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Rapid 2 ms Interrogation of Electrochemical, Aptamer-Based Sensor Response using Intermittent Pulse Amperometry

Mirelis Santos-Cancel[†], Robert A. Lazenby[†], and Ryan J. White^{†,§,*}

[†]Department of Chemistry, University of Cincinnati, Cincinnati, OH, USA

[§]Department of Electrical Engineering and Computer Science, University of Cincinnati, Cincinnati, OH, USA

Abstract

In this manuscript, we employ the technique intermittent pulse amperometry (IPA) to interrogate equilibrium and kinetic target binding to the surface of electrochemical, aptamer-based (E-AB) sensors, achieving as fast as 2 ms time resolution. EAB sensors comprise an electrode surface modified with a flexible nucleic acid aptamer tethered at the 3'-terminus with a redox-active molecule. The introduction of a target changes the conformation and flexibility of the nucleic acid which alters the charge transfer rate of the appended redox molecule. Typically, changes in charge transfer rate within this class of sensor are monitored via voltammetric methods. Here, we demonstrate that the use of IPA enables the detection of changes in charge transfer rates (*i.e.* current) at times <100 μ s after the application of a potential pulse. Changes in sensor current are quantitatively related to target analyte concentration and can be used to create binding isotherms. Furthermore, the application of IPA enables rapid probing of the electro-chemical surface with a time resolution equivalent to as low as twice the applied potential pulse width, not previously demonstrated with traditional voltammetric techniques employed with E-AB sensors (alternating current, square wave, cyclic). To visualize binding, we developed false-color plots analogous to those used in the field of fast-scan cyclic voltammetry. The use of IPA is universal as demonstrated with two representative small molecule E-AB sensors directed against the aminoglycoside antibiotic tobramycin and adenosine triphosphate (ATP). Intermittent pulse amperometry exhibits an unprecedented sub-ms temporal response and is a general method for measuring rapid sensor performance.

Graphical Abstract

*Corresponding author: ryan.white@uc.edu, † If an author's address is different than the one given in the affiliation line, this information may be included here.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

ASSOCIATED CONTENT

Supporting Information

This material is available free of charge via the Internet at <http://pubs.acs.org>. Example raw current time trajectories, equilibrium binding curves obtained using traditional square wave voltammetry, and reverse pulse color plots (PDF). The Supporting Information is available free of charge on the ACS Publications web-site.

Keywords

aptamers; sensors; binding kinetics; electrochemistry; intermittent pulse amperometry

There is a high demand for analytical tools able to quantitate molecules of interest with high specificity, sensitivity, and temporal resolution. Electrochemical, aptamer-based (E-AB) sensors offer an avenue for this type of analytical detection of an extended range of targets, from small molecules to proteins.¹ EAB sensors employ target-induced conformational changes of a redox-active species-labeled aptamer sequence (modified at the distal end of the oligonucleotide sequence) to report on the presence of a specific molecular analyte in a quantitative manner (Scheme 1).² This type of sensor demonstrates specific detection in relatively complex media with nanomolar (nM) to micromolar (μM) binding affinities.³

Traditionally, E-AB sensor signal transduction relies on the use of voltammetric methods. These methods include square wave voltammetry (SWV), alternating current voltammetry (ACV),⁴ or cyclic voltammetry (CV),^{3,5} all of which offer sensitive detection of target analytes. Differential techniques like SWV offer additional advantages such as control over signaling polarity and sensitivity of the aptamer-based sensor by selecting optimal interrogation frequencies.⁶ In short, this class of sensor can operate in two modes. First, when interrogated at the appropriate square wave frequency, the measured voltammetric peak current increases in the presence of target. This type of response is termed “signal on.” Alternatively, a decrease in voltammetric peak current upon the addition of target is termed “signal-off” behavior.

Although the aforementioned voltammetric techniques offer distinct advantages, the time resolution of these techniques is typically limited to, at best, a few seconds (limited by the time scale of the voltammetric sweep). In all cases, a linear potential sweep is applied to the electrode surface (as is the case for CV) or is superimposed with a square wave or sinusoidal waveform in SWV or ACV respectively. Consequently, the experimental time scale, and thus

time resolution of the sensor measurement, is limited by the sweep rate. For example, a SWV scan between 0.0 and -0.5 V usually takes $\sim 2\text{--}3$ s for frequencies of ~ 200 Hz. The same potential window can be scanned in 10 seconds using CV with a scan rate of 100 mV s^{-1} . While the time resolution can be dramatically improved using fast-scan cyclic voltammetry (FSCV),⁷ to the best of the authors' knowledge this technique has not been applied to interrogate E-AB sensors.

Chronoamperometry offers enhanced temporal resolution compared to voltammetric techniques because it only relies on the application of a static potential pulse.⁸ As such, chronoamperometry has long been a powerful technique in electroanalytical chemistry in measuring fast processes. For example, chronoamperometry allows temporally-resolved measurements of biological processes such as vesicular exocytosis (e.g., catecholamine release) using carbon fiber microelectrodes.^{9,10}

With only one notable example discussed below, chronoamperometry has seen limited application in interrogation of EAB sensors. The limited application is because of the nature of the E-AB sensors surface. More specifically, E-AB sensors have a finite number of redox molecules on the electrode surface. In chronoamperometry, when a potential pulse is applied to a surface with a limited supply of bound redox molecules, current decays to zero after all molecules are reduced/oxidized.¹¹ This contrasts to an experiment where the redox molecules are in solution. In this case, the redox molecules are continuously replenished at the surface from surrounding solution leading to a steady-state current related to bulk redox molecule concentration. The current can thus change as bulk concentration changes. With a finite number of redox molecules, once the current reaches zero, the measurement is complete. One way around this limitation was reported by Arroyo-Currás *et al.* In their report, Arroyo-Currás *et al.* use the current decay rate after the application of a potential pulse as opposed to absolute current. Specifically, the authors demonstrate that exponential fits of the current decay profile are quantitatively related to the concentration of target analyte. Using this methodology, Plaxco and coworkers achieve sub-second time resolution (300 ms) and quantitative measure of target analyte.¹²

In this manuscript, we report the use of intermittent pulse amperometry (IPA) to achieve rapid ($< \text{ms}$) E-AB sensor interrogation. IPA uses repetitive alternating potential pulses to reduce and oxidize a reversible redox-active species. In our system, a methylene blue (MB)-labeled aptamer, we alternate between a sufficient negative overpotential to reduce MB (-0.4 V vs. Ag/AgCl; defined as a forward pulse) and 0.0 V, or potential at which the methylene blue is reoxidized (defined as a reverse pulse), thus restoring the sensor surface. We demonstrate that absolute current values at a defined time after the application of a potential pulse (δt) are quantitatively related to the concentration of target analyte in solution. Using this method, we demonstrate that equilibrium binding isotherms can be obtained at times as low as $50\text{--}200\text{ }\mu\text{s}$ after a potential pulse. Furthermore, we show that IPA allows kinetic measurements of E-AB sensor response in sub 100 ms time scales. To visualize kinetic binding data, we generate 3-dimensional (3D), false-color plots that are analogous to those used in FSCV developed by Wightman and coworkers to visualize exocytosis events.¹³ Using the square wave potential pulse waveform, time resolution is ultimately limited by the potential pulse width or the binding kinetics of the aptamer-target interaction. We believe

that the application of IPA to E-AB sensors marks a new method of rapid quantification of the E-AB sensor binding to new, yet unrealized time regimes.

EXPERIMENTAL SECTION

Chemicals and Solutions

Tris-2-carboxyethyl-phosphine (TCEP), 6-mercapto-1-hexanol (99%), Trizma (tris) base (2-amino-2-(hydroxymethyl)-1,3-propanediol, magnesium chloride (MgCl_2), gold (III) chloride (HAuCl_4) trihydrate, potassium bromide (KBr), sulfuric acid (H_2SO_4), hydrogen peroxide (H_2O_2), hydrochloric acid (HCl) sodium chloride (NaCl), tobramycin, adenosine triphosphate (ATP), and 10 $\times$ Tris-EDTA were used as received from Sigma Aldrich. All solutions were prepared using autoclaved, ultrapure water (18.0 M Ω cm at 25 °C) using a Biopak Polisher Millipore ultra-purification system (Millipore, Billerica, MA). The DNA tobramycin aptamer sequence (5'-GGGACTTGGTTTAGGTAATGAGTCCC-MB-3')¹⁴ and DNA destabilized ATP aptamer (5'-CTGGGGGAGTATTGCGGAGGAAA-3')¹⁵ oligonucleotide sequences were purified using dual HPLC (Biosearch Technologies, CA) and used as received.

Electrochemical Aptamer-Based (E-AB) Sensor Fabrication for Equilibrium Measurements

Before sensor fabrication, each macro (2 mm diameter poly-crystalline gold) electrode (CH Instruments, Austin, TX) was hand polished on a microcloth in a 1 μm diamond suspension, followed by polishing in 0.05 μm aluminum oxide slurry (microcloth, diamond suspension and alumina from Buehler). All electrodes were rinsed and electrochemically cleansed using sodium hydroxide and sulfuric acid as previously described.¹⁶ After cleaning, electrodes were incubated in a 200 nM parent tobramycin or 200 nM destabilized ATP aptamer solution in autoclaved 20 mM tris buffer (pH $\sim$ 7.4) with 100 mM NaCl and 5 mM MgCl_2 for 1 h. Prior to incubation, all oligonucleotide sequences were reacted with 2 μL 100 mM TCEP for 1 hour to reduce the 5'-disulfide bond. Following aptamer self-assembled monolayer formation, electrodes were passivated in a 30 mM 6-mercapto-1-hexanol solution for 1 h. Sensors were equilibrated in autoclaved 20 mM tris buffer (pH = 7.4) with 100 mM NaCl and 5 mM MgCl_2 for 1 h prior to use.

Electrochemical Aptamer-Based (E-AB) Sensor Fabrication for Kinetic Measurements

E-AB sensors to perform kinetic measurements were prepared on a thin film electrode (ED-SE1-AuPt) containing one 1 mm diameter gold working electrode, one platinum counter electrode, and one platinum quasi-reference electrode purchased from MicruX Fluidic (Oviedo, Spain). All electrodes were hand polished in an alumina slurry for 2 minutes using a disposable cotton swab, followed by electrochemical reductive desorption using sodium hydroxide as previously described.¹⁶ Gold nanoparticles were electrodeposited following a previously described method by Ivanova and Zamborini,¹⁷ to increase the surface area of the working electrode and therefore improve electrochemical signaling.¹⁸ Briefly, bulk electrolysis was performed for 10 seconds at -0.15 V in a solution containing 20 mM HAuCl_4 , 0.1 M KBr and 0.5 M HCl. E-AB sensors on nanostructured thin film electrodes were fabricated in the same way as 2 mm diameter polycrystalline gold electrodes.

Electrochemical Measurements

Electrochemical measurements were performed using a CH Instruments 620D Electrochemical Workstation (CH Instruments, Austin, TX) in a three-electrode cell system using Ag/AgCl (3 M NaCl) and a platinum wire as a reference and counter electrode, respectively. Square wave voltammograms were obtained using a pulse amplitude of 25 mV with a constant frequency (100 Hz) and a step width of 1 mV. IPA measurements were done by applying a -0.4 V potential with a 1 sec pulse width with a sample interval equivalent to 70 μ s.

Kinetics measurements were obtained by applying alternating potential steps between 0.0 and -0.4 V (vs. Ag/AgCl) with a 1 ms pulse width (1000 Hz) and 10 μ s sample interval, using a CH instruments 660D Electrochemical Workstation controlled via in-house written LabVIEW code (LabVIEW 2014). Electrochemical measurements were performed on the thin film electrode assembly housed on a commercial aluminum base (AIO Drop Cell-Base) with a poly(methylmethacrylate) (PMMA) cover (AIO Cell Add-On Batch Cell PMMA-2.0), both from MicruX Technologies (Oviedo, Spain). The target was added by manual injection of 0.8 μ L into the solution to reach the desired concentration.

RESULTS AND DISCUSSION

Intermittent Pulse Amperometry (IPA) and E-AB Sensors

IPA is an electroanalytical technique that involves a series of repetitive potential pulses between a potential at which no faradaic electrochemistry occurs to a sufficient overpotential to oxidize/reduce a redox couple at a diffusion-limited rate (Figure 1). For E-AB sensors modified with MB, the potential is pulsed at a sufficient overpotential (-0.4 V vs. Ag/AgCl) to reduce MB to leucomethylene blue, followed by time intervals where the working electrode is held at 0.0 V to oxidize leucomethylene blue, thus resetting the sensor baseline. The continuous, current-time profile shows a series of sharp pulses in the forward and reverse direction (schematically shown in Figure 1 and representative data in supporting information (SI) Figure S1).

To better understand the IPA current-time response at the surface of E-AB sensors, we approximate the surface of E-AB sensors as a redox polymer film in which there is a finite number of discrete localized redox sites (methylene blue). The thickness of the film (d) at a maximum is defined by the fully extended length of the nucleic acid tether (~ 15 nm).^{19,20} When the motion of the redox sites are appreciable, the amperometric response of redox polymer films behaves similarly to a thin layer electro-chemical cell.²¹ The current response depends on the relationship of the diffusion layer thickness ($d = D t_e$) and the thickness of the film (d). This relationship can be described by comparing the diffusion coefficient of the redox probe (D), the experimental time scale (t_e), and the film thickness through the relationship $D t_e / d^2$. When $D t_e / d^2 \gg 1$, or the film thickness is much smaller than the diffuse layer, the current response to a potential perturbation will follow thin layer behavior – essentially the redox molecule is reduced/oxidized rapidly, and current will decay exponentially. However, if $D t_e / d^2 \ll 1$, then the current behavior will exhibit semi-infinite diffusion behavior.

When a thin layer cell or redox polymer exhibits a current response in the semi-infinite diffusion regime ($Dt_e/d^2 \ll 1$), the current-time decay will follow that of a freely diffusing species described in equation 1 (the Cottrell equation):

$$i = \frac{nFAC\sqrt{D}}{\sqrt{\pi t}} \quad (\text{eq. 1})$$

where A is the electrode area and C (mol/cm³) is the concentration of redox species (G/d —where G is the surface concentration in mol/cm²). Finally, n is the number of electrons transferred per equivalent, and F is Faraday's constant.

When considering a potential pulse applied to thin a layer cell, another term must be added to account for a longer time regime in which a finite diffusion boundary is observed, and the concentration of active redox molecule depletes to zero. Equation 2 describes the current-time response to a sufficient over-potential pulse:²¹

$$i_t = \frac{nFAC\sqrt{D}}{\sqrt{\pi t}} \left[\sum_{k=0}^{\infty} (-1)^k \left\{ e^{\left(\frac{-k^2 d^2}{Dt} \right)} - e^{\left(\frac{-(k+1)^2 d^2}{Dt} \right)} \right\} \right] \quad (\text{eq. 2})$$

At longer time scales, the current decay rate deviates from the $t^{-1/2}$ behavior described by the Cottrell expression, described by the latter portion of the expression to account for the depletion of the redox centers.

Finally, at short time scales, or when the film thickness is thin, and $Dt_e/d^2 \gg 1$, the current response is likely a convoluted mixture of double layer capacitive charging and faradaic pseudocapacitance.^{22–24} In our case, the application of a potential pulse to the surface of E-AB sensors results in a current-time response exhibiting a double exponential decay (Figure 2). Since the thickness of the layer is thin (~15 nm, assuming 6.3 Å per nucleotide length), we do not observe a region of Cottrell-like behavior, rather the current response is dictated by the latter term of equation 2. Additionally, after ~0.30 ms, the current decays to zero as is expected for a surface-confined reaction. *Interestingly, and what serves as the basis for this manuscript, we observe quantitative changes in both portions of the current-time trace upon target (here tobramycin) addition (Figure 2), which we explore in more detail below.*

IPA Enables Rapid and Quantitative Measure of E-AB Sensor Performance

To demonstrate the applicability of IPA for a quantitative measure of target analyte concentration, we fabricated sensors using two representative small molecule aptamers: an amino-glycoside (tobramycin) binding aptamer and adenosine triphosphate (ATP) binding aptamer, reported by Rowe *et al.*¹⁴ and White *et al.*,¹⁵ respectively. Traditionally, the aminoglycoside-based sensor functions as a signal-on sensor when interrogated with SWV at high frequencies (> 400 Hz, SI Figure S2).

Sensors fabricated to detect aminoglycoside antibiotics (*e.g.* tobramycin) exhibit an apparent binding affinity (K_D) $\sim 5.0 \pm 0.6 \mu\text{M}$ and maximum signal change of $\sim 40\%$ when interrogated voltammetrically (SI Figure S2). Similarly, sensors fabricated with the aptamer specific to ATP detection yield a K_D of $0.4 \pm 0.2 \text{ mM}$ and signal changes of $\sim 120\%$ when at saturation, when interrogated at high SWV frequencies ($\sim 400 \text{ Hz}$) (SI Figure S2).

Monitoring current after the application of a potential pulse provides quantitative information about tobramycin concentrations (Figures 3 and 4). To perform IPA, we monitor current after a forward or reverse pulse with $7 \mu\text{s}$ resolution (Figure 3). Qualitatively, we observe a difference in the instantaneous current and the rate of current decay when comparing a current time trace with 1 mM tobramycin and without target (Figure 3) after the application of a forward pulse. Similarly, we observe differences in the current-time trace after the application of a reverse pulse (Figure 3).

With careful evaluation, we find that the magnitude and polarity of the change in current with and without a target is a function of when current is sampled after the application of a potential pulse, or dt (Figure 4). To quantify E-AB sensor response, we use the traditionally used “percent signal change” defined as: $(i_{\text{Target}} - i_{\text{No target}} / i_{\text{No target}}) \times 100$, where i_{Target} and $i_{\text{No target}}$ represent the current response of the sensor with and without target in solution, respectively. The polarity of percent signal change is a function of dt and changes from positive (signal-on) to negative (signal-off). At dt values $< \sim 0.05 \text{ ms}$, the signal change is positive in the presence of target whereas, at longer sampling times, $> 0.05 \text{ ms}$, the signal change is negative. We also observe that the magnitude of the signal change is quantitatively related to tobramycin concentration (Figure 4). For example, when current is sampled at three different dt values (0.084 , 0.203 , and 0.301 ms), the percent signal changes follows Langmuir-like binding isotherms related to tobramycin concentration. This trend holds for both forward and reverse pulses (Figure 4), albeit with slightly different sensitivities. We find the highest sensitivity for tobramycin at a dt value of 0.084 ms . (Figure 4). The binding curve begins to approach a saturating level with a maximum signal change value of $\sim 30 - 35\%$, typical to what has been observed previously. Fitting the data to a Langmuir-like binding isotherm,¹ we obtained an apparent binding affinity, $K_D = 5.0 \pm 0.6 \mu\text{M}$, which is comparable to that achieved using voltammetric methods (SI Figure S2). Sampling at dt values of 0.203 and 0.301 ms yields reproducible maximum signal changes of $\sim 12\%$ and $\sim 8\%$, respectively (Figure 4).

Current recorded after the application of a reverse pulse also yields quantitative binding isotherms. Similar to the forward pulse, the magnitude of the current difference is a function of tobramycin concentration (Figure 4). We see the highest sensitivity ($\sim 30\%$ maximum signal change) at $dt = 0.035 \text{ ms}$ (Figure 4). Fitting the sensor response at dt values of 0.035 , 0.203 and 0.301 ms gives Langmuir-like isotherms with observed K_D values of $2.2 \pm 0.4 \mu\text{M}$, similar to that achieved with voltammetric detection (SI Figure S2).

The use of IPA for quantitative determination of target analyte concentration with E-AB sensors is a general strategy. To demonstrate this, we interrogated E-AB sensors specific for ATP detection. We found that the polarity of the current change is always positive, as opposed to the tobramycin sensor (Figure 5). Similar to what we observed with the

tobramycin sensor, we find the magnitude of signal change is quantitatively related to ATP concentration. Specifically, we plot the average percent signal change (of three sensors) at three different dt values, 0.035, 0.105 and 0.154 ms (Figure 5). The sensor response yielded the highest sensitivity at a dt value of 0.035 ms with a $K_D \sim 0.7 \pm 0.3$ mM, comparable to the value obtained using SWV (SI Figure S2). Similar to results obtained with the tobramycin sensor, we see no signal change after 0.30 ms, when the current approaches zero. Of note, unlike the response of the tobramycin aptamer sensor, we do not observe as robust of signal changes in current for the reverse pulse compared to tobramycin data (SI Figure S3). Nonetheless, IPA appears to be a general electrochemical method for probing E-AB sensor response.

IPA Enables Monitoring of Rapid Binding Kinetics at E-AB Sensor Surfaces

The nature of the repetitive forward and reverse pulses employed in IPA allows rapid and continuous interrogation of the signaling of E-AB sensors, and thus grants the ability to monitor binding kinetics electrochemically with a time resolution not yet demonstrated. With this in mind, we set out to demonstrate the ability to achieve as low as 2 ms time-resolved measurements of E-AB sensor response to explore aptamer (A)-target (T) binding kinetics which have been typically been limited to surface plasmon resonance-based measurements.²⁵ For aptamer-target interaction, the rate of aptamer-target complex (AT) formation is given by the bimolecular association constant (k_{on}) and the monomolecular dissociation constant (k_{off}) to obtain $K_D = k_{off}/k_{on}$ (equation 3), describing a 1:1 interaction between aptamer and target.

IPA enables a rapid measure of the binding rate of tobramycin and ATP (both small molecules) to the aptamer sensor surface (Figures 6 and 7). To perform binding kinetic experiments, we utilized a square wave potential pulse profile between 0.0 and -0.4 V, with a 1 ms pulse width. This ultimately establishes the temporal resolution of continuous measurements. The current was recorded without filtering at 10 μ s resolution. To better visualize the IPA data, we have developed false-color plots similar to those used in FSCV (Figure 6).¹³ The color plots are created by stacking individual forward-pulse current responses along the x -axis with dt plotted on the y -axis.

Color, in the z -axis, represents the change in absolute current (Δ Current) with respect to the current recorded at the same δt . The baseline for this change was set to the current value before target addition. Note that for the Tobramycin sensor response, Δ Current has been inverted so that the maximum change is a positive value. This allows for a more direct comparison with the ATP sensor.

The addition of 200 μ M target can be clearly visualized for both tobramycin and ATP sensors (Figure 6). All data are plotted as a positive Δ current value for ease of visualization, however, the polarity of the signal changes match what was observed in the equilibrium

measurements. In the case of the tobramycin sensor, reverse pulse color plots also illustrate changes in sensor signal with the addition of target (SI Figure S4). Individual line scans at various dt values (30, 70, 190, and 490 μ s for the tobramycin sensor and 10, 30, 50, and 490 μ s for the ATP sensor) allow visualization of sampled-current-time traces or trajectories of sensor response with respect to time (Figure 6). Since only the forward pulses are plotted, each data point in the sampled-current-time trace represents the current sampled at the respective dt every 2 ms. This time resolution is ultimately limited by the duration of one complete forward and reverse applied potential pulse. Boxcar averaging of the current smooths the data, at the cost of temporal resolution (10 ms/pt in Figure 6, right).

IPA enables measurement of observed binding rates to E-AB sensor surfaces (Figure 7). The sampled-current-time trace should follow the following exponential rise in current (equation 4)

$$i_t = i_{eq, [T]} \left[1 - e^{(-k_{obs}t)} \right] \quad (\text{eq. 4})$$

where $i_{eq, [T]}$ is the equilibrated current change at equilibrium for a specific T, and the observed binding rate is $k_{obs} = k_{on}[T] + k_{off}$ where k_{on} ($M^{-1} s^{-1}$) and k_{off} (s^{-1}), are the on and off binding rates, respectively. The tobramycin sensor equilibrates to a maximum signal change rapidly after the addition of 200 μ M tobramycin, as shown when Dcurrent is sampled at $\delta t = 30, 70$, and 190 μ s (Figure 7, left). More specifically, the sampled-current-time response reaches a rapid increase to an equilibrium current. Fitting the sampled-current-time trace following equation 4, we find $k_{obs} = 0.72 \pm 0.04 s^{-1}$. Similarly, when the ATP sensor is challenged with 200 μ M target, the sensor equilibrates faster than the tobramycin counterpart with $k_{obs} = 1.4 \pm 0.4 s^{-1}$. These data mark the fastest electrochemical interrogation to the authors' best knowledge. Traditional voltammetric interrogation methodologies would typically not be able to capture the observed k_{obs} responses due their potential sweep limitation that at best reaches temporal resolution of seconds. Also, IPA showed good rapid binding kinetics analysis for both small molecule targets when compared with other techniques.²⁵

CONCLUSION

In this manuscript, we demonstrate the use of IPA to monitor E-AB sensor response with 2 ms time resolution. Using IPA represents a potential new electroanalytical technique to push electrochemical aptamer-based sensors into new time regimes, on a par with what is achievable using FSCV. While the data shown in this manuscript reach a time resolution as fast as 2 ms, the time resolution of the technique is ultimately limited to two times the potential pulse width used in the IPA waveform. Presumably, because our current decays to zero after $\sim 200 \mu$ s, it is conceivable that we could achieve 400 μ s time resolution. Nonetheless, the data provided here are the first demonstration of <5 ms time-resolved measurements of E-AB sensor response. The change in current is quantitatively related to the concentration of the target analyte in solution. Finally, we developed false-color plots as a means to visualize the change in sensor response.

IPA is a potential general strategy to probe both the equilibrium binding and kinetic regimes of E-AB sensor response with unprecedented time resolution. This powerful combination of the IPA technique with E-AB sensors could be applied to a range of analytical applications including real-time measurement of biological processes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

IPA	intermittent pulse amperometry
ATP	adenosine triphosphate
E-AB sensor	electrochemical, aptamer-based sensor
MB	methylene blue
SWV	square wave voltammetry
ACV	alternating current voltammetry
CV	cyclic voltammetry
FSCV	fast-scan cyclic voltammetry
SPR	surface plasmon resonance

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Figure 1.

Intermittent pulse amperometry (IPA) involves (top left) the application of repetitive potential pulses to the working electrode, alternating between a potential sufficient to reduce/oxidize a redox-active molecule and a potential to reversibly oxidize/reduce the molecule (*i.e.* either side of the standard electrode potential E°). (Bottom left) The resulting continuous amperometric time trace is represented as a series of forward and reverse current pulses. In sampling E-AB sensors, we sample current at a given time after the pulse (δt). (Top right) If the rates of charge transfer (k_{et}) differ (e.g., free aptamer without or with the target), the current sampled (Δi) will be different, thus provides the basis for signal transduction of a target binding. (Bottom right) A plot of sampled-current vs. time yields a “sampled” current-time, or amperometric trace.

Figure 2.

Comparison of the amperometric current-time trace with and without target (tobramycin) reveals a difference in absolute current measured. (Left) The current-time response using a representative forward pulse of a tobramycin E-AB sensor with and without 1 mM tobramycin present exhibits notable changes in current. These data represent the current-time response with the application of a potential pulse from 0.0 V to -0.4 V (vs. Ag/AgCl) at $\Delta t = 0$. (Right) Current plotted on a log-log scale better illustrates the difference in current with and without target analyte. The current-time response in both cases appears to follow a double exponential decay.

Figure 3.

Differences in current are observed at both the forward and reverse potential pulse. Current response after both a (top) forward and (bottom) reverse pulse show a different response to the presence and absence of target analyte. E-AB sensors for the detection of tobramycin show marked differences in absolute current and decay rates for both (top) forward (-0.4 V vs. Ag/AgCl) and (bottom) reverse (0.0 V vs. Ag/AgCl) pulses. Plots on the right show the zoomed-in regions of interest from the pulses on the left, where this difference in current is most apparent.

Figure 4.

Amperometric current response is quantitatively related to the concentration of tobramycin present, and the magnitude and polarity of that response is a function of when the current is measured after the application of a pulse (δt) for both forward (left) and reverse (right) pulses. (Top) Evaluation of current after a potential pulse shows a significant signal change in times as fast as 50 μ s upon target addition, with the most significant changes occurring at 84 μ s after the potential pulse. (Middle) Zoom-ins of the shaded regions show the sampling times that give greatest percent signal change. (Bottom) Plotting percent signal change at different δt values creates quantitative binding curves that can be fitted to Langmuir-like

isotherms for quantitation of target concentrations. The current was sampled at three dt values, marked as dashed lines in the top plots.

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Figure 5.

The use of IPA interrogation is a general means of probing E-ABs. (Left) ATP-specific E-AB sensor interrogated using IPA exhibited sensitive target-induced responses within tens of μs timeframe. Changes in current are dependent on ATP concentration as well as δt with an optimal sensor response at δt values between 10 – 140 μs . (Right) Data obtained at multiple δt values (dashed lines on the left plot) fit Langmuir-like isotherms for quantitative analysis of ATP concentration.

Figure 6.

False color plots allow visualization of IPA current response with the addition of target analyte (tobramycin and ATP). 2D and 3D color plots (zoom-in region from false color plots) align each forward pulse along the x -axis, δt along the y -axis, and the change in current (Δ Current) in the out of plane, or z -axis. Current is plotted as positive (signal-on) for both ATP and Tobramycin (where the sign of the Tobramycin change has been inverted), so that the two sensors can more easily be compared. Line scans, indicated by the white dashed lines, provide sampled-current time traces with 2 ms time resolution or box-car averaged to yield 10 ms time resolution. Consistent with equilibrium binding data, the sensitivity of the measurement is a function of which δt value is used.

Figure 7.

E-AB sensor response follows a single exponential rise as illustrated by sampled-current time traces after the addition of 200 μ M tobramycin (left) and ATP (right). Tobramycin sensors respond with a $k_{\text{obs}} = 0.72 \pm 0.04 \text{ s}^{-1}$ whereas the ATP sensor responds in about half the time with a $k_{\text{obs}} = 1.4 \pm 0.4 \text{ s}^{-1}$. Data shown are boxcar averaged and thus represent 10 ms/point time resolution.

Scheme 1.

Electrochemical, aptamer-based sensors employ target-induced conformational changes of a redox active-labeled aptamer sequence to quantitatively report on the presence of a specific molecular analyte. Changes in charge transfer rates (k_{et}) between the redox moiety with (k'_{et}) and without (k_{et}) target lead to changes in measured current.