Systematic Design of a Quorum Sensing-Based Biosensor for Enhanced Detection of Metal Ion in Escherichia Coli

Chih-Yuan Hsu, Bing-Kun Chen, Rei-Hsing Hu, and Bor-Sen Chen, *Life Fellow, IEEE*

***Abstract*—With the recent industrial expansion, heavy metals and other pollutants have increasingly contaminated our living surroundings. The non-degradability of heavy metals may lead to accumulation in food chains and the resulting toxicity could cause damage in organisms. Hence, detection techniques have gradually received attention. In this study, a quorum sensing (QS)-based amplifier is introduced to improve the detection performance of metal ion biosensing. The design utilizes diffusible signal molecules, which freely pass through the cell membrane into the environment to communicate with others. Bacteria cooperate via the cell-cell communication process, thereby displaying synchronous behavior, even if only a minority of the cells detect the metal ion. In order to facilitate the design, the ability of the engineered biosensor to detect metal ion is described in a steady state model. The design can be constructed according to user-oriented specifications by selecting adequate components from corresponding libraries, with the help of a genetic algorithm (GA)-based design method. The experimental results validate enhanced efficiency and detection performance of the quorum sensing-based biosensor of metal ions.**

***Index Terms*—Metal ion detection, quorum sensing (QS)-based biosensor, synthetic biology, systematic design methodology.**

I. Introduction

METAL ions play important roles in biological metabolic pathways and affect cellular processes through a wide variety of reactions. Organisms require metal ions, which are involved in processes, such as bacterial respiration, electron transport, and peroxide reduction [1]. The uptake and efflux systems in biology regulate ion homeostasis to prevent a lack or excess of metal ions. With the recent industrial expansion, there has been either an excess of metal ions in wastewater [2]–[6] or the intentional or careless discharge of other contaminants [7] into the natural environment. Metal ions can be classified based on their effects and toxicity on cells into essential ions, trace ions, and heavy metals. The stronger redox ability of heavy metals allows them to compete with essential ions and trace ions in redox actions, leading to superoxide or hydroxyl radicals [8]. Superoxide and hydroxyl radicals may damage lipids and proteins [9], [10], disrupt DNA oxidation [8], and even cause cell death [8]. Furthermore, heavy metals are difficult to remove via metabolism and can accumulate in organs, causing permanent damage. Hence, detection techniques have gradually received great attention. In this study, we introduce a synthetic genetic circuit that can enhance the detection performance of metal ion biosensors and has the potential to be applied for environmental detection and environmental bioremediation.

Recent progress in research on synthetic biology [11]–[20] has provided an alternative means for metal ion detection aided by specific promoter elements such as P$pbrA$ derived from *R. metallidurans* [21], [22] or P$cusC$ and P$pcoE$ found in *E. coli* [23]. In order to improve the detection performance, a metal ion-induced promoter connects to bacterial quorum sensing (QS) system. Quorum sensing is a mechanism which regulates gene expression for many functions through cell-cell communication [24]–[28]. Bacteria produce diffusible signal molecules, generally known as autoinducers (AIs) [29]–[33], and release them into the environment to communicate with others. Through this process, bacteria cooperate and thereby display synchronous behavior. The most extensively studied quorum sensing system is the autoinduction of luminescence in the marine bacterium, *Vibrio fischeri*, which is symbiotic with squids and some marine fishes [32]–[34]. The luminescence here is produced by the lux operon acquired from *V. fischeri*, of which the LuxR protein is the transcriptional activator of luminescence, and the LuxI protein synthesizes the specific N-acylated homoserine lactone (AHL) [35]–[37]. After the LuxR protein binds AHL to form a complex, the complex can bind the target promoter sequence and activate downstream gene transcription, even if only some of the bacteria can detect the metal ion. Thus, it can be employed to enhance the detection performance by signal amplification.

To make the design of the QS-based metal ion biosensor easier, we introduce a mathematical model to describe the dynamic and steady state regulatory behavior, which is related

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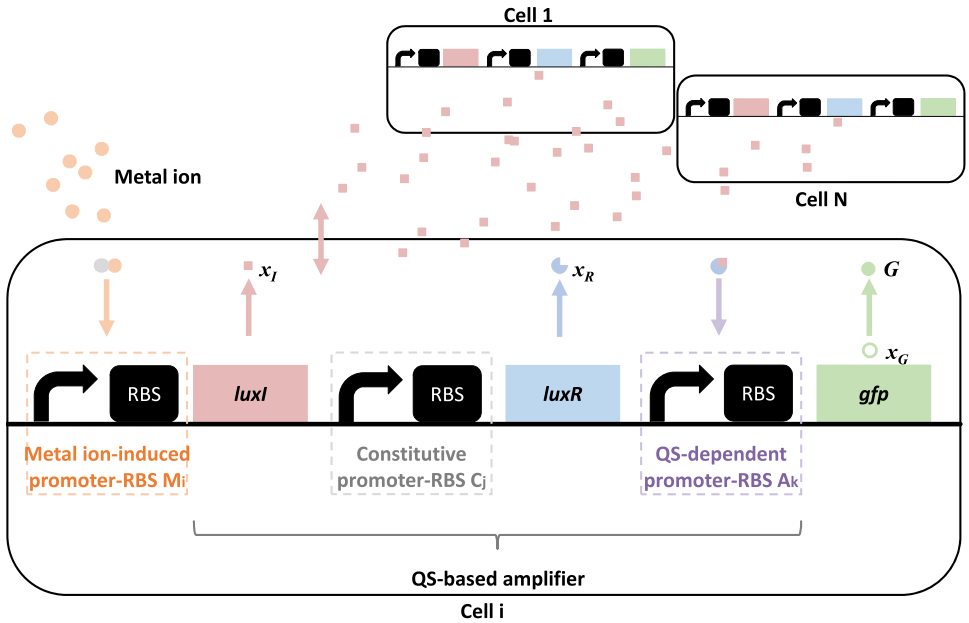

Fig. 1. The QS-based metal ion biosensor. A metal ion-induced promoter-RBS connects with a quorum sensing system found in the marine bacterium, *V. fischeri*. Via the transmission of signal molecules, the output signal will be produced significantly by the activation of the QS-dependent promoter.

to transcriptional and translational processes [38]. A promoter allows RNA-polymerase molecules to latch onto a DNA strand and initialize transcription of a downstream gene into mRNA, and a RBS allows ribosome to bind and translate mRNA. A promoter combined with a RBS thus in this study is viewed as a component, of which characterizations can be identified with a reporter protein by measuring the fluorescence intensity. One can therefore use kinetic parameters as component libraries to design the circuit for QS-based metal ion detection. The design can be constructed according to user-oriented specifications by selecting adequate promoter-RBS components within a feasible range of metal ion concentrations. A long computation time is required in general when the component libraries become large. Hence, a genetic algorithm-based design method proposed here provides a simple and useful tool that saves time in evaluating and selecting components. So in summary, this study provides a systematic methodology for developments in next-generation synthetic biology, from component library construction to gene circuit assembly. When the libraries are more complete, more precise detection can be achieved.

The contributions of this paper are fourfold: (a) A QS-based amplifier is introduced into a biosensor to enhance the ability for metal ion detection. (b) Based on the promoter-RBS kinetic strengths, we establish three kinds of component libraries. (c) According to user-oriented specifications, the quorum sensing (QS)-based metal ion biosensor can be constructed by selecting adequate promoter-RBS components from the corresponding libraries to achieve a desired ability for metal ion detection. (d) The proposed methodology could provide synthetic biologists with a useful tool to design metal ion biosensors.

II. Methods

A. Construction of QS-Based Metal Ion Biosensor

Recent research on synthetic biology offers an alternative means for metal ions detection using specific promoter elements derived from microorganisms. For example, P$cusC$ and P$pcoE$ are copper-inducible promoters regulated by CusRS, which are a part of the *E. coli* chromosome cus system and pco system, respectively [39]. The cus system (*cusCFBA*) found in *E. coli* is a tetrapartite system endowing resistance to copper ion and is involved in periplasmic copper detoxification [40]. The pco system (*pcoABCDRSE*) is discovered in bacteria that survive in copper-rich environments; plasmid-encoded operons in this system are known to be responsible for resistance to copper toxicity [41]–[43]. Another example of a metal ion-regulated promoter is the lead-resistance promoter P$pbrA$ [21]. The promoter P$pbrA$ is acquired from *Ralstonia metallidurans* strain CH34 isolated from lead-contaminated soil, containing at least seven determinants encoding resistances to toxic heavy metals [21], [22]. Combining functions involved in uptake, efflux and accumulation of Pb(II) ion, the lead resistance operon *pbr* (*pbrABCDRT*) is regulated by PbrR, which belongs to the MerR family of metal regulatory proteins [44], [45].

To improve the detection performance, a metal ion-induced promoter-RBS M_i is connected to the LuxI protein coding sequence (in Fig. 1). This allows the LuxI protein to be translated by the activation of the metal ion-induced promoter. The $luxI$ component derived from the lux operon in *V. fischeri* is involved in the production of the LuxI protein, which catalyzes S-adenosylmethionine and acyl-acyl carrier protein into a specific AHL (referred to as HSL) as signal molecules [36], [37]. HSLs may be classified into several types according to acyl group length (C4–C18). Here, the type of HSL synthesized by LuxI protein is C6-HSL. Because the lux operon is an exogenous DNA sequence for *Escherichia coli* (*E. coli*), it is required

to supply LuxR protein for the activation of promoter Plux. The LuxR protein coding sequence is connected to a constitutive promoter-RBS component C_i. When sufficient amounts of the LuxR protein is produced using a constitutive promoter-RBS component with the presence of C6-HSL, C6-HSL can then bind LuxR protein to form the LuxR complex. The complex targets the cognate QS-dependent promoter-RBS component A_k and activates the transcription of the green fluorescent protein (GFP) coding sequence, even if only some of the bacteria can detect the metal ion. This mechanism is helpful to enhance the detection ability by signal amplification.

B. Mathematical Model of QS-Based Metal Ion Biosensor

To construct the dynamic model, promoter-RBS regulation must be introduced. The promoter-RBS regulation function is first defined by $P(P_u, P_l, TF, I)$, in which P_u and P_l denote the maximum and minimum promoter-RBS strengths, respectively, TF denotes transcription factor concentration, and I is inducer concentration. This function describes the biochemical aspect of the transcription and translation process. The dynamic model of the metal ion biosensor with QS-based amplifier in Fig. 1 is thus described as follows. Note that the QS-based metal ion biosensor is divided into three stages.

The first stage involves a metal ion-induced promoter-RBS M_i for producing autoinducer synthase LuxI, which catalyzes S-adenosylmethionine and acyl-acyl carrier protein into a specific AHL. The dynamics is described in the following equation [46]:

$$\dot{x}_E(t) = P_M(P_{u,i}, P_{l,i}, x_S, I_M) - (d + r_E) \cdot x_E(t) \qquad (1)$$

in which

$$P_M(P_{u,i}, P_{l,i}, x_S, I_M) = P_{u,i} + \frac{P_{u,i} - P_{l,i}}{1 + \left(\frac{K_{SI}}{x_{SI}(x_S, I_M)}\right)^{n_{SI}}}$$

$$x_{SI}(x_S, I_M) = \frac{x_S}{1 + \left(\frac{K_M}{I_M}\right)}$$

where x_E denotes the concentration of autoinducer synthase. I_M is the concentration of metal ion; x_S is the total concentration of metal ion-dependent regulatory protein. x_{SI} denotes the complex of x_I and I_M. i denotes the ith metal ion-induced promoter-RBS component in Table A1. $P_M(P_{u,i}, P_{l,i}, x_S, I_M)$ denotes the promoter-RBS regulation activity of the metal ion-induced promoter-RBS components. $P_{u,i}$ and $P_{l,i}$ denote the maximum and minimum promoter-RBS strengths of the ith metal ion-induced promoter-RBS component in supplementary Table A1. r_E denotes the degradation rate for autoinducter synthase. d is the dilution rate due to cell growth. K_{SI} and n_{SI} denote the binding affinity and binding cooperativity between the complex x_{SI} and the corresponding promoter-RBS component, respectively. K_M is the dissociation rate between the metal ion I_M and the metal regulatory protein x_S. Then, the dynamics for the catalysis of S-adenosylmethionine and acyl-acyl carrier protein into a specific autoinducer is given by

$$\dot{x}_I(t) = a x_E(t) - (d + r_I) \cdot x_I(t) \qquad (2)$$

where x_I is the concentration of autoinducer. a is autoinducer synthesis rate; r_I denotes the degradation rate for autoinducter.

The second stage involves a constitutive promoter-RBS part C_i for producing protein, LuxR. The change in concentration of regulatory protein LuxR with respect to time is given by [46]

$$\dot{x}_R(t) = P_C(P_{u,j}, 0, 0, 0) - (d + r_R) \cdot x_R(t) \qquad (3)$$

and

$$P_C(P_{u,j}, 0, 0, 0) = P_{u,j}$$

where x_R denotes the concentration of transcriptional activator protein, LuxR. j is the jth constitutive promoter-RBS component in Table A2. $P_C(P_{u,i}, 0, 0, 0)$ is the regulation activity of the constitutive promoter-RBS components. $P_{u,j}$ is the promoter-RBS strength of the jth constitutive promoter-RBS component in Table A2. r_R denotes the degradation rate for transcriptional activator protein.

The third stage contains a QS-dependent promoter-RBS part A_k for driving the expression of the immature reporter protein. When complex of x_R and x_I accumulates, it activates the QS-dependent promoter and enhances the expression of reporter gene. Therefore, the dynamics is governed by the following equation [46]:

$$\dot{x}_G(t) = P_A(P_{u,k}, P_{l,k}, x_R, x_I) - (d + r_G) \cdot x_G(t) \qquad (4)$$

and

$$P_A(P_{u,k}, P_{l,k}, x_R, x_I) = P_{u,k} + \frac{P_{u,k} - P_{l,k}}{1 + \left(\frac{K_{RI}}{x_{RI}(x_R, x_I)}\right)^{n_{RI}}}$$

$$x_{RI}(x_R, x_I) = \frac{x_R}{1 + \left(\frac{K_I}{x_I}\right)}$$

where x_G is the concentration of immature reporter protein. x_{RI} represents the complex of x_R and x_I. k is the kth QS-dependent promoter-RBS component in Table A3. $P_A(P_{u,k}, P_{l,k}, x_R, x_I)$ is the activity of the QS-dependent promoter-RBS components. $P_{u,k}$ and $P_{l,k}$ are the maximum and minimum promoter-RBS strengths of the kth QS-dependent promoter-RBS component in Table A3. r_G is the degradation rate for immature reporter protein. K_{RI} and n_{RI} are the binding affinity and binding cooperativity between the complex x_{RI} and the promoter-RBS part, respectively. K_I is the dissociation rate between the autoinducer $x_I$and the transcriptional activator protein x_R. Finally, the dynamics for the maturation of x_G into the mature reporter protein G is given by

$$\dot{G}(t) = m x_G - (d + r) G(t) \qquad (5)$$

where m is the maturation rate for the reporter protein and r is the degradation rate for mature reporter protein.

The dynamic model for the QS-based metal ion biosensor is then transformed into steady-state model by assuming that the dynamics in (1)–(5) are equal to zero. The steady-state model of the QS-based metal ion biosensor with promoter-RBS parts M_i, C_j, and A_k being selected from the corresponding libraries in Supplementary Appendix A, respectively, is derived as follows:

$$\begin{cases} x_{ISS} = \frac{P_M(P_{u,i},P_{l,i},x_S,I_M)}{d+r_E} \cdot \frac{a}{d+r_I} \\ x_{RSS} = \frac{P_C(P_{u,j},0,0,0)}{d+r_R} \\ x_{GSS} = \frac{P_A(P_{u,k},P_{l,k},x_R,x_I)}{d+r_G} \\ G_{SS} = \frac{m}{d+r} x_G \end{cases} \tag{6}$$

where $x_{ISS}, x_{RSS}, x_{GSS}$ and G_{SS} are the steady-state concentrations of autoinducter, transcriptional activator protein, and immature reporter protein, as well as steady state mature reporter protein, respectively. The steady-state expression of the QS-based metal ion biosensor can be obtained from the steady-state model in (6) with the regulation functions $P_M(P_{u,i}, P_{l,i}, x_S, I_M)$, $P_C(P_{u,i}, 0, 0, 0)$ as well as $P_A(P_{u,k}, P_{l,k}, x_R, x_I)$, respectively.

In general, a synthetic genetic circuit *in vivo* also suffers from extrinsic environmental cellular noise, such as transmitted noise from upstream and global noise affecting all cells [47]–[49]. Thus, the equation in (6) should be modified as follows:

$$\begin{cases} x_{ISS} = \frac{P_M(P_{u,i},P_{l,i},x_S,I_M)}{d+r_E} \cdot \frac{a}{d+r_I} + v_1 \\ x_{RSS} = \frac{P_C(P_{u,j},0,0,0)}{d+r_R} + v_2 \\ x_{GSS} = \frac{P_A(P_{u,k},P_{l,k},x_R,x_I)}{d+r_G} + v_3 \\ G_{SS} = \frac{m}{d+r} x_G + v_4 \end{cases} \tag{7}$$

where the Gaussian random noise v_p, $p = 1, 2, 3$, with a zero mean and variance of σ_p^2, denotes cellular noise for both the transcriptional and translational gene expression processes at the steady state. v_4 denotes cellular noise in mature protein expression at the steady state. Besides, biological components are inherently uncertain in a molecular biological system [47]. For example, the intrinsic kinetic parameters of the components including the processes of transcription and translation, the degradation rates of regulatory proteins, dilution rates of the cells, and the maturation rate for the reporter proteins, are all stochastically uncertain *in vivo*, as a result of gene expression noise from biochemical processes, thermal fluctuations, DNA mutation, and evolution. These perturbations are defined as follows:

$$\begin{aligned} &P_{u,i} \to P_{u,i} + \Delta P_{u,i} n_1(t),\ P_{l,i} \to P_{l,i} + \Delta P_{l,i} n_2(t), \\ &P_{u,j} \to P_{u,j} + \Delta P_{u,j} n_3(t), \\ &P_{u,k} \to P_{u,k} + \Delta P_{u,k} n_4(t),\ P_{l,k} \to P_{l,k} + \Delta P_{l,k} n_5(t), \\ &r_E \to r_E + \Delta r_E n_6(t), \\ &r_R \to r_R + \Delta r_R n_7(t), \\ &r_G \to r_G + \Delta r_G n_8(t), \\ &m \to m + \Delta m n_9(t), \\ &r \to r + \Delta r n_{10}(t), \\ &d \to d + \Delta d n_{11}(t) \end{aligned} \tag{8}$$

where $\Delta P_{u,i}$, $\Delta P_{l,i}$, $\Delta P_{u,j}$, $\Delta P_{u,k}$, $\Delta P_{l,k}$, Δr_E, Δr_R, Δr_G, Δr, Δm, and Δd are the standard deviations of the corresponding stochastic parameters, and $n_q(t)$, $q = 1, 2, \ldots, 11$, denote Gaussian noise, which have zero mean and unit variance, and account for random fluctuation sources. If the kinetic parameters in the steady state model in (7) are replaced by the parameter perturbations shown in for robust design of the gene circuit, then the QS-based metal ion biosensor can tolerate these fluctuations *in vivo*, i.e., a design that accounts for (8) should be able to tolerate the parameter fluctuations.

C. Design Specifications for QS-Based Metal Ion Biosensor

The purpose of our design is to construct a QS-based metal ion biosensor by selecting a set of suitable components from the corresponding libraries to achieve optimal tracking of a desired I/O response within a feasible range of metal ion concentrations. To achieve this, the following design specifications are needed:

- Desired I/O response $G_{ref}(I_M)$ of the synthetic genetic QS-based metal ion biosensor
- A feasible range of metal ion concentrations
- Standard derivations of cellular disturbances and parameter fluctuations to be tolerated *in vivo*
- A cost function between the desired reference steady state fluorescence intensity G_{ref} and the steady state fluorescence intensity G_{SS} in within a specified range of metal ion concentrations is given to be minimized as follows:

$$J(S) = E \int \left(G_{ss}(S, I_M) - G_{ref}(I_M)\right)^2 dI_M \tag{9}$$

where S denotes the set of promoter-RBS components M_i, C_j, and A_k to be selected from the corresponding libraries. If the cost function in is minimized by choosing the most appropriate set of components under design specifications, the fluorescence intensity of an engineered metal ion biosensor will be as close as possible to the specified steady state fluorescence intensity under parameter fluctuations and environmental noise optimally. Although the cost function $J(S)$ can be minimized by traditional conventional search methods, it will require long computation times and several trial-and-error experimentations when component libraries become large. Thus, a more effective and efficient genetic algorithm (GA)-based searching method is proposed here to save time in evaluating and selecting adequate promoter-RBS components.

D. Design Procedure for the QS-Based Metal Ion Biosensor

The design procedure for a QS-based metal ion biosensor is summarized as follows:

1) Provide user-defined design specifications $G_{ref}(I_M)$ for the quorum sensing-based metal ion biosensor.
2) Select an initial set S of promoter-RBS components.

3) Calculate the cost value $J(S)$ in for S. The relationship between the fitness value $F(S)$ and the cost value $J(S)$ is inversely proportional to (10)

$$F(S) = \frac{1}{J(S)}. \tag{10}$$

The fitness value plays a key role in natural selection for selecting a set S of promoter-RBS components to maximize the fitness value $F(S)$, or equivalently to minimize the cost function $J(S)$ in (9). For example

$$\max_S F(S) = \frac{1}{\min_S J(S)}. \tag{11}$$

4) Create an offspring set S, using GA operators such as reproduction, crossover, and mutation [50].
   1) Make copies of possible solutions, on basis of their fitness.
   2) Swap values between two possible solutions.
   3) Randomly alter the value in a possible solution.
5) Calculate the cost value of the new set S obtained by natural selection. Stop when the design goal is achieved or an acceptable solution is obtained due to a limited number of components available from the libraries. Otherwise, create the next generation and return to step 3.

III. Results

For the convenience of description and explanation, as shown in Fig. 1, a QS-based metal ion biosensor is assembled by selecting a set of components from Supplementary Appendix A, namely, a metal ion-induced promoter-RBS component M_i, a constitutive promoter-RBS component C_j, and a QS-dependent promoter-RBS component A_k. The design specifications are as follows:

- The desired fluorescence intensity to different metal ion concentrations is described as follows:

$$G_{ref}(I_M) = 65 + \frac{5000}{1 + \left(\frac{10^{-1}}{I_M}\right)^2}. \tag{12}$$

- Input operation range: 10^{-3} to 10^{0} μM [51].
- The standard deviations of parameter fluctuations that are supposed to be tolerated *in vivo* are given by

$$\begin{aligned} &\Delta P_{u,i} = 0.05P_{u,i},\ \Delta P_{l,i} = 0.05P_{l,i},\ \Delta P_{u,j} = 0.05P_{u,j},\\ &\Delta P_{u,k} = 0.05P_{u,k},\ \Delta P_{l,k} = 0.05P_{l,k},\ \Delta r_E = 0.05r_E,\\ &\Delta r_R = 0.05r_R,\ \Delta r_G = 0.05r_G,\ \Delta r = 0.05r,\\ &\Delta m = 0.05\text{ m},\ \Delta d = 0.05d \end{aligned} \tag{13}$$

as well as the external environmental noise $v_p, p = 1, 2, 3$, for transcription and translation processes, and noise v_4 for mature reporter protein expression, are all Gaussian, with zero mean and unit variance.

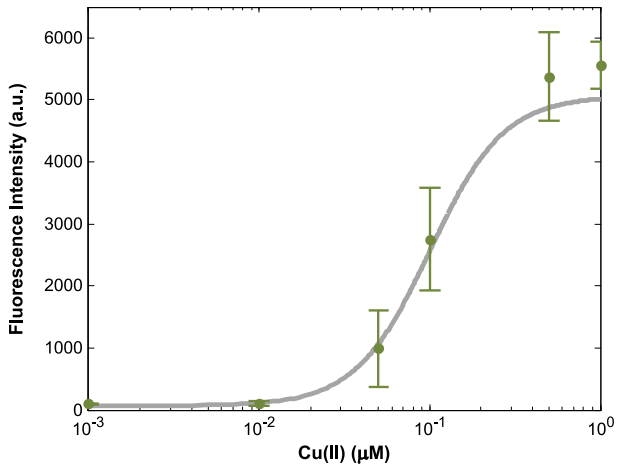

Fig. 2. Results for the QS-based metal ion biosensor. By minimizing the cost function in for the metal ion biosensor in Fig. 1, an adequate set $S = (M_1, C_6, A_3)$ is selected from the corresponding libraries in Supplementary Appendix A. The green points are the experimental results (mean of three trials) by $S = (M_1, C_6, A_3)$. The gray solid line is the desired I/O response in (12).

- The cost function is given by

$$J(S) = E\int_{10^{-3}}^{10^{0}} \left(G_{ss}(S, I_M) - G_{ref}(I_M)\right)^2 dI_M. \tag{14}$$

In order to efficiently solve the constrained optimal matching design problem of the metal ion biosensor, a GA-based library search method is employed to search a set S from corresponding libraries in Supplementary Appendix A to minimize the cost function (14). The adequate promoter-RBS components from the corresponding libraries are found to be M_1, C_6 and A_3. The desired response is shown in Fig. 2, with the fluorescence intensity values taken from Fig. 3 under different Cu(II) ion concentrations. Clearly, the metal ion biosensor can be as close as possible to the specified I/O response despite the parameter fluctuations and environmental noise. Besides, the detection performance is better than that without quorum sensing-based amplifier in Fig. 4 (see Appendix for mathematical details) at the same copper ion concentrations (see Fig. 5 and Fig. 6). In particular, the QS-based metal ion biosensor increases the sensitivity to Cu(II) ion by roughly one order of magnitude and the dynamic range by roughly fourfold. It confirms the quorum sensing-based metal ion biosensor is useful for enhancing the detection ability.

IV. Conclusion

In this study, a QS-based biosensor is constructed to improve the performance of metal ion detection. Based on four design specifications, the QS-based biosensor can be constructed by selecting adequate promoter-RBS components in combination with a feasible range of metal ion concentrations. The

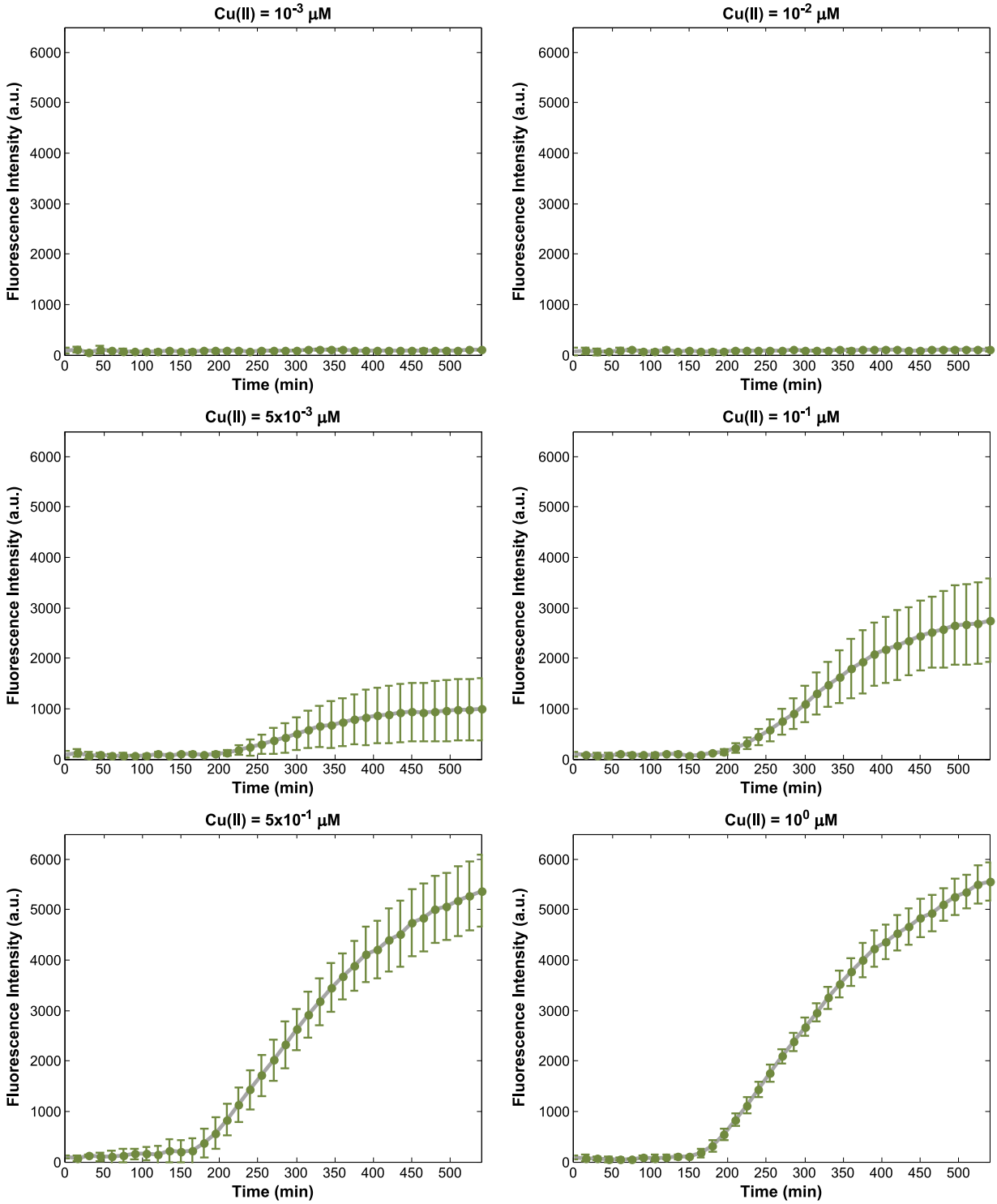

Fig. 3. Time profiles for the QS-based metal ion biosensor under different concentrations of Cu(II) ion, with measurements taken every 15 min. The green points are the experimental results by $S = (M_1, C_6, A_3)$.

proposed GA-based searching method could provide synthetic biologists with a useful tool to save time in selecting adequate promoter-RBS components to meet a specified detection performance. The experimental results confirm that the quorum sensing-based biosensor can efficiently enhance the performance in metal ion detection. In the future, the mechanism of metal ions detection can be used for environmental bioremediation [52]. Bioremediation, which utilizes the ability of biosorption (e.g., protein) to bind the ions using biomass or biopolymers, is a promising approach for environmental treatment. Proteins for biosorption are extensively studied for their specific binding regions and are engineered as biosorbent because of their higher adsorption capacity, low cost, and reusability [52], [53]. Research indicates that biosorbents contain a variety of functional sites including carboxyl, phosphate, amino groups, etc., and biology materials, like bacteria, yeast,

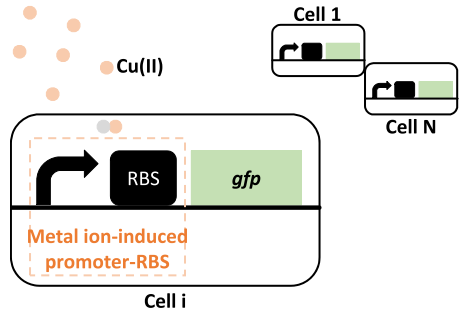

Fig. 4. Copper ion biosensor without quorum sensing-based amplifier, using metal ion-induced promoter-RBS component M_1 selected from the library in Supplementary Appendix A.

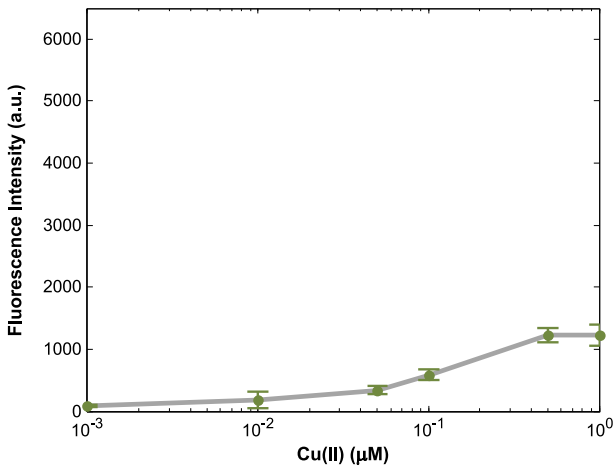

Fig. 5. Results for the Cu(II) ion biosensor without QS-based amplifier. The green points are the experimental results (mean of three trials) by M_1 from the library in Supplementary Appendix A.

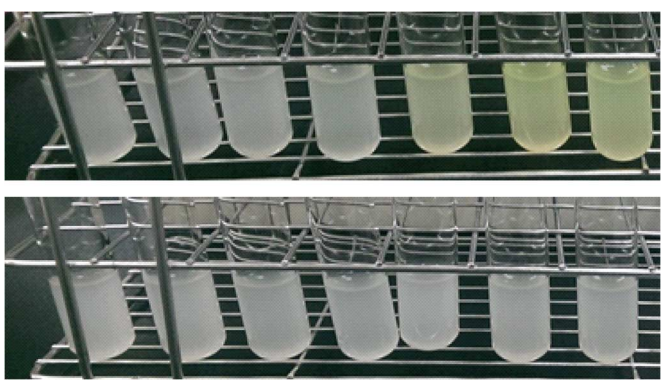

Fig. 6. Comparison of Cu(II) ion biosensors with (top) and without (bottom) QS-based amplifier. The Cu(II) concentrations from left to right are 0, 10^{-3}, 10^{-2}, 5×10^{-2}, 10^{-1}, 5×10^{-1}, and 1 μM.

and fungi have gained attention for their removal and recovery abilities [52], [53]. Ravikumar *et al.* used a recombinant strain based on the specific interaction of Cu(II) with metal binding peptide, which displayed selective adsorption of Cu(II) from aqueous solutions [54]. Thus, the QS-based biosensor can be combined with the Cu(II) bioadsorption system to promote bioremediation (see Fig. 7).

V. Materials and Methods

A. Biosensor Reagents

All restriction enzymes and DNA ligation kit are purchased from New England Biolabs. The chemicals used here are from Sigma-Aldrich. The oligonucleotides are from Integrated DNA Technologies.

B. Bacteria Strains and Medium

Escherichia coli strain DH5α cells from Yeastern biotech (ECOS) are used for the construction of the procedure and for the fluorescence measurement experiments. An M9 working medium (34 g/L Na2HPO4, 15 g/L K2HPO4, 2.5 g/L NaCl, 5 g/L NH4Cl, 2 g/L casamino acids, 2 μM vitamin B1, 2 mM MgSO4, 0.2% glucose) with proper antibiotics is used for *E. coli* cultivation at 37°C and 200 r.p.m..

C. Plasmid Construction

The DNA parts used in this study are selected from BioBrick or synthesized by MDBio Biotech Co. Ltd. All DNA parts are assembled into the backbone pSB3K3 (20–30 copies per cell), via the BioBrick standard assembly method.

D. Fluorescence Measurement

The cells containing the genetic circuit are inoculated in the M9 working medium with 50 mg/mL Kanamycin. The culture is incubated for approximately 14–16 hours, after which it is diluted 250-fold and further incubated until the OD600 reaches 0.1. Different concentrations of $CuSO_4$ are then added to the medium. The fluorescence intensity of the culture is measured subsequently by the microplate reader (BioTek, Synergy H1, GFP settings are 490/510 nm for excitation and emission).

Appendix

The steady-state model of the metal ion biosensor without QS-based amplifier is as follows:

$$\begin{cases} x_{GSS} = \frac{P_M(P_{u,i},P_{l,i},x_S,I_M)}{d+r_G} \\ G_{SS} = \frac{m}{d+r} x_G \end{cases} \tag{15}$$

where x_{GSS} and G_{SS} are the steady-state concentrations of immature and mature reporter proteins, respectively.

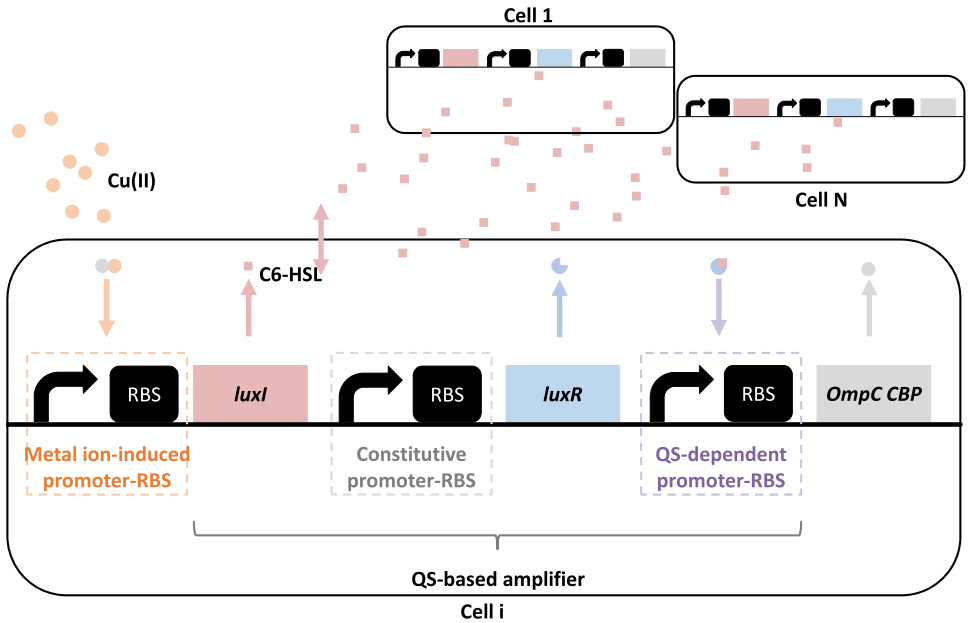

Fig. 7. A copper sensing and adsorbing system in E. coli.

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