Biosensors and Bioelectronics 146 (2019) 111710

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

YestroSens, a field-portable *S. cerevisiae* biosensor device for the detection of endocrine-disrupting chemicals: Reliability and stability

Nadine Lobsiger[a], Jonathan E. Venetz[b], Michele Gregorini[a], Matthias Christen[b], Beat Christen[b], Wendelin J. Stark[a,*]

[a] Department of Chemistry and Applied Biosciences, Institute for Chemical and Bioengineering, ETH Zürich, 8093, Zürich, Switzerland
[b] Department of Biology, Institute of Molecular Systems Biology, ETH Zürich, 8093, Zürich, Switzerland

ARTICLE INFO

Keywords:
Biosensor device
Estrogen
S. cerevisiae
Field application
Storage

ABSTRACT

Farming, industry and urbanization lead to increases in the concentrations of potentially harmful compounds in waste, surface and drinking waters. One example of such pollution are estrogens, the steroidal female reproductive hormones. Already at a few nanograms per litre, these hormones can trigger endocrine disruption and cause acute and chronic health problems in humans and wildlife. Here, we present a *Saccharomyces cerevisiae* estrogen biosensor capable of detecting estradiol, as well as ethinylestradiol, at concentrations of 1 nM. After an initial characterization of the sensor strain performance in an optimal laboratory setting, we focused on developing a biosensor device. We addressed current limitations of biosensors, such as the requirement of the cells for a liquid growth matrix, controlled storage conditions required to preserve cell viability, and the usually required bulky, as well as expensive, laboratory equipment. Our study provides significant new insights into the field of applied biosensors. The system presented in this work takes microorganism-based analytics one step closer to field application in decentralized locations.

1. Introduction

Human activities such as farming, industry and urbanization lead to increases in the concentrations of potentially harmful compounds in water. This study is concerned with the detection of estrogens, the steroidal female reproductive hormones mainly produced by humans and livestock. Estrogens are constituted by 18 carbon atoms in three hexagonal and one pentagonal ring. The compounds of particular interest are either of natural origin, such as estradiol (E2), estrone (E1), or estriol (E3) or of synthetic origin such as 17α-ethinylestradiol (EE2), the oral contraceptive (Combalbert and Hernandez-Raquet (2010)). Apart from increased estrogen concentrations in sewage treatment plants, the manure from livestock, which is spread on land, represents a major source of steroids in soil and water (Shrestha et al. (2012)). Already at a very low concentration of a few nanograms per litre, these molecules are known to trigger endocrine disruption (EDCs, endocrine-disrupting chemicals) and cause acute and chronic health problems in humans and wildlife (Adeel et al. (2017); Daston et al. (1997)). The high estrogenic potency of natural hormones is caused by their high affinity for binding to nuclear estrogen receptors (ERs) (Combalbert and Hernandez-Raquet (2010)). Concentrations and exact effects vary depending on species and developmental stage. For example, in wild roach (*Rutilus rutilus*) an EE2 concentration as low as 0.5 ng/L already significantly affects gene expression during early development. In such aquatic species, continuous long-term exposure to EE2 at low concentrations (sub ng/L to ng/L) have been reported to change gene expression and induce intersex and reproduction deficiencies (Nikoleris et al. (2016)).

There is a growing demand for reliable analytic methods for EDCs detection at sub-nanomolar concentrations from environmental matrices. In laboratories, LC-MS/MS is routinely employed to detect the presence of EDCs and cell-based bioassays are used to detect and quantify biological activity. The main drawbacks of laboratory-based assays are the laborious sample preparation involving extraction and pre-concentration, as well as expensive analytic devices. To globally monitor the spreading of environmental pollution and raise awareness of the ecosystem disrupting consequences of EDCs, there is an increased need for on-site analysis of EDC levels. Ideally, there would exist a low cost, facile assessment of the pollution level employing a first-line bioactivity monitoring. Upon confirmation of the presence of EDCs, the samples could be sent to a laboratory for a more thorough analysis by LC-MS/MS.

In the discipline of biomonitoring, living organisms and their

* Corresponding author.
E-mail address: wendelin.stark@chem.ethz.ch (W.J. Stark).

https://doi.org/10.1016/j.bios.2019.111710
Received 5 July 2019; Received in revised form 26 August 2019; Accepted 16 September 2019
Available online 19 September 2019
0956-5663/

physiological responses to stimuli are used to determine changes in an environment such as water, air or soil. Microbiologists, synthetic biologists and ecotoxicologists have collaborated and constructed bioreporter systems in pro- and eukaryotes. In whole-cell biosensors, a target compound interacts with the cellular biorecognition element and a physicochemical transducer converts the interaction into a measurable signal. A comprehensive overview of different bacterial bioreporter designs is provided by van der Meer and Belkin (van der Meer and Belkin (2010)). The biosensor systems can be classified according to their detection systems and it is distinguished between amperometric, colourimetric, luminometric or fluorometric biosensors (Jarque et al. (2016)). Most previously reported whole-cell biosensors are based on bacterial cells due to short cultivation times and thorough genetic characterization. However, due to their eukaryotic and robust nature, the interest in *Saccharomyces cerevisiae* (*S. cerevisiae*) as a biosensor organism has increased (Adeniran et al. (2015)). It is hypothesized that eukaryotic cells might even provide information on the effects of direct relevance to other eukaryotes (Baronian (2004)). Additionally, the genome of *S. cerevisiae* is completely sequenced and strategies, as well as vectors to genetically modify it, are readily available (Gnügge et al. (2016); Mewes et al. (1997); Sikorski and Hieter (1989)).

Yeast cells, which respond to a broad range of different molecules have been engineered and reported in literature (Cahill et al. (2004); Tag et al. (2007); Wang et al. (2013)). With the intrinsic ability of *S. cerevisiae* to correctly express transfected vertebrate nuclear receptors and the subsequent generation of transgenic yeast strains, yeast biosensors targeting EDCs have become particularly prominent (Beck et al. (2005); Jarque et al. (2016); Lyttle et al. (1992)).

For example the strain of *S. cerevisiae* used in this study does not naturally react to estrogenic compounds Ottoz et al. (2014). have engineered it to use E2 as a transcription factor. A fusion between the bacterial *LexA* gene and the human estrogen receptor (ER) (Kumar et al. (1986)) was stably integrated into the genome. Upon binding of E2 to the ER, LexA can bind to specific LexA-binding sites, inducing the expression of a downstream reporter gene such as citrine (Griesbeck et al. (2001)) Fig. 1. With a higher number of LexA binding-sites, more LexA can bind upstream of the promoter and thus the resulting expression is stronger. The system was developed to precisely control gene expression in biotechnological applications (Ottoz et al. (2014)). The stable genetic integration of the gene fusions into the genome of the yeast cell is an advantage, as it makes antibiotic selection obsolete. The absence of antibiotics facilitates the usage of the system, as it reduces the handling steps and use of thermally fragile compounds (Berendsen et al. (2011)) necessary for cell cultivation. Several yeast-based *in vitro* assays for estrogens have been developed and they are commonly known under the name yeast estrogen screen (YES). Several studies have assessed estrogenic potency in waste, surface and drinking waters employing YES (Escher et al. (2014); Fang et al. (2000); Mehinto et al. (2015); Murk et al. (2002); Neale and Escher (2019)). Furthermore, Jarque et al. (2016) have described standardization and validation methods that are essential for regulatory acceptance when moving from research towards practical monitoring. A recent example of a yeast-based biosensor, which is routinely employed in a standardized fashion is the ISO certification for the determination of the estrogenic potential of water and wastewater (ISO 19040–1:2018).

Lateral flow assays, such as a home pregnancy test, are the prime example for market-implemented, standardized and validated point of care devices. We observe that these systems require minimal sample preparation and no instrumentation.

However, comparing these devices to currently existing whole-cell biosensors, the following limitations have been identified: 1) Cell embedding matrix enabling long-term ambient temperature storage while preserving cell activity and viability, 2) Scalability of the sensor material production, 3) Laborious pre-analytical steps such as extraction and concentration, 4) Analyte toxicity on sensor cells, 5) Lack of on-site application and user-friendliness and 6) Compliance with the national and international legislation controlling the application of genetically modified organisms.

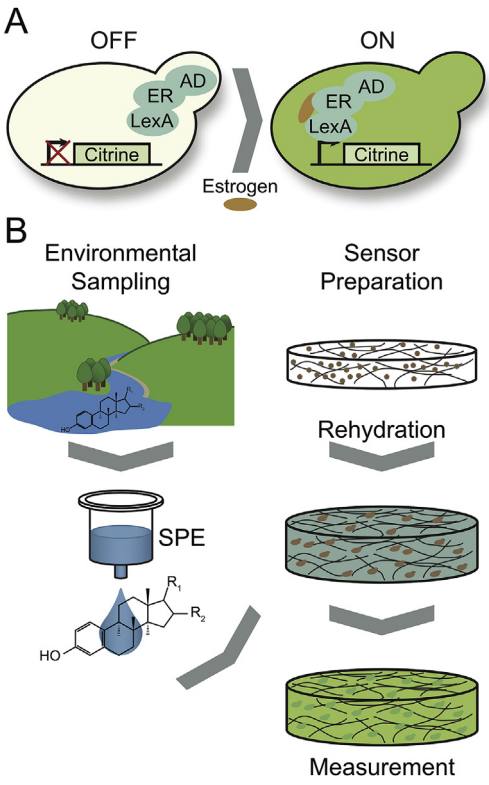

Fig. 1. (A) Schematic representation of the mechanism of action of the LexA-ER-AD system with ER being the hormone-binding domain of the human estrogen receptor and AD the activation domain. Adapted from Ottoz et al. (2014) (B) Schematic visualizing environmental sampling followed by pre-concentration using solid-phase extraction (SPE). The dried sensor material is rehydrated, incubated and induced the environmental sample. The sensor material subsequently detects EDCs and show a dose-dependent fluorescent response.

The requirements for a polymeric material to entrap the yeast cells should permit diffusion of molecules, be non-toxic, biodegradable and provide good mechanical stability (van der Meer and Belkin (2010)). For example, Vopálenská et al. (2015) have presented the immobilization of yeast cells in alginate beads for the detection of copper ions. Similarly, the enclosure of sensor cells in agarose for the detection of diclofenac in wastewater has been described (Schirmer et al. (2018)).

All these requirements are envisioned to facilitate on-site handling steps, ensure cell viability, sensor stability and reproducibility. In this work, we report a modified version of the Lentikats system using polyvinyl alcohol to create favourable microenvironments for the sensor cells (Krasňan et al. (2016)). The resulting biocompatible hydrogel matrix can be manufactured at low-costs to facilitate scalability of the sensor material production. The combination of the matrix with the efficient approach of lyophilizing, preserves yeast cells in a dormant state that facilitates long-term storage, addresses aforementioned limitations 1 and 2. The final device will be designed in a way to prevent the direct contact of the biosensor matrix with natural water bodies to avoid escape and contamination of the environment.

Addressing limitation 3, which is concerned with laborious pre-analytical steps such as extraction and concentration, different methods have been described. Most methods to extract estrogens from environmental assays for bioassay analysis rely on solid-phase extraction and the components are commercially available as kits greatly facilitating the use (Abbas et al. (2019); Escher et al. (2014); Kase et al. (2018); Neale et al. (2018); Simon et al. (2019); Tušil et al. (2018)). Heub et al. presented a platform to perform SPE for the detection of E2 in environmental water samples by immunoassays. The system is fully

automated, portable, user-friendly and efficient for the pre-concentration of E2 at ng/L levels in water samples and immunoassays (Heub et al. (2015)).

In this work, the challenge of extraction was omitted as artificially prepared estrogen samples were used to prove the concept of sensor cell performance in liquid culture, within the material and after lyophilization and storage.

To improve on-site applicability and user-friendliness, we present an integrated smartphone-compatible sensor-device based on a fluorescent reporter system, avoiding yeast cell lysis and the addition of temperature-sensitive external substrates. Combining the sensor material enclosed in a device with a smartphone equipped with a custom-designed filter set for fluorescence detection, we can substitute bulky and expensive bench-top instrumentation within a simple chip. Recently, Cevenini et al. (2018) have presented an example of a cell-based mobile platform employing a novel bioluminescent yeast-estrogen screen (nanoYES) in combination with a low-cost compact camera as the light detector. Using such a camera instead of a smartphone increases the applicability as it can wireless connect with any smartphone model. Lopreside et al. (2019) have recently presented a smartphone-based device to monitor bioluminescence in an upgraded version of the widely used yeast estrogen screening (YES) assays. This paper demonstrates the potential integration of cell cultivation, sensor function and signal detection within a stimulus-responsive material incorporated into a field-portable device. Further, it shows that the handling steps are minimized to facilitate the application by an untrained user in a resource-limited setting. The limitation concerning the legislation has not been addressed in this work as all experiments were conducted within a laboratory setting. To facilitate the prospective use of the sensor device outside of a laboratory, yeast, a well-characterized and non-pathogenic organism was used.

2. Material and methods

2.1. Chemicals and reagents

Unless stated otherwise, all chemicals were purchased from Sigma Aldrich (St. Louis, MO, US). The H_2O used was MQ-H_2O.

2.1.1. Strains and their construction

The strains used in this study have all been constructed and published by Ottoz et al. (2014). All strains were kindly gifted by Fabian Rudolf. The positive control (PC) corresponds to strain FRY757, the negative control (NC) to strain FRY11 and the sensor strain is FRY666.

2.2. Microbial growth conditions

Standard culturing conditions were used to grow microbial strains. *S. cerevisiae* strains were cultured in synthetic defined medium (SD) (Fred (2002); Venetz et al. (2019)) at 30 °C. The SD-Full medium contained all the amino acids essential for *S. cerevisiae* growth. For agar plates, 20 g/L agar granulated (Difco) were added to the SD medium. "High-density cultures" describes cultures, which have reached stationary phase, where the rate of cell death equals the rate of cell division and the optical density of the suspension no longer changes.

2.3. Estrogen induction

A 10 mM stock of E2 in absolute ethanol was prepared. Dilutions of 10, 1, 0.1 and 0.001 μM were prepared subsequently (Ottoz et al. (2014)).

2.4. Alternative inducers

Stock solutions at a concentration of 10 mM of EE2, E1 and E3 were prepared followed by subsequent dilutions to working solutions of 10, 1, 0.1 and 0.001 μM.

2.5. Lyophilization

Cell cultures were prepared for lyophilization by removing culture medium (centrifugation, 5 min, 2543 RCF). The supernatant was discarded and replaced by the equal volume of a sterile 5% sodium glutamate solution. The resuspended cells were incubated for 5 min before freezing. Frozen samples were immediately transferred to the lyophilizer unit at −49 °C and 0.007 mbar and dried for 16 h.

2.6. Storage

Tubes containing lyophilized samples were sealed with parafilm and stored at room temperature until use.

2.7. Assessment of viability after lyophilization

2.7.1. Viability analysis in liquid cultures

Viability of the lyophilized yeast strains was assessed by rehydrating the cultures in the same volume of nutrient medium from which they were lyophilized. Subsequently, the cells were diluted 250x in fresh nutrient medium, transferred to the wells of a 96-well plate and inducer added in the corresponding concentrations. Growth and induction curves were recorded with the microplate reader.

2.8. Viability analysis in liquid cultures after storage

The tubes containing the lyophilized cells were stored at room temperature until use and rehydrated as described in paragraph 2.7.1. Growth and induction curves were recorded with the microplate reader. As bacterial growth can be approximated with a logistic function, the following equation was used and fitted with the least-squares method:

$$f(x) = \frac{L}{1 + e^{-k(x-x_0)}} \quad (1)$$

A threshold value of $OD_{600} = 0.2$ was defined. The cycle number, at which the threshold was crossed, was determined to be a measure for the viability of the cells after lyophilizing, as it reflects the duration of the lag phase. The growth curves were evaluated by this method at different time points.

2.8.1. Yeast spotting assay

Spotting assays were used to visualize the survival of yeast after lyophilization. The colonies were grown overnight to full density and serially diluted five times at a 1:10 ratio in a 96-well plate (Thermo Fisher, Waltham, MA, US). Alternatively, lyophilized dense cultures were used and treated the same way as the fresh cultures. To create the spots, 3.5 μl of the diluted cells were pipetted onto SD-Full plates. This theoretically resulted in between 3 and 5 cells in the lowest concentrated spot. Following incubation of the serial dilutions, the plates were visually inspected and cell counts of the spots were compared. Spots of a similar count were identified and the mortality rate was determined under consideration of the dilution factor.

2.9. Microplate reader

Growth of yeast cultures was monitored by measuring the optical density at a wavelength of 600 nm. GFP fluorescence (excitation = 488 nm, emission = 530 nm) was measured from the bottom at 30 °C in a 96-well well plate with a microplate reader (TECAN, Spark). The data were initially analyzed in Excel (Office 2010; Microsoft).

2.10. Preparation of PEG-PVA hydrogel

Solutions of poly(vinyl alcohol) (PVA, Mowiol 28–99; Mw

145,000 g/mol) at 10% (w/w) were prepared by dissolving PVA in tap water at 80 °C and followed by repeated heating to 80 °C and stirring on a magnetic stirrer plate. Once dissolved polyethylene glycol 600 (PEG, abcr GmbH), at 6% (w/w), was added while continuously heating and stirring (Krasňan et al. (2016)). The hydrogel solution was stored at room temperature until use.

2.11. Material production

The biosensor material containing the yeast strains was produced using high-density cultures. After growth, 5 ml of cells were harvested by centrifugation (5 min, 2543 RCF). The supernatant was discarded and the pellet resuspended in 500 µl sterile 5% sodium glutamate solution and the suspension was incubated for 5 min before adding it to 9.5 ml of previously described PEG-PVA hydrogel (Schulz-Schönhagen et al. (2019)). To ensure homogeneous mixing, material and cells were mixed in a SpeedMixer DAC 150 FVZ (Hauschild Engineering, Germany) at 2000 rpm for 10 s. The homogeneously mixed hydrogel was applied to the UV-sterilized aluminium mould (10 × 10 × 0.1 cm) and frozen at −20 °C for 1 h. Frozen samples were immediately transferred to the lyophilizer unit (CHRIST ALPHA 1–2, Germany) at −49 °C and 0.007 mbar and dried for 16 h.

2.12. Material cost analysis

1 l of PVA-PEG hydrogel solution is required to produce 1 m^2 of sensor material (60 g PEG, 100 g PVA). Polyethylene glycol 600 is available at CHF 60.50/kg and the PVA (Mowiol® 28–99; Mw 145,000) at CHF 128.40/kg). 1 l of hydrogel solution, therefore, amounts to CHF 16.40.

2.13. Data analysis

All experiments were carried out in biological triplicates and data was analyzed with Origin (OriginLab Corporation). For the evaluation of the sensor performance within the hydrogel material, a two-sample *t*-test for unequal variance was conducted with an assumed alpha level of 0.05.

2.14. Design of the field-portable device and the smartphone adapter

The 3D design of the field-portable device was created using the software SOLIDWORKS 2016 and printed with a ProJet 2500 Plus (3D Systems, Inc.). The material used for the 3D print was VisiJet M2R-WT, an ABS-like material providing a rigid function with moderate flex. Using this workflow, the field-portable device, as well as the smartphone adapter prototypes, were manufactured.

3. Results and discussion

3.1. Analytical performance of the sensor

The analytical performance of the yeast biosensor in a laboratory setting has been evaluated. In an initial screen, the limit of quantification (LoQ) for E2 was determined (Ottoz et al. (2014)). Subsequently, the sensor was tested for cross-inducibility by the different estrogenic compounds E1, E3 and EE (Beck et al. (2005)). To pave the way for a real-world application within a device, the viability and E2-inducibility of the cells after lyophilization were characterized.

3.1.1. Limit of quantification and induction specificity in liquid cultures

We have characterized the sensor strain in terms of inducibility by E2 and compared the performance to a positive (constitutive reporter gene expression) and a negative control ('empty' strain) in a dose-response curve as visualized in Fig. 2A.

To characterize the biosensor in terms of induction specificity, several steroidal estrogens were tested at concentrations between 0 and 10 nM. Strong induction was observed for E2 and EE2. E1 also induced the sensor strain at a concentration of 10 nM. E3 did not cause a measurable induction of the sensor.

3.1.2. Viability and growth recovery of sensor cells after lyophilization

The biosensor strain is strongly induced by E2 and EE2 and weaker at higher concentrations of E1 when tested with a fresh, non-lyophilized culture. The sensor material fabrication process involves freeze-drying to enable storage. To assess the compatibility of the yeast sensor strain with such a process, high-density cultures were subjected to lyophilization in a 5% sodium glutamate solution.

Following lyophilization growth and induction, curves were measured in regular, seven-day intervals to assess a potential decrease in viability after extended storage. Cultures were rehydrated in SD-medium and diluted to an OD_{600} of 0.001. By monitoring the growth over 60 h, we have learned that the lag phase of the lyophilized yeast cells was extended. The growth after lyophilization was approximated by fitting a logistic function. Subsequently, the cycle at which the growth curve crosses the arbitrarily defined threshold value of OD_{600} = 0.2 was determined. For a fresh culture, an OD_{600} of 0.2 was reached after about 8 h. Compared to a lyophilized sample, the extension of the lag phase caused the culture to only cross the OD_{600} threshold of 0.2 after 29 h if not stored, which is offsetting it to 43 h (RT storage, 35 days). Currently, we are testing the storage behaviour over several months.

To qualitatively check the impact of lyophilization on the survival of *S. cerevisiae*, a yeast spotting assay was conducted. Two time points post-lyophilization were tested. The first colony was lyophilized and subsequently stored at room temperature for one month and the second colony was plated 24 h after lyophilization. As a positive control, a freshly grown colony was plated. As expected, the survival rate of the lyophilized colonies was lower than the positive control. The survival rate after the lyophilization process was determined to be about 1 in 10^4. Visual inspection did not yield a major observable difference in survival for the two samples stored for different periods of time as shown in Fig. 3. Furthermore, no difference between the different strains (sensor, constitutive and empty) was observed. In line with the dose-response curves reported for the LOQ, the inducibility of the sensor cells after lyophilization was checked and confirmed to be preserved. As soon as growth was observed, the sensor cells produced citrine in the presence of E2 and the positive control indicated viability by showing inducer-independent fluorescence signal.

After characterization of the cell survival following lyophilization, the robustness of the sensor cells was exploited, the cells embedded in a polymeric matrix and the material quantitatively tested.

3.2. Fabrication of the biosensor material

3.2.1. Production of sensor material

Briefly, cell culture was resuspended in a small volume of a 5% sodium glutamate solution. This cell suspension was mixed with the hydrogel solution resulting in a 10%, w/w and PEG 6%, w/w biosensor material precursor (Schulz-Schönhagen et al. (2019)). In the previous section, we have demonstrated the yeast survival after lyophilizing in presence of sodium glutamate as a cryoprotectant. Combining lyophilization with the hydrogel platform, we have established an easily scalable production process for a yeast biosensor material.

3.2.2. Low cost of sensor material manufacturing with current setup

Considering the low costs of the polymers constituting the growth matrix for the *S. cerevisiae* sensor strains, we have calculated the material cost for the production of one batch of sensor discs. With a lyophilizer chamber diameter of 23 cm and a height of 27 cm, square aluminium moulds with a diagonal of 15 cm can be used for sample preparation. The moulds are 1 cm thick, potentially resulting in 27

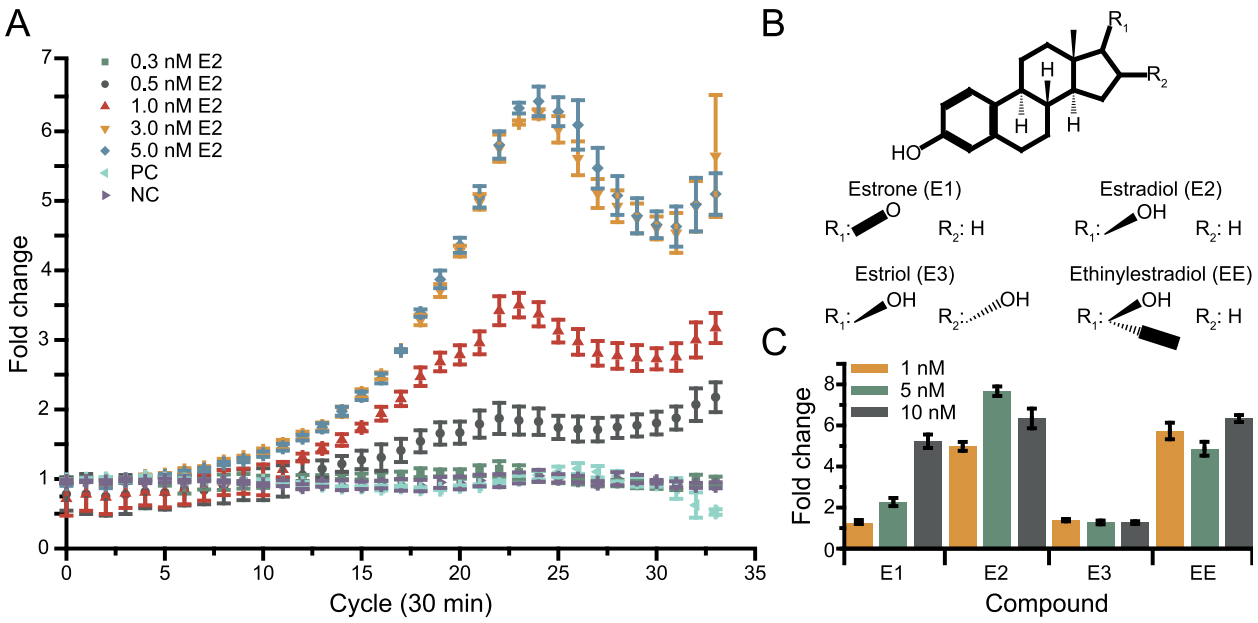

Fig. 2. (A) The fold change in fluorescence expression upon E2-induction was plotted as a function of growth time. One cycle corresponds to 30 min. Symbols represent the mean and error bars the standard deviation. It is observed that the increase in fluorescence is higher than background fluorescence starting from 0.5 nM. (B) Chemical structures used for the determination of induction specificity. (C) Comparison of sensor inducibility by E1, E3, E2 and EE2 at 1, 5 and 10 nM evaluated after 24 cycles (12 h), when the cells had reached an OD_{600} of approximately 0.3.

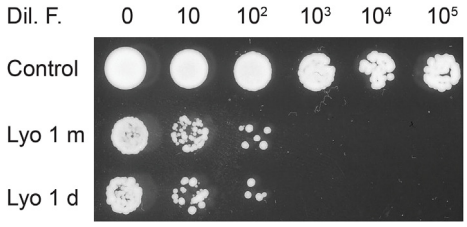

Fig. 3. (A) Results of the yeast spotting assay. The top row indicates the dilution factor and the left column the different conditions tested. The non-lyophilized culture shows growth at all dilutions. For the lyophilized and stored samples, reduced viability was observed.

layers to be stacked within the chamber. Assuming a sensor disc diameter of 0.5 cm, 900 discs can fit per layer. A total number of 24'300 discs can be produced per lyophilizer batch. A full run requires approximately 600 ml of hydrogel and the material cost amount to CHF 9.95. The production of an individual sensor disc therefore costs CHF 0.0004. As four discs are necessary for the analysis of one sample, the cost of the sensor material within a single device amount to of CHF 0.001. In the future, fabrication of the device housing the sensor discs will be performed by injection moulding allowing for very low prices per unit and paving the way further for cheap and robust on-site applications.

3.2.3. Qualitative evaluation of cell-recovery and sensor performance

Up to this point, this work has described the production of a low-cost, ambient temperature storable and cell-containing material. The sensor performance of the material was evaluated. The sensor material discs were rehydrated and incubated in nutrient medium. For cell recovery, 48 h were allowed as determined from the lyophilization growth assays. Subsequently, E2 was added at concentrations ranging from 0 to 5 nM. After 5 h, the sensor discs were transferred to a sample holder and images were taken (Fig. 4A). Fig. 4B compares the mean pixel intensities of the citrine fluorescence readout of the positive control (fluorescent protein is driven by a constitutive promoter), the negative control (yeast wildtype strain containing no reporter gene construct), and the sensor strain with the E2-inducible element. As

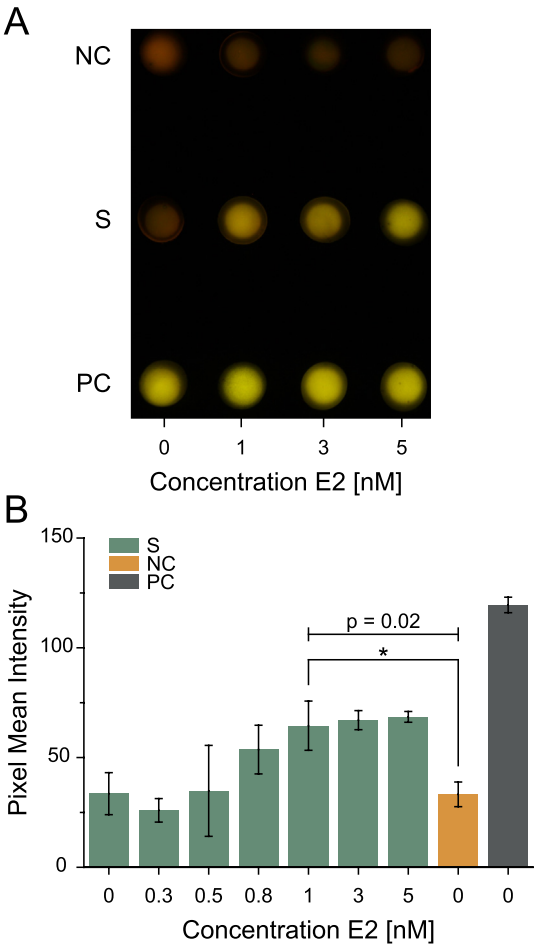

Fig. 4. (A) Visualization of the effect of E2 on the sensor material. PC: positive control, NC: negative control, S: sensor material. (B) Quantitative evaluation of the sensor discs depicted in (A) after 48 h of growth followed by 5 h of induction.

expected, the positive control exhibited fluorescence independent of the inducer concentration and the negative control has only shown a background signal. For the sensor strain, the finding of Fig. 2B of a limit of quantification of 1 nM. Fluorescence of the induced sensor strain at 1 nM was significantly different from the negative control ($p = 0.02$). To enable quantification, the experiment was carried out in triplicates and Fig. 2B shows the mean intensities of the different strains at different E2 concentrations. In summary, two different detection limits have been observed depending on the experimental conditions. In liquid culture, where fluorescence is monitored with a highly sensitive microplate reader, the signal caused by 0.5 nM E2 could be detected. On the other hand, within the hydrogel material, the fluorescence signal is captured with a single-lens reflex camera with lower sensitivity and only 1 nM E2 could be detected.

3.3. A smartphone-readable sensor device to enable on-site analysis and field use

3.3.1. Device integrating cell cultivation, sample application and sensor readout

Current biosensor systems come at the limitations of lacking the capability of controlled storage, which directly impacts sensor cell survival and therefore sensor performance. Ideally, a biosensor device would facilitate cell handling to an extent where a biology laboratory was made obsolete and sensor readout can be conducted without expensive laboratory equipment(Cevenini et al. (2018); Lopreside et al. (2019)). We have developed a device integrating cell cultivation and sample application, while at the same time providing a platform for imaging of the sensor material after a period of incubation. The device was 3D-printed with a ProJet 2500 Plus, which is a state of the art professional-grade 3D printer capable of printing rigid and elastomeric parts with high precision. The biosensor device features four sterile, independent chambers, which are sealed from the environment with a septum. Within each chamber, the sensor material disc is mounted on support pillars, which hold it in position underneath a glass window. During assembly, nutrient medium powder is immobilized on the walls of the device.

Fig. 5A graphically shows the steps to use the sensor device. The device is delivered to the customer in a sachet providing a controlled atmosphere for longterm storage. Upon unpacking from a sachet, each chamber is injected with a given volume of water provided with the kit. Together with the immobilized nutrient powder, the water constitutes the medium required for bacterial growth. The device is flipped to incubation position to ensure optimal nutrient access as depicted in Fig. 5B. The inducer is added 48 h after of incubation, the device is incubated for another 5 h and subsequently flipped by 180° back to imaging position. The disc will no longer be covered by nutrient medium and is optimally presented in the window.

3.3.2. Smartphone adapter enabling sensor readout

To fully detach the sensor device from a laboratory environment and enable facilitated readout of the signal, a smartphone filter adapter was designed and fabricated using 3D printing technology. The last part of Fig. 5A shows the adapter mounted to a smartphone. The adapter features two filters. The first one is a blue excitation filter (peak at 460 nm) covering the area of the flash. When the flash is operated, the excitation filter produces light at a wavelength smaller than 460 nm. Subsequently, the orange emission filter (bandpass filter, 520 nm), mounted in front of the camera, allows capturing light at the emitted wavelength. After incubation in the field at slightly elevated ambient temperature conditions, the device is flipped to the imaging position, the adapter is fixed to the smartphone camera and images are taken. In the future, we plan to develop a smartphone application to automate the processing of the fluorescent signal quantification. The results will be reported in a future publication.

4. Conclusion

Here we report the development of a portable and storable *S. cerevisiae* biosensor platform for the detection of E2. The sensor cells embedded in a polymer matrix can reliably detect the presence of E2 at concentrations of 1 nM. After storage, the sensor material discs are incubated in nutrient medium for 48 h, the inducer is added and the signal reported after another 5 h of incubation. Looking at the specificity of the sensor, we have observed that E2 and EE2 cause induction of the sensor already at a concentration of 1 nM, E1 at 10 nM and there

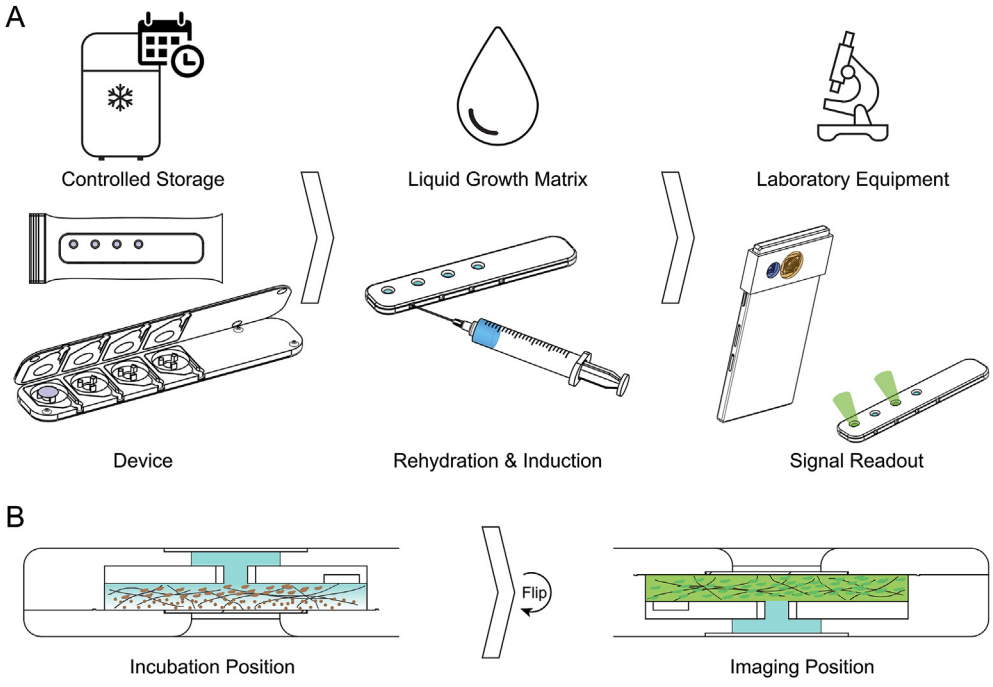

Fig. 5. (A) The top row summarizes the challenges of current biosensor applications. Sensor cell survival is closely linked to controlled storage conditions, usually in a freezer to stop the metabolism or at least in a fridge to slow it down. The cells require a liquid growth matrix for optimal growth and usually expensive and bulky laboratory equipment is necessary for signal readout. The bottom row of (A) describes how this biosensor tackles the aforementioned challenges. With lyophilization of the sensor cells and incorporation within a device, storage is addressed. The cells are rehydrated and induced within the sterile environment of the sensor chip and the use of expensive laboratory equipment is made obsolete by employing a smartphone with a filter set for signal readout. (B) The device is supposed to be used in two different orientations. A cross-section through the device showing the polymer disc housing dried cells with nutrient medium penetrating the disc in incubation position is presented. In a real-world scenario, the incubation with the analyte takes place at room temperature in the incubation position. Subsequently, the device is flipped to imaging position and images are taken.

was no measurable fluorescence signal in the presence of E3.

Conventionally, the detection of environmental pollutants is conducted in specialized analytical laboratories. Such facilities are equipped with devices for analytical chemistry, enabling a very precise determination of sample composition. In a biological laboratory, biological detection methods are employed to a similar outcome. Assays conducted in laboratory facilities deliver extremely precise results but come at the main disadvantage of the high cost of maintenance, labour and consumables, resulting in a high cost per analysis. With the biosensor platform described in this study, we have taken laboratory analytics one step closer to the application in the field. The lyophilized polymeric matrix enables longterm storage of the sensor-cells within the sensor device and facilitates sensor operation by minimizing the handling steps. By the means of rapid prototyping, we have developed a field-portable analytical biosensor device integrating cell cultivation, sensor function and signal detection within a stimulus-responsive material. Signal readout and quantification are conducted with a nowadays omnipresent electronic device and maximally facilitated. With methods from analytical chemistry, a well-equipped laboratory and educated staff, very precise determination of a chemical sample is possible. If the bioactivity of compounds in a sample matrix is of interest, bioassays are the tools of choice but with similar drawbacks as mentioned above. With an analytical task at hand, one always has to balance the pros and cons of different methods. In the case of a biosensor, the cons usually are slow growth, a narrow range of optimal assay conditions and the requirement for sterile handling conditions. The system presented in this work takes microorganism-based analytics one step closer to application in decentralized locations. Future efforts should be centred around improving the limit of detection either by biologically improving the sensor strain or by facilitating the extraction and pre-concentration methods. Once these challenges have been mastered, the true performance of the bioreporter assay can be examined by comparing its results to chemical analytics conducted in parallel.

Funding

Financial support by ETH Zurich is kindly acknowledged. The authors gratefully acknowledge the financial funding by the Gebert Rüf Stiftung (GRS-056/16).

CRediT authorship contribution statement

Nadine Lobsiger: Conceptualization, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization, Project administration. **Jonathan E. Venetz:** Conceptualization, Validation, Investigation, Writing - original draft, Writing - review & editing, Visualization, Project administration. **Michele Gregorini:** Conceptualization, Formal analysis, Writing - review & editing. **Matthias Christen:** Conceptualization, Writing - review & editing, Supervision. **Beat Christen:** Conceptualization, Writing - review & editing, Supervision. **Wendelin J. Stark:** Conceptualization, Writing - review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to thank F. Rudolf for the gifted *S. cerevisiae* strains. Many thanks also to R. Walker from the ETH Zurich, D-CHAB, ICB workshop for support with rapid prototyping.

References

Abbas, A., Schneider, I., Bollmann, A., Funke, J., Oehlmann, J., Prasse, C., Schulte-Oehlmann, U., Seitz, W., Ternes, T., Weber, M., Wesely, H., Wagner, M., 2019. Water Res. 152, 47–60.
Adeel, M., Song, X., Wang, Y., Francis, D., Yang, Y., 2017. Environ. Int. 99, 107–119.
Adeniran, A., Sherer, M., Tyo, K.E., 2015. FEMS Yeast Res. 15, 1–15.
Baronian, K.H., 2004. Biosens. Bioelectron. 19, 953–962.
Beck, V., Pfitscher, A., Jungbauer, A., 2005. J. Biochem. Biophys. Methods 64, 19–37.
Berendsen, B.J., Elbers, I.J., Stolker, A.A., 2011. Food Addit. Contam. Part A Chem Anal Control Expo Risk Assess 28, 1657–1666.
Cahill, P.A., Knight, A.W., Billinton, N., Barker, M.G., Walsh, L., Keenan, P.O., Williams, C.V., Tweats, D.J., Walmsley, R.M., 2004. Mutagenesis 19, 105–119.
Cevenini, L., Lopreside, A., Calabretta, M.M., D'Elia, M., Simoni, P., Michelini, E., Roda, A., 2018. Anal. Bioanal. Chem. 410, 1237–1246.
Combalbert, S., Hernandez-Raquet, G., 2010. Appl. Microbiol. Biotechnol. 86, 1671–1692.
Daston, G.P., Gooch, J.W., Breslin, W.J., Shuey, D.L., Nikiforov, A.I., Fico, T.A., Gorsuch, J.W., 1997. Reprod. Toxicol. 11, 465–481.
Escher, B.I., Allinson, M., Altenburger, R., Bain, P.A., Balaguer, P., Busch, W., Crago, J., Denslow, N.D., Dopp, E., Hilscherova, K., Humpage, A.R., Kumar, A., Grimaldi, M., Jayasinghe, B.S., Jarosova, B., Jia, A., Makarov, S., Maruya, K.A., Medvedev, A., Mehinto, A.C., Mendez, J.E., Poulsen, A., Prochazka, E., Richard, J., Schifferli, A., Schlenk, D., Scholz, S., Shiraishi, F., Snyder, S., Su, G., Tang, J.Y.M., van der Burg, B., van der Linden, S.C., Werner, I., Westerheide, S.D., Wong, C.K.C., Yang, M., Yeung, B.H.Y., Zhang, X., Leusch, F.D.L., 2014. Environ. Sci. Technol. 48, 1940–1956.
Fang, H., Tong, W., Perkins, R., Soto, A.M., Prechtl, N.V., Sheehan, D.M., 2000. Environ. Health Perspect. 108, 723–729.
Fred, S., 2002. Methods Enzymol. 350, 3–41.
Gnügge, R., Liphardt, T., Rudolf, F., 2016. Yeast 33, 83–98.
Griesbeck, O., Baird, G.S., Campbell, R.E., Zacharias, D.A., Tsien, R.Y., 2001. J. Biol. Chem. 276, 29188–29194.
Heub, S., Tscharner, N., Monnier, V., Kehl, F., Dittrich, P.S., Follonier, S., Barbe, L., 2015. J. Chromatogr. A 1381, 22–28.
Jarque, S., Bittner, M., Blaha, L., Hilscherova, K., 2016. Trends Biotechnol. 34, 408–419.
Kase, R., Javurkova, B., Simon, E., Swart, K., Buchinger, S., Könemann, S., Escher, B.I., Carere, M., Dulio, V., Ait-Aissa, S., Hollert, H., Valsecchi, S., Polesello, S., Behnisch, P., di Paolo, C., Olbrich, D., Sychrova, E., Gundlach, M., Schlichting, R., Leborgne, L., Clara, M., Scheffknecht, C., Marneffe, Y., Chalon, C., Tusil, P., Soldan, P., von Danwitz, B., Schwaiger, J., Morán, A., Bersani, F., Perceval, O., Kienle, C., Vermeirssen, E., Hilscherova, K., Reifferscheid, G., Werner, I., 2018. Trends Anal. Chem. 102, 343–358.
Krasňan, V., Stloukal, R., Rosenberg, M., Rebroš, M., 2016. Appl. Microbiol. Biotechnol. 100, 2535–2553.
Kumar, V., Green, S., Staub, A., Chambon, P., 1986. EMBO J. 5, 2231–2236.
Lopreside, A., Calabretta, M.M., Montali, L., Ferri, M., Tassoni, A., Branchini, B.R., Southworth, T., D'Elia, M., Roda, A., Michelini, E., 2019. Anal. Bioanal. Chem. 1–13.
Lyttle, C.R., Damian-Matsumura, P., Juul, H., Butt, T.R., 1992. J. Steroid Biochem. Mol. Biol. 42, 677–685.
van der Meer, J.R., Belkin, S., 2010. Nat. Rev. Microbiol. 8, 511–522.
Mehinto, A.C., Jia, A., Snyder, S.A., Jayasinghe, B.S., Denslow, N.D., Crago, J., Schlenk, D., Menzie, C., Westerheide, S.D., Leusch, F.D., Maruya, K.A., 2015. Water Res. 83, 303–309.
Mewes, H., Albermann, K., Bähr, M., 1997. Nature 387, 7–65.
Murk, A.J., Legler, J., Van Lipzig, M.M., Meerman, J.H., Belfroid, A.C., Spenkelink, A., Van der Burg, B., Rijs, G.B., Vethaak, D., 2002. Environ. Toxicol. Chem. 21, 16–23.
Neale, P.A., Brack, W., Aït-Aïssa, S., Busch, W., Hollender, J., Krauss, M., Maillot-Maréchal, E., Munz, N.A., Schlichting, R., Schulze, T., Vogler, B., Escher, B.I., 2018. Environ Sci Process Impacts 20, 493–504.
Neale, P.A., Escher, B.I., 2019. Curr. Opin. Env. Sci. Health 7, 1–7.
Nikoleris, L., Hultin, C.L., Hallgren, P., Hansson, M.C., 2016. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 180, 56–64.
Ottoz, D.S., Rudolf, F., Stelling, J., 2014. Nucleic Acids Res. 42 e130–e130.
Schirmer, C., Posseckardt, J., Kick, A., Rebatschek, K., Fichtner, W., Ostermann, K., Schuller, A., Rödel, G., Mertig, M., 2018. J. Biotechnol. 284, 75–83.
Schulz-Schönhagen, K., Lobsiger, N., Stark, W.J., 2019. Adv. Mater. Technol. 1900266.
Shrestha, S.L., Casey, F.X.M., Hakk, H., Smith, D.J., Padmanabhan, G., 2012. Environ. Sci. Technol. 46, 11047–11053.
Sikorski, R.S., Hieter, P., 1989. Genetics 122, 19–27.
Simon, E., Schifferli, A., Bucher, T.B., Olbrich, D., Werner, I., Vermeirssen, E.L., 2019. Anal. Bioanal. Chem. 411, 2057–2069.
Tag, K., Riedel, K., Bauer, H.J., Hanke, G., Baronian, K.H., Kunze, G., 2007. Sens. Actuators B Chem. 122, 403–409.
Tušil, P., Vermeirssen, E., Schwaiger, J., Dulio, V., Marneffe, Y., Könemann, S., Kase, R., Escher, B.I., Werner, I., Schlichting, R., Scheffknecht, C., Di Paolo, C., Simon, E., von Danwitz, B., Reifferscheid, G., Leborgne, L., Clara, M., Perceval, O., Javurkova, B., Valsecchi, S., San Martín Becares, M.I., Olbrich, D., Soldàn, P., Behnisch, P., Chalon, C., Bersani, F., Carere, M., Schlüsener, M., Hilscherová, K., Sychrova, E., Buchinger, S., Swart, K., Ternes, T., Aït-Aïssa, S., Polesello, S., Hollert, H., 2018. Trends Anal. Chem. 102, 225–235.
Venetz, J.E., Del Medico, L., Wölfle, A., Schächle, P., Bucher, Y., Appert, D., Tschan, F., Flores-Tinoco, C.E., van Kooten, M., Guennoun, R., Deutsch, S., Christen, M., Christen, B., 2019. Proc. Natl. Acad. Sci. U.S.A. 116, 8070–8079.
Vopálenská, I., Váchová, L., Palková, Z., 2015. Biosens. Bioelectron. 72, 160–167.
Wang, H., Lang, Q., Li, L., Liang, B., Tang, X., Kong, L., Mascini, M., Liu, A., 2013. Anal. Chem. 85, 6107–6112.