchable magnetic resonance imaging realized by c(RGDyK) modified ultrasmall Fe3O4 nanoparticles, Adv. Funct. Mater. 28 (2018) 1802821.
E. P. T.-J. Yoon, H. Lee, H. Shao, S.A. Hilderbrand, R. Weissleder, Multicore assemblies for liver tumor imaging, Adv. Mater. 23 (2011) 4753–4759.

Z. Zhou, R. Tian, Z. Wang, Z. Yang, Y. Liu, G. Liu, et al., Artificial local magnetic field inhomogeneity enhances T2 relaxivity, Nano. Commun. 8 (2017) 15468.

J. Liu, C. Lin, J.-S. Chen, S.-J. Huang, J.-H. Ko, Y.-M. Wang, T.-L. Chen, et al., Folic acid–pluronic F127 magnetic nanoparticle clusters for combined targeting, diagnosis, and therapy applications, Biomaterials 30 (2009) 5114–5124.

K.C. Barick, M. Aslam, Y.-P. Lin, D. Bahadur, P.V. Prasad, V.P. Dravid, Novel and efficient MR active aqueous colloidal Fe3O4 nanoparticles, J. Mater. Chem. 19 (2009) 7029–7032.

Z. Zhao, X. Chi, L. Yang, R. Wang, B.W. Ren, X. Zhu, et al., Cation exchange of anisotropic-shaped magnetite nanoparticles generates high relaxivity contrast agents for live tumor imaging, Chem. Med. Chem. 6 (2011) 1391–1396.

P.C. Liang Zpl, Principles of Magnetic Resonance Imaging: a Signal Processing Perspective, Wiley-IEEE Press, 1999.

M.R.J. Carroll, R.C. Woodward, M.J. House, W.Y. Teoh, R. Amal, T.L. Hanley, et al., Experimental validation of proton transverse relaxation rate mapping for superparamagnetic nanoparticle MRI contrast agents, Nanootechnology 21 (2009), 035103.

Z. Zhou, R. Tian, Z. Wang, Z. Yang, Y. Liu, G. Liu, et al., Artificial local magnetic field inhomogeneity enhances T2 relaxivity, Nano. Commun. 8 (2017) 15468.

J. Zeng, J. Ling, J. You, H. Miao, J. Rao, Q. Jia, et al., Anchoring group effects of surface ligands on magnetic properties of Fe3O4 nanoparticles: towards high performance MRI contrast agents, Adv. Mater. 26 (2014) 264–2698.

H.W. de Haan, C. Pugnet, Enhancement and optimization of the relaxation rate resulting from the encapsulation of magnetic particles with hydrophilic coatings, Magn. Reson. Med. 66 (2011) 1759–1766.

J. Liu, Z. Sun, Y. Deng, Y. Zou, C. Li, X. Guo, et al., Highly water-dispersible biocompatible magnetic nanoparticles with low cytotoxicity stabilized by citrate groups, Angew. Chem. Int. Ed. (2009) 5875–5879.

K.B. Vargo, A.A. Zaki, R. Warden-Rothman, A. Tsourkas, D.A. Hammer, Superparamagnetic iron oxide nanoparticle micelles stabilized by recombinant oléosin for targeted magnetic resonance imaging, Small 11 (2015) 1409–1413.

E. Schellenberger, J. Schorr, C. Ruestlingsperger, L. Ungethüm, W. Meyer, M. Taupitz, et al., Linking proteins with anionic nanoparticles via protamine: ultrasmall protein-coupled probes for magnetic resonance imaging of apoptosis, Small 4 (2008) 225–230.

A.G. Roca, J.F. Marco, M.D. Morales, C.J. Serna, Effect of nature and particle size on properties of uniform magnetite and maghemite nanoparticles, J. Phys. Chem. C 111 (2007) 18599–18604.

H.M. Joshi, M. De, F. Richter, J. He, P.V. Prasad, V.P. Dravid, Effect of silica shell thickness of Fe3O4–SiO2 core–shell nanostructures on MRI contrast, J. Nanoparticle Res. 15 (2013) 1448.

S. Tong, S. Hsu, Z. Zheng, J. Zhou, G. Bao, Coating optimization of superparamagnetic iron oxide nanoparticles for high T2 relaxivity, Nano Lett. 10 (2010) 4607–4613.

J. Huang, L. Wang, R. Lin, A.Y. Wang, L. Yang, et al., Canein-coated iron oxide nanoparticles for high MRI contrast enhancement and efficient cell targeting, ACS Appl. Mater. Interfaces 5 (2013) 4632–4639.

F. Reynolds, T. O’Loughlin, R. Weisleder, L. Josephson, Method of determining nanoparticle core width, Anal. Chem. 77 (2005) 814–817.

I. Solomon, Relaxation processes in a system of two spins, Phys. Rev. (1995) 559–565.

N. Bлюберген, L.O. Morgan, Proton relaxation times in paramagnetic systems. Effects of electron spin relaxation, J. Phys. Chem. 34 (1961) 842–850.

A. Bleszyn, F. Foglia, E. Furet, L. Hahn, A.E. Merbach, J. Weber, Second coordination shell water exchange rate and mechanism:experiments and modeling on hexaquachromium(III), J. Am. Chem. Soc. 118 (1996) 1219–1225.

C.S. Bonnet, P.H. Fries, S. Crouxey, P. Delarge, Outer-sphere investigation of MRI relaxation contrast agents. Example of a cyclododecapodine gadolinium complex with second-sphere water, J. Phys. Chem. B 114 (2010) 8770–8781.

E.J. Werner, A. Dabrowski, C.J. Coker, K.N. Raymond, High-field relaxivity MRI contrast agents: when chemistry meets imaging, Angew. Chem. Int. Ed. (2007) 8568–8580.

Chapter 3 Relaxation, Coord. Chem. Rev. 150 (1996) 77–110.

A.-J. Yoon, A. Urrutia, M. Silva, F. Palacio, V.S. Amaral, E. Snoek, et al., Surface effect on magnetic nanoparticle eels, J. Magn. Magn. Mater. 312 (2007) L59–L63.

Z. Zhao et al.
[219] N.D. Thornton, R.A. Bohara, S.A.M. Tofail, Z.A. Shiddiky, M.S. Zhou, et al., Superparamagnetic gadolinium ferrite nanoparticles with controllable core–shell structure: thermoresponsive and drug delivery systems for MR-imaging guided chemotherapy, Eur J Inorg Chem (2016) 2016:4586–4597.

[220] Z. Zhou, L. Wang, X. Chi, J. Bao, L. Yang, W. Zhao, et al., Engineered iron-oxide-based nanoparticles as enhanced T1 contrast agents for efficient tumor imaging, Biomater Sci 7 (2019) 1236–1256.

[221] Z. Zhou, C. Hu, H. Xiu, Z. Zhu, L. Wang, et al., Surface and interfacial engineering of iron oxide nanoparticles for highly efficient magnetic resonance angiography, ACS Nano (2013) 7 (2013) 4973–4982.

[222] I. Fernández-Barahona, L. Gutiérrez, S. Veintemillas-Verdaguer, J. Pellico, MDP, Morales, M. Catala, et al., Cu-decorated extremely small iron oxide nanoparticles with large longitudinal relaxation: one-pot synthesis and in vivo targeted molecular imaging, ACS Omega 3 (2018) 2719–2727.

[223] O. Perlmutter, I.S. Weitz, H. Azhari, Copper oxide nanoparticles as contrast agents for MRI and ultrasound dual-modality imaging, Phys. Med. Biol. 60 (2015) 5767–5783.

[224] M. Zhou, M. Tian, C. Li, Copper-based nanomaterials for cancer imaging and therapy, Bioconjugate Chem. 27 (2016) 1188–1199.

[225] J. Mou, C. Liu, P. Li, Y. Chen, H. Xu, et al., A facile synthesis of versatile controllable curie temperature T1 contrast agents for MRI and CT, Biomed Mater. 8 (2013) 115601.

[226] Y.-K. Peng, C.N.P. Lui, Y.-W. Chen, S.-W. Chou, E. Raine, P.-T. Chou, et al., Facile synthesis of ultrasmall PEGylated iron oxide nanoparticles: a chitosan-based nanotheranostic system, Chem. Commun. 52 (2016) 378–381.

[227] D. Hu, C. Liu, L. Song, H. Cui, G. Gao, P. Liu, et al., Indocyanine green-loaded polyolpaper-iron oxide coordination nanoparticles for photodynamic/photomechanical therapy and imaging-guided cancer thermal therapy, Nanoscale 8 (2016) 17150–17158.

[228] R. Madru, P. Kjellman, F. Olsson, K. Wingårdh, C. Ingvar, P. Stähleberg, et al., Te-labeled superparamagnetic iron oxide nanoparticles for multimodal SPECT/MRI of sentinel lymph nodes, J. Nucl. Med. 53 (2012) 1456–1463.

[229] J.T.W. Wang, L. Cabana, M. Bourgogne, H. Kafa, A. Protti, K. Venner, et al., Magnetically decorated twovaken carbon nanotubes as dual MRI and SPECT contrast agents, Adv. Funct. Mater. 24 (2014) 1880–1894.

[230] P. Wang, Y. Shi, Z. Zhang, Y. Zhang, et al., Hydrogen peroxide responsive iron-based nanoplatform for multimodal imaging-guided cancer therapy, Small 15 (2019) 1803791.

[231] J. Choi, J.C. Park, H. Nam, S. Woo, J. Oh, K.M. Kim, et al., A hybrid nanoparticle probe for dual-modality positron emission tomography and magnetic resonance imaging, Angew. Chem. Int. Ed. 47 (2008) 6259–6262.

[232] Y. Peng, X. Wang, Y. Wang, G. Guo, X. Shi, et al., Macrophage-laden gold nanoflowers embedded with ultrasmall iron oxide nanoparticles for enhanced dual-mode CT/MRI imaging of tumors, Pharmaceuticals 13 (2021) 995.

[233] H. Cai, K. Li, J. Li, S. Wen, Q. Chen, M. Shen, et al., Dendrimer-assisted formation of Fe3O4/Au nanoparticle composite for targeted dual mode CT/MRI imaging of breast cancer, Small 11 (2015) 2278–2286.

[234] A. Sood, V. Arora, J. Shah, R.K. Kottala, T.K. Jain, Multifunctional gold coated iron oxide core-shell nanoparticles stabilized using thiolated sodium alginate for biomedical applications, Mater. Sci. Eng. C 80 (2018) 274–281.

[235] S. Veintemillas-Verdaguer, C.J. Serna, J. Oku, C. Vergez, M. Varela, M. Calero, et al., Bismuth labeling for the CT assessment of local administration of nanocarriers, Biomaterials 26 (2005) 10312–10320.

[236] H.Y. Zhao, S. Liu, J. He, C.C. Pan, H. Li, Z.Y. Zhou, et al., Synthesis and application of strawberry-like Fe3O4@Au nanoparticles as CT-MR dual-modality contrast agents in accurate detection of the progressive liver disease, Biomaterials 51 (2015) 194–207.

[237] N. Lee, H.R. Cho, M.H. Oh, S.H. Lee, K.M. Kim, B.H. Kim, et al., Multifunctional Fe3O4/Ta2O5 core/shell nanoparticles for simultaneous in vitro magnetic resonance and X-ray computed tomography, J. Am. Chem. Soc. 134 (2012) 10309–10312.

[238] M. Ma, H. Zhu, J. Liang, S. Gong, Y. Zhang, X. Yia, et al., Quasiamorphous and Hierarchical Fe2O3 nanocrystals: active T1-weighted magnetic resonance imaging in vivo and renal clearance, ACS Nano 14 (2020) 4036–4044.

[239] X. Xu, X. Zhou, B. Xiao, H. Xu, D. Yu, Y. Qian, et al., Glutathione-responsive magnetic nanoparticles for highly sensitive diagnosis of liver metastases, Nano Lett. 21 (2021) 2199–2206.

[240] Y. Liu, Z. Yang, X. Huang, G. Yu, S. Wang, Z. Zhou, et al., Glutathione-responsive self-assembled magnetic gold nanorow for enhanced tumor imaging and imaging-guided photothermal therapy, ACS Nano 12 (2018) 8129–8137.

[241] S. Fu, Z. Cui, H. Ai, Stimulus-responsive magnetic resonance imaging contrast agents: design considerations and applications, Adv. Healthc Mater 10 (2021) 2001091.

[242] Z. Xiao, P. Park, S-J Park, Y. Lu, K.S. Kim, M.J. Hackett, et al., Multifunctional tumor pHi-sensitive self-assembled nanoparticles for bimodal imaging and treatment of resistant heterogeneous tumors, J. Am. Chem. Soc. 136 (2014) 5647–5655.

[243] H. Zhou, M. Guo, J. Li, L. Qin, Y. Wang, T. Liu, et al., Hypoxia triggered self-assembly of ultrasmall iron oxide nanoparticles to amplify the imaging signal of a tumor, J. Am. Chem. Soc. 143 (2021) 1846–1853.
