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Paper-Based Progesterone Sensor Using an Allosteric Transcription Factor

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Cite This: *ACS Omega* 2022, 7, 5804−5808

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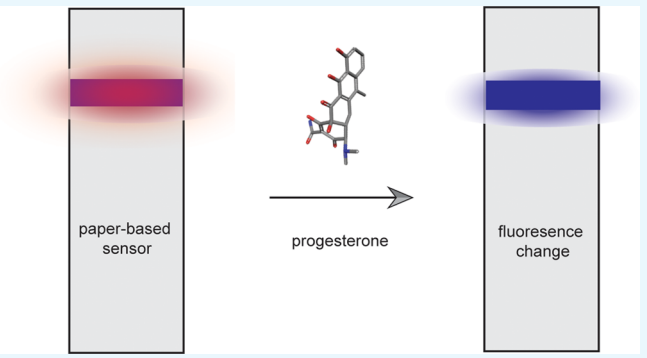
ABSTRACT: Progesterone monitoring is an essential component of in vitro fertilization treatments and reproductive management of dairy cows. Gold-standard biosensors for progesterone monitoring rely on antibodies, which are expensive and difficult to procure. We have developed an alternative transcription factor-based sensor that is superior to conventional progesterone biosensors. Here, we incorporate this transcription factor-based progesterone sensor into an affordable, portable paperfluidic format to facilitate widespread implementation of progesterone monitoring at the point of care. Oligonucleotides labeled with a fluorescent dye are immobilized onto nitrocellulose via a biotin−streptavidin interaction. In the absence of progesterone, these oligonucleotides form a complex with a transcription factor that is fluorescently labeled with tdTomato. In the presence of progesterone, the fluorescent transcription factor unbinds from the immobilized DNA, resulting in a decrease in tdTomato fluorescence. The limit of detection of our system is 27 nm, which is a clinically relevant level of progesterone. We demonstrate that transcription factor-based sensors can be incorporated into paperfluidic devices, thereby making them accessible to a broader population due to the portability and affordability of paper-based devices.



INTRODUCTION

Biosensors are widely used in molecular diagnostics to detect analytes of interest; a biosensor uses a biorecognition element on a transducer that elicits a signal change upon binding to the analyte.[1] However, the scarcity of biorecognition elements restricts the number of possible biosensors that can be created.[2] Recently, our group has used bacterial mining to screen for allosteric transcription factors that reversibly bind to hormones and other small molecules.[3] Transcription factors are proteins that influence the gene expression by binding to DNA. Transcription factors have been implicated in diseases, and there are numerous assays that screen for them.[4−6] Instead of screening for the transcription factors, we use them as sensor components to detect small molecules. These transcription factors have been isolated and used in fluorescent-based biosensors to detect anhydrotetracycline[7,8] and progesterone.[3,9] These sensor components have been suspended in solution[3,7] and in hydrogels[10] and immobilized on gold surfaces,[11] demonstrating their versatility and ease of adaptation to various sensing modalities. However, these sensors have yet to be incorporated into paper-based diagnostics, which are favorable for point-of-care testing. Paper-based diagnostics are popular for point-of-care testing due to their ease of use, affordability, and portability.[12] One common configuration of paper-based assays is the dipstick test.[13] Dipsticks typically contain antibodies immobilized on a paper membrane; these antibodies interact with the analyte of interest to produce a visual readout.[13] Dipsticks have been used for blood type determination[14] and urine analysis;[15] they have also been used to detect pathogens,[16,17] hormones,[18] and nucleic acids.[19] However, the vast majority of these assays have been immunosensors.[3] Immunosensors require antibodies, which are expensive and difficult to produce.[20] Similar to antibodies, transcription factors are proteins. However, unlike antibodies, transcription factors can be isolated and purified from bacteria without needing to inoculate an animal with antigens to induce the expression of antibodies that can be recovered from the animal's serum. To the best of our knowledge, dipstick assays have yet been used to detect hormones using transcription factor-based sensors. We will demonstrate that we can incorporate our transcription factor-based biosensors into a dipstick assay using progesterone as a model target. Progesterone is a sex hormone that is necessary

Received: October 15, 2021
Accepted: January 25, 2022
Published: February 7, 2022

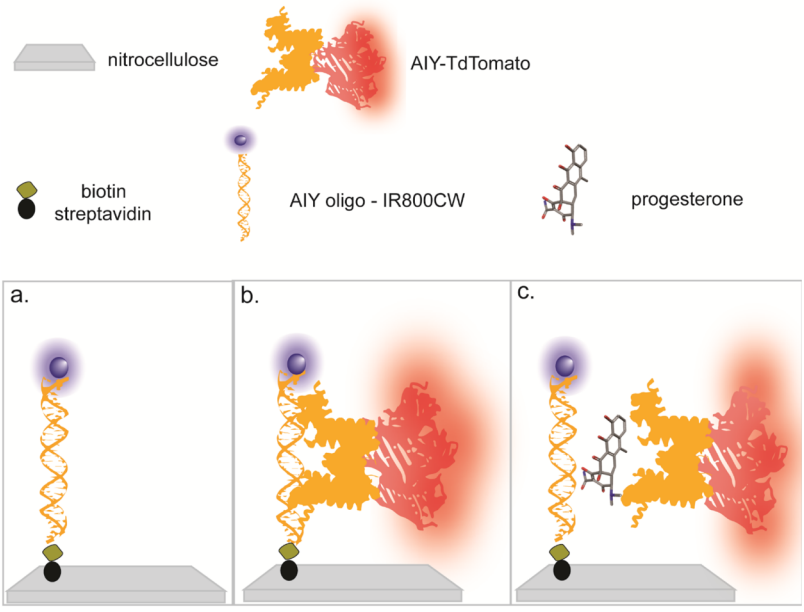


Figure 1. Sensor design. (a) IR-labeled oligonucleotides bind to nitrocellulose via a biotin−streptavidin interaction. (b) SRTF1-tdTomato specifically binds to the immobilized oligos. (c) Exposure to progesterone causes the unbinding of the SRTF1-tdTomato from the immobilized oligonucleotide.

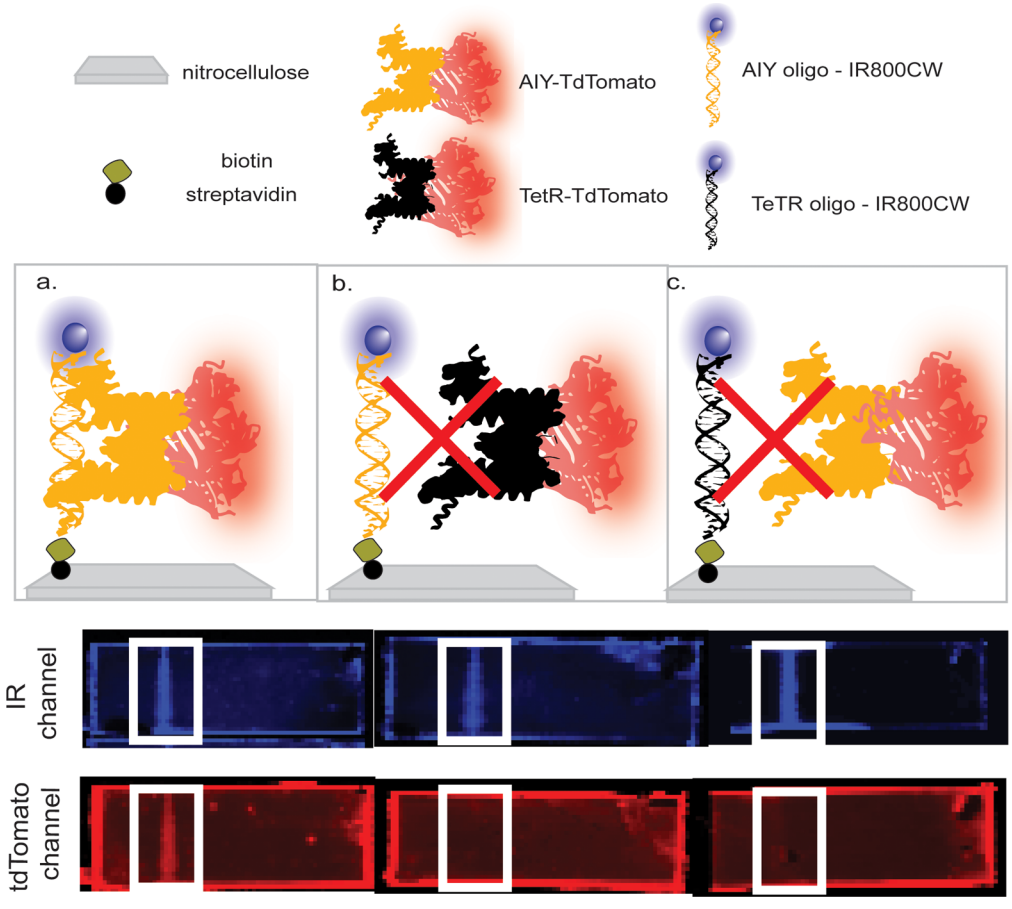


Figure 2. Binding of SRTF1-tdTomato to the immobilized oligonucleotide is specific. (a) SRTF1-tdTomato binds to an immobilized oligo containing the binding sequence of SRTF1. (b) TeTR-tdTomato does not bind to an immobilized oligo containing the binding sequence of SRTF1. (c) SRTF1-tdTomato does not bind to an immobilized oligo containing the binding sequence of TeTR-tdTomato.

for pregnancy.[21] Monitoring of progesterone levels may be beneficial in humans undergoing in vitro fertilization (IVF) and cattle used to produce dairy products. In individuals undergoing IVF, elevated levels of progesterone on the day of

https://doi.org/10.1021/acsomega.1c05737
ACS Omega 2022, 7, 5804−5808

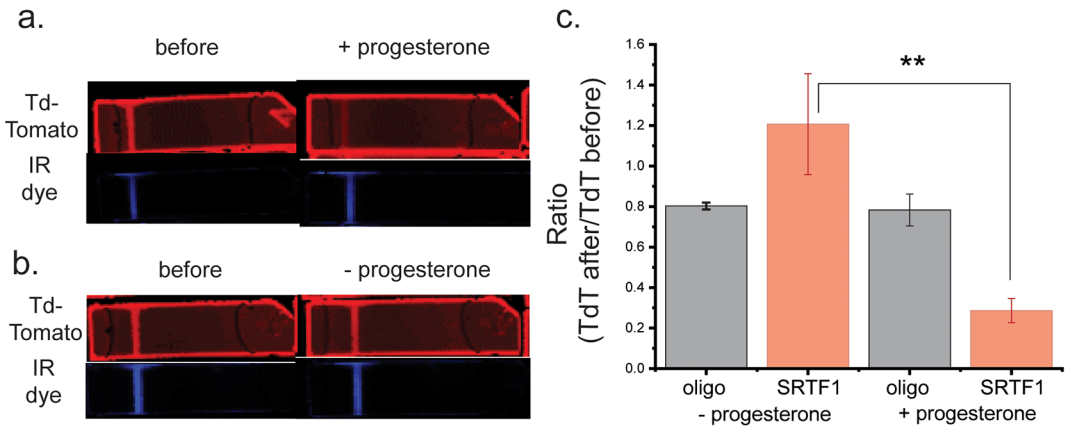


Figure 3. Progesterone detection. (a) Treatment with progesterone causes a decrease in tdTomato fluorescence but not in IR fluorescence. (b) Treatment with buffer does not cause a decrease in either tdTomato or IR fluorescence. (c) Decrease in tdTomato fluorescence caused by progesterone is statistically significant according to a *t*-test ($p < 0.01$). The error bars represent the standard deviation for $n = 3$ biological replicates.

human chorionic gonadotropin administration may decrease the chance of pregnancy.[22] In cattle, monitoring progesterone levels is helpful for successful reproduction management.[23] Commercial point-of-care progesterone tests require expensive benchtop equipment. A paper-based progesterone sensor that could be coupled with off-the-shelf electronics[3] to detect changes in fluorescence would be much more affordable and portable, allowing for more widespread and frequent progesterone testing.

RESULTS AND DISCUSSION

Sensor Design. We have created a paper-based progesterone sensor using an allosteric transcription factor, called as SRTF1 (Genbank ID AIY20223), for low-cost progesterone monitoring at the point of care. In the absence of progesterone, SRTF1 binds to a specific sequence of double-stranded DNA. In the presence of progesterone, SRTF1 is released from this DNA, given its higher affinity for binding progesterone. In order to make a paper-based progesterone sensor using SRTF1, oligonucleotides containing the SRTF1 binding sequence (Table S1) were immobilized on nitrocellulose via a biotin−streptavidin linkage (Figure 1a). The oligos are terminated with a fluorescent infrared (IR) dye that we used to monitor the oligonucleotide deposition. The SRTF1 is tagged with tdTomato, a fluorescent protein to monitor the binding to (Figure 1b) and progesterone-induced unbinding (Figure 1c) of the SRTF1 from the oligonucleotides. As seen in Figure 2a, the complete surface-bound SRTF1−oligonucleotide complex results in fluorescence in the both the IR channel and the tdTomato channel.

Specificity of SRTF1-DNA Binding. The interaction between the immobilized oligos and SRTF1 is specific. TeTR-tdTomato, which is a transcription factor that binds to anhydrotetracycline, does not bind to the oligonucleotides, despite the fact that SRTF1 is a TeTR family transcription factor.[24] This is shown in Figure 2b, where we observe fluorescence in the IR channel but not in the tdTomato channel. The reverse is also true—SRTF1-tdTomato does not bind to oligonucleotides that are specific to TeTR-tdTomato (Figure 2c). This ensures that a transcription factor that does not bind to progesterone will not be nonspecifically bound to the immobilized DNA, which would result in a false-negative test result.

Progesterone Detection. Fluorescent signals were used to monitor progesterone-induced disassociation of SRTF1-tdTomato from the immobilized oligos. Treatment with progesterone decreases the fluorescence intensity of tdTomato on the strips (Figure 3a), indicative of progesterone-induced disassociation of SRTF1-tdTomato from the immobilized oligonucleotides. Treatment with progesterone does not affect the fluorescence intensity of the IR dye because the IR-tagged oligos are still immobilized on the nitrocellulose. Treatment with buffer does not decrease the fluorescence intensity of tdTomato on the strips (Figure 3b); in the absence of progesterone, SRTF1-tdTomato remains bound to the oligos immobilized on the strips. The ratio of tdTomato fluorescence of the strips after treatment with progesterone (or buffer) to that before treatment was calculated; the progesterone-induced decrease in tdTomato fluorescence was statistically significant ($p < 0.01$ according to a *t*-test) (Figure 3c), confirming that these test strips can be used for progesterone monitoring.

Limit of Detection. In order to determine the limit of detection (LOD) of the progesterone biosensor, individual strips were exposed to either 0 nM, 25 nM, 75 nM, 100 nM, 1 μM, or 10 μM progesterone. As seen in Figure 4, the results were plotted and fit using a Hill function to reflect the

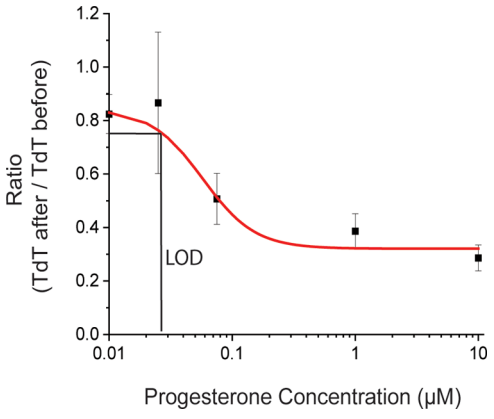


Figure 4. Progesterone sensor LOD. The paper-based progesterone sensor was tested using various concentrations of progesterone. The LOD of the sensor was 27 nM. Error bars represent the standard deviation of $n = 3$ biological replicates.

https://doi.org/10.1021/acsomega.1c05737
ACS Omega 2022, 7, 5804−5808

cooperative binding of the transcription factor.[3] In order to determine the LOD, the following equations were used[25]

$$\mathrm{LOB} = \mu_{\mathrm{blank}} - 1.645\sigma_{\mathrm{blank}} \quad (1)$$

$$\mathrm{LOD} = \mathrm{LOB} - 1.645\sigma_{\mathrm{low\ concentration\ sample}} \quad (2)$$

σ_{blank} represents the standard deviation of the blank sample and $\sigma_{\mathrm{low\ concentration\ sample}}$ represents the standard deviation of the low-concentration sample. Our sensor had an LOD of 27 nM or 8.22 ng/mL, which is a physiologically relevant level in both premenopausal women[26] and cows.[23] The sensor sensitivity could further be improved using oligonucleotides with a lower affinity for SRTF1[3] or by altering the ratio of SRTF1 to oligos on the surface, similar to altering the ratio of SRTF1 to quantum dots in a previous sensor design.[3]

CONCLUSIONS

We have fabricated a paper-based progesterone dipstick sensor that can detect physiologically relevant levels of progesterone. The test is portable and low cost and can be paired with inexpensive, off-the-shelf phototransistors[3] to achieve point-of-care progesterone monitoring. The transcription factor-based progesterone sensor is a proof of concept and just one of many possible transcription factor-based sensors that have yet to be discovered.[3] By developing a paper-based progesterone sensor, we demonstrate that other transcription factor-based sensors can similarly be adapted into a paperfluidic format. This approach could enable widespread, affordable point-of-care testing of a wide variety of hormones and small molecules for disease monitoring.

MATERIALS AND METHODS

Chemicals and Consumables. TRIS buffer was purchased from Fisher (BP152-1). Sodium chloride (S5886-1 kg), sodium citrate (51904−500 g), Tween-20 (P1379), and Ponceau (P7170-1 L) were purchased from Sigma-Aldrich. Buffer EB was purchased from Qiagen (19086). Instant nonfat dry milk was purchased from the grocery store. 10× blocker bovine serum albumin (BSA) in TBS was purchased from Thermo (37,520). Backed nitrocellulose was purchased from GE Healthcare (10,547,002). Double-sided tape was purchased from Scotch (34-8716-0599-3). Microscope slides were purchased from Fisherbrand (12-550-A3). Oligonucleotides (Table S1) were purchased from IDT.

Instrumentation. An Epilog Zing 16 Laser cutter (Epilog) was used to laser-cut nitrocellulose strips with the speed, power, and frequency settings set to 100%/7%/5000 Hz, respectively. A sciFLEXARRAYER S3 (Scienion) was used to spot streptavidin on the nitrocellulose strips in a horizontal line. Each drop contained 10,000 pL of streptavidin, and the spot pitch in both the x and y directions was 250 μm. A Sapphire Biomolecular Imager (Azure Biosystems) was used to image the strips. A VWR advanced digital shaker (890-32-096) was used during Ponceau staining and BSA blocking. A Labquake rotisserie shaker (Barnstead International, 415,110) was used for all other washes and incubations.

Plasmid Preparation. The TetR plasmid production was previously reported.[7]

SRTF1-tdTomato was cloned using the previously reported SRTF1 and TetR-tdTomato expression plasmids.[3,4] The linker-tdTomato coding sequence was amplified from T7mTetR; tdTmSTII_AE using primers pri_tDT_F (TACTTGCGGACGACGACGCCgctgaagctgctgctaAAGAag) and pri_tdT_R (TAGTGATGGTGGTGATGATGgccgcccttgtacagct). Primer pri_tdT_R replaced the strep-tag used to purify TetR-tdTomato with a 6×His tag. Plasmid pRham_SRTF1_C-His_Kan was linearized for tdTomato insertion using primers pri_SRTF1vect_F (acgagctgtacaagggcggcCATCATCACCACCATCACTAATAGAGCG) and pri_SRTF1vect_R (TCTTtagcagcagcttcagcGGCGTCGTCGTCCG). PCRs were purified using the NEB Monarch PCR & DNA Cleanup, and plasmid was constructed using the NEB Gibson Assembly kit.

Protein Purification. The protocol for TeTR protein purification was previously reported.[7]

SRTF1-tdTomato was purified as previously reported.[3]

Dipstick Sensor Fabrication. Oligonucleotides were first hybridized to each other in-tube. A 500 μL working solution of 1 μM SRTF1-oligonucleotide and 1.5 μM CSRTF1-oligonucleotide in hybridization buffer (10 mM Tris pH 8.0, 66.7 mM NaCl) was prepared in a 1.5 mL Eppendorf tube. The solution was heated at 95 °C for 5 min and then left to sit at room temperature for at least 1 h. While the DNA was cooling, the nitrocellulose strips were prepared.

Backed nitrocellulose was taped to a microscope slide using double-sided tape. The nitrocellulose was laser-cut into rectangular strips. The microscope slide was then inserted into a spotter. Streptavidin was spotted onto the nitrocellulose. The strips were left to dry for a few minutes. A Ponceau stain was used to visually confirm successful streptavidin deposition. They were then blocked with 1× BSA in TBS for 30 min with agitation. After 30 min, the strips were washed 3× with TBS-T.

Next, six of the streptavidin-coated strips were submerged in the hybridized oligonucleotide solution for 20 min. The oligo-immobilized strips were then washed 1× with TBS-T, 1× with 2×SSC, and 1× with 0.1× SSC; each wash was done with a Rotoquake for 5 min. Next, the strips were blocked with 5% nonfat milk at room temperature for 2 h using the Rotoquake. After 2 h, the strips were washed 3× with TBS-T for 5 min each. Finally, the oligonucleotides immobilized on the strips were exposed to 0.1 μM SRTF1-tdTomato for 15 min. The strips were then washed 3× with TBS-T, for 5 min each time on the Rotoquake. The strips were then imaged in both the IR and tdTomato wavelengths.

Progesterone Assay. Each strip was submerged in a 1 mL progesterone solution on the Rotoquake for 30 min. The strips were washed 3× with TBS for 5 min each on the Rotoquake.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c05737.

Progesterone detection (TIF)

Sensor design (TIF)

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https://doi.org/10.1021/acsomega.1c05737
ACS Omega 2022, 7, 5804−5808

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Notes

The authors declare the following competing financial interest(s): A patent application has been filed with the US Patent Office through Boston University. Uros Kuzmanovic is CEO of Biosens8, Mark W. Grinstaff is a board member of Biosens8, James E. Galagan is President of Biosens8 and Catherine M. Klapperich is a co-founder of Biosens8.

■ ACKNOWLEDGMENTS

This work was supported by a DARPA grant (W911NF-16-C-0044), the BU Precision Diagnostics Center (C.M.K.) and the USUHS\TTW grant (HU00011810022).

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