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# A Chimeric Two-Component Regulatory System-Based *Escherichia coli* Biosensor Engineered to Detect Glutamate

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**Abstract** In this study, we constructed amino acid biosensors that can be used as a high-throughput system to screen microorganisms that produce glutamate. The biosensors are based on two-component regulatory systems (TCRSs) combined with green fluorescent protein (GFP) as a reporter. A chimeric DegS/EnvZ (DegSZ) TCRS was constructed by fusing the N-terminal domain of the sensor kinase DegS from *Planococcus* sp. PAMC21323 with the catalytic domain of the osmosensor EnvZ from *Escherichia coli* to control expression of *gfp* in response to glutamate. *gfp* was controlled by the *ompC* promoter through the activated response regulator OmpR-P. The chimeric TCRS-based biosensors showed a 4-fold increase in the fluorescent signal after adding glutamate. A linear correlation was observed between fluorescence intensity and exogenously added glutamate concentration. The chimeric TCRS-based biosensor was used to determine glutamate concentration at the single-cell level by fluorescence-activated cell sorting. Therefore, this biosensor can be used to isolate novel gene products and optimize pathways involved in amino acid production.

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## Introduction

Currently, the amino acid industry constitutes a significant portion of the world's chemical industry. The commercial availability of these amino acids is mainly based on four processes: protein hydrolysates, chemical synthesis, enzymatic synthesis, and microbial fermentation [1]. Amino acids, such as glutamate and lysine, are important sources of food additives, pharmaceuticals, cosmetics, feed supplements, polymer materials, and agricultural chemicals [2, 3].

In the past few decades, microorganisms have been continuously engineered for an efficient and sustainable amino acid production. *Corynebacterium glutamicum* has extensively been studied for the production of large amounts of glutamate, a key intermediate in the production of gamma-aminobutyric acid (GABA) that is used to synthesize Nylon-4 [4–6]. However, isolating the ideal set of novel genes and identifying the best conditions to produce target molecules such as glutamate and GABA are difficult and time-consuming. Thus, high-throughput screening is necessary to rapidly and efficiently screen active heterologous genes.

Quantifying an amino acid-overproducing strain or monitoring intermediate cellular metabolites is often challenging for several reasons, particularly specificity and measurability. Nature has provided numerous specific molecular biosensors that employ different transcriptional regulatory mechanisms in bacteria. Among these, the two-component regulatory system (TCRS) is the main signal transduction mechanism in bacteria, which regulates transcription through a sensor histidine kinase (SK) and response regulator (RR). Therefore, the use of a TCRS or chimeric TCRS as a biosensor to correlate the detection of a target molecule with the expression of a reporter protein is a promising method that can be used in developing microbial cell factories. These chimeric TCRS-based biosensors were engineered to use SK to detect the amino acid through a domain shuffling strategy, using the natural signal transduction mechanism of the TCRS for reporter protein expression [7, 8]. Similarly, other chimeric TCRS-based biosensors were recently developed to detect various biomolecules such as fumarate and malate [9, 10]. In two-component-based biosensor, fluorescence-activated cell sorting (FACS) could be used for high-throughput screening when determining the novel heterologous enzymes, optimized conditions, and fine-tuned metabolic pathways for the robust and efficient production of target molecules. These bio-based sensors can be employed in screening billions of mutant libraries to detect cells with increased levels of target molecule [11]. Cost-effective strategies, such as the use of cheap and renewable substrates combined with systems metabolic engineering of host strains, have been extensively studied in the synthesis of desired products [12–21]. However, along with the development of these strategies, it is also needed to develop high-throughput screening methods to develop host strains for robust and efficient synthesis of desired products. For instance, a spectrophotometric method was recently developed for fast detection of lysine using a specific ninhydrin-ferric reagent [22]. Similarly, microbial biosensors have been employed in evaluating amino acid production. We have recently developed a recombinant *E. coli* strain capable of sensing amino acid in its environment. A chimeric two-component system was constructed by fusing the sensor domain *Pseudomonas putida*

KT2440 AauS and the catalytic domain of EnvZ, which phosphorylates OmpR to activate OmpC promoter-based translation of GFP reporter protein [7]. Similarly, the same strategy was employed in constructing a two-component system for the detection of malate and fumarate [9, 10].

The aim of this study was to develop chimeric TCRS-based biosensors for glutamate-overproducing strains and detect key intermediates, specifically glutamate, in GABA production [23]. Previously, DegS-DegU TCRS from *Bacillus subtilis* was found to sense osmotic stress-related stimuli [24]. The DegS, a sensor kinase localized at the cytoplasm due to the absence of hydrophobic domain, was also identified to sense glutamate with high specificity [25]. Careful analysis of the Pfam domain database revealed that DegS-DegU TCRS is also present in the genome of *Planococcus* sp. PAMC21323 (GenBank accession no. CP009129) that was isolated from King George Island in Antarctica [26]. Thus, we took advantage of this newly identified system to engineer a chimeric DegSZ TCRS by fusing sensor histidine kinase DegS from PAMC21323 with the catalytic domain EnvZ from *E. coli* to generate a biosensor for glutamate detection. Here, we developed a new TCRS-based biosensor in *E. coli* for the detection of endogenous glutamate at the single-cell level.

## Materials and Methods

### Bacterial Strains, Media, and Culture Conditions

*Escherichia coli* DH5α was used for recombinant DNA manipulation, while Top10 was used for recombinant protein expression and whole-cell biosensors. General molecular biology techniques, including PCR, gel electrophoresis, and transformation, were performed using standard protocols [27]. The bacterial strains and plasmids used in this study are listed in Table 1. *Escherichia coli* strains were grown in Luria-Bertani (LB) broth (10 g bacto-tryptone $L^{-1}$, 5 g bacto-yeast extract $L^{-1}$, and 5 g NaCl $L^{-1}$) and potassium minimal medium (46 mM $K_2HPO_4$, 23 mM $KH_2PO_4$, 8 mM $(NH_4)_2SO_4$, 0.4 mM $MgSO_4$, 6 μM $FeSO_4$, 0.66 mM citric acid, 0.33 mM tri-sodium citrate, 1% thiamine HCl, 2 g glucose $L^{-1}$, and 0.2% casein hydrolysate) [28]. Liquid cultures were routinely incubated in 250-mL Erlenmeyer flasks overnight at 37 °C at 200 rpm. Transformed *E. coli* TOP10 cells were selected by addition of the antibiotics ampicillin and chloramphenicol to the nutrient medium at final concentrations of 50 and 34 μg $mL^{-1}$, respectively, according to the drug resistance of the plasmids.

### Plasmid Construction

The plasmids pDegSZ and pOGFP express DegSZ chimeric SK under control of the $P_{BAD}$ promoter and *gfp* under control of the *ompC* promoter, respectively. Before cloning *DegS* sensor domain fragments, *envZ* (684 bp) was amplified from the *E. coli* TOP10 genome (GenBank NC010473) using the primers envZ_F and envZ_R. PCR was performed with the Veriti 96-well thermal cycler (Applied Biosystems, Foster City, CA, USA) using the Expand High Fidelity PCR system (Roche Molecular Biochemicals, Mannheim, Germany). The amplified product was ligated into the low-copy number plasmid pBAD30C (a gift from Prof. Soon Ho Hong) to produce pBAD-EnvZ, which contains a chloramphenicol resistance gene. An open reading frame of 1146 base pairs (bp) was previously reported as the sensor histidine

**Table 1** Strains and plasmids used in this study

| Strain, plasmid, or oligonucleotides | Relevant characteristic/sequence (5′-3′) | Source or reference |
|---|---|---|
| Strains | | |
| TOP10 | F-mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZΔM15 ΔlacX74 nupG recA1 araD139 Δ(ara-leu)7697 galE15 galK16 rpsL($Str^R$) endA1 $\lambda^-$ | Invitrogen |
| DH5α | supE44 DlacU169 (~80lacZDM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1 | Invitrogen |
| TOP-DegSZ1 | TOP 10 with pBAD-DegSZ1 and pG-OGFP | This study |
| TOP-DegSZ2 | TOP 10 with pBAD-DegSZ2 and pG-OGFP | This study |
| Plasmids | | |
| pUC18 | $Amp^R$ | New England Biolabs[a] |
| pUEup1 | pUC19 (250 bp *ompC*-EnvZ intergenic region), containing *ompC*promoter region, $Amp^r$ | This work |
| pOGFP | pUC19 (250 bp *ompC*-EnvZ intergenic region), *ompC′-gfp* transcriptional fusion vector, containing *ompC*promoter region, $Amp^r$ | This work |
| pBAD30C | pBAD30 ($Amp^r$ was replaced with $Cm^r$) | [12] |
| pBAD-EnvZ | pBAD30C derivative, containing the *EnvZ* gene | This work |
| pDegSZ1 | pBAD-EnvZ derivative, containing the truncated *DegS I* gene fused to HK of *EnvZ* | This work |
| pDegSZ2 | pBAD-EnvZ derivative, containing the truncated *DegS 2* gene fused to HK of *EnvZ* | This work |
| Oligonucleotides | | |
| ompC_F | GAATTCTCCCTTGCATTTACATTTTGAAACAT CTATAGCGATCTCTTGAGTTGGA | |
| ompC_R | TCTAGTAAGTCCATTCTCCCCAAAAATGCAGAATAATCCAACTCAAGAG | |
| gfp_F | ACTTACTAGAGAAAGAGGAGAAATACTAGATGCAGTCTAAAGGA | |
| gfp_R | GGATCCTTATTAATGGTGATGGTGATGGTG | |
| envZ_F | TCTAGAATGAGGCGATTGCGCTTCTCG | |
| envZ_R | AAGCTTTTACCCTTCTTTTGTCGTGCC | |
| degS_F | TAAGCATCTAGAATGACAAAAAAAATCAGTGGA | |
| degS1_R | TGCTTACATATGTTCTTCTTGCACCTCGAT | |
| degS2_R | TGCTTACATATGGATAATACGGAGGCTATA | |

kinase DegS in the *Planococcus* sp. PAMC21323 (GenBank CP009129.1). The truncated sensor kinase sequences of DegS, which were 540 bp (*DegS1*) and 513 bp (*DegS2*), were optimized based on the *E. coli* codon preference. Synthetic*DegS1* and *DegS2* were synthesized by Macrogen, Inc. (Seoul, Korea), and the sequences were submitted to the GenBank database under accession numbers KX137119 (DegS1) and KX137120 (DegS1). The N-terminal domain of the sensor kinases *DegS1* and *DegS2* were amplified from the synthetic DNA construct using the primers degS_F and degS1_R or degS2_R. Truncated DegS1 or DegS2 was cloned into pBAD-EnvZ at the *Xba*I and *Nde*I restriction sites to generate chimeric pDegSZ1 or pDegSZ2 TCRS, respectively (Fig. 1b). Expression of pDegSZ1 or pDegSZ2 was induced under control of the $P_{BAD}$ promoter by the addition of arabinose.

The mutant *ompC* promoter of 108 bp was amplified using the primers ompC_F and ompC_R, which were designed based on the sequences upstream of *ompC* in the *E. coli* TOP10 genome. This sequence consists of a single ompR binding site and the *ompC* promoter, whereas the natural site contains three binding sites for OmpR-P [29]. *gfp*, encoding GFP-mut3.1b, was amplified from the pET28a plasmid [30] with oligonucleotides gfp_F and gfp_R. The two PCR products were integrated by overlap PCR and cloned into pUC18 to

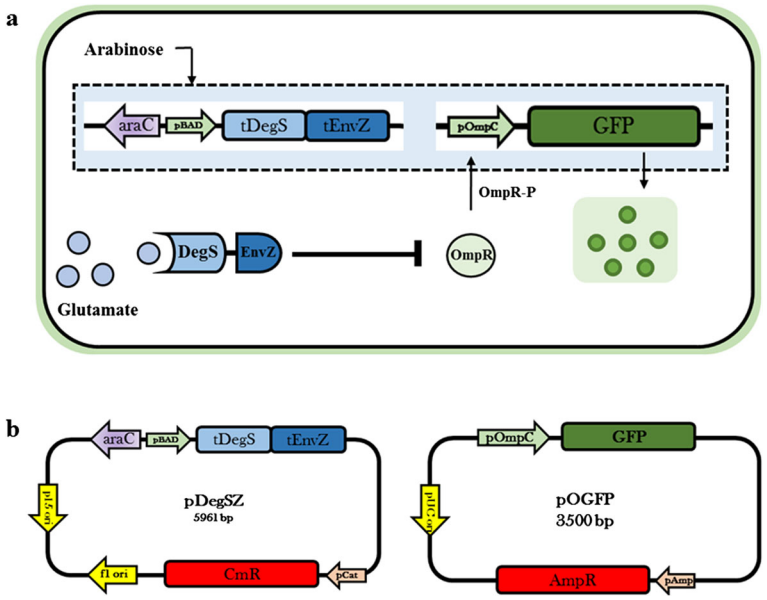


**Fig. 1** Schematic outline of glutamate-sensing chimeric TCRS-based biosensor system. **a** The sensor domain of DegS from *Planococcus* sp. PAMC21323 and catalytic domain of EnvZ from *Escherichia coli* were fused to form a DegSZ chimeric TC regulatory protein. The presence of glutamate induces a phosphorylation cascade, leading to binding by OmpR to the mutant *ompC* promoter and GFP-mut3.1b protein expression. **b** Plasmid construction. Chimeric DegSZ sensor kinase under transcriptional control of the tightly regulated $P_{BAD}$ promoter. Plasmid pOGFP with *gfpmut3.1b* under transcriptional control of the *ompC* promoter

generate the pOGFP biosensor plasmid, which contains an ampicillin resistance gene (Fig. 1b). Oligonucleotides were synthesized by Macrogen, Inc. and are listed in Table 1.

## Cultivation Conditions

For gene cloning and manipulation, LB liquid or agar (1.5%, *w*/*v*) was routinely used. For analysis of the whole-cell biosensor, a single colony of *E. coli* TOP10 cells, transformed with pOGFP and either pDegSZ1 or pDegSZ2, was grown overnight at 37 °C in LB medium. The overnight cultures were diluted by 100-fold in fresh potassium minimal medium containing 50 μg $mL^{-1}$ampicillin and 34 μg $mL^{-1}$ chloramphenicol. The cells were incubated aerobically in an orbital shaker at 37 °C and grown to an approximate optical density at 600 nm ($OD_{600}$) of 0.5. Arabinose was added to the medium at a concentration of 0.1 mM to induce expression of chimeric SK. Cell density and fluorescence were measured at different concentrations of glutamate over 8 h. In addition to glutamate, the amino acids aspartate, methionine, and glutamine were also tested. Cell density was monitored by measuring $OD_{600}$ on the Versamax Microplate Reader (Molecular Devices, Sunnyvale, CA, USA).

## SDS-PAGE Analysis

*Escherichia coli* TOP10 cells, harboring pOGFP, and either pDegSZ1 or pDegSZ2, were grown at 37 °C in LB medium to an $OD_{600}$ of approximately 0.5. Expression of chimeric TCRS was induced by adding 0–1 mM arabinose, followed by addition of 1 mM glutamate.

After 4-h incubation at 37 °C, cells were harvested and lysed by sonication and then centrifuged at 10,000×*g* at 4 °C for 20 min. The supernatant was saved as the soluble protein fraction and analyzed by SDS-PAGE (10% acrylamide gel). Aliquots of the cultures, induced by different concentrations of arabinose, were subjected to 10% SDS-PAGE without thermal denaturation. The gel was rinsed with distilled water and exposed to a UV trans-illuminator to detect fluorescent bands, followed by staining with Coomassie blue.

### Quantification and Imaging of GFP Expression

Fluorescence intensity of the cell suspension was determined using a Synergy Mx plate reader (Bio-Tek, Winooski, VT, USA). GFP fluorescence was measured at an excitation of 485 ± 10 nm and emission of 515 ± 10 nm. The relative fluorescence intensity was calculated (fluorescent emission/OD) and normalized to the intensity of cells harboring pOGFP and either pDegSZ1 or pDegSZ2. Fluorescence images of the cells were taken with a Zeiss AX10 fluorescence microscope (Carl Zeiss Microscopy, Göttingen, Germany).

### Fluorescence-Activated Cell Analysis

To perform fluorescence-activated cell analysis, *E. coli* TOP10 cells harboring pOGFP and either pDegSZ1 or pDegSZ2 were grown at 37 °C in potassium minimal medium to an optical $OD_{600}$ of approximately 0.5. Next, arabinose was added at a concentration of 0.1 mM to induce chimeric TCRS expression, followed by addition of 0, 0.5, or 1.0 mM glutamate. After 2 h of incubation at 37 °C, the cells were centrifuged and re-suspended in 0.02 mM PBS to a final density of $10^6$ cells $mL^{-1}$ and analyzed using the BD FACS Aria (BS Biosciences, San Jose, CA, USA) with an FL1-H channel at an excitation of 488 nm and emission of 515 nm. A total of 20,000 recombinant cells were gated for analysis.

## Results

### Two-Component-Based Biosensor Design and Construction

A schematic diagram of DegSZ signaling system used as biosensor is shown in Fig. 1. DegSZ mediates both phosphorylation of OmpR and dephosphorylation of OmpR-P. OmpR-P activates the *ompC* promoter-driven transcription of the reporter protein. In the presence of glutamate, the phosphatase activity of DegSZ decreases, resulting in increased OmpR-P levels sufficient to activate transcription of the reporter protein [31]. To design a biosensor that responds to elevated intracellular concentrations of glutamate, we constructed a chimeric TCRS-based biosensor. In *B. subtilis*, the DegS sensor domain of TCRS can sense intracellular glutamate. Thus, we examined the DegS identified from the *Planococcus* sp. PAMC21323 (GenBank accession no. CP009129) DegS-DegU TCRS. Since *E. coli* does not contain a glutamate sensing TCRS, the constructed synthetic TCRS was introduced to generate an *E. coli*-based glutamate biosensor. To create the chimera, we aligned members of the N-terminal domain of the sensor kinase DegS from PAMC21323 with the catalytic domain of EnvZ from *E. coli* TOP10, and identified potential functional fusion points between PAMC21323 DegS and *E. coli* EnvZ. Therefore, we constructed two chimeric HKs, designated as DegSZ1 and DegSZ2, with different linker lengths of 513 and 540 bp, respectively,

from the N-terminus of DegS. Although it was expressed in the cytosolic fraction, DegSZ was cloned into the vector pBAD30C, which contains the tightly regulated $P_{BAD}$ promoter that is highly expressed upon induction. The pOGFP reporter plasmid was constructed to monitor the output signal of DegSZ chimeric HK in response to intracellular glutamate concentrations. In pOGFP, GFP-mut3.1 was expressed under control of the mutant *ompC* promoter. This part of the promoter contains only one binding site for ompR-P, which is induced by activated ompR (Fig. 1b) [32]. One of the two resulting chimeric plasmids and sensor plasmid were transformed together into *E. coli* TOP10 cells to generate the biosensor.

## Expression Analysis of Chimeras DegSZ1 and DegSZ2

Using SDS-PAGE analysis, a protein band was detected at approximately 36 kDa in the lysates from arabinose-induced cells harboring one of the two biosensors. The proteins were undetectable in non-induced cells because of the tightly regulated $P_{BAD}$ promoter (Fig. 2a, b). The apparent molecular weight of each chimera was consistent with the predicted molecular weight for the polypeptide. The predicted polypeptide was extremely soluble, indicating cytoplasmic localization. Both chimeras appeared to localize in the cytoplasm as they do not possess hydrophobic domains within their amino acid sequences, as analyzed by TMHMM 2.0 (Fig. S1). SDS-PAGE without thermal denaturation was used to determine the sensing ability of the chimeras DegSZ1 and DegSZ2. In this experiment, recombinant cells were grown in the

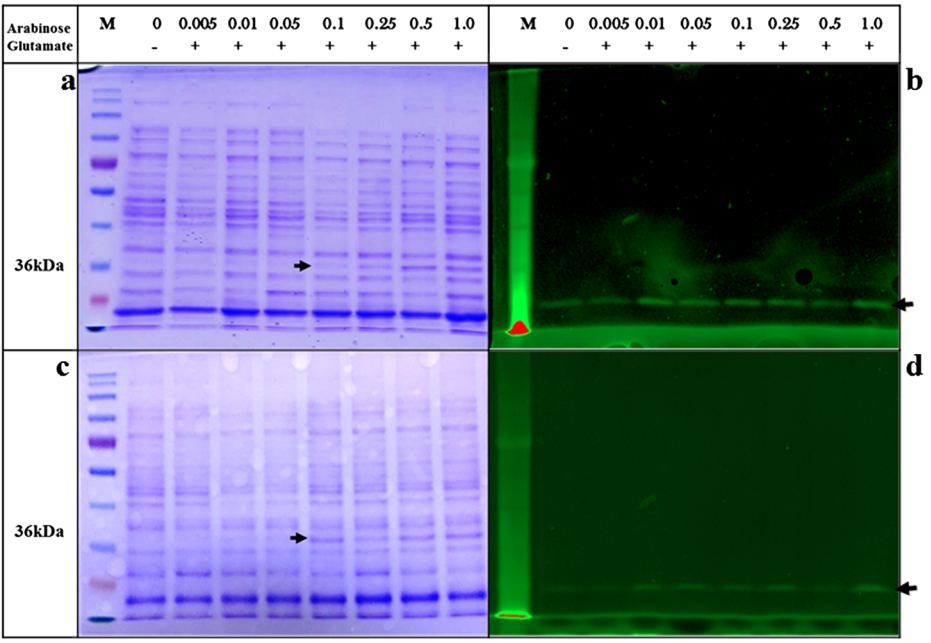


**Fig. 2** SDS-PAGE expression analysis. Reporter gene expression from the chimeric DegSZ1 (**a**) and pDegSZ2 (**b**) with pOGFP after addition of 1 mM glutamate. Induced recombinant plasmid at the indicated arabinose concentrations (lanes 3–9) and uninduced recombinant plasmid (lane 2). The gels were exposed to a UV transilluminator for visualization of fluorescence bands (GFP) and then stained with Coomassie brilliant blue G-250. GFP expression in strains harboring biosensor DegSZ1 (**c**) and GFP expression in strains harboring biosensor DegSZ2 (**d**). The arrows in **a** and **b** indicate DegSZ1 and DegSZ2 (~ 36 kDa), respectively, and arrows in **c**, **d** indicate GFP-mut3.1b

presence of different concentrations of arabinose and a fixed concentration of 1.0 mM glutamate. The concentration profiles of induced cells showed that the protein fluorescence in the gel increased as arabinose concentration increased. A low level of fluorescence was observed for the lysate from the control, uninduced cells, which may have been due to basal levels of *ompC* promoter expression in the LB medium (Fig. 2c, d). These results suggest that reporter protein expression was precisely regulated by the two chimeras in response to the intracellular glutamate concentration.

## Quantitative Characterization of the Chimeric TCRS-Based Biosensor

To demonstrate the feasibility of using the chimeric TCRS to sense intracellular glutamate concentrations, we analyzed the fluorescence intensity distribution of cells in the presence of 1 mM glutamate and varying arabinose concentrations (0, 0.01, 0.1, and 1.0 mM at 8 h after induction) using a fluorescent microplate reader at 480-nm excitation and 515-nm emission. The results suggest that expression of the two chimeras, DegSZ1 and DegSZ2, increased the expression of the reporter protein by approximately 4- and 2-fold, respectively (Fig. 3). Moreover, both chimeric TCRS-based biosensors showed optimal activation of the *ompC* promoter and GFP expression when induced with 0.1 mM arabinose.

To determine the effector specificity and range of the constructed chimeric TCRS-based biosensors, *E. coli* cells harboring the respective chimeric TCRS and reporter plasmid were grown in modified potassium minimal medium for 8 h in the presence of 1 mM glutamate, aspartate, methionine, or glutamine. The fluorescence of the cells was measured *in vivo* at 8 h after amino acid addition (Fig. 4). The two biosensor plasmids showed high expression of the reporter protein in the presence of glutamate and aspartate, but low expression in the presence of methionine and glutamine. The sensitivity of the biosensors was also tested by measuring fluorescence in cells exposed to a range of glutamate concentrations (Fig. 5). The minimum concentration of glutamate required for detection by both biosensors was 0.25 mM. Moreover, there was a strong correlation between relative fluorescence intensity and glutamate concentrations up to 2 mM. Finally, we compared the temporal expression profiles of the two

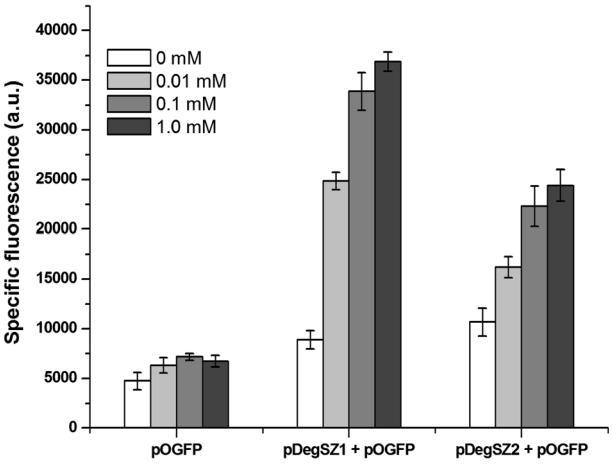


**Fig. 3** TCRS-based biosensor as a function of arabinose concentration. Fluorescence intensity of the culture 8 h after induction using a fluorescence microplate reader (emission at 488 nm, excitation at 512 nm). Error bars indicate the means of three independent cultures

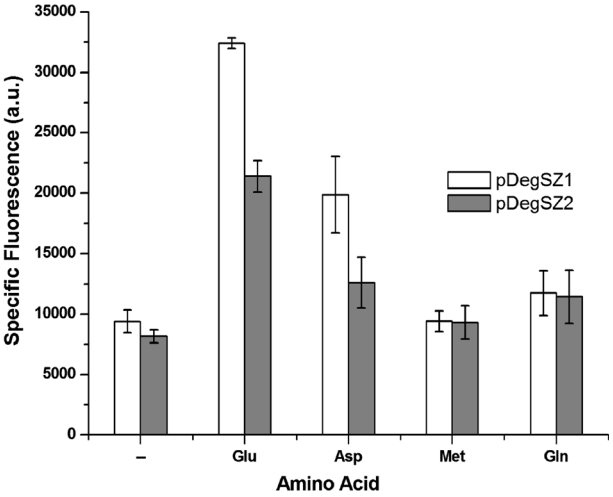


**Fig. 4** Effector spectrum of the constructed biosensors. The fluorescence of *E. coli* cells harboring the biosensor after cultivation in the presence of 1.0 mM of the indicated amino acid using a fluorescence microplate reader (emission at 488 nm, excitation at 512 nm). Mean values and standard deviations of three independent experiments are shown

biosensors to compare their dynamic responses to glutamate, using cells transformed with only pOGFP as a negative control (Fig. 6). Fluorescence increased as culture time increased; the fluorescence intensity was stronger for the DegSZ1 biosensor than for the DegSZ2 biosensor. These cells were imaged by fluorescence microscopy (Fig. 6, inset). As shown, the

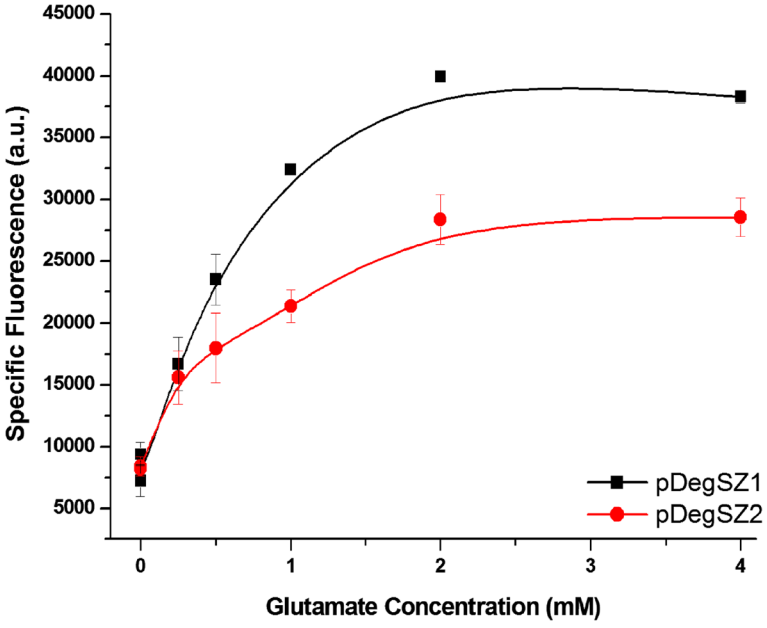


**Fig. 5** TCRS-based biosensor as a function of glutamate concentration. The fluorescence of the culture was measured 8 h after induction using a fluorescence microplate reader (emission at 488 nm, excitation at 512 nm). Error bars indicate the means of three independent cultures

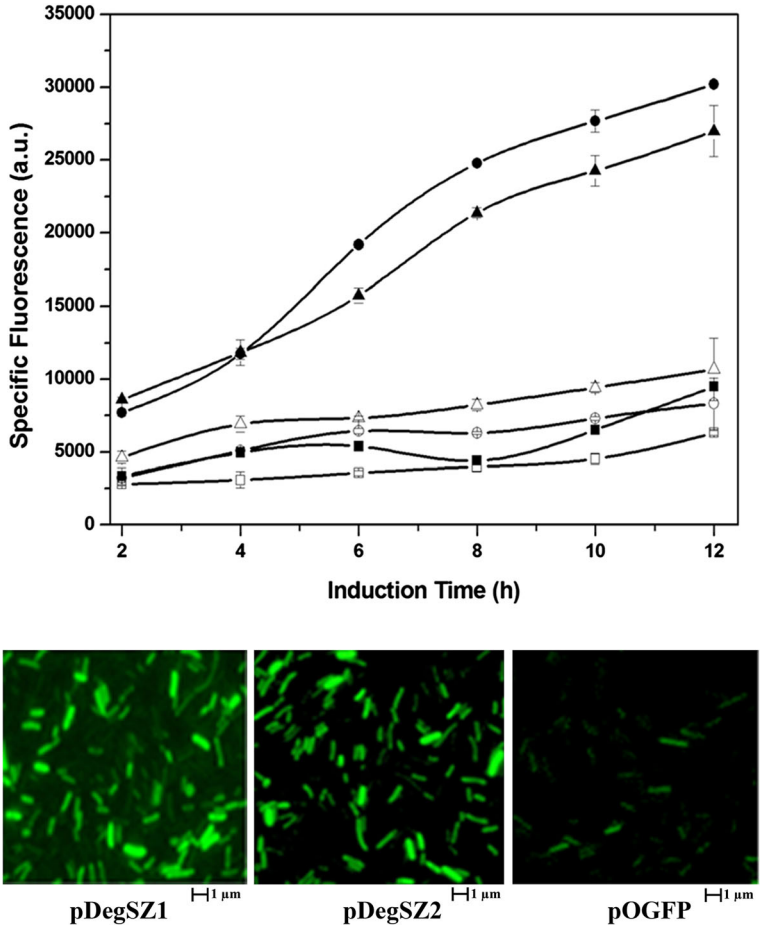


**Fig. 6** TCRS-based biosensor as a function of time. Dynamic response curve of the strains harboring pOGFP (square), pDegSZ1/pOGFP (circle), and pDegSZ2/pOGFP (triangle) in the presence (closed) and absence (open) of 1.0 mM of glutamate and 0.1 mM of arabinose. Specific fluorescence was measured after induction at different time intervals using a fluorescence microplate reader (emission at 488 nm, excitation at 512 nm). Error bars indicate the means of three independent cultures. GFP-mut3.1b expression was imaged by fluorescence microscopy (inset)

DegSZ1 TCRS-based biosensor was rapidly activated and expressed the reporter protein within 4 h, with a 4-fold increase in fluorescence compared to that of DegSZ2.

## Measurement at the Single-Cell Level

To measure the heterogeneity of the cell population and test the potential to use this chimeric TCRS-based biosensor for high-throughput screening at the single-cell level, *E. coli* cultures harboring one of the two biosensors were analyzed by FACS. With increasing glutamate concentration, there was a clear increase in cellular fluorescence intensity (Fig. 7). Specifically, if the fluorescent intensity of the control strain was adjusted to 1% of the threshold

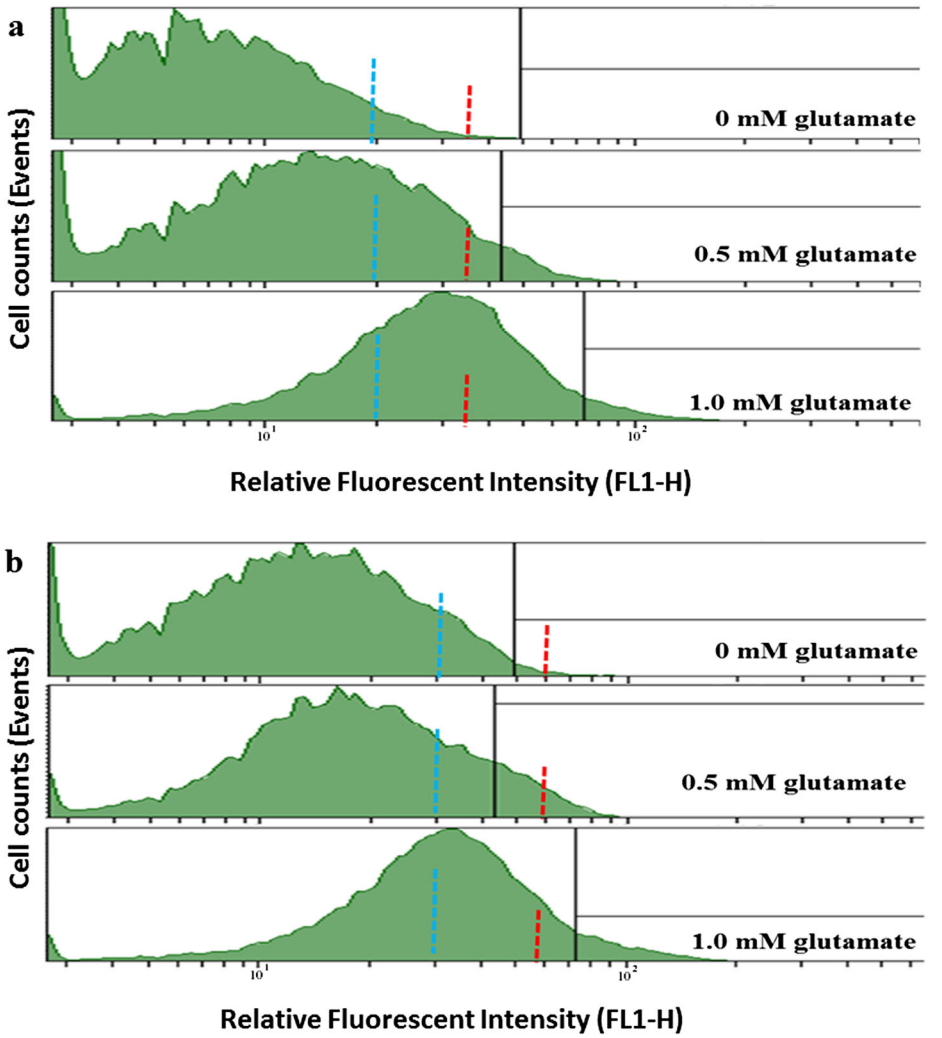


**Fig. 7** Fluorescence response of chimeric TCRS-based recombinant biosensor. Fluorescence-activated cell analysis of cells exposed to different glutamate concentrations by a flow cytometer. The blue lines indicate the green fluorescence threshold level of uninduced cells (10%; 0 mM glutamate), while the red lines indicate the green fluorescent threshold level of 1% of uninduced cells. **a** pDegSZ1. **b** pDegSZ2

fluorescence level, cells harboring the pDegSZ1 biosensor reached fluorescence intensities of nearly 35 and 75% when induced by 0.5 and 1.0 mM glutamate, respectively. In cells harboring the pDegSZ2 biosensor, compared to the threshold fluorescence level, less than 35 and 75% cells were induced by 0.5 and 1.0 mM of glutamate, respectively. Additionally, while there were very few control cells with FL1-H greater than $10^2$, nearly 1% of the total cells induced by 1.0 mM glutamate showed an FL1-H greater than $10^2$. This result demonstrates that cells harboring the pDegSZ1 biosensor show relatively stronger fluorescence than those harboring the pDegSZ2 biosensor.

## Discussion

In this study, we used a chimeric TCRS-based strategy by genetic modification and assembly to develop a glutamate biosensor for measuring intracellular glutamate concentrations. In this system, the expression of the reporter protein was directly proportional to external glutamate levels existing in culture medium, which were determined based on relative fluorescence intensities. The development of metabolically engineered bacterial cells that can efficiently produce glutamate has provided a resource for the production of food additives and pharmaceuticals. Therefore, the development of a simple, convenient, and high-throughput method to quickly evaluate or estimate glutamate production levels is needed. This high-throughput screening would facilitate identification of novel enzymes or optimize biomolecule production. The chimeric signal transduction apparatuses were constructed by integrating DegS from PAMC21323 belonging to the NarL family and EnvZ from *E. coli* TOP10 belonging to the ompR family. In previous studies, similar chimeric TCRS-based biosensors were developed from the CitB [9, 10] and chemotaxis [8, 33] families. These studies demonstrated that different two-component sensory proteins can be fused to the catalytic domain of EnvZ TCRS and used as biosensors [34–37]. Up to date, there are at least 100 TCRS that have been reported to recognize a variety of substrate from small molecules to aromatic compounds [38, 39]. However, it is necessary to further exploit new two-component sensory proteins from diverse organisms in order to develop new biosensors that can be employed in various purposes.

The TCRS biosensor constructed in this study was capable of sensing intracellular glutamate with arabinose concentrations ranging from 0 to 0.1 mM, and the resulting increase in chimeric TCRS in the cytosolic fraction led to increased cellular fluorescence. We also found a correlation between the response of the TCRS biosensor generated in this study to the concentrations of glutamate and inducer arabinose, which was similar to the previously reported response of a chimeric TCRS biosensor to the acidic amino acid aspartate [7, 40]. Our results suggest that the synthetic TCRS-based biosensor can be used to estimate externally added glutamate concentrations in the medium. Further studies are needed to introduce this biosensor into strains that overproduce glutamate concentration (> 10 mM), which would greatly facilitate monitoring of glutamate production in cells.

Notably, the linear detection range of the synthetic TCRS-based biosensor was 0–2.0 mM glutamate, with saturation occurring between 2.0 and 4.0 mM. This biosensor also allowed the use of FACS to identify cells producing glutamate for high-throughput screening when determining the novel fine-tuned metabolic pathways for the robust and efficient production of target molecules [11]. The chimeric cytoplasmic two-component biosensors, designated as DegSZ1 and DegSZ2, consist of a chimeric sensor kinase (pDegSZ1 or pDegSZ2) and reporter gene (pOGFP). The biosensor was rapidly activated and strongly expressed, showing as much as a 4-fold increase in fluorescence levels within 4 h. Cells harboring the pDegSZ1 biosensor showed higher fluorescence levels than cells harboring the pDegSZ2 biosensor, likely because of the impact of the linker length on our ability to isolate an active chimera.

## Conclusion

Our results indicate that the glutamate biosensor based on the chimeric TCRS strategy can be used to isolate novel gene products and optimize pathways involved in amino acid production.

Additional improvements to these biosensors are needed to increase the fluorescence response and reduce variability in the fluorescent signals at the single-cell level. Furthermore, coupling these biosensors to FACS enables evaluation of libraries containing up to a billion cells. This technology is expected to identify novel enzymes catalyzing glutamate formation, thereby accelerating the development of next-generation cell factories for amino acid production [25, 41]. Moreover, the system demonstrated in this study can be further extended in the development of strategies for the synthesis of industrially valuable products. The TCRS can be used in screening novel enzymes and optimized conditions that can be further employed in the production of target compounds.

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**Compliance with Ethical Standards**

**Conflict of Interest** The authors declare that they have no conflict of interest.

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