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Quorum Sensing Communication: Molecularly Connecting Cells, Their Neighbors, and Even Devices

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Abstract

Quorum sensing (QS) is a molecular signaling modality that mediates molecular-based cell–cell communication. Prevalent in nature, QS networks provide bacteria with a method to gather information from the environment and make decisions based on the intel. With its ability to autonomously facilitate both inter- and intraspecies gene regulation, this process can be rewired to enable autonomously actuated, but molecularly programmed, genetic control. On the one hand, novel QS-based genetic circuits endow cells with smart functions that can be used in many fields of engineering, and on the other, repurposed QS circuitry promotes communication and aids in the development of synthetic microbial consortia. Furthermore, engineered QS systems can probe and intervene in interkingdom signaling between bacteria and their hosts. Lastly, QS is demonstrated to establish conversation with abiotic materials, especially by taking advantage of biological and even electronically induced assembly processes; such QS-incorporated biohybrid devices offer innovative ways to program cell behavior and biological function.

1. QUORUM SENSING

1.1. History and Background

Quorum sensing (QS): a cell–cell communication process discovered in bacteria

Autoinducer (AI): small molecule secreted by quorum sensing bacteria as a measure of population density

AHL: acyl-homoserine lactone

Although they are considered primitive, microbes have been found to be social creatures, just like humans. Whereas we use words and body gestures, gregarious bacteria converse through secretion and perception of small signal molecules. Greenberg and colleagues termed this phenomenon quorum sensing (QS). Hints of microbial social interaction through extracellular molecules had been found in early studies in which scientists discovered that both (*a*) luminescence in two species of marine bacteria (1, 2) and (*b*) genetic competence in *Streptococcus pneumoniae* (3) required production of hormone-like small molecules. The big breakthrough in QS studies came with two landmark discoveries: One identified the genes that control (*luxI*, *luxR*) and produce (*lux*) luminescence in *Vibrio fischeri* (4, 5), and the other unveiled the QS-signal molecule to be *N*-(3-oxohexanoyl)-L-homoserine lactone (3OC6-HSL) (6). Soon after, the dawn of genomic profiling allowed the discovery of an explosion of systems that are homologous to the *lux* QS system in different species (7, 8). Since then, many and widely disparate scientific efforts have been dedicated to understanding how bacteria communicate.

1.2. Quorum Sensing Systems and Networks

In general, QS bacteria produce and release chemical signal molecules termed autoinducers (AIs), the external concentrations of which increase as a function of increasing cell-population density within a particular niche. Once the bacteria detect that AIs have reached a threshold concentration, they will respond by altering their gene expression and behavior. AIs, playing a vital role in QS, are the cues by which QS bacteria communicate and synchronize particular behaviors on a population-wide scale, thus gaining the ability to function as a multicellular organism. In this section, two well-characterized QS systems and their signals, receptors, mechanisms of signal transduction, and target outputs are reviewed.

1.2.1. LuxI/R system. For most QS systems in Gram-negative bacteria, the LuxI/R system of *V. fischeri* (**Figure 1*a***) serves as an underlying paradigm (9). In this system, proteins LuxI and LuxR control expression of the luciferase operon (*luxICDABE*) required for luminescence. *luxI* encodes for an AI synthase that produces the acyl-homoserine lactone (AHL) AI *N*-3-oxododecanoyl-HSL ($3OC_{12}$HSL). Following its production, the AHL will accumulate—its concentration increasing as the cell density increases. Upon reaching a critical level, LuxR, the cytoplasmic AI receptor/DNA-binding transcriptional activator, will bind to AHL, and this complex will initiate the expression of the luciferase operon. This actuates a positive feedback loop, as *luxI* is encoded in the operon, and soon the environment is flooded with AHL, which, in turn, switches all bacteria nearby to the QS-active, light-shedding mode (10). All other LuxI/R systems share a general mechanism: LuxI homologs synthesize AHL as AIs, and LuxR homologs recognize, specifically, their cognate AHL. This specificity makes LuxI/R QS systems ideal for enabling intraspecies communication. In Gram-positive bacteria, such as the aforementioned *S. pneumoniae*, the modified oligopeptides are used as AIs, and the two-component-type membrane-bound sensor histidine kinases are used as receptors. Like the Gram-negative LuxI/R system, each Gram-positive bacterium uses a signal different from that used by other bacteria, and the cognate receptors are exquisitely sensitive to the signals' structures. Because AHLs from different species have been characterized extensively, they can be used for synthetic cell–cell communication. In addition, components of the LuxI/R system can be used as modules within heterologous gene circuits, as they are often ported to non-native strains. Particularly importantly, the broad class of signals comprising the

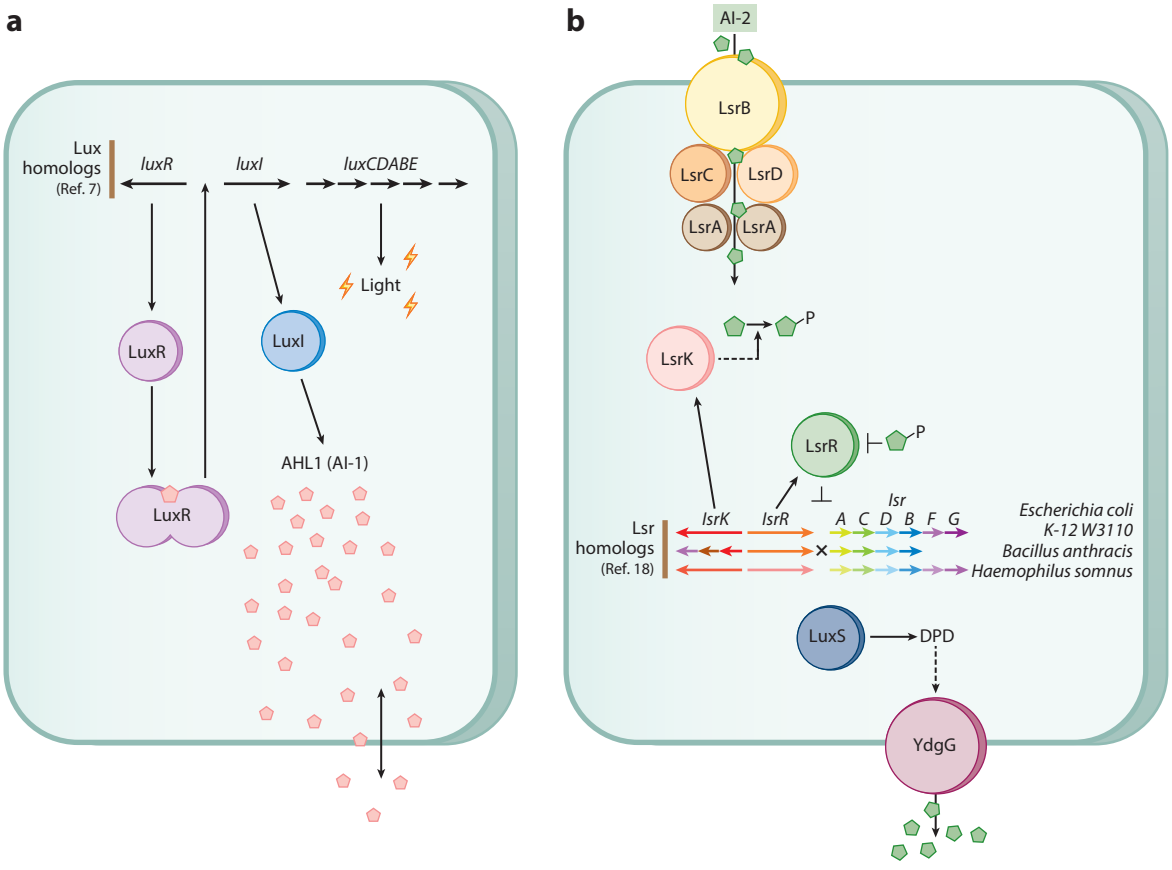


Figure 1

Canonical quorum sensing (QS) systems. (*a*) QS in *Vibrio fischeri*. Pink pentagons denote the acyl-homoserine lactone (AHL) autoinducers (AIs) that LuxI produces (3OC6-homoserine lactone). Transcriptional regulator LuxR modulates expression of AHL synthase, LuxI, and the lux operon, leading to luciferase-mediated light emission. Homologous *lux*-like systems are described in Reference 7. (*b*) Regulatory mechanisms of the LuxS/AI-2 circuit in *Escherichia coli*. AI-2 (*green pentagons*) is imported into the cell by the Lsr transporter (LsrACDB) and is then phosphorylated by LsrK. When AI-2P binds LsrR and releases LsrR from the promoter region (thus modulating Lsr gene expression), AI-2 uptake is increased. LuxS produces DPD, the precursor to AI-2. The AI is exported by YdgG (TqsA). *Lsr* homologs and their organizational structures are found in both Gram-positive and Gram-negative bacteria (18).

AHLs freely diffuse through Gram-negative membranes so that active signal transduction motifs are not needed for synthetic communications systems. Also, bacteria rarely rely on one exclusive LuxI/R QS system but often employ multiple signaling systems in parallel. This biochemical diversity of AHL signaling pathways can be leveraged for circuits controlled by combinations of unique signals (11). Together, these characteristics make the LuxI/R system particularly attractive to fields like synthetic biology, as they can be translated to function in engineered systems and environments.

LuxS: *S*-ribosylhomocysteine lyase, breaks down *S*-ribosylhomocysteine into DPD, precursor of AI-2

1.2.2. LuxS/AI-2 system. The LuxS/AI-2 system (**Figure 1*b***) was first observed in yet another bioluminescent marine bacterium, *Vibrio harveyi*, which can communicate via multiple QS signals, including those secreted by other species (12). The QS signal AI-2, which is actually a family of cyclic furanones (13), serves as the "bacterial Esperanto" (14), as it can be generated by more than

80 species of both Gram-negative and Gram-positive bacteria (10, 15). During central metabolism, the reactive methyl moieties of *S*-adenosylmethionine are transferred to various substrates, yielding by-product *S*-adenosylhomocysteine (SAH). LuxS-containing bacteria have two enzymes (Pfs and LuxS) acting sequentially to convert SAH to adenine, homocysteine, and the signal molecule 4,5-dihydroxy-2,3-pentanedione (DPD). This, in turn, is exported and cyclized to AI-2, enabling both the synthesis of AI-2 and the detoxification of the toxic by-product SAH (16).

SAH: *S*-adenosylhomocysteine

Pfs: 5′-methylthioadenosine/*S*-adenosylhomocysteine nucleosidase, catalyzes SAH into *S*-ribosylhomocysteine

Lsr transporter: LuxS-regulated transporter

Remarkably, AI-2 is found to be actively transported into the cell by the *luxS*-regulated (Lsr) transporter in *Enterobacteriaceae* and several other taxa (17, 18). In *Escherichia coli*, the AI is imported by the Lsr transporter (LsrACDB) and in turn is phosphorylated by LsrK to AI-2P. As AI-2P binds LsrR, it relieves the repression of LsrR on the Lsr genes and accelerates AI-2 intake. Interestingly, through modeling and experimental studies, alternative, less prominent routes of AI-2 synthesis (19) and uptake (20, 21) have been postulated. Also interestingly, LsrACDB is also regulated via glucose and cyclic AMP/cyclic AMP receptor protein, as well as other common carbon sources (20, 22). Based on these discoveries, the LuxS/AI-2 system has two noteworthy features: (*a*) the desynchronization of the LuxS/AI-2 QS system caused by AI-2 intake via the *lsr* operon, which allows the display of bimodal Lsr signaling and fractional induction (23), and (*b*) the ability to endow cell population–dependent behavior while interacting with central metabolism and regulating cell fitness through the intracellular activated methyl cycle and intervention of the aforementioned *S*-adenosylmethionine metabolism pathway. Moreover, in **Figure 1*b***, we also show that the AI-2 uptake mechanism (Lsr) is phylogenetically dispersed among Gram negatives and Gram positives (18). That is, although bacteria possessing the AI-2 signal transduction mechanisms are believed to have the ability to sense the general bacterial population density in a multispecies consortium, this diversity suggests the ability to self-report. The prevalence of LuxS among bacteria (and AI-2 in proximal microenvironments) has fueled speculation about the role of AI-2 as a QS-signal molecule, yet its diverse uptake and signal translocation mechanisms enable species-specific ability to respond to AI-2. Interestingly, some question whether AI-2 and the LuxS/AI-2 QS system can be defined as a true interspecies QS-signaling pathway, or in some cases a non-QS-related cue (24), but this system possesses many attributes and components that can be rewired and incorporated into engineered systems.

1.3. Global Quorum Sensing Regulons

The prevalence of genomic fingerprinting has revealed that QS can control gene expression in a global manner. QS mutants of *S. pneumoniae* and related streptococci show defects in multiple pathways, including biofilm formation, acid tolerance, bacteriocin production, and virulence (7). *E. coli*, too, have been reported to elicit broad QS activities. For example, the quantity and architecture of biofilms are regulated by *lsrR/K*, as well as the generation of several small RNAs (25, 26). Transcriptome analyses of an *E. coli luxS* mutant, which showed that 242 genes (5.6% of the whole genome) exhibited significant transcriptional changes upon a 300-fold AI-2 signaling differential (8, 21, 27), suggest that QS coordinates the control of a large subset of genes. Although we are not entirely certain whether these *luxS* mutant phenotypes are a result of the lack of QS signaling or may simply be due to metabolic perturbations, these findings surely demonstrate that QS allows bacteria to alternate between distinct genome-wide programs by activating numerous genes both directly and indirectly. For example, AI-2 also serves as a chemoattractant for *E. coli* (28, 29). Chemotaxis studies have revealed that both LsrB and Tsr, a serine chemoreceptor, are involved in AI-2 sensing (30). As a signal molecule, AI-2 is not a nutrient, unlike other known chemoattractants of *E. coli*; therefore, chemotaxis toward AI-2 may not involve the narrow dose ranges that are characteristic of most indirectly binding chemoattractants (31). This provides

opportunities to enable programmed motility toward user-selected features on nearby surfaces. Antigen 43–dependent autoaggregation of *E. coli* is also mediated by AI-2. Hence, the AI-2 chemotactic response will lead to active aggregation, and in turn, autoaggregation enhances AI-2-mediated signaling, and subsequently, biofilm formation and stress resistance (32).

Since long before the formal recognition of metabolic engineering in 1991 (33), scientists and engineers had been developing microbial strains for the production of valuable chemicals. The emergence of metabolic engineering and synthetic biology was predicated on the multidimensional value associated with biological synthesis processes for chemical products—a natural progression was the desire to program cells to carry out these functions. QS regulons, having the potential to connect inter- and intraspecies communication systems with genome-spanning regulatory processes, dramatically simplify what otherwise might be the de novo engineering of gene circuits (34, 35). QS serves as an excellent platform for many technologies, particularly if one understands the regulatory reach of the genetic circuits. In the past two decades, the rewiring of native QS networks has enabled novel ways to engineer cell behavior, exemplified by such advances as programmed population controllers (36), synchronized genetic clocks (37), and population-based autonomous gene actuators (38, 39). These studies have set the stage for the future development of a variety of innovative biotechnological applications, which are discussed in the following sections.

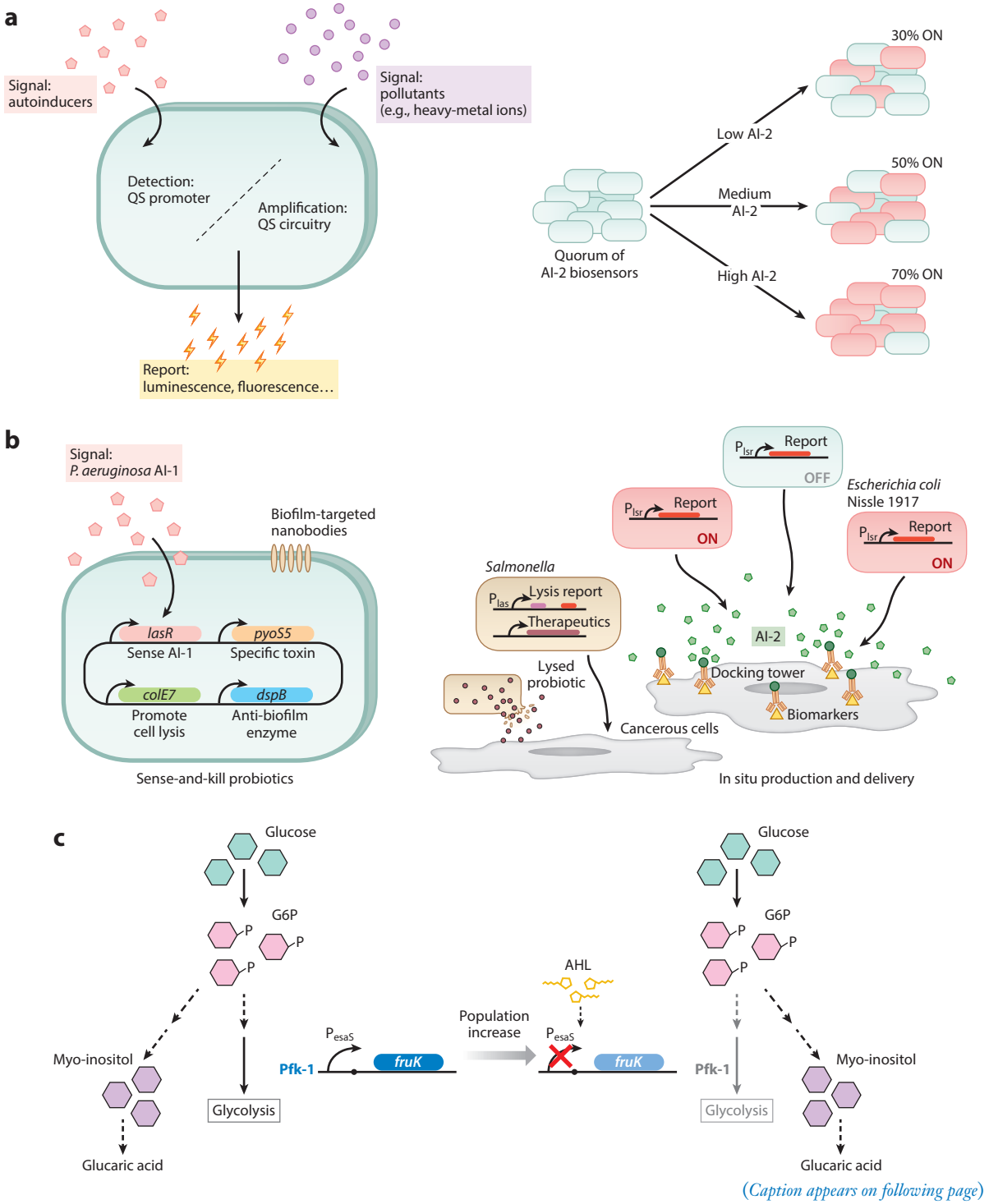
2. MANIPULATION OF QUORUM SENSING SYSTEMS: ENDOWING CELLS WITH NEW FUNCTION

Owing to their diversity and versatility, QS systems are perfect candidates as platforms for facilitating the endowment of bacterial strains with unique and increasingly complex functions. In this section, we explore recent endeavors to excerpt QS mechanisms and pathways to enable cells with advanced functions for various applications.

2.1. Biosensors

Whole-cell biosensors, as the name suggests, are native or engineered cells that detect and report on a target or condition of interest (40, 41). Biocompatible and renewable, they make good substitutes for current chemical or electrical sensors. Originally, cells that elicit QS behavior were simply rewired to detect their own AIs (42). This was typically accomplished by deleting the terminal AI-synthase gene and replacing the native QS-induced gene system with a reporter gene. Perhaps the most well-characterized and used sensor is the *V. harveyi* strain BB170, of Bassler and coworkers (43). Although they were developed more than two decades ago, these biosensor cells continue to benefit science; for example, they are frequently used in studies to probe for new QS inhibitors (44–47). Analogously, by fusing QS promoters with fluorescence genes, *Pseudomonas aeruginosa* were given the ability to report on the genetic expression of four QS networks (48). Owing to the wide diversity of natural QS systems, various pathogens and infection markers can also be detected by engineered QS-based biosensors (49–51). These bacterial sentinels were further enhanced to perform with higher sensitivity (52) or to encode and distribute therapeutic payload upon detection, which is discussed in the following section.

QS-based biosensors are also employed to probe pollutants (i.e., heavy metal ions) (see the left side of **Figure 2*a***) in the environment, in which the positive feedback loop of the LuxI/R system is used for signal amplification (53, 54). On top of enhancing signal amplitude, QS circuits have been shown to resolve some common problems met by biosensors. Genetic noise, or specifically variation in phenotype between cells, can be assisted by QS systems (55, 56). Early work showed that by coupling LuxI/R circuitry to the expression of a toxic protein (CcdB), it was possible to



(Caption appears on following page)

Figure 2 (*Figure appears on preceding page*)

Quorum sensing (QS)-enabled cell functions. (*a*, *left*) Paradigm of QS biosensors. A wide range of signals can be detected and amplified through QS circuitry and reported via optical or biological means. (*right*) A quantified quorum of biosensors is engineered to respond to different levels of signal and produce a collective response. (*b*) Examples of QS therapeutics. (*left*) Sense-and-kill probiotics (*green*) can sense pathogenic signals, specifically acyl-homoserine lactone (AHL) produced by pathogens, and express toxin (pyocin S5) and cell-lysis proteins (lysin E7) to eliminate *Pseudomonas aeruginosa* (63). Anti-biofilm enzyme (DspB) and biofilm-targeted nanobodies can be incorporated to facilitate biofilm penetration. These smart probiotics can either autoaggregate toward tumor cells (e.g., *Salmonella*) or be programmed to actively seek target cells. (*right*) Nanofactories consisting of Pfs, LuxS, protein G, and anti-EGFR antibody will specifically bind to selected biomarkers (EGFR) and produce AI-2. Probiotics (*Escherichia coli* Nissle 1917) recognize and then chemotax toward AI-2 (produced by targeted cells) (80). Upon arriving at their destination, smart probiotics use the same AI-2 level to report (via red/green fluorescence) (*green/red*, *E. coli*) or lyse when the population threshold is reached and release genetic-encoded cargo (*tan*, *Salmonella*). (*c*) Example of QS-facilitated biosynthesis (85). G6P flux is split into three pathways: (*i*) myo-inositol (MI) production (which leads to glucaric acid production), (*ii*) glycolysis, and (*iii*) the pentose phosphate pathway (deleted in study and not shown in figure). Dynamic, autonomous downregulation of Pfk-1 is achieved by placing *fruK* under the P*esaS* promoter, and this integrates the signaling from *esaI* in the genome. As the population and AHL concentration increase, the P*esaS* promoter will be repressed by AHL-bound EsaR, which then switches off Pfk-1 expression. This biases the G6P-to-MI (and glucaric acid) metabolic flux, increasing the yield of glucaric acid.

program population dynamics irrespective of the variability in individual cells (36). With the same notion in mind, a synchronized genetic clock was engineered based on both the LuxI/R system and the QS system of *Bacillus thuringiensis* (37). Here, colony-level synchronized oscillation could diminish single-cell variability and increase the sensitivity and robustness of the response to external signals. These frontier endeavors have paved the way for building better, more effective macroscopic biosensors. Interestingly, one further study chose to dwell on the heterogeneity observed within bacterial populations that, despite the group showing collective behavior, could still be observed. In this work, a stable quorum wherein a specific fraction of the whole exhibits the desired collective behavior is generated (see the right side of **Figure 2*a***); for instance, a group of cells could be programmed to have 65% of the total population express DsRed, a red fluorescent protein (57). This was done by manipulating the native *E. coli* AI-2 transduction cascade along with an AI signal amplification vector that makes the strain hypersensitive to AI-2; therefore, it overexpresses the marker protein and, with this QS mechanism, leads a subgroup of cells to exhibit the same behavior. We believe studies like this also anticipated many future works that focus, beyond AI-2 biosensors, on intentional control of group behavior, which is discussed further below. Owing to the development of the many tools of synthetic biology, QS components and signaling mechanisms have helped to streamline biosensor development. The adaptable and well-defined nature of QS systems will surely continue to benefit the improvement of biosensors, even broadening their capabilities.

2.2. Therapeutics

Although the use of bacteria as therapeutics dates to more than a century ago (58, 59), smart bacteria with programmed therapeutic functions are now a tangible reality owing to the recent strides made in synthetic biology. By leveraging the species specificity of different native quorum signals, bacteria that are able to detect infection caused by *P. aeruginosa* (49, 60, 61) and *Enterococcus faecalis* (62) have been tested in vitro. These engineered sentinels not only can serve as diagnostics but are capable of eliminating pathogens through expression and excretion of bacteriocins (60) or antimicrobial peptides (49, 61, 62).

2.2.1. QS-regulated sense-and-kill probiotics. In an extension of previous work (61), Hwang et al. (63) have endowed probiotic *E. coli* strain Nissle 1917 to seek the pathogen through AI

($3OC_{12}HSL$)-regulated motility; induce self-lysis (driven by lysin E7); and release pyocin c5, an anti–*P. aeruginosa* toxin (**Figure 2*b***). The engineered strain exhibits in vivo prophylactic and therapeutic activity against *P. aeruginosa* during gut infection. Recently, BeQuIK (Biosensor Engineered Quorum Induced Killing), a newly proposed design that aims to combat recalcitrant biofilms, adopted the same sense-and-kill premise but with a twist for aiding in the targeting and penetration of biofilms (64). Biofilms are 3D structures in which a cluster of bacteria resides within a self-produced matrix primarily composed of proteins, polysaccharides, and extracellular DNA (65). Mature biofilms are notoriously difficult to penetrate or degrade. Because QS activity governs biofilm formation, the sense-and-kill system can be adopted to clear these 3D structures; however, success depends on localization of the engineered *E. coli* because this system relies heavily on the diffusion of AIs. Better eradication of biofilms could perchance be achieved through surface display of one or more biofilm-targeting nanobodies (Nbs), which are single-domain antibodies derived from the heavy-chain antibodies of camelids (66), to recognize and bind to components of the extracellular polymeric substance or biofilm-mediated proteins. Additionally, fusing one or more biofilm-degrading enzyme domains to the Nbs would possibly allow for more effective diffusion of AIs for activating the killing mechanism, as well as better permeation of the therapeutic agents.

2.2.2. QS-regulated in situ production and delivery. It is perhaps inevitable that some bacteria would evolve to preferentially grow in environments that harbor disease, and hence these cells may serve as a natural platform for the development of engineered therapies (67–69). *Salmonella*, for instance, are one of the ideal candidates because they preferentially accumulate in tumors, actively penetrate tumor tissue, and can be engineered to produce anticancer drugs in situ (70–73). Notably, however, nonspecific expression can damage healthy tissue. Engineering QS signaling offers the opportunity to restrict expression of the therapeutic compounds to relevant body sites. For example, the LuxI/R QS system was excerpted to build a density-dependent switch in *Salmonella* so that the engineered bacteria express the to-be-delivered proteins only in tightly packed colonies within tumors (74). This proof-of-concept study using an in vitro 3D tumor-on-a-chip device and in vivo mouse models showed that QS *Salmonella* specifically initiates green fluorescent protein expression within cancerous tissue while remaining uninduced in liver; hence, this study provided a road map for limiting systemic toxicity caused by unwanted expression in healthy tissues. In addition, such therapies could also benefit from bacteria that are programmed to maintain relatively low overall colonization levels in the body while continually producing and releasing cytotoxic agents (75, 76). Using coupled positive and negative feedback loops that had previously been used to generate robust oscillatory dynamics (37, 77), Din et al. (78) constructed a synchronized lysis circuit to allow engineered *Salmonella* to deliver therapeutic cargo and lyse synchronously at a threshold population. In this system, both *luxI* and *hlyE* (which encodes haemolysin E, an antitumor toxin) were constitutively expressed. As the AIs slowly built up to reach a threshold level, the bacteriophage lysis gene (*ϕX174E*) was induced, thus triggering cell lysis and simultaneously releasing the therapeutic toxin. This genetic synchronized lysis circuit was also used in a recent study to enable a nonpathogenic *E. coli* strain to lyse specifically within tumor microenvironments and release an encoded Nb antagonist of CD47, an antiphagocytic receptor commonly overexpressed in human cancers (79). This approach confers multiple advantages over the conventional immunotherapy, in that the engineered *E. coli* provide a way to increase the local concentration of Nbs while preventing systemic toxicity. Alternatively, targeted therapeutic cargo delivery has also been made possible in *E. coli* via incorporation of the native AI-2/Lsr signaling pathway (80) (**Figure 2*c***). Here, bacteria were modified to enable programmed motility, sensing, and actuation based on the density of user-selected features on nearby surfaces. Specifically, bacterial enzymes LuxS and Pfs were introduced onto cancerous eukaryotic cells as a nanofactory, where they were

used to synthesize chemoattractant AI-2. That is, the LuxS-Pfs fusion protein also contained a bacterial protein G domain that enabled the assembly of targeting antibodies. After expression and purification from *E. coli* and incubation with an antibody targeting epidermal growth factor receptor (which is upregulated on cancer cells), these nanofactories were used to synthesize AI-2 on the cell surfaces at EGFR sites, thus directing engineered *E. coli* to swim toward the cancer cell line (SCCHN). Once in place, the same AI-2, which is differentially accumulated above cancer cells, enabled QS computation to induce red fluorescent protein (RFP), a marker for therapeutic or other compounds.

2.3. Biosynthesis

Metabolic engineering often takes inspiration from natural regulatory mechanisms in microbes and repurposes them to maximize productivity; QS systems offer many suitable components that can be exploited for this purpose. One of the more severe problems met by the most productive metabolic engineering methods is the heavy metabolic burden that accompanies the genetic modification. Because QS networks are capable of reporting the metabolic state of a bacterial population and the metabolic burden is self-indicated by this network (27), Tsao et al. (38) created a system in which the system itself can autonomously induce protein expression and achieve metabolically balanced coordination through rewiring native AI-2/LuxS QS circuitry in *E. coli*. Further, a LuxI/R-based, semiautonomous induction circuit was assembled in *E. coli* and employed for isopropanol production (81); it was later modified to create a fully self-induced system for synthesis of a biofuels compound, bisabolene (82). These approaches allow cells to grow with less metabolic burden in early stages and transition to a production mode after the population growth phase has completed. Notably, these systems can ensue without exogenous addition of inducers or perhaps even user input.

In addition to gene actuation, QS-based circuits can also direct metabolic flux via repression of essential genes in endogenous metabolic pathways. *Saccharomyces cerevisiae* were programmed to autonomously trigger RNA interference, hence silencing genes that compete with p-hydroxybenzoic acid production at a high population density via a synthetic QS network built by modifying the native pheromone communication system (83). Whereas gene expression is induced upon reaching a threshold concentration of AIs in both LuxI/R and AI-2/LuxS systems, the Esa QS system found in *Pantoea stewartii* does the opposite—originally EsaR binds at the promoter region and serves as an activator, but until the AI produced by EsaI reaches a certain level and disrupts the binding of EsaR, the promoter is in turn deactivated (84). Gupta et al. (85) rewired this Esa QS system and successfully switched off phosphofructokinase-1 and shikimate kinase autonomously at a certain cell density, which siphoned carbon into D-glucaric acid production and led to an increase in glucaric acid, *myo*-inositol, and shikimic acid production (**Figure 2**). This system was further enhanced by layering a pathway-dependent regulation strategy, a *myo*-inositol biosensor, to allow the cells to accumulate a sufficient amount of *myo*-inositol before converting to the final product, glucaric acid (86). Another recent study integrated both cell density–controlled upregulation and downregulation to assemble a bifunctional metabolic switch via a synthetic QS system and applied it for dynamic fine-tuning of menaquinone-7 synthesis in *Bacillus subtilis* (87). Although this study witnessed a 40-fold increase in end-product formation, this setup employed only downregulation of cell-growth genes and not a bimodal balancing of metabolic flux that would simultaneously upregulate genes in the product synthesis pathway, perhaps leading to an even higher yield. Further, a recent study combined the power of directed evolution of critical enzymes along with metabolic pathway optimization in product synthesis via Esa-P_{EsaR} (a modified version of the native Esa circuitry that in lieu of repression activates target gene expression) QS

activation of said engineered enzymes to amplify de novo production of 4-hydroxyphenylacetic acid (88). Compared with other strategies exploiting dynamic pathway engineering, QS-based regulatory mechanisms provide process- and pathway-independent control of the metabolic state, which makes them highly applicable to different bioprocesses. That said, there remains a general lack of quantitative understanding of exactly how much burden the additional QS logic gates bring to these engineered systems; perhaps computational modeling or fundamental understanding of resource competition within cells could bring about a more robust, productive cell factory in the future.

3. MANIPULATION OF QUORUM SENSING SYSTEMS: OPENING LINES OF COMMUNICATION

In the previous section, we witnessed how QS regulons could be taken apart and reassembled into novel genetic circuits and how these endow cells with various advanced functions. These strategies mostly made use of the QS information/control paradigm in which cells autonomously regulate gene expression after detecting self-generated or synthetically produced molecular cues in their immediate microenvironments. Most often these systems have employed diffusible AIs, such as the large family of AHLs. That said, QS derives from its well-established function as a means for conveying and coordinating social behavior (89, 90); hence, it is also expedient to apply and further engineer QS systems to do what they do best: launch and promote communication between groups of bacteria.

3.1. Microbial Consortium: A Prospective Platform

As the saying goes, "Two heads are better than one." Microbial consortia have long proven their abilities to outperform single microorganisms at multiple tasks, as evidenced by the evolutional development of natural communities. The gut microbiota, for example, plays multiple vital roles in human health, from regulating metabolism to influencing the immune system and even guiding maturation of the enteric nervous system, all while varying in composition across time, location, and individuals (91, 92). Besides their versatility, native consortia (such as the gastrointestinal microbiome) are also robust; they respond to environmental challenges, display cooperation and exchange of public goods, and communicate (chemically or physically) between species. In light of these beneficial traits, microbial communities present themselves as an attractive platform for synthetic biologists who aim to modify microorganisms for biotechnological applications. From an engineering perspective, the division of labor, in which different populations are charged to perform different tasks, represents a key to overall effectiveness. Some potential advantages born by such a division of labor include improving functionality via specialization, reducing metabolic burden via function distribution, and reducing engineering complexity (93). Within consortia, complex tasks can be segmented, and each part can be delegated to a subpopulation. This allows subgroups to specialize and together display sophisticated multifunctionality that cannot be achieved in a single clonal population. Because cells are now completing only part of the overall function, they can be relieved from the responsibility of carrying all of the modified genetic machineries and hence ameliorate their heavy metabolic burden. Lastly, compartmentalizing cellular processes into different populations increases modularity. Therefore, engineering design becomes more facile, as each module (or subpopulation) can be tuned, modified, or even replaced in a plug-and-play manner (94). That said, synthetic microbial communities are significantly more complex to engineer than monocultures. With each additional member, the size of the interaction matrix increases geometrically, so that the transition from static monoculture to dynamic consortia

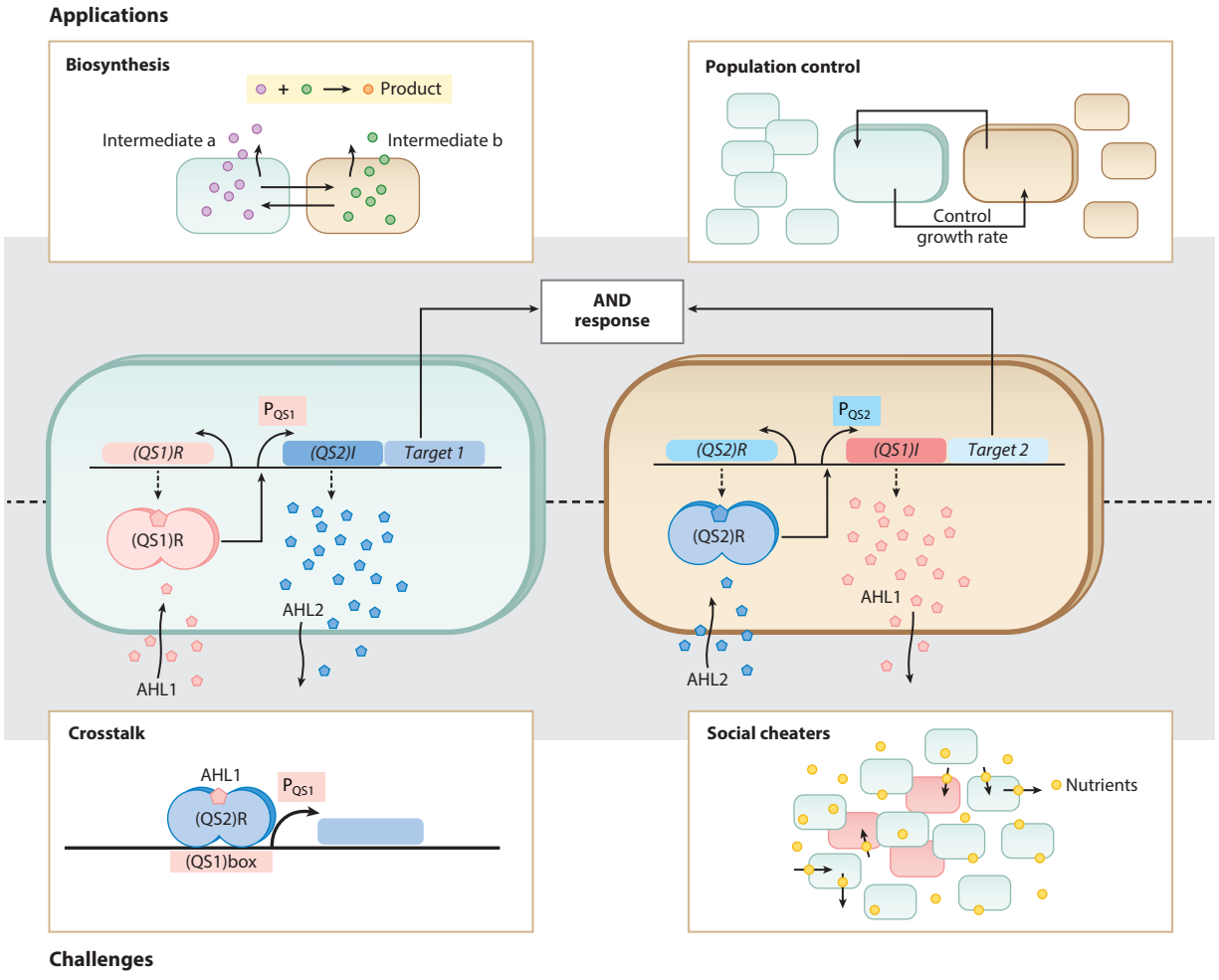


Figure 3

Applications and challenges of quorum sensing (QS)-based synthetic consortia. The middle images provide an example in which QS-communicative consortia are used to provide a collective response (95). Population 1 (*green*) expresses regulator (QS1)R and secretes AHL2 and target 1 under the control of promoter PQS1, which activates upon AHL1-bound QS1R. Population 2 (*tan*) does the reverse. Because one population makes the required signal for the other's gene expression, both cells and genetic circuits are needed to generate a response. Hence, this is an AND logic switch. Such synthetic consortia can be applied to metabolic engineering for distributing tasks among multiple strains (*top left*). Such a system can be fine-tuned by controlling the growth rate of one of the populations relative to the other (*top right*). Challenges must be overcome, however, for these systems to work effectively. One involves signal crosstalk (*bottom left*), and another, social cheaters (*bottom right*).

presents a new challenge for us to conquer. In the following sections, we discuss how QS networks may contribute to the synthetic biology toolbox that assists in the construction and deployment of synthetic microbial communities.

3.1.1. Engineering QS-based communication. Brenner et al. (95) first demonstrated a synthetic coculture composed of two engineered *E. coli* strains that was able to communicate bidirectionally and reach a consensus (see the middle panel of **Figure 3**). Two populations conversed through secretion and detection of 3-oxododecanoyl-HSL ($3OC_{12}HSL$) and butanoyl-HSL

(C_4HSL), which are AIs made by enzymes LasI and RhlI, respectively, from the QS networks of *P. aeruginosa*. In this consortium, one population relied on the signal from the other population to activate gene expression, and a consensus could be attained when both populations reached their cell-density thresholds. Remarkably, the responses were sustained for up to several days when the consortium was cultured as a biofilm. This success took scientists one step closer to engineering a living film—the consensus response could potentially be replaced into an enzyme and prodrug pair or two inactive fragments of a toxin. Complex metabolic tasks could be divided into two or more, and the intermediate pieces could be assembled to reach consensus.

Soon after, the concept of synthetic consortia with bidirectional communication became a paradigm for many similarly programmed consortia. Balagaddé et al. (96) reported an artificial ecosystem that aims to mimic the canonical predator–prey systems in terms of logic and dynamics. Here, communication was fostered by *lux* and *las* QS networks, and similarly the two populations regulated each other's gene expression via QS-rewired circuits. The predator population could kill the prey population by secreting AIs, which, in turn, induced a killer protein (CcdB) expression within the prey; meanwhile, the prey revived the predator, in which the killer protein was constitutively expressed, but with an induced antidote protein (CcdA). Recently, it was proposed and modeled in silico that this predator-and-prey architecture, or a slightly altered version in which two groups rescue each other, could turn into a population-controlled consortium (97, 98). A similar *lux*-based population control strategy was later applied to a synthetic three-species consortium for vitamin C fermentation (99). A subsequent study built a symbiotic microbial ecosystem with the aid of *lux* and *rhl* QS networks to examine the interplay between the environment and the ecosystem (100). Many population dynamics, such as extinction, mutualism, and commensalism, can be observed by tuning different levels of environmental factors (represented in this work by antibiotics) and initial cell densities. Genetic oscillations, previously made possible in monocultures, were now shown to be generated by an activator and repressor coculture (101). Two strains conversed through QS networks: *rhl* (from *P. aeruginosa*), providing the additional positive feedback loop, and *cin* (from *Rhizobium*), providing the additional negative feedback loop, with both containing an inherent AiiA-mediated negative feedback loop. Experimental and modeling data together had shown that this network topology displays more robust oscillations than those generated by just one negative feedback loop. AHL-mediated communication can also be coupled with AI-2 signaling to create an autonomously regulated consortium (102). In particular, this system consists of two populations of *E. coli*, one of which generates AHL based on nearby AI-2 levels, and the AHL concentration, in turn, affects the growth rate of the other population, resulting in a change in coculture composition. Subsequent programming enables composition trajectories and control. Together, these studies portend engineering of complex synthetic populations, perhaps bacteria in combination with tissues and even organs composed of multiple cell types.

Cocultured cellular networks can also serve as effective biosensors. Terrell et al. (103) described assembly of a nano-guided QS information processor, which included two cell populations that independently interrogate natural microbial communities and autonomously generate information about QS activity by accessing AI-2. They were used to eavesdrop on the dialogue initiated by Gram-positive *Listeria innocua* in complex media. Populations displayed either red or green fluorescent proteins on their outer surfaces and were designed to detect lower and higher levels of AI-2, respectively. With the help of streptavidin-binding protein that was expressed on their outer surfaces and exogenously added magnetic nanoparticles, both populations reported on AI-2 secreted by *L. innocua* and were binned by their fluorescence responses. The magnetic nanoparticles enabled an unbiased collection of the sensing cells and at the same time focused the signal responses. This multidimensional setup combined biotic and abiotic features for the active probing of molecular space and translated the molecular dialogue into light signals that were easy to bin

and interpret. The success of these studies has proven QS-based communication to be an effective, modular, and robust icebreaker for initiating communication in a microbial society.

3.1.2. Engineering a microbial consortium: challenges. Despite their utility for engineering microbial consortia, QS systems have several drawbacks that limit their use as field-deployable communication networks. First and foremost is the crosstalk (see challenges in **Figure 3**) between different QS networks at both the promoter and signal levels (104). Signal crosstalk occurs when a receptor can bind its noncanonical AI. LuxR is known to bind $3OC_{12}HSL$, the AI native to another QS system, the *las* system. Promoter crosstalk occurs when activated receptors can bind a noncanonical promoter. For instance, Brenner et al. (95) encountered this issue when pairing QS networks *rhl* and *las*. Specifically, high levels of activated LasR were found to initiate the *rhl* promoter. A combination of signal and promoter crosstalk is also possible, whereby a receptor that is activated by a noncanonical AI binds to a noncanonical promoter.

That said, if parts are well-characterized, such crosstalk can be harnessed to create unique dynamic circuits. Initially, most endeavors were made to avoid crosstalk; for example, a positive-feedback loop on the I proteins was incorporated into the design of the bidirectional communication circuit to mitigate promoter crosstalk between *LasR* and *rhl* (95). However, it is critical that additional QS systems with complete orthogonality be developed. Via rational promoter and protein engineering, Scott & Hasty (104) adapted two new QS systems, the *rpa* system from *Rhodopseudomonas palustris* and the *tra* system from *Agrobacterium tumefaciens*, into *E. coli* to expand upon the extensively used *lux* and *las* systems. Notably, engineered *rpa* and *tra* systems displayed complete orthogonality, while signal and promoter orthogonality were observed between *rpa/lux* and *tra/las* QS systems, respectively. Another recent study systematically characterized six commonly used QS systems, the *lux*, *las*, *tra*, *rpa*, *rhl*, and *cin* networks, and developed a software tool that automatically identifies combinations of receptors and AIs that behave orthogonally within a given AI concentration regime (105). The software predictions were carefully validated through experimental characterization of synthetic *E. coli* consortia that, in turn, implemented three orthogonal communication channels: *rhl*, *lux*, and *las*. Use of different classes of QS systems, such as the AI-2/LuxS system or Gram-positive signaling oligopeptide systems, in parallel with the *lux*-like systems has also demonstrated orthogonality (23, 106).

Another challenge in consortia engineering would be the presence of cheaters in a microbial community (see challenges in **Figure 3**). Microbial social cheaters rely on public goods or other beneficial collective actions to survive, but they do not contribute. These noncooperating populations pose a potential threat to the robustness of consortia. Studies have shown, however, that native consortia can stably maintain coexistence with and prevent proliferation of these external populations compared with high-fitness monocultures that are more prone to be exploited by cheaters (107). While it was postulated that employing QS could also be a way to reward cooperators and thus aid in eliminating cheaters (108), QS was also shown to be exploitable in many laboratory cultures (109). One illustrative case concerned the opportunistic pathogen *P. aeruginosa*, which relies on QS to induce the production of extracellular proteases that are required for growth on proteins. It was observed that QS mutants were able to survive when in coculture with QS-competent cells (110). This phenomenon subsequently led to an ongoing debate as to why there remain numerous functional QS systems that are maintained in nature, especially if QS systems are so easily exploited or circumvented. Policing, the ability of cooperators or hosts to hinder the fitness of cheaters, could be one of the possible reasons; this idea was used to introduce the concept of punishing freeloaders (111). Majerczyk et al. (112) described how QS regulation of pairs of genes coding for toxins and toxin immunity serve as a policing mechanism. In *Burkholderia thailandensis*, those that are QS competent deliver toxins to other individuals. Although the

QS-competent cells are immune, QS mutants are not. Perhaps this scheme can be incorporated into future circuit and consortium designs to help create more robust communities that repress or eliminate social cheaters.

3.2. Interkingdom Consortia: Engineering Communication Networks

As described earlier, the AI-2/LuxS system was shown to be distinct relative to the *lux* systems in many Gram-negative bacteria, and it is been dubbed the bacterial Esperanto because a plethora of both Gram-negative and Gram-positive bacteria have been reported to synthesize AI-2 and putatively use AI-2 as a signal molecule. Because this LuxS-mediated AI-2 synthesis system is so widespread, it is only natural to think of its perception as a way of delineating its role as a signal molecule. That is, there is significant diversity in the uptake/signal transduction systems across many genera, including Gram positives and Gram negatives. The canonical *lsr* operon, found in *E. coli*, consists of the genes noted in **Figure 1*b***. Quan & Bentley (18) showed how some of these genes (e.g., *lsrRK*, *tam*) are absent in some strains, whereas their order and regulatory regions are different in others, providing great diversity in the way AI-2 is perceived. The net result of this is that evolutionary pressures may have led to distinct patterns by which AI-2 could be taken up or processed and thereby used as a signal molecule. This is completely orthogonal to its synthesis as a metabolic by-product. As such, it may be possible that next-generation antimicrobials could be created by intercepting intra- and interspecies bacterial communication for the creation of smart, disease-fighting bacteria. Instead of targeting the viability of pathogenic strains, interruption of their communication is proposed, as there may be less selective pressure to develop resistance if instead one targets the mechanisms that key pathogenicity (113). This idea is not new for small-molecule drugs, but to our knowledge not as mediated by bacteria. Because it is an AI, inhibition of the signal AI-2 could possibly lead to decreased virulence in a variety of bacterial species. Many parts of the AI-2/LuxS system, from signal generators (Pfs and LuxS) to signal receptors (LsrK, LsrR), are likely targets for inhibition, especially because many synthesized AI-2 analogs are available for quorum quenching (45–47, 114). For example, commensal *E. coli* were engineered to increase AI-2 levels in the mouse gut. During streptomycin-induced dysbiosis, these AI-2-producing *E. coli* promoted gut colonization by *Firmicutes* over *Bacteroidetes* (115), whereas added streptomycin massively favored the *Bacteroidetes* and inhibited *Firmicutes*. This offered an exciting possibility that by altering AI-2, one could ameliorate the effect of an applied antibiotic on microbiota-derived functions. This success suggests that continued efforts to engineer strains with the intent to bias microbiome signaling (52) will surely emerge. Additionally, AI-2-producing *Ruminococcus obeum* were also shown to be vital for defeating *Vibrio cholerae* infection and facilitating recovery (116). *R. obeum* restricted colonization of *V. cholerae* through upregulation of the *luxS* gene to produce more AI-2, and in turn, AI-2 displayed QS-mediated repression of several *V. cholerae* colonization factors. Whereas AI-2 signaling was found to be critical in native gut environments, LuxI/R-type systems have not been detected in the normal, healthy gut. This provides another possible opportunity to interrogate and manipulate communication for positive gain. A recent study constructed an information-transfer system to probe whether the *lux* QS system can be repurposed into a functional, artificially established language in the mammalian gut (117). Both interspecies and intraspecies communication were made possible despite some complexities in in vivo studies. Together, these studies promise to underpin many valuable uses of QS networks in the future to either promote or interrupt communication, not just at the species level but to affect a whole microbiome.

3.2.1. Interkingdom and beyond. QS bacteria are also observed reaching out to eukaryotes. Indeed, QS-communicating bacteria and their components are emerging at many interfaces,

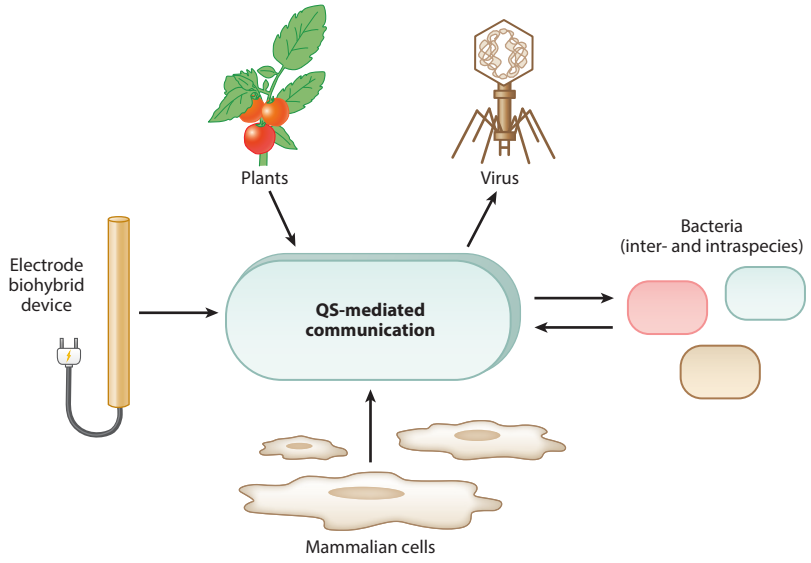


Figure 4

Native and engineered quorum sensing (QS)-mediated communication can be used to target a variety of areas. QS bacteria not only are observed to display both intra- and interspecies communication (*right*) but also are capable of interpreting signals from eukaryotes, such as plants (*top left*) and mammalian cells (*bottom*). Recently, viruses (*top right*) have been found to respond to and make decisions based upon host-produced acyl-homoserine lactones (AHLs). Communications between abiotic materials and QS-communicating bacteria (*left*) by QS-component assembly on gold electrodes or on/within artificial cells.

including in interactions with viruses, eukaryotic cells and organisms (e.g., plants), artificial cells, biomaterials, and even electronic devices (**Figure 4**). Orphan, or solo, LuxR homologs were first discovered in *Salmonella typhimurium*, a bacterium that was reported as lacking a LuxI homolog and the ability to produce AHL-type AIs. It was further discovered that an *E. coli* LuxR homolog, *sdiA*, responds to mammalian host-produced small molecules (118). This discovery hinted at the possibility that LuxR homologs, instead of acting as QS receptors, were sensors of the host environment. Many orphan LuxR homologs were then found in plant-associated bacteria, regulating plant–bacterial interactions through detection of small molecules secreted by the host (119). Contrarily, host cells could respond to AIs secreted by their commensal bacteria. RNA-sequencing technology revealed that human colonic cell line HCT-8 expresses inflammatory cytokine interleukin 8 in response to AI-2 secreted by nonpathogenic *E. coli* (120). Surprisingly, Silpe & Bassler (121) recently revealed that vibriophage VP882 can respond to a *V. cholerae*–produced QS AI (DPO). Once bound to DPO, the phage QS receptor $VqmA_{Phage}$ in turn activates the phage lytic program. Activated $VqmA_{Phage}$ can even recognize the host *vqmR* promoter and influence its QS behavior. This is the first case reported to show that viruses can eavesdrop on their hosts and decide their actions based on what they have heard.

Finally, when considering more biotechnological objectives, we note that QS systems have also enabled cell signaling to pass from biological niches to abiotic and even microelectromechanical systems. Although this could be the topic of a far more extensive review, we note a few examples that are logical extensions of the above work in that the molecular components of AI-2 QS are abstracted and put in play to mediate bio-/device signaling. For example, to understand the interplay between QS signal molecules and human epithelial cells, a nanofactory consisting of the two

terminal AI-2 synthases, Pfs and LuxS, and a targeting antibody was created and electroassembled into microfluidic devices, where it was used to capture cells and stimulate their QS responses (122, 123). Analogously, the construct was loaded onto receptor molecules of human intestine epithelial cells, where they stimulated QS activity among nearby commensal *E. coli* (123, 124). The same enzyme construct was later shown to be grafted onto spider silk and subsequently wound into place in a microfluidic device, where its molecular signaling activity could be localized with minimal machine guidance or intervention (125). In another example, Lentini et al. (126) engineered minimal artificial cells capable of expressing AI-2 synthesizing fusion protein HLPT (His_6-LuxS-Pfs-Tyr_5) (122), wherein newly synthesized AI-2 was proven to induce luminescence in nearby cells. The same HLPT fusion was shown to be electrically assembled onto gold electrodes, where its activity was controlled electrochemically (127) by simple applied voltage and redox actuation. In all of these systems, AI-2 is synthesized in carefully controlled environments and in ways that are programmed by external inputs—some even electronic. That is, biomolecular synthesis reactions, even pathways, are carried out in well-controlled microsystems so that the kinetics and mass transfer processes can be controlled, designed, and even optimized. Such complex microfluidic environments are recreated on chips for preclinical drug development and toxicity screening. These studies ultimately suggest that, by bridging with nonbiological materials, there are many opportunities to build novel microelectronic, biohybrid devices that can alter the complex networks of natural cells without tampering with the original genetic makeup. In turn, these can be used in drug development studies as well as detailed biological studies in which distances and dynamics are of the same scale as the cells and molecules themselves.

4. CONCLUSION AND FUTURE OUTLOOK

QS has provided researchers with a variety of novel platforms or techniques from which to address biotechnological problems. In this article, we addressed the versatility of native QS and the numerous strategies aiming to repurpose QS systems to program the behavior of a single cell, a cell consortium, or even a microbiome. QS advances synthetic biology by offering various genetic building blocks that can be reassembled into functional circuits to regulate gene expression and biological phenotype. Owing to the engineered QS circuitry, cells are endowed with smart functions, such as user-specified sensing and reporting, in situ drug delivery, and sophisticated biosynthesis processes. Further, as researchers have attempted to build more complex functions into a single cell, they have realized that these may be too much responsibility for one microbe to carry. Hence, looking again to nature for guidance, many groups have turned to engineering multispecies consortia that are considered to be more robust than monocultures. In this way, rewired QS networks can help create a synthetic microbial community in which members actively interact with each other with end user–designed guidance. These activities feed well into the emergence of systems biology tools that enable detailed interrogation of various microbiomes. Finally, QS systems and their components can allow direct interaction with abiotic materials, creating biohybrid, microelectronic devices that may integrate with our daily lives. We believe these innovative QS-based methods will no doubt continue to generate impactful applications in the future.

SUMMARY POINTS

1. Quorum sensing (QS), a cell–cell communication process in bacteria, can be repurposed to facilitate many engineering applications.

2. QS-based genetic circuits can endow cells with smart functions that can serve many purposes.
3. QS-mediated cell–cell communication can aid in the interrogation of natural microbial communities and the engineering of synthetic consortia.
4. Rewired QS systems can also allow communication with abiotic materials to create biohybrid, microelectronic devices.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Errata

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