Supporting Information for:

Facile detection of aminoglycoside antibiotics using RNA aptamers and gold nanoparticle probes.

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Further Information for Materials and Methods

AMG biotinylation and immobilisation and purification

Apramycin, gentamicin, kanamycin, neomycin, paromomycin and tobramycin were incubated at five-fold molar excess over the NHS-PEG_{12}-Biotin in PBS for 30 min, at room temperature on a roller. Streptomycin was incubated at a ten-fold molar excess with hydrazide-PEG_4-biotin overnight, under the same conditions. Reactions were terminated through the addition of a ten-fold molar excess of glycine. All reactions were analysed by LC-ToF-MS (Agilent 1200 series LC connected to an Agilent 6224 ToF-MS) using a Waters (Hertfordshire, UK) Acquity HSS T3 50 x 2.1mm 1.8 µm column with mobile phases of a) 0.1% formic acid in water and b) 0.1% formic acid in methanol and a gradient elution of 5% B to 99% B in 15 minutes. Positive-mode electrospray ionisation was used. Data analysis was carried out using Agilent MassHunter software. As a confirmatory measure a small portion of the biotinylated AMGs were incubated with an excess of streptavidin magnetic beads (SA-MB) and bound AMGs partitioned then eluted in a 100 fold excess of free biotin at room temperature for three days on a roller. The AMG samples were analysed pre and post bead incubation, and post biotin competition elution in order to confirm immobilised. For SELEX, SA-MBs were saturated with biotinylated AMG at 4°C, overnight on a roller. Mono-biotinylated aminoglycosides were isolated to a purity >95% using an Agilent 1260 LC Prep system (Waters XBridge Prep 5 um OBD 19 x 100mm column) connected to an Agilent 6120 Quadropole MS with Agilent Chemstation detection software. A gradient of 5-95%(v/v), water/methanol plus 0.1%(v/v) formic acid, over 8 min with a flow-rate of 20 ml/min and detection was in positive ion mode. Expected and observed masses for mono-biotinylated AMGs are shown in Supporting Information Table S2.

SELEX and cloning

The initial dsDNA library consists of approximately 10^{15} sequences, each containing an N_{30} random region, flanked by fixed primer regions; one of which carries the T7 RNA polymerase promoter. Aptamer selections were carried out on a Biomek 2000

liquid handling system, using protocols based on those described in detail elsewhere[4,36]. In order to isolate aptamers which recognise the features common amongst the AMGs, a series of toggle selections were performed, alternating the selection target between pairs of structurally similar AMGs. The toggle pairs were as follows; gentamicin with apramycin, kanamycin with tobramycin and paromomycin with neomycin. The toggle partner for streptomycin (dihydrostreptomycin) could not be successfully immobilised, so no toggle was carried out during that selection. PBS supplemented with 10% (v/v) methanol was used for all binding and washing steps. In the first 5 rounds, the aptamer pool was counter selected against underivatised streptavidin beads, prior to incubation with the selection target. Bound species were eluted by thermal denaturation before being amplified by RT-PCR, to create an enriched library for the subsequent round. In rounds 6 to 11, bound RNA species were eluted by a competition wash with a high concentration (1 mg ml^{-1}) of non-biotinylated AMG.

Aptamer pools eluted from round 11 were cloned into the pGEM-T Easy Vector system (Promega UK) and subsequently used to transform XL10-Gold Ultracompetent *E. coli* (Stratagene UK) following manufacturers protocols. For each aptamer pool, 2x96 well culture plates were inoculated using an automated colony picker and the inserted sequence amplified out using primers specific to regions of the pGEM-T vector to yield a ~350 bp PCR product that contains the aptamer sequence. This was then amplified using aptamer specific primers to 'release' the 100 bp aptamer template. PCR products were checked using the 5K DNA assay kit on the Caliper GXII capillary electrophoresis system. Aptamer clones were identified by the presence of the ~350bp product in the pGEM-T primer amplification and the 100 bp product in the aptamer amplification. Sequences cloned successfully were transcribed using the Y639F variant T7 RNA polymerase and 2'-F CTP and UTP and purified using Rnaclean resin ready for analysis.

Preparation of GNPs

26 nm GNPs were prepared from $HAuCl_4$ by the citrate reduction method. All glassware was initially washed and submerged for 10 mins in aqua rega which is a

3:1 mixture of hydrochloric and nitric acid. This was followed by thorough rinses with pure water. A 1.05 mM $HAuCl_4$ aqueous solution (100 ml) was prepared , adjusted to pH 7 using 1 M NaOH and then heated to reflux in a round bottomed flask on a stirrer hotplate . A solution containing 294 mg of tri-sodium citrate dissolved in 10 ml water was then added to the $HAuCl_4$ solution. The resulting solution was then refluxed for a further 30 minutes until a constant wine red colour emerged. The solution was then allowed to cool to room temperature to form the 26 nm GNP stock. The stock concentration of the 26 nm GNP particles was on average 0.5 nM (this varied a little from batch to batch), calculated from the peak plasmon absorbance (530 nm) using the Beer-Lambert law (extinction coefficient 3.0×10^9 $M^{-1}.cm^{-1}$). We found that each batch of gold nanoparticles was different. Each batch required different concentrations of NaCl to cause aggregation and that the sensitivity of the AMG-GNP assay altered with each batch.

Structure probing

10 µg of LGA11 and LS13 aptamers (2'-OH transcripts) were dephosphorylated using Antartic phosphatise (New England Biolabs (NEB) M0289S) following manufacturer's instructions. 5 µg of the dephosphorylated RNA was then 5'- labelled with ATP-γ-P using T4 polynucleotide kinase (NEB M0201S) following manufacturer's instructions. A decade marker (Ambion, 7778) was radio labelled in the same manner and cleaved following manufacturer's instructions. The radio labelled RNA were gel purified by electroelution, ethanol precipitated and resuspended in 25 µL of nuclease free water. The two radio labelled aptamers were cleaved with gradients of RNase A, T, V and S (all sourced from Ambion) in the presence of un-labelled yeast RNA then ethanol precipitated and resuspended in 5 µL of RNA loading buffer. Samples were analysed by 20 % denaturing PAGE and the cleavage patterns compared to the secondary structure predictions made by Mfold.

Tables

AMG	No. of rings	pKa experimental (theoretical)	pI	Approx charge at pH 7	H-bond acceptors	H bond donors	Rotatable bonds
A	4	8.5(13.01)	10.92	5	16	11	6
D	2	7.8	11.79	3	15	13	9
G (C_1)	3	8.2(13.16)	11.38	5	12	8	7
G (C_2)	3				12	8	
G (C_{1a})	3	(13.16)			12	8	6
K (A)	3	7.2(12.75)	10.81	4	15	11	6
K (B)	3				15	11	
K (C)	3				15	11	
N (B)	4	(13.19)	10.97	5	19	13	9
P	4	(12.90)	10.94	4	19	13	9
S	3		10.72	3	19	14	9
T	3	(13.13)	11.11	5	14	10	6

Table S1. Physical and chemical properties of AMGs

(http://www.drugbank.ca, http://www.chemicalize.org, http://www.chemspider.com, Reference 44)

Aminoglycoside	Expected mass (Da)	Observed (m/z)	Assignment
Mono-biotinylated-A	1365	1388.0	(M+Na) 705.4 (M+2Na)
Mono-biotinylated-G	C1 - 1303 C1a – 1275 C2 – 1289	1325.9 1299.0 1312.7	(M(C1)+Na) (M(C1a)+Na) (M(C2)+Na)
Mono-biotinylated-K	A – 1310 B – 1309 C – 1310	1332.9 1310.8 656.1 678.0	(M(A or C)+Na) (M(B)+H) (M(A or C)+2H) (M(A or C)+2Na)
Mono-biotinylated-N	1440	1463.0	(M+Na)
Mono-biotinylated-P	1441	1464.0 721.5	(M+Na) (M+2H)
Mono-biotinylated-S	1068	1069 546.3	(M+H) (M+Na+H)
Mono-biotinylated-T	1293	1315.6 647.4	(M+Na) (M+2H)

Table S2. Mass spectrometry analysis of AMG biotinylation reactions

Aptamer	Random region sequence (5'-3')	Aptamer	Random region sequence (5'-3')
LGA1	ACUUCGGCUUGAACUCGAACCUAUCAGGCG	LPN1	AACGUUUCUUUUUCGGCGGUGCUUGUUGU
LGA 2	CUUGACGUCGACCACUAUUACUUUUUUCG	LPN2	AGUAUCGCCUGUUUUAUAAGUCUCAAAGUU
LGA 3	CCGGGCGAUCUCUAAAUAUGUUUUUUCUGU	LPN3	GUUAAGUAUACCGUCUAUGGUUUUGGGGGC
LGA 4	CUAAUCAACAUUGAUCUGUUAUGGUGAU	LPN4	UGAGGUAUCUCAUCGAGUUGGUUAUUUUGC
LGA 5	CUACCCUUUAUUUUGUAAAAGGUGUUCAAU	LPN5	CACGCCCUCACCUUAAAUUGCCCAUGAGUG
LGA 6	AGGACAUUAAUCCGUCCUUUAUCGCGGGUA	LPN6	GGGGCUUCUAUUAUUUGAUGUGGUUCUGAG
LGA 7	UUUUCAGGAGUUAGUGGAUUGCUCUUCGCU	LPN7	AGUUUUUAUAUGGGGUCUUUUUGUUGGGGC
LGA 8	GUAUAACGAUUUCUAGCCAGGGAACGUUUG	LPN8	UUGCACCGCCAAUCGCUUUAUUAUCUACUC
LGA 9	CCUUGGUACCGUUUGUUCCUUCGCCCUCAC	LPN9	UUCAGUGGCUUACGUACUCAAUUAGUCAGU
LGA 10	CUUAUUACUUAUGGGCCUGACCCCAUGUUU	LPN10	UCCAUGUUUGAUUUUUAAUCCGAGCAUCGU
LGA 11	GGCCCGACAUUCCCCUAAAAAAGCUUGUUC	LPN11	CUGUAGGUUCGGGCGUCCAACCUUUGAUUA
LGA12	CUACCGUUCUUGCGUUGUUGGAGGUGUAAA	LPN12	GUCCAUGCCGCGUUAUGGGAAUUUACUUUA
LGA13	CCCUAAUGGACACAAUGAGGCCCCACCGU	LPN13	CUAUGCGAUACGAGUACUUGGCACAAUUUU
LGA14	CAGGAUUUGGGGUCUCCGAAAUAUUUGUCA	LPN14	CUCUCCAAAUGCCGUUAAUGAUCUAUUCUA
LGA15	UAAUCUUCUUAUCGAAUCCCCCCCUCCCAA	LPN15	CAAUGAAUUCGAGUUCCCAUUUGCUUUCUU
LGA16	GCUUGCUUUUACUCCGAAGUUUAAGCCAUC	LPN16	CUGCGAGGAAUUCUUUGGUUAUGGUCCCAU
LGA17	AUUAUUUUGUCCGUAGUUUUUUGACACGAU	LPN17	UAUCCAGAUUUGCGUUAAAGUCUGUGGGAC
LGA18	CCUCUUCGCUAGUGUGUACGCGGAGUCUUG	LPN18	AGCCAUAGAUGUCCCGCGUGCGUUGUGAAU
LGA19	CUAUUUCUCUUUCAUUGUUUGCGUUGGCUU	LPN19	UCAGCUAAAGCAUUCUGAGUUCUCUUUCGU
LGA20	AUUCCCACCGGGUCUCUAGGGCAACUACAAC	LPN20	CCGGUGUAUGGCCUGCUAUUAAAGAUGUAC
LKT1	UCUCUCUUUUUUCUUUCUCCCCCGCUUUAA	LS1	CCAUACUUCCUUCCCGCGGUAUUGCGCAUA
LKT2	UUUUGGCGUAGUUACUAGUUCGUGUUAACC	LS 2	CCGCAGUGGAAAGAAUGAGGCCAAUAAGGU
LKT3	UAUAUUCGUUUAAAUAGAAUUUUAGUACA	LS 3	AGGCGUCUGCUCCGGGCACCACAGCCUCGU
LKT 4	GCCACAGACGAAAAAUGGAGUGGAACACGG	LS 4	UAUGUGAUCCGAUGGUGGUUUUUUAUCUUG
LKT 5	ACUUGUUUGCCACCUAGUUUAGGUAUUGGA	LS 5	UCCCCAGUUGAGUGAUGACCGACGUUCAUU
LKT 6	UUUCCGAGGUUCUAGUUAUCGUCUCUCUUU	LS 6	AGGCCCACACACUACAUCUGAUUUUAGUUA
LKT 7	CCAGUCUGAUCUUUUUAUUUUUGUCGCUCC	LS 7	CGUGAUAGGGGGGGUUGUUUGUCUGAGUUG
LKT 8	GUUAGUACUCAUGCUUUGAUGUCCCAUUAA	LS 8	AUGCUUUCUCCCUAUUUUUGCAACGUUAUC
LKT 9	CUCGGUUUCGCCUAUUUUAUUGUUUGACUG	LS 9	CGGAGAAUCUAAGUUAAUUAUCAUAAGAUU
LKT 10	UCUUCCAUCUCAGUCUAUUUGUCCGGCAUC	LS 10	UGACUGAUCCUUGCAUAUAUCGGUUUCCAU
LKT 11	GUUUUGGAACCUUUUCGGCUUUUUCUGUG	LS 11	CGUGCGUACUCCAUGAUUCUUACGGUUUUG
LKT 12	ACAGUCCUAUUCAAUUAUAACUGCCAUGAA	LS 12	CCCGGACCGAGCUCGGCGGGUCAGAUCUUU
LKT 13	GGUAAGUUGAACAAGUUAGUAUUAGUCAUC	LS 13	CCAGUUUUAUUUGUUUUAUUGUUAUAUGCU
LKT 14	ACCCUAUCGGGUUUCAUAUUCAUUUCUGUA	LS 14	CACAUUGCUCUGCAUUUACAUUAAGAGGAA
LKT 15	GGUUCUACUUGAAUACCCUGCAGUAAGUUC	LS 15	UUUUCCGUUGUUUACCAUUACGUUAUUGUA
LKT 16	UUGUGGCUUUCCCUGGACCGAUGCUUUAGG	LS 16	CGUGCGUUCUCAAGUCCGCCUCAUCCCUCA
LKT 17	CGCCUCCAGAUCAGCUGUUCUGUAUUCUCU	LS 17	GUUCUUGGCUUUUUUAUUUUUAAUCGCAUG
LKT 18	CGGGGUUUCCUAACAGCACAGUCUUUAUCA	LS 18	CGUACCUAUGUCGGAAUUGUAUAGCACU
LKT 19	GCGCCUGUUUUCCCCUGCUGGUUACUUCUC	LS 19	GAAAUCUUCCAGGAAUCAGCCGUCCCUCUGA
LKT 20	ACACCUUCGAAGUCUCGGUUUUUGAAGUUU	LS 20	GGCCCCCUUUAAUUGCUUUGUAGUUUCGAU

Table S3. Aptamer sequences. The random region sequences of 20 of the aptamers cloned from each selection pool are shown.

Aptamer	L	G	A	K	T	P	N	S	Aptamer	L	G	A	K	T	P	N	S
GA1	-	+	++	++	++	+++	+++	++	PN1	-	-	-	-	-	-	-	-
GA2	-	++	++	+++	+++	+++	++	-	PN2	-	-	-	-	-	-	-	-
GA3	++	+++	-	+++	+++	+++	+++	+	PN3	-	-	-	-	-	-	-	-
GA4	++	-	++	++	++	++	++	++	PN4	-	-	-	-	-	-	-	-
GA5	++	+++	++	++	++	++	++	-	PN5	-	-	-	-	-	-	-	-
GA6	+	+	++	++	++	++	++	++	PN6	-	-	-	-	-	-	-	-
GA7	-	-	+	+	-	+	+	+	PN7	-	-	-	-	-	-	-	-
GA8	+	-	-	-	-	-	-	+	PN8	-	-	-	-	-	-	-	-
GA9	+++	+++	+++	+++	+++	+++	++	+++	PN9	-	-	-	-	-	-	-	-
GA10	+	+	+	-	-	+	++	++	PN10	-	-	-	-	-	-	-	-
GA11	-	-	+	-	-	+	+++	-	PN11	++	-	-	-	-	+	-	-
GA12	-	-	-	-	-	+	++	++	PN12	-	-	-	-	-	-	-	-
GA13	-	-	-	-	-	+	++	++	PN13	-	+	-	-	-	-	-	-
GA14	-	-	-	-	+	+	++	++	PN14	-	-	-	-	-	+	-	-
GA15	-	-	+	-	++	++	++	++	PN15	-	-	-	-	-	-	-	-
GA16	-	-	++	+	+	+	++	+	PN16	-	-	-	-	-	-	-	-
GA17	++	++	++	++	++	++	+++	++	PN17	-	-	-	-	-	-	-	-
GA18	-	-	-	-	-	-	-	-	PN18	-	-	-	-	-	-	-	-
GA19	-	-	+	-	+	+	+	+	PN19	-	-	-	-	-	-	-	-
GA20	+	++	++	+	-	++	++	++	PN20	-	-	-	-	-	-	-	-
KT1	-	+	++	-	+	+++	-	+	SD1	-	-	-	-	-	-	-	-
KT2	-	+	++	-	++	++	+++	+	SD2	-	-	-	-	-	-	-	-
KT3	-	-	-	-	+	+	+++	++	SD3	-	-	-	-	-	-	-	-
KT4	-	-	-	-	-	+	+	+	SD4	-	-	-	-	-	-	-	-
KT5	-	-	-	+	+	+	+	+	SD5	-	-	-	-	-	-	-	-
KT6	-	+	+	+	+	+	+	-	SD6	-	-	-	-	-	-	-	-
KT7	++	+++	+++	+++	+++	+++	+++	-	SD7	-	-	-	-	-	-	-	-
KT8	-	+	+	-	+	-	+	+	SD8	-	-	-	-	-	-	+	-
KT9	++	++	++	++	++	++	+	++	SD9	-	-	-	-	-	-	-	-
KT10	+	++	++	++	++	++	-	++	SD10	-	-	-	-	-	++	++	++
KT11	-	-	+	+	++	+	++	++	SD11	-	-	-	-	-	++	-	-
KT12	-	-	-	+	+	-	++	++	SD12	-	-	-	-	-	-	-	-
KT13	-	-	-	-	-	-	+	+	SD13	-	++	++	++	-	++	++	++
KT14	-	-	-	-	-	+	-	+	SD14	-	-	-	-	-	-	-	-
KT15	-	-	-	-	-	-	-	-	SD15	-	-	-	++	++	++	++	++
KT16	-	-	-	-	+	-	++	+	SD16	-	-	-	-	-	-	-	-
KT17	-	-	-	+	+	+	++	++	SD17	-	-	-	-	-	-	-	-
KT18	-	-	-	-	+	+	++	+	SD18	-	-	-	-	-	-	-	-
KT19	-	-	-	-	-	+	+	+	SD19	-	-	-	-	-	-	-	+
KT20	-	-	-	+++	+++	+++	-	-	SD20	-	-	+	-	+	+	++	+

Table S4. Pull down assay data from all 80 clones.

Figures

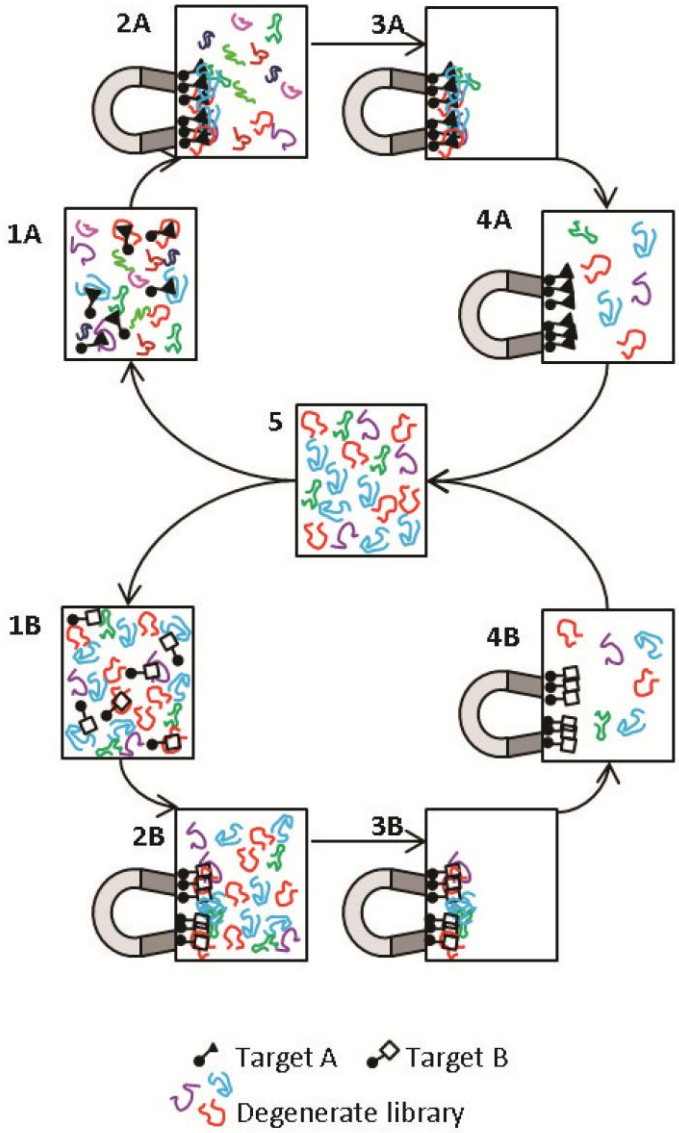


Figure S1. Outline of Toggle SELEX. Toggle SELEX alternates a pair targets at the start of each round to isolate aptamers with affinity for both targets. A degenerate library of oligonucleotides is incubated with the targets immobilised on streptavidin magnetic beads (Steps 1). Binders are partitioned (Steps 2) and non-binders removed by washing (Steps 3). Bound sequences are eluted by thermal denaturation (rounds 1-5) or competition with free target (rounds 6-11) (Steps 4) and reverse transcribed and amplified (Steps 5).

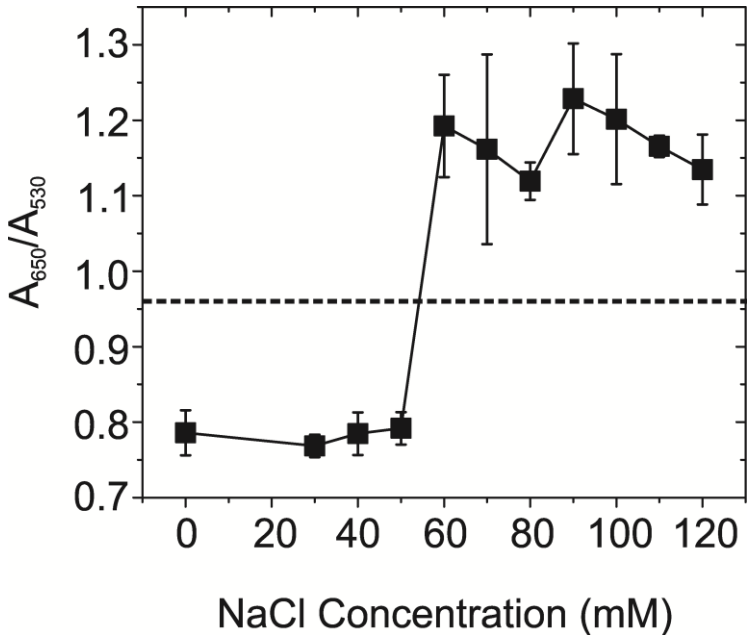


Figure S2. Salt stabilisation assay. 1M NaCl was titrated against gold nanoparticles with no RNA aptamer present. The dashed line in the graph highlights the ratio at which the visible colour change occurs: above the line the solutions are blue and below it they are red.

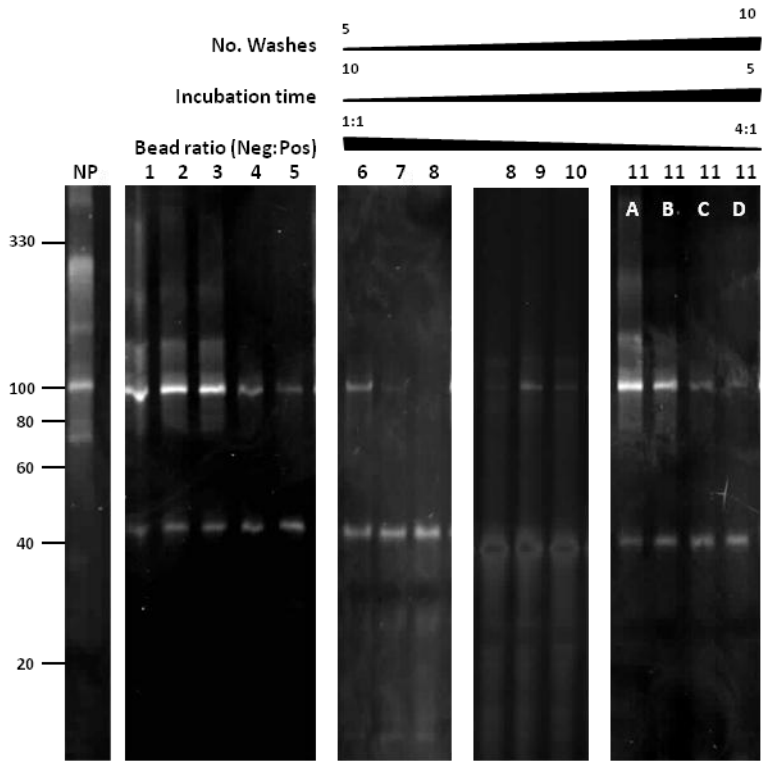
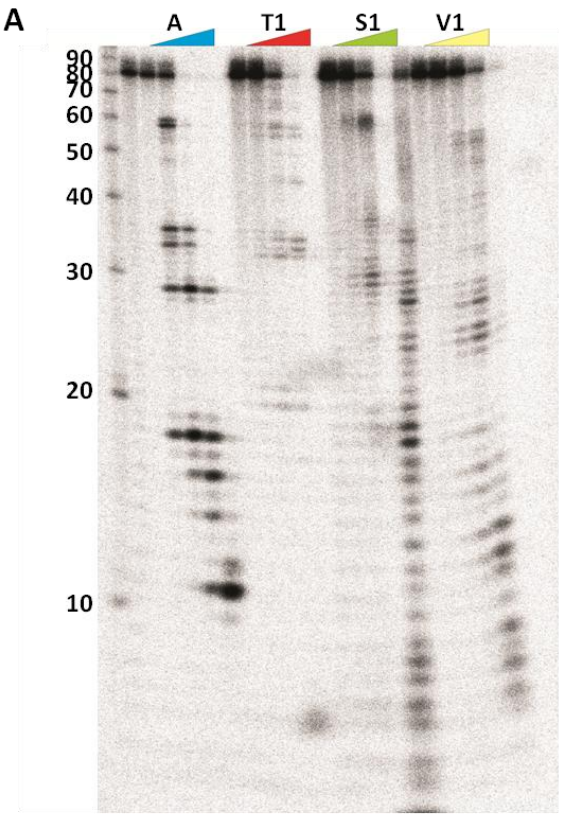
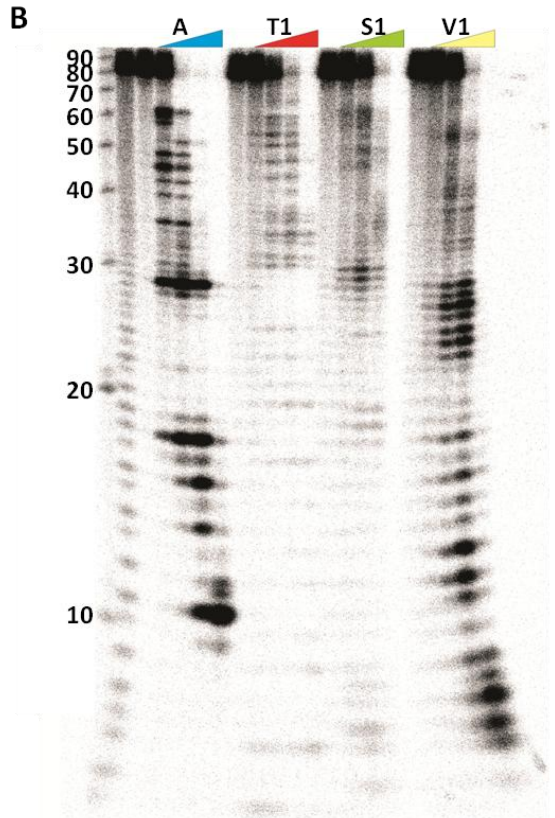


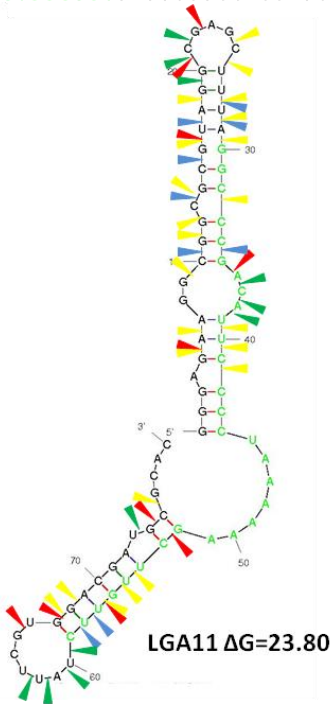
Figure S3. Example gel electrophoresis images from a single toggle selection sample (paromomycin/neomycin toggle pair). NP – naive pool, numbers indicates round number. In the final eleventh round A) is the R10 pool following amplification prior to SELEX, and elutions with B) a non-target pair AMG, C) alternate AMG of the toggle pair, and D) cognate AMG target.

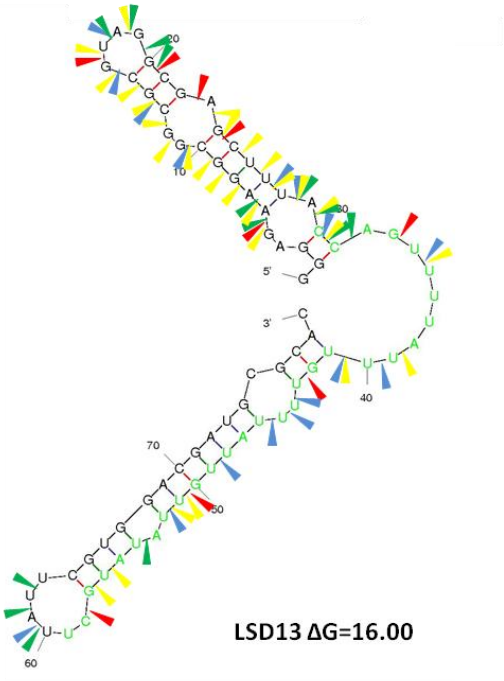




5′GGAGAAGGCGGCGCGUAGGCGAGCUUUAGGCCCGACAUU
CCCCUAAAAAAGCUUGUUCUAUUCGUGGACGAUGCGCAC3′

5′GGGAGAAGGCGGCGCGUAGGCGAGCUUUACCAGUUUUAUU
UGUUUUAUUGUUAUAUGCUUAUUCGUGGACGAUGCGCAC 3′

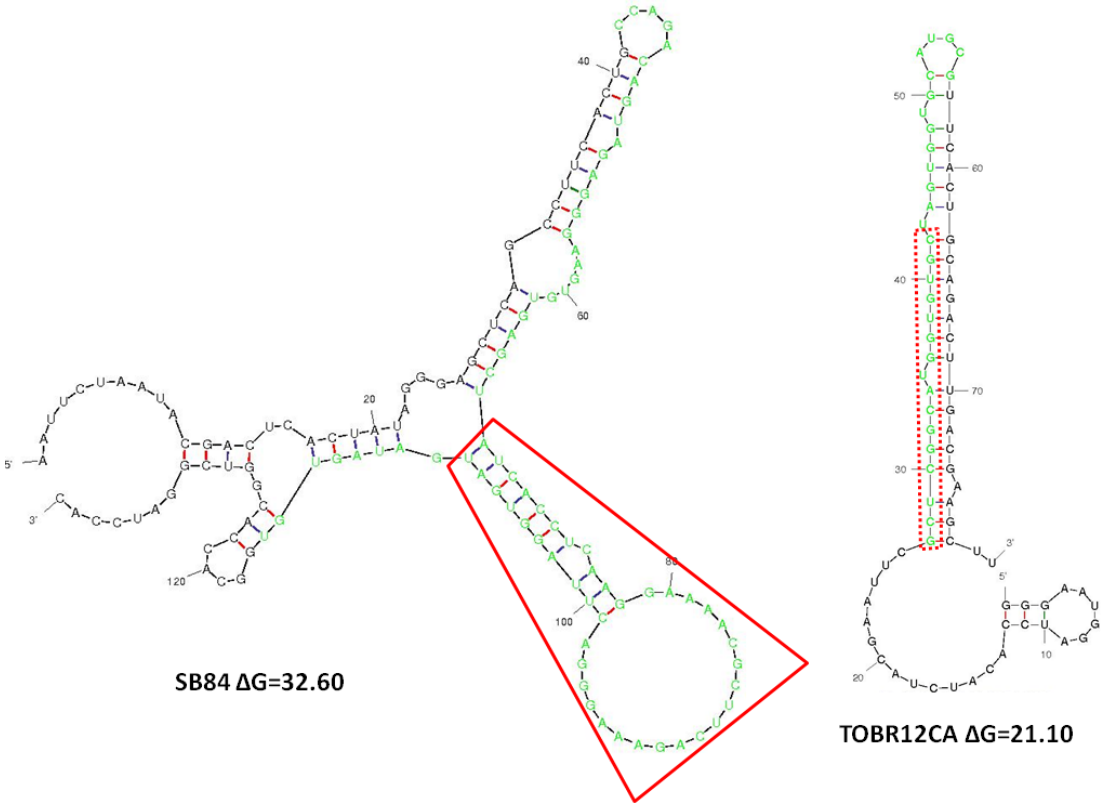




C

SB84 5′AAUUCUAAUACGACUCACUAUAGGGAGCUCAGCCUUCACUGCCAGACAGUAGAGGGAAGUGUGAGCU
AUCACCUCAAGGAAAACGCUUCAGAAAGGGACUUAGGUGAUGAUAGUGUGGCACCACGGUCGGAUCC
AC3′

TOBR12CA 5′GGGAAUGGAUCCACAUCUACGAAUUCGCUCGGCAUGGUGUGCUAGUGGUGCAUGCGUUCACUGCAG
ACUUGACGAAGCUU3′



Supplementary Figure S4. Secondary structures AMG binding aptamers. Structure probing gels[45] of LGA11 and LS13 (A and B respectively) also showing the aptamer sequence with the selected sequence region shown in green. Nuclease cleavage sites are indicated by blue, red, green and yellow arrows, for the C- and U-specific RNase A, the G-specific RNase T1, the single-strand-specific nuclease S1, and ds/helical-specific nuclease V1, respectively. These results are consistent with the predicted secondary structures from MFold[46] shown here with the mapped structure probing cleavage sites. Sequences and MFold predictions for SB84 and TOBR12CA are shown (C). Random regions in green and the mapped streptomycin binding region of SB84 highlighted by a solid red box and the conserved motif from the TOBR12CA selection highlighted by a dashed red box.

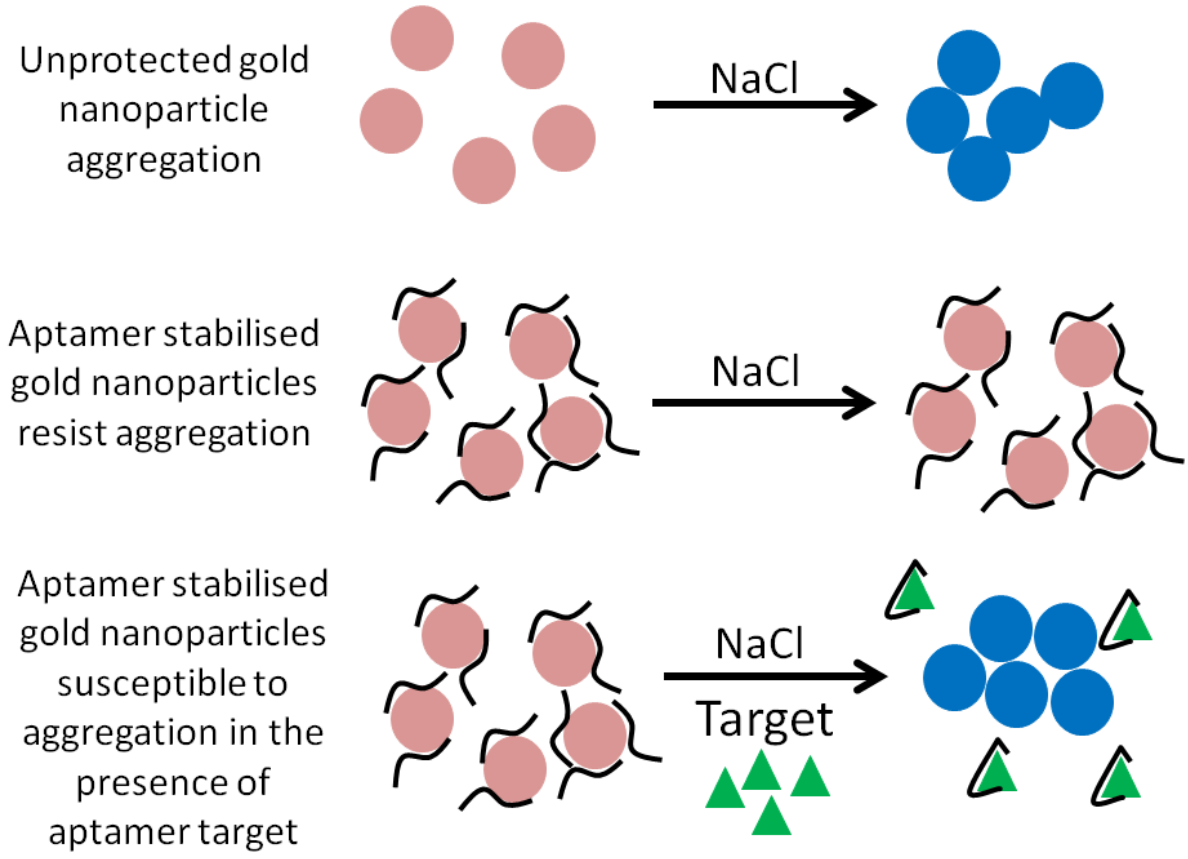


Figure S5. Schematic of GNP assay.

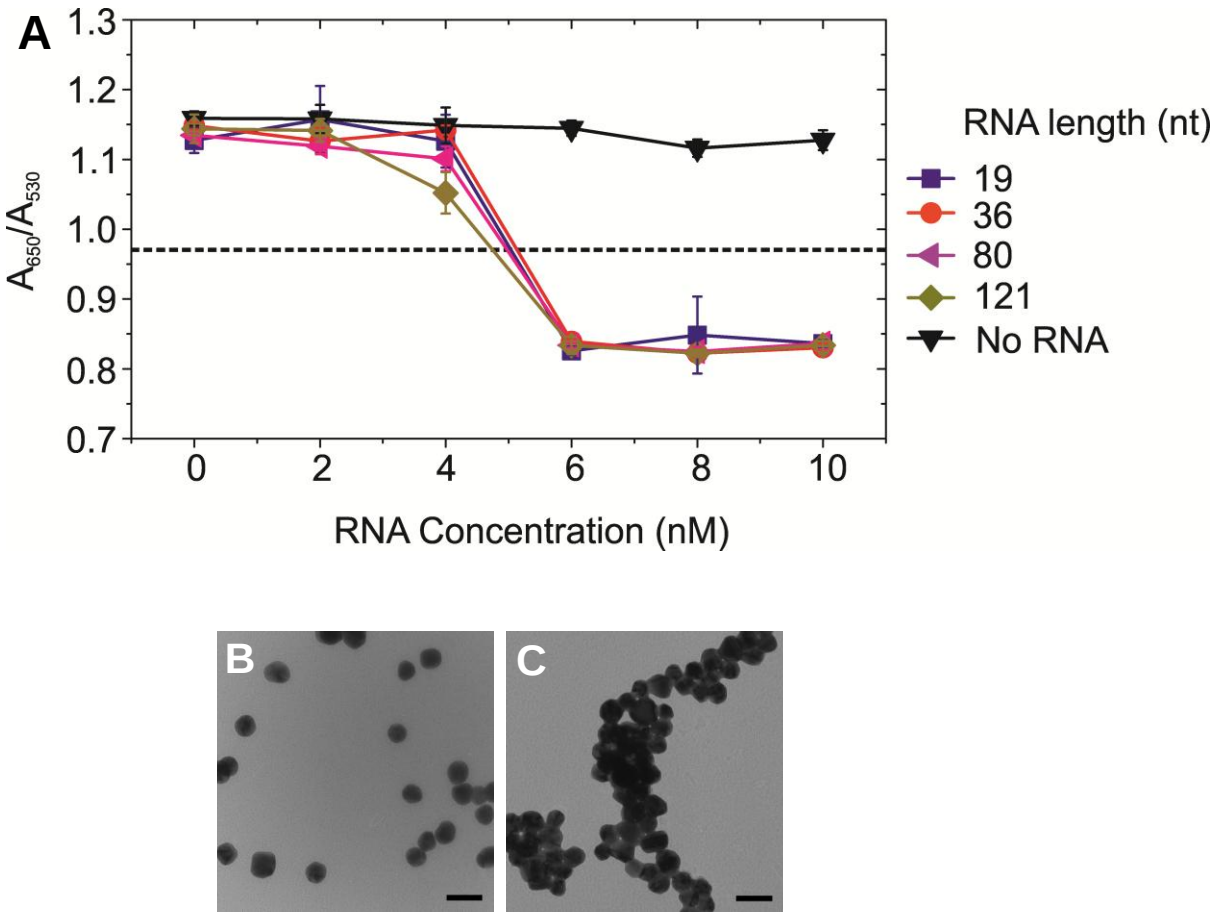


Figure S6. RNA stabilisation of GNPs. RNAs of different lengths (A) were titrated against gold nanoparticles and incubated for 1 minute before 1 M NaCl was added (61 mM final concentration). The dashed line in the graph highlights the ratio at which the visible colour change occurs: above the line the solutions are blue and below it they are red. Also shows TEM images of GNPs exposed to salt with (B) and without (C) physi-sorbed 2'-F LGA11 aptamer. Scale bars = 50 nm.

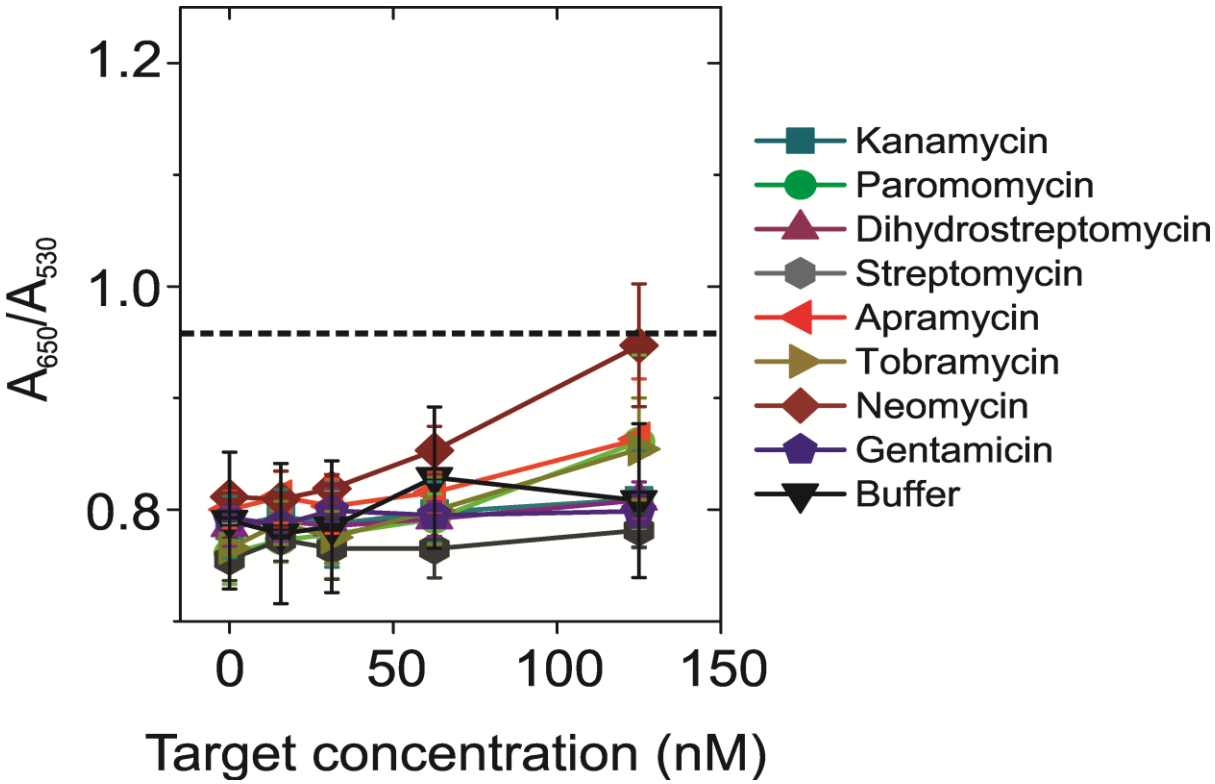


Figure S7. Naive RNA library: GNP assay against the range of AMGs. 61 mM NaCl (final concentration was used). The dashed line in the graph highlights the ratio at which the visible colour change occurs: above the line the solutions are blue and below it they are red.

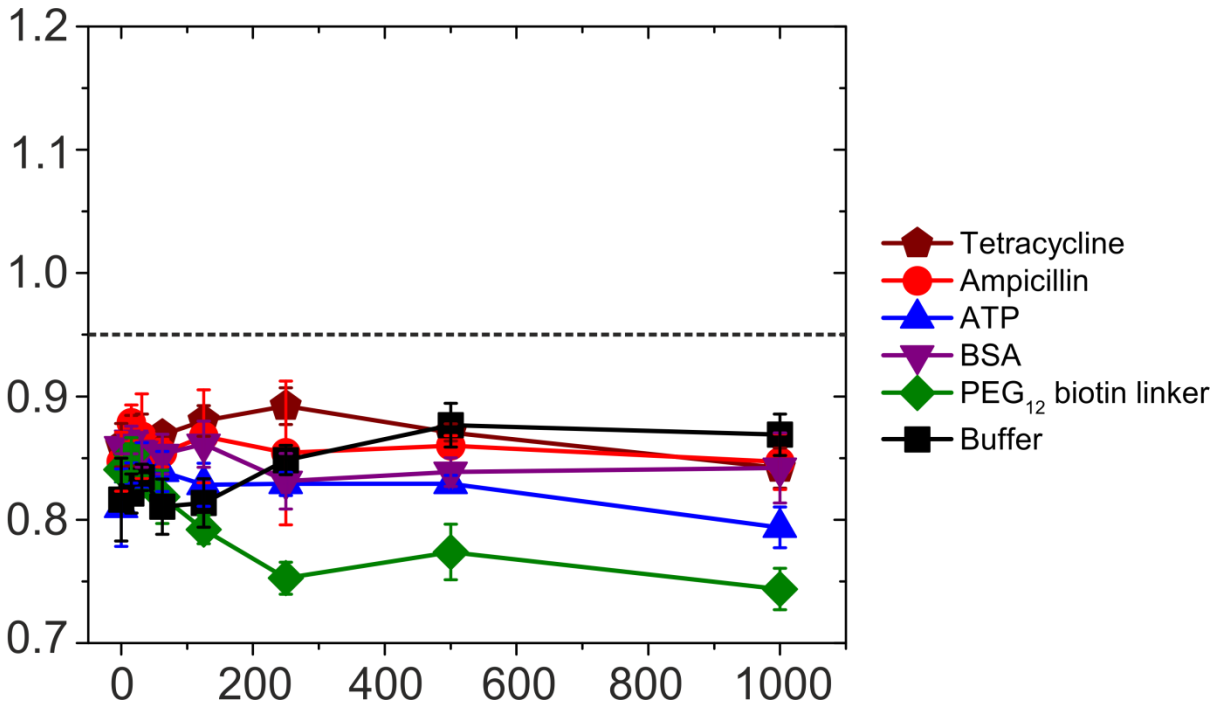


Figure S8. GNP detection to non- AMG compounds. Shows the results of the GNP-LGA11 aptamer assay against tetracycline, ampicillin, ATP, BSA and PEG_{12} biotin linker. 61 mM NaCl (final concentration was used). The dashed line in the graph highlights the ratio at which the visible colour change occurs: above the line the solutions are blue and below it they are red.

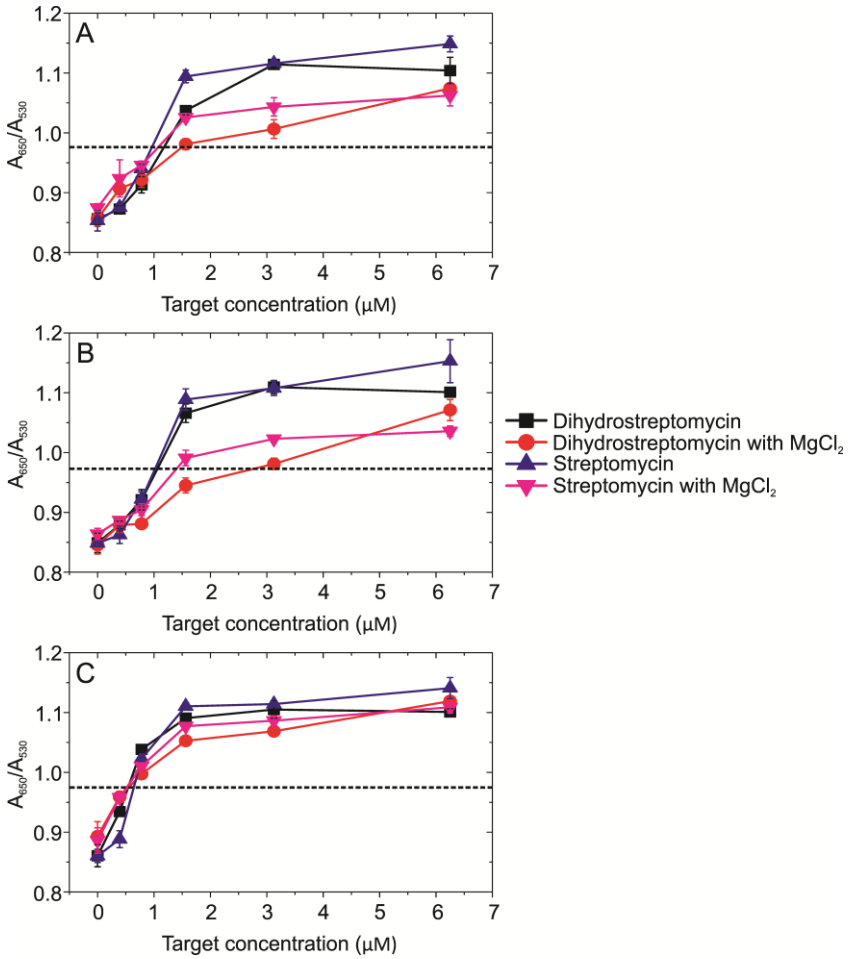


Figure S9. GNP detection of streptomycin and dihydrostreptomycin. Shows the results of the GNP-LS13 (A), GNP-SB84 (B) and GNP-TOBR12CA (C) aptamer assays against streptomycin and dihydrostreptomycin in the presence/absence of 0.5 mM $MgCl_2$. 61 mM NaCl (final concentration was used).

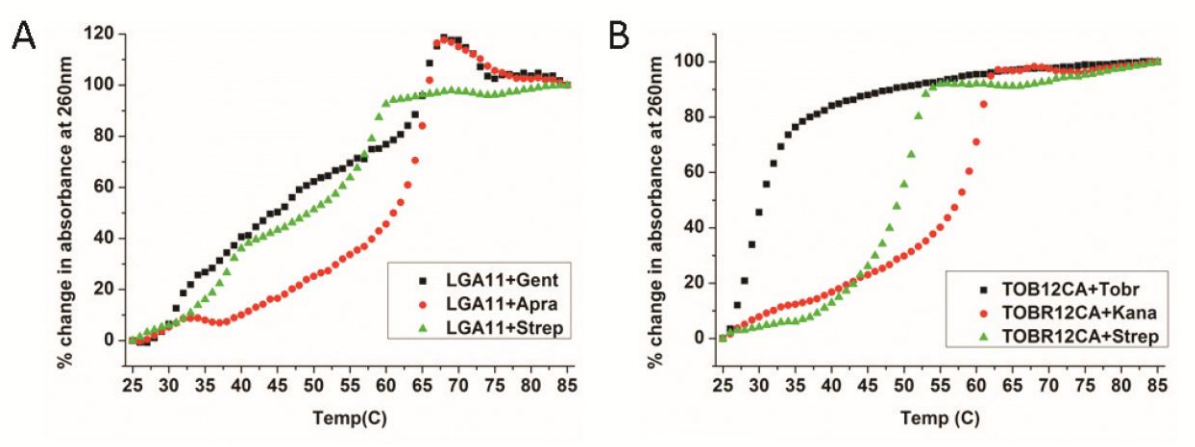


Figure S10. Solution ligand binding by the anti-AMG aptamers. LGA11 (**A**) and the 2'-F version of TOBR12CA (**B**) were incubated with their selection targets and other AMGs (Gent- gentamicin, Apra- apramycin, Strep- streptomycin, Tobr- tobramycin, and Kana- kanamycin) at equimolar concentration then diluted to 400 nM in urea (6 M final concentration) and heated from 25 to 85ºC and back in 3 cycles. The absorbance at 260 nm was observed and plotted as the % change vs. temperature.