Solution-Phase Electrochemical Aptamer-Based Sensors

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***Abstract*—Electrochemical aptamer-based sensors (EABs) using self-assembled monolayers on gold working-electrodes have now been in-vivo demonstrated for multiple-analytes, demonstrating their sensitivity and specificity even in a continuous sensing format. However, longevity has been demonstrated for only 24 hours and sensitivity has been challenging for highly dilute analytes (nM regime). A novel approach is reported here using electrochemical aptamer-based sensing that is not covalently-bound to a gold-working electrode but where aptamers are freely mobile in solution. This alternative approach has the potential to improve longevity by reducing electrode surface degradation and improving sensitivity using aptamer binding constructs that are not available for aptamers when covalently bound to the electrode. Specifically, a molecular-beacon (fluorescent) cortisol aptamer was adapted into an amperometry solution-phase cortisol EAB sensor, demonstrating ∼5% signal gain starting at only 10 nM and a saturated signal gain of ∼70% at several μM. A robust signal was achieved due to use of methylene-blue redox-tagged aptamer that was measured through amperometry with interdigitated electrodes. While this result demonstrates the basic feasibility of solution-phase EAB sensors, the result also required a self-assembled monolayer alkylthiolate blocking-layer on the gold working electrode which restricts potential device longevity. These results cumulatively suggest that initial significance of solution-phase EAB sensors may be strongest for point-of-care type testing applications and further development would be required for long-lasting continuous sensing applications.**

***Index Terms*—Amperometry, electrochemical sensor, aptamer, interdigitated electrode, redox-recycling.**

I. Introduction

THE use of continuous glucose monitors for diabetes management is a historical achievement in modern diagnostics, but unfortunately, despite acute needs for the real-time monitoring of many other molecules across the broader field of human disease management, it remains an isolated success [1]. The problem is that glucose sensors are enzymatic (i.e., rely on the enzymatic oxidation of their target), limiting their generalizability to other analytes. Unlike enzymatic sensors, electrochemical aptamer-based (EAB) sensors are broadly generalizable, as shown by multiple examples of real-time molecular monitoring such as cocaine [2], procaine [3], doxorubicin [4], gentamicin [5], phenylalanine [6], irinotecan [7], vancomycin [8], serotonin [9], tobramycin [10], and ATP [11], [12]. However, unfortunately for the clinical adoption EAB sensors, in-vivo device longevity and sensitivity remain significant challenges [13].

Conventional EAB sensors rely on a redox-tagged aptamer that is chemically bound to the electrode used for measurement. This electrode-bound-aptamer design imparts a double penalty by: (1) requiring sustained monolayer attachment to the electrode; and (2) being unable to fully leverage the greater variety of aptamer switching mechanisms that exist for aptamers in solution (e.g., molecular beacons [14]). Given this, significant benefits could be realized if a novel aptamer biosensor approach were developed that resolves both these challenges.

Therefore, we hypothesized two innovative advantages that solution-phase EAB sensors could potentially provide: (1) they can access a larger tool kit of aptamer design strategies used broadly across the field of aptamers in general which have the potential to improve sensitivity and detection limits; (2) the aptamers do not require fragile attachment to the gold electrode, which can enable greater sensor longevity. Specifically, a molecular-beacon (fluorescent) cortisol aptamer was adapted into an amperometric solution-phase cortisol EAB sensor, demonstrating ∼5% signal gain starting at only 10 nM and a saturated signal gain of ∼70% at several μM. A robust signal was achieved due to use of methylene-blue redox-tagged aptamer that was inherently measurement amplified by redox-recycling between interdigitated electrodes. While this result demonstrates the basic feasibility of solution-phase EAB sensors, the result also requires a self-assembled monolayer alkylthiolate blocking-layer on the gold working electrode which restricts potential device longevity. These results cumulatively suggest that initial significance of solution-phase EAB sensors may be for

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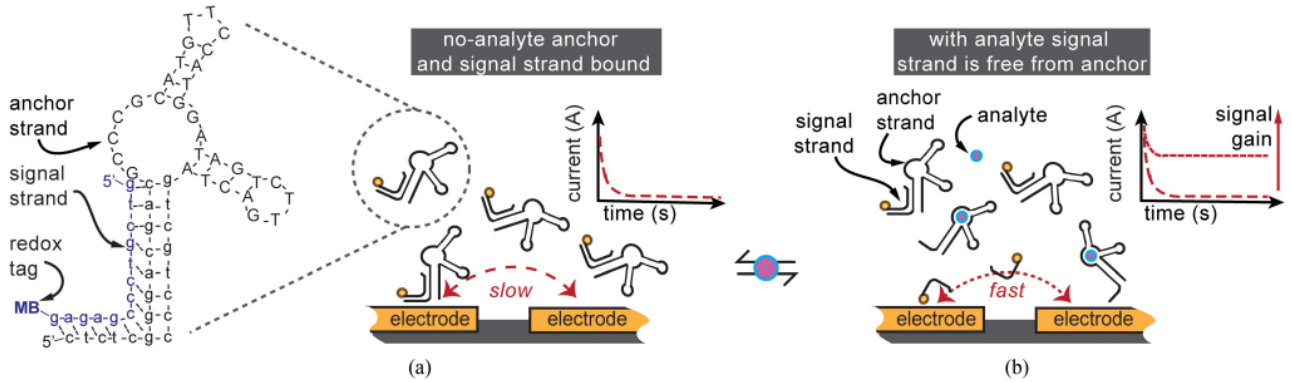

Fig. 1. (a) predicted secondary structure of the cortisol aptamer; (b) the discussed aptamer releases a small signal strand with a redox tag with binding to the target analyte. The freed signal strand is capable of faster diffusion (greater redox recycling) which can be detected using chronoamperometry.

point-of-care type testing applications and further development would be required for long-lasting continuous sensing applications.

II. Device Description

Later in the paper, the discussion section presents a general description of multiple possible solution-phase EAB constructs, but the discussion here will begin specifically with a two-strand aptamer amperometric approach that is demonstrated for cortisol sensing. As shown in Fig. 1, a two-strand aptamer construct for cortisol is utilized, containing a 'anchor' strand of aptamer (51 bases) and a smaller 'signal' strand of aptamer (13 bases). The signal strand is tagged via an amino modifier with a methylene blue redox reporter. This aptamer is adapted for cortisol detection from a previously demonstrated molecular beacon aptamer with a binding affinity with K_d of 2.54 μM for cortisol, where the aptamer is tagged with a fluorescent beacon and fluorescence quencher [15]. With no cortisol, the signal and anchor strand form the geometry shown in Fig. 1, bound using complimentary base-pairing between the aptamers. As shown in Fig. 1, as cortisol binds to the anchor strand, the signal strand is released, similar to that of the molecular beacon switch [15]. Upon releasing, the signal strand experiences two fundamental changes that impact its electrochemical detection: firstly, it is now smaller in size in solution, and therefore it is more probable that the redox tag can pass close enough to an electrode to experience electron-transfer [16], [17]; secondly, it is now smaller in size and mass which allows it to diffuse faster in solution which should increase its rate of collision with an electrode. Both of these fundamental changes can then be directly exploited and measured amperometrically using redox-recycling between interdigitated electrodes as shown in Fig. 2.

Interdigitated working/counter electrodes are utilized to amplify the measured signal by allowing redox recycling of the methylene blue tagged aptamers as they diffuse between two electrodes. The stabilized/steady-state current of interdigitated electrodes under chronoamperometry is proportional to the diffusion coefficient of the oxidized/reduced species as described

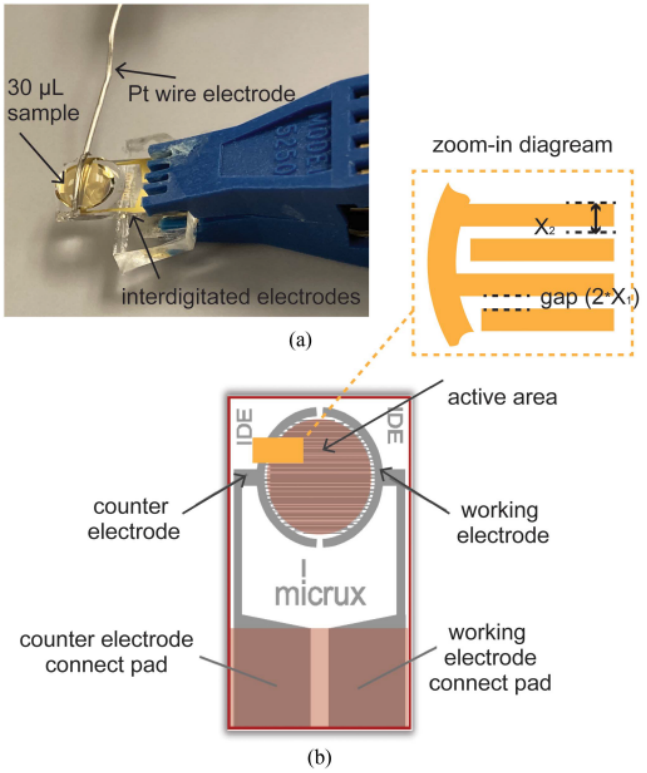

Fig. 2. (a) picture of the experiment setup using interdigitated electrodes; (b) diagram of the IDE used in this work.

in equation [18]:

$$I = \frac{2nFDc^b}{\pi\left(X_2 - X_1\right)} \ln\left[\frac{X_2}{X_1} + \left(\left(\frac{X_2}{X_1}\right)^2 - 1\right)^{1/2}\right] \quad (1)$$

where n is the unit of electron charge that the redox species carries, F is Faraday constant, D is diffusion coefficient of the redox species, c^b is the concentration of the redox species, X_1 is the half width of the gap between the working electrode and

the counter electrode, and X_2 is the sum of the width of a single electrode finger/band and half width of the gap. These dimensions are labeled in the Fig. 2(b). As previously described, when the signal strand is released from the anchor strand due to binding of cortisol, it achieves a smaller hydrodynamic radius and therefore higher diffusion coefficient. According to (1) the resulting gain in current should therefore be directly proportional to the percentage of signal strand that is freed from the anchor strand as cortisol binds with the anchor strand. Because the gain in current is directly proportional to cortisol binding, the gain therefore should exhibit a Langmuir isotherm response.

III. Sensor Solution and Device Preparation Process

The anchor strand and the signal strand of the cortisol aptamer were separately synthetized by Integrated DNA Technologies, INC (IA, USA) using a sequence for the anchor-strand of 5'-/5Acryd/CTC TCG GGA CGA CGC CCG CAT GTT CCA TGG ATA GTC TTG ACT AGT CGT CCC -3' and for the signal strand of 5'- GTC GTC CCG AGA G/3MeBIN/ -3'. The dry aptamers were dissolved separately in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer solution to obtain 20 μM concentration. The HEPES buffer solution contained 20 mM HEPES, 1 M NaCl, 10 mM $MgCl_2$ and 5 mM KCl. Then this solution was vortexed for over 30 seconds to ensure full dissolution. The solution was stored at -20 °C until testing. Upon testing, the aptamer solution was brought to room temperature in a dark environment. The anchor aptamer solution and signal aptamer solution were then combined at a 1:1 ratio to prepare 10 μM of the cortisol responding aptamer, first by placing the combined solution in a bath of boiling water for 5 minutes, and then cooling the solution in the dark over 45 minutes to room temperature. This process enabled the anchor and signal strand to take on the paired form as illustrated in Fig. 1(a).

The interdigitated electrodes shown in Fig. 2 were purchased from MicruX Technologies (Spain). Each electrode has 120 fingers. Each finger of the counter and working electrode is 10 nm wide with a 5 nm gap. Prior to testing, the electrodes were rinsed with Acetone for 1 minute, and DI water for 30 seconds. Then the electrodes were electrochemically cleaned in 0.05 M H_2SO_4 solution using cyclic voltammetry by sweeping the potential range from 0 to 1.5 V for 20 scans. Before being tested, the electrodes were rinsed with DI water and incubated in 5 *mM* mercaptohexanol (MCH) solution for over 2 hours to create an electrochemical blocking layer which reduces oxygen reduction current.

For the titration test, the interdigitated electrodes were used as working and counter electrode, and a 0.5 mm diameter Pt wire was used as the reference electrode. The test setup is shown in the photo of Fig. 2(a). The 10 μM cortisol aptamer solution described previously was used as the testing solution.

For separate tests on stability and longevity, gold disk electrodes with 2 mm diameter were used as the working electrode, a 0.5 mm diameter and 32 mm long Pt wire as the counter electrode, and a glass-frit sealed 0.5 mm Ag/AgCl electrode used as the reference electrode. All electrodes were purchased from CH Instruments, INC (TX, USA). The gold disk working electrode was polished with 3 μm and 0.5 μm Alumnia Slurry on a polishing pad for 1 minute. After rinsing with DI water, the gold rod working electrode was electrochemically cleaned in 0.5 M NaOH solution using cyclic voltammetry with 0 to -1.6 V potential range for 300 scans, and then in 0.05 M H_2SO_4 solution with 0 to 1.5 V potential range for 300 scans. The cleaned gold disk working electrode was then incubated in 5 mM MCH solution for over 2 hours.

IV. Testing and Results

All titration tests for measuring sensor response to cortisol concentration were performed at room temperature. The reduction peak of the methylene blue tag between the working and counter electrode was observed at approximately -500 mV using cyclic voltammetry. Therefore, for each chronoamperometric measurement, 500 mV voltage was applied for 10 seconds, and the response quickly saturated into a steady-state diffusion-limited response. The resulting current was therefore measured as the average current recorded between the 9th second to the 10th second (Fig. 3(a)). This measured current should represent a diffusion-limited current response, where increased cortisol results in increased freely diffusing signal strands which will exhibit a greater rate of diffusion and therefore greater measured current.

Before the sensor titration data was measured, a control test was performed with addition of buffer solution with no cortisol using the same volume of solution added when cortisol solution was added. No change in measured current was generated in the control test (Fig. 3(b)). Since the aptamer has been well studied for its specificity to cortisol [15], [19] and has been shown by our group in others work to respond to cortisol even in whole serum, no selectivity work was performed against other likely interferents such as other steroid hormones.

For testing sensor response to cortisol, increasing cortisol concentrations were pipetted (titrated) into the test setup with the resulting data plotted in Fig. 3(b). The resulting titration curve was further analyzed by Igor Pro (WaveMatrics Inc) software to fit a Langmuir-isotherm response. We obtain a similar but slightly stronger binding affinity of $K_d = 1.82\ \mu M$ compared to the original florescent cortisol aptamer ($K_d = 2.54\ \mu M$) and other reported cortisol aptamers ($K_d = 6-10\ \mu M$) [20], [21]. The data for 4 sensors also reveals a 5% and 13% signal increment for 10 nM and 25 nM cortisol concentration additions. The response to as little as 10 nM of cortisol could be because interdigitated electrodes with redox recycling provides a signal amplifying effect that enhances limit of detection, similar to other locally amplified aptamer sensors such as those based on field-effect transduction [22], but any signal amplification will also amplify noise and redox interferents and is not fully defendable until it is shown to operate in a complex in-vivo environment. Alternately, a low concentration response could also be due to intermittent binding of cortisol to the aptamer resulting in a fast release of the signal strand while re-attachment of the signal strand is a slower process. Both of these speculations remain unknown and are beyond the scope of this work. It is also worth noting that the cortisol concentration added in is lower than the aptamer

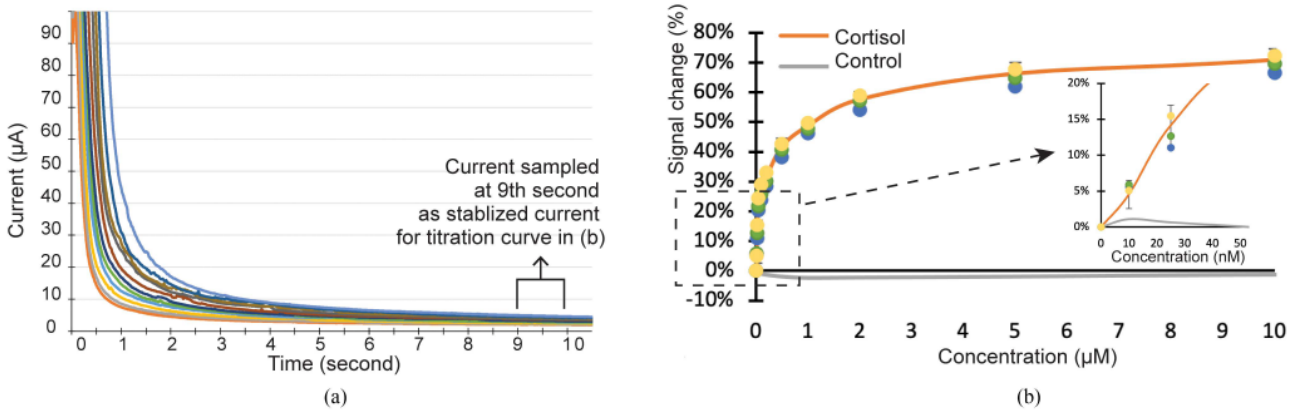

Fig. 3. (a) the measured I-t curve using chronoamperometry for titration test; (b) titration curve of solution-phase cortisol aptamer (For the control test group, in each data point 1 μL of the PBS buffer solution was added to the initial 30 μL aptamer droplet over the electrodes).

concentration, and therefore the aptamer can act as a 'sink' or reservoir which lowers the freely diffusing cortisol which could further shift the observed binding affinity (K_d) to higher concentrations than the actual binding affinity. Regardless, the results exhibit a K_d similar to the original fluorescently labeled cortisol aptamer, and clearly demonstrate for the first time the working principles of a solution-phase EAB sensor.

Next, sensor stability was tested since longevity could be a potential advantage of solution-phase EAB sensors. The purpose of the longevity test here was to simply determine what may be the longevity-limiting mechanism for the device, not to demonstrate a long-lasting device. Like a conventional EAB sensor, the solution-phase EAB sensor benefits from the use of a blocking layer such as MCH, otherwise background current such as oxygen reduction current can dominate over the redox tag current [23], [24] and significantly increase the required concentration of aptamers in solution. Therefore, stability of a potential electrode blocking layer was next explored with redox-tagged aptamers in solution.

To initially test the stability of MCH as a blocking layer for solution-phase EAB sensors, a 2-day longevity test was performed as illustrated in Fig. 4(a). In these tests the aptamer concentration was 10 μM as used for the data in Fig. 3(b). One testing group was operated under 20 °C room temperature, while the other was at 37 °C. The reasons we chose to also test in 37 °C are that: (1) it has been reported that temperature would cause structural changes to a self-assembled monolayer (SAM) and its reorganization and/or desorption over time [23], [25]; (2) 37 °C is human body temperature and therefore of interest for wearable and implantable sensors. Here, we applied square wave voltammetry with potential range from 0 to -0.65 V at 120 Hz to sample the peak current. The analyzed results are shown in Fig. 4(d) and (e) where signal current is measured as the absolute peak current, and background current is measured as oxygen reduction current. The results in Fig. 4(d) and (e) clearly show that a stand-alone blocking layer of MCH is insufficient for more than roughly one day of operation at 37 °C. We suspect that at 37 °C at near 28-30 hours a large amount of self-assembled monolayer desorbed from the surface, which led to increased O_2 reduction reaction. The quickly elevated background current which dominated over the signal peak. This result is consistent with our own recent observations for conventional electrode-bound EAB sensors and has been cited by others as a source of sensor degradation [26]. Although it is feasible that longer-term stability for blocking layers could be achievable with alternate materials and chemistries, these blocking layers will also need to deal with electrode fouling which may be equally challenging in raw biofluids such as blood or interstitial fluid.

V. Discussion

As hypothesized in the introduction, solution-phase EAB sensors have the potential to provide: (1) a larger tool kit of aptamer design strategies used broadly across the field of aptamers in general which have the potential to improve sensitivity and detection limits; (2) the ability to resolve fragile attachment to the gold electrode, which can enable greater sensor longevity. Based on the preliminary results demonstrated here (Figs. 3 and 4), the latter advantage in longevity will clearly require development of more stable blocking layers, an effort in progress by our research group and others [13], [26]. For a continuous sensing applications, the device will be membrane sealed to retain the aptamer in the device. Membrane sealing could increase lag times by limiting the diffusive flux of analytes to the aptamers, and especially so if the aptamers are at high concentration and inside a large solution volume inside the membrane. However, a membrane will also block many of the largest and most problematic foulants such as albumin, thus increasing the remaining focus on the need for more stable and long-lasting blocking layers to reduce background current such as oxygen reduction current. Alternately, different redox tags could be potentially used as well to find a window of electrochemical operation with minimum redox-active background current, as implemented in the latest generation of enzymatic glucose sensors [27]. Worst case, solution-phase EABs may not offer a significant longevity advantage but may still provide advantage in simple point-of-care testing type devices where reagents are solubilized in the test fluid itself during use of the test.

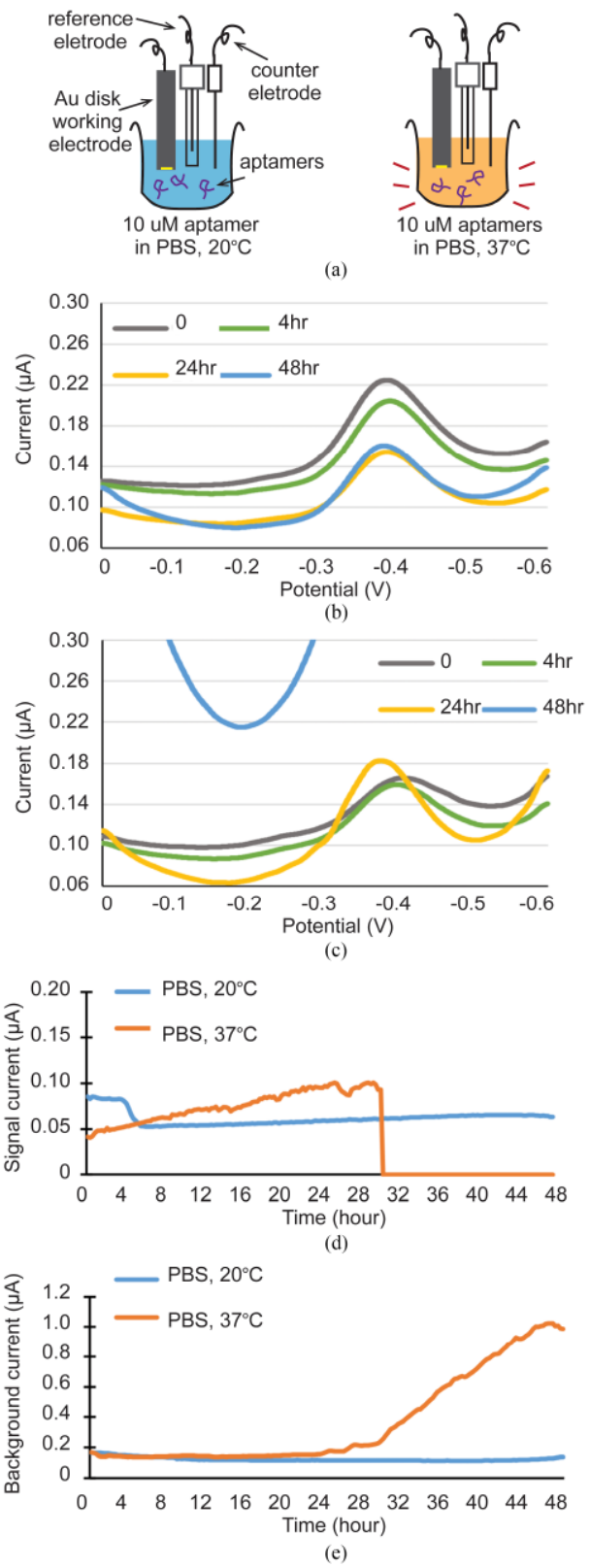

Fig. 4. (a) longevity test setup at different temperatures; voltammogram of (b) test at room temperature and (c) test at 37 °C.; test results comparison of (d) redox peak current change; (e) the background current change.

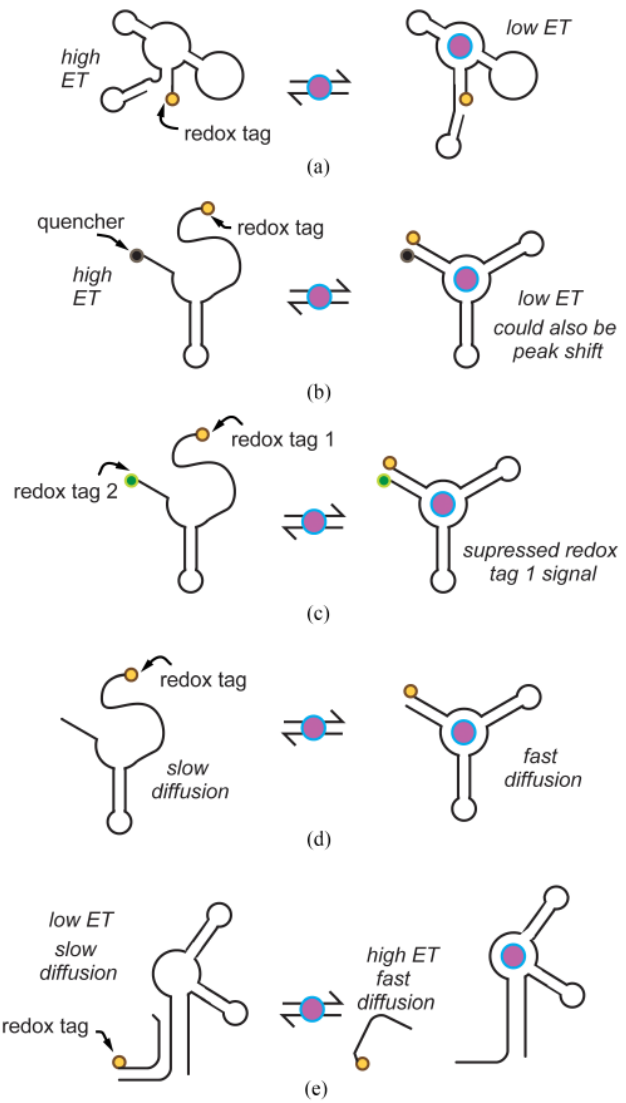

Fig. 5. Specific available aptamers that can be utilized for solution-phase aptamer-based sensor: (a) single chain aptamer modified from a phenylalanine aptamer [6], (b) single chain aptamer with redox quencher tag and (c) single chain aptamer with two-step electron transfer process modified from a cocaine aptamer [28], (d) single chain aptamer modified from a cocaine aptamer leading to diffusion change while binding target and (e) two-chain aptamer leveraging a cortisol aptamer [15].

The other hypothetical advantage of solution-phase EABs is accessing a larger tool kit of aptamer design strategies. This larger tool kit is worth additional discussion. By leveraging fully characterized and modeled aptamers, there are many potential working mechanisms for solution-phase EAB sensors. With reference to Fig. 5, the basic principle of operation for a solution-phase EAB sensor is that analyte binding should cause a change in electron transfer between a working electrode and the redox tag on the aptamer.

Several examples are now taught. Fig. 5(a) illustrates a single chain aptamer based on the phenylalanine aptamer [6]. The operation is similar to electrode-bound EAB sensors where

the electron transfer changes when binding with target analyte. Here, without the presence of analyte, the redox tag is potentially more exposed which could enable higher electron transfer (ET) with the working electrode. Fig. 5(b) shows a single chain aptamer with a quencher of the redox couple on the other end of the stem [28]. This proposed construct directly leverages molecular beacon type aptamers that utilize a fluorescent tag and an optical quencher [29]. In an electrochemical form, one could, for example, tag both stem terminals of an aptamer with carminic acid. The carminic acid is redox active when isolated with an open aptamer stem, but for a closed aptamer stem it acts as a carminic acid dimer which is redox inactive [30]. Fig. 5(c) displays a single chain aptamer with two-step electron transfer which could for example be adapted to work with a cocaine aptamer [28]. The terminal of one stem can be tagged with a first redox couple, and another stem with a second distinct redox couple with a slightly shifted redox potential. When those two redox couples are brought close to each other, electron transfer could experience a two-step electron transfer process. Two step electron-transfer could then result in suppression of one of the redox couple peaks [30]. In addition to providing a change of the redox tag exposure to the electrode, solution-phase aptamers can also take advantage of the diffusion constant change of the aptamer after it binds with the target (Fig. 5(d), example aptamer [28]), similar to the example demonstrated earlier in this manuscript (Fig. 1). This diffusion-constant based construct represents a highly novel switching method that in theory could result in a large change in signal with analyte binding. Lastly, as illustrated in Fig. 5(d) and demonstrated in this paper, a two-chain anchor and signal strand aptamer [15] has even greater potential as both hydrodynamic radius and mass changes significantly with analyte binding which then changes the diffusion coefficient [32] and therefore the measured current.

VI. Conclusion

In this manuscript, a new signal transduction methodology of solution-phase EAB sensor is introduced to improve the detection limit and the longevity of EABs. We successfully demonstrate an amperometric solution-phase EAB mechanism utilizing the diffusion coefficient change of a cortisol aptamer. This solution-phase cortisol EAB sensor is able to measure as low as 10 nM cortisol concentration in buffer solution. However, in order to bring this technology to clinical applications, there are still at least two remaining technological limitations: potential long sensor lag time and a short-lived blocking layer. A long lag time could be caused by a high aptamer concentration which acts as a sink for analyte, which could potentially be improved by miniaturizing the device. The longevity of the sensor is also challenged by the instability of the blocking layer on the working electrode surface, which is being studied by many groups including us to create blocking layers which last for weeks or more. These two major issues of lag-time and longevity may initially limit the use of solution-phase EAB sensors for continuous sensing applications, but we further conclude that solution-phase EAB sensors may already be appropriate for point-of-care applications.

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References

[1] J. Heikenfeld et al., "Accessing analytes in biofluids for peripheral biochemical monitoring," *Nature Biotechnol.*, vol. 37, no. 4, pp. 407–419, 2019, doi: 10.1038/s41587-019-0040-3.

[2] J. S. Swensen et al., "Continuous, real-time monitoring of cocaine in undiluted blood serum via a microfluidic, electrochemical aptamer-based sensor," *J. Amer. Chem. Soc.*, vol. 131, no. 12, pp. 4262–4266, Apr. 2009, doi: 10.1021/ja806531z.

[3] M. A. Pellitero, S. D. Curtis, and N. Arroyo-Currás, "Interrogation of electrochemical aptamer-based sensors via Peak-to-Peak separation in cyclic voltammetry improves the temporal stability and Batch-to-Batch variability in biological fluids," *ACS Sensors*, vol. 6, no. 3, pp. 1199–1207, Mar. 2021, doi: 10.1021/acssensors.0c02455.

[4] N. Bahner et al., "An aptamer-based biosensor for detection of doxorubicin by electrochemical impedance spectroscopy," *Anal. Bioanalytical Chem.*, vol. 410, no. 5, pp. 1453–1462, 2018, doi: 10.1007/s00216-017-0786-8.

[5] A. A. Rowe, E. A. Miller, and K. W. Plaxco, "Reagentless measurement of aminoglycoside antibiotics in blood serum via an electrochemical, ribonucleic acid aptamer-based biosensor," *Anal. Chem.*, vol. 82, no. 17, pp. 7090–7095, Sep. 2010, doi: 10.1021/ac101491d.

[6] A. Idili et al., "Calibration-Free measurement of phenylalanine levels in the blood using an electrochemical aptamer-based sensor suitable for Point-of-Care applications," *ACS Sensors*, vol. 4, no. 12, pp. 3227–3233, Dec. 2019, doi: 10.1021/acssensors.9b01703.

[7] A. Idili et al., "Seconds-resolved pharmacokinetic measurements of the chemotherapeutic irinotecan in situ in the living body," *Chem. Sci.*, vol. 10, no. 35, pp. 8164–8170, 2019, doi: 10.1039/C9SC01495K.

[8] P. Dauphin-Ducharme et al., "Electrochemical aptamer-based sensors for improved therapeutic drug monitoring and high-precision, feedback-controlled drug delivery," *ACS Sensors*, vol. 4, no. 35, pp. 2832–2837, Oct. 2019, doi: 10.1021/acssensors.9b01616.

[9] J. L. Chávez, J. A. Hagen, and N. Kelley-Loughnane, "Fast and selective plasmonic serotonin detection with aptamer-gold nanoparticle conjugates," *Sensors*, vol. 17, no. 4. 2017, Art. no. 681, doi: 10.3390/s17040681.

[10] J. Nie et al., "Electrochemical detection of tobramycin based on enzymes-assisted dual signal amplification by using a novel truncated aptamer with high affinity," *Biosensors Bioelectron.*, vol. 122, pp. 254–262, 2018, doi: https://doi.org/10.1016/j.bios.2018.09.072.

[11] Y. Huo et al., "A sensitive aptasensor for colorimetric detection of adenosine triphosphate based on the protective effect of ATP-aptamer complexes on unmodified gold nanoparticles," *Biosensors Bioelectron.*, vol. 78, pp. 315–320, 2016, doi: 10.1016/j.bios.2015.11.043.

[12] F. R. J. White, A. A. Rowe, and K. W. Plaxco, "Re-engineering aptamers to support reagentless, self-reporting electrochemical sensors," *Analyst*, vol. 135, no. 3, pp. 589–594, 2010, doi: 10.1039/B921253A.

[13] A. Shaver and N. Arroyo-Currás, "The challenge of long-term stability for nucleic acid-based electrochemical sensors," *Curr. Opin. Electrochemistry*, vol. 32, 2022, Art. no. 100902, doi: https://doi.org/10.1016/j.coelec.2021.100902.

[14] A. Marx and O. Seitz, *Molecular Beacons: Signalling Nucleic Acid Probes, Methods, and Protocols. Preface*, vol. 429. Totowa, NJ, USA: Humana Press, 2008.

[15] K. A. Yang et al., "High-Affinity nucleic-acid-based receptors for steroids," *ACS Chem. Biol.*, vol. 12, no. 12, pp. 3103–3112, 2017, doi: 10.1021/acschembio.7b00634.

[16] M. Van Megen, *Redox Cycling at Nanospaced Electrodes*. Wohrmann Print Service, 2013.

[17] C. Ma et al., "Redox cycling in nanoscale-recessed ring-disk electrode arrays for enhanced electrochemical sensitivity," *ACS Nano*, vol. 7, no. 6, pp. 5483–5490, Jun. 2013, doi: 10.1021/nn401542x.

[18] B. J. Seddon, H. H. Girault, and M. J. Eddowes, "Interdigitated microband electrodes: Chronoamperometry and steady state currents," *J. Electroanalytical Chem. Interfacial Electrochem.*, vol. 266, no. 2, pp. 227–238, 1989, doi: 10.1016/0022-0728(89)85070-3.

[19] Y. Yuan et al., "Oil-membrane protection of electrochemical sensors for Fouling- and pH-insensitive detection of lipophilic analytes," *ACS Appl. Mater. Interfaces*, vol. 13, no. 45, pp. 53553–53563, Nov. 2021, doi: 10.1021/acsami.1c14175.

[20] A. S. Zainol Abidin et al., "Current and potential developments of cortisol aptasensing towards Point-of-Care diagnostics (POTC)," *Sensors*, vol. 17, no. 5. 2017, doi: 10.3390/s17051180.

[21] A. Kaushik et al., "Recent advances in cortisol sensing technologies for point-of-care application," *Biosensors Bioelectron.*, vol. 53, pp. 499–512, 2014, doi: 10.1016/j.bios.2013.09.060.

[22] C.-A. Vu and W.-Y. Chen, "Predicting future prospects of aptamers in field-effect transistor biosensors," *Molecules*, vol. 25, no. 3, Feb. 2020, Art. no. 680, doi: 10.3390/molecules25030680.

[23] X. Xu et al., "Structural changes of mercaptohexanol self-assembled monolayers on gold and their influence on impedimetric aptamer sensors," *Anal. Chem.*, vol. 91, no. 22, pp. 14697–14704, Nov. 2019, doi: 10.1021/acs.analchem.9b03946.

[24] M. C. Brothers et al., "Impact of self-assembled monolayer design and electrochemical factors on impedance-based biosensing," *Sensors*, vol. 20, no. 8. 2020, Art. no. 2246, doi: 10.3390/s20082246.

[25] E. Delamarche et al., "Thermal stability of self-assembled monolayers," *Langmuir*, vol. 10, no. 11, pp. 4103–4108, Nov. 1994, doi: 10.1021/la00023a033.

[26] A. Shaver, S. D. Curtis, and N. Arroyo-Currás, "Alkanethiol monolayer end groups affect the long-term operational stability and signaling of electrochemical, aptamer-based sensors in biological fluids," *ACS Appl. Mater. Interfaces*, vol. 12, no. 9, pp. 11214–11223, Mar. 2020, doi: 10.1021/acsami.9b22385.

[27] S. Phetsang et al., "A redox Cu(II)-graphene oxide modified screen printed carbon electrode as a cost-effective and versatile sensing platform for electrochemical label-free immunosensor and Non-enzymatic glucose sensor," *Front. Chem.*, vol. 9, May 2021, Art. no. 671173, doi: 10.3389/fchem.2021.671173.

[28] M. N. Stojanovic, P. de Prada, and D. W. Landry, "Aptamer-Based folding fluorescent sensor for cocaine," *J. Amer. Chem. Soc.*, vol. 123, no. 21, pp. 4928–4931, May 2001, doi: 10.1021/ja0038171.

[29] J.-U. Park et al., "Nanoscale patterns of oligonucleotides formed by electrohydrodynamic jet printing with applications in biosensing and nanomaterials assembly," *Nano Lett.*, vol. 8, no. 12, pp. 4210–4216, Dec. 2008, doi: 10.1021/nl801832v.

[30] G. Cheng et al., "A new electrochemically active–inactive switching aptamer molecular beacon to detect thrombin directly in solution," *Biosensors Bioelectron.*, vol. 25, no. 10, pp. 2265–2269, 2010, doi: 10.1016/j.bios.2010.03.008.

[31] Y. Wu, S. Ali, and R. J. White, "Electrocatalytic mechanism for improving sensitivity and specificity of electrochemical nucleic acid-based sensors with covalent redox tags—Part I," *ACS Sensors*, vol. 5, no. 12, pp. 3833–3841, Dec. 2020, doi: 10.1021/acssensors.0c02362.

[32] S. Araki et al., "Hydrodynamic radius of circular DNA is larger than that of linear DNA," *Chem. Phys. Lett.*, vol. 418, no. 1, pp. 255–259, 2006, doi: 10.1016/j.cplett.2005.10.115.