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A Cell-free Protein Synthesis Approach to Biosensing hTRβ-Specific Endocrine Disruptors

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ABSTRACT: Here we introduce a Rapid Adaptable Portable In-vitro Detection biosensor platform (RAPID) for detecting ligands that interact with nuclear hormone receptors (NHRs). The RAPID platform can be adapted for field use, allowing rapid evaluation of endocrine disrupting chemicals (EDC) presence or absence in environmental samples, and could also be applied for drug screening. The biosensor is based on an engineered, allosterically-activated fusion protein, which contains the ligand binding domain from a target NHR (human thyroid receptor β in this work). In vitro expression of this protein using cell-free protein synthesis (CFPS) technology in the presence of an EDC leads to activation of a reporter enzyme, reported through a straightforward colorimetric assay output. In this work, we demonstrate the potential of this biosensor platform to be used in a portable "just-add-sample" format for near real-time detection. We also demonstrate the robust nature of the cell-free protein synthesis component in the presence of a variety of environmental and human samples, including sewage, blood, and urine. The presented RAPID biosensor platform is significantly faster and less labor intensive than commonly available technologies, making it a promising tool for detecting environmental EDC contamination and screening potential NHR-targeted pharmaceuticals.

Biosensors can be life-changing devices, with uses ranging from daily glucose monitoring for diabetes patients to the rapid detection of toxins in the environment[1,2]. When biosensors provide the required degree of specificity and sensitivity in combination with more rapid assay times, they are excellent alternatives to traditional detection methods[3]. Biosensing systems are available in various formats, from cell-based systems with complex metabolic pathways to less complex *in vitro* systems. Cell-based systems can have a broader spectrum of detection capabilities, however, they are hindered by transmembrane transport limitations, the need to maintain cell viability and stability, time-consuming preparation, and protracted assay times[4,5]. In contrast, *in vitro* methods are commonly faster, more straightforward, simpler to store, and less expensive. Here we present a versatile, near-real time *in vitro* biosensor for detecting ligands that bind nuclear hormone receptors (NHRs)

NHRs help regulate vital functions of the cells and organisms, such as metabolism, homeostasis, differentiation, development, and reproduction[6-8]. NHRs interact with many natural and synthetic ligands and about 4% of all currently marketed therapeutics interfer with the activity of one or more NHRs[9]. NHRs also can interact with environmental endocrine disrupting chemicals (EDCs), which have become a public safety concern due to their ability to disrupt naturally occurring endocrine control. EDCs affect the endocrine system in humans and animals, commonly by mimicking natural hormones and binding to specific NHR ligand binding domains[10]. Examples of these include medical and industrial xenoestrogens, such as diethylstilbestrol and bisphenol-A, as well as naturally occurring phytoestrogens, such and genistein and daidzein[8,11,12]. EDCs have been found in common dietary, environmental, and household chemicals and have been linked to diverse diseases and disorders, including multiple cancers, developmental disorders, and other epigenetic dysfunction[13,14]. Unfortunately, due to their large numbers, most chemicals and mixtures in commerce worldwide remain largely uncharacterized for endocrine disrupting activity[15].

In order to deliver faster detection of NHR-ligand interactions, we previously developed an EDC biosensor platform where the presence of an EDC is reported through a change in growth phenotype of an engineered *Escherichia coli* strain[16,17]. This platform relies on a multi-domain engineered allosteric fusion protein, which reports ligand binding to a given NHR through the activation of a fused thymidylate synthase reporter enzyme. In practice, the biosensor protein is constitutively expressed in an engineered *E. coli* thymidine-auxotroph strain, leading the growth phenotype of the strain to be dependent on the presence of an NHR-targeting ligand. Binding of the ligand to the NHR ligand binding domain activates the thymidylate synthase reporter enzyme and enables cell growth, allowing the presence and activity of a specific NHR ligand to be readily ascertained by a simple turbidity measurement after overnight incubation. A critical aspect of this multi-domain biosensor protein is that it is modular, potentially allowing new biosensors based on alternate human and animal NHRs to be generated by

swapping NHR ligand binding domains[18-20]. However, the system still relies on bacterial growth phenotypes for activity quantification, and thus requires a minimum overnight incubation to produce a sufficient signal. Also, this assay and other cell-based assays (i.e. bacterial, yeast, and mammalian) for detecting NHR-binding ligands can be affected by the presence of cytotoxic chemicals in samples and poor cellular uptake rates.

In this work, we introduce the *R*apid *A*daptable *P*ortable *In-vitro D*etection biosensor (RAPID). This assay system combines our existing multi-domain biosensor protein design with rapid and efficient CFPS technology to overcome specific limitations of both *in vitro* and cell-based assays. In this system, the biosensor fusion protein is expressed using a CFPS system in the presence or absence of an unknown EDC sample. An engineered reporter enzyme domain on the biosensor protein exhibits ligand-dependent activity, resulting in a simple, colorimetric readout. Unique CFPS characteristics, including its chemically accessible reaction environment, robustness, scalability, and control[21-23], make this technology a powerful biosensing platform for both simple and complex detection applications. In addition, the ability to lyophilize the CFPS components enables this type of biosensor to be stockpiled for emergencies and biothreat situations. Further, the robustness of the sensor design and simplicity of its visual readout could facilitate field-deployment, where assays of environmental samples could be carried out by minimally trained personnel in the absence of any conventional laboratory equipment. By leveraging the advantageous traits of CFPS, we have generated a highly practical and effective CFPS biosensor for uses in detecting toxic EDCs, as well as potentially valuable therapeutics against this important drug target class.

EXPERIMENTAL SECTION

Materials. The ligands used for this paper, 3,3',5-triiodothyroacetic acid (TRIAC, 95%), 17-β-estradiol (E2), and 3,3′,5-triiodo-L-thyronine sodium salt hydrate (T_3, 95%), were purchased from Sigma-Aldrich.

Biosensor Design and Construction. The pET-based plasmid encoding the biosensor protein (MBP-I_N-hTRβ-I_C-βlac as illustrated in Figure 1A) is based on our previously reported biosensor design for thyroid receptor (TR) ligands[18]. The biosensor fusion protein was inserted into the DHFR control plasmid supplied with the PureExpress® *In Vitro* Protein Synthesis Kit (New England Biolabs), which includes a T7 promoter to regulate expression of the target protein. Construction of the biosensor gene was accomplished by stepwise insertion of DNA segments encoding the maltose-binding domain (MBP), the intein-human TR fusion (I_N-hTRβ-I_C), and the β-lactamase reporter protein (β-lac), where the resulting biosensor fusion gene replaces the DFHR expression control gene. In this case, the MBP was taken from the commercially available pMal-c2 expression vector (New England Biolabs), the I_N-hTRβ-I_C segment was taken from our previously reported TR biosensor plasmid[18], and the β-lac reporter protein was taken from a previously reported intein fusion expression plasmid[24].

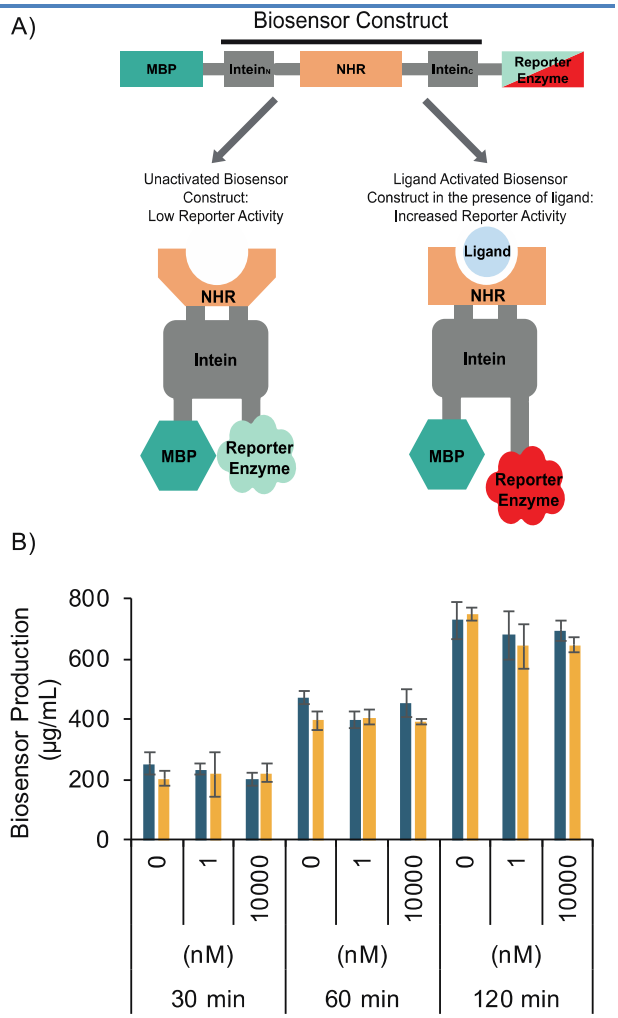


Figure 1. (A) The protein construct for the RAPID biosensor. It includes the Maltose Binding Protein (MBP) at its N-terminus, a mini-intein splicing domain with an inserted NHR ligand binding domain (from hTRβ in this work), and a C-terminal reporter enzyme (β-lac in this work).The presence of ligand during expression of the protein changes the structure of the biosensor and improves accessibility of the reporter enzyme. (B) CFPS of the biosensor fusion protein with protein production yields reported for increasing reaction times and in the presence of three levels of the ligand T3 (total protein = dark bars, soluble protein = light bars, reaction volume was 20µl). The error bars represent one standard deviation and n=3.

Cell Extract Preparation. Cell extract preparation was performed as previously described[25]. Briefly, 5 ml of LB media was inoculated using *E. coli* BL21.DE3* strain in a cell culture tube. The culture was incubated overnight at 37 °C while shaking at 280 rpm. The culture was transferred to 100 ml LB media and upon reaching OD 2.0, it was transferred to 1 liter LB media in Tunair flask. T7 RNA polymerase was overexpressed by inducing the culture with 1 mM Isopropyl β-D-1-thiogalactopyranoside (IPTG) at OD 0.6. The cells were harvested at the end of the exponential phase by centrifugation at 6000 RCF for 10 min at 4 °C. The cells were washed by suspending in pre-chilled Buffer A (10 mM Tris-acetate pH 8.2, 14 mM magnesium acetate, 60 mM potassium glutamate, and 1 mM dithiothreitol (DTT)), and subsequently centrifuged at 6000 RCF for 10 min 4 °C. The cells were resuspended in 1 ml Buffer A per gram

cells and homogenized using EmulsiFlex French Press homogenizer at 20000 psi. The lysate was clarified by centrifugation at 12000 RCF for 30 min at 4 °C. The supernatant was incubated at 37 °C for 30 min while shaking at 280 rpm, flash frozen in liquid nitrogen, and then stored at -80 °C for later use as cell extract for CFPS.

Lyophoilizing biosenoser system. For lyophilized biosensor systems, CFPS reagents were mixed and lyophilized as described previously[26,27] with slight modifications including that all reagents necessary for CFPS were combined and lyophilized together. Briefly, CFPS components were added to a prechilled tube in the following order while the tube rested on the ice: deionized water, magnesium glutamate, PANOxSP, and lastly the plasmid. The reaction mixture was mixed gently and transferred to 1.5 ml Eppendorf tubes in 250 µl aliquots. Tubes was quickly placed in liquid nitrogen container to flash freeze the reaction. The samples were lyophilized using FreeZone 2.5 Liter Benchtop Freeze Dry System (LABCONCO, Kansas City, MO) with the operation condition of -50°C and <120 mTorr for 8 hr.

Cell-free Protein Synthesis Reaction. The CFPS reactions were performed in 96 well plate using PANOxSP system for 20 to 180 min at 37 °C[28]. The reactions contained 25 volume percent cell extract, 1.20 nM plasmid and following components all from Sigma-Aldrich (St. Louis, MO): 10 to 15 mM magnesium glutamate, which was optimized based on the extract, 1 mM 1,4-Diaminobutane, 1.5 mM Spermidine, 33.33 mM phosphoenolpyruvate (PEP), 10 mM ammonium glutamate, 175 mM potassium glutamate, 2.7 mM potassium oxalate, 0.33 mM nicotinamide adenine dinucleotide (NAD), 0.27 mM coenzyme A (CoA), 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 the canonical amino acids except glutamic acid. For experiments requiring measurement of protein production yield using a scintillation counter, 5 µM l-[U-14C] Leucine (PerkinElmer, Waltham, MA) was added to the reaction, and protein yield was calculated based on total and washed counts described previously[29].

Environmental and Human Samples Tested in Cell-free Protein Synthesis. Tap water, storm water, and pond water were collected at various locations in Utah County, USA. Soil and snow samples were collected in Salt Lake County, Utah, where soil samples were extracted into ddH_2O at a one to one (weight to volume) ratio. All of the wastewater treatment samples were collected from the Provo city water reclamation facility. Raw sewage was influent of the plant. Post clarifier sample was after primary sedimentation basins. Post biological sample was the effluent of aeration basins with activated sludge. Post filter sample was the activated sludge process effluent (final clarifier effluent) passed through anthracite filters. The effluent sample was the final product of the plant after chlorination and dechlorination treatments. Single donor human whole blood-Na Heparin sample was obtained from Innovative Research (Peary Ct, Novi, MI). Urine samples were obtained from volunteers.

Hormone Biosensor Assay. The Hormone biosensor assay was performed in 2 stages. Stage 1: CFPS of the biosensor protein in 96 well plate for 20 min in the presence of 0 to 10 µM TRIAC, T3, or E2 dissolved in Dimethyl sulfoxide (DMSO). For consistency all CFPS reactions were adjusted to have 5 volume percent DMSO. Stage 2: After 20 min, the reactions were diluted 104-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 to the wells at the same time to achieve a final nitrocefin concentration of 200 µM. The plates were then directly quantified via plate reader (BioTek Synergy Mx) for a nitrocefin-based beta-lactamase activity assay [30]. 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 15 min. At the end of the assay, the absorbance was read at 760 nm to provide a relative background level for the assay. The rate of nitrocefin conversion was determined at each ligand concentration using the time course measurements, and the resulting rates were used to determine the half maximal effective ligand concentration (EC50).

Analysis of Hormone Biosensor Assay Results. The nitrocefin conversion value (NCV) was calculated using Equation 1. The A_{390} is λ_{max} of the yellow substrate nitrocefin, while A_{490} is the λ_{max} of the red nitrocefin conversion product, and A_{760} is background absorbance of each well. In order to maximize the signal-to-noise ratio, the time point with the maximum difference between the NCVs of the negative control (zero ligand) and maximum ligand concentration was selected to calculate the dose-response curves. The Four-Parameter Logistic Function (Equation 2) was fitted to this data to yield the half maximal effective concentration (EC_{50})[31]. Parameters "a" and "b" define lower and upper plateau value of the function, respectively, while "k" is the slope factor.

$$\text{Nitrocefin Conversion Value (NVC)} = \frac{A_{490} - A_{760(median\ of\ all\ reaction\ wells)}}{A_{390} - A_{760(median\ of\ all\ reaction\ wells)}} \quad (1)$$

$$\text{Predicted NVC} = a + \frac{b-a}{1+\left(\exp\left(k(\log(ligand\ concentration) - \log(EC_{50}))\right)\right)} \quad (2)$$

To generate percentage dose-response graphs, values and predicted values from the fitted function were normalized based on the equation 3.

$$\text{Normalized Dose Response} = \frac{(NCV) - Min(NCV)}{Max(NCV) - Min(NCV)} * 100\% \quad (3)$$

The overall quality of the assays was assessed using Z' factor, signal-to-noise ratio (S/N), and signal to background ratio (S/B) parameters. The parameters were calculated using a previously described method[19,32]. Also, the limit of detection (LOD) was calculated based on IUPAC methodology by finding the corresponding concentration value for blank measurement added to its three times standard deviation[33]

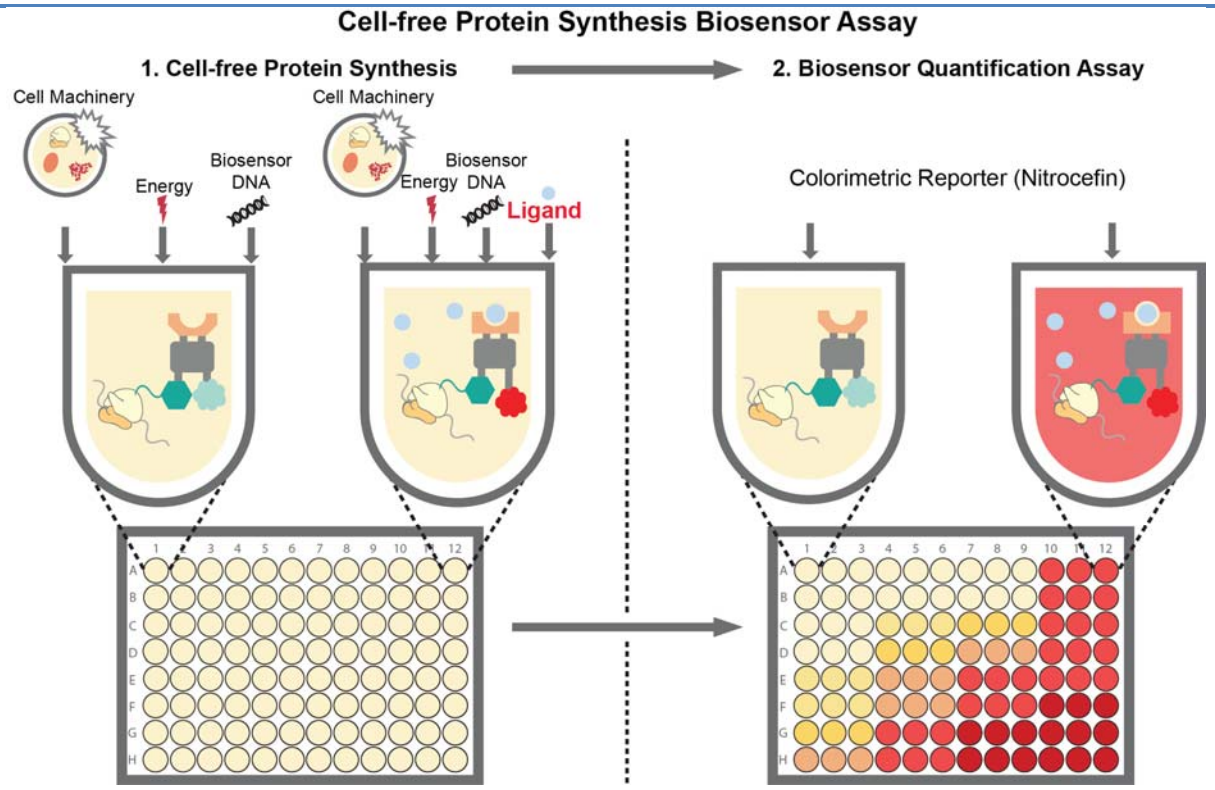


Figure 2. Scheme of the RAPID biosensor to detect chemicals that target NHRs. The biosensor assay includes two steps: 1- CFPS reaction to produce the biosensor, 2- colorimetric assay to quantify the biosensor protein activation. The presence of ligand during protein synthesis activates the biosensor by altering the conformation of biosensor enzyme and increasing the nitrocefin assay signal.

■ RESULT AND DISCUSSION

RAPID Biosensor Design and Rationale. Here we report the RAPID (*R*apid *A*daptable *P*ortable *In-vitro D*etection) biosensor for NHR-binding ligands. The goal of this work was to create a near real-time biosensor platform by combining our previous cell-based allosterically activated, fusion protein approach[18] with the open flexibility of CFPS systems[34]. The fusion protein consists of four domains including: 1) maltose binding domain, which improves the solubility of the fusion protein[16]; 2) mini-intein domain, which acts as a stabilizing domain for the NHR domain[16]; 3) NHR ligand binding domain, which is the heart of biosensor and acts as a switch to activate the reporter enzyme; and 4) the reporter enzyme. An interaction between a ligand/chemical and the NHR ligand binding domain causes a conformation change which results in improved reporter protein activity as preciously described[35]. Hence, a signal results from the presence of a chemical/ligand that binds the NHR ligand binding domain during protein synthesis (Figure 1A, 2). Due to the cell-free nature of CFPS, there is no membrane transfer limitation for chemicals that might target NHRs[36], while the direct translation of the sensor protein provides a fast, inexpensive, and convenient assay for the presence of EDC activity in unknown test chemicals.

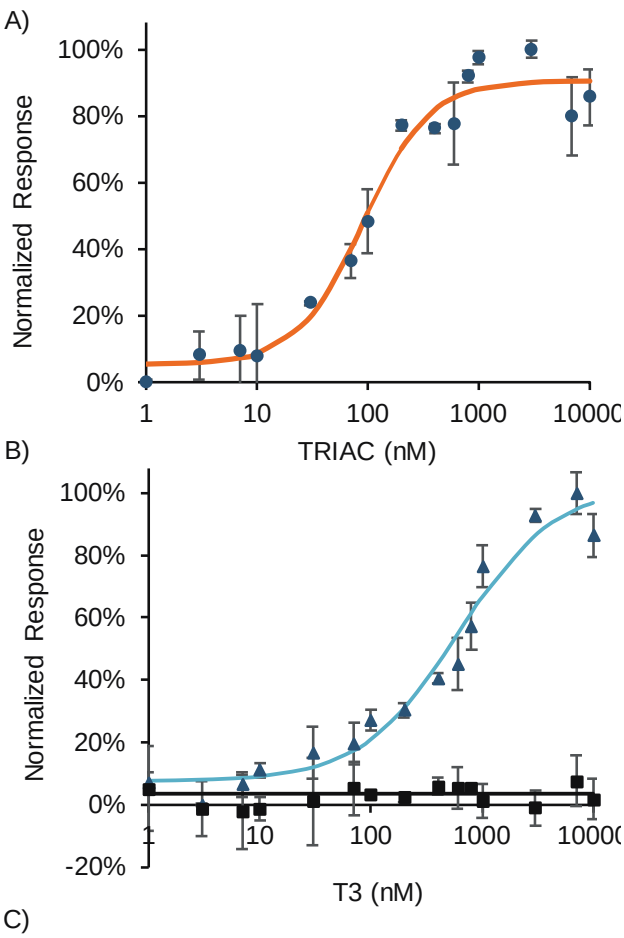
The initial step in creating the CFPS-based RAPID biosensor was to re-engineer the reporter protein domain for a rapid and straightforward colorimetric assay readout. Our previous bacterial biosensor platform employed the thymidylate synthase reporter enzyme to enable growth phenotype changes[16]. Unfortunately, *in vitro* assays for thymidylate synthase activity are cumbersome and require oxygen-sensitive reagents. For these reasons, the β-lactamase (β-lac) enzyme was selected to replace the thymidylate synthase enzyme due to its similarity in size and commercially available colorimetric activity assay.

To characterize our RAPID biosensor, the human thyroid receptor β (hTRβ) was chosen for the initial ligand binding domain due to its robust behavior in our bacterial biosensor[18]. It also has high sensitivity and selectivity to TRIAC, a potent agonist, with a half-maximal effective concentration value (EC_{50}) reported at 70 nM. Cloning work to incorporate the β-lac reporter and hTRβ ligand binding domain into the fusion protein is described in the methods section, with the final fusion protein sequence illustrated in Figure 1A.

Cell-free Protein Synthesis of the Reporter Fusion Protein. The resulting fusion protein, containing the hTRβ ligand binding domain and β-lac, was expressed in an *E. coli*-based CFPS system as detailed in the methods section. To elucidate the mechanism of activation, total protein titer and protein solubility were measured by tracking the incorporation of C-14 radiolabeled leucine (Figure 1B). The 92 kD MBP-I_N-hTRβ-I_C-β-lac fusion protein was expressed at yields up to 700 μg/mL in 3 hr and the expression level was unaffected by the presence of T3 ligand (Figure 1B). Also, the protein solubility yields were consistently greater than 85% (Figure 1B).

Hormone Biosensor Assay. The hormone biosensor assay consists of two steps as illustrated in Figure 2. First, cell-free expression of the MBP-I_N-hTRβ-I_C-β-lac reporter fusion protein is performed in the presence of the sample to be tested. The resulting protein is then subjected to a colorimetric reporter enzyme activity assay, where NHR-ligand binding is reflected in

the activity of the reporter enzyme domain (β-lac). The hormone sensing capability of this assay was assessed with 3 known endocrine disrupting chemicals; two chemicals that are known to target hTRβ (TRIAC and T3), and a negative control (estrogen) that targets the human estrogen receptor NHR but does not target hTRβ. The results are reported in Figure 3, where the EC_{50}, Z' factor, signal-to-noise ratio (S/N), and signal to background ratio (S/B) are calculated for each chemical. The Z' factor was between 0.5 to 1 for all assays, indicating "an excellent assay" for screening and sensing[19,32]. The measured EC_{50} for TRIAC and T3 were 90 and 607 nM, respectively, which correspond well to the EC_{50} from our previous studies with the bacterial biosensor, 70 and 580 nM respectively (Figure 3)[18]. Also, the calculated LOD were 48 and 75 nM, respectively for TRIAC and T3. As expected, a statistically significant signal was not observed with the estrogen negative control (Figure 3B, Square markers, p-value of 0.84). TRIAC was 7-fold more potent than T3 against TRβ which is similar to our bacterial biosensor at 8-fold and other reported sensors at 6-fold[37]. Although some *in vitro* binding and transactivation assays can detect ligands with higher sensitivity, the simplicity, speed, and the lack of toxicity or cell-uptake complications make the RAPID system a strong candidate for screening of NHR-binding ligands[37-39].



C)

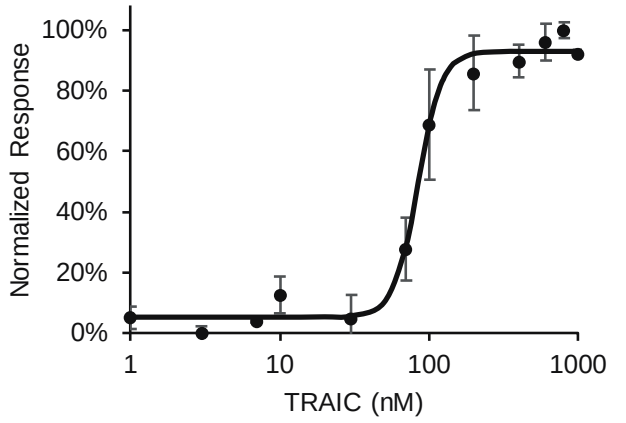
Ligand	EC50 (nM)	k	Z'	S/N	S/B
TRIAC	90	-1.5	0.75	8	1.8
T3	607	-1.0	0.61	23	3.1
E2	N/A	N/A	0.33	0.26	1.0

Figure 3. A) Dose-response curve for the hTRβ biosensor in the presence of TRIAC. B) Dose-response curves of the hTRβ biosensor in the presence of T3 (triangles), and E2 (squares). C) The half-maximal effective concentration (EC50), slope factor (k), Z' factor, signal to noise ratio (S/N), and signal to background ratio (S/B) for the responses against TRIAC, T3, and E2. The solid lines represent fitted nitrocefin conversion values, the markers represent the average measured values, and the error bars represent one standard deviation for n=2.

One considerable strength of our RAPID biosensor is speed of the assay, with the total time needed to generate clear results being less than 30 min. Alternatively, mammalian-based assays may take days to weeks to complete and bacterial-based assays take 24-36 hours[18,40]. Another strength of the cell-free system is the elimination of confounding issues associated with membrane transport of test chemicals, unknown or unintended side effects related to cell growth or toxicity, or cross activation of NHRs[34]. In contrast to other *in vitro* techniques, a further advantage of our system is that there is no need for any purification or complex enzyme stabilization steps[41]. Furthermore, the

modular nature of the fusion protein opens the possibility of optimizing the system by rapidly incorporating new reporter enzymes, while also expanding the RAPID biosensor to include diverse nuclear hormone receptors for human and animal applications[42].

Lyophilized Biosensor. To develop our RAPID biosensor platform for potential field use (*i.e.* outside of the laboratory), we assessed the possibility of lyophilizing the CFPS biosensor components. Previously, we reported lyophilized cell extracts remained active after 90 days of storage at room temperature, and demonstrated the potential for CFPS to be used in biotherapeutic protein production[43]. For this work, all essential elements, including cell extract, plasmid encoding the fusion protein, and necessary small molecule additives were combined and lyophilized at the same time, to create a "just-add-sample" CFPS assay. The results illustrate that lyophilized CFPS performed similarly to freshly prepared CFPS in detecting TRIAC (85 nM EC50, -5.5 k, 0.81 Z', 35 S/N, 1.6 S/B, 59 nM LOD) (Figure 4). Thus, the RAPID biosensor has the potential to be used as a field assay for *in situ* real-time detection of EDCs in essential infrastructure, such as watersheds.



Ligand	EC50 (nM)	k	Z	S/N	S/B
TRIAC	85	-5.5	0.81	35	1.6

Figure 4. Dose-response graph and statistical analysis results for the RAPID biosensor with lyophilized CFPS components in the presence of TRIAC. The solid line represents fitted nitrocefin conversion values, while circle markers represent the measured values. Error bars represent one standard deviation for n=2.

CFPS Performance in Different Environmental Samples. To understand the utility of this new NHR biosensor for evaluating environmental samples, we tested the performance of the CFPS system – a sensitive component of the RAPID biosensor – in various untreated water sources, raw sewage, and human bodily fluids (Figure 5). For all of the samples, CFPS produced a model protein GFP at sufficient protein production levels necessary for the biosensor assay. The water samples (tap, pond, snow, storm) and samples from various stages of a wastewater treatment plant did not significantly effect CFPS levels, with the exceptions being raw sewage wastewater and post clarifier wastewater. However, even after adding 47% by volume raw sewage or post clarifier wastewater to CFPS reactions, greater than 50% of protein production level was maintained. The robustness of CFPS across diverse environmental samples indicates the potential for use in diverse environmental monitoring situations.

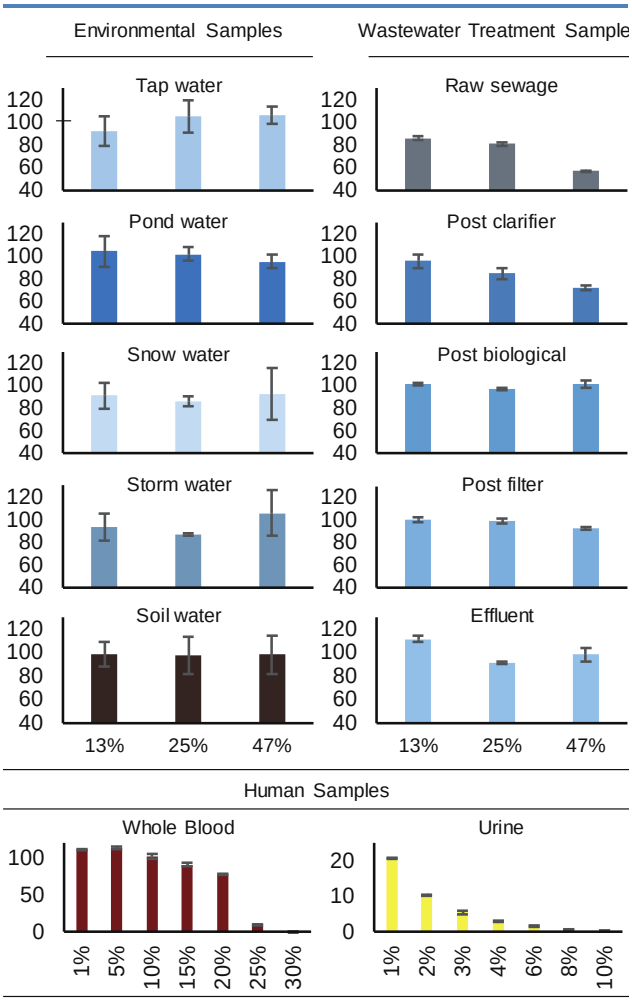
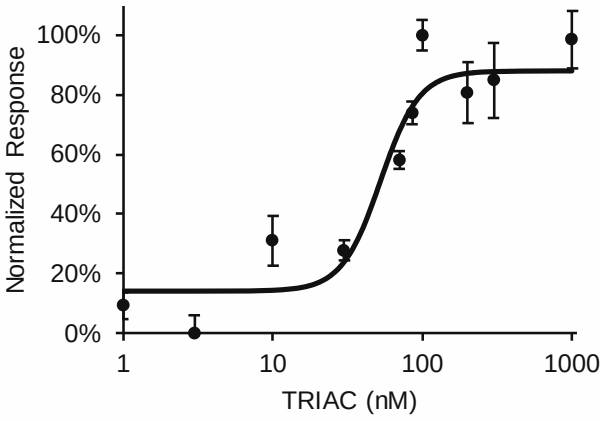


Figure 5. Protein production capability of CFPS in the presence of environmental and human samples. In all cases model protein GFP is expressed and the production level (y-axis) is normalized to GFP production in a standard CFPS with 100 corresponding to 100% of the GFP production level in standard CFPS. Each sample type is described in the methods section and the x-axis corresponds to the final concentration for the sample in the CFPS reaction by volume percent. The error bars represent one standard deviation for n=3.

Beyond environmental and wastewater samples, we examined CFPS tolerance to human medical samples, including blood and urine. Greater than 60% of the original CFPS activity was retained in reactions containing up to 20% by volume human blood. Additionally, we note that the blood we used in this work contained heparin as anticoagulant in lieu of EDTA, because EDTA at high concentrations can sequester magnesium and inactivate CFPS[28]. Expectedly, human urine, which contains a significant concentration (~280 mM) of the protein denaturant urea[44], had the greatest impact on CFPS activity. However, CFPS activity remained detectable at up to 8% by volume urine (1% original activity, with a standard deviation of 0.05 %). To account for significant yield changes cause by urine samples, a control CFPS reaction with a model protein such as GFP could be used in combination with the biosensor to ensure consistent dilution of the CFPS biosensing protein in the second

colorimetric stage of the biosensor assay. Overall, the ability of CFPS to tolerate high levels of various contaminants, such as organic matter, bacteria, blood, urine and wastewater demonstrates its robustness as a biosensing platform.

NHR RAPID Biosensor Performance in an Environmental Sample. Raw sewage was chosen to investigate how the composite biosensor was affected by the presence of an actual environmental sample. CFPS reactions containing 40% final volume raw sewage and TRIAC at varying concentrations were reacted for 20 mins. Subsequently, the reactions were diluted and assayed using the described colorimetric assay. The resulting RAPID biosensor maintained its sensitivity for TRIAC (53 nM EC50, -3.4 k, 0.63 Z', 40 S/N, 1.7 S/B, 28 nM LOD) (Figure 6).



Ligand	EC50 (nM)	k	Z	S/N	S/B
TRIAC	53	-3.4	0.63	40	1.7

Figure 6. Dose-response graph and statistical analysis results for the RAPID biosensor in the presence of TRIAC and 40% by volume raw sewage. The solid line represents fitted nitrocefin conversion values, the circle markers represent the measured values, and the error bars represent one standard deviation for n=2.

■ CONCLUSION

Here we have developed a new RAPID biosensor platform for chemicals that target nuclear hormone receptors using a quick, versatile cell-free protein synthesis approach. The developed biosensor has some key advantages over existing biosensors, including near real-time readout, the potential for portable field use, and reduced labor and cost requirements. This biosensor is also a promising tool for studying various NHR-binding ligands in a high-throughput manner. Additionally, the ability of CFPS to perform protein synthesis in different human and environmental samples, showed strong potential of the biosensor for detecting NHR-targeting compounds directly, without requiring purification or modification of the sample. Overall the RAPID biosensor is an attractive alternative to currently available technology and provides a fast, versatile platform for detecting potential NHR-binding ligands including EDCs and therapeutics.

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