Emerging strategies of activatable MR imaging probes and their advantages for biomedical applications

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Abstract
Magnetic resonance imaging (MRI) is a highly valuable diagnostic tool as it is a noninvasive technique that offers high spatial resolution. The use of contrast agents (CAs) can enhance the precision and specificity of MRI for disease diagnosis, but their imaging signals are “always on” regardless of whether they interact with target tissues or cells. Hence, a poor target-to-background signal ratio (TBR) is inevitably produced. In contrast, activatable CAs with high performance have been used to significantly improve the TBR, thus these CAs have also received extensive attention and undergone in-depth research. In this review, we summarized the recent advances in design strategies and principles of activatable MR CAs, including ion conversion, self-assembly, and disassembly. Additionally, we analyzed the advantages of these strategies in biomedical applications from in vitro biodetection to in vivo disease diagnosis compared to the outcomes of conventional MR CAs. Finally, we discussed the potential limitations, proposed solutions, and future perspectives of these activatable CAs.

KEYWORDS
activatable MR imaging, design strategies, target-to-background signal ratio, relaxivity, biomedical applications

1 | INTRODUCTION

Magnetic resonance imaging (MRI), as an excellent technique for noninvasive disease diagnosis and therapeutic monitoring, has received extensive attention and has undergone in-depth research due to the key advantages it offers, such as a lack of radiation, the absence of a tissue penetration depth limit, and high resolution in soft tissues.1–6 MRI can provide invaluable details of anatomical structures, but it is difficult to distinguish anatomical lesions and healthy tissues in the images.7,8 Thus, MR contrast agents (CAs) have been developed to enhance...
contrast and improve sensitivity. Currently, MRI CAs are clinically used in almost half of all MRI examinations.\(^9\)\(^{10}\)

Currently, there are two categories of MR CAs that have been used in clinics—Gd-based\(^{11,12}\) or Mn-based\(^{13,14}\) compounds (positive CAs) and iron-based nanostructures (negative CAs).\(^{15,16}\) However, these CAs are still insufficient, and there is huge room for improvement.\(^{17}\) Therefore, in the past few decades, researchers have focused on developing MR CAs with high relaxation properties, good stability, and excellent biocompatibility.\(^{18}\) Consequently, a series of MR CAs, such as paramagnetic metal (Gd\(^{3+}\), Mn\(^{2+}\), and Fe\(^{3+}\)) chelates/nanostructures and magnetic nanoparticles (NPs), were synthesized and used for in vivo T\(_1\)- or T\(_2\)-weighted MRI to evaluate diseases.\(^{19–28}\) Nevertheless, the MR signal of H\(^+\) induced by these CAs is “always on” regardless of whether they interact with target tissues or cells.\(^{29}\) Hence, a low target-to-background signal ratio (TBR) inevitably develops, which makes the tissue or anatomical features that we interested in more difficult to distinguish.\(^{30}\) Generally, these CAs need targeted modification and high-dose injections to improve the concentration and imaging contrast of the lesion, but this approach often induces toxic side effects in the body.

In recent years, activatable MR CAs with signals that can be switched from off to on have received widespread attention due to the reduction of background signals and the resulting increase in TBR, which can significantly improve diagnostic efficiency.\(^{31–40}\) For instance, by leveraging the significantly different microenvironments between lesions and healthy tissues, such as the presence of acid,\(^{41–44}\) overexpressed reactive oxygen species (ROS)\(^{45}\) and glutathione (GSH),\(^{46–48}\) the relaxivity of probes can be significantly enhanced in response to these specific biological stimulations, but is the signal would still be low in normal tissues. Thus, background signals can be minimized, which, in turn, improves the specificity and sensitivity of MR. In recent years, a series of new strategies for designing and synthesizing activatable probes based on the principles of MRI have been reported successively, and their advantages are reflected in biomedical applications that cover in vitro biodetection and in vivo diagnosis. Thus, a systematic summary and review is necessary to guide us in the creation of new activatable MRI probes and in the exploration of their new biomedical applications.

In this review, we presented the latest progress on the design strategies and advantages of activatable MRI probes in biomedical applications. First, three design strategies, including ion extraction (activatable T\(_1\)-weighted MRI), self-assembly (activatable T\(_1\)- or T\(_2\)-weighted MRI), and disassembly (activatable T\(_1\)-weighted MRI), were summarized systematically and compared for the first time. Meanwhile, some key factors involved in the designing activatable MR probes were also summarized to inspire researchers to learn more about the creation of activatable probes (Figure 1). Second, the advantages of activatable MR probes over common MR CAs in biomedical applications, including more accurate biodetection in vitro, imaging with higher TBR in vivo and monitoring drug release, were introduced. Finally, the challenges and prospects of activatable MRI probes were deeply discussed.

## 2 | STRATEGIES TO DESIGN ACTIVATABLE PROBES FOR MRI

The TBR is a key factor in determining image quality, which in turn affects the diagnosis of diseases. In most previous studies, researchers tried to improve relaxation performance or magnetic properties and altered the biodistribution and pharmacokinetics of MR CAs, but they often ignored the interference of the background signal, resulting in a low TBR.\(^{53}\) In contrast, the emerging activatable MR probes respond specifically to the microenvironment of the lesion but exhibit no signal or a slight signal in normal tissues, thus this difference in signal can significantly decrease the background noise and increase the TBR.\(^{50}\) In recent years, there have been many reports on improving the TBR for MRI of diseases, whereas a systematic summary of these strategies is lacking. In this section, we summarized and named three strategies to design activatable MR probes, including ion conversion, self-assembly, and disassembly strategies; these strategies will be discussed in detail below. Moreover, the related principles behind the three design strategies will also be explained. Finally, the biodistribution and clearance way of these activatable probes before and after activation will be introduced if related work was mentioned in their reports, which could be helpful for us to choose a better type of design for different biomedical applications.

### 2.1 | Ion conversion strategy

#### 2.1.1 | Ion extraction

The proton relaxation rate of water protons is much slower than the associated electron orbital motion; however, the relatively slower electron optional motion can interact more closely with water protons.\(^{54}\) Therefore, only paramagnetic metal ions with high electron spin angular momentum(S),\(^{55}\) such as gadolinium ions (Gd\(^{3+}\)), manganese ions (Mn\(^{2+}\)), and iron ions (Fe\(^{3+}\)) can effectively decrease the longitudinal relaxation time (T\(_1\)) of water protons.\(^{56}\) In contrast, when these elements exist in non-ionic forms, such as oxides or sulfides, relaxation ability becomes relatively weak. According to this principle, the
strategy of releasing paramagnetic ions from NPs to activate $T_1$-weighted MR signals in the special microenvironment of the lesions was developed.

**1) Mn$^{2+}$ ion extraction.** Mn$^{2+}$ is a typical paramagnetic ion with five unpaired electrons and high longitudinal relaxivity ($r_1$); thus, it has been widely used for MRI. In contrast, Mn-based NPs, such as MnO$_2$ and Mn-doped NPs, usually exhibit a weak MR contrast effect due to Mn shielding from aqueous environments; thus, they do not contribute to proton longitudinal or transverse relaxation. Nonetheless, Mn-based NPs have still attracted research interest in recent decades due to their response to various conditions, such as acidic, GSH, and H$_2$O$_2$ conditions, which can significantly activate the MR signal by Mn$^{2+}$ release. Furthermore, released Mn$^{2+}$ ions can interact with surrounding proteins, forming a Mn$^{2+}$-protein complex and then amplifying the $T_1$-weighted MR signal.

For instance, Mi et al. synthesized PEGMnCaP by confining Mn$^{2+}$ ions to pH-responsive calcium phosphate (CaP) NPs followed by capping them with a poly(ethylene glycol) shell (Figure 2A). In the acidic environment of solid tumors, PEGMnCaP NPs were degraded, resulting in Mn$^{2+}$ release, which could be detected by the result of inductively coupled plasma mass spectrometry (ICP-MS) (Figure 2B). Simultaneously, the $r_1$ was significantly enhanced after Mn$^{2+}$ release, especially binding with proteins like human serum albumin (HSA) (Figure 2C). In their experiment of MRI of liver metastasis, they found the contrast of liver decreased and the contrast of the gallbladder increased, which indicated the PEGMnCaP was cleared from liver and this phenomenon also could be deduced by the biodistribution result of PEGMnCaP.

In addition to an acidic environment, H$_2$O$_2$ is considered to be overproduced in tumors. In another work, Li and his coworkers conjugated ultrasmall manganese...
ferrite NPs (UMFNPs) with a class of pentapeptide Cys-Arg-Glu-Lys-Ala (CREKA) with tumor targeting capacity to synthesize an ultrasensitive T₁-weighted MRI probe (UMFNP-CREKA) (Figure 2D). In the presence of mild acidity conditions (pH = 6.5) and H₂O₂, Mn²⁺ ions were extracted successfully from UMFNPs, and the T₁-weighted MR signal was significantly activated (Figure 2E). Moreover, the addition of fetal bovine serum (FBS) further enhanced r₁, which suggests that the surrounding proteins of a tumor can further amplify the MR signal after binding with Mn²⁺ (Figure 2F). In their pharmacokinetic study of these probes, they found probes mainly accumulated in the tumor, liver, and kidney after injection. Finally, they also found continuous enhanced contrast in the MR images of kidney, which revealed an effective renal clearance of UMFNP-CREKA due to their small size.

(2) Fe³⁺ ion extraction. Fe³⁺ is another typical paramagnetic ion that can significantly reduce the relaxation time of H₂O, thus exhibiting a good T₁-weighted MR contrast effect due to 5 unpaired electrons. As it is one of the important trace elements needed in the human body, Fe³⁺ displayed better biocompatibility than Mn²⁺. Similar to the design discussed above, activating the MR signal by extracting Fe³⁺ from Fe-based NPs is also a reasonable approach.

For instance, Qiu et al. synthesized novel FeP NPs with a pH-activatable T₁-weighted MR signal. In the presence of an acidic microenvironment (pH = 5.5), nearly 60% of Fe³⁺ ions were extracted, which is almost ten-fold that under neutral conditions (pH = 7.4). As expected, the release of Fe³⁺ ions led to higher r₁ and a brighter T₁-weighted MR signal. After incubation with MCF-7 cells, the T₁-weighted MR images of FeP NPs became brighter over time, which further confirmed the pH-activatable MR signal. Their biodistribution analysis of FeP NPs confirmed that over half of probes accumulated in the liver and spleen and almost ten percent of FeP NPs accumulated in the tumor, which could produce an amplified MR signal after being activated.

Additionally, Yu et al. reported pH-responsive core-shell iron carbide NPs (Fe₅C₂@Fe₃O₄). Due to their high magnetization and relatively large size (20 nm), the
Fe₅C₂@Fe₃O₄ NPs mainly exhibited T₂-weighted MRI capability (Figure 3A). In the presence of acid in the tumor microenvironment, Fe₅C₂@Fe₃O₄ NPs were degraded, and the size of Fe₅C₂@Fe₃O₄ NPs decreased gradually. As a result, the magnetization of Fe₅C₂@Fe₃O₄ NPs decreased, which resulted in a relatively lower transverse relaxivity (r₂) and weaker T₂-weighted MR signal. In contrast, as iron ions (Fe²⁺ and Fe³⁺) were released from Fe₅C₂@Fe₃O₄ NPs and diamagnetic Fe²⁺ ions could be oxidized into Fe³⁺ by H₂O₂, the r₁ was significantly enhanced, and the T₁-weighted MR signal was activated (Figure 3B–C). In order to further investigate the biodistribution of probes, IR-783 labeled Fe₅C₂@Fe₃O₄ NPs were intravenously injected into mice, which could be helpful to study the accumulation of probes in various tissues through NIR imaging. These results showed that probes could effectively accumulate in the tumor, and a much stronger fluorescence signal was observed with the assistance of magnet.

### 2.1.2 Valance conversion

In addition to the strategy of ion extraction from NPs, valance conversion is an emerging strategy to activate MR signals. According to previous reports, the same element has different magnetic properties at different valences. For example, Fe³⁺, Mn²⁺, and Cu²⁺ ions are usually paramagnetic and can affect the r₁ of protons, whereas Cu⁺, Mn³⁺, Mn⁴⁺, and Fe²⁺ ions display diamagnetic properties; therefore, they are difficult to use in MRI. Hence, some designs based on valance changes of these diamagnetic ions have emerged. For instance, Liu et al. synthesized copper (I) phosphide nanocrystals (CP NCs) to generate T₁-weighted MR signals in situ (Figure 4A). Initially, the Cu⁺ ions on the surface of CP NCs exhibited a weak MR signal due to their completely filled 3d orbital-induced diamagnetic property. After reacting with H₂O₂, the diamagnetic Cu⁺ ions were oxidized into paramagnetic Cu²⁺ ions.
FIGURE 4  (A) Schematic illustration of CP NC in situ self-generation MRI. (B) Magnetic hysteresis loop of CP NCs treated with/without \( \text{H}_2\text{O}_2 \). (C) \( T_1 \)-weighted MR images of CP NCs in different Cu concentrations with and without \( \text{H}_2\text{O}_2 \). Reproduced with permission.\(^68\) Copyright 2019, Wiley-VCH. (D) Schematic illustration of biomarker detection through interconversion between \( \text{Fe}^{2+} \) and \( \text{Fe}^{3+} \). (E) Schematic illustration of \( T_1 \)-based assay for detecting glucose. (F) The change in the \( T_1 \) signal in response to the concentration of \( \text{H}_2\text{O}_2 \). (G) The \( T_1 \)-based assay for the detection of glucose in serum, urine, and saliva with different concentrations of glucose. Reproduced with permission.\(^49\) Copyright 2018, American Chemical Society.

(Figure 4B), and the valance change of Cu significantly activated the \( T_1 \)-weighted MR signal (Figure 4C).

In addition to the valance change between \( \text{Cu}^+ \) and \( \text{Cu}^{2+} \), Chen et al.\(^{49}\) explored \( \text{Fe}^{3+}/\text{Fe}^{2+} \) interconversion as an activatable strategy (Figure 4D). In one work of their report, they took advantage of the reaction between glucose and glucose oxidase to produce \( \text{H}_2\text{O}_2 \), which can oxidize diamagnetic \( \text{Fe}^{2+} \) ions into paramagnetic \( \text{Fe}^{3+} \) ions (Figure 4E); then, the \( T_1 \)-weighted MR signal was activated (Figure 4F). In different samples, this \( T_1 \)-based assay can also detect glucose through the change in \( T_1 \) relaxation time (Figure 4G).

2.2 Self-assembly strategy

2.2.1 Self-assembly of nanoparticles

In addition to activating signals based mainly on ion conversion, there are many other designs that can be used to achieve a higher TBR by increasing the size of small molecules and NPs through self-assembly in the presence of certain pathological and physiological stimuli.

As one of the most widely used MR CAs, \( \text{Fe}_3\text{O}_4 \) NPs have attracted much attention and in-depth research.\(^{69}\) As research progressed, researchers found that as the particle size of a gradually increased or as NPs self-assembled into nanoclusters, the \( r_1 \) of \( \text{Fe}_3\text{O}_4 \) NPs gradually decreased, and the \( r_2 \) increased.\(^{70}\) This phenomenon is induced by dynamic changes between its surface-to-volume ratio (SVR) and magnetization properties. When the size of \( \text{Fe}_3\text{O}_4 \) NPs decreases, the SVR increases, and magnetization is weakened at the same time. Decreased magnetization produces a lower \( r_2 \). In contrast, a higher SVR means that more \( \text{Fe}^{3+} \) ions on the surface of NPs interact with surrounding water protons and produce a higher \( r_1 \). Therefore, the self-assembly of \( \text{Fe}_3\text{O}_4 \) NPs in response to pathological and physiological stimuli can effectively enhance the efficiency of \( T_2 \)-weighted MRI and even provide a good \( T_1/T_2 \) switchable imaging effect in some cases. As a result, many activatable probes have been developed that take advantage of this self-assembly strategy to influence the dynamic changes between its SVR and magnetization properties.\(^{71}\)

Wang et al.\(^{72}\) synthesized the Ac-Arg-Val-Arg-Arg-Cys(StBu)-Lys-CBT molecule compound (compound 1),
followed by coupled with carboxyl-decorated superparamagnetic iron oxide (SPIO) NPs to form SPIO@1NPs. When SPIO@1NPs entered tumor cells overexpressing furin, high concentrations of GSH reacted with the disulfide bond of the Cys motif, and the RVRR substrate was cleaved by furin. Moreover, a condensation reaction occurred between the cyano groups of the CBT motifs and the free 1,2-aminothiol groups. Finally, SPIO NPs aggregated into nanoclusters and resulted in an activatable T2-weighted MR signal. With the aid of furin together with GSH, SPIO@1NPs aggregated, and the size of SPIO@1NPs greatly increased to 139.72 ± 24.69 nm. Along with the change in size, the T2-weighted MR images of SPIO@1NPs became darker, and the r2 also increased. In this study, they also found that the aggregation of SPIO@1NPs could enhance and prolong the accumulation of probes in the tumor.

Additionally, Yuan et al. designed the Ac-Asp-Glu-Val-Asp-Cys(StBu)-Lys-CBT (1) molecule to covalently modify uSPIO NPs to prepare monodisperse Fe3O4@1 NPs. In response to GSH and caspase-3/7, 1 could yield a cyclized dimer, whereas 1-Scr only exhibited a reduction in GSH but was impervious to Casp3. Hence, with the aid of GSH and caspase-3/7, aggregated Fe3O4 nanoclusters formed (Figure 5A–B). As the size of the material increased, T2 decreased and r2 increased (Figure 5C–D). According to the result of inductively coupled plasma atomic emission spectroscopy (ICP-AES), higher concentration of Fe was found in the apoptotic tumor, which indicated that the aggregation is helpful to the accumulation of probes in tumor.

In addition to smart probes activating T2-weighted MR signals as discussed above, a T1/T2 switchable MRI probe was reported by Wang et al. They synthesized ultrafine iron oxide NPs (uIONPs), which could simply extravasate from the tumor vasculature. Moreover, uIONPs could easily diffuse into the tumor tissue and self-assemble into clustered uIONPs in response to a mildly acidic environment.
microenvironment (Figure 5E). The change in size could be shown by the results of dynamic light scattering (DLS) and zeta potential (Figure 5F). With the formation of clustered uIONPs, both $T_1$-weighted and $T_2$-weighted MR images became darker, and this phenomenon represented that the $r_2$ increased and the $r_1$ decreased, respectively (Figure 5G). In their report, they also explored the variation in the enrichment of uIONPs (3.5 nm) and big particles (20 nm) and their result indicated that the extravasation of uIONPs from vessel to the tumor tissue is deeper than larger sized counterparts. In the study of biodistribution and clearance of uIONPs, they showed that the blood half-life time of uIONPs was almost 10 hours and uIONPs was cleared from bodies through kidney due to its small size.

### 2.2.2 Self-assembly of small molecules

Compared to small molecules, NPs have the potential to limit molecular rotation and increase rotational correlation time due to their larger size, which is significant in enhancing $r_1$ relaxivity. Therefore, the design of small molecules self-assembling into NPs to activate $T_1$-weighted MR signals in response to specific biological stimuli has emerged.\(^7^5\)

Liang et al.\(^7^6\) took advantage of the controllable condensation reaction between 1,2-aminothiol and CBT discussed above and designed a new activatable probe 1. In their work, when probes entered into locations rich in GSH, a condensation reaction was initiated, followed by the formation of gadolinium-containing NPs (Gd NPs). The authors found that the relaxivity of these assembled NPs was higher than that of small molecules at the same concentration of Gd.

The self-assembly of small molecules can also be stimulated by some enzymes, such as caspase-3/7, which can be seen as early markers of cell apoptosis. Ye et al.\(^7^7\) reported a caspase-3/7-activatable MRI probe based on Gd (C-SNAM) that included a 2-cyano-6-hydroxyquinoline (CHQ) and a D-cysteine residue for effective biocompatible cyclization, a DEVD peptide that could be recognized by active caspase-3/7, a disulfide bond that could have a reduction reaction within intracellular GSH and a Gd-DOTA chelate with MR signal enhance function (Figure 6A).

Under the combined action of GSH and caspase-3/7, the intramolecular cyclization of C-SNAM was triggered and formed macrocyclic product 2, which is more rigid and hydrophobic, so that C-SNAM could further self-assemble into Gd NPs. As shown in Figure 6B, the Gd NPs were formed successfully, and the $T_1$ relaxation time decreased at the same time (Figure 6C).

Additionally, Li et al.\(^5^0\) reported a caspase-3/7 responsive bimodal fluorescence-MR (FL-MR) caspase probe 1 (CP1) to evaluate the effectiveness of cancer therapy.
FIGURE 7 (A) Schematic illustration of the DEP/GdCuB clusters. (A) Larger clusters could increase the half-lifetime of circulation and tumor accumulation and block the T₁-weighted MRI enhancement contrast effect. (B) In the tumor microenvironment, excessive ATP reverses the charge of DEP and then GdCuB 121 d, which activates the T₁-weighted MRI signal. (B) TEM images, (C) T₁-weighted MR images, and (D) r₁ curve of GdCuB, DEP/GdCuB, and DEP/GdCuB with 1×10⁻⁴ M ATP. Reproduced with permission. Copyright 2019, Wiley-VCH.

(E) Schematic illustration of the bright-to-dark T₂-T₁ MRI switchable contrast effect of iron oxide NP assemblies (IONAs). (F) TEM images of IONAs in water at pH 7.4 and 5.5. Inset: hydrodynamic size distribution of IONAs at pH 7.4 and 5.5. (G) T₁-/T₂-weighted MR images of IONAs in water at pH 7.4 and 5.5. (H) r₁/r₂ curve of IONAs in water at pH 7.4 and 5.5. Reproduced with permission. Copyright 2019, American Chemical Society.

and that could be used to screen preclinical anticancer drugs (Figure 6D). CPI contains the following main components: Gd-DTPA, which provides MR signal enhancement effect; tetrakis(4-methoxyphenyl)porphyrin, as the aggregation-induced emission luminogen (AIEgen); and DEVD peptide, which can react with caspase-3/7. In response to caspase-3/7, the hydrophilic peptide DEVD was cleaved, and Gd-AIEgen gradually aggregated and formed Gd-containing NPs that had a lower and suitable tumbling rate, leading to an activatable T₁-weighted MR signal (Figure 6E–F).

2.3 Disassembly strategy

2.3.1 Disassembly of nanoclusters

As discussed above, increasing NP size or self-assembly could enhance the T₂-weighted MRI ability of CA. In contrast, decreasing NP size or disassembly could activate the T₁-weighted MR signal of CAs. Hence, after nanoclusters disassembled into small NPs in the lesion, r₁ was obviously improved; thus, the T₁-weighted MR signal could also be activated at the same time.

For instance, Zhou et al. prepared Gd³⁺ and CuS-coloaded small bovine serum albumin (BSA) NPs (GdCuB) and then assembled them into large-sized DEP/GdCuB nanoclusters (120 nm) by using polyethylene glycol (PBA) and ethylenediamine (EDA)-decorated dextrin (DEP). Because of the formation of DEP/GdCuB nanoclusters and the consequent reduced interaction between Gd³⁺ and water protons, the T₁-weighted MR signal of GdCuB was largely weakened (Figure 7A). After blood circulation and accumulation in tumors, the DEP/GdCuB nanoclusters disassembled into small-sized GdCuB NPs (<10 nm) in response to excessive adenosine triphosphate (ATP) levels in tumors (Figure 7B). Simultaneously, the tumor permeability of DEP/GdCuB NPs and T₁-weighted MRI performance could be significantly improved (Figure 7C–D).
Figure 8 (A) Schematic illustration of the degradable polymer matrix determining the interaction of water with Gd oxide NPs. (B) The $r_1$ of the smart probe in an environment at different pH values. (C) The $r_1$ value of the smart probe in response to different concentrations of H$_2$O$_2$. Reproduced with permission. Copyright 2013, American Chemical Society. (D) Schematic illustration of mesoporous silica NPs (MSNPs). (E) $r_1$ value of MSNPs versus the Gd concentration. (F) The corresponding in vitro T$_1$-weighted MR images of the solution containing MSNPs in phosphate buffered saline (PBS) buffer at different pH values. Reproduced with permission. Copyright 2019, Wiley-VCH.

In this report, they also showed that the size switching strategy could improve the accumulation of DEP/GdCuB NPs in the tumor. Finally, they also found that DEP/GdCuB (120 nm) had higher half-life time in blood than small-sized GdCuB NPs (<10 nm) through measuring samples.

Additionally, Li et al. synthesized iron oxide NP assemblies (IONAs) that were cross-linked by aldehyde derivative ligands (Figure 7E). IONAs showed a low $r_1$ value of 3.2 mM$^{-1}$s$^{-1}$ and a high $r_2$ value of 108 mM$^{-1}$s$^{-1}$. After the disassembly of IONAs in response to the mildly acidic microenvironment in tumors, extremely small iron oxide NPs (ESIONs) with a strong ability to enhance T$_1$-weighted MRI data appeared (Figure 7F). The enhancement of the T$_1$-weighted MR capacity of ESIONs was evident due to the higher $r_1$ and brighter T$_1$-weighted MR images (Figure 7G–H).

2.3.2 Disassembly of potent interactions with water protons

CAs decrease T$_1$ or T$_2$ relaxation time by interacting with surrounding water protons. Therefore, numerous designs of activatable probes that can first weaken or even block the interaction between CAs and water and then recover in response to specific microenvironments or biomolecules have emerged. The recovery of interactions with water protons could largely activate the MR enhancement effect of probes.

Viger and his coworkers designed an activatable probe by encapsulating ultrasmall gadolinium oxide NPs (Gd oxide NPs) into biodegradable polymer capsules; this activatable probe was responsive to mild acid and H$_2$O$_2$. Due to the hydrophobic polymeric matrices, Gd oxide NPs were protected from water protons to the greatest extent. In response to the mildly acidic microenvironment and excessive H$_2$O$_2$ present in tumor tissue, these polymer capsules degraded, and gadolinium oxide NPs were released, which generated a strong MR enhancement effect leading to higher $r_1$ and brighter T$_1$-weighted MR images (Figure 8A–C).

Additionally, Yi et al. also took advantage of the same principles to construct NaGdF$_4$-CaCO$_3$ nanoconjugates and wrapped them with a cell membrane and other polymers to separate NaGdF$_4$ NPs from water protons so that they could not produce their own T$_1$-weighted MR enhancement effect (Figure 8D). In response to acidic conditions, the CO$_2$ was produced by the decomposition of CaCO$_3$ led to the degradation of NaGdF$_4$-CaCO$_3$ nanoconjugates; then these NaGdF$_4$ NPs released and
interacted with water protons. Thus, the $r_1$ of this smart probe increased from 0.79 to 8.23 s$^{-1}$·mM$^{-1}$ (Figure 8E). The $T_1$-weighted MR images became much brighter after probes encountered acid (Figure 8F).

These two examples of disassembly for better interaction with water protons seem to be similar to smart probes based on ion extraction. Nevertheless, there are two advantages of this disassembly strategy compared to direct ion extraction from activatable probe systems. First, metal ion release directly in our bodies will lead to disease, and these disassembly processes release NPs on the surface; then, ions interact with water protons safely because of the reduced ion release. Additionally, compared to ions that react with water directly, NPs with larger sizes and higher molecular weights have lower tumbling rates, which are relatively close to those of water in our body. Because of these two reasons discussed above, we divided these two similar strategies into two classes.

### 2.3.3 Disassembly to remove magnetic field perturbations

In 2010, Choi et al. synthesized core-shell NP CAs named DMCA (Figure 9A). Additionally, the authors found that the thickness of SiO$_2$ between the MnFe$_2$O$_4$ core and Gd$_2$O$_4$(CO$_3$)$_2$ shell could obviously affect the $T_1$-weighted MR signal of DMCA. When the thickness of SiO$_2$ increased in the order 4, 8, 16, and 20 nm, the $r_1$ of DMCA was enhanced in the order 2.0, 4.0, 25.1, and 32.5 s$^{-1}$·mM$^{-1}$ (Figure 9B–C). As shown in Figure 9C, 94% of the $T_1$-weighted MR enhancement effect of Gd$_2$O$_4$(CO$_3$)$_2$ was quenched when the thickness of SiO$_2$ was 4 nm and then recovered completely as the distance exceeded 16 nm.

In 2017, Choi et al. reported the distance-dependent MR tuning (MRET) phenomenon occurring between a $T_2$-weighted CA quencher and a $T_1$-weighted CA enhancer. A
TABLE 1 Summary of different designs of activatable MRI probes

| Design strategies | Types | Advantages | Disadvantages |
|-------------------|-------|------------|---------------|
| Ion conversion    | Mn$^{2+}$ extraction | Strong MR enhancement effect | Highly toxic |
|                   | Fe$^{3+}$ extraction | Low toxicity | Weak MR enhancement effect |
|                   | Valance change | Quantified signal activation in vitro | Easily to be reduced to Fe$^{2+}$ in vivo |
| Self-assembly     | Small particle self-assembly | Switchable MRI signal | Uncertain source of activated signal |
|                   | Small molecule self-assembly | High sensitivity to stimuli | Relatively difficult to metabolize |
| Disassembly       | Nanocluster disassembly | Controlled assembly in vitro | Large size lead to more RES absorption |
|                   | Passivation layer disassembly | Good signal shielding effect | Difficulty in design and coating of the layer |
|                   | MRET system disassembly | Excellent signal shielding effect | Unclear shield effect between different CAs |

smart probe based on this MRET phenomenon emerged (Figure 9D–F).

Li et al. used carboxyl group-modified iron oxide NPs (Fe$_3$O$_4$ NPs) as the quencher (Q) and polyethylene glycol-coated gadolinium oxide (PEG-Gd$_2$O$_3$) NPs as the enhancer (E). Moreover, the authors applied Cys containing a disulfide bond to link Fe$_3$O$_4$ NPs and PEG-Gd$_2$O$_3$ NPs (Figure 9G). The T$_1$-weighted MR enhancement effect of Gd$_2$O$_3$ was largely disturbed by Fe$_3$O$_4$. In the presence of GSH, the disulfide bond between Fe$_3$O$_4$ NPs and PEG-Gd$_2$O$_3$ NPs was cleaved and then led to a decreased T$_1$ relaxation time and brighter T$_1$-weighted MR images compared to the outcomes of the group of Fe$_3$O$_4$ NPs only, the PEG-Gd$_2$O$_3$ group and a simple blend of Fe$_3$O$_4$ NPs and PEG-Gd$_2$O$_3$ group (Figure 9H).

As a summary of the above design of activatable MRI probes, a table is provided to show the advantages and disadvantages of various designs (Table 1). The judgment on the advantages and disadvantages of materials mainly focuses on the toxicity of activatable probes, signal hiding effect, sensitivity to microenvironment, signal enhancement ability, practical application, distribution, and clearance in vivo. First, the signal hiding effect, sensitivity to microenvironment, and signal enhancement ability of activatable probes is the main research direction at present. Second, the toxicity and more practical application in biomedicine of activatable probes is now gradually emerging and developing. Finally, the distribution and clearance investigation, which are always included in the reports of traditional MRI probes are relatively fewer according to reports discussed above. Therefore, the criteria for evaluating the biodistribution and clearance of activatable probes are mainly based on previous work.

1. Activatable probes with small size could be cleared by kidney (< 5 nm) and rapid liver uptake (10–20 nm) which might mean low half-lifetime in blood circulation and high individual probability to enter the tumor by EPR effect.
2. Activatable probes with big size (>200 nm) are filtered in the sinusoidal spleen and recognized and cleared by RES, which mean medium half-lifetime in blood circulation and low individual probability to enter the tumor by EPR effect.
3. Activatable probes with medium size (20–200 nm) might mean long half-lifetime in blood and medium individual probability to enter the tumor by EPR effect.

Based on the summary in Table 1, we think that these design strategies of activatable probes still have room for improvement. First, the size of activatable probes is significant for the accumulation, which might mean higher intensity of the signal after excitation and clearance, which is important for the metabolic organs such liver and kidney. For longer half-lifetime in blood circulation and higher accumulation in the lesion, probes with medium size (20–200 nm) is more suitable than probes with small size (<20 nm) and big size (>200 nm). Additionally, the clearance of activatable probes through should be deeper in morphology and pathology even genetics for further application in the future. Second, activatable probes need to be less toxic or even nontoxic. Such as strategies based on Mn$^{2+}$ extraction, small molecule assembly and MRET system seems to be more toxic because of the use of Mn and Gd, which can be more dangerous than Fe with same concentration. Activatable probes using these designs based on Fe may be worth a try.
These discussions above may be helpful for researchers to choose optimal design strategies for different biomedical applications. First of all, for biomedical application in vitro such as molecule detection, strategy (valance change) may be a good candidate because the change of the signal of MR could be converted to precise values. Second, to diagnose diseases with rare and specific markers such as caspase-3, furin and ALP, strategy (small molecule/particle assembly and nanocluster disassembly) could be suitable because they are more sensitive to stimuli. Finally, some diseases with relative obvious lesion characteristic such as acid, ROS, and GSH, strategy (passivation layer/MRET system disassembly and ion extraction) could be better because their initial signal is very low and the signal will be stronger after more stimuli, which could produce a high TBR.

3 | BIOMEDICAL APPLICATIONS OF ACTIVATABLE MRI PROBES

3.1 | In vitro molecule detection

Biomolecules such as ascorbic acid (AA) or GSH play important roles in many metabolic processes. Therefore, it is significant for us to develop methods to detect these biomolecules in pharmaceutical preparations, food products, and medical diagnosis. Currently, due to its high precision and sensitivity, optical detection methods such as fluorometry and colorimetry have become the mainstream biomolecule detection methods. However, they usually suffer from low spatial resolution and interference during the analysis of complex samples. As a noninvasive technique, MRI is considered to be suitable for a combination with FL because of its high tissue penetration and spatial resolution. After reacting with biomolecules, activated signals of those two detection modes provide cross-validation dual-modal accurate detection.

Our group synthesized blended Au@BSA and MnO$_2$@BSA NP mixtures that could be denoted Au/MnO$_2$@BSA through a BSA-mediated reaction strategy (Figure 10A). In this nanosystem, the MR enhancement effect of MnO$_2$ was shielded because MnO$_2$ was wired in the compounds. After reacting with AA, the Mn$^{4+}$ ions were released and reduced into Mn$^{2+}$ ions, which have stronger MR enhance effect and produced a high T$_1$-weighted MR signal (Figure 10B). Through the curve we obtained, we could accurately detect the concentration of AA depending on the change of the T$_1$ value, and the limit of detection (LOD) for AA was calculated to be 0.4 $\mu$M in MR mode and 0.6 $\mu$M in fluorescence mode (Figure 10C–D). In this dual “turn on” mode for the detection of AA, fluorescence mode provided more accurate results, and MR mode provided results with higher sensitivity and spatial resolution. An excellent cross-validation dual-modal accurate detection configuration was built.

In addition to detecting AA in vitro, the calculation of other biomolecules, such as GSH, in vitro was also researched by Xu et al. The authors designed a novel MR/fluorescence dual-modal molecule sensor based on a polyethyleneimine (PEI)-passivated carbon dot-MnO$_2$ platform (pCDs-MnO$_2$). In this platform, the fluorescence of pCDs was quenched effectively by MnO$_2$ at the beginning. Then, with the addition of GSH, MnO$_2$ became Mn$^{2+}$ ions, which were extracted from the platform, and the fluorescence shielding effect of MnO$_2$ on pCDs was no longer present (Figure 10E). Finally, the released Mn$^{2+}$ ions activated the MR signal, and the fluorescence of pCDs was restored. The variation in MR images and fluorescence also had a close linear relationship with the concentration of GSH that reacted with the pCD-MnO$_2$ platform, and this relationship could help us to deduce the concentration of GSH in vitro (Figure 10F–G). The LOD for GSH was calculated to be 2.8 $\mu$M in MR mode and 0.6 $\mu$M in fluorescence mode. In this dual “turn on” mode for the detection of AA, fluorescence mode provided more sensitive results, and MR mode provided results with higher spatial resolution.

From these two examples discussed above, we can see that the combination of activatable MR probes and fluorescence probes can significantly improve the quality of the detection results and further increase the LOD of biomolecules in vitro.

3.2 | Disease diagnosis in vivo

As one of the most important activatable applications, early diagnosis of diseases in vivo can effectively reduce the damage and death rate due to human diseases. In the process of diagnosis, if we could design an activatable probe that could only be activated by biomolecules or the microenvironment in lesions, it would be easier for us to distinguish healthy tissue and lesions because of the higher TBR compared to that of common molecule or NP CAs.

One of the shortcomings of common inorganic NP CAs for MRI is their large amount of enrichment in the liver, which results in MR images of the liver being so intense that they influence the diagnosis result. To solve this problems, Lin et al. prepared Fe$_3$O$_4$-ZIF-8 assemblies by applying pH- and GSH-responsive ZIF-8 assemblies to assemble small Fe$_3$O$_4$ NPs. The microenvironment in tumors with more acid and GSH can degrade Fe$_3$O$_4$-ZIF-8 assemblies into Fe$_3$O$_4$ NPs with an average size of 6
These small Fe$_3$O$_4$ NPs displayed a remarkable T$_1$ enhancement effect compared to the T$_2$ enhancement contrast effect of Fe$_3$O$_4$-ZIF-8 assemblies. Different from the switchable MR enhancement contrast effect in tumors, Fe$_3$O$_4$-ZIF-8 assemblies aggregated in the liver, which has a high concentration of GSH and a neutral pH. The aggregation phenomenon led to darker T$_1$-weighted MR images of the liver, which was obviously different from the brighter T$_1$-weighted MR images of tumors (Figure 11B). The design of this activatable probe not only solved the signal interference caused by the deposition of NP probes in the liver but also obviously increased the TBR of the MR images. As we can detect in Figure 11B, the T$_1$-weighted signal increased by a maximum of approximately 31% at 5 hours, which was the best time to judge the location of the lesion.
FIGURE 11  (A) Schematic illustration of a pH- and GSH-responsive Fe$_3$O$_4$-ZIF-8 assembly as an MRI CA for $T_2$ contrast enhancement in a normal liver and $T_2$ to $T_1$ switchable contrast enhancement in a tumor. (B) $T_1$-weighted MR images and relative $T_1$ values of tumors and livers in mice acquired at different time points. Reproduced with permission. Copyright 2019, Royal Society of Chemistry. (C) Schematic illustration of P-CyFF-Gd for NIR FL/MR bimodality imaging of ALP-positive tumor cells in vivo. (D-E) $T_1$-weighted MR images of P-CyFF-Gd (I), P-Cy-Gd (II), or P-CyFF-Gd together with Na$_3$VO$_4$ (III) in vivo. Reproduced with permission. Copyright 2019, American Chemical Society.

Yan et al. designed alkaline phosphatase (ALP)-activated near-infrared (NIR) fluorescence and MR bimodal imaging probes P-CyFF-Gd that consisted of a pro-quenched NIR fluorophore (merocyanine, Cy-Cl) capped with an ALP recognition phosphate group (-PO$_3$H), DOTA-Gd for MR enhancement, and a hydrophobic dipeptide Phe-Phe (FF) linker.

After the ALP recognition phosphate group (-PO$_3$H) was hydrolyzed by ALP on tumor cells, CyFF-Gd was released and then self-resembled into NPs with the aid of an FF linkage that can offer efficient hydrophobic interactions and $\pi-\pi$ stacking (Figure 11C). The formation of NPs not only promoted cellular uptake and localization in lysosomes but also increased the $r_1$ of the activatable probe by prolonging the tumbling time to a greater extent than that of DOTA-Gd. As shown in Figure 11D–E, after P-CyFF-Gd was intravenously injected into HeLa tumor-bearing mice, the fluorescence and $T_1$-weighted MR images became brighter than those of mice injected with P-Cy-Gd (which was unable to self-assemble). From this phenomenon, we can infer that this activatable probe, which can assemble in response to ALP, has a better imaging effect and higher TBR than common CAs based on Gd$^{3+}$. (DOTA-Gd).
In addition to tumor diagnosis, smart probes can also evaluate other diseases through the detection of a specific biomarker. Although we can identify numerous biomarkers in serum, the necessary sensitivity and reliability requirements have not yet been met. As a non-invasive technique without the limitation of tissue penetration depth, MRI is considered a good candidate method for obtaining an early diagnosis of disease. As an early indicator of sepsis, excessive ROS can be used to detect the location of sepsis. Wang et al. prepared DTPA-HA-SPION nanoprobes by linking Gd-DTPA \( T_1 \) E and SPION \( T_2 \) Q by using HA as a linker. As a result, the \( T_1 \) enhancement contrast effect of Gd-DTPA was quenched by SPION. When the DTPA-HA-SPION nanoprobes encountered sepsis, excessive concentrations of ROS cleaved HA, resulting in the separation of Gd-DTPA and SPION. Thus, the \( T_1 \) signal enhancement effect of Gd-DTPA could be activated swiftly. In a mouse model of severe sepsis, ROS CA-treated septic mice showed stronger MR signals than healthy mice and ROS CA-treated septic mice. Moreover, the authors also found that an injection of GSH could sufficiently block the \( T_1 \)-weighted MR enhancement effect of an ROS CA in mice; this phenomenon also confirmed that the \( T_1 \)-weighted MR enhancement contrast effect was the result of an ROS CA signifying sepsis.

### 3.3 Monitoring drug release

Currently, the design of nanosystems tends to merge the functions of diagnosis and treatment to treat diseases more accurately and effectively. Additionally, the
diagnostic function of the system is not only aimed at the location of the disease but can also be used to monitor the release of the carried therapeutic drugs to determine whether the therapeutic effect of a nanosystem is truly exerted. As a noninvasive technique without the limitation of tissue penetration depth, MRI is considered a good candidate method for monitoring drug release. Therefore, designing smart probes with the function of monitoring drug release has been a good choice to merge diagnosis with therapy more closely.

Ding et al.91 designed smart probes (SMARTs) and then loaded them with the drug doxorubicin (DOX). Finally, the authors took advantage of tetradecanol (TD) to encapsulate DOX. After NIR-II light irradiation, the temperature of this system was higher than the melting point of TD, and then TD degraded. Therefore, DOX was released, and the T1-weighted MRI signals recovered (Figure 12A–B). Additionally, the authors found a linear relationship between MRI signal strength and drug release (Figure 12C). Moreover, an in vivo study was also conducted by incubating SMART-DOX with CT26 cells. After three NIR-II light irradiation cycles, an excitation-dependent MR signal was observed, whereas cells in the control group did not present an obvious MR signal (Figure 12D). Finally, an in vivo study was conducted in mice. Before NIR-II light irradiation, mice were kept at room temperature, and obvious changes in MR signals could not be found. Upon NIR-II light irradiation, the T1-weighted MR signal increased rapidly (Figure 12E).

Moreover, Dong et al.92 used Mn2+-chelated chlorin [Ce6 Ce6(Mn)] as a photosensitizer and DOX as a chemotherapy drug (Figure 12F). Then, the authors applied polyethylene glycol (PEG)-modified CaCO3 NPs as carriers to load Ce6 (Mn) and DOX [CaCO3@Ce6 (Mn)-PEG(DOX)]. As shown in Figure 12G, the CaCO3 carrier decomposed, and the release of Ce6 (Mn) made the T1-weighted MR image brighter in the mildly acidic environment, which resulted in T1-weighted MR signals that were activated. Meanwhile, the curve of the percent of released DOX was similar to the corresponding change curve of the intensity of the MR signal. Twenty-four hours after intravenous injection of NPs, the enhanced intensity of the MR signal was as high as eightfold compared with the time point before injection (Figure 12H). Moreover, to further study the drug release ability and the TBR of NPs, the authors administered NPs within tumor and normal subcutaneous tissue. In Figure 12H, obvious enhancement of the MR signal and TBR is shown; this enhancement is a result of the release of the drug, and the enhancement of the MR signal only occurred in tumor tissue.

As a summary of the comments of activatable probes in various biomedical applications, a table is provided to show the advantages of various designs in biomedicine based on some previous report (Table 2).93,94

| Biomedical application | Suitable design strategies | Advantages                      |
|------------------------|---------------------------|---------------------------------|
| Biomolecule detection in vitro | Valance change, MRET system disassembly, Ion extraction | Quantified signal activation, Highly compatible with optical methods, Low initial signal |
| Disease diagnosis in vivo (marker: Caspase-3/furin/ALP) | Small particle self-assembly, Small molecule self-assembly, Nanocluster disassembly | Higher sensitivity to stimuli, High TBR, Long blood circulation time |
| Disease diagnosis in vivo (microenvironment: acid/ROS/GSH) | MRET system disassembly, Ion extraction | Low initial signal, More activated signal, High TBR |
| Drug release | Passivation layer disassembly | Low initial signal, Ability to encapsulate drug |

### SUMMARY AND OUTLOOK

Based on a large number of studies on high-performance MRI CAs and the clinical need to increase the TBR, activatable probes have received increasing attention and investment. To summarize what we have presented above, we show recent progress in the design and biomedical applications of activatable MRI probes. First, we compare recent articles and reports with each other and summarize three main strategies for the design of smart probes to guide us in the creation of better and more practical activatable probes. These three design strategies include ion conversion, self-assembly, and disassembly. In addition, the distribution, clearance way, advantages, disadvantages, and related improvement measures and suggestions for researchers of activatable probes based on various design strategies are discussed. Moreover, the advantages of these design strategies in biomedical applications from in vitro...
biodetection to in vivo disease diagnosis compared to conventional MR CAs were introduced. In summary, progress in the design of activatable probes effectively accelerates the pace of activatable probes to be applied in the clinic and benefits all human beings.

Nevertheless, these smart probes seem to be slightly difficult to apply to the clinic quickly because of several problems in their designs. These problems can be divided into three parts: microenvironment, designs of probes, distribution, and clearance way in vivo. These three plates complement each other.

First of all, our comprehension of microenvironment such as specific marker, marker content, vascular permeability, extracellular matrix is significant to guide the design of activatable probes. Hence, we should learn more about different diseases before designing activatable probes. To achieve this goal, activatable probes could be helpful because we can design different probes, which could be activated specific markers or microenvironment to research on different diseases. Moreover, we can use other activatable probes to measure the concentration of markers. So, the progress of understanding microenvironment and designing activatable probe could be a spiral process.

Additionally, based on the information about microenvironment, the design of activatable probes should focus on some points below. First, the initial signals of activatable probes are maximally suppressed or even quenched, which could be a guarantee of high TBR. Second, whether smart probes are precise enough that they can be used for a specific disease. Moreover, whether smart probes are sensitive enough to endogenous ROS (GSH, Caspase-3, etc.) or exogenous stimuli (light irradiation, ultrasound, magnetic field, etc.) remains to be seen. Furthermore, whether the MR signal enhancement effect of a smart probe is maximized after being activated is unknown. Finally, the problem whether the activated MR signals come from only stimuli and not from other interference, such as a larger amount of activatable probe with certain initial signal in a certain location, also needs to be further evaluated. To solve these five significant problems, more effort should be devoted to the design of smart probes. First, to minimize the MR enhancement effect before being activated and to maximize the MR signal enhancement effect in response to stimulation, we can mix several strategies in the design of activatable probes. Second, to make smart probes specific enough to one disease, we can design smart probes that can only be activated by two or more molecules of this disease appearing at the same time, such as the Fe₃O₄·ZIF-8 construct discussed above. To eliminate signal interference from the probe itself to accurately quantify MR signal changes, we could use other imaging modalities that have stable signals to compare the amount of MR probe present. These design methods can make the diagnosis of smart probes much more accurate and closer to being ready for clinical applications.

Third, the biodistribution and clearance way of activatable probes is a promise for further biomedical application. First, information about vascular permeability, extracellular matrix of microenvironment is important for the enrichment in the lesion. Second, based on the design of probes, the half-lifetime in blood circulation could be helpful for more accumulation in the lesion. In addition, the assembly in the microenvironment could also be helpful to increase the accumulation and residence time in the lesion. Finally, the clearance way of probes after activation should be investigate in morphology and pathology even genetics for further application in the future.

There are still many challenges in the field of designing a smart probe. Nevertheless, the starting point for designing smart probes is to improve the TBR, which is a general trend of future development of MRI CAs in the clinic. With the development of the study of microenvironment, designs of probes, distribution, and clearance way in vivo, activatable MR probes will be a good candidate to change the world.

CONFLICT OF INTEREST
The authors declare no conflict interest.

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