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NOTE

BIOTECHNOLOGY PROGRESS

Engineering *Escherichia coli* for enhanced sensitivity to the autoinducer-2 quorum sensing signal

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Abstract

The autoinducer-2 (AI-2) quorum sensing system is involved in a range of population-based bacterial behaviors and has been engineered for cell–cell communication in synthetic biology systems. Investigation into the cellular mechanisms of AI-2 processing has determined that overexpression of uptake genes increases AI-2 uptake rate, and genomic deletions of degradation genes lowers the AI-2 level required for activation of reporter genes. Here, we combine these two strategies to engineer an *Escherichia coli* strain with enhanced ability to detect and respond to AI-2. In an *E. coli* strain that does not produce AI-2, we monitored AI-2 uptake and reporter protein expression in a strain that overproduced the AI-2 uptake or phosphorylation units LsrACDB or LsrK, a strain with the deletion of AI-2 degradation units LsrF and LsrG, and an "enhanced" strain with both overproduction of AI-2 uptake and deletion of AI-2 degradation elements. By adding up to 40 μM AI-2 to growing cell cultures, we determine that this "enhanced" AI-2 sensitive strain both uptakes AI-2 more rapidly and responds with increased reporter protein expression than the others. This work expands the toolbox for manipulating AI-2 quorum sensing processes both in native environments and for synthetic biology applications.

KEYWORDS
AI-2, autoinduction, quorum sensing

1 | INTRODUCTION

The "universal" quorum sensing signal autoinducer-2 (AI-2) is produced and recognized by many species of bacteria and influences important cell processes including biofilm formation, persistence, and virulence.[1-4] AI-2 is likely also important in the gastrointestinal (GI) tract, as the AI-2 signal can influence mammalian cell gene expression,[5] and manipulation of AI-2 signaling influences the microbiome composition of the mouse GI tract after antibiotic treatment.[6] As the fields of synthetic biology and metabolic engineering have advanced, an emerging area of focus is on engineering cell–cell communication, both amongst a single population (intra-) of bacteria or between communities (inter-).[7,8] Developing strains of *Escherichia coli* able to sense and respond to a range of AI-2 concentrations could be useful both for interrogating and manipulating native populations of bacteria and as tools for regulating communication in engineered ecosystems.

Previously, we developed inducible AI-2 controller cells with enhanced ability to uptake and respond to AI-2 by manipulating the AI-2 quorum sensing network through overexpression of proteins responsible for uptake and phosphorylation of AI-2, specifically LsrACDB and LsrK.[9-11] We have also engineered cells that autonomously activate protein expression based on accumulation of AI-2[12] and demonstrated that deleting genes responsible for degrading and altering AI-2, *lsrF* and *lsrG*, results in activation of protein expression

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at lower AI-2 levels.[13,14] In this paper, we demonstrate that combining these two strategies, use of an enhanced feedback loop through overexpression of LsrK and LsrACDB and deletion of *lsrF* and *lsrG* from the genome, can be used to engineer *E. coli* cells with enhanced sensitivity and ability to uptake and report on the presence of biologically relevant extracellular AI-2 concentrations. We envision these cells could be further engineered to genetically actuate "programmed" responses based on AI-2 level, enabling their activation at lower levels or with less delay than possible based on current AI-2 sensing capabilities.

2 | MATERIALS AND METHODS

2.1 | Bacterial strains and growth conditions

The bacterial strains and plasmids used in this study are listed in Table S1. Unless otherwise noted, all cultures were inoculated from single colonies grown on LB-agar plates into LB media with 50 μg/mL ampicillin and 50 μg/mL kanamycin at 37°C with 250 rpm shaking and grown to approximately OD 0.4, at which time AI-2 was added (ranging from 0 to 40 μM AI-2). Chemically synthesized AI-2 was kindly provided by Dr. Herman Sintim (Purdue University).[15]

2.2 | qPCR analysis

RNA was extracted with the RNAqueous kit (Invitrogen). qPCR was performed using SensiFAST SYBR Hi-ROX One-Step Kit (Bioline, Taunton, MA). Analysis was performed using an Applied Biosystems HT7900. The transgene immediately following the T7 promoter on the pET plasmid and various plasmid derivatives as well as 16sRNA (e.g., reference gene) were quantified. Results are shown as a fold change relative to the control samples (either MDAI2 pCT6 pET or CT104 pCT6 pET) for each AI-2 concentration. Each sample was performed in triplicate. Averages are reported.

2.3 | AI-2 analysis

To measure AI-2 activity, *Vibrio harveyi* BB170 cells were used in a reporter assay.[16] Briefly, conditioned media (CM) samples were collected from experimental cultures by filtration through a 0.2 μm filter and frozen until they were made ready for analysis. BB170 cultures were inoculated into AB medium with 10 μg/mL kanamycin and grown overnight for 16 hr at 30°C with 250 rpm shaking. The overnight BB170 cultures were diluted 5,000 times into fresh AB medium with 10 μg/mL kanamycin. To measure AI-2 activity, CM samples were added to dilute BB170 cultures in a 1:9 ratio and grown at 30°C and 250 rpm shaking. For a negative control, fresh LB media was added in place of the CM. Luminescence was recorded using a luminometer (EG&G Berthold LB 9509 Jr) and values were normalized to the negative control. Each sample was tested in triplicate.

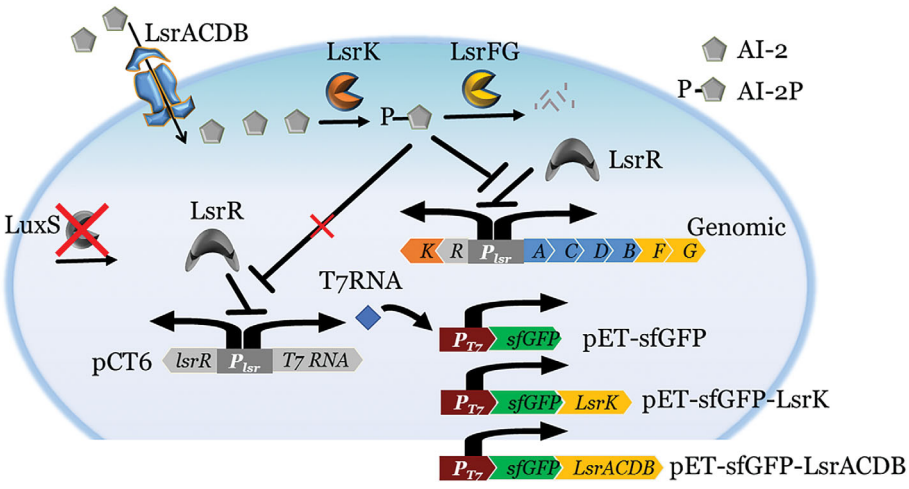
2.4 | sfGFP protein expression

Flow cytometry was used to analyze sfGFP expression. A BD FACSCanto II flow cytometer with a 488 nm laser and 530/30 green filter was used to measure both the percent of fluorescing cells and the average intensity of the fluorescence of each cell.

3 | RESULTS AND DISCUSSION

3.1 | Design of enhanced AI-2 reporter cell

The native *E. coli* response to AI-2 and the enhanced AI-2 reporter cell design are illustrated in Scheme 1. In the native system, AI-2 is imported by the transporter, LsrACDB, and phosphorylated by the kinase, LsrK. Phosphorylated AI-2 (AI-2P) relieves repression of the *lsr* operon resulting in additional transcription of the AI-2 uptake and processing genes. In our previously designed system, phosphorylated AI-2 also activates transcription of the florescent reporter sfGFP encoded on amplifier plasmid pET-sfGFP using a two plasmid system wherein the first transducer plasmid (pCT6) uses the *lsr* promoter to express T7



SCHEME 1 Enhanced *E. coli* AI-2 sensor. AI-2 is imported by LsrACDB and phosphorylated by the kinase LsrK. AI-2P activates expression of the native *lsr* operon causing production of additional LsrACDB and LsrK. AI-2P also activates transcription of T7 RNA polymerase on plasmid pCT6 followed by transcription of the reporter gene on plasmid pET-sfGFP. On plasmids pET-sfGFP-LsrK and pET-sfGFP-LsrACDB, AI-2P activates additional production of LsrK or LsrACDB resulting in an enhanced feedback loop and further uptake and processing of AI-2. The AI-2 degrading units LsrF and LsrG are deleted from the genome. AI-2, autoinducer-2; AI-2P, phosphorylated AI-2

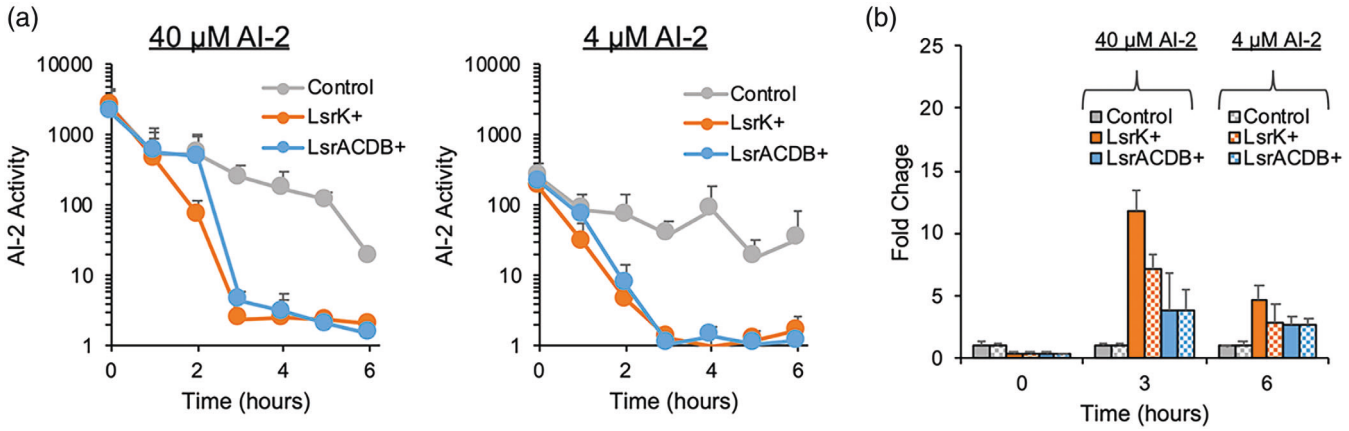


FIGURE 1 AI-2 uptake profiles and transgene expression for MDAI2 pCT6 transformed with either pET (control), pET-LsrK (LsrK+), or pET-LsrACDB (LsrACDB+). Each culture was grown to approximately OD_{600} 0.4 at which time cultures were supplemented with 4 or 40 μM AI-2 (*t* = 0 hr). (a) AI-2 activity in the extracellular media for cultures, with 4 μM (left panel) or 0.4 μM (right panel) added AI-2 is shown over time. (b) qPCR was used to measure pET transgene mRNA levels for each culture at 0, 3, and 6 hr from AI-2 addition. Fold change is shown relative to "Control" levels. Error bars represent *SD* of biological triplicates. AI-2, autoinducer-2

polymerase, which then directs sfGFP expression from the second—the net result being significant amplification of the native *lsr*-mediated signal.[12] In a *luxS* knockout (cells lacking the ability to produce AI-2) host strain such as MDAI2, this systems allows for uptake and reporting of AI-2 in the extracellular environment.[13] For the design of the "enhanced" cells, we build on this system by manipulating the response and sensitivity to AI-2 by also overexpressing LsrK or LsrACDB in addition to sfGFP in response to AI-2 (using plasmids pET-sfGFP-LsrK or pET-sfGFP-LsrACDB), and by using a host strain CT104 that lacks the AI-2 degrading proteins, LsrF and LsrG.

3.2 | Enhanced feedback loop in MDAI2 results in accelerated AI-2 uptake and increased AI-2 driven transcription

We first tested the enhanced feedback loop in host stain MDAI2. Plasmids pET-LsrK or pET-LsrACDB were tested and compared to the control plasmid pET in MDAI2 pCT6 (Figure 1). AI-2 concentrations of 40 μM and 4 μM were exogenously added and extracellular AI-2 levels and pET transgene transcription levels were observed over 6 hr. Use of plasmids pET-LsrK and pET-LsrACDB resulted in accelerated AI-2 uptake over the control plasmid (Figure 1a). When 40 μM AI-2 was added, it was rapidly cleared within 3 hr using either enhanced feedback plasmid. Using the control plasmid, AI-2 was more slowly removed over the course of 6 hr. When a lower concentration of 4 μM AI-2 was added, the control population was unable to clear the AI-2 within the time tested, while the cells with the enhanced feedback plasmids still cleared the AI-2 within 3 hr, illustrating that these cells have an increased sensitivity to AI-2. qPCR results also indicated that the enhanced plasmids result in greater expression of the pET transgene at both AI-2 concentrations (Figure 1b). This effect is seen within 3 hr of addition of AI-2, in agreement with the AI-2 uptake data. Growth rates and optical densities were similar among all strains (Figure S1a).

We note here that the use of either of the enhanced feedback loops (pET-LsrK or pET-LsrACDB) significantly increased AI-2 uptake over the control strain and that the LsrK+ strain appeared to have a slightly increased uptake rate compared to the LsrACDB+ strain. This suggests that LsrK is the bottleneck in the AI-2 uptake process in this experimental setup. That is, phosphorylation and sequestration of AI-2 within the cell is the limiting step in comparison to AI-2 uptake from the extracellular environment by the LsrACDB transporter. There is likely an ideal balance between LsrK and LsrACDB levels that maximizes AI-2 uptake and this balance may change over time or at different stages of growth. For instance, we have previously seen that overexpression of LsrACDB results in increased AI-2 uptake compared to overexpression of LsrK when expressed via an IPTG inducible promoter (as opposed to induction from the *lsr* promoter as illustrated here).[9] It may be that early expression of LsrACDB has a disproportionately significant effect, reflecting the fact that LsrK acts downstream of LsrACDB in the AI-2 signal transduction pathway. For instance, in the IPTG inducible system, LsrACDB can be expressed prior to the cell taking up any AI-2 in order to jump start the process. In the autonomous induction system used here, the cell would have already taken up some AI-2 in order to activate the *lsr* promoter and subsequent overexpression of either LsrACDB or LsrK. Overexpression of LsrACDB may be less important (relative to overexpression of LsrK) at that point. Also, the activity of LsrK can be inhibited based on the phosphorylation state of the phosphotransferase protein HPr,[17] which is involved in sugar transport. The state of HPr, too, likely changes over the course of the growth curve and may necessitate the need for more or less LsrK for optimal AI-2 uptake.

3.3 | Enhanced feedback loop in CT104 further increases sensitivity to AI-2

We next tested the enhanced feedback loop in host strain CT104. CT104 has previously been shown to be more sensitive to AI-2 than

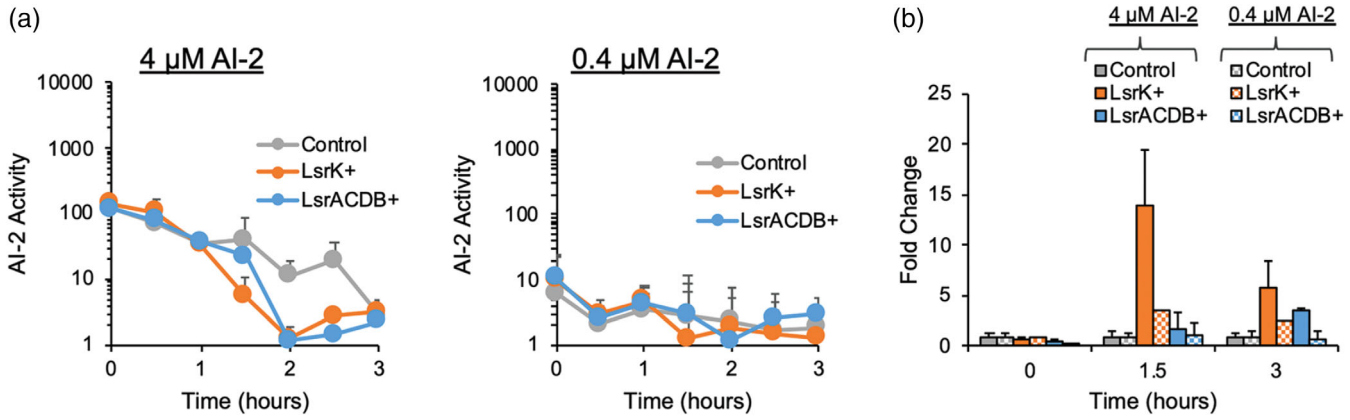


FIGURE 2 AI-2 uptake profiles and transgene expression for CT104 pCT6 transformed with either pET (control), pET-LsrK (LsrK+), or pET-LsrACDB (LsrACDB+). Each culture was grown to approximately OD_{600} 0.4 at which time cultures were supplemented with 4 or 0.4 μM AI-2 (t = 0 hr). (a) AI-2 activity in the extracellular media for cultures with 4 μM (left panel) or 0.4 μM (right panel) added AI-2 is shown over time. (b) qPCR was used to measure pET transgene mRNA levels for each culture at 0, 1.5, and 3 hr from AI-2 addition. Fold change is shown relative to "Control" levels. Error bars represent *SD* of biological triplicates. AI-2, autoinducer-2

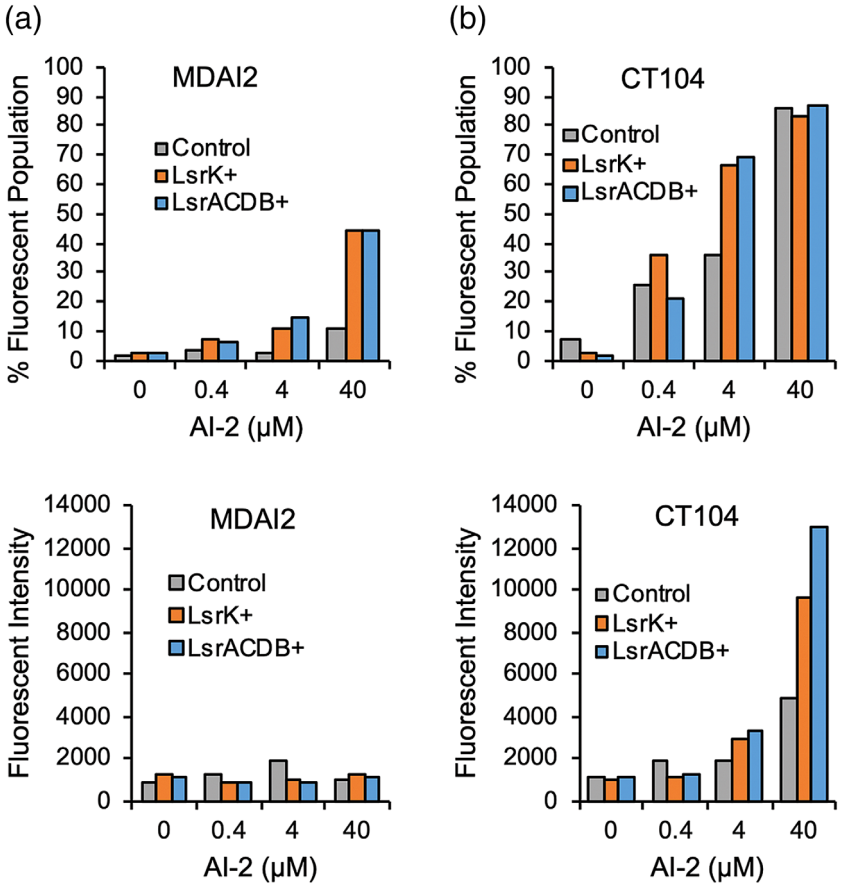


FIGURE 3 Fluorescent population percentage and average fluorescence intensity for MDAI2 (a) and CT104 (b) containing pCT6 and either pET-sfGFP (control), pET-sfGFP-LsrK (LsrK+), or pET-sfGFP-LsrACDB (LsrACDB+). Each culture was grown to approximately OD_{600} 0.4 and supplemented with AI-2 ranging from 0 to 40 μM. Samples were taken 4 hr after AI-2 addition for flow cytometry analysis. AI-2, autoinducer-2

MDAI2.[13,14] Here, we tested whether use of the enhanced feedback loop could further increase AI-2 sensitivity in these cells. We tested AI-2 concentrations of 4 and 0.4 μM and observed results over 3 hr. When 4 μM AI-2 was added, AI-2 was rapidly cleared within 2 hr using either enhanced feedback plasmid (Figure 2a). We note that this is a shorter time for AI-2 clearance than observed in the MDAI2 cells with the enhanced plasmids at the same AI-2 concentration (Figure 1a). Experiments with both cell lines were started at cell densities of approximately 0.4 (MDAI2 and CT104 starting densities ranged from 0.40 to 0.60 and 0.36 to 0.46, respectively) and growth curves were similar between the two strains (Figure S1). Therefore, the increased rate of AI-2 clearance in the CT104 strain is not an artifact

of using more cells in the culture. Using the control plasmid in CT104, AI-2 was removed over the course of 3 hr (Figure 2a). When adding 0.4 μM AI-2, AI-2 uptake was difficult to observe for both the control and enhanced cell lines, likely due to the sensitivity of the assay. The qPCR results largely agree with the AI-2 uptake data (Figure 2b). The enhanced plasmids result in an increase in transgene levels over the control plasmid when 4 μM AI-2 was added. This effect was especially significant when using the enhanced LsrK plasmid, and could be observed 1.5 hr after addition of AI-2. Growth rates were similar in all CT104 strains (Figure S1).

3.4 | Enhanced feedback loop in MDAI2 and CT104 result in greater expression of reporter genes

We then tested whether the enhanced feedback loops could improve expression of a fluorescent reporter protein in response to AI-2. sfGFP expression was tested in both MDAI2 and CT104 containing plasmid pCT6 and either pET-sfGFP, pET-sfGFP-LsrK, or pET-sfGFP-LsrACDB for AI-2 concentrations ranging from 0 to 40 μM (Figure 3). As expected, both the number of fluorescing cells and the intensity of fluorescence were generally higher in CT104 than MDAI2.[13,14] The lack of AI-2 degrading proteins likely allows prolonged activation of the *lsr* promoter resulting in measurable levels of sfGFP even in cells with low intracellular AI-2 levels. MDAI2 cells with the enhanced feedback loops showed a trend toward higher fractions of fluorescing cells over the controls across all tested AI-2 concentrations, although an increase in fluorescence intensity (per cell) was not observed (Figure 3a). The control cells showed a noticeable increase in fluorescent population only when adding 40 μM AI-2, the highest concentration tested. Even at this high AI-2 concentration, the fluorescent population reached a maximum of less than 20%. In contrast, in the cells with enhanced expression of LsrK or LsrACDB, 40 μM AI-2 addition resulted in a fluorescent population of over 40%. We note that distributed responses to AI-2 and other signaling or inducer molecules is common[10,13,18] and can be controlled.[10,19]

For example, here, using the enhanced plasmids in the host strain CT104 resulted in an increase in fluorescence over use of the control plasmids at AI-2 concentrations of 4 μM or higher (Figure 3b). At the 4 μM AI-2 concentration, the fluorescent population was about twice as high in the cells with the enhanced feedback loops. An increase in the fluorescent population was not seen at the 40 μM AI-2 concentration, but the percentages were already high (~90%) across all cells including the control strain. We note, however, that the intensity of fluorescence was significantly higher in these cells with the enhanced feedback plasmids.

4 | CONCLUSIONS

In conclusion, we developed an *E. coli* strain that consumes AI-2 more rapidly and responds with higher protein output to physiological ranges of AI-2 by combining two previous strategies. We overexpressed the AI-2 uptake and phosphorylation units, LsrK or LsrACDB (using the enhanced feedback loop plasmids), to increase uptake of AI-2, and we deleted the AI-2 degrading units LsrF and LsrG (through use of host strain CT104), to increase sensitivity to AI-2. We show that these cells uptake AI-2 at an accelerated rate and respond with increased protein expression when biologically relevant concentrations of 4 μM AI-2 or higher are added. We envision these cells will allow for more precise interrogation of and response to AI-2 in natural environments, such as the GI tract.

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REFERENCES

1. Waters CM, Bassler BL. Quorum sensing: cell-to-cell communication in bacteria. *Annu Rev Cell Dev Biol*. 2005;21:319-346.
2. Rutherford ST, Bassler BL. Bacterial quorum sensing: its role in virulence and possibilities for its control. *Cold Spring Harb Perspect Med*. 2012;2(11):1-25.
3. Pereira CS, Thompson JA, Xavier KB. AI-2-mediated signalling in bacteria. *FEMS Microbiol Rev*. 2013;37(2):156-181.
4. Vendeville A, Winzer K, Heurlier K, Tang CM, Hardie KR. Making 'sense' of metabolism: autoinducer-2, LuxS and pathogenic bacteria. *Nat Rev Microbiol*. 2005;3(5):383-396.
5. Zargar A, Quan DN, Carter KK, et al. Bacterial secretions of non-pathogenic *Escherichia coli* elicit inflammatory pathways: a closer investigation of interkingdom signaling. *MBio*. 2015;6(2):e00025.
6. Thompson JA, Oliveira RA, Djukovic A, Ubeda C, Xavier KB. Manipulation of the quorum sensing signal AI-2 affects the antibiotic-treated gut microbiota. *Cell Rep*. 2015;10(11):1861-1871.
7. Shong J, Jimenez Diaz MR, Collins CH. Towards synthetic microbial consortia for bioprocessing. *Curr Opin Biotechnol*. 2012;23(5):798-802.
8. Brenner K, You L, Arnold FH. Engineering microbial consortia: a new frontier in synthetic biology. *Trends Biotechnol*. 2008;26(9):483-489.
9. Zargar A, Quan DN, Emamian M, et al. Rational design of 'controller cells' to manipulate protein and phenotype expression. *Metab Eng*. 2015;30:61-68.
10. Zargar A, Quan DN, Bentley WE. Enhancing intercellular coordination: rewiring quorum sensing networks for increased protein expression through autonomous induction. *ACS Synth Biol*. 2016;5(9):923-928.
11. Zargar A, Quan DN, Abutaleb N, et al. Constructing "quantized quorums" to guide emergent phenotypes through quorum quenching capsules. *Biotechnol Bioeng*. 2017;114(2):407-415.
12. Tsao CY, Hooshangi S, Wu HC, Valdes JJ, Bentley WE. Autonomous induction of recombinant proteins by minimally rewiring native quorum sensing regulon of *E. coli*. *Metab Eng*. 2010;12(3):291-297.
13. Servinsky MD, Terrell JL, Tsao CY, et al. Directed assembly of a bacterial quorum. *ISME J*. 2016;10(1):158-169.
14. Terrell JL, Wu HC, Tsao CY, et al. Nano-guided cell networks as conveyors of molecular communication. *Nat Commun*. 2015;6:8500.
15. Smith JA, Wang J, Nguyen-Mau SM, Lee V, Sintim HO. Biological screening of a diverse set of AI-2 analogues in *Vibrio harveyi* suggests that receptors which are involved in synergistic agonism of AI-2 and analogues are promiscuous. *Chem Commun*. 2009;7033-7035.

16. Bassler BL, Greenberg EP, Stevens AM. Cross-species induction of luminescence in the quorum-sensing bacterium *Vibrio harveyi*. *J Bacteriol*. 1997;179(12):4043-4045.
17. Ha JH, Hauk P, Cho K, et al. Evidence of link between quorum sensing and sugar metabolism in *Escherichia coli* revealed via cocrystal structures of LsrK and HPr. *Sci Adv*. 2018;4(6):eaar7063.
18. Siegele DA, Hu JC. Gene expression from plasmids containing the araBAD promoter at subsaturating inducer concentrations represents mixed populations. *Proc Natl Acad Sci U S A*. 1997;94(15):8168-8172.
19. McKay R, Hauk P, Quan D, Bentley WE. Development of cell-based sentinels for nitric oxide: ensuring marker expression and unimodality. *ACS Synth Biol*. 2018;7(7):1694-1701.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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