# Aminoglycoside Microarrays To Explore Interactions of Antibiotics with RNAs and Proteins

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**Abstract:** RNA is an important target for drug discovery efforts. Several clinically used aminoglycoside antibiotics bind to bacterial rRNA and inhibit protein synthesis. Aminoglycosides, however, are losing efficacy due to their inherent toxicity and the increase in antibiotic resistance. Targeting of other RNAs is also becoming more attractive thanks to the discovery of new potential RNA drug targets through genome sequencing and biochemical efforts. Identification of new compounds that target RNA is therefore urgent, and we report here on the development of rapid screening methods to probe binding of low molecular weight ligands to proteins and RNAs. A series of aminoglycosides has been immobilized onto glass microscope slides, and binding to proteins and RNAs has been detected by fluorescence. Construction and analysis of the arrays is completed by standard DNA genechip technology. Binding of immobilized aminoglycosides to proteins that are models for study of aminoglycoside toxicity (DNA polymerase and phospholipase C), small RNA oligonucleotide mimics of aminoglycoside binding sites in the ribosome (rRNA A-site mimics), and a large (≈400 nucleotide) group I ribozyme RNA is detected. The ability to screen large RNAs alleviates many complications associated with binding experiments that use isolated truncated regions from larger RNAs. These studies lay the foundation for rapid identification of small organic ligands from combinatorial libraries that exhibit strong and selective RNA binding while displaying decreased affinity to toxicity-causing proteins.

**Keywords:** antibiotics · bioorganic chemistry · high-throughput screening · RNA · small molecule microarrays

## Introduction

Microarrays are powerful platforms for conducting of high-throughput screens, and a vast number of applications using this technology have been described. For example, DNA oligonucleotide arrays permit monitoring of the expression levels of nearly every gene in an entire organism.[1–3] Protein arrays can be used to detect protein–protein interactions and enzymatic modification of proteins.[4] Small molecule arrays have aided the rapid identification of compounds that bind to proteins, thus enabling chemical genetics experiments.[5–7] More recently, carbohydrate arrays to probe carbohydrate–protein interactions have been described.[8–10] The potential to screen a large number of events in parallel with minuscule amounts of both ligand and analyte has resulted in the widespread use of this technology.

Although interactions of proteins and small molecules have been probed by use of microarrays, binding of small molecules to RNAs has not been examined in this format. The ability to screen compounds rapidly for binding to RNAs would allow the identification of new RNA-binding ligands. These molecules may serve as improved therapeutics that target RNA or, alternatively, as biochemical probes of RNA function inside cells. There is increased interest in examining RNA functions, since new roles for RNA in biochemical events have recently been discovered. These include microRNAs,[11–13] interfering RNAs[14] and RNAs controlling translation.[15–17] Identification of selective ligands for a particular RNA can aid in analysis of its biological roles inside of cells, thus leading to more complete understanding of its contribution to cellular processes. In addition, several clinically used antibiotics elicit their antibacterial effect by binding to bacterial ribosomes and inhibiting protein synthesis.[18,19] These antibiotics, however, are losing their efficacy due to increasing antibiotic resistance[20] and their inherent toxicity. Rapid screens for identifying new compounds that bind RNA tightly and exhibit decreased af-

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finity towards resistance- and toxicity-causing proteins should greatly facilitate the discovery of improved antibiotics.

We have developed an assay that allows for aminoglycoside–RNA and aminoglycoside–protein interactions to be detected in a microarray format. Aminoglycoside antibiotics have been arrayed onto glass microscope slides, and these compounds' abilities to bind both RNAs and proteins have been probed by incubating arrays with these ligands. Unbound RNAs and proteins are washed from the surface, and binding is detected with fluorescence. The RNAs used are oligonucleotide mimics of two rRNA A-sites and a group I intron. The proteins used are phospholipase C and DNA polymerase, which are potential models for testing aminoglycoside toxicity.[21–23] The use of aminoglycoside arrays may allow the discovery of new RNA-binding ligands with enhanced binding affinities for their desired target sites diminished binding to toxicity- and resistance-causing[24] proteins.

## Results and Discussion

**Optimization of immobilization chemistry**: The aminoglycoside amikacin (see below) was spatially arrayed onto glass slides with a robotic arrayer to test several different types of immobilization chemistries (Figure 1). Three types of immobilization were explored: 1) amine-coated glass slides treated with tetraethyleneglycol disuccinimidyl disuccinate (polyethyleneglycol, PEG), 2) slides coated with a layer of bovine serum albimin (BSA) that had been treated with *N*,*N*′-disuccinimidyl carbonate, and 3) aldehyde-coated slides. For each immobilization, the arrayer delivered ≈2 nL of a 5 mM aminoglycoside solution to defined positions on the surface. After arraying, slides were incubated at room temperature overnight, and unreacted succinimide esters or aldehydes were quenched with ethanolamine. Several different quenchers including butylamine, glycine, and ethanolamine were tested. Quenching with ethanolamine yielded the highest signal with the lowest background (data not shown).

To determine if immobilized compounds retain their ability to bind to RNA, slides were incubated with 100 pmoles of a fluorescently labeled oligonucleotide mimic of the bacterial rRNA A-site (Figure 2). Unbound oligonucleotide was washed from the slides, before the slides were scanned. Signal from aminoglycoside bound to the bacterial rRNA A-site is well above background for each of these surfaces. The strongest signal was observed with succinimide ester immobilized aminoglycosides. Within this series, slides coated with the PEG linker gave the strongest fluorescence (Figure 1). Immobilization onto aldehyde slides resulted in significantly less signal than observed for immobilization onto the other surfaces (≈tenfold).

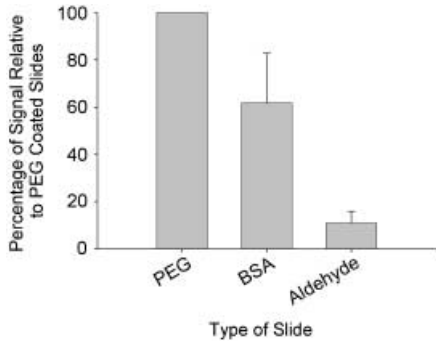



Figure 1. Testing of different immobilization chemistries for detection of RNA binding to immobilized aminoglycosides. The plot is the amount of fluorescent signal exhibited from immobilized amikacin binding to the bacterial RNA.

**Binding of RNA to immobilized aminoglycosides**: A series of aminoglycosides and other small molecules (Figure 3) was arrayed onto PEG-coated glass slides, and the compounds were tested for their abilities to bind oligonucleotide mimics of rRNA A-sites (Figure 2). All immobilized aminoglycosides bind to the bacterial oligonucleotide (Figure 3). Amikacin has the highest fluorescence intensity, followed by lividomycin, paromomycin, neomycin, and tobramycin, which all have similar intensities. For the human rRNA mimic, amikacin also has the highest spot intensity, followed by kanamycin, neomycin, bekanamycin, and tobramycin, respectively. In each of these experiments, little or no signal was observed from 2′-aminoethyl α-D-mannopyranoside, spermine, and spermidine negative controls (Table 1).

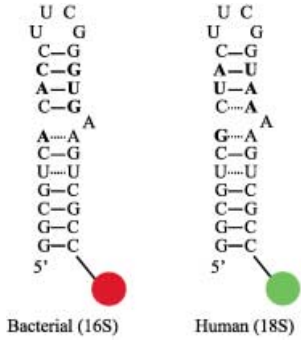



Figure 2. The oligonucleotide mimics of rRNA A-sites that were incubated with the aminoglycoside arrays. The bacterial oligonucleotide (16S) has been shown to be the binding site for some aminoglycosides in the ribosome.[19] The human oligonucleotide (18S) has been tested for aminoglycoside binding in MS experiments.[25] Each oligonucleotide was fluorescently labeled. The bacterial RNA is labeled with TAMARA and the human with fluorescein.

Binding of some of the aminoglycosides used here to various A-site mimics has been studied in detail by mass spectroscopy (MS),[25] surface plasmon resonance spectroscopy (SPR),[26] fluorescence anisotropy and quenching,[27,28] and chemical mapping of RNA.[29] In the MS assay, tobramycin and bekanamycin bound with similar affinities to both the bacterial and human RNAs ($K_d$ values of ≈2 μM). Paromomycin, neomycin, and lividomycin bound with $K_d$ values in the low nanomolar range. For experiments here, lividomycin, neomycin, paromomycin, and tobramycin have similar fluorescence intensities. The fact that the signal intensities do not correlate exactly with these results suggests that the immobilization onto the slide affects the binding of each

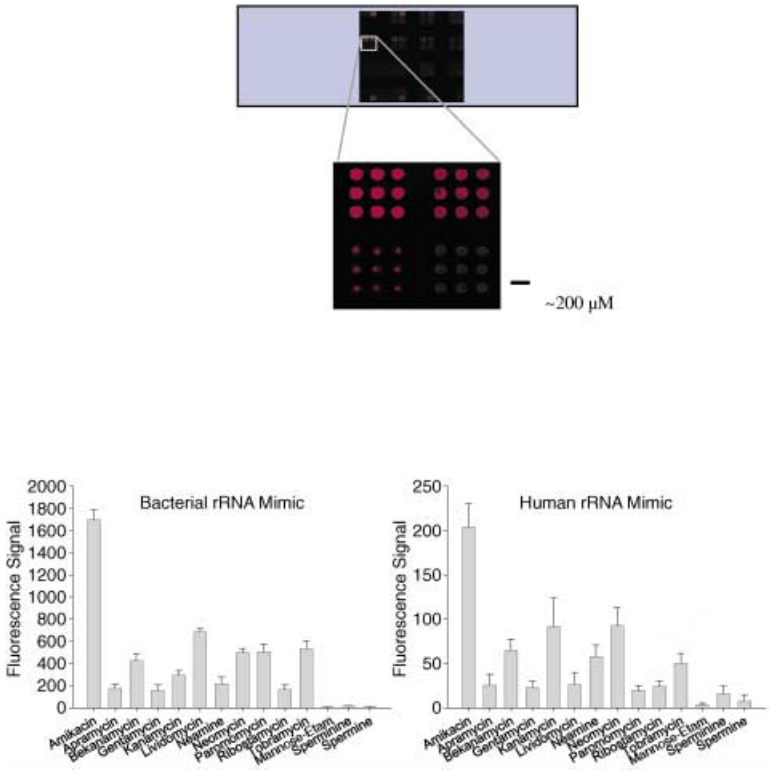
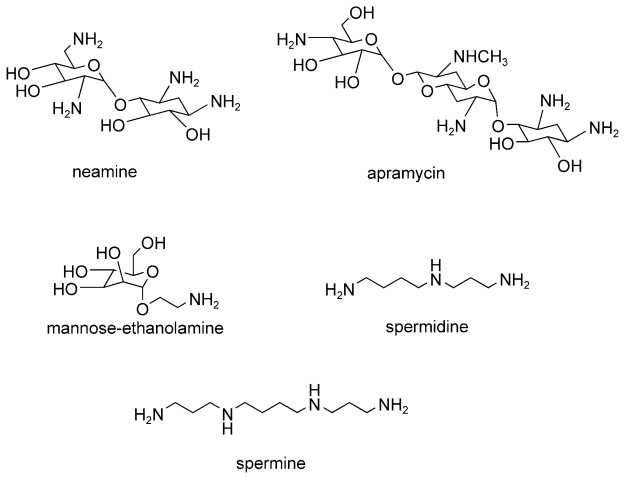



Figure 3. Binding of several immobilized aminoglycosides to rRNA A-site mimics. Top: a picture of an array hybridized with the bacterial RNA. Bottom: plots of the amount of fluorescence signal observed for each arrayed aminoglycoside binding to the different rRNA A-site mimics. Each point is the average of signals from 18 spots acquired from at least two different slides.

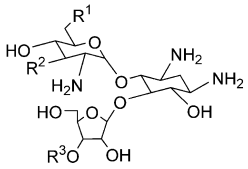



compound in a different way, most probably to do with the number and type (secondary and primary) of amines present in these compounds. Amikacin has the highest fluorescence signal for both oligonucleotides tested. Immobilization of this compound through the primary amine on the 2,4-deoxystreptamine ring may diminish the effect of immobilization on RNA binding since it leaves the A-ring, which is important for RNA binding, unchanged.

Specificity is another critical issue for the development of new therapeutics. Several studies have determined the specificities of aminoglycosides for other RNAs.[25–29] The general consensus is that most aminoglycosides display little specificity in binding to different RNA sequences. The exceptions are some of the 4,5-linked 2-deoxystreptamine derivatives, which include lividomycin, paromomycin, and neomycin. These compounds have binding affinities to the bacterial A-site approximately ten times stronger than those of other RNAs.[25,26,28]

In the array method, specificities can be estimated by comparing relative fluorescence intensities of each aminoglycoside binding to different RNA sequences. Lividomycin and paromomycin bind at least an order of magnitude more weakly to the human RNA than to the bacterial RNA ($K_d$ values are $>20\,\mu M$ and $\approx 100\,nM$, respectively).[25,26] In contrast, bekanamycin and tobramycin bind to these RNAs with similar affinities ($\approx 2\,\mu M$).[25,26] Array data show that the relative fluorescence intensities for aminoglycoside binding change depending on the oligonucleotide incubated with the array. For example, lividomycin and paromomycin exhibit $\approx 1.5$ to two times more signal with the bacterial RNA than bekanamycin and kanamycin. For the human RNA, on the other hand, bekanamycin and kanamycin gave signals around three to

Table 1. Aminoglycoside used to construct the aminoglycoside arrays: 4,5-linked 2-deoxystreptamine derivatives (below) and other small molecules (left).



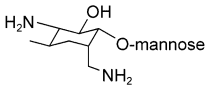
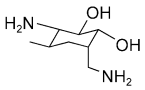
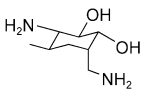
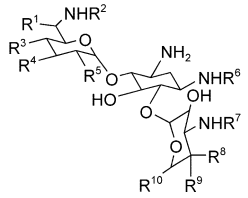
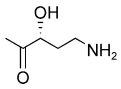
| Aminoglycoside | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| lividomycin | HO | H |  |
| neomycin | $NH_2$ | OH |  |
| paromomycin | OH | H |  |
| ribostamycin | $NH_2$ | OH | H |

Table 2. Aminoglycoside used to construct the aminoglycoside arrays: 4,6-linked 2-deoxystreptamine derivatives.



| Aminoglycoside | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ | $R^6$ | $R^7$ | $R^8$ | $R^9$ | $R^{10}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| kanamycin | H | $NH_2$ | OH | OH | OH | H | H | H | OH | $CH_2OH$ |
| bekanamycin | H | $NH_2$ | OH | OH | $NH_2$ | H | H | H | OH | $CH_2OH$ |
| tobramycin | H | $NH_2$ | OH | H | $NH_2$ | H | H | H | OH | $CH_2OH$ |
| gentamycin C1 | $CH_3$ | $NHCH_3$ | H | H | H | H | $CH_3$ | OH | $CH_3$ | H |
| gentamycin C1a | H | $NH_2$ | H | H | H | H | $CH_3$ | OH | $CH_3$ | H |
| gentamycin C2 | $CH_3$ | $NH_2$ | H | H | H | H | $CH_3$ | OH | $CH_3$ | H |
| amikacin | H | $NH_2$ | OH | OH | H |  | H | H | OH | $CH_2OH$ |

five times stronger. These results are consistent with the previously reported binding specificities.[25,26]

**Binding of arrayed aminoglycosides to a group I intron**: After initially focusing on binding of aminoglycosides to short RNA oligonucleotide mimics of a larger RNA, we sought to detect hybridization of large RNAs to arrayed aminoglycosides. A group I ribozyme derived from the *Candida albicans* self-splicing group I intron was chosen because this RNA is a potential drug target and is about 400 nucleotides in length.[30] In addition, several aminoglycosides bind to and inhibit group I intron self-splicing.[31–34]

Ribozyme was refolded under conditions previously shown to fold >90% of the intron into an active conformation.[30] Aminoglycoside arrays were then incubated with 10 picomoles of ribozyme, and unbound RNA was removed from the slide by washing. This amount of RNA is less than what is typically obtained from a single in vitro transcription.[35] Bound RNA was detected by incubation of the slide with a solution containing the nucleic acid dye SYBR green II, which has been used to detect oligonucleotide hybridization on DNA arrays.[36,37] As shown in Figure 4, amikacin, kanamycin, lividomycin, and tobramycin gave the highest fluorescence intensities. The negative controls 2′-aminoethyl $\alpha$-D-mannopyranoside, spermidine, and spermine gave little signal, further suggesting that specific interactions are being detected.

These results show that binding of large RNAs to arrayed compounds can be detected. This advancement alleviates many of the potential problems associated with current screening methods that use short oligonucleotides to mimic a part of a larger RNA. These complications are due to improper folding of the shorter oligonucleotide, because tertiary contacts that dictate the exact structure of the RNA cannot always be accounted for when designing shorter RNAs. Furthermore, post-hybridization staining of bound RNA with a dye eliminates having to attach a fluorophore covalently to the RNA, which greatly simplifies sample preparation.

**Binding of proteins to aminoglycosides**: Although aminoglycosides elicit their antibiotic effect by binding to rRNA, they also interact with proteins and lipids, and these interactions have been implicated in causing toxicity. Toxicity is mainly manifested in the kidney and the ear (ototoxicity).[38] Aminoglycoside binding to proteins may be involved in causing these side effects. Previous experiments have shown that phospholipase C[22] and DNA polymerase[23] activities are modulated by aminoglycosides, and these two enzymes have been

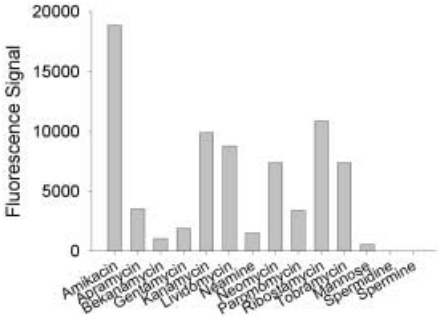



Figure 4. Fluorescence intensities from binding of aminoglycoside arrays to the group I intron from *C. albicans*. Arrays were incubated with 10 picomoles of group I intron RNA. After unbound RNA had been washed away from the slide, bound RNA was stained with SYRB green II nucleic acid stain.

used to study how aminoglycosides interact with proteins, since they may serve as models to probe aminoglycoside toxicity.

Incubation of phospholipase C with the aminoglycoside arrays shows that every aminoglycoside binds this protein. Amikacin exhibits the strongest interactions, followed by paromomycin, gentamycin, and tobramycin. Previously, several of the aminoglycosides studied here had been shown to activate phospholipase C.[22] Fluorescence intensities (gentamycin > neomycin > kanamycin) do not correlate with the order of activation (kanamycin > gentamycin > neomycin). This discrepancy, however, may be due to interaction of these compounds with different sites of the enzyme. In addition, binding affinity may not always correlate with inhibition of enzymatic activity.

The activity of Klenow DNA polymerase is inhibited by several aminoglycosides[23], and its inhibition has also been implicated in causing side effects associated with aminoglycosides. Inhibition of DNA polymerase activity is due to interaction of these compounds with divalent metal ion binding sites. This enzyme thus serves as a model system with which to test the interactions of aminoglycosides with metal ion binding sites in proteins. While several aminoglycosides

interact with Klenow DNA polymerase, however, the rather weak signals in the assay indicates low affinity between aminoglycosides and DNA polymerases (Figure 5). This is corroborated by $K_i$ values of $>100\,\mu M$ for aminoglycoside inhibition.[23]

**Comparison of aminoglycoside arrays with other techniques for study of RNA–small molecule interactions**: Techniques for screening for RNA–small molecule interactions, including a high-throughput mass spectroscopy assay, have been developed previously.[25] This method allows for binding constants to be determined and for several RNAs to be screened at once, and also provides information about the binding sites for small molecules to a particular RNA by determining sites of protection. Such experiments, however, require specialized and expensive instrumentation, unavailable to most laboratories interested in this area, and this limits the widespread use of this technique.

A SPR method to screen for RNA–small molecule interactions has also been reported.[26,39,40] The SPR experiments can be completed with immobilized RNA oligonucleotides, and binding constants for these interactions can be determined. This assay, however, is not amenable to screening large numbers of compounds at once, since only a small number of compounds can be tested in parallel.

The strategy described here has several potential advantages over these methods. For example, arrays allow many more interactions to be probed in parallel than can be completed by using either SPR or MS assays. These include the binding of several thousand small molecules to many different RNAs at once. The only modification that has to be made to a particular RNA to allow for parallel screening of sequences is the placement of a different fluorescent tag on each sequence to be probed. In addition, both weak and strong interactions can be detected by use of the arrays. For example, binding of lividomycin and neomycin ($K_d$ values are about 10 nM) and ribostamycin ($K_d$ of about 20 μM) to the bacterial RNA are all detected and have intensities that correlate with this trend.[25] Current investigations in this area include the development of an orthogonal immobilization chemistry that does not use amino groups for immobilization. Use of these amino functional groups for immobilization probably affects the A-rings in most of the aminoglycosides, which would prevent strong interactions with the bacterial RNA.[27]

**Implications for therapeutic and biochemical probe discovery**: Selectivity and toxicity are critical issues for clinical use and development of new antibiotics. Studies have implicated interactions of aminoglycosides with the negatively charged phospholipids in cellular membranes cause toxicity.[21] Additionally, aminoglycosides have been shown to inhibit or activate phospholipase C, depending on the aminoglycoside and its concentration.[22,41] Here we provide evidence that aminoglycosides directly bind phospholipase C,[22] since every aminoglycoside tested interacts with this enzyme. Aminoglycosides also inhibit several different DNA polymerases,[23] and interactions of aminoglycosides to these enzymes were detected by use of the arrays. Since side effects often limit the clinical use of aminoglycosides as antibacterial agents, compounds found to bind tightly to RNA but weakly to toxicity-causing proteins may allow for development of improved therapeutics.

The discovery of new RNA binding ligands is essential for the development of new therapeutics that target RNA and of biochemical probes for RNA function inside cells. New functions and roles of RNA in biology have been discovered recently, and include micro-RNAs and RNAs that control translation.[11–13,16,17] In addition to these targets, a plethora of other RNA targets are being identified through genome sequencing efforts. Screening of these RNAs is expected to allow discovery of new targets for RNA-based drug discovery. Further development of combinatorial libraries and screening techniques for binding of biomolecules should facilitate discovery of molecules that attenuate RNA function.

## Summary and Outlook

Several crystal structures of the ribosome or parts of the ribosome have recently appeared in the literature[42–54]. Some of these structures have been solved with antibiotics bound.[53,55–57] This information has led to the development of improved antibiotics that evade resistance.[58] Rapid screening for RNA binding—including fluorescence quenching experiments,[27,59] surface plasmon resonance (SPR),[26]

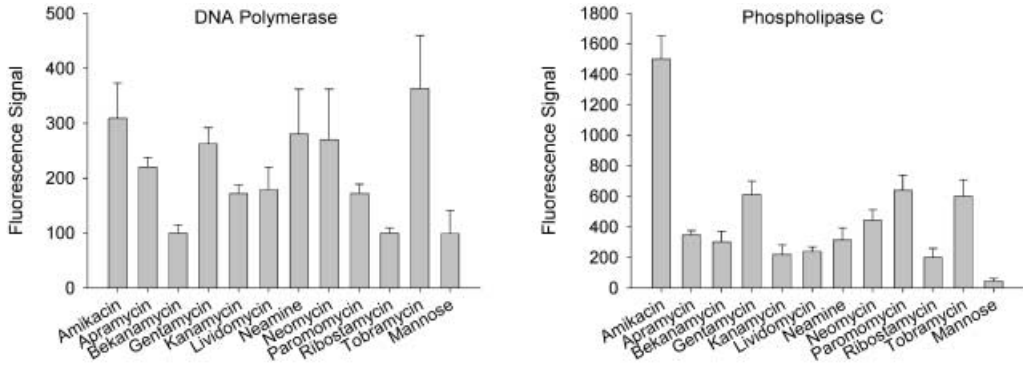



Figure 5. Binding of immobilized aminoglycosides to proteins that may model aminoglycoside-induced toxicity. Arrays were hybridized with fluorescently labeled Klenow DNA polymerase and phospholipase C.

and mass spectrometry[25]—is often difficult. The method described here can be used for rapid screening of compounds synthesized from combinatorial libraries that make use of this recent structural information for high-affinity binding to desired RNA targets and weak binding to undesired targets, which include bystander RNA and resistance-causing enzymes.[24] A major limitation of this technique, however, is the nonspecific manner in which the aminoglycosides are arrayed onto the surface. This is illustrated by the fact that amikacin binds most strongly to the RNA tested here despite the fact that, from solution measurement, lividomycin and neomycin would be expected to bind most tightly. New libraries that are immobilized through functional groups that are not known to interact with RNA will next be tested. These methods should greatly facilitate the discovery of new RNA binding ligands.

## Experimental Section

**Materials**: Neomycin was purchased from Fluka. Neamine was synthesized by methanolysis of neomycin as described.[60] The mannose derivative was synthesized as described.[61] All other aminoglycosides and small molecules were purchased from Sigma and were used without purification. BSA was purchased from Roche. Tetraethylene glycol disuccinimidyl disuccinate was synthesized as described.[62] All aqueous solutions used for chip hybridizations and washings were filtered through a 0.2 μm syringe filter prior to use.

**Oligonucleotide synthesis**: Oligonucleotides were synthesized on an Applied Biosystems automated RNA/DNA synthesizer on a 1 μmole scale. All monomers were purchased from Glen Research (Baltimore, MD). The RNA monomers contained 2′-hydroxy groups protected as their triisopropylsilyloxymethyl (TOM) ethers. Samples were deprotected by the manufacturer's standard procedure, with the 2′-TOM groups removed by incubation with TEAHF at 55 °C for 48 h. After the samples had been deprotected, they were purified from failure sequences by gel electrophoresis (20% polyacrylamide, 8 M urea). Full-length product was isolated from the gel by the crush and soak method (gel slice stirred in sterile water with a sterile stir bar). The solution containing the RNA was then applied to a Sephadex NAP 25 prepacked column to remove salts. Samples were lyophilized, resuspended in sterile water, and stored at −20 °C. Oligonucleotide concentration was determined by use of the extinction coefficients of the fluorescent dyes. The *Candida albicans* ribozyme was synthesized by run-off transcription from a DNA template, purified, and renatured as described.[30]

**Protein labeling**: Phospholipase C and Klenow DNA polymerase were purchased from Sigma. Proteins were labeled in a solution containing $\approx 1\ mg\,mL^{-1}$ of protein and 1 mg of succinimide ester fluorescent probe (Molecular Probes) dissolved in anhydrous DMF in 0.1 M sodium bicarbonate pH 8.8 buffer, as recommended by the manufacturer. Reaction mixtures were incubated for at least 2 h and were then quenched by addition of 100 mM ethanolamine. To remove the uncoupled fluorescent probe, the solution was loaded onto a Sephadex G25 size exclusion column. Fractions that contained labeled protein were placed in a buffer that contained 50 mM Tris·HCl and 20% glycerol. Proteins were stored at −20 °C until use.

**Preparation of glass slides**: GAPS II amine-coated slides (Corning, NY) were placed in a solution containing 10 mM disuccinimide (PEG or carbonate) linker and 100 mM *N,N′* diisopropylamine in DMF. BSA-coated slides were prepared by incubation of slides previously treated with disuccinimde carbonate linker with a solution containing BSA (1%) in sodium bicarbonate buffer (pH 8.8, 100 mM) for 12 h. After BSA had been covalently attached to the surface, the slides were then again treated with *N,N′*-disuccinimidyl carbonate as described above. Slides were removed from the linker solution and washed several times with ethanol or methanol, dried, and stored in vacuum. Aldehyde slides were purchased from Arayit Brand Products and were arrayed as described for DNA.[36]

Aminoglycosides were spatially arrayed onto glass slides by use of an automated arraying robot (MicroGrid II, Biorobotics) in an aqueous solution of DMF (25% *v/v*). Each spot on the slide contained ≈2 nL. After arraying, the slides were incubated in a humidity chamber overnight at room temperature. Aminoglycosides that had not reacted with the slides were washed away from the slides with water. Unreacted succinimide esters on the slides were quenched by placing the slides in a solution of ethanolamine (100 mM) and *N,N*-diisopropylamine (100 mM) in DMF for at least 3 h at room temperature. Slides were then finally washed several times with water and ethanol and stored under vacuum until use.

**Hybridization of RNAs and proteins**: The RNA oligonucleotides that are mimics of the rRNA A-site were refolded by placing the oligonucleotides in a buffer (pH 7.4) containing NaCl (200 mM) and Hepes (20 mM) and heating the solution to 60 °C for 5 min, followed by slow cooling to room temperature, as previously described.[26,39] Oligonucleotides were then placed on the slide (100 pmol) in a volume of 10 μL. A glass cover slip was placed over the slide to allow the solution to be distributed evenly. Arrays were incubated at room temperature for 1 h. To remove unbound oligonucleotide, chips were placed in a solution containing hybridization buffer, with or without Tween 20 (0.1%) as supplement, for at least 5 min. Slides were removed from the buffer, briefly dipped in water, and centrifuged to dryness, and the backs of the slides were cleaned with 70% ethanol. Slides were scanned by use of an Array WorX fluorescent chip scanner and quantified with Molecular Ware software.

For slides incubated with the *C. albicans* ribozyme, ribozyme was refolded as described, by heating the ribozyme in 1X H10Mg buffer (50 mM Hepes, 135 mM KCl, and 10 mM $MgCl_2$, pH 7.5) for 10 min at 55 °C, followed by slow cooling to room temperature.[30] Chips were incubated with 10 picomoles of ribozyme for 1 h at room temperature. After incubation, slides were placed in a solution containing 1X TBE with $^{1}/_{10000}$ diluted SYBR green II nucleic acid stain (Molecular Probes) for ≈2 min to stain the bound RNA.[36,37] Slides were then washed in TBE buffer with Tween 20 (0.1%) for 5 min, briefly rinsed with water, dried, scanned, and quantified as described above.

For experiments with proteins, the protein was placed onto the slides in a buffer containing Tris·HCl (pH 7.5, 50 mM), 2-mercaptoethanol (0.1%), NaCl (50 mM), and Tween 20 (0.01%). Slides were incubated for 1 h at room temperature, and unbound protein was removed from the slides by incubating them in same buffer as above, plus an additional 0.1% Tween 20, for 5 min. For experiments with DNA polymerase, slides were briefly incubated in hybridization buffer to remove unbound protein.

**Analysis of the array data**: Slides were scanned with an Array WorX fluorescent chip scanner and quantified by use of Molecular Ware software. The amount of signal was determined from the spot-normalized intensity. This was chosen because it corrects for the amount of signal that is outside of the spot area, thus normalizing data for noise. Data presented are the average of 18 spots, two sets of nine spots on the same array. Errors are the standard deviations for each measurement.

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