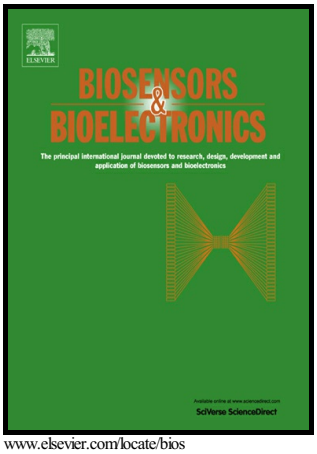

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Amplification of electrochemical signal by a whole-cell redox reactivation module for ultrasensitive detection of pyocyanin

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Abstract:

A bioelectrochemical sensing system based on a novel whole-cell redox reactivation module for ultrasensitive detection of pyocyanin (the biomarker of *Pseudomonas aeruginosa* infections) was developed. The electroactive bacteria mediated redox reactivation module was constructed using *Shewanella oneidensis* MR-1 cells as the bioelectro-catalyst and lactate as the electron donor and was employed to regenerate reductive pyocyanin from its oxidative state, which enabled pyocyanin molecule repeatedly registered by the electrode. Uniquely, with this redox reactivation module, the electrochemical response of pyocyanin was amplified about 405 times (1.3 μA/nM *vs.* 3.2 nA/nM). Thus, an ultrasensitive bioelectrochemical sensing system for pyocyanin quantification was developed by integrating the pyocyanin reactivation module with conventional electrochemical detection system.

Remarkably, with the developed biosensing system, an extremely low LOD of 47±1 pM was reached. Additionally, this biosensing system showed excellent resistance to interferences from human fluids or bacterial contamination. This work provided a simple, ultrasensitive and robust tool for pyocyanin detection, and more importantly, demonstrated a new dimension for electrochemical signal amplification in biosensing.

Key words: Bioelectrochemistry; Biosensor; *Pseudomonas aeruginosa*; Pyocyanin; Signal amplification

1. Introduction

Pseudomonas aeruginosa is one of the most ubiquitous pathogens that cause serious diseases including cystic fibrosis, sepsis, pneumonia and fester otitis media (Folkesson et al. 2012; Hatchette et al. 2000; Laughlin et al. 2000; Neeff et al. 2016). With its inherited and acquired resistance to a wide range of antibiotics, *P. aeruginosa* stands for the stubborn nosocomial pathogen that causes severe infection with high mortality rate among hospitalized patient, especially for those receive organ transplants (Chua et al. 2016; Deschaght et al. 2011; Khan and Cerniglia 1994; Mohanty et al. 2014; Obritsch et al. 2004; Tam et al. 2010). Direct detection of *P. aeruginosa* cells by microbiological identification of bacterial colony or DNA

identification with PCR were the most conventional and widely adopted diagnosis method in hospital (Dong et al. 2015; Weiser et al. 2014; Workentine et al. 2016). However, these methods suffered with low sensitivity, high labor and time cost, or expensive equipment/reagents (He et al. 2017; Velusamy et al. 2010). Even worse, *P. aeruginosa* cells usually firmly anchor on the tissues with biofilm (Chua et al. 2016), only small portion of free cells could be found in the excreta of patients. Thus, detection of *P. aeruginosa* cells from the excreta was difficult and could not reflect the real infection status. Thus, accurate detection usually depends on getting tissue samples instead of excreta, which caused injury and great hurt to the patients.

Instead, detection of biomarkers in human excreta or fluids was considered to be a promising alternative, which was more suitable for early diagnosis due to the minimum injury and hurt caused to the patients. Fortunately, pyocyanin (PYO), a kind of blue dye belonging to phenazine derivatives, was found to be exclusively secreted by the *P. aeruginosa* and was proved to be the distinct diagnostic biomarkers of *P. aeruginosa* infections (Alatraktchi et al. 2016; Bianchi et al. 2004; Dietrich et al. 2006; Kanthakumar et al. 1993; Usher et al. 2002). Thus, it is imperative to develop methods for sensitive and fast detection of PYO. To date, various methods including high performance liquid chromatography (HPLC or HPLC-MS), surface-enhanced Raman spectrometry (SERS) and immunochemical method have been developed for PYO detection (Pastells et al. 2016; Wu et al. 2014; Yong et al. 2011). However,

complicated sample pretreatment or expensive instruments/reagents are usually required. Interestingly, the substituted phenazine structure endows PYO excellent electrochemical activity and makes it applicable for simple determination by cyclic voltammetry (CV) analysis (Wang and Newman 2008). Meanwhile, the respective substituted groups on the phenazine ring remarkably shift the standard redox potential of phenazine derivatives, which resulted in separated redox peaks when PYO is electrochemical analyzed with other phenazines (Bellin et al. 2014; Bellin et al. 2016). Thus, simple electrochemical methods for PYO detection were developed (Chen et al. 2015; Sharp et al. 2010; Webster et al. 2014). However, the sensitivity of these electrochemical assays still needed to be improved.

Redox reactivation/cycling is an efficient method to amplify the electrochemical signal and offers opportunity for the ultra-sensitive detections of diagnostic biomarkers, pathogens, toxic heavy metal ions and pesticides have been demonstrated (Chen et al. 2016; Lu et al. 2015; Park et al. 2014; Wang et al. 2016; Xie et al. 2016). For example, chemical reductants assisted p-aminophenol redox cycling was widely applied as signal amplification module and remarkably improved the detection sensitivity of various biomolecules (Liu et al. 2014a; Liu et al. 2014b; Xia et al. 2013). However, most of these redox cycling systems composed of multiple enzymes or nanomaterials, coupled with sequenced enzymatic, electrochemical and chemical reactions. In this work, a novel whole-cell redox reactivation/cycling module based on the

bioelectrocatalysis of electroactive bacteria (EAB) was constructed and employed as a new signal amplification module for electrochemical detection of PYO. Based on this signal amplification module, a bioelectrochemical sensing system with high sensitivity for PYO detection was developed and optimized. Moreover, the analytical performance of this biosensing system was characterized in details and it was applied for analysis of PYO in several different kinds of clinical samples. The high sensitivity and robust performance towards clinical samples indicated integrating such whole-cell reactivation module is promising for electrochemical signal amplification and extreme sensitive biosensor development.

2. Materials and methods

2.1. Bacteria strains and culture conditions

Shewanella oneidensis MR-1 and mutants were routinely cultivated in LB broth (peptone 10g/L, yeast extract 5g/L, NaCl 5g/L, pH 7.0) at 30 ^{o}C with shaking (150 rpm).(Sun et al. 2017; Yong et al. 2014) *Pseudomonas aeruginosa* PAO1 or *Escherichia coli* BL21 was cultivated in LB broth at 37 ^{o}C with shaking (220 rpm).

2.2. Sample collection and pretreatment

Saliva samples are obtained from health volunteers and directly used without pretreatment. Sputum samples were collected from health volunteers and were

solubilized by stirring with DTT solution (600 μl DDT solution (0.1% w/v) was added to 150 mg sputum sample) for 30 min at room temperature.(Pastells et al. 2016) Human serum and blood plasma samples were purchased from Guangzhou Hongquan Bio-tel (Guangdong, China) and directly used without further treatment.

2.3. Electrochemical measurement

All electrochemical measurements were performed with three-electrode system by CHI 660E electrochemical workstation (Chenhua Instruments Co., Ltd., Shanghai, China). The three-electrode system was composed of a platinum wire counter electrode, a saturated calomel electrode (SCE, +0.243 V *vs*. SHE) reference electrode and a plain carbon cloth (1 cm × 2 cm) or biofilm colonized carbon cloth (1 cm × 2 cm) working electrode. All potentials are reported versus SCE. A 15 mL cylindrical borosilicate glass bottle was used as the electrochemical cell for biosensing and electrochemical analysis.

2.4. Preparation of biofilm colonized electrode

Biofilm colonized working electrode was prepared as described elsewhere with modifications.(Ross et al. 2011) Briefly, overnight cultures (*S. oneidensis* MR-1) in LB broth (OD600 is about 5.0) were collected and the cells were harvested by centrifugation. The harvested cells were resuspended in the electrolyte (95% M9 mineral medium plus 5% LB and 10 mM electron donor (lactate used for *S.*

oneidensis)(Si et al. 2016) to an OD600 of about 1.5. Then, cell suspension (12 ml) was injected into the electrochemical cell and a three-electrode system was equipped. The working electrode (carbon cloth (1 cm × 2 cm)) was poised at a potential of 0 V (*vs.* SCE) for 36-48 hours to facilitate the biofilm formation (Baron et al. 2009; Ross et al. 2011), and the current was monitored by CHI660E workstation. To make sure the biofilm with high electroactivity, the biofilm colonized working electrode was collected when the current output reached its maximum value (~100 μA), rinsed with PBS buffer for three times and used for biosensing system assembly.

2.5. Biosensing system assembly and PYO detection

For biosensing system assembly, a three-electrode system was equipped (platinum counter electrode, SCE reference electrode, biofilm colonized carbon cloth or plain carbon cloth was used as the working electrode) in the electrochemical cell. Then, 12 ml of fresh electrolyte (for biofilm-based biosensor) or cell suspension (suspension cell based biosensor, OD600=1.5) (purged with N_2 to remove the dissolved oxygen) was added into the electrochemical cell, and tightly sealed to maintain anaerobic condition. The assembly process was operated in an anaerobic workstation (Ruskinn, UK). Next, the PYO containing sample (water sample or human fluids sample, 60 μl) was injected by using syringe with sharp needle from the tightly sealed rubber cap, and cyclic voltammetry was performed.

3. Results and discussion

3.1. Biosensing system design and PYO reactivation module construction

As PYO is a typical redox active molecule, traditional CV analysis would result in a pronounced redox peaks (Fig. 1 and Fig. S1). For the anodic reaction, the reduced PYO at the electrode surface was quickly oxidized and an oxidative current was then generated (Fig. 1a). The peak current (i_p) can be described by Randles–Sevcik equation and is proportional to PYO concentration (Fig. 1c). Thus, direct CV or similar voltammetry analysis was used as traditional method for PYO detection.(Sharp et al. 2010; Webster et al. 2014) However, the oxidized PYO cannot be immediately reduced during the single scanning process (meaning one PYO molecule was only registered for one time by the electrode), the responding current and PYO sensor performance is restricted by the local PYO concentration. Once the reductive PYO can be immediately regenerated after the electrode oxidation (Fig. 1b), the PYO might be repeatedly registered by the electrode and could generate much higher oxidative current, which might dramatically improve the sensitivity for PYO detection (Fig. 1d). Thus, a PYO reactivation/cycling module (Fig. 1b) that can efficiently reduce the electrode-oxidized PYO was expected to be constructed.

However, PYO reactivation module should have appropriate reduction activity that did not disrupt the main structure of PYO to maintain its redox recyclability. Moreover, the reactivation process should be efficient enough to make sure the

re-reduced PYO can be re-registered by the electrode during the fast CV scanning. In addition, low cost and continuous electron source is also important for the cost consideration. EAB that capable of electron exchange between cells and the electrode was considered to be an environment friendly and cost-effective electron generation system (can degrade various organic compounds and continuously generate electrons).(Logan 2009) More strikingly, some EABs showed high efficiency to gently reduce some redox molecules and retained their redox recyclability for shuttling electron transfer between cells and the electrode.(Lovley 2006) Thus, we speculated to use the EAB to construct a PYO reactivation module. It is supposed that, with the continuous electron supply to reduce the electrode-oxidized PYO, the CV curve of PYO would transform from typical symmetrical redox peak to sigmoid shape, which can be described by a revised Nernst-Monod Equation (Fig. 1e, with detailed description in supporting information).

S. oneidensis MR-1 was a typical EAB and has already been used for bioelectrochemical system construction by wiring the intracellular electron consumption system with the extracellular electrode and chemical electron shuttling system (Si et al. 2016). However, this EAB was more well-known as an electron producer than as an electron consumer (Yong et al. 2013), and was expected to be used for PYO reactivation module development. So, this strain was selected for biosensor development. Another typical EAB (*P. aeruginosa*) was also selected due to

it was reported to use phenazine as electron shuttle (Liu et al. 2012; Yu et al. 2017). The most intensively studied model bacteria *E. coli* with low electroactivity was selected as the control. As shown in Fig. 2a, the CV curve of *E. coli* cell suspension did not show any significant redox waves, while *P. aeruginosa* only showed a slight redox wave corresponding to redox compounds secreted by cells. The CV curve of *S. oneidensis* showed a sigmoid shape redox wave centered at about -0.41 V, which could be assigned to the well-defined redox response from riboflavin secreted by *S. oneidensis* cells.(Yong et al. 2013) As illustrated in Fig. 2b, the CV curve of PYO with *E. coli* or *P. aeruginosa* only showed typical symmetrical redox peaks corresponding to the redox reaction of PYO molecule, which showed little difference from that without cells, indicating the anticipated PYO reactivation process was not achieved with these two bacteria strains. Impressively, two sigmoid shape CV waves with extremely high and stable oxidative current were obtained when *S. oneidensis* cells were added into the PYO solution (Fig. 2b). The sigmoid shape redox wave centered at about -0.41 V (started from -0.45 V) was corresponding to the response of riboflavin secreted by the *S. oneidensis* cells (Fig. 2a, 2b). The other sigmoid-shape redox wave centered at about -0.24 V (started from -0.28 V) should be assigned to the response of PYO. Considering the large potential difference (about 0.17 V) and the sigmoid-shape of the redox waves, the CV response of PYO could be easily discriminated from that of riboflavin. The stable oxidative current obtained by PYO with *S. oneidensis* was about 31 μA, while the peak current of PYO with *E. coli* and *P.*

aeruginosa was 1.6 μA and 1.8 μA, respectively. The sigmoid shape CV response of PYO with *S. oneidensis* implied that *S. oneidensis* cells could reduce the electrode-oxidized PYO, which continuously provided electrons for enhanced and stable current output.

The PYO bioreduction by *S. oneidensis* cells was further confirmed by UV-vis analysis (Fig. S2). The oxidative PYO showed blue color and had four typical adsorption peaks at about 236 nm, 310 nm, 375 nm, and 700 nm. After *S. oneidensis* cells were added into the PYO solution, the blue color was immediately disappeared and distinguished adsorption peaks emerged (249 nm, 335 nm), which corresponding to the reductive status of PYO. More interestingly, the bioreduced PYO could be easily re-oxidized to its oxidative status by vigorous stirring in air (Fig. S2), which confirmed its redox cycling ability.

To further understand the mechanism of PYO bioreduction by *S. oneidensis*, the electron transfer pathway between bacteria and PYO was investigated. As cytochrome C proteins were considered to be the conduit (MtrA-MtrB-MtrC/OmcA) for transferring the intracellular electrons to the outer cell membrane surface (Fig. S3) (Hartshorne et al. 2009), the effect of cytochrome C mutation on PYO bioreduction was determined. As shown in Fig. 3a, PYO with MtrA or MtrB mutation only showed CV curves similar to that without cells, indicating no electron transfer between cells and PYO. This is due to the indispensable roles of MtrA and MtrB in the extracellular

electron transfer pathway of *S. oneidensis*.(Ross et al. 2011) PYO with MtrC mutant showed sigmoid shape CV curve but with much lower oxidative current (~11 μA) than the wild type strain (~31 μA). The result is also reasonable as the role of MtrC could be partially substituted by OmcA.(Ross et al. 2011) These results identified Mtr cytochrome C conduit of *S. oneidensis* cells was responsible for PYO reduction, and substantially indicated that *S. oneidensis* cells could be used as reduction system for redox cycling of PYO. Thus, it is expected to using *S. oneidensis* cells as PYO reactivation module to amplify the electrochemical response of PYO, which would greatly improve the sensitivity for PYO detection.

3.2. Biosensing system development and optimization

Thus, a biosensing system with the signal amplification module of *S. oneidensis* cells was constructed in a three-electrode electrochemical cell. The efficiency of cell-PYO-electrode interaction at the electrode surface was assumed to determine the sensitivity of the biosensing system. It was reported that biofilm with high density cells attached on the electrode surface is superior to suspension cells for electron transfer between cells and electrode.(Borole et al. 2011) So, an electroactive biofilm colonized electrode was prepared through continuous anodic discharge in a three-electrode system under constant potential (Fig. S4). This biofilm colonized electrode was used as the working electrode and its CV response to PYO was compared with the suspension cells. As shown in Fig. 3b, the CV curve of PYO with

biofilm showed pronounced sigmoid shape with the oxidative current of about 130 μA, which is about 4 times of that from PYO with suspension cells (31 μA) and 260 times of that from PYO with plain carbon cloth electrode. Furthermore, the anodic curve was ideally fitted with the Monod term and Nernst term of the revised Nernst-Monod equation (Fig. S5 & S6**)**, implying that when a giving PYO concentration was provided to this system, the overall electron transfer chain (in the sequence of lactate, bacteria, PYO and electrode) was thermodynamically (electrode potential) controlled. These results also gave strong support to our assumption that electron transfer both at *Shewanella*/PYO and PYO/electrode interface are fast and PYO microbial reactivation would greatly enhance its response current. Thus, the biosensing system was developed, i.e., three-electrode system with biofilm colonized carbon cloth as working electrode, platinum wire as counter electrode, SCE as the reference electrode and lactate as the electron donor (Fig. S4).

Then, the biosensor response to different concentrations of PYO was determined to evaluate its feasibility for PYO quantification. As shown in Fig. 4a, at the concentration lower than 300 nM, the current output gradually increased along with the increasing PYO concentration. However, once the concentration exceeded 300 nM, the current output increment was largely suppressed and reached its highest output of about 250 μA at 1000 nM (Fig. 4b). Furthermore, significant negative shift of midpoint potential was observed when the PYO concentration was higher than 300

nM (Fig. 4a and Fig. S7). This phenomena might be caused by the fact that the microbial recharging (PYO reduction) rate could not compete with the electrode oxidization rate when PYO concentration is high, which in turn resulted in a transformation of CV curve from sigmoid shape to typical symmetrical shape and negative shift of corresponding peak location (Nicholson and Shain 1964). Impressively, a significant current output of about 4.3 μA could be discriminated at extremely low concentration of 100 pM (Fig. 4b). The relationship between current output and PYO concentration (100 pM to 100 nM) was analyzed and a linear calibration curve was obtained. As shown in Fig. 4c, the calibration curve showed good linear relationship (R^2=0.9996) at the concentration range from 0.1 nM to 100 nM. The limit of detection (LOD, *S/N*=3) estimated from the calibration curve is 47±1 pM, which is much lower than the PYO concentrations reported in the clinical samples from the patients infected by *P. aeruginosa*.(Hunter et al. 2012) The limit of quantification (LOQ, *S/N*=10) determined is 155±3 pM. Strikingly, the sensitivity obtained here was 1314±27 nA/nM PYO, which is over 400 times higher than that obtained by CV analysis without *S. oneidensis* cells (3.18 nA/nM) (Fig. S8). Compared with the recently reported electrochemical assay for PYO, the method developed here showed about three orders of magnitude lower LOD and with much higher sensitivity (Table S1). The featured low LOD and high sensitivity indicated that it holds great promise for early diagnosis of *P. aeruginosa* infection.

3.3. Analytic performance

Next, the analytical performance of the developed biosensing system was evaluated before its application to real samples. Thus, the selectivity, stability, accuracy and reproducibility were determined.

The clinical samples might contain various organics (such as glucose, lactic acid) in different human fluids (such as blood, saliva, sputum). Therefore, possible interference from these substrates was tested. Coexistence with the organic compounds did not significantly affect the biosensor output (Fig. S9a). Although the biosensor output was slightly repressed by the human fluids, the output with human fluid was 94.7±0.6% of the control without interference, suggesting the interferences from human fluids including blood, saliva and sputum were also marginal, which may not significantly affect the analytical performance of the developed biosensing system. Another concern with biosensing system is the contamination from other bacteria. *P. aeruginosa* cells are inevitable in the infected samples. So, the possible interference from this bacterium should be taken into account. *E. coli* was also selected as another model bacterium as it is ubiquitous in different clinical samples. It was found that the biosensor output showed no significant difference (102±0.4 % for *P. aeruginosa* contamination, 101±0.7 % for *E. coli* contamination) from the output of the control without contamination (Fig. S9b). In addition, the biosensing system also showed good stability under low temperature storage (over 85% signal output could be

retained after 1 week storage at 4 ^{o}C (Fig. S10)) owing to the excellent low temperature resistance of *S. oneidensis* MR-1 (Si et al. 2016). These results indicated that the developed biosensing system was robust enough with excellent resistance to possible interferences and good stability, which confirmed its feasibility for practical applications.

To establish the creditability of this newly developed biosensing system, the accuracy and reproducibility were tested by using synthetic PYO water samples (Table S2). The recovery of the biosensing detection (ranging from 1 to 80 nM) was between 93% and 105%, which indicated good accuracy of this assay. Moreover, this biosensing system also showed good reproducibility as evidenced by low coefficient of variation achieved (1% to 8.9%). These results substantiated that this *S. oneidensis* biofilm based biosensing system was highly reliable for PYO detection in aqueous samples.

3.4. Detection of pyocyanin in human fluids

For early diagnosis, different human fluids such as blood, saliva or sputum might be the ideal samples for testing the infection of *P. aeruginosa*, owing to the good availability, minimum hurt and injury caused to the patients. Thus, a set of blind samples prepared by spiking blank human fluids with PYO were used to test the possibility for application in clinical samples.

Commercial human serum or blood plasma purchased was used to simulate the human blood sample. After spiking with different concentrations of PYO, these samples were subjected to biosensing analysis. As shown in Table 1, the accuracy of biosensing assay was excellent with the recovery between 88.0% and 95.6%, and also showed good reproducibility (the COV ranges from 3.4% to 8.7%). The saliva samples were directly collected from a healthy volunteer and different concentration of PYO was spiked before biosensing analysis. It was also found that the accuracy and reproducibility were both excellent (Table 1). Sputum was usually used as the sample for early diagnosis of lung infection by *P. aeruginosa* (cystic fibrosis) as high content of PYO usually detected in the sputum sample of patients(Hunter et al. 2012). Thus, sputum samples were collected from several healthy volunteers and analyzed (Table 2). The low coefficient of variation (0.6% to 3.8%) indicated excellent reproducibility of this assay in this sputum samples. It is worth to note that the recovery for samples from different people showed small variation, although the sputum from different people was very different in the physicochemical properties such as viscosity and chemical ingredient. However, the recovery of the biosensing assay for all samples was ranged from 89% to 99%, indicating excellent accuracy regardless of the origin of the samples.

4. Conclusion

A new and simple whole-cell based bioelectrochemical sensing system for the diagnostic of *P. aeruginosa* infection was developed. It was composed of a PYO reactivation module and a three-electrode detection module. The *Shewanella* cells of PYO reactivation module could continuously generate electrons from lactate metabolism and injected the electrons to the electrode-oxidized PYO, which made PYO molecule registered by the electrode again. Based on this design, the PYO could be repeatedly registered by the electrode, which greatly amplified electrochemical output signal. Thus, the developed bioelectrochemical sensing system showed ultrahigh sensitivity (1.3 μA/nM, several orders of magnitude higher than other electrochemical methods), low LOD (47±1 pM), excellent accuracy and reproducibility, and was robust enough for various clinical samples analysis. The analytical tool developed here would be helpful to reduce the cost and pain to patients for early diagnosis of *P. aeruginosa* infection, and would in turn reduce the mortality and improve the life quality of patients suffered from *P. aeruginosa* infection. The idea of using exoelectrogens for redox reactivation and signal amplification would be promising for new electrochemical biosensor design.

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Table 1. Analysis of human serum, human blood plasma, and human saliva samples spiked with authentic PYO

PYO spiked (nM)*	Determined by biosensor (nM)	COV (%)**	Recovery (%)
Human serum			
5	4.78	3.5	95.6
20	19.1	8.2	95.5
80	71.2	5.2	89.0
Human blood plasma			
5	4.73	3.6	94.6
20	17.8	8.7	89.0
80	70.4	3.4	88.0
Human saliva			
5	5.01	2.1	100
20	18.6	6.1	92.8
80	71.0	4.5	88.8

* Samples without spiked PYO did not showed significant biosensor output.

**COV: coefficient of variation (n= 3)

Table 2. Analysis of different sputum samples spiked with authentic PYO

Sample No.*	PYO spiked (nM)	Determined by biosensor (nM)	COV (%)**	Recovery (%)
1	5	4.48	0.9	89.6
2	5	4.83	0.6	96.6
3	20	18.3	2.1	91.5
4	20	19.9	1.4	99.5
5	60	56.3	3.8	93.8

*All samples without spiked PYO did not showed significant biosensor output.

** COV: coefficient of variation (n= 3).

Figure 1. Schematic of the conventional PYO electrochemical detection system (EC detection) (a), EAB based redox reactivation module (b), typical CV curve obtained by EC detection (c), bioelectrochemical sensing system (integration of PYO reactivation module with conventional electrochemical detection system) (d) and typical CV curve obtained by bioelectrochemical detection system (e). The red arrows

indicate the pathway for electron transfer. $[CH2O]_X$ represents the electron donor (organic compound) for EAB (e.g., lactate used for *S. oneidensis* MR-1). PYO_{RE} indicates reductive PYO; PYO_{OX} indicates oxidative PYO.

Figure 2. CV curves of different cell suspension without (a) or with PYO (b). The scan rate is 5 mV/s, the added PYO is 100 nM, the OD600 of cell suspension is 1.5. The inset indicates the larger view of the selected part. Glucose (10 mM) is added in the electrolyte as the electron donor for *P. aeruginosa* or *E. coli*; lactate (10 mM) is added in the electrolyte as the electron donor for *S. oneidensis*.

Figure 3. (a) CV analysis of PYO (100 nM) with different cytochrome C mutations of *S. oneidensis* MR-1 (suspension mode). (b) CV analysis of PYO (100 nM) with plain carbon cloth, *Shewanella* biofilm colonized electrode, and PYO (100 nM) with *Shewanella* biofilm colonized electrode. The scan rate is 5 mV/s, lactate (10 mM) is added in the electrolyte as the electron donor. Inset of (b) indicates the larger view of the selected part for the CV of PYO.

Figure 4. (a, b) Dose-dependent response of bioelectrochemical sensing system to PYO. Inset of (b) indicates the enlarged view of the selected low concentration part. (c) Calibration curve based on the relationship between the CV current response and the PYO concentration.

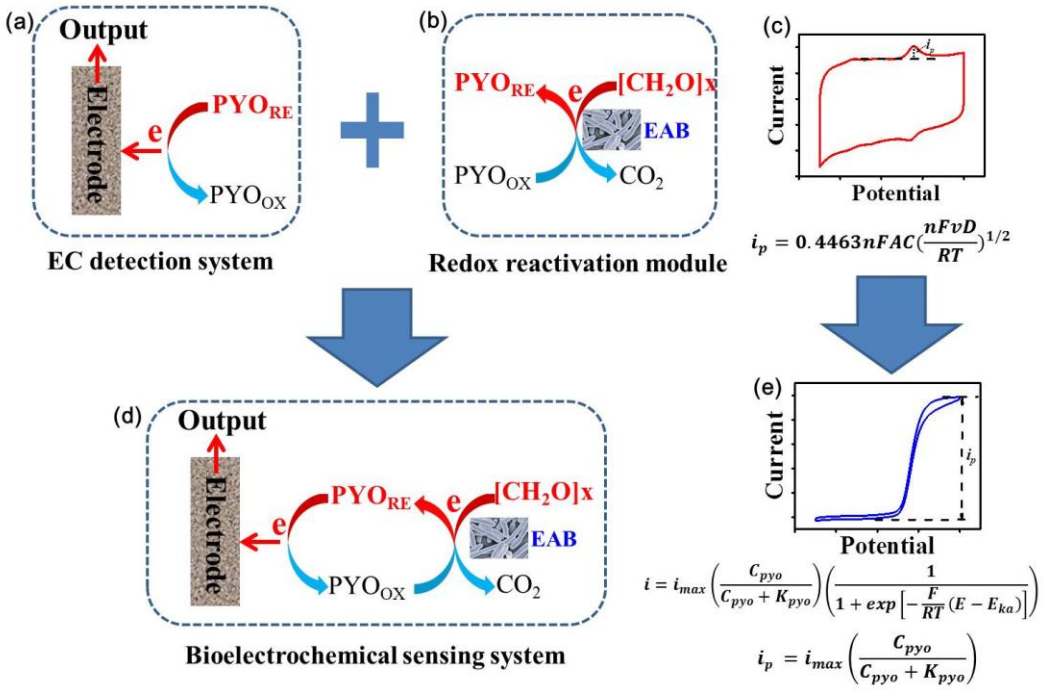


Fig. 1.

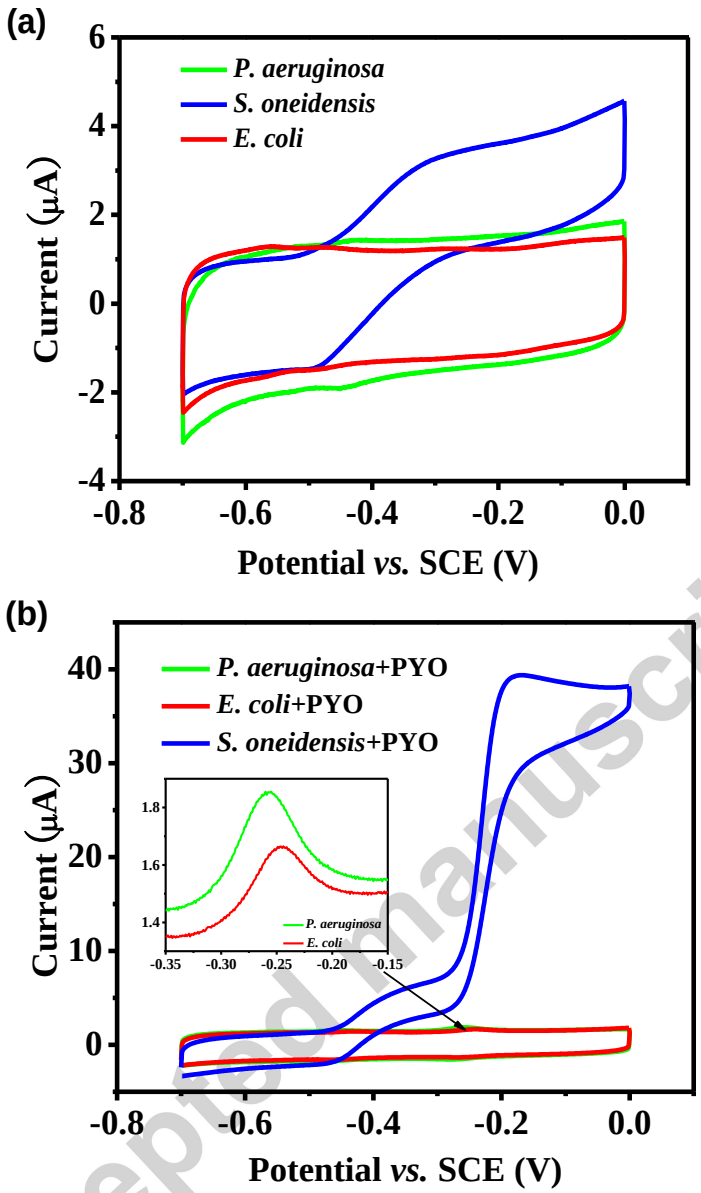


Fig. 2.

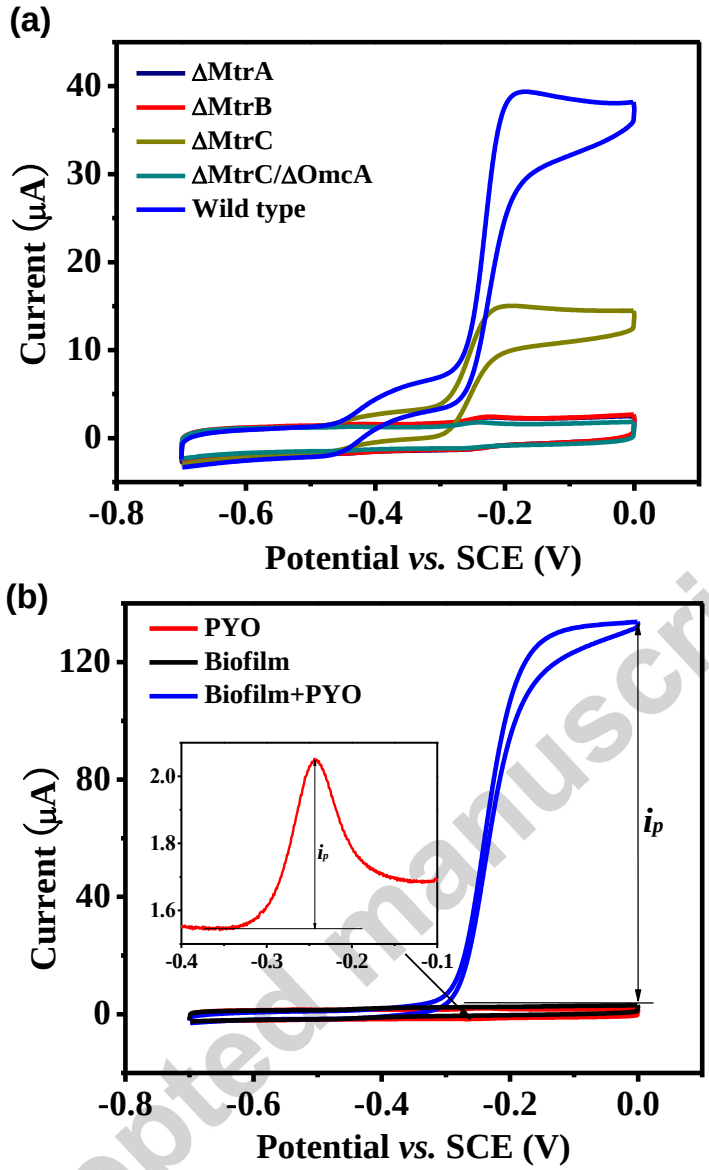


Fig. 3.

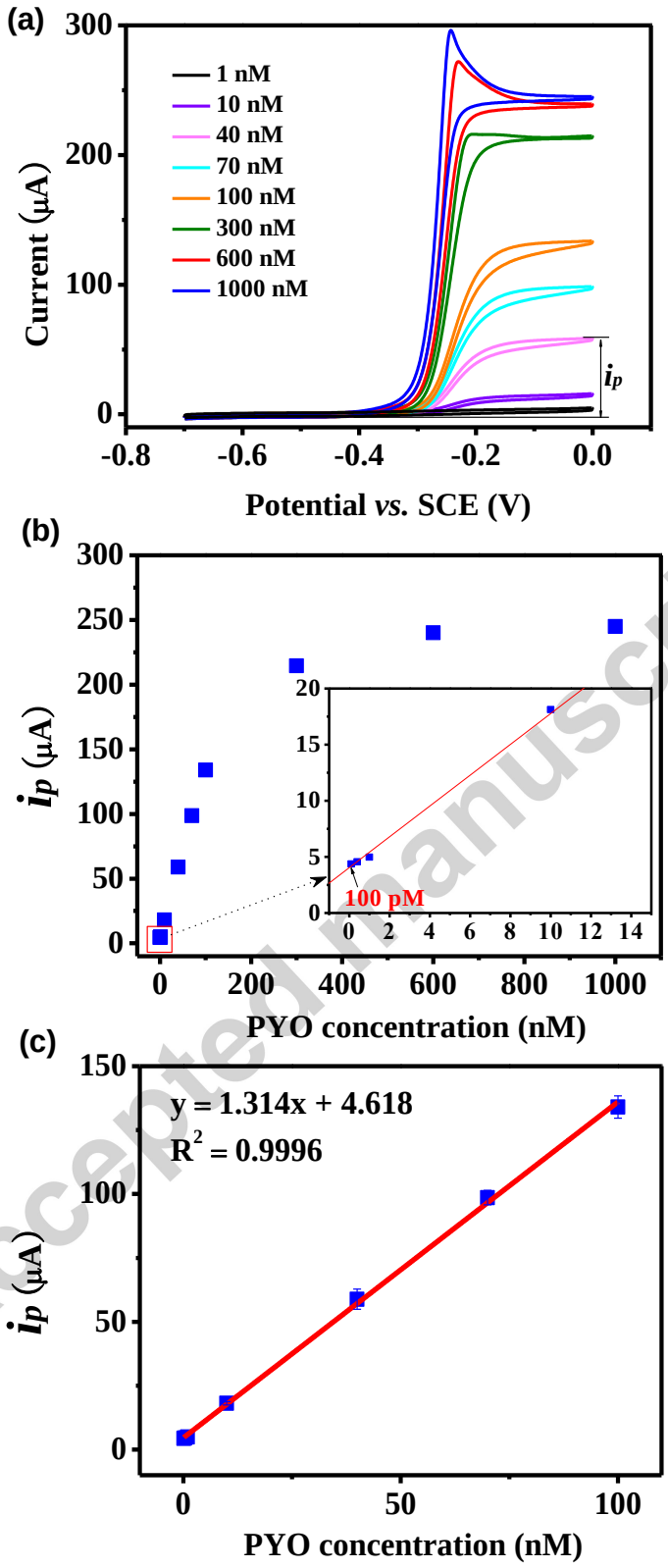
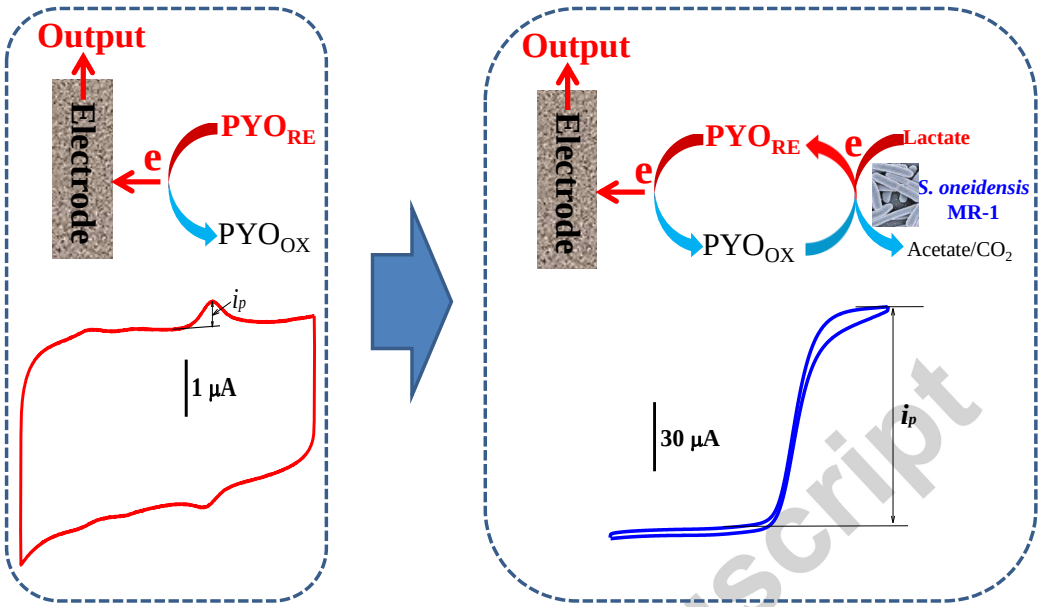


Fig. 4.

Graphical Abstract



Traditional EC detection system

Bioelectrochemical sensing system

Highlights

-A whole-cell redox reactivation module based on exoeletrogen was developed

-Whole-cell signal amplification for bioelectrochemical sensing was developed

-Pyocyanin detection was achieved by using this signal amplification strategy

-The electrochemical response of pyocyanin was amplified about 405 times

-An extremely low LOD of pM level for pyocyanin detection was obtained

-The biosensing system was robust enough for clinic sample analysis