Published in final edited form as:

Electroanalysis. 2017 March ; 29(3): 873–879. doi:10.1002/elan.201600548.

A Single Electrochemical Probe Used for Analysis of Multiple Nucleic Acid Sequences

Dawn M. Mills^[a], Percy Calvo-Marzal^[a], Jeffer M. Pinzon^[a], Stephanie Armas^[a], Dmitry M. Kolpashchikov^{[a],[b],[c]}, and Karin Y. Chumbimuni-Torres^{[a],*}

^[a]Department of Chemistry, University of Central Florida, 4000 Central Florida Blvd., Orlando, FL 32816, United States

^[b]National Center for Forensic Science, University of Central Florida, Orlando, FL 32816, United States

^[c]Burnett School of Biomedical Science, University of Central Florida, Orlando, FL 32816, United States

Abstract

Electrochemical hybridization sensors have been explored extensively for analysis of specific nucleic acids. However, commercialization of the platform is hindered by the need for attachment of separate oligonucleotide probes complementary to a RNA or DNA target to an electrode's surface. Here we demonstrate that a single probe can be used to analyze several nucleic acid targets with high selectivity and low cost. The universal electrochemical four-way junction (4J)-forming (UE4J) sensor consists of a universal DNA stem-loop (USL) probe attached to the electrode's surface and two adaptor strands (m and f) which hybridize to the USL probe and the analyte to form a 4J associate. The m adaptor strand was conjugated with a methylene blue redox marker for signal ON sensing and monitored using square wave voltammetry. We demonstrated that a single sensor can be used for detection of several different DNA/RNA sequences and can be regenerated in 30 seconds by a simple water rinse. The UE4J sensor enables a high selectivity by recognition of a single base substitution, even at room temperature. The UE4J sensor opens a venue for a re-useable universal platform that can be adopted at low cost for the analysis of DNA or RNA targets.

Keywords

Universal probe; nucleic acid; four-way junction; methylene blue

1 Introduction

Nucleic acid detection using hybridization techniques has received significant attention due to its valuable applications in clinical diagnostics, national defense and forensics [1–3].

Inspired by the undoubted success of the blood glucose meter, electrochemical methods have

* Karin.ChumbimuniTorres@ucf.edu.

Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/elan.201601548>.

been explored as a valuable approach for nucleic acid detection due to the potential for *on-site* testing while offering a fast, simple and inexpensive analysis [4–12]. Fan and colleagues initiated the field of electrochemical nucleic acid analysis by introducing a DNA stem-loop (SL) probe conjugated with a redox marker [13]. The DNA SL probe remains in a hairpin configuration until the stability is disrupted upon hybridization with a fully matched sequence, forming a thermodynamically favored duplex structure. Varying sensor strategies using DNA SL probes have achieved limits of detection as low as aM [14–15]. Despite this success, to the best of our knowledge no commercial electrochemical sensor for nucleic acid analysis is available up to date. One significant challenge is the absence of a universal probe which could be used for the analysis of many nucleic acid sequences while maintaining high selectivity with minimum modifications of the assay conditions [16]. Optimizing the performance of a universal platform could lead to low cost bulk manufacturing and use of the same electrode in a variety of applications. Universal platforms for recognition of nucleic acids have received ever growing attention for fluorescent formats [17–21]. Previously, we demonstrated how a DNA four-way junction (4J)-forming multicomponent probe can be used for analysis of multiple nucleic acid analytes [18]. The approach is based on a molecular beacon (MB) probe, a fluorophore and a quencher labeled hairpin DNA strand (Scheme 1A) [22,23]. Two adaptor strands hybridized to both a universal MB probe and the target DNA or RNA sequences to form a 4J structure [17,18]. In this structure, the MB probe acquires an elongated conformation with the fluorophore separated from the quencher, resulting in high fluorescence. The MB probe does not hybridize directly to the nucleic acid analyte and therefore, can be used for analysis of potentially any sequence if the adaptor strands are tailored for each new analyte. Importantly, this approach enabled high selectivity of nucleic acid recognition, even at ambient temperatures [17,18]. The high selectivity can be attributed to the short hybrid of one of the adaptor strands to the target which is extremely sensitive to a single mismatched base pairing. This allows the detection of single nucleotide polymorphisms (SNPs) in folded target analytes, which is not possible by the conventional DNA SL probe, e.g. MB probe [23,25]. The fluorescent platform was then adopted for electrochemical nucleic acid analysis [26,27]. The first sensor used a redox reaction of electrochemically active markers which was inhibited (signal OFF) upon 4J complex formation on the surface of the electrode [26]. The major drawback of this approach is the signal OFF sensing format, which can easily be affected by false positive responses caused by interactions other than the target binding [8,13,28,29]. A second sensor used a signal ON format via electrocatalysis of glucose oxidase with covalently bound methylene blue (MeB) redox markers [27]. This sensor was shown to recognize target analytes with impressively low detection limits. Here we demonstrate how the multicomponent design enables detection of multiple analytes by utilizing the same electrode-bound probe. This study in combination with low limits of detection (LOD) demonstrated previously [26,27], will eventually enable widespread use of electrochemical techniques in nucleic acid analysis.

We have chosen a signal ON format (Scheme 1B). The sensor utilized a thiol bond between a universal-stem loop probe (USL) and a gold substrate. Two adaptor strands, m and f, were introduced along with the target during hybridization to form a 4J structure. The m adaptor strand was conjugated with MeB for electrochemical detection using square wave

voltammetry (SWV). The following parameters were optimized using electrochemical techniques in conjunction with spectroscopic ellipsometry: optimal USL probe and adaptor strand concentrations, immobilization and hybridization time, selectivity and conditions for sensor regeneration. The focus of this work is to demonstrate for the first time the ability of the UE4J sensor to be re-used for cost effective analyses of multiple analytes using a USL probe.

2 Experimental

2.1 Reagents and Solutions

The USL, f adaptor strands (f1 and f2), miRNA-122, target DNA (T-DNA) and mismatch sequences were purchased from Integrated DNA Technologies (Coralville, USA) and used as received (Table 1). The m adaptor strand modified with a MeB redox marker (seven carbon (C7) linker) was purchased from Biosearch Technologies, Inc. (Petaluma, USA) and used as received (Table 1). Trizma hydrochloride (Tris-HCl), tris(2-carboxyethyl) phosphine hydrochloride (TCEP), sodium phosphate dibasic (Na_2HPO_4), sodium phosphate monobasic dihydrate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$), ethylenediaminetetraacetic acid (EDTA), 6-mercapto-1-hexanol (MCH) and magnesium chloride (MgCl_2) were purchased from Sigma Aldrich (St. Louis, USA). Sodium chloride (NaCl), potassium chloride (KCl) sodium hydroxide (NaOH) and sulfuric acid (H_2SO_4) were obtained from Fisher Scientific (Pittsburgh, USA). Gold screen printed electrodes (SPE) and gold disc electrodes (GDE) were purchased from DROPSSENS (Spain) and CH Instruments (Austin, USA), respectively. Alumina slurry (1.0 μm , 0.3 μm and 0.05 μm) was obtained from Buehler (Lake Bluff, USA). All aqueous solutions were prepared with deionized water (18 M Ω cm resistivity) using a Siemens PURELAB Ultra system (Lowell, USA). A phosphate buffer saline (PBS) solution was used as the immobilization buffer (IB) and was prepared with 50 mM Na_2HPO_4 , 50 mM $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 250 mM NaCl and adjusted to a pH of 7.4 using 1 M sodium hydroxide (NaOH). The hybridization buffer (HB) was prepared with 50 mM Tris-HCl, 25 mM NaCl, 50 mM MgCl_2 and adjusted to pH 7.4 using 1 M NaOH.

2.2 Preparation of Electrodes

Gold screen printed electrodes (SPE) and gold disc electrodes (GDE) were used as substrates for USL probe immobilization. GDEs were chemically cleaned by immersion in a piranha solution (1:3 ratio of H_2O_2 : H_2SO_4) for ten minutes. Then, the electrodes were manually polished on a microcloth with alumina slurry (1.0 μm , 0.3 μm and 0.05 μm), rinsed with water and sonicated in ethanol to remove any residual alumina particles trapped at the surface of the electrode. The GDEs and SPEs were finally activated in 0.5 M H_2SO_4 via cyclic voltammetry from 1.6 to -0.1 V at a scan rate of 100 mV/s and their corresponding areas were calculated as described previously [31].

2.3 Immobilization and Hybridization

The USL probe was immobilized on the electrode's surface via a gold-thiol bond. The disulfide bonds of the USL probe were reduced with 1 mM TCEP by shaking the solution at room temperature for 1 hour. The solution was then diluted with IB to yield 0.1 μM of the USL probe and 15 μL of this solution was drop casted on the electrode and incubated at

room temperature for 1.5 hours. The electrodes were rinsed with IB and dried with nitrogen. Then, 15 μL of 2 mM MCH in IB was drop casted on the electrode and incubated for 30 minutes to minimize nonspecific adsorption on the electrode surface. Hybridization was performed using the desired concentration of target miRNA-122 or T-DNA by preparing with an equimolar of m and f adaptor strands (1 μM) in HB. Next, 15 μL of this solution was drop casted to the electrode and incubated for 2 hours at room temperature.

2.4 Characterization and Optimization of USL Probe, Adaptor Strand Concentrations and Experimental Timing

The concentrations of USL probe were varied from 0.05 μM to 1 μM and incubated with 1 μM m-MeB, 1 μM f1, and 50 nM miRNA-122. Next, the concentration of the adaptor strands (m-MeB and f1) was varied in equimolar concentrations (0.1 to 1.0 μM) and incubated with 50 nM miRNA-122 upon immobilization of 0.1 μM USL probe. Finally, the concentration of the m-MeB adaptor strand was held constant at 0.25 μM and the concentration of the f1 strand was changed from 0.1 μM to 1 μM in the presence of 50 nM miRNA-122. Immobilization time was varied from 15 to 75 min whereas hybridization time was varied from 15 to 120 min.

2.5 Electrochemical Measurements

Square wave voltammetry (SWV) was performed with a CHI660D Electrochemical Workstation (CH Instruments, Austin, USA). A typical 3-electrode system was used where the modified gold (Au) SPE or GDE served as the working electrode, a platinum wire was used as the counter electrode, and Ag/AgCl (3 M KCl) was used as a reference electrode (CH Instruments, Austin, USA). SWV measurements were recorded in a buffer solution at a potential range from 0.0 to -0.5 V, frequency of 100 Hz and amplitude of 0.07 V. Nitrogen was bubbled in the electrochemical cell to remove oxygen before measurements were performed at room temperature. At least three electrodes were used for each experiment to provide statistically significant results.

2.6 Ellipsometric Measurements

Ellipsometric measurements were performed with a V-Vase ellipsometer from J. A. Woollam Co. (Lincoln, USA). Spectroscopic ellipsometry has previously been used to calculate the thickness of DNA layers before and after hybridization [32,33]. The experimental data obtained for the thickness of the USL probe was modeled using the WVASE software package (J. A. Woollam Co.) and assessed using the mean square error (MSE). Generally, a MSE <10 is acceptable and smaller MSE values indicate good agreement of the model with the experimental data. A spectroscopic scan from 300 to 800 nm was first performed on clean glass slides (1.254 mm) coated with 5 nm titanium oxide (TiO) and 100 nm Au (Infolab Inc., Herndon, USA) at incident angles of 65°, 70°, and 75°. Then, measurements were recorded after USL probe immobilization, MCH backfilling and hybridization with adaptor strands (m and f1) and miRNA-122 on the clean Au slides. A Cauchy layer was used to model the DNA layer thickness on the gold slides and measurements were performed in triplicate.

3 Results and Discussion

3.1 Thickness of the USL

Ellipsometry was used to monitor the thickness of the DNA layer on Au coated glass slides. The thickness of immobilized USL probe on the electrode's surface was 11.16 ± 0.32 Å. This value is about half of the value reported for a linear single stranded DNA probe of similar length, which correlates with our assumption of the stem-loop folded conformation of the USL probe shown in Scheme 1B [32,33]. Upon addition of MCH, the thickness was 10.78 ± 1.12 Å, indicating an insignificant change of the DNA orientation. Hybridization with the adaptor strands (m and f1) and the miRNA-122 yielded a measured thickness of 37.85 ± 1.31 Å, demonstrating a significantly increased thickness caused by 4J structure formation. This thickness is comparable to previously studied conventional double-stranded complexes of a similar length [34]. In the presence of the target containing a SNP the thickness was 9.77 ± 0.26 Å, which is comparable to the thickness of the original USL probe. This reflects negligible interaction of the USL probe with the adaptor strands in the presence of a mismatched target. In contrast, a previous ellipsometric study with a conventional hybridization probe yielded a slight increase of thickness upon exposure to a mismatched target when compared to the thickness of the probe [33]. It is worth mentioning that the ellipsometric measurements provide only about 20% of the theoretical film thickness. For example, if the USL probe was extended to a linear conformation the theoretical thickness would yield about 235 Å [35]. This could indicate that both the USL probe alone and in complex with a target do not form closely packed monolayers [33].

3.2 Optimization of the Concentrations of the USL Probe and Adaptor Strands

The blank response of the sensor in the absence of the USL probe, SWV frequency dependence of the current density and buffer optimizations are presented in the Supporting Information (Figures S1–S3). The concentrations of the USL probe during the immobilization step was optimized to achieve highest signal in the presence of analyte. As illustrated in Figure 1A, the current density (j) as the concentration of the USL probe during the immobilization step increased from 0.05 μM to its maxima at 0.1 μM . A decrease in current density was observed at higher concentrations than 0.1 μM , likely due to a steric crowding effect and electrostatic repulsion of the neighboring DNA structures. Thus, using 0.1 μM USL probe for immobilization produced near optimal density of the USL probe on the surface of the electrode for formation of the 4J structure. Next, concentrations of m-MeB and f1 adaptor strands were optimized during the detection step using 50 nM miRNA-122 as a target analyte. The concentration of m-MeB and f1 adaptor strands were varied simultaneously in equimolar concentrations (0.10 μM , 0.25 μM , 0.50 μM , 0.75 μM and 1.0 μM) followed by the detection of current from the MeB redox marker (Figure 1B). The current density increased as the equimolar concentration of adaptor strands m-MeB and f1 increased from 0.1 μM to 0.25 μM reaching a plateau at high concentrations. Consequently, the m adaptor strand concentration was held constant at 0.25 μM and the concentration of the f strand was changed from 0.1 μM to 1 μM in the presence of 50 nM miRNA-122. The highest signal observed was with 0.25 μM m-MeB and 0.5 μM f1 (Figure 1C), which is comparable to the signal observed in Figure 1B and was subsequently used for further studies.

3.3 Experimental Timing

Next, the immobilization time of the USL probe was varied in intervals from 15–75 min. The purpose of this experiment was to minimize electrode preparation time without significant loss of the signal intensity. Following the immobilization, 6-mercepto-1-hexanol (MCH) was added for 30 min and hybridization with adaptor strands and target was carried out for 120 min. As seen in Figure 2A, the current increased from immobilization times of 15 to 30 min where the signal remained relatively constant up to 75 min. The purpose of this experiment was to minimize analysis time without compromising the signal. Therefore, an immobilization time of 30 min was adequate for further analyses. Similarly, the hybridization time was investigated from 15 to 120 min. The current increased from hybridization times of 15 to 90 min until it reached saturation to 120 min (Figure 2B). Consequently, 90 min was a sufficient incubation time to produce a high signal and was used for subsequent experiments.

3.4 Sensor Regeneration

It is known that DNA probes can be regenerated from their complexes with analytes by heating or changing the pH [6]. In contrast, Lubin et al. reported the regeneration of a DNA SL probe sensor with high reproducibility by rinsing with water for 30 sec [36]. This is most likely due to the DNA SL probe conformation which shifts thermodynamic equilibrium from the duplex to dissociated state. Similarly, we demonstrate here that the 4J structure dissociates at room temperature after rinsing with water, likely due to the incorporation of the DNA SL probe. To demonstrate the distinctive DNA SL probe regeneration characteristic, the sensor was re-used up to seven times after the original hybridization with over 97% recovery (Figure 3) by rinsing the sensor with deionized water for 30 sec following hybridization. The electrodes showed stability over one week when stored in IB at 4°C. The representative voltammograms before and after regeneration are presented in the Supporting Information (Figure S4).

3.5 Sensor Selectivity

The selectivity of the sensor was studied with a target containing a SNP (Table 1). The current density was close to the background (Figure 4, curve c) when compared to the fully matched target at the same concentration (50 nM; Figure 4, curve b). Furthermore, even when the mismatched target was used in four-fold excess (200 nM) the current density still remained low (Figure 4d), thus reflecting the capability of the sensor to detect a fully matched target even in four times excess amount of a single base mismatched analyte, a property important in practice [36]. The high selectivity is attributed to the sensor design: the m strand with a shorter target binding arm does not hybridize to the mismatched target and has the capability to discriminate a SNP [17,18,24].

These results are in agreement with our previous findings using the 4J sensor in a fluorescent format, which offers a higher selectivity at ambient temperatures than the DNA SL probe alone [17,18,30]. This performance is hard to achieve by conventional hybridization sensors when analyzing folded target analytes such as miRNAs used in this study [24,25,38]. The hybridization between a DNA SL probe and folded target analyte is inefficient because the hairpin structure is energetically favored over the formation of a duplex, which can be

circumvented by using a multicomponent sensing approach such as the 4J structure [24,25]. Therefore, the UE4J sensor would be ideal for a highly selective analysis of folded structures.

3.6 LOD and the Universality of UE4J

Although we did not focus on achieving a low LOD, we determined the detection limit of the UE4J sensor as follows. The current density (j) increased with increasing concentrations of miRNA-122 from 5 to 75 nM as shown in Figure 5A. The linear dynamic range (LDR) was from 5 nM to 50 nM and the response became nonlinear beyond the upper level, indicating electrode saturation. The limit of detection (LOD) was calculated to be 3.2 nM as three times the standard deviation of the blank divided by the slope from the calibration curve (S_b/m).

Finally, the target-binding arms of strands m and f can be changed to tailor the sensor to each new target sequence using one optimized USL probe. Previously, the 4J sensor was shown to successfully detect different miRNAs but its affinity for DNA detection was not explored using the same probe [26,27]. To demonstrate this ability, the sensor was incubated with a new target DNA (T-DNA: 5'-CTGGATACGGATATGGTGTGTTG-3') sequence using the same USL probe with the same m adaptor strand (m-MeB) and the new adaptor strand, f2, equipped with analyte binding arms complementary to T-DNA (Table 1). As shown in Figure 5B, the current density increased with increasing concentrations of T-DNA from 5 to 75 nM. The LDR was from 5 to 50 nM and the response became nonlinear beyond the upper level, indicating electrode saturation. The LOD was calculated to be 0.65 nM. In addition, a longer DNA target (60 nt) and target containing a SNP were analyzed and are discussed further in the Supporting Information to demonstrate the applicability of long analyte detection (Figure S5). These results illustrate that the same USL probe can be used for the detection of various nucleic acids by only modifying the target binding arm of the adaptor strands. The universal character of the 4J design in conjunction with the ability to regenerate the DNA SL probe, provides a user friendly format for analyzing a variety of nucleic acids. This multicomponent format eliminates the labor intensive immobilization of a new probe for each new target and in conjunction with the low cost of synthetic oligonucleotides, the costs for detection of many analytes with a single UE4J will be reduced compared to conventional formats.

4 Conclusions

A systematic characterization of a UE4J sensor has been performed in this study to provide new insights for the future use of a portable universal electrochemical sensor for SNP discrimination at room temperature. The next step is to focus on achieving a lower LOD for the analysis of biological samples as it is a current limitation of this sensor. We also envision that the sensor reported here has advantages over conventional designs for the analysis of folded nucleic acids such as bacterial 16S rRNAs. To achieve unfolding, RNA strand f can be designed with a long analyte-binding arm. This will unwind possible secondary structures and liberate an RNA fragment to bind the SNP specific m strand, as it was reported earlier for fluorescent sensors [24,25]. The novelty of this sensor is that (i) enables signal ON

detection with zero signal background in the absence of the target; (ii) it is highly selective toward a SNP at room temperature; (iii) it is able to be regenerated by simply rinsing with water due to the incorporation of the DNA SL probe, and (iv) it provides a universal format allowing a cost effective analysis of multiple analytes. Indeed, changing the target-binding arms of strands m and f is sufficient to tailor the sensor to each new target sequence. The characteristics of the UE4J sensor reported here with SNP discrimination at room temperature and the possibility to detect folded endogenous RNA in a re-usable format are highly significant since it promises to deliver a new efficient tool for a highly specific and cost-effective detection of DNA and RNA analytes in a portable electrochemical format.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors acknowledge the College of Sciences and the Department of Chemistry at the University of Central Florida for financial support of this research. DMK was supported by NSF CCF grants #1117205 and 1423219 and NIH R15AI10388001A1.

References

1. Li W, Wu P, Zhang H, Cai C. Chem Commun. 2012; 48:7877–7879.
2. Rodriguez-Mozaza S, Lopez de Aldaa MJ, Marco MP, Barcelo D. Talanta. 2005; 65:291–297. [PubMed: 18969798]
3. Yáñez-Sedeño P, Agüí L, Villalonga R, Pingarrón JM. Anal Chim Acta. 2014; 823:1–19. [PubMed: 24746348]
4. Lai G, Yan F, Ju H. Anal Chem. 2009; 81:9730–9736. [PubMed: 19863072]
5. Lai RY, Plaxco KW, Heeger AJ. Anal Chem. 2007; 79:229–233. [PubMed: 17194144]
6. Li F, Peng J, Zheng Q, Guo X, Tang H, Yao S. Anal Chem. 2015; 87:4806–4813. [PubMed: 25874968]
7. Wu X, Chai Y, Zhang P, Yuan R. ACS Appl Mater Interfaces. 2015; 7:713–720. [PubMed: 25495913]
8. Yang C, Dou B, Shi K, Chai Y, Xiang Y, Yuan R. Anal Chem. 2014; 86:11913–11918. [PubMed: 25369729]
9. Yin H, Zhou Y, Chen C, Zhu L, Ai S. Analyst. 2012; 137:1389–1395. [PubMed: 22311172]
10. Malmstadt HV, Pardue HL. Anal Chem. 1961; 33:1040.
11. Heller A, Feldman B. Chem Rev. 2008; 108:2482–2505. [PubMed: 18465900]
12. Lan T, Zhang J, Lu Y. Biotechnology Advances. 2016; 34:331–241. [PubMed: 26946282]
13. Fan C, Plaxco KW, Heeger AJ. Proc Natl Acad Sci USA. 2003; 100:9134–9137. [PubMed: 12867594]
14. Abi A, Ferapontova EE. Anal Bioanal Chem. 2013; 405:3693–3703. [PubMed: 23263518]
15. Xiong EZ, Liu X, Zhou Y, Yu J, Li P, Chen X. J Anal Chem. 2015; 87:7291–7296.
16. Labib M, Sargent EH, Kelley SO. Chem Rev. 2016
17. Kolpashchikov DM. J Am Chem Soc. 2006; 128:10625–10628. [PubMed: 16895431]
18. Gerasimova YV, Hayson A, Ballantyne J, Kolpashchikov DM. ChemBioChem. 2010; 11:1762–1768. [PubMed: 20665615]
19. Faltin B, Zengerle R, von Stetten F. Clin Chem. 2013; 59:1567–1582. [PubMed: 23938456]
20. Ravan H. Trends in Analytical Chemistry. 2015; 65:97–106.

21. French DJ, Richardson JA, Howard RL, Brown T, Debenham PG. Mol Cell Probes. 2015; 29:228–236. [PubMed: 25981184]
22. Tyagi S, Kramer FR. Nat Biotechnol. 1996; 14:303–308. [PubMed: 9630890]
23. Kolpashchikov DM. Scientifica (Cairo). 2012; 2012:928783. [PubMed: 24278758]
24. Nguyen C, Grimes J, Gerasimova YV, Kolpashchikov DM. Chemistry. 2011; 17:13052–13058. [PubMed: 21956816]
25. Grimes J, Gerasimova YV, Kolpashchikov DM. Angew Chem Int Ed. 2010; 49:8950–8953.
26. Labib M, Ghobadloo SM, Khan N, Kolpashchikov DM, Berezovski MV. Anal Chem. 2013; 85(20):9422–9427. [PubMed: 24047131]
27. Labib M, Khan N, Berezovski MV. Anal Chem. 2015; 87:1395–1403. [PubMed: 25495265]
28. Guerreiro GV, Zaitouna AJ, Lai RY. Anal Chim Acta. 2014; 810:79–85. [PubMed: 24439508]
29. Yang W, Lai RY. Langmuir. 2011; 27:14669–14677. [PubMed: 21981414]
30. Stancescu M, Fedotova TA, Hooyberghs J, Balaeff A, Kolpashchikov DM. J Am Chem Soc. 2016 in press.
31. Carvalhal RF, Freire RS, Kubota LT. Electroanalysis. 2005; 17:1251–1258.
32. Gupta G, Atanassov P. Electroanalysis. 2011; 23:1615–1622.
33. Herne TM, Tarlov MJ. J Am Chem Soc. 1997; 119:8916–8920.
34. Steel AB, Herne TM, Tarlov MJ. Anal Chem. 1998; 70:4670–4677. [PubMed: 9844566]
35. Peterlinz KA, Georgiadis RM. J Am Chem Soc. 1997; 119:3401–3402.
36. Lubin AA, Lai RY, Baker BR, Heeger AJ, Plaxco KW. Anal Chem. 2006; 78:5671–5677. [PubMed: 16906710]
37. Diehl F, Schmidt K, Choti MA, Romans K, Goodman S, Li M, Thornton K, Agrawal N, Sokoll L, Szabo SA, Kinzler KW, Vogelstein B, Diaz LA Jr. Nature Med. 2008; 14:985–990. [PubMed: 18670422]
38. Farjami E, Clima L, Gothelf K, Ferapontova EE. Anal Chem. 2011; 83:1594–1602. [PubMed: 21314139]

Fig. 1.

(A) Current density (j) obtained from SWV in the presence of 1 μM of adaptor strands (m-MeB and f1) and 50 nM miRNA-122 obtained with different concentrations of the USL probe during immobilization. (B) Optimization of the adaptor strand concentration, which are simultaneously analyzed at equimolar concentrations, at fixed concentration of USL probe (0.1 μM). (C) 0.25 μM of m-MeB strand fixed and variable f strand concentrations along with 50 nM miRNA-122.

Fig. 2. Optimization of time for (A) immobilization of 0.1 μM USL probe and (B) hybridization with 0.25 μM m-MeB strand, 0.50 μM f strand and 50 nM miRNA-122 target.

Fig. 3.
Regeneration of the UE4J sensor using Au SPEs.

Fig. 4. Selectivity of UE4J sensor. Sensor response after (a) immobilization of USL probe and backfilling with MCH (b) incubation with m-MeB, f1 and miRNA-122 (5'-UGG-AGU-GUG-ACA-AUG-GUG-UUU-G-3'; 50 nM) (c) addition of a single base mismatch (50 nM) and (d) a four times excess of single base mismatch (200 nM).

Fig. 5.

(A) Calibration curve for miRNA-122. (B) Calibration curve for T-DNA. Insets: Respective SWVs corresponding to each concentration of miRNA-122 and T-DNA at 1, 5, 15, 25, 30, 40, 50, and 75 nM. SWVs were performed in HB. The errors bars represent the standard deviation of the signal generated from three separate electrodes. The standard deviation is represented by the error bars, which accounts for the current density generated from three separate electrodes.

Scheme 1.

Schematic illustration of the (A) MB fluorescence sensor and (B) UE4J sensor used for nucleic acid detection.

Table 1

Oligonucleotides used in the study.

Name ^[a]	Sequences ^[b]
USL probe	5'-S-S (CH ₂) ₆ TTTTTTTTTT <i>CGCGTTAACATACAATAGATCGCG</i> -3'
f1	5'-GATCTATTGTGTCACACTCCA-3'
f2	5'-GATCTATTGATCCGTATCCAG-3'
m-MeB	5'-CAAACACCATTTATGTAACTTTTTTTT TT-MeB-3'
T-DNA	5'-CTGGATACGGATATGGTGTGTTG-3'
miRNA-122	5'-UGGAGUGUGACAAUGGUGUUUG-3'
Mismatch	5'-UGGAGUGUGACAAUGGUCUUUG-3'

^[a]MeB, methylene blue, redox label.

^[b]SNP sites are underlined; self-complementary regions of USL probe are in italic.