Using Gd-DOTA and NP attached to DNA at Different Distances

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Abstract

By using EDC and sulfo-NHS coupling we can attach Gd-DOTA to a strand of ssDNA and attach gold-coated nanoparticles to a partner strand of ssDNA. Then by coupling the DNA we can get a sensing contrast that can be set at different lengths and be able to identify tumors easier. MRI contrast has been a powerful and transformative diagnostic tool in modern medicine. However, as contrast is currently used it can only enhance contrast between tissue types, presenting relief of anatomical features, or localization of cancer cells.

1 Gold-Coated Iron Oxide Nanoparticles

The use of iron oxide nanoparticles for biomedical applications has been relevant for some time. This is due to the ability to target either passively or actively and can avoid toxicity in the healthy tissue. They also can be designed and altered as either pH-sensitive or temperature-sensitive to establish and regulate the drug release. The pH-sensitive drug delivery system can deliver drugs within the acidic TME. Similarly, the temperature-sensitive NPs release the drugs in the target site due to changes in temperature brought in by sources like magnetic fields and ultrasound waves [G]. Nanoparticles can also have their molecular mass, size, surface chemistry, and shape altered to target tumors directly.

We coat these nanoparticles in gold for the properties they possess and because they allow the iron oxide nanoparticles to be used in humans due to the gold being safe inside the human body. In comparison with Gd alone, GNPs can persist in the tumor mass for hours and potentially days after a single injection. Injected GNPs allow for accurate delineation of the tumor mass. The selectivity of GNPs for brain tumor cells, their prolonged retention in the tumor itself, as well as their effectiveness as therapeutic agents provides the theoretical basis for the "theranostic cycle." The theranostic cycle has four steps. The first is that the brain tumor diagnosis is achieved by advanced brain magnetic resonance imaging with gadolinium-conjugated gold nanoparticles (GNPs). Then, after the usual surgical debulking of the tumor mass, the loco-regional invasion is identified and removed in two steps: a macroscopic phase and a microscopic phase involving GNPs suitable for Raman imaging, fluorescent imaging, photoacoustic imaging, photoacoustic flow cytometry. Finally, the GNPs can be loaded with

therapeutic agents, such as chemotherapy agents, that can target and destroy potential tumor residuals. If the tumor appears again then the cycle can be repeated.

2 Background

We started our research based off a paper that studied distance-dependent magnetic resonance tuning for sensing biological targets, which in our case is cancer cells, through MRI. This paper describes how distance between a superparamagnetic nanoparticle and an enhancer can affect the enhancer by turning it on or off. They also describe the multiple ways that these two can be bonded and how they can alter the distance based on these bonds. The paper is finished off by showing scans and data collected from experiments tested in mice.

From this, our research involves producing a system where multiple gadolinium ions can be attached to a superparamagnetic nanoparticle. This is done by having ligands attached to both the gadolinium and nanoparticle and then as many ssDNA strands attached to those ligands as we can get. When we anneal the DNA we plan to have these systems combine and form one unit as shown in Figure 1. This bond will then be broken when a tumor is present, and the contrast will be separated, causing it to turn on and show readings. This is using the cleavage technique that the paper discusses.

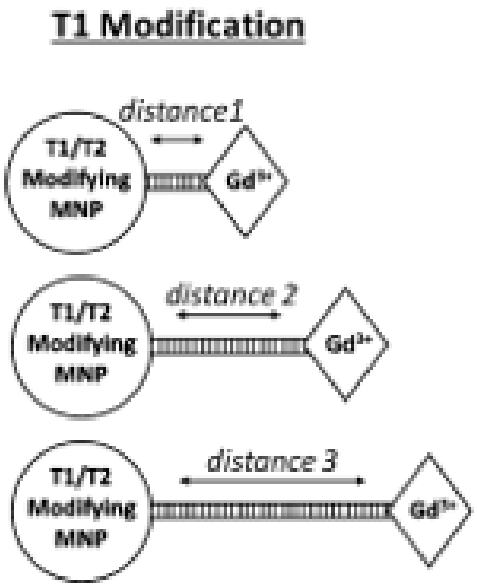

Fig. 1

Visual representation of NP&Gd-DOTA conjugation at different lengths. (Note: I was not constructing different length in the lab, but it is the final product that we were hoping to achieve.)

2 MRI

MRI stands for magnetic resonance imaging and works by producing a strong magnetic field that forces the protons in our body to align with it. The MRI machine will send out a pulse disrupting the alignment with this field and when it ends the protons realign with the field. The time it takes to realign, as well as the dephasing of spins that occurs are labeled as T1 and T2. T1 relaxation is the process by which the net magnetization grows/returns to its initial maximum value parallel to Bo (E). In Figure 2, this phenomenon known as T1 relaxation is shown. The time that it takes for the protons to realign is affected by the type of tissue and the environment they are in. T2 relaxation is the process by which the transverse components of magnetization decay or dephase. When net magnetization is tipped, not all the spins are locked in phase. Some of these rotate into the transverse plane. The initial Boltzmann distribution "ordering" of spins in the z-direction has been preserved and transformed by the rotation into what can be considered "phase coherence" in the xy-plane(E). This phenomenon is shown in Figure 3.

In MRI, contrasts are used to enhance the T1 signal allowing for tumors to be brighter when showing up on scans. The scans are made up of a couple million voxels that generate their own MRI signal depending on a couple factors. These are differences in frequency, phase, and signal timing, as well as the distance from the receivers. When hit with the pulse from the receiver, the protons will realign and depending on those four factors, certain areas of the scan will be brighter than others. This helps locate where an abnormality is and when a contrast is used, these areas come up even brighter. This is because the contrast will accumulate where the tumors are, and when they are hit with the pulse they affect the spin of the protons and make

them closer to the Larmor frequency, basically it shortens the T1 values, which shows up brighter on the scans.

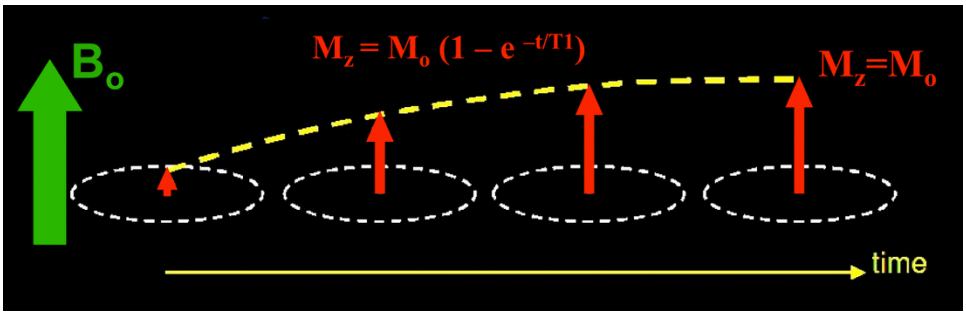

Fig. 2

Shows how the net magnetization returns to its initial maximum value.

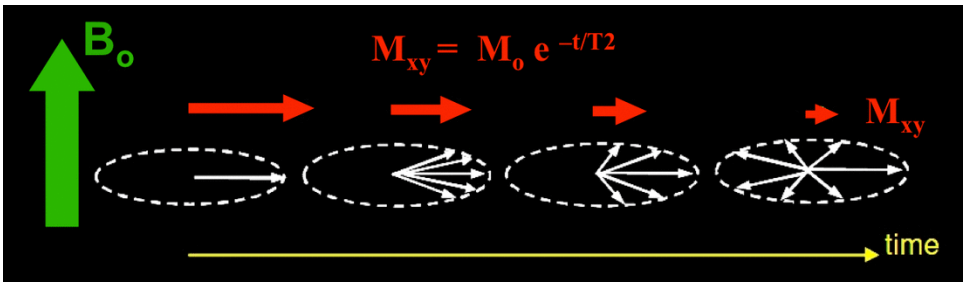

Fig. 3

Showing loss of Mxy component of magnetization and T2. The dephasing that occurs

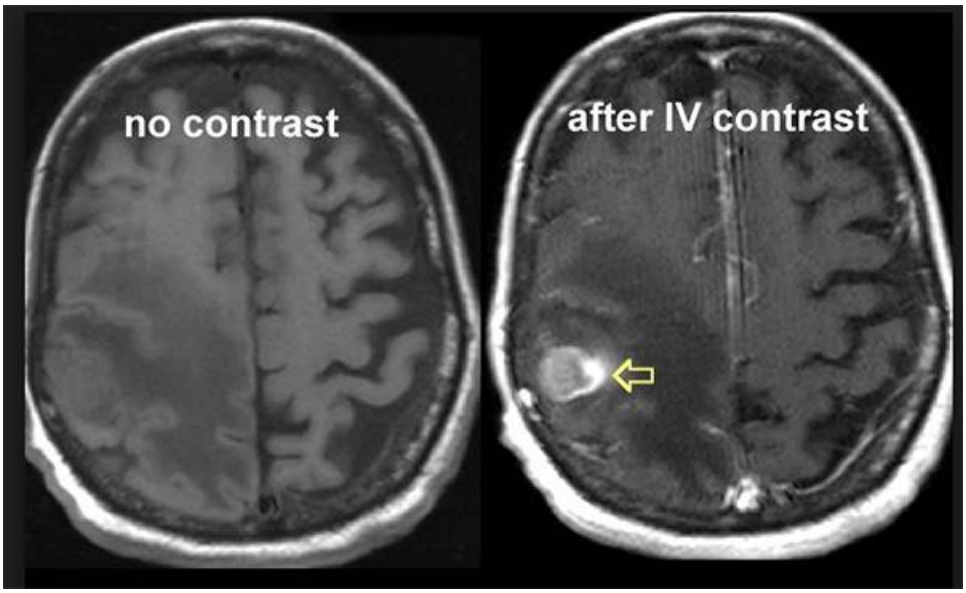

Fig. 4

Difference in what shows up on an MRI scan normally and in the presence of a contrast.

3 Theory

Our research is based on the dipole-dipole interactions that occur between the free electrons in the gadolinium and the protons in the water. These are affected by the spin and distance of the ions. The interaction of a proton and electron is much larger due to the smaller size of the electron and it having a high gyromagnetic ratio, as well as it being able to be closer to the proton. The strength of the interaction is inversely proportional to the sixth power of distance, so the farther apart they are, the weaker the interaction.

With this, water tumbles too fast to be seen for T1 readings, but when the free electrons in gadolinium get close to these water molecules, the proton from the hydrogen atom in water has an interaction and the spin slows down allowing for the readings to show up for T1. When combined with the nanoparticle, the spin of gadolinium is affected. The spin is then too slow to show up for T1 readings and therefore the gadolinium is quenched. When they separate enough, the gadolinium is no longer quenched and can interact with the water around it. This is how you get bright areas in MRI scans and why the contrast is used to enhance.

4 Experiments and Goals

Our experiments involved trying to find a way to attach multiple strands of ssDNA to a nanoparticle and to Gd-DOTA, anneal the two strands of ssDNA together, and obtain at least twenty Gd-DOTA per nanoparticle. We tried two main ways to attach the ssDNA to the nanoparticle which are by using EDC and Sulfo-NHS, as well as using HATU. We used the basic method for annealing, combining the two solutions that had complementary ssDNA and heating them up, and we ran across some issues. This was mainly loss of DNA which was our main issue throughout all of our experiments. Whether this was due to the annealing or the prior conjugation steps is still unknown. Overall, the more contrast per nanoparticle, should result in better and brighter images, to an extent. By getting at least twenty, we are able to get a brighter image and when combining that with the distance-controlled system that we would like to achieve, it would result in a more precise image for where the cancer is even when it is in the small, early stages.

Our goal is to make a system that involves quenching and unquenching that can have their distance altered to better locate tumors. By adding on to something that is widely used and safe, if done right, we can allow for an easy entry and implementation of a new way to detect cancer early on and hopefully reduce the number of cancer patients that are too late at catching it before it becomes severe.

5 Results

As far as results go, we did not get any results that would show up on MRI scans while I was in the lab. To get a good visible reading on the MRI you need to have about twenty DNA strands attached to each nanoparticle. The most we achieved was around fourteen as shown in Figure 5. The main issue we had was that we could not keep the DNA attached and it was washed out throughout the procedure. We had tried two main methods to conjugate the DNA and neither seemed to work enough to produce results visible in MRI. We also lost DNA during the annealing process, which we are unsure of the reason why. The consistent loss of DNA lead to not nearly enough DNA per nanoparticle, although fourteen per was much better than our earlier experiments where we could not get more than five DNA per nanoparticle.

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