Self-Assembling DNA Quadruplex Conjugated to MRI Contrast Agents

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Experimental section

Materials and instruments

DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) was purchased from Sigma-Aldrich and used without further purification. The core oligonucleotides sequences were synthesized by W. M. Keck Foundation Biotechnology Resource Laboratory, Yale University using automated solid phase synthesis. The modified phosphoramidite 5'-amino-modifier C6 (6-(4-Monomethoxytritylamino)hexyl-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite), 5'-amino-modifier 5 (2-[2-(4-Monomethoxytrityl) -aminoethoxy]ethyl-(2-cyanoethyl)-N,N-diisopropyl)-phosphoramidite), and trebler phosphoramidite (Tris-2,2,2-[3-(4,4'-dimethoxytrityloxy)propyloxymethyl]ethyl-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite) were obtained from Glen Research. The oligonucleotides were first subjected to gel filtration using Microspin G-25 columns (GE health care), then purified by reverse-phase HPLC (Varian Prostar) equipped with a timberline TL-105 column heater. The concentrations of the purified oligonucleotides were determined based on their absorption at 260 nm using an Agilent A453 UV-vis spectrometer. The molecular weights of the purified oligonucleotides were obtained by MALDI-TOF (Matrix-Assistant Laser Desorption Ionization-Time of Flight) using an Applied Biosystems Voyager-DE PRO workstation. The Formation of DNA quadruplexes was confirmed by circular dichroism (CD) spectroscopy. ICP analysis was done by Bodycote testing group.

Standard preparation of oligonucleotides

To 4 mg of DOTA (12 mg for the preparation of dendrimers **1Y** and **2Y**) in a vial was added 4mg Pybop (12 mg for the preparation of dendrimers **1Y** and **2Y**), 1 ml of anhydrous DMF and 60 µl DIPEA and the mixture was stirred for 1 min. Then the solution was transferred into syringes and attached to the cartridge for solid-phase coupling. The cartridge contained the resin tethered with the activated amino modified oligonucleotide (1.0 µmol). The activated DOTA solution was pushed through the cartridge 3 times and agitated for 1 hr. This procedure was repeated three times and then the cartridge was allowed

to shake overnight. The solution was removed and the cartridge was washed two times with 2 ml DMF and 3 times with 2 ml acetonitrile. The resin was dried by blowing argon through the cartridge for 1 hr, and then the DNA was cleaved from the solid support with 30% NH_4OH for 2h at RT, followed by global deprotection with 30% NH_4OH (3 ml) at 55 ℃ for 16 h. The gadolinium (III) was introduced by adding 15 mol equiv of gadolinium (III) citrate and the mixture was stirred overnight at ambient temperature.

The GOTA tethered oligonucleotides were purified by an HPLC column maintained at 65 ℃. The molecular weights of the de-salted purified oligonucleotides were analyzed by MALDI-TOF using 9:1:1 mixture of 2,4,6-trihydroxyacetopheone (THAP) (10 mg/ml in 50% CH_3CN/water), ammonium citrate (50 mg/ml in water), and oligonucleotide solution, repectively.

Oligonucleotide **1X**. MALDI-TOF Calculated: 4153.0, found: 4150.1

Oligonucleotide **2X**. MALDI-TOF Calculated: 4315.01, found: 4311.4

Oligonucleotide **1Y**. MALDI-TOF Calculated: 5920.9, found: 5921.5

Oligonucleotide **2Y**. MALDI-TOF Calculated: 6088.0, found: 6085.7

Exposure to quadruplex self-assembly conditions

Typically, the purified aliquot of ODNs in an Eppendorf tube was diluted to the desired concentration by using the buffer (10 mM Tris-HCl, 80 mM KCl, pH 7.5). Then the tube was tightly capped and sealed with parafilm, and heated at 95 ℃ for 15 min. The solution was slowly cooled to room temperature and then incubated at 4 ℃ for 48 hr. The formation of quadruplexes was assessed by CD spectroscopy.

The measurements of relaxivity

Molar relaxivity was measured at the field strength of 4.0 Tesla at 20.5 ^{o}C on a Bruker Biospec MRI instrument. Four concentrations were used for both the single strand construct and the quadruplex, including a zero concentration control. A saturation recovery T_1 mapping protocol was used employing spin echo readouts, incorporating ten TRs ranging from 50 msec to 10 seconds. T_1s were calculated for each concentration and inversely plotted in seconds versus concentration in millimolar, the slope yielding molar relaxivity. All concentrations were verified by ICP emission spectroscopy.

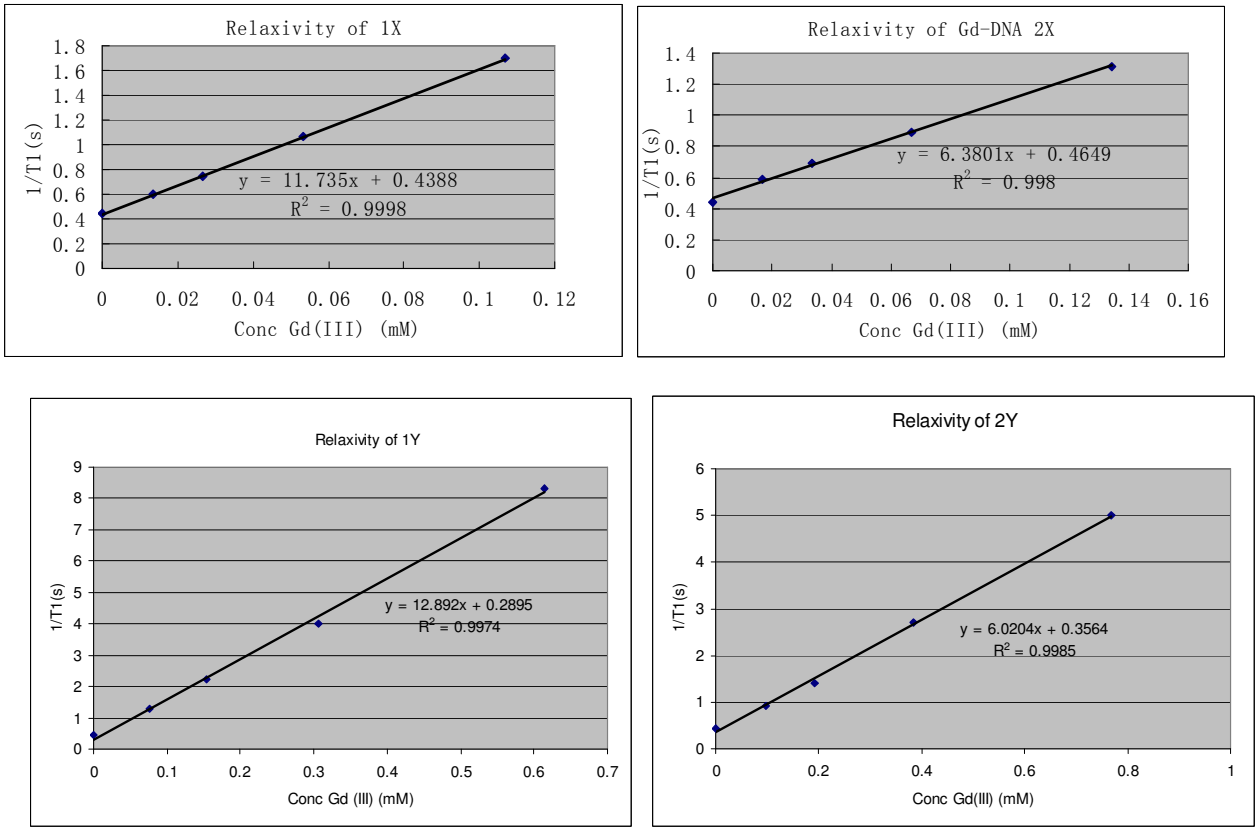

Figure S1. Relaxivity calculations for **1X, 2X, 1Y** and **2Y**.

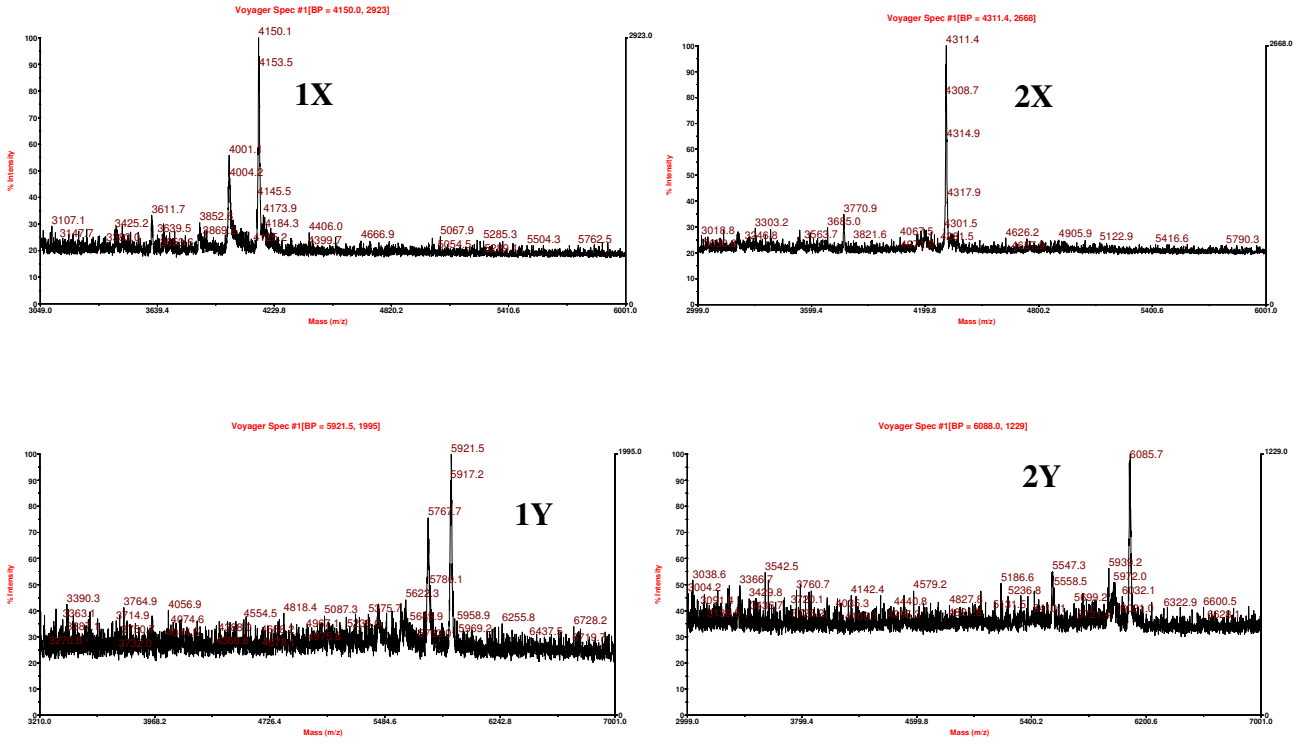

Figure S2. MALDI data of **1X**, **2X, 1Y** and **2Y**.