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A Sensing Platform Based on Electrodissolution of a Ag Bipolar Electrode

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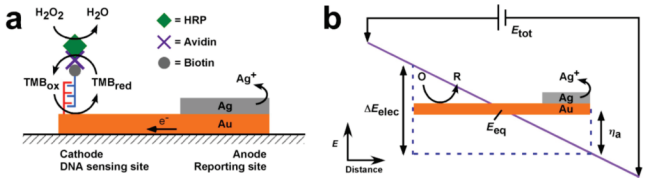
Abstract: Here we report a new type of sensing platform that is based on electrodissolution of a metallic bipolar electrode (BPE). When the target DNA binds to the capture probe at the cathodic pole of the BPE, it triggers the oxidation and dissolution of Ag metal present at the anodic pole. The loss of Ag is easily detectable with the naked eye or a magnifying glass and provides a permanent record of the electrochemical history of the electrode. More importantly, the decrease in the length of the BPE can be directly correlated to the number of electrons passing through the BPE and hence to the sensing reaction at the cathode.

Here we report a new type of sensing platform that is based on electrodissolution of a metallic bipolar electrode (BPE). The concept is illustrated in Scheme 1a. Hybridization of DNA labeled with horseradish peroxidase (HRP) activates the oxidation of tetramethylbenzidine (TMB). When a sufficiently high electric field is applied across the BPE, oxidized TMB is reduced at its cathodic pole, and this triggers simultaneous oxidation and dissolution of Ag metal present at the anodic pole. The loss of Ag is easily detectable with the naked eye or a magnifying glass. More importantly, the decrease in the length of BPE as it begins to dissolve can be directly correlated to the number of electrons passing through the BPE and hence to the DNA binding process at the cathode. Important properties of this device include the following: (1) Ag dissolution provides a physical record of the sensing event; (2) because no direct connection to the BPEs is required, very large arrays of electrodes can be managed simultaneously with no additional instrumentation or device fabrication required;[1] (3) the only instrumentation required for running this device is a power supply or 12 V battery; and most importantly (4) because the Ag layer can have any desired thickness, this device has the potential to be highly sensitive and have a very low limit of detection.

Scheme 1



The theory of bipolar electrochemistry has been described in several recent publications,[2−5] and a number of interesting applications have been reported.[1,6−9] The specific operating principles

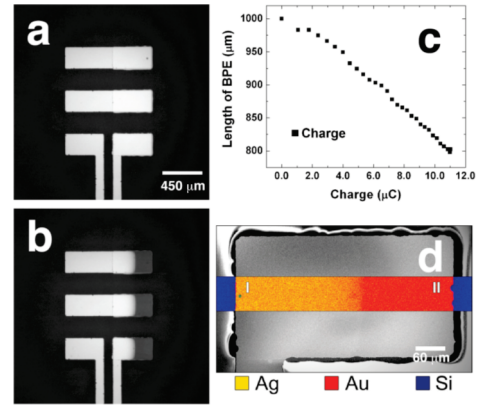


Figure 1. Optical micrographs of bipolar and split BPEs (a) before and (b) after application of $E_{tot} = 12.0$ V for 290 s. (c) Calibration plot showing the linear relationship between Ag film length and charge passed. (d) Electron micrograph of the anodic pole of the split BPE overlaid with an EDS element map.

used in this study are illustrated in Scheme 1b. When a driving voltage (E_{tot}) is applied across the two ends of a microchannel, a linear potential drop develops in the buffer solution filling the channel. This causes the potential of the BPE to float to an equilibrium value (E_{eq}) relative to the solution. When the solution potential difference (ΔE_{elec}) between the two ends of the BPE is larger than the cell potential for a pair of faradaic reactions, electrochemical reactions occur at the cathodic and anodic poles of the BPE. Importantly, the reduction and oxidation reactions are exactly correlated; that is, the rate of one is identical to the rate of the other. Accordingly, a sensing event, such as DNA hybridization (Scheme 1a), triggers an amount of Ag dissolution that is directly correlated to the triggering event.

Figure 1a shows two types of BPEs housed inside a microchannel (1.2 cm long × 2.5 mm wide × 0.5 mm high).[2] Both the continuous and split electrode configurations had an edge-to-edge length of 1.00 mm and were 0.25 mm wide. The BPEs were fabricated on glass by depositing 5 nm of Cr and 20 nm of Ag over 0.45 mm of the distal anodic pole of the Au BPE. The Cr layer enhances the optical contrast between the Ag and Au layers, making it easier to observe the dissolution of Ag. The two halves (each 0.45 mm × 0.25 mm) of the split BPE were separated by 0.10 mm, and their inner edges were connected to an ammeter via contacts that extend out of the channel. We have previously demonstrated that a pair of split BPEs connected with an ammeter is equivalent to a continuous bipolar electrode of the same overall length.[2] Two Ag/AgCl driving electrodes were placed in the reservoirs at either end of the microchannel, and an external field was applied via a power supply. Complete details about the fabrication procedures can be found in the Supporting Information.

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The microchannel housing the BPEs shown in Figure 1a was filled with 1.00 mM *p*-benzoquinone (a sacrificial oxidant) in 0.10 M acetate buffer at pH 5.5. When 12.0 V (E_{tot}) was applied to the driving electrodes, *p*-benzoquinone was reduced at the cathodic poles of the BPEs. This triggered oxidation of Ag at the anodic pole, which (as shown in the movie in the Supporting Information) started at the distal ends of the anodic poles and proceeded toward the center of the BPE. Figure 1b shows that after 290 s the lengths of the BPEs had uniformly decreased to 0.797 ± 0.005 mm (Figure 1b).

The key observation that enables this BPE configuration to be used for chemical sensing is that Ag electrodissolution starts at the distal end of the anodic pole and proceeds inward toward the center. This finding was anticipated from our earlier studies,[2] which showed that the anodic overpotential (η_a) increases from the position of E_{eq} to the outer edge of the BPE (Scheme 1b). Because the overpotential is always highest at the outer edge of the electrode, dissolution preferentially occurs there. Electrodissolution of the BPE eventually stops when the electrode length has decreased to a point where ΔE_{elec} (i.e., the electric field strength times the electrode length) is no longer high enough to drive oxidation of Ag and reduction of *p*-benzoquinone.[3]

Because it was possible to measure the current flowing through the split BPE, the visually determined shortening of the electrode could be directly correlated to the total charge passed at any time during the experiment. Results representing this experiment are shown in Figure 1c. For example, 10.6 μC had been passed through the BPE after 290 s. When the cross-sectional area of the BPE and the density of Ag are taken into account, this corresponds to a calculated decrease of 0.22 mm in the length of the electrode. This value corresponds closely to the measured reduction in length: 0.203 ± 0.005 mm (Figure 1b).

Figure 1d shows an electron micrograph and element map of the anodic pole of the split BPE shown in Figure 1b taken after electrodissolution of Ag. The Ag, Au, and Si signals were recorded horizontally along the electrode and quantified at the indicated locations. For example, comparison of the element EDS spectra at spots I and II indicated that the Ag signal was reduced by 99%. Additional analysis of the EDS intensities is provided in the Supporting Information. Specifically, Figure S1 shows that the dissolving Ag edge has a finite width, which might be due to the electric field gradient over the BPE.[5]

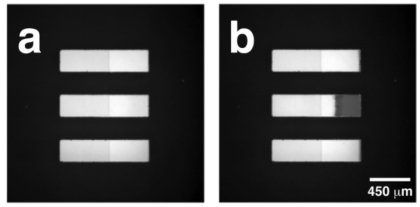

Figure 2. Optical micrographs of the DNA-sensing device (Scheme 1a) showing (middle) DNA-modified and (top and bottom) thiol-modified electrodes (a) before and (b) after E_{tot} = 12.0 V was applied for 90 s.

To demonstrate the biosensing function illustrated in Scheme 1a, the cathodic pole of the middle BPE shown in Figure 2a was modified with 20 nucleotide long, thiol-modified capture DNA, and the other two electrodes were modified with 6-mercaptohexanol (complete experimental details are provided in the Supporting Information). After immobilization, all three BPEs were exposed to 30.0 μL of 1.00 μM biotin-modified complementary target DNA and subsequently to 30.0 μL of 3 μg/mL avidin-functionalized HRP. Under these conditions, hybridization of DNA should occur only on the middle BPE. The channel was then filled with 0.10 M acetate buffer [containing 1.6% (v/v) dimethyl sulfoxide at pH 5.5] plus 0.42 mM TMB and 1.3 mM H_2O_2. HRP catalyzes the reduction of H_2O_2 to H_2O while simultaneously oxidizing the reduced form of TMB (TMB_{red}) to the oxidized form (TMB_{ox}).[10] If ΔE_{elec} is sufficiently high, then enzyme-generated TMB_{ox} can be reduced back to TMB_{red} at the cathodic pole of the BPE. This process triggers Ag oxidation at the anodic pole, which serves to signal DNA hybridization by visual inspection.

Figure 2b shows that for E_{tot} = 12.0 V, the length of the Ag on the middle BPE decreased by 0.29 mm after 90 s. The length of the unmodified electrodes did not change, indicating that DNA hybridization did not occur on the top and bottom BPEs.

In summary, we have shown that BPE dissolution can be used to provide a permanent record of a sensing/recognition event. This method provides a number of key advantages over our previously reported approach based on electrogenerated chemiluminescence signaling. Specifically, the sensitivity and limit of detection of the method are simply controlled by the cross-sectional area of the Ag patch on the BPE. Moreover, no optical readout is required: just the naked eye or a magnifying glass provides sufficient resolution to determine the state of the sensor. In addition to these advantages, the many desirable attributes of BPE sensing are retained: very simple instrumentation and simultaneous control and readout of thousands[1] or perhaps millions of BPEs.

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Supporting Information Available: Information about the BPE fabrication, electrode modification, and EDS analysis and a video file (MPG) corresponding to Figure 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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