Gd(III)-based Enzyme-Responsive Nanoprobes as MRI Contrast Agents

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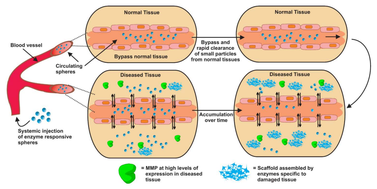
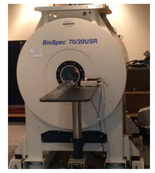
Abstract

The goal of this work is to develop Gd^{III}-based T_1 contrast agents (CA) engineered to accumulate in tumor tissue in response to elevated matrix metalloproteinase (MMP) activity. MMPs are chosen as targets for stimuli-responsive diagnostic probes due to their higher concentrations in cancerous tissues.[1-3] To date, the Gianneschi group has demonstrated the site-specific accumulation of fluorescently-labeled probes in both tumors[4,5] and diseased cardiac tissue.[6] However, to make these materials clinically relevant, new detection strategies are necessitated. Magnetic resonance imaging (MRI) is a suitable method for probe detection because it is non-evasive, clinically translatable, and can provide molecular level diagnostics. Therefore, in an effort to achieve MRI detectable probes, Gd^{III} chelates that could be readily incorporated into existing nanoparticle architectures were targeted.



Mechanism of Probe Accumulation in Cancerous Tissue

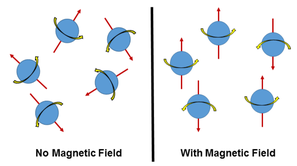


MRI Instrument at UCSD

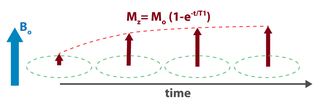
Contrast Agents

MRI is a frequently used imaging tool routinely used to obtain anatomical and functional information on soft tissue, and has become increasingly important in the diagnosis of human diseases.[7] In principle, MRI relies on the excitation and relaxation of magnetic moments of the nuclei of hydrogen atoms in water.

Importantly, when protons are exposed to a strong magnetic field, their nuclear spins will either align parallel or antiparallel to the magnetic field vector. When equilibrium between these spin populations is reached, a small excess of protons aligned parallel to the magnetic field remains. When a radio frequency pulse equal to the energy difference between the two populations is applied perpendicular to the magnetic field, the populations will begin to invert, and over time these populations will return to equilibrium accompanied by a release of energy that can be measured, and detected in an MRI experiment.



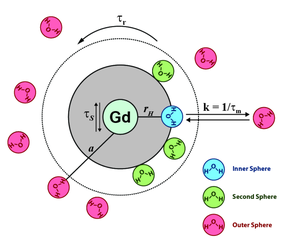
The magnetic fields effect on nuclear spin



T_1: The longitudinal relaxation time

The time in which this magnetization recovery occurs along the magnetic field vector is referred to as T_1, and is highly dependent on the water molecules' environment. The differences between these recovery times of water molecules in different regions of the body provide the contrast of an MR image. Contrast agents can enhance the MR image by speeding up T_1-recovery through magnetization transfer of the magnetic moment.

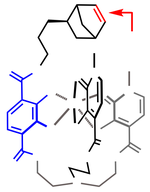
Gadolinium is an especially good CA because it possesses seven unpaired electrons and therefore has a very strong magnetic moment. Selectively delivering enzyme-responsive Gd^{III} CAs to a tumor will allow detection of the tumor at the molecular level because it will appear brighter in the MR image.



Magnetization transfer by contrast agents

Synthesis of the Gadolinium Chelate

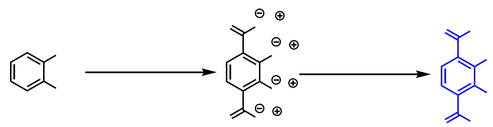
The target Gadolinium chelate is shown to the right. This is a rather complex molecule that takes over 20 steps to synthesize. This molecule is incorporated into the nanoparticle through polymerization of the alkene group highlighted in red. The objective in our short time together was to synthesize the 2,3-dihydroxyterephthalic acid portion of the molecule highlighted in blue.



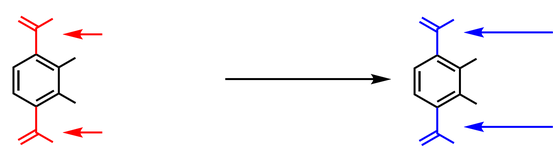
Gadolinium Chelate

The first step in this synthesis involved a Kolbe-Schmidt reaction. Under Kolbe-Schmidt conditions, (high CO_2 pressures and elevated temperatures), carbonylation of the position *ortho* (or adjacent) to the phenolic (arene-OH) group occurs. Crucial to this reaction is the presence of an alkaline base, which activates both the phenol functionality of the arene ring and CO_2 towards addition. Here, catechol is converted to 2,3-dihydroxyterephthalic acid via substitution of the *ortho* positions of catechol with carbonyl groups. More precisely, the procedure employed here involved mortaring and pestling catechol together with five equivalents of K_2CO_3 and placing this mixture into a high-pressure bomb reactor. The reactor was then pressurized to 800 PSI with CO_2, and heated at 200˚ C for one day. The resulting material is the potassium salt of the molecule. This salt was dissolved in hot water, stirred over activated charcoal and filtered. Once cooled, the terephthalic acid was precipitated from solution via the addition of 6 M HCl, which was filtered, washed with water, and dried under reduced pressure with heating to 100° C.

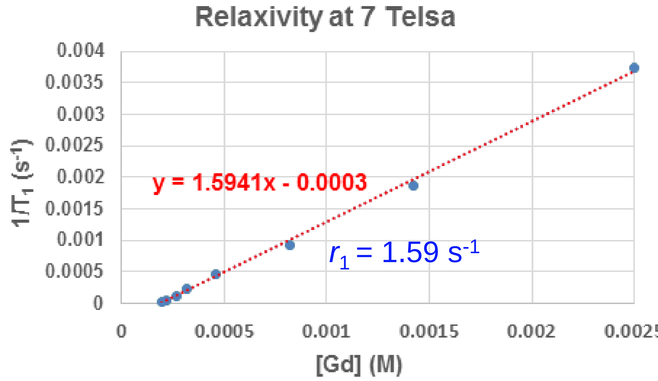
Bomb Reactor



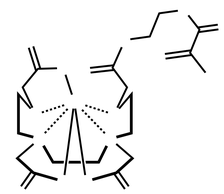
In order to protect the carboxylic acid groups in later steps, they were protected by the formation of methylester groups. The terephthalic acid was dissolved in methanol and an excess of H_2SO_4 was added to catalyze the reaction. The solution was heated for 12 hours, during which the OH groups were replaced with OCH_3 (methyl) groups. The solution was then cooled and the majority of the methanol was removed using a rotovap. The remaining liquid was then neutralized with saturated sodium bicarbonate ($NaCO_3H$). The aqueous solution was then extracted three times with chloroform, using a separatory funnel. In the funnel, the top layer consisted of the aqueous solutions and the bottom layer contained chloroform and the desired product. The three chloroform fractions were combined, and sodium sulfate (Na_2SO_4) was added to remove any remaining water. After filtering away the sodium sulfate from the chloroform solution, the white crystalline product was obtained through removal of the chloroform using a rotovap.



MRI Characterization of Gadolinium Chelates



Concentration



Gadolinium Chelate

The measure of a contrast agent's ability to speed up the rate of proton relaxation is referred to as its relaxivity (r_1). To determine this value, a series of dilutions of the contrast agent dissolved in water were prepared. These solutions were then placed in an MRI instrument and the T_1 values were determined. By plotting the inverse of T_1 against the concentration of the contrast agent in water, and then performing a linear fit of the data, the slope of this line equals the relaxivity of the contrast agent. Given that the target contrast agent could not have reasonably been synthesized in the duration of my time in the lab, this procedure was performed on a previously synthesized contrast agent.

References

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Acknowledgments

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