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Article

An Allosteric Transcription Factor DNA-Binding Electrochemical Biosensor for Progesterone

Karthika Sankar, R. Baer, Chloé Grazon, Robert C. Sabatelle, Sébastien Lecommandoux, Catherine M. Klapperich, James E. Galagan,* and Mark W. Grinstaff*

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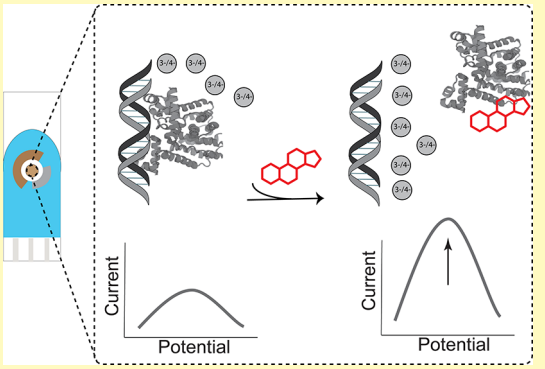
ABSTRACT: We describe an electrochemical strategy to transduce allosteric transcription factor (aTF) binding affinity to sense steroid hormones. Our approach utilizes square wave voltammetry to monitor changes in current output as a progesterone (PRG)-specific aTF (SRTF1) unbinds from the cognate DNA sequence in the presence of PRG. The sensor detects PRG in artificial urine samples with sufficient sensitivity suitable for clinical applications. Our results highlight the capability of using aTFs as the biorecognition elements to develop electrochemical point-of-care biosensors for the detection of small-molecule biomarkers and analytes.



KEYWORDS: *transcription factor, progesterone, impedance, DNA, electrochemical biosensor, ferrocyanide/ferricyanide, detection*

Biosensors play a pivotal role in medicine as well as food, environmental, and agricultural industries, and their potential to positively impact society is increasing as our world becomes more digital.[1−3] Analytes for detection widely vary based on applications, and our particular interest is in hormones. Progesterone (PRG) is a steroid hormone secreted by the corpus luteum and is central to fertility planning, assisted reproductive technologies, and endocrine disorders. Among women, one million in the US suffer from infertility, approximately 7.5 million suffer from reduced fertility, and about 15 million miscarry, with similar numbers in the EU. In developing countries, the statistics are even higher, and the rates of infertility approach 30%, with only half of the people in developed and developing countries seeking infertility care.[4] As simple as tracking PRG levels during an ovulation cycle increases fertility odds. Furthermore, during pregnancy, PRG balance helps nurture and develop the fetus. Determining ovulation windows is challenging because of fluctuating menstrual cycles and inaccurate interpretations of hormone levels. Currently, PRG is measured from serum via blood collection at the clinic/hospital or at home, followed by analysis at a commercial laboratory with results provided in a few days to a week.

Today, at-home urine tests measure pregnanediol glucuronide (PDG), a metabolic product of PRG. However, PDG levels are more variable than PRG levels, and up to 12% of women do not metabolize PRG sufficiently to produce detectable PDG.[5] A rapid, facile at-home fertility planning and a monitoring point-of-care (POC) device will alleviate the apprehensiveness associated with routine screenings and give couples the privacy desired during fertility planning. With a mindset toward this goal and recognizing that conventional antibody[6−10]- or aptamer[11−15]-based sensors (summarized in Table S1) have not yet provided a solution tailored toward the POC approach, we evaluate transcription factors as the biorecognition elements for sensors. Recently, our group successfully mined the microbial microverse for a bacterial allosteric transcription factor (aTF), SRTF1, responsive to PRG.[16] Bacteria have evolved over 3 billion years to respond to virtually all classes of stimuli, including chemical analytes of interest, and represent an almost limitless database to search for unique aTFs. Like antibodies, aTFs recognize analytes with high sensitivity and specificity. In contrast to antibodies, aTFs respond to analyte binding by changing their affinity for a cognate DNA sequence. This change in DNA-binding affinity provides an intrinsic transduction mechanism that we capture, electrochemically, for the development of a novel aTF-based PRG biosensor.

Specifically, our biosensor comprises a gold electrode with a surface-immobilized DNA-aTF complex (Figure 1). In the

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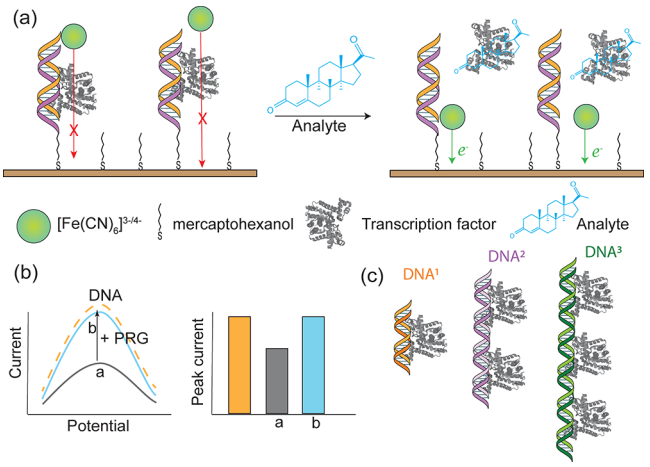

Figure 1. (a) Overview of the electrochemical biosensor: DNA-TF complex on the gold electrode blocks mediator access, giving low current output. (b) Presence of hormone is detected by a higher current output using square wave voltammetry. (c) DNA with 3 (DNA^3), 2 (DNA^2), and 1 (DNA^1) binding pockets.

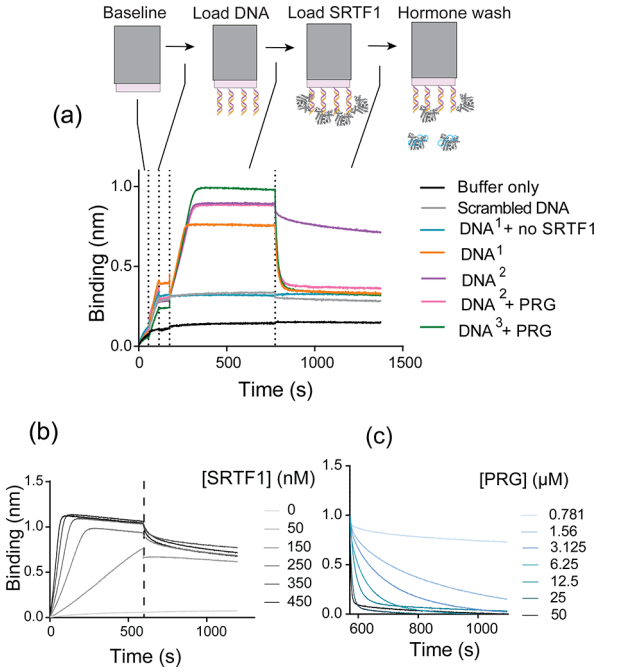

Figure 2. Biolayer interferometry (a) of $DNA,^1$ $DNA,^2$ and DNA^3 (1, 2, and 3 binding sites, respectively). (b) To calculate K_D, different [SRTF1] were added to DNA^2 to measure the association constant and thereafter dipped in buffer solution for the determination of the dissociation constant. (c) DNA^2–SRTF1 system responds to PRG in a dose-dependent manner. Please see the Supporting Information page 3 for additional details.

absence of an analyte (e.g., progesterone), the overall negative charge of the DNA-aTF complex blocks the negatively charged mediator ($Fe(CN)_6^{3-/4-}$) approaching the gold electrode and affords a low current output. The addition of the analyte binds the aTF, releasing it from the DNA strand on the surface and decreasing the barrier for the mediator to reach the electrode, thereby resulting in an amplified current output. As binding of the analyte to the DNA-aTF complex is a dose-dependent reaction, the difference in the current output before and after the addition of the analyte correlates to the analyte concentration. Herein, we demonstrate the above biosensor concept with a working prototype. We further explore the effect of increasing the number of aTF-binding pockets on DNA to improve the sensor output.

First, we performed biolayer interferometry (BLI) experiments using synthetic DNAs containing SRTF1 binding site(s), SRTF1, and PRG (Table S2). SRTF1 rapidly binds to the DNA, conjugated to the probe via streptavidin–biotin interactions, and we observe an increase in the BLI binding signal that corresponds to an increase in the thickness from the sensing tip. (Figure 2a). The DNA strand possessing two or three SRTF1 binding sites (DNA^2 and $DNA,^3$ respectively) affords a larger BLI signal in the presence of SRTF1, consistent with a greater binding signal with no significant change in the binding constant ($K_D \sim 4$ nM) between DNA and SRTF1 (Figures 2b and S1; Table S3). The scrambled DNA, lacking a binding site for SRTF1, shows no change in the BLI signal and highlights the importance of aTF recognition and specificity. In the presence of PRG, SRTF1 rapidly dissociates from the DNA-SRTF1 complex in comparison to the buffer control, where no significant change in the BLI signal occurs. Varying the concentration of PRG yields a dose-dependent BLI signal (Figures 2c, S1, and S2). Together, these data confirm that (i) SRTF1 binds to the cognate DNA sequence in the absence of PRG; (ii) SRTF1 allosterically unbinds from the DNA in the presence of PRG; and (iii) adding an additional SRTF1 binding site to the DNA increases the amount of aTF bound. This is crucial for the electrochemical sensor design as an increase in the amount of aTF will result in higher impedance changes.

The electrochemical biosensor utilizes ferrocyanide/ferricyanide as the mobile electrochemically active mediator and thereby avoids the requirement to covalently conjugate a redox probe to the protein or DNA. The biosensor consists of three electrodes: gold working (diameter 1.6 mm) and counter electrodes along with a Ag/AgCl reference electrode. Following the electrode cleaning by cyclic voltammetry in 0.5 M H_2SO_4 (Figure S3), we immobilized the 5′- thiol-modified DNA and 1 mM mercapto-6-hexanol, to prevent nonspecific interactions, on the working electrode following published procedures.[17,18] The DNA surface coverage is $\approx 3 \times 10^{-11}$ mol/cm^2 (Figures S4 and S5), as determined by cyclic voltammetry measurements with tris-ruthenium hexamine, and the value is in agreement with previous reports.[5] Electrochemical and AFM studies support the DNA immobilization and SRTF1 binding. Upon immobilization of the DNA and protein to the electrode, the resistance increases as does the height from the electrode surface from ~700 pm to 8 nm (Figures S6–S8). DNA-derivatized electrodes are widely used in biosensors with elegant strategies employing DNA-mediated charge transport,[19,20] conformational changes[21−24] during binding events, and electrocatalysis events by covalent and noncovalent association of redox mediators.[18,25] These strategies are used to detect target molecules,[26−28] hybridization events,[29,30] single base mismatches,[31] as well as for catalytic oxidation of guanine.[32]

Following DNA immobilization, we measured the current by square wave voltammetry (0–0.6 V, step size: 4 mV, amplitude: 50 mV, frequency: 50 Hz) in the absence and presence of aTF. The current is the greatest for the DNA-modified sensor and decreases upon the addition and formation of the DNA-SRTF1 complex (Figure 3b). Upon

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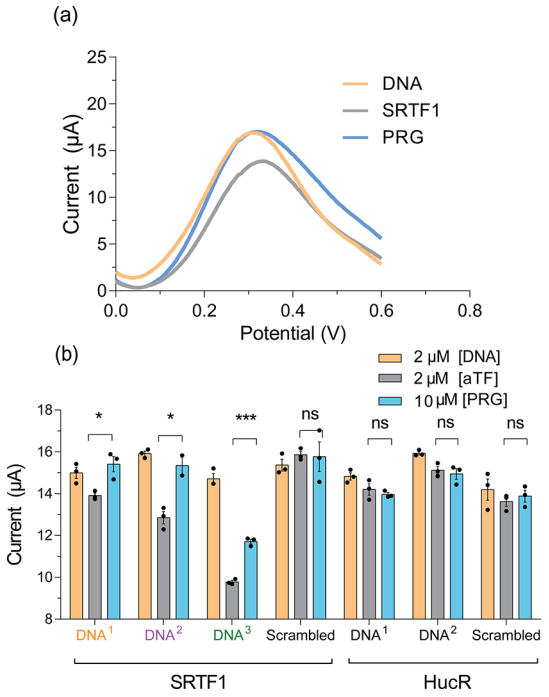

Figure 3. A typical square wave voltammogram (a). Current drops on SRTF1 addition and increases on PRG addition. Peak current of square wave voltammogram plotted (b) for DNA,[1] DNA,[2] DNA,[3] and scrambled DNA with the addition of aTF and PRG. The error bars represent the standard deviation for $N = 3$ biological replicates, and statistical significance was established according to a t-test. (* $p < 0.05$, *** $p < 0.001$).

the addition of PRG, the current increases and returns to its DNA baseline. Use of the scrambled/nonspecific DNA results in no change in the current in the presence of SRTF1 or PRG (Figure 3b). Similarly, the use of DNA with a nonspecific aTF (HucR, a uric acid transcription factor), which does not recognize the DNA-binding site, affords no change in the current in the presence of this aTF or PRG. Furthermore, in the absence of SRTF1, no significant change in current is observed on the addition of PRG to DNA-functionalized electrodes (Figure S9). Increasing the number of SRTF1 binding sites on the DNA from one to two and three increases the magnitude of the current change upon the addition of SRTF1. The biosensors with DNA^2 and DNA^3 exhibit a 20 and 35% decrease in current upon aTF complexation, respectively. This result is consistent with greater protein surface coverage impeding ferro/ferricyanide mobility to the electrode surface for electron transfer and a lower background signal. However, only in the DNA^2 system, does the current return to a baseline level in the presence of PRG. We suspect that the larger DNA^3-SRTF1 complex on the surface inhibits PRG binding, and thus, this reaction does not readily go to completion.

Next, we evaluated the analytical response of the system toward PRG by quantifying the current changes of the biosensor system in the presence of varying concentrations of PRG (0−10 μM) in Tris buffered saline, pH 7.4, and purchased artificial urine spiked with 2 μM bovine serum albumin (BSA) to better mimic the physiological conditions. We plotted the peak currents before and after the addition of PRG. The difference in peak currents positively correlates with the PRG concentration. The limit of detection (LOD) for the DNA^1-SRTF1 biosensor in buffer is 47 nM. In artificial urine, the LOD increases to 56 nM (Figure 4a; Table S4). There is no significant effect due to the addition of BSA and PRG in the

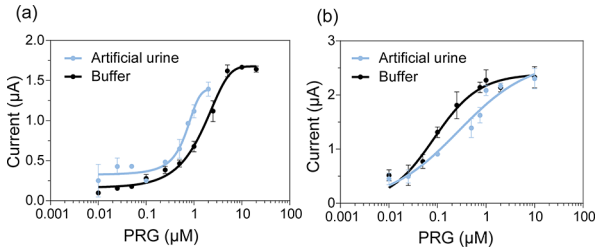

Figure 4. Calibration curve of the electrochemical sensor system. DNA^1 (a) and DNA^2 (b). Difference in current output before and after the addition of PRG is plotted against concentrations of PRG in artificial urine and buffer. The error bars represent the standard deviation for $N = 3$ biological replicates.

absence of SRTF1 to the DNA-functionalized electrode (Figures S9 and S10). A bottleneck for achieving a lower LOD of the sensor is the relatively small change in the current signal from the baseline observed at a lower PRG concentration. We addressed this challenge using the DNA^2-aTF biosensor, which possesses two aTF binding sites per DNA, that is, increased number of binding sites for the same electrode surface area. At a low concentration of PRG, more SRTF1 unbinds from the DNA^2 than DNA,[1] affording a greater current change from the baseline and lowering the LOD. The LOD for the DNA^2-SRTF1 decreases to 17 nM in buffer and to 29 nM in artificial urine (Figure 4b; Table S4). From a woman's health perspective, the current PRG detection range is suitable for monitoring all trimesters of fetus development, which span a urine PRG concentration from 30 nM to 1 μM (Table S5). These results are comparable to our previously developed fluorescence resonance energy transfer (FRET)-based sensor that had an LOD of 15 nM in artificial urine (Table S1).[16] Efforts are ongoing to further advance the design and components as well as the type of electrochemical measurement to lower the LOD so as to identify optimal times for conception based on a PRG measurement (Table S5).

Biologically, transcription factors control gene expression and are a mainstay of many synthetic biology experiments performed in whole cell (WCB) and cell-free platforms.[33−35] Transformation of these strategies to biosensing of small analytes is of significant interest.[36−38] Successes in WCB include the detection of environmental pollutants[37,39−41] and diagnostic biomarkers[33,42] via the regulation of a reporter gene followed by the subsequent expression of, for example, green fluorescent protein (GFP).[37,43,44] WCBs offer signal amplification through gene expression, high selectivity, and high reproducibility. A recent study describes a PRG WCB based on the transcription of GFP because of a slight conformational change of a non-natural TF upon PRG binding.[45] However for WCB platforms, challenges include maintenance of cells, costs, biocontainment, and regulatory concerns, as well as difficulty in the detection of analytes that are toxic to cells. Cell-free systems hold promise of safety, high sensitivity, and fast response time with examples of detecting virus, nucleic acids, antibiotics, and biomarkers.[38,46−48] The majority of the cell-free systems are based on protein synthesis using crude or purified components with optical output signals based on fluorescence, colorimetric, and bioluminescence reporters.[33,49]

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On the electrochemical front, Barton et al.[50] describe a transcriptional activator TATA binding protein (TBP) that binds and kinks a redox molecule-labeled DNA, which significantly attenuates charge transport through DNA. Recently, Mousavi et al., report an electrochemical sensor to detect antibiotic genes by using a cell-free expression of restriction enzymes mediated by transcription factor-analyte binding. The restriction enzyme cleaves the reporter genes that binds to capture genes on the electrode, producing a detectable electrochemical signal.[51] To date, the work on TFs based electrochemical sensors includes the capture of binding events between DNA and aTFs to detect transcription factors (example, NF-$\kappa\beta$)[50,52−59] or interaction between the analyte and transcription factor that results in conformational changes. Finally, Sode et al. report a glutamine-binding protein tagged with a redox probe that undergoes conformational changes in the presence of L-glutamine, altering the electrochemical current output.[60]

In summary, we report an electrochemical transcription factor-based biosensor for the detection of small molecule analytes. This aTF-DNA sensor joins the family of electrochemical-based biosensors, which are being increasingly investigated for health monitoring.[61−67] The advantages of such biosensors, generally, include rapid test-to-results, high selectivity/sensitivity, and low-cost of detection. Our sensor demonstrates clinically relevant sensitivity required for adaptation into a prototype POC biosensor. The key findings of this work are (1) translation of transcription factor binding affinities (to target DNA and analyte) into an electrical readout; (2) increasing the number of aTF binding sites on DNA improves sensitivity; and (3) detection of physiological levels of PRG in buffer and artificial urine samples. Our results hold promise for the development of a new suite of electrochemical biosensors based on natural or engineered bacterial aTFs.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssensors.2c00133.

Materials and methods, DNA sequences, expression and assay conditions, electrode characterization (AFM, impedance spectroscopy), and binding kinetics (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

James E. Galagan − *Department of Microbiology and Department of Biomedical Engineering, Boston University, Boston, Massachusetts 02215, United States*; Email: jgalag@bu.edu

Mark W. Grinstaff − *Division of Materials Science and Engineering, Department of Chemistry, and Department of Biomedical Engineering, Boston University, Boston, Massachusetts 02215, United States*; orcid.org/0000-0002-5453-3668; Email: mgrin@bu.edu

Authors

Karthika Sankar − *Division of Materials Science and Engineering, Boston University, Boston, Massachusetts 02215, United States*

R. Baer − *Department of Microbiology, Boston University, Boston, Massachusetts 02215, United States;* Present Address: Stemloop, Inc., Evanston, Illinois 60204, United States (R.B.)

Chloé Grazon − *Department of Chemistry and Department of Biomedical Engineering, Boston University, Boston, Massachusetts 02215, United States; CNRS, Bordeaux INP, LCPO, UMR 5629, University Bordeaux, F-33600 Pessac, France; Institut des Sciences Moléculaires (CNRS UMR 5255), University Bordeaux, 33405 Talence, France*

Robert C. Sabatelle − *Department of Biomedical Engineering, Boston University, Boston, Massachusetts 02215, United States*

Sébastien Lecommandoux − *CNRS, Bordeaux INP, LCPO, UMR 5629, University Bordeaux, F-33600 Pessac, France*

Catherine M. Klapperich − *Division of Materials Science and Engineering and Department of Biomedical Engineering, Boston University, Boston, Massachusetts 02215, United States;* orcid.org/0000-0001-6103-849X

Complete contact information is available at: https://pubs.acs.org/10.1021/acssensors.2c00133

Author Contributions

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Notes

The authors declare the following competing financial interest(s): K.S., R.B., C.G., C.M.K., J.E.G., and M.W.G. are inventors on a patent describing this technology filed by Boston University, which is available for license.

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■ ABBREVIATIONS

aTF, allosteric transcription factor; BLI, biolayer interferometry; LOD, limit of detection; PDG, pregnanediol glucuronide; POC, point-of-care; PRG, progesterone; SWV, square wave voltammetry; WCB, whole cell biosensor

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