A progesterone biosensor derived from microbial screening

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Bacteria are an enormous and largely untapped reservoir of biosensing proteins. We describe an approach to identify and isolate bacterial allostERIC transcription factors (aTFs) that recognize a target analyte and to develop these TFs into biosensor devices. Our approach utilizes a combination of genomic screens and functional assays to identify and isolate biosensing TFs, and a quantum-dot Förster Resonance Energy Transfer (FRET) strategy for transducing analyte recognition into real-time quantitative measurements. We use this approach to identify a progesterone-sensing bacterial aTF and to develop this TF into an optical sensor for progesterone. The sensor detects progesterone in artificial urine with sufficient sensitivity and specificity for clinical use, while being compatible with an inexpensive and portable electronic reader for point-of-care applications. Our results provide proof-of-concept for a paradigm of microbially-derived biosensors adaptable to inexpensive, real-time sensor devices.

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Biosensors underlie applications ranging from medical diagnostics, environmental monitoring, food and water safety, to the detection of chemical or biological threats. A typical biosensor utilizes a biorecognition element coupled to a transduction mechanism. Yet the number and types of biorecognition elements are limited. The gold standard for clinical hormone analysis uses antibodies as the biorecognition element which are expensive to design and manufacture, do not provide an intrinsic read-out, and do not readily support repeated or continuous measurements.

Bacteria have evolved over 3 billion years to detect and respond to a wide range of stimuli. One common molecular sensing mechanism is an allosteric transcription factor (aTF). Binding of ligand to aTFs leads to differential binding of the TF to its cognate DNA binding sites and an alteration in gene expression. Allosteric TFs are used as biosensors in whole cell applications, but the use of bacterial cells in sensors is limited by slow response times, biosafety concerns, and the practical limitations of using a cellular host. Moreover, characterized TFs are a small fraction of the hundreds of thousands of known TFs that have been sequenced, and sequenced genes are only a tiny sampling of the diversity of microbes. Here, we report an approach to identify and harvest microbial aTFs specific to a target analyte, and a transduction strategy for engineering these into sensor devices. We show the application of our approach to develop an optical progesterone sensor based on a previously uncharacterized microbial aTF identified with our screening approach.

**Results**

**A progesterone sensing aTF.** Our screening approach employs a combination of genomic and functional assays for the targeted identification, validation, and characterization of TF biosensors for specific analytes (Supplementary Fig. 1). Our approach (Supplementary Fig. 1) is based on three observations: bacterial TFs commonly bind upstream of their genes to regulate their own promoters or those of adjacent genes, genes for the metabolism of analytes are often found in genome clusters, and these clusters are often induced by their substrates via TFs in genomic proximity. We applied our approach to identify bacterial aTFs responsive to steroid hormones owing to their central roles in human physiology, wellness, and health. Steroids are also found widely in nature due to contamination by human activity, and steroids are also found in plants, fungi, and animals. Consequently, a host of environmental bacteria are able to use steroids as carbon and energy sources, and one annotated steroid metabolizing bacterium is *Pimelobacter simplex* (also *Nocardioides simplex* and formerly *Corynebacterium simplex*) which biotransforms a range of steroids. Though *P. simplex* is employed in industry for the metabolism of steroids, little is known about the corresponding regulatory mechanisms.

*P. simplex* was screened to identify aTFs capable of sensing steroid hormones. Cultures of *P. simplex* were exposed to aldosterone, cortisol, estrone, estradiol, progesterone and control media (Supplementary Fig. 2). RNA Sequencing (RNA-Seq) was used to identify gene clusters and associated TFs induced by steroids (Fig. 1). One such gene cluster was significantly upregulated on exposure to progesterone, aldosterone, and cortisol and was termed the Steroid Responsive Genomic Island (SRGI). The SRGI overlaps a previously reported cluster of genes experimentally associated with steroid metabolism and includes one annotated 3-ketosteroid-9α-hydroxylase, two annotated 3-ketosteroid-Δ1-dehydrogenases, and one annotated steroid 8-isomerase. Importantly, the SRGI includes three predicted but functionally uncharacterized TF genes (Fig. 1f, Supplementary Table 1): (1) a predicted TetR-family TF in a divergent promoter with a predicted ketosteroid-dehydrogenase gene, (2) a predicted IclR-family TF adjacent to a second ketosteroid-dehydrogenase gene, and (3) a predicted MarR-family TF upstream of the IclR-TF. Only the first two TFs (whose proteins were termed SRTF1 and SRTF2, respectively) display induction by steroids in our experiments (Fig. 1f). Analysis of the divergent intergenic region upstream of the SRTF1 gene revealed a 22 bp palindromic sequence immediately upstream of the adjacent ketosteroid-dehydrogenase gene (Fig. 1h). We confirmed binding to this palindromic by SRTF1 using a method for in vitro chromatin-immunoprecipitation followed by sequencing (in vitro ChiP-Seq). In vitro ChiP-Seq discovered two additional binding sites in the cluster including one upstream of SRTF2 (Supplementary Fig. 3). These data suggest that SRTF1 mediates the steroid responsiveness of both ketosteroid-dehydrogenase genes and SRTF2. We thus focused additional analysis on SRTF1.

We quantitatively confirmed that SRTF1 is a sterol responsive aTF using biolayer interferometry (BLI) (Fig. 2). Synthetic oligonucleotides containing either the predicted palindromic site found upstream of SRTF1 or a shuffled control sequence were conjugated to the BLI probe (Fig. 2a). The probe was placed in a solution with purified SRTF1 protein and no steroids. SRTF1 rapidly bound to the oligo containing the predicted binding sequence, while no binding was observed to the scrambled sequence, further confirming the specificity of the predicted binding motif (Fig. 2a). The probe was then moved into a solution with no protein and either vehicle or 5 μM steroid hormones (Fig. 2a, b, d). Minimal unbinding was observed with vehicle, and a quantitative analysis indicated nanomolar affinity of SRTF1 to the oligonucleotide in the absence of steroids (Supplementary Fig. 8, Supplementary Table 2). In contrast, SRTF1 rapidly dissociated after exposure to progesterone, with 81% dissociation at 30 s after exposure compared to 4.5% in vehicle (Fig. 2d, e). This change in DNA binding was highly specific to progesterone. Exposure to cholesterol or the estrogens beta-estradiol or estrone resulted in no SRTF1 dissociation relative to control, while exposure to aldosterone or cortisol yielded only 10 and 13% dissociation, respectively (Fig. 2d, e). Moreover, no differences in dissociation were observed after exposure to 5β-Pregnan-3α,20α-diol (pregnanediol) and 5β-Pregnan-3α,20α-diol-glucuronide (pregnanediol-glucuronide) (Fig. 2d, e). Both are urine metabolites of progesterone, and antibodies against pregnanediol-glucuronide are the basis of many tests for the indirect measurement of progesterone. Experiments with varying concentrations of progesterone demonstrated that progesterone-induced dissociation was dose-dependent (Fig. 2b, c). Together, these data confirm that SRTF1 is an aTF that binds its cognate DNA site in the absence of steroid hormones and allosterically rapidly unbinds in the presence of progesterone. To our knowledge, this is the first example of a progesterone-sensing bacterial transcription factor.

**A QD-TF-FRET framework for aTF biosensors.** Using aTFs as an affinity-based biorecognition element in a biosensor requires converting analyte binding into a detectable signal. Antibodies are the benchmark for affinity-based biosensors. However, antibodies bound to an analyte are inert. Detection of binding typically requires the addition of secondary antibodies, and this additional step prevents real-time and continuous measurements and prohibits multiple measurements. In contrast, aTFs combine affinity recognition with a built-in mechanism for the real-time transduction of reversible analyte binding: the reversible binding to their cognate DNA binding sites.
**Fig. 1 Screening of P. simplex for steroid sensing aTFs.** a–e Log2-fold change expression in response to each hormone relative to control. Each triangle is a gene (red = differentially expressed TF, black = non-differentially expressed TF, blue = gene with annotated sterol related function, green = differentially expressed other gene, gray = non-differentially expressed other gene). The Steroid Responsive Gene Island (SRGI) that is differentially expressed in response to PRG, ALD, and CRT is boxed in red. SRTF1 and SRTF2 are two steroid responsive TFs. f Zoomed in view of SRGI. Yellow peaks are experimentally validated binding sites for SRTF1. g SRGI region around SRTF1. h SRTF1 palindromic binding site upstream of SRTF1 gene. RNA-seq data are deposited in the Gene Expression Omnibus with accession number GSE141603.

**Fig. 2 SRTF confirmed as a progesterone responsive aTF.** a Top panel: BioLayer Interferometry (BLI) experimental approach. Bottom panel, binding layer thickness on probe normalized to baseline. Probes were loaded with oligos containing the SRTF1 binding site (red line) or a scrambled sequence (gray line). Addition of progesterone resulted in rapid unbinding of SRTF1 (red line) that was not observed with vehicle control (cyan line). b Unbinding of SRTF1 is dose-responsive. Dissociation curves in duplicate were normalized such that binding at time 0 was equal to 1. c Fraction SRTF1:DNA complex from curves shown in panel b dissociated at 5 s. d Dissociation curves in triplicate of SRTF1 in the presence of 5 μM various steroids showing strongest induction by progesterone normalized as in panel b. e Fraction SRTF1:DNA complex from curves shown in panel d dissociated at 30 s. Error bars are standard error over three experiments. Data underlying bar graphs in panels c and e are available in the Source Data file.
We have developed a framework for coupling the molecular transduction mechanism of aTFs to an optical output (Fig. 3a). Importantly, this approach can be used to develop optical biosensors with any aTF for which a cognate binding site is known. Our framework uses QD-TF-FRET. Quantum dots are widely used in bioimaging and biosensing and provide high photostability, color tunability, and abundant surface area for biofunctionalization. In our approach, QD FRET donors are decorated with purified aTFs and a DNA oligonucleotide with the TF binding site is conjugated to a FRET acceptor. TF-hormone binding alters TF-DNA binding resulting in changes in fluorescence. When TFs are bound to the DNA probe, the donor and acceptors are close enough to enable energy transfer. FRET reduces the emission intensity from the QD (donor fluorescence intensity, $F_D$) and increases emission intensity from the acceptor (acceptor fluorescence intensity, $F_A$, Fig. 3b). When TFs are not bound to the DNA probe FRET is reduced leading to increased $F_D$ and decreased $F_A$. Sensor output is a normalized ratio of donor to acceptor emission and measuring this ratio increases signal relative to either $F_A$ or $F_D$ alone. We describe here the comprehensive characterization and validation of this framework through the development of a QD-TF-FRET biosensor.

An optical progesterone biosensor. We used our QD-TF-FRET framework to develop an optical biosensor for progesterone based on SRTF1. Specifically, CdSe/CdS/ZnS QDs were coated via a biphasic ligand exchange with a zwitterionic polymer containing histamine groups for chelation-based binding to the QD surface. The SRTF1 gene was cloned with a C-terminal 6x His-tag, purified, and self-assembled onto the QD surface at specified average ratios of TFs to QD. Oligonucleotides containing SRTF1 binding sites were labeled with Cy5 as the FRET acceptor and with QD-TFs at specified ratios of DNA to QD. The QD emission spectrum overlapped the Cy5 excitation spectrum to enable FRET (Supplementary Fig. 4).

As a first sensor test (sensor 1), oligonucleotides containing the strong SRTF1 binding site upstream of the SRTF1 promoter were used. SRTF1 was combined with QDs at a ratio of 4 TFs per QD and oligonucleotides were combined with QD-TFs at a ratio of 18 oligos per QD. The fluorescence emission of QD-TF/DNA in...
Published reports have demonstrated the ability to dramatically alter the specificity profile of aTFs with a combination of random mutagenesis, directed evolution, and targeted protein modifications. These methods can be used to further increase the specificity of SRTF1-based sensors to progesterone. Thus, we have generated a mutant library of SRTF1. As a first step in characterizing this library, 10 mutants were randomly selected and characterized with a reporter gene assay (Supplementary Fig. 10). One mutant, SRTF1_MUT1, demonstrated a relative decrease in sensitivity to cortisol. Sequencing of the SRTF1_MUT1 gene revealed two mutations located in a region of SRTF1 with the potential to contain the steroid binding domain (Supplementary Fig. 11). We constructed a version of sensor 3 using SRTF1_MUT1 and characterized its response to the panel of steroids. Remarkably, we observed a 6-fold decrease in cross-reactivity to cortisol of 5.7%, with no change in cross-reactivity to other tested sterols (Fig. 3d, Supplementary Table 5, Supplementary Fig. 12). While only preliminary, and based on a randomly selected handful of mutants, these results suggest that directed evolution can further improve both the specificity and sensitivity of SRTF1 to progesterone.

A key advantage of aTF sensors relative to antibodies is the potential for multiple and continuous measurements. This stems from the fact that no secondary assays are required that result in fixation of analyte binding. All interactions associated with the QD-TF-FRET sensor are reversible. As an initial demonstration of this capability, we tested sensors with repeated cycles of progesterone exposure and removal by overnight dialysis (Supplementary Fig. 13). Measurements were performed after each exposure and dialysis. The results confirm that repeated exposures to the same progesterone concentration resulted in nearly identical sensor output (Fig. 3e, Supplementary Fig. 14). While greater variability was observed between measurements after dialysis, this was likely due to limitations in the ability of dialysis to completely remove progesterone. These data confirm the ability of our sensor to reliably measure repeat exposures of progesterone without the need for surface immobilization or additional procedures to reverse binding.

The development of practical devices requires manufacturing and storage reproducibility. To assess this, we tested three independent sensor batches constructed over the span of 6 months. In addition, the sensors were developed from two independent purifications of SRTF1, and a QD with a modified surface treatment and emission spectrum was used for the third sensor batch. As shown in Fig. 3f, the sensor responses to progesterone for all three batches were identical. We further tested the stability of our sensors to storage in different conditions: room temperature, at 4 °C, and at room temperature after freeze-drying. For up to a week. In all cases, we observed consistent responses when sensors were retrieved or rehydrated at all tested progesterone concentrations (Fig. 3g, Supplementary Fig. 15). We also tested long-term storage for ten months after freeze-drying. Rehydration restored full sensor responsiveness (Supplementary Fig. 15) although altered dose-response characteristics would necessitate re-calibration. These results are consistent with reports demonstrating the stability of cell-free gene circuits containing transcription factors for over a year.

Point-of-care urine testing. Progesterone sensing has applications in fertility planning. The detection of surges in luteinizing hormone (LH) and the rise in estrogen prior to the LH surge predict but do not verify ovulation. Detecting progesterone surges confirms ovulation but typically requires blood testing. Two at-home urine tests are currently being marketed to measure pregnenadiol-glucuronide. However, pregnenadiol-glucuronide...
levels are more variable than progesterone levels, and up to 12% of women do not metabolize progesterone sufficiently to produce detectable amounts\textsuperscript{34}. To our knowledge, no urine test for the direct detection of progesterone is available. As a first test of our sensor for urine testing, we measured responses to varying concentrations of progesterone in artificial urine. We tested three different conditions using sensor 3: artificial urine at room temperature, artificial urine warmed to body temperature, and sensors freeze-dried and then rehydrated in artificial urine at room temperature. In all cases, we see responses identical to buffer, verifying that common interferents in urine do not affect the sensing of progesterone by the sensor (Fig. 3b).

Use of our sensor at home or at point of care requires a portable electronic reader. As proof-of-principle, we built a prototype device using inexpensive off-the-shelf electronics (Fig. 4a, Supplementary Fig. 16). A 10 mW UV LED controlled by an Arduino was used for excitation. Two phototransistors amplified using a standard low-voltage common emitter circuit detected fluorescence emissions. A 600 nm bandpass filter was used to isolate QD fluorescence emission while a 665 nm long pass filter was used to isolate Cy5 emission (Supplementary Fig. 16). Despite its simplicity, tests of the progesterone sensor in this low-cost device confirmed the same degree of sensor accuracy as with a state-of-the-art laboratory device (Fig. 4b). This proof-of-principle device can be reduced in size and cost through printed circuit board fabrication for the electronics and 3D printing for the housing. Any similar device could be used for low-cost sensing with any bacterial aTF sensor developed with the QD-TF-FRET optical biosensor framework.

Discussion

Together, our data provide the first demonstration of a microbial screening-to-sensor approach to develop new sensing devices for specific target analytes. The progesterone sensor provides proof-of-concept for a class of sensors based on bacterial aTFs. It demonstrates sensitivity, reproducibility, stability, and signal strength applicable for point-of-care use. It also possesses two key advantages over current antibody-based assays. First, the intrinsic transduction mechanism of aTFs enables real-time and repeat sensing. Our QD-TF-FRET framework converts this transduction to an optical readout. Methods for converting this transduction into a direct electrical readout could also be developed. Second, bacterial proteins are inexpensive to produce, modify, and evolve. The specificity of the progesterone sensor could be further improved through directed evolution\textsuperscript{41}, also providing an opportunity to evolve variants specific to other steroids. More broadly, the identification of a progesterone sensing aTF highlights the diverse and largely untapped reservoir of biorecognition elements latent within bacteria. To more fully capture this diversity, our approach can be extended to uncharacterized and unculturable organisms with metagenomic screening\textsuperscript{12}, and expanded to target other biorecognition elements, including redox enzymes for electrochemical sensors\textsuperscript{3,46,47}. Our results thus provide a paradigm for the targeted development of a diverse range of sensor devices.

Methods

Materials. All DNA oligonucleotides were purchased from IDT Technologies. Progesterone (PRG), cholesterol (CHL), cortisol (CORT), aldosterone (ALD), estrone (ESN), 5β-pregnane-3α-20α-diol (PRE), 5β-pregnane-3α,20β-diol-glucuronide (PRE-Glu), and lysozyme were bought from Sigma Aldrich. Artificial urine DIN EN1616:199 was bought from Pickering Laboratories. RNAProtect Bacteria Reagent was bought from Qiagen. Proteinase K was bought from Roche. Cadmium oxide (CdO; 99.95%, Alfa Aesar), sulfur (99.95%, ACSRO Organics), 1-octadecene (ODE; 90% ACSRO Organics), and oleylamine (80–90%) were bought from Fisher Scientific and used as purchased. Zinc acetate (Zn(AC)\textsubscript{2}, 99.99%), selenium pellets (Se, 99.99%), triptycholipin (TOP, 97%), triptycholipin oxide (TOPO, 98%), oleic acid (OA, 98%), poly(isobutylene-alt-maleic anhydride)–6000 g mol\textsuperscript{-1} (PI(MA)), (2-aminoethyl)trimethylammonium chloride, histamine, triethylamine, and HEPES (4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid) were obtained from Sigma-Aldrich. HPLC-grade solvents including hexanes (Fisher Scientific), methanol (Honeywell), anhydrous dimethyl sulfoxide (Sigma Aldrich) and chloroform (J.T. Baker) were bought and used without further purification. HEPES 1× is a solution of 25 mM of HEPES and 150 mM of NaCl, adjusted to pH 7.6.

Strain selection. Pseudomonas simplex strain 6946 was purchased from ATCC and referenced with a corresponding GenBank accession number (CP009896.1). The strain is an obligate aerobe and was grown in media and conditions as recommended by ATCC.

Strain characterization. To determine the doubling time of the strain, growth curves were generated (Supplementary Fig. 18). All growth curves were done in 100 µL per well volumes in 96 well flat bottom black polystyrene TC-treated microplates which were individually wrapped with a lid and sterile (Corning). Measurements were done by an Infinite M200 Pro (TECAN) spectrophotometer at the temperature suited for P. simplex. Readings were performed over 96 cycles of 15 min each at 600 nm absorbance with 25 flashes in a 3 × 3 (XY-Line) type reads per well. In between reads there was orbital shaking at 150 rpm frequency for a total of 10 min. To first characterize the growth alone, a ½ serial dilution of 9 concentrations from 0.5–0.0020 OD\textsubscript{600} nm were prepared in the respective media.
Then each concentration was measured as previously described by the TECAN microplate reader and normalized against a media background control in technical triplicate.

Solvent exposure. Once an appropriate starting cell concentration was chosen, a secondary growth curve was performed to test the toxicity levels of the solvents used to suspend the pellets. Fifty milliliters overnight LB + kanamycin culture was diluted 1:250 of fresh LB + kanamycin, then allowed to grow to an OD600 of approximately 0.6 shaking at 37 °C. Two and a half milliliters of filter sterilized 20% v/v rhhamnose was then added to induce protein expression, and culture was shaken for 4 h at 37 °C. Culture was then removed from the incubator and stored at 4 °C overnight. Culture was then pelleted by centrifugation and frozen overnight.

Protein expression and purification. SRT1F1 coding sequence codon-optimized for expression in *Escherichia coli* was ordered from Integrated DNA technologies with appropriate upstream and downstream fusion sequences being ligated into the pRham C-His Kan vector (Lucigen), resuspended in 1× TE buffer, and 45 ng heat shock transformed into chemically competent E. coli 10 G (Lucigen) along with 25 ng of linearized pRham C-His Kan Vector (Lucigen). Cells were grown on LB agar + 50 μg mL⁻¹ kanamycin overnight at 37 °C. Resulting colonies were grown in 0.5 mL LB + kanamycin, plated purified using Qiagen MaxiPrep Kit and CDS insertion verified through Sanger Sequencing using forward primer Rham F and reverse primer pET11 R (Lucigen).

Protein was expressed in E. coli 10 G cells grown overnight from a singly colony. Five milliliters overnight LB + kanamycin culture was diluted 1:250 of fresh LB + kanamycin, then allowed to grow to an OD600 of approximately 0.6 shaking at 37 °C. Two and a half milliliters of filter sterilized 20% v/v rhhamnose was then added to induce protein expression, and culture was shaken for 4 h at 37 °C. Culture was then removed from the incubator and stored at 4 °C overnight. Culture was then pelleted by centrifugation and frozen overnight.

Protein was purified using the Ni-NTA Fast Start kit (Qiagen). Cells were lysed using 10 mL of the provided Native Lysis Buffer supplemented with 10 mg lysosome and 250 U benzonase as specified in the manufacturer’s protocol. Pellet was resuspended by stirring and pipetting up and down with a 25 mL graduated pipette. Cells were lysed on ice for 1 h, with gentle swirling every 20 min. Some preparations were highly viscous after the 1 h incubation, likely due to incomplete nuclease activity. Viscous lysates had an additional 250 U benzonase added to them and were incubated for twenty more minutes. All samples treated with additional benzonase had their viscosity reduced to normal after this additional incubation. Lysate was centrifuged at 14,000 × g for 30 min at 4 °C. Lysate supernatant was applied to pre-cleaned Ni-NTA column and allowed to run through at room temperature. Column was washed three times with 4 mL of 3 mL of Native Wash Buffer (NEB), then eluted twice with 1 mL each of Elution Buffer (NEB).

Protein was present in both elution fractions off the column, so elutions 1 and 2 were pooled for desalting. Protein was either dialyzed against the destination buffer using 0.5–3 mL 3500 MWCO Slide-a-Lyzer (ThermoFisher), twice for 2 h at 4 °C temperature with magnetic stirring, then overnight at 4 °C without stirring, or using Amicon Ultra-0.5 centrifugal filters with a 10kD MW cutoff. Elutions were added to the column 0.5 mL at a time, at spun 14,000 × g at 4 °C until only approximately 75 μL remained, around 30 min. Destination buffer was added to the 0.5 mL mark, and centrifugation repeated. This process was repeated 3 additional times, for a total of 4 times.

Destination buffer for protein used in BLI, iv-Chip-seq, and QD-TF-FRET assays was Tris-buffered saline pH 7.4. Protein concentration was quantified using a Qubit Protein Assay kit (ThermoFisher), then aliquoted and frozen at −80 °C until used.

In vitro Chip-Seq. We developed an in vitro Chip-Seq assay similar to previously published in-vitro DNA precipitation protocols.53, P. *simplex* picked from a single colony on M3 Agar was grown in M3 Broth at room temperature with gentle shaking for 3 days until culture was turbid. One milliliter aliquots of culture were pooled extracted using the QuickExtract reagent. RNA was used for RNA 6000 Pico Kit (Agilent) and run in a Bioanalyzer (Agilent). RNA samples were further immediately used for RNA-Seq library preparation or stored long-term at −80 °C.

**RNA-Seq library preparation.** After RNA samples have been quantified and analyzed they were DNase treated using a TURBO DNase 2 U 1 μL (Thermo Fisher Scientific) and cleaned using Agencourt RNAClean XP SPRI beads (Beckman Coulter). RNA-Seq libraries were then produced from these samples using a slightly modified ScriptSeq v2 RNA-Seq Library Preparation Kit (Illumina) ensuring use of unique index primers through ScriptSeq Index PCR Primers (Sets 1–4) and cleaned using Agencourt RNAClean XP SPRI beads (Beckman Coulter) and further quality bases trimmed from both ends using Cutadapt.48 Reads were aligned to the reference genome with Bowtie2.49 BAM files were sequenced and indexed using SAMtools.40 Transcript assembly and expression quantification was performed using Cufflinks.51. All resulting raw expression counts were normalized as a group using edgeR.52 A custom multal script was then used to calculate fold changes of normalized counts for each gene between each steroid exposure experiment and its corresponding vehicle control.

**RNA-Seq data analysis.** Adapter sequences were removed from reads and low quality bases trimmed from both ends using Cutadapt.48 Reads were aligned to the reference genome with Bowtie2.49 BAM files were sorted and indexed using SAMtools.40 Transcript assembly and expression quantification was performed using Cufflinks.51. All resulting raw expression counts were normalized as a group using edgeR.52 A custom multal script was then used to calculate fold changes of normalized counts for each gene between each steroid exposure experiment and its corresponding vehicle control.
BioLayer interferometry. Single stranded DNA oligos containing SRTF1 binding sites or control sequences were ordered from IDT. Forward strands with 5’ biotin were annealed to reverse strands by mixing to a final concentration of 10 mM in Annealing Buffer (10 mM Tris-HCl pH 8.0, 50 mM NaCl), heating to 95 °C, then slowly cooling to room temperature over 1 h. All BLI steps were performed in 1× BLI Buffer (28 mM Tris-HCl pH 8.0, 5 mM MgCl₂, 4.25% Glycerol, 25 mM NaCl, 1.67 mg mL⁻¹ BSA) at 30 °C in a ForteBio OctetRed96. For DNA association assays, SA tips were baseline in buffer, then dsDNA was loaded on by dipping into wells containing 75 nM DNA in buffer. DNA for shellning, a sure biase in buffer, then dipped in buffer containing varying concentrations of SRTF1 and allowed to reach equilibrium. Tips were then dipped in buffer for complex dissociation. Dissociation of DNA from the tip was controlled by subtracting the trace from a DNA-coated tip that was not exposed to SRTF1. Trace Y-Axes were aligned to the last 5 s of the second baseline step, and noise removed by Savitzky-Golay filtering. Association and dissociation data were fit to a Mass Transport model.

Hormone-induced dissociation was assayed using BLI by dipping DNA-coated SA tips in buffer containing 150 nM SRTF1 and allowing the reaction to reach equilibrium. Complex was then dipped in buffer containing varying amounts of hormone. Curve traces for each sensor were rescaled such that the binding level at equilibrium was equal to 1.

Quantum dots synthesis. Core/shell/shell quantum dots were made according to a previously reported procedure56 and is described briefly here. The precursors used for this synthesis included: 0.2 M Cd(OAc)₂, 0.2 M Zn(OAc)₂, 0.2 M sulfur in ODE, and 1 M TOP. For the sulfur and selenium precursors, the appropriate amount of anion was weighed and dissolved into either ODE or TOP at the desired concentration. Once the solutions were fully dissolved, the curators were heated under vacuum at 120 °C for at least 1 h before use to remove traces of water. For Cd(OAc)₂ and Zn(OAc)₂, CdO or Zn(Ac)₂ were weighed and added to oleic acid at a 1:4 molar ratio. The solutions were heated under vacuum at 120 °C until fully dissolved and diluted to a final concentration of 0.2 M with ODE. All precursors were stored under argon at room temperature. Both Cd(OAc)₂ and Zn(OAc)₂ are waxy solids at room temperature and were therefore heated to 120 °C for use in the QD synthesis.

For nucleation of CdSe cores, we used an air-free hot injection method. In brief, 1 g of TOPO, 8 mL of ODE, and 1.9 mL of 0.2 M Cd(OAc)₂ were loaded into a 100 mL round bottom flask (r/b) and placed under vacuum at room temperature for 30 min. The flask was heated to 80 °C and degassed by backfilling with argon and switching back to vacuum 3× over the course of 1 h. Once the solution had been sufficiently degassed, the flask was placed under argon flow and heated to 300 °C. In an argon-filled glovebox, we pre-mixed 4 mL of 1 M TOPSe, 3 mL of oleylamine, and 3 mL of ODE for injection into the Cd solution at 300 °C. The reaction temperature was set to 270 °C. After 3 mins, the flask was taken off of the heating element and allowed to cool to room temperature. The CdSe cores were precipitated from solution under air-free conditions using ethanol and methanol and re-dispersed in hexane.

For shellning, a successive ion layer adsorption reaction (SILAR) was used as previously described. In brief, 5 mL ODE and 5 mL oleyamine were added to a 100 mL r/b and heated under vacuum at 120 °C for 1 h before 200 nmol of CdSe cores in hexane were added to the flask, and the hexanes removed via low-pressure evaporation. For each shell material, a single monolayer of the corresponding surfactant was added at a time. The amount of precursor needed to add each monolayer was calculated on a volume basis using the density and lattice constants for wurtzite CdS and ZnS. For the CdS shell, 1 monolayer of CdS was added. The first Cd addition was added dropwise at 160 °C to the core solution under argon and annealed for 2.5 h. The temperature was then increased to 240 °C and the corresponding amount of sulfur precursor added dropwise and annealed for 1 h. All additional monolayers were added and annealed at 240 °C. After CdS shellning, 2 monolayers of ZnS were added in a similar fashion. After 2 full monolayers of ZnS were added, an additional layer of Zn was added to ensure that the QD surface was Zn-rich.

Polymer synthesis. The polymer capping the QDs (P1) was synthesized using a slightly modified version of a previously reported procedure57. In a typical experiment, 47.5 µL QDs (5.03 mmol, 1 equivalent) was dissolved in 3 mL anhydrous dimethyl sulfoxide at 45 °C. In parallel, 116 mg (2-aminoethyl)trimethylammonium chloride (0.66 mmol, 22 equivalents), 73 mg histamine (0.66 mmol, 22 equivalents), and 193 µl triethylaniline (1.39 mmol, 46 equivalents) were dissolved in 1.5 mL anhydrous dimethyl sulfoxide at 50 °C. After complete dissolution of both solutions, the solution containing the amine was added with a syringe to the P1 solution. The reaction was kept overnight at 45 °C. The polymer was purified by several precipitations in ethyl acetate. A white powder was obtained with 67% yield. 1 H NMR (500 MHz, D₂O): δ (ppm) = 8.33 (s, 0.35 H, imidazole), 7.14 (s, 0.52 H, imidazole), 3.61 (0.63 H), 3.37 (2.24 H), 3.08 (s, 5.14 H), 2.49 (1.26 H), 2.15 (1.01 H), 2−1 (m, 2.45 H), 0.86 (m, 6.7 H, CH₂). FTIR shows the disappearance of the C=O stretch band of the anhydride at 1770 cm⁻¹ and appearance of the C=O stretch of carboxylic acid and amide bond at 1710 cm⁻¹ and 1650 cm⁻¹, respectively.
The limit of detection (LOD) was calculated using the parameters of the fit with the non-linear equation for $y = Lx$

$$LOD = IC_{50} \times \frac{A_1 - A_2}{V_{D1} - V_{D2}} - 1$$

The 95% Confidence Interval was calculated using Origin Pro Software.

**Artificial urine assays.** Artificial urine composition: pH 6.6 ± 0.1, urea 250.0 g L⁻¹, sodium chloride 9.0 g L⁻¹, disodium hydrogen orthophosphate anhydrous 2.5 g L⁻¹, potassium dihydrogen orthophosphate 2.5 g L⁻¹, ammonium chloride 3.0 g L⁻¹, creatinine 2.0 g L⁻¹, sodium sulphite hydrated 3.0 g L⁻¹.

For the artificial urine assays, QDs, TF, and DNA were mixed together in 1x HEPEs before artificial urine and artificial urine + PRG was added such that 50% of the final volume comprised artificial urine.

Artificial urine at 37°C: the sensor was assembled at RT then artificial urine and artificial urine + PRG were added at 37°C. For tests in artificial urine following lyophilization, QDs, TF and DNA were assembled in 1% BSA for QDs and ultra-pure water (no salts) and lyophilized. The sensor was recovered in artificial urine (same volume as sublimated during lyophilization process). Then artificial urine + PRG was added to the sensor.

**Portable electronic reader.** A UV-LED (LED405E, 10 mW, Thorlabs) was attached to an SM1-threaded LED mount (S1LEDM, Thorlabs) that was screwed into one of the ports of the cuvette holder. The LED was powered with 3.3 V using an Arduino Uno. Two phototransistors (Digi-Key, 751-1057-ND) were used to detect the emitted light, one for each channel. In order to detect the Cy5 emission (670 nm), a 665 nm LP filter (Chromatech, ET655lp) was placed in front of one of the phototransistors to filter the light emitted by the Cy5 dye. In order to detect the QD emission (605 nm), a 600 nm BP filter (Edmund Optics, P/N 84875, 600 nm, FWHM 50 nm) was placed in front of the second phototransistor to filter the light emitted by the QDs.

Each one of the phototransistors was placed in a common-emitter phototransistor circuit (Supplementary Fig. 16). A DC power supply was used to power the circuit (BK Precision, 1760A). During an experiment, the voltage drop across the phototransistor was monitored with a multimeter (Agilent34410A) that was connected to LabView, which enabled real-time recording of measurements. The device was encased in a metal Faraday cage.

The LED was turned on and allowed to warm up for at least 20 min prior to the experiment. One hundred microliter of sensor with a given concentration of target was pipetted into the cuvette. The cuvette was inserted into the cuvette holder such that the 10 mm path length was parallel to the filters. Upon inserting the cuvette, a timer was started. A black cover was placed over the cuvette and a lid placed on the Faraday cage. At $t = 10, 30$ and 50 s, a Labview program was started to begin data collection for a duration of 10 s each. Each 10 s long period of data collection is counted as one technical replicate.

The following equations were used to calculate $F_{1,i}$/$F_{0,i}$ for the benchtop device:

$$F_{1,i} = \frac{Cy_{signal,i}}{QD_{signal,i}}$$

$$F_{0,i} = \frac{Cy_{signal,i}}{QD_{signal,i}}$$

where

$$QD_{signal,i} = 5 - \varepsilon$$

$$Cy_{signal,i} = 5 - \varepsilon$$

$\varepsilon =$ the concentration of prostegere in the sample $i$ = the replicate number for a given concentration (i.e., 1 is the first technical replicate, etc.)

The standard deviation of each ratio is calculated using the following equation:

$$\sigma_{Ri} = \frac{1}{\sqrt{(\frac{\sigma_{QD_{signal,i}}}{QD_{signal,i}})^2 + (\frac{\sigma_{Cy_{signal,i}}}{Cy_{signal,i}})^2}}$$

In order to calculate the error bars associated with the average of the ratio, the following equation was used:

$$\sigma_{Ri} = \sqrt{\frac{1}{N} \left(\frac{\sigma_{R1}}{\sigma_{R2}} \right)^2 + (\frac{\sigma_{R2}}{\sigma_{R3}})^2 + (\frac{\sigma_{R3}}{\sigma_{R4}})^2}$$

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

All RNA-seq data that support this paper have been submitted to the Gene Expression Omnibus with the accession number GSE141603. All iv-ChIP-seq data that support this paper have been submitted to the Gene Expression Omnibus with the accession number GSE131041. Source data from which Figs. 2c, e, 3c, e, f, g, h, ab, and Supplementary Figs. 5, 9, 10, 12, 14, and 15 are generated are located in the Source Data file. Other relevant data are available on request.

**Code availability**

Custom software, and analysis scripts will be made available on request.

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Author contributions

C.G. developed, characterized, and optimized the final progesterone QD-TF-FRET sensor. R.C.B. identified, isolated, and characterized SRTP1, its binding sites, and the mutant. U.K. developed the protocol for, and performed, bacterial screening. T.N. established the initial QD-TF-FRET framework and developed the first progesterone sensor. M.C. performed the repeat measurement experiments. M.Z. developed the low-cost electronic reader. Margaret C. contributed to the development of the QD-TF-FRET framework. P.A. contributed to the screening and developed the initial in vitro Chip-Seq protocol. X.Z. contributed to the screening. A.F. contributed to analyses and designs. Mario C. contributed to analyses and designs. S.L. contributed to the design of the QD polymer coating, C.K. co-led the project, and contributed to all analyses. M.W.G. co-led the project, contributed to all analyses, and wrote the paper.

Competing interests

The authors declare no competing interests.

Additional information

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