Supplementary Data

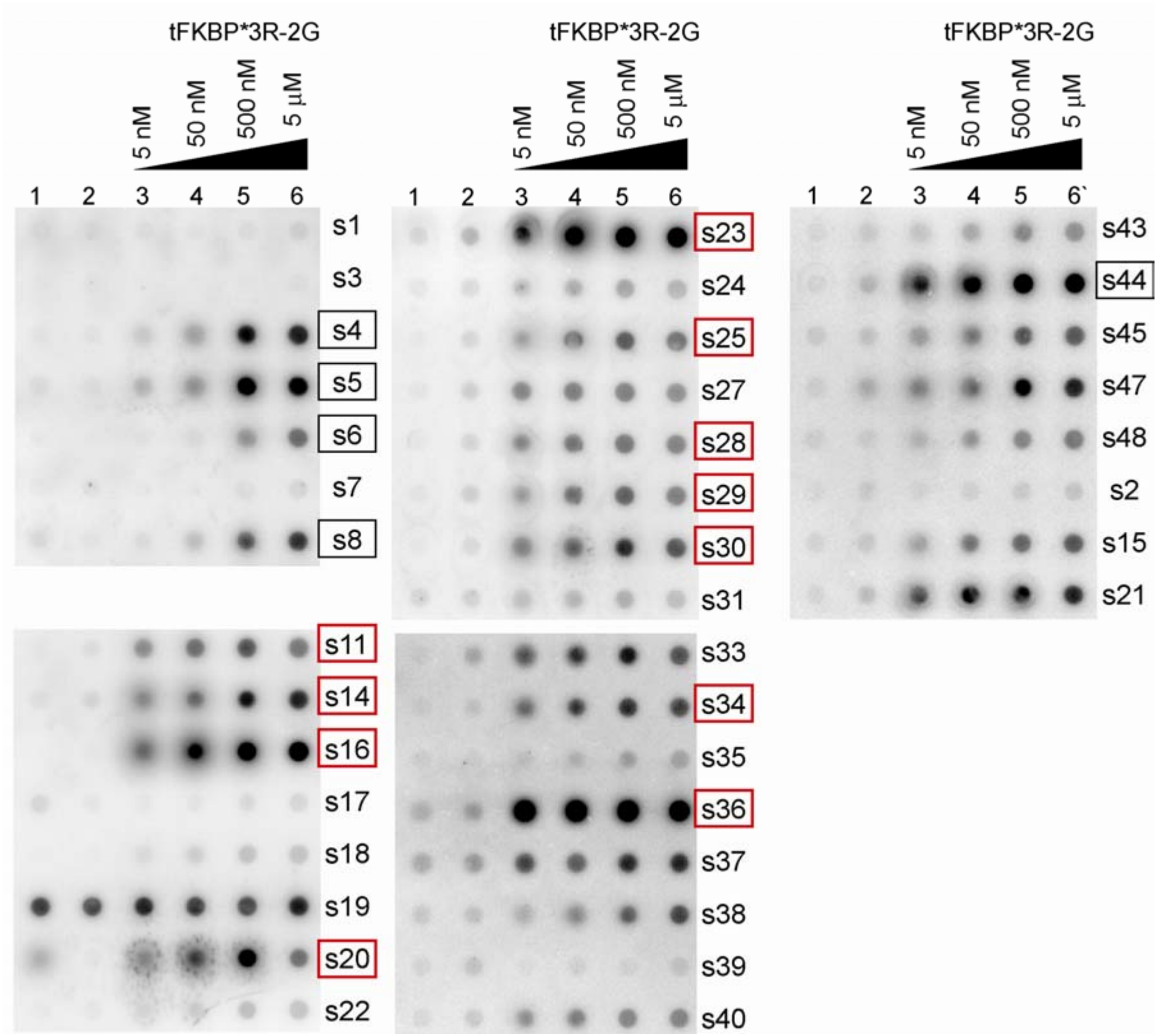
Characterization of small molecule ligands.

Compound 2G: R_f = 0.15 (10% CH_3OH in dichloromethane); ^1H NMR (400 MHz $CDCl_3$) (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 $C_{45}H_{55}N_7O_{11}$ ($M + H^+$) 870.4038, found 870.3992.

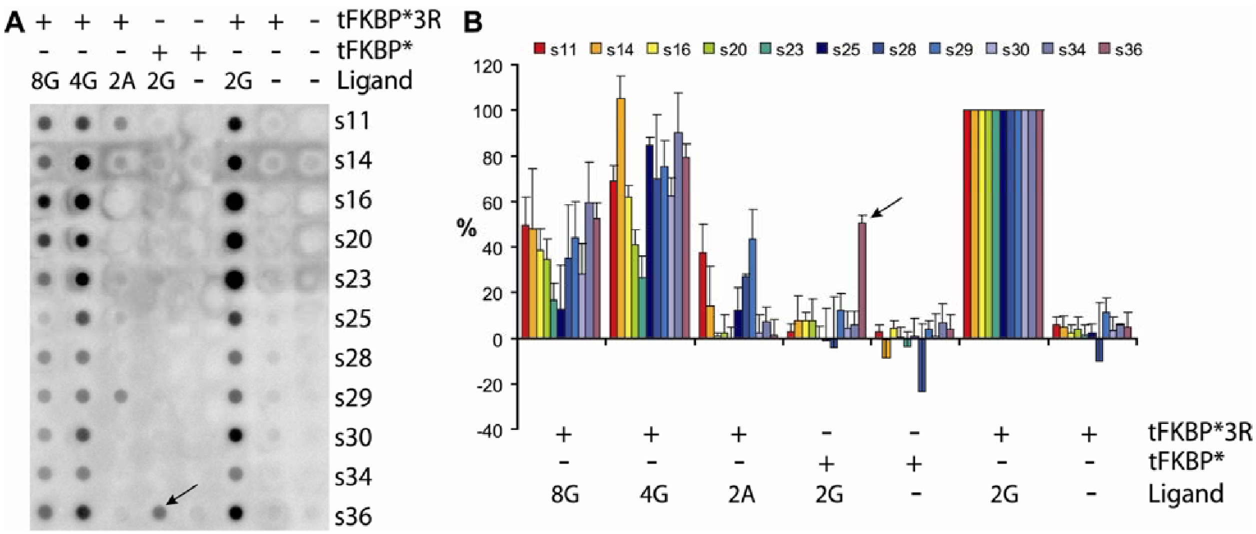
Compound 4G: R_f = 0.15 (10% CH_3OH in dichloromethane); ^1H NMR (400 MHz $CDCl_3$) (mixture of rotamers) δ 0.90 (t, 3H, J = 7.0 Hz), 1.28-1.46 (m, 2H), 1.52-1.75 (m, 10H), 2.00-2.31 (m, 2H), 2.40-2.60 (m, 2H), 2.86 (m, 2H), 3.26-3.64 (m, 5H), 3.68 (s, 6H), 3.79 (s, 3H), 3.82 (s, 3H), 3.83 (s, 3H), 4.51-4.80 (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 $C_{47}H_{59}N_7O_{11}$ ($M + H^+$) 898.4351, found 898.4318.

Compound 8G: R_f = 0.13 (10% CH_3OH in dichloromethane); ^1H NMR (400 MHz $CDCl_3$) (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 $C_{49}H_{63}N_7O_{13}$ ($M + H^+$) 958.4562, found 958.4563.

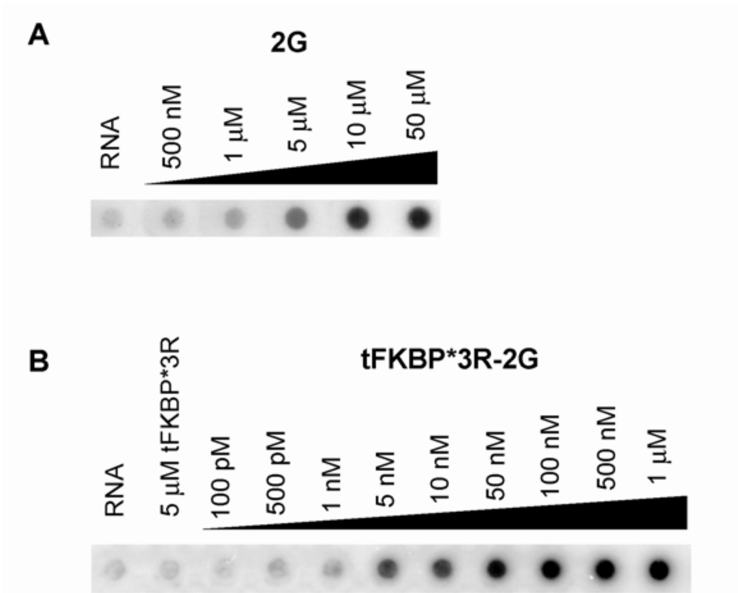
Compound 2A: R_f = 0.15 (10% CH_3OH in dichloromethane); ^1H NMR (400 MHz $CDCl_3$) (mixture of rotamers) δ 0.91 (t, 3H, J = 6.8 Hz), 1.24-1.50 (m, 2H), 1.60-1.80 (m, 4H), 1.86-2.14 (m, 4H), 2.32-2.60 (m, 2H), 2.88 (m, 2H), 3.60-3.70 (m, 5H), 3.71 (s, 6H), 3.79 (s, 3H), 3.83 (s, 3H), 3.84 (s, 3H), 4.38-4.46 (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 $C_{45}H_{55}N_7O_{10}$ ($M + H^+$) 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 μM tFKBP*3R-2G over background and at least a 2.5-fold greater signal at 5 μM 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 ± 0.8 µM. 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 µM 2G, binding of G72A is barely detectable, and at 10 µM 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 ± 0.5 nM is the average of three independent measurements. Note that no significant binding of s23 to 5 µM 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	GAAAAUUUAAUGCGUUGGUAAUGGUUGGGGAUUGUCAAUGUUACUCAGGAAUACGUACGGG
s8	132 ± 29	UCGCGUUAGACGUGGUAAAUGGGACUCGCUCUGAGUUUAGACCGUGGGUGGUGCGUUGGCGG
s11	< 5	GAUUUAAGUUAAGAGGGAGGGUCAUGUGUGCCGGUGUGAUGCGGUACUGAUCCAAG
s14	< 5	UUGGUGUGGGCGUGACUAUCUCGGGUCUUUUGGGGAUAGUAUGGAAUGAUGAUCCUUGUGC
s16	10.5 ± 1.9	GGUGACUGCGGCGGCGGCGAUUCGCUAUCCCCGGGUAUUAUGCUUCUGGAUCUACUCUGU
s20	8.9 ± 4.9	UAGAGUGGUGAAGCGGGAUGUGAUUUUCAAGUUCGUUGGGACCAAGAGGCAGAGCCAAC
s23	< 5	UCAUUUCAUUGUGGCGGUGCAGUCAAAUGUGAUGCUUCGUUCAGUGACGGGAGGGUUGUGAG
s25	< 5	AGCGGUGGGUUGCUGAGGCUUUGGAAAGUGUGCAAAGUGCUUUGUGUCGUAUCCGGUGU
s28	< 5	AAGAUGGCGGGUGGAGUAUGGCUUUCGGCGGUAUGCCGUGUGGAGAAGUAUGUUCAGUGGUCCU
s29	< 5	AGUAUGCAUCCGGAGUUAGACUCCGAUGGUGGCGAGGGCGUGGUAGACUGGGUGCGAU
s30	< 5	UCACGGAUGCAGAGGACGGAUGGAGCCAGGUGAAAGGAUUGGCUGAGUAGCUGUGACGA
s34	< 5	AAUGGUGGUAAGUGGGUGGCUGCUUAGGGGCUUGAGAAACGAGUUCCAAGGCGGAUGUGU
s36	< 5	AUUUGAUGUUUUGGUUAUGUCCAGACCGUACCCUACAGUGGUGGCAUGUGGCGNGUGGUUA
s44[a]	< 5	UUCGCGAGCCUAACGUUUAGGAGGGUAUGAAGUAUAUGGUGGAAGGGGACAGUUUUCAUA

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