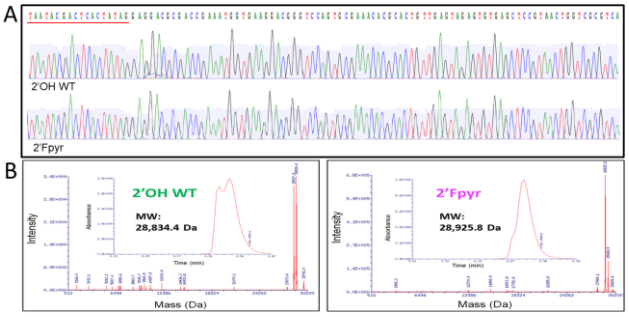
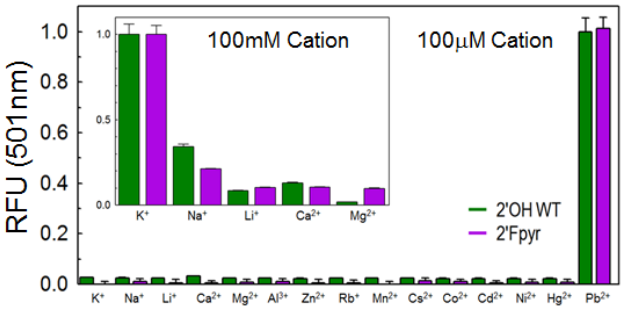
Electronic Supplementary Material (ESI) for Chemical Communications.

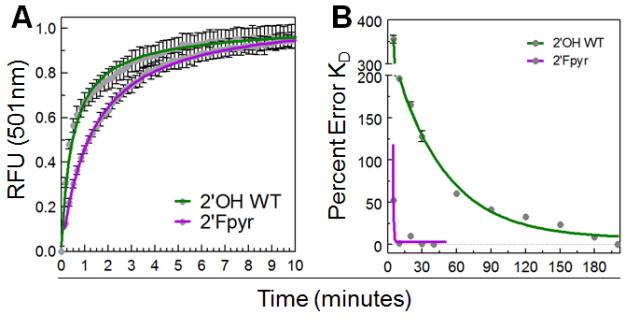
Supplemental Material



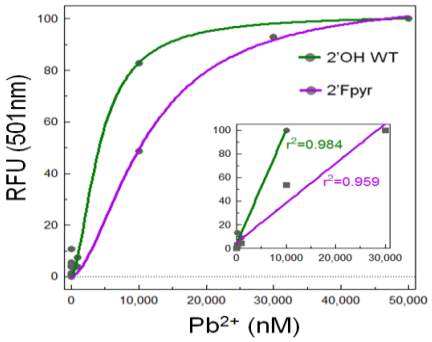
Supplemental S1: A) Sequence Identity: cDNA of Spinach constructs were sequenced by Sanger sequencing validating integrity of the transcripts generated by T7 RNA polymerase variants and modified nucleotides (T7 promoter sequenced is underlined in red). **B) LC-MS:** Spinach Constructs analysed by Liquid Chromatography—Mass Spectrometry to validate sample identity. 2'OH WT sample composed of two species differing by a single 5' terminal guanosine, likely due to T7 RNA polymerase slipping. Mass shift between 2'OH WT and 2'Fpyr constructs corresponds to fluorination of pyrimidine residues.



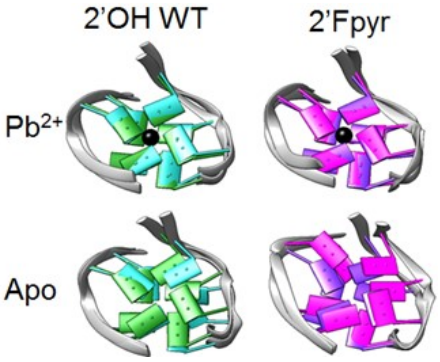
Supplemental S2: Cation Selectivity: Spinach constructs were folded in the presence of DFHBI and various cations. Both constructs show similar fluorescent yield with each cation, showing the strongest preference for Pb^{2+} in the micromolar range. *Inset*: physiologically relevant cations were also investigated in 100mM concentrations. Both constructs show similar preferences. All cation solutions prepared from Cl^- salts for consistent background.



Supplemental S3: A) Binding Kinetics of DFHBI: Fluorescent gain after 20μM DFHBI mixing with folded 0.5μM RNA pre-incubated with Pb^{2+}. Colored lines are monoexponential fits. **B) Percent error of fitted K_D vs. Time.** K_D values determined (K_D^{Thr}) at specified time windows 5, 10, 20, 30, 40 (2'Fpyr and 2'OH WT), 60, 90 120, 150, 180, 200 min (2'OH WT only). Deviation from calculated K_D (K_D^{Calc}; calculated at saturation for 2'Fpyr and 2'OH WT, 40 and 200min, respectively) at each time point is presented as percent error of K_D^{Calc}, and is calculated as ($|K_D^{Thr} - K_D^{Calc}|/K_D^{Calc}$)*100. Lines are monoexponential fits.



Supplemental S4: Detection Limit: Pb^{2+} was detectable by fluorescence down to 10nM by both Spinach constructs. *Inset*: Linear regression of Pb^{2+} detection by both constructs for Pb^{2+} concentrations between 50nM and 30μM.



Supplemental S5: Structural Comparison of G-quadruplex at 0 and 10ns: Images correspond to 2'OH WT and 2'Fpyr both in coordination with Pb^{2+} and in Apo states. Green and purple correspond to 0ns, and cyan and magenta correspond to 10ns.

Methods

***In vitro* Transcription:** RNA transcripts were prepared using linear dsDNA PCR templates, using the forward strand sequence (T7 promoter is underlined): 5'—GCGCGCGAATTCTAATACGACTCACTATAGGAGGACGCGACCGAAATGGTGAAGGACGGGTCCAGTGCGAAACACGCACTGTTGAGTAGAGTGTGAGCTCCGTAACTGGTCGCGTC—3'. Reactions assembled at room temperature, in the following order, to final concentrations of: water, 1X T7 RNAP transcription buffer (NEB), 2.5-5% DMSO, 24mM $MgCl_2$, 4mM each rNTP (2'OH purines and 2'OH pyrmidines from NEB, 2'F pyrimidines from Trilink Biotechnologies), 15-25ng/μl dsDNA, 5mM DTT, 1U/μl murine RNase inhibitor (NEB), 2.5mU/μl yeast inorganic pyrophosphatase

(NEB), 5U/μl WT T7 RNA polymerase (NEB) or 1.25U/μl mutant T7 R&D polymerase (Lucigen, Inc). RNA was transcribed at 42°C for ≥4 hours, and observed on a 4% agarose TBE gel. Target transcript band was purified by gel excision and electro-elution in dialysis tubing, and dialyzed against ≥5,000X volume of 10mM Tris (pH 8.0) for ≥2 hours at room temp. RNA was cleaned with three successive mixes with *acidified* phenol:chloroform:isoamyl alcohol (Ambion), then precipitated in 0.3M sodium acetate (pH 5.2) with either ≥50% isopropanol or ≥75% ethanol at -20°C for ≥2 hours. RNA was pelleted by centrifugation (16.1k *x g* for ≥30 minutes at 4°C), washed with ice-cold 70% ethanol and centrifuged again (16.1k *x g* for ≥10 minutes at 4°C). RNA pellets dried at 37°C and suspended in RNase free 20mM Tris (pH 8.0), quantified by UV-absorbance at 260nm or fluorescence (Qubit, Invitrogen), and stored at -80°C. Based on current reagent prices and our average recovery efficiency at a low scale of transcription, we calculate 2'Fpyr modified RNA to be ~2-4 fold more expensive per unit mass than 2'OH unmodified. Unless otherwise stated, all RNA experimentation was performed in a standard base buffer solution of 20mM Tris (pH 8.0), 5mM $MgCl_2$, 20μM DFHBI. Prior to experimental use of frozen samples, RNA was folded following a basic protocol: Dilution in buffer then successive thermocycler incubations at 85°C (20sec), 50°C (1min), addition of DFHBI, 37°C (1min), 25°C (≥30min).

RT.PCR: RNA constructs were reverse transcribed and amplified using NEB OneTaq OneStep RT.PCR kit following manufacturer protocol. PCR cycling conditions as follows: 48°C (30min), 95°C (1min), 25cyles at 95°C (20sec) 63°C (30sec) 68°C (30sec), 68°C (10min), 4°C. RT.PCR amplicons were gel purified as described above and ethanol precipitated, before ligation into a TOPO.pca2.1 TA sequencing vector (Invitrogen) and used for transformation of DH5α E. coli. Sanger sequencing was performed commercially by Eurofins Scientific.

Liquid Chromatography—Mass Spectrometry: LC-MS experimentation and analysis performed commercially by Novatia, LCC, using a Dionex UltiMate HPLC and Thermo Finnigan LTQ mass spectrometer.

Circular Dichroism Spectroscopy: Measurements were performed on an Aviv 202 CD Spectrometer at 20°C. RNA samples were folded and prepared similarly to fluorescence based experiments. 5μM RNA samples were scanned in 1mm quartz CD cuvette from 320 to 185nm by 0.25nm steps, with a 2 second integrated read per step. Data presented as average of three individual scans. Control scans of DFHBI in buffer subtracted from RNA spectra. Values plotted in GraphPad Prism 5.

Chemical Stability Measurements: *RNase-dependent:* 0.5μM RNA was folded as described in standard spinach buffer without lead. RNA was incubated with purified RNase A (Qiagen) at a final concentration of 1.25mU/μl at 25°C. 10μl samples were removed from pooled incubation at designated time points quenched with 10mM EDTA, and 2X DNA loading dye (Thermo Scientific). Samples were run on a 3% agarose TBE gel, stained with Sybr Gold nucleic acid dye. Band intensities were quantified by densitometry with Image Lab software and plotted in GraphPad Prism 5. *Pb^{2+}-dependent:* 0.5μM RNA was folded as described in standard Spinach buffer solutions, and incubated with or without 500μM Pb^{2+} for 4 hours at 37°C. 10μl samples were removed from pooled incubation at designated time points, quenched with 10mM EDTA, and frozen. Samples were run on a 3% agarose TBE gel, stained with Sybr Gold nucleic acid dye. Band intensities were quantified by densitometry with Image Lab software, and plotted in Graph Pad 5.

Thermal Stability Measurements: Thermal melting was observed using an ABI StepOne Real Time PCR Thermocycler. 0.5μM RNA was folded as described in 20mM Tris (pH 8.0), 5mM $MgCl_2$, 50μM $PbCl_2$, 20μM DFHBI, 1% DMSO, and loaded in 20μl volumes in triplicate wells in an ABI brand 96 well QPCR plate. Fluorescence was measured in the Sybr Green Ex/Em channel from 4°C to 70°C at a 2% gradient. Control scans of DFHBI in buffer subtracted from RNA spectra. Fluorescent values averaged and plotted in GraphPad Prism 5.

Fluorescence Measurements: All fluorescent measurements were performed on a Photon Technologies International QM-1 steady state fluorescent spectrophotometer. Samples were prepared at stated concentrations and excited at 468nm, at 20°C held constant by a circulating water bath, in a 2mm quartz fluorescence cuvette. Kinetic experimentation was performed by rapid, in-cuvette mixing of folded Apo-state RNA in buffer and 10% vol. of 10X $PbCl_2$ in water. Emission at 501nm was read every 29.5 seconds with a 0.5 sec shutter exposure. K_D^{Calc} values were determined using k_{on} and k_{off} determined under steady state fluorescence conditions, at 200min and 40min for 2'OH WT and 2'Fpyr, respectively. K_D^{Thr} values were determined by excluding data beyond stated time points, and refitting to generate new theoretical k_{obs} at those time points. Control scans of DFHBI in buffer subtracted from RNA spectra. Fluorescence data was plotted and fit in GraphPad Prism 5.

Modelling and Molecular Dynamic Simulations: Spinach aptamer (PDB 4kzd) without co-crystalized Fab was used as the template for modelling and MD simulations. 2'Fpyr construct was modelled by direct chemical replacement of the C2 hydroxyl group with fluorine on every pyrimidine ribose using YASARA *Structure* modelling software. A revised AMBER ff14 RNA force field was used for energy minimizations, using an explicit solvent TIP3P water model. Nucleotide bases were frozen and the backbone was subject to an energy minimization, after which the backbone was frozen and the bases were minimized. The structure was then subject to a final, full-structure minimization with no constraints. Potassium was replaced with a lead atom, in both constructs, and full-structure energy minimizations were performed with no constraints. MD simulations were run for 10ns, with snapshots taken every 100ps, using the YASARA md_run macro with explicit solvent conditions at 298K with a physiological intracellular NaCl concentration. Hydrogen bonding atoms present in the bases of the starting aptamer structure were identified and the distances between them were catalogued for every snapshot. A distance over 2.5Å was defined as a broken hydrogen bond. YASARA hydrogen bond interpolation was then used to count the number of hydrogen bonds present within the aptamer over time, within the G-Quadruplex, duplex sections, and the transition between the duplex and the G-Quadruplex core. These values were obtained for every snapshot. Hydrogen bond values were plotted in Sigma Plot, and structural images presented with Chimera.