Recent Advances in Peptide- and Protein-Based MRI Contrast Agents

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Magnetic resonance imaging (MRI) is a common noninvasive and nonradioactive medical imaging technique that allows for diagnosis of many medical conditions from sports injuries to cancer. MRI contrast agents are used to create higher-resolution images by increasing the rate of water proton relaxation in certain tissues to provide better contrast in images. The limited resolution of current clinical standard agents, which allows small anatomical features such as early-stage tumors to go undetected, has motivated a search for improved contrast agents.

Gadolinium(III), due to its 7 unpaired electrons and high magnetic moment, is the most common material for contrast agents. Several considerations must be taken in designing Gd-based contrast agents, including high stability constants to avoid free Gd(III) and nephrotoxicity and high relaxivity values. All the currently approved Gd-based contrast agents are created from small molecule chelates. Two of the most common Gd-based contrast agents are Gd-DTPA (Magnevist) (Figure 1A), which has the highest relaxivity ($r_1$ and $r_2$) values on the market, and Gd-DOTA (Dotarem) (Figure 1B), which is considered the safest of the Gd-based contrast agents due to its high binding constant.

![Figure 1. Structures of Gd-DTPA (A) and Gd-DOTA (B) with log K_a and relaxivity data.](image)

Although Magnevist and Dotarem are the clinical gold standard agents, neither of them approach the theoretical maximum possible relaxivity, defined as the ability of a contrast agent to reduce the relaxation rate of water protons. Relaxivity can be most improved by increasing the number of coordinated water molecules ($q$) and the rotation correlation constant ($\tau_R$) leading to higher relaxivity values.

![Figure 2. Model peptide contrast agent Gd(MB1-2)_3.](image)

![Figure 3. Protein contrast agent CA1.CD2/ProCA1 (A) and ProCA32 (B).](image)
better image contrast. Peptides and proteins have been considered as a scaffold for MRI imaging agents because of their increased size and potential to engineer a higher q value within the design. While multiple groups have attempted to link existing gadolinium chelates to proteins, only modest improvements in relaxivity have been made\(^4\), likely due to the free motion of the chelate compared to the motion of the whole agent\(^5\).

Peacock et. al. published a paper in 2014 in which a three-stranded Gd(III)-binding coiled coil was created de novo\(^6\). A lanthanide binding site was engineered into the middle of this peptide complex, called Gd(MB1-2)\(^3\) in later papers (Figure 2), by mutating selected amino acids into oxygen binding chelators. The number of coordinating water molecules determined by the Horrocks and Sudnick Equation\(^7\) was found to be 0.4; the number of inner-sphere water molecules was determined by the Beeby and Parker Equation\(^8\) to be 0.1. While no coordinated waters is known to lead to a reduced relaxivity value, the relaxivity values for this complex were 6.3 ± 2.1 mM\(^{-1}\)s\(^{-1}\) and 18.9 ± 1.5 mM\(^{-1}\)s\(^{-1}\) for r\(_1\) and r\(_2\), respectively, a slight improvement over Magnevist (see Figure 1) likely due to the increased size.

The group then performed a systematic study in which the Gd(III) binding site was systematically moved to each possible position in the peptide complex\(^9\). In this, they found that the systems which had the binding site close to the ends of the complex had higher numbers of inner-sphere waters than those binding sites places in the middle. The number of inner-sphere waters was found to positively correlate with both the r\(_1\) and r\(_2\) values. In addition, by systematically increasing the bulkiness of the second peptide in the chain, shown to sit directly above the binding site, the group was able to reduce the number of inner-sphere waters via steric crowding to tune the relaxivity values\(^10\). Overall, these complexes are the first Gd-binding de novo peptide systems but will not be developed as clinical agents because their Gd binding constants (log \(K_s\) approximately 5) are much too low.

The Yang group, in 2008, rationally designed a Gd(III) binding site to make the chemical contrast agent CA1.CD2 (known later as ProCA1)\(^11\) (Figure 3A). Later, the group improved upon this structure by the addition of long-chain PEG to various sites around the protein that enhanced relaxivity and bioretention\(^12\). While these invented protein contrast agents had too low binding constants (log \(K_s\), approximately 12) to be seriously considered as clinical agents, the relaxivities were greatly improved compared to Gd-DTPA, especially once the PEG groups were added to make a higher relaxivity. Part of this effect could be due to the number of coordinated waters in the inner sphere (>2.4).

The group created a second designed protein contrast agent, ProCA32, based upon the rat parvalbumin protein\(^13\) (Figure 3B). This protein featured a comparable binding constant to clinical agents; moreover, this system had higher metal selectivity over calcium, zinc, and magnesium compared to Gd-DTPA. No waters were found to be bound to the inner sphere and the resulting relaxivity values were lower than ProCA1; however, the group went forward with improving this structure due to its high binding constant and higher chemical safety. When a collagen-targeting moiety was added, this system demonstrated the ability to show the stage of liver disease and provide resolution of collagen fibroids at 0.24 mm, much lower than the previous 0.5 mm\(^14\).

The research of peptide- and protein-based contrast agents is expanding in both the knowledge of these systems’ structure-functional relationships and the application in the clinic. By continuing to improve on these designed systems, the knowledge can be translated into a clinical setting.
References:


