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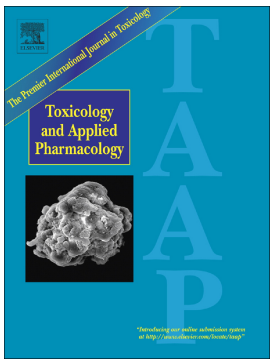

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Biosensing Estrogenic Endocrine Disruptors in Human Blood and Urine: A RAPID Cell-free Protein Synthesis Approach

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ABSTRACT: Many diseases and disorders are linked to exposure to endocrine disrupting chemicals (EDCs) that mimic the function of natural estrogen hormones. Here we present a Rapid Adaptable Portable In-vitro Detection biosensor platform (RAPID) for detecting chemicals that interact with the human estrogen receptor β (hERβ). This biosensor consists of an allosteric fusion protein, which is expressed using cell-free protein synthesis technology and is directly assayed by a colorimetric response. The resultant biosensor successfully detected known EDCs of hERβ (BPA, E2, and DPN) at similar or better detection range than an analogous cell-based biosensor, but in a fraction of time. We also engineered cell-free protein synthesis reactions with RNAse inhibitors to increase production yields in the presence of human blood and urine. The RAPID biosensor successfully detects EDCs in these human samples in the presence of RNAse inhibitors. Engineered cell-free protein synthesis facilitates the use of protein biosensors in complex sample matrices without cumbersome protein purification.

Unintentional as well as intentional discharge of harmful chemicals into the environment has been the conventional reality of industrialized society for hundreds of years. In recent decades, an increasing wealth of evidence has shown a certain class of chemicals known as Endocrine Disrupting Chemicals (EDCs) to be of concern (Gore *et al.*, 2015). Studies have detected significant levels of EDC activity in air (Holmes, 2016), soil (Aitkenhead *et al.*, 2014), drinking water (Padhye *et al.*, 2014), food (Minta *et al.*, 2013), personal care products (De Coster and van Larebeke, 2012), pharmaceuticals (Scognamiglio *et al.*, 2016), and synthetic hormones (Scognamiglio *et al.*, 2016). These studies suggest that EDC exposure likely contributes to acute and chronic conditions including cancer (Soto and Sonnenschein, 2010), diabetes (Attina *et al.*, 2016), obesity (De Coster and van Larebeke, 2012; Nalbone *et al.*, 2013), metabolic syndrome(Philips *et al.*, 2017), infertility (De Coster and van Larebeke, 2012), and permanent brain damage (De Coster and van Larebeke, 2012). A recent report estimated an EDC-exposure health burden of $340 billion USD in the United States and $209 billion USD in the EU (Attina *et al.*, 2016).

One class of EDC's known as xenoestrogens (XEs) interferes specifically with the function of estrogen receptors. XEs originate from both natural (e.g. soy plants) and unnatural (e.g. BPA) sources. Research has linked exposure to XEs with obesity (Teixeira *et al.*, 2015), birth defects (Titus-Ernstoff *et al.*, 2010) including DNA methylation and placental alteration (Vilahur *et al.*, 2014a), cancer (Morgan *et al.*, 2016), reproductive impairment (N'Tumba-Byn *et al.*, 2012), cognitive disabilities (Elsworth *et al.*, 2015; Zwart *et al.*, 2015), and developmental disorders (Vilahur *et al.*, 2014b). Thus, public chemical safety would be enhanced with rapid, reliable, and cost-effective methods to screen chemicals, environmental samples, and human/animal samples for high levels of XE activity.

Characterizing XE interactions with estrogen receptors also benefits medical technology. Pharmaceuticals called selective estrogen receptor modulators are currently used to treat a variety of conditions including infertility, breast cancer, and postmenopausal complications, and are one of the World Health Organization's "essential medicines" (Moens *et al.*, 2012; Johansen *et al.*, 2013). Rapid screening technologies for ER modulators are valuable tools in drug discovery and characterization. Detection of estrogens and their derivatives in blood and urine samples is also an important diagnostic tool (Venners *et al.*, 2006; Hormann *et al.*, 2014).

Long-standing methods for detecting XEs utilize yeast and human cell lines (Soto *et al.*, 1995; Routledge and Sumpter, 1996; Balaguer *et al.*, 1999; Legler *et al.*, 1999; Sonneveld *et al.*, 2005; Leusch *et al.*, 2010). While these are reliable and sensitive, their complicated laboratory procedures and long assay durations prohibit rapid screening and in-field detection (Alvarez *et al.*, 2013; Scognamiglio *et al.*, 2016; Conley *et al.*, 2017). LC/MS and GC/MS are likewise popular techniques, but require trained technicians and significant equipment (~$190,000) (Ye *et al.*, 2005; Tomaszewski *et al.*, 2014; Covaci *et al.*, 2015). Strategies employing biosensor proteins have been investigated in whole-cell (McLachlan *et al.*, 2011) and purified-protein formats (De *et al.*, 2005), but these methods require mammalian cell culturing and protein purification, both of which are cumbersome processes. There is a need for rapid and inexpensive methods for identifying XEs.

In a recent study, we introduced our Rapid Adaptable Portable In-vitro Detection biosensor platform (RAPID) for determining EDC activity (Salehi *et al.*, 2017). This biosensor platform relies on exploiting the basic cellular mechanism of EDC activity in the following manner. An allosterically activated fusion protein containing the ligand-binding domain of a nuclear hormone receptor (NHR) and the reporter enzyme β-lactamase is synthesized in a cell-free protein synthesis (CFPS) reaction in the presence of a sample. If the sample contains NHR binding ligands, an increase in colorimetric signal is generated real-time. Unlike many emerging biosensor technologies, the RAPID biosensor is not analyte specific. Instead, the sensor detects binding interactions with a NHR.

Our previous work demonstrated the utility of this biosensor to detect and screen EDCs that interact with the Human thyroid receptor β (hTRβ). In this work, we demonstrate the modular nature of the RAPID biosensor by replacing the hTRβ domain with that of the human estrogen receptor β (hERβ) to detect XEs. The CFPS reaction allows direct utilization of the biosensor protein without cell culture or protein purification. We further engineer the CFPS biosensing reaction using RNAse inhibitors to achieve significantly enhanced protein synthesis, and thereafter XE detection, in human blood and urine. To our knowledge, this is the first report of biosensor proteins produced in CFPS reactions engineered to overcome adverse sample matrix effects.

■ EXPERIMENTAL SECTION

Materials. All EDC ligands were purchased from Sigma-Aldrich (St. Louis, Missouri USA): diarylpropionitrile (DPN), bisphenol A (BPA), β-estradiol (E2), and TRIAC (3,3′,5-triiodothyroacetic acid).

Blood samples were purchased from Innovative Research (Novi, Michigan, USA). The human donor urine was donated anonymously by the BYU health center. Nitrocefin was purchased from Cayman Chemical (Ann Arbor, Michigan USA), and murine RNAse inhibitor was purchased from New England Biolabs (Ipswich, Massachusetts).

Biosensor Design and Construction. The pDB-MI-hERβ-β-lac construct was created in a similar manner as our previous biosensor construct (Salehi *et al.*, 2017). The pDB vector encodes for the biosensor protein complex that comprises four domains: maltose binding domain (MBD), human estrogen receptor β (hERβ), β-lactamase, and two split intein segments between MBD and hERβ, and hERβ and β-lactamase. The gene encoding this protein is under control of the T7 promoter. The construction of the vector was detailed previously (Salehi *et al.*, 2017).

Cell Extract Preparation. Cell extract was prepared as described previously (Smith *et al.*, 2014). Briefly, an inoculum of *E. coli* BL21* (DE3) strain was grown overnight in 5 mL of LB media at 37 ˚C and 280 rpm. The culture was added to 100 mL of LB media and grew until OD_{600} reached 2.0. At 2.0 OD_{600}, the 105 mL culture was transferred to 1 L of LB media in a tunair baffled flask. At 0.6 OD_{600}, isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to achieve a final concentration of 1 mM. Cells were harvested at the end of the exponential growth phase using centrifugation at 6000 RCF for 10 min at 4˚C. These were then washed with 4˚C Buffer A (10 mM Tris-acetate pH 8.2, 14 mM magnesium acetate, 60 mM potassium glutamate, and 1 mM dithiothreitol (DTT)) and then collected using an identical centrifugation cycle. Next, the pellet was re-suspended in Buffer A (1g of cell/mL of buffer A), and homogenized with 3 passes through an EmulsiFlex French Press at 20000 psi. The homogenized cells were centrifuged at 12000 RCF for 30 min at 4 ˚C to clear the lysate. The supernatant was incubated in a shaking incubator for 30 min at 280 rpm and 37 ˚C. The extract was flash-frozen in liquid nitrogen for 60 seconds before being stored at - 80 ˚C.

Cell-free Protein Synthesis Reaction. CFPS reactions took place in a 96-well plate at 37 ˚C for 2 hours. PANOxSP was used as an energy source (25% reaction volume of the cell extract, 1.2 nM plasmid, 12 to 15 mM magnesium glutamate, 1 mM 1,4-diaminobutane, 1.5 mM Spermidine, 33.3 mM phosphoenolpyruvate, 10 mM ammonium glutamate, 175 mM potassium glutamate, 2.7 mM potassium oxalate, 0.33 mM nicotinamide adenine dinucleotide, 0.27 mM coenzyme A, 1.2 mM ATP, 0.86 mM CTP, 0.86 mM GTP, 0.86 mM UTP, 0.17 mM folinic acid, 2 mM of all canonical amino acids with the exception of glutamic acid(Salehi *et al.*, 2016)).

CFPS protein yield was quantified by addition of 5 μM radiolabeled (U-14C) leucine (PerkinElmer, Waltham, MA) to the CFPS reaction. Protein solubility was determined with TCA (trichloroacetic acid) precipitation while tracking radioactive ^{14}C. Varying concentrations (0 to 200 μM) of ligands (dissolved in DMSO) were added to the CFPS reaction, and a DMSO control was used with every test. In case of human sample testing, 20% and 10% of whole blood and urine (v/v), respectively were added to the CFPS in addition to the EDC ligands.

RAPID Hormone Biosensor Assay. The biosensor assay was performed in 2 stages as described previously (Salehi *et al.*, 2017). Stage 1: CFPS of the biosensor protein in 96 well plate for 2 hours in the presence of 0 to 10 μM DPN, BPA, TRIAC, or E2 dissolved in dimethyl sulfoxide (DMSO). The presence of the hormone receptor ligand is thought to enhance correct folding of the biosensor protein, leading to increased activity upon expression. For consistency, all CFPS reactions were adjusted to have final 5% (v/v) DMSO concentration. Stage 2: After 2 hours, CFPS reactions first were diluted 13-fold into PBS buffer, of which 25 μl was transferred into each well of a UV-transparent Corning® 96 well plate. To each well, 175 μl of 228.6 μM nitrocefin in PBS was additionally added at the same time to achieve a final nitrocefin concentration of 200 μM and overall 104-fold dilution of CFPS to eliminate signal overflow. The plates were then directly quantified via plate reader (BioTek Synergy Mx) for a nitrocefin-based beta-lactamase activity assay. Specifically, the absorbance was read at 390 and 490 nm wavelengths for unreacted and reacted substrate nitrocefin, respectively. Measurements were repeated at 1 min intervals, with 10 sec shaking at each interval to mix, for 40 min. At the end of the assay, the absorbance was read at 760 nm to provide a relative background level for the assay. Nitrocefin conversion was determined for each ligand concentration at each point in time, and the data from the time point with the largest difference in nitrocefin conversion was fit to a four-parameter logistic function to obtain the effective ligand concentration (EC_{50}) (Salehi *et al.*, 2017). The log-linear response range is the logarithm of ligand concentrations that produces a linear response from the sensor.

The quality of the assays was assessed using the signal-to-noise ratio (S/N) and signal to background ratio (S/B) which were calculated using previously described methods (Zhang *et al.*, 1999; Gierach *et al.*, 2013). The limit of detection (LOD) was calculated according to IUPAC methodology which is the mean of the zero-ligand measurement added to three times its standard deviation (Vareiro *et al.*, 2005).

Detection of Endocrine Disrupting Chemicals (EDCs) in Human Samples Using Biosensor Protein. Each CFPS reaction containing EDCs and human blood or urine was diluted 8-fold with PBS. This diluted CFPS reaction was further diluted with PBS and nitrocefin mixture (final 32- and 64-fold dilution for urine and blood, respectively). The final nitrocefin concentration was 200 μM. The activity of the reporter protein (β-lactamase) was measured as stated above. RNase inhibitor was added to each reaction at 32 U per 40 uL reaction.

■ RESULTS

RAPID Biosensor Design for the hERβ. The modular, flexible nature of the RAPID biosensor is demonstrated for the first time by adapting it to detect hERβ-specific endocrine disruptors. The RAPID biosensor, as illustrated in Figure 1A, was constructed by replacing the human thyroid receptor β ligand-binding domain in our previously reported sensor (Salehi *et al.*, 2017) with the hERβ ligand-binding domain.

Cell-free Protein Synthesis of the Reporter Fusion Protein. The RAPID biosensor protein (Figure 1A) was expressed using an *E. coli*-based CFPS system. The total protein production and solubility percentage were measured by incorporating ^{14}C radiolabeled leucine as described in the method section. As illustrated in Figure 2, the 120 kD RAPID biosensor protein was expressed up to 650 μg/mL in 2 hr with an average solubility of 75%. These results were similar to the hTRβ construct which achieved 700 μg/mL in 3 hrs of protein production (Salehi *et al.*, 2017).

RAPID Biosensor Assay. The biosensor assay was performed as detailed in the methods section. Briefly, the two-step process includes: 1) expressing of the RAPID biosensor protein construct using CFPS in the sample to be tested and 2) performing nitrocefin colorimetric enzyme activity assay (Figure 1B).

Biosensor performance was evaluated in the presence of four known endocrine disrupting compounds: E2, DPN, BPA, and TRIAC (Figure 3). E2, DPN, and BPA are known XEs (EDCs for the human estrogen nuclear hormone receptor) with different binding affinities, while TRIAC is a negative control (EDC for hTRβ). All assays with XEs produced dose-response graphs with excellent quality. The EC_{50} for E2, DPN, and BPA were 124, 71, and 1443 nM, respectively, which correspond well to the values obtained in our previous bacterial biosensor studies: 150, 190, and 1700 nM, respectively (Gierach *et al.*, 2013). As expected the negative control, TRIAC, did not generate a statistically significant result, which provides an indication of the estrogenic-chemical specificity of the assay. The total time to perform the biosensor assay was 2.5 hr (2 hr performing CFPS and 30 reading colorimetric assay), which is an order of magnitude less than the analogous cell based biosensors (Skretas and Wood, 2005; Skretas *et al.*, 2007; Gierach *et al.*, 2012).

Engineering CFPS for Human Bodily Samples. To expand the applications of the biosensor, we previously showed that CFPS reactions maintain protein synthesis capability in the presence of various environmental samples including up to 45% by volume raw sewage. Although we illustrated that CFPS reactions can produce

green florescent protein (GFP) in presence of human blood and urine, the protein yield was lower in these samples, especially for urine (Salehi *et al.*, 2017).

In this work, we engineered the CFPS reaction environment to improve protein yield in the presence of urine and blood. Although only small amounts of protein are necessary for the biosensor assay, increasing the protein yield reduces the quantity of reagents required to achieve the same sensitivity.

Initially, we hypothesized that urea is the main component to hinder protein synthesis in urine samples. The urea concentration in human samples is in a range of 0.1 to 0.8 M (Putnam, 1971; Taylor and Vadgama, 1992; Shaykhutdinov, 2009) with the average being 0.22 M (Putnam, 1971). To understand the effect of urea on CFPS, we synthesized the model protein GFP in the presence of urea at concentrations between 0.001 to 1 M (Figure 4). The results revealed that 0.1 M urea doesn't have a significant effect on CFPS performance. Even reactions with 0.5 M urea retained 40% protein synthesis capability. We should point out that our previous work showed 10% urine, or about ~0.02 M urea, effectively eliminated CFPS (Salehi *et al.*, 2017). These results suggested that urea is not the main component in urine to negatively impact the CFPS.

We next hypothesized that RNase activity hinders protein synthesis in both urine and blood samples (Blank and Dekker, 1981; Iwama *et al.*, 1981; Landre *et al.*, 2002). To test this hypothesis, we performed CFPS of GFP in the presence of urine and blood, and added murine RNase inhibitor as shown in Figure 5. Results revealed significant improvement in protein synthesis. For instance, for 20% by volume urine sample, the GFP production yield increased from less than 10% to more than 30%. The improvement was more significant for blood samples, as 10% by volume blood eliminated protein synthesis, but even 45% by volume blood in CFPS containing RNase inhibitor retained 50% protein synthesis yield. The results suggest significant RNase activity in the blood samples and to a lesser extent in urine samples. Adding RNase inhibitors to CFPS reactions facilitates synthesis of sufficient biosensor protein to detect ligands in up to 25% and 45% by volume urine and blood, respectively. These higher volume percentages help to detect lower concentrations of ligands in the samples.

RAPID Biosensor Performance in Human Urine and Blood. We chose 10% and 20% by volume of urine and blood, respectively, to investigate the robustness of the RNAse-inhibited biosensor assay in the presence of these human medical samples. As illustrated in Figure 6, the biosensor assay generated a dose-response with

EC_{50} of 15 and 35 nM for E2 in presence of urine and blood, respectively. Both assays showed excellent signal to noise ratios (21 and 12 for urine and blood, respectively). The EC_{50} is lower in the presence of urine and blood (Figure 3), and this increased sensitivity could be attributed to a lower background signal as less biosensor protein was produced (Figure 5). Previously, an increase in RAPID sensitivity was also observed in the presence of raw sewage which similarly lowered the biosensor protein production yields (Salehi *et al.*, 2017). Additional engineering of the biosensor protein and CFPS reaction environment may further increase assay sensitivity.

■ DISCUSSION

This work demonstrates that CFPS detection of hERβ ligands contributes several key advantages to estrogenic EDC detection technology. First, CFPS detection of estrogenic EDCs is rapid, requiring 2.5 hours, while conventional cell-based biosensors require days or weeks of mammalian, yeast, or bacterial cell culturing before readout. The use of CFPS also removes cell culturing steps from the detection workflow and enables rapid execution. This characteristic makes the RAPID biosensor especially well-suited to high-throughput screening and portable detection applications. Already, the RAPID biosensor has been deployed from lyophilized cell-free protein synthesis components (Salehi *et al.*, 2017), an important step toward achieving a portable, shelf-stable biosensor (Hunt *et al.*, 2017). Second, CFPS detection facilitates manipulation of the biochemical detection platform. This means that the relative abundance of components can be optimized, or that disadvantageous components can be inhibited. In this work, expression of the receptor construct was enhanced by the addition of RNAse inhibitors to the reaction environment, limiting human sample matrix effects. This is significant because cell-based sensors can be adversely affected by the toxicity of the sample matrix (Gawrys *et al.*, 2009). CFPS biosensors naturally overcome sample toxicity effects because they lack viable cells. Lastly, CFPS detection is potentially very economic. Conventional protein biosensors require protein expression followed by expensive purification. In contrast, no protein purification is necessary to deploy the RAPID biosensor. The detection limits of the RAPID biosensor are comparable to or better than a similar bacterial cell-based mechanism (Skretas *et al.*, 2007). Nevertheless, yeast and mammalian cell-based assays generally provide higher sensitivity detection of estrogenic EDCs (Cevenini *et al.*, 2017). Yet, coupling the RAPID biosensor with sample concentration techniques could produce a very rapid and sensitive detection tool.

■ CONCLUSION

Here we expanded the application of our RAPID biosensor to detect chemicals that interact with the human estrogen receptor β (XEs). Specifically, we demonstrated the modular, flexible nature of this biosensor can be exploited to expand the RAPID biosensor to additional nuclear hormone receptors and the detection of other types of endocrine disrupting chemicals. The RAPID biosensor has the same level of sensitivity as the analogous *E. coli* cell-based assays and many other reported cell-based methods, but achieves this result in a fraction of the time needed for these assays. Finally, we demonstrated for the first time that the RAPID biosensor can detect EDCs directly in human blood and urine matrices, without sacrificing sensitivity, by adding RNAse inhibitors to the biosensor assay.

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■ Figure Captions

Figure 1. Scheme of RAPID biosensor assay and its mechanism of action. A) Estrogen receptor β RAPID biosensor construct. The conformation of the human estrogen receptor β changes upon interaction with hERβ-specific ligands and the reporter enzyme (β-lactamase) is activated. B) The RAPID biosensor assay. The biosensor assay can be performed in the presence of human bodily samples (urine and blood). The presence of

estrogen hERβ-specific ligands in the sample triggers a color change in the assay, which can be observed visually or more accurately measured using a spectrometer.

Figure 2. CFPS of the RAPID biosensor protein. Protein production yield increases with increasing reaction time. The solubility level remains relatively constant with increasing protein concentration. Bars represent total protein yield and dashed line represents solubility percentage. The error bars represent one standard deviation for n=3.

Figure 3. Dose-response graphs and statistical analysis results for the Estrogen receptor β RAPID biosensor in the presence of E2, DPN, BPA, and TRIAC (negative control, normalized based on the E2 maximum response). The EC_{50} represents half-maximal effective concentration, k is the slope factor, S/N is the signal to noise ratio, S/B is the signal to background ratio, and LOD is the limit of detection. The solid line represents fitted nitrocefin conversion values, the square markers represent the measured values, and the error bars represent one standard deviation for n=2. The concentrations over which the sensor exhibits a log-linear response are 30-1000 nM, 300-10000 nM, and 10-700 nM for E2, BPA, and DPN, respectively.

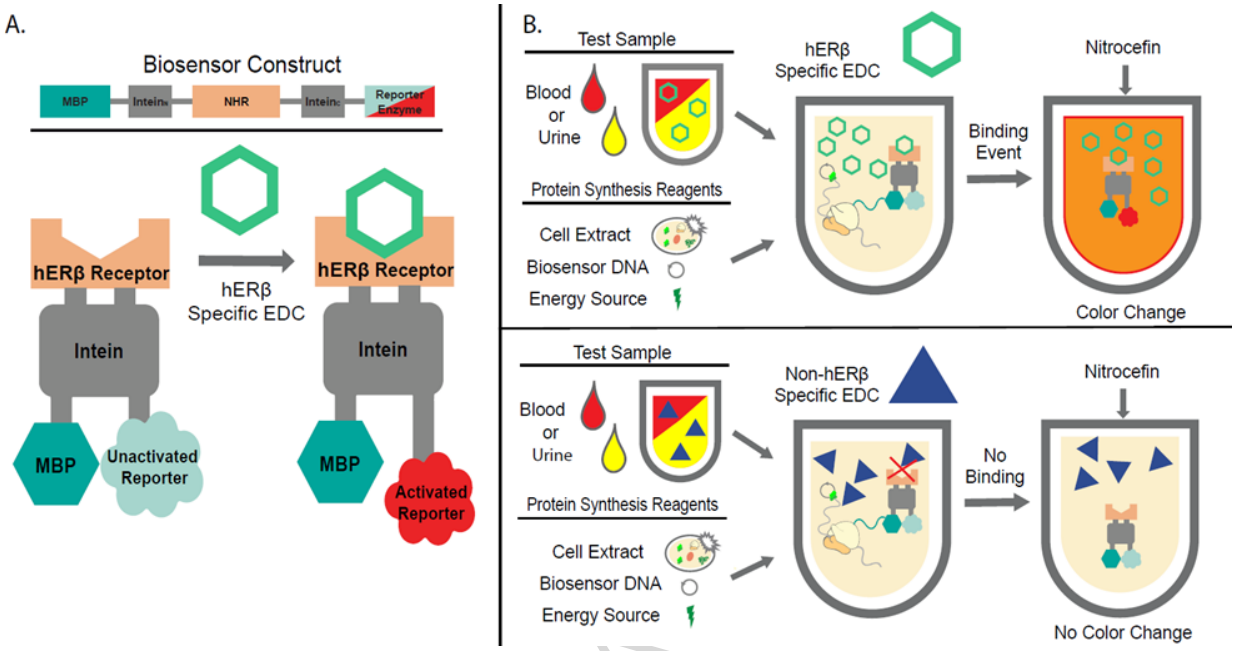
Figure 4. Effect of urea on CFPS of GFP. Error bars represent one standard deviation for n=3.

Figure 5. Effect of RNase inhibitor on CFPS of GFP in the presence of urine (top) and blood (bottom). The blue bars in both graphs represent the improvements in protein production in the presence of urine or blood with the addition of RNase inhibitor. The error bars represent one standard deviation for n=3.

Figure 6. Dose-response graph and statistical analysis results for the RAPID biosensor in the presence of E2 and 20% by volume blood and 10% by volume urine. RNAse inhibitors were added to both reactions. The solid line represents fitted nitrocefin conversion values, the square markers represent the measured values, and the error bars represent one standard deviation for n=2. The concentrations of E2 over which the sensor exhibits a log-linear response are 4-100 nM, and 8-300 nM, in urine and blood, respectively.

■ Figures

Figure 1:



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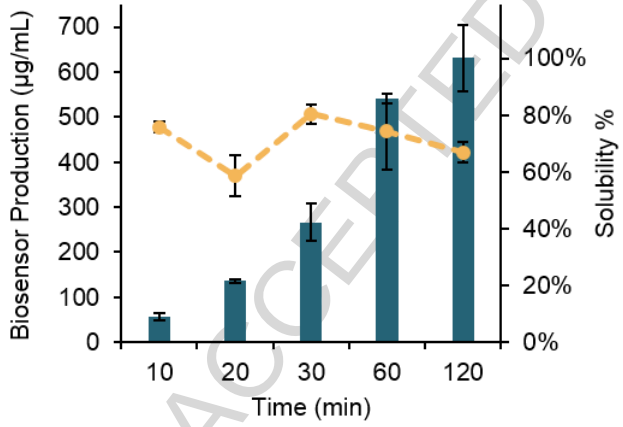

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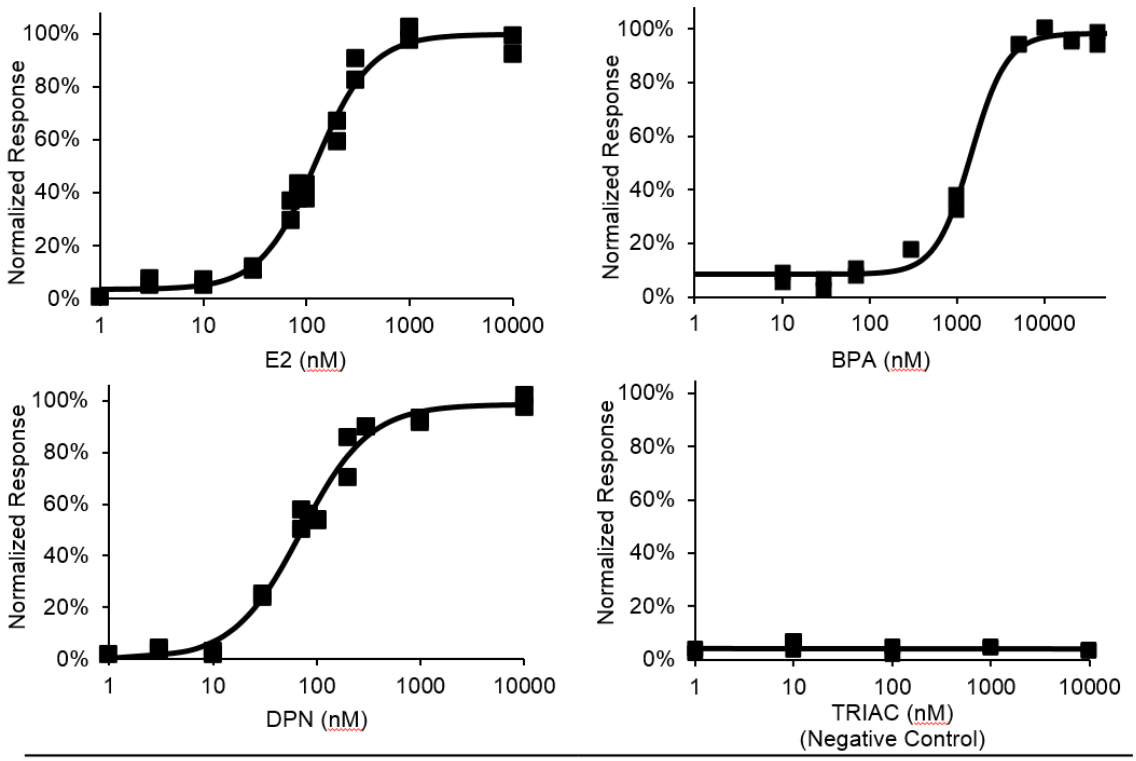
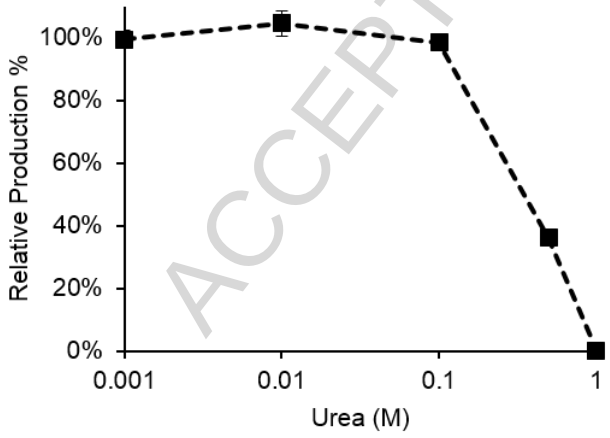


Ligand	EC50 (nM)	k	S/N	S/B	LOD (nM)	Ligand	EC50 (nM)	k	S/N	S/B	LOD (nM)
E2	124	-1.6	179	3.1	26	**BPA**	1443	-2.1	32	2.1	330
DPN	71	-1.3	36	2.9	9	**TRIAC**	N/A	-0.1	0.3	1.1	N/A

Figure 4:



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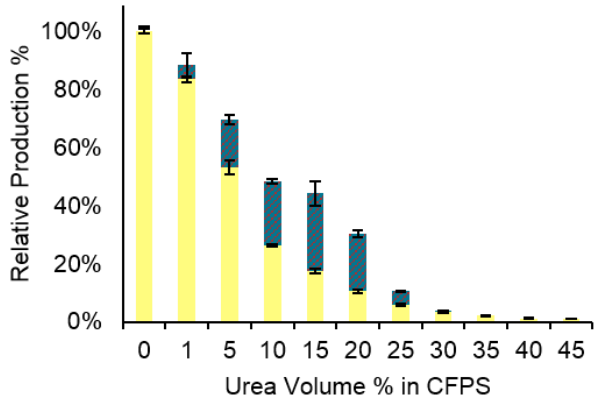

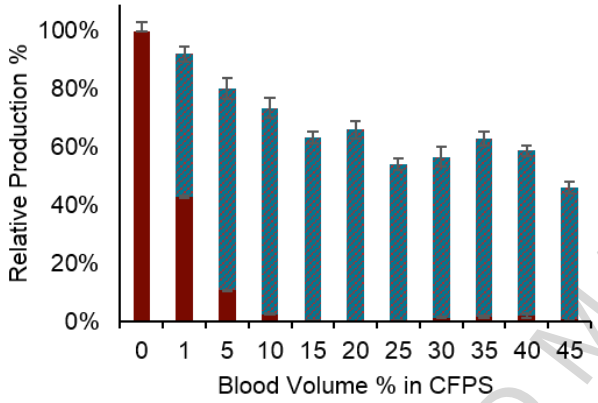

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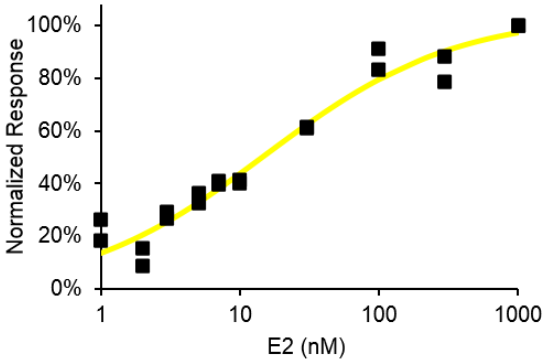
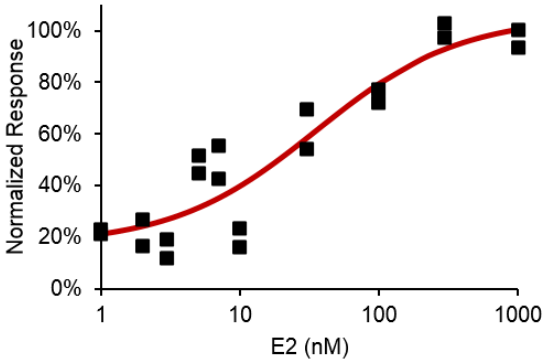




Sample	EC50 (nM)	k	S/N	S/B	LOD (nM)
Urine	15	-0.6	21	1.5	4
Blood	35	-0.8	12	1.5	8

Conflict of Interest Statement October 11, 2017

"Biosensing Estrogenic Endocrine Disruptors in Human Blood and Urine: A RAPID Cell-free Protein Synthesis Approach"

The authors report funding from NIH, NSF, and DARPA, and two patents pending regarding detection of endocrine disrupting chemicals using cell-free protein synthesis. The authors have no other conflicts of interest to declare.

Specifically:

Dr. Salehi reports grants from NSF, grants from DARPA, during the conduct of the study.

Dr. Yang reports grants from NSF, grants from DARPA, during the conduct of the study; .

Conner Earl reports grants from NSF, grants from DARPA, during the conduct of the study.

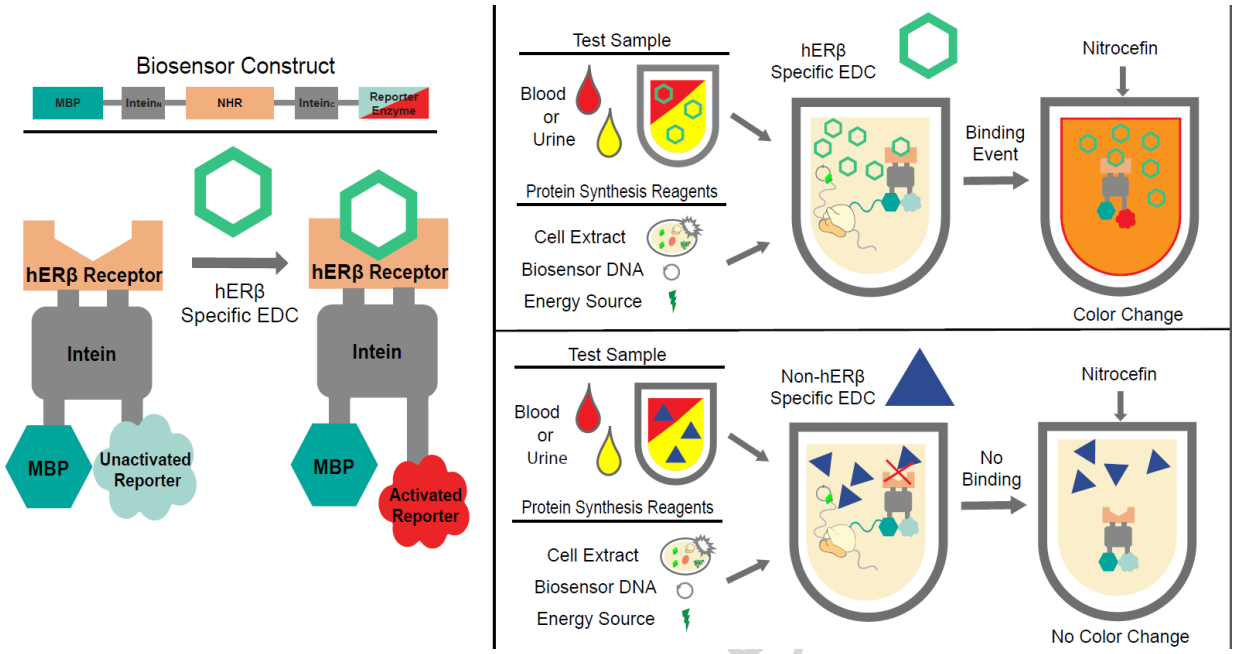
Dr. Shakalli Tang reports grants from NIH, during the conduct of the study.

Porter Hunt reports grants from NSF, grants from DARPA, during the conduct of the study; In addition, Dr. Hunt has a patent Transcriptionally activated cell-free protein synthesis biosensor for nuclear hormone receptor ligands. pending.

Dr. Smith reports grants from NSF, grants from DARPA, during the conduct of the study; In addition, Dr. Smith has a patent Cell-free protein synthesis Approach to Biosensing hTRbeta-specific endocrine disruptors pending.

Dr. Wood reports grants from NIH, during the conduct of the study; In addition, Dr. Wood has a patent Cell-Free Protein Synthesis Approach to Biosensing hTRβ-Specific Endocrine Disruptors pending, and a patent Bacterial Ligand-Binding Sensor issued.

Dr. Bundy reports grants from NSF, grants from DARPA, during the conduct of the study; In addition, Dr. Bundy has a patent Cell-Free Protein Synthesis Approach to Biosensing hTRβ-Specific Endocrine Disruptors pending, and a patent Transcriptionally activated cell-free protein synthesis biosensor for nuclear hormone receptor ligands. pending.



Graphical abstract

"Biosensing Estrogenic Endocrine Disruptors in Human Blood and Urine: A RAPID Cell-free Protein Synthesis Approach."

Highlights

- Cell-free protein synthesis (CFPS) detection of estrogenic endocrine disruptors.
- CFPS in blood and urine improved with addition of RNAse inhibitors.
- Rapid CFPS detection of the E2 in human blood and urine.