

# Nanoparticle-based Cancer Biomarker Sensor

G. Y. Kim<sup>1</sup>, K. D. Daniel<sup>2</sup>, C. C. Vassiliou<sup>3</sup>, N. M. Elman<sup>4</sup>, L. Josephson<sup>5</sup>, M. J. Cima<sup>6</sup>

<sup>1</sup> Harvard-MIT Health Sciences & Technology, Cambridge, MA, graceyk@mit.edu

<sup>2</sup> Dept of Chemical Engineering, MIT, Cambridge, MA, kdaniel@mit.edu

<sup>3</sup> Dept of Electrical Eng & Computer Sci, MIT, Cambridge, MA, chrisv@mit.edu

<sup>4</sup> Dept of Materials Science & Engineering, MIT, Cambridge, MA, nelman@mit.edu

<sup>5</sup> Center for Molecular Imaging, MGH, Charlestown, MA, josephso@helix.mgh.harvard.edu

<sup>6</sup> Dept of Materials Science & Engineering, MIT, Cambridge, MA, mjcima@mit.edu

## ABSTRACT

Magnetic relaxation switches (MRS) are being developed for local monitoring of cancer biomarkers. MRS are functionalized magnetic iron oxide nanoparticles that exhibit analyte-induced aggregation and a corresponding change in transverse spin-spin relaxation time ( $T_2$ ), which can be measured through NMR spectroscopy or MRI. MRS were functionalized with a matched pair of antibodies to detect the beta subunit of human chorionic gonadotrophin (hCG), which is elevated in testicular and ovarian cancers. These MRS have been shown to aggregate specifically in the presence of the hCG- $\beta$  and hCG in a concentration dependent manner as confirmed by  $T_2$  changes and particle sizing measurements. Here, we report further characterization of this sensor system. The aggregates were found to dissociate with the addition of large excesses of hCG as governed by the dissociation constant of the antibodies. The effect of valency on the formation of aggregates was also studied.

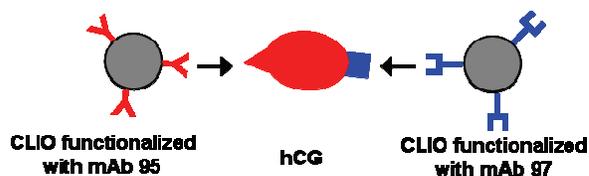
**Keywords:** magnetic; nanoparticle; sensor; cancer; human chorionic gonadotrophin

## 1 INTRODUCTION

Molecular imaging has been playing an increasing role in cancer detection and treatment through the development of magnetic relaxation switches (MRS), which can measure specific molecular targets, including DNA and ions [1,2,3]. MRS are also being developed to monitor the efficacy of treatments to bulky cancers with restricted delivery or to cancers requiring optimized and personalized approaches. The target molecule causes the MRS to aggregate, causing a change in the transverse spin-spin relaxation time ( $T_2$ ), which can be measured by MRI or NMR relaxivity. The use of a device to contain and expose the MRS to the tumor milieu allows localized measurements of soluble cancer analytes, which have different concentrations than measured in systemic circulation [4,5].

Human chorionic gonadotrophin (hCG) is secreted as an  $\alpha\beta$  heterodimer or its subunits. Elevated hCG- $\beta$  levels are associated with several pathologies, including testicular and ovarian cancers. MRS to detect hCG- $\beta$  using crosslinked

iron oxide (CLIO) nanoparticles has been fabricated [6]. These nanoparticles have been shown to aggregate specifically in the presence of the hCG- $\beta$  and hCG in a concentration dependent manner as confirmed by  $T_2$  changes and particle size measurements. Aggregation occurs because of the conjugation of CLIO nanoparticles to a matched pair of antibodies (Figure 1). The multivalency of the functionalized CLIO nanoparticle enables the crosslinking required for networks of aggregated nanoparticles to form. Here, we report further characterization of this hCG- $\beta$  sensor.



**Figure 1:** Aggregation schematic  
CLIO nanoparticles were conjugated to a matched pair of monoclonal antibodies (mAb) specific to hCG.

## 2 MATERIALS AND METHODS

### 2.1 Nanoparticle Conjugation

Magnetic iron oxide nanoparticles with amine terminated dextran shell (CLIO-NH<sub>2</sub>) were produced as described previously[7]. Two separate CLIO nanoparticle conjugations were made to one of a matched pair of antibodies. CLIO-NH<sub>2</sub> was treated with sulfo-SMCC (Pierce, Rockford, IL) to create a maleimide functional group. Monoclonal antibodies to hCG- $\beta$  (Scripps Laboratories, San Diego, CA) were activated with (N-succinimidyl-S-acteylthioacetate) (Pierce) to generate a blocked sulfydryl group which was deprotected with hydroxylamine. The CLIO-SMCC was incubated with the prepared antibody solutions for 4 to 8 hours at 4°C. The reaction was quenched with mercaptoethanol and purified with a Sephacryl column. The conjugated CLIO nanoparticles are referred to by their product identification, C95 and C97 for short. C95 and C97 were determined to have approximately 2 and 3 antibodies, respectively, per CLIO by BCA (Pierce).

## 2.2 Relaxation Time Measurements

Proton relaxation time measurements were performed at 0.47 T and 40°C (Bruker NMR Minispec, Billerica, MA). Samples were incubated at 40°C for one hour for thermal equilibration before measurements were taken. Relaxivities were determined by plotting  $1/T_2$  or  $1/T_1$  ( $\text{sec}^{-1}$ ) as a function of the iron concentration (mM).

Aggregation experiments were performed by mixing equal volumes of CLIO nanoparticle solutions and analyte solutions. Dilutions of hCG- $\beta$  (Scripps Laboratories, 28 kDa) or hCG (Sigma, 37.9 kDa) were prepared in phosphate buffered saline with 0.1 or 1% bovine serum albumin (PBS-BSA) using the theoretical molecular weight for calculations. PBS-BSA was added for negative controls. Reported values are the final iron and analyte concentrations obtained after mixing. Relaxation time measurements were performed at 0.47 T and 40°C. Relaxation times are reported either measured or normalized by the negative control and plotted as a percent change.

## 3 RESULTS AND DISCUSSION

Relaxation time is sensitive to the state of aggregation and the iron concentration of the CLIO nanoparticles. Measurements must be compared to a control value of the same iron concentration in order to attribute  $T_2$  changes to the aggregation state. The addition of hCG to the MRS resulted in aggregation induced  $T_2$  reductions compared to the addition of PBS (Figure 2). The  $T_2$  relaxation time of an equal mixture of C95 and C97 was 110.1 ms in the absence of hCG and dropped to 79.8 ms when hCG was added (8  $\mu\text{g/ml}$  Fe, 13.5 nM hCG, final concentration). Aggregation was hypothesized to be reversible as governed by dissociation constant of the antibody ( $K_d$ ). An excess of hCG at concentrations far above the  $K_d$ , which was 0.5 and 1.0 nM for these antibodies, would cause the aggregates to dissociate as all the antibodies become saturated with hCG and very few hCG form crosslinks (Figure 1). PBS or a concentrated hCG solution were added to the aggregated solution (\*, Figure 2). The addition of PBS to the aggregated solution does not cause dissociation, but the  $T_2$  increases due to dilution of the nanoparticles. The addition of concentrated hCG solution (4  $\mu\text{g/ml}$  Fe, 919 nM, final concentration) causes the aggregates to fall apart, resulting in a higher  $T_2$  than when PBS is added to the aggregated solution. The reversibility of this sensor may be further explored using a semi-permeable membrane system, such as a dialysis cassette, if proper reference values are available. As volumetric fluctuations are often observed in dialysis,  $T_2$  changes due to iron concentration would need to be carefully isolated from that induced by aggregation. Presumably, this system is reversible as governed by antibody-antigen binding kinetics, and in a fixed volume device, the  $T_2$  would reflect the dynamically aggregating

and dissociating CLIO in response to the hCG concentration in the local environment.

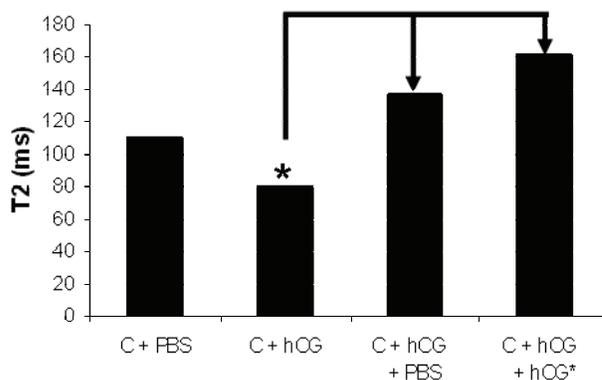


Figure 2: Reversible hCG induced  $T_2$  change

The stoichiometric relationship among the aggregating species (two populations of functionalized CLIO antibodies and the hCG crosslinker) was also explored by varying the relative ratios of antibodies. A scarcity of one of the matched pairs of antibody hinders aggregation formation when the total amount of CLIO was fixed. The valencies of C95 and C97 are not matched, and it was hypothesized that the largest change in  $T_2$  would occur when an equal number of 95 and 97 antibodies were present as shown by:

$$n = V_{C95} * C_{C95} = V_{C97} * C_{C97} \quad (1)$$

where  $n$  is the total number of antibodies,  $V$  is the valency or number of antibodies per particle, and  $C$  is the concentration of particles. The total number of antibodies was calculated to match when the CLIO mixture contains 0.7 C97 per C95 nanoparticle. The relative concentration of C97 to C95 was varied while keeping the total iron concentration fixed at 8  $\mu\text{g/ml}$ . Final analyte concentrations were 5  $\mu\text{g/ml}$  for both hCG and hCG- $\beta$ . The percent  $T_2$  change was found to vary with relative ratios of antibody with the most pronounced change in  $T_2$  found when there was less C97 compared to C95 (Figure 3). This is in agreement with our calculation as C97 has a greater valency than C95.

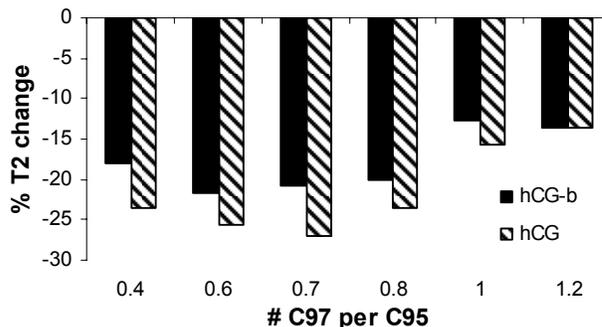


Figure 3: Stoichiometric dependence of aggregation

## 4 CONCLUSION

Detection of hCG and hCG- $\beta$  in solution was performed by measuring the aggregation-dependent  $T_2$  of functionalized CLIO. A stoichiometric dependence of analyte-induced  $T_2$  changes was observed. The presence of hCG caused the CLIO to aggregate as measured by a decrease in  $T_2$ . The aggregates were made to dissociate by introducing a large excess of analyte. The most pronounced change in  $T_2$  was observed when the number of 95 and 97 antibodies was equalized. This particle based sensor can be adapted to detect other soluble cancer biomarkers.

## 5 ACKNOWLEDGEMENT

This project is sponsored by the NCI Centers of Cancer Nanotechnology Excellence No. 5 U54 CA119349-12 grant.

## REFERENCES

- [1] Weissleder R. Molecular imaging in cancer. *Science*, *312*: 1168-1171, 2006.
- [2] Perez JM, O'Loughin T, Simeone FJ, Weissleder R, Josephson L. DNA-based magnetic nanoparticle assembly acts as a magnetic relaxation nanoswitch allowing screening of DNA-cleaving agents. *J Am Chem Soc*, *124*: 2856-2857, 2002.
- [3] Atanasijevic T, Shusteff M, Fam P, Jasanoff A. Calcium-sensitive MRI contrast agents based on superparamagnetic iron oxide nanoparticles and calmodulin. *Proc Natl Acad Sci U S A*, *103*: 14707-14712, 2006.
- [4] Grossmann M, Hoermann R, Gocze PM, Ott M, Berger P, Mann K. Measurement of human chorionic gonadotropin-related immunoreactivity in serum, ascites and tumour cysts of patients with gynaecologic malignancies. *Eur J Clin Invest*, *25*: 867-873, 1995.
- [5] Sedlaczek P, Frydecka I, Gabrys M, Van Dalen A, Einarsson R, Harlozinska A. Comparative Analysis of CA125, Tissue Polypeptide Specific Antigen, and Soluble Interleukin-2 Receptor  $\alpha$  Levels in Sera, Cyst, and Ascitic Fluids from Patients with Ovarian Carcinoma. *Cancer*, *95*, 1886-1893, 2002.
- [6] Kim G, Josephson L, Cima MJ. Magnetic relaxation switch detection of human chorionic gonadotrophin. (In progress).
- [7] Josephson L, Tung CH, Moore A, and Weissleder R. High-efficiency intracellular magnetic labeling with

novel superparamagnetic-Tat peptide conjugates. *Bioconjug Chem*, *10*: 186-191, 1999.