Paper-Based Sensor for Electrochemical Detection of Silver Nanoparticle Labels by Galvanic Exchange

Josephine C. Cunningham,† Molly R. Kogan,† Yi-Ju Tsai,† Long Luo,† Ian Richards,‡ and Richard M. Crooks†

†Department of Chemistry and the Texas Materials Institute, The University of Texas at Austin, 10S East 24th Street, Stop A5300, Austin, Texas 78712, United States
‡Interactives Executive Excellence LLC, 201 North Weston Lane, Austin, Texas 78733, United States

ABSTRACT: Here we report a three-dimensional paper fluidic device configured for electrochemical detection of biomolecules labeled with silver nanoparticles (AgNPs). This new sensor, which we call a NoSlip, represents a major improvement of our previously reported oSlip system. Specifically, detection of AgNPs in the NoSlip is based on galvanic exchange rather than a chemical oxidant (bleach or MnO4− in the oSlip). Galvanic exchange is implemented by depositing a very small amount of gold onto the working electrode. Once the AgNP labels are brought into the proximity of the electrode through the use of magnetic force, a fraction of the Au0 is electrochemically oxidized to Au3+. The Au3+ reacts with the AgNPs to form Ag+ and Au0. The Ag+ is then detected by anodic stripping voltammetry. This new methodology resolves three shortcomings of the oSlip while simultaneously simplifying the basic sensor form factor. First, the NoSlip resolves an oxidant instability issue because of the inherent stability of the Au0 coating on the electrode that is used to electrogenerate the oxidant (Au3+). Additionally, Au3+ is a milder oxidizing agent than bleach or MnO4−, so it does not attack the major components of the NoSlip. Finally, the NoSlip eliminates the need for a slip layer because the oxidant (Au3+) is electrogenerated on demand. The NoSlip is able to detect AgNP labels down to concentrations as low as 2.1 pM, the time to result is ~7 min, and the cost at the laboratory scale, not including application-specific reagents, is $0.30.

KEYWORDS: electrochemical sensor, silver nanoparticles, anodic stripping voltammetry, paper analytical device, galvanic exchange

Lateral flow assays (LFAs) were first demonstrated in the 1950s as semiquantitative, colorimetric glucose sensors.1 Their low cost and simplicity were ideally suited for many applications, and at the present time they dominate the point-of-care (PoC) sensing market.2,3 LFAs do have limitations that restrict their applications, however. For example, the vast majority provide binary (yes/no) or, at best, semiquantitative output. They are also restricted to simple assays that do not require timed reaction steps, chemical amplification (e.g., polymerase chain reaction), or high degrees of multiplexing.

In 2007, Whitesides and co-workers published a seminal paper following year the same group showed that this same basic design rule could be expanded to three dimensions,14 thereby further increasing the number of potential applications.15–19

Over the past four years, we have expanded on Whitesides’ original multidimensional paper sensing ideas by introducing more convenient fabrication methods based on the principles of origami,20,21 quantitative detection of analytes at subpicomolar concentrations,22 using on-chip electrochemical methods,23 hollow channels for faster, more flexible assays,22–24 and slip layers for timing reactions.22,25,26 Subsequently, these four developments, in combination with an indirect oxidation strategy,27–29 came together in a three-dimensional, paper-based sensing device we call an oSlip ("o" for origami and "Slip" to indicate that it incorporates a "slip layer").25,30 The oSlip has been used to detect targets, including a model analyte,22 DNA,31 and the biological warfare agent ricin.32 It uses two stages of amplification to achieve detection limits in the low picomolar range at a cost per-sensor of ~$0.30 at the laboratory scale, not including application-specific reagents.32

The oSlip strategy relies on the target linking together antibody-functionalized magnetic microbeads (MµBs) and silver nanoparticles (AgNPs). The MµBs can be directed

Received: August 19, 2015
Accepted: September 29, 2015
toward an electrochemical detection zone by a magnetic force, and the AgNPs can then be detected by indirect oxidation of Ag.

As alluded to above, there are some very desirable aspects of the oSlip: low limits of detection (LODs), low cost, scalable fabrication, robust labels (AgNPs, rather than enzymes), time-to-result of <5 min, and relatively simple reconfiguration for different targets. There are, however, three key problems with the oSlip. First, a chemical oxidant is required to oxidize the AgNPs, and although we have screened many candidates, none have the necessary stability when dried on paper. Second, the slip layer, which is used for timed delivery of the chemical oxidant, is not user-friendly. Third, although both bleach and permanganate oxidant, is not user-friendly. Third, although both bleach and permanganate effectively oxidize the AgNP labels, they are very powerful oxidizing agents that also react with other components in real samples and even the oSlip itself. We have found this to be problematic for a number of reasons. Accordingly, we sought to develop an alternative design that retains the positive aspects of the oSlip while minimizing or eliminating its deficiencies.

The device design that has emerged from our work is called a NoSlip (Scheme 1) to indicate that the additional slip layer has been eliminated. The fabrication and operation of the NoSlip will be described in detail later, but a few key points are briefly mentioned now. The main shortcomings of the oSlip all relate to the presence of a predispensed chemical oxidant, such as permanganate or bleach, so we eliminated the chemical oxidant and now electrogenerate the oxidant on demand. Specifically, Au metal is stored on the device, and it is electrochemically converted to the oxidant Au\(^{3+}\) at the precise time and location needed. This resolves the instability issue, because of the inherent stability of zerovalent Au (Au\(^0\)), and it also eliminates the need for the slip layer, because the oxidant is now introduced electrochemically rather than by physical manipulation of a piece of paper. Importantly, the improvements associated with the NoSlip do not come at the expense of any aspect of device or assay performance.

### EXPERIMENTAL SECTION

#### Chemicals and Materials.

All solutions were prepared using deionized (DI) water (>18.0 MΩ·cm, Milli-Q Gradient System, Millipore, Bedford, MA). NaCl, NaOH, HCl, H\(_2\)AuCl\(_4\), KNO\(_3\), Na\(_2\)SO\(_4\), sodium citrate dihydrate, citric acid monohydrate, urea, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), Whatman grade 1 chromatography paper (180 \(\mu\)m thick, 20 cm \(\times\) 20 cm, linear flow rate (water) of 13 cm/30 min), and siliconized low retention microcentrifuge tubes were all purchased from Fisher Scientific (Pittsburgh, PA). NaHCO\(_3\), K\(_2\)HPO\(_4\), and borax were purchased from EM Science (Gibbstown, NJ). MgSO\(_4\) and KH\(_2\)PO\(_4\) were purchased from EMD Chemicals (Gibbstown, NJ).

A 0.10 M borate solution containing 0.10 M NaCl (referred to hereafter as BCI) was prepared by dissolving the appropriate amount of boric acid and NaCl in DI water, and then adjusting the pH to 7.5 with NaOH. Artificial urine was prepared according to a previously published procedure\(^\text{33}\) with a slight modification (1.0 mM of ascorbic acid instead of lactic acid). Citrate-capped AgNPs (nominal 20 nm diameter) and conductive Cu tape (30 mm wide) were purchased from Ted Pella (Redding, CA). Erioglaucine disodium salt (blue dye), ascorbic acid, and NH\(_4\)Cl were obtained from Acros Organics (Pittsburgh, PA).

Conductive carbon paste (CI-2042) was purchased from Engineered Conductive Materials (Delaware, OH). Cylindrical neodymium magnets (1/16 in. \(\times\) 1/2 in., N48) were acquired from Apex Magnets (Petersburg, WV). Streptavidin-coated M\(_2\)Bs (2.8 \(\mu\)m diameter) were obtained from Bangs Laboratories (Fishers, IN). Thiol-DNA-biotin (5′-d Thiol C6 SS-ACATTAAAATTC-Biotin 3′) was acquired as a powder from Biosearch Technologies (Petaluma, CA) and, before use, was dissolved in an appropriate volume of DI water to yield a concentration of 1.0 mM. Gold was deposited onto the working electrode of the NoSlip using a recessed electrochemical cell made of polytetrafluoroethylene (PTFE) equipped with a saturated Hg/Hg\(_2\)SO\(_4\) reference electrode (RE), and Pt wire counter electrode (CE) from CH Instruments (Figure S-1).

#### Instrumentation.

All electrochemical measurements were made using a model 700E bipotentiostat from CH Instruments (Austin, TX). A BioShake iQ (Q Instruments) was used to control mixing during incubation. UV−vis measurements were made with a Hewlett-Packard HP8453 spectrometer and a quartz cell (l = 10.0 mm, 50.0 nm) and processed using Origin Pro8 SR4 v 8.0951 (Northampton, MA).

A Sorvall Legend Micro 21R centrifuge (Thermo Scientific) was used for washing steps during the synthesis of biotinylated AgNPs. A Hitachi S550 scanning electron microscope was used for imaging. The electrode stencil was cut using an Epilog laser engraving system (Zing 16). The 3D-printed holder that encases the NoSlip (Figure S-2) was designed using Autodesk 123D and printed using polyactic acid (PLA) on a Modified Makerbot Replicator 2s. Adobe Illustrator CS6 (version 16.0.0) was used for the design of the NoSlip and electrode stencil. The charge under the ASVs was determined by baseline correcting the ASVs using Origin Pro8 SR4 v 8.0951 (Northampton, MA), integrating the area under the peaks, and then dividing by the scan rate.

#### NoSlip Fabrication.

NoSlips were fabricated as follows. First, a sheet of chromatography paper was patterned with wax (using the wax printer) with multiple NoSlips (Scheme 1 and Figure S-3a). Second, the sheet of paper was placed (with the wax pattern facing up) into the oven at 130 °C for 30 s to melt the wax through the thickness of the paper to create hydrophobic barriers. Note that the hemichannel on
Layer 4 of the NoSlip (Scheme 1 and Figure S-3a) is made using 60% yellow wax (specified in Adobe Illustrator CS6) that does not melt through the entire thickness of the paper, and therefore creates a hydrophilic floor to drive capillary flow in the NoSlip hollow channel.24 Third, the individual devices were cut around the exterior edge and the void spaces on Layers 2 and 3 were removed with a laser cutter. Additional details relating to the fabrication of the electrodes are provided in the Supporting Information.

Preparation of the MμB-AgNP Composite. Complete details relating to the conjugation of AgNPs to biotin, as well as formation of the AgNP-biotin-streptavidin-MμB composite (referred to hereafter as the “MμB-AgNP composite”) are provided in the Supporting Information.

Scheme 2

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>$3\text{Ag}^{(s)} + \text{Au}^{3+} \rightarrow 3\text{Ag}^{+} + \text{Au}^{(s)}$</td>
<td>$\Delta E = 0.70$ V</td>
</tr>
</tbody>
</table>

Under standard conditions, the driving force for eq 1 is just the difference in the standard potentials of the individual half reactions: $\Delta E = 0.70$ V. This is sufficient overpotential to drive eq 1 to completion.

We are interested in developing assays for specific targets that rely on detection of AgNP labels via a galvanic exchange mechanism. As a preliminary step in that direction, we carried out a proof-of-concept assay using the NoSlip and a model analyte consisting of AgNPs linked to MμBs via biotin–streptavidin conjugation: the MμB-AgNP composite. In the future, the conjugate will be an antibody or DNA sandwich, or any other specific binding reaction of interest. The general operation of the NoSlip is shown in Scheme 2. Once the device is assembled, the sample (50 μL) is loaded at the inlet (Scheme 2b). Capillary flow, driven by the hydrophilic hemichannel, commences immediately, driving the sample down the open channel toward the sink (Scheme 2c). As the sample passes the working electrode, the magnet localizes the MμB-AgNP composite under the working electrode. When the sink becomes full, upward flow is initiated through the paper reservoir at the end of Layer 3 and toward the outlet. This rehydrates the dye on Layer 2 and a blue color appears at the outlet, indicating that flow has stopped (Scheme 2e) and that galvanic exchange detection can be initiated.

Scheme 2d–g illustrates how the galvanic exchange process plays out. Scheme 2d shows the MμB-AgNP composite trapped at the working electrode. The first step in the detection process is the application of a 50.0 s potential step (from the open circuit potential (OCP) to 0.30 V vs CQRE) to the Au-modified working electrode. This results in conversion of $\text{Au}^{0}$ to $\text{Au}^{3+}$. A key feature of the NoSlip is that this oxidant is formed exactly where it is required: adjacent to the trapped AgNPs. Moreover, the oxidizing power of $\text{Au}^{3+}$ is sufficient to oxidize Ag, but, as far as we know, it is not so strong as to interact with other components of the NoSlip or the buffer present in the channel. Scheme 2e shows that eq 1 now proceeds spontaneously to yield Ag$^{+}$. A second potential step from 0.30 V to −0.70 V for 200.0 s results in electrodeposition of metallic Ag onto the working electrode (Scheme 2f). Residual (i.e., unreacted) $\text{Au}^{3+}$ is also electrodeposited as $\text{Au}^{0}$ at this potential. In the last detection step (Scheme 2g), bulk, $\text{Ag}^{0}$ is electrochemically oxidized at the working electrode surface by scanning the WE potential from −0.70 to 0.20 V vs CQRE. Integration of the area under the peak in the resulting current transient corresponds to the charge contained in the AgNP labels, and hence it reflects the original concentration of the...
analyte. One final point: In the absence of galvanic exchange, no Ag signal is observed even at high AgNP concentrations. In other words, there is no direct oxidation of AgNPs at the working electrode, and therefore this galvanic exchange approach is a zero-background detection method.

The NoSlip detection approach retains the double-amplification methodology we previously introduced in the oSlip.22,31,32 The first amplification step corresponds to preconcentration of the AgNP labels at the electrode surface using magnetic force. The second corresponds to the 250 000 equiv of charge present in each 20-nm-diameter AgNP. Importantly, however, the use of galvanic exchange, in place of chemical oxidation by (for example) KMnO₄ greatly increases the robustness of the assay. This is because Au is stored in its highly stable metallic form until it is required to oxidize the AgNPs. Even more important, this approach removes the need for the slip layer, thereby eliminating a user-initiated step in the assay. In short, the NoSlip represents another significant advance in the field of paper fluidics.

**Galvanic Exchange Detection Performance on the NoSlip.** Using the process described in the previous section, we collected anodic stripping voltammograms (ASVs) after injecting different concentrations of the M₄B-AgNP composite into NoSlip sensors (Figure 1a). For reasons we will discuss later, we collected two ASVs for each concentration and used the second one for the analysis. The NoSlips are disposable, so each experiment was carried out using a different device. The observed shifts in ASV peak potentials are likely due to the CQRE, which is not as stable as a real reference electrode. This is not a big problem for the NoSlip, however, because there are no other species oxidized within the potential range of the ASV peak positions.

The dose–response curve in Figure 1b shows the relationship between charge (measured under ASV peaks like those in Figure 1a) and the concentration of AgNPs. Between 2.1 and 33.8 pM the plot is linear, but at higher AgNP concentrations the dose–response curve plateaus, suggesting that insufficient Au⁴⁺ was created to fully oxidize the AgNPs during galvanic exchange. The important point, however, is that even at this very early stage of development, the pre-prototype NoSlip is able to detect 2.1 pM of the AgNP labels with a device-to-device coefficient of variation (CV) of 15.8% (average of the standard deviation divided by the mean for all AgNP concentrations in the M₄B-AgNP composite), a sample-to-result time of ∼7 min, and a collection efficiency of 16.8% (charge collected divided by charge-equivalents injected into the NoSlip).

As mentioned earlier, one of the problems we experienced with the oSlip was that the strong chemical oxidant (e.g., bleach...
or permanganate) oxidized components of the target matrix in addition to the AgNPs. In contrast, Au^{3+} is a milder oxidizing agent, so we reasoned that this problem would be minimized in the NoSlip. To test this hypothesis, we carried out NoSlip experiments using artificial urine as the matrix rather than BCI solution. The composition of artificial urine is described in the Experimental Section, but briefly, the four principal components are urea (170 mM), NaCl (90 mM), NaHCO_{3} (25 mM), and NH_{4}Cl (25 mM). None of these are electroactive in the potential range used in the galvanic exchange/ASV analysis, so they do not interfere with the assay. However, ascorbic acid is present at a low concentration of 1.0 mM and, as shown in Figure S-4, its oxidation onset potential is close to that of Au^{0}. This could be problematic, because co-oxidation of ascorbic acid and Au^{0} results in less than 100% current efficiency for Au^{0} oxidation. By careful selection of the potential used for Au^{0} oxidation (0.30 V vs CQRE) this possible problem is largely avoided.

For the artificial urine experiments, the same procedure used for the buffer experiments was followed, except the MμB-AgNP composite was resuspended in 50.0 μL of artificial urine after the third waking step. Figure 2a shows the resulting ASVs for a range of AgNP label concentrations. As discussed earlier, the location of the ASV peak is not constant due to the instability of the quasi-reference electrode, but this has no practical effect on the assay. Figure 2b is a dose–response curve that was generated by integrating ASVs like those in Figure 2a. The linear range in artificial urine is from 3.4 to 53.8 pM AgNPs, which is comparable to that found in buffer: 2.1 to 33.8 pM AgNPs (Figure 1b). The lowest detectable concentrations of AgNPs in artificial urine and buffer are also comparable: 3.4 pM and 2.1 pM, respectively. Note that because the NoSlip is a zero-background method it is not possible to calculate a well-defined limit of detection.

A Closer Look at Galvanic Exchange. The galvanic exchange process is somewhat more complicated than we have thus far alluded to. This additional complexity does not affect the NoSlip assay to any great degree, but it is interesting and relevant and therefore requires some further explanation.

During the electrochemical detection procedure, an excess number of equivalents of Au^{3+} (relative to Ag^{0}) are electro-generated to ensure that the galvanic exchange process goes to completion. This means that after the AgNPs, which are localized near the electrode surface, are fully oxidized to Ag^{+}, Au^{3+} is also present in the vicinity of the working electrode. Accordingly, when the electrode potential is stepped negative to reduce Ag^{0}, Au^{0} is codeposited resulting in the likely formation of a AgAu alloy. Moreover, due to the inhomogeneous distribution of Au^{3+} and Ag^{0} in the diffusion layer, and because of the large excess of Au^{3+}, Au^{0} preferentially deposits on the electrode toward the end of the electrodeposition period. This results in formation of a (primarily) Au^{0} shell capping the electrodeposited AgAu alloy. As shown in Scheme 3a, this renders the underlying Ag^{0} electrochemically inaccessible. Note that the innermost core of Au^{0} shown in Scheme 3a was deposited during initial device fabrication (see Experimental Section and also Figure S-5).

The presence of this structure means that during the first ASV scan following codeposition of Ag^{0} and Au^{0}, the Ag ASV peak is either small or nonexistent as illustrated by the black voltammogram in Figure 3. However, after obtaining the first ASV, the working electrode potential is stepped back to −0.70 V, and a second ASV scan is initiated using the same parameters as for the first. The result is the red trace in Figure 3, which exhibits a much larger current than the first ASV. The second peak is larger than the first because during the last part of the first scan (between −0.20 and 0.20 V) a little of the Au^{0} overlayer is oxidized (see Figure S-6) allowing the underlying Ag^{0} to be accessed electrochemically. This situation is illustrated in Scheme 3b.

The really interesting finding is that if a third ASV is obtained (blue trace, Figure 3) using the same procedure as for the first and second, another Ag ASV peak is observed, and it has nearly the same shape and height as the second. This result is surprising, because one would anticipate that after each scan some Ag^{0} would be lost due to diffusion away from the working electrode and the corresponding incomplete redeposition of Ag^{0}. We hypothesized that this strange result is a consequence of the presence of 0.10 M Cl⁻ in the electrolyte solution, and hence precipitation of AgCl(s) (K_{sp} = 1.8 \times 10^{-10}) onto the electrode surface.

Clearly, it is crucial to understand the underlying mechanism of the galvanic exchange procedure, because it is at the heart of the NoSlip methodology. Accordingly, we undertook a number of experiments to confirm or refute the AgCl(s) precipitation hypothesis. In the first of these, a series of experiments was carried out in which the electrodeposition time was varied between 5.0 and 200.0 s, and then the first and second ASVs were recorded. The methodology for these experiments was very similar to those used to obtain the data in Figure 1: a separate NoSlip device for each experiment and injection of 5.0 μL of 33.8 pM MμB-AgNP composite present in 0.10 M BCI solution.

Figure 4 shows the results of this experiment, and our interpretation is illustrated on the left side of the figure. The charge under the ASV peak of the first scan increases up to electrodeposition times of 100.0 s, and then it decreases. As shown on the left side of Figure 4, we believe that codeposition of Ag^{0} and Au^{0} occurs at shorter times, but at longer times the...
Ag⁺ is largely depleted and primarily only Au³⁺ is electrodeposited. This results in deposition of a protective shell of mostly Au⁰ (bottom frame of Figure 4) that limits the amount of Ag⁰ that can be oxidized during the first ASV. As discussed earlier, however, some of the Au⁰ shell is oxidized at the end of the first ASV scan (between −0.20 and 0.20 V), and this leaves some Ag⁰ exposed that can be oxidized during the second scan. Therefore, there is a consistent increase in the charge due to Ag⁰ oxidation as a function of the electrodeposition time during the second scan (right side of Figure 4).

Notice also that, at all times (50.0−200.0 s), the Ag ASV peaks are noticeably sharper for the second scans compared to the first. The broad peaks in the first scans indicate that Ag is more difficult to oxidize, which may be due to Ag⁰ being in the form of an AgAu alloy. We hypothesize that immediately following galvanic exchange, some of the Ag⁰ will precipitate as AgClₐ(s), but a large portion of the Cl⁻ is consumed by the excess gold as AuCl₄⁻; therefore, the majority of the Ag⁰ will deposit onto the electrode as a AgAu alloy. Following codeposition of Ag⁰ and Au⁰ on the WE, the bulk concentration of free Cl⁻ is re-established in the vicinity of the electrode. Accordingly, a large percentage of the Ag⁰ that is oxidized during the first ASV scan likely forms AgClₐ(s). The second-scan Ag ASV peaks are sharper due to the fast kinetics of the Ag⁰ to AgCl redox reaction.¹²

Recall that we invoked the importance of aqueous Cl⁻ in our interpretation of the ASVs in Figures 3 and 4, and we claimed that the limited solubility of AgCl is responsible for the observation that ASVs subsequent to the first scan are nearly identical. To test this hypothesis, a 50.0 μL aliquot of 0.75 mM citrate-stabilized (no Cl⁻ present) AgNPs (i.e., not conjugated to thiol-DNA-biotin) were injected into a NoSlip and the galvanic exchange electrochemical procedure was followed as described in the Experimental Section with a single modification: the first 50.0 s potential step was from OCP to 0.60 V vs CQRE rather than to 0.30 V. This modification was necessary because in the absence of Cl⁻ a more positive potential is required to oxidize Au⁰.

The first and second ASVs resulting from this experiment are displayed in Figure 5. Clearly, both ASV peaks are small, which is a consequence of the absence of the M₂Bs and hence absence of AgNP localization near the WE. The more important point, however, is that the second Ag ASV peak is significantly smaller than the first. This is because AgClₐ(s) cannot form in the absence of Cl⁻, and therefore Ag⁰ is able to diffuse away from the electrode rather than precipitate in its proximity. The data in Figure 5 confirm the original hypothesis and clearly illustrate the importance of Cl⁻ for this assay. Additional information that supports the mechanism of the galvanic exchange detection strategy is provided in the Supporting Information.

#### SUMMARY AND CONCLUSION

Our original origami paper sensor, the oSlip, suffered from three problems: (1) it was necessary to use chemical oxidants (e.g., bleach or permanganate), which have poor stability when dried on paper; (2) bleach and permanganate are very strong oxidizing agents that react with other components in the system, including the oSlip itself; and (3) the slip layer, which is needed for timed delivery of the chemical oxidant, was not user-friendly. By changing the means by which the AgNP labels are oxidized (galvanic exchange vs chemical oxidation with a reagent like MnO₄⁻), all three of these issues have been resolved with only minor (simplifying) changes to the basic form factor of the platform and no significant change in performance. Specifically, the NoSlip resolves the oxidant instability issues because of the inherent stability of the Au⁰ coating on the electrode that is used to electrogenerate the oxidant (Au³⁺). Additionally, Au³⁺ is a milder oxidizing agent than bleach or permanganate, so it does not noticeably react with other components of the NoSlip. Finally, the NoSlip eliminates the need for the slip layer because the oxidant (Au³⁺) is electrogenerated on demand.

![Figure 4](image1.png)

![Figure 5](image2.png)
The NoSlip is inexpensive (not including application-specific reagents, the laboratory-scale cost is ~$0.30 per device), the on-chip assay time is ~7 min, it requires no user intervention other than sample placement, and it is able to detect label concentrations as low as 2.1 pM. Importantly, the NoSlip sensor can be configured to detect a variety of target molecules, including proteins, DNA, bacteria, and viruses, if appropriate capture agents are available. Looking to the future, we plan to devise specific assays that take advantage of the sensitivity and design flexibility of the NoSlip, we are working with collaborators to develop a dedicated reader that eliminates concentrations, and it is able to detect label reagents, the laboratory-scale cost is including proteins, DNA, bacteria, and viruses, if appropriate capture agents are available. Looking to the future, we plan to devise specific assays that take advantage of the sensitivity and design flexibility of the NoSlip, we are working with collaborators to develop a dedicated reader that eliminates the need for a research-grade potentiostat, and we are developing methods for incorporating assay reagents directly onto the NoSlip to eliminate the need for off-chip sample manipulation. The results of these experiments will be reported in due course.

**ASSOCIATED CONTENT**

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acssensors.5b00051.

RevSI-Model NoSlip-092315. Electrochemical cell for gold electrodeposition on the NoSlip working electrode; 3D printed holder design and dimensions; NoSlip and electrode stencil dimensions; NoSlip electrode fabrication; co-oxidation of Au and ascorbic acid; scanning electron micrographs of working electrode; oxidation of Au overlay to expose Ag; protocol for AgNP-biotin conjugation; protocol for binding AgNP-biotin to MAb-streptavidin; ASVs in smaller potential windows prevent Ag reoxidation; rededeposition of Ag in between ASVs; and effect of scan rate on Ag ASVs. (PDF)

**AUTHOR INFORMATION**

Corresponding Author
*E-mail: crooks@cm.utexas.edu. Tel: 512-475-8674.

Notes
The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

This project is sponsored by the Department of the Defense, Defense Threat Reduction Agency (contract number HDTRA-1-13-1-0031) and the National Science Foundation (Grant No. 1402242). R.M.C. thanks the Robert A. Welch Foundation (Grant F-0032) for sustained research support. J.C.C. thanks the NASA Harriett G. Jenkins Graduate Fellowship Program, a NASA Office of Education Minority University Research and Education Program (MUREP). We gratefully acknowledge Xiang Li and the Innovation Station in the Cockrell School of Engineering at The University of Texas at Austin for printing the 3D device holder.

**ABBREVIATIONS**

AgNP, silver nanoparticle; LFAs, lateral flow assays; PoC, point-of-care; MfBs, magnetic microbeads; LODs, limits of detection; BCl, borate and chloride solution; PTFE, polytetrafluoroethylene; RE, reference electrode; CE, counter electrode; WE, working electrode; PLA, polyactic acid; CQRE, carbon quasi-reference electrode; OCP, open circuit potential; ASVs, anodic stripping voltamograms; CV, coefficient of variation

**REFERENCES**