Published in final edited form as:

Anal Chem. 2010 September 1; 82(17): 7090–7095. doi:10.1021/ac101491d.

Reagentless Measurement of Aminoglycoside Antibiotics in Blood Serum via an Electrochemical, Ribonucleic Acid Aptamer-Based Biosensor

Aaron A. Rowe[†], Erin A. Miller[†], and Kevin W. Plaxco^{*,†,‡}

Department of Chemistry and Biochemistry and Biomolecular Science and Engineering Program, University of California, Santa Barbara, California 93106

Abstract

Biosensors built using ribonucleic acid (RNA) aptamers show promise as tools for point-of-care medical diagnostics, but they remain vulnerable to nuclease degradation when deployed in clinical samples. To explore methods for protecting RNA-based biosensors from such degradation we have constructed and characterized an electrochemical, aptamer-based sensor for the detection of aminoglycosidic antibiotics. We find that while this sensor achieves low micromolar detection limits and subminute equilibration times when challenged in buffer, it deteriorates rapidly when immersed directly in blood serum. In order to circumvent this problem, we have developed and tested sensors employing modified versions of the same aptamer. Our first effort to this end entailed the methylation of all of the 2'-hydroxyl groups outside of the aptamer's antibiotic binding pocket. However, while devices employing this modified aptamer are as sensitive as those employing an unmodified parent, the modification fails to confer greater stability when the sensor is challenged directly in blood serum. As a second potentially naive alternative, we replaced the RNA bases in the aptamer with their more degradation-resistant deoxyribonucleic acid (DNA) equivalents. Surprisingly and unlike control DNA-stem loops employing other sequences, this DNA aptamer retains the ability to bind aminoglycosides, albeit with poorer affinity than the parent RNA aptamer. Unfortunately, however, while sensors fabricated using this DNA aptamer are stable in blood serum, its lower affinity pushes their detection limits above the therapeutically relevant range. Finally, we find that ultrafiltration through a low-molecular-weight-cutoff spin column rapidly and efficiently removes the relevant nucleases from serum samples spiked with gentamicin, allowing the convenient detection of this aminoglycoside at clinically relevant concentrations using the original RNA-based sensor.

Unintentional drug overdoses are among the most common and costly medical crises. For example, the narrow therapeutic indices of coumadin and other blood thinners cause uncontrolled bleeding events that send 58 000 Americans per year to the emergency room.¹ Aminoglycoside antibiotics, likewise, can cause hearing loss, tinnitus, and kidney failure.^{2,3} To administer these drugs properly, physicians must monitor their patients between doses by drawing blood for fluorescence polarization immunoassay or turbidimetry analysis.^{4,5} However, while these tests can achieve a turnaround time of under 1 h, they require dedicated instruments that are largely confined to clinical laboratories. A point-of-care

*To whom correspondence should be addressed., kwp@chem.ucsb.edu. Phone: (805) 893-5558. Fax: (805) 893-4120.

[†]Department of Chemistry and Biochemistry.

[‡]Biomolecular Science and Engineering Program.

SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

device may, thus, better serve both patients and physicians by eliminating the follow-up visits that are often necessary in order to achieve proper dosing.

In response to the largely unmet need for rapid, point-of-care diagnostics, our laboratory^{6,7} and others⁸ have developed a reagentless, electrochemical platform capable of quantifying a range of molecular analytes in blood, urine, and other complex media.^{9,10} These electrochemical, aptamer-based sensors (E-AB sensors; see Figure 1) are rapid, sensitive, reagentless, reusable, and convenient.⁶ They are also versatile, with E-AB sensors having been reported against targets ranging from proteins^{11,12} to inorganic ions^{13,14} to small molecules.^{8,6,15} With a single exception,^{15–17} all of these E-AB sensors employed deoxyribonucleic acid (DNA) aptamers as their recognition elements, presumably because of the significantly greater stability of this probe. Unfortunately, however, the only oligonucleotides reported to date that bind aminoglycoside antibiotics are composed of ribonucleic acid (RNA), which renders them vulnerable to degradation by nucleases in blood serum samples and, thus, would likely limit the utility of E-AB sensors fabricated using them. With the aim of solving this problem, we have explored several strategies for protecting RNA probes from degradation. In doing so, we have developed a reagentless, electrochemical approach that can be used to measure the concentrations of aminoglycoside antibiotics in serum.

MATERIALS AND METHODS

Probe Sequences

RNA: 5'-HS- GGGACUUGGUUUAGGUAAUGAGUCCC-NH-MethyleneBlue-3'

DNA: 5'-HS-GGGACTTGGTTTAGGTAATGAGTCCC-NH-MethyleneBlue-3'

Control DNA: 5'-HS-AGCCCATTTATCCGTTCTCCTAGTGGTGGGC-NH-MethyleneBlue-3'

OMe1: 5'-HS-(G)(G)(G)(A)(C)(U)UGGU(U)(U)(A)(G)G(U)(A)(A)(U)(G)(A)(G)(U)(C)(C)(C)-NH-MB-3'

2'F-RNA: 5'-HS- GGGACUUGGUUUAGGUAAUGAGUCCC-NH-MethyleneBlue-3'

Tris Buffer: 20 mM Tris, 100 mM NaCl 5mM MgCl₂, pH 7.5

All of the probes were synthesized, modified, and purified by BioSearch Technologies (Novato, CA) and used as received. An unmodified version of the RNA probe was synthesized by Sigma Genosys (Saint Louis, MO) and purified by high performance liquid chromatography. Tris-2-carboxyethyl-phosphine, TCEP, was obtained from Invitrogen (Carlsbad, CA). Tobramycin, gentamicin, 6-mercapto-1-hexanol, and urea were obtained from Sigma Aldrich (Saint Louis, MO). Kanamycin was obtained from Fischer Scientific (Waltham, MA). Newborn calf serum was obtained from Sigma Aldrich (Saint Louis, MO). Millipore YH-3 spin columns, with a 3000 Da cutoff, were obtained from Fisher Scientific (Waltham, MA). Seradyn calibrator kits containing gentamicin in human serum were obtained from Polymedco (Chicago, IL). All reagents, unless otherwise mentioned, were used as received. Multiplex sensor chips were obtained from Osmetech (Pasadena, CA) and modified according to the following procedure.

Sensor Fabrication

All probe molecules were hydrated with Tris buffer to a final concentration of 200 μ M and stored at -20°C until use. To reduce the disulfide bond on each probe molecule, a 2 μ L

aliquot of the stock was combined with 8 μ L of 10 mM TCEP solution, mixed gently, and stored at 4 °C for 1 h. Following that reaction, the probe was diluted to 200 nM and a 200 μ L droplet was placed over each set of electrodes on a sensor chip, which was stored under vacuum with minimal handling up until this point. The chips were allowed to incubate in the dark for 1 h at room temperature and then rinsed with deionized water. The bottom side and edges of each chip were dried with a folded kimwipe, and 200 μ L of a 3 mM 6-mercapto-1-hexanol solution was applied for another hour. After that passivation procedure, the chips were immersed in deionized water and stored in the dark overnight.

Electrochemical Measurements

Sensor experiments were performed with a CHI 730C Electrochemical Workstation (CH Instruments, Austin, TX) and a 32-channel multiplexer made by the same manufacturer. Each square wave voltammogram was acquired at 60 Hz, over a potential range of -0.1 to -0.5 V. In serum, that window was extended to -0.6 V. The sensor chips contained an integrated gold counter electrode and an Ag/AgCl reference electrode. Measurements were conducted in 1 mL of buffer (20 mM Tris, 100 mM NaCl, 5 mM MgCl₂, pH 7.5), newborn calf serum, or ultrafiltered newborn calf serum. Prior to each experiment, the chips were immersed in 1 mL of Tris buffer and subjected to square wave test measurements. All experimental measurements were made no less than 4 min after the addition of the analyte. Upon completion of an experiment, the sensors were rinsed and stored in deionized water for reuse later. After storage, but before reuse, reused sensors were subjected to an hour of test scans in order to re-equilibrate their baseline signals.

Human Serum Measurements and Ultrafiltration

Gentamicin calibrator kits obtained from Polymedco contain six 1 mL human serum samples, at concentrations ranging from 0 to 10 mg/L with $<0.1\%$ sodium azide added as a preservative. Since they are of low molecular weight (Tobramycin, 467.5 Da; Kanamycin B, 483.5 Da; Gentamicin C, 477.6 Da) and generally do not adhere to serum proteins, the aminoglycosides easily passed through a 3000 Da molecular weight cutoff membrane. For each experiment, two samples of a given concentration were pooled and added to a Millipore YM-3 Centriprep spin column and then spun at 5100 rpm for 30 min in a Fisher Centrifuge 225 centrifuge (Waltham, MA). After spinning, the exterior of each ultrafiltrate reservoir was carefully dried with a kimwipe before its contents were transferred to a centrifuge tube for storage. The ultrafiltrate was a clear liquid, and 400 μ L was used for each electrochemical measurement. The sensor was immersed in those solutions and allowed to equilibrate for 4 min in advance of each recording.

Sensor Degradation Experiments

The surface of each chip was mounted onto the bottom of a 3 mL polycarbonate well. The well was filled with 1 mL of buffer. Square wave measurements were made on eight channels, and the channel with the flattest baseline was selected for repetitive measurements. The plastic reservoir was drained and refilled with buffer or serum. Immediately upon addition of the fresh liquid, square wave voltam-mograms were acquired every minute for 30 min.

Circular Dichroism

The spectrum of a 2 mM RNA aptamer solution was measured before and after the addition of tobramycin. Background signals of buffer and buffer with 4 mM tobramycin were subtracted from the spectra of their corresponding RNA solutions to remove any background circular dichroism caused by the drug itself.¹⁸ All measurements were made from 220 to 330 nm on an Aviv 202 spectrometer, at room temperature, in a 3 mL quartz cuvette. In this

and many other circular dichroism (CD) studies of aptamers [data not shown], we have noticed that it is necessary to bring the analyte concentration up well above the reported dissociation constant (here 13.2 μM) in order to obtain a pronounced signal change; CD measurements make use of aptamers at very high concentrations, and it is impossible to saturate them without reaching comparably high concentrations of analyte.

Surface Plasmon Resonance

Tobramycin was coupled to a Biacore CM 5 chip using a Biacore EDC coupling kit (Piscataway, NJ). Oligonucleotides were injected over the sample surface with a flow rate of 40 μL per minute at a series of concentrations. Measurements were conducted in 20 mM Tris buffer (100 mM NaCl, 5 mM MgCl_2 , pH 7.5).

RESULTS AND DISCUSSION

E-AB sensors are made by affixing an aptamer capped at one end by a reduction and oxidation-active (redox) reporter group and attached via its other end to the surface of an interrogating electrode (Figure 1). In this set of experiments, our aptamers were bound to gold electrodes via a thiol modification at their 5'-ends and labeled at their 3'-ends with the redox-active reporter methylene blue. For the first generation sensors fabricated here, we employed the 26-base RNA aptamer of Wang and Rando,^{19,20} which adopts a stem-loop configuration that binds aminoglycosides in its loop region.²¹ Circular dichroism studies indicate that this aptamer undergoes a significant conformational change upon target binding (Figure 2); specifically, when 4 mM tobramycin is added to a solution of the aptamer, the intensity of the positive ellipticity band at 260 nm increases significantly. This band is often attributed to the formation of A-RNA structure, and thus, the observed signal change may be linked to an increase in the helical content of the aptamer. Solution NMR studies indicate, however, that the loop in the stem-loop aptamer wraps around the tobramycin molecule,²¹ suggesting that the observed change in ellipticity may signal a more complex structural transition. Irrespective of the precise nature of this conformational change, it is sufficient to support E-AB signaling: the binding of aminoglycosides to our electrode-bound aptamer causes a large, readily measurable decrease in Faradaic current from the attached redox tag (Figure 3).

The aminoglycosides comprise a large family of closely related antibiotics. For example, gentamicin, tobramycin, and kanamycin share similar hydroxyl- and primary amine-rich structures that form hydrogen bonds with compatible groups on the nucleobases of their target RNA (Figure 1). Indeed, tobramycin differs from kanamycin A by the addition of one hydroxyl group, the replacement of a second with a primary amine, and the absolute configuration of a single stereocenter. Thanks to these structural similarities, the aptamer that we have employed binds all three of these aminoglycosides with similar affinities.^{19,20} Consistent with this, we find that our sensor responds robustly to all three of the aforementioned antibiotics, achieving signal suppressions of ~60% at saturating drug concentrations (Figure 3). Of note, while this cross reactivity could confound the measurement of samples taken from patients who are on more than one member of this family of drugs, the aminoglycosides are relatively toxic and exhibit very similar spectra of coverage, and thus, the simultaneous administration of multiple aminoglycosides is medically contraindicated.

Our sensors are capable of monitoring aminoglycoside concentrations over the therapeutically relevant,²² 2–6 μM (4–10 $\mu\text{g}/\text{mL}$) range. Two of the three antibiotics we have investigated, tobramycin and kanamycin, produce the hyperbolic saturation curves expected for single-site binding and yield dissociation constants of 319 and 281 μM . The latter values are orders of magnitude higher than solution-phase values reported (12 nM to

13.2 μM) for longer aptamers bearing the same core sequence^{19,20} These previous reports, however, employed a pyrene-modified tobramycin molecule, and not tobramycin itself, as a reporter, and thus, this discrepancy is perhaps not unexpected. In contrast to tobramycin and kanamycin, gentamicin produces a biphasic binding curve in which the biosensor current decreases at low concentrations and then increases at higher concentrations. (See Figure SI.1 in Supporting Information.) The origins of this biphasic behavior are unclear, but it is possible that, at high concentrations, gentamicin causes the hairpin to denature, liberating the redox tag to collide rapidly with the sensor surface and, thus, producing the observed increase in current at very high drug concentrations. Alternatively, at high concentrations, the binding of additional drug molecules may force the aptamer to undergo additional conformational changes that bring that tag into proximity with the sensor surface.

Because RNA is relatively unstable against chemical and enzymatic degradation, our first-generation, RNA-based sensor is far less stable than E-AB sensors fabricated with more robust DNA probes. In Tris buffer, for example, the RNA-based sensor deteriorates significantly within a few days. (See Figure SI.2 in Supporting Information.) The problem of RNA stability is particularly acute when the sensor is deployed in blood serum (Figure 4). Indeed, even in RNase inhibitor-treated serum, the sensor's signal diminishes to unacceptably low levels within an hour of immersion and the sensor cannot be regenerated. (See Figure SI.3 in Supporting Information.) These deficiencies contrast sharply with the attributes of previously reported sensors composed of DNA probes, which are stable in buffered saline for months at room temperature and even withstand more than a week of storage in blood serum under the same conditions.²³

The problem of serum nucleases digesting unprotected, RNA-based probes is general, thus motivating our interest in protecting these delicate sensing elements without altering their ability to bind their specific targets. To this end, we have explored several probes lacking the reactive 2'-hydroxyl group of RNA. As a first approach, we used the published solution NMR structure of the aptamer as a guide while replacing all the aptamer's 2'-hydroxyl groups with 2'-methoxy groups save for five positions that are closest to the aptamer's drug-binding site. We find, however, that while this modified aptamer retains the high affinity of its parent RNA aptamer (Figure 5), it fails to offer significant protection against nucleases when deployed in blood serum. (See Figure SI.3 in Supporting Information.) Likewise, we explored aptamers in which all of the 2' positions were replaced with fluorines. This modification, however, significantly degrades the aptamer's affinity and conveys only modest protection in serum. (See Figure SI.4 in Supporting Information.) Moving forward, we decided to attempt an experiment in which we simply removed all 26 of the 2'-hydroxyl groups from the RNA aptamer; that is, we converted the RNA aptamer into the equivalent DNA sequence. While this is perhaps naive, as converting an RNA sequence into the equivalent DNA sequence is clearly a major alteration of the chemistry of the recognition probe, several arguments suggested that this approach was at least worth attempting. First, the RNA aptamer adopts a simple hairpin structure that does not require the formation of a complex, presumably RNA-specific tertiary structure. Second, the aptamer's drug binding pocket is located in its major groove, far from the 2' hydroxyls, which are uniformly oriented away from the bound aminoglycoside. Indeed, the nearest 2'-hydroxyl, located on base G14, is more than 4 Å from the nearest hydrogen bond acceptor on the drug. Furthermore, the conversion of RNA aptamers to DNA is not without precedent. Walsh et al. found that a DNA translation of a dopamine binding aptamer^{24,25} retains the ability to bind this target. Finally, Szostak et al. have shown that the DNA translation of a flavin-binding RNA aptamer binds flavin with just over a 10-fold reduction in affinity²⁶ (albeit this aptamer forms a G-quadruplex that may be readily formed from DNA). Consistent with these simple arguments, surface plasmon resonance experiments indicate that a DNA probe with the same sequence as the original RNA aptamer readily binds a tobramycin-coated

surface (see Figure SI.4 in Supporting Information), an observation that further encouraged us to pursue this approach. In doing so, we find that sensors fabricated using this DNA probe respond to our target aminoglycosides, albeit with affinities against tobramycin, kanamycin, and gentamicin that are reduced 4-, 2-, and 3-fold, respectively, relative to those of the equivalent RNA aptamer (Figure 5). In contrast, control sensors fabricated using an unrelated DNA stem-loop do not exhibit any evidence of binding to the aminoglycoside antibiotics. (See Figure SI.5 in Supporting Information.) Unfortunately, however, while these DNA-based sensors are significantly more stable than our first generation RNA-based sensors (see Figure SI.3 in Supporting Information), their reduced affinities impede their ability to detect aminoglycosides at medically relevant concentrations.

Due to our failure to identify modified probes that are of sufficiently high affinity and yet remain stable under clinically relevant conditions, we have adopted an alternative approach for the use of RNA-based sensors with clinically relevant samples. Because the aminoglycosides are of low molecular weight (and generally do not form complexes with high-molecular weight serum proteins), they readily pass through molecular weight cutoff membranes. Ultrafiltration of serum through a 3000 Da cutoff spin column, thus, effectively separates the antibiotic from serum nucleases. When immersed in the clear ultrafiltrate obtained from newborn calf serum (a safe and convenient proxy for human samples), sensors fabricated using the unmodified RNA aptamer remain quite stable (Figure 4). Moreover, under these conditions, we can readily quantify gentamicin over the therapeutic range both when spiked into newborn calf serum (see Figure SI.6 in Supporting Information) and as present in commercial human serum calibration samples (Figure 6). Although this sample protocol is effective, it is slow; the need for ultrafiltration adds more than 30 min onto an otherwise short workflow. For that reason, we believe that the advantages of this RNA biosensor would best be realized in an integrated microfluidic system that rapidly prepares the serum sample before feeding it onto the E-AB sensor.

The reported E-AB sensor has many attributes that would make it an ideal component within a point of care medical diagnostic device. It is reusable, reagentless, and compact. It is also rapid: when aminoglycosides are introduced to a sensor, the resulting current change equilibrates within the 10 s dead time of a single voltammetric scan. (See Figure SI.7 in Supporting Information.) Similarly swift results have been observed with aptamer-based devices meant to detect cocaine, adenosine triphosphate, and theophylline.^{6,15} Because all of the sensor components are firmly adsorbed onto the electrode surface (Figure 1), the sensor is readily regenerable: a simple rinse with deionized water disrupts the target–aptamer complex, allowing for the sensor's reuse. (See Figure SI.8 in Supporting Information.) Finally, our sensors are fabricated on submillimeter scale electrodes and driven by simple, desktop electronics (Figure 1, right), further speaking to their compatibility with microfluidics or a hand-held device format.

CONCLUSIONS

Here, we have demonstrated a reusable RNA aptamer-based electrochemical biosensor that supports the rapid (<10 s) measurement of aminoglycoside concentrations over the therapeutically relevant, low-micromolar range. Unfortunately, it is quite vulnerable to degradation by nucleases. Our first effort to improve the sensor's stability, which involved modification of selected nucleotides with 2'-methoxy groups, did not increase the stability of the aptamer in newborn calf serum and, thus, does not appear to be a promising solution to this problem. Likewise, replacement of the RNA probe with an equivalent DNA sequence yields a sensor that is stable in newborn calf serum but that transformation significantly reduces the affinity of our probe. Thus, while the substitution of deoxyribose for ribose may prove useful in some applications, this obviously does not represent a general solution to the

problem of stabilizing RNA aptamers. The approach we finally adopted, ultrafiltration, is an effective means of removing nucleases from serum samples. It allows us to make reliable measurements in filtered serum calibrators and may prove to be a general approach for the use of RNA probes for the detection of at least low molecular weight targets. While this pretreatment slows the overall workflow of the sensor, the process is complete in less than 30 min and requires only an inexpensive, desktop centrifuge.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the NIH through Grant GM062958-01 and by the Institute for Collaborative Biotechnologies through Grant DAAD19-03-D-0004 from the U.S. This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344. We are grateful to Osmetech for providing us with several batches of their electrode arrays.

References

1. Lesko L. Clin Pharmacol Ther. 2008; 84:301–303. [PubMed: 18714317]
2. Lu CC, James SH, Lien YH. Am J Kidney Dis. 1996; 28:767–771. [PubMed: 9158219]
3. Black RE, Lau WK, Weinstein RJ, Young LS, Hewitt WL. Antimicrob Agents Chemother. 1976; 9:956–961. [PubMed: 938024]
4. Stead DA. J Chromatogr, B: Biomed Sci Appl. 2000; 747:69–93. [PubMed: 11103900]
5. Begg EJ, Barclay ML, Kirkpatrick CMJ. Br J Clin Pharmacol. 2001; 52:35–43. [PubMed: 11453888]
6. Baker BR, Lai RY, Wood MS, Doctor EH, Heeger AJ, Plaxco KW. J Am Chem Soc. 2006; 128:3138–3139. [PubMed: 16522082]
7. Cash KJ, Ricci F, Plaxco KW. J Am Chem Soc. 2009; 131:6955–6957. [PubMed: 19413316]
8. Zuo X, Song S, Zhang J, Pan D, Wang L, Fan C. J Am Chem Soc. 2007; 129:1042–1043. [PubMed: 17263380]
9. Xiao, Y.; Plaxco, KW. Functional Nucleic Acids for Analytical Applications. 2009. p. 179-197.
10. Lubin AA, Lai RY, Baker BR, Heeger AJ, Plaxco KW. Anal Chem. 2006; 78:5671–5677. [PubMed: 16906710]
11. Lai RY, Plaxco KW, Heeger AJ. Anal Chem. 2007; 79:229–233. [PubMed: 17194144]
12. Xiao Y, Piorek BD, Plaxco KW, Heeger AJ. J Am Chem Soc. 2005; 127:17990–17991. [PubMed: 16366535]
13. Radi A, O'Sullivan CK. Chem Commun. 2006:3432.
14. Xiao Y, Rowe AA, Plaxco KW. J Am Chem Soc. 2007; 129:262–263. [PubMed: 17212391]
15. Ferapontova EE, Olsen EM, Gothelf KV. J Am Chem Soc. 2008; 130:4256–4258. [PubMed: 18324816]
16. Ferapontova EE, Gothelf KV. Langmuir. 2009; 25:4279–4283. [PubMed: 19301828]
17. Ferapontova EE, Gothelf K. Electroanalysis. 2009; 21:1261–1266.
18. Jezowska-Bojczuk M, Karaczyn A, Kozłowski H. Carbohydr Res. 1998; 313:265–269. [PubMed: 10209869]
19. Wang Y, Killian J, Hamasaki K, Rando RR. Biochemistry. 1996; 35:12338–12346. [PubMed: 8823168]
20. Wang Y. Chem Biol. 1995; 2:281–290. [PubMed: 9383430]
21. Jiang L, Patel DJ. Nat Struct Mol Biol. 1998; 5:769–774.
22. Setia U, Gross PA. J Infect Dis. 1976; 134:S125–S129. [PubMed: 972270]
23. Swensen JS, Xiao Y, Ferguson BS, Lubin AA, Lai RY, Heeger AJ, Plaxco KW, Soh HT. J Am Chem Soc. 2009; 131:4262–4266. [PubMed: 19271708]

24. Walsh R, DeRosa MC. *Biochem Biophys Res Commun.* 2009; 388:732–735. [PubMed: 19699181]
25. Mannironi C, Di Nardo A, Fruscoloni P, Tocchini-Valentini GP. *Biochemistry.* 1997; 36:9726–9734. [PubMed: 9245404]
26. Lauhon CT, Szostak JW. *J Am Chem Soc.* 1995; 117:1246–1257. [PubMed: 11539282]
27. Clark C, Cecil P, Singh D, Gray D. *Nucleic Acids Res.* 1997; 25:4098–4105. [PubMed: 9321664]

Figure 1. Illustration of the E-AB sensor, shown left, in which the RNA aptamer is affixed to a gold electrode and the binding of tobramycin draws a methylene blue reporter molecule farther from the electrode surface. The sensors were fabricated on a 32-electrode array, shown right, which was surrounded by a circular plastic reservoir and filled with the sample.

Figure 2.

Circular dichroism spectra indicate that the antiaminoglycoside aptamer undergoes a substantial conformational change upon binding to tobramycin. Specifically, a positive band at 260 nm that is often attributed to the formation of A-RNA structure²⁷ increases substantially upon drug binding.

Figure 3.

(left) E-AB sensor robustly responds to aminoglycoside antibiotics via the loss in the faradaic current observed at the -0.35 V potential characteristic of the reduction of methylene blue. (right) Saturation curves indicate that the biosensor has nearly equal affinity for kanamycin and tobramycin and slightly greater affinity for gentamicin. The error bars on this and the following figures reflect the standard error of triplicate measurements taken with independently fabricated and measured sensors.

Figure 4.

Sensors constructed with an RNA probe can endure hours of exposure in buffered saline but decompose rapidly in (untreated), room temperature newborn calf serum. Ultrafiltration of the serum through a 3000 Da molecular weight cutoff membrane, however, removes the nucleases, rendering it possible to measure aminoglycoside concentrations in realistic clinical samples.

Figure 5.

Probes composed of deoxyribose or 2'-O-methyl modified ribose also bind aminoglycosides. All three of the aminoglycosides we have investigated, however, bind both RNA and 2'-O-methyl-RNA probes (left and right bars, respectively) with greater affinity than they bind the equivalent DNA probe (central bars).

Figure 6. Sensor responds well to gentamicin that was present in spiked serum samples. Each measurement was made 4 min after the sensor was immersed in the ultrafiltrate from samples that were intended for calibrating the Innofluor system, a widely used clinical assay.