Electronic Supplementary Material (ESI) for ChemComm.

ELECTRONIC SUPPLEMENTARY INFORMATION

Materials and Methods

Preparation of Agarose Resin for SELEX

For SELEX experiments using O^6-benzylguanine (bG) derivitizedagarose (SNAP capture pull-down resin, New England Biolabs), a mock resin was prepared to deplete RNAs that would non-specifically bind to the agarose. Dry *N*-hydroxysuccinimidylester (NHS)-activated agarose resin (33 mg, Life Technology) was first resuspended in 500 µL of phosphate buffered saline (PBS) by mixing end-over-end at 4°C overnight. Tris buffer was then added (500 µL 1 M Tris-HCl, pH 7.4) and mixing was continued for an additional 2 h at room temperature. The resin was then washed with PBS and stored at 4°C.

Preparation of Dynabeads for SELEX

For SELEX experiments using magnetic Dynabeads both bG-derivitized and mock dynabeads were prepared. For synthesis of the bG resin, tosyl-activated Dynabeads (600 µL, Life Technology) were prewashed and equilibrated in borate buffer (600 µL; 0.1 M sodium borate, pH 9.5). Amine-derivitizedbG (360 µg; BG-PEG-NH2, New England Biolabs) was dissolved in dimethylsulfoxide (DMSO) to a final concentration of 20 µg/µL. This was then diluted by addition of 432 µL of borate buffer. The resulting solution was then combined with the Dynabeads and incubated with end-over-end mixing at 37°C for 48h.

After incubation, the beads were then isolated with a magnet and washed twice with 600 µL of DMSO and once with 600 µL of 0.2 M Tris, pH 8.5. The beads were then resuspended in an additional 600 µL of 0.2 M Tris, pH 8.5, and incubated with mixing at 37°C for an additional 4 h. Beads were then isolated with a magnet and washed five times with PBS buffer. The derivitized beads were stored in 600 µL of PBS at 4°C.

Mock Dynabeads were prepared using the same protocol as above except that an amine-derivitized PEG molecule was used in place of bG. NH2-PEG_3-OH (360 µg, ChemPep Inc.) was dissolved in DMSO to a final concentration of 60 µg/µL before dilution with 444 µL of borate buffer. Subsequent steps for preparation of the

mock Dynabeads were identical to those used for preparation of the bG-derivitizedDynabeads.

Preparation of the Starting RNA Library for SELEX

The initial pool of RNAs for SELEX experiments was prepared using a partially structured N24-2 DNA library, in which two 24-base stretches of random nucleotides were separated by a fixed sequence of 12 bases. The fixed sequence (5'-CTG CCG AAG CAG-3') encoded a 4-base pair RNA stem topped by a UUCG tetraloop. The DNA library containing approximately 10^{14} molecules was prepared with a Klenow extension reaction (166 µL) that contained 1 µM of template DNA oligo (5'-GTG ACG CGA CTA GTT ACG GA(N:25:25:25;25)-N24-CTG CCG AAG CAG-N24-TTC ATT CAG TTG GCG CCT CC-3', HPLC purified; IDT), 1 µM of N24-2 reverse primer containing a T7 RNA polymerase promoter sequence (5'-ATG TAA TAC GAC TCA CTA TAG GAG GCG CCA ACT GAA TGA A-3', HPLC purified; IDT), 0.5 mMdNTPs, 0.1 mg/mL BSA, 50 mMNaCl, 10 mMTris-HCl pH 7.9, 10 mM $MgCl_2$, 1 mM DTT, and 0.05 U/µL of Klenow Fragment (exo-, New England Biolabs).

The entire Klenow extension reaction was then further amplified by large-scale polymerase chain reaction (PCR, 12 mL total volume) in a reaction containing 1 µM of the reverse primer, 1 µM of forward primer (5'-GTG ACG CGA CTA GTT ACG GA-3', HPLC purified; IDT), 0.2 mMdNTP, 1x GoTaq buffer (Promega), and 0.025 U/µL GoTaq DNA Polymerase (Promega). PCR was carried out in 50 µL aliquots with five cycles of 95^{o}C denaturation for 1 min, 54.5^{o}C annealing for 1 min, and 72^{o}C extension for 1 min. The resulting PCR products were combinedbefore being extracted twice with chloroform:phenol:isoamyl alcohol (25:24:1), precipitated with 2 volumes of ethanol, and resuspended in water.

The initial RNA pool was prepared from 100µg the PCR-amplified DNA product in a 400µL T7 RNA polymerase transcription reactionusing the T7-flash Kit (Epicentre Biotechnologies).120 µCiof [^{32}P]-α-UTP (10 µCi /µl，PerkinElmer) was included to aid in RNA quantification and selection monitoring. Subsequent to transcription,RNase-free DNase I (100 U, New England Biolabs)was added to digest

the DNA templates for 1 h at 37°C. The RNA productswerethen extracted twice with chloroform:phenol:isoamyl alcohol (25:24:1), precipitated with 3 volumes of ethanol and further purified on a 8% denaturing polyacrylamide (PAGE) gel. The RNA was excised from the gel and eluted in 0.3M NaOAc (pH5.2) overnight before being ethanol precipitated. RNA pellets were washed with 70% ethanol and dissolved in water before SELEX.

SELEX using bGAgarose Resin

RNAs prepared from the random DNA library were diluted to a final concentration of ~1 μM and volume of 1 mL in Selection Buffer (SB; 40 mM HEPES pH 7.4, 125 mMKCl, 5 mM $MgCl_2$, and 5% DMSO). The RNAs were then heat denatured by incubation at 75°C for 5 min before cooling at room temperature for 10 min.

RNAs were then incubated with 1 mL of the mock agarose resin for 30 min at room temperature with end-over-end mixing. The mixture was then placed into a Micro Bio-Spin column (Bio-Rad) and the flowthrough collected by gravity flow. The flowthrough was then incubated with 1 mL of SNAP capture pull-down resin (New England Biolabs) for 30 min at room temperature with mixing. The mixture was then placed into a second Micro Bio-spin column and washed with 6 x 1 mL of Selection Buffer. RNAs were eluted by incubation of the resin with 1 mL of 5 mMbG (Matrix Scientific) overnight at room temperature with end-over-end mixing and collecting the eluate. The resin was subsequently incubated with another 1 mL of 5 mMbG for 1 h. Both eluates from the bG incubation were then combined.

The eluted RNAs were then ethanol precipitated using glycogen (1 μg, Fermentas)as a coprecipitant before reverse transcription (RT). cDNAs were prepared using the Maxima H Minus First Strand cDNA Synthesis Kit (Thermo Scientific) in a reaction containing 2.5 μM of the N24-2forward primer and 0.5 mMdNTP. This mixture was then heated to 65°C for 5 min. After cooling on ice, the kit buffer was added followed by 1 μL of the enzyme mix. The reaction was then incubated at 50°C for 45 min before being terminated by heat denaturation at 85°C

for 5 min. RT products were then amplified by PCR in total volumes of 200-400 µL using GoTaq DNA polymerase before subsequent *in vitro* transcription in the presence of 10 µCiof [^{32}P]-α-UTP for the next round of SELEX. In total, ten rounds of RNA transcription, bGagarose binding and elution, cDNA synthesis, and PCR were carried out.

SELEX using bGDynaBeads

SELEX using magnetic bGDynaBeads was carried out essentially as described for bGagarose except that 1 mL of mock or bG-derivitizedDynaBeads were used.

Cloning and Sequencing of DNAs Encoding RNA Aptamers

PCR-amplified DNAsobtained after 10 rounds of SELEX using either the bGagarose or bGDynabeadswereligatedinto the pJET1.2/blunt cloning vector (Thermo scientific CloneJET PCR Cloning Kit). Sequencing results were aligned using Clustal Omega (EMBL). Prior to SELEX samples of the initial DNA pool were also sequenced to confirm library diversity.

Preparation of Aptamers JX1-6 and JX1 derivatives

Aptamers for structural and binding studies were prepared by *in vitro* transcription. Templates for transcription of aptamers JX1-6 were prepared by PCR amplification of the corresponding regions from the pJET1.2 plasmids used for sequencing. The resulting DNAs were then used as templates for transcription with T7 RNA polymerase. RNAs were then purified by HiTrap Q column (GE Healthcare), precipitated with ethanol, resuspended in RNase free H_2O, and quantified by measuring the UV absorbance at 260 nm. Transcription templates for derivatives of JX1 were prepared by PCR amplification of ssDNA templates (IDT).

Column binding assay

[^{32}P]-labeled RNA (100 pmol)wasadded to SB (50 µL) and then incubated with either bGagarose or mock resin (50 µL) for 30 min. The resin was then washed 20 times

with SB (50 µL for each wash). Bound RNA was then eluted by addition of 10 mMbG. RNA concentration in the wash and elution buffers were determined by scintillation counting.

Fluorescence Polarization Assays to Determine Aptamer Binding Affinity

Binding assays (60 µL) were carried out with a fixed concentration of SNAP-Surface ® 488 (Atto488-bG, 50 nM, New England Biolabs) or SNAP-Surface Dy549 ® (Dy549-bG, 50 nM, New England Biolabs) and addition of RNA aptamers from 0.1-10 µM. Prior to each assay, the RNA aptamers were diluted into SB, unfolded by heating to 75°C for 5 min, and then cooled at room temperature for 10 min. Binding reactions were carried out at room temperature by addition of the fluorophore followed by incubation at room temperature for 30 min prior to collection of fluorescence data.

Fluorescence polarization (FP) data were collected using a Infinite M1000 Pro plate reader (Tecan) in 96-well flat bottom, black polystyrol plates (Corning Incorporated). For monitoring binding of Atto488-bG, excitation and emission wavelengths were set to 470 and 520 nm, respectively, with a 5 nm bandwidth. Binding of Dy549-bG was monitored with excitation and emission wavelengths set to 530 and 575 nm, respectively, with a 5 nm bandwidth. The instrument gain was set to 100 and 10 flashes were collected from each well with a 30 ms settle time between wells. Data were background corrected using a buffer blank and collected in triplicate. Reported values represent the mean and standard deviation. FP data were analyzed and fit to Equation (1) using Origin 8.0 (OriginLab Corporation) where FP_{max}represents the maximum change in fluorescence polarization, FP_{min} the FP value in the absence of aptamer, $E_o$represents the aptamer concentration, and ΔFP represents the measured change in FP at each aptamer concentration.

$$\Delta FP = \frac{(FP_{max} - FP_{min}) \times E_0}{K_d + E_0} \quad (1)$$

Competitive Binding Assay

Binding of non-fluorescent molecules was measured by their ability to compete with Atto488-bG (50 nM) in the FP assay. FP experiments were carried out as described above except for addition of the competitor during binding. Guanine (Tokyo Chemical Industry Co., LTD.) and guanosine (Chem-IMPEX International, Inc.) were added to a final concentration of 250 μM due to their limited solubility.

RNA Aptamer Structural Prediction

Prediction of RNA secondary structure by freeenergy minimization was done by the MFold program.

RNase T1 digestion assay

1 pmol of 5'- ^{32}P labeled JX1 RNA were dissolved in a volume of 10μl containing 40mMHepes buffer (pH7.4), 125 mMKCl, 5 mM $MgCl_2$, in the presence of 1μg Yeast tRNA (Life Technology). After unfolding for 5min at 75℃, the RNA was left at room temperature for 10min. Then the binding reaction was performed at room temperature for 30min with the addition of 1μL DMSO or 1μl of 2mM BG. The RNase T1 (Thermo Scientific) digestion reactions were performed at 37℃ for 10min by adding 0.1 U (1μL) RNase T1 and subsequent immediate quenching of the reaction on ice and addition of gel loading buffer.

G-specific ladder:

G-specific sequencing ladders were generated by incubation of 1pmol of 5'- ^{32}P labeled JX1 RNA with 1 μg yeasttRNA in 10 μl 12.5 mM sodium citrate buffer (pH 4.5) containing 3.5 M urea and 0.5 mM EDTA. After denaturation of the RNA for 10 min at 55°C, 0.1 U (1μl) RNase T1 were added and the sample was incubated for additional 10 min at 55°C. Then the reaction was terminated by addition of gel loading buffer.

Native Purification of JX1 from Yeast Total RNA

JX1 (10 nM) was combined with yeast total RNA (1 µg, prepared by hot phenol extraction of *S. cerevisiae*) in Selection Buffer in a final volume of 50 µL. The mixture was then heated to 75°C for 5 min and then cooled at room temperature for 5 min. This mixture was then incubated while mixing end-over-end at room temperature for 30 min with bGagarose (50 µL) that had been prewashed with selection buffer. The flowthrough was collected and the resin washed five times with selection buffer in column format. Bound RNAs were eluted by incubating the resin with 50 µL of Selection Buffer containing 5 mM benzyl guanine with end-over-end mixing at room temperature for 1 h and then collecting the eluate. Samples were combined with an equal volume of 95% deionized formamide before being loaded onto a denaturing 10% acrylamide gel. After electrophoresis, bands were identified by ethidium bromide staining.

RNA Name	Sequences	AE	BE
JX1 RNA	GGAGGCGCCAACUGAAUGAACGAGUUGAAAUGCGGAUCAAGUUACUGCUUCGGCAGUACCUAGUGG GAAAAGUUCUUGGGUCCGUAACUAGUCGCGUCAC	23	5
JX2 RNA	GGAGGCGCCAACUGAAUGAAACCGUGCUAAGGAGUUGGACUCCGCUGCUUCGGCAGAGCGAUAUAG UGCCAGCUGAUACAUCCGUAACUAGUCGCGUCAC	20	7
JX3 RNA	GGAGGCGCCAACUGAAUGAAAGGUUACGCUUCAACGCCGUGCAGCUGCUUCGGCAGGCUUGUGAG UACAUGGCCCAUUGAUCCGUAACUAGUCGCGUCAC	3	
JX4 RNA	GGAGGCGCCAACUGAAUGAAUGUUCUGGAUCUGCUAGAGACUAUCUGCUUCGGCAGCACUCUCCGU UAACAACACGUACAUCCGUAACUAGUCGCGUCAC	5	
JX5 RNA	GGAGGCGCCAACUGAAUGAAAGAGGUAACGAAAGAUGAUUGUGCCUGCUUCGGCAGUACAACUUCU CGGAUAAAUGAUUAUCCGUAACUAGUCGCGUCAC	2	
JX6 RNA	GGAGGCGCCAACUGAAUGAAGGUGUAUUUCAGUUAAAGAACUUGCUGCUUCGGCAGCGUUCUGAGU UACUAGUCCAAUAAUCCGUAACUAGUCGCGUCAC		9

Table S1. Nucleotide sequences of the JX1-6 RNA aptamers. AE and BE indicate the number of occurrences of each aptamer sequence in the sequencing results from RNAs eluted from agarose (AE) or DynaBeads (BE). Red nucleotides indicate the primer binding regions common to all aptamers, while green nucleotides indicate the common UUCG tetraloop region.

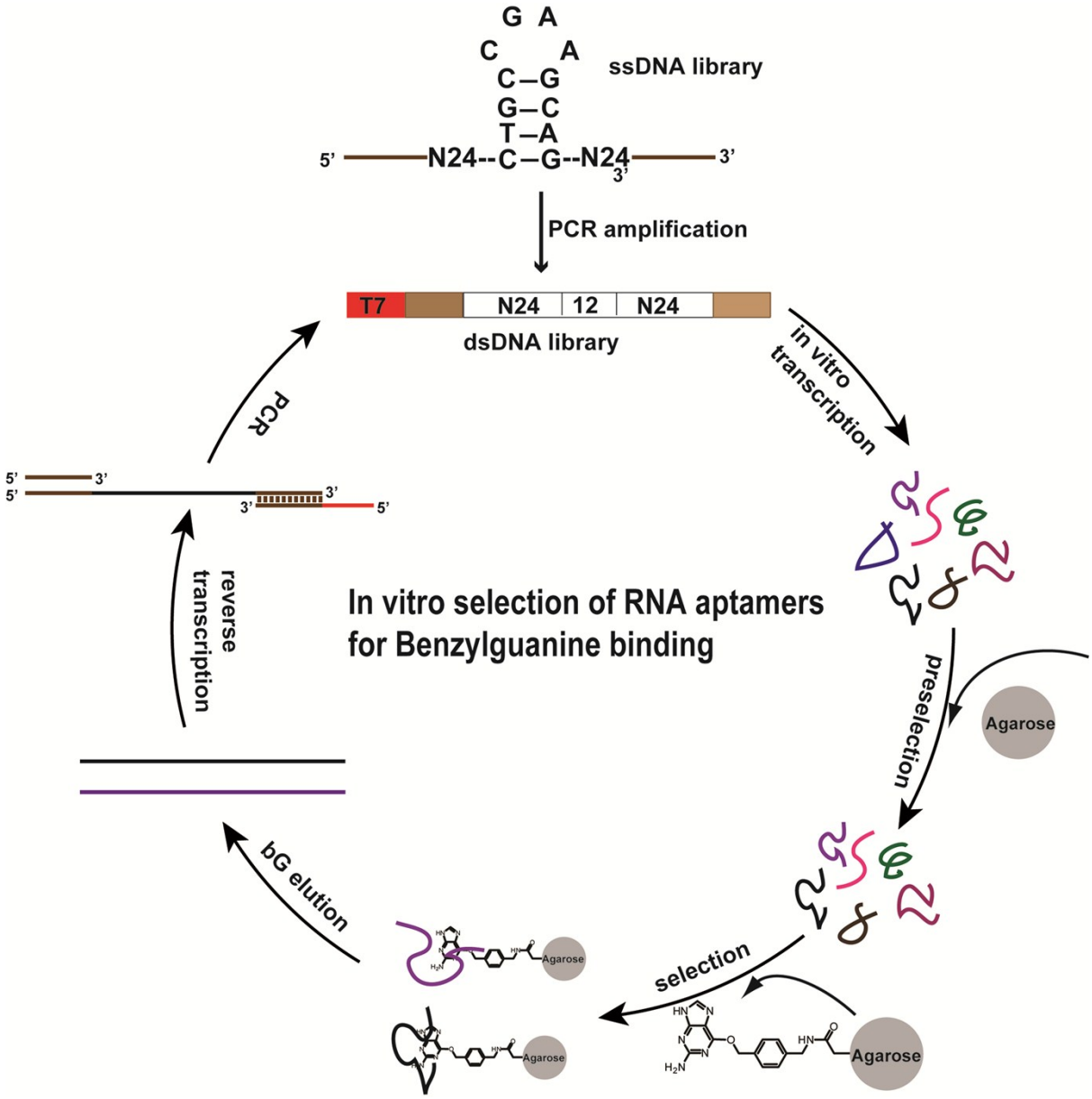

Figure S1. Scheme for carrying out SELEX for evolution of the bG binding aptamer. The SELEX experiment began by PCR amplication of assDNA library containing two, 24-nt regions of random sequence separated by a common hairpin-forming region. RNAs were then prepared by transcription, non-specific interactors removed by incubation with a mock agarose resin, and incubated with bG-agarose. RNAs were eluted by incubation with bG before cDNAs were prepared and amplified. A similar protocol was used when DynaBeads were used in place of agarose.

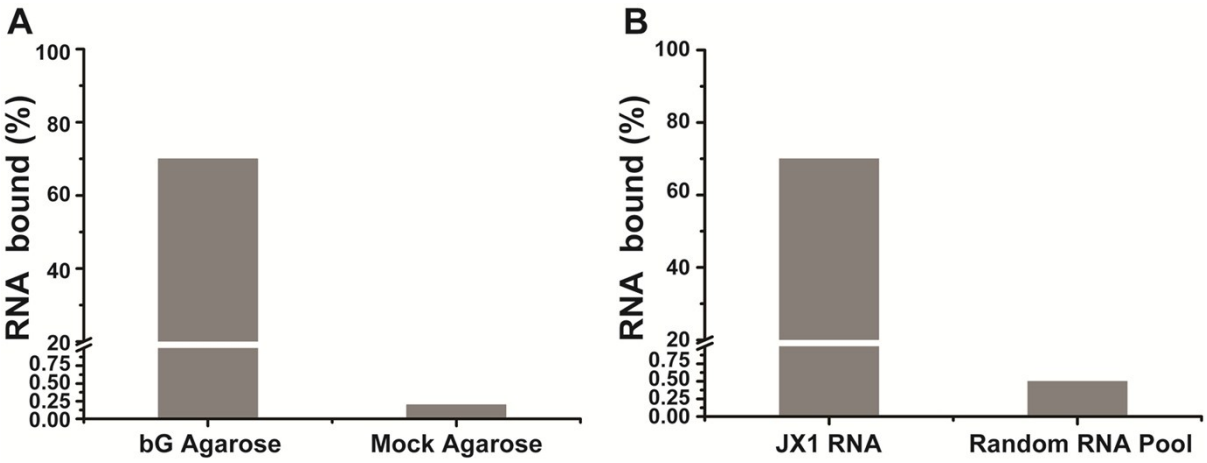

Figure S2. Binding of the JX1 aptamer to bG agarose. (A) The JX1 aptamer specifically binds t mo bG agarose but not to a mock agarose resin lacking bG. (B) JX1 binds to bG agaroseuch more efficiently than the starting random RNA pool used in SELEX.

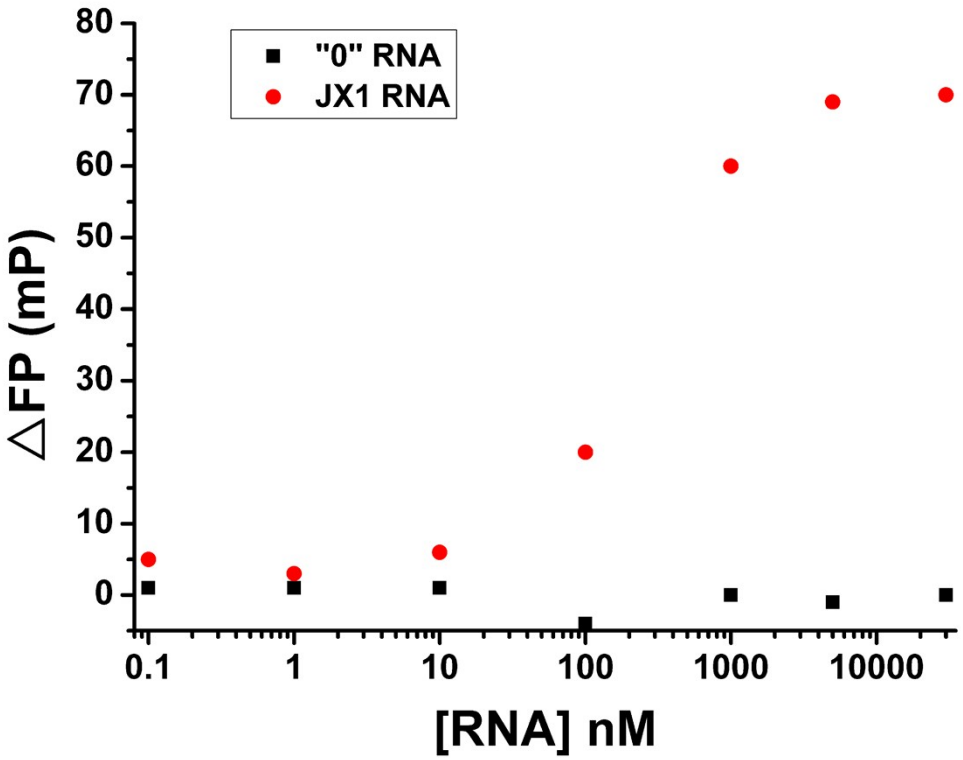

Figure S3. The starting, random RNA pool (black squares) shows no change in FP when incubated with Atto488-bG compared with the JX1 aptamer (red circles).

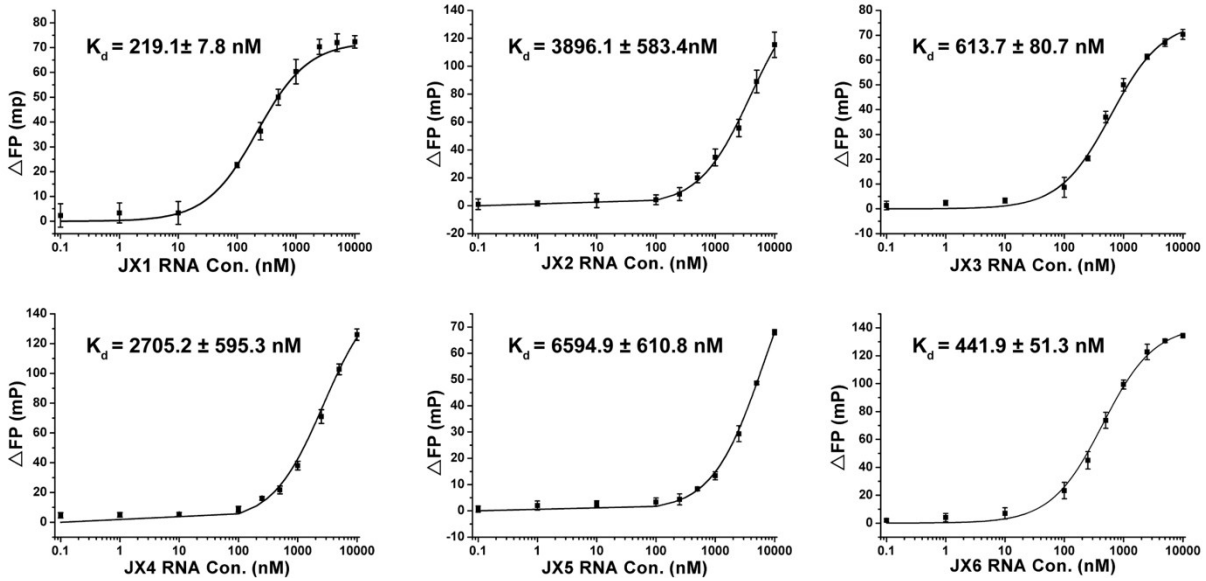

Figure S4. Binding affinity of the JX1-6 aptamers for Atto488-bG as measured by FP. Each point represents the average of three measurements ±S.D.