



Triple functional small-molecule-protein conjugate mediated optical biosensor for quantification of estrogenic activities in water samples

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ABSTRACT

Establishing biosensors to map a comprehensive picture of potential estrogen-active chemicals remains challenging and must be addressed. Herein, we describe an estrogen receptor (ER)-based evanescent wave fluorescent biosensor by using a triple functional small-molecule-protein conjugate as a signal probe for the determination of estrogenic activities in water samples. The signal probe, consisting of a Cy5.5-labelled streptavidin (STV) moiety and a 17 β -estradiol (E₂) moiety, acts simultaneously as signal conversion, signal recognition and signal report elements. When xenoestrogens compete with the E₂ moiety of conjugate in binding to the ER, the unbound conjugates are released, and their STV moiety binds with desthiobiotin (DTB) modified on the optical fiber via the STV-DTB affinity interactions. Signal probe detection is accomplished by fluorescence emission induced by an evanescent field, which positively relates with the estrogenic activities in samples. Quantification of estrogenic activity expressed as E₂ equivalent concentration (EEQ) can be achieved with a detection limit of 1.05 μ g/L EEQ by using three times standard deviation of the mean blank values and a linear calibration range from 20.8 to 476.7 μ g/L EEQ. The optical fiber system is robust enough for hundreds of sensing cycles. The biosensor-based determination of estrogenic activities in wastewater samples obtained from a full-scale wastewater treatment plant is consistent with that measured by the two-hybrid recombinant yeast bioassay.

1. Introduction

Endocrine disruption in various organisms caused by exposure to chemicals with hormonal activities has attracted increasing attention worldwide (Kortenkamp et al., 2011). These chemicals can bind to the organism's endocrine receptors to activate, block or alter natural hormone synthesis and degradation; as a result, these 'false' or abnormal hormonal signals may cause various health problems, such as reproductive damage, developmental impairment and cancer in females and males (Adeel et al., 2017; Giulivo et al., 2016). Although endocrine disruption can occur from various biological mechanisms, more data exist for estrogens than for the other classes of activities (Fang et al., 2000). Estrogens are found in wastewater, surface water and even drinking water worldwide (Conley et al., 2017; Ferguson et al., 2013; Hashimoto et al., 2007). Chemicals that may act as estrogens show a broad structural diversity (Fang et al., 2000), which indicates that the large-scale instrument-based analytical techniques, such as gas and

liquid chromatography with mass spectrometry, fail to detect all chemicals with estrogenic activities, especially previously unrecognised varieties. To thoroughly address the possible adverse estrogenic effects, reliable technologies that can detect and identify the chemicals with diverse structural classes remain highly in demand.

The estrogen receptor (ER), including its two isoforms (ER α and ER β), is a nuclear hormone receptor that can selectively bind to its ligand (i.e. estrogens) and subsequently initiates a series of molecular events culminating in the activation or repression of target genes (Brzozowski et al., 1997). The activities of estrogens that bind ER can be detected using animal- or cell-based bioassays (Hunt et al., 2017). However, the majority of these established bioassays require days-long operations and laboratory equipment and are possibly limited by transmembrane transport or cytotoxicity with exposure to environmental water matrix (Hunt et al., 2017; Rodriguez-Mozaz et al., 2004). Therefore, cell-free bioassays based on ligand-ER interaction have been extensively explored to achieve the accurate screening of the possible

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adverse estrogenic effects of water environmental samples. These methods are simple and easy to perform and allow the identification of all possible endocrine disruptors that act through the ER (Rodríguez-Mozaz et al., 2004; Scognamiglio et al., 2016). Moreover, they are not limited by transmembrane transport or cytotoxicity due to the lack of cellular metabolic processes and cell wall (Hunt et al., 2017; Rodríguez-Mozaz et al., 2004). The ER competitive-binding assay established by this principle has been adopted in Tier 1 screening assays in the US EPA's Endocrine Disruptor Screening Program (Fenner-Crisp et al., 2000), which can rapidly ascertain whether an environmental contaminant can act through the same binding mechanism as endogenous estradiol. However, most of these assays remain confined in the homogenous system, which are not considered to be true sensors due to the lack of solid surfaces.

Biosensors, which serve as a beneficial supplement to large-scale analytical instruments, are devices incorporating biological recognition elements with transducers to deliver measurements with simple and easy-to-use formats (Turner, 2013). They harness the exquisite sensitivity and specificity of biology. More importantly, they can facilitate online and in-field detection via the construction of a reusable solid surface. Currently, different biological recognition elements (including enzymes, antibodies and functional nucleic acids) have been used to construct various biosensors for several estrogenic substances. Such structure-based biosensing methods can only determine the concentrations of single known estrogenic compounds but fail to detect a broad range of estrogens, especially unrecognised ones (Du et al., 2017; Hunt et al., 2017; Liu et al., 2017b; Peng et al., 2018). Without much conceptual dispute, biosensors can work as independent portable devices, serving as an efficient and cost-effective way to detect estrogens in the environment (Du et al., 2017; La Spina et al., 2018; Liu et al., 2017a). They usually consist of bioactive transducer surfaces, which are regenerable, thus achieving cost-effective, automated, facile and online detection (Wang et al., 2015b). To date, numerous ER-based biosensors have been reported, and the sensing element most commonly used is the human ER α (hER α) (Liu et al., 2017a; Shang et al., 2014). However, the majority of them are limited by poor surface regeneration due to the possible damage of severe elution conditions to the surface-tethered ER protein (Carmon et al., 2005; Fechner et al., 2009). The immobilisation of 17 β -estradiol (E₂)-protein conjugates rather than ER protein may compensate the weakness because of the chemical stability of the organic compound. However, considering the innermost ligand-binding portion of ER (Brzozowski et al., 1997), long spacer arms, which are difficult to synthesise, are needed (Garrett et al., 1999; Hock et al., 2002; Seifert et al., 1999; Usami et al., 2002). To circumvent the above-mentioned disadvantages, our previous studies reported a fluorescein-labelled anti-E₂ antibody-mediated evanescent wave fluorescent (EWF) biosensor with a facile regenerable sensing surface (Liu et al., 2017a). However, this method requires a two-step bioaffinity conversion process, which weakens the detection sensitivity. Moreover, the system cannot distinguish exogenous E₂ with a pre-added amount. Consequently, it will lose function when facing the water samples with unknown contaminants. Therefore, establishing a rational design of ER-based biosensors to map a comprehensive picture of potential estrogenic active chemicals remains highly desirable yet challenging.

Herein, we describe an improved ER-based optical fiber fluorescent biosensor technology for the facile detection of the estrogenic activities of environmental water samples. A specially designed fluorescein-small-molecule (E₂)-protein conjugate acts simultaneously as signal conversion, signal recognition and signal report elements. The strategy utilises the rationally designed affinity pair of desthiobiotin (DTB) and streptavidin (STV) as the surface recognition mechanism, which exhibits high surface regeneration ability (Hirsch et al., 2002; Wang et al., 2015a; Wang et al., 2015b; Wang et al., 2016). The E₂ moiety of the conjugate competes with the estrogenic chemicals in the samples to bind the ER, leaving the unbound conjugate to pass into the biosensor flow cell and interact with the DTB-modified fiber via the DTB-STV

interaction. The Cy5.5 fluorescein grabbed on the fiber and acting as the signal report element will then be excited by the evanescent field, resulting in the fluorescence signal that is proportional to the estrogenic activity of test sample. To validate the bioassay, we tested the wastewater samples from a full-scale wastewater treatment plant (WWTP) in Beijing and compared the results with those by the yeast two-hybrid system.

2. Experiment

2.1. Materials

The chemicals, buffers and biosensor system used in this work are listed in Supplementary Information (Pages 2–3, Notes S1–2). Additional information regarding the basic experimental steps, including the construction and characterisation of recombinant hER α in the form of resin-hER α complex, preparation and optimisation of DTB-modified optical fiber, are also listed in the Supplementary Information (Pages 4–5, Notes S3–4). Moreover, the protocols of recombinant yeast assay for measuring estrogenic activity, as the cell-based assay counterpart of ER biosensor, can be found in the Supplementary Information (Page 6, Note S5).

2.2. Sensing mechanism

Fig. 1a depicts a schematic of the optical fiber-based EWF biosensing platform used in this work. Additional details can be found in the Supplementary Information (Page 4). The sensing schematic of ER-based biosensor using triple functional fluorescein-labelled E₂-STV conjugates for estrogenic activity detection is shown in Fig. 1b. In brief, a E₂-STV conjugate consisting of a fluorescein-labelled STV moiety and an E₂ moiety is designed. This conjugate competes with the estrogen-active chemicals in the test samples to bind with the hER α in the form of bound state (resin-hER α complex). Once more estrogen-active chemicals exist, more E₂-STV conjugates are released. After a simple centrifugal separation, the supernatant with unbound conjugates is fed into the flow cell of biosensor. The STV moiety of conjugates binds with the DTB molecules modified on the optical fiber via the STV-DTB affinity interactions (Garlick and Giese, 1988), which is followed with an increase in the fluorescent intensity with evanescent field excitation. Normally, the more the estrogenic activities, the higher the fluorescent intensity, demonstrating a typical 'turn-on' sensing mode.

To realise the conjugate-mediated optical biosensor for the quantification of estrogenic activities in test samples, we synthesised the fluorescein (Cy5.5)-labelled E₂-STV conjugate as follows (Fig. 1c, Supplementary Information, Page 7, Note S6). An estradiol derivative (E₂-COOH) is a customer-designed product synthesised by Shanghai Qianyan Biotechnology Co., Ltd. (China) by using beta-estradiol 17-hemisuccinate and 6-aminocaproic acid to increase the spacer arm between E₂ and STV in the E₂-STV conjugate. The nuclear magnetic resonance identification report of the synthesised compound is shown in the Supplementary Information (Page 8, Note S7). As indicated in the sensing mechanism, the conjugate simultaneously acts as signal recognition, conversion and report elements.

2.3. Quantification of estrogenic activities

The optimal procedures for estrogenic activity detection are as follows: 500 μ L of 5 μ g/mL Cy5.5-labelled E₂-STV conjugate is added to 58 pmol resin-hER α , and the obtained mixture is kept at 4 $^{\circ}$ C overnight for affinity reaction. After centrifugation at 3000 rpm for 2 min, 450 μ L of supernatant is discarded. Subsequently, 500 μ L of E₂ standard solution or test sample is added, mixed and rotated for 8 h at 4 $^{\circ}$ C. The dissociated conjugate then binds to the DTB-modified optical fiber, and is detected by fluorescence emission induced by evanescent field. The Cy5.5-labelled E₂-STV conjugate is then eluted using 10 mL of 0.5%

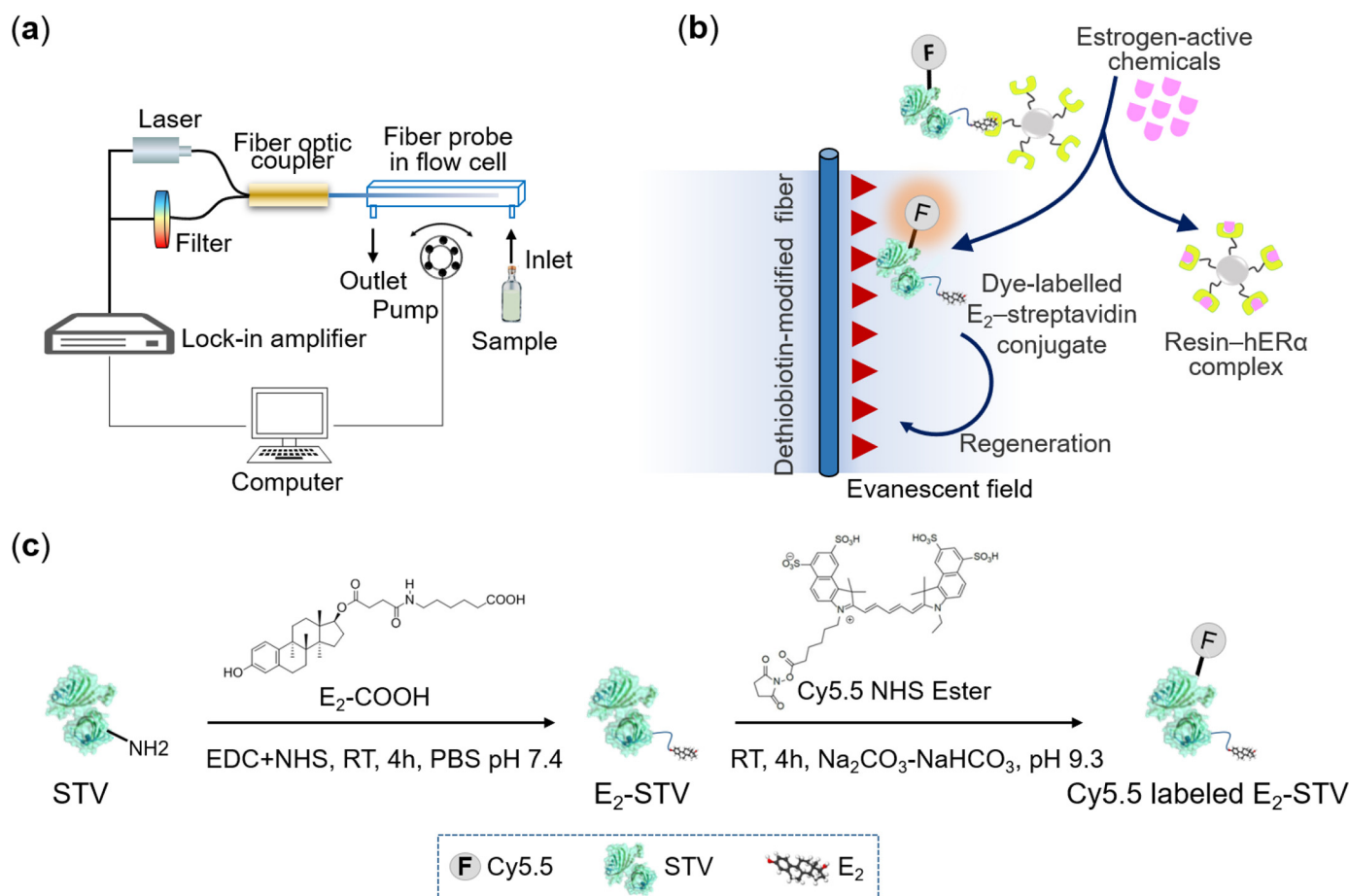


Fig. 1. (a) Schematic of the optical fiber EWF biosensing platform. (b) Sensing scheme of the ER-based biosensor using triple functional fluorescein-labelled E₂-STV conjugates for estrogenic activities quantification. In brief, the optical fiber surface (in blue-black) is tethered with the DTB molecules (in triangles), to which the E₂-STV conjugates with fluorescein labeling are captured via the ligand-ER interaction, thus grabbing the Cy5.5 fluorescein in the evanescent wave (in light blue). The fluorescein is then excited by the evanescent field, resulting in the fluorescence signal that is proportional to the estrogenic activity of the test sample. After measurement, the conjugate is eluted by using 0.5% SDS (pH 1.9) to regenerate the sensing surface for further use. (c) Schematic of the synthesis of Cy5.5-labelled E₂-STV conjugate. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

SDS (pH 1.9) for 25 min to ensure the regeneration of sensing surface. The whole EWF detection procedure takes less than 40 min.

To prove that hERα may retain biorecognition capabilities towards the conjugated E₂ with STV, we used the denatured ER (heating at 95 °C for 30 min) instead of the original ER for comparison. Two buffers, including PBS-B buffer (i.e. 10 mM PBS buffer, 5 mg/mL BSA, pH 7.4) and PBS buffer (i.e. 10 mM PBS buffer, pH 7.4), are compared for their response. All of the experiments are investigated in accordance with the abovementioned procedures.

2.4. Sample collection and extraction in WWTP

Wastewater samples are obtained from a full-scale reclaimed WWTP treating the bathing water in Tsinghua Campus, Beijing, China. The capacity of the WWTP is 1200 m³/d. The treatment processes consist of screen, hair filter, membrane bioreactor and disinfection (Fig. 2). The samples include the mixed liquor in membrane bioreactor (Sample A) and effluent (Sample B). Approximately 2.5–3.5 L of samples were collected in brown glass bottles and processed within 2 h in accordance with previous methods with slight modification (Li et al., 2010). In brief, the water sample firstly passes through a 0.7 μm glass fiber filter. The filtrate is further extracted by the solid-phase extraction cartridges, rinsed with 5 mL of methanol and then rinsed with a mixture of n-hexane and acetone (1:1) instead of the previously used methanol and acetyl acetate (1:1) mixture. Subsequently, the raw extract is filtered

through anhydrous Na₂SO₄ to remove water, followed by blowing until dry under nitrogen. The residue after nitrogen blowing is dissolved in 0.2 mL of dimethyl sulfoxide for subsequent dilution. A small portion of the residue is further diluted 10 times with 10 mM PBS (pH 7.4), which serves as the stock solution for biosensor detection. Another small portion of the residual elute is reconstituted with 10 mM PBS (pH 7.4) and prepared in a twofold dilution series for the two-hybrid recombinant yeast assay (Routledge and Sumpter, 1996). Additional information regarding the use of recombinant yeast cells for estrogen screening assay can be found in our previous report (Jiang et al., 2012; Li et al., 2008; Li et al., 2010).

3. Results and discussion

3.1. Optimisation and detection performance

Given that the reported recombinant hERα in the form of resin-hERα complex exhibits high E₂ binding affinities in the appearance of competitive estrogens (Liu et al., 2017a; Shang et al., 2014), when exposing to the conjugated E₂ with STV, hERα may retain biorecognition capabilities because the space arms induced the structural flexibility of conjugate as described above. To test this hypothesis, we utilised the optical fiber evanescent wave biosensor to reveal the ER-E₂ binding affinity-induced conjugate release and the DTB-STV surface recognition activities (Fig. 3). Before the tests, a DTB-modified

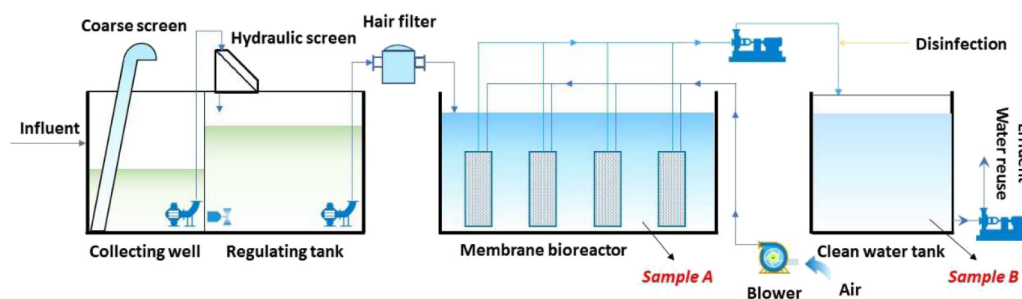


Fig. 2. Diagram of WWTP treating the bathing water at Tsinghua Campus, Beijing, China.

combination tapered fiber is synthesised based on earlier reported strategies (Wang et al., 2015a; Wang et al., 2015b; Wang et al., 2016), which is embedded into the flow cell of the biosensor. The biosensor signal traces are recorded automatically when the E_2 moiety in the conjugate is exposed to the denatured and original ER. As expected, the fluorescent intensity increased significantly when shifting the exposure of raw ER to denatured ER. We define the biosensor signal responding to 0.5 $\mu\text{g/mL}$ Cy5.5 labelled E_2 -STV conjugate in PBS-B buffer (i.e. 10 mM PBS buffer, 5 mg/mL BSA, pH 7.4) as the control. The ratio of the peak value under each condition (Fig. 3a) to the peak value of the control is regarded as the signal recovery ratio in Fig. 3b. Quantitatively, Fig. 3b shows that approximately onefold increase in the peak of fluorescent intensity is achieved. Such results suggest that the conjugated E_2 preserves the affinity capability to bind the recombinant hER α in the form of resin-hER α complex, which enables us to further utilise the conjugate to develop a facile estrogenic activity sensing biosensor. Considering that the surface blocking is always challenging when designing the surface-based affinity bioassay (Wang et al., 2017), the interaction between the denatured resin-hER α complex and the conjugate in PBS buffer (pH 7.4, 10 mM) is evaluated. The heavy un-specific adsorption of STV protein on the resin surface is observed, thus causing the false positive signals (Fig. 3a). Therefore, 5 mg/mL BSA is added into PBS buffer to serve as the surface blocking agent, which is overdosed to fully cover the surface of resin through a rough estimation (Supplementary Information, Page 9, Note S8). As a result, a remarkable increase in the fluorescent intensity is found, as observed from curves 3 to 1 (Fig. 3a).

To achieve an optimal detection performance, we optimised the experimental conditions, including the E_2 /STV ratio in mole concentration, the conjugate concentration and the affinity binding time. For E_2 /STV ratio optimisation, by selecting the mole concentration ratios for synthesising reaction, that is, $n(c_{(E_2)}:c_{(STV)})$, to be 10, 20, 50 and 100, the corresponding E_2 /STV conjugate coupling ratios can be

measured using the matrix-assisted laser desorption/ionisation-time-of-flight (MALDI-TOF) mass spectrometry, as shown by the black line in Fig. 4a. The MALDI-TOF results and details of the coupling ratio calculation are shown in Supplementary Information, Pages 10–11, Note S9. Approximately 0.5 mL of 0.5 $\mu\text{g/mL}$ Cy5.5-labelled conjugates with different coupling ratios are mixed with equimolar resin-hER α at 4 °C overnight, the supernatant is discarded, and equimolar E_2 standard solution is subsequently added to compete with the resin-hER α . The competing and initially added conjugates are separately introduced into the sensing surface, and the signals are recorded (F and F_0). The signal recovery efficiencies, that is, F/F_0 , reach a maximum at $n(c_{(E_2)}:c_{(STV)})$ of 20, which is selected as the optimised value for synthesis. For the conjugate concentration optimisation, to ensure that the resin-hER α fully combined with the Cy5.5-labelled E_2 -STV conjugate, we tested the signals of the Cy5.5-labelled E_2 -STV conjugate at different incubating concentrations with 0 pmol resin-hER α (red curve) and 58 pmol resin-hER α (black curve) (Fig. 4b). The inset of Fig. 4b shows the difference of fluorescence signals (ΔS) under both conditions, which is used for the optimisation of the Cy5.5-labelled E_2 -STV conjugate concentration. The fluorescence difference increases to a maximum and slightly changes when the Cy5.5-labelled E_2 -STV conjugate concentration reaches 5 $\mu\text{g/mL}$, indicating that the resin-hER α binding sites are fully saturated. Thus, 5 $\mu\text{g/mL}$ conjugate is used to ensure the highest sensitivity. Fig. 4c shows the effect of affinity binding time on the calibration curves of EWF for the detection of E_2 . All calibration curves accord well with the logistical model, and the quantitative detection ranges and sensitivities under five binding times are demonstrated in Supplementary Information, Pages 12–13, Table S2. The response of EWF towards E_2 exhibits a relatively wide linear range and low linear interval when the affinity binding time is 8 h. Therefore, 8 h is adopted as the appropriate affinity binding time for the following experiments.

As described above, the added external estrogens compete with the

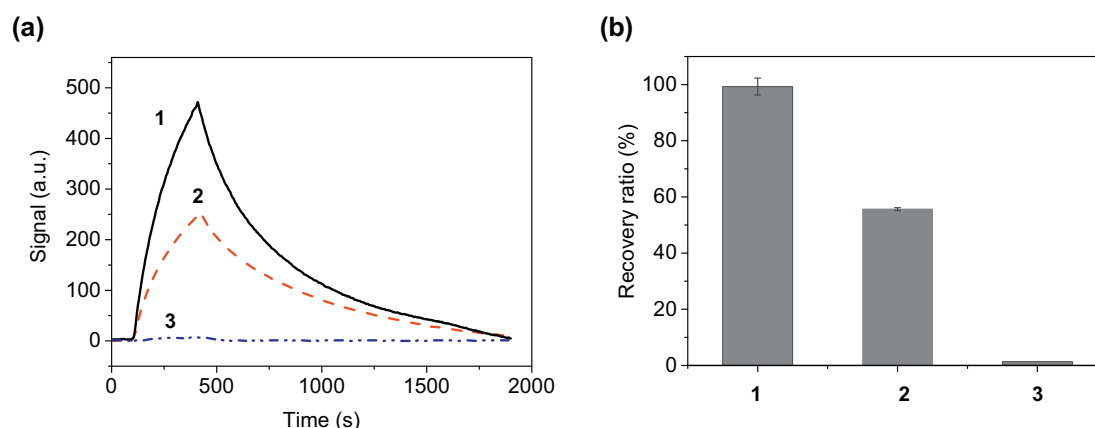


Fig. 3. (a) Original sensorgrams and (b) signal recovery ratio of EWF biosensor under different conditions: 1. Incubation of denatured hER α in the form of resin-hER α complex with Cy5.5-labelled E_2 -STV conjugate in PBS-B buffer; 2. Incubation of raw hER α in the form of resin-hER α complex with Cy5.5-labelled E_2 -STV conjugate in PBS-B buffer; 3. Incubation of denatured hER α in the form of resin-hER α complex with Cy5.5-labelled E_2 -STV conjugate in PBS buffer.

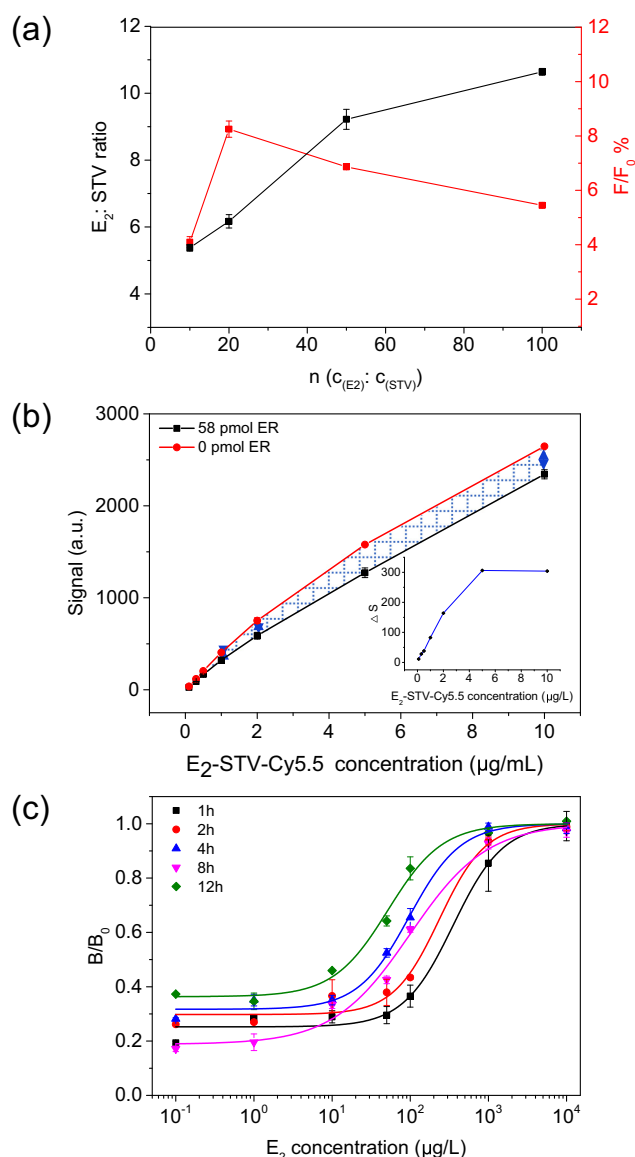


Fig. 4. Optimisations of detection performance: (a) E_2 /STV ratio (signal recovery efficiency equals F/F_0 , where F_0 and F represent the initial fluorescent intensity and fluorescent intensity after adding E_2 , respectively), (b) conjugate concentration and (c) affinity binding time.

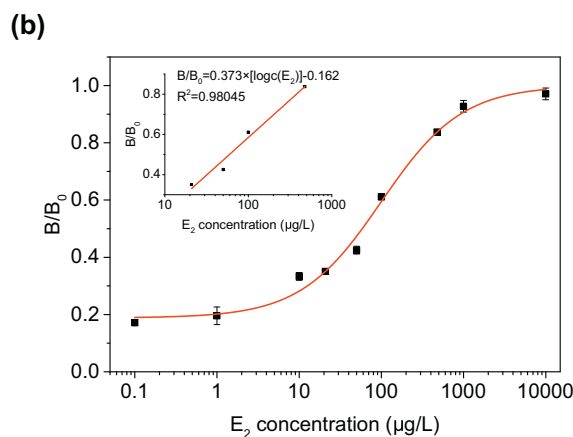
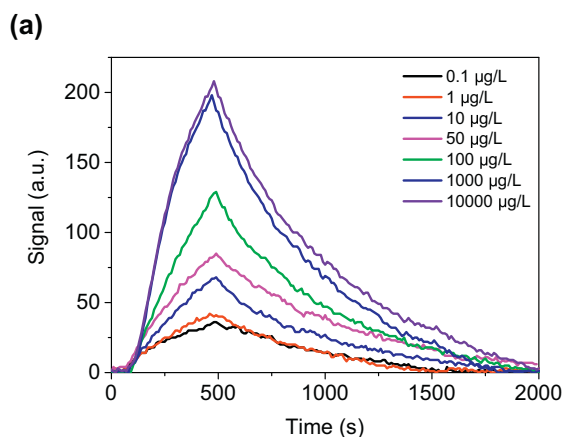


Fig. 5. (a) Original sensor grams of biosensor signals under different E_2 concentrations and (b) variations of corresponding peak values and logarithmic fitted calibration plot. The inset depicts the linear range of E_2 and corresponding calibration plot with the regression coefficient of $R^2 = 0.98$.

Table 1

Summary of the ER-based biosensor developed for estrogen compound analysis.

Transducer used	Detection range ($\mu\text{g/L}$) ^a	LOD ($\mu\text{g/L}$)	Reusability
Electrochemical (Murata et al., 2001)	Screening	/	/
Fluorescent (Liu et al., 2017a)	Screening	/	300 times
Piezoelectric (Zhihong et al., 1999)	2.72–27.2	2.12	30 times
Surface plasmon resonance (La Spina et al., 2018)	2–6	0.2	/
Surface plasmon resonance (Hock et al., 2002)	/	1	/
Surface plasmon resonance (Habauzit et al., 2008)	/	1.4	/
Fluorescent (This work)	20.8–476.7	1.05	200 times

^a "/" means data was not available in the reference.

^a Screening means the biosensor can only use for the estrogenic activity screening rather than quantification.

E_2 moiety of conjugate to bind hER α . The released Cy5.5-labelled E_2 -STV conjugate are left into the supernatant, which is fed into the embedded flow cell of the evanescent wave biosensor. The STV moiety of conjugate binds the DTB molecule modified on the optical fiber surface, increasing the fluorescent intensity with the excitation of evanescent field. Fig. 5a shows the typical real-time biosensor signals traced by the built-in biosensor software. By taking E_2 as the reference compounds, high E_2 concentrations are accompanied with large fluorescent intensity after treatment with various concentrations. To quantify the targets, we recorded the net signals in fluorescent peak and related them to the concentrations of target (Fig. 5b), leading to a logistic function-fitted calibration curve. As shown in Fig. 5b, a linear relationship between the E_2 concentrations and net peak values, which is defined as 20%–80% inhibition, is observed in the range of 20.85–476.73 $\mu\text{g/L}$. As shown in the inset of Fig. 5b, the linear fitted calibration plot for E_2 exhibits the regression coefficient of $R^2 = 0.98$. According to the rule of three times standard deviation of the mean blank values, the calculated limit of detection (LOD) of E_2 is 1.05 $\mu\text{g/L}$. A comparison of the analytical performance of other ER-based biosensors for estrogen compounds is shown in Table 1. The LOD of this proposed method is comparable with the previous reported techniques and possesses high surface regeneration. However, more efforts are needed to further decrease the LOD of this biosensor, considering that the environmental estrogens are usually at ng/L level in most natural water environments. Moreover, three potential xenoestrogens belonging to phthalate esters are investigated by using the optical biosensor platform (Supplementary Information, Page 14, Note S11). The response variations of biosensor under different phthalate concentrations and logarithmic fitted calibration plots are depicted in Fig. S4. The

Table 2

Estrogenic activities of real wastewater samples tested by the established biosensor and two-hybrid recombinant yeast assay.

Method	Sample	E ₂ (μg/L)			Recovery %
		Found	Spiked	Found Mean ^a ± SD ^b	
Biosensor	Sample A	/	30.0	29 ± 2	97
		/	50.0	55 ± 0.5	108
	Sample B	/	30.0	27 ± 3	90
Two-hybrid recombinant yeast assay	Sample A	/	50.0	59 ± 10	113
		/	30.0	28 ± 1	93
	Sample B	/	50.0	50 ± 0.3	100
		/	30.0	32 ± 5	107
		/	50.0	50 ± 0.3	100

^a Mean value of three individual determinations.

^b Standard deviation.

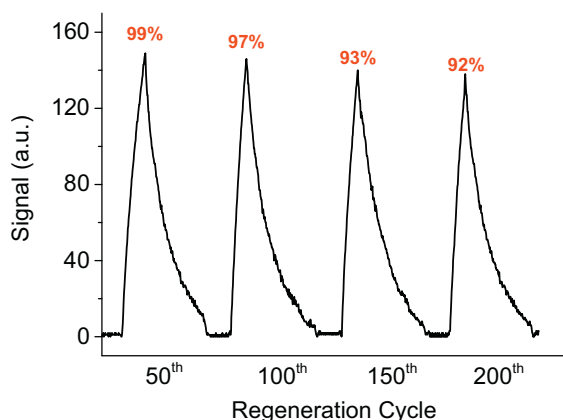


Fig. 6. Traced signals every 50 times of regeneration with the signal recovery percentages.

relative estrogen-agonist potencies decreased in the order butyl benzyl phthalate > dibutyl phthalate, whereas di-2-ethylhexyl phthalate showed no evidence of estrogenic activities as measured by this ER-based biosensor. The results obtained above are consistent with some previous studies (Harris et al., 1997; Takeuchi et al., 2005).

3.2. Determination of estrogenic activities of wastewater samples

To verify the applicability of this technology, we tested the ER-based biosensor for estrogenic activity determination with wastewater samples collected from WWTP. Although the enrichment factor of both samples A and B reached 1000-fold, the estrogenic activities by using the biosensor and two-hybrid recombinant yeast assay were not detectable. We attributed the low estrogenic activities to the influent type and the biodegradation process. Normally, human wastes are a primary source of steroid estrogens (Bertin et al., 2009). Other synthetic chemicals, including many environmental persistent organic compounds, are also estrogen-active (Braga et al., 2005; Mu et al., 2015; Tohyama et al., 2015). However, these ingredients rarely exist in bathing water. Moreover, the membrane bioreactor exhibits high removal efficiency for estrogens, such as 89% for E₂ (Yang et al., 2012). Therefore, 30 and 50 μg/L E₂ are spiked into the wastewater samples and detected by using both methods. As shown in Table 2, the E₂ concentrations calculated on the basis of the calibration curve are similar with the spiked values in both test samples, confirming the reliability of the established biosensor for the detection of estrogenic activities in real wastewater samples. The recovery ratios are similar to those measured by the traditional two-hybrid yeast bioassay.

3.3. Reusability of the DTB-modified optical fiber

Reusable biosensor promises advantages such as high accuracy and precision and increased functionality compared with its disposable counterpart (Choi and Chae, 2009). Samples can be detected continuously or semi-continuously, paving the way towards automated and online environmental monitoring (Wang et al., 2016). In light of the easily reversible binding between STV and DTB (Wang et al., 2015a; Wang et al., 2016; Wang et al., 2015c), the DTB-modified fiber surface is regenerable by utilising the suitable washing buffer (0.5% SDS, pH 1.9). Fig. 6 shows four representative signal traces tested approximately every 50 times to evaluate the regeneration capability of this sensing surface. In the whole testing, the sensing fiber is reused for at least 200 times with signal recoveries ranging from 92% to 99%. These results confirm that the biosensor is reliable for estrogenic activity detection with satisfactory reusability and accuracy.

4. Conclusions

A facile method for the quantification of estrogenic activities is developed by utilising the triple functional small-molecule-protein conjugates as the sensing elements. After the optimisation of detection conditions, the exposure of estrogens causes the fluorescein-labelled conjugates to be released in the supernatant. This increases the amount of fluorescein attached on the fiber surface by separation, which is observed by an increase in the fluorescent signal. Through this approach, the estrogenic activity of water samples is detected at a LOD of 1.05 μg/L by taking E₂ as reference. The biosensor promises reliable applicability for estrogenic activity detection in real wastewater samples. Moreover, such biosensor is easy-to-use and efficient with high reusable capability.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2019.105091>.

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