

## MAGNETIC NANOSENSORS FOR DNA ANALYSIS

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**INTRODUCTION:** Sensitive and cost-effective biosensors are required for the high throughput detection of DNA in un-modified biological samples and potentially even *in vivo*. To facilitate these studies, we have developed biocompatible magnetic nanoparticles capable of detecting specific DNA sequences upon structured self-assembly of the disperse nanoparticles into a stable assembly (clusters). This target-induced assembly occurs with a concomitant change in the proton relaxivity of neighboring water molecules, rendering these nanoparticles as magnetic nanoswitches capable of sensing DNA as a function of changes in water relaxivity. We have shown that these magnetic nanoparticles can be used to read out complementary DNA sequences and that the resulting changes in water relaxivity can be detected by NMR. The developed system could be useful as generic biosensors in a variety of other applications such as affinity ligand determination for rapid magnetic resonance imaging of arrays, as probes for magnetic force microscopy, and potentially, for *in vivo* imaging.

### METHODS:

**Synthesis of magnetic nanosensors.** A dextran-coated iron oxide colloid was cross-linked with epichlorohydrin, and treated with ammonia to yield amino-derivitized, cross-linked iron oxide nanoparticles (CLIO-NH<sub>2</sub>), as described [1]. These superparamagnetic nanoparticles were further derivitized with the cross-linking agent N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) to yield 2 pyridyldithiopropionate-derivitized CLIO (CLIO-SPDP). The CLIO-SPDP preparation had an iron concentration of 2.8 mg Fe/mL and an average of 48 pyridyl disulfide groups per particle. CLIO-SPDP (3 mg of Fe in 0.1 M phosphate buffer, pH 8.0) was reacted with the corresponding alkanethiololigonucleotide (HS-(CH<sub>2</sub>)<sub>6</sub>-CGC-ATT-CAG-GAT or TCT-CAA-CTC-GTA-(CH<sub>2</sub>)<sub>3</sub>-SH) and incubated overnight at room temperature. The next day the mixture was applied to a magnetic separation column (Miltenyi Biotec, Auburn, CA) equilibrated with 0.1 M phosphate buffer, pH 7.5, and the retained material was washed with phosphate buffer to remove any non-bound oligonucleotide. To recover the purified conjugate, the column is removed from the magnet and the

material is eluted with buffer. The pooled volume, containing either CLIO-S-S-(CH<sub>2</sub>)<sub>6</sub>-CGC-ATT-

CAG-GAT (P1) or TCT-CAA-CTC-GTA-(CH<sub>2</sub>)<sub>3</sub>-S-S-CLIO (P2), had an iron concentration of 0.58 mg of Fe/mL and 3 oligonucleotides per particle, on average [2].

**Synthesis of Target Oligonucleotides.** The complementary (TAC-GAG-TTG-AGA-ATC-CTG-AAT-GCG), and non-complementary (ATG-CTA-AAT-GAC-GAC-TGC-CCA-CAT)

oligonucleotides were synthesized on a 50-nmol scale using standard phosphoramidite chemistry. Stock solutions (10 OD per mL) were prepared by resuspending the sample in deionized water.

**Relaxation Times (T<sub>2</sub>) Measurements.** The T<sub>2</sub> relaxation times of P1, P2 or P1/P2 (10 µg Fe/mL, each) were recorded at 0.47 Tesla (Bruker NMR Minispec, Billerica, MA). To study the effect that hybridization might have on the magnetic properties of these nanoparticles, the T<sub>2</sub> was recorded before and after addition of complementary oligonucleotide target (390 ng) to a mixture of P1 and P2 (10 µg Fe/mL, total iron). Control experiments were performed using the half-complementary and non-complementary oligonucleotides.

**Atomic force microscopy.** An atomic force microscope (Dimension 3100, Digital Instrument) was used to collect images of P1/P2 with and without target DNA. The samples containing either equal amounts of each nanoparticle (P1/P2) alone or with target oligonucleotide were spotted on freshly cleaved mica at a total iron concentration of 10 µg/mL. This iron concentration is identical to the one used in relaxation time measurements. Images were taken by mounting the sample on a fluid cell containing a silicon nitride cantilever (20-40 nm tip size). Images were recorded using a tapping/lift mode.

### RESULTS:

The magnetic nanoparticles used in these studies consist of a 3 nm superparamagnetic iron oxide core caged with a cross-linked and aminated dextran coating, resulting in 50-nm amine derivitized magnetic nanoparticles. We coupled an average of 3 oligonucleotides per particle with sequences complementary to a target DNA

sequence. The oligonucleotide sequences attached to the nanoparticles were chosen to recognize adjacent sequences on the DNA target. For each target sequence we made two particle populations (termed P1 and P2) recognizing adjacent sequences covering 24 base pairs (Figure 1). These nanoparticles were stable in solution without precipitation for months and were present in a monodisperse state when viewed by atomic force microscopy (Figure 1a). However, upon hybridization with a target sequence, the particles oligomerized into larger assemblies of approximately 200 nm (Figure 1b). Such oligomerization was not observed when the nanoparticles were incubated with a non-complementary oligonucleotide.

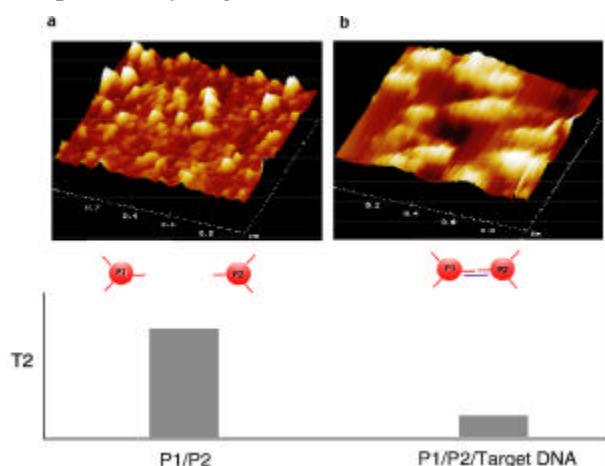


Fig. 1: Clustering of magnetic nanoparticles (P1 and P2) upon hybridization to a target DNA as shown by an AFM image of the particles before (a) and after (b) addition of target DNA. Such clustering correlates with a decrease in T2 relaxation times observed upon addition of target DNA.

These magnetic nanoparticles are potent enhancers of the spin-spin and spin-lattice relaxation process. Oligomerization of these nanoparticles (P1 and P2) into larger assemblies resulted in significant changes in the spin-spin relaxation times (T2) of neighboring water molecules as determined by relaxation measurements using a NMR bench top relaxometer. Within several minutes after oligonucleotide addition to a P1/P2 mixture (10  $\mu\text{g}$  Fe/mL), the T2 relaxation times decreased by over 20 msec and remained constant. No visible precipitate formed at this concentration over a 24-hour observation period indicating that the clusters are stable in solution. When dithiothreitol (DTT) was added, T2 relaxation times returned to baseline values, as the hybridized oligonucleotides were cleaved from the nanoparticles and the particle

nanoassembly was converted back to a monodisperse state. (Data not shown).

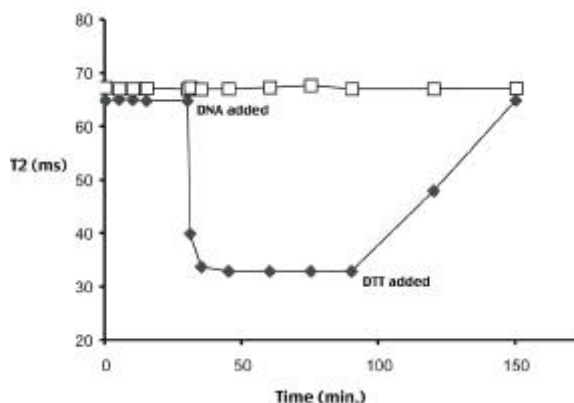


Figure 2: Time course of water T2 relaxation times for a mixture of P1 and P2 (10  $\mu\text{g}/\text{mL}$ , total iron) with complementary ( $\blacklozenge$ ) and non-complementary target oligonucleotide ( $\ast$ ).

**CONCLUSION:** We have shown that the hybridization-induced assembly of magnetic nanoparticles results in a decrease in the spin-spin relaxation time of neighboring water protons. Using a simple bench top NMR relaxometer (0.47T), we have observed a fast and reversible decrease in T2 relaxation time when complementary oligonucleotide is added to P1 and P2. Using this technique, we have been able to detect as low as 10 fmoles of DNA while lower amounts (0.5 fmoles) have been detected using a 1.5 T MR imager (data not shown). The developed magnetic nanosensors will have considerable applications in the detection of oligonucleotides (DNA/RNA) and studies are underway to expand the use of these nanoproboscopes to study other types of molecular interactions.

**REFERENCES:** <sup>1</sup>L. Josephson, C.H. Tung, A. Moore, R. Weissleder (1999) *Bioconjugate Chem.*, 10, 186-191. <sup>2</sup>L. Josephson J.M. Perez, R. Weissleder (2001) *Angew. Chemie Int. Ed. Engl.* 40/17: 3204-3206.

**ACKNOWLEDGEMENTS:** This work was supported in part by a grant from the National Cancer Institute at NIH. JMP is a recipient of a fellowship from the National Cancer Institute.