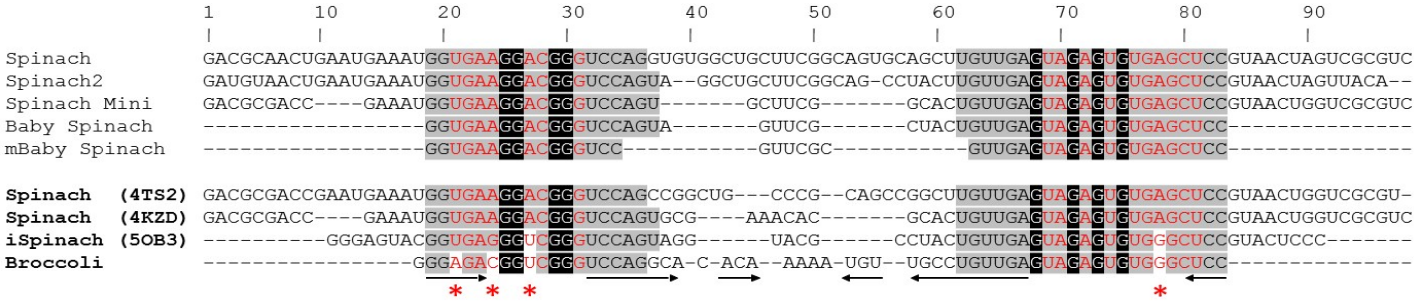
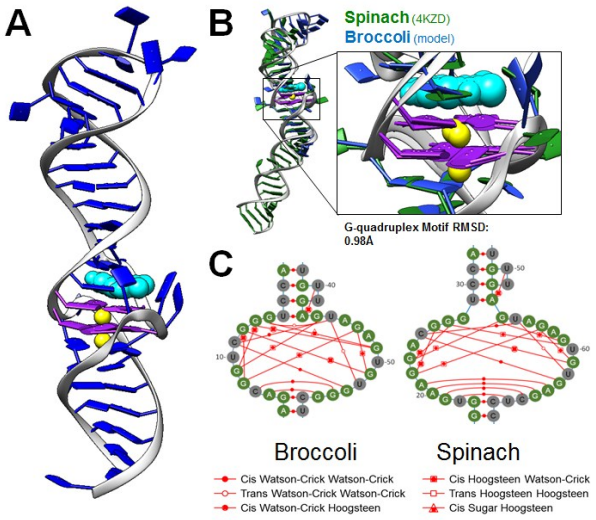
Supplemental Material

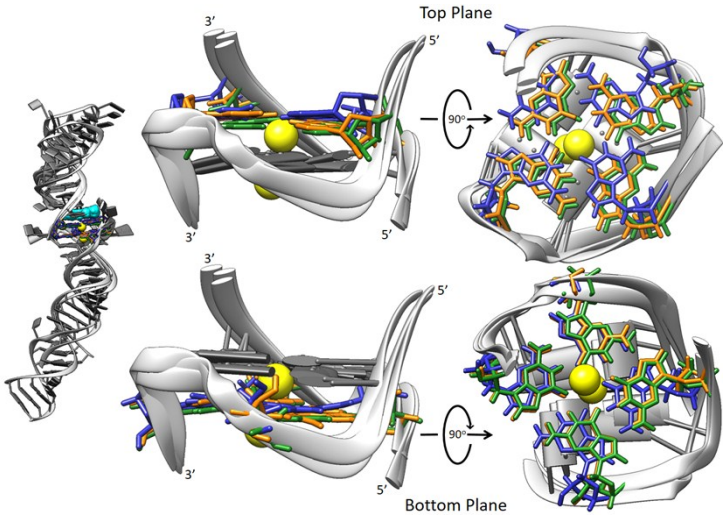
Figures



Supplemental 1: Full sequence alignment: Comparison of Spinach derivative family members and Broccoli. Color coding: Red letters/grey background are G-Quad core motif residues; White letters/Black background are G-Quad guanine residues; Black Lettering/Grey background are conserved non-G-Quad residues; arrows indicated complementary regions that point towards the hairpin. Bottom and bold sequences correspond to crystalized sequences (or in the case of Broccoli, modelled here).



Supplemental 2: (A) Broccoli model: Cartoon representation of the homology model of Broccoli; DFHBI, G-Quad guanine bases, and K^+ ions are colored cyan, cyan, and yellow, respectively **(B) Spinach v Broccoli comparison:** The crystal Structure of Spinach (4KZD) and model Broccoli are aligned. The two show significant structural similarity, as would be expected based on the similarity between Spinach and iSpinach. G-Quad bases are purple, DFHBIs are cyan, and K^+ ions are yellow. **(C) Residue interaction map:** The G-Quad core residues are presented in a 2D flattened projection, with corresponding nucleotide interactions.



Supplemental 3: G-Quadruplex Structural Alignment: Comparison of G-quadruplex planes of Spinach (4KZD), iSpinach (5OB3), and Broccoli model, colored green, orange, and blue, respectively. DFHBI colored in cyan, and K^+ ions in yellow. Top and bottom planes are individually colored against a grey neutral background for visual clarity.

Methods

In vitro Transcription and purification: RNA transcripts were prepared using linear dsDNA PCR templates. Sequences of the forward DNA strand are as follows (T7 promoter is underlined, construct in bold font): Spinach 5′—GCGCGCGAATTCTAATACGACTCACTATA**GGAGGACGCGACCGAAATGGTGAAGGACGGGTCCAGTGCGAAACACGCACTGTTGAGTAGAGTGTGAGCTCCGTAACTGGTCGCGTC**—3′; and Broccoli 5′—GCGCGCGAATTCTAATACGACTCACTATA**GGAGACGGTCGGGTCCAGGCACACAAAAATGTTGCCTGTTGAGTAGAGTGTGGGCTCC**—3′. Prior to transcription, PCR templates were gel purified, phenol:chloroform cleaned, and desalted, similar to RNA clean up (described below). Transcription reactions were assembled at room temperature in the following order, to final concentrations of: water, 1X T7 RNAP transcription buffer (NEB), 24mM $MgCl_2$, 4mM

each rNTP (NEB), 15-25ng/μl dsDNA, 5mM DTT, 1U/μl murine RNase inhibitor (NEB), 2.5mU/μl Yeast Inorganic Pyrophosphatase (NEB), 5U/μl T7 RNA polymerase (NEB). RNA was transcribed at 42°C for 4-10 hours. Transcriptions were quenched with DNase I treatment, and observed on a 4% agarose sodium borate gel, stained with Sybr Gold nucleic acid dye. Target transcript bands were purified by gel excision and electro-eluted into 3.5kDa MWCO dialysis tubing. RNA was concentrated with a 3.5KDa MWCO concentrating spin column (Millipore) and cleaned with acidified phenol:chloroform:isoamyl alcohol (Ambion). RNA was then desalted into 5mM Tris (pH 8.0) using a Zeba 7kDa MWCO desalting spin column (Thermo), and quantified by UV-absorbance at 260nm and fluorescence (Qubit HS RNA kit; Invitrogen), and stored at -80°C. Prior to experimental use of frozen samples, RNA was folded following a basic protocol: Dilution into desired buffer, then successive thermocycler incubations at 85°C (20sec), 50°C (10sec), 37°C (10sec), addition of DFHBI, 4°C (≥30min).

Fluorescence: All fluorescent measurements were performed on a Photon Technologies International QM-1 steady state fluorescent spectrophotometer. All samples were read under the following solution conditions unless otherwise stated: 0.5μM RNA, 20mM Tris (pH 8.0), 100mM KCl, 5mM MgCl2, 10μM DFHBI. Samples were 20°C held constant by a circulating water bath, excited at 468nm in a 2mm quartz fluorescence cuvette. Kinetic experimentation was performed by rapid, in-cuvette mixing of folded Apo-state RNA in buffer and a 10% volume of 1M KCl in water. Emission was then read at 501nm every 29.75 seconds with a 0.25 second shutter exposure. KCl titrations were performed by folding RNA in the presence of various KCl concentrations in individual reaction tubes. Samples were then incubated on ice for 2hr to ensure equilibrium was reached. Fluorescent of each sample was individually scanned (468/501nm). Fluorescence data was plotted and fit in GraphPad Prism 5.

Circular Dichroism: Measurements were collected on an Aviv 202 CD Spectrometer at 20°C. RNA samples were assayed in 10mM Cacodylate (pH 7.4), 100mM KCl, 5mM $MgCl_2$. RNA was folded as described above. 135ng/μl RNA samples were scanned in 1mm quartz CD cuvette from 320 to 190nm by a 0.2nm step, with a 3 second integrated read per step. Data presented as average of three individual scans. Control scans of buffer were subtracted from spectra. Values plotted in GraphPad Prism 5.

Thermal Stability: 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 $MgCl_2$, 100mM KCl, 20μM DFHBI. 20μl sample volumes were scanned in triplicate wells in an ABI brand 96 well white opaque 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 were subtracted from RNA spectra. Fluorescent values averaged and plotted in GraphPad Prism 5.

Homology modelling: Initial homology models for Broccoli were produced using automated 3D RNA structural prediction servers, RNA Composer[1], ModeRNA[2], and SimRNA[3]. While these servers provided 3D predictions consistent with their structural templates, a complete and fully automated predictive model for Broccoli was only partially generated. Hence, a local multiple sequence alignment (MSA) of the target RNA, Broccoli, with derivatives of Spinach, and multiple structural templates 4TS2[5], 4KZD[6], and 5OB3[8] was obtained using ClustalW, and edited to ensure optimal base complementarity indicated by arrows pointing towards the hairpin (Fig.2A). The G-Quad motif for Broccoli was generated from 5OB3 based on the MSA that established this region of the target RNA is almost identical within all available structural templates. An extensive fragment library search identified a loop of 16-nucleotides from a zinc finger RNA (1UN6, residues 13-28) as an optimal fragment to bridge the G-quad motif in Broccoli from residues 20-35[4]. The model was energy minimized using a modified Amber14ff RNA force field[16] in YASARA modelling suite, and the quality of the model was assessed using RNAssess[5], a computational server that discriminates between potentially correct and incorrect conformations by comparing RNA 3D models with the reference structures.

MD simulation: Generated Broccoli model and Spinach (4KZD) without co-crystalized Fab were used in MD simulations. A revised AMBER ff14 RNA force field[19] was used for energy minimizations, using an explicit solvent TIP3P water model. MD simulations were run for 50ns, 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. Hydrogen bond values were plotted in Sigma Plot, and structural images presented with Chimera.

References:

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4 D. Lu, M. A. Searles and A. Klug, *Nature*, 2003, **426**, 96–100.

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