Electronic Supplementary Material (ESI) for Sensors & Diagnostics.

SUPPLEMENTARY INFORMATION

pH-responsive i-motif-conjugated nanoparticles for MRI analysis

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General Methods

Unless otherwise noted, reagents were obtained from Sigma-Aldrich or Fisher Scientific and used as received. Gadolinium (III) S-2-(4-isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (Gd-p-SCN-Bn-DOTA) was obtained from Macrocyclics. 10 nm Gold Nanoparticles were obtained from Sigma-Aldrich. GelRed® Nucleic Acid Gel Stain was purchased from Biotium. Dry solvents were obtained from Aldrich as anhydrous Sure-Seal bottles. Absorbance spectroscopy was performed on a spectrophotometer (NanoDropTM 2000, ThermoScientific). The final concentrations of gadolinium (Gd) and gold (Au) were verified by inductively coupled plasma-mass spectrometry (ICP-MS XSERIES 2, Thermo Fisher Scientific) at Center of Materials and Sensor Characterization at University of Toledo. Thermo Scientific Orion Micro pH Electrode was used to measure the pH of buffer solutions.

Experimental Procedures

Gel electrophoresis. Polyacrylamide gels were prepared (12% PA, 1× TAE) using Novex™ Bolt mini cassettes (1.0 mm) according to manufacturer specifications and run on an Invitrogen™ XCell SureLock™ Mini-Cell system (Thermo Fisher) at 180 V for 30 min at room temperature. Sample bands were visualized by staining the gels for 15 min in 1× GelRed® DNA staining solution, then imaged using an Axygen Gel Documentation System (Corning).

Dynamic Light Scattering & Zeta Potential Measurements. The hydrodynamic diameters (d) of the iM-GNP and the T_{33}-GNP were characterized using a Zetasizer Nano ZS (Malvern Instruments). The particle size was measured using dynamic light scattering (DLS) where the scattered light was collected at 173°. Each sample was measured 3 times in a ZEN0040 cell at 25 °C. The zeta (ζ) potential was calculated using the Smoluchowski model. Each sample was measured 3 times in 1x PBS buffer (pH 7.4) using the DTS1060 dip-cell at 25 °C.

Serum Sample Preparation & Stability Study (1.4 T NMR). Single-donor whole human serum (HS) was obtained fresh from ZenBio Inc. Aliquots were prepared immediately and frozen at −80 °C. Frozen HS aliquots were thawed on ice directly before use. All HS dilutions were prepared using in 20 mM MOPS/MES/EPPS buffer at pH 4.99 and 8.79 prior to adding in iM-GNPs to reach the final effective HS concentration. Relaxivity measurements were made with 30 nM iM-GNP across 10 timepoints over a 48 hour period. 20% HS were prepared without iM-GNP to serve as our baseline (T_0). 20% HS with iM-GNP were prepared in the acidic and neutral condition and measured on the 1.4 T Bruker Minispec mq60 NMR analyzer at 37°C at the following time points: 0, 2, 4, 6, 8, 10, 24, 30, 34 and 48 h.

Cell Culture & Media Preparation. HEK293T cells (**ATCC** CRL-3216) were cultured in media composed of Dulbecco's Modified Eagle's Medium (DMEM), 10% Fetal Bovine Serum (FBS) and 1% antibiotics (penicillin 100 µg/mL, streptomycin 100 µg/mL. Cells were incubated at 37 °C, 5% CO_2 in T-75 flasks. Flasks that reach 70-90% confluency were detached with StemPro Accutase and passaged into new flasks. **MTT-DMEM.** For the MTT assay, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was added to 1x Phosphate Buffered Saline (pH 7.4) and filter sterilized to generate a stock solution (5 mg/mL). Stock solution was added to DMEM to generate MTT-DMEM (0.2 mg/mL). **Neutral Red Medium.** For the Neutral Red assay, Neutral Red Powder was added to 1X Phosphate Buffered Saline (pH 7.4) and filter sterilized to generate a stock solution (4 mg/mL). Stock solution was added to cell media composed of DMEM, 10% FBS and 1% antibiotics (penicillin 100 µg/mL, streptomycin 100 µg/mL) to generate neutral red medium (50 µg/mL). Medium was incubated overnight at 37 °C before use.

Table S1. Oligonucleotides

Sequences (5’ to 3’) and the functional groups on the terminal ends of the oligonucleotide strands

Strand	Sequence (5’ to 3’) and functional groups
i-motif	NH2-C3-CCC TAA CCC TAA CCC TAA CCC TTT TTT TTT TTT -C3-S-S
control	NH2-C3- TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT -C3-S-S

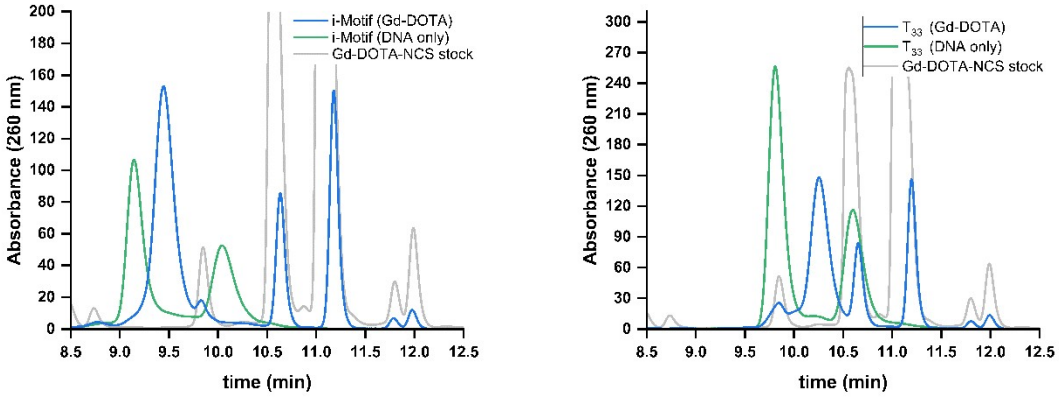

Figure S1. Overlayed chromatograms for the HPLC data from Figure 1 for the analysis of the Gd-DOTA coupling reaction onto the i-motif and T_{33} oligonucleotides. Included in grey is the chromatogram from the analysis of the Gd-DOTA stock solution under the same conditions to demonstrate the residual peaks present in the Gd-DOTA reaction samples.

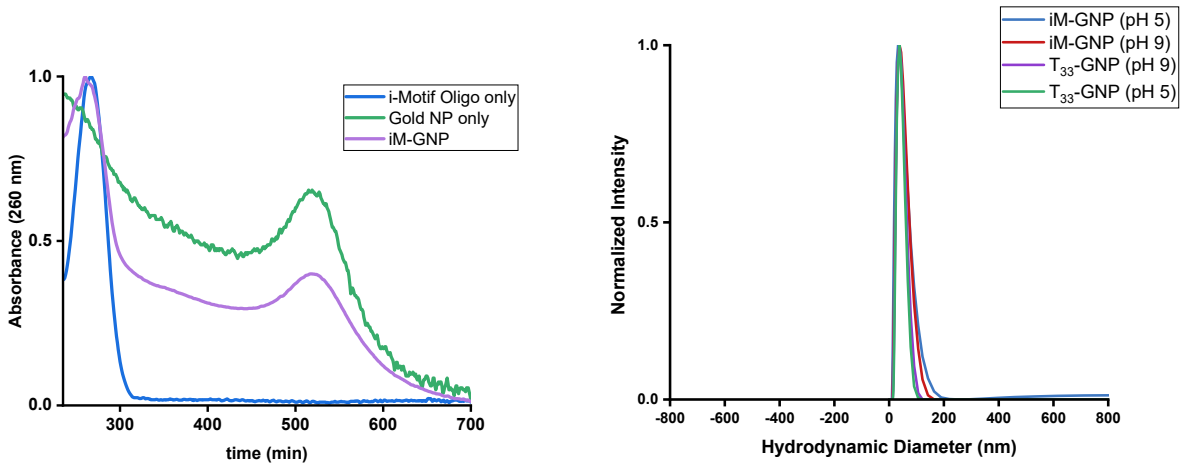

Figure S2. **Absorption spectra of the i-motif DNA oligo, GNP, and iM-GNP and DLS of the iM-GNP & T_{33}-GNP at different pH solutions.** Absorption spectra of the i-motif DNA oligo shows a strong absorption peak at 260 nm which is typical for DNA oligos. The GNP show a maximum absorption peak around 530 nm, which is often seen for GNPs with a diameter of around 10-40 nm. And for the iM-GNP, you can see absorption peaks are 260 nm and 530 nm. DLS analysis shows the average size of both the iM-GNP and the T_{33}-GNP, 35.2 ± 2.5 nm.

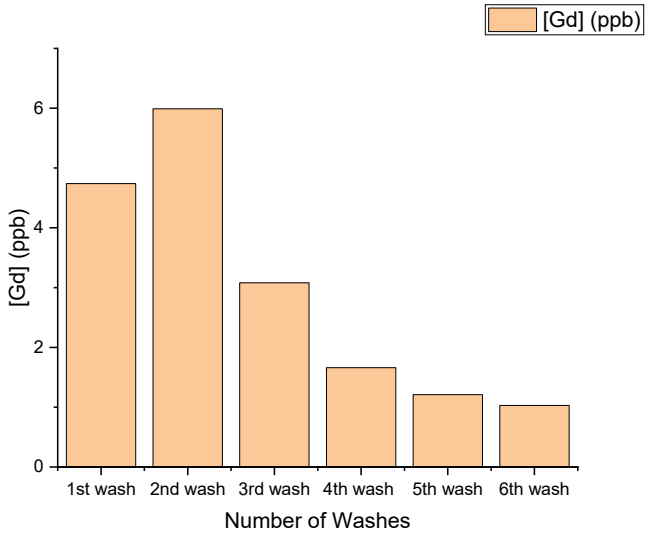

Figure S3. **Gd content analysis in filtrates: iM-GNP solution at various wash steps**. ICP-MS analysis of Gd content (ppb) in the filtrates after spinning down iM-GNP with 100K molecular weight cut-off spin filters to wash off excess i-Motif+Gd-DOTA.

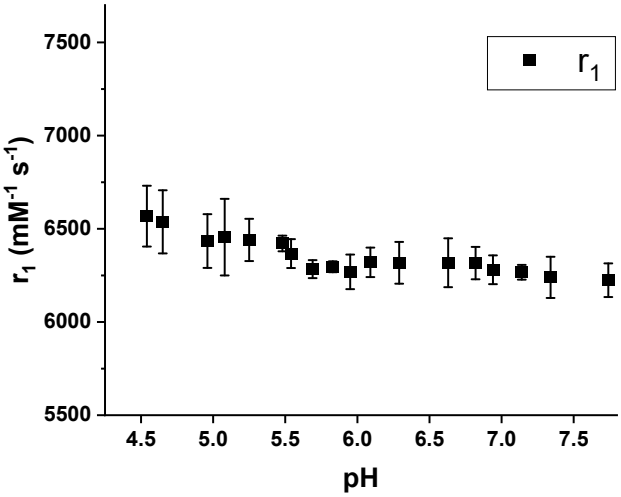

Figure S4. **Relaxivity Curve vs pH measured at 1.4 NMR for control (T33-GNP).** Relaxivity profiles of T_{33}-GNP in MES/HEPPS/HEPES as a function of pH. Relaxivity (r_1) was calculated from GNP concentration and buffer relaxation times, n = 3. Measured at 30 nM T_{33}-GNP at 37 ℃ .

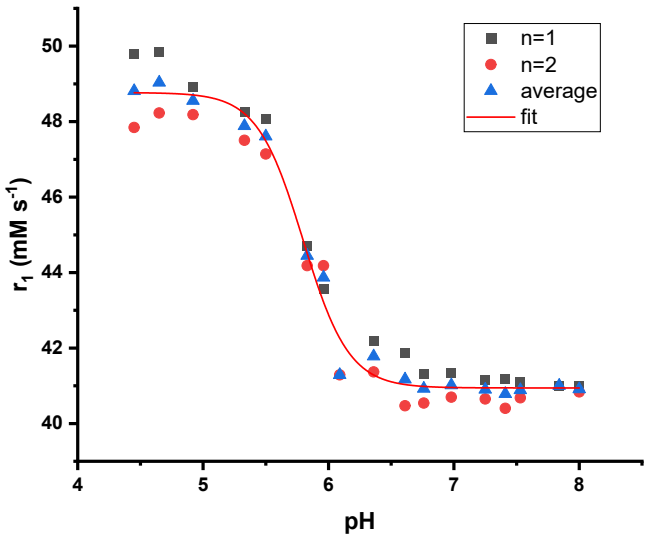

Figure S5. Relaxivity profiles of ssDNA + Gd-DOTA as a function of pH on 1.4 T. Relaxivity profiles of i-motif+spacer+Gd-DOTA (not immobilized on gold nanoparticles) in MES/HEPPS/HEPES as a function of pH. Relaxivity (r_1) was calculated from DNA concentration and buffer relaxation times, n = 2 with a reported pKa value of 5.79 ± 0.03. Measured at 5 µM ssDNA with Gd-DOTA at 37 ℃ .

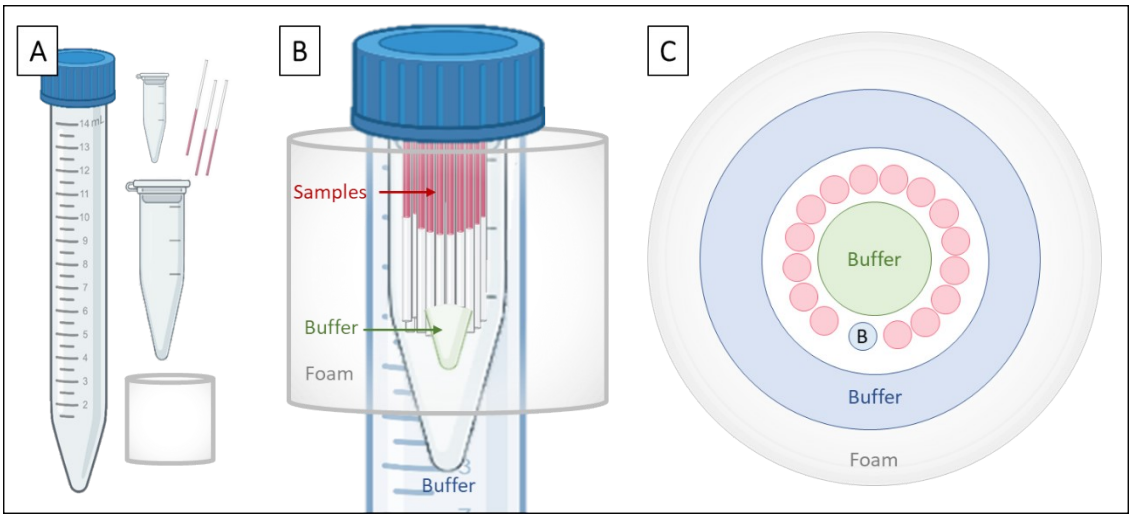

Figure S6. In vitro test bed[2] with capillaries to hold contrast agent samples (microliter scale) in a concentric orientation. Inner and outer rings designated to hold buffer solutions. A) Materials, B) Coronal orientation, C), Axial orientation.

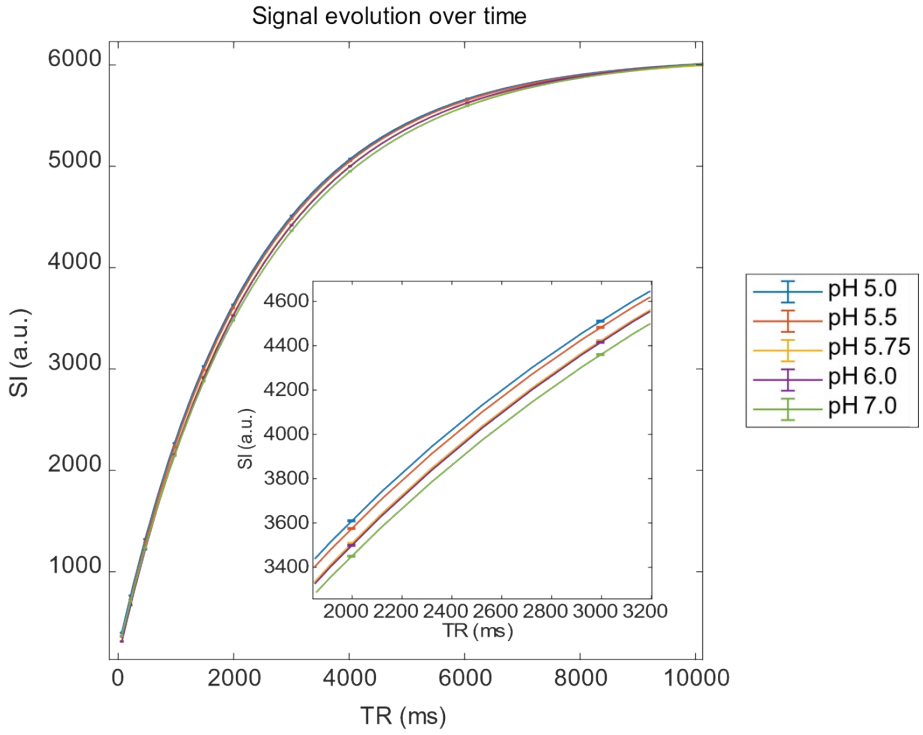

Figure S7. Representative raw 3.0 T MRI signal intensity (SI) over repetition time (TR) measurements used to calculate iM-GNP T_1 values under different pH conditions, acquired at 25 °C. The lines represent the mean values for each pH, the error bars represent the standard deviation. A zoomed-in view is included for the region around the T_1 range.

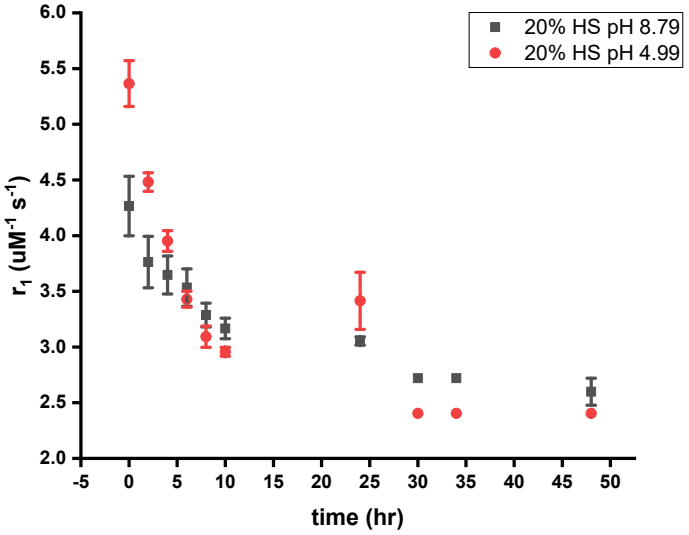

Figure S8. Relaxivity (r_1 $\mu M^{-1}s^{-1}$) profiles of iM-GNP in 20% HS over 48 hours measured by 1.4 T Bruker Minispec mq60 NMR. 30 nM of iM-GNP suspended in 20% human serum and MOPS/MES/EPPS buffer at pH 4.99 and pH 8.79. Graph displays change in r_1 (n=2).

References:

1. Elisé P. Wright, Julian L. Huppert, Zoë A. E. Waller, Identification of multiple genomic DNA sequences which form i-motif structures at neutral pH, Nucleic Acids Research, Volume 45, Issue 6, 7 April 2017, Pages 2951–2959, https://doi.org/10.1093/nar/gkx090

2. Zanchet, D.; Micheel, C. M.; Parak, W. J.; Gerion, D.; Alivisatos, A. P., Electrophoretic Isolation of Discrete Au Nanocrystal/DNA Conjugates. *Nano Letters* **2001,** *1* (1), 32-35.

3. Perera-Gonzalez, M.; Ma, K; Flask, C; Clark, H; In Vitro Testbed Platform for Evaluating Small Volume Contrast Agents Via Magnetic Resonance Imaging, 2022 DOI: 10.1109/DSD57027.2022.00082