Supporting Information: An in solution assay for parallel interrogation of structure and affinity of small molecule-binding aptamers

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CONTENTS:

(1) Variable temperature UV-Visible experimental melting temperature determination for FB_1 39 *minimers*

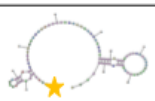
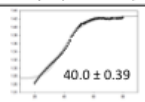
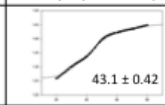
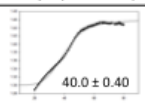
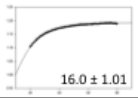
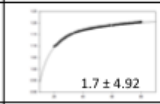
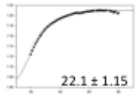
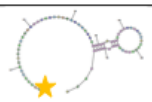
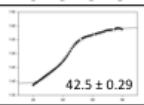
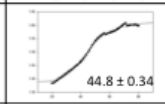
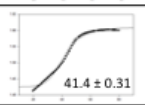
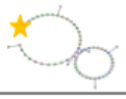
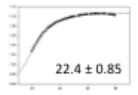
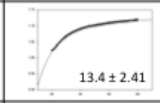
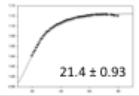
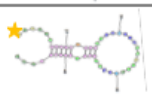
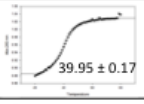
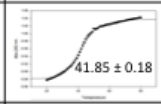
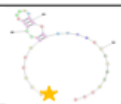
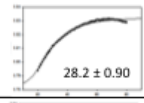
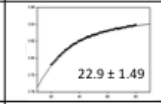
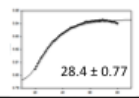
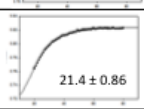
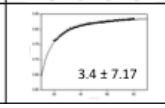
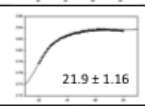
FB1 aptamer	RNA structure fold	Melting studies			
		Ramp 1 (80°C - 20°C)	Ramp 2 (20°C - 80°C)	Ramp 3 (80°C - 20°C)	Average Tm (°C)
FB1_39		 40.0 ± 0.39	 43.1 ± 0.42	 40.0 ± 0.40	41.0 ± 0.4
FB1_39t3		 16.0 ± 1.01	 1.7 ± 4.92	 22.1 ± 1.15	13.3 ± 2.4
FB1_39t5		 42.5 ± 0.29	 44.8 ± 0.34	 41.4 ± 0.31	42.9 ± 0.1
FB1_39t3-5		 22.4 ± 0.85	 13.4 ± 2.41	 21.4 ± 0.93	19.1 ± 1.1
FB1_39m3		 39.95 ± 0.17	 41.85 ± 0.18	N.D.	40.9 ± 0.2
FB1_39m5		 28.2 ± 0.90	 22.9 ± 1.49	 28.4 ± 0.77	27.7 ± 4.6
FB1_39cm		 21.4 ± 0.86	 3.4 ± 7.17	 21.9 ± 1.16	19.0 ± 6.6

Figure S1. Sequence, RNA Structure fold[1] and UV-Vis thermal denaturation melting studies (T_m) for *FB_1 39* and minimers (*FB1_39t3*, *FB1_39t5*, *FB1_39t3-5*, *FB1_39m3, FB1_39m5* and *FB1_39cm*). T_m studies were monitored at 260 nm with three temperature ramps at 0.5°C / min. R1 80°C - 20°C; R2 20°C - 80°C; R3 80°C - 20°C with a 5 min hold between each ramp.

(2) Proof of concept study: correlation of DNase I digestion patterns to secondary structure predictions of DNA

A proof-of-concept study was designed to examine the relationship between secondary structure and DNase I digestion fragment patterns. Three sequences with rationally designed hairpins were synthesized. The three sequences have varying patterns of structural complexity: 2-way junction, a 3-way junction, and a long hairpin bulge (Supplemental Figure 1). The secondary structure of the sequences were analyzed by RNA Structure to visualize the predicted motifs (Figure 1).[1] All sequences are labeled with 5'-fluorescein. After digestion by DNase I, the 5'-fluorescein labeled fragments were separated by denaturing PAGE . As expected, each band (or cluster of bands) corresponds with a site of digestion cleavage at a region of stable secondary structure within the sequence. For the 2-way junction, four bands are present that represent digestion at each of the hairpins (1 and 2; 3 and 4), with 5'-labeled fragments originating from digestion cleaving at each side of the hairpins. The full-length sequence (4) could have a small amount of digestion

from the 3' end. The 3-way junction shows three regions of digestion (2, 3, 4) as well as the full-length sequence (5), and very short 5' fragments (1). The long bulge hairpin shows multiple digestion bands as much of the structure is comprised of duplex DNA; bands 3, 4, and 5 represent digestion from the 3' end hairpins; bands 1 and 2 represent regions of digestion from the structure at the 5' end. These results support the concept that predominant DNase I digestion occurs at sites of stable duplex DNA. This supports the selective digestion at duplex DNA.

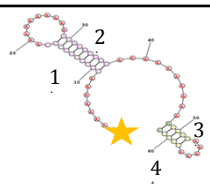
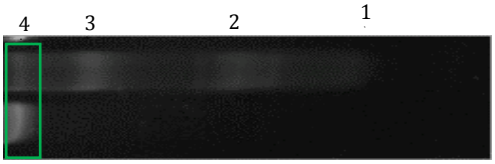
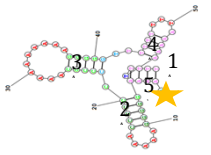
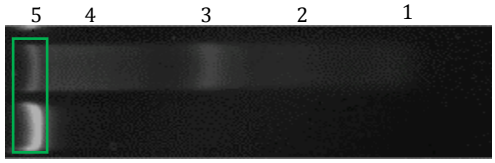
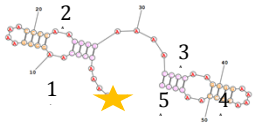
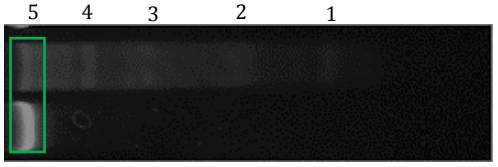
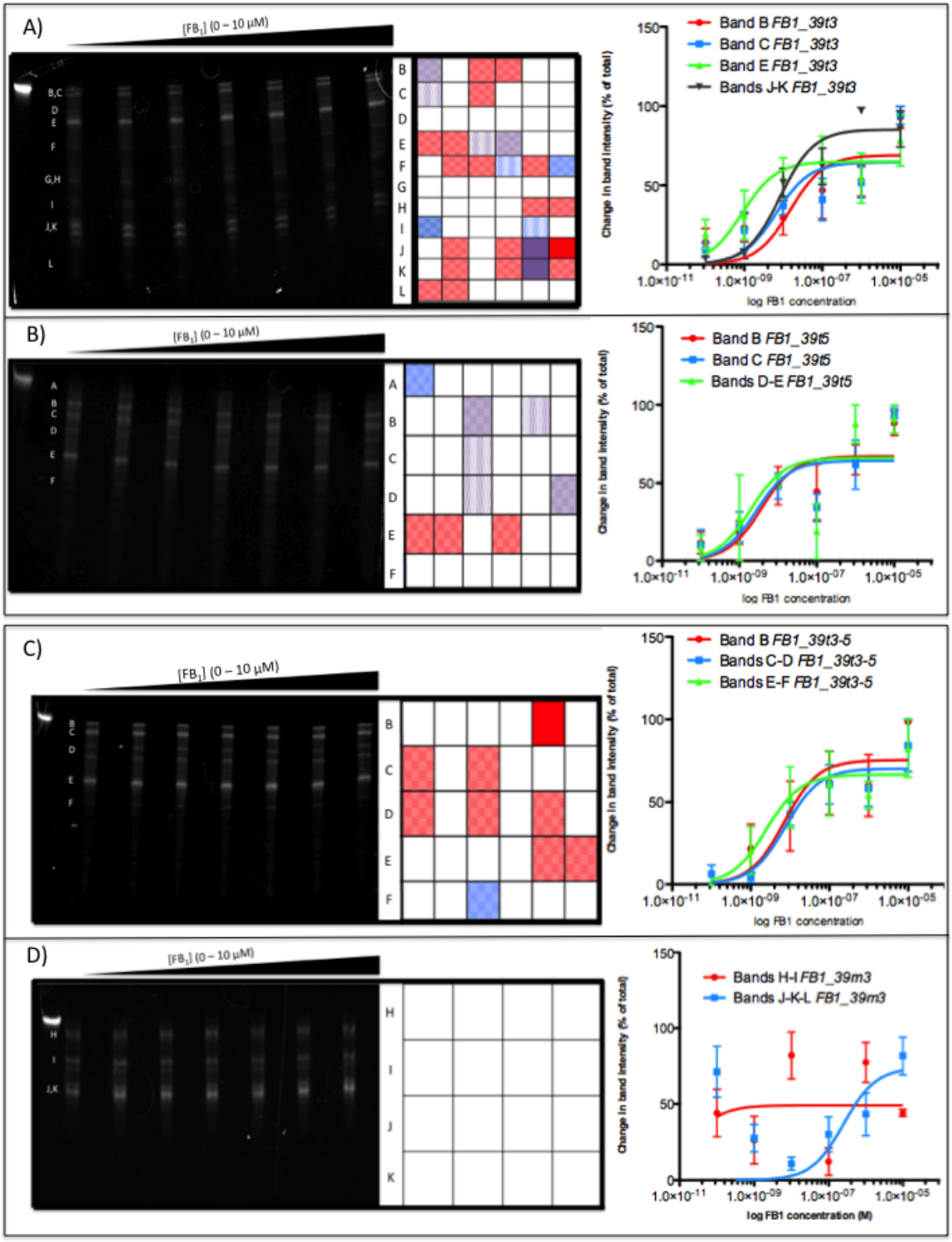
Structure	DNA sequence (5' – 3')	RNA Structure	DNase I digestion on denaturing PAGE
2-way junction	FAAAAAAAACCCCCCCCCAAAA AAAAAAGGGGGGGGGAAAAAA AAAAGGGGGAAAAACCCCC		
3-way junction	FAAAAAGGGGGAAAAACCCCC CCCCCCAAAAAAAAAAGGG GGTTTTTTTTTAAAAATTTTT		
Long bulge hairpin	FAAAACCCCAAGGGGAAAAG GGGAACCCCAAAACCCCAAGGG GAAAAGGGGAACCCCAAAA		

Figure S2. Sequences designed with known secondary structure, shown with predicted structure (RNA structure), and separated fragments produced by DNase I digestion for 1 min at 37°C of 5'-fluorescein labeled aptamer separated by 19% denaturing PAGE. DNase I digestion regions (1 – 5) are assigned to putative digestion sites within the aptamer sequences based on preferential endonuclease activity of DNase I on duplex DNA. The full-length sequences are indicated by a green box outline.

(3) DNase I assay and analysis of FB_1 39 *minimers to determine aptamer affinity*



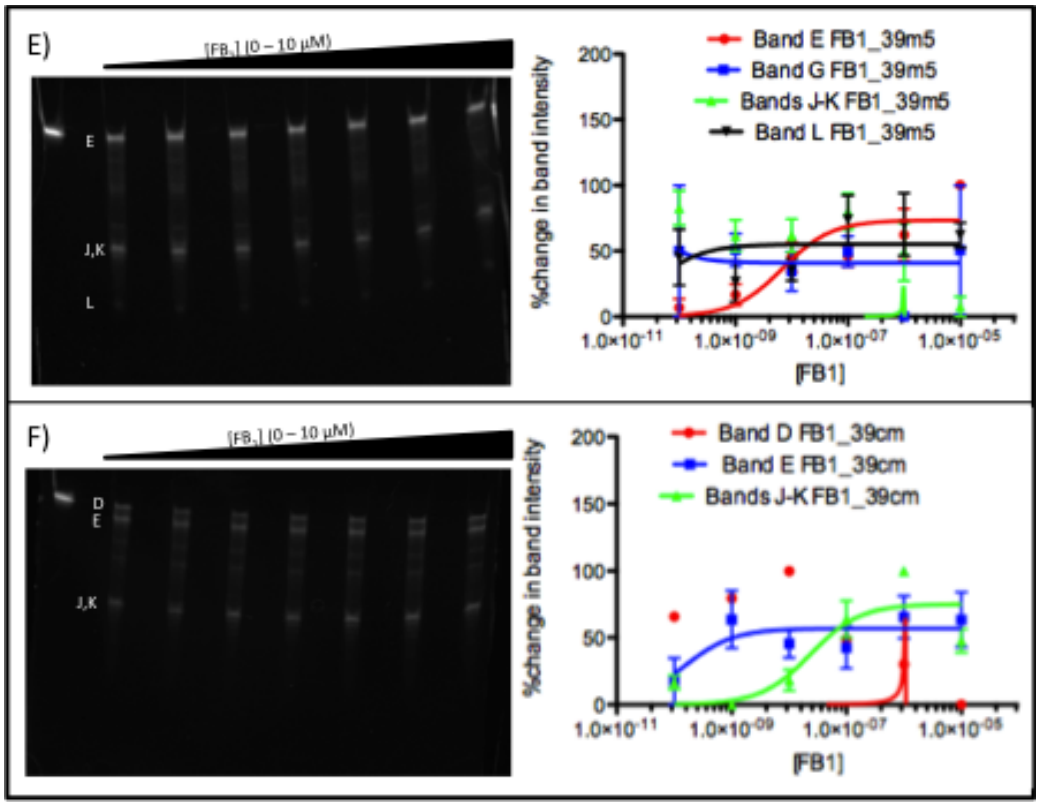
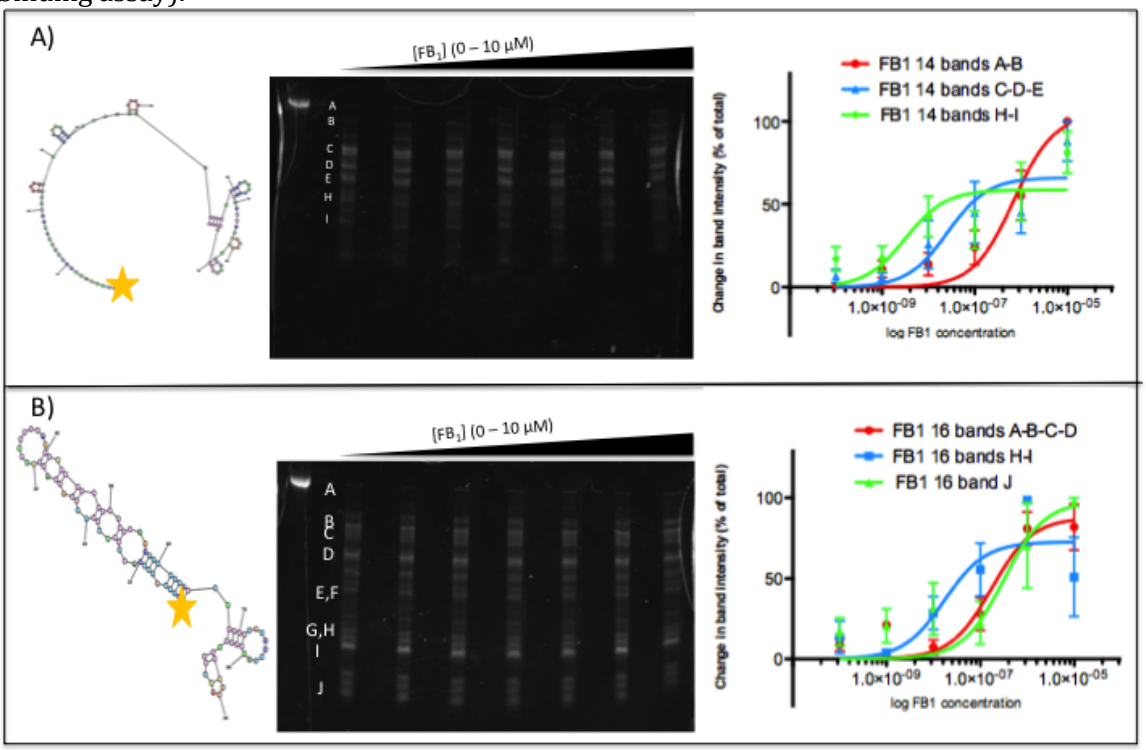


Figure S3. Representative gel for DNase I assay of *FB_1 39* minimers (minimum of 3 replicates each) analyzed using a 19% denaturing polyacrylamide gel, FB_1 concentration increasing from 0 – 10 µM with a corresponding heat map of the DNase I assay. K_ds were determined with GraphPad Prism non-linear regression one site specific binding. In the heat map, colour indicates K_d range (Red <100 nM; Purple 100 nM – 1000 nM, Blue > 1000 nM). Intensity of colour indicates error associated with K_d (solid colour corresponds to lower error; lighter colour with higher error). **A)** *FB1_39t3*: band B (15.1 ± 11.5 nM), band C (6.5 ± 4.6 nM), band E (0.9 ± 0.8 nM), bands J-K (8.3 ± 3.2 nM); **B)** *FB1_39t5*: band B (3.7 ± 2.8 nM), band C (2.8 ± 2.2 nM), bands D-E 2.0 ± 2.7 nM); **C)** *FB1_39t3-5*: band B (7.1 ± 5.9 nM), bands C-D (7.6 ± 4.4 nM), bands E-F (2.5 ± 2.2 nM) ; **D)** *FB1_39m3*: bands H-I (no binding), bands J-K-L (no binding). **E)** *FB1_39m5*: band E (6.9 ± 4.6 nM), band G (no binding), bands J-K (no binding), band L (no binding). **F)** *FB1_39cm*: band D (no binding); band E (0.2 ± 0.2 nM); bands J-K (23.3 ± 16.8 nM).

(4) DNase I assay and analysis of additional FB_1 aptamers (FB_1 14, FB_1 16, FB_1 23, FB_1 31, FB_1 32*)[3] to determine aptamer affinity*

DNA aptamer	Sequence (5'-3')	Reported K_d (nM)
FB_1 39	FATACCAGCTTATTCAATTAATCGCATTACCTTATACCAGCTTATTCAATTACGTCTG CACATACCAGCTTATTCAATTAGATAGTAAGTGCAATCT	100 ± 30[3]
FB_1 32	FATACCAGCTTATTCAATTAATGTACGATGTGTGGGCAACATGAGTATGTCGTGTG ATATCTAGATGAGGTAGCGGTGGAGATAGTAAGTGCAATCT	400 ± 100[4]
FB_1 31	FATACCAGCTTATTCAATTCGGGGACGTGTATACCAGCTTATTCAATTC ACAGTTATGTCCTATACCAGCTTATTCAATTAGATAGTAAGTGCAATCT	470 ± 60[4]
FB_1 23	*F*ATACCAGCTTATTCAATTGCGGATGCGTAAATGACGATAAACATAGATGGGGTA TATCGCGATGCGACAGGGTGTAGATAGTAAGTGCAATCT	1000 ± 20[4]
FB_1 14	FATACCAGCTTATTCAATTCTATACGGAGTGGATATCGATCTGTAACGTGAGTGAG ATAATGTGATGCATAGTCGTGGAGATAGTAAGTGCAATCT	920 ± 40[4]
FB_1 16	FATACCAGCTTATTCAATTCATCCAGTAACAAACACATAAGTAACGGCGATATGTC AAAGCGGTATCGGCTACAGATGAGATAGTAAGTGCAATCT	200 ± 100[4]

Table S1. FB_1 aptamer sequences (F = 5'-fluorescein) and reported K_d (magnetic bead binding assay).[3,4]



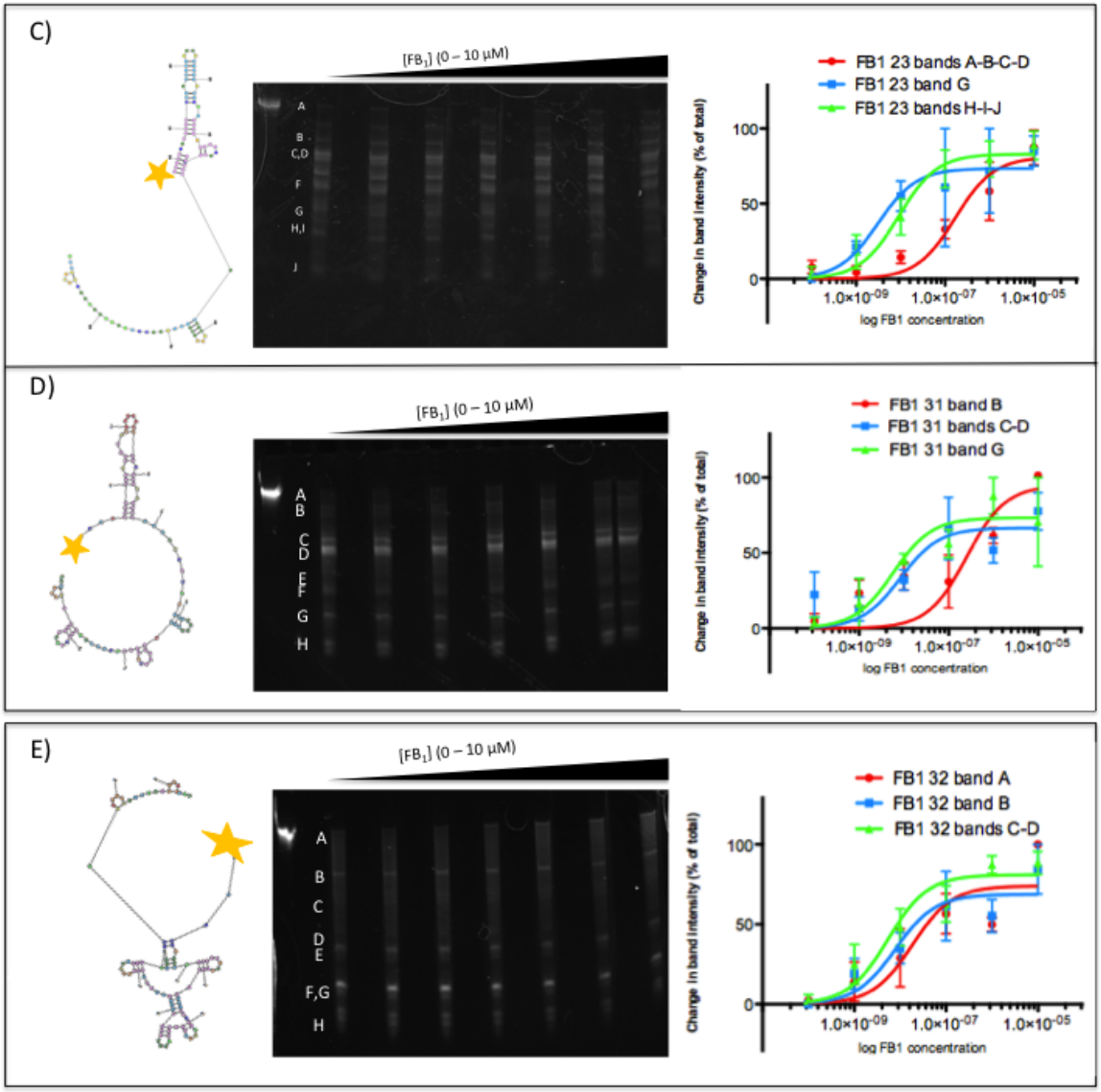


Figure S4. Representative gel for DNase I assay of FB_1 14, 16, 23, 31 and 32 (minimum of 3 replicates each) separated on a 19% denaturing polyacrylamide gel, FB_1 concentration increasing from 0 – 10 µM. The K_ds were determined with GraphPad Prism non-linear regression one-site specific binding. RNA Structure predictions are shown, yellow star indicates 5' Fluorescein modification. **A)** *FB_1 14*: bands A-B (698.6 ± 297.3 nM); bands C-D-E (25.9 ± 22.3 nM); bands H-I (3.2 ± 3.0 nM) **B)** *FB_1 23*: bands A-B-C-D (174.1 ± 14.9 nM); bands H-I (17.2 ± 14.9 nM); band J (311.5 ± 257.7 nM) **C)** *FB_1 23*: bands A-B-C-D (170.0 ± 94.8 nM); band G (3.1 ± 3.0 nM); bands H-I-J (89.9 ± 4.4 nM) **D)** *FB_1 31*: band B (254.4 ± 161.2 nM); bands C-D (8.4 ± 6.7 nM); band G (5.7 ± 4.4 nM) **E)** *FB_1 32*: band A (18.3 ± 13.5 nM); band B (7.8 ± 5.9 nM); bands C-D (5.4 ± 2.6 nM)

(5) PAGE gel of FB_1 39 DNase I digestion for fragment size estimate

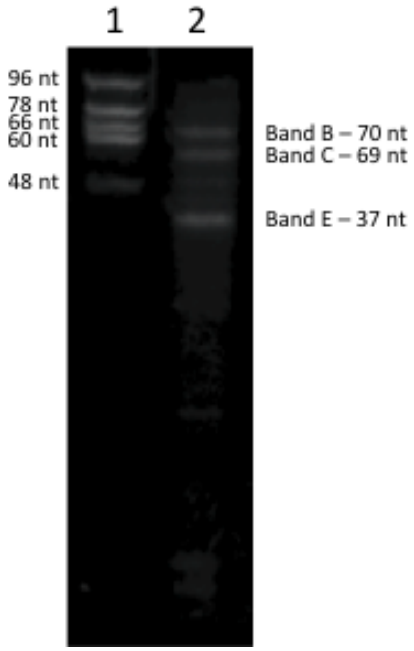


Figure S5. 19% PAGE gel of *FB_1 39* DNase I digestion to estimate size of digestion fragments. Lane 1: 5'-fluorescein labelled DNA ladder. Lane 2: *FB_1 39* DNase I digest with fragment size estimates of prominent bands calculated by Molecular Weight calculator, AlphaImager, AlphaInnotech.

(6) DNase I K_d comparison of additional FB_1 39 minimers (FB1_39cm and FB1_39m5)

Aptamer	DNase I assay (Band E) (nM)
$FB_1$39	2.8 ± 2.4
FB1_39t3	0.6 ± 0.5
FB1_39t5	0.5 ± 0.4
FB1_39t3-5	2.8 ± 2.7
FB1_39m3	N.B
FB1_39m5	6.9 ± 4.6
FB1_39cm	0.2 ± 0.2

Figure S6. Apparent K_d of additional *FB_1 39* minimers (*FB1_39m5* and *FB1_39cm*) at Band E compared to other *FB_1 39* minimers studied.

(7) DNase I assay and analysis of FB_1 39 *for control targets (OTA, FB_2)*

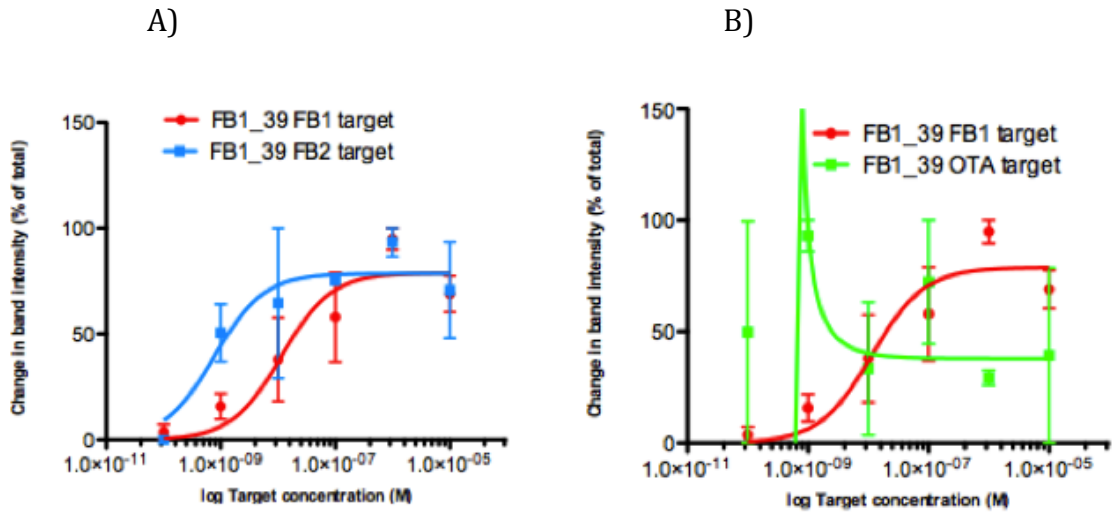


Figure S7. A) Binding isotherms generated from Band E of the DNase I digestion assay showing the K_d of *FB_1 39* with FB_1 and FB_2, and **B)** Binding isotherms generated from Band E of the DNase I digestion assay showing the K_d of *FB_1 39* with FB_1 and OTA. Binding isotherms were generated using GraphPad Prism non-linear regression one-site specific binding. K_d of binding to FB_2 was 0.77 ± 0.65 nM; no binding observed to OTA.

(8) Magnetic bead binding assay, comparing affinity of FB1_39t3 *and a control aptamer for Ochratoxin A (A08)[5] to FB_1-modified magnetic beads*

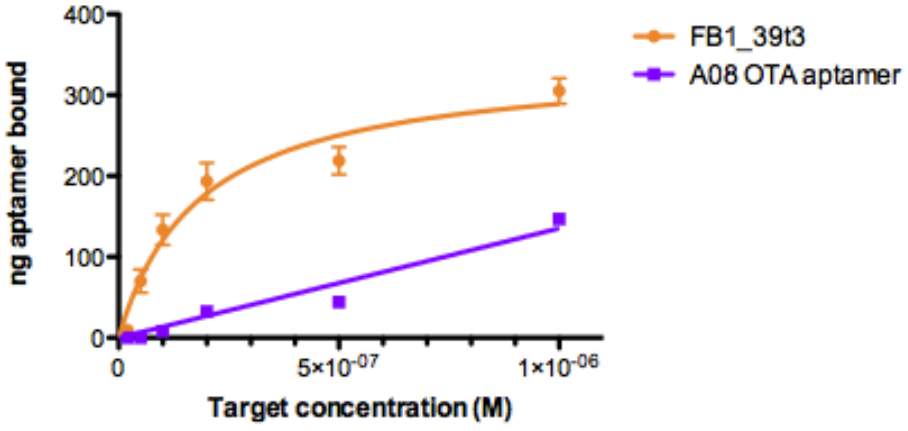


Figure S8. Magnetic bead affinity assays for *FB1_39t3* (K_d 184 ± 43 nM) and A08 control aptamer (no binding).[5] Binding isotherms generated by GraphPad Prism non-linear regression one site specific binding.

(9) REFERENCES:

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