

Whole-cell *Escherichia coli* lactate biosensor for monitoring mammalian cell cultures during biopharmaceutical production[†]

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Abstract

Many high-value added recombinant proteins, such as therapeutic glycoproteins, are produced using mammalian cell cultures. In order to optimise the productivity of these cultures it is important to monitor cellular metabolism, for example the utilisation of nutrients and the accumulation of metabolic waste products. One metabolic waste product of interest is lactic acid (lactate), overaccumulation of which can decrease cellular growth and protein production. Current methods for the detection of lactate are limited in terms of cost, sensitivity, and robustness. Therefore, we developed a whole-cell *Escherichia coli* lactate biosensor based on the *lldPRD* operon and successfully used it to monitor lactate concentration in mammalian cell cultures. Using real samples and analytical validation we demonstrate that our biosensor can be used for absolute quantification of metabolites in complex samples with high accuracy, sensitivity and robustness. Importantly, our whole-cell biosensor was able to detect lactate at concentrations more than two orders of magnitude lower than the industry standard method, making it useful for monitoring lactate concentrations in early phase culture. Given the importance of lactate in a variety of both industrial and clinical contexts we anticipate that our whole-cell biosensor can be used to address a range of interesting biological questions. It also serves as a blueprint for how to capitalise on the wealth of genetic operons for metabolite sensing available in Nature for the development of other whole-cell biosensors. This article is protected by copyright. All rights reserved

Keywords: Synthetic Biology, Whole-cell bacterial biosensor, Biopharmaceutical processing, *lldPRD* operon , lactate / lactic acid

Introduction

Biopharmaceuticals are protein-based drugs, such as monoclonal antibodies and enzymes, often with sugars attached that affect their efficacy and mechanism of action. They include blockbuster drugs such as Humira, Avastin, and Herceptin, with the top five selling drugs generating nearly 70 billion USD in revenue in 2013 alone. Currently, biopharmaceuticals are predominantly produced using mammalian cell cultures to ensure appropriate glycosylation, with Chinese Hamster Ovary (CHO) cells the most prevalent host for the production of glycoproteins in industry today (Kyriakopoulos and Kontoravdi 2013; Walsh 2014).

The measurement of key metabolites during biopharmaceutical production is essential to inform the design of medium and feeds and to optimise cell culture conditions for increased productivity. It is also routinely used to monitor and control the trajectory of cultures during production to prolong the life of cells and maximise the yield of the product (Constantinou and Polizzi 2013; Wuest et al. 2012). One particular metabolite of interest is lactic acid (lactate), a metabolic waste product produced as a byproduct of glycolysis. High concentrations of lactate can negatively affect cell growth and productivity (Li et al. 2010; Zhou et al. 2011) and can, via acidification of the medium, cause changes in glycosylation patterns (Yoon et al. 2005).

Lactate concentrations can exhibit very complicated dynamics across different culture phases as it is both produced, and later, consumed by Chinese Hamster Ovary cells (Bertels et al. 2012). Recent work has also shown that lactate metabolism can also be an indicator of bioreactor performance during an individual run, with ability to discriminate between low and high productivity runs, even as early as the inoculum train. This suggests lactate accumulation could be a target for remedial action if cells appear to be headed towards a low productivity run. Interestingly, the analysis suggests that interventions must occur before 70 hours into the production run to have the most chance of being successful (Le et al. 2012).

Given the importance of lactate as a metabolite, the development of biosensors to detect lactate in samples has been an active area of research. In particular, a number of biosensors based on enzymes such as lactate dehydrogenase or lactate oxidase have been developed. The output signals from these biosensors vary, but include spectroscopic (Shah et al. 2007), fluorescent (D'Auria et al. 2000; Li et al. 2009; Trettnak and Wolfbeis 1989), chemiluminescent (Martinez-Olmos et al. 2009; Roda et al. 2014; Zhou et al. 2014), or amperometric detection (Haccoun et al. 2006; Hasebe et al. 2005; Male et al. 1997; Parra et al. 2006; Weber et al. 2006), with the latter as the dominant detection modality (recently reviewed in (Rathee et al. 2016)). Novel methods of immobilisation to improve detection have also been explored (Haccoun et al. 2006; Hasebe et al. 2005; Pagan et al. 2015; Tu et al. 2016; Xu et al. 2005; Yang et al. 2008). Although whole-cell biosensors for lactate have not been previously reported, San Martin et al (2013) developed a Förster Resonance Energy Transfer (FRET) protein-based biosensor, which was expressed in mammalian cells in order to directly monitor changes in intracellular lactate (San Martin et al. 2013). The disadvantage of this approach is that it requires cells to express an additional protein, which would not be desirable in a biopharmaceutical process as it may negatively impact the ability of cells to express the recombinant product.

Current standard analytical methods for measuring lactate concentrations rely largely on HPLC and electrochemical methods, which are limited in terms of cost, sensitivity, and robustness (Bracewell and Polizzi 2014). The industry standard instrument, the Bioprofile Analyzer, quantifies lactate concentration using an amperometric immobilised enzyme biosensor (Biomedical 2016). Even though this analysis can be automated (Deraus et al. 2010), a large sample volume is required (at least 1 mL) and the lower limit of detection is in the millimolar range, limiting its application to later stages of culture. Enzymatic lactate detection kits, based on the lactate oxidase enzyme are also commercially available. These have a higher sensitivity for detection of lactate, but are very costly, largely due to the costs associated with purification of the enzyme.

Nature offers a potential solution in the form of bacterial genetic operons, which are designed to sense the concentration of important metabolites in the environment and activate gene expression in response (Goers et al. 2013). The sensitivity of such systems is very high—often compounds are detected at micromolar or even nanomolar concentrations (Goers et al. 2013) and a wealth of such systems that can detect important metabolites for mammalian cell culture such as sugars, amino acids, and metabolic waste products have been identified (e.g. (Engels et al. 2008; Pittard et al. 2005; Senior 1975)). Therefore, bacterial genetic operons represent a rich source for designing new whole-cell biosensors for application in industrial bioprocessing. In particular, operons to utilise lactate as a carbon source have been described in *E. coli* (Aguilera et al. 2008), *Corynebacterium* (Gao et al. 2008), *Pseudomonas* (Gao et al. 2012) and *Bacillus subtilis* (Chai et al. 2009). Any of these can be used to design a whole-cell biosensor by co-opting the existing genetic elements to sense lactate and activate gene expression and using them to express a reporter protein in response to lactate induction.

Research in whole-cell biosensors began in the 1970s (Ames et al. 1973), but has recently undergone a resurgence through the application of synthetic biology methodologies to rapidly and reliably engineer new sensors (van der Meer and Belkin 2010). The simplest whole-cell biosensors are microorganisms, either natural (e.g. *Vibrio fischeri*, which is naturally luminescent (Bulich and Isenberg 1981) or engineered (Belkin et al. 1996; Quillardet et al. 1982), used to identify the presence of toxic molecules via a disturbance in the expression of a reporter protein. More sophisticated whole-cell biosensors can be designed to sense particular compounds of interest including heavy metals (Aleksic et al. 2007; Prindle et al. 2012; Wong et al. 2015), medically relevant molecules (Auslander et al. 2014), and other small molecules (Mustafi et al. 2011; Rogers et al. 2015; Tessaro et al. 2012) and activate the expression of a reporter gene in response (thereby 'lighting up' in the presence of the analyte). Cell arrays can be used to detect multiple compounds from the same sample or to distinguish between related compounds (Kabessa et al. 2013; Kim et al. 2013). Although they have largely been discussed in the context of sensing environmental

pollutants (Aleksic et al. 2007; Belkin 2003; Checa et al. 2012; Prindle et al. 2012), in principle, whole-cell biosensors can be designed to detect any compound of interest. Whole-cell biosensors are not limited to bacteria, but can include any type of cell such as mammalian (Auslander et al. 2014; Hillger et al. 2015), plant (Wong et al. 2015), algae (Podola and Melkonian 2003), fungi (Weitz et al. 2002), etc.

Whole-cell biosensors have several advantages over other types of sensing formats. As a living organism, they are self-renewing and obviate the need for purification of enzymes, leading to low reagent costs. They can be grown in large quantities from a single cell if required, meaning that they are portable. Crucially, because they are based on genetic operons, the sensitivity of whole-cell biosensors is often very high (Goers et al. 2013).

However, applying whole-cell biosensors in complex media is difficult due to interference from other compounds in the mixture and low signal-to-noise ratios (Courbet et al. 2015).

Previous reports of whole-cell biosensors often lack cross-validation with existing measurement methods (Mustafi et al. 2011) or focus on the detection of compounds in 'mock' samples of low complexity (Prindle et al. 2012). Moreover, to date there have been very few reports of whole-cell biosensors to measure metabolites that are also made by the biosensor host (Bertels et al. 2012; Mustafi et al. 2011; Tessaro et al. 2012). Instead, efforts have primarily focused on the detection of exogenous compounds (e.g. heavy metals or pollutants) (Aleksic et al. 2007; Belkin 2003; Checa et al. 2012; Prindle et al. 2012) in order to avoid crosstalk with the host organism. However, with the advent of synthetic biology tools and techniques such as rapid and cost-effective DNA assembly and automated part characterisation, it is now possible to design biosensor variants where cross-talk with endogenous metabolites is minimised (Wang et al. 2013).

The application of whole-cell biosensors to bioprocess monitoring would require quantification of the concentration of metabolites as obtained by the Bioprofile Analyser, HPLC and other analytical methods. However, this type of characterisation is not routinely conducted in the development of new biosensors and is complicated by the complexity of the sample background. Using iterative cycles of characterisation in increasingly complex

environments, we developed a workflow to achieve robust quantification of lactate in mammalian cell culture samples. Crucially, we used automation to control the environment, which will increase reproducibility and limit the effects of crosstalk with the host organism by keeping cells in the same metabolic state across different experiments. We used two genetic variations of the natural *E. coli* lldPRD lactate operon (Figure 1) to construct our biosensor and tested its performance in a wide range of conditions. We were able to demonstrate that our whole-cell bacterial biosensor shows a measurable response to lactate in a variety of different cell culture media. Further, we show that the concentration of lactate in real cell culture samples can be accurately quantified when validated against existing analytical methods. Finally, our biosensor has a lower limit of detection than the Bioprofile Analyser and is less expensive per sample analysed than commercially available lactate oxidase assay kits. Therefore, it can be used for routine monitoring of cell culture trajectories, even in early phases of culture where lactate concentrations may be less than 1 mM.

Materials and Methods

Molecular Biology

E. coli DH5 α (α -Select, Bioline) cells were used for expression and characterisation of all bacterial constructs. All plasmids were based on the pSB1A2 plasmid backbone and pre-existing BioBrick parts were obtained from the distributions of the iGEM Registry of Standard Biological Parts (partsregistry.org). The lldPRD promoter and the lldR gene (Supplementary Table 1) were amplified from the *E. coli* genome via PCR using the primers listed in Supplementary 2, which incorporate the BioBrick prefix and suffix. Constructs were assembled using the BioBrick Assembly standard. Plasmids were verified by DNA sequencing (Source Biosciences).

Biosensor Characterisation

Biosensors were characterized using an Aviso-GmbH Theonyx robotic platform linked to a plate reader (Synergy HT, Biotek) and a shaking incubator (Ventura 2000, Mikura). Single colonies containing the biosensor plasmid or a control plasmid lacking the lldPRD promoter

were used to inoculate 1 ml of LB medium containing 100 µg/mL ampicillin (Sigma Aldrich) and grown for approximately 6 hours during the day at 37°C with shaking at 250 rpm. The starter culture was diluted 1:100 into 5 ml of M9 minimal medium (1x M9 salts, 2 mM MgSO₄, 0.1 mM CaCl₂, 0.34 g/L thiamine hydrochloride, 1 g/L NH₄Cl and 0.4% glycerol or glucose as appropriate) containing 100 µg/mL ampicillin and grown overnight at 37°C with shaking. The next morning, cultures were transferred to 96-well plate format with 200 µl per well. Using the Theonyx platform, the cells then underwent dilution into fresh M9 medium containing 100 µg/mL ampicillin at a cell density of 140 ng/µl (OD₆₀₀ of 0.15 in the attached plate reader) in a total volume of 100 µl. This was followed by an outgrowth phase of approximately 2.5 h to allow cells to attain exponential growth. Subsequently, 25 µl of sample were added. For characterisation, mock samples containing varying concentrations of L-lactate, D-lactate, or pyruvate (all Sigma Aldrich) were mixed in appropriate background solvent of M9 minimal medium, CD-CHO (Invitrogen, UK), CD-CHO + HT supplement (11067-030, Gibco) + 8 mM glutamine (Invitrogen, UK), DMEM (SIGMA, 51449C), DMEM containing phenol red (Sigma, D6546), or DMEM containing 10% (v/v) calf bovine serum (ATCC, 30-2030). For quantification of mammalian cell culture samples, culture supernatant taken from growing cultures described below were added and a standard curve containing known concentrations of lactate in the same background solution was included to create a transfer function from which lactate concentration was calculated. The OD₆₀₀ and GFP fluorescence ($\lambda_{\text{excitation}}$ 485/20 nm, $\lambda_{\text{emission}}$ 528/20 nm) were measured every 10 min. All cell growth and characterisation on the robotic platform was done at 37°C with shaking at 700 rpm.

Analysis of Biosensor Data and Calculation of Concentration Estimates

Raw fluorescence data was processed in R 3.1.2 as follows. A spline interpolation was fit to each fluorescent time course to reduce the impact of equipment error on the values. To prevent over-fitting of the spline and ensure that the interpolation calculated was a strong predictor of the original time course, an exhaustive cross validation was carried out to

choose the ideal smoothing value for each. Leave-p out cross validation was used, where $p=2$. Subsequently, Microsoft Excel was used for linear regression of the fluorescence versus corrected OD_{600} of the control cells was used to fit an equation, which was used to subtract cellular autofluorescence from biosensor fluorescence measurements as follows:

$$Fl_{i,corrected} = Fl_i - ((m * OD_i) + c)$$

where m and c represent the slope and intercept of the trendline, respectively

The fluorescence rate of change (GFP synthesis rate) was calculated by dividing the difference in fluorescence between adjacent time points by one half of the difference in OD_{600} between the same time points. All samples were normalised to the biosensor cultures without addition of lactate. The transfer functions were created using data from 150 min post addition of the sample to the biosensor cultures using the GraphPad Prism nonlinear regression (sigmoidal, 4PL) function to fit the data.

To compare the biosensors under different conditions, the fold-induction, goodness-of-fit of the transfer function, limit of detection, and sensitivity were calculated. All values were calculated at 150 minutes, except for the experiment where cells were grown directly in CD-CHO medium, where 90 minutes was used due to lack of separation of the lactate concentrations at 150 minutes. The fold-induction was calculated as the fluorescence rate of change of samples containing 14 mM lactate divided by the fluorescence rate of change of samples with no added lactate. The goodness-of-fit and the sensitivity (also called the 'Hill slope' or the gradient of the Hill function) were calculated using GraphPad Prism. The limit of detection reported is the lowest lactate concentration that gave a response different from background at the 150 min timepoint. The actual lowest limit of detection may be lower, but was not tested here or appears at a different time point.

To calculate lactate concentrations in mammalian cell culture samples, GFP synthesis rate values from a set of standards from the same experiment were chosen to make a standard curve and the GFP synthesis rate values from biosensors incubated with the cell culture

samples were used to interpolate (sigmoidal, 4PL) the lactate concentrations using the GraphPad Prism software.

Mammalian cell culture

Hybridoma flask cultures: ATCC-CRL1606 murine hybridoma, producing a mAb (HFN7.1) against human fibronectin were cultured in 500 ml Erlenmeyer flasks (Corning, UK) at 37°C in an atmosphere containing 5% CO₂ on an orbital shaking platform rotating at 125 rpm. The basal growth medium was 100 ml glutamine-free DMEM (Sigma, D6546) supplemented with 10% (v/v) calf bovine serum (ATCC, 30-2030) and 0 mM -10 mM Gln (Sigma, G3126), which was sterilised by filtration.

CHO-S flask cultures: The CHO-S cell line expressing a truncated glycosyltransferase-ECFP-EYFP fusion protein was a kind gift from Antony Constantinou (CSynBI, Imperial College London). The CHO-S cells were cultured in a 250 ml Erlenmeyer flask (Corning, UK) at 37°C in an atmosphere containing 5% CO₂ on an orbital shaking platform rotating at 125 rpm. The basal growth medium was 100 ml CD-CHO medium (Invitrogen,UK), supplemented with 8 mM glutamine (Invitrogen, UK) and 1X HT supplement (11067-030, Gibco).

GS-CHO bioreactor cultures: The GS-CHO 46 cell line, kindly provided by Lonza Biologics (Slough, U.K.), was cultured at 140 rpm in humidified 37°C incubator with 8% CO₂ supply. Cells were sub-cultured in fresh CD-CHO medium (Life Technologies, Paisley, UK) every three days at a seeding density of 2×10^5 viable cells/mL and transferred into the bioreactor system on the fourth cell passage. 25 µM L-Methionine sulfoximine (MSX) (Sigma-Aldrich, Dorset, UK) was added to the medium in first and second passages only.

The 3L Mobius® CellReady bioreactor (EMD Millipore, Bedford, MA) was inoculated at a seeding density of 3×10^5 cells/mL in an initial culture volume of 1.2 L. The culture was mixed using an in-house up-pumping marine impeller rotating at 100 rpm. The culture was controlled with the my-control unit (Applikon Biotechnology, Schiedam, Netherlands) at a temperature of $37 \pm 0.5^\circ\text{C}$ using a heating blanket, and at a pH of 7.0 ± 0.1 using CO₂

supplementation and 100 mM NaHCO₃/ 100 mM Na₂CO₃ alkali solution (Sigma-Aldrich, Dorset, UK). Dissolved oxygen tension (DOT) was set at a minimum of 30% with oxygen supply. Culture temperature, pH and DOT were monitored online using BioExpert software, version 1.1X (Applikon Technology, Schiedam, Netherlands). The culture was supplemented with CD EfficientTM Feed C AGTTM (Life Technologies, Paisley, UK) at 10% of culture volume on alternate days starting from day 2. 5% w/v antifoam C (Sigma-Aldrich, Dorset, UK) was added when foaming was observed. The working volume was maintained between 1.0 and 1.3 L by drawing out excess culture fluid. The cell culture was harvested on day 16.

Cross validation of lactate concentration

Samples of 1-5 ml as required were taken from the culture, the cells were removed by centrifugation at 800 rpm for 5 minutes and the supernatant transferred to a fresh tube and stored at -20°C before further analysis. The Bioprofile[®] 400 Analyzer (Nova Biomedical) was used to measure ammonia, glucose, glutamine, glutamate and lactate concentrations according to the manufacturer's guidelines. Lactate concentrations in samples were also analysed using a colourimetric, enzymatic lactate kit (MAK064, Sigma) according to the manufacturer's instructions.

Results

Initial testing of biosensor designs

We designed and constructed two whole-cell lactate biosensors (Figure 2) and compared their performance in samples of M9 minimal medium with glycerol as the carbon source spiked with different concentrations of L-lactate. The first biosensor expressed green fluorescent protein (GFP) under the control of the *lldPRD* promoter (Aguilera et al. 2008). Characterisation of this construct showed a very limited lactate response (Figure 2a, Table 1) with only 2-fold induction and a high level of leaky expression in the absence of lactate. For example, the fluorescence rate of change of the biosensor at 150 minutes is 1402.8 arbitrary units versus 25.3 arbitrary units in the improved biosensor design discussed below (data not shown), indicating that the genomically-expressed *lldR* is not sufficient to repress

reporter expression in the absence of inducer. Negative values for fluorescence and GFP synthesis rates result from the data analysis procedure where values are normalised to the biosensor cultures with no added lactate. In the case of this biosensor design, low concentrations of lactate are not distinguishable from zero lactate and, by random chance, can give fluorescence values below that of the culture without added lactate.

An improved version of the biosensor contained an additional module overexpressing the lldR transcription factor under a constitutive promoter in order to lower the baseline GFP expression. Characterisation of this biosensor in M9 minimal medium with added lactate showed a clear lactate response with good separation between different concentrations (Figure 2b, Table 1). The new biosensor had a 60-fold induction and a lower limit of detection that was an order of magnitude lower. Thus, the version of the biosensor with the additional module for overexpression of lldR was used in all further studies.

The biosensor sensitivity to the growth environment was also tested (Supplementary Figure 1a, Table 1). When cells were grown in M9 minimal medium with glucose instead of glycerol as the carbon source, the biosensor is still able to detect lactate with very similar lower limit of detection. However, the induction of the response is nearly 70% lower suggesting the environment does influence sensing capabilities.

We also analysed the biosensor response to two structurally similar metabolites. The biosensor was found to show a very small response to D-lactate (Supplementary Figure 1b, Table 1) with a sensitivity decreased by 91% and a lower limit of detection of 0.5 mM.

However, its response to pyruvate was within the inherent measurement error and therefore negligible (Supplementary Figure 1c, Table 1). It is possible that the response to D-lactate is due to small amounts of L-lactate contamination in the D-lactate, which is only 99% pure.

The fact that the biosensor can distinguish between L-lactate and pyruvate is important for cell culture studies where pyruvate is often present either as a medium additive or additional metabolite produced by the mammalian cells during metabolism.

Developing a workflow for quantification

When biosensor cells were cultured directly in CD-CHO, a widely used chemically defined mammalian cell culture medium, the lower limit of detection was decreased by a factor of 4 and the GFP synthesis profile showed only a short burst which quickly returned to baseline (Supplementary Figure 2a), most likely due to the rapid growth rate of the cells in this medium. In contrast to the robust signals in M9 medium, the low signal-to-noise ratio of cells cultured in CD-CHO (fold induction of 2.8 versus 60, Figure 3A, Table 1) made it difficult to distinguish between low concentrations of lactate. This is analogous to the observations of Courbet et al. where signals were damped in urine and serum due to the complexity of the medium (Courbet et al. 2015). Although CD-CHO is chemically defined, it contains more than 70 different compounds in its formulation (European Patent Application, EP 1 482 031 A1, 2004) including sugars, amino acids, and lipids.

Therefore, we developed a workflow where the biosensor cells were initially cultured in M9 medium and a mock sample of mammalian cell culture medium supplemented with a known concentration of lactate was added to the cells. By minimising the volume of the cell culture sample to 20% of the total volume (5-fold dilution), the biosensor cells were able to detect lactate spiked into CD-CHO medium (Figure 3b, Table 1), CD-CHO medium containing HT supplement and glutamine (Figure 3c, Table 1), Dulbecco's Modified Eagle Medium (DMEM) (Figure 3d), DMEM containing the pH indicator phenol red (Figure 3e, Table 1) and DMEM containing 10% serum, a common culture additive (Figure 3f, Table 1). Although the dynamic (Supplementary Figure 2a-f) and static (Figure 3a-f) response of the biosensor to lactate changes in different media, a lactate response is always measurable, and therefore, quantifiable using a standard curve.

In all conditions, the lactate response initially increases, but levels off over time. Further investigation suggests that this is as a result of lactate concentration in the biosensor cultures decreasing over time (Supplementary Figure 3), most likely due to lactate being taken up and metabolised by the bacterial cells as part of the sensing process. The *E. coli* strain used as a biosensor host retains the genomic copy of LldP (lactate permease) and

LldD (lactate dehydrogenase), which allow for the transport and utilisation of lactate, respectively, by the biosensor cells when the operon expression is induced (Figure 1).

Utility Testing with Cell Culture Samples and Cross Validation

We then undertook a series of cell culture experiments to see if the biosensor could be used to quantify lactate metabolism in real cultures. When cells are inoculated into the medium, they use the nutrients provided, grow, produce additional metabolites, and express recombinant protein, further increasing the complexity of the sample. To test the performance of the biosensor in a range of industrial conditions, we used three experimental scenarios: (i) hybridoma cells grown in shake flasks in DMEM containing phenol red and supplemented with 10% serum and different concentrations of glutamine in the medium to optimise antibody expression level, mimicking the type of medium optimisation experiment that is commonly employed in industry, (ii) a CHO-S cell line expressing a fusion protein grown in batch culture in CD-CHO medium in shake flasks, and (iii) a GS-CHO cell line expressing a monoclonal antibody grown in a fed-batch bioreactor with CD-CHO medium and fed with CD Efficient™ Feed C AGT™ on alternate days beginning on day 2. Samples were taken over time and simultaneously analysed using our biosensor workflow, the Bioprofile® Analyzer, and a lactate oxidase enzymatic assay to enable cross validation of the results (Figure 4a).

Significantly, our results show that the biosensor can accurately determine lactate concentrations in samples from a range of different conditions relevant to the biopharmaceutical industry (Figures 4b-4d, Table 1). Figure 4b shows a snapshot of lactate concentration after 100 hours of hybridoma culture in DMEM supplemented with different concentrations of glutamine. Similar types of experiments are done in industry to optimise the formulation of media. Cell number and antibody production vary across the media with different glutamine concentrations (Supplementary Figure 4) leading to different sample backgrounds, yet the biosensor accurately quantifies the extracellular concentration of lactate when compared with the other assay methods. Similarly, the biosensor provides accurate quantification of lactate accumulation over time in batch culture of CHO-S in shake

flasks (Figure 4c, Table 1) despite changing concentrations of other metabolites (Supplementary Figure 5). Finally, samples from GS-CHO cell cultures grown in fed-batch bioreactor (Figure 4d, Table 1) accumulated much higher concentrations of extracellular lactate over the course of a 16 day culture, which when combined with pH control would lead to very high osmolarity of the samples (Supplementary Figure 6). Nonetheless, quantification with the biosensor was in good agreement with the other two methods, except for the final sample from 16 days of culture, where the biosensor overestimated the concentration of lactate by a factor of ~1.5 compared to the average value from the two established methods (Figure 4d). This overestimate is likely due to the effects of high osmolarity (> 700 mmol/L) on cells. Indeed antibody titres have actually decreased on day 16 compared to their maximum at day 12 (Supplementary Figure 6), suggesting significant amounts of cell lysis have occurred, which would vastly increase the complexity of the sample. Overall, our biosensor has a lower limit of detection for lactate than the BioProfile[®] Analyzer, which is the current method of choice for metabolite analysis of bioprocessing samples in industry. It is able to measure lactate concentrations as low as 50 μ M in M9 medium, which is approximately 100-fold lower than the lower limit of detection of the BioProfile[®] Analyzer. Even when samples are in complex backgrounds such as CD-CHO or DMEM with phenol red, the lower limit of detection was still 100 μ M.

Discussion

We have created a functional whole-cell lactate biosensor based on the *E. coli* lldPRD operon and have demonstrated that this biosensor can be used for monitoring lactate in complex industrial contexts, specifically mammalian cell cultures producing a recombinant protein, with high accuracy and a lower limit of detection than the industry standard method.

In fact, our biosensor was able to detect lactate at a concentration up to two orders of magnitude lower than the Bioprofile analyser (100 μ M versus 1-2 mM) in samples from real cell cultures. To our knowledge this is the first demonstration of robust quantification of samples in complex backgrounds using a whole-cell biosensor. In addition, our biosensor

has a cheaper cost per sample analysed than the commercial lactate oxidase enzyme assay kit at (approximately 3% of the cost at £13 versus £403.50 per 96-well plate analysed, Supplementary Methods).

The work described here capitalises on the synthetic biology principles of automation and characterisation. We used iterative cycles of characterisation of biosensor behaviour in increasingly complex conditions to develop a workflow for robust quantification of metabolites in real samples. The workflow we developed can serve as a blueprint for developing whole-cell biosensors for other metabolites, many of which are important in health and disease (Wishart 2016), as well as industrial contexts. In this respect, we have shown that biosensors need not be limited to sensing exogenous compounds, which will enable biologists to fully capitalise on the wealth of natural operons available for biosensing to design biosensors for broader variety of compounds. Automation is useful to increase the reproducibility of measurements, permit the detection of small responses, and facilitate the comparison of datasets across different experiments. We also found that culturing the cells in a single base medium (M9 medium) and adding samples of interest gives more robust growth dynamics and better detection ability across many different kinds of samples.

Our whole-cell lactate biosensor can be applied towards a broad range of biological questions, as lactate is widely used in the food and pharmaceutical industries, and also in the production of polylactic acid, a biodegradable plastic alternative made from renewable resources (Wang et al. 2015). It is also an important clinical biomarker for altered metabolism and many physiological conditions, including inflammation (Haas et al. 2015), cardiovascular diseases (Zhang et al. 2015) and cancer (San Martin et al. 2013; Vlachostergios et al. 2015). Finally, in combination with advances in 3D printing of cells (Cao et al. 2016), we envision that the biosensor could also be used to gain spatial information from adherent cell cultures, which would facilitate the detection of differences in metabolism within individual cells, potentially enabling the diagnosis of diseases such as cancer, arteriosclerosis, and diabetes based on metabolic signatures within cells (Galluzzi et al. 2013; Wishart 2016).

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Nomenclature

CD-CHO- Chemically defined medium for Chinese Hamster Ovary cell lines

CHO- Chinese Hamster Ovary cells

DMEM- Dulbecco's Modified Eagle Medium

GFP- Green Fluorescent Protein

HPLC- High Performance Liquid Chromatography

MSX- L-Methionine sulfoximine

PBS- Phosphate buffered saline

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List of Figures and Figure Legends

Figure 1: Schematic of the LldPRD operon and biochemical mechanism (a)

Organisation of the LldPRD operon. O1 and O2 represent the operator sites in the LldPRDp promoter. The three genes in the operon are (from left to right) LldP: lactate permease to allow lactate transport, LldR: regulatory protein, LldD: lactate dehydrogenase for lactate utilisation. (b) Diagram of the mechanism of lactate-dependent induction of LldPRD operon in *E. coli* cells. Top: In the absence of lactate, dimers of LldR bind to the operator sites in the LldPRDp promoter and form a tetramer, sequestering the DNA and preventing transcription of the operon. Bottom: Lactate enters the cell via the glycolate permease (GlcA) or LldP and interacts with the LldR regulator protein. The LldR dimer bound to O2 dissociates when bound to lactate, but the dimer bound to O1 becomes a transcriptional activator that promotes transcription of the operon when lactate binds.

Figure 2: Characterisation of two different biosensor designs (a) Biosensor without overexpression of LldR (b) Biosensor with constitutive overexpression of LldR regulator. In each panel: (Top): Diagram of the genetic construct. (Bottom): Characterisation data at different concentrations of lactate: Fluorescence normalised by OD600, Fluorescence rate of change, and Transfer function from data at 150 minutes. Error bars represent the standard deviation of 6 measurements (2 technical replicates of 3 biological replicates). The negative fluorescence and fluorescence rate of change values are a result of the normalisation of data to the biosensor culture without added lactate.

Figure 3: Measurement of lactate in mammalian cell culture media (a) Biosensor response to L-lactate when grown in CD-CHO medium. (b-f) Biosensor response to L-lactate spiked into different mammalian cell culture media (b) CD-CHO medium, (c) CD-CHO medium with added HT supplement and 8 mM glutamine, (d) DMEM medium, (e) DMEM medium with added phenol red, (f) DMEM medium with added serum. Data are presented as transfer functions from data at 150 minutes. For the time course data please see

Supplementary Figure 2. Error bars indicate the standard deviation of 6 measurements (2 technical replicates each of 3 biological replicates).

Figure 4: Quantitative analysis of cell culture samples using lactate biosensor with

cross-validation against existing methods. (a) Experimental workflow. Samples were collected from mammalian cell cultures and analysed for lactate content using the lactate

biosensor (blue), a BioProfile® Analyzer (green) and a lactate oxidase enzymatic assay

(orange). (b) Samples from CRL1606 hybridoma cultures in DMEM containing phenol red

and 10% serum. Six hybridoma cultures were run in parallel with varying starting

concentrations of glutamine starting as indicated in order to maximise antibody titre. The

samples shown here were collected after 100 h of mammalian cell culture. (c) Time course

samples from a CHO-S batch shake flask culture in CD-CHO medium supplemented with 1X

HT supplement and 8 mM glutamine. (d) Time course samples from a GS-CHO fed-batch

bioreactor culture in CD-CHO supplemented with Feed C every two days. Error bars

indicate the standard deviation of 6 measurements (2 technical replicates each of 3

biological replicates) for biosensor measurements, 2 technical replicates for the enzyme

assays and a single measurement for the BioProfile® Analyzer. For further metabolite data

for these mammalian cell cultures please see Supplementary Figures 4-6. Missing values

are below the limit of detection of the BioProfile® Analyzer.

Table 1: Comparison of biosensor across different growth media and sample composition

Biosensor	Growth condition	Sample background	Fold-Induction	Transfer function Goodness-of-fit	Limit of Detection	Sensitivity (Hill Slope)
Without IIdR	M9 with glycerol	M9 with glycerol	2.02	0.905	0.5	1.54
With IIdR	M9 with glycerol	M9 with glycerol	59.67	0.999	0.05	4.00
With IIdR	M9 with glucose	M9 with glucose	18.63	0.981	0.05	4.62
With IIdR	M9 with glycerol	D-lactate in M9 with glycerol	1.95	0.974	0.5	0.34
With IIdR	M9 with glycerol	pyruvate in M9 with glycerol	0.73	0.837	0.5	-11.57
With IIdR	CD-CHO	CD-CHO*	2.78	0.971	0.2	0.45
With IIdR	M9 with glycerol	CD-CHO	8.25	0.999	0.1	0.09
With IIdR	M9 with glycerol	CD-CHO + HT supplement, 8 mM gln	11.96	0.992	0.05	0.28
With IIdR	M9 with glycerol	DMEM	16.75	0.771	0.001	0.66
With IIdR	M9 with glycerol	DMEM + phenol red	23.11	0.950	0.1	0.29
With IIdR	M9 with glycerol	DMEM + serum	7.90	0.947	0.05	0.50

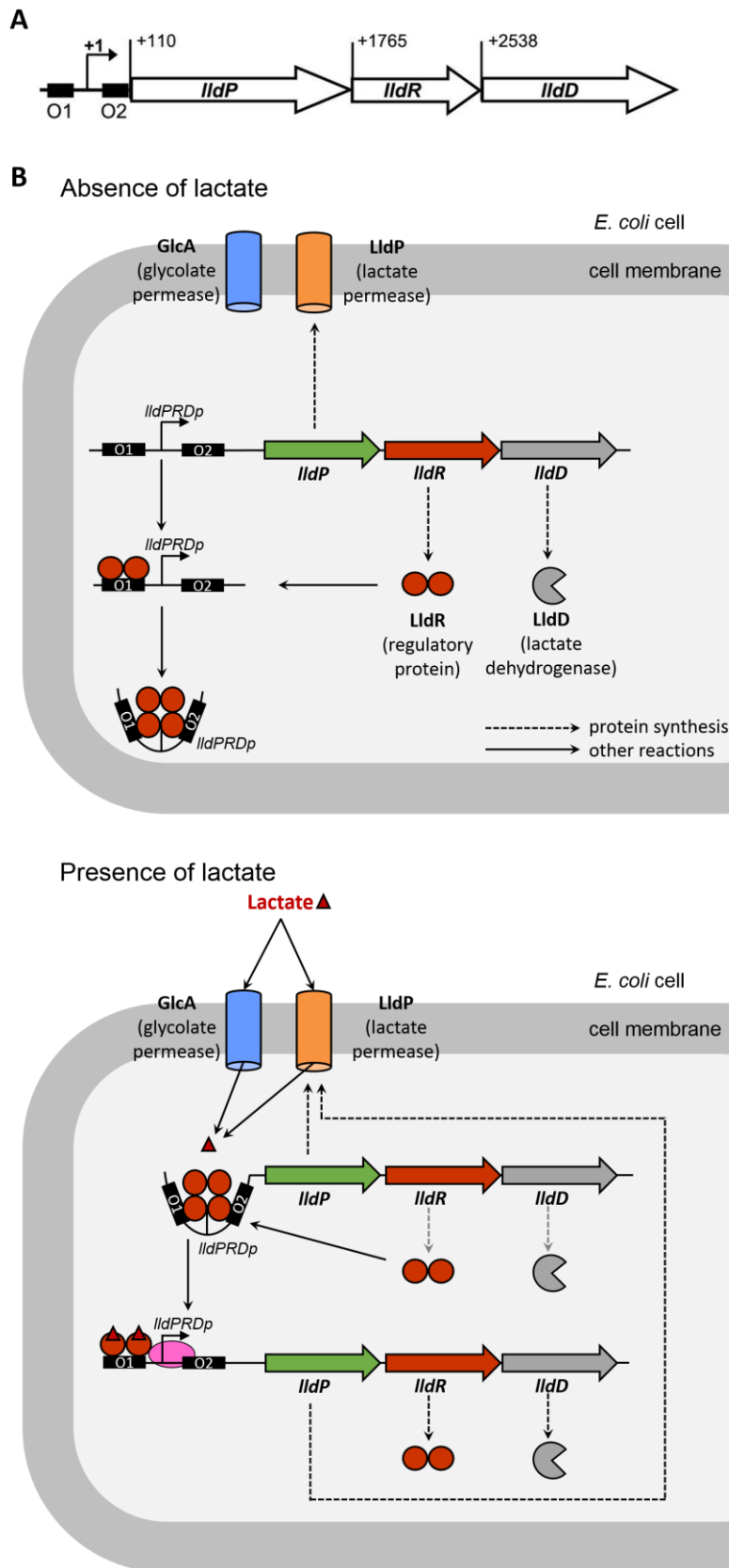


Figure 1

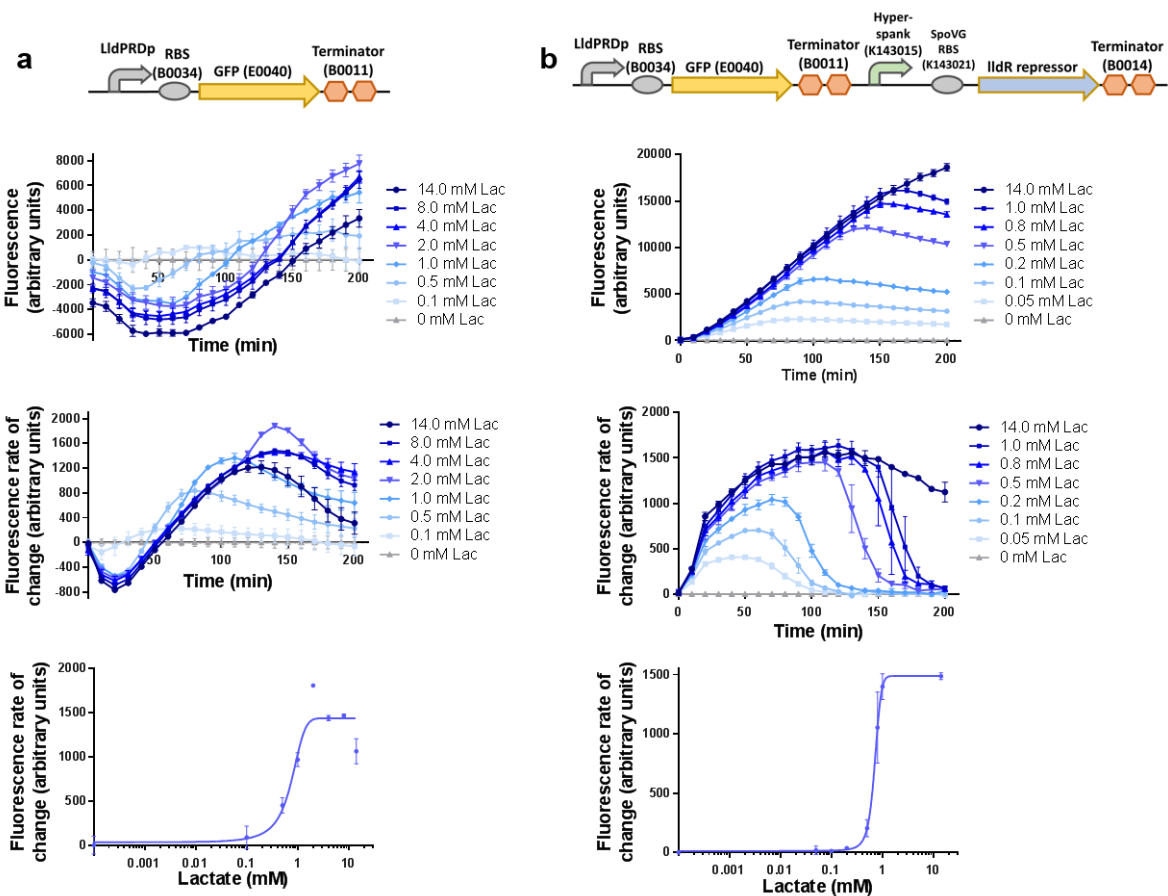


Figure 2

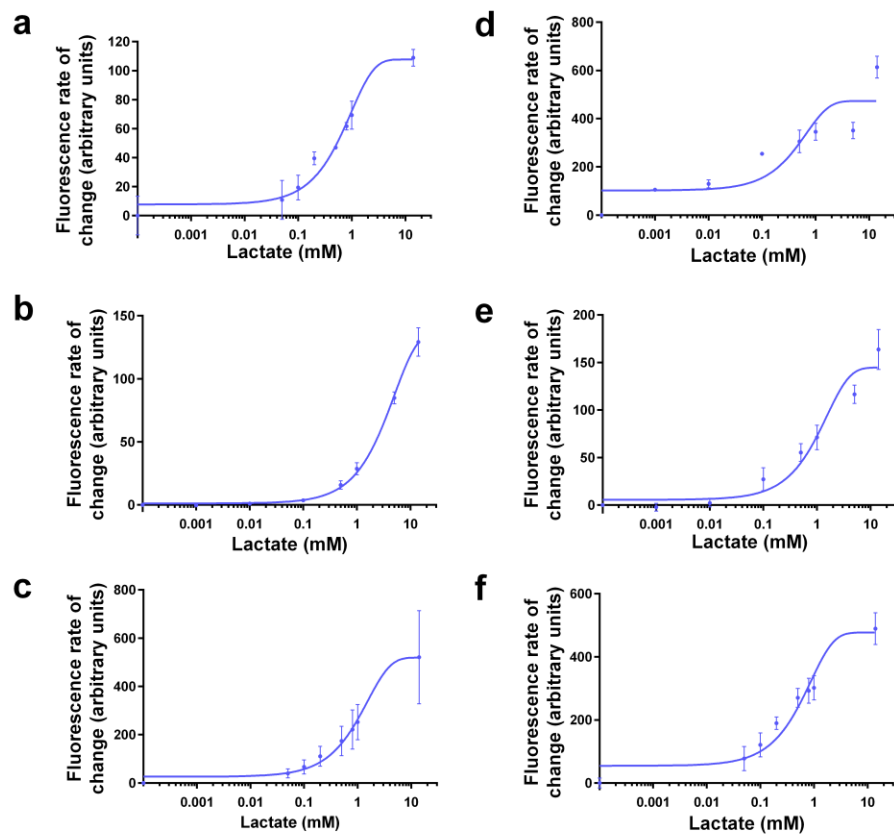


Figure 3

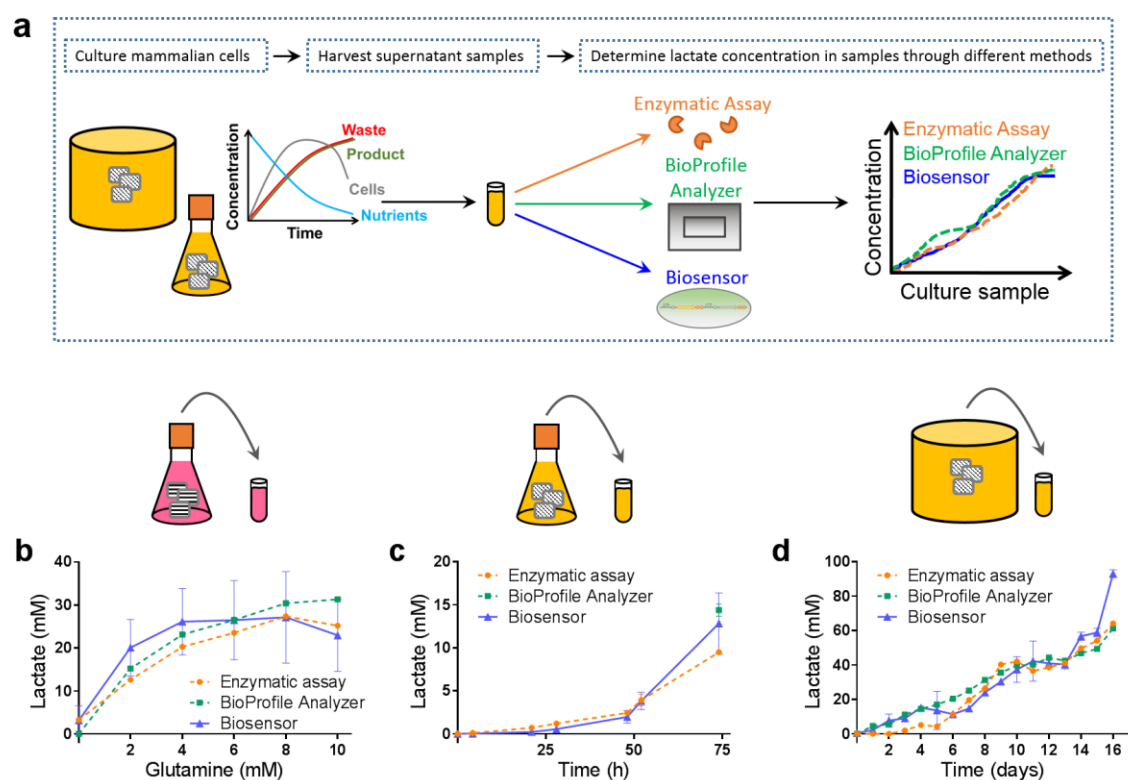


Figure 4