

Supplementary Data

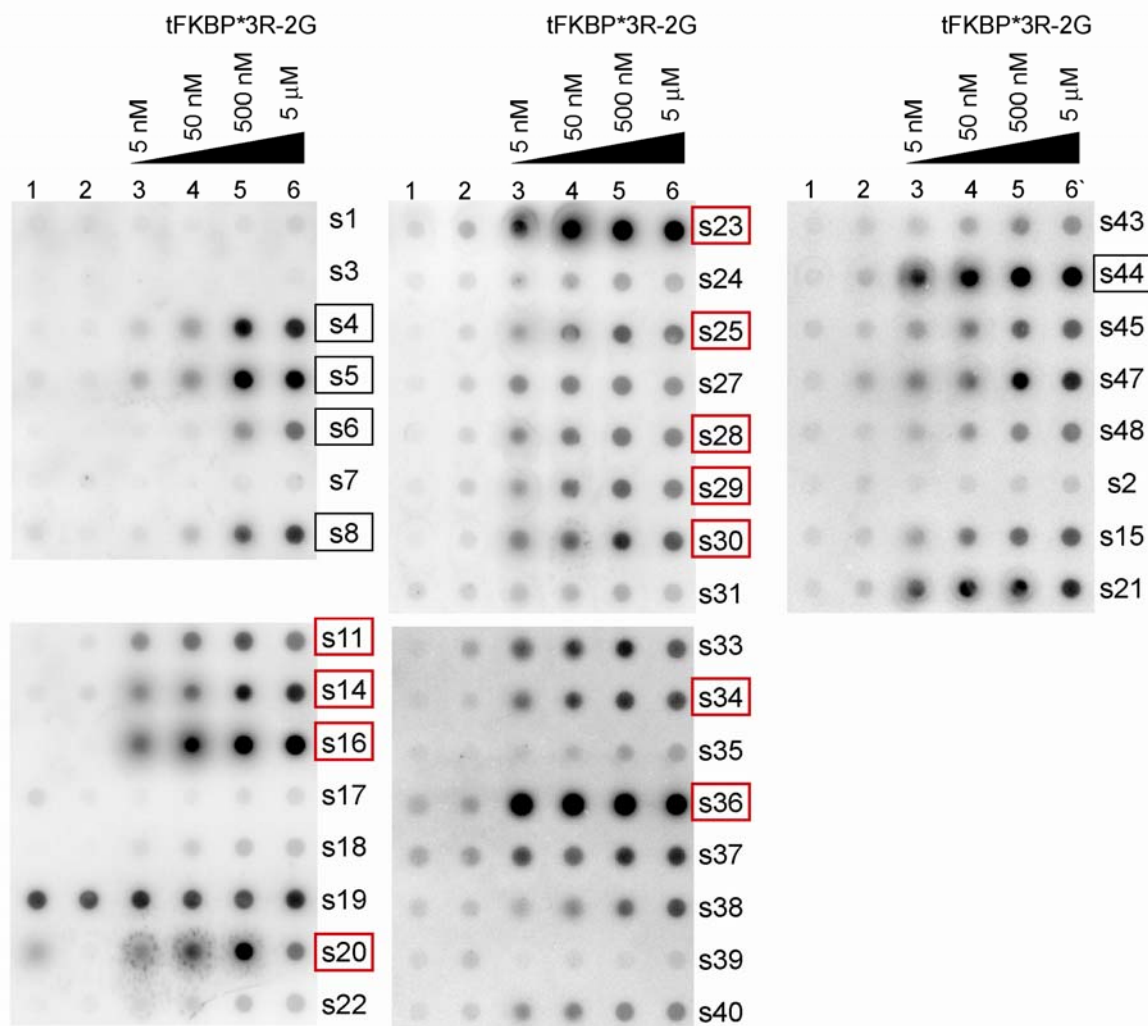
Characterization of small molecule ligands.

Compound 2G: $R_f = 0.15$ (10% CH₃OH in dichloromethane); ¹H NMR (400 MHz CDCl₃) (mixture of rotamers) δ 0.94 (t, 3H, $J = 6.5$ Hz), 1.18-1.38 (m, 2H), 1.46-2.36 (m, 8H), 2.38-2.58 (m, 2H), 2.95 (m, 2H), 3.50-3.68 (m, 5H), 3.71 (s, 6H), 3.79 (s, 3H), 3.80 (s, 3H), 3.81 (s, 3H), 4.36-4.48 (m, 2H), 5.40 (m, 1H), 5.50-5.60 (m, 1H), 6.41 (s, 2H), 6.58-7.20 (m, 7H), 7.56 (br, 1H); ES⁺ HRMS calcd for C₄₅H₅₅N₇O₁₁ (M + H⁺) 870.4038, found 870.3992.

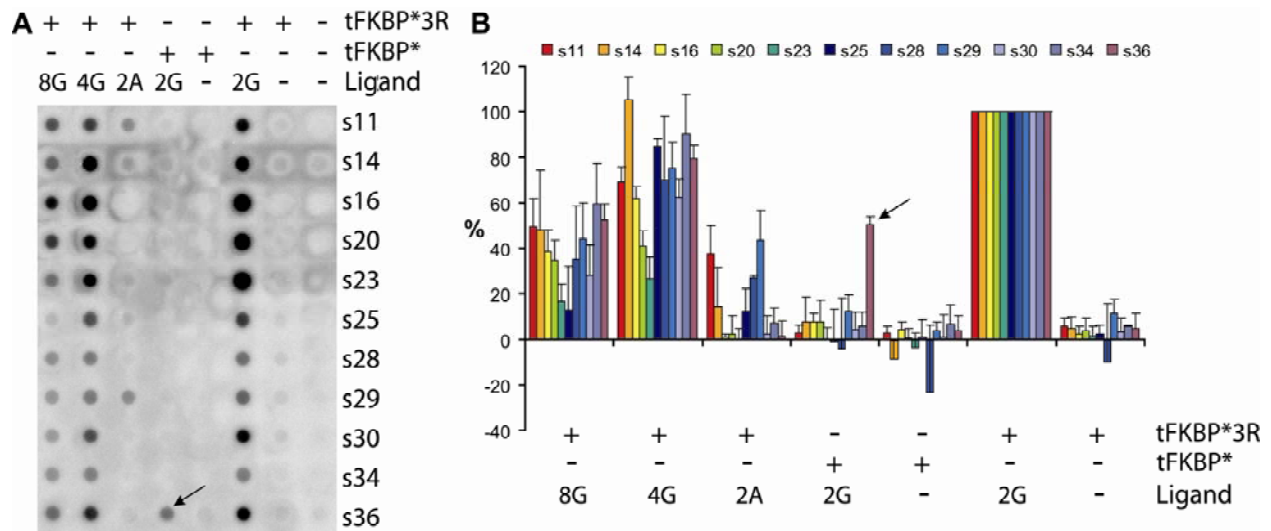
Compound 4G: $R_f = 0.15$ (10% CH₃OH in dichloromethane); ¹H NMR (400 MHz CDCl₃) (mixture of rotamers) δ 0.90 (t, 3H, $J = 7.0$ Hz), 1.28-1.46 (m, 2H), 1.52-1.75 (m, 10H), 2.00-2.31 (m, 2H), 2.40-2.60 (m, 2H), 2.86 (m, 2H), 3.26-3.64 (m, 5H), 3.68 (s, 6H), 3.79 (s, 3H), 3.82 (s, 3H), 3.83 (s, 3H), 4.51-4.80 (m, 2H), 5.43 (m, 1H), 5.59-5.62 (m, 1H), 6.39 (s, 2H), 6.58-7.20 (m, 7H), 7.38 (br, 1H); ES⁺ HRMS calcd for C₄₇H₅₉N₇O₁₁ (M + H⁺) 898.4351, found 898.4318.

Compound 8G: $R_f = 0.13$ (10% CH₃OH in dichloromethane); ¹H NMR (400 MHz CDCl₃) (mixture of rotamers) δ 0.90 (t, 3H, $J = 7.5$ Hz), 1.22-1.46 (m, 2H), 1.52-1.76 (m, 4H), 1.88-2.36 (m, 4H), 2.40-2.62 (m, 2H), 2.81 (m, 2H), 3.50-3.66 (m, 13H), 3.68 (s, 6H), 3.80 (s, 3H), 3.83 (s, 3H), 3.85 (s, 3H), 4.48-4.66 (m, 2H), 5.45 (m, 1H), 5.56-5.65 (m, 1H), 6.41 (s, 2H), 6.60-7.20 (m, 7H), 7.46 (s, 1H); ES⁺ HRMS calcd for C₄₉H₆₃N₇O₁₃ (M + H⁺) 958.4562, found 958.4563.

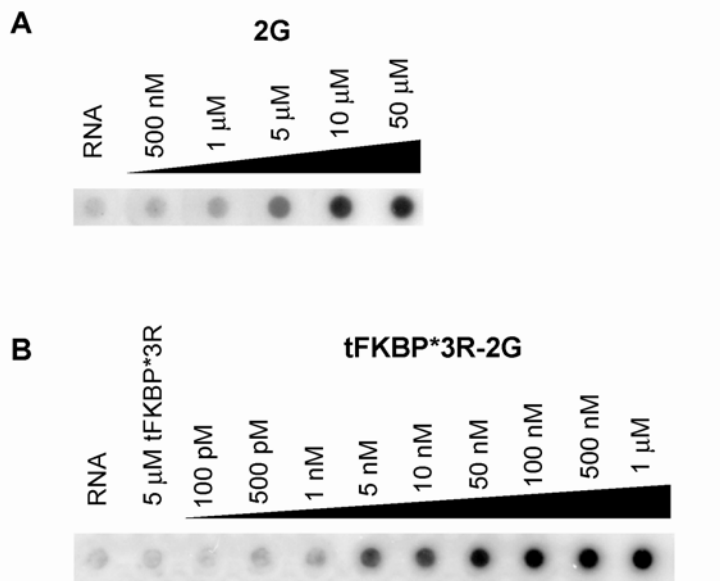
Compound 2A: $R_f = 0.15$ (10% CH₃OH in dichloromethane); ¹H NMR (400 MHz CDCl₃) (mixture of rotamers) δ 0.91 (t, 3H, $J = 6.8$ Hz), 1.24-1.50 (m, 2H), 1.60-1.80 (m, 4H), 1.86-2.14 (m, 4H), 2.32-2.60 (m, 2H), 2.88 (m, 2H), 3.60-3.70 (m, 5H), 3.71 (s, 6H), 3.79 (s, 3H), 3.83 (s, 3H), 3.84 (s, 3H), 4.38-4.46 (m, 2H), 5.55 (m, 1H), 5.60-5.65 (m, 1H), 6.43 (s, 2H), 6.58-7.10 (m, 7H) 7.80 (s, 1H), 8.28 (s, 1H).; ES⁺ MS calcd for C₄₅H₅₅N₇O₁₀ (M + H⁺) 854.40, found 854.50.



Supplementary Figure 1. Screening round 7 clones by nitrocellulose filter binding assays. Each labeled RNA was incubated with buffer (lane 1), 500 nM tFKBP*3R (lane 2) or varying concentrations of tFKBP*3R-2G complex (lanes 3-6), and the resulting solution was applied to the filter. Boxed sequences display small molecule-enhanced binding. Specifically, these sequences show at least a two-fold greater signal at 5 μ M tFKBP*3R-2G over background and at least a 2.5-fold greater signal at 5 μ M tFKBP*3R-2G over protein alone in at least one of duplicate measurements. Sequences in red have an average K_d (duplicate measurement) below 50 nM (see Supplementary Table 1).



Supplementary Figure 2. Specificity of high-affinity aptamers for tFKBP*3R and 2G. Nitrocellulose filter-binding experiments were used to determine whether structurally similar small molecules would induce protein-small molecule-RNA complex formation as efficiently as 2G. Aptamers were also tested for their ability to differentiate between tFKBP* and tFKBP*3R. (A) A representative nitrocellulose filter-binding assay. Note the increased level of tFKBP*-2G-s36 binding (arrow). (B) Quantification of the data in (A), as a percent of tFKBP*3R-2G-aptamer binding. Values reflect the average of quadruplicate measurements.



Supplementary Figure 3 2G-s23 and tFKBP*3R-2G-s23 K_d measurements. **(A)** The 2G-s23 affinity was determined by nitrocellulose filter binding assays, and a representative blot is shown. Triplicate measurements indicate a K_d of $4.6 \pm 0.8 \mu\text{M}$. Binding of 2G to one of the point mutant s23 RNA molecules (G72A) that has a greatly diminished capacity to form a ternary complex was also assayed. At $50 \mu\text{M}$ 2G, binding of G72A is barely detectable, and at $10 \mu\text{M}$ is not detectable, implying that binding of s23 to 2G is specific. **(B)** Representative blot of tFKBP*3R-2G-s23 binding. The K_d for ternary complex formation of $4.3 \pm 0.5 \text{ nM}$ is the average of three independent measurements. Note that no significant binding of s23 to $5 \mu\text{M}$ tFKBP*3R can be seen.

Supplementary Table 1. Estimate of aptamer affinities for tFKBP*3R-2G

Aptamer	K_d (nM)	Sequence of Variable Region
s4	79.6 ± 2.6	UACUAUAGGAUGGUCAGGGUCUGACCAACGGUGGGUGGUGAGAGCUAUAGUUGGUGGUC
s5	57.2 ± 6.9	UUGCAGGCCGGUAUGAACGGUCGGGGGGUUGGUACGCGUCGCGGUAGGCGGGCCGGUCAU
s6	314 ± 3	GAAAAUUUAAUGCGUUGGUAUUGGUUGGGGAUUGUCAUUGUACUCAGGAAUACGUACGGG
s8	132 ± 29	UCGCGUUAGACGUGGUAAAUGGGACUCGCUCUGAGUUUAGACCUGGGUGGUGCGUUGGCCG
s11	< 5	GAUUUAAGUUAAAGAGGGAGGGUCAUGUGUGCCGGUGUGAUGCGGUACUGAUCCAAG
s14	< 5	UUGGUGUGGGCGUGACUAUCUCGGGUCUUUUGGGGAUAGUAUGGAAUGAUGAUCCUUGUGC
s16	10.5 ± 1.9	GGUGACUGCGGCGGCGGCGAUUCGCUAUCCCCGGGUAUUAUGCUUCUGGAUCUACUCUGU
s20	8.9 ± 4.9	UAGAGUGGUGAAGCGGGAUGUGAUUUUCAAGUUCGUUGGGACCAAGAGGCAGAGCCAAC
s23	< 5	UCAUUUCAUUGUGGGCGGUGCAGUCAAAUGUGAUGCUUCGUUCAGUGACGGGAGGGUUGUGAG
s25	< 5	AGCGGUGGGUUGCUGAGGCUUUGGAAAGUGUGCAAAGUGCUUUGUGUCGUAUCCGGUGU
s28	< 5	AAGAUGGCGGGUGGAGUAUGGCUUUCGGCGGUAUGCCGUGUGGAGAAGUAUGUUCAGUGGUCCU
s29	< 5	AGUAUGCAUCCGGAGUUAGACUCCGAUGGUGGCGAGGGCGUGGUAGACUGGGUGCGAU
s30	< 5	UCACGGAUGCAGAGGACGGAUGGAGCCAGGUGAAAAGGAUUGGCUGAGUAGCUGUGACGA
s34	< 5	AAUGGUGGUAAGUGGGUGGCUGCUUAGGGGCUUGAGAAACGAGUUCCAAGGCAGGUGUGU
s36	< 5	AUUUGAUGUUUUGGUUAUGUCCAGACCGUACCCUACAGUGGUGGCAUGUGGCGNGUGGUUA
s44 ^a	< 5	UUCGCGAGCCUAACGUUUAGGAGGGUAUGAAGUAUAUGGUGGAAGGGGACAGUUUCAUA

Duplicate nitrocellulose filter binding assays with the 16 RNA aptamers and tFKBP*3R-2G were quantified and binding affinities were calculated. ^aThe K_d for s44 represents only one measurement.