Ultrasensitive DNA detection in biological systems using Magnetic fluorochrome nanoparticles

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Abstract

We hypothesized that a magnetic nanoparticle (NP) surface functionalized with multiple DNA binding fluorochromes might react with DNA through a multivalent, avidity-type reaction and yield a highly sensitive method for detecting DNA by the T2 relaxation time of water protons measured by Magnetic Resonance (MR) techniques. Although oligonucleotides have been used to target NP's to specific sequences on nucleic acids, the considerable literature on fluorochrome/nucleic acid interactions has not been mined for potential nanoparticle (NP) targeting strategies. Our studies showed how a NP displaying a DNA-binding fluorochrome, bind in a multivalent fashion to yield a highly sensitive, T2-based method of detecting DNA. Furthermore, fluorochrome-functionalized NPs are a novel class of nanomaterials which, based on their recognition of DNA in biological systems, can serve as vital fluorochromes.

We synthesized the DNA-binding NPs by attachment of the DNA-binding fluorochrome TO-PRO 1 to the Feraheme (FH) NP using a "TO- PRO1 NHS ester". TO-PRO 1 fluoresces when bound to DNA by intercalation. The resulting NPs, termed FH-TO, had variable TO-PRO 1's per NP attached through a 6-carbon, flexible linker. FH-TO NPs had r_1 and r_2 relaxivities between 23.3 and 122 $(mM\ Fe\ sec)^{-1}$, and a size between 18.2 and 42 nm. The parent FH nanoparticle had a zeta potential of -37.8±3 mV (pH 6) that was largely preserved with the attachment of TO-PRO 1 to the NPs.

A PCR reaction was monitored by MR using FH-TO NPs as intercalant agent. Either light scattering or relaxometry can be used to determine aggregate formation in the low DNA concentration range. However, relaxometry has two advantages: T_2 is a hyperbolic function of DNA concentration (no hook effect) and T_2 is a radiofrequency-based method (no light based interferences). With light scattering and hook effects, some aggregate sizes (e.g. 200 nm) can reflect low or high concentrations and additional measurements with diluted samples are required. The estimated sensitivity of DNA detection by T_2 was 27 fM DNA per a T_2 change of 2.6 msec.[1]

On the other hand fluorochrome-functionalized NPs have a series of properties that make them far different from vital fluorochromes. First, fluorochrome-functionalized NPs are far larger than DNA binding fluorochromes. TO-FHs with different numbers of fluorochromes attached had diameters of 18 to 42 nm, corresponding to proteins with molecular weights in excess of 750 kDa, while vital fluorochromes have molecular weights of less than about 1000 Da. Second, when injected, TO-FH had a blood half-life similar to the parent Feraheme NP, rather than the far more rapid clearance seen with low molecular weight materials. Third, fluorochrome-functionalized NPs have superparamagnetic cores that allowed their reaction with DNA to be determined by relaxometry (or potentially by MRI). Finally, the conjugation of fluorochromes to NP surfaces provides a means of synthesizing DNA binding materials with valencies far above the divalency obtained with fluorochromes like TO-TO. High valency fluorochrome-functionalized NPs exhibit strong multivalent effects when binding DNA.

Attaching multiple DNA binding fluorochromes to magnetic nanoparticles provides a way of generating DNA binding NPs that can be used to detect DNA by microaggregate formation in vitro, for imaging the DNA of necrotic cells in culture, and for imaging the DNA of a tumor treated with a chemotherapeutic agent. Fluorochrome functionalized NPs are a multimodal (magnetic and fluorescent), highly multivalent (n ≈ 10 fluorochromes/NP) nanomaterials useful for imaging the DNA of biological systems.[2]

References

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Figures

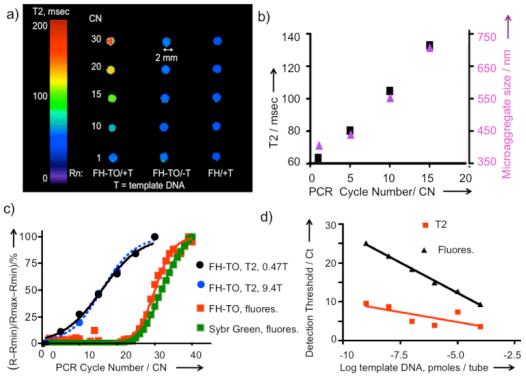

Fig. 1. (a) PCR reactions with FH-TO added were run in sealed PCR tubes and imaged by MR at 9.4T. Omission of template DNA or use of FH yielded no changes in T2. (b) PCR reaction checked by relaxometry and DLS. (c) Comparison of the response by T2 and fluorescence. (d) Comparison of threshold cycle (Ct) by fluorescence and T2.

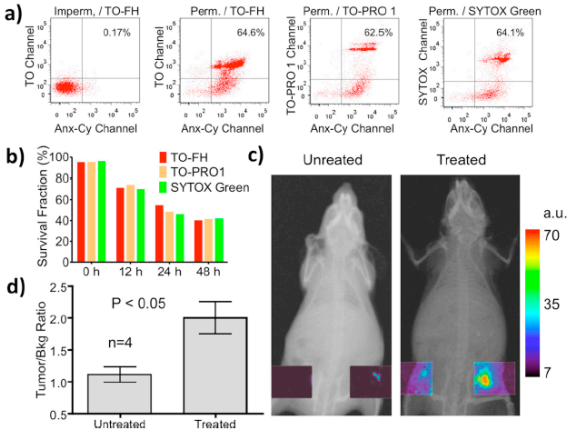

Fig. 2. Interaction of TO-FH with HT-29 cells after permeabilization or after exposure to 5-FU/oxaliplatin treatment to induce cell death. (a) Normal or permeabilized cells were reacted with Anx-Cy and TO-FH or TO-PRO 1 or Sytox Green. (b) Cells were treated with 5-FU/oxaliplatin and exposed to Anx-Cy plus the indicated fluorochrome. Data are plotted as the survival fraction versus time of expose. Survival fraction is the percent of cells failing to bind both Anx-Cy and a second fluorochrome, i.e., the lower left-hand quadrant of the scatter plot. With an increasing duration of treatment, the survival fraction falls. Survival fraction falls similarly with Anx-Cy and any of the three vital fluorochromes, TO-FH, TO-PRO 1, Sytox Green. (c) Tumor surface fluorescence after TO-FH injection with untreated and treated (5-FU/oxaliplatin) HT-29 xenografts. (d) Tumor fluorescence, measured as tumor/bkg fluorescence ($p < 0.05$), $n = 4$.